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FINAL TECHNICAL REPORT
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Unraveling Photosystems

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1. A residue substitution in phosphoribulokinase of *Synechocystis* PCC 6803 renders the mutant light-sensitive

We isolated a light-sensitive mutant (BRLS) of the photosynthetic cyanobacterium *Synechocystis* 6803 (*S.* 6803) that does not survive exposure to bright light: 70% of BRLS cells die upon exposure to light of >3000 lux for 2 h. A complementing DNA fragment from wild-type cells and the corresponding DNA from the BRLS cells were cloned and sequenced. An open reading frame was found to encode phosphoribulokinase, a key enzyme in the enzyme system for photosynthetic carbon reduction (ES-PCR). The deduced peptide sequence of this enzyme is highly homologous to eukaryotic phosphoribulokinases but is not similar to known prokaryotic phosphoribulokinases. The mutation responsible for the phenotype of BRLS is a single nucleotide change that results in substitution of phenylalanine for Ser-222 in the phosphoribulokinase. The catalytic activity and the apparent affinity for ATP of the mutated kinase are about one-tenth and one-seventh those of the wild-type kinase, respectively. Furthermore, the mutated kinase is selectively degraded in BRLS cells in bright light. Degradation of the mutated kinase and cell death in bright light can be suppressed by inhibiting photosynthetic electron flow (PS-EF) with 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The data indicate that PS-EF is not impeded by an impaired ES-PCR although the ES-PCR activity is controlled by the rate of PS-EF. Continued PS-EF in the absence of the normal substrates for carbon reduction appears to result in damage to cellular components essential for life or in the generation of lethal components.

2. Excitation energy transfer from phycocyanin to chlorophyll in an *apcA*-defective mutant of *Synechocystis* sp. PCC 6803

A greenish mutant of the normally blue-green cyanobacterium *Synechocystis* sp. PC 6803, designated UV6p, were isolated and characterized. UV6p possesses functional photosystems I and II (PSI and PSII) but lacks normal light harvesting phycobilisomes because allophycocyanin is absent and core-specific linker proteins and almost entirely absent. The mutation responsible for the UV6p phenotype was identified; it is a base substitution which results in the creation of a termination codon within the coding region of the *apcA* gene. Phycocyanin (PC) and phycobilisome rod linker proteins are present in UV6p and, despite the absence of core components, at least 35% of the PC is associated with rod linker proteins. At 77 K, light absorbed by PC of UV6p elicits PSI florescence comparable to that of wild type cells but produces greatly diminished PSII florescence. The results indicate that the assembly of rods is independent of cores and that light energy absorbed by rods is independent of cores and that light energy absorbed by rods can be transferred principally and directly to PSI. This energy pathway,

which may also be present in wild type, may have a regulatory role in maintaining the balance of input excitation energy in PSI *versus* PSII during photosynthesis.

3. Deletion of the *psbG1* gene of the cyanobacterium *Synechocystis* sp. PCC6803 leads to the activation of the cryptic *psbG2* gene.

The genes *psbG1* and *psbG2* in cyanobacterium *Synechocystis* sp. PCC6803 are homologous. The *psbG1* gene is located on the chromosome and is part of the *ndhC-psbG1-ORF157* operon, while *psbG2* is located on a plasmid and is not flanked by equivalent *ndhC* or *ORF157* genes. Mutants in which *psbG1* is deleted grow well under autotrophic conditions, while their growth is impeded in mixotrophic medium. These results argue against a functional role for *psbG1* in photosynthesis, i.e. photosystem II, and are more compatible with a function in respiration. The psbG2 gene is not transcribed in wild-type cells, but in *psbG1* mutants the insertion of DNA sequences in close proximity to the *psbG2* reading frame has led to transcriptional activation of *psbG2*. Thus, *psbG2* represents an example of a cryptic gene, similar to those found in other bacteria.

4. Deletion of the structural gene for the NADH-dehydrogenase subunit 4 of *Synechocystis* 6803 alters respiratory properties

Chloroplasts and cyanobacteria contain genes encoding polypeptides homologous to some subunits of the mitochondrial respiratory NADH-ubiquinol oxidoreductase complex (NADH dehydrogenase). Nothing is known of the role of the NADH dehydrogenase complex in photosynthesis, respiration, or other functions in chloroplasts, and little is known about there specific roles of the perhaps 42 subunits of this complex in the mitochondrion. Inactivation of a gene for subunit 4 (*ndh*D-2, *ndh*4) of this complex in the cynaobacterium *Synechocystis* 6803 has no effect on photosynthesis, judging from the rate of photoautotrophic growth of mutant cells, but the mutant's respiratory rate is about 6 times greater than that of wild-type cells. Respiratory electron transport activity in cyanobacterium is associated both with photosynthetic thylakoid membranes and with the outer cytoplasmic membrane of the cell. Cytoplasmic membranes of mutant cells have much greater NADH-dependent cytochrome reductase activity than preparations from wild-type cells; this activity remains at wild-type levels in isolated thylakoid membranes. It is suggested that the 56.6-kD product of *ndh*D-2 is not essential for the activity of a cytoplasmic membrane-bound NADH dehydrogenase but that it regulated the rate of electron flow through the complex, establishing a link between this *ndh* gene and respiration. The activity of molecularly distinct thylakoid-bound NADH dehydrogenase is apparently unaffected by the loss of *ndh*D-2.

Publications

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