Module 7

Cellular Processes

Synopsis

Signalling pathways use different messenger systems acting through specific sensors and effectors to control a great variety of cell types (Module 7: Table cell inventory). Each specialized cell type has developed control mechanisms to suit their particular functions. For example, skeletal and cardiac muscle cells have very rapid signalling mechanisms capable of driving contraction within milliseconds. Smooth muscle cells tend to act more slowly, and are far more diverse in both their function and control mechanisms. Some cells contract in a conventional way in response to an external agonist, while others have more complex control mechanisms responsible for maintaining the tonic contraction of the smooth muscle cells surrounding blood vessels.

Another example is cell secretion, where there is similar diversity in function and control. Preformed secretory products packaged into vesicles are released through a process of exocytosis. In exocrine glands and the intestine, there is a process of fluid secretion that depends upon the active transport of ions to provide the driving force for a parallel flow of water. Secretion is evident in many different cell types and cellular processes, such as transmitter release during presynaptic events in neurons, enzyme and fluid secretion by pancreatic acinar cells, release of insulin by insulin-secreting β-cells and inflammatory mediators by mast cells.

Many of these mammalian cell types are controlled by more than one signalling pathway, and the object here is to understand how signalling pathways and their downstream sensors and effectors function to bring about a change in cellular activity in some typical examples of specialized cell types.

Mammalian cell types

There are a large number of mammalian cell types (Module 7: Table cell inventory). They are adapted to perform highly specific functions, which are controlled by signalling systems uniquely adapted to carry out their particular functions. These cell-specific signalsomes are a selection from the diverse array of intracellular signalling pathways (Module 2: Figure cell signalling pathways). During the process of cell differentiation, each primary cell type expresses those signalling pathways that are particularly suited to control their particular functions as illustrated by some of the main mammalian cell types shown below:

Gametes
- Oocytes
- Spermatozoa

Stem cells
- Intestinal stem cells
- Satellite cells
- Haematopoietic stem cells (HSCs)
- Epidermal stem cells
- Melanocyte stem cells
- Mesenchymal stem cells
- Mammary gland stem cells
- Male germ cells
- Neural stem cells

Nervous system
- Cortex
  - Dorsolateral prefrontal cortex (DLPFC)
    - Cortical pyramidal neuron
    - Cortical inhibitory interneurons
    - Cajal-Retzius neuron
    - Chandelier neurons
    - Double bouquet neurons
    - Martinotti interneurons
    - Wide arbor (basket) interneurons
  - Hippocampus
    - Hippocampal CA1 neurons
    - Hippocampal CA3 neurons
    - Granule cells
    - Hippocampal interneurons
      - Axo-axonic cell
      - Basket cell (PV^+ CCK^-)
      - Basket cell (PV^- CCK^+)
      - Basket cell (PV^- CCK^+ VIP^+)
      - Bis-stratified cell
      - Oriens-lacunosum molecular (O-LM) cell
      - Schaffer-collateral-associated cell
      - Lacunosum-moleculare-perforant path (LM-PP)

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- Lacunosum-moleculare-radiatum-perforant path (LM-R-PP)
- Trilaminar cell
- Back-projecting cell
- Hippocampal septal cell
- Cerebellum
  - Cerebellar Purkinje neurons
  - Cerebellar climbing fibre
  - Basket cells
- Neocortical neurons
- Starburst amacrine cells
- Thalamic interneurons
- Suprachiasmatic nuclei (SCN)
- Medium spiny neurons
  - D1 dopamine receptor-expressing MSNs (D1+ MSNs)
  - D2 dopamine receptor-expressing MSNs (D2+ MSNs)
- Hypothalamus
  - Magnocellular (MCN) neurons
    - Oxytocin neurons
  - Vasopressin neurons
- Anterior pituitary
  - Corticotrophs
  - Gonadotrophs
  - Lactotrophs
  - Somatotrophs
  - Thyrotrophs
  - Folliculostellate (FS) cells
- Pineal gland

Sensory systems

- Hearing
  - Outer hair cells
  - Inner hair cells
- Hypoxia-sensing
  - Glomus cells (see carotid body Module 10: Figure carotid body chemoreception)
  - Sustentacular cell
  - Neuroepithelial bodies
- Nociception
  - Aα sensory fibres
  - Aβ sensory fibres
  - Aδ sensory fibres
  - C fibres
- Olfaction
  - Olfactory receptor cells
- Osmoreception
  - Organum vasculosum of the lamina terminalis (OVLT) neuron
  - Subfornical organ (SFO) neuron
- Photoreception
  - Cones
  - Rods
- Taste
  - Type I glial-like cells
  - Type II receptor cells
  - Type III presynaptic cells
- Touch
  - Merkel cells
- Glial cells
  - Astrocytes
  - Bergmann glia
  - Müller cells
  - Microglia
  - Pituicytes
  - Oligodendrocytes
  - Satellite glial cells
  - Schwann cells

Endothelium

- Endothelial cells

Skeletal muscle

- Slow-twitch muscle
- Fast-twitch muscle IIa
- Fast-twitch muscle IId/x
- Fast-twitch muscle IIba
- Satellite cells

Cardiac cells

- Sinoatrial node pacemaker cells
- Purkinje fibres
- Atrial cells
- Ventricular cells

Smooth muscle

Smooth muscle pacemaker cells

- Interstitial cells of Cajal
- Atypical smooth muscle cells

Smooth muscle cells

- Airway smooth muscle cells
- Corpus cavernosum smooth muscle cell
- Detrusor smooth muscle cell
- Gastrointestinal smooth muscle cells
- Ureter smooth muscle cells
- Urethral smooth muscle cells
- Uterine smooth muscle cells
- Vascular smooth muscle cells
- Vas deferens

Parathyroid gland

- Chief cells

Thyroid gland

- Thyroid follicular cells
- C cells

Bone/cartilage

- Osteoblasts
- Osteoclasts
- Osteocytes
- Chondrocytes

Skin

- Arrector pilli muscle
- Keratinocytes
- Melanocytes
- Sebaceous gland
- Sweat gland secretory coil cells
- Sweat gland reabsorptive duct cells

Alimentary canal

- Stomach
  - Parietal cells
  - D cells
  - G cells
  - Enterochromaffin-like cell (ECL cell)
  - X/A-like cells
- Intestine

- Small intestine
  - Enterochromaffin cell
  - L cell
  - Colon
- Kidney
  - Kidney tubule cells
    - Proximal convoluted tubule (PCT)
    - Loop of Henle
    - Distal convoluted tubule (DCT)
    - Collecting duct cells
  - Juxtaglomerular apparatus
    - Extraglomerular mesangial cells (lacis cells)
    - Intraglomerular mesangial cells
    - Macula densa cells
    - Renin-producing glomerular cells
- Salivary gland
- Pancreas
  - Islets of Langerhans
    - Glucagon-secreting α-cells
    - Insulin-secreting β-cells
  - Exocrine pancreas
    - Exocine pancreatic acinar cells
    - Exocrine pancreatic centroacinar cells
- Liver
  - Liver cells
  - Kupffer cells
  - Hepatic stellate cells
- Adrenal gland
  - Adrenal cortex
    - Zona glomerulosa cells
    - Zona fasciculata/reticularis cells
  - Adrenal medulla
    - Chromaffin cells
- Adipose tissue
  - White fat cells
  - Brown fat cells
- Haematopoietic cells
  - Blood platelets
  - B cells
  - T cells
  - Neutrophils
  - Macrophages
    - Tingible body macrophages
  - Mast cells

In many cases, different cell types are collected together into complex functional networks:

- Neural circuits in the brain are built up from interactions between different neurons, as occurs in the cerebellum (Module 10: Figure cerebellar neural circuit).
- Astrocytes can provide a platform for interactions with both neurons and smooth muscle cells (Module 7: Figure astrocyte structure).
- The metabolic energy network brings together cells that function in energy storage (liver and white fat cells), energy consumption (skeletal muscle, neurons and white fat cells) and cells that provide the hormones that regulate energy usage (insulin-secreting β-cells, glucagon-secreting α-cells and adrenal cells) (Module 7: Figure metabolic energy network).
- Control of food intake and body weight depends upon interactions between endocrine and neural signalling systems that act on the feeding and satiety centres in the brain (Module 7: Figure control of food intake).
- Sites of inflammation and wounding draw together many of the cells such as macrophages, neutrophils, mast cells and endothelial cells that co-operate with each other to fight off infections and to restore tissue integrity (Module 11: Figure inflammation).
- A number of cell types contribute to Ca^{2+} homoeostasis (Module 7: Figure Ca^{2+} homoeostasis).
- Blood pressure is regulated by a complex cellular and hormonal network responsible for blood Na^{+} regulation (Module 7: blood pressure control).
- Bone remodelling depends upon an interaction between the different bone cells (Module 7: Figure bone cells).

Metabolic energy network

The primary function of the metabolic energy network is to manage the energy status of the cell (Module 7: Figure metabolic energy network). Energy is derived from the intake of food, which is processed in the intestine to form the high-energy metabolites such as amino acids, free fatty acids and glucose that then circulate in the plasma to be dealt with by cells that participate in the metabolic network. In addition, there are other cells that monitor various parameters of the energy supply and then release appropriate hormonal signals to make adjustments to different components of the network. The energy supply is dealt with in two ways: most of the energy is consumed by a variety of cellular processes, while any excess is then stored either as triacylglycerols in the white fat cells or as glycogen in the liver cells. Metabolic homoeostasis depends on energy consumption closely matching energy supply. If consumption exceeds supply, then energy is mobilized from the energy stores to maintain equilibrium. Since the energy stores are finite, such an imbalance in energy supply begins to cause severe wasting as the process of autophagy kicks in to begin to mobilize energy from the structural components of cells. Conversely, if the supply of energy exceeds energy consumption, the excess is diverted into the energy stores, resulting in obesity. This balance between energy uptake and expenditure is determined by the control of food intake and body weight.

The numbers on Module 7: Figure metabolic energy network refer to some of the major components of the network that determines energy homoeostasis:

1. The alimentary canal deals with the intake of food and produces the high-energy components such as fatty acids and glucose, which constitute the energy supply that is then either consumed to maintain cellular processes or stored in the white fat cells and liver.
2. An increase in the level of glucose following a meal provides a signal to activate the insulin-secreting β-cells (Module 7: Figure β-cell signalling). In addition, subsets of neurons in the brain respond to glucose, with some increasing and others decreasing their firing rates.

Module 7: | Table cell inventory
An inventory of mammalian primary cells that have specific cell functions.

Tissue and cell	Comments
Gametes	Male and female gametes participate in the process of fertilization (Module 8: Figure mammalian fertilization)
Oocytes	
Spermatozoa	
Stem cells	
Intestinal stem cells	Located in the crypt region of the intestine (Module 12: Figure colon cancer)
Satellite cells	Skeletal muscle stem cells that function in muscle repair and regeneration (Module 8: Figure Satellite cell function)
Haematopoietic stem cells (HSCs)	Located in the bone marrow and gives rise to common lymphoid progenitor (CLP) and the common myeloid progenitor (MLP) cells (Module 8: Figure haematopoiesis)
Epidermal stem cells	Function in the hair follicle cycle (Module 8: Figure hair follicle cycle)
Melanocyte stem cells	Provide melanocytes during the hair follicle cycle (Module 8: Figure hair follicle cycle)
Mesenchymal stem cells	The precursor of a wide range of primary cells Module 8: Figure MSC differentiation
Mammary gland stem cells	
Male germ cells	
Neural stem cells	
Skeletal muscle	See Module 7: Figure skeletal muscle E-C coupling
Slow-twitch muscle	MyHC I; SERCA2a
Fast-twitch muscle IIa	MyHC IIa; SERCA1
Fast-twitch muscle IId/x	MyHC IId/x; SERCA1
Fast-twitch muscle IIba	MyHC IIb; SERCA1
Satellite cells	Skeletal muscle stem cells
Cardiac muscle	
Sinoatrial node cells	Responsible for the cardiac pacemaker (Module 7: Figure cardiac pacemaker)
Purkinje fibres	Specialized cells that conduct action potentials to the ventricular cells
Atrial cells	Responsible for atrium contraction (Module 7: Figure atrial Ca^{2+} domains)
Ventricular cells	Responsible for ventricle contraction (Module 7: Figure ventricular cell E-C coupling)
Smooth muscle	Responsible for contraction of smooth muscle (Module 7: Figure smooth muscle cell E-C coupling)
Atypical smooth muscle cell	Smooth muscle pacemaker cell (Module 7: pelviureteric organization)
Interstitial cells of Cajal	Smooth muscle pacemaker cell (Module 7: Figure ICC pacemaker)
Airway smooth muscle cells	See Module 7: Figure bronchiole-arteriole contraction
Corpus cavernosum smooth muscle cell	See Module 7: Figure corpus cavernosum
Detrusor smooth muscle cell	See Module 7: Figure bladder SMC activation
Gastrointestinal smooth muscle cells	See Module 7: Figure ICC pacemaker
Urethral smooth muscle cells	See Module 7: Figure ICC pacemaker
Uterine smooth muscle cells	See Module 7: Figure uterus activation
Vascular smooth muscle cells	See Module 7: Figure SMC cytosolic oscillator
Vas deferens	See Module 7: Figure vas deferens activation
Nervous system	
Cortex	
Dorsolateral prefrontal cortex (DLPFC)	See Module 10: Figure dorsolateral prefrontal cortex
Cortical pyramidal neuron	
Cortical inhibitory interneurons	
Cajal–Retzius neuron	
Chandelier neurons	
Double bouquet neurons	
Martinotti interneurons	
Wide arbor (basket) interneurons	
Hippocampus	See trisynaptic hippocampal circuit (Module 10: Figure hippocampus)
Hippocampal CA1 neurons	
Hippocampal CA3 neurons	See Module 10: Figure CA3 hippocampal neurons
Granule cells	
Hippocampal interneurons	See Module 10: Figure hippocampal interneurons
Axo-axonic cell	See Module 10: Figure CA3 hippocampal neurons
Basket cell (PV^+ CCK^-)	
Basket cell (PV^- CCK^+)	
Basket cell (PV^- CCK^+ VIP^+)	
Bis-stratified cell	
Oriens-lacunosum molecular (O-LM) cell	
Schaffer-collateral-associated cell	See Module 10: Figure CA3 hippocampal neurons
Lacunosum-moleculare-perforant path (LM-PP)	
Lacunosum-moleculare-radiatum-perforant path (LM-R-PP)	
Trilaminar cell	
Back-projecting cell	
Hippocampal septal cell	
Hypothalamus	See Module 10: Figure hypothalamic pituitary regulation
Magnocellular (MCN) neurons	See Module 10: Figure magnocellular neurons
Oxytocin neurons	See Module 10: Figure oxytocin neuron

Module 7 | Table continued

Tissue and cell	Comments
Vasopressin neurons	See Module 10: Figure vasopressin neuron
Cerebellum	See Module 10: Figure cerebellar neural circuit
Cerebellar Purkinje neurons	See Module 10: Figure Purkinje cell input-specific Ca^{2+} signals
Cerebellar climbing fibre	See Module 10: Figure cerebellar climbing fibres
Basket cells	See Module 10: Figure basket cell Ca^{2+} transients
Starburst amacrine cells	Directionally selective retinal ganglion cells
Thalamic interneurons	See Module 10: Figure dendritic GABA release
Suprachiasmatic nuclei	Location of the circadian clock neurons (Module 6: Figure circadian clock location)
Medium spiny neurons	See Module 10: Figure medium spiny neuron signalling
D1+ MSN	
D2+ MSN	
Hypothalamic neurons	See Module 10: Figure hypothalamic Ca^{2+} syntilla
Pineal gland	
Pinealocytes	Synthesize and secrete melatonin
Sensory systems	
Hearing	
Outer hair cells	
Inner hair cells	See Module 10: Figure hair cell
Hypoxia-sensing	Functions in O_2 sensing (Module 10: Figure carotid body chemoreception)
Glomus cells	Associates with the glomus cell
Sustentacular cell	
Neuroepithelial bodies	
Nociception	See Module 10: Figure inflammatory soup
Aα sensory fibres	Function in proprioception
Aβ sensory fibres	Function in proprioception
Aδ sensory fibres	Function in nociception
C fibres	Function in nociception
Olfaction	
Olfactory receptor cells	See Module 10: Figure olfaction
Osmoreception	
Organum vasculosum of the lamina terminalis (OVLT) neuron	
Subfornical organ (SFO) neuron	
Photoreception	See Module 10: Figure rod and cone structure
Cones	
Rods	
Taste	See Module 10: Figure taste receptor cells
Type I glial-like cells	
Type II receptor cells	
Type III presynaptic cells	
Touch	
Merkel cells	See Module 10: Figure Merkel cell
Glial cells	
Astrocytes	See Module 7: Figure astrocyte structure
Bergmann glia	See Module 7: Figure Ca^{2+} microdomains in Bergmann glia
Microglia	See Module 7: Figure microglia interactions
Müller cells	
Pituicytes	See Module 10: Figure magnocellular neurons
Oligodendrocytes	
Satellite glial cells	
Schwann cells	
Anterior pituitary	See Module 10: Figure hypothalamic pituitary regulation
Corticotrophs	See Module 10: Figure corticotroph regulation
Gonadotrophs	See Module 10: Figure gonadotroph regulation
Lactotrophs	See Module10: Figure lactotroph regulation
Somatotrophs	See Module 10: Figure somatotroph regulation
Thyrotrophs	See Module 10: Figure thyrotroph regulation
Folliculostellate (FS) cells	
Adrenal gland	See Module 7: Figure adrenal gland
Zona glomerulosa	See Module 7: Figure glomerulosa cell signalling
Zona fasciculata/reticularis cells	See Module 7: Figure adrenal gland
Chromaffin cell	See Module 7: Figure chromaffin cell secretion
Parathyroid gland	
Chief cell	See Module 7: Figure parathyroid gland
Stomach	See Module 7: Figure stomach structure
Parietal cell	See Module 7: Figure parietal cell
D cell	See Module 7: Figure stomach structure
G cell	See Module 7: Figure stomach structure
Enterochromaffin-like cell	See Module 7: Figure stomach structure
X/A-like cells	See Module 7: Figure stomach structure
Intestine	See Module 7: Figure small intestine
Small intestine	See Module 7: Figure intestinal secretion
Enterochromaffin cell	See Module 7: Figure small intestine
L cell	See Module 7: Figure L cell
Colon	See Module 7: Figure colon function

Module 7 | Table continued

Tissue and cell	Comments
Pancreas	
Islets of Langerhans	See Module 7: Figure pancreas
α-Cells	See Module 7: Figure α-cell signalling
Insulin-secreting β-cells	See Module 7: Figure β-cell signalling
Exocrine pancreas	See Module 7: Figure exocrine pancreas
Exocrine pancreatic acinar cells	See Module 7: Figure control of pancreatic secretion
Exocrine pancreatic centroacinar cells	
Salivary gland	See Module 7: Figure salivary gland secretion
Liver	See Module 7: Figure liver cell structure
Liver cells	See Module 7: Figure liver cell signalling
Hepatic stellate cell	See Module 7: Figure hepatic stellate cell
Kupffer cell	
Adipose tissue	
White fat cells	See Module 7: Figure lipolysis and lipogenesis
Brown fat cells	See Module 7: Figure brown fat cell
Endothelium	
Endothelial cells	See Module 7: Figure endothelial cell
Haematopoietic cells	Many of these cells participate in inflammation (Module 11: Figure inflammation)
Blood platelets	See Module 11: Figure platelet activation
B cells	See Module 9: Figure B cell activation
T cells	See Module 9: Figure TCR signalling
Neutrophils	See Module 11: Figure neutrophil chemotactic signalling
Macrophages	See Module 11: Figure macrophage signalling
Tingible body macrophages	See Module 8: Figure germinal centre
Mast cells	See Module 11: Figure mast cell signalling
Kidney	See Module 7: Figure kidney tubule
Proximal convoluted tubule	See Module 7: Figure kidney tubule
Loop of Henle	See Module 7: Figure kidney tubule
Distal convoluted tubule	See Module 7: Figure kidney tubule
Collecting duct cells	See Module 7: Figure collecting duct function
Juxtaglomerular apparatus	See Module 7: Figure juxtaglomerular apparatus
Mesangial cell	See Module 7: Figure mesangial cell
Macula densa	See Module 7: Figure macula densa
Renin-producing glomerular cell	See Module 7: Figure renin secretion
Bone/cartilage	
Osteoblasts	See Module 7: Figure osteoblast function
Osteoclasts	See Module 7: Figure bone cells
Stromal cells	
Chondrocytes	See Module 7: Figure bone cells
Skin	See Module 7: Figure skin cells
Arrector pilli muscle	See Module 7: Figure skin cells
Keratinocytes	See Module 7: Figure melanogenesis
Melanocytes	See Module 7: Figure melanogenesis
Merkel cells	See Module 10: Figure Merkel cell
Sebaceous gland	See Module 7: Figure skin cells
Sweat gland secretory coil cells	See Module 7: Figure sweat gland function
Sweat gland reabsorptive duct cells	See Module 7: Figure sweat gland function

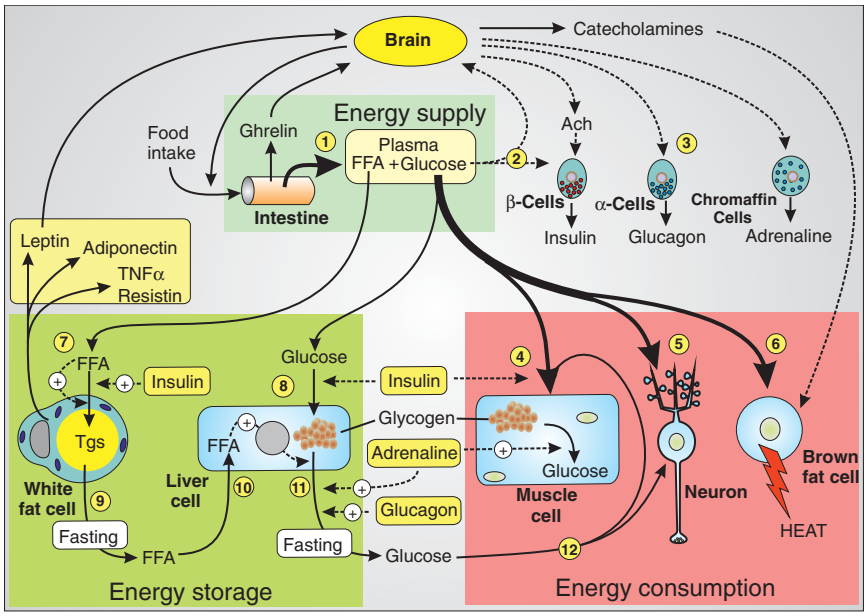
Each cell type has a unique signalling system that is put in place during development. There are a large number of intracellular signalling pathways (Module 2: Figure cell signalling pathways) and each cell type selects out those pathways to establish a cell-specific signalsome that enables it to precisely regulate its activity.

3. When plasma glucose levels are low, the glucagon-secreting α-cells are stimulated to release glucagon (Module 7: Figure α-cell signalling), which then act to mobilize glucose from liver cells (Step 11 in Module 7: Figure metabolic energy network).

All cells have to consume energy to survive and to carry out their different functions. Three examples of energy-consuming cells are illustrated in Module 7: Figure metabolic energy network:

4. Skeletal muscle uses glucose for contraction. While it is shown here as an energy consumer, it also plays a role in energy storage because it is able to take up excess glucose and store it as glycogen. This storage of glycogen is regulated by insulin-induced glycogen synthesis. There are sophisticated feedback loops, such as the Ca^{2+}-dependent breakdown of glycogen, that ensure that ATP levels remain relatively constant during normal contraction (Module 7: Figure skeletal muscle E-C coupling). During intense activity, usually associated with stress, energy metabolism can be enhanced further through a process of adrenaline (epinephrine)-induced glycogenolysis (Steps 6–8 in Module 7: Figure skeletal muscle E-C coupling).
5. Neurons are an example of typical energy-consuming cells that are totally reliant for their function on a continuous supply of energy. It is one reason why blood glucose levels have to be regulated so carefully.
6. Brown fat cells consume energy to produce heat. This process of thermogenesis depends on the expression of uncoupling protein 1 (UCP1), which provides a leak pathway for protons to enter the mitochondria without producing ATP, and the energy consumed then appears as heat (Module 7: Figure brown fat cell).

Module 7: | Figure metabolic energy network



Metabolic homoeostasis depends upon interactions between cell types that either carry out energy metabolism or provide the hormones responsible for regulation.

Energy derived from food intake enters the plasma from the intestine and is then distributed to cells involved in energy consumption, while any excess is dealt with by cells that function in energy storage. The brain plays a critical role in relaying information to endocrine cells that release the hormones that function in regulating this energy network. The neural and endocrine control of food intake and body weight is described in Module 7: Figure control of food intake.

The two major energy storage cells are the white fat cells and the liver cells. They are specialized to store energy when it is in excess and then deliver it to the energy consumers during periods of fasting (Module 7: Figure metabolic energy network):

7. White fat cells are specialized to store energy as fat in the form of triacylglycerols. Fat can be synthesized from lipids, carbohydrates or amino acids, but the main source is dietary fat, with about 10% coming from the uptake of glucose. Lipoprotein lipase on the surface of endothelial cells hydrolyses triacylglycerols to free fatty acids (FFAs), which are metabolic messengers. These FFAs are taken up by the white fat cells, and to a lesser extent by the liver cells. FFAs are also carried to these storage cells as a complex with albumin. The uptake and storage of these fatty acids is enhanced by insulin (Module 7: Figure lipolysis and lipogenesis). In addition, there is a positive-feedback loop based on the ability of fatty acids to stimulate the peroxisome-proliferator-activated receptor γ (PPARγ), which up-regulates the components responsible for the uptake of FFAs and their conversion into triacylglycerol. This strong positive-feedback loop is responsible for the onset of obesity, which usually results in insulin resistance and metabolic syndrome.
8. Liver cells play a major role in glucose metabolism. When plasma glucose levels rise, the increase in insulin released from the β-cells (Step 2) promotes the uptake of glucose and its conversion into glycogen.
9. During fasting, the white fat cells begin to release FFAs.
10. The FFAs act on the liver to stimulate PPARα to increase the expression of genes involved in synthesis of glucose (gluconeogenesis) from lactate and pyruvate (Module 7: Figure liver cell signalling).
11. Release of glucose from the liver during fasting is also enhanced by release of glucagon from the α-cells (Step 3), or adrenaline (epinephrine) from the chromaffin cells of the adrenal gland promotes the hydrolysis of glycogen (glycogenolysis) (Module 7: Figure liver cell signalling)
12. The release of glucose from the liver is then used to maintain the function of energy-consuming cells during the fasting period.

Control of food intake and body weight

Energy homoeostasis is achieved by balancing energy supply and energy consumption (Module 7: Figure metabolic energy network). The latter depends on a variety of processes such as basal metabolisim, physical activity and heat production through adaptive thermogenesis. Energy supply comes from food intake, which is usually balanced against energy consumption to protect against weight gain or loss. Control of food intake and hence body weight depends upon endocrine and neural signalling systems interacting with each other to control the feeding and satiety centres in the brain. Meals are usually terminated by satiation signals released from the gastrointestinal tract. The stomach responds mainly to mechanical distension to provide some of the earliest hormones whereas the

chemical composition of the food provides the main signals for the enteroendocrine cells in the intestine. The release and function of these gut hormones is outlined in the following sequence of events (Module 7: Figure control of food intake):

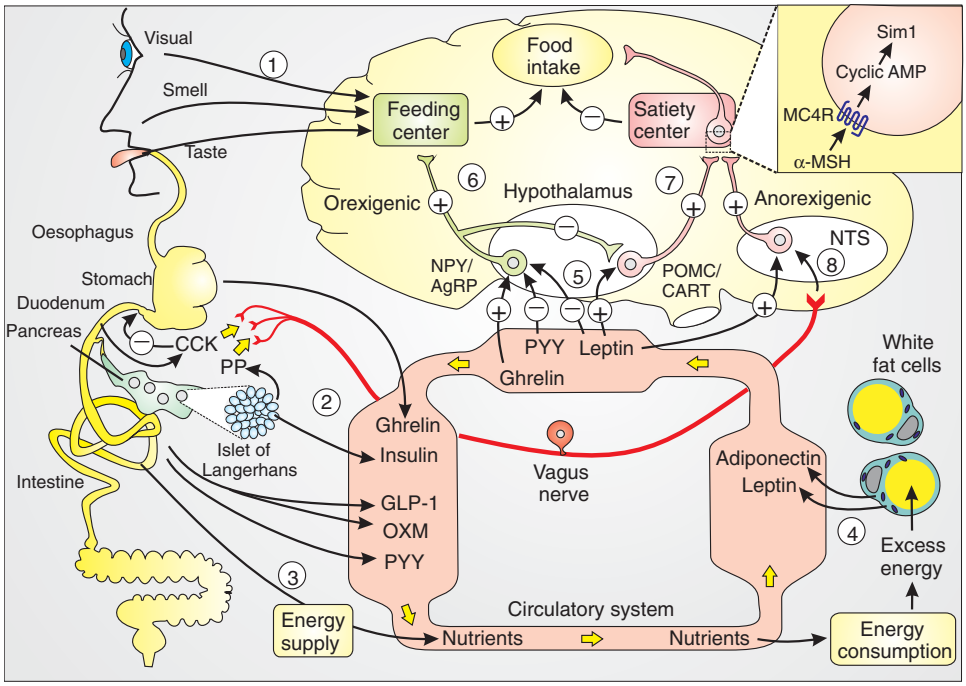
1. Favourable visual, smell and taste sensory information feeds information into the feeding centre that initiates the process of food intake.
2. As food enters the gastrointestinal tract it triggers the release of numerous gut hormones [ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), oxyntomodulin (OXM), peptide YY (PYY)]. In addition, the islets of Langerhans release both insulin and pancreatic polypeptide (PP). These gut and pancreatic hormones then function to control the feeding response through different mechanisms:
   - Ghrelin enters the blood stream and is carried to the brain where it activates the feeding centre to increase food intake.
   - Cholecystokinin (CCK) has a local action to inhibit gut emptying. CCK also activates the afferent endings of the vagus nerve that carries information to the nucleus of the solitary tract (NTS) to inhibit feeding.
   - Glucagon-like peptide 1 (GLP-1) enters the blood stream and one of its primary targets is the insulin-secreting β-cell where it facilitates the glucose-induced release of insulin (Module 7: Figure β-cell signalling). GLP-1 also inhibits food intake through mechanisms that remain to be established. There are indications that it may act to enhance the anorexigenic neurons in the hypothalamus and NLS.
   - Oxyntomodulin (OXM) functions much like GLP-1 to inhibit food intake through actions at both the hypothalamus and NLS.
   - Peptide YY (PYY) enters the blood stream and is carried to the brain where it inhibits the feeding centre to decrease food intake.
   - Pancreatic polypeptide (PP) activates the afferent endings of the vagus nerve that carries information to the nucleus of the solitary tract (NTS) to inhibit feeding.
3. The intestine also provides the nutrients, which represent the energy supply component of the metabolic energy network that is then balanced by energy consumption (Module 7: Figure metabolic energy network). If feeding provides energy that is surplus to requirements, the excess is stored in the fat cells resulting in an increase in body weight. The white fat cells turn out to be particularly important with regard to regulating the energy supply part of the equation by contributing to the mechanisms that control food intake (Module 7: Figure control of food intake).
4. The white fat cell has a capacity to 'sense' the energy content of their fat store and to relay this information to the brain by functioning as an endocrine cell by releasing various adipokine hormones such as leptin and adiponectin. The level of leptin declines during starvation but increases with overfeeding when the excess energy is stored as lipid in the fat cells.
5. The primary action of leptin occurs in the brain where it reduces food intake through mechanisms that suppress the feeding centres (orexigenic action) and activate the satiety centres (anorexigenic actions) (Step 5 in Module 7: Figure control of food intake). The neural circuits responsible for carrying out these two processes, which appear to be concentrated in the hypothalamus and nucleus of the solitary tract (NTS), are complex and are still been worked out. The following simplified description summarizes some of the neural mechanisms that have been identified in the arcuate nucleus (ARC) within the hypothalamus that functions to integrate many of the signals that control the feeding response and is one of the primary locations of leptin-sensitive neurons. The ARC is located close to the median eminence where the blood–brain barrier is incomplete and thus enables the circulating hormones to gain access to the energy-sensitive neurons.
6. One group of neurons located in the ARC express the orexigenic neuropeptides Agouti-related protein (AgRP) and neuropeptide Y (NPY) and are referred to as NPY/AgRP neurons. NPY is a potent orexigenic neuropeptide and its levels and release from the NPY/AgRP neurons increases after feeding. Application of NPY to this region results in hyperphagia and obesity.

   These NPY/AgRP neurons thus provide an important positive input to activate the feeding centre and appear to be the target for many of the hormones that regulate food intake. Ghrelin, which is released in anticipation of eating, is a powerful activator of the feeding centre and this might be mediated by activating the NPY/AgRP neurons. On the other hand, the satiety signal peptide YY (PYY) and the adipokine leptin strongly inhibit these orexigenic neurons.

   These NPY/AgRP neurons also have collaterals that innervate the pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) neurons. These collaterals release the inhibitory neurotransmitter γ-aminobutyric acid (GABA), which then acts on GABA receptors to inhibit the anorexigenic POMC/CART neurons. In this way, the NPY/AgRP neurons simultaneously activate the feeding centre and inhibit the satiety centre. The terminals of these NPY/AgRP neuronal collaterals express CB1 receptors that operate the endocannabinoid retrograde signalling mechanism that switches off the inhibitory inputs to the POMC/CART neurons

   These NPY/AgRP neurons also express 5-HT_{1D}Rs which act to hyperpolarize these neurons thereby reducing there orexigenic action to reduce food intake.
7. Another set of neurons within the ARC, which are anorexigenic, express pro-opiomelanocortin (POMC) and cocaine and amphetamine-related transcript (CART) and are referred to as POMC/CART neurons. POMC is the precursor for the melanocortins such as α-melanocyte stimulating hormone (α-MSH), β-MSH, γ-MSH and adrenocorticotropic hormone (ACTH), which is released by the corticotrophs in the anterior pituitary (Module 1: Figure pro-opiomelanocortin).

Module 7: | Figure control of food intake



Neural and endocrine control of food intake.
In response to sensory stimulation (visual, smell and taste), a feeding centre in the brain initiates food uptake that is then terminated by a satiety centre. Both the gastrointestinal tract and the white fat cells are responsible for releasing signals that are integrated by brain regions such as the hypothalamus and nucleus of the solitary tract (NTS) to control the neural circuits that make up the feeding and satiety neural circuits that regulate food intake. See text for further details.

These melanocortins are produced in the anterior and intermediate lobes of the pituitary, the ARC and in the nucleus of solitary tract (NTS). The POMC/CART neurons in the hypothalamus release α-MSH, which acts on the melanocortin 4 receptor (MC4R) (Module 1: Table G protein-coupled receptors) on the second order neurons, to activate the satiety centre thus decreasing food intake and weight loss (see inset in Module 7: Figure control of food intake). The MC4Rs are coupled to the cyclic AMP signalling pathway resulting in activation of the hypothalamic transcription factor Single-minded 1 (Sim1). Haploinsufficiency of the Sim1 gene causes hyperphagic obesity. In addition to inhibiting the NPY/AgRP neurons, the anorexigenic action of leptin also depends on its ability to stimulate the POMC/CART neurons.

These POMC/CART neurons express 5-HT_{2C}Rs, which might explain the action of the appetite regulating neurotransmitter 5-hydroxytryptamine (5-HT). 5-HT depolarizes these neurons resulting in increased anorexigenic activity.

8. The nucleus of the solitary tract (NTS) in the hind brain has anorexigenic neurons, which are sensitive to satiety factors such as leptin. The cholycystokinin (CCK) and pancreatic polypeptide (PP), which is released from the intestine and pancreas respectively, activates the vagus nerve that carries satiety signals to the NTS. In the NTS, these vagal inputs then activate pro-opiomelanocortin (POMC) neurons that release α-MSH to act on melanocortin 4 receptors (MC4Rs) the in the satiety centres.

Mutations in MC4R, which carry out the anorexigenic action of the POMC/CART neurons (step 7 in Module 7: Figure control of food intake), account for approximately 2% of adult obesity. Subjects carrying this mutation eat excessively and display increases in fat and lean mass as well as increases in bone mineral density.

The neurotransmitter 5-hydroxytryptamine (5-HT) is a potent suppressor of appetite. Some of its action occurs in the arcuate nucleus (ARC) where it simultaneously can inhibit the orexigenic NPY/AgRP neurons while activating the POMC/CART neurons. Analogues of 5-HT have been used to treat obesity.

Glial cells

The brain is composed of two principal cell types: neurons and glial cells. The neurons form the neural circuits that are responsible for carrying out the computational functions of the brain. The glial cells, which are present in the brain in approximately equal numbers as the neurons, have a variety of functions. The first point to recognize is that there are a number of glial cell types (Module 7 Table cell inventory) with different functions. There are oligodendrocytes and Schwann cells that are responsible for myelination in the central nervous system and the peripheral nervous system respectively. The microglia are phagocytic cells that function in host defence. Finally, there are the astrocytes, which are the major glial cells of the brain. There are many

different astrocytes and some of these have been given special names, such as the Bergmann glia found in the cerebellum, the Müller cell found in the retina and the satellite glial cells. It is the astrocytes that will be described in more detail below.

Astrocytes

For a long time, astrocytes were thought to play a secondary role in the brain by supporting neuronal activity by carrying out a number of essential housekeeping duties. They are known to provide neurons with nutrients and the redox buffer glutathione (GSH) (Module 12: Figure astrocyte-induced neuronal cell death). They provide a mechanisms for the spatial buffering of K^+ and they remove neurotransmitters and other waste products. Recently, however, it has become apparent that glial cells play a much more active role in regulating neural function because they not only respond to information coming from the neurons, but also can transmit information back to the neurons. This two-way dialogue, which depends upon the neuronal–astrocyte communication and the reciprocal astrocyte–neuronal communication systems, may play a critical role in modulating neuronal activity and information processing in the brain. It is becoming evident that this active participation in brain function depends upon a process of astrocyte excitability. This excitability has two important functional consequences. First, it results in the formation of intra- and inter-cellular Ca^{2+} waves that spread information throughout individual cells or between groups of cells respectively. For example, this excitability is critical for the astrocyte regulation of cerebral blood flow, where astrocytes integrate the degree of neural activity and use this information to regulate contractile tone in arteriole smooth muscle cells. Secondly, astrocyte excitability can lead to the formation of spontaneous astrocyte Ca^{2+} oscillations that may have a critical role to play in the astrocyte–neuronal communication system. The complex astrocyte structure reveals the intimate associations with both neurons and blood vessels and shows how these cells can perform these different functions.

The astrocytes appear to be particularly sensitive to the β-amyloid peptides and may contribute to the pathology of Alzheimer's disease through a process of astrocyte-induced neuronal death.

Astrocyte structure

The problem with trying to deal with astrocyte function is that astrocytes come in many different shapes and sizes (Module 7: Figure astrocyte diversity). Some of these astrocytes have precise locations, such as the Bergmann glia (cell II in Module 7: Figure astrocyte diversity) and the Müller cells found in the retina. These astrocytes are also defined by the kinds of connections that they make within the brain. For example, the tanycytes have their cell bodies facing the ventricles, and then have long extensions connecting to either the pia or the blood vessels. A characteristic feature of many of the astrocytes is a long extension with an endfoot that is attached to a blood vessel (e.g. cells Ib and IV–VII in Module 7: Figure astrocyte diversity). It is this cell type that co-ordinates neural activity with cerebral blood flow.

The most important structural feature of these astrocytes is their extraordinary degree of branching. Each of the major arms that radiate out from the cell body divides repeatedly to produce a highly complex tree-like arrangement (Module 7: Figure astrocyte organization). The long extensions of each astrocyte are often connected to those of other astrocytes through gap junctions made up from connexion 43 (Cx43) (Module 7: Figure astrocyte structure). Astrocytes are often mistaken for neurons because the branching pattern can closely resemble the dendritic tree found on neurons (Module 10: Figure Purkinje cell input-specific Ca^{2+} signals). This analogy is apt because it is this highly branched structure that makes contact with the neuronal synaptic endings. The reason for this high degree of branching is that each astrocyte makes contact with an enormous number of synaptic endings. It is estimated that each astrocyte makes contact with approximately 150 000 synaptic connections. Since each neuron has roughly the same number of synaptic connections, there has to be a similar number of astrocytes, and this seems to be the case because it is estimated that there are approximately 1.4 astrocytes for every neuron in the human brain. It is quite remarkable to find that all the synaptic endings in the brain are closely associated with astrocytic processes to form tripartite junctions (Module 10: Figure synaptic organization).

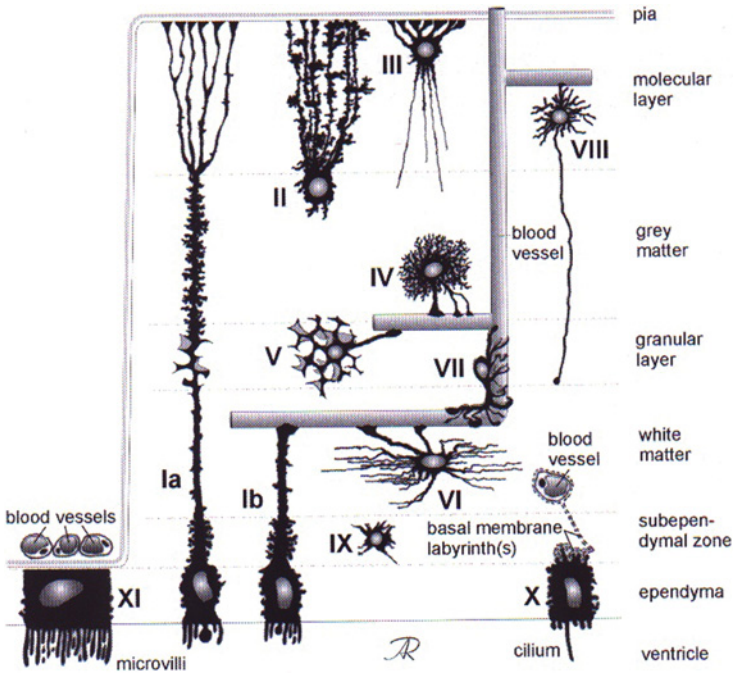
The structural organization of the astrocyte is dominated by this close relationship with the synaptic connections between neurons (Module 7: Figure astrocyte structure). The presynaptic neuron (shown in green) sends its axons into the astrocyte domain, where they make connections with the synaptic endings of another set of neurons, the postsynaptic neurons (shown in red). Each synaptic ending comes into close contact with one of the myriad astrocytic processes to form the tripartite junction. Only two such junctions are shown, but one has to imagine that within each astrocyte domain there are approximately 150 000 similar junctions. In addition to this intimate contact with the neuronal synaptic connections, many of the astrocytes also make intimate contact with the cerebral blood vessels. Some of the branches extend out towards the blood vessels, where they end in an endfoot that is planted on the surface to make a junctional contact with the smooth muscle cells.

These astrocytes with their connections to neuronal synapses and blood vessels are packed together to form pseudo-crystalline arrays, which is an important organizing principle within the nervous system (see panel b in Module 7: Figure astrocyte organization). This structural organization forms the basis for the spatial buffering of K^+, neuronal–astrocyte communication, astrocyte–neuronal communication and the astrocyte regulation of cerebral blood flow.

Spatial buffering of K^+ by astrocytes

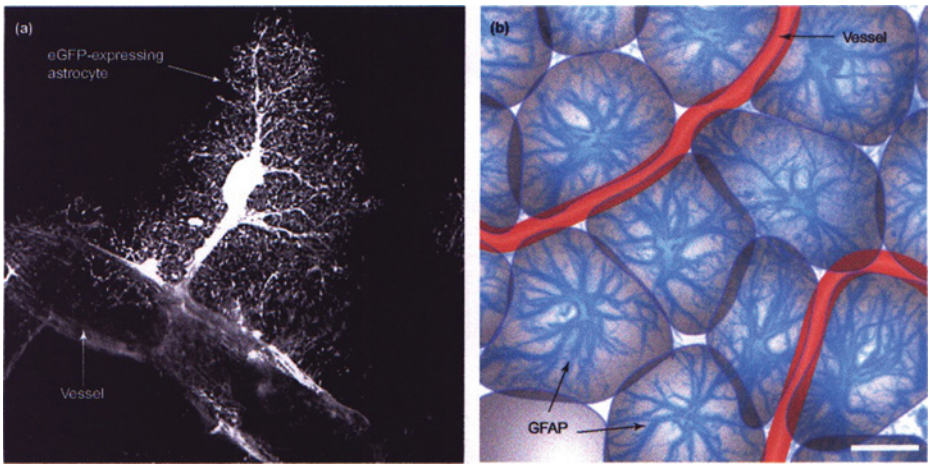
The astrocytes have a key role in maintaining a constant concentration of K^+ in the narrow extracellular spaces

Module 7: | Figure astrocyte diversity



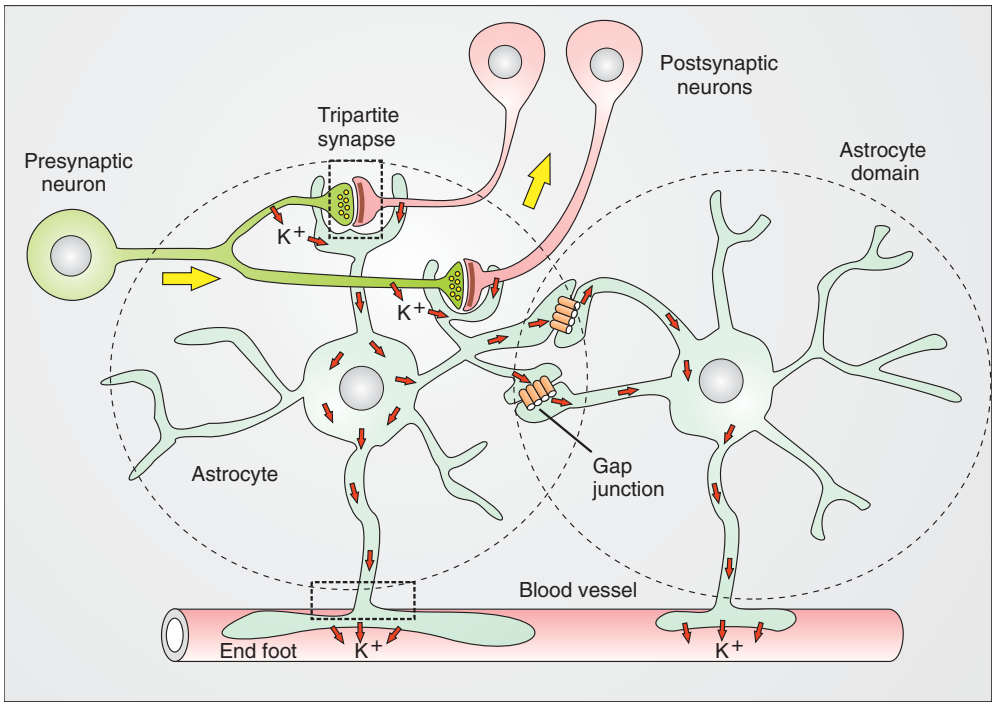
Structural diversity of astrocytes.
This drawing summarizes the structural diversity of astrocytes. A consistent feature are the variable number of extensions that radiate out from the cell body and then anatomize into an elaborate branching tree-like structure. Many of the astrocytes have a longer extension containing an endfoot that makes contact with blood vessels. Details of the branching pattern of a typical astrocyte are shown in the left-hand panel of Module 7: Figure astrocyte organization. Reproduced from Reichenbach, A. and Wolburg, H. (2005) Astrocytes and ependymal glia. In Neuroglia, Kettenmann, H. and Bruce, R.R., eds, pp. 19–35, by permission of Oxford University Press; http://www.oup.com; see Reichenbach and Wolburg 2005.

Module 7: | Figure astrocyte organization



The structure and arrangement of astrocytes.
a. The stellate organization of astrocytes is illustrated in the enhanced green fluorescent protein (eGFP)-expressing astrocyte shown on the left. Note the foot processes on the surface of the blood vessel. In the section on the right, astrocytes have been stained with an antibody against the glial fibrillary protein (GFAP), which picks out the main branches. The outline of each cell has been drawn in as transparent polyhedra to illustrate that there is very little overlap between the individual astrocytes. The blood vessels run in between the columns of astrocytes. For details of the relationship between the astrocytes and the blood vessels, see Module 7: Figure astrocyte structure. Reproduced from *Trends Neurosci.*, Vol. 26, Nedergaard, M., Ransom, B and Goldman, S.A., New roles for astrocytes: redefining the functional architecture of the brain, pp. 523–530. Copyright (2003), with permission from Elsevier; see Nedergaard et al. 2003.

Module 7: | Figure astrocyte structure



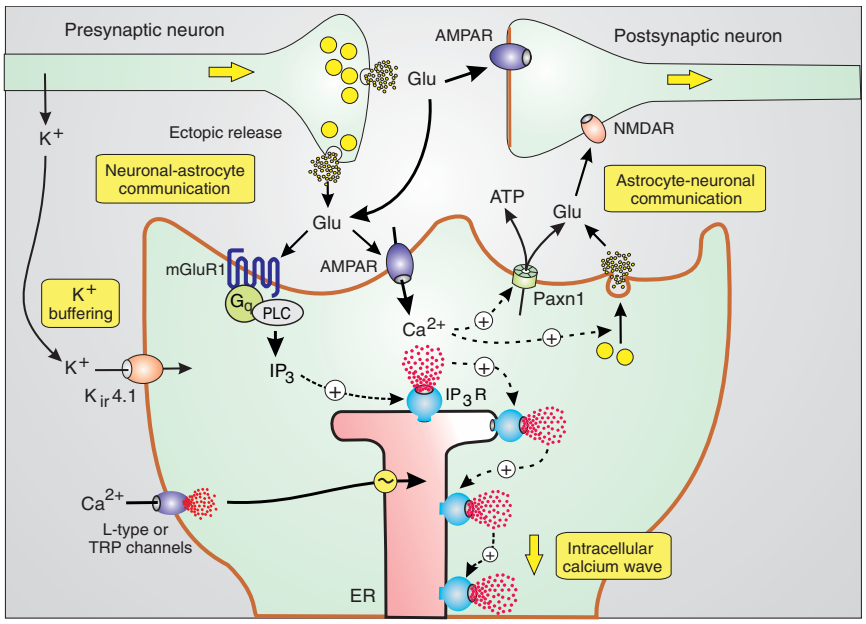
Relationship between astrocytes, neurons and blood vessels.
Astrocytes are stellate cells with numerous branches. Only a few of the branches are shown, and the two circles illustrate astrocyte domains that show very little overlap. In the regions of overlap, the astrocytes are connected to each other by gap junctions. Some of the branches also have endfoot processes that spread out over the blood vessels (arterioles). Many of the branches make intimate connections with neuronal synapses to form a tripartite synapse. Here only two such synapses are shown, but in reality each astrocyte can form 150 000 such synapses. The elaborate branching pattern is shown in Module 7: Figure astrocyte organization and the function of the tripartite synapse is described in more detail in Module 7: Figure astrocyte tripartite synapse. The red arrows represent the spatial buffering of K^+. The K^+ released during neuronal activity is taken up by the astrocytes and flows down towards the blood vessels where it is released at the end feet.

that surround each neuron. The K^+ released during periods of neuronal activity is buffered by a 'K^+ siphoning' process whereby the K^+ is taken up by astrocytic process near the neurons and is then transferred throughout the astrocytic syncytial system finally to be released from the end foot processes on the blood vessels (see red arrows on Module 7: Figure astrocyte structure). The flow of K^+ in and out of the astrocytes is regulated by a number of different types of K^+ channels. The K^+ that is taken up at the astrocytic processes near the synapses enters the astrocytes through the glial-specific $K_{ir}4.1$ (Module 7: Figure astrocyte tripartite synapse). $K_{ir}4.1$ is one of the inward rectifier K^+ (K_{ir}) channel family that form either homo- or heteromeric channels. In the case of the uptake of K^+ by the astrocytes, this seems to depend on the strongly rectifying heteromeric channels formed by $K_{ir}4.1$ combining with either $K_{ir}2.1$ or $K_{ir}5.1$(Module 3: Table inward rectifier K^+ (Kir) channel). At the other end of the siphon, the K^+ leaves the end foot processes that line up on the blood vessels through two types of channel. It can leave through either the weakly rectifying homomeric $K_{ir}4.1$ channels or through the large-conductance (BK) channels (Module 7: Figure astrocyte endfoot signalling). The role of the latter is described in more detail in the section on the astrocyte regulation of cerebral blood flow.

Neuronal–astrocyte communication

Astrocytes are sensitive to a large number of stimuli such as glutamate, γ-aminobutyric acid (GABA), noradrenaline (also known as norepinephrine), ATP and dopamine. Many of these stimuli are released from the synaptic endings of the tripartite synapse (Module 7: Figure astrocyte tripartite synapse). The transmitter that escapes from the synaptic cleft diffuses out to make contact with the astrocyte. The amount of transmitter spilling out of the synapse will depend on the level of synaptic activity. At low levels of neural activity, the astrocyte receives little stimulation, but this increases markedly when there is rapid neural communication. In addition to this indirect supply of transmitter, there may be specialized ectopic release sites on the presynaptic ending that release transmitter directly on to the astrocytes. The response of the astrocytes to these transmitters depends on the receptors that they express, and this can vary between the different astrocytes. In most cases, however, there is an increase in the intracellular level of Ca^{2+} achieved by activating different Ca^{2+} signalling modules. For many of the astrocytes, there are metabotropic receptors that respond to glutamate by activating the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette (i.e. Ca^{2+} module 6 in Module 2: Figure Ca^{2+} modules). In addition,

Module 7: | Figure astrocyte tripartite synapse



Functional interactions between neurons and astrocytes at the tripartite synapse.
Action potentials travelling down the presynaptic ending release glutamate that activates postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (AMPARs) during the typical process of synaptic transmission. Some of the glutamate spills out from the synapse and diffuses across to the astrocyte surface, where it activates either metabotropic glutamate receptors (mGluRs) to stimulate the formation of inositol 1,4,5-trisphosphate ($InsP_3$) to release Ca^{2+} from the endoplasmic reticulum (ER) or AMPARs to increase Ca^{2+} entry. This Ca^{2+} can stimulate the release of glutamate from the astrocyte and this feeds back to the neuronal synapse, where it has two actions: it can either inhibit or activate transmission. In the example provided by this figure, it is shown to be stimulating *N*-methyl-D-aspartate (NMDA) receptors (NMDARs) to enhance transmission. Ca^{2+} can also trigger a regenerative Ca^{2+} wave that spreads throughout the neuron. To see what happens when this wave reaches the endfoot, see Module 7: Figure astrocyte endfoot signalling.

astrocytes such as the Bergmann glia have ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (AMPARs) composed of the glutamate receptor 1 (GluR1) and 4 (GluR4) subunits that enable them to gate Ca^{2+} (i.e. Ca^{2+} module 1 in Module 2: Figure Ca^{2+} modules).

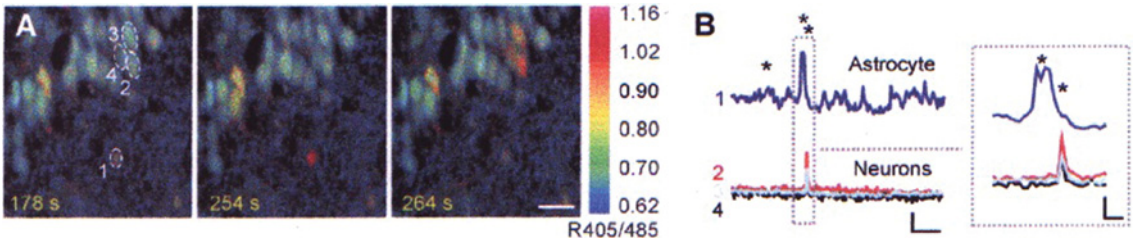
The increase in Ca^{2+} within the astrocyte that results from this neuronal–astrocyte communication system can have at least two functions. Firstly, it is responsible for triggering the reciprocal astrocyte–neuronal communication system by releasing glutamate (Module 7: Figure astrocyte tripartite synapse). The mechanism of glutamate release is still not absolutely certain: both vesicular- and channel-mediated release mechanisms have been proposed. With regard to release by exocytosis, typical vesicles have been observed in astrocytes. Since there are no large membrane depolarizations, astrocytes may use a mechanism of exocytosis triggered by Ca^{2+} release from internal stores (Module 4: Figure Ca^{2+}-dependent exocytosis). Alternatively, the release of Ca^{2+} may activate hemichannels formed from pannexin 1 (Panx1) to release ATP and glutamate (See Module 3: Figure hemichannels and gap junctions). The glutamate then triggers astrocyte–neuronal communication.

The second function of the astrocytic Ca^{2+} signal is to set up a regenerative intracellular Ca^{2+} wave that spreads the local signal generated near the synaptic sites to more distant parts of the astrocyte. This signalling mechanism is described in more detail in the section on astrocyte excitability.

Astrocyte–neuronal communication

Astrocytes have the ability to initiate neural activity. The stimulus, which is an increase in intracellular Ca^{2+}, is induced either as a consequence of the neuronal–astrocyte communication pathway (Module 7: Figure astrocyte tripartite synapse) or it can occur spontaneously through the generation of astrocyte Ca^{2+} oscillations. In both cases, the increase in the intracellular level of Ca^{2+} within the astrocytic processes, which are a part of the tripartite synapse, release stimuli such as glutamate that then act on receptors on the neuronal endings to either excite or inhibit neuronal activity. An example of how an astrocytic Ca^{2+} can lead to the activation of neural activity has been described in the hippocampus (Module 7: Figure astrocytic synchronization of hippocampal neurons). A spontaneous Ca^{2+} transient in the astrocyte was able to activate increases in Ca^{2+} in three neurons. The implication of this observation is that the astrocyte administers the synaptic connections of more than one output neuron. This organization is depicted in Module 7: Figure astrocyte structure, which illustrates how a single astrocyte can make contact with the synapses that go to two neurons. This arrangement can account for the observation that astrocytes are able to synchronize the activity of groups of neurons. This synchronization is not due to coupling between the

Module 7: | Figure astrocytic synchronization of hippocampal neurons



Synchronization of hippocampal neural activity by spontaneous astrocytic Ca^{2+} transients.
The arrangement of the astrocyte (cell 1) and the three neurons (cells 2, 3 and 4) are outlined in panel A. Panel B reveals how a spontaneous transient in the astrocyte resulted in a delayed, but synchronous elevation in all three neurons. Reproduced from *Neuron*, Vol. 43, Fellin, T., Pascual, O., Gobbo, S., Pozzan, T., Haydon, P.G. and Carmignoto, G., Neuronal synchrony mediated by astrocyte glutamate through activation of extrasynaptic NMDA receptors, pp. 729–743. Copyright (2004), with permission from Elsevier; see Fellin et al. 2004.

neurons because current injection into one of the synchronized neurons fails to elicit a response in the other neuron. Also, blocking the ability of neurons to generate action potentials with tetrodotoxin failed to inhibit this synchronicity. The astrocyte-evoked responses in the neuron tend to have a very slow time course, which is consistent with the fact that they are driven by the relatively slow spontaneous transients in the astrocytes and they also occur with a frequency that matches those observed in the astrocytes. The next question to consider is how the astrocyte Ca^{2+} transient can trigger these neuronal responses.

The increase in Ca^{2+} within the astrocytic process is thought to trigger the release of glutamate from the astrocyte (Module 7: Figure astrocyte tripartite synapse). The release of glutamate then acts on receptors on the neuronal synapse to activate neuronal activity. In the case of the synchronized Ca^{2+} transients just described in hippocampal neurons, the glutamate acts on extrasynaptic ionotropic N-methyl-d-aspartate (NMDA) receptors that gate Na^{+} and Ca^{2+} to provide sufficient inward current to depolarize the neurons to give the Ca^{2+} signals recorded in the experiment shown in Module 7: Figure astrocytic synchronization of hippocampal neurons.

The astrocyte–neuronal communication system indicates that the excitability of astrocytes can extend to regulation of neural activity. The astrocyte regulation of cerebral blood flow is another example of how astrocytes can regulate another cell type, in this case the smooth muscle cells.

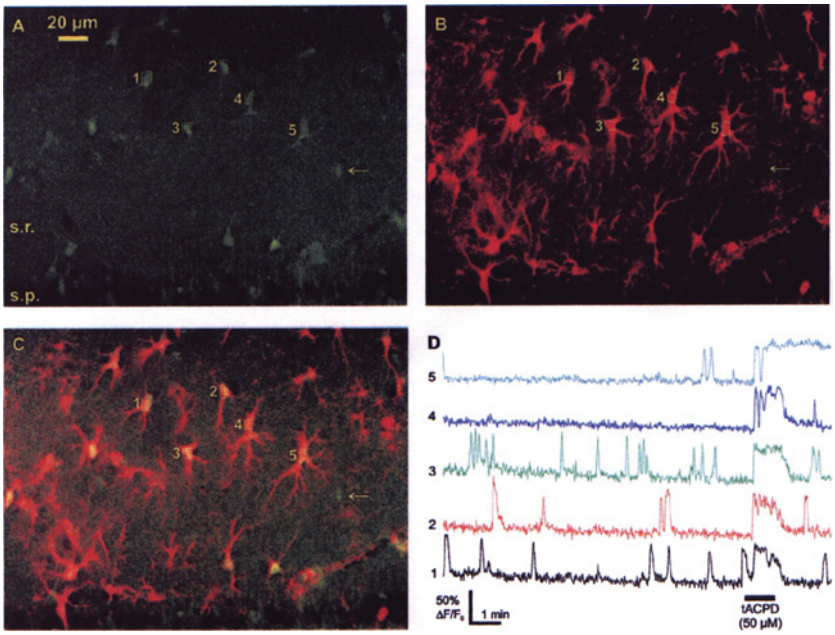
Astrocyte excitability

Astrocytes are not excitable in the classical sense of being able to conduct action potentials as occurs in neurons and muscle. However, they have an element of excitability in that they are able to generate and conduct Ca^{2+} signals. This depends upon the role of the endoplasmic reticulum (ER) being an excitable system that can generate propagating Ca^{2+} waves. This property is similar to that described in neurons, where it is the basis of the neuron-within-a-neuron hypothesis. The ER is a continuous network that has release channels that have the Ca^{2+}-induced Ca^{2+}-release (CICR) mechanism to enable them to set up regenerative Ca^{2+} waves (Module 2: Figure Ca^{2+}-induced Ca^{2+} release). Astrocytes appear to have a similar excitable system in that they can generate both intracellular and intercellular Ca^{2+} waves (Module 7: Figure astrocyte Ca^{2+} signalling). These waves appear to initiate at the tripartite synapses, from where they spread through the astrocyte and down to the endfoot that sits on the muscle. Since the astrocytes are linked together through gap junctions, such waves can also spread from one cell to the next to create a long-range communication system. Such long-range communication depends on the neighbouring cells being sufficiently excitable to detect and propagate the wave.

Astrocytes have different modes of Ca^{2+} signalling that depend on the intensity of the signals they receive from the neuronal–astrocyte communication pathway at the tripartite synapse. When neuronal signalling at the synapses is at a low level, there is little stimulation, and the resulting Ca^{2+} signal is restricted to local microdomains in the astrocytic processes in immediate contact with the active synapses. Such local Ca^{2+} microdomains have been observed in Bergmann glial cells following stimulation of the parallel fibres (Module 7: Figure Ca^{2+} microdomains in Bergmann glia). Since these astrocytic processes are at the end of fine branches, the increase in Ca^{2+} is effectively isolated within a small compartment. This is exactly analogous to the isolation of Ca^{2+} signals in the individual spines on the dendritic tree of neurons (see Module 10: Figure hippocampal input-specific Ca^{2+} signals). This is an example of how the astrocyte endings appear to be matched to the neighbouring synaptic region.

When the neuronal synapse is stimulated at higher intensities, the Ca^{2+} in the microdomains spreads out to the rest of the astrocyte and this usually travels as an intracellular Ca^{2+} wave (Module 7: Figure astrocyte Ca^{2+} signalling). It is this propagating wave that determines astrocyte excitability. The initiation and wave propagating mechanism is shown in Module 7: Figure astrocyte tripartite synapse. The initiation is triggered by the formation of inositol 1,4,5-trisphosphate ($InsP_3$) during the operation of the neuronal–astrocyte communication pathway. The $InsP_3$ then releases Ca^{2+}, which can then excite neighbouring $InsP_3$ receptors to set up the regenerative wave that spreads through the process of Ca^{2+}-induced Ca^{2+} release (CICR). In order for the $InsP_3$ receptors to participate in CICR, they must be excitable, and there are a number of factors that determine this excitability. One

Module 7: | Figure astrocyte Ca^{2+} oscillations



Ca^{2+} oscillations in astrocytes located within the CA1 region of the hippocampus.

Astrocytes loaded with Calcium Green acetoxymethyl ester (AM) were found to display periodic Ca^{2+} transients. Cells 1 and 3 (as labelled in the figure) displayed fairly regular oscillations, whereas cell 4 was quiescent throughout the recording period. Note that all of the cells displayed a large Ca^{2+} transient when the preparation was treated with the metabotropic glutamate receptor agonist 1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (tACPD). Reproduced from Nett, W.J., Oloff, S.H. and McCarthy, K.D. (2002) Hippocampal astrocytes *in situ* exhibit calcium oscillations that occur independent of neuronal activity. J. Neurophysiol. 87:528–537, with the permission of the American Physiological Society; see Nett et al. 2002.

of these is the level of $InsP_3$ and the other is the luminal level of Ca^{2+}. The lumen must be charged up with Ca^{2+}, and the level of this loading can regulate the excitability of the $InsP_3$ receptors. This loading is very dependent on the entry of external Ca^{2+}, and astrocytes have a number of entry channels, such as L-type Ca^{2+} channels and members of the transient receptor potential (TRP) ion channel family, that may function to load the intracellular store.

The intracellular waves are often restricted to single astrocytes, but there are numerous examples of intercellular Ca^{2+} waves (lower panel in Module 7: Figure astrocyte tripartite synapse). The Ca^{2+} spreads from one cell to the next to co-ordinate the activity of a large population of cells. The function of such intercellular waves is still not clear. However, they are most often observed during extremes of cell stimulation and have been linked with pathological states, such as spreading depression.

Another aspect of astrocyte excitability is the generation of astrocyte Ca^{2+} oscillations.

Astrocyte Ca^{2+} oscillations

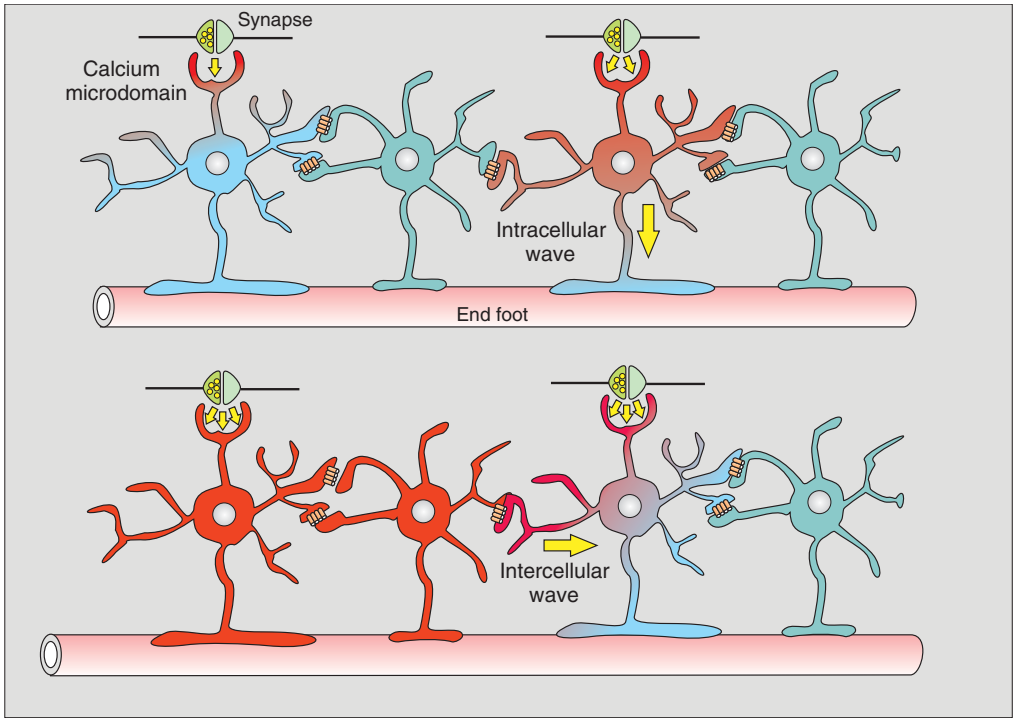
One of the remarkable aspects of astrocytes is that they can generate spontaneous Ca^{2+} oscillations apparently independently of external stimulation (Module 7: Figure astrocyte Ca^{2+} oscillations). While there may be no obvious stimulation, it is important to stress that such oscillations usually depend upon the intracellular release channels such as the inositol 1,4,5-trisphosphate ($InsP_3$) receptors being in an excitable state. Despite this reservation, what is evident is that these oscillations can occur in the absence of neural activity at the tripartite synapse because they can occur under conditions where neuronal action potentials are suppressed with tetrodotoxin. When groups of astrocytes are analysed simultaneously, it is evident that the individual cells oscillate independently of each other. When these oscillations were analysed in finer detail, it was discovered that they could be restricted to individual processes, thus providing further evidence that the individual astrocytic processes can function independently of each other.

The spontaneous oscillations generated by the astrocytes are of particular significance with regard to astrocyte–neuronal communication because they indicate that the astrocytes are able to initiate a signalling process to control neural activity.

Astrocyte regulation of cerebral blood flow

The vasculature tone of the cerebral arterioles, like those found elsewhere in the body, depends on the contractile state of smooth muscle cells (SMCs). SMC tone depends upon the cell being in a dynamic condition, poised between states of contraction and relaxation. There are indications that this dynamic state may be driven by an endogenous smooth muscle cell cytosolic oscillator that delivers the regular pulses of Ca^{2+} to maintain SMC tone (Module 7: Figure smooth muscle cell Ca^{2+} oscillations). This Ca^{2+} oscillator generates pulses of Ca^{2+} with a frequency of about 0.2 Hz. Such oscillations can be generated by either a membrane or a cytosolic oscillator (Module 6: Figure membrane and cytosolic oscillators). It has not been established which one of these oscillatory mechanisms is

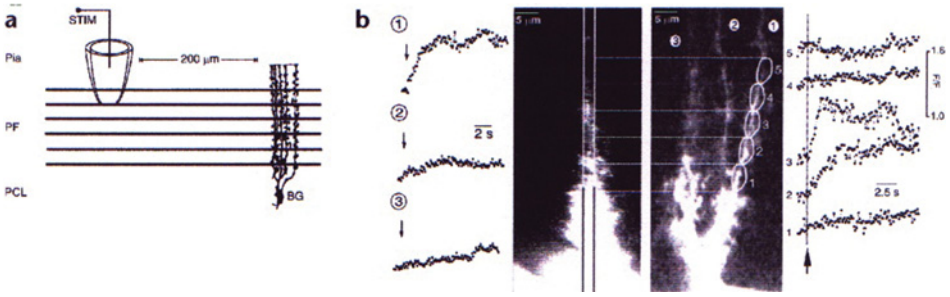
Module 7: | Figure astrocyte Ca^{2+} signalling



Different modes of Ca^{2+} signalling in astrocytes.

Astrocytes display different modes of Ca^{2+} signalling depending on the intensity of synaptic activity in the associated nerve endings. At low levels of synaptic transmission, the astrocytes receive little stimulation, and the resulting Ca^{2+} signal is confined to microdomains within the branches close to the synapse (top left). At higher levels of stimulation, these microdomains tend to spawn an intracellular Ca^{2+} wave that spreads the signal throughout the astrocyte and down to the endfoot (top right). In general, these regenerative waves tend to be confined to single astrocytes, but during more intense stimulation and under more pathological conditions, intercellular waves can spread from astrocyte to astrocyte, as shown at the bottom.

Module 7: | Figure Ca^{2+} microdomains in Bergmann glia

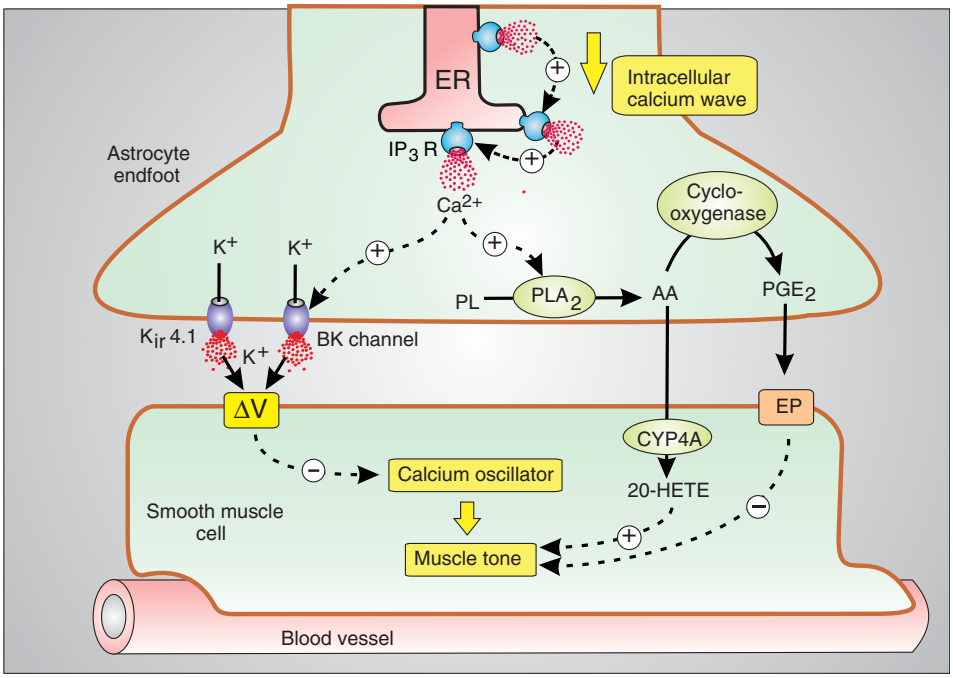


Microdomains of Ca^{2+} in a Bergmann glial cell following stimulation of the parallel fibre inputs.

The arrangement of the recording system is shown in panel a. Parallel fibres (PFs) were stimulated while Ca^{2+} responses were recorded in three of the major processes of the Bergmann glial (BG) cell, as shown in the three traces on the left of panel b. Process 1 showed the largest increase in Ca^{2+}. When this process was subdivided into five regions of interest, the increase in Ca^{2+} was restricted to regions 2 and 3. Reproduced by permission from Macmillan Publishers Ltd: *Nat. Neurosci.*, Grosche, J., Matyash, V., Möller, T., Verkhratsky, A., Reichenbach, A. and Kettermann, H. (1999) Microdomains for neuron–glia interaction: parallel fibre signalling to Bergmann glial cells. 2:139–143. Copyright (1999); http://www.nature.com/neuro; see Grosche et al. 2002.

operating in these arteriole SMCs, but it is likely to be a cytosolic oscillator similar to that found in other vascular smooth muscle cells (Module 7: Figure SMC cytosolic oscillator). However, what is clear is that this Ca^{2+} oscillator is rapidly switched off when the Ca^{2+} wave invades the endfoot. It has been suggested that this Ca^{2+} activates the large-conductance (BK) channels to release K^+, which then acts to depolarize the SMC, resulting in inhibition of the oscillator, a decrease in contraction and dilation of the vessel (Module 7: Figure astrocyte endfoot signalling). Some of the other mechanisms that have been proposed to explain how a Ca^{2+} signal in the endfoot causes the SMC to relax are based on the formation of lipid messengers. The idea is that the Ca^{2+} activates phospholipase A_2 (PLA_2) to hydrolyse phospholipids to release arachidonic acid (AA), which is then converted

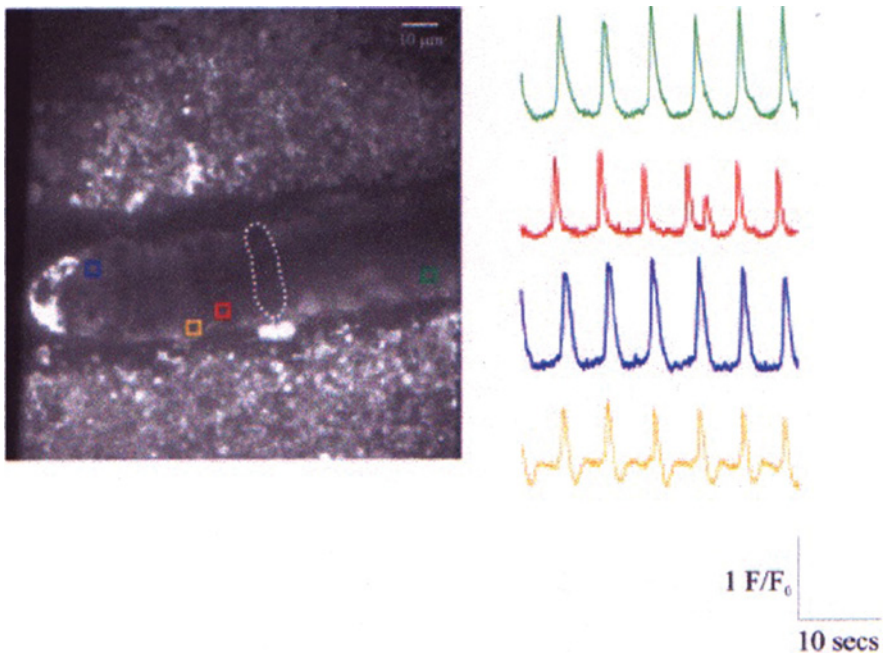
Module 7: | Figure astrocyte endfoot signalling



Astrocyte regulation of cerebral blood flow.

When the intracellular Ca^{2+} wave spreads down into the astrocyte endfoot, it activates a number of signalling mechanisms that can alter smooth muscle cell (SMC) tone and hence the flow of blood through the arterioles. The nature of these signalling pathways are still being worked out, and the different astrocytes may use different mechanisms. One mechanism seems to depend on the activation of the large-conductance (BK) Ca^{2+}-dependent channels, which release K^+ that depolarizes the SMC to inhibit the Ca^{2+} oscillator, resulting in vessel dilation. Another mechanism is for Ca^{2+} to activate phospholipase A_2 (PLA_2) that hydrolyses phospholipids (PLs) to produce arachidonic acid (AA), which can have two actions. It can diffuse across to the SMC to be converted into the vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE), or it can be converted by cyclooxygenase into PGE_2, which then acts on PGE_2 receptors (EP receptors) to inhibit muscle tone.

Module 7: | Figure smooth muscle cell Ca^{2+} oscillations



Ca^{2+} oscillations recorded from the smooth muscle cells surrounding cortical arterioles.

The dotted line indicates the position of one of the smooth muscle cells (SMCs) that wrap around the arteriole. The Ca^{2+} signals shown on the right were recorded from the regions indicated by the coloured boxes on four separate SMCs. The oscillations had a mean frequency of 0.18 Hz. Reproduced from Filosa, J.A., Bonev, A.D. and Nelson, M.T. (2004) Calcium dynamics in cortical astrocytes and arterioles during neurovascular coupling. Circ. Res. 95:e73–e81, with permission from Lippincott Williams & Wilkins; http://www.lww.com/; see Filosa et al. 2004.

into agents such as 20-hydroxyeicosatetraenoic acid (20-HETE) or prostaglandin E_2 (PGE_2) (Module 1: Figure eicosanoids) that are known to influence muscle tone.

Although the signalling mechanisms operating between the endfoot and the SMCs remain to be fully established, what seems certain is that an astrocytic Ca^{2+} signal initiated by neural activity at the tripartite synapse spreads down to the endfoot to bring about a change in blood flow through the cerebral arterioles.

Satellite glial cells

The satellite glial cells, which surround the primary sensory neurons located in the sensory ganglia, have a very similar function to the astrocytes in the brain. For example they have a mechanism that resembles the spatial buffering of K^+ in astrocytes. The inward rectifier K^+ (K_{ir}) channel family, particularly the $K_{ir}4.1$ channel, contribute to both the entry and release of K^+ that is a feature of this buffering of the extracellular concentration of K^+ that controls neuronal excitability. A decrease in the expression of this $K_{ir}4.1$ channel may contribute to the onset of neuropathic pain.

Microglia

The microglia, which resemble macrophages, are phagocytic cells located specifically in the central and peripheral nervous systems. The microglia express many of the markers normally associated with macrophages such as colony-stimulating factor 1 receptor (CSF1R), CD11b, CD14 and EGF-like module-containing mucin-like hormone receptor-like-1 (EMR1). Their main function within the nervous system is to maintain the status quo by responding to adverse changes such as the death of neurons as occurs following ischaemia or due to localized breaches of the blood–brain barrier that allows entery of plasma components and pathogens (Module 7: Figure microglia interactions). They have two functional states. For most of their life they exist as star-shaped cells with numerous long processes that are moving constantly as they survey their local environment. This surveillance mode switches into an activation mode during injury to the nervous system or in response to stimuli received from the surrounding neurons. The activated microglia transform into amoeboid cells that can proliferate and migrate thereby producing a cohort of cells capable of releasing neurotrophic factors and pro-inflammatory mediators [chemokines, cytokines, reactive oxygen species (ROS) and nitric oxide (NO)]. Like macrophages, the microglia removes debris through phagocytosis. This phagocytotic response appears to be induced by the G protein-coupled receptor $P2Y_6$ (Module 1: Table G protein-coupled receptors) that respond to the UDP produced by damaged and dying cells.

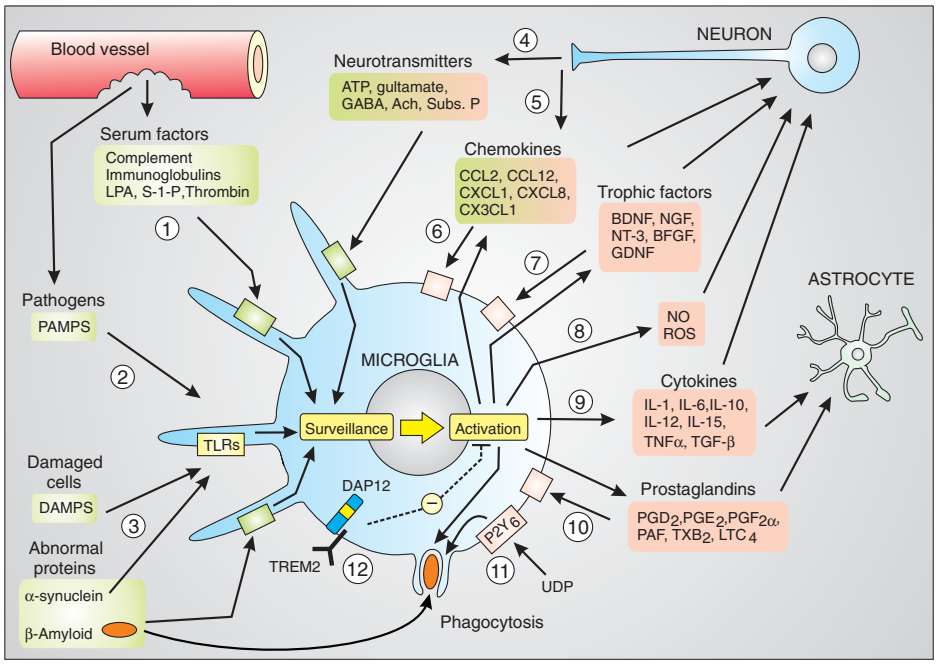
In addition to this sentinel role, microglia are tightly integrated into neuronal activity through a two-way communication network of neuronal–microglia interactions largely convened by chemokine signalling pathways.

A characteristic feature of microglia is their very large receptor repertoire and their capacity to express and release an enormous number of stimuli (Module 7: Figure microglia interactions):

1. In keeping with their role as the immune cells of the brain, microglia have receptors capable of detecting deleterious chemicals and pathogens. While the internal milieu of the brain is normally protected by the blood–brain barrier, this defence can be breached especially if this barrier is damaged. Microglia have receptors capable of detecting a number of serum factors such as complement factors (C1q and C5a), immunoglobulins, lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P) and thrombin.
2. Pathogens such as bacteria, viruses and fungi are dealt with by the microglia using the pathogen-associated molecular patterns (PAMPs), which are specific components of these invading pathogens (Module 11: Figure formation and action of PAMPs).
3. Microglia are also sensitive to endogenous damage-associated molecular patterns (DAMPS). As occurs in macrophages, microglial cells respond to the DAMPS and PAMPS using Toll-like receptors (TLRs) that initiate an inflammatory response during the activation process. These TLRs then recruit various downstream signalling pathways, such as the Toll receptor signalling pathway (Module 2: Figure Toll receptor signalling) or the mitogen-activated protein kinase (MAPK) signalling pathway, to generate an inflammatory response to match the kind of pathogens that are invading the organism.

   Microglia are also on the look out for abnormal proteins, such as β-amyloid and α-synuclein, that not only activate the microglia but are also taken up and destroyed by the phagocytotic pathway that is induced during activation (Module 7: Figure microglia interactions). Phagocytosis is also used to clear away the debris left behind when neurons die. The microglia cells are also capable of destroying β-amyloid by releasing the insulin-degrading enzyme (IDE) (Module 12: Figure amyloid cascade hypothesis).
4. Microglia express a large number of neurotransmitter receptors that enable them to tune in to neuronal activity. Excessive neuronal activity can then contribute to microglial cell activation and this is part of the neuronal–microglia interactions that may have important implications for the onset of neuropathic pain (Module 7: Figure neuronal chemokine function).
5. Neurons also release chemokines (CCL2, CCL12, CXCL1, CXCL8 and CX3CL1), which are then able to activate corresponding chemokine receptors on the microglia (paracrine response) (Module 7: Figure microglia interactions).
6. The microglia also express and release chemokines as part of the activation process and thus can feed information back to themselves (autocrine response) and can also act on the neurons (paracrine response). This chemokine signalling network is a key component of neuronal–microglia interactions.
7. Microglia express and release a number of neurotrophic factors such as brain-derived

Module 7: | Figure microglia interactions



Microglial surveillance and activation functions.

Microglial cells have two main functional states: surveillance and activation phases. During their surveillance mode (shown on the left) long fine processes monitor the environment and respond to many different stimuli such as pathogens and serum factors that invade the brain from ruptured blood vessels, abnormal proteins such as the β-amyloid and neurotransmitters released from the neurons. These different stimuli activate the microglia that then release a wide range of stimuli such as chemokines, trophic factors, cytokines and prostaglandins. Many of these stimuli act in an autocrine manner to feedback on to the microglia or they have a paracrine function by acting on the neurons or astrocytes. See text for further details and a description of the abbreviations.

neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), basic fibroblast growth factor (BFGF) and glial-derived neurotrophic factor (GDNF). These trophic factors function in a paracrine manner to promote both neuronal survival and regeneration. In addition, these factors have an autocrine function in that they can act on the microglia to prolong the activation state and this could have implications for the prolongation of neuropathatic pain.

8. As part of the inflammatory response, microglia secrete both nitric oxide (NO) and reactive oxygen species (ROS).
9. During the activation phase, microglial cells secrete a number of pro-inflammatory cytokines such as interleukins -1, -6, -10, -12 and -15 (IL-1, IL-6, IL-10, IL-12 and IL-15) and tumour necrosis factor (TNF) (Module 1: Figure cytokines). They also release anti-inflammatory transforming growth factor-β (TGF-β). These cytokines have paracrine actions on both neurons and astrocytes.
10. The microglia produce a number of eicosanoids such as prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), platelet-activating factor (PAF), thromboxane B_2 (TXB_2) and leukotriene C_2 (LTC_2). The microglia also express many of the receptors used to respond to these eicosanoids such as EP2, EP3 and TP (Module 1: Figure eicosanoids).
11. Activated microglia remove cell debris by a process of phagocytosis, which is activated by the UDP released from damaged and dying cells. This UDP acts on $P2Y_6$ receptors that increase the intracellular level of Ca^{2+} by recruiting the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette (Module 1: Table G protein-coupled receptors).
12. Microglia express the triggering receptor expressed in myeloid cells 2 (TREM-2), which is coupled to DAP12 to activate various signalling pathways (Module 1: Figure cytokines) that act as a negative regulator of innate immunity. Loss of function mutations in either the DAP12 gene (*TYROBP*) or in the TREM-2 gene have been linked to polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy syndrome (PLOSL), which is also known as Nasu-Hakola disease (NHD).

The signalling mechanisms used by microglial cells have not been established in the sort of detail described for macrophages. Since both cell types have many receptors in common and seem to display similar inflammatory responses, it is likely that the microglia will have signalling pathways very similar to those used by macrophages (Module 11: Figure macrophage signalling). However, there is a major difference between these two cell

types and that concerns the special relationship that exists between the microglia and neurons and this is explored further in the section on neuronal–microglia interactions.

A number of neurodegenerative diseases, such as Alzheimer's disease and Nasu-Hakola disease (NHD), have been linked to the inflammatory conditions created by excessive and prolonged activation of microglial cells.

Neuronal–microglia interactions

There is a two-way communication operating between the neurons and the microglia (Steps 4–6 in Module 7: Figure microglia interactions). Much of the interaction between these two cells seems to be carried out through a chemokine signalling network (Module 7: Figure neuronal chemokine function). The basis of this network is that both neurons and microglia can release and respond to chemokines. It has been proposed that this network might be responsible for a long-term up-regulation of neuronal activity within the pain transmission pathway leading to the onset of neuropathic pain. One of the current ideas is that the excessive stimulation during the acute phase of injury or disease results in a heightened sensitization of the participating neurons that can persist for a long time. In response to peripheral nerve injury, for example, this sensitization reaches a peak after about a week and can then persist for many weeks before declining. The following hypothesis, based on the idea of phenotypic signalsome remodelling (Module 12: Figure signalsome remodelling), attempts to describe how this switch in neuronal sensitization may develop. The basis of the hypothesis is that excessive neuronal activation due to peripheral injury brings about a phenotypic remodelling of the neurons that become sufficiently sensitized that they begin to conduct painful stimuli long after the original painful stimuli have gone. This remodelling may occur through the following reciprocal interactions operating between the neurons and microglia (Module 7: Figure neuronal chemokine function):

1. The remodelling begins when the neurons operating within the pain pathway are stimulated excessively resulting in the release of conventional neurotransmitters such as glutamate as well as chemokines such CCL2.
2. The CCL2 has both autocrine and paracrine actions in that it binds to CCR2 receptors, which are G protein-coupled receptors (Module 1: Figure chemokines), located on the neurons and microglia respectively.
3. These CCR2 receptors are coupled to G_q (Module 1: Table G protein-coupled receptors) and this can account for the generation of the Ca^{2+} signals and the activation of the nuclear factor of activated T cells (NFAT) that has been observed in these neurons. In addition, the CCR2 are also capable of activating ion channels that bring about membrane depolarization (ΔV).
4. It is known that NFAT acts on the promoter site for both CCL2 and CCL5 and this will result in an increased expression of these chemokines. At the same time, the membrane depolarization will sensitize neurons by acting presynaptically to enhance synaptic activity.
5. Neurons also express large amounts of the chemokine CX3CL1 (fractalkine), which is tethered to the cell surface (Module 7: Figure neuronal chemokine function). During intense nerve stimulation, proteases such as a disintegrin and the ADAM protease family cleave off the membrane tether and the CX3CL1 is then free to interact with the CX3CR1 receptors that are strongly expressed on the microglia and are thought to act through Ca^{2+}.
6. The microglial cells also have receptors that can respond to neurotransmitters such as glutamate and ATP that spill out of the neuronal synapses during intense stimulation. Microglia express both P2X and P2Y receptors and these would also contribute Ca^{+} signals that will enhance the expression and release of chemokines by activating the NFAT system (see Step 3 above Module 7: Figure neuronal chemokine function).
7. The increased formation of chemokines by microglial cells will thus increase the ambient level of CCL2 and this will further enhance neuronal excitability.
8. As described in Step 4, activation of CCR2 causes neuronal membrane depolarization (ΔV) that not only increases presynaptic release, but can also have postsynaptic effects to enhance neuronal excitability.
9. Conventional excitatory transmitters excite neurons by opening channels that pass inward current to depolarize the membrane sufficiently to trigger action potentials. Activation of the CCR2 receptors will enhance excitability by decreasing the resting membrane potential thus enhancing neuronal sensitivity.
10. A separate endocannabinoid retrograde signalling mechanism (Module 10: Figure endocannabinoid retrograde signalling) may have an analgesic effect by inhibiting pain transmission.

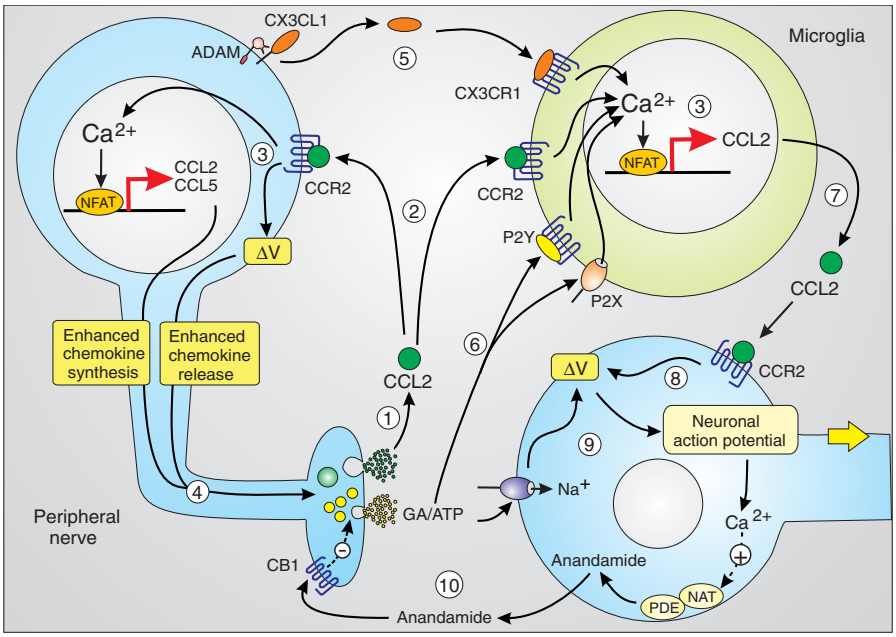
Up-regulation of the chemokine signalling network as outlined above provides a plausible mechanism to describe both the onset and persistence of neuropathic pain.

Endothelial cells

Endothelial cells are flattened cells that line the inside of blood vessels and provide a variable barrier between the circulating blood and the interstitial space. In larger vessels, they are surrounded by smooth muscle cells, but in the finer capillaries, they often are the only cell type. Endothelial cells have a number of signalling pathways that regulate their numerous functions in vascular biology:

- Endothelial control of vasoconstriction and vasodilation regulates blood pressure by controlling smooth muscle tone. This action is illustrated by the control of corpora cavernosum smooth muscle cell relaxation during penile erection (Module 7: Figure corpus cavernosum).
- There is endothelial regulation of paracellular permeability, especially during inflammatory responses, when there is a marked increase in paracellular permeability.

Module 7: | Figure neuronal chemokine function



Neuronal–microglia chemokine signalling interactions.

Neurons and microglia interact with each other within both the peripheral and central nervous systems. Much of this interaction is carried out by chemokines and both cell types not only synthesize and release chemokines but they can also respond to them thus setting up a two-way interacting network that has led to the development of a hypothesis to explain the neuronal sensitization responsible for neuropathic pain.

- Contribution to the processes of platelet aggregation during blood clotting (Module 11: Figure inflammation).
- Proliferation of endothelial cells contributes to angiogenesis.

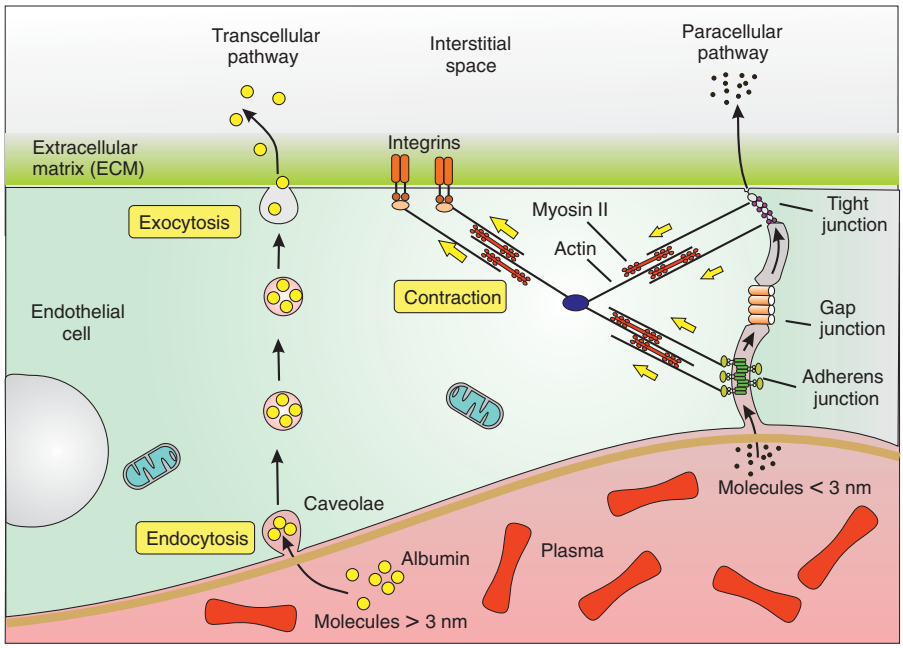
Endothelial regulation of paracellular permeability

The endothelial cells that line the inside of blood vessels provide a semi-permeable barrier that separates the plasma from the interstitial space (Module 7: Figure endothelial cell). This barrier is made up of two components. Firstly, there is an extracellular matrix (ECM) composed of a meshwork of different proteins (collagen IV, fibronectin, entactin, laminin, chondroitin sulphate, heparin sulphates, perlecan and syndecan). Endothelial cells are attached to this ECM through focal adhesion complexes based on integrin receptors. The second component of the barrier is the interendothelial junction (IEJ), which contains different junctional complexes. The tight junction is particularly important in restricting the movement of substances with molecular radii above about 3.0 nm. Ions and small molecules can pass through this paracellular pathway. However, anything above this radius, such as plasma proteins including albumin, are restricted and cross the endothelium through a transcellular pathway that is based on caveolae. Endothelial cells have a large number of caveolae, which pinch off at the luminal surface to form vesicles containing a cargo of proteins (endocytosis) that are then carried across to the basal surface. The vesicles fuse with the basal surface through an exocytotic process to release proteins to the interstitial space. Both the transcellular and paracellular pathways can be regulated. Considerable attention has focused on this paracellular pathway.

The paracellular pathway can be opened up by a contractile mechanism that displays some remarkable similarities to events in smooth muscle cells. This increase in endothelial permeability is particularly evident during inflammation, when inflammatory mediators such as thrombin stimulate the endothelial cells to contract and pull apart the cell junctions (Step 4 in Module 11: Figure inflammation). The contractile event is carried out by a cytoskeletal network of actin and non-muscle type II myosin filaments that are attached to cell junctions and to the cell adhesion complexes that face the ECM (Module 7: Figure endothelial cell). The myristoylated alanine-rich C-kinase substrate (MARCKS) protein may play a role in attaching the actin filaments to the membrane. Vascular endothelial growth factor (VEGF), which plays a critical role in inducing angiogenesis, can also increase vascular permeability by opening up the junctions between the endothelial cells. The signalling pathways that control permeability resemble those used to induce endothelial cell proliferation (Module 9: Figure VEGF-induced proliferation). The proteins that function to hold endothelial cells together are the targets for these signalling pathways and once phosphorylated the junctions weaken and the cells can be pulled apart.

At the tight junctions, occludin and claudin molecules function to fuse the two membranes at small patches to form the barrier. In addition, the C-terminal regions of occludin and claudin are attached to the zona occludens protein (ZO-1), which is linked to actin fibres through

Module 7: | Figure endothelial cell



Endothelial structure and mechanisms of transcellular and paracellular pathways.
One of the primary functions of the endothelial cell is to function as a semi-permeable barrier between the plasma and the interstitial space. Substances can cross this barrier via two separate pathways. Larger molecules such as albumin move through a transcellular pathway, where they are carried across the cell by caveolae that are pinched off from the luminal side (endocytosis) and then released to the basal side by exocytosis. Ions and small molecules with molecular radii smaller that 3 nm use a paracellular pathway by passing between the cells through the interendothelial junction (IEJ). This junctional region can be opened up in response to inflammatory mediators to increase the passage of plasma proteins and inflammatory cells to the interstitial space (Module 7: Figure regulation of paracellular permeability).

α-catenin and spectrin. Similarly, one of the classical cadherins (Module 6: Figure classical cadherin signalling) called vascular endothelial cadherin (VE-cadherin), which forms the adherens junction, is also linked to actin through β-catenin and p120 catenin. It seems likely that the actin filaments coming out from the tight junction and the adherens junction form an interconnected meshwork by interacting with the actin filaments coming from the integrin receptors. The VEGF receptors appear to be associated with the VE-cadherins and are thus positioned within the junction where they can control adhesive properties through phosphorylation of components such as β-catenin, p120 catenin, occluding and ZO-1.

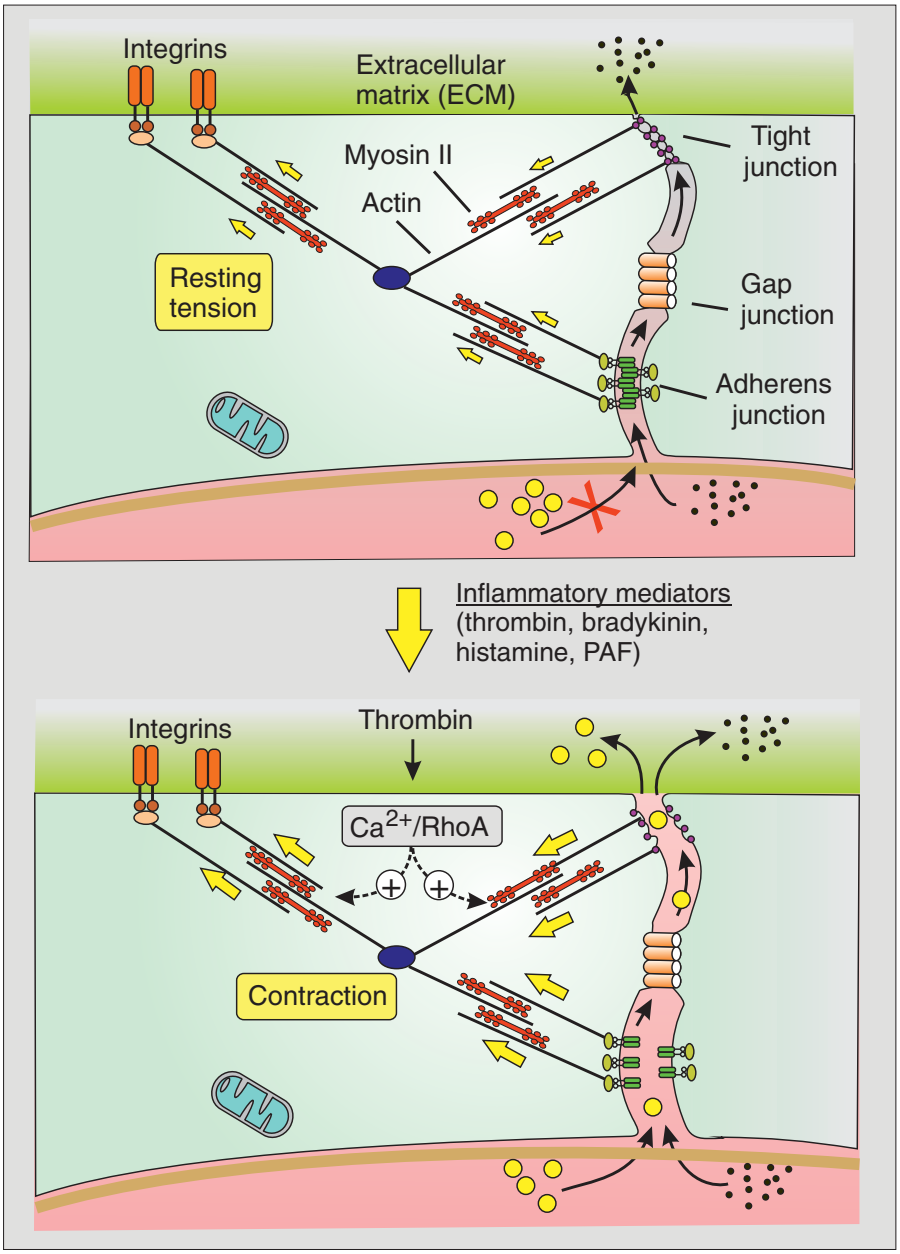
It has been suggested that there is a dynamic tension within this network, with the adhesive properties of the junctional complexes resisting the forces within the cytoskeleton trying to pull the junctions apart. Under normal conditions, the resting tension is relatively small and the junctions can resist the separation forces within the cytoskeleton to maintain the semi-permeable barrier (Module 7: Figure regulation of paracellular permeability). However, in response to mediators such as thrombin, the cytoskeletal network contracts to pull the junctions apart, thus opening up the barrier. The ability of thrombin to stimulate contraction depends upon the combined action of both the Ca^{2+} and RhoA signalling mechanisms (Module 7: Figure endothelial cell contraction).

The control of contraction in endothelial cells is remarkably similar to that found in smooth muscle cells (Module 7: Figure smooth muscle cell E-C coupling). In both cases, contraction is activated by two separate signalling pathways, one controlled by Ca^{2+} and the other by the RhoA signalling mechanism (Module 2: Figure Rho signalling).

The endothelial cells that line blood vessels are constantly subjected to fluctuating haemodynamic forces resulting from the pulsatile flow of blood. Mechanosenstive channels, which remain to be properly characterized, respond to these shear stresses by opening to allow entry of external Ca^{2+}. Ca^{2+} signals can also be generated by thrombin receptors that are coupled through the heterotrimeric protein G_q to phospholipase C (PLC) to generate inositol 1,4,5-trisphosphate ($InsP_3$), which then releases Ca^{2+} from the endoplasmic reticulum (Module 7: Figure endothelial cell contraction). Endothelial cells also express a number of transient receptor potential (TRP) ion channel family members. The canonical TRP 1 (TRPC1) channel may function as a store-operated channel (SOC) in endothelial cells to provide the prolonged Ca^{2+} signal that has been observed during the action of thrombin. This increase in Ca^{2+} acts through calmodulin (CaM) to stimulate the CaM-dependent myosin light chain kinase (MLCK) to phosphorylate the myosin light chain (MLC) that then enables the non-muscle myosin II to interact with actin to induce contraction.

Endothelial contraction can also be activated by the RhoA signalling mechanism. Thrombin is also known to act through $G_{12/13}$, which is one of the G protein subunits capable of activating RhoA (Module 2: Figure Rho signalling). The active RhoA/GTP complex has been implicated in a number of endothelial processes

Module 7: | Figure regulation of paracellular permeability



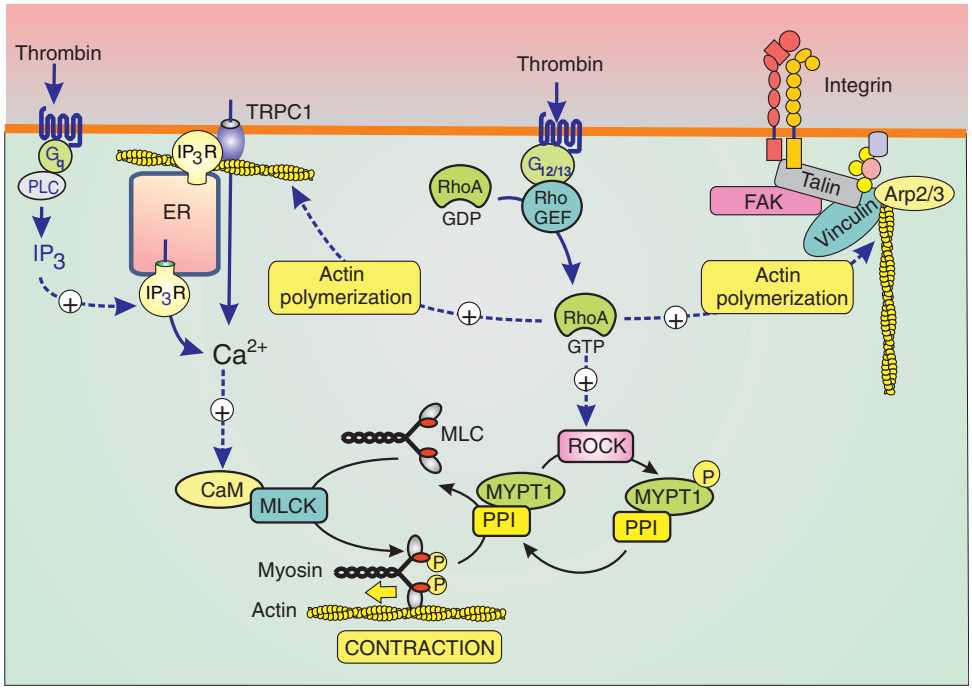
Regulation of paracellular permeability by contraction of the cytoskeleton.
Under resting conditions, the endothelial cells provide a semi-permeable barrier by denying access to large molecules such as albumin. Inflammatory mediators induce contraction of the cytoskeleton that pulls on the molecules of the tight and adherens junctions to open up the permeability barrier to large molecules and inflammatory cells, such as neutrophils and monocytes. The signalling mechanism that controls contraction depends on the activation of contraction by both Ca^{2+} and RhoA, as illustrated in Module 7: Figure endothelial cell contraction.

(Module 7: Figure endothelial cell contraction). It can stimulate the Rho kinase (ROCK) to phosphorylate the myosin phosphatase targeting subunit (MYPT1), which results in inhibition of protein phosphatase 1 (PP1). RhoA thus facilitates contraction by reducing the dephosphorylation of MLC. In addition, RhoA can increase actin polymerization and appears to act by converting cortical actin into the stress fibres responsible for contraction. Finally, there also are indications that the ability of RhoA to induce actin polymerization seems to enhance the entry of Ca^{2+} through the TRPC1 channels, perhaps by facilitating the mechanism of store-operated channel (SOC) activation (Module 3: Figure STIM-induced Ca^{2+} entry).

Skeletal muscle

Skeletal muscle is responsible for locomotion and for moving various parts of the body, such as the diaphragm. Skeletal muscle structure is characterized by the high degree of organization of the contractile proteins actin and myosin that are arranged into striated myofibrils. The coordinated activation of these myofibrils is mostly under

Module 7: | Figure endothelial cell contraction



Control of endothelial permeability by contraction of actomyosin.
Opening of the endothelial permeability barrier is regulated by contraction of the actomyosin filaments making up the cytoskeletal network. One of the major inflammatory stimuli is thrombin, which seems to act through both Ca^{2+} and RhoA. Details of these two pathways are described in the text.

voluntary nervous control, as opposed to the involuntary control of smooth muscle cells. Control of contraction is regulated by motor neurons that release acetylcholine to initiate the process of excitation–contraction (E-C) coupling in skeletal muscle. In addition to this control over contraction, there also is a tight regulation of energy metabolism in skeletal muscle. A process of excitation–metabolism coupling in skeletal muscle ensures that the levels of ATP remain constant despite variations in demand. There are sophisticated feedback loops, such as the Ca^{2+}-dependent breakdown of glycogen, that ensure that ATP levels remain relatively constant during normal contraction. During intense activity usually associated with stress, energy metabolism can be enhanced further through a process of adrenaline (epinephrine)-induced glycogenolysis. Storage of glycogen in skeletal muscle is regulated by insulin-induced glycogen synthesis.

Skeletal muscle structure

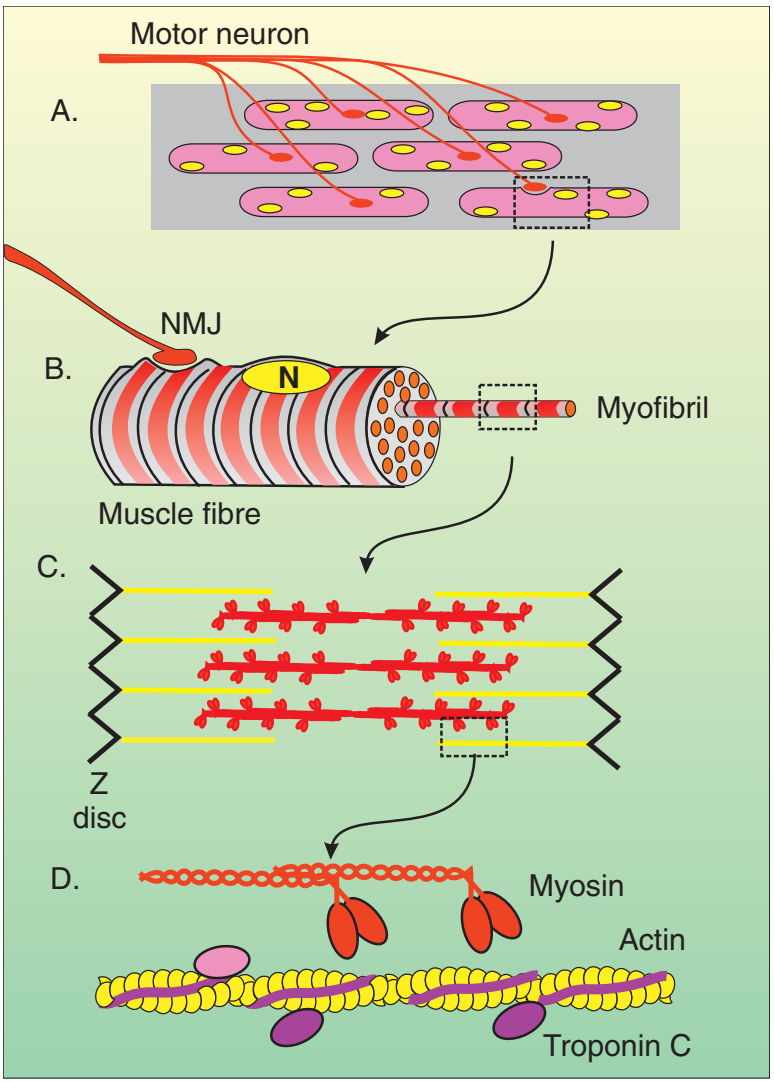
The basic contractile unit in skeletal muscle is the muscle fibre (Module 7: Figure skeletal muscle structure). This fibre is not a conventional cell, but is a syncytium formed by the fusion of myoblasts during the differentiation of skeletal muscle (Module 8: Figure skeletal muscle myogenesis). The individual muscle fibres are held together by connective tissue to form the compact muscles that connect to different parts of the skeleton. The individual fibres are often very long, and can stretch from one end of the muscle to the other, but usually the fibres are shorter than the muscle as a whole and are bound together by connective tissue septa. The contractile machinery, which makes up the bulk of the fibre, is arranged in the form of myofibrils that have characteristic striations due to the precise organization of the contractile proteins actin and myosin (Module 7: Figure skeletal muscle structure). The process of excitation–contraction (E-C) coupling in skeletal muscle functions to deliver the global Ca^{2+} signal that drives the interaction between these two contractile proteins (Module 7: Figure skeletal muscle E-C coupling).

Excitation–contraction (E-C) coupling in skeletal muscle

The process of excitation–contraction (E-C) coupling in skeletal muscle cells concerns the way in which an electrical signal in the sarcolemma is transduced into the Ca^{2+} signal that activates contraction. In addition to the control of contraction, there are signalling mechanisms to regulate energy metabolism. The way in which all these signalling events are co-ordinated to regulate skeletal muscle function are outlined in the following steps, as shown in Module 7: Figure skeletal muscle E-C coupling:

1. The process begins when the motor neuron transmits an action potential that travels down the motor neuron to the neuromuscular junction, where it triggers the release of acetylcholine (ACh) into the narrow synaptic cleft.
2. The ACh diffuses across the narrow synaptic cleft (a 40–50 nm gap separates the presynaptic ending from the sarcolemma). During each action potential,

Module 7: | Figure skeletal muscle structure



Structure of skeletal muscle fibres.
A. The multinucleate muscle fibres are held together in a connective tissue matrix. The nuclei (N) are located close to the cell surface. Each fibre is innervated by a motor neuron that terminates at the neuromuscular junction (NMJ). B. Each muscle fibre has characteristic striations that reflect the precise organization of the contractile proteins actin and myosin along the length of the myofibril. C. The basic contractile unit is the sarcomere. At either end of each sarcomere are the Z discs to which the actin filaments (yellow) are attached. D. Actin has a helical structure made up of spherical subunits [globular actin (G-actin)] joined together into two strands that wind around each other to form the F-actin helix with a diameter of 8 nm. The other major contractile protein is myosin, which is organized into filaments that lie in the middle of the sarcomere between the actin fibres. Each filament contains about 200 myosin molecules. Each myosin molecule has a head region and a long tail. The long tails of two myosins wrap around each other to form a dimer, which in turn aggregates to form the myosin filament, with the head regions lined precisely opposite the actin filaments. When viewed in transverse sections, the actin filaments form a hexagonal pattern around each myosin filament.

approximately 10 000 molecules of ACh are showered into this restricted region of the muscle surface, which contains about 2×10^7 nicotinic acetylcholine receptors (nAChRs). The nAChRs are typical receptor-operated ion channels (Module 3: Figure nicotinic acetylcholine receptor) that respond to ACh by gating approximately 50 000 cations (mostly Na^+, but also some Ca^{2+}), which results in a small depolarization (0.3 μV). The corresponding voltage changes in neighbouring receptors are summed to give the large end-plate potential (ΔV) that elicits the all-or-none action potential.

3. The muscle action potential spreads over the entire muscle membrane and down the T-tubules, where the depolarization activates the $Ca_V1.1$ voltage-operated Ca^{2+} channel (Module 3: Figure $Ca_V1.1$ L-type channel), which interacts directly with the type 1 ryanodine receptor (RYR1) (module 4 in Module 2: Figure Ca^{2+} modules). The coupling between depolarization and the release of Ca^{2+} from the sarcoplasmic reticulum (SR) occurs at the triadic junction, where the SR makes close contact with the T-tubule. These two membranes are connected together by foot structures, which are the aggregates of the $Ca_V1.1$ L-type channels and RYR1, which are functionally coupled together, to transmit information across the 20 nm gap that separates the membranes (Module 3: Figure L-type channel/RYR1 complex). The structural organization of the triadic

junction appears to be maintained by junctophilin-1 (JP1). The $Ca_V1.1$ L-type channel contains a specific RYR1-binding region located in the cytoplasmic loop located between domains II and III (Module 3: Figure $Ca_V1.1$ L-type channel).

4. The transduction process begins when the $Ca_V1.1$ L-type channel responds to depolarization by undergoing a voltage-dependent conformational change that activates the RYR1 to release Ca^{2+} from the SR (Module 7: Figure skeletal muscle E-C coupling).
5. Ca^{2+} released from the SR diffuses towards the sarcomeres, where it acts on troponin C (TnC) to trigger the interaction between actin and myosin that is responsible for contraction. Muscle relaxation occurs when this Ca^{2+} is pumped back into the SR by the sarco/endo-plasmic reticulum Ca^{2+} ATPase 1 (SERCA1). Inactivating mutations of SERCA1 are the cause of Brody disease.

Excitation–metabolism coupling in skeletal muscle

The energy for muscle contraction comes from glucose, which is stored within the muscle in the form of glycogen. An important control of energy supply depends upon the rate at which glycogen is hydrolysed to glucose by the enzyme phosphorylase. Inactive phosphorylase *b* is converted into active phosphorylase *a* by the enzyme phosphorylase kinase, which is the focal point for two signalling systems. One operates through cyclic AMP, the other through Ca^{2+}, as illustrated in Steps 6–8 in Module 7: Figure skeletal muscle E-C coupling:

6. The control of energy production by adrenaline (epinephrine) begins with the activation of the cyclic AMP signalling pathway. Adrenaline binds to the β-adrenergic receptor that acts through the heterotrimeric G protein (G_S) to activate adenylyl cyclase (AC) to produce the second messenger cyclic AMP.
7. Cyclic AMP then diffuses into the cell to bind to protein kinase A (PKA), which acts to phosphorylate the phosphorylase kinase that converts inactive phosphorylase *b* into active phosphorylase *a*. Phosphorylase kinase is also sensitive to Ca^{2+}, and this provides an elegant feedback mechanism because Ca^{2+} can simultaneously activate both energy metabolism and contraction.
8. Phosphorylase *a* hydrolyses glycogen to glucose, which is fed into glycolysis to provide the ATP necessary to power contraction.

Insulin control of skeletal muscle glycogen synthesis

Skeletal muscle is an important component of the metabolic energy network (Module 7: Figure metabolic energy network). It is able to take up glucose and synthesize glycogen through processes that are regulated by insulin through Steps 9–13, as shown in Module 7: Figure skeletal muscle E-C coupling:

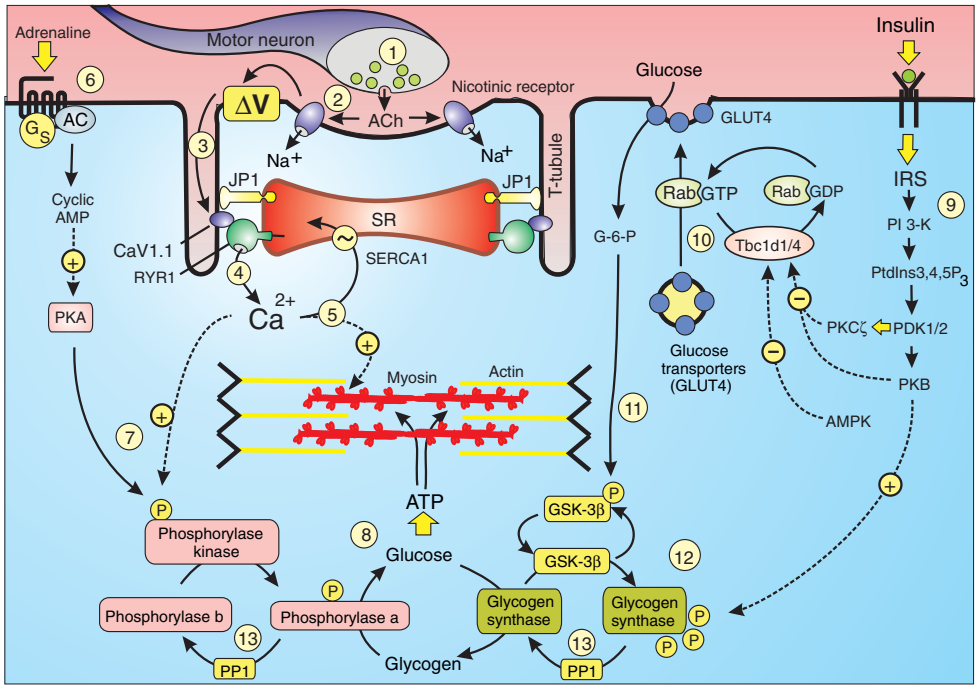
9. Insulin acts on skeletal muscle to regulate both glucose uptake and glycogen synthesis. This control is exerted through the PtdIns 3-kinase (PI 3-K) signalling pathway that is activated by the insulin receptor (Module 2: Figure insulin receptor).
10. GLUT4-containing vesicles (GSVs) containing the insulin-sensitive glucose transporter 4 (GLUT4) are induced to fuse with the sarcolemma following activation by either protein kinase Cζ (PKCζ), protein kinase B (PKB) or AMPK. These kinases act by phosphorylating various Rab GTPase-activating proteins (GAPs) such as Tbc1d1 and Tbc1d4/AS160, which normally inactivate the Rab signalling mechanism (Module 2: Figure Rab signalling). Once phosphorylated, these GAPs are inhibited, which thus promotes signalling through the Rabs responsible for promoting insertion of the GSVs into the plasma membrane (Module 7: Figure skeletal muscle E-C coupling).
11. Once the vesicles fuse with the sarcolemma, the glucose transporters begin to feed glucose into the cell, where it is converted into glucose 6-phosphate (G6P) by hexokinase, before being passed on to glycogen synthase to be converted into glycogen.
12. The activity of glycogen synthase is regulated by its level of phosphorylation: it is active when dephosphorylated. When the PtdIns 3-kinase pathway is activated by insulin, the PKB phosphorylates glycogen synthase kinase-3β (GSK-3β), thereby inactivating the enzyme and preventing it from phosphorylating glycogen synthase.
13. Protein phosphatase 1 (PP1) contributes to the activation process by dephosphorylating glycogen synthase. Glycogen functions as a scaffold to hold together many of these enzymes that function in its metabolism (Module 5: Figure PP1 targeting to glycogen).

Cardiac cells

The heart is an organ superbly designed to pump blood around the body with a remarkable degree of regularity and adaptability. The regularity is controlled by a pacemaker system located in the sinoatrial node pacemaker cell that generates the repetitive action potentials that travel through gap junctions to excite all the contractile cells to drive each heartbeat (Module 7: Figure heart summary). This communication through electrical signals is used to spread the action potentials from the sinoatrial node throughout the atrium, where it triggers atrial cell contraction. The action potential then invades the atrial–ventricular node that is coupled to the Purkinje fibres responsible for transmitting action potentials to the ventricles to stimulate ventricular cell contraction.

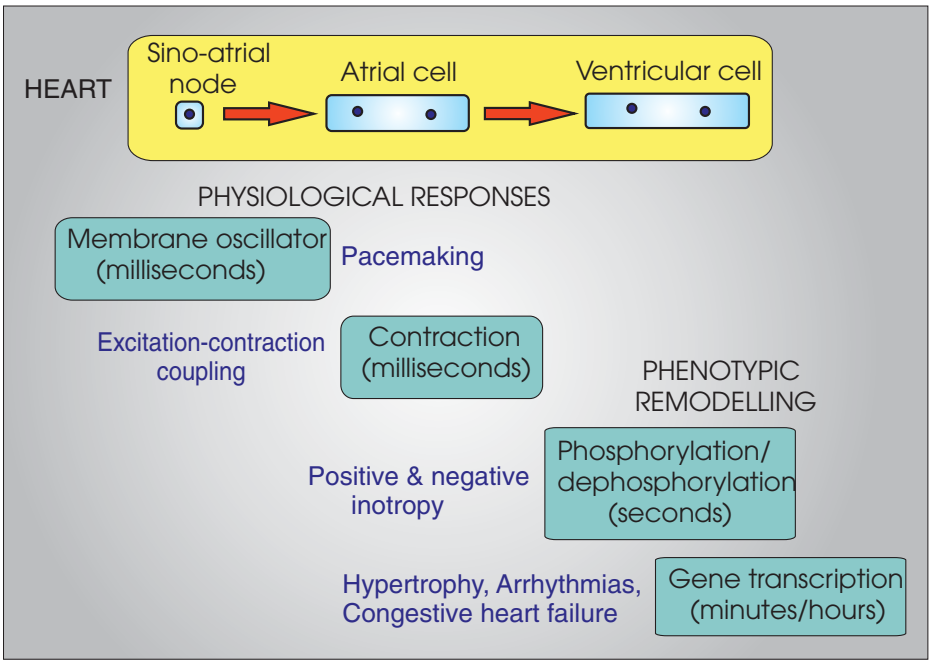
One of the remarkable properties of the heart is its ability to adapt to changing demands. In the short term, the modulation of ventricular Ca^{2+} signals in response to different hormones can account for both positive and negative inotropy. In the longer term, ventricular cells can adapt to persistent modulator signals by undergoing a process of phenotypic remodelling of the signalsome. For example, an increased load on the heart results in cardiac hypertrophy that can lead to congestive heart failure (CHF).

Module 7: | Figure skeletal muscle E-C coupling



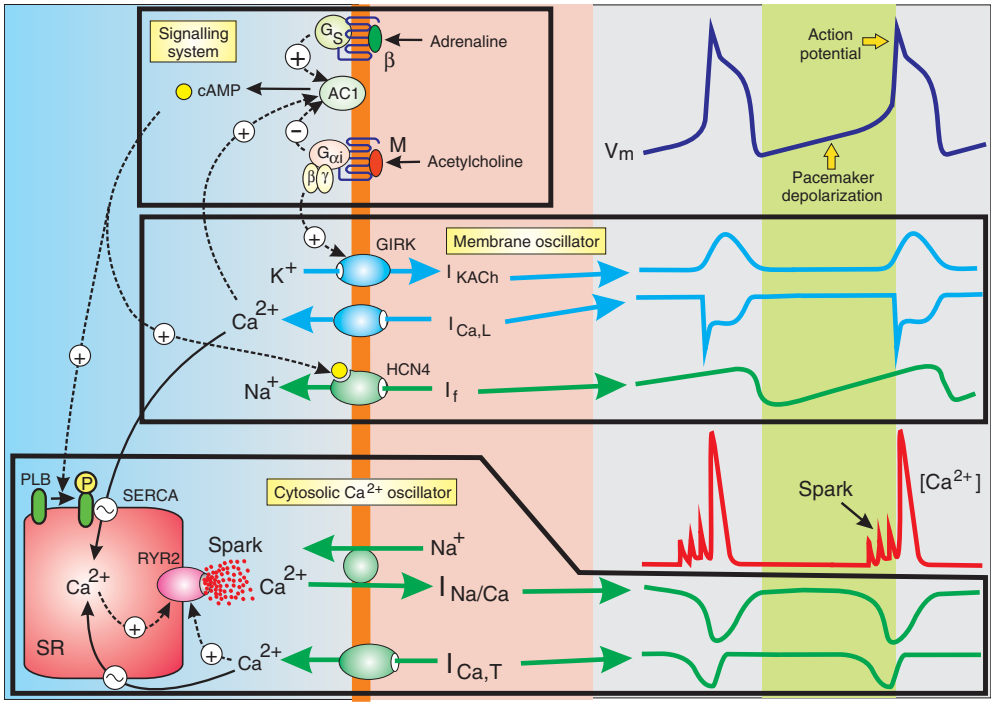
Skeletal muscle control mechanisms of contraction and energy metabolism.
A number of different signalling pathways co-operate to regulate the activity of skeletal muscle contraction and metabolism. The processes of excitation–contraction (E-C) coupling is the sequence of events that transduce the electrical signal arriving via the motor neurons into the Ca^{2+} signal that triggers contraction (Steps 1–5). The supply of energy is increased both by Ca^{2+} (Step 7) and by the adrenaline-induced activation of the cyclic AMP signalling pathway (Steps 6–8). Insulin promotes energy storage by stimulating glycogen synthesis (Steps 9–13).

Module 7: | Figure heart summary



Summary of major physiological and pathophysiological processes in the heart.
The sinoatrial node cell, which has a typical membrane oscillator, is responsible for pacemaking by generating an action potential that spreads to the atrial and ventricular cells to initiate excitation–contraction coupling that occurs on a millisecond time scale. Phenotypic remodelling through phosphorylation/dephosphorylation processes can change the force of contraction (positive and negative inotropy) over a time scale of seconds. Phenotypic remodelling operating over minutes/hours or longer can enhance the size of the heart (hypertrophy) and can also lead to pathophysiological changes resulting in arrhythmias and congestive heart failure (CHF).

Module 7: | Figure cardiac pacemaker



The major ionic channels and conductances that contribute to the cardiac pacemaker located in the sinoatrial node of the heart.
The membrane potential (V_m) undergoes two main changes: a slow depolarization (pacemaker depolarization) that occurs during diastole (the green-shaded region) that sets the stage for the periodic action potentials. There are three main components of the oscillator (boxes): the signalling system that regulates oscillator frequency, the membrane oscillator and the cytosolic Ca^{2+} oscillator. The channels shown in green generate the pacemaker potential as described in the text.

Sinoatrial node pacemaker cells

The rhythmical contraction of the heart is driven by the cardiac pacemaker located in the sinoatrial (SA) node. This SA node consists of a small group of specialized muscle cells that has an oscillatory mechanism, which consists of a membrane oscillator that is coupled to a cytosolic Ca^{2+} oscillator, that spontaneously generates repetitive action potentials (Module 7: Figure cardiac pacemaker). The electrical activity of these cells is characterized by a slow pacemaker depolarization of the membrane potential (V_m), which is responsible for triggering each cardiac action potential. The steepness of this pacemaker potential determines the frequency of the rhythm. The main pacemaker channel currents operating during the pacemaker depolarization can be divided into two groups depending on whether they are driven by the membrane oscillator or the cytosolic Ca^{2+} oscillator.

Pacemaker channel currents

The slow depolarization that occurs during the pacemaker phase (the green shaded area in Module 7: Figure cardiac pacemaker) depends upon a number of currents that are generated during the operation of two interacting oscillators: a membrane oscillator that is driven by various voltage-sensitive channels and a cytosolic Ca^{2+} oscillator that depends on Ca^{2+} release from the RYRs. The fact that these two oscillators act together, means that the pacemaker mechanism has some degree of redundancy.

In the case of the membrane oscillator, the primary pacemaker channel is I_f (f stands for funny) that is carried by Na^+ that provides the inward current that slowly depolarizes the membrane. This I_f current is carried by the HCN4 member of the hyperpolarizing-activated cyclic nucleotide (HCN)-gated channels (Module 3: Figure HCN channels). The key properties of this channel is that it is activated by hyperpolarization, which means that I_f switches on when the action potential hyperpolarizes and then slowly decays throughout the rest of the pacemaker period (the green shaded area in Module 7: Figure cardiac pacemaker). The channels are somewhat non-selective, and since they tend to carry Na^+ rather than K^+, I_f generates an inward current that slowly depolarizes the membrane potential (V_m) towards the threshold for activating the next action potential.

In the case of the cytosolic Ca^{2+} oscillator, the $I_{Na/Ca}$ current comes into play towards the end of the final stages of the pacemaker phase to speed up the onset of the action potential (Module 7: Figure cardiac pacemaker). Release of Ca^{2+} from the SR close to the sarcolemma plays a primary role in this pacemaker mechanism by controlling the onset of this $I_{Na/Ca}$ current by activating the Na^+/Ca^{2+} exchanger (NCX). Opening of the RYRs during the action potential contributes to the global elevation in Ca^{2+}. During the interspike interval, the SERCA pumps replenish the store and there is a slow build-up of Ca^{2+} within the SR lumen and this may contribute to the pacemaker mechanism by sensitizing the RYRs to generate the periodic Ca^{2+} sparks.

When pacemaker cells are imaged, a series of Ca^{2+} sparks is evident in the run up to the action potential (Module 7: Figure pacemaker Ca^{2+} sparks). These sparks occur immediately below the membrane, where there is close apposition between the sarcoplasmic reticulum (SR) and the sarcolemma (Module 7: Figure SA node junctional zone). The appearance of these sparks appears to be facilitated by the activity of the Ca_V3 family of T-type Ca^{2+} channels. As I_f depolarizes the membrane, the T-type channels begin to open and the Ca^{2+} that flows into the cell will help to load the store and may also activate the ryanodine receptor 2 (RYR2) channels in the SR to create localized Ca^{2+} sparks (Module 7: Figure pacemaker Ca^{2+} sparks). Some of the Ca^{2+} from these sparks is extruded from the cell via the Na^+/Ca^{2+} exchanger (NCX). As three Na^+ ions enter for each Ca^{2+} that is extruded, the $I_{Na/Ca}$ is a net inward current that acts to further depolarize the membrane and to activate more T-type channels. The T-type channel, the RYR2 and the NCX thus function as a feedback amplifier that comes in to play towards the end of the pacemaker phase to initiate the onset of the action potential (Module 7: Figure cardiac pacemaker).

In summary, the activation of $I_{Na/Ca}$ amplifies the unitary T-type currents, thereby prolonging the inward current flow. It is estimated that only a few sparks are necessary to enhance the pacemaker towards the threshold for the onset of the action potential. This is a feedback amplifier based on the release of internal Ca^{2+} that is coupled to the plasma membrane pacemaker mechanism.

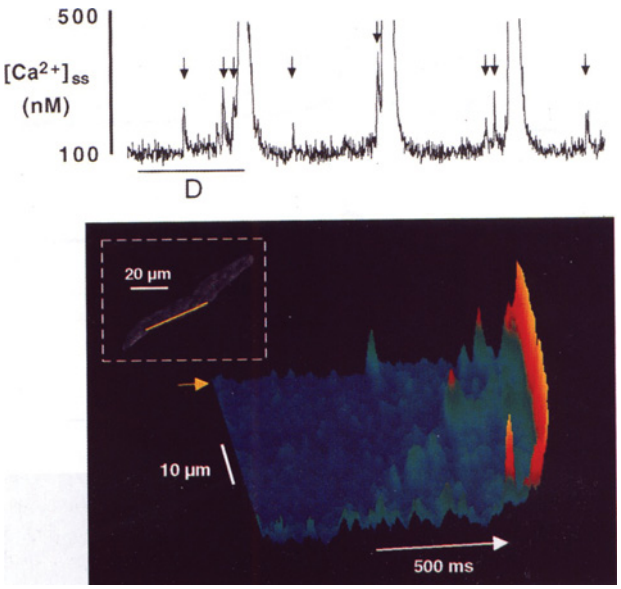
The frequency of the oscillator is regulated by a signalling system that can be increased by adrenaline or reduced by acetylcholine both of which act through the cyclic AMP signalling pathway. Adrenaline acts through β-adrenoceptors and G_S to stimulate type 1 adenylyl cyclase (AC1) (Module 2: Table adenylyl cyclases). AC1 is one of the isoforms that is activated by Ca^{2+}. The increase in cyclic AMP accelerates the oscillator in two ways. Firstly, cyclic AMP can increase membrane depolarization by binding to the cyclic nucleotide-binding domain (CNBD) on the C-terminal tail to stimulate the hyperpolarizing-activated cyclic nucleotide (HCN)-gated channels (Module 3: Figure HCN channels) responsible for I_f. Secondly, cyclic AMP stimulates the phosphorylation of phospholamban (PLB) to accelerate the SERCA pump to enhance the loading of the SR with Ca^{2+} to initiate the Ca^{2+} sparks.

Acetylcholine acting through G_i reduces the frequency through two mechanisms. First, $G_{\alpha i}$ inhibits AC1 to reduce the level of cyclic AMP thereby reducing the activation of HCN4 channels responsible for I_f. Secondly, the $\beta\gamma$ subunit activates the GIRK channel to hyperpolarize the membrane.

Action potential currents

The sinoatrial (SA) node action potential is driven by two main currents, the $I_{Ca,L}$ that flows through the $Ca_V1.2$ L-type channel and I_K that flows through K^+ channels (blue traces in Module 7: Figure cardiac pacemaker). When the pacemaker depolarization reaches the activation threshold for the L-type channel, the latter opens and the inward $I_{Ca,L}$ rapidly depolarizes the membrane to give the rapid upstroke of the action potential. The rapid depolarization then switches on the K^+ channel, and this strong outward current (I_K) rapidly curtails the action potential by hyperpolarizing the membrane. It is this strong hyperpolarization that then activates I_f to begin the next pacemaker phase, as described earlier.

Module 7: | Figure pacemaker Ca^{2+} sparks



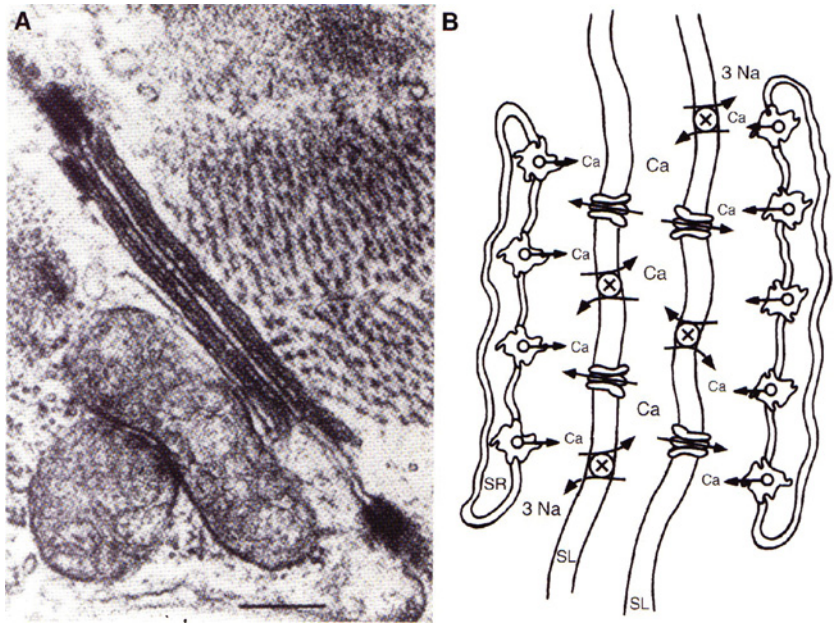
Appearance of Ca^{2+} sparks in an atrial pacemaker cell.

The measurement of Ca^{2+} in the top trace illustrates the appearance of small sparks that precede the onset of the large Ca^{2+} spike that occurs during the action potential. The spatiotemporal plot shown on the bottom provides a visual record of the Ca^{2+} signals that occur in a region close to the plasma membrane (indicated by the orange line in the inset where the scan was made) during the final period of the pacemaker phase. Note the small Ca^{2+} sparks that occur before the onset of the large uniform elevation of Ca^{2+} that occurs when the L-type channels open during the action potential. Reproduced from Hüser, J., Blatter, L.A. and Lipsius, S.L. (2000) Intracellular Ca^{2+} release contributes to automaticity in cat atrial pacemaker cells. J. Physiol. 524:415–422, with permission from Blackwell Publishing; see Hüser et al. 2000.

Ventricular cells

The large striated muscle cells that make up the ventricles are collectively responsible for generating the force that pumps blood out of the heart. This pumping action depends on the co-ordinated activation of all the ventricular cells. This co-ordination is achieved by the action potential that sweeps through all the cells very quickly to ensure that they all contract in unison. In addition to co-ordinating the activity of cells throughout the ventricle, the action potential also serves to synchronize the large number of contractile units within each cell. This internal synchronization is achieved through the highly ordered ventricular and atrial cell structure that ensures that the 20000 individual dyadic junctions (the signalling units that generate Ca^{2+} signals) are located in register with the contractile units. The dyadic junctions are responsible for the process of excitation–contraction (E-C) coupling during which the electrical signal in the plasma membrane triggers ventricular cell Ca^{2+} release from the sarcoplasmic reticulum. One

Module 7: | Figure SA node junctional zone



Sinoatrial node junctional zone between the sarcolemma and sarcoplasmic reticulum (SR).
The electron micrograph on the left illustrates a contact between two sinoatrial cells. Note the thin stacks of sarcoplasmic reticulum (SR) membranes closely apposed to the sarcolemma. The diagram on the right shows how Ca^{2+} released from the SR is directed towards the sarcolemma, which has an array of channels and Na^+/Ca^{2+} exchangers. Reproduced from Lipsius, S.L., Hüser, J. and Blatter, L.A. (2001) Intracellular Ca^{2+} release sparks atrial pacemaker activity. News Physiol. Sci. 16:101–106, with permission from the American Physiological Society; see Lipsius et al. 2001.

of the remarkable properties of the ventricular signalling system is its ability to adapt to changing demands. In the short term, the modulation of ventricular Ca^{2+} signals in response to different hormones can account for both positive and negative inotropy. In the longer term, ventricular cells can adapt to persistent modulator signals by undergoing a process of phenotypic remodelling of the signalsome. For example, an increased persistent load on the heart results in cardiac hypertrophy that can lead to congestive heart failure (CHF).

Ventricular and atrial cell structure

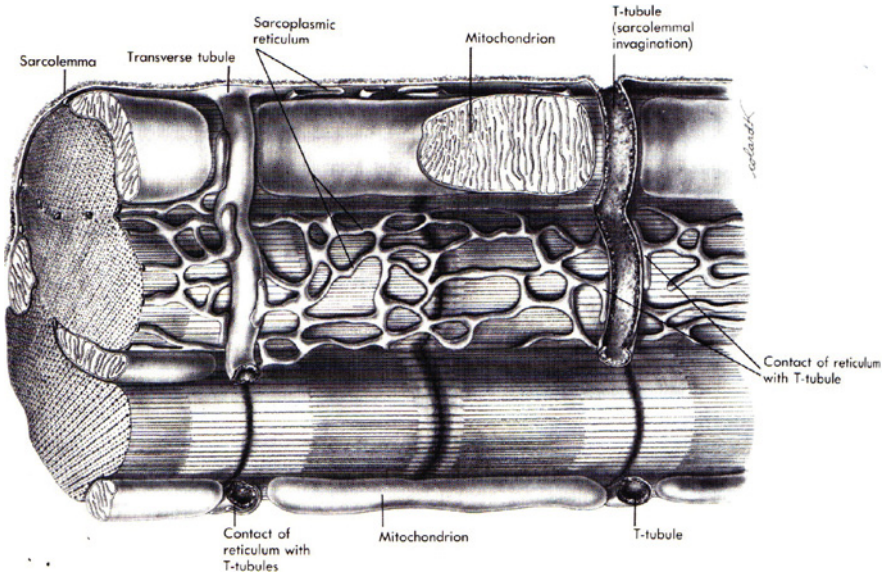
The two major contractile cells in the heart (ventricular and atrial cells) differ markedly both in their structure and in the way that electrical stimulation induces the release of Ca^{2+} by the type 2 ryanodine receptors (RYR2s) on the sarcoplasmic reticulum (SR). This difference results from the way the SR is organized relative to the plasma membrane (Module 7: Figure ventricle and atrial cell organization). In ventricular cells, the plasma membrane has tubular invaginations (T-tubules) that make regular contacts with specialized regions of the SR at the junctional zones (Module 7: Figure ventricle cell). Atrial cells lack T-tubules, and the junctional SR makes contact with the sarcolemma at the cell surface, and the rest forms the non-junctional SR that projects into the cell interior (Module 7: Figure ventricle and atrial cell organization). The junctional SR and the non-junctional regions are separated by a gap that is populated by mitochondria, which may function as a firewall to restrict Ca^{2+} released by the junctional SR from diffusing in to excite the RYR2s on the internal non-junctional SR. This mitochondrial firewall may feature significantly in the modulation of atrial Ca^{2+} signals. The difference in the structural organization between these two cell types is clearly apparent in sections that have been stained with an antibody against RYR2 (Module 7: Figure RYR2 location in cardiac cells).

The critical region of the ventricular cell with regard to excitation–contraction (E-C) coupling is the junctional zone where the ryanodine receptors 2 (RYR2s) on the junctional SR face the T-tubule (Module 7: Figure ventricular junctional zone). Note how the junctional zone makes close contact with the T-tubule. A critical component of the E-C coupling mechanism is the relationship between the Ca^{2+} channels on the two membranes (Module 7: Figure junctional zone Ca^{2+} channels). There are approximately 10000 junctional zones in each ventricular cell. The T-tubule region that faces each junctional zone contains about ten $Ca_V1.2$ L-type channels, which provide the trigger Ca^{2+} responsible for activating the RYR2s. There are about 100 RYRs located in each junctional zone arranged in a pseudo-crystalline array. These L-type Ca^{2+} channels and RYRs are critical components of ventricular cell Ca^{2+} release. This release of Ca^{2+} is activated by the ventricular cell action potential, which is generated by the sinoatrial node pacemaker cells.

Ventricular cell action potential

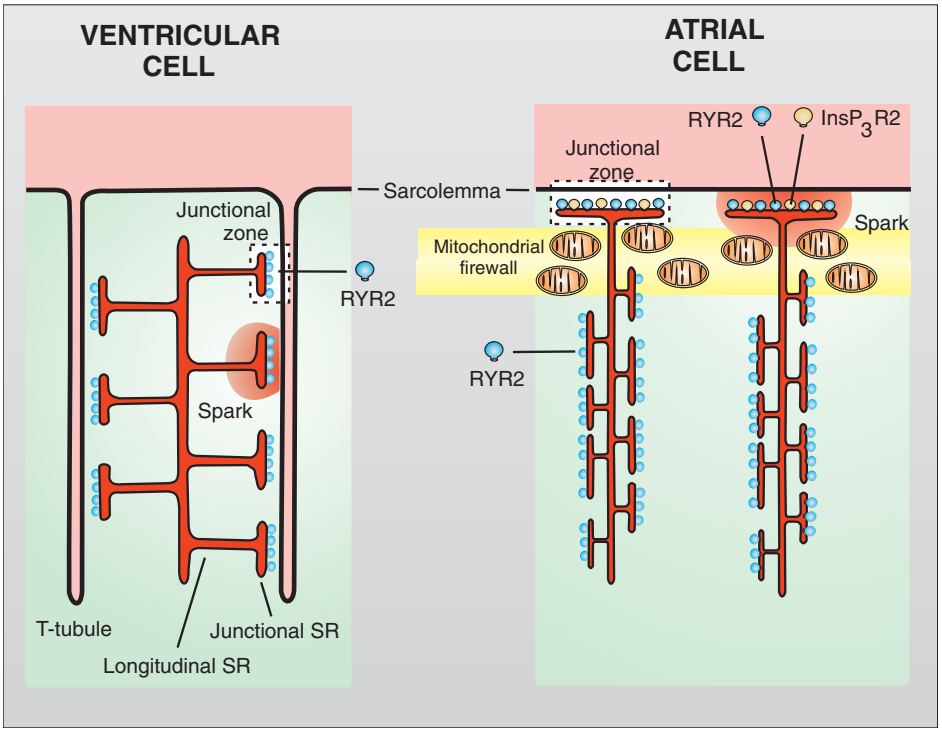
The ventricular cell action potential depends on the sequential activation and inactivation of a number of inward and outward currents (Module 7: Figure ventricular action potential). The membrane potential passes through a series

Module 7: | Figure ventricle cell



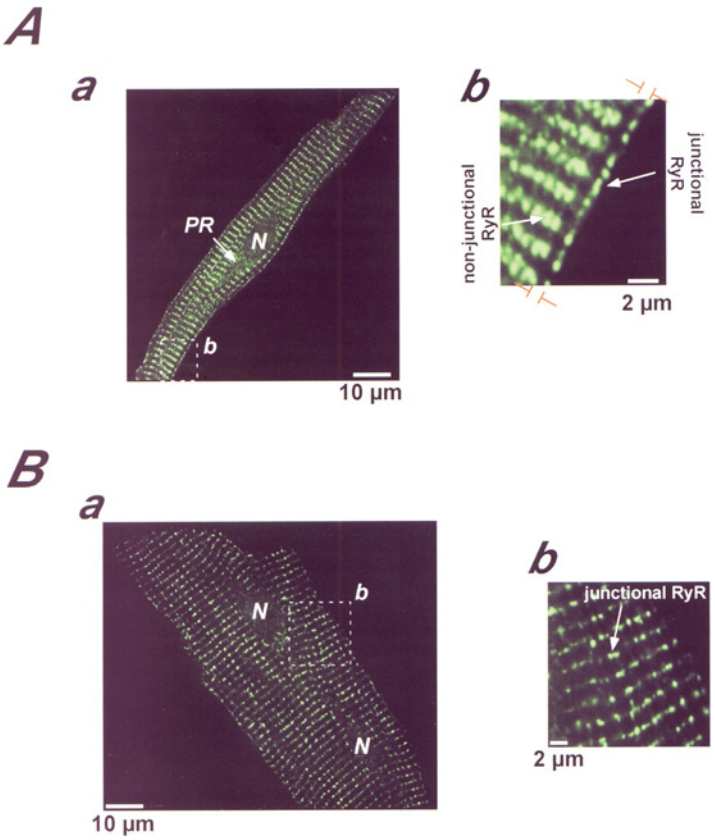
Structural organization of the cardiac ventricular cell.
The plasma membrane has periodic transverse tubules (T-tubules), which extend deep into the cell at regular intervals that coincide with the Z-band of the contractile myofibrils. The bulk of the cell is composed of these striated myofibrils, which are surrounded by mitochondria and by the sarcoplasmic reticulum (SR). The SR has two parts: the longitudinal SR that forms a meshwork around the myofibrils and the junctional SR that make closes contact with the T-tubules to form the dyadic junction. It is at this dyadic junction where the electrical signal travelling down the T-tubule triggers the release of Ca^{2+} from the junctional SR during the process of excitation–contraction (E-C) coupling. Reproduced from William Bloom and Don W. Fawcett, *A Textbook of Histology*, 10th edition (W.B. Saunders, 1975), © 1975 by W.B. Saunders Company, reproduced by permission of Edward Arnold (D.W. Fawcett and S. McNutt, *Journal of Cell Biology*, 42:1, 1969); see Bloom and Fawcett 1975.

Module 7: | Figure ventricle and atrial cell organization



Comparison of the structural organization of cardiac ventricular and atrial cells.
In ventricular cells, T-tubules extend deep into the cell, where they make regular contact with the junctional sarcoplasmic reticulum (SR) to form junctional zones (see boxed area) that are responsible for excitation–contraction (E-C) coupling. The blue spheres are the type 2 ryanodine receptors (RYR2s) that release a Ca^{2+} spark that can occur spontaneously when the cells are at rest, as shown here. In atrial cells, there are no T-tubules, and the junctional zones are lined up on the sarcolemma at the cell surface. This junctional zone contains both RYR2s and type 2 inositol 1,4,5-trisphosphate receptors ($InsP_3R2$s). In addition to the RYR2s in the junctional zone, the SR that extends into the atrial cell also contains these release units that are critical for amplifying the Ca^{2+} emanating from the sparks. This activation of ventricle and atrial cells is shown in Module 7: Figure ventricular and atrial cell kinetics.

Module 7: | Figure RYR2 location in cardiac cells



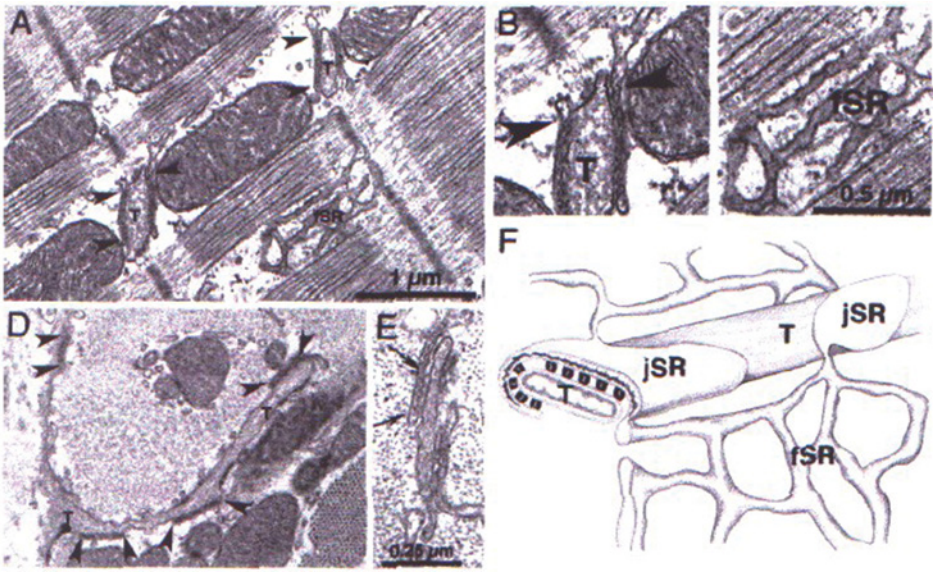
The distribution of ryanodine receptor 2 (RYR2) in atrial and ventricular cells.

A. Immunolocalization of ryanodine receptor 2 (RYR2) in atrial myocytes. The RYR2s are located in two main areas: immediately below the sarcolemma, where they have a punctate appearance (shown more clearly in the higher magnification image A,b) and as non-junctional RYRs that run in parallel strands. B. In the ventricular myocyte, the RYR2s are located in junctional regions on the T-tubules that invade the interior of the cell. Note the staining of the perinuclear region (PR) surrounding the nuclei. A diagram illustrating the localization of RYR2 is shown in Module 7: Figure ventricle and atrial cell organization. Reproduced from MacKenzie, L., Bootman, M.D., Berridge, M.J. and Lipp, P. (2001) Predetermined recruitment of calcium release sites underlies excitation-contraction coupling in rat atrial myocytes. J. Physiol. 530:417–429, with permission from Blackwell Publishing; see MacKenzie et al. 2001.

of distinct phases during the course of the action potential: 1. resting potential; 2. rapid depolarization; 3. brief notch; 4. prolonged plateau; 5. repolarization back to the resting potential. The inward and outward currents responsible for these different phases are generated by a number of ion channels:

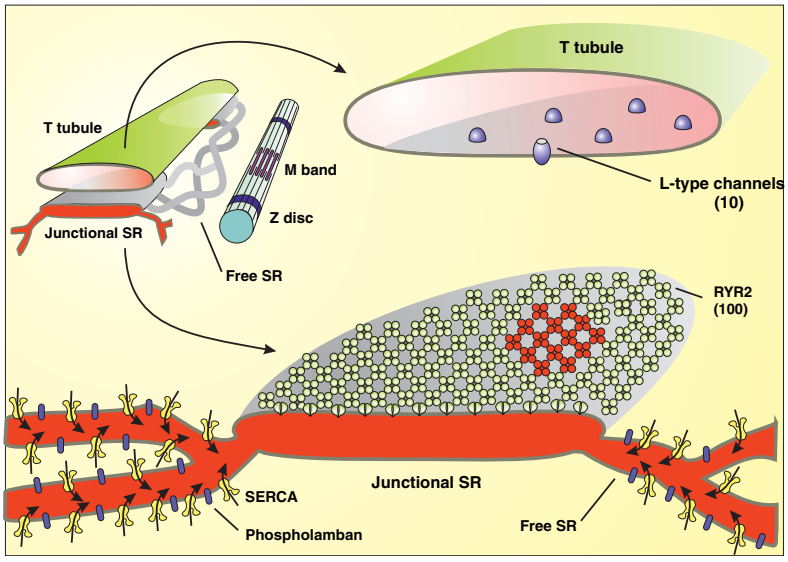
1. The inward rectifier K^+ (K_{ir}) channel $K_{ir}2.1$ (encoded by the KCNJ2 gene), provides the I_{K1} current that functions to hold the resting potential in its hyperpolarized state. It shuts off when depolarization begins, but comes on again later to contribute to the repolarizing phase. A loss-of-function in the KCNJ2 gene causes Andersen-Tawil syndrome.
2. The I_{Na} current responsible for the rapid depolarization is driven by activation of a voltage-dependent sodium channel. This current is very brief because the channel inactivates very quickly but it does conduct enough inward current to depolarize the membrane sufficiently to activate the all important Ca^{2+} channel ($Ca_V1.2$ L-type channels).
3. The cardiac action potential often has a notch resulting from the activation of one or more of the voltage-dependent K^+ (K_v) channels that gives rise to the transient outward current (I_{to}). The $K_v4.2$ channel is thought to be responsible for generating I_{to}.
4. The plateau phase depends on the activation of the $Ca_V1.2$ L-type channels that gives rise to the I_{Ca} current. The prolonged entry of Ca^{2+} generates a Ca^{2+} sparklet, which is the brief burst of trigger Ca^{2+} that initiates the process of ventricular cell Ca^{2+} release (Module 7: Figure ventricular cell E-C coupling).
5. The activation of the rapid delayed rectifier K^+ current (I_{kr}) and the slow delayed rectifier K^+ current (I_{ks}) are jointly responsible for the membrane repolarization that brings the potential back to the hyperpolarized resting potential. The activation of these currents, which are carried by channels belonging to the family of voltage-dependent K^+ (K_v) channels, thus determines the duration of the action potential. The I_{kr} current is carried by $K_v11.1$ (encoded by the KCNH2 gene) whereas the I_{ks} current is carried by $K_v7.1$ (encoded

Module 7: | Figure ventricular junctional zone



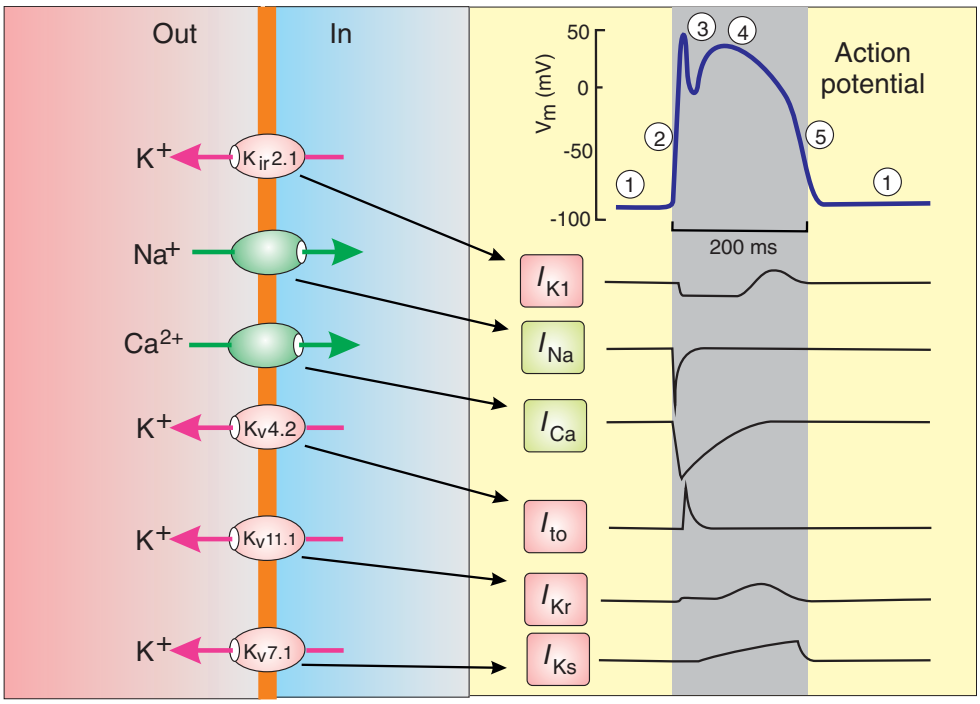
Structural organization of the junctional zones in ventricular cardiac cells.
These electron micrographs illustrate the structural organization of the junctional zone and its relationship to the striated contractile fibres and mitochondria. (A) The arrowheads point to the T-tubules (T) lined up with the dark Z disc of the contractile elements. (B) Detail of a T-tubule with flattened sacs of junctional sarcoplasmic reticulum (jSR) indicated by the arrowheads. (C) Details of the free sarcoplasmic reticulum (fSR) running between the contractile fibres. (D) A tangential view at the level of the Z line illustrates how the contractile fibres are surrounded by jSRs (bracketed by arrowheads). (E) The arrows point to striations that are the ryanodine receptors that span the gap between the jSR and T tubule. (F) This drawing illustrates the relationship between the jSR, fSR and the T-tubule (T). Reproduced from Brochet, D.X.P., Yang, D., Di Maio, A.D., Lederer, W.J., Franzini-Armstrong, C. and Cheng, H. (2005) Ca^{2+} blinks: rapid nanoscopic store calcium signaling. Proc. Natl. Acad. Sci. U.S.A. 102:3099–3104. Copyright (2005) National Academy of Sciences, U.S.A; see Brochet et al. 2005.

Module 7: | Figure junctional zone Ca^{2+} channels



Distribution of Ca^{2+} channels in the ventricular cell junctional zone.
The top left of the figure illustrates the organization of the junctional zones that are distributed along the length of the T-tubule in register with the Z disc of the myofibrils. Junctional zones are connected to each other by the tubular network of free sarcoplasmic reticulum (SR). Approximately ten L-type channels are found in each T-tubule region that faces a junctional zone. The latter has approximately 100 type 2 ryanodine receptors (RYR2s) that are packed together in a pseudo-crystalline array. During excitation–contraction (E-C) coupling, approximately one L-type channel opens to create a sparklet, which then activates 10–15 RYR2s (e.g. those coloured red). The free SR contains the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps that transfer Ca^{2+} into the lumen, from which it diffuses towards the junctional zone.

Module 7: | Figure cardiac action potential



Cardiac action potential.

The action potential in cardiac cells, as shown here for the ventricular cells, is driven by ion channels that conduct either the inward Na^+ and Ca^{2+} currents (shown in green) to depolarize the membrane or outward K^+ currents (shown in red) that hyperpolarize the membrane. The time course of these ionic currents generated by these channels determines the shape of the action potential.

by the KCNQ1 gene). Mutations in these channels are responsible for the heritable long QT syndrome.

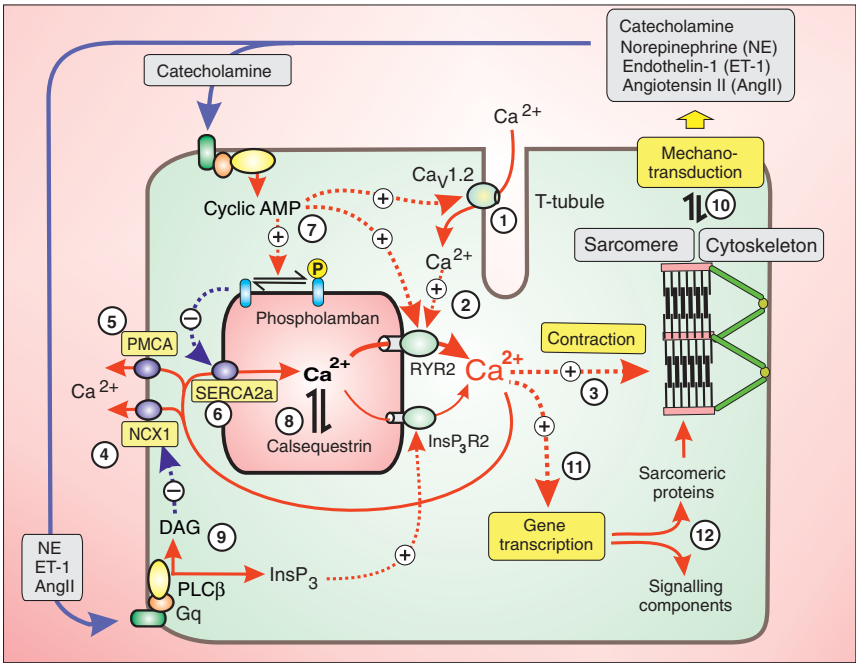
The cardiac action potential that sweeps through the heart initiates the process of ventricular cell Ca^{2+} release (Module 7: Figure ventricular Ca^{2+} signalling).

Ventricular cell Ca^{2+} release

The action potential generated by the sinoatrial node pacemaker cells sweeps through the heart to initiate the process of excitation–contraction (E-C) coupling in the ventricular cells (Module 7: Figure heart summary). The function of the heart is dominated by Ca^{2+}, which not only drives contraction during each heartbeat, but also plays a role in stimulating gene transcription. The latter is particularly important in cardiac hypertrophy, where alterations in Ca^{2+} signals can lead to congestive heart failure. The following overview of ventricular Ca^{2+} signalling will include information on how Ca^{2+} signalling changes in the diseased heart. Ventricular cells have a complex signalsome, and the control of ventricular Ca^{2+} signalling is summarized in the following sequence of steps as outlined in Module 7: Figure ventricular Ca^{2+} signalling:

1. The cardiac action potential induces the membrane depolarization that spreads down the T-tubule to activate the $Ca_V1.2$ L-type channels to produce a Ca^{2+} sparklet, which is a brief burst of trigger Ca^{2+} that initiates the process of ventricular cell E-C coupling (Module 7: Figure ventricular cell E-C coupling).
2. Trigger Ca^{2+} diffuses into the cell to activate the type 2 ryanodine receptors (RYR2s) in the sarcoplasmic reticulum (SR) to release Ca^{2+} into the cytosol in the form of a Ca^{2+} spark. This is the process of Ca^{2+}-induced Ca^{2+} release (CICR), which represents the gain in the system.
3. Ca^{2+} acts on the sarcomeres to induce contraction. This activation of contraction completes the process of E-C coupling, which is followed by various OFF mechanisms responsible for the recovery phase when Ca^{2+} is removed from the cytoplasm.
4. The Na^+/Ca^{2+} exchanger 1 (NCX1) on the plasma membrane extrudes some of the Ca^{2+}. Agonists that act through G_q-coupled receptors activate this exchanger through the diacylglycerol (DAG)/protein kinase C (PKC) signalling pathway.
5. The plasma membrane Ca^{2+}-ATPase (PMCA) on the plasma membrane also extrudes some of the Ca^{2+}.
6. The sarco/endo-plasmic Ca^{2+}-ATPase 2a (SERCA2a) pump on the SR takes up most of the released Ca^{2+}, which is reused to trigger subsequent contractions. This SERCA2a pump, which has a central role in maintaining the store of releasable Ca^{2+}, features significantly in the development of heart disease because it is severely down-regulated during heart failure, resulting in a decline in the Ca^{2+} content of the SR.
7. Catecholamines acting through cyclic AMP play a major role in the modulation of ventricular Ca^{2+} signals by regulating a number of the Ca^{2+} signalling

Module 7: | Figure ventricular Ca^{2+} signalling



Summary of Ca^{2+} signalling in cardiac ventricular cells.

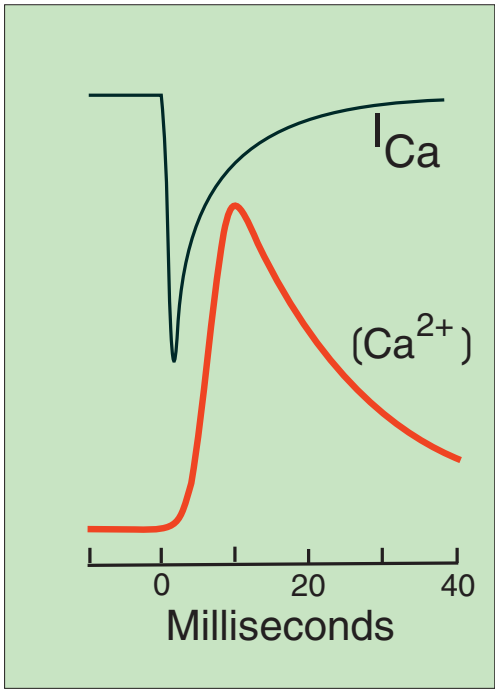
There are a large number of components responsible for Ca^{2+} signalling in ventricular cells. In addition to activating the periodic contractions of the heart, Ca^{2+} also contributes to the stimulation of the gene transcription events responsible for remodelling the cardiac signalsome during hypertrophy. The numbers on the figure summarize the main processes of this signalling network that are described in the text.

components. Cyclic AMP phosphorylates phospholamban, thus relieving its inhibition of SERCA2a to allow the latter to enhance Ca^{2+} signalling by increasing the amount of stored Ca^{2+}. Cyclic AMP phosphorylates $Ca_V1.2$ to increase Ca^{2+} entry into the cell. Catecholamines contribute to the hypertrophic response in that chronic stimulation of the β-adrenergic receptor that occurs during hypertrophy results in inactivation of the receptor induced by the β-adrenergic receptor kinase 1 (βARK1). This inactivation of cyclic AMP signalling in the heart plays a major role in the development of congestive heart failure.

8. Calsequestrin (CSQ), a Ca^{2+}-binding protein, functions as a Ca^{2+} buffer within the lumen of the SR, and thus regulates the amount of Ca^{2+} released through the RYR2s.
9. Activation of phosphoinositide metabolism through G_q-coupled receptors for noradrenaline (norepinephrine), endothelin-1 and angiotensin II contribute to the onset of hypertrophy. The significance of this pathway is evident from the observation that the expression of an active form of G_q in the heart can lead to hypertrophy. Just how this signalling system operates is unclear. An obvious possibility is for inositol 1,4,5-trisphosphate ($InsP_3$) to contribute to the Ca^{2+} signal directly. Alternatively, DAG may act through PKC to stimulate the NCX to bring about an increase in Ca^{2+} extrusion.
10. The structural organization of the contractile and cytoskeletal elements of cardiac cells is shown in Module 12: Figure cardiac contractile elements. Alterations in the contractile system can cause hypertrophy in two ways. Firstly, a pressure overload exerted on the cardiac cell will alter the tension between the fibrillar network and the membrane, and this can generate signals through a process of mechanotransduction, which can feed information into the Ca^{2+} signalling pathway. Secondly, alterations in the organization of the sarcomeres or the cytoskeleton will decrease the contractile efficiency of the cardiac cell, and this will activate feedback mechanisms such as the release of catecholamines, endothelin-1 and angiotensin II that can contribute to the onset of hypertrophy.
11. Changes in Ca^{2+} signalling can activate distinctive programmes of gene transcription. A key element of this transcriptional activation is the Ca^{2+}-dependent activation of calcineurin (CaN) (Module 12: Figure hypertrophy signalling mechanisms).
12. The activation of gene transcription remodels the cardiac cell with changes occurring in both the contractile machinery and, to a lesser extent, in the signalling system. An example of the former is an increase in the production of sarcomeric proteins, which leads to enlarged cardiac cells that are capable of generating greater force. An example of a change in a signalling component is an increase in the mRNA and expression levels of NCX1.

The sequences of events outlined above provide an overview of Ca^{2+} signalling in the ventricular cell. To better

Module 7: | Figure cardiac Ca^{2+} kinetics



Kinetics of the ventricular cell Ca^{2+} transient.
Contraction of the ventricular cell is driven by a brief Ca^{2+} transient that is generated by the small amount of trigger Ca^{2+} that flows into the cell and can be measured as an electrical current (I_{Ca}). Note that the onset of this I_{Ca} precedes the rising phase of the Ca^{2+} transient, which reaches its peak in 10 ms before returning back to the resting level.

understand how Ca^{2+} controls contraction it is necessary to consider in more detail the ventricular cell excitation—contraction (E-C) coupling process at a single junctional complex.

Ventricular cell excitation–contraction (E-C) coupling

The key event in ventricular cell excitation–contraction (E-C) coupling is the link between Ca^{2+} entry across the sarcolemma and the release of Ca^{2+} from the sarcoplasmic reticulum (SR) (Steps 1 and 2 in Module 7: Figure ventricular Ca^{2+} signalling). This process is critically dependent on the structural organization of the individual diadic junctions (Module 7: Figure junctional zone Ca^{2+} channels). Each junctional zone is an autonomous signalling unit that depends upon a close association of approximately ten $Ca_V1.2$ L-type channels in the T-tubule and about 100 type 2 ryanodine receptors (RYR2s) in the junctional SR membrane. The integrity of this diadic junction seems to be maintained by junctophilin 2 (JP2). The way in which these two channels communicate with each other during E-C coupling is shown in Module 7: Figure ventricular cell E-C-coupling.

As the action potential invades the T-tubule, it depolarizes the sarcolemma, which then activates the L-type channels to allow Ca^{2+} to enter the cell to form a sparklet. Rapid imaging studies have been able to visualize a sparklet at one of these junctional zones (Module 3: Figure Ca^{2+} sparklet). At each of the 10 000 junctional zones, the individual sparklets contribute a flow of charge that sums to produce a measurable electrical current (I_{Ca}) (Module 7: Figure Ca^{2+} cardiac kinetics). Notice how this inward Ca^{2+} current precedes the Ca^{2+} transient because it is this entry of Ca^{2+} that is responsible for activating the RYR2s to release Ca^{2+} from the junctional SR, which is the basis of E-C coupling. It is estimated that each sparklet will activate about ten of the 100 available RYR2s in each junctional zone to produce a spark, which then diffuses out to activate myofibrils in the immediate vicinity to complete the process of E-C coupling.

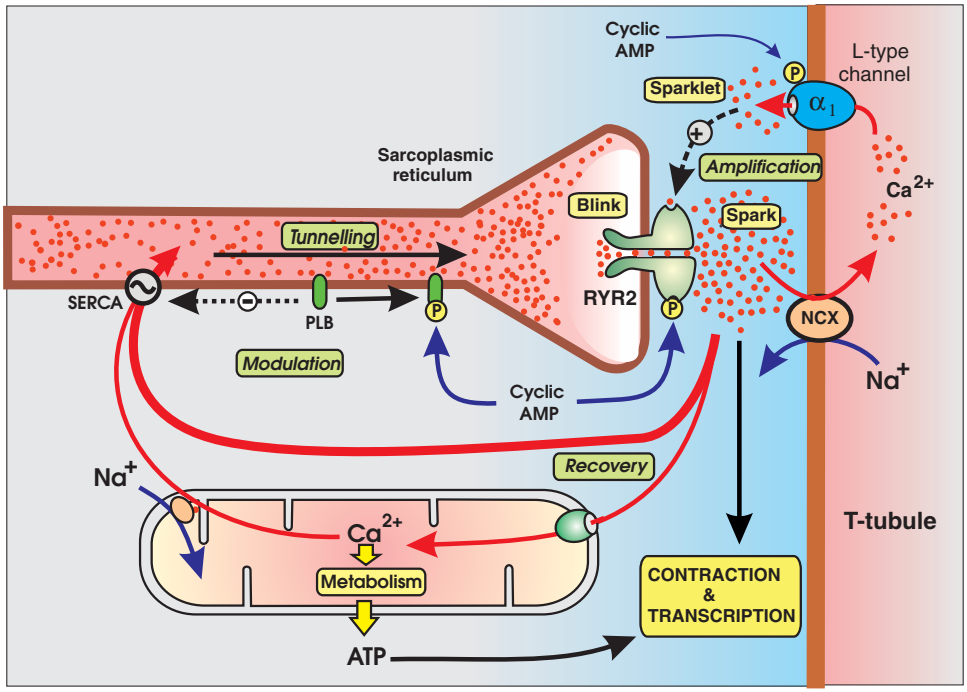
The next problem to consider is how these individual sparks are recruited to provide the global Ca^{2+} signal that activates contraction. There are approximately 10 000 junctional zones in each ventricular cell. In order to get a fast contraction, it is necessary to activate sparks in all of them as synchronously as possible, and this is achieved through a process of electrical recruitment. The action potential provides the rapid synchronization signal to trigger all these autonomous units simultaneously (Module 7: Figure ventricular and atrial cell kinetics). A critical aspect of this E-C mechanism is that each of the junctional zones is an autonomous unit that produces its spark independently of its neighbours. Evidence for this autonomy can be seen in line scans of ventricular cells activated by increasing levels of depolarization (Module 7: Figure autonomous Ca^{2+} sparks). At low membrane depolarizations, when relatively few junctional complexes are being recruited, the individual sparks are clearly evident. This is an example of how the SR can be divided into modular Ca^{2+} signalling units that produce local Ca^{2+} signals that can then be recruited to provide the rapid global Ca^{2+} signal necessary to contract a large muscle cell.

A critical question in cardiac physiology concerns the inactivation of ventricular RYR2s. What causes the channels to suddenly close once they have delivered sufficient Ca^{2+} to activate a contraction? A related question arises when one considers the sequence of events that occur in a junctional zone during the formation of a spark. An obvious question is why only about 10% of the available RYR2s contribute to a spark. Why do the majority of RYRs remain quiescent even though they must be experiencing a high level of Ca^{2+} as the spark spreads out of the junctional zone? It seems that rapid inactivation of ventricular type 2 ryanodine receptors (RYR2s) may be caused by depletion of Ca^{2+} within the lumen of the SR.

Inactivation of ventricular type 2 ryanodine receptors (RYR2s)

During the course of a ventricular Ca^{2+} transient, the type 2 ryanodine receptors (RYR2s) open for a brief period, then they inactivate, and the recovery process begins. The nature of this inactivation mechanism is also relevant to the question of why only a small proportion of the available RYR2s are activated during each spark. It has been estimated that each junctional zone contains about 100 RYR2s packed together in the sarcoplasmic reticulum (SR) membrane (Module 7: Figure junctional zone Ca^{2+} channels). Only about 10% of these channels seem to be employed

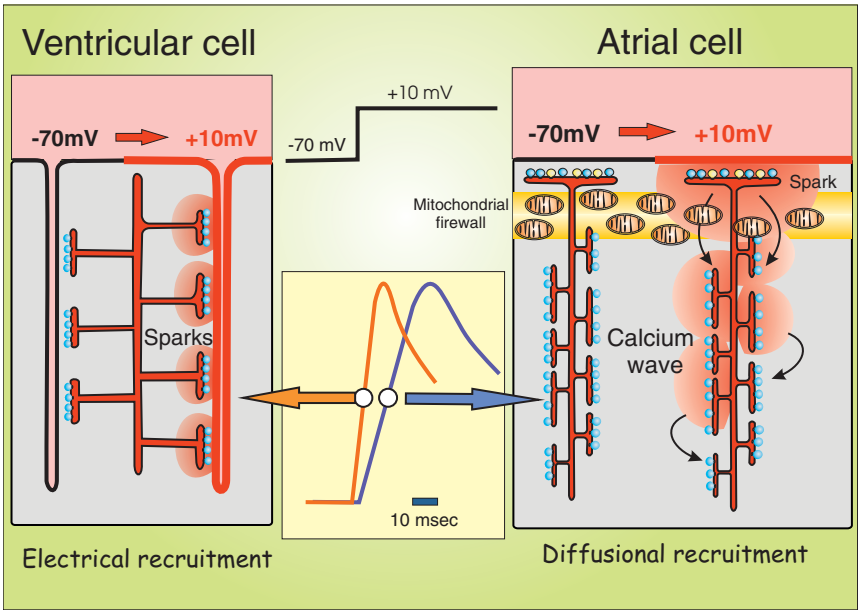
Module 7: | Figure ventricular cell E-C coupling



Excitation–contraction coupling in the junctional zone of ventricular cells.

Depolarization of the T-tubule activates the α1 subunit of the $Ca_v1.2$ L-type channel that introduces a small amount of Ca^{2+} to form a sparklet. Ca^{2+} diffuses across the 20 nm gap in the junctional zone to activate the type 2 ryanodine receptors (RYR2s) that release Ca^{2+} to form a much larger spark. The latter then diffuses away from the junctional zone to activate contraction in the neighbouring myofibrils. After releasing a package of Ca^{2+}, the RYRs inactivate, and the released Ca^{2+} is then removed. During this recovery phase, a proportion similar to that entering through the $Ca_v1.2$ channel is removed from the cell by the Na^+/Ca^{2+} exchanger (NCX), while the remainder is taken back into the sarcoplasmic reticulum (SR) by the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps distributed over the longitudinal free SR. The Ca^{2+} entering the latter is then transferred back to the junctional SR through a process of tunnelling. Ca^{2+} homoeostasis is maintained by virtue of the fact that the Ca^{2+} fluxes across the T-tubule and the SR are balanced during the excitation and recovery phases. An animated version of this figure is available.

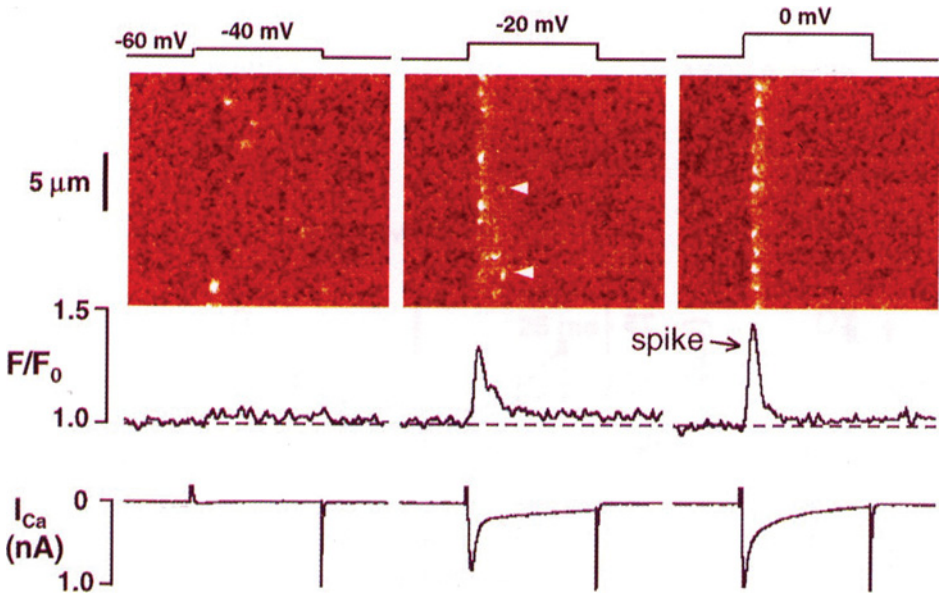
Module 7: | Figure ventricular and atrial cell kinetics



Ventricular and atrial cardiac cells have different modes of Ca^{2+} signalling.

The resting condition of the sarcolemma at –70 mV is shown in black, whereas the active state that occurs when the membrane is depolarized to + 10 mV is shown in red. Ventricular cells use electrical recruitment to co-ordinate the release of Ca^{2+} sparks from the autonomous junctional zones. Depolarization simultaneously activates all these junctional zones to create a whole series of sparks that sum to produces the fast global Ca^{2+} transient (see inset) to drive contraction. In atrial cells, a process of diffusional recruitment results in a slower Ca^{2+} transient (blue trace). The junctional zones at the cell surface of atrial cells are activated by membrane depolarization to generate a spark, which then spreads into the cell in the form a wave through a process of Ca^{2+}-induced Ca^{2+} release (CICR).

Module 7: | Figure autonomous Ca^{2+} sparks



Electrical recruitment of Ca^{2+} sparks in ventricular cells.
The line scan images at the top record the appearance of sparks in response to three different depolarizations. There were very few sparks for the depolarization to −40 mV. The current trace at the bottom recorded the inward Ca^{2+} current (I_{Ca}), which shows that there was very little entry of trigger Ca^{2+} for this small depolarization. When depolarization was increased to −20 mV, a distinct line of sparks appeared. However, there were some gaps, and some sparks showed a long latency (white arrowheads) that resulted in a sluggish transient, as seen in the middle trace, which shows the spatially averaged F/F_0 values. At the larger depolarization to 0 mV, the response was uniform and much sharper. Reproduced from Song, L.-S., Sham, J.S.K., Stern, M.D., Lakatta, E.G. and Cheng, H. (1998) Direct measurement of SR release flux by tracking "Ca^{2+} spikes" in rat cardiac myocytes. J. Physiol. 512:677–691, with permission from Blackwell Publishing; see Song et al. 1998.

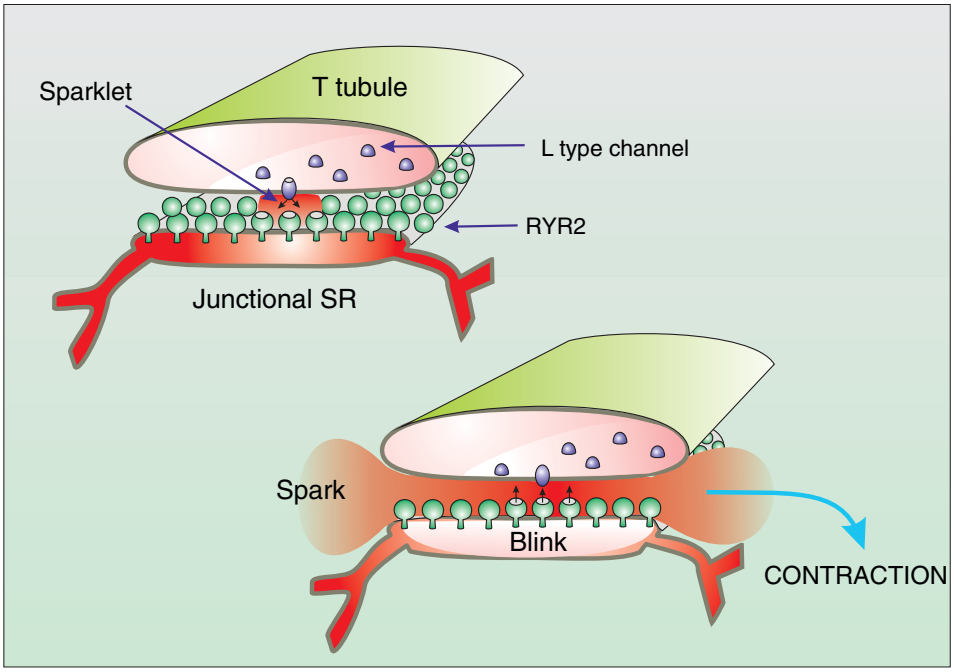
to produce the individual Ca^{2+} sparks at each junctional zone. It is not clear why the majority of the RYR2s remain quiescent. The luminal regulation of Ca^{2+} release channels may provide an explanation for how the RYR2s might be rapidly inactivated. The idea is that the Ca^{2+} spark inactivates the RYR2s by causing a rapid depletion of Ca^{2+} within the lumen of the junctional zone (Module 7: Figure ventricular cell Ca^{2+} blink). When the Ca^{2+} sparklet activates the RYR2s to produce a spark, the amount of Ca^{2+} leaving the lumen of SR is so large that it causes a temporary depletion, and this has been called a blink. The effect of this blink is to inactivate the RYR2s because the sensitivity of these channels is very dependent on the level of Ca^{2+} within the internal store. If indicators are used to simultaneously monitor Ca^{2+} in the cytoplasm and within the lumen of the SR, it is possible to visualize the appearance of a spark and the simultaneous appearance of a blink (Module 7: Figure sparks and blinks).

Modulation of ventricular Ca^{2+} signals

A remarkable feature of cardiac function is the way that contractility can be modulated rapidly to change the force of contraction. The sympathetic nervous system controls the positive inotropic response, which is an increase in the force of contraction that is activated through the β-adrenergic receptors acting through the cyclic AMP signalling pathway (Step 7 in Module 7: Figure ventricular Ca^{2+} signalling). The β-adrenergic receptors are located on the T-tubules, where they appear to produce two cyclic AMP microdomains (Module 6: Figure ventricular cyclic AMP microdomains). These microdomains are responsible for phosphorylating some of the key components responsible for regulating both the generation and action of the Ca^{2+} transients that control each heartbeat (Module 7: Figure ventricular cell E-C coupling):

- Activity of the $Ca_V1.2$ L-type channels is enhanced by phosphorylation of both the α and β subunits by protein kinase A (PKA), which is positioned close to the channel through its association with A-kinase-anchoring protein 18 (AKAP18) (Module 3: Figure $Ca_V1.2$ L-type channel). The phosphorylated channels feed more Ca^{2+} into the heart, and this contributes to the larger contractions by producing larger sparks by recruiting more type 2 ryanodine receptors (RYR2s) and by increasing the load of Ca^{2+} within the SR.
- Loading of the SR is also enhanced by increasing the activity of the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pump that returns Ca^{2+} to the SR after each heartbeat (Module 7: Figure ventricular cell E-C coupling). Cyclic AMP stimulates PKA to phosphorylate phospholamban (PLN), which is an inhibitor of the pump (Module 5: Figure phospholamban mode of action). Inactivation of PLN greatly enhances the activity of the SERCA pump to increase the amount of Ca^{2+} within the lumen of the SR, which not only increases the amount of Ca^{2+} that leaves during each spark, but also enhances the sensitivity of RYRs to the stimulatory action of Ca^{2+}.

Module 7: | Figure ventricular cell Ca^{2+} blink



Ventricular cell sparklets, sparks and blinks.

This figure illustrates the organization of a junctional zone with the L-type Ca^{2+} channels in the T-tubule facing the type 2 ryanodine receptors (RYR2s) in the sarcoplasmic reticulum (SR). The top diagram shows how opening of one of the L-type channels forms a sparklet, which acts within a limited domain to activate a few RYR2s to trigger a spark (bottom diagram). The release of Ca^{2+} to form the spark depletes Ca^{2+} within the SR lumen, resulting in a blink. It is this depletion of luminal Ca^{2+} that may inactivate the RYR2s.

- The sensitivity of the RYRs is also increased by a cyclic AMP-dependent phosphorylation of Ser-2809 by a resident PKA that is attached through mAKAP (Module 3: Figure ryanodine receptor structure).
- The sensitivity of the contractile filaments is controlled by the phosphorylation of troponin I (Module 6: Figure ventricular cyclic AMP microdomains).

These three effects of cyclic AMP on the L-type entry channels, the SERCA pump and the RYR2s all contribute to the positive inotropic effect by increasing the amount of Ca^{2+} that is released during each beat.

Atrial cells

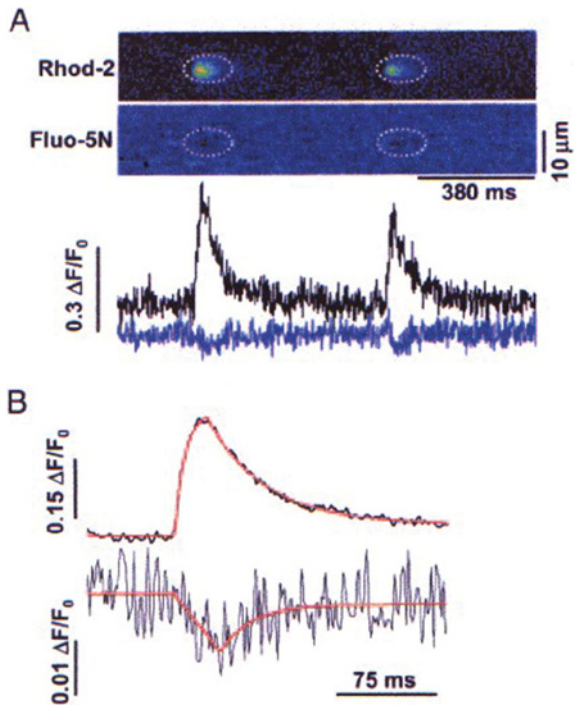
The striated atrial cells responsible for contracting the atrial chambers of the heart have a superficial resemblance to the ventricular cell, but there are a number of marked differences. The first obvious difference concerns atrial cell structure in that there are no T-tubules. In ventricular cells, the T-tubules are used to carry the electrical signal deep into the cell to trigger a rapid synchronous release of Ca^{2+}. In atrial cells, the electrical signal activates a Ca^{2+} signal that is normally confined to the cell surface, but under some circumstances it can be amplified by a process of Ca^{2+}-induced Ca^{2+} release (CICR), which is much slower because it spreads through a Ca^{2+} wave. The mechanism of atrial cell Ca^{2+} release is thus very different from that in ventricular cells. The modulation of atrial Ca^{2+} signals depends on whether or not the sparks at the cell surface can activate the global Ca^{2+} wave.

Atrial cell structure

Atrial cells have a long elongated structure and are somewhat smaller than the ventricular cells (Module 7: Figure RYR2 location in cardiac cells). The type 2 ryanodine receptors (RYR2s) have two distinct locations: they lie along the surface sarcolemma and they also form parallel strands at right angles to the surface. This arrangement is shown diagrammatically in Module 7: Figure ventricle and atrial cell organization. As there are no T-tubules, like those in the ventricular cells, the junctional zones are restricted to the sarcolemma at the cell surface, and it is here where the process of atrial cell Ca^{2+} release begins. There also are non-junctional sarcoplasmic reticulum (SR) sheets that spread perpendicularly into the cell, and these are lined with RYR2s that can amplify the initial signal at the cell surface. Whether or not these non-junctional RYR2s are brought into play seems to depend upon a mitochondrial firewall located between the junctional and non-junctional SR.

Atrial cells also express type 2 inositol 1,4,5-trisphosphate receptors ($InsP_3R2$), which have very specific locations. One group is located together with the RYR2s in the junctional zone, and the other group is clustered around the nucleus. The former have an important role in mediating the positive inotropic response to

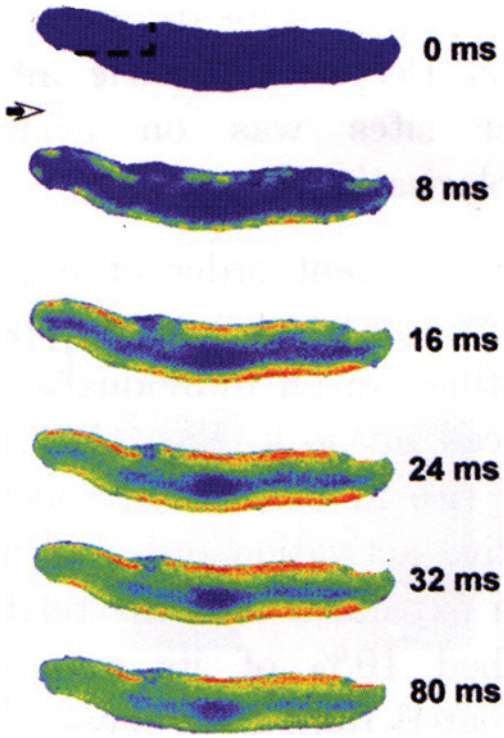
Module 7: | Figure sparks and blinks



Simultaneous measurement of sparks and blinks in a cardiac ventricular cell.

The level of Ca^{2+} with the cytoplasm and lumen of the sarcoplamsic reticulum (SR) was measured simultaneously using Rhod-2 and Fluo-5N respectively. A. In the scans in the upper two panels, Rhod-2 detected two sparks that were associated with two blinks detected with Fluo-5N. The traces below show the time course for the sparks (black) and the blinks (blue). B. These two traces illustrate that the two events occur simultaneously. Reproduced from Brochet, D.X.P., Yang, D., Di Maio, A.D., Lederer, W.J., Franzini-Armstrong, C. and Cheng, H. (2005) Ca^{2+} blinks: rapid nanoscopic store calcium signaling. Proc. Natl. Acad. Sci. U.S.A. 102:3099–3104. Copyright (2005) National Academy of Sciences, U.S.A; see Brochet et al. 2005.

Module 7: | Figure atrial cell Ca^{2+} signalling



Development of a global Ca^{2+} signal in the isolated rat atrial cell.

The development of a Ca^{2+} signal in an atrial myocyte in response to an electrical depolarization given at the time of the arrow. The successive images indicate how the signal begins with Ca^{2+} sparks at the periphery that appear by 8 ms. These early sparks then trigger a wave that spreads the signal in towards the centre of the cell. The sequence of events is shown diagrammatically in Module 7: Figure ventricular and atrial cell kinetics). Reproduced from MacKenzie, L., Bootman, M.D., Berridge, M.J. and Lipp, P. (2001) Predetermined recruitment of calcium release sites underlies excitation-contraction coupling in rat atrial myocytes. J. Physiol. 530:415–429, with permission from Blackwell Publishing; see MacKenzie et al. 2001.

stimuli such as endothelin-1 during the modulation of atrial Ca^{2+} signals.

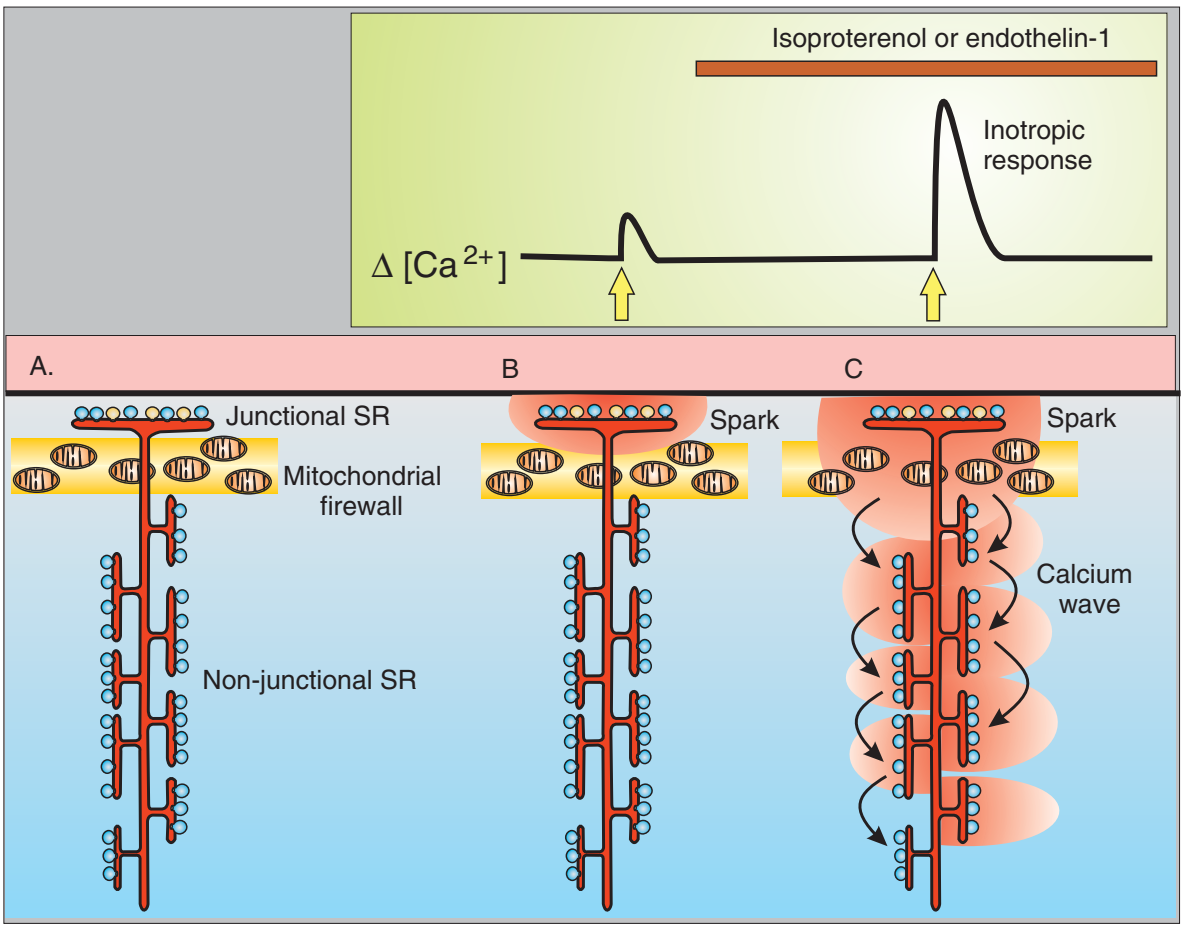
Atrial cell Ca^{2+} release

Atrial cells use two distinct mechanisms of Ca^{2+} release. The junctional zones on the sarcolemma generate Ca^{2+} sparks using a mechanism similar to that described for ventricular cells (Module 7: Figure ventricular and atrial cell kinetics). L-type Ca^{2+} channels in the sarcolemma produce a sparklet that then triggers a spark in the junctional zone at the cell surface. These sparks at the surface then activate the second mechanism of Ca^{2+}-induced Ca^{2+} release (CICR) that spreads the Ca^{2+} signal into the cell. The gradual development of this atrial Ca^{2+} signal can be visualized using fluorescent imaging, which clearly reveals how the signal originates at the periphery and then spreads into the cells (Module 7: Figure atrial cell Ca^{2+} signalling). Globalization is achieved by the regenerative process of CICR that forms a wave to activate contraction as it spreads into the cell from the plasma membrane (Module 7: Figure ventricular and atrial cell kinetics). This mode of diffusional recruitment in atrial cells is much slower than the electrical recruitment mechanism found in ventricular cells. It also is the basis for the modulation of atrial Ca^{2+} signals that depend upon stimuli such as β-adrenergic agents and endothelin-1 being able to couple the sparks at the cell surface with the release of Ca^{2+} from the non-junctional sarcoplasmic reticulum (SR).

Modulation of atrial Ca^{2+} signals

The modulation of Ca^{2+} signals in atrial cells seems to depend upon the coupling of cell-surface Ca^{2+} sparks to the release of Ca^{2+} from the non-junctional sarcoplasmic reticulum (SR) (Module 7: Figure atrial Ca^{2+} domains). In response to membrane depolarization, the L-type channels in the junctional zone introduce a small pulse of Ca^{2+} that then triggers Ca^{2+} release from the type 2 ryanodine receptors (RYR2s) to create a Ca^{2+} spark. Under normal conditions, this spark fails to breach the mitochondrial firewall because the Ca^{2+} is rapidly removed by uptake into the mitochondria and by uptake into the SR by the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps. Not enough Ca^{2+} gets across the firewall to activate the internal RYR2s. This small signal spatially localized to the cell periphery accounts for why the normal atrial contractions are very weak. However, stronger

Module 7: | Figure atrial Ca^{2+} domains



Modulation of atrial cell contractility by manipulating Ca^{2+} microdomains.
A. Organization of the junctional sarcoplasmic reticulum (SR) and non-junctional SR separated by the mitochondrial firewall. B. Under control conditions, depolarization (yellow arrows) triggers Ca^{2+} sparks in the junctional SR that fail to spread to the non-junctional SR, and this results in a small Ca^{2+} transient. C. In the presence of either isoproterenol or endothelin-1, the initial spark is larger and is able to breach the firewall to ignite the non-junctional type 2 ryanodine receptors (RYR2s) to set up a Ca^{2+} wave to give a much larger global signal that is responsible for the inotropic response. (Information for this figure was derived from Mackenzie et al. 2004.)

contractions are induced following stimulation of the atrial cells by either β-adrenergic agonist or by endothelin (Module 7: Figure atrial arrhythmias). These two stimuli induce a large positive inotropic response in two ways. Firstly, they enable the cell-surface sparks to breach the mitochondrial firewall so as to stimulate the non-junctional RYR2s to ignite the Ca^{2+} wave that produces the global signal responsible for the stronger contractions. Secondly, they enhance the sensitivity of the RYR2s so that they can respond to the signal coming through the firewall.

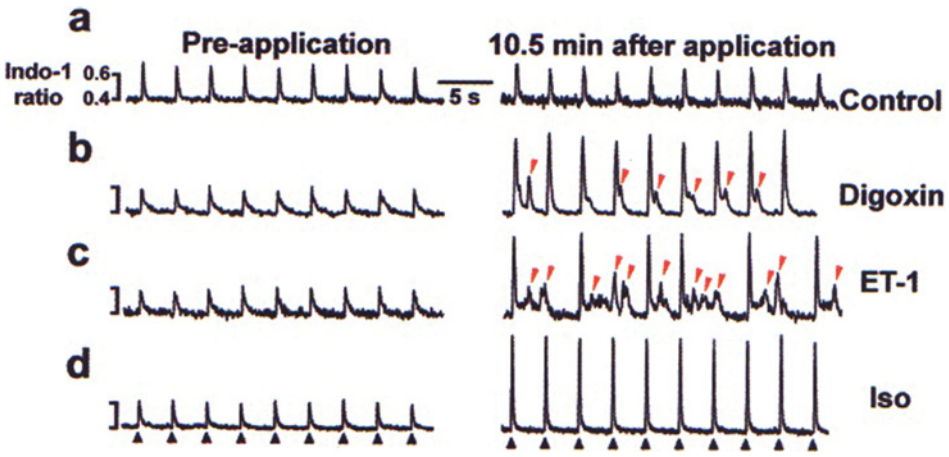
The action of β-adrenergic agonists such as isoproterenol (isoprenaline) seems to depend on the same mechanisms that have been described for the modulation of ventricular Ca^{2+} signals. By activating the L-type channels and the SERCA pumps, cyclic AMP increases the load of Ca^{2+} within the SR, and this is responsible for creating the larger Ca^{2+} sparks to breach the mitochondrial firewall and for sensitizing the RYR2s to respond to this signal.

The ability of endothelin-1, which acts through the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette, is still not fully understood. $InsP_3$ receptors in the junctional zone (Module 7: Figure ventricle and atrial cell organization) are appropriately positioned to contribute to the initial Ca^{2+} spike. Since the $InsP_3$ receptors are sensitive to both $InsP_3$ and Ca^{2+}, they may make little contribution to the spark under normal conditions. However, when endothelin-1 increases the level of $InsP_3$, the $InsP_3$ receptors will be brought into play and may augment the Ca^{2+} spark sufficiently to break through the firewall to recruit the RYR2s on the non-junctional SR. This enhancement of Ca^{2+} signalling by endothelin often leads to the development of atrial arrhythmias (Module 7: Figure $InsP_3$-induced arrhythmias).

Atrial arrhythmias

Atrial arrhythmias are the most common form of long-lasting arrhythmias that carry a high risk of stroke. However, little is known about the cause of these arrhythmias. Recently, it has been shown that the type 2 inositol 1,4,5-trisphosphate receptors ($InsP_3R2s$), which are strongly expressed in atrial cells, may play a role in

Module 7: | Figure atrial arrhythmias

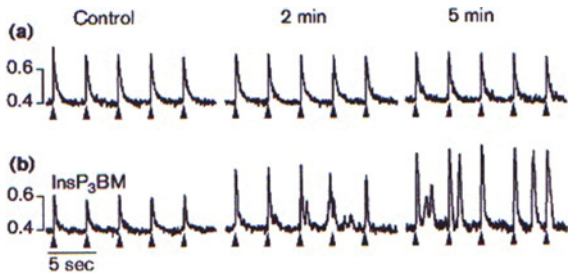


Modulation of atrial cell contractility.
Responses of electrically paced atrial cells to digoxin (1 μM), endothelin-1 (ET-1; 0.1 μM) and isoproterenol (Iso; 0.1 μM). The black arrowheads indicate when the electrical stimulation was applied. The pre-application recordings on the left illustrate the regular control Ca^{2+} transients. The recordings on the right are responses 15 min after applying the different agents. From MacKenzie, L., Bootman, M.D., Laine, M., Brig, J., Thuring, J., Holmes, A., Li, W.-H. and Lipp, P. (2002) The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. J. Physiol. 541:395–409, with permission from Blackwell Publishing; see MacKenzie et al. 2002.)

initiating this arrhythmic activity. If atrial myocytes are stimulated with endothelin-1, which acts to increase the level of $InsP_3$, there is a gradual increase in extra Ca^{2+} transients in the intervals between the depolarization-induced transients (see traces c in Module 7: Figure atrial arrhythmias). These arrhythmias seem to be caused by $InsP_3$, because they can be triggered simply by adding a membrane-permeant $InsP_3$ ester (Module 7: Figure $InsP_3$-induced arrhythmias). What is remarkable about this action of $InsP_3$ is that some of the transients it induces are as large as those produced by the membrane depolarization that was applied at each of the arrowheads in this figure. Just how $InsP_3$ acts to trigger these extra transients is still unclear.

The fact that the $InsP_3$ receptors are located in the atrial cell junctional zone intermingling with the type 2 ryanodine receptors (RYR2s) means that they are in the right place to have a role in excitation–contraction (E-C) coupling (Module 7: Figure ventricle and atrial cell organization). A likely scenario therefore is that high levels of $InsP_3$ in the region of the junctional zone can induce sufficient Ca^{2+} release to trigger the RYR2s to produce a Ca^{2+} spark. This spark will then act to open non-specific cation channels to depolarize the sarcolemma sufficiently to initiate an action potential to produce the large Ca^{2+} transients. This action of $InsP_3$ thus constitutes an alternative mechanism that comes into play during pathological conditions to produce atrial arrhythmias.

Module 7: | Figure $InsP_3$-induced arrhythmias

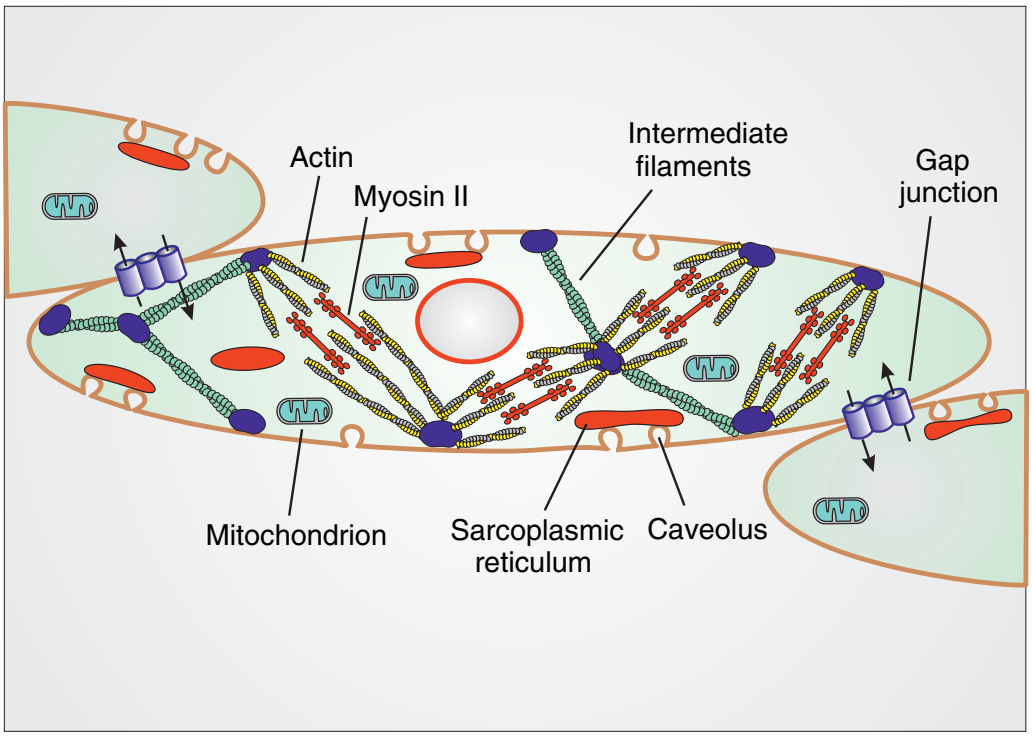


Inositol trisphosphate ($InsP_3$)-induced arrhythmias in atrial myocytes.
The arrowheads represent the times when the cells were depolarized. a. There was no change in the control cells recorded after 2 and 5 min. b. The cells treated with 10 μM $InsP_3$BM, which is a membrane-permeant inositol 1,4,5-trisphosphate ($InsP_3$) ester, began to develop arrhythmias. By 5 min, the spontaneous Ca^{2+} transients were as large as those induced by electrical stimulation. Reproduced from *Curr. Biol.*, Vol. 10, Lipp, P., Laine, M., Tovey, S.C., Burrell, K.M., Berridge, M.J., Li, W.-H. and Bootman, M.D., Functional $InsP_3$ receptors that may modulate excitation–contraction coupling in the heart, pp. 939–942. Copyright (2000), with permission from Elsevier; see Lipp et al. 2000.

Smooth muscle cells

Smooth muscle cells are a heterogeneous collection of cell types that function in many different organs, where they regulate a variety of functions such as vascular smooth muscle control of blood vessel tone, emptying of the bladder, tonic contractions of sphincters such as the urethra and the rhythmical contraction of the gastrointestinal tract, uterus and ureter. In order to carry out these multiple functions, a number of different signalling mechanisms have evolved to control smooth muscle contractility. There is a certain degree of uniformity with regard to smooth muscle cell structure and there also are some general mechanisms that are responsible for smooth muscle cell excitation—contraction coupling. In addition to this excitatory mechanism, there also are signalling pathways responsible for smooth muscle cell relaxation. However, when it comes to the smooth muscle activation mechanisms, each muscle has to be considered separately especially with regard to the way in which excitatory stimuli bring about the increase in Ca^{2+} necessary to trigger contraction. Most smooth

Module 7: | Figure smooth muscle cell structure



Structure of a typical smooth muscle cell.
One end of the actin filament is tethered to a dense body, which is either attached to the plasma membrane or lies free within the cytoplasm. The dense bodies, which provide the anchorage points between the contractile filaments and the plasma membrane, contain α-actinin and vinculin and appear to be analogous to the Z lines in skeletal muscle.

muscle cells are excited by membrane depolarization that then activates VOCs to provide the Ca^{2+} signal to induce contraction. For many of these muscles, this depolarization is induced by the interstitial cells of Cajal that have a pacemaker function resembling that of the sino-atrial node in the heart. Other smooth muscles have an endogenous pacemaker mechanism while others are activated directly by neurotransmitters released by nerves.

Smooth muscle cell structure

Smooth muscle cells have a spindle shape, and are usually lined up alongside each other and are connected through gap junctions (Module 7: Figure smooth muscle cell structure). They are about 100 μm long and 5–10 μm wide. The cell surface has numerous caveolae and many of these make close contact with the sarcoplasmic reticulum (Module 6: Figure smooth muscle caveolae). By comparison with skeletal and cardiac muscle, the contractile system composed of actin and myosin is somewhat disorganized, with actin filaments radiating away from localized densities dotted around the plasma membrane. Smooth muscle cell contraction also differs from that found in skeletal and cardiac muscle in that there are two different mechanisms for regulating the contractile processes (Module 7: Figure smooth muscle cell E-C coupling):

Smooth muscle cell excitation–contraction coupling

Excitation–contraction coupling refers to the mechanism whereby the smooth muscle cell contracts in response to excitatory stimuli. Most smooth muscle activation mechanisms result in depolarization of the membrane that then initiates the smooth muscle Ca^{2+} signalling cascades that lead to contraction. In addition, there is a smooth muscle Rho/Rho kinase signalling pathway that bring about contraction.

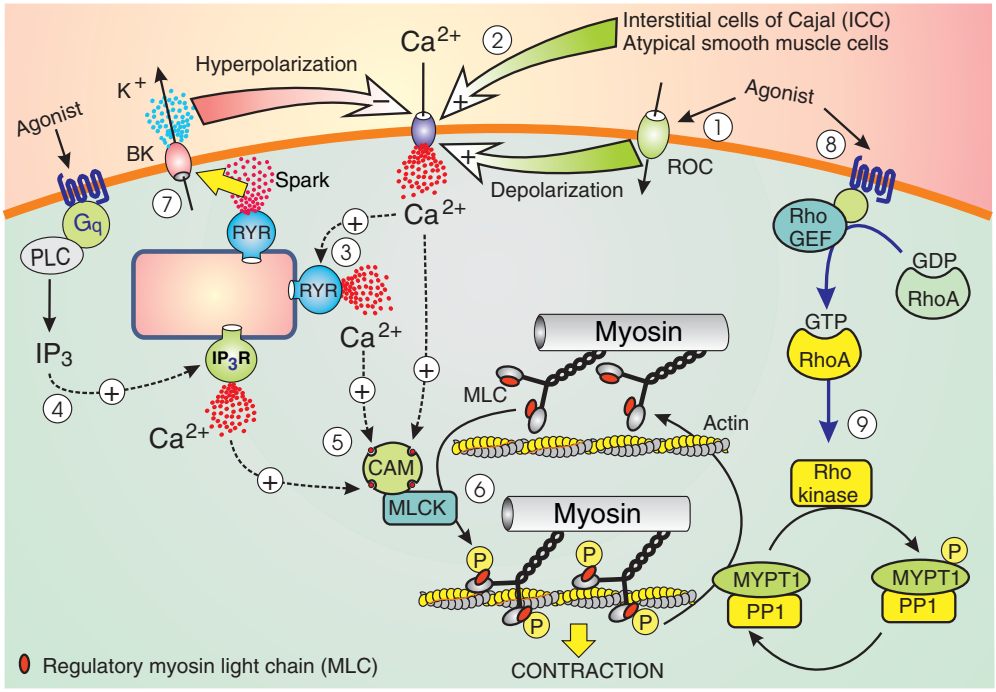
Smooth muscle Ca^{2+} signalling

Like other muscle cells, smooth muscle cells use actomyosin to generate force. However, the way in which the interaction between actin and myosin is controlled in smooth muscle is very different to that in other muscles. The sensor is not troponin C (TnC), as it is in skeletal and cardiac muscle, but is calmodulin (CaM) that responds to Ca^{2+} by stimulating myosin light chain kinase (MLCK) to phosphorylate the myosin light chains (MLCs) enabling myosin and actin to interact and drive contraction (Module 7: Figure smooth muscle cell E-C coupling).

The way in which smooth muscle cells produce their Ca^{2+} signals varies between the different cell types (Module 7: Figure smooth muscle cell E-C coupling):

1. In those muscles that are under neural control, the arrival of transmitters that act on receptor-operated

Module 7: | Figure smooth muscle cell E-C coupling



Smooth muscle cell excitation–contraction (E-C) coupling mechanisms.

A network of signalling pathways is responsible for smooth muscle excitation–contraction (E-C) coupling. Ca^{2+} is derived from three sources: it can enter the cell through L-type voltage-operated channels (VOCs), it can be released by ryanodine receptors (RYRs) from internal stores or it can be released by inositol 1,4,5-trisphosphate (IP_3). Ca^{2+} acts through calmodulin (CaM) to stimulate a myosin light chain kinase (MLCK), which phosphorylates the regulatory myosin light chain (MLC) to enable the myosin heads to interact with actin to initiate the contraction cycles. Sensitization of the Ca^{2+} signalling system is carried out through a Rho/Rho kinase signalling pathway that phosphorylates a myosin phosphatase targeting subunit 1 (MYPT1), which then inactivates the catalytic protein phosphatase 1 (PP1) to prevent it from dephosphorylating the regulatory light chains.

channels (ROCs) can depolarize the membrane to open L-type voltage-operated channels (VOCs) that then allows external Ca^{2+} to flood into the cell to trigger contraction as occurs in detrusor smooth muscle cells (Module 7: Figure bladder SMC activation).

2. Other smooth muscle cells either develop their own depolarization signal as occurs in uterine smooth muscle cells (Module 7: Figure uterus activation) or they rely on pacemaker cells, such as the interstitial cells of Cajal (ICC) and atypical smooth muscle cells, that provide a depolarizing drive to open VOCs as occurs in the gastrointestinal smooth muscle cells, urethral smooth muscle cell and ureter smooth muscle cells (Mechanism C in Module 7: Figure SMC activation mechanisms).
3. The influx of external Ca^{2+} can be amplified by triggering the activation of ryanodine receptors (RYRs) to release more Ca^{2+} from the internal store.
4. Some of the cell-surface receptors produce inositol 1,4,5-trisphosphate ($InsP_3$), which then acts on $InsP_3$ receptors to releases internal Ca^{2+}.
5. The increase in Ca^{2+} induced by the different mechanisms then acts through calmodulin (CaM) to stimulate myosin light chain kinase (MLCK).
6. The MLCK phosphorylates the myosin light chains (MLCs), thus enabling the myosin and actin to interact to drive contraction.
7. Under conditions where the internal store is heavily loaded with Ca^{2+}, the RYRs spontaneously produce Ca^{2+} sparks that act on large-conductance (BK) channels to hyperpolarize the membrane and inhibit excitation–contraction coupling. Such activation of sparks occurs during the refractory period in ureter smooth muscle contraction (Module 7: Figure pelviureteric organization)

A feature of the Ca^{2+} signalling system in smooth muscle cells is that there is considerable variation with regard to the spatial and temporal properties of their Ca^{2+} signals. In some cells, there are global signals where Ca^{2+} rises uniformly throughout the cell. In other cell types, the signal appears as waves that sweep regularly through the cell to drive a corresponding wave of contraction. Finally, there are highly localized elementary events that resemble the Ca^{2+} sparks found in cardiac cells. These smooth muscle cell Ca^{2+} sparks are one of the mechanisms used to control smooth muscle relaxation, since they can act on cell-surface large-conductance (BK) channels to hyperpolarize the membrane, thereby reducing the entry of Ca^{2+} through the VOCs.

Smooth muscle cell relaxation occurs when these Ca^{2+} signalling mechanisms are inhibited either by the cyclic GMP signalling pathway, as outlined in the section on

nitric oxide (NO)/cyclic GMP and smooth muscle relaxation, or through the action of smooth muscle cell Ca^{2+} sparks.

Smooth muscle Rho/Rho kinase signalling

In addition to the direct Ca^{2+}-dependent control of contraction, smooth muscle cells can also regulate contraction through a Rho signalling mechanism, as illustrated in Module 2: Figure Rho signalling. In the case of smooth muscle cells, activation of various receptors can contribute to the contraction through Steps 8 and 9 in Module 7: Figure smooth muscle cell E-C coupling. Like the Ca^{2+} signalling pathway, this Rho/Rho kinase signalling pathway is also directed towards the phosphorylation status of the myosin light chain (MLC). However, instead of acting on the myosin light chain kinase (MLCK), it acts to inhibit the protein phosphatase 1 (PP1) that dephosphorylates MLC (Module 7: Figure smooth muscle cell E-C coupling).

Smooth muscle cell relaxation

Many smooth muscle cells maintain a tone whereby they are poised between states of contraction and relaxation. To understand this tone, it is necessary to understand how the contractile mechanisms described above are balanced by processes that cause relaxation. There are two main mechanisms for controlling smooth muscle relaxation:

- Nitric oxide (NO)/cyclic GMP and smooth muscle relaxation
- Smooth muscle cell Ca^{2+} sparks

Nitric oxide (NO)/cyclic GMP and smooth muscle relaxation

Smooth muscle tone depends upon a balance between the contractile mechanisms and the mechanisms that promote relaxation. The nitric oxide (NO)/cyclic GMP signalling pathway plays an important role in controlling relaxation by acting at a number of sites, and particularly those concerned with Ca^{2+} signalling (Module 7: Figure smooth muscle cell cyclic GMP signalling):

1. Phosphorylation of the large-conductance (BK) channels enhances their activity, and the resulting hyperpolarization decreases the activity of the L-type channels that bring Ca^{2+} into the cell.
2. The production of inositol 1,4,5-trisphosphate (IP_3) is reduced by the cyclic GMP-dependent phosphorylation of phospholipase Cβ3 (PLCβ3), which is sensitive to G protein-coupled receptors (Module 2: Figure PLC structure and function).
3. Cyclic GMP acting through cyclic GMP-dependent protein kinase type Iβ (cGKIβ) phosphorylates inositol 1,4,5-trisphosphate receptor-associated cGKI substrate (IRAG), which then acts to reduce the release of Ca^{2+} by the $InsP_3$ receptor (IP_3R).
4. By associating with the myosin phosphatase targeting subunit 1 (MYPT1) of the protein phosphatase 1 (PP1) catalytic subunit, which dephosphorylates the myosin light chains (MLCs), cGKIα enhances the activity of the smooth muscle myosin phosphatase, thus reducing the Ca^{2+}-sensitivity of the contractile apparatus.

The ability of cyclic GMP to relax smooth muscle has been exploited to develop the drug Viagra, which is used in the treatment of male erectile dysfunction.

A decrease in the operation of this cyclic GMP-dependent relaxation of smooth muscle might be one of the causes of hypertension.

Viagra

Viagra (sildenafil) is the drug used in the treatment of male erectile dysfunction. It acts to enhance the level of cyclic GMP by inhibiting the cyclic GMP-specific phosphodiesterase PDE5, which hydrolyses cyclic GMP in smooth muscle cells (Module 7: Figure smooth muscle cell cyclic GMP signalling). By stimulating an increase in the level of cyclic GMP, Viagra will enhance the nitric oxide (NO)/cyclic GMP and smooth muscle relaxation mechanisms.

Rho-mediated Ca^{2+}-sensitization contributes to the flaccid state of the corpus cavenosum, and this can be reversed by a Rho kinase inhibitor (Y-27632), which might be suitable for therapy of erectile dysfunction.

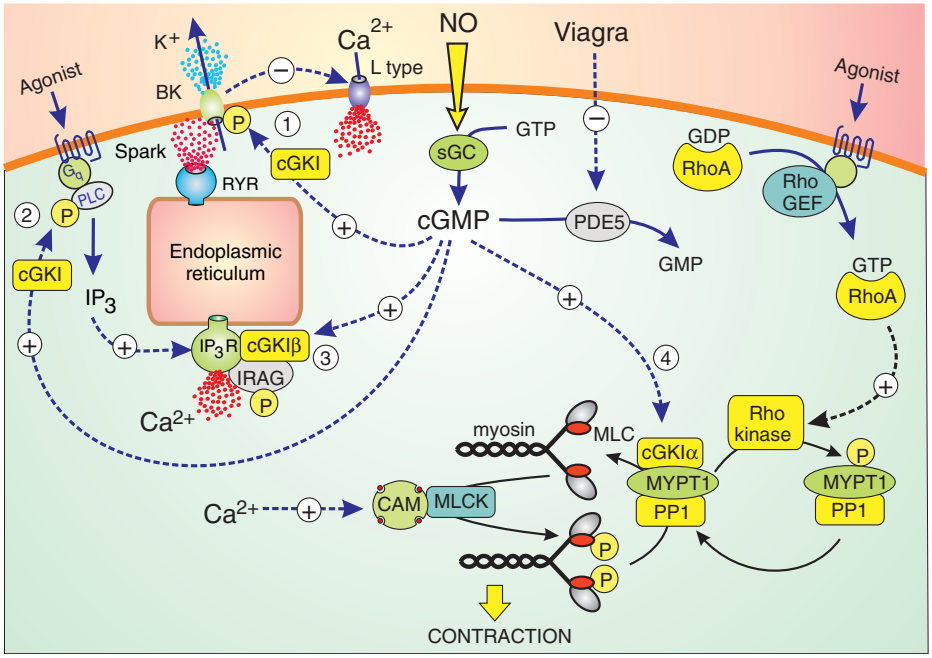
Smooth muscle cell Ca^{2+} sparks

Smooth muscle cell relaxation can also be activated by Ca^{2+}, which is somewhat counterintuitive because an increase in Ca^{2+} is usually associated with contraction. An explanation of how Ca^{2+} can control both contraction and relaxation can be found in the spatial organization of smooth muscle Ca^{2+} signalling. When the cell is activated to give global Ca^{2+} signals through the entry and release of internal Ca^{2+}, then contraction occurs (Module 7: Figure smooth muscle cell spark). Smooth muscle cells also produce Ca^{2+} sparks that act locally to switch on either Cl^- or K^+ channels. The relationship between sparks and the activation of a K^+ current carried by large-conductance (BK) channels is shown in Module 3: Figure smooth muscle cell Ca^{2+} sparks. If sparks occur in the vicinity of Cl^- channels, they will depolarize the membrane, resulting in global Ca^{2+} signals and contraction. However, if the sparks activate large-conductance (BK) channels, the resulting hyperpolarization prevents Ca^{2+} entry through the L-type channels and the muscle relaxes.

Smooth muscle activation mechanisms

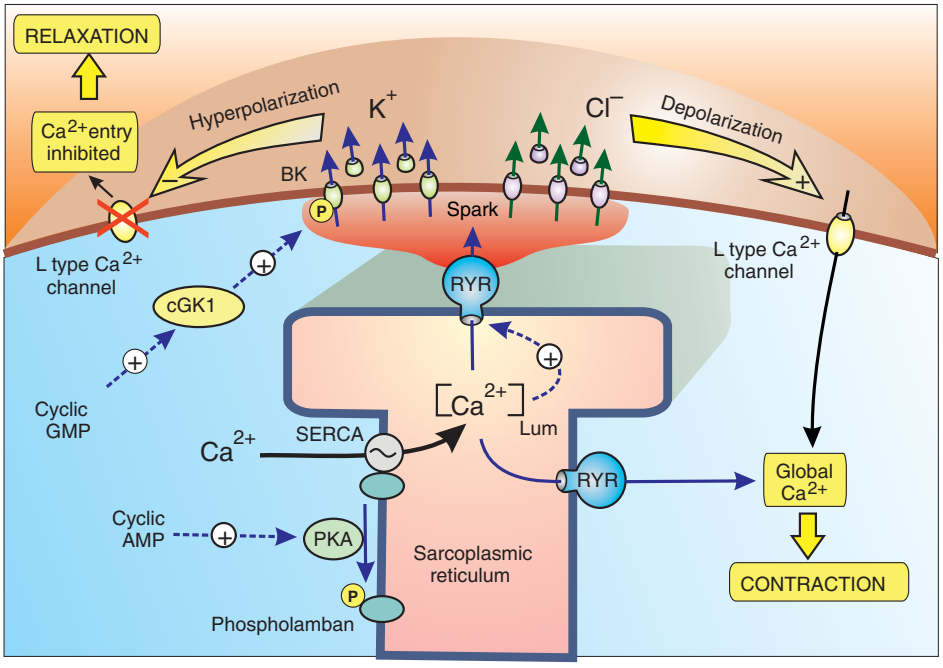
The activation mechanisms responsible for generating the Ca^{2+} signal that controls contraction varies considerably between different smooth muscle cell (SMC) types. Smooth muscle Ca^{2+} signalling plays a major role in the process of smooth muscle cell excitation–contraction coupling (Module 7: Figure smooth muscle cell EC coupling). The different cell types put in place a Ca^{2+} activation mechanism that suits their particular function. Those muscles such as the bladder and vas deferens, which have to produce rapid phasic contractions at a particular point in time, have activation mechanisms that respond quickly to nerve stimulation. In other smooth muscles, which have to maintain a particular tone over an extended period of time, are more dependent on pacemaker mechanisms that generate regular pulses of Ca^{2+}.

Module 7: | Figure smooth muscle cell cyclic GMP signalling



Function of the nitric oxide (NO)/cyclic GMP signalling pathways in controlling relaxation of smooth muscle cells.
Nitric oxide (NO) acts on the soluble guanylyl cyclase (sGC) to form cyclic GMP (cGMP), which acts through a number of targets to dampen both the Ca^{2+}- and Rho-dependent signalling mechanisms that induce contraction by increasing the phosphorylation of the myosin light chain attached to the myosin.

Module 7: | Figure smooth muscle cell spark

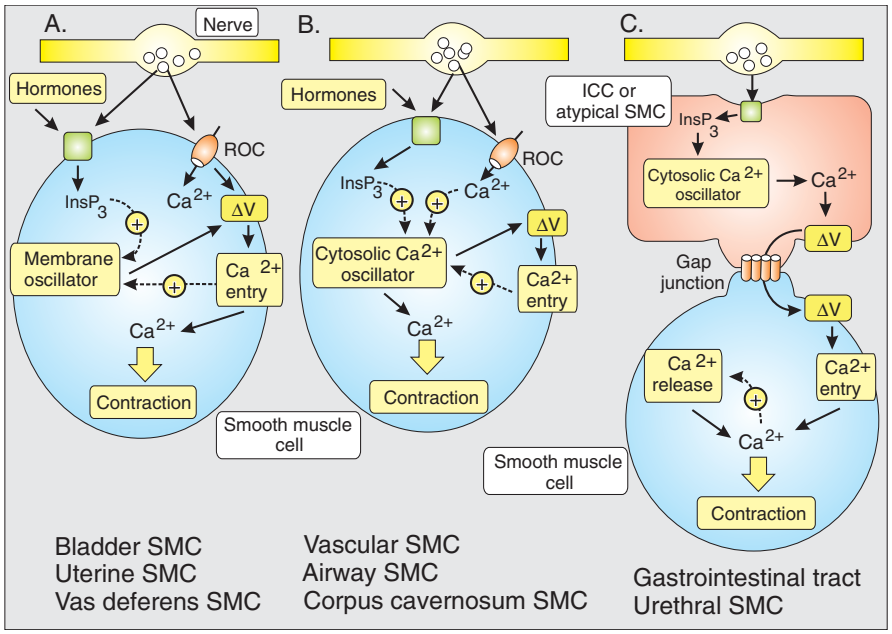


Function of smooth muscle cell Ca^{2+} sparks in the regulation of membrane potential and contraction/relaxation.
Local and global Ca^{2+} signalling in smooth muscle cells can determine whether the muscle contracts or relaxes, as described in the text.

Some muscles increase Ca^{2+} by opening of VOCs whereas others have a cytosolic oscillator that generates the Ca^{2+} pulses to drive the contractions that maintain smooth muscle tone. There are three main smooth muscle activation mechanisms (Module 7: Figure SMC activation mechanisms):

A. In some phasic muscles, such as the vas deferens, activation depends upon the release of neurotransmitters such as ATP that induce the depolarization (ΔV) necessary to trigger contraction. In other cases (bladder and uterus), the depolarization depends on the operation of a membrane oscillator. The way in which

Module 7: | Figure SMC activation mechanisms



Smooth muscle cell Ca^{2+} activation mechanisms.
There are three main mechanisms responsible for generating the Ca^{2+} transients that trigger contraction. See text for further details.

this oscillator is modulated varies between smooth muscles. In the bladder, neurotransmitters released from neural varicosities stimulate two main Ca^{2+} signalling pathways. First, ATP acts through receptor-operated channels (ROCs) such as the P2X receptors that gate an inward current resulting in membrane depolarization and the activation of VOCs. These VOCs can activate contraction directly and can also recruit further Ca^{2+} by stimulating release from internal stores through a process of Ca^{2+}-induced Ca^{2+} release (CICR). Secondly, transmitters such as acetylcholine can also stimulate the $InsP_3/Ca^{2+}$ signalling pathway that accelerates the oscillator. In the uterus, the hormone oxytocin acts through the $InsP_3/Ca^{2+}$ signalling pathway to accelerate the membrane oscillator (Module 7: Figure uterus activation).

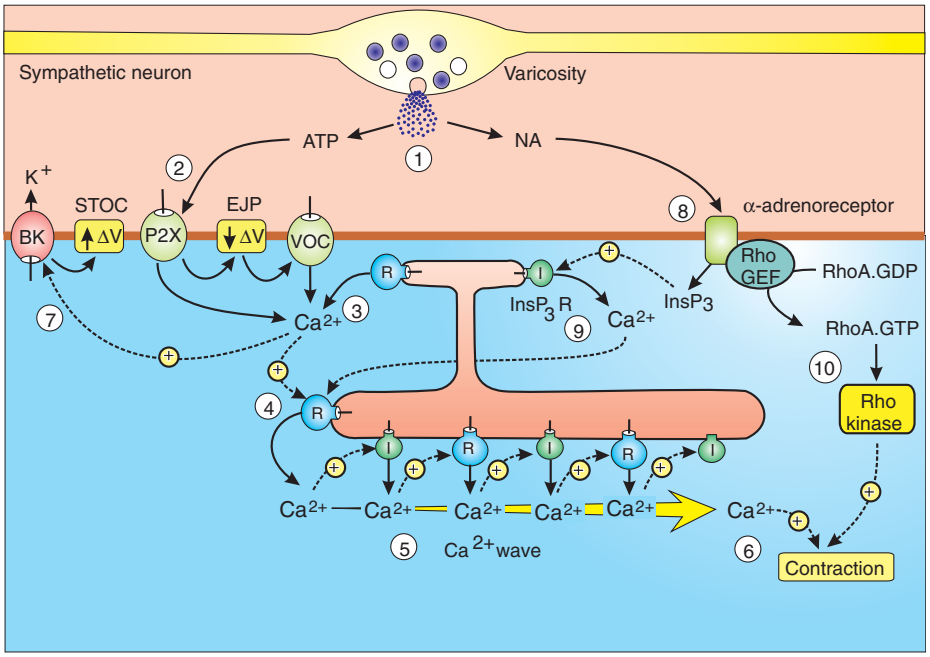
B. Some smooth muscle cells have an endogenous pacemaker driven by a cytosolic oscillator. In vascular, airway and corpus cavernosum smooth muscle, which have to maintain a variable tone, this cytosolic oscillator generates a myogenic rhythm consisting of Ca^{2+} oscillations. If the repetitive transients are coupled to membrane depolarization, the activation of VOCs can augment each Ca^{2+} transient by enhancing Ca^{2+} entry. Release of neurotransmitters does not activate contraction directly but it can influence tone by modulating the frequency of the endogenous pacemaker that is a central feature of this smooth muscle cell cytosolic oscillator (Module 7: Figure SMC cytosolic oscillator).

C. Certain smooth muscles (gastrointestinal tract and urethra) depend on the Interstitial cells of Cajal (ICC), which provide the pacemaker depolarization necessary to drive contraction (Module 7: Figure SMC activation mechanisms). The ICC, which are closely connected to the smooth muscle cells through gap junctions, generate a pacemaker rhythm (slow waves) that is responsible for providing the periodic depolarizations responsible for smooth muscle cell excitation—contraction coupling (Module 7: Figure smooth muscle cell EC coupling). The ICC cytosolic Ca^{2+} oscillator produces repetitive Ca^{2+} transients that activate an inward current by opening Ca^{2+}-sensitve chloride channels resulting in spontaneous transient depolarization (STD) (ΔV in Module 7: Figure ICC pacemaker). The current flow that causes this depolarization spreads electrotonically through the gap junctions to induce a near simultaneous depolarization in the smooth muscle cell to activate the VOCs that then triggers contraction.

These different smooth muscle activation mechanisms are illustrated by describing some specific examples from the many different smooth muscle and pacemaker cell types listed below:

- Pacemaker cell types
  - Interstitial cells of Cajal
  - Atypical smooth muscle cells
- Smooth muscle cell types
  - Airway smooth muscle cells
  - Gastrointestinal smooth muscle cells
  - Genital tract
    - Corpus cavernosum smooth muscle cell
    - Uterine smooth muscle cells
    - Vas deferens
  - Urinary tract
    - Detrusor smooth muscle cell

Module 7: | Figure vas deferens activation



Activation of the vas deferens.
The smooth muscle cells that surround the vas deferens are controlled by sympathetic neurons that have numerous varicosities that release ATP and noradrenaline (NA) to control the peristaltic contractions that transport sperm from the testis to the urethra. See text for details of the signalling pathways used by ATP and NA.

Ureter smooth muscle cell
Urethral smooth muscle cells
Vascular smooth muscle cells

Vas deferens

The vas deferens functions to propel spermatozoa into the ejaculate. The peristaltic contractions of the smooth muscle cells that surround the vas deferens are activated by sympathetic neurons (Module 7: Figure urinogenital tract). These neurons have numerous varicosities that release ATP and noradrenaline (NA) that activate contraction. These transmitters act through mechanism A in Module 7: Figure SMC activation mechanisms. Unlike some of the other smooth muscle cells that use this mechanism, such as the bladder and uterus, the vas deferens shows little evidence of the pacemaker activity characteristic of muscles that have to maintain contractile activity over a protracted period. In the case of the vas deferens, contractile activity has to be switched on rapidly to induce sperm transfer during the brief period of ejaculation. This rapid activation is achieved by the transmitters released from the sympathetic neurons that form an extensive network throughout the vas deferens with numerous varicosities that make close contact with each muscle cell. The signalling pathways activated by ATP and NA are illustrated in Module 7: Figure vas deferens activation:

1. Action potentials passing down the sympathetic neurons induce an increase in Ca^{2+} within the varicosities to release ATP and NA on to the surface of the vas deferens smooth muscle cells.
2. ATP acting on the P2X1 receptor induces an influx of Ca^{2+} that result in an excitatory junctional potential (EJP).
3. The EJPs sum to depolarize the membrane sufficiently to activate the L-type voltage-operated channels (VOCs).The entry of Ca^{2+} then triggers the release of Ca^{2+} from the internal stores near the surface.
4. The Ca^{2+} ions coming in from the outside spreads inwards to create a global Ca^{2+} signal.
5. Globalization of the Ca^{2+} signal depends on the formation of an intracellular Ca^{2+} wave. It is unclear how this wave spreads but it probably depends on the regenerative release of Ca^{2+} by both the ryanodine receptors (R) and the inositol 1,4,5-trisphosphate receptors (I).
6. The global Ca^{2+} signal then triggers contraction.
7. In addition, the Ca^{2+} near the membrane activates the large-conductance (BK) channels to form the spontaneous transient outward current (STOC) that hyperpolarizes the membrane and helps to terminate the activation processes.
8. The noradrenaline (NA) that is released from the varicosities can also activate contraction by stimulating α_1-adrenoreceptors.
9. The $InsP_3$ releases Ca^{2+} and this may induce a global response by feeding into the same mechanism used by ATP (Steps 4 and 5).
10. Another important mechanism activated by the α_1-adrenoreceptors is the smooth muscle Rho/Rho kinase signalling pathway that serves to increase the Ca^{2+} sensitivity of the contractile machinery.

Alterations in the signalling pathways that control vas deferens contraction and particularly the α_1-adrenoreceptor signalling pathway may be one of the causes of ejaculatory dysfunction.

Uterine smooth muscle cells

The uterus is made up of three parts: the body that makes up the major part, a domed-shape fundus at the top and the cervix at the bottom (Module 7: Figure urinogenital tract). Most of the uterus is composed of the myometrium, which is a thick layer of longitudinal and circular smooth muscle cells. Within each layer, the smooth muscle cells are arranged in individual bundles (fasciculus) that lie parallel to each other and are connected through gap junctions.

During early pregnancy, the uterus is relatively quiescent. There are a few weak twitches resulting from unsynchronized smooth muscle cell contractions. With the onset of labour, the uterus begins to develop stronger and more frequent contractions that appear first in the fundus and then spread rapidly through the body of the uterus. This remarkable switch from quiescence to a cycle of strong contractions is an example of the phenotypic remodelling of the signalsome, which in this case is the endogenous uterine smooth muscle activation mechanism. For example, there is an increase in gap junctional coupling just before labour. There is an increase in the expression of PDE4B, which decreases the effect of tocolytic agents such as β-adrenergic agents that use cyclic AMP to inhibit uterine contractions. There is an increase in the expression of the oxytocin receptors that act by inducing and accelerating contractions. The expression of the ryanodine receptor 2 (RYR2) is up-regulated during pregnancy and this may facilitate the process of excitation–contraction coupling. A decline in the activity of the Na^+-K^+-ATPase pump during labour may accelerate the uterus smooth muscle cell membrane oscillator and thus contribute to the increase in the frequency of contractions. There may also be a decrease in the activity of potassium channels such as TREK-1 and the large-conductance (BK) channels that contributes to the quiescence of the uterus during pregnancy. The next aspect to consider is how this remodelled signalsome switches the uterus out of its quiescent state such that it begins to generate the repetitive electrical impulses responsible for driving contractions during labour.

The uterine contractions that occur during labour have a periodicity of approximately 3–5 min (see Module 7: human uterine contractions and Module 7: Figure Ni^{2+} slows uterine oscillations). They have a characteristic shape; there is a slow rising phase (15 s), a plateau that lasts for about 30 s that is followed by a gradual relaxation phase that is roughly symmetrical with the rising phase. Each of these protracted uterine contractions depends upon the co-ordinated activation of most of the smooth muscle cells and this synchronicity is achieved by an action potential spreading through the smooth muscle cell layer with a conduction velocity of approximately 4 cm/s. The wave of excitation appears to begin at the fundus and then spreads through the body of the uterus towards the cervix. Even though all smooth muscle cells in the uterus are capable of generating a rhythm, those in the fundus are inherently more rhythmical and thus initiate each contraction wave.

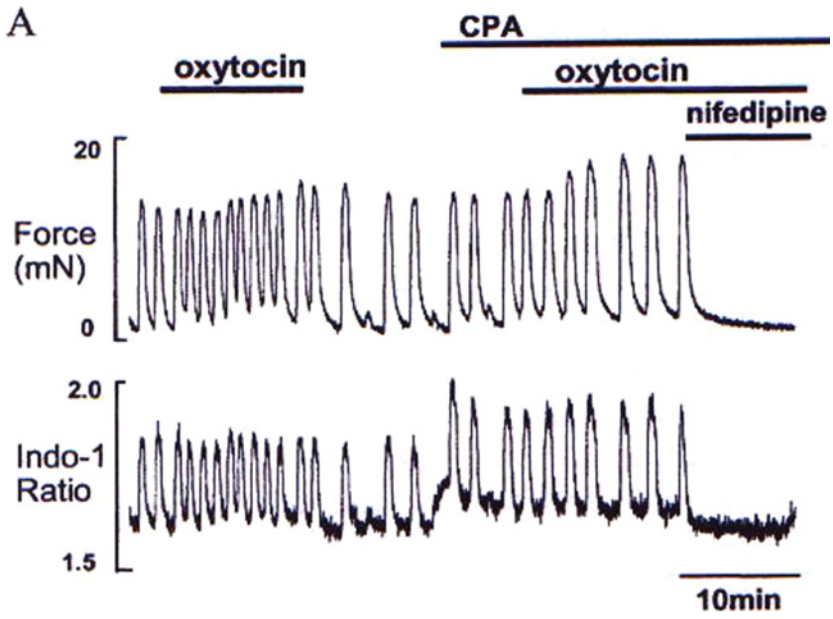
Since there appear to be no interstitial cells of Cajal and there is little neural innervation, the uterine myometrium is driven by an endogenous pacemaker mechanism (see A in Module 7: Figure SMC activation mechanisms). Some of the features of this pacemaker mechanism are illustrated in Module 7: human uterine contractions. The regular rhythm is accelerated reversibly by oxytocin. Perhaps the most interesting feature of the rhythm is that it continues and is accelerated by the drug cyclopiazonic acid (CPA) that blocks the sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pump and thus empties the internal store of Ca^{2+}. This observation rules out the presence of a cytosolic oscillator and suggests that the pacemaker is driven by a membrane oscillator. The activation mechanism thus has two main components (Module 7: Figure uterus activation). A membrane oscillator (horizontal box), which generates a pacemaker depolarization (ΔV), and L-type VOCs that respond to depolarization with a fairly typical smooth muscle cell excitation–contraction coupling mechanism (vertical green box). The primary signal for triggering contraction is the Ca^{2+} that enters the cell through the L-type VOC. There appears to be little role for release of Ca^{2+} from the internal stores since contractions continue when these stores have been depleted. However, there is an increased expression of RYR2s during the end of pregnancy and these may open in response to the entry of external Ca^{2+} to provide an additional boost of Ca^{2+} to increase the force of contraction (Module 7: Figure uterus activation). However, the major activation mechanism for contraction is the opening of the L-type channels by the membrane depolarization provided by the action potential that sweeps through the uterine smooth muscle cell syncytium.

While the nature of the excitation–contraction coupling mechanism is fairly well established, there is less information on the nature of the uterus smooth muscle cell membrane oscillator that is responsible for generating the periodic action potentials.

Uterus smooth muscle cell membrane oscillator

The nature of the pacemaker mechanism responsible for generating the action potentials that drive the smooth muscle contraction cycles during labour is still a mystery. The following membrane oscillator model attempts to explain the mechanism responsible for the slow pacemaker depolarization that triggers each contraction and how the frequency of this oscillator is modulated by stimuli such as oxytocin (Module 7: Figure uterus activation). The shape of the membrane potential fluctuation that drives each contractile cycle has two main components: there is a slow pacemaker depolarization that brings the membrane potential to the threshold (dashed line in Module 7: Figure uterus activation) for the action potential that is driven by the opening of the L-type VOCs. At the end of the action potential, the membrane potential switches back to a hyperpolarized state before beginning the next pacemaker depolarization phase. The critical question with regard to

Module 7: | human uterine contractions



Spontaneous contractions of human uterine smooth muscle.
Strips of human smooth muscle respond to oxytocin by a marked acceleration in the rhythmic contractions. Each contraction is driven by transient pulses of Ca^{2+} measured with the indicator Indo-1. Upon addition of cyclopiazonic acid (CPA), the basal level of Ca^{2+} increased and the oscillator continued. Oscillations ceased immediately after adding 10 μM nifedipine that inhibits Ca^{2+} entry through VOCs. Reproduced from Kupittayanant et al. (2002).

uterine contractility is the nature of the membrane oscillator that drives this slow pacemaker depolarization.

At any moment in time, membrane potential depends upon the co-ordinated activity of ion channels, pumps and exchangers that can either depolarize or hyperpolarize the membrane. At the end of the action potential, the hyperpolarizing components are dominant but as their influence wanes the depolarizing components begin to take over to produce the pacemaker depolarization. There are a number of candidates that could contribute to this hyperpolarizing/depolarizing switch (see the membrane oscillator box in Module 7: Figure uterus activation where the components that contribute to depolarization or hyperpolarization are coloured green and red respectively). At present, it is not clear how or when these possible contenders might contribute to the membrane oscillator during the course of the pacemaker depolarization. These different candidates have been included in the model based on either physiological evidence or the fact that they are expressed in human uterine or other smooth muscle cells as outlined below (Module 7: Figure uterus activation).

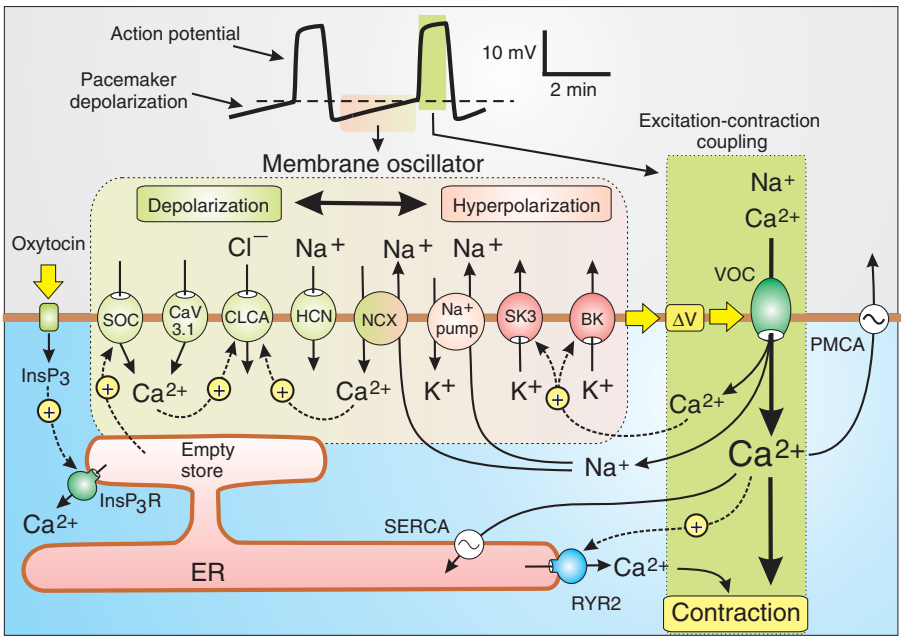
K^+ channels

A number of K^+ channels regulate uterine contraction. Both the large-conductance (BK) channels and the small-conductance (SK) channels are expressed in uterine smooth muscle and may contribute to the membrane oscillator. Activation of the BK and SK3 channels by Ca^{2+} contribute to the repolarizing phase of the action potential and may thus determine the maximal hyperpolarizing potential at the start of the pacemaker phase. As Ca^{2+} is pumped out of the cell, their hyperpolarizing influence will wane and this will contribute to the gradual pacemaker depolarization. Inhibition of the SK3 channel with apamin results in an acceleration of the oscillator. Conversely, if SK3 is overexpressed in rat uterus, there is a drastic reduction in oscillator frequency.

In addition to contributing to the membrane oscillator during labour, the BK channel may also play a role in maintaining the quiescent state of the uterus during pregnancy. These BK channels appear to be concentrated in the numerous caveolae that are a characteristic feature of smooth muscle cells (Module 7: Figure smooth muscle cell structure). These infoldings of the surface membrane, which often come into close contact with the endoplasmic reticulum (ER) (Module 6: Figure smooth muscle caveolae), have numerous signalling components and are rich in lipids such as cholesterol and sphingomyelin (SM) (Module 6: Figure caveolae organization). The cholesterol appears to function by enhancing the activity of the BK channels to reduce membrane excitability and thus contribute to the quiescent state. The increases in cholesterol that accompany obesity may increase the risk of complications in pregnancy by reducing the uterine contractility during labour and may account for the increased incidence of Caesarean sections in obese women.

The normal uterus contracts when stretched, but this excitability is reduced during pregnancy through the expression of stretch-sensitive TREK-1 channels and this enanles the uterus to distend. At the end of pregnancy, a decrease in TREK-1 expression enhances excitability and thus contributes to the onset of labour.

Module 7: | Figure uterus activation



A model for the activation mechanism of human uterus contractility.

Contractions of the uterus during labour are driven by an endogenous membrane oscillator (horizontal green to pink box) that induces the slow pacemaker depolarization responsible for triggering the excitation–contraction coupling mechanism (vertical green box).The oscillator depends on an interaction between ion channels, pumps and exchangers that are colour-co-ordinated to indicate their contribution to either depolarization (green) or hyperpolarization (red). When the pacemaker depolarization (ΔV) reaches the threshold for the activation of the L-type VOCs (dashed line in the membrane potential trace at the top), excitation–contraction coupling is triggered and the muscle contracts. See text for further details.

Na^+-K^+-ATPase pump

During the course of the action potential, opening of the L-type VOC results in Na^+ entering the cell together with Ca^{2+}. A hyperpolarization is generated when the Na^+ is removed by the Na^+ pump, which is electrogenic because it extrudes $2Na^+$ for $3K^+$. The pump will be activated immediately after an action potential to extrude Na^+ and this will contribute to the early hyperpolarizing phase. But as the Na^+ concentration returns to resting levels, this hyperpolarizing effect will decline and this may contribute to the depolarizing pacemaker.

Na^+/Ca^{2+}-exchanger (NCX)

The Na^+/Ca^{2+}-exchanger (NCX) has a somewhat ambiguous role because it can operate in both a forward and reverse mode (Module 5: Figure Na^+/Ca^{2+}-exchangers) and thus can contribute to either depolarization or hyperpolarization. The resting membrane potential of the uterus as it begins to oscillate is approximately −50 mV, which is probably more positive than the equilibrium potential for Na^+, so it is likely that the exchanger will be operating in its reverse mode as shown in Module 7: Figure uterus activation . When operating in its reverse mode, $1Ca^{2+}$ is exchanged for $3Na^+$ so this stoichiometry may also favour hyperpolarization.

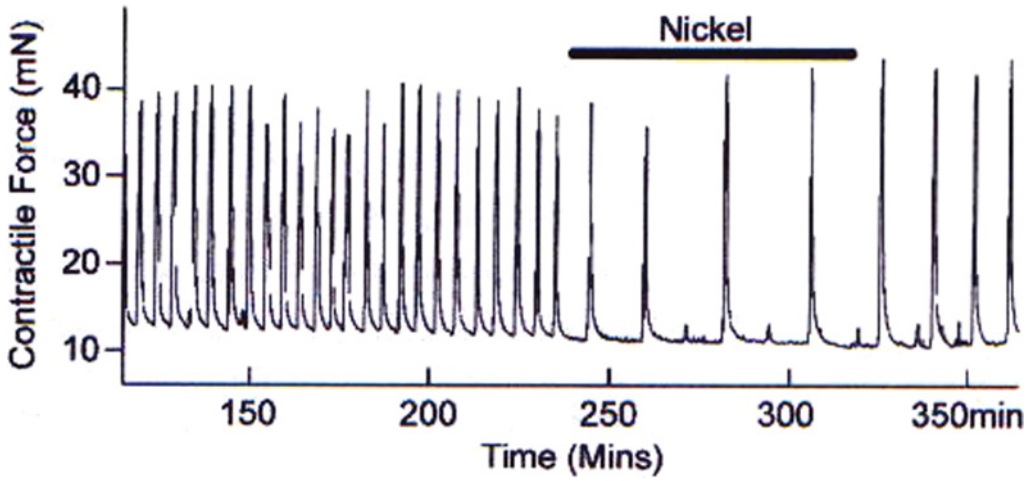
Store-operated Ca^{2+} channel (SOC)

Smooth muscle cells are known to express store-operated channels (SOCs) and these have been included as one of the possible channels for providing the inward current that is necessary to provide the depolarizing drive during the pacemaker depolarization. In addition, such a SOC mechanism may help to describe how oxytocin can accelerate the membrane oscillator. The suggestion is that oxytocin stimulates the formation of $InsP_3$ that then acts to empty part of the endoplasmic reticulum (ER) store that lies near the membrane to promote the opening of the SOC and this increase in inward current will steepen the pacemaker depolarization to accelerate the oscillator. Another possibility is that the Ca^{2+} that enters the cell may act to switch on channels such as the Ca^{2+}-sensitive chloride channels (CLCA). Ca^{2+} may also activate TRPM4 or TRPM5 that gate an inward monovalent current and have been implicated in the operation of other membrane oscillators.

Ca_V3 T-type channels

Uterine smooth muscle cells express $Ca_V3.1$, which is one of the isoforms of the $Ca_V3.1$ family of T-type channels (Module 3: Table VOC classification), which have been implicated in the pacemaker activity of other excitable cells. At the low membrane potential of activated uterine smooth muscle cells, these T-type channels are probably inactivated. However, at these inactivation potentials, a small population may still contribute to a pacemaker depolarization by gating an inward flux of Ca^{2+}. Such a role for the T-type channel is consistent with the observation that low concentrations of Ni^{2+} slow the oscillator (Module 7: Figure Ni^{2+} slows uterine oscillations). The entry of Ca^{2+} through these T-type channels may also contribute to the depolarization by activating other inward currents such as those gated by TRPM4 or TRPM5 as mentioned above.

Module 7: | Figure Ni^{2+} slows uterine oscillations



Human uterine smooth muscle contractile oscillations are slowed by Ni^{2+}.

Strips of human smooth muscle contract spontaneously with a period of about 5 min. Upon addition of 100 μM Ni^{2+}, the periodicity increased to 20 min. Reproduced from Blanks et al (2007).

Ca^{2+}-sensitive chloride channels

A proportion of human uterine cells express Ca^{2+}-sensitive chloride channels (CLCA), which could play an important role in providing the inward current to drive pacemaker depolarization (Module 7: Figure uterus activation).

TRPM4 and TRPM5

TRPM4 and TRPM5, which are members of the melastatin-related transient receptor potential (TRPM) family of ion channels, respond to an increase in Ca^{2+} by gating an inward current carried by monovalent cations such as Na^{+}. Although there is no information yet on their expression in uterine smooth muscle, they are known to occur in other smooth muscle cell types. These channels are not shown in Module 7: Figure uterus activation, but they should also be considered in the model because they could play a critical role in the pacemaker mechanism by providing an inward current to drive the pacemaker depolarization.

Hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels

A number of cells that display pacemaker activity express members of the hyperpolarizing-activated cyclic nucleotide-gated (HCN) channels, which have been described in rat uterine smooth muscle. They have been included in the model (Module 7: Figure uterus activation) because they are one of the major pacemaker channel currents in the sinoatrial (SA) node pacemaker cells that has a similar membrane oscillator (Module 7: Figure cardiac pacemaker).

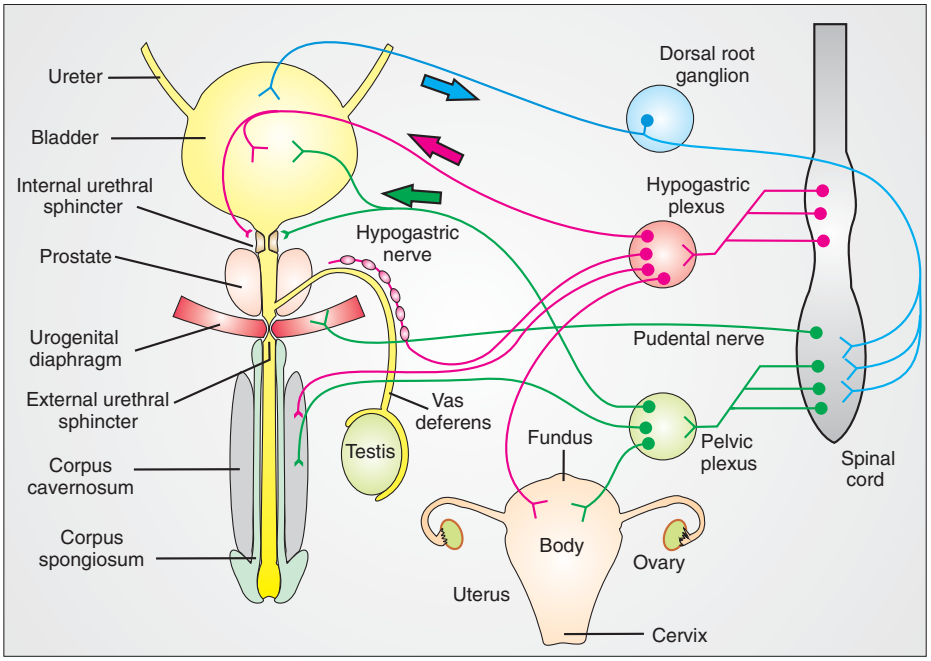
In conclusion, uterine contraction is driven by a membrane oscillator that consists of multiple components that can either depolarize or hyperpolarize the membrane. At the end of the action potential, the hyperpolarizing elements predominate but as these wane and the depolarizing components begin to take over the membrane gradually depolarizes to the point where the next action potential and contraction are triggered. Many of the hormones that modulate contractility are likely to influence some of these oscillatory components. For example, it is argued that oxytocin may act to switch on a SOC to provide an inward Ca^{2+} current that not only contributes a depolarizing signal but it also may act to switch on some of the TRPM channels. Oxytocin may have additional actions because it can increase the force of contraction in muscle cells where the internal stores have been depleted of Ca^{2+} following treatment with CPA (Module 7: human uterine contractions). In this case, it seems likely that oxytocin may switch on the smooth muscle Rho/Rho kinase signalling pathway to enhance the sensitivity of the contractile mechanism.

It is difficult to speculate further on how contractility is modulated until the nature of the membrane oscillator has been established. This is an important priority because problems remain concerning an effective treatment of controlling premature labour, which is one of the major causes of neonatal morbidity.

Detrusor smooth muscle cell

The bladder, which functions to store and expel urine, is lined with an uroepithelium that is surrounded by a lamina propia and layers of detrusor smooth muscle cells. These detrusor cells line up in bundles called fascicles that are arranged in a circular layer sandwiched between two longitudinal layers. The concerted contraction of these detrusor smooth muscle cells during micturition, is regulated by an extensive neural innervation (Module 7: Figure urinogenital tract) that orchestrates both the slow refilling of the bladder and the periodic and co-ordinated contraction of the detrusor muscles during micturition. Sensory neurons (shown in blue), which respond to stretch as the bladder fills, sends information into the spinal cord as part of the micturition reflex. Sympathetic nerves originating from the hypogastric plexus have two functions: they inhibit the detrusor muscles to enable the bladder to fill and

Module 7: | Figure urinogenital tract



Organization and neural innervation of the urinogenital tract.
Smooth muscle cells (SMCs) feature prominently in the function of the urinogenital tract where they control urine transfer to the bladder (ureter SMCs), bladder emptying (detrusor SMCs), contraction of the urethral sphincter (urethral SMCs), penile erection (corpus cavernosum SMCs), sperm transfer (vas deferens SMCs) and uterine contraction during labour (uterine SMCs). Many of these SMCs are under neural control either by parasympathetic (green) or sympathetic (red) nerves. Sensory nerves (blue) carry information into the spinal cord.

they excite the urethral smooth muscle cells that keep the internal urethral sphincter closed to prevent urine escaping from the bladder. Parasympathetic pelvic nerves from the pelvic plexus provide the major excitatory input to trigger detrusor smooth muscle cell contraction during micturition.

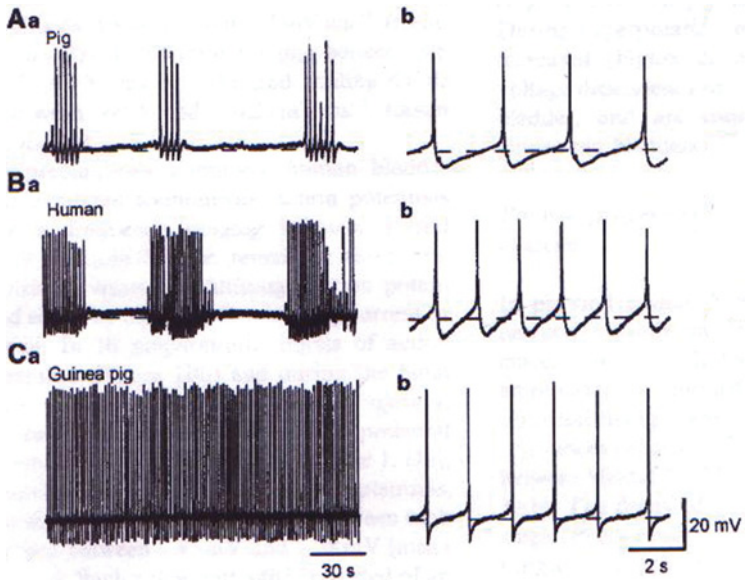
The detrusor smooth muscle cells thus have two operational modes. During bladder filling the muscle cells remain relaxed for an extended period to enable the bladder wall to expand. During micturition, they respond to parasympathetic stimulation by contracting vigorously to increase the intraluminal pressure to expel urine. This bladder contraction phase must be co-ordinated with relaxation of the urethral smooth muscle cells that control the internal urethral sphincter and the opening of the external urethral sphincter by contraction of the striated muscles that make up the urogenital diaphragm. To understand detrusor smooth muscle cell activation, therefore, it is necessary to consider both the control of the relaxation phase and how this is switched into the active contraction phase by the neurotransmitters released from the parasympathetic neural innervation.

Detrusor smooth muscle cell activation

Detrusor muscle seems to be activated by mechanism A in Module 7: Figure SMC activation mechanisms. They have an endogenous membrane oscillator capable of producing repetitive trains of action potentials (Module 7: Figure detrusor action potentials). The pattern of action potentials varies between species. In pig and human, the action potentials are arranged in bursts whereas in guinea-pig and some other mammals they appear as a continuous train. This electrical activity is present during both the resting state when the bladder is filling and during the active contraction phase. The major difference is that this activity and associated contractions are restricted to small areas of the bladder during the resting phase. During the active phase, neurotransmitters released from the parasympathetic neurons not only induces such pacemaker activity in most of the detrusor muscles but it also results in an increase in frequency (Module 7: Figure bladder pacemaker acceleration). With regard to detrusor smooth muscle cell activation, therefore, there are two main questions: how is this pacemaker generated and how is it induced or accelerated during the contractile phase of bladder emptying?

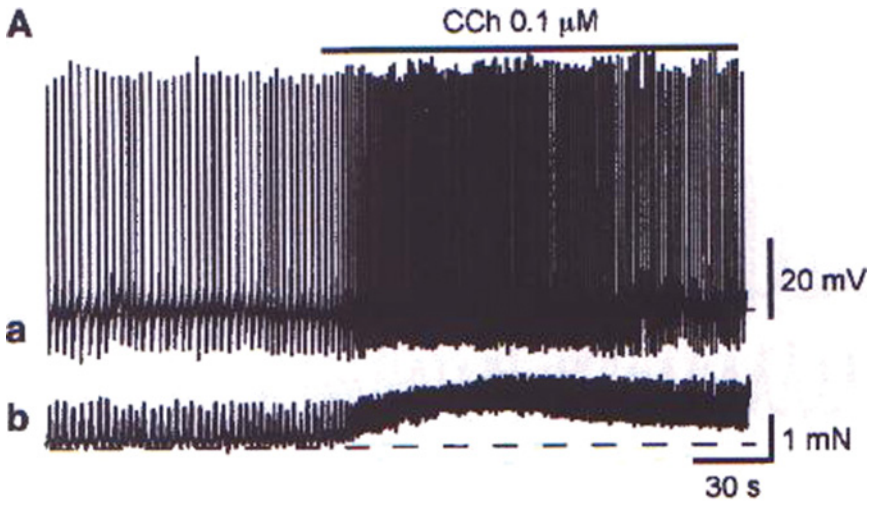
The pacemaker mechanism that generates the repetitive action potentials is driven by a membrane oscillator, which has not been fully characterized. However, it contains many of the ionic components found in the oscillator in the uterus (Module 7: Figure uterus activation). An L-type VOC is responsible for the upstroke of the action potential, which is terminated by both Ca^{2+}-induced desensitization of the VOC and by the activation of the Ca^{2+}-sensitive large-conductance (BK) channels and the small-conductance (SK) channels. Activation of BK and SK3 set the potential at its maximum hyperpolarizing potential at the start of the pacemaker phase following each action potential. As Ca^{2+} is pumped out of the cell, their hyperpolarizing influence will wane and this will contribute to the gradual pacemaker depolarization. The frequency of the

Module 7: | Figure detrusor action potentials



Action potential patterns recorded from the bladders of different species.
Spontaneous action potentials recorded from pig, human and guinea-pig bladder. The faster time course recordings shown on the right illustrates the typical after hyperpolarizations (AHPs) that follow each action potential. Reproduced from Hashitani and Brading (2003).

Module 7: | Figure bladder pacemaker acceleration

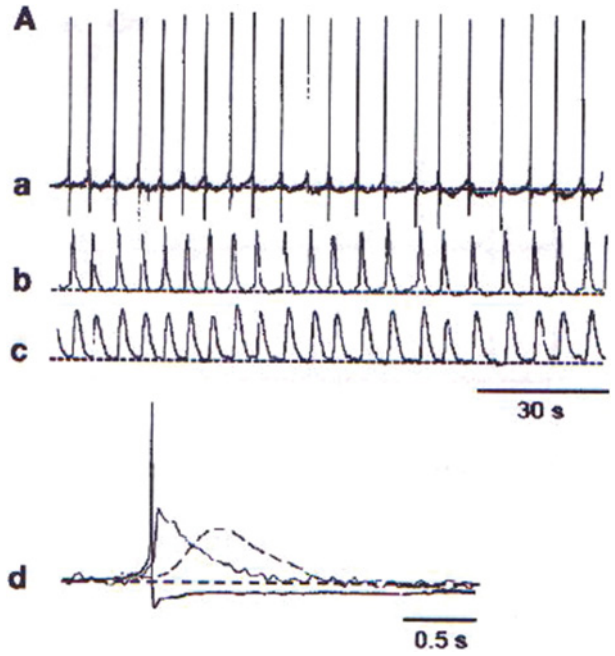


Carbachol accelerates electrical and contractile activity of guinea-pig bladder.
The rhythmical action potentials (trace a) and contractions (trace b) of guinea-pig bladder were markedly accelerated by a low dose of carbachol. Note the tonic contraction that develops slowly during the action of carbachol. Reproduced from Hashitani et al (2004).

oscillator is acutely sensitive to changes in membrane potential being accelerated by depolarization and slowed or stopped when the membrane is hyperpolarized. The role of inward depolarizing currents in this pacemaker depolarization has not been fully determined. The main point is that the detrusor has an endogenous membrane oscillator that generates the spontaneous action potential that occurs in the resting muscle.

Each of these spontaneous action potentials is capable of inducing an increase in intracellular Ca^{2+} and contraction, which develop with much slower time courses (Module 7: Figure bladder E-C coupling). Each of the action potentials (trace a) is associated with an increase in Ca^{2+} (trace b) and contraction (trace c). The temporal relationship between these three events is shown in trace d. The Ca^{2+} signal lags behind the action potential because it spreads into the cell in the form of a Ca^{2+} wave (see panel Aa in Module 7: Figure bladder Ca^{2+} wave). About 10 ms after the depolarization, Ca^{2+} appears in the form of a spark at two sites (α and β) near the membrane. These sparks then develop into waves that spread into the cell to produce a global signal after about 200 ms. If the ryanodine receptors are blocked by 100 μM ryanodine, the Ca^{2+} signal is restricted to a smaller elevation immediately below the membrane, which might represent Ca^{2+} sparklets. The signalling components responsible for these spatiotemporal

Module 7: | Figure bladder E-C coupling



Excitation–contraction coupling in bladder smooth muscle cells.
Action potentials (a), intracellular Ca^{2+} (b) and contractions (c) were measured simultaneously in guinea-pig smooth muscle. The temporal relationship between these three events is shown on a faster time scale in panel (d). Reproduced from Hashitani et al (2004).

patterns are illustrated in Module 7: Figure bladder SMC activation. During this process of excitation–contraction coupling, membrane depolarization (ΔV) activates the L-type VOCs to produce sparklets, which provide the Ca^{2+} signal to activate ryanodine receptors (RYRs) located near the membrane to produce sparks. These sparks then recruit RYRs deeper in the cell to initiate the regenerative Ca^{2+} wave responsible for the global Ca^{2+} signal that triggers contraction. Ryanodine inhibits both the sparks and the wave leaving behind the sparklets that produce the small Ca^{2+} signal that appears as a ring immediately below the membrane (see panel Ba in Module 7: Figure bladder Ca^{2+} wave).

It is likely that the excitation–contraction (E-C) coupling process associated with the spontaneous action potentials are similar to those that occur when the whole bladder contracts during the emptying process. In the latter case, neural stimulation induces action potentials throughout the bladder thus recruiting the contractile activity of most of the bladder cells. This neural activation process is complicated by the fact that the parasympathetic neurons that innervate the bladder (Module 7: Figure urinogenital tract) release both acetylcholine (ACh) and ATP. These two transmitters have different effects on action potential frequency and contractile behaviour (Module 7: Figure neural evoked contractions). If the nerves are activated by a brief train of stimuli (solid bar), there is rapid acceleration of action potential frequency both during the burst and after the burst following a brief delay (see trace A). A strong contraction began during the neural stimulus. In the presence of α,β-methylene ATP, which inhibits ATP to reveal the action of ACh (trace B), there were no action potentials during the 1 s stimulus period, but thereafter the membrane depolarized and there was a marked increase in the frequency of action potentials similar to those seen in trace A. During the action of ACh, the onset of an oscillatory period of contraction was delayed. A similar delayed action potential acceleration and enhanced contraction is observed following stimulation of the cholinergic pathway with carbachol (Module 7: Figure bladder pacemaker acceleration). The acceleration of the membrane oscillator resulted in a tonic contraction that probably resembles what happens *in vivo* during bladder emptying. The ATP and ACh released during neural stimulation thus have similar effects but with different time courses: ATP acts quickly whereas ACh acts more slowly.

The next aspect to consider is how ACh and ATP act to induce/accelerate the membrane oscillator to stimulate contraction (Module 7: Figure bladder SMC activation):

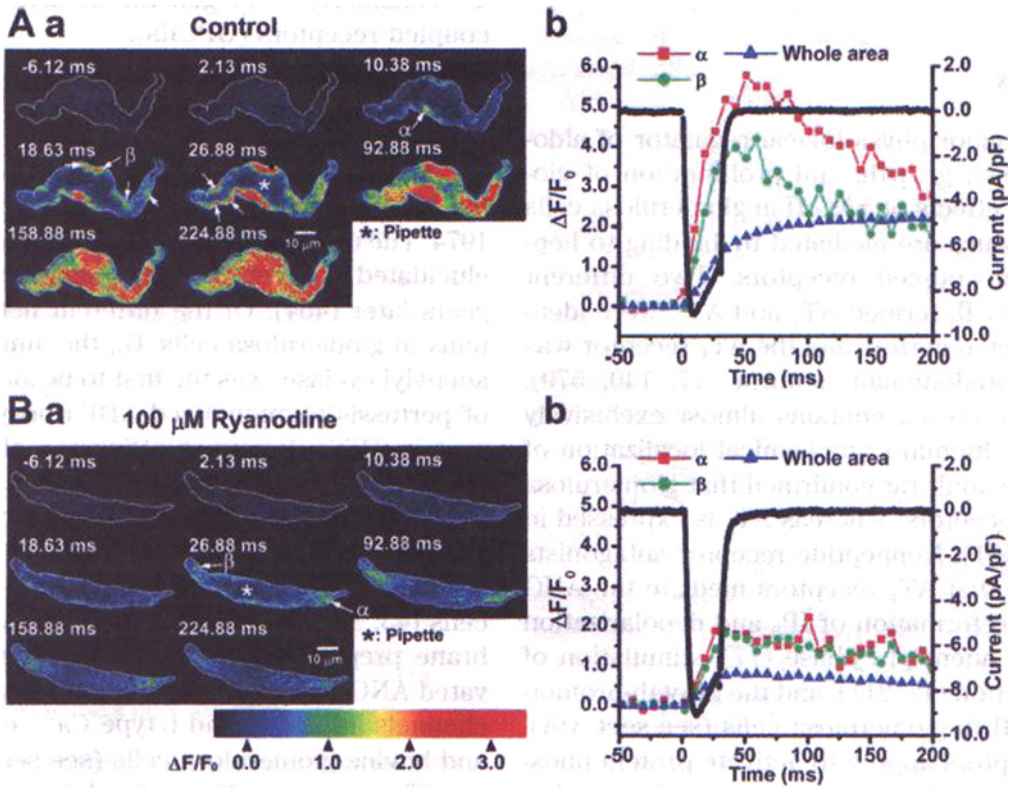
ATP-induced activation

1. The parasympathetic neurons release both ATP and acetylcholine (ACh).
2. ATP acts through a P2X receptor to increase the entry of Ca^{2+}, which produces an excitatory junction potential (EJP) and this depolarization (ΔV) then either accelerates an ongoing rhythm or induces inactive cells to oscillate. The activation of the voltage-operated channels (VOCs) produces the periodic action potentials.
3. During each action potential, opening of the L-type VOC produces a sparklet as Ca^{2+} enters the cell. This localized pulse of Ca^{2+} then diffuses across to activate ryanodine receptors 2 (RYRs) (R in Module 7: Figure bladder SMC activation) on the endoplasmic reticulum lying close to the plasma membrane.
4. The Ca^{2+} in the spark then diffuses out to ignite RYR2s deeper in the cell to produce a Ca^{2+} wave that spreads inwards (Module 7: Figure bladder Ca^{2+} wave). In the absence of ACh stimulation and hence $InsP_3$, the $InsP_3$ receptors (I in Module 7: Figure bladder SMC activation) probably fail to respond so the wave will be carried along primarily by the RYR2s. These waves provide the global Ca^{2+} signal to trigger contraction.
5. The large global elevation of Ca^{2+} also provides a signal to activate the large-conductance (BK) channels and small-conductance (SK) channels that contribute to the repolarization phase of the action potential.

ACh-induced activation

6. ACh stimulates an increase in the second messenger inositol 1,4,5-trisphosphate ($InsP_3$) that acts through the $InsP_3$ receptors (I, shown in green) to release Ca^{2+}.
7. Just how this early action of ACh brings about the membrane depolarization necessary to activate/accelerate the membrane oscillator is not clear. One possibility is that $InsP_3$ depletes stores near the

Module 7: | Figure bladder Ca^{2+} wave



Spatiotemporal patterns of Ca^{2+} signals in mouse bladder smooth muscle cells.

Mouse bladder smooth muscle cells voltage clamped at −60 mV were depolarized to 0 mV for 30 ms. The spatial characteristic of the Ca^{2+} signal recorded with fluo-4 is shown under control conditions (Aa) or in the presence of 100 μM ryanodine to block Ca^{2+} release from the ryanodine receptors (Ba). In panels b, the black line is the current trace, the red and green traces record Ca^{2+} at sites α and β near the membrane whereas the blue trace records Ca^{2+} over the whole cell. Reproduced from Morimura et al (2005).

membrane to activate store-operated Ca^{2+} channels (SOCs) to provide the inward current necessary to depolarize the membrane. Once action potentials are triggered, the opening of the VOCs will ignite another wave as described above (steps 3–5).

8. In this case, where waves are generated by ACh, the presence of $InsP_3$ will sensitize the $InsP_3$ receptors enabling them to function together with the RYR2s to generate the wave.

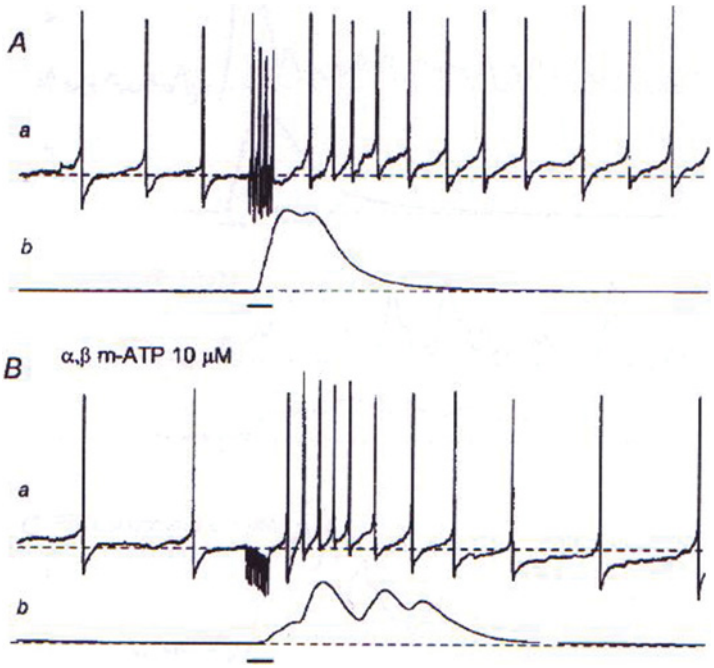
Ureter smooth muscle cell

Transfer of urine from the kidney to the bladder is carried out by the pelviureteric system that has two components, the renal pelvis and the ureter (Module 7: Figure pelviureteric organization). Urine emerging from the kidney tubules is passed via the pelvicalyceal junction (PCJ) to the proximal renal pelvis and then into the distal renal pelvis and finally into the ureter. Urine is propelled through the pelvis and ureter by peristaltic smooth muscle contractions. This peristalsis is initiated in the proximal region and then spreads through the distal renal pelvis and ureter by an action potential that triggers smooth muscle contraction. Within the renal pelvis, a wave of Ca^{2+} sweeps through the typical smooth muscle cells at a rate of approximately 1mm/s (Module 7: Figure renal pelvis SMC Ca^{2+} wave), which is very much faster than classical intercellular Ca^{2+} waves that have an upper propagation rate of about 100 μm/s. In the case of the pelvis, the faster rate depends on the fact that the Ca^{2+} signal in each cell is driven by an action potential that travels through the smooth muscle cell syncitium. With regard to cell signalling in ureter smooth muscle cells, therefore, there are two main questions. First, what is the nature of the pelviureteric pacemaker mechanism that initiates the propagating action potential? Secondly, how does the action potential drive the process of ureter smooth muscle contraction?

Pelviureteric pacemaker mechanism

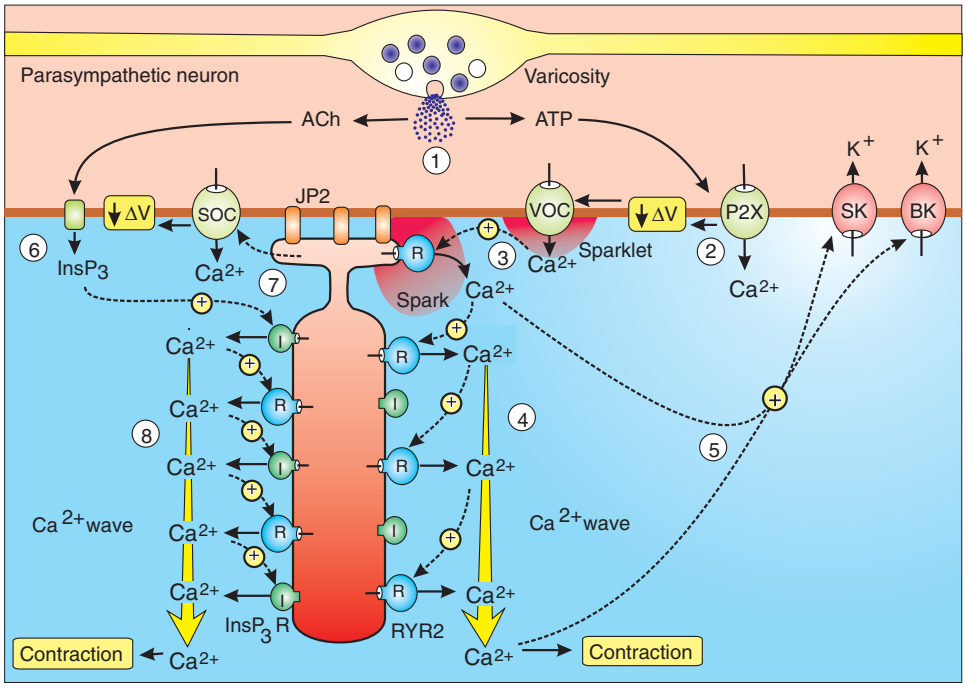
Activation of smooth muscle cells in the renal pelvis and ureter resembles most closely mechanism C in Module 7: Figure SMC activation mechanisms, but with some minor variations. In this mechanism, smooth muscle cells (SMCs) are activated by depolarization produced by current flow from other cells, such as the interstitial cells of Cajal (ICC). In the case of the renal pelvis, where pacemaker activity is located, there are two cell types that could function as pacemakers to drive contraction (Module 7: Figure pelviureteric organization). One is the interstitial cells of Cajal-like cells that resemble the classical interstitial cells of Cajal (ICC) both in their structure and their expression of c-KIT. They are spontaneously active and generate rhythmical action potentials that have very long plateau phases.

Module 7: | Figure neural evoked contractions



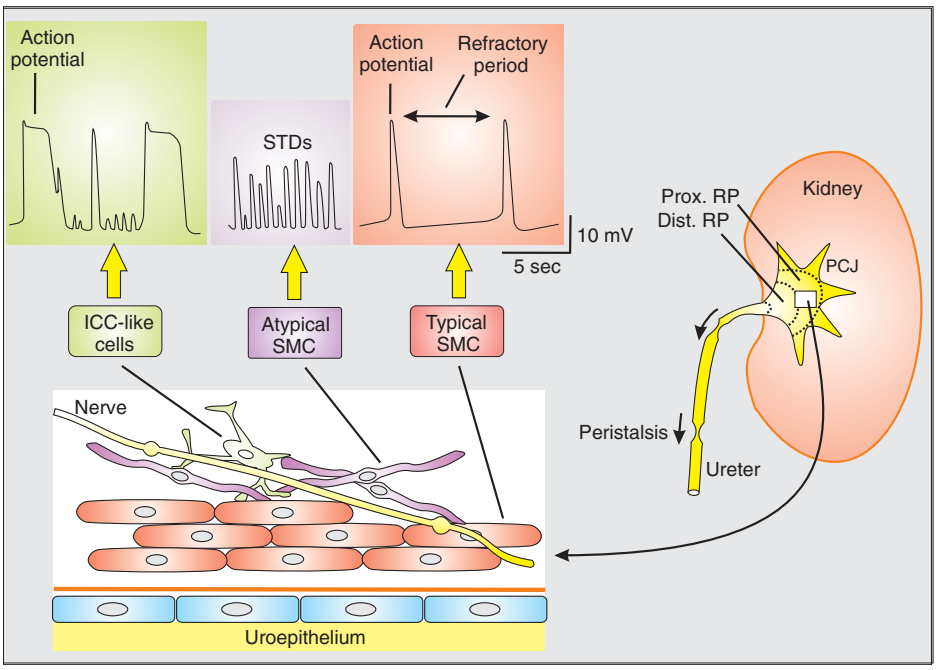
Electrical and mechanical responses to neural stimulation of guinea-pig bladder.
Strips of smooth muscle cells were given a train of stimuli at 20 Hz for 1 s (black bar), which would have released both ATP and acetylcholine (ACh). A. The spontaneous single action potentials (trace a) preceding the stimulation failed to induce a contraction (trace b). During the stimulation period there was a rapid acceleration of action potentials and a large contraction. Following the stimulation the increased rate of action potentials continued for a while. B. The same procedure was repeated in the presence of α,β-methylene ATP, which inhibits ATP to reveal the action of ACh. There were no action potentials during the 1 s stimulus period, but thereafter the membrane depolarized and there was a marked increase in the frequency of action potentials associated with an oscillatory period of contractions. Reproduced from Hashitani et al (2000).

Module 7: | Figure bladder SMC activation



Neural activation of bladder smooth muscle cells (SMCs).
Smooth muscle cells of the bladder are activated by neurotransmitters such as ATP and acetylcholine (ACh) released from varicosity that occur along the axons of the nerves that innervate the bladder. These two transmitters induce increases in Ca^{2+} through two separate pathways as described in the text.

Module 7: | Figure pelviureteric organization



Organization and function of the pelviureteric system.
Urine formed by the kidney tubules is collected by the pelvicalyceal junction (PCJ) and is then passed towards the proximal renal pelvis (Prox. RP), distal renal pelvis (Dist. RP) and then to the ureter. The pelvis and ureter use a peristaltic pumping mechanism to transfer urine towards the bladder. In the pelvic region, this pumping is carried out by the typical smooth muscle cells (SMCs). In addition, there are atypical SMCs and interstitial cells of Cajal-like cells, which appear to function as pacemakers to drive the peristaltic contractions of the typical SMCs. The characteristic electrical activity of these three cells types is described in the text. Information for this drawing was taken from Figures 1 and 11 in Lang et al (2007).

At present, most evidence indicates that they may not be the primary pacemakers. Rather, attention has focused on the atypical smooth muscle cells, which form a loose network lying on top of the smooth muscle layer. These atypical muscle cells produce regular spontaneous transient depolarizations (STDs) that are thought to provide the depolarizing drive that triggers the action potentials to drive the Ca^{2+} waves that spread through the layer of typical smooth muscle cells (Module 7: Figure renal pelvis SMC Ca^{2+} wave).

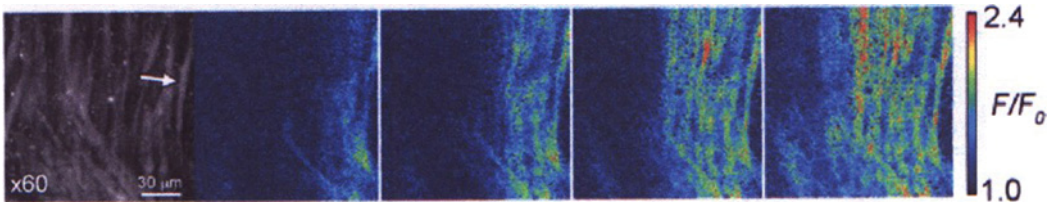
Atypical smooth muscle cells

The atypical smooth muscle cells, which are restricted to the proximal renal pelvis (Prox. RP), are long thin cells that lie on the surface of the typical smooth muscle cells (**Module 7: Figure pelviureteric organization**). The contractile filaments are rather sparse and separated by various organelles such as the Golgi, endoplasmic reticulum and many mitochondria. With regard to their pacemaker role, the atypical cells form numerous gap junctions with the typical smooth muscle cells, which presumably enables them to pass depolarizing current to the smooth muscle cells (see mechanism C in **Module 7: Figure SMC activation mechanisms**). The nature of the pacemaker mechanism in these atypical smooth muscle cells, which depends upon a tonic production of prostaglandins and the release of tachykinins from sensory neurons, is still being worked out but seems to resemble the **ICC cytosolic Ca^{2+} oscillator** (**Module 7: Figure ICC pacemaker**). One important difference is that the oscillations in cytosolic Ca^{2+} do not activate Ca^{2+}-sensitive Cl^- channels in these atypical cells. The spontaneous transient depolarizations (STDs), which create the current flow to excite **ureter smooth muscle contraction**, depend upon the activation of an inward Na^+ current carried by channels that remain to be determined. In addition, there appears to be a role for L-type Ca^{2+} channels that function to synchronize STDs into large enough pacemaker events capable of triggering the action potentials in the typical smooth muscle cells to drive **ureter smooth muscle contraction**.

Ureter smooth muscle contraction

The current flow created by the spontaneous transient depolarizations (STDs) in the atypical smooth muscle cells provides the depolarization drive responsible for activating the periodic action potentials that travel through the typical smooth muscle cells (Module 7: Figure pelviureteric organization). Despite the fact that the atypical smooth muscle cells generate STDs at a high frequency (10–40/min), the action potentials that they initiate in the typical smooth muscle cells occur at a much lower frequency (6–15/min) and this is caused by a prolonged refractory period induced by the Ca^{2+} that enters the cell during the action potential. During the recovery period, much of this Ca^{2+} is sucked up by the endoplasmic reticulum and this loading of the store sensitizes the ryanodine receptors 2 (RYR2s), which immediately begin to generate smooth muscle cell Ca^{2+} sparks. These sparks then activate

Module 7: | Figure renal pelvis SMC Ca^{2+} wave



Ca^{2+} wave in the mouse renal pelvis.
The typical smooth muscle cells of the renal pelvis were loaded with the Ca^{2+} indicator fluo-4. The arrow in the first image points to a single smooth muscle cell. The other four images taken at 66 ms intervals show a wave of Ca^{2+} traveling from the right to the left. Reproduced from Lang et al (2007).

large-conductance (BK) channels; the resulting hyperpolarization prevents the activation of action potentials thus resulting in a refractory period. Once the Ca^{2+} level in the stores declines back to normal, the sparks decline and the membrane can now respond to the depolarizing drive coming from the atypical smooth muscle cells to initiate another action potential.

This action potential then drives the process of smooth muscle cell excitation–contraction coupling (Module 7: Figure smooth muscle cell E-C coupling). To what extent contraction depends upon the entry or the release of internal Ca^{2+} remains to be determined, but it has to be fast to account for the rapid rates of Ca^{2+} wave propagation (Module 7: Figure renal pelvis SMC Ca^{2+} wave) that establish the peristaltic waves necessary to propel urine to the bladder.

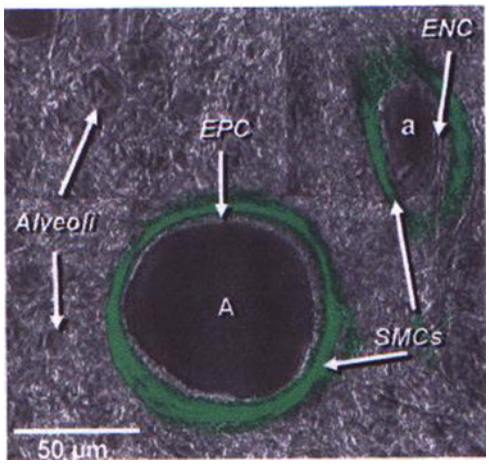
Airway smooth muscle cells

Maximal gas exchange in the lung depends upon a precise matching of ventilation to blood perfusion. Smooth muscle cells (SMCs), which have been coloured green, surround the airways and arterioles that contribute to this gas exchange (Module 7: Figure lung morphology). Contraction of these SMCs can be activated by a number of stimuli (Module 7: Figure bronchiole-arteriole contraction). The lung arterioles, which contracted strongly in response to 5-hydroxytryptamine (5-HT), endothelin-1 (ET-1) and an increase in KCl but not to acetylcholine (ACh), are described in the section on vascular smooth muscle cells. The airway bronchioles responded to ACh and 5-HT. The action of ACh on the muscarinic receptors is modulated by GRK3 and GRK5, which are members of the family of G protein receptor kinases (GRKs). At the single-cell level, these airway smooth muscle cell contractions are driven by oscillations in the intracellular level of Ca^{2+}, which is driven by a smooth muscle cell cytosolic oscillator (Module 7: Figure SMC cytosolic oscillator). Airway smooth muscle cell activation thus conforms to mechanism B in Module 7: Figure SMC activation mechanisms.

Hydrogen sulfide (H_2S) is turning out to be an important bronchodilator of airway smooth muscle cells. The relaxing action of H_2S might depend on it being an inositol 1,4,5-trisphosphate receptor ($InsP_3R$) antagonist (Module 3: Figure $InsP_3R$ regulation). By inhibiting the $InsP_3$-induced release of Ca^{2+}, H_2S reduces the Ca^{2+} oscillations that trigger contraction.

Alterations in airway smooth muscle function are important for a number of respiratory disorders such as asthma.

Module 7: | Figure lung morphology

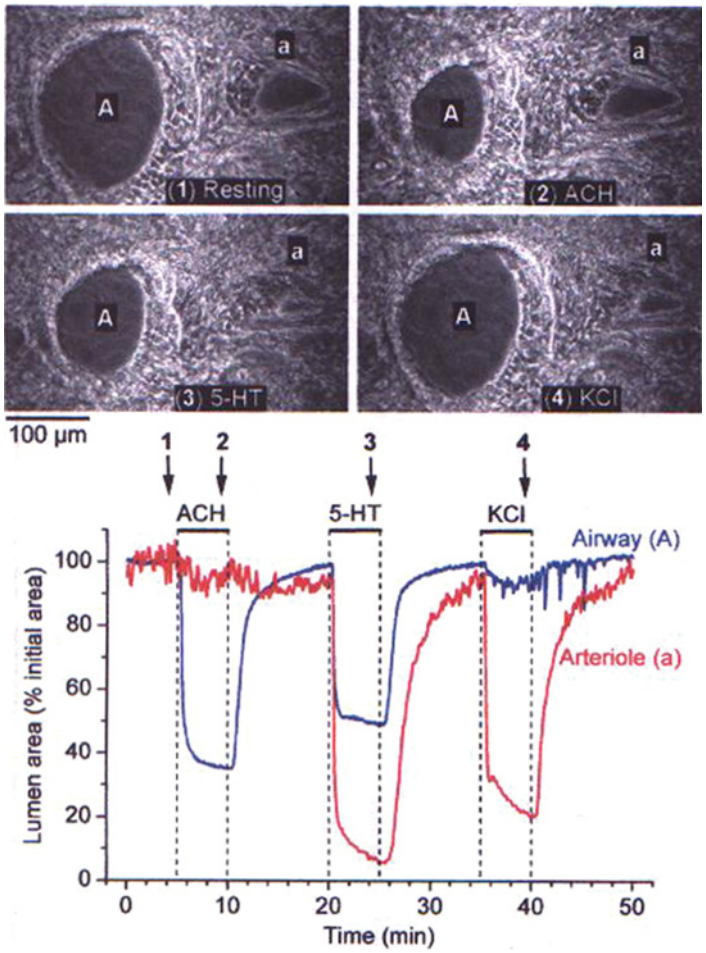


Smooth muscle cell (SMC) location in the lung.
A lung slice was stained with an antibody against smooth muscle cell α-actin 9 (green) and the fluorescent image was superimposed on a phase-contrast image to show the ring of smooth muscle cells (SMCs) that surround both the bronchiole airway (A) and an associated arteriole (a). The airway is lined with epithelial cells (EPC) whereas endothelial cells (ENC) line the arterioles. The response of these two vessels to different agonists is shown in Module 7: Figure bronchiole-arteriole contraction. Reproduced from Perez and Sanderson (2005b).

Vascular smooth muscle cells

The blood vessels that make up the vasculature are surrounded by a layer of smooth muscle cells (SMCs). Vascular SMCs have been studied in a number of different vessels such as mesenteric arteries, cerebral arteries, coronary arteries and arterioles located in the lung. In the case of the lung, the arterioles are located close to the bronchioles (Module 7: Figure lung morphology). The SMCs, which have been coloured green, surround both vessels. Contraction of these SMCs can be activated by a number of stimuli (Module 7: Figure bronchiole-arteriole contraction). The lung arterioles contracted strongly to 5-hydroxytryptamine (5-HT), endothelin-1 (ET-1) and

Module 7: | Figure bronchiole-arteriole contraction

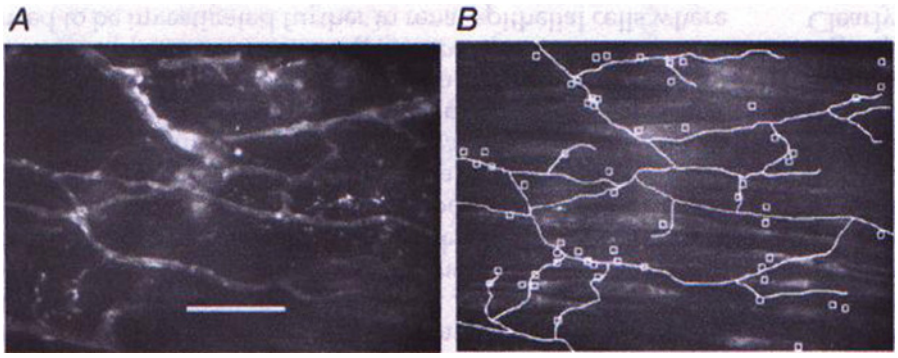


Contraction of an airway and arteriole in the mouse lung.
The panels at the top are phase-contrast images showing the airway bronchiole (A) and arteriole (a) used for the measurements of the luminal area. The lower panel illustrates the changes in luminal area in response 1 µM acetylcholine (ACh), 1 µM 5-hydroxytryptamine (5-HT) and 100 mM KCl. The four arrows indicate the times when the phase-contrast pictures were taken. Reproduced from Perez and Sanderson (2005a).

an increase in KCl but not to acetylcholine (ACh). On the other hand, the airway bronchioles responded to both ACh and 5-HT but not KCl. These experiments illustrate that the SMCs express different receptors and are likely to have slightly different signalling pathways. Despite these differences, most vascular SMCs are responsible for maintaining the myogenic tone of blood vessels. By constricting or dilating the resistance arteries, these SMCs regulate the flow of blood and thus blood pressure. Many of these vascular SMCs have a myogenic vasoconstriction response that enables them to contract when the intraluminal pressure increases and then relax when the pressure falls. Blood pressure is determined by this myogenic tone. In addition, neurotransmitters released from the nerves that innervate the blood vessels can also control these vascular SMCs. Before considering how these various transmitters act to generate muscle contraction, it is appropriate to consider the neural mechanisms responsible for delivering these transmitters on to the surface of the vascular smooth muscle cells.

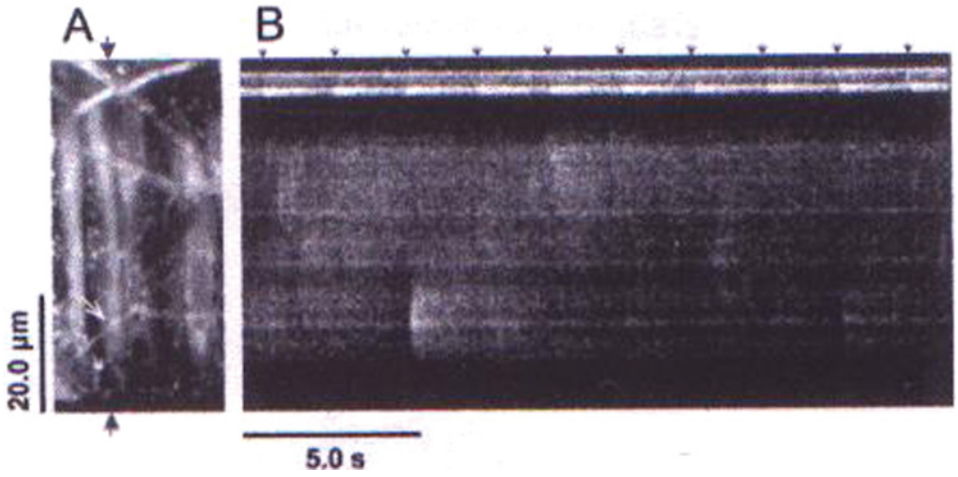
In the case of mesenteric arteries, the perivascular nerves that ramify over the smooth muscle surface (Module 7: Figure arterial nerves) release both ATP and noradrenaline. When these nerves are stimulated, small Ca^{2+} signals appeared in the underlying muscle cells at discrete points close to the nerve tracts (white boxes in panel B Module 7: Figure arterial nerves). These small signals in the muscle cells are called junctional Ca^{2+} transients (jCaTs) and are best seen in spatiotemporal plots (Module 7: Figure junctional Ca^{2+} transient). An image of one of these small jCaTs was captured at 4.8 s in the line-scan image in panel B. The fine line running along the length of the plot is the Ca^{2+} signal in the fine nerve indicated by the white arrow in panel A. In these experiments, the effect of noradrenaline was inhibited by prazosin so the appearance of the jCaTs was the result of ATP acting on the smooth muscle cells to open P2X receptor channels to introduce a local pulse of Ca^{2+} (Module 7: Figure SMC cytosolic oscillator). There is little contraction in response to these small ATP-dependent jCaTs. Larger contractions are seen when prazosin is removed so that the muscle can respond to both ATP and noradrenaline (Module 7: Figure neurogenic contractions). The ATP acts first to produce a small initial contraction that is then followed by a much larger

Module 7: | Figure arterial nerves



Perivascular nerves in rat mesenteric arteries.
This segment of rat mesenteric artery was loaded with the Ca^{2+} indicator fluo-4. Panel A shows the nerve bundles spread over the surface of the smooth muscle layer. These nerves were visualized by focusing 1.2 μm above the level of the smooth muscle cell layer (B). The boxes in B show the location of junctional Ca^{2+} transients (jCaTs) in the smooth muscle cells during nerve stimulation in the presence of prazosin to inhibit activation of the α1-adrenoreceptors. Note that these muscle Ca^{2+} signals are located close to the white lines that indicate the nerves shown in B. When these nerves are stimulated in the absence of prazosin, α1-adrenoreceptor activation induces a global oscillatory Ca^{2+} signal that triggers contraction (Module 7: Figure neurogenic contraction). Reproduced from Lamont et al (2003).

Module 7: | Figure junctional Ca^{2+} transient

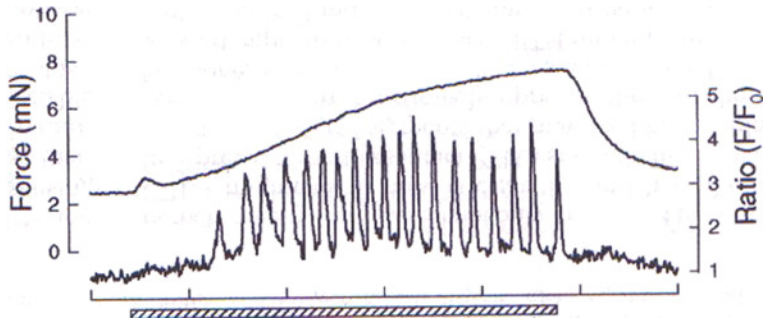


Junctional Ca^{2+} transients in rat mesenteric arteries.
A. This image of the surface of a mesenteric artery illustrates smooth muscle cells lying longitudinally and a number of fine nerves that cross over the surface of the fibres. The two black arrows represent the column of pixels that were scanned repeatedly to create the line-scan image shown in B. The row of black arrowheads at the top of B indicates the times when the nerves were stimulated. The nerves, which accumulate more of the Ca^{2+} indicator, show up as bright lines streaking across the spatiotemporal plot. A junctional Ca^{2+} transient (jCaT) appears at about 5 s along one of these lines that corresponds to a point where a fine nerve crosses a smooth muscle cell (white arrow in A). Reproduced from Lamont and Wier (2002).

contraction when noradrenaline initiates a series of Ca^{2+} transients, which are a characteristic feature of vascular SMC contractility. In the case of the astrocyte regulation of cerebral blood flow, the activity of cerebral arterioles is controlled by factors coming from the astrocytes (Module 7: Figure astrocyte endfoot signalling).

Contractile activity of many vascular SMCs depends on oscillatory Ca^{2+} signals. In response to stimuli such as noradrenaline (NA), 5-hydroxytryptamine (5-HT) and endothelin-1 (ET-1), individual cells are found to generate repetitive Ca^{2+} transients (Module 7: Figure neurogenic contractions). Another example is the rhythmical contractions of cerebral arterioles (Module 7: Figure smooth muscle cell Ca^{2+} oscillations). In some blood vessels, a specific tone is maintained by the spatial averaging of asynchronous oscillations. However, there are some vessels where the oscillations in individual cells are synchronized resulting in pulsatile contractions known as vasomotion. Another important feature of this oscillatory activity is that variations in transmitter concentration are translated into a change in contractile tone through a frequency modulation mechanism (Module 7: Figure frequency modulation of SMCs). Frequency modulation is one of the mechanisms used for the encoding and decoding of Ca^{2+} oscillations. The signalling mechanisms that generate these oscillations conform to mechanism B in Module 7: Figure SMC activation mechanisms. The primary activation mechanism for vascular smooth muscle is a cytoplasmic Ca^{2+} oscillator that generates periodic pulses of Ca^{2+} that then drives contraction. Transmitters operate by

Module 7: | Figure neurogenic contractions



Neural stimulation of contraction in mesenteric arteries.
Neural stimulation of rat mesenteric arteries for 3 min (hatched bar at the bottom) induced the onset of Ca^{2+} oscillations (recorded from a single cell, bottom trace) and an increase in force (upper trace). Reproduced from Lamont et al (2003).

controlling the frequency of the oscillator. Entry of Ca^{2+} also plays an important role in modulating the oscillator. In some cells, the oscillator can alter membrane potential (ΔV) and this can act both as a feed-forward signal and as a way of entraining the oscillators in neighbouring cells to create the synchronicity responsible for vasomotion. The central feature of vascular SMC activation is a smooth muscle cell cytosolic oscillator.

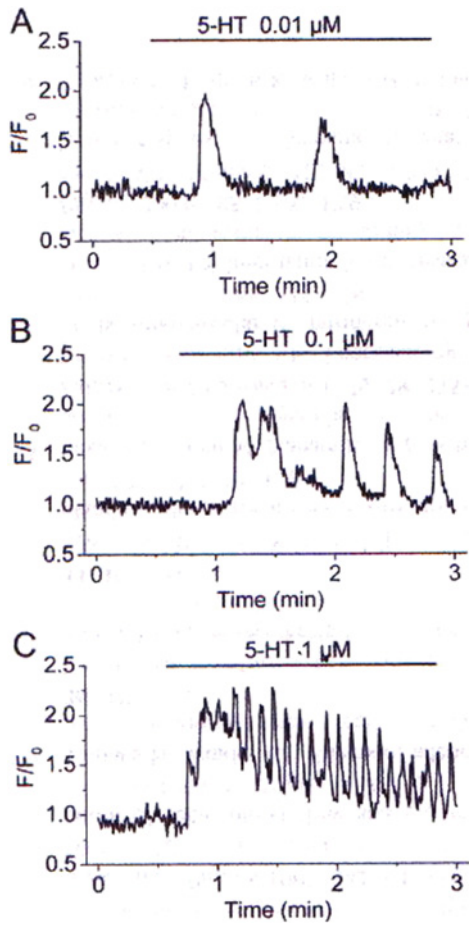
Vascular SMCs thus have sophisticated signalling mechanisms that maintain precise control over blood pressure. Since an alteration in the delicate balance between contraction and relaxation is one of the defects leading to hypertension, there is a clear imperative to understand how these cells are activated.

Smooth muscle cell cytosolic oscillator

A smooth muscle cytosolic Ca^{2+} oscillator drives the contractile activity of both vascular smooth muscle cells, airway smooth muscle cells and perhaps also in the corpus cavernosum smooth muscle cells. The Ca^{2+} oscillation mechanism depends upon a sequential series of events: store loading, spike initiation, spike development and spike recovery (Module 6: Figure Ca^{2+} oscillation model). The way in which these components function in the smooth muscle cell oscillator is illustrated in Module 7: Figure SMC cytosolic oscillator:

1. The operation of this oscillator depends upon the action of transmitters such as acetylcholine (ACh), 5-hydroxytryptamine (5-HT) and noradrenaline (NA) and hormones such as endothelin-1 (ET-1) that act through the $InsP_3/Ca^{2+}$ signalling pathway. The primary action of $InsP_3$ is to increase the sensitivity of the $InsP_3$ receptors (I), which together with the ryanodine receptors (R), are responsible for releasing stored Ca^{2+} that often appears as a cytosolic Ca^{2+} wave. The process of Ca^{2+}-induced Ca^{2+} release (CICR), which is responsible for orchestrating the release of Ca^{2+} from the endoplasmic reticulum depends upon these channels being sensitive to Ca^{2+}. An important determinant of this sensitivity is the luminal concentration of Ca^{2+} and as this builds up the release channels become sensitive to Ca^{2+}.
2. The nature of the mechanism responsible for initiating Ca^{2+} release is unclear. There are suggestions that ryanodine receptors sensitized by store loading initiate the first release event.
3. This initial release of Ca^{2+} is then amplified by regenerative Ca^{2+} release by the other ryanodine and $InsP_3$ receptors to generate the global Ca^{2+} signal that often appears as a wave travelling down the length of the smooth muscle cell.
4. The global Ca^{2+} signal then activates contraction. The recovery phase depends on both the plasma membrane Ca^{2+} ATPase (PMCA), which pumps Ca^{2+} out of the cell, and the sarco/endo-plasmic reticulum Ca^{2+} ATPase (SERCA) that pumps some of the Ca^{2+} back in to the endoplasmic reticulum.
5. Continuation of the cytosolic oscillator depends upon Ca^{2+} entry to provide the Ca^{2+} necessary to re-charge the stores for the next oscillatory cycle. The nature of these entry channels (yellow panel in Module 7: Figure SMC cytosolic oscillator) may vary between cell types and are still being characterized. Some of the main contenders are one of the isoforms of the Ca_V3 family of T-type channels, the Na^+/Ca^{2+}-exchanger (NCX) operating in its reverse mode, the ATP-sensitive P2X receptor responsible for generating the jCaTs (Module 7: Figure junctional Ca^{2+} transient) or various isoforms of the transient receptor potential (TRP) ion channel family such as TRPC3 and TRPC7. These two TRP channels are of interest because they are sensitive to DAG that is formed together with $InsP_3$ during the action of the transmitters that drive the cytosolic oscillator (Module 7: Figure SMC cytosolic oscillator).
6. The entry of external Ca^{2+} functions to charge up the endoplasmic reticulum to sensitize the ryanodine and $InsP_3$ receptors prior to the next phase of release.
7. One of the effects of the released Ca^{2+} is to stimulate Ca^{2+}-sensitive K^+ channels such as the large-conductance (BK) channels and the small-conductance (SK) channels that will lead to membrane hyperpolarization.

Module 7: | Figure frequency modulation of SMCs



Pulmonary artery smooth muscle cell frequency modulation.
Arterial smooth muscle cells in the lung display frequency modulation in response to changes in 5-hydroxytryptamine (5-HT) concentration. Reproduced from Perez and Sanderson (2005b).

8. Another action of Ca^{2+} is to stimulate Ca^{2+}-sensitive chloride channels (CLCA) that result in membrane depolarization to activate voltage-operated channels (VOCs) to introduce Ca^{2+} into the cell resulting in further membrane depolarization (ΔV).
9. This depolarization can spread to neighbouring cells by current flow through the gap junctions and this may provide a synchronization mechanism in those cases where the oscillators are coupled together to provide vasomotion. The membrane depolarization will activate VOCs in the neighbouring cells and thus provide a Ca^{2+} pulse that will initiate Ca^{2+} transients in phase with the cell with the fastest rhythm.

Myogenic vasoconstriction

Myogenic vasoconstriction, which is also known as the Bayliss effect, is a dynamic mechanism that enables small-resistance arterial blood vessels to contract in response to an increase in intraluminal pressure. This response is important for maintaining vascular tone and peripheral vascular resistance that ensures that organs such as the brain and kidney retain a normal perfusion. This myogenic response seems to be carried out by the smooth muscle cells that have a mechanotransduction mechanism that enables them to detect mechanical deformation and to generate the internal signals necessary to activate contraction. It has been proposed that the mechanosensor might be a G protein-coupled receptor (GPCR) such as the angiotensin II AT_1 receptor. In response to mechanical deformation, the GPCR activates $G_{q/11}$, which then stimulates phospholipase β (PLCβ) to generate $InsP_3$ and diacylglycerol (DAG). The DAG then activates the TRPC6 to provide the inward current necessary to depolarize the membrane such that the L-type Ca^{2+} channel can open to bring about contraction.

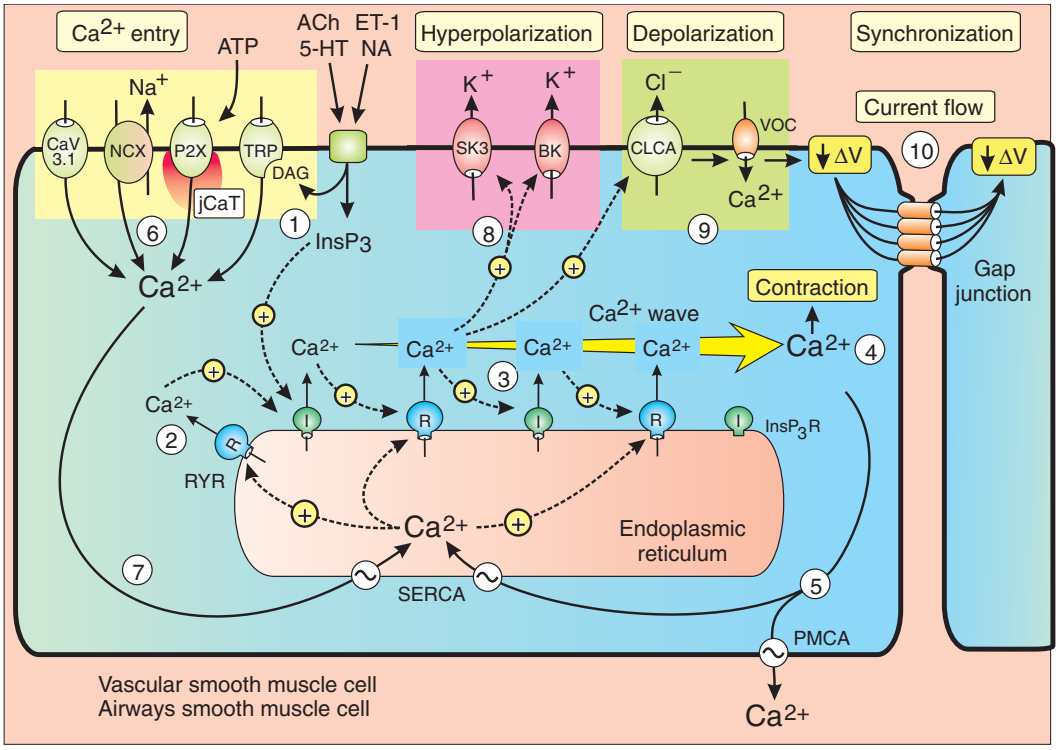
Corpus cavernosum smooth muscle cells

The corpus cavernosum, which are two elongated sponge-like bodies that lie on either side of the penis, fill up with blood during an erection (Module 7: Figure urinogenital tract). The interconnected cavernous spaces, which give the corpora cavernosum a sponge-like appearance, are connected to the penile arteries. Both the arteries and the spaces are lined with smooth muscle cells that regulate the flow of blood into the corpora cavernosum. The penis is flaccid when these muscles are contracted, but becomes rigid when these muscles relax to allow blood to flow into the cavernous spaces. The tunica albuginea that surrounds this erectile tissue is relatively inextensible and resists the increased entry of blood resulting in an increase in pressure within the corpora cavernosa. As the tissue distension continues, the emissary veins that carry blood out of the penis are compressed resulting in further engorgement and penile rigidity. In summary, penile erection is critically dependent on the contractile state of the corpora cavernosum smooth muscle cells.

The corpora cavernosum smooth muscle cells are regulated by a large number of stimuli that determine whether the muscle is relaxed or contracted (Module 7: Figure corpus cavernosum). Like other vascular smooth muscle cells, contraction seems to depend on mechanism B in Module 7: Figure SMC activation mechanisms. The central feature of this mechanism is a smooth muscle cytosolic Ca^{2+} oscillator that resembles that found in both vascular and airway smooth muscle cells (Module 7: Figure SMC cytosolic oscillator). There is some debate as to where this oscillator is located. Interstitial Cells of Cajal (ICC), which have a pacemaker role in other tissues such as the gastrointestinal tract, have been described in the corpus cavernosum but whether they play a direct role in regulating contraction is still an open question. However, the ICC may play an indirect role by providing stimuli such as the eicosanoid prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). In the model depicted in Module 7: Figure corpus cavernosum, the oscillator is located in the corpus cavernosum.

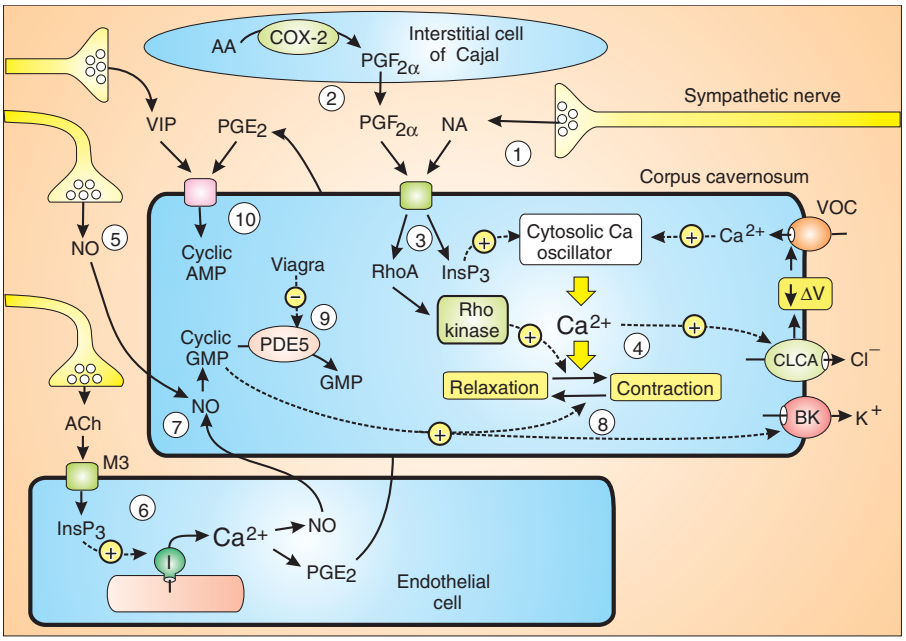
This oscillator generates the pulses of Ca^{2+} that maintains the contractile state (Module 7: Figure corpus cavernosum oscillations). The pulses of Ca^{2+} spread through the muscle cells as fast waves traveling at about 800μm/s. This wave may spread to neighbouring cells by current flow through the gap junctions to synchronize the oscillators of neighbouring cells. The membrane

Module 7: | Figure SMC cytosolic oscillator



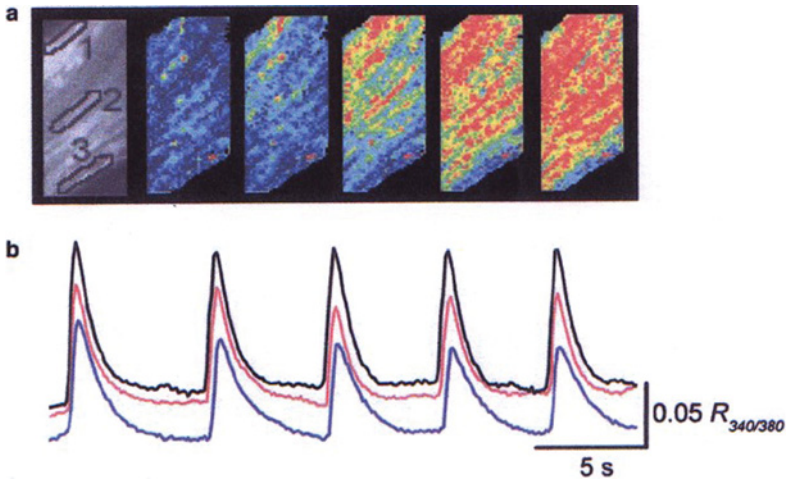
Vascular smooth muscle cell oscillatory mechanism.
Activation of certain smooth muscle cells (SMCs), such as those in the vascular or airways are driven by an endogenous cytosolic oscillatory mechanism that generates periodic pulses of Ca^{2+} that usually appear as a propagating Ca^{2+} wave. The pacemaker is induced/modulated by neurotransmitters such as acetylcholine (ACh), 5-hydroxytryptamine (5-HT), noradrenaline (NA) and endothelin-1 (ET-1), which act through inositol 1,4,5-trisphosphate ($InsP_3$) that is an essential feature of the cytosolic oscillator that generates the Ca^{2+} waves. See text for further details of the pacemaker mechanism and how it is modulated by a variety of ion channels.

Module 7: | Figure corpus cavernosum



Corpus cavernosum signalling pathways.
The corpus cavernosum smooth muscle cells are poised between contraction and relaxation that depend on multiple stimuli released from neurons, endothelial cells and interstitial cells of Cajal. Some of the stimuli such as noradrenaline (NA) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) drive contraction, whereas nitric oxide (NO), prostaglandin E_2 (PGE_2) and vasoactive intestinal peptide (VIP) control relaxation. See text for details of the signalling pathways used by these stimuli.

Module 7: | Figure corpus cavernosum oscillations



Synchronous Ca^{2+} oscillations in the corpus cavernosum.

A meshwork of corpus cavernosum smooth muscle cells loaded with the fluorescent dye fura-2 display regular fluctuations in Ca^{2+}. The response of three cells (black outlines in the first panel in a), which were located 40 μm from each other, reveal the existence of a wave that began in cell 1 (black trace) and then spread to cell 2 (red trace) and cell 3 (blue trace). The delay between each transient was 200 ms, which means that the wave travelled at about 800 μm/s. Reproduced from Hashitani and Suzuki (2004).

depolarization will activate voltage-operated channels (VOCs) in neighbouring cells and thus provide a Ca^{2+} pulse that will initiate Ca^{2+} waves in phase with the cell with the fastest rhythm. The following description provides further details of how this central oscillator is controlled during both contraction and relaxation (Module 7: Figure corpus cavernosum):

Corpus cavernosum contraction (penile detumesence)

1. One of the primary regulators of contraction is the noradrenaline (NA) released from the sympathetic nerves that innervate the corpus cavernosum (Module 7: Figure urinogenital tract).
2. The interstitial cells of Cajal express large amounts of cyclooxygenase 2 (COX-2), which functions in the synthesis of eicosanoids such as the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Module 1: Figure eicosanoids). $PGF_{2\alpha}$ diffuses across to activate the corpus cavernosum smooth muscle cells (Module 7: Figure corpus cavernosum).
3. NA and $PGF_{2\alpha}$ have two main actions. First, they act by generating the second messenger $InsP_3$, which has a major role in activating and maintaining the smooth muscle cytosolic Ca^{2+} oscillator (details of how this oscillator functions is shown in Module 7: Figure SMC cytosolic oscillator). This oscillator produces regular Ca^{2+} transients that sweep through the smooth muscle cells as a rapid wave (Module 7: Figure corpus cavernosum oscillations). Secondly, they can activate the smooth muscle Rho/Rho kinase signalling pathway that serves to increase the Ca^{2+} sensitivity of the contractile machinery (Module 7: Figure corpus cavernosum).
4. The Ca^{2+} transients drive the corpus cavernosum contraction responsible for penile detumesence. The transients also activate Ca^{2+}-sensitive chloride channels (CLCA) that result in membrane depolarization to activate voltage-operated channels (VOCs), which not only introduce Ca^{2+} to modulate the oscillator but also creates a flow of current to entrain the oscillatory activity of neighbouring cells to account for the way these corpora cavernosa cells contract in near unison with each other.

Corpus cavernosum relaxation (penile erection)

5. One of the major stimuli for relaxation is nitric oxide (NO) derived from nonadrenergic noncholinergic (NANC) neurons.
6. NO is also synthesized by the endothelial cells following stimulation by acetylcholine (ACh) released from the parasympathetic neurons. The ACh acts through muscarinic M3 receptors to stimulate $InsP_3$ formation and the release of Ca^{2+}, which then activates endothelial nitric oxide synthase (eNOS) to produce NO (Module 2: Figure eNOS activation).
7. NO released from the NANC neurons and endothelial cells diffuses into the muscle cells to activate the nitric oxide (NO)/cyclic GMP signalling pathway (Module 7: Figure corpus cavernosum).
8. Cyclic GMP induces relaxation by acting on various targets as illustrated in the section on NO/cyclic GMP and smooth muscle relaxation (Module 7: Figure smooth muscle cell cyclic GMP signalling).
9. The action of cyclic GMP is terminated by PDE5, which is the target of Viagra (Module 7: Figure corpus cavernosum).
10. Prostaglandin E_2 (PGE_2), which is also synthesized in the endothelial cells in response to cholinergic stimulation, and vasoactive intestinal peptide (VIP) released from neurons act by increasing the formation of cyclic AMP that can also promote relaxation,

but the mechanisms are less well understood than those mediated by cyclic GMP.

Alterations in the signalling systems that control the contractile activity of corpus cavernosum smooth muscle cells are responsible for erectile dysfunction.

Gastrointestinal smooth muscle cells

The intestine and stomach that make up the gastrointestinal tract maintain a peristaltic rhythm. The waves of contraction are driven by a pacemaker mechanism located within the Interstitial cells of Cajal (ICC) (Module 7: Figure ICC morphology). These ICC cells have characteristic positions within the gastrointestinal tract (Module 7: Figure ICC subtypes). The stomach and intestinal smooth muscle ICCs have smooth muscle activation mechanisms that depends on the ICC generating slow waves of membrane depolarization that spread through the gap junctions to drive the periodic contractions (Module 7: Figure SMC activation mechanisms). There are two main components of the pacemaker mechanism: an ICC cytosolic Ca^{2+} oscillator, which generates the slow waves, and the modulation of ICC pacemaker activity by hormonal and neural stimuli (Module 7: Figure ICC pacemaker).

A remarkable feature of the ICC network is its ability to co-ordinate the contractile activity of large groups of smooth muscle cells by synchronizing their individual oscillators (Module 7: Figure ICC network synchrony). This synchronicity, which has been referred to as a phase wave, might be achieved by coupling oscillators together through a depolarizing signal spreading through the gap junctions (Module 7: Figure ICC coupling). The nature of this synchronicity and how the separate oscillators are coupled together is described in the section on phase waves.

Urethral smooth muscle cells

Urethral smooth muscle cells control the internal urethral sphincter located at the base of the bladder (Module 7: Figure urinogenital tract). There is a reciprocal relationship between the operation of the bladder and the urethra. During urine storage, the detrusor smooth muscle cells of the bladder are relatively relaxed whereas the urethral smooth muscle cells are contracted to close off the sphincter to prevent urine leaving the bladder. These roles are reversed when urine is voided, the urethra relaxes and the bladder contracts. These different functions are under neural control. Like the bladder, the urethra receives a parasympathetic (cholinergic) and a sympathetic (adrenergic) innervation. The latter releases noradrenaline (NA) responsible for maintaining the contractile tone whereas acetylcholine (ACh) released from the parasympathetic nerves provides the nitric oxide (NO) that mediates relaxation.

The neurogenic-dependent contractile tone of the urethral smooth muscle is a dynamic process driven by regular oscillations in the level of intracellular Ca^{2+}. These oscillations depend on a pacemaker mechanism located within a population of interstitial cells of Cajal (ICC) that are connected to the smooth muscle cells by gap junctions. The ICC come in two shapes, bipolar and multipolar (Module 7: Figure ICC morphology). The urethral smooth muscle thus have smooth muscle activation mechanisms that depend on the ICCs generating slow waves of membrane depolarization that spread through the gap junctions to drive the periodic contractions (mechanism C in Module 7: Figure SMC activation mechanisms). When Ca^{2+} is recorded simultaneously in these two cells, each ICC slow wave is associated with a Ca^{2+} transient in the urethral smooth muscle cell (Module 7: Figure ICC–SMC synchronization). There are two main components of the pacemaker mechanism that drives these slow waves: an ICC cytosolic Ca^{2+} oscillator, which generates the slow waves and the modulation of ICC pacemaker activity by hormonal and neural stimuli (Module 7: Figure ICC pacemaker). Activation of this pacemaker by noradrenaline induces the urethral cells to contract to close off the sphincter to prevent urine from escaping from the bladder. Stress urinary incontinence may result from a defect in the control of this closure mechanism.

Relaxation of contraction during bladder emptying depends both on the sympathetic nerves being switched off to reduce the release of noradrenaline and the activation of the parasympathetic nerves that increase the release of NO. The latter then acts through the nitric oxide (NO)/cyclic GMP and smooth muscle relaxation mechanisms (Module 7: Figure smooth muscle cell cyclic GMP signalling).

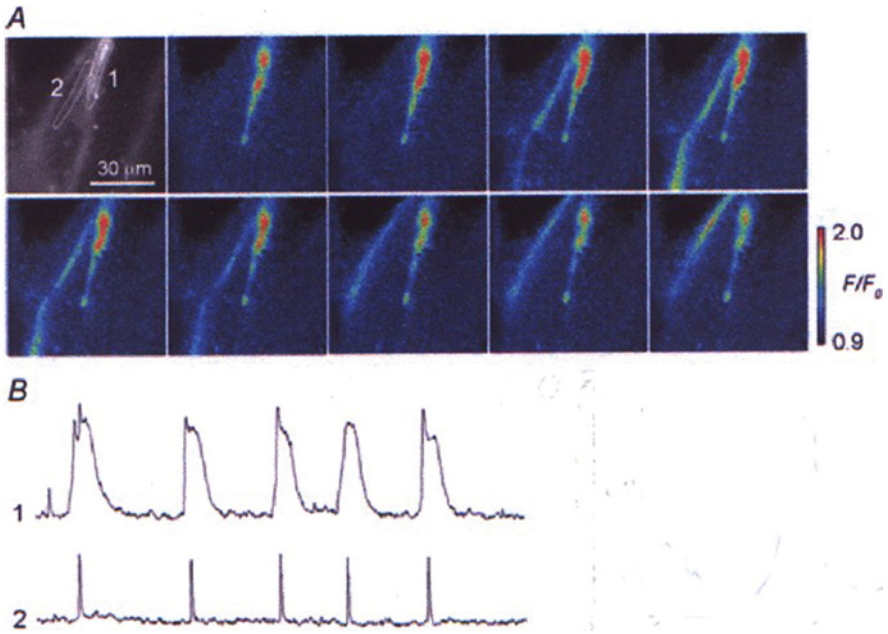
Interstitial cells of Cajal

The Spanish histologist Ramon y Cajal first described the elongated cells that are located in many organs and have been well-characterized in various smooth muscles. These Interstitial Cells of Cajal (ICC) are particularly important in the gastrointestinal tract and urethra where they function as pacemaker cells to provide one of the major smooth muscle activation mechanisms (see C in Module 7: Figure SMC activation mechanisms). The ICC has two distinct morphologies, bipolar and multipolar (Module 7: Figure ICC morphology). Since the bipolar ICC closely resemble the spindle-shaped smooth muscle cells, it has proved difficult to distinguish the two cell types *in situ*. However, the finding that the ICC can be picked out by using an antibody against the Kit receptor has greatly facilitated their identification in the gastrointestinal tract, urethra, lymphatic system, prostate and fallopian tube.

The ICC have characteristic distributions within smooth muscle. Much of the work on these cells has been done on the gastrointestinal tract where the terminology of the different cell types is based on their characteristic regional locations (Module 7: Figure ICC subtypes):

- ICC-SM: the ICC of submucosa (ICC-SM) are multipolar cells that have a number of branches that divide to form projections that interact with those of neighbouring ICC-SM to form a network. They are located in the connective tissue that separates the mucosal and inner circular muscle layers in the pyloric region of the stomach.
- ICC-SMP: The ICC of the submucosal plexus (ICC-SMP) in the colon resembles the ICC-SM in the stomach.

Module 7: | Figure ICC–SMC synchronization



Simultaneous recording of Ca^{2+} transients in a urethral interstitial cell of Cajal (ICC) and a smooth muscle cell (SMC).
A. The first panel shows the location of an ICC (cell 1) and a smooth muscle cell (cell 2) in a rabbit urethral preparation. The remaining frames taken at 0.2 s intervals illustrate the Ca^{2+} response of the ICC slightly preceding that of the smooth muscle cell. B. The two traces illustrate the close temporal relationship between the ICC (1) and smooth muscle (2) responses. The Ca^{2+} transient in the smooth muscle is much briefer than that in the ICC. Reproduced from Hashitani and Suzuki (2007).

- ICC-DMP: the ICC of the deep muscular plexus (ICC-DMP) of the small intestine are multipolar, but their branches tend to be orientated along the circumference where they associate with the nerve bundles of circular muscle cells.
- ICC-CM: the ICC of the circular smooth muscle (ICC-CM) are also know as intramuscular ICC (ICC-IM) because these bipolar cells intermingle with the circular smooth muscle cells. They tend to lie along the main axis and are often closely associated with nerve bundles (see panels c and d in Module 7: Figure ICC tissue location).
- ICC-MY: the ICC of the myenteric plexus (ICC-MY) are bipolar cells with multiple branches that form a network (see panel 2a in Module 7: Figure ICC tissue location) located in the region between the circular and longitudinal muscle layers.
- ICC-LM: the ICC of longitudinal muscle (ICC-LM) resemble the ICC-CM and are also referred to as ICC-IM because they too intermingle with the smooth muscle cells as do the ICC-CM (Module 7: Figure ICC subtypes).

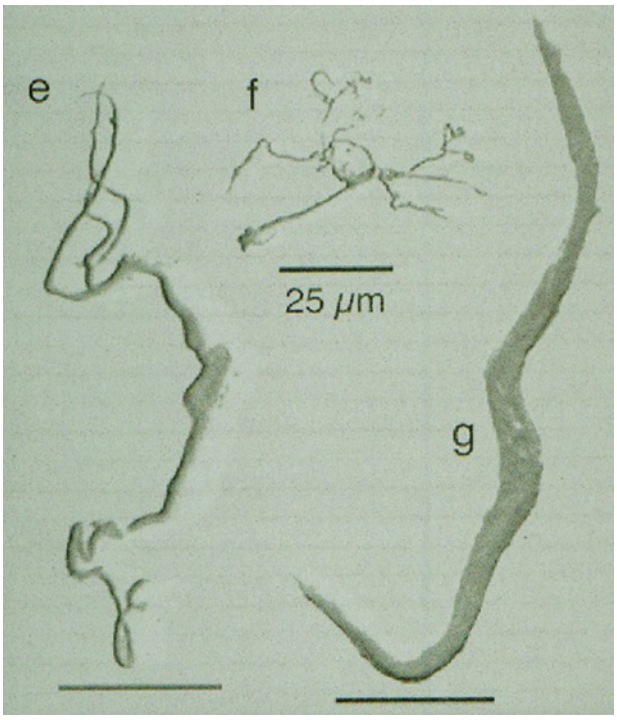
It remains to be determined whether these ICC subtypes, as defined by their tissue locations, are functionally different. There are indications that ICC can carry out a variety of functions. They are pacemaker cells responsible for activating many smooth muscle cells. As part of this pacemaker activity they also function to propagate electrical activity to the smooth muscle cells. The close association between ICC and neurons supports physiological evidence that the ICC can also function as neuroeffector cells to receive and to relay excitatory and inhibitory signals to the smooth muscle cells (see C in Module 7: Figure SMC activation mechanisms).

The ICC pacemaker activation mechanism generates the stimuli that maintain repetitive smooth muscle cell contractility. This activation process depends upon an ICC cytosolic Ca^{2+} oscillator that generates periodic pulses of Ca^{2+}, which are known as slow waves, that then activates membrane channels that carry inward currents. The resulting current flow and depolarization spreads through the gap junctions to the smooth muscle cells. Since the ICC form an interconnected network that extends through the smooth muscle cell layer, they can co-ordinate the activity of large populations of smooth muscle cells to function in near synchrony. This co-ordinated activity is evident in the form of the peristaltic waves of contraction that spread through the gastrointestinal tract. It is important to emphasize, however, that this excitation is not propagated through an action potential because the ICC are not excitable. Rather, the near synchronous contraction of large populations of smooth muscle cells, which has been referred to as a phase wave, depends on the synchronization of the ICC cytosolic Ca^{2+} oscillators located in each of the ICC that make up the excitatory network.

ICC pacemaker activation mechanism

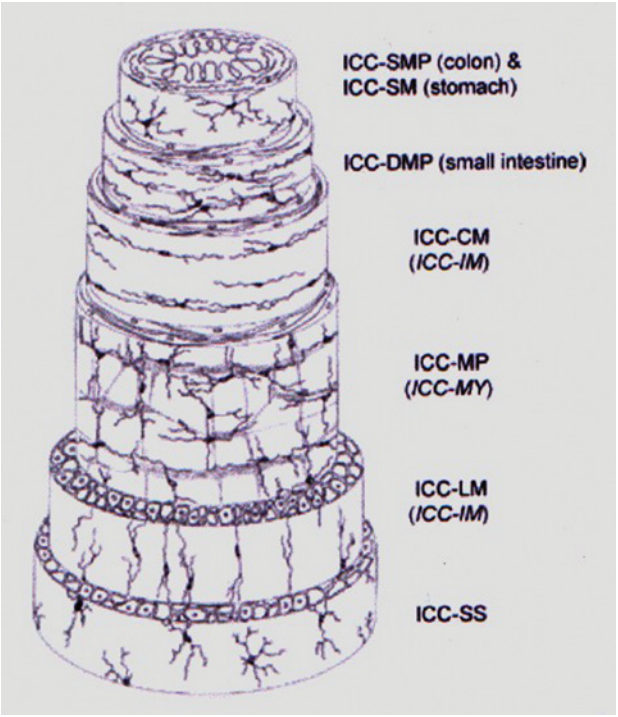
Studies on ICC in both the gastrointestinal tract and urethra have indicated that pacemaker activity depends upon a typical cytosolic oscillator (Module 6: Figure membrane and cytosolic oscillator). The oscillator in the ICC provides the depolarizing drive responsible for triggering

Module 7: | Figure ICC morphology



Urethral interstitial cells of Cajal (ICC) and smooth muscle cell shapes. The interstitial cells of Cajal (ICC) (e and f) have two basic shapes: bipolar (e) or multipolar (f). Smooth muscle cells (g) are long and thin and thus resemble the bipolar ICC. Reproduced from Sergeant et al. (2000).

Module 7: | Figure ICC subtypes



Location of ICC subtypes in the gastrointestinal tract. The interstitial cells of Cajal (ICC) are either bipolar or multipolar cells that have characteristic locations within the gastrointestinal tract. The morphology of the two ICC types (bipolar or multipolar) is shown in Module 7: Figure ICC morphology. Reproduced from Komuro (2006).

smooth muscle cell contraction (see C in Module 7: Figure SMC activation mechanisms). There are two main components of this pacemaker mechanism: the ICC cytosolic Ca^{2+} oscillator, which provides the depolarization to activate smooth muscle contraction and the modulation of ICC pacemaker activity by hormonal and neural stimuli (Module 7: Figure ICC pacemaker).

ICC cytosolic Ca^{2+} oscillator

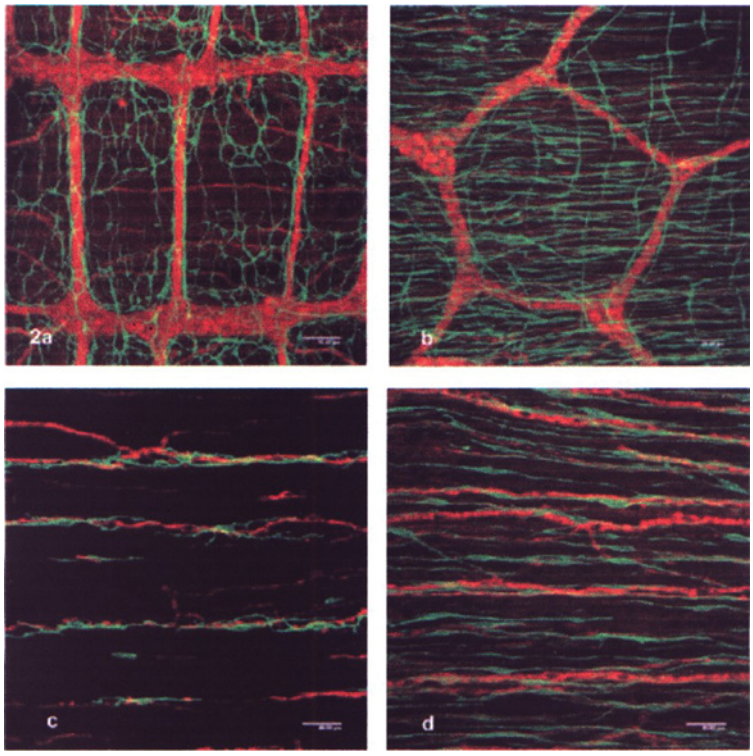
The following hypothesis, which attempts to integrate the main features of this ICC oscillator, is based on the basic mechanism of Ca^{2+} oscillations with a few additional features. This ICC cytosolic Ca^{2+} oscillator resembles that of the smooth muscle cell cytosolic oscillator but there are enough differences for them to be described separately. These differences may well relate to how they function in the activation process. The muscle oscillator provides the internal Ca^{2+} signal that triggers contraction (Module 7: Figure SMC cytosolic oscillator), whereas the ICC oscillator is designed to generate a depolarizing signal to activate a local population of muscle cells (Module 7: Figure ICC pacemaker).

The basis of this ICC oscillator is that Ca^{2+} is periodically released from $InsP_3$-sensitive stores (Module 6: Figure Ca^{2+} oscillation model). The timing of each release depends upon the entry of external Ca^{2+}, which loads up the stores resulting in the gradual sensitization of the internal release channels such as the ryanodine receptors (RYR) and possible the $InsP_3$ receptors to a point where they trigger the next phase of Ca^{2+} release. The enhanced sensitivity of the channels prior to each release is often accompanied by the appearance of Ca^{2+} puffs, which then coalesce to form a global Ca^{2+} signal that activates the repetitive membrane depolarizations that constitute the slow wave.

This basic oscillatory model has a central role in driving ICC pacemaker activity as outlined in the following sequence of events (Module 7: Figure ICC pacemaker):

1. There is little information on what initiates the oscillatory cycle. In many other cell types, such oscillations are induced by stimuli acting to increase the formation of $InsP_3$, but in the case of ICC such an input of this messenger seems to be part of the modulatory mechanism (see Step 11). The most likely possibility is that the onset of each transient is a stochastic process that is triggered by the loading of the store that then sensitizes the release channels. The resting activity of $InsP_3$ may be sufficient to maintain oscillatory activity. In addition, the ryanodine receptor 3 (RYR3), which is also dependent on store loading, may also provide an initial Ca^{2+} pulse to activate the $InsP_3$ receptor-dependent cascade.
2. Once the release process begins, it spreads through the Ca^{2+}-induced Ca^{2+} release (CICR) process to initiate a Ca^{2+} wave.
3. The wave terminates when release stops, perhaps because the decrease in Ca^{2+} concentration within the store inactivates the release channels, and Ca^{2+} is then

Module 7: | Figure ICC tissue location

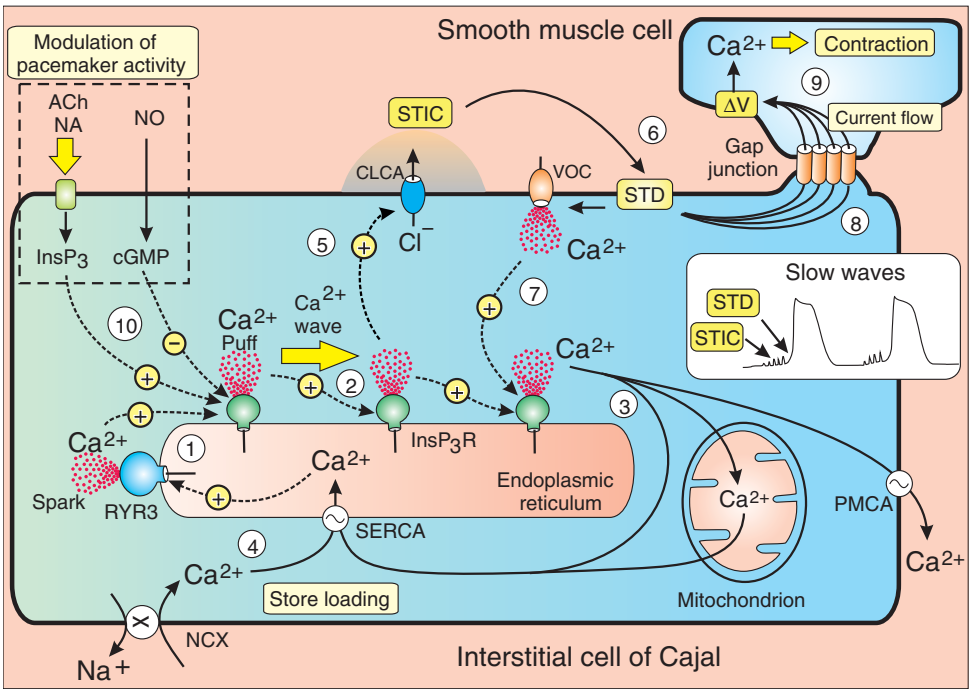


Location of ICC in the guinea-pig gastrointestinal tract.
Immunohistochemical techniques were used to locate ICCs (green fluorescence) and nerves (red fluorescence). a. A network of ICC-MY located in the myenteric plexus of the small intestine. Some of the ICC are closely associated with the regular neural network. b. Most of the ICC-IM run along the smooth muscle cells orientated in the horizontal direction. A few ICC-IM in the longitudinal muscle are shown running in the perpendicular direction. c. ICC-IM closely associated with nerve tracts located in the circular muscle of the small intestine. d. ICC-CM located in the corpus region of the stomach. Note how many of the ICC-IM run in parallel with nerve fibres. Reproduced from Komuro (2006).

extruded from the cell by the plasma membrane Ca^{2+} ATPase (PMCA) and is taken up by the mitochondria or returned to the ER by the SERCA pump. The mitochondria play an important role in maintaining the activity of the oscillator by ensuring that a sufficient amount of the Ca^{2+} that is released during each transient is returned to the ER.

4. During the recovery period, Ca^{2+} is pumped back into the ER by the sarco/endo-plasmic reticulum Ca^{2+} ATPase (SERCA) to reload the store. The entry of external Ca^{2+} is of central importance to load up the stores and this accounts for why ICC Ca^{2+} oscillations are critically dependent on the external concentration of Ca^{2+} (Module 7: Ca^{2+}-dependent ICC oscillations). Note that when the concentration of external Ca^{2+} was either elevated (trace A) or lowered (trace B) the baseline level of Ca^{2+} increased or decreased respectively. Such marked changes in the resting level of Ca^{2+} are indicative of a high resting level of Ca^{2+} entry, which is essential for maintaining oscillatory activity. The nature of the ICC Ca^{2+} entry mechanisms is still being determined. In the case of the urethral ICC, entry might be maintained by the Na^{+}/Ca^{2+} exchanger (NCX) operating in its reverse mode (Module 7: Figure ICC pacemaker), but there may be other entry mechanisms.
5. The Ca^{2+} that is released during each transient activates Ca^{2+}-sensitive chloride channels (CLCAs) that set up the slow waves of membrane depolarization (see inset in Module 7: Figure ICC pacemaker). The early Ca^{2+} puffs generate the spontaneous transient inward currents (STICs) that occur during the pacemaker depolarization.
6. Summation of these STICs then results in the spontaneous transient depolarization (STD) that initiates each slow wave (see panel in Module 7: Figure ICC pacemaker).
7. In many cases, this initial pacemaker depolarization provided by the STD is enhanced by the opening of a voltage-operated channel (VOC), which accounts for the rapid rising phase observed for many slow waves. Opening of the VOCs is not essential for the operation of this ICC oscillator, which can continue when the channels are inhibited by nifedipine (Module 7: Figure ICC slow waves). However, this additional depolarizing and the resulting entry of external Ca^{2+} has two important functions. First, it can provide trigger Ca^{2+} to increase the release of Ca^{2+} from the internal stores distributed down the length of the long thin ICC. If the recruitment of all the release channels depended solely on CICR, the rate of rise of the slow wave would be much slower. This is evident when the

Module 7: | ICC pacemaker



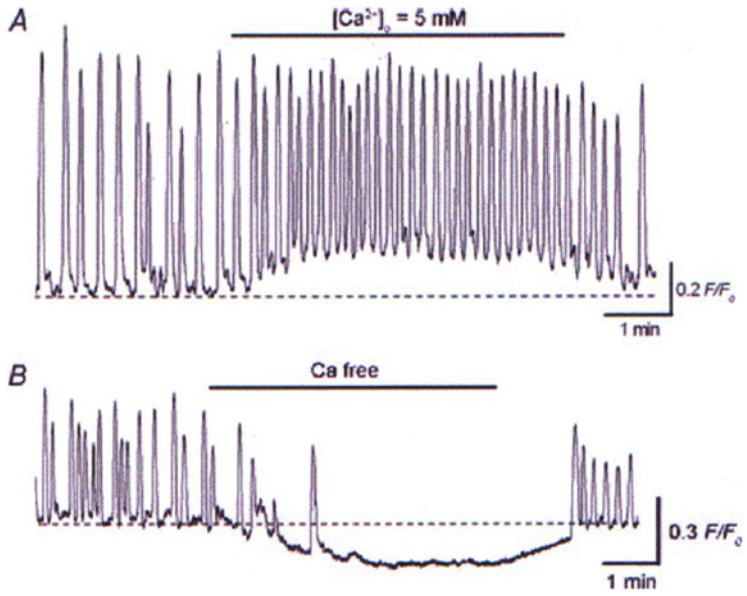
Cytosolic Ca^{2+} oscillator responsible for pacemaker activity in interstitial cells of Cajal.

Interstitial cells of Cajal (ICC) release periodic pulses of Ca^{2+} that begin as puffs and progress into global Ca^{2+} waves. Each puff activates a Cl^- channel to give a spontaneous transient inward current (STIC) that sum to form the spontaneous transient depolarizations (STD). The STD spreads into neighbouring smooth muscle cells through current flow through gap junctions to activate contraction. See text for a description of the oscillator that drives this activation process.

opening of the VOCs is prevented by depolarizing the membrane progressively by raising the external concentration of potassium (Module 7: Figure K^+ slows ICC pacing). At 20 mM K^+, the amplitude of each transient is decreased and there is a very slow rate of rise and this can be accounted for by the fact that the ICC have long thin extensions and it probably takes time for the Ca^{2+} wave to engulf the whole cell. The opening of the VOC thus serves to sharpen the wave front by speeding up the recruitment of the intracellular release channels located down the length of the long ER store. This sharpening of the release phase relates to the second role for this rapid depolarization which is to drive the phase wave that synchronizes oscillatory activity throughout the ICC network. The next question to consider is how this ICC slow wave carries out its pacemaker role to activate smooth muscle contraction.

8. The pacemaker slow wave generated in the ICC provides the activation stimulus to depolarize smooth muscle cells to produce the Ca^{2+} transient that triggers contraction (see C in Module 7: Figure SMC activation mechanisms). Transfer of the activation stimulus from ICC to smooth muscle cell is achieved through a passive flow of current through the gap junctions (Module 7: Figure ICC pacemaker). Since there are multiple gap junctions between the ICC and the smooth muscle cells, current flow in one cell will rapidly spread to its neighbours. During the pacemaker slow wave, therefore, the inward current flow that discharges the membrane of the ICC simultaneously draws current from the smooth muscle cells that also begin to depolarize (Module 7: Figure ICC pacemaker).
9. Smooth muscle cell contraction begins when this depolarization reaches the activation threshold for the L-type Ca^{2+} channels that open to initiate the process of smooth muscle cell excitation–contraction coupling (Module 7: Figure smooth muscle cell E-C coupling). The relationship between the Ca^{2+} transient in the ICC, which initiates the slow wave, and the resulting Ca^{2+} transient in the smooth muscle cell is illustrated in Module 7: Figure ICC-SMC synchronization. There is a striking difference in the time course of the two responses. The ICC Ca^{2+} response (cell 1) lasted very much longer than the brief Ca^{2+} transient in the smooth muscle cell (cell 2). The functional significance of this difference may relate to the way in which ICC have to transmit their pacemaker signal to the smooth muscle cells. Since the ICC network is not excitable, it has to transmit its message through a passive electrotonic mechanism that decays rapidly as it spreads into the large population of smooth muscle cells. By generating a prolonged Ca^{2+} transient, the ICC maximizes its chances of spreading a meaningful signal to the smooth muscle cells. Another way of maximizing pacemaker activity is for all the ICC within the network to operate together and this synchronization of oscillatory activity creates a phase wave.

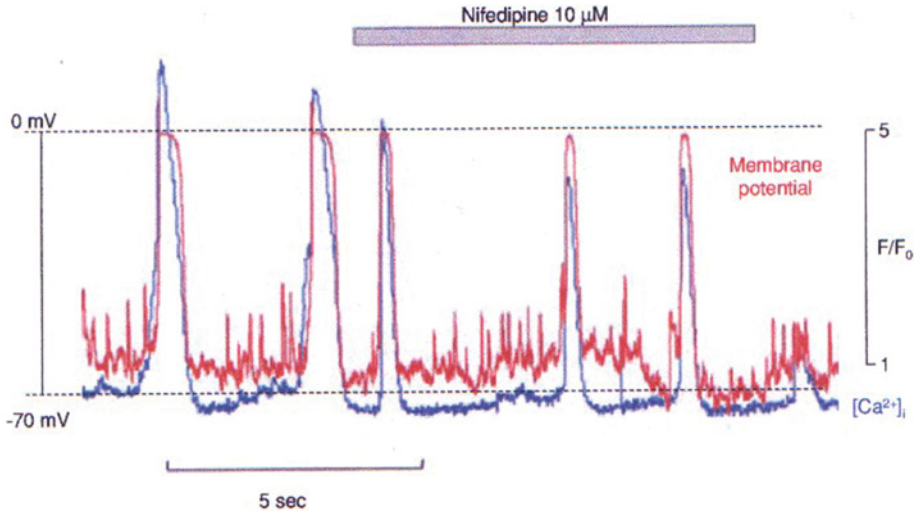
Module 7: | Figure Ca^{2+}-dependent ICC oscillations



ICC Ca^{2+} oscillations depend on the entry of external Ca^{2+}.

A. Spontaneous Ca^{2+} oscillations of rabbit urethral interstitial cells of Cajal (ICC) are accelerated by an elevation of external Ca^{2+} from 2.5 to 5 mM. B. A decrease in external [Ca^{2+}] from 2.5mM to a nominally Ca^{2+}-free solution resulted in a cessation of oscillatory activity. Note the changes in the baseline (resting level of Ca^{2+}) that occurs during the changes in oscillation frequency. Reproduced from Hashitani and Suzuki (2007).

Module 7: | Figure ICC slow waves



Simultaneous recording of Ca^{2+} transients and membrane potential slow waves.

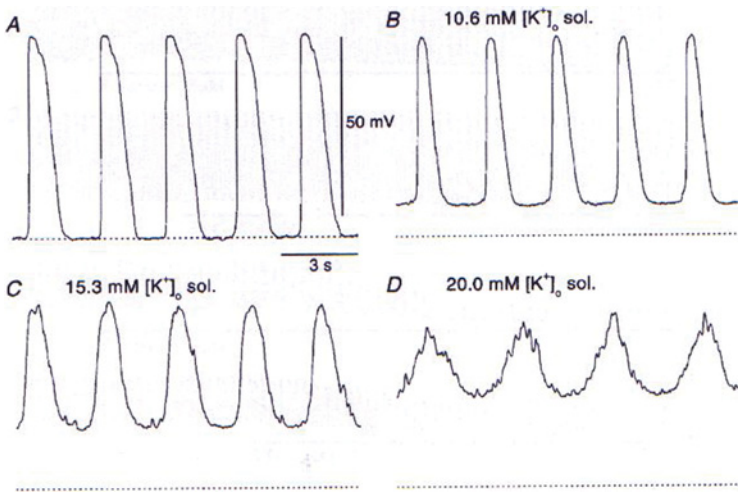
The intracellular Ca^{2+} transients (red trace) recorded in urethral cells coincide with the slow wave fluctuations in membrane potential (blue trace). Pacemaker activity continued in the presence of nifedipine that inhibits voltage-operated Ca^{2+} channels. The brief depolarizations that precede each slow wave correspond to the Ca^{2+}-dependent spontaneous transient inward currents (STICs) shown in Module 7: Figure ICC pacemaker. Reproduced from Seargent et al. (2006).

10. Agonists, such as acetylycholine (ACh) and noradrenaline (NA), and nitric oxide (NO) are responsible for the modulation of ICC pacemaker activity.

Modulation of ICC pacemaker activity

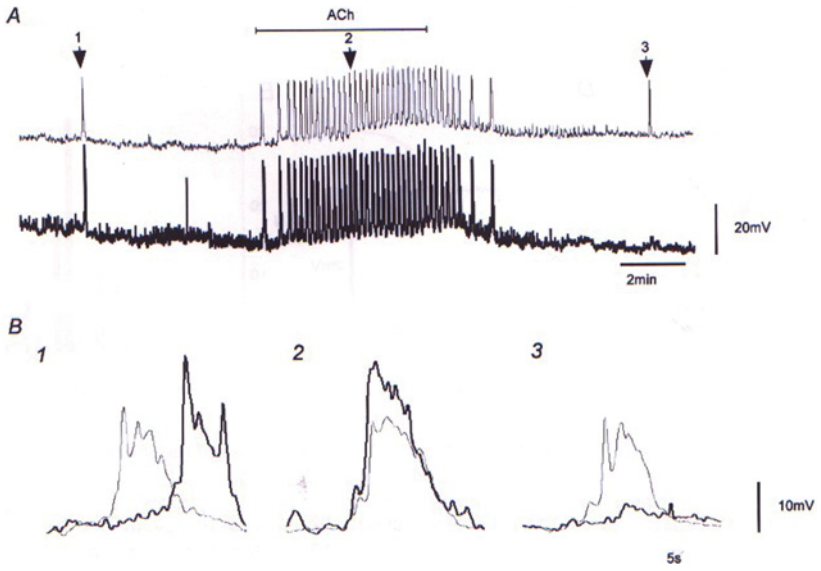
Pacemaker activity can be modulated by the neural input that forms synaptic junctions with the ICC cells (Module 7: Figure SMC activation mechanisms). Many of the neurotransmitters released on to the ICC, such as acetylcholine (ACh) and noraderenaline (NA), accelerate oscillator frequency (Module 7: Figure ICC modulation). In the absence of ACh, there are very few transients but after the addition of 100 nM ACh there is a reversible acceleration of frequency. These transmitters act through the phosphoinositide signalling pathway to increase the formation of $InsP_3$ that then increases the sensitivity of the $InsP_3$ receptors to the stimulatory action of Ca^{2+} (Step 11 in Module 7: Figure ICC pacemaker). Conversely, inhibitory nerves that release nitric oxide (NO) had the opposite effect of reducing both the amplitude and the frequency

Module 7: | Figure K^+ slows ICC pacing



Depolarization alters the ICC pacemaker waveform.
The pacemaker of ICC in the mouse small intestine is altered as the resting membrane potential is depolarized by increasing the concentration of K^+ in the bathing medium. Note the progressive decline in frequency, amplitude and rate of rise of the transients. The rate of rise indicates the rate at which the wave spreads along the long thin ICC cells. As the membrane depolarizes, the contribution of the voltage-operated channels (VOCs) to synchronize the internal release declines thus slowing down the Ca^{2+} waves and hence the rate of rise. Reproduced from Kito and Suzuki (2003).

Module 7: | Figure ICC modulation



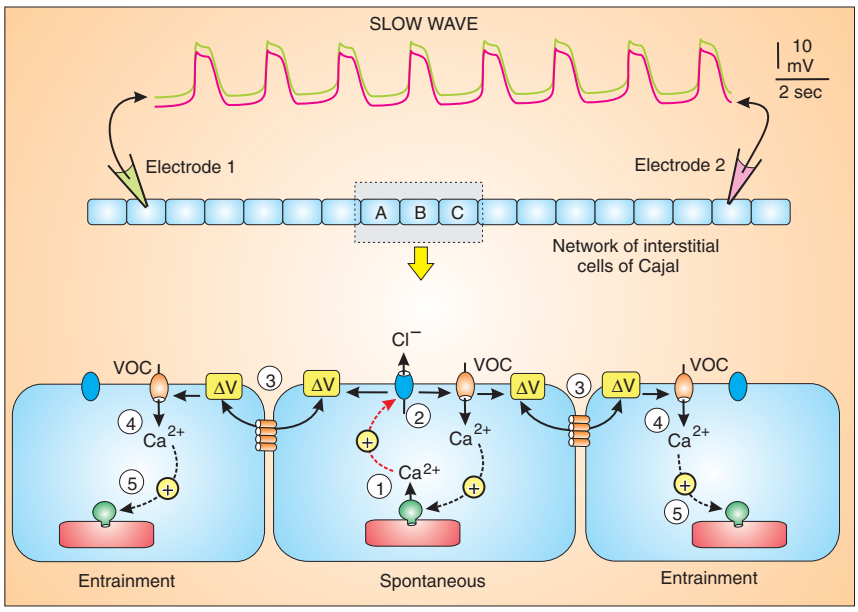
Acetylcholine accelerates slow wave activity in guine-pig gastric pylorus.
A. The two traces were recorded from two electrodes placed 4.5 mm apart. Upon addition of acetylcholine (ACh) (100 nM), there was a reversible acceleration of slow wave activity. B. Under resting conditions there was little synchrony (regions 1 and 3), but this increased markedly when the frequency was accelerated by ACh (region 2). Reproduced from van Helden and Imtiaz (2003).

of slow waves. This overall reduction in the slow wave reduces the pacemaking drive responsible for smooth muscle contraction. This action of the nitric oxide (NO)/cyclic GMP signalling pathway also seems to be directed towards the $InsP_3$ receptors. In this case, the $InsP_3R$ modulation by protein phosphorylation depends upon the action of cyclic GMP-dependent protein kinase Iβ (cGKIβ), which reduces the Ca^{2+} sensitivity of the receptor.

Phase wave

A remarkable feature of the network of interstitial cells of Cajal (ICC), which co-ordinate the contractile activity of large groups of smooth muscle, is that their oscillatory activity can be closely synchronized (Module 7: Figure ICC modulation). Slow waves in cells that are 4.5 mm apart occur at the same time. The basis of this experiment is shown in Module 7: Figure ICC coupling. If the slow wave is recorded from two cells widely separated from each other (e.g. electrodes 1 and 2), the regular waves of membrane depolarization occur in phase with each other. This synchronicity has been referred to as a phase wave to indicate that the repetitive Ca^{2+} transients responsible for the slow wave occur closely in phase with each other within a large population of cells. In reality, there are small

Module 7: | Figure ICC coupling



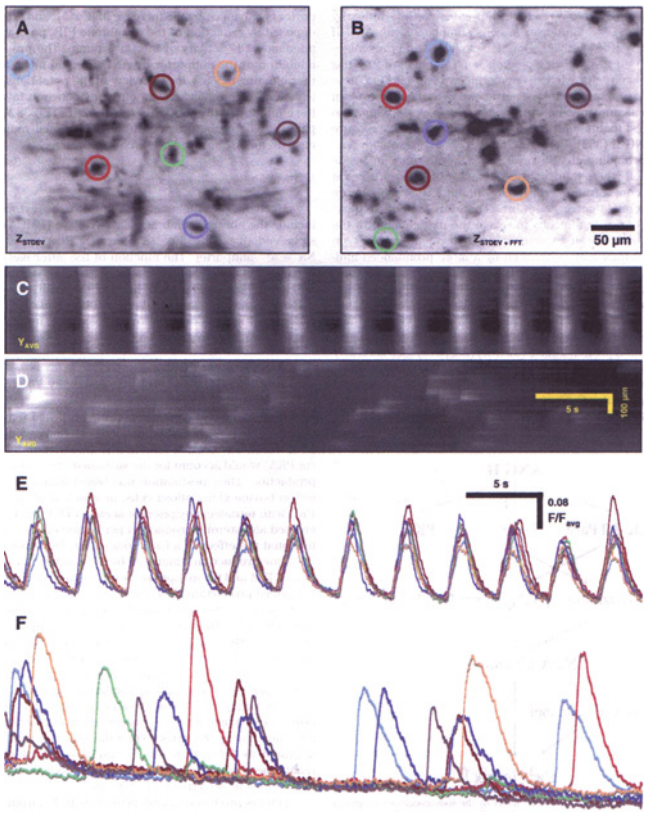
Coupling of slow wave activity within a network of interstitial cells of Cajal.
The slow waves generated by each cell of the ICC network are tightly coupled together. The waves recorded from one cell (green trace recorded by electrode 1) are synchronized with those many millimeters away (red trace recorded by electrode 2). The hypothesis to explain this long range synchronization is illustrated by reference to the three cells (A, B and C) shown on the bottom. Each cell has an endogenous cytosolic Ca^{2+} oscillator and is capable of releasing Ca^{2+} spontaneously. In this case, cell B releases Ca^{2+} first and this results in membrane depolarization (ΔV), which then spreads to its neighbours (A and C) to entrain their oscillatory activity as described in the text. The nature of the oscillator in each cell is described in Module 7: Figure ICC pacemaker.

phase shifts that result from the fact that excitation spreads quickly down the network at 5–40 mm/s to account for the waves of peristalsis. Phase waves can also be observed when the Ca^{2+} transients responsible for these slow waves are simultaneously recorded from a number of ICC cells that form a network (Module 7: Figure ICC network synchrony). Trace E shows that all the cells highlighted by the coloured circles in panel A produced Ca^{2+} transients in close synchrony with each other. However, when the gap junctions are blocked, this synchrony is lost and the individual cells produce Ca^{2+} transients at random (Panel F). It is clear, therefore that the ICC cytosolic Ca^{2+} oscillator in each cell is tightly coupled to that of its neighbours and this phase locking seems to depend on a signal being passed through the gap junctions.

Just how the oscillatory activity of ICCs are coupled together to form a phase wave is still somewhat of a mystery. The phase wave spreads too fast (5–40 mm/s) to be explained by an intercellular Ca^{2+} wave (Module 6: Figure intercellular Ca^{2+} wave), which has an upper propagation rate of about 100 μm/s. Most attention is now focused on the idea that coupling depends upon membrane depolarization spreading through the gap junctions and is thus an example of communication through electrical signals (Module 1: Figure cell communication). This depolarization is initiated by the Ca^{2+} oscillator in each ICC (Module 7: Figure ICC pacemaker). The upstroke of each slow wave is initiated by the release of Ca^{2+} that then opens the chloride and Ca^{2+} channels to produce each depolarization. The idea is that this depolarization spreads quickly to entrain the oscillatory activity of neighbouring cells. Since this oscillatory activity depends upon the spontaneous activation of $InsP_3$ receptors, depolarization may entrain oscillations by enhancing the supply of either the Ca^{2+} or $InsP_3$ necessary to trigger release. It seems unlikely that a depolarization-induced synthesis of $InsP_3$ would be fast enough to be the coupling mechanism. In the following hypothesis, therefore, it is suggested that a depolarization-induced influx of Ca^{2+} provides a mechanism for coupling together the oscillators in separate cells (Module 7: Figure ICC coupling):

1. All the ICC cells within the network have a Ca^{2+} oscillator that pulse at similar frequencies. During the pacemaker period prior to the next slow wave, one of the cells (i.e. cell B in Module 7: Figure ICC coupling) spontaneously releases Ca^{2+}.
2. The Ca^{2+} released from the ER then activates the chloride channels to provide the initial depolarization that then switches on the voltage-operated channels (VOCs) to give the rapid depolarization (ΔV) that characterizes the upstroke of the slow wave. The importance of VOC activation is that it provides a global elevation of Ca^{2+} to synchronize release of all the $InsP_3$ receptors within the cell (see Step 8 in Module 7: Figure ICC pacemaker).
3. The Ca^{2+} release and subsequent depolarization (ΔV), which arises spontaneously in cell B, spreads electronically very rapidly to neighbouring cells A and C, and perhaps further a field depending on the space constant, to cause a similar depolarization.

Module 7: | Figure ICC network synchrony



Gap junctions maintain ICC network slow wave synchrony.

Recordings of intracellular Ca^{2+} were made from cells (coloured rings) that make up the ICC network in mouse ileum. A, Control cells. B, Cells treated with β-glycyrrhetinic acid (10 µM) to disrupt the gap junctions. C. A spatiotemporal plot obtained by averaging fluorescence intensity down a column of pixels in the Y axis revealed a synchronous elevation of Ca^{2+} throughout the network. D, After disruption of gap junctions, individual cells fired independently of each other. E and F, These recordings revealed that the Ca^{2+} transients were tightly synchronized (E) whereas this synchronization disappeared when electrical coupling through the gap junctions was inhibited (F). Reproduced from Park et al. (2006).

4. Membrane depolarization in cells A and C induces the rapid opening of VOCs that provide an influx of Ca^{2+}.
5. This input of external Ca^{2+} provides an entrainment pulse to initiate the oscillatory release of Ca^{2+} in the neighbouring cells so that they contribute their Ca^{2+} pulse in phase with everyone else.

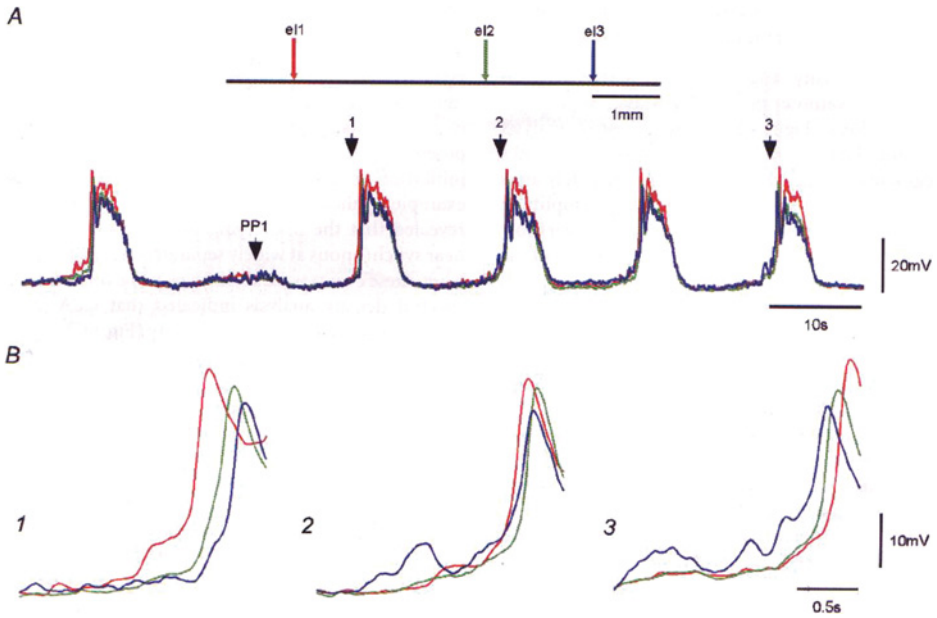
The critical feature of this mechanism is that all the oscillators in the network are coupled together through a voltage-dependent mechanism. At any moment in time, an individual cell is either functioning as a pacemaker to initiate a Ca^{2+} transient (e.g. cell B in Module 7: Figure ICC coupling) or it is responding to the depolarization emanating from a neighbouring pacemaker cell (e.g. cells A and C). It seems that these two functions are not fixed and cells can switch roles on a beat by beat basis. This switching is clearly evident when three cells are monitored during successive slow waves (Module 7: Figure ICC phase switching). During wave 1, the cell recorded by electrode 1 (el1, red trace) fired first and this was followed by el2 (green trace) and then el3 (blue trace). This sequence was reversed during wave 3. The main point to make, therefore, is that this system of coupled oscillators is highly dynamic in that each cell is not only capable of initiating each wave but it is also finely tuned to respond to the entrainment signal coming from any of its neighbours and this ensures that each beat is synchronous within a large population of cells.

Ca^{2+} homoeostasis

The concentration of Ca^{2+} in plasma is maintained within a narrow physiological range by a number of interacting hormonal and organ systems (Module 7: Figure Ca^{2+} homoeostasis). This constancy of the level of serum Ca^{2+} is crucial not only to maintain the skeleton but also because the Ca^{2+} signalling system that controls so many cellular processes is very sensitive to variations in extracellular Ca^{2+} concentration. A number of cell types located in different tissues contribute to Ca^{2+} homoeostasis.

The average human skeleton contains about 1000 g of Ca^{2+} and is the major reservoir from which Ca^{2+} can be added or removed. When the dietary supply of Ca^{2+} is adequate, the rate of bone formation and bone resorption are approximately equal at about 400 mg/day. Bone is not a static organ, and bone remodelling occurs continuously in response to changing skeletal requirements. Control over this ebb and flow of Ca^{2+} between bone and plasma is a major component of the control mechanisms that regulate

Module 7: | Figure ICC phase switching



Temporal patterns of individual ICC slow waves.
A. Three electrodes were used to record slow waves from individual interstitial cells of Cajal (ICC). B. An expanded time course of the three waves labeled 1 to 3. Note that the activation sequence varies from beat to beat. Reproduced from van Helden and Imtiaz (2003).

Ca^{2+} homoeostasis. The kidney, which filters almost the total plasma content of Ca^{2+} each day, is another important site of hormone regulation. Almost all of this Ca^{2+} is reabsorbed, with a small amount (200 mg/day) being lost in the urine. Control over this Ca^{2+} reabsorption by the kidney is a major component of Ca^{2+} homoeostasis. There also are large fluxes of Ca^{2+} associated with the intestine. There is an input of Ca^{2+} from food and the secretions coming from the salivary glands and pancreas. A little less than half of the Ca^{2+} entering the intestine is reabsorbed and slightly more is lost in the faeces, resulting in a net uptake of approximately 200 mg/day, which thus balances the urinary losses (Module 7: Figure Ca^{2+} homoeostasis). Control over this Ca^{2+} reabsorption by the intestine is another major component of Ca^{2+} homoeostasis.

All the systems that participate in Ca^{2+} homoeostasis are carefully regulated by three main hormones:

- Parathyroid hormone (PTH)
- Calcitonin
- 1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]

PTH is released from chief cells in the parathyroid glands, whereas calcitonin is released from C cells in the thyroid (Module 7: Figure parathyroid gland). Circulating PTH plays a major role in regulating $1,25(OH)_2D_3$ formation, which is synthesized by a series of steps located in the skin, liver and kidney (Module 7: Figure vitamin D metabolism). The hormonal regulation of Ca^{2+} homoeostasis is based on the ability of these three hormones to regulate Ca^{2+} fluxes in tissues, such as bone intestine and kidney.

Hormonal regulation of Ca^{2+} homoeostasis

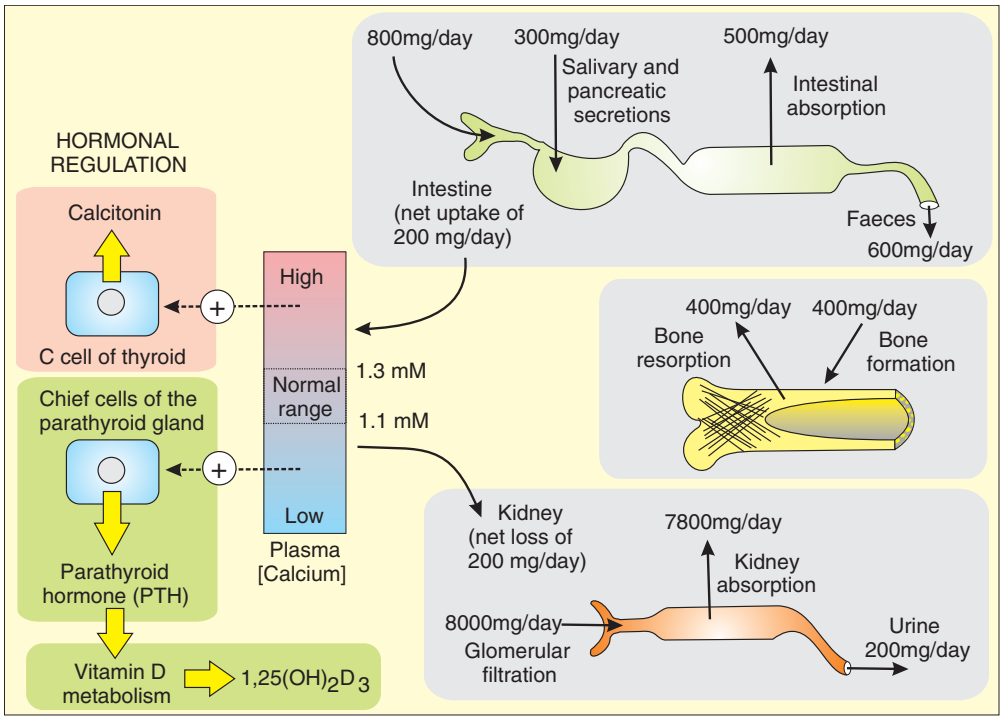
The nicely balanced system of Ca^{2+} homoeostasis is regulated by three main hormones: parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] and calcitonin. PTH and $1,25(OH)_2D_3$ act primarily to increase the level of Ca^{2+} in the plasma, whereas calcitonin has the opposite effect. PTH is formed and released by the parathyroid gland, which is located alongside the thyroid gland (Module 7: Figure parathyroid gland). C cells dispersed throughout the thyroid are responsible for forming and releasing calcitonin. $1,25(OH)_2D_3$ is formed through a series of steps located in different cells (Module 7: Figure vitamin D metabolism). There are three major processes that are regulated by these hormones:

- Bone remodelling
- Ca^{2+} reabsorption by the intestine
- Ca^{2+} reabsorption by the kidney

Parathyroid gland

The parathyroid gland, which synthesizes and releases parathyroid hormone (PTH), has a somewhat varied location in mammals. In humans, it has four separate parts, each about the size of an apple seed, that are located in close association with the thyroid gland (Module 7: Figure parathyroid gland). A thick capsule of connective tissue surrounds the sheets of chief cells that synthesize and release PTH. Chief cells are typical protein secretory cells with the usual stacks of rough endoplasmic reticulum and Golgi bodies that function to package PTH into storage vesicles. An unusual feature of these chief cells is that they have relatively few storage granules, which means that the store of hormone is rather limited in relation to the amount being released. PTH thus turns over quickly and the level

Module 7: | Figure Ca^{2+} homoeostasis



Regulation of Ca^{2+} homoeostasis by multiple hormonal and organ effector systems.

The concentration of Ca^{2+} within the plasma is tightly regulated within a normal range between 1.1 and 1.3 mM. On a daily basis, there is a net input of approximately 200 mg from the intestine, and this is balanced by a similar loss in the urine. The rates of bone resorption and bone formation are normally balanced at about 400 mg/day. Hormonal regulation is based on the release of hormones that respond to either low (hypocalcaemia) or high (hypercalcaemia) plasma Ca^{2+} concentrations. Low Ca^{2+} together with the resulting release of parathyroid hormone (PTH) can also activate vitamin D metabolism to form the hormone 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which also functions in the regulation of Ca^{2+} homoeostasis.

of Ca^{2+} in the plasma is one of the main determinants of parathyroid hormone (PTH) synthesis and release.

Excessive stimulation of the parathyroid gland results in parathyroid gland hyperplasia, which is particularly evident during the secondary hyperparathyroidism brought on by kidney disease.

C cells

The calcitonin-producing parafollicular cells (C cells) are located amongst the thyroid follicular cells (Module 7: Figure parathyroid gland). These C cells express the Ca^{2+}-sensing receptor (CaR) that respond to elevations in the level of serum Ca^{2+} by releasing calcitonin.

Parathyroid hormone (PTH)

Parathyroid hormone (PTH), which is the most important hormonal regulator of Ca^{2+} homoeostasis, is synthesized and released from the parathyroid gland (Module 7: Figure parathyroid gland). PTH is released as a linear protein of 84 amino acids. It shares considerable homology with parathyroid hormone (PTH)-related peptide (PTHrP), which also has a function in regulating bone, especially during development.

One of the main actions of PTH is to elevate blood Ca^{2+} levels by affecting bone remodelling by increasing bone resorption by the osteoclasts, by stimulating Ca^{2+} reabsorption by the kidney and by stimulating the kidney synthesis of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] (Module 7: Figure vitamin D metabolism). With regard to bone resorption, one of the actions of PTH is to stimulate the osteoblasts to increase the expression of receptor activator of nuclear factor κB (NF-κB) ligand and to decrease the release of osteoprotegerin (OPG), which combine to increase the differentiation of the osteoclasts (Module 8: Figure osteoclast differentiation).

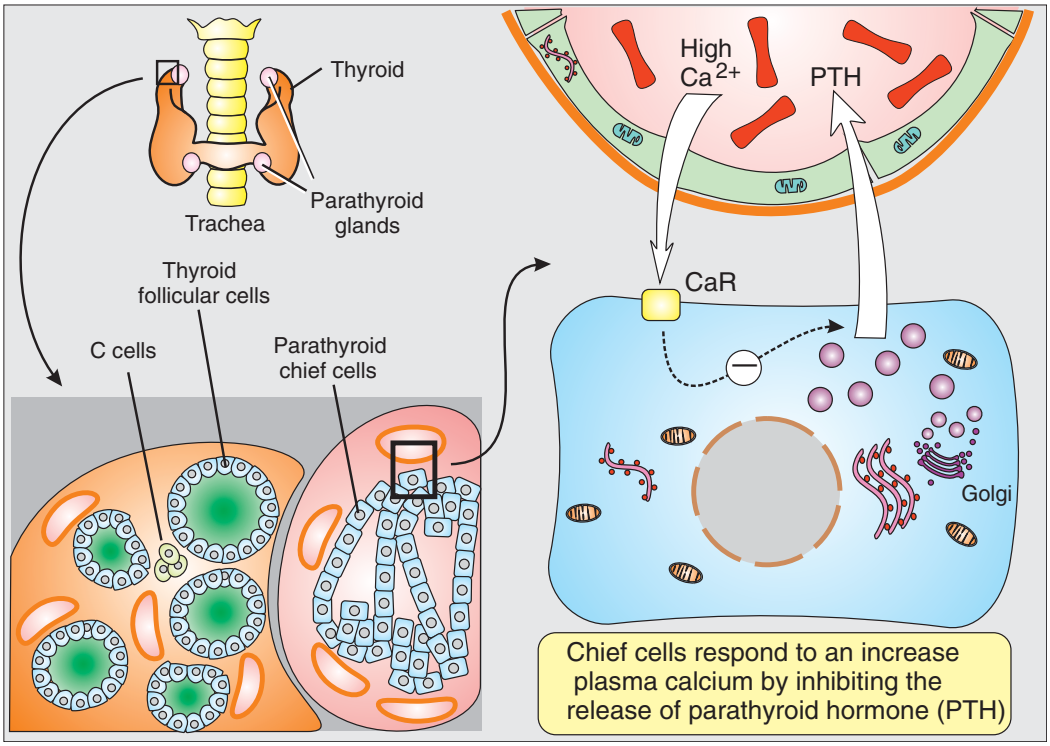
Parathyroid hormone (PTH)-related peptide (PTHrP)

Parathyroid hormone (PTH)-related peptide (PTHrP) has some homology with parathyroid hormone (PTH), especially with regard to the first 13 amino acids. The similarity between these two molecules extends to the fact that they both act on the same PTH receptor 1 (PTHR1). Like PTH, PTHrP plays a role in bone remodelling, especially during embryogenesis. In adult life, PTHrP has also been implicated in the regulation of a number of cellular processes, such as control of cell proliferation, smooth muscle cell relaxation and the transplacental flux of Ca^{2+}. With regard to pathology, PTHrP appears to be one of the main components of humoral hypercalcaemia of malignancy (HHM).

Parathyroid hormone (PTH) synthesis and release

The synthesis and release of parathyroid hormone (PTH) is regulated by a number of factors. The primary control is through the plasma level of Ca^{2+}, which is monitored by a Ca^{2+}-sensing receptor (CaR) located on the surface

Module 7: | Figure parathyroid gland



Location and structure of parathyroid glands.
In humans, there are four separate parathyroid glands, which are closely associated with the thyroid gland. The main cell type in the parathyroid gland is the chief cells that are arranged into sheets that lie close to blood vessels. High levels of Ca^{2+} acts through a Ca^{2+}-sensing receptor (CaR) to inhibit the release of parathyroid hormone (PTH).

of the chief cells (Module 7: Figure parathyroid gland). The parathyroid gland is richly supplied with blood vessels, thus enabling the CaR on the chief cells to constantly monitor the level of Ca^{2+} in the plasma. The Ca^{2+} in the plasma thus functions as an agonist by acting on the CaR to regulate the release of PTH. There is an inverse relationship between the plasma level of Ca^{2+} and the release of PTH (Module 7: Figure PTH secretion). PTH secretion is maximal at low levels of Ca^{2+} and declines steeply over the narrow range of Ca^{2+} concentrations that occur normally in the plasma. This relationship is somewhat unusual in that it is opposite to what might be expected. Since the CaR is coupled to the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette, it might be expected that an increase in the stimulation of CaR should increase the level of Ca^{2+} signalling and hence the level of PTH secretion, but the opposite is the case. Since the CaR acts to increase the level of intracellular Ca^{2+}, it seems reasonable to assume that Ca^{2+} somehow acts to inhibit exocytosis contrary to its normal stimulatory effect. This paradoxical situation is currently unresolved.

The CaR is also known to be coupled to the cyclic AMP signalling pathway, where it acts through $G\alpha_i$ (Module 2: Table heterotrimeric G proteins) to reduce the level of cyclic AMP. Cyclic AMP does appear to play a role in modulating the parathyroid gland, which is richly innervated by a sympathetic neural input. Like Ca^{2+}, cyclic AMP may also exert a negative-feedback effect on PTH secretion because inhibition of the β-receptor that acts by increasing cyclic AMP enhances PTH secretion.

The secretory activity of the parathyroid gland is also regulated by 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which is the hormonally active form of vitamin D (Module 7: Figure PTH secretion). The $1,25(OH)_2D_3$ acts on a longer time scale in that it regulates the synthesis of PTH by controlling the expression of the PTH gene. $1,25(OH)_2D_3$ enters the nucleus, where it binds to the vitamin D receptor (VDR), which binds to the VDR element (VDRE) to inhibit transcription of preproPTH and hence the synthesis of PTH.

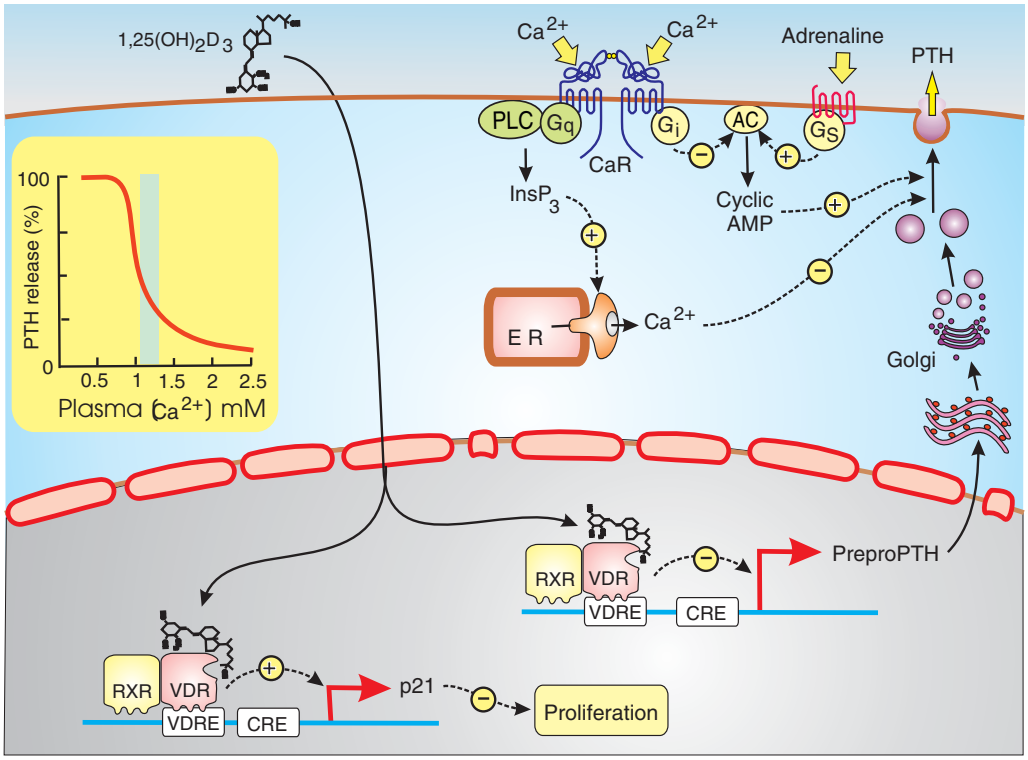
Many of the disorders of Ca^{2+} homoeostasis arise from an increase in the release of PTH. There are two main types of hyperparathyroidism:

- Primary hyperparathyroidism
- Secondary hyperparathyroidism

Changes in PTH secretion are also a feature of the inherited disorders of Ca^{2+} that arise from mutations in CaR:

- Familial hypocalciuretic hypercalcaemia (FHH)
- Neonatal severe hyperparathyroidism (NSHPT)
- Autosomal dominant hypocalcaemia (ADH)

Module 7: | Figure PTH secretion



Control of parathyroid gland secretion and proliferation.
The primary function of the parathyroid gland is to synthesize and release parathyroid hormone (PTH). This PTH release has an inverse relationship to the plasma level of Ca^{2+} (see inset). The bar represents the normal range of plasma Ca^{2+} concentration. A Ca^{2+}-sensing receptor (CaR) responds to changes in the plasma level by activating phospholipase C (PLC) to increase the level of inositol 1,4,5-trisphosphate ($InsP_3$) and Ca^{2+}. It can also inhibit adenylyl cyclase (AC) to reduce the level of cyclic AMP. Since an increase in these messengers is associated with a decrease in secretion, it is assumed that they somehow inhibit exocytosis. The vitamin D-derived hormone 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] acts on the vitamin D receptor (VDR) to inhibit both secretion and proliferation.

Calcitonin

The hormone calcitonin, which is a polypeptide containing 32 amino acids, is released from the parafollicular cells, which are also known as C cells, located in the thyroid gland (Module 7: Figure parathyroid gland). Work on some of the lower vertebrates such as fish seemed to indicate that calcitonin might function by counteracting the effects of parathyroid hormone (PTH) by reducing the level of Ca^{2+} in the plasma. For example, it can reduce Ca^{2+} absorption by the intestine and kidney tubules. It can also act by decreasing the activity of the osteoclasts (Step 7 in Module 7: Figure osteoclast function). In humans, there is less evidence for a role of calcitonin in regulating Ca^{2+} homoeostasis. For example, when calcitonin levels are increased (i.e. in medullary thyroid cancer) or decreased following thyroidectomy, there is little change in the level of serum Ca^{2+}. However, there are indications that calcitonin may have a role in maintaining Ca^{2+} homoeostasis during the physiological mineral stress associated with the reproductive cycle. This is especially the case during lactation, when there is a large requirement for Ca^{2+} during milk production. It seems that calcitonin may help to protect the skeleton by reducing mineral loss during pregnancy and lactation.

1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]

The hormone 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which is the active component of vitamin D3, has an important role in the feedback mechanisms that regulate Ca^{2+} homoeostasis. $1,25(OH)_2D_3$ formation and metabolism depends upon a series of reactions that take place in a number of different tissues (Module 7:Figure vitamin D metabolism). The role of $1,25(OH)_2D_3$ in the hormonal regulation of Ca^{2+} homoeostasis occurs in a number of tissues. One of its main actions is to promote Ca^{2+} reabsorption by the kidney (Module 7: Figure kidney Ca^{2+} reabsorption) and Ca^{2+} reabsorption by the intestine (Module 7: Figure intestinal Ca^{2+} reabsorption). It also plays an import role in reducing the expression of PTH synthesis by the parathyroid gland (Module 7: Figure PTH secretion). Vitamin D deficiencies, which result in a decline of $1,25(OH)_2D_3$, removes this inhibitory effect on PTH synthesis resulting in secondary hyperparathyroidism. The relationship between vitamin D and Ca^{2+} regulation in neurodegeneration suggest that deficiencies in vitamin D may contribute to a number of the major neural diseases in humans such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.

1,25-Dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] formation and metabolism

Formation of 1,25-dihydroxyvitamin D_3 [1,25-dihydroxycholecalciferol or 1,25(OH)$_2$D$_3$], which is the hormonally active form of vitamin D_3, depends upon a series of reactions, most of which are carried out by the cytochrome P-450-containing (CYP) enzymes, and take place in different tissues (Module 7: Figure vitamin D metabolism):

1. The first reaction begins in the skin, where the precursor 7-dehydrocholesterol is photolysed by UV light to form cholecalciferol (vitamin D_3). Vitamin D_3 can also be obtained from the diet.
2. Vitamin D_3 enters the circulation and is transferred to the liver, where it undergoes the first of two hydroxylation reactions. A hydroxy group is added to the C-25 position by a vitamin D-25 hydroxylase (encoded by the *CYP27A1* gene) to form 25-hydroxyvitamin D_3 [25(OH)D_3].
3. The 25(OH)D_3 is then released to the blood, where it circulates as a complex with a vitamin D-binding protein (DBP). This 25(OH)D_3/DBP complex is filtered by the glomerulus and, as it passes down the lumen, it interacts with the endocytic receptor megalin, which facilitates its uptake into the kidney tubule cells. The latter have a 25(OH)D_3-1α-hydroxylase (encoded by the *CYP27B1* gene), which adds another hydroxy group to the 1 position to form the active hormone 1,25(OH)$_2$D$_3$. This active hormone can also be produced by a number of other tissues such as the brain, colon, endothelium, mammary gland skin, immune cells and prostate.
4. A rate limiting step for the metabolism of both 1,25(OH)$_2$D$_3$ and its precursor 25(OH)D_3 is a 24-hydroxylation step carried out by 1α,25-(OH)2D3 24-hydroxylase (encoded by the *CYP24A1*) that convert them into 1,24,25(OH)$_2$D$_3$ and 24,25(OH)$_2$D$_3$ respectively. Expression of *CYP24A1* is enhanced by 1,25(OH)$_2$D$_3$ thus providing a negative feedback loop that checks on the level of this hormone. Loss of function mutations in *CYP24A1* have been identified in idiopathic infantile hypercalcemia.
5. The 1,25(OH)$_2$D$_3$ functions as a hormone to regulate a number of Vitamin D signalling pathways to regulate processes such as calcium homoeostasis, proliferation, inflammation and bone formation (Module 7: Figure vitamin D receptor activation). Most of these actions are carried out by 1,25(OH)$_2$D$_3$ acting through a vitamin D receptor (VDR).

These metabolic pathways responsible for forming this hormone are carefully regulated depending on the level of Ca^{2+} in the plasma. One of the actions of parathyroid hormone (PTH), which is released when Ca^{2+} levels are low, is to stimulate the activity of the 1α-hydroxylase to increase the formation of 1,25(OH)$_2$D$_3$ (Module 7: Figure Ca^{2+} homoeostasis). On the other hand, 1,25(OH)$_2$D$_3$ operates a negative-feedback loop whereby it can activate the 24-hydroxylase to reduce its level should it become too high.

The formation of 1,25(OH)$_2$D$_3$, often acting together with PTH, plays a major role in the hormonal regulation of Ca^{2+} homoeostasis.

Vitamin D receptor (VDR)

The vitamin D receptor (VDR) is a member of the nuclear receptor family (Module 4: Table nuclear receptor toolkit) and is widely distributed in many different cell types. The VDR functions as a heterodimer together with the retinoid X receptor (RXR), which is another nuclear receptor (Module 7: Figure vitamin D receptor activation). The VDR has a number of functional domains: a DNA-binding (DNB) domain, which has two Zn fingers, a nuclear localization signal (NLS), which directs the receptor into the nucleus, a ligand-binding domain (LBD), and a transactivation domain that binds to activation factor-2 (AF-2).

When VDR binds 1,25(OH)$_2$D$_3$, it is phosphorylated on serine-51 in the DNB domain by protein kinase C (PKC) and on serine-208 by casein kinase II and this alters its conformation resulting in the removal of co-repressors such as nuclear receptor co-repressor (N-CoR) and the closely related silencing mediator for retinoid and thyroid hormone receptor (SMRT). The liganded VDR then interacts with Retinoid X receptor (RXR) and the heterodimer binds to the vitamin D response element (VDRE). A number of coactivators such as CBP/p300, steroid receptor coactivator 1 (SRC-1) and the vitamin D receptor interacting protein 205 (DRIP205) contribute to this gene transcription. The DRIP205 is part of the mediator complex that links transcription factors to the initiation factor II (TFII) that are associated with RNA polymerase II (pol II) at the core promoter where the target genes are transcribed. There are a large number of vitamin D-sensitive target genes most of which are activated by 1,25(OH)$_2$D$_3$, but there are some that are repressed. The transcription of these genes contributes to the control of many different cellular processes:

- Vitamin D control of bone function
- Vitamin D control of cell proliferation
- Vitamin D control of Ca^{2+} homoeostasis
- Vitamin D control of inflammation

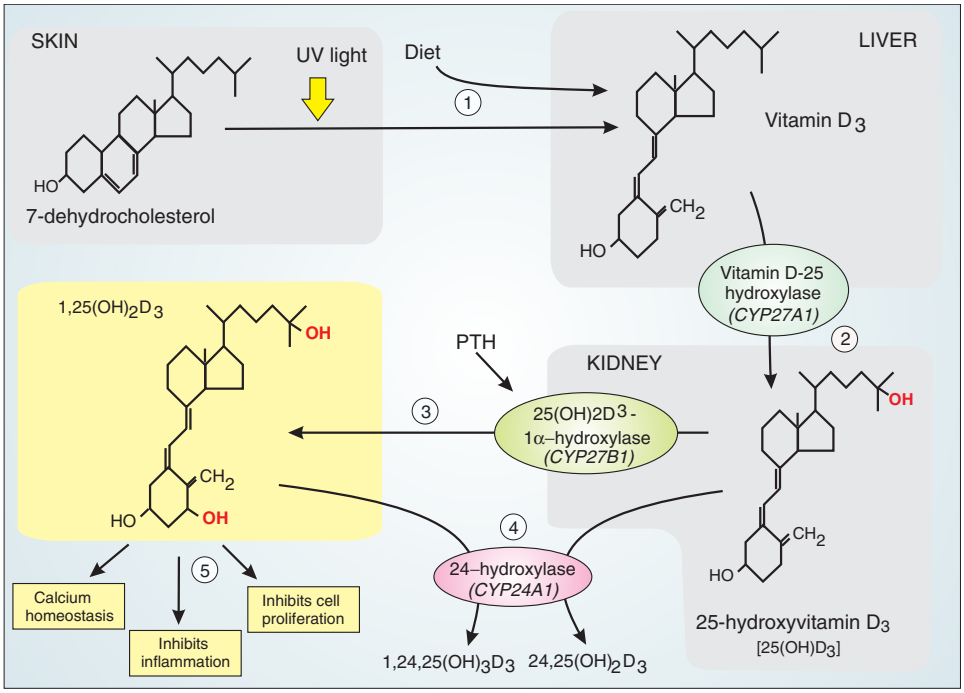
There are multiple polymorphisms of the VDR gene and some of these have been associated with various disorders including autoimmune diseases and cancer. Expression of the VDR is reduced in a large proportion of the population in the Mediterranean island of Sardinia that suffer from Multiple sclerosis (MS). These MS patients have reduced expression of the *Ifng* gene that encodes Interferon-γ (IFN-γ), which plays a role in regulating the expression of the VDR.

The cancer drug bexarotene acts by stimulating RXR and may thus increase the activity of vitamin D. Such an action might explain the reported beneficial effects on both Parkinson's disease and Alzheimer's disease, but these findings are somewhat controversial.

Vitamin D control of bone function

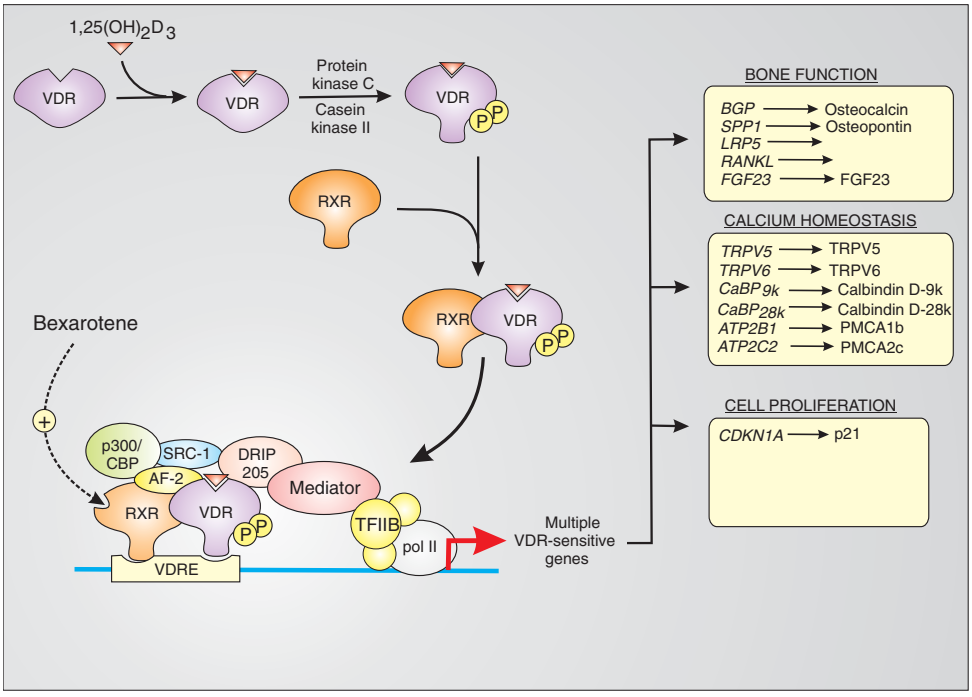
One of the important actions of vitamin D is to regulate bone remodelling by regulating the expression of many of

Module 7: | Figure vitamin D metabolism



Metabolism of vitamin D to form the Ca^{2+} homoeostatic hormone 1,25-dihydroxyvitamin D_3 [1,25-dihydroxycholecalciferol or $1,25(OH)_2D_3$]. Synthesis of 1,25-dihydroxyvitamin D_3 [1,25-dihydroxycholecalciferol or $1,25(OH)_2D_3$] occurs through three steps carried out in separate tissues. The sequence begins in the skin with the UV-dependent photolysis of 7-dehydrocholesterol to Vitamin D_3 (chlolecalciferol). The next step occurs in the liver, where a vitamin D-25 hydroxylase adds a hydroxy group to chlolecalciferol to form $25(OH)D_3$. The final reaction occurs in the kidney where a 1α-hydroxylase adds another hydroxy group to form the active hormone $1,25(OH)_2D_3$.

Module 7: | Figure vitamin D receptor activation



Vitamin D receptor activation.
The activation of the vitamin D receptor (VDR) begins with the binding of the vitamin D_3 hormone 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] and this is followed by its phosphorylation by protein kinase C and casein kinase II that then induces a conformational change enabling it to bind its partner retinoid X receptor (RXR). The VDR/RXR heterodimer then binds to the vitamin D response element (VDRE). Once in place, the complex binds to a number of co-activators enabling the transcriptional complex to activate RNA polymerase II (Pol II) to initiate the expression of a large number of genes that encode proteins that function in a number of cellular processes.

the genes that control the function of the osteoblasts and osteoclasts and thus regulates both bone formation and bone resorption (Module 7: Figure bone cells).

The vitamin D hormone $1,25(OH)_2D_3$ (Module 7: Figure vitamin D metabolism) acting through the vitamin D receptor (VDR) activates a number of genes that regulate bone formation (Module 7: Figure vitamin D receptor activation):

- *FGF23* gene expression is increased to provide the fibroblast growth factor 23 (FGF23), which is produced by osteocytes and released to act on the kidney to reduce the reabsorption of phosphate.
- *RANKL* gene expression is increased to provide the receptor activator of nuclear factor κB (NF-κB) ligand (RANKL), which plays an important role in osteoclastogenesis to stimulate bone resorption (Module 8: Figure osteoclastogenesis).
- *Runx2* gene expression is reduced in osteoblasts to slow down osteoblastogenesis to increase bone resorption (Module 8: Figure bone cell differentiation).
- *LRP5* gene expression is increased to provide the LRP5, which is a member of the low-density lipoprotein (LDL) receptor superfamily, which acts through the Wnt signalling pathway to control the proliferation and differentiation of the osteoblasts (Module 7: Figure osteoblast function).
- *BGP* gene expression is increased to provide the osteocalcin that is released from osteoblasts to function both as a circulating hormone and as part of the bone matrix (Module 7: Figure osteoblast function).

Vitamin D control of cell proliferation

Vitamin D (calcitriol) can have a profound effect on cellular proliferation. Deficiencies of vitamin D have been identified in many cancers and supplementation with this vitamin D has been used as a preventive measure to reduce cancer risk and prevalence.

Vitamin D control of Ca^{2+} homoeostasis

One of the important functions of vitamin D is to contribute to the mechanisms responsible for whole body Ca^{2+} homoeostasis (Module 7: Figure Ca^{2+} homoeostasis). The vitamin D hormone $1,25(OH)_2D_3$ (Module 7: Figure vitamin D metabolism) acting through the vitamin D receptor (VDR) activates a number of genes that regulate different aspects of the mechanisms that regulate the serum level of Ca^{2+} (Module 7: Figure vitamin D receptor activation):

- *PTH* gene expression is reduced resulting in a decline in the release of parathyroid hormone (PTH), which is released from the parathyroid gland by $1,25(OH)_2D_3$ (Module 7: Figure PTH secretion). PTH is one of the most important hormonal regulators of Ca^{2+} homoeostasis.
- A number of vitamin D-regulated genes are associated with Ca^{2+} reabsorption by the intestine (Module 7: Figure intestinal Ca^{2+} reabsorption) and Ca^{2+} reabsorption by the kidney (Module 7: Figure kidney Ca^{2+} reabsorption):

TRPV5

Expression of this gene is increased to provide the TRPV5 channel in the apical membrane of kidney tubule cells (Module 7: Figure kidney Ca^{2+} reabsorption).

TRPV6

Expression of this gene is increased to provide the TRPV6 channel in the apical membrane of intestinal cells (Module 7: Figure intestinal Ca^{2+} reabsorption).

$CaBP_{9k}$

Expression of this gene is increased in intestinal cells to provide the calbindin D-9k, which acts as a buffer to facilitate the diffusion of Ca^{2+} from the apical membrane across the cell to the basolateral surface where it is pumped out of the cell by the plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) pumps that are also regulated by $1,25(OH)_2D_3$ (Module 7: Figure intestinal Ca^{2+} reabsorption).

$CaBP_{28k}$

Expression of this gene is increased in kidney cells to provide the calbindin D-28k, which acts as a buffer to facilitate the diffusion of Ca^{2+} from the apical membrane across the cell to the basolateral surface where it is pumped out of the cell by the plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) pumps that are also regulated by $1,25(OH)_2D_3$ (Module 7: Figure kidney Ca^{2+} reabsorption).

ATB2B1

This gene regulates the expression of the plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) that extrudes Ca^{2+} from cells.

ATP2C2

This gene regulates the expression of the plasma membrane Ca^{2+}-ATPase 2c (PMCA2c) that extrudes Ca^{2+} from cells.

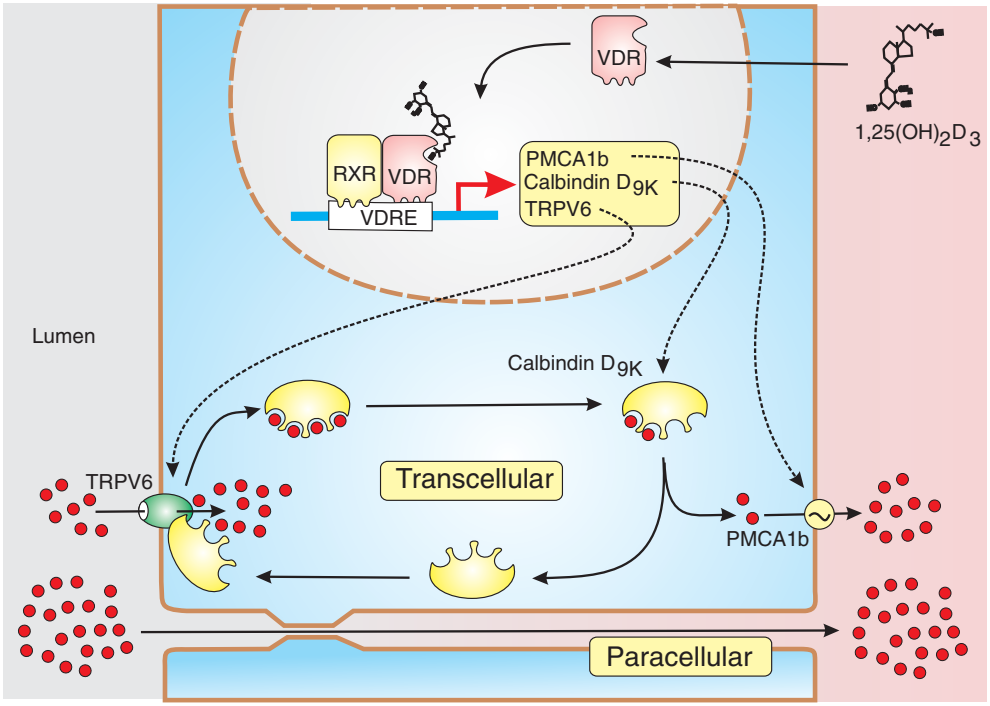
Vitamin D control of inflammation

Vitamin D can exert a beneficial effect by dampening down inflammatory responses to reduce the formation of inflammatory cytokines such as Tumour necrosis factor (TNF). The primary action of Vitamin D seems to depend on the suppression of T lymphocyte-mediated inflammation as is the case in Multiple sclerosis (MS). Vitamin D may also act on microglia in the brain to reduce inflammation in Alzheimer's disease (AD) (Module 12: Figure Inflammation and Alzheimer's disease).

Ca^{2+} reabsorption by the intestine

Absorption of Ca^{2+} by the intestine is a key component of Ca^{2+} homoeostasis (Module 7: Figure Ca^{2+} homoeostasis). Most of this absorption takes place in the small intestine. Movement across the intestinal epithelium occurs through either a passive paracellular pathway or an active transcellular pathway (Module 7: Figure intestinal Ca^{2+} reabsorption). The paracellular pathway seems to occur down the length of the intestine, whereas the transcellular mechanism is found mainly in duodenum and upper jejunum.

Module 7: | Figure intestinal Ca^{2+} reabsorption



Control of Ca^{2+} reabsorption by intestinal cells.

Transport of Ca^{2+} across intestinal cells occurs through both active and passive transport mechanisms. The paracellular pathway depends upon the passive flow of Ca^{2+} through the tight junctions. The active transcellular pathway depends upon entry across the apical membrane through vanilloid transient receptor potential 6 (TRPV6) channels, facilitated diffusion across the cell associated with calbindin D-9k and then extrusion from the cell by plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) pumps located on the basolateral membrane. 1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] enhances this transcellular pathway by increasing the expression of these three main components.

The active transcellular pathway consists of three main steps. Firstly, there is the passive entry of Ca^{2+} from the lumen through vanilloid transient receptor potential 6 (TRPV6), which is a member of the transient receptor potential (TRP) ion channel family (Module 3: Figure TRP channel phylogeny). TRPV6 functions in much the same way as TRPV5 does in Ca^{2+} reabsorption by the kidney (Module 7: Figure kidney Ca^{2+} reabsorption). An important property of TRPV5 and TRPV6 is that they inactivate rapidly when the intracellular level of Ca^{2+} increases. As occurs in the kidney, this inactivation of TRPV6 may be alleviated by a Ca^{2+} buffer, in this case calbindin D-9k. As it charges up with Ca^{2+}, the calbindin D-9k is thought to leave the channel and then diffuse across the cell to the basolateral surface, where it gives up its cargo to the plasma membrane Ca^{2+}-ATPase 1b (PMCA1b) pumps that extrude Ca^{2+}, thus completing the transcellular flux of Ca^{2+} (Module 7: Figure kidney Ca^{2+} reabsorption).

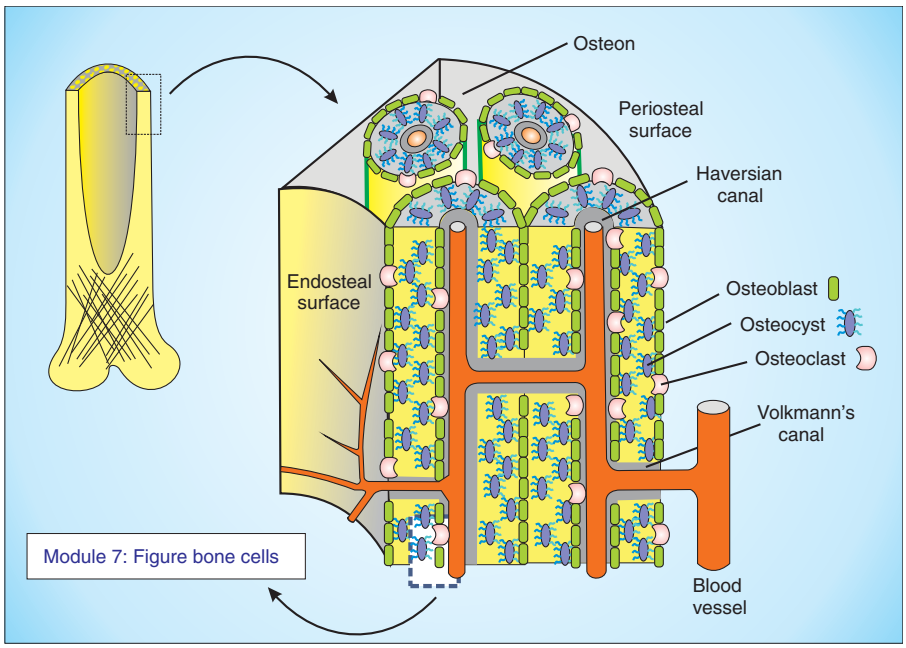
The transcellular reabsorption of Ca^{2+} by the intestinal cells is regulated by 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which acts by increasing the expression of all of the main components responsible for transporting Ca^{2+} across the cell. The $1,25(OH)_2D_3$ binds to the vitamin D receptor (VDR), which then acts on the VDR response element (VDRE) to increase the expression of TRPV6, calbindin D-9k and PMCA1b.

Bone

Bone is a living organ that serves both mechanical and physiological functions. The mechanical function is obvious; bone is the main structural component of the skeleton. The physiological function is mainly concerned with mineral metabolism and particularly the maintenance of Ca^{2+} homoeostasis (Module 7: Figure Ca^{2+} homoeostasis). The average human skeleton contains about 1000 g of Ca^{2+} and is the major internal reservoir from which Ca^{2+} can be added or removed. When the dietary supply of Ca^{2+} is adequate, the rate of bone formation and bone resorption are approximately equal at about 400 mg/day. Bone is not a static organ, but is constantly being remodelled in response to changing skeletal requirements. During bone remodelling, there is an ebb and flow of Ca^{2+} between bone and plasma that is carried out by the bone cells.

A cross-section through a piece of compact bone reveals that much of the matrix is organized into long circular rods called osteons (Module 7: Figure bone structure). Blood vessels ramify through the central Haversian canals, which are connected to the Volkmann's canals. The matrix is composed of proteins (e.g. collagen, fibronectin and osteopontin) that provide a framework for the deposition of Ca^{2+} and phosphate. The main matrix protein is collagen, which is cross-linked in such a way that the individual fibrils retain their ordered structure despite the massive impregnation with minerals.

Module 7: | Figure bone structure



Structural organization of bone and bone cells.
A small section of compact bone illustrates the microarchitecture of bone, which is composed of osteons where the three major bone cell types are located. The osteoblasts (green) and the osteoclasts (pink) line the surfaces of the osteons, whereas the osteocytes (blue) are embedded within the bone matrix. The white box indicates the close structural relationship that exists between the bone cells as illustrated in more detail in Module 7: Figure bone cells.

Bone remodelling

During growth, bones increase in diameter by resorbing matrix material from the endosteal surface while depositing new material at the periosteal surface. Bone growth is thus an example of how the skeleton is constantly been remodelled by the addition and removal of the matrix. This constant turnover of bone is regulated by three main bone cell types (Module 7: Figure bone cells):

- Osteoblasts are the bone-forming cells.
- Osteoclasts are the bone-resorbing cells.
- Osteocytes are modified osteoblasts that become buried within the bone matrix where they may function as mechanosensitive cells that detect bone deformation.

An important aspect of bone remodelling is the way the processes of bone formation and resorption are coordinated through a process of bone cell coupling. During development, growth of the skeleton occurs through an increase in the rate of bone formation over that of bone resorption. In adulthood, these processes come into balance, and this accounts for the rate of Ca^{2+} flux in and out of the bone being equal at 400 mg/day. Osteoporosis occurs when this balance is perturbed so that the rate of bone resorption exceeds that of bone formation, whereas the opposite occurs in osteopetrosis.

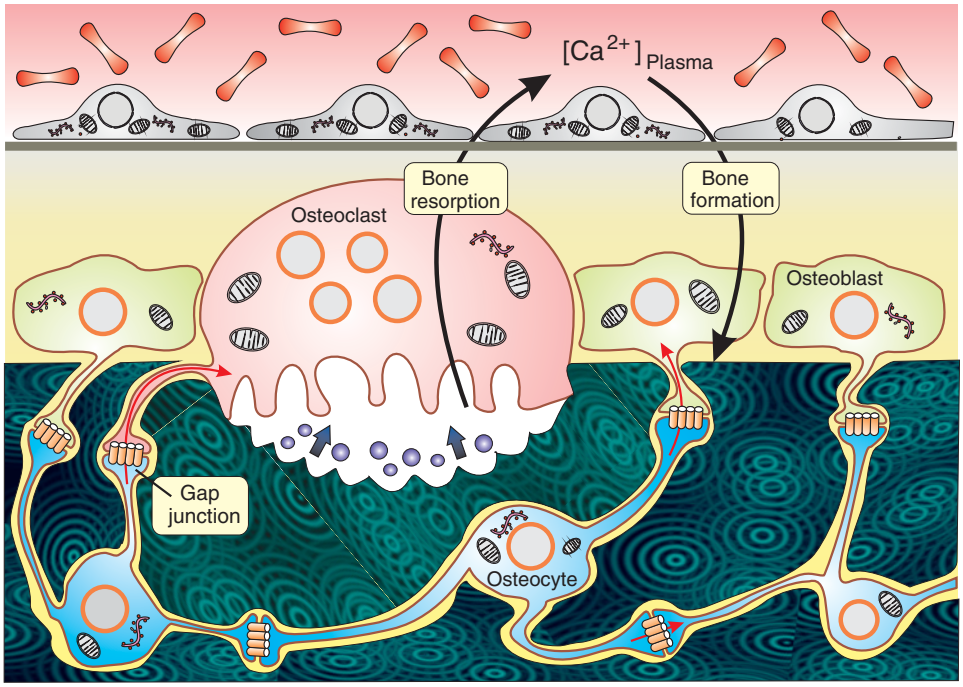
Osteoblasts

Osteoblasts are derived from mesenchymal stem cells (MSCs) and their maturation into mature bone forming cells (a process of osteoblastogenesis) is driven by the canonical Wnt signalling pathway (Module 8: bone cell differentiation). Osteoblasts cover the bone-forming surfaces (Module 7: Figure bone structure), where they are responsible for synthesizing and secreting the collagen fibrils and osteocalcin, which constitute the organic phase of the bone matrix (Module 7: Figure osteoblast function). They are typical protein-secreting cells with extensive arrays of rough endoplasmic reticulum (Module 7: Figure bone cells). As these secrete bone, they become trapped in the forming bone and gradually transform into the osteocytes.

A large number of signalling pathways have been implicated in the control of bone formation by the osteoblasts. There appear to be two main mechanisms. First, there are the signalling pathways that drive osteoblastogenesis to promote bone formation by increasing the number of cells participating in this activity. Secondly, there are mechanisms that modulate the activity of fully differentiated osteoblasts. With regard to the former, the canonical Wnt signalling pathway acting through β-catenin has a primary role in driving osteoblast proliferation and differentiation (Module 7: Figure osteoblast function). In addition, the insulin-like growth factor 1 (IGF-1), which acts through the PtdIns 3-kinase signalling pathway, promotes osteoblast survival.

A number of pathways have been invoked with regard to the regulation of the osteoblasts once they have differentiated. The cyclic AMP signalling pathway, which is activated or inhibited by different agonists, controls the expression of the extracellular matrix components, such as collagen I and osteocalcin responsible for bone formation. They also control the expression of RANKL that is

Module 7: | Figure bone cells



Functional organization of bone cells

Bone remodelling depends upon a functional interaction between the three major cell types. Bone formation is carried out by the osteoblasts (green) that lie on the cell surface. As the bone forms, it traps the osteoblasts that gradually transform into osteocytes (blue), which have long extensions that make contact via gap junctions both with themselves and with the other two cell types (red arrows). Bone resorption is carried out by the multinucleated osteoclasts.

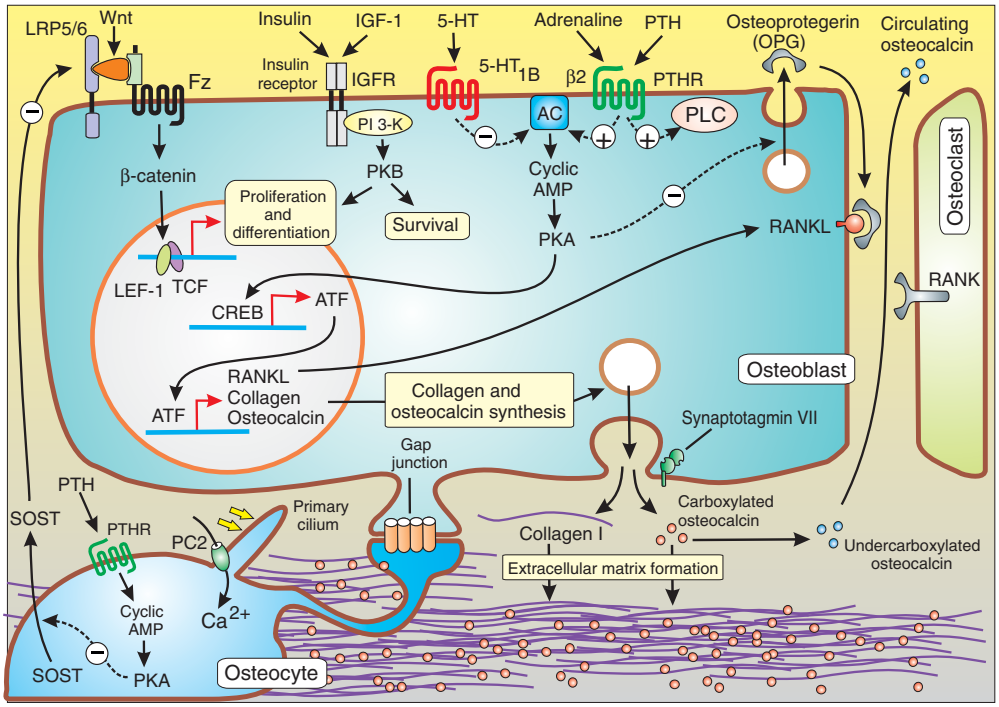
part of a feedback interaction with the osteoclasts (Module 8: bone cell differentiation). Positive signals for bone formation are provided by β2 adrenoreceptors that activate adenylyl cyclase (AC) and parathyroid hormone (PTH) that also stimulates AC, but may also be coupled to phospholipase C (PLC). The cyclic AMP signalling pathway, which results from AC activation, stimulates protein kinase A (PKA) that then phosphorylates various transcription factors. Phosphorylation of CREB increases expression of activating transcription factor (ATF), which then increases expression of RANKL, collagen I and osteocalcin. The collagen I and osteocalcin are released to form the bone matrix. The RANKL is inserted into the membrane where it functions as an agonist to stimulate RANK in the osteoclasts. In addition, the PKA inhibits the formation and release of osteoprotegerin (OPG), which inhibits this interaction between RANKL and RANK and thus enhances the activation of osteoclasts. This RANKL/RANK pathway, which enables osteoblasts to excite osteoclasts, enhances bone resorption and opposes the bone forming function of the osteoblasts. This interaction between the osteoblasts and osteclasts is thus an example of the dynamic nature of the bone cell coupling mechanisms that ensure that bone formation and bone resorption are precisely balanced.

Osteoblasts also express 5-HT_{1B} receptors that respond to 5-hydroxytryptamine (5-HT, also known as serotonin) (Module 1: Figure G protein-coupled receptors) by inhibiting the cyclic AMP signalling pathway thus resulting in a decrease in bone resorption (Module 7: Figure osteoblast function). The circulating 5-HT is released by the enterochromaffin cells located in the gut (Module 7: Figure small intestine).

Mutations in *LRP5* which codes for the LRP5 co-receptor that operates in the Wnt signalling pathway, can cause either extremely high or low bone mass traits. A high bone mass syndrome (HBM) is caused by gain-of-function mutations of LRP5. On the other hand, loss-of-function LRP5 mutations are linked to osteoporosis pseudoglioma (OPPG). The effect of the LRP5 mutations may either influence the operation of the osteoblasts directly or they act indirectly by altering the way that the enterochromaffin cells release 5-HT.

Osteoblasts can also be considered as an endocrine cell in that the osteocalcin released during bone formation (Module 7: Figure osteoblast function) can function as a circulating hormone. Osteocalcin is released from the osteoblast in a carboylated form in that three of the Gla residues are carboxylated and this enhances its affinity for the bone matrix during extracellular matrix formation. A portion of this carboxylated osteocalcin is decarboxylated to form undercarboxylated osteocalcin that enters the circulation to function as a hormone. This circulating osteocalcin can enhance the release of insulin by the insulin-secreting β-cells, it can enhance insulin sensitivity by increasing the release of adiponectin by white fat cells and it has been

Module 7: | Figure osteoblast function



Osteoblast function in bone formation.
Bone formation is regulated by a large number of signalling pathways. The Wnt signalling pathway acts through β-catenin to control the proliferation and differentiation of the osteoblasts. The insulin-like growth factor 1 (IGF-1) acts through the PtdIns 3-kinase pathway to promote survival of the osteoblasts. The cyclic AMP signalling pathway, which is activated or inhibited by different agonists regulates the expression of the extracellular matrix components such as collagen I and osteocalcin responsible for bone formation.

reported to support the survival of germ cells by boosting testosterone production. The ability of osteocalcin to stimulate insulin release by the insulin-secreting β-cells sets up a positive-feedback loop in that the osteoblasts provide a signal that then enhances the release of insulin which then acts to increase bone formation.

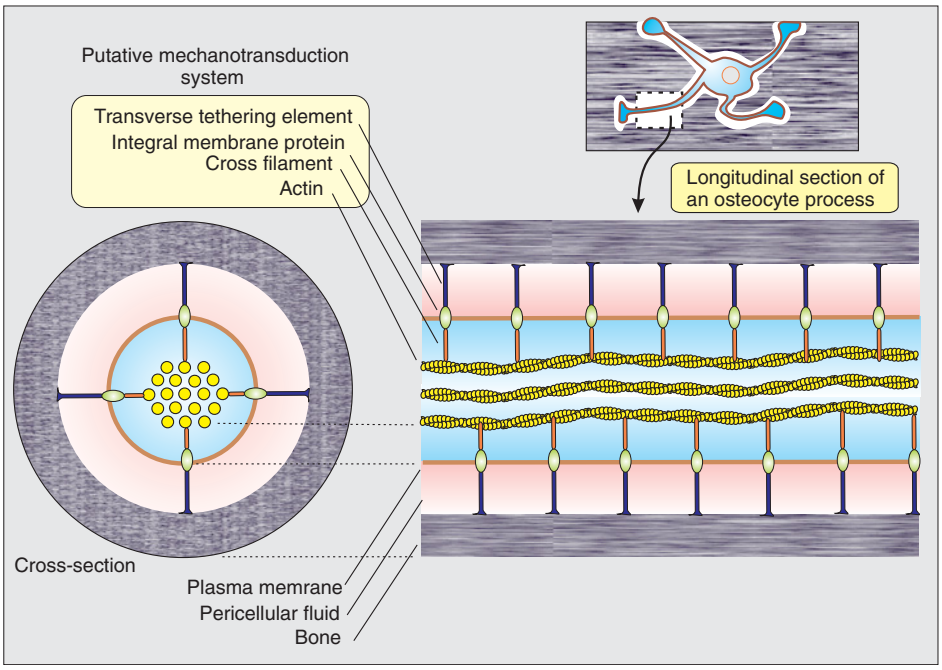
Osteocytes

Osteocytes, which are by far the most numerous of the bone cells, are derived from osteoblasts that are buried by the growing bone (Module 8: bone cell differentiation). As the osteoblasts form bone, they transform into the osteocytes that are embedded within the bone matrix. As they transform, the volume of the cell body declines and they develop long radiating extensions that lie within an interconnected canalicular system (Module 7: Figure bone cells). A tracer molecule such as horseradish peroxidase, when added to the blood, can permeate throughout this system of canals, implying that plasma can penetrate throughout the matrix. The finger-like extensions of individual osteocytes lying within this fluid-filled space communicate with the other bone cells on the surface through gap junctions. It is this interaction with the other bone cells that appears to be crucial for the function of these osteocytes and may contribute to bone cell coupling.

The osteocytes are not able to form or resorb bone, but they do appear to play a critical role in bone remodelling by modulating the activity of the osteoblasts and osteoclasts. The osteocytes appear to function as strain sensors to provide information about the degree of mechanical loading. When the loading stress on bone is increased, more bone is formed, but when loading declines, bone is removed. Osteocytes may thus play a key role in bone remodelling by regulating the balance between bone formation and resorption by the osteoblasts and osteoclasts respectively. Since they are embedded in the bone matrix, the osteocytes are in a unique position to detect minute deformations and can relay this information through their physical contacts with the osteoblasts and osteoclasts.

Just how the osteocytes detect minute bone deformations and relay this information to the other bone cells is still not clear. These cells are difficult to study because they are embedded within bone. A strain amplification hypothesis proposes that there is an elaborate interconnected strain sensory system running down the length of the long osteocyte processes (Module 7: Figure osteocyte mechanotransduction). The core of these processes contains hexagonally packed actin filaments that are connected to integral membrane proteins, which in turn, are connected to rigid transverse tethering elements that are attached to the bone lining the canals. The basic idea is that bone deformation displaces the pericellular fluid which then deflects the transverse elements to create a 'hoop strain' that will be transmitted to the actin core. Amplification might be achieved by virtue of having numerous transduction elements dispersed throughout the interconnected protein network. Just how this molecular deformation creates an intracellular signal is unclear, but one can imagine that

Module 7: | Figure osteocyte mechanotransduction



A proposed osteocyte mechanotransduction mechanism.
The irregularly shaped osteocyte has long processes lying within the canalicular system containing pericellular fluid. The processes contain a core of hexagonally packed actin filaments, which have cross filaments connecting them to an integral membrane protein. The latter are connected to transverse tethering elements that are attached to the bone wall lining the canaliculi. These different elements may provide a rigid interconnected network to provide the mechanotransduction system that responds to bone deformation (see the text for further details). This figure is based on information contained in Figure 1 in Han et al. 2004, which describes the structural basis of the strain-amplification hypothesis.

tension on the integral membrane proteins might act to generate messengers, perhaps from the opening of Ca^{2+} channels. Such a mechanotransduction process is very reminiscent of the mechanoelectrical transduction process present on hair cells, where an ion channel is also connected to both the extracellular matrix and an internal actin core (Module 10: Figure tip link).

The nature of the osteocyte signals that are used to communicate with the other bone cells is uncertain. Given the apparent importance of the gap junctions, it is conceivable that small messengers such as Ca^{2+} or cyclic AMP might be passed directly to the osteoblasts and osteoclasts. Alternatively, the osteocyte might release factors such as prostaglandin E_2 (PGE_2), ATP or nitric oxide (NO). The osteocytes respond to parathyroid hormone (PTH) by decreasing the release of sclerostin (SOST) that acts to inhibit the binding to the LRP5/6 co-receptors (Module 7: Figure osteoblast function).

Osteoclasts

Osteoclasts are large multinucleated cells whose function is to resorb bone (Module 7: Figure bone cells). Osteoclasts originate from a haematopoietic stem cell (HSC) line located in the bone marrow by a process of osteoclastogenesis (Module 8: bone cell differentiation). An important determinant of bone remodelling is the balance between the number of osteoblasts and osteoclast. The rate of osteoclast formation is thus a critical factor in determining the rate of bone resorption. Once the mature osteoclasts have been formed, they are activated to begin bone resorption once they attach and spread out on the bone surface. The process of bone resorption by the osteoclast depends upon two major control mechanisms:

- Osteoclast formation
- Osteoclast activation

Osteoclast formation

An important aspect of bone remodelling is the balance between the activity of the osteoclasts (bone resorption) and osteoblasts (bone formation). One way of enhancing the contribution of the former is to increase the number of osteoclasts by stimulating their differentiation. The process of osteoclastogenesis is responsible for providing a constant supply of osteoclasts (Module 8: bone cell differentiation). The orderly development of a mature osteoclast is tightly regulated by a number of cytokines such as colony-stimulating factor-1 (CSF-1), which acts on the colony-stimulating factor (CSF-1) receptor (CSF-1R). Another key component is the tumour necrosis-related factor called receptor activator of nuclear factor κB (NF-κB) ligand (RANKL), which is located on the surface of the osteoblasts (Module 8: Figure osteoclastogenesis). The section on osteoclastogenesis contains details of the signalling pathways responsible for orchestrating the transcriptional events responsible for carrying out this developmental process (Module 8: Figure osteoclast differentiation). Once the mature osteoclasts are formed, they are highly mobile and can migrate over the bone

surface until they find an area where the bone has to be resorbed. The interaction with the bone surface triggers an osteoclast activation process that converts the mobile cell into a sedentary cell that can begin to resorb bone (Module 7: Figure osteoclast function).

Osteoclast activation

The mature osteoclast is unusual in being able to rapidly switch from being a highly mobile cell, which can migrate to different bone regions, to a sedentary cell that is polarized to carry out the process of bone resorption (Module 7: Figure osteoclast podosome). The switch to a polarized cell occurs when osteoclasts attach themselves to the bone surface. The signalling mechanisms have to control both the movement of the migrating cell and the events that occur when the cell attaches and spreads out on the bone to become a sedentary resorbing cell.

The signalling pathways responsible for activating osteoclasts are complex and not fully established. An important feature of osteoclast activation seems to depend on integrin signalling, which can generate a number of intracellular messengers (Module 1: Figure integrin receptor). In the case of osteoclasts, integrin signalling takes place in the podosome, which is a specialized adhesion site that is used by the cell both when it is migrating and as it spreads out to begin resorbing bone (Module 7: Figure osteoclast podosome). The signalling events that occur within this attachment site are described in the section on the osteoclast podosome. Here we will concentrate on the events that occur when the osteoclast begins to settle down on the bone surface to begin the process of resorption as outlined in the following sequence of events Module 7: Figure osteoclast function:

1. Integrin receptors appear to play a central role in activating osteoclast bone resorption. An interesting feature of this integrin signalling is that it can be bidirectional (Module 1: Figure integrin receptor). The two components of *inside-out* and *outside-in* signalling may be particularly relevant to osteoclast activation. When the osteoclast begins to mature, the colony-stimulating factor-1 receptor (CSF-1R) may activate the integrin receptors, and this inside-out signalling may increase their sensitivity and hence their ability to interact with bone (Module 7: Figure osteoclast function). The initial interaction between the osteoclast and bone depends on the osteoclast podosome (Module 7: Figure osteoclast podosome).
2. As the podosomes form, the integrin receptors may switch to an outside-in signalling mode (Module 7: Figure osteoclast function), and this appears to provide the signals that trigger the release of proteolytic enzymes and the fusion of the lysosome with the ruffled membrane resulting in the insertion of the vacuolar H^+ pump and CLC-7, which is a H^+-Cl^- exchanger. The latter is associated with its auxiliary β-subunit Ostm1 that regulates the trafficking and stability of CLC-7. The vacuolar H^+ pump and CLC-7 combine to form the hydrochloride acid (HCl) that initiates the resorption of the mineral component of bone. This removal of Ca^{2+} and PO_4^{2-} precedes the hydrolysis of the organic matrix by enzymes such as matrix metalloproteinase 9 (MMP-9) and cathepsin K. The process of exocytosis and subsequent uptake of degradation products by endocytosis results in the formation of a ruffled membrane that characterizes the apical surface of the osteoclast.
3. The podosomes are also responsible for remodelling the actin cytoskeleton to form an actin ring that runs around the periphery. During this final attachment phase, a tight seal is formed that functions to isolate the resorption pit.
4. The low pH within the resorption pit begins to dissolve the mineral content of the bone, and the enzymes hydrolyse the extracellular matrix proteins.
5. Uptake of these degradation components by endocytosis is the first event of a process of transcytosis whereby the various products of bone resorption are ferried across the osteoclast in vesicles that then fuse with the basal surface.
6. The ephrin (Eph) receptor signalling system plays a critical role in the way the osteoclasts and the osteoblasts communicate with each other as part of the bone cell coupling mechanism. A feature of Eph signalling is that it can operate in both forward and reverse modes (Module 1: Figure Eph receptor signalling). In the case of these bone cells, ephrin-B2 blocks osteoclast differentiation and function, whereas the EphB4 receptors promote osteoblast differentiation (Module 7: Figure osteoclast function). In this way, the osteoblasts are recruited in to the resorption pit to begin the process of bone formation to replace that recently removed by the osteoclasts.
7. Calcitonin acting through the calcitonin receptor (CTR), which is coupled to the cyclic AMP signalling pathway, seems to act to inhibit osteoclast activity.

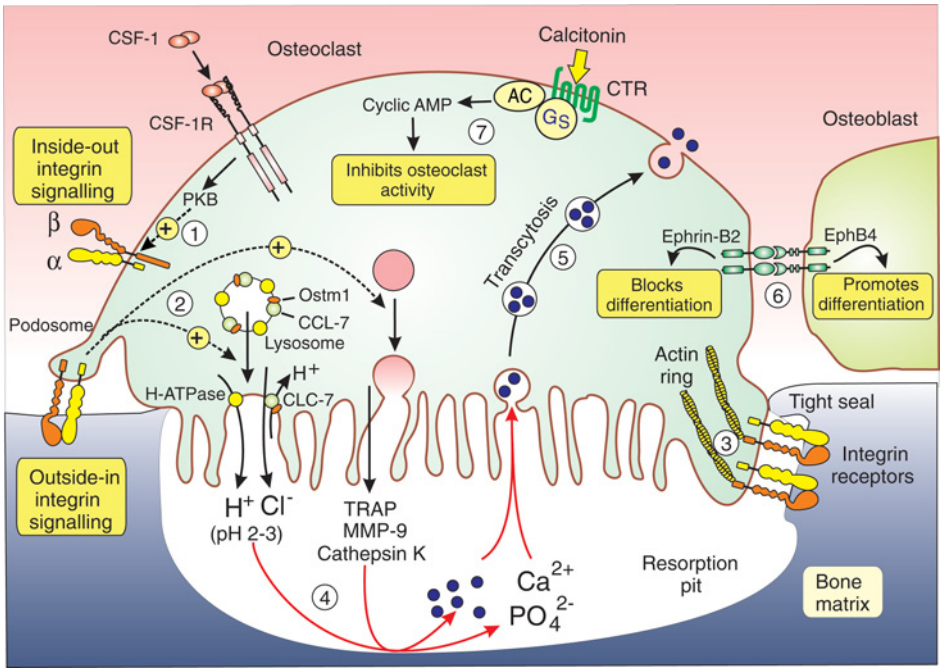
Bone cell coupling

Bone remodelling is a dynamic process where the activity of the bone forming osteoblasts is balanced by the bone resorbing activity of the osteoclasts. There is a positive balance during growth when there is net bone formation and a negative balance during ageing resulting in osteoporosis. During most of adult life, however, these two processes are balanced, and this is achieved through a tight coupling of the activities of the osteoblasts and osteoclasts. Osteopetrosis is a rare inherited disorder caused by mutations that interfere with the osteoclast activation mechanism. Although there is considerable information on osteoblast-to-osteoclast signalling, there is less information on the reverse process of osteoclast-to-osteoblast signalling. These two cell types use a number of mechanisms to communicate with each other.

Osteobast-to-osteoclast signalling

The osteoblasts exert a profound effect on the process of osteoclastogenesis where they provide the receptor activator of nuclear factor κB (NF-κB) ligand (RANKL). They also release osteoprotegerin (OPG), which is a soluble

Module 7: | Figure osteoclast function



Control of osteoclast-dependent bone resorption.
The mature osteoclast is a polarized cell that functions to dissolve the bone matrix to form a resorption pit. The early phases of attaching to the bone surface through a podosome is shown on the left (Steps 1 and 2), whereas the more structured attachment site with its tight seal and actin ring is shown on the right (Step 3). Both the early and later attachment sites are controlled by integrin signalling. The different steps are described in more detail in the text.

RANKL-binding decoy receptor that inhibits the action of RANKL. Parathyroid hormone (PTH) promotes bone resorption by inhibiting the release of OPG and enhancing the expression of RANKL (Module 8: Figure osteoclastogenesis).

Osteoclast to osteoblast signalling

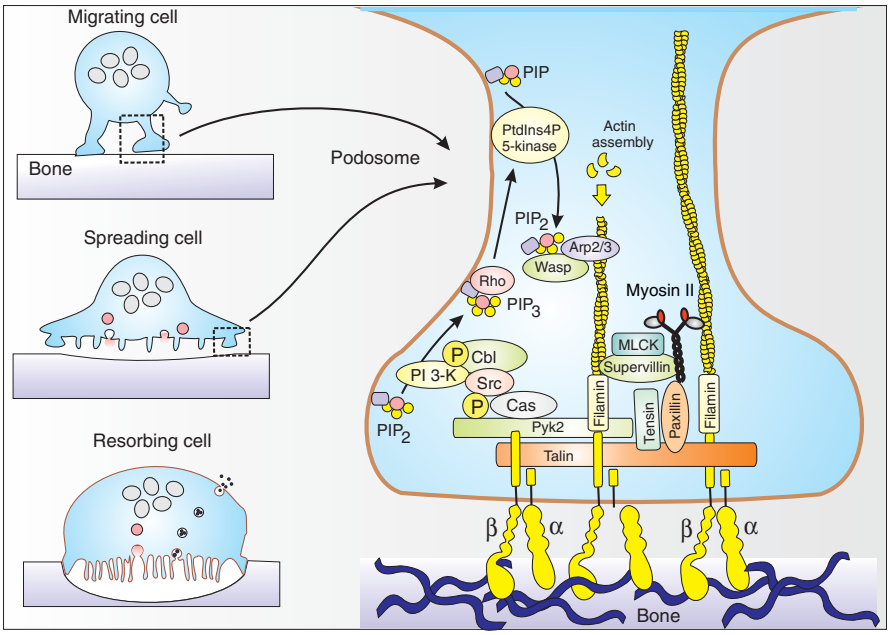
There is evidence that once osteoclasts have been activated to resorb bone, there is a simultaneous activation of the osteoblasts to replace the bone that has been resorbed. It appears that the osteoclasts orchestrate this activation of the osteoblasts through mechanisms that are still being worked out. One suggestion is that the osteoclasts may communicate with the osteoblasts through the ephrin (Eph) receptor signalling pathway (Module 1: Eph receptor signalling). The osteoclast expresses ephrin-B2, which then activates forward signalling through the EphB4 receptors to enhance osteoblast differentiation (Module 7: Figure osteoclast function). Since Eph receptor signalling is bidirectional, the reverse signalling received through ephrin-B2 simultaneously switches off the osteoclasts, thus further enhancing the ascendancy of the osteoblasts to replace the previous phase of bone resorption with bone formation. The fact that this cell communication is taking place through close cell contacts necessitated by the nature of Eph signalling means that the coupling between resorption is achieved within a highly localized region.

Osteoclast podosome

The podosome is one of the cell adhesion complexes that are used by cells to attach to the extracelluar matrix (ECM) or to interact with other cells. Podosomes contain many of the same components that are found in the focal adhesion complex (Module 6: Figure integrin signalling), but they differ from the latter in that they are much more labile. While the adhesion complexes can last for hours, podosomes turn over within minutes. The osteoclast podosome is a typical example of how this structure can rapidly remodel the actin cytoskeleton to enable both movement of the migrating cell and the formation of the actin ring that contributes to the tight seal that forms as the astrocyte spreads out to form the resorbing cell (Module 7: Figure osteoclast podosome). The podosomes are highly dynamic structures that can assemble and disassemble in a few minutes.

The rapid formation of the podosome is driven by integrin signalling pathways. Of the multiple known combinations of the α- and β-integrin subunits (Module 1: Figure integrin heterodimeric complexes), osteoclasts mainly express the α_V/β_3 heterodimer. Once the α_V subunit recognizes the RGD motif on bone matrix proteins, such as osteopontin and bone sialoprotein, the receptor undergoes a conformational change enabling the cytoplasmic regions to bring together the macromolecular complex that assembles actin filaments (Module 7: Figure osteoclast podosome). One of the earliest events is the activation of non-receptor protein tyrosine kinases such as

Module 7: | Figure osteoclast podosome



Structure and regulation of the osteoclast podosome.
The osteoclast undergoes periodic changes in its functional activity. It can migrate rapidly over the bone surface until it finds a site where it begins to attach and spreads out to form the polarized bone resorbing cell. The podosomes (dashed box), which are used by migrating cells and by cells as they spread on to the bone surface, contain the actin remodelling proteins and associated signalling components that are responsible for the periodic processes of actin assembly and disassembly. The activation and function of the highly polarized resorbing cell is shown in Module 7: Figure osteoclast function. See the text for further details.

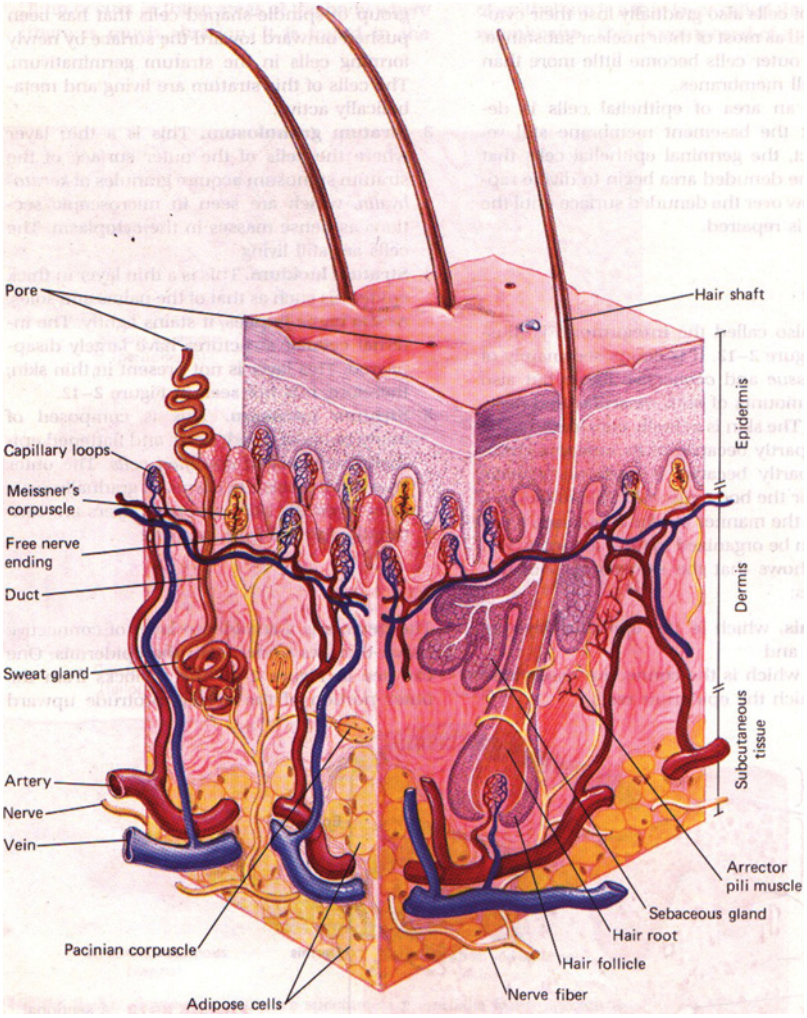
proline-rich tyrosine kinase 2 (Pyk2) and Src. Following integrin receptor activation, Pyk2 is phosphorylated on Tyr-402, which provides a binding site for the Src homology 2 (SH2) domain of Src. This binding of Src removes its autoinhibition, allowing it to phosphorylate and recruit the adaptor protein Cbl, which has two functions. Firstly, the Cbl down-regulation of signalling components (Module 1: Figure receptor down-regulation) will begin to apply the brakes to the signalling complex by initiating the proteasomal degradation of key transducing elements such as Src. Secondly, Cbl acts together with Src to recruit PtdIns 3-kinase that generates the lipid messenger PtdIns3,4,5P_3, which contributes to the processes that control actin assembly.

The monomeric G protein Rho, which is responsible for a number of signalling events related to actin remodelling (Module 2: Figure Rho signalling), is activated by PtdIns3,4,5P_3 (Module 7: Figure osteoclast podosome). One of the functions of Rho is to activate the PtdIns4P 5-kinase (PtdIns4P 5-K) that converts PtdIns4P into PtdIns4,5P_2. The latter has an important role in the process of PtdIns4,5P_2 regulation of actin remodelling that is responsible for the activation of Wiskott-Aldrich syndrome protein (WASP) and the actin-related protein 2/3 complex (Arp2/3 complex) that control actin assembly. The podosomes contain proteins such as talin, paxillin and filamin that function to attach the growing actin filaments to the β-integrin subunit. In the case of the resorbing cell, these filaments form the actin ring that contributes to the tight seal (Module 7: Figure osteoclast function). Another key feature of the podosome is a complex formed between supervillin and the cytoskeletal proteins such as actin, non-muscle myosin II (NMII) and myosin light chain kinase (MLCK). Activation of this contractile unit may result in the dissolution of the podosome.

Chondrocytes

Chondrocytes are derived from mesenchymal stem cells (MSCs) that split into two cell lineages early in developmental (Module 8: Figure MSC differentiation). The skeletal precursor line then undergoes two separate processes: chondrogenesis, which results in the formation of the chondrocytes and osteoblastogenesis that gives rise to the osteoblasts. Chondrocytes are located in articular cartilage, where they function to maintain the dynamic equilibrium between matrix synthesis and degradation. Alterations in this equilibrium result in rheumatoid arthritis (RA) and osteoarthritis (OA). These pathological changes may depend upon pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumour necrosis factor α (TNF-α), which act on chondrocytes to increase the release of matrix proteinases and to inhibit the synthesis of cartilage proteoglycans and type II collagen. ATP may also play a role because chondrocytes release this nucleotide, which then acts in an autocrine manner to enhance the release of prostaglandin E_2 (PGE_2).

Module 7: | Figure skin



Skin morphology.
Structure of the skin illustrating the organization of the different layers (dermis, epidermis and subcutaneous tissue), the blood supply and neural innervation. The hair root and follicle, sebaceous gland and sweat gland are located mainly in the subcutaneous tissue. Some of the major skin cell types are illustrated in Module 7: Figure skin cells. Reproduced from Guyton (1985).

Skin

Skin is the largest organ of the body. Its primary function is to provide a protective barrier against many environmental insults such as temperature changes, UV radiation and pathogens. It is composed of two main layers: the epidermis (composed of keratinocytes) and the dermis that contains the sensory nerve endings and blood vessels (Module 7: Figure skin). In addition, the skin also has a number of specialized cells and structures:

- Arrector pilli muscle
- Epidermal stem cells
- Hair follicle
- Melanocytes
- Melanocyte stem cells
- Merkel cells
- Sebaceous gland
- Sweat gland

In performing its barrier role, the skin suffers numerous assaults such as harmful radiation, scratches and wounds that can be repaired by a remarkable capacity for self-renewal. The adult processes of skin regeneration and repair have much in common with the process of skin development that occurs when the skin is formed in the embryo.

Epidermis

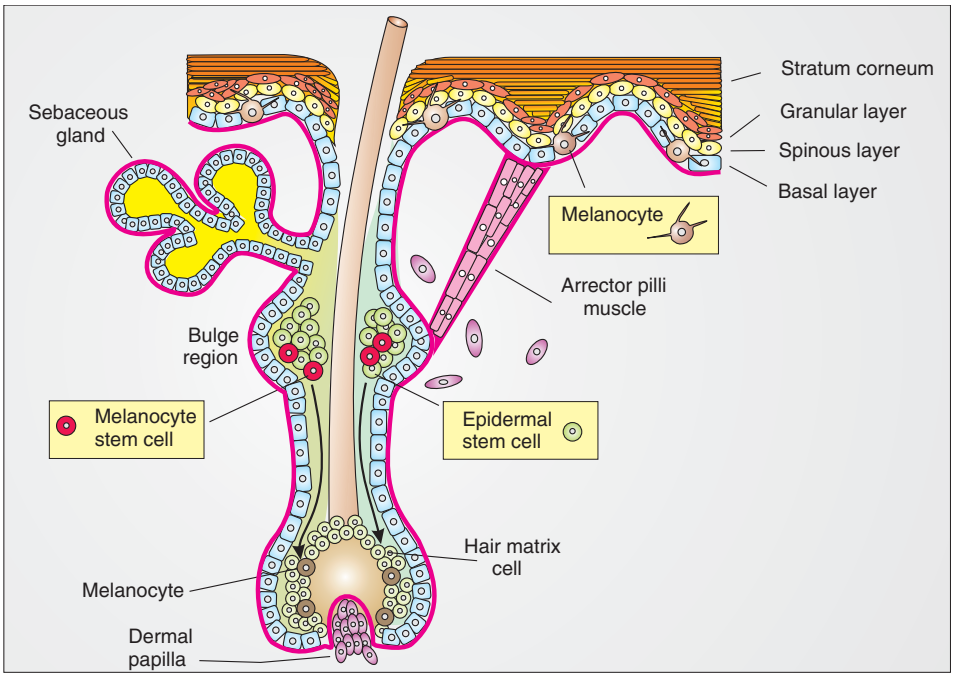
The epidermis, which is the outer epithelial layer of the skin, is a stratified squamous epithelium composed of keratinocytes (Module 7: Figure skin cells).

Keratinocytes

Keratinocytes are the cells that make up the multi-layered epidermis. The different layers consist of keratinocytes that are in different stages of the differentiation that occurs during the process of skin development and this gives rise to the different layers.

The basal layer that rests on the extracellular matrix is mitotically active and is responsible for generating the continuous supply of keratinocytes that then move

Module 7: | Figure skin cells



Location of skin cells.
The epidermis is composed of multiple layers of differentiating keratinocytes (basal layer, spinous layer, granular layer and stratum corneum). The basal layer invaginates into the hair follicle and contributes to the formation of the sebaceous gland and hair follicle. Epidermal and melanocyte stem cells reside in the bulge region and are used for skin regeneration and repair. During the hair follicle cycle, these two stem cells migrate down into the hair bud where they contribute to the hair matrix that form new hairs during the anagen phase (Module 8: Figure hair follicle cycle).

outwards and gradually transform into the other layers of the epidermis (Module 7: Figure skin cells).

The spinous layer is formed from spindle-shaped spinous cells that stop growing once they move away from the basal layer. Having withdrawn from the cell cycle, they initiate a terminal differentiation programme resembling that which occurs during skin development.

To form the granule layer, cells of the spinous layer move outwards, they begin to flatten and accumulate numerous granules.

The stratum corneum forms as the granule layers die to form the flattened cornified layers that make up the upper surface of the skin.

The way in which the keratinocytes are constantly being formed and then transformed to form the outer layers of the skin is first seen during skin development and continues to operate during skin regeneration and repair.

The keratinocytes release thymic stromal lymphopoietin (TSLP), which has been linked to the development of atopic dermatitis (AD). The TSLP then acts on sensory neurons in the skin to activate Itch (Module 10: Figure Itch signal transduction mechanism).

Dermis

The dermis is a connective tissue layer that lies beneath the epidermis (Module 7: Figure skin). The dermis contains the mesenchymal cells that contribute to the control of skin regeneration and repair. The dermis also contains the blood vessels and nerve fibres that serve the skin.

Hair follicle

Hairs are produced by the hair follicles that are a self-contained organ, which is formed from a specialized region of the epidermis that invaginates deep into the dermis (Module 7: Figure skin cells). The hair follicle is divided into two discrete regions: a permanent non-cycling upper follicle (sebaceous gland and the bulge region containing the stem cells) and a lower cycling bulb region that produces the hair and undergoes a regular hair follicle cycle (Module 8: Figure hair follicle cycle).

Arrector pilli muscle

The arrector pilli muscle functions to adjust the position of the hair shaft. This bundle of smooth muscle cells forms an attachment between the hair follicle and an infolding of the epidermis (Module 7: Figure skin cells). The neural innervation controls the contraction of this muscle, which causes the hair to 'stand on end'.

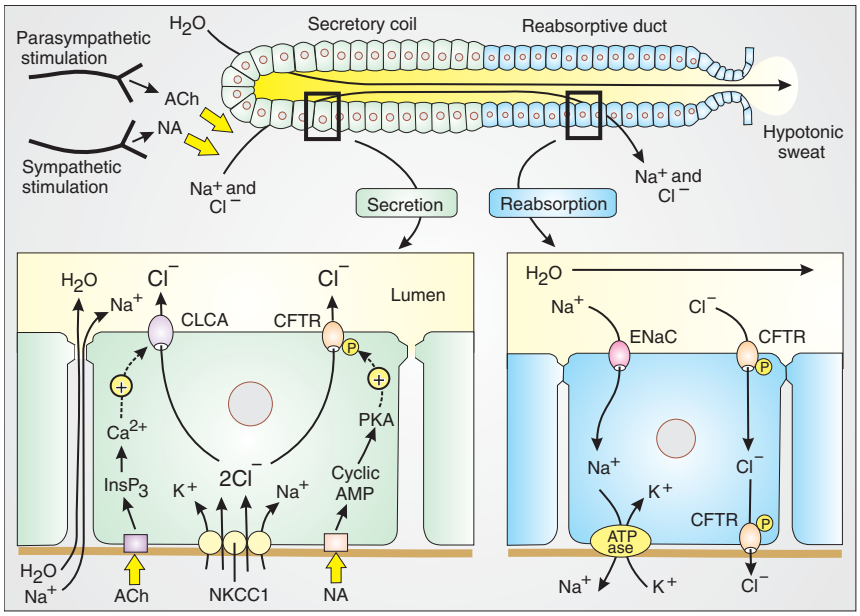
Sebaceous gland

The branched sebaceous gland is made up of epithelial cells that secrete an oily secretion called sebum that oozes out through a duct on to the hair shaft before moving out to the skin surface (Module 7: Figure skin cells).

Sweat gland

The surface of the body has approximately 20000 small tubular epithelial sweat glands (Module 7: Figure skin). They consist of two parts: about half of the 3–5mm long tube is the secretory coil that is located deep in the dermis. The

Module 7: | Figure sweat gland function



Structure and function of sweat glands.
The tubular sweat gland consists of a secretory coil that secretes an isotonic NaCl solution and a reabsorptive duct that reabsorbs Na^+ and Cl^- to produce the hypotonic sweat that flows on to the surface of the skin. The secretory coil is innervated by both parasympathetic and sympathetic neurons. See text for details of the mechanism responsible for secretion and reabsorption in the two regions of the gland. Information for this figure was obtained from Quinton (2007). Paul Quinton also provided information on the secretory and reabsorptive mechanisms.

sweat gland secretory coil cells are responsible for secreting an isotonic NaCl solution (Module 7: Figure sweat gland function).This primary secretion then passes through the straighter sweat gland reabsorptive duct cells, which reabsorbs much of the Na^+ and Cl^- resulting in the hypotonic sweat that passes out through pores on the surface of the skin.

Sweat glands are innervated by both parasympathetic and sympathetic neurons that function to regulate the formation of sweat. An increase in body temperature induces the anterior hypothalamus to stimulate the sympathetic neurons that release noradrenaline (NA), which is one of the sweat-promoting stimuli. The NA acts through the cyclic AMP signalling pathway to control fluid secretion by the sweat gland secretory coil cells. These cells are also activated by acetylcholine (ACh) released from the parasympathetic neurons.

Sweat gland secretory coil cells

The secretory coil cells, which are responsible for secreting the primary isotonic NaCl solution, are regulated by both the parasympathetic and sympathetic nervous systems (Module 7: Figure sweat gland function).

Fluid secretion by the secretory coil cells is driven by a net flux of Cl^- from the plasma into the lumen. Entry of Cl^- across the basolateral membrane is carried out by the $Na^+/K^+/2Cl^-$ 1 (NKCC1) cotransporter with the driving force supplied by the Na^+ entering the cell down its electrochemical gradient that is maintained by the classical ouabain-sensitive Na^+ pump (Na^+/K^+-ATPase). Any accumulation of K^+ is leaked back to the plasma through a K^+ channel. The accumulation of Cl^- within the cell increases the electrochemical gradient and this enables this anion to pass into the lumen through two channels: the cystic fibrosis transmembrane conductance regulator (CFTR) channel and one of the Ca^{2+}-sensitive chloride channels. The flow of Cl^- into the lumen then provides the electrical gradient for a parallel flow of Na^+ and this movement of NaCl then provides the osmotic gradient for the flow of water.

This fluid secretion can be regulated by both noradrenaline (NA) released from the sympathetic neurons or by acetylcholine (ACh) coming from the parasympathetic neurons. These two stimuli use different signalling pathways to regulate the mechanism of fluid secretion. NA acts through the cyclic AMP signalling pathway to open the CFTR that gates Cl^- across the apical membrane. On the other hand, ACh uses the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette to open the Ca^{2+}-sensitive chloride channels (CLCAs).

In patients with cystic fibrosis, the defect in the CFTR channel results in a marked reduction in the secretory response to NA, but there is no defect in the response to ACh, which uses the Ca^{2+}-sensitive chloride channels instead of CFTR.

Sweat gland reabsorptive duct cells

The reabsorptive duct cells dilute the isotonic solution produced by the secretory coil cells by removing Na^+ and Cl^- to produce hypotonic sweat (Module 7: Figure sweat gland function). The driving force for this flux of ions depends upon the Na^+/K^+-ATPase located on the

basolateral membrane that extrudes Na^+ from the cell and this provides the driving force for the uptake of Na^+ through the epithelial Na^+ channels (ENaCs) in the apical membrane. The balancing negatively charged ion is Cl^-, which enters and leaves the cell through the cystic fibrosis transmembrane conductance regulator (CFTR) channels located in the apical and basolateral membranes.

Melanocytes

Melanocytes are dendritic cells located in the basal layer of the skin epidermis (Module 7: Figure skin cells) and are also found in the uvea in the eye and also in the ear. Early in development these melanocytes, which are derived from the neural crest, migrate out into the skin where they comprise about 5–10% of the cells in the basal layer. Melanocytes are responsible for pigmentation of the hair and skin. Skin colour depends upon three main chromophores: melanin, carotenoids and haemoglobin. The red tinge of the skin is provided by haemoglobin in red blood cells circulating in the profuse blood supply in the dermis (Module 7: Figure skin). Dietary carotenoids can also appear in the skin often evident on the palms of the hand. However, the most important chromophore is melanin, which comes in two forms: pheomelanin (red/yellow) and eumelanin (brown/black). The wide variation in pigmentation within the human population is largely dependent on variations in the production of these two major forms of melanin.

Melanin plays a primary role in protecting the skin against ultraviolet (UV) radiation. This photoprotection is adaptive in that the skin darkens in response to UV radiation. This tanning response depends on a process of melanogenesis, which is controlled by an interaction between the keratinocytes and melanocytes (Module 7: Figure melanogenesis). The keratinocytes respond to UV radiation by releasing various stimuli that diffuse out to activate the melanocytes to increase their synthesis of melanin that is then packaged into melanosomes and transported down the dendrites where it is released from the tips. These melanosomes are then taken up by the keratinocytes and tend to congregate over the top of the nucleus to form an apical cap that protects DNA from harmful UV radiation.

Modifications of the complex series of biochemical events that occur during melanogenesis and their orchestration by various signalling pathways are responsible for a number of disorders:

Albinism
- Oculocutaneous albinism type 1 (OCA1)
- Oculocutaneous albinism type 2 (OCA2)
- Oculocutaneous albinism type 3 (OCA3)

Waardenburg syndrome
- Waardenburg syndrome 1 (WS1)
- Waardenburg syndrome 2a (WS2a)
- Waardenburg syndrome 3 (WS3)
- Waardenburg syndrome 4 (WS4)

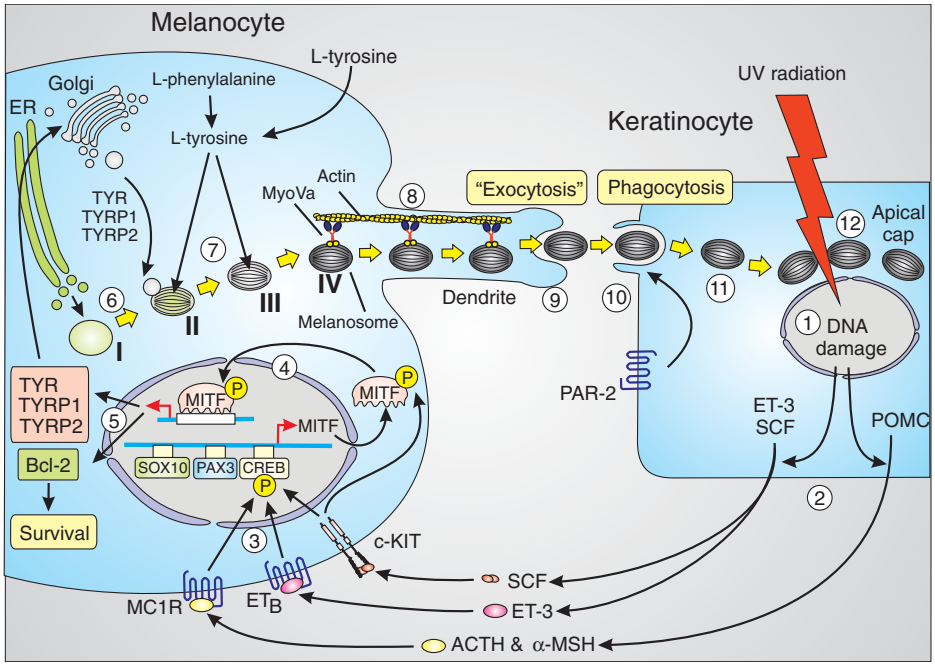
Piebaldism

Melanogenesis

Melanogenesis is the tanning process that occurs in the skin in response to ultraviolet (UV) irradiation. It depends on an interaction between the keratinocytes, which provide the stimuli to induce the melanocytes to synthesize melanin that is then transferred to the keratinocytes. The following sequence of events summarizes the key events that occur during this two-way communication between the keratinocytes and the melanocytes (Module 7: Figure melanogenesis):

1. UV radiation, which causes DNA damage, is responsible for triggering melanogenesis. Just how this occurs is unclear but it may depend upon the development of thymidine breaks.
2. As a result of UV radiation, the keratinocyte releases various stimuli. There is an increase in the formation of pro-opiomelanocortin (POMC), which is then processed to form α-melanocyte-stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH). The keratinocyte also synthesizes and releases stem cell factor (SCF) and endothelin-3 (ET-3). Mutations in SCF or its receptor c-KIT cause piebaldism.
3. The melanocytes have receptors that respond to the stimuli released from the keratinocyte. Both α-MSH and ACTH act on the melanocortin-1 receptor (MC1R), which is one of the G protein-coupled receptors (GPCRs) that are coupled to the cyclic AMP signalling pathway (Module 1: Figure stimuli for cyclic AMP signalling). Cyclic AMP acts through protein kinase A (PKA) to phosphorylate CREB. Activated CREB together with a number of other transcription factors including paired box 3 (Pax3), sex-determining region Y (SRY)-box 10 (SOX10) and lymphoid enhancer-binding factor 1 (LEF1) increase the transcription of the microphthalamia-associated transcription factor (MITF). SCF enhances both the transcription and activation of MITF by acting through the mitogen -activated protein kinase (MAPK) signalling pathway to phosphorylate CREB and MITF. Endothelin-3 (ET-3) acting through the ET_B receptor can also influence MITF transcription and this is particularly important during development. Mutations in MITF are responsible for Waardenburg syndrome 2a (WS2a).
4. MITF acts to increase the transcription of a number of the key proteins such as enzymes [e.g. tyrosinase (TYR), tyrosinase-related protein 1(TYRP1) and tyrosinase-related protein 2 (TYRP2)], which are responsible for the production of melanin and the anti-apoptotic factor Bcl-2. TYRP2 codes for dopachrome tautomerase.
5. Transcripts of the genes activated by MITF are translated at the endoplasmic reticulum (ER). Some of these proteins are then packaged into vesicles by the Golgi before being transferred to the developing melanosome. Other proteins such as Bcl-2 contribute to melanocyte survival.
6. The melanosome is a modified lysosome that has different shapes depending on the type of melanin they

Module 7: | Figure melanogenesis



Keratinocyte-melanocyte interactions during melanogenesis.
In response to UV irradiation, keratinocytes release stimuli such as α-MSH and SCF that act in a paracrine manner to stimulate melanogenesis by the melanocytes. The sequence of events (1–12) that occur during melanogenesis are described in the text. Adrenocorticotropic hormone (ACTH); ETB, endothelin receptor B: ET-3, endothelin-3; MC1R, melanocortin-1 receptor; α-MSH, α-melanocyte-stimulating hormone; MITF, microphthalamia-associated transcription factor; PAR3, proteinase-activated receptor 3; Pax3, paired box 3; POMC, pro-opiomelanocortin; SCF, stem cell factor; SOX10, sex-determining region Y (SRY)-box 10; TYR, tyrosinase; TYRP1, tyrosinase-related protein 1; TYRP2, tyrosinase-related protein 2.

synthesize. Those that form eumelanin (as shown in Module 7: Figure melanogenesis) are elliptical characterized by a fibrillar matrix. On the other hand, those that form pheomelanin are usually rounded and have a globular matrix. There are four distinct phases of melanogenesis. Stage I depends on the fusion of vesicles coming from the endoplasmic reticulum (ER). During stage II, the fibrillar matrix appears and vesicles containing enzymes for melanin synthesis are provided by vesicles coming from the Golgi. Melanin is formed during stages III and IV.

7. Melanin is synthesized from L-tyrosine derived from two sources. It is either taken up from the plasma or it is synthesized by hydroxylation of L-phenylalanine. The L-tyrosine is taken up by the developing melanosome where it is converted into melanin to finally form the mature stage IV melanosome.
8. The mature melanosomes attache to actin and moves out along the dendrites driven by the myosin Va motor (see panel C in Module 4: Figure myosin motor).
9. Melanosomes are released from the cell at the tips of the dendrites through an unknown mechanism. The most likely mechanism may be a form of exocytosis because the melanophores express components of the classical exocytotic machinery such as the vSNAREs and tSNAREs.
10. The melanosomes released from the melanocytes are engulfed by the keratinocytes through a phagocytic mechanism. The proteinase-activated receptor 2 (PAR_2) (Module 1: Table G protein-coupled receptors), which is linked to phosphoinositide signalling, plays a role in the transfer into the keratinocytes.
11. Once inside the keratinocytes, the melanocytes migrate towards the nucleus (Module 7: Figure melanogenesis).
12. The melanocytes congregate to form an apical cap over the nucleus that functions like an umbrella to shield the DNA against UV radiation.

Mutations in MITF are responsible for Waardenburg syndrome 2a (WS2a).

Alimentary canal

The alimentary canal is a complex organ system responsible for digestion and the absorption of nutrients. It is composed of two main parts: the stomach and the intestine. The arrival of food in the stomach triggers a battery of neural and endocrine functions of the stomach designed to co-ordinate the control of acid secretion by the parietal cell. The intestine is divided into the small intestine and the colon. The small intestine has a major role in the absorption of nutrients, but it also has a role in maintaining the fluid content of the intestinal lumen by adjusting the balance between NaCl absorption and secretion. There is a small intestine neural and endocrine system that regulates ion and water transport across the small intestine. Similar mechanisms operate in the colon to regulate the

bidirectional flux of NaCl. The small intestine also contributes to Ca^{2+} homoeostasis (Module 7: Figure Ca^{2+} homoeostasis). Ca^{2+} reabsorption by the intestine is regulated by 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$].

Stomach

The stomach functions as a storage organ where food is processed into a liquid form called chyme. Secretion of acid by parietal cells located in the lining of the stomach (Module 7: Figure stomach structure) creates conditions for the early stages of digestion and also plays an important role in sterilizing the upper part of the gastrointestinal tract. The neural and endocrine functions of the stomach are responsible for providing the neural (acetylcholine), endocrine (gastrin) and paracrine (histamine and somatostatin) systems that co-ordinate the control of parietal cell secretion.

The stomach is divided into three parts, the fundus, the body and the antrum, which contain different cell types. In the fundus and body, the gastric epithelium of the stomach invaginates to form gastric glands, which are composed of mucous neck cells that secrete a mucous glycoprotein, chief (zymogen) cells that secrete pepsinogen and the parietal cells that secrete acid. There are no parietal cells in the epithelium of the antrum, but this region does have G cells that synthesize and release gastrin, which is one of the major stimuli of acid secretion.

Secretion of hydrochloric acid (HCl) by the parietal cells maintains the very low pH in the stomach and the gastric mucosa, which has developed special devices for protecting itself against acid. If this defensive system breaks down or if it is overwhelmed by excessive acid production, the outer epithelium begins to leak acid into the underlying interstitial layers causing bleeding and the development of peptic ulcers. There is thus considerable interest in understanding the neural and endocrine functions of the stomach that release the excitatory and inhibitory stimuli that regulate acid secretion.

Neural and endocrine functions of the stomach

Control of acid secretion depends upon neural, endocrine and paracrine mechanisms that are activated by different aspects of feeding (Module 7: Figure stomach structure). There is a cephalic phase (control of acid secretion by the brain) during which the smell of food and the process of ingestion generates neural signals that are conveyed to the stomach by the vagus nerve. These neural signals may be responsible for releasing the gut hormone ghrelin from the X/A-like cells. The vagus does not innervate parietal cells directly, but the signals are relayed through a network of nerve cells in the myenteric and submucous plexus, which contain the postganglionic neurons that then innervate the parietal cells. These cholinergic neurons release acetylcholine (ACh), which is a potent parietal cell stimulus. Postganglionic neurons also innervate the enterochromaffin-like cells (ECLs) to stimulate the release of histamine, which is another major activator of parietal cell secretion.

Another part of the cephalic phase that is mediated through the vagus nerve is the release of gastrin from the G cells located in the pyloric antrum. As described above, the vagus terminates at the myenteric and submucous plexus from which a postganglionic neuron releases gastrin-releasing peptide (GRP) to activate the G cells to release gastrin. This gastrin enters the bloodstream and is carried to the fundic region, where it has multiple functions to activate the parietal cells, the enterochromaffin-like cells (ECLs) and the somatostatin-secreting D cells. Separate populations of D cells, which are located in the antral region, are part of the epithelium and have an apical surface that can sample the level of H^+ as part of a negative-feedback loop. If the acid content of the stomach gets too high, there is an increase in the release of somatostatin that then switches off the release of gastrin by the G cells. This cephalic phase is controlled solely by the brain and can occur independently of any food entering the stomach.

In addition to the cephalic phase, there is a gastric phase of secretion that is initiated by the arrival of food in the stomach. Sensory neurons in the wall of the mucosa respond to stretching of the stomach, and this triggers a simple reflex through the submucous plexus to activate the postganglionic neurons that activate both the parietal and G cells as described above. Another aspect of the gastric phase is the direct stimulation of the G cells by the products of digestion such as the amino acids.

G cell

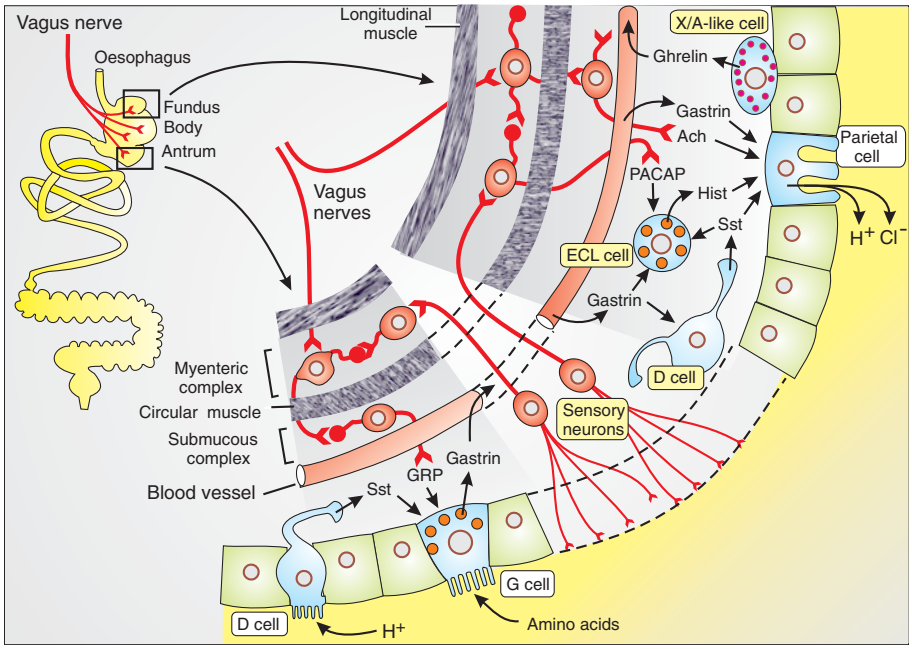
G (gastrin) cells are located in the gastric epithelium in the antral region of the stomach (Module 7: Figure stomach structure). These are unusual endocrine cells, as they are part of the gastric epithelium. They have apical microvilli that increase the surface area to enhance their ability to detect food components in the stomach such as amino acids, which act to increase the release of gastrin. The G cell is also activated via the vagus nerve, which makes contact with the myenteric and submucous complex to activate a postganglionic neuron that releases gastrin-releasing peptide (GRP) that functions to stimulate the release of gastrin.

This antral region also contains D cells that are a part of the epithelium and they function to secrete somatostatin (Sst), which inhibits the G cells. The apical membrane has microvilli, whereas the basal region has characteristically long projections that release Sst in the vicinity of the G cell.

Gastrin is an amidated heptapeptide that is synthesized and released by the G cells. Gastrin is synthesized as a large preprogastrin that undergoes a number of post-translational modifications as it passes through the endoplasmic reticulum/Golgi/granule secretory pathway:

1. The signal peptide of preprogastrin is cleaved to progastrin by a signal peptidase located on the inner membrane of the endoplasmic reticulum.
2. Progastrin enters the Golgi stacks where it is sulphated on tyrosine residues.
3. While in the Golgi, the sulphated protein also undergoes phosphorylation of Ser-96 before it is packaged into secretory granules.

Module 7: | Figure stomach structure



Neural and endocrine control of stomach acid secretion.
The stomach has three main regions: fundus, body and antrum. The two boxes on the left are expanded on the right to illustrate the neural and endocrine cells that regulate the function of the stomach. ACh, acetylcholine; GRP, gastrin-releasing peptide; Hist, histamine; PACAP, pituitary adenylyl cyclase-activating peptide (PACAP); Sst, somatostatin.

4. While in the granules, the progastrin undergoes dibasic cleavage at several sites by various prohormone convertases resulting in the formation of either G34 or G17. The next step is the removal of basic residues by carboxypeptidase E (CPE).
5. The final step is C-terminal amidation to form the active G17-NH_2, which is the active gastrin. In the case of the G34-Gly precursor, this may be amidated to G34-NH_2 which is then converted into G17-NH_2.

Gastrin released from the G cells enters the surrounding blood vessels and is carried to the fundal region where it has three main targets: it stimulates HCl secretion by the parietal cells, it stimulates histamine release by the enterochromaffin-like cells (ECLs) and it stimulates somatostatin release by the D cells.

Infection of the stomach by *Helicobacter pylori* enhances the secretory activity of these G cells, and this may contribute to the enhanced secretion of acid that causes peptic ulcer.

Zollinger-Ellison syndrome is caused by the overproduction of gastrin.

D cell

D cells are endocrine cells that release somatostatin (Sst). They are found in tissues such as the islets of Langerhans, intestine and in different regions of the stomach (Module 7: Figure stomach structure). They have long cytoplasmic processes that make intimate contact with both the parietal and G cells. Such a paracrine arrangement allows Sst to exert its inhibitory effect by being released directly on to the G cells and the parietal cells. In the case of the latter, somatostatin acts to inhibit acid secretion (Module 7: Figure HCl secretion).

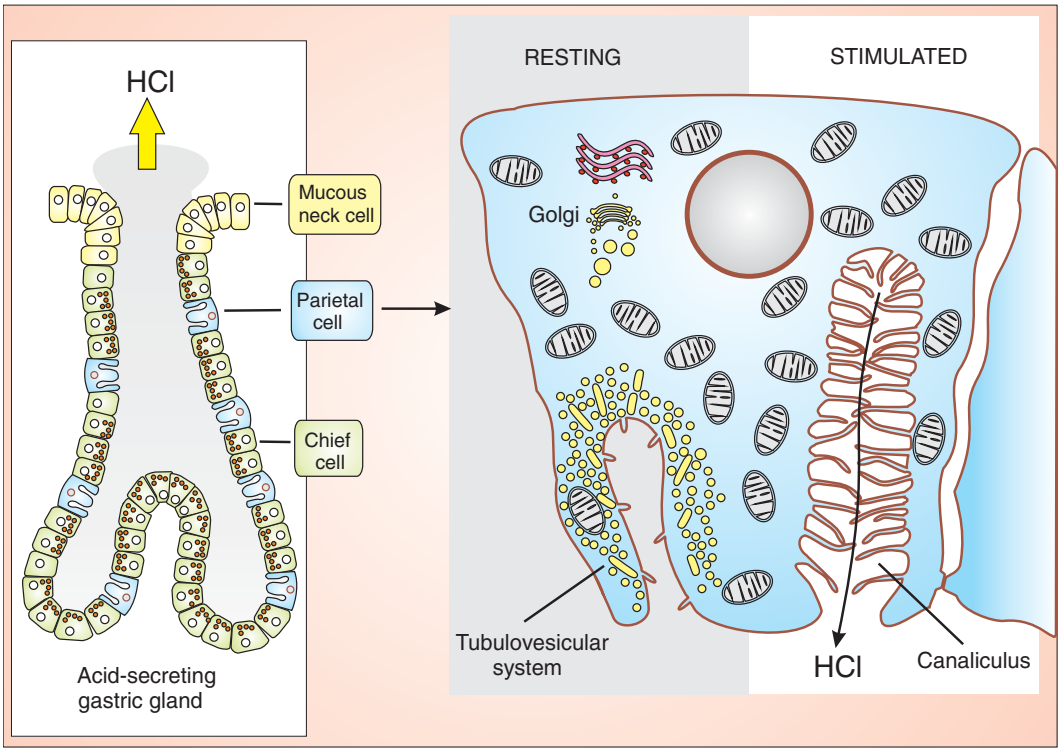
Infection of the stomach by *Helicobacter pylori* inhibits the secretory activity of these D cells and this may contribute to the enhanced secretion of acid that causes peptic ulcers.

Enterochromaffin-like cells (ECLs)

Enterochromaffin-like cells (ECLs), which resemble mast cells, are endocrine cells located within the gastric mucosa, where they contribute to the control of acid secretion by releasing histamine (Module 7: Figure stomach structure). Histamine is synthesized from histidine by a decarboxylation reaction carried out by histidine decarboxylase (HDC). The histamine is stored within large secretory vesicles that are released by a variety of stimuli. The main stimuli are gastrin, which is produced by the antral G cells and reaches the ELCs through the circulation and pituitary adenylyl cyclase-activating peptide (PACAP) that is released from the nerve endings of postganglionic neurons.

Release of histamine is controlled by Ca^{2+} signalling pathways. Gastrin acts through the cholecystokinin-B (CCK_B) receptors that are coupled to the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. In addition to driving histamine secretion, the increase in Ca^{2+} also activates transcription of genes for HDC, the vesicular monamine transporter of subtype 2 (VMAT-2), which transfers histamine into the vesicular store, and chromogranin A. The action of PACAP is unclear; the most likely possibility is for it to act through the PACAP type 1 (PAC_1)

Module 7: | Figure parietal cell



Parietal cell structure.
The epithelium of the stomach folds into gastric glands that contain mucous neck cells that communicate with the surface, HCl-secreting parietal cells and chief (zymogen) cells that secrete pepsinogen. The apical region of the resting parietal cell (shown on the left of the right-hand panel) contains a large number of vesicles and tubules of variable length. Following stimulation, this tubulovesicular system fuses with the apical membrane to form an extensive canaliculus containing a large number of thin microvilli.

receptor that is coupled to the same signalling pathway used by the gastrin receptor (CCK_B).

Prolonged stimulation of these cells can result in hyperplasia and this can contribute to Zollinger-Ellison syndrome. Such a scenario occurs in gastrinoma when there are tumours that cause an excessive secretion of gastrin.

X/A-like cell

X/A-like cells, which are located mainly in the stomach, are endocrine cells specialized for the synthesis and release of the gut hormone ghrelin. They constitute about 20% of the endocrine cell population in the stomach but are also found in other gut regions (duodenum, ileum and colon). X/A-like cells have a large population of dense-cored vesicles containing stored ghrelin, which is released on anticipation of feeding and plays a major role in the control of food intake and body weight (Module 7: Figure control of food intake).

Parietal cell

Parietal cells function to secrete acid into the stomach. They are located in the main body and, to a lesser extent, in the fundic region of the stomach (Module 7: Figure stomach structure). The gastric mucosa contains a large number of gastric glands, which open to the surface through gastric pits (Module 7: Figure parietal cell). The glands are composed of parietal cells interspersed amongst the chief (zymogen) cells that release pepsinogen. The mucous neck cells at the surface secrete mucous, which protects the surface against the harsh environment in the stomach.

The ultrastructure of the parietal cell reflects their function in fluid secretion. The surface area of the luminal surface is greatly enlarged through an extensive system of canaliculi that are lined with microvilli. These canaliculi are highly dynamic, depending on the degree of stimulation. In a resting cell, the canaliculi collapse in on themselves as the membrane is withdrawn into the cell to form tightly packed vesicles and short tubules known as the tubulovesicular system. When cells are stimulated to secrete acid, the collection of tubules and vesicles fuse with the apical membrane to reform the canaliculi. This fusion process seems to occur through the classical exocytotic/endocytotic cycle because the vesicles contain the usual components of the exocytotic machinery such as the *N*-ethylmaleimide-sensitive fusion protein-attachment protein receptors (SNAREs), synaptobrevin [also known as vesicle-associated membrane protein 2 (VAMP2)] and syntaxin 3.

The canaliculi are the sites where H^+ and Cl^- are secreted to provide the osmotic gradient for a parallel flow of water. The control of parietal cell secretion is carried out by neural (acetylcholine), endocrine (gastrin) and

paracrine (histamine and somatostatin) stimuli (Module 7: Figure HCl secretion).

Control of parietal cell secretion

Hydrochloric acid (HCl) secretion by the parietal cell is regulated by neural (acetylcholine), endocrine (gastrin) and paracrine (histamine and somatostatin) stimuli (Module 7: Figure HCl secretion). The key component of the ionic mechanisms responsible for acid secretion is the H^+/K^+-ATPase, which exists in two states. In the resting cell, the inactive enzyme is located on the tubulovesicular system (Module 7: Figure parietal cell). Following stimulation, canaliculi are formed when the vesicles and tubules fuse with the apical membrane and results in the activation of the H^+/K^+-ATPase that begins to transport H^+ into the lumen of the canaliculi in exchange for K^+ (Module 7: Figure HCl secretion). The extrusion of H^+ provides the driving force for the parallel flux of Cl^- and water. A continuous supply of H^+ is provided by carbonic anhydrase that combines CO_2 and water to form H^+ and HCO_3^-. The latter is then extruded across the basolateral membrane by a Cl^-/HCO_3^- exchanger, which also supplies the influx of Cl^- used for acid secretion at the canaliculus. This elegant fluid secretory mechanism is controlled by the fusion of the tubulovesicular system with the canaliculus that results in the activation of the HCO_3^-/Cl^- exchanger.

The stimuli that control acid secretion all activate signalling pathways that converge on this fusion event. The neural signal acetylcholine acts through the muscarinic M3 receptor to induce the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. Exactly the same signalling mechanism is employed by gastrin acting through cholecystokinin-B (CCK_B) receptors. The increase in Ca^{2+} then acts to stimulate the fusion events that result in the transfer of tubulovesicles to the apical membrane. The role of Ca^{2+} is still not clear, but one of its actions is to stimulate Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) to phosphorylate the Ca^{2+}-sensitive phosphoprotein of 28 kDa (CSPP-28).

Histamine, which is one of the major secretagogues, acts on H_2 receptors that are coupled to the cyclic AMP signalling pathway. Cyclic AMP then acts through protein kinase A (PKA) to phosphorylate a range of substrates that may contribute to the translocation and fusion of vesicles with the apical membrane. Two of these phosphorylated proteins (ezrin and lasp-1) function in actin remodelling. Lasp-1 appears to be an adaptor protein, which contains an N-terminal LIM domain, two internal nebulin repeats and a C-terminal Src homology 3 (SH3) domain. When lasp-1 is phosphorylated by PKA, it binds to actin and may thus contribute to reorganization of the cytoskeleton during membrane fusion. PKA also phosphorylates parchorin, which then translocates from the cytosol to the apical membrane, where it may contribute to the onset of acid secretion.

Somatostatin (Sst), which is released from the D cells (Module 7: Figure stomach structure), is a paracrine regulator that functions to inhibit acid secretion. Sst binds to an sstR2 receptor that acts through the G protein G_i to inhibit cyclic AMP formation by adenylyl cyclase (Module 7: Figure HCl secretion).

Small intestine

The main function of the small intestine is to absorb nutrients from the chyme that is periodically squirted out of the stomach into the duodenum. As the liquid chyme passes through the intestine, there are very large fluxes of ions and water in both directions. The structure of the small intestine reveals the presence of a highly infolded epithelium that is backed up by a complex interstitium containing layers of smooth muscle cells and a small intestine neural and endocrine system that controls small intestine fluid secretion (Module 7: Figure small intestine).

Structure of the small intestine

The intestine is divided into a number of regions. The chyme that leaves the stomach passes first into the duodenum, which together with the jejunum and ileum constitutes the small intestine. The ileum connects to the colon. The small intestine is a complex tissue consisting of outer layers of longitudinal and circular muscle surrounding the convoluted mucosa, which is highly infolded to produce long villi interspersed between the crypts of Lieberkühn (Module 7: Figure small intestine). Both the crypts and villi are lined with columnar epithelial cells that are responsible for nutrient absorption and the regulation of fluid absorption and secretion.

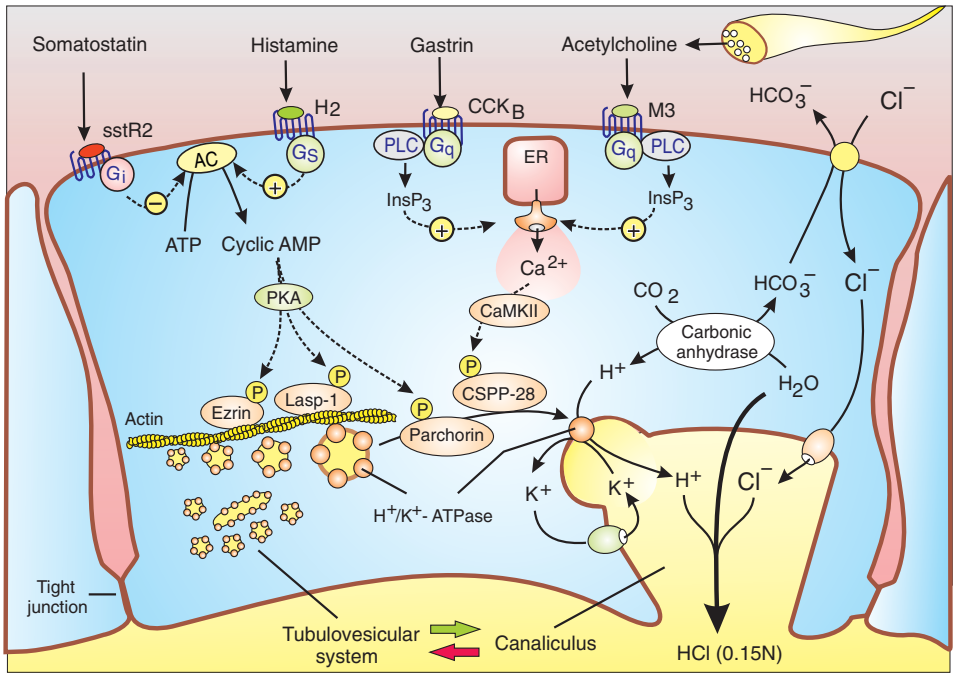
Each epithelial cell has approximately 1000 microvilli, which serve to increase the surface area available for absorption. This delicate absorptive surface is protected by a dense fluffy mucous coat of fine filaments attached to the outer surface of the microvilli. The regular organization of the microvilli is maintained by an actin cytoskeleton. Each microvillus has a bundle of actin filaments, which is attached to a cap at the tip of the microvillus. The other end of the actin bundle protrudes into the cytoplasm, where it makes contact with the terminal web.

The small intestine neural and endocrine system functions to control both intestinal motility and the transport functions of the mucosa.

Small intestine neural and endocrine system

Ion and water transport by the small intestine is regulated by stimuli originating from both neural and endocrine sources (Module 7: Figure small intestine). The intestine contains a nervous system often referred to as the enteric nervous system that rivals in complexity many parts of the central nervous system. Similar to what is found in the stomach (Module 7: Figure stomach structure), the enteric nervous system consists of a complex network of interconnecting neurons organized into the myenteric plexus and the submucous plexus (Module 7: Figure small intestine). They receive a cholinergic input from the vagal nerve and an adrenergic input from the sympathetic nervous system. This enteric neural complex functions as a local nervous system to control all of the major processes in the intestine. It regulates the activity of the longitudinal and circular layers of smooth muscle that are responsible for intestinal motility. It also contains postganglionic neurons that

Module 7: | Figure HCl secretion



Control of HCl secretion by the parietal cell.

The fluid secretory mechanism (shown on the right) depends on the transfer of H^+ and Cl^- into the canaliculus through the operation of the H^+/K^+-ATPase and a Cl^- channel respectively. These two ions then provide the osmotic gradient for a parallel flow of water. In resting cells, the H^+/K^+-ATPase is kept inactive by being withdrawn into the tubulovesicular system. During stimulation by a range of stimuli (see the text for details) the vesicles and tubules bearing the H^+/K^+-ATPase fuse with the apical membrane and secretion begins.

send out axons to release transmitters such as acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP) in the vicinity of the mucosa to regulate ion transport across the epithelium.

The small intestine is also regulated by endocrine cells such as the enterochromaffin cells that synthesize and release 5-hydroxytryptamine (5-HT). These cells are an integral part of the epithelium in that they communicate with both the lumen (mucosal) and plasma (serosal) surface. Release of 5-HT, which is controlled by stimuli arriving at both surfaces, can have a number of actions. It can stimulate the epithelial cells to increase fluid secretion. 5-HT can also provide an input into the nervous system with two important consequences. By activating neurons that are connected to the enteric nervous system, it can induce a local feedback loop resulting in the release of both ACh and VIP. Termination of the 5-HT signal is carried out by a serotonin-selective reuptake transporter (SERT). All of the epithelial cells that line the mucosa express SERT, which means that the intestine has an enormous capacity to remove 5-HT once it has been released.

The small intestine contains a population of enteroendocrine L cells that release various gut hormones such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), which contribute to the control of food intake and body weight (Module 7: Figure control of food intake).

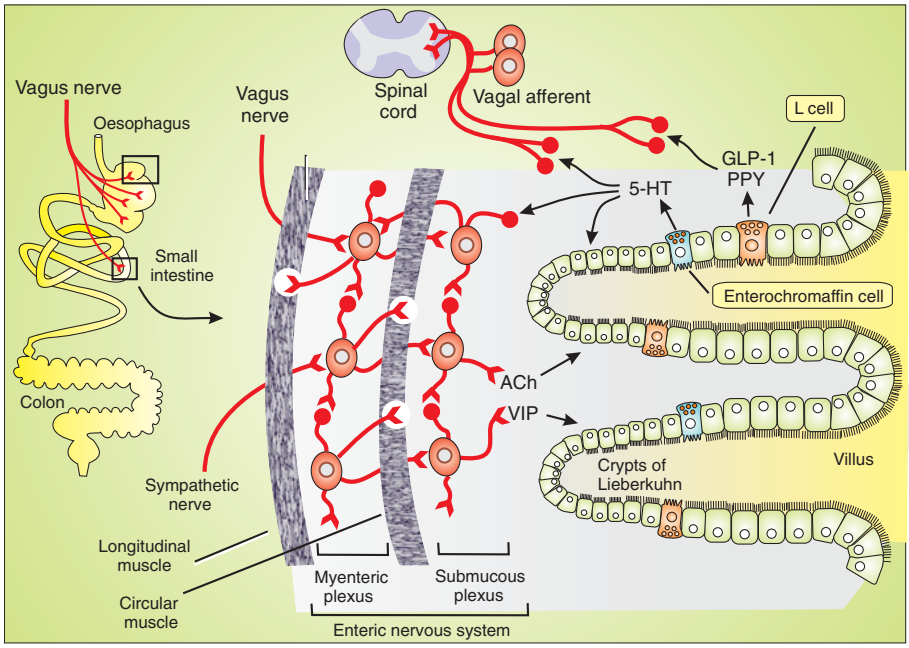
Alterations in the operation of this 5-HT signalling system might be one of the causes of irritable bowel syndrome. In addition, 5-HT can excite 5-HT_3 receptors on the endings of vagal afferents that convey information back to the brain to induce nausea and vomiting.

Enterochromaffin cells

Enterochromaffin cells (ECs) are located in the mucosa of the small intestine (Module 7: Figure small intestine) and colon. The apical surface has microvilli that project out from the mucosal surface and are thought to monitor the chemical composition of the lumen. They synthesize large amounts of 5-hydroxytryptamine (5-HT), which is accumulated in secretory vesicles by a vesicular monoamine transporter I (VMAT-1). The ECs also secrete the peptide guanylin, which functions to control intestinal fluid secretion. These vesicles are located in the basolateral pole. 5-HT is released through a Ca^{2+}-dependent mechanism that is activated by a number of different stimuli acting from both the apical and basolateral surfaces. The latter include mechanical forces, acidification of the lumen and noxious stimuli that cause nausea. The basolateral membrane appears to have muscarinic and nicotinic receptors, suggesting that the ECs are sensitive to the stimulatory action of acetylcholine. However, they also have receptors that may inhibit secretion in response to 5-HT, γ-aminobutyric acid (GABA), adenosine and somatostatin (Sst).

5-HT has an important role in regulating the intestine by acting on the enteric nervous system. It can control peristalsis, secretion and can activate vagal afferents. The latter send signals to the spinal cord that communicate the signals that lead to nausea. In addition, the released 5-HT

Module 7: | Figure small intestine



Small intestine neural and endocrine control systems.
Control of fluid secretion by the small intestine is controlled by a combination of neural and endocrine systems. The vagus and sympathetic inputs interact with the myenteric and submucous neural networks that send postganglionic endings to release vasoactive intestinal polypeptide (VIP) and acetylcholine (ACh) on to the surface of the intestinal cells. Enterochromaffin cells in the intestinal epithelium release 5-hydroxytryptamine (5-HT), which has a number of functions. L cells release various gut hormones such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1). See the text for further details.

enters the circulation where it can have peripheral actions such as the inhibition of bone resorption by the osteoblasts (Module 7: Figure osteoblast function).

There is growing evidence that changes in 5-HT signalling, which is an integral part of the small intestine neural and endocrine system, may be responsible for many of the symptoms associated with irritable bowel syndrome.

L cells

L cells are enteroendocrine cells that are located in the intestinal tract where they function to synthesize and release various gut hormones such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) (Module 7: Figure small intestine). GLP-1 and PYY contribute to the control of food intake and body weight (Module 7: Figure control of food intake). Most of the L cells are located in the lower intestine and colon. L cells are activated by stimuli arriving at either the basolateral or apical membranes (Module 7: Figure L cell). The activation that occurs after feeding takes place in two phases. The first phase is triggered by acetylcholine released from neurons innervating the foregut regions. The second phase is activated by nutrients that begin to appear in the lumen especially in the lower regions of the gut. In both cases, the stimuli induce hormone release by acting on G protein-coupled receptors (GPCRs) to produce the Ca^{2+} necessary to trigger exocytosis. The nutrient receptors on the apical surface are coupled to α-gustducin ($G\alpha_{gust}$) (Module 2: Table heterotimeric G proteins), which also functions in detecting bitter compounds by type II receptor cells (Module 10: Figure taste receptor cells).

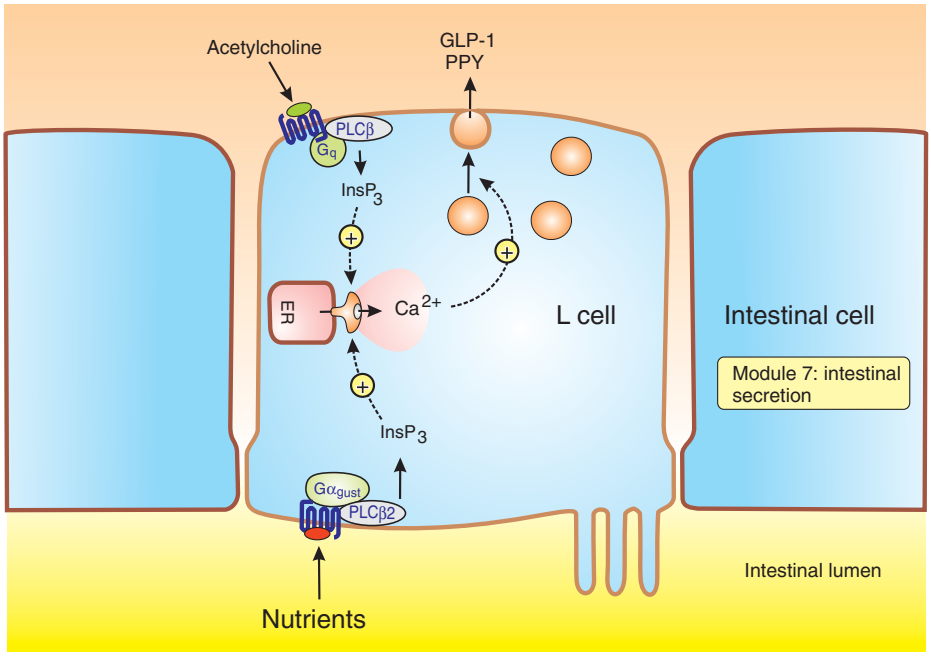
Small intestine fluid secretion

The intestine can both absorb and secrete fluid, and the amount of water lost from the intestine depends upon a balance between these two processes. Under normal conditions, the small intestine receives approximately 10 litres of water every 24 h. As this fluid passes down the intestinal lumen, water is absorbed and secreted, but the former predominates, which means that there is a net absorption of 7.5 litres. The remaining 2.5 litres enters the colon, where most of the water is absorbed, leaving only 100 ml in the faeces.

Unlike many other secretory systems, which are either active or quiescent, the intestine is continuously active, during which absorption and secretion occur continuously. The absorptive mechanism predominates and may be considered to be the resting default mode. Originally, it was thought that secretion occurred in the crypts, whereas absorption was restricted to the villi. However, recent work suggests that these two processes occur throughout the epithelium. The absorption of water is linked to the uptake of NaCl, and this absorptive mechanism is similar to that described in more detail in the colon (see Module 7: Figure colon function). Here attention will focus on fluid secretion and how it is controlled by a variety of factors.

Fluid secretion by the small intestine is driven by a net flux of Cl^- from the plasma into the lumen (Module 7: Figure intestinal secretion). Entry of Cl^- across the

Module 7: | Figure L cell



Control of L cell secretory function.
L cells are enteroendocrine cells located in the wall of the intestine where they function to release various gut hormones such as peptide YY (PYY) and glucagon-like peptide 1 (GLP-1). Acetylcholine on the basal side or nutrients in the lumen act on G protein-coupled receptors to induce the formation of inositol 1,4,5-trisphosphate that then releases Ca^{2+} to trigger hormone release. The neighbouring intestinal cells function in nutrient absorption and fluid secretion (Module 7: Figure intestinal secretion).

basolateral membrane is carried out by the Na^+-K^+-Cl^- cotransporter 1 (NKCC1) with the driving force supplied by the Na^+ entering the cell down its electrochemical gradient that is maintained by the classical ouabain-sensitive Na^+ pump (Na^+/K^+-ATPase). Any accumulation of K^+ is leaked back to the plasma through a K^+ channel. The accumulation of Cl^- within the cell increases the electrochemical gradient, and this enables this anion to pass into the lumen through two channels: the cystic fibrosis transmembrane conductance regulator (CFTR) channel and one of the Ca^{2+}-sensitive Cl^- channels (CLCAs). The flow of Cl^- into the lumen then provides the electrical gradient for a parallel flow of Na^+, and this movement of NaCl then provides the osmotic gradient for the flow of water.

This secretion of fluid by the small intestine is controlled by a number of stimuli. Of particular importance are acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP) released from the enteric nervous system (Module 7: Figure small intestine). ACh acts through the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette, and the increase in Ca^{2+} activates the K^+ and Cl^- channels responsible for fluid secretion (Module 7: Figure intestinal secretion). On the other hand, VIP acts through the cyclic AMP signalling pathway, which acts through protein kinase A (PKA) to enhance both the entry and exit of Cl^-. It phosphorylates the Na^+-K^+-Cl^- cotransporter 1 (NKCC1), which is activated to enhance the entry of Cl^-. In addition, PKA phosphorylates the R domain of the CFTR channel (Module 3: Figure CFTR channel) to increase Cl^- flux into the lumen.

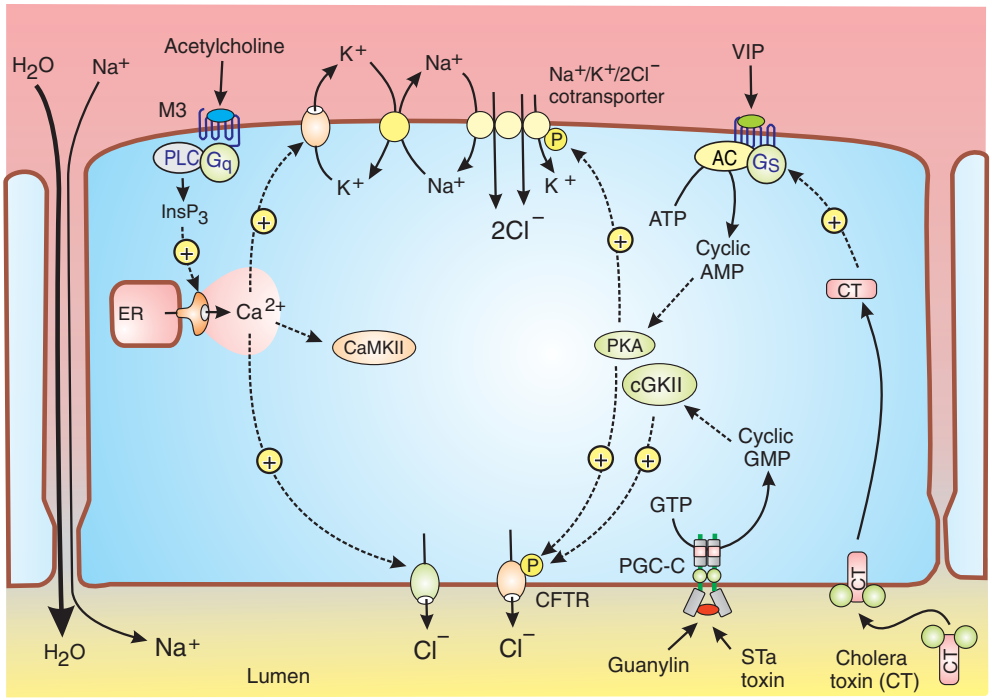
The cyclic GMP signalling pathway is switched on by the intestinal hormone guanylin, which acts on pGC-C, which is one of the particulate guanylyl cyclases (pGCs). Cyclic GMP then acts through cyclic GMP-dependent protein kinase II (cGKII) to phosphorylate CFTR (Module 7: Figure intestinal secretion).

Bacteria such as *Escherichia coli* and *Vibrio cholerae* produce toxins that induce diarrhoea by taking over some of the signalling pathways that control intestinal secretion. In the case of cholera, cholera toxin (CT) produced by *V. cholerae* stimulates intestinal secretion by activating the cyclic AMP signalling pathway.

Colon

Each day approximately 2.5 litres of fluid leaves the small intestine to enter the colon, which removes most of the water, leaving only 100 ml in the faeces. The colon is divided into proximal and distal segments that have subtly different uptake mechanisms. The overall structure of the mucosa is similar to that found in the small intestine in that it contains epithelial cells, mucous cells and a small number of enterochromaffin cells (Module 7: Figure small intestine). As for the small intestine, there is a dynamic balance between absorption and secretion, with the system biased towards net absorption to account for the almost complete removal of ions and water as fluid moves down the colon. Pathological conditions such as irritable bowel syndrome or diarrhoea are caused by alterations in this balance between absorption and secretion. For the sake of simplicity, the major ion transport mechanisms

Module 7: | Figure intestinal secretion



Control of fluid secretion by the small intestine.

Acetylcholine controls secretion by stimulating the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling pathway that controls two channels: K^+ efflux across the basolateral membrane and Cl^- flux into the lumen. Vasoactive intestinal polypeptide (VIP) uses the cyclic AMP signalling pathway to control the co-transporter that brings Cl^- into the cell and the cystic fibrosis transmembrane conductance regulator (CFTR) channel that allows it to pass into the lumen. Guanylin acts through the particulate guanylyl cyclase (pGC) to produce cyclic GMP, which also acts to open CFTR. Various toxins such as STa and cholera toxin (CT) induce secretion by activating the cyclic AMP and cyclic GMP signalling systems respectively.

responsible for both secretion and absorption will be described in the context of a generic colon cell (Module 7: Figure colon function).

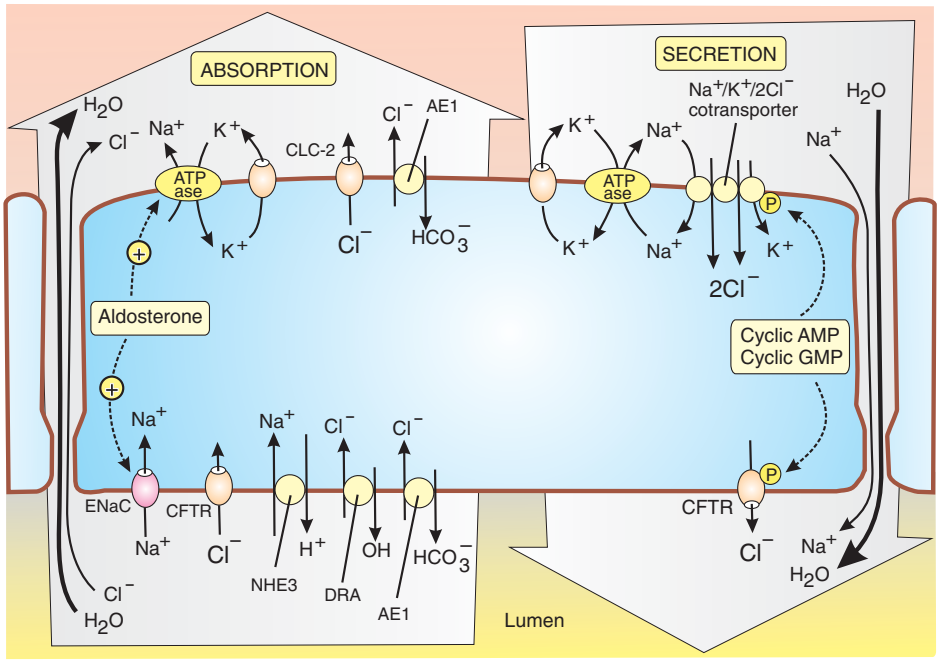
Absorption depends upon the active transport of NaCl from the lumen to the serosal side. The driving force for this flux of ions depends upon the Na^+/K^+-ATPase located on the basolateral membrane that extrudes Na^+ from the cell, and this provides the driving force for the uptake of Na^+ across the apical membrane. This uptake of Na^+ can occur through two mechanisms. Firstly, there is an electroneutral mechanism based on the type 3 Na^+/H^+ exchanger (NHE3), which is responsible for the bulk transport of Na^+. This electroneutral mechanism is enhanced by an electrogenic mechanism based on the epithelial Na^+ channel (ENaC), which is particularly prevalent in the posterior colon. The balancing negatively charged ion is Cl^-, which enters the cell through the anion exchanger type 1 (AE1) Cl^-/HCO_3^- exchanger, the Cl^-/OH^- exchanger or the cystic fibrosis transmembrane conductance regulator (CFTR) channel. A CLC-2 channel located in the basolateral membrane is also responsible for Cl^- transport across the serosal surface. In addition, Cl^- and water can move passively through the paracellular pathway.

The Cl^-/OH^- exchanger is thought to be the down-regulated in colonic adenoma (DRA) protein, which is mutated in congenital chloride diarrhoea.

Fluid secretion by the colon is driven by a net flux of Cl^- from the plasma into the lumen by mechanisms that are very similar to those in the small intestine (Module 7: Figure intestinal secretion). Entry of Cl^- across the basolateral membrane is carried out by the Na^+-K^+-Cl^- cotransporter 1 (NKCC1) with the driving force supplied by Na^+ entering the cell down its electrochemical gradient that is maintained by the classical ouabain-sensitive Na^+ pump (Na^+/K^+-ATPase) (Module 7: Figure colon function). Any accumulation of K^+ is leaked back to the plasma through a K^+ channel. The accumulation of Cl^- within the cell increases the electrochemical gradient, and this enables this anion to pass into the lumen through the CFTR. The transepithelial flow of Cl^- then provides the electrical gradient for a parallel flow of Na^+ through the paracellular pathway, and this movement of NaCl then provides the osmotic gradient for the flow of water.

A variety of signalling mechanism control the absorptive and secretory mechanisms. Aldosterone is the main regulator of Na^+ absorption in the colon, where it acts in a manner very similar to that found in the distal convoluted tubule (DCT) of the kidney. It enters the cell and binds to a mineralocorticoid receptor (MR) in the cytosol before translocating into the nucleus, where it acts to increase the expression of the basolateral Na^+/K^+-ATPase and the luminal amiloride-sensitive ENaC that act together to absorb Na^+ from the lumen. Control of fluid secretion is regulated by the cyclic AMP and cyclic GMP signalling pathways through control mechanisms that closely

Module 7: | Figure colon function



Control of fluid absorption and secretion by the colon epithelial cell.
There is a dynamic balance between fluid absorption and secretion across the epithelial cells of the colon. Aldosterone enhances absorption by increasing the expression of the Na^+ /K^+-ATPase and the epithelial Na^+ entry channel (ENaC). Secretion is activated by various intracellular messengers that control the activity of the co-transporter that brings Cl^- into the cell and the cystic fibrosis transmembrane conductance regulator (CFTR) channel that passes Cl^- into the lumen (for details, see Module 7: Figure intestinal secretion).

resemble those found in the small intestine (Module 7: Figure intestinal secretion).

Kidney

The mammalian kidney is a complex organ that is responsible primarily for excretion and osmoregulation. It removes metabolic waste products and foreign compounds taken up in the diet. With regard to osmoregulation, the kidney is responsible for blood Na^+ regulation, which is critical for maintaining normal blood pressure (Module 7 blood pressure control). These various functions are carefully regulated by hormones such as vasopressin, parathyroid hormone (PTH), angiotensin II, aldosterone and 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. For example, water balance is regulated through vasopressin-induced antidiuresis. In addition to these hormonal controls, the kidney is innervated by the sympathetic nervous system, which apparently can modulate certain processes such as blood flow. Another important regulatory system in the kidney is the tubuloglomerular feedback (TGF) mechanism, which is a local control network operated by the juxtaglomerular apparatus (JGA).

In addition to its role in excretion and osmoregulation, the kidney also functions as an endocrine organ by producing various hormones:

- It is a major player in the renin–angiotensin system (RAS), which functions in regulating blood Na^+. Through this regulation of blood Na^+ levels, the kidney controls blood volume and hence blood pressure.
- The kidney proximal tubule cells synthesize erythropoietin (EPO), which is one of the haematopoietic cytokines that stimulates stem cells in the bone marrow to produce erythrocytes (Module 8: Figure haematopoietic cytokines).
- The kidney is also responsible for 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] formation and metabolism (Module 7: Figure vitamin D metabolism). One of the main functions of 1,25$(OH)_2D_3$ is to promote Ca^{2+} reabsorption by the kidney, which occurs in the distal convoluted tubule (DCT) (Module 7: Figure kidney Ca^{2+} reabsorption).

The architectural organization of tubules and accompanying blood vessels is a major feature of kidney structure, which determines how it carries out its many functions (Module 7: Figure kidney tubule). Each tubule contains a primary cilium, which functions in mechanotransduction in kidney cells. Mutations in the polycystin channels located on these cilia cause polycystic kidney disease.

Blood Na^+ regulation

The kidney tubule is at the centre of a hormonal network that plays a major role in ensuring that the blood Na^+ level remains normal. The kidney functions as an endocrine system to release signals that regulates the processes of Na^+ filtration and reabsorption through the following sequence of events (Module 7: blood pressure control):

1. The primary function of the kidney is to filter the blood to remove waste products. The primary filtrate formed at the glomeruli contains large amounts of Na^+ and unless a large proportion of this is reabsorbed, the plasma level of Na^+ would rapidly decline.
2. As the filtrate passes down the tubule, much of the Na^+ is reabsorbed by the proximal convoluted tubule (PCT) (Module 7: Figure kidney tubule function). Much of the remaining Na^+ is taken up by the distal convoluted tubule (DCT) through a process that is carefully regulated by the hormone aldosterone.
3. A tubuloglomerular feedback (TGF) mechanism functions to control the amount of Na^+ that is reabsorbed by the DCT. The feedback mechanism is activated by the concentration of Na^+ and Cl^-, which is detected by specialized macula densa cells located in the lower regions of the tubule that forms part of the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus). If the concentration of Na^+ in the tubule is too high, resulting in large net losses of Na^+ in the urine, the macula densa cells are activated to release ATP (Module 7: Figure macula densa).
4. One of the targets of ATP released from the macula densa is the renin-producing granular cells that release renin (Module 7: blood pressure control). Another target is the smooth muscle cells that surround arteries.
5. Renin acts to stimulate the renin–angiotensin system (RAS) to produce angiotensin II, which has many functions (Module 7: blood pressure control).
6. Angiotensin II activates the zona glomerulosa cells of the adrenal cortex to increase the formation and release of aldosterone (Module 7: Figure glomerulosa cell signalling).
7. One of the actions of aldosterone is to stimulate the reabsorption of Na^+ by the DCT to reduce the urinary loss of Na^+ (Module 7: Figure kidney tubule function).
8. Angiotensin II operates a negative-feedback loop by inhibiting the release of renin by the renin-producing granular cells (Module 7: Figure renin secretion).
9. Angiotensin acts on thirst centres in the brain to increase drinking.
10. Angiotensin acts on the posterior pituitary to enhance the release of vasopressin, which increases water retention through vasopressin-induced antidiuresis (Module 7: Figure collecting duct function).
11. Angiotensin acts on smooth muscle cells to increase blood vessel constriction (Module 7: blood pressure control).

An important component of this regulation of blood Na^+ levels is the hormone angiotensin II, which is critical for the control of blood pressure. Abnormal elevation in the levels of this hormone is one of the main contributors to hypertension.

Blood pressure

Blood pressure, which provides the driving force to move blood through the vascular system, varies during the cardiac cycle of contraction (systole) and relaxation (diastole). 'Normal' blood pressure is considered to be a systolic pressure of 120 mmHg and a diastolic pressure of 80 mmHg (i.e. 120/80). However, blood pressure can vary considerably to meet different demands for oxygen and nutrients, as occur during strenuous exercise or thermogenesis. This physiological regulation of blood pressure is complex and depends upon a number of interacting systems (Module 7: blood pressure control). The pressure within the vascular system can be varied either by altering the volume of fluid or by altering the force exerted on this fluid by the blood vessels. With regard to the former, changes in blood volume result from an alteration in blood Na^+ regulation. If the Na^+ level rises, it will draw water out of the cells leading to a large blood volume and hypertension. With regard to regulation of blood vessel contractility, baroreceptors located in the aortic arch and carotid sinus send information to the brain to modify the neural input to the smooth muscle cells that surround the blood vessels.

Varying the peripheral resistance of the blood vessels is a particularly important parameter, because their diameter is a major determinant of peripheral vascular resistance, as revealed by Poiseuille's Law:

$$R = 8\eta L/\pi r^4$$

where R is the resistance to laminar flow down a cylindrical tube of length L, η is the fluid viscosity, and r is the radius of the tube.

Since the resistance is inversely proportional to the fourth power of the tube radius, it is clear that blood pressure is very dependent on the radius of the blood vessels, which can be modified by many factors such as hormones [e.g. angiotensin II and adrenaline (epinephrine)], neurotransmitters [e.g. noradrenaline (norepinephrine), ATP and acetylcholine released from the neuronal innervation] and factors released from the endothelium [e.g. nitric oxide (NO) and endothelin].

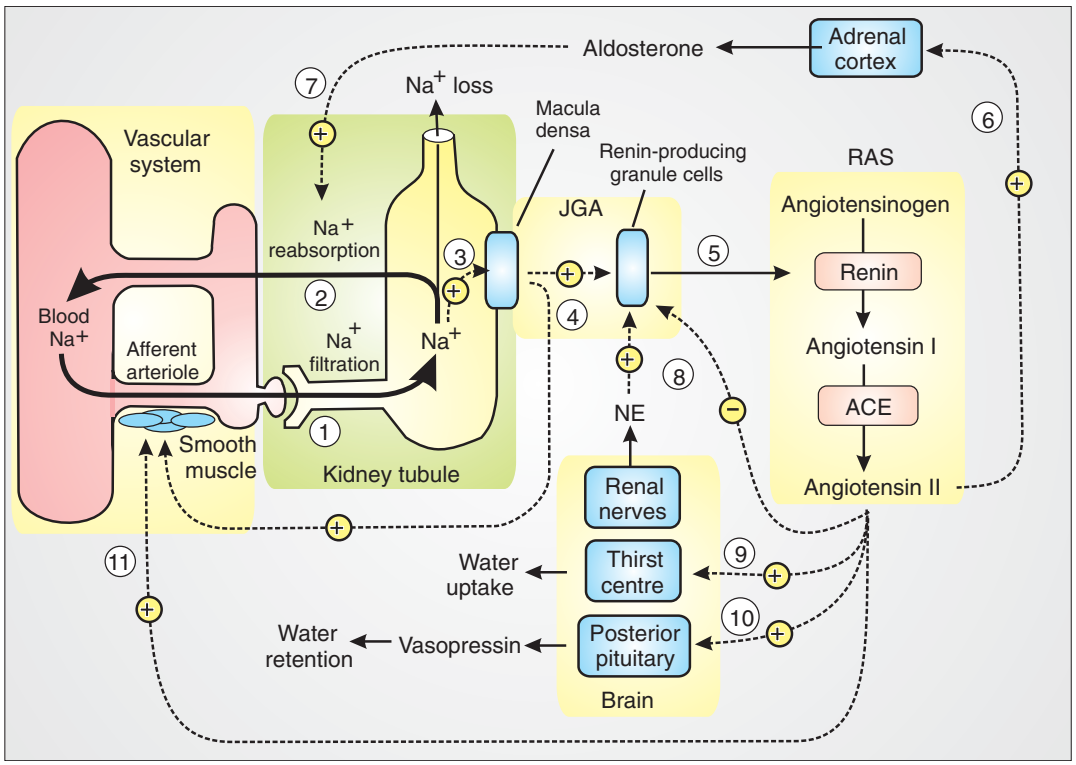
An abnormal decrease in the diameter of the blood vessels or an increase in blood volume resulting from a change in blood Na^+ regulation, results in raised blood pressure and hypertension.

Kidney structure

The two kidneys in mammals are bean-shaped organs located on the posterior wall on either side of the vertebral column. The indented region is known as the hilum, which marks the point of exit of the ureter. The renal artery and vein are also connected through the hilum. The kidney is subdivided into a series of pyramids whose apices face the calyxes that are fan-shaped collecting funnels that channel urine towards the ureter. The pyramids, which have an outer cortex and an inner medulla, contain the kidney tubules. Each kidney contains approximately one million tubules, which have a fairly consistent structure, which is crucial for their function (Module 7: Figure kidney tubule). Each kidney tubule is an autonomous unit that has its own in-built control system based on the tubuloglomerular feedback (TGF) mechanism.

A critical feature of kidney structure is the relationship of tubules to the renal arterial system. Each tubule receives

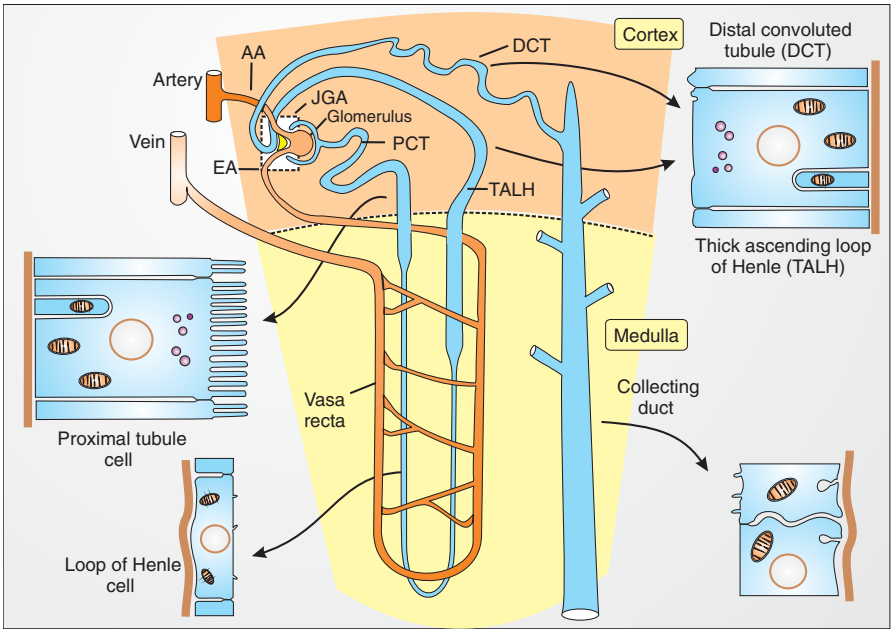
Module 7: | Figure blood pressure control



Hormonal regulation of blood Na^+ levels.

Regulation of blood Na^+ levels within the vascular system depend upon the control of Na^+ filtration and reabsorption by the kidney by a number of systems. A major player is the renin–angiotensin system (RAS) which is activated by the release of renin from renin-producing cells within the juxtaglomerular apparatus (JGA). The resulting formation of angiotensin II then acts on a number of systems as described in the text.

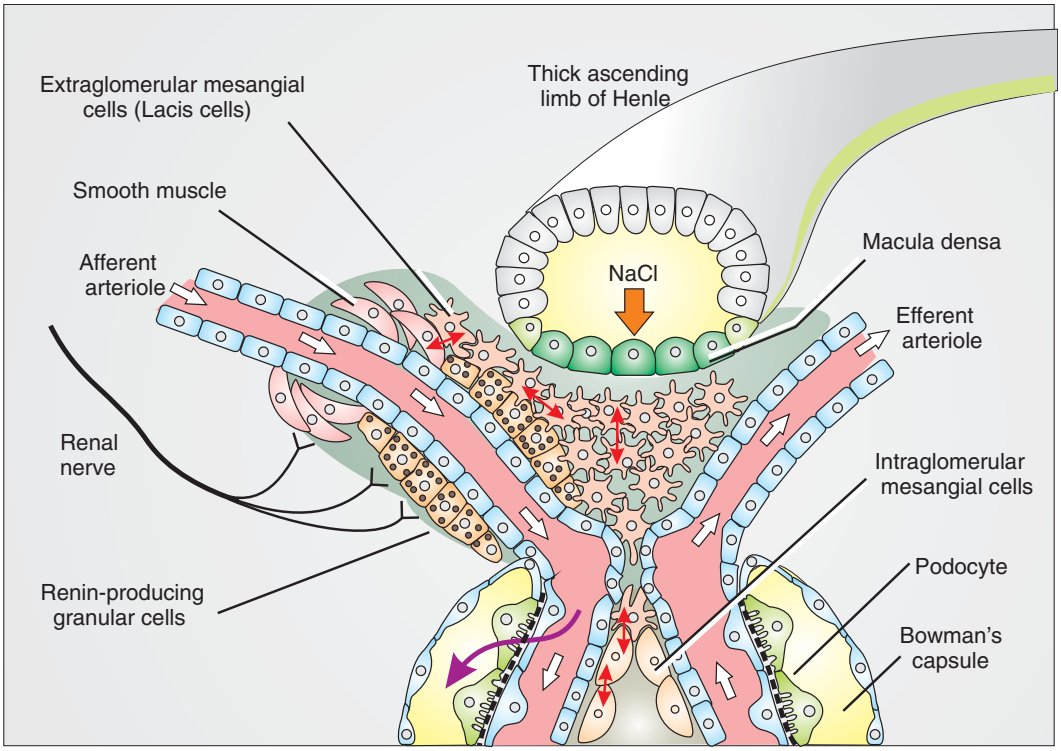
Module 7: | Figure kidney tubule



Structural organization of a kidney tubule.

The kidney tubule begins at the glomerulus where filtration occurs. The primary urine is then passed down the proximal convoluted tubule (PCT) before entering the descending segment of the loop of Henle, followed by the thin ascending limb and then the thick ascending limb of Henle (TALH). A portion of this TALH bends back and makes close contact with the glomerulus, afferent arteriole (AA) and efferent arteriole (EA) to form the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus). The TALH leads into the distal convoluted tubule (DCT) that connects to the collecting duct.

Module 7: | Figure juxtaglomerular apparatus



Structural organization of the juxtaglomerular apparatus (JGA).
Each kidney glomerulus has a juxtaglomerular apparatus (JGA) consisting of different cells that interact with each other to regulate the flow of blood through the glomerulus (white arrows). A band of smooth muscle cells control the flow of blood that enters through the afferent arteriole and leaves via the efferent arteriole. The NaCl level within the distal convoluted tubule is monitored by the macula densa cells that send stimuli to adjust the activity of smooth muscle cells, which control blood flow through the afferent arterioles. The intraglomerular mesangial cells control blood flow through the glomerular arterioles. The renin-producing granular cells release renin as part of the renin–angiotensin system (RAS). The mauve arrow illustrates the ultrafiltration pathway while the double-headed red arrows illustrate the cells that are connected via gap junctions.

an afferent arteriole that forms a glomerulus, which is a network of capillaries surrounded by a Bowman's capsule, which marks the beginning of the tubule. Urine formation begins with ultrafiltration into Bowman's capsule to form the primary urine that then flows into the proximal convoluted tubule (PCT), which then straightens out as it passes towards the medulla where it abruptly changes into the thin descending segment of the loop of Henle. This descending segment then loops back on itself and begins to move back towards the cortex where the thin ascending loop changes into the thick ascending loop of Henle (TALH). During its return passage, the TALH comes into close contact with the afferent arteriole (AA) and efferent arteriole (EA) to form the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus). Finally, the TALH leads into the distal convoluted tubule (DCT) that is connected to the collecting duct. A number of ducts drain into these collecting tubules that finally open at the tips of the pyramids.

The different parts of the tubule receive a vascular supply, which is unusual in that each artery is associated with two capillary networks arranged in series (Module 7: Figure kidney tubule). The first capillary network is that found in the glomerulus and is responsible for the process of ultrafiltration that forms the primary urine. The EA that leaves the glomerulus then divides into another capillary network which surrounds different parts of the tubule. Not shown in the figure is the extensive capillary network in the cortex. In addition, efferent vessels dip down into the medulla to form the vasa recta that extend deep into the pyramid to serve the lower parts of the loop of Henle.

Juxtaglomerular apparatus (JGA)

A consistent structural feature of each tubule is the juxtaglomerular apparatus (JGA), which is formed by the close association between the thick ascending loop of Henle (TALH) and the afferent and efferent arterioles in the immediate vicinity of the glomerulus (Module 7: Figure juxtaglomerular apparatus). In this area of close contact, there are considerable modifications in both the vascular and tubular elements. Cells of the TALH that face the JGA have a denser cytoplasm and are known as the macula densa. There are smooth muscle cells surrounding the afferent arteriole that regulate the flow of blood in to the glomerulus. These smooth muscle cells can be transformed into secretory cells and are the origin of the renin-producing granular cells. These typical secretory cells, which surround the afferent arteriole next to the glomerulus, are responsible for synthesizing and secreting renin, which is an essential component of the renin–angiotensin system (RAS). These renin-producing granular cells and

the macula densa are connected by a network of extraglomerular mesangial cells, which are also known as lacis cells, which have long ramifying extensions. These extraglomerular mesangial cells are connected to other cells of the JGA (smooth muscle cells, renin-producing granular cells and the intraglomerular mesangial cells) through gap junctions (double-headed red arrows in Module 7: Figure juxtaglomerular apparatus) that may be responsible for an intercellular Ca^{2+} wave that spreads throughout this community of cells during the operation of the tubuloglomerular feedback (TGF) mechanism.

Urine formation

Urine formation begins at the glomerulus with ultrafiltration of the blood into Bowman's capsule (mauve arrow in Module 7: Figure juxtaglomerular apparatus). The primary filtrate is selectively modified as it passes down the tubule (Module 7: Figure kidney tubule function). It is this modification of the primary filtrate by different regions of the tubule which is particularly important for homoeostasis and represents some of the main sites of action of the various hormones.

The human kidney filters about 125 ml of fluid every 1 min. This ultrafiltrate, which contains all soluble components in the plasma up to a molecular mass of 5000 Da, is formed by the first capillary network located in the glomerulus. Most plasma proteins are too large to pass through the filter and are retained to pass out through the efferent arteriole (EA). The filtration process is passive, deriving its energy from the hydrostatic pressure of blood which forces fluid through the filter. Despite changes in arterial pressure, the kidney maintains a constant rate of glomerular filtration through the operation of an autoregulatory tubuloglomerular feedback (TGF) mechanism. The primary filtrate leaves Bowman's capsule and begins its journey down the tubule by first passing through the proximal convoluted tubule (PCT).

Proximal convoluted tubule (PCT)

As described earlier, the tubules filter about 125 ml/min, which means that during the course of a day 180 litres of water will have entered the kidney tubules. Since the total blood volume is approximately 3 litres, the entire blood volume would be lost in less than 30 min, unless most of the fluid is returned to the blood. The proximal convoluted tubule (PCT) is responsible for reabsorbing almost 99% of the water in the primary filtrate. Proximal convoluted tubule cells are ideally suited for isotonic fluid absorption (Module 7: Figure kidney tubule). There is an extensive brush border, which greatly facilitates the entry of solutes and water by enlarging the surface area of the mucosal surface.

The permeability to water depends on aquaporin 1 (AQP1) (Module 3: Table aquaporin family), which is expressed in large amounts in both the apical and basolateral membranes of the PCT (Module 7: Figure proximal tubule function). The movement of water through these AQP1 channels is linked to the active transport of Na^+ (Module 7: Figure kidney tubule function). The latter enters passively across the brush border and is then extruded by the Na^+/K^+-ATPase located on the basolateral membranes. As this extrusion of Na^+ is carried out in exchange for K^+, the cell will gradually fill up with K^+ and this will begin to reduce the trans-epithelial flux of Na^+ unless this K^+ is recycled back across the basolateral membrane by the TASK-2 channel.

Some of the Na^+ that enters through the luminal membrane drives the Na^+/H^+ exchanger 3 (NHE3). The Na^+ is exchanged for H^+ that is extruded into the lumen as part of the mechanism for reabsorbing bicarbonate (HCO_3^-). Once in the lumen, the H^+ interacts with HCO_3^- to form CO_2 through an interaction catalysed by a luminal carbonic anhhdrase IV (AC IV) (Module 7: Figure proximal tubule function). An intracellular carbonic anhydrase II (AC II) converts this CO_2 into HCO_3^- and H^+. The latter is then available to carry out another HCO_3^- transport cycle across the luminal membrane. The intracellular HCO_3^- then leaves the cell via a Na^+/HCO_3^- cotransporter (KNBC1). The alkalinisation of the fluid outside the cell will activate the TASK-2 channel to increase the efflux of K^+.

The filtrate/urine leaves the PCT and enters the loop of Henle (Module 7: Figure kidney tubule function).

Loop of Henle

The transition between the proximal convoluted tubule and the descending thin limb of the loop of Henle is usually abrupt. The cells are very thin and have little membrane elaboration (Module 7: Figure kidney tubule). Cells in this descending portion of the loop of Henle play little active role in modifying the urine, but they allow passive equilibration with the interstitial fluid. Water is pulled out of the fluid, thus greatly concentrating the luminal contents (Module 7: Figure kidney tubule function). The cells in this descending portion have a high water permeability due to the expression of aquaporin 1 (AQP1) (Module 3: Table aquaporin family), which facilitates this equilibration.

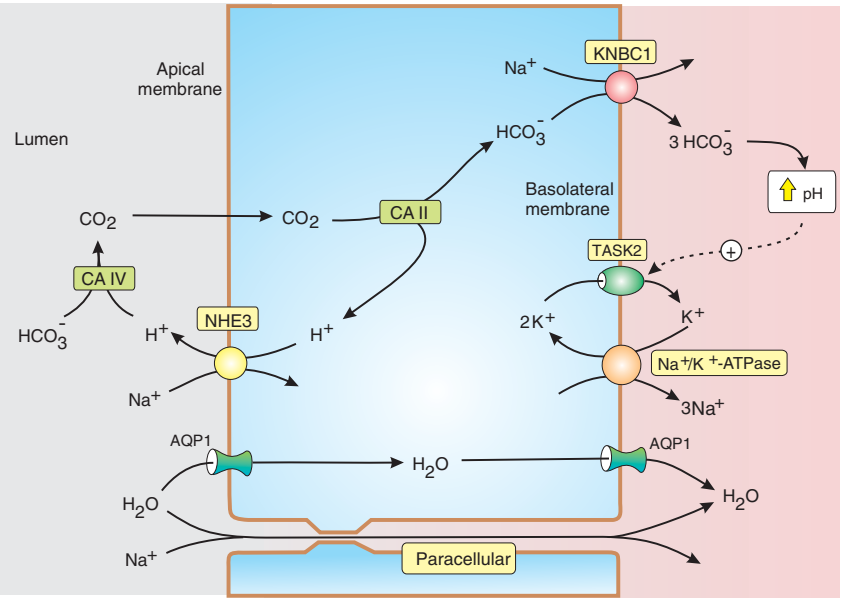
As the fluid rounds the U-bend and begins to move up the ascending thin limb, it comes into contact with cells that have a much lower water permeability because they lack the aquaporin channels. The thin cells are tightly linked together by numerous tight junctions further increasing their impermeability. This thin segment then connects to the thick ascending loop of Henle (TALH).

Thick ascending loop of Henle (TALH)

Cells of the thick ascending loop of Henle (TALH) are much larger than those in the thin segment (Module 7: Figure kidney tubule). There is little change in the structure of the apical surface, but the basal surface is much more elaborate, with numerous very-long infoldings, of which many are formed by interdigitation with cytoplasmic processes from neighbouring cells. The TALH is impermeable to water, but can absorb Na^+ and Cl^-, and this serves to dilute the urine that is passed on to the distal convoluted tubule (DCT).

The apical surface of the TALH contains the electroneutral Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2), which is the target of the loop diuretics and transports Na^+, K^+ and $2Cl^-$ from the lumen into the cell

Module 7: | Figure proximal tubule function



Proximal tubule transport of salt and water.
Absorption of ions and water by the proximal tubule is largely driven by the active transport of Na^+ that is extruded across the basolateral membrane by the Na^+/K^+-ATPase. See the text for further details.

(Module 7: Figure kidney salt reabsorption). The K^+ is returned to the lumen by the $K_{ir}1.1$ (ROMK) channel and perhaps also through the TWIK-1 channel. This recycling of K^+ is essential in order to maintain the activity of NKCC2. The Na^+ leaves the cell via the Na^+/K^+-ATPase whereas the Cl^- passes out through the CLC-Kb channel. The Ca^{2+}-sensing receptor (CaR), which is strongly expressed on the basolateral membrane of the TALH cells, responds to an increase in extracellular Ca^{2+} to produce an intracellular signal, either an increase in Ca^{2+} or a decline in the level of cyclic AMP that then inhibits NKCC2 and ROMK.

Mutations in different components of this TALH mechanism of salt reabsorption results in Bartter's disease.

A small section of the TALH comes into contact with glomerulus, where some of the tubule cells are modified to form the macula densa, which is part of the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus).

Distal convoluted tubule (DCT)

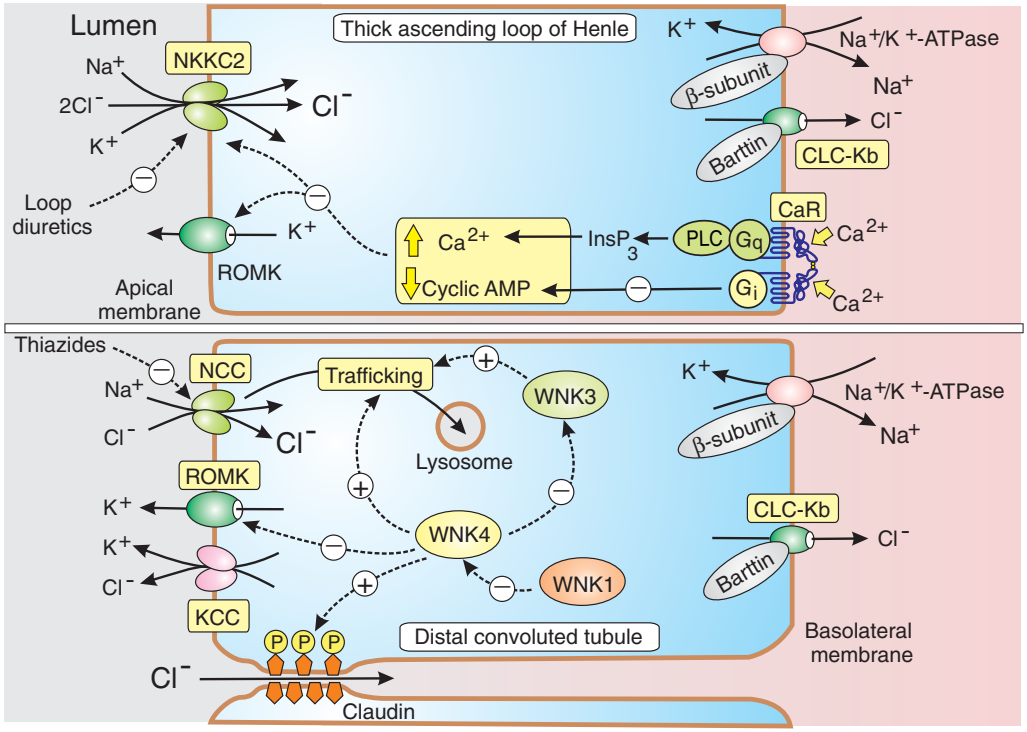
Cells of the distal convoluted tubule (DCT) resemble those of the thick ascending loop of Henle (TALH) (Module 7: Figure kidney tubule), but the infoldings of the basal membrane are less elaborate. The DCT is the site of K^+ secretion, and it also continues the process of Na^+ and Cl^- reabsorption, thus diluting the urine even further. The mechanism of salt reabsorption by cells of the DCT differs from that found in the thick ascending loop of Henle (TALH) (Module 7: Figure kidney salt reabsorption). The Na^+-Cl^- cotransporter (NCC), which is inhibited by the thiazides, transports Na^+ and Cl^- from the lumen into the DCT cell. Na^+ also enters through the amiloride-sensitive epithelial Na^+ channel (ENaC) especially in the lower reaches of the DCT and in the collecting duct. The K^+ that is secreted into the lumen crosses the apical membrane through both the $K_{ir}1.1$ (ROMK) channel and the K^+-Cl^- cotransporter 1 (KCC1). Na^+ leaves the cell via the Na^+/K^+-ATPase, which also provides some of the K^+ that is secreted into the lumen. The WNK protein kinases control the activity of both NCC and ROMK. This regulation seems to depend on the WNK4 isoform controlling the expression of these carriers in the apical membrane. For example WNK4 reduces the activity of NCC by activating its internalization and trafficking to the lysosome. WNK1 is part of the signalling cascade because it acts to inhibit the activity of WNK4.

Mutations in WNK1 and WNK4 result in the increased NCC activity responsible for the hypotension seen in Gordon's disease. On the other hand, mutations in NCC that result in a decrease in salt absorption cause the hypertension seen in Gitelman's disease.

The steroid hormone aldosterone, which is released from the adrenal cortex, regulates this uptake of Na^+ and is critical for regulating Na^+ balance. Aldosterone acts through a genomic pathway (Module 1: Figure steroid stimuli). It enters the cell and binds to a mineralocorticoid receptor (MR) in the cytosol before translocating into the nucleus, where it acts to increase the expression of the basolateral Na^+/K^+-ATPase and the luminal amiloride-sensitive epithelial Na^+ channel (ENaC) that act together to absorb Na^+ from the lumen.

The DCT is the main site for Ca^{2+} reabsorption by the kidney, which is regulated by 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] (Module 7: Figure kidney Ca^{2+} reabsorption). Fluid leaving the DCT is then passed into the collecting duct.

Module 7: | Figure kidney salt reabsorption



Reabsorption of Na^+ and Cl^- by the kidney.
The thick ascending loop of Henle (TALH) and the distal convoluted tubule (DCT) reabsorb salt using different transport mechanisms.

Mutations in proline-rich segments of ENaC cause Liddle's disease, which is a salt-sensitive form of hypertension.

Epithelial Na^+ channel (ENaC)

The epithelial Na^+ channel (ENaC) is expressed in a number of epithelia such as the kidney, intestine, pancreas, testis, lung and sweat glands. In the kidney, ENaC is expressed mainly in the cortical and distal collecting ducts. ENaC consists of three subunits (α, β and γ). The α-subunit is responsible for conducting Na^+ whereas the β and γ subunits have a regulatory function. ENAC expression is regulated by oestrogen.

The activity of ENaC depends upon its rate of membrane insertion. ENaC is ubiquitinylated by the ubiguitin ligase Nedd4-2, which binds to a PPPXY motif on the cytoplasmic tail of the α-subunit, which is then degraded by proteasomes. In Liddle's disease, this cytoplasmic region is removed thus reducing the ENaC degradation that then builds up in the membrane resulting in enhanced Na^+ reabsorption and hypertension.

Collecting duct

The collecting ducts, which are connected to a number of distal convoluted tubules (DCTs), are responsible for passing fluid down to the pyramid. The apical surface has numerous short microvilli. The basal membrane is also slightly enlarged by having short infoldings, while the lateral borders are fairly tortuous (Module 7: Figure kidney tubule). The upper regions continue the process of Na^+ reabsorption, thus contributing further to Na^+ retention and dilution of the urine. This hypotonic urine then passes through the lower regions, where water may or may not be absorbed depending on the water balance.

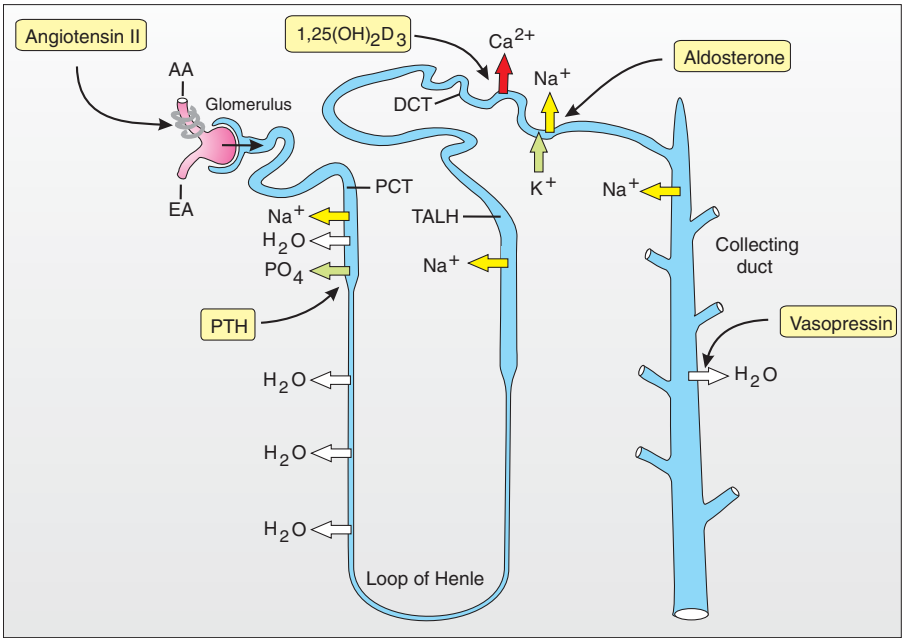
If there is an excess of water, the collecting duct cells are almost totally impermeable, thus preventing reabsorption and water is lost in the urine. During dehydration, vasopressin-induced antidiuresis conserves water by increasing the permeability of the apical membrane to draw water in from the final urine (Module 7: Figure collecting duct function). The driving force for water movement is the high concentration of solutes maintained in the interstitial space through the operation of the countercurrent mechanism.

Vasopressin-induced antidiuresis

Water excretion is regulated by the antidiuretic hormone vasopressin, which is released from the posterior pituitary. The urine entering the collecting duct is hypotonic as a result of Na^+ and Cl^- absorption by the distal convoluted tubule (DCT) and the upper regions of the collecting duct, which are impermeable to water (Module 7: Figure collecting duct function). In the absence of vasopressin, this dilute urine passes down the collecting duct, resulting in rapid diuresis. An increase in the circulating level of vasopressin reverses this diuresis by increasing the permeability of the apical membrane of the collecting duct, which is the rate-limiting barrier to water movement.

Vasopressin acts through the V_2 vasopressin receptor, which is a G protein-coupled receptor (GPCR) that acts

Module 7: | Figure kidney tubule function



Functional organization and control of kidney tubule urine formation.
The primary filtrate is formed by ultrafiltration (black arrow) at the glomerulus. Angiotensin II controls the filtration rate by acting on smooth muscle cells that control blood flow through the afferent arteriole (AA). This primary filtrate is modified as it flows through the tubule by the absorption of ions and water that are regulated by various hormones as described in the text. DCT, distal convoluted tubule; PCT, proximal convoluted tubule; PTH, parathyroid hormone; TALH, thick ascending loop of Henle.

through the cyclic AMP signalling pathway (Module 1: Table G protein-coupled receptors). The messenger cyclic AMP acts through protein kinase A (PKA) to phosphorylate Ser-256 on the C-terminal cytoplasmic tail of aquaporin 2 (AQP2) (Module 3: Figure aquaporin structure). The vesicles containing phosphorylated AQP2 molecules are linked through the dynein adaptor dynactin to the molecular motor dynein, which propels them along microtubules to the apical membrane. Once they reach the apical surface, they fuse to allow water to enter the cell. Water then leaves the cell through both AQP3 and AQP4 channels in the basolateral membrane (Module 7: Figure collecting duct function). Just how the fusion process is carried out is not clear. Many of the classical fusion proteins seem to be involved, and there also is some suggestion that there might be a localized Ca^{2+} signal to induce the fusion event. Alterations in the terminal web of actin filaments may also play a role.

Vasopressin can also increase the transcription of the AQP2 and AQP3 channels through stimulation of the transcription factor cyclic AMP response element-binding protein (CREB), which is phosphorylated on Ser-133 by protein kinase A (PKA).

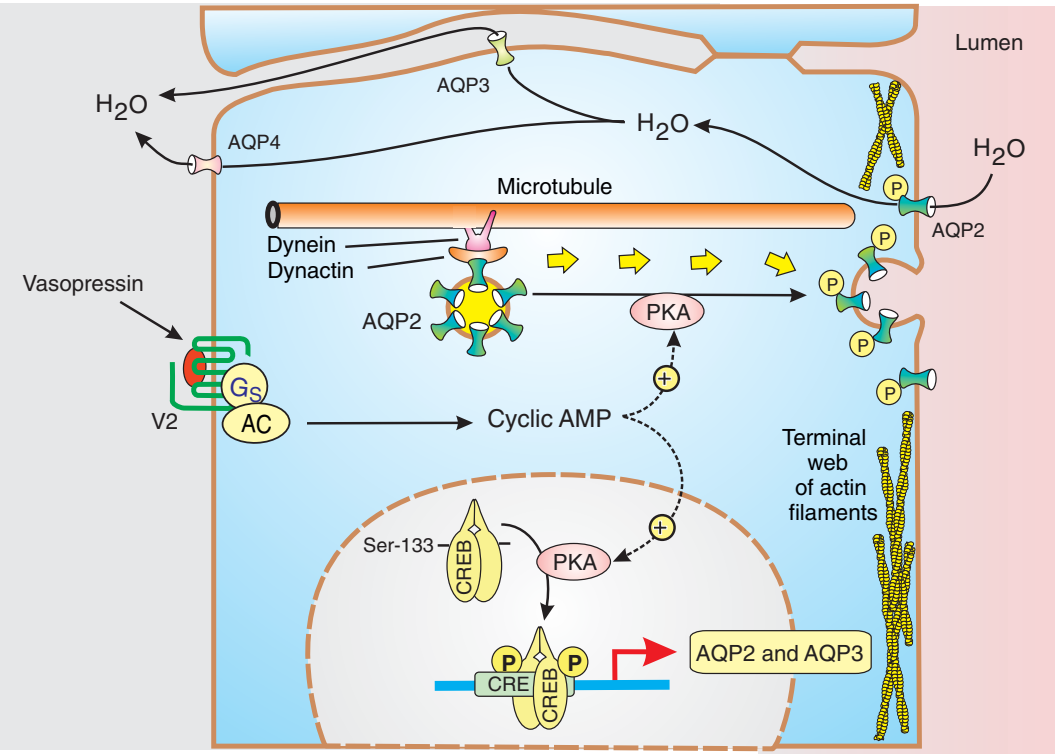
Diabetes insipidus (DI), which can have multiple causes, results from a defect in this role of the collecting ducts to reabsorb water.

Ca^{2+} reabsorption by the kidney

On a daily basis, the kidney filters about 8 g of Ca^{2+}, and all but 200 mg are reabsorbed by the kidney (Module 7: Figure Ca^{2+} homoeostasis). Reabsorption takes place in different regions down the length of the kidney tubule (Module 7: Figure kidney tubule function). Approximately 70% of this Ca^{2+} is taken back across the proximal convoluted tubule (PCT). Most of this Ca^{2+} is reabsorbed passively along a paracellular pathway (i.e. moves between the cells) driven by the reabsorption of Na^+ (Module 7: Figure kidney Ca^{2+} reabsorption). There is no absorption in the thin limbs of Henle, but reabsorption through the paracellular pathway resumes along the thick ascending limb of Henle (TALH). In these regions where paracellular transport occurs, the tight junctions are fairly permeable to enable this passive flux to occur. A protein called paracellin 1 (PCLN-1) may play a role in regulating this passive permeability to both Ca^{2+} and Mg^{2+}. Mutations in PCLN-1 may be responsible for hypomagnesaemia hypercalciuria syndrome.

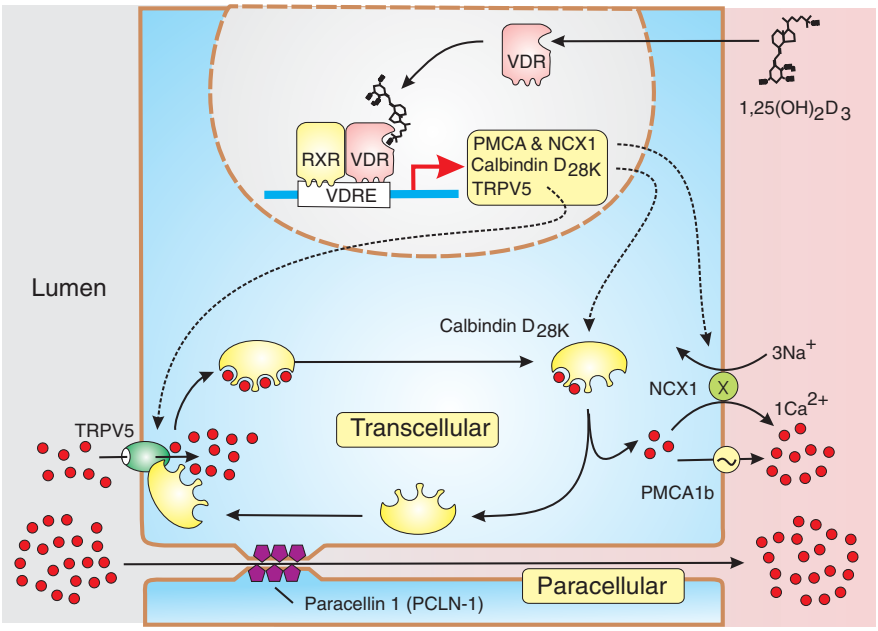
The final stages of Ca^{2+} reabsorption are completed in the distal convoluted tubule (DCT). This reabsorption occurs against the electrochemical gradient, and this active transport is carried out by the transcellular pathway, which has three main stages: Ca^{2+} entry from the lumen, diffusion across the cell and then active extrusion by pumps in the basolateral membrane. The main entry channel on the apical membrane is TRPV5, which is a member of the vanilloid transient receptor potential (TRP) ion channel family (Module 3: Figure TRP channel phylogeny). In these kidney tubules, the Ca^{2+}-dependent inactivation of TRPV5 appears to be alleviated by the Ca^{2+} buffer calbindin D-28k (CB). When the internal Ca^{2+}

Module 7: | Figure collecting duct function



Vasopressin control of water flux across the kidney collecting duct.
Vasopressin acts on V2 receptors to increase the level of cyclic AMP that then works through protein kinase A (PKA) to phosphorylate aquaporin 2 (AQP2) located in vesicles. These are transported on microtubules to the apical membrane where they fuse, enabling the phosphorylated AQP2to take up water, which leaves the cell through AQP3 and AQP4 channels in the basolateral membrane. Cyclic AMP can also phosphorylate Ser-133 on cyclic AMP response element-binding protein (CREB) to increase the transcription of both AQP2 and AQP3.

Module 7: | Figure kidney Ca^{2+} reabsorption



Control of Ca^{2+} reabsorption by kidney cells.
Transport of Ca^{2+} across kidney cells occurs through both paracellular and transcellular pathways. The paracellular pathway carries much of the initial reabsorption that occurs across the proximal convoluted tubule, whereas the transcellular pathway is responsible for the final active stage that occurs in the distal convoluted tubules. (See the text for more details.)

concentration is low, calbindin D-28k associates with TRPV5 and is thus positioned to rapidly buffer Ca^{2+} to keep the channel open. As it charges up with Ca^{2+}, the calbindin D-28k leaves the channel to diffuse across the cell to the basolateral membrane, where it gives up its cargo to the pumps that extrude Ca^{2+} thus completing the transcellular flux of Ca^{2+} (Module 7: Figure kidney Ca^{2+} reabsorption). The active transport step is the extrusion of Ca^{2+} by the Na^+/Ca^{2+} exchanger (NCX) and the plasma membrane Ca^{2+}-ATPase 1b (PMCA1b). NCX1 and PMCA1b seem to be the main isoforms in these kidney cells.

The transcellular reabsorption of Ca^{2+} is regulated by 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which acts by increasing the expression of all the main components responsible for transporting Ca^{2+} across the cell. The $1,25(OH)_2D_3$ binds to the vitamin D receptor (VDR), which then acts on the VDR-response element (VDRE) to increase the expression of TRPV5, CB, NCX1 and PMCA1b.

Tubuloglomerular feedback (TGF) mechanism

Each kidney tubule has a built-in tubuloglomerular feedback (TGF) mechanism that functions to balance glomerular filtration rate to the reabsorptive capacity of the tubule. The feedback mechanism is located within the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus) where a portion of the thick ascending loop of Henle (TALH) comes into close contact with the afferent arteriole. The composition of the fluid passing down the TALH is monitored by the macula densa cells, and any change is converted into signals that are sent back to the glomerular region to adjust the rate of fluid filtration and renal haemodynamics. As such, the TGF mechanism is an important component of blood Na^+ regulation (Module 7: Figure blood pressure control). For example, if the filtration rate is too high, the flow of solutes down the tubule overwhelms the reabsorptive capacity of the tubule resulting in a large loss of solutes, particularly Na^+ and Cl^-. The high concentration of Na^+ and Cl^- in the JGA stimulates the macula densa cells to release signals, such as ATP, to contract the arteriole smooth muscle cells, which then reduces the flow of blood and the filtration rate is reduced to match the reabsorptive capacity of the tubule. In addition, the macula densa cells also release prostaglandin E_2 (PGE_2), which regulates the activity of the renin-producing granular cells (Module 7: Figure renin secretion).

The role of the extraglomerular mesangial cells, which face the macula densa (Module 7: Figure juxtaglomerular apparatus) is not absolutely clear. They may play a critical role by initiating an intercellular Ca^{2+} wave that relays information to other cells within the JGA such as the smooth muscle cells, the renin-producing granular cells and intraglomerular mesangial cells.

Macula densa

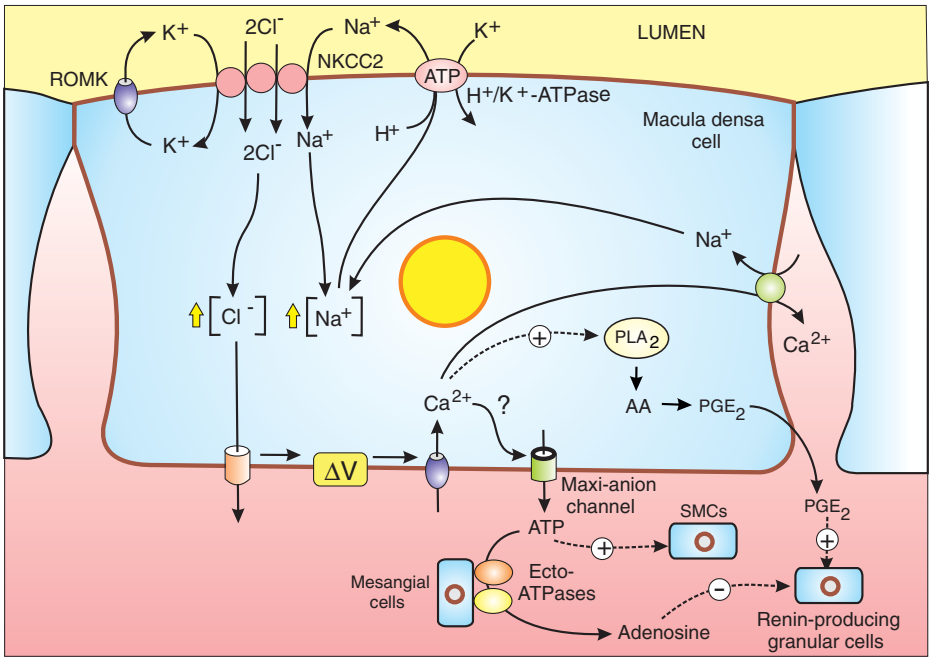
The macula densa is made up of a specialized group of cells within the thick ascending loop of Henle (TALH) that is a part of the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus). There are about 20 macula densa cells, which are part of the tubuloglomerular feedback (TGF) mechanism that respond to an increase in the concentration of Na^+ and Cl^- in the lumen of the TALH by releasing ATP that then activates the other cells of the JGA (Module 7: Figure macula densa). The signal transduction mechanism is unusual because the cells have to respond to a change in the level of ions within the lumen by stimulating the release of ATP across the opposite surface of the cell. There is general agreement that transduction begins with the furosemide-sensitive Na^+-K^+-$2Cl^-$ cotransporter 2 (NKCC2) located within the apical plasma membrane facing the lumen. In order for this transporter to continue to operate, K^+ is returned to the lumen through an inwardly rectifying K^+ channel, which seems to be a renal outer medullary K^+ channel (ROMK). The removal of Na^+ is somewhat more problematical because these macula densa cells are not very effective at extruding Na^+. They have a very weak conventional Na^+ pump (Na^+/K^+-ATPase) activity, but they do have a ouabain-sensitive colonic form of H^+/K^+-ATPase. The latter is able to extrude some Na^+ together with H^+. However, the decreased capacity to extrude Na^+ means that the intracellular level is very sensitive to fluctuations in the luminal level of this ion.

In the region of the TALH where it forms part of the JGA, the concentration of NaCl fluctuates around a value of about 25mM,which is close to the equilibrium value of the $Na^+/K^+/2Cl^-$ co-transporter. This means that the NaCl uptake mechanism is poised at a value that optimizes its ability to respond to fluctuations in the luminal level of NaCl. If the level of NaCl increases, there is an immediate increase in the uptake of Na^+ and Cl^-, resulting in an increase in the intracellular level of these two ions. Just how these increases in the intracellular levels of Na^+ and Cl^- trigger the release of ATP is still debated, but there does appear to be a requirement for an increase in the level of Ca^{2+}. One possibility is that the increase in Cl^- enhances its efflux through a basolateral Cl^- channel to cause membrane depolarization (ΔV) and that this then triggers an influx of Ca^{2+} through a channel that remains to be identified. Another possibility is that the accumulation of Na^+ slows down the Na^+/Ca^{2+} exchanger, and this will reduce Ca^+ extrusion and allow the intracellular level of this ion to increase. It remains to be seen whether or not this increase in Ca^{2+} is responsible for stimulating the release of ATP through the maxi-anion channel.

The increase in Ca^+ may also function to activate phospholipase A_2 (PLA_2) to release arachidonic acid, which is converted into prostaglandin E_2 (PGE_2) (Module 1: Figure eicosanoids). PGE_2 is released from the macula densa and is one of the stimuli responsible for activating the renin–producing granular cells.

The ATP released from the macula densa cells can function as an extracellular messenger, but it can also be converted by various ecto-ATPases into AMP and then to adenosine, which can also function as an extracelullar signal to regulate other components of the JGA such as the mesangial cells, renin-producing granular cells and the

Module 7: | Figure macula densa



Macula densa signal transduction mechanism.

Macula densa cells respond to an increase in the level of Na^+ and Cl^- in the lumen of the tubule by releasing ATP from the basolateral membrane. This ATP release through maxi-anion channels may be triggered by Ca^{2+}, which may also act through phospholipase A_2 (PLA_2) to produce prostaglandin E_2 (PGE_2), which is released to stimulate the renin-producing granular cells. ATP functions to activate smooth muscle cells that control blood flow into the glomerulus and it can also be converted by ecto-ATPases into adenosine, which acts to inhibit renin secretion (Module 7: Figure renin secretion). NKCC2, Na^+-K^+-$2Cl^-$ cotransporter 2; SMCs, smooth muscle cells; PLA_2, phospholipase A_2.

afferent arterial smooth muscle cells (Module 7: Figure renin secretion).

Renin–angiotensin system (RAS)

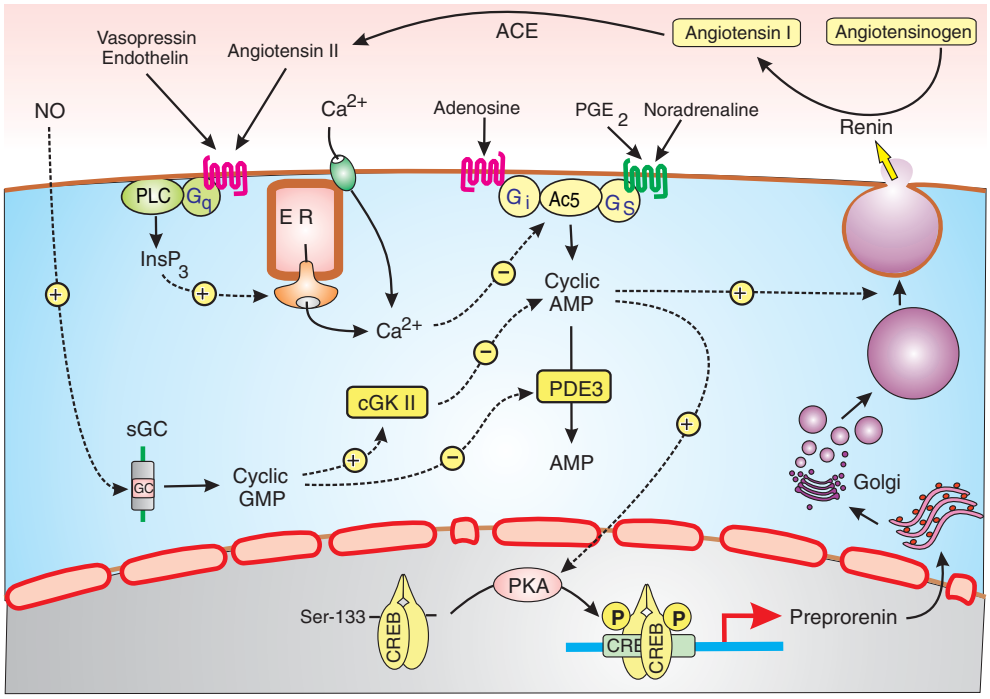
The renin–angiotensin system (RAS) plays a major role in blood Na^+ regulation (Step 5 in Module 7: Figure blood pressure control). The renin-producing granular cells secrete the enzyme renin that acts on angiotensinogen in the plasma to release angiotensin I. The latter is then converted into the hormone angiotensin II by the angiotensin-converting enzyme (ACE), which is particularly rich in the lung. Angiotensin II is a multifunctional hormone capable of stimulating a number of different cellular processes (Module 7: Figure blood pressure control):

- Angiotensin II stimulates the adrenal cortex to release aldosterone, which acts to increase Na^+ reabsorption by the kidney distal convoluted tubule (Module 7: Figure kidney tubule function).
- Angiotensin II operates a negative-feedback loop to limit the release of renin (Module 7: Figure renin secretion).
- Angiotensin II can control thirst centres to control drinking (Step 9 in Module 7: Figure blood pressure control).
- Angiotensin II can activate the posterior pituitary to release vasopressin, which then acts to increase water reabsorption by the collecting ducts (Module 7: Figure collecting duct function).
- Angiotensin II is a major activator of smooth muscle contraction (Module 7: Figure blood pressure control) and thus contributes to blood pressure control.
- The contraction and proliferation of mesangial cells is controlled by angiotensin II (Module 7: Figure mesangial cell).

Renin-producing granular cells

The synthesis and release of renin is a major component of the renin–angiotensin system (RAS) (Module 7: Figure blood pressure control). These renin-producing granular cells are also known as juxtaglomerular cells, but the latter term can be confusing because it could apply equally as well to the other cells within the juxtaglomerular apparatus (JGA) (Module 7: Figure juxtaglomerular apparatus). Therefore the more functional terminology will be used, as it relates directly to their role in synthesizing and releasing renin. The cells that release renin are modified smooth muscle cells located in the media of the afferent arteriole that feeds blood into the glomerulus. The phenotypic transformation of smooth muscle cells into the renin-secreting cells is a gradual process, and there are intermediate cells that retain the ability to contract as they begin to assemble the large granules containing renin. These granules are unlike most other Golgi-derived secretory granules in that they are modified lysosomal vesicles. The fact that these renin-containing granules are derived from lysosomes may explain there unusual secretory mechanism, which occurs independently of the classical

Module 7: | Figure renin secretion



Control of renin secretion by renin-producing granular cells.

Renin-producing granular cells are regulated by a large number of signalling systems that all seem to focus on cyclic AMP. Both the Ca^{2+} and the nitric oxide (NO)/cyclic GMP signalling pathways modulate the level of cyclic AMP. Released renin acts to hydrolyse angiotensinogen to angiotensin I, which is then converted into angiotensin II through angiotensin-converting enzyme (ACE). The angiotensin II is part of a negative-feedback loop, since it acts to increase Ca^{2+} that inhibits adenylyl cyclase 5 (AC5), thereby reducing the level of cyclic AMP.

Ca^{2+}-dependent exocytosis mechanism (Module 4: Figure Ca^{2+}-induced membrane fusion). Not only is secretion independent of Ca^{2+}, but also it is inhibited by elevations in Ca^{2+}, which is a most unusual situation. Another example of such an inverse arrangement operates in parathyroid hormone (PTH) synthesis and release (Module 7: Figure PTH secretion).

In the case of the renin-producing granular cells, this Ca^{2+} paradox seems to have been resolved by finding that the secretion of renin is controlled by the cyclic AMP signalling pathway (Module 7: Figure renin secretion). A large number of external signals regulate renin secretion and many of these interact in one way or another with cyclic AMP signalling. Noradrenaline (norepinephrine) released from the renal nerves (Module 7: Figure juxtaglomerular apparatus) and prostaglandin E_2 (PGE_2) released from the macula densa cells (Module 7: Figure macula densa) act through the heterotrimeric G protein $G\alpha_S$ to stimulate the type 5 adenylyl cyclase (AC5) (Module 2: Table adenylyl cyclases). By contrast, the adenosine derived from the ATP released from the macula densa cells (Module 7: Figure macula densa) acts through $G\alpha_i$ to inhibit AC5.

A number of stimuli (angiotensin II, endothelin and vasopressin), which act through the G protein-coupled receptors (GPCRs) that induce the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette (Module 1: Figure stimuli for $InsP_3$/DAG signalling) all function to increase the level of Ca^{2+}, which then acts to reduce the activity of the Ca^{2+}-inhibitable AC5 (Module 7: Figure renin secretion). This Ca^{2+}-dependent inhibition of cyclic AMP formation can explain why an increase in intracellular Ca^{2+} reduces the secretion of renin. The inhibitory action of angiotensin II is part of a negative-feedback loop because the released renin is part of the renin–angiotensin system (RAS) responsible for generating angiotensin II, which then feeds back to inhibit the renin-producing granular cells (Step 8 in Module 7: Figure blood pressure control).

The nitric oxide (NO)/cyclic GMP signalling pathway has a bimodal effect on renin secretion. At low levels of stimulation, cyclic GMP inhibits phosphodiesterase PDE3, which enhances the level of cyclic AMP to promote renin secretion (Module 7: Figure renin secretion). On the other hand, high levels of cyclic GMP result in the activation of cyclic GMP-dependent protein kinase II (cGKII), which then inhibits the action of cyclic AMP resulting in a reduction in renin secretion.

The cyclic AMP signalling pathway acting through the cyclic AMP response element-binding protein (CREB) transcription factor also plays an important role in regulating transcription of the preprorenin gene.

Mesangial cells

There are two types of mesangial cells. The extraglomerular mesangial cells, which are also known as lacis cells, are located below the macula densa in the cleft formed by the afferent and efferent arterioles (Module 7: Figure juxtaglomerular apparatus). The function of these mesangial

cells is not clear, but they may function to relay information to the afferent arteriole smooth muscle cells and to the renin-producing granular cells. The other mesangial cell type is the intraglomerular mesangial cells, which are specialized pericytes that function to control the flow of blood through the glomerular capillaries and thus can modulate glomerular filtration rate. They closely resemble vascular smooth muscle cells in both their morphology and embryological origins. These intraglomerular mesangial cells are highly versatile cells that regulate the flow of blood, can proliferate and also play a structural role as one of the major contributors to the formation and degradation of the extracellular matrix proteins, such as fibronectin, type IV collagen, percalan and laminin, that make up the renal filter (broken line in Module 7: Figure juxtaglomerular apparatus).

The different mesanglial cell functions are regulated by a number of signalling pathways (Module 7: Figure mesangial cell). Their contractile tone, which determines the glomerular filtration rate, depends upon a balance between contractile and relaxing hormones. The major contractile hormones are angiotensin II and endothelin-1 that act through the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. Release of Ca^{2+} from the internal stores provides an initial burst of Ca^{2+} that can initiate contraction. However, this initial release of Ca^{2+} may trigger a subsequent entry of Ca^{2+} to maintain contractile activity for longer periods. The Ca^{2+} can activate a Ca^{2+}-sensitive Cl^- channel (CLCA), which then depolarizes the membrane sufficiently to activate a voltage-operated channel (VOC) to allow Ca^{2+} to enter from the outside. Excessive increases in Ca^{2+} are avoided through a negative-feedback loop whereby Ca^{2+} activates the large-conductance (BK) channel, which increases the efflux of K^+ to hyperpolarize the membrane and thus reduce entry through the VOC. Depletion of stored Ca^{2+} can also activate store-operated channels (SOCs) that remain to be properly defined in mesangial cells.

The major relaxing stimuli are atrial natriuretic factor (ANF) and nitric oxide (NO), which act through the nitric oxide (NO)/cyclic GMP signalling pathway (Module 2: Figure NO and cyclic GMP signalling). The increase in cyclic GMP appears to reduce Ca^{2+} signalling in mesangial cells as it does in smooth muscle cells (Module 7: Figure smooth muscle cell cGMP signalling). One of the actions of cyclic GMP is to activate cyclic GMP-dependent protein kinase I (cGKI), which phosphorylates the BK channel to enhance its sensitivity to Ca^{2+} and this increases the negative-feedback loop.

An important feature of the mesangial cells is that they are readily induced to proliferate following stimulation with either hormones such as angiotensin II and endothelin-1 or growth factors such as platelet-derived growth factor (PDGF) (Module 7: Figure mesangial cell). Such mesangial proliferation plays an important function in maintaining the cell population and hence the integrity of the glomerulus. For example, such proliferation is an important adaptive response to restore the glomeruli that are damaged after streptococcal infections or lupus nephritis.

Mesangial cell proliferation is curbed by the local release of transforming growth factor β1 (TGF-β1), which acts through the Smad signalling pathway (Module 2: Figure Smad signalling). Activation of Smad signalling also acts to increase the expression of various extracellular matrix components such as collagen IV, which are components of the filtration filter (Module 7: Figure mesangial cell).

The propensity of the mesangial cell to proliferate does have serious pathological consequences because it is one of the major causes of a number of kidney diseases such as diabetic nephropathy that then lead to end-stage renal disease (ESRD).

Mechanotransduction in kidney cells

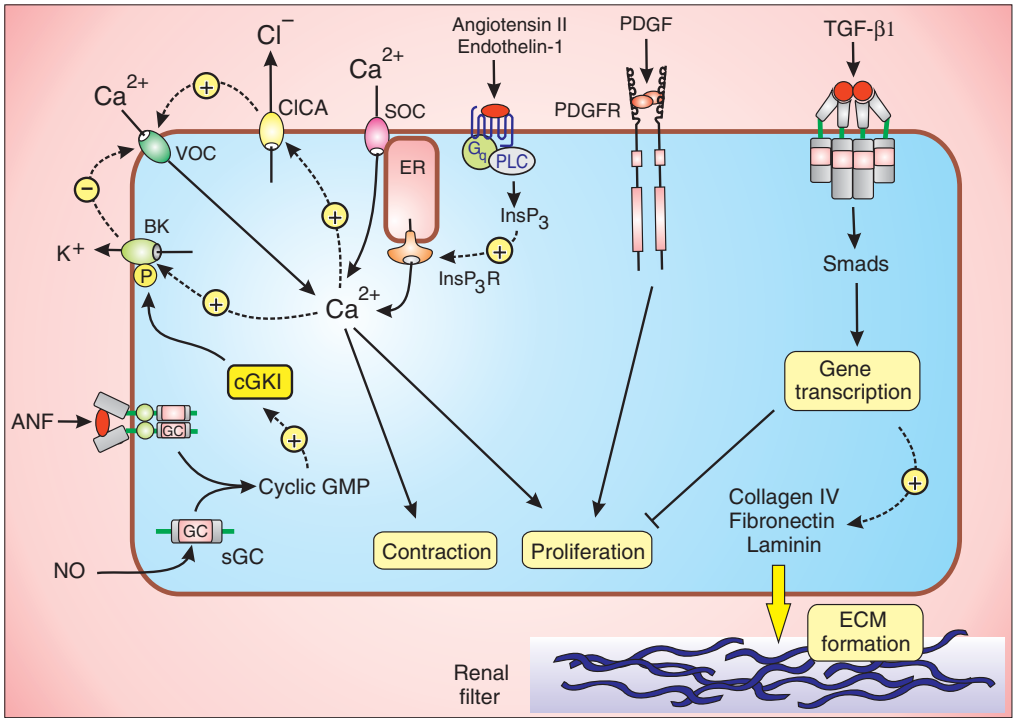
All of the cells of the kidney tubule, except the collecting duct, contain a single primary cilium about 2–3 μm long, which functions in mechanotransduction in kidney cells. The single primary cilium on each kidney cell is located on the apical surface and extends into the lumen. The suggestion is that this cilium has a sensory role in monitoring the flow of fluid in the tubule lumen, and this information is used to control the rate of ion transport. Whatever its function turns out to be, it is clear that mechanical deformation of the cilium will generate a Ca^{2+} signal (Module 7: Figure cilia deformation and Ca^{2+} signalling). The increase in Ca^{2+} seems to depend on the activation of the polycystins using mechanisms 1 and 2 in Module 3: Figure polycystin channel location.

The Ca^{2+} that enters through the polycystin channel may also be responsible for maintaining phenotypic stability, especially with regard to the expression of the mitogen-activated protein kinase (MAPK) signalling pathway. Polycystic kidney disease results from mutations of these channels, which decrease Ca^{2+} entry and may result in kidney cell proliferation and cyst formation through alterations in MAPK signalling (Module 12: Figure polycystins and polycystic kidney disease).

Salivary gland

Mammalian salivary glands elaborate fluid that moistens the oral cavity between meals and also prepares food for ingestion and digestion. The first function is carried out by numerous small labial, buccal and palatal glands, which secrete continuously to moisten the mouth. The copious flow of saliva secreted during feeding originates from three large glands (submandibular, parotid and sublingual), which secrete in response to mechanical, thermal or chemical stimuli. In humans, as much as 0.75 litres of fluid is secreted by the salivary glands each day. In addition to producing the watery saliva, these glands also synthesize and secrete amylase, which functions in the digestion of starch and glycogen (Module 7: salivary gland structure). Salivary gland structure reflects this dual role of fluid and enzyme secretion. This dual function is also reflected in the salivary gland control mechanisms in that the parasympathetic system controls fluid secretion, whereas the sympathetic system regulates the release of amylase.

Module 7: | Figure mesangial cell



Control of intraglomerular mesangial cell functions.
Intraglomerular mesangial cells respond to a wide range of stimuli to control contraction, proliferation and formation of the extracellular matrix (ECM). See the text for further details. ANF, atrial natriuretic factor; BK, large-conductance K^+ channel; cGKI, cyclic GMP-dependent protein kinase I; CLCA, Ca^{2+}-sensitive Cl^- channel; NO, nitric oxide; sGC, soluble guanylyl cyclase; VOC, voltage-operated channel.

Salivary gland structure

Salivary glands have a racemose organization consisting of numerous bulbous acini connected to a central collecting duct by a variety of tubular elements (Module 7: salivary gland structure). The organization of the ducts can vary between the different glands. In the case of rat submandibular glands, the terminal acini drain into the main tubular system via short thin intercalated ducts. The more proximal parts of the main duct system, which often contains numerous granules, merge into a region characterized by elaborate striations. These striated ducts then connect to the main ducts that carry fluid into the oral cavity.

The acini have a pyramidal structure with their apical surfaces clustered around a narrow central lumen. The basal plasma membrane has numerous short infoldings, and the lateral membranes have similar infoldings to form intercellular canaliculi that function in fluid secretion. The acini also have all the structural features associated with protein secretion. There is an elaborate system of rough endoplasmic reticulum, Golgi and a number of large amylase-containing granules, which take up a large volume of the cell.

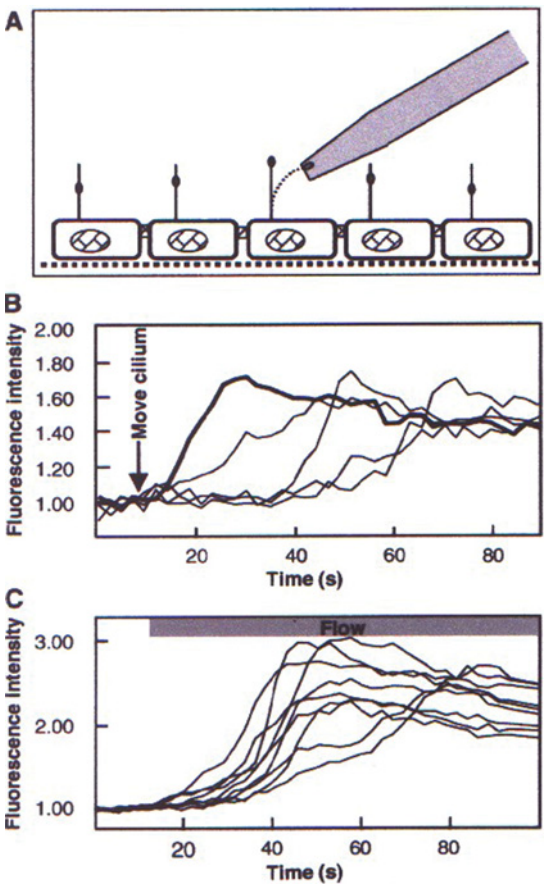
The granular and striated ducts, which function in ion reabsorption, have elaborate infoldings of the basal plasma membrane including an elaborate interdigitation of the infoldings of neighbouring cells. These basal structures are responsible for the striations of the striated ducts. By contrast, the apical surface is almost completely unspecialized with occasional short bulbous microvilli. The simplicity of the apical membrane may enable these duct cells to reabsorb ions without a concomitant flow of water, thus leading to dilute saliva.

Each acinus has a single contractile myoepithelial cell, which has a starfish-shaped structure with the long arms forming a basket around the acinus. Numerous bipolar spindle-shaped myoepithelial cells are associated with the intercalated ducts. These myoepithelial cells are thought to facilitate the flow of saliva from the acini into the main ducts. Contraction of the single basket-shaped cell squeezes the acinus, whereas contractions of the longitudinal cells of the intercalated ducts shorten and widen the lumen to allow secreted products access to the main ducts.

Salivary glands have a rich blood supply. Fenestrated capillaries ramify throughout the gland and come into close contact with both the secretory acini as well as the reabsorptive cells of the ducts. The presence of fenestrae permits ions and water to leave the capillary lumen rapidly enough to maintain high rates of salivary secretion.

Salivary secretion is controlled by transmitters released from both the parasympathetic and sympathetic nervous systems. Nerve bundles ramify throughout the gland and single neurons come to lie close to the surface. In some cases, neurons lie in grooves between two epithelial cells enabling transmitter to be released directly on to the surface of the epithelial cells (white arrows in Module 7: salivary gland structure). Transmitters such as

Module 7: | Figure cilia deformation and Ca^{2+} signalling



Mechanical deformation of a primary cilium generates a Ca^{2+} signal. The effect of deforming the primary cilium on intracellular Ca^{2+} was investigated in Madin–Darby canine kidney (MDCK) cells, a cultured cell line derived from the collecting duct of the kidney. A. A micropipette was used to bend the cilium on one of the cells. B. Shortly after bending the cilium, there was a rapid rise in the intracellular level of Ca^{2+} (thick line), which was followed later by an increase in neighbouring cells (thinner lines). C. An increase in Ca^{2+} was observed in all of the cells when fluid flowed over the cells at a rate of 8 μl/s. Reproduced from Praetorius, H.A. and Spring, K.R. (2001) Bending the MDCK cell primary cilium increases intracellular calcium. J. Membr. Biol. 184:71–79, with kind permission from Springer Science and Business Media; see Praetorius and Spring 2001.

acetylcholine (ACh) and noradrenaline (norepinephrine) activate the salivary gland control mechanisms responsible for regulating fluid and enzyme secretion.

Salivary gland control mechanisms

A characteristic of salivary gland function is that long periods of quiescence are interrupted with short periods of intense secretory activity. These short bursts of secretion are regulated by both components of the autonomic nervous system (Module 7: Figure salivary gland function). The parasympathetic nervous system primarily regulates fluid secretion, whereas the sympathetic system controls the release of amylase. The distinction between these two control mechanisms is not completely clear-cut in that there is some enzyme release during parasympathetic stimulation. Similarly, some fluid is stimulated by the sympathetic system.

Fluid secretion depends upon a transepithelial flux of Cl^- (Module 7: Figure salivary gland secretion). Entry across the basolateral membrane is carried out by the Na^+-K^+-Cl^- cotransporter 1 (NKCC1) with the driving force supplied by the Na^+ entering the cell down its electrochemical gradient that is maintained by the classical ouabain-sensitive Na^+ pump (Na^+/K^+-ATPase). Any accumulation of K^+ is leaked back to the plasma through a Ca^{2+}-sensitive large-conductance (BK) channel. Another mechanism for Cl^- entry depends on the paired Cl^-/HCO_3^- and Na^+/H^+ exchangers. Fluid secretion is very dependent on the activity of carbonic anhydrase (CA), which catalyses a reaction between water and CO_2 to give the HCO_3^- that is exchanged for Cl^-. The other product of this reaction is expelled by the Na^+/H^+ exchanger. The accumulation of Cl^- within the cell increases the electrochemical gradient and this enables this anion to pass into the lumen through Ca^{2+}-sensitive Cl^- channels (CLCAs). The flow of Cl^- into the lumen then provides the electrical gradient for a parallel flow of Na^+, and this movement of NaCl then provides the osmotic gradient for the flow of water. Water flow into the lumen occurs through two pathways: by passing either through the cell (transcellular) or via the intercellular junctions (paracellular). The transcellular flow is carried out by aquaporins with AQP3 facilitating entry into the cell and AQP5 in the apical membrane enabling water to flow into the lumen.

Control of fluid secretion by the parasympathetic neurons depends on the release of acetylcholine (ACh) that acts through muscarinic (M3) receptors that are coupled to the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette (Module 7: Figure salivary gland secretion). The increase in Ca^{2+}, which depends both on the release from internal stores and the entry through a store-operated channel (SOC), has two main functions. Firstly, Ca^{2+} activates the Ca^{2+}-sensitive Cl^- channels (CLCAs) that pass Cl^- into the lumen, which then provides the driving force for Na^+ and water movement. Secondly, Ca^{2+} activates the BK channel on the basolateral membrane and thus acts to hyperpolarize the membrane to maintain the electrochemical gradient that drives Cl^- efflux into the lumen.

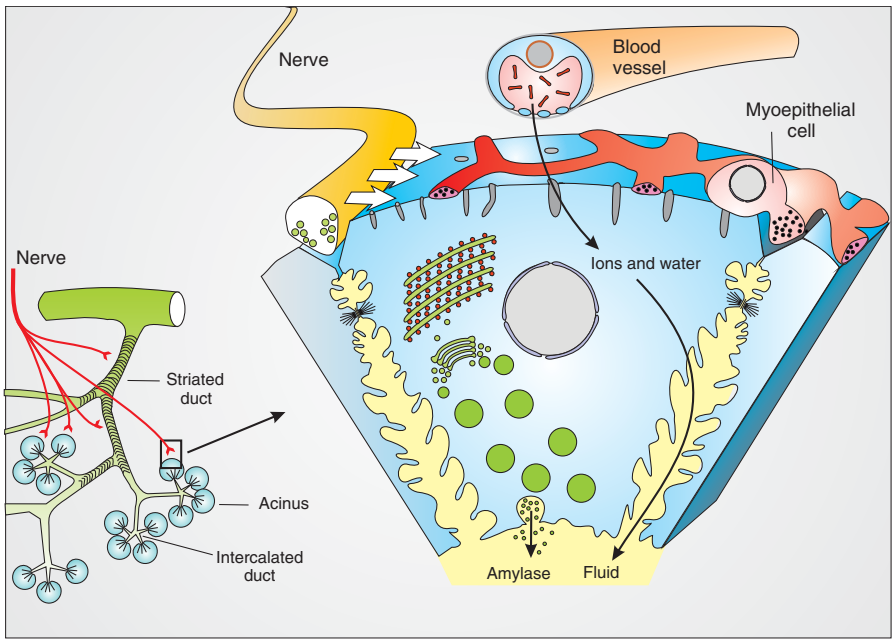
The control of amylase release by the adrenergic pathway is not fully established. The cyclic AMP pathway clearly has a major role, but exactly how it functions and whether there is a role for Ca^{2+} is still not clear.

Exocrine pancreas

The main function of the exocrine pancreas is to synthesize and secrete a range of digestive enzymes. These enter the duodenum in a flow of pancreatic juice that is rich in HCO_3^-, which plays an essential role in the control of duodenal pH by neutralizing the H^+ ions coming from the stomach. The latter periodically releases some of its contents into the duodenum, and this gastric emptying is co-ordinated with pancreatic secretion by a complex interplay between various hormonal and neural pathways.

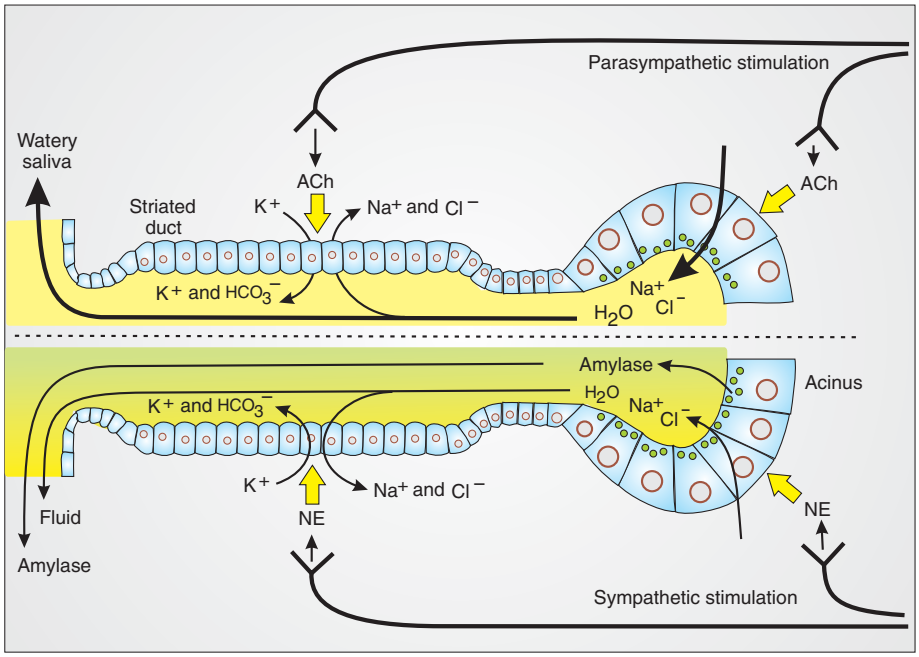
The pancreas consists of numerous lobules held together with connective tissue containing blood vessels and nerves

Module 7: | Figure salivary gland structure



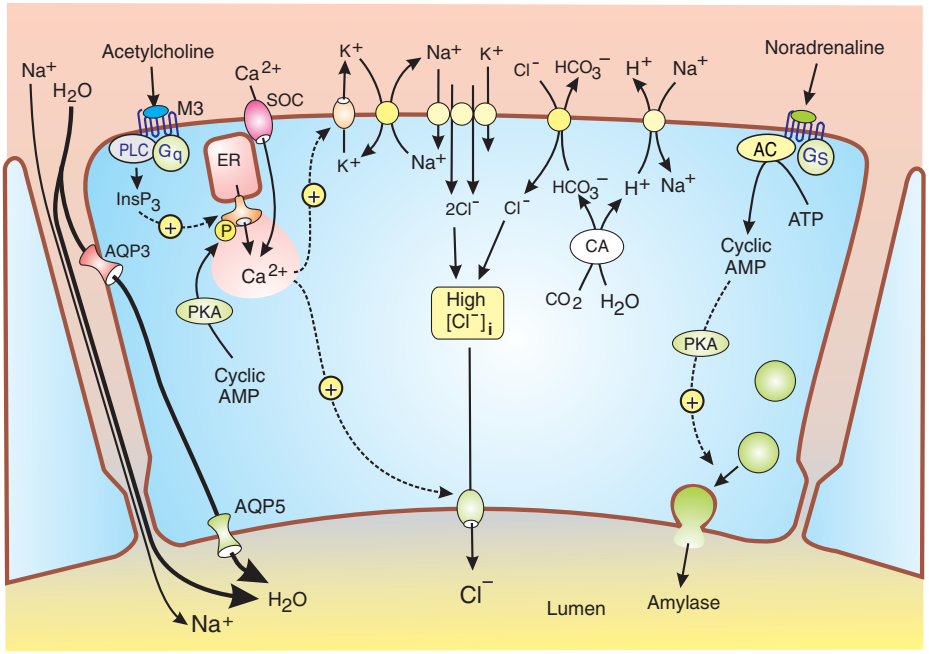
Structural organization of the salivary gland.
The diagram on the left shows the racemose structure of the gland. Numerous acini are attached to the terminal branches of the duct system. Each acinus is made up of epithelial cells that secrete both amylase and fluid. Secretion is controlled by parasympathetic and sympathetic nerves that lie close to the surface where transmitters are released (white arrows). The gland is surrounded by blood vessels that contain numerous fenestrae that allow ions and water to pass easily from the blood to the surface of the epithelial cells. A single basket shaped contractile myoepithelial cells surrounds each acinus.

Module 7: | Figure salivary gland function



Neural control of salivary gland secretion and reabsorption.
Acetylcholine (ACh) released from the nerve endings of parasympathetic neurons induces the secretion of an isotonic solution of Na^+ and Cl^-. As this solution passes down the ducts it is modified by the reabsorption of Na^+ and the secretion of K^+, resulting in a dilute salivary solution containing little amylase. By contrast, the release of noradrenaline (norepinephrine; NE) from sympathetic neurons activates the acini to release amylase together with a small amount of Na^+ and Cl^-. Na^+ is exchanged for K^+ as this fluid passes down the ducts resulting in a smaller volume of fluid that contains large amounts of amylase. The mechanisms that control enzyme and fluid secretion by the acinus are shown in Module 7: Figure salivary gland secretion.

Module 7: | Figure salivary gland secretion



Control of fluid and enzyme secretion by the salivary gland.

Acetylcholine acts through the Ca^{2+} signalling system to increase the ionic fluxes that drive Cl^- into the lumen with Na^+ and water following passively. Noradrenaline (norepinephrine) acts through the cyclic AMP signalling system to stimulate the release of vesicles containing amylase. Cyclic AMP can also potentiate the Ca^{2+} signalling system by sensitizing the inositol 1,4,5-trisphosphate ($InsP_3$) receptors to the stimulatory action of $InsP_3$. CA, carbonic anhydrase; PKA, protein kinase A.

(Module 7: Figure exocrine pancreas). The exocrine pancreatic acinar cells, which are most conspicuous because of their profuse rough endoplasmic reticulum and large accumulation of zymogen granules, have a pyramidal shape, with their apices facing the lumen where the zymogen granules are released. The exocrine pancreatic centroacinar cells, which merge into the ducts, are much lighter due to the low density of intracellular organelles. These exocrine pancreatic centroacinar cells are responsible for much of the fluid produced by the pancreas. Their activity is controlled by secretin, which acts to stimulate the secretion of ions such as Na^+ and HCO_3^-.

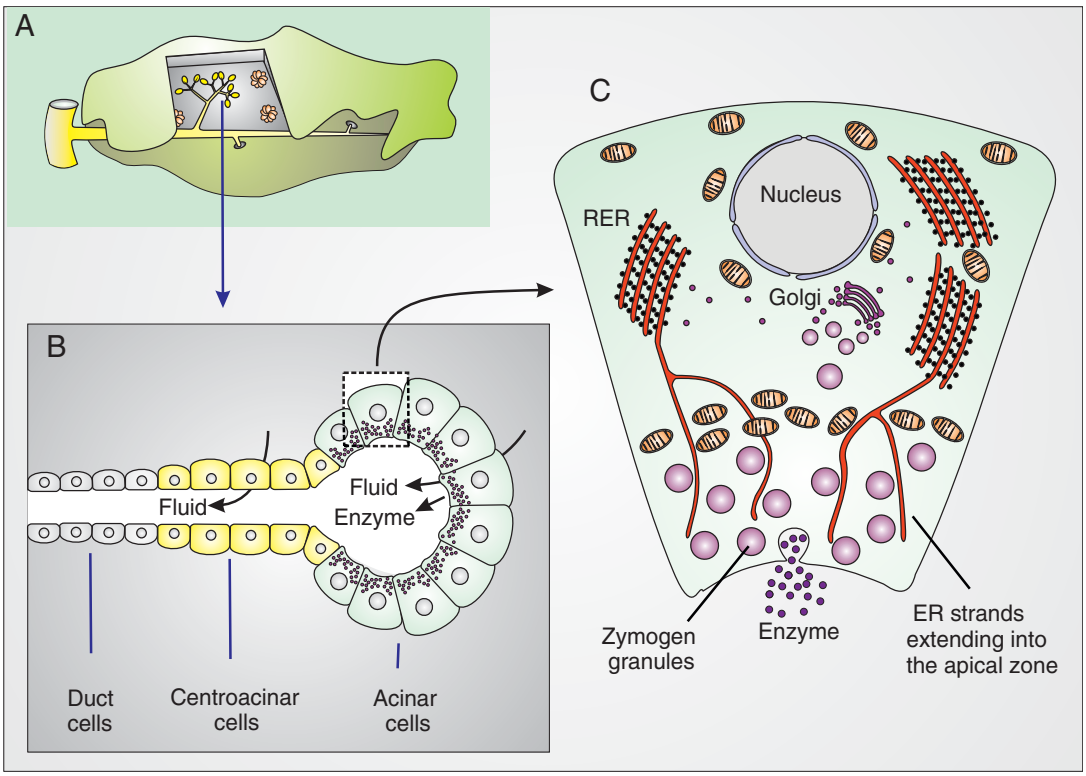
Exocrine pancreatic acinar cells

Most attention has been focused on the pancreatic acinar cells, which are typical secretory cells designed to release both proteins and fluid. The former are the digestive enzymes stored in the zymogen granules located in the apical part of the cell. The human pancreas secretes a wide range of proteins such as lipases, ribonuclease, deoxyribonuclease, proelastase, chymotrypsinogen and trypsinogen. This secretory activity is regulated by secretagogues such as acetylcholine (ACh) and cholecystokinin (CCK), which generate Ca^{2+} signals with complex spatial and temporal profiles. At low doses, both ACh and CCK generate rapid Ca^{2+} transients localized to the apical region. A mitochondrial firewall that surrounds this apical zone may help to restrict this apical microdomain. At higher doses, these stimuli can also produce stronger Ca^{2+} signals that start in the apical zone, but then breach the mitochondrial firewall to propagate outwards into the basal region of the cell in the form of an intracellular Ca^{2+} wave. The generation of these Ca^{2+} signals is very dependent on release from the endoplasmic reticulum (ER), which is very extensive in these pancreatic cells. Most of the rough ER that synthesizes proteins is located in the basal region, but there are thin strands that percolate down into the apical region, where it plays a critical role in producing the localized Ca^{2+} signals that regulate both exocytosis and fluid secretion (Module 7: Figure control of pancreatic secretion). Another source of Ca^{2+} is the bafilomycin-sensitive acid compartment that consists of the zymogen granules, endosomes and granules that are located exclusively in the apical granular pole.

Release of Ca^{2+} from the ER is replenished by uptake of external Ca^{2+} carried out by a store-operated entry mechanism. As the store empties, the decrease in Ca^{2+} is detected by the ER Ca^{2+} sensor STIM that activates the Orai channels located in the baso-lateral membrane through conformational coupling mechanism as outlined in Module 3: Figure conformational coupling hypothesis. The Ca^{2+} that enters is then pumped into the ER by sarco/endo-plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps.

The localized transients of Ca^{2+} in the apical region not only function to trigger the exocytosis of the zymogen granules, but also are responsible for stimulating fluid secretion. The latter depends on Ca^{2+} activating a Ca^{2+}-sensitive Cl^- channel (CLCA) located in the apical membrane, and this allows Cl^- to enter the lumen. This

Module 7: | Figure exocrine pancreas



Structural organization of the exocrine pancreas.

A. The pancreas has two components: the endocrine pancreas composed of the islets of Langerhans and the exocrine pancreas made up of branched lobules that drain their contents into the duodenum. B. Organization of the acinar and centroacinar cells in one of the lobules. C. Polarized structure of a pancreatic acinar cell. Rough endoplasmic reticulum (RER) in the basal region synthesizes digestive enzymes, which are packaged into zymogen granules that accumulate in the apical region ready for release. Thin strands of endoplasmic reticulum (ER) extend down into the apical region where they have channels that release pulses of Ca^{2+} to control secretion. Regulation of secretion is described in Module 7: Figure control of pancreatic secretion.

influx of Cl^- makes the lumen negative, thus providing a driving force to allow Na^+ to flow through junctions to create the osmotic gradient for a parallel flow of water. The increase in Ca^{2+} is also important for activating the large-conductance (BK) K^+ channel, which keeps the membrane hyperpolarized and thus maintains the driving force for Cl^- to flow into the lumen. In addition, the efflux of K^+ from the cell helps to maintain the operation of the Na^+-K^+-Cl^- cotransporter 1 (NKCC1) that is responsible for providing the ionic gradient that maintains this mechanism of fluid secretion. With regard to control, both exocytosis and the two Ca^{2+}-sensitive channels are driven by Ca^{2+} signals localized to the apical region. A number of intracellular Ca^{2+} release channels contribute to the apical microdomain of Ca^{2+}. The mechanisms for acetylcholine-induced pancreatic secretion differ from that of cholecystokinin (CCK)-induced pancreatic secretion, and are thus considered separately.

Non-oxidative fatty acid ethyl ester (FAEE) metabolites of ethanol, which release Ca^{2+} from both the ER and acidic Ca^{2+} stores, have been implicated in pancreatitis.

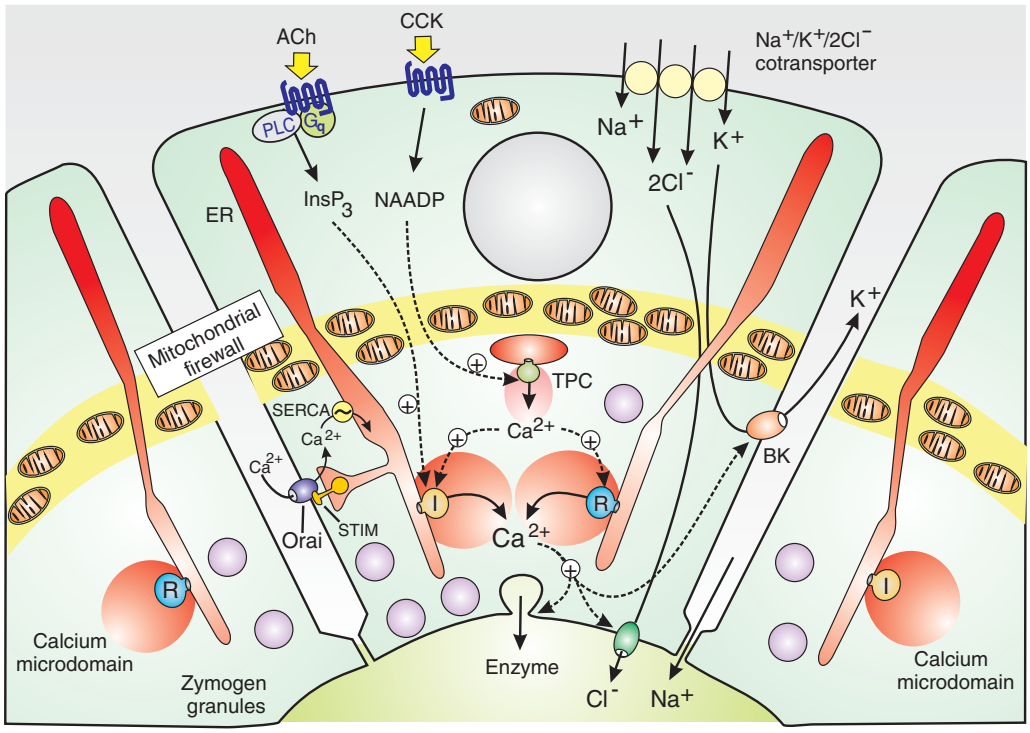
Acetylcholine-induced pancreatic secretion

Acetylcholine (ACh) acts on muscarinic receptors to produce inositol 1,4,5-trisphosphate ($InsP_3$), which diffuses into the cell to stimulate release of Ca^{2+} by $InsP_3$ receptors located in the apical region of the cell. The Ca^{2+} signal may be amplified by recruiting neighbouring ryanodine receptors (RYRs) to create a wave that spreads to the basal region (Module 7: Figure control of pancreatic secretion). During continuous stimulation, ACh can set up regular Ca^{2+} oscillations (Module 6: Figure pancreatic Ca^{2+} oscillations).

Cholecystokinin (CCK)-induced pancreatic secretion

The action of cholecystokinin (CCK)-induced pancreatic secretion seems to depend on nicotinic acid-adenine dinucleotide phosphate (NAADP) signalling (Module 2: Figure cADPR/NAADP function). Low doses of CCK stimulate the formation of NAADP by a mechanism that remains to be fully characterized. This NAADP then acts on a putative receptor located on a lysosomal-related organelle to provide a small amount of trigger Ca^{2+}, which is amplified by stimulating the inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) and the ryanodine receptors (RYRs) (Module 7: Figure control of pancreatic secretion).

Module 7: | Figure control of pancreatic secretion



Control of secretion by the exocrine pancreatic acinar cells.

Acetylcholine (ACh) and cholecystokinin (CCK) are two of the major regulators of enzyme and fluid secretion. Control of these two secretory processes occurs through Ca^{2+} signals that are localized to the apical zone. A mitochondrial firewall helps to ensure that the apical microdomain of Ca^{2+} is restricted to this apical zone. The Ca^{2+} signal is generated by a number of release channels: inositol 1,4,5-trisphosphate ($InsP_3$) receptors, ryanodine receptors (RYRs) and the putative nicotinic acid–adenine dinucleotide phosphate (NAADP) receptor (NAADPR). The way in which these different receptors are activated is described in the text.

Exocrine pancreatic centroacinar cells

The exocrine pancreatic centroacinar cells and duct cells are responsible for secreting the bicarbonate-rich fluid that helps to carry the enzymes secreted by the acinar cells down into the intestine (**Module 7: Figure exocrine pancreas**). The prime mover for fluid secretion is the trans-epithelial transport of HCO_3^-, which enters across the basolateral membrane through the pancreatic Na^+-HCO_3^- cotransporter (pNBC1) and is secreted across the apical membrane into the lumen through two members of the solute carrier family 26 (SLC26): SLC26A3 and SLC26A6, which are coupled Cl^-/HCO_3^- exchangers. The secretion of HCO_3^- may be maintained by the **cystic fibrosis transmembrane conductance regulator (CFTR)** channel that provides the Cl^- in the lumen that is exchanged for HCO_3^-. The entry of HCO_3^- across the basolateral membrane and its exit from the cell into the lumen appears to be coordinated by **$InsP_3$R-binding protein released with inositol 1,4,5-trisphosphate (IRBIT)** acting to enhance the activity of both pNBC1 and CFTR.

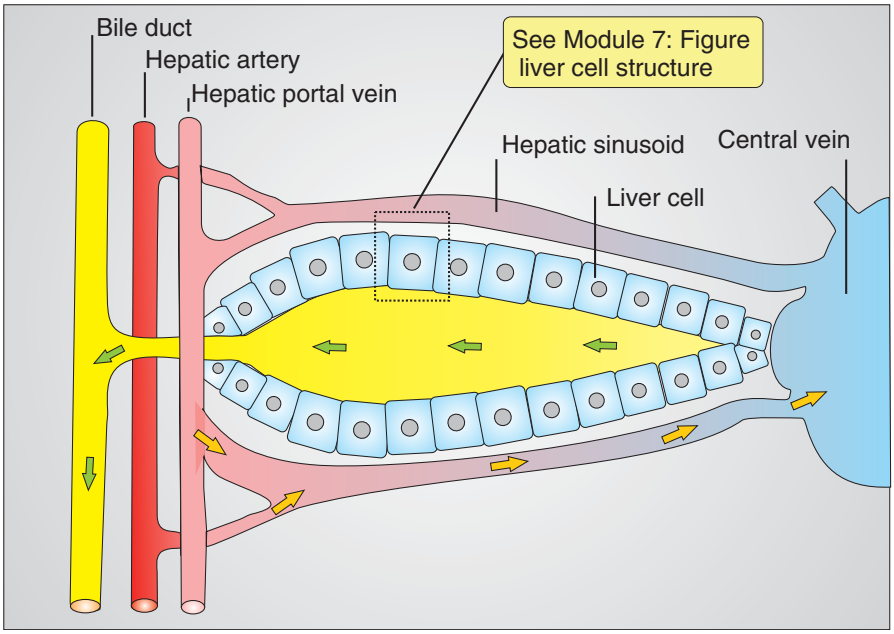
Liver cells

The liver carries out a number of important functions. It maintains the level of circulating metabolites, particularly glucose (Module 7: Figure metabolic energy network). There is a constant ebb and flow of oxidative substrates between the plasma and liver cells. They contain a major store of glycogen, and the process of glycogen synthesis during periods when glucose is plentiful is controlled by insulin, whereas glucagon activates a process of glycogenolysis to release glucose back into the plasma when glucose levels are low. Liver cells thus occupy a critical position in the overall metabolic energy network. In addition to this role in glucose metabolism, the liver has a number of other functions:

- It removes the products resulting from the breakdown of red blood cells (e.g. bilirubin).
- It synthesizes urea.
- It detoxifies and conjugates poisonous and harmful substances, and inactivates many hormones.
- It synthesizes a large number of plasma proteins.
- It secretes bile acids and various other components necessary for digestion.

The organization of the liver reveals how the liver cells receive a constant flow of blood from both the hepatic artery cells and hepatic portal vein (Module 7: Figure liver lobule). About 70% of the blood enters from the portal vein, which comes from the digestive tract, whereas the remainder enters via the hepatic artery. Most of the nutrients absorbed by the intestine pass directly to the liver. Immediately after a meal, there is a rapid rise in the level of glucose in the hepatic portal vein, whereas the level

Module 7: | Figure liver lobule



Organization of the liver cell parenchymal sheets and their relationship to the blood supply and the bile duct system.
Liver cells function as an epithelium organized into parenchymal sheets. The blood supply comes from both the hepatic artery and the hepatic portal vein, which combine to form the hepatic sinusoid that allows plasma to percolate over one surface. The opposite surface secretes bile, which collects in a sac and is carried away in the bile duct (green arrows). The structural organization of a typical liver cell is shown in Module 7: Figure liver cell structure.

in the central hepatic venous system changes very little. This is because much of the glucose is taken up from the plasma as it percolates along the hepatic sinusoid. Liver cells are arranged in epithelial sheets, with one surface facing the space of Disse that is open to the sinusoid where the metabolic exchanges occur, whereas the other side is responsible for secreting bile (Module 7: Figure liver cell structure). Liver cells, which are arranged as cell sheets radiating out from the central vein, comprise about 85% of the total cell mass of the liver. The other main cells in the liver are the endothelial cells that line the sinusoids, the Kupffer cells that are resident macrophage-like cells and the hepatic stellate cells that have a variety of functions.

Most attention will be focused on the regulation of glucose metabolism, which is the main function of liver cells.

Regulation of glucose metabolism

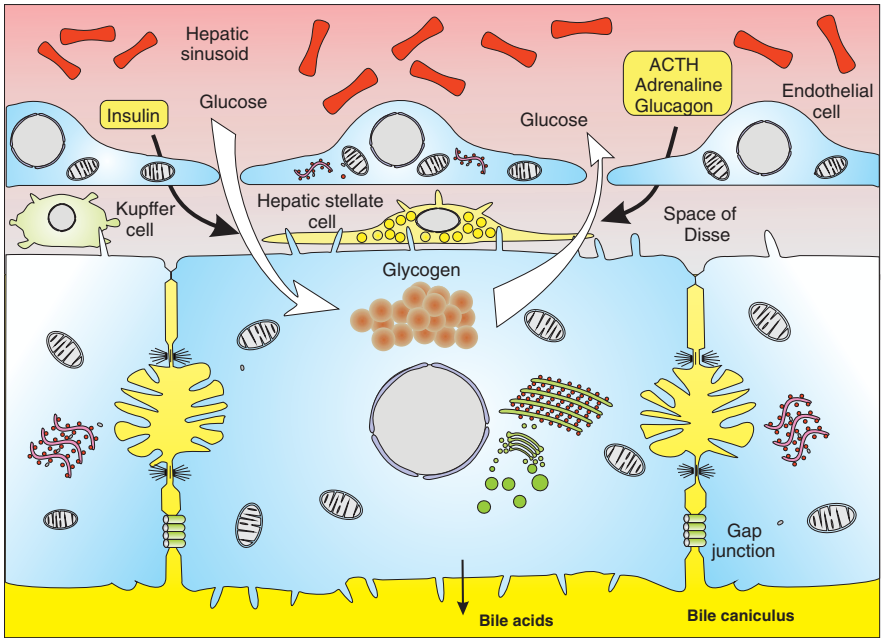
The regulation of glucose metabolism by liver cells is dictated by the energy balance within the metabolic energy network (Module 7: Figure metabolic energy network). If energy is required during fasting or stress, catabolic hormones such as glucagon and adrenaline (epinephrine) stimulate the release of glucose (Step 11 in Module 7: Figure metabolic energy network). However, when glucose levels are high after feeding, the hormone insulin shifts the emphasis from catabolism to anabolism and the liver begins to synthesize and store glycogen (Step 8 in Module 7: Figure metabolic energy network). Control of the two processes of liver cell glycogenolysis and liver cell gluconeogenesis are at the centre of the regulation of glucose metabolism.

Liver cell glycogenolysis

Liver cell glycogenolysis is the process whereby the cell regulates its glycogen store. The level of glycogen is controlled by a balance between glycogen hydrolysis carried out by the enzyme phosphorylase and glycogen synthesis carried out by the enzyme glycogen synthase (Module 7: Figure glycogenolysis and gluconeogenesis). Phosphorylase exists in two states: an active phosphorylase *a* form and an inactive phosphorylase *b* form. Conversion into the active state is carried out by phosphorylation through another enzyme, phosphorylase kinase, which in turn exists in an inactive *b* form and an active *a* form. Phosphorylase kinase is a Ca^{2+}-sensitive enzyme that is controlled by Ca^{2+} acting through a resident calmodulin (CaM) molecule. The enzyme is also stimulated by the cyclic AMP signalling pathway, which acts through protein kinase A (PKA) to phosphorylate the enzyme to enhance its sensitivity to Ca^{2+}. Cyclic AMP also promotes glycogen breakdown by simultaneously phosphorylating glycogen synthase to convert it into the inactive *b* form. The phosphorylation events are reversed by protein phosphatase 1 (PP1), which is targeted to the surface of glycogen particles by the glycogen targeting subunit G_L (Module 5: Figure PP1 targeting to glycogen).

Hydrolysis of glycogen gives glucose 1-phosphate (G1P), which is converted by a phosphoglucomutase into glucose 6-phosphate (G6P), which is then hydrolysed by the enzyme glucose-6-phosphatase (G6Pase) to form glucose. G6Pase is an enzyme unique to liver cells, where it also serves as the final step in the process of liver cell gluconeogenesis.

Module 7: | Figure liver cell structure



Structural organization of a liver cell.
Liver cells have a typical epithelial structure, with one surface facing the hepatic sinusoid and the other facing the bile canaliculi (shown in yellow). There are gaps between the Kupffer cells, which form the endothelial layer lining the hepatic sinusoid, to allow liver cells free access to metabolites and hormones in the plasma. The surface area of the liver cells facing the space of Disse is increased by having numerous small microvilli. When glucose is high, insulin promotes the uptake of glucose, which is then synthesized into glycogen. During fasting or stress, hormones such as glucagon, adrenaline (epinephrine) and corticotropin [or adrenocorticotrophic hormone (ACTH)] stimulate the hydrolysis of glycogen, and glucose is returned to the bloodstream. Further details of the hormonal regulation of glucose metabolism are provided in Module 7: Figure liver cell signalling.

Liver cell gluconeogenesis

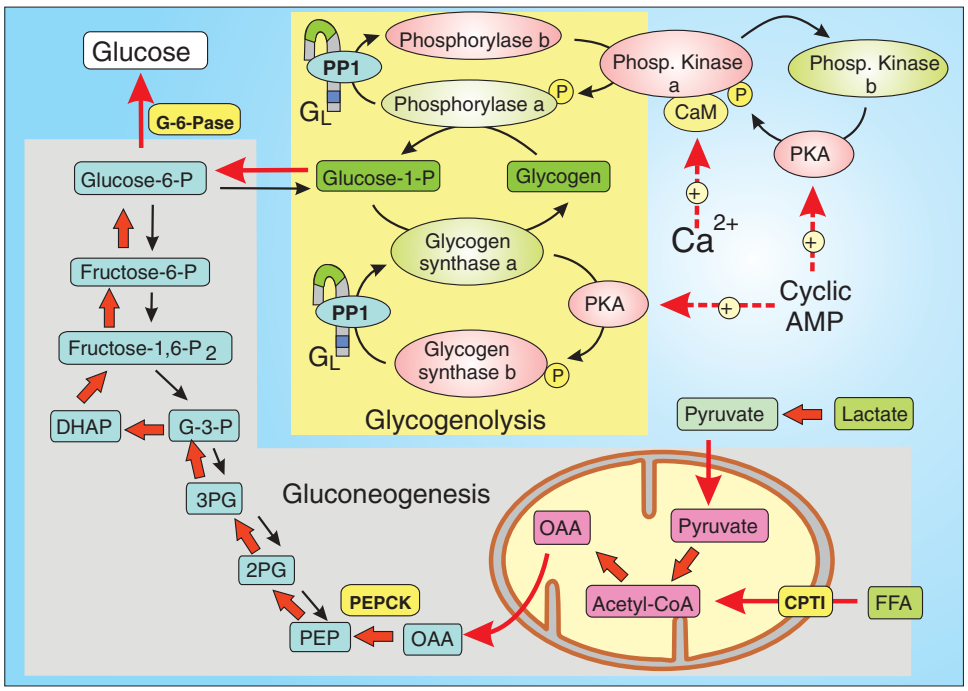
The liver cell is also capable of synthesizing glucose from various precursors, such as the lactate it receives from skeletal muscle and the free fatty acids and glycerol that come from the white fat cells (Module 7: Figure metabolic energy network). It is also able to use amino acids, and this source becomes particularly relevant during extreme starvation, when autophagy begins to degrade structural proteins to maintain glucose supplies. These precursors enter the mitochondrion, where they are converted into oxaloacetic acid (OAA), which is the common precursor used during gluconeogenesis (Module 7: Figure glycogenolysis and gluconeogenesis). This process of gluconeogenesis is particularly important during periods of fasting when glycogen reserves have been exhausted. In humans, glycogen reserves can satisfy the demand for glucose for about 12 h; thereafter, the liver has to switch on gluconeogenesis. This is a remarkably efficient process, because it can maintain the normal blood glucose levels that are essential for the energy-consuming tissues, such as the brain, kidney, testis and red blood cells, as occurs during calorie restriction (CR) or during periods of starvation.

Many of the reactions of gluconeogenesis are simply a reversal of glycolysis. Most of the glycolytic reactions are close to equilibrium, so the whole process can be reversed simply by varying the input of substrate at either end. However, there are several non-equilibrium reactions that are not easily reversed and have to be bypassed. Two of these steps, namely those mediated by hexokinase and phosphofructokinase (PFK-2), are bypassed by glucose-6-phosphatase (G6Pase) and fructose bisphosphatase. The interconversion of phosphoenolpyruvate (PEP) and pyruvate by pyruvate kinase is also by passed by two separate reactions, which are important sites for the hormonal control of gluconeogenesis. In the first reaction, pyruvate is converted into OAA by a carboxylation step that requires ATP and is catalysed by pyruvate carboxylase. OAA is then decarboxylated and phosphorylated to PEP by phosphoenolpyruvate carboxykinase (PEPCK). The significant point about some of these bypass enzymes is that their expression is regulated by liver cell signalling mechanisms.

Liver cell signalling mechanisms

The liver cell signalling mechanisms are responsible for switching the liver cell from its energy storage mode into its role of delivering energy in the form of glucose during fasting (Module 7: Figure metabolic energy network). The former is controlled by insulin, whereas the release of glucose is a somewhat more complicated process, as it is sensitive to a number of factors, including hormones [e.g. adrenaline (epinephrine), glucagon and vasopressin], lipid signals such as the fatty acids derived from white fat cells, or the energy status of the cell (Module 7: Figure liver cell signalling). Vasopressin and noradrenaline (norepinephrine) act through the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette, whereas glucagon and adrenaline act through the cyclic AMP signalling pathway. The way in which these two pathways regulate

Module 7: | Figure glycogenolysis and gluconeogenesis



Liver cell glycogenolysis and gluconeogenesis.
The liver cell can produce glucose through two processes: glycogenolysis (highlighted in the yellow box) and gluconeogenesis (highlighted in the grey box). Glycogen synthesis and hydrolysis are controlled by phosphorylase *a* and glycogen synthase *a* respectively. Cyclic AMP stimulates glycogen breakdown by inhibiting glycogen synthase and by enhancing the sensitivity of the Ca^{2+}-sensitive enzyme phosphorylase kinase. Gluconeogenesis is the process for converting various substrates such as lactate (derived from skeletal muscle) or free fatty acids (FFA; from white fat cells) into glucose by reversing many of the steps of glycolysis (red arrows). This process is markedly enhanced by an increase in the expression of some of the key enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), and the carnitine palmitoyltransferase 1 (CPT1) that brings FFAs into the mitochondrion. The transcriptional control of these proteins is shown in Module 7: Figure liver cell signalling). Other abbreviations for gluconeogenic intermediates: DHAP, dihydroxyacetone phosphate; G-3-P, glyceraldehyde 3-phosphate; 2PG, 2-phosphoglycerate; 3PG, 3-phosphoglycerate.

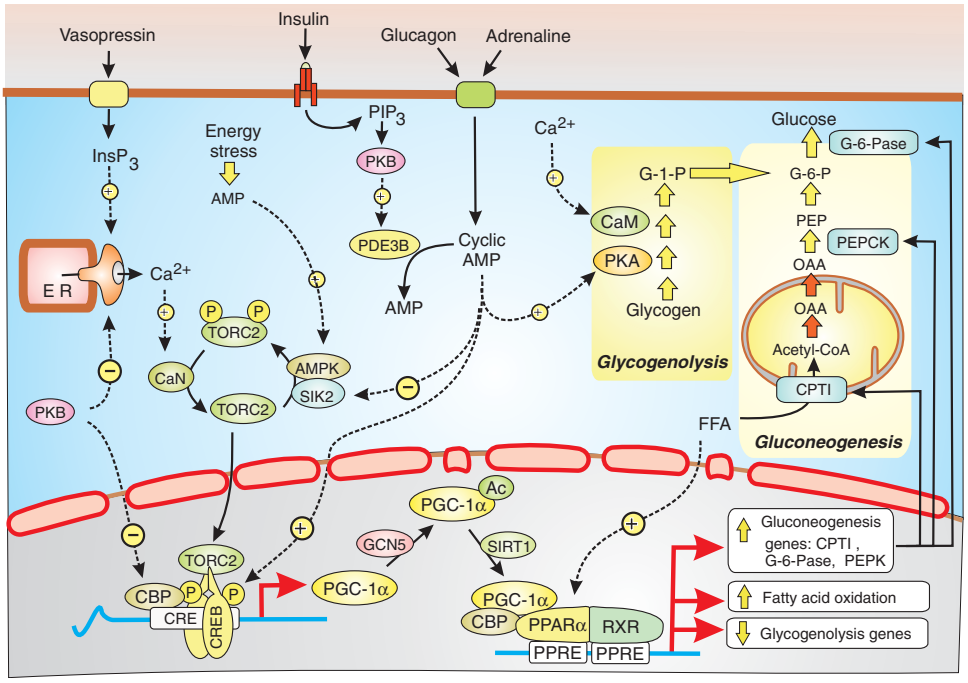
glycogenolysis is shown in more detail in Module 7: Figure glycogenolysis and gluconeogenesis. They both act to stimulate the enzyme phosphorylase kinase, which is responsible for switching on the enzyme phosphorylase that hydrolyses glycogen to glucose. Cyclic AMP enhances this breakdown of glycogen by simultaneously inhibiting glycogen synthesis.

In addition to activating this process of liver cell glycogenolysis, these signalling mechanisms are also able to activate liver cell gluconeogenesis, which is a somewhat more complicated process because it depends upon a phenotypic remodelling of the liver cell signalsome. The different signalling pathways activate a transcriptional cascade that results in an increase in the expression of some of the key rate-limiting steps in glycogenolysis (Module 7: Figure liver cell signalling). Control of this cascade has two main components. Firstly, there is activation of transducer of regulated CREB 2 (TORC2) and the transcription factor cyclic AMP response element-binding protein (CREB), which act to increase the expression of peroxisome-proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α). Secondly, the PGC-1α then acts together with PPARα to induce the transcription of some of the rate-limiting enzymes and transporters, such as glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK) and carnitine palmitoyltransferase 1 (CPT1), which are responsible for liver cell gluconeogenesis (Module 7: Figure glycogenolysis and gluconeogenesis). In addition, this transcriptional cascade can also activate genes that increase fatty acid oxidation or reduce the expression of genes that control glycolysis. An increase in the activity of all these components accelerates the formation of glucose.

The activity of PGC-1α is regulated by acetylation: the histone acetylase GCN5 inactivates PGC-1α, whereas its activation depends on the deacetylase sirtuin SIRT1. This activation by SIRT1 plays an important role in the maintenance of energy metabolism and antioxidant defences (Module 12: Figure ageing mechanisms) and has been implicated in the process of ageing.

The key event in this transcriptional cascade is the increased expression of PGC-1α induced by the CREB transcription factor. A number of signalling pathways are known to activate CREB (Module 4: Figure CREB activation), and two of these are evident in liver cells. Cyclic AMP has two actions. It acts not only by phosphorylating CREB in the nucleus, but also by regulating the activity of the CREB cofactor TORC2. A number of control mechanisms regulate the shuttling of TORC2 between the nucleus and cytoplasm.

Module 7: | Figure liver cell signalling



Hormonal control of liver cell function.
Liver function is regulated by a number of signalling pathways. Some of these, which use Ca^{2+} and cyclic AMP as messengers, activate glycogenolysis. The same messengers can also activate a transcriptional cascade initiated by the transcription factor CREB that results in an up-regulation of some of the key enzymes involved in gluconeogenesis. Insulin provides an inhibitory pathway to switch off the expression of peroxisome-proliferator-activated receptor γ (PPARγ) coactivator-1α (PGC-1α), which exerts a major control over gluconeogenesis. Free fatty acids (FFAs) contribute to this transcriptional cascade by activating the PPARα. Insulin counteracts the cyclic AMP signalling pathway by stimulating the phosphodiesterase PDE3B that hydrolyses cyclic AMP.

Glucagon or fasting stimulates a rapid translocation of TORC2 into the nucleus (within about 10 min), whereas it remains inactive in a phosphorylated state during the action of insulin or when energy reserves are high. An increase in cyclic AMP inhibits the salt-inducible kinase 2 (SIK2), which normally acts to phosphorylate TORC2, thereby preventing it from entering the nucleus (Module 7: Figure liver cell signalling). Ca^{2+} may also inhibit TORC2 by activating calcineurin (CaN) to dephosphorylate TORC2 so that it can enter the nucleus to carry out its role as a CREB coactivator.

The AMP signalling pathway also plays a role in regulating the CREB/TORC transcriptional mechanism. The AMP-activated protein kinase (AMPK), which is activated during energy stress when the level of AMP rises, phosphorylates TORC2, thereby preventing it from activating the transcriptional cascade responsible for switching on the gluconeogenic pathway. By shutting down gluconeogenesis, the cell is able to conserve energy instead of sending it off to the energy-consuming cells.

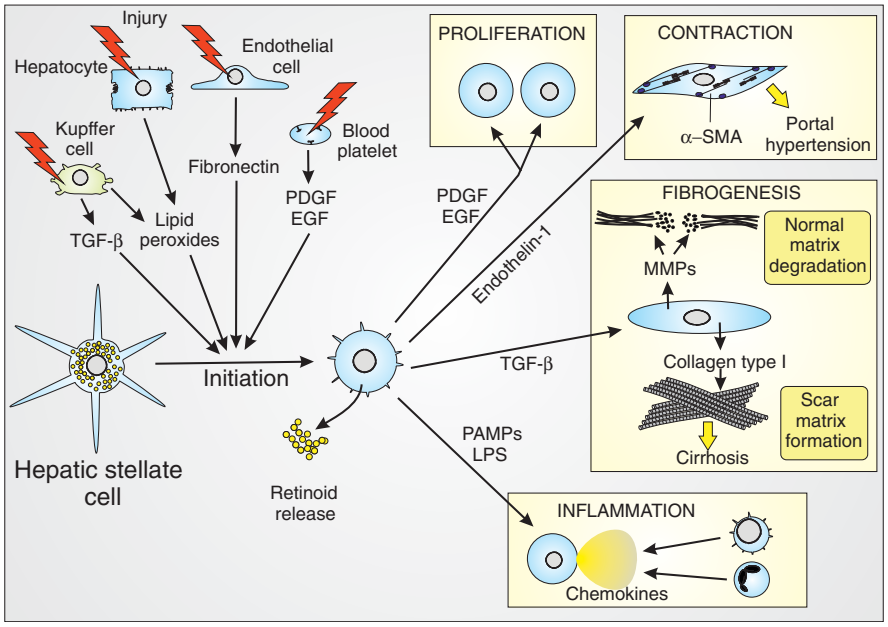
Insulin promotes energy storage by counteracting many of the glycolytic and gluconeogenic signalling pathways. Insulin stimulates the PtdIns 3-kinase signalling pathway, which uses the lipid second messenger PtdIns3,4,5P_3 (PIP_3) to activate protein kinase B (PKB), which then stimulates the phosphodiesterase (PDE3B) that hydrolyses cyclic AMP, thus reducing the stimulatory action of this second messenger on both glycogen breakdown and glucose synthesis (Module 7: Figure liver cell signalling). The PKB can exert some additional inhibitory effects. PKB is one of the protein kinases that can phosphorylate the inositol 1,4,5-trisphosphate receptor ($InsP_3R$) to reduce Ca^{2+} signalling. In addition, it can inactivate the CREB-binding protein (CBP), thereby reducing the action of CREB in inducing the transcriptional cascade.

Hepatic stellate cell

The hepatic stellate cells, which make up about 15% of the cells in the liver, have had many names such as perisinusoidal cell, Ito cell, lipocyte and fat-storing cell. They are located in the subendothelial space between the hepatocytes and the endothelial cells (Module 7: Figure liver cell structure) and are characterized by their long processes and by their numerous lipid droplets that store large amounts of vitamin A. They are the main storage site for retinoids, which are vitamin A compounds.

Apart from their role in storing the retinoids, these stellate cells are normally quiescent. However, following injury to the liver they undergo a remarkable transformation that enables them to help in organ repair (Module 7: Figure hepatic stellate cell). The hepatic stellate cells respond to stimuli released from the other liver cells. Kupffer cells release transforming growth factor β (TGF-β) and lipid peroxides; hepatocytes release lipid peroxides; endothelial cells release fibronectin and the blood platelets release platelet-derived growth factor (PDGF) and

Module 7: | Figure hepatic stellate cell



Hepatic stellate cell function.
The hepatic stellate cells are characterized by a large deposit of lipid droplets containing vitamin A. They are normally quiescent but are strongly activated during liver injury when they respond to a variety of stimuli released from damaged cells. The hepatic stellate cells undergo a process of phenotypic remodelling during which they lose their lipid (retinoid droplets) and are transformed to carry out a variety of functions that include proliferation, contraction, fibrogenesis and inflammation.

epidermal growth factor (EGF). These stimuli induce a process of phenotypic remodelling whereby the hepatic stellate cells switch from a resting vitamin A-rich cell to a much more active cell capable of carrying out process such as proliferation, contraction, fibrogenesis and inflammation (Module 7: Figure hepatic stellate cell).

The PDGF released from blood platelets is one of the main growth factors responsible for activating hepatic stellate cell proliferation. PDGF acts through the PDGFR to induce many of the typical growth factor signalling pathways such as the Ca^{2+} signalling and MAP kinase signalling pathways, which are responsible for inducing cell proliferation (Module 9: Figure growth factor signalling).

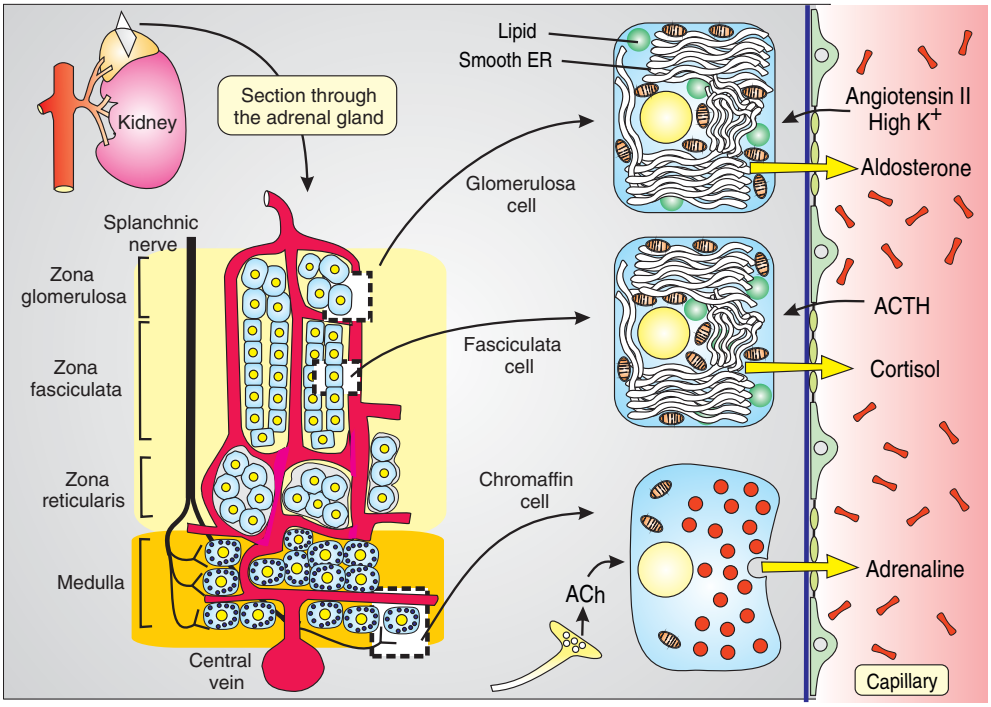
A diagnostic feature of the transformation of hepatic stellate cells into myofibroblast-like cells is the expression of α-smooth muscle cell actin (α-SMA). Contraction of these cells, which is activated by endothelin-1, appears to be regulated by two mechanism that resemble the smooth muscle Ca^{2+} signalling cascade and the smooth muscle Rho/Rho kinase signalling pathway. This contraction constricts the sinusoids and thus increases the portal resistance and contributes to the portal hypertension associated with cirrhosis of the liver.

One of the major consequences of hepatic stellate cell activation is the onset of fibrogenesis to form hepatic scars that are mainly caused by the secretion of collagen type I, which is responsible for scar matrix formation. The appearance of these scars results from a complex process of extracellular matrix remodelling that disrupts the normal liver matrix that is then replaced by the collagen-based scar matrix (Module 7: Figure hepatic stellate cell). This matrix degradation and synthesis, which is orchestrated by the fibroblasts, is stimulated mainly by TGF-β. Degradation of the normal liver matrix is carried out by secretion of matrix metalloproteinases (MMPs) such as MMP2, MMP9 and stromelysins (Module 1: Table MMPs and their inhibitors). The formation of the scar matrix begins with the synthesis and secretion of collagen type I. TGF-β acts through the Smad signalling pathway to induce the transcription of collagen type I. The fibroblasts also function to stabilize the scar matrix by secreting TIMP-1 and TIMP-2, which are the tissue inhibitors of metalloproteinases. If the formation of the scar matrix becomes excessive it can disrupt liver cytoarchitecture resulting in cirrhosis of the liver.

Hepatic stellate cells play an active role in immunoregulation where they contribute to inflammatory responses through a number of mechanisms. Like other immune cells, they express the Toll-like receptors (TLRs) that respond to the pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), which are derived from various pathogens. These TLRs then act through the Toll receptor signalling pathway that induces the activation of the NF-κB and p38 pathway to stimulate the formation of inflammatory cytokines and chemokines (Module 2: Figure Toll receptor signalling). The latter provide a chemotactic gradient to attract various inflammatory cells such as neutrophils (Module 7: Figure hepatic stellate cell).

Once the infection or injury has been resolved, the activity of the hepatic stellate cells is curtailed by various processes that seem to begin with a process of stress-induced senescence whereby the activated cells cease proliferating and withdraw from their various activities such as the

Module 7: | Figure adrenal gland



Adrenal gland structure and function.
The orange-coloured adrenal glands are located on the top of each kidney. A section through the gland illustrates the outer cortex, which is divided into three zones and an inner medulla that has the chromaffin cells. The latter are innervated by the splanchnic nerve. Shown on the right are the functions of the cells specialized to synthesize and secrete hormones (yellow arrows). Angiotensin II and high K^+ stimulate aldosterone release (Module 7: Figure glomerulosa cell signalling), corticotropin [or adrenocorticotrophic hormone (ACTH)] stimulates the release of cortisol, while release of acetylcholine (ACh) from the splanchnic nerve endings activates the chromaffin cells to release adrenaline (epinephrine) (Module 7: Figure chromaffin cell secretion).

formation of the scar matrix. The senescent cells then attract natural killer cells resulting in the hepatic stellate cells being eliminated from the liver. The onset of senescence thus helps to protect the liver from the excessive fibrogenesis that can progress to cirrhosis of the liver

Adrenal gland

The two adrenal glands are compact, endocrine organs, which lie immediately above each kidney (Module 7: Figure adrenal gland). They consist of two main parts, which differ both in function and embryological origin. The outer adrenal cortex, which arises from the mesoderm and is part of the hypothalamic–pituitary–adrenal axis, makes up most of the gland and secretes both mineralocorticoids (e.g. aldosterone) and glucocorticoids (e.g. corticosterone). The inner adrenal medulla, which is derived from the neural crest, contains chromaffin cells that secrete catecholamines such as adrenaline (epinephrine) and noradrenaline (norepinephrine), especially under stressful situations.

The adrenal gland is richly supplied with blood from vessels arising from the inferior phrenic artery, aorta and renal artery. These incoming arteries subdivide into smaller branches that enter the capsule. There are long parallel vessels running down the length of the cortex that join a reticular plexus in the region where the cortex and medulla meet. Vessels that leave the reticular plexus ramify throughout the medulla before draining into the large central veins, which then unite to form the adrenal vein. Each hormone-secreting cell is intimately associated with a blood vessel, and this ensures that released hormones are rapidly transferred into the circulatory system. This rapid transfer is facilitated further by the fact that the endothelial cells that line the blood vessels have numerous fenestrae.

Adrenal cortex

The outer cortical region of the adrenal gland is divided into three main regions: zona glomerulosa, zona fasciculata and the zona reticularis (Module 7: Figure adrenal gland). The cells in all three regions share many structural similarities. The cells are arranged in radial cords running alongside the parallel blood capillaries. The well-developed system of tightly packed smooth endoplasmic reticulum (ER) is closely associated with a large population of mitochondria. There also are large lipid droplets, which contain a store of cholesterol that is the precursor for hormone synthesis. During aldosterone and cortisol biosynthesis, the synthetic intermediates alternate between the

ER and the mitochondria and co-operate with each other during steroid biosynthesis (Module 1: Figure aldosterone and cortisol biosynthesis).

The three cortical regions are specialized to secrete separate steroid hormones in response to different stimuli. Most attention has focussed on the zona glomerulosa cells that secrete aldosterone and the zona fasciculata cells that secrete the corticosteroids such as cortisol (hydrocortisone) and corticosterone. The zona reticularis secretes androgen hormones.

Zona glomerulosa cells

Zona glomerulosa cells, which are located in the outer region of the adrenal cortex, function to synthesize and release aldosterone (Module 7: Figure adrenal gland). These cells contain large amounts of smooth endoplasmic reticulum (ER) that function in aldosterone and cortisol biosynthesis (Module 1: Figure aldosterone and cortisol biosynthesis). The various synthetic steps are located on either the inner membrane of the mitochondria or on the surface of the smooth ER (Module 7: Figure glomerulosa cell signalling). The major physiological stimuli of glomerulosa cells are angiotensin II and small elevations in the plasma level of K^+, both of which seem to act by increasing the intracellular level of Ca^{2+}.

Angiotensin II stimulates the AT_1 receptor to induce the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette. Following the formation of $InsP_3$, Ca^{2+} is released from the internal ER store to provide the initial signal to stimulate aldosterone synthesis and release. During prolonged periods of stimulation, the cell has to switch to an external source of Ca^{2+} as the internal store loses its Ca^{2+}. There appear to be a number of channels responsible for Ca^{2+} entry, but just how they are activated has been a matter of considerable debate. Glomerulosa cells appear to have a capacitative Ca^{2+} entry mechanism based on the opening of store-operated channels (SOCs).

Glomerulosa cells express voltage-operated channels (VOCs) such as the Ca_V1 family of L-type channels and the Ca_V3 family of T-type channels (Module 3: Table VOC classification). The $Ca_V1.2$ and $Ca_V1.3$ L-type and the $Ca_V3.2$ T-type channels seem to be the major isoforms used to generate the prolonged Ca^{2+} signal in response to both angiotensin II and high K^+. There has been a problem in understanding how these channels are activated by such small elevations in the plasma level of K^+ (secretion is maximally stimulated between 8 and 10 mM), which causes relatively small membrane depolarizations. All the evidence seems to point to the T-type channel being particularly important since they have activation thresholds in the -60 to -70 mV range (Module 3: Table VOC classification). They are thus ideally suited to respond to the relatively small elevations in serum K^+ that will produce correspondingly small membrane depolarizations. The L-type channels have also been shown to contribute to Ca^{2+} signalling, but they may only come into play during more intense bouts of stimulation.

In addition to responding to fluctuations in external K^+, the VOCs are also recruited by the angiotensin II AT_1 receptors to maintain Ca^{2+} signalling during prolonged stimulation (Module 7: Figure glomerulosa cell signalling). Several mechanisms have been proposed for how the AT_1 receptors activate these Ca^{2+} entry channels. The most likely is an indirect mechanism that depends upon depolarization of the membrane by switching off various K^+ channels using a variety of regulatory signalling pathways. The TREK-1 channels are inhibited by cyclic AMP. The glomerulosa cells express TASK channels, both TASK-1 and TASK-3 isoforms, which may function as heterodimers. Finally, there are $K_v7.1$ channels. These TASK and KV7.1 channels appear to be under the $PtdIns4,5P_2$ regulation of ion channels and exchanger mechanism (Module 2: Figure $PtdIns4,5P_2$ regulation of K^+ channels) whereby this inositol lipid keeps the channel open, but when $PtdIns4,5P_2$ is hydrolysed by angiotensin II, the channel closes (Module 7: Figure glomerulosa cell signalling).

Glomerulosa cells are also sensitive to the high levels of adrenocorticotrophic hormone (ACTH) [or corticotropin] that occur during severe Na^+ or fluid loss. ACTH acts through the cyclic AMP signalling pathway and its action appears to be closely integrated with that of Ca^{2+}. L-type Ca^{2+} channels are known to be phosphorylated and activated by cyclic AMP (Module 3: Figure $Ca_V1.2$ L-type channel), and such a mechanism may explain how ACTH can bring about an increase in the level of Ca^{2+} in glomerulosa cells. Another control system is carried out by atrial natriuretic factor (ANF) that inhibits the secretion of aldosterone by using cyclic GMP to increase the activity of PDE2 to reduce the level of cyclic AMP, which drives the release of this steroid

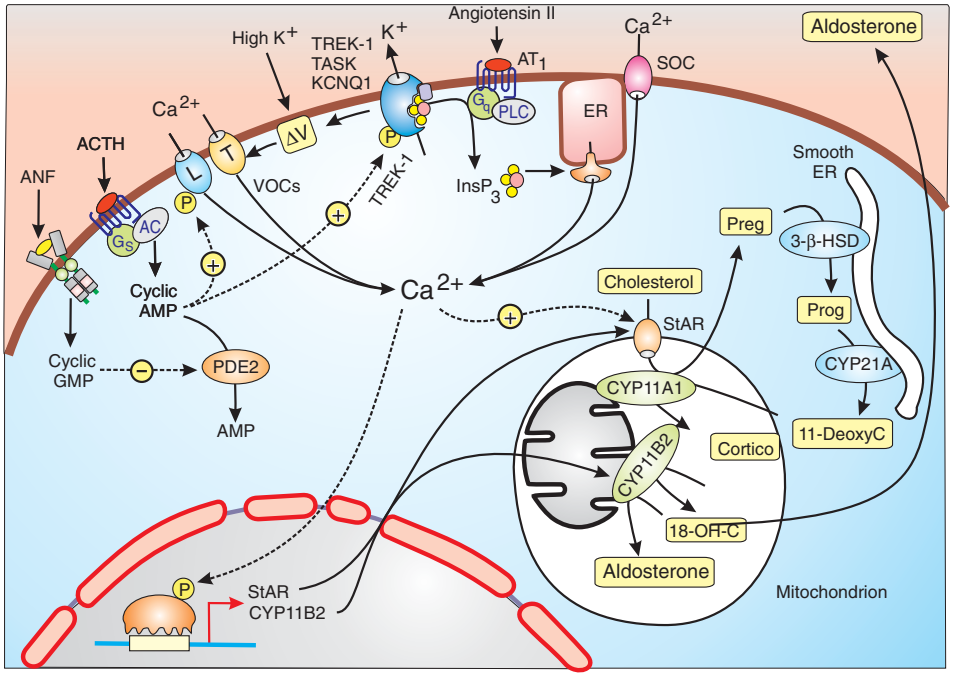
All of the evidence on the control of glomerulosa cells thus indicates a central role for Ca^{2+} in regulating aldosterone synthesis and release (Module 7: Figure glomerulosa cell signalling). The primary action of Ca^{2+} seems to be directed towards an early step in steroid biosynthesis, since it activates the steroidogenic acute regulator (StAR) protein located in the outer mitochondrial membrane. StAR appears to be a rate-limiting step in steroid synthesis because it controls the access of cholesterol to the first enzyme CYP11A1 located on the inner mitochondrial membrane where it functions in aldosterone and cortisol biosynthesis . There is a longer term action of Ca^{2+} to stimulate the transcription of genes encoding components of the biosynthetic pathway such as StAR and the aldosterone synthase CYP11B2

Mutation of the StAR protein has been linked to congenital lipoid adrenal hyperplasia.

Zona fasciculata cells

The zona fasciculata cells, together with the zona reticularis cells, are responsible for secreting the glucocorticoid cortisol (Module 7: Figure adrenal gland). These cells contain large amounts of smooth endoplasmic reticulum that function in aldosterone and cortisol biosynthesis (Module 1: aldosterone and cortisol biosynthesis). The fasciculata cells differ from the glomerulosa cells by expressing the enzyme CYP17, which is a steroid 17α-hydroxylase and 17,20-lyase that converts progesterone

Module 7: | Figure glomerulosa cell signalling



Control of aldosterone synthesis and secretion by adrenal zona glomerulosa cells.
Adrenal zona glomerulosa cells synthesize aldosterone through a series of steps carried out within the mitochondrion and on the smooth endoplasmic reticulum (ER). Angiotensin II and an elevation in plasma K^+ levels act through Ca^{2+} to control the transfer of cholesterol into the mitochondrion through the steroidogenic acute regulator (StAR). The increase in Ca^{2+} can also enhance synthesis by activating the expression of some of the components of aldosterone biosynthesis. Location of the glomerulosa cells within the adrenal is described in Module 7: Figure adrenal gland and the biosynthesis of aldosterone is described in Module 1: Figure aldosterone and cortisol biosynthesis.

into 17α-progesterone that begins the synthetic sequence that results in the formation of cortisol. Cortisol synthesis is stimulated by adrenocorticotrophic hormone (ACTH) that acts through receptors that are coupled to the cyclic AMP signalling pathway.

Excessive release of cortisol causes Cushing's syndrome.

Adrenal medulla

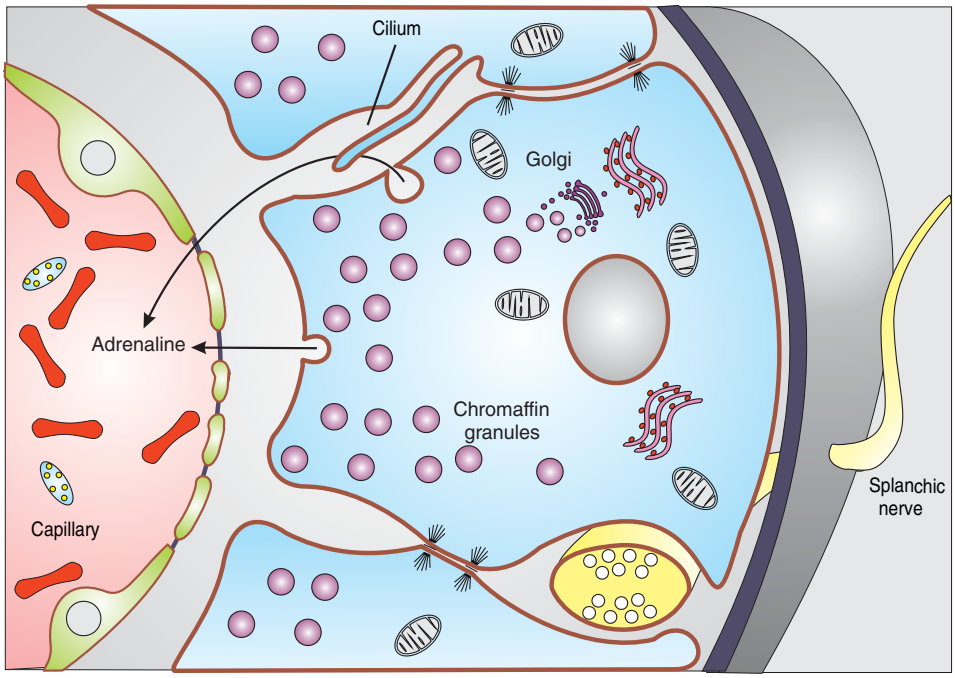
The region of the adrenal gland known as the adrenal medulla contains the chromaffin cells that function to release catecholamines (Module 7: Figure adrenal gland). The most common cells, which have 10 000–20 000 round or oval opaque granules, secrete adrenaline (epinephrine). The cells are polarized in that most of the granules are located in the apical region of the cell facing the blood vessels. Exocytosis can occur from both the apical and lateral surfaces. The scarcer cell type, which either can form aggregates or are dispersed among the predominant adrenaline-secreting cells, releases noradrenaline (norepinephrine) contained in granules with a higher electron density. This medullary region is richly innervated with preganglionic sympathetic nerves, of which most originate from the thoracic splanchnic nerve that passes through the cortex before dividing repeatedly to form individual axons that innervate the chromaffin cells. From a signalling perspective, both cell types seem to be regulated in much the same way: an action potential travelling down the splanchnic neurons activate the chromaffin cell to release their stored catecholamines.

Chromaffin cell

Chromaffin cells located within the adrenal medulla are arranged in a compact epithelial fashion surrounding the blood vessels that permeate through the adrenal gland (Module 7: Figure adrenal gland). The intercellular membranes are held together by desmosomes in the basal region of the cell. There do not appear to be any gap junctions or tight junctions. Where there are no desmosomes, the intercellular space can be quite open and can contain a primary cilium or a nerve ending. It has been proposed that the cilium may stir up the contents of this intercellular space. Since the chromaffin cells do not secrete fluid, there is no flow of fluid to carry secreted products towards the blood vessels. Having a cilium thrashing about in this confined space would serve to waft secreted products towards the blood vessels. The endothelial cells lining these blood vessels contain numerous fenestrae that will also enhance the movement of secreted catecholamines into the blood vessel.

Chromaffin cells are innervated by branches of the splanchnic nerve and single axons pass through the basement membrane to lie within the intercellular space where they form typical synaptic endings capable of activating both cells (Module 7: Figure chromaffin cell secretion).

Module 7: | Figure chromaffin cell



Structural organization of the chromaffin cell.
Chromaffin cells located in the adrenal medulla are specialized to stimulate catecholamines [adrenaline (epinephrine) and noradrenaline (norepinephrine)] into the neighbouring capillaries. Secretion is stimulated by acetylcholine released from splanchnic nerve endings that often insinuate themselves in between the chromaffin cells. Many of the chromaffin cells contain a single primary cilium that extends into the intercellular space facing the capillaries.

The splanchnic nerve endings release acetylcholine that acts on nicotinic acetylcholine receptors (nAChRs) to initiate the process of stimulus–secretion coupling in chromaffin cells. The mechanism of nicotinic acetylcholine receptor (nAChR) signalling through membrane depolarization sets up action potentials that spread around the cell surface to recruit voltage-operated channels (VOCs) such as the P/Q-type (55%), N-type (25%) and L-type (20%) (Module 3: Table VOC classification). The proportion of these channels expressed in chromaffin cells is species-specific, and the ratios shown above are for human chromaffin cells. The opening of these channels during the course of the action potentials creates microdomains of Ca^{2+}, which occur immediately below the cell surface. These microdomains can be enhanced further by recruitment of Ca^{2+} from the internal stores by activation of the ryanodine receptors (RYRs) through a process of Ca^{2+}-induced Ca^{2+} release (CICR) (Module 2: Figure Ca^{2+}-induced Ca^{2+} release). The localized high intensity Ca^{2+} microdomains immediately below the surface of the plasma membrane are responsible for activating two processes. Firstly, it activates adseverin, which is also known as scinderin (SCIN), that severs the cortical actin filaments thus allowing the chromaffin granules to access the membrane. Secondly, the increase in Ca^{2+} triggers chromaffin granule exocytosis (Module 7: Figure chromaffin cell secretion).

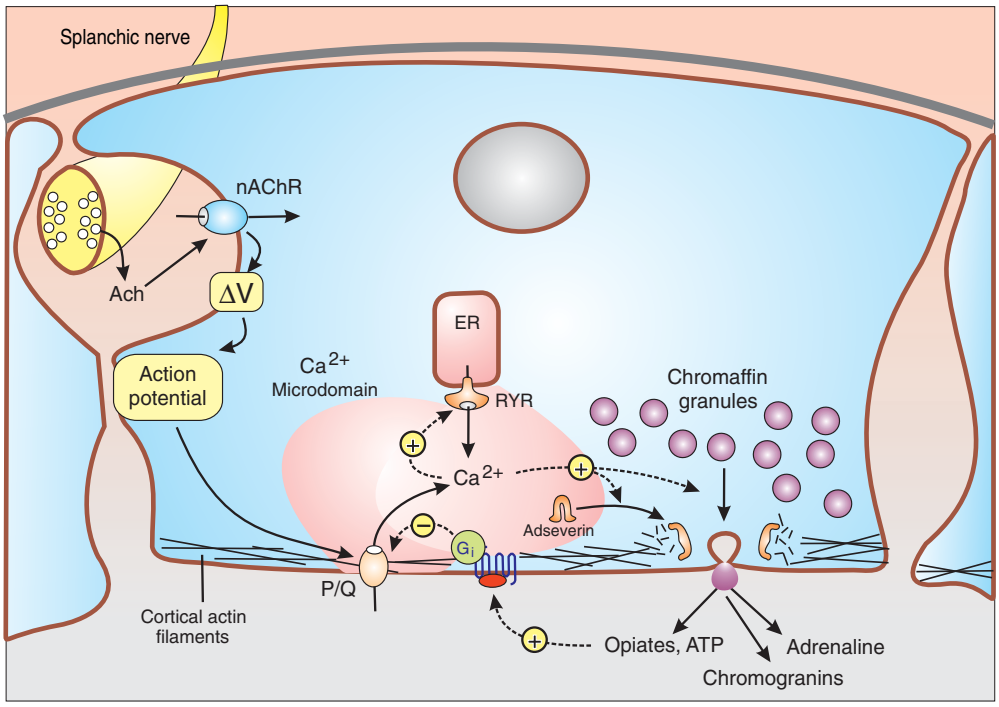
There are two type of exocytosis in chromaffin cells that depend on the intensity of stimulation (Module 7: Figure chromaffin cell exocytosis). Under resting or low intensity stimulus conditions, kiss-and-run vesicle fusion occurs. The brief narrow pore that forms during this exocytotic process allows a small part of the adrenaline (epinephrine) to be released, whereas the remaining adrenaline and larger molecules are retained within the granule that can then participate in multiple kiss-and-run vesicle fusion events. During intense stimulation, when there are higher levels of Ca^{2+}, the classical exocytotic/endocytotic cycle is activated where a complete fusion event occurs, resulting in the release of the entire contents of the granule. Once fusion is complete, the process of endocytosis retrieves the membrane as part of the vesicle cycle (Module 4: Figure vesicle cycle).

The increased release of opiates and perhaps also ATP that occur during intense stimulation contribute to an autoreceptor negative-feedback modulation of the VOCs (Module 7: Figure chromaffin cell exocytosis). For example, the purinergic P2Y and opioid μ and δ receptors inhibit the predominant P/Q-type channels through the G protein-dependent modulation mechanisms (Module 3: Figure Ca_V2 channel family).

Insulin-secreting β-cells

Carbohydrate metabolism is regulated through the antagonistic action of two polypeptide hormones: glucagon and insulin (Module 7: Figure metabolic energy network). The former stimulates the hydrolysis of glycogen,

Module 7: | Figure chromaffin cell secretion



Chromaffin cell stimulus–secretion coupling.
The splanchnic nerve that innervates chromaffin cells release acetylcholine (ACh) that activates the nicotinic acetylcholine receptor (nAChR) that gates Na^+, causing the membrane to depolarize (ΔV) sufficiently to trigger action potentials that sweep around the cell to activate voltage-operated channels (VOCs) such as the P/Q channels. Entry of external Ca^{2+} creates a microdomain of Ca^{2+} that triggers exocytosis and the release of chromaffin granule contents that contain adrenaline (epinephrine), opiates, ATP and chromogranins. The opiates and ATP can feed back to activate receptors that use the G protein subunit $G_{\alpha i}$ to inhibit the entry channels. The Ca^{2+} signal is amplified by stimulating the ryanodine receptors (RYRs) to release Ca^{2+} from the internal stores.

particularly in the liver, resulting in elevated plasma glucose levels, whereas insulin has the opposite effect by increasing the conversion of glucose into glycogen in liver and muscle. A number of external signals can release insulin, including an increase in plasma glucose. There is considerable clinical interest in the mechanism and control of insulin secretion because it often declines during the onset of insulin resistance (Module 12: Figure insulin resistance) that leads to the widespread and increasing occurrence of diabetes.

β-Cells, which are located together with the glucagon-secreting α-cells in the islets of Langerhans, have a structure that reflects their specialized role in protein secretion. The primary stimulus for insulin secretion from pancreatic β-cells is glucose and is part of a feedback loop between the metabolite and the hormone that regulates its metabolism. Glucose has a bifunctional role in that it controls both insulin release and biosynthesis.

β-Cells are also sensitive to circulating hormones and to neural influences that function to adjust their responsiveness to glucose. For example, glucagon appears to sensitize β-cells, whereas noradrenaline (norepinephrine) released from the autonomic nervous system inhibits insulin release. Therefore, the main control features that must be considered are the primary action of glucose, together with the way in which release is modulated by hormonal (glucagon, somatostatin) and neural (adrenergic and cholinergic) pathways.

With regard to control mechanisms, the main problem is therefore to understand how glucose functions in both insulin release and biosynthesis.

Islets of Langerhans

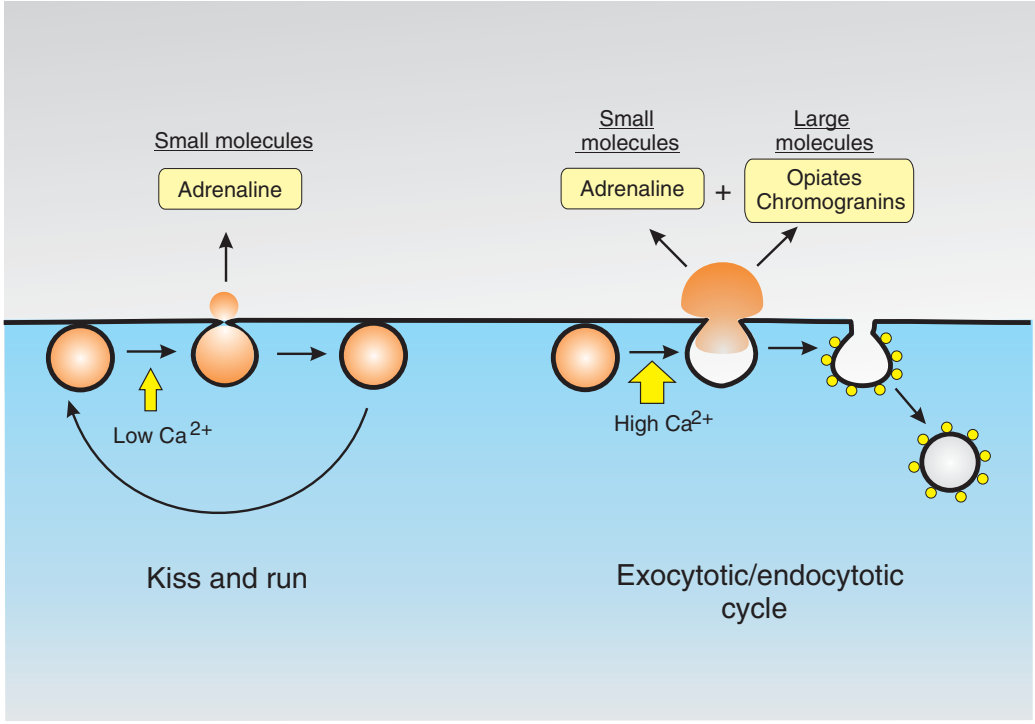
The islets of Langerhans are small groups of glucagon-secreting α-cells (30–40%) and insulin-secreting β-cells (60–70%) (Module 7: Figure pancreas).

Insulin release and biosynthesis

In response to an elevation in blood glucose, there is an oscillatory release of insulin. In addition to triggering the release of insulin, glucose also regulates the biosynthesis of insulin (Module 7: Figure β-cell signalling). The oscillatory response to glucose is driven by slow Ca^{2+} oscillations with a periodicity of 4–6 minutes. On the crest of each Ca^{2+} elevation there are faster Ca^{2+} spikes with a period of 10-–20 seconds.

The generation of these two oscillatory modes can be explained by the 'dual oscillator model' consisting of a glycolytic oscillator operating together with a Ca^{2+} oscillator (-cell">Module 7: Figure β-cell signalling).

Module 7: | Figure chromaffin cell exocytosis



Two modes of chromaffin cell Ca^{2+}-dependent exocytosis.
Release of chromaffin granules occurs through either a kiss-and-run mechanism or through a classical exocytotic/endocytotic cycle. The former mechanism, which is triggered by low levels of Ca^{2+}, creates a small fusion pore that can rapidly reverse, resulting in a small release of small molecules such as adrenaline. At higher levels of Ca^{2+}, a full fusion event occurs, resulting in the releases of both small molecules and the larger molecules in the granule, such as the opiates and chromogranin. This exocytosis is then followed by the membrane retrieval process of endocytosis (see Module 4: Figure vesicle cycle).

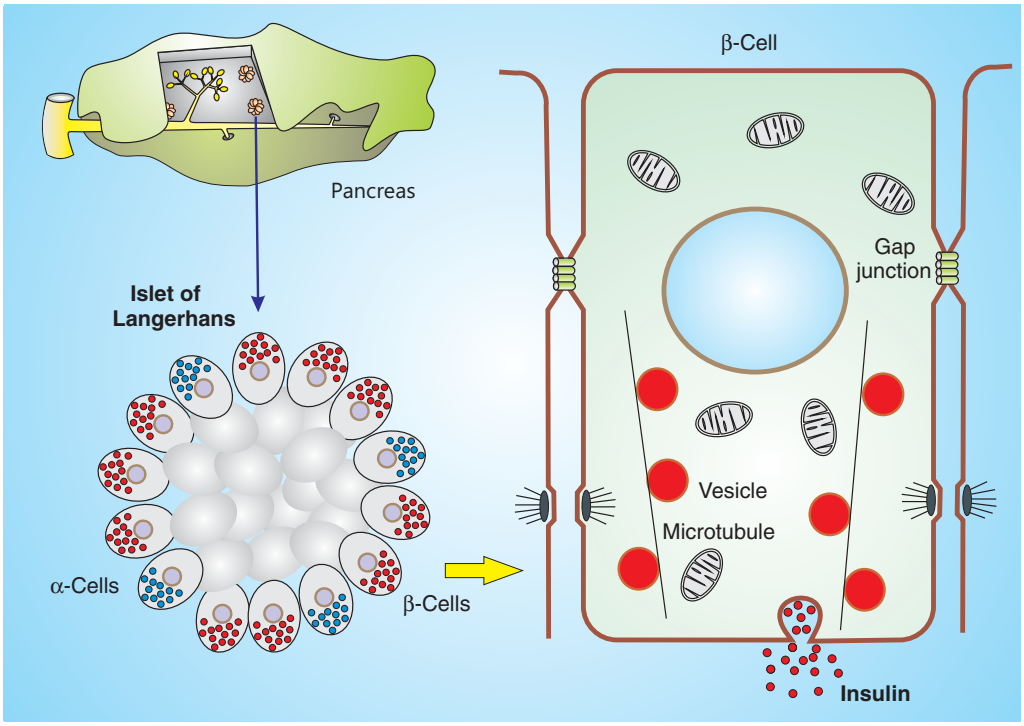
The glycolytic oscillator is driven by glucose entering the cell through the glucose transporter 2 (GLUT2) and is then rapidly phosphorylated by hexokinase IV (also known as glucokinase) to form glucose-6-phosphate (G-6-P), which is then converted into fructose-6-phosphate (F-6-P). The latter may play a role in the oscillatory mechanism by accelerating glycolysis as part of a feed forward mechanism whereby it is converted into fructose-2,6-P_2 (F-2,6-P_2) by phosphofructose kinase-2 (PFK2). The F-2,6-P_2 then stimulates PFK1 to convert F-6-P into fructose-1,6-P_2 (F-1-6-P_2). The latter then feeds into glycolysis and the tricarboxylic acid (TCA) cycle to increase the level of ATP while reducing the level of AMP, which function as a metabolic messenger to control insulin biosynthesis. The F-1-6-P_2 also has a role in that it acts to stimulate pyruvate kinase M2 (PKM2) to increase the formation of pyruvate that enters the mitochondrion to fuel the formation of ATP. The existence of such a glycolytic oscillator is supported by oscillations in many of the components and related metabolic variables such as oxygen consumption, mitochondrial membrane potential, NAD(P)H and ATP levels. The most important variable is the level of ATP that provides the link to the faster Ca^{2+} oscillator.

The increase in ATP acts to inhibit an ATP-sensitive K^+ (K_{ATP}) channel, which then depolarizes the membrane, resulting in the opening of L-type voltage-operated channels (VOCs) that provide the localized pulses of Ca^{2+} to trigger exocytosis of the preformed insulin vesicles. The membrane potential of β-cells is very sensitive to glucose (the membrane depolarizes by 8.5 mV for each 10-fold increase in glucose concentration). This opening of the Ca^{2+} channels appears as frequent bursts of action potentials, which constitutes the Ca^{2+} oscillator, that depend on a rapid feedback effect operating on a Ca^{2+}-sensitive K^+ channels, mostly likely to be BK channels, that rapidly terminates each action potential. The oscillatory spiking depends on a rapid switching between the L-type Ca^{2+} channels and the Ca^{2+}-sensitive K^+ channels (Module 7: Figure β-cell signalling). The intense activity of the Ca^{2+}-induced events may begin to deplete ATP and this may then slow down the glycolytic oscillator to terminate each oscillatory phase.

A second action of ATP may occur through the cyclic ADP-ribose (cADPR) signalling or nicotinic acid-adenine dinucleotide phosphate (NAADP) signalling pathways to release Ca^{2+} from internal stores. Ca^{2+} derived from internal stores via the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette has also been implicated in controlling release, and this can account for the way in which secretagogues such as acetylcholine (ACh) activate release.

Control of insulin biosynthesis depends on the ability of the AMP signalling pathway to activate gene transcription. Under resting conditions, a high level of the

Module 7: | Figure pancreas



Structure of insulin-secreting β-cells located in the islets of Langerhans.
The islets of Langerhans, which make up about 2% of the pancreas volume, are richly supplied with blood vessels. They consist of a nest of closely packed insulin-secreting β-cells (60–70%), together with fewer glucagon-secreting α-cells. These islets are distinct from the surrounding exocrine pancreatic acinar cells that release enzymes and fluid into pancreatic ducts. The β-cells contain granules lined up on cytoskeletal elements and directed towards the surface facing the capillaries, which have fenestrae similar to those seen in other endocrine organs, i.e. the kidney and intestine. These fenestrae ensure a rapid dispersal of insulin into the blood, and also enable the β-cells to continuously monitor the blood level of glucose and circulating hormones. Individual β-cells are connected by both desmosomes and by gap junctions. The latter ensure that the β-cells are electrically coupled, and this is critical for secretory efficiency.

metabolic messenger AMP normally represses gene transcription by phosphorylating and inactivating the hepatocyte nuclear factor 4α (HNF4α). When glucose is present and metabolism is enhanced, the decline in the level of AMP removes this inhibition, resulting in transcription of preproinsulin, which results in an increase in the synthesis of insulin. Ca^{2+} acting through the Ca^{2+}-sensitive transcription factor nuclear factor of activated T cells (NFAT) also provides an important stimulus for increasing insulin transcription (-cell">Module 7: Figure β-cell signalling).

The survival and proliferation of β-cells is also regulated by glucose and by various hormones, such as glucagon-like peptide-1 (GLP-1), which appear to act synergistically to activate gene transcription of factors such as cyclic AMP response element-binding protein (CREB) (Module 4: Figure CREB activation). The basis of this synergism seems to depend upon the CREB co-activator called transducer of regulated CREB (TORC). When phosphorylated, TORC remains in the cytoplasm, but upon dephosphorylation, it enters the nucleus, where it acts together with CREB to promote transcription (Module 7: Figure β-cell signalling). TORC phosphorylation depends upon a Ca^{2+}-dependent dephosphorylation mediated by calcineurin (CaN) and the cyclic AMP/protein kinase A (PKA)-dependent inhibition of the salt-inducible kinase 2 (SIK2) that phosphorylates TORC.

Insulin-like growth factor I (IGF-I) and leptin may reduce insulin secretion in response to GLP-1 by stimulating the activity of phosphodiesterase PDE3B, thereby reducing the level of cyclic AMP.

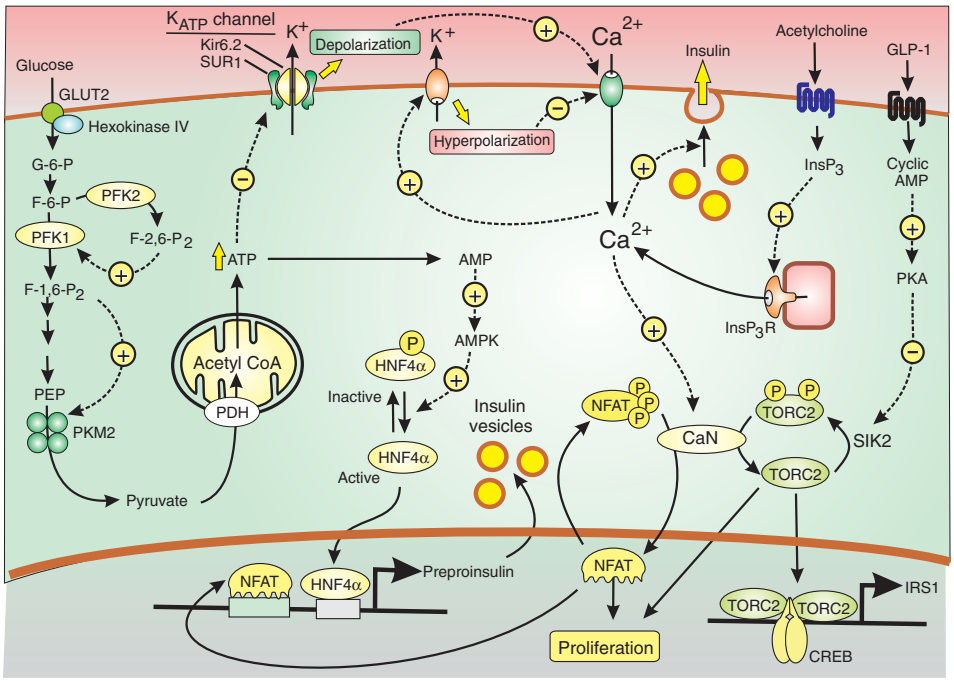
Insulin-secreting β-cell proliferation is also regulated by glucose and by various hormones, such as GLP-1. The signalling pathways responsible for activating proliferation are unclear, but seem to depend upon TORC, and perhaps also on Ca^{2+} signalling mechanisms. There is evidence that Ca^{2+} activates proliferation by stimulating the Ca^{2+}-sensitive transcription factor nuclear factor of activated T cells (NFAT).

Autoimmune destruction of these insulin-secreting β-cells is the cause of Type 1 diabetes.

Glucagon-secreting α-cells

Glucagon-secreting α-cells found within the islets of Langerhans (Module 7: Figure pancreas) play an essential role in maintaining blood glucose levels. In addition to releasing glucagon, they also have a sophisticated signalling system to ensure that the synthesis of glucagon is maintained at a level to satisfy the demands of the secretory system. These two processes of release and biosynthesis are synchronized by being coupled to the same Ca^{2+} signalling pathway (Module 7: Figure α-cell signalling). One

Module 7: | Figure β-cell signalling



Control of insulin release and biosynthesis by insulin-secreting β-cells.
β-Cells are controlled by multiple signalling pathways that regulate both the synthesis and the release of insulin. One important regulator is glucose itself, which enters through the glucose transporter (GLUT2) and is rapidly phosphorylated by hexokinase IV to form glucose 6-phosphate (G6P). G6P then feeds into glycolysis and the tricarboxylic acid (TCA) cycle to increase the level of ATP and reduce the level of AMP, which function as metabolic messengers to control both insulin release and biosynthesis. Information on the dual oscillation model depicted in this figure was taken from Bertram et al. (2007).

of the important actions of Ca^{2+} is to activate transcription factors such as nuclear factor of activated T cells (NFAT) (Module 4: Figure NFAT activation) and cyclic AMP response element-binding protein (CREB), which bind to the glucagon gene promoters.

Airway epithelial cells

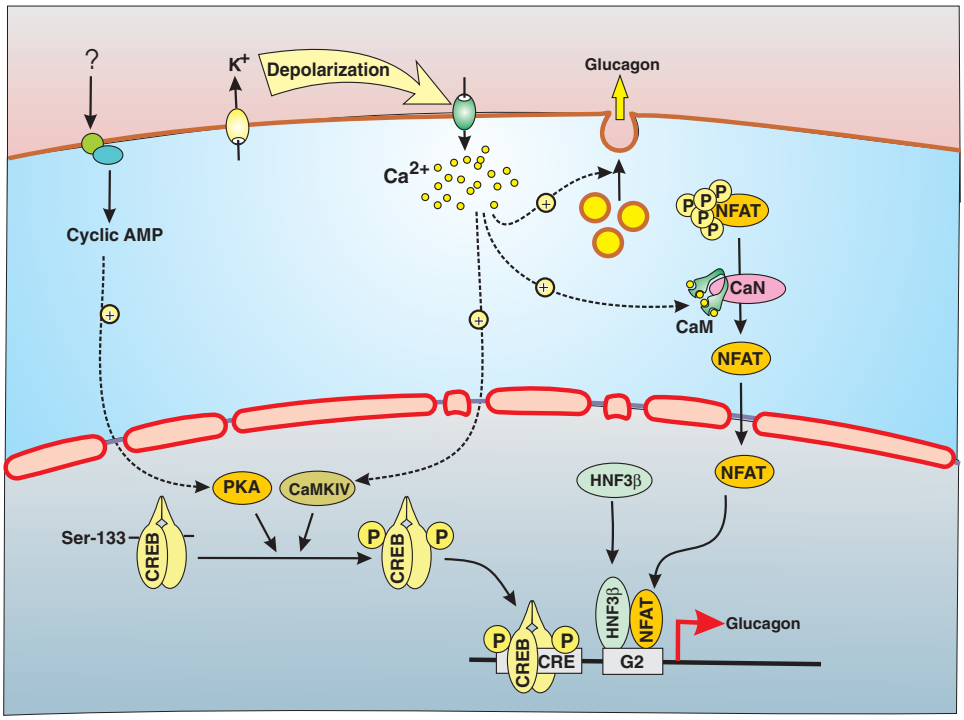
The cilia lining the airway epithelia are responsible for removing mucus from the lung. The operation of this mucociliary transport mechanism is determined by the tip velocity of the cilia and their co-ordinated activity to set up metachronal waves. The latter develop because successive cilia start their beat in sequence so that each one is slightly more advanced in its cycle, thereby setting up waves that can sweep over the epithelial surface to expel mucus from the lung.

The cilia beat very quickly with a basal frequency of about 14 Hz, but this ciliary beat frequency (CBF) can be increased to rates as high as 35 Hz following stimulation of the cells by ATP (Module 7: Figure airway cell oscillations). The ATP acts by binding to cell-surface P2Y receptors, which recruit the inositol 1,4,5-trisphosphate ($InsP_3$)/Ca^{2+} signalling cassette to generate the Ca^{2+} signal responsible for accelerating the CBF. The Ca^{2+} signal appears as oscillations whose frequency changes with agonist concentration, indicating that they use a frequency modulation (FM) mode of information coding (Module 6: Figure encoding oscillatory information). The very tight coupling between the Ca^{2+} oscillations and CBF is an example of a digital tracking mechanism for decoding FM Ca^{2+} signals (Module 6: Figure decoding oscillatory information), which must mean that Ca^{2+} has a direct action on the ciliary axoneme.

White fat cells

White fat cells are a key component of the metabolic energy network (Module 7: Figure metabolic energy network). They are found in white adipose tissue, which makes up most of the body fat distributed throughout the subcutaneous tissue, the omentum, mesenteries and retroperitoneal regions. They are characterized by having a large fat droplet made up of triacylglycerols; the constituent fatty acids and glycerol can be either removed or added to the droplet, depending on nutritional status. Lipolysis occurs during fasting when the white fat cell mobilizes its triacylglycerol store to release fatty acids and glycerol into the blood. During lipogenesis, free fatty acids are removed from the blood and converted back into triacylglycerol to reconstitute the lipid reservoir (Module 7: Figure white fat cells). The metabolism of white fat cells is thus poised

Module 7: | Figure α-cell signalling



Control of glucagon release and biosynthesis by glucagon-secreting α-cells.

Membrane depolarization opens L-type Ca^{2+} channels that introduce Ca^{2+} into the cell, which is responsible for both glucagon release and biosynthesis. The immediate effect is to stimulate the exocytosis of vesicles containing glucagon. The longer-term changes in biosynthesis depend upon Ca^{2+} activation of glucagon gene transcription. It activates the calcineurin (CaN)/nuclear factor of activated T cells (NFAT) transcription cascade. The dephosphorylated NFAT that enters the nucleus combines with hepatocyte nuclear factor 3β (HNF3β) to bind to a G2 site. In addition, Ca^{2+} enters the nucleus to activate Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV), which phosphorylates cyclic AMP response element-binding protein (CREB). The latter is also activated by hormonal inputs operating through cyclic AMP, and this co-operation between the Ca^{2+} and cyclic AMP pathways enables the cell to regulate biosynthesis through multiple inputs.

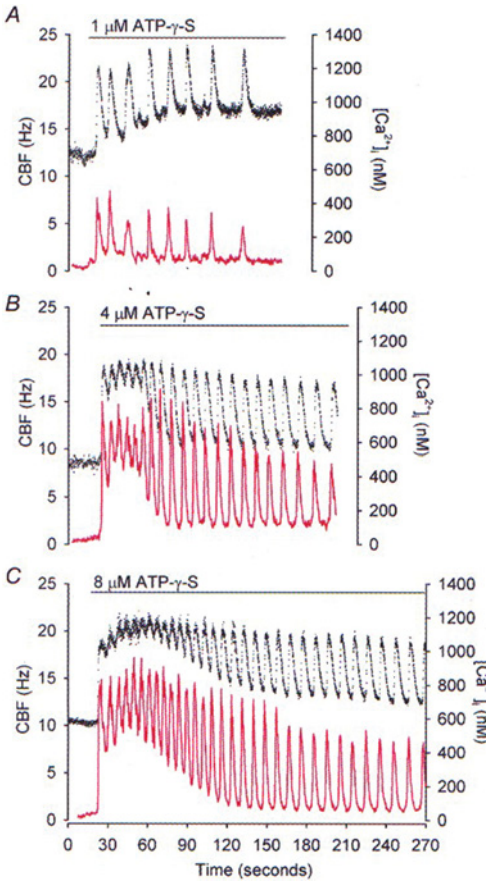
between anabolism and catabolism, depending on the energy requirements of the organism. A number of signalling mechanisms contribute to the hormonal control of white fat metabolism. In addition to its role in lipid storage, there also is an important white fat cell endocrine function.

Lipogenesis

Lipogenesis is the process of conjugating three free fatty acid (FFA) molecules to a glycerol backbone to form triacylglycerol (TGA) that is stored in the large lipid droplets (Module 7: Figure white fat cell metabolism). There are two sources of the FFA precursor. Firstly, it can enter across the plasma membrane through entry pathways such as fatty acid transport protein 1 (FATP1) or CD36, which is a fatty acid translocase (FAT) also known as FAT/CD36. Secondly, a process of FFA synthesis using pyruvate derived from glycolysis as a precursor. The pyruvate is formed from the glucose that enters through the GLUT4 channel that is regulated by insulin. When it enters the mitochondrion, pyruvate is converted into acetyl-CoA and then to citrate that enters the cytoplasm where an ATP citrate lyase converts it back into acetyl-CoA, which is then carboxylated to malonyl CoA by an acetyl-CoA carboxylase (ACC). The malonyl CoA is then converted into the fatty acid palmitate by a fatty acid synthase (FAS) complex. A variety of enzymes then extends the length of this palmitate and a stearoyl CoA desaturase 1 (SCD-1) introduces double bonds. The resulting fatty acylCoA precursors are now conjugated to glycerol 3-phosphate through a series of steps: glycerol 3-phosphate acyltransferase (GPAT) combines the two precursors to form lysophosphatidic acid (LPA), which is converted into phosphatidic acid (PA) by acyl-CoA:1-acylglycerol-sn-3-phosphate acyltransferase (AGPAT) and diacylglycerol:acyl-CoA acyltransferase (DGAT) completes the process by converting DAG into TAG (Module 7: Figure white fat cell metabolism). The movement of fatty acids about the cell during these metabolic processes is facilitated by adipocyte protein 2 (aP2).

Most of the stored lipid is composed of neutral triacylglycerols, which are non-polar and aggregate by van der Waals forces. Triacylglycerols consist of three fatty acids (R-COOH, R'-COOH and R''-COOH) connected by ester linkages to glycerol. The nature of the fatty acid components shows considerable variation from species to species. Palmitic and oleic acids are the major components in human fat, which also contains appreciable quantities of linoleic, stearic, myristic and palmitoleic acids. These fatty acids are a very efficient way of storing energy. The carbon atoms are fully reduced (-CH_2-) and, because they are hydrophobic, they attract each other, and by excluding

Module 7: | Figure airway cell oscillations



Ca^{2+} control of ciliary beat frequency (CBF) in airway epithelial cells. When the ciliary beat frequency (CBF) (black line) and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (shown in red) were measured simultaneously, there was a tight coupling between these two parameters. In response to the non-metabolizable ATP analogue adenosine 5-[γ-thio]triphosphate (ATPγS), the $[Ca^{2+}]_i$ was found to oscillate. The three panels illustrate that when the frequency increased as the concentration of ATPγS increased, there was a parallel change in CBF. Reproduced from Zhang, L. and Sanderson, M.J. (2003) Oscillations in ciliary beat frequency and intracellular calcium concentration in rabbit tracheal epithelial cells induced by ATP. J. Physiol. 546:733–749, with permission from Blackwell Publishing; see Zhang and Sanderson 2003.)

water, they thus occupy much less space than other energy stores such as glycogen.

Lipolysis

During lipolysis, the triacylglycerol (TAG) stored in lipid droplet is hydrolysed to glycerol and fatty acids that then leave the cell through specific transporters (Module 7: Figure white fat cell metabolism). Glycerol is transported out of the cell following lipolysis by aquaporin 7, which is strongly expressed in white fat cells. The fatty acid leaves through the fatty acid transport protein 1 (FATP1) or CD36, which is a fatty acid translocase (FAT) also known as FAT/CD36. The TAG is hydrolysed by both hormone-sensitive lipase (HLS) and by adipose triglyceride lipase (ATGL).

Hormonal control of white fat cell metabolism

The dynamic nature of this lipid reservoir depends on hormones such as insulin, which drives lipogenesis, and catecholamine, adrenocorticotrophic hormone (ACTH) and glucagon, which act through the cyclic AMP signalling pathway to promote lipolysis (Module 7: Figure lipolysis and lipogenesis).

The activation of lipolysis depends on receptors that are coupled to the formation of cyclic AMP (cAMP) that acts on protein kinase A (PKA) to phosphorylate the hormone-sensitive lipase (HLS) and the adipose triglyceride lipase (ATGL) that initiates the hydrolysis of triacylglycerol to free fatty acids (FFA) and glycerol. These hydrolytic products then diffuse out of the fat cell and enter the blood vessels, where they bind to albumin (Ab).

Insulin opposes this lipolytic process by activating the PtdIns 3-kinase signalling pathway to form the lipid second messenger PtdIns3,4,5P_3 (PIP_3), which activates phosphoinositide-dependent kinase 1 (PDK1) and protein kinase B (PKB). The latter induces lipogenesis through a number of mechanisms. It can phosphorylate phosphodiesterase 3B (PDE3B), and thus attenuates the lipolytic response by enhancing the hydrolysis of the pro-lipolytic second messenger cyclic AMP. The insulin signalling pathway can also enhance lipogenesis by activating the entry of glucose through the GLUT4 transporter and by facilitating the entry of fatty acids by increasing the insertion of the fatty acid transporters FATP1 and FAT/CD36. In addition, insulin enhances the activation of peroxisome-proliferator-activated receptor γ (PPARγ), which acts to enhance the expression of those genes that function in lipid synthesis (Module 4: Figure PPARγ activation).

White fat cell endocrine function

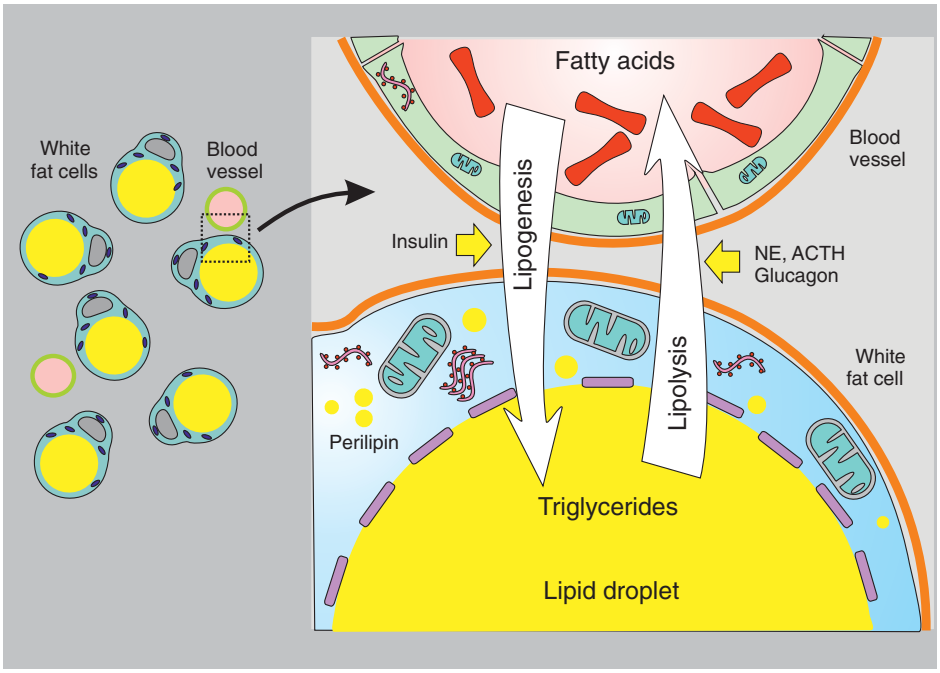
In addition to its role in lipid metabolism and storage, the white fat cell also has an endocrine function in that it releases various adipokine hormones such as leptin and adiponectin (Module 7: Figure lipolysis and lipogenesis). The release of adiponectin is enhanced by the osteocalcin released from osteoblasts. The release of leptin contributes to the mechanisms that control food intake and body weight (Module 7: Figure control of food intake).

White fat cell insulin resistance plays a central role in the development of metabolic syndrome and the onset of diabetes that occurs in obesity.

Brown fat cells

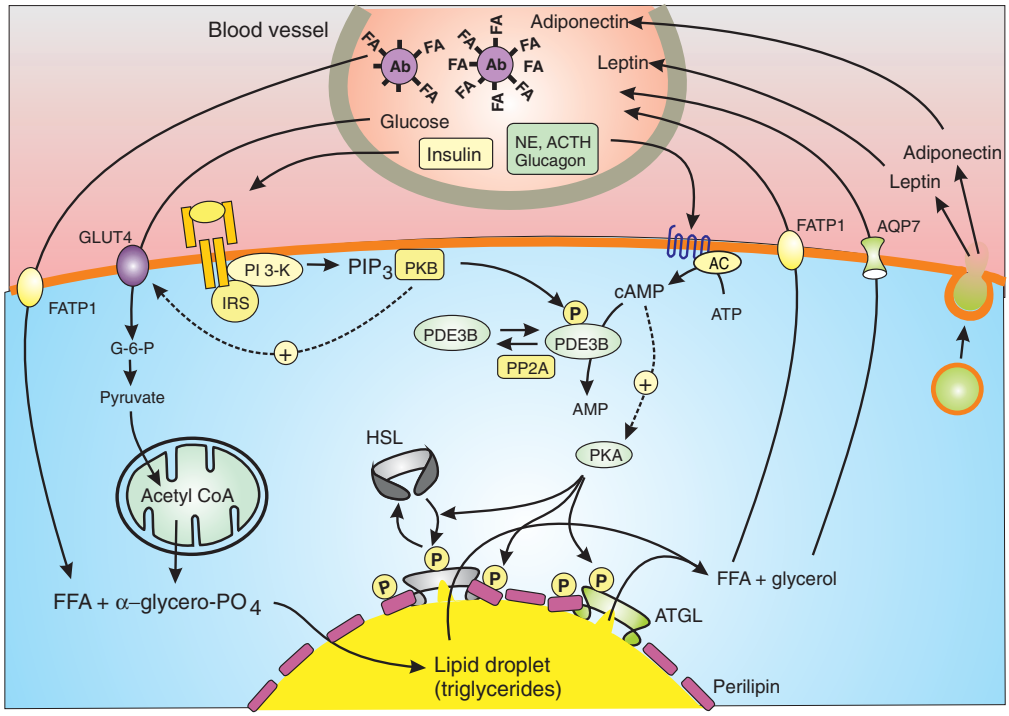
Brown adipose tissue is much less abundant than white adipose tissue. It is restricted to specific areas such as the back between the shoulder blades, along blood vessels in the neck and surrounding the adrenals and kidney. The brown colour results from the cytochromes packed in to the large number of mitochondria, which are specialized to produce heat. Heat can be generated by animals either through shivering (muscular activity) or by an increase in metabolic rate that has been called nonshivering thermogenesis and is largely confined to the brown fat cells. Unlike white adipose tissue, brown fat cells are organized to form distinct lobules, which are richly supplied

Module 7: | Figure white fat cells



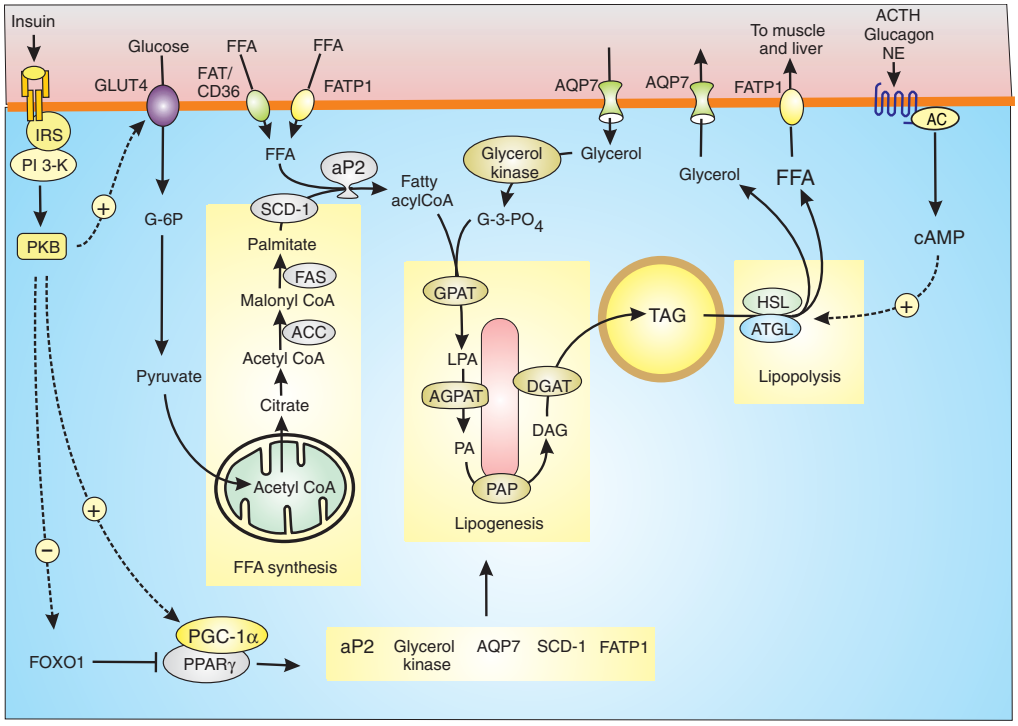
The functional organization of white fat cells.
The white fat cells are tightly packed to form a compact tissue with a sparse blood supply. Each cell contains a large lipid droplet, which can displace the cytoplasm to the periphery, where it forms a thin ring containing mitochondria and some smaller lipid droplets with a consistency resembling that of the large droplet. A conventional membrane does not surround the lipid droplet, but there is a structural web at the surface mainly composed of a protein called perilipin, which appears to provide a structural framework for organizing the enzymes that control lipid deposition and mobilization. ACTH, adrenocorticotrophic hormone; NE, noradrenaline (norepinephrine).

Module 7: | Figure lipolysis and lipogenesis



Hormonal regulation of lipogenesis and lipolysis in white fat cells.
The process of lipolysis, which is shown on the right side of the figure, is controlled by hormones such as noradrenaline (norepinephrine; NE), adrenocorticotrophic hormone (ACTH) and glucagon. The action of these hormones is mediated by the cyclic AMP signalling pathway. The lipogenic action of insulin is carried out by the PtdIns 3-kinase signalling pathway that stimulates the activity of phosphodiesterase PDE3B, which thus removes cyclic AMP and reduces the ability of this signalling pathway to promote lipolysis.

Module 7: | Figure white fat cell metabolism



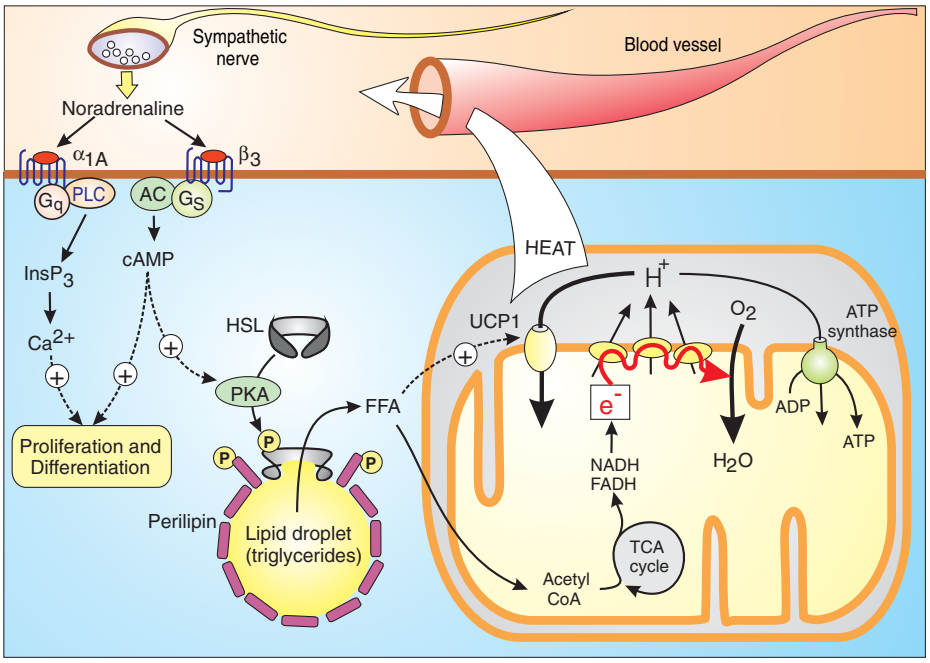
White fat cell metabolism.
The metabolism of white fat cells is poised between lipogenesis and lipolysis that are regulated through separate signalling pathways. The free fatty acid (FFA) used to synthesize triacylglycerol (TAG) is either synthesized from pyruvate or is taken up across the plasma membrane through a fatty acid transport protein 1 (FATP1). During lipolysis, the TAG is hydrolysed by lipases such as adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HLS) to release glycerol and FFA. See text for further details.

with blood vessels and by nerves originating from the sympathetic nervous system. These nerves are responsible for releasing the noradrenaline that regulates heat production (Module 7: Figure brown fat cell). Information regarding temperature, feeding states and energy reserves are thought to be collated by the ventromedial hypothalamic nucleus (VMN), which then send information through to the sympathetic nerves to release the noradrenaline that stimulates heat production by the brown fat cells.

The fuel used for heat production is stored in the form of triacylglycerols within numerous small lipid droplets that are coated with the protein perilipin that protects the lipid from hydrolysis by hormone-sensitive lipase (HLS). Activation of heat production depends upon the cyclic AMP signalling pathway that is induced when noradrenaline binds to a β-adrenoceptor (β_3) (Module 1: Table G protein-coupled receptors) that is coupled through G_S to adenylyl cyclase. The resulting formation of cyclic AMP then has various actions. It acts through protein kinase A (PKA) to phosphorylate and activate HLS. In addition, PKA phosphorylates perilipin causing it to dissociate from the surface of the lipid that then allows HLS access to its substrate.

Hydrolysis of the triacylglycerols by HLS liberates free fatty acids that enter the mitochondria on the carnitine shuttle and then undergo β-oxidation to release acetyl CoA that enters the tricarboxylic acid (TCA) cycle to fuel heat production. Under normal circumstances, cells exercise a tight control over mitochondrial respiration such that oxidation of fuel reserves is curtailed when energy demands are low. The mitochondria in brown fat cells have evolved a mechanism to overcome this control by developing a proton leak that enables fuels to be oxidized continuously thereby releasing large amounts of heat (Module 7: Figure brown fat cell). This leak depends upon the expression of one of the uncoupling proteins, which in the case of the brown fat cells is uncoupling protein 1 (UCP1) that enables H^+ to return to the mitochondrial matrix without producing ATP. During normal mitochondrial metabolism, the generation of ATP depends on fuels such as the FFA entering the tricarboxylic acid (TCA) cycle to produce NADH and FADH, which then provides the electrons that travel down the electron transport chain, during which protons are ejected into the cytoplasm (Module 5: mitochondrial Ca^{2+} signalling). The inner mitochondrial membrane is exceedingly impermeable to H^+ so the removal of H^+ creates a very large membrane potential of –180 mV. There are a few components in the inner mitochondrial membrane that are capable of dissipating this energy reservoir. Protons flowing through the ATP synthase energize ATP synthesis. Mitochondrial Ca^{2+} uptake is driven by this energy gradient. Finally, protons can leak back into the matrix through UCP1 and enables the mitochondria to generate heat (Module 7: Figure brown fat cells).

Module 7: | Figure brown fat cell



Brown fat cell thermogenesis.
Sympathetic nerves release noradrenaline that acts through the cyclic AMP (cAMP) signalling pathway to control heat production by brown fat cells. Cyclic AMP acts through cyclic-AMP-dependent protein kinase A (PKA) to phosphorylate and stimulate hormone-sensitive lipase (HSL) to release free fatty acids (FFA) that are then metabolized by the tricarboxylic acid (TCA) cycle in the mitochondria. FFA also provides the stimulus to activate uncoupling protein 1 (UCP1) that dissipates the H^+ gradient resulting in the production of heat that is carried away by the blood vessels.

Just how noradrenaline activates heat production is still somewhat uncertain but all the evidence suggests that the mechanism is indirect and depends on the release of free fatty acid, which then functions as a typical metabolic messenger to activate UCP1.

Uncoupling proteins

Uncoupling proteins (UCPs) are integral membrane proteins located in the inner mitochondrial membrane. The UCP family contains five homologues with most information available for uncoupling protein 1 (UCP1), uncoupling protein 2 (UCP2) and uncoupling protein 3 (UCP3), uncoupling protein 4 (UCP4) and uncoupling protein 5 (UCP5). These three UCPs functions as channels to transport H^+ that flow out of the mitochondrial matrix to the cytoplasm. In the case of UCP1, which is strongly expressed in the mitochondria of brown fat cells, this leak serves to dissipate the proton gradient that normally functions to produce ATP through oxidative phosphorylation and the energy is lost as heat (Module 7: Figure brown fat cells).

Uncoupling protein 1 (UCP1)

Uncoupling protein 1 (UCP1) is restricted to brown fat cells where it functions in thermogenesis (Module 7: Figure brown fat cells). The expression of UCP1 during the differentiation of brown fat cells is controlled by noradrenaline operating through the cyclic AMP signalling pathway (Module 8: Figure brown fat cell differentiation).

Uncoupling protein 2 (UCP2)

Uncoupling protein 2 (UCP2) is fairly widely expressed and its function has been studied in some detail in insulin-secreting β-cells. The function of UCP2 remains somewhat problematic. It seems clear that UCP2 can transport H^+ to provide a proton leak across the mitochondrial membrane. However, this leak is not as large as that caused by UCP1 so there is less uncoupling of oxidative phosphorylation and less heat production. Nevertheless, any leak will reduce the efficiency of energy production. There is considerable interest in the finding that the activity of UCP2 is increased by superoxide ($O_2^{-\bullet}$), which is one of the reactive oxygen species (ROS) that can function as intracellular messengers (Module 2: Figure summary of redox signalling).

The mitochondrial reactive oxygen species (ROS) formation of superoxide ($O_2^{-\bullet}$) will thus activate UCP2 and this will serve to dissipate the proton gradient and reduce the formation of ATP. In effect, this sets up a negative-feedback loop because the activation of UCP2 by superoxide ($O_2^{-\bullet}$) will reduce the mitochondrial membrane potentials that drive ROS formation. This negative-feedback loop may have serious consequences for the insulin-secreting β-cells because the decrease in ATP production attenuates the main stimulus responsible for insulin secretion (-cell">Module 7: Figure β-cell signalling). A polymorphism in the promoter region of the gene encoding UCP2, which results in an increased expression of this coupling protein, results in defective

insulin secretion and contributes to the onset of Type 2 diabetes.

Uncoupling protein 3 (UCP3)

Uncoupling protein 3 (UCP3) is expressed mainly in skeletal and heart muscle. It is highly homologous with uncoupling protein 2 (UCP2) and these two proteins share similar properties. The function of the UCP3-dependent leak of protons across the inner mitochondrial membrane is still not known. Like UCP2, UCP3 activity can be enhanced by superoxide ($O_2^{-\bullet}$), which will reduce the mitochondrial membrane potential, ATP production and the formation of reactive oxygen species (ROS). In effect, this control through superoxide ($O_2^{-\bullet}$) will establish a negative-feedback loop that could protect cells against excessive ROS formation by the mitochondria.

Uncoupling protein 4 (UCP4)

Uncoupling protein 4 (UCP4) is expressed in the dopaminergic neurons of the substantia nigra pars compacta. Like the other UCPs, it can leak protons across the inner mitochondrial membrane and this may provide a mechanism to protect these neurons from oxidative stress (Module 10: Figure tonic oscillation in DA neurons). An increase in mitochondrial ROS stimulates the opening of uncoupling proteins 4 to leak H^+ that will depolarize the inner mitochondrial membrane potential thus reducing respiration and the entry of Ca^{2+}.

Uncoupling protein 5 (UCP5)

Uncoupling protein 5 (UCP5) is expressed in the dopaminergic neurons of the substantia nigra pars compacta. Like the other UCPs, it can leak protons across the inner mitochondrial membrane and this may provide a mechanism to protect these neurons from oxidative stress (Module 10: Figure tonic oscillation in DA neurons). An increase in mitochondrial ROS stimulates the opening of uncoupling proteins 5 to leak H^+ that will depolarize the inner mitochondrial membrane potential thus reducing respiration and the entry of Ca^{2+}.

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