PostDoctoral Research Project: The dynamic and function of Supercomplexes in the Photosynthetic Electron Transfer Chain

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The structural and functional organization of bioenergetic electron transfer chains has been a matter of long-standing, intense debates with fluctuating outcomes. The solid-state model, in which electron transfer reactions occur within a single supramolecular edifice[1] has been challenged by the random collision model[2] that relied on the finding that the enzymatic activities involved in the chain are born by individual membrane bound complexes and on functional studies supporting the notion that these membrane bound complexes are linked by soluble electron carriers which encounter no constraint to their diffusion. Yet, the beginning of this century saw the revival of the solid state model with the observation by Schägger et al. that non-denaturing conditions lead to the purification of supercomplexes assembling, in a single biochemical unit, several components of the respiratory chain[3]. Since then, numerous and circumstantial evidences supported the notion that the structural clustering of the various complexes of the mitochondrial electron transfer chain can shape its function[4-8] and this reached its acme with the observation that "respirasomes" made of all the enzymes and soluble electron carriers required to funnel electron transfer from NADH to molecular oxygen do respire[9] and are dynamic structures that determine electron flux from different substrates[10].

As expected, similar concepts apply to the photosynthetic electron transfer chain. Protein crowding has been shown to constraint the diffusion of plastoquinone in the thylakoid membrane and of plastocyanin in the lumen[11,12]. Interestingly, the latter limitation to the diffusion of plastocyanin is alleviated upon the light-induced swelling of the lumen thereby providing a possible regulation mechanism[13]. Along similar lines, the dynamic assembly or disassembly of supercomplexes that would sequester the soluble electron carrier, ferredoxin, has provided a tempting model to rationalize the switch between two essentially different electron transfer modes: linear and cyclic electron flow[14,15]. Again, the isolation of the elusive supercomplex comprising, among others complexes, Photosystem I, cytochrome b_6f[16] has provided experimental and molecular support to the notion that structural remodeling of bioenergetic electron transfer chain can shape their function by (re)routing the electron flux.

The injection of energy into the biosphere relies on photosynthesis. Solar energy is transiently stored in the form of carbohydrates ensuing from the assimilation of carbon dioxide by the Benson-Calvin cycle[17]. To turnover, this enzymatic cycle demands its substrates, ATP and NADPH, in a strict stoichiometry of 3/2. It is commonly accepted that, to comply with this requirement, the photosynthetic electron transfer operates according to two different modes, linear (LEF) and cyclic electron flow (CEF) both being indispensable[14,15,18]. While the former provides ATP and NADPH in a 2.6/2 ratio, the latter exclusively drives phosphorylation[19] and thus supplies with the remaining ATP. The fine tuning of these two modes is thus vital in essence but its mechanism remains poorly understood.

We recently identified, in the model organism *Chlamydomonas reinhartdii*, the intracellular redox poise as the main determinant of the switch between LEF and CEF[20]. Yet, the mechanistic rationales behind the switch still need to be unravelled. In addition we found that the promotion of CEF correlates with a massive structural rearrangement which includes, in agreement with previous findings[16,21], the formation of supercomplexes comprising Photosystem I, cytochrome b_6f, Ferredoxin NADP Reductase (FNR) and several other proteins which functions remain elusive, but also a dramatic increase of the amount of the membrane bound fraction of the, otherwise soluble protein, FNR. Thus the redox state of some still unidentified molecular entities may tune the affinity of FNR

for a membrane-bound protein complex that may contain one of the proteins, of unknown function, found in the supercomplexes. In this project we will:

i) Characterize the interactions that determine the dynamic assembly and disassembly of the PS1-b6f supercomplexes. To this aim we will down-regulate the expression of the genes encoding subunits of unknown function, found in the supramolecular edifice and characterize the consequences of this attenuation in terms of stability and functional efficiency of the complex.

i) Rely on the knowledge gained from our previous studies which allows mastering the switch between LEF and CEF, to impose fluctuating changes of the determining parameter and follow the transient activation/deactivation of CEF.

ii) Characterize the function, in vitro, and structure of the purified supercomplex to decipher the sequence of electron transfer reactions that allows the redox cycle within the supercomplex. To start with, the structure of the supercomplex will be studied by cryo-electron microscopy in collaboration with Roman Kouril (Palacký University of Olomouc, CZ).

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(1) Chance, B.; Williams, G. R. *Nature* 1955, *176*, 250.

(2) Hackenbrock, C. R.; Chazotte, B.; Gupte, S. S. *J Bioenerg Biomembr* 1986, *18*, 331.

(3) Schagger, H.; Pfeiffer, K. *EMBO J* 2000, *19*, 1777.

(4) Bianchi, C.; Genova, M. L.; Parenti Castelli, G.; Lenaz, G. *J Biol Chem* 2004, *279*, 36562.

(5) Krause, F.; Reifschneider, N. H.; Vocke, D.; Seelert, H.; Rexroth, S.; Dencher, N. A. *J Biol Chem* 2004, *279*, 48369.

(6) Dudkina, N. V.; Heinemeyer, J.; Sunderhaus, S.; Boekema, E. J.; Braun, H. P. *Trends Plant Sci* 2006, *11*, 232.

(7) Bultema, J. B.; Braun, H. P.; Boekema, E. J.; Kouril, R. *Biochim Biophys Acta* 2009, *1787*, 60.

(8) Althoff, T.; Mills, D. J.; Popot, J. L.; Kuhlbrandt, W. *EMBO J* 2011.

(9) Acin-Perez, R.; Fernandez-Silva, P.; Peleato, M. L.; Perez-Martos, A.; Enriquez, J. A. *Mol Cell* 2008, *32*, 529.

(10) Lapuente-Brun, E.; Moreno-Loshuertos, R.; Acin-Perez, R.; Latorre-Pellicer, A.; Colas, C.; Balsa, E., . . . Enriquez, J. A. *Science* 2013, *340*, 1567.

(11) Lavergne, J.; Joliot, P. *Trends Biochem Sci* 1991, *16*, 129.

(12) Kirchhoff, H. *Trends Plant Sci* 2008, *13*, 201.

(13) Kirchhoff, H.; Hall, C.; Wood, M.; Herbstova, M.; Tsabari, O.; Nevo, R., . . . Reich, Z. *Proc Natl Acad Sci U S A* 2011, *108*, 20248.

(14) Allen, J. F. *Cell* 2002, *110*, 273.

(15) Foyer, C. H.; Neukermans, J.; Queval, G.; Noctor, G.; Harbinson, J. *J Exp Bot* 2012, *63*, 1637.

(16) Iwai, M.; Takizawa, K.; Tokutsu, R.; Okamuro, A.; Takahashi, Y.; Minagawa, J. *Nature* 2010, *464*, 1210.

(17) Arnon, D. I. *Nature* 1959, *184*, 10.

(18) Munekage, Y.; Hashimoto, M.; Miyake, C.; Tomizawa, K.; Endo, T.; Tasaka, M.; Shikanai, T. *Nature* 2004, *429*, 579.

(19) Arnon, D. I.; Whatley, F. R.; Allen, M. B. *Science* 1958, *127*, 1026.

(20) Takahashi, H.; Clowez, S.; Wollman, F. A.; Vallon, O.; Rappaport, F. *Nature Commun* 2013, *4*, 1954.

(21) Terashima, M.; Petroutsos, D.; Hudig, M.; Tolstygina, I.; Trompelt, K.; Gabelein, P., . . . Hippler, M. *Proc Natl Acad Sci U S A* 2012, *109*, 17717.