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Review

The ancient roots of calcium signalling evolutionary tree

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ABSTRACT

Molecular cascades of calcium homeostasis and signalling (Ca^{2+} pumps, channels, cation exchangers, and Ca^{2+}-binding proteins) emerged in prokaryotes and further developed at the unicellular stage of eukaryote evolution. With progressive evolution, mechanisms of signalling became diversified reflecting multiplication and specialisation of Ca^{2+}-regulated cellular activities. Recent genomic analysis of organisms from different systematic positions, combined with proteomic and functional probing invigorated expansion in our understanding of the evolution of Ca^{2+} signalling. Particularly impressive is the consistent role of Ca^{2+}-ATPases/pumps, calmodulin and calcineurin from very early stages of eukaryotic evolution, although with interspecies differences. Deviations in Ca^{2+} handling and signalling are observed between vertebrates and flowering plants as well as between protists at the basis of the two systematic categories, Unikonta (for example choanoflagellates) and Bikonta (for example ciliates). Only the B-subunit of calcineurin, for instance, is maintained to regulate highly diversified protein kinases for stress defence in flowering plants, whereas the complete dimeric protein, in vertebrates up to humans, regulates gene transcription, immune-defence and plasticity of the brain. Calmodulin is similarly maintained throughout evolution, but in plants a calmoldulin-like domain is integrated into protein kinase molecules. The eukaryotic cell has inherited and invented many mechanisms to exploit the advantages of signalling by Ca^{2+}, and there is considerable overall similarity in basic processes of Ca^{2+} regulation and signalling during evolution, although some details may vary.

1. Bacterial inheritance: Ca^{2+} regulation and primaeval Ca^{2+} signalling

Early life may have emerged in the ocean or in local parts of it under alkaline conditions that favoured relatively low (in a 100 s nM range) Ca^{2+} concentrations ([1,2] this special issue). At this early stage Ca^{2+} permeation into ancestral cells, Ca^{2+} handling and Ca^{2+} influence on energetic (and in particular the requirement of low free Ca^{2+} for ATP metabolism [3]) made Ca^{2+} ions critical for life and for signalling processes.

Bacteria maintain Ca^{2+} homeostasis [4,5] although resting levels of free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) are somewhat higher than in eukaryotes [6] and although specific mechanisms employed by different bacterial species are yet to be characterised in detail. Some bacteria express primary and secondary active transporters including P-type Ca^{2+}-transport ATPases of which several resemble the Sarcoplasmic and Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) of eukaryotes [7,8]. These pumps, together with mechanosensitive channels [9], Ca^{2+}-activated channels [10], cation exchangers, such as Ca^{2+}/H^+ and Ca^{2+}/Na^+ exchangers, an array of Ca^{2+}-binding proteins (CaBP), and a battery of Ca^{2+}-activated enzymes, which all are present in bacteria ([11] this special issue) formed the primordial Ca^{2+} homeostatic and Ca^{2+} signalling system. In bacteria which live today, changes in $[Ca^{2+}]_i$, regulate numerous functions such as, for example, chemotaxis [6,12]. Calmodulin-like proteins are found in the genome of certain Gram-positive bacteria [13–15], in addition to other CaBPs [16]. However, fast Ca^{2+} sensors with C2 domains (such as for example synaptotagmins [17]) have not been reported.

Considering the frequent occurrence of gene transfer between ancestral organisms, the early period of evolution of molecular cascades responsible for control over cellular Ca^{2+} remains rather vague and speculative. Even genuine Ca^{2+} signalling in bacteria has been debated [10]. Apart from these restrictions it appears that

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bacteria prophesied several important mechanisms that have been advanced and refined throughout the evolutionary ladder.

2. From bacteria to the eukaryote cell: requirement of Ca^{2+} for trafficking

There is considerable uncertainty about the origin of the eukaryotic cell from archaebacteria or eubacteria [18], with different scenarios being proposed [19,20]. Even the age of eukaryotes is disputed; the classical (and prevailing) view of their emergence (as witnessed by fossils) ~2 billion years ago [21–24], is not universally acknowledged with the data on the presence of eukaryotic markers in much older (~3 to ~3.5 billion years) fossils [25,26]. Based on the analysis of eukaryotic signature proteins, the emergence of a "chronocyte", the intermediate distinct from Archaea and eubacteria has been contemplated [27]. The textbook view highlights an archaebacterial ancestor whose genome has been sequestered by another cell through invagination of the cell membrane. Integration of an archaebacterium into an eubacterium is another hypothetic scenario. The Ca^{2+} regulating and Ca^{2+} regulated proteins outlined above are essentially known from eubacteria, whereas important proteins of the nucleus have orthologues in some archaebacteria [28].

By infolding of the cell membrane with ribosomes attached, the Endoplasmic Reticulum (ER) could have formed, followed by controlled blebbing and fusion of vesicular compartments, also a Ca^{2+}-dependent process, wherever it has been accessible to analysis. This must have been prerequisite to any further differentiation and trafficking. This capability, together with a cytoskeleton, has been ascribed to the chronocyte [27]. In contrast, a LAECA-(latest archaeal-eukaryote common ancestor) type organisms, endowed with high internal complexity, has been postulated to precede genuine eukaryotes [29]. Endocytosis and intracellular digestion are thought to have become important for further complexity of the LAECA-type ancestor, and the early eukaryotic cell [30,31]. The acquisition of endomembranes and intracellular compartments that could emerge in some prokaryotes was certainly associated with the transition from prokaryotes to eukaryotes and prompted new developments in Ca^{2+} signalling.

Regulation of intracellular trafficking become the special function of Ca^{2+}, which generally assumes a key role in membrane-membrane interactions, and hence complex $[Ca^{2+}]_i$ dynamics, regulated in space and time, provided a canvass for ubiquitous and versatile Ca^{2+} signalling. Ca^{2+} has outstanding properties that make it an almost ideal second messenger [32]. Since too high $[Ca^{2+}]_i$ is toxic, strict regulation and "taming" of Ca^{2+} movements is required, which have made it a molecule suitable for signalling at a low additional energy costs. The human body contains up to 1.4–2 kg of Ca^{2+} (of which 99% is present in the form of insoluble phosphates accumulated in bones). Concentration of Ca^{2+} is ~10 mM in the ocean; total Ca^{2+} concentration reaches 25 mM in plants; in mammalian cells, total Ca^{2+} concentration in the cytoplasm (free and bound) is in the millimolar range [32], whereas free concentrations of $[Ca^{2+}]_i$ are, as a rule, below ~0.1 micromolar in the resting cell [33]. The existence of a continuous concentration gradient aimed at the cytosol allows $[Ca^{2+}]_i$ to be rapidly and locally increased for signalling at defined sites, which rise is followed by reversible binding to CaBPs. These latter proteins are generally characterised by rapid binding kinetics and widely different affinity (expressed as a binding constant, K_D). High capacity/low affinity binding makes some CaBPs suitable for Ca^{2+} binding inside the organelles (universally known as dynamic Ca^{2+} stores) and for signal inactivation in the cytosol, whereas rapid activation of dynamic processes is a responsibility of low capacity/high affinity binding with a variety of membrane-bound and cytosolic CaBPs. Local regulation of Ca^{2+} controls selective and spatially restricted specific processes [33], avoids toxicity and keeps energetic costs for re-establishing homeostasis low. For the latter purpose, some high capacity/low affinity CaBPs are also present in the cytosol [34].

3. An evolutionary time scale and diversification of Ca^{2+} signalling

Ancestral eukaryotes diversified into two main branches, Unikonta (that eventually evolved into vertebrates) and Bikonta (that are at the root of angiosperms, or flowering plants [35,36]). The founders of these branches are two unicellular groups whose current main representatives date back to ~760–960 million of years (choanoflagellates [37]) and ~800 million of years (ciliates [23]). Choanoflagellates are considered as the founding group of metazoa and, therefore, deserve special interest with regard to Ca^{2+} signalling (Cai et al. [48], this special issue). Myxamoebae (*Dictyostelium*) are another well analysed unikont; their phylogenetic age is somewhat ambiguous, although they can be younger than the other groups [38]. Mammals are more than 200 million of years old being therefore older than flowering plants that are believed to emerge 130–190 million of years ago [23,39].

Molecules participating in trafficking have greatly diversified during evolution, which could be extrapolated from comparative genomic studies. There are about 20 SNAREs (soluble N-ethyl maleimide sensitive attachment protein receptors) in the Ur-eukaryote, whereas they are about twice as many in mammals [40]. The number of Rab-type GTPases increased from an estimated 20 in ancestral eukaryote [41] to 163 in human [42]. Insights from cells living today suggests that already in early times Ca^{2+} must have been "hired" for signalling purposes. Considering rapid diffusion, binding and deactivation of Ca^{2+}, increasingly elaborate intracellular trafficking required a strict localisaton of Ca^{2+} signals. This in turn requires Ca^{2+} stores, with high capacity/low affinity CaBPs in their lumen, and mechanisms for Ca^{2+} uptake and local release [43,44]. All these components, including primary and secondary active Ca^{2+} transport mechanisms and Ca^{2+} release channels (CRC) are abundant in protozoa in one or the other form, as found in *Paramecium* [45,46], and to some extent in *Dictyostelium* [47] and in choanoflagellates ([48], this special issue). The first two genera represent the major phylogenetic lines and are frequently used for studies in cell biology, whereas choanoflagellates are currently only analysed by molecular biology, although with important predictions. The increasing importance of Ca^{2+} during evolution is highlighted by an increase in the number of CaBPs, which rises from ~70 in bacteria to 3640 in mammals [16,49]. Substantial increase in numbers and diversity of CaBP in eukaryotes reflects a rising capability of fine tuning Ca^{2+} signals [50]. A rather different way of diversification of Ca^{2+} signalling, however, is observed in plants ([51–53] in this special issue).

It is now generally acknowledged that evolutionary improvement of cell energetic is associated with endocytosis and domestication of eubacteria with respiratory activity, that become mitochondria, about 1.5 billion years ago [54]. Considering the high proportion of energy investment in ionic balance in modern eukaryotes, one may assume that acquisition of mitochondrial precursors was an important step in advancement of Ca^{2+} signalling ([2], this special issue). The uptake of Ca^{2+} by mitochondria, achieved in modern eukaryotes by an uniporter [55] is swift and it stimulates ATP production by activating dehydrogenases in the mitochondrial matrix [56]. A homologue of Ca^{2+} uniporter is present already in bacteria [57], and in choanoflagellates ([48], this special issue), while a mitochondrial calcium uniporter (MCU) is conserved from protozoa to human, no MCU homologues, however, were found in various parasitic protozoa [57,58]. The essential