S10.L3

Chloroplast ion channels affect photosynthetic efficiency

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While ion channels are well characterized in mammalian mitochondria [1], much less is known about the molecular identity and the function of ion channels in the bioenergetic organelles in plants. We have recently identified three channels in the chloroplasts and studied their impact on photosynthesis. In particular, TPK3, a two-pore potassium channel was found to be located in the thylakoid membrane stromal lamellae and to modulate the composition of the pmf through ion counterbalancing. Recombinant TPK3 exhibited potassium-selective channel activity sensitive to Ca^{2+} and H^{+}. *Arabidopsis* plants silenced for the TPK3 gene display reduced growth and altered thylakoid membrane organization. This phenotype reflects an impaired capacity to generate a normal pmf, which results in reduced CO_2 assimilation and deficient nonphotochemical dissipation of excess absorbed light. Thus, the TPK3 channel manages the pmf necessary to convert photochemical energy into physiological functions [2]. In addition, two glutamate receptors of clade III were found to be located in the chloroplast envelope membrane, including AtGLR3.4 [3]. Plants lacking only one of these putative calcium channels exhibit a mild photosynthetic deficit, while double-knock-out organism is characterized by a more severe phenotype.

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Visualizing functional motions of membrane transporters at high temporal and spatial resolutions

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Membrane transporters are the principal mediators of active exchange of materials across the cellular membrane. These complex molecular machines constitute highly sophisticated, fine-tuned molecular pumps that efficiently couple various sources of energy in the cell to vectorial transport of a wide range of molecules across the membrane, often against the electrochemical gradient. Substrate binding and translocation along the transport pathway in membrane transporters are closely coupled to numerous, largely unknown protein conformational changes of varying magnitude and nature that are induced by and/or coordinated with the energy-providing chemical steps. A detailed description of the mechanism of membrane transporters, therefore, relies on high-resolution methodologies offering simultaneous spatial and temporal resolutions that can describe the dynamics of the process at an atomic level. In this talk, some of the recent methodological advances in our lab and latest results from employing molecular dynamics simulations performed on a number of membrane transporters and the molecular events involved in their function revealed by these simulations will be presented. In particular, we have been able to find the most optimal pathways for large-scale structural transitions of several key membrane transporters and quantify the energetics associated with these transitions using advanced free energy calculations.

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S10.O1

Structural mechanics and regulation of the Ca^{2+}-ATPase

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The Ca^{2+}-ATPase of the sarcoplasmic reticulum (SR) membrane is responsible for active pumping of Ca^{2+} ions from the cytosol into the SR lumen, fueled by ATP hydrolysis. The 110 kDa multi-pass transmembrane protein consists of multiple domains which have to move in concert in order to accomplish the challenging task of translocating ions across the lipid bilayer against a concentration gradient. ATP hydrolysis and vectorial ion transport occur at sites that are about 50 Å apart, which makes a tight coupling of domain movements and a fail-safe communication between the sites necessary. Recent crystal structures of the Sarcolipin-bound Ca^{2+}-ATPase [1,2] and an occlusion-defective mutant form E309Q [3] have revealed new insight about both the mechanism of Ca^{2+} loading and occlusion, and the long-distance communication between the sites of Ca^{2+} binding and ATP-hydrolysis: both mechano-chemical and electrostatic effects are fine-tuned to translate large conformational changes into the punctual formation and distortion of selective Ca^{2+}- and ATP binding sites. Furthermore, transient access of Ca^{2+} to its high affinity binding-sites, followed by tight occlusion within the hydrophobic transmembrane region, which is crucial for unidirectional transport, requires a balanced interaction between the enzyme and its lipid environment.

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