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Chloroplast and Mitochondrial DNAs of *Arabidopsis thaliana:* Conventional Genomes in an Unconventional Plant

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Many of the unusual features that make *Arabidopsis thaliana* such an attractive model system for flowering plants relate, either directly or indirectly, to its unconventional nuclear genome. Of paramount importance, the notably small size of this genome, together with the relative lack of repeated sequences and reduced size of many introns and gene families, directly facilitates efforts to map, isolate, and characterize genes. Indirectly, the small size of the nuclear genome may be related to, perhaps in some measure responsible for, the reduced size of the plant and length of its life cycle, features that considerably enhance its genetic tractability.

The chloroplast and mitochondrial genomes of *Arabidopsis* have not shared the limelight with its nuclear genome. This is in part because these organellar genomes have not, until recently, been subject to much experimental study and also because they appear, in distinct contrast to the nuclear genome, to be utterly conventional in all important respects. The primary goal of this chapter is to show how the chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) of *Arabidopsis* are typical of flowering plant organellar genomes in terms of size, organization,

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Arabidopsis
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content of repeats and genes, gene structure, and, probably, modes of expression and regulation. In terms of direct molecular characterization, the *Arabidopsis* mitochondrial genome should soon assume primacy among flowering plant mtDNAs, thanks to a genome-sequencing project that is now more than half complete.

A secondary goal of this chapter is to describe what we view as the proper and rightful swing of the nuclear spotlight onto the organellar genomes of *Arabidopsis*, a swing that is just starting and which should soon accelerate. This swing is a natural reflection of the intricate and extensive interdependency of the nuclear and organellar genomes in controlling organellar biogenesis and function: The great majority of chloroplast and mitochondrial proteins are encoded by nuclear genes, and virtually all regulation of organellar gene expression is mediated by nuclear gene products. Hence, the power of *Arabidopsis* nuclear molecular genetics ought to be fully unleashed onto questions of organellar function and biogenesis.

The first two sections of this chapter summarize our current knowledge of *Arabidopsis* cpDNA and mtDNA with respect to their organization, gene content, and expression. This information is placed in the context of our overall knowledge of organelle genomes in land plants. Finally, we review published studies and describe promising future directions of integrated work on nuclear/organellar genetic interactions in *Arabidopsis*.

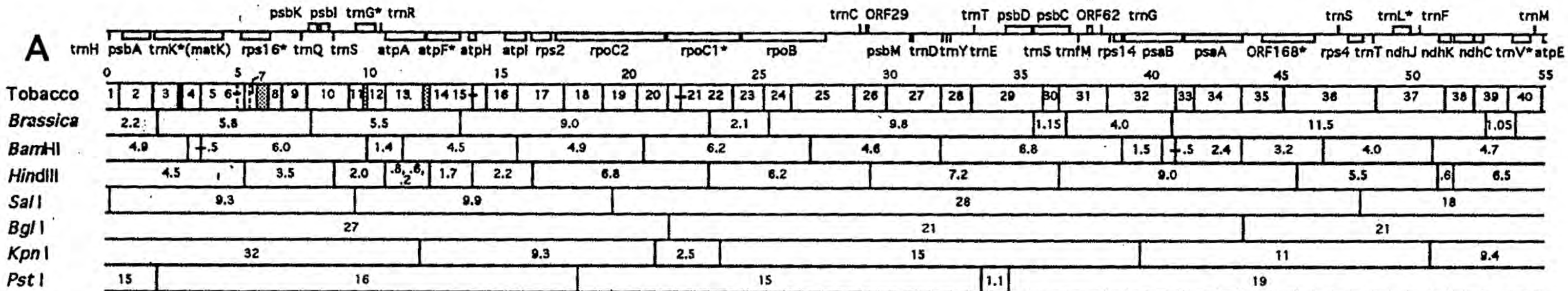
THE CHLOROPLAST GENOME

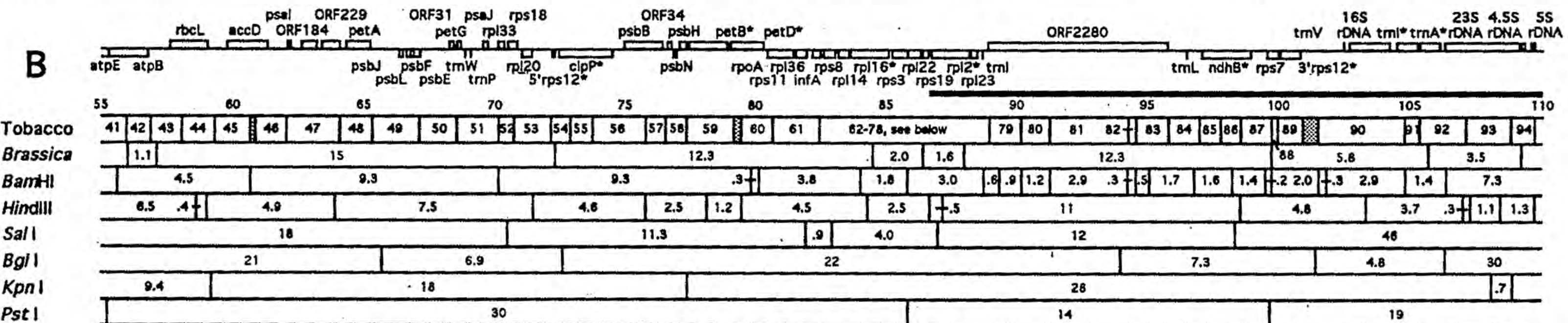
Tobacco as Paradigm

The best perspective for viewing the *Arabidopsis* chloroplast genome is as a tobacco chloroplast genome only slightly modified by the roughly 100 million years of evolution that separate the two plants. This statement follows from three observations: First, with some exceptions, land plant chloroplast genomes are highly conserved in all important aspects of size, organization, and sequence (Palmer 1991, 1992; Wolfe et al. 1991; Downie and Palmer 1992; Sugiura 1992). Second, the tobacco chloroplast genome is the type chloroplast genome for seed plants by virtue of being the only completely sequenced genome (Shinozaki et al. 1986; Shimada and Sugiura 1991) that has the ancestral gene order for this group (Palmer and Stein 1986; Raubeson and Jansen 1992). Third, a series of comprehensive hybridizations between the two genomes shows that *Arabidopsis* and tobacco cpDNAs are virtually identical in size, gene order, and gene content (Fig. 1).

The tobacco chloroplast genome is 156 kb in size and contains the characteristic cpDNA "inverted repeat," an inverted duplication of 25 kb that separates large and small single-copy regions of 87 kb and 18 kb, respectively (Fig. 1) (Shinozaki et al. 1986). The 113 known genes in the tobacco genome can be grouped into four classes according to function (Shimada and Sugiura 1991; Sugiura 1992). More than half of the genes encode components of the machinery for gene expression, including 4 subunits of RNA polymerase, a putative splicing factor, 4 ribosomal and 30 transfer RNAs, 21 ribosomal proteins, and a translation initiation factor (although the gene for the latter may be a pseudogene in tobacco; Wolfe et al. 1992b). Another 40 genes encode either known components of the photosynthetic apparatus (29 genes) or subunits of a chlororespiratory NADH dehydrogenase (11 genes) thought to be associated with photosynthetic metabolism (dePamphilis and Palmer 1990; Wolfe et al. 1992a). The functions of another 10 genes are completely unknown. Finally, only 2 genes, encoding subunits of the clpP protease and acetyl-CoA carboxylase of fatty acid synthesis, are known to specify proteins that function in some process other than gene expression or photosynthesis. In this respect, tobacco and other angiosperm cpDNAs are notably poor; even the bryophyte *Marchantia polymorpha* contains several additional chloroplast genes, for sulfate transport and light-independent chlorophyll biosynthesis (Wolfe et al. 1991), whereas the genomes of nongreen algae encode a remarkable number and diversity of proteins involved in both genetic and metabolic activities (see, e.g., Reith and Munholland 1993).

The chloroplast genes of tobacco and other land plants are organized and expressed in an intriguing melange of ways, some primitive and prokaryotic, others derived subsequent to the endosymbiotic origin of the chloroplast some 1 billion years ago (Gray 1989, 1992). On the one hand, most genes are tightly packed into eubacterial-like operons (Fig. 1). Many transcription units contain eubacterial-like "-35" and "-10" promoter elements, and the genome encodes all four core subunits of a eubacterial-like RNA polymerase (Igloi and Kössel 1992). Chloroplast ribosomes are highly similar to eubacterial 70S ribosomes in size, subunit composition, and antibiotic resistance properties (Subramanian et al. 1991). On the other hand, some genes, including tRNA genes with internal promoters, do not contain obviously bacterial promoters, and there is increasing evidence that a second, nuclear-encoded RNA polymerase of probable nonbacterial ancestry is also active in transcribing chloroplast genes (Morden et al. 1991; Igloi and Kössel 1992; Wolfe et al. 1992a; Hess et al. 1993; Lerbs-Mache 1993). RNA processing is much more complex in plastids than in bacteria. Tobacco and most angiosperms con-


A
psbK psbI trnG* trnR
trnC ORF29
trnT psbD psbC ORF62 trnG
trnS trnL* trnF
trnM
trnH psbA trnK*(matK) rps16* trnQ trnS atpA atpF* atpH atpI rps2 rpoC2 rpoC1* rpoB
psbM trnD trnY trnE trnS trnfM rps14 psaB psaA ORF168* rps4 trnT ndhJ ndhK ndhC trnV* atpE
Tobacco
Brassica
BamHI
HindIII
SalI
BglI
KpnI
PstI


B
psaI ORF229
rbcL accD ORF184 petA
ORF31 psaJ rps18
petG rpl33
ORF34
psbB psbH petB* petD*
ORF2280
16S rDNA
trnV trnI* trnA*
23S rDNA 4.5S rDNA 5S rDNA
atpE atpB
psbJ psbF trnW
psbL psbE trnP
rpl20 5'rps12* clpP*
psbN
rpoA rpl36 rps8 rpl16* rpl22 rpl2* trnI
rps11 infA rpl14 rps3 rps19 rpl23
trnL ndhB* rps7 3'rps12*
62-78, see below
Tobacco
Brassica
BamHI
HindIII
SalI
BglI
KpnI
PstI

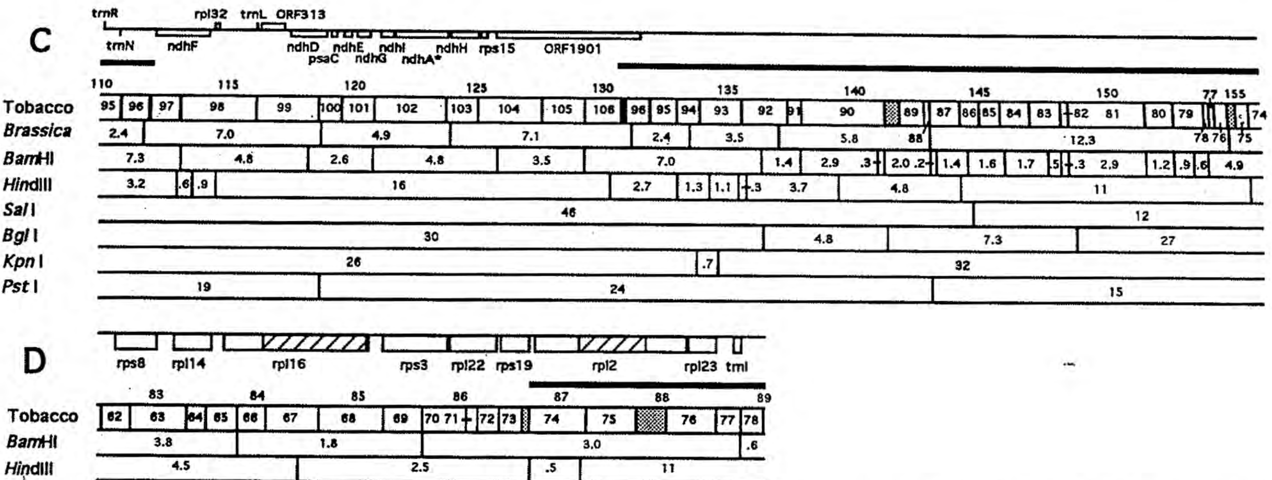


Figure 1 Physical and gene maps of the 153-kb chloroplast genome of *A. thaliana* cv. Columbia. Parts *A–C* comprise the entire genome; *D* is a blow-up of the region corresponding to tobacco probes 62–78. Top map of *A–D* is a gene map of tobacco cpDNA (Shinozaki et al. 1986; as modified by Sugiura 1992 and Wolfe et al. 1992b); genes above the map are transcribed from left to right and vice versa. Asterisks in *A–C* indicate genes with introns (all marked genes contain one intron, except *clp*P, *ORF168*, and the *trans*-spliced *rps12*, which have two). Introns in *D* are hatched. Within experimental limits, the tobacco gene map is the same as the *Arabidopsis* gene map, i.e., the 106 tobacco probes in the maps labeled "tobacco" hybridized in a colinear manner to *Bam*HI and *Hin*dIII fragments of *Arabidopsis* cpDNA and without any evidence of major insertion or deletion. Stippled boxes in the tobacco probe maps indicate short regions not used as probes. Numbers above the tobacco probe maps give the coordinates of the tobacco genome in kb (Shinozaki et al. 1986). Maps labeled "*Brassica*" give sizes of cloned cpDNA fragments from *B. campestris* (Nugent and Palmer 1988) that were used as hybridization probes to digests of *Arabidopsis* cpDNA with *Sal*I, *Bgl*I, *Kpn*I, and *Pst*I. The bottom six maps in *A–C* and the bottom two maps in *D* show restriction site maps for *Arabidopsis* cpDNA for the indicated enzymes. Fragment sizes are in kb; the maps are not drawn precisely to scale, but are slightly adjusted so that the *Arabidopsis* genome is drawn to the exact scale of the tobacco genome, with the two genomes aligned according to results from the hybridization experiments described above. Long black bars indicate the extent of the 25-kb inverted repeat. Maps portray these chloroplast genomes in only one of their two orientations (in the other, the single copy regions are reversed in polarity; Palmer 1983, 1991).

tain 21 introns; 20 of these are group II introns of likely postendosymbiotic acquisition by the green alga ancestors of land plants (Palmer 1991), whereas only a single intron, a group I intron in a tRNA-Leu gene, appears to be of cyanobacterial ancestry (Kuhsel et al. 1990; Xu et al. 1990). In addition to splicing, chloroplast transcripts are subject to complex pathways of 5′and 3′trimming and, for polycistronic transcripts, internal cutting (Sugiura 1991, 1992; Herrmann et al. 1992). In contrast to bacteria, where most control of gene activity is exerted at the level of transcription, chloroplast gene activity is also thought to be regulated extensively at posttranscriptional levels of RNA processing, RNA stability, and translation (Link 1991; Rochaix 1992a,b; Gruissem and Tonkyn 1993).

Arabidopsis and Tobacco cpDNAs Compared

The only published studies on *Arabidopsis* cpDNA report the unremarkable sequences of a few of its genes (Chen et al. 1988; Luschnig and Schweizer 1992) and observations on duplicated cpDNA sequences in *Arabidopsis* mtDNA (Nugent and Palmer 1988; discussed below in The Mitochondrial Genome). In addition, the chapter by Price et al. (this volume) describes phylogenetic studies that have used restriction site and *rbc*L sequence data from *Arabidopsis* cpDNA. In this chapter, we report the first complete restriction site map for the *Arabidopsis* chloroplast genome and a complete gene map as deduced from a comprehensive series of hybridizations between tobacco gene probes and *Arabidopsis* cpDNA. This gene map was constructed as part of a much larger study on the evolution of chloroplast genome organization and gene and intron content in angiosperms (Downie and Palmer 1992). We are using this chapter as the primary and original place of publication of these physical and gene maps; i.e., they will not be reported in any journal article, and, hence, this chapter constitutes the proper place for citation of these results. These mapping studies were conducted by S.R. Downie, J.M. Nugent, and J.D. Palmer.

A cleavage site map of *Arabidopsis* cpDNA for six restriction enzymes was constructed in two ways. Sites for *Bam*HI and *Hin*dIII were mapped by hybridizing the 106 tobacco cpDNA probes shown in Figure 1 to filter-blots containing digests of *Arabidopsis* cpDNA with these two enzymes. Sites for *Sal*I, *Bgl*I, *Kpn*I, and *Pst*I were mapped by hybridizing the 22 *Brassica* probes also shown in Figure 1 to digests with these four enzymes. The use of two unconnected sets of hybridizations means that there is some uncertainty in the exact placement of sites for *Bam*HI and *Hin*dIII relative to those for the other four enzymes. Furthermore,

because all hybridizations were to single digests of *Arabidopsis* cpDNA, there is also uncertainty in the exact registry of the single enzyme maps within each of the two sets of maps. However, this uncertainty is minimal for *Bam*HI and *Hin*dIII compared to the other four enzymes, due to the small sizes of the tobacco fragments used as probes and of the *Arabidopsis* fragments being mapped.

The restriction site map (Fig. 1) shows that the *Arabidopsis* chloroplast genome is a circular chromosome 153 kb in length. Thus, the *Arabidopsis* genome is very similar in size to genomes in *Brassica* and related crucifers (Palmer et al. 1983; Warwick and Black 1991) and less than 3 kb smaller than the tobacco genome (Shinozaki et al. 1986). The four sectors of the *Arabidopsis* chloroplast genome—the two inverted repeats and the large and small single-copy regions—are also of a typical, tobacco-like size.

The 106 tobacco probes contain 98.1% of the 130.5-kb sequence complexity of the tobacco genome and average 1.2 kb in size. Fifty-five probes are internal to or contain part of but a single gene, 30 contain all or part of two genes, and 21 probes contain parts of more than two genes (Fig. 1). This level of coverage of the entirely sequenced tobacco genome allows a fine level of inference about gene content and location in *Arabidopsis* cpDNA (Fig. 1). Each of the 106 tobacco probes showed significant hybridization to *Arabidopsis* cpDNA as normalized relative to 15 other angiosperm cpDNAs present on the same set of replica filter blots. That is, under the same conditions, a number of probes failed to hybridize to one or more of the other cpDNAs, leading to inferences (in some cases proven by subsequent sequence analysis) of specific losses of individual genes or introns (see, e.g., Gantt et al. 1991; Downie et al. 1991; for review, see Downie and Palmer 1992). Moreover, the 106 tobacco probes hybridized in a colinear manner to *Arabidopsis* cpDNA (Fig. 1). Thus, gene order and content appear to be the same in tobacco and *Arabidopsis* cpDNAs.

The limits of these experiments must be emphasized. Part or all of a small gene that comprises only a short region of a particular tobacco probe fragment could in fact be absent from the *Arabidopsis* genome, and yet we would have no basis for suspecting such an absence. Even a gene that occupies the entire length of a strongly hybridizing tobacco fragment could be nonfunctional, if it contained a frameshift or other subtle mutation that would only be detectable by sequencing. However, because all tobacco probes hybridize so well, because the two genomes are so similar in size, and because their aligned maps show no evidence of gene deletion in *Arabidopsis*, our estimate is that few if any genes present in tobacco cpDNA have been lost from or are nonfunctional

Arabidopsis cpDNA.

The 21 introns in tobacco cpDNA range in size from 503 bp to 1148 bp. Only 2 of these, from *rpl2* (also see Downie et al. 1991) and *rpl16*, were represented by intron-specific probes among the 106 probes shown in Figure 1. In other experiments, we have also tested specifically for the presence of the *cis*-spliced introns in *trnI* and *rps12*. All 4 of these introns are present in their respective genes in *Arabidopsis* cpDNA. These results, together with the above-stated conclusion that there is no evidence for any major deletions from the *Arabidopsis* chloroplast genome (relative to tobacco), lead us to tentatively conclude that intron content in *Arabidopsis* cpDNA is likely to be very similar, possibly identical, to that in tobacco.

In summary then, within the limits of detection of these mapping studies, the *Arabidopsis* chloroplast genome is identical to that of tobacco in gene order, gene content, and intron content. This conclusion should come as no surprise, given the large body of evidence indicating that land plant cpDNAs are highly conserved in their evolution (see beginning of preceding section).

An *Arabidopsis* Chloroplast Genome Project?

It seems inevitable that the *Arabidopsis* chloroplast genome will eventually be entirely sequenced, so the relevant question is not whether, but when. Given the inherent conservatism of cpDNA evolution and the (unremarkable) similarity between *Arabidopsis* cpDNA and the fully sequenced genome of tobacco, we would argue that sequencing of the former genome should not be a high priority. Instead, it would seem appropriate to sequence this 153-kb genome only as part of a much larger project to sequence the nuclear genome of *Arabidopsis*; i.e., it would be a poor investment to sequence the chloroplast genome until sequence technologies and funding allow efficient sequencing of the approximately 1000 times larger nuclear genome. In the meantime, any sequencing of *Arabidopsis* cpDNA should continue to be carried out in a piecemeal fashion by those scientists who need to know more about a particular region of the genome.

THE MITOCHONDRIAL GENOME

The situation with respect to the mitochondrial genome of *Arabidopsis* and other land plants stands in direct contrast to that for the corresponding chloroplast genomes. Plant mtDNAs are spectacularly variable in size and structure and are relatively poorly understood (only a single

genome, from the bryophyte *Marchantia polymorpha*, is completely sequenced [Oda et al. 1992b], and little is known about mitochondrial gene expression), whereas *Arabidopsis* mtDNA is now one of the best-characterized genomes. These differences dictate a completely different organization of this section compared to the preceding one. Here, we present a straightforward review of what is known about *Arabidopsis* mtDNA—its structure, genes, and expression—and interweave this material with general comparisons to other plant mitochondrial genomes.

The *Arabidopsis* Mitochondrial Genome Project: Overview of Genome Structure

The laboratory of A. Brennicke is now about 60% through a project to determine the entire sequence of the approximately 372-kb mitochondrial genome of *A. thaliana*. This is likely to be the first complete mtDNA sequence from any flowering or even vascular plant. The ultimate goal of this project is to provide a major part of the necessary background information for studies on plant mitochondrial gene expression and regulation. Indeed, as described below, a substantial start in this direction has already been made. An ancillary goal is to further our understanding of the processes that have led to the extraordinarily large sizes of plant mtDNAs, some of which are more than 100 times larger than animal mtDNAs.

Analysis of more than 300 individual cosmids has allowed construction of a single, circular linkage map that includes their entire sequence complexity (Fig. 2). This map contains two different pairs of large (several kb) repeated sequences that meet the definition (Stern and Palmer 1984) of "recombination repeats," i.e., sequences that recombine at a high enough frequency to generate substantial levels of genomic isomers that differ only with respect to the combinations of unique sequences that flank the repeats. Cosmids containing all four possible combinations of repeat-flanking sequences have been isolated for each set of repeats. The particular representation of the *Arabidopsis* mitochondrial genome shown in Figure 2 features one pair of direct repeats interspersed with one pair of inverted repeats. All four genomic isomers resulting from this arrangement of recombination repeats are formally diagramed in Figure 1C of Lonsdale (1984). For *Arabidopsis*, these would include three different master chromosomes of 372 kb, which interconvert with each other via recombination between inverted repeats, and one set of subgenomic circles (of ~234 kb and ~138 kb), which are generated from two of the master circles via recombination between direct repeats. In complexity of organization, *Arabidopsis* mtDNA ranks midway between the

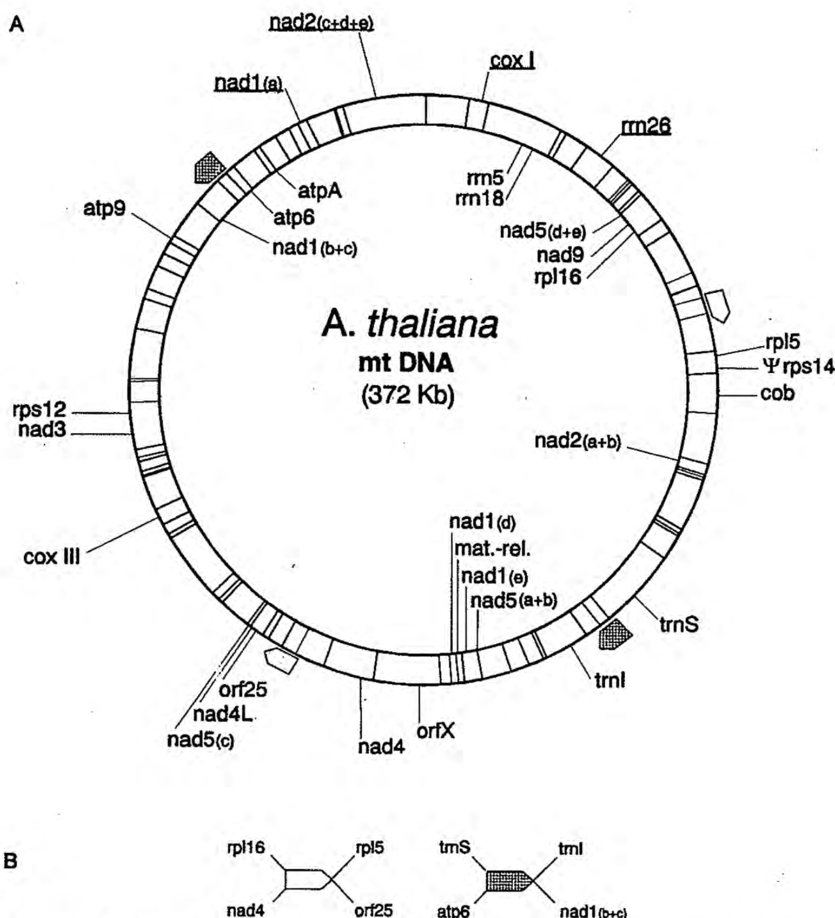


Figure 2 Physical and gene maps of the 372-kb mitochondrial genome of *A. thaliana* line C24. (*A*) Circular map containing the entire sequence content of the genome. Radial lines indicate *Bam*HI sites. Stippled and cross-hatched thickened arrows indicate the positions of the two pairs of large (several kb) repeated elements in the genome. Recombination between members of each pair, present in either direct or indirect orientation, leads to three other isomeric forms of the genome besides the one shown (see text and Fig. 1C of Lonsdale et al. 1984). Only those genes whose positions on the map are precisely known are shown (cf. Table 1). The positions of genes and of exons of three *trans*-spliced genes are marked outside the circle for sequences transcribed clockwise and vice versa. (*B*) Arrangements of the four unique sequences flanking each of the two different major mtDNA repeats. For example, the genome representation in *A* shows the *nad4-orf25* and *rpl16-rpl5* configurations of the stippled repeat, whereas the other two configurations of this repeat (flanked by *nad4-rpl5* and *rpl16-orf25*) are present in other isomeric forms of the genome that result from recombination between the two copies of this repeat.

fairly simple genomes of spinach, sunflower, and most other crucifers, which exist as one master chromosome and one pair of subgenomes, and the highly complex genomes of maize, wheat, sugar beet, and petunia (Palmer 1992).

For purposes of discussion, the *Arabidopsis* mitochondrial genome can be broken down into five overlapping categories of sequences: genes, introns, repeated elements, sequences of chloroplast origin, and everything else. Genes and introns are discussed in the following two sections. The two largest repeats, highly active in recombination, are discussed in the preceding paragraph. A large number of other, much shorter repeats (up to 160 bp in size) are dispersed throughout the genome. These include duplications of both noncoding regions and fragments of genes, such as portions of *nad3*, exon A of *nad5*, and the 18S and 26S rRNA genes (A. Brennicke et al., unpubl.). The potential function of these short repeats with respect to transcription and translation signals needs further investigation (Wissinger et al. 1991a).

Extrapolation from the sequenced portions of the *Arabidopsis* mitochondrial genome suggests that roughly 15% of the genome exists as repeat sequence DNA. Similar estimates have been made for the much larger genomes of cucurbits by reassociation kinetics (Ward et al. 1981) and for the smaller, 218-kb *Brassica campestris* genome by comprehensive Southern hybridization and partial sequencing studies (M. Shirzadegan and J. Palmer, unpubl.). In the latter case, an estimated 66 distinct families of repeats are present, with each kilobase of the genome containing, on average, one repeat element. Overall then, although sequence duplication contributes a significant fraction to the inordinately large sizes of angiosperm mitochondrial genomes, additional forces must also be responsible.

One well-known way in which plant mtDNAs grow larger is by the fairly frequent and stable uptake of cpDNA-derived sequences (Schuster and Brennicke 1988; Palmer 1992). A low-resolution, hybridization study showed that *Arabidopsis* mtDNA contains only about half as many cpDNA sequences (5–7 kb; ≤2% of the mitochondrial genome) as most other crucifer genomes of substantially smaller size (Nugent and Palmer 1988). These sequences include portions of the chloroplast *rbc*L and *psa*A genes. Several cpDNA-derived regions have been sequenced, including $tRNA^{Ser}$, $tRNA^{Met}$, and part of 23S rRNA (Wintz et al. 1988; Brandt et al. 1993; P. Brandt and M. Unseld, unpubl.). Unlike all other cpDNA-derived sequences, most chloroplast tRNA genes appear to be functional within angiosperm mitochondria (see, e.g., Joyce and Gray 1989).

Although exemplary of flowering and, perhaps, vascular (Palmer et

al. 1992) plants in all of the above respects, *Arabidopsis* mtDNA differs substantially from the fully sequenced genome of the bryophyte *Marchantia polymorpha* (Oda et al. 1992b). At 187 kb, the *Marchantia* genome is almost exactly half the size of the *Arabidopsis* genome; although this size is large for non-plants, it is smaller than that of any flowering plant. Although it contains "quite a few" repetitive sequences (Oda et al. 1992b), *Marchantia* mtDNA lacks any large recombination repeats and exists as a simple, unicircular chromosome, in contrast to all but one characterized angiosperm mtDNA (Palmer and Herbon 1987; Palmer 1992). Finally, *Marchantia* mtDNA does not appear to contain any cpDNA-derived sequences (Oda et al. 1992a,b).

Gene Content and Organization

About 50 genes have been identified thus far on the *Arabidopsis* mitochondrial genome. Most have been identified by complete sequencing of the gene locus, a few by partial sequencing, and several by heterologous hybridization only. These include a full set of three rRNA genes and about a dozen tRNA genes (Table 1). As in other angiosperms, translation in *Arabidopsis* mitochondria probably uses three sets of tRNAs of distinct genetic origins, a set of about 15 tRNAs of mitochondrial origin, several tRNAs encoded by mtDNA sequences of chloroplast origin (see above), and several imported tRNAs encoded by nuclear genes of eukaryotic origin (Joyce and Gray 1989; Dietrich et al. 1992).

Although only approximately 60% sequenced, *Arabidopsis* mtDNA is already known to contain substantially more protein genes (>30) than the fully sequenced genomes of animals (12–13), fungi (7–14), the green alga *Chlamydomonas reinhardtii* (7), the ciliate *Paramecium aurelia* (16), the trypanosome *Trypanosoma brucei* (12), and the apicomplexan *Plasmodium falciparum* (3) (Gray 1992). The only mitochondrial genome known to encode many more proteins (60–70) is that of *Marchantia* (Oda et al. 1992b). However, we expect that *Arabidopsis* will turn out to contain a similar number of mitochondrial protein genes, given that so much of the genome remains to be sequenced. The mystery of why plant mtDNAs encode so many proteins is really just part of the larger mysteries of why gene content is so extraordinarily variable among mitochondria as a whole (see above), and why so many different lineages (e.g., *Saccharomyces*, *Chlamydomonas*, *Plasmodium*, with only 3–7 encoded metabolic proteins) have independently experienced near-extinctions of their mitochondrial genomes. This unusual abundance of genes is clearly an additional factor responsible for the large baseline size of plant mitochondrial genomes.

Table 1 Gene and intron content of *Arabidopsis* mtDNA

Class of gene product	Gene name
Ribosomal RNA	*rrn26, rrn18, rrn5*
Transfer RNA	*trnE, trnF, trnF, trnG, trnH, trnI, trnK, trnN, trnS, trnS, trnT, trnY*
Small subunit ribosomal protein	*rps3*(1), *rps7, rps12, ψrps14, ψrps19*
Large subunit ribosomal protein	*rpl2, rpl5, rpl16*
ATP synthase subunit	*atpA, atp6, atp9, ψatp9*
Cytochrome *c* oxidase subunit	*coxI, coxII, coxIII*
Cytochrome *b* subunit	*cob*
NADH dehydrogenase subunit	*nad1*(4), *nad2*(4), *nad3, nad4*(3), *nad4L, nad5*(4), *nad6, nad7*(4), *nad9*
Open reading frames	*orfX, orf25, ψorf86A, orf169, orf206, orf322*(1), *orf509, mat-r*

For all genes with introns, the number of introns is given in parentheses after the gene name. References for published gene sequences: Wintz et al. 1988; Chen et al. 1989; Binder et al. 1991; Knoop et al. 1991; Wissinger et al. 1991a; Aubert et al. 1992; Brandt et al. 1992, 1993; Martínez-Zapater et al. 1992; Schuster et al. 1993. All other sequences are unpublished data of A. Brennicke et al. Also see Grohmann et al. 1993; Wissinger et al. 1991b.

In terms of function, the *Arabidopsis* mitochondrial protein genes can be conveniently divided into three groups (Table 1). Sixteen genes encode subunits of four respiratory chain complexes; it is members of this class of genes that are most commonly found throughout the spectrum of mitochondrial genomes. Eight genes are homologous to eubacterial ribosomal protein genes. However, two of these (*rps14* and *rps19*) are pseudogenes, and no sequences at all for a third (*rps13*) were detectable by hybridization (Aubert et al. 1992; Brandt et al. 1993; Nugent and Palmer 1993; A. Brennicke, unpubl.). Functional copies of all three of these ribosomal protein genes may have been recently relocated to the nucleus in *Arabidopsis*, as has been demonstrated for *coxII* in legumes (Nugent and Palmer 1991; Covello and Gray 1992) and *rps12* in *Oenothera* (Grohmann et al. 1992). Finally, eight genes are open reading frames (most with conserved counterparts known in other mitochondrial genomes) of poorly known or unknown function (Table 1). One of these (*orf509*) is homologous to a bacterial gene whose product is involved in cytochrome *c* biogenesis (Schuster et al. 1993).

As in other angiosperm mtDNAs, genes in *Arabidopsis* mtDNA are scattered randomly in the genome and, for the most part, transcribed singly. Reflecting the extremely rapid rate of inversions and other rearrangements in plant mtDNA, the order of genes in *Arabidopsis* is quite different from that in other plants (Palmer et al. 1992). Compared to *Marchantia*, where 14 of 16 ribosomal protein genes are organized into

two clusters (putative operons) of eubacterial organization (Takemura et al. 1992), most ribosomal protein genes are scattered in *Arabidopsis*, with the only organizational trace of their eubacterial ancestry being the cotranscribed *rpl5* and *ψrps14* genes.

Introns and *cis*- and *trans*-Splicing

Little is known about the transcription of *Arabidopsis* mitochondrial genes, although some progress has been made on this subject in other plant systems (for review, see Gray et al. 1992). However, as described in this and the following section, much is known about the unusual RNA processing of transcripts in mitochondria of *Arabidopsis* and other plants. A total of 21 introns have been identified in *Arabidopsis* mtDNA, most by gene sequencing (Knoop et al. 1991; Wissinger et al. 1991b; A. Brennicke, unpubl.) and a few by heterologous hybridization (Nugent and Palmer 1993 and unpubl.). All 21 introns are group II introns, compared to 25 group II introns and 7 group I introns in *Marchantia* mtDNA (Ohta et al. 1993). Only one of these introns, intron C in *nad2*, is homologous in position in *Arabidopsis* and *Marchantia* mtDNAs (Nozato et al. 1993), yet most of these introns are present broadly in all monocots and dicots examined (Nugent and Palmer 1993 and unpubl.). It thus seems likely that angiosperms acquired most of their introns after their divergence from a common ancestor with bryophytes.

The mitochondrial introns in *Arabidopsis* and other angiosperms are largely restricted to a specific subset of protein genes (Table 1). No introns are present in any tRNA or rRNA genes or in 27 of 34 completely sequenced protein genes, and 2 genes (*rps3* and *orf322*) each contain a single intron. On the other hand, the remaining 19 introns are clustered within five NADH dehydrogenase genes, four of which have four introns each (Table 1). *Marchantia* mtDNA has a more random distribution of introns in both respects (Oda et al. 1992b), and the significance, if any, of the angiosperm pattern is unclear.

The most striking aspect of angiosperm mitochondrial introns is the extent to which they have been fractured by rearrangement events that have created *trans*-spliced introns from formerly *cis*-spliced ones. Only two *trans*-spliced group II introns are known outside of angiosperm mitochondria (one in *rps12* of land plant chloroplasts [Fig. 1] and the other in *psa*A of *Chlamydomonas* chloroplasts [Kück et al. 1987]), whereas 5 of the 12 group II introns in the *Arabidopsis nad1*, *nad2*, and *nad5* genes are *trans*-spliced (Knoop et al. 1991; Wissinger et al. 1991b; A. Brennicke, unpubl.), and an additional *nad1* exon is *trans*-spliced in wheat and petunia (Chapdelaine and Bonen 1991; Conklin et al. 1991).

With the exception of this *nad1* intron, the *trans*-spliced arrangements of these three genes are highly conserved among monocots and dicots (Knoop et al. 1991; Wissinger et al. 1991b; Nugent and Palmer 1993 and unpubl.), indicating that these introns were fractured prior to the emergence of angiosperms. Particularly striking is the structure of the *nad5* gene, which requires two *trans*-splicing events to join the central exon of only 22 nucleotides to its two flanking exons (Knoop et al. 1991).

The mechanism of *trans*-splicing of group II introns is thought to be essentially the same as for *cis*-splicing, with the folded secondary structure of the uninterrupted *cis*-spliced intron (Michel et al. 1989) mimicked by the noncovalent association of base-paired halves of the interrupted group II intron (Wissinger et al. 1992). Interestingly, all of the *trans*-spliced fractures in *Arabidopsis* mitochondrial genes occur within domain IV of the group II intron, and the specificity of *trans*-splicing may be associated with a somewhat longer base-pairing stem of this interrupted domain. The RNA editing events that characterize most plant mitochondria mRNAs (see next section) also occur within introns, and some of these edits may improve the extent of intronic base-pairing and thereby facilitate *trans*-splicing (Wissinger et al. 1991b, 1992; Binder et al. 1992). The relative timing of splicing and editing events has been investigated for *nad1* in petunia (Sutton et al. 1991) and *coxII* in maize (Yang and Mulligan 1991). It appears that intron excision is not required for editing; editing can precede splicing at any editing site, yet unspliced RNAs are generally less completely edited than spliced RNAs. Thus splicing and editing, although independent, are temporally correlated.

RNA Editing

Extensive mRNA editing has been observed in mitochondria of all flowering plants and at least one conifer. Seed plants again differ, and again in a derived manner, from *Marchantia*, in which mtRNA editing is thought to be absent or very infrequent (Oda et al. 1992b). Sequence comparisons of cDNAs and genomic DNA show that transcripts from each of eight *Arabidopsis* protein genes investigated (*nad3*, *nad4L*, *nad5*, *cob*, *rps12*, *ψrps14*, *rpl5*, and *orf25*) are edited. All edits found to date in *Arabidopsis* mitochondria are C to U transitions; in other flowering plants a few U to C changes have also been observed (Bonnard et al. 1992; Wissinger et al. 1992; Gray and Covello 1993). Most editings occur at replacement positions, i.e., they change the encoded amino acid. For example, all 9 *nad4L* edits in *Arabidopsis* lead to amino acid changes, whereas only 1 of 10 edits for *rpl5* is silent (Brandt et al. 1992).

With the exception of cytochrome *b*, for which all examined cDNA

clones show identical, apparently complete, editing patterns, each of the other seven *Arabidopsis* genes studied shows variably complete editing among sequenced cDNAs. Editing frequencies at conserved sites vary between *Arabidopsis* and other plant species. In *Arabidopsis*, approximately 90% of the transcripts containing the *nad3* and the *rps12* reading frames are edited at all identified positions, whereas some sites in the *nad3* gene are altered in only about 50% of cDNA clones from *Oenothera* (Schuster et al. 1990). The *Arabidopsis rps14* pseudogene, although interrupted by a stop codon and a genomic frameshift, is transcribed and edited at three positions (Aubert et al. 1992). A gene requiring unusually little RNA editing is *atp6*, for which all 20 non-silent editing positions described for *Oenothera* (Schuster and Brennicke 1991) are already encoded as Ts in *Arabidopsis* mtDNA. However, the *Arabidopsis atp6* mRNA is expected to be edited at one additional site that is edited in *Brassica* (Handa and Nakajima 1992) but not *Oenothera*.

The mechanism(s) of and sources of information and specificity for RNA editing in plant mitochondria are entirely unknown. Determination of the complete sequence of the *Arabidopsis* mitochondrial genome should allow searches for potential *trans*-acting factors (e.g., something akin to guide RNAs of trypanosomes; Cattaneo 1991; Stuart 1991) of the RNA editing process.

ORGANELLE DNA MUTATIONS AND NUCLEAR MUTATOR GENES

It is well established that alterations in angiosperm mtDNA may lead—in combination with a certain nuclear genotype—to cytoplasmic male sterility (CMS). Although CMS mutations are known from a large number of plants (most prominently, maize and petunia [Hanson 1991; Braun et al. 1992; Levings and Siedow 1992]), including several cultivated crucifers (Makaroff and Palmer 1988; Makaroff et al. 1989; Singh and Brown 1991; Bonhomme et al. 1992), none has yet been reported in *Arabidopsis*. Until recently, the only other phenotypic class of plant mtDNA mutations were the nonchromosomal stripe (NCS) mutants of maize, which have variegated, striped leaves (Newton et al. 1989). In contrast, numerous cpDNA mutants have been analyzed. These may occur spontaneously, may be induced by mutagens, or may occur with usually high frequency in response to certain nuclear mutations (for review, see Börner and Sears 1986; Hagemann 1992).

In *Arabidopsis*, a large number of nuclear mutants have been selected that affect morphology, specific components, development, differentiation, and functions of plastids (Rédei 1992; Chory and Susek, this volume). This contrasts with a surprisingly low number of cpDNA

mutants. No spontaneous mutation is known in *Arabidopsis* that is inherited in the uniparental, maternal manner typical for mutations in the plastid (and mitochondrial) genome. However, Röbbelen (1962) reported on the appearance of cpDNA mutants after irradiation of *Arabidopsis* with X-rays.

The way in which a nuclear gene induces mutations in organellar DNA is unknown. However, the available data indicate the existence of at least two mechanisms. One category of nuclear mutator genes always induces a single type of organellar mutation (deduced from identical phenotypes), such as *iojap* and *chloroplast mutator* of maize (Walbot and Coe 1982; Thompson et al. 1983; Han et al. 1992); *Okina-muki*, *albostrians*, and *striata 4* of barley (Imai 1928; von Wettstein 1961; Hagemann and Scholz 1962; Hess et al. 1993); and, probably, *albomaculans* of *Arabidopsis* (Röbbelen 1966). Another category of nuclear mutator genes induces various kinds of mutations (again deduced from the phenotype, which in this case is variable). To this group belong the *mp1* and *mp2* alleles of *Epilopium hirsutum* (Michaelis 1968a,b), the cpDNA mutator of *Oenothera hookeri* (Epp 1973; Chui et al. 1990), and the *chm* locus of *A. thaliana* (Rédei 1973). As described in the following section, mutations at this last locus have been extensively studied and have led to some recent surprises.

The Choroplast Mutator of *Arabidopsis* Causes mtDNA Rearrangements

Rédei (1973) discovered a nuclear gene (*chm*) that acts as a chloroplast mutator in *A. thaliana*. He showed that the spontaneous rate of appearance of new plastid phenotypes in leaf cells was increased about 10^6-fold in plants that were homozygous for certain mutant alleles at the *chm* locus. Once induced, the new phenotypes were inherited independently of the mutator gene in the uniparental, maternal manner characteristic of cytoplasmic mutations.

The mutator gene induces a variety of phenotypes: discolored leaf sectors (white, yellow, pale green), distorted leaf differentiation ("rough and ragged" leaves), and impaired fertility (Rédei and Plurad 1973). Electron microscopy revealed an almost continuous series of plastid aberrations, affecting both overall plastid morphology and internal membrane architecture, in cells of the discolored sectors (Rédei and Plurad 1973; Rédei 1974; Mourad and White 1992). The occurrence of two or more types of plastids (both normal chloroplasts and variously aberrant plastids) within a single, "mixed" cell is regarded as good evidence that the plastid genome is the site of the mutation (Rédei and Plurad 1973;

Mourad and White 1992). Nuclear (and mitochondrial) mutations that affect plastids should lead to the same aberration in all plastids of the affected cells (Hagemann, 1964; Rédei, 1967). In addition, the ultrastructure of mitochondria was normal in chm cells with aberrant plastids (Rédei and Plurad 1973).

As pointed out by Rédei and Plurad (1973), the observation of variable plastid phenotypes does not necessarily prove that different plastid mutations were responsible for each type of alteration. Therefore, the isolation of different, apparently homoplastidic mutants with color variations or rough-leaf phenotypes provides not only excellent material for further investigations into the molecular basis of the mutations, but also evidence for the induction of different organellar DNA mutations (Rédei 1973; Mourad and White 1992).

Martínez-Zapater et al. (1992) studied a new variegated mutant of *Arabidopsis* that results from mutation at the *chm* locus. As in Rédei's (1973) analyses, the new allele (designated *chm-3*) induces clearly cytoplasmic mutations in all homozygous plants. Southern hybridizations of total DNA from normal and variegated plants with several cpDNA probes showed identical band patterns. This is consistent with studies on cpDNA isolated from normal and homoplastidic mutant leaves (Mourad and White 1992; G.P. Rédei, pers. comm.). Surprisingly, however, two of four mtDNA probes hybridized to additional mtDNA fragments in variegated plants compared to normal plants (Martínez-Zapater et al. 1992). Furthermore, these new bands cosegregate with the mutant phenotype, and their abundance appears to correlate with the extent of variegation. Sequence analysis of one of the new mtDNA fragments showed that it is rearranged relative to wild-type mtDNA (Martínez-Zapater et al. 1992). A number of examples of specific nuclear gene effects on mitochondrial genome sorting-out and/or rearrangement have also been described in maize and bean (see Martínez-Zapater et al. 1992).

Mitochondria and chloroplasts are known to be intimately related metabolically and sometimes even physically, and therefore mutations in the DNA of one organelle might be expected to affect the other organelle. The NCS mutants of maize (see above) are prime examples in which various mutations in mtDNA lead to altered ultrastructure and physiology of chloroplasts (see, e.g., Roussell et al. 1991). Conversely, a nuclear-gene-induced cpDNA mutant of barley that lacks plastid ribosomes has altered levels of mtDNA and mitochondrial transcripts (Börner and Hess 1993 and unpubl.).

The data of Martínez-Zapater et al. (1992) clearly support the idea that the observed changes in the *Arabidopsis* mitochondrial genome are

due to the action of the *chm* gene. However, it is less clear whether these mtDNA changes are responsible for leaf variegation and the underlying plastid alterations. One possibility that cannot be ruled out is that the *chm* locus induces mutations in both mtDNA and cpDNA (e.g., point mutations or small insertions/deletions in cpDNA would have gone undetected in the studies cited above). As stated above, the existence of mixed cells with different types of plastids is more readily explained by a direct effect of the *chm* locus on the plastid genome than by an indirect effect on the mitochondrial genome. This intriguing set of nuclear/cytoplasmic mutants clearly deserves further study.

CONCLUSIONS AND PERSPECTIVES

Precisely because they are so conventional in organization, gene content, and, apparently, expression, the organelle genomes of *Arabidopsis* should serve as exemplars for angiosperms in general and, in the case of the chloroplast, for land plants as a whole. Although we currently have relatively little direct knowledge about the molecular biology of the *Arabidopsis* chloroplast, the studies newly presented in this chapter suggest that much of the extensive information already available about the chloroplast genomes of tobacco and other land plants will be directly transferable to *Arabidopsis*. Conversely, as the progress report in this chapter documents, *Arabidopsis* mtDNA is rapidly becoming one of the best-characterized mitochondrial systems in angiosperms: The genome seems likely to become the first of the large angiosperm mitochondrial genomes to be completely sequenced and is also extensively characterized in terms of basic aspects of RNA editing and splicing.

Two developments must be realized, however, in order for the study of organelle genomes in *Arabidopsis* to truly blossom. First, the full power of the facile molecular genetics of the *Arabidopsis* nuclear genome must be brought to bear on the organelles. More than 80–90% of chloroplast and mitochondrial proteins are nuclear gene products. These include virtually all proteins of primary organelle metabolism except for a limited number of photosynthetic and respiratory polypeptides, and many of these proteins and metabolic systems have already been investigated in some detail in *Arabidopsis* (see various chapters in this volume). However, the parallel application of nuclear molecular genetics to the study of basic processes of organelle molecular biology—such as organelle DNA replication, recombination, transcription, splicing, RNA editing, translation, and the whole control of gene expression during development—is barely touched. The recent study by Martínez-Zapater et al. (1992), on a nuclear mutator gene that seems to affect both chloro-

plasts and mitochondria, is a promising start in this direction. An impressive number of nuclear gene products control and interact with mitochondrial and chloroplast genetic systems in yeast and *Chlamydomonas*, respectively (Gillham 1994), and one can expect a still greater complexity of nuclear/cytoplasmic genetic interactions in a more complex, multicellular eukaryote.

The second development that must be realized is a technical one: the establishment of efficient systems for the transformation of both organelle genomes of *Arabidopsis*. Organellar transformation is currently facile only for the two above-mentioned microbial systems, to the extent that many workers on land plant chloroplasts have expanded their efforts to include the *Chlamydomonas* system (Boynton et al. 1992). A promising start on chloroplast transformation has, however, been made in tobacco (Maliga 1993; Svab and Maliga 1993), and with luck, the *Arabidopsis* organelles will not be far behind.

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