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Higher Plant Mitochondria

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INTRODUCTION

Over the past 20 years, researchers investigating the mitochondria of plants have been astonished by the phenomenal variation these organelles display relative to their mammalian and fungal counterparts. Plant mitochondria have evolved distinct strategies for genome maintenance, genetic decoding, gene regulation, and organelle segregation. Their physiological and biochemical functions have similarly evolved to meet the specific demands of photosynthetic organisms "rooted" in place. Unfortunately, making sense of the great number of variations inherent to plant mitochondria has been a slow process. This has been made more difficult by the fact that geneticists and biochemists have traditionally formed two distinct and often poorly communicating research groups in mitochondrial biology. The productive merging of these two bodies of information has begun only recently. With this review, we attempt to provide perspective to the recent developments in this field and their implications for our understanding of organellar biogenesis and mitochondrial integration into whole-plant physiology.

Mitochondrial genomes encode only a fraction of the genetic information required for their biogenesis and function; the vast majority is nuclear derived. Consequently, it can be assumed that the large number of unique genetic and biochemical features displayed in plant mitochondria arose in the context of a nuclear–mitochondrial coevolution particular to the plant kingdom. Plant mitochondria are compelled to coordinate gene functions with other organelles, including plastids. Likewise, tissues demanding high rates of metabolism during reproduction and fruiting, or in the case of nitrogen fixation, requiring low oxygen concentrations, represent processes peculiar to plants. Due to an inability to mobilize so as to avoid environmental stresses, plants have evolved unique adaptations to stress, some of which involve the mitochondrion. For some species, these unusual evolutionary demands may have been exacerbated by thousands of years of genetic manipulation by breeders. Given this perspective, it is not so surprising that nuclear–mitochondrial interactions within the plant kingdom are highly specialized and unusual.

Organelles communicate by means of essential polypeptides and bidirectional information flow, allowing for organogenesis and responses to the environment. In plants, regulatory models from bacterial energy transduction have been extended to photosynthesis in plastids (Allen, 1993; Allen et al., 1995), including regulation by redox poise (Escoubas et al., 1995) and translation of the chloroplast-encoded proteins (Danon and Mayfield, 1994; Yohn et al., 1996). Similarly, yeast has served as an excellent model system for nuclear–mitochondrial interaction (reviewed in Poyton and McEwen, 1996). In studies concerning plant mitochondrial–nuclear interaction—where a third powerful organelle, the chloroplast, is present—we have basic genetic paradigms such as cytoplasmic male sterility (CMS), nonchromosomal stripe mutations, and nuclear mutations affecting heritable phenotypes. However, a dynamic model for plant mitochondrial–nuclear interaction, one differentially responding to environmental and growth challenges, has not risen above a rudimentary level. Such model systems will likely be crucial to the in-depth investigation of mitochondrial integration with overall plant cellular processes.

In this review, we describe the current understanding of specialized genetic and biochemical features unique to plant mitochondria. We also address the more speculative but exciting aspects of interorganellar interaction, namely, the recent efforts to identify molecules mediating nuclear–mitochondrial and plastid–mitochondrial communication.

THE PLANT MITOCHONDRIAL GENOME

Mitochondrial genome structure and size are more highly variable within the plant kingdom than in most other eukaryotes (reviewed in Wolstenholme and Fauron, 1995). Recently, marked progress has been made in our interpretation of this unusual variation in plants. One important advance came with the sequencing of the entire mitochondrial genomes of liverwort (Oda et al., 1992) and Arabidopsis (Unseld et al., 1997). With these genomes elaborated, it is clear that much of the size variation can be accounted for by

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coding redundancy and changes in genome structure brought about by high levels of recombination and extraneous DNA integration.

Recombinationally active repeated sequences are present within the mitochondrial genomes of nearly all plant species examined, and in direct orientation, they subdivide the genome into a number of different, highly redundant, subgenomic molecules. A second class of repeat, much smaller in size and seldom active, can effect recombinations intragenically, resulting in novel open reading frames (reviewed in Andre et al., 1992; Vedel et al., 1994).

Much of the data available regarding the mitochondrial genome in plants is derived from physical mapping efforts. The predicted physical structures, previously assumed to be circular in form, have yet to be confirmed. In fact, contrary to the model of a circular genome replicating bidirectionally via theta structures in mammalian systems, recent evidence suggests that plant mitochondrial genomes may replicate by a rolling circle mechanism (Backert et al., 1996, 1997) inasmuch as they exist to a large extent as linear and branched molecules (Bendich, 1993; Oldenburg and Bendich, 1996, 1998).

The unusual recombination activity detected in plant mitochondria surely adds to the complexity of genome structure in plants, but what biological advantage does it serve? Subgenomic DNA molecules can be maintained at unusually low copy number, far fewer than one copy per cell (Small et al., 1987). This apparent genomic heterogeneity may relate to the detection of different phenotypic subpopulations within plant cells (Dai et al., 1998). It has been suggested that the subdividing of the genome, and subsequent differential distribution of the distinct forms, might provide the organelle with a genetic advantage in maintaining variation (Small et al., 1989). Substoichiometric retention of mitochondrial DNA molecules is widely reported in plants (Bonhomme et al., 1992; Kanazawa et al., 1994; Yesodi et al., 1995; Suzuki et al., 1996; Gutierres et al., 1997; Janska et al., 1998), and incidents of apparently spontaneous genomic rearrangements in vitro (Vitart et al., 1992; Kanazawa et al., 1994) and in vivo (Janska et al., 1998) have been attributed to the ability of these molecules to undergo sudden changes in copy number. Furthermore, suppression of copy number can apparently result in the effective silencing of encoded genes (Laser et al., 1997; Janska et al., 1998).

Copy number regulation and transmission of the mitochondrial genome is, no doubt, under nuclear control. This assertion is supported not only by work in yeast (Zweifel and Fangman, 1991; Piskur, 1994; Lockshon et al., 1995) but by the identification of two plant nuclear genes, *CHM* in Arabidopsis and *Fr* in common bean, that influence the copy number/transmission of particular mitochondrial DNA molecules. In both cases, the target molecules contain mutations that affect plant phenotype (Mackenzie and Chase, 1990; Martinez-Zapater et al., 1992; Sakamoto et al., 1996; Janska et al., 1998), and in the case of bean, the copy number suppression appears reversible (Janska et al., 1998).

With regard to the coding capacity of the plant mitochondrial genome, 57 genes have been identified in Arabidopsis to encode components of complexes I to V and cytochrome c biogenesis, rRNAs, ribosomal proteins, tRNAs, and a few additional open reading frames (Unseld et al., 1997). Interorganellar DNA exchanges involving the mitochondrion appear to be common. In recent times, evolutionarily speaking, the plant mitochondrial genome has been targeted for horizontal transfer of a single group I intron sequence (Adams et al., 1998; Cho et al., 1998). Moreover, within given plant families, it has been persuasively demonstrated that individual plant species likely represent evolutionary intermediates in an ongoing process of gene transfer from the mitochondrion to the nucleus (Brennicke et al., 1993; Gray, 1995). Such gene transfer apparently occurs via RNA intermediates, presumably a vestige of earlier endosymbiotic processes. If this is the case, how would a newly introduced nuclear form of a gene then derive a means of transferring its product back to the mitochondrion? Analysis of "recently" transferred mitochondrial genes within the nucleus of rice has established the integration of introduced genes at duplicated sites already encoding mitochondrial proteins to allow, essentially, the requisitioning of the previous transit sequence (Kadowaki et al., 1996).

PLANT MITOCHONDRIAL GENE EXPRESSION

Transcription

A curious feature of gene expression particular to plant mitochondria is the complex pattern of transcripts arising from a given mitochondrial gene-coding region. Variation in transcript size arises from multiple transcription initiation and termination sites as well as post-transcriptional cleavage and splicing (reviewed in Gray et al., 1992). Plant mitochondrial transcription is mediated by at least one nuclear-encoded RNA polymerase that bears striking similarity to the RNA polymerases of bacteriophages T7, T3, and SP6 (Hedtke et al., 1997). Presumably, this similarity represents a feature acquired by plant mitochondria subsequent to the endosymbiotic event (Gray and Lang, 1998). Essential features of mitochondrial promoters have been identified for specific genes in both monocot (Hanic-Joyce and Gray, 1991; Rapp and Stern, 1992; Rapp et al., 1993) and dicot (Binder and Brennicke, 1993; Binder et al., 1995) species; however, a truly conserved promoter consensus sequence has not emerged. Rather, it appears that different types of promoters might exist for particular genes or groups of genes, perhaps requiring their own specificity factors. In this regard, a single nuclear gene designated *Mct* has been identified to influence promoter selection upstream to the mitochondrial cytochrome oxidase subunit II (*coxII*) gene in a maize alloplasmic line (Cooper et al., 1990; Newton et al.,

1995). Recent approaches to characterize DNA binding proteins associated with transcription initiation offer promise for the elucidation of such specificity factors (Hatzack et al., 1998).

Transcriptional modulation does not appear to represent the primary means of gene regulation in plant mitochondria, although evidence exists to suggest tissue-specific differences in transcript levels for particular loci. In in situ hybridization studies of maize seedling tissues, particular mitochondrial transcripts are detected at different levels, depending on tissue type (Li et al., 1996). Likewise, studies in developing anthers of sunflower demonstrate a marked accumulation of *atpA*, *atp9*, *cob*, and *rrn26* transcripts in young meiotic cells with a concomitant increase in their respective protein products (Smart et al., 1994).

Unlike yeast and mammalian systems, plant mitochondrial genomes have a tendency to accumulate dominant mitochondrial mutations as a consequence of intragenic recombination events (Bonen and Brown, 1993). Several such mutations have been associated with pollen sterility (Hanson, 1991; Schnable and Wise, 1998), although many others appear to have no phenotypic consequences (Marienfeld et al., 1997). These transcriptionally active, chimeric mutations generally share 5′ promoter regions in common with their wild-type counterparts. It is perhaps in response to this circumstance that several unique post-transcriptional means of suppressing gene activity have evolved in plants.

Transcript Processing

Nuclear-directed mitochondrial transcript processing (reviewed in Gray et al., 1992) apparently represents an effective means of gene regulation in plant mitochondria. In fact, several distinct nuclear fertility restorer loci, identified based on their ability to suppress the sterility phenotype in CMS mutant lines, have been shown to directly influence transcript processing within mitochondrial CMS-associated regions.

In CMS-T maize, a well-investigated example, restoration of fertility is effected by two dominant nuclear loci, *Rf1* and *Rf2* (Duvick, 1965; Laughnan and Gabay-Laughnan, 1983). The product of *Rf1*, essential although not sufficient to restore fertility, appears to promote transcript splicing of the T-*urf13* mitochondrial region (Dewey et al., 1987; Kennell et al., 1987; Wise et al., 1996). The degree of T-*urf13* transcript splicing correlates with a dramatic reduction in the corresponding 13-kD T-URF13 polypeptide (Forde et al., 1978; Dewey et al., 1987). In sorghum line IS1112C, CMS may be caused by the expression of an open reading frame designated *orf107* (Tang et al., 1996). Again, restoration of fertility occurs with the internal splicing of *orf107* transcripts and a concomitant reduction in a 12-kD polypeptide presumed to be the product of this gene (Tang et al., 1996). Interestingly, the transcript processing sites described in both CMS-T maize and CMS sorghum share sequence features (Dill et al., 1997), implying that particular sequence motifs within plant mitochondrial genes can serve as targets for nuclear-directed gene modulation.

This suggested means of gene regulation is further extended by the CMS system in the oilseed rape *Polima* cytoplasm. In this case, CMS is associated with the expression of a sequence nearby to ATPase subunit 6 (*atp6*) (Singh and Brown, 1991). Suppression of the sterility phenotype accompanies transcript splicing of the sterility-associated sequence that cosegregates with a single dominant nuclear locus, *Rfp1* (Singh et al., 1996). The alternate allele at this locus, *rfp1*, or a second locus tightly linked to *rfp1*, promotes transcript processing of two additional, unrelated mitochondrial genes, *nad4* and a *ccl1*-like gene. At all four processing sites associated with *Rfp1* or *rfp1* activity, a similar sequence motif, UUGUGG or UUGUUG, was located very near to the site of splicing. This sequence does not bear obvious similarity to that reported in the maize and sorghum examples. However, these observations, taken together, suggest that sequence motifs that influence the splicing process are recognizable and should facilitate the biochemical characterization of the process involved.

Transcript Editing

Observed most often as C to U conversions, post-transcriptional editing occurs in nearly all plant mitochondrial transcripts. Although its role in gene regulation remains unclear, the incidence and biochemical features of this process are well described (Smith et al., 1997). Evidence to date suggests that features of the local editing site are important (e.g., Covello and Gray, 1990; Gualberto et al., 1990; Wilson and Hanson, 1996; Williams et al., 1998), as well as nuclear genotype (Lu and Hanson, 1992). Furthermore, some editing events may be cell-type specific (Howad and Kemken, 1997).

An intriguing feature of the editing process is that transcripts for a given gene are not all fully edited at the same rate. The extent of transcript editing is significantly influenced by plant tissue type, developmental stage, and growth conditions (Grosskopf and Mulligan, 1996). Consequently, for a particular gene, one observes both fully edited and partially edited transcripts within a cell type. How, then, does the translational apparatus distinguish between these two transcript forms? It appears that both edited and unedited transcripts are translationally competent, as was first suggested upon observation of both edited and partially edited transcripts within the polysomal fractions of mitochondrial mRNAs (Gualberto et al., 1988; Lu and Hanson, 1996). Recent studies using specific antibodies confirm that polypeptides are produced from partially edited and unedited transcripts, as well as edited forms, in both petunia and maize mitochondria (Lu et al., 1996; Phreaner et al., 1996). In the case of a ribosomal protein gene, however, the aberrant polypeptides arising from unedited or partially

edited transcripts are unassembled and are not incorporated to functional ribosomes (Phreaner et al., 1996).

Post-Translational Regulation

The detected translational activity of unedited or partially edited transcripts implies a crucial role for post-translational regulation in the management of aberrant gene products. Similarly, this role is implied from the large number of translationally active transcripts of chimeric gene mutations existing within the plant genome. Unfortunately, relatively little information is currently available regarding plant mitochondrial proteolysis.

To date, mitochondrial proteases have been characterized best in yeast, in which several proteases, both matrix and membrane localized, are described (reviewed in Rep and Grivell, 1996). Aside from proteases that participate in proteolytic processing (Szigyarto et al., 1998), no protease activity has been detected in the matrix of plant mitochondria. However, limited activity is detected within the inner membrane, and there is some indication that this activity may be involved in the proteolysis of unassembled, imported proteins (Knorpp et al., 1995).

In the CMS system of common bean, a convincing argument can be made for the post-translational regulation in vegetative tissues of the sterility-associated mitochondrial protein ORF239. Mitochondria isolated from young seedling tissues of the male-sterile bean line produce the ORF239 protein only when incubated in the presence of protease inhibitors (Sarria et al., 1998). In maize (Barakat et al., 1998) and Arabidopsis (Sarria et al., 1998), nuclear genes bearing striking homology to the mitochondrial *lon* homolog of yeast (Suzuki et al., 1994; Van Dyck et al., 1994) and human (Wang et al., 1993) have been cloned. In yeast, this matrix-localized, serine-type protease is known to serve functions essential to the organelle (Suzuki et al., 1994; Van Dyck et al., 1994). The plant LON protease, located on the inner mitochondrial membrane, also demonstrates proteolytic activity characteristic of a serine-type protease and appears to be involved in post-translational turnover of an aberrant protein (Sarria et al., 1998).

A second nuclear-directed process for regulating mitochondrial protein function is likely by phosphorylation. In mammals, several mitochondrial proteins, including a complex I subunit (Papa et al., 1996) and the cytochrome c oxidase subunit IV (Steenaart and Shore, 1997), have been shown to be phosphorylated by endogenous kinases, presumably nuclear encoded. In plants, the mitochondrial HSP70 demonstrates calcium-stimulated autophosphorylation (Vidal et al., 1993), and two subunits of the F_0F_1-ATPase on the inner mitochondrial membrane are shown to be phosphorylated (Struglics et al., 1998). The role of this observed phosphorylation is not yet defined, but the detected activity of several kinases within mitochondria suggests that it is significant for interorganellar regulation.

Mitochondrial Protein Import in Plants

The primary work toward formal genetic dissection of the components involved in mitochondrial protein import has been performed elegantly in yeast and *Neurospora* systems over several years (Pfanner et al., 1994; Lithgow et al., 1995; Schatz and Dobberstein, 1996). Although the same genetic approaches have not been feasible in plant systems, it has been possible to demonstrate several of the corresponding processes in plant cells. An in-depth review of protein import into plant mitochondria can be found in Whelan and Glaser (1997). Here, we point out some of the features recently identified to be distinct in plants.

Over 80 presequences required to direct proteins from the cytosol to the mitochondrion have been reported in plants, and features of these have been summarized elsewhere (Whelan and Glaser, 1997). In common with those presequences identified in other eukaryotes, most transit peptides for plant proteins have the potential to form amphiphilic alpha helices, with the N-terminal portion of the transit peptide indispensable for proper mitochondrial targeting. Unlike the case in fungal systems, plant cytosolic proteins destined for the mitochondrion must be distinguished from those destined for the plastid. The means for this discrimination is not completely clear, although it is likely a function of the transit peptide (Whelan et al., 1990; see also Keegstra and Cline, 1999, in this issue).

Several of the components of the plant mitochondrial import machinery appear to represent functional homologs to those identified in fungi (Perryman et al., 1995; Heins and Schmitz, 1996). One distinguishing factor in plant protein import is the mitochondrial processing peptidase (MPP), responsible for removing the presequence upon import via proteolytic cleavage. Unlike in fungal systems, in which the majority of the processing activity is detected within the matrix, most of the MPP activity in plant systems cofractionates with the cytochrome bc_1 complex of the respiratory chain. This complex is located within the inner membrane and demonstrates a dual role in processing imported proteins and electron transport (Eriksson et al., 1994, 1996; Braun and Schmitz, 1995). Recent investigations have shown a second source of MPP activity, with metalloprotease features similar to its membrane-bound counterpart, located within the matrix (Szigyarto et al., 1998).

As in fungal protein import, cytosolic chaperones play an essential role in the plant import process. That HSP70 is localized on the outer mitochondrial membrane has been shown in various plant species (Mooney and Harmey, 1996). Curiously, the purification of respiratory-competent mitochondria from plant cells does not ensure import competence in vitro. The efficiency of import using isolated tobacco mitochondria is largely dependent on the timing of tissue harvest; mitochondria from leaves harvested during the dark period of the growth cycle produce more efficient import than do light-harvested samples (Dessi and Whelan, 1997). Moreover, levels of mitochondrial HSP70 protein de-

cline as the plant ages, and this decline is concomitant with the decrease in mitochondrial protein import in mature plant tissues (Dudley et al., 1997). Presumably, mitochondrial protein import in plants is not a constitutive process.

The plant mitochondrial genomes investigated all contain an incomplete set of tRNA genes; the balance is nuclear encoded and imported into the mitochondrion. It has been established that tRNAs are imported in association with their corresponding aminoacyl tRNA synthases (Dietrich et al., 1996), although it is not clear what features distinguish those tRNAs that are to be targeted from those that will function in the cytoplasm (Ramamonjisoa et al., 1998).

Recently, attention has been drawn to the possibility that mitochondria might also export macromolecules. This speculation arises from a few important observations. First, mitochondria catalyze the final step in heme biosynthesis via a nuclear-derived protoporphyrinogen IX oxidase (Lermontova et al., 1997). This oxidative reaction is thought to occur in the mitochondrial matrix. Proper assembly to c-type cytochromes, however, requires that heme be exported to the intermembrane space (Goldman et al., 1998). Over the past few years, evidence of an ABC-type transporter within the mitochondria of fungi (Leighton and Schatz, 1995) and plants (Bonnard and Grienenberger, 1995) has accumulated. This evidence, as yet limited in plants, is suggestive of active export.

In CMS of common bean plants, sterility is associated not only with the expression of ORF239, the mitochondrial protein discussed above, but also the aberrant deposition of callose on the wall of the pollen mother cell (Abad et al., 1995). Immunocytological examinations of pollen development in this CMS mutant, using anti-ORF239 antibodies, show a surprisingly large amount of the ORF239 protein to reside within the callose layer, implying transport of this mitochondrial protein to the periphery of the cell. The mechanism of transport is not known.

UNIQUE BIOCHEMICAL FUNCTIONS IN PLANT MITOCHONDRIA

Our general understanding of mitochondria is as organelles in mammalian cells that produce cellular ATP through an electron transport chain containing four respiratory complexes. These four complexes are depicted in Figure 1: complex I, NADH-dehydrogenase; complex II, succinate dehydrogenase; complex III, bc_1; and complex IV, cytochrome oxidase. Mitochondria produce carbon dioxide through the tricarboxylic acid (TCA) cycle as well as cellular biosynthetic substrates. By way of contrast, plant mitochondria exist in cells/organisms that (1) contain chloroplasts, thus producing ATP and synthesizing a large portion of their own respiratory substrates; (2) lack the ability to escape many environmental stresses; (3) produce a wealth of primary and secondary metabolites, some in response to specific stresses, all of which require carbon skeletons; and (4) photorespire. To meet these novel demands and through little-understood mechanisms, plant mitochondria have evolved to function in dramatic contrast to their nonphotosynthetic counterparts.

Function of a Second Plant Respiratory Pathway for Metabolic Flexibility

The mitochondria of plants (along with those of some protists, fungi, and algae) possess an alternative respiratory pathway composed of a single terminal oxidase (Vanlerberghe and McIntosh, 1997). One key to understanding this pathway comes in learning that it is not linked to a proton gradient, does not produce ATP, and as such, serves a regulatory function. Why? Our best understanding, originally described as the overflow hypothesis (Lambers, 1982), is that the normal cytochrome respiratory pathway can become saturated, causing increased ratios of ATP/ADP and NADH/NAD^+. In turn, the TCA cycle slows, limiting the number of carbon skeletons produced. Alternative oxidase can be thought of as a clutch that, when depressed, allows the TCA cycle to "spin off" carbon skeletons. The regulation of alternative pathway expression fits this hypothesis inasmuch as it is upregulated, in general, through many types of stress, possibly indicating the need for increased biosynthesis of stress-related compounds. These stresses include cold, pathogen attack, drought, and wounding. The hypothesis that increased alternative pathway activity allows increased carbon flow is difficult to prove, but the question is being addressed through the use of transgenic plants with altered levels of the alternative oxidase.

Animal cells use the TCA cycle primarily to catabolize the breakdown products of proteins, lipids, and carbohydrates. Plants produce many of their own substrates and accumulate higher concentrations of organic acids siphoned off from the TCA cycle to be employed in anabolic processes (Ap Rees et al., 1983; Hill, 1997). The TCA cycle in plants operates, in some part, as a shuttle for carbon skeletons upon demand. This conclusion has been supported by data illustrating the low activities of enzymes for the decarboxylative portion of the cycle (isocitrate dehydrogenase through α-ketoglutarate dehydrogenase) relative to the remaining reductive portion of the cycle (Wiskich, 1980; Millhouse et al., 1983; Oliver and McIntosh, 1995). The cycle is not depleted, however, due to the synthesis of oxaloacetic acid by phosphoenolpyruvate carboxylase in the cytosol with subsequent transport into mitochondria. The alternative oxidase thus appears to work in concert with the TCA cycle to satisfy the plant's needs for increased carbon skeletons.

If this additional alternative pathway functions, in part, to feed anabolic reactions, then how is it controlled? Posttranslational regulation of this "linked" alternative oxidase/TCA cycle appears to provide the "fine control" via a redox mechanism. Alternative oxidase is a homodimer existing in two forms: an oxidized, or less active form; and a more active,

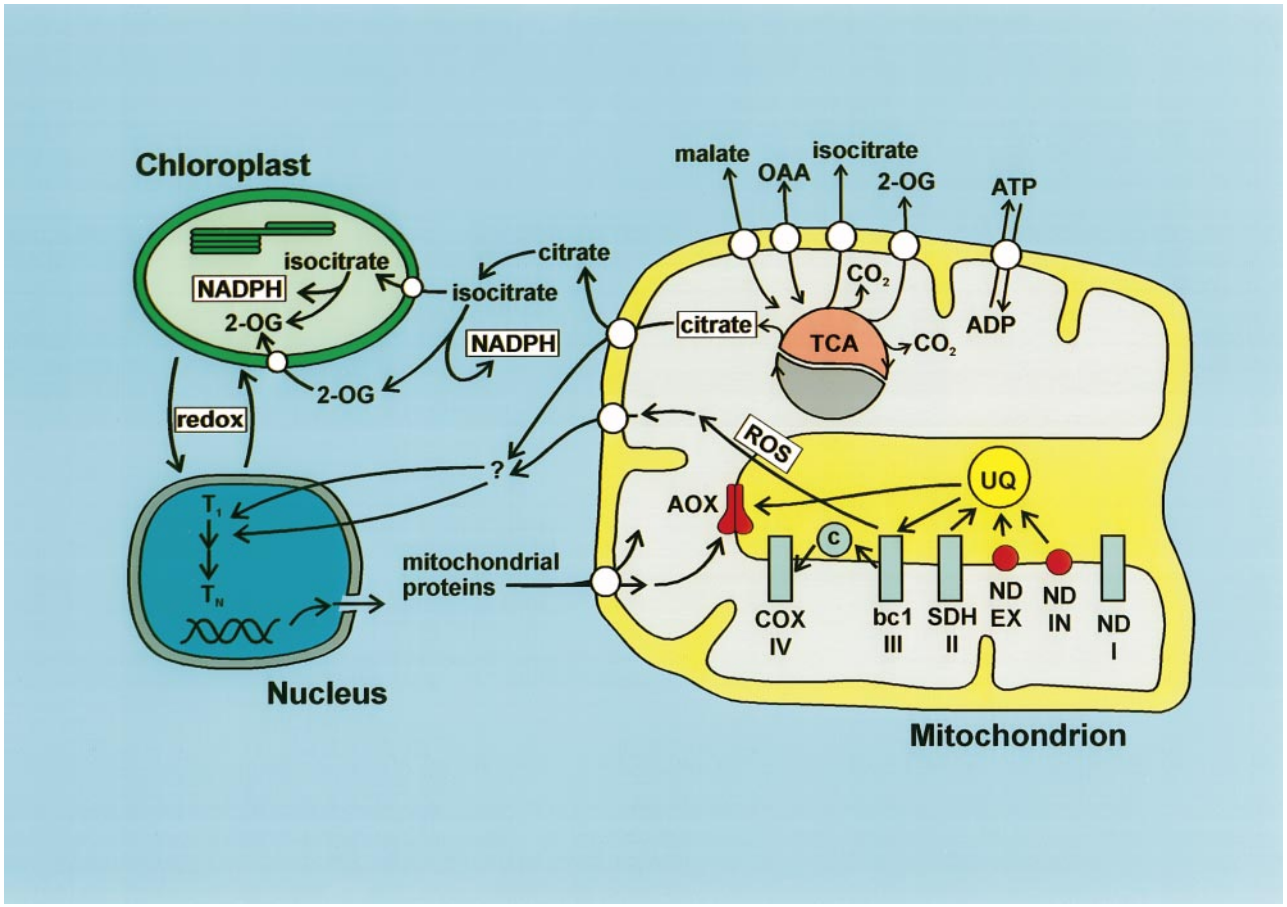

Figure 1. Interorganellar Interactions That Involve Plant Mitochondrial Biochemistry.

Three organelles are involved in the regulation of mitochondrial biogenesis and energy transduction: the nucleus, plastid, and mitochondrion. Plant mitochondria contain two terminal oxidases: complex IV (cytochrome oxidase; COX), and alternative oxidase (AOX). The energy-transducing inner membrane includes complex I (NADH-dehydrogenase; ND), several proposed internal (IN) and external (EX) NAD(P)H-dehydrogenases; complex II (succinate dehydrogenase); complex III (the cytochrome bc1 complex), and cytochrome c (c). Reactive oxygen species (ROS) arise in part from this electron transport pathway; along with citrate, it is known to be involved in the induction of the nuclear-encoded alternative oxidase. A number of compounds, including TCA cycle intermediates and adenylates, have, as yet, ill-defined translocators (open circles) that allow passage between cytosol and plastid with mitochondrial compartments.

reduced enzyme (Umbach and Siedow, 1993; Umbach et al., 1994; Day et al., 1995). Following the "coarse control" of gene expression and enzyme synthesis, fine control appears to be affected by the reduction of the oxidase to its active form. Additionally, pyruvate is an allosteric activator of the enzyme (Day and Wiskich, 1995; Day et al., 1995). The reducing equivalents necessary for alternative oxidase activity apparently arise from NADPH produced by the TCA cycle, possibly from the matrix-associated NADPH-isocitrate dehydrogenase (Vanlerberghe et al., 1995; Vanlerberghe and McIntosh, 1997). It is not known what mediates this reduction, although thioredoxin (Bodenstein-Lang et al., 1989) and glutathione (Jimenez et al., 1997) have been reported in plant mitochondria and may be involved in the process.

Alternative Pathway and Reactive Oxygen Species

Mitochondria are major producers of cellular reactive oxygen species (ROS) (Gonzalez-Flecha and Boveris, 1995; Poyton and McEwen, 1996). A number of environmental stresses that increase ROS in plants also induce alternative path respiration. This has led to proposals that this type of respiration may function to mitigate ROS damage in plant cells (Purvis and Shewfelt, 1993; Wagner and Moore, 1997). Further evidence also has come from the observation that addition of hydrogen peroxide (H_2O_2) to cultured plant cells and fungi induces alternative pathway respiration (Minagawa et al., 1992; Wagner, 1995; Vanlerberghe and McIntosh, 1996). Furthermore, experiments with isolated

soybean and pea mitochondria have shown that additions of alternative pathway inhibitors such as salicylhydroxamate and propyl gallate stimulate H_2O_2 production (Popov et al., 1997). Recent experiments in transgenic tobacco cell lines with repressed or overexpressed alternative pathway have given further support to the above hypothesis. In these experiments, oxidase stress with accompanying H_2O_2 production was induced in cells by addition of the cytochrome oxidase pathway inhibitor antimycin A. Cells lacking alternative oxidase had dramatic increases in H_2O_2 over controls. Interestingly, cells overexpressing alternative oxidase had little detectable H_2O_2 compared with controls (McIntosh et al., 1998).

Recently, another antioxidant pathway, an ascorbate–glutathionine cycle, has been identified in plant mitochondria (Jimenez et al., 1997). This cycle consists of ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase (GR), as well as ascorbate and glutathione. GR is an NADPH-dependent enzyme that, as alternative oxidase, may act to lessen ROS damage to cells.

Specialized Plant Mitochondrial Functions and $NAD^+/NADP^+$

Unique to plant mitochondria is a multiplicity of $NAD^+/NADP^+$ carriers in the electron transport chain. Besides complex I (NADH-dehydrogenase), at least four other $NAD(P)^+$ dehydrogenases, two "internal" (facing the intermembrane space) and two "external" (facing the matrix; Figure 1), have been proposed for plants (Moller and Rasmusson, 1998). Similar to the alternative oxidase, none of these dehydrogenases contribute to a proton gradient. At the experimental level, the "external" NAD(P)H dehydrogenase adds a distinct function to plant mitochondria, allowing isolated mitochondria to oxidize exogenously added NAD(P)H. At the molecular level, both complex I subunits and these additional dehydrogenases are now just beginning to be characterized (Menz and Day, 1996a, 1996b; Moller and Rasmusson, 1998; Rasmusson et al., 1998). In addition, other NAD(P)H-associated matrix enzymes that may be critical for plant mitochondrial function are present: $NADP^+$-isocitrate dehydrogenase, $NAD^+/NADP^+$ malic enzyme, and delta^1-pyrroline-5-carboxylate dehydrogenase (discussed below).

It may be that an increased role for $NADP^+$-linked enzymes is associated with increased anabolic functions of plant mitochondria. The example given above involved alternative oxidase, reduction state, and efflux of TCA cycle–derived carbon skeletons. Another example comes from recent studies demonstrating that plant mitochondria are sites of folate and thymidylate synthesis (Neuburger et al., 1996; Rebeille et al., 1997). Photorespiration, as light-dependent CO_2 evolution and O_2 uptake, involves the chloroplast, mitochondrion, and peroxisome and depends on glycine decarboxylase (GDC) (Oliver, 1994; Oliver and Raman, 1995). GDC is present in many organisms, serving to convert glycine to serine; however, in plants its activity is intimately connected to photosynthesis. GDC is the major protein in leaf mitochondria, which may indicate why folate biosynthesis is localized to the mitochondrion. GDC is composed of four activities/subunits: H, T, L, and P (Canvin and Salon, 1997). Both dihydrofolate reductase and methylenetetrahydrofolate dehydrogenase are $NADP^+$-linked enzymes. $NADP^+$-dependent isocitrate dehydrogenase (NADP-ICDH), responsible for the oxidative decarboxylation of isocitrate to α-ketoglutarate, is present in most living organisms, but its function, except in *Escherichia coli*, is unknown. NADP-ICDH in *E. coli* is the TCA cycle enzyme and a controlling enzyme in the distribution of carbon at the branch point of the TCA cycle and the glyoxylate shunt (Walsh et al., 1989). The TCA cycle enzyme in other organisms is NAD^+ dependent. In plants, NADP-ICDH isozymes exist in the plastid (ICDH2), cytosol (ICDH1), and mitochondrion (mtICDH) (Galvez and Gadal, 1995). Thus, the plant TCA cycle contains a second isocitrate dehydrogenase, one that is $NADP^+$ linked and whose function has not been proven. As described above, previous work implicated mtICDH as part of a regulatory mechanism to siphon excess reducing equivalents to alternative oxidase under stress conditions and use carbon outflow from the TCA cycle (Vanlerberghe et al., 1995; Vanlerberghe and McIntosh, 1997). In mitochondria from the axes of germinating sunflower seeds (Attucci et al., 1994) and pea leaf (McIntosh and Oliver, 1992), the specific activities of NAD-ICDH and mtICDH were similar, whereas in potato tubers the NAD-ICDH was higher than that of mtICDH (Rasmusson and Moller, 1990). It is interesting to note that in nonphotosynthesizing, heterotrophically grown, cultured tobacco cells, mtICDH specific activity is 11-fold higher than is NAD-ICDH activity (G. Gray and L. McIntosh, unpublished results). These reports indicate that mtICDH activity is significant in plant mitochondria, even though a specific role for this enzyme has yet to be described.

Other functions of importance to plant mitochondria tend to arise from their unique alternative oxidase and coenzyme ($NADP^+$). For example, proline accumulates under some types of stress, such as salt stress, and is ultimately synthesized from TCA cycle carbon skeletons (Delauney and Verma, 1993). Delta^1-pyrroline-5-carboxylate dehydrogenase, an enzyme involved in the catabolism of proline, can readily use $NADP^+$ and is localized to plant mitochondria (Forlani et al., 1997). All of the enzymes necessary for fatty acid biosynthesis, requiring large quantities of NADPH, reside in plant mitochondria (Wada et al., 1997), but why? It was suggested that the majority of the octanoic acid (one of the major intermediates in the mitochondrial synthesis of fatty acids) synthesized was incorporated into the H-protein of GDC as lipoic acid (Wada et al., 1997). The GDC activity and fatty acid synthesis are thus dependent on NADPH concentrations in the mitochondrion. Is photorespiration therefore dependent on NADPH levels in plant mitochondria?

INTERORGANELLAR COMMUNICATION WITHIN THE CELL

One of the most important emerging areas of mitochondrial research involves the identification of molecules mediating interorganellar communication. Although this area is not yet well understood, it is already clear that multiple interorganellar interactions likely occur. Cytological evidence exists to suggest that physical contacts may exist between organelles, including mitochondrion–endoplasmic reticulum (Staehelin, 1997), mitochondrion–chloroplast (inferred from observations by Kohler et al., 1997), and mitochondrion–nucleus (Smart et al., 1994; Southworth et al., 1997) interactions at particular stages in cell development. Such contacts may provide a means for the transfer of genetic information to and from the mitochondrial genome (see Unseld et al., 1997), as well as the exchange of membrane components and the delivery of interorganellar signals.

A compelling argument has been made for an evolutionary process of gene transfer from the mitochondrion to the nucleus (Brennicke et al., 1993), in some cases occurring via RNA intermediates. Reverse transcriptase sequence homology (Wahleithner et al., 1990; Moenne et al., 1996) and enzyme activity (Moenne et al., 1996) are detected in plant mitochondria, implying that such RNA-mediated transfer remains feasible.

Apoptosis, or programmed cell death, comprises a fairly well-defined series of cellular processes triggered by mitochondrial events (reviewed in Hirsch et al., 1997). Although most research has been conducted to date in animal systems, evidence has accumulated to suggest that similar processes occur in plant cells. In plants, apoptotic events have been linked to the hypersensitive response to pathogens (Greenberg, 1997; Morel and Dangl, 1997) and various forms of terminal cellular differentiation (Beers, 1997; Nooden et al., 1997). A primary cellular trigger for programmed cell death is the release of cytochrome c from mitochondria; this process is inhibited by a nuclear gene designated *Bcl-2* (Kluck et al., 1997; Yang et al., 1997). Recently, a BCL-2 homolog has been detected in plant cells immunologically and has been associated with mitochondria, plastids, and nuclei (Dion et al., 1997). Moreover, an Arabidopsis clone has been identified that shows significant similarity to the mammalian *defender against apoptotic death 1* (*DAD1*) (Gallois et al., 1997).

Regulation of Nuclear Genes Encoding Mitochondrial Proteins

In fungal and animal systems, models have emerged for the expression of mitochondrial proteins encoded in the nucleus. One of the best studied has been the expression of the nuclear-encoded subunits of cytochrome oxidase in yeast, serving as a paradigm of anaerobic versus aerobic regulation of expression (reviewed in de Winde and Grivell, 1993; Grivell, 1995; Poyton and McEwen, 1996). In nonphotosynthetic eukaryotes, cytochrome c oxidase is the critical regulator of cellular energy production, and in yeast, oxygen and carbon sources are the main environmental effectors of cytochrome c oxidase levels (Zitomer and Lowry, 1992). Gene regulation for the mitochondrial-encoded subunits (COX1, COX2, and COX3) occurs, in part, through modulation of the mitochondrial RNA polymerase (Ulery et al., 1994), whereas regulation of nuclear-encoded proteins occurs through cascades of response elements, including transcription factors primarily responsive to oxygen tension and carbon source. In mammals, carbon source plays no apparent role, whereas oxygen concentration and hormone levels are the main effectors of specific transcription factors (Silve et al., 1992; Poyton and McEwen, 1996; Burke et al., 1997). These studies have identified classes of nuclear respiratory factors (NRFs), such as mammalian NRF-1 and NRF-2 (Virbasius et al., 1993; Scarpulla, 1997; Au and Scheffler, 1998) and the yeast retrograde (RTG) factors RTG1 and RTG2 (Liao and Butow, 1993; Jia et al., 1997). Indeed, NRFs have been linked to numerous classes of genes regulating mitochondrial functions (Scarpulla, 1997).

Plants present a more complex challenge for understanding nuclear-encoded mitochondrial gene regulation. They contain plastids capable of producing both oxygen and carbon as substrates for respiration, and their mitochondria have functions both different and possibly more elaborate than their mammalian and fungal counterparts (Vanlerberghe and McIntosh, 1997). A number of nuclear-encoded mitochondrial proteins have been characterized, including those coding for the alternative oxidase (Rhoads and McIntosh, 1991), citrate synthase (Unger et al., 1989), subunits of GDC (Srinivasan and Oliver, 1995; Vauclare et al., 1996), adenine nucleotide transporter (Winning et al., 1991), HSP70 (Watts et al., 1992), NAD-ICDH (Behal and Oliver, 1998), Mn-superoxide dismutase, aconitase (Bowler et al., 1989), E1α subunit of pyruvate dehydrogenase (Grof et al., 1995), the Rieske iron-sulfur protein (Huang et al., 1994), the apoprotein of cytochrome c (Kemmererj et al., 1991), and 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (Rebeille et al., 1997). Expression of the gene(s) encoding alternative oxidase is induced by conditions of general stress (Vanlerberghe and McIntosh, 1996, 1997) and demonstrates differential tissue-specific expression of the small gene family in soybean (Finnegan et al., 1997). Genes encoding citrate synthase (Landschutze et al., 1995a, 1995b), the Reiske-Fe-S protein (Huang et al., 1994), the E1α subunit of pyruvate dehydrogenase (Grof et al., 1995), and alternative oxidase (Rhoads and McIntosh, 1992) have all demonstrated higher levels of mRNA accumulation in flower tissues, indicating increased mitochondrial activity in these tissues (Huang et al., 1994). Transcripts of GDC subunits are developmentally regulated and increase in a light-dependent manner (Srinivasan and Oliver, 1995; Vauclare et al., 1996). Oxidative stress or cold causes increased transcript accumulation of the alternative

oxidase (Vanlerberghe and McIntosh, 1996, 1997). It is possible that the nuclear gene *Rf2*, encoding an aldehyde dehydrogenase in maize (Cui et al., 1996) and present throughout development, may act to influence mitochondrial function in a developmentally regulated fashion during pollen formation.

It is clear that our understanding of the regulation of nuclear-encoded mitochondrial proteins in plants is at a rudimentary stage. Signals that induce transcription, for example, flower formation and oxidative/cold stress, have been only superficially addressed. No signal pathways have been brought to the more comprehensive level of the mammalian NRF or the yeast RTG systems, in which transcription factors have been isolated, cloned, and found responsible for suites of genes and their regulation. The classes of transcription regulators presumed to exist for plant mitochondria are still to be discovered.

Redox Passage and Metabolite Exchange: Mitochondrion–Chloroplast Interaction

An enigmatic phenotype that has been associated with mitochondrial mutation is green-white variegation, perhaps illustrating the pronounced interdependence of the plastid and mitochondrion. This phenotype, presumably induced mitochondrially, is observed in the chloroplast mutator (*chm*) mutants of Arabidopsis as well as the nonchromosomal stripe mutants of maize (Newton, 1995). Although genetic dissection of mitochondrion–chloroplast interaction has not yet been feasible, it is possible, to some extent, to examine essential interorganellar biochemical associations likely contributing to these unusual phenotypes.

Chloroplast–mitochondrion interaction, one of redox passage and metabolite exchange, has, in large part, been investigated indirectly at the physiological level. Aside from the intertwining of chloroplast, mitochondrial, and peroxisomal functions during photorespiration (Oliver and McIntosh, 1995), the fundamental bioenergetic observation has been that photosynthetic activity in the light is dependent on oxidative phosphorylation (reviewed in Kromer, 1995). Approximately 25 to 50% of the NADH, or redox equivalents, formed in vivo in plant mitochondria are oxidized in extramitochondrial processes via the malate oxaloacetate shuttle (Hanning and Heldt, 1993). These results are obtained with the use of oligomycin, an inhibitor of the F_0/F_1-ATP synthases of inner mitochondrial and plastid thylakoid membranes. The mitochondrial ATP synthase is sensitive to concentrations of oligomycin 400-fold lower than that required to inhibit the plastid enzyme (Maury et al., 1981; Kromer et al., 1988). Kromer et al. (1988) have shown that oligomycin concentrations that inhibit oxidative phosphorylation produce a net photosynthetic decrease in oxygen evolution, indicating that mitochondrial ATP is required for photosynthesis in the light (Kromer et al., 1988; Kromer, 1995). Oxidative phosphorylation has also been proposed to operate in the opposite direction to lessen photoinhibitory effects by preventing over-reduction of the cytosol through mitochondrial oxidation of NAD(P)H (Raghavendra et al., 1994). With the ability to use reconstitution assays (Raghavendra et al., 1998), a more quantitative assessment of interorganellar interdependence may now be feasible.

Redox passage between organelles can occur through transport of organic acids and their concomitant interconversions and in conjunction with oxidation and reduction of specific coenzymes (Figure 1). Plant mitochondria certainly participate in redox passage, although the cellular machinery for such communication is relatively unknown. We know little concerning specific transporters other than through indirect physiological/biochemical measurements. Molecular approaches aimed at isolating these specific transporters, linked with the production of transgenic plants altered in transporter functions, are likely to facilitate understanding of how metabolites are important to whole-cell metabolism and energy distribution.

FUTURE PERSPECTIVES

Clearly, the plant mitochondrion is a highly unusual and complex organelle; in light of its intricacy, progress toward understanding its many unique features has been impressive over the past few years. An understanding of mitochondria must come from approaches that reveal their unique features in the context of whole-plant biology. Recent biochemical advances have given us many new and important targets for molecular intervention that we hope will lead to new understanding. In many other areas, however, the primary impediments to future major insights are technical; the development of appropriate genetic and in vitro systems for investigating editing mechanisms, mitochondrial DNA replication, and the signals mediating bidirectional interorganellar communications are just a few. As chloroplast transformation in higher plants is becoming routine, mitochondrial transformation eludes us. Whereas a strikingly detailed picture of the mitochondrial genome and its expression emerges, relatively little attention has been paid to those nuclear components so essential to its function. Assuredly, new initiatives under way in genomics and expressed sequence tag databasing will offer exciting avenues for untangling the myriad of essential cellular signals that couple mitochondrial, chloroplast, and nuclear functions throughout development.

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