8 Light-responsive gene expression

8.1 PsbA

As the thermophilic organism *T. elongatus* grows photoautotrophically in hot springs, it is not surprising that its light environment directly regulates expression of genes that encode key components of the photosynthetic protein-pigment complexes. Whereas in higher plants and algae, the expression of the chloroplast encoded *psbA* gene is mainly regulated at post-transcriptional steps, for instance mRNA stability and translation (Rochaix, 1992) the transcription of the *psbA* genes (gene families) in cyanobacteria are modulated by light (Golden, 1995). The light-responsive regulation has been best studied for PSII and phycobilisomes. Wavelength-specific photoreceptors are controlling gene transcription of latter two proteins. In the genome of *T. elongatus* three copies of the *psbA* gene have been identified encoding two distinct forms of the D1 protein (Golden *et al.*, 1986) The three genes are regulated differentially in response to changes in light intensity. In the laboratory, this is demonstrated by a low- to high-light shift. At low light, more than 80% of the *psbA* transcripts are from *psbA1*, and the only D1 protein detectable in the thylakoid membrane is form PsbA1 (Golden, 1995). Immediately upon a shift to high light, the *psbA2* and *psbA3* genes are induced, and the *psbA1* message is actively degraded (reviewed in (Golden, 1995)).

The PsbA1 product of the *psbA1* gene is different from the PsbA2 product of the *psbA2/3* genes (Golden *et al.*, 1986). The sequences of PsbA1 and PsbA2 show a sequence identity of 90% and similarity of 95% (Fig. 8.2). In total 36 residues of the 360 amino acid long polypeptide differs. Table 8.1 summarises the most significant mutations and gives a short description about their location within D1. The most variant region is found from residue 144 to 158 upstream of Tyr_Z on TMH-**c**. Within this15 residues segment, comparing all three copies for D1, 4 variant residues are located. This might have an influence on the electron and proton transfer properties of Tyr_Z. Considering a higher degree of conservation of amino acids in the transmembrane-spanning region, this variance of this sequence patch is significant. PsbA1 and PsbA3 show a sequence identity of 94% and similarity of 98%. Compared to PsbA1, PsbA3 differs in 22 residues. The polypeptides of PsbA2 and PsbA3 show sequence identity of 91% and similarity of 98% (Fig. 8.2).

Table 8.1: Variant residues, restricted to significant exchanges, within the different gene copies of D1 are listed. Their location and the neighbourhood to cofactors are given.

residue	PsbA1	PsbA2/PsbA3	location	neighbourhood
4	Thr	Val	N-terminus	
80	Gly	Ala	loop	
93	Phe	Leu	loop	
124	Ser	Phe	TMH-**b**	Chl*a*41 of CP43
130	Gln	Glu	TMH-**b**	$Pheo_{D1}$/H-bond donor
144	Cys	Pro	TMH-**c**	
147	Tyr	Phe	TMH-**c**	$Pheo_{D1}$/H-bond donor
155	Phe	Thr	TMH-**c**	Chl*a* 41 of CP43
158	Phe	Leu	TMH-**c**	Chl_{D1}
172	Met	Leu	loop	Chl_{D1}
173	Pro	Met	loop	Mn-Ca-cluster
199	Gln	Met	TMH-**d**	Chl_{D2}/H-bond donor
212	Cys	Ala/Ser	TMH-**d**	non-haem Fe^{2+}
233	Ala	Thr	loop	
270	Ser	Ser/Ala	TMH-**e**	Fe^{2+}
286	Thr	Ala	TMH-**e**	P_{D1}
310	Lys	Gln	loop	contact to PsbV
328	Met	Ile	eC(1)	P_{D1}
346	Ala	Gly	C-terminus	
348	Ser	Leu	C-terminus	
359	Asn	Glu	C-terminus	
360	Gly	Ala	C-terminus	

Interestingly, a number of variant residues are located on TMH-**b**, -**c**, -**d** and -**e** of D1 (Fig. 8.1). A closer look on the positions within the TMH reveals that residues are partially located in close neighbourhood of redox-active cofactors ($P_{D1,}$ $Pheo_{D1}$, Chl_{D1}, non-haem Fe^{2+} and Mn-Ca-cluster) or in the vicinity of Chl*a*41 or even might have an influence of the binding affinity to other subunits (PsbV; Table 8.1). $Pheo_{D1}$ has to be highlighted as two variant residues are in its binding pocket (see 6.2.3 and Fig. 8.2). D1-130 is possibly strengths the H-bond pattern as in PsbA2 due to D1-Gln130 to D1-Glu130 exchange. This is supported by spectroscopic studies on a *Synechocystis* mutant with the same exchange, where a shift of the Q_x absorption band in the $Pheo_{D1}$ spectrum was observed (Giorgi *et al.*, 1996). An even more drastic change of D1-Tyr147 eliminates a potential H-bond to the chlorin moiety of $Pheo_{D1}$. All these changes affecting the H-bonding pattern could have an influence on the redox potential of

$Pheo_{D1}$. A weakening or loss of the H-bond to this Pheo*a* is expected to destabilise Pheoa^- and thus make its redox potential more negative (Cuni *et al.*, 2004). This was shown in *Synechocystis* by measurements of the equilibrium constant between the excited singlet state P* and the P^+Pheoa^- radical pair state and of the quantum yield for nanosecond stabilisation of the radical pair (Giorgi *et al.*, 1996; Merry *et al.*, 1998).

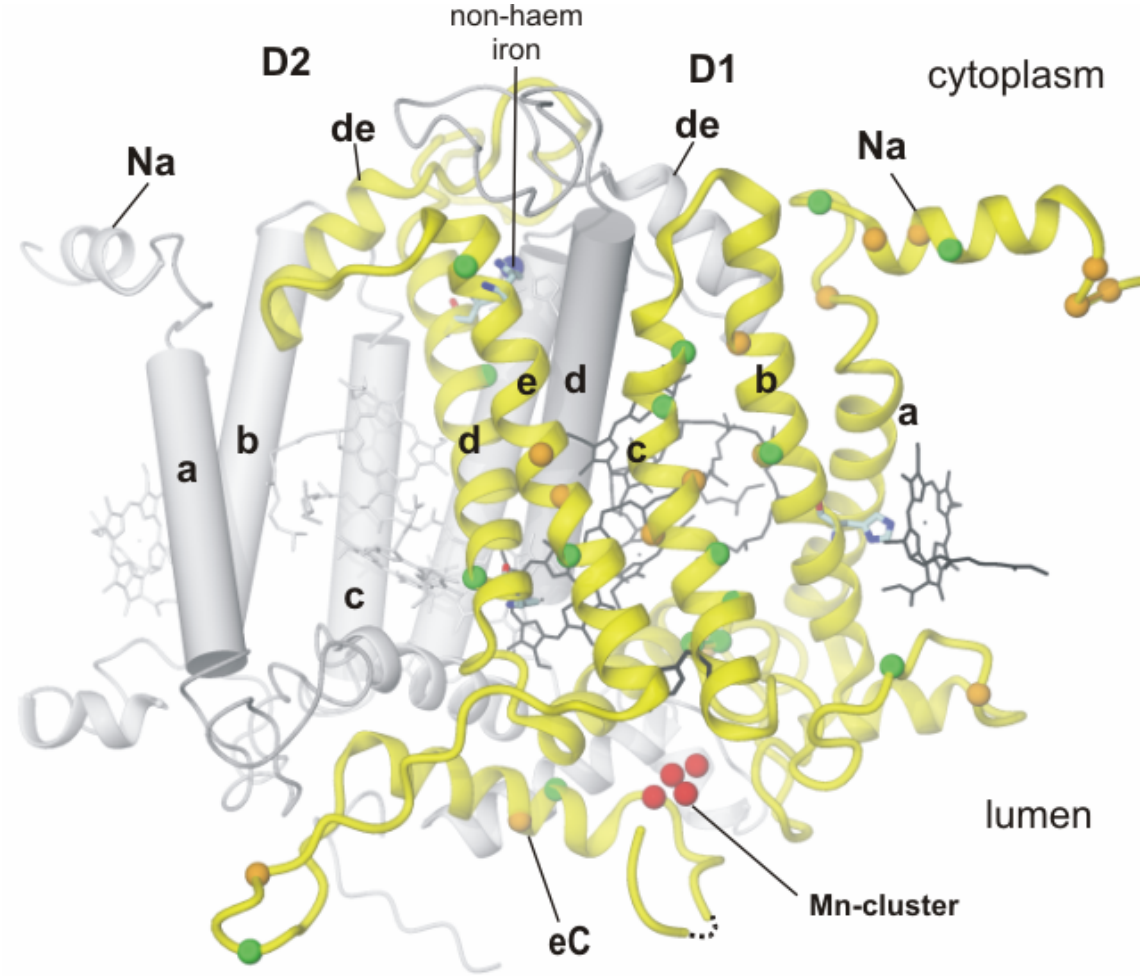

Fig. 8.1: View along the membrane plane. Variant residues within the D1 subunit are indicated by spheres. D2 is shown in grey Significant changes are indicated by green spheres, whereas minor changes in the sequence are shown by orange spheres. The cofactors of D1 are drawn in black, whereas cofactor embedded in D2 are shown in grey. The Mn-Ca-cluster and the non-haem Fe^{2+} (blue) are represented by red and blue spheres.

Amino acid sequence analysis reveals that higher plants exclusively have glutamate at position D1-130, whereas cyanobacteria have either glutamate or glutamine (Fig. 8.2). Only the genes *psbA1* of *T. elongatus*, *psbA1* of *Synechococcus* sp. WH_8102, *psbA* of *Prochlorococcus marinus* subsp. *pastoris* str. CCMP1378, *psbA1*, *psbA2*, *psbA3* of *Synechocystis* sp. PCC 6803 as well as *psbA* and *psbA1 Nostoc* sp. PCC 7120, *Anacystis*

nidulans and *Chloroflexus aurantiacus* have glutamine at this position. It seems that this property is restricted to cyanobacteria. In the PbRC of *Rhodobacter capsulatus* L-Glu104, equivalent to D1-Tyr147 in *T. elongatus*, was mutated to Leu and Gln and the variants investigated by spectroscopy. The electron transfer in both variants was slightly slower than for the wild type (Bylina *et al.*, 1988). In PbRC this residue is responsible for the spectroscopic red shift of the bacterial Pheo*b*.

The thermoluminescence analyses indicated that the reversible shift between the *psbA1* and *psbA2* forms in *Synechococcus* has a major influence on the redox potential of Q_B (Sane *et al.*, 2002). Since the major changes in the amino acid residues of *psbA1*and *psbA2* are found in the N terminus (Golden *et al.*, 1986), certain unspecific long-range effects of the modified N-terminus in *psbA2* on the Q_B-binding pocket and the redox properties of Q_B might be responsible for this observation.

To date no other changes in redox potential or electron transfer of cyanobacteria have been described in the literature. It appears that the translation of the different copies of *psbA* is monitoring the environmental conditions and latter adjustment has an influence on the electron transfer within the ETC.

8.2 PsbD

There are two *psbD* genes that encode an identical D2 protein and are arranged in different transcriptional units (Golden and Stearns, 1988). Gene *psbD1* overlaps and is co-transcribed with gene *psbC* encoding for CP43 (Golden and Stearns, 1988). In chloroplast genomes the same arrangement is found for genes *psbD* and *psbC* (Ohyama *et al.*, 1986). *T. elongatus* has an additional monocistronic *psbD* gene designated *psbD2*. It was shown that the monocistronic *psbD2* gene is functional and no growth impairment was noted in mutants lacking PsbD2 (Golden *et al.*, 1989). However, under special growth conditions the two different genes may serve different purpose.

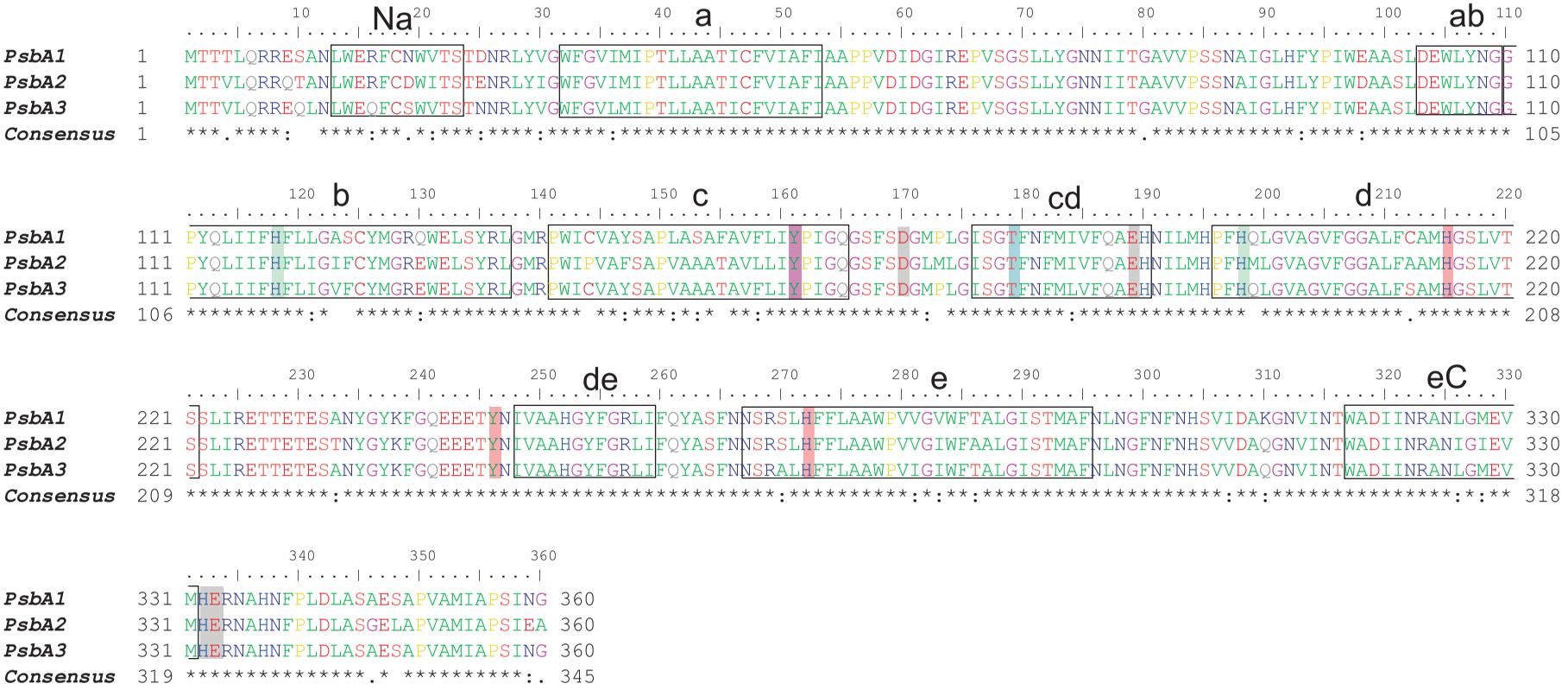

Fig. 8.2: Sequence alignment of the three gene copies *psbA1*, *psbA2* and *psA3* encoding the D1 protein. Rectangles define the positions of α-helices. The consensus sequence for all three gene copies is given: star indicates conserved residues, two dots highly similar and one dot similar amino acids. Colouring scheme of amino acids according to chapter 4.2. In the consensus sequence amino acids are resembled as follows: identical by asterix, similar by two dots, less similar by one dot and not conserved by spacing.