Subunit organisation of the FtsH complexes in the cyanobacterium *Synechocystis* sp. PCC 6803

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1. FtsH proteases in *Synechocystis* sp. PCC 6803

FtsH proteases are universally conserved in bacteria, mitochondria and plastids. They are ATP-dependent, Zn^{2+}-dependent metalloproteases involved in degrading soluble and membrane-bound proteins. Studies on over-expressed soluble domains of FtsH suggest that FtsH can form hexameric complexes. However structural information on the intact complex is sparse. The substrate is believed to be pulled via ATP hydrolysis through a central pore to be degraded into oligopeptides within the protease domain (Ito and Akiyama, 2005). There are four FtsH homologues found in the cyanobacterium *Synechocystis* sp. PCC 6803: FtsH1 (Slr1390), FtsH2 (Slr0228), FtsH3 (Slr1604) and FtsH4 (Sll1463). FtsH2 has been shown to be involved in the degradation of damaged D1 during PSII repair (see Figure 2; Nixon et al., 2005). FtsH1 and 3 are essential for cell viability (Mann et al., 2000); however their specific functions as well as that of FtsH4 remain unclear.

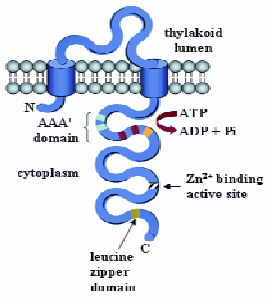


Figure 1: Schematic representation of the FtsH domains (Nixon *et al.*, 2005)

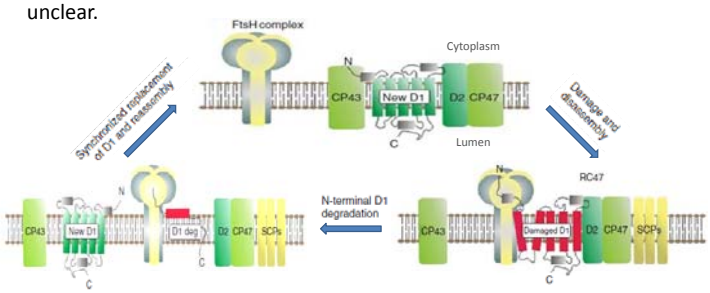


Figure 2: Model for the FtsH-mediated PSII repair cycle (adepted from Nixon *et al.*, 2010). After photoinhibition, CP43 is released from the damaged PSII complex, which enables the FtsH2/FtsH3 protease complex to gain access to the N-terminus of D1 protein. The damaged D1 protein is then degraded processively and a newly synthesised copy of the D1 protein is incorporated into the reassembling PSII complex.

2. Aims of study

1- Isolate each of the 4 FtsH homologues from *Synechocystis* sp. PCC 6803 using an affinity-tagging approach
2- Characterise the subunit composition of the FtsH complexes through immunoblotting and/or protein sequencing
3- Assess the 3-D structures of the FtsH complexes through transmission electron microscopy (TEM) of negatively stained particles

3. Affinity tagging and isolation of FtsH from *Synechocystis* sp. PCC 6803

Each of the four FtsH homologues was tagged in *Synechocystis* sp. PCC 6803 with a glutathione *S*-transferase (GST) affinity tag and purified by a one-step affinity chromatography procedure using glutathione sepharose.

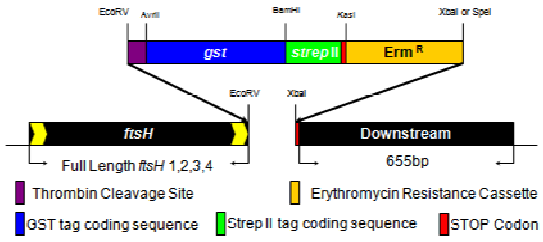


Figure 3: Cloning strategy and construct scheme. A DNA tagging cassette which contains a thrombin cleavage site, GST and Strep II tag coding sequences as well as the erythromycin resistance cassette was ligated at the 3' end of each FtsH coding sequence.

The FtsH-GST fusion proteins (~97 kDa) were expressed and were stable to purification. A smaller protein band (~68 kDa) consistently co-purified with GST-tagged FtsH1, FtsH2 and FtsH3 protein but not with FtsH4-GST (see Figure 4A). Western blots using global FtsH antibodies suggested that the 68-kDa band contained WT FtsH homologues (Figure 4C). The identity of the co-purified FtsH was revealed using FtsH-specific antibodies (Figure 4D-G).

Immunoblotting indicated the presence of FtsH1/FtsH3 and FtsH2/FtsH3 heterocomplexes, as well as FtsH4 homocomplexes.

As FtsH2 is known to be involved in D1 degradation, the structure of the FtsH2-GST/FtsH3 complex was assessed first (see panel 4).

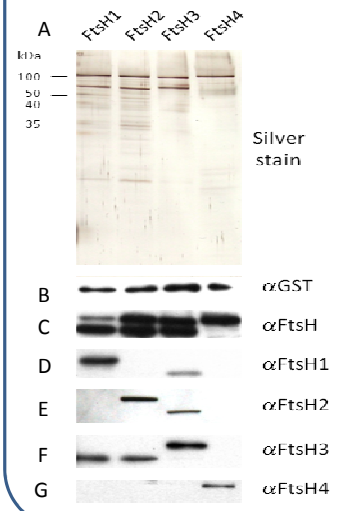
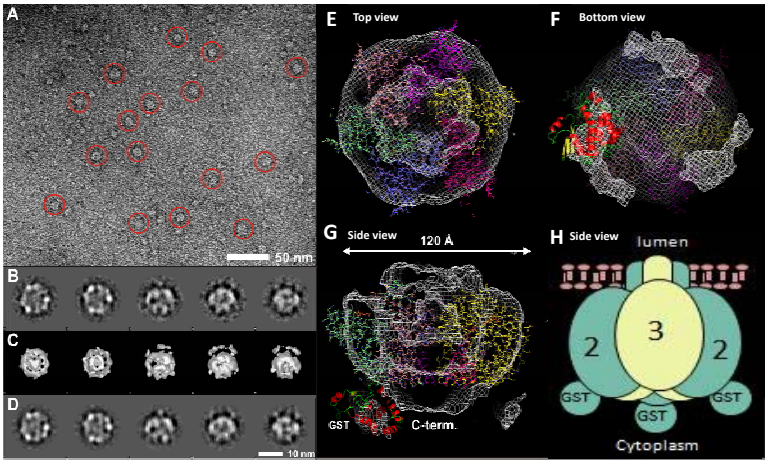


Figure 4: Isolation of FtsH complexes. Purified FtsH-GST proteins were analysed by SDS-PAGE (A) and by immunoblotting using GST and FtsH specific antibodies (B to G).

4. Electron microscopy and single particle analysis of the FtsH2-GST/FtsH3 complex



A 3D model for the structure of the FtsH2-GST/FtsH3 complex was obtained by modeling in the crystal structures of the hexameric cytosolic region from *T. thermophilus* FtsH (Suno *et al.*, 2006) and GST tag within the surface rendered molecular envelope, thresholded at 2.5 sigma. The final structure (non-symmetrised, as shown) was calculated to have a resolution of approx 26 Å (by Fourier Shell Correlation), revealing a defined hexameric ring of ~12 nm in diameter. The alternating arrangement of FtsH2-GST and FtsH3 (non-tagged) subunits was evident through locating the GST tag densities on one side of the map. This structural model supports the presence of a transmembrane region that acts to anchor the complex in the thylakoid membrane, a small lumenally exposed domain and a larger domain exposed to the cytoplasm.

Figure 5: Negatively stained FtsH2-GST/FtsH3 particles imaged by TEM (A, B, C and D), 3D reconstruction of the protein complex (E, F and G) and the schematic representation of the side view of an FtsH2-GST/FtsH3 complex (H). (A) A region of the micrograph with FtsH2-GST/FtsH3 particles (circled, red). (B) Selection of five characteristic 2D views. (C) 3D reconstruction of the protein complex using 263 different class averages from 2,964 particles (at approx 26 Å resolution). (D) Surface-rendered views of the final 3D map calculated by angular reconstitution. (E to G) The 3D model of FtsH2-GST/FtsH3 complex.

5. Conclusions

1- FtsH1/FtsH3 and FtsH2/FtsH3 heterocomplexes are present in *Synechocystis* sp. PCC 6803
2- FtsH4 probably exists as a homocomplex
3- The FtsH2-GST/FtsH3 complex is a hexameric complex of alternating subunits

6. References

Ito and Akiyama (2005) *Annu Rev. Microbiol.* ***59***: 211-31
Mann *et al.* (2000) *FEBS Lett.* **479**: 72-77
Nixon *et al.* (2005) *J Exp Bot.* **56**: 357-63
Nixon *et al.* (2010) *Ann Bot.***106**: 1-16
Suno *et al.* (2006) *Mol Cell.* **22**: 575-85