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Inseparable tandem: evolution chooses ATP and Ca^{2+} to control life, death and cellular signalling

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From the very dawn of biological evolution, ATP was selected as a multipurpose energy-storing molecule. Metabolism of ATP required intracellular free Ca^{2+} to be set at exceedingly low concentrations, which in turn provided the background for the role of Ca^{2+} as a universal signalling molecule. The early-eukaryote life forms also evolved functional compartmentalization and vesicle trafficking, which used Ca^{2+} as a universal signalling ion; similarly, Ca^{2+} is needed for regulation of ciliary and flagellar beat, amoeboid movement, intracellular transport, as well as of numerous metabolic processes. Thus, during evolution, exploitation of atmospheric oxygen and increasingly efficient ATP production via oxidative phosphorylation by bacterial endosymbionts were a first step for the emergence of complex eukaryotic cells. Simultaneously, Ca^{2+} started to be exploited for short-range signalling, despite restrictions by the preset phosphate-based energy metabolism, when both phosphates and Ca^{2+} interfere with each other because of the low solubility of calcium phosphates. The need to keep cytosolic Ca^{2+} low forced cells to restrict Ca^{2+} signals in space and time and to develop energetically favourable Ca^{2+} signalling and Ca^{2+} microdomains. These steps in tandem dominated further evolution. The ATP molecule (often released by Ca^{2+}-regulated exocytosis) rapidly grew to be the universal chemical messenger for intercellular communication; ATP effects are mediated by an extended family of purinoceptors often linked to Ca^{2+} signalling. Similar to atmospheric oxygen, Ca^{2+} must have been reverted from a deleterious agent to a most useful (intra- and extracellular) signalling molecule. Invention of intracellular trafficking further increased the role for Ca^{2+} homeostasis that became critical for regulation of cell survival and cell death. Several mutually interdependent effects of Ca^{2+} and ATP have been exploited in evolution, thus turning an originally unholy alliance into a fascinating success story.

This article is part of the themed issue 'Evolution brings Ca^{2+} and ATP together to control life and death'.

1. Introduction: the early pairing of ATP and Ca^{2+}

The course of biological evolution was preordained from the very beginning, when nucleosides become elementary units of genetic code and ATP emerged as a universal energy substrate. These events defined the molecular canvass of living cell and shaped major signalling cascades; however diverse are the living forms, the basic property of life lies in tight control over its internal milieu that has to be compatible with phosphate-based energetics. Of course, our contemplation of the habitat at the dawn of biological evolution is purely speculative, and yet the survival and diversification of all life forms including the most early ones (be these common ancestral progenotes, bacteria or else unknown forms)

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resulted directly from their interaction with the immediate environment. In this interaction, cells used what was readily available to them; and by whatever chance, purines and inorganic phosphates were readily available, and so ATP become central for energy metabolism.

The pyrophosphate bonds, phosphorylated nucleosides and ATP most likely occurred during the prebiotic period, being fundamental for subsequent organic evolution and RNA/DNA formation [1,2]. The exact origin of organic phosphates remains enigmatic–the most exotic under consideration invokes an extraterrestrial source: phosphates have been discovered in meteorites [3]. Of course, even accepting the extraterrestriality of ATP, we still face the question of its genesis, however distal from our planet. Conceptually, nucleotide triphosphates were available for biological evolution from the very early stage because of their requirement for genetic code, first in a RNA world [4] and then for DNA and for translationally active molecules (tRNA, mRNA, etc.). Historically, the pairing of ATP and Ca^{2+} could reflect an idiosyncrasy of the primordial alkaline ocean that contained only a minute concentration of Ca^{2+}; therefore, low Ca^{2+} concentration was natural for the cytosol of early organisms. Rapid decrease in ocean alkalinity coincided with a similarly rapid rise in seawater Ca^{2+} concentration ($[Ca^{2+}]$), which arguably could represent the environmental switch that triggered the emergence of complex ion homeostasis, evolutionary diversity and even multicellularity [5]. It should be noted that this hypothesis is by no means accepted by all palaeobiologists, and many believe in a slightly acid primeval ocean containing high Ca^{2+} [6], so the truth is yet to be established.

The natural question is: why ATP, rather than other nucleotide triphosphates? Possible driving factors in eukaryote evolution may reflect the fact that ATP had been preset for energy conservation already in bacteria before Ca^{2+} has been established as a signalling molecule; that Ca^{2+} stimulates ATP synthesis by activating dehydrogenases of the tricarbonic acid (Krebs) cycle [7]; and that ATP prevails several fold over GTP, which is formed and used in only one step by transphosphorylation from ATP to GDP [8]. Acquisition of mitochondrial precursors is considered an early and critical step of eukaryote evolution [9,10]. Formation of an endomembrane system is attributed to the first eukaryotic common ancestor, which emerged in combination with formation of multiple paralogues of molecules dedicated to signalling and trafficking [11–13]. Production of ATP by mitochondria may have been a prerequisite because it greatly increased energy available for vesicle trafficking, be it for fuelling motor proteins for vesicle transport, for activation of the triple-A ATPase *N*-ethylmaleimide sensitive factor [14], a SNARE chaperone, or for increasingly sophisticated Ca^{2+} regulation. It is of note, however, that binding of ATP and GTP to F1-ATPase is similar [15]. All in all, there are only weak arguments for the selection of ATP, rather than other nucleotide triphosphates, for energy metabolism [16]. In contrast, the high free energy of GTP hydrolysis is proposed to be advantageous in allowing efficient transition from the active to the inactive state, as it occurs with GTPases [17] when they mediate specific vesicle interactions [18,19].

Be this all as it may, ATP remains quite a unique molecule, which arguably participates in more chemical reactions than any other compound on the Earth's surface, except water. At the same time, ATP metabolism is highly demanding, being operational only at very low levels of free Ca^{2+}. This in turn stipulates that free Ca^{2+} inside the cells using ATP for energetics must be exceedingly low; and very low it is set indeed, being, as a rule, around 100 nM [20–22]. This low Ca^{2+} requirement shaped the biological evolution and linked ATP with ion composition of the intracellular milieu, which has minute amounts of Ca^{2+} and relatively low concentration of Na^{+}; maintaining the ion composition of the cytosol, in turn, became the main energy expenditure of all living cells. Thus, ATP needs control over Ca^{2+} and control over Ca^{2+} needs ATP, hence making the two an inseparable tandem.

Evolution perfected ion-controlling mechanisms and used ion gradients for many purposes, most notably for cellular signalling. Calcium as a signalling ion is employed by the most primitive bacteria [23,24] and by protozoa [25,26] before the system of Ca^{2+} signalling became highly elaborated in higher eukaryotes. Some bacteria already contain a Ca^{2+}–ATPase/pump that is important for Ca^{2+} export and bacterial [27–29] and protozoan [30,31] survival. Most importantly, all Ca^{2+} signalling and homeostatic molecules are controlled by Ca^{2+} ions themselves, which imposes multiple feedbacks thus making the system highly versatile and adaptable [32]. Eukaryotic evolution proceeded along with a significant increase in the number of Ca^{2+}-binding proteins [33,34] as well as in the number of signalling and metabolic processes that are regulated by Ca^{2+} [20,35]. Exocytotic SNARE proteins, Ca^{2+} signalling and directionality of organelle-specific GTPases must have been early evolutionary achievements maintained throughout eukaryotic kingdoms [11,36,37]. Figure 1 gives an overview of the widely branched regulation mechanisms and effects of Ca^{2+} that are exerted in cooperation with ATP as an energy source, from normal cell life to programmed cell death (apoptosis).

2. Some evolutionary preconditions and basic considerations of having Ca^{2+} and ATP as crucial cell regulators

Signalling mediated by Ca^{2+} has developed early in evolution because of sheer omnipresence and abundance of this ion in the environment, whereas the ATP-centric metabolism made free Ca^{2+} toxic inside cells. Nonetheless, Ca^{2+} permeation through biomembranes, though variable and tightly controlled, is unavoidable. Like with oxygen which, when emerging in the atmosphere instigated the extinction of almost all living forms, evolution turned a disadvantage into an advantage by taming toxicity through spatial and temporal restriction [25,38,39]. Ionized Ca^{2+} has to be kept low in the cytosol to avoid interference with phosphate groups in free and bound form. On the other side, low Ca^{2+} concentration offers the advantage of economic handling. Assuming that Ca^{2+} was required to govern emerging intracellular trafficking, which may have developed only after full development of aerobic energy production with the consequence of high energy conservation by ATP, these two aspects represented a revolution in eukaryote evolution. Furthermore, considering the requirement of ATP for intracellular movement and of Ca^{2+} for membrane–vesicle interactions, the availability of both molecules in adequate concentrations was mutually interdependent from the very

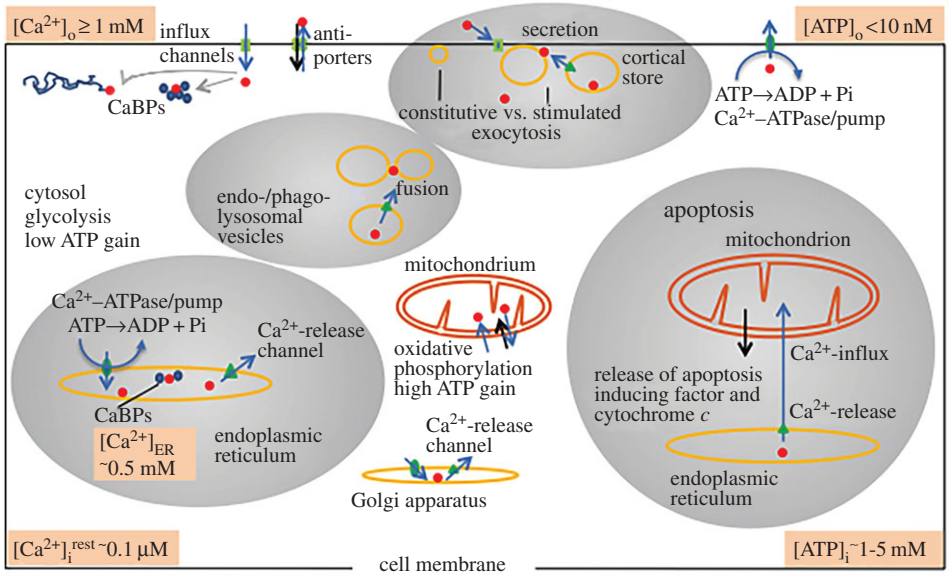

Figure 1. Regulatory principles in Ca^{2+}-based signalling and ATP-based energetics. As $[Ca^{2+}]_o$ is much higher than $[Ca^{2+}]_i$, Ca^{2+} steadily leaks into the cell (not drawn), but this is counterbalanced by the Ca^{2+} – ATPase/pump and by antiporters. Upon stimulation, Ca^{2+} can flow into the cell by influx channels of different types. Rapid downregulation is executed by Ca^{2+}-binding proteins (CaBP) and by sequestration mainly into the endoplasmic reticulum (endowed with an organellar Ca^{2+}-pump), but also into other organelles, such as cortical stores, the Golgi apparatus, different trafficking organelles and mitochondria. In their inner membrane, mitochondria have available a uniporter for Ca^{2+} uptake and a antiporter system for its rapid release. Ca^{2+}-release channels, mainly of the type $InsP_3R$ and RyR, are available in the different organelles. In the endophago/lysosomal trafficking system, such channels can mediate short-range Ca^{2+} signalling. Requirement of Ca^{2+} is also ascertained for stimulated exocytosis, whereby Ca^{2+} may originate from influx and/or release from cortical stores. Regulation of apoptosis (regulated cell death), among a plethora of molecules, involves release of Ca^{2+} from the endoplasmic reticulum and release of an 'apoptosis-inducing factor' as well as of cytochrome *c* from mitochondria. In summary, in the eukaryotic cell, there are many local signalling pathways, with many interactions, for a variety of subcellular processes.

beginning of eukaryote evolution about 2.5×10^9 years ago. As a rule, cytosolic concentrations are kept at millimolar range for ATP and at submicromolar range for Ca^{2+}, the $[Ca^{2+}]_i$ being about 1000 times less than ambient concentration in the extracellular space [20]. From protists to humans, concentrations of total (free and bound) and free Ca^{2+} in the cytosol are very similar, being approximately 1 mM and approximately 0.1 µM, respectively. After stimulation, $[Ca^{2+}]_i$ usually rises by up to, or even above, 10 µM. In Ca^{2+}-storing organelles, before release upon stimulation, both total [Ca] and ionized $[Ca^{2+}]$ are substantially higher compared with the cytosol, from protozoa [40] to mammals [41,42]. Sequestration into stores for trapping by binding to luminal proteins inactivates Ca^{2+}, but also makes it available for rapid release upon stimulation.

3. Availability of Ca^{2+} for early evolution, and its properties predestining it for intracellular functions

The prevalence of divalent cations in the earth crust and in the ocean is: $Ca^{2+} > Mg^{2+} > Ba^{2+} > Sr^{2+}$. While Ca^{2+} is mainly used for signalling, Mg^{2+} binds to many proteins and serves as a cofactor in biochemical reactions. For instance, together with GTP, Mg^{2+} activates the protein initiation factor or, as MgATP, frequently serves as a substrate. The preference of MgATP may reside in the highest binding constant for Mg^{2+} over other divalent cations [43,44]. In contrast to Mg^{2+}, Ba^{2+} and Sr^{2+} drive several subcellular functions, similarly (albeit not always) to Ca^{2+} (e.g. exo–endocytosis in fish neurons [45] or ciliary movements in ciliates [46]), however their effects are, as a rule, weaker. Sometimes, however, Ba^{2+} can surpass the effects of Ca^{2+}, for instance in chromaffin cells and in ciliated protozoa (the 'barium dance' [47]). It is plausible that Ba^{2+} and Sr^{2+} may have been overlooked by evolution because of their minor availability in nature. An alternative view considers different coordinated binding of Mg^{2+}, Ca^{2+} and Ba^{2+} to peptide fragments, depending on polar aliphatic residues and ion radius [48], thus reflecting some gross dependency from the primary protein structure. Binding is much more restricted to Ca^{2+} when proteins possess specific Ca^{2+}-binding motifs. Here specificity can be quite selective. For example, different paralogues and isoforms of the Ca^{2+}-sensor synaptotagmin bind Ca^{2+} considerably better than Ba^{2+}, Mg^{2+} or Sr^{2+} [49]. By these different aspects, during evolution, Ca^{2+} may have been selected as the main signalling molecule, while the other abundant divalent metal, Mg^{2+}, was chosen for other functions.

Binding to proteins is widely different for Ca^{2+} and Mg^{2+}: relatively loose and reversible coordinative binding of Ca^{2+} to specific domains of dedicated Ca^{2+}-binding proteins at low concentrations is an important factor. This enables low-capacity/high-affinity Ca^{2+}-binding proteins for rapid and efficient signal transfer by conformational change. The archetypal and most widely distributed, multifunctional example is calmodulin [50], whose four EF-hand loops are hierarchically occupied, the lowest-affinity loop IV also binding Mg^{2+}. Gradual conformational changes can thus serve for the transmission of a chemical signal into a mechanical one, be it at a subunit of plasmalemmal ion channels in protozoa [51] or intracellular Ca^{2+}-release channels in mammals [52]. The Ca^{2+}-sensor for membrane fusion, synaptotagmin, possesses distinct C2 domains for cooperative Ca^{2+}-binding [53]. These C2 domains stick out from β-barrel structures

and increase lipophilic properties upon Ca^{2+} binding. This mediates membrane binding and perturbation of adjacent membranes for Ca^{2+}-dependent fusion [54].

An unusually high number of acidic amino acid residues in cytosolic Ca^{2+}-binding proteins allows Ca^{2+} binding even at relatively high concentration. These high-capacity/low-affinity Ca^{2+}-binding proteins, such as parvalbumin, serve as immobile Ca^{2+} buffers for rapid downregulation of cytosolic Ca^{2+} [55,56]. Centrin can operate not only by high-affinity binding sites belonging to the EF-hand motif, but also by its negative charges [57]. Centrin is the dominant cytosolic Ca^{2+}-buffer in the cortex of ciliated protozoa [58]. In Ca^{2+} stores, high-capacity/low-affinity Ca^{2+}-binding proteins are defined by their excess of acidic amino acids residues for Ca^{2+} storage and rapid release upon stimulation [59]. Calreticulin and calnexin in the endoplasmic reticulum are examples; they also exert a Ca^{2+}-dependent chaperone function for folding newly synthesized proteins [60].

4. The specific case of magnesium

Magnesium is the fourth most abundant ion in eukaryotic cells, and, incidentally, it is the most abundant free divalent cation (total Mg^{2+} content of the body is approx. 24 g or approx. 1100 mmol, whereas ionized cytosolic $[Mg^{2+}]$ fluctuates between 0.25 and 1 mM [61,62]). This high cytosolic concentration could be another explanation for why evolution favoured Ca^{2+} over Mg^{2+} for signalling purposes. Well-defined domain structures evolved for Ca^{2+} binding, whereas Mg^{2+} buffers remain generally unknown; identifying Mg^{2+}-binding domains is a tedious task and they generally are considered elusive. A similar conclusion arose from the bioinformatic analysis of Mg^{2+}-binding proteins, the so-called magnesome, which includes rather diffuse Mg^{2+}-binding motifs [63]. This reflects essential differences between the Mg^{2+} and the Ca^{2+} ion (and some other earth alkali metals, with the exception of Ba^{2+} and Sr^{2+} that closely resemble Ca^{2+}). These differences [62,64] are in ionic radii (smaller for Mg^{2+}), charge density, water shell binding (higher hydration for Mg^{2+}), binding by inner and outer sphere coordination, etc. Coordination geometry is particularly important for binding to DNA, RNA and to various proteins and their substrates [65]. Several hundred enzymes are known to interact with Mg^{2+}, mainly through using soluble MgATP, rather than ATP *per se* as a substrate. A large part of the cellular effects of Mg^{2+} are because of its abundance in ionized form, absence of toxicity (as opposed to Ca^{2+}), less strict and less specific binding.

Data mining and molecular dynamics analyses have been applied to identify possible coordination structures based on crystal data for actin, myosin, DNA polymerase, RNA polymerase, DNA helicase and mitochondrial F_1-ATPase [66]. These analyses identified a broad range of molecules being affected by Mg^{2+} and revealed a diversity of both transitory and stable coordination arrangements between Mg^{2+} and ATP. In eubacteria, Mg^{2+} stabilizes the structure of 16S rRNA in a conformation favourable for binding to the ribosomal 30S subunit [67]. Similarly, the binding of transcriptional activator protein c of bacteriophage Mu to bacterial DNA is enhanced by Mg^{2+}-induced change from a more β-sheath-like to a more α-helical conformation, as evidenced by circular dichroism analysis [68]. All these processes are not related to cell signalling.

However, indirectly, Mg^{2+} may contribute to intercellular signalling, because Mg^{2+} (and not Ca^{2+}) controls interaction between the subunits of trimeric GTP-binding proteins (G-proteins) with GTP and GDP, respectively, thus determining their signal transduction activity [69]. As alluded to above, in the calmodulin molecule, which acts as an activator of many enzymes and ion channels, four Ca^{2+}-binding EF-hand loops show hierarchical Ca^{2+} binding with different binding constants, which depend on $[Mg^{2+}]_i$ [70] and is different for each loop [71]. In addition, Ca^{2+} and Mg^{2+} often exert different, in part even antagonistic, effects on enzymes, such as protein kinases and protein phosphatases. For example, Ca^{2+} and Mg^{2+} differentially regulate cAMP-dependent protein kinase A [72]. Another example is associated with protein phosphatase type 2C, which is activated by physiological $[Mg^{2+}]_i$, in contrast to Ca^{2+}, which is inhibitory at micromolar concentrations [73].

5. Why ATP has been selected as an energy-storing nucleotide?

Interactions of Ca^{2+} and Mg^{2+} with ATP are different, with CaATP being much less soluble. As mentioned, Mg^{2+} binds to ATP to form a complex that serves as a substrate for many enzymes, such as ATPases including Ca^{2+}–ATPases/pumps for extrusion or sequestration of Ca^{2+}. Why among nucleotide triphosphates did ATP prevail over GTP, CTP, TTP, UTP, ITP? There may be evolutionary ('historical') reasons, e.g. because ATP had already been established in energy conservation. If so—why? Any other nucleotide triphosphate, if available in similar concentration, might do equally well because of similar free energy [74]. However, functions of the different nucleotide triphosphates may have been diversified already at the early evolutionary stages. As we see now, ATP has been selected preferably for energy conservation, whereas GTP is mainly used for signalling, e.g. for membrane interactions. This mainly concerns monomeric GTP-binding proteins (Rab-type GTPases) in the context of vesicle/membrane interaction [18,19,75], in addition to nucleo-cytoplasmic transport and microtubule dynamics. Only rarely is GTP used in bioenergetics, e.g. in only one step of the tricarbonic acid cycle. The reduction of nicotinamide dinucleotide (NAD) to NADH for subsequent exploitation by oxidative phosphorylation in the mitochondria greatly increases energy gain. Other nucleotide triphosphates are used for specific biosynthetic processes, such as N-glycosylation of proteins in the endoplasmic reticulum. Thus, ATP prevalence in energy metabolism may result from rather stochastic early evolutionary specialization, although the early pairing of ATP and Ca^{2+} could also play a role. Using different nucleotide triphosphates beyond ATP and GTP, including UTP and CTP, for different purposes may be advantageous for specific regulation of a range of processes.

In summary, it may be due to the evolutionary priority to have energetics and metabolism based on ATP, rather than on other nucleotide triphosphates. During evolution, ATP and Ca^{2+} became intimately connected in functional terms for many activation and deactivation processes (protein

phosphorylation and dephosphorylation), whereas GTP has been assigned other functions.

6. Intracellular Ca^{2+} fluxes and Ca^{2+} regulation

Cellular Ca^{2+} fluxes are mediated by uniporters (influx- and release channels), primary active (Ca^{2+}–ATPase/pump) and secondary active transport systems (e.g. H^+/Ca^{2+} exchangers or related antiporters), all to be found in the plasmalemma and in the endomembranes [20,22,76,77]; intracellular Ca^{2+} dynamics is further regulated by Ca^{2+} buffers [56]. All these mechanisms were invented early in evolution, be it in ancestors of unikonts, such as choanoflagellates [78], or be it in bikonts, such as ciliates [38,40]. Cytosol contains both mobile and immobile Ca^{2+} buffers of different capacity and affinity. Mobile high-affinity Ca^{2+} buffers hinder Ca^{2+} diffusion, thus favouring localization of Ca^{2+} signalling events that often occur in a form of microdomains. In the endoplasmic reticulum, in contrast, low-affinity Ca^{2+} buffers allow diffusion and rapid equilibration of Ca^{2+} within the lumen through Ca^{2+} tunnels [79]. From immobile buffers, including high-capacity/low-affinity binding proteins, Ca^{2+} can slowly dissociate to be fed into active transport systems. For primary active transport, the ATP → ADP system is energetically roughly the same as it would be for pyrophosphatase activity The preference for the ATP → ADP system can be explained by the requirement of only one energy limiting step for ATP re-synthesis, rather than two for the pyrophosphatase system, whereas the same amount of free energy is available for both [80]. Thus, pyrophosphatase-driven Ca^{2+}-sequestration is used only exceptionally, for example in acidocalcisomes of trypanosome parasites [81].

Furthermore, to keep energy expenditure low, Ca^{2+} influx and/or release from internal stores is frequently restricted to selective sites. The textbook example is the nerve terminal, where voltage-dependent Ca^{2+}-influx channels are restricted to the active zone where transmitter is released by exocytosis [82,83]. In ciliated protozoa, this type of channels is restricted to cilia [84]. In the central nervous system of mammals and in ciliates, these Ca^{2+} channels are inactivated by formation of a Ca^{2+}/calmodulin complex [46,85,86] using the very same Ca^{2+} they had conducted. This is another example of a principle conserved from ciliates to the human brain: it is generally appropriate to limit $[Ca^{2+}]_i$ increase to a small volume and short time, with values just supporting activation and, thus, greatly reducing ATP consumption for re-establishing $[Ca^{2+}]_i$.

The principle of local restriction is also often applied to intracellular signalling. For intracellular Ca^{2+} release, the cell possesses different types of Ca^{2+}-release channels activated by distinct compounds represented by (i) inositol 1,4,5-trisphosphate ($InsP_3$) formed from phosphatidyl inositol 4,5-bisphosphate ($PInsP_2$, a component of lipid membranes) and binding to $InsP_3$-receptor-type Ca^{2+}-release channels ($InsP_3R$, [87]); (ii) by Ca^{2+} ions themselves, which activate ryanodine-receptors (RyR) evolutionarily related to the $InsP_3$-receptor at the protozoan level where intermediates of the two channel types are also found [26,38,39,88,89]; and (iii) by nicotinic acid adenosine dinucleotide phosphate (NAADP), which activates two-pore channels (TPCs) of acidic Ca^{2+} stores [90]. In metazoans, RyRs are activated by Ca^{2+} [91] and by cyclic adenosine diphosphoribose (cADPR) formed from the glycolytic H^+ acceptor NAD [92]. Both NAD and nicotinamide adenine dinucleotide phosphate (NADP), but probably also $PInsP_2$, have been available in early eukaryote evolution—at least the key components [93] and enzymes [94] are found in protozoa. Both channel types, $InsP_3R$ and RyR, are also predicted by database mining for choanoflagellates [78,95]. An intermediate form between $InsP_3R$ and RyR was suggested from the nucleotide sequence in Purkinje cells [96]. Similar to *Paramecium*, the two types or Ca^{2+}-release channels coexist in Ascidian egg cells [97]. In conclusion, there are diverse ways to release Ca^{2+} locally by different activators, only some being considered here. Considerable similarities between $InsP_3Rs$ and RyRs from ciliated protozoa [88] to mammals [98] indicate that both may possibly have evolved from a common ancestral molecule, assembled from six transmembrane domains [38,89].

Another set of intracellular channels is represented by the TPCs that are members of the voltage-gated ion channel superfamily; the TPCs contain 12 transmembrane domains, being in essence a half of a voltage-gated channel [99–101]. These channels reside in acidic compartments (which form acidic Ca^{2+} stores), notably in the endo/lysosomal compartments and in the plant vacuoles [90,102], and are activated by NAADP, derived from the metabolite, NADP [90,99,103,104].

In evolution, TPCs are present in monokonts, from choanoflagellates and the social amoeba *Dictyostelium* [105]) to mammals [100], and in bikonts, from protozoa to green plants [101,106]. In plants, TPCs can be activated by osmotic stress [107] as well as by Ca^{2+} ions (this Ca^{2+}-dependent activation contributes to regulation of seed germination and stomatal movement [108]). *Paramecium*, which is another bikont, also seems to possesses TPCs [109]. In the parasitic monokont, *Trypanosoma*, NAADP does not trigger Ca^{2+} response in contrast to the bikont apicomplexan parasite *Toxoplasma gondii*, a relative of ciliates [110]. The *T. gondii* also possesses a plant-like vacuole and acidocalcisomes, which may bear TPC channels encoded by orthologous genes that are absent in Apicomplexa [106].

Different types of Ca^{2+}-release channels frequently coexist within the membrane of Ca^{2+} storage organelles [111,112]. The RyRs and $InsP_3Rs$ localized in the ER membrane may be functionally linked to TPCs in neighbouring acidic organelles [90,113–116]; Ca^{2+} release from TPCs can trigger Ca^{2+}-induced Ca^{2+} release (CICR) [115]. From the fact that $InsP_3Rs$ and RyRs can be activated by TPC activity, it has been speculatively concluded that TPCs are evolutionarily older than other Ca^{2+}-release channels [90]. According to this hypothesis, channels that allegedly emerged later in evolution (i.e. $InsP_3Rs$ and RyRs) may serve for signal amplification through consecutive channel activation [90,107,113–115]. In addition, NAADP can also activate Ca^{2+}-influx through TPCs localized in the plasmalemma [115], which was confirmed in patch-clamp studies [90]. The TPCs were also proposed to be responsible for topologically restricted Ca^{2+} signals, which may locally ignite phosphoinositol turnover, store-operated Ca^{2+} entry (SOCE) activity and possibly also cAMP signalling [117]. From a more conventional perspective, Ca^{2+} signals arising from TPCs have been considered relevant for membrane fusions along the endolysosomal pathway [118]. For the time being, however, all these effects remain hypothetical. In summary, TPCs are universally distributed in eukaryotes, with the exception of some

unicellular parasites. Otherwise, however, our knowledge about TPCs remains rather incomplete.

Transient receptor potential (TRP) channels owe their name to a phenotype of a *Drosophila* mutant whose electrical photoreceptor response is abnormally short (i.e. transient). Mammals possess 28 TRP channels classified into six subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), TRPML (mucolipin) and TRPP (polycystin) [119,120]. The TRP channels are widely distributed between different cell types and may be present in plasmalemma as well as in endomembranes (with only nucleus and mitochondrion being devoid of them). The TRP channels are strictly cationic with highly variable Ca^{2+} permeability; some TRP channels can conduct Mg^{2+} [119,120]. The intracellular TRP channels may mediate Ca^{2+} release from the stores; plasmalemmal TRP channels can be linked to intracellular Ca^{2+} release through a store-operated mechanism [120]. The TRP channels are broadly distributed throughout many species along the phylogenetic ladder. In protozoa, systematic analysis is scant and assignment to eukaryotic, specifically mammalian types is difficult. For the bikont *Paramecium*, originally no TRP channel genes had been found [106], but then three genes were retrieved [121]. Parasitic relatives of *Paramecium*, *Apicomplexa*, *Toxoplasma gondii* and probably also *Plasmodium* spp. all possess genes encoding TRP channels [106]. Parasitic flagellates *Leishmania* and *Trypanosoma* also possess TRP channel, but for all unicellular parasites, molecular characterization is yet to be done [110]. The green flagellate *Chlamydomonas reinhardtii* contains 19 genes encoding TRP channels [122], one of which shows considerable similarity to a human form and is localized probably in the flagellar membrane, contributing to sensory transduction [121]. Data mining revealed TRP channels encoding genes in *Dictyostelium* [121], where one of the members, PKD2, is localized in the cell surface and governs rheotaxis [123]. Choanoflagellates are endowed with TRP channels genes [124]; expanded molecular data mining revealed TRPA, TRPC, TRPM, TRPML and TRPV types [78]. Higher plants (viriplantae) apparently do not express TRP channels [122,125].

There is a crosstalk between intracellular Ca^{2+} stores, exemplified, for example, by variable coupling of endoplasmic reticulum and mitochondria [126,127], as well as between stores and plasmalemmal Ca^{2+} influx. In cardiomyocytes, Ca^{2+} entering the cytosol through voltage-gated channels directly activates RyRs, thus initiating CICR [128]. In skeletal muscle, activation of L-type plasmalemmal Ca^{2+} channels directly activates RyR type II, causing depolarization-induced Ca^{2+} release. Emptying the ER Ca^{2+} stores triggers plasmalemmal store-operated Ca^{2+}-entry, SOCE [128], which ubiquitously occurs in non-excitable cells [129,130]. Activation of SOCE involves intraluminal Ca^{2+}-sensor (Stim) and an influx channel (Orai and associated proteins or TRPC channels) in the plasmalemma [131,132]. Although operational SOCE is well characterized in the ciliate, *Paramecium* [40], Orai and Stim have not been detected (as yet), in contrast to informatics data obtained from choanoflagellates [78] that are placed at the basis of metazoans. However, several alternative modes of cell membrane–cortical store coupling are discussed, for instance by extended synaptotagmins with super-numerary C2 domains [133] which also occur in ciliates [38]. Altogether, SOCE appears to be an evolutionarily old mechanism.

The intracellular Ca^{2+} dynamics in the nerve terminal has to be exceptionally fast, in contrast to stimulus–secretion coupling in most other cells where no specific intimate structural and functional coupling exists and where the Ca^{2+} response, therefore, is less precise (an obvious example being the difference in vesicular secretion between neurons and neuroglia [134]). An exception is the ciliate, *Paramecium*, where a rapidly activated SOCE underlies the fastest known exocytosis of dense core-secretory vesicles important for predator defence [40,135]. The final target of Ca^{2+} during any type of secretion is the C2-domain-bearing protein synaptotagmin, and closely related Ca^{2+}-sensors, such as extended synaptotagmin isoforms of which several (probably with different kinetics) are known [53].

Elaborated Ca^{2+} signalling is also required for intracellular trafficking from organelle to organelle, which can be concluded from the distribution of synaptotagmins [136,137]. The signal may come from the very same trafficking organelles, as they contain Ca^{2+}-release channels of different types, including $InsP_3Rs$ and RyRs. This is true from protozoa [38,88] to mammals [98], even though other types of Ca^{2+}-release channels can also be involved (not considered here). Organelles of the endophagocytotic/lysosomal pathway contain Ca^{2+} at different concentrations [138]; release of Ca^{2+} drives local vesicle–vesicle interactions and fusion. Requirement for local availability of Ca^{2+} has been shown for instance for endosomes [139], endoplasmic reticulum and Golgi vesicles [140,141], as well as for lysosome–phagosome fusion [142]. Besides influx channels, endocrine cells also possess $InsP_3Rs$ in their secretory vesicles [143]. Again, limiting the signal to the organelles involved is a prerequisite for avoiding toxicity and to keep energy cost low.

Which Ca^{2+}-release channel is the oldest? Different types of Ca^{2+}-release channels, i.e. $InsP_3R$, RyR, TPCs and TRPCs are all present at the lowest evolutionary stage when monokonts separated from bikonts, which happened approximately 1.6×10^9 years ago [144]. This makes it difficult to decide upon the nature of ancestral Ca^{2+}-release channel, although low selectivity of TRPCs [120] may argue in their favour. The mechanosensor TRPY1 of yeast may be such a prototype [145] (of note however, yeasts emerged by secondary reduction). Channels with high selectivity for Ca^{2+}, such as $InsP_3Rs$ and RyRs, require Ca^{2+} stores endowed with uptake mechanisms for specific sequestration of Ca^{2+}; therefore, high selectivity of Ca^{2+}-release channels reflects availability of Ca^{2+} in the store lumen (for TPCs, see above). Whichever is the oldest, Ca^{2+}-release channels appear as inevitable prerequisite for 'constructing' large eukaryotic cells with abundant vesicle trafficking.

7. Molecular targets of Ca^{2+} in cells

Salient features of targets for Ca^{2+} signals are disparate in different cell types. For membrane–vesicle interaction, various SNARE proteins and synaptotagmin-type Ca^{2+}-sensors are present in spatially separated cellular compartments [146,147], in contrast to non-stimulated (constitutive) exocytosis that may not depend on Ca^{2+} signals, if one makes painstaking scrutiny [148]. Endocytosis via clathrin-coated pits or uncoated vesicles is synchronized with exocytosis by the pulse of Ca^{2+} required for both processes in mammalian [149,150] as well as in protozoan cells [151]. Endocytosis requires

dephosphorylation of the large GTPase dynamin, by the Ca^{2+}/calmodulin-activated phosphatase 2B/calcineurin [152]—an enzyme maintained from protozoa [153] to human, where it is essential for many signalling processes, including long-term potentiation and immune defence [154–156]. Calcineurin is a true multipurpose enzyme [157]. Nevertheless, in evolution, only one of the two calcineurin subunits is maintained in plants, where it serves for coping with ionic stress [125]. Unfortunately, no data are available for characaean algae placed at the roots of higher plant evolution. The situation with trafficking vesicles deep inside a cell is different because it requires locally restricted and finely tuned Ca^{2+} signals. The availability of synaptotagmin at the surface of vesicles of the endophagolysosomal vesicles [136,137] and of Ca^{2+} in their lumen allows for local signalling and fusion. Conceptually, Ca^{2+} ions have to be provided 'at the place', because diffusion from the far remote influx channels at the cell surface would be too inefficient, as Ca^{2+} would be trapped on its way through the cell by Ca^{2+}-binding proteins.

The Ca^{2+}/CaM-activated protein kinase ('CaM kinase') is important for neurotransmission, because it regulates, by phosphorylation of synapsin, the release of vesicles from the actin web, thus facilitating their access to the cell membrane [158]. During evolution, CaM kinase has been maintained only in monokonts from myxamoebae *Dictyostelium* to man [159]. This is different for bikonts, from ciliates up to viridiplantae, where CaM kinase proper does not exist. Here, Ca^{2+}-dependent protein kinases are kinases with integrated CaM-like motifs [125,160]. The situation is again different for cyclases for cyclic nucleotide formation. These are Ca^{2+}-dependent enzymes from ciliates [161,162] to humans [163]. Thus, via cyclases, cyclic nucleotide-activated protein kinases at the end of a signalling cascade also depend (indirectly) on Ca^{2+}.

The multiple ways in which Ca^{2+}-dependent cell motility is regulated are well characterized. The Ca^{2+}-sensitive molecules involve severing proteins (gelsolin), actin bundling proteins (caldesmon), troponin, etc. In muscle cells, Ca^{2+} binds to troponin C; the resulting conformational change of the troponin–tropomyosin complex allows the interaction of the myosin head with the actin filaments, which causes cell contraction by tilting of the myosin [164]. During stimulus–contraction coupling, this movement from molecular to macroscopic scale is fuelled by ATP hydrolysis. Ca^{2+} exerts pleiotropic effects on ciliary activity, especially in ciliated protozoa. The availability of a minimum of cytosolic ionized Ca^{2+} is required for ciliary/flagellar activity [165], which can be further modulated by Ca^{2+} [166]. In ciliates, normal ciliary beat depends on Ca^{2+} [165], as does any change in beat activity. For instance, ciliary beat is reversed when intraciliary $[Ca^{2+}]$ increases by activation of voltage-gated Ca^{2+} channels, as described for ciliated protozoa [166], and in ctenophores (comb jellies). In both systems, these channels are restricted to the cilia [84,167]. In ciliates, Ca^{2+} mediates dynein subunit phosphorylation by Ca^{2+} followed by calmodulin [168]; this type of regulation appears to be absent in cilia at a higher evolutionary level. In ciliates, the Ca^{2+}-regulatory mechanism involves activation of a cGMP-activated protein kinase [169] formed by a Ca^{2+}-dependent cyclase [170]. It also should be mentioned that many basic metabolic processes, such as glycogen turnover, are regulated by Ca^{2+}. In metazoans, the same is true of intercellular communication through gap junctions (which close when $[Ca^{2+}]$ rises in the cytosol [171]). This may contribute to epigenetic effects achieved during evolution by non-structural RNA species, which can be transferred from one cell to another, thus harmonizing tissues.

Intimate links between ATP and Ca^{2+} are observed in mitochondria [172,173]. Mitochondrial Ca^{2+} is connected with ATP production through stimulation of some mitochondrial dehydrogenases [7], of oxidative phosphorylation [174] and of ATP synthase [175]. This requires rapid crosstalk between the cytosol and the organelle. The key player is the mitochondrial calcium uniporter (MCU) for rapid Ca^{2+} influx [176,177]; the MCU and its regulators are present at the protozoan level. An MCU-encoding gene is found in *Trypanosoma* [31]. *Paramecium* also possesses MCU and associated MIUC protein, as well as the MCU regulator, EMRE [176,178]. Accordingly, in *Paramecium*, during synchronous exocytosis, Ca^{2+} rapidly (on a subsecond time scale) flushes into mitochondria; most of the Ca^{2+}, however, is released back into the cytosol within seconds, with only a minor fraction remaining in the organelle [109,179]. Mitochondrial exchangers, H^+/Ca^{2+} and Na^+/Ca^{2+} (NCLX) are known in higher eukaryotes [172,173], but they have not been identified in protozoa.

8. Ca^{2+} as a regulator of programmed cell death

In mammalian cells, apoptosis is triggered by release of Ca^{2+} from the endoplasmic reticulum, influx of Ca^{2+} into mitochondria [180] and release of cytochrome *c*, parallelled by a rise in $[Ca^{2+}]_i$, and further Ca^{2+} entry into mitochondria. A protease (caspase) cascade is activated and DNA is cut to nucleosome-size fragments (or multiples, called DNA ladder). Breakdown of subcellular structures, autophagy and phagocytosis of cell fragments [181] are additional events. Altogether, there are multiple effects of Ca^{2+} during apoptosis in mammalian cells [182], and mitochondria, before degeneration, play a key role in this process [183]. Toxic levels of $[Ca^{2+}]$ and failure of energy supply in conjunction with cytochrome *c* release [183], functional and structural disturbance of mitochondria and phagocytosis are final events.

Some molecules pertinent to apoptosis can be encountered already in unikont protists, such as choanoflagellates [184]. In bikont protists, such as ciliates, the macronucleus (equivalent to the soma, as opposed to the generative micronucleus), rather than the whole cell, is degraded in a process called 'nuclear death' [185]. This includes DNA fragmentation and autophagy of mitochondria, although the role for ATP and Ca^{2+} remain unsettled. The apoptotic stratagem is only slightly more complex in lower metazoans [186], including secondary reduction of the process in the nematode *C. elegans* [184]. Altogether, many important details of apoptosis at the roots of unikonts and bikonts are not yet well understood, as summarized recently [187]. It appears that these crucial aspects of apoptosis, including mitochondrial function and involvement of Ca^{2+}, have only successively evolved in the course of metazoan evolution.

9. Intercellular signalling: purinergic transmission links to Ca^{2+} signals

The concentration gradient for ATP (aimed extracellularly) is likely the highest existing in living cells. Indeed, intracellular

concentration of ATP is set at approximately 1–5–10 mM, whereas the extracellular concentration does not exceed 1–10 nM creating thus a difference of approximately 1 million times. An extremely low concentration of extracellular ATP results from constant operation of ectonucleotidases [188], which degrade ATP to ADP, AMP and adenosine. This ATP degrading system has ancient evolutionary roots, being present in bacteria, in early eukaryotes and in protists [188–190]. The steep concentration gradient favours ATP exit upon opening of transmembrane pores and following plasmalemmal damage; it should not be surprising therefore, that evolution rapidly began to use ATP as an intercellular signalling molecule. Already some prokaryotes are known to release ATP, which could be used for intercellular communications [191], whereas the plasmalemmal channels-mediated ATP release is described in polyphenic fungi, type *Candida albicans* [192].

Biological effects of extracellular ATP are many, and they are widespread throughout virtually all life forms [190]. In prokaryotes, exposure to ATP affects bacterial growth and development, sporulation and germination, changes ion fluxes and alters gene expression [193–196]. In protozoans, extracellular ATP controls multiple responses; in social amoeba, *Dictyostelium discoideum*, ATP inhibits amoeboid movement, and triggers depolarization and Ca^{2+} influx; in the ciliates, *Paramecium* and *Tetrahymena*, ATP induces avoidance reactions through altering the rate of cilia beating and swimming [47]; in *Trypanosoma cruzi*, ATP induces parasitosis [193]. Purinergic signalling operates in all kingdoms of life, in plants, in fungi and in animals [190,197,198]. Chemical signalling by ATP, adenine nucleotides and adenosine is present in various forms virtually in all organisms and contributes to a wide variety of functions, from food detection (purinoceptors are an important part of food search behaviour in crustaceans such as lobsters and in insects such as mosquitoes [199,200]) to neurotransmission [201]. ATP-mediated transmission is operative in most (if not in all) organs and systems in vertebrates [197].

At the molecular level, purinergic chemical transmission is mediated by several systems responsible for transmitter release, and by specific plasmalemmal purinoceptors. Purinergic receptors are represented by: (i) ionotropic P2X receptors that are ATP-gated cationic plasmalemmal channels [202,203] and (ii) by metabotropic, G-protein-coupled seven-transmembrane receptors classified as P1 adenosine receptors, P2Y nucleotide (ATP, ADP, UTP, UDP and UDP-sugar) receptors and P0 adenine receptors [204,205]. The ATP-gated ion channels are similar in their architecture to P2X receptors, and had appeared already in protozoa; hitherto, P2X receptors have been identified and characterized in *D. discoideum*, in the marine green alga *Ostreococcus tauri*, in the choanoflagellate *Monosiga brevicollis*, in trematode *Schistosoma mansoni* and in a tardigrade *Hypsibius dujardini* [206–210]. The P2X receptors retain their structure throughout evolution, being composed of three subunits with each subunit containing two transmembrane domains.

Purinergic metabotropic receptors generally emerged at later evolutionary stages. Although the rather unique cAMP metabotropic receptors (defined as CAR1–4) are present in *D. discoideum* [211], the proper metabotropic purinoceptors appeared later. The most evolutionarily ancient adenosine receptors were identified in the sea anemone *Nematostella vectensis*, and they are abundant in insects, in bivalves (mussels) and in echinoderms [212]. The P2Y metabotropic receptors seem to emerge in vertebrates: the earliest homologues have been found in sharks and skate, *Raja erinacea* [213]. The evolutionary roots of P0 adenine receptors are unknown; these have been cloned only from mouse and hamster [205].

Release of ATP seems to be a common feature of life forms, and it has been identified in the earliest life forms: ATP is secreted by bacteria, by protozoa, by yeasts and plants, and by many types of cells in all multicellular animals [193]. The pathways for ATP release are represented by (i) transmembrane diffusion via ATP-permeable channels; (ii) active transport; and (iii) exocytosis [214,215]. Arguably, diffusional ATP release that uses plasmalemmal ion (usually anion) channels or unpaired hemichannels of connexin and innexin family, or pannexins (which though related to connexins/innexins do not form gap junctional channels and hence cannot be properly called hemichannels; there are indications that pannexins act as anion channels [216]) is the most ancient and general mechanism; indeed, diffusional ATP release is found throughout phylogeny and is present in many types of cells in mammals, from kidney and osteoclasts to neurons and neuroglia [134,217–220]. Active plasmalemmal transport of ATP is associated with ABC transporters that are expressed already in protists [221]. Finally, exocytotic ATP release that uses vesicles bearing specific nucleotide transports (VNUT/SLC17A9) allows spatially restricted and tightly regulated ATP release characteristic for nervous system, the VNUT-bearing vesicles are found in invertebrates and characterized in the nematode *C. elegans*, in sea anemone, in sea urchin and in some insects [222,223]; they are present in vertebrates, where they are expressed in various tissues including the brain [215].

In its physiological action, ATP transmission is tightly connected with Ca^{2+} signalling. Indeed, generation of Ca^{2+} signals seems to be the general consequence of activation of purinoceptors. All P2X receptors are permeable to Ca^{2+}: the $P_{Ca}/P_{monovalent}$ is approximately 5 for $P2X_1$ receptors, approximately 2.5 for $P2X_2$ receptors, approximately 1.5 for $P2X_3$ receptors, approximately 4.2 for $P2X_4$ receptors and approximately 1.5 for $P2X_5$ receptors; the heteromeric receptors similarly had an appreciable Ca^{2+} permeability. The $P2X_7$ receptors' permeability for Ca^{2+} may vary depending on the state of the pore: dilation of the latter increases $P_{Ca}/P_{monovalent}$ [193,224–226]. Fractional Ca^{2+} currents through P2X receptors measured directly varied between 3 and 15 depending on channel composition (with rat $P2X_1$ and human $P2X_4$ receptors having maximal fractional Ca^{2+} currents at 12.4% and 15%, respectively [227]). This is remarkable, because Ca^{2+} fluxes through P2X receptors are fully comparable to Ca^{2+} current through NMDA receptors (fractional Ca^{2+} permeability approx. 14% [227]) and hence P2X-mediated Ca^{2+} signals may seriously contribute to postsynaptic plasticity. Activation of ionotropic purinergic receptors triggers Ca^{2+} signals in many types of cells [228–232]. Incidentally, P2X receptors may also serve as intracellular Ca^{2+}-release channels, as has been shown in *D. discoideum* [233], further corroborating an intimate relation between ATP and Ca^{2+} from the very early evolution of cellular signalling. The P2X receptors seem to operate in the membranes of other organelles, including mitochondria, lysosomes and nuclei [234], although whether activation of these intracellular receptors is linked to Ca^{2+} movements remains to be found.

Metabotropic purinoceptors are distributed even more abundantly than P2X channels; the P2Y receptors and A1 adenosine receptors are expressed virtually in every cell type in mammals [197,235]. In many cells, these receptors are coupled, via several G proteins, to phospholipase C that produces $InsP_3$ and initiates Ca^{2+} release from the endoplasmic reticulum. The ATP-induced Ca^{2+} signalling mediated through the metabotropic route seems to be almost universal in non-excitable cells, being present in immune cells [236], in neuroglia [237], in kidney [238], in cells of urinary tract [239], in skin, bone and cartilages [240], in endocrine glands [241], etc. The P2Y receptors and their downstream Ca^{2+} signalling can be activated not only by ATP, but also by other nucleotides including ADP, UTP, UDP and UDP-glucose [242,243]. The adenosine-mediated signalling and A1 receptors are similarly widely distributed, being abundant in the nervous system, in the heart, in the lungs, in the blood vessels and so on [244–246] and in many cases, these receptors are coupled with cellular Ca^{2+} signalling. Adenosine is the product of ATP catabolism and is also released (through equilibrating transports) when cells experience high ATP breakdown (for example under stress or in pathology), thus directly coupling ATP biochemistry with ATP/Ca^{2+} intercellular signalling.

10. Conclusion

During evolution, inevitable leakage of Ca^{2+} into the eukaryotic cell was not only a problem—because of toxicity even in moderate concentrations—but also a chance. A compromise has been developed between ATP-based energetics and very low solubility of CaATP. However, considerable amounts of ATP have to be invested to keep [Ca^{2+}] low in the cell, thus enabling Ca^{2+} to serve as a low cost second messenger. As a result, ATP is used by the cell preferably as an energy-storing molecule, whereas Ca^{2+} mainly serves for intracellular signalling. Intracellular Ca^{2+} signals can be generated by Ca^{2+} influx, by Ca^{2+} release from stores or by a combination of both. The termination of signal is achieved by binding to cytosolic buffers, sequestration and extrusion. There are many intracellular targets for Ca^{2+}, the number of Ca^{2+}-binding proteins increasing considerably during evolution. Another ancient strategy of the eukaryotic cell is to exploit the unavoidable leakage of nucleotide triphosphates from the cell as an extracellular messenger by purinergic receptors. Incidentally, this mirrors the stratagem of how the cell exploits leakage of Ca^{2+}, but in opposite direction. Again, Ca^{2+} signals triggered by activation of purinoceptors provide for intracellular signalling. Considering the legion of cellular activities that depend on, or are modulated by Ca^{2+}, this is an essential signal for life and death of a cell.

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References

1. Galimov EM. 2009 Concept of sustained ordering and an ATP-related mechanism of life's origin. *Int. J. Mol. Sci.* **10**, 2019–2030. (doi:10.3390/ijms10052019)
2. Ponnamperuma C, Sagan C, Mariner R. 1963 Synthesis of adenosine triphosphate under possible primitive earth conditions. *Nature* **199**, 222–226. (doi:10.1038/199222a0)
3. Bryant DE *et al.* 2013 Hydrothermal modification of the Sikhote–Alin iron meteorite under low pH geothermal environments. A plausibly prebiotic route to activated phosphorus on the early Earth. *Geochim. Cosmochim. Acta* **109**, 90–112. (doi:10.1016/j.gca.2012.12.043)
4. Eigen M. 1971 Selforganization of matter and the evolution of biological macromolecules. *Naturwissenschaften* **58**, 465–523. (doi:10.1007/BF00623322)
5. Kazmierczak J, Kempe S, Kremer B. 2013 Calcium in the early evolution of living systems: a biohistorical approach. *Curr. Org. Chem.* **17**, 1738–1750. (doi:10.2174/13852728113179990081)
6. Fralick P, Riding R. 2015 Steep Rock Lake: sedimentology and geochemistry of an archean carbonate platform. *Earth-Sci. Rev.* **151**, 132–175. (doi:10.1016/j.earscirev.2015.10.006)
7. Denton RM. 2009 Regulation of mitochondrial dehydrogenases by calcium ions. *Biochim. Biophys. Acta* **1787**, 1309–1316. (doi:10.1016/j.bbabio.2009.01.005)
8. Bazil JN, Buzzard GT, Rundell AE. 2010 Modeling mitochondrial bioenergetics with integrated volume dynamics. *PLoS Comput. Biol.* **6**, e1000632. (doi:10.1371/journal.pcbi.1000632)
9. Speijer D. 2015 Birth of the eukaryotes by a set of reactive innovations: new insights force us to relinquish gradual models. *Bioessays* **37**, 1268–1276. (doi:10.1002/bies.201500107)
10. Blackstone NW. 2015 The impact of mitochondrial endosymbiosis on the evolution of calcium signaling. *Cell Calcium* **57**, 133–139. (doi:10.1016/j.ceca.2014.11.006)
11. Dacks JB, Field MC. 2007 Evolution of the eukaryotic membrane-trafficking system: origin, tempo and mode. *J. Cell Sci.* **120**, 2977–2985. (doi:10.1242/jcs.013250)
12. Field MC, Dacks JB. 2009 First and last ancestors: reconstructing evolution of the endomembrane system with ESCRTs, vesicle coat proteins, and nuclear pore complexes. *Curr. Opin. Cell Biol.* **21**, 4–13. (doi:10.1016/j.ceb.2008.12.004)
13. Patel S, Cai X. 2015 Evolution of acidic Ca^{2+} stores and their resident Ca^{2+}-permeable channels. *Cell Calcium* **57**, 222–230. (doi:10.1016/j.ceca.2014.12.005)
14. Whiteheart SW, Schraw T, Matveeva EA. 2001 N-ethylmaleimide sensitive factor (NSF) structure and function. *Int. Rev. Cytol.* **207**, 71–112. (doi:10.1016/S0074-7696(01)07003-6)
15. Volkan-Kacso S, Marcus RA. 2015 Theory for rates, equilibrium constants, and Bronsted slopes in F1-ATPase single molecule imaging experiments. *Proc. Natl Acad. Sci. USA* **112**, 14 230–14 235. (doi:10.1073/pnas.1518489112)
16. Milo R, Philips R. 2016 *Cell biology by the numbers*, 400 p. New York and Abingdon: Garland Science.
17. Goody RS. 2003 The significance of the free energy of hydrolysis of GTP for signal-transducing and regulatory GTPases. *Biophys. Chem.* **100**, 535–544. (doi:10.1016/S0301-4622(02)00304-6)
18. Novick P, Zerial M. 1997 The diversity of Rab proteins in vesicle transport. *Curr. Opin. Cell Biol.* **9**, 496–504. (doi:10.1016/S0955-0674(97)80025-7)
19. Zhen Y, Stenmark H. 2015 Cellular functions of Rab GTPases at a glance. *J. Cell Sci.* **128**, 3171–3176. (doi:10.1242/jcs.166074)

20. Berridge MJ, Bootman MD, Roderick HL. 2003 Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* **4**, 517–529. (doi:10.1038/nrm1155)
21. Berridge MJ. 2006 Calcium microdomains: organization and function. *Cell Calcium* **40**, 405–412. (doi:10.1016/j.ceca.2006.09.002)
22. Clapham DE. 2007 Calcium signaling. *Cell* **131**, 1047–1058. (doi:10.1016/j.cell.2007.11.028)
23. Shemarova IV, Nesterov VP. 2005 Evolution of mechanisms of calcium signaling: the role of calcium ions in signal transduction in prokaryotes. *Zh. Evol. Biokhim. Fiziol.* **41**, 12–17.
24. Case RM, Eisner D, Gurney A, Jones O, Muallem S, Verkhratsky A. 2007 Evolution of calcium homeostasis: from birth of the first cell to an omnipresent signalling system. *Cell Calcium* **42**, 345–350. (doi:10.1016/j.ceca.2007.05.001)
25. Plattner H, Verkhratsky A. 2015 Evolution of calcium signalling. *Cell Calcium* **57**, 121–122. (doi:10.1016/j.ceca.2015.02.007)
26. Plattner H, Verkhratsky A. 2015 The ancient roots of calcium signalling evolutionary tree. *Cell Calcium* **57**, 123–132. (doi:10.1016/j.ceca.2014.12.004)
27. Ambudkar SV, Lynn AR, Maloney PC, Rosen BP. 1986 Reconstitution of ATP-dependent calcium transport from streptococci. *J. Biol. Chem.* **261**, 15 596–15 600.
28. Raeymaekers L, Wuytack E, Willems I, Michiels CW, Wuytack F. 2002 Expression of a P-type Ca^{2+}-transport ATPase in *Bacillus subtilis* during sporulation. *Cell Calcium* **32**, 93–103. (doi:10.1016/S0143-4160(02)00125-2)
29. Rosch JW, Sublett J, Gao G, Wang YD, Tuomanen EI. 2008 Calcium efflux is essential for bacterial survival in the eukaryotic host. *Mol. Microbiol.* **70**, 435–444. (doi:10.1111/j.1365-2958.2008.06425.x)
30. Lourido S, Moreno SN. 2015 The calcium signaling toolkit of the Apicomplexan parasites *Toxoplasma gondii* and *Plasmodium* spp. *Cell Calcium* **57**, 186–193. (doi:10.1016/j.ceca.2014.12.010)
31. Docampo R, Huang G. 2015 Calcium signaling in trypanosomatid parasites. *Cell Calcium* **57**, 194–202. (doi:10.1016/j.ceca.2014.10.015)
32. Burdakov D, Petersen OH, Verkhratsky A. 2005 Intraluminal calcium as a primary regulator of endoplasmic reticulum function. *Cell Calcium* **38**, 303–310. (doi:10.1016/j.ceca.2005.06.010)
33. Williams DB. 2006 Beyond lectins: the calnexin/calreticulin chaperone system of the endoplasmic reticulum. *J. Cell Sci.* **119**, 615–623. (doi:10.1242/jcs.02856)
34. Morgan RO, Martin-Almedina S, Iglesias JM, Gonzalez-Florez MI, Fernandez MP. 2004 Evolutionary perspective on annexin calcium-binding domains. *Biochim. Biophys. Acta* **1742**, 133–140. (doi:10.1016/j.bbamcr.2004.09.010)
35. Berridge MJ, Bootman MD, Lipp P. 1998 Calcium: a life and death signal. *Nature* **395**, 645–648. (doi:10.1038/27094)
36. Stenmark H. 2012 The Rabs: a family at the root of metazoan evolution. *BMC Biol.* **10**, 68. (doi:10.1186/1741-7007-10-68)
37. Kloepper TH, Kienle CN, Fasshauer D. 2008 SNAREing the basis of multicellularity: consequences of protein family expansion during evolution. *Mol. Biol. Evol.* **25**, 2055–2068. (doi:10.1093/molbev/msn151)
38. Plattner H. 2015 Molecular aspects of calcium signalling at the crossroads of unikont and bikont eukaryote evolution: the ciliated protozoan *Paramecium* in focus. *Cell Calcium* **57**, 174–185. (doi:10.1016/j.ceca.2014.12.002)
39. Plattner H. 2015 Calcium signalling in the ciliated protozoan model, *Paramecium*: strict signal localisation by epigenetically controlled positioning of different Ca^{2+}-channels. *Cell Calcium* **57**, 203–213. (doi:10.1016/j.ceca.2014.09.003)
40. Plattner H. 2014 Calcium regulation in the protozoan model, *Paramecium tetraurelia*. *J. Eukaryot Microbiol.* **61**, 95–114. (doi:10.1111/jeu.12070)
41. Verkhratsky A. 2005 Physiology and pathophysiology of the calcium store in the endoplasmic reticulum of neurons. *Physiol. Rev.* **85**, 201–279. (doi:10.1152/physrev.00004.2004)
42. Solovyova N, Veselovsky N, Toescu EC, Verkhratsky A. 2002 Ca^{2+} dynamics in the lumen of the endoplasmic reticulum in sensory neurons: direct visualization of Ca^{2+}-induced Ca^{2+} release triggered by physiological Ca^{2+} entry. *EMBO J.* **21**, 622–630. (doi:10.1093/emboj/21.4.622)
43. Storer AC, Cornish-Bowden A. 1976 Concentration of $MgATP^{2-}$ and other ions in solution. Calculation of the true concentrations of species present in mixtures of associating ions. *Biochem. J.* **159**, 1–5. (doi:10.1042/bj1590001)
44. Garfinkel L, Altschuld RA, Garfinkel D. 1986 Magnesium in cardiac energy metabolism. *J. Mol. Cell Cardiol.* **18**, 1003–1013. (doi:10.1016/S0022-2828(86)80289-9)
45. Neves G, Neef A, Lagnado L. 2001 The actions of barium and strontium on exocytosis and endocytosis in the synaptic terminal of goldfish bipolar cells. *J. Physiol.* **535**, 809–824. (doi:10.1111/j.1469-7793.2001.t01-1-00809.x)
46. Brehm P, Eckert R. 1978 Calcium entry leads to inactivation of calcium channel in *Paramecium*. *Science* **202**, 1203–1206. (doi:10.1126/science.103199)
47. Hennessey TM, Kuruvilla HG. 2000 Electrophysiology of *Tetrahymena*. *Methods Cell Biol.* **62**, 363–377. (doi:10.1016/S0091-679X(08)61543-5)
48. Dilger JM, Valentine SJ, Glover MS, Clemmer DE. 2013 A database of alkaline-earth-coordinated peptide cross sections: insight into general aspects of structure. *J. Am. Soc. Mass Spectrom.* **24**, 768–779. (doi:10.1007/s13361-013-0579-z)
49. Kee Y, Scheller RH. 1996 Localization of synaptotagmin-binding domains on syntaxin. *J. Neurosci.* **16**, 1975–1981.
50. Park HY, Kim SA, Korlach J, Rhoades E, Kwok LW, Zipfel WR, Waxham MN, Webb WW, Pollack L. 2008 Conformational changes of calmodulin upon Ca^{2+} binding studied with a microfluidic mixer. *Proc. Natl Acad. Sci. USA* **105**, 542–547. (doi:10.1073/pnas.0710810105)
51. Saimi Y, Kung C. 2002 Calmodulin as an ion channel subunit. *Annu. Rev. Physiol.* **64**, 289–311. (doi:10.1146/annurev.physiol.64.100301.111649)
52. Taylor CW, da Fonseca PC, Morris EP. 2004 IP_3 receptors: the search for structure. *Trends Biochem. Sci.* **29**, 210–219. (doi:10.1016/j.tibs.2004.02.010)
53. Pang ZP, Sudhof TC. 2010 Cell biology of Ca^{2+}-triggered exocytosis. *Curr. Opin. Cell Biol.* **22**, 496–505. (doi:10.1016/j.ceb.2010.05.001)
54. Rizo J, Chen X, Arac D. 2006 Unraveling the mechanisms of synaptotagmin and SNARE function in neurotransmitter release. *Trends Cell Biol.* **16**, 339–350. (doi:10.1016/j.tcb.2006.04.006)
55. Neher E. 1998 Usefulness and limitations of linear approximations to the understanding of Ca^{++} signals. *Cell Calcium* **24**, 345–357. (doi:10.1016/S0143-4160(98)90058-6)
56. Schwaller B. 2010 Cytosolic Ca^{2+} buffers. *Cold Spring Harb. Perspect. Biol.* **2**, a004051. (doi:10.1101/cshperspect.a004051)
57. Huang B, Mengersen A, Lee VD. 1988 Molecular cloning of cDNA for caltractin, a basal body-associated Ca^{2+}-binding protein: homology in its protein sequence with calmodulin and the yeast CDC31 gene product. *J. Cell Biol.* **107**, 133–140. (doi:10.1083/jcb.107.1.133)
58. Sehring IM, Klotz C, Beisson J, Plattner H. 2009 Rapid downregulation of the Ca^{2+}-signal after exocytosis stimulation in *Paramecium* cells: essential role of a centrin-rich filamentous cortical network, the infraciliary lattice. *Cell Calcium* **45**, 89–97. (doi:10.1016/j.ceca.2008.06.004)
59. Cho JH *et al.* 2000 Calsequestrin, a calcium sequestering protein localized at the sarcoplasmic reticulum, is not essential for body-wall muscle function in *Caenorhabditis elegans*. *J. Cell Sci.* **113**, 3947–3958.
60. Williams RJ. 2006 The evolution of calcium biochemistry. *Biochim. Biophys. Acta* **1763**, 1139–1146. (doi:10.1016/j.bbamcr.2006.08.042)
61. Grubbs RD. 2002 Intracellular magnesium and magnesium buffering. *Biometals* **15**, 251–259. (doi:10.1023/A:1016026831789)
62. Jahnen-Dechent W, Ketteler M. 2012 Magnesium basics. *Clin. Kidney J.* **5**, 3–i14. (doi:10.1093/ndtplus/sfr163)
63. Piovesan D, Profiti G, Martelli PL, Casadio R. 2012 The human 'magnesome': detecting magnesium binding sites on human proteins. *BMC Bioinformatics* **13**(Suppl. 14), S10. (doi:10.118/1471-2105-13-S14-S10)
64. Cowan JA. 1998 Magnesium activation of nuclease enzymes—the importance of water. *Inorg. Chim. Acta* **275–276**, 24–27. (doi:10.1016/S0020-1693(98)00073-5)
65. Black CB, Huang HW, Cowan JA. 1994 Biological coordination chemistry of magnesium, sodium, and potassium ions. Protein and nucleotide binding sites. *Coord. Chem. Rev.* **135/136**, 165–202. (doi:10.1016/0010-8545(94)80068-5)

66. Bojovschi A, Liu MS, Sadus RJ. 2014 Mg^{2+} coordinating dynamics in Mg:ATP fueled motor proteins. *J. Chem. Phys.* **140**, 115102. (doi:10.1063/1.4867898)
67. Ivanov AV, Malygin AA, Karpova GG. 2013 Mg^{2+} ions affect the structure of the central domain of the 18S rRNA in the vicinity of the ribosomal protein S13 binding site. *Mol. Biol.* (*Mosk*) **47**, 157–166.
68. De A, Ramesh V, Mahadevan S, Nagaraja V. 1998 Mg^{2+} mediated sequence-specific binding of transcriptional activator protein C of bacteriophage Mu to DNA. *Biochemistry* **37**, 3831–3838. (doi:10.1021/bi972171v)
69. Higashijima T, Ferguson KM, Sternweis PC, Smigel MD, Gilman AG. 1987 Effects of Mg^{2+} and the bg-subunit complex on the interactions of guanine nucleotides with G proteins. *J. Biol. Chem.* **262**, 762–766.
70. Nelson MR, Chazin WJ. 1998 Calmodulin as a calcium sensor. In *Calmodulin and signal transduction* (eds LJ van Eldik, MD Watterson), pp. 17–64. San Diego, MA: Academic Press.
71. Malmendal A, Linse S, Evenas J, Forsen S, Drakenberg T. 1999 Battle for the EF-hands: magnesium-calcium interference in calmodulin. *Biochemistry* **38**, 11 844–11 850. (doi:10.1021/bi9909288)
72. Knape MJ, Ahuja LG, Bertinetti D, Burghardt NC, Zimmermann B, Taylor SS, Herberg FW. 2015 Divalent metal ions Mg^{2+} and Ca^{2+} have distinct effects on protein kinase A activity and regulation. *ACS Chem. Biol.* **10**, 2303–2315. (doi:10.1021/acschembio.5b00271)
73. Klumpp S, Selke D, Hermesmeier J. 1998 Protein phosphatase type 2C active at physiological Mg^{2+}: stimulation by unsaturated fatty acids. *FEBS Lett.* **437**, 229–232. (doi:10.1016/S0014-5793(98)01237-X)
74. Brüssow H. 2007 *The quest for food. A natural history of eating*, 870 p. Heiedelberg, Germany: Springer.
75. Grosshans BL, Ortiz D, Novick P. 2006 Rabs and their effectors: achieving specificity in membrane traffic. *Proc. Natl Acad. Sci. USA* **103**, 11 821–11 827. (doi:10.1073/pnas.0601617103)
76. Petersen OH, Michalak M, Verkhratsky A. 2005 Calcium signalling: past, present and future. *Cell Calcium* **38**, 161–169. (doi:10.1016/j.ceca.2005.06.023)
77. Booth IR, Miller S, Muller A, Lehtovirta-Morley L. 2015 The evolution of bacterial mechanosensitive channels. *Cell Calcium* **57**, 140–150. (doi:10.1016/j.ceca.2014.12.011)
78. Cai X, Wang X, Patel S, Clapham DE. 2015 Insights into the early evolution of animal calcium signaling machinery: a unicellular point of view. *Cell Calcium* **57**, 166–173. (doi:10.1016/j.ceca.2014.11.007)
79. Petersen OH, Verkhratsky A. 2007 Endoplasmic reticulum calcium tunnels integrate signalling in polarised cells. *Cell Calcium* **42**, 373–378. (doi:10.1016/j.ceca.2007.05.012)
80. Cooper GM. 2000 *The cell: a molecular approach*, 2nd edn. Sunderland, MA: Sinauer Associates.
81. Docampo R, Ulrich P, Moreno SN. 2010 Evolution of acidocalcisomes and their role in polyphosphate storage and osmoregulation in eukaryotic microbes. *Phil. Trans. R. Soc. B* **365**, 775–784. (doi:10.1098/rstb.2009.0179).
82. Robitaille R, Adler EM, Charlton MP. 1990 Strategic location of calcium channels at transmitter release sites of frog neuromuscular synapses. *Neuron* **5**, 773–779. (doi:10.1016/0896-6273(90)90336-E)
83. Dunlap K, Luebke JI, Turner TJ. 1995 Exocytotic Ca^{2+} channels in mammalian central neurons. *Trends Neurosci.* **18**, 89–98. (doi:10.1016/0166-2236(95)80030-6)
84. Machemer H, Ogura A. 1979 Ionic conductances of membranes in ciliated and deciliated *Paramecium*. *J. Physiol.* **296**, 49–60. (doi:10.1113/jphysiol.1979.sp012990)
85. Xia XM *et al.* 1998 Mechanism of calcium gating in small-conductance calcium-activated potassium channels. *Nature* **395**, 503–507. (doi:10.1038/26758).
86. Levitan IB. 1999 It is calmodulin after all! Mediator of the calcium modulation of multiple ion channels. *Neuron* **22**, 645–648. (doi:10.1016/S0896-6273(00)80722-9)
87. Mak DO, Foskett JK. 2015 Inositol 1,4,5-trisphosphate receptors in the endoplasmic reticulum: a single-channel point of view. *Cell Calcium* **58**, 67–78. (doi:10.1016/j.ceca.2014.12.008)
88. Ladenburger EM, Plattner H. 2011 Calcium-release channels in *Paramecium*. Genomic expansion, differential positioning and partial transcriptional elimination. *PLoS ONE* **6**, e27111. (doi:10.1371/journal.pone.0027111)
89. Plattner H, Verkhratsky A. 2013 Ca^{2+} signalling early in evolution: all but primitive. *J. Cell Sci.* **126**, 2141–2150. (doi:10.1242/jcs.127449)
90. Galione A. 2015 A primer of NAADP-mediated Ca^{2+} signalling: from sea urchin eggs to mammalian cells. *Cell Calcium* **58**, 27–47. (doi:10.1016/j.ceca.2014.09.010)
91. Verkhratsky A, Shmigol A. 1996 Calcium-induced calcium release in neurones. *Cell Calcium* **19**, 1–14. (doi:10.1016/S0143-4160(96)90009-3)
92. Lee HC. 2012 Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization. *J. Biol. Chem.* **287**, 31 633–31 640. (doi:10.1074/jbc.R112.349464)
93. Leondaritis G, Siokos J, Skaripa I, Galanopoulou D. 2013 Genome-wide analysis of the phosphoinositide kinome from two ciliates reveals novel evolutionary links for phosphoinositide kinases in eukaryotic cells. *PLoS ONE* **8**, e78848. (doi:10.1371/journal.pone.0078848)
94. Nusblat AD, Bright LJ, Turkewitz AP. 2012 Conservation and innovation in *Tetrahymena* membrane traffic: proteins, lipids, and compartments. *Methods Cell Biol.* **109**, 141–175. (doi:10.1016/B978-0-12-385967-9.00006-2)
95. Mackrill JJ. 2012 Ryanodine receptor calcium release channels: an evolutionary perspective. *Adv. Exp. Med. Biol.* **740**, 159–182. (doi:10.1007/978-94-007-2888-2_7)
96. Mignery GA, Sudhof TC, Takei K, De Camilli P. 1989 Putative receptor for inositol 1,4,5-trisphosphate similar to ryanodine receptor. *Nature* **342**, 192–195. (doi:10.1038/342192a0)
97. Albrieux M, Sardet C, Villaz M. 1997 The two intracellular Ca^{2+} release channels, ryanodine receptor and inositol 1,4,5-trisphosphate receptor, play different roles during fertilization in ascidians. *Dev. Biol.* **189**, 174–185. (doi:10.1006/dbio.1997.8674)
98. Ponting CP. 2000 Novel repeats in ryanodine and IP_3 receptors and protein *O*-mannosyltransferases. *Trends Biochem. Sci* **25**, 48–50.
99. Calcraft PJ *et al.* 2009 NAADP mobilizes calcium from acidic organelles through two-pore channels. *Nature* **459**, 596–600. (doi:10.1038/nature08030)
100. Rahman T, Cai X, Brailoiu GC, Abood ME, Brailoiu E, Patel S. 2014 Two-pore channels provide insight into the evolution of voltage-gated Ca^{2+} and Na^{+} channels. *Sci. Signal.* **7**, ra109. (doi:10.1126/scisignal.2005450)
101. Zhu MX, Ma J, Parrington J, Calcraft PJ, Galione A, Evans AM. 2010 Calcium signaling via two-pore channels: local or global, that is the question. *Am. J. Physiol. Cell Physiol.* **298**, C430–C441. (doi:10.1152/ajpcell.00475.2009)
102. Patel S, Docampo R. 2010 Acidic calcium stores open for business: expanding the potential for intracellular Ca^{2+} signaling. *Trends Cell Biol.* **20**, 277–286. (doi:10.1016/j.tcb.2010.02.003)
103. Lee HC. 2005 Nicotinic acid adenine dinucleotide phosphate (NAADP)-mediated calcium signaling. *J. Biol. Chem.* **280**, 33 693–33 696. (doi:10.1074/jbc.R500012200)
104. Malavasi F, Deaglio S, Funaro A, Ferrero E, Horenstein AL, Ortolan E, Vaisitti T, Aydin S. 2008 Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. *Physiol. Rev.* **88**, 841–886. (doi:10.1152/physrev.00035.2007)
105. Watanabe K. 2011 The localization and functional analysis of two-pore channel proteins. PhD Thesis. Oxford, Oxford University.
106. Prole DL, Taylor CW. 2011 Identification of intracellular and plasma membrane calcium channel homologues in pathogenic parasites. *PLoS ONE* **6**, e26218. (doi:10.1371/journal.pone.0026218)
107. Kilpatrick BS, Eden ER, Schapira AH, Futter CE, Patel S. 2013 Direct mobilisation of lysosomal Ca^{2+} triggers complex Ca^{2+} signals. *J. Cell Sci.* **126**, 60–66. (doi:10.1242/jcs.118836)
108. Peiter E, Maathuis FJ, Mills LN, Knight H, Pelloux J, Hetherington AM, Sanders D. 2005 The vacuolar Ca^{2+}-activated channel TPC1 regulates germination and stomatal movement. *Nature* **434**, 404–408. (doi:10.1038/nature03381)
109. Plattner H, Sehring IM, Mohamed IK, Miranda K, De Souza W, Billington R, Genazzani A, Ladenburger EM. 2012 Calcium signaling in closely related

protozoan groups (Alveolata): non-parasitic ciliates (Paramecium, Tetrahymena) vs. parasitic Apicomplexa (Plasmodium, Toxoplasma). *Cell Calcium* **51**, 351–382. (doi:10.1016/j.ceca.2012.01.006)

110. Docampo R, Moreno SN, Plattner H. 2014 Intracellular calcium channels in protozoa. *Eur. J. Pharmacol.* **739**, 4–18. (doi:10.1016/j.ejphar.2013.11.015)
111. Xu H, Martinoia E, Szabo I. 2015 Organellar channels and transporters. *Cell Calcium* **58**, 1–10. (doi:10.1016/j.ceca.2015.02.006)
112. Solovyova N, Verkhratsky A. 2003 Neuronal endoplasmic reticulum acts as a single functional Ca^{2+} store shared by ryanodine and inositol-1,4,5-trisphosphate receptors as revealed by intra-ER $[Ca^{2+}]$ recordings in single rat sensory neurones. *Pflugers Arch.* **446**, 447–454. (doi:10.1007/s00424-003-1094-z)
113. Guse AH. 2012 Linking NAADP to ion channel activity: a unifying hypothesis. *Sci. Signal.* **5**, ppe18. (doi:10.1126/scisignal.2002890)
114. Guse AH, Lee HC. 2008 NAADP: a universal Ca^{2+} trigger. *Sci. Signal.* **1**, re10. (doi:10.1126/scisignal.144re10)
115. Patel S, Marchant JS, Brailoiu E. 2010 Two-pore channels: regulation by NAADP and customized roles in triggering calcium signals. *Cell Calcium* **47**, 480–490. (doi:10.1016/j.ceca.2010.05.001)
116. Penny CJ, Kilpatrick BS, Eden ER, Patel S. 2015 Coupling acidic organelles with the ER through Ca^{2+} microdomains at membrane contact sites. *Cell Calcium* **58**, 387–396. (doi:10.1016/j.ceca.2015.03.006)
117. Levine TP, Patel S. 2016 Signalling at membrane contact sites: two membranes come together to handle second messengers. *Curr. Opin. Cell Biol.* **39**, 77–83. (doi:10.1016/j.ceb.2016.02.011)
118. Patel S. 2015 Function and dysfunction of two-pore channels. *Sci. Signal.* **8**, re7. (doi:10.1126/scisignal.aab3314)
119. Gees M, Colsoul B, Nilius B. 2010 The role of transient receptor potential cation channels in Ca^{2+} signaling. *Cold Spring Harb. Perspect. Biol.* **2**, a003962. (doi:10.1101/cshperspect.a003962)
120. Nilius B, Owsianik G. 2011 The transient receptor potential family of ion channels. *Genome Biol.* **12**, 218. (doi:10.1186/gb-2011-12-3-218)
121. Arias-Darraz L, Cabezas D, Colenso CK, Alegria-Arcos M, Bravo-Moraga F, Varas-Concha I, Almonacid DE, Madrid R, Brauchi S. 2015 A transient receptor potential ion channel in *Chlamydomonas* shares key features with sensory transduction-associated TRP channels in mammals. *Plant Cell* **27**, 177–188. (doi:10.1105/tpc.114.131862)
122. Wheeler GL, Brownlee C. 2008 Ca^{2+} signalling in plants and green algae: changing channels. *Trends Plant Sci.* **13**, 506–514. (doi:10.1016/j.tplants.2008.06.004)
123. Lima WC, Vinet A, Pieters J, Cosson P. 2014 Role of PKD2 in rheotaxis in *Dictyostelium*. *PLoS ONE* **9**, e88682. (doi:10.1371/journal.pone.0088682)
124. Cai X. 2008 Unicellular Ca^{2+} signaling 'toolkit' at the origin of metazoa. *Mol. Biol. Evol.* **25**, 1357–1361. (doi:10.1093/molbev/msn077)
125. Edel KH, Kudla J. 2015 Increasing complexity and versatility: how the calcium signaling toolkit was shaped during plant land colonization. *Cell Calcium* **57**, 231–246. (doi:10.1016/j.ceca.2014.10.013)
126. Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G. 2006 Structural and functional features and significance of the physical linkage between ER and mitochondria. *J. Cell Biol.* **174**, 915–921. (doi:10.1083/jcb.200604016)
127. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. 1998 Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. *Science* **280**, 1763–1766. (doi:10.1126/science.280.5370.1763)
128. Protasi F. 2002 Structural interaction between RYRs and DHPRs in calcium release units of cardiac and skeletal muscle cells. *Front Biosci* **7**, d650–d658. (doi:10.2741/A801)
129. Lewis RS. 1999 Store-operated calcium channels. *Adv. Second Messenger Phosphoprotein Res.* **33**, 279–307. (doi:10.1016/S1040-7952(99)80014-7)
130. Hofer AM, Fasolato C, Pozzan T. 1998 Capacitative Ca^{2+} entry is closely linked to the filling state of internal Ca^{2+} stores: a study using simultaneous measurements of I_{CRAC} and intraluminal $[Ca^{2+}]$. *J. Cell Biol.* **140**, 325–334. (doi:10.1083/jcb.140.2.325)
131. Dellis O, Dedos SG, Tovey SC, Taufiq Ur R, Dubel SJ, Taylor CW. 2006 Ca^{2+} entry through plasma membrane IP_3 receptors. *Science* **313**, 229–233. (doi:10.1126/science.1125203)
132. Lewis RS. 2007 The molecular choreography of a store-operated calcium channel. *Nature* **446**, 284–287. (doi:10.1038/nature05637)
133. Giordano F *et al.* 2013 $PI(4,5)P_2$-dependent and Ca^{2+}-regulated ER-PM interactions mediated by the extended synaptotagmins. *Cell* **153**, 1494–1509. (doi:10.1016/j.cell.2013.05.026)
134. Verkhratsky A, Matteoli M, Parpura V, Mothet JP, Zorec R. 2016 Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion. *EMBO J.* **35**, 239–257. (doi:10.15252/embj.201592705)
135. Plattner H, Kissmehl R. 2003 Dense-core secretory vesicle docking and exocytotic membrane fusion in *Paramecium* cells. *Biochim. Biophys. Acta* **1641**, 183–193. (doi:10.1016/S0167-4889(03)00092-2)
136. Haberman Y, Grimberg E, Fukuda M, Sagi-Eisenberg R. 2003 Synaptotagmin IX, a possible linker between the perinuclear endocytic recycling compartment and the microtubules. *J. Cell Sci.* **116**, 4307–4318. (doi:10.1242/jcs.00719)
137. Czibener C, Sherer NM, Becker SM, Pypaert M, Hui E, Chapman ER, Mothes W, Andrews NW. 2006 Ca^{2+} and synaptotagmin VII-dependent delivery of lysosomal membrane to nascent phagosomes. *J. Cell Biol.* **174**, 997–1007. (doi:10.1083/jcb.200605004)
138. Hay JC. 2007 Calcium: a fundamental regulator of intracellular membrane fusion? *EMBO Rep.* **8**, 236–240. (doi:10.1038/sj.embor.7400921)
139. Mayorga LS, Beron W, Sarrouf MN, Colombo MI, Creutz C, Stahl PD. 1994 Calcium-dependent fusion among endosomes. *J. Biol. Chem.* **269**, 30 927–30 934.
140. Chen JL, Ahluwalia JP, Stamnes M. 2002 Selective effects of calcium chelators on anterograde and retrograde protein transport in the cell. *J. Biol. Chem.* **277**, 35 682–35 687. (doi:10.1074/jbc.M204157200)
141. Porat A, Elazar Z. 2000 Regulation of intra-Golgi membrane transport by calcium. *J. Biol. Chem.* **275**, 29 233–29 237. (doi:10.1074/jbc.M005316200)
142. Jaconi ME, Lew DP, Carpentier JL, Magnusson KE, Sjogren M, Stendahl O. 1990 Cytosolic free calcium elevation mediates the phagosome–lysosome fusion during phagocytosis in human neutrophils. *J. Cell Biol.* **110**, 1555–1564. (doi:10.1083/jcb.110.5.1555)
143. Yoo SH. 2010 Secretory granules in inositol 1,4,5-trisphosphate-dependent Ca^{2+} signaling in the cytoplasm of neuroendocrine cells. *FASEB J.* **24**, 653–664. (doi:10.1096/fj.09-132456)
144. Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. 2004 A molecular timeline for the origin of photosynthetic eukaryotes. *Mol. Biol. Evol.* **21**, 809–818. (doi:10.1093/molbev/msh075)
145. Su Z, Zhou X, Loukin SH, Haynes WJ, Saimi Y, Kung C. 2009 The use of yeast to understand TRP-channel mechanosensitivity. *Pflugers Arch.* **458**, 861–867. (doi:10.1007/s00424-009-0680-0)
146. Rothman JE. 2014 The principle of membrane fusion in the cell (Nobel lecture). *Angew. Chem. Int. Ed. Engl.* **53**, 12 676–12 694. (doi:10.1002/anie.201402380)
147. Sudhof TC. 2014 The molecular machinery of neurotransmitter release (Nobel lecture). *Angew. Chem. Int. Ed. Engl.* **53**, 12 696–12 717. (doi:10.1002/anie.201406359)
148. Jaiswal JK, Rivera VM, Simon SM. 2009 Exocytosis of post-Golgi vesicles is regulated by components of the endocytic machinery. *Cell* **137**, 1308–1319. (doi:10.1016/j.cell.2009.04.064)
149. Thomas P, Lee AK, Wong JG, Almers W. 1994 A triggered mechanism retrieves membrane in seconds after Ca^{2+}-stimulated exocytosis in single pituitary cells. *J. Cell Biol.* **124**, 667–675. (doi:10.1083/jcb.124.5.667)
150. Artalejo CR, Henley JR, McNiven MA, Palfrey HC. 1995 Rapid endocytosis coupled to exocytosis in adrenal chromaffin cells involves Ca^{2+}, GTP, and dynamin but not clathrin. *Proc. Natl Acad. Sci. USA* **92**, 8328–8332. (doi:10.1073/pnas.92.18.8328)
151. Plattner H, Braun C, Hentschel J. 1997 Facilitation of membrane fusion during exocytosis and exocytosis-coupled endocytosis and acceleration of 'ghost' detachment in *Paramecium* by extracellular calcium. A quenched-flow/freeze-fracture analysis. *J. Membr. Biol.* **158**, 197–208. (doi:10.1007/s002329900257)
152. Lai MM, Hong JJ, Ruggiero AM, Burnett PE, Slepnev VI, De Camilli P, Snyder SH. 1999 The calcineurin-

dynamin 1 complex as a calcium sensor for synaptic vesicle endocytosis. *J. Biol. Chem.* **274**, 25 963–25 966. (doi:10.1074/jbc.274.37.25963)

153. Fraga D, Sehring IM, Kissmehl R, Reiss M, Gaines R, Hinrichsen R, Plattner H. 2010 Protein phosphatase 2B (PP2B, calcineurin) in *Paramecium*: partial characterization reveals that two members of the unusually large catalytic subunit family have distinct roles in calcium-dependent processes. *Eukaryot Cell* **9**, 1049–1063. (doi:10.1128/EC.00322-09).
154. Yakel JL. 1997 Calcineurin regulation of synaptic function: from ion channels to transmitter release and gene transcription. *Trends Pharmacol. Sci.* **18**, 124–134. (doi:10.1016/S0165-6147(97)01046-8)
155. Malleret G *et al.* 2001 Inducible and reversible enhancement of learning, memory, and long-term potentiation by genetic inhibition of calcineurin. *Cell* **104**, 675–686. (doi:10.1016/S0092-8674(01)00264-1)
156. Aramburu J, Heitman J, Crabtree GR. 2004 Calcineurin: a central controller of signalling in eukaryotes. *EMBO Rep.* **5**, 343–348. (doi:10.1038/sj.embor.7400133)
157. Rusnak F, Mertz P. 2000 Calcineurin: form and function. *Physiol. Rev.* **80**, 1483–1521.
158. Cesca F, Baldelli P, Valtorta F, Benfenati F. 2010 The synapsins: key actors of synapse function and plasticity. *Prog. Neurobiol.* **91**, 313–348. (doi:10.1016/j.pneurobio.2010.04.006)
159. Goldberg JM, Manning G, Liu A, Fey P, Pilcher KE, Xu Y, Smith JL. 2006 The *Dictyostelium* kinome: analysis of the protein kinases from a simple model organism. *PLoS Genet.* **2**, e38. (doi:10.1371/journal.pgen.0020038)
160. Kim K, Messinger LA, Nelson DL. 1998 Ca^{2+}-dependent protein kinases of *Paramecium:* cloning provides evidence of a multigene family. *Eur. J. Biochem.* **251**, 605–612. (doi:10.1046/j.1432-1327.1998.2510605.x)
161. Kudo S, Muto Y, Nozawa Y. 1985 Regulation by calcium of hormone-insensitive adenylate cyclase and calmodulin-dependent guanylate cyclase in *Tetrahymena* plasma membrane. *Comp. Biochem. Physiol. B* **80**, 813–816.
162. Kudo S, Muto Y, Inagaki M, Hidaka H, Nozawa Y. 1985 Interaction of calcium-binding proteins with calmodulin-dependent guanylate cyclase in *Tetrahymena* plasma membrane. *Comp. Biochem. Physiol. B* **80**, 495–498. (doi:10.1016/0300-9629(85)90403-7)
163. Imanishi Y, Li N, Sokal I, Sowa ME, Lichtarge O, Wensel TG, Saperstein DA, Baehr W, Palczewski K. 2002 Characterization of retinal guanylate cyclase-activating protein 3 (GCAP3) from zebrafish to man. *Eur. J. Neurosci.* **15**, 63–78. (doi:10.1046/j.0953-816x.2001.01835.x)
164. Coluccio LMEE. 2008 *Myosin. A superfamily of molecular motors*, 477 p. Dordrecht, The Netherlands: Springer.
165. Naito Y, Kaneko H. 1972 Reactivated triton-extracted models of paramecium: modification of ciliary movement by calcium ions. *Science* **176**, 523–524. (doi:10.1126/science.176.4034.523)
166. Eckert R, Brehm P. 1979 Ionic mechanisms of excitation in paramecium. *Annu. Rev. Biophys. Bioeng.* **8**, 353–383. (doi:10.1146/annurev.bb.08.060179.002033).
167. Tamm SL. 2015 Ctenophores and termites - systems for motility. In *Cilia and ciliates, flagella and flragellates* (eds K Hausmann, R Radek), pp. 147–171. Stuttgart, Germany: Schweizerbart Science Publishers.
168. Blum JJ, Hayes A, Jamieson Jr GA, Vanaman TC. 1980 Calmodulin confers calcium sensitivity on ciliary dynein ATPase. *J. Cell Biol.* **87**, 386–397. (doi:10.1083/jcb.87.2.386)
169. Miglietta LA, Nelson DL. 1988 A novel cGMP-dependent protein kinase from *Paramecium*. *J. Biol. Chem.* **263**, 16 096–16 105.
170. Schultz JE, Klumpp S. 1991 Calcium-regulated guanylyl cyclases from *Paramecium* and *Tetrahymena*. *Methods Enzymol.* **195**, 466–474. (doi:10.1016/0076-6879(91)95193-N)
171. Peracchia C. 1978 Calcium effects on gap junction structure and cell coupling. *Nature* **271**, 669–671. (doi:10.1038/271669a0)
172. Carafoli E. 2003 Historical review: mitochondria and calcium: ups and downs of an unusual relationship. *Trends Biochem. Sci.* **28**, 175–181. (doi:10.1016/S0968-0004(03)00053-7)
173. Jacobson J, Duchen MR. 2004 Interplay between mitochondria and cellular calcium signalling. *Mol. Cell Biochem.* **256–257**, 209–218. (doi:10.1023/B:MCBI.0000009869.29827.df)
174. Glancy B, Willis WT, Chess DJ, Balaban RS. 2013 Effect of calcium on the oxidative phosphorylation cascade in skeletal muscle mitochondria. *Biochemistry* **52**, 2793–2809. (doi:10.1021/bi3015983)
175. Balaban RS. 2009 The role of Ca^{2+} signaling in the coordination of mitochondrial ATP production with cardiac work. *Biochim. Biophys. Acta* **1787**, 1334–1341. (doi:10.1016/j.bbabio.2009.05.011)
176. Sancak Y *et al.* 2013 EMRE is an essential component of the mitochondrial calcium uniporter complex. *Science* **342**, 1379–1382. (doi:10.1126/science.1242993)
177. Murgia M, Rizzuto R. 2015 Molecular diversity and pleiotropic role of the mitochondrial calcium uniporter. *Cell Calcium* **58**, 11–17. (doi:10.1016/j.ceca.2014.11.001)
178. Kamer KJ, Mootha VK. 2014 MICU1 and MICU2 play nonredundant roles in the regulation of the mitochondrial calcium uniporter. *EMBO Rep.* **15**, 299–307. (doi:10.1002/embr.201337946)
179. Hardt M, Plattner H. 2000 Sub-second quenched-flow/X-ray microanalysis shows rapid Ca^{2+} mobilization from cortical stores paralleled by Ca^{2+} influx during synchronous exocytosis in *Paramecium* cells. *Eur. J. Cell Biol.* **79**, 642–652. (doi:10.1078/0171-9335-00087)
180. Rizzuto R *et al.* 2009 Ca^{2+} transfer from the ER to mitochondria: when, how and why. *Biochim. Biophys. Acta* **1787**, 1342–1351. (doi:10.1016/j.bbabio.2009.03.015)
181. Pinton P, Giorgi C, Siviero R, Zecchini E, Rizzuto R. 2008 Calcium and apoptosis: ER–mitochondria Ca^{2+} transfer in the control of apoptosis. *Oncogene* **27**, 6407–6418. (doi:10.1038/onc.2008.308)
182. Orrenius S, Nicotera P, Zhivotovsky B. 2011 Cell death mechanisms and their implications in toxicology. *Toxicol. Sci.* **119**, 3–19. (doi:10.1093/toxsci/kfq268)
183. Wang X. 2001 The expanding role of mitochondria in apoptosis. *Genes Dev.* **15**, 2922–2933.
184. Zmasek CM, Godzik A. 2013 Evolution of the animal apoptosis network. *Cold Spring Harb. Perspect. Biol.* **5**, a008649. (doi:10.1101/cshperspect.a008649)
185. Endoh H, Kobayashi T. 2006 Death harmony played by nucleus and mitochondria: nuclear apoptosis during conjugation of *Tetrahymena*. *Autophagy* **2**, 129–131. (doi:10.4161/auto.2.2.2368)
186. Rosenberg SH. 2011 Mammalian apoptosis in a parasitic worm. *Proc. Natl Acad. Sci. USA* **108**, 6695–6696. (doi:10.1073/pnas.1104151108)
187. Plattner H. 2015 Signalling in ciliates: long- and short-range signals and molecular determinants for cellular dynamics. *Biol. Rev. Camb. Philos. Soc.* (doi:10.1111/brv.12218)
188. Zimmermann H, Zebisch M, Strater N. 2012 Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signal.* **8**, 437–502. (doi:10.1007/s11302-012-9309-4).
189. Vivian JP *et al.* 2010 Crystal structure of a *Legionella pneumophila* ecto-triphosphate diphosphohydrolase, a structural and functional homolog of the eukaryotic NTPDases. *Structure* **18**, 228–238. (doi:10.1016/j.str.2009.11.014)
190. Verkhratsky A, Burnstock G. 2014 Biology of purinergic signalling: its ancient evolutionary roots, its omnipresence and its multiple functional significance. *Bioessays* **36**, 697–705. (doi:10.1002/bies.201400024)
191. Ivanova EP, Alexeeva YV, Pham DK, Wright JP, Nicolau DV. 2006 ATP level variations in heterotrophic bacteria during attachment on hydrophilic and hydrophobic surfaces. *Int. Microbiol.* **9**, 37–46.
192. Vylkova S, Sun JN, Edgerton M. 2007 The role of released ATP in killing *Candida albicans* and other extracellular microbial pathogens by cationic peptides. *Purinergic Signal.* **3**, 91–97. (doi:10.1007/s11302-006-9040-0)
193. Burnstock G, Verkhratsky A. 2012 *Purinergic signalling and the nervous system*. Heidelberg, Germany: Springer.
194. Choi SB, Kim JU, Joo H, Min CK. 2010 Identification and characterization of a novel bacterial ATP-sensitive K^{+} channel. *J. Microbiol.* **48**, 325–330. (doi:10.1007/s12275-010-9231-9)
195. Li M, Kim TJ, Kwon HJ, Suh JW. 2008 Effects of extracellular ATP on the physiology of *Streptomyces coelicolor* $A3_2$. *FEMS Microbiol. Lett.* **286**, 24–31. (doi:10.1111/j.1574-6968.2008.01248.x)
196. Naseem R, Wann KT, Holland IB, Campbell AK. 2009 ATP regulates calcium efflux and growth in *E. coli*.

J. Mol. Biol. **391**, 42–56. (doi:10.1016/j.jmb.2009.05.064)

197. Burnstock G, Verkhratsky A. 2009 Evolutionary origins of the purinergic signalling system. *Acta Physiol.* (*Oxf.*) **195**, 415–447. (doi:10.1111/j.1748-1716.2009.01957.x)
198. Roux SJ, Steinebrunner I. 2007 Extracellular ATP: an unexpected role as a signaler in plants. *Trends Plant Sci.* **12**, 522–527. (doi:10.1016/j.tplants.2007.09.003)
199. Galun R, Kabayo JP. 1988 Gorging response of *Glossina palpalis palpalis* to ATP analogues. *Physiol. Entomol.* **13**, 419–423. (doi:10.1111/j.1365-3032.1988.tb01125.x)
200. Zimmer-Faust RK. 1993 ATP: A potent prey attractant evoking carnivory. *Luminol. Oceonogr.* **38**, 1271–1275. (doi:10.4319/lo.1993.38.6.1271)
201. Backus KH, Braum S, Lohner F, Deitmer JW. 1994 Neuronal responses to purinoceptor agonists in the leech central nervous system. *J. Neurobiol.* **25**, 1283–1292. (doi:10.1002/neu.480251009)
202. Khakh BS, North RA. 2006 P2X receptors as cell-surface ATP sensors in health and disease. *Nature* **442**, 527–532. (doi:10.1038/nature04886)
203. North RA. 2002 Molecular physiology of P2X receptors. *Physiol. Rev.* **82**, 1013–1067. (doi:10.1152/physrev.00015.2002)
204. Ralevic V, Burnstock G. 1998 Receptors for purines and pyrimidines. *Pharmacol. Rev.* **50**, 413–492.
205. Thimm D, Knospe M, Abdelrahman A, Moutinho M, Alsdorf BB, von Kugelgen I, Schiedel AC, Muller CE. 2013 Characterization of new G protein-coupled adenine receptors in mouse and hamster. *Purinergic Signal.* **9**, 415–426. (doi:10.1007/s11302-013-9360-9)
206. Agboh KC, Webb TE, Evans RJ, Ennion SJ. 2004 Functional characterization of a P2X receptor from *Schistosoma mansoni*. *J. Biol. Chem.* **279**, 41 650–41 657. (doi:10.1074/jbc.M408203200)
207. Baines A, Parkinson K, Sim JA, Bragg L, Thompson CR, North RA. 2013 Functional properties of five *Dictyostelium discoideum* P2X receptors. *J. Biol. Chem.* **288**, 20 992–21 000. (doi:10.1074/jbc.M112.445346)
208. Bavan S, Straub VA, Blaxter ML, Ennion SJ. 2009 A P2X receptor from the tardigrade species *Hypsibius dujardini* with fast kinetics and sensitivity to zinc and copper. *BMC Evol. Biol.* **9**, 17. (doi:10.1186/1471-2148-9-17)
209. Fountain SJ. 2013 Primitive ATP-activated P2X receptors: discovery, function and pharmacology. *Front. Cell Neurosci.* **7**, 247. (doi:10.3389/fncel.2013.00247)
210. Fountain SJ, Cao L, Young MT, North RA. 2008 Permeation properties of a P2X receptor in the green algae *Ostreococcus tauri*. *J. Biol. Chem.* **283**, 15 122–15 126. (doi:10.1074/jbc.M801512200)
211. Meima M, Schaap P. 1999 *Dictyostelium* development-socializing through cAMP. *Semin. Cell Dev. Biol.* **10**, 567–576. (doi:10.1006/scdb.1999.0340)
212. Kukulski F, Levesque SA, Sevigny J. 2011 Impact of ectoenzymes on p2 and p1 receptor signaling. *Adv. Pharmacol.* **61**, 263–299. (doi:10.1016/B978-0-12-385526-8.00009-6)
213. Hoyle CH. 2011 Evolution of neuronal signalling: transmitters and receptors. *Auton. Neurosci.* **165**, 28–53. (doi:10.1016/j.autneu.2010.05.007)
214. Pankratov Y, Lalo U, Verkhratsky A, North RA. 2007 Quantal release of ATP in mouse cortex. *J. Gen. Physiol.* **129**, 257–265. (doi:10.1085/jgp.200609693)
215. Pankratov Y, Lalo U, Verkhratsky A, North RA. 2006 Vesicular release of ATP at central synapses. *Pflugers Arch.* **452**, 589–597. (doi:10.1007/s00424-006-0061-x)
216. Ma W, Compan V, Zheng W, Martin E, North RA, Verkhratsky A, Surprenant A. 2012 Pannexin 1 forms an anion-selective channel. *Pflugers Arch.* **463**, 585–592. (doi:10.1007/s00424-012-1077-z)
217. Fields RD. 2011 Nonsynaptic and nonvesicular ATP release from neurons and relevance to neuron–glia signaling. *Semin. Cell Dev. Biol.* **22**, 214–219. (doi:10.1016/j.semcdb.2011.02.009)
218. Sabirov RZ, Dutta AK, Okada Y. 2001 Volume-dependent ATP-conductive large-conductance anion channel as a pathway for swelling-induced ATP release. *J. Gen. Physiol.* **118**, 251–266. (doi:10.1085/jgp.118.3.251)
219. Samuels SE, Lipitz JB, Dahl G, Muller KJ. 2010 Neuroglial ATP release through innexin channels controls microglial cell movement to a nerve injury. *J. Gen. Physiol.* **136**, 425–442. (doi:10.1085/jgp.201010476)
220. Suadicani SO, Brosnan CF, Scemes E. 2006 $P2X_7$ receptors mediate ATP release and amplification of astrocytic intercellular Ca^{2+} signaling. *J. Neurosci.* **26**, 1378–1385. (doi:10.1523/JNEUROSCI.3902-05.2006)
221. Sauvage V, Aubert D, Escotte-Binet S, Villena I. 2009 The role of ATP-binding cassette (ABC) proteins in protozoan parasites. *Mol. Biochem. Parasitol.* **167**, 81–94. (doi:10.1016/j.molbiopara.2009.05.005)
222. Sawada K, Echigo N, Juge N, Miyaji T, Otsuka M, Omote H, Yamamoto A, Moriyama Y. 2008 Identification of a vesicular nucleotide transporter. *Proc. Natl Acad. Sci. USA* **105**, 5683–5686. (doi:10.1073/pnas.0800141105)
223. Sreedharan S, Shaik JH, Olszewski PK, Levine AS, Schioth HB, Fredriksson R. 2010 Glutamate, aspartate and nucleotide transporters in the SLC17 family form four main phylogenetic clusters: evolution and tissue expression. *BMC Genomics* **11**, 17. (doi:10.1186/1471-2164-11-17)
224. Pankratov Y, Lalo U, Krishtal OA, Verkhratsky A. 2009 P2X receptors and synaptic plasticity. *Neuroscience* **158**, 137–148. (doi:10.1016/j.neuroscience.2008.03.076)
225. Lalo U, Pankratov Y, Parpura V, Verkhratsky A. 2011 Ionotropic receptors in neuronal–astroglial signalling: what is the role of 'excitable' molecules in non-excitable cells? *Biochim. Biophys. Acta* **1813**, 992–1002. (doi:10.1016/j.bbamcr.2010.09.007)
226. Egan TM, Samways DS, Li Z. 2006 Biophysics of P2X receptors. *Pflugers Arch.* **452**, 501–512. (doi:10.1007/s00424-006-0078-1)
227. Egan TM, Khakh BS. 2004 Contribution of calcium ions to P2X channel responses. *J. Neurosci.* **24**, 3413–3420. (doi:10.1523/JNEUROSCI.5429-03.2004)
228. Palygin O, Lalo U, Verkhratsky A, Pankratov Y. 2010 Ionotropic NMDA and $P2X_{1/5}$ receptors mediate synaptically induced Ca^{2+} signalling in cortical astrocytes. *Cell Calcium* **48**, 225–231. (doi:10.1016/j.ceca.2010.09.004)
229. White SM, Imig JD, Kim TT, Hauschild BC, Inscho EW. 2001 Calcium signaling pathways utilized by P2X receptors in freshly isolated preglomerular MVSMC. *Am. J. Physiol. Renal Physiol.* **280**, F1054–F1061.
230. Pubill D, Dayanithi G, Siatka C, Andres M, Dufour MN, Guillon G, Mendre C. 2001 ATP induces intracellular calcium increases and actin cytoskeleton disaggregation via P2x receptors. *Cell Calcium* **29**, 299–309. (doi:10.1054/ceca.2000.0194)
231. Zhao X, Falck JR, Gopal VR, Inscho EW, Imig JD. 2004 P2X receptor-stimulated calcium responses in preglomerular vascular smooth muscle cells involves 20-hydroxyeicosatetraenoic acid. *J. Pharmacol. Exp. Ther.* **311**, 1211–1217. (doi:10.1124/jpet.104.070797)
232. Surprenant A, North RA. 2009 Signaling at purinergic P2X receptors. *Annu. Rev. Physiol.* **71**, 333–359. (doi:10.1146/annurev.physiol.70.113006.100630)
233. Sivaramakrishnan V, Fountain SJ. 2013 Intracellular P2X receptors as novel calcium release channels and modulators of osmoregulation in *Dictyostelium*: a comparison of two common laboratory strains. *Channels* (*Austin*) **7**, 43–46. (doi:10.4161/chan.22737)
234. Burnstock G. 2015 Intracellular expression of purinoceptors. *Purinergic Signal.* **11**, 275–276. (doi:10.1007/s11302-015-9455-6)
235. Burnstock G, Knight GE. 2004 Cellular distribution and functions of P2 receptor subtypes in different systems. *Int. Rev. Cytol.* **240**, 31–304. (doi:10.1016/S0074-7696(04)40002-3)
236. Burnstock G, Boeynaems JM. 2014 Purinergic signalling and immune cells. *Purinergic Signal.* **10**, 529–564. (doi:10.1007/s11302-014-9427-2)
237. Verkhratsky A, Krishtal OA, Burnstock G. 2009 Purinoceptors on neuroglia. *Mol. Neurobiol.* **39**, 190–208. (doi:10.1007/s12035-009-8063-2)
238. Burnstock G, Evans LC, Bailey MA. 2014 Purinergic signalling in the kidney in health and disease. *Purinergic Signal.* **10**, 71–101. (doi:10.1007/s11302-013-9400-5)
239. Burnstock G. 2014 Purinergic signalling in the urinary tract in health and disease. *Purinergic Signal.* **10**, 103–155. (doi:10.1007/s11302-013-9395-y)
240. Burnstock G, Verkhratsky A. 2010 Long-term (trophic) purinergic signalling: purinoceptors control cell proliferation, differentiation and death. *Cell Death Dis.* **1**, e9. (doi:10.1038/cddis.2009.11)

241. Burnstock G. 2014 Purinergic signalling in endocrine organs. *Purinergic Signal.* **10**, 189–231. (doi:10.1007/s11302-013-9396-x)
242. Abbracchio MP *et al.* 2003 Characterization of the UDP-glucose receptor (re-named here the $P2Y_{14}$ receptor) adds diversity to the P2Y receptor family. *Trends Pharmacol. Sci.* **24**, 52–55. (doi:10.1016/S0165-6147(02)00038-X)
243. Abbracchio MP *et al.* 2006 International union of pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol. Rev.* **58**, 281–341. (doi:10.1124/pr.58.3.3)
244. Fredholm BB, AP IJ, Jacobson KA, Klotz KN, Linden J. 2001 International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* **53**, 527–552.
245. Burnstock G, Fredholm BB, Verkhratsky A. 2011 Adenosine and ATP receptors in the brain. *Curr. Top. Med. Chem.* **11**, 973–1011. (doi:10.2174/156802611795347627)
246. Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. 2000 Structure and function of adenosine receptors and their genes. *Naunyn Schmiedebergs Arch. Pharmacol.* **362**, 364–374. (doi:10.1007/s002100000313)