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Probing the role of the Band 7 protein superfamily in the cyanobacterium *Synechocystis sp. PCC 6803*

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Introduction

The Band 7 protein superfamily is found throughout nature and features the SPFH domain (**S**tomatin, **P**rohibitin, **F**lotillin and **H**flK/C) as a common motif [1]. In both, *E. coli* and *S. cerevisiae*, Band 7 proteins form large, hetero-multimeric complexes with a FtsH protease and are implicated in the assembly of membrane protein complexes [2,3]. In the cyanobacterium *Synechocystis* sp. PCC 6803 the FtsH homologue (Slr0228) has been shown to be important for the repair of the photosystem two (PSII) complex and the protection of the cell from the damaging effects of light [4,5]. In a recent publication, the stomatin homologue of *Synechocystis* sp. PCC 6803 (Slr1128) has been implied to be associated with the high light-inducible proteins (HLIPs) A and B and to possibly help stabilising trimeric photosystem I (PSI) complexes [6].

Identification and Characterisation of the Band 7 proteins of *Synechocystis* sp. PCC 6803

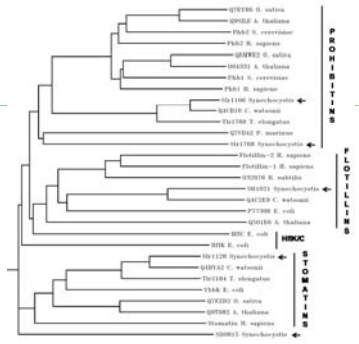


Fig.1: Phylogenetic analysis of the Band 7 proteins of *Synechocystis* sp. PCC 6803 (marked with arrows). Annotation of subfamilies is according to the InterPro DB.

According to database annotations (InterPro DB) and a phylogenetic analysis (Fig.1), the identified five Band 7 proteins of *Synechocystis* sp. PCC 6803 (Slr1106, Slr1768, Slr1128, Sll0815 and Sll1021) were assigned to different Band 7 protein subfamilies. The phylogenetic analysis further suggested that the Band 7 proteins of *Synechocystis* sp. PCC 6803 are only distantly related among themselves and to other known Band 7 proteins.

In order to be able to characterise the Band 7 proteins of *Synechocystis* sp. PCC 6803, antibodies were raised from *E. coli* over-expressed protein. With these antibodies, it could be shown that Slr1106, Slr1768 and Slr1128 are membrane-bound proteins (Fig.2A). Further localisation experiments demonstrated that Slr1106 is present in both the thylakoid as well as the plasma membrane, while the Slr1768 and Slr1128 proteins are solely present in the plasma membrane (Fig.2B).

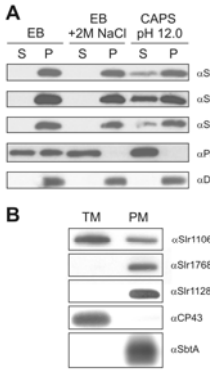


Fig.2: Immunoblotting analyses of (A) a differential membrane protein extraction and (B) a membrane localisation experiment. (A) extraction buffer (EB), soluble (S) and pellet (P) fractions. (B) Thylakoid (TM) and plasma membrane (PM). Membrane samples were provided by Prof. Eva-Mari Aro and had been prepared by two-phase partitioning.

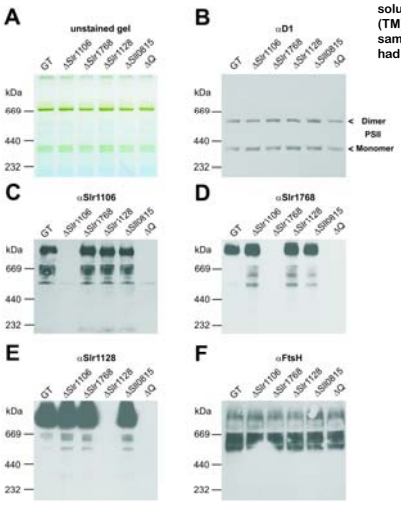


Fig.3: Blue-native (BN) and immunoblotting analyses of *Synechocystis* sp. PCC 6803 wild-type glucose-tolerant (GT) as well as of single and multiple Band 7 gene inactivation mutants (ΔQ is a quadruple mutant with the Slr1106, Slr1768, Slr1128 and Sll0815 genes disrupted).

BN-PAGE and immunoblotting analyses on crude membrane isolations from wild-type as well as from single and multiple Band 7 gene inactivation mutants were performed, in order to assess the sizes and potential interactions of the Band 7 protein complexes in *Synechocystis* sp. PCC 6803 (Fig. 3). The results of these analyses suggest, that the proteins form large and possibly homo-multimeric complexes with an apparent molecular mass of more than 669 kDa. Furthermore, in the single or multiple Band 7 gene inactivation mutants, the Slr1106, Slr1768 and Slr1128 protein complexes still form, even though other respective Band 7 proteins are not present. FtsH protein complexes also did not seem to be affected by the inactivation of Band 7 genes (Fig.3F).

The Band 7 proteins of *Synechocystis* sp. PCC 6803 do not seem to be involved in PSII repair

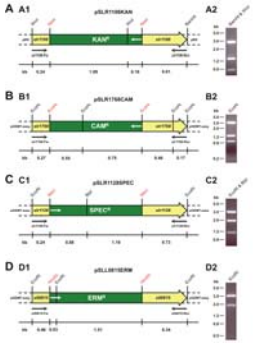


Fig.4: (A-D) Schematic drawing and restriction digest analyses of the plasmids used for the generation of the Band 7 quadruple mutant (ΔQ) and (E) PCR analysis of the Band 7 genes using genomic DNA from the wild-type and ΔQ mutant strains.

To probe the physiological relevance of the Band 7 proteins and to assess their potential role in the PSII repair cycle, a quadruple mutant (ΔQ) was constructed in which 4 of the 5 Band 7 genes were disrupted (see Fig.4). However, even though various growth conditions were tested (e.g. high-light, cold, oxidative stress, DCMU), no different phenotype compared to wild-type strain could be observed for the segregated mutant. Pulse-chase (data not shown) as well as photoinhibition analyses (Fig.5) did not reveal any changes in the rate of D1 protein turnover or in the capability to evolve oxygen after high-light treatment. Thus, it appears unlikely that the Band 7 proteins of *Synechocystis* sp. PCC 6803 play a crucial role for PSII maintenance under high-light conditions.

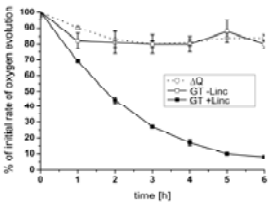


Fig.5 (left): Photoinhibition experiment using the *Synechocystis* sp. PCC 6803 wild-type glucose-tolerant (GT) and the Band 7 quadruple mutant (ΔQ) strains. Cells were exposed to ~1,200 µE m^{-2} s^{-1} in the absence (-) or presence (+) of the protein synthesis inhibitor lincomycin (Linc).

The stomatin homologue (Slr1128) of *Synechocystis* sp. PCC 6803 forms a ring-like structure

Synechocystis sp. PCC 6803 Band 7 protein specific antibodies were affinity-purified and covalently crosslinked to Protein A sepharose. Subsequently performed immunoprecipitation experiments provided further evidence that these Band 7 proteins do not interact with each other (Fig.6 and Fig.3). In the case of Slr1128, the sample was of sufficient quantity and quality to allow low-resolution structural studies by negative-stain electron-microscopy followed by single-particle analysis (Fig.7). The visualised Slr1128 complexes displayed a ring-like structure with an approximate diameter of 16 nm. Overall, a subpopulation of 499 particles (classified as the largest projections observed within a total of 3,708 particles) was assigned as a "top view" and further distinguished into 7 classes.

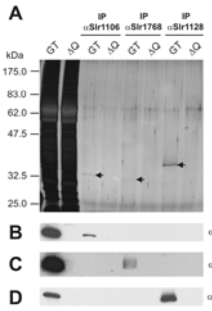
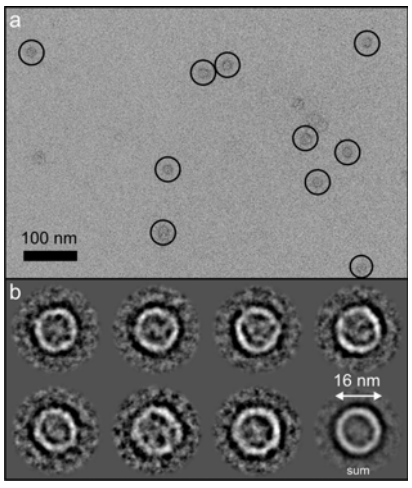


Fig.6 (above): Immunoprecipitations of *Synechocystis* sp. PCC 6803 wild-type glucose-tolerant (GT) and quadruple mutant (ΔQ) crude membrane samples and Band 7 protein specific antibodies.

Fig.7 (right): (A) A typical micrograph section of Slr1128 complexes generated by negative-stain electron-microscopy and (B) single-particle analysis class averages of immunoprecipitated Slr1128 protein complexes shown in Fig. 6.



Conclusions

The Band 7 proteins of *Synechocystis* sp. PCC 6803 are:
- only distantly related among themselves and to other Band 7 proteins.
- not essential for cell viability.
- membrane-bound, with Slr1768 and Slr1128 solely localised in the plasma membrane and Slr1106 present in both the thylakoid and plasma membrane.
- large, possibly homo-multimeric protein complexes (>669 kDa).
- unlikely to play a crucial role in PSII maintenance under high-light conditions.

The Slr1128 protein forms ring-like complexes with a diameter of around 16 nm.

Acknowledgements:

We thank Prof. Eva-Mari Aro and Dr. Pengpeng Zhang for their help with the localisation and BN-PAGE experiments that were performed during Marko Boehm's stay at the University of Turku in 2006.

References:

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