Imperial College London

Structure and function of FtsH complexes in *Synechocystis* 6803

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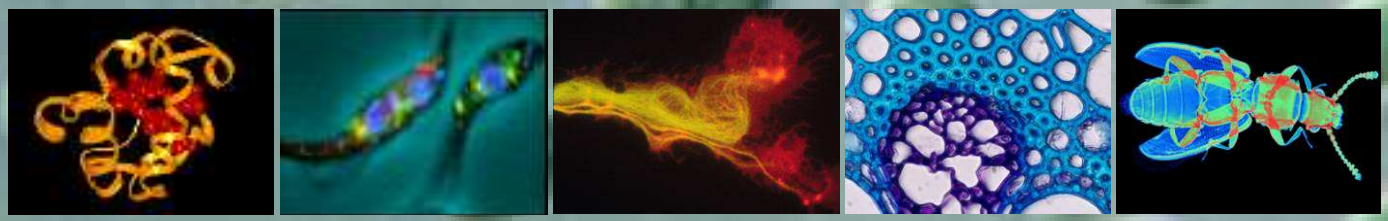
Division of Biology



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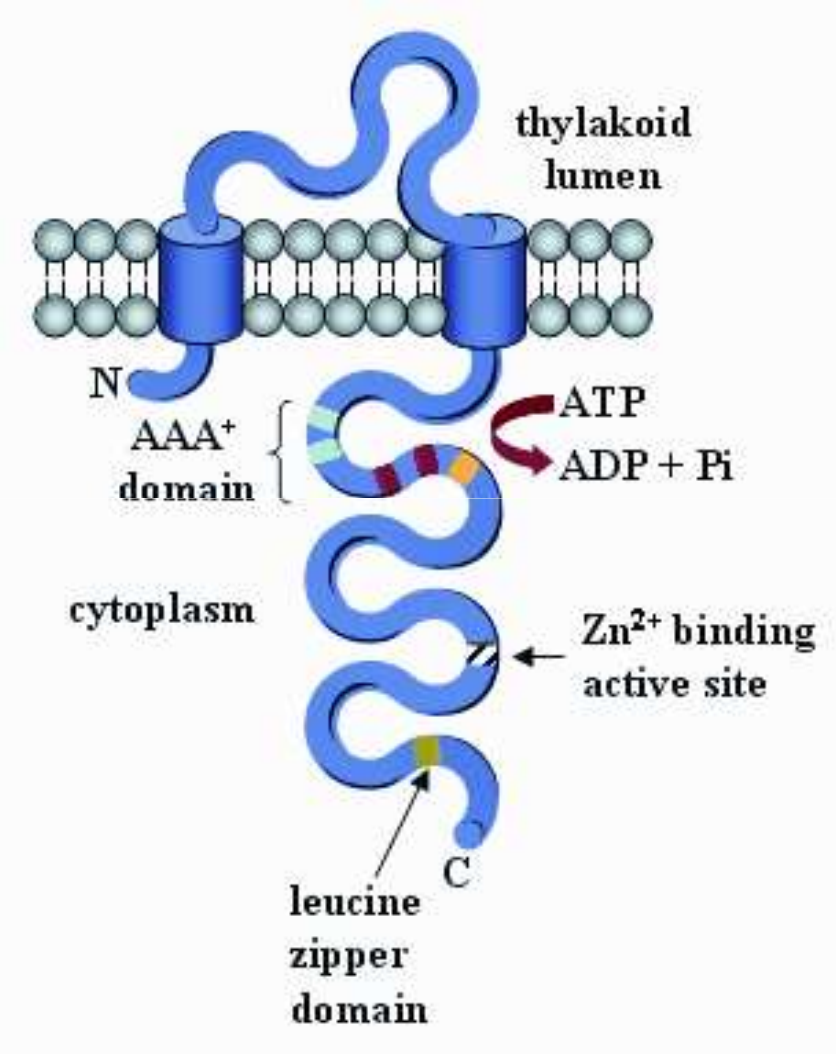
FtsH proteases



- **Universally conserved in bacteria and found in chloroplasts and mitochondria**

- **Involved in degradation, assembly and dislocation**

- **4 homologues in *Synechocystis* 6803:**

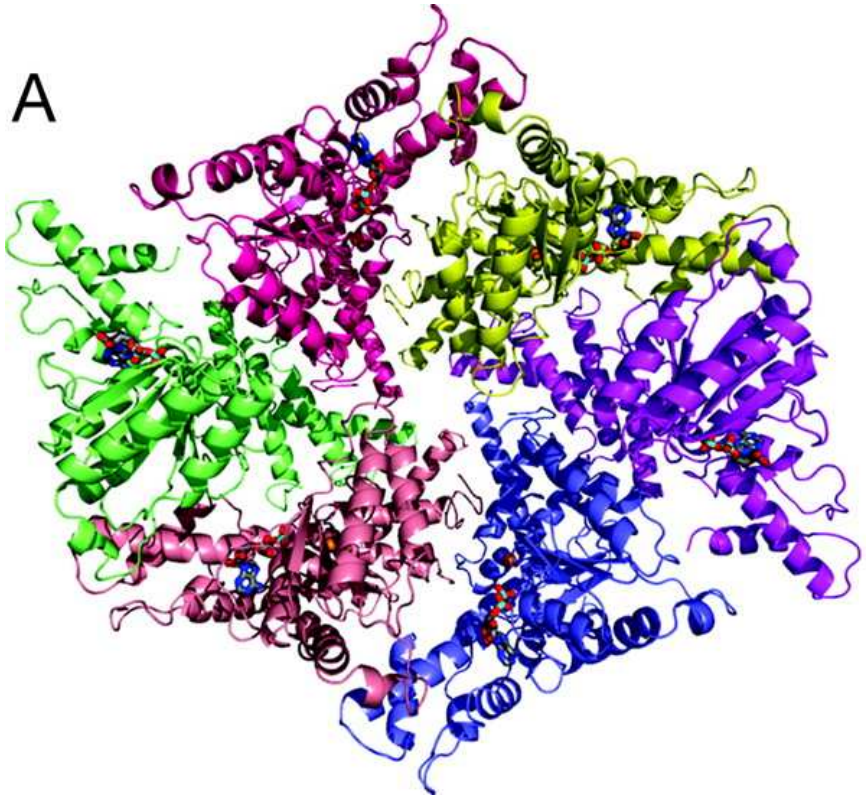
Slr1390 (FtsH1) vital
Slr0228 (FtsH2) dispensible
Slr1604 (FtsH3) vital
Sll1463 (FtsH4) dispensible

(Mann et al., 2000)

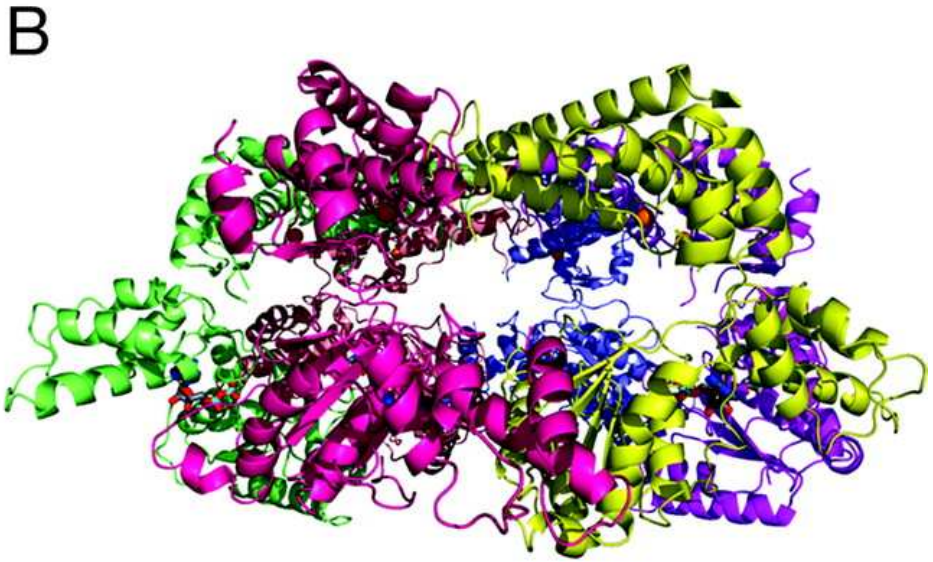
- **9 FtsH homologues targeted to *Arabidopsis* chloroplast**

(Sakamoto et al., 2003)

The crystal structure of the soluble cytosolic region from *Thermotoga maritima*



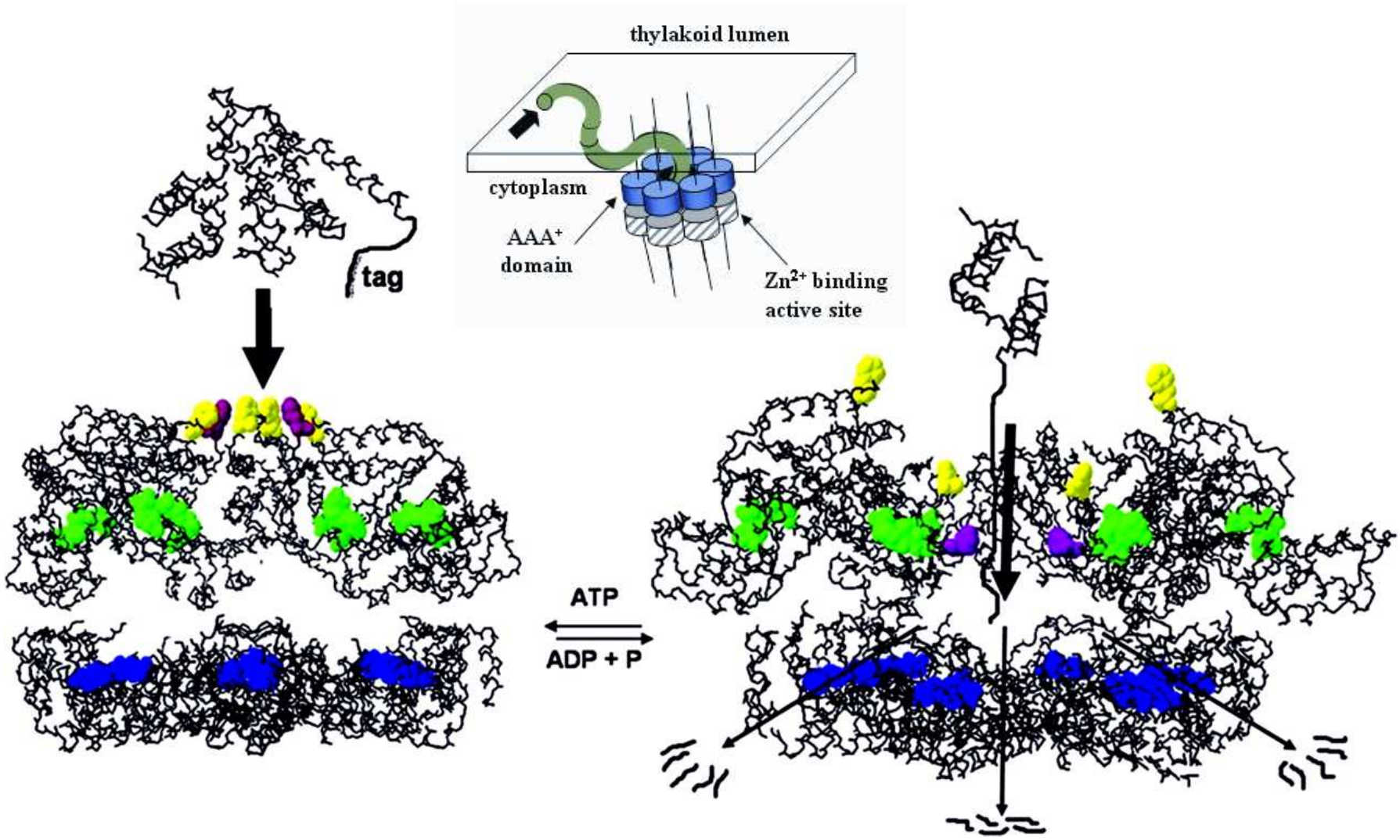
Top view looking onto hexameric AAA ring



Side view: AAA ring on bottom, protease ring on the top

Bieniossek et al (2006) PNAS **103**, 3066-3071

Possible degradation mechanism: ‘Pulling model’



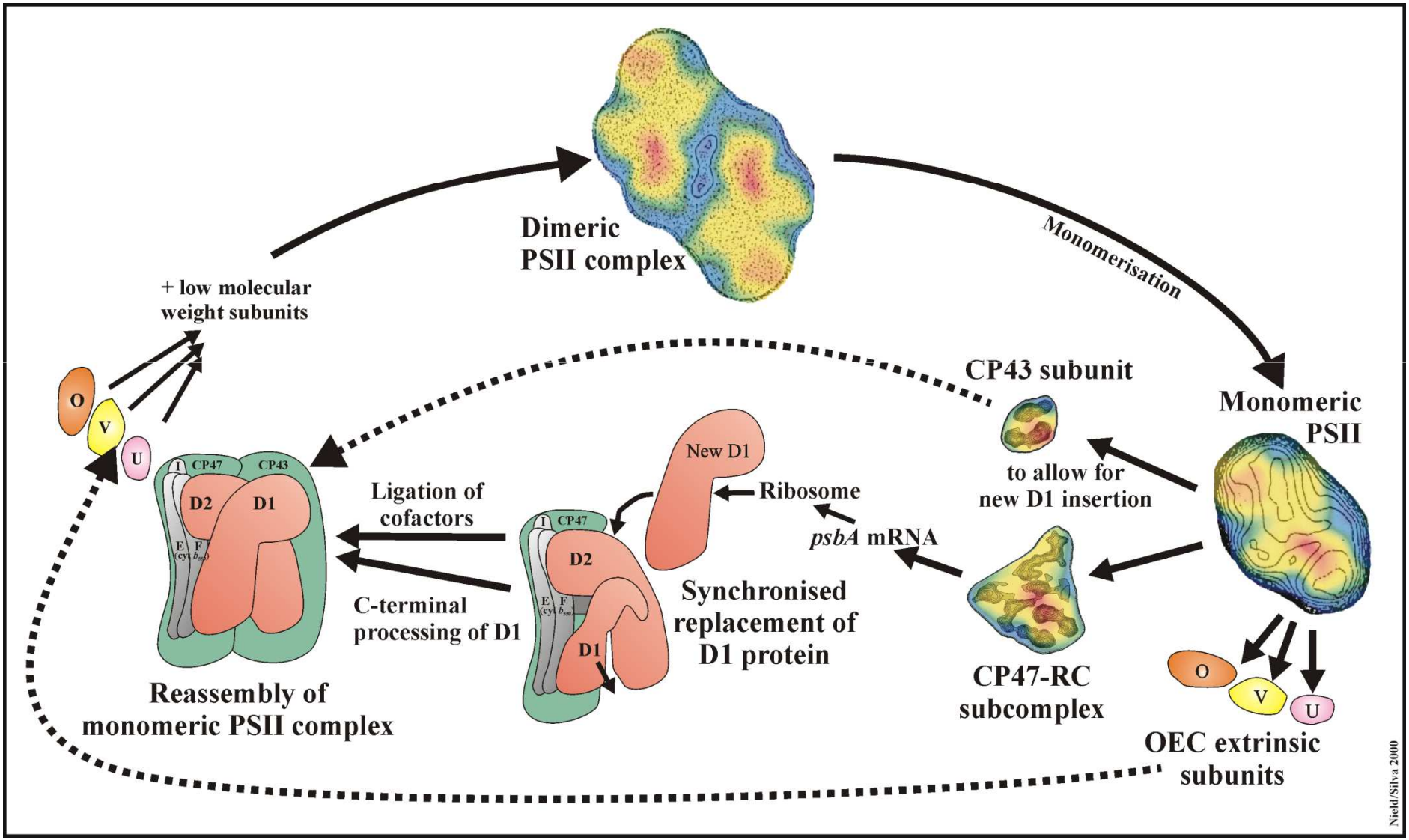
Bieniossek et al (2006) PNAS **103**, 3066-3071

FtsH is involved in the response to:

- Visible light stress (Silva et al., 2003)
- UV-B stress (Cheregi et al., 2007)
- Heat stress (Kamata et al., 2005)
- Low levels of inorganic carbon (Zhang et al., 2007)
- Osmotic (salt) stress (Stirnberg et al., 2007)

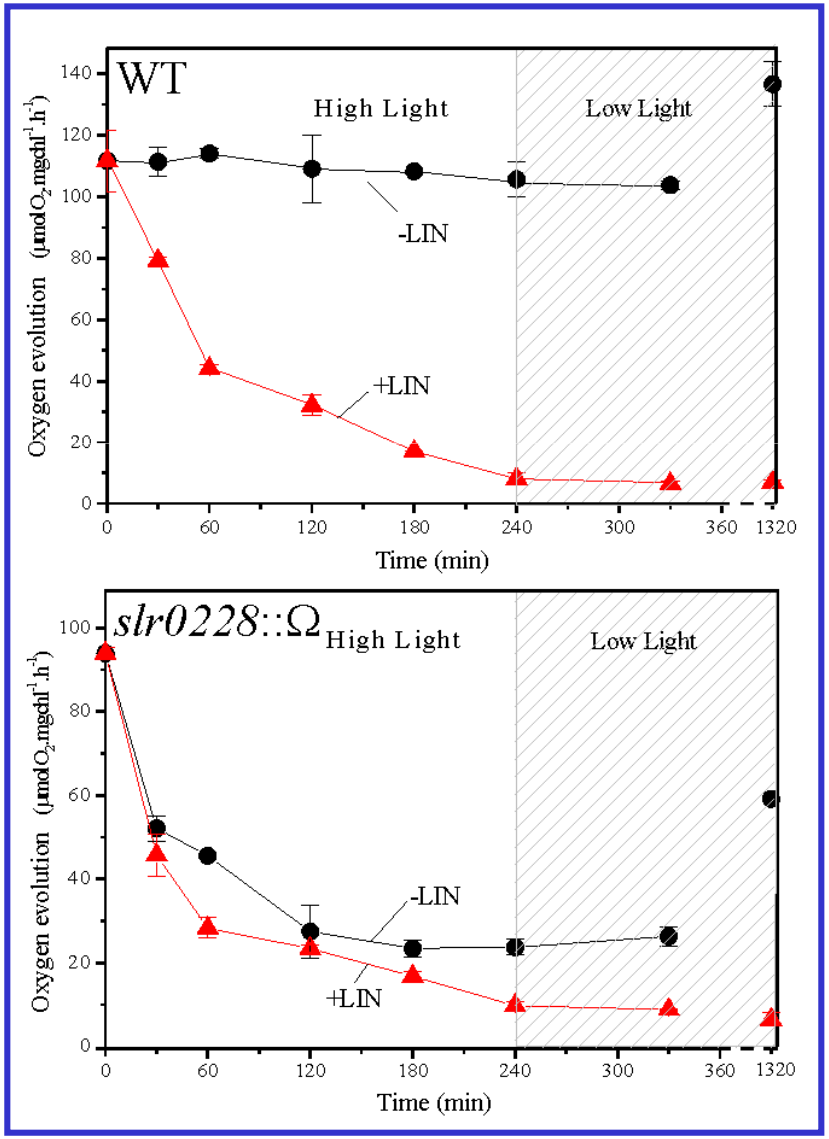
Evidence that FtsH2 (slr0228) plays a direct role in the early stages of D1 degradation during PSII repair

A scheme for the PSII repair cycle in cyanobacteria

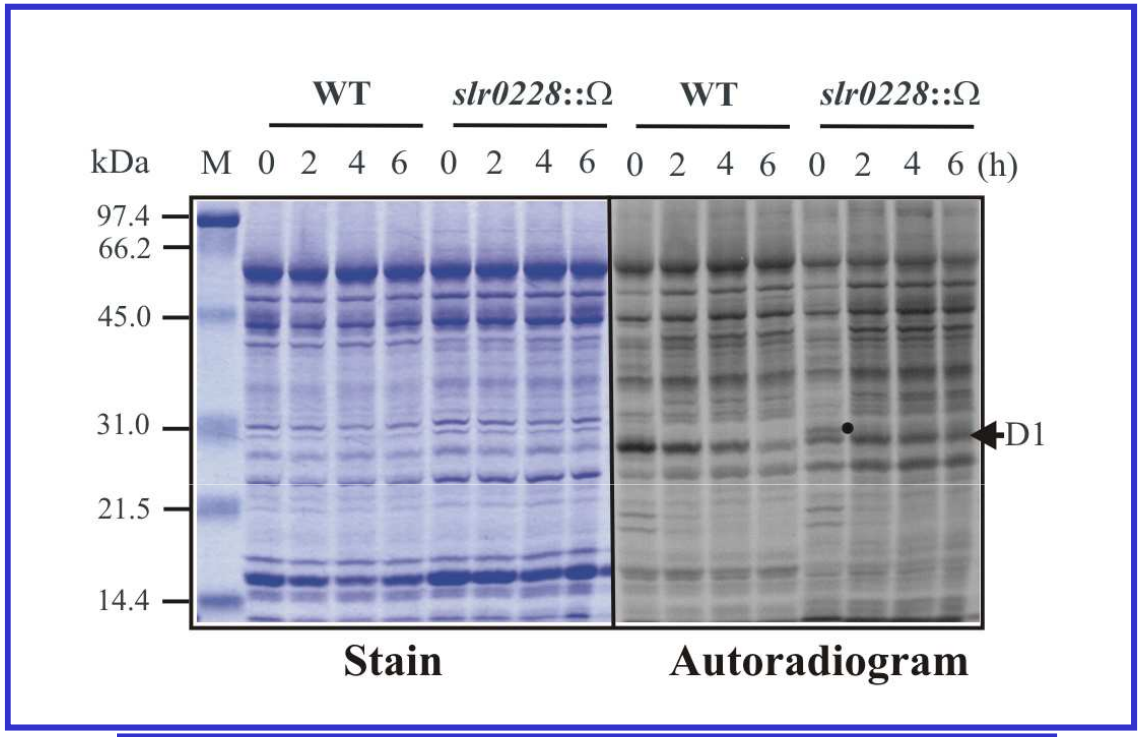


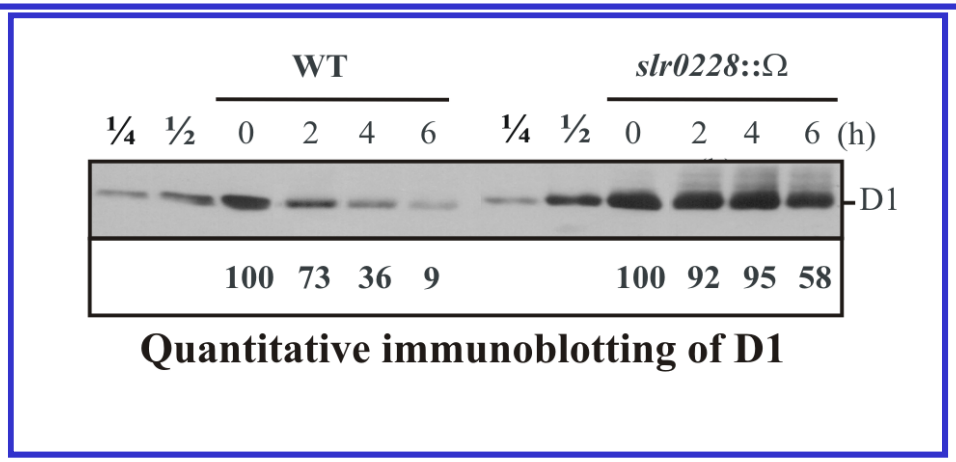
1. Growth of *ftsH2* (slr0228) insertion mutant is sensitive to light stress

2. Repair of PSII is impaired



3. D1 degradation is slower in *ftsH* (slr0228) insertion mutant

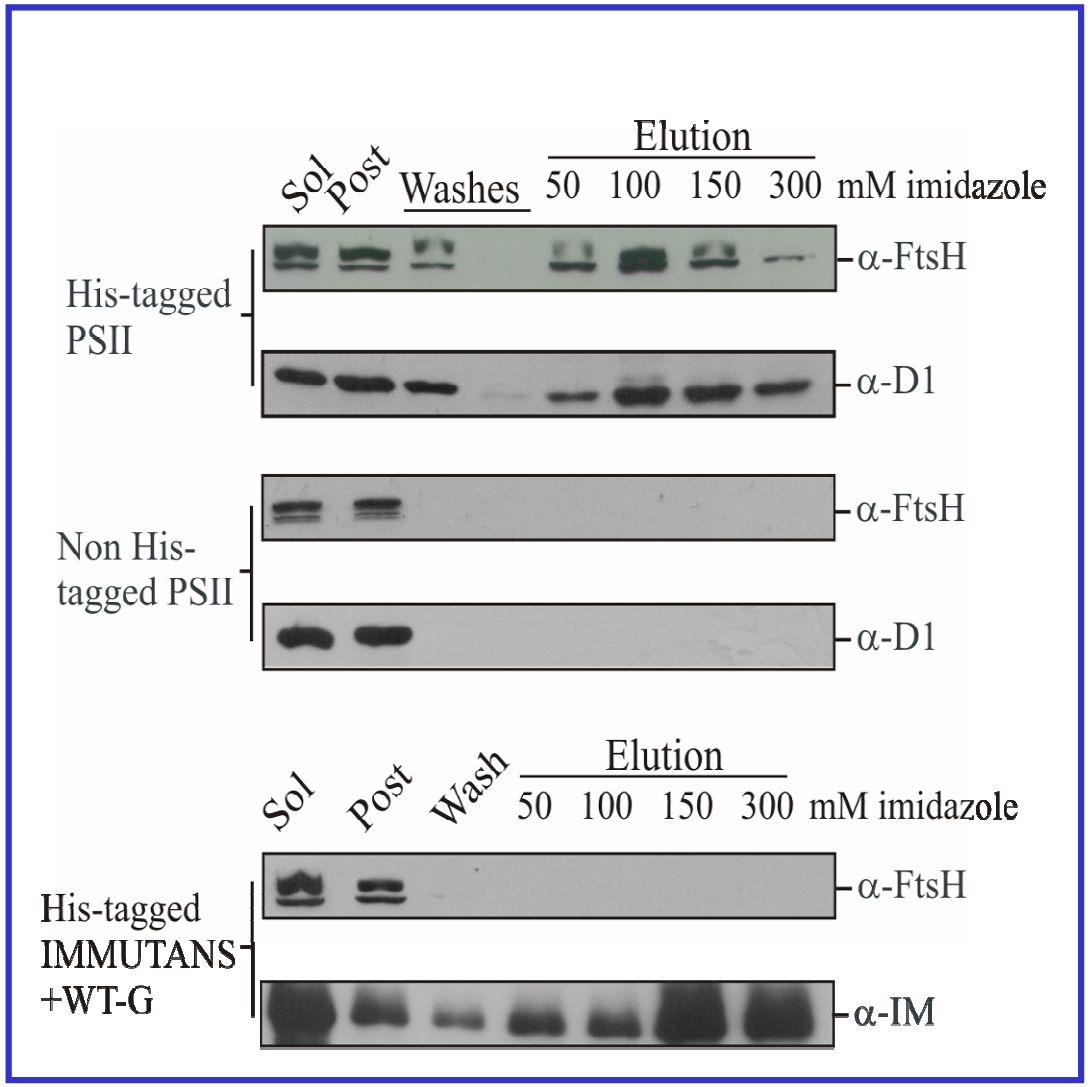


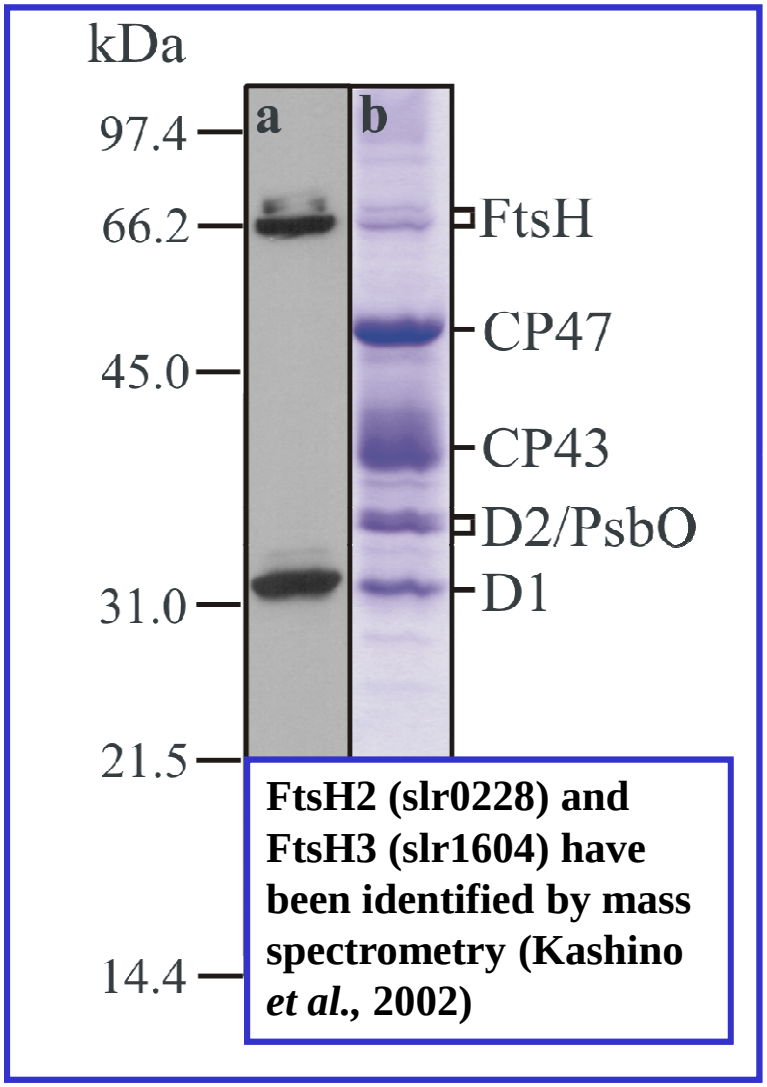


No fragments!

Silva et al. (2003)

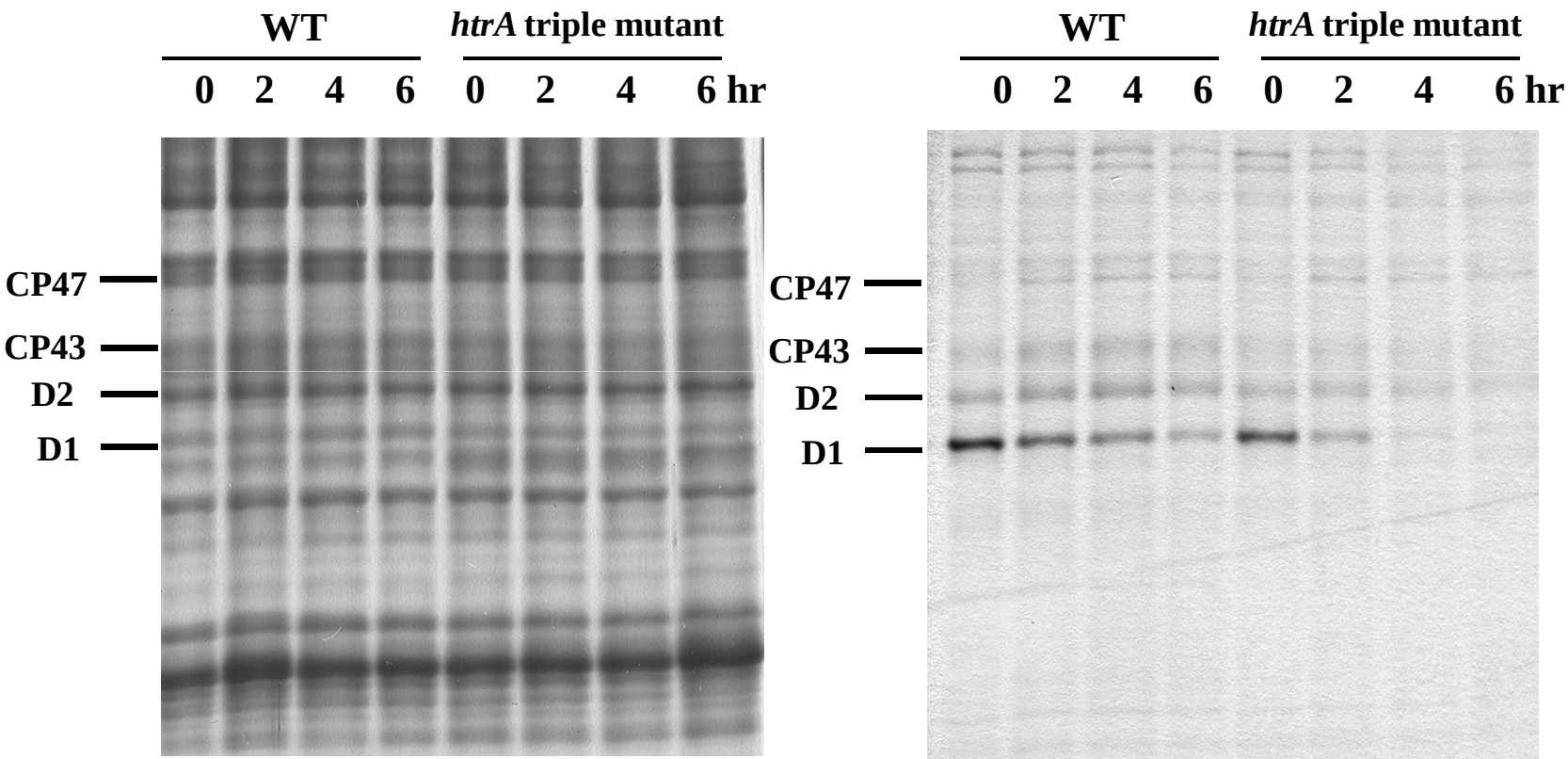
4. FtsH interacts with His-tagged PSII





FtsH2 (slr0228) and FtsH3 (slr1604) have been identified by mass spectrometry (Kashino *et al.*, 2002)

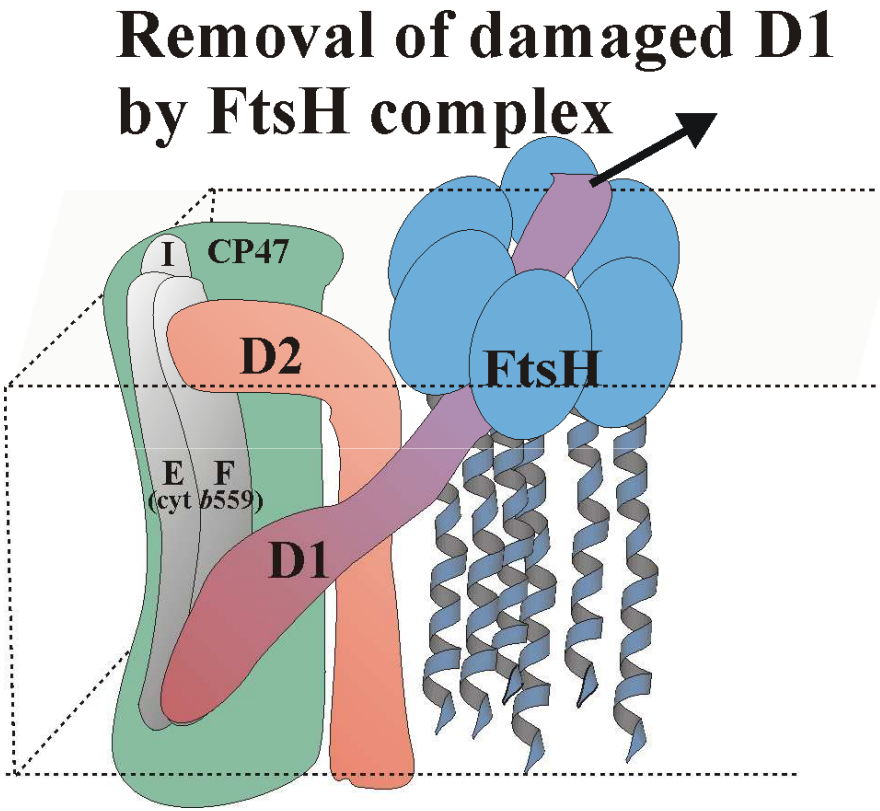
D1 turnover is unimpaired in a *degP*/*htrA* triple null mutant in a pulse-chase experiment



Coomassie stain **Autorad**

Barker et al (2006) J Biol Chem **281**, 30347-30355

‘FtsH-only’ model for D1 degradation

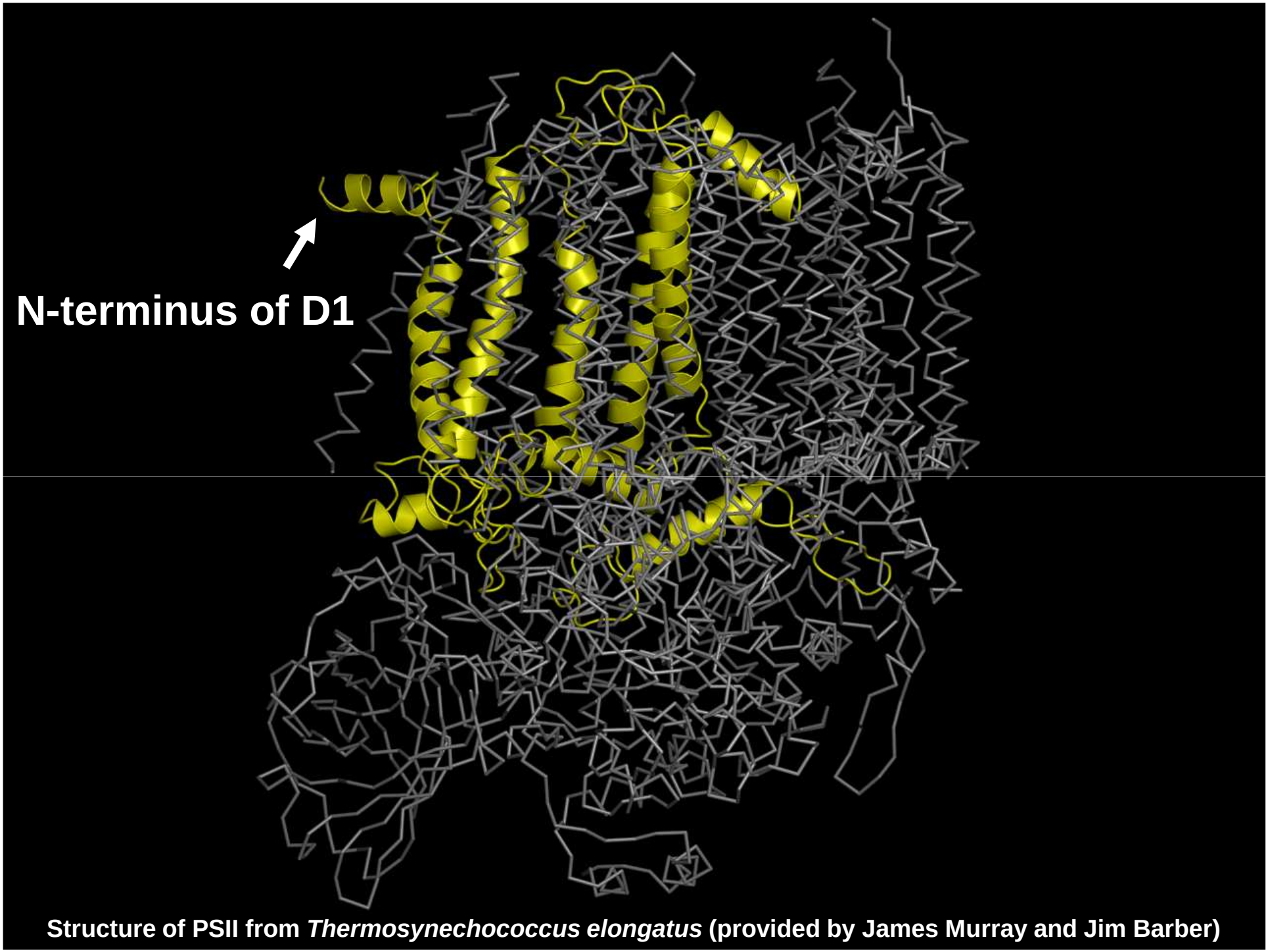


Some predictions :

- Proteolysis is highly processive and is driven by ATP hydrolysis. No breakdown intermediates.
- Deg proteases not needed for D1 degradation (Barker et al., 2006)
- D1 is triggered for degradation by destabilisation/partial unfolding. Common pathway for both donor-side and acceptor-side damage? (Cheregi et al., 2007)
- Housekeeping role
- Degradation can be initiated at N-terminus (as long as greater than 20 residues)
- FtsH complex is probably hexameric and might be homo- or hetero-oligomeric
- Forms supercomplex with Prohibitins

Nixon et al (2005) J Exp Bot **56**, 357-363

Mechanism of D1 degradation: Evidence for N-terminal mediated D1 degradation in *Synechocystis* 6803



Structure of PSII from *Thermosynechococcus elongatus* (provided by James Murray and Jim Barber)

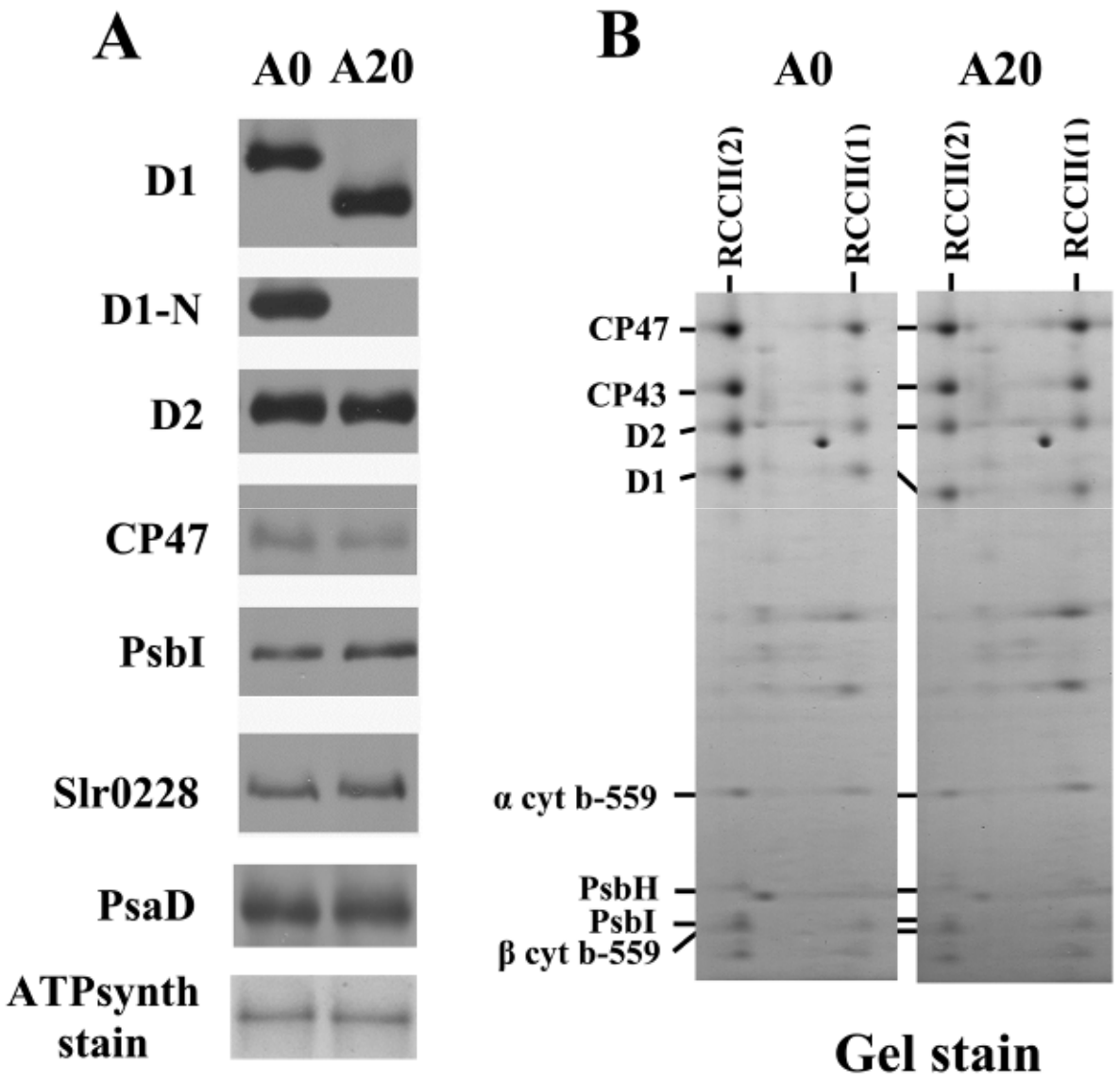
Mutant A20 of *Synechocystis* 6803 lacks 20 amino-acid residues from the exposed N-terminal tail

```
D1 TOBACCO:      MTAILERRES ESLWGRFCNW ITSTENRLYI GWFGVLMIPT LLTATSVFII
                 **  *  ***  ***  ** *  *** ** *  **** ***** *****  ***
D1 S.6803 A0:    MTTTLQQRES ASLWEQFCQW VTSTNNRIYV GWFGTLMIPT LLTATTCFII
D1 S.6803 A5:         MQRES ASLWEQFCQW VTSTNNRIYV GWFGTLMIPT LLTATTCFII
D1 S.6803 A10:              MSLWEQFCQW VTSTNNRIYV GWFGTLMIPT LLTATTCFII
D1 S.6803 A20:                        MTSTNNRIYV GWFGTLMIPT LLTATTCFII
```

Surface α-helix

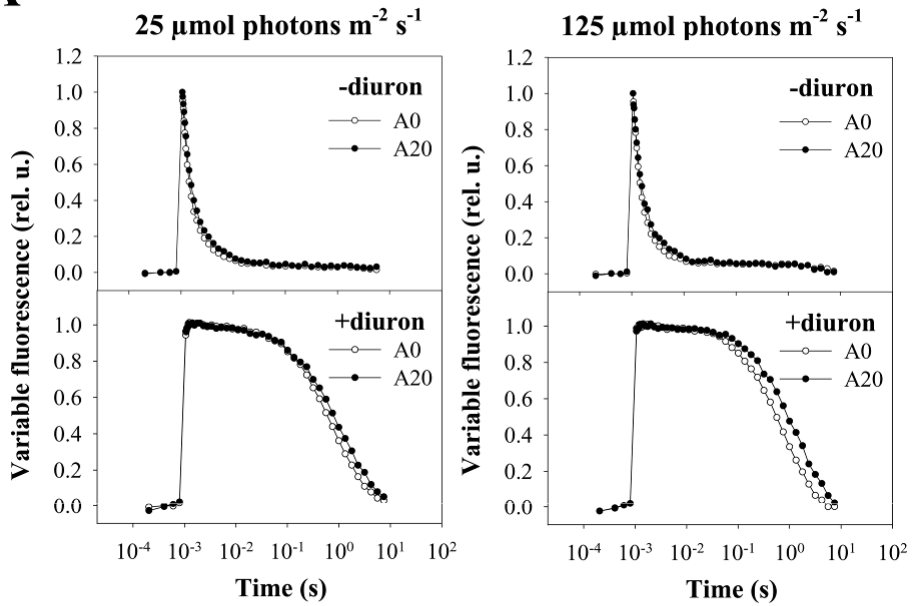
Start of the first transmembrane α-helix

A20 still assembles dimeric PSII complexes

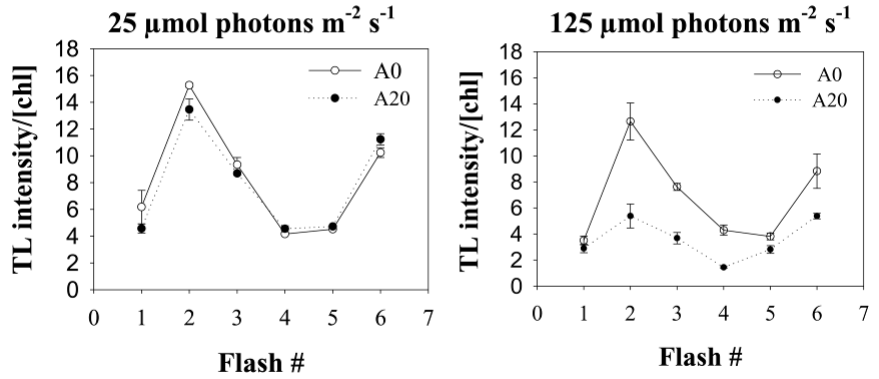


PSII activity in A20 is sensitive to high irradiance

A



B

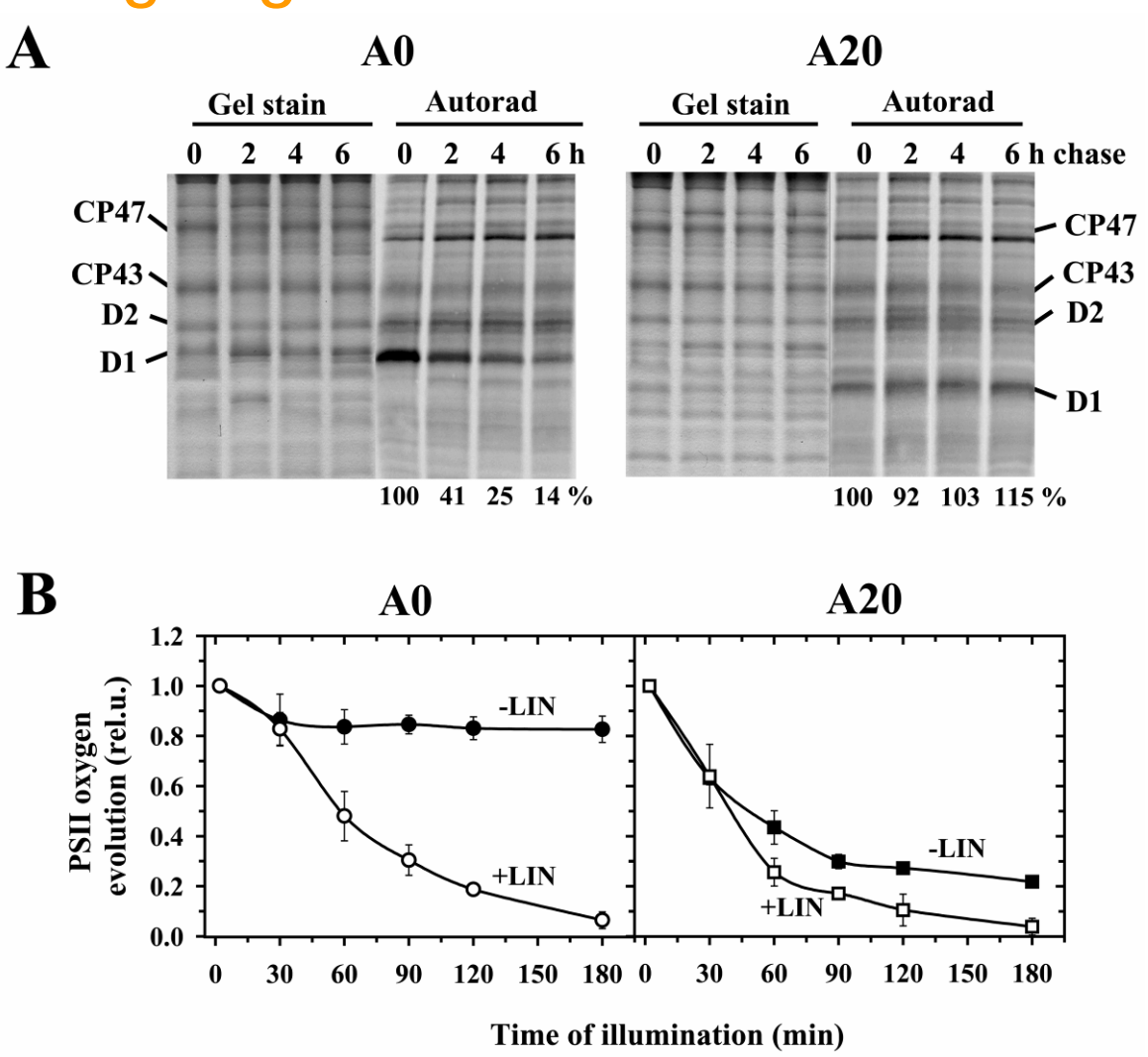


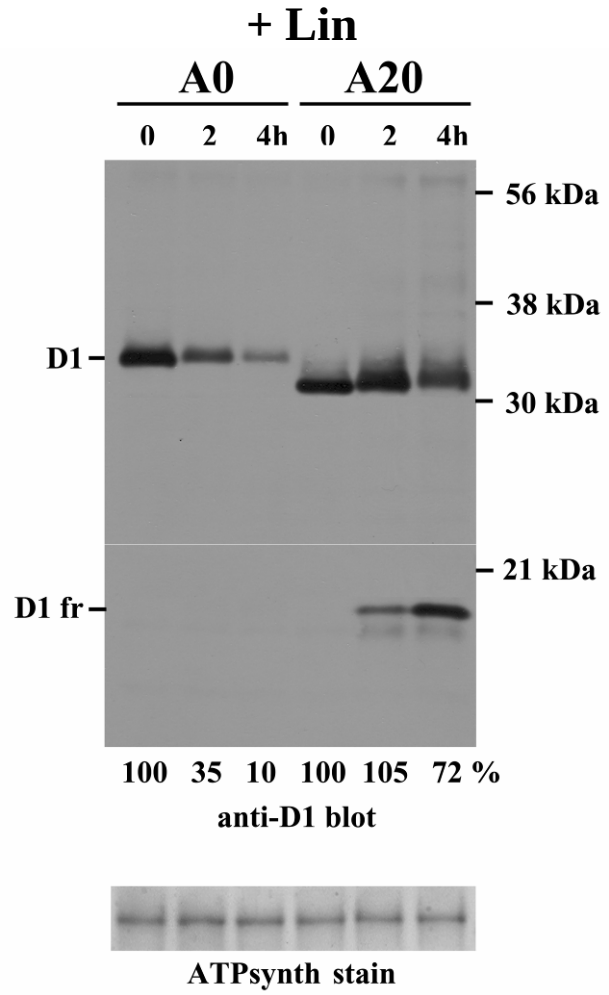
Strains	Autotrophic doubling time (hours) using low inoculum	Oxygen evolution (µmol O_2. mg $Chl^{-1}.h^{-1}$) from cultures obtained using high inocolum
A0 LL	9.8 ± 0.1	640 ± 20
A0 ML	10.4 ± 0.2	820 ± 20
A20 LL	13.9 ± 0.2	560 ± 30
A20 ML	no growth	430 ± 40

LL - 25 µmol photons.$m^{-2}.s^{-1}$

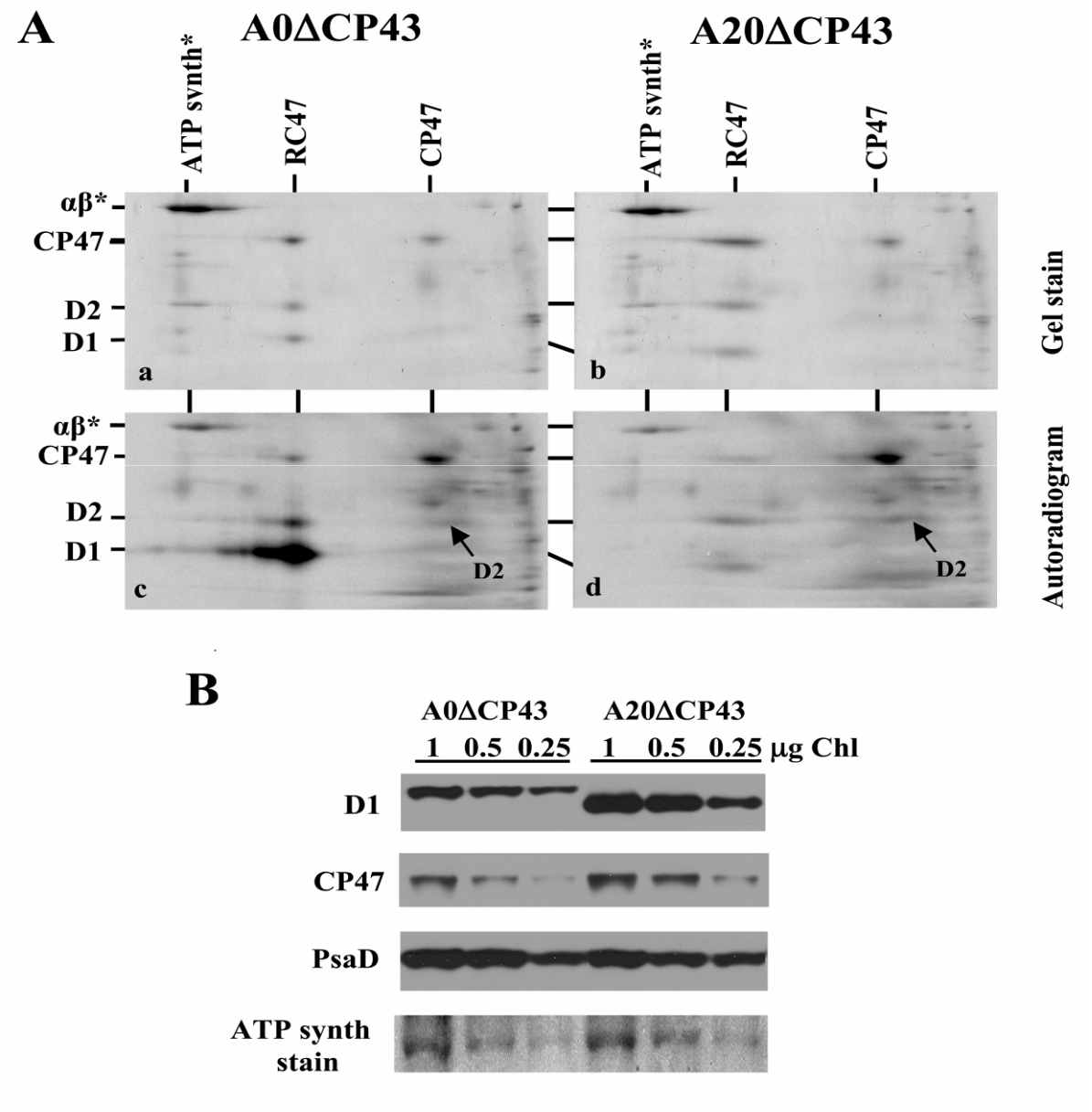
ML - 125 µmol photons.$m^{-2}.s^{-1}$

D1 degradation and PSII repair is inhibited in A20 at high light intensities

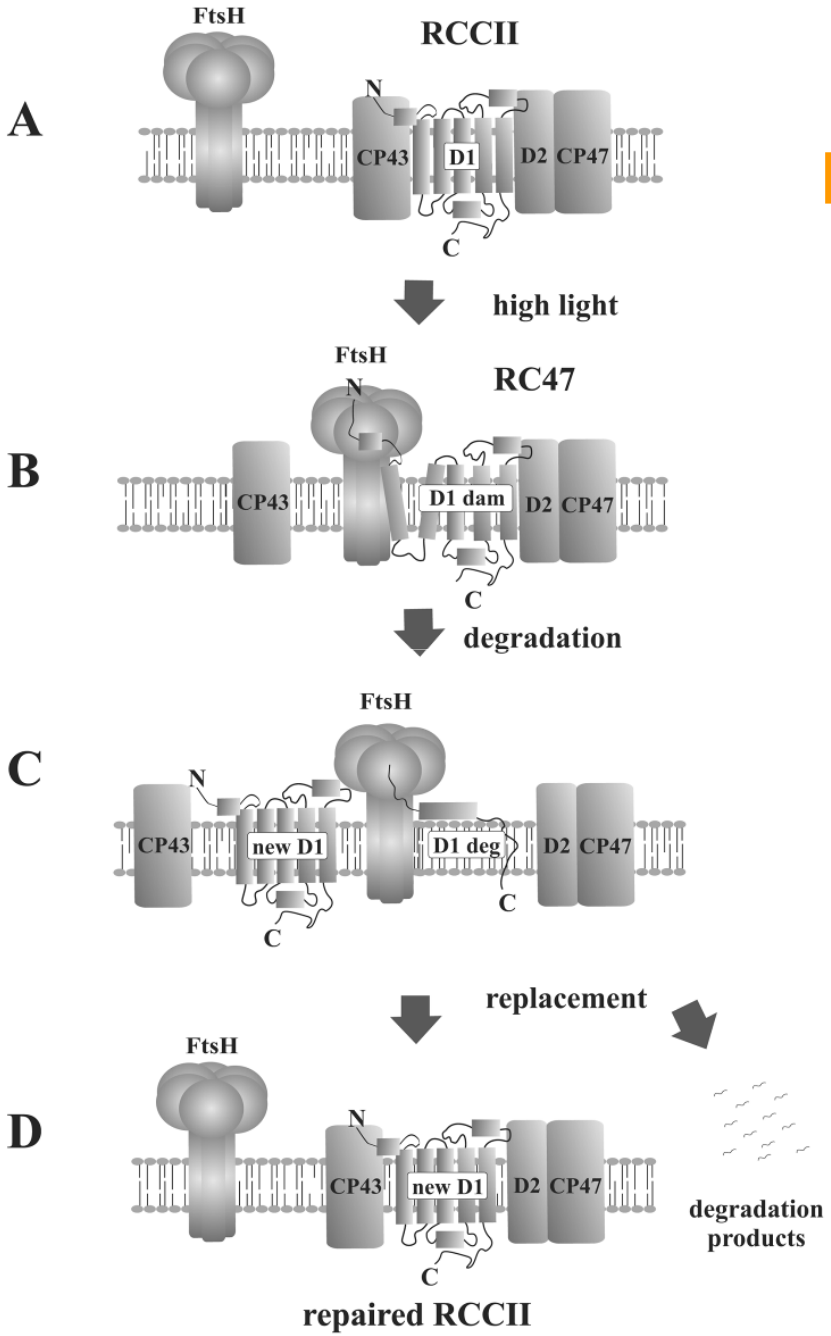




Truncation of D1 in A20 blocks degradation in the RC47 complex

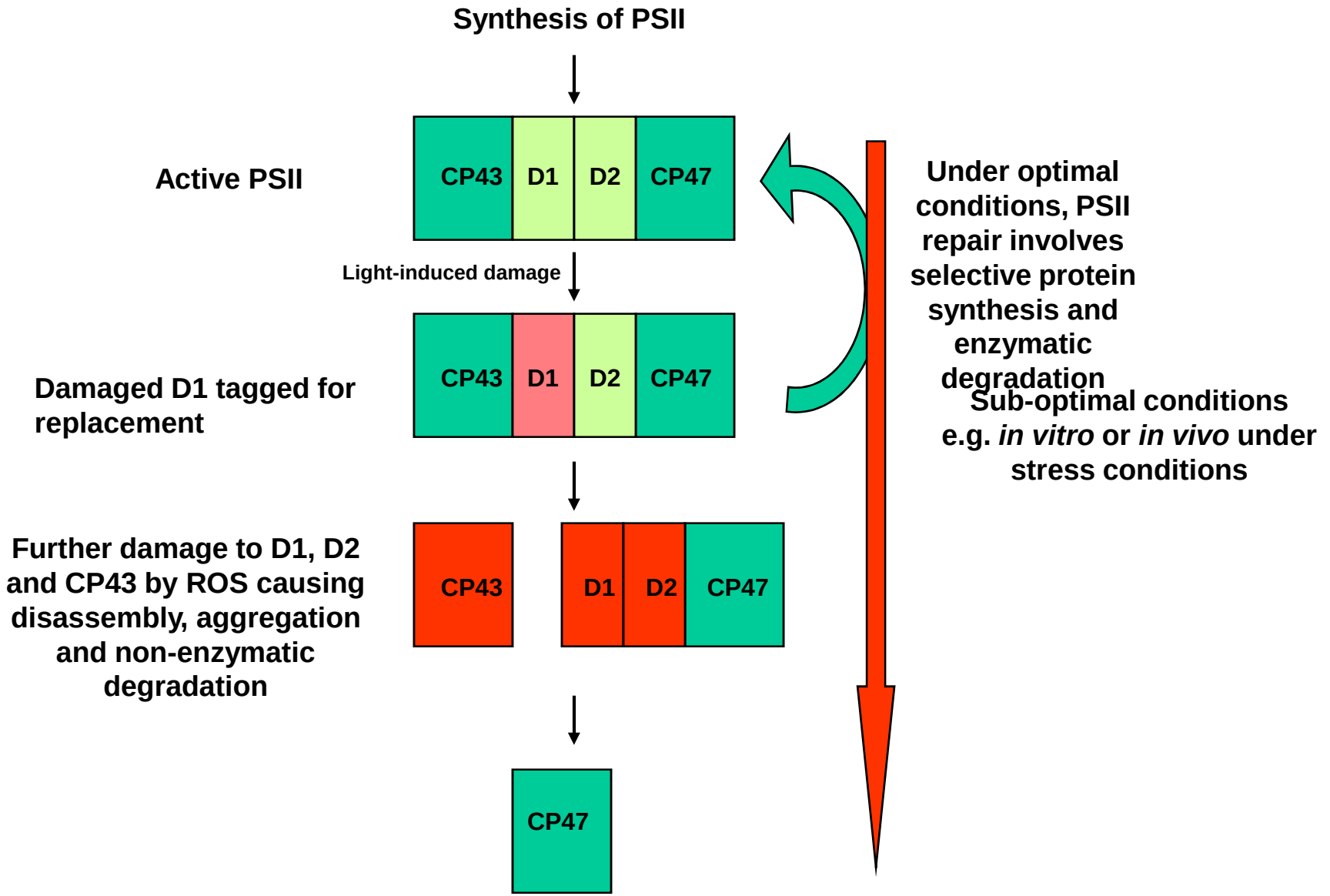


Model of D1 degradation



Komenda et al (2007) *Plant Cell* **19**, 2839-2854

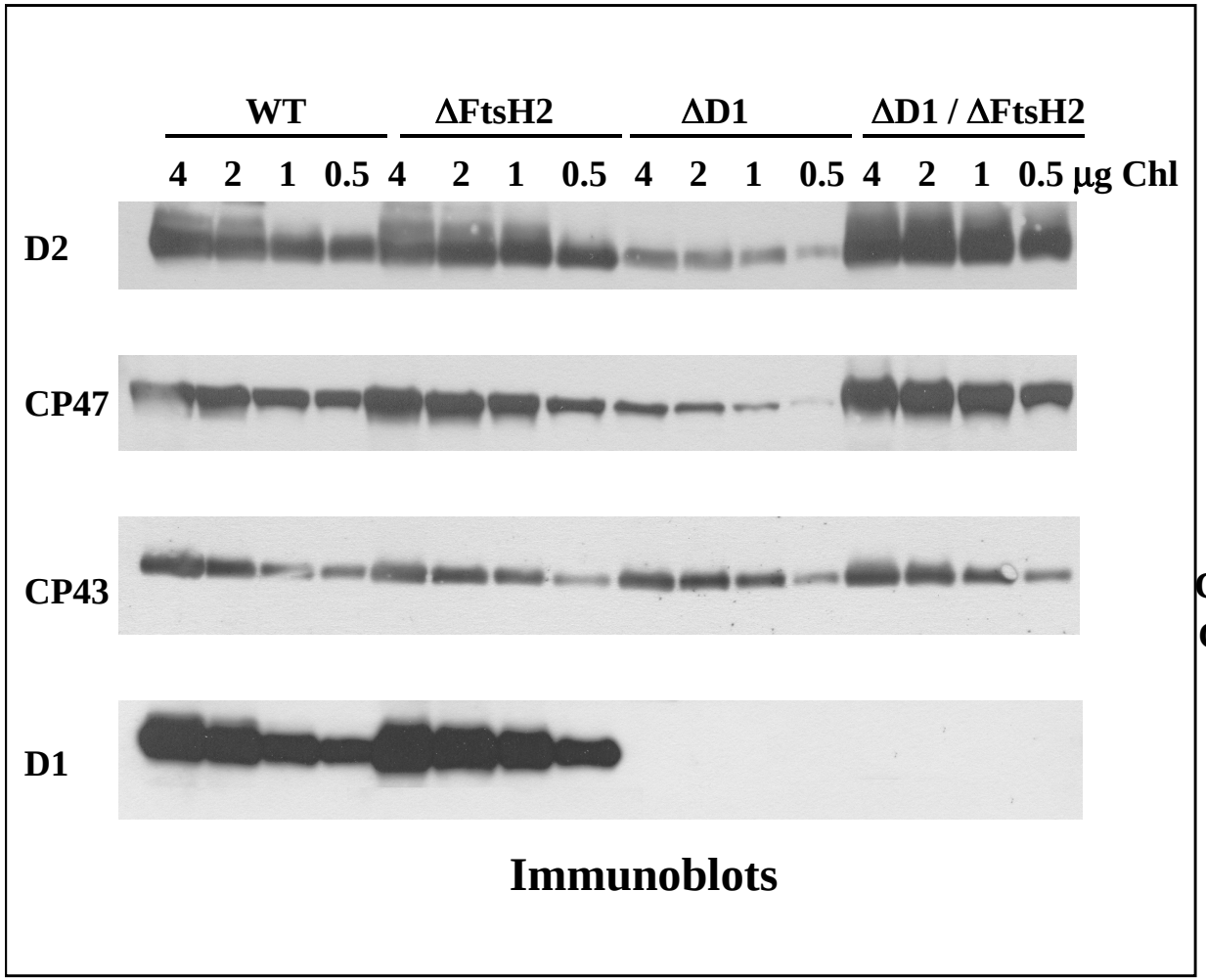
What about the D1 fragments and D1 aggregates?

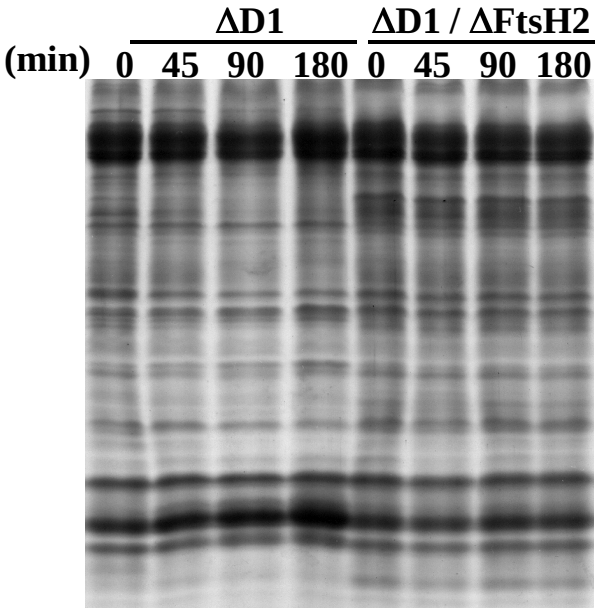


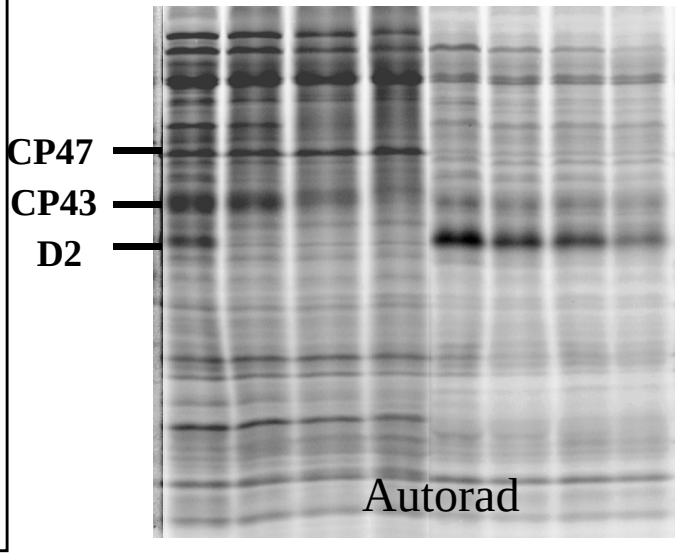
Adapted from Komenda and Masojídek (1995) Eur J Biochem **233**, 677-682

Does FtsH2 play a general role in quality control?

FtsH2 is involved in removal of unassembled PSII subunits in D1 deletion strain



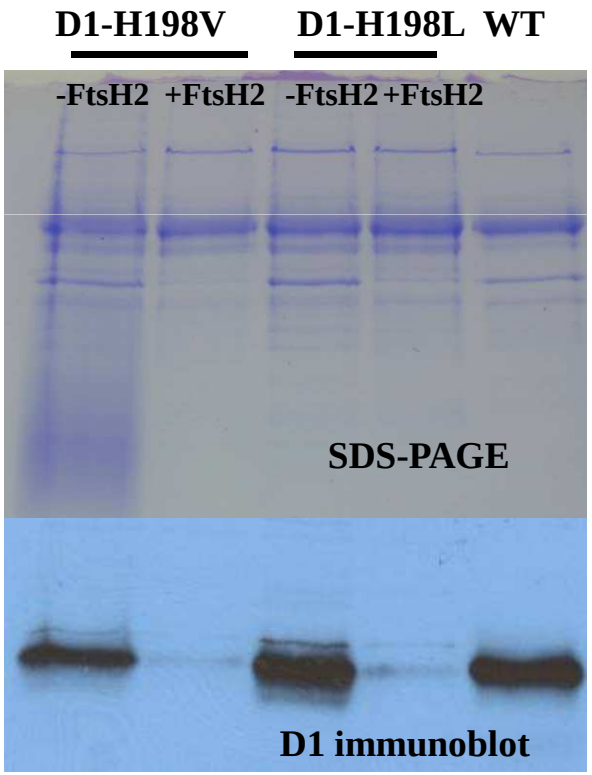


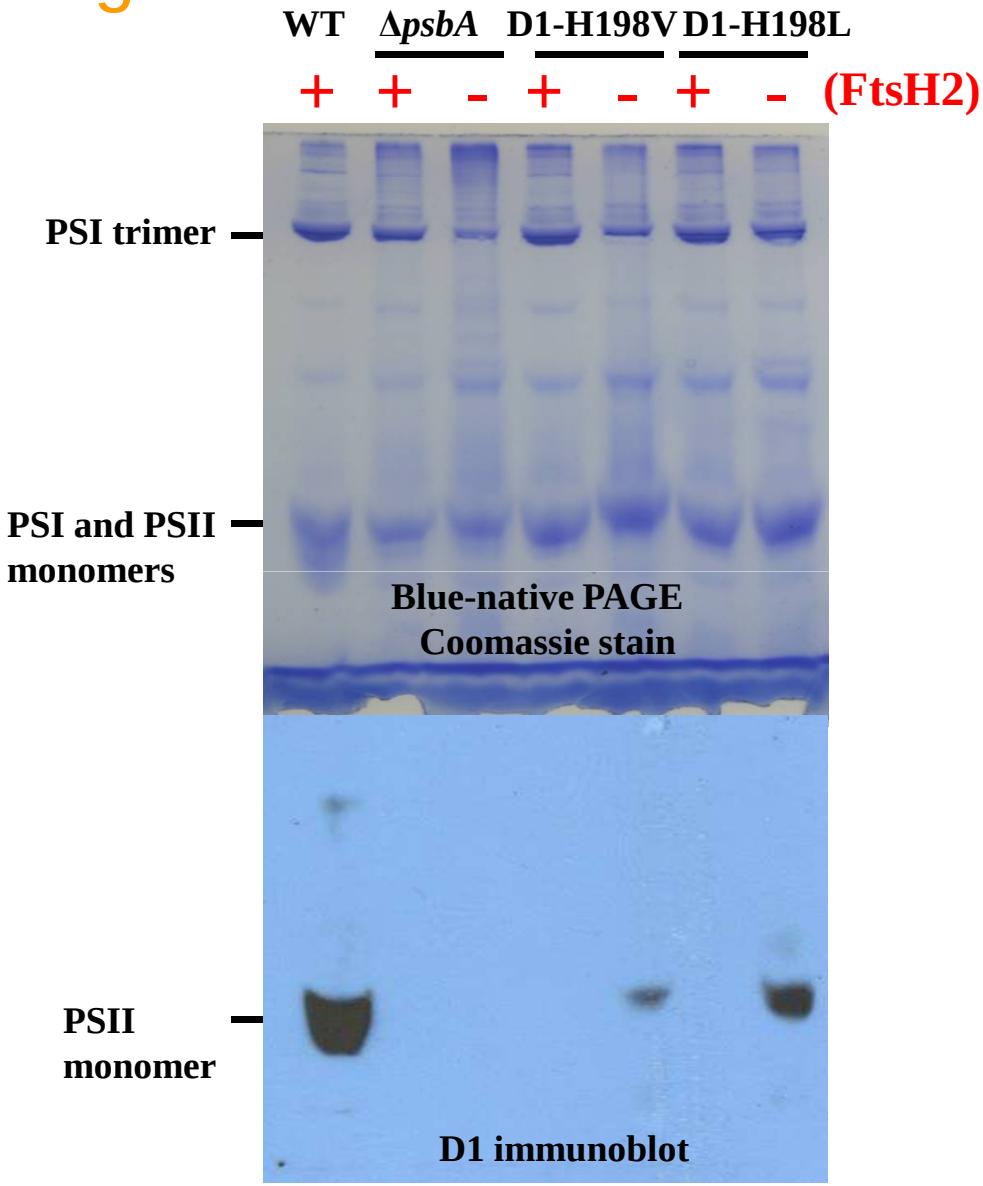


Pulse-chase experiment

Komenda et al. (2006) J. Biol. Chem. **281**, 1145-1151

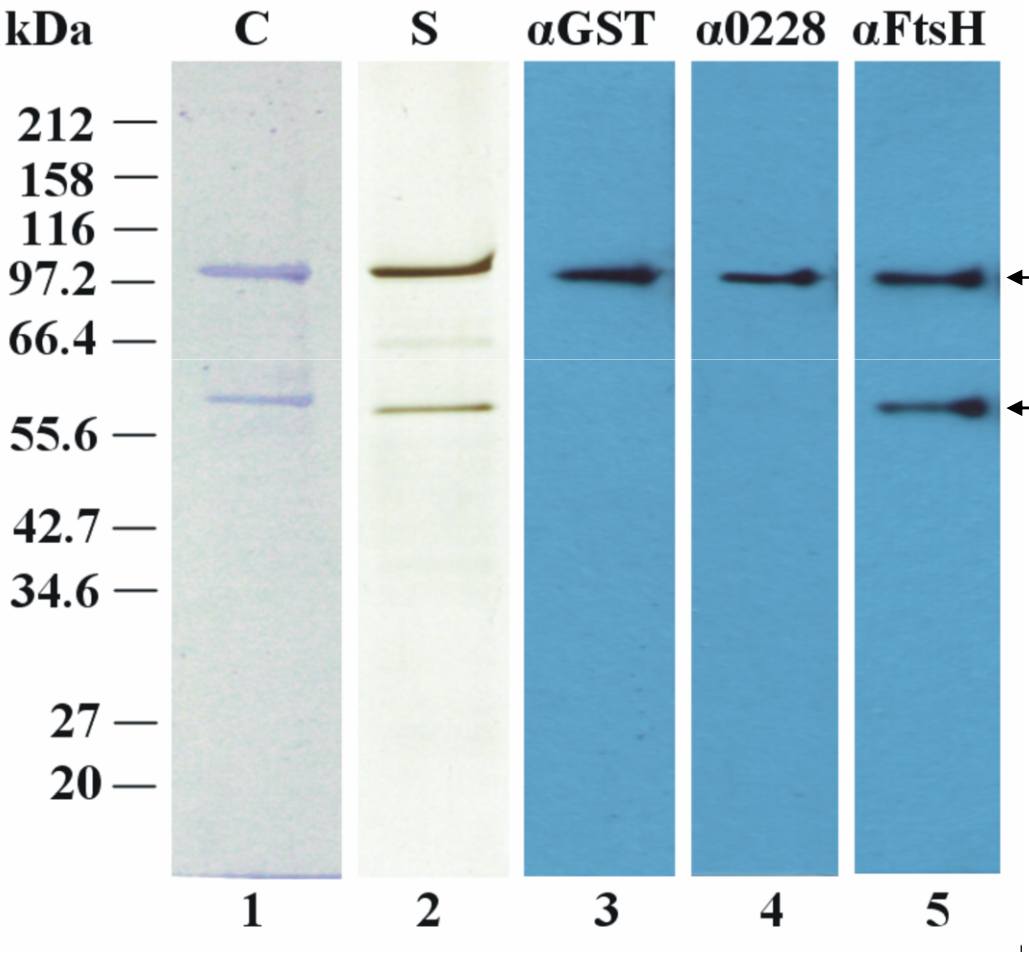
FtsH2 is involved in removing mutant D1 proteins





Isolation of FtsH2 complex

Isolation of FtsH2-Glutathione-*S*-transferase (GST) fusion protein

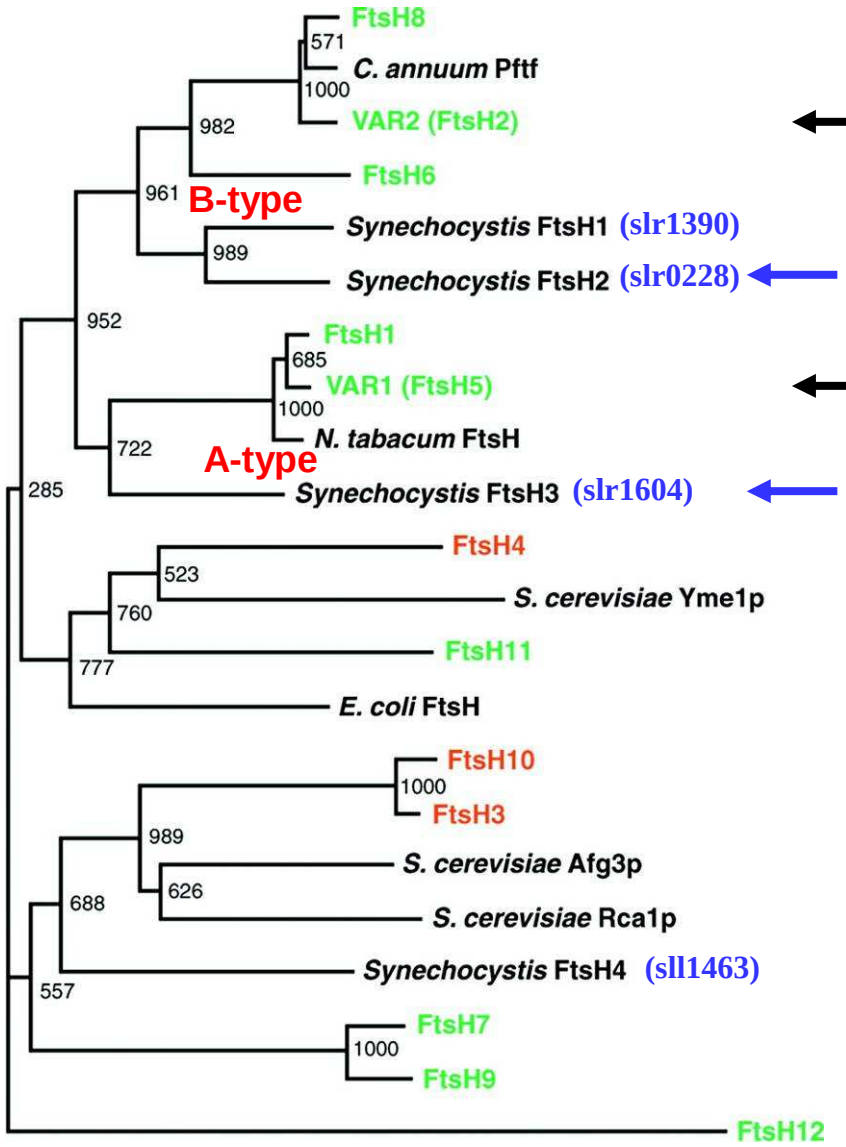


← FtsH2-GST fusion

← SKNNKKXXNA N-terminal sequence
MSKNNKKWRNA FtsH3 (slr1604)

Barker et al (2007)

Phylogenetic Tree of FtsH Proteins from *Arabidopsis*, *Synechocystis 6803* and Other Organisms



VAR1 (A-type subunit)
VAR2 (B-type subunit)
heterocomplex detected
in chloroplasts

Sakamoto W., Zaltsman, A., Adam, Z. and Takahashi, Y. (2003) Plant Cell **15**, 2843-2855
Zaltsman, A., Ori, N. and Adam, Z. (2005) Plant Cell **17**, 2782-2790

Summary

- **FtsH2 is involved in PSII repair in *Synechocystis* sp. PCC 6803 following damage by visible light**
- **FtsH2 acts at an early stage in D1 degradation and is not restricted to the removal of D1 fragments**
- **We propose an 'FtsH-only' model for PSII repair and propose that the main pathway for D1 degradation proceeds via the N-terminus (Komenda et al., 2007)**
- **FtsH2 also appears to play a 'house-keeping' role in the removal of unassembled subunits and misassembled PSII complexes within the thylakoid membrane (Komenda et al., 2006)**
- **FtsH2 forms hetero-hexameric complex with FtsH3 (Barker et al., 2007)**
- **FtsH complexes probably play similar roles in chloroplasts**