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The Characterization of a Nuclear Locus Affecting the Function of Protochlorophyllide Oxidoreductase

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Poster Presentation 46

THE CHARACTERIZATION OF A NUCLEAR LOCUS AFFECTING THE FUNCTION OF PROTOCHLOROPHYLLIDE OXIDOREDUCTASE

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Protochlorophyllide oxidoreductase, which catalyzes the conversion of protochlorophyllide to chlorophyllide, is a key enzyme in the biosynthetic pathway of chlorophyll. Currently there are seven known nuclear loci that affect function of the enzyme. The chloroplast itself contains three genes coding for catalytic subunits of protochlorophyllide oxidoreductase, yet disruption of the nuclear loci inhibits formation of the enzyme by the chloroplast. Completion of this project will provide a better understanding of nuclear-chloroplast communication, since the evidence suggests that the nuclear loci play a significant role in the regulation of cellular levels of chlorophyll, the molecule that harvests light in plants. To accomplish this goal, a mutant strain of the algae *Chlamydomonas reinhardtii* that contains a defective Arg 7 gene (phenotype Arg 7-) had a functional Arg 7 gene (phenotype Arg 7+) inserted randomly into the genome. In a certain percentage of transformed cells, the Arg 7+ gene becomes inserted such that it disrupts a nuclear locus affecting the expression of protochlorophyllide oxidoreductase. The disruption of the locus leads to a yellow phenotype in the dark. It is yellow only in the dark because the light-dependent biosynthetic pathway is not operating and the light independent pathway has been inhibited by the disruption of the locus. The strain TY 5, a strain where the Arg 7+ gene has inserted successfully, is being analyzed. The goals for the project include the determination of which nuclear locus was disrupted, followed by the cloning and sequencing of the locus. The project will contribute to the understanding of this vitally important gene. The determination of the disrupted locus is being conducted using allesism tests which cross the TY 5 strain with strains of a known mutation. The locus will be isolated by the creation of genomic library followed by the cloning of the locus using standard hybridization techniques. Finally, the disrupted locus will be sequenced.