"Genomic studies of chimeric mitochondria in cybrids from Solanaceae" 2009.

Project Narrative

Genome rearrangements, nucleotide substitutions, and the introduction of foreign DNA shape the mitochondrial genomes of eukaryotes and often have severe consequences for human health, as evidenced by several important mitochondrially-inherited diseases. In addition, the key experimental organism of this study (tobacco) is widely used as an expression system for biopharmaceutical production of vaccines, antibiotics, and a number of therapeutic proteins. Understanding the molecular mechanisms of mitochondrial DNA evolution is therefore of considerable importance to human health and disease.

Project Summary

The study of mitochondrial genome evolution, dynamics, uptake of foreign DNA, and interactions with the nuclear genome is essential to a deep understanding of the eukaryotic cell. Mitochondrial genomes provide an excellent system to increase our knowledge of genome rearrangements, chimeric genes, nuclear-cytoplasmic incompatibilities, and horizontal gene transfer (HGT). Plant mitochondria are unique in their propensity to acquire genes by HGT. The most pervasive example of HGT in eukaryotes involves the *cox1* intron, which encodes a putative homing endonuclease that increases the frequency of the intron's fixation via horizontal transfer. We propose to study aspects of the interactions between two distinct mitochondrial genomes that recombine in a cybrid plant obtained by protoplast fusion experiments. During cybrid formation, the two mitochondrial parental types and their genomes (only one containing the *cox1* intron) will fuse, resulting in a hybrid mitochondrial genome. We will establish some 20 cybrid lines derived from somatic crosses between *cox1* intron-containing and -lacking species in order to address the following two aims: First, we will test the hypothesis that the *cox1* intron encodes a functional homing endonuclease in plants, assess rates of intron colonization, and measure lengths of exonic coconversion tracts that accompany intron insertion. Second, we will sequence and analyze the entire mitochondrial genomes of up to 20 cybrid lines. This will serve as an experimental model for recapitulating the natural process of HGT in plant mitochondria and will provide a rich picture of the process and pattern of mitochondrial genome recombination at various levels. Maintenance of an intact, functioning mitochondrial genome is critical for survival. Given the many molecular parallels between plants and animals, this project will be valuable for the development of both animal gene therapy and plant genetic engineering.

Specific Aims:

Horizontal gene transfer (HGT), the exchange of genetic material between more or less distantly related, “non-mating” organisms, is widespread in prokaryotes and, to a lesser extent, unicellular eukaryotes. Over the past few years, however, HGT involving multicellular eukaryotes has been increasingly reported. Most notably, plant mitochondria stand out for their exceptionally high rates of HGT. HGT in plant mitochondria often results in the presence of two intact copies of a particular mitochondrial gene, one native and one foreign copy. Chimeric genes formed by recombination of native and foreign copies of a mitochondrial gene have also been described. Little is known about the expression and functional implications of these horizontally transferred genes.

The most frequently documented case of HGT in eukaryotes involves an intron present in the mitochondrial *cox1* of flowering plants (angiosperms). This group I intron was originally transferred to angiosperms from a fungal donor and subsequently spread among many diverse angiosperms via hundreds if not thousands of plant-to-plant transfer events. Group I introns often encode a family of homologous proteins that are site-specific DNA endonucleases and which facilitate intron propagation (Figure 1). The high mobility of these “homing” introns is promoted by the intron-encoded homing endonuclease, which catalyzes the integration of the intron, via the double-strand-break-repair pathway, into its target, cleavage sequence (termed the “homing site”) that is present in intron-lacking alleles of the intron’s target gene. The remarkably high frequency of horizontal transfer of the *cox1* intron in plants, and the presence of an intact homing endonuclease-like reading frame in almost all plant *cox1* introns, predict that the plant *cox1* intron encodes an active homing endonuclease, but there is no direct, genetic or biochemical evidence to support this inference.

The overall goal of this proposal is develop the first genetic, experimental system in which to investigate aspects of HGT in plant mitochondria. In the absence of any system for directly transforming plant mitochondrial genomes, we will instead take advantage of the well-established fact that somatic hybrid (cybrid) plants invariably contain recombinant mitochondrial genomes resulting from the fusion of parental mitochondria following protoplast fusion. We will use these cybrid plants to:

1) Test the hypothesis that the *cox1* intron encodes a functional homing endonuclease in plants, assess rates of intron colonization, and measure lengths of exonic coconversion tracts that accompany intron insertion.

2) Sequence and analyze the entire mitochondrial genomes of up to 20 cybrid (somatic hybrid) lines. This will serve as an experimental model for recapitulating the natural process of HGT in plant mitochondria and will provide a rich picture of the process and pattern of mitochondrial genome recombination at various levels.