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DNA Transfer from Organelles to the Nucleus: The Idiosyncratic Genetics of Endosymbiosis

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Annu. Rev. Plant Biol. 2009. 60:115–38

First published online as a Review in Advance on November 17, 2008

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

This article's doi: 10.1146/annurev.arplant.043008.092119

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1543-5008/09/0602-0115$20.00

Key Words

gene evolution, gene transfer, genome evolution, plastid, mitochondrion, NUMTs, NUPTs

Abstract

In eukaryotes, DNA is exchanged between endosymbiosis-derived compartments (mitochondria and chloroplasts) and the nucleus. Organelle-to-nucleus DNA transfer involves repair of double-stranded breaks by nonhomologous end-joining, and resulted during early organelle evolution in massive relocation of organelle genes to the nucleus. A large fraction of the products of the nuclear genes so acquired are retargeted to their ancestral compartment; many others now function in new subcellular locations. Almost all present-day nuclear transfers of mitochondrial or plastid DNA give rise to noncoding sequences, dubbed nuclear mitochondrial DNAs (NUMTs) and nuclear plastid DNAs (NUPTs). Some of these sequences were recruited as exons, thus introducing new coding sequences into preexisting nuclear genes by a novel mechanism. In organisms derived from secondary or tertiary endosymbiosis, serial gene transfers involving nucleus-to-nucleus migration of DNA have also occurred. Intercompartmental DNA transfer therefore represents a significant driving force for gene and genome evolution, relocating and refashioning genes and contributing to genetic diversity.

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INTRODUCTION

In eukaryotes, DNA molecules are found in several distinct cellular compartments: the nucleus, mitochondria, and, in the case of algae and plants, plastids. Mitochondria and plastids descended respectively from α-proteobacteria-like and cyanobacteria-like prokaryotes by endosymbiosis, but they contain much less DNA than their contemporary prokaryotic relatives. This loss of DNA is caused, over evolutionary time, by the redistribution of genetic material between nucleus, mitochondria and plastids via intercompartmental DNA transfer—a phenomenon discovered more than 25 years ago (34, 127, 135, 141). Of the six types of DNA transfer that are theoretically possible among the three genetic compartments present in plants, at least five have been observed (**Figure 1**).

NUMTs: nuclear mitochondrial DNAs

NUPTs: nuclear plastid DNAs

Transfer of DNA from mitochondrion or plastid to the nucleus has significantly shaped eukaryotic genomes; during the early phase of organelle evolution, transfer of DNA from organelle to nucleus resulted in a massive relocation of organelle genes. Conversely, the apparent absence of sequences of nuclear origin in plastid DNAs (ptDNAs) implies that nucleus-to-plastid transfer occurs extremely rarely, if at all, whereas evidence for mitochondrion-to-plastid gene transfer in a green alga was provided recently. Among the promiscuous DNA sequences originating from nucleus-to-mitochondrion and plastid-to-mitochondrion transfer only plastid-derived mitochondrial tRNA genes are functional (see section below entitled Transfer of Entire Genes to the Nucleus).

Although the movement of certain mitochondrial and plastid genes to the nucleus has occurred quite frequently in flowering plants during evolutionarily recent times (1), in many eukaryotes (including animals) the transfer of functional genes is now rare or has ceased altogether (17). Nevertheless, DNA transfer from organelle to nucleus is an ongoing and ubiquitous process. Almost all present-day nuclear transfers of mitochondrial DNA (mtDNA) and ptDNA give rise to noncoding sequences, dubbed nuclear mitochondrial DNAs (NUMTs) (72) and nuclear plastid DNAs (NUPTs) (134). Analysis of such sequences has allowed the reconstruction of many aspects of the mechanisms of DNA integration into the nucleus and thrown much light on the evolutionary forces acting on noncoding DNA sequences (see section below entitled DNA Transfer Resulting in Acquisition of Noncoding Nuclear Sequences: NUMTs and NUPTs). More recently, researchers discovered an intermediate between the transfer of entire genes and the generation of noncoding sequences: NUMTs and NUPTs can be recruited as novel exons for preexisting nuclear genes (see section below entitled Generation of Novel Nuclear Exons).

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In algae derived from secondary or tertiary endosymbiosis, complex plastids with additional genetic compartments were initially present. This presence adds an extra level of complexity to intercompartmental DNA transfer and permits serial DNA transfers, involving the remobilization of previously transferred DNA (see section below entitled Nuclear DNA Transfer in Organisms Derived from Secondary and Tertiary Endosymbiosis). The systematic analyses of insertions of organelle DNA in the nuclear genomes of humans, *Arabidopsis*, and rice, as well as experiments designed to trace nuclear gene transfer under laboratory conditions, have provided insights into the mode of origin and divergence of nuclear organelle DNA (see section below entitled Mechanisms). The role of nuclear DNA transfer in gene and genome evolution was obviously crucial in the era of large-scale gene transfer from organelles to the nucleus, whereas the evolutionary impact of NUMTs and NUPTs is less well defined (see section below entitled Evolutionary Consequences). In this review, we summarize recent progress in the field of organelle-to-nucleus transfer of DNA, with particular reference to the transfers associated with the cyanobacterial endosymbiosis that led to the evolution of the green lineage of photoautotrophs, and we emphasize the underlying cellular and genetic mechanisms and their evolutionary consequences.

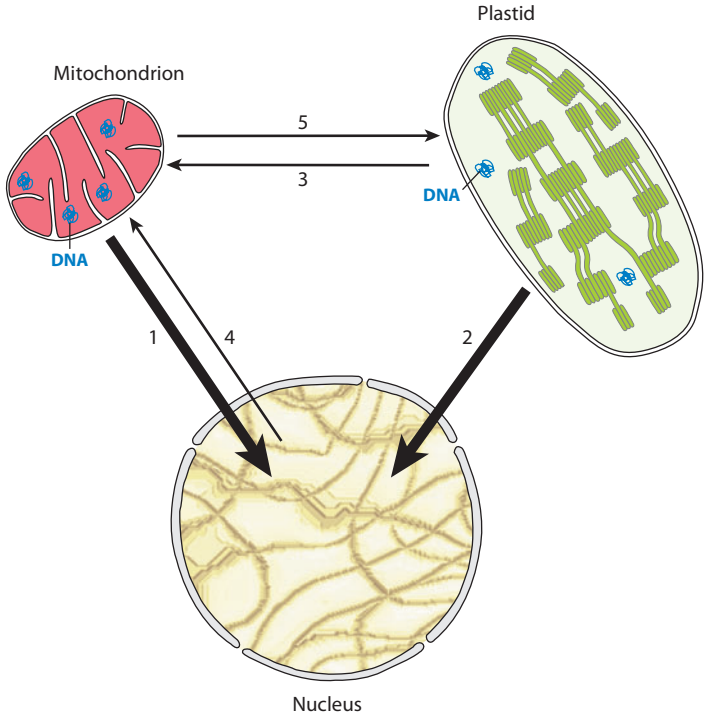


Figure 1

Overview of known types of interorganelle DNA transfer: (1) mitochondrion-to-nucleus, (2) plastid-to-nucleus, (3) plastid-to-mitochondrion, (4) nucleus-to-mitochondrion, and (5) mitochondrion-to-plastid. The thickness of the lines indicates the frequency of events detected in contemporary plant genomes.

GENETIC AND GENOMIC CONSEQUENCES OF INTERCOMPARTMENTAL DNA TRANSFER

Transfer of Entire Genes to the Nucleus

Both mitochondria and plastids are of endosymbiotic origin: They are descendants of α-proteobacterium-like (4) and cyanobacterium-like (104) [more specifically, heterocyst-forming (31)] progenitors, respectively. Although the organelles have retained much of their prokaryotic biochemistry (134), their genomes now encode only a small fraction of the organelle's proteins, ranging from 3 to 67 in mitochondria and from 15 to 209 in plastids (reviewed in Reference 58). This reduction is the consequence of the loss or transfer of endosymbiotic genes to the nucleus of the host, a special form of horizontal (or lateral) gene transfer that is associated with the gradual loss of the organelle's genetic autonomy (66, 68, 78, 134). On the basis of the composition of the gene sets that remain in plastids and mitochondria, most organelle genes are thought to have been transferred in early (and perhaps rapid) migrations, whereas subsequent transfers are thought to have been highly discontinuous (reviewed in Reference 58). A nuclear copy of an organelle gene must acquire additional genetic elements if it is to remain functional in its new environment.

Secondary (tertiary) endosymbiosis: engulfment and retention by another free-living eukaryote of a product of primary (secondary) endosymbiosis

Complex plastids: plastids that derive from secondary or tertiary endosymbiosis; typically surrounded by more than two membranes

The nuclear copy needs (*a*) appropriate promoter and terminator sequences to drive its expression and (*b*) presequences to target its protein product to the appropriate subcellular compartment (reviewed in Reference 77).

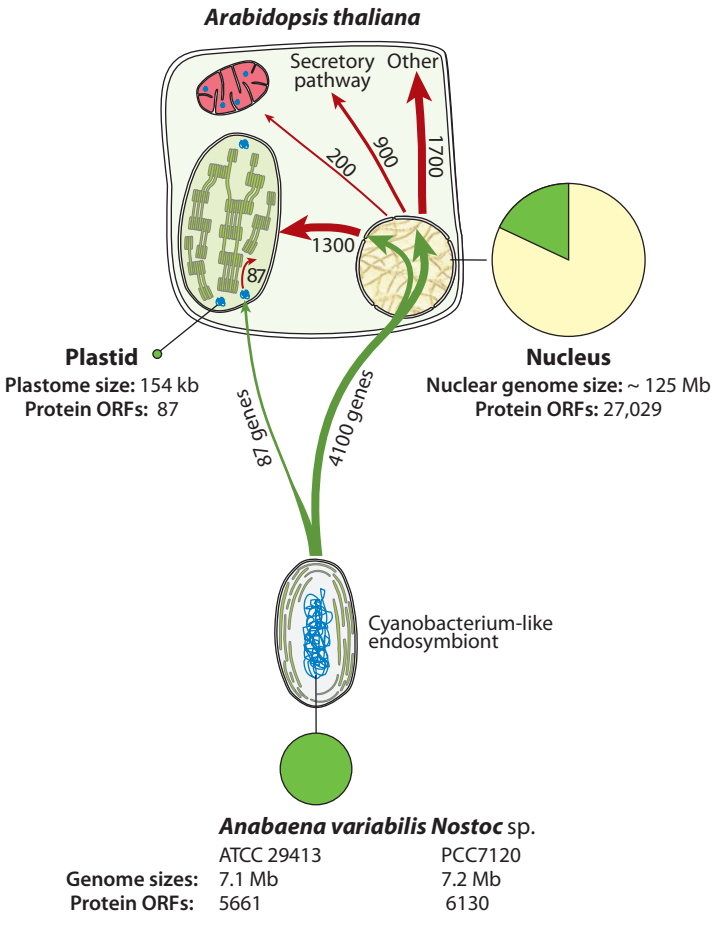


Figure 2

Fate of cyanobacterial genes and the intracellular targeting of their products in the flowering plant *Arabidopsis thaliana*. Chloroplasts such as those in *Arabidopsis* are descended via primary endosymbiosis from a cyanobacterium-like endosymbiont. On the basis of genome-wide sequence comparisons, the ancestral endosymbiont was most probably similar to contemporary filamentous, heterocyst-forming (nitrogen-fixing) cyanobacteria (31). Numbers and intracellular targeting are shown according to References 31, 78, and 110. Green arrows indicate the origins of the 87 plastid and more than 4000 nuclear genes of cyanobacterial origin, whereas red arrows indicate the predicted intracellular targets of the products of genes of cyanobacterial origin. The relative sizes of the three genomes studied (in terms of gene number) are represented by the areas of the circles; the fraction of nuclear genes that is of cyanobacterial origin in *Arabidopsis* is represented by the green sector.

The transfer of a gene from an endosymbiont to the nucleus of its host was originally postulated to always be accompanied by retargeting and import of its product to the original compartment, whether chloroplast or mitochondrion (reviewed in Reference 77). Many of the transferred genes are now recognized to have evolved further to encode proteins involved in extraorganellar functions (31, 78, 110; reviewed in References 67 and 134) (see below). In this respect, nuclear copies of organelle genes serve as raw material for evolutionary tinkering in the nuclear genome, which can in turn lead to evolutionary innovation.

Extent of nuclear gene transfer. To what extent has endosymbiotic gene transfer shaped the nuclear genomes of eukaryotes, specifically those of plants? Assessment of the significance of the endosymbiotic contribution to the evolution of nuclear genes became possible when the first prokaryote and eukaryote genomes were completely sequenced. In particular, the cyanobacterial heritage discernible in the genomes of *Arabidopsis* (31, 78) and three other photosynthetic eukaryotes (31) has been characterized in detail. On the basis of comparisons of the *Arabidopsis* genome with those of three cyanobacterial species, 16 other prokaryotes, and baker's yeast, 18% (or 4500) of all nuclear genes in *Arabidopsis thaliana* are estimated to be of cyanobacterial origin, and approximately one-half of these genes now provide nonplastid functions (78) (**Figure 2**). In an extension of this approach, the genomes of *Arabidopsis*, rice, *Chlamydomonas reinhardtii*, and the red alga *Cyanidioschyzon* were compared with nine cyanobacterial, 215 other prokaryotic, and 13 eukaryotic genomes. On average, approximately 14% of the proteins examined in the nuclear genomes of each of the four photosynthetic eukaryotes were estimated to be of cyanobacterial origin, with higher proportions (17–25%) observed among alignments with better sequence conservation [that is, alignments whose site patterns were independent of the order in which amino acids were

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aligned (31)]. As in the earlier study (78), the list of genes transferred to plant nuclei encompasses sequences coding for proteins that belong to virtually all functional categories (31). Another study identified 630 nucleus-encoded proteins that originate from protomitochondria, of which 22% and 32% were predicted to be targeted to mitochondria in humans and yeast, respectively (36). Indeed, as many as 75% of all nuclear genes in yeast might derive from protomitochondria (35). Numerous other examples of genes that have been transferred from mitochondria to nuclei and then recruited for functions outside of the original organelle can be found in protein databases (11). Taken together, these data allow us to conclude that the transfer of DNA from mitochondrion or plastid to the nucleus has significantly shaped eukaryotic genomes, and that this transfer resulted in the massive relocation of organelle genes during the early phase of organelle evolution.

Constraints on endosymbiotic gene transfer. Given the extent of gene transfer these findings reveal, one question immediately presents itself. Why have any genes at all been retained in plastid or mitochondrial genomes? A core set of genes present in all plastids and mitochondria has apparently resisted successful transfer to the nucleus. One feature these genes have in common is that they all code for hydrophobic proteins involved in energy-generating processes. Therefore, two major explanations for their sedentariness appear plausible. First, the hydrophobicity of certain organellar proteins might interfere with their efficient retargeting to and import into their designated workplace (the hydrophobicity-importability hypothesis) (29). However, this explanation is not applicable to all members of the core set of retained plastid genes. The large subunit of RubisCO (RbcL) and the D1 protein of photosystem II (equipped with chloroplast transit peptides) can be expressed experimentally from nucleus-encoded gene copies in functional form, demonstrating that at least these large hydrophobic proteins can be successfully retargeted to and imported into chloroplasts (26, 57). An alternative explanation focuses on the fact that the genes retained in the organelles have more in common than just the hydrophobicity of their products: These products function predominantly in energy transduction. Therefore, organelles likely need to be able to control directly the expression of genes for components of their electron transport chain so that they can synthesize these components as needed to maintain redox balance and avoid the production of toxic reactive oxygen species (2, 3, 76). Thus, safe and efficient production of ATP might require tight regulation of the expression of mitochondrial and chloroplast genes for functions directly involved in energy transduction, and thus favor rapid, on-site regulation within the organelle. In contrast, more leisurely modes of regulation, such as acclimation responses that operate on a long timescale, involve both regulation of organelle gene expression and modification of the expression of nuclear gene products (101). More recently, two additional explanations for the retention of plastid genomes, particularly in non-photosynthetic organisms, have been provided: the essentiality of certain plastid tRNAs and a principal reduction in plastid-nucleus DNA transfer in species with only one plastid per cell (9).

Evolutionary mosaics and their consequences for intercompartmental signaling. The respiratory chain in mitochondria and the photosynthetic machinery in chloroplasts are essentially very similar to the energy-generating systems found in their prokaryotic relatives (12). However, in the eukaryotic systems, one finds that the crucial multiprotein complexes contain both organelle- and nucleus-encoded proteins (**Figure 3**). In addition, novel proteins that have no counterparts in prokaryotes are present in the eukaryotic complexes (e.g., reviewed in References 88 and 119). Therefore, the expression of the different sets of proteins encoded in the nucleus and the plastid needs to be tightly coordinated to ensure appropriate and energy-saving

Horizontal (or lateral) gene transfer: movement of genes across normal mating barriers, in contrast to the standard vertical transmission of genes from parent to offspring

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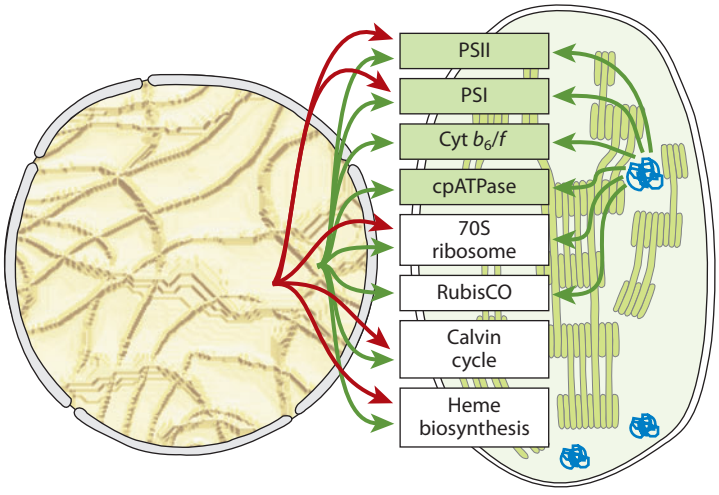


Figure 3

Evolutionary mosaics resulting from organelle-to-nucleus gene transfer and compartmental redirection of gene products. The thylakoid multiprotein complexes cytochrome b_6/f (Cyt b_6/f) and ATP synthase (cpATPase), as well as the stroma-located Calvin cycle enzyme RubisCO, consist of subunits homologous to cyanobacterial proteins, some of which are still encoded in the plastid, whereas others are now encoded in the nucleus. Photosystem II (PSII) and I (PSI), as well as the chloroplast ribosome, also contain nucleus-encoded proteins that lack cyanobacterial homologs. Other metabolic pathways, such as heme biosynthesis and the Calvin cycle (with the exception of RubisCO), depend solely on nucleus-encoded proteins of mixed origin. Green arrows depict proteins encoded by genes of cyanobacterial origin; red arrows depict proteins of noncyanobacterial origin.

assembly of the multiprotein complexes. A number of mechanisms have evolved to provide for direct control of organellar gene expression by nuclear genes (anterograde mechanisms); these mechanisms in turn rely on signals that convey information from the organelle to the nucleus (retrograde signaling) (reviewed in References 70, 100, and 143). The identity of the signalling molecule remains unknown; for some time the tetrapyrrole Mg-protoporphyrin IX has been thought to transmit information on the status of the plastid to the nucleus (5, 128), but more recent data strongly argue against this function (84, 86). In addition, mechanisms that act at the level of transcriptional coregulation in the nucleus have evolved to enable indirect upregulation of organellar gene expression when levels of abundant nucleus-encoded organelle proteins increase (13, 118).

In addition to the multiprotein complexes that mediate photosynthesis and respiration, various other metabolic pathways have turned out to be evolutionary mosaics (**Figure 3**). Examples include the Calvin cycle in the chloroplast and glycolysis in the cytosol, both of which exploit enzymes of diverse evolutionary origin (reviewed in Reference 79). Moreover, the evolution of the heme biosynthesis pathway in photosynthetic eukaryotes has been influenced by gene fusion, horizontal gene transfer, and endosymbiotic replacement (93). Furthermore, phylogenetic analyses strongly suggest a proteobacterial origin for metacaspases and the HtrA-like proteases, which are key components of the apoptotic machinery (64); several central components of the nuclear pore complex most probably also have an endosymbiotic origin (75).

Recent events. How relevant has organelle-to-nucleus transfer of functional genes been in evolutionarily recent times? In animals, functional gene transfer from mitochondria to the nucleus has entirely ceased (17). In contrast, transfer of certain mitochondrial and plastidic genes in flowering plants is now known to have occurred frequently in the evolutionarily recent past (1). For example, 26 instances of transfer of the mitochondrial *rps*10 gene to the nucleus, with concomitant loss from the mtDNA, were observed among 277 diverse angiosperms (1). As in the case of *rps*10, the chloroplast translation initiation factor 1 gene (*infA*) is also strikingly mobile on the basis of a survey of more than 300 angiosperm species (82). In four species with nonfunctional chloroplast *infA* genes, transferred and expressed copies of the gene, complete with putative chloroplast transit peptide sequences, were found to be present in the nucleus. Phylogenetic analysis of *infA* sequences and assessments of transit peptide homology indicate that the four nuclear *infA* genes probably derive from four independent chloroplast-to-nucleus gene transfers during angiosperm evolution (82).

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Other Types of Intercompartmental Gene Transfer

Transfer of plastidic or nuclear genes to mitochondria. In addition to DNA transfers from organelle to nucleus, researchers have observed cases of plastid-to-mitochondrion and nucleus-to-mitochondrion migration (**Figure 1**). In 1982, chloroplast genes were detected for the first time in mitochondria (34). The set of tRNA genes present in the mitochondrial genomes of higher plants turns out to represent a mixture of sequences of proteobacterial and cyanobacterial origin. The chloroplast-derived tRNA genes still retain high sequence similarity (95–100%) with their chloroplast analogs and at least a fraction of them is now actively transcribed in mitochondria of higher plants (116, 140). However, not all the plastid DNA sequences that were transferred to the mitochondrion gave rise to functional tRNA genes; for instance, the $tRNA^{Val}$ gene is part of a 417-bp DNA insertion of chloroplast origin in the mitochondrial genome of sunflower, but the gene is not transcribed (23).

Furthermore, researchers have detected several distinct cases of transfer of chloroplast *rbcL* genes to mitochondria. The mitochondrial copies of *rbcL*, however, exhibit insertion and/or deletion mutations that disrupt the reading frame, and all show enhanced frequencies of nonsynonymous substitutions, providing clear evidence that these sequences represent pseudogenes (28).

In the transcriptionally active regions of the mitochondrial genome of *Oenothera*, a 528-bp stretch shows 91% homology to a nuclear 18S ribosomal RNA (rRNA) sequence in maize and includes an open reading frame with significant sequence homology to reverse transcriptases (120). But to our knowledge, no examples of the formation of a functional (protein-coding) mitochondrial gene from nuclear DNA have been reported to date, and there is no evidence for the incorporation of nuclear DNA into the mitochondrial genome of maize (27). Moreover, in the mitochondrial genome of the nonvascular plant *Marchantia*, no sequences with homology to chloroplast or nuclear DNA have been identified so far (94). Therefore, when and under what circumstances plants are capable of accepting foreign DNA insertions into their mitochondrial genomes remains unclear (63).

Nonsynonymous substitutions: nucleotide changes that alter the encoded amino acid sequence

Transfer of nuclear or mitochondrial genetic elements to plastids. Until recently, nucleus-to-plastid and mitochondrion-to-plastid gene transfers were thought to occur extremely rarely or not at all (68, 134). A number of plausible explanations for the rarity of such events can be postulated. For instance, the compactness of the plastid genomes makes it likely that foreign DNA will disrupt some vital function upon integration (123). Alternatively, chloroplast genomes might be protected against invasion by foreign DNA because their potential to undergo nonhomologous recombination is limited. Another possibility is that plastids, unlike mitochondria, lack both (*a*) an efficient uptake system for exogenous DNA and (*b*) a propensity to fuse with one another (reviewed in Reference 58).

However, in the exceptionally large inverted repeat of the chloroplast genome of the green alga *Oedogonium cardiacum*, Brouard and coworkers (19) identified two open reading frames (ORFs), *int* and *dpoB*, that show no sequence similarity with any of the genes usually present in chloroplast genomes. The *int* and *dpoB* genes code for a site-specific tyrosine recombinase and a DNA-directed DNA polymerase of the B family, respectively, and are most similar to proteins of unknown function encoded by the mitochondrial genomes of two other green algae (in the case of *int*) and the DNA polymerase encoded by a linear mitochondrial plasmid found in the fungus *Neurospora intermedia* (*dpoB*). This result strongly suggests that *Oedogonium* acquired the *int* and *dpoB* genes through horizontal transfer of mobile element(s) originating from the mitochondria of an unknown donor—most probably

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Apicoplast: the reduced plastid of Apicomplexa

in a single event, because the two genes are clustered in the same region of the *Oedogonium* inverted repeat (19).

In addition, researchers have reported the ancient transfer of the RubisCO operon (*rbcL* and *rbcS*) from a proteobacterium into the common ancestor of red algal plastids and their secondary derivatives (30), transfer of a bacterial *rpl36* gene into the ancestor of the cryptophyte and haptophyte plastids (109) (**Figure 4*a***), and acquisition of *dnaX* by lateral gene transfer in an ancestor of *Rhodomonas*—most likely from a firmicute bacterium (60). In contrast to gene transfers, the acquisition of new introns may be relatively common in plastids. Examples include a mitochondrion-to-plastid transfer in the ulvophyceate *Pseudendoclonium akinetum* (102), as well as cases of interkingdom horizontal transfer of homing group II introns from a cyanobacterial donor to the chloroplast genome (95, 122).

DNA Transfer Resulting in Acquisition of Noncoding Nuclear Sequences: NUMTs and NUPTs

Numbers and diversity. In almost all eukaryotes analyzed so far, noncoding nuclear DNA sequences exist that are homologous to mt or ptDNA (111, 112). NUMTs vary in copy number: No NUMTs have been detected in the mosquito genome; a few in *Caenorhabditis*, *Drosophila*, and dog; several hundreds in rice, some teleost fishes, and hominid species; and more than a thousand in honeybee nuclear DNA (6, 46, 96, 98, 111) (**Table 1**). Similarly, NUPTs are rare in *Chlamydomonas* and *Plasmodium* (both of which possess only one plastid/apicoplast), but are frequent in the flowering plants *Arabidopsis* and rice (112). The largest stretches of nuclear organelle DNA known have been found in the domestic cat (72) and in flowering plants (53, 91, 129). NUMT/NUPT repertoires can differ markedly even between closely related species, particularly with respect to the average length of inserts and the relative size of their contribution to the total nuclear genome (40, 65, 73) (**Table 1**), implying that the evolution of NUMTs and NUPTs is a continuous and dynamic process. Moreover, no obvious correlation exists between the abundance of nuclear organelle DNA and the size of either the nuclear or organelle genomes, or the gene density in the nuclear genome (111).

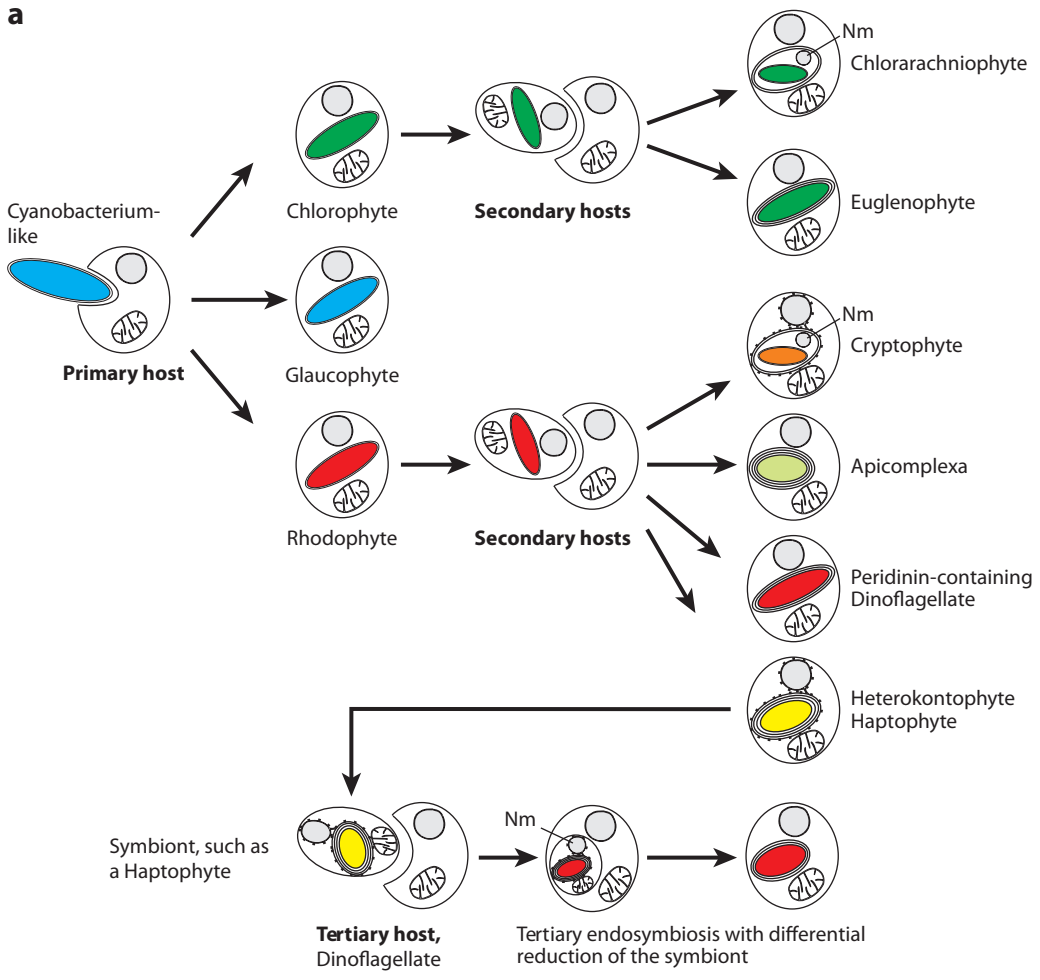
Structure and genomic distribution. Three main types of nuclear organelle DNAs have been observed in animals and plants (reviewed in Reference 68): (*a*) continuous stretches of NUMTs and NUPTs that are colinear with mt or ptDNA, (*b*) rearranged and scrambled nuclear organelle DNAs derived from different regions of one organelle chromosome, and (*c*) rearrangements involving different chromosomes. The latter have been detected in plants and can contain dozens of different fragments of highly variable sizes (91).

The most detailed studies on the genomic organization of NUMTs and NUPTs have been

Figure 4

Primary, secondary, and tertiary endosymbiosis and their consequences for gene transfer and protein targeting. (*a*) Primary endosymbiosis of a cyanobacterium-like prokaryote, possibly similar to modern filamentous, heterocyst-forming cyanobacteria (31), gave rise to chlorophytes (with chloroplasts), glaucophytes (with cyanelles), and rhodophytes (with rhodoplasts). Secondary endosymbiosis involving chlorophytes or rhodophytes and secondary hosts resulted in the case of plastid/apicoplast-harboring groups in chlorarachniophytes and euglenophytes on the one hand, and cryptophytes, apicomplexa, peridinin-containing dinoflagellates, heterokontophytes, and haptophytes on the other hand. Finally, cases of tertiary endosymbiosis ensued when secondarily evolved organisms were engulfed by some dinoflagellate forms and integrated into their cellular architecture as tertiary endosymbionts, which eventually evolved into tertiary plastids. Nm, nucleomorph. (*b*) The different modes of gene transfer (GT) are indicated on the left. Primary gene transfer involved migration of protoplastid or plastid genes to the nucleus of the host. During secondary and tertiary gene transfer, genes moved not only from the symbiont's evolved organelles, but also from the endosymbiont nucleus, to the host nucleus. The typical composition of precursor proteins targeted to the (complex) plastid is shown. TP, transit peptide; SP, signal peptide.

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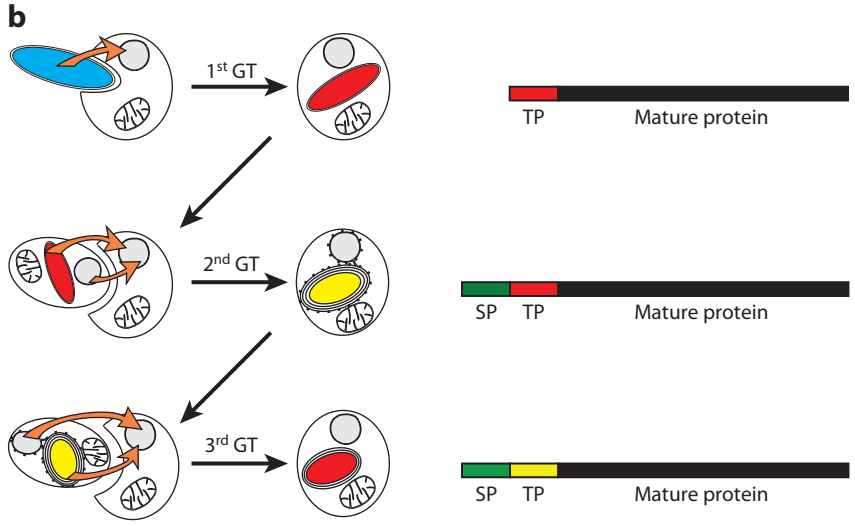


Table 1 Amount and density of nuclear mitochondrial DNAs (NUMTs) and nuclear plastid DNAs (NUPTs) in the nuclear genomes of selected species

Species	Total length (bp)	Density (bp/kb)	Reference
NUMTs			
Arabidopsis	625,000	5.42	7, 129
Rice	852,913	2.19	55
Honeybee	237,325	1.0	96
Human	475,269	0.17	46
Baker's yeast	2121	0.17	106
Zebrafish	32,454	0.021	6
Chicken	8869	0.008	98
Drosophila	777	0.005	96
NUPTs			
Rice	900,741	2.32	80
Arabidopsis	35,235	0.31	112
Chlamydomonas reinhardtii	2461	0.026	112

performed in humans, *Arabidopsis*, and rice. In *Homo sapiens*, approximately 450 NUMTs have been identified (46)—with locus sizes of up to ~15 kb (87, 136)—that are evenly distributed within and among chromosomes (142). More than half of the human NUMTs localize to repetitive sequences, in particular to mobile elements (83). The genomes of the flowering plants *Arabidopsis* and rice are particularly rich in nuclear organelle DNA (111, 112). These genomes also contain the largest insertions of nuclear organelle DNA reported so far, namely a 620-kb NUMT [*A. thaliana* (129)] and a 131-kb NUPT [rice (53, 108)]. In both species, nuclear organelle DNA is frequently organized in clusters of insertions that are physically linked to varying degrees (loose clusters and tight clusters; 112). Interestingly, approximately 25% of nuclear organelle DNAs are located within genes (112) (see below). In rice, large NUPTs preferentially localize to the pericentromeric regions of the chromosomes; such insertions should be less deleterious than integrations in other gene-rich chromosomal regions (80).

Evolutionary dynamics. Cross-species comparisons and phylogenetic analyses have allowed us to distinguish between old and recent NUMTs and NUPTs. Alignments of the human and chimpanzee genomes have revealed nonorthologous NUMTs that are derived mostly from novel insertions acquired after the divergence of the two species (46). Loss of an ortholog through deletion in one species and creation of paralogous NUMTs by the tandem duplication of a preexisting NUMT appear to have played minor roles in generating nonorthologous NUMTs in these two species (46). On average, 5.7 NUMTs per 1 million years have been inserted and retained in the human germline.

In *Arabidopsis* and rice, the degree of sequence similarity between NUPTs/NUMTs and their donor pt/mtDNAs on the one hand, and the size of the integrant on the other hand, are correlated, implying that the primary insertions were large, but decayed over evolutionary time into smaller fragments with more divergent sequences via tight and loose clusters as intermediates (112). The insertion of transposable elements and other DNA sequences unrelated to organelle DNA into NUPTs and NUMTs contributed significantly to this fragmentation process (91). In rice, at least 47 NUPT integration events have occurred over the past one million years (80). Indeed, most large rice NUPTs are of relatively recent origin (53, 91) and seem to decay within one million years (80).

Organelle DNA that has been incorporated into the nuclear genome is inevitably exposed to the evolutionary forces that act on this compartment. In humans and other animals, the mutation rate in the nucleus is much lower than that in mitochondria. NUMTs therefore represent molecular fossils of mtDNAs. This fact has been exploited to trace ancestral states of mtDNAs and improve mtDNA-based phylogenies by providing suitable outgroups (10, 99). Conversely, in plants, nucleotide substitutions generally occur much less frequently in organelle DNA than in the nuclear genome. Here, NUMTs and NUPTs can serve as probes for the types of mutation that predominate in nuclear DNA. In two independent studies, a

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preponderance of C → T and G → A transition mutations was observed in large, recently acquired insertions of nuclear organelle DNA in *Arabidopsis* and rice (53, 91). The prevalence of these mutations can be interpreted in the context of the nonfunctional nature of NUMTs and NUPTs, which favors transcriptional silencing by, among other means, hypermethylation of cytosine residues. The G-T mismatch created by spontaneous deamination of 5-methylcytosine can be repaired to G-C, but repair will also create an A-T pair with the same probability, resulting in C → T transitions (and G → A transitions on the opposite strand). Deletion of DNA from large nuclear organelle DNA loci in rice and *Arabidopsis* tends to occur between perfect repeats, and is thought to originate by replication slippage (53, 91). However, deletions are more than compensated for by insertions of nonorganelle DNA (see above), leading to local expansion at the site of insertion (91).

Generation of Novel Nuclear Exons

A certain fraction of NUPTs and NUMTs is associated with genes. Thus, old NUMTs in several fish species, as well as recent NUMTs in humans, seem to show a bias for insertion into known or predicted genes (6, 107). Two of the 30 NUMTs in the yeast genome have been described as genic insertions (106), whereas approximately 25% of NUMTs and NUPTs are located within genes in *Arabidopsis* and rice. However, this value must be considered in the context of the fact that exons and introns together make up between 40% and 50% of the genomes of *Arabidopsis* and rice (112).

Recently, Noutsos and colleagues (90) analyzed in detail the number and organization of genic nuclear insertions of organelle DNA in yeast, *H. sapiens*, *Arabidopsis*, and rice. A total of 474 NUMTs and NUPTs were identified in, or next to, annotated genes. Of these, approximately 70% were located in introns or untranslated regions. Interestingly, a set of 45 insertions contributed sequences to a total of 49 protein-coding exons in 34 genes. Functionality was demonstrated for a subset of these exons on the basis of their mRNA expression and their mutational spectra. In-depth sequence comparisons of the protein-coding NUPTs/NUMTs, their transcripts, and the sequences of the donor organelle DNAs showed that the generation of novel protein-coding sequences from previously noncoding mt or ptDNA is favored over the co-option of preexisting organelle protein domains for remodeling of nucleus-encoded proteins. In the few cases where preexisting organelle reading frames were incorporated as nuclear protein-coding exon sequences, rates of nonsynonymous substitution (K_A) exceeded those of synonymous substitution (K_S), i.e., $K_A/K_S > 1$, reflecting adaptation of the NUMT/NUPT-derived protein sequences to their new cellular function, as expected for the evolution of new genes (71).

Moreover, interspecific sequence comparisons indicated that NUMT/NUPT-derived protein-coding exon sequences exist that are not directly detectable by conventional sequence searches, because sequence divergence has reduced their similarity to the query sequence below the minimum necessary for statistical recognition. This finding suggests that ancient organelle-derived DNA insertions might be responsible for many more instances of functional exon acquisition than the relatively few cases found so far (90).

Nuclear DNA Transfer in Organisms Derived from Secondary and Tertiary Endosymbiosis

The principles of secondary and tertiary endosymbiosis. Plastids originated by primary endosymbiosis, in which a eukaryote captured a cyanobacterium-like cell (prokaryote-eukaryote endosymbiosis), which was reduced to an organelle surrounded by two membranes in the course of subsequent evolution (e.g., 105, 134) (**Figure 4*a***). Some of the photoautotrophic organisms that arose in this way later served as endosymbionts for new hosts (eukaryote-eukaryote endosymbiosis), a process known as secondary endosymbiosis, which

Replication slippage: occurs at repetitive sequences when the new strand mispairs with the template strand; can result in deletion or duplication of the sequence flanked by the repeats

Synonymous (or silent) substitutions: nucleotide changes that do not alter the encoded amino acid sequence

Primary endosymbiosis: engulfment and retention of a bacterium by another free-living organism; led to the DNA-containing organelles (mitochondria and plastids)

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established the photosynthetic machinery in new groups of eukaryotes (e.g., 21, 74). Via secondary endosymbiosis, complex plastids originated in which the stroma is separated from the host cytosol by either three or four membranes (**Figure 4*a***). Furthermore, secondarily evolved organisms were in some cases engulfed by dinoflagellates, which integrated them as tertiary endosymbionts, creating tertiary plastids with highly complex morphologies (e.g., 22, 41).

Dinoflagellates: ecologically important protists; approximately 50% of the taxa are photosynthetically active, and most of these harbor plastids surrounded by three membranes

Nucleomorph: highly reduced remnant of a eukaryotic nucleus found in complex plastid-bearing compartments of certain organisms derived by secondary endosymbiosis

The evolution of secondarily evolved organisms is associated with different degrees of morphological reduction—or even elimination—of the secondary endosymbiont. Although in some cases all compartments dating back to the secondary endosymbiont may have been eliminated—at least in the case of oomycetes (138)—plastids of peridinin-containing dinoflagellates and phototrophic euglenophytes are surrounded by three membranes and represent the most reduced morphotype of a secondary plastid in terms of the loss of surrounding membranes (**Figure 4*a***). Other secondarily evolved organisms, such as heterokontophytes, haptophytes, and the apicomplexa, harbor plastids/apicoplasts that are surrounded by four membranes, which may trace back to the following structures (proceeding from host cytosol to stroma): an endomembrane of the host, the remnants of the cytoplasmic membrane of the secondary endosymbiont, and the two plastid envelope membranes of the symbiont (21, 48); for an alternative view see Reference 81.

Two other algal groups, the chlorarachniophytes and cryptophytes, demonstrate the step-by-step reduction of the secondary endosymbiont within the host cell. In both, four membranes surround the plastid (**Figure 4*a***). However, between the outer and inner membrane pair the remnant cytoplasm of the symbiont is still maintained, harboring its own expression apparatus, as indicated by the presence of 80S ribosomes and a tiny cell nucleus (74). This compacted nucleus, the nucleomorph, is the descendant of the nucleus of the secondary endosymbiont and encodes only a very small number of plastid-targeted proteins, the genes for which have already been transferred in the host nucleus in other related, secondarily evolved organisms (32, 37).

Genomic sequencing projects on phototrophic algae are either ongoing or annotation and analysis are under way. Thus, little is currently known regarding the general distributions of NUMTs and NUPTs in the nuclei of secondarily evolved organisms. Expressed sequence tag (EST) libraries will, however, provide a powerful resource for studying transferred genes that encode plastid or mitochondrial functions.

From long jump to triple jump: gene transfer in secondary and tertiary endosymbiosis. In primary endosymbiosis, most of the genomic information introduced by the cyanobacterium-like symbiont was apparently removed relatively rapidly from the plastid compartment, either by the loss of dispensable genes or by transfer of the genetic material into the host nucleus (see section above entitled Transfer of Entire Genes to the Nucleus). In cases of secondary endosymbiosis, therefore, many of the genes required for photoautotrophism already have been resident in the symbiont's nucleus. Because this nucleus has either been reduced to a nucleomorph or (as in most cases) completely eliminated, genes must have been transferred from the symbiont's nucleus to the nuclear genome of the host. Because this transfer represents a transfer of genetic material from one cell nucleus to another, the mechanism(s) of gene migration in secondarily evolved organisms may differ from those that facilitate organelle-to-nucleus gene transfer in primarily evolved organisms. In particular, it is conceivable that wholesale DNA-based transfer of symbiont chromosome(s), either as intact or fragmented molecules, could have led to their elimination from the symbiont. Alternatively, as others have postulated, only a minor portion of the symbiont's nuclear genome, such as genes encoding components of the photosynthesis apparatus, may have been transferred from one nucleus to the other (e.g., 45), because, according to that hypothesis, the host already harbored a plastid.

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One characteristic of the genomes of secondary plastids is that their gene content is reduced relative to the plastid genomes of free-living relatives of the secondary endosymbionts (see References 78, 114, and 115). This characteristic is not only seen in the apicoplast genome, but also reflected in the photosynthetically active plastids of cryptomonads, haptophytes, heterokontophytes, euglenophytes, and chlorarachniophytes. In addition, the enigmatic peridinin-containing dinoflagellates, which are thought to harbor so-called minicircles instead of a classical plastid genome (50), express the largest set of nucleus-encoded plastid proteins among secondarily evolved organisms (8, 42). Thus, a second round of gene loss or gene transfer from the plastid genome into the host's cell nucleus coincides with secondary endosymbiosis. One of the smallest plastid genomes known in a photosynthetic organism is found in the model chlorarachniophyte *Bigelowiella natans* (114). Apart from implicating gene compaction, this finding may indicate either that gene transfer occurred at comparatively high rates after the establishment of a green alga as a eukaryotic symbiont, or that gene transfer is still ongoing. The latter possibility could contribute to the extensive gene loss seen in *B. natans*, because this species harbors several complex plastids, which may be a prerequisite for the efficient transfer of genetic material from plastids to the cell nucleus (69).

In primary plastids, nucleus-encoded plastid proteins are mostly targeted to the organelle by their N-terminally located transit peptides (56, 59, 124). For successful delivery to secondary plastids, nucleus-encoded proteins must traverse additional membranes. Analysis of the respective genes has indicated that, irrespective of their evolutionary history and cell biology, all proteins are encoded as preproteins with an N-terminally located, bipartite signal sequence composed of a signal peptide followed by a transit peptide (summarized in References 39 and 48) (**Figure 4*b***). Thus, genes transferred from the nucleus of a eukaryotic symbiont, which already encodes a transit peptide, must be provided with a sequence encoding a signal peptide in the target nucleus if the gene product is needed in the symbiont. If such a transcriptional unit evolved as a consequence of the insertion of a gene proximal to a small reading frame with certain signal peptide characteristics, one might expect to find an intron separating the sequences for signal and transit peptides. Interestingly, such exon/intron structures have been discovered recently (61). As in the case of signal peptides, a transit peptide may evolve by random insertion of a gene that was previously located in the plastid genome into a nuclear chromosome. The presence of an intron separating the topogenic signal from the gene encoding the mature protein would imply the involvement of such a mechanism (61).

Tertiary endosymbiosis has given rise to organisms in which several phylogenetically unrelated, eukaryotic phototrophs were intracellularly established in a dinoflagellate host, providing a veritable solar powerhouse (e.g., 41). Recent work on such organisms, in which the eukaryotic symbiont is reduced to a plastid, has indicated that the plastid proteome is expressed in part from nuclear genes derived from the eukaryotic endosymbiont (e.g., 89, 97). Thus, triple gene transfers have occurred: first from the prokaryotic symbiont to the host nucleus during primary endosymbiosis, then from nucleus to nucleus in secondary endosymbiosis, and finally from the nucleus of the tertiary endosymbiont to the nucleus of the dinoflagellate host (**Figure 4*b***).

MECHANISMS

The Nature of the Migrant Nucleic Acid

Because nuclear *coxII* sequences more closely resemble edited mitochondrial transcripts than the *coxII* genes encoded in mitochondrial DNA, researchers initially thought that organelle-to-nucleus transfer of genes must involve an (edited) RNA intermediate that undergoes reverse transcription (92). However, other interpretations of these sequence patterns are possible. For instance, a priori, it appears more likely

that cDNA copies of spliced and edited transcripts of higher-plant mitochondria would recombine with mitochondrial DNA than with nuclear DNA and, in consequence, erase editing sites and introns in the mitochondrial genes (49); this argues in favor of DNA rather than RNA as the migrating intermediate. Moreover, experimental and bioinformatics studies carried out in yeast and other eukaryotes have shown that (*a*) any segment of an organellar genome can be transferred to the nucleus and (*b*) large segments of organellar DNAs that span several genes or even entire organellar chromosomes exist in the nucleus (reviewed in References 16, 68, and 134). Furthermore, analysis of human NUMTs has failed to provide evidence for splicing or polyadenylation of organellar nucleic acids prior to insertion (142), indicating that migration of mtDNA sequences to the nucleus is predominantly DNA mediated.

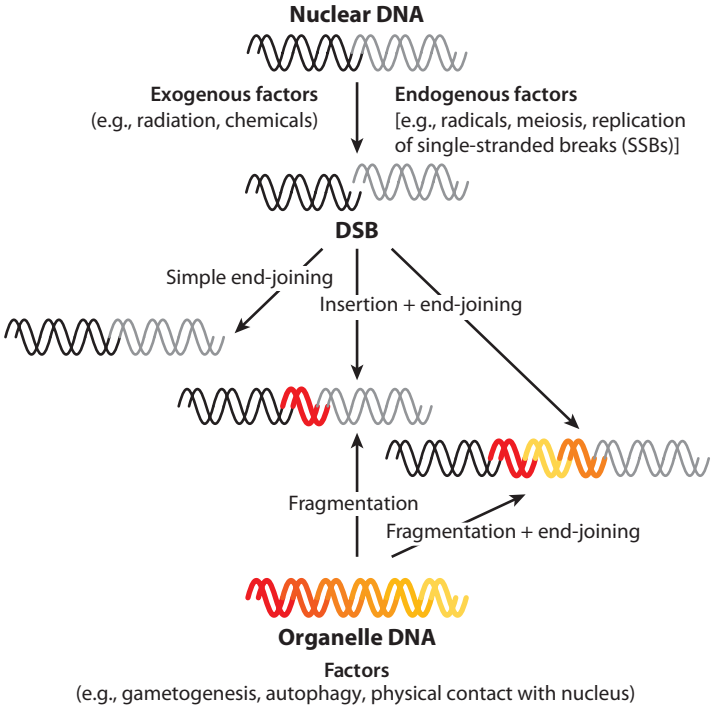


Figure 5

Model of generation of nuclear insertions of organelle DNA. Double-stranded breaks (DSBs) are induced by exogenous and endogenous sources. Other possible endogenous sources of DSBs not listed in the figure are somatic hypermutation, transposon excision, and endonucleolytic cleavage. The release of organelle DNA may also be one consequence of various cellular stresses. According to this model, any increase in the frequency of DSBs should influence the rate of nuclear insertion of organelle DNA. In this context it should be noted that a case of de novo mitochondrial DNA (mtDNA) insertion in the human germline has been associated with high-level radioactive contamination (137). In addition to mtDNA, cDNA intermediates of the yeast retrotransposon *Ty1* have been found to repair DSBs by nonhomologous end-joining (145).

Escape of organelle DNA and its uptake into the nucleus have now been experimentally demonstrated in yeast (130) and tobacco (51, 52, 125, 126; reviewed in Reference 16). The experimental studies were based on integration into the mitochondrial or plastid genome of a marker gene that would function only in the nucleus, thus allowing phenotypic selection for nuclear acquisition of the marker. A refined genetic screen in tobacco was designed to select specifically for activation of a transferred spectinomycin-resistance (*aadA*) gene in the nuclear genome (125). Spectinomycin-resistant lines with a functionally activated *aadA* gene in the nucleus were successfully obtained, demonstrating that DNA-mediated gene transfer can give rise to functional nuclear genes if appropriate mutations or rearrangements in the nuclear genome follow (125).

Escape of DNA from Organelles

How does DNA escape from organelles? Several possibilities have been proposed. Disruption of organelle membranes can occur during autophagy, organelle fusion or division, and cell stress. These processes could therefore make organelle DNA accessible for illegitimate uptake via the nuclear import machinery (20, 130, 132) (**Figure 5**). Inactivation of Yme1p, an ATP-dependent metalloprotease located in mitochondria of the yeast *Saccharomyces cerevisiae*, results in the degradation of abnormal mitochondria in the vacuole and also increases the incidence of DNA escape from mitochondria to the nucleus (133). Moreover, inactivation of Yme2p, an integral inner mitochondrial membrane protein, leads to increased rates of relocation of mitochondrial DNA to the nucleus (44). These findings suggest that perturbation

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of the structural integrity of organellar membranes provides opportunities for DNA to escape from organelles—an idea that is supported by studies of DNA movement across the membranes of isolated chloroplasts from pea (24).

Because the incidence of complex nuclear loci containing DNA from both mitochondria and plastids is high in plants (91), it can be concluded that the concomitant release of plastid and mitochondrial DNA is not all that rare an occurrence. Such occasions probably arise under conditions that affect both organelles simultaneously, such as when cells are stressed or during gametogenesis-associated organelle degradation (112, 134). Direct physical association of the nucleus with mitochondria (85) or chloroplasts (33), as well as the uptake of whole mitochondria by nuclei, as occurs for instance in tobacco sperm cells (144), might also contribute to DNA exchange. In many eukaryotes, including humans and various flowering plants, organelles are maternally inherited. Therefore, organelle-to-nucleus transfer of DNA is thought to occur preferentially when programmed degeneration of organelles takes place during male gametogenesis: during pollen development in flowering plants and in mammals when DNA is released from degenerating sperm mitochondria shortly after penetration of the egg by the sperm cell (reviewed in Reference 68) (**Figure 5**). Recently, a screen carried out on transplastomic tobacco plants confirmed that relocation of chloroplast DNA to the nucleus occurs in both somatic and gametophyte tissue; however, the male germline shows a markedly increased frequency of transposition (121).

In the haploid unicellular green alga *C. reinhardtii*, a screen similar to those performed in tobacco and yeast, and designed to detect the transfer of DNA from the mitochondrion or chloroplast to the nucleus (51, 126, 130), failed to identify transfer events (69; reviewed in Reference 76). Among several billion homoplastomic cells tested, not a single instance of stable nuclear integration of chloroplast DNA was detected, neither under normal nor stressful conditions. One possible explanation for this result is suggested by the fact that, unlike angiosperms, *Chlamydomonas* has only a single large chloroplast. If the mechanism of DNA transfer to the nucleus involves chloroplast rupture (as discussed above), lysis of its sole chloroplast would very likely be a lethal event for *Chlamydomonas*, because the organelle is the site of several essential biosynthetic pathways, including photosynthesis. This theory might explain why extensive gene transfer from the plastid to the nucleus has not been observed in *Chlamydomonas* (16, 69, 112). Conversely, in flowering plants, where each cell has many chloroplasts, rupture of one or more need not result in cell death.

Mechanisms of DNA Integration at the Molecular Level

Analyses of the results of the DNA transfer experiments in tobacco showed that microhomologies (2–5 bp) were often found adjacent to the integration sites (125), suggesting that in tobacco, as in yeast (106), organelle DNA can integrate into the nuclear genome by nonhomologous end-joining (NHEJ) repair (illegitimate repair) of double-stranded breaks (DSBs). DSBs are regarded as potentially the most deleterious form of DNA damage that can be induced in vivo by exogenous and endogenous sources (i.e., 103, 139) (**Figure 5**). Repair of DSBs by NHEJ requires little or no sequence homology (0–4 bp; microidentities) between the termini, enabling the noncomplementary ends of DSBs and organelle DNA to be pasted to one another (106, 139). In tobacco, the spectrum of sequences inserted at chromosomal breaks is broad (62), whereas no insertions could be detected in *Arabidopsis* (62), implying that DSB repair mechanisms can show major differences even among closely related eukaryotes. However, similar patterns of terminal microidentities have been observed for NUMT insertions in yeast and humans (15, 107), indicating that DSB-repair-mediated insertion of organelle DNA by NHEJ might be a phenomenon common

NHEJ: nonhomologous end-joining

DSBs: double-stranded breaks

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to all eukaryotes. Furthermore, random end-joining of linear organelle DNA fragments, or of T-DNA (transfer DNA) sequences during *Agrobacterium*-mediated T-DNA transformation of plants, can apparently take place before or during insertion into the nuclear genome (15, 103, 106) and likely also involves the NHEJ mechanism.

Chan and coworkers (25) recently demonstrated that ionizing radiation increases the frequency of microhomology-mediated DNA integration. Irradiated yeast cells displayed 77% microhomology-mediated integration, compared with 27% in nonirradiated cells. In consequence, certain genotoxic stresses, in addition to facilitating the release of plastidic DNA caused by plastid damage and decay, could also promote the integration of the released DNA into the nuclear genome (16).

EVOLUTIONARY CONSEQUENCES

The transfer of DNA from the proto-organelles to the nucleus is now recognized to have led not only to the successful establishment of mitochondria and plastids, but also to substantial changes in the composition of the proteomes of other compartments in the plant cell (reviewed in Reference 67). Thus, as many as half of the 3500–4500 proteins encoded by cyanobacterial genes in *A. thaliana* localize to compartments other than the chloroplast (31, 78, 110), implying that products of genes derived from organelles are not automatically redirected back to their original compartment. This finding uncovers a fascinating facet of eukaryotic evolution, i.e., that any endogenous or introduced gene can be tested and selected for its usefulness by redirecting it to different compartments of the cell. Consequently, many metabolic pathways represent evolutionary mosaics.

Although in most eukaryotes transfer of functional genes is now a rare occurrence or has ceased altogether, organelle DNAs are nevertheless still being ubiquitously and continuously transferred to the nucleus. Until recently, NUMTs and NUPTs, which result from this ongoing transfer, were considered to be harmless, forming non-protein-coding sequences or pseudogenes (10, 47). But given the remarkably high rate of invasion of nuclear genomes by organelle DNA (51, 130), organelle DNA invasion has been proposed to be potentially detrimental to nuclear genes (43, 113). In accordance with this proposal, recent NUMT insertions were found to modify exon-intron patterns in predicted human genes (107), and potentially harmful mutagenic effects of organelle DNA are supported by examples of an association between NUMTs and inherited diseases in humans (18, 137).

The high rate of ongoing organelle-to-nucleus DNA transfer, together with the association of recent NUMTs/NUPTs insertions with genic regions, has raised the question of whether invasion of the nucleus by NUMTs and NUPTs has been exclusively neutral or deleterious for gene evolution or whether beneficial effects have accrued over evolutionary time. The recent identification of genes containing NUMT/NUPT-derived exons in yeast, humans, *Arabidopsis*, and rice (90), as well as genes containing old NUMTs in several fish species (6), provides evidence for the view that DNA transfer from organelles to the nucleus represents a novel mechanism for remodeling nuclear genes by inserting entirely new modules into preexisting proteins.

One provocative idea is that transposition of organelle DNA to the nucleus might confer a selective advantage at the species level, even though most ptDNA transpositions are likely to be nonfunctional or detrimental (121). Because inheritance and replication of a functional chloroplast genome are essential for survival, the uniparental (parental) mode of organelle inheritance that allows transfer of organellar DNA to the nucleus in the male germline, while suppressing transposition in the female germline, would maximize the benefits associated with transposition while maintaining the essential functions of the plastid genome, and hence would become a characteristic of successful species. If true, this idea would provide a

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new explanation for the widespread incidence of uniparental inheritance in eukaryotes. The argument can, however, be reversed: Biparental inheritance might be positively selected for during evolution as a means to reduce the detrimental (mutational) effects of organelle-to-nucleus DNA transfer.

CONCLUDING REMARKS

Two major consequences of organelle-to-nucleus DNA transfer for nuclear genome evolution are well known: the ancient relocation of entire plastid and mitochondrial genes to the nucleus, and the ongoing generation of NUMTs and NUPTs, noncoding sequences or pseudogenes, that are deleterious or selectively neutral (68). In addition, organelle DNA can clearly also refashion nuclear genes by providing new exon modules (121), revising the view that new nuclear genes are exclusively created by exon shuffling, gene duplication, retroposition, mobile element recruitment, gene fusion/fission, or de novo derivation from previously noncoding genomic regions (71). Indeed, nuclear insertions of organelle DNA might occur relatively frequently in genes (90, 107), because transcription promotes DNA breakage (38), which, in turn, stimulates the integration of other DNA sequences by NHEJ (68). Although the vast majority of such insertion events should have deleterious effects, as observed in the case of recent human NUMTs (107), a certain proportion will generate novel ORFs coding for proteins with improved, or even novel, functions. Sequence changes within the new protein module can then optimize the chimeric protein for its new cellular function. Cases in which exons derived from organelle DNA show a ratio of nonsynonymous versus synonymous substitution rates of >1 argue for the adaptation of the chimeric protein sequences to their new biochemical function in the cell (90). This theory is compatible with the current view that the products of recently created genes tend to undergo rapid evolution (71). How often might nuclear genes be productively remodeled by organelle DNA? The relatively small number of cases observed in the four species that have been investigated extensively (90) might represent only the tip of the iceberg of organelle DNA–mediated formation of novel exons, comprising only the most recent fraction of the longest nuclear insertions of organellar DNA sequences recruited successfully as coding nuclear gene sequences.

Taken together, the data so far accumulated demonstrate that organelle-to-nucleus DNA transfer is a multifaceted phenomenon. Organelle-to-nucleus DNA transfer (*a*) provides the nuclear genome with novel genes derived from endosymbionts, (*b*) complements the mutational spectrum in the nucleus by a steady rate of DNA insertions, and (*c*) remodels preexisting nuclear genes by providing novel exon sequences. But there might be additional effects on the regulation of nuclear genes. Instances of organelle DNA sequences inserted into noncoding regions of nuclear genes have been associated with changes in their regulation (14). Such insertions can function as autonomous replication sequences (54), promoter elements (117), introns, or novel splice sites (107). Therefore, future analyses will need to clarify the extent and variety of the effects of organelle-to-nucleus DNA transfer on gene activity.

The high frequency of plastid transgene relocation to the nucleus also has profound consequences for biosafety issues (51, 121). Plastid transgenesis alone clearly does not provide complete transgene containment, and additional safeguards will be necessary to eliminate all possibility of transgene escape. This concept leads again to the question of what mechanisms mediate intercompartmental DNA transfer and what factors influence its frequency. The answers may emerge from genetic screens for mutants with increased rates of nuclear DNA relocation. Although initial attempts in yeast failed to identify components that are required specifically for organelle-to-nucleus DNA transfer (44, 131, 133), novel large-scale screens promise to define sets of genes (and their products) that are required for intercompartmental DNA transfer or that have the

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potential to be used in transgenic approaches to suppress DNA transfer, for instance in transgenic crops.

In summary, the inevitable (adventitious or controlled) release of DNA from intracellular compartments, in combination with omnipresent mechanisms that can integrate pieces of DNA into chromosomes, makes intercompartmental DNA transfer an unavoidable attendant consequence of the eukaryotic grade of cellular organization. The current composition of the different genomes in eukaryotic cells therefore reflects the outcome of selective forces that have acted on the results of such cases of DNA transfer. Almost all organellar genes now reside in the nucleus, where they can be regulated in a complex and integrated manner (13, 77). Some genes still reside in the organelles, because their products either cannot be efficiently reimported from the cytosol or must be retained in the organelle to ensure efficient regulation of their expression. Last but not least, intercompartmental DNA transfer is ongoing and continues to reshape eukaryotic genomes. Given the frequency and wide spectrum of nuclear insertions of organelle DNA, one can no longer consider organelle-to-nucleus DNA transfer as an evolutionary force that was relevant only in ancient times, when it enabled the transfer of entire genes en masse to the nucleus. Instead, the discovery of chimeric genes with single exons derived from organelle DNA, and the association of NUMTs and NUPTs with regulatory elements of genes, teaches us that transfer of organelle DNA still has a major impact on the evolution of nuclear genes and genomes, albeit on a rather more subtle level than in the distant past.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Jeremy Timmis and Paul Hardy for critical comments on the manuscript. The authors' work was supported by the Deutsche Forschungsgemeinschaft (grants SFB-TR1 TP A7 and A10) and the Max-Planck Society.

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