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CREATION OF CHLOROPHYLL BIOSYNTHETIC MUTANTS OF
Chlamydomonas reinhardtii BY INSERTIONAL MUTAGENESIS

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Nuclear transformation of the green alga Chlamydomonas reinhardtii is a useful technique for studying various cellular processes, including chlorophyll biosynthesis. The mutations resulting from the transformation occur because of the integration of foreign DNA into the nuclear genome, disrupting the function of the gene at the point of insertion. We are attempting to isolate chlorophyll biosynthesis mutants of Chlamydomonas reinhardtii using two separate selectable markers as inserting DNA. One DNA fragment contains an A, a prokaryotic antibiotic resistance gene, which makes chloroplast ribosomes resistant to spectinomycin. The other DNA fragment contains the Arg7 gene, coding for the enzyme arginosuccinate lyase, allowing the organism to produce the amino acid arginine. Transformants are selected and screened for their ability to synthesize chlorophyll in the dark. Creation of the mutants in this manner allows for the analysis of the genes that were disrupted. The isolated genes, coding for proteins, enzymes, or subunits of enzymes important in light-independent chlorophyll biosynthesis can then be cloned and characterized, leading to a better understanding of the process of chlorophyll biosynthesis in Chlamydomonas reinhardtii.