NUCLEAR CONTROL OF PLASTID AND MITOCHONDRIAL DEVELOPMENT IN HIGHER PLANTS

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ABSTRACT
The nucleus must coordinate organelle biogenesis and function on a cell and tissue-specific basis throughout plant development. The vast majority of plastid and mitochondrial proteins and components involved in organelle biogenesis are encoded by nuclear genes. Molecular characterization of nuclear mutants has illuminated chloroplast development and function. Fewer mutants exist that affect mitochondria, but molecular and biochemical approaches have contributed to a greater understanding of this organelle. Similarities between organelles and prokaryotic regulatory molecules have been found, supporting the prokaryotic origin of chloroplasts and mitochondria. A striking characteristic for both mitochondria and chloroplast is that most regulation is posttranscriptional.

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INTRODUCTION

It is generally believed that higher plant evolution occurred through two different endosymbiotic events, both involving prokaryotic organisms. Eukaryotic cells initially acquired mitochondria through endosymbiosis of a bacterium. Later, the chloroplast was derived from a cyanobacteria-type organism (149). Mitochondria and plastids each contain an autonomous genome, yet the nucleus now plays a major role in determining organelle properties, because it encodes the majority of the genes required for function and maintenance. The presence of organelle genes in two different cellular compartments creates an obvious regulatory problem. The cell must coordinate the expression of nuclear-encoded genes, present in only one or a few copies per cell, with the expression of organellar-encoded genes present in several hundred or even thousands of copies. Coordinate control is a necessity for mitochondrial and chloroplast function and likely requires new regulatory pathways not present in the original endosymbionts (48).

Many of the structural genes required for the photosynthetic and respiratory reactions have been cloned and sequenced. We have much less information about the many nuclear genes required in development. This is a broad subject for study, as higher plants contain a variety of differentiated plastids types, reflecting cell and tissue type (85). This review concentrates primarily on recent advances in organelle development and function in vascular plants. Recent published reviews cover other photosynthetic organisms such as *Chlamydomonas* and *Euglena* (45, 54, 142, 160).
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A DEVELOPMENTAL CONSIDERATION

Plastids exhibit a very clear developmental program. All plastids are derived from proplastids present in meristematic cells (85). Upon cell differentiation, proplastids also differentiate. Plastids can also redifferentiate in response to external environmental signals (85). The best characterized differentiation process results in a chloroplast.

Almost every step of plastid development depends on the direct action of nuclear-encoded molecules. Molecules required in the initial stages of organelle differentiation could affect all plastid types, whereas molecules necessary at later stages should affect only one plastid type.

Chloroplast differentiation appears to start very early during plant development. The leaf primordia that develop from the apical meristem contain mesophyll tissue from which most of the chloroplasts will be derived. Mesophyll chloroplasts can then undergo further differentiation, as is well-documented for differentiation of mesophyll and bundle sheath chloroplasts of C4 plants (124).

Microscopic studies have provided a sequential picture of the events during plastid differentiation (95). Further refinement of these events at the molecular level has been difficult. It is possible that many events during chloroplast differentiation are concurrent or occur in such a brief period that they cannot be dissected with the available techniques. In monocot leaves initial differentiation events include DNA replication and production of the chloroplast decoding apparatus (11, 123). Production of thylakoid membranes and accumulation of specific photosynthetic complexes are detected in later stages (85, 123).

The study of the participation of nuclear genes in plastid development has benefited from the isolation and characterization of an enormous number of mutants, many of which will be described in this review. In contrast to the well-defined developmental program of the chloroplast, the mitochondrion does not have an obvious developmental program. However, it is clear that mitochondria play essential roles throughout cellular differentiation and take on a pronounced role at particular points in higher plant development.

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Initial Steps

Transcription is a central regulatory point during the early stages of chloroplast differentiation (54, 123). Transcription is low in the proplastid, and is activated in the immature chloroplast (11). In parallel, the synthesis of both the transcription and translation apparatus takes place (63). Several lines of evidence suggest a nuclear origin for the enzyme responsible for initial transcriptional activity in
the chloroplast (69, 70, 74, 122). For example, in the parasitic plant _Epifagus virginiana_, which lacks the chloroplast-encoded subunits of RNA polymerase, and in the _albostrians_ barley mutant, which lacks ribosomes, transcription of particular plastid genes has been detected (70, 122).

Allison et al (2) have shown that a subset of the chloroplast-encoded genes are transcribed by a second RNA polymerase in which at least some subunits are nuclear-encoded. The role of this nuclear-encoded RNA polymerase (NEP polymerase) seems to be primarily, but not exclusively, the expression of housekeeping genes during the early phases of chloroplast development (54, 123, 160). Among the genes transcribed by this enzyme are the rRNA genes (2), ribosomal proteins (70), and the _rpoB_ operon (72), which encodes the subunits of the plastid-encoded RNA polymerase. Candidates for the NEP enzyme have been purified from spinach (97). NEP is probably encoded by the _RpoPt_ gene recently isolated from _Arabidopsis_ (68); it shares important similarities with phage-type RNA polymerases.

Concomitant with transcription of the rRNA genes, nuclear-encoded ribosomal proteins are synthesized (63) and imported into the chloroplast to form ribosomes, in preparation for translation in later stages of development. The current view is that the nucleus initiates chloroplast differentiation and provides key components of the transcriptional and translational machinery required for later stages of development (123).

### MUTANTS THAT AFFECT EARLY CHLOROPLAST DEVELOPMENT

#### Early Developmental Mutants

Although many chloroplast developmental mutants have been isolated, few of them affect initial events in organelle biogenesis. Because the plastid produces many essential compounds such as hormones, lipids, and cofactors, mutations that interrupt these pathways will probably disrupt both plastid biogenesis and plant function. The _amidophosphoribosyl-transferase deficient_ (atd) mutant from _Arabidopsis_ is an example; chloroplast development is arrested at an early stage in _atd_ lines because one of the genes encoding the ATase enzyme, _ATase2_ gene) is disrupted; a key enzyme of purine biosynthesis. This nuclear gene provides the only ATase activity in photosynthetic tissue, but a second gene might permit purine biosynthesis in other tissues (170).

Similar examples of fundamental mutants include _dcl_ from tomato (80), _dag_ from _Antirrhinum_ (18), and the _Arabidopsis_ albino T-DNA tagged _cla1-1_ (110). Plastids present in these plants are very small, with almost no thylakoid
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membrane, and thus resemble proplastids. mRNA analyses showed that CLA1, DAG, and DCL genes are normally expressed not only in photosynthetic but also in nonphotosynthetic tissue, suggesting that these genes are probably required for the development of different plastid types. In both cla1-1 (J Estevez, A Arroyo, A Cantero & P Leon, unpublished results) and dag (18) plants, chloroplasts and etioplasts are abnormal. Each of these genes encodes a novel plastid-localized protein of unknown function. Null mutations of the DCL gene cause embryo abortion (JS Keddie & W Gruissem, unpublished data). Proteins homologous to CLA1 are found in photosynthetic and nonphotosynthetic prokaryotes; recent data indicate that this protein may be involved in the biosynthesis of a novel isoprenoid pathway conserved in evolution (J Estevez, A Arroyo, A Cantero & P Leon, unpublished results). At the morphological level, dag and dcl plants apparently lack palisade cells (18, 80).

Similar alterations occur in plastids after disruption of the PALE CRESS (PAC), IMMUTANTS (IM), and ATD genes of Arabidopsis (139, 170, 178). Because it seems unlikely that all these genes directly regulate leaf differentiation, a novel signaling system during early plastid development has been hypothesized that would affect the final divisions of mesophyll cells in the prepalisade stage (18). Compelling evidence indicates that a factor originating in the chloroplast signals its developmental stage to the nucleus, coordinating the expression of many nuclear-encoded genes (reviewed in 162, 165). Alterations in this signal transduction pathway do not have any obvious impact on mesophyll morphology. These results suggest that multiple signals might exist to ensure proper coordination between cell and organelle during plastid development.

Genes involved in early plastid development have also been described in C4 maize plants. Phenotypic characteristics of the bsd3 (R Roth & J Langlade, unpublished data) mutant resemble those described above, with no thylakoid formation and alterations in different plastid types. However, in the bsd3 mutant, neither the morphology of the photosynthetic tissue nor accumulation of the C4 nuclear-encoded photosynthetic genes is altered. These results suggest that, in contrast to what it is found in C3 plants, leaf development in C4 plants such as maize might not rely on chloroplast signals for final differentiation.

Pigment Mutants

Direct participation of pigment biosynthesis in chloroplast development has been difficult to establish because of pleiotropy. Several mutants with altered chlorophyll accumulation have been described (158). In all cases, chloroplast development was arrested at initial membrane assembly, suggesting that chlorophyll synthesis and chloroplast development may be interdependent (174). Because these kinds of mutations can disrupt different metabolic activities, loss of
chlorophyll along with other plastid components might represent secondary effects (62). Recent data that support the hypothesis that chlorophyll production has the potential to influence organelle development come from work with mutants altered in chlorophyll \( a/b \) ratio (40) and the \( \text{olive} (\text{oli}) \) mutant from \textit{Antirrhinum} (73). The \( \text{OLI} \) gene seems to encode a key enzyme of the chlorophyll biosynthetic pathway, the porphyrin IX Mg-chelatase. \( \text{oli} \) conditionally affects chlorophyll synthesis and organelle development, and the chloroplasts in this mutant contain thylakoids but no grana. This phenotype seems to be the direct result of a block in chlorophyll biosynthesis, not a result of photodamage (73). Chlorophyll \( b \)-deficient mutants usually result in smaller photosynthetic unit sizes (115), possibly because of LHC destabilization and an abnormal thylakoid membrane system (39). Chlorophyll appears to be necessary for either translation or stability of the nuclear-encoded apoproteins of the light-harvesting complex and the formation of grana-deficient thylakoid membranes (64, 88). Gene disruptions at other steps of chlorophyll biosynthesis display alterations in plastid development even in the dark, where photodamage does not exist, supporting the hypothesis that chlorophyll is involved directly (39, 64, 146, 158).

**NUCLEAR REGULATION DURING CHLOROPLAST DEVELOPMENT**

\textit{Division}

Organelle division is a fundamental process in chloroplast and mitochondrial biogenesis. With the recent isolation of several mutants from \textit{Arabidopsis thaliana}, Pyke and coworkers have contributed to our understanding of the molecular basis of control of chloroplast division (135, 136). These mutants, named \textit{arc} for “accumulation and replication of the chloroplast,” demonstrate that several nuclear genes are required. Interestingly, all mutants analyzed so far show a reduction in chloroplast number per mesophyll cell, but no obvious plant morphological alterations (134a, 135). It is likely that the total amount of chloroplast material within a cell is the important parameter for cell function rather than chloroplast number.

Physiological analysis of most \textit{arc} mutants shows plastid division to be altered only in chloroplast (134a). An exception is the \textit{arc6} mutant, the most extreme mutation isolated, which may affect division at the proplastid state (134a) the gene affected in the \textit{arc6} mutant appears to be a homolog of the \textit{FtsZ} protein, implicated in bacterial cell division (12, 31, 131). \textit{FtsZ}, which polymerizes similarly to eukaryotic cytoskeletal elements, may be a progenitor of tubulin.
(38), which raises the intriguing possibility that the mechanism of organelle division may be, to some degree, conserved between prokaryotes and plants.

**Transcription**

Although transcriptional control is the key element setting expression of nuclear-encoded plastid-localized gene products (90), the relative transcription rates of many chloroplast-encoded genes are constant in different tissues and at different developmental stages. This suggests that posttranscriptional events may predominate in chloroplast gene regulation (29). Evidence suggests, however, that stage-specific transcriptional regulation occurs during leaf maturation. For example, some plastid-encoded genes fluctuate in transcription levels in response to factors such as light and plastid type (103, 114, 123, 138). This is the case for the light-activated genes such as *psbA* in barley and maize (86), *psbD-psbC* in barley (150), and *petG* from maize (59). Similarly, the transcription rates of *rbcL*, *psbA*, and *atpB/E* genes from *Arabidopsis* are specifically reduced in amyloplasts (77). All these genes are preferentially transcribed by the better-characterized plastid-encoded RNA polymerase (74). It is tempting to attribute the selectivity of the transcriptional response during chloroplast development to the interaction of the core RNA polymerase with specific regulatory molecules (103). These factors might be available only under certain conditions and in this way might regulate gene transcription during development.

Two types of factors seem to play a role in the regulation of chloroplast transcription: factors that bind DNA only in the presence of the RNA polymerase (sigma-like factors) and sequence-specific proteins acting as activators or repressors. Several sigma-like factors have been recognized (102, 167). For example, a 90-kDa sigma-like factor from mustard is present in young but not in mature spinach leaves (96). In contrast, the transcriptional activity of chloroplasts and etioplasts in mustard appears to be modulated by differential phosphorylation of three sigma factors in response to light (166). In this way only dephosphorylated factors permit efficient transcription initiation of promoters like that of *psbA*. The most likely candidate responsible for phosphorylation of these sigma-like factors is a serine/threonine kinase found to associate with the active transcription complex (103).

Evidence has emerged recently for a number of sequence-specific binding factors that might also modulate expression of photosynthetic genes under specific conditions. A nuclear factor involved in differential light-mediated transcription of the *psbD* gene has been isolated (84). This element (AGF) interacts at a specific site within the promoter region of the *psbD* gene and shares similarity with a specific DNA binding factor, CDF2 (for chloroplast DNA binding factor 2), isolated recently (76). Both factors have similar properties,
suggesting that they may belong to a larger family of DNA-binding proteins, but their specific function remains to be seen.

Posttranscriptional Processing

Posttranscriptional and translational events play a major role in regulating the differential accumulation of many plastid-encoded mRNAs during chloroplast development (for extensive reviews, see 53, 54, 114, 142). Most chloroplast-encoded genes are transcribed as polycistronic units (53). These precursors undergo a series of complex maturation events that include processing of the mature mRNAs, although only in Chlamydomonas (98, 141, 142) the importance of posttranscriptional processing understood. One of the few exceptions in higher plants is the CRP1 gene from maize, which seems to be required for the efficient translation of two chloroplast mRNAs (pet A and pet D) (9). This gene affects processing of the petD mRNA in such a way that the monocistronic transcript is absent, implying a mechanistic link between RNA processing and translation. It is hypothesized that in this mutant the petD protein is no longer synthesized, because the ribosome cannot initiate within the polycistronic mRNA, due to masking of the translation initiation region. The CRP1 gene has been cloned, but the encoded protein shows no significant similarity to any known protein (M Walker & A Barkan, unpublished data).

In contrast to processing, mRNA stability seems to be important for the accumulation of several photosynthetic mRNAs required during the transition from proplastid to chloroplast. For example, as measured by run-on experiments, steady state levels of the psbA and atpB transcripts increase during greening of etiolated spinach cotyledons without changes in the transcription rate (29). In addition, the stability of several chloroplast-encoded transcripts seems to be dramatically increased during chloroplast development in barley (83) and spinach (51). Stabilization seems to occur by the correct processing of the polycistronic mRNAs in the differentiated chloroplasts, whereas in proplastids these mRNAs appear to be rapidly degraded (53). It has recently been established that nuclear-encoded enzymes are required for correct mRNA processing (66). Both exo- and endoribonuclease activities are found in a high-molecular-weight complex that binds near stem and loop sequences found in the 3′ region of chloroplast transcripts (53, 114, 142). Although this complex has not been fully characterized, similarities have been postulated with a ribonucleolytic complex ("degradosome") in Escherichia coli (66). In addition to the action of the chloroplast high-molecular-weight complex, stabilization of transcripts requires additional factors that inhibit rapid degradation (53, 66). One of these factors is a nuclear-encoded 28-kDa protein (28RNP) (53) that interacts with both the transcript and the high-molecular-weight complex (66). This protein is differentially expressed during plant development, is modified by
phosphorylation, and is likely necessary to direct the correct 3′-end processing of plastid transcripts under particular developmental conditions (66).

Unexpectedly, polyadenylation is the signal for degradation of specific chloroplast transcripts. Following the endonucleolytic cleavage events in the petD mRNA by components of the high-molecular-weight complex, the products are then polyadenylated (89). By increasing susceptibility to degradation in response to conditions such as light, polyadenylation seems to play a critical role in regulating chloroplast transcript stability. For example, in dark-adapted plants, the extent of polyadenylated petD-specific transcripts is higher than in plants grown in light (89). This unexpected process is probably not restricted to a particular transcript and will most likely require the action of additional nuclear regulatory factors.

The modulation of transcript stability during the differentiation of chloroplasts in C4 plants has also been documented. In the dimorphic cells of the C4 plant, the photosynthetic genes are expressed in a cell-specific manner. The characterization of plants altered in bundle sheath cell differentiation in maize has led to identification of a nuclear gene (Bsd2) that influences the regulation of transcript stability and/or translation of rbcL mRNA. In this mutant, chloroplast development is prevented in bundle sheath cells; this is likely the result of photo-oxidative damage in the absence of the RuBPCase protein in these cells (145). It is conceivable that the product of the Bsd2 gene might interact in a sequence-specific manner with the rbcL mRNA in bundle sheath cells to stabilize this transcript.

A few other mutants have been described that alter stability of specific RNA transcripts, including the maize hcf2 and hcf6 mutations (118). Still others have a more general effect on the stability of multiple transcripts such as hcf38 from maize (7, 118) and the hcf109 and hcf5 mutants from Arabidopsis (35, 116).

In both mitochondria and chloroplasts unspliced transcripts can accumulate to high levels (177). The role of splicing in the regulation of chloroplast development in higher plants has not been extensively studied, but recent studies in maize show that the relative abundance of spliced and unspliced transcripts differs among plastid types. Compared to chloroplasts, unspliced forms predominate in both amyloplasts and proplastids (6). RNA splicing could exert control in plastid development by regulating the abundance of plastid-encoded proteins.

Probably the best evidence for participation of splicing in chloroplast development comes from the work with the crs (chloroplast RNA splicing) mutants in maize. Chloroplast genes in higher plants contain multiple group II introns, from both the A and B subgroups (117). These introns are capable of self-splicing in vitro; genetic evidence indicates that trans-acting factors are required for efficient splicing in vivo (148). Recently, two nuclear genes, crsl
and crs2, were reported to be required for the proper splicing of group II introns in maize chloroplasts (79). Mutation at crs1 specifically blocks splicing of the atpF type IIA intron that encodes subunit I of the chloroplast ATP synthase, whereas mutation at the recently cloned crs2 gene blocks the splicing of many group IIB introns. Disruption of chloroplast development in these mutants provides important biological evidence for the structural division of type II introns because different splicing factors are required to process IIA and IIB introns. Previously described mutants might also reflect splicing deficiencies. For example, in plants with the nuclear gene iojap (ij) splicing of most IIA introns is defective (16). Whether there is a direct relationship between ij and crs1 remains to be deduced.

Translational and posttranslational regulation has been documented in Chlamydomonas, Euglena, and higher plants (45, 54, 142, 160). This control could underlie the rapid changes in protein accumulation prominent in several photosynthetic factors during chloroplast development; for example, the product of the psbA transcripts does not accumulate during senescence of bean leaves (10), in etiolated leaves (159), in roots, and in cultured cells (71). Translational control is possible at several steps, but the best analyzed so far is during ribosome binding to the mRNA. Both 5′ and 3′ untranslated leader (UTR) sequences play important roles during translation in eukaryotes, and several cis-acting sequences have been identified (45). Nuclear-encoded proteins have been identified that interact with specific stem-loop sequences in the 5′ UTR of chloroplast transcripts in several organisms (27, 65, 71). These 5′ UTR binding complexes seem to modulate ribosome binding, acting as translational activators or repressors (28, 114). The nuclear-encoded factors identified to date are mostly from Chlamydomonas; these factors can regulate the translation of either individual or a group of transcripts (45, 142). In higher plants, a specific protein factor(s) was recently demonstrated to be required for the efficient translation of psbA mRNA in an in vitro translation system from tobacco chloroplasts. This factor interacts with specific elements of the 5′ UTR of the psbA mRNA (71). This approach has an enormous potential to analyze the molecular mechanisms of translational regulation in vascular plants.

Coordinate protein accumulation for different photosynthetic protein complexes depends on translational control. It is known that in order to maintain the stoichiometric levels of different plastid complexes, their subunits are rapidly degraded when prevented from assembling (142). Until now, the molecular mechanism has been little studied, but evidence currently exists to suggest it as an important point of translational regulation. For example, in Chlamydomonas mutants, the accumulation of cytochrome f decreases in the absence of other genes of the cytochrome b_{6}f complex (petB or petD), although its transcript level remains unchanged. The most likely interpretation of this phenomenon
is the direct autoregulation of the cytochrome f so that when unassembled, its translation is attenuated (160).

A similar mechanism appears to be present in higher plants. It has been shown that the translation of the large subunit protein (LS) of the Rubisco enzyme is controlled by levels of the small subunit (SS). The levels of the rbcL transcript remain unchanged, again suggesting translational regulation. Though the mechanism underlying this mode of regulation is still unclear, one might postulate that proteins such as SS may act as activators during translation of the rbcL transcript (144).

Posttranslational proteolysis is important during plastid differentiation. For example, during chloroplast to chromoplast differentiation, massive degradation of the photosynthetic complexes and the accumulation of new sets of proteins must occur (111). Likewise, photosystem D1 protein is specifically degraded after damage by oxygen radicals during electron transport (92), and this degradation is mediated by a specific protease (75). Several nuclear proteins involved in proteolytic degradation in chloroplasts have been identified. The gene homologous to ClpA, a subunit of the ATP-dependent serine protease (Clp) (47), is nuclear-encoded and transported into the chloroplast (81), where it may be involved in the degradation of soluble and thylakoidal proteins (151). This offers a means by which the nucleus may regulate protein accumulation during specific stages in the biogenesis of the organelle. This subject, though not yet extensively studied, likely represents a very important point in plastid developmental regulation.

Concomitant with the accumulation of the photosynthetic complexes, extensive proliferation of thylakoid membranes and the formation of granal stacks occurs in maturing chloroplast. It has been suggested that incorporation of the light-harvesting complex (LHCII) into the thylakoid membrane plays an important role in thylakoid stacking in Chlamydomonas and higher plants (35, 158). Only recently has participation of other nuclear genes been analyzed (36). One interesting example is development of transgenic Arabidopsis plants with low levels of a dynamin-like gene (ADL1), which show a greatly reduced thylakoid membranes and an increase in lipid granules. Because dynamin-like proteins in other systems are involved in the formation of membrane vesicles (180), it is proposed that the nuclear-encoded ADL1 gene participates in thylakoid biogenesis in higher plants (JM Park, JH Cho, SG Kang, HJ Jang, KT Pih, et al, unpublished data).

**Nuclear Regulation of Targeting**

The proper allocation of the nuclear-encoded subunits is required for organelle functionality and development. Plastids have developed an elaborate sorting system to ensure proper targeting of multimeric protein complexes. The presence
of three distinct membranes requires that proteins are incorporated into the chloroplast and later reallocated within the organelle (21). General aspects of chloroplast protein import have been known for some time, but the mechanisms for sorting and import are just beginning to be elucidated. Most genes involved in this process are nuclear-encoded, and their proper expression regulates correct targeting during chloroplast development (21).

A fairly detailed picture has emerged recently for the integration of proteins into the thylakoid membrane. Mutations in four nuclear genes of maize interfere with plastid biogenesis by disrupting the translocation of thylakoid proteins (8). Mutations in the *tha1* and *tha5* genes affect a SecA-type pathway, whereas mutations in the *hcf106* and *tha4* genes disrupt a DpH pathway that depends on pH (112, 172). These nuclear mutations are recessive and result in pale-green, nonphotosynthetic seedlings that die after endosperm depletion. The *tha1* mutant affects chloroplast development specifically in the mesophyll cells in which giant grana are found, but does not apparently affect bundle sheath chloroplasts that look normal (173). In the *hcf106* mutant, both plastid types are abnormal (112). *tha1* encodes a chloroplast-localized SecA protein homologous to the SecA protein from *E. coli*. This suggests that at least one of the plastid targeting mechanisms is similar to the prokaryotic secA/Y/E pathway (173). Recently the *tha4* gene has also been cloned; it is closely related to an open reading frame of unknown function of *Synechocystis*. In *tha4* plants the DpH pathway is disrupted; this pathway may have evolved from a cyanobacterial progenitor (M Walker & A Barkan, unpublished data). It is unknown why two distinct translocation pathways exist for the tylakoidal proteins and what the differences are between both mechanisms. Given the recent identification of the proteins involved in tylakoidal protein import, important progress in the future is expected.

**NUCLEAR SIGNALS FOR CHLOROPLAST DEVELOPMENT**

**Light Regulation**

Organelle development and plant development respond to external signals, particularly light. Light regulation is mediated by the photoreceptors: a phytochromes, blue/UV-A cryptochromes, and the UV-A and -B receptors. Several nuclear genes are part of the signal transduction system coupling light to plastid and plant development (19, 37, 137). Mutants with a de-etiolated phenotype—*det, fus, and cop* classes—have a morphology when grown in the dark similar to light-grown wild-type seedlings (91, 137, 173a); these mutants
have altered expression of several nuclear- and chloroplast-encoded photosynthetic genes (119, 137). The plastids in det1, cop1, and cop9 develop a mature thylakoid system when grown in the dark, even though they do not accumulate chlorophyll. These mutants together with several fus mutants also affect the amyloplast, by promoting differentiation of a chloroplast, resulting in a constitutive default photomorphogenic developmental pathway (91, 119). In contrast, other de-etiolated mutants such as det2 show no impact in chloroplast development (20). Several of the genes affected in these mutants have been cloned (30, 100, 132, 176), and the characteristics and localization of their gene products suggest that they may be nuclear regulators (17). The COP1 protein is probably one of the best studied light regulators; it is a negative transcriptional regulator capable of direct interaction with upstream DNA sequences of its target genes (137). Other genes such as COP8, COP9, and COP11, seemed to act in the same pathway (176a).

The cue mutants, were selected to identify elements that play a positive role during de-etiolation in Arabidopsis (99, 105). Most cue genes influence chloroplast development in the light and appear to modulate expression levels of particular nuclear-encoded photosynthetic genes. CUE1 encodes a phosphate/phosphoenolpyruvate translocator (PPT) of the chloroplast inner envelope membrane (42, 161). PPT is likely required early in chloroplast development; loss of this function probably directly influences signals from the chloroplast that affect nuclear expression of light-regulated genes.

MUTATIONAL ANALYSIS OF PLANT MITOCHONDRIA

Unlike the chloroplast case, there are few mutations in mitochondrial development. Most loss of function mutation in mitochondria are lethal unless maintained in a heteroplasmonic state (125). Some dominant mitochondrial mutations, resulting from expression of novel open reading frames in mtDNA, survive in higher plants. For the most part, these mutations lead to a similar phenotype of pollen inviability known as cytoplasmic male sterility (cms) (61). This maternally inherited trait has been reported in over 150 plant species, and in all cases examined in detail results from expression of novel mitochondrial polypeptides. Usually, these novel polypeptides contain at least one hydrophobic domain, likely facilitating membrane association. Each cms-associated polypeptide identified has been unique, and their modes of action are unclear. Cms mutations have proven particularly useful in defining nuclear regulation of mitochondrial functions. Nuclear suppressors of the cms phenotype are readily identified as nuclear fertility restorer (Rf) genes. In the majority of cases, Rf
genes act as single, dominant loci, though examples exist of polygenic restorer systems. Nuclear-directed mitochondrial functions identified via the analysis of fertility restoration mechanisms include mitochondrial transcript processing (154, 164, 182), posttranscriptional functions (157, 185), possible modes of biochemical detoxification (26), and alteration of mitochondrial genome organization (108).

NUCLEAR REGULATION OF MITOCHONDRIAL GENE EXPRESSION

Regulation of Transcription

A particularly unusual feature of plant mitochondria is the variable and complex pattern of transcripts arising from a given mitochondrial gene. Variation in transcript size can arise from use of multiple transcription start sites and termination sites (50). Complexity is compounded by posttranscriptional processing, described below.

Transcriptional regulation of mitochondrial gene expression does occur, although it does not appear to be the predominant means of gene modulation. For example, tissue-specific differences in transcript accumulation have been detected in various tissues of the maize seedling using in situ hybridization (101). It is unknown whether these differences reflect cell-specific changes in transcription rate, as opposed to posttranscriptional processes; it is clear that cell-to-cell modulation of mitochondrial expression can occur. Plant mitochondrial transcription appears to be mediated by at least one nuclear-encoded RNA polymerase bearing striking similarity to the RNA polymerases of bacteriophages T7, T3, and SP6 (68).

Data to date suggest that variation in promoter strength is likely a primary influence on relative transcription rates (15, 41, 50). Nuclear factors regulating transcription rate or promoter selection are difficult to detect. Perhaps the most compelling demonstration of nuclear influence on mitochondrial transcription is described in a Zea mays/Zea perennis alloplasmic line. A single nuclear gene, designated Mct, influences promoter selection in the cytochrome oxidase subunit II (coxII) gene in maize (22, 126). Transcriptional initiation at position −907 produces the predominant coxII transcript of ~1900 nucleotides. The dominant Mct allele apparently directs transcriptional initiation at a second site (−347) upstream to the coxII locus. Interestingly, this alternate initiation site, detected as a shorter coxII transcript, does not conform to the consensus promoter sequence described for maize. This provides the first evidence that specific cofactors may influence promoter selection in plant mitochondria, unlike yeasts and mammalian systems.
Certain nuclear-directed changes in mitochondrial transcript levels appear to be tissue-specific. This has been suggested by in situ hybridization studies of maize seedling tissues, where several mitochondrial transcripts appeared to be present at different levels depending on tissue type (101). Similar studies in developing anthers of sunflower demonstrated a marked accumulation of \textit{atpA}, \textit{atp9}, \textit{cob}, and \textit{rrn26} transcripts in young meiotic cells, with a concomitant increase in their respective protein products (157). Moreover, \textit{orf522} transcripts decrease in \textit{cms} sunflower; the encoded 15-kDa protein is observed in anthers when the nuclear \textit{Rf} gene, likely acting posttranscriptionally, is present (121). These results imply that nuclear-directed modulation of mitochondrial gene expression may occur in a cell-specific pattern. Further support comes from observations in \textit{cms} Petunia where restoration of fertility by a nuclear \textit{Rf} gene is associated with the tissue-specific reduction in a particular transcript derived from the \textit{pcf} mitochondrial region (185).

\textbf{Transcript Processing}

Nuclear background influences mitochondrial transcription patterns in particular genomic regions. For example, nuclear background alters transcription of \textit{atpA} in the Ogura cytoplasm of radish (109) and the \textit{cms} gene (\textit{T-urf 13}) in the T cytoplasm of maize. The \textit{T-urf13} sterility-associated sequence is co-transcribed with the \textit{orf 221} open reading frame. Specific lines of maize revealed a marked influence of nuclear background on its pattern of transcription (82). Rocheford & Pring (1994) demonstrated that the transcript changes were not only a function of the mitochondrial genomic environment encompassing the \textit{T-urf13/orf221} region but also of dominant nuclear gene action influencing the pattern of posttranscriptional processing these transcripts undergo (143).

Transcript processing is a widespread phenomenon in plant mitochondria though not yet well understood mechanistically. It was first deduced by the observation that unusually complex transcript in certain regions of the plant mitochondrial genome were produced not only by multiple transcription start and stop sites, but also by internal transcript processing (reviewed in 50). Although the role of processing in gene regulation is not yet clear, nuclear regulation of transcript processing could be an important means of mitochondrial gene suppression. This conclusion is based on the observation that three different nuclear \textit{Rf} loci directly influence transcript processing within the respective mitochondrial \textit{cms}-associated regions.

In \textit{cms-T} maize, perhaps the most well-investigated example of cytoplasmic male sterility, fertility restoration requires dominant alleles of two unlinked nuclear loci, \textit{Rf1} and \textit{Rf2} (94). Compelling evidence demonstrates that the product of \textit{Rf1}, essential though not sufficient to restore fertility, directly influences
transcript processing of the T-urf13 mitochondrial region (182). Processing of T-urf13 transcripts appears to be directly associated with a marked reduction in the expression of the encoded 13-kDa T-URF13 polypeptide (43). The action of Rf2 will be discussed in a later section. In sorghum line IS1112C, cms is associated with expression of the orf107 open reading frame (164). Again, fertility restoration is associated with altered processing of orf107 transcripts, and the concomitant reduction in the accumulation of a 12-kDa polypeptide presumed to be the gene product (164). Of particular interest is the observation that the transcript processing sites described in both cms-T maize and cms sorghum share sequence features (34), implying that sequence motifs exist within plant mitochondrial genes that may act as targets for nuclear-directed gene modulation.

cms in the Brassica napus (oilseed rape) Polima cytoplasm is associated with aberrant expression of a region encoding the atp6 gene cotranscribed with a downstream chimeric sequence, orf224 (152). Fertility restoration, conditioned by either of two dominant single nuclear loci, results in a transcript processing event that generates predominantly monocistronic atp6 transcripts (152, 153). Generation of monocistronic atp6 transcripts cosegregates with a single dominant fertility restorer locus, Rfp1 (154). Most intriguing, however, is the observation that an alternate recessive allele at this locus, (rfp1) or a second locus tightly linked to rfp1, influences transcript processing of two other mitochondrial genes not associated with sterility, nad4 and a ccl1-like gene that may be involved in cytochrome c biogenesis. At all four processing sites under Rfp1 or rfp1 control, there is UUGUGG or UUGUUG, a sequence motif, located very near the processing site.

**Transcript Editing**

One of the more surprising features distinguishing plant mitochondrial gene expression from that of yeast and mammals is the extensive editing of plant mitochondrial transcripts. The biochemistry and pattern of transcript editing has been reviewed recently (49, 60). The mechanisms regulating the rate of editing, as well as the local sequence features determining sites to be edited, are not yet understood. Evidence to date indicates that local features of the editing site are important (24, 55, 56) and that sites that alter codons may be preferentially edited (181). Nuclear genotype (181) as well as tissue type, developmental stage, and growth conditions may influence the extent of transcript editing (52). Perhaps the most definitive evidence of nuclear influence on transcript editing involves differential nad3 editing in a particular Petunia mitochondrial genotype when combined with different nuclear backgrounds. The extent of nad3 editing changes dramatically in response to different nuclear backgrounds, with a high extent of editing segregating
as a single dominant nuclear locus. Interestingly, this genetic variation for extent of transcript editing pertained exclusively to nad3; this implies specificity in nuclear control of editing and predicts that additional nuclear factors exist.

**Translational/Posttranslational Regulation**

Relatively little evidence currently exists for nuclear-directed, translational regulation in plant mitochondria. In contrast, several nuclear factors influence translation of specific mitochondrial genes in yeast (44). In plants, sequence conservation exist upstream of some mitochondrial start codons, but it is not clear whether these sequences actually function as specific binding sites for translational components (134). Furthermore, although particular mitochondrial polypeptide differences have been associated with changes in plant nuclear background (23), it is not yet determined whether the protein differences result from differential transcription or translation.

Perhaps more provocative at this stage is the accumulating evidence suggesting surprisingly limited regulation of translation in mitochondria, particularly in light of extensive translational control in plastids. One unexpected observation of transcript editing is that both edited and unedited transcripts are translationally competent. Both edited and partially edited transcripts appear to be represented in association with polysomes (57, 106). Moreover, little discrimination by the ribosome occurs with regard to untranslated leader sequences or transcript splicing status (184).

It may be argued that polysome association does not, in itself, demonstrate nonselective translation. Using antibodies against the predicted polypeptide product of the rps12 unedited transcript, compelling evidence demonstrates that polypeptides are produced from partially edited or unedited transcripts, as well as edited forms (107, 133). Not unexpectedly, these aberrant polypeptides are not incorporated into functional ribosomes (133).

These observations raise the obvious general question about the fate of aberrant translation products, and why they are not more commonly detected within plant mitochondria. One explanation may be differences in half-life for products of edited vs unedited transcripts. Although protein import and the concomitant processing events associated with import have been well described (reviewed in 46, 179), little is yet known about posttranslational proteolysis of mitochondrial gene products. Mitochondrial proteolysis has been best detailed in yeast, where several proteases, both matrix and membrane-localized, have been characterized (reviewed in 140). In plants, no proteolytic activity has been detected in the matrix of mitochondria, but 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 (87).
In common bean, posttranslational regulation of the cms-associated mitochondrial protein appears to occur in vegetative tissues. In bean, cms is associated with the expression of a 239–amino acid polypeptide, (ORF239) (1, 67) that accumulates only in reproductive tissues, with no detectable ORF239 in vegetative (seedling, leaf, or root) tissues (1). More extensive investigation shows that the ORF239 protein is subject to proteolysis in vegetative tissues, dependent in part on a mitochondrial protease related to the lon homologs of yeast (163, 171) and human (175; R Sarria, A Lyznik, E Vallejos & S Mackenzie, submitted manuscript). Whether this newly identified plant protease is involved in the degradation of other aberrant mitochondrial gene products remains an active area of inquiry.

Detoxification of metabolic poisons is a quite unexpected means of influencing mitochondrial functions posttranslationally. The Rf2 fertility restorer gene in cms-T maize encodes a putative aldehyde dehydrogenase (26). Although the function of this gene in fertility restoration is not yet clear, recent biochemical studies have confirmed its enzymatic activity identity (P Schnable, personal communication). It has been speculated that the Rf2 gene product may play a role in the detoxification of a pollen-specific product that interacts with the T-URF13 protein to cause premature tapetal breakdown.

NUCLEAR INFLUENCE ON THE MITOCHONDRIAL GENOME

The plant mitochondrial genome, now fully sequenced in Arabidopsis (169) and Marcantia (128), is organized in a much more complex and variable structure than is observed in other higher eukaryotes (reviewed in 183). In most higher plants, this organization is defined by the presence of recombinationally active repeated sequences that allow for high- and low-frequency inter- and intramolecular recombination events to occur (3). The physical organization of the mitochondrial genome in plants has been difficult to define. Although most genomes map as circular molecules defined by overlapping clones, direct physical observation by pulsed field gel electrophoresis, electron microscopy, and other procedures has indicated that the genome may consist of both linear and circular forms, with molecules much larger than the multiple circles constructed by clone analysis (3, 14, 130).

The nucleus definitely affects mitochondrial genome organization. One value of the remarkable mitochondrial DNA variation existing within plant families is the information it provides regarding evolution of this unusual genome and the cellular forces molding current organization. Examination of variation within the legume family (Fabaceae) provides striking evidence for the ongoing evolutionary transfer of functional genetic information from the mitochondrion.
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to the nucleus via RNA intermediates (25, 127). This evolutionary transfer of mitochondrial genes to the nucleus has presented some intriguing problems, most notably the requirement to move tRNA into mitochondria (33). There is evidence that this transfer likely requires association with the appropriate aminoacyl tRNA synthase to mediate transmembrane import (32).

A multipartite genome organization exists in the mitochondrion of most plant species, with each molecule containing only a portion of the genetic information. Overall structure is further complicated by the variable stoichiometry of specific subgenomic regions. Remarkably, in several plant species, a subset of mitochondrial DNA molecules, atypical genomic organizations termed “sublimons,” can be retained indefinitely in nearly undetectable levels (155, 156). The relative copy number of the various mitochondrial DNA forms and their recombinational activity appears to be under nuclear control. One of the most pronounced examples is the observed loss of a mitochondrial genomic molecule in response to a single nuclear gene in common bean. The \textit{cms}-associated mitochondrial mutation in common bean, \textit{pvs-orf239}, appears to be maintained on a single 210-kb molecule within a tripartite mitochondrial genome organization (78). Introduction of a single dominant nuclear factor, \textit{Fr}, results in a genomic shift of the \textit{pvs-orf239}–containing molecule to substoichiometric levels within the genome, thus restoring pollen fertility (H Janska, R Sarria, M Woloszynska, M Arrieta-Montiel & S Mackenzie, manuscript submitted).

The development of alloplasmic lines, derived by recurrent backcrossing strategies or protoplast fusion to combine different mitochondrial and nuclear genotypes, routinely gives rise to changes in relative stoichiometries and mitochondrial genomic rearrangements in \textit{Nicotiana} (5, 13, 58), \textit{Brassica} (93, 104), and \textit{Triticum} (120, 129, 168). In some cases, it has been possible to identify specific nuclear loci essential to establishing compatibility in individual nuclear-cytoplasmic combinations (4). Moreover, particular nuclear-cytoplasmic genetic combinations in maize can be predicted to give rise to a high frequency of specific mitochondrial mutations. These mutations, referred to collectively as nonchromosomal stripe mutations, generally result in loss of mitochondrial gene function, leaf striping, severe growth impairment, and infertility; \textit{ncs} mutations are maintained in a heteroplasmic state with wild-type, functional mitochondria (125). Several \textit{ncs} mutations affect in distinct loci and have arisen by what appear to be different molecular events.

In \textit{Arabidopsis}, the appearance of mitochondrial mutations is likewise associated with modification of the nuclear genotype. In the case of \textit{Arabidopsis}, mutation at a single dominant nuclear gene, \textit{CHM}, yields mitochondrial genomic rearrangements (113). Notably, the mutant mitochondrial forms arising upon \textit{CHM} mutation are already present in the wild-type lines at substoichiometric levels (147), implying that the role of the \textit{CHM} locus, like that of the \textit{Fr} locus
in bean, may be to suppress copy number of mutant mitochondrial forms. Efforts are ongoing to determine whether the Fr locus in cms bean involve homologous functions (B Li & S Mackenzie, personal communication).

PERSPECTIVES

Chloroplast and mitochondrial development are regulated by complex intracellular interactions. In the case of the plastids, it is likely that the discrete nuclear factors set the program that determines to a large degree the stage of organelle development. In contrast, plastid signaling to the nucleus may be continual throughout development. In the next few years, we will likely see important advances in the characterization of chloroplast signals that trigger changes in nuclear expression.

In the past years, we have seen an important number of breakthroughs in several aspects of the chloroplast field as protein import, transcription, and posttranscriptional events. Now a clearer picture is emerging of the regulatory events that take place in each of these processes. Collectively, in the near future, it will be possible to understand the surprising variety of regulatory mechanisms that in concert permit chloroplast development. In the years to come, the implementation of new approaches will contribute to the identification of molecules required for alternative plastid development pathways. Novel classes of genetic loci in Arabidopsis define unsolved steps of early chloroplast differentiation. Detailed study of these genes during plant development will certainly help us understand the mechanisms operating in early chloroplast development.

In mitochondrial research, several laboratories are now quite close to cloning additional fertility restorers involved in transcript processing, as well as the CHM and Fr loci involved in differential amplification of the genome. A surprising picture emerges as we investigate mitochondrial regulation; namely, that the mitochondrial genome is permitted to expend what would appear to be tremendous energy in the execution of transcription and, in most cases, translation of products destined for rapid turnover. These observations imply that, unlike prokaryotes, factors other than energy conservation may be most influential in plant mitochondria. We predict that future investigations will discover the impetus for retaining functions as perplexing as substoichiometric genomic forms and prolific editing functions. Until such time, the plant mitochondrial genome remains an enigma.

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