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Analysis of mutations to suppress the chloride requirement of the *psbV*-disruptant of the cyanobacterium *Synechocystis* sp. PCC 6803

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Introduction

It is known that the H_2O-splitting reaction requires the inorganic cofactors Ca^{2+} and Cl^- to exhibit optimal activity in oxygenic photosynthesis. As yet, however, little is known about precise location or function of these cofactors in PSII. Requirement of these ions is attenuated by the presence of the extrinsic proteins termed OEEs. Cytochrome c550 is one of the cyanobacterial OEEs and has been demonstrated to be critical for photoautotrophic growth in the Cl^--free medium, while the role of low potential c-type heme is not known. We attempted to gain an insight into the Cl^- homeostasis that is critical for PSII activity by analyzing spontaneous suppressor mutants derived from the *psbV*-disruptant.

Materials and methods

The *psbV*-disruptant of unicellular cyanobacterium *Synechocystis* sp. PCC 6803 was a kind gift from Dr. J.R. Shen (RIKEN). *Synechocystis* cells were grown in the liquid BG11 medium at 31 °C with continuous light and bubbled with air containing 1% (v/v) CO_2. Alternatively, cells were grown on 1.5 % (w/v) agar plates containing BG11 and 0.3% (w/v) sodium thiosulfate. Since *psbV* was disrupted by an erythromycin-resistant cassette, the mutant was maintained in BG11 with 20 μg/ml erythromycin. Spontaneous non-Cl^--requiring mutants were isolated from the *psbV*-disruptant that was incubated in the Cl^--free BG11 medium under illumination. Genomic DNA library of the mutants was digested by *Eco*RI and cloned into pUC118. Photoautotrophic growth of the *psbV*-disruptant on the Cl^--free BG11 plate was restored by addition of DNAs from genomic DNA library. Since agar (Bacto-Agar, Difco) contains residual chloride, extensive washing is essential for the growth arrest of the *psbV*-disruptant on the Cl^--free BG11 plate. Agar was sequentially washed with water, ethanol and acetone and finally dried up before preparation of agar plates. The complementing DNA fragments were sequenced to identify the mutation.

Results

The *psbV*-disruptant does not grow photosynthetically at all without Cl^-. However, during incubation in the Cl^--free medium with erythromycin for weeks, we found growing cells, which were derived from it. They retained capability of photoautotrophic growth in the absence of Cl^- whenever transferred to fresh Cl^--free medium with or without erythromycin. Their growth rate was about 2/3 of wild type (Fig. 1). This implies that another mutation suppressed the Cl^- requirement in the *psbV*-disruptant instead of simple reversion.

Genomic DNA library from the non-Cl^--requiring mutants was screened by growth complementation of the *psbV*-disruptant on the Cl^--free agar plate. Three positive clones containing 3~6kbp genome fragments were obtained. One was a deletion of 481 bp harboring the initiation codon of *slr0753*. The other two contained related but not identical insertion sequence ISY203 in *slr0753* at different loci. They were ISY203x (1175b) and ISY203f (1175bp). Sequence comparison of the mutant clones with wild type revealed that insertion of these ISs generated a direct repeat of 9 bp in *slr0753*. Nucleotide sequence of ISY203x was different from ISY203f at only three base positions. In any case, insertion of IS disrupted the coding frame of *slr0753*. These suggest that inactivation of *slr0753* strictly coupled with escape from the Cl^- requirement of the *psbV*-disruptant.

According to CyanoBase, the deduced product of *slr0753* was annotated as "*p* protein" but its function remains unknown. Slr0753 has twelve hydrophobic segments potentially to span the membrane. Blastp search of the protein database revealed that Slr0753 is highly homologous to a group of conserved ORFs in *Pyrococcus*, *Mycobacterium* and *Thermotoga* and significantly homologous to the arsenic efflux pump protein of *Escherichia coli* (Dey and Rosen 1995). These results suggest that the *slr0753* product acts as an efflux pump of Cl^- or some anions that are critical for water oxidation in the PSII. On the genetic background of wild type, *slr0753* was successfully disrupted by a kanamycin-resistant cassette. The *slr0753* mutant showed a slightly retarded growth in the Cl^--free medium. This suggests that Slr0753 is not essential but contribute to the growth of wild type probably by regulation of Cl^- homeostasis.

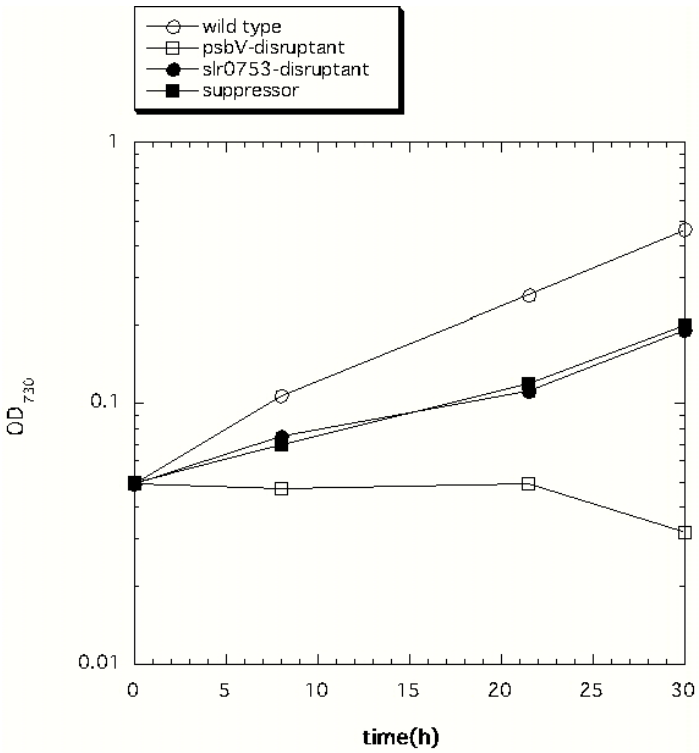

Fig. 1: Growth of wild type and mutants in the Cl^--free BG11 medium under the photoautotrophic conditions. Wild-type, open circle; *psbV*-disruptant, open square; the suppressor mutants, closed square; the *slr0753*-disrupted mutant, closed circle.

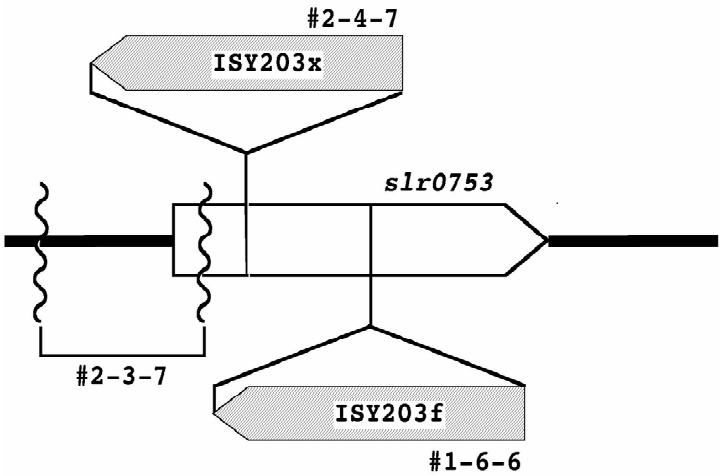

Fig. 2: Diagram of three independent mutations in *slr0753*.
#2-3-7, #2-4-7 and #1-6-6 are the three independent suppressor mutants. Mutation in #2-3-7 was a deletion harboring the initiation codon of *slr0753*. #2-4-7 and #1-6-6 contained IS in *slr0753* at different loci.

Discussion

In this study, we identified mutations of the non-Cl^--requiring mutants isolated from the *psbV*-disruptant. The three independent mutations were found in the same ORF *slr0753*. Slr0753 is a hydrophobic protein without any ATPase motif. Homology search revealed that Slr0753 is highly homologous to a group of conserved ORFs in *Pyrococcus*, *Mycobacterium* and *Thermotoga* and significantly homologous to the arsenic efflux pump protein ArsB of *E. coli* (Dey and Rosen 1995) and a group of *p* protein of mouse (Puri et al. 2000) and *Drosophila*. In the case of ArsB, the arsenic efflux is probably driven by proton motive force. Nothing is known about substrates for the other bacterial conserved ORFs. Homology of deduced amino acid sequences suggests that Slr0753 is closest to the bacterial ORFs, although it is definitely not the member of this group including some eubacteria and archaea. It is assumed that the *p* protein is a transporter to keep optimum pH of melanosomes. These facts suggest that Slr0753 is a novel class of an efflux pump of Cl^- or some anions at the expense of high energy state of the cytoplasmic membrane or the thylakoid. A mechanism to suppress the Cl^- requirement of the *psbV*-disruptant can be rationalized by assuming that inactivation of Cl^- efflux pump resulted in higher concentration of Cl^- in the cytoplasm and thylakoid lumen.

We demonstrated here that inactivation of *slr0753* can be positively screened on the genetic background of *psbV*-disruption under the low Cl^- conditions. Transposition of ISY203x was described in our previous report (Okamoto et al. 1999). The sequence identical to ISY203x is present in the complete genome of Kazusa strain (Kaneko et al. 1996) but is absent in the wild-type genome. Very likely, it was originated from a yet unidentified plasmid. On the other hand, ISY203f that is present in the wild-type genome has not yet been demonstrated as an active mobile element before. Although the nucleotide sequence of ISY203f is different from the active ISY203x by 3 bp, the amino acid sequence of transposase in ISY203f is identical to that in ISY203x. Thus, ISY203 is an active mobile element in *Synechocystis* sp. PCC 6803. It is suggested that the coding region of *slr0753* in the *psbV*-disruptant can be used as an efficient positive trap for IS transposition that has not been analyzed systematically in Sy*nechocystis*.

Acknowledgments

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