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Final Report

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DOE/ER/20185--T1

**REGULATION OF CHLOROPLAST NUMBER AND DNA SYNTHESIS
IN HIGHER PLANTS**

Final Report

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June 17, 1997

**PREPARED FOR THE U.S. DEPARTMENT OF ENERGY
UNDER GRANT NUMBER DE-FG03-95ER20185**

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FINAL REPORT

TITLE: Regulation of Chloroplast Number and DNA Synthesis in Higher Plants
GRANT NO.: DE-FG05-ER14054
PERIOD: 8/95-8/96 plus a no cost extension until 1/97
AGENCY: Department of Energy
P.I.: Dr. John E. Mullet

A. OVERALL OBJECTIVE

The long term objective of this research is to understand the process of chloroplast development and its coordination with leaf development in higher plants. This is important because the photosynthetic capacity of plants is directly related to leaf and chloroplast development. This research focused on obtaining a detailed description of leaf development and the early steps in chloroplast development including activation of plastid DNA synthesis, changes in plastid DNA copy number, activation of chloroplast transcription and increases in plastid number per cell. The research focused on the isolation of the plastid DNA polymerase, and identification of genetic mutants which are altered in their accumulation of plastid DNA and plastid number per cell.

B. SUMMARY OF RESULTS

Objective 1: Isolate the plastid DNA polymerase which increases in activity early in chloroplast development.

The aim of this objective was to isolate the plastid DNA polymerase in order to obtain protein sequence information and antibodies which in turn would allow isolation of the corresponding genes. We have followed the purification procedure described by McKnown and Tewari (9) and Heinhorst et al. (8) with some success. In brief, plastids are isolated from pea plants on Percoll gradients. The intact plastids are lysed in 2% Triton X-100. The mixture of membranes and soluble phase is treated with ammonium sulfate and the DNA polymerase activity is precipitated between 30% and 70%. The precipitate is dialyzed and loaded on a DEAE column and the DNA polymerase is eluted with 150 mM KPO₄. The DNA polymerase fractions are concentrated, loaded on a glycerol gradient and centrifuged. Fractions from the glycerol gradient are loaded on a P-11 column and eluted with a salt gradient. At this point the DNA polymerase has been purified approximately 3000-fold with 10% recovery. One additional purification step will be tested to see if we are approaching homogeneity (heparin sepharose or Mono-S). Once a nearly homogeneous preparation was obtained, we proceeded to antibody preparation and partial sequence analysis. The antibodies were tested for reactivity against the purified DNA polymerase preparation and subsequently used to identify clones expressing the corresponding proteins. The inserts of several clones were sequenced. This analysis indicated that the proteins we isolated were not the DNA polymerase but were most likely activators of DNA synthesis.

Objective 2: Screen mutagenized *Arabidopsis* for mutants varying in plastid number per mesophyll cell.

To begin to understand the molecular mechanisms regulating plastid division and maturation, we screened X-ray deletion and T-DNA tagged *Arabidopsis* lines for mutants

exhibiting altered plastid numbers, chloroplast size and shape. Cells from primary leaves of young mutant seedlings were screened visually under a microscope for changes in chloroplast number and size. Approximately 900 seedlings derived from X-ray treated M2 lines and 1,500 T-DNA tagged seedlings have been screened to date. Two chloroplast division mutants, *cdm1* and *cdm2*, have been characterized so far. *cdm1* has greatly reduced numbers of chloroplasts per mesophyll cell whereas *cdm2* has nearly twice the normal plastid number per cell.

cdm1, derived from a T-DNA tagged line, contains a significantly reduced number of chloroplasts per cell. In primary leaves of the mutant, only one to about five large chloroplasts are apparent in contrast to 40-50 plastids observed in wild type mesophyll cells. Large variations in plastid size are also observed. *cdm1* plants and plastids do not display a morphology similar to any of the arc mutants described by Pyke and coworkers. The plants are pale green compared to their wild type counterparts. Otherwise, all events in the life cycle of *cdm1* plants are similar to wild type.

The genetic basis for the *Cdm1*-phenotype was determined by crossing *cdm1* and wild type lines. This demonstrated that the *cdm1* mutation segregated as a single recessive locus, but independently from the T-DNA insert. A map-based cloning strategy was initiated, employing Bulk Segregant Analysis and RAPDs, to identify the *CDM1* gene. Three RAPD markers were identified that were closely linked to the *CDM1* locus, covering an interval of approximately four cMs. Using the RAPD markers as starting points, a chromosome walk was initiated using a combination of YAC and BAC clones. Two PCR based markers were identified during the walk, mapping to either side of one BAC in close proximity to the *CDM1* locus. The BAC insert was found to be approximately 120 kbp in size.

Sixteen open reading frames were identified on the BAC by random shotgun sequencing. The mRNA from one ORF is expressed in wild type leaves but not the mutant. A 16 bp deletion was identified in the mutant coding region of this ORF, causing a translational shift and premature termination of the encoded protein. This deletion is completely linked with the *CDM1* locus and phenotype. Complementation assays are currently being carried out to determine if this ORF can rescue the mutant phenotype. Other genes with significant similarity to the ORF have not been identified in any species except for ESTs in Arabidopsis and rice. A protein with a low, but significant, level of similarity was identified in cyanobacteria.

Objective 3: Determine if CPFTSZ functions in chloroplast division.

The plant gene, *CPFTSZ* is similar in sequence to the bacterial cell division gene, *ftsZ*. To test if *CPFTSZ* is involved in chloroplast division, plants expressing antisense *CPFTSZ* were constructed. These plants have a uniform decrease in chloroplast number and increase in size. This result confirms a role for *CPFTSZ* in chloroplast division.

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