**Molecular basis of cyclic electron flow revealed**

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Oxygenic photosynthesis is a fundamental process performed by cyanobacteria, algea and higher plants that transforms the energy of sunlight into chemically stored energy. It is driven by two main electron transport pathways. The linear electron flow (LEF) involves two photosynthetic reaction centers that act in series and generates an electrochemical proton gradient, that fuels the chloroplast ATP synthase. First, Photosystem II releases electrons from water by using energy derived from harvested photons. The second complex, Photosystem I, functions as an electron pump, which further increases the energy of the transmitted electron and uses it to reduce ferredoxin (Fd). Amongs others, a subsequent enzymatic reaction uses Fd to generate NADPH. Both NADPH and ATP are mainly used for carbon fixation in the Calvin-Benson-Bassham (CBB) cycle. LEF produces ATP and NADPH in a ratio of about 9:7, however the CBB cycle requires 3 ATP per 2 NADPH. Furthermore, many other cellular processes need ATP or NADPH in different amounts, dependent on the metabolic status of the cell. To adjust the ATP to NADPH ratio, the cyclic electron flow (CEF) evolved, which involves only PSI. The electrons generated by PSI are transferred back into the electron transfer chain via photosynthetic complex I (NDH-1) and hence produce only ATP.

Complex I functions in all domains of life as a redox driven proton pump exist in all domains of life. The generated proton gradient is used to fuel ATP synthases. The structure and function of the bacterial and mitochondrial respiratory complex I systems are well studied, whereas photosynthetic complex I lacks mechanistic insights. Respiratory complex I systems use NADH as the electron source. For this they have evolved a specialized NADH oxidation module that is not present in photosynthetic complex I, which carries a set of oxygenic-photosynthesis-specific (OPS) subunits instead. These structural differences raised the question how electron transfer is realized in the photosynthetic system. Fd was identified as a reasonable candidate, as photosynthetic complex I evolved from membrane bound NiFe hydrogenases that oxidize reduced Fd to drive proton pumping.

To test this hypothesis, we mimicked CEF in vitro by mixing isolated PSI with purified Fd and photosynthetic complex I from the thermophilic cyanobacterium *Thermosynechococcus elongatus*. After excitation of PSI by a short laser pulse we could monitor electron transfer from PSI via Fd to photosynthetic complex I by fast optical spectroscopy. Thus, we could for the first time demonstrate unequivocally that Fd directly mediates electron transfer between PSI and photosynthetic complex I, instead of using intermediates such as NADPH.

To provide a structural basis for the interaction with Fd and to clarify the role the OPS subunits in this reaction, we solved a high resolution cryo-EM single particle structure of photosynthetic complex I. Thereby, purified protein is embedded in a layer of vitrious ice to preserve their native conformation and protect them from damage in the electron microscope. After several thousands of images are collected they are reconstructed in the computer to generate a three-dimensional representation. These reconstructions rivals the details that can be observed using X-ray crystallography. Based on the structural analysis, we could identify a putative ferredoxin binding site on the peripheral, non-membrane embedded part of the complex and that the OPS subunit NdhS plays a central role in Fd recruitment. This was further corroborated by NMR chemical shift perturbation experiments. These experiments measured changes in the resonant frequency of atomic nuclei in the protein dependent on its environment. They revealed NdhS’s flexible C-terminus as the main interaction side to reel in Fd like a molecular fishing rod.

This combination of structural and in-vitro spectroscopic analysis allowed us to explain the molecular details of the interaction between Fd and photosynthetic complex I and uncovers a highly efficient recruitment process.

In contrast to all other trees of life, cyanobacteria have evolved different variants of photosynthetic complex I. Under carbon limiting conditions, they can express paralog complex I subunits that – additionally to proton pumping -  drive vectorial CO2hydration to store CO2that has leaked out from carboxysomes, the main compartment for CO2-fixation. Thus, a full understanding of the cyanobacterial complex I variants does not only greatly enhance our understanding of prokaryotic environmental adaption, it furthermore may serve as a blueprint for engineering of photosynthetic organisms to increase productivity, thereby promoting solutions for future food production and biomimetic CO2 mitigation.