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Review

Increasing complexity and versatility: How the calcium signaling toolkit was shaped during plant land colonization

Kai H. Edel [a], Jörg Kudla [a,b,*]

[a] Institut für Biologie und Biotechnologie der Pflanzen, Universität Münster, Schlossplatz 4, 48149 Münster, Germany
[b] College of Science, King Saud University, Riyadh 11451, Kingdom of Saudi Arabia

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ABSTRACT

Calcium serves as a versatile messenger in adaptation reactions and developmental processes in plants and animals. Eukaryotic cells generate cytosolic Ca^{2+} signals *via* Ca^{2+} conducting channels. Ca^{2+} signals are represented in form of stimulus-specific spatially and temporally defined Ca^{2+} signatures. These Ca^{2+} signatures are detected, decoded and transmitted to downstream responses by an elaborate toolkit of Ca^{2+} binding proteins that function as Ca^{2+} sensors.

In this article, we examine the distribution and evolution of Ca^{2+}-conducting channels and Ca^{2+} decoding proteins in the plant lineage. To this end, we have in addition to previously studied genomes of plant species, identified and analyzed the Ca^{2+}-signaling components from species that hold key evolutionary positions like the filamentous terrestrial algae *Klebsormidium flaccidum* and *Amborella trichopoda*, the single living representative of the sister lineage to all other extant flowering plants.

Plants and animals exhibit substantial differences in their complements of Ca^{2+} channels and Ca^{2+} binding proteins. Within the plant lineage, remarkable differences in the evolution of complexity between different families of Ca^{2+} signaling proteins are observable. Using the CBL/CIPK Ca^{2+} sensor/kinase signaling network as model, we attempt to link evolutionary tendencies to functional predictions. Our analyses, for example, suggest Ca^{2+} dependent regulation of Na^{+} homeostasis as an evolutionary most ancient function of this signaling network. Overall, gene families of Ca^{2+} signaling proteins have significantly increased in their size during plant evolution reaching an extraordinary complexity in angiosperms.

1. Introduction

Spatially and temporally defined changes in calcium (Ca^{2+}) concentration fulfill important signaling and regulatory functions in all eukaryotes [1,2]. Fundamental for the evolution of Ca^{2+} as a messenger ion was likely its cellular toxicity as it precipitates with phosphates if present in high enough intracellular concentration. Ca^{2+} ions are highly abundant in seawater and terrestrial environments, while cytosolic Ca^{2+} concentration in eukaryotes is maintained at very low levels of 100–200 nM. It is generally assumed that the evolution of ancient cellular detoxification systems that actively extruded Ca^{2+} ions out of the cell or sequestered this ion into membrane-bonded organelles set the stage for the emergence of Ca^{2+} signaling systems. The active extrusion of Ca^{2+} from the cytoplasm resulted in a massive concentration gradient across the plasma membrane (PM; up to 20,000-fold) and endomembranes [3]. In this situation Ca^{2+} fluxes *via* Ca^{2+} conducting channels could rapidly increase the cytosolic Ca^{2+} concentration. Conversely, since only relative minor amounts of Ca^{2+} ions are required for efficient concentration increases, membrane resident energy-driven Ca^{2+} pumps could not only promptly restore non-toxic pre-elevation Ca^{2+} concentrations but also likely contribute to the shape of stimulus specific Ca^{2+} signatures [4]. Evolutionary factors that drove the development of Ca^{2+} as an important messenger have been explored in more detail in several recent reviews [5–8].

In response to specific Ca^{2+} signatures, a wealth of downstream effector proteins is modulated to further transmit and decode the primary signal [2]. Plants are equipped with an elaborate toolkit of Ca^{2+} binding proteins that function as Ca^{2+} sensors in signal decoding and propagation [9–13]. Such sensors include the ubiquitously conserved Calmodulins (CaM) and CaM-like proteins (CMLs) [9,10]. Through the function of Ca^{2+}-regulated kinases Ca^{2+} signals can be directly translated into protein phosphorylation events that orchestrate further downstream responses [3]. Ca^{2+}-dependent protein kinases (CDPKs) and Ca^{2+}/CaM activated kinases (CCaMKs)

* Corresponding author. Tel.: +49 251 83 24813.
E-mail addresses: kai.edel@uni-muenster.de (K.H. Edel), jkudla@uni-muenster.de (J. Kudla).

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are important representatives of such signaling molecules in plants [13–16]. A special case is provided by plant Calcineurin B-like proteins (CBLs) that were first identified in Arabidopsis [17]. In animals and fungi the Phosphatase 2B holoenzyme, designated as Calcineurin, is formed by interaction of its Ca^{2+}-binding regulatory subunit Calcineurin B with the enzymatic phosphatase subunit Calcineurin A [18]. Calcineurin has been shown to regulate a wide range of fundamental processes and targets from brain activity modulation in mammals to salt tolerance in yeast [19]. Despite their structural conservation, plant CBLs appear not to interact with a similar phosphatase but instead interact with and activate a specific family of serine-threonine kinases designated as CBL-interacting kinases (CIPKs) [20]. CBLs and CIPKs have been reported to form complex signaling networks in plants [11,12]. The function and evolution of plant Ca^{2+} signal-decoding systems has also been discussed in several recent publications [8,21]. Special features of the Ca^{2+} ion like its so-called diffusion anomaly also contribute to its versatility as signaling molecule during the signal decoding and transmission processes [22]. In simple saline solutions, hydrated Ca^{2+} can diffuse 40 μm/s (40 nm/ms), a mobility that is never achieved in the cytoplasm of eukaryotic cells [3,23]. Here, a free molecule of Ca^{2+} has been determined to have a half-life of around 25 μs and an ability to diffuse no further than 100–500 nm at maximum [22,24]. These features on one hand allowed the establishment of spatial and temporal specificity in Ca^{2+}-based signaling processes and on the other hand defined the frame within which Ca^{2+} signaling systems and processes can evolve. In this article, we survey the currently existing knowledge about the evolution of Ca^{2+} signaling systems in plants and comparatively discuss the occurrence and function of Ca^{2+} conducting channels in the animal and plant kingdoms. Moreover, based on recently published genome sequences we extend this analysis by including our genomics analyses of Ca^{2+} signaling components in key reference species that reflect crucial events in plant evolution like the colonization of terrestrial habitats and the evolution of angiosperms. Using the CBL/CIPK Ca^{2+} sensor-kinase network as a model, we place special emphasis on emerging themes like evolution of signaling network complexity and versatility.

2. Being special: differences in animal and plant Ca^{2+} signature formation

All eukaryotic cells show a remarkably high dependency on Ca^{2+} ions as a second messenger. Shape, amplitude and duration of Ca^{2+} influx that are presented as stimulus specific Ca^{2+} signatures, likely encode the information needed for the cellular response [4]. A unifying feature of Ca^{2+} signaling in the eukaryotic domain lies in the requirement of Ca^{2+} conductive channels that are responsible for the Ca^{2+} influx into the cytoplasm. Quite remarkably, already previous studies reported significant differences in the equipment of such channels between plant and animal species [6,25]. Together these analyses already revealed that Ca^{2+} conductive channels that play prominent roles in Ca^{2+} signal generation in ophistokonts, like voltage dependent Ca^{2+} channels (VDCCs), transient receptor potential (TRP) channels and IP_3 receptors (IP_3Rs) might have been lost sometime during the evolution of land plants for diverse reasons [7,25].

To extend the resolution and coverage of the evolutionary analyses of Ca^{2+} conducting channels we considered the published analyses of model species genomes like the higher plant *Arabidopsis thaliana*, the moss *Physcomitrella patens* and the algae *Ostreococcus tauri* and *Chlamydomonas reinhardtii* [26–29]. Moreover, we included reference data from humans (*Homo sapiens*), fruit flies (*Drosophila melanogaster*) and yeast (*Saccharomyces cerevisiae*) [30–32]. To extend this data set we analyzed recently published plant genomes to produce a comprehensive overview of Ca^{2+} channels within the Viridiplantae lineage (Fig. 1). These representatives involved the charophyte algae *Klebsormidium flaccidum* that evolutionary marks the transition to terrestrial habitats and represents the sister group to all embryophytes [33]. We also included the lycophyte *Selaginella moellendorffii* [34]. Moreover, we analyzed the genomes of *Pinus taeda* and *Picea abies* as representatives of gymnosperms and included *Amborella trichopoda* as the single living representative of the sister lineage to all other extant flowering plants [35–37]. To extend the data set on angiosperms we also analyzed the monocot *Oryza sativa* and the eudicot *Solanum lycopersicum* [38,39]. It should be noted that some of the examined genomes are only early release versions leaving a degree of uncertainty about the absolute number of identified genes.

In agreement with previous studies we noticed that homologous sequences to classic animal Ca^{2+} channels such as IP_3Rs, VDCCs, TRPs, purinergic P2X receptors (P2XRs) and cysteine loop channels (Cys-loop) are in principle also present in algal genomes but cannot be identified in higher plants [6,25]. This supports the conclusion that these channels have been lost during the evolution of plants and that plants have evolved specific Ca^{2+} release components. Importantly, IP_3Rs, P2XRs, Cys-loop and TRPs are already absent in the charophyte *K. flaccidum* indicating a loss of these channels before divergence of Embryophyta and Charophyta (Fig. 1). However, this interpretation still requires to consider that solid electro-physiological evidence supports the existence of Ca^{2+}-specific voltage-gated and mechano-activated channel currents in higher plants, despite our lack of knowledge about the molecular identity of the channels underlying these activities [40,41]. Remarkably, we also observed significant differences in the endowment of different algae and plant lineages with Ca^{2+} conducting channels. In addition to a brief introduction into the function of the channel classes, these differences in channel distribution across the plant kingdom are detailed in the following paragraphs.

2.1. Voltage dependent Ca^{2+} channels (VDCCs)

Animal VDCCs form extensive gene families in humans and fruit fly and have been reported to be crucially involved in many processes such as neurotransmission, secretion or regulation of gene transcription. In yeast the VDCC Ca^{2+} channel homologue 1 (CCH1) is involved in abiotic stress responses and mating [42–44]. A complement of nine potential VDCCs is encoded in the genome of *C. reinhardtii* [25]. Of these CAV2 is to date the only characterized voltage dependent channel in the Viridiplantae group. CAV2 is required for flagella waveform conversion and mutated forms (*e.g.* ppr2) show for example defects in the photophobic response [45]. Previous studies already reported the presence of VDCCs in many other taxa including diatoms, brown algae and oomycetes, that all harbor at least one representative VDCC [6].

Homologue sequences for VDCCs or voltage gated Ca^{2+} channels (Ca_Vs) appear to be absent in all embryophyte species. They are however present in green algae (Fig. 1) up to the charophytes (represented by *K. flaccidum*) which are the sister group of land plants. This situation suggests that VDCCs have been lost during the colonization of land but before the evolution of more advanced Streptophyta. In animal VDCCs, the selectivity for Ca^{2+} ions is conferred by four conserved aspartate residues in the conserved pore-forming P-loop domains [46]. These residues are conserved in some but not all VDCCs from algae, suggesting that these channels may vary in ion selectivity [6]. However, this aspect of VDCC function in algae requires further experimental investigation.

2.2. Mechanosensitive ion channel (MSLs)

Mechanosensitive ion channels are named after the bacterial mechanosensitive channel of small conductance (MscS). In bacteria, this diverse family of ion channels is required for

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