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Kai H. Edel, Elodie Marchadier, Colin Brownlee, Joerg Kudla, Alistair M. Hetherington

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The Evolution of Calcium-Based Signalling in Plants

Kai H. Edel[1], Elodie Marchadier[2,3], Colin Brownlee[4,5], Jörg Kudla[1], and Alistair M. Hetherington[2,*]
[1]Institut für Biologie und Biotechnologie der Pflanzen, Universität Münster, Schlossplatz 7, 48149 Münster, Germany
[2]School of Biological Sciences, Life Sciences Building, University of Bristol, Tyndall Avenue, Bristol BS8 1TQ, UK
[3]Génétique Quantitative et Evolution - Le Moulon, INRA, Univ. Paris-Sud, CNRS, AgroParisTech, Université Paris-Saclay, 91190 Gif-sur-Yvette, France
[4]Marine Biological Association of the UK, The Laboratory, Citadel Hill, Plymouth PL1 2PB, UK
[5]School of Ocean and Earth Sciences, University of Southampton, Southampton, SO14 3ZH, UK
*Correspondence: Alistair.Hetherington@bristol.ac.uk
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The calcium-based intracellular signalling system is used ubiquitously to couple extracellular stimuli to their characteristic intracellular responses. It is becoming clear from genomic and physiological investigations that while the basic elements in the toolkit are common between plants and animals, evolution has acted in such a way that, in plants, some components have diversified with respect to their animal counterparts, while others have either been lost or have never evolved in the plant lineages. In comparison with animals, in plants there appears to have been a loss of diversity in calcium-influx mechanisms at the plasma membrane. However, the evolution of the calcium-storing vacuole may provide plants with additional possibilities for regulating calcium influx into the cytosol. Among the proteins that are involved in sensing and responding to increases in calcium, plants possess specific decoder proteins that are absent from the animal lineage. In seeking to understand the selection pressures that shaped the plant calcium-signalling toolkit, we consider the evolution of fast electrical signalling. We also note that, in contrast to animals, plants apparently do not make extensive use of cyclic-nucleotide-based signalling. It is possible that reliance on a single intracellular second-messenger-based system, coupled with the requirement to adapt to changing environmental conditions, has helped to define the diversity of components found in the extant plant calcium-signalling toolkit.

Introduction

The calcium ion (Ca^{2+}) is a ubiquitous intracellular second messenger used extensively in plants, animals and microorganisms to couple extracellular stimuli to their characteristic intracellular responses and to coordinate a wide range of endogenous processes. Over 15 years ago, Berridge and coworkers proposed a framework, known as the 'Ca^{2+} signalling toolkit', around which to build our current understanding of the operation of Ca^{2+}-based signalling [1,2]. Central to the operation of Ca^{2+}-based signalling, in any organism, is the concentration of free Ca^{2+} in the cytosol ($[Ca^{2+}]_{cyt}$). In the unstimulated, or resting cell, bulk $[Ca^{2+}]_{cyt}$ is in the region of 10^{-7} M. However, upon stimulation, this increases approximately 10-fold to reach low-μM levels [3]. Because $[Ca^{2+}]_{cyt}$ is low relative to the extracellular fluid, or intracellular compartments (such as the endoplasmic reticulum or plant vacuole), generating an increase in $[Ca^{2+}]_{cyt}$ can be achieved by allowing the controlled entry of Ca^{2+} into the cytosol from these locations. The key here is that entry into the cytosol needs to be tightly regulated because above 10^{-4} M sustained increases in $[Ca^{2+}]_{cyt}$ are cytotoxic. Having said this, highly localised microdomain $[Ca^{2+}]_{cyt}$ is well tolerated and is an important effector. The collective mechanisms responsible for generating the increase in $[Ca^{2+}]_{cyt}$ have been termed the 'on mechanisms' [2]. To operate as an effective signalling system, there is a requirement to return $[Ca^{2+}]_{cyt}$ to its pre-stimulus levels. This is achieved by the so-called 'off mechanisms' [2], which encompass a diverse suite of membrane proteins that move Ca^{2+}, against a concentration gradient, into intracellular stores, such as the vacuole, or expel it from the cell. The net result of the off and on mechanisms is to form or shape the Ca^{2+} signature in the sense that they define its spatial and temporal characteristics [4]. In the context of plant calcium signalling evolution, it is important to note that the plant vacuole deserves special attention. Indeed, it has been proposed that, by making use of the large Ca^{2+}-storing vacuole, plants have to deal with two extracytoplasmic compartments — the vacuole and the apoplast [5]. This opens the possibility for a second suite of Ca^{2+}-signalling components that act independently or in conjunction with the cytosolic toolkit to facilitate plant Ca^{2+} signalling.

Cells contain a suite of proteins whose Ca^{2+}-binding properties allow them to respond to stimulus-induced increases in $[Ca^{2+}]_{cyt}$. Typically, this involves an alteration in protein conformation. If the protein is an enzyme, this is likely to be reflected in an alteration in activity. Ca^{2+}-induced changes in conformation may also allow the Ca^{2+}-binding proteins to interact with other targets, or in the case of cytoskeletal proteins, allow them to perform work. The overall role of these proteins is to decode and respond to the stimulus-induced increases in $[Ca^{2+}]_{cyt}$. The former of these roles should not be overlooked, because it has a bearing on the question of how specificity is encoded in Ca^{2+}-based signalling systems. It is in the context of this role that, in plants, the spatio-temporal pattern of stimulus-induced increases in $[Ca^{2+}]_{cyt}$ has been referred to as the 'Ca^{2+} signature' [4]. In plants, it has been proposed that specificity in Ca^{2+}-based signalling is achieved through the interplay of Ca^{2+} signatures with cognate Ca^{2+}-binding proteins that act to decode or interpret these increases [4,6–11]. The complement of (mostly) proteins that comprise the on mechanisms, the off mechanisms and the Ca^{2+}-responsive proteins that interpret

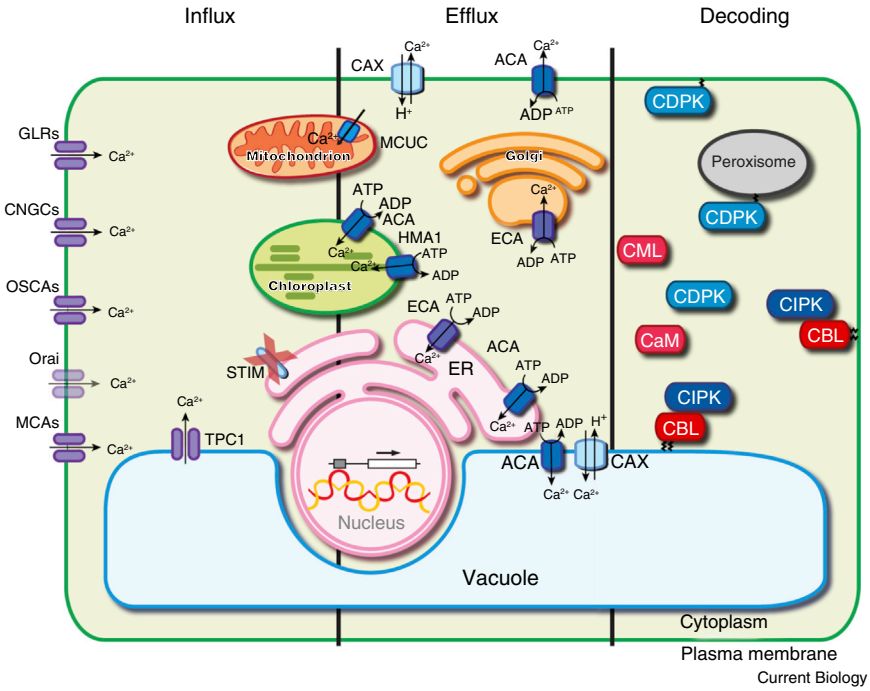

Figure 1. The plant Ca^{2+}-signalling toolkit.
Three major elements influence the generation and translation of a stimulus-induced Ca^{2+} signal: influx, efflux and decoding. Ca^{2+} influx is mediated by Ca^{2+} channels, namely cyclic nucleotide-gated channels (CNGCs), glutamate receptor-like channels (GLRs), two-pore channels (TPCs), mechanosensitive channels (MCAs), reduced hyperosmolality-induced Ca^{2+} increase channels (OSCAs) and potentially by Orai channels (at least in plants outside of the angiosperm group). To shape a Ca^{2+} influx into an informative signature, plants employ Ca^{2+}-efflux systems: autoinhibited Ca^{2+}-ATPases (ACAs), ER-type Ca^{2+}-ATPases (ECAs), P1-ATPases (HMA1), mitochondrial calcium uniporter complex (MCUC) and Ca^{2+} exchangers (CAX). The decoding is brought about by many different protein families (at least 250 proteins encoded in the *Arabidopsis* genome harbour EF-hands). Here, we represent the three major groups, consisting of calcium-dependent protein kinases (CDPKs), calcineurin B-like (CBL) protein kinases (CIPKs) as well as calmodulin (CaM) and CaM-like proteins (CMLs). See text for details.

(decode) the Ca^{2+} signal form the basic family of tools within the plant Ca^{2+} signalling toolkit (Figure 1).

Ca^{2+}-based signalling in plants has been the subject of several authoritative recent reviews, and the reader is directed towards these for more information [8–10,12,13]. However, in contrast to the situation in metazoan (including animal) cells [14–20], the evolution of the Ca^{2+}-based signalling system in plants has received rather less attention [21–26].

In this Review, we will discuss the evolution of plant Ca^{2+} signalling. What is clear from genomic and physiological investigations is that while the basic elements in the Ca^{2+} toolkit are common between plants and animals, evolution has acted in such a way that, in plants, some components have diversified with respect to their animal counterparts, while others have either been lost or have never evolved in the plant lineages. This is likely to have fundamental consequences for how Ca^{2+}-signalling modules are composed, and especially how these are integrated with other cellular signalling systems. In this Review, our objectives are to use insights from genomic datasets to highlight differences between the animal and plant Ca^{2+}-signalling toolkits. Rapid 'action-potential'-based electrical signalling is a phenomenon central to the evolution and success of animals, and yet this was largely lost during plant evolution. We will describe how electrical, Ca^{2+}-based signalling evolved separately in plants. Finally, we will take the first steps towards identifying the nature of the selective pressures, operating over the evolutionary timescale, which have helped to dictate the complement of proteins present in the plant Ca^{2+} toolkit.

Overview of the Evolution of Ca^{2+}-based Signalling in Plants and Animals

Ca^{2+}-based signalling was present at the unikont–bikont split, as it is used in prokaryotes and represents a common feature of life [27]. A recent study investigated the evolution of Ca^{2+} signaling based on conserved structural protein domains. Combinations of these domains form protein architectures that are characteristic of protein function. Using this approach, Marchadier *et al.* (2016) reported that the last eukaryote common ancestor (LECA) was potentially able to generate and decode Ca^{2+} signatures [28]. They also concluded that evolution of the proteins in the Ca^{2+}-signalling toolkit was radically different from other proteins. Intra-genome diversity of Ca^{2+} toolkit components increased at a far greater rate than other proteins. Moreover, in comparison with other proteins, the Ca^{2+} toolkit components were markedly less duplicated [28].

Comparing inter-genome Ca^{2+} signalling evolution in different eukaryote lineages highlights a progressive growth of the Ca^{2+}-signalling toolkit from that present in LECA. Overlaid on this pattern, there are also lineage-specific evolution profiles whose diversity increases with organismal complexity. Looking at the Ca^{2+} toolkit as a whole, the strongest difference between lineages is observed between animals and plants, while flowering and lower plants present similar evolutionary profiles. The overall trend is an increase in the diversity of Ca^{2+}-binding protein architecture in animals, with a lower diversity in plants, and even lower in the algae. However, when this is looked at in greater detail, it becomes apparent that, within Ca^{2+}-binding proteins, protein architectures associated with Ca^{2+} influx and Ca^{2+} decoding have expanded more in plants than in animals [24,28]. This suggests that evolutionary pressures, operating in plants, have resulted in the differential expansion of these components of the Ca^{2+}-signalling toolkit.

The detailed phylogenetic analysis by Marchadier *et al.* also suggests that animals differentially lost proteins specialized for Ca^{2+} efflux during evolution, while in plants influx proteins have been predominantly lost [21,22,24,28]. We will return to the

possible significance of this observation later. Interestingly, the algae present a different case with an increase in the proteins involved in Ca^{2+} efflux observed during evolution [28]. However, they did not expand the repertoire of Ca^{2+}-binding proteins potentially capable of responding to or decoding Ca^{2+} signals. In contrast, the development of decoding mechanisms appears to have been favoured very early during plant evolution. Surprisingly, a parallel loss of decoding mechanisms is observed in animals [28]. Having pointed to the trends in the evolution of Ca^{2+} signalling across multiple lineages and highlighted similarities and areas of divergence, we will now concentrate on the evolution of Ca^{2+} signalling in plants. Our emphasis will be to concentrate on elements of the Ca^{2+}-signalling toolkit, where the most significant differences between animals and plants are apparent. In particular, we will focus on the loss of Ca^{2+}-influx systems and the diversification of proteins responsible for decoding Ca^{2+} signatures in plants.

Ca^{2+} Influx — Diversity Lost along the Way

Based on the evidence currently available, comparisons among animals, algae and plants suggest that during evolution, plants show a trend towards reduced diversity of mechanisms responsible for Ca^{2+} influx [21,22,24,28]. There is, of course, an important caveat associated with this statement, which is that there could be additional Ca^{2+}-influx mechanisms and components still to be discovered in plants. However, on the basis of what we currently know, it is apparent that this general loss is also associated with amplification of a limited array of specific mechanisms, including gene families for cyclic nucleotide gated channels (CNGCs), glutamate receptors (GLRs) and reduced hyperosmolality-induced $[Ca^{2+}]_{cyt}$ increase (OSCAs) channels. The genome of *Arabidopsis thaliana* and of other sequenced higher plants do not contain genes for the homologues of 4-domain voltage-dependent cation channels (VDCCs), inositol trisphosphate receptors (IP_3Rs), ATP-gated purinergic channels (P2XRs), the cys-loop superfamily of ligand-gated ion channels (Cys-loops) or transient receptor potential channels (TRPs) (though the plant 2-pore domain channel TPC1 has sequence homology with the 4-domain VDCCs) [29]. However, most of these Ca^{2+}-permeable channels are present in chlorophyte algae, indicating that they were part of the last common ancestor of chlorophytes and streptophytes (within which the evolution of land plants initiated), and were subsequently lost in plant evolution. Quite remarkably, most of these channels, with the exception of the VDCCs, were already lost in the charophyte *Klebsormidium flaccidum*, an algal lineage that together with the embryophytes (which includes all land plants) forms the streptophytes [24]. The charophytes, as a sister lineage to all land plants, are understood as the last evolutionary step towards land colonization, and therefore already harbour many important characteristics of land plants [24]. A more detailed analysis including more green algal species would be needed to link these losses of animal-like Ca^{2+} channels to specific evolutionary events. Nevertheless, the early loss of these channels indicates that it did not coincide with the colonization of the terrestrial environment since it occurred in the charophytes well before the move to land took place.

In terms of Ca^{2+} influx, so far, five protein families have been shown to transport Ca^{2+}in land plants, namely: CNGCs (20 genes in the *Arabidopsis* genome) [30], GLRs (20 genes in *Arabidopsis*) [31], two-pore channels (TPCs; one gene in *Arabidopsis*) [29], mechanosensitive channels (MCAs; two genes in *Arabidopsis*) [32], and the most recently identified (OSCAs;15 in *Arabidopsis*) [33]. OSCAs were identified as an important component of the early osmotic response in *Arabidopsis,* and are a well-conserved family of channel proteins present in all eukaryotes that have been analysed [24]. However, it is notable that in plants they have particularly diversified. Phylogenetic analyses of fully sequenced genomes revealed four major OSCA clades (I–IV), of which only clade IV is present in genomes outside of the plant kingdom. So far, two OSCA genes (OSCA1.1 and OCAS1.2) have been characterized and their potential to transport Ca^{2+} proven experimentally [33,34]. Further research and higher-order mutants are needed to elucidate the functions of additional members of the OSCA family.

In animals, the immune response represents a well-characterized Ca^{2+}-based signalling machinery involving a drop in ER Ca^{2+} content, which triggers sensor-dependent opening of plasma membrane Ca^{2+} channels [35]. In molecular terms, this involves the stromal interaction molecules (STIMs; which sense ER Ca^{2+} concentration using EF-hands) and the pore-forming Orai proteins (which form the hexameric Ca^{2+} release-activated Ca^{2+} (CRAC) channel) [36–39]. Activation of CRAC leads to the prolonged low capacity, high selectivity Ca^{2+} influx associated with the immune response [40]. Attempts to identify Orai sequences in Viridiplantae genomes have failed [41]. A recent study identified one Orai sequence in the genomes of the green alga *Chlamydomonas reinhardtii* and the moss *Physcomitrella patens*, showing the preservation of many important sequence features [42]. However, the activating ER-localized STIMs appear to be missing in both species. This might suggest that STIMs have been lost in these species, and that there may be an alternative role for the Orai proteins.

We extended the search for Orai and STIM sequences to additional plant species that represent informative evolutionary snapshots in the plant tree (Figure 2). Our analysis indicates that Orai genes are indeed absent in angiosperms, but are still present in most species up to gymnosperms. Additionally, we confirmed that STIM proteins are absent from the green lineage, indicating that the functional regulation of Bikonta Orai proteins may be independent of ER Ca^{2+} concentration, and their role thereby likely different [42]. Additionally, Orai but not STIM sequences were identified in the Heterokonta phylum (here represented by different oomycete species). Heterokonta and Archaeplastida are both part of the Bikonta supergroup, and are believed to have a common ancestor. This is interesting in the context of previous studies that have already established commonalities in terms of Ca^{2+} signalling between plants and oomycetes [43]. Moreover, lack of STIM proteins in all analysed Bikonta species supports the hypothesis that STIM evolved only in the Unikonta supergroup, and that the regulation of the Orai Ca^{2+} channels by STIMs is a secondary feature [42]. This raises questions concerning the ancient function and regulation of Orai proteins. These questions are particularly interesting in the context of plants, where the presence of Orai proteins without STIM represents the usual situation.

Animal CRAC channels show a remarkably high Ca^{2+} selectivity that is mainly brought about by a selectivity ‘ring’ in the

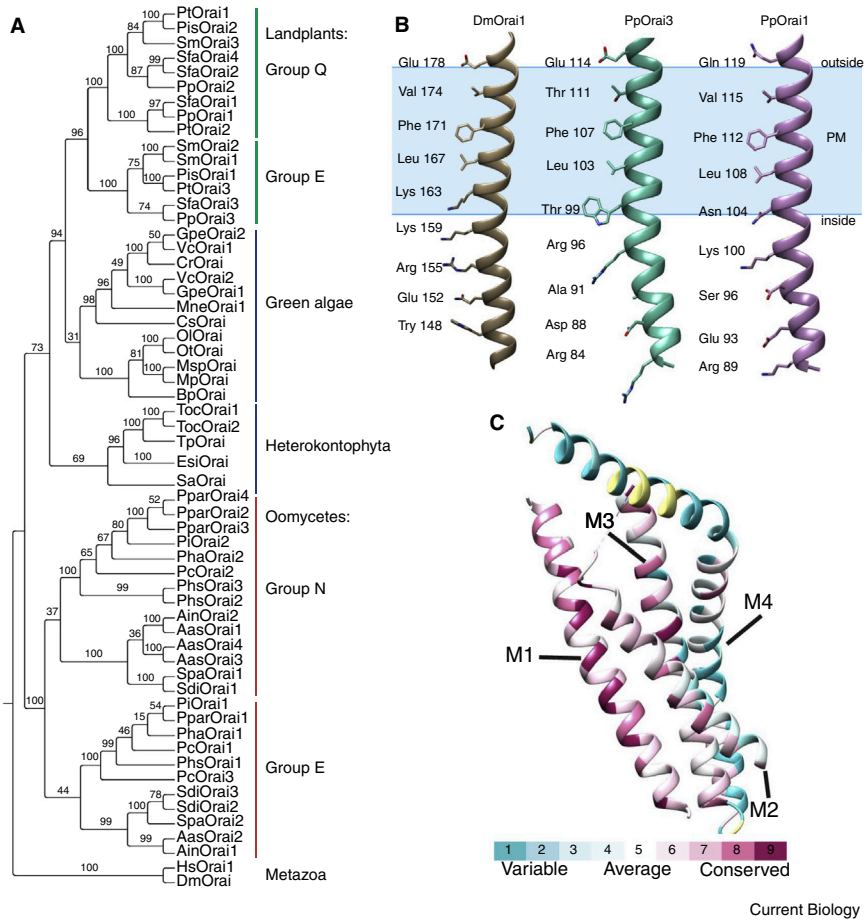

Figure 2. Phylogeny and structure of Bikonta Orai proteins.

(A) Maximum likelihood tree of Orai amino acid sequences. Numbers on branches indicate bootstrap probabilities. Multiple-sequence alignment was performed with MaffT (version. 7), maximum likelihood analyses were conducted with RAxML (version 8.2.9) and GTR amino acid substitution matrix under default parameters. Ain: *Aphanomyces invadans*; Aas: *Aphanomyces astaci*; Bp: *Bathycoccus prasinos*; Cr: *Chlamydomonas reinhardtii*; Cs: *Coccomyxa subellipsoidea*; Dm: *Drosophila melanogaster;* Esi: *Ectocarpus siliculosus*; Gpe: *Gonium pectoral*; Hs: *Homo sapiens*; Mne: *Monoraphidium neglectum*; Mp: *Micromonas pusilla*; Msp: *Micromonas* sp. RCC299; Ot*: Ostreococcus tauri*; Ol: *Ostreococcus lucimarinus*; Pis: *Picea sitchensis*; Pp: *Physcomitrella patens*; Pha: *Phytophthora alni*; Pc: *Phytophthora capsici*; Pi: *Phytophthora infestans*; Phs: *Phytophthora sojae*; Ppar: *Phytophthora parasitica*; Pt: *Pinus taeda*; Sm: *Selaginella moellendorffii*; Sa: *Schizochytrium aggregatum*; Sfa: *Sphagnum fallax*; Sdi: *Saprolegnia diclina*; Spa: *Saprolegnia parasitica*; Tp: *Thalassiosira pseudonana*; Toc: *Thalassiosira oceanica*; Vc: *Volvox carteri*.

(B) Comparison of the M1 helix from *D. melanogaster* Orai1 and *P. patens* Orai1 and Orai3. Blue indicates lipid membrane. Homology modelling was conducted with Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2) and visualized using Chimera (version 1.10).

(C) Overall sequence homology among all identified Orai sequences calculated using Consurf [45] and plotted on the DmOrai1 crystal structure (chain A - 4hkr - http://www.ebi.ac.uk/pdbe) in Chimera (version 1.10).

pore opening [36,44]. This ring is formed by six glutamic acids (E). Phylogenetic combined with structural analyses indicate that the E at the extracellular site of the first α-helix represents the primordial state. Interestingly, land plant Orai genes have evolved into two phylogenetically distinct groups of which one still harbours the E at the homologous site. The other group evolved a glutamine (Q) at this site, thereby potentially changing the charge at the pore selectivity filter (Figure 2A). Moreover, a similar trend is visible within the oomycete Orai proteins, where two distinct groups are present, one harbouring the preserved E residue, whereas the other group evolved an asparagine (N), again changing the charge of the pore. It would be of great interest to identify the function and ion selectivity of the ancient 'E' as well as the derived 'Q' or 'N' Orai versions for the respective species. The fact that these two different gating amino acids (Q and N) evolved independently, twice, and remained conserved in the Bikonta may suggest that it represents the solution to an effective selective pressure enforced on the Bikonta.

A detailed inspection of the M1 helix of two *P. patens* Orai proteins reveals a strong conservation of the pore-lining amino acids (Figure 2B) [36,45]. The major differences are found in the cytosolic region of the helix. Since this is the region of interaction with STIM proteins in animals, the general absence of STIMs in plants and oomycetes creates the need for a different mechanism of regulation [37]. Alternative regulation (activation) is also discussed in the metazoan context. Here, two STIM-independent mechanisms have been reported that could also function in plants. Arachidonic acid is discussed as an alternative activation mechanism for ARC (arachidonate-regulated Ca^{2+}) channels (very similar to CRAC channels, but formed by different Orai subunits) [46]. Interaction between ARC channels and the fatty acid arachidonic acid leads to a prolonged low-capacity Ca^{2+} influx. Studies in seed plants have indicated that arachidonic acid treatment can increase resistance of plants to pathogens [47,48].

Arachidonic acid is not present in plants, but it can be found in several plant pathogens, including oomycetes like *Phytophthora*. Orai proteins in lower plants, therefore, could be associated with plant defence signalling. A second STIM-independent regulation mechanism revolves around the extracellular redox state. Orai proteins were reported to be regulated by their redox state [49]. H_2O_2 was found to inactivate Orai1 and Orai2 (but not Orai3), and a conserved site was identified (Cys 195 in HsOrai1; part of the second transmembrane domain (TM)). Although this specific site is not conserved in Bikonta Orai proteins, there is an alternative highly conserved Cys site that also belongs to TM2 (as Cys 195 does). This site is strictly conserved in all Bikonta species, and Orai sequences that have been analysed. This might enable a similar negative regulation by increased extracellular reactive oxygen species (ROS) concentration. Taking these two regulatory mechanisms together, it is tempting to hypothesize a model in which pathogens trigger, through arachidonic acid, an Orai-transmitted Ca^{2+} signal. Ca^{2+}, as well as

pathogen triggers, are well known to regulate plasma membrane NADPH oxidases (RBOHs) leading to an increase in extracellular ROS to stimulate appropriate defence mechanisms and counteract the pathogen attack. Additionally, extracellular H_2O_2 has been shown to activate unidentified plant Ca^{2+} channels through membrane hyperpolarization, providing the potential means for a positive feedback on ROS production, potentially enhancing the proposed pathogen defence response [50]. However, ROS in high concentrations can have detrimental effects, and its production must be tightly regulated. A negative feedback loop terminating the RBOH-activating Ca^{2+} signal through redox regulation of the Orai proteins would provide such a mechanism.

All Orai proteins show a remarkably high conservation, particularly for the pore-forming M1 helix (Figure 2C). Whether these still form Ca^{2+}-selective channels in plants will be the topic of future research. In fact, no plant Orai protein has been experimentally characterized so far. However, the loss of Orai genes after the separation of angiosperms and gymnosperms would make them the most recently lost Ca^{2+} channel family in the plant lineage. It would be of great interest to identify their function in lower plants and how their loss was compensated for in higher plants.

Ca^{2+} Efflux — Shaping Ca^{2+} Transients into Informative Signatures

To form $[Ca^{2+}]_{cyt}$ transients into informative signatures, plants employ a sophisticated set of efflux proteins that help to shape and terminate a cellular Ca^{2+} signal, and also to maintain the low basal Ca^{2+} levels needed for fast influx. Antiporters, uniporters and Ca^{2+}-ATPases on all major cellular membranes enable plant cells to expel Ca^{2+} from the cytosol in a fast and effective way. The *Arabidopsis* genome encodes five different Ca^{2+} efflux systems, namely autoinhibited Ca^{2+}-ATPases (ACAs), ER-type Ca^{2+}-ATPases (ECAs), P1-ATPases (HMA1), the mitochondrial calcium uniporter complex (MCUC) and Ca^{2+} exchangers (CAX) (Figure 1). These are discussed in a number of recent authoritative reviews [51–57].

Calcium Decoding — One Messenger, Many Translators

Ca^{2+} signatures are the net result of the operation of the 'on' and 'off' systems [2]. In the previous section, we highlighted some of the differences in the complement of proteins that contribute towards the on and off mechanisms in plants and animals. We shall now turn to a consideration of how Ca^{2+} signatures are decoded in plants and animals.

Like animals, plants have many Ca^{2+} effector proteins but their Ca^{2+}-decoding 'tools' are remarkably different [28]. Plants possess specific decoder proteins that are absent from the animal lineage. It would appear that plants either expanded the diversity of selected protein families during evolution, or new specialised functions evolved in existing protein families. One remarkable feature of plant Ca^{2+}-decoding proteins is that they are represented by relatively few distinct families, which, during evolution, expanded greatly. Examples of this phenomenon are calcineurin B-like (CBL) interacting protein kinases (CIPKs) and Ca^{2+}-dependent protein kinases (CDPKs) that expanded from 1 and 3, respectively, in algae (e.g., in *Ostreococcus tauri*) to around 30 in higher plants [24,25,58]. Approximately 250 proteins in plants contain EF-hands, which are often combined in certain architectures with other functional enzymatic domains, such as in plant NOXs (NADPH oxidases, 10 members in *Arabidopsis*) [59]. Still, it is remarkable that three major Ca^{2+}-decoder families (CDPKs, 34 in *Arabidopsis*; CBLs–CIPKs, 10/26 in *Arabidopsis*; calmodulins (CaMs)/CaM-like (CMLs), 7/50 in *Arabidopsis*) alone account for more than one-third of all EF-hand-containing Ca^{+2}-sensor proteins encoded in plant genomes [24,26,58,60]. This scenario suggests that after a (hypothetical) bottleneck within the evolutionary line to higher plants, a restricted toolkit of Ca^{2+} sensors (namely CBLs–CIPKs CDPKs, CaMs/CMLs) was diversified to acquire multiple distinct functions.

The increasing complexity of this Ca^{2+}-decoding system coincides with increasing morphological complexity of plants and the increasing ability to live in habitats with fluctuating environments. It is also worth noting that CDPKs were already quite abundant in many algal species (15 in *Chlamydomonas reinhardtii* and 12 in *Klebsormidium flaccidum*), and that particularly CBLs and CIPKs were amplified during land plant evolution (1 CIPK and 3 CBLs in *K. flaccidum*), perhaps suggesting a prominent role for CBL–CIPKs after land colonization (compare Figure 3) [24]. An important characteristic of CBL–CIPK-dependent Ca^{2+} signalling is represented by their cellular targeting (Figure 1). Unlike CDPKs, interaction of CIPKs with specific CBLs allows for a directed targeting to the plasma membrane (e.g., interaction with CBL1 or 9) or to the vacuolar membrane (e.g., interaction with CBL2 or 3) [61–64]. Thereby, CBL–CIPKs are the only known Ca^{2+}-regulated sensor–kinase modules that can directly perceive Ca^{2+} signals at the vacuolar membrane, a characteristic recently shown to be important for pH regulation, magnesium stress as well as pollen tube growth [65–68]. The strong phenotypes of mutations of multiple tonoplast-localized CBLs (like *cbl2*/*cbl3*) are in agreement with their unique function in vacuolar Ca^{2+} release [69]. However, this is not to say that CBL–CIPKs are the only Ca^{2+}-receiving proteins at the vacuolar membrane. In fact, the above-mentioned Ca^{2+} channel TPC1 is regulated and activated by cytosolic Ca^{2+}, harbours EF-hands within its structure and is an integral component of the vacuolar membrane [70,71].

Among the Ca^{2+} effectors, some are unique to plants, like the CDPK-related kinases (CRKs) and the phospho*enol*pyruvate carboxylase kinase-related kinases (PEPRKs) [72,73]. Others, like CDPKs and CBL–CIPKs, were long assumed to be plant specific. However, the recent availability of sequenced genomes of key species at informative positions within the tree of life places the origin of CBLs and CIPKs firmly at the root of the Bikonta tree [43]. CBLs and CIPKs were identified in a wide variety of species, including chromalveolate-like *Phytophthora infestans*, excavate-like *Naegleria gruberi* or the parabasalid *Trichomonas vaginalis*. Most of these species show very little amplification of the CBL–CIPK signalling system, and often retained only one pair of CBL–CIPKs. However, characteristic sequence features and enzymatic properties of CBLs (number and structure of EF-hands, phosphorylation sites, Ca^{2+}-binding abilities) and CIPKs (NAF domain, cofactor preference) were mostly retained, indicating the importance of the system [43]. Remarkably, only in land plants is the abundance of CBL and CIPK genes dramatically increased, and this increase can be correlated with the increase in complexity during plant evolution. On the other hand, species harbouring only single or

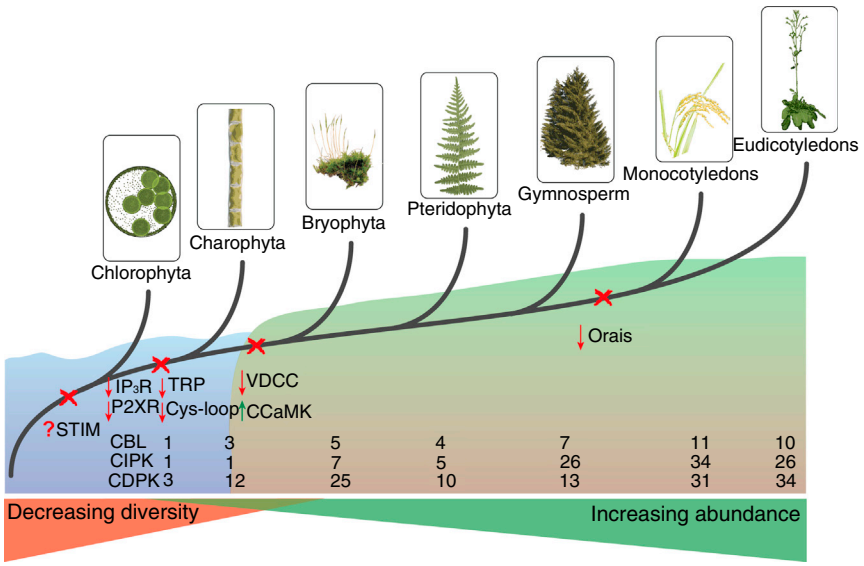

Figure 3. Evolution of the plant Ca^{2+}-signalling toolkit.
Significant losses (red arrow) and gains (green arrow) of Ca^{2+} signalling components are associated with their respective position in the tree of plant evolution. Number of identified *CBL*, *CIPK* and *CDPK* genes of representative species (Chlorophyte: *Ostreococcus tauri*; Charophyte: *Klebsormidium flaccidum*; Bryophyte: *Physcomitrella patens*; Pteridophyte: *Selaginella moellendorffii*; Gymnosperm: *Pinus taeda*; Monocotyledons: *Oryza sativa*; Eudicotyledons: *Arabidopsis thaliana*) for each phylum are indicated. The two prevailing trends are a loss of diversity of Ca^{2+}-influx components during algal evolution and an increase in gene abundance of decoding components during land plant evolution. Image credits: *Ostreococcus*, Zappys Technology Solutions; *Klebsormidium*, Katz lab, Tatiana Mikhailyuk; *Physcomitrella*, A.J. Cann; *Selaginella*, Alex Popovkin; *Pinus*, MPF, Wikimedia Commons; Rice, 영철이 Flickr; and *Arabidopsis*, Dr. Stefan Weinl, IBBP, Universität Münster.

sometimes duplicated CBL-CIPK pairs mostly represent unicellular species [24,43].

In addition to CBL–CIPKs, CDPKs were also identified in non-plant species. In the case of CDPKs, they are found in apicomplexan parasites [74,75]. Here, the evolutionary events that led to the occurrence of CDPKs outside of the plant lineage are less clear, and a potential early horizontal gene transfer has been suggested as an explanation for their presence. Regardless of the evolution of the individual families in the Bikonta supergroup, all of the above protein families are absent from the Unikonta, including the metazoan lineage [23,24]. Considering the importance of CBL–CIPKs, CDPKs, CRKs and PEPRKs for the signalling capability of plants, it is striking that compared with animals, the majority of the decoding system is composed of Bikonta-specific proteins.

Two things can be distilled from these findings: firstly, the loss of diversity and the low abundance of the remaining Ca^{2+} sensors may indicate a bottleneck in plant evolution that resulted in a net loss of diversity in the Ca^{2+} toolkit. Secondly, the increase in complexity inherent during land plant evolution forced a radical conversion of the plant Ca^{2+}-signalling toolkit in which many new functions had to be fulfilled by a limited number of sensors, leading to their amplification and functional diversification.

Another important group of Ca^{2+}-effector proteins that illustrate an increase in diversity as a function of plant complexity are CaM and CMLs [26,76]. CaMs are well conserved in all eukaryotes, whereas CMLs are mainly found in plants and have not been identified in the Unikonta supergroup [77]. Zhu *et al.* described correlations between major CaM/CML evolutionary steps in the green lineage, and the acquisition of new traits that could have contributed to the adaptation to selective pressure during land colonization. The two main increases in CaM/CML gene numbers correlate with the move to terrestrial environments (Charophyceae to Bryophyta) and the extension of multicellularity (gymnosperms to monocots and dicots) [26].

Diverging and Converging — Evolution of Fast Electrical Excitability in Plants

The previous sections have outlined how Ca^{2+}-signalling mechanisms in plants have evolved in parallel with losses and expansions of gene families associated with generation of $[Ca^{2+}]_{cyt}$ signatures, and their subsequent decoding. Electrical excitability in the form of fast action potentials (APs), intimately associated with $[Ca^{2+}]_{cyt}$ signal generation and underpinned by 4-domain Na^+/Ca^{2+} VDCCs, is widespread among basal protists (including unicellular members of the green lineage such as *Chlamydomonas*), and indicates an ancient function that was largely lost in multicellular plants, but that evolved into sophisticated, fast neuromuscular and other signalling networks in metazoans [21,22]. The typical fast animal AP lasts for less than 50 ms and travels along nerves at between 3 and 100 ms^{-1} [78]. The *Chlamydomonas* photoshock AP is a fast depolarization (duration <10 ms) mediated by light-gated plasma membrane cation channels (channelrhodopsins) localized above the eyespot. Subsequent membrane depolarization leads to activation of a VDCC (*CAV2*) in the flagellar membrane and the associated AP [79]. Multicellular plants do not generally display fast electrical excitability of this type, consistent with the absence of Na^+/Ca^{2+} VDCCs [21,22]. However, there are several examples of rapid communication and Ca^{2+}-dependent responses in multicellular plant systems based on electrical excitability, raising the question of how this may be achieved in the absence of the key components that underlie the canonical AP.

The charophyte AP, induced by wounding or electrical stimulation, is several hundred times slower than the typical animal AP, with a duration of several seconds and a propagation velocity of 10–20 mms^{-1} (see [80,81] for reviews). The initial phase of the *Chara* AP involves Ca^{2+} influx across the plasma membrane, while the main depolarization current is brought about by Cl^- efflux though Ca^{2+}-activated Cl^- channels. There is also a role for release of Ca^{2+} from intracellular stores in augmenting the associated $[Ca^{2+}]_{cyt}$ elevation [82]. While the molecular counterparts of these currents remain to be identified, it is clear

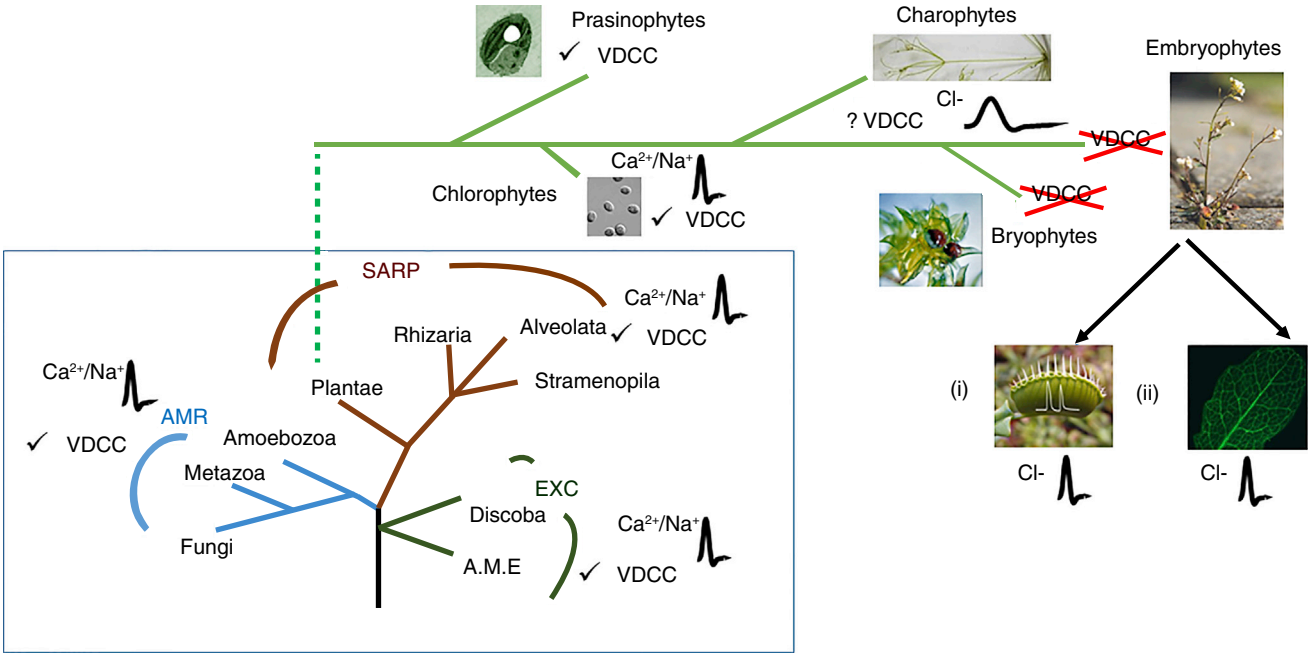

Figure 4. Distributions of canonical 4-domain VDCC channels and electrical excitability throughout the eukaryotes.
The box illustrates the widespread distribution of fast electrical excitability and VDCCs throughout the eukaryote megagroups (tree adapted from [132]); AMR: Amorphea; SARP: Stramenopila, Alveolata, Rhizaria, Plantae; EXC: Excavata; A.M.E.: Amitrochondriate excavates). In the Plantae lineage, both fast APs and VDCCs occur in the chlorophytes. While VDCCs may be present in the charophytes, they do not carry the major depolarization current during the slow AP. In the absence of VDCCs, land plants have evolved alternative methods of generating fast electrical and/or Ca^{2+} signals, exemplified by the fast APs of the Venus flytrap (i) and long-distance electrical signalling in phloem (ii). See text for details. Image acknowledgements: Prasinophyte *Ostroecoccus tauri* courtesy of H. Moreau and ML Escande, Oceanological Observatory, Banyuls; *Arabidopsis* and *Physcomitrella* from Marcus Roepers and Jean-Pierre Zryd (respectively), Wikimedia Commons; images (i) and (ii) reproduced with permission from [89] and [133].

that a VDCC, identified in the charophyte *Klebsormidium* (see above) does not carry the major depolarization current, though it may have a role in mediating the initial Ca^{2+} influx.

Significantly faster electrical signals can be found in multicellular plants. The Venus flytrap *Dionaea muscipula* displays arguably the fastest electrical signalling in plants. Stimulation of sensory hair cells by insect prey leads to very rapid depolarization of the plasma membrane of trap cells, and an AP that propagates across the gland tissue [83,84]. These APs comprise a sophisticated counting mechanism, closely linked to Ca^{2+} signalling and downstream processes that underlie prey digestion and nutrient absorption [85]. Two or more APs resulting from hair cell displacement cause rapid closure of the trap, but fail to generate a Ca^{2+} elevation in the gland cells. Further hair cell displacements by the trapped insect result in Ca^{2+} elevations and the triggering of a series of downstream responses. The APs are very rapid — direct microelectrode monitoring of membrane potential in trap lobe cells reports $\tau_{1/2}$ of 0.3 s [86], though a study using surface electrode recording reports shorter duration (1.5 ms) action potentials [87]. Generation of a flytrap AP is thought to be preceded by the activation of mechanosensitive ion channels [84,86], and the most likely candidate for the fast AP depolarization has been proposed to be rapid R-type Cl^- channels [86], providing another example of substitution of Na^+ with Cl^- for fast electrical depolarization, consistent with the absence of VDCCs in Venus flytrap [88]. How this electrical activity is converted into Ca^{2+} signatures, in a dose-dependent manner, remains to be determined. This electrical Ca^{2+}-signalling mechanism is further modulated by plant-specific hormonal signals like ABA and jasmonic acid [85].

While plants do not possess structures homologous to animal nerves, rapid long-distance propagation of electrical and Ca^{2+} signals does occur in multicellular plants (Figure 4). Electrical signals comprising fast and slow components propagate through the phloem in response to cold and wounding in *Arabidopsis* [89]. The fast component lasts around 15 s and travels at a velocity of about 1.0 mms^{-1}. While this is considerably slower than a typical animal AP-propagation velocity, it presents the possibility of long-range electrical signalling through specialized phloem 'green cables' [89]. It is proposed that R-type *QUAC1* voltage-regulated anion channels, likely also activated by Ca^{2+} influx through Ca^{2+} channels, have biophysical properties consistent with their role in long-range electrical propagation [89].

Arabidopsis also displays rapidly propagating long-distance Ca^{2+} elevations in response to salinity stress that travel through the innermost cortex and endodermal tissues from root to shoot, and which play a role in bringing about systemic responses to salt stress [90]. These Ca^{2+} elevations travel from cell to cell at velocities up to 400 μms^{-1}. If these elevations propagate through cells as waves of $[Ca^{2+}]_{cyt}$ they are extremely rapid. In animals, a typical 'fast' trans-cellular Ca^{2+} wave, based on Ca^{2+}-induced Ca^{2+} release from intracellular stores, travels at

around 10–50 μms^{-1} [91], although fast inter-cellular Ca^{2+} waves do travel through animal smooth muscle tissues at velocities around 2 mms^{-1} [92]. A clue to the mechanism of generation of cell–cell $[Ca^{2+}]_{cyt}$ signals is found in their dependence on the expression of two-pore Ca^{2+} channels (TPCs) [90]. TPCs occur on animal and plant intracellular membranes, and in plants are thought to allow Ca^{2+} release from vacuoles. Mutants defective in the vacuolar channel *TPC1* were also defective in propagation of rapid long-distance Ca^{2+} signals. Simultaneous global vacuolar activation of TPC1-mediated Ca^{2+} release could potentially bring about very rapid elevation of Ca^{2+} in individual cells that may propagate from cell to cell by an as yet unexplained mechanism. It remains to be seen whether there is link between the electrical signals travelling through the phloem and rapid long-distance Ca^{2+} signals.

Selective Pressures Driving the Evolution of Ca^{2+} Signalling in Plants

One of the most striking features to emerge from the recent investigations into the evolution of plant Ca^{2+}-based signalling is the loss of various classes of components that occurred prior to the conquest of the terrestrial environment [21,22,24]. As described above, this relates to the loss of influx components. Worthy of note, however, is an expansion of components associated with Ca^{2+} signature decoding and processing [24,28]. Before thinking about the possible consequence and significance of these losses and expansions, it is first necessary to consider the selection pressures likely to operate on the evolution of intracellular signalling in plants.

At the cellular level, plants face the same set of challenges as all other organisms do. However, they also face plant-specific challenges. To complete their life cycle and reproduce, it is advantageous for land plants to grow towards the light, exhibit positive geotropism, to capture water and nutrients from the soil and to respond appropriately when these resources become limiting. It is also beneficial to flower at the appropriate time and, at other times in the life cycle, to lose leaves or plant parts through abscission. Reproduction is an imperative (although the timescale varies), as is the ability to mount effective defences against attack by predators and pathogens. The successful operation of intra- and intercellular signalling networks underlies all these processes, and in many, Ca^{2+} is known to act as an intracellular second messenger [3,93].

The possibility that the evolution of plant-specific Ca^{2+}-signalling components was associated with colonization of the land has been suggested in the context of ion channels by some of us [21,22]. When these suggestions were made, the evolutionary ancestors of plants were assumed to be marine green algae, and one of the major selective pressures would have been the transition from the saline to the freshwater environment. However, as recent papers have proposed a possible freshwater origin for the green plants, it may well be that the importance of the saline–freshwater transition in shaping the evolution of the Ca^{2+}-signalling toolkit needs to be re-examined [94,95]. At this stage, it seems safest to conclude that a complex set of evolutionary drivers have been important in shaping the Ca^{2+}-signalling machinery in plants. These may include a specialised 'low Ca^{2+}' apoplastic environment, very negative membrane potentials, a H^+-based (rather than a Na^+-based) energization, and the need for sophisticated signalling associated with maintaining cellular water balance, being the most obvious. However, additional work will be required to shed new light on this aspect of the evolution of the Ca^{2+}-signalling toolkit.

It has been postulated that loss of VDCCs coincides with the loss of flagella-mediated motility in widely divergent eukaryotes, including streptophytes [96]. Interestingly, these authors report a putative BLAST hit of a VDCC in the moss *Physcomitrella patens,* which produces motile sperm, though Wheeler and Brownlee (2008) reported the absence of 4-domain VDCCs in this species [21]. A more detailed study of VDCC occurrence in mosses, liverworts and ferns, all of which possess motile sperm, will be needed to resolve whether loss of motile sperm was a major driver in the loss of VDCCs in land plants.

In the context of this Review, perhaps the key point we would like to make is that plant Ca^{2+}-based intracellular signalling serves the plant well, despite using a toolkit which, compared with animals, is generally reduced in diversity. The apparent success, in the sense that it is fit for purpose, of Ca^{2+}-based signalling in plants suggests that reductions in component diversity have not translated into reductions in service or capacity. Part of this must be because, despite the overall reductions in diversity, some components, such as the decoding proteins, have expanded, diversified and neo-functionalized [23–26,43]. In this way, expansion and functional diversification of specific protein families could compensate for the reduction in the overall number of functional protein families. Before discussing the possible significance, or otherwise, of these plant-specific reductions, diversifications and expansions, it is necessary to pause and examine the intracellular signalling landscape in plants more generally. This is because the presence or absence of other, potentially alternative, intracellular signalling systems can be viewed as applying either positive or negative selective pressures on the evolution of the Ca^{2+}-based signalling system.

A significant point to bear in mind is that plants either lack, or fail to make extensive use of, canonical cyclic-nucleotide signalling systems based on a toolkit made up of adenylyl (or guanylyl) cyclases, cyclic nucleotide phosphodiesterases, protein kinases A or G, and the intracellular second messengers, cAMP and cGMP [97]. Interestingly, as pointed out above, plants do possess ion channels that can be gated by cyclic nucleotides. However, lack of the canonical cyclic-nucleotide signalling systems that feature prominently in algae, fungi and animals suggests that in plants, if cyclic nucleotides are regulatory molecules, they are not operating in the same way. In animals, fungi and algae, cyclic-nucleotide signalling is a system which, just like Ca^{2+}-based signalling, serves to couple extracellular stimuli to their intracellular responses [98,99] and includes signal amplification, ensuring response specificity (fidelity) and, in concert with other signalling pathways, co-ordination and integration [100].

The point here is that, unless there are yet-to-be discovered intracellular signalling systems capable of contributing to amplification, signalling fidelity, coordination and integration in plants, then the Ca^{2+}-signalling system must shoulder a greater burden in terms of fulfilling these functions. Might this help to explain the way that evolution has shaped Ca^{2+} signalling in plants?

On the basis of the available evidence, it would seem that plants have lost diversity of Ca^{2+}-influx mechanisms while

increasing the number of different types of proteins involved in decoding. At its simplest, a plethora of influx systems coupled with efflux systems provides the organism with the capacity, in spatial and temporal terms, to generate highly complex, information-rich, Ca^{2+} signatures. Is there any evidence that plants generate less complex Ca^{2+} signatures than animals? At this point we don't have enough experimental data to provide an authoritative answer to this question. In plants, Ca^{2+}-imaging studies suggest that complex patterns are produced; however, there are not enough data to determine whether these are more or less complex than those found in, for example, mammals [90,101–111]. Rather, what is striking is that, in general, where measurements of stimulus-induced increases in $[Ca^{2+}]_{cyt}$ have been measured, at the level of the single cell, in plants they are often characterised by slower kinetics than in mammalian cells [7]. It is possible, with one exception, that plants have not experienced the selective pressures which might result in the development of influx and efflux systems capable of generating rapid Ca^{2+} signatures. An intriguing possible exception, as discussed above, are rapid long-distance Ca^{2+} signals that may rely on explosive Ca^{2+} release through vacuolar TPC channels [90]. Taken together, it would appear that, even without cyclic-nucleotide signalling, the loss of influx mechanisms has left plants with capacity to amplify signals and to generate Ca^{2+} signatures that are sufficiently information rich.

The second observation to emerge from investigations of the evolution of Ca^{2+}-based signalling in plants is that there has been a divergence of proteins capable of decoding the Ca^{2+} signature. Seen in the context of the lack of cyclic-nucleotide signalling, this expansion is perhaps explicable because of an increased intracellular workload for Ca^{2+}-based signalling. If this is the case, expansion of the repertoire of downstream signature decoding proteins would help to ensure that intracellular signalling exhibits fidelity and robustness and, when new selective pressures arise, has the capacity to evolve and produce changed signalling responses. Intracellular second-messenger-based signalling systems are ideally placed to play a key role in the orchestration or co-ordination of the multiple individual reactions that together result in the final cellular response. A classic example in plants would be stomatal closure induced by ABA [112–114]. This involves the control of ion channel activity, membrane trafficking, cytoskeletal movements, metabolic processes and changes in gene expression — all of which combine to bring about stomatal closure. Experiments with EGTA and BAPTA reveal that an increase in $[Ca^{2+}]_{cyt}$ is required during this process and additional investigations uncovered that it depends on the presence and activity of CPK and CIPK–CBL decoders targeting guard cell anion channels [114–117]. In the absence of the cyclic-nucleotide-based signalling system, it is possible that, in this example, the primary responsibility for orchestration falls to Ca^{2+}. If this assumption is correct, then expansion of decoding proteins would be explicable and advantageous in order to fulfil this function. Related to this is the requirement to generate graded responses. Again, lack of an alternative second-messenger-based system might have resulted in selection for the expansion of signature decoding proteins exhibiting differing Ca^{2+} affinities, thereby facilitating graded or nuanced responses.

Currently, the available information suggests that, while plants use Ca^{2+} signatures, they, in most cases, fail to exhibit the rapid kinetics of their mammalian counterparts. Whether this is as a result of a lack of diversity in Ca^{2+} influx systems is not known. However, the lack of the rapidly generated signals would suggest that these have not been selected for during plant evolution. The selective pressure to diversify the repertoire of signature-decoding proteins to provide plants with opportunities for signalling co-ordination may have resulted from the absence of other second-messenger-based intracellular signalling systems. Likewise, it is tempting to assume that the abundance of different signal decoders (CDPKs, CBL–CIPKs, CMLs) that we see in extant plants is reflective of an increase in the ability to colonise a diverse array of environmental niches that have occurred over evolutionary time. In this scenario, the ability to respond appropriately to an increasing range of environmental stimuli would be of selective advantage to evolving plants.

Unanswered Questions and Opportunities

In the preceding sections, we have sought to describe differences between animal and plant Ca^{2+}-based intracellular signalling, and have taken some first steps to identify the selective pressures that might have operated to shape the plant Ca^{2+}-signalling toolkit. At this stage our approach has been, of necessity, broad brush, comparing plants with animals and attempting to account for the tools as represented by the Ca^{2+} toolkit of today. With the addition of more sequenced genomes, it will be possible to drill deeper into the evolution of the plant Ca^{2+}-signalling network. In particular, an increase in the number of genome sequences will provide the increase in the granularity required to investigate whether there is a correlation between, for example, the increased diversity in the Ca^{2+} signature-decoding proteins and the appearance of key innovations in plant morphology and physiology. Likewise, increased granularity will permit the overlaying of paleoclimate data on the timeline describing the evolution of the Ca^{2+}-signalling toolkit and the evolution of plant morphology. Mapping major losses to, or expansions of, the plant Ca^{2+}-signalling toolkit onto a timeline of plant innovations and significant changes to climate and environment might reveal the identity of the key selective pressures that shaped the evolution of Ca^{2+} signalling in plants. Ideally, such approaches should be paralleled by experimental determination of quantitative Ca^{2+}-binding characteristics and enzymatic kinetics of the Ca^{2+}-signalling components to aid understanding of their functional differentiation and diversification during evolution.

In seeking to understand what factors have shaped the evolution of the Ca^{2+}-signalling toolkit in plants, it might be fruitful to concentrate more attention on photosynthesis, and in particular the chloroplast. In this context, it is important to note that in 1987, Miller and Sanders observed a light-induced reduction in $[Ca^{2+}]_{cyt}$ in the characean alga *Nitellopsis*, which they proposed "constitutes a fundamental signal which enables the rate of extra-chloroplastic metabolism to be geared to photosynthetic processes in the chloroplast" [118]. The importance of organelles, including the chloroplast and the mitochondria, in influencing cellular Ca^{2+} signals has been proposed in a number of studies (see [119] for review). Indeed, it has been proposed that the chloroplast plays a key role in Ca^{2+} and ROS signalling underlying stomatal closure through facilitation of the primed

state of guard cells to respond to closing stimuli [120]. The discovery of the plant-specific chloroplast thylakoid-localized Ca^{2+} sensor, CAS, lends further evidence to the pivotal role of the chloroplast in the evolution of plant Ca^{2+} signalling [121,122]. In *Arabidopsis*, CAS is responsible for stress related stromal as well as cytosolic Ca^{2+} transients and is involved in Ca^{2+}-modulated MAPK regulation of ABI4 [121,123,124]. However, it is clear that retention of 'animal-like' Ca^{2+}-toolkit components persisted through the evolution of the chloroplast CAS-related Ca^{2+}-signalling machinery, at least in the unicellular green algae, as evidenced by the presence of CAS signalling in photoadaptation responses in *Chlamydomonas* [125].

In *Chlamydomonas*, CAS contributes to the transcriptional regulation of LHCSR3 as well as to components of carbon concentrating mechanisms [125,126]. Besides chloroplasts, mitochondria have become a recent focus for Ca^{2+}-related research [57]. Due to the occurrence of mitochondria in both the animal as well as the plant lineage, their Ca^{2+}-signalling toolkit is of particular interest from an evolutionary point of view. The mitochondrial Ca^{2+} uniporter complex MCUC was first identified in animals, but recently components of this transport machinery were also found in *Arabidopsis*, indicating conservation of the pore-forming protein MCU (mitochondrial calcium uniporter) as well as the EF-hand-containing regulatory component MICU1 (mitochondrial Ca^{2+} uptake 1) [127–129]. Absence of MICU1 in mutant *Arabidopsis* lines resulted in higher mitochondrial Ca^{2+} content, indicating that MICU1 may be involved in sensing and restricting matrix Ca^{2+} levels [129]. Mitochondria have been reported to influence cytosolic Ca^{2+} signatures in animals, and the noticeable homologies between the animal and the plant system in terms of the mitochondrial Ca^{2+}-import machinery may help identify similar mechanisms in plants [130,131]. The emerging contributions of organelles to cytosolic Ca^{2+} transients will have to be taken into account in future models of plant intracellular Ca^{2+} signalling.

In summary, in this Review we have highlighted some of the major differences between the way that evolution has shaped the Ca^{2+}-signalling toolkit between animals and plants and begun the process of seeking to understand the origin and significance of these changes. With the increasing availability of genomic information, the scene is set to make significant advances in our understanding of this fundamental process.

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REFERENCES

1. Berridge, M.J., Lipp, P., and Bootman, M.D. (2000). The versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. *1*, 11–21.
2. Berridge, M.J., Bootman, M.D., and Roderick, H.L. (2003). Calcium signalling: dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. *4*, 517–529.
3. Clapham, D.E. (1995). Calcium signaling. Cell *80*, 259–268.
4. Webb, A.A.R., McAinsh, M.R., Taylor, J.E., and Hetherington, A.M. (1996). Calcium ions as intracellular second messengers in higher plants. Adv. Bot. Res. *22*, 45–96.
5. Hedrich, R. (2012). Ion channels in plants. Physiol. Rev. *92*, 1777–1811.
6. McAinsh, M.R., and Hetherington, A.M. (1998). Encoding specificity in Ca^{2+} signalling systems. Trends Plant Sci. *3*, 32–36.
7. Hetherington, A.M., and Brownlee, C. (2004). The generation of Ca^{2+} signals in plants. Annu. Rev. Plant Biol. *55*, 401–427.
8. Batistič, O., and Kudla, J. (2012). Analysis of calcium signaling pathways in plants. Biochim. Biophys. Acta. *1820*, 1283–1293.
9. Kudla, J., Batistič, O., and Hashimoto, K. (2010). Calcium signals: the lead currency of plant information processing. Plant Cell *22*, 541–563.
10. Dodd, A.N., Kudla, J., and Sanders, D. (2010). The language of calcium signaling. Annu. Rev. Plant Biol. *61*, 593–620.
11. Steinhorst, L., and Kudla, J. (2013). Calcium and reactive oxygen species rule the waves of signaling. Plant Physiol. *163*, 471–485.
12. Hepler, P.K. (2016). The cytoskeleton and its regulation by calcium and protons. Plant Physiol. *170*, 3–22.
13. Steinhorst, L., and Kudla, J. (2014). Signaling in cells and organisms - calcium holds the line. Curr. Opin. Plant Biol. *22*, 14–21.
14. Cai, X., and Clapham, D.E. (2012). Ancestral Ca^{2+} signaling machinery in early animal and fungal evolution. Mol. Biol. Evol. *29*, 91–100.
15. Cai, X. (2008). Unicellular Ca^{2+} signaling "toolkit" at the origin of metazoa. Mol. Biol. Evol. *25*, 1357–1361.
16. Cai, X., Wang, X., and Clapham, D.E. (2014). Early evolution of the eukaryotic Ca^{2+} signaling machinery: conservation of the CatSper channel complex. Mol. Biol. Evol. *31*, 2735–2740.
17. Cai, X., Wang, X., Patel, S., and Clapham, D.E. (2015). Insights into the early evolution of animal calcium signaling machinery: a unicellular point of view. Cell Calcium *57*, 166–173.
18. Plattner, H., and Verkhratsky, A. (2015). The ancient roots of calcium signalling evolutionary tree. Cell Calcium *57*, 123–132.
19. Plattner, H., and Verkhratsky, A. (2013). Ca^{2+} signalling early in evolution–all but primitive. J. Cell Sci. *126*, 2141–2150.
20. Verkhratsky, A., and Parpura, V. (2014). Calcium signalling and calcium channels: evolution and general principles. Eur. J. Pharmacol. *739*, 1–3.
21. Wheeler, G.L., and Brownlee, C. (2008). Ca^{2+} signalling in plants and green algae–changing channels. Trends Plant Sci. *13*, 506–514.
22. Verret, F., Wheeler, G., Taylor, A.R., Farnham, G., and Brownlee, C. (2010). Calcium channels in photosynthetic eukaryotes: implications for evolution of calcium-based signalling. New Phytol. *187*, 23–43.
23. Hamel, L.-P., Sheen, J., and Séguin, A. (2014). Ancient signals: comparative genomics of green plant CDPKs. Trends Plant Sci. *19*, 79–89.
24. Edel, K.H., and Kudla, J. (2015). Increasing complexity and versatility: how the calcium signaling toolkit was shaped during plant land colonization. Cell Calcium *57*, 231–246.
25. Kleist, T.J., Spencley, A.L., and Luan, S. (2014). Comparative phylogenomics of the CBL-CIPK calcium-decoding network in the moss Physcomitrella, Arabidopsis, and other green lineages. Front. Plant Sci. *5*, 187.
26. Zhu, X., Dunand, C., Snedden, W., and Galaud, J.-P. (2015). CaM and CML emergence in the green lineage. Trends Plant Sci. *20*, 483–489.
27. Case, R.M., Eisner, D., Gurney, A., Jones, O., Muallem, S., and Verkhratsky, A. (2007). Evolution of calcium homeostasis: from birth of the first cell to an omnipresent signalling system. Cell Calcium *42*, 345–350.
28. Marchadier, E., Oates, M.E., Fang, H., Donoghue, P.C.J., Hetherington, A.M., and Gough, J. (2016). Evolution of the calcium-based intracellular signaling system. Genome Biol. Evol. *8*, 2118–2132.

29. Morgan, A.J., and Galione, A. (2014). Two-pore channels (TPCs): current controversies. Bioessays *36*, 173–183.

30. Zelman, A.K., Dawe, A., Gehring, C., and Berkowitz, G.A. (2012). Evolutionary and structural perspectives of plant cyclic nucleotide-gated cation channels. Front. Plant Sci. *3*, 95.

31. Price, M.B., Jelesko, J., and Okumoto, S. (2012). Glutamate receptor homologs in plants: functions and evolutionary origins. Front. Plant Sci. *3*, 235.

32. Kurusu, T., Kuchitsu, K., Nakano, M., Nakayama, Y., and Iida, H. (2013). Plant mechanosensing and Ca^{2+} transport. Trends Plant Sci. *18*, 227–233.

33. Yuan, F., Yang, H., Xue, Y., Kong, D., Ye, R., Li, C., Zhang, J., Theprungsirikul, L., Shrift, T., Krichilsky, B., *et al*. (2014). OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in Arabidopsis. Nature *514*, 367–371.

34. Hou, C., Tian, W., Kleist, T., He, K., Garcia, V., Bai, F., Hao, Y., Luan, S., and Li, L. (2014). DUF221 proteins are a family of osmosensitive calcium-permeable cation channels conserved across eukaryotes. Cell Res. *24*, 632–635.

35. Di Capite, J., and Parekh, A.B. (2009). CRAC channels and Ca^{2+} signaling in mast cells. Immunol. Rev. *231*, 45–58.

36. Hou, X., Pedi, L., Diver, M.M., and Long, S.B. (2012). Crystal structure of the calcium release-activated calcium channel Orai. Science *338*, 1308–1313.

37. Rothberg, B., Wang, Y., and Gill, D. (2013). Orai channel pore properties and gating by STIM: implications from the Orai crystal structure. Sci. Signal. *6*, pe9.

38. Prakriya, M., Feske, S., Gwack, Y., Srikanth, S., Rao, A., and Hogan, P.G. (2006). Orai1 is an essential pore subunit of the CRAC channel. Nature *443*, 230–233.

39. McNally, B.A., Somasundaram, A., Yamashita, M., and Prakriya, M. (2012). Gated regulation of CRAC channel ion selectivity by STIM1. Nature *482*, 241–245.

40. Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S.-H., Tanasa, B., Hogan, P.G., Lewis, R.S., Daly, M., and Rao, A. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature *441*, 179–185.

41. Cai, X. (2007). Molecular evolution and structural analysis of the Ca^{2+} release-activated Ca^{2+} channel subunit. Orai. J. Mol. Biol. *368*, 1284–1291.

42. Collins, S.R., and Meyer, T. (2011). Evolutionary origins of STIM1 and STIM2 within ancient Ca^{2+} signaling systems. Trends Cell Biol. *21*, 202–211.

43. Beckmann, L., Edel, K.H., Batistič, O., and Kudla, J. (2016). A calcium sensor – protein kinase signaling module diversified in plants and is retained in all lineages of Bikonta species. Sci. Rep. *6*, 31645.

44. McNally, B.A., and Prakriya, M. (2012). Permeation, selectivity and gating in store-operated CRAC channels. J. Physiol. *590*, 4179–4191.

45. Celniker, G., Nimrod, G., Ashkenazy, H., Glaser, F., Martz, E., Mayrose, I., Pupko, T., and Ben-Tal, N. (2013). ConSurf: using evolutionary data to raise testable hypotheses about protein function. Isr. J. Chem. *53*, 199–206.

46. Shuttleworth, T.J. (2009). Arachidonic acid, ARC channels, and Orai proteins. Cell Calcium *45*, 602–610.

47. Savchenko, T., Walley, J.W., Chehab, E.W., Xiao, Y., Kaspi, R., Pye, M.F., Mohamed, M.E., Lazarus, C.M., Bostock, R.M., and Dehesh, K. (2010). Arachidonic acid: an evolutionarily conserved signaling molecule modulates plant stress signaling networks. Plant Cell *22*, 3193–3205.

48. Dedyukhina, E.G., Kamzolova, S.V., and Vainshtein, M.B. (2014). Arachidonic acid as an elicitor of the plant defense response to phytopathogens. Chem. Biol. Technol. Agric. *1*, 18.

49. Bhardwaj, R., Hediger, M.A., and Demaurex, N. (2016). Redox modulation of STIM-ORAI signaling. Cell Calcium *60*, 142–152.

50. Pei, Z.-M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G.J., Grill, E., and Schroeder, J.I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature *406*, 731–734.

51. Bose, J., Pottosin, I.I., Shabala, S.S., Palmgren, M.G., and Shabala, S. (2011). Calcium efflux systems in stress signaling and adaptation in plants. Front. Plant Sci. *2*, 85.

52. Pittman, J.K., and Hirschi, K.D. (2016). CAX-ing a wide net: Cation/H^+ transporters in metal remediation and abiotic stress signalling. Plant Biol. *18*, 741–749.

53. Manohar, M., Shigaki, T., and Hirschi, K.D. (2011). Plant cation/H^+ exchangers (CAXs): biological functions and genetic manipulations. Plant Biol. *13*, 561–569.

54. Spalding, E.P., and Harper, J.F. (2011). The ins and outs of cellular Ca^{2+} transport. Curr. Opin. Plant Biol. *14*, 715–720.

55. Pittman, J.K. (2011). Vacuolar Ca^{2+} uptake. Cell Calcium *50*, 139–146.

56. Peiter, E. (2011). The plant vacuole: emitter and receiver of calcium signals. Cell Calcium *50*, 120–128.

57. Wagner, S., De Bortoli, S., Schwarzländer, M., and Szabò, I. (2016). Regulation of mitochondrial calcium in plants versus animals. J. Exp. Bot. *67*, 3809–3829.

58. Weinl, S., and Kudla, J. (2009). The CBL-CIPK Ca^{2+}-decoding signaling network: function and perspectives. New Phytol. *184*, 517–528.

59. Day, I.S., Reddy, V.S., Shad Ali, G., and Reddy, A.S.N. (2002). Analysis of EF-hand-containing proteins in Arabidopsis. Genome Biol. *3*, RESEARCH0056.

60. Cheng, S., Willmann, M.R., Chen, H., and Sheen, J. (2002). Update on calcium signaling through protein kinases. The Arabidopsis calcium-dependent protein kinase gene family *1* (129), 469–485.

61. Batistič, O., Rehers, M., Akerman, A., Schlücking, K., Steinhorst, L., Yalovsky, S., and Kudla, J. (2012). S-acylation-dependent association of the calcium sensor CBL2 with the vacuolar membrane is essential for proper abscisic acid responses. Cell Res. *22*, 1155–1168.

62. Batistič, O., Sorek, N., Schültke, S., Yalovsky, S., and Kudla, J. (2008). Dual fatty acyl modification determines the localization and plasma membrane targeting of CBL/CIPK Ca^{2+} signaling complexes in Arabidopsis. Plant Cell *20*, 1346–1362.

63. Schlücking, K., Edel, K.H., Drerup, M.M., Köster, P., Eckert, C., Leonie, S., Waadt, R., Batistič, O., Kudla, J., Drerup, M.M., *et al*. (2013). A new β-estradiol-inducible vector set that facilitates easy construction and efficient expression of transgenes reveals CBL3-dependent cytoplasm to tonoplast translocation of CIPK5. Mol. Plant *6*, 1814–1829.

64. Batistič, O., Waadt, R., Steinhorst, L., Held, K., and Kudla, J. (2010). CBL-mediated targeting of CIPKs facilitates the decoding of calcium signals emanating from distinct cellular stores. Plant J. *61*, 211–222.

65. Mogami, J., Fujita, Y., Yoshida, T., Tsukiori, Y., Nakagami, H., Nomura, Y., Fujiwara, T., Nishida, S., Yanagisawa, S., Ishida, T., *et al*. (2015). Two distinct families of protein kinases are required for plant growth under high external Mg^{2+} concentrations in Arabidopsis. Plant Physiol. *167*, 1039–1057.

66. Steinhorst, L., Mähs, A., Ischebeck, T., Zhang, C., Zhang, X., Arendt, S., Schültke, S., Heilmann, I., and Kudla, J. (2015). Vacuolar CBL-CIPK12 Ca^{2+}-sensor-kinase complexes are required for polarized pollen tube growth. Curr. Biol. *25*, 1475–1482.

67. Tang, R.-J., Zhao, F.-G., Garcia, V.J., Kleist, T.J., Yang, L., Zhang, H.-X., and Luan, S. (2015). Tonoplast CBL-CIPK calcium signaling network regulates magnesium homeostasis in Arabidopsis. Proc. Natl. Acad. Sci. USA *112*, 3134–3139.

68. Tang, R.-J., Liu, H., Yang, Y., Yang, L., Gao, X.-S., Garcia, V.J., Luan, S., and Zhang, H.-X. (2012). Tonoplast calcium sensors CBL2 and CBL3 control plant growth and ion homeostasis through regulating V-ATPase activity in Arabidopsis. Cell Res. *22*, 1650–1665.

69. Eckert, C., Offenborn, J.N., Heinz, T., Armarego-Marriott, T., Schültke, S., Zhang, C., Hillmer, S., Heilmann, M., Schumacher, K., Bock, R., *et al*. (2014). The vacuolar calcium sensors CBL2 and CBL3 affect seed size and embryonic development in *Arabidopsis thaliana*. Plant J. *78*, 146–156.

70. Peiter, E., Maathuis, F.J.M., Mills, L.N., Knight, H., Pelloux, J., Hetherington, A.M., and Sanders, D. (2005). The vacuolar Ca^{2+}-activated channel TPC1 regulates germination and stomatal movement. Nature *434*, 404–408.

71. Hedrich, R., and Neher, E. (1987). Cytoplasmic calcium regulates voltage-dependent ion channels in plant vacuoles. Nature *329*, 833–836.

72. Nagata, T., Iizumi, S., Satoh, K., Ooka, H., Kawai, J., Carninci, P., Hayashizaki, Y., Otomo, Y., Murakami, K., Matsubara, K., *et al*. (2004). Comparative analysis of plant and animal calcium signal transduction element using plant full-length cDNA data. Mol. Biol. Evol. *21*, 1855–1870.

73. Hrabak, E.M., Chan, C.W.M., Gribskov, M., Harper, J.F., Choi, J.H., Halford, N., Kudla, J., Luan, S., Nimmo, H.G., Sussman, M.R., *et al*. (2003). The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol. *132*, 666–680.

74. Zhang, X.S., and Choi, J.H. (2001). Molecular evolution of calmodulin-like domain protein kinases (CDPKs) in plants and protists. J. Mol. Evol. *53*, 214–224.

75. Wernimont, A.K., Amani, M., Qiu, W., Pizarro, J.C., Artz, J.D., Lin, Y.-H., Lew, J., Hutchinson, A., and Hui, R. (2011). Structures of parasitic CDPK domains point to a common mechanism of activation. Proteins *79*, 803–820.

76. McCormack, E., Tsai, Y.-C., and Braam, J. (2005). Handling calcium signaling: Arabidopsis CaMs and CMLs. Trends Plant Sci. *10*, 383–389.

77. Mohanta, T.K., Kumar, P., and Bae, H. (2017). Genomics and evolutionary aspect of calcium signaling event in calmodulin and calmodulin-like proteins in plants. BMC Plant Biol. *17*, 38.

78. Debanne, D., Campanac, E., Bialowas, A., Carlier, E., and Alcaraz, G. (2011). Axon physiology. Physiol. Rev. *91*, 555–602.

79. Fujiu, K., Nakayama, Y., Yanagisawa, A., Sokabe, M., and Yoshimura, K. (2009). Chlamydomonas CAV2 encodes a voltage- dependent calcium channel required for the flagellar waveform conversion. Curr. Biol. *19*, 133–139.

80. Fromm, J., and Lautner, S. (2007). Electrical signals and their physiological significance in plants. Plant. Cell Environ. *30*, 249–257.

81. Beilby, M.J. (2007). Action potential in charophytes. Int. Rev. Cytol. *257*, 43–82.

82. Plieth, C., Sattelmacher, B., Hansen, U.-P., and Thiel, G. (1998). The action potential in Chara: Ca^{2+} release from internal stores visualized by Mn^{2+}-induced quenching of fura-dextran. Plant J. *13*, 167–175.

83. Volkov, A.G., Adesina, T., Markin, V.S., and Jovanov, E. (2007). Kinetics and mechanism of *Dionaea muscipula* trap closing. Plant Physiol. *146*, 694–702.

84. Volkov, A.G., Adesina, T., and Jovanov, E. (2008). Charge induced closing of *Dionaea muscipula* Ellis trap. Bioelectrochemistry *74*, 16–21.

85. Böhm, J., Scherzer, S., Krol, E., Kreuzer, I., von Meyer, K., Lorey, C., Mueller, T.D., Shabala, L., Monte, I., Solano, R., *et al*. (2016). The Venus flytrap *Dionaea muscipula* counts prey-induced action potentials to induce sodium uptake. Curr. Biol. *26*, 286–295.

86. Escalante-Perez, M., Krol, E., Stange, A., Geiger, D., Al-Rasheid, K.A.S., Hause, B., Neher, E., and Hedrich, R. (2011). A special pair of phytohormones controls excitability, slow closure, and external stomach formation in the Venus flytrap. Proc. Natl. Acad. Sci. USA *108*, 15492–15497.

87. Volkov, A.G., Adesina, T., and Jovanov, E. (2007). Closing of Venus flytrap by electrical stimulation of motor cells. Plant Signal. Behav. *2*, 139.

88. Bemm, F., Becker, D., Larisch, C., Kreuzer, I., Escalante-Perez, M., Schulze, W.X., Ankenbrand, M., Van de Weyer, A.-L., Krol, E., Al-Rasheid, K.A., *et al*. (2016). Venus flytrap carnivorous lifestyle builds on herbivore defense strategies. Genome Res. *26*, 812–825.

89. Hedrich, R., Salvador-Recatal, V., and Dreyer, I. (2016). Electrical wiring and long-distance plant communication. Trends Plant Sci. *21*, 376–387.

90. Choi, W.-G., Toyota, M., Kim, S.-H., Hilleary, R., and Gilroy, S. (2014). Salt stress-induced Ca^{2+} waves are associated with rapid, long-distance root-to-shoot signaling in plants. Proc. Natl. Acad. Sci. USA *1*, 1–6.

91. Jaffe, L.F. (1991). The path of calcium in cytosolic calcium oscillations: a unifying hypothesis. Proc. Natl. Acad. Sci. USA *88*, 9883–9887.

92. Halidi, N., Boittin, F.-X., Bény, J.-L., and Meister, J.-J. (2011). Propagation of fast and slow intercellular Ca^{2+} waves in primary cultured arterial smooth muscle cells. Cell Calcium *50*, 459–467.

93. Clapham, D.E. (2007). Calcium signaling. Cell *131*, 1047–1058.

94. Ponce-Toledo, R.I., Deschamps, P., López-García, P., Zivanovic, Y., Benzerara, K., and Moreira, D. (2017). An early-branching freshwater cyanobacterium at the origin of plastids. Curr. Biol. *27*, 386–391.

95. Blank, C.E. (2013). Origin and early evolution of photosynthetic eukaryotes in freshwater environments: Reinterpreting proterozoic paleobiology and biogeochemical processes in light of trait evolution. J. Phycol *49*, 1040–1055.

96. Brunet, T., and Arendt, D. (2016). From damage response to action potentials: early evolution of neural and contractile modules in stem eukaryotes. Phil. Trans. R. Soc. B Biol. Sci. *371*, 20150043.

97. The Arabidopsis Genome Initiative. (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature *408*, 796–815.

98. Lucas, K.A., Pitari, G.M., Kazerounian, S., Ruiz-Stewart, I., Park, J., Schulz, S., Chepenik, K.P., and Waldman, S.A. (2000). Guanylyl cyclases and signaling by cyclic GMP. Pharmacol. Rev. *52*, 375–414.

99. D'Souza, C.A., and Heitman, J. (2001). Conserved cAMP signaling cascades regulate fungal development and virulence. FEMS Microbiol. Rev. *25*, 349–364.

100. Dyachok, O., Isakov, Y., Sågetorp, J., and Tengholm, A. (2006). Oscillations of cyclic AMP in hormone-stimulated insulin-secreting beta-cells. Nature *439*, 349–352.

101. Behera, S., Wang, N., Zhang, C., Schmitz-Thom, I., Strohkamp, S., Schültke, S., Hashimoto, K., Xiong, L., and Kudla, J. (2015). Analyses of Ca^{2+} dynamics using a ubiquitin-10 promoter-driven Yellow Cameleon 3.6 indicator reveal reliable transgene expression and differences in cytoplasmic Ca^{2+} responses in Arabidopsis and rice (*Oryza sativa*) roots. New Phytol. *206*, 751–760.

102. Bonza, M.C., Loro, G., Behera, S., Wong, A., Kudla, J., and Costa, A. (2013). Analyses of Ca^{2+} accumulation and dynamics in the endoplasmic reticulum of Arabidopsis root cells using a genetically encoded cameleon sensor. Plant Physiol. *163*, 1230–1241.

103. Loro, G., Wagner, S., Doccula, F.G., Behera, S., Weinl, S., Kudla, J., Schwarzländer, M., Costa, A., and Zottini, M. (2016). Chloroplast-specific in vivo Ca^{2+} imaging using Yellow Cameleon fluorescent protein sensors reveals organelle-autonomous Ca^{2+} signatures in the stroma. Plant Physiol. *171*, 2317–2330.

104. Behera, S., Long, Y., Schmitz-Thom, I., Wang, X.-P., Zhang, C., Li, H., Steinhorst, L., Manishankar, P., Ren, X.-L., Offenborn, J.N., *et al*. (2017). Two spatially and temporally distinct Ca^{2+} signals convey *Arabidopsis thaliana* responses to K^+ deficiency. New Phytol. *213*, 739–750.

105. Knight, H., Trewavas, A.J., and Knight, M.R. (1996). Cold calcium signaling in Arabidopsis involves two cellular pools and a change in calcium signature after acclimation. Plant Cell *8*, 489–503.

106. Knight, M.R., Campbell, A.K., Smith, S.M., and Trewavas, A.J. (1991). Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. Nature *352*, 524–526.

107. Whalley, H.J., and Knight, M.R. (2013). Calcium signatures are decoded by plants to give specific gene responses. New Phytol. *197*, 690–693.

108. Monshausen, G.B., Messerli, M.A., and Gilroy, S. (2008). Imaging of the Yellow Cameleon 3.6 indicator reveals that elevations in cytosolic Ca^{2+} follow oscillating increases in growth in root hairs of Arabidopsis. Plant Physiol. *147*, 1690–1698.

109. Keinath, N.F., Waadt, R., Brugman, R., Schroeder, J.I., Grossmann, G., Schumacher, K., and Krebs, M. (2015). Live cell imaging with R-GECO1 sheds light on flg22- and chitin-induced transient $[Ca^{2+}]_{cyt}$ patterns in Arabidopsis. Mol. Plant *8*, 1188–1200.

110. McAinsh, M.R., Webb, A., Taylor, J.E., and Hetherington, A.M. (1995). Stimulus-induced oscillations in guard cell cytosolic free calcium. Plant Cell *7*, 1207–1219.

111. McAinsh, M.R., Brownlee, C., and Hetherington, A.M. (1992). Visualizing changes in cytosolic-free Ca^{2+} during the response of stomatal guard cells to abscisic acid. Plant Cell *4*, 1113–1122.

112. Hetherington, A.M., Gray, J.E., Leckie, C.P., McAinsh, M.R., Ng, C., Pical, C., Priestley, A.J., Staxen, I., and Webb, A.A.R. (1998). The control of specificity in guard cell signal transduction. Phil. Trans. R. Soc. B Biol. Sci. *353*, 1489–1494.

113. Kim, T.-H., Böhmer, M., Hu, H., Nishimura, N., and Schroeder, J.I. (2010). Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. Annu. Rev. Plant Biol. *61*, 561–591.

114. Munemasa, S., Hauser, F., Park, J., Waadt, R., Brandt, B., and Schroeder, J.I. (2015). Mechanisms of abscisic acid-mediated control of stomatal aperture. Curr. Opin. Plant Biol. *28*, 154–162.

115. Webb, A.A.R., Larman, M.G., Montgomery, L.T., Taylor, J.E., and Hetherington, A.M. (2001). The role of calcium in ABA-induced gene expression and stomatal movements. Plant J. *26*, 351–362.

116. Allen, G.J., Chu, S.P., Harrington, C.L., Schumacher, K., Hoffmann, T., Tang, Y.Y., Grill, E., and Schroeder, J.I. (2001). A defined range of guard cell calcium oscillation parameters encodes stomatal movements. Nature *411*, 1053–1057.

117. Chen, Z.-H., Hills, A., Lim, C.K., and Blatt, M.R. (2010). Dynamic regulation of guard cell anion channels by cytosolic free Ca^{2+} concentration and protein phosphorylation. Plant J. *61*, 816–825.

118. Miller, A.J., and Sanders, D. (1987). Depletion of cytosolic free calcium induced by photosynthesis. Nature *326*, 397–400.

119. Stael, S., Wurzinger, B., Mair, A., Mehlmer, N., Vothknecht, U.C., and Teige, M. (2012). Plant organellar calcium signalling: an emerging field. J. Exp. Bot. *63*, 1525–1542.

120. Wang, W.-H., He, E.-M., Chen, J., Guo, Y., Chen, J., Liu, X., and Zheng, H.-L. (2016). The reduced state of the plastoquinone pool is required for chloroplast-mediated stomatal closure in response to calcium stimulation. Plant J. *86*, 132–144.

121. Weinl, S., Held, K., Schlücking, K., Steinhorst, L., Kuhlgert, S., Hippler, M., and Kudla, J. (2008). A plastid protein crucial for Ca^{2+}-regulated stomatal responses. New Phytol. *179*, 675–686.

122. Han, S., Tang, R., Anderson, L.K., Woerner, T.E., and Pei, Z.-M. (2003). A cell surface receptor mediates extracellular Ca^{2+} sensing in guard cells. Nature *425*, 196–200.

123. Nomura, H., Komori, T., Uemura, S., Kanda, Y., Shimotani, K., Nakai, K., Furuichi, T., Takebayashi, K., Sugimoto, T., Sano, S., *et al*. (2012). Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nat. Commun. *3*, 926.

124. Guo, H., Feng, P., Chi, W., Sun, X., Xu, X., Li, Y., Ren, D., Lu, C., David Rochaix, J., Leister, D., *et al*. (2016). Plastid-nucleus communication involves calcium-modulated MAPK signalling. Nat. Commun. *7*, 12173.

125. Petroutsos, D., Busch, A., Janßen, I., Trompelt, K., Bergner, S.V., Weinl, S., Holtkamp, M., Karst, U., Kudla, J., and Hippler, M. (2011). The Chloroplast Calcium Sensor CAS Is Required for Photoacclimation in *Chlamydomonas reinhardtii*. Plant Cell *23*, 2950–2963.

126. Wang, L., Yamano, T., Takane, S., Niikawa, Y., Toyokawa, C., Ozawa, S., Tokutsu, R., Takahashi, Y., Minagawa, J., Kanesaki, Y., *et al*. (2016). Chloroplast-mediated regulation of CO_2 -concentrating mechanism by Ca^{2+} -binding protein CAS in the green alga *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA *113*, 12586–12591.

127. Baughman, J.M., Perocchi, F., Girgis, H.S., Plovanich, M., Belcher-Timme, C.A., Sancak, Y., Bao, X.R., Strittmatter, L., Goldberger, O., Bogorad, R.L., *et al*. (2011). Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature *476*, 341–345.

128. De Stefani, D., Raffaello, A., Teardo, E., Szabò, I., and Rizzuto, R. (2011). A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature *476*, 336–340.

129. Wagner, S., Behera, S., De Bortoli, S., Logan, D.C., Fuchs, P., Carraretto, L., Teardo, E., Cendron, L., Nietzel, T., Füßl, M., *et al*. (2015). The EF-hand Ca^{2+} binding protein MICU choreographs mitochondrial Ca^{2+} dynamics in Arabidopsis. Plant Cell *27*, 3190–3212.

130. Rizzuto, R., De Stefani, D., Raffaello, A., and Mammucari, C. (2012). Mitochondria as sensors and regulators of calcium signalling. Nat. Rev. Mol. Cell Biol. *13*, 566–578.

131. Foskett, J.K., and Philipson, B. (2015). The mitochondrial Ca^{2+} uniporter complex. J. Mol. Cell. Cardiol. *78*, 3–8.

132. He, D., Fiz-Palacios, O., Fu, C.-J., Fehling, J., Tsai, C.-C., and Baldauf, S.L. (2014). An alternative root for the eukaryote tree of life. Curr. Biol. *24*, 465–470.

133. Scherzer, S., Krol, E., Kreuzer, I., Kruse, J., Karl, F., von Rüden, M., Escalante-Perez, M., Müller, T., Rennenberg, H., Al-Rasheid, K.A.S., *et al*. (2013). The *Dionaea muscipula* ammonium channel DmAMT1 provides NH_4^+ uptake associated with Venus flytrap's prey digestion. Curr. Biol. *23*, 1649–1657.