CALCIUM SIGNALLING: DYNAMICS, HOMEOSTASIS AND REMODELLING

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Ca^{2+} is a highly versatile intracellular signal that operates over a wide temporal range to regulate many different cellular processes. An extensive Ca^{2+}-signalling toolkit is used to assemble signalling systems with very different spatial and temporal dynamics. Rapid highly localized Ca^{2+} spikes regulate fast responses, whereas slower responses are controlled by repetitive global Ca^{2+} transients or intracellular Ca^{2+} waves. Ca^{2+} has a direct role in controlling the expression patterns of its signalling systems that are constantly being remodelled in both health and disease.

CALCIUM

Ca^{2+} is a highly versatile intracellular signal that can regulate many different cellular functions[1,2]. To achieve this versatility, the Ca^{2+}-signalling system operates in many different ways to regulate cellular processes that function over a wide dynamic range (FIG. 1). At the synaptic junction, for example, Ca^{2+} triggers exocytosis within microseconds, whereas at the other end of the scale Ca^{2+} has to operate over minutes to hours to drive events such as gene transcription and cell proliferation. One of the challenges is to understand how these widely different Ca^{2+}-signalling systems can be set up to control so many divergent cellular processes.

At any moment in time, the level of intracellular Ca^{2+} is determined by a balance between the 'on' reactions that introduce Ca^{2+} into the cytoplasm and the 'off' reactions through which this signal is removed by the combined action of buffers, pumps and exchangers (FIG. 1). During the on reaction, a small proportion of the Ca^{2+} binds to the effectors that are responsible for stimulating numerous Ca^{2+}-dependent processes (FIG. 1). These heterogeneous Ca^{2+}-signalling systems are assembled from an extensive Ca^{2+}-signalling toolkit[1] (BOX 1). Through alternative splicing, many of the toolkit components have different isoforms with subtly different properties, which further expands the versatility of Ca^{2+} signalling.

This review concentrates on the nature of the Ca^{2+}-signalling toolkit and how it is exploited to create many different Ca^{2+}-signalling systems. We have gathered emerging evidence that the expression patterns of the different signalling components might be regulated by Ca^{2+} itself to set up a 'self-assessment system' to ensure that these signalling systems remain constant. Alterations in this Ca^{2+}-dependent homeostatic mechanism might be the cause of many prominent diseases, as exemplified by end-stage heart failure.

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Ca^{2+}-signalling toolkit and signalling dynamics

Each cell type expresses a unique set of components from the Ca^{2+}-signalling toolkit to create Ca^{2+}-signalling systems with different spatial and temporal properties. Almost all Ca^{2+}-signalling systems have one thing in common — they function by generating brief pulses of Ca^{2+}. These Ca^{2+} transients are created by variations of the basic on/off reactions that are outlined in FIG. 1.

Signal Ca^{2+} is derived either from internal stores or from the external medium (FIG. 1). In the case of the latter, there are many different plasma-membrane channels (BOX 1) that control Ca^{2+} entry from the external medium in response to stimuli that include membrane depolarization, stretch, noxious stimuli, extracellular agonists, intracellular messengers and the depletion of intracellular stores. The release of Ca^{2+} from the internal store — usually the endoplasmic reticulum (ER) or its muscle equivalent, the sarcoplasmic reticulum (SR) — is controlled by Ca^{2+} itself, or by an expanding group of messengers[3], such as inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), cyclic ADP ribose (cADPR), nicotinic

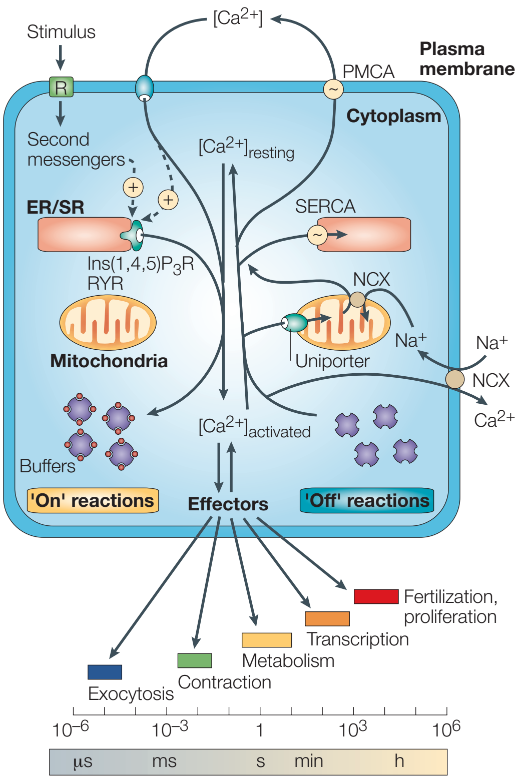


Figure 1 | **Calcium-signalling dynamics and homeostasis.** During the 'on' reactions, stimuli induce both the entry of external Ca^{2+} and the formation of second messengers that release internal Ca^{2+} that is stored within the endoplasmic/sarcoplasmic reticulum (ER/SR). Most of this Ca^{2+} (shown as red circles) is bound to buffers, whereas a small proportion binds to the effectors that activate various cellular processes that operate over a wide temporal spectrum. During the 'off' reactions, Ca^{2+} leaves the effectors and buffers and is removed from the cell by various exchangers and pumps. The Na^+/Ca^{2+} exchanger (NCX) and the plasma-membrane Ca^{2+}-ATPase (PMCA) extrude Ca^{2+} to the outside, whereas the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) pumps Ca^{2+} back into the ER. Mitochondria also have an active function during the recovery process in that they sequester Ca^{2+} rapidly through a uniporter, and this is then released more slowly back into the cytosol to be dealt with by the SERCA and the PMCA. Cell survival is dependent on Ca^{2+} homeostasis, whereby the Ca^{2+} fluxes during the off reactions exactly match those during the on reactions. [Ca^{2+}], Ca^{2+} concentration; Ins(1,4,5)P_3R, inositol-1,4,5-trisphosphate receptor; RYR, ryanodine receptor.

acid adenine dinucleotide phosphate (NAADP) and sphingosine-1-phosphate (S1P), that either stimulate or modulate the release channels on the internal stores.

Inositol-1,4,5-trisphosphate. Many stimuli function through PHOSPHOLIPASE C (PLC) to generate Ins(1,4,5)P_3 that functions to release Ca^{2+} from an internal store[1] (FIG. 2). There are several PLC isoforms (BOX 1) that are activated by different mechanisms, such as G-protein-coupled receptors (PLCβ), tyrosine-kinase-coupled receptors (PLCγ), an increase in Ca^{2+} concentration (PLCδ) or activation through Ras (PLCε)[4]. The Ins(1,4,5)P_3 that is responsible for triggering the Ca^{2+} oscillations that are required to activate mammalian eggs during fertilization is generated by a newly discovered PLC, PLCζ, that is injected into the egg by the sperm[5] (FIG. 2).

PHOSPHOLIPASE C (PLC). A phosphodiesterase that splits the bond between the phosphorus atom and the oxygen atom at C-1 of the glycerol moiety.

The dynamics of Ins(1,4,5)P_3 production can be very different depending on the receptor type being activated[6]. Bradykinin and neurokinin A receptors give large rapid Ca^{2+} transients, whereas lysophosphatidic acid (LPA), thrombin and histamine receptors give smaller responses that develop slowly but persist for much longer. Some of this variability might arise from the fact that receptors engage different transducing elements and PLC isoforms in a cell-type-specific manner[7,8]. For example, at the parallel fibre/Purkinje cell synapse, glutamate operates through a metabotropic glutamate receptor type I (mGluR1)→$G_{q\alpha}$→PLCβ4→Ins(1,4,5)P_3→ Ins(1,4,5)P_3 receptor 1 (Ins(1,4,5)P_3R1) →Ca^{2+} cascade, whereas hippocampal neurons respond to the same agonist using a mGluR5→$G_{11\alpha}$→PLCβ1→Ins(1,4,5)P_3→Ins(1,4,5)P_3R→Ca^{2+} sequence. The reason why neurons use these different signalling cascades is not known, but there is evidence from other cell types that mGluR1 and mGluR5 can result in radically different Ca^{2+} signals[9]. mGluR1 produces a single Ca^{2+} transient, whereas mGluR5 generates an oscillatory pattern.

Another example is found in pancreatic acinar cells in which muscarinic receptors generate small, highly localized Ca^{2+} transients, whereas the cholecystokinin (CCK) receptors produce much larger global Ca^{2+} transients. These different outputs might depend on the action of the regulators of G-protein signalling (RGS)[10]. In comparison to CCK receptors, muscarinic receptors are much more sensitive to the inhibitory action of RGS proteins (RGS2, RGS4 and RGS16), and this might limit the supply of Ins(1,4,5)P_3. Qualitatively different Ca^{2+} signals might also arise if receptors combine Ins(1,4,5)P_3 with other Ca^{2+}-mobilizing messengers/modulators such as cADPR or NAADP[11–13], as discussed in the next section.

cADPR and NAADP. These two nucleotides mobilize intracellular Ca^{2+} through different mechanisms, even though they are generated through the same enzymatic pathway[14] (FIG. 2). Mammalian cells express CD38, which is a multifunctional ADP ribosyl cyclase with both synthase and hydrolase activity. The synthase component of CD38 can use either NAD to produce cADPR or NADP to generate NAADP. CD38 has been located both on the cell surface and on intracellular membranes. On the cell surface, CD38 has been suggested to both produce cADPR and NAADP and to transport them into cells. Different activation mechanisms have been proposed for the cytosolic enzyme. External agonists might activate it, but a consistent mechanism for the transduction process is still lacking. An alternative possibility is that the formation of cADPR and NAADP is sensitive to cellular metabolism (FIG. 2). In other words, cADPR and NAADP might be metabolic messengers that can relay information about the state of cellular metabolism to the Ca^{2+}-signalling pathways. Such an idea is supported by the fact that cADPR metabolism by the hydrolase is inhibited by either ATP[15] or NADH[16] (FIG. 2).

Box 1 | Calcium-signalling toolkit

The Ca^{2+}-signalling system has a very large toolkit of signalling components that can be mixed and matched to create a diverse array of signalling units that can deliver Ca^{2+} signals with very different spatial and temporal properties. The following list is by no means inclusive but it summarizes some of the main toolkit components in mammalian cells:

Receptors
G-protein-coupled receptors: muscarinic receptors (M1–3) | adrenoceptors ($\alpha_{1A–C}$) | angiotensin receptor (AT_1) | bombesin receptors (BRS-1, BRS-2) | bradykinin receptors (B_1, B_2) | cholecystokinin receptors (CCK_1, CCK_2) | endothelin receptors (ET_A, ET_B) | metabotropic glutamate receptors (mGlu1, mGlu5) | luteinizing receptor (LSH) | histamine receptor (H_1) | 5-Hydroxytryptamine receptors ($5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, $5\text{-}HT_{2C}$) | leukotrine receptors (BLT, $CysLT_1$, $CysLT_2$) | neurotensin receptor (NTS1) | oxytocin receptor (OT) | extracellular Ca^{2+}-sensing receptor (CaR) | prostanoid receptor ($PGF_{2\alpha}$) | thrombin receptor (PAR1) | substance P receptor (NK_1) | substance K receptor (NK_2) | substance B receptor (NK_3) | thyrotropin-releasing hormone receptor (TRHR) | vasopressin receptors (V_{1A}, V_{1B})

Tyrosine-kinase-linked receptors: platelet-derived growth factor receptors (PDGFRα, PDGFRβ) | epidermal growth factor receptors (ERBB1–4)

Transducers
G proteins: $G_{q\alpha}$, $G_{11\alpha}$, $G_{14\alpha}$, $G_{16\alpha}$ | $G_{\beta\gamma}$

Regulators of G-protein signalling (RGS): RGS1 | RGS2 | RGS4 | RGS16

Phospholipase C (PLC): PLCβ1–4 | PLCγ1, PLCγ2 | PLCδ1–4 | PLCε | PLCζ

ADP ribosyl cyclase: CD38

Channels
Voltage-operated channels (VOCs): $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$, $Ca_V1.4$ (L-type) | $Ca_V2.1$ (P/Q-type) | $Ca_V2.2$ (N-type) | $Ca_V2.3$ (R-type) | $Ca_V3.1$, $Ca_V3.2$, $Ca_V3.3$ (T-type)

Receptor-operated channels (ROCs): NMDA receptors (NR1, NR2A, NR2B, NR2C, NR2D) | ATP receptor ($P2X_7$) | nACh receptor

Second-messenger-operated channels (SMOCs): cyclic nucleotide gated channels (CNGA 1–4, CNGB 1, CNGB 3) | arachidonate-regulated Ca^{2+} channel (I_{ARC})

Transient receptor potential (TRP) ion-channel family: TRPC1–7 | TRPV1–6 | TRPM1–8 | TRPML (mucolipidin 1,2) | TRPNI (ANKTM1)

Inositol-1,4,5-trisphophate receptors ($Ins(1,4,5)P_3Rs$): $Ins(1,4,5)P_3R1–3$

Ryanodine receptors (RYRs): RYR1–3

Polycystins: PC-1 | PC-2

Channel regulators: triadin | junctin | sorcin | FKBP12 | FKBP12.6 | phospholamban | IRAG | IRBIT

Calcium buffers
Cytosolic buffers: calbindin D-28 | calretinin | parvalbumin

ER/SR buffers and chaperones: calnexin | calreticulin | calsequestrin | GRP 78 | GRP 94

Calcium effectors
Ca^{2+}-binding proteins: calmodulin | troponin C | synaptotagmin | S100A1–12, S100B, S100C, S100P | annexin I–X | neuronal Ca^{2+} sensor family (NCS-1) | visinin-like proteins (VILIP-1, VILIP-2, VILIP-3) | hippocalcin | recoverin | Kv-channel-interacting proteins (KchIP1–3) | guanylate-cyclase-activating proteins (GCAP1–3)

Calcium-sensitive enzymes and processes
Ca^{2+}-regulated enzymes: Ca^{2+}/calmodulin-dependent protein kinases (CaMKI–IV) | myosin light chain kinase (MLCK) | phosphorylase kinase | $Ins(1,4,5)P_3$ 3-kinase | PYK2 | protein kinase C (PKC-α, PKC-βI, PKC-βII, PKC-γ) | cyclic AMP phosphodiesterase (PDE1A–C) | adenylyl cyclase (AC-1, AC-III, AC-VIII, AC-V, AC-VI) | nitric oxide synthase (endothelial NOS, eNOS; neuronal NOS, nNOS) | calcineurin | Ca^{2+}-activated proteases (calpain I and II)

Transcription factors: nuclear factor of activated T cells (NFATc1–4) | cyclic AMP response element-binding protein (CREB) | downstream regulatory element modulator (DREAM) | CREB-binding protein (CBP)

Ca^{2+}-sensitive ion channels: Ca^{2+}-activated potassium channels (SK, small conductance Ca^{2+}-sensitive channel; IK, intermediate conductance Ca^{2+}-sensitive channel; BK, large conductance Ca^{2+}-sensitive channel) | Human Cl^- channel, Ca^{2+}-activated (HCLCA1)

Calcium pumps and exchangers
Na^+/Ca^{2+} exchangers (NCXs): NCX1–3

Mitochondrial channels and exchangers: permeability transition pore | Na^+/Ca^{2+} exchanger | Ca^{2+} uniporter | H^+/Ca^{2+} exchanger

Plasma membrane Ca^{2+}-ATPases (PMCAs): PMCA1–4

Sarco(endo)plasmic reticulum Ca^{2+}-ATPases (SERCAs): SERCA1–3

Golgi pumps: SPCA1, SPCA2

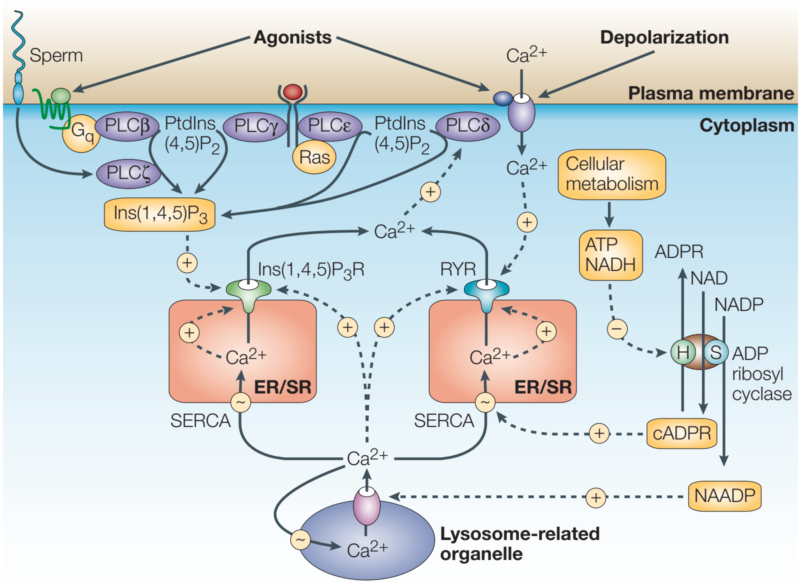


Figure 2 | **Calcium-mobilizing messengers and modulators.** Various second messengers or modulators regulate the release of Ca^{2+} from internal stores by the inositol-1,4,5-trisphosphate receptor ($Ins(1,4,5)P_3R$) or the ryanodine receptor (RYR). These release channels are sensitive to factors that function from the cytosol and from within the lumen of the endoplasmic/sarcoplasmic reticulum (ER/SR). The $Ins(1,4,5)P_3R$ is regulated by inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), which is generated by various signalling pathways using different isoforms of phospholipase C (PLC; β, δ, ε, γ and ζ). The nucleotides cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) are generated by ADP ribosyl cyclase, which has both synthase (S) and hydrolase (H) activity. This dual-function enzyme might be sensitive to the cellular metabolism, as ATP and NADH inhibit the hydrolase. Just how cADPR and NAADP function is still unclear, but they seem to have an indirect action. cADPR might function by stimulating the sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) pump, which increases the luminal level of Ca^{2+}, which results in sensitization of the RYR. NAADP functions through a channel that is located on a lysosomal-like organelle to release Ca^{2+} that can either stimulate the $Ins(1,4,5)P_3R$ or the RYR directly, or might function indirectly, like cADPR, to increase the lumenal level of Ca^{2+}. $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate.

RYANODINE RECEPTOR (RYR). A Ca^{2+}-release channel that is located in the membrane of the endoplasmic/sarcoplasmic reticulum and is regulated by several factors including Ca^{2+} itself, as well as the intracellular messenger cyclic ADP ribose.

Ca^{2+}-INDUCED Ca^{2+} RELEASE (CICR). An autocatalytic mechanism by which cytoplasmic Ca^{2+} activates the release of Ca^{2+} from internal stores through channels such as inositol-1,4,5-trisphosphate receptors or ryanodine receptors.

VOLTAGE-OPERATED CHANNEL (VOC). A plasma-membrane ion channel that is activated by membrane depolarization.

NAADP functions by releasing Ca^{2+} from an internal store, which was recently identified in sea urchin eggs as a reserve granule store. This store is distinct from the stores that are regulated by $Ins(1,4,5)P_3Rs$ and RYANODINE RECEPTORS (RYRs), because the latter stores can be depleted without affecting the ability of NAADP to release Ca^{2+}. This separate reserve granule store might be equivalent to a lysosome-related organelle in mammalian cells[17] (FIG. 2). In contrast to the $Ins(1,4,5)P_3Rs$ and RYRs, the NAADP release mechanism is not sensitive to Ca^{2+} and therefore does not support the process of Ca^{2+}-INDUCED Ca^{2+} RELEASE (CICR). NAADP seems to have a role in both initiating and coordinating various Ca^{2+}-signalling systems[11,12]. NAADP might sensitize the $Ins(1,4,5)P_3Rs$ and RYRs through two mechanisms (FIG. 2): it might function directly by providing trigger Ca^{2+}, or indirectly by releasing a bolus of Ca^{2+}, which is then taken up by the other stores in which it can sensitize the release channels by functioning from the lumen.

The function of cADPR resembles that of a modulator rather than a messenger. When cADPR is introduced into cells there usually is no immediate effect[18,19]. In those cases in which it elicits a Ca^{2+} response, there usually is a long latency[20,21], which indicates that it might be functioning indirectly by increasing the Ca^{2+} sensitivity of RYRs, as has been shown in neurons[18,19,22] and in the heart[21,23]. In these two excitable cells, VOLTAGE-OPERATED CHANNELS (VOCs) respond to membrane depolarization by admitting a small pulse of Ca^{2+}, which then stimulates the RYRs to release further Ca^{2+} through CICR (FIG. 2). The degree to which the initial entry signal is amplified by CICR, which is referred to as the 'gain' of the signalling system, can be regulated by cADPR. This might have pathological consequences, as cardiac arrhythmias can develop if cADPR sets the gain too high[24].

Just how cADPR functions to modulate the sensitivity of the RYRs is still unclear. One view is that cADPR functions as a messenger to stimulate Ca^{2+} release by the RYRs[14]. However, cADPR does not seem to bind directly to the RYR, instead it seems to function through some intermediary — the FK506-binding protein 12.6 (FKBP12.6), which is a subunit that is associated with the RYR (BOX 2, part a), has been suggested as a possible candidate[25]. An alternative view is that cADPR functions by activating the SARCO(ENDO)PLASMIC RETICULUM Ca^{2+}-ATPASE (SERCA) pump[23]. Such an indirect action is consistent with many of the properties of cADPR. By stimulating the SERCA pump, cADPR would enhance the load of Ca^{2+} within the lumen of the ER — a process that is known to increase the Ca^{2+} sensitivity of RYRs[23,26] (FIG. 2).

Sphingolipid-derived messengers. Some agonist-evoked Ca^{2+} signals might be controlled by a sphingolipid-activated Ca^{2+}-release pathway that functions independently of $Ins(1,4,5)P_3Rs$ or RYRs[27]. In mast cells, the sphingolipid-derived messenger S1P functions together with $Ins(1,4,5)P_3$ to generate the Ca^{2+} signals that underlie the synthesis and release of inflammatory mediators. The dual activation of these pathways leads to a Ca^{2+} signal with a rapid peak (S1P dependent) and a sustained plateau ($Ins(1,4,5)P_3$ dependent)[28]. Exactly how S1P stimulates Ca^{2+} release from intracellular stores is unclear. Until recently, the best candidate for the S1P receptor was a widely expressed protein known as SCaMPER (sphingolipid Ca^{2+}-release-mediating protein of endoplasmic reticulum). However, it has no similarity to any known intracellular Ca^{2+} channel and is a small (~20-kDa) protein with only one transmembrane domain. A recent reinvestigation of SCaMPER showed that there was little correlation between its intracellular location and that of known intracellular Ca^{2+} stores. Furthermore, expression of SCaMPER was found not to confer sensitivity to sphingolipids or to affect Ca^{2+} homeostasis, but could lead to cell death[29].

Ca^{2+}-entry mechanisms. Ca^{2+} that enters the cell from the outside is a principal source of signal Ca^{2+} during the on reaction (FIG. 1). Entry of Ca^{2+} is driven by the presence of a large electrochemical gradient across the plasma membrane. Cells use this external source of signal Ca^{2+} by activating various entry channels with widely different properties. We know the most about the VOCs, which are found in excitable cells

Box 2 | Multimolecular complexes of calcium-signalling components

Like many other signalling systems, the components of the different Ca^{2+}-signalling systems are often grouped together into large complexes.

The RYR2 Ca^{2+}-release complex in cardiac cells

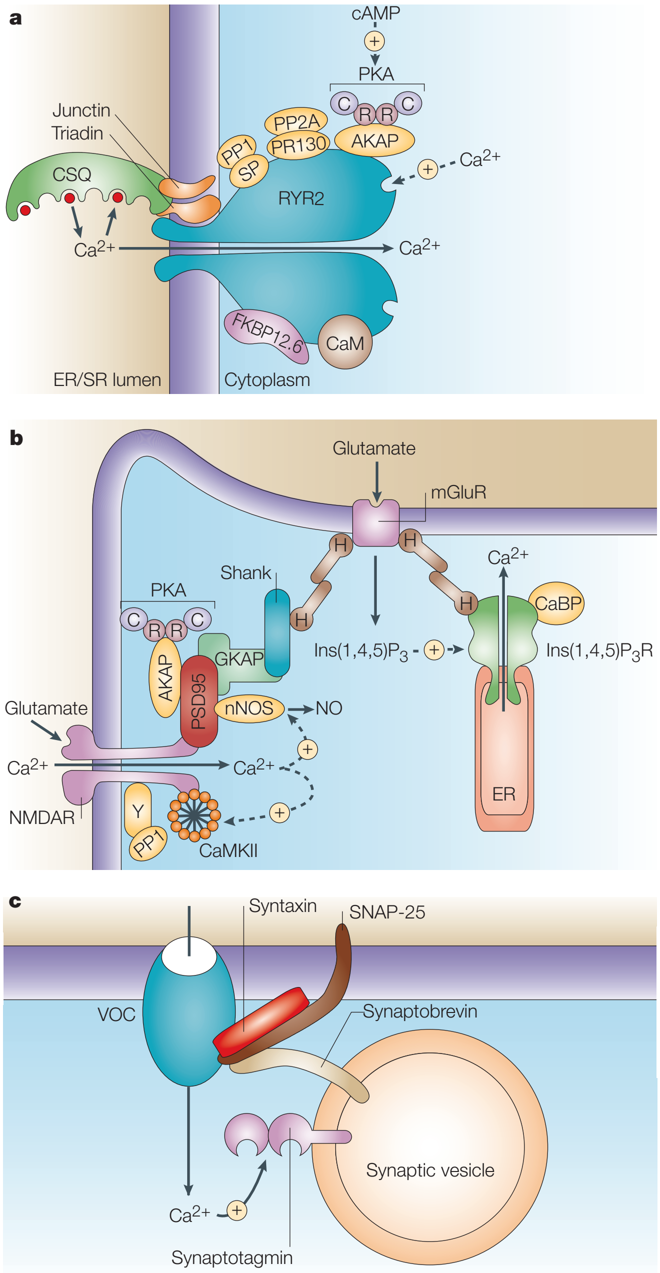
Ryanodine receptor 2 (RYR2) is composed of four subunits that form the channel, which is associated with various proteins that function to modulate its opening (see figure, part **a**). The endoplasmic/sarcoplasmic reticulum (ER/SR) luminal Ca^{2+}-binding protein calsequestrin (CSQ) modulates the sensitivity of RYR2 (FIG. 2). The interaction between CSQ and RYR2 is facilitated by the transmembrane proteins triadin and junctin. The reversible phosphorylation of RYR2 by cyclic AMP (cAMP) is controlled by protein kinase A (PKA), which is composed of regulatory (R) and catalytic (C) subunits that are attached through an A kinase anchoring protein (AKAP). Dephosphorylation depends on protein phosphatase 2A (PP2A), which is attached through the isoleucine-zipper-binding scaffolding protein PR130, and on protein phosphatase 1 (PP1), which is attached through spinophilin (SP). RYR2 is also modulated by calmodulin (CaM) and by FK506-binding protein 12.6 (FKBP12.6).

NMDA and Ins(1,4,5)P_3 receptor complexes in neurons

Glutamate-induced Ca^{2+} entry is carried out by NMDA (*N*-methyl-D-aspartate) receptors (NMDARs) that are linked to other signalling components (see figure, part **b**), some of which are Ca^{2+} sensitive, such as Ca^{2+}/CaM-dependent kinase II (CaMKII) and neuronal nitric oxide synthase (nNOS). NMDARs are also associated with other proteins such as yotiao (Y), which binds PP1, and the scaffolding protein PSD95, which links into other signalling components such as the AKAP–PKA complex and guanylate kinase-associated protein (GKAP). Proteins such as shank and Homer (H) might link the metabotropic glutamate receptor (mGluR) to both the NMDAR and the inositol-1,4,5-trisphospate receptor (Ins(1,4,5)P_3R), which is also associated with a Ca^{2+}-binding protein (CaBP).

Synaptic-vesicle complex

The exocytotic release of synaptic vesicles, which occurs within microseconds of membrane depolarization, is activated by an influx of Ca^{2+} through the Ca_v2 voltage-operated channels (see figure, part **c**), for example, the P/Q-, N- and R-type VOCs (BOX 1). These entry channels have a binding site that anchors them to components of the exocytotic machinery such as syntaxin or SNAP-25. In this way, the Ca^{2+} that enters through the channel has immediate access to the synaptotagmin Ca^{2+} sensor that is thought to initiate the fusion event that is carried out by syntaxin, SNAP-25 and synaptobrevin.



and generate the rapid Ca^{2+} fluxes that control fast cellular processes such as muscle contraction or exocytosis at synaptic endings (BOX 2, part c).

There are many other Ca^{2+}-entry channels (BOX 1) that open in response to different external signals, such as the RECEPTOR-OPERATED CHANNELS (ROCs), for example the NMDA (*N*-methyl-D-aspartate) receptors (NMDARs) that respond to glutamate (BOX 2, part b). There also are SECOND-MESSENGER-OPERATED CHANNELS (SMOCs) that are controlled by internal messengers, such as the cyclic-nucleotide-gated channels that are found in sensory systems and the arachidonic-acid-sensitive channel[30]. In addition to these more clearly defined channel-opening mechanisms, there are many other channel types that are sensitive to a diverse array of stimuli, such as the STORE-OPERATED CHANNELS (SOCs), thermosensors and stretch-activated channels. Many of these channels belong to the large transient receptor protein (TRP) ion-channel family[31–34], which are encoded by up to 23 different genes. This family consists of three groups — the canonical TRPC family, the vanilloid TRPV family and the melastatin TRPM family (BOX 1). TRP channels tend to have low conductances and therefore can operate over much longer time scales without swamping the cell with too much Ca^{2+}. Members of the TRP family are particularly important in controlling slow cellular processes such as smooth-muscle contractility and cell proliferation.

SARCO(ENDO)PLASMIC RETICULUM Ca^{2+}-ATPASE (SERCA). A pump located in sarcoplasmic or endoplasmic reticulum membranes that couples ATP hydrolysis to the transport of Ca^{2+} from cytosolic to lumenal spaces.

RECEPTOR-OPERATED CHANNEL (ROC). A plasma-membrane ion channel that opens in response to the binding of an extracellular ligand.

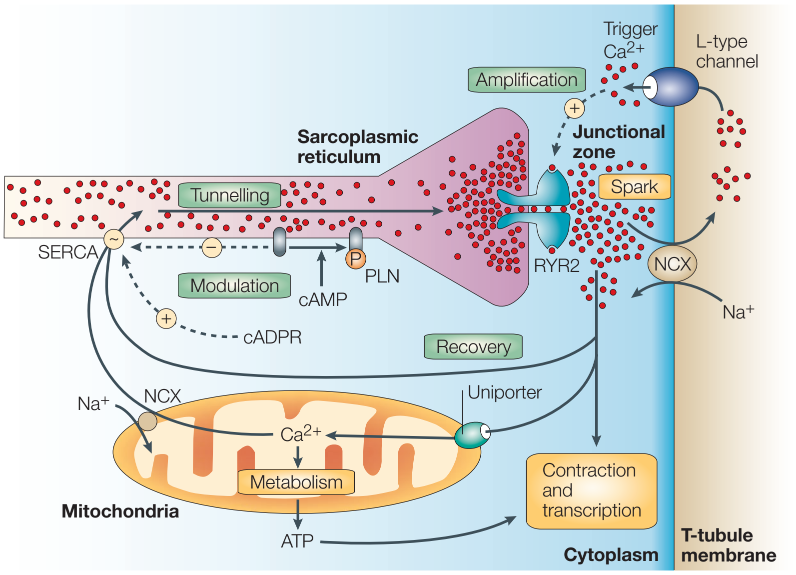


Figure 3 | **Cardiac calcium-signalling module.** This self-contained module generates the localized Ca^{2+} 'sparks' that are responsible for activating contraction and perhaps also gene transcription (Ca^{2+} is shown as red circles). Signalling begins with an amplification step in the junctional zone, where the L-type channel on the T-tubule membrane responds to depolarization by introducing a small pulse of trigger Ca^{2+}, which then diffuses across the narrow gap of the junctional zone to activate ryanodine receptor 2 (RYR2) (BOX 2, part a) to generate a spark. Ca^{2+} from this spark diffuses out to activate contraction. Recovery occurs as Ca^{2+} is pumped out of the cell by the Na^+/Ca^{2+} exchanger (NCX) or is returned to the sarcoplasmic reticulum (SR) by sarco(endo)plasmic Ca^{2+}-ATPase (SERCA) pumps on the non-junctional region of the SR. A proportion of this Ca^{2+} travels through the mitochondria, during which time it stimulates the metabolism to provide the ATP that is necessary to maintain contraction and transcription. The Ca^{2+} that is returned to the SR 'tunnels' back to the junctional zone to be used again for subsequent heart beats. This circulation of Ca^{2+} is modulated by second messengers such as cyclic AMP (cAMP), which removes the inhibitory action of phospholamban (PLN), or by cyclic ADP ribose (cADPR) that activates the pump to increase the amount of releasable Ca^{2+} in the SR.

SECOND-MESSENGER-OPERATED CHANNEL
(SMOC). A plasma-membrane ion channel that opens in response to the binding of intracellular second messengers such as diacylglycerol, cyclic nucleotides or arachidonic acid.

STORE-OPERATED CHANNEL
(SOC). A plasma-membrane ion channel, of uncertain identity, that opens in response to the depletion of internal Ca^{2+} stores.

FIGHT-OR-FLIGHT RESPONSE
This response occurs in the hypothalamus, which, when stimulated by stress, initiates a sequence of nerve-cell firing and chemical release (including adrenaline and noradrenaline) that prepares our body for running or fighting.

Ca^{2+} release from internal stores. The other principal source of Ca^{2+} for signalling is the internal stores that are located primarily in the ER/SR, in which Ins(1,4,5)P_3Rs or RYRs regulate the release of Ca^{2+} (FIG. 2). These two channels are sensitive to Ca^{2+}, and this CICR process contributes to the rapid rise of Ca^{2+} levels during the on reaction and the development of regenerative Ca^{2+} waves. In addition to Ca^{2+}, these channels are regulated by many different factors that operate on both the luminal and cytosolic surfaces of the channel (BOX 2, part a).

In the case of the Ins(1,4,5)P_3Rs, the primary determinants are Ins(1,4,5)P_3 and Ca^{2+}. The binding of Ins(1,4,5)P_3 increases the sensitivity of the receptor to Ca^{2+}, which has a biphasic action (that is, it activates at low concentrations, but becomes inhibitory at the higher concentrations that occur after Ca^{2+} release). This Ca^{2+} regulation is mediated by the direct action of Ca^{2+} on the receptor, as well as indirectly through calmodulin (CaM), which can be activating or inhibitory[35,36]. Recently, a Ca^{2+}-binding protein (CaBP) was shown to activate Ca^{2+} release in the absence of Ins(1,4,5)P_3 (REF 37). However, this new function for CaBP is contentious[38]. In addition to these cytosolic actions, Ca^{2+} can also sensitize the Ins(1,4,5)P_3R by functioning from the lumen (FIG. 2). This luminal sensitivity might be conferred by the ER luminal lectin chaperones calreticulin and calnexin, which are Ca^{2+}-binding proteins that are known to interact with the Ins(1,4,5)P_3R. The Ca^{2+}-binding sites on calreticulin have affinities that are sufficiently low to enable them to regulate Ins(1,4,5)P_3Rs through luminal Ca^{2+} levels.

The InsP_3R can also be modulated by other signalling pathways, including phosphorylation by Ca^{2+}/CaM-dependent kinase II (CaMKII), cGMP-dependent protein kinase (PKG), protein kinase C (PKC) and cAMP-dependent protein kinase (protein kinase A; PKA)[2]. For some of these kinases, the scaffolding proteins that mediate recruitment to their site of action on the Ins(1,4,5)P_3R have been identified. For example, PKG is recruited by the Ins(1,4,5)P_3R-associated cGMP kinase substrate (IRAG), which results in Ins(1,4,5)P_3R phosphorylation and a decrease in receptor activity[39]. In B cells, the Ins(1,4,5)P_3R is phosphorylated by the tyrosine kinase Lyn, which results in increased activity. This phosphorylation event is facilitated by the scaffolding protein Bank that links together Lyn, the Ins(1,4,5)P_3R and the B-cell receptor[40]. Similarly, in neuronal cells Ins(1,4,5)P_3Rs are tethered to mGluRs by the Homer protein, thereby linking the source of Ins(1,4,5)P_3 production to its site of action (BOX 2, part b). The protein phosphatases 1 and 2a (PP1 and PP2a) have been found to co-purify with PKA and the Ins(1,4,5)P_3R, which is reminiscent of their interaction with the RYR[41]. This protein complex of phosphatase, kinase and substrate allows the rapid regulation of Ins(1,4,5)P_3R activity by phosphorylation/dephosphorylation.

RYRs are also controlled by several signalling pathways, as illustrated by the RYR2 that is found in cardiac cells. Like the Ins(1,4,5)P_3R, RYR2 responds to Ca^{2+} in a bell-shaped fashion (that is, RYR2 is inactive at low nM concentrations of Ca^{2+}, active at low μM concentrations of Ca^{2+} and inactivated by high concentrations of Ca^{2+} that are in the mM range)[42]. In the cardiac myocyte, Ca^{2+} that enters through the L-type Ca^{2+} channel activates RYR2 to create the 'spark' that triggers contraction (FIG. 3). This role of RYR2 in excitation–contraction (E–C) coupling is a highly regulated process that involves many accessory factors that are bound to both its luminal and cytosolic domains (BOX 2, part a).

Opening of RYR2 is inhibited by CaM, which is present in Ca^{2+}-bound and non-bound forms, which are known as CaCaM and apoCaM, respectively[43]. Several accessory proteins contribute to the control of heart function by the sympathetic nervous system, through the FIGHT-OR-FLIGHT RESPONSE[44]. After β-adrenergic stimulation, RYR2 is phosphorylated by PKA, which is attached through an A kinase anchoring protein (AKAP)[45] (BOX 2, part a). Phosphorylation of RYR2 by PKA results in the dissociation of the 12.6-kDa FKBP12.6, which normally stabilizes the RYR in a closed conformation[45]. Furthermore, FKBP12.6 is required for the coupled gating between neighbouring receptors that coordinates the activation and inactivation of physically linked receptors during E–C coupling[44]. PKA also phosphorylates sorcin, which is another regulator of RYR2 (REF. 46).

The RYR2 macromolecular complex also includes the phosphatases PP1 and PP2a, which interact with RYR2 through the leucine/isoleucine-zipper-binding scaffolding proteins spinophilin and PR130, respectively (BOX 2, part a)[44]. The presence of these phosphatases in the same protein complex as the kinase and substrate ensure that there is a tight regulation of the phosphorylation status of the receptor and, therefore, its activity. The membrane and luminal region of the RYR2 is present in a complex with three other proteins: junctin, triadin and calsequestrin (BOX 2, part a)[47,48]. Calsequestrin is the principal Ca^{2+}-binding protein of muscle cells and is highly concentrated in the junctional region of the SR. In the lumen of the SR, calsequestrin does not bind directly to the RYR, but is anchored adjacent to the Ca^{2+}-release site through junctin and triadin, which are both membrane-bound proteins (BOX 2, part a)[48]. Triadin and junctin interact with calsequestrin in a Ca^{2+}-dependent manner[48], and this interaction might account for the sensitivity of RYR2s to Ca^{2+} in the lumen[23,26]. Indeed, transgenic studies have shown that there is a significant role for calsequestrin, junctin and triadin in cardiac Ca^{2+} signalling and hypertrophy (see below).

Emerging Ca^{2+} channels. AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE (ADPKD) has been linked to mutations in two membrane-spanning proteins, which are known as polycystin-1 (PC-1) and polycystin-2 (PC-2)[49]. PC-1 has a large extracellular domain and might function in transducing sensory information, such as shear stress during fluid flow[49]. PC-2 has been shown to function as an intracellular Ca^{2+}-release channel[50] and to form a non-selective cation channel when it is inserted into the plasma membrane[51]. PC-2 has homology with VOCs and TRP channels, and when expressed by itself it shows spontaneous channel activity. Mutations in either protein somehow corrupts the PC-1–PC-2 complex, which leads to abnormal Ca^{2+} signalling and, consequently, altered rates of cell proliferation and function. These proteins seem to have a widespread expression, although their role outside the kidney is not well understood. Interestingly, PC-2 is localized to the ER in some cell types[52], which indicates that it might be a ubiquitous CICR channel.

Ca^{2+}-binding proteins. During the on reaction (FIG. 1), Ca^{2+} flows into the cell and interacts with different Ca^{2+}-binding proteins, of which there are ~200 encoded by the human genome that function either as Ca^{2+} effectors or buffers[2].

The buffers, which become loaded with Ca^{2+} during the on reaction and unload during the off reaction, function to fine-tune the spatial and temporal properties of Ca^{2+} signals. They can alter both the amplitude and the recovery time of individual Ca^{2+} transients. These buffers have different properties and expression patterns. For example, calbindin D-28 (CB) and calretinin (CR) are fast buffers, whereas parvalbumin (PV) has much slower binding kinetics and a high affinity for Ca^{2+}. These are mobile buffers that increase the diffusional range of Ca^{2+} (REF. 53). Of the Ca^{2+} that enters the cytosol, only a very small proportion ends up being free, because most of it is rapidly bound to the buffers and, to a lesser extent, the effectors (FIG. 1). The Ca^{2+}-BINDING RATIO (K_S) is used to compare the buffering capacity of cells. Some cells, such as motoneurons and adrenal chromaffin cells, have K_S values of approximately 40, but this can increase to values as high as 2,000 in Purkinje neurons[54]. The low buffering capacity of motoneurons enables them to generate rapid Ca^{2+} signals, but this does make them much more susceptible to excitotoxic stress, which might contribute to motoneuron disease[54].

The importance of buffers in Ca^{2+} signalling has emerged from studying transgenic animals in which individual buffers have been deleted. When the *PV* gene that encodes PV is knocked out, the relaxation of fast-twitch muscles is impaired, but they become fatigue resistant through a remarkable compensatory mechanism that involves an upregulation of the mitochondria, which increases their capacity to sequester Ca^{2+}, such that they can partially replace the loss of PV[55]. These $PV^{-/-}$ animals also have defects in their short-term synaptic plasticity[56] owing to defects in Ca^{2+} signalling.

Several different effectors, such as troponin C, CaM, synaptotagmin, S100 proteins and the annexins (BOX 1), are responsible for activating different Ca^{2+}-sensitive cellular processes. For those processes that respond rapidly, there is a close juxtaposition of the signalling and effector components as occurs for the pre-synaptic and post-synaptic events in neurons (BOX 2, parts b,c). For those processes that operate over longer time scales, such as cell proliferation, Ca^{2+} functions more globally (see below).

Pumps and exchangers. During the course of a typical Ca^{2+} transient, the on reactions are counteracted by the off reactions, during which time various pumps and exchangers remove Ca^{2+} from the cytoplasm (FIG. 1). The pumping mechanisms also have important homeostatic functions in that they maintain the resting level of Ca^{2+} at approximately 100 nM and ensure that the internal stores are kept loaded. Four different pumping mechanisms are responsible for the off reaction — the PLASMA-MEMBRANE Ca^{2+}-ATPASE (PMCA), the Na^+/Ca^{2+} EXCHANGER (NCX), SERCA and the MITOCHONDRIAL UNIPORTER (FIG. 1).

These pumping mechanisms have different thresholds for activity. PMCA and SERCA pumps have lower transport rates but high affinities, which means that they can respond to modest elevations in Ca^{2+} levels and set basal Ca^{2+} levels. The NCX and mitochondrial uniporter have much greater transport rates, and can limit Ca^{2+} transients over a wider dynamic range. For example, mitochondria accumulate Ca^{2+} even when presented with modest nM global Ca^{2+} changes, but the rate of mitochondrial Ca^{2+} uptake is optimal at μM Ca^{2+} concentrations[57,58].

The diverse PMCA, SERCA and NCX molecular toolkit (BOX 1) enables cells to select the combination of off reactions that exactly meets their Ca^{2+}-signalling requirements. For example, cells, such as stereocilia, skeletal and cardiac muscle, that generate rapid Ca^{2+} transients have PMCA isoforms (PMCA2a and PMCA3f) that pump at fast rates, whereas cells that produce slower Ca^{2+} transients to activate cell proliferation express PMCA4b that pumps much more slowly[59].

AUTOSOMAL-DOMINANT POLYCYSTIC KIDNEY DISEASE
(ADPKD). A fatal disease that is characterized by the progressive development of fluid-filled cysts in the kidney, liver and pancreas.

Ca^{2+}-BINDING RATIO
(K_S). The ratio between the amount of Ca^{2+} that is bound compared to the Ca^{2+} that is free in the cytosol.

PLASMA-MEMBRANE Ca^{2+}-ATPASE
(PMCA). A pump on the plasma membrane that couples ATP hydrolysis to the transport of Ca^{2+} from cytosolic to extracellular spaces.

Na^+/Ca^{2+} EXCHANGER
(NCX). A plasma-membrane enzyme that exchanges three moles of Na^+ for one mole of Ca^{2+}, either inward or outward, depending on the ionic gradients across the membrane.

MITOCHONDRIAL UNIPORTER
A 'channel' that is located in the inner mitochondrial membrane that transports Ca^{2+} from the cytosol into the mitochondrial matrix.

Recent studies have highlighted the contribution of a hitherto unrecognized family of Ca^{2+} pumps in regulating Ca^{2+} stores. These Ca^{2+} pumps, which are known as secretory-pathway Ca^{2+}-ATPases (SPCAs), are related to SERCAs but have distinct functional properties and cellular roles[60]. It seems that SPCAs might be responsible for Ca^{2+} sequestration into Golgi compartments. The expression of these pumps increases in lactotrophs before parturition, and mutations can lead to HAILEY–HAILEY DISEASE, which indicates that the maintenance of Golgi Ca^{2+} levels by SPCAs is crucial in regulating secretion and cellular contacts.

Spatial and temporal organization of Ca^{2+} signalling

In addition to the extensive Ca^{2+}-signalling toolkit, another factor that contributes to the versatility of Ca^{2+} signalling is its high degree of spatial and temporal diversity[1]. Spatial properties are particularly relevant for rapid responses when components of the on reactions and their downstream effectors are closely associated. This spatial contiguity is less apparent for slower responses such as gene transcription and cell proliferation when Ca^{2+} signals are usually presented in the form of repetitive Ca^{2+} transients and waves.

Spatial aspects of Ca^{2+} signalling. Many Ca^{2+}-signalling components are organized into macromolecular complexes (BOX 2) in which Ca^{2+} signalling functions within highly localized environments. These complexes can function as autonomous units, or modules, that can be multiplied, or mixed and matched, to create larger, more diverse signalling systems. For example, the cardiac Ca^{2+}-release unit (FIG. 3) can be recruited independently of its neighbours to produce graded contractions. Similarly, the individual spines on neurons operate as autonomous Ca^{2+}-signalling units, which greatly increases the computational capacity of neurons. For example, individual spines can undergo input-specific, Ca^{2+}-dependent synaptic modifications during the process of learning and memory.

Temporal aspects of Ca^{2+} signalling. Almost all Ca^{2+}-sensitive processes are tuned to respond to Ca^{2+} transients that are generated by the on/off mechanisms that are summarized in FIG. 1. At the fast end of the scale, for example, synaptic transmission or cardiac contraction, the effector systems respond to pulses within the microsecond to millisecond range. Moving up the timescale, the Ca^{2+} transients tend to last for longer (seconds to minutes) and the resulting signal usually spreads out as a Ca^{2+} wave (see below) to reach targets that are distributed throughout the cell. During prolonged stimulation, these Ca^{2+} transients are repeated to set up regular Ca^{2+} oscillations that have been implicated in the control of many different processes, such as oocyte activation at fertilization[61], growth-cone migration[62], growth-cone turning[63], axonal growth of cortical neurons[64], neuronal-cell migration[65], development of neurotransmitter phenotypes[66], formation of nodules in plant root hairs[67], contributing to astrogliosis and epilepsy in neocortical slices[68], development of muscle[69], release of cytokines from renal epithelial cells[70] and the disassembly of adhesive structures during cell migration[71].

Cells respond to such oscillations using highly sophisticated mechanisms, including an ability to interpret changes in frequency. Such frequency-modulated Ca^{2+} signalling occurs in many cells (for example, hepatocytes, salivary glands, endothelial cells and smooth muscle cells), in which it can regulate specific responses such as exocytosis[72], mitochondrial redox state[73] and differential gene transcription[74–78]. The molecular machines that are responsible for decoding frequency-modulated Ca^{2+} signals include CaMKII[79] and PKC[80]. The function of repetitive Ca^{2+} spiking in differential gene transcription is explored more fully in the section on cardiac hypertrophy.

Ca^{2+} waves. The Ca^{2+} signal that makes up Ca^{2+} oscillations often spreads through the cytoplasm as a regenerative wave[1], and depends on successive rounds of Ca^{2+} release and diffusion from clusters of Ins(1,4,5)P_3Rs or RYRs that are located on the ER/SR. In the case of Ins(1,4,5)P_3Rs, the channel clusters give rise to local signals that are known as Ca^{2+} 'puffs', whereas RYRs generate Ca^{2+} 'sparks'. Typically, these elementary Ca^{2+} signals produce a modest elevation of the cytosolic Ca^{2+} concentration (~50–600 nM), with a limited spatial spread (~2–6 μm), and reflect the transient opening of channels (duration of <1 s)[81,82]. Ca^{2+} that is released by one channel cluster can diffuse to a neighbouring site and activate it. This SALTATORIC PROPAGATION mechanism allows the initial local signal to trigger global Ca^{2+} waves and oscillations[1].

In some cases, these waves are highly localized, for example in starburst amacrine cells in the retina, in which localized dendritic signals are used to compute the direction of motion[83]. In addition to such intracellular waves, information can also spread from cell to cell through intercellular waves as has been described in endocrine cells[84], the vertebrate gastrula[85] and the intact perfused liver[86]. In some cases, intercellular waves can cross from one cell type to another as occurs between endothelial and smooth muscle cells[87].

On and off reactions in cardiac cells. The highly organized cardiac Ca^{2+}-signalling module illustrates several of the important dynamic aspects of Ca^{2+} signalling, such as amplification, homeostasis, tunnelling and modulation through crosstalk with other signalling pathways (FIG. 3). Activation begins when the cardiac action potential depolarizes the T-TUBULE to open the L-type VOC to introduce a small pulse of trigger Ca^{2+} — a 'sparklet'[88] — that diffuses across the JUNCTIONAL ZONE to stimulate RYR2s (BOX 2, part a) to generate a spark. As the sparklet activates a cluster of 4–6 RYR2s, the spark is much larger and results in a considerable amplification of the initial sparklet. The Ca^{2+} within the spark then diffuses away from the junctional zone to induce contraction by activating sarcomeres that are situated in the immediate vicinity. During the recovery phase, the off reactions that were described earlier (FIG. 1) begin to remove Ca^{2+} from the cytoplasm (FIG. 3).

An important feature of Ca^{2+} signalling is homeostasis — cells avoid a net loss or gain of Ca^{2+} by ensuring that the fluxes occurring during the on and off reactions

HAILEY–HAILEY DISEASE
A rare autosomal-dominant skin disease that is characterized by disturbed keratinocyte adhesion.

SALTATORIC PROPAGATION
A mechanism by which Ca^{2+} signals leap from one group of Ca^{2+} channels to the next.

T-TUBULE
(transverse tubule). A tubular invagination of the plasma membrane in a muscle fibre, the function of which is to pass the excitation signal from the muscle-cell surface to the sarcomeres, to ensure rapid and synchronous activation.

JUNCTIONAL ZONE
The narrow space that is located between the T-tubule and sarcoplasmic reticulum in cardiac cells in which the process of excitation–contraction coupling occurs.

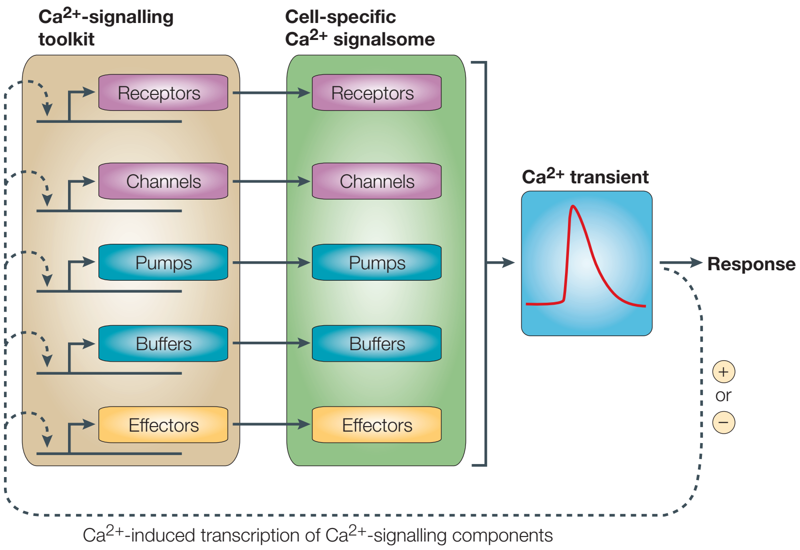


Figure 4 | **A calcium-induced calcium-signalling remodelling hypothesis.** Cell-specific Ca^{2+} signalsomes that are created by selecting components from the Ca^{2+}-signalling toolkit (BOX 1) generate Ca^{2+} transients that are characteristic for each cell type. It is proposed that the Ca^{2+} transient has two functions. In addition to activating cellular responses, it also functions as part of a feedback mechanism to regulate the transcriptional events that are responsible for maintaining the signalsome. This Ca^{2+}-dependent transcriptional regulation might have a central role in the compensatory mechanisms that enable cells to adapt to any modifications of their Ca^{2+}-signalling systems. It seems that Ca^{2+} can adjust transcriptional activity both positively and negatively. Diseases such as manic depression, hypertension, diabetes and congestive heart failure might develop from a failure of this proposed phenotypic remodelling system.

INOTROPIC
Affecting the force of cardiac contractions.

SIGNALSOME
The collection of components that constitute the different signalling pathways found in specific cell types.

are always balanced. Homeostasis is particularly important in cardiac cells, in which there is a large circulation of Ca^{2+} with every heart beat (FIG. 3). For homeostasis to occur, the same amount of Ca^{2+} that enters through the L-type channel during the on reaction must be removed by the NCX during the off reaction. Likewise, the same amount of Ca^{2+} that is released by the RYR2s is returned to the SR by the SERCA pump. As the SERCA pumps are distributed over the non-junctional regions of the SR, the newly pumped Ca^{2+} has to 'tunnel' its way through the SR lumen to reach the junctional region to become available for subsequent release cycles. This part of the recovery process is complicated by the fact that a proportion of the released Ca^{2+} travels through the mitochondria while *en route* to the SERCA pump, and this is particularly evident in neonatal cells[89]. While in transit, Ca^{2+} within the mitochondrial matrix activates the metabolism that ensures that the formation of ATP is maintained at the required rate to sustain contraction.

The force of contraction can be adjusted by varying the amount of Ca^{2+} that circulates during each on/off cycle. The positive INOTROPIC response that is produced by β-adrenergic stimulation is mediated by cyclic AMP/PKA, which has three main actions on Ca^{2+} signalling. First, it stimulates the L-type VOCs to increase the amount of Ca^{2+} that enters during each action potential. Second, it phosphorylates phospholamban to reduce its inhibitory effect on the SERCA pump, which is then able to increase the luminal Ca^{2+} concentration so that more Ca^{2+} is released from the SR — as described earlier, an increase in the activity of the SERCA pump is also enhanced by cADPR[23]. Third, cAMP/PKA phosphorylates the RYRs, thereby enhancing their ability to release Ca^{2+} to form sparks[45].

The expression of the individual components of this cardiac Ca^{2+}-signalling module, as for Ca^{2+}-signalling systems in other cells, is constantly under review. The next section describes how Ca^{2+} has a direct role in remodelling its signalling pathways in both health and disease.

Remodelling Ca^{2+}-signalling systems

The full exploitation of the Ca^{2+}-signalling toolkit depends on differential gene transcription to assemble diverse Ca^{2+}-signalling systems. However, such cell-specific Ca^{2+} SIGNALSOMES are not fixed in stone, in that they are constantly being remodelled to ensure that each cell type continues to deliver the Ca^{2+} signals that characterize its unique function. It seems that each cell has a signalling blueprint and 'knows' exactly what kind of signal it is expected to deliver. If the spatio-temporal properties of this output signal change because of a loss or defect of a key component, compensatory mechanisms come into play to restore the normal output signal. This remodelling process indicates that there is an element of quality assessment, in that the output of the signalling system is constantly monitored. We propose the hypothesis that Ca^{2+} itself has an important function in this internal assessment mechanism by remodelling its own signalling pathway (FIG. 4).

Ca^{2+}-induced Ca^{2+}-signalling remodelling. The central pillars of this Ca^{2+}-induced Ca^{2+}-signalling remodelling hypothesis are that Ca^{2+} is a potent activator of gene transcription[2,90–93], and that some of the genes that are activated code for components of the Ca^{2+}-signalling toolkit. The various Ca^{2+}-dependent transcription factors (BOX 1) are activated by different mechanisms. In the case of downstream regulatory element modulator (DREAM), which functions as a repressor, Ca^{2+} that enters the nucleus functions by removing DREAM from its DNA-binding site[91]. Ca^{2+} can also function indirectly through protein kinases (CaMKII and CaMKIV) or protein phosphatases (for example, calcineurin) to alter the phosphorylation state of various transcription factors (FIG. 5). Alternatively, Ca^{2+} can promote gene transcription by recruiting either the Ras/mitogen-activated protein kinase (MAPK)- or cAMP-signalling pathways.

There is increasing evidence that Ca^{2+} can alter the expression level of Ca^{2+}-signalling components such as pumps and channels[94–98] (FIG. 4). The Ca^{2+}-dependent development of cerebellar granular neurons was marked by an upregulation of Ins(1,4,5)P_3R and two PMCA isoforms, PMCA2 and PMCA3. On the other hand, PMCA4 and the type 2 NCX (NCX2) were rapidly downregulated[94,96]. Some of these changes, such as the expression of Ins(1,4,5)P_3R, are mediated through the calcineurin/nuclear factor of activated T cells (NFAT) transcriptional cascade[97,98].

This ability of Ca^{2+} to regulate its signalling pathways might help to explain the ability of the system to compensate for changes in the activity of individual

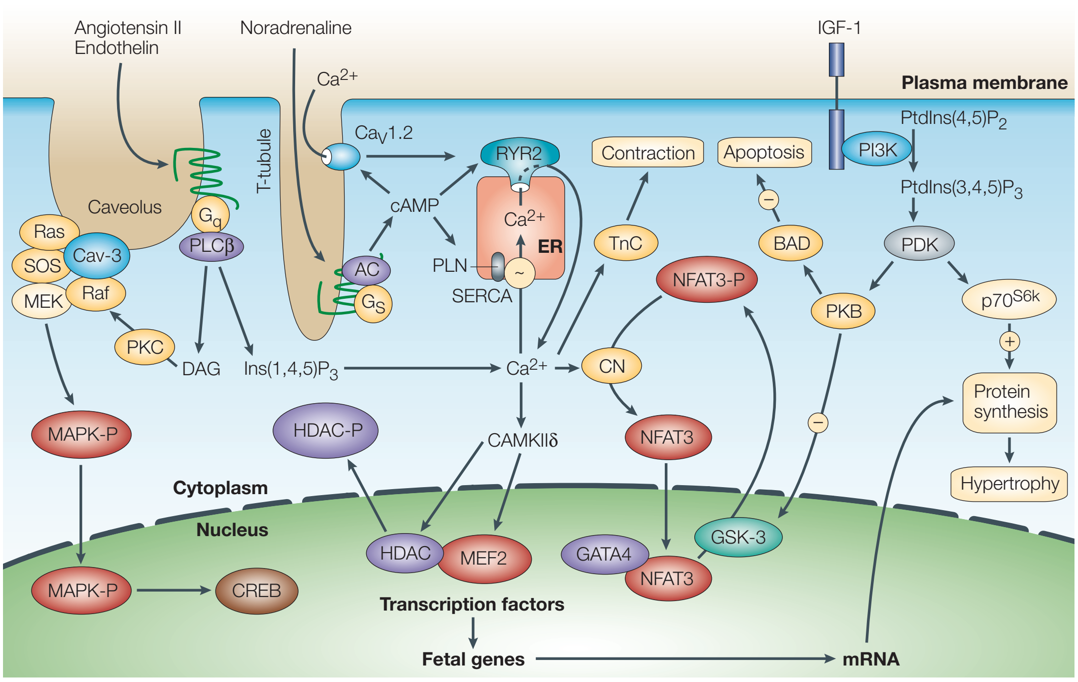


Figure 5 | **Signalling pathways that participate in the control of compensatory hypertrophy.** A key element of this remodelling process is the Ca^{2+} that is released by the cardiac signalling module (FIG. 3). This Ca^{2+} signal is enhanced during hypertrophy by the adrenergic pathway that functions through adenylyl cyclase (AC) to increase cyclic AMP (cAMP), which enhances signalling by phosphorylating the entry channel ($Ca_V1.2$), ryanodine receptor 2 (RYR2) and phospholamban (PLN). Ca^{2+} functions through calcineurin (CN) to dephosphorylate the transcription factor NFAT3, thereby enabling it to enter the nucleus to induce the transcription of genes, some of which are fetal genes. Hypertrophy can also be enhanced by angiotensin II and endothelin that function through G_q and phospholipase C-β (PLCβ) to generate inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$), which could enhance Ca^{2+}, and diacylglycerol (DAG), which seems to function by recruiting the mitogen-activated protein kinase (MAPK)-signalling pathway to stimulate cAMP response element-binding protein (CREB). The phosphatidylinositol 3-kinase (PI3K)-signalling pathway, which is activated by insulin-like growth factor (IGF-1), contributes to hypertrophy by inhibiting the glycogen synthase kinase 3 (GSK-3) that inactivates NFAT3, by stimulating Bcl-associated death promoter (BAD) to inhibit apoptosis and by stimulating $p70^{S6k}$ (70-kD ribosomal S6 kinase) to stimulate the expression of the newly transcribed genes. Cav-3, caveolin-3; GATA4, zinc-finger transcription factor; HDAC, histone deacetylase; MEF2, myocyte enhancer factor 2; MEK, MAPK kinase; NFAT, nuclear factor of activated T cells; PDK, phosphoinositide-dependent protein kinase; PKB, protein kinase B; PKC, protein kinase C; $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; $PtdIns(3,4,5)P_3$, phosphatidylinositol-3,4,5-trisphosphate; SOS, Son of sevenless; TnC, troponin C.

components[99]. Compensatory changes in Ca^{2+} signalling have also been recorded in cells from mice that carry a null mutation in one copy of the *SERCA2* gene, which resembles the mutation that is found in DARIER'S DISEASE. Despite a 40–60% decline in the Ca^{2+} content of the intracellular store, the mouse cardiac cells were still able to generate moderate Ca^{2+} spikes through compensatory changes in the expression levels of phospholamban and NCX[100]. Exocrine glands adjusted to the same mutation by upregulating specific PMCA isoforms (PMCA3 and PMCA4) and by adjusting the levels of its Ca^{2+} sensors to increase the sensitivity of exocytosis[101]. Each cell type adopted a different strategy to overcome the same mutation, thereby emphasizing the flexibility of Ca^{2+}-signalling remodelling systems.

Cardiac hypertrophy. Several important disease states (hypertension, heart disease, diabetes, manic depression and Alzheimer's disease) might result from abnormal remodelling of the Ca^{2+} signalsome. A good example is CONGESTIVE HEART FAILURE, which begins when the heart adapts to stress by increasing in size and by showing an altered phenotype owing to the expression of neonatal genes. This initial hypertrophy is a compensatory change, because the heart returns to its original phenotype and size if the abnormal inputs are reduced. However, if the stresses persist, this compensated hypertrophy shifts to a state of congestive heart failure, which is more difficult to reverse than compensatory hypertrophy. The phenotypic remodelling that occurs during cardiac hypertrophy and congestive heart failure is controlled by several signalling pathways in which Ca^{2+} has a prominent role (FIG. 5).

This central role of Ca^{2+} signalling in cardiac hypertrophy is apparent from studies on transgenic mice in which the overexpression of genes encoding components of this pathway (FIG. 5), such as the L-type Ca^{2+} channel[102], calcineurin and NFAT[103], triadin 1 (REF 104), junctin[105], calsequestrin[106] and $G_{q\alpha}$ (REF. 107), all result in hypertrophy. Transgenic mice that overexpress calsequestrin have a particularly marked phenotype that is characterized by severe cardiac hypertrophy and decreased depolarization-induced Ca^{2+} release from the SR. The overexpression of calsequestrin resulted in a compensatory decrease in the

DARIER'S DISEASE
An autosomal-dominant skin disorder.

CONGESTIVE HEART FAILURE
A syndrome that is characterized by the failure of the heart to maintain the circulation of the blood adequately.

expression of its binding partners (junctin, triadin and RYR2) within the RYR2 complex (BOX 2, part a)[106,108]. Conversely, hypertrophy can be prevented by either inactivating components such as $G_{q\alpha}$ (REF. 109) or by promoting the activity of glycogen synthase-3 (GSK-3), which inactivates the Ca^{2+}-sensitive transcription factor NFAT[110] (FIG. 5). Ablating phospholamban was able to rescue the hypertrophy that resulted from the overexpression of calsequestrin[111], but not hypertrophy caused by overexpressing $G_{q\alpha}$ (REF. 112). The effect of manipulating phospholamban seems to depend on the initial cause of hypertrophy, and this might explain why the effects of ablating this regulator in mice are very different to those seen in humans[113]. In humans, hypertrophy and heart disease developed in individuals carrying a mutation that results in the deletion of phospholamban, thereby emphasizing the significance of alterations in Ca^{2+} signalling as a cause of heart failure in humans[113].

A major problem in trying to understand how Ca^{2+} controls cardiac hypertrophy is the fact that the heart is continuously subjected to large periodic Ca^{2+} signals (FIG. 3) that flood through the cytoplasm and nucleus every time the heart contracts. Why is it then that cardiac cells subjected to this constant barrage of Ca^{2+} avoid triggering a hypertrophic response? It has been suggested that the normal functioning heart might not be transcriptionally silent, but might be under constant Ca^{2+}-dependent control[114]. In other words, the transcriptional events that are responsible for regulating the expression of the cardiac signalsome are continuously assessed as proposed earlier for the Ca^{2+}-induced Ca^{2+}-signalling remodelling hypothesis (FIG. 4). If this is the case, then the increase in transcription during hypertrophy might result from subtle alterations in the spatio-temporal properties of the individual Ca^{2+} spikes. Indeed, a broadening of the Ca^{2+} transient or an increase in its amplitude has been recorded in cases in which hypertrophy is induced by modifying the levels of proteins that associate with RYR2 (BOX 2, part a), such as triadin[104] or FKBP12.6 (REF. 115), respectively. The hypertrophy that occurred in the *FKBP12.6*$^{-/-}$ mice failed to develop in the females, who might be protected against the hypertrophic effect of Ca^{2+} by female sex hormones such as oestrogen. The failure of phospholamban ablation to prevent hypertrophy[112] might be explained by the fact that these transgenic animals had a large increase in the amplitude of the Ca^{2+} transients. In all of these cases, the rising phase that is responsible for triggering contraction remains the same, but the increase in amplitude or spike broadening enhances the extent of the Ca^{2+} pulse. These subtle changes in the individual spikes might be integrated over time to stimulate a distinct programme of gene transcription, and some of the genes transcribed will be fetal genes. It is this change in the dynamics of the Ca^{2+} transient that seems to carry the information that is responsible for inducing hypertrophy.

A different phenotypic remodelling process occurs during the onset of congestive heart failure when the amplitudes of the Ca^{2+} transients are much reduced owing to a severe downregulation of the Ca^{2+}-signalling system. One of the most noticeable changes is a decline in the activity of the SERCA pump that is due, in part, to the enhanced inhibition through phospholamban[116], which results in the severe depletion of the SR store that characterizes congestive heart disease[117]. One reason for this decline in Ca^{2+} signalling is a marked downregulation of β-adrenergic-receptor signalling[118]. During compensatory hypertrophy, an increased release of catecholamines contributes to the hypertrophic response by activating the cAMP pathway that enhances Ca^{2+} signalling (FIG. 5). This enhancement of Ca^{2+} signalling is seriously compromised when the β-adrenergic-signalling system is downregulated during congestive heart failure.

The severity of congestive heart failure might arise when this downregulation of the β-adrenergic-signalling system coincides with a Ca^{2+}-induced Ca^{2+}-signalling remodelling process to switch off the expression of key components such as the SERCA pumps. It is the coincidence of these two regulatory events that might explain the severe disfunction of the Ca^{2+} uptake and storage capacity, which results in the weak transients that characterize the failing human heart[117].

Conclusion

There are numerous Ca^{2+}-signalling systems that are designed to regulate many different cellular processes. This versatility is achieved by the existence of an extensive Ca^{2+}-signalling toolkit that is used to assemble these cell-specific signalsomes that can deliver Ca^{2+} signals with the spatial and temporal characteristics that are necessary for its many control functions. A major challenge for the future is to determine the differential transcription and expression mechanisms that are responsible for putting together these different signalling pathways. In addition, it will be important to establish the quality-assessment mechanisms that are responsible for maintaining the integrity of these signalling pathways. There is already some indication that Ca^{2+} itself might function in regulating the stability of its signalling pathways. This Ca^{2+}-dependent quality-assessment mechanism warrants further attention, because there are indications that several pathologies, such as cardiac disease, might develop after the abnormal remodelling of Ca^{2+} signalling.

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DATABASES

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