REVIEW PAPER

Plant organellar calcium signalling: an emerging field

Simon Stael1, Bernhard Wurzinger1, Andrea Mair1, Norbert Mehlmer2, Ute C. Vothknecht2,3 and Markus Teige1,*

1 Department of Biochemistry and Cell Biology, MFPL, University of Vienna, Dr Bohrgasse 9, A-1030 Vienna, Austria
2 Department of Biology I, Botany, LMU Munich, Großhaderner Str. 2, D-82152 Planegg-Martinsried, Germany
3 Center for Integrated Protein Science (Munich) at the Department of Biology of the LMU Munich, D-81377 Munich, Germany
* To whom correspondence should be addressed. E-mail: markus.teige@univie.ac.at

Received 25 August 2011; Revised 2 November 2011; Accepted 8 November 2011

Abstract

This review provides a comprehensive overview of the established and emerging roles that organelles play in calcium signalling. The function of calcium as a secondary messenger in signal transduction networks is well documented in all eukaryotic organisms, but so far existing reviews have hardly addressed the role of organelles in calcium signalling, except for the nucleus. Therefore, a brief overview on the main calcium stores in plants—the vacuole, the endoplasmic reticulum, and the apoplast—is provided and knowledge on the regulation of calcium concentrations in different cellular compartments is summarized. The main focus of the review will be the calcium handling properties of chloroplasts, mitochondria, and peroxisomes. Recently, it became clear that these organelles not only undergo calcium regulation themselves, but are able to influence the Ca2+ signalling pathways of the cytoplasm and the entire cell. Furthermore, the relevance of recent discoveries in the animal field for the regulation of organellar calcium signals will be discussed and conclusions will be drawn regarding potential homologous mechanisms in plant cells. Finally, a short overview on bacterial calcium signalling is included to provide some ideas on the question where this typically eukaryotic signalling mechanism could have originated from during evolution.

Key words: Bacteria, calcium flux, calcium signalling, CAS, chloroplast, EF-hand protein, ER, mitochondria, peroxisome.

Introduction

Plants react to changing environmental conditions through immediate signal transduction pathways. One integral part of many signal transduction pathways, in both plant and animal cells, is the usage of free calcium ions (Ca2+) as secondary messengers. As Ca2+ forms insoluble precipitates with phosphate, which would interfere with phosphate-based metabolism, cells actively translocate Ca2+ from their cytoplasm to organelles and extracellular compartments. This principle has been conserved throughout evolution from bacteria to eukaryotes. The resulting ~10 000-fold difference between cytoplasmic (~100 nM) and non-cytoplasmic (mM) Ca2+ concentrations enables the generation of calcium signals by fast changes of cytoplasmic Ca2+ levels via membrane-localized Ca2+-permeable channels. A wide variety of signals, including abiotic, biotic, and developmental stimuli, were observed to evoke specific spatiotemporal calcium transients which are further transduced by Ca2+ sensor proteins into a transcriptional and metabolic response. So far in plants, most of the research on Ca2+ signalling has been focused on the transport mechanisms for Ca2+ into and out of the cytoplasm as well as the components involved in decoding of cytoplasmic Ca2+ signals, which has been extensively reviewed (Clapham, 2007; McAINISH and Pitman, 2009; DeFalco et al., 2010; Dodd et al., 2010; Kudla et al., 2010). However, recent advances demonstrate how different organelles are involved in the process of Ca2+ signalling. Therefore, this review starts with a brief summary of the role of the main Ca2+ storage compartments [the vacuole, endoplasmic reticulum (ER) and apoplast] and the main focus is on the emerging role of Ca2+ signalling processes in plastids, mitochondria, and peroxisomes. Finally, considering the endosymbiotic origin of chloroplasts and mitochondria, the
review will end with a glimpse at bacterial Ca\textsuperscript{2+} signalling in order to propose some ideas regarding the evolutionary background of Ca\textsuperscript{2+} signalling processes in plants.

**Main plant calcium stores: the vacuole, the ER, and the apoplast**

The vacuole

The main storage compartment of calcium in plants is the central vacuole. Consequently, plants become hypersensitive to Ca\textsuperscript{2+} if vacuolar uptake is hampered (Cheng et al., 2005). In dicotyledonous plants Ca\textsuperscript{2+} is preferentially stored in the leaf mesophyll rather than in the epidermal vacuole (Storey and Leigh, 2004; Conn et al., 2011). Estimates of the free vacuolar Ca\textsuperscript{2+} concentration range from 0.2 mM to 1–5 mM and can reach a maximum of 80 mM of total Ca\textsuperscript{2+} (free and bound Ca\textsuperscript{2+} combined, Fig. 1) (Conn and Gilliham, 2010). Most of the Ca\textsuperscript{2+} is tightly bound to chelating agents, such as malate, citrate, and isocitrate, and, therefore, is not readily available for calcium signalling. Calcium ions might also be transiently bound to proteins, in a fashion comparable with the classical Ca\textsuperscript{2+}-binding storage proteins of the ER (e.g. calreticulin). For example, a radish vacuolar Ca\textsuperscript{2+}-binding protein (RVCaB) was found to improve the calcium storage capacity of the vacuole (Yuasa and Maeshima, 2001), whereas similar proteins in Arabidopsis were only found associated with the plasma membrane and probably do not play a role in calcium storage (Kato et al., 2010).

Interestingly, evidence is emerging that calcium is not only stored in the vacuole, but plays an important role in signalling there as well, mainly by influencing the activity of tonoplast (vacuolar membrane) localized ion transporters (Peiter, 2011). Various Ca\textsuperscript{2+} channels and transporters were identified in the tonoplast (Pottosin and Schonknecht, 2007). In the case of ligand-gated channels, Ca\textsuperscript{2+} fluxes were measured upon the addition of the signalling molecules inositol 1,4,5-triphosphate (InsP\textsubscript{3}) and cyclic ADP-ribose (cADPR) (Allen et al., 1995), although this could not be repeated in a more recent study (Pottosin et al., 2009). Voltage-dependent Ca\textsuperscript{2+} channels and ligand-gated Ca\textsuperscript{2+} channels release Ca\textsuperscript{2+} into the cytoplasm and have been characterized mainly during the 1990s through electrophysiological studies (Sanders et al., 2002). Although they have been well characterized for almost 20 years now, no molecular identity has been found for these channels so far, except for TPC1. AtTPC1, which has no other homologues in Arabidopsis, was confirmed to be the slow vacuolar (SV, named after its voltage-gated characteristics) channel of the tonoplast (Hedrich and Neher, 1987; Peiter et al., 2005). Despite being the major vacuolar calcium release channel (Pottosin et al., 2009), debate surrounds the physiological contribution of the SV channel to calcium signalling, since knockout or overexpression of AtTPC1 has no effect on the calcium signals of a whole range of biotic and abiotic stresses (Ranf et al., 2008). Moreover, the robust phenotype of ipc1 mutants—impaired stomatal closure upon extracellular Ca\textsuperscript{2+} elevation—is not due to impaired Ca\textsuperscript{2+} homeostasis in guard cells (Peiter et al., 2005; Islam et al., 2010), and evidence for...
a physiologically relevant release of Ca$^{2+}$ from the vacuole still needs to be provided (Hedrich and Marten, 2011).

The ER

The calcium storage role of the ER is probably best known from human and animals, where the molecular mechanisms of calcium release from and uptake by the sarcoplasmic endoplasmic reticulum (SR; the ER of the muscle cell) during muscle contractions have been described in detail (Rossi and Dirksen, 2006). In animals, the total Ca$^{2+}$ concentration in the ER is estimated to be 2 mM, while the free Ca$^{2+}$ concentration varies between 50 μM and 500 μM (Fig. 1) (Coe and Michalak, 2009). In contrast, in plants, few data are available that describe the calcium storage properties of the ER. Research is hampered mainly due to the absence of direct homologues of the well-characterized mammalian InsP3 and ryanodine receptors that are responsible for ER Ca$^{2+}$ efflux in higher plant genomes. Interestingly though, the genomes of several algae species, including Volvox and Chlamydomonas, do contain these receptor proteins, suggesting they were present in ancestral eukaryotes and were lost to land plants after their divergence from the chlorophyte algae (Wheeler and Brownlee, 2008). Although higher plant genomes lack these canonical receptors, calcium fluxes have been measured from ER membrane preparations upon stimulation with InsP3 and cADPR (Muir and Sanders, 1997; Navazio et al., 2001). Moreover, the signalling molecule nicotinic acid adenine dinucleotide phosphate (NAADP), that was originally described to evoke calcium release from the ER in marine invertebrate species, is active in higher plants as well (Navazio et al., 2000). Voltage-gated Ca$^{2+}$ fluxes were also measured in ER membrane preparations of the tactile organs of Bryonia dioica (Klusener et al., 1995; Klusener et al., 1997) and in root-tip endomembranes of Lepidium sativum L. (Klusener and Weiler, 1999). However, whether this is a more widespread phenomenon in the ER of higher plants still needs to be established.

Calreticulin, a luminal Ca$^{2+}$-binding protein of the ER, was found to be important for Ca$^{2+}$ homeostasis in plants, but also serves as a chaperone for protein folding (Christensen et al., 2010). The overexpression of maize calreticulin in tobacco cells lead to increased Ca$^{2+}$ retention in the ER, and the down-regulation of calreticulin expression in Arabidopsis resulted in enhanced sensitivity of the plants to low Ca$^{2+}$ (Persson et al., 2001). A comparison of Ca$^{2+}$-binding proteins between animals and plants suggests that calreticulin is the major Ca$^{2+}$ storage protein in plants (Nagata et al., 2004). In conclusion, little is known about plant ER Ca$^{2+}$ storage and release compared with the animal field, which is reflected by the fact that the molecular identity of the plant ER Ca$^{2+}$ release channel(s) is still unknown to date.

The apoplast

The apoplast is another major plant Ca$^{2+}$ store. At the same time it acts as the ‘highway’ through which Ca$^{2+}$ is trafficked to the cells by means of the water transpiration stream. Furthermore, the Ca$^{2+}$ concentration in the apoplast needs to be tightly regulated as a high apoplastic Ca$^{2+}$ concentration impairs stomatal movement (Kim et al., 2010) and plant cell wall rigidity depends on Ca$^{2+}$ for pectate cross-linking (Hepler, 2005). That these two reasons can drastically affect plant growth was recently demonstrated by the analysis of a mutant plant that is deficient in the main vacuolar Ca$^{2+}$ importers AtCAX1 and AtCAX3 (Conn et al., 2011). The authors reasoned that deficient Ca$^{2+}$ sequestration in the vacuole led to an increase in free Ca$^{2+}$ in the apoplast, with the above-mentioned defects as a consequence. Most of the Ca$^{2+}$ in the apoplast is bound to negatively charged carboxyl groups of pectin and oxalates, and reports on the concentration of Ca$^{2+}$ vary from 10 μM to 10 mM (Hepler, 2005).

In conclusion, it should be noted that being large calcium stores does not automatically mean that the apoplast and the vacuole are the only relevant organelles for calcium signalling in plants. The vacuole and apopoplast, but also the ER, each contain an equally high potential for unloading Ca$^{2+}$ into the cytoplasm, include the cyclic nucleotide-gated channels (CNGCs) and glutamate receptor-like channels (GLRs). Both gene families in Arabidopsis contain 20 members and they can carry a diversity of ions, but some members have been implicated in mediating cytosolic increases of Ca$^{2+}$ (Ali et al., 2006, 2007; Hua et al., 2003; Qi et al., 2006; Kaplan et al., 2007). All CNGCs and GLRs studied so far are targeted to the plasma membrane.

Organellar calcium signalling

**Chloroplast calcium signalling**

Already some decades ago it was found that Ca$^{2+}$ modulates the metabolic reactions of the chloroplast. Elevated Ca$^{2+}$...
concentrations effectively inhibit the Calvin–Benson cycle enzymes fructose-1,6-bisphosphatase and sedoheptulose bisphosphatase, leading to a halt of photosynthetic CO₂ fixation (Racker and Schroeder, 1958; Portis and Heldt, 1976; Charles and Halliwell, 1980). The total concentration of Ca²⁺ in the chloroplast has been estimated at 15 mM or higher (Nobel, 1969; Portis and Heldt, 1976) and increases upon illumination, by the uptake of Ca²⁺ from the external medium during the daylight (Kreimer et al., 1985; Roh et al., 1998). Because a high amount of free chloroplastic Ca²⁺ would inhibit photosynthesis and precipitate abundant chloroplastic phosphate, most of the Ca²⁺ is bound to thylakoid membranes or to stromal proteins (Gross and Hess, 1974; Davis and Gross, 1975; Kreimer et al., 1987).

Accordingly, the resting free Ca²⁺ concentration in the stroma during the day was estimated to be ~150 nM (Fig. 1) (Johnson et al., 1995). Calcium also affects the photosynthetic reactions from the luminal side of the thylakoid. It is an essential cofactor of the oxygen-evolving complex and binds the 8 kDa subunit of the ATP synthase, thereby regulating the photosynthetic proton flow and ATP production (Zakharov et al., 1993; Ifuku et al., 2010). So, the chloroplast has an essential requirement for Ca²⁺, but needs tight control over its distribution, as indicated in Fig. 2.

Using chloroplast-targeted Aequorin reporter lines, calcium fluxes were detected in chloroplasts in daily rhythms after the transition from light to dark (Johnson et al., 1995; Sai and Johnson, 2002). This stromal Ca²⁺ flux after the light–dark transition is proposed to be responsible for inhibiting photosynthetic CO₂ fixation during the night and could help to entrain the circadian clock. The characteristics of these Ca²⁺ fluxes suggest that chloroplasts take up Ca²⁺ from the cytosol during the light period and store it in the thylakoid membrane or in another as yet unknown store. Upon transition from light to dark the Ca²⁺ is subsequently released from the store back into the cytosol. Light, via the thylakoid proton gradient, seems to drive Ca²⁺ uptake into the thylakoid lumen as well, through the activity of a Ca²⁺/H⁺ exchanger (Ettinger et al., 1999). However, the inhibition of the photosynthetic electron transport chain, and correspondingly the proton gradient, resulted in a slight increase of stromal Ca²⁺ during the light period, but did not inhibit the charging of the Ca²⁺ store that was discharged by lights off (Sai and Johnson, 2002). Hence, the authors proposed the existence of an unknown alternative stromal Ca²⁺ store.

The molecular nature of the chloroplast Ca²⁺ store is a long-standing question, and the massive difference between the total and free resting Ca²⁺ concentration (Fig. 1) calls for a strong Ca²⁺-buffering mechanism in the chloroplast. It has long been known that the net negative charge of the thylakoid surface, which has been referred to as ‘a diffuse electrical layer’ (Barber et al., 1977), is balanced by the positive charge of magnesium ions (Mg²⁺) (Nakatani et al., 1979; Jennings et al., 1981). Recently,

**Fig. 2.** Overview of proteins involved in calcium signalling in chloroplasts, mitochondria, and peroxisomes of plants. Calcium and calmodulin (CaM) are highlighted by red boxes. Calcium transporters are depicted in yellow, and calcium/CaM-binding proteins in blue. EF-hand-containing proteins are indicated by the presence of an ‘EF’-box. Proteins of uncertain nature (lack of protein identity or uncertain function) are indicated with a (?). The central ‘Ca²⁺’ symbol indicates the possibility of calcium exchange between organelles and the contribution of organelles to the cytoplasmic calcium concentration. Full names for protein abbreviations can be found in the text. Other abbreviations are Calvin (Calvin–Benson cycle), TCA (tricarboxylic acid cycle), IE (inner envelope), OE (outer envelope), IM (inner membrane), OM (outer membrane).
Fristedt and colleagues reported that the negative charge is mainly due to the gross phosphorylation of the photosynthetic complexes and that mutation of the involved kinases, STN7 and STN8 (State Transition 7 and 8), influences the Mg²⁺-dependent stacking of the thylakoid (Fristedt et al., 2009, 2010). Furthermore, the STN7 and STN8 kinases regulate phosphorylation of the photosynthetic complexes in a light-dependent manner (Depege et al., 2003). Since Ca²⁺ has highly similar metal properties to Mg²⁺, it is feasible that phosphorylated protein residues of the thylakoid account for much of the Ca²⁺-buffering capacity of the chloroplast. Considering Ca²⁺ transients on light to dark transitions, the link with thylakoid protein phosphorylation seems an interesting route for further investigation. Furthermore, the influence of pH should also be considered, as pH is known to influence the binding capacity of Ca²⁺ storage proteins (Hidalgo et al., 1996), due to protonation of the acidic residues involved in Ca²⁺ binding. Particularly in chloroplasts, where dramatic differences in pH are present during light conditions and change in the transition from light to dark, this is expected to impact on calcium signalling significantly.

Active Ca²⁺ transport has also been measured across the chloroplast inner envelope and could account for the observed Ca²⁺ uptake of the chloroplast during the light period. A negative inside membrane potential-driven Ca²⁺ transport (uniport) was measured from inner envelope membrane vesicles of *Pisum sativum*, confirming previous experiments with intact chloroplasts (Kreimer et al., 1985; Roh et al., 1998). This observation is strengthened by measurements with a calcium ion-selective microelectrode in intact algal cells that recorded a photosynthesis-dependent decrease in cytoplasmic Ca²⁺ levels (Miller and Sanders, 1987). However, the molecular identity of this channel has not been described. On the other hand, two potential Ca²⁺ ATPases were identified in the chloroplast envelope. The first is AtACA1 from the autoinhibited Ca²⁺-ATPases (Hidalgo et al., 1996), due to protonation of the acidic residues involved in Ca²⁺ binding. Particularly in chloroplasts, where dramatic differences in pH are present during light conditions and change in the transition from light to dark, this is expected to impact on calcium signalling significantly.

From more recent work, it became apparent that calmodulin (CaM) has regulatory roles in chloroplasts. The best example is the import of nuclear-encoded chloroplast proteins via the TOC (translocon at the outer envelope of chloroplasts) and TIC (translocon at the inner envelope of chloroplasts) complexes. Calcium/CaM was found to promote chloroplast import, and this is most likely to be due to the direct interaction of calmodulin with the stromal side of Tic32 (TIC protein of 32 kDa) (Chigiri et al., 2005, 2006). Furthermore, in a different study, electrophysiological measurements revealed that the gating properties of the main pore-forming subunit of the TIC complex, Tic110, were affected in a specific manner by Ca²⁺ (Balsera et al., 2009). Taken together, Ca²⁺ seems to influence chloroplast protein import at these closely linked sites. Another function of the chloroplast that was found to be modulated by Ca²⁺ is the chloroplast inner vesicle transport system (Morre et al., 1991). This system is proposed to have a role in thylakoid membrane biogenesis and was found to be disrupted by CaM inhibitors as well as calcium depletion (Westphal et al., 2001, 2003). Chloroplast division might also be regulated in a Ca²⁺-dependent manner by AtMinD1 (*Arabidopsis* Minicell D1), which is a part of the chloroplast division machinery, because its ATPase activity depends on Ca²⁺, rather than Mg²⁺ as is the case in bacteria (Aldridge and Moller, 2005). Furthermore, two chloroplastic mechanosensitive ion channels MSL2 and 3 [homologues of the bacterial mechanosensitive (MS) channel MscS: MscS-Like 2 and 3] were found to influence chloroplast division and act in concert with the Min proteins (Haswell and Meyerowitz, 2006; Wilson et al., 2011). It would be interesting to see if MSL2 and 3 can directly influence the Ca²⁺-dependent activity of AtMinD1, either by mediating Ca²⁺ fluxes or through a depolarization of the chloroplast envelope.

Chloroplast movement in response to fluctuating light conditions is another Ca²⁺-dependent process shown to occur in different species, such as *Lemma trisulca* and tobacco (Tilarka and Fricker, 1999; Anielska-Mazurek et al., 2009). Chloroplasts move along the actin cytoskeleton in plant cells (Kong and Wada, 2011), and accordingly the use
of Ca\(^{2+}\) chelators and CaM inhibitors revealed the stabilizing effect of Ca\(^{2+}\) on actin polymerization and its importance for chloroplast movement. However, chloroplast movement was influenced even in the presence of an intact actin network, thereby evoking a signalling function of Ca\(^{2+}\) in light-induced chloroplast movements.

NAD kinase (NADK) is an intriguing case for chloroplastic calcium-dependent regulations. Through the use of CaM inhibitors, NADK was the first plant enzyme found to be activated by CaM, and the majority of its activity was found to localize to the chloroplast (Jarrett et al., 1982). Jarrett and colleagues purified a CaM-containing fraction from pea chloroplasts to near homogeneity, but did not identify the protein responsible for NADK activation. The initial increase of Ca\(^{2+}\) in the chloroplast upon illumination was proposed to activate NADK via the interaction with CaM. NADK catalyses the light-dependent conversion of NADPH to NADP\(^{+}\). Calcium-dependent NADK activity in chloroplasts (Turner et al., 2004; Waller et al., 2010). A region of 45 amino acids within the long N-terminal extension of AtNADK2 was found to be sufficient and necessary to bind to CaM. Other examples of chloroplast proteins that were found to interact with CaM are AtPsaN (a subunit of photosystem I), the chaperonin AtCPN10, and AtAFG1L1, an AAA+-ATPase (Yang and Poovaiah, 2000; Reddy et al., 2002; Bussemmer et al., 2009). However, the functional relevance of these interactions needs further study. To conclude, although a chloroplastic CaM or CaM-like protein is still unknown, various stromal proteins are able to bind to CaM, leading to a change of their activity. In the same context, a Ca\(^{2+}\)-dependent protein kinase activity and cross-talk between Ca\(^{2+}\) signalling and protein phosphorylation is described in two accompanying papers in this issue (Bayer et al., 2012; Stael et al., 2012). Therefore, the identification of CaMs or other Ca\(^{2+}\)-binding proteins in chloroplasts is expected greatly to advance the understanding of the physiological relevance of these calcium/CaM-dependent regulations.

So far there are two reports of EF-hand-containing proteins in the chloroplast. The best documented is the Ca\(^{2+}\)-activated RelA/SpoT homologous protein (CRSH), that is novel both for being a chloroplastic EF-hand protein and for its alleged function. CRSH contains two EF-hands and a RelA/SpoT enzymatic domain, which is responsible for the calcium-dependent production of a small signalling nucleotide, guanosine 5’-diphosphate 3’-diphosphate (ppGpp) (Masuda et al., 2008). ppGpp signalling was discovered in bacteria as a response to stress conditions, such as nutrient deprivation, in a process called ‘the bacterial stringent response’, and homologous proteins have since been found in various plant species (Tozawa et al., 2007; Masuda et al., 2008). The bacterial RelA and SpoT proteins do not contain EF-hands, which seems to be an exclusive trait of CRSH in higher plants. ppGpp was found mainly in the chloroplast, and the levels changed after different stress and hormonal treatments and upon the transition from light to dark (Takahashi et al., 2004). Similar to its function in transcription and translation in bacteria, ppGpp was found to modulate exclusively the function of the bacterial type plastid-encoded plastid RNA polymerase (PEP), but not the nuclear-encoded plastid RNA polymerase (NEP) (Sato et al., 2009). It seems that the bacterial stringent response has been conserved in chloroplasts from its cyanobacterial origin, but more experimental work is needed to elucidate its physiological function and the interplay with Ca\(^{2+}\) signalling, in plants. The second chloroplastic EF-hand protein is a substrate carrier (AtSAMTL, for SAMT-like) that contains a single EF-hand and belongs to the mitochondrial carrier protein family. It was recently found in a targeted proteomics screen and was confirmed to reside in the chloroplast envelope by yellow fluorescent protein (YFP) fusion analysis (Bayer et al., 2011). Based on a detailed functional prediction, it is proposed to import S-adenosylmethionine (SAM) into the chloroplast in addition to the better described SAM transporter, SAMT (Stael et al., 2011b).

Reports on a chloroplast-localized protein involved in ‘calcium sensing’ (AtCAS) evoke the idea that chloroplasts may modulate cytoplasmic Ca\(^{2+}\) signalling. AtCAS was first reported as a plasma membrane-localized Ca\(^{2+}\)-sensing receptor, important for inducing stomatal closure provoked by elevation of the extracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)\(_{ext}\)]—a hallmark of stomatal movement (Han et al., 2003). The protein was found to bind Ca\(^{2+}\) with a low affinity and high capacity, and down-regulation of its expression impaired the production of [Ca\(^{2+}\)\(_{ext}\)]-induced cytoplasmic Ca\(^{2+}\) oscillations. However, subsequent reports identified AtCAS to be targeted to the thylakoid membrane (Friso et al., 2004; Nomura et al., 2008; Vainonen et al., 2008; Weinl et al., 2008). Nevertheless, knock-out of AtCAS was still confirmed, by different labs, to impair [Ca\(^{2+}\)\(_{ext}\)]-induced stomatal closure, whereas overexpression of CAS promoted stomatal closure in the absence of externally applied Ca\(^{2+}\) (Nomura et al., 2008; Weinl et al., 2008). Disruption of AtCAS in Arabidopsis retarded plant growth, and AtCAS was found to be increasingly phosphorylated by the state transition kinase AtSTN8 under increasing light intensities (Vainonen et al., 2008). Similarly, CAS is required for photoacclimation in the process of non-photochemical quenching (NPQ) in Chlamydomonas where cas-knockdown lines displayed a high-light-sensitive phenotype (Petroutos et al., 2011). Stomata of cas Arabidopsis plants displayed normal closure after the application of externally imposed cytoplasmic Ca\(^{2+}\) oscillations, indicating that the guard cells are still responsive to Ca\(^{2+}\) signals but most probably have a defect in the generation of Ca\(^{2+}\) fluxes (Weinl et al., 2008). This suggests that the chloroplast can sense and influence cytoplasmic Ca\(^{2+}\) levels; however, the molecular mechanisms behind these processes still await discovery.
Mitochondrial calcium signalling

In contrast to the animal and human field, where mitochondrial calcium uptake and release are well studied and known to play important cellular roles, relatively few data are available on calcium signalling in plant mitochondria. It is now well established that mitochondria in animals function as transient calcium stores that can produce Ca$^{2+}$ micro-domains through a close interaction with the ER, thereby modulating Ca$^{2+}$ signatures (Clapham, 2007; Laude and Simpson, 2009). On the other hand, the elevation of Ca$^{2+}$ inside the mitochondria positively affects ATP production by up-regulating the major limiting enzymes of the citric acid cycle (similar to the up-regulation of the Calvin cycle in chloroplasts) (McCormack et al., 1990). The overaccumulation of Ca$^{2+}$ in the mitochondria is linked to the induction of apoptosis by opening of the mitochondrial permeability transition pore (mPTP) and the subsequent release of mitochondrial apoptosis markers, such as cytochrome c (Giacomello et al., 2007; Szabadkai and Duchen, 2008). This process apparently occurs in plants as well (Arpagaus et al., 2002; Tiwari et al., 2002; Virolainen et al., 2002).

In plants, the resting free Ca$^{2+}$ concentration in the mitochondria was estimated to be ~200 nM (Logan and Knight, 2003), with most of the mitochondrial Ca$^{2+}$ probably being bound in the form of a ready-releasable amorphous phosphate precipitate (Fig. 1) (Chalmers and Nicholls, 2003; Starkov, 2010). Ca$^{2+}$ fluxes in Arabidopsis mitochondria have been observed upon various stimulations (Logan and Knight, 2003). Whereas most mitochondrial Ca$^{2+}$ fluxes were similar, H$_2$O$_2$ and touch stimulation produced a signal that was different from the concurrently occurring cytosolic Ca$^{2+}$ fluxes. This indicates that mitochondria are not just passive calcium sinks, but are able to regulate their own Ca$^{2+}$ fluxes as depicted in Fig. 2.

Surprisingly little information about Ca$^{2+}$ transporters of plant mitochondria exists, compared with the knowledge on this topic in the animal and human field (Collins and Meyer, 2010; Starkov, 2010). Animal mitochondria take up high or low concentrations of Ca$^{2+}$ differently, and various Ca$^{2+}$ channels have recently been described (Hajnoczky and Csordas, 2010; Hoppe, 2010). The mitochondrial calcium uniporter (MCU) imports Ca$^{2+}$ from ‘microdomains’ with highly elevated cytosolic Ca$^{2+}$ concentrations. MCU has been extensively studied and its properties are well delineated: (i) electrogenic transport that is driven by a negative inside membrane potential; (ii) sensitivity to ruthenium red; (iii) low affinity for Ca$^{2+}$; and (iv) regulation by Ca$^{2+}$. After almost 50 years, the molecular nature of the uniporter has been discovered in animals in a series of recent studies (Perocchi et al., 2010; Baughman et al., 2011; De Stefani et al., 2011). In an integrative genomics and proteomics search, Perocchi and colleagues found an EF-hand-containing protein, which is targeted to the mitochondrial inner membrane, to induce a Ca$^{2+}$-dependent calcium influx into the mitochondria. They called it MICU1, for mitochondrial Ca$^{2+}$ uptake 1, and reasoned that it is most probably not the uniporter itself, but the sensor for Ca$^{2+}$ that regulates the MCU. In two independent follow-up experiments, based on the phylogenetic profile, protein distribution, and characteristics of MICU1, a 40 kDa protein was identified as the actual Ca$^{2+}$ pore and therefore it was called MCU. The MCU protein has two transmembrane helices, connected by a conserved loop containing the amino acids DIME, and most probably oligomerizes in the mitochondrial inner membrane. Intriguingly, the Arabidopsis genome contains six uncharacterized genes with a rather low homology to the human MCU, which share the pore-forming domain with two transmembrane helices connected by a conserved DVME motive (Fig. 3A). These potential Arabidopsis MCU isoforms are highly predicted to localize to the mitochondria, except for At5g66650, which is equally predicted to target to the chloroplast according to Aramemnon (Schwacke et al., 2003) (Fig. 3B). A quick survey of the expression profiles by GENEVESTIGATOR (Zimmermann et al., 2004) suggests a differential tissue expression of the various isoforms, for example to the flower, root, or guard cell. The possibility that an MCU isoform is located in the chloroplast is supported by the fact that the light-dependent Ca$^{2+}$ influx in chloroplasts, reported by Kreimer et al. (1985), is inhibited by ruthenium red and that the overall characteristics are similar to the mitochondrial Ca$^{2+}$ uniport. Moreover, the potential chloroplastic localization and preferential guard cell expression of At5g66650 are interesting in the light of stomatal movement, since chloroplasts have been implicated in ‘sensing’ extracellular calcium transients through CAS protein and the modulation of stomatal closure.

In addition to the low-affinity MCU system, mammalian mitochondria take up Ca$^{2+}$ at lower cytoplasmic concentrations via the high-affinity Ca$^{2+}$/H$^+$ antiporter, LETM1 (Jiang et al., 2009). Interestingly, the closest Arabidopsis homologue of LETM1, the mitochondrial high-affinity Ca$^{2+}$/H$^+$ antiporter, contains two EF-hands and was recently found to localize to the mitochondria by green fluorescent protein (GFP) fusion analysis (Van Aken et al., 2009). In this study the authors attempted to delineate the mitochondrial stress response by searching for mitochondrial proteins that show the greatest expression variation upon 16 selected stress treatments. The fact that the AtLETM1-like protein was included in this set further prompts the study of this protein for its Ca$^{2+}$/H$^+$ activity and the involvement of mitochondrial calcium signalling upon stress conditions in Arabidopsis. In mammals, calcium efflux from mitochondria can occur through a reversal of LETM1 or through the Na$^+$/Ca$^{2+}$ exchanger NCLX, that was recently found to be targeted to the mitochondria (Palty et al., 2010). To conclude, the presence of homologues in the Arabidopsis genome of all the above-mentioned proteins raises the interesting possibility of translating the mammalian findings directly into plants.

Calcium/CaM was reported to promote mitochondrial protein import in a manner similar to how it occurs in chloroplasts (Kuhn et al., 2009). Furthermore, it was found to be a plant-specific trait, because yeast mitochondria were not susceptible to the CaM inhibitors and calcium ionophores used in the study. Pyruvate dehydrogenase activity
was also found to decline with the use of CaM inhibitors (Miernyk et al., 1987). The pyruvate dehydrogenase complex (PDC) assists in the conversion of pyruvate into acetyl-CoA and, therefore, an important connection between glycolysis and the citric acid cycle might be regulated by Ca2+ in plants. The finding of a CaM-binding AAA+-ATPase, AtAFG1L1, with dual localization to chloroplasts and mitochondria further suggests the presence of CaM in the plant mitochondria (Bussemer et al., 2009). So far, the only report on CaM in the mitochondria came from Biro and colleagues, who found an oat CaM in the intermembrane space that was lost upon removal of the outer mitochondrial membrane (Biro et al., 1984).

The glutamate dehydrogenase β subunit (GD-β) localizes to the mitochondrial matrix, contains a single EF-hand, and its activity was reported to be stimulated by the addition of Ca2+ in plants. The finding of a CaM-binding AAA+-ATPase, AtAFG1L1, with dual localization to chloroplasts and mitochondria further suggests the presence of CaM in the plant mitochondria (Bussemer et al., 2009). So far, the only report on CaM in the mitochondria came from Biro and colleagues, who found an oat CaM in the intermembrane space that was lost upon removal of the outer mitochondrial membrane (Biro et al., 1984).

Ample evidence for the influence of mitochondrial Ca2+ on cytoplasmic Ca2+ fluxes exists in animals (Laude and Simpson, 2009), and these observations probably hold true for plants, given that plant mitochondria exhibit a similar handling of Ca2+ (Silva et al., 1992). Maize mitochondria have been shown to release Ca2+ upon hypoxia, and their internal Ca2+ concentration changes rapidly and in a reversible manner when returning back to normoxia (Subbaiah et al., 1994, 1998). The recently described mitochondrial carrier proteins APC1, 2, and 3 (short for ATP-phosphate carrier1, 2, and 3) could be immediate targets of these mitochondrial Ca2+ fluxes (Stael et al., 2011b). APC1–APC3 each contain four EF-hands and are better known from yeast and mammals to import ATP into mitochondria in a Ca2+-dependent manner (Chen, 2004; Traba et al., 2008). Mitochondrial ATP import might be a first line of defence against hypoxia, in order to maintain the mitochondrial membrane potential and cell viability by a reversal of the F0F1 H+-ATP synthase. Other evidence for mitochondrial Ca2+ efflux comes from the measurement of Ca2+ changes in the root hair in response to the disruption of actin polymerization by latrunculin B (Lat-B) and Jasplakinolide (Jas) (Wang et al., 2010). Root hair cells produce a tip-focused Ca2+ gradient, with Ca2+ oscillation lagging behind growth oscillation, in a similar fashion to growing pollen tubes (Cardenas et al., 2008; Monshausen et al., 2008). Modulation of actin polymerization caused a release of Ca2+ from the mitochondria, probably via the mPTP, and a concurrent increase in cytosolic Ca2+ concentrations. Furthermore, Wang and colleagues showed that the concentration of free Ca2+ in mitochondria displays a gradient from a high concentration at the growing tip (~500 nM) to low at the base (~200 nM). Together with the observation that mitochondria shuttle up

---

**Fig. 3.** Conserved regulators of mitochondrial Ca2+ import between humans and plants. (A) Alignment of the transmembrane domain of the human mitochondrial calcium uniporter (MCU, gi24308400) with its six homologues from Arabidopsis thaliana displays the two transmembrane helices (TM1 and TM2) connected by a highly conserved loop domain containing the amino acids DIME in human and DVME in Arabidopsis. (B) Overview of targeting and expression of the MCU homologues. The cladogram displays the homology relationship between the Arabidopsis proteins. The blast e-value for each protein relative to the human MCU is written in parentheses after the Arabidopsis gene identifier (AGI). Protein localization was predicted by Aramemnon (Schwacke et al., 2003) and the meta-score is indicated in parentheses. Organ-related RNA expression profiles were examined with the help of the GENEVESTIGATOR browser (Zimmermann et al., 2004) and the presence of expressed sequence tags (ESTs) in the TAIR 10 database (www.arabidopsis.org).
Peroxisomal calcium signalling

Information on calcium signalling in the peroxisome is scarce. It was only recently that the occurrence of calcium fluxes in the peroxisome was recognized in mammals (Raychaudhury et al., 2006; Drago et al., 2008; Lasorsa et al., 2008). Two different resting free Ca²⁺ concentrations have been reported from these experiments, namely 150 nM (Drago et al., 2008) and 2 μM (Lasorsa et al., 2008). A similar study, based on the expression of a peroxisomal-targeted chameleon probe (a Ca²⁺ reporter protein construct), showed that plant peroxisomes undergo Ca²⁺ fluxes as well (Costa et al., 2010). Furthermore, the peroxisosomal Ca²⁺ increase was found to enhance the detoxification of the reactive oxygen species (ROS) H₂O₂ in vivo through the activity of the catalase isofrom 3 (AtCAT3), as was proposed earlier. In 2002, Yang and Poovaiah demonstrated the in vitro stimulation of AtCAT3 activity by the calcium-dependent binding of CaM, and provided evidence for the presence of CaM in peroxisomes. However, they did not find its molecular identity (Yang and Poovaiah, 2002). A member of the Arabidopsis calcium-dependent protein kinase family, AtCPK1, was found to bind to the external surface of peroxisomes (Dammann et al., 2003; Coca and San Segundo, 2010) and lipid bodies, which is most likely to be due to an N-terminal myristoylation signal, which determines the localization of many calcium-dependent protein kinases (CDPKs) and other kinases (Lu and Hrabak, 2002; Benetka et al., 2008; Stael et al., 2011a). AtCPK1 was shown to mediate pathogen resistance (Coca and San Segundo, 2010). Given the few reports, the already presented interplay between calcium and ROS prompts the further study of the molecular nature of calcium handling and signalling in and around peroxisomes.

Nuclear calcium signalling

Nuclear Ca²⁺ in plants has recently been reviewed comprehensively (Mazars et al., 2009, 2011) and will therefore be discussed only briefly. Calcium signals in the nucleus enable the cell to react to environmental changes by alteration of gene expression in animals and plants (Ikura et al., 2002; M.C. Kim et al., 2009; Galon et al., 2010; Reddy et al., 2011). This may sound straightforward; however, only recently were direct target genes of Ca²⁺-dependent gene expression reported in Arabidopsis (Kaplan et al., 2006). The main problem is to distinguish them from non-Ca²⁺-dependent gene expression mediated via different nuclear signalling pathways in response to stress treatment (Finkler et al., 2007; Wurzinger et al., 2011). Various studies revealed that many of the Ca²⁺-regulated genes contained abscisic acid-responsive element (ABRE)-related cis-elements and were already implicated in the abiotic stress response before (Hirayama and Shinozaki, 2010). Ca²⁺ can influence transcription through Ca²⁺-binding transcription factors, CaM-binding transcription activators [AtCAMTAs, six members in Arabidopsis (Bouche et al., 2002)], or phosphorylation of transcription factors by CDPKs, of which quite a number are present in the nucleus (Dammann et al., 2003; Choi et al., 2005; Zhu et al., 2007; Boudsocq et al., 2010; Mehmler et al., 2010).

Various stimuli such as abiotic or biotic stresses as well as symbiotic signals elicit nuclear Ca²⁺ fluxes (Pauly et al., 2000; Lecourieux et al., 2005; Oldroyd and Downie, 2006; Sieberer et al., 2009). A lot of the research on nuclear Ca²⁺ signalling has been focused on the question whether nuclei can generate Ca²⁺ fluxes autonomously from the cytosol. The nuclear resting free Ca²⁺ concentration was measured at ~100 nM (Brini et al., 1993; Mazars et al., 2009), which is similar to the cytosolic value (Fig. 1). The fact that the nucleoplasm and cytosol are connected by relatively large pores in the nuclear envelope suggests that nuclear Ca²⁺ fluxes are the result of passive influx from the cytosol. However, the delays that have been measured between cytosolic and nuclear Ca²⁺ fluxes implicate the opposite. Furthermore, when tobacco cells were treated with a biologically active derivate of jasmonate (jasmonate-isoleucine), nuclei were able to generate Ca²⁺ fluxes without any measurable cytosolic Ca²⁺ responses (Walter et al., 2007). Experiments with isolated nuclei further emphasized the autonomous nature of plant nuclei from the extranuclear environment with regards to Ca²⁺ signalling (Xiong et al., 2004, 2008).

If the nucleus is able to produce its own Ca²⁺ fluxes, than the nuclear envelope is most likely to serve as the responsible Ca²⁺ store. Analysis of its protein components has been hampered by the difficult extraction of intact nuclei and the contamination by ER membranes (Matzke et al., 2010). Nonetheless, several Ca²⁺ channels and transporters have been found at the inner and outer membrane of the nuclear envelope, and it is hypothesized that the nuclear pores can act as Ca²⁺-selective channels. Two interesting examples of nuclear ion channels are Castor and Polux. Initially they had been reported to localize to chloroplasts (Imaizumi-Anraku et al., 2005), but more recent work showed that they are cation channels in the nuclear envelope (Charpentiier et al., 2008). Mutation of Castor and Polux abolishes perinuclear Ca²⁺ spiking and, concurrently, root symbioses with arbuscular mycorrhizal fungi and rhizobial bacteria in legume species, such as Lotus japonicas, or non-legume species, such as rice (Chen et al., 2009). Root symbiosis in legumes is probably the best example to demonstrate the importance of nuclear calcium signalling and has been extensively reviewed elsewhere (Oldroyd and Downie, 2006; Murray, 2011).

Bacterial calcium signalling

The observation that Ca²⁺ signalling is present in organelles of endosymbiotic origin leads to the question of whether some basic mechanisms of generation or decoding of Ca²⁺ signals can also be found in bacteria. Ca²⁺ signalling is generally
considered to be a typical eukaryotic invention. However, bacteria also need to exclude Ca$^{2+}$ actively from the cytosol in order to operate their phosphate-based metabolism. Similar to eukaryotes, the resulting unequal distribution of free Ca$^{2+}$ between the cytosol and the extracellular space would provide the basis to use Ca$^{2+}$ as a signalling molecule. Furthermore, the discovery of various Ca$^{2+}$ transporters, Ca$^{2+}$-binding proteins, and Ca$^{2+}$-dependent processes all argue in favour of some calcium signalling taking place in bacteria.

It has been shown that the intracellular free Ca$^{2+}$ concentration in bacteria is actively maintained in the range of 100–300 nM (Knight et al., 1991; Herbaud et al., 1998; Jones et al., 1999; Torrecilla et al., 2000). Like in eukaryotes, distinct transient changes in the cytoplasmic Ca$^{2+}$ concentration were also observed in bacteria (Domínguez, 2004) for different stimuli such as heat, cold (Torrecilla et al., 2000), salt, osmotic stress (Torrecilla et al., 2001), and oxidative stress (Herbaud et al., 1998), or in response to nitrogen deprivation (Torrecilla et al., 2004; Zhao et al., 2005; Shi et al., 2006; Leganes et al., 2009).

An early bioinformatic investigation revealed the presence of Na$^+$, K$^+$, and Ca$^{2+}$ selective voltage-gated ion channels, Ca$^{2+}$ cation antiporters, and P-type Ca$^{2+}$-ATPases in 18 bacterial genomes based on sequence homology to eukaryotic counterparts (Paulsen et al., 2000). Since then, experimental evidence for Ca$^{2+}$ transporters in bacteria has accumulated (Raeymaekers et al., 2002; Fujisawa et al., 2009; Faxen et al., 2011). In the cyanobacteria, Synechococcus elongatus PCC 7942, the PacL gene product was identified as a P-type Ca$^{2+}$-ATPase (Berkelman et al., 1994), and in Synechocystis sp. PCC 6803 the mechanosensitive ion channel MscL was reported to be responsible for major Ca$^{2+}$ effluxes observed after membrane depolarization and high temperature (Nazarenko et al., 2003). Finally, Ca$^{2+}$/H$^+$ antiporters, homologous to the CAX gene family in Arabidopsis, have also been identified in cyanobacterial genomes (Waditee et al., 2004). The CAX protein from Synechocystis sp. PCC 6803 was shown to be biochemically functional, localized at the plasma membrane, and required for salt tolerance, as well as for adaptation to an alkaline milieu in this strain. In contrast, molecular identities of Ca$^{2+}$-specific influx systems in bacteria are largely missing so far.

If intracellular Ca$^{2+}$-mediated signalling should take place in bacteria like in eukaryotic cells, then calcium sensors in the form of Ca$^{2+}$-binding proteins must exist. During the last decade various successful attempts have been made to identify EF-hand-containing proteins in bacterial genomes (Michiels et al., 2002; Zhou et al., 2006; Rigden et al., 2011). Globally, it can be said that EF-hand-containing proteins are found in most bacterial genomes and that they are evenly distributed throughout families and genera. In addition, new calcium-binding motifs are predicted and found in bacterial proteins, such as the Dx[DN]xDG motif, which is part of the EF-hand motif. This further increases the number of potential Ca$^{2+}$-binding proteins in bacteria (Morgan et al., 2006; Hu et al., 2011; Rigden et al., 2011). For example, the calmodulin-related protein CasA from the symbiotic bacterium Rhizobium etli is secreted during infection and colonization of its plant host (Xi et al., 2000).

How calcium signalling can impact on bacterial physiology is best illustrated by the effect of a Ca$^{2+}$-binding protein on nitrogen fixation. CcbP, a non-EF hand-containing protein from Nostoc (Anabaena) sp. PCC7120, has been demonstrated to be an intracellular Ca$^{2+}$-binding protein which is involved in heterocyst differentiation upon limitation of combined nitrogen (Zhao et al., 2005; Hu et al., 2011). Several genera of cyanobacteria grow heterocysts, which are specialized cells capable of fixing N$_2$, and they make up a considerable part of the global nitrogen cycle. At the initial stage of differentiation, CcbP becomes degraded by hetR, a serine protease specifically expressed at that stage of development, resulting in a rise of intracellular free [Ca$^{2+}$] and subsequently heterocyst formation (Shi et al., 2006). Furthermore, alterations of the nitrogen depletion-induced Ca$^{2+}$ transient led to an arrest of heterocyst differentiation (Torrecilla et al., 2004).

Except for the Ca$^{2+}$ transporters, none of the already described bacterial Ca$^{2+}$-binding proteins in the literature has clear homologues in higher plant species. The apparent lack of conservation of calcium signalling components is most probably due to the rather different physiology of bacteria and higher plants, whereas the active extrusion of Ca$^{2+}$ by transporters from the cytosol seems to be conserved and universal. Moreover, the eukaryotic cell acquired more complexity in terms of compartmentalization than a bacterial cell, and consequently needs a signal transduction system, such as calcium signalling, to orchestrate all processes in those different subcellular compartments. Interestingly, highly conserved bacterial processes governed by proteins such as MinD and the RelA/SpoT homologue CRSH (Givens et al., 2004; Aldridge and Moller, 2005) might have acquired Ca$^{2+}$ regulation to be able to function coordinately in the cellular environment of higher plants. Table 1 lists 19 conserved proteins between Arabidopsis thaliana and Nostoc (Anabaena) sp. PCC7120, containing the Ca$^{2+}$-binding Dx[DN]xDG motif. It is interesting that most of these proteins localize, or are predicted to localize to different compartments other than to the plastid. Nevertheless, together with two other proteins, CML41 is predicted to localize to the plastid, which may suggest that the so far ‘missing’ plastidal CaM might be a CaM-like protein.

**Conclusions and future perspectives**

A central question in Ca$^{2+}$ signalling is how such a simple ion can mount an appropriate and specific physiological response, when almost every stress or developmental process elicits calcium fluxes in the cell. While the answer to the specificity question is manifold (Sanders et al., 2002), it can partly be explained by the compartmentalized nature of calcium signalling. In other words, the possibility to mobilize
Ca\textsuperscript{2+} from various subcellular stores, including chloroplasts and mitochondria, combined with its rapid release and uptake, implies that the effect of a Ca\textsuperscript{2+} flux can be highly localized to microdomains in the cytosol (Laude and Simpson, 2009). Furthermore, through the physical separation of organelles from the cytosol, Ca\textsuperscript{2+}-dependent regulation inside organelles, such as chloroplasts, mitochondria, peroxisomes, and nuclei, can be exerted in a highly localized and specific manner.

The question arises then: what is the physiological relevance of the organelles to cellular Ca\textsuperscript{2+} signalling? At least in chloroplasts, the impact of impaired organellar Ca\textsuperscript{2+} handling on plant physiology has already been demonstrated by CAS and PPF1. The mutation of, respectively, a putative chloroplastic Ca\textsuperscript{2+} sensor and a transporter led to impaired stomatal movement and impaired plant growth. To provide further insight into the physiological relevance of organellar Ca\textsuperscript{2+} signalling, it will be crucial to identify the proteins that are responsible for calcium transport (channels and transporters), storage, and decoding of the Ca\textsuperscript{2+} signals that have been observed. Likewise, more should be known on the stimuli that provoke Ca\textsuperscript{2+} signals in mitochondria and peroxisomes, and especially in chloroplasts, where until now only light and dark were found to induce Ca\textsuperscript{2+} fluxes. For future research, the combination of the molecular players and the elicitors of calcium signalling in organelles together with newly generated detection systems for measuring organellar Ca\textsuperscript{2+} concentrations in planta (Krebs et al., 2012; Mehlmer et al., 2012) should provide fruitful grounds for further discoveries.

Interplay between organelles can greatly affect Ca\textsuperscript{2+} signalling. In human and animal cells, the intimacy of ER and mitochondria was found to be beneficial for movement of Ca\textsuperscript{2+} between the two stores, and this was further strengthened by the identification of the molecular bridge between them (Kornmann et al., 2009; de Brito and Scorrano, 2010). Similarly in plants, Ca\textsuperscript{2+} might be exchanged between chloroplasts, mitochondria, and peroxisomes, while they are required to be closely connected for photorespiration and the exchange of metabolites (Majeran et al., 2010; Gowik et al., 2011). Moreover, Ca\textsuperscript{2+} exchange between chloroplasts and the ER cannot be excluded. The co-localization of the ER with stromules—stroma-filled tubules that extend from plastids (Fig. 1)—considerably increases the interactive surface between the plastid and the ER (Schattat et al., 2011a, b). The observation that sugars and abiotic stresses induce stromule formation (Gray et al., 2012; Schattat and Klosgen, 2011) further supports the idea that Ca\textsuperscript{2+} signals could be modulated by the interplay between organelles.

The data on bacterial calcium signalling clearly indicate that the prerequisites for eukaryotic Ca\textsuperscript{2+} signalling, an actively maintained Ca\textsuperscript{2+} gradient across membranes and Ca\textsuperscript{2+} sensor proteins, are already present in bacteria. The even distribution and frequent occurrence of Ca\textsuperscript{2+} transporters and EF-hand-containing proteins throughout genomes of bacterial families (Paulsen et al., 2000; Zhou et al., 2006) further indicate a prokaryotic origin of these core elements in eukaryotic Ca\textsuperscript{2+} signalling. For this, it is reasonable to search for conserved Ca\textsuperscript{2+} signalling processes.
between bacteria, eukaryotes, and their endosymbionts, mitochondria and plastids, too.

Acknowledgements

Work in the author’s lab has been funded by the Austrian GEN-AU program in the ERA-PG project CROP (FFG Project No. 818514), the Austrian Science Foundation (FWF) (P19825-B12), and by the EU in the Marie-Curie ITN COST (ITN 2008 GA 215-174).

References


Chai MF, Chen QJ, An R, Chen YM, Chen J, Wang XC. 2005. NADK2, an Arabidopsis chloroplastic NAD kinase, plays a vital role in both chlorophyll synthesis and chloroplast protection. Plant Molecular Biology 59, 553–564.


Coe H, Michalak M. 2009. Calcium binding chaperones of the endoplasmic reticulum. General Physiology and Biophysics 28 Spec No Focus, F96–F103.


Gross EL, Hess SC. 1974. Correlation between calcium ion binding to chloroplast membranes and divalent cation-induced structural changes.
changes and changes in chlorophyll a fluorescence. Biochimica et Biophysica Acta 339, 334–346.


Shattat M, Barton K, Mathur J. 2011b. Correlated behavior implicates stromules in increasing the interactive surface between plastids and ER tubules. Plant Signal Behav 6, 715–718.


