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Identification of a *Chlamydomonas reinhardtii* Chloroplast Gene with Significant Homology to Bacterial Genes Involved in Cytochrome c Biosynthesis

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As entire chloroplast genomes are sequenced, the structure of chloroplast genes, open reading frames, and spacer regions can be compared. Shimada and Sugiura (1991) identified 11 open reading frames that were conserved in the rice, *Marchantia*, and tobacco chloroplast genomes. These genes have been designated *ycf* genes (Hallick, 1989). There are also 11 additional conserved genes that are thought to encode subunits of a putative chloroplast NADPH dehydrogenase. *Chlamydomonas reinhardtii* offers a suitable experimental system to test the physiological function of these genes because chloroplast genes can be disrupted by insertional mutagenesis. We have located the *ycf5* gene on the C. *reinhardtii* chloroplast genome and report the sequence of this gene and the flanking DNA in this communication (Table I).

* C. reinhardtii chloroplast DNA was obtained from strain CC-400, a cell-wall-deficient strain carrying the cw-15 mutation. Chloroplast DNA was obtained by density gradient centrifugation and probed with tobacco DNA probes carrying the *ndhD* gene or the *ycf5* gene. These genes lie adjacent to each other on the tobacco, rice, and *Marchantia* genomes. Although no significant hybridization was observed using the *ndhD*-specific probe, a probe carrying the *ycf5* gene hybridized with both isolated C. *reinhardtii* chloroplast DNA and the cloned BamHI 13 fragment obtained from the *Chlamydomonas reinhardtii* Genetics Center. Sequencing of the BamHI 13 fragment indicated that, although the 5' end of the gene was on the BamHI 13 fragment, part of the gene must be on the adjacent BamHI 13 fragment. The 3' end of the gene was obtained by polymerase chain amplification of the BamHI 17 fragment using primers from the flanking BamHI 4 and BamHI 13 fragments. The open reading frame that spanned these two BamHI fragments was 1062 bp and would encode a protein of 353 amino acids.

A comparison of the deduced amino acid sequence with other known sequences revealed that the *Chlamydomonas* gene is very homologous to *ycf5* genes found in higher plants. It has 74.5% identity with the *Marchantia* *ycf5* gene (Ohyama et al., 1986) and 66% identity with the tobacco *ycf5* gene (Shinozaki et al., 1986). In addition, like other *ycf5*

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membrane with the heme attached (Thony-Meyer, 1994). If that is the case, a protein analogous to the Ccl1 gene product must be present in mitochondria and chloroplasts. Although this function has not been physiologically demonstrated in C. reinhardtii or other plants, we have detected a 3.3-kb transcript using a ycf5-specific probe, implying that this is a functional chloroplast gene. In the C. reinhardtii chloroplast, both Cyt f and Cyt c-552 (Merchant and Bogorad, 1987) are c-type cytochromes and would require the proteins necessary to covalently bind the heme to the apoprotein.

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