Studying photosynthetic organisms from different angles

- Photosynthesis - Chloroplast transformation - Hydrogen production -

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I. The PSII repair cycle in cyanobacteria

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Too much light irreversibly damages the photosystem II (PSII) protein complex (site of the water-splitting reaction in photosynthesis). However, oxygenic photosynthetic organisms have evolved an elaborate mechanism to counteract this damage and to maintain their photosynthetic activity (see Figure I.1).

Our lab has identified the FtsH2 protease (Slr0228) as a key component for the synchronised replacement of the D1 protein of PSII in *Synechocystis* sp. PCC 6803 (Silva et al., 2003).

Recently, we have localised FtsH2 of *Synechocysits* sp. PCC 6803 in the thylakoid membrane, GST-tagged and affinity purified the protein as well as determined a low resolution structure of its complex by single particle analysis (see Figure I.2).

Currently, our aims are to perform ATPase activity assays on the purified protease and to identify and purify potential repair complexes that FtsH2 might form with its substrates.

Silva, P., Thompson, E., Bailey, S., Kruse, O., Mullineaux, C.W., Robinson, C., Mann, N.H., and Nixon, P.J. (2003), Plant Cell, 15, 2152-64.

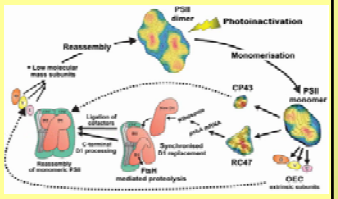

Figure I.1: Schematic representation of the PSII repair cycle in cyanobacteria.

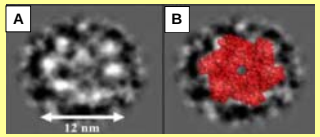

Figure I.2: Single particle analysis of the purified FtsH2-GST protein complex of *Synechocystis* sp. PCC 6803. (A) Magnified FtsH2-GST average. (B) Overlay of the average with an AAA domain (PDB: 1D2N).

II. The role of cytochrome *b*-559 in PSII

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Cytochrome *b*-559 (Cyt *b*-559) is an essential part of photosystem II (PSII), the light driven water-plastoquinone oxidoreductase found within the thylakoid membrane of oxygenic photosynthetic organisms (Figure II.1). Deletion of the alpha subunit leads to a loss of PSII (Morais et al., 1998).

Cyt *b*-559 is a heterodimer composed of alpha and beta subunits. The function of Cyt *b*-559 is still unknown, although most hypotheses have centred on a role in photoprotection.

To investigate the physiological role of Cyt *b*-559, we have constructed a PsbE-H23C mutant of *Chlamydomonas reinhardtii* in which the His-ligand to the haem provided by the the α subunit has been replaced by Cys. The green alga *Chlamydomonas reinhardtii* (Figure II.2) is an excellent model organism in which to investigate PSII function as it is able to survive heterotrophically when supplied with a carbon source.

The mutant assembles PSII at 20% of WT levels, it is more susceptible to light damage than WT cells and has an impaired PSII repair cycle. We aim to use this mutant to explore the precise role of Cyt *b*-559 in photoprotection.

Morais, F., Barber, J., Nixon, P., (1998), JBC, 273, 29315-20
Ferreira, K., Iverson, T., Maghlaoui, K., Barber, J., Iwata, S., (2004), Science, 303, 1831-8

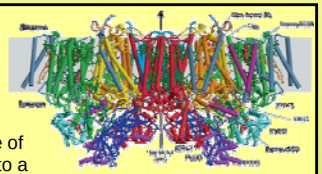

Figure II.1: Crystal structure of the PSII protein complex (Ferreira *et al.*, 2004).

Figure II.2: *Chlamydomonas reinhardtii* cells under the light microscope.

III. Chloroplast transformation

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Chloroplast transformation marks a new era in the field of plant biotechnology using the chloroplast as a cellular compartment that offers attractive advantages over other plant transgene expression methods:

- high level of transgene expression (up to 46 % of tsp)
- strong natural gene containment
- some posttranscriptional modifications (disulfide bonds, lipidation)
- no gene silencing or position effects
- allows expression of entire prokaryotic operons

We are investigating chloroplast transformation in the following species:

- *N. tabacum (Tobacco)*
- *Coffea canephora (Coffee)*
- *C. reinhardtii (Green Alga)*

~~Our aim is to evaluate the potential application of this technique to provide:~~

- Cost-effective production of biopharmaceuticals
- Abiotic and biotic stress-resistance in Coffee
- Photosynthesis-related mutants

Tregoning, J., Maliga, P., Dougan, G., Nixon, P., Phytochemistry, (2004), 65, 8, 989-94.

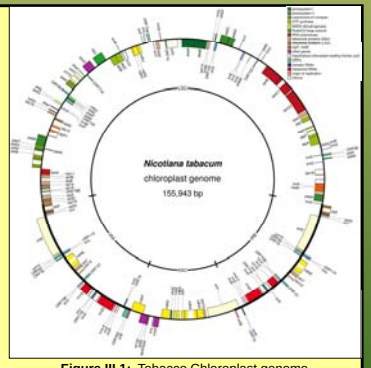

Figure III.1: Tobacco Chloroplast genome

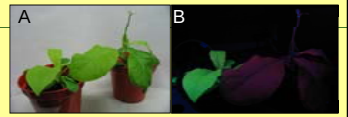

Figure III.2: Transplastomic plant expressing GFP and wild-type under (A) visible light (B) UV light.

IV. H_2 production in Green Algae

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To counteract climate change and because of an ever increasing demand for energy, it is vital for our future to develop new clean energy sources. H_2 can be such an alternative.

Interestingly, certain Green Algae produce H_2 under anaerobic conditions, in order to maintain ATP production. This metabolic pathway operates at ambient temperatures and might be coupled to the desalination of seawater or the production of oil from fermentation waste.

In our lab, we are currently pursuing forward and reverse genetics approaches to improve H_2 yields in the model organism *C.reinhardtii* by:

- screening algal strains for increased H_2 production.
- lowering internal, cellular O_2 levels.
- increasing electron flow to the H_2ase.
- utilizing amiRNAi vectors to knockdown competing pathways (see Figure IV.2).

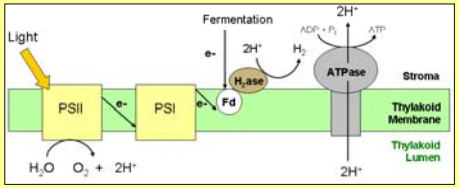

Figure IV.1: Schematic representation of the H_2 production process in green algae. PSI, PSII;Photosystems I and II; Fd, Ferredoxin; H_2ase, Fe-hydrogenase.

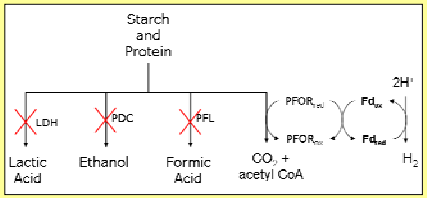

Figure IV.2: Knockdown targets in *C. reinhardtii* fermentative pathways. LDH, Lactate dehydrogenase; PDC, Pyruvate Decarboxylase; PFL, Pyruvate Formate Lyase; PFOR, Pyruvate Ferredoxin Oxidoreductase.