FINAL REPORT

Project Title - "Post-Transcriptional Regulation of Chloroplast Gene Expression by Nuclear Encoded Gene Products"

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The research supported by this grant sought to understand how proteins required for photosynthesis in green algae and higher plants are made, and to understand how the synthesis of these proteins is regulated. The principal goal of the project was to identify nuclear genes whose products are required for the synthesis of chloroplast-encoded photosynthetic proteins, and to determine how these nuclear gene products function in the organelle. We focused our work on the synthesis of an important photosystem II protein called D2. This protein is required for electron transfer reactions within photosystem II and for oxygen evolution. D2 is encoded by a chloroplast gene called psbD. In the unicellular green alga *Chlamydomonas reinhardtii*, the synthesis of D2 is dependent on the products of at least three nuclear genes. One gene (NAC2) codes for a protein which stabilizes the psbD messenger RNA. Two other nuclear genes (NAC1 and AC115) are required for D2 protein synthesis. The majority of our research focused on these latter two genes. Our results are summarized below.

1. We have isolated and completely sequenced the AC115 gene of the unicellular green alga *Chlamydomonas reinhardtii*. The AC115 gene codes for a protein of 113 amino acids. The protein has at its amino terminal end a sequence which resembles a chloroplast transit signal which could direct it to the chloroplast. The carboxy terminus of the AC115 protein is very hydrophobic. This region may serve either to embed the protein into the thylakoid membranes of the chloroplast or to associate AC115p with other proteins involved in D2 synthesis and/or photosystem II assembly. The genetic lesion in the ac115 mutant was determined to be a nonsense mutation at the fourth codon of the open reading frame. This mutation completely blocks AC115p synthesis, and as a consequence, the D2 protein is not made in the chloroplast. This work is described in publication 2.
2. We have characterized the expression of the D2 protein in cells mutated in the AC115 and NAC1 genes. We find that in mutant cells, the psbD mRNA encoding D2 is present at normal quantities, the message is polysome bound, and is associated with thylakoid membranes, the site of D2 protein synthesis. This indicates that the AC115 and NAC1 gene products are required for D2 synthesis *after* the translation of the psbD message has begun. The AC115 and NAC1 gene products may function in the folding and/or the stabilization of the D2 protein, or they may be involved in the assembly of D2 into the photosystem II complex. The manuscript describing this

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work (number 4) is in preparation.

3. The NAC1 gene was genetically mapped to the short arm of chromosome IX of *Chlamydomonas reinhardtii*. We have isolated DNA sequences closely linked to NAC1 and are using these sequences to clone the gene. Recent success in cloning genes from a BAC library of *Chlamydomonas* genomic DNA is prompting us to follow this approach. This work is ongoing.
4. We have isolated and characterized a bypass suppressor mutation which works to suppress the phenotypes caused by mutations within both the NAC1 and AC115 genes. This gene may encode another protein involved in D2 synthesis. This work is described in reference 1.
5. Together with our collaborators, we have generated a collection of photosynthetic mutant strains of *Chlamydomonas reinhardtii* by an insertional mutagenesis strategy. In several of these mutant strains, the mutated gene is genetically linked to the phenotype attributed by the inserted sequence and so is "tagged" with this sequence and is accessible to cloning. The cloning of these genes is ongoing work. A subset of these mutants was determined to be deficient in the synthesis of a chloroplast-encoded photosystem II protein called D1. Genetic analysis revealed that these mutants identified at least four nuclear loci whose products are required for the translation of the psbA mRNA encoding D1. This work is described in reference 3.

Overall, the work accomplished in this grant period has served to identify a number of nuclear genes of *Chlamydomonas reinhardtii* whose products are needed for the expression of genes within the chloroplast. With these genes in hand, we are poised to understand how their products function in the chloroplast. This will lead to a better understanding of how the proteins involved in photosynthesis are made and how their synthesis is regulated.

Publications Resulting from Research

1. Wu, H. Y. and Kuchka, M. R. (1995) A nuclear suppressor overcomes defects in the synthesis of the chloroplast psbD gene product caused by mutations in two distinct nuclear genes of *Chlamydomonas*. *Current Genetics*, 27: 263-269.
2. Rattanachaikunsopon, P., Rosch, C., and Kuchka, M. R. (1998) Cloning and characterization of the nuclear AC115 gene of *Chlamydomonas reinhardtii*. *Plant Molecular Biology*, in press.
3. Yohn, C. B., Cohen, A., Rosch, C., Kuchka, M. R., and Mayfield, S. P. (1998) Translation of the chloroplast psbA mRNA requires the nuclear encoded poly(A) binding protein RB47. *Journal of Cell Biology*, 142: 435-442.
4. Wu, H. Y., He, R., and Kuchka, M. R. (1998) The nuclear AC115 gene product is involved in the synthesis of the chloroplast-encoded D2 protein of photosystem II after translation initiation. Manuscript in preparation.