

## Final Report

Project Title: *Proteomic Dissection of the Mitochondrial DNA Metabolism Apparatus in Arabidopsis*  
Principal Investigator: Sally A. Mackenzie  
Period of Report: 9/1/03-8/31/04  
Recipient Organization: Beadle Center for Genetics Research, University of Nebraska, Lincoln, NE 68588-0660  
DOE Award Number: DE-FG02-03ER15440  
Unexpended Budget: none

### Project Aims:

1. To functionally characterize genetic components of mitochondrial DNA maintenance in Arabidopsis.
2. To begin investigation of interactions among components of the mitochondrial DNA maintenance apparatus.

### Research Progress:

1. During the first year of this study, investigations were focused primarily on three nuclear-encoded genetic components: the gamma-type DNA polymerase (*AtPolγ*), the *MutS*-related gene *AtMsh1*, and the *RecA*-related gene *AtRecA3*. These genes were selected because they are completely uncharacterized in higher plants, appear to have undergone unique evolutionary paths in plants, and represents components derived from three primary DNA metabolic functions of replication, mismatch repair and recombination.

*AtPolγ*: Two highly homologous loci are identified within the Arabidopsis genome predicted to encode a polymerase of the gamma type characteristic of mitochondrial DNA polymerase. Our long-term goal is to conduct mutational analysis of the organellar DNA polymerase in order to create a series of mitochondrial DNA point mutations for investigation of mitochondrial influence on overall plant growth and development. However, in our first year, we have focused our attention on characterizing the subcellular localization and expression properties of the two encoded forms of organellar polymerase, AtPolγ1 and AtPolγ2.

Detailed analysis suggests that At Polγ1 is dual targeted to mitochondrial and chloroplasts, with targeting conferred by sequences located immediately following the initiator MET. AtPolγ2 is also dual targeted, but appears to initiate translation upstream to the annotated initiator MET. Extensive truncation, deletion and site mutation analysis has allowed us to deduce a non-AUG start of translation and to locate distinct mitochondrial and chloroplast-targeting domains, implying that the original gene may have encoded a strictly plastid-targeting form that later acquired mitochondrial targeting capacity by relaxing translation initiation to permit initiation further upstream by approximately 29 amino acids. A similar organization appears to exist in rice, implying that this dual-domain, dual targeting feature has been conserved. We have detailed these findings for publication. The following manuscript was submitted to Plant Cell for publication. Revisions were requested following review, and the revised manuscript is pending.

Christensen, A., Lyznik, A., Elo, A., Yule, R., Mohammed, S., Elowsky, C., Huo, G., Mackenzie, S. Dual Domain, dual targeting organellar protein presequences within higher plants may utilize non-AUG start codons. Manuscript submitted.

*AtMsh1*. A novel feature of the plant mitochondrial genome is its rapid and reproducible rearrangement in response to nuclear genotype. The process, termed substoichiometric shifting,

results in the dramatic suppression or amplification of particular portions of the mitochondrial genome in response to alterations in nuclear gene expression. In the preceeding DOE-sponsored grant period, we cloned a nuclear gene that appears to directly influence mitochondrial substoichiometric shifting (Abdelnoor et al. 2003). The AtMSH1 gene, when mutated, results in a reproducible amplification of a portion of the mitochondrial genome, together with the appearance of green-white variegation of the mutant plant.

During the past year, ongoing characterization of the MSH1 locus in Arabidopsis, a single gene, has allowed us to arrive at three important conclusions. (1) The MSH1 locus encodes a highly conserved protein present throughout the plant kingdom, but apparently absent from mammalian and fungal lineages. A distantly related mitochondrial MSH1 form exists (Reenan and Kolodner, 1992), but shares less than 17% amino acid identity with the plant form. (2) Secondly, the plant MSH1 protein contains six conserved domains, including a DNA binding domain, an ATP binding and hydrolysis domain, and a domain that is not found in any mismatch repair component identified previously (Figure 1). This domain, designated domain VI, encodes a GIY-YIG endonuclease motif (Figure 2). Of the 11 plant species (barley, maize, wheat, tomato, potato, bean, soybean, tobacco, Arabidopsis, millet, rice) from which the MSH1 gene sequence has been identified to date, all contain the six domains. Most significant about this observation is the fact that one other example of a 3' endonuclease domain associate with a mismatch repair-related protein has been reported. In the coral genus *Sarcophyton* a distinct form of endonuclease, containing the HNH motif, was found associated with a mismatch repair component encoded within the mitochondrial genome (Pont-Kingdon et al. 1998). No other animal lineage outside of the corals has been found to have either a mitochondrial encoded mismatch repair component, or a mismatch repair component fused to an endonuclease domain at the COOH end of the protein.

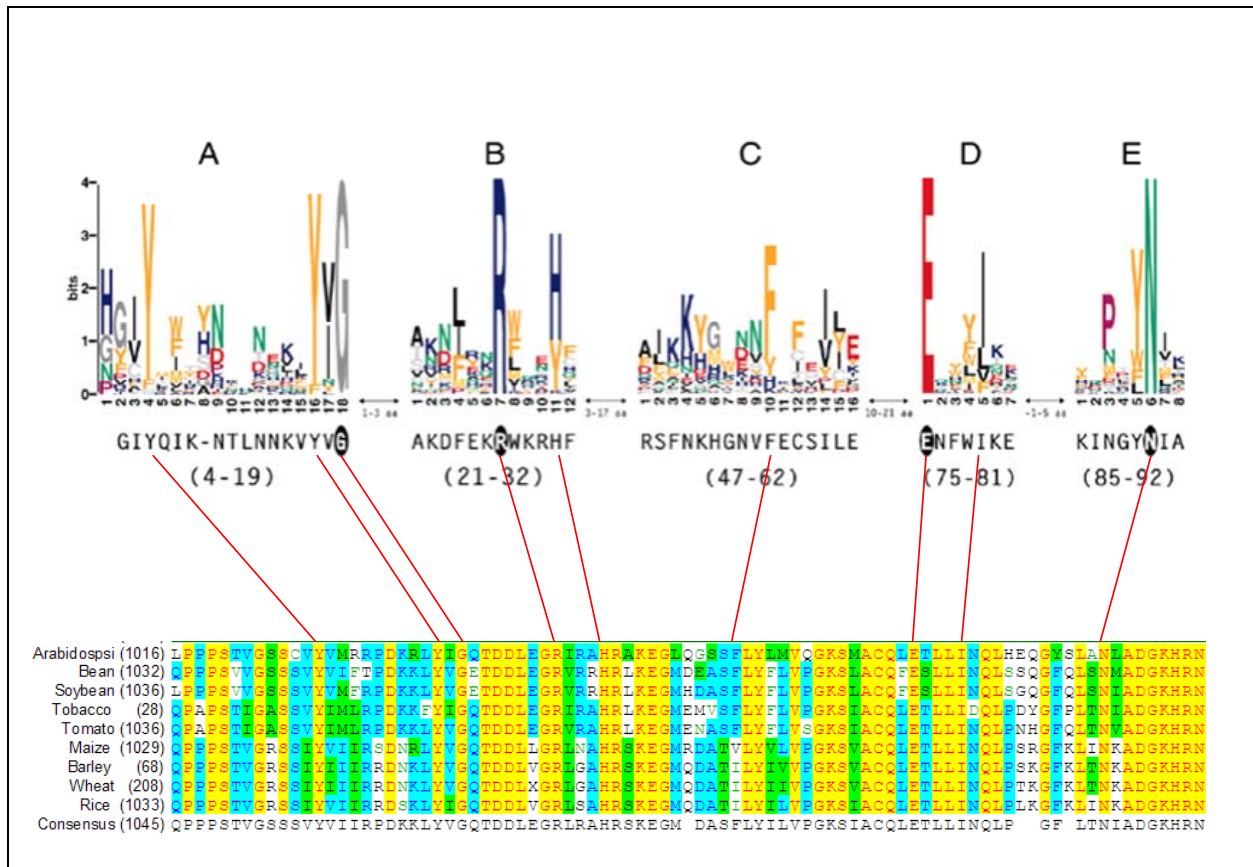
We postulate that this "coincidence" linking distinct animal and plant lineages is the consequence of convergent evolution. We suggest that the driving force for the convergent pattern is an adaptation provided by the novel mitochondrial MSH1-endonuclease gene functioning to expand reproductive capacity to the organism. Both coral and plants represent lineages of sessile organisms that, as a consequence, have established some form of sexual dioecy. In the case of plants, gynodioecy is often established to permit both self- and cross-pollination within a single species. The dynamic equilibrium between these two reproductive forms is maintained by induction of male sterility, often cytoplasmically conferred. In nature, mitochondrial substoichiometric shifting permits the cytoplasmic male sterile plant to undergo spontaneous reversion to fertility, re-establishing the hermaphrodite condition (Arrieta-Montiel et al. 2001). Substoichiometric shifting activity is regulated by the nuclear gene MSH1.

Likewise in the sessile coral *Sarcophyton*, both self- and cross-fertilization is effected by sexual dimorphism, again in a dynamic equilibrium. We suggest that this sexual dimorphism is likewise influenced by the MSH1 locus and mitochondrial influence on male and/or female fertility. Data from this study are being prepared for manuscript submission. More detailed function analysis of MSH1 is the focus of a currently funded DOE grant to our laboratory.

Abdelnoor, R., Christensen, A., Moriyama, H., Mackenzie, S. Evidence of convergent evolution in animal and plant mitochondrial mismatch repair components. Manuscript in preparation.

**Figure 1. MSH1 alignments from six plant species reveals six distinct gene domains.**





**Figure 2. The GIY-YIG domain (VI) of the Arabidopsis MSH1 gene.** Multiple alignment of the C-terminal domain of MSH1 proteins from *Arabidopsis*, common bean, soybean, tobacco (EST), tomato, maize, barley (EST), wheat (EST) and rice. The conserved residues of the domain are identified by red line. The top part of the figure is an insert representing the conserved motifs of the GIY-YIG domain based on the alignment of several protein sequences containing this domain. The height of each amino acid is proportional to its conservation at that position (Kowalski et al., 1999). Beneath each motif is the I-TevI sequence.

**RecA.** Three gene forms demonstrating significant homology to bacterial RecA are present within the Arabidopsis genome. We have designated these RecA1, RecA2 and RecA3. The product of RecA1 was reported by another group to target strictly to plastids (Cerutti et al. 1992). We have confirmed this report, and have found that the products of RecA2 and RecA3 target dually and to mitochondria, respectively. Mutational analysis reveals that only loss of RecA2 is lethal, implying that it is this gene that is involved in the essential recombination functions of both organelles. Intriguingly, mutation of RecA3 results in the identical mitochondrial substoichiometric shifting observed with mutation of MSH1, suggesting that the two gene products interact functionally. Secondly, loss of RecA3 function does not result in leaf variegation, suggesting that substoichiometric shifting and the variegation phenotype associated with *msh1* mutants are distinct phenomena. Finally, RecA3 appears to be truncated at its COOH end relative to other RecA genes, and this appears to be the case for the RecA3 form in rice as well, suggesting novel adaptation of the gene for its specialized function. Currently we are in the process of testing for protein:protein interaction between RecA3 and MSH1, and are attempting to identify additional components of this putative complex.

**References cited:**

- Abdelnoor, R.V., Yule, R., Elo, A., Christensen, A.C., Meyer-Gauen, G., and Mackenzie, S.A. (2003). Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. *Proc Natl Acad Sci U S A* 100, 5968
- Arrieta-Montiel, M., Lyznik, A., Woloszynska, M., Janska, H., Tohme, J., and Mackenzie, S. (2001). Tracing evolutionary and developmental implications of mitochondrial stoichiometric shifting in the common bean. *Genetics* 158, 851-64.
- Cerutti, H., Osman, M., Grandoni, P., and Jagendorf, A. (1992). A Homolog of Escherichia coli RecA Protein in Plastids of Higher Plants. *PNAS* 89, 8068-8072.
- Kowalski, J., Belfort, M., Stapleton, M., Holpert, M., Dansereau, J., Pietrokovski, S., Baxter, S., and Derbyshire, V. (1999). Configuration of the catalytic GIY-YIG domain of intron endonuclease I-TevI: coincidence of computational and molecular findings. *Nucl. Acids. Res.* 27, 2115-2125.
- Mackenzie, S.A. (2004). The mitochondrial genome of higher plants: A target for natural adaptation. In *Plant Diversity*. R. Henry, ed. (CAB Publ), pp. in press
- Pont-Kingdon, G., Okada, N.A., Macfarlane, J.L., Beagley, C.T., Watkins-Sims, C.D., Cavalier-Smith, T., Clark-Walker, G.D., and Wolstenholme, D.R. (1998). Mitochondrial DNA of the coral *Sarcophyton glaucum* contains a gene for a homologue of bacterial MutS: a possible case of gene transfer from the nucleus to the mitochondrion. *J Mol Evol* 46, 419-31.
- Reenan, R.A. and Kolodner, R.D. (1992). Characterization of insertion mutations in the *Saccharomyces cerevisiae* MSH1 and MSH2 genes: evidence for separate mitochondrial and nuclear functions. *Genetics* 132, 975-85.