ORIGIN AND EVOLUTION OF MITOCHONDRIAL DNA

Michael W. Gray

Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

INTRODUCTION

In contemplating how the mitochondrial genome originated and what has happened to it since, one is immediately confronted with the extraordinary variation in size, structure, organization, and mode of expression of mitochondrial DNA (mtDNA) in different eukaryotes (Gray 1982, 1988; Gray & Doolittle 1982; Wallace 1982; Sederoff 1984). This diversity has made it very difficult to discern the pathway(s) of mitochondrial genome evolution. Only within groups of relatively closely related organisms have comparative studies (and in particular sequence analysis) begun to reveal the mode and tempo of mtDNA evolution, permitting reasoned speculation about the mechanisms by which mtDNA divergence has occurred.

Underlying the present review are many questions: What is the evolutionary origin of the mitochondrial genome? What was its ancestral form?
What has happened to it since its appearance in the eukaryotic cell? By what mechanism(s) has mtDNA evolved in different eukaryotes? Did the range of mitochondrial genomes in contemporary eukaryotes originate in a single event, or have they had several, independent origins (i.e. is mtDNA monophyletic or polyphyletic)? Is it possible to decide between these two alternatives, and if so, how? Although much progress has been made in defining the structural and evolutionary basis of mtDNA diversity, we are still some way from being able to provide satisfactory answers to all but perhaps the first of these questions.

Several excellent reviews dealing with the genetics, molecular biology, and biogenesis of mitochondria have appeared recently (Tzagoloff & Myers 1986; Chomyn & Attardi 1987; Attardi & Schatz 1988); only limited reference to these topics, sufficient to frame the evolutionary perspective of the present review, will be made here. Considerable emphasis will, however, be placed on the nature and extent of mtDNA structural variation, as this is basic to an ultimate understanding of mtDNA evolution. Because we will take a broad look at the subject, a great deal of relevant data and many insights pertaining to intraspecific mtDNA divergence and intra-individual mtDNA variation [heteroplasmy; see Solignac et al (1987) and references therein] must be omitted. Much of this information has come from the increasing application of mtDNA analysis to questions of systematics and population genetics; again, detailed critical reviews of these topics may be found elsewhere (Wilson et al 1985; Avise et al 1987; Moritz et al 1987). Finally, in order to keep within reasonable bounds, this review will be selective in focus and will preferentially highlight topics from the most recent literature.

**STRUCTURAL AND ORGANIZATIONAL FLUIDITY OF MITOCHONDRIAL DNA**

Mitochondrial DNA has the same basic role in all eukaryotes that contain it: it encodes rRNA and tRNA components of a mitochondrial protein synthesizing system, whose function is to translate a small number of mtDNA-encoded mRNAs that specify essential polypeptide components of the mitochondrial electron transport chain (Tzagoloff & Myers 1986; Attardi & Schatz 1988). In animal mitochondria, the thirteen translation products (their genes indicated in parentheses) include seven subunits of NADH dehydrogenase \((ndh1,2,3,4,4L,5,6)\), the apocytochrome \(b\) component of ubiquinol cytochrome \(c\) reductase \((cob)\), three subunits of cytochrome \(c\) oxidase \((cox1,2,3)\), and two subunits of ATP synthase \((H^+\text{-ATPase})\) \((atp6,8)\). Not all of these genes are found in all mtDNAs: in fact, only \(cox1\) and \(cob\) and the genes encoding large subunit (LSU) and small subunit (SSU) rRNAs are common to all those mitochondrial genomes
whose coding capacity has been completely defined. On the other hand, some mitochondrial genomes carry extra genes (e.g. for proteins associated with mitochondrial ribosomes or involved in mitochondrial RNA processing or intron transposition) (Chomyn & Attardi 1987).

Although conservative in basic genetic function, mtDNA differs radically in structure among and even within the four traditional eukaryotic kingdoms (Animalia, Plantae, Fungi and Protista) (Gray & Doolittle 1982; Gray 1982, 1988; Wallace 1982; Sederoff 1984; Clark-Walker 1985). Mitochondrial genome size varies over more than a 150-fold range, from a current low of 14.3 kbp in the nematode worm, *Ascaris suum* (Wolstenholme et al. 1987) to an estimated high of 2400 kbp in the cucurbit, *Cucumis melo* (muskmelon) (Ward et al. 1981). Most mtDNAs are circular, as deduced from their form when isolated or from the fact that they have circular genetic and/or physical maps. However, linear mtDNAs have also been identified in the animal *Hydra* (Warrior & Gall 1985), the fungi *Hansenula mrakii* (Wesolowski & Fukuhara 1981) and *Candida rhagii* (Kováč et al. 1984), the ciliate protozoans *Paramecium* (Pritchard et al. 1986) and *Tetrahymena* (Suyama et al. 1985), and the green alga *Chlamydomonas reinhardtii* (Grant & Chiang 1980).

**Animal Mitochondrial DNA**

Initially, animal mtDNA was considered to be not only “an extreme example of genetic economy” (Attardi 1985), but a paradigm of structural stability. This perception was based on the finding that four vertebrate mtDNAs (human, mouse, bovine and *Xenopus laevis*) are very similar in size (16.5–17.6 kbp), have an invariant gene order, and are organized and expressed in an essentially identical manner. These mtDNAs mostly consist of structural genes, which are butt-joined one to another, with few or no spacer nucleotides between them. What little size variation exists among vertebrate mtDNAs is mainly confined to a 1–2 kbp noncoding region (the D-loop region), which contains the promoters of heavy (H) and light (L) strand transcription as well as the origin of H-strand replication (Clayton 1984).

More recently, this view of structural uniformity has been challenged by studies of additional animal mtDNAs, particularly nonvertebrate ones. Size variation now extends from 14.3 kbp in *A. suum* (Wolstenholme et al. 1987) to as much as 32.1–39.3 kbp in *Plactopecten magellanicus* (sea scallop) (Snyder et al. 1987). The smaller size of *Ascaris* mtDNA reflects the absence of the *atp8* gene found in vertebrate mtDNA, while expansion in some of the larger animal mtDNAs is due to localized sequence amplification, resulting in large (0.8–8.0 kbp) direct tandem duplications of both coding and noncoding portions of the genome (Moritz & Brown 1987; Bentzen et al. 1988; Hyman et al. 1988).
A different mitochondrial gene order has been found in each animal phylum studied to date (vertebrates, insects, echinoderms, nematodes, platyhelminths) (Clary & Wolstenholme 1984; Wolstenholme et al 1987; Jacobs et al 1988; Garey & Wolstenholme 1989). Even within phyla, variation in gene order may exist: e.g. between the mtDNAs of birds and other vertebrates (Yang & Zhou 1988), echinoid and asteroid echinoderms (Jacobs et al 1989), and orthopteran and dipteran insects (Haucke & Gellissen 1988). Nevertheless, vertebrate and invertebrate mtDNAs both maintain the extremely compact arrangement of genetic information first described in human and mouse mtDNA.

In several cases, gene order is seen to be related. Human mtDNA differs from that of sea urchin by only two transpositions in the order of protein and rRNA genes (Jacobs et al 1988), and from that of Drosophila by three inversions (Clary & Wolstenholme 1984). In other instances (e.g. Ascaris vs human) there is little evident relationship (Wolstenholme et al 1987). The positions of tRNA genes are much more variable. In both sea urchin (Jacobs et al 1988; Cantatore et al 1988) and starfish (Jacobs et al 1989), 15 of 22 tRNA genes are clustered together, whereas tRNA genes are more uniformly distributed throughout vertebrate, insect, and A. suum mtDNAs, although in different orders relative to each other and other genes.

**Fungal Mitochondrial DNA**

Among ascomycetes, mtDNA size varies from 17.6 kbp in Schizosaccharomyces pombe EFI to 115 kbp in Cochliobolus heterostrophus (Wolf & Del Guidice 1988). Almost as great a variation (28–101 kbp) has been reported in a group of much more closely related yeasts (Hoeben & Clark-Walker 1986) and even within a single genus of basidiomycetes (36–121 kbp in Suillus; Bruns et al 1988). Features contributing to this large size variation have been extensively documented and are discussed in detail elsewhere (Clark-Walker 1985; Wolf & Giudice 1988). They include intergenic spacer length, presence or absence of optional introns, presence and number of short repetitive sequences, duplication of portions of the genome, presence of novel open reading frames (ORFs), and length polymorphisms within coding regions. Changes in the amount of sequence between and within coding regions (i.e. spacers and introns, respectively) are chiefly responsible for the wide intraspecies as well as interspecies variation in fungal mitochondrial genome size. Comparison of the mitochondrial genomes of Torulopsis glabrata (18.9 kbp) and Saccharomyces cerevisiae (68–81 kbp) graphically illustrates this point: although their coding regions share >80% sequence identity (see Clark-Walker et al 1983), these two genomes differ in size by 50–60 kbp, most of which is accounted for by A+T-rich intergenic DNA.

The smallest fungal mtDNAs, those of S. pombe and T. glabrata, are
MITOCHONDRIAL DNA EVOLUTION

only slightly larger than typical animal mtDNAs. Not surprisingly, they also exhibit a compact arrangement of genetic material, although economy of organization is not taken to the extreme seen in animal mtDNA. Thus, whereas animal mtDNA entirely lacks spacers, genes in the smallest fungal mtDNAs are still separated by A+T-rich spacers of from a few to a few hundred bp in length (Lang et al 1983; Clark-Walker et al 1985b).

Intron content underlies major differences in the size of homologous genes in fungal mtDNA. In *S. cerevisiae*, cox1 may contain up to seven introns, and as such span about 10 kbp, half the size of the entire *S. pombe* mitochondrial genome. Strain-dependent variation in intron number, indeed their complete dispensability (Séraphin et al 1987), characterizes fungal mitochondrial introns as optional. Although introns are reduced in number in the smallest fungal mitochondrial genomes, they are not entirely absent (Wolfe & Del Giudice 1988); in contrast, introns have not been found in animal mtDNAs.

Is intron variation a consequence of progressive intron loss from a larger, intron-containing progenitor, or have introns been progressively acquired starting with a small, intron-less mitochondrial genome? Clark-Walker et al (1983) have argued that because *S. cerevisiae* and *Neurospora crassa* mtDNAs share (at exactly the same positions) introns that are lacking in *T. glabrata* mtDNA, and because *S. cerevisiae* and *T. glabrata* are more closely related at the level of mtDNA sequence than either is to *N. crassa*, it is likely that introns have been selectively lost from *T. glabrata* mtDNA during its evolution, rather than selectively gained by *S. cerevisiae* mtDNA. However, the recognition of mitochondrial introns as mobile genetic elements (Dujon et al 1986), and the demonstration that duplicative transposition of a yeast mitochondrial intron is a highly site-specific event (Colleaux et al 1988), weaken this argument and make it likely that introns have both come and gone in the course of fungal mtDNA evolution. Recent evidence is, in fact, consistent with two independent acquisitions of an intron by an originally intron-less cob gene in *S. pombe* (Zimmer et al 1987). Other considerations have prompted speculation about the possibility of horizontal transfer of mitochondrial introns (Lang 1984; Dujon et al 1986; Michel & Dujon 1986; Wolf & Del Giudice 1987; Cummings & Domenico 1988).

Unique mitochondrial gene orders have been reported in every fungal genus examined, although conserved blocks of genes are evident in pairwise comparisons (Wolf & Del Giudice 1988). Of particular note is a five gene cluster that is common to the mtDNAs of *T. glabrata* and *Saccharomyces exiguis* (23.7 kbp), and that includes juxtaposed *atp6* and *atp9* genes in the same order and orientation as their homologues in the *Escherichia coli* unc operon. These two genes are dispersed in the larger yeast mtDNAs, which led Clark-Walker et al (1983) to suggest that the latter are descended
from smaller ancestral forms bearing some resemblance to *T. glabrata/S. exiguus* mtDNAs.

Comparisons at intermediate levels of divergence have begun to reveal common or closely related patterns of fungal mtDNA organization. A common mitochondrial gene order is shared by three ascomycetes (two *Brettanomyces*, one *Eniella*) whose mtDNAs range between 28.5 and 41.7 kbp (Hoeben & Clark-Walker 1986). Genes are also arrayed identically in four species of basidiomycetes (genus *Suillus*) having mtDNAs differing more than 3-fold in size (36–121 kbp) (Bruns et al 1988; Bruns & Palmer 1989). In both of these studies, similar mitochondrial gene orders, interconvertible by either one or two single-step rearrangements, were identified in related species or genera. Consideration of what appear to be single-step large scale rearrangements, coupled with intensive restriction site mapping of small size-conserved regions, permits the construction and evaluation of putative pathways of divergence, and has provided suggestive evidence for an expansion of the mitochondrial genome within *Suillus* (Bruns & Palmer 1989).

**Plant Mitochondrial DNA**

Plant mitochondrial genomes are by far the largest and most complex mtDNAs known (Pring & Lonsdale 1985; Newton 1988). The smallest plant mtDNA (208 kbp in *Brassica hirta*, white mustard; Palmer & Herbon 1987) is already larger than the largest fungal mtDNA (176 kbp, *Agaricus bitorquis*; Hintz et al 1985) and larger than most chloroplast DNAs (ctDNAs) (Palmer 1985a,b). Plant mitochondrial genome size varies over a 10-fold range (up to 2400 kbp in *C. melo*), and shows a 4-fold difference even within a single family (Ward et al 1981). Although a few novel genes have been described in plant mtDNA, and some plant mitochondrial genes contain introns, these two features do not contribute in a major way either to the large size or the size variation of plant mtDNA. Rather, most of the plant mitochondrial genome appears to consist of noncoding DNA. In contrast to fungal mtDNAs, putative spacer DNA is not A+T-rich in plant mtDNA, but has much the same base composition as the bulk mtDNA (ca. 47% G+C).

Genes in plant mtDNA are scattered and for the most part solitary; only in a few cases are coding sequences within close proximity to one another and either potentially or actually cotranscribed (Gray & Spencer 1983; Bland et al 1986; Wissinger et al 1988; Gualberto et al 1988). Gene maps have been published for the mtDNAs of maize (Dawson et al 1986), spinach (*Stern & Palmer* 1986), and turnip (*Brassica campestris*) (Makaroff & Palmer 1987); these are completely different from one another. The conserved juxtaposition of 18S and 5S rRNA genes (Huh & Gray 1982) is so far the only feature common to all angiosperm mtDNAs examined.
Other than this, species as closely related as wheat and maize (members of the same family) show no evident commonality in sequence arrangement. On the other hand, within species structural variation can be quite low (Palmer 1988).

A major step in understanding the physical organization of plant mtDNA has been the recognition that it contains directly repeated sequences that are recombinationally active (Palmer 1985a; Pring & Lonsdale 1985). This has led to the formulation of a multicircular genome model, in which the entire genetic complexity is represented in a circular molecule (the master chromosome) that can be resolved into a number of subgenomic circular molecules by intramolecular recombination (Palmer 1985a; Lonsdale et al 1988). In the simplest case, exemplified by the tripartite turnip mitochondrial genome, a 218 kbp master circle is in equilibrium with 135 and 83 kbp subgenomic circles generated by recombination between a single pair of 2 kbp direct repeats in the master chromosome (Palmer & Shields 1984). Direct evidence for the existence of the predicted circular forms has recently been obtained (Palmer 1988). As the number of recombinationally active repeats increases (as in the 570 kbp maize mtDNA), so do the possibilities for complex recombination (Lonsdale et al 1984). This model largely accounts for the physical heterogeneity displayed by isolated plant mtDNA (as seen by restriction and hybridization analysis; Bonen & Gray 1980; but see Palmer & Herbon 1987). Sublimons, substoichiometric restriction fragments presumed to result from infrequent recombination events between very short regions of sequence similarity (Small et al 1987), may also contribute to the physical heterodispersity of plant mtDNA. Judging from the results of sequence analysis (Gualberto et al 1988; Joyce et al 1988b; D. F. Spencer & M. W. Gray, unpublished), plant mtDNA appears to contain an abundance of short dispersed repeats, which are obvious candidates as foci for rearrangement.

Another hallmark of the plant mitochondrial genome is its acceptance of sequence information from other genomes, notably chloroplast (Stern & Lonsdale 1982) but also nuclear (Schuster & Brennicke 1987). Promiscuous chloroplast sequences are widely distributed in plant mtDNA, seemingly in random fashion both with respect to their variation in different plant species and the portion of the chloroplast genome incorporated (Stern & Palmer 1984). Sequential transfer of genetic information from chloroplast to mitochondrion in the course of evolution, and the persistence of these sequences in plant mtDNA over long periods of evolutionary time, has recently been reported (Nugent & Palmer 1988). Prominently represented among the promiscuous chloroplast sequences in plant mtDNA are tRNA genes, and there is increasing evidence that some of these are transcribed and matured and may actually function in translation in plant mito-
Thus, the plant mitochondrial genome may be considered an evolutionary mosaic (Schuster & Brennicke 1988), with evidence for the acquisition of genetic information (and possibly even active genes) from several distinct sources in the course of evolution.

**Protist Mitochondrial DNA**

Considering the evolutionary diversity and antiquity of the unicellular eukaryotes, the true magnitude of which we are just now beginning to appreciate (Sogin et al 1989), it is a bit of an understatement to say that relatively little is known about protist mitochondrial genomes. Only within 5 of the 27 protistan (protoctist) phyla described by Margulis & Schwartz (1988) has mtDNA been characterized in any detail: Zoomastigina (genera *Crithidia, Leishmania, Trypanosoma*), Ciliophora (*Paramecium, Tetrahymena*), Chlorophyta (*Chlamydomonas*), Chytridiomycota (*Allomyces, Blastocladiella*), and Oomycota (*Achlya*).

To date, the most intensively studied protist mitochondrial genome has been the 20–40 kbp maxicircle DNA of the kinetoplastid (trypanosomid) protozoa (Benne 1985; Simpson 1986, 1987). In this genome, a relatively well conserved transcribed region (15–17 kbp) contains several of the classical mitochondrial genes (rRNA, *cox1,2,3, cob*, several *ndh*) and a few unidentified ORFs, but seemingly lacks *atp* or tRNA genes. The remainder of the mtDNA is an A+T-rich divergent (apparently nontranscribed) region containing a complex set of repetitive sequences (Muhich et al 1985). Coding information, although compactly organized (de la Cruz et al 1984), is not as condensed as in animal mtDNA. Maxicircle genes are conserved among the trypanosomids, but their order is entirely different from that seen in other organisms (Benne 1985). Consistent with the deep branching of the trypanosomid lineage (Sogin et al 1989), trypanosomid mitochondrial genes are less similar in sequence to the homologous yeast or human genes than the latter are to each other (de la Cruz et al 1984). Particularly unusual are the trypanosomid mitochondrial 9S (SSU) and 12S (LSU) rRNAs, which display only the barest resemblance to conventional rRNAs (Benne 1985; Simpson 1987). RNA editing, an intriguing phenomenon recently discovered in trypanosomid mitochondria, is reviewed elsewhere in this volume (see L. Simpson).

Unique features are also found in the linear mtDNAs of the ciliates *Paramecium* (41 kbp; Pritchard et al 1986) and *Tetrahymena* (55 kbp; Suyama et al 1985). *T. pyriformis* mtDNA contains duplicate LSU rRNA genes, located in 3 kbp sub-terminal inverted repeats and well separated from an internally localized, single copy SSU rRNA gene. The rRNA genes consist of two modules, encoding the two subunits (α and β) of split SSU and LSU rRNAs (Schnare et al 1986; Heinonen et al 1987); the α
cistrons specify the 5′-terminal 208 (SSU) or 280 (LSU) nucleotides of the respective rRNAs. A striking feature of the LSU gene is that the α module is rearranged relative to its expected position: in the direction of transcription, it is found downstream of the β coding sequence, and separated from it by a tRNA\textsubscript{Leu} gene (Heinonen et al 1987). Paramecium mtDNA does not possess a terminal duplication, although its single LSU rRNA gene is still located at one end of the mtDNA and also specifies a split LSU rRNA (Seilhamer et al 1984). In this case, however, the α module is located conventionally, upstream of the β module in the direction of transcription. It appears that duplication of the mitochondrial LSU rRNA coding region took place in the Tetrahymena lineage after its divergence from Paramecium, and was accompanied by rearrangement of the LSU rRNA gene. Interestingly, one copy of the LSU\textsubscript{α} and tRNA\textsubscript{Leu} genes is missing in several species of Tetrahymena (Morin & Cech 1988b). Other notable features of Tetrahymena mtDNA are (a) the presence of heterogeneous telomeric ends, composed of short (30–50 bp), species-specific tandem repeats (Morin & Cech 1986, 1988a), and (b) the apparent specification of fewer than the minimal number of tRNA genes required for mitochondrial protein synthesis (Suyama 1986). Ciliate mitochondrial protein genes are the most divergent known, more so even than their trypanosomid counterparts (Pritchard et al 1986); this is puzzling in view of the fact that (a) ciliates are supposed to have diverged later than trypanosomids in eukaryotic evolution (Sogin et al 1989), and (b) ciliate mitochondrial rRNA genes are much more conventional than their trypanosomid counterparts (Gray 1988).

At 15.8 kbp, the linear mtDNA of Chlamydomonas reinhardtii is the smallest protist mitochondrial genome described to date. It displays extensive physical and transcriptional linkage of genes, and in organization and mode of expression more closely resembles animal than plant mtDNA (Gray & Boer 1988). Notable features include (a) the apparent absence not only of any atp genes but of the otherwise ubiquitous cox2 and cox3 genes, (b) the presence of two novel ORFs, one of which specifies a reverse transcriptase-like polypeptide (Boer & Gray 1988a), (c) the presence of only three tRNA genes in 80% of the genome sequenced (Boer & Gray 1988c), and (d) a scrambled arrangement of rRNA coding modules for highly split SSU and LSU rRNAs (Boer & Gray 1988b).

Within the Chytridiomycota (phycomycetes), the size of the mtDNA ranges from 35.5 kbp in Blastocladiella emersonii to 56 kbp in Allomyces macrogynus (see Borkhardt & Olson 1986). Borkhardt et al (1987) reported pronounced differences in the restriction profiles of mtDNA from seven species of Allomyces, as well as of the (same size) mtDNAs from four isolates of A. arbuscula. In spite of this variation, available evidence suggests that gene order is the same in B. emersonii, A. arbuscula, and A. macrogynus.
(Borkhardt et al 1988), with single SSU and LSU rRNA genes widely separated in the circular mtDNA, as in some fungi. In contrast, rRNA genes are localized within 10–12 kbp inverted repeats in the 50 kbp circular mitochondrial genomes of the oomycetes Achlya ambisexualis (Hudspeth et al 1983) and A. klebsiana (Boyd et al 1984). This arrangement, so far unique among mtDNAs, is found in most ctDNAs (Palmer 1985b); like the latter, Achlya mtDNA exists as isomeric forms that are generated by a flip-flop recombination mechanism involving the inverted repeats (Palmer 1985b).

RAPID VS SLOW EVOLUTION OF MITOCHONDRIAL DNA

A remarkable feature of mammalian mtDNA is its rapid rate of evolution (Brown 1985; Wilson et al 1985; Cantatore & Saccone 1987). Brown et al (1979, 1982) first reported that mtDNA in primates undergoes sequence divergence at a 5–10-fold higher rate than primate single copy nuclear DNA (nDNA), a finding subsequently extended to other mammals (Miyata et al 1982). Salient features of this accelerated rate of sequence change are (a) an exceptionally high proportion of silent (>90%) vs replacement (<10%) substitutions, (b) a high transition/transversion ratio (on the order of 10:1), and (c) a strong bias toward C → T transitions in the L-strand (see Brown & Simpson 1982; Brown et al 1982). In pairwise comparisons, the transition/transversion ratio falls as the divergence times of the compared species increase, probably as a result of obliteration of the record of transitions by multiple substitutions at the same site (see Wilson et al 1985). Rates of sequence divergence for different functional portions of the mtDNA decrease in the order D-loop region > protein coding genes > rRNA and tRNA genes (Brown 1985). Although tRNA genes are changing in sequence at about one-half the rate of protein genes in primate mtDNA, they are evolving on the order of 100 times faster than their nuclear counterparts. This has been attributed to relaxed functional constraints on mitochondrial compared with nuclear tRNA genes (Brown et al 1982). Each of the 13 protein coding genes in mammalian mtDNA exhibits its own characteristic rate of change, which appears to be the same among mammals (Brown 1985). One striking exception is the cox2 gene, which in the primate lineage has undergone at least a 5-fold acceleration in rate of divergence at the amino acid level (Brown & Simpson 1982; Cann et al 1984), concomitant with a parallel acceleration in the rate of change of cytochrome c, a nucleus-encoded partner protein.

At the present time there is some debate about whether other animal mtDNAs sustain the rapid rate of sequence evolution observed in mammalian mtDNA. Virtually equivalent rates of mtDNA and nDNA divergence have been reported for Drosophila (Powell et al 1986; Solignac et al
1986) and sea urchin (Vawter & Brown 1986), as well as an equivalent frequency of transitions and transversions in Drosophila mtDNA (Wolstenholme & Clary 1985). More recent work, however, indicates that the mtDNAs of Hawaiian Drosophila species evolve quickly and with a strong transition bias, but, intriguingly, do not become very different, i.e. mtDNA change saturates at a low ceiling (DeSalle et al 1987). One complicating factor in the case of Drosophila is the nucleotide composition bias of the mtDNA (74–80% A + T), which may place particular constraints on mtDNA sequence divergence. Through continuous selection for A/T nucleotides at all sites at which such selection is compatible with function (Wolstenholme & Clary 1985), an A + T-rich mtDNA may be prevented from becoming very different even at silent sites (i.e. the third position of codons) (DeSalle et al 1987). On the other hand, Vawter & Brown (1986) contend that the rapid rate of vertebrate mtDNA evolution is, in part, an artifact of a widely divergent rate of nuclear DNA evolution. To reconcile these divergent interpretations will require additional mtDNA and nDNA sequence comparisons of both closely and distinctly related species of both vertebrates and invertebrates.

The large amount of attention focused on rapid evolution of animal mtDNA has tended to foster the impression that mtDNA evolves this way in all eukaryotes. While some cases of rapid evolution of nonanimal mtDNA have been reported (e.g. in Tetrahymena; Morin & Cech 1988b), this phenomenon is by no means universal. In marked contrast to animal mtDNA, plant mtDNA diverges in sequence at an extremely slow rate. This difference was apparent in the earliest comparisons of protein coding (e.g. Bonen et al 1984) and rRNA (Chao et al 1984) genes, and has been verified and emphasized by subsequent work (Gwynn et al 1987; Sederoff 1987; Wolfe et al 1987; Palmer & Herbon 1988). Wolfe et al (1987) have estimated that angiosperm mtDNA evolves at least five times more slowly than nuclear sequences and even has a synonymous substitution rate at least 3-fold lower than ctDNA. In Brassica mtDNA this low rate extends over the entire genome, including noncoding as well as coding regions (Palmer & Herbon 1988). Thus, while animal mtDNA is one of the most rapidly evolving genomes known, plant mtDNA is one of the slowest!

MECHANISMS OF MITOCHONDRIAL GENOME DIVERGENCE

Animal Mitochondrial DNA

To account for the high rate of both point and length (Cann & Wilson 1983) mutations in animal mtDNA, an enhanced mutation pressure has been postulated (Brown et al 1982; Cann et al 1984; Wilson et al 1985). Factors that might contribute to such a pressure include (a) greater expo-
sure of mtDNA to oxidative damage (e.g. superoxide; see Richter 1988); 
(b) a more error-prone system of DNA replication (poor fidelity at the level 
of dNTP selection and/or lack of editing); (c) absence or deficiency of DNA 
repair functions; (d) relative lack of a recombinational mechanism by which 
natural selection could eliminate mildly deleterious mutations (see Cann & 
Wilson 1983; Cann et al 1984), and (e) a high rate of turnover of mtDNA. 

Mammalian mitochondria apparently lack both excision and recom­ 
bination repair capacity (Clayton et al 1974; Lansman & Clayton 1975). 
However, enzymes implicated in DNA repair in other systems, such as 
uracil-DNA glycosylase and AP endonuclease, have been found in mam­ 
malian mitochondria (see Richter 1988; Tomkinson et al 1988). Moreover, 
the high fidelity of chick embryo pol-γ (mitochondrial DNA polymerase), 
attributable to a 3′ → 5′ exonuclease (proofreading) activity (Kunkel & 
Soni 1988), seems at odds with the postulate of a high rate of error 
introduction during mtDNA replication. At this point, therefore, it is not 
clear to what extent the biochemistry of mtDNA replication and repair 
plays a role in its rapid evolution.

On the other hand, a role for relaxed selection and/or relaxed functional 
constraints seems indicated. In animal mitochondria an expanded codon 
recognition pattern means that a single tRNA species is able to decode all 
four codons in those quartets that specify a given amino acid. Third posi­
tion codon changes are therefore effectively silent in these cases. In fact, 
an exceptionally high proportion of silent to replacement substitutions has 
been found in animal mitochondrial protein genes (Brown & Simpson 1982; 
Brown et al 1982), which supports the contention that the rapid evolution 
of mtDNA relative to nDNA is due only to silent changes and that amino 
acid altering substitutions accumulate in nDNA and mtDNA at compar­
able rates (Brown & Simpson 1982). Relaxed functional constraints have 
been invoked to account for the 10-fold greater (overall) rate of evolution 
of tRNA than protein coding genes in animal mtDNA (Brown et al 1982).

There is a general consensus that animal mtDNA does not undergo 
recombination (Wilson et al 1985; but see Horak et al 1974; Olivo et al 
1983). On the other hand, although major rearrangements seem to have 
ocurred relatively infrequently in the evolution of animal mtDNA, they 
obviously have occurred. It is not clear how rearrangement happens in 
such a tightly packed genome, or, equally, how it is tolerated. Recently, a 
role for tRNAs as agents of genomic change in animal mitochondria has 
been proposed (Moritz & Brown 1987; Cantatore et al 1987; Jacobs et al 
1988). One model (in which tRNAs occasionally serve as illegitimate 
primers for DNA replication) predicts a relocation of tRNA genes adjacent 
to the replication origin, as is seen in sea urchin mtDNA (Jacobs et al 
1988). However, in starfish mtDNA, an identical cluster of 13 tRNA genes 
is dissociated from the replication origin, which led Jacobs et al (1989) to
propose that clustering of tRNA genes represents the ancestral situation and that evolutionary dispersal (rather than gathering together) of tRNA genes has taken place in the animal mitochondrial genome. Supporting this view is the presence of conserved juxtaposed clusters of tRNA genes in the mtDNAs of vertebrates, insects, echinoids, nematodes, and fungi (Jacobs et al 1989).

**Fungal Mitochondrial DNA**

Few studies of fungal mtDNA evolution have assessed the rapidity of sequence change (Weber et al 1986); instead, the focus has been almost exclusively on genome rearrangement. The petite mutation in *S. cerevisiae*, which involves the formation of altered, functionally defective mitochondrial genomes (Bernardi 1979), has provided the basis for models of how evolutionary rearrangement of yeast mitochondrial genomes may occur. In *S. cerevisiae*, excision of portions of the wild-type mtDNA is mediated by repetitive elements within the A + T-rich spacer regions, via a mechanism involving site-specific recombination events between homologous sequences (de Zamaroczy et al 1983); short G+C-rich clusters within the A + T-rich spacers seem to be particularly recombinogenic (Dieckmann & Gandy 1987; Zinn et al 1988). As in the case of plant mtDNAs, intramolecular recombination between direct repeats results in excision of the DNA segment between them as a circular molecule, which in petite mutants may then be amplified as a tandem array to generate a replication-competent (but respiratory-deficient) petite mitochondrial genome. Mitochondrial DNA in other fungi undergoes similar excision-amplification events characterized by intramolecular recombination between repeated sequences (De Vries et al 1981;)

Features of yeast mtDNA that appear to contribute to evolutionary rearrangement include *(a)* a high rate of mtDNA recombination, *(b)* circularity of the mitochondrial genome, *(c)* a high proportion of intergenic, noncoding spacer sequences, and *(d)* the existence within spacers of short repeated sequences, dispersed throughout the genome (Clark-Walker & Miklos 1974). Elegant models of rearrangement, based on illegitimate recombination between short repetitive elements, have been elaborated by Clark-Walker & co-workers (Evans & Clark-Walker 1985; Clark-Walker et al 1985a). Direct evidence in support of the postulated rearrangement pathway comes from sequence analysis of novel junctions in rearranged recombinant molecules (G. D. Clark-Walker, personal communication).

Slonimski & co-workers (Kotylak et al 1985) have emphasized a key role for intron-encoded proteins in fungal mitochondrial genome evolution. Proteins encoded by yeast mitochondrial introns have been implicated in clean deletion of introns, duplicative intron transposition, and homologous recombination of exons with gene restructuring (Kotylak et al 1985).
Intron-encoded “nucleic acid wielding” proteins (Kotylak et al 1985) include putative or demonstrated reverse transcriptase (Michel & Lang 1985), DNA recombinase (Kotylak et al 1985), and DNA transposase (double-strand endonuclease) (Colleaux et al 1988) activities. Recently, evidence pointing to an independent evolution of structural and ORF regions of an intron has been reported (Mota & Collins 1988; Cummings & Domenico 1988). In one instance, highly homologous introns interrupt the ndh1 genes of Neurospora crassa and N. intermedia Varkud at exactly the same position; however, these introns contain ORFs that differ both in sequence and location (Mota & Collins 1988).

Given the large size range of contemporary fungal mitochondrial genomes, can we make any conjecture about the likely size and organization of the ancestral form, and about the direction (large to small or vice versa) in which fungal mtDNA evolution has proceeded? Clark-Walker et al (1985a) suggest that the original yeast mtDNA may have had a size near the present median value (37 kbp), with smaller and larger yeast mtDNAs representing the evolutionary consequences of contraction and expansion processes, respectively. Similarities in size and gene arrangement are not, however, necessarily indicative of close evolutionary relationship (Clark-Walker et al 1987). For example, the mtDNAs of T. glabrata (18.9 kbp) and S. exiguus (23.7 kbp), although sharing considerable similarity in gene order (Clark-Walker et al 1983), are no more closely related to each other at the level of cox2 gene sequence than each is to the larger (68–81 kbp) S. cerevisiae mitochondrial genome, which has a much different order and arrangement of genes (Clark-Walker et al 1985a). As the proportion of nonessential spacer sequence increases, we would expect that fungal mitochondrial genomes should become more prone to rearrangement by the mechanisms discussed above. There are observations both in support of (Hoeben & Clark-Walker 1986) and in conflict with (Bruns et al 1988) this expectation. Lability or resistance to rearrangement may depend on the extent to which the various features that promote evolutionary rearrangement of yeast mtDNA (outlined above) are present in other fungi.

We do not yet know how spacer sequences and their imbedded G+C-rich elements (Prunell & Bernardi 1977, Yin et al 1981) are introduced into (or deleted from) fungal mtDNAs and why these genomes are so AT-rich. As suggested by Wolfe & Del Giudice (1988), evolution toward A+T-rich genomes may reflect lack of a mechanism for repairing C to U delaminations (e.g. absence of a mitochondrial uracil N-glycosylase).

**Plant Mitochondrial DNA**

It is clear that plant mtDNA evolves rapidly in structure, but slowly in sequence (Palmer & Herbon 1988). It might be anticipated, therefore, that factors invoked to explain the rapid evolution of animal mtDNA, such
as an enhanced mutation pressure, lack of recombination, and relaxed functional constraints (Cann et al 1984), might not be operating in plant mitochondria. We have no basis for judging the fidelity of DNA repair and the existence and efficiency of post-replication repair in plant mitochondria; there simply are no data that address these issues. Available information suggests that there is not a relaxed codon recognition pattern in plant mitochondria. Relative to their nuclear-encoded counterparts, plant mitochondrial rRNAs (Gray 1988) and tRNAs (Joyce et al 1988a) are much more highly conserved in primary sequence and secondary structure than are animal mitochondrial tRNAs and rRNAs. This might be an indication that translation is more constrained in plant than in animal mitochondria and less able to accommodate rapid change in protein coding sequences, even at nominally silent sites. However, an appeal to functional constraint cannot explain the slow rate of sequence change over the entire genome (noncoding as well as coding sequences; Palmer & Herbon 1988). Lonsdale et al (1988) have suggested that plant mtDNA exists as a panmictic population (due to fusion of mitochondria) in recombinational equilibrium, which suppresses nucleotide sequence change through processes such as copy correction.

The formation of novel mitochondrial genotypes as a result of somatic cell fusion provides direct evidence of mitochondrial fusion and active mtDNA recombination in plants (reviewed in Lonsdale 1987). Similarly, the discovery of rearranged (mosaic) regions in plant mtDNA (Dewey et al 1986) implicates recombination in the generation of evolutionary diversity. The structural determinants of rearrangement/recombination processes in plant mitochondria still have to be elucidated. Detailed comparative studies of rearranged regions may provide some clues (e.g. Joyce et al 1988b). Recently, this approach has provided evidence that models elaborated for the evolutionary rearrangement of yeast mtDNA (Evans & Clark-Walker 1985; Clark-Walker et al 1985a) may also apply to plant mtDNA (Small et al 1989). Even at this stage, it is evident that the plant mitochondrial genome is a highly plastic entity that is able to tolerate considerable structural variation with little or no effect on function. This implies that sequence context is largely immaterial to the expression of genetic information in plant mtDNA.

ORIGIN OF MITOCHONDRIAL DNA:
THE ENDOSYMBIONT HYPOTHESIS

The endosymbiont hypothesis is generally regarded as the best explanation of the origin of the mitochondrial genome, as well as of the structural and functional complexity of the mitochondrion itself (Gray & Doolittle 1982; Gray 1983, 1988; Taylor 1987; but see Mikelsaar 1987). This hypothesis
(see Margulis 1981) proposes that mitochondria originated in evolution as bacterial endosymbionts that were ultimately integrated into a host cell that provided the nuclear genome. Mitochondria remain semi-autonomous in the sense that they retain a distinctive genome that is replicated and expressed, but they are incapable of independent existence. According to the endosymbiont hypothesis, the contemporary mitochondrial genome is a bacterial remnant of the original endosymbiotic event(s) that created the ancestor of the eukaryotic cell in which it now resides. Most if not all of the genes now found in mtDNA are considered to represent genetic information retained from the original endosymbiont. Two assumptions implicit in this hypothesis are (a) that nuclear and mitochondrial genomes derive from distinctly separate lineages that enjoyed a long period of independent existence and evolutionary divergence before they were united within a single cell, and (b) that in the course of evolution there was massive transfer to the nucleus and/or loss of genetic information from the endosymbiont genome.

In posing the question, "Has the endosymbiont hypothesis been proven?", W. F. Doolittle and I argued, "If the evolutionary histories of nuclear genomes and one of the organellar genomes were known with certainty, and were with certainty different—that is, if the two could be shown to derive from genomic lineages which were phylogenetically distinct before the formation of the eucaryotic cell—then the endosymbiotic origin of that organelle could be taken as proven" (Gray & Doolittle 1982). In the case of mitochondria, there is now a satisfying concordance of structural, biochemical, and genetic evidence not only supporting an origin of this organelle from eubacteria, but, indeed, tracing this origin to a specific subdivision, the \( \alpha \)-purple bacteria (Yang et al 1985; Villanueva et al 1985; John 1987; Cedergren et al 1988). Although the evolutionary origin of the nuclear genome is still uncertain, it is clearly distinct from that of the eubacterial genome (Woese 1987). Moreover, although the burden of evidence indicates that chloroplasts and mitochondria are both direct descendents of eubacteria, they have quite clearly arisen from separate and distinct phyla: cyanobacteria and \( \alpha \)-purple bacteria, respectively (see Gray 1988). As emphasized by Doolittle (1980), this makes it impossible to maintain an autogenous evolutionary scenario for both organelles; in an autogenous scheme, the progenitor of the nuclear genome would have had to have been either a cyanobacterium or an \( \alpha \)-purple bacterium, but quite obviously could not have been both.

TRACING THE PATHWAYS OF MITOCHONDRIAL GENOME EVOLUTION

Arguments for (Raven 1970; Dayhoff & Schwartz 1981; Stewart & Mattox 1984) and against (Cavalier-Smith 1987) multiple origins of mitochondria
have been made. At least part of the vast structural diversity summarized in this review might be attributable to a polyphyletic origin of mitochondrial genomes; on the other hand, one of the strongest arguments in favor of a monophyletic origin is that the basic function of mtDNA (as reflected in the genes it encodes) is fundamentally the same in all eukaryotes. Assuming that contemporary mitochondrial genomes are the result of a massive trimming down of a much larger protomitochondrial genome, the fact that they contain much the same genetic information is hard to reconcile with a polyphyletic origin, since it would imply a process of gene loss and retention that has been highly convergent. It is not at all clear what the evolutionary forces operating in such a process would be (but see von Heijne 1986).

In the quest to explore the evolutionary history of mitochondria, and to distinguish between monophyletic and polyphyletic scenarios of mitochondrial origin, much emphasis is being placed on rRNA, the ultimate molecular chronometer (Woese 1987). The rationale for using rRNA sequence comparisons to establish global phylogenetic relationships among organisms and organelles has been discussed elsewhere (Gray et al 1984; Gray 1988; Cedergren et al 1988). One particular consideration is that the genes for SSU and LSU rRNAs are the only ones that are encoded by all organellar genomes as well as by nuclear and prokaryotic (eubacterial and archaeabacterial) genomes. Thus, it is possible to use rRNA sequence information to construct phylogenetic trees that simultaneously reveal the evolutionary descent of nuclear and mitochondrial genomes. This allows one not only to determine relationships within each lineage, but to assess the degree of correspondence between nuclear and mitochondrial tree topologies (e.g. Morin & Cech 1988b). In the case of an early, monophyletic origin of mitochondria, nuclear and mitochondrial phylogenies are expected to be congruent, reflecting a parallel descent and evolution of the two genomes. In a polyphyletic scenario, major incongruities in nuclear and mitochondrial phylogenies may be anticipated, which reflects the fact that whereas nuclear genomes will have shared a common line of descent, mitochondrial genomes within these same eukaryotes will not.

It should be emphasized that there are a number of inherent difficulties in using rRNA sequence comparisons to determine mitochondrial phylogenies. Firstly, because of the extreme structural diversity displayed by homologous mitochondrial rRNAs (Gray 1988), one is constrained to use only the most conservative regions in such comparisons. This means that close-range relationships may not be well defined, because there are insufficient differences between the compared sequences. Secondly, because of widely differing rates of mtDNA sequence divergence in different eukaryotes (e.g. animals vs plants), the observed branching patterns
may be subject to methodological artifacts (see Cedergren et al 1988). Thus, it is important to test for such artifacts and to interpret the results of rRNA phylogenetic analysis in the light of comparative information about mitochondrial genome organization and expression. That said, rRNA sequence comparisons currently provide the only generally applicable and objective method of establishing long-range evolutionary connections within the mitochondrial lineage.

CONCLUSIONS AND PERSPECTIVE: MITOCHONDRIAL DNA, AN EVOLUTIONARY MOSAIC?

In summing up this admittedly incomplete review of a very complex and controversial topic, we should perhaps return to some of the questions posed in the introduction.

WHAT IS THE EVOLUTIONARY ORIGIN OF THE MITOCHONDRIAL GENOME? At least in the case of rRNA genes, a definite answer can be given. Mitochondrial rRNA genes are of direct eubacterial ancestry (Spencer et al 1984; Gray et al 1984; Yang et al 1985; Cedergren et al 1988). To what extent this fact establishes the origin of the mitochondrial genome as a whole remains to be seen. Certainly, the eubacterial character of the mitochondrial respiratory chain, and its affiliation with one particular phylum of eubacteria (the same phylum to which the ancestry of mitochondrial rRNA genes has been traced), is evident (John 1987). Mitochondrially encoded genes for respiratory proteins are clear homologues of their eubacterial counterparts (Raitio et al 1987). Thus, an endosymbiotic origin of the mitochondrial genome is most consistent with the available evidence. It is true that, in contrast to rRNA genes, we do not have available a range of eubacterial and archaeabacterial respiratory gene sequences for comparison with the range of available mitochondrial sequences. Nor do we know very much about genes that we presume were transferred to the nucleus as a consequence of a eubacterial endosymbiosis. Nuclear genes that we should particularly examine are those encoding mitochondrial ribosomal proteins, translation factors, and aminoacyl-tRNA synthetases—proteins having cytosolic homologues that are also encoded by nuclear genes.

WHAT WAS THE ANCESTRAL FORM OF THE MITOCHONDRIAL GENOME? This is particularly difficult to answer. The protomitochondrial genome may have been organized quite differently than contemporary eubacterial genomes; it may, for example, have contained introns and/or much more intergenic spacer sequence. It is reasonable to suppose that the original mitochondrial genome was a large, spaciously arrayed one, containing
much noncoding sequence—perhaps not unlike present-day plant mitochondrial genomes. In such a genome considerable evolutionary restructuring could presumably occur with minimal effect on function. In this context we would regard the small compactly organized mtDNAs, such as that in animals, as derivative rather than ancestral forms. Certainly, it is difficult to accept that something resembling the highly condensed animal mitochondrial genome, an exquisite example of economy of organization and expression, could have existed as such at the very earliest stages of mtDNA evolution.

**BY WHAT MECHANISM(S) HAS mtDNA EVOLVED IN DIFFERENT EUKARYOTES?**

As we have seen, comparative studies have started to reveal characteristic patterns of mitochondrial genome evolution, patterns that are quite distinct in the various eukaryotic phyla and that presumably account for much of the structural diversity we see among present-day mtDNAs. In some cases, both the mode and tempo of mtDNA evolution have become apparent as a result of careful, extensive studies of this kind. We can expect that as these studies are extended within a phylum, e.g. from vertebrate to invertebrate animals, the evolutionary basis for differences in mitochondrial genome arrangement and expression will become clearer. What will still be missing are the evolutionary connections between the mtDNAs in the major kingdoms (e.g. plants and animals); these connections may lie within the largely unexplored group of unicellular eukaryotes, the protists. Here again, answers can only come through judicious comparative studies. Equally, we are at a rather primitive stage in our understanding of the biochemistry of enzymes that act on mtDNA, and that surely have played a major role in setting the mitochondrial genome on different evolutionary pathways in different eukaryotes.

**IS mtDNA MONOPHYLETIC OR POLYPHYLETIC?**

As outlined above, one way to address this question is through phylogenetic tree analysis. A recent application of this approach (Cedergren et al 1988) has uncovered a discordance with respect to the branching position of higher plants in nuclear and mitochondrial phylogenies, determined from separate SSU and LSU rRNA databases. Coupled with the fact that green algae (chlorophytes) and higher plants (metaphytes) do not appear to have shared a common mitochondrial ancestor as recently as they have shared a common nuclear ancestor (Gray & Boer 1988), this observation has raised the possibility that the rRNA genes of plant mitochondria have been derived more recently (in a separate endosymbiotic event) than the rRNA genes of other mitochondria (Gray et al 1989). An implication of this suggestion is that mtDNA may be an evolutionary mosaic, having derived (and lost) genetic information through various processes of lateral gene transfer in the course of its evolutionary history. Solid evidence that this has occurred in plant
mtDNA already exists (Schuster & Brennicke 1988). This leads us to a new perspective on mtDNA evolution. Perhaps some of the genetic information in mtDNA (e.g. the genes encoding components of the respiratory chain) does derive from a single endosymbiotic event, whereas information encoding other components (e.g. of the mitochondrial transcription, translation, and/or RNA processing machinery) has been more labile in the course of evolution. More extensive phylogenetic analysis (involving mitochondrial protein coding as well as rRNA genes) may provide a critical test of this possibility. The concept that eukaryotic cells are evolutionary mosaics is now well established (Margulis 1981); the proposal that eukaryotic genomes are also, to varying extents, evolutionary mosaics, is one worth careful consideration.

Acknowledgments

I am most grateful to colleagues around the world who provided reprints and preprints of unpublished data and observations. This information was invaluable in the preparation of this review. At the same time, I apologize to all those whose contributions to this topic could not be cited within the confines of this particular presentation. Finally, I greatly appreciate the assistance of Lisa Laskey in the preparation of the final manuscript. Support in the form of an Operating Grant from the Medical Research Council of Canada (MT-4124) and a Fellowship from the Canadian Institute for Advanced Research (Evolutionary Biology Program) is also gratefully acknowledged.

Literature Cited

Boer, P. H., Gray, M. W. 1988a. Genes encoding a subunit of respiratory NADH dehydrogenase (ND1) and a reverse transcriptase-like protein (RTL) are linked to ribosomal RNA gene pieces in Chlamydomonas reinhardtii mitochondrial DNA. EMBO J. 7: 3501–8
Bonnen, L., Boer, P. H., Gray, M. W. 1984. The wheat cytochrome oxidase subunit II gene has an intron insert and three radical
mitochondrial DNA evolution

Amino acid changes relative to maize. EMBO J. 3: 2531-36

Borkhardt, B., Brown, T. A., Thim, P., Olson, L. W. 1988. The mitochondrial genome of the aquatic phycomycete Allo-

Borkhardt, B., Olson, L. W. 1986. The mito-

chondrial genome of the aquatic phyco-
mycete Blastocladiella emersonii. Curr. Genet. 11: 139-43


Cann, R. L., Brown, W. M., Wilson, A. C. 1984. Polymorphic sites and the mecha-
nism of evolution in human mito-

chondrial DNA. Genetics 106: 479-99


Cantatore, P., Roberti, M., Rainaldi, G., Saccone, C., Gadaleta, M. N. 1988. Clus-
tering of tRNA genes in Paracentrotus lividus mitochondrial DNA. Curr. Genet. 13: 91-96


Cavalier-Smith, T. 1987. The simultaneous symbiotic origin of mitochondria, cho-

loroplasts, and microbodies. In Endo-


Clark-Walker, G. D., McArthur, C. R., Srip-

rakash, K. S. 1983. Order and orientation of genic sequences in circular mito-

chondrial DNA from Saccharomyces exiguus: implications for evolution of yeast mtDNAs. J. Mol. Evol. 19: 333-41

Clark-Walker, G. D., McArthur, C. R., Srip-

rakash, K. S. 1985b. Location of transcriptional control signals and transfer RNA sequences in Torulopsis glabrata mitochondrial DNA. EMBO J. 4: 465-73


Evans, R. J., Clark-Walker, G. D. 1985. Elevated levels of petite formation in strains of *Saccharomyces cerevisiae* restored to respiratory competence. II: Organization of mitochondrial genomes in strains having high and moderate frequencies of petite mutant formation. *Genetics* 111: 403–32


Joyce, P. B. M., Spencer, D. F., Bonen, L., Gray, M. W. 1988a. Genes for tRNAAsp, tRNAPro, tRNA Tyr and two tRNAsSec in wheat mitochondrial DNA. Plant Mol. Biol. 10: 251–62

Joyce, P. B. M., Spencer, D. F., Gray, M. W. 1988b. Multiple sequence rearrangements accompanying the duplication of a tRNAPro gene in wheat mitochondrial DNA. Plant Mol. Biol. 11: 833–43


Lang, B. F. 1984. The mitochondrial genome of the fission yeast Schizosaccharomyces pombe: highly homologous introns are inserted at the same position of the otherwise less conserved cox1 genes in Schizosaccharomyces pombe and Aspergillus nidulans. EMBO J. 3: 2129–36


Lonsdale, D. M. 1987. The molecular


DNA sequences and RNA transcripts for cytochrome oxidase subunit I, URF1, and three ORFs adjacent to the replication origin. Gene 44: 243–53


Small, I. D., Isaac, P. G., Leaver, C. J. 1987. Stoichiometric differences in DNA molecules containing the atpA gene suggest mechanisms for the generation of mitochondrial genome diversity in maize. EMBO J. 6: 865–69


Taylor, F. J. R. 1987. An overview of the


