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Review

Calcium-dependent regulation of photosynthesis ☆

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ABSTRACT

The understanding of calcium as a second messenger in plants has been growing intensively over the last decades. Recently, attention has been drawn to the organelles, especially the chloroplast but focused on the stromal Ca^{2+} transients in response to environmental stresses. Herein we will expand this view and discuss the role of Ca^{2+} in photosynthesis. Moreover we address of how Ca^{2+} is delivered to chloroplast stroma and thylakoids. Thereby, new light is shed on the regulation of photosynthetic electron flow and light-dependent metabolism by the interplay of Ca^{2+}, thylakoid acidification and redox status. This article is part of a Special Issue entitled: Chloroplast biogenesis.

1. Introduction

Calcium is an essential plant nutrient. It is required for structural roles in the cell wall and membranes, as counter-cation for inorganic and organic anions in the vacuole and plays an essential role as intracellular messenger in the cytosol [1]. The concentration of cytoplasmic calcium $[Ca^{2+}]_{cyt}$ in plant cells increases in response to various developmental conditions and environmental factors. It is considered that alterations in $[Ca^{2+}]_{cyt}$ are crucial for the physiological response of the plant. More than 30 distinct developmental processes or environmental challenges initiate perturbation in the $[Ca^{2+}]_{cyt}$ (for review see [2]). Such perturbation signals include plant hormones, light, stress factors, and pathogenic or symbiotic elicitors [3–10]. In the green alga *Chlamydomonas reinhardtii*, fast phototactic movements require both light-induced H^+ and Ca^{2+} signaling events that are associated with the eyespot region of *Chlamydomonas* and mediate the regulation of flagellar bending and appropriate swimming direction [11,12], thereby providing photoprotection by avoiding excess exposure to light.

The calcium signaling network consists of distinct modules responsible for i) the generation of the Ca^{2+} signature, i.e. an elevation of the $[Ca^{2+}]$ which is stimulus-specific in regard to its amplitude, frequency, and shape, in response to a signal, ii) recognition of the signature by Ca^{2+} sensors and iii) transduction of the Ca^{2+} signature message to targets that mediate signal-specific responses [9,13–15]. Molecular and bioinformatic analyses of *Arabidopsis* genes and genome revealed the presence of about 80 polypeptides at the level of Ca^{2+} signature, about 400 sensors and about 200 target proteins, indicating an intricate Ca^{2+} signaling network [14]. Herein a central question is: how do plants decode and distinctively transmit perturbations in cytosolic Ca^{2+} signatures to downstream targets? Two principle types of Ca^{2+}-decoding signaling components are known in plants. Type I components are "sensor-responder" proteins that possess both Ca^{2+}-binding and enzymatic "effector" domains. Important examples are the Calcium-Dependent Protein Kinases (CDPKs) [16–18] and the Calcium–Calmodulin-Dependent Kinases (CCaMKs) [19]. Type II components are "sensor-relay" proteins, such as calmodulin (CaM), which have a Ca^{2+}-binding domain but do not exhibit an enzymatic activity [20]. When Ca^{2+} is bound, these proteins are turned on to interact with respective target proteins and to alter their biological activity. Another example for type II components are Calcineurin B-like calcium sensor proteins (CBLs) from *Arabidopsis* [21], which specifically interact with a family of protein kinases designated as CBL-Interacting Protein Kinases (CIPKs) [22]. A prime example for such a type of regulation is the phosphorylation of the K^+ channel AKT1 via CIPK23 and up-regulation of its activity under limiting K^+-supply conditions [23,24].

Dark-induced increases of chloroplast stromal Ca^{2+} levels precede the generation of cytosolic Ca^{2+} transients in tobacco leaf cells [25], suggesting that the chloroplast represents an element of the cellular Ca^{2+} network and contributes to the cytosolic Ca^{2+} signaling [26–31]. Commonly, it has been described that Ca^{2+} uptake into the chloroplast occurs in the light while Ca^{2+} is released into the cytosol in the dark (as reviewed in [30]). Furthermore, there is emerging evidence that chloroplasts may contribute to cytosolic Ca^{2+} signaling via the chloroplast localized calcium sensor protein (CAS) [32–34]. While the exact mechanisms have not been resolved, cytosolic Ca^{2+} signals induced by

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external Ca^{2+} could not be generated in CAS knockout mutants [32,35]. Changes in chloroplast Ca^{2+} might influence enzymatic functions of Ca^{2+}-binding proteins in the organelle and might regulate oxygen-evolving capacity of PSII and properties of photosynthetic electron transfer and photo-protection mechanism. This raises the question how is Ca^{2+} taken up and released from chloroplasts?

2. Organellar Ca^{2+} dynamics, transporters and signaling

Chloroplasts are plant organelles that possess a high concentration of Ca^{2+}. The predominant portion of the chloroplastic Ca^{2+} (~15 mM [68]) is bound to the negatively charged thylakoid membranes or to calcium-binding proteins [69] keeping the resting free $[Ca^{2+}]_{stroma}$ as low as 150 nM to avoid the precipitation of phosphates (Fig. 1A).

Since more than 40 years it has been known, that a light-dependent uptake of Ca^{2+} occurs in isolated chloroplasts [36,37,70]. However, the molecular mechanisms behind this process are still poorly understood (for recent reviews see [31,34]). Based on the work of Kreimer et al. [37,70], the Ca^{2+} influx is mediated by a uniport-type carrier and linked to photosynthetic electron transport via the membrane potential.

Furthermore, in 1995 Johnson et al. [71] observed a Ca^{2+} flux in the chloroplast upon the change from light to darkness (Fig. 1B). This dark-stimulated Ca^{2+} flux was shown to peak 20–30 min after the offset of light with a magnitude that was proportional to the previous duration of light exposure and it was not prevented by the inhibition of photosynthetic electron flow with DCMU [25]. They concluded that Ca^{2+} was taken up during illumination, sequestered in the thylakoid lumen or by Ca^{2+} storage proteins and released from these stores rather than imported from the cytosol after lights-off.

It should be noted, that specific Ca^{2+} signals can also be observed in the chloroplast in response to pathogen-associated molecular patterns (PAMPs) [34,72] as well as cold, high-salt and hyperosmotic stimuli [34].

2.1. Import of Ca^{2+} across the chloroplast envelope and thylakoid membrane

Despite the analysis of Ca^{2+} signals in the chloroplast and their importance, the identification of Ca^{2+} transporters in the chloroplast envelope and thylakoid membrane remains an unresolved challenge. For the import of Ca^{2+} through membrane potential-driven Ca^{2+} transporters [73] two potential chloroplast envelope membrane Ca^{2+}-ATPases have been proposed (Fig. 1A). In the first place, the autoinhibited Ca^{2+}-ATPase AtACA1 was identified by Huang et al. in the *Arabidopsis* chloroplast envelope but its higher abundance in roots, the lack of Ca^{2+}-ATPase activity at the envelope [39] as well as the proteomic assignment of AtACA1 to the ER and plasma membrane [74,75] question the role of AtACA1 in chloroplastidial Ca^{2+} import. Secondly, the heavy metal P-type ATPase AtHMA1 was identified in the chloroplast envelope by a proteomic approach by Ferro et al. [40] and its localization was confirmed by GFP fusion [41]. However, conflicting studies concerning its transport of Ca^{2+} [76] and/or heavy ions like Cu^{2+} and Zn^{2+} [41,77] exist.

The family of mechanosensitive channel of small conductance-like (MSL) proteins exhibits two members in the chloroplast envelope of *Arabidopsis thaliana*: MSL2 and MSL3 [44,45]. They have been shown to regulate the plastid size, shape [44] and division [78]. However, Ca^{2+}-permeability can be only inferred from their bacterial homolog MscS [79].

Furthermore, the glutamate receptor AtGLR3.4 was localized to the chloroplast as well as the plasma membrane [42]. GLRs can form nonselective cation channels and have putative roles in Ca^{2+} signaling [43]. Interestingly, GLRs as well as cyclic nucleotide-gated channels (CNGCs) and two-pore channels (TPCs) were found in green algae, higher plants and animals. This is in contrast to the four-domain voltage-dependent Ca^{2+} channels (VDCCS), transient receptor potential channels (TRPs) and inositol (1,4,5)-trisphosphate receptors, which are absent from land plants (reviewed in [80]) but present in green algae such as *C. reinhardtii* [81].

The import of Ca^{2+} across the thylakoid membrane has been shown to be dependent on a light- or ATP-induced transthylakoid proton gradient [82]. Overexpression and knockdown of the thylakoid protein Post-Floral-specific gene 1 (PPF1) led to an increased and decreased calcium storage capacity of *Arabidopsis* guard cells, respectively [46]. Furthermore, its expression in human hepatoma cells led to inward Ca^{2+} currents. Therefore, PPF1 represents a candidate for the transport of Ca^{2+} into the thylakoid lumen.

2.2. Import of nuclear encoded proteins into the chloroplast

Ca^{2+} signals also regulate the import of proteins into the chloroplast via the TOC–TIC complex (translocon of the outer/inner envelope membrane of chloroplasts) (see review [51]). TIC20 and TIC110 have been proposed for the translocation of preproteins across the inner membrane [83,84] and recently the main components of the TIC20 complex have been identified: TIC56, TIC100, and TIC214 (YCF1) [85]. The import of proteins was shown to be affected by the CaM inhibitor Ophiobolin A as well as the calcium ionophores A23187 and Ionomycin [86]. Ca^{2+} influences the channel activity of TIC110 [83] and TIC32, an interaction partner of TIC110, was identified as a CaM-binding protein in vitro [87]. Interestingly, the interaction between TIC32 and TIC110 is inhibited in the presence of NADPH [87] providing a link between Ca^{2+} signaling and the redox status of the chloroplast.

2.3. Mitochondrial Ca^{2+} import

Mitochondria can sequester Ca^{2+} as well and mitochondrial Ca^{2+} signals not only regulate the rate of mitochondrial energy (ATP) production in animals [88] but are also a response to cold stress, hyperosmotic stress and mechanical stimuli in plants [89]. The search for mitochondrial Ca^{2+} import proteins took nearly 50 years and resulted in the identification of the Mitochondrial Calcium Uniporter (MCU) in 2011 [90,91], which is associated with several proteins (MICU1, MICU2, EMRE) in a uniporter complex [92–94]. MCU has a high-selectivity but low-affinity for Ca^{2+} [95] and interestingly, it is inhibited by ruthenium red, which was shown for the light-dependent Ca^{2+} influx in chloroplasts as well [37]. The identification of mitochondrial Ca^{2+} transporter in plants is missing so far. However, six potential MCU homologs have been identified in *A. thaliana*, one of which is predicted to be targeted not only to mitochondria but also to chloroplasts (At5g66650,) [30].

3. The impact of Ca^{2+} on chloroplast metabolism

The following section will introduce the importance of Ca^{2+} towards chloroplast metabolism and highlight on the reactions, which are closely linked to photosynthesis. These processes are illustrated in Fig. 1A and Table 1 summarizes the function of the involved proteins in regard to photosynthesis as well as their Ca^{2+} dependency. Besides the light-induced redox poise a low resting free $[Ca^{2+}]$ (~150 nM [71]) is needed for the activation of the photosynthetic carbon fixation. During light-to-dark transition, large Ca^{2+} fluxes into the stroma lead to the deactivation of carbon fixation (Fig. 1B) [25,82]. It is remarkable that the activation and deactivation of certain enzymes are regulated by the same signal molecule.

3.1. Light-independent reactions of photosynthesis

The light-independent reactions represent the known Calvin–Benson–Bassham (CBB) cycle which takes place in the stroma of chloroplasts and is the primary pathway of carbon fixation of C3 plants [119]. The CBB cycle proceeds in three main stages: carboxylation, reduction and regeneration. In the first stage, carbon dioxide is

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