Effect of *crhR* mutation on low temperature induced changes in photosynthetic pigment protein complexes

Chapter 4
4.1. Introduction

4.1.1. Adaptation of cyanobacteria to environmental changes

One of the intriguing features of thylakoid membranes and the photosynthetic apparatus in general is the capability of adaptation during or following environmental changes. Fluctuations of various environmental factors including levels of specific nutrients, the intensity of the incident irradiation and temperature alters growth rates of phototrophic organisms. Dramatic changes in pigment content, activities of various metabolic processes and cell morphology may be observed under extreme conditions (Kehoe and Grossman, 1996; Grossman et al., 1993; He et al., 2001).

4.1.2. Light adaptation

Acclimation to light regimes is one of the most essential and complex responses of photosynthetic organisms to varying environmental conditions. Light adaptation is often accompanied by changes in content of pigments and in the composition of thylakoid proteins (Allen, 1995; Anderson et al., 1995) with following modifications in the composition of antenna complexes (Lorimier et al., 1991), redistribution of excitation energy between the photosystems (Murakami and Fujita, 1991), changes in the composition of reaction centres or in CO₂ fixation activity (Schmetterer, 1994; Murakami et al., 1997). Under light-limiting conditions cyanobacteria increase their concentration of PBPs and chlorophyll a. Increasing antenna sizes occurs by elongation of the phycobilisome rods and by an increase in the number of PBS per unit area of the thylakoid membrane. Under light-saturating conditions cyanobacteria reduce their antenna size and photosystem content which is accompanied with a marked decrease in the chlorophyll a and phycobiliprotein levels and a slight decrease in total carotenoid levels (Hihara et al., 1998; He et al., 2001; Havaux et al., 2003). Simultaneously, phycobilisome sizes and photosystem contents are reduced to avoid absorption of excess light energy.
Expression analysis in *Synechocystis* cells showed that chlorophyll *a* and PC contents decline drastically within 3 h of acclimation to high light. These changes could originate from down-regulation of the genes that encode enzymes for biosynthesis of photosynthetic pigments: chlorophyll genes and structural components of the PBS (aps and cpc genes) and from synthesis of proteins that are directly or not directly involved in the degradation of chlorophyll *a* and PBPs (Lorimier et al., 1991; Hihara et al., 2001). Expression analysis of genes encoding PBS subunits uncovered that transcription of APC genes is down-regulated by light, whereas the mRNA levels of linker proteins are not affected at high light regimes (Lorimier et al., 1991). Simultaneously, the remodeling of the PSII antenna complex is accompanied by a shortening of the PBS rods via detachment of external rod segments or whole rods from the PBS core. Various studies indicated that peptidases participate in post translational modification of the PBS antenna (Yamanaka et al., 1980). Later, it was shown that Lhc-like polypeptides (HLIP; high light inducible polypeptides) accumulate during adaptation to light stress in plants and cyanobacteria (Adamska et al., 1999; Funk and Vermaas, 1999; He et al., 2001). Cyanobacterial cells encode five genes (*hliA, hliB, hliC, hliD* and *hemH*) for polypeptides with similarity to Lhc polypeptides of vascular plants. Levels of all Hli polypeptides were elevated in high light and during nitrogen limitation (van Waasbergen et al., 2002). The transcripts of three genes, *hliA, hliB, hliC*, accumulate to high levels following exposure to sulphur deprivation and low temperature. Therefore, the products of these genes can be involved in the protection against different types of stress, including light stress. The initial accumulation of Hli polypeptides occurs during the phase of acclimation in which cells are unable to divide. The role of HliA protein in photosynthetic electron flow is unclear, although the polypeptides may be involved in the dissipation of excess absorbed light energy or function in a complex that shuttles chlorophylls to sites of degradation and/or pigment protein complex biosynthesis (He et al., 2001; Havaux et al.,
2003). Expression studies of *Synechocystis* peptidase genes under acclimation to different light regimes showed that three genes, *clpB2, ftsH1* and *ftsH2*, are induced within 15 min after transfer to high levels of light and become down-regulated within next 15 h (Hihara et al., 2001). As it was noted before, the products of *ftsH1* and *ftsH2* genes were found to be essential for cells since no knock-out mutants for these peptidases could be obtained. FtsH2 protein is essential for controlling the turnover of D1 protein (Bailey et al., 2002; Silva et al., 2003). In cyanobacterial cells about 110 - 140 molecules of chlorophyll *a* are attached with the reaction centre of PSI, but only 35 - 70 molecules reside in PSII reaction center. As chlorophyll *a* is mainly associated with PSI, the acclimation to high light is accompanied by a decrease in PSI relative to PSII (Fujita et al., 1994; Murakami et al., 1997a; McConnell et al., 2002). Such adaptation serves to regulate the distribution of excitation energy between photosystems and to correct any energy imbalances (Hihara et al., 1998). Changes in photosystem contents are regulated by decreasing mRNA levels of genes encoding subunits of photosystems (Hihara et al., 1998; Hihara et al., 2001; Muramatsu and Hihara, 2003) and simultaneously, by activation of proteolytic components involved in the degradation of photosynthetic proteins during light stress (Lindahl et al., 2000; Bailey et al., 2001; Lensch et al., 2001; Kanervo et al., 2003; Silva et al., 2003). Recently, it has been shown that transcript levels of photosystem I genes rapidly decrease to less than 10% of initial levels within 1 h after a shift to HL, whereas the response of PSII transcript levels was not coordinated (Hihara et al., 1998; Muramatsu and Hihara, 2003). Fast changes in the transcription of PSI genes, but not PSII genes, seem to be important for *Synechocystis* cells to regulate their photosystem contents in response to high light acclimation. Probably, the content of PSII can be promptly reduced under HL conditions due to an accelerated turnover rate of its reaction centre subunits (Mohamed and Jansson, 1989; Komenda et al., 2000). The turnover process of PSII includes the degradation of damaged D1 polypeptide, de novo synthesis of D1 and assembly
of the heterodimeric complex with other PSII polypeptides (Melis, 1991; Komenda et al., 2000). There are various proteases and chaperones that are involved in D1 turnover in vivo. Two families of proteases are being studied: the FtsH family of Zn$^{2+}$-activated nucleotide-dependent proteases; and the HtrA (or DegP) family of serine-type proteases. Recent evidence showed that the HtrA family of proteases is involved in the resistance of *Synechocystis* to light stress and play a part, either directly or indirectly, in the repair of PSII in vivo (Bailey et al., 2001). Chloroplast FtsH showed light-inducible gene expression (Lindahl et al., 2000). It was proposed that this peptidase is involved in the degradation of unassembled thylakoid proteins (Ostersetzer and Adam, 1997) and perhaps, in the second step of the degradation of PSII reaction center core protein D1 (Lindahl et al., 2000; Silva et al., 2003).

### 4.1.3. Heat stress

Response to elevated temperature is an environmental factor that is also studied in cyanobacteria. Exposure of cyanobacterial cells to temperatures exceeding 60 – 65°C even for 10 min results in a bleached phenotype. Such alternation is the result of a breakdown of components of PBS (Zhao and Brand, 1989; Nishiyama et al., 1993). Higher temperatures bleach bilin-containing pigments fast, but also resulted in a gradual bleaching of chlorophyll and carotenoids. PSII has been shown to be the most sensitive thylakoid assembly to heat among the photosynthetic activities (Berry and Bjorkman, 1980). The dissociation of two of the four Mn atoms from the PSII complex by heat results in complete inactivation of oxygen evolution without significant loss of proteins (Nash et al., 1985). Therefore, the mechanism of photosynthetic adaptation to high temperature is related to ability to protect the PSII oxygen evolving complex against heat-induced inactivation.

Different factors, which are regulated by growth temperature, may contribute to observed adaptation. Early investigations suggested that high temperature increases the level
of saturated fatty acids in membrane lipids and enhances the thermal stability of photosynthesis (Shneyour et al., 1973). Later, it has been shown that thermal stability is not only affected by changes in the saturation level of membrane lipids, but other factors are also responsible for adaptation of photosynthesis to high temperature. There are a number of heat stress proteins, chaperones and peptidases that are involved in the refolding or degradation of polypeptides misfolded by heat. One of the best studied peptidases functioning during heat stress is the bacterial HtrA peptidase (Strauch et al., 1989; Lipinska et al., 1990; Spiess et al., 1999).

### 4.1.4. Low temperature

Research on temperature stress in cyanobacteria has mainly focused on heat stress while less attention has been paid to low temperature. Nevertheless, some mechanisms of cold responses and acclimation have been identified (Phadtare, 2004). At low temperatures chaperones act to protect proteins that are susceptible to damage. Lower temperatures make cell membranes more rigid, and as a consequence the desA and desB genes encoding desaturase-enzymes in *Synechocystis* are activated (Sakamoto and Bryant, 1997). Desaturases increase the number of double bonds in fatty acid hydrocarbon chains thus increasing membrane fluidity. While its role in cellular sensing encompasses a variety of stresses, the membrane-bound Hik33 histidine kinase also perceives the degree of membrane fluidity and regulates the expression of the desaturases (Suzuki et al., 2000; Suzuki et al., 2001; Kanesaki et al., 2007).

The main aim of this objective is to study the low temperature induced changes in the photosynthetic machinery and also study the impact of mutation of *crhR* on the cell during low temperature.
4.2. Results and Discussion

4.2.1. Effect of low temperature on *Synechocystis* wild-type and ΔcrhR

Acclimation responses to stress conditions include changes in the cell morphology, metabolism and pigment content. Changes in the pigment protein complexes can be analysed by absorption spectra of whole cells. The absorption spectra of the wild-type and ΔcrhR cells grown under optimal and low temperature stress were taken in order to compare the pigment content alterations. Absorption spectra of wild-type cells and ΔcrhR cells were recorded to further examine the changes in pigment protein complexes at low temperature incubation. Both strains exhibited absorption peaks corresponding to chlorophyll *a* at 685 nm and 446 nm, as well as an absorption peak of PC at 625 nm. An increase in the absorption maximum of both chlorophyll *a* and PC peaks were observed in the WT-24°C-cells when compared to the WT-34°C-cells (Fig.4.1). Absorption spectra of ΔcrhR-24°C-cells exhibited a decrease in the chlorophyll *a* absorption maximum when compared to the ΔcrhR-34°C-cells. This further supports the observation that in ΔcrhR-24°C cells the cellular concentration of chlorophyll binding proteins is significantly lower when compared to ΔcrhR-34°C cells (Fig.4.1).

4.2.2. Blue shift of Chlorophyll absorption peak in wild-type cells at low temperature

Absorption spectra were recorded for wild-type and ΔcrhR cells that were grown under optimum growth conditions (34°C) and then shifted to low temperature conditions (24°C). Spectra were recorded at 3, 6, 23, 29, 47, 53 and 72 h after shift from 34°C to 24°C. This lead to the identification of low temperature induced alteration in the pigment protein complexes during the onset of incubation at 24°C. After 72 h of low temperature treatment, we observed a shift in the chlorophyll absorption peak from 685 to 680 nm in the wild-type cells (Fig.4.2A). However this blue shift was not observed in the absorption spectra of ΔcrhR cells during the course of low temperature incubation (Fig.4.2B). Previous reports indicated that the expression of IsiA (Iron stress inducible protein A) lead to the peak shift under iron stress.
Fig. 4.1. Changes in absorption spectra of wild-type and ΔcrhR cells at 34°C and during incubation at 24°C. (A) Absorption spectra of wild-type cells grown at 34°C for 16 h and the wild type cells that had been grown at 34°C for 16 h and then incubated at 24°C for 72 h (B) ΔcrhR mutant cells grown at 34°C for 16 h and the ΔcrhR cells that had been grown at 34°C and then incubated at 24°C for 72 h. The spectra were corrected for light scattering at 700 nm.
Fig.4.2. Changes in chlorophyll absorption spectra to monitor blue shift in wild-type during low temperature (A) Absorption spectra of wild-type cells grown at 34°C for 16 h and the wild type cells that had been grown at 34°C for 16 h and then incubated at 24°C for 72 h: (B) ∆crhR mutant cells grown at 34°C for 16 h and the ∆crhR cells that had been grown at 34°C and then incubated at 24°C for 72 h. (C) and (D) Chlorophyll Absorption peak of wild-type and ∆crhR at 685 nm.
conditions. It has also been reported that the monomerisation of PSI contributes to the peak shift under iron stress (Sandstrom et al., 2002). The blue shift could be due to changes in the pigment-protein interactions that might have undergone various modifications like loss of longer wavelength chlorophylls to perform optimal photosynthesis upon prolonged exposure to low temperature. However in ΔcrhR blueshift was not observed when the culture was shifted to low temperature.

4.2.3. Loss of chlorophyll is prominent in ΔcrhR cells at low temperature

In order to monitor changes in pigment protein complexes in more detail, further examined the effect of low temperature on pigment content of the ΔcrhR cells in comparison with wild-type cells. From the results presented in Fig.4.3A, a significant increase in the chlorophyll content was observed during incubation of wild-type cells at 24°C, suggesting incubation of wild-type cells at low temperature enhances the levels chlorophyll containing protein complexes. Almost similar chlorophyll content was observed between wild-type and ΔcrhR cells at 34°C. However, during incubation of cells at 24°C, ΔcrhR cells contained relatively lower chlorophyll content, as compared to the wild-type cells suggesting that loss of chlorophyll protein complexes in the ΔcrhR cells at low temperature (Fig.4.3A).

Results presented in Fig.4.3B shows PC content in wild-type and ΔcrhR during low temperature incubation. A significant increase in the PC content during incubation of wild-type cells at 24°C, suggests an increase in phycobilisome content during incubation of cells at 24°C. A slight but significant increase in the PC content was observed ΔcrhR cells at low temperature (Fig.4.3B). Fig.4.3B indicates that there is fourfold increase in the PC content (at 72 h time point) of the wild-type cells at 72 h of 24°C incubation. However ΔcrhR cells displayed only two fold increase in the PC content. The observed loss in the PC content is mainly from the reduced cell density of the ΔcrhR cells.
Fig. 4.3. Pigment analysis at 34°C and during low temperature treatment in wild-type (black circles) and ∆crhR (grey circles). (A) Chlorophyll content; (B) PC content.
These results indicate that there is a predominant loss of chlorophyll rather than the PC in the ΔcrhR cells under low temperature.

4.2.4. Effect of low temperature on oligomerization of photosystems

The extent of changes in photosystems in *Synechocystis* wild-type and ΔcrhR cells under low temperature were monitored by sucrose density gradient separation of solubilized thylakoid complexes. Fractionation of solubilized thylakoid membranes on sucrose density gradient resulted in three pigment protein containing fractions (Fig. 4.4). According to previous reports the orange coloured fraction on the top of sucrose density gradient (F1) comprises of free pigments, upper green fraction (F2) enriched with PSII and PSI monomer mixture and lowest green fraction (F3) contains PSI trimer (Fuhrmann et al., 2009). The level of F2 containing PSII and PSI monomer was increased in WT-24°C-cells (Fig. 4.4). Fractionation of thylakoid membranes isolated from ΔcrhR-24°C-cells showed a significant decrease in the PSI trimer and an increase in the PSII/PSI monomer fraction as compared to the ΔcrhR-34°C-cells (Fig. 4.4). However, the relative increase in the PSII/PSI monomer fraction and decrease in the PSI trimer fraction does not reflect the concentrations within the living cells because, the sucrose density gradients were loaded on equal chlorophyll basis, even though there was significantly low chlorophyll in the ΔcrhR-24°C-cells (see Fig. 3.9 bleached phenotype). Thus, the loss in PSI trimers may be much more in reality than observed and the increase in PSII/PSI monomers may not be occurring in vivo.

Since the Sucrose density gradient centrifugation was carried out on equal chlorophyll basis and the relative amount of PSII and PSI of F2 seemed to be increased in the ΔcrhR when compared to wild-type, to rule out this sucrose density gradient centrifugation of the solubilised membranes were carried out on equal protein basis (Fig. 4.5). The F2 comprising of PSI monomer and PSII dimer were not affected under low temperature in both
Fig.4.4. Fractionation of the thylakoid protein complexes by sucrose density ultracentrifugation. (A) Thylakoid protein complexes of wild-type cells grown at 34°C for 16 h and wild type cells that had been grown at 34°C for 16 h and then incubated at 24°C for 72 h. (B). Thylakoid protein complexes of ΔcrhR cells grown at 34°C for 16 h and ΔcrhR cells that had been grown at 34°C for 16 h and then incubated at 24°C for 72 h. The thylakoid protein complexes were then separated by ultracentrifugation at 160,000 x g for 16 h at 4°C on a step gradient of sucrose density. Samples were loaded on equal chlorophyll basis.
**Fig. 4.5.** Fractionation of the thylakoid protein complexes by sucrose density gradient ultracentrifugation (A) Thylakoid protein complexes of wild-type cells grown at 34°C for 16 h and wild type cells that had been grown at 34°C for 16 h and then incubated at 24°C for 72 h.  (B) Thylakoid protein complexes of ∆crhR cells grown at 34°C for 16 h and ∆crhR cells that had been grown at 34°C for 16 h and then incubated at 24°C for 72 h.  The thylakoid protein complexes were then separated by ultracentrifugation at 160,000 x g for 16 h at 4°C on a step gradient of sucrose density.  Samples were loaded on equal protein basis.
wild-type and ΔcrhR formation of trimeric form of PSI was found to be hugely effected in the mutant.

The changes observed in Sucrose density ultracentrifugation were further confirmed by Green Gel electrophoresis of the solubilised thylakoid membranes after resolving on to 10% LiDS gel. Green gel electrophoresis showed four distinct bands on the slab gel (Fig.4.6). From top to bottom of the gel the pigment band corresponds to the PSI trimer, PSI monomer, PSII and free pigment. In consistent with sucrose density gradient separation of the solubilized membranes there was no significant difference in the trimer between wild-type 34°C cells and wild-type 24°C cells. A significant decrease in the PSI trimer content was observed in crhR-24°C cells when compared to crhR 34°C cells, accompanied by a significant increase in the PSI monomer content. This data suggests that mutation in RNA helicase crhR leads to monomerisation of PSI trimer at low temperature and drastic decrease in the overall content of PSI. A slight but significant decrease in PSI trimer content was observed in the wild-type cells-24°C when compared to wild-type-34°C cells.

4.2.5. Enhanced PSII dimer content in wild-type cells was observed at low temperature

In sucrose density centrifugation changes in PSII were not resolved hence the pigment protein complexes were further separated by BN-PAGE. To further confirm the oligomeric states of the photosystems, BN PAGE was carried out for the solubilised thylakoids (Fig.4.7). The pattern of PSI trimeric and monomeric complexes on the gel was identical in both the wild-type and ΔcrhR cells maintained under optimal conditions exhibiting the PSI trimers as predominant species (Fig.4.7). PSII dimer content was increased in wild-type 24°C when compared to wild-type 34°C cells. In contrast, no significant change in the PSII dimer content was observed on incubation of ΔcrhR cells at 24°C for 72 h. As observed by Sucrose density gradient separation of PSI complexes and by green gel electrophoresis, PSI trimer content was significantly reduced in ΔcrhR-24°C when compared to ΔcrhR-34°C cells. Thus
Fig.4.6. Mild denaturing green gel electrophoresis analysis of chlorophyll pigment complexes in thylakoid membranes. Native PAGE (10%) of thylakoids isolated from WT-34°C cells, WT-24°C cells, ΔcrhR-34°C cells and, ΔcrhR-24°C cells. Sample preparation was done by solubilising thylakoid membrane by mixing equal volume of 2X sample buffer and 10% w/v DDM at 4°C under constant rotation for 25 min. Unsolubilized membranes were pelleted out by ultracentrifugation at 45,000 x g for 30 min at 4°C. Supernatant containing solubilised thylakoid membranes equivalent of chlorophyll was loaded on each lane of the 8% SDS-PAGE gel (4% stacking gel, 10% resolving gel).
**Fig. 4.7.** Pigment protein complexes of wild-type and ∆crhR at 34°C and 24°C separated by 8% BN-PAGE. To the solubilised thylakoid complexes added 1/10 volume of 5% serva blue G (100 mM Bis Tris-HCl, pH 7.0, 0.5 M 6-amino-n-caproic acid, 30% glycerol) and loaded on equal chlorophyll basis.
it is clear that PSI trimer content was significantly reduced in ΔcrhR at low temperature. PSII dimers formed in the wild-type at low temperature could be an acclimative response to low temperature stress. Such an acclimative oligomerization of PSI was not detected in ΔcrhR mutant at 24 °C.

4.2.6. PBS uncoupling at low temperature in wild-type cells and effect of ΔcrhR mutation

Since, PBS are the major light-harvesting complexes involved in cell acclimation, the degradation of the PBS can reflect one of the protection mechanisms for controlling photosynthetic activity during stress conditions. The reduction of the PBS can lead to a decrease of absorbed energy, thus preventing cells from photo damage. The adaptation of the PBS to stress occurs through a decrease of the rod size, the number of rods and the number of PBS that are controlled at the transcriptional and post-translational levels. The PBS are mobile structures on the surface of the thylakoid membrane. The association of PBS with PS II and PSI is transient and PBS can be found in a membrane-bound and non-associated form with PSI/PSII. PBS efficiently harvest light energy and transfer it to the chlorophyll a of PSII or PSI when they are attached to these complexes. Thereby the membrane-bound and free PBS (soluble in the cytoplasm) may provide information on energy absorption and transfer through PBS to the reaction centres of the two photosystems.

Fig.4.8 shows the room temperature fluorescence emission spectra at of wild-type and ΔcrhR cells, when excited at 580 nm (Fig.4.8A, B) or 436 nm (Fig.4.8C, D). As shown in Fig.4.8A, there was a significant rise in PBS emission peak at ~660 nm, in WT-24°C-cells when compared to wild-type-34°C-cells. However, there was no difference in the fluorescence emission at 660 nm in ΔcrhR -34°C-cells and ΔcrhR -24°C-cells when excited cells excited at 580 nm (Fig.4.8B).

In chlorophyll emission spectra, significant increase in the emission spectral properties at 605, 645, and 680 nm which corresponds to PC, APC and terminal emitters of PBS,
**Fig. 4.8.** Changes in fluorescence emission spectrum of wild-type and ∆crhR cells at 34°C and 24°C. Fluorescence emission spectra of wild-type (A) and ∆crhR cells (B) excited at 580 nm. Fluorescence emission spectra of wild-type (C) and ∆crhR mutant cells (D) excited at 436nm. Spectrum of cells grown at 34°C (solid line) and cells incubated at 24°C for 72 h (dashed line).
respectively was monitored in wild-type-24°C-cells when compared to wild-type-34°C-cells (Fig.4.8C). There was no difference in ΔcrhR-34°C-cells and ΔcrhR-24°C-cells (Fig.4.8D). Taken together absorption and fluorescence emission spectra it is clear that the increase in fluorescence emissions at 605, 645 and 680 nm were may be due to uncoupling of PBS from PSII in wild-type cells at low temperature. Such uncoupling of PBS was not seen in the ΔcrhR mutant.

4.3. Conclusion

Different environmental parameters can modify the activities of photosynthetic complexes and the levels of pigments and proteins associated with these complexes (Grossman et al., 1993). The photosynthetic apparatus is highly dynamic and alteration of light harvesting complex synthesis and degradation occurs in response to environmental stimuli including changes in light quality (Tandeau de Marsac, 1993), light intensity (Horton et al., 1996), and nutrient availability (Allen and Smith, 1969). Such an alteration helps to efficiently balance the absorption of excitation energy.

Here in the present study with wild-type and ΔcrhR cells grown at low temperature conditions revealed several acclimatory mechanisms. Changes in pigment protein complexes indicated a preferential loss of chlorophyll over PC in ΔcrhR upon low temperature treatment. An alteration in PC/Chl ratio was also observed in the wild-type and ΔcrhR indicating that the association of PBS to chlorophyll has altered. However in ΔcrhR the ratio of PBS to chlorophyll has not altered but overall photosystems were altered. Loss of longer wavelength chlorophylls was evidenced by a blue shift in the red region of absorption spectrum was observed in the wild-type cells upon low temperature treatment. However such shift was not seen in ΔcrhR-24°C-cells. Uncoupling of PBS from PSII in the wild-type cells was observed upon low temperature. Such uncoupling was not seen in ΔcrhR. An increase in the PSI monomer content and PSII dimer content upon low temperature was observed in the wild-
type indicating changes in the oligomerisation of the photosystems. However increase in PSII dimer content is not seen in the mutant while the loss of PSI trimer is more significant.

All the above results indicate that the wild-type cells adopt different mechanisms to cope up the low temperature stress while ΔcrhR was unable to exhibit these adaptive responses. Since ability of cyanobacteria to sense and respond to changes in their environment through a complex series of regulatory pathways is initiated by changes in electron flow and RNA helicase was reported to be redox regulated, we assumed that redox regulation of crhR expression allows Synechocystis to respond rapidly to environmental changes, on the order of minutes, similar to the PQ redox state-mediated response observed for the photosynthetic genes, psaAB and psbA, in mustard chloroplasts (Pfannschmidt et al., 1999). Hence we further aimed to study the redox regulated changes in the wild-type and ΔcrhR upon shift to low temperature in the next objective.