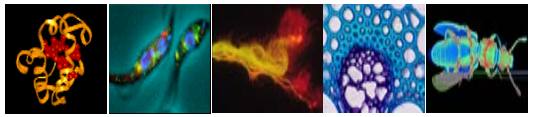

Imperial College London

The role of prohibitins in *Synechocystis* sp. PCC 6803

Are prohibitins involved in photoprotection?

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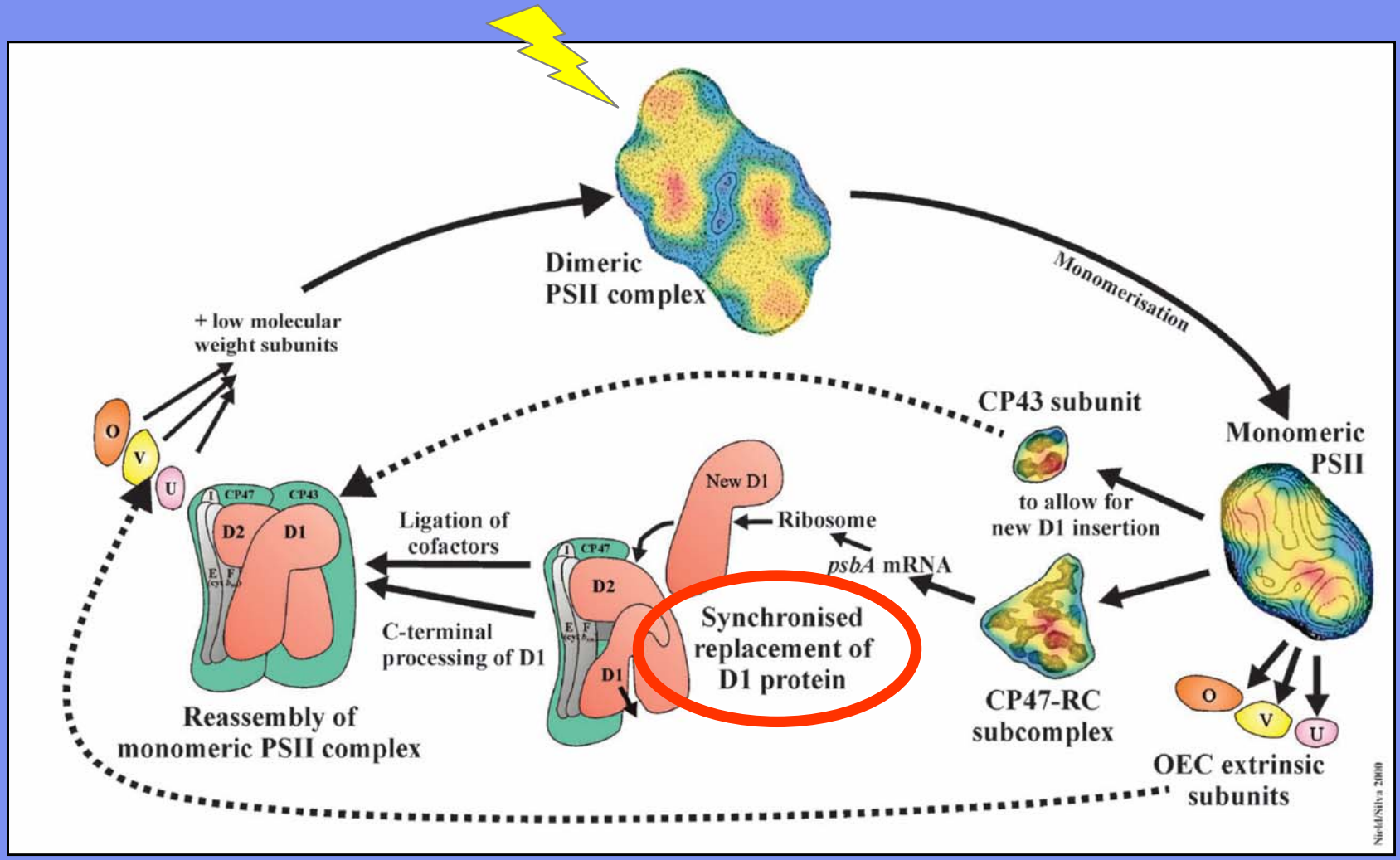
The PSII repair cycle



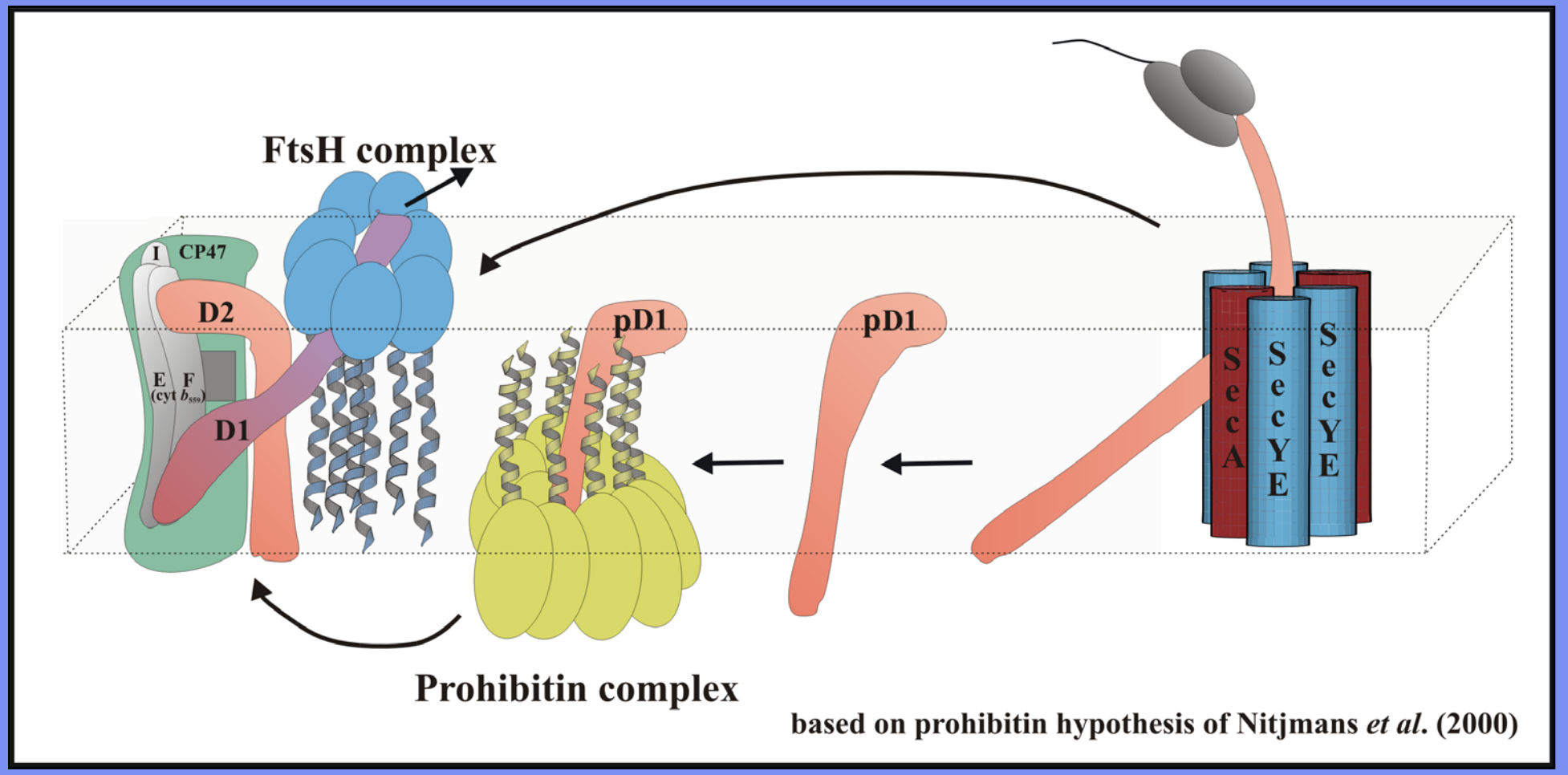
(provided by Paulo Silva and Jon Nield)

Background

- **In *Synechocystis* sp. PCC 6803 FtsH has recently been found to be involved in the removal and degradation of damaged D1 protein (Silva et al., 2003).**
- **In *S. cerevisiae* and *E. coli* FtsH homologues have been found to be associated with prohibitin homologues (Steglich et al., 1999; Saikawa et al., 2004).**
- **The prohibitin homologues in *S. cerevisiae* form large, multimeric complexes (Tatsuta et al., 2005).**
- **These complexes have been reported to negatively regulate an FtsH homologue by binding newly synthesised membrane proteins (Nijtmans et al., 2000).**

Hypothesis and working model

- for synchronised replacement of the D1 protein -



(Silva et al., 2002)

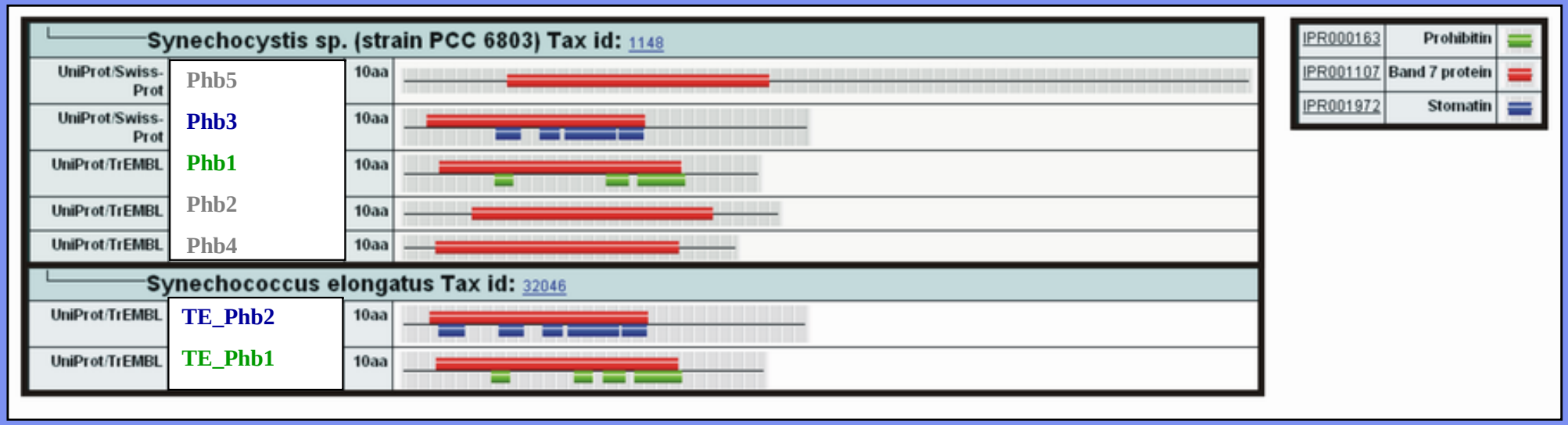
Aims

- **Identification of potential prohibitin homologues in *Synechocystis* sp. PCC 6803 and the thermophilic cyanobacterium *Thermosynechococcus elongatus*.**
- **Bioinformatic analysis of the identified cyanobacterial prohibitin homologues.**
- **Identification and characterisation of possible prohibitin complexes in *Synechocystis* sp. PCC 6803 *in vivo*.**
- **Generation of prohibitin inactivation mutants in *Synechocystis* sp. PCC 6803.**
- **Test the involvement of the prohibitin homologues in the PSII repair cycle.**

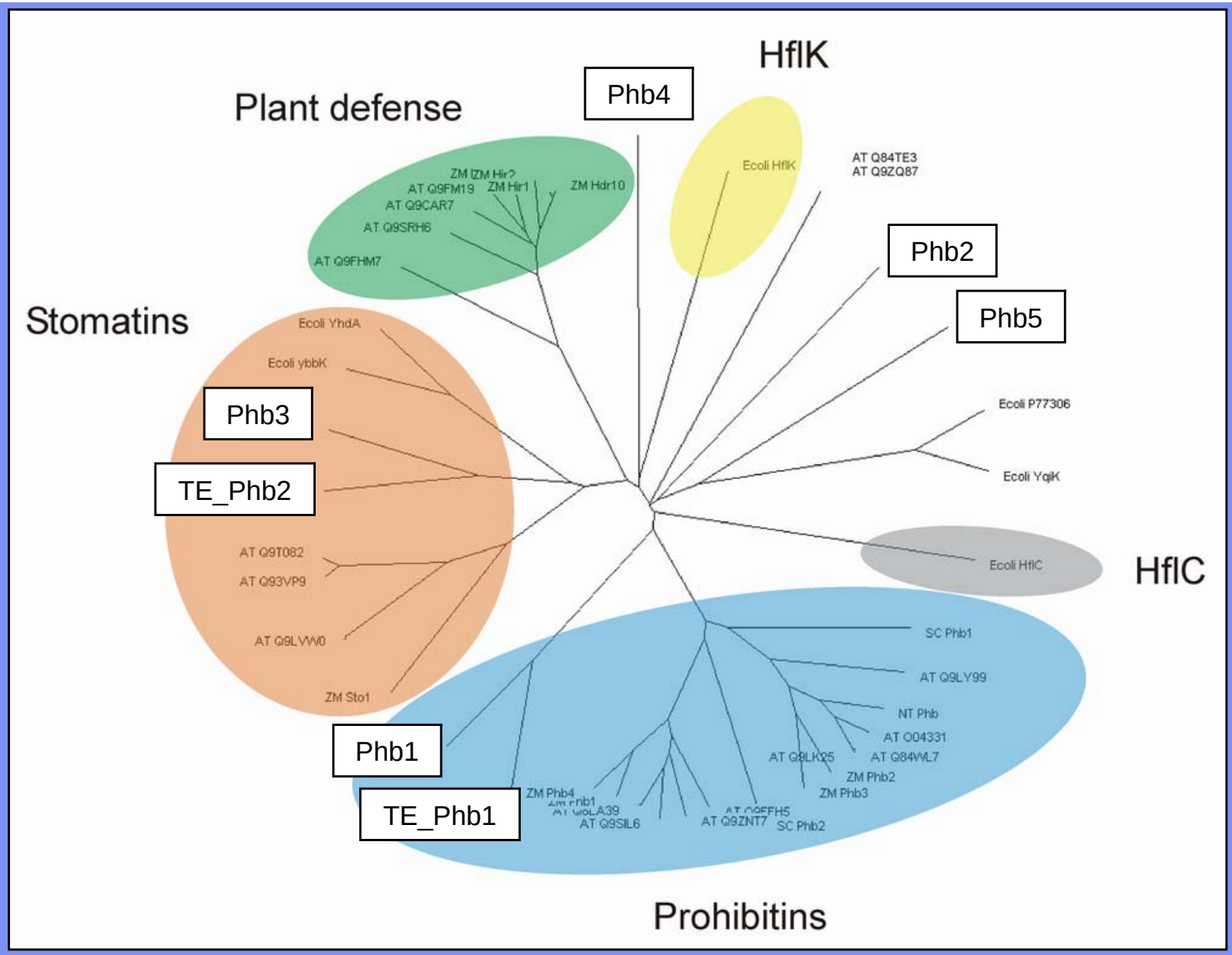
Prohibitin homologues in cyanobacteria

- Prohibitins are members of the Band 7 protein superfamily, that share the SPFH domain as a common motif (Tavernarakis et al., 1999). (SPFH = stomatin, prohibitin, flotillin and HflK/C).

- An Interpro database search identified five Band 7 proteins in *Synechocystis* sp. PCC 6803 and two in *Thermosynechococcus elongatus*.



Dendrogram of selected prohibitin homologues



(based on Nadimpalli et al., 2000)

Prohibitin homologue complexes I

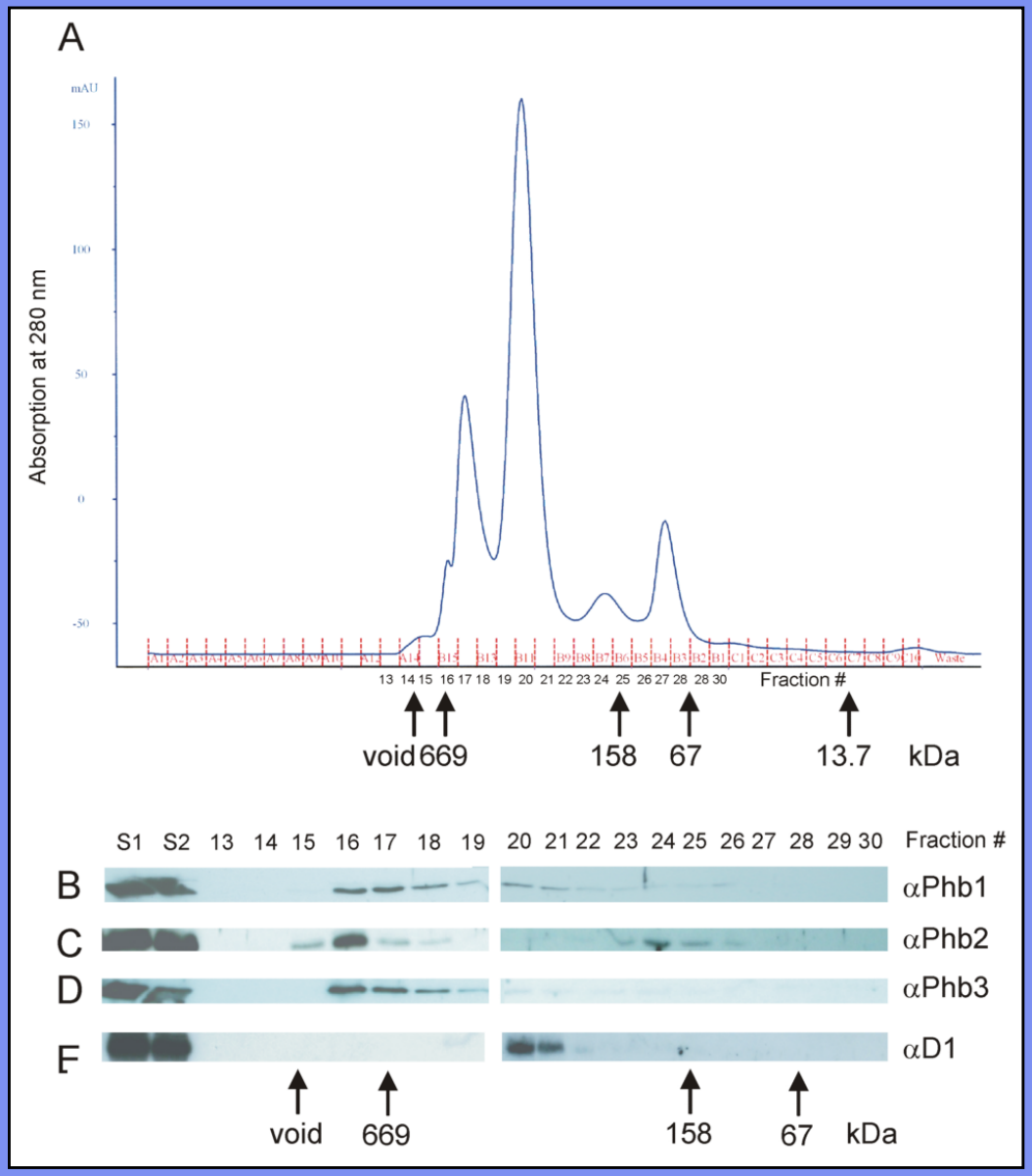
Aims:

Identification and characterisation of prohibitin complexes in membrane extracts from *Synechocystis* sp. PCC 6803 wildtype and mutant cells.

Methods:

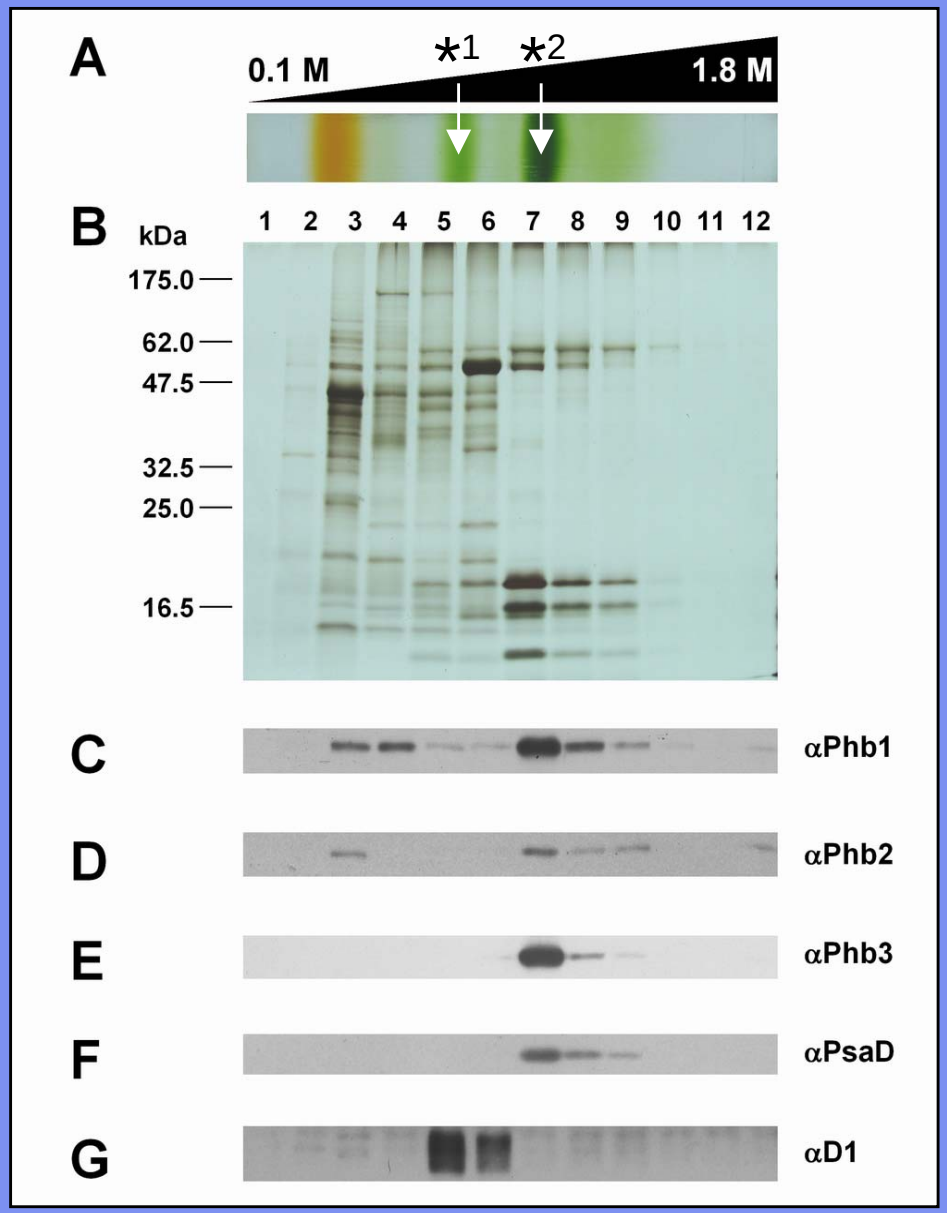
- Raise polyclonal antibodies against *E. coli* overexpressed proteins.
- Generate prohibitin inactivation mutants.
- Separate and identify prohibitin homologue complexes under native conditions by FPLC, BN and sucrose density gradient centrifugation followed by immunoblotting.

Prohibitin homologue complexes II



The prohibitin homologues 1, 2 & 3 form large, multimeric complexes (~669 kDa).

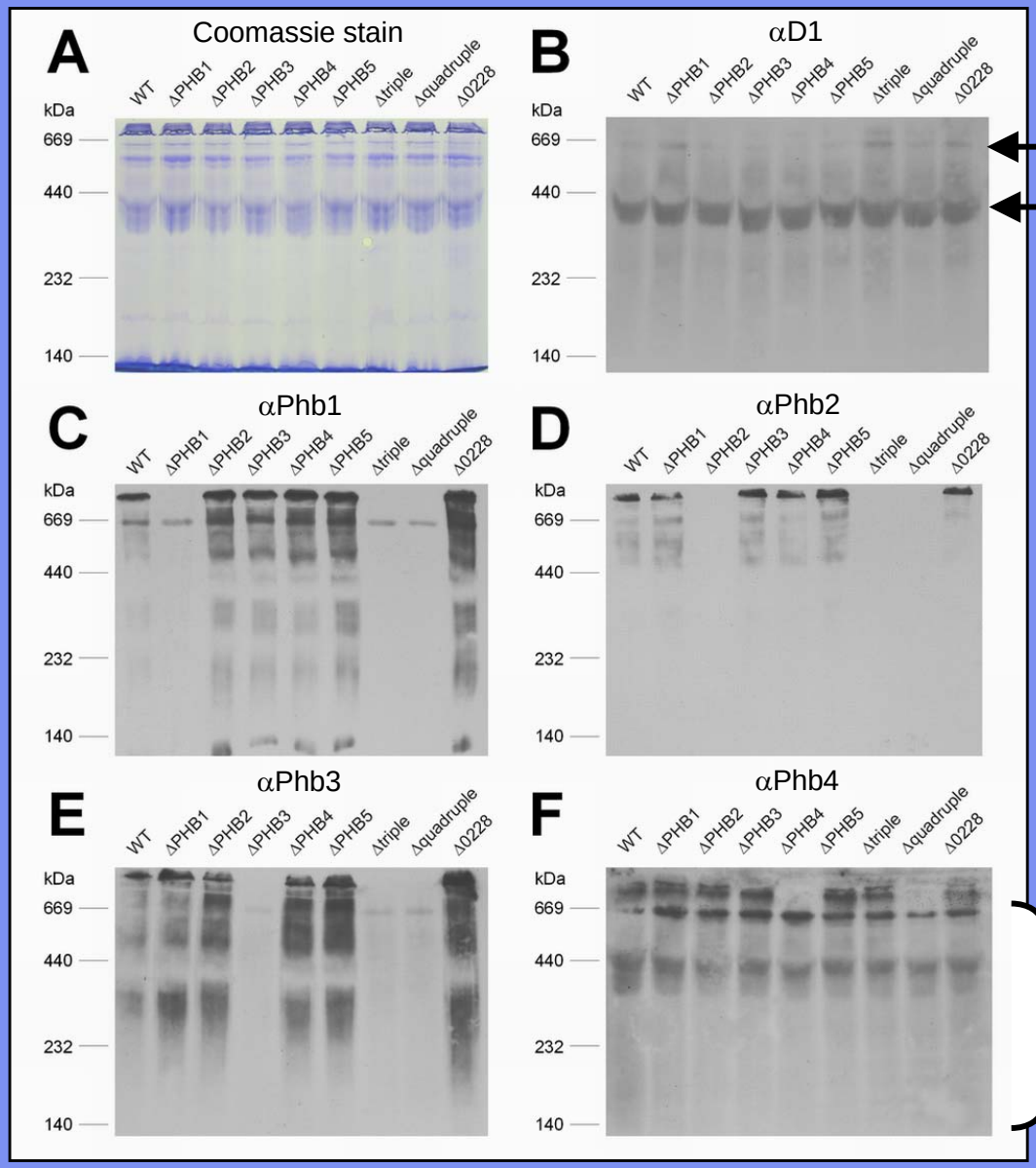
Prohibitin homologue complexes III



The prohibitin homologues 1, 2 & 3 form large, multimeric complexes (~900 kDa).

*1 = monomeric PSI and PSII (~300 kDa)
*2 = trimeric PSI (~900 kDa)

Prohibitin homologue complexes IV



dimeric PSII

monomeric PSII

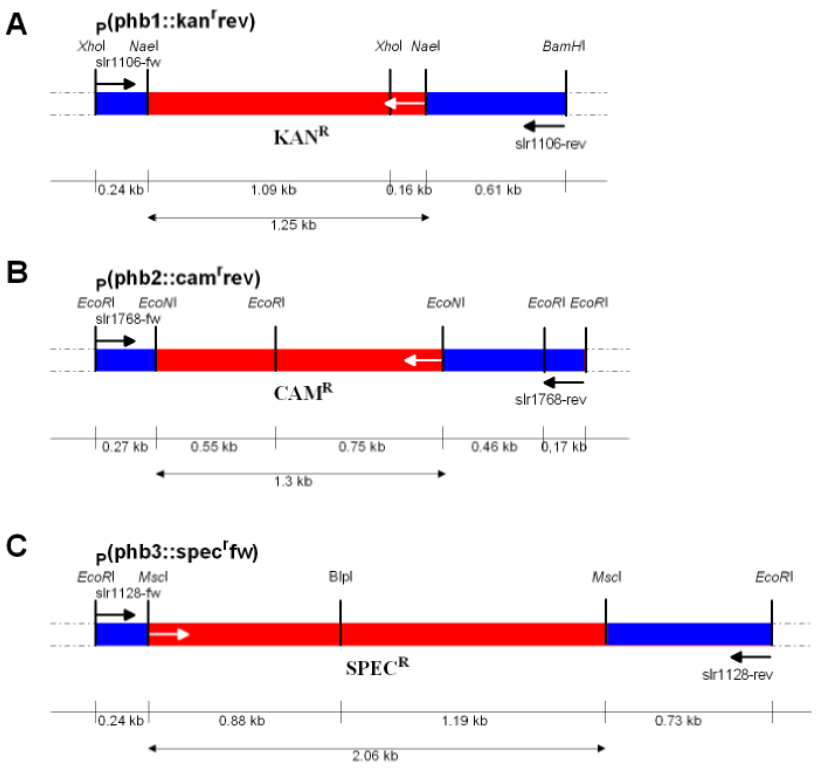
nonspecific

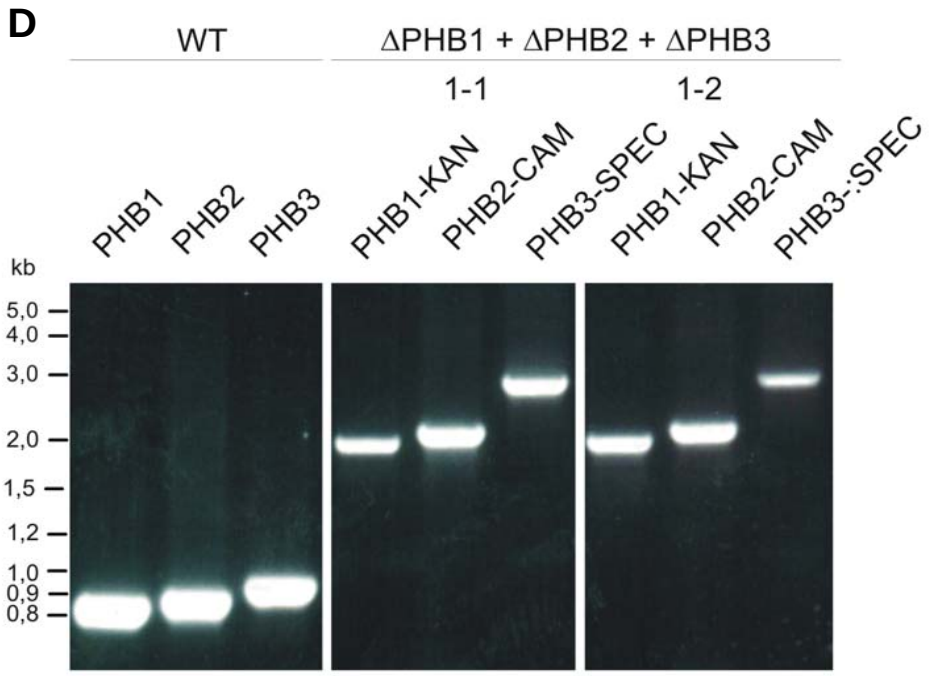
The prohibitin homologues 1, 2, 3 & 4 form large complexes (>669 kDa).

Each complex still forms, even if one of the other prohibitin homologues is inactivated.

The prohibitin triple mutant generation

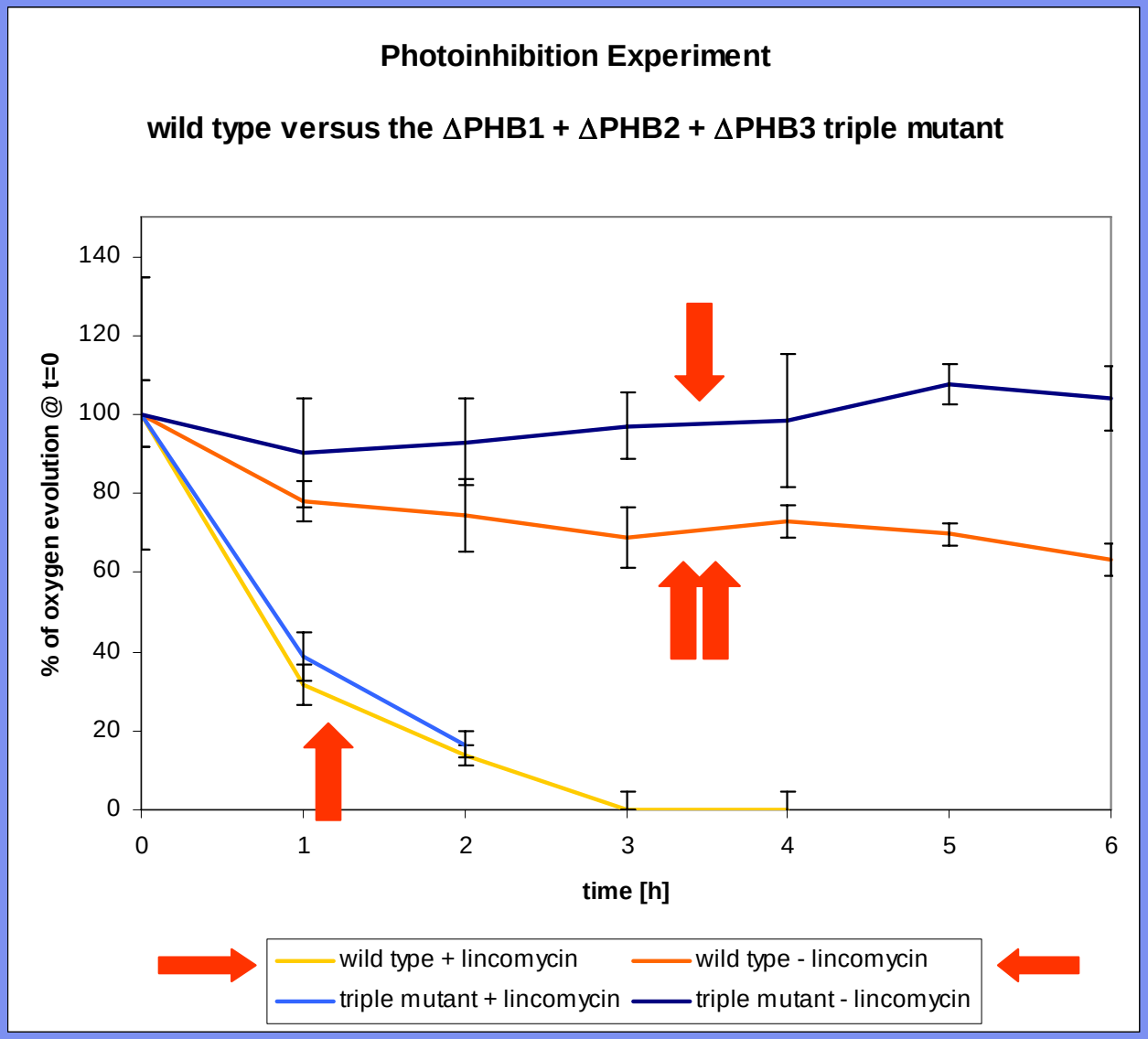
Phb1, Phb2 & Phb3 were insertionally inactivated by transforming *Synechocystis* sp. PCC 6803 with recombinant DNA constructs.





PCR analysis of wildtype and mutant strains

Photoinhibition experiment



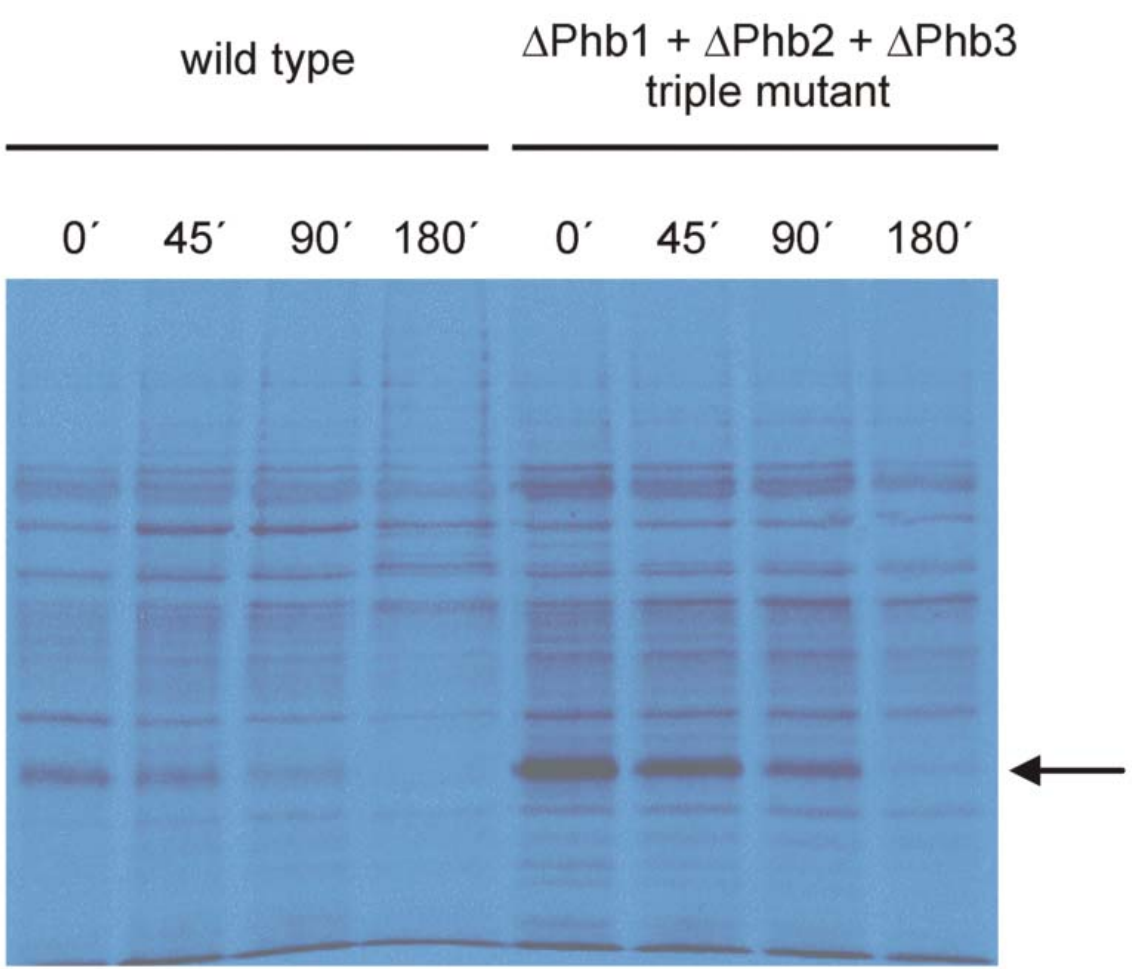
Aim:

Test if prohibitins are involved in the PSII repair cycle.

- Wildtype cells maintain PSII activity (oxygen evolution).
- PSII activity decreases in wild-type cells in the presence of a protein synthesis inhibitor.
- The triple mutant acts similar to wildtype cells.

PSII repair cycle is not impaired in the triple mutant!

Pulse-chase experiment



Aim:

Monitor selective D1 protein turnover in wildtype and mutant *Synechocystis* sp. PCC 6803 cells.

- The initial D1 protein labeling appears to be higher in the triple mutant.
- Nevertheless the rate of D1 protein degradation is similar in wildtype and mutant cells.

Similar rate of D1 protein turnover in the triple mutant!

Conclusions

- ✓ **Five prohibitin homologues were identified in *Synechocystis* sp. PCC 6803 and two in *Thermosynechococcus elongatus*.**
- ✓ **Cyanobacterial prohibitin homologues are only distantly related to other known, eukaryotic prohibitin homologues.**
- ✓ **The prohibitin homologues Phb1, Phb2, Phb3 and Phb4 form large and possibly homomultimeric protein complexes.**
- ✓ **The prohibitin homologues Phb1, 2, 3, 4 & 5 are not essential for cell viability under laboratory growth conditions.**
- ✓ **The prohibitin homologues Phb1, Phb2 and Phb3 seem not to be involved in the PSII repair cycle or affect the rate of D1 protein turnover under the conditions tested.**

Future work

- **Elucidating the physiological relevance of the prohibitins in *Synechocystis* sp. PCC 6803 by growing the generated mutants under various stress conditions.**
- **Further characterisation of the prohibitin homologues and their respective complexes.**
  - **Purification of the prohibitins to analyse possible interaction partners (pD1?).**
  - **Single particle analysis on purified prohibitin complexes.**

Aknowledgements

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