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Robert Graham  
*Illinois Wesleyan University*

David Bollivar, Faculty Advisor  
*Illinois Wesleyan University*

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Oral Presentation 3.2

**THE CHARACTERIZATION OF THE TY5 STRAIN OF *Chlamydomonas reinhardtii*, A CHLOROPHYLL BIOSYNTHETIC MUTANT**

Robert Graham and David Bollivar\*

Department of Biology, Illinois Wesleyan University

Currently, there are seven nuclear loci known to affect the function of protochlorophyllide oxidoreductase, the enzyme which catalyses the conversion of protochlorophyllide to chlorophyllide in the alga *Chlamydomonas reinhardtii*. The conversion of protochlorophyllide to chlorophyllide is one of the final steps in the biosynthetic pathway of chlorophyll, the light harvesting molecule for many photosynthetic organisms. The chloroplast genome of *C. reinhardtii* contains three genes coding for the catalytic subunits of protochlorophyllide oxidoreductase. Yet, disruption of the nuclear loci is believed to result in the inhibition of chloroplast production of the enzyme. This evidence suggests that nuclear loci play a vital role in the regulation of chlorophyll levels in the cell by regulating the products or expression of chloroplast genes, a possible example of nuclear-chloroplast communication.

The strain of the alga *C. reinhardtii* that was studied is a transformed strain, TY5. The TY5 strain probably contains a mutation in one of the seven known nuclear loci affecting protochlorophyllide oxidoreductase function and was formed by insertional mutagenesis. The insertion of a functional Arg7 gene causes a yellow phenotype in the dark that is indicative of the strain's inability to produce chlorophyll by light independent pathways. The inability to produce chlorophyll is most likely the result of the disruption of a nuclear encoded regulatory protein that affects the function of protochlorophyllide oxidoreductase.

The specific goals for the project included the determination of which nuclear locus was disrupted, followed by the isolation and sequencing of the gene disrupted in the TY5 strain. The determination of which nuclear locus is mutated in the TY5 strain was attempted by complementation mating tests. Preliminary evidence suggests the mutated locus in the TY5 strain is the  $\gamma$ -6 locus.

The isolation of the  $\gamma$ -6 locus will be accomplished by creating a size-selected genomic library of the TY5 strain. The size of the 1000 bp fragment which contains the functional Arg7 gene was determined using Southern Blot analysis. Since the DNA flanking the functional Arg7 gene is part of the  $\gamma$ -6 locus, the isolated fragment of TY5 genomic DNA can be used to screen a genomic library of *C. reinhardtii*. This process will allow the isolation of a complete  $\gamma$ -6 locus, making the sequencing and further analysis of this important locus possible.

It is suspected that the sequencing of the gene will lead to the identification of a regulatory protein important in chlorophyll biosynthesis, specifically affecting the function of protochlorophyllide oxidoreductase. Isolation of this protein may provide significant insight into how the nucleus regulates the chloroplast and the mechanism by which the cell regulates levels of chlorophyll. This information will help our understanding of the complex process of nuclear-chloroplast communication.