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Review

Ions channels/transporters and chloroplast regulation

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ABSTRACT

Ions play fundamental roles in all living cells and their gradients are often essential to fuel transports, to regulate enzyme activities and to transduce energy within and between cells. Their homeostasis is therefore an essential component of the cell metabolism. Ions must be imported from the extracellular matrix to their final subcellular compartments. Among them, the chloroplast is a particularly interesting example because there, ions not only modulate enzyme activities, but also mediate ATP synthesis and actively participate in the building of the photosynthetic structures by promoting membrane-membrane interaction. In this review, we first provide a comprehensive view of the different machineries involved in ion trafficking and homeostasis in the chloroplast, and then discuss peculiar functions exerted by ions in the frame of photochemical conversion of absorbed light energy.

1. Introduction

Ions play key roles in all living organisms being involved in all metabolic and cellular functions. Therefore, ions are found in all subcellular compartments and have to be imported from the extracellular matrix to their final localization within cells. In Arabidopsis, acquisition and translocation of ions within plant organs, cells and subcellular compartments involve large families of ionic transporters with various substrate specificities, expression patterns, and subcellular localization. These families were classified into three major categories: channels/porins, primary transporters/pumps and secondary transporters (according to the Transport Classification system [1]). Channels transport solutes down their concentration gradient without consuming energy and display the fastest transport rates among transporters. Primary transporters (*e.g.* ATPases) directly use energy to transport molecules across membranes. Secondary transporters (also named electrochemically-driven transporters) use the concentration gradient of co-transported molecules and therefore include antiporters and symporters.

Transmembrane proteins have an essential role in the regulation of ions homeostasis and in biological functions. In chloroplasts, a large variety of ions are found, at micro to millimolar concentrations. Since metal ions (zinc -Zn-, copper -Cu-, iron -Fe-, *etc*) can be toxic for the cell, they are not found as free ions, but are always chelated by proteins or biomolecules. Conversely, ions like potassium (K), magnesium (Mg), can be free and reach millimolar concentrations. In the last 40 years, the existence of ionic fluxes across the chloroplast envelope or the thylakoid membranes has been mostly deduced from physiological measurements or from knowledge of the chloroplast metabolism. However, most of the proteins responsible for these fluxes have not been identified yet. Indeed, their low abundance, their localization in intracellular membranes, their hydrophobicity and the difficulties encountered when trying to produce them in heterologous systems have strongly limited their identification and functional characterization using classical approaches. Several chloroplast transporters have been identified in the last years, thanks to proteomic approaches targeted to the chloroplast and its sub-compartments (*e.g.* [2–6]), and to reverse genetic studies. Nonetheless, controversies still exist about the sub-plastidial localization or function of some of these transporters, and others are still missing. Again, proteomics

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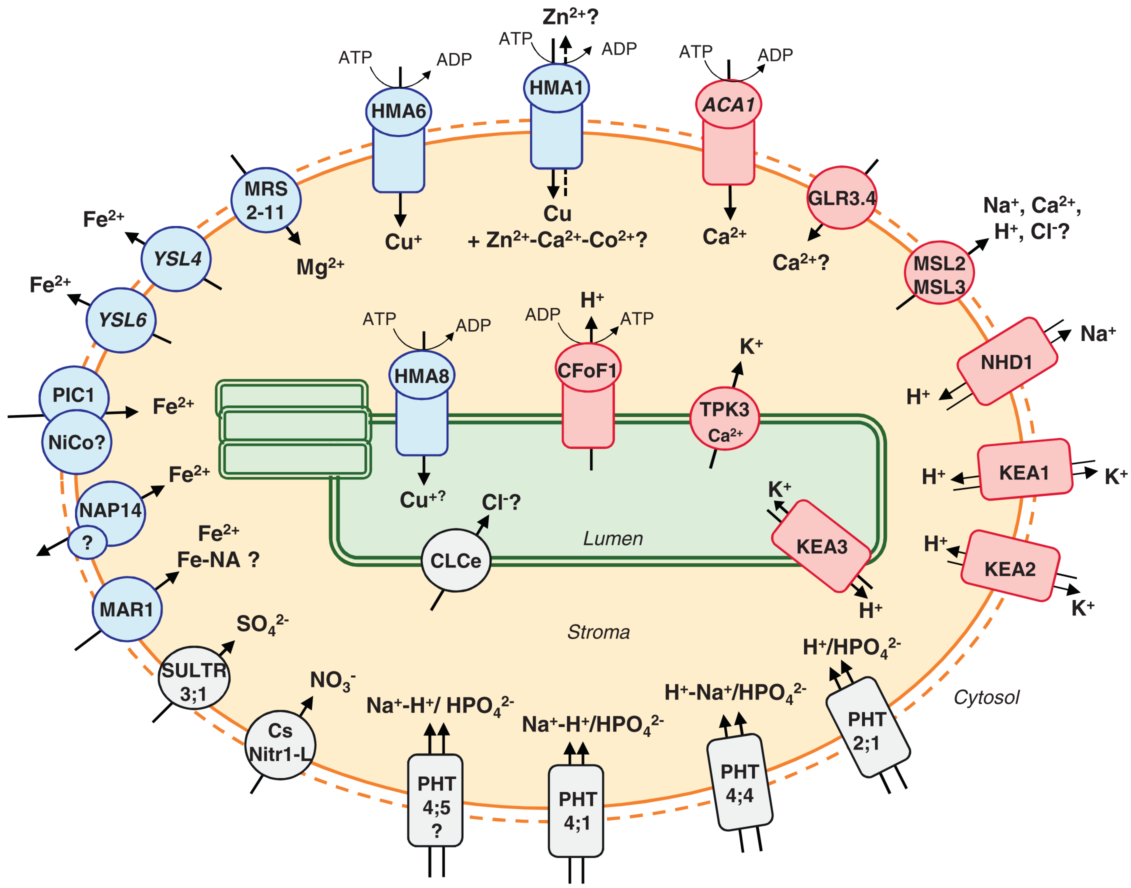

Fig. 1. Overview of Arabidopsis chloroplast ions transporters/channels. Metals transporters are represented in blue, anions transporters in grey and other ions in orange. Transporters whose chloroplast localization is controversial are noted in italic (YSL4–YSL6 and ACA1). Substrates, protein or chloroplast localization that need to be further validated are noted "?". The regulation of TPK3 by Ca^{2+} is highlighted.

data have provided a list of unknown proteins that, based on sequence similarities, could be involved in ion transport. However, no functional characterization exist in most cases. Moreover, while not identified through large-scale approaches, members of well-characterized ion transporters families have also been predicted to be localized in the chloroplast using bioinformatics tools (for recent reviews on chloroplast ion transporters see [7,8]). Overall, when considering the known, hypothetical and missing transporters, a scenario emerges where a plethora of transporters is involved in ion fluxes across the membranes to facilitate exchanges between the cytosol, the stroma and the thylakoid lumen (Fig. 1).

In the first part of this review, we will provide a comprehensive description of transporters identified in Arabidopsis chloroplast membranes, including those that are still only incompletely characterized. In the second part, we will discuss the implication of ions concentration and fluxes on the optimization of the photosynthetic process.

2. A global overview of chloroplast ion channels/transporters

2.1. Transporters involved in metal homeostasis

Metal ions are essential cofactors for numerous chloroplast proteins involved in photosynthesis (Cu, Mg, manganese -Mn-, Fe), oxidative stress detoxification (Cu, Zn, and Fe), nutrient assimilation (Fe), biosynthesis of aminoacids (*e.g.* Zn for cysteine and methionine), *etc.* Cu (estimated chloroplast concentration ~60 μM, Fig. 2 [9]) plays a key role in the photosynthetic process and can exist under a reduced (Cu^{+}) or oxidized form (Cu^{2+}). In its Cu^{2+} form, it constitutes the redox cofactor of plastocyanin (PC), a protein required for electron transport from the cytochrome b_6f complex (b_6f) to photosystem I (PSI) [10]. Cu is also required for the activity of the Cu/Zn superoxide dismutase (Cu/Zn-SOD), a soluble enzyme that scavenges reactive oxygen species produced by photosynthesis under stress conditions, which is found in eucaryotes and some procaryotes. In Arabidopsis, Cu delivery to chloroplasts and thylakoids requires three P_{IB}-type ATPases: AtHMA1, AtHMA6 and AtHMA8 (Fig. 1). AtHMA1 (At4g37270) and AtHMA6 (At4g33520) are localized in the chloroplast envelope [5,11,12] and AtHMA8 (At5g21930) in the stroma-lamellae, *i.e.* non-appressed fractions, of the thylakoid membranes [6]. Genetic approaches have shown that both AtHMA6 and AtHMA8 are Cu transporters, AtHMA6 being the main route of Cu supply to the Cu/Zn SOD and to the thylakoid transporter AtHMA8, as required for PC biosynthesis [11,13]. More recent biochemical studies have demonstrated that AtHMA6 is a high affinity Cu^{+} transporter [14] while the biochemical properties of AtHMA8 still remain unknown. AtHMA1, the second envelope transporter, provides an additional way to import Cu into the chloroplast, which would provide Cu to the Cu/Zn SOD, and which is essential under light stress conditions [12,15]. AtHMA1 could also transport other metal/divalent ions like Zn, cobalt (Co), calcium (Ca) [16] and was also proposed to be involved in Zn or Cu/Zn export from Arabidopsis and Barley chloroplasts [17,18]. The ionic specificity of AtHMA1 is still controversial, strongly suggesting that this protein could transport a broad range of divalent cations, probably depending on the physiological conditions. As for AtHMA8, there