Commentary

Optimal photosynthesis requires a balanced diet of ions

Optimal macro- and micronutrient supply is crucial for the efficient growth of plants. The chloroplast, a key organelle in plant cells, is essential for producing energy, amino acids, fatty acids, and hormones. It requires a well-balanced ion homeostasis to function effectively. Understanding the mechanisms of chloroplast ion homeostasis is therefore crucial for optimizing plant growth and maximizing crop yield, especially regarding its impact on photosynthesis. Published in this issue of *New Phytologist*, the Tansley review by Kunz *et al.* (2024; pp. 543–559), provides a comprehensive update on the relevance of mineral ions and their respective transport mechanisms in chloroplasts (Fig. 1), along with recommendations on areas of research, which should be prioritized in the future.

'…the rise of molecular biology and deciphering of the Arabidopsis genome has ushered in a new era of discovery of the molecular players, for example transporters and channels of chloroplast ion homeostasis.'

Forty years ago, before the emergence of genomic tools and before Arabidopsis was designated as the model organism for plant biology, the elemental ion composition from isolated chloroplasts and thylakoids was measured in peas and spinach (Demmig & Gimmler, 1983). These early studies still form the basis of our current understanding of chloroplast ion homeostasis (Pottosin & Shabala, 2016). However, the rise of molecular biology and deciphering of the Arabidopsis genome has ushered in a new era of discovery of the molecular players, for example transporters and channels of chloroplast ion homeostasis.

This breakthrough paved the way for the identification of several ion transporters, almost 20 years after the first work in this field, triggering a wave of research on ion transport in the chloroplast and its effects on photosynthesis functioning. In their review, Kunz *et al.* highlight the different ways in which chloroplast ion homeostasis impacts photosynthesis.

Ions are necessary for the structure and functioning of pigments and proteins involved in photosynthesis. For example, magnesium (Mg^{2+}) is the central ion in the porphyrin structure of chlorophyll (Wang & Grimm, 2021). The oxygen-evolving complex contains a cluster (Mn_4CaO_5) made of manganese (Mn^{2+}) and calcium

(Ca^{2+}), and chloride anions ($Cl^−$) are located in the vicinity of this cluster (Umena *et al.*, 2011). Iron (Fe^{2+}) is also essential for the electron transport chain (Kroh & Pilon, 2020).

An imbalance in plastid ion homeostasis can also negatively affect plastid gene expression. This was demonstrated in *kea1 kea2* mutants, where altered secondary structures of stromal mRNAs and decreased plastid gene expression were observed. Disrupted plastid gene expression leads to a delay in chloroplast development, in part explained by a reduced production of photosynthetic proteins (DeTar *et al.*, 2021).

During photosynthesis, absorbed light energy generates a reducing power, which drives electron transfer reactions, these are coupled to the transfer of protons into the thylakoid lumen, generating a proton motive force (pmf) necessary for the synthesis of ATP (Armbruster *et al.*, 2017) (Fig. 1b). When photosynthesis is challenged by excessive light exposure, the pH of the thylakoid lumen decreases significantly as more protons are transferred there. This decrease in pH is a key signal that triggers certain photoprotective mechanisms in the photosynthetic chain (also known as nonphotochemical quenching, NPQ) (Bassi & Dal'osto, 2021). NPQ allows the conversion of excess light energy into heat, preventing the production of reactive oxygen species. Ion homeostasis is of paramount importance in this process: during the transition from high to low light conditions, it has been shown that KEA3 K^+/H^+ exchange is necessary to inactivate the NPQ, as conditions are again favourable for photosynthesis (Wang *et al.*, 2017). However, a more active KEA3 leads to a faster inactivation of NPQ (Armbruster *et al.*, 2016), because less ΔpH is maintained in this line. Another ion involved in NPQ regulation is chloride ion ($Cl^−$), which based on studies on the thylakoid BESTROPHIN-LIKE PROTEIN/voltage-dependent $Cl^−$ channel 1 (VCCN1) suggested a role in dissipating membrane potential to enable timely NPQ activation during transition from low to high light (Herdean *et al.*, 2016). Excitingly, accelerating NPQ relaxation has already found practical application in optimizing photosynthesis in crops, thereby boosting yield (Leister, 2023).

The Tansley review by Kunz *et al.* also serves as a comprehensive guide to understanding the main challenges that researchers will have to face to better understand ion homeostasis within the chloroplast.

First is the study of ion concentrations in the chloroplast, which could be revolutionized thanks to genetically encoded ion sensors, as suggested by the authors. Starting with the development of Ca^{2+} sensors, now sensors for K^+, Mg^{2+}, $Cl^−$, and other ions have been successfully tested in various organisms (Sadoine *et al.*, 2023). Sensors that function within the correct concentration range can be titrated and expressed in both WT and mutant plant cells, and they can then be targeted to the plastid stroma and thylakoid lumen. Therefore, using genetically encoded ion sensors for imaging will

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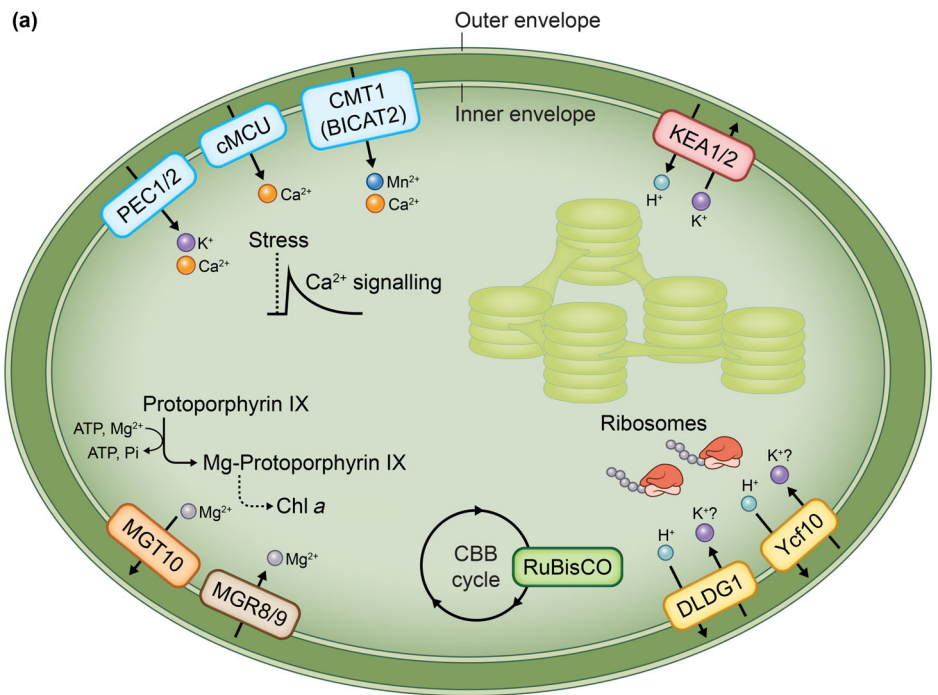
Fig. 1 Physiological relevance of mineral ions and their respective transport mechanisms in chloroplasts (adapted from Kunz *et al.*, 2024, in this issue of *New Phytologist*, pp. 543–559). (a) The proteins and pathways mentioned in this Commentary on Kunz *et al.* with the names and locations based on work in *Arabidopsis thaliana*. Question marks (?) indicate unknown genes, locations, or substrates. BICAT2, BIVALENT CATION TRANSPORTER 2; CBB, Calvin-Benson-Basham cycle; cMCU, CHLOROPLAST MITOCHONDRIAL CALCIUM UNIPORTER; CMT1, CALCIUM/MANGANESE CATION TRANSPORTER 1; DLDG1, DAY-LENGTH-DEPENDENT DELAYED-GREENING 1; KEA1/2, K EFFLUX ANTIPORTER 1/2; MGR8/9, Magnesium Release 8/9; MGT10, MAGNESIUM TRANSPORTER 10; PEC 1/2, plastid envelope channel 1/2; RuBisCO, Ribulose-1,5-bisphosphate carboxylase/oxygenase; Ycf10, homolog of the nuclear-encoded DLDG1. (b) Simplified overview of the generation and dynamics of proton motive force (*pmf*) and role of ion carriers. A, antheraxanthin; ATP Synt, ATP synthase; b_6f, cytochrome b6f complex; F, Faraday constant; Fd, ferredoxin; FNR, ferredoxin-NADP$^+$ reductase; OEC, oxygen-evolving complex; PbsS, photosystem II subunit S; PC, plastocyanin; PQ, plastoquinone/plastoquinol pool; PSI, photosystem I; PSII, photosystem II; R, ideal gas constant; SOD, superoxide dismutase; T, temperature; V, violaxanthine; VDE, violaxanthin de-epoxydase; Z, zeaxanthine; ZE, zeaxanthine Epoxydase; ΔpH, proton gradient; $\Delta\Psi$, membrane potential.
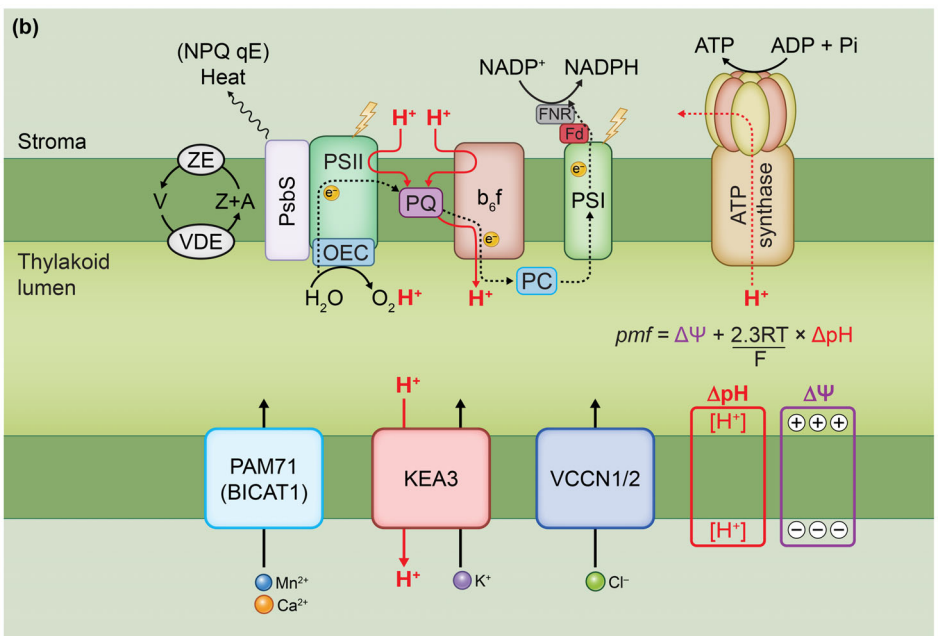
increasingly provide a valuable method to investigate plastid transport proteins directly within plants.

Second, to better understand the dynamics of ions flux into the stroma and the lumen of chloroplast, new techniques will have to emerge. For identifying new candidate genes encoding plastid ion transporters or channels, the authors suggested that developed genome-wide multi-targeted artificial micro-RNA and CRISPR libraries could be a solution. However, the newly created libraries should exclusively target the plastid proteome to improve the plant ionome (Baxter, 2010).

Third, structural knowledge of plastidial ion transporters is also an area in need of improvement. For that, the authors recommend more frequent use of solid-state membrane electrophysiology: this method allows multiple recordings of liposomal or membrane samples adsorbed on a sensor, and it is sufficiently sensitive to detect the transport currents of moderate flux transporters that are inaccessible to traditional electrophysiology techniques (Thomas *et al.*, 2021). For *in vivo* analysis, x-ray microscopy is recommended to visualize the spatial distribution of ions within the chloroplast and understand their dynamics in real time (Fittschen *et al.*, 2017).

One drawback is that it is necessary to work at the synchrotron facilities to obtain the highest resolutions. Another strategy would be the combination of cryo-electron microscopy and structural prediction software such as ALPHAFOLD. This approach will not only deepen our understanding of predicted protein structures but also provide clues about their substrates, potential regulatory mechanisms and functions. Studying the regulatory mechanisms of ion transporters is important, as these mechanisms appear to contribute to maintaining ionic balance in the chloroplast. Some of these ion transporters exist in large complexes, increasing the likelihood of regulation. Proximity labelling with TurboID fusions can be used for stromal targeting and has already produced promising results in Arabidopsis (Wurzinger *et al.*, 2022).

Finally, understanding the roles of chloroplast ion homeostasis during stress conditions and its impact on plant growth will be crucial. Furthermore, ion homeostasis may also play a role in molecular signalling pathways within plastids. The most extensively studied ion, as a signalling molecule to date, is Ca^{2+}. It has been shown to fluctuate in response to changes in light intensity, temperature, osmotic pressure, and pathogen infection (Costa *et al.*, 2018). We have also started identifying sensors for calcium in the chloroplasts (such as the Ca^{2+}-sensing receptor or the calredoxin in *Chlamydomonas reinhardtii*) (Petroutsos *et al.*, 2011; Hochmal *et al.*, 2016).

The insights gained from these advancements will not only enhance our understanding of chloroplast ion homeostasis but also hold immense potential for practical applications in agriculture.

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