Imperial College London

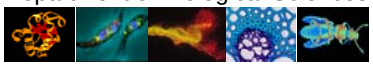
Degradation prohibited?

The role of prohibitins in cyanobacteria

Marko Boehm, Myles Barker, Remco de Vries and Peter Nixon

Wolfson Laboratories, Department of Biological Sciences, Imperial College London, South Kensington, Campus, SW7 2AZ, UK

Department of Biological Sciences



Background

- Under excess light the D1 protein of photosystem II (PSII) is irreversibly damaged (Barber and Andersson, 1992).
- Damaged D1 protein is removed and degraded by the FtsH protease in *Synechocystis sp.* PCC 6803 (Silva et al., 2003).
- A new copy of the D1 protein is integrated into the reassembling PSII complexes.

- Prohibitins and their homologues in various organisms have been found to:
  a. form a large complex with FtsH (Saikawa et al., 2004).
  b. have a negative, regulatory effect on the turnover of membrane proteins (Steglich et al., 1999).

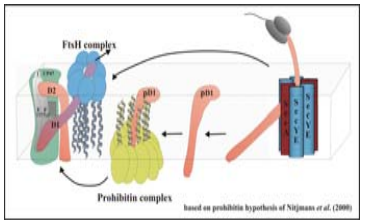


Figure 1 (from Silva et al., 2002): Working hypothesis for D1 protein turnover. D1 degradation is catalysed by a large FtsH complex. The replacement D1 protein may be inserted into PSII co-translationally via the Sec translocon or post-translationally via a large prohibitin complex. This model is based on the prohibitin hypothesis for assembly of respiratory complexes in yeast (Nijtmans *et al.*, 2000).

- Prohibitins belong to a protein family that shares the SPFH domain as a common feature (Tavernarakis et al., 1999).
- We have identified five prohibitin homologues in *Synechocystis sp.* PCC 6803 and two in *Thermosynechococcus elongatus*.

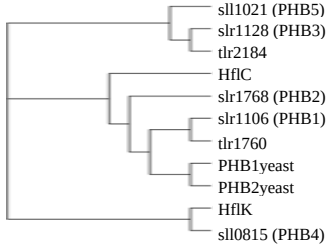


Figure 2: Phylogenetic tree of prohibitins and their homologues from various organisms. slr1106, slr1768, slr1128, sll0815 and sll1021 *Synechocystis sp.* PCC 6803; tlr1760 and tlr2184 *Thermosynechococcus elongatus*; PHB1yeast and PHB2yeast *S. cerevisiae*; HflK and HflC *E. coli*. The tree is based on a bootstrapped CLUSTALW alignment.

Aims

- to test the importance of prohibitins for cell viability.
- to investigate the role of prohibitins in D1 turnover.
- to identifiy and characterize prohibitin complexes.

Construction of a prohibitin triple mutant

Aim: As a first step, generate a prohibitin triple mutant.

Method: Directed mutagenesis by transformation and homologous recombination; confirmation by PCR.

Result: A segregated and viable *phb1*::KAN^R, *phb2*::CAM^R, *phb3*::SPEC^R mutant has been generated.

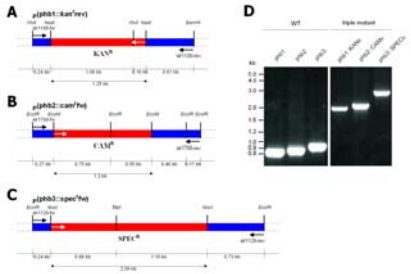


Figure 3: Generation of a prohibitin triple mutant. The three prohibitin genes (slr1106 = *phb1*; slr1768 = *phb2* and slr1128 = *phb3*) of *Synechocystis sp.* PCC 6803 wild type cells were knocked out by insertion of an antibiotic resistance cassette via homologous recombination. (A-C) Schematic drawings of the constructs that were used for transformation. (D) Complete segregation of the mutant was confirmed by PCR.

The prohibitin triple mutant is not sensitive to high light illumination

Aim: To test whether the prohibitin triple mutant is susceptible to light stress.

Method: WT, slr0228::CAM^R (positive control; mutant dies at high irradiance) and the prohibitin triple mutant were restreaked on BG11 +/- glucose plates and grown under high and low light conditions.

Result: The prohibitin triple mutant survives high light illumination.

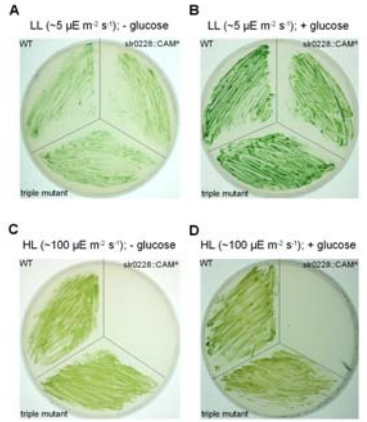


Figure 4: Growth of the prohibitin triple mutant under high and low light. (A-D) WT, slr0228::CAM^R and the prohibitin triple mutant were restreaked on BG11 +/- 5mM glucose plates and grown under high (~100 µE m^{-2} s^{-1}) and low (~5 µE m^{-2} s^{-1}) light.

D1 replacement is unimpaired in the prohibitin triple mutant

Aim: To investigate the role of PHB1,2,3 in D1 turnover.

Method: Comparative pulse-chase analysis under high light conditions with WT and the prohibitin triple mutant.

Result: No dramatic changes in the pattern of D1 protein turnover can be observed in the prohibitin triple mutant.

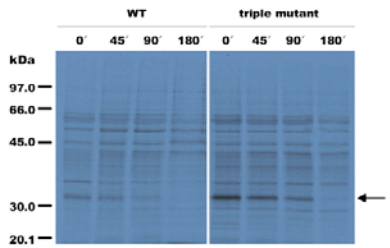


Figure 5: Comparative pulse-chase analysis of WT and the prohibitin triple mutant. Cells were pulsed with radio-labelled methionine and then exposed to high light conditions (~1000 µE m^{-2} s^{-1}). Thylakoids were isolated at indicated time points (0, 45, 90 and 180 min) and separated by SDS-PAGE. The gel was dried and exposed to a film for 1 day. The arrow on the right indicates the position of the D1 protein.

Identification of a prohibitin complex in *Thermosynechococcus elongatus*

Aim: To identify a prohibitin complex in cyanobacteria.

Method: 2D-PAGE (Blue-Native- followed by SDS-PAGE) and western analysis of thylakoids isolated from the thermophilic cyanobacterium *Thermosynechococcus elongatus*.

Result: A large (~1 MDa) PHB1 homologue complex has been identified in *Thermosynechococcus elongatus*. The FtsH homologue has been identified in two large complexes of about 450 kDa and ~1MDa in size.

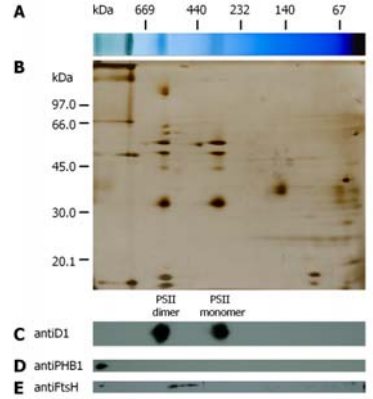


Figure 6: 2D-PAGE and western analysis of thylakoid membranes isolated from *Thermosynechococcus elongatus*. (A) The protein complexes were separated by Blue-Native-PAGE and (B) resolved into their subunits on a SDS gel. Various proteins have been identified with specific antibodies in western analysis: (C) D1; (D) prohibitin 1 homologue (antibody against PHB1 from *Synechocystis sp.* PCC 6803) and (E) FtsH homologue (antibody against FtsH from *E. coli*).

Conclusions

- slr1106, slr1768 and slr1128 are not essential even when the cells are grown under high light conditions.
- slr1106, slr1768 and slr1128 are not absolutely needed for D1 replacement after photoinhibition.
- PHB1 in *Synechocystis sp.* PCC 6803 (data not shown) and its homologue in *Thermosynechococcus elongatus* form a large complex (~1MDa).

References

Barber, J. and Andersson, B. (1992) *Trends Biochem Sci* **17**, 61-6

Nijtmans, L. G., de Jong, L., Artal Sanz, M., Coates, P. J., Berden, J. A., Back, J. W., Muijsers, A. O., van der Spek, H., and Grivell, L. A. (2000) *EMBO J* **19**, 2444-51

Saikawa, N., Akiyama, Y., and Ito, K. (2004) *J Struct Biol* **146**, 123-9

Silva, P., Choi, Y. J., Hassan, H. A., and Nixon, P. J. (2002) *Philos Trans R Soc Lond B Biol Sci* **357**, 1461-7; discussion 1467-70

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

Steglich, G., Neupert, W., and Langer, T. (1999) *Mol Cell Biol* **19**, 3435-42

Tavernarakis, N., Driscoll, M., and Kyrpides, N. C. (1999) *Trends Biochem Sci* **24**, 425-7