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# Structure and Evolution of Organelle Genomes

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## INTRODUCTION

Recent molecular biological analyses of mitochondrial deoxyribonucleic acids (mtDNAs) and chloroplast deoxyribonucleic acids (chlDNAs) have revealed many interesting and unexpected and some totally unique features of these genomes. The mtDNAs of unicellular organisms vary greatly in size, shape, and gene arrangement, with some of the genes having organizations not previously observed in bacterial or eucaryotic genomes. By contrast, animal mtDNAs are strikingly uniform in structure and contain an extraordinary gene arrangement which permits maximum exploitation of their coding capacity. Similarly, the chlDNAs of unicellular eucaryotes are more variable in size and organization than the chlDNAs of vascular plants. Nevertheless, the organization and sequence of many chlDNA genes are highly homologous to the organization and sequence of bacterial genes, and some cloned chloroplast genes have even been expressed in *Escherichia coli*.

Analyses of the amino acid coding sequences of organelle DNAs have yielded an even greater surprise. The genetic codes of mtDNAs differ from those of all other organisms and even from each other. However, chlDNAs retain the universal code present in bacteria.

These unique and varied characteristics have rekindled interest in the origin and evolution of mitochondria and chloroplasts. Are these organelles highly evolved endosymbionts (6, 253, 264, 389) which still retain remnants of their original DNAs and associated replication, transcription, and translation systems (154, 275–277, 309, 313, 315), or are they simply advanced procaryotic algae in which the energy production membranes have been elaborated and have trapped cellular DNA, enzymes, and ribosomes (5, 85, 201)?

I have collated the data on organelle genomes available up to October 1981 and used these data to propose empirical generalizations and evolutionary relationships about organelle DNAs. The combined conclusions have been integrated into a scenario for the evolution of these unique and essential eucaryotic DNAs.

## ORGANELLE GENOME SIZE AND SHAPE

Substantial data have been accumulated on the sizes and shapes of organelle genomes (57, 154, 155, 200, 249). Generally, organelle DNAs

are much smaller than DNAs of bacteria (272, 377) and are incapable of coding for all of the mitochondrial and chloroplast proteins. Many key enzymes, including mitochondrial DNA polymerase (32, 45, 316, 384) and mitochondrial and chloroplast ribonucleic acid (RNA) polymerases (16, 17, 91), ribosomal proteins, and translation factors (17, 91, 96, 211, 217, 322) are synthesized in the cytosol and are presumably encoded in the nucleus.

### Size and Shape of Mitochondrial Deoxyribonucleic Acids (DNAs)

The mitochondrial genomes of unicellular eucaryotes exist in three alternative forms: closed circular, linear, and aggregates of large and small circles (Table 1). The closed circular monomer is the most common form.

Circular mtDNAs are found in fungi, slime molds, algae, and certain protozoa. These DNAs range from 31 μm long (62 megadaltons [Md]) in the fungus *Podospora anserina* to approximately 5 μm long (about 10 Md) in *Chlamydomonas reinhardtii*. Certain *Neurospora* strains also contain 3.3- to 3.4-kilobase circular mitochondrial plasmids (104). Linear mtDNA molecules with sizes between 25 and 35 Md have been found primarily in the ciliates *Paramecium* and *Tetrahymena*, although one linear mtDNA has been described for the yeast *Hansenula*

TABLE 1. Genome sizes of unicellular eucaryotes[a]

| Organism | Shape | Length (μm) | Mol wt ($\times 10^6$) | Reference(s) |
|---|---|---|---|---|
| Fungi | | | | |
| *Brettanomyces custersii* | | | 71.5 | 99 |
| *Podospora anserina* | Circle | 31 | 61–63 | 112 |
| *Ustilago cynodontis* | | | 50 | 265 |
| *Saccharomyces carlsbergensis* | Circle | 25.6 | 45–51 | 154 |
| *Saccharomyces cerevisiae* | Circle | 21–25 | 46–52 | 154 |
| *Neurospora crassa* | Circle | 19–20 | 40 | 154 |
| *Brettanomyces anomalus* | Circle | 18 | 37.6 | 98 |
| *Hansenula mrakii* | Linear | | 36.4 | 383 |
| *Saprolegnia* sp. | Circle | 14 | 28 | 57 |
| *Kluyveromyces lactis* | Circle | 11.4 | 24 | 154, 286 |
| *Candida parapsilosis* | Circle | 11.1 | 23 | 154, 286 |
| *Aspergillus nidulans* | Circle | 10.4 | 21 | 242 |
| *Kloeckera africana* | Circle | 9.3 | 17.9 | 99 |
| *Hansenula wingei* | Circle | 8.2 | 17.3 | 286 |
| *Schizosaccharomyces pombe* | Circle | 6.0 | 12.5 | 154, 286 |
| *Torulopsis glabrata* | Circle | 6.0 | 12.8 | 98, 101, 154 |
| Slime molds | | | | |
| *Physarum polycephalum* | Circle | 19.1 | 41 | 41 |
| *Dictyostelium discoideum* | | | 35, 40 | 249 |
| Algae | | | | |
| *Euglena gracilis* | | | 16–45, 100 | 155, 249 |
| *Chlamydomonas reinhardtii* | Circle | 4.5 | 9.8 | 154, 249 |
| Protozoa | | | | |
| Ciliata | | | | |
| *Tetrahymena pyriformis* | Linear | 15 | 32.6, 31.0, 28.4, 25.8 | 154, 162, 249 |
| *Paramecium aurelia* | Linear | 13.8 | 35, 30 | 154, 158, 249 |
| Rhizopoda | | | | |
| *Acanthamoeba castellanii* | Circle | 12.8 | 27–28 | 154, 265 |
| Sporozoa | | | | |
| *Plasmodium lophurae* | Circle | 10.3 | 21 | 57 |
| Mastigophora | | | | |
| *Crithidia fasciculata* | Circle (maxi) | 12 | ~23 | 61 |
| | Circle (mini) | 0.79 | 1.59 | |
| *Crithidia luciliae* | Circle (maxi) | 11.3 | 23 | 61 |
| | Circle (mini) | 0.76 | 1.48 | |
| *Leishmania tarentolae* | Circle (maxi) | 9.9 | ~20 | 61, 88 |
| | Circle (mini) | 0.28 | 0.56 | |
| *Trypanosoma mega* | Circle (maxi) | 9.0 | 16.1 | 61 |
| | Circle (mini) | 0.74 | 1.49 | |
| *Trypanosoma brucei* | Circle (maxi) | 6.3 | 13 | 56, 61, 62 |
| | Circle (mini) | 0.32 | 0.56 | |

[a] Constants for changing the units of genome size have been described previously (272).

*mrakii*. mtDNAs composed of complex aggregates of large and small circles (maxicircles and minicircles) are found exclusively in the kinetoplasts of the Trypanosomatidae. Minicircles lack long amino acid coding frames (20), and only the 13- to 25-Md maxicircles are transcribed (62, 186).

The extensive variation in mitochondrial genome size found in the unicellular eucaryotes is not present in multicellular animals. Of 33 animal mtDNAs examined (from flatworms to humans), all are circles of approximately 5 μm (10 Md), or 16 to 17 kilobase pairs (Table 2) (75, 177, 360). Only *Drosophila* mtDNAs deviate from this size, due to a variable adenine-plus-thymine (A+T)-rich region around the replication origin (393).

The coding capacities of plant mtDNAs are much greater than those of any other mtDNA, although the exact sizes and shapes of these DNAs remain unclear (Table 3). Electron microscope and gel electrophoresis studies have revealed a wide variety of circular and linear molecules, which vary in number and size between species, although 30-μm circular elements seem to be a common feature. Cesium chloride-ethidium bromide gradients have revealed large amounts of open circular or linear DNA (115, 116). This complexity has been confirmed by both DNA renaturation kinetics and restriction

TABLE 2. Genome sizes of animal cell mtDNAs[a]

| Organism | Shape | Length (μm) | Mol wt ($\times 10^6$) | Reference(s) |
|---|---|---|---|---|
| Platyhelminthes | | | | |
| *Hymenolepis diminuta* | Circle | 4.8 | 9.6 | 57 |
| Nematoda | | | | |
| *Ascaris lumbricoides* | Circle | 4.8 | 9.6 | 57 |
| Annelida | | | | |
| *Urechis caupo* | Circle | 5.9 | 11.8 | 57 |
| Arthropoda | | | | |
| Crustacea | | | | |
| *Artemia salina* | Circle | 5.1 | 10.2 | 57 |
| Insecta | | | | |
| *Drosophila melanogaster* | Circle | 6.2 | 12.35 | 142 |
| *Drosophila simulans* | Circle | 6.0 | 11.96 | 142 |
| *Drosophila takahashii* | Circle | 5.4 | 10.75 | 142 |
| *Drosophila kikkawai* | Circle | 5.4 | 10.68 | 142 |
| *Drosophila funebris* | Circle | 5.2 | 10.34 | 142 |
| *Drosophila tripunctata* | Circle | 5.2 | 10.32 | 142 |
| *Drosophila grimshawi* | Circle | 5.0 | 10.00 | 142 |
| *Drosophila silvarentis* | Circle | 5.0 | 10.01 | 142 |
| *Drosophila erecta* | Circle | 5.0 | 9.92 | 142 |
| *Drosophila balioptera* | Circle | 5.0 | 9.91 | 142 |
| *Musca domestica* | Circle | 5.2 | 10.4 | 57 |
| Echinodermata | | | | |
| *Lytechinus pictus* | Circle | 4.7 | 9.4 | 57 |
| Chordata | | | | |
| Pisces | | | | |
| *Ictalurus punctatus* | Circle | 5.1 | 10.2 | 57 |
| *Carrassius carrassius* | Circle | 5.4 | 10.8 | 57 |
| Amphibia | | | | |
| *Rana pipiens* | Circle | 5.8 | 11.6 | 57 |
| *Xenopus laevis* | Circle | 5.8 | 11.7 | 57, 249 |
| *Siredon mexicanum* | Circle | 4.7 | 9.4 | 57 |
| Reptilia | | | | |
| *Terrapene ornata* | Circle | 5.3 | 10.6 | 57 |
| Aves | | | | |
| *Gallus domesticus* | Circle | 5.4 | 10.8 | 156 |
| *Anas platyrhynchos* | Circle | 5.1 | 10.2 | 57 |
| Mammalia | | | | |
| *Mus musculus* | Circle | 4.7–5.5 | 9.4–11.0 | 23, 33, 57, 297 |
| *Rattus norvegicus* | Circle | 4.9–5.4 | 9.8–10.8 | 57, 75 |
| *Ovis aries* | Circle | 5.4 | 10.8 | 57, 360 |
| *Homo sapiens* | Circle | 4.8–5.1 | 9.6–10.2 | 57, 75, 288 |

[a] Constants for changing the units of genome size have been described previously (272).

TABLE 3. Genome sizes of plant cell mtDNAs[a]

| Organism | Shape | Length (μm) | Mol wt (×10⁶) | | | Reference(s) |
|---|---|---|---|---|---|---|
| | | | Contour length | Renaturation kinetics | Restriction endonuclease fragments | |
| Subclass Monocotyledoneae | | | | | | |
| *Zea mays* | Circles | 30, 22, 16 | | 320 | 120–130, 388 | 226, 337, 378 |
| | Other | 0.8, 0.6, 0.5, 0.47 | | | | |
| *Triticum aestivum* | Circles | 1–30 | | | 140–230 | 49, 301, 302 |
| Subclass Dicotyledoneae | | | | | | |
| *Glycine max* L. | Circles | 29.9, 24.5, 20.4, 16.6, 12.9, 10.0, 5.9 (mini)[b] | 241 | | 150 | 226, 345 |
| *Pisum sativum* | Circles | 5, 30 | 60–70 | 74, 240 | 231 | 207, 378 |
| *Vicia faba* | Circle | | | 70 | | 207 |
| *Spinacia oleracea* | Circles | 30 | | 70 | | 207 |
| *Solanum tuberosum* | Linear | | 58–60 | 100 | 90 | 301, 371 |
| *Nicotiana tabacum* | Circles | 0.5–42, 9[c] | | | | 335 |
| *Lactuca sativa* | Circles | 30 | | 70, 140 | | 207, 381 |
| *Parthenocissus tricuspidata* | Linear and circles | 5–30 | 60–70 | | 165 | 301, 302 |
| *Oenothera berteriana* | Circles | ~30 | 66 | | 120–130 | 70 |
| | Linear | 0–22 | ≥45 | | | |
| *Cucumis melo* | | | | 1,600 | >477 | 378 |
| *Cucumis sativus* | | | | 1,000 | 120, >497 | 301, 378 |
| *Cucurbita pepo* | | | | 560 | 475 | 378 |
| *Citrullus vulgaris* | | | | 220 | 231 | 378 |

[a] Constants for changing the units of genome size have been described previously (272). Molecular weights were determined by contour length measurements, renaturation kinetics, and summation of restriction endonuclease fragments. All of the organisms listed are members of the class Angiospermae. These plants belong to the following orders: *Zea mays* (corn) and *Triticum aestivum* (wheat), Graminales; *Glycine max* (soybean), *Pisum sativum* (pea), and *Vicia faba* (broad bean), Leguminales; *Spinacia oleracea* (spinach), Chenopodiales; *Solanum tuberosum* (potato) and *Nicotiana tabacum* (tobacco), Solanales; *Lactuca sativa* (lettuce), Asterales; *Parthenocissus tricuspidata* (Virginia creeper), Rhamnales; *Oenothera berteriana* (evening primrose), Onagrales; *Cucumis melo* (muskmelon), *Cucumis sativus* (cucumber), *Cucurbita pepo* (zucchini squash), and *Citrullus vulgaris* (watermelon), Cucurbitales.

[b] The 16.6-μm DNA is the predominant size class.

[c] The 9-μm DNA is the predominant size class.

enzyme analyses. Although initial kinetic analysis suggested that plant mtDNAs were 70 Md (207), subsequent studies have yielded values ranging from 140 Md for lettuce (381) to 1,600 Md for muskmelon (378). Several plant mtDNAs have also been found to contain repetitive sequences of 2 to 4 Md (378).

Digestion of plant mtDNAs with restriction endonucleases yields numerous fragments, many in greater than equimolar quantities. The sum of these fragments yields genome sizes ranging from 90 to 230 Md when overlapping bands are ignored and from 231 to 495 Md (378) when multiple fragment bands are counted (Table 3). The variety of fragment sizes is not due to restriction site methylation (50).

The smaller closed circular molecules of tobacco, bean, and corn mtDNAs have been found to contain sequences homologous to the larger discrete mtDNA elements of the corresponding cells. Further, in beans and tobacco, the second smallest element is a dimer of the smallest element. The tobacco monomer hybridizes to most tobacco mitochondrial RNAs, and the bean monomer hybridizes to two RNAs (both longer than the DNA). In contrast, the two smallest corn molecules do not share homology, and they hybridize to one common transcript and one unique transcript (115). Even so, each cloned restriction fragment from corn generally hybridizes only to its fragment of origin (337). These results suggest that plant mtDNAs are composed of a series of discrete sequences which can be located on a variety of genomic elements of different sizes.

Plant mtDNAs appear to be relatively fluid. Two different cultured cell lines from a single tobacco strain yielded the same mtDNA restriction fragments, but contained different populations of mtDNA molecules (116). Many species of plants are subject to cytoplasmic male sterility (132), which correlates with the gain or loss of specific mtDNA elements in corn and sugar beet (197, 224, 299). In corn, reversion from S

strain male sterility to normal results in the loss of two linear elements, S-1 and S-2. These sequences become integrated into other mtDNA genomic elements (224, 225), perhaps by transposition (80, 224–226, 337). Both S-1 and S-2 are located beside the same 26-kilobase repeated sequence in normal strains (239). Since changes between normal corn and T and S strain male-sterile corn are associated with alterations in the mitochondrially synthesized polypeptides (145, 146, 222), it is tempting to speculate that these changes may regulate mtDNA gene expression.

Thus, plant mtDNA sequences appear to be complex and fluid. The genomic sequences could be distributed among a number of separate genome elements, or they could be located primarily in one large genomic DNA with which a number of smaller genetic elements are associated.

### Size and Shape of Chloroplast DNAs

All green alga and higher plant chlDNAs are single closed circular molecules. The chlDNAs of the algae differ greatly in size from each other. The alga *Acetabularia* (Chlorophyta) has the largest chlDNA (1,500 Md), which is equivalent to a medium-sized bacterial genome (Table 4) (377). This chlDNA can be isolated as a nucleoid

TABLE 4. Genome sizes of chlDNAs[a]

| Organism | Shape | Length (μm) | Mol wt (×10⁶) | Reference(s) |
|---|---|---|---|---|
| Subkingdom Thallophyta (algae) | | | | |
| Division Chrysophyta (golden algae) | | | | |
| *Olisthodiscus luteus* | Circle | 44 | 88 | 2 |
| | Circle | 22 | 44 | |
| | Circle | 11 | 22 | |
| Division Chlorophyta (green algae) | | | | |
| *Acetabularia cliftonii* | | | 1,500 | 294 |
| *Acetabularia mediterranea* | | | 1,100 | 169 |
| *Chlorella pyrenoidosa* | | | 210–230 | 200 |
| *Chlamydomonas reinhardtii* | Circle | 63 | 170–200 | 200, 294 |
| *Codium fragile* | Circle | 27.32 | 56 | 182 |
| Division Euglenophyta (green algae) | | | | |
| *Euglena gracilis* | Circle | 44.5 | 92–150 | 200 |
| Subkingdom Embryophta | | | | |
| Division Bryophyta (nonvascular plants) | | | | |
| Class Hepaticae (liverworts) | | | | |
| *Sphaerocarpos donnellii* | Circle | 38.5 | 84.5 | 185 |
| Division Tracheophyta (vascular plants) | | | | |
| Class Filicinae (ferns) | | | | |
| *Asplenium nidus* | Circle | 44.8 | 93 | 185 |
| *Pteris vittata* | Circle | 43.8 | 91 | 185 |
| Class Angiospermae | | | | |
| Subclass Monocotyledoneae | | | | |
| *Zea mays* | Circle | 43 | 87 | 200, 208, 352 |
| *Narcissus pseudonarcissus* | Circle | 44 | 90 | 200, 208 |
| Subclass Dicotyledoneae | | | | |
| *Spirodela oligorrhiza* | Circle | | 120 | 366 |
| *Sinapis alba* | Circle | | 105 | 236 |
| *Lactuca sativa* | Circle | | 98 | 200, 352 |
| *Nicotiana tabacum* | Circle | | 99–114 | 144, 193, 200 |
| *Spinacia oleracea* | Circle | 43.8–45.7 | 89–100 | 200, 208, 352 |
| *Beta vulgaris* | Circle | 44.9 | 97–100 | 200 |
| *Antirrhinum majus* | Circle | 45.9 | 99–100 | 200, 208 |
| *Petunia hybrida* | Circle | | 96–101 | 64 |
| *Oenothera hookeri* | Circle | 45.2 | 98–100 | 200 |
| *Phaseolus vulgaris* | Circle | | 90 | 208 |
| *Pisum vulgaris* | Circle | 37–42 | 87–95 | 200, 208, 352 |
| *Vigna radiata* | Circle | | 99.3 | 296 |
| *Vicia faba* | Circle | | 79.8 | 206 |
| *Pisum sativum* | Circle | | 79.4 | 296 |

[a] See Table 3, footnote *a*. The angiosperms not listed in Table 3 belong to the following orders: *Narcissus pseudonarcissus*, (daffodil), Amaryllidales; *Spirodela oligorrhiza* (duckweed), Arales; *Sinapis alba* (mustard), Rhoedales; *Beta vulgaris* (sugar beet), Chenopodiales; *Antirrhinum majus* (snapdragon) and *Petunia hybrida* (petunia), Personales; *Oenothera hookeri* (evening primrose), Onagrales; *Phaseolus vulgaris* (red bean), *Pisum vulgaris*, and *Vigna radiata* (mung bean), Leguminales.

which, during gentle lysis, releases 200-μm fragments, suggesting a large circular molecule. Closed circular molecules 0.1 to 4.4 μm long are also found (167, 260, 294). The chlDNAs of the chlorophyte algae *Codium fragile* and *Chlamydomonas* are much smaller, having as little as 1/27th the coding capacity of *Acetabularia* chlDNA (182).

By contrast, vascular plant chlDNAs are all approximately 45 μm long (100 Md) (Table 3). Liverwort, pea, and broad bean chlDNAs are slightly smaller.

The chlDNA of the golden alga *Olisthodiscus* (Chrysophyta) differs from other chlDNAs. This DNA contains three genomic elements which are 44, 22, and 11 μm long, giving a total genome complexity of 154 Md (2). This genome and the genomes of the chloromonadophyte algae *Gonyostomum* and *Vacuolaria* are packaged in ring-shaped beaded structures. The *Gonyostomum* nucleoid is 55 μm in circumference (103).

## GENE ARRANGEMENT IN ORGANELLE DNAs

Organelle gene arrangements have now been determined for a variety of organisms. Early studies identified organelle translation products by differential labeling in the presence of cytosol ribosome inhibitors. Cytosol ribosomes are sensitive to cycloheximide, emetine, and anisomycin, whereas organelle ribosomes are sensitive to chloramphenicol (67, 154, 238, 298). Subsequently, the genes for the organelle-synthesized polypeptides, as well as the ribosomal RNA (rRNA) and transfer RNA (tRNA) genes, were mapped on organelle DNAs by genetic, biochemical, and molecular methods.

### Mitochondrial DNA Gene Organizations

**Comparative organization of unicellular eucaryotic mitochondrial DNAs.** The available data on the arrangement of the mtDNAs in unicellular eucaryotic organisms are summarized in Fig. 1. All of these mtDNAs encode a complete set of tRNAs, a large rRNA, and a small rRNA, but no 5S rRNA. The arrangement of these genes varies greatly among species. The mtDNAs of *Tetrahymena* and *Paramecium* contain a central small rRNA gene, but *Tetrahymena* has two terminal large rRNA genes, whereas *Paramecium* has only one. All yeast mtDNAs have only one functional large rRNA gene and one functional small rRNA gene. *Kloeckera africana* mtDNA is exceptional in that it also has an additional large rRNA gene fragment located in an inverted repeat (99). The *Saccharomyces cerevisiae* RNA genes are widely separated on the genome; genes for COII, COIII, and several tRNAs map between them. In contrast, the rRNA genes of *H. mrakii* and *Torulopsis glabrata* are close together and are separated by only tRNA genes. The rRNA genes of the trypanosome mtDNAs lie close together, with the large rRNA gene transcribed first. All of these rRNA arrangements differ from the arrangement found in bacteria, in which the rRNAs are located in a single operon and are transcribed in the following order: promoter-16S-23S-5S (72, 271, 285). The tRNA genes of the fungal mtDNAs also have a variety of arrangements, although there is a tendency for some clustering around the rRNA genes.

The sizes of the mitochondrial rRNA genes also vary among species. *S. cerevisiae*, which has one of the largest fungal mtDNAs, codes for mitochondrial rRNAs of 3,100 and 1,460 nucleotides (338); these are similar in size to the *E. coli* 2,904-nucleotide 23S rRNA (71) and 1,541-nucleotide 16S rRNA (73). In contrast, *K. africana* and *T. glabrata*, which have the smallest yeast mtDNAs, code for mitochondrial rRNAs which are 2,700 and 1,450 to 1,400 nucleotides long (101, 338). *Tetrahymena* and *Paramecium* mitochondrial rRNAs are about this same size (2,700 and 1,500 nucleotides) (114). *Trypanosoma brucei* rRNAs are the smallest known: the large 12S rRNA is between 1,085 and 1,230 nucleotides long, whereas the small 9S rRNA is between 590 and 640 nucleotides long (60, 62).

The unicellular eucaryotic mtDNAs also differ in the type and arrangement of their polypeptide-encoding genes (Fig. 1). *S. cerevisiae* mitochondrial ribosomes synthesize at least nine polypeptides, only seven of which have been identified (343, 359). These include the three largest cytochrome *c* oxidase subunits (COI, COII, and COIII), the cytochrome *b* apoprotein, mitochondrial adenosine triphosphatase (mtATPase) subunits 6 and 9 (subunit 9 is the dicyclohexylcarbodiimide [DCCD]-binding protein), and a mitochondrial ribosomal protein (var1) (58, 78, 237, 320, 343, 359, 373). The genes for mtATPase subunit 9 (184, 243, 244, 359) and subunit 6 (245), COI (52, 53), COII (105), COIII (353), cytochrome *b* (282, 283), var1 (78, 372), and the mitochondrial tRNAs (30, 54, 233, 256, 257, 267, 268, 281) have been mapped (58, 60, 237) and sequenced by using petite mutants. Yeast petite (respiration-deficient) mutants have large deletions in their mtDNAs and, in some instances, retain amplified sequences of only one gene. These single-gene petite mtDNAs have been used as probes to map homologous sequences in other yeast mtDNAs (Fig. 1). Although the same genes have been found consistently, they are arranged differently in each mtDNA.

Essentially the same polypeptides are synthesized on *Neurospora* ribosomes (216, 320). By using the yeast petite probes, these genes have

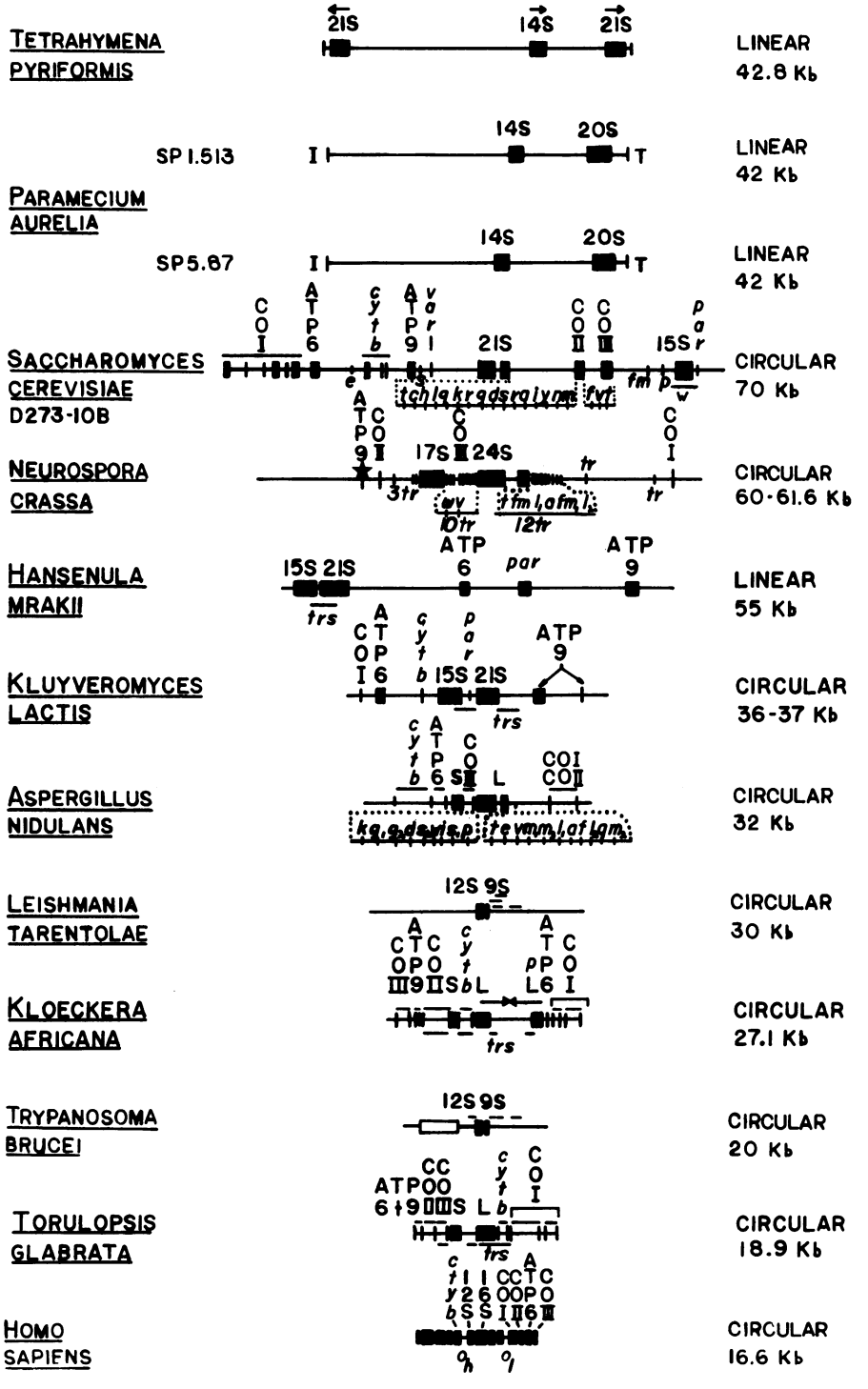


FIG. 1. Protist mtDNAs. Circular maps of protist mtDNAs were linearized, with the 5′ end of the large rRNA transcript (when known) positioned in the middle and the direction of transcription toward the right. Linear maps were positioned by using the midpoint of the DNA. *T. pyriformis* and *P. aurelia* are ciliates; *S. cerevisiae*, *H. mrakii*, *K. lactis*, *K. africana*, and *T. glabrata* are yeasts; *N. crassa* and *A. nidulans* are filamentous fungi; and *L. tarentolae* and *T. brucei* are Trypanosomatidae. *H. sapiens* is included for comparison. The arrows over the *T. pyriformis* rRNA genes indicate the orientations of these genes. Two *P. aurelia* maps are given, one for species 1

also been mapped on *Neurospora* and *Aspergillus* mtDNAs. However, in both *Aspergillus* and *Neurospora* the DCCD-binding protein is synthesized in the cytosol (189, 331, 358), and this gene has been located on *Neurospora* nuclear linkage group VII (331). Since *Neurospora* mtDNA still retains sequences homologous to the yeast subunit 9 gene, these sequences must represent a subunit 9 pseudogene (1). *Aspergillus* mitochondria also synthesize a polypeptide with partial sequence homology to the yeast DCCD-binding protein (1, 212).

Stable transcripts have also been found for *T. brucei* mtDNA. However, the translation products of these transcripts have not been identified yet (61, 62, 334).

Yeast mtDNA genes are flanked by regions high in A+T-containing DNA (31). Few, if any, consistent sequences have been found adjacent to the 5′ or 3′ ends of the genes. Most of the genes are composed of uninterrupted sequences, with the polypeptide genes initiating at a methionine and ending with a termination codon (30, 54, 105, 184, 233, 243–245, 256, 257, 268, 281, 353, 359). However, other genes contain some surprising anomalies. All of the tRNA genes lack the 3′ CCA sequence, indicating that these nucleotides are added post-transcriptionally, similar to the situation for nuclear tRNAs (256, 267). Some mitochondrial tRNA transcripts require other nonprotein mtDNA gene products to be processed from their precursors (258). The probable mRNA for the var1 protein is homologous to only 250 base pairs of mtDNA at the var1 locus (78, 372). The genes for the 21S rRNA (128), cytochrome *b* (282, 283), and COI (52, 53) are split into coding regions (exons) and noncoding intervening sequences (introns), and the introns contain sequences which could code for other proteins.

The presence of introns in fungal large rRNA genes seems to be a common but not consistent feature. Introns are found in the large rRNAs of *S. cerevisiae* $\omega^+$ strains, *Neurospora*, and *Aspergillus* (Fig. 1), but they are absent in *S. cerevisiae* $\omega^-$ and $\omega^n$ strains and *Saccharomyces carlsbergensis* (63, 128, 174, 220). The $\omega$ locus of *S. cerevisiae* maps at or near the intron and controls the direction of gene conversion in the yeast mitochondrial 21S rRNA gene (58, 128, 129). The sequence of the *S. cerevisiae* $\omega^+$ rRNA intron contains an unidentified reading frame (amino acid coding region) of unknown function (128). This reading frame is apparently not required for processing since the intron is spliced out in petite mutants, which lack mitochondrial protein synthesis (128, 346), and mitochondrial rRNA splicing mutants in *Neurospora* are encoded by the nucleus (216, 252).

The structures of the *S. cerevisiae* genes for cytochrome *b* and COI are much more complex. Different strains have different numbers of introns and exons in both genes. In the extreme cases, cytochrome *b* is separated into six exons and five introns, whereas COI is composed of six to eight exons and five to seven introns. Some of the introns contain open reading frames of considerable length that are continuous with the 3′ end of the adjacent exons on the mRNA (53, 59, 221, 282, 368). It is now clear that the splicing of these genes is at least partially under the control of proteins encoded by some of the exons and by one of the intron sequences. It has been proposed that these sequences code for *trans*-acting splicing enzymes, termed maturases. These enzymes would be self-regulating since they would destroy their own mRNA by catalyzing the next splicing step (3, 24, 221, 321). It is interesting that the yeasts *K. africana* and *T. glabrata*, which have much smaller mtDNAs,

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stock 513 (SPI.513) and one for species 5 stock 87 (SP5.87). *P. aurelia* species 4 rRNAs have also been mapped and have been found to be in the same positions as species 1 rRNAs, except that they are separated by an additional 2 kilobases (Kb) (113). I and T indicate the positions of initiation and termination of replication, respectively. Where known, genes are delineated by solid boxes. Otherwise, gene locations are indicated by vertical lines. Abbreviations for the proteins are defined in the text (cyt *b*, cytochrome *b*). ATP9★ on the *N. crassa* map indicates the silent gene homologous to yeast ATPase subunit 9. tRNA genes (*tr*) are designated as follows: *a*, alanine; *r*, arginine; *n*, asparagine; *d*, aspartic acid; *c*, cysteine; *q*, glutamine; *e*, glutamic acid; *g*, glycine; *h*, histidine; *i*, isoleucine; *l*, leucine; *k*, lysine; *m*, methionine; *fm*, formyl methionine; *f*, phenylalanine; *p*, proline; *s*, serine; *t*, threonine; *w*, tryptophan; *y*, tyrosine; and *v*, valine. The numbers after the tRNA designations indicate isoaccepting species. The genetic designations for the *S. cerevisiae* genes are as follows: COI, oxi3; ATP 6, oli2; cyt *b*, cob-box; ATP 9, oli1; *var1*, mitochondrial ribosomal protein; COII, oxi1; COIII, oxi2; and *par*, paromomycin resistance (occurring either within or beside the 15S rRNA gene). The position of the *K. lactis* ATPase subunit 9 gene (ATP9) seems to differ in the two available reports. The preliminary map positions of hemoflagellate mtDNA transcripts are indicated by lines above the map. The box in the *T. brucei* map indicates the A+T-rich region. The data are from the following references: *T. pyriformis*, references 161 through 163; *P. aurelia*, references 113 and 114; *S. cerevisiae*, references 30, 51, 52, 58, 59, 60, 237, and 382; *N. crassa*, references 1, 122, 170, 174, 179, and 181; *H. mrakii*, reference 383; *K. lactis*, references 172 and 383; *A. nidulans*, references 205, 212, 220, 242, and 358; *L. tarentolae*, reference 334; *K. africana*, references 99 and 100; *T. brucei*, references 62 and 187; and *T. glabrata*, references 100 and 101.

lack cytochrome *b* introns but retain introns in COI. At least one of the COI introns is not homologous to the introns of the *S. cerevisiae* gene (100).

The structure of the origin of replication and the mode of replication also differ among unicellular eucaryotic mtDNAs. Replication of the linear *Tetrahymena* mtDNA is initiated in the center of the genome and continues bidirectionally to the ends. The terminal inverted large rRNA repeats are believed to permit completion of the ends (161, 162). The linear *Paramecium* mtDNA initiates replication at one end, where the two strands of the double helix have become linked. DNA synthesis opens a loop which expands outward in a lariat to the end, and the resulting dimer is then cleaved (113, 158, 159, 300). The circular yeast mtDNA initiates replication from at least seven separate origins containing highly conserved sequences (36, 123). Replication of the trypanosomal mtDNA mini- and maxicircles seems to proceed in a coordinated manner from the outside of the kinetoplast DNA aggregate inward by the incorporation of precursors into nicked or gapped minicircle DNA (61, 139). The sequences of three different minicircles contain regions of high homology, which may be replication origins (20).

**Comparative organization of animal cell mitochondrial DNAs.** In contrast to unicellular eucaryotic mtDNAs, all multicellular animal mtDNAs have essentially the same gene organization. In almost every case where human (8, 9, 12, 22, 23, 289) and mouse (21, 22, 33) mtDNA genes have been identified by sequencing or molecular mapping, comparable transcripts have been mapped on *Xenopus laevis* mtDNA (Fig. 2) (287, 304, 305). The ribosomal gene order (5′-$tRNA^{Phe}$-12S rRNA-$tRNA^{Val}$-16S rRNA-$tRNA^{Leu}$-3′) of human and mouse mtDNAs has also been found in the mtDNAs of *Drosophila*, birds, rats, and cows (9, 21–23, 33, 108, 140, 156, 177, 202, 210, 289, 304, 314, 367).

It has been found that mammalian mitochondrial ribosomes synthesize between 7 and 25 polypeptides (12, 106, 219, 394). Of these, COI, COII, COIII, and two mtATPase subunits have been identified (120, 176). The DCCD-binding protein appears to be synthesized in the cytosol (119). Since the DCCD-binding protein is also

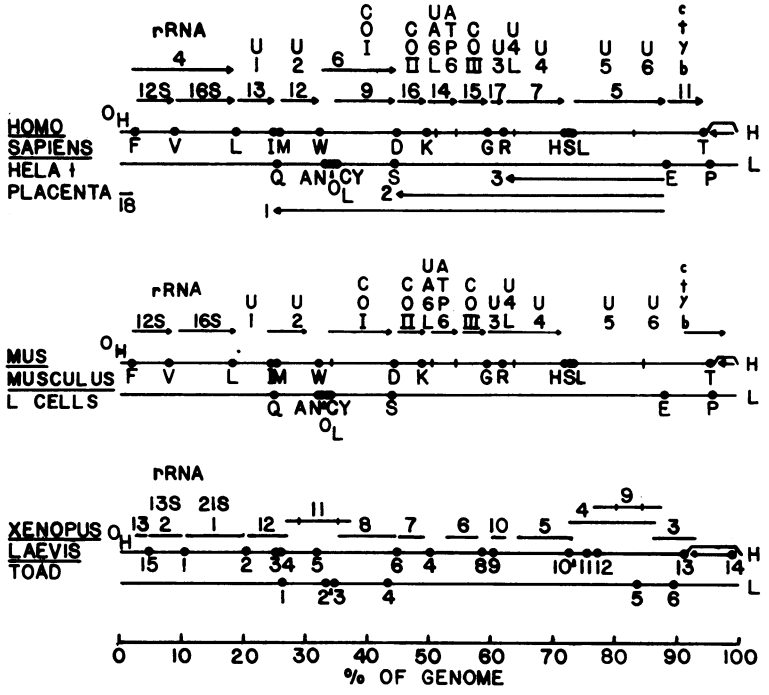


FIG. 2. Animal mtDNAs: linear maps of closed circular animal mtDNAs. Human mtDNA is 16.569 kilobases long (8), mouse mtDNA is 16.295 kilobases long (33), and *X. laevis* mtDNA is 17.2 kilobases long (304). rRNA genes are indicated by their sedimentation values. U, Unidentified reading frame; $O_H$ and $O_L$, H- and L-strand origins, respectively. The D-loops of the H-strand are diagrammed at the extreme right of each map. The tRNA abbreviations are as defined in the legend to Fig. 1, but are indicated here by uppercase letters. The arrowheads in the *X. laevis* map indicate possible additional tRNA genes. The data are from the following references: human, references 8, 9, 12, 22, 23, and 140; mouse L cells, references 21, 23, and 33; and toad, references 287, 304, and 305.

important in conferring sensitivity to oligomycin, this result is consistent with the recent assignment of an mtATPase oligomycin resistance locus to human chromosome 10 (380). Sequence analyses of human (8) and mouse (33) mtDNAs have permitted the assignment of most of these mitochondrially synthesized polypeptides to mtDNA. The mtATPase subunit 9 gene has not been found in the mtDNA.

Most of the mtDNA genes are encoded by the heavy strand (H-strand). These include the genes for most tRNAs, the rRNAs, COI, COII, COIII, mtATPase subunit 6, cytochrome *b*, and seven unidentified reading frames (URFs) (8, 10, 19, 33, 108, 140, 317). The light strand (L-strand) codes for some tRNAs and one URF, URF 6 (8, 10, 33). In human mtDNA, three short-lived RNAs have been observed which encompass URF 6, as well as one stable transcript, transcript 18. Transcript 18 maps immediately adjacent to the D-loop and is the only mature human mitochondrial messenger RNA (mRNA) with an extended 5′ noncoding end (12). Transcript 13 has also been mapped to the same position on the H-strand of *Xenopus* mtDNA (305), although no comparable transcript or open reading frame has been located in mouse mtDNA (21, 22, 33).

The most striking feature of animal mtDNAs is their highly efficient organization. Like *T. brucei*, animal mitochondrial rRNAs are smaller than *E. coli* rRNAs. Human and mouse mitochondrial 16S rRNAs are 1,559 to 1,582 nucleotides long, and their 12S rRNAs are 953 to 956 nucleotides long (140, 367). The 12S rRNAs lack the mRNA binding sequence ACCUCC found at the 3′ end of the *E. coli* 16S rRNA (140, 333, 367).

Animal mtDNAs are strikingly devoid of sequences without coding functions. Intervening sequences, the 3′ CCA of the tRNA genes, and the 5′ and 3′ noncoding regions of polypeptide genes are all absent (8, 21, 22, 33, 289, 305). It has been proposed that animal mtDNA transcription starts at single sites on the H- and L-strands and continues symmetrically around the genome. The tRNAs, which are interspersed between most of the genes, then fold within the transcripts, and the resulting secondary structures are used as recognition sites for processing of the transcripts into mature tRNAs, mRNAs, and rRNAs (8, 10, 11). The tRNA genes at the 5′ ends of polypeptide genes are consistently found within a few nucleotides of the polypeptide start codon. Hence, in the mature mRNAs there are few, if any, nucleotides 5′ with respect to the AUG start codon (7, 8, 10, 11, 33, 90, 270, 290, 291). Since mammalian mitochondrial mRNAs lack 5′ cap structures (171), the mitochondrial ribosomes must initiate translation in a unique fashion. Similarly, the tRNA genes located at the 3′ ends of amino acid coding regions lie close to the termination codons. In several instances, the tRNA gene sequence begins before the TAA termination codon is complete. Presumably, the termination codon is completed in the mRNA by the post-transcriptional addition of the approximately 55-nucleotide-long polyadenylate tail (7, 8, 10, 11, 33, 291).

Unlike many viruses (318), this efficient utilization of sequences does not include the extensive overlap of genes. The longest overlap which has been found in human and mouse mtDNAs is 43 nucleotides long and is between the mtATPase subunit 6 gene and URF A6L. URFs 4 and 4L and URFs 5 and 6 also overlap by a few nucleotides (8, 33). Thus, animal mtDNAs are characterized by a uniform genetic arrangement among species and by a highly efficient organization.

The variable A+T-rich region around the origin of replication of *Drosophila* mtDNA is the only known exception to the conservation of animal mtDNA organization. Mammalian and *Xenopus* mtDNAs replicate by a distinctive D-loop mechanism. Replication of the H-strand begins at an origin shared by the 7S DNA. The nascent DNA is base paired to the L-strand, and replication progresses by displacement synthesis away from the rRNA genes. After approximately two-thirds of the H-strand is replicated, L-strand replication initiates at the newly exposed L-strand origin and continues back in the opposite direction (28, 29, 194, 310). The H- and L-strand origins have been located at the same sites in human, mouse, and hamster mtDNAs (8, 33, 255, 274, 350). The sequences around the mouse and human L-strand origins show significant homology (255, 317), as do the sequences around the rat and mouse H-strand origins. Rodent and human H-strand origins are much less homologous (109, 332). In contrast, *Drosophila* mtDNA replication initiates within the A+T-rich region, and no D-loop structure has been observed. H-strand replication proceeds toward the rRNA genes, and the displaced strand does not initiate replication until the synthesis of the first strand is essentially complete (160). The genetic basis of these differences remains to be determined.

**Organization of vascular plant cell mitochondrial DNAs.** A molecular analysis of wheat mtDNAs has revealed genes for mitochondrial 26S, 18S, and 5S rRNAs (49). The wheat mitochondrial 18S rRNA shows substantial homology to the bacterial 16S rRNAs (46). Wheat mtDNA rRNA genes have been found to have a unique arrangement; the 18S and 5S rRNAs are tightly linked, whereas the 26S rRNA is separate (49). Wheat mtDNA also codes for tRNAs, some of which are clustered (49).

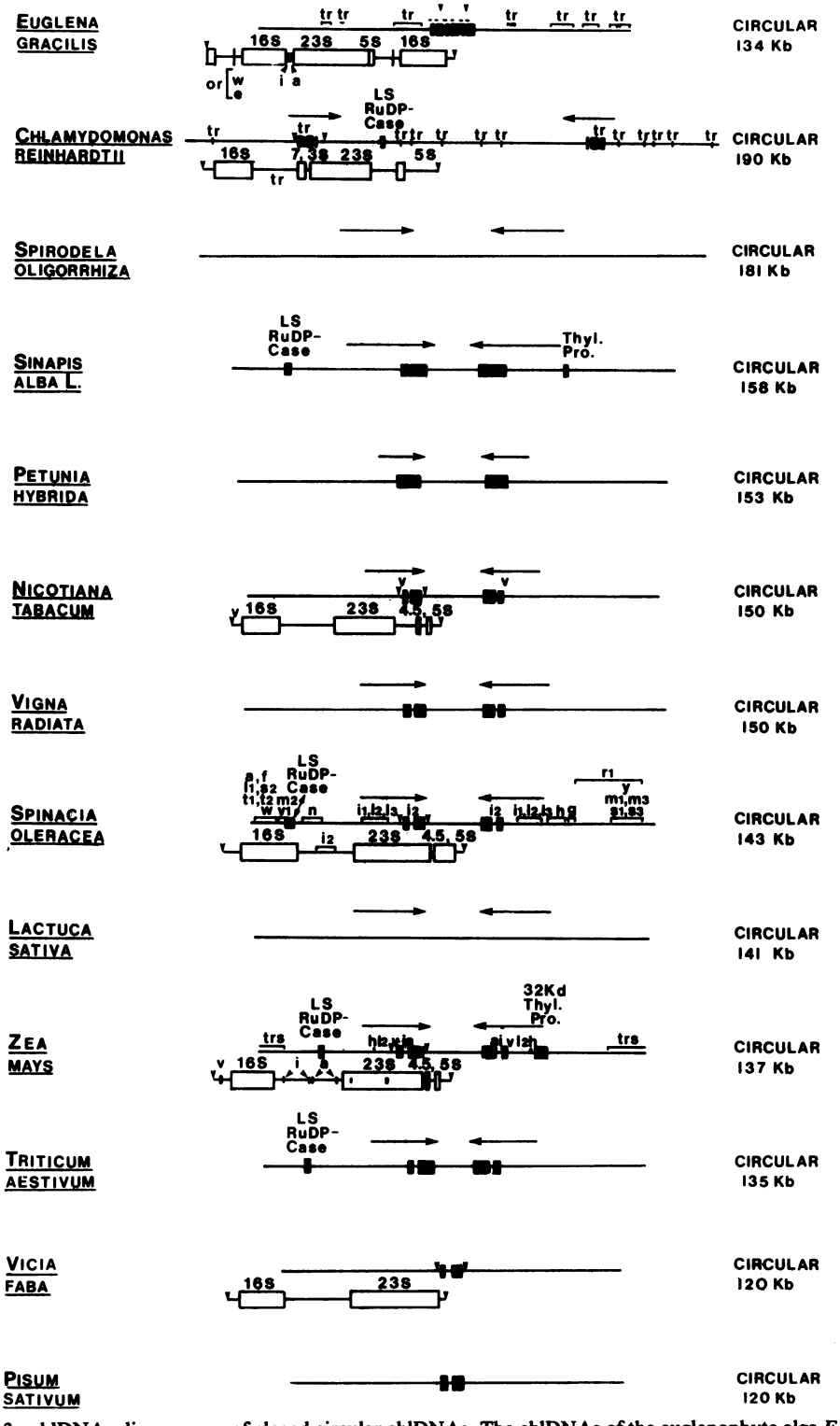


FIG. 3. chlDNAs: linear maps of closed circular chlDNAs. The chlDNAs of the euglenophyte alga *E. gracilis* and the chlorophyte alga *C. reinhardtii* are diagrammed at the top. The chlDNAs of vascular plants are drawn below *C. reinhardtii* in order of decreasing molecular weight. The *E. gracilis*, *V. faba*, and *P. sativum* maps were centered at the 5′ ends of their 23S rRNAs. For *Euglena*, the 23S rRNA of the central rRNA operon was used. All other maps were aligned at the center of the smaller single-copy DNA region, as defined by the inverted repeats (indicated by the arrows above the maps). The abbreviations are defined in the legends to Fig. 1 and 2 and

At least 20 polypeptides are synthesized in corn mitochondria (145, 146, 222). Of these, COI and COII have been tentatively identified (222), and the COII gene has been cloned and sequenced. This gene is 47% homologous in amino acid sequence to the yeast COII gene and 40% homologous to the bovine COII gene. The gene is split by a 794-base pair intervening sequence which contains two overlapping open reading frames. Neither of these reading frames is initiated by AUG, nor are these frames contiguous with the first exon. A number of RNA species which hybridize with the exons and intron have been observed, clearly indicating that transcripts of this gene are synthesized and spliced (150).

### Chloroplast DNA Gene Organization

**Organization of unicellular eucaryotic chloroplast DNAs.** Analyses of the chloroplast genes of the single-celled algae have revealed considerable variation among species. The *Chlamydomonas* chlDNA contains an inverted repeat, with each repeat containing one set of rRNA genes. These genes are organized and transcribed in the same order as *E. coli* rRNA operons (Fig. 3) and contain tRNAs in the 16S to 23S spacer. Unlike *E. coli*, the *Chlamydomonas* 23S rRNA contains an intron (4) and additional 3S and 7S RNA species. The inverted repeats define two regions of single-copy DNA roughly comparable in size. Loose clusters of tRNA genes have been located in both of these regions (Fig. 3).

The chloroplast ribosomes of the chlorophyte algae *Chlamydomonas* and *Acetabularia* synthesize the larger of the two ribulose-1,5-diphosphate carboxylase (RuDPCase) subunits, a major thylakoid protein of about 32 kilodaltons, the apoprotein of chlorophyll-protein complex I, and probably components of chloroplast adenosine triphosphatase (chlATPase) coupling factor 1 and ribosomal proteins (40, 154, 168, 251). Chloroplast elongation factor G of *Chlorella* is also synthesized on chloroplast ribosomes (93, 94, 97). In contrast, the small subunit of RuDPCase and the apoprotein of chlorophyll-protein complex II of *Acetabularia* and *Chlamydomonas* are synthesized on cytosol ribosomes (154, 168). The gene for the large subunit of RuDPCase has been cloned and mapped on *Chlamydomonas* chlDNA to the single-copy DNA region bounded by the 5S rRNA genes (251).

Unlike *Chlamydomonas*, the chlDNA of the euglenophyte alga *Euglena* codes for three rRNA operons which are tightly clustered in a tandem array. Some strains may also have an extra 16S rRNA gene (191). These rRNA operons are organized in the same manner as the operons of *E. coli*, and no introns have been found in these genes (Fig. 3). The nucleotide sequence of the 3′ end of the *Euglena* chloroplast 16S rRNA and the 16S to 23S rRNA spacer has revealed a high degree of homology to the *E. coli* rRNA operons. The 3′ end of the *Euglena gracilis* 16S rRNA ends in AACAACUCN, the terminal five nucleotides of which are similar to the *E. coli* mRNA recognition sequence ACCUCC. In addition, the *Euglena* rRNA spacers contain isoleucine and alanine tRNAs in the same order as *E. coli* rRNA operons, and the tRNAs and spacers have a high degree of homology to the tRNAs and spacers of *E. coli* (164, 293, 396). The *E. coli* and *Euglena* spacer tRNAs differ in one respect. The *Euglena* tRNA genes do not code for the 3′ CCA, even though three different nucleotides are still present in the appropriate locations (164). Thus, the *Euglena* chloroplast tRNA 3′ CCA must be added post-transcriptionally. The *Euglena* chlDNA tRNA genes outside the rRNA operons are loosely clustered.

*Euglena* differs from the chlorophyte algae in that bacterium-like chloroplast elongation factors G and T are synthesized on cytosol ribosomes rather than chloroplast ribosomes (68, 69, 149, 336). No polypeptide genes have been mapped on the *Euglena* chlDNA.

**Organization of vascular plant chloroplast DNAs.** Like animal mtDNAs, higher plant chlDNAs have a relatively uniform gene organization (Fig. 3). Virtually all vascular plant chlDNAs contain two copies of the rRNA genes located within inverted repeats, which define two unequal regions of single-copy DNA. The pea (*Pisum sativum*) and the broad bean (*Vicia*

the text. The rRNA regions of several chlDNAs are expanded 10-fold below the left-hand portions of the maps to show more detail. The expanded portions are indicated by arrows on the map. Both the $tRNA^{His}$ gene (328) and the gene for the large subunit of RuDPCase (LS RuDPCase) (235) of *Z. mays* have unidentified genes immediately adjacent to their 5′ ends, but in the opposite orientation. The dots in the *Z. mays* 23S rRNA gene represent inverted repeats not found in the *E. coli* gene (131). A heteroduplex analysis has shown that the *P. sativum* rRNA operon is highly homologous to the rRNA operon of *Z. mays* throughout its length. Presumably then, the gene order is identical in these two chlDNAs (218). The data are from the following references: *E. gracilis*, references 77, 111, 136, 155, 164, 166, 175, 191, 196, 292, 293, and 307; *C. reinhardtii*, references 4, 250, 251, 311, and 312; *S. oligorrhiza*, reference 366; *S. alba* L., references 234 and 236; *N. tabacum*, references 144, 193, 214, 347, 348, and 356; *V. radiata*, reference 296; *S. oleracea*, references 42, 43, 127, 141, and 342; *L. sativa*, references 208, 209, and 352; *Z. mays*, references 25, 26, 39, 40, 77, 130, 131, 204, 235, 327, 328, and 329; *T. aestivum*, references 65, 74, and 77; *V. faba*, reference 206; and *P. sativum*, references 218 and 296.

*faba*) are exceptions; the genomes of these plants contain only one of the repeats, resulting in correspondingly smaller chlDNAs (206, 208, 218, 296, 352). Liverwort chlDNA, which is similar in size to pea chlDNA, may also contain a single rRNA operon.

The size and organization of the vascular plant chlDNA rRNA genes are very similar to the size and organization of the *E. coli* rRNA genes. However, vascular plant chlDNAs contain a 4.5S rRNA distal to the 23S rRNA which corresponds to the 3′ end of *E. coli* 23S rRNA (157, 241, 246). The similarity in gene organization has been powerfully illustrated by the recently published sequences of the corn chlDNA rRNA operon. Corn chloroplast and *E. coli* large and small rRNAs share approximately 70% homology (131, 326). In addition, the corn chlDNA 16S to 23S rRNA spacer contains isoleucine and alanine tRNAs in the same order found in *E. coli* (204, 271, 396).

Introns have not been found in corn chloroplast rRNA genes, but the rRNA spacer isoleucine and alanine tRNA genes do contain 949- and 806-base pair introns, respectively. These introns are homologous to each other and contain open reading frames and inverted terminal repeats (204). DNA heteroduplexes between a cloned corn rRNA operon and pea chlDNA have revealed nearly perfect homology. This suggests that the rRNA operons that include tRNA introns may be common to all plants (218).

The arrangement of tRNA genes also appears to be conserved within vascular plant chlDNAs. The 3′ CCA sequence is added post-transcriptionally, and except for the rRNA spacer tRNAs, chloroplast tRNAs lack introns (204, 327, 329, 356). The tRNA genes of spinach and corn are primarily clustered in the center of the large region of single-copy DNA. Some tRNAs, including those for isoleucine and leucine, are located in the same positions within the inverted repeats (Fig. 3). In both corn and tobacco chlDNAs, a valine tRNA has been identified within 200 to 300 nucleotides of the 5′ end of the 16S rRNA (329, 356).

Chloroplasts of higher plants synthesize more than 80 polypeptides. These include all of the proteins which have been found to be translated on the chloroplast ribosomes of the chlorophyte algae. In addition, vascular plant chloroplasts synthesize three or four of the chlATPase coupling factor 1 subunits, the DCCD-binding protein, possibly a second chlATPase membrane polypeptide, cytochrome *f*, cytochrome $b_{559}$, nicotinamide adenine dinucleotide phosphate-linked malate dehydrogenase, chloroplast elongation factors G and T, and possibly the apoproteins of chlorophyll-protein complexes III and IV (91, 92, 95, 97, 137, 138, 152, 279, 280, 355, 398). As in the chlorophyte algae, the small subunit of RuDPCase and the apoprotein of chlorophyll-protein complex II are synthesized on cytosol ribosomes (27, 91).

The genes for the large subunit of RuDPCase and the 32-kilodalton thylakoid protein have been mapped to similar locations on a number of plant chlDNAs (large subunit of RuDPCase on corn, mustard, spinach, and wheat, and the 32-kilodalton thylakoid protein on corn and mustard) (Fig. 3). Mung bean chlDNA, which has two rRNA operons, and pea chlDNA, which has one, have a high degree of sequence homology but different sequence arrangements. Since chlDNAs having the inverted repeat seem to have a relatively conserved gene order, it has been proposed that the inverted repeat helps stabilize the chlDNA. If so, the rearrangements in pea chlDNA could have occurred after a loss of an rRNA operon repeat (295).

Like the rRNA genes, the transcription and translation signals of vascular plant chlDNAs are highly homologous to those of *E. coli*. The corn (151, 263) and spinach RuDPCase genes (401) and the tobacco chlDNA rRNA operon (356) are all preceded by sequences that are homologous to bacterial promoters. The 3′ end of the chlDNA 16S rRNA gene has the same secondary structure as *E. coli* 16S rRNA and ends in the bacterial ACCUCCUUA-OH sequence (328, 333, 367). The complementary AGGAGG sequence is located in the 5′ noncoding region of the genes for the large subunit of RuDPCase (263, 401). In fact, when plasmids carrying the corn and wheat RuDPCase genes were transferred into *E. coli*, the RuDPCase gene was expressed (151). In conclusion, the chlDNAs of various vascular plants seem to code for similar genes and have similar gene arrangements, and their genes retain standard eubacterial structures and controlling sequences.

## CONCLUSIONS ABOUT THE ORGANIZATION AND EVOLUTION OF ORGANELLE DNAs

### Impetus of and Constraints on Organelle Genome Evolution

From the above analysis a clear distinction between the organelle DNAs of unicellular and multicellular eucaryotes has emerged. The organelle DNAs of unicellular eucaryotes vary greatly in size, shape, and gene arrangement even among closely related species, whereas the organelle DNAs of multicellular animals and plants are comparatively uniform.

This difference might be the product of two factors: (i) a general evolutionary trend toward a reduction in organelle DNA genome size and (ii)

a reduction in the rate of organelle genome evolution when lineages switch from unicellular to multicellular. The selective advantages which might result in genome reduction could include an increase in organelle efficiency by loss of genes not required in the rich cytosolic environment and an increase in the organelle DNA replication rate, resulting in increased copy numbers and better partitioning. The reduced rate of organelle DNA evolution in multicellular organisms could reflect a shift in adaptive strategies from changes in intracellular gene organization in unicellular organisms to the elaboration of tissues and organs in multicellular organisms. According to this model, the organelle DNAs of the unicellular organisms would have remained free to evolve up to the present, whereas those of the multicellular organisms would retain primitive traits present at the onset of multicellularity in the late Precambrian.

Evidence that genome reduction is a major factor in organelle DNA evolution comes from a number of observations. First, there seems to be a continuum of chlDNA and mtDNA sizes in the unicellular eucaryotes from large to small, with chlDNAs starting in the range of bacterial genomes. Second, reductions in genome size are often accompanied by reductions in gene sizes, particularly for the rRNAs. Third, the animal mtDNAs have lost almost all of their noncoding sequences, implying that they have been subjected to extreme pressure for genome reduction. Finally, the *Neurospora* DCCD-binding protein gene appears to be at an intermediate stage in transfer from the mtDNA to the nucleus (1, 248). This demonstrates that transfer of mtDNA genes to the nucleus probably does occur.

The evidence that the organelle DNAs of multicellular organisms are stable comes from their uniform size, shape, and organization and the fact that the organelle DNAs of multicellular organisms are often larger than those of their most likely phylogenetic progenitors. For example, the chlorophyte algae are felt to be the most likely progenitors of higher plants (340, 341), yet *Chlamydomonas* mtDNA is between 1/10th and 1/200th the complexity of plant mtDNA and *Codium* chlDNA is 1/27th the complexity of plant chlDNAs. This anomaly is readily explained if it is assumed that the organelle DNAs of multicellular organisms became stabilized soon after divergence from the unicellular lineage, whereas the organelle DNAs of their unicellular relatives continued to evolve by genome reduction.

### Introns: Old or New?

The discovery of introns in organelle genes raises the question of whether organelle introns are the products of ancient or recent events (59). The presence of introns in tRNAs and rRNAs might best be explained as having arisen after the origin of the organelles, possibly by the insertion of transposable elements (80, 204) from the nucleus. This could explain the open reading frames found within the rRNA gene introns of yeast mtDNA and the tRNA gene introns of corn chlDNA, as well as the inverted terminal repeats found in the tRNA gene introns of corn chlDNAs. Site-specific insertion would also explain why corn chloroplast tRNA (204) and yeast nuclear tRNA (203) introns, as well as yeast mitochondrial 21S rRNA (128) and *Physarum* nuclear 26S rRNA (284) introns, are in the same relative locations. Insertion of these sequences into the organelle rRNA genes of some cells but not others would explain the variable appearance of these introns within phylogenetic lineages. If the sequences had existed previously in the nucleus, then nuclear splicing enzymes would have already been present to splice out the new organelle insertions. This might explain why the mitochondrial rRNA intron splicing enzymes are encoded in the nucleus and why the introns of the *Chlamydomonas* chloroplast 23S rRNA gene (4), the yeast mitochondrial 21S rRNA gene (63, 128), and the *Tetrahymena* and *Physarum* nuclear rRNA genes (284) all have common junction nucleotides and possibly related splicing recognition mechanisms (284). Adaptation of preexisting nuclear enzymes by organelles would also explain why all organelle tRNA genes do not encode the 3′ CCA sequence.

The origin of the introns in the organelle polypeptide genes is more ambiguous. Since introns have not yet been found in chlDNA polypeptide genes, they may be specific for mtDNA. In mtDNA, introns have been found in the corn mitochondrial COII gene (150), as well as in the yeast mitochondrial cytochrome *b* and COI genes (53, 59, 221, 282). All of these introns contain open reading frames. Shuffling of yeast exon and intron protein coding regions to provide new enzymatic functions is reminiscent of proposals for gene organization in primitive cells (34, 125, 153). Since no comparable splicing mechanisms have been observed in eucaryotic nuclei (227, 228), it is possible that these splicing systems were a component of the protomitochondrial genome before the formation of the organelle.

## ORGANELLE GENETIC CODES

The genetic code has generally been considered to be universal (110, 379). However, the genetic codes of mtDNAs from various organisms have been found to differ from those of all

other organisms and even from each other.

Mammalian and fungal mtDNAs code for far fewer tRNAs than the 32 tRNAs required by the wobble hypothesis (110). Human and mouse mtDNAs code for 22 tRNAs (8, 33), whereas yeast mtDNA codes for 24 tRNAs (51). This major reduction in the number of tRNAs is accomplished by having a single tRNA recognize all of the codons in each of the four codon family boxes: leucine (CUN), valine (GUN), serine (UCN), proline (CCN), threonine (ACN), alanine (GCN), arginine (CGN), and glycine (GGN) (N is defined as any of the four bases in the third position). In yeast, *Neurospora*, *Aspergillus*, mouse, and human mtDNAs, this reduction correlates with the presence of a uridine in the 5′ wobble position of the tRNA anticodon. This uridine is unmodified in *Neurospora* mitochondrial tRNAs (18, 33, 51, 180, 205). In the universal code, a 5′ anticodon uridine is modified and can only read an adenine or a guanine in the third (3′) codon position (110). This decreased constraint on the 5′ anticodon nucleotide is found in those mitochondrial tRNAs which recognize codon families with the stronger guanine-cytosine base pairs in the first two positions. This discovery has revived the two-out-of-three reading hypothesis (215).

Mammalian and fungal mitochondria have also been found to utilize both the tryptophan codon (UGG) and the opal stop codon (UGA) to code for tryptophan (18, 33, 51, 180, 205, 395). This is accomplished by the alteration of the tryptophan tRNA anticodon from 3′-ACC-5′ to 3′-ACU-5′ (8, 180, 205, 257).

Although sharing these common features, the mitochondrial genetic codes of fungal and animal species differ. Unlike human, bovine, mouse, and *Aspergillus* mitochondria, yeast mitochondria use the CUN family for threonine instead of leucine (51, 205, 233). *Aspergillus* mtDNA has three methionine tRNAs, yeast has two, and mouse and human mtDNAs may have only one. In yeast and *Aspergillus*, all methionine tRNAs have 3′-UAC-5′ anticodons, which recognize only AUG. The adjacent codon, AUA, is translated into isoleucine. The methionine tRNA of animal mtDNAs has the anticodon 3′-UAC-5′, yet it is likely that certain URFs are initiated at the isoleucine codons AGA and AUU in human mtDNA and AUA, AUU, and AUC in mouse mtDNA. The molecular mechanism by which these codons are recognized by the methionine tRNA is not known (8, 10, 18, 33, 51).

Yeast and animal mtDNAs also differ in their use of arginine codons. Animal mtDNAs lack a tRNA to translate the arginine codons AGA and AGG; this makes these codons termination codons. Animal mtDNAs do have a tRNA with a 3′-GCU-5′ anticodon for the arginine CGN four-member family box (18, 33, 395). Yeasts have two arginine tRNAs, one (3′-UCU-5′) to read codons AGA and AGG and a second (3′-GCA-5′) to read codons of the CGN family (52). However, the anticodon 3′-GCA-5′ should only recognize codons CGU and CGC, not CGA and CGG (110). Since CGA and CGG are never used in yeast mtDNA (51, 53, 282), they are probably also termination codons. Thus, by slightly different means, animal and yeast mtDNAs have converted two arginine codons to stop codons.

Why should yeast mtDNA have two arginine tRNAs and animal mtDNA have only one? The only arginine codon that is used in yeast mtDNA structural genes is AGA; the CGN codons are used exclusively in the introns of cytochrome *b* and COI (53, 282). It is possible that the charged yeast 3′-GCA-5′ arginine tRNA is limiting and thus provides translational control for the intron-encoded splicing enzymes. Since animal mtDNA genes lack introns, retention of two differently controlled arginine tRNA genes would be unnecessary.

What is the significance of these novel coding systems? The alteration of the recognition rules of the base in the third position permits a simpler translation system. This might be a more primitive code, or it might be the result of the strong selective pressure to reduce the mtDNA genome size. Since the universal code can be converted directly into the simpler mitochondrial code by cessation of modification of the wobble position uridine in certain tRNAs, it seems most likely that the mitochondrial code evolved from the universal code by loss of this constraint. This alteration then became established because it permitted deletion of tRNA genes, which resulted in genome reduction. The retention of two glycine tRNAs (3′-CCU-5′ and 3′-CCA-5′) in *Aspergillus* mitochondria (205) confirms this proposal, for it implies that at one time all four codon families employed two tRNAs.

Once the tRNA redundancy was reduced, further alterations in the mtDNA codon recognition rules could have occurred by simple mutations or deletions of the remaining tRNA genes. These mutations may have become established because they minimized the deleterious effects of accidental transfer of mRNAs between the mitochondria and the cytosol.

The mitochondria occupy up to 20% of the cytoplasm of eucaryotic cells (223), and each mitochondrion contains high molar concentrations of only a few mRNAs. If the mitochondrial membranes were damaged, these mRNAs would be released into the cytosol. Before changes in the mitochondrial translation system and genetic code, the mitochondrial mRNAs would have competed with the less prevalent cytosol mRNAs for the cytosol ribosomes. This would

have greatly decreased the efficiency of cytosol translation and, in extreme cases, would have killed the cell and its resident mitochondria. Alteration of the initiation signals of the mitochondrial mRNAs would have minimized the binding of mitochondrial mRNAs to cytosol ribosomes. This might partially explain the absence of a 5′ noncoding region in animal mitochondrial mRNAs and the high adenine and uridine contents of the flanking regions of yeast mitochondrial polypeptide genes. By also including opal stop codons for tryptophan in the mitochondrial mRNAs, the translation of mitochondrial mRNAs would have been prematurely terminated in the cytosol. Once the tryptophan tRNA anticodon mutated to recognize both UGG and UGA, the mitochondrial tryptophan codons would have been free to mutate to opal. Selective pressure would then have favored the use of opal codons. This might explain why the opal codon UGA is used for tryptophan three times more frequently than UGG in human mtDNA (18), 14 times more frequently in mouse mtDNA (33), and exclusively in yeast mtDNA (51). It has been observed that *Xenopus* oocytes cannot charge mitochondrial tryptophan tRNA when it is microinjected into the cytosol (259). This is consistent with the hypothesis proposed above. If the cytosol tryptophan tRNA synthetase could charge the mitochondrial tryptophan tRNA, released mitochondrial tRNA would both suppress essential opal codons in cytosol mRNAs and permit translation of released mitochondrial mRNAs.

Leakage of cytosol mRNAs into the mitochondrion could similarly decrease the efficiency of mitochondrial translation. If the mitochondrion lacked a tRNA for one or more of the commonly used cytosol codons, then mitochondrial translation of cytosolic mRNAs would be prematurely terminated. The amino acids serine, isoleucine, and arginine are unique in the genetic code. Each has six codons and, even with the simplified mtDNA code, must have at least two tRNAs. The loss of one of these tRNAs would create new mtDNA stop codons without forfeiting the use of an amino acid. Of the three amino acids, arginine is used least frequently in mitochondrial polypeptides (18, 51). The loss of two arginine codons would create an effective translational block for the cytosol mRNAs and would have the least deleterious effect on mitochondrial translation.

Because of these reciprocal translation barriers, any mRNAs that were exchanged between the mitochondria and cytosol would have had to use the coding system in which the mRNA was to be read. It has been proposed that most of the var1 mRNA is encoded in the nucleus (78, 372). If so, this nuclear gene would have to use the mitochondrial coding system. Similarly, human mtDNA transcript 18 uses the coding system characteristic of the cytosol and not the mitochondrion. The transcript 18 sequences of HeLa mtDNA (nucleotides 241 to 104) (12) and the placental mtDNA (nucleotides 342 to 212) (8) contain the mitochondrial arginine stop codons AGA and AGG. These genes also contain three tryptophan codons (UGG), but no opal codons. Transcript 18 is the only mitochondrial mRNA which has a 65-nucleotide 5′ noncoding sequence. Since no obligatory 5′ sequence for cytosol translation is known (15, 133) and the necessary polyadenylate tail is added in the mitochondrion, this mRNA could be read on cytosol ribosomes. Such an mtDNA gene might be involved in the assembly of cytosol organelles or in nuclear-cytoplasmic interactions (16, 17, 247, 369).

The genetic code of higher plant mtDNAs is much more closely related to the universal code than the codes of the fungal and animal mtDNAs are. The sequence of the corn mitochondrial COII gene includes virtually all of the universal codons, and these codons correlate with the expected amino acids. Five tryptophan UGG codons are used, but no UGA codons. Thus, UGA must still be a termination codon. The use of the CGG arginine codon is the only deviation from the universal code; this codon codes for tryptophan instead of arginine. This creates a 3:1 split in the arginine CGN family, similar to the isoleucine-methionine split found for the AUN codons (150). A single mutation in a tryptophan tRNA anticodon (from 3′-ACC-5′ to 3′-GCC-5′) could account for this change.

The observed variation in the mtDNA genetic code among organisms suggests that the changes were established at different times during the evolution of the mtDNAs. Since plant mtDNAs use an arginine codon and animal and fungal mtDNAs use the opal codon for tryptophan, these changes must have occurred after the divergence of the plant and animal-fungal mtDNA lineages. The animal and fungal mtDNAs, in turn, differ in their use of arginine, isoleucine, and leucine codons. Hence, these changes probably occurred after the divergence of animal and fungal mitochondria.

The chlDNAs of higher plants do not seem to have the alterations in codon usage found in mtDNAs. The complete nucleotide sequence of the RuDPCase large subunit gene of spinach and maize has 475 amino acids containing six universal UGG tryptophan codons but no opal codons (263, 401). The tryptophan tRNA from spinach has been sequenced and has the anticodon 3′-ACC-5′, which according to normal rules would not recognize the UGA opal codon (82). Almost all of the universal codons are used in the

RuDPCase gene and correlate with the standard amino acids. With normal wobble rules this would require 32 tRNAs, more than the estimated 22 to 28 tRNAs encoded in corn and *Euglena* chlDNAs (173, 262, 326). Thus, it remains possible that the uridine wobble rule used by mtDNAs may also be used by plant chlDNAs.

## ORIGIN OF ORGANELLE DNAs

Presently, there are two major hypotheses on the origin of organelle DNAs: progressive filiation and endosymbiosis. Recent biochemical and molecular biological analyses have provided substantial support for the endosymbiosis hypothesis.

### Current Formulations of the Hypotheses

The progressive filiation hypothesis contends that all eucaryotic cell organelle DNAs evolved from fragmentation and compartmentalization of the genome of an advanced photosynthetic procaryotic alga. This presumably occurred by a series of small changes, possibly motivated by increased needs for energy, which caused the expansion of the photosynthetic and oxidative membranes. Genome fragments generated by genome duplication (308), episomes (266, 303), or clusters of genes called cluster clones (38, 361) became associated with the membranes and were encapsulated due to membrane invagination, budding, and fusion. The resulting eucaryotic alga with mitochondria and chloroplasts then gave rise to oxidative eucaryotic cells by loss of its chloroplasts (5, 38, 85, 201, 266, 303, 308, 361, 362).

The endosymbiosis hypothesis proposes that the eucaryotic cell was assembled by a series of symbiotic events. As a result, the nucleus-cytosol component, mitochondrion, and chloroplast were each derived from a different phylogenetic lineage. The nucleus-cytosol component is generally thought to have been derived from a glycolytic, probably predatory, microorganism (102, 143, 192, 253, 254, 306, 339, 340, 351, 387). Ancestors of *Thermoplasma* spp. have been proposed recently (330, 370). These organisms are archaebacteria, procaryotes whose 16S rRNA sequences differ as much from those of the eubacteria as they do from the 18S rRNAs of eucaryotic nuclei. The archaebacteria (third primary biological kingdom) are considered to be primitive because of their small genomes, their variable cell wall compositions, the anaerobic methane metabolism of certain genera, their unique lipids, and the nucleotide sequence of the *Thermoplasma* methionine initiation tRNA. This tRNA is highly homologous to the proposed ancestral tRNA quasispecies (14, 135, 147, 148, 199, 269, 330, 377, 390–392). The thermoacidophilic archaebacteria also have a number of features which are characteristic of eucaryotic cells. These include absence of a cell wall, an actin-like protein, ability to invaginate membranes, a chromatin protein with homology to histone H2A, ribosomes sensitive to anisomycin but not chloramphenicol, and protein synthesis elongation factors sensitive to diphtheria toxin (198, 240, 330, 370, 390, 399).

It has been proposed that mitochondria originated from symbiosis of an organism having a physiology comparable to that of modern *Paracoccus denitrificans* (192, 385). Each type of eucaryotic chloroplast was derived from a symbiotic relationship with a different procaryotic algal lineage (254, 306, 340, 351, 386, 387). A symbiotic origin has also been proposed for the spindle and cilia (254). Although structural RNAs have been found in the basal bodies of cilia (183), there is no evidence that these structures are genetically autonomous, and they are not discussed here.

Both hypotheses envision further organelle evolution by progressive deletion of organelle DNA genes and acquisition of the deleted functions by the nucleus. Proposed mechanisms include deletion of organelle genes duplicated in the nucleus, duplication and adaptation of nuclear genes to fulfill functions within the organelle, and direct transfer of genes from the organelle DNA to the nucleus. The transfer of the fungal mtATPase subunit 9 to the nucleus is an example of this latter process. Not surprisingly, mtATPase subunit 9 is the only yeast mtDNA protein which lacks tryptophan and, therefore, would contain no UGA stop codons (184, 243, 248, 359).

### Analysis of the Theories

**Interpretation of the data.** Since both hypotheses propose a bacterial origin for mitochondria and chloroplasts (361, 362), there are only two ways by which the two theories can be distinguished. The first is the point of divergence of the nuclear and organelle DNAs. Progressive filiation predicts that the nuclear and organelle DNAs diverged at the same time from the same advanced procaryotic algal DNA. In contrast, endosymbiosis predicts that each eucaryotic DNA was derived from a different DNA lineage, with the nuclear DNA diverging from the organelle DNAs long before eucaryotic cells arose. The second distinction is the number of independent origin events proposed for the organelles. Progressive filiation predicts only one origin for all organelle types, whereas endosymbiosis predicts independent origins for mitochondria and chloroplasts and is compatible with multiple origins for each organelle type.

The following two assumptions have been

used in this analysis: (i) the ancient divergence of the archaebacterial and eubacterial lineages can be used as a reference point to determine the relative sequence of events leading to the eucaryotic nuclear DNA, chlDNA, and mtDNA lineages, and (ii) the chloroplasts and mitochondria probably originated by the same mechanism. This second assumption is consistent with the numerous features which the two types of organelles have in common: self-replicating DNA, independent transcription and translation of mRNAs, characteristic ribosomes, double membranes, etc. Such common features would not be expected if one type of organelle formed by progressive filiation and the other formed by endosymbiosis, for this would require an unlikely degree of convergent evolution.

**Divergence patterns of organelle and nuclear DNAs.** The most direct means for determining the branch points of phylogenetic trees is to compare the amino acid or nucleotide sequences of genes with identical functions encoded in the genomes of interest. Analyses of sequences from nucleus-encoded organelle proteins, such as cytochrome *c* and ferredoxin (118, 124, 323, 325), are inadequate due to the absence of comparable genes in one of the compartments. This makes comparisons between the two genomes and a third reference genome impossible. The interpretation of such results also requires a priori assumptions of how the organelle genes got to the nucleus.

Optimally, an analysis of the sequence data should permit the reconstruction of the ancestral sequences at the nodes and the definition of the exact sequence of events which led to the different genomic lineages. Unfortunately, such predictions become increasingly difficult as the sequences become more divergent, even though mathematical procedures have been developed to optimize the reliability of tree construction (117, 118, 323). Deduction of secondary structures and analysis of highly conserved sequences can often assist in refining trees. On the basis of these considerations, the best comparative data for organelle and nuclear DNAs come from the nucleotide sequences of the tRNAs, 5S rRNAs, small rRNAs, and large rRNAs and the biochemical data on protein synthesis elongation factors.

Extensive sequence data for the methionine initiation tRNA and the phenylalanine tRNA are available. The methionine initiation tRNAs of the eubacteria, chloroplasts, and mitochondria are formylated, whereas those of eucaryotic nuclei and archaebacteria are not (390, 399). The tRNA sequences of chloroplasts are most homologous to those of the blue-green algae (Cyanophyta). These sequences lie within the cluster of eubacterial sequences. The eucaryotic nuclear tRNAs cluster in a separate group, as do the available archaebacterial sequences and the mitochondrial sequences (8, 33, 79, 81, 83, 86, 134, 135, 178, 261, 324).

The sequences of the 120-nucleotide 5S rRNAs have been determined for numerous eubacteria, chloroplasts, mitochondria, eucaryotic nuclei, and archaebacteria. The 5S gene sequences of chloroplasts are closely associated with those of the blue-green algae and within the more diverse, but distinctive, eubacterial lineage. Eucaryotic nuclear 5S rRNAs form a separate group, as do the archaebacterial and wheat mitochondrial 5S rRNA sequences (121, 165, 213, 240, 323, 349).

Data on small rRNAs have been obtained by comparing the sequences of T1 oligonucleotides and by direct sequence analysis. T1 oligonucleotide sequences have separated the eubacterial, eucaryotic nuclear, and archaebacterial small rRNAs into separate kingdoms and have revealed extensive homologies between the chloroplast and blue-green alga small rRNAs (47, 48, 126, 148, 391, 397). Similarly, the wheat mitochondrial 18S rRNA has been found to be much more homologous to the bacterial 16S rRNAs than to the wheat cytosol 18S rRNA (46). The 3'-terminal sequences of chloroplast 16S rRNAs are also closely related to those of the blue-green algae (55), and the complete nucleotide sequences of the corn chloroplast and *E. coli* 16S RNAs have revealed a level of homology of approximately 70% (328). Analysis of the secondary structure of small rRNA sequences has confirmed the homology between chloroplast and *E. coli* small rRNAs. Many sequence differences between corn chloroplasts and *E. coli* occur in double-stranded regions, and these differences are associated with complementary changes in the other strand (84, 344, 402). The 18S cytosolic rRNA sequences of *S. cerevisiae* and *Xenopus laevis* form a highly homologous group distinct from the sequences of corn chloroplasts and *E. coli*. The human and mouse mitochondrial 12S rRNAs form a third group and lack many of the stems and loops found in the other two groups of small rRNAs. Certain single-stranded regions of the mitochondrial 12S rRNAs are highly conserved and show substantial homology between bacterial and mitochondrial small rRNAs (402).

The complete nucleotide sequences and their deduced secondary structures are also available for the 23S rRNAs of *E. coli* and corn chloroplasts and the 16S rRNAs of human and mouse mitochondria. To compare the sequences of these rRNAs, it is essential that the associated small rRNAs be included. The eucaryotic cytosol 5.8S rRNA is equivalent to the 5' end of the bacterial 23S rRNA (66, 107, 190, 278, 374). The

chloroplast 4.5S RNA is equivalent to the 3′ ends of the bacterial 23S rRNA and the eucaryotic 28S rRNA (157, 241, 246), although previously thought otherwise (388). The combined corn 23S and 4.5S rRNAs have approximately 71% homology with the *E. coli* 23S rRNA (131). The secondary structures of the two species are identical and reveal numerous compensatory mutations in double-stranded regions. The mouse and human mitochondrial 16S rRNAs can be arranged in secondary structures having central features similar to those of *E. coli*, but with many structures deleted. Again, the sequences of the single-stranded regions are the most highly conserved (66, 157).

Additional information has been obtained by analysis of a highly conserved region of the large rRNA gene near the 3′ end of the rRNA. This region lies between nucleotides 2,042 and 2,625 of the *E. coli* 23S rRNA sequence (66, 157) and encompasses all of the nucleotide changes identified by sequencing chloramphenicol-resistant mutants in yeast (129), mouse (76), and human (375, 376) mtDNAs (Fig. 4) (35, 37, 128, 195). Chloramphenicol blocks peptidyl transfer, and together with other data (13), the localization of chloramphenicol resistance mutations in this region suggests that this sequence is associated with the peptidyl transferase active site. A comparison of the sequences in this region from three mtDNAs, corn chlDNA, *E. coli* DNA, and *Physarum* nuclear DNA revealed two highly conserved single-stranded regions, a 13-nucleotide "left-hand box" and a 10-nucleotide "right-hand box." In these regions, the sequences for the three mtDNAs and the chlDNA are identical and are also closely related to the bacterial sequence, differing by only two nucleotides in the left-hand box. The organelle and *E. coli* sequences differ from the *Physarum* nuclear gene sequence by four to five nucleotide changes dispersed over both conserved regions. These results are consistent with the hypothesis that the *E. coli* and organelle rRNA genes diverged from each other more recently than the bacterial and eucaryotic nuclear rRNA genes.

It is intriguing that one of the nucleotide differences in the *Physarum* sequence in the left-hand box (uridine instead of adenine) is adjacent to the nucleotide change in the chloramphenicol-resistant human mitochondrial mutant HT102W. It is possible that this change imparted chloramphenicol resistance to the ancestral 80S ribosome. Since the archaebacteria are also chloramphenicol resistant (390, 399), it will be interesting to determine whether the archaebacterial sequence in this region is comparable to the sequence in *Physarum*.

The elongation factors of vascular plants and chlorophyte algae can also be used to determine the order of branching for the eubacteria, archaebacteria, eucaryotic nuclei, and chlDNAs since these polypeptides are encoded on the chlDNAs (92, 94, 95, 97, 355). The archaebacterial and eucaryotic nuclear elongation factors react to diphtheria toxin and nicotinamide adenine dinucleotide, whereas the analogous eubacterial elongation factor G does not (198). Since chloroplast elongation factor G is known to function in *E. coli* in vitro protein-synthesizing

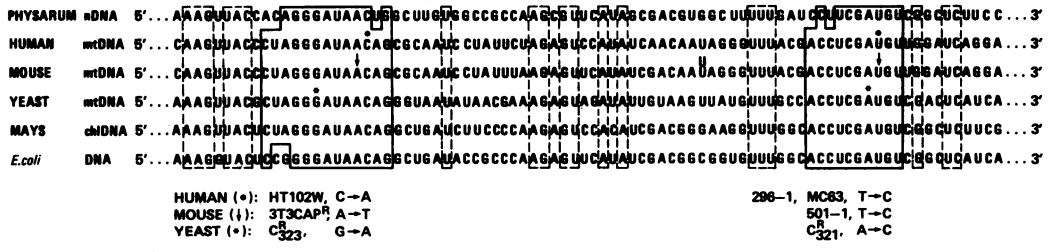


FIG. 4. Chloramphenicol resistance mutations and sequence homologies occurring at the 3′ ends of different large rRNAs. The rRNA sequences presented were deduced from the corresponding DNA sequences. The *Physarum* nuclear 28S rRNA sequence (284) corresponds to nucleotides 149 to 165 and to nucleotides 1,157 to 1,223 (nucleotides 166 to 1,156 represent the intron present in the gene but absent at the RNA level). The human 16S rRNA mtDNA sequence (8) corresponds to nucleotides 2,920 to 3,003, the mouse L A9 mtDNA sequence (367) corresponds to nucleotides 2,486 to 2,570, the yeast 21S rRNA mtDNA sequence (128) corresponds to nucleotides −77 to +7 of the $\omega^-$ strain, the *Z. mays* (mays) chlDNA sequence (131) corresponds to nucleotides 2,528 to 2,612, and the *E. coli* 23S rRNA sequence (71) corresponds to nucleotides 2,433 to 2,516. The two largest homologous sequences observed between the three mtDNA rRNAs (human, mouse, and yeast) and the corn chlDNA are enclosed in solid boxes (13 nucleotides on the left and 10 nucleotides on the right). The corresponding homologous regions of the *Physarum* and *E. coli* sequences are also included. The dotted boxes show additional complete sequence homologies among the five organisms. The changes in the mtDNA sequences associated with chloramphenicol resistance in human cell lines HT102W, 296-1, and MC63 (35, 195) are indicated by solid dots, the changes in mouse cell lines 3T3CAP$^R$ and 501-1 (37, 195) are indicated by arrows, and the changes in yeast strains $C^R_{323}$ and $C^R_{321}$ (128) are indicated by asterisks.

systems and share sensitivity to fusidic acid and common antigenic determinants with *E. coli* elongation factor G (69, 93, 336), it is likely that they are also resistant to diphtheria toxin. Therefore, the chloroplast and eubacterial elongation factors cluster in a group separate from the elongation factors of the archaebacteria and eucaryotic nuclei.

All of these data are consistent with the hypothesis that the chlDNA genes diverged from the blue-green algae well after the divergence of the eubacterial and eucaryotic nuclear lines. Therefore, chlDNAs must have originated by a symbiotic event between members of the eubacteria and eucaryotic nuclear lineages. Similarly, sequence data have indicated that the mtDNA rRNA genes are more homologous to bacterial rRNA genes than to rRNA genes of eucaryotic nuclei. They too probably also had an endosymbiotic origin.

**Monophyletic origin versus polyphyletic origin.** The progressive filiation and endosymbiotic hypotheses can also be distinguished by the number of independent organelle origins predicted. Analyses of chloroplast and procaryotic algal pigments have provided a test for this distinction. The chloroplasts of extant eucaryotic algae have at least four different types of photosynthetic pigments (44, 306, 340). The red algae (Rhodophyta), including *Porphyridium*, contain chlorophyll *a* and phycobilins. The green algae (Euglenophyta, Charophyta, and Chlorophyta) contain chlorophylls *a* and *b*. The golden algae and the dinoflagellates (Chrysophyta and Pyrrhophyta) contain chlorophylls *a* and *c*. The cryptomonads (Crytophyta) contain chlorophylls *a* and *c* and phycobilins.

Progressive filiation attributes this variation to the divergence of pigments from the chlorophyll *a* and phycobilins present in the protoeucaryotic blue-green alga. However, a new procaryotic alga, *Prochloron* (Prochlorophyta), has been discovered recently, which contains chlorophylls *a* and *b* (229–232, 354, 387). An explanation of this coincidence within the progressive filiation hypothesis requires convergent evolution in the procaryotic and eucaryotic lines. It is more readily explained as two separate symbiotic events by the endosymbiotic hypothesis (306).

Studies of chloroplast 16S rRNAs also suggest independent origins for the chloroplasts of red and green algae. The T1 oligonucleotides of *Porphyridium* chloroplast 16S rRNA are more homologous to the T1 oligonucleotides of the blue-green algae than they are to the T1 oligonucleotides of *Euglena* chloroplast 16S rRNAs (47, 48, 126), although this conclusion has been disputed (397).

The hydrogenosomes of the anaerobic protozoan *Tritrichomonas foetus* contain circular DNAs about 3 μm long. Hydrogenosomes generate adenosine triphosphate from glycolytic pyruvate by a substrate level phosphorylation pathway which uses coenzyme A and releases $H_2$, $CO_2$, and acetate (87, 273, 385, 387). This pathway is reminiscent of the pathways found in strict anaerobes and is more likely the product of symbiosis with an anaerobe than the terminal evolutionary stages of an advanced oxidative and photosynthetic procaryote. Thus, eucaryotic organelles and their associated DNAs have a polyphyletic origin, which is consistent only with the endosymbiotic hypothesis.

## TENTATIVE PHYLOGENIES OF ORGANELLE DNAs

With this background, it is now possible to propose tentative scenarios for the evolution of chlDNAs and mtDNAs. These scenarios assume an endosymbiotic origin for organelle DNAs. They also assume that the primary trend in organelle DNA evolution has been toward genome reduction, that organelle DNAs of multicellular organisms have retained primitive traits due to genome stabilization, and that the mtDNA genetic codes diverged from the universal code by a sequential series of mutations which occurred after the mitochondrial symbiont became established.

### Evolutionary History of Chloroplast DNAs

The chloroplasts of each algal group were derived from a separate endosymbiotic event. The chloroplasts of red algae are descendants of the blue-green algae. The chloroplasts of green algae (Chlorophyta and Euglenophyta) and the higher plants are descendants of the Prochlorophyta (306). The chlorophyte algae gave rise to the vascular plants (340, 341), and the vascular plant organelle DNAs became stabilized at an intermediate stage in chlorophyte organelle DNA evolution. The protochloroplasts were highly evolved eubacterial cells with 2,000- to 4,000-Md unicircular genomes and standard bacterial rRNA operons and transcription and translation signals.

Once established, the chlDNAs of the chlorophyte algae began to evolve by genome reduction, both by deletion of duplicated functions and by transfer of genes to the nucleus. The chlDNAs of *Acetabularia* (1,500 Md), *Chlamydomonas* (170 to 200 Md), vascular plants (100 Md), and *C. fragile* (56 Md) represent intermediates in this process. By the time that the 200-Md *Chlamydomonas* chlDNA had developed, the number of rRNA operons had been reduced to two, and these were arranged in an inverted repeat. This arrangement was preserved in vascular plant chlDNAs, perhaps because it helped stabilize the genome (295). It is possible that this

may be a general mechanism for stabilizing small circular genomes, since the 500-Md genome of *Mycoplasma capricolum* is known to contain two rRNA operons (319, 377). Further reductions in chlDNAs probably resulted in the loss of one of the rRNA operons. Throughout the progression from prochlorophyte bacteria to vascular plant chlDNAs, the gene organization of the rRNAs and the nucleotide sequences of the transcription and translation signals were conserved. In certain organisms, however, additional DNA elements were inserted into the rRNA and tRNA genes. The rRNA spacer tRNAs in the chlDNA of the progenitor of the vascular plants picked up transposable elements from the nucleus, which have been preserved in their modern descendants.

Although the chlDNAs decreased 15-fold from *Acetabularia* to vascular plants, the distribution of photosynthetic genes between the nucleus and the chlDNAs remained relatively constant. The genes for the small subunit of RuDPCase and the polypeptides of chlorophyll-protein complex II must have been among the first genes to be transferred from the chlDNA to the nucleus, since they are located in the nucleus of *Acetabularia* (168), as well as in all other chlorophyte algae and vascular plants. A number of other key biosynthetic and photosynthetic genes were retained by the chlDNA. These include the rRNA and tRNA genes, the large subunit of RuDPCase, the apoprotein of chlorophyll-protein complex I, and a major thylakoid protein. Thus, the transfer of a few key photosynthetic genes to the nucleus appears to have been an early event in the stabilization of the chloroplast symbiosis. By the time that the vascular plant chlDNAs evolved, the chloroplast RNA polymerase and ribosomal proteins had been transferred to the nucleus. Further studies on *Acetabularia* and *Chlamydomonas* chlDNAs should clarify when these genes were transferred.

The chloroplasts of the Euglenophyta also originated from a prochlorophyte bacterium. The rRNA gene sequences and transcription and translation signal sequences have been highly conserved in *Euglena* chlDNA. The unique organization of *Euglena* rRNA operons suggests that this algal line either diverged early from the chlorophyte line or had an independent origin (386). The latter possibility is supported by the observation that the *Euglena* cytosolic phenylalanine tRNA is more homologous to animal cytosolic phenylalanine tRNAs than to the cytosolic phenylalanine tRNAs of plant cells (89).

The chlDNA of the golden alga *Olisthodiscus* is composed of three circles and thus is totally different from the chlDNAs of the green algae. Whatley and co-workers (386, 387) have proposed that the chloroplasts of the red algae, green algae, euglenoids, and land plants evolved after ingestion of procaryotic algae, whereas the chloroplasts of the remaining eucaryotic algal groups evolved after ingestion of other eucaryotic algae. If this proposal is correct, then the photosynthetic organelles of *Olisthodiscus* might be expected to retain three DNA components, one each from the nuclear genome, the chlDNA, and the mtDNA.

### Evolutionary History of Mitochondrial DNAs

The diversity of mtDNAs and their small genome size make it more difficult to propose an internally consistent evolutionary scenario for mitochondria. Both the number of individual symbiotic events and the nature of the original symbiont are unclear. On the basis of cytochrome *c* data, three independent origins of mitochondria have been proposed: one yielding modern *Tetrahymena* mitochondria; another yielding *Crithidia* and *Euglena* mitochondria; and a third yielding the fungal, animal, and plant mitochondria (118). An analysis of 5S rRNA sequences has led to the alternative suggestion that the mitochondria of green algae and vascular plants had a separate origin from the mitochondria of fungi and animals (213). The molecular data on mtDNAs do little to clarify this question. It could be argued that closed circular, linear, and multiple circular mtDNAs had separate origins. However, at least one yeast contains a linear genome, making the significance of these differences unclear. The simplest hypothesis is that all mitochondria diverged from a single symbiotic event, which occurred early in the evolution of the eucaryotic nuclear line (253, 254). This supposition is used for the discussion below.

The nature of the protomitochondrial genome is not known. The small and varied mtDNAs of extant organisms indicate that modern mtDNAs have evolved far from their procaryotic progenitors. Cytochrome *c* sequences, T1 oligonucleotide sequences of wheat mitochondrial 18S rRNA, and sequences of conserved regions of the large rRNA gene all suggest that the protomitochondrion was an advanced oxidative bacterium. However, given the fluid gene arrangement found in plant and yeast mtDNAs, the absence of bacterial rRNA operon organization in plant, fungal, and protozoan mtDNAs, the unique intron-encoded mRNA splicing system of yeast, the low level of rRNA methylation, and the methionine initiation tRNA with similarities to the archaebacterial initiation tRNA (248), it is possible that the protomitochondrion had a very different genome organization. Regardless of the structure, the protomitochondrial DNA need not have been large. Oxidative *Mycoplas-*

*ma* strains have genomes of only 500 Md (363–365, 377).

Regardless of the nature of the genome of the symbiont, it is clear that after it became established, the mtDNA genome became highly fluid. This stage has been preserved in the complex organization of vascular plant cell mtDNAs. This early mtDNA structure may also have included the A+T-rich spacer arrangement and the exon-intron splicing system found in modern yeast mtDNAs. Such a dynamic mtDNA would have facilitated the loss of extraneous genetic material and the rapid transfer of genes to the nucleus.

During this period, the constraints on tRNA codon recognition were relaxed, permitting a substantial reduction in the number of tRNA genes and a loss of tRNA redundancy. The resulting coding system with one or a few tRNA genes per amino acid became highly susceptible to mutations and started to drift away from the universal code.

The chlorophyte alga and vascular plant mtDNA lineage diverged from the animal and fungal lineage at about this time. This may have been due to the acquisition of a prochlorophyte algal symbiont in the chlorophyte line. Soon thereafter, the mtDNA of one member of the chlorophyte algae acquired a mutation in the anticodon of one of its tryptophan tRNAs, resulting in a tRNA which recognized the CGG codon instead of UGG. Along with the accompanying bacterium-like chlDNA, this mtDNA became stabilized by the development of multicellularity in this line and the evolution of higher plants. Organelle DNAs of the remaining chlorophyte algae continued to experience mtDNA and chlDNA reduction.

Presumably, the animal and fungal mtDNA lineage retained the A+T-rich gene spacers and intron-encoded splicing enzymes and continued to experience rapid mtDNA reduction. Eventually, sufficient genes were lost so that a mutation in the anticodon of the tryptophan tRNA to recognize both the tryptophan codon and the opal stop codon did not destroy any vital genes. This change permitted subsequent mutation of the mtDNA tryptophan codons to opal, which yielded a natural barrier to the translation of mitochondrial mRNAs by the cytosol translational system. This increased cellular efficiency and was fixed by selection. As an increasing proportion of the tryptophan codons mutated to opal, the genes containing these codons could no longer function within the nucleus. This slowed the nuclear transfer of mitochondrial genes. During this period, other algal symbioses occurred, yielding some of the current algal groups. Ultimately, the mtDNAs retained only the genes for rRNAs, tRNAs, a ribosomal protein, cytochrome *b*, COI, COII, COIII, mtATPase subunits 6 and 9, and eight or nine other polypeptides.

The fungal and animal mtDNA lineages then diverged. The fungal line continued to retain the A+T-rich spacer and the intron splicing systems, the latter being confined to the cytochrome *b* and COI genes. Additional gene reductions resulted in the loss of most of the polypeptide genes, except the genes for ribosomal protein, cytochrome *b*, COI, COII, COIII, and mtATPase subunits 6 and 9. The filamentous fungal line then diverged and lost the mtATPase subunit 9 gene. The yeast mtDNA lineage continued to modify its genetic code, one change resulting in the specialization of its arginine tRNA (3′-GCU-5′) for use in intron sequences. The anticodon of this tRNA mutated to 3′-GCA-5′, thus creating two new stop codons. This provided protection for the mitochondrion from the influx of cytosolic mRNAs.

In the animal mtDNA lineage, gene rearrangements continued to occur and the mtATPase subunit 9 gene was transferred to the nucleus. Ultimately, virtually all of the protein-encoding sequences and rRNAs were separated by tRNA genes. This arrangement permitted the use of the tRNA secondary structure as a signal for processing of the mtDNA transcripts. Thus, the A+T-rich spacers were unnecessary and were deleted, resulting in a two- to fivefold reduction in genome size. The 3′-UCU-5′ arginine tRNA was also deleted, creating two new stop codons and blocking the translation of cytosolic mRNA which entered the mitochondrion. A cell harboring this mtDNA developed multicellularity, which fixed the number of polypeptides coded in the mtDNA at nearly twice the number found in yeast.

Analyses of the mtDNAs of other organisms might be useful in defining the phylogenetic relationships of these organisms. A determination of the codons used for tryptophan in *Euglena* mtDNA might permit the placement of the oxidative host cell in either the plant or animal lineage and thus resolve the question of whether the chloroplasts of the euglenoid and chlorophyte algae had the same or independent origins. Similarly, the phylogenetic affinities of the other eucaryotic algae, the Trypanosomatidae, and the Ciliata might be clarified by analyses of the genetic codes and polypeptides of their mtDNAs.

These chlDNA and mtDNA phylogenies are admittedly tentative and, in many cases, quite speculative. However, they do provide a valuable framework for organizing information and designing experiments. One fact is clear: the genomes of extant mitochondria and chloro-

plasts represent unique biological entities which are highly adapted to their intracellular environments. As such, we have much to learn from them about the evolution of eucaryotic cells and the biology of symbiosis (357).

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LITERATURE CITED

1. **Agsteribbe, E., J. Samallo, H. de Vries, L. A. M. Hensgens, and L. A. Grivell.** 1980. Sequence homologies between the mitochondrial DNAs of yeast and *Neurospora crassa*, p. 51–60. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
2. **Aldrich, J., and R. A. Cattolico.** 1979. Characterization of the chloroplast genome of the marine alga, *Olisthodicus luteus*. J. Supramol. Struct. Suppl. 3:142.
3. **Alexander, N. J., P. S. Perlman, D. K. Hanson, and H. R. Mahler.** 1980. Mosaic organization of a mitochondrial gene: evidence from double mutants in the cytochrome b region of *Saccharomyces cerevisiae*. Cell 20:199–206.
4. **Allet, B., and J.-D. Rochaix.** 1979. Structure analysis at the ends of the intervening DNA sequences in the chloroplast 23S ribosomal genes of *C. reinhardii*. Cell 18:55–60.
5. **Allsopp, A.** 1969. Phytogenetic relationships of the procaryota and the origin of the eukaryotic cell. New Phytol. 68:591–612.
6. **Altmann, R.** 1890. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Verlag von Veit and Co., Leipzig.
7. **Amalric, F., C. Merkel, R. Gelfand, and G. Attardi.** 1978. Fractionation of mitochondrial RNA from HeLa cells by high-resolution electrophoresis under strongly denaturing conditions. J. Mol. Biol. 118:1–25.
8. **Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young.** 1981. Sequence and organization of the human mitochondrial genome. Nature (London) 290:457–465.
9. **Angerer, L., N. Davidson, W. Murphy, D. Lynch, and G. Attardi.** 1976. An electron microscope study of the relative positions of the 4S and ribosomal RNA genes in HeLa cell mitochondrial DNA. Cell 9:81–90.
10. **Attardi, G.** 1981. Organization and expression of the mammalian mitochondrial genome: a lesson in economy. Trends Biochem. Res. 6:86–89.
11. **Attardi, G.** 1981. Organization and expression of the mammalian mitochondrial genome: a lesson in economy. Trends Biochem. Res. 6:100–103.
12. **Attardi, G., P. Cantatore, E. Ching, S. Crews, R. Gelfand, C. Merkel, J. Montoya, and D. Ojala.** 1980. The remarkable features of gene organization and expression of human mitochondrial DNA, p. 103–119. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
13. **Baer, R. J., and D. T. Dubin.** 1981. Methylated regions of hamster mitochondrial ribosomal RNA: structural and functional correlates. Nucleic Acids Res. 9:323–337.
14. **Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe.** 1979. Methanogens: re-evaluation of a unique biological group. Microbiol. Rev. 43:260–296.
15. **Baralle, F. E., and G. G. Brownlee.** 1978. AUG is the only recognizable signal sequence in the 5′ non-coding regions of eukaryotic mRNA. Nature (London) 274:84–87.
16. **Barath, Z., and H. Küntzel.** 1972. Induction of mitochondrial RNA polymerase in *Neurospora crassa*. Nature (London) New Biol. 240:195–197.
17. **Barath, Z., and H. Küntzel.** 1972. Cooperation of mitochondrial and nuclear genes specifying the mitochondrial genetic apparatus in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. 69:1371–1374.
18. **Barrell, B. G., S. Anderson, A. T. Bankier, M. H. L. De Bruijn, E. Chen, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young.** 1980. Different pattern of codon recognition by mammalian mitochondrial tRNAs. Proc. Natl. Acad. Sci. U.S.A. 77:3164–3166.
19. **Barrell, B. G., A. T. Bankier, and J. Drouin.** 1979. A different genetic code in human mitochondria. Nature (London) 282:189–194.
20. **Barrois, M., G. Riou, and F. Galibert.** 1981. Complete nucleotide sequence of minicircle kinetoplast DNA from *Trypanosoma equiperdum*. Proc. Natl. Acad. Sci. U.S.A. 78:3323–3327.
21. **Battey, J., and D. A. Clayton.** 1978. The transcription map of mouse mitochondrial DNA. Cell 14:143–156.
22. **Battey, J., and D. A. Clayton.** 1980. The transcription map of human mitochondrial DNA implicates transfer RNA excision as a major processing event. J. Biol. Chem. 255:11599–11606.
23. **Battey, J., P. Nagley, R. A. Van Etten, M. W. Walberg, and D. A. Clayton.** 1980. Expression of the mouse and human mitochondrial DNA genome, p. 277–286. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
24. **Bechmann, H., A. Haid, R. J. Schweyen, S. Mathews, and F. Kaudewitz.** 1981. Expression of the "split gene" COB in yeast mtDNA: translation of intervening sequences in mutant strains. J. Biol. Chem. 256:3525–3531.
25. **Bedbrook, J. R., D. M. Coen, A. R. Beaton, L. Bogorad, and A. Rich.** 1979. Location of the single gene for the large subunit of ribulosebisphosphate carboxylase on the maize chloroplast chromosome. J. Biol. Chem. 254:905–910.
26. **Bedbrook, J. R., R. Kolodner, and L. Bogorad.** 1977. *Zea mays* chloroplast ribosomal RNA genes are part of a 22,000 base pair inverted repeat. Cell 11:739–749.
27. **Bedbrook, J. R., S. M. Smith, and R. J. Ellis.** 1980. Molecular cloning and sequencing of cDNA encoding the precursor to the small subunit of chloroplast ribulose-1, 5-bisphosphate carboxylase. Nature (London) 287:692–697.
28. **Berk, A. J., and D. A. Clayton.** 1974. Mechanism of mitochondrial DNA replication in mouse L-cells: asynchronous replication of strands, segregation of circular daughter molecules, aspects of topology and turnover of an initiation sequence. J. Mol. Biol. 86:801–824.
29. **Berk, A. J., and D. A. Clayton.** 1976. Mechanism of mitochondrial DNA replication in mouse L-cells: topology of circular daughter molecules and dynamics of catenated oligomer formation. J. Mol. Biol. 100:85–102.
30. **Beriani, R. E., S. G. Bonitz, G. Coruzzi, M. Nobrega, and A. Tzagoloff.** 1980. Transfer RNA genes in the cap-oxil region of yeast mitochondrial DNA. Nucleic Acids Res. 8:5017–5030.
31. **Bernardi, G., A. Prunell, G. Fonty, H. Kopecka, and F. Strauss.** 1976. The mitochondrial genome of yeast: orga-

nization, evolution and the petite mutation, p. 185–198. *In* C. Saccone and A. M. Kroon (ed.), The genetic function of mitochondrial DNA. Elsevier/North-Holland Biomedical Press, Amsterdam.
32. **Bertazzoni, U., and A. I. Scovassi.** 1980. Mitochondrial DNA polymerase of eukaryotic cells, p. 287–290. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
33. **Bibb, M. J., R. A. Van Etten, C. T. Wright, M. W. Walberg, and D. A. Clayton.** 1981. Sequence and gene organization of mouse mitochondrial DNA. Cell **26**:167–180.
34. **Blake, C. C. F.** 1978. Do genes-in-pieces imply proteins-in-pieces? Nature (London) **273**:267.
35. **Blanc, H., C. A. Adams, and D. C. Wallace.** 1981. Different nucleotide changes in the large rRNA gene of the mitochondrial DNA confer chloramphenicol resistance on two human cell lines. Nucleic Acids Res. **9**:5785–5795.
36. **Blanc, H., and B. Dujon.** 1980. Replicator regions of the yeast mitochondrial DNA responsible for suppressiveness. Proc. Natl. Acad. Sci. U.S.A. **77**:3942–3946.
37. **Blanc, H., C. T. Wright, M. J. Bibb, D. C. Wallace, and D. A. Clayton.** 1981. Mitochondrial DNA of chloramphenicol-resistant mouse cells contains a single nucleotide change in the region encoding the 3′ end of the large ribosomal RNA. Proc. Natl. Acad. Sci. U.S.A. **78**:3789–3793.
38. **Bogorad, L.** 1975. Evolution of organelles and eukaryotic genomes. Science **188**:891–898.
39. **Bogorad, L.** 1979. The chloroplast, its genome and possibilities for genetically manipulating plants, p. 181–203. *In* J. K. Setlow and A. Hollaender (ed.), Genetic engineering: principles and methods, vol. 1. Plenum Press, New York.
40. **Bogorad, L., J. R. Bedbrook, D. M. Coen, R. Kolodner, and G. Link.** 1978. Genes for chloroplast proteins and rRNAs, p. 541–551. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
41. **Bohnert, H. J.** 1977. Size and structure of mitochondrial DNA from *Physarum polycephalum*. Exp. Cell Res. **106**:426–430.
42. **Bohnert, H. J., A. J. Driesel, E. J. Crouse, K. Gordon, R. G. Herrmann, A. Steinmetz, M. Mubumbila, M. Keller, G. Burkard, and J. H. Weil.** 1979. Presence of a transfer RNA gene in the spacer sequence between the 16S and 23S rRNA genes of spinach chloroplast DNA. FEBS Lett. **103**:52–56.
43. **Bohnert, H. J., K. Gordon, A. J. Driesel, E. J. Crouse, and R. G. Herrmann.** 1978. Mapping of genes on the restriction endonuclease site map of *Spinacia oleracea* chloroplast DNA, p. 569–572. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
44. **Bold, H. C., and M. J. Wynne.** 1978. Introduction to the algae: structure and reproduction. Prentice-Hall, Inc., Englewood Cliffs, N.J.
45. **Bolden, A., G. P. Noy, and A. Weissbach.** 1977. DNA polymerase of mitochondria is a γ-polymerase. J. Biol. Chem. **252**:3351–3356.
46. **Bonen, L., R. S. Cunningham, M. W. Gray, and W. F. Doolittle.** 1977. Wheat embryo mitochondrial 18S ribosomal RNA: evidence for its prokaryotic origin. Nucleic Acids Res. **4**:663–671.
47. **Bonen, L., and W. F. Doolittle.** 1975. On the prokaryotic nature of red algae chloroplasts. Proc. Natl. Acad. Sci. U.S.A. **72**:2310–2314.
48. **Bonen, L., and W. F. Doolittle.** 1976. Partial sequences of 16S rRNA and the phylogeny of blue-green algae and chloroplasts. Nature (London) **261**:669–673.
49. **Bonen, L., and M. W. Gray.** 1980. Organization and expression of the mitochondrial genome of plants. I. Genes for wheat mitochondrial ribosomal and transfer RNA: evidence for an unusual arrangement. Nucleic Acids Res. **8**:319–335.
50. **Bonen, L., T. Y. Huh, and M. W. Gray.** 1980. Organization and expression of the mitochondrial genome of plants. II. Can partial methylation explain the complex fragment patterns observed when plant mitochondrial DNA is cleaved with restriction endonucleases? FEBS Lett. **111**:340–346.
51. **Bonitz, S. G., R. Berlani, G. Coruzzi, M. Li, G. Macino, F. G. Nobrega, M. P. Nobrega, B. E. Thalenfeld, and A. Tzagoloff.** 1980. Codon recognition rules in yeast mitochondria. Proc. Natl. Acad. Sci. U.S.A. **77**:3167–3170.
52. **Bonitz, S. G., G. Coruzzi, B. E. Thalenfeld, A. Tzagoloff, and G. Macino.** 1980. Assembly of the mitochondrial membrane system. Physical map of the Oxi3 locus of yeast mitochondrial DNA. J. Biol. Chem. **255**:11922–11926.
53. **Bonitz, S. G., G. Coruzzi, B. E. Thalenfeld, A. Tzagoloff, and G. Macino.** 1980. Assembly of the mitochondrial membrane system. Structure and nucleotide sequence of the gene coding for subunit 1 of yeast cytochrome oxidase. J. Biol. Chem. **255**:11927–11941.
54. **Bonitz, S. G., and A. Tzagoloff.** 1980. Assembly of the mitochondrial membrane system: sequences of yeast mitochondrial tRNA genes. J. Biol. Chem. **255**:9075–9081.
55. **Borbely, G., and A. Simoncsits.** 1981. 3′-Terminal conserved loops of 16S rRNAs from the cyanobacterium *Synechococcus* AN PCC 6301 and maize chloroplast differ only in two bases. Biochem. Biophys. Res. Commun. **101**:846–852.
56. **Borst, P., and F. Fase-Fowler.** 1979. The maxi-circle of *Trypanosoma brucei* kinetoplast DNA. Biochim. Biophys. Acta **565**:1–12.
57. **Borst, P., and R. A. Flavell.** 1976. Properties of mitochondrial DNAs, p. 363–374. *In* G. D. Fasman (ed.), Handbook of biochemistry and molecular biology, 3rd ed. Nucleic acids, vol. 2. CRC Press, Cleveland, Ohio.
58. **Borst, P., and L. A. Grivell.** 1978. The mitochondrial genome of yeast. Cell **15**:705–723.
59. **Borst, P., and L. A. Grivell.** 1981. One gene's intron is another gene's exon. Nature (London) **289**:439–440.
60. **Borst, P., and L. A. Grivell.** 1981. Small is beautiful—portrait of a mitochondrial genome. Nature (London) **290**:443–444.
61. **Borst, P., and J. H. J. Hoeijmakers.** 1979. Kinetoplast DNA. Plasmid **2**:20–40.
62. **Borst, P., J. H. J. Hoeijmakers, A. C. C. Frasch, A. Snijders, J. W. G. Janssen, and F. Fase-Fowler.** 1980. The kinetoplast DNA of *Trypanosoma brucei*: structure, evolution, transcription, mutants, p. 7–19. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
63. **Bos, J. L., K. A. Osinga, G. Van der Horst, N. B. Hecht, H. F. Tabak, G.-J. B. Van Ommen, and P. Borst.** 1980. Splice point sequence and transcripts of the intervening sequence in the mitochondrial 21S ribosomal RNA gene of yeast. Cell **20**:207–214.
64. **Bovenberg, W. A., A. J. Kool, and H. J. J. Nijkamp.** 1981. Isolation, characterization and restriction endonuclease mapping of the *Petunia hybrida* chloroplast DNA. Nucleic Acids Res. **9**:503–517.
65. **Bowman, C. M., B. Koller, H. Delius, and T. A. Dyer.** 1981. A physical map of wheat chloroplast DNA showing the location of the structural genes for the ribosomal RNAs and the large subunit of ribulose 1,5-bisphosphate carboxylase. Mol. Gen. Genet. **183**:93–101.
66. **Branlant, C., A. Krol, M. A. Machatt, J. Pouyet, J.-P. Ebel, K. Edwards, and H. Kossel.** 1981. Primary and secondary structures of *Escherichia coli* MRE600 23S ribosomal RNA. Comparison and models of secondary structure for maize chloroplast 23S rRNA and for large

portions of mouse and human 16S mitochondrial rRNAs. Nucleic Acids Res. 9:4303–4324.
67. **Brega, A., and C. Baglioni.** 1971. A study of mitochondrial protein synthesis in intact HeLa cells. Eur. J. Biochem. 22:415–422.
68. **Breitenberger, C. A., M. C. Graves, and L. L. Spremulli.** 1979. Evidence for the nuclear location of the gene for chloroplast elongation factor G. Arch. Biochem. Biophys. 194:265–270.
69. **Breitenberger, C. A., and L. L. Spremulli.** 1980. Purification of *Euglena gracilis* chloroplast elongation factor G and comparison with other prokaryotic and eukaryotic translocases. J. Biol. Chem. 255:9814–9820.
70. **Brennicke, A.** 1980. Mitochondrial DNA from *Oenothera berteriana*. Plant Physiol. 65:1207–1210.
71. **Brosius, J., T. J. Dull, and H. F. Noller.** 1980. Complete nucleotide sequence of a 23S ribosomal RNA gene from *Escherichia coli*. Proc. Natl. Acad Sci. U.S.A. 77:201–204.
72. **Brosius, J., T. J. Dull, D. D. Sleeter, and H. F. Noller.** 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J. Mol. Biol. 148:107–127.
73. **Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller.** 1978. Complete nucleotide sequence of a 16S ribosomal gene from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 75:4801–4805.
74. **Brown, C. M., B. Koller, H. Delius, and T. A. Dyer.** 1981. A physical map of wheat chloroplast DNA showing the location of the structural genes for the ribosomal RNAs and the large subunit of ribulose 1,5-biphosphate carboxylase. Mol. Gen. Genet. 183:93–101.
75. **Brown, W. M., M. George, Jr., and A. C. Wilson.** 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. U.S.A. 76:1967–1971.
76. **Bunn, C. L., D. C. Wallace, and J. M. Eisenstadt.** 1974. Cytoplasmic inheritance of chloramphenicol resistance in mouse tissue culture cells. Proc. Natl. Acad. Sci. U.S.A. 71:1681–1685.
77. **Burkard, G., J. Canaday, E. Crouse, P. Guillemaut, P. Imbault, G. Keith, M. Keller, M. Mubumbila, L. Osoria, V. Sarantoglou, A. Steinmetz, and J. H. Weil.** 1980. Transfer RNAs and aminoacyl-tRNA synthetases in plant organelles, p. 313–320. *In* C. J. Leaver (ed.), Genome organization and expression in plants. NATO Advanced Study Institute Series, vol. 29. Plenum Press, New York.
78. **Butow, R. A., I. C. Lopez, H.-P. Chang, and F. Farrelly.** 1980. The specification of var1 polypeptide by the var1 determinant, p. 195–205. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
79. **Calagan, J. L., R. M. Pirtle, I. L. Pirtle, M. A. Kashdan, H. J. Vreman, and B. S. Dudock.** 1980. Homology between chloroplast and prokaryotic initiator tRNA: nucleotide sequence of spinach chloroplast methionine initiator tRNA. J. Biol. Chem. 255:9981–9984.
80. **Calos, M., and J. H. Miller.** 1980. Transposable elements. Cell 20:579–595.
81. **Canaday, J., G. Dirheimer, and R. P. Martin.** 1980. Yeast mitochondrial methionine initiator tRNA: characterization and nucleotide sequence. Nucleic Acids Res. 8:1445–1457.
82. **Canaday, J., P. Guillemaut, R. Gloeckler, and J.-H. Weil.** 1981. The nucleotide sequence of spinach chloroplast tryptophan transfer RNA. Nucleic Acids Res. 9:47–53.
83. **Canaday, J., P. Guillemaut, and J.-H. Weil.** 1980. The nucleotide sequences of the initiator transfer RNAs from bean cytoplasm and chloroplasts. Nucleic Acids Res. 8:999–1008.
84. **Carbon, P., J. P. Ebel, and C. Ehresmann.** 1981. The sequence of the ribosomal 16S RNA from *Proteus vulgaris*. Sequence comparison with *E. coli* 16S RNA and its use in secondary structure model building. Nucleic Acids Res. 9:2325–2333.
85. **Cavalier-Smith, T.** 1975. The origin of nuclei and of eukaryotic cells. Nature (London) 256:463–468.
86. **Cedergren, R. J., B. LaRue, D. Sankoff, G. Lapalme, and H. Grosjean.** 1980. Convergence and minimal mutation criteria for evaluating early events in tRNA evolution. Proc. Natl. Acad. Sci. U.S.A. 77:2791–2795.
87. **Čerkasovová, A., J. Čerkasov, J. Kulda, and J. Reischig.** 1976. Circular DNA and cardiolipin in hydrogenosomes, microbody-like organelles of trichomonads. Folia Parasitol. (Prague) 23:33–37.
88. **Challberg, S. S., and P. T. Englund.** 1980. Heterogeneity of minicircles in kinetoplast DNA of *Leishmania tarentolae*. J. Mol. Biol. 138:447–472.
89. **Chang, S. H., L. I. Hecker, C. K. Brum, J. J. Schnabel, J. E. Heckman, M. Silberklang, U. L. RajBhandary, and W. E. Barrett.** 1981. The nucleotide sequence of *Euglena* cytoplasmic phenylalanine transfer RNA. Evidence for possible classification of *Euglena* among the animal rather than the plant kingdom. Nucleic Acids Res. 9:3199–3204.
90. **Chomyn, A., M. W. Hunkapiller, and G. Attardi.** 1981. Alignment of the amino terminal amino acid sequence of human cytochrome c oxidase subunits I and II with the sequence of their putative mRNAs. Nucleic Acids Res. 9:867–877.
91. **Ciferri, O.** 1978. The chloroplast DNA mystery. Trends Biochem. Sci. 3:256–258.
92. **Ciferri, O., G. Di Pasquale, and O. Tiboni.** 1979. Chloroplast elongation factors are synthesized in the chloroplast. Eur. J. Biochem. 102:331–335.
93. **Ciferri, O., and O. Tiboni.** 1973. Elongation factors for chloroplast and mitochondrial protein synthesis in *Chlorella vulgaris*. Nature (London) New Biol. 245:209–211.
94. **Ciferri, O., and O. Tiboni.** 1976. Evidence for the synthesis in the chloroplast of elongation factor G. Plant Sci. Lett. 7:455–466.
95. **Ciferri, O., O. Tiboni, G. Di Pasquale, and D. Carbonera.** 1980. Sites of synthesis and codification of chloroplast elongation factors, p. 373–379. *In* C. J. Leaver (ed.), Genome organization and expression in plants. NATO Advanced Study Institute Series, vol. 29. Plenum Press, New York.
96. **Ciferri, O., O. Tiboni, G. Lazar, and J. Van Etten.** 1974. Elongation factors for organellar protein synthesis in *Chlorella vulgaris*, p. 107–115. *In* A. M. Kroon and C. Saccone (ed.), The biogenesis of mitochondria: transcriptional, translational and genetic aspects. Academic Press, Inc., New York.
97. **Ciferri, O., O. Tiboni, M. L. Munoz-Calvo, and G. Camerino.** 1977. Protein synthesis in plants: specificity and role of the cytoplasmic and organellar systems, p. 155–166. *In* L. Bogorad and J. H. Weil (ed.), Nucleic acids and protein synthesis in plants. NATO Advanced Study Institute Series, vol. 30. Plenum Press, New York.
98. **Clark-Walker, G. D., and C. R. McArthur.** 1978. Structural and functional relationships of mitochondrial DNAs from various yeasts, p. 255–272. *In* M. Bacila, B. L. Horecker, and A. O. M. Stoppani (ed.), Biochemistry and genetics of yeasts. Academic Press, Inc., New York.
99. **Clark-Walker, G. D., C. R. McArthur, and K. S. Sriprakash.** 1981. Partial duplication of the large ribosomal RNA sequence in an inverted repeat in circular mitochondrial DNA from *Kloeckera africana*: implications for mechanisms of the petite mutation. J. Mol. Biol. 147:399–415.
100. **Clark-Walker, G. D., and K. S. Sriprakash.** 1981. Sequence rearrangements between mitochondrial DNAs of *Torulopsis glabrata* and *Kloeckera africana* identified by hybridization with six polypeptide encoding regions from *Saccharomyces cerevisiae* mitochondrial DNA. J. Mol. Biol. 151:367–387.
101. **Clark-Walker, G. D., K. S. Sriprakash, C. R. McArthur, and A. A. Azad.** 1980. Mapping of mitochondrial DNA from *Torulopsis glabrata*: location of ribosomal and

transfer RNA genes. Curr. Genet. 1:209–217.
102. **Cohen, S. S.** 1970. Are/were mitochondria and chloroplasts microorganisms? Am. Sci. **58**:281–289.
103. **Coleman, A. W., and P. Heywood.** 1981. Structure of the chloroplast and its DNA in chloromonadophycean algae. J. Cell Sci. **49**:401–409.
104. **Collins, R. A., L. L. Stohl, M. D. Cole, and A. M. Lambowitz.** 1981. Characterization of a novel plasmid DNA found in mitochondria of *N. crassa*. Cell **24**:443–452.
105. **Coruzzi, G., and A. Tzagoloff.** 1979. Assembly of the mitochondrial membrane system. DNA sequence of subunit 2 of yeast cytochrome oxidase. J. Biol. Chem. **254**:9324–9330.
106. **Costantino, P., and G. Attardi.** 1975. Identification of discrete electrophoretic components among the products of mitochondrial protein synthesis in HeLa cells. J. Mol. Biol. **96**:291–306.
107. **Cox, R. A., and J. M. Kelly.** 1981. Mature 23S rRNA of prokaryotes appears homologous with the precursor of 25-28S rRNA of eukaryotes. FEBS Lett. **130**:1–6.
108. **Crews, S., and G. Attardi.** 1980. The sequences of the small ribosomal RNA gene and the phenylalanine tRNA are joined end to end in human mitochondrial DNA. Cell **19**:775–784.
109. **Crews, S., D. Ojala, J. Posakony, J. Nishiguchi, and G. Attardi.** 1979. Nucleotide sequence of a region of human mitochondrial DNA containing the precisely identified origin of replication. Nature (London) **277**:192–198.
110. **Crick, F. H. C.** 1966. Codon-anticodon pairing: the wobble hypothesis. J. Mol. Biol. **19**:548–555.
111. **Crouse, E. J., J. M. Schmitt, H.-J. Bohnert, K. Gordon, A. J. Driesel, and R. G. Herrmann.** 1978. Intramolecular compositional heterogeneity of *Spinacia* and *Euglena* chloroplast DNAs, p. 565–569. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
112. **Cummings, D. J., L. Belcour, and C. Grandchamp.** 1979. Mitochondrial DNA from *Podospora anserina*. II. Properties of mutant DNA and multimeric circular DNA from senescent cultures. Mol. Gen. Genet. **171**:239–250.
113. **Cummings, D. J., and J. L. Laping.** 1981. Organization and cloning of mitochondrial deoxyribonucleic acid from *Paramecium tetraaurelia* and *Paramecium primaurelia*. Mol. Cell. Biol. **1**:972–982.
114. **Cummings, D. J., R. A. Maki, P. J. Conlon, and J. Laping.** 1980. Anatomy of mitochondrial DNA from *Paramecium aurelia*. Mol. Gen. Genet. **178**:499–510.
115. **Dale, R. M. K.** 1981. Sequence homology among different size classes of plant mtDNAs. Proc. Natl. Acad. Sci. U.S.A. **78**:4453–4457.
116. **Dale, R. M. K., J. H. Duesing, and D. Keene.** 1981. Supercoiled mitochondrial DNAs from plant tissue culture cells. Nucleic Acids Res. **9**:4583–4593.
117. **Dayhoff, M. O.** 1978. Atlas of protein sequence and structure, vol. 5, suppl. 3. National Biomedical Research Foundation, Georgetown University Medical Center, Washington, D.C.
118. **Dayhoff, M. O., and R. M. Schwartz.** 1981. Evidence on the origin of eukaryotic mitochondria from protein and nucleic acid sequences. Ann. N.Y. Acad. Sci. **361**:92–103.
119. **DeJong, L., M. Holtrop, and A. M. Kroon.** 1980. The biogenesis of rat-liver mitochondrial ATPase: evidence that the N,N'-dicyclohexyl carbodiimide-binding protein is synthesized outside the mitochondria. Biochim. Biophys. Acta **606**:331–337.
120. **DeJong, L., M. Holtrop, and A. M. Kroon.** 1980. The biogenesis of rat mitochondrial ATPase: two subunits are synthesized inside the mitochondria. Biochim. Biophys. Acta **608**:32–38.
121. **Delihas, N., J. Andersen, H. M. Sprouse, and B. Dudock.** 1981. The nucleotide sequence of the chloroplast 5S ribosomal RNA from spinach. Nucleic Acids Res. **9**:2801–2805.
122. **de Vries, H., J. C. De Jong, H. Bakker, H. Meurs, and A. Kroon.** 1979. The anatomy of the tRNA-rRNA region of the *Neurospora crassa* mitochondrial DNA. Nucleic Acids Res. **6**:1791–1803.
123. **de Zamaroczy, M., R. Marotta, G. Faugeron-Fonty, R. Goursot, M. Mangin, G. Baldacci, and G. Bernardi.** 1981. The origins of replication of the yeast mitochondrial genome and the phenomenon of suppressivity. Nature (London) **292**:75–78.
124. **Dickerson, R. E.** 1980. Cytochrome c and the evolution of energy metabolism. Sci. Am. **242**:136–153.
125. **Doolittle, W. F.** 1978. Genes in pieces: were they ever together? Nature (London) **272**:581–582.
126. **Doolittle, W. F., and L. Bonen.** 1981. Molecular sequence data indicating an endosymbiotic origin for plastids. Ann. N.Y. Acad. Sci. **361**:248–256.
127. **Driesel, A. J., E. J. Crouse, K. Gordon, H. J. Bohnert, R. G. Herrmann, A. Steinmetz, M. Mubumbila, M. Keller, G. Burkard, and J. H. Weil.** 1979. Fractionation and identification of spinach chloroplast transfer RNAs and mapping of their genes on the restriction map of chloroplast DNA. Gene **6**:285–306.
128. **Dujon, B.** 1980. Sequence of the intron and flanking exons of the mitochondrial 21S rRNA gene of yeast strains having different alleles at the ω and rib-1 loci. Cell **20**:185–197.
129. **Dujon, B., A. M. Colson, and P. P. Slonimski.** 1977. The mitochondrial genetic map of *Saccharomyces cerevisiae*: compilation of mutations, genes, genetic and physical maps, p. 579–669. *In* W. Bandlow, R. J. Schweyen, K. Wolf, and F. Kaudewitz (ed.), Mitochondria 1977: genetics and biogenesis of mitochondria. W. de Gruyter, Berlin.
130. **Dyer, T. A., and J. R. Bedbrook.** 1980. The organization in higher plants of the genes coding for chloroplast ribosomal RNA, p. 305–311. *In* C. J. Leaver (ed.), Genome organization and expression in plants. NATO Advanced Study Institute Series, vol 29. Plenum Press, New York.
131. **Edwards, K., and H. Kossel.** 1981. The rRNA operon from *Zea mays* chloroplasts: nucleotide sequence of 23S rRNA and its homology with *E. coli* 23S rDNA. Nucleic Acids Res. **9**:2853–2869.
132. **Edwardson, J. R.** 1970. Cytoplasmic male sterility. Bot. Rev. **36**:341–420.
133. **Efstratiadis, A., J. W. Posakony, T. Maniatis, R. M. Lawn, C. O'Connel, R. A. Spritz, J. K. DeRiel, B. G. Forget, S. M. Weissman, J. L. Slightom, A. E. Blechl, O. Smithies, F. E. Baralle, C. C. Shoulders, and N. J. Proudfoot.** The structure and evolution of the human β-globin gene family. Cell **21**:653–668.
134. **Eigen, M., W. Gardiner, P. Schuster, and R. Winkler-Oswatitsch.** 1981. The origin of genetic information. Sci. Am. **244**:88–118.
135. **Eigen, M., and R. Winkler-Oswatitsch.** 1981. Transfer-RNA: the early adapter. Naturwissenschaften **68**:217–228.
136. **El-Gewely, M. R., M. I. Lomax, E. T. Lau, R. B. Helling, W. Farmerie, and W. E. Barnett.** 1981. A map of specific cleavage sites and tRNA genes in the chloroplast genome of *Euglena gracilis bacillaris*. Mol. Gen. Genet. **181**:296–305.
137. **Ellis, R. J., and R. Barraclough.** 1978. Synthesis and transport of chloroplast proteins inside and outside the cell, p. 185–194. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
138. **Ellis, R. J., S. M. Smith, and R. Barraclough.** 1980. Synthesis, transport and assembly of chloroplast proteins, p. 321–335. *In* C. L. Leaver (ed.), Genome organization and expression in plants. NATO Advanced Study Institute Series, vol. 29. Plenum Press, New York.
139. **Englund, P. T.** 1978. The replication of kinetoplast DNA networks in *Crithidia fasciculata*. Cell **14**:157–168.

140. **Eperon, I. C., S. Anderson, and D. P. Nierlich.** 1980. Distinctive sequence of human mitochondrial ribosomal RNA genes. Nature (London) **286**:460–467.
141. **Erion, J. L., J. Tarnowski, H. Weissbach, and N. Brot.** 1981. Cloning, mapping, and *in vitro* transcription-translation of the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase from spinach chloroplasts. Proc. Natl. Acad. Sci. U.S.A. **78**:3459–3463.
142. **Fauron, C. M.-R., and D. R. Wolstenholme.** 1976. Structural heterogeneity of mitochondrial DNA molecules within the genus *Drosophila*. Proc. Natl. Acad. Sci. U.S.A. **73**:3623–3627.
143. **Flavell, R.** 1972. Mitochondria and chloroplasts as descendants of prokaryotes. Biochem. Genet. **6**:275–291.
144. **Fluhr, R., and M. Edelman.** 1981. Physical mapping of *Nicotiana tabacum* chloroplast DNA. Mol. Gen. Genet. **181**:484–490.
145. **Forde, B. G., and C. J. Leaver.** 1980. Nuclear and cytoplasmic genes controlling synthesis of variant mitochondrial polypeptides in male-sterile maize. Proc. Natl. Acad. Sci. U.S.A. **77**:418–422.
146. **Forde, B. G., R. J. C. Oliver, and C. J. Leaver.** 1978. Variation in mitochondrial translation products associated with male-sterile cytoplasms in maize. Proc. Natl. Acad. Sci. U.S.A. **75**:3841–3845.
147. **Fox, G. E., L. J. Magrum, W. E. Balch, R. S. Wolfe, and C. R. Woese.** 1977. Classification of methanogenic bacteria by 16S ribosomal RNA characterization. Proc. Natl. Acad. Sci. U.S.A. **74**:4537–4541.
148. **Fox, G. E., E. Stackebrandt, R. B. Hespell, J. Gibson, J. Maniloff, T. A. Dyer, R. S. Wolfe, W. E. Balch, R. S. Tanner, L. J. Magrum, L. B. Zablen, R. Blakemore, R. Gupta, L. Bonen, B. J. Lewis, D. A. Stahl, K. R. Luehrsen, K. N. Chen, and C. R. Woese.** 1980. The phylogeny of prokaryotes. Science **209**:457–463.
149. **Fox, L., J. Erion, J. Tarowski, L. Spremulli, N. Brot, and H. Weissbach.** 1980. *Euglena gracilis* chloroplast EF-Ts: evidence that it is a nuclear-coded gene product. J. Biol. Chem. **255**:6018–6019.
150. **Fox, T. D., and C. J. Leaver.** 1981. The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TGA codons. Cell **26**:315–323.
151. **Gatenby, A. A., J. A. Castleton, and M. W. Saul.** 1981. Expression in *E. coli* of maize and wheat chloroplast genes for large subunit of ribulose bisphosphate carboxylase. Nature (London) **291**:117–121.
152. **Geetha, V., A. H. Mohamed, and A. Gnanam.** 1981. Identification of chloroplast NADP-linked malate dehydrogenase as a product of chloroplast protein synthesis. FEBS Lett. **126**:223–226.
153. **Gilbert, W.** 1978. Why genes in pieces? Nature (London) **271**:501.
154. **Gillham, N. W.** 1978. Organelle heredity. Raven Press, New York.
155. **Gillham, N. W., and J. E. Boynton.** 1981. Evolution of organelle genomes and protein-synthesizing systems. Ann. N.Y. Acad. Sci. **361**:20–40.
156. **Glaus, K. R., H. P. Zassenhaus, N. S. Fechheimer, and P. S. Periman.** 1980. Avian mtDNA: structure, organization and evolution, p. 131–135. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
157. **Glotz, C., C. Zwieb, R. Brimacombe, K. Edwards, and H. Kossel.** 1981. Secondary structure of the large subunit ribosomal RNA from *Escherichia coli*, *Zea mays* chloroplast, and human and mouse mitochondrial ribosomes. Nucleic Acids Res. **9**:3287–3306.
158. **Goddard, J. M., and D. J. Cummings.** 1975. Structure and replication of mitochondrial DNA from *Paramecium aurelia*. J. Mol. Biol. **97**:593–609.
159. **Goddard, J. M., and D. J. Cummings.** 1977. Mitochondrial DNA replication in *Paramecium aurelia*. Cross-linking of the initiation end. J. Mol. Biol. **109**:327–344.
160. **Goddard, J. M., and D. R. Wolstenholme.** 1980. Origin and direction of replication in mitochondrial DNA molecules from the genus *Drosophila*. Nucleic Acids Res. **8**:741–757.
161. **Goldbach, R. W., J. E. Bollen-De Boer, E. F. J. Van Bruggen, and P. Borst.** 1978. Conservation of the sequence and position of the ribosomal RNA genes in *Tetrahymena pyriformis* mitochondrial DNA. Biochim. Biophys. Acta **521**:187–197.
162. **Goldbach, R. W., J. E. Bollen-De Boer, E. F. J. Van Bruggen, and P. Borst.** 1979. Replication of the linear mitochondrial DNA of *Tetrahymena pyriformis*. Biochim. Biophys. Acta **562**:400–417.
163. **Goldbach, R. W., P. Borst, J. E. Bollen-De Boer, and E. F. J. Van Bruggen.** 1978. The organization of ribosomal RNA genes in the mitochondrial DNA of *Tetrahymena pyriformis* strain ST. Biochim. Biophys. Acta **521**:169–186.
164. **Graf, L., H. Kossel, and E. Stutz.** 1980. Sequencing of 16S-23S spacer in a ribosomal RNA operon of *Euglena gracilis* chloroplast DNA reveals two tRNA genes. Nature (London) **286**:908–910.
165. **Gray, M. W., and D. F. Spencer.** 1981. Is wheat mitochondrial 5S ribosomal RNA prokaryotic in nature? Nucleic Acids Res. **9**:3523–3529.
166. **Gray, P. W., and R. B. Hallick.** 1978. Physical mapping of the *Euglena gracilis* chloroplast DNA and ribosomal RNA gene regions. Biochemistry **17**:284–289.
167. **Green, B. R.** 1976. Covalently closed minicircular DNA associated with *Acetabularia* chloroplasts. Biochim. Biophys. Acta **447**:156–166.
168. **Green, B. R.** 1980. Protein synthesis by isolated *Acetabularia* chloroplasts. *In vitro* synthesis of the apoprotein of the P-700–chlorophyll a–protein complex (CPI). Biochim. Biophys. Acta **609**:107–120.
169. **Green, B. R., B. L. Muir, and U. Padmanabhan.** 1977. The *Acetabularia* chloroplast genome: small circles and large kinetic complexity, p. 107–122. *In* C. F. L. Woodcock (ed.), Progress in *Acetabularia* research. Academic Press, Inc., New York.
170. **Green, M. R., M. F. Grimm, R. R. Goewert, R. A. Collins, M. D. Cole, A. M. Lambowitz, J. E. Heckman, S. Yin, and U. L. RajBhandary.** 1981. Transcripts and processing patterns for the ribosomal RNA and transfer RNA region of *Neurospora crassa* mitochondrial DNA. J. Biol. Chem. **256**:2027–2034.
171. **Grohmann, K., F. Amalric, S. Crews, and G. Attardi.** 1978. Failure to detect "cap" structures in mitochondrial DNA-coded poly(A)-containing RNA from HeLa cells. Nucleic Acids Res. **5**:637–651.
172. **Groot, G. S. P., and N. van Harten-Loosbroek.** 1980. Genetic organization of mitochondrial DNA of *Kluyveromyces lactis*, p. 61–64. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
173. **Gruol, D. J., and R. Haselkorn.** 1976. Counting the genes for stable RNA in the nucleus and chloroplasts of *Euglena*. Biochim. Biophys. Acta **447**:82–95.
174. **Hahn, U., C. M. Lazarus, H. Lunsdorf, and H. Kuntzel.** 1979. Split gene for mitochondrial 24S ribosomal RNA of *Neurospora crassa*. Cell **17**:191–200.
175. **Hallick, R. B., P. W. Gray, B. K. Chelm, K. E. Rushlow, and E. M. Orozco, Jr.** 1978. *Euglena gracilis* chloroplast DNA structure, gene mapping and RNA transcription, p. 619–622. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
176. **Hare, J. F., E. Ching, and G. Attardi.** 1980. Isolation, subunit composition, and site of synthesis of human cytochrome c oxidase. Biochemistry **19**:2023–2030.
177. **Hauswirth, W. W., P. J. Laipis, M. E. Gilman, T. W. O'Brien, G. S. Michaels, and M. A. Rayfield.** 1980. Genetic mapping of bovine mitochondrial DNA from a single animal. Gene **8**:193–209.

178. **Heckman, J. E., L. I. Hecker, S. D. Schwartzbach, W. E. Barnett, B. Baumstark, and U. L. RajBhandary.** 1978. Structure and function of initiator methionine tRNA from the mitochondria of *Neurospora crassa*. Cell **13**:83–95.
179. **Heckman, J. E., and U. L. RajBhandary.** 1979. Organization of tRNA and rRNA genes in *N. crassa* mitochondria: intervening sequence in the large rRNA gene and strand distribution of the RNA genes. Cell **17**:583–595.
180. **Heckman, J. E., J. Sarnoff, B. Alzner-DeWeerd, S. Yin, and U. L. RajBhandary.** 1980. Novel features in the genetic code and codon reading patterns in *Neurospora crassa* mitochondria based on sequences of six mitochondrial tRNAs. Proc. Natl. Acad. Sci. U.S.A. **77**:3159–3163.
181. **Heckman, J. E., S. Yin, B. Alzner-DeWeerd, and U. L. RajBhandary.** 1979. Mapping and cloning of *Neurospora crassa* mitochondrial transfer RNA genes. J. Biol. Chem. **254**:12694–12700.
182. **Hedberg, M. F., Y.-S. Huang, and M. H. Hommersand.** 1981. Size of the chloroplast genome in *Codium fragile*. Science **213**:445–447.
183. **Heidemann, S. R., G. Sander, and M. W. Kirschner.** 1977. Evidence for a functional role of RNA in centrioles. Cell **10**:337–350.
184. **Hensgens, L. A. M., L. A. Grivell, P. Borst, and J. L. Bos.** 1979. Nucleotide sequence of the mitochondrial structural gene for subunit 9 of yeast ATPase complex. Proc. Natl. Acad. Sci. U.S.A. **76**:1663–1667.
185. **Herrmann, R. G., H. K. Palta, and K. V. Kowallik.** 1980. Chloroplast DNA from three archegoniates. Planta **148**:319–327.
186. **Hoeijmakers, J. H. J., and P. Borst.** 1978. RNA from the insect trypanosome *Crithidia luciliae* contains transcripts of the maxi-circle and not of the mini-circle component of kinetoplast DNA. Biochim. Biophys. Acta **521**:407–411.
187. **Hoeijmakers, J. H. J., A. Snijders, J. W. G. Janssen, and P. Borst.** 1981. Transcription of kinetoplast DNA in *Trypanosoma brucei* bloodstream and culture forms. Plasmid **5**:329–350.
189. **Jackl, G., and W. Sebald.** 1975. Identification of two products of mitochondrial protein synthesis associated with mitochondrial adenosine triphosphatase from *Neurospora crassa*. Eur. J. Biochem. **54**:97–106.
190. **Jacq, B.** 1981. Sequence homologies between eukaryotic 5.8S rRNA and the 5′ end of prokaryotic 23S rRNA: evidence for a common evolutionary origin. Nucleic Acids Res. **9**:2913–2932.
191. **Jenni, B., and E. Stutz.** 1978. Physical mapping of the ribosomal DNA region of *Euglena gracilis* chloroplast DNA. Eur. J. Biochem. **88**:127–134.
192. **John, P., and F. R. Whatley.** 1975. *Paracoccus denitrificans* and the evolutionary origin of the mitochondrion. Nature (London) **254**:495–498.
193. **Jurgenson, J. E., and D. P. Bourque.** 1980. Mapping of rRNA genes in an inverted repeat in *Nicotiana tabacum* chloroplast DNA. Nucleic Acids Res. **8**:3505–3516.
194. **Kasamatsu, H., and J. Vinograd.** 1974. Replication of circular DNA in eukaryotic cells. Annu. Rev. Biochem. **43**:695–719.
195. **Kearsey, S. E., and I. W. Craig.** 1981. Altered ribosomal RNA genes in mitochondria from mammalian cells with chloramphenicol resistance. Nature (London) **290**:607–608.
196. **Keller, M., G. Burkard, H. J. Bohnert, M. Mubumbila, K. Gordon, A. Steinmetz, D. Heiser, E. J. Crouse, and J. H. Weil.** 1980. Transfer RNA genes associated with the 16S and 23S rRNA genes of *Euglena* chloroplast DNA. Biochem. Biophys. Res. Commun. **95**:47–54.
197. **Kemble, R. J., and J. R. Bedbrook.** 1980. Low molecular weight circular and linear DNA in mitochondria from normal and male-sterile *Zea mays* cytoplasm. Nature (London) **284**:565–566.
198. **Kessel, M., and F. Klink.** 1980. Archaebacterial elongation factor is ADP-ribosylated by diphtheria toxin. Nature (London) **287**:250–251.
199. **Kilpatrick, M. W., and R. T. Walker.** 1981. The nucleotide sequence of the $tRNA_M^{Met}$ from the archaebacterium *Thermoplasma acidophilum*. Nucleic Acids Res. **9**:4387–4390.
200. **Kirk, J. T. O.** 1976. Chloroplast nucleic acids, p. 356–362. *In* G. D. Fasman (ed.), Handbook of biochemistry and molecular biology, 3rd ed. Nucleic acids, vol. 2. CRC Press, Cleveland, Ohio.
201. **Klein, R. M.** 1970. Relationships between blue-green and red algae. Ann. N.Y. Acad. Sci. **175**:623–633.
202. **Klukas, C. K., and I. B. Dawid.** 1976. Characterization and mapping of mitochondrial ribosomal RNA and mitochondrial DNA in *Drosophila melanogaster*. Cell **9**:615–625.
203. **Knapp, G., R. C. Ogden, C. L. Peebles, and J. Abelson.** 1979. Splicing of yeast tRNA precursors: structure of the reaction intermediates. Cell **18**:37–45.
204. **Koch, W., K. Edwards, and H. Kossel.** 1981. Sequencing of the 16S-23S spacer in a ribosomal RNA operon of *Zea mays* chloroplast DNA reveals two split tRNA genes. Cell **25**:203–213.
205. **Köchel, H. G., C. M. Lazarus, N. Basak, and H. Kuntzel.** 1981. Mitochondrial tRNA gene cluster in *Aspergillus nidulans*: organization and nucleotide sequence. Cell **23**:625–633.
206. **Koller, B., and H. Delius.** 1980. *Vicia faba* chloroplast DNA has only one set of ribosomal RNA genes as shown by partial denaturation mapping and R-loop analysis. Mol. Gen. Genet. **178**:261–269.
207. **Kolodner, R., and K. K. Tewari.** 1972. Physiochemical characterization of mitochondrial DNA from pea leaves. Proc. Natl. Acad. Sci. U.S.A. **69**:1830–1834.
208. **Kolodner, R., and K. K. Tewari.** 1975. The molecular size and conformation of the chloroplast DNA from higher plants. Biochim. Biophys. Acta **402**:372–390.
209. **Kolodner, R., and K. K. Tewari.** 1979. Inverted repeats in chloroplast DNA from higher plants. Proc. Natl. Acad. Sci. U.S.A. **76**:41–45.
210. **Kroon, A. M., G. Pepe, H. Bakker, M. Holtrop, J. E. Bollen, E. F. J. Van Bruggen, P. Cantatore, P. Terpstra, and C. Saccone.** 1977. The restriction fragment map of rat-liver mitochondrial DNA: a reconsideration. Biochim. Biophys. Acta **478**:128–145.
211. **Küntzel, H.** 1969. Proteins of mitochondrial and cytoplasmic ribosomes from *Neurospora crassa*. Nature (London) **222**:142–146.
212. **Küntzel, H., N. Basak, G. Imam, H. Kochel, C. M. Lazarus, H. Lunsdorf, E. Bartnik, A. Bidermann, and P. P. Stepien.** 1980. The mitochondrial genome of *Aspergillus nidulans*, p. 79–86. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
213. **Küntzel, H., M. Heidrich, and B. Piechulla.** 1981. Phylogenetic tree derived from bacterial, cytosol and organelle 5S rRNA sequences. Nucleic Acids Res. **9**:1451–1461.
214. **Kusuda, J., K. Shinozaki, F. Takaiwa, and M. Sugiura.** 1980. Characterization of the cloned ribosomal DNA of tobacco chloroplasts. Mol. Gen. Genet. **178**:1–7.
215. **Lagerkvist, U.** 1981. Unorthodox codon reading and the evolution of the genetic code. Cell **23**:305–306.
216. **Lambowitz, A. M.** 1980. Mitochondrial ribosome assembly and RNA splicing in *Neurospora crassa*, p. 291–300. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
217. **Lambowitz, A. M., N.-H. Chua, and D. J. L. Luck.** 1976. Mitochondrial ribosome assembly in *Neurospora*. Preparation of mitochondrial ribosomal precursor particles, site of synthesis of mitochondrial ribosomal proteins and studies on the *poky* mutant. J. Mol. Biol. **107**:223–253.
218. **Lamppa, G. K., and A. J. Bendich.** 1981. Fine scale interspersion of conserved sequences in the pea and corn

chloroplast genomes. Mol. Gen. Genet. **182**:310–320.
219. **Lansman, R. A., and D. A. Clayton.** 1975. Mitochondrial protein synthesis in mouse L-cells: effect of selective nicking on mitochondrial DNA. J. Mol. Biol. **99**:777–793.
220. **Lazarus, C. M., H. Lunsdorf, U. Hahn, P. P. Stepien, and H. Kuntzel.** 1980. Physical map of *Aspergillus nidulans* mitochondrial genes coding for ribosomal RNA: an intervening sequence in the large rRNA cistron. Mol. Gen. Genet. **177**:389–397.
221. **Lazowska, J., C. Jacq, and P. P. Slonimski.** 1980. Sequence of introns and flanking exons in wild-type and box 3 mutants of cytochrome b reveals an interlaced splicing protein coded by an intron. Cell **22**:333–348.
222. **Leaver, C. J., and B. G. Forde.** 1980. Mitochondrial genome expression in higher plants, p. 407–425. *In* C. J. Leaver (ed.), Genome organization and expression in plants. NATO Advanced Study Institute Series, vol. 29. Plenum Press, New York.
223. **Lehninger, A. L.** 1970. Biochemistry. Worth Publishers, Inc., New York.
224. **Levings, C. S., III, B. D. Kim, D. R. Pring, M. F. Conde, R. J. Mans, J. R. Laughnan, and S. J. Gabay-Laughnan.** 1980. Cytoplasmic reversion of cms-S in maize: association with a transpositional event. Science **209**:1021–1023.
225. **Levings, C. S., III, and D. R. Pring.** 1979. Molecular bases of cytoplasmic male sterility in maize, p. 171–193. *In* J. G. Scandalios (ed.), Physiological genetics. Academic Press, Inc., New York.
226. **Levings, C. S., III, and D. R. Pring.** 1979. Mitochondrial DNA of higher plants and genetic engineering, p. 205–222. *In* J. K. Setlow and A. Hollander (ed.), Genetic engineering: principles and methods, vol. 1. Plenum Press, New York.
227. **Lewin, B.** 1980. Alternative for splicing: recognizing the ends of introns. Cell **22**:324–326.
228. **Lewin, B.** 1980. Alternative for splicing: an intron-coded protein. Cell **22**:645–646.
229. **Lewin, R. A.** 1976. Prochlorophyta as a proposed new division of algae. Nature (London) **261**:697–698.
230. **Lewin, R. A.** 1977. *Prochloron*, type genus of the Prochlorophyta. Phycologia **16**:217.
231. **Lewin, R. A.** 1981. *Prochloron* and the theory of symbiogenesis. Ann. N.Y. Acad. Sci. **361**:325–328.
232. **Lewin, R. A., and N. W. Withers.** 1975. Extraordinary pigment composition of a prokaryotic alga. Nature (London) **256**:735–737.
233. **Li, M., and A. Tzagoloff.** 1979. Assembly of the mitochondrial membrane system: sequences of yeast mitochondrial valine and an unusual threonine tRNA gene. Cell **18**:47–53.
234. **Link, G.** 1981. Cloning and mapping of the chloroplast DNA sequences for two messenger RNAs from mustard (*Sinapis alba* L.). Nucleic Acids Res. **9**:3681–3694.
235. **Link, G., and L. Bogorad.** 1980. Sizes, locations, and directions of transcription of two genes on a cloned maize chloroplast DNA sequence. Proc. Natl. Acad. Sci. U.S.A. **77**:1832–1836.
236. **Link, G., S. E. Chambers, J. A. Thompson, and H. Falk.** 1981. Size and physical organization of chloroplast DNA from mustard (*Sinapis alba* L.). Mol. Gen. Genet. **181**:454–457.
237. **Linnane, A. W., and P. Nagley.** 1978. Mitochondrial genetics in perspective: the derivation of a genetic and physical map of the yeast mitochondrial genome. Plasmid **1**:324–345.
238. **Lizardi, P. M., and D. J. L. Luck.** 1972. The intracellular site of synthesis of mitochondrial ribosomal proteins in *Neurospora crassa*. J. Cell Biol. **54**:56–74.
239. **Lonsdale, D. M., R. D. Thompson, and T. P. Hodge.** 1981. The integrated forms of the S1 and S2 DNA elements of maize male sterile mitochondrial DNA are flanked by a large repeated sequence. Nucleic Acids Res. **9**:3657–3669.
240. **Luehrsen, K. R., G. E. Fox, M. W. Kilpatrick, R. T. Walker, H. Domdey, G. Krupp, and H. J. Gross.** 1981. The nucleotide sequence of the 5S rRNA from the archaebacterium *Thermoplasma acidophilum*. Nucleic Acids Res. **9**:965–970.
241. **Machatt, M. A., J.-P. Ebel, and C. Branlant.** 1981. The 3′-terminal region of bacterial 23S ribosomal RNA: structure and homology with the 3′-terminal region of eukaryotic 28S rRNA and with chloroplast 4.5S rRNA. Nucleic Acids Res. **9**:1533–1549.
242. **Macino, G., C. Scazzocchio, R. B. Waring, M. M. Berks, and R. W. Davies.** 1980. Conservation and rearrangement of mitochondrial structural gene sequences. Nature (London) **288**:404–406.
243. **Macino, G., and A. Tzagoloff.** 1979. Assembly of the mitochondrial membrane system: the DNA sequence of a mitochondrial ATPase gene in *Saccharomyces cerevisiae*. J. Biol. Chem. **254**:4617–4623.
244. **Macino, G., and A. Tzagoloff.** 1979. Assembly of the mitochondrial membrane system: partial sequence of a mitochondrial ATPase gene in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **76**:131–135.
245. **Macino, G., and A. Tzagoloff.** 1980. Assembly of the mitochondrial membrane system: sequence analysis of a yeast mitochondrial ATPase gene containing the oli-2 and oli-4 loci. Cell **20**:507–517.
246. **MacKay, R. M.** 1981. The origin of plant chloroplast 4.5S ribosomal RNA. FEBS Lett. **123**:17–18.
247. **Macklin, W. B., D. J. Meyer, D. O. Woodward, and S. K. Erickson.** 1977. Chloramphenicol-sensitive labelling of protein in microsomes of *Neurospora crassa*. Nature (London) **269**:447–449.
248. **Mahler, H. R.** 1981. Mitochondrial evolution: organization and regulation of mitochondrial genes. Ann. N.Y. Acad. Sci. **361**:53–75.
249. **Mahler, H. R., and P. S. Perlman.** 1979. Mitochondrial biogenesis: evolution and regulation, p. 11–33. *In* D. J. Cummings, P. Borst, I. B. Dawid, S. M. Weissman, and C. F. Fox (ed.), Extrachromosomal DNA. ICN-UCLA Symposia on Molecular and Cellular Biology, vol. 15. Academic Press, Inc., New York.
250. **Malnoë, P., and J.-D. Rochaix.** 1978. Localization of 4S RNA genes on the chloroplast genome of *Chlamydomonas reinhardii*. Mol. Gen. Genet. **166**:269–275.
251. **Malnoë, P., J.-D. Rochaix, N. H. Chua, and P.-F. Spahr.** 1979. Characterization of the gene and messenger RNA of the large subunit of ribulose 1,5-diphosphate carboxylase in *Chlamydomonas reinhardii*. J. Mol. Biol. **133**:417–434.
252. **Mannella, C. A., R. A. Collins, M. R. Green, and A. M. Lambowitz.** 1979. Defective splicing of mitochondrial rRNA in cytochrome-deficient nuclear mutants of *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **76**:2635–2639.
253. **Margulis, L.** 1968. Evolutionary criteria in thallophytes: a radical alternative. Science **161**:1020–1022.
254. **Margulis, L.** 1970. Origin of eukaryotic cells. Yale University Press, New Haven, Conn.
255. **Martens, P. A., and D. A. Clayton.** 1979. Mechanism of mitochondrial DNA replication in mouse L-cells: localization and sequence of the light-strand origin of replication. J. Mol. Biol. **135**:327–351.
256. **Martin, N. C., D. Miller, J. Hartley, P. Moynihan, and J. E. Donelson.** 1980. The $tRNA^{Ser}_{AGY}$ and $tRNA^{Arg}_{CGY}$ from a gene cluster in yeast mitochondrial DNA. Cell **19**:339–343.
257. **Martin, N. C., H. D. Pham, K. Underbrink-Lyon, D. L. Miller, and J. E. Donelson.** 1980. Yeast mitochondrial $tRNA^{Trp}$ can recognize the nonsense codon UGA. Nature (London) **285**:579–581.
258. **Martin, N. C., and K. Underbrink-Lyon.** 1981. A mitochondrial locus is necessary for the synthesis of mitochondrial tRNA in the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. U.S.A. **78**:4743–4747.
259. **Martin, R. P., A.-P. Sibler, G. Dirheimer, S.de Henau, and H. Grosjean.** 1981. Yeast mitochondrial $tRNA^{Trp}$ injected with *E. coli* activating enzyme into *Xenopus*

oocytes suppresses UGA termination. Nature (London) 293:235–237.
260. **Mazza, A., A. Casale, P. Saccone-Corsi, and S. Bonotto.** 1980. A minicircular component of *Acetabularia acetabulum* chloroplast DNA replicating by the rolling circle. Biochem. Biophys. Res. Commun. 93:668–674.
261. **McCoy, J. M., and D. S. Jones.** 1980. The nucleotide sequence of *Scenedesmus obliquus* chloroplast $tRNA_f^{MET}$. Nucleic Acids Res. 8:5089–5093.
262. **McCrea, J. M., and C. L. Hershberger.** 1976. Chloroplast DNA codes for transfer RNA. Nucleic Acids Res. 3:2005–2018.
263. **McIntosh, L., C. Poulsen, and L. Bogorad.** 1980. Chloroplast gene sequence for the large subunit of ribulose biphosphatecarboxylase of maize. Nature (London) 288:556–560.
264. **Mereschkowsky, C.** 1910. Theorie der zwei Plasmaarten als Grundlage der Symbiogenesis, einer neuen Lehre von der Entstehung der Organismen. Biol. Zentralbl. 30:353–367.
265. **Mery-Drugeon, E., E. J. Crouse, J. M. Schmitt, H.-J. Bohnert, and G. Bernardi.** 1981. The mitochondrial genomes of *Ustilago cynodontis* and *Acanthamoeba castellanii*. Eur. J. Biochem. 114:577–583.
266. **Meyer, R. R.** 1973. On the evolutionary origin of mitochondrial DNA. J. Theor. Biol. 38:647–663.
267. **Miller, D. L., N. C. Martin, H. D. Pham, and J. E. Donelson.** 1979. Sequence analysis of two yeast mitochondrial DNA fragments containing the genes for $tRNA_{UCR}^{Ser}$ and $tRNA_{UUY}^{Phe}$. J. Biol. Chem. 254:11735–11740.
268. **Miller, D. L., C. Sigurdson, N. C. Martin, and J. E. Donelson.** 1980. Nucleotide sequence of the mitochondrial genes coding for $tRNA_{GGR}^{Gly}$ and $tRNA_{GUR}^{Val}$. Nucleic Acids Res. 8:1435–1443.
269. **Mitchell, R. M., L. A. Loeblich, L. C. Klotz, and A. R. Loeblich III.** 1978. DNA organization of *Methanobacterium thermoautotrophicum*. Science 204:1082–1084.
270. **Montoya, J., D. Ojala, and G. Attardi.** 1981. Distinctive features of the 5'-terminal sequences of the human mitochondrial mRNAs. Nature (London) 290:465–470.
271. **Morgan, E. A., T. Ikemura, and M. Nomura.** 1977. Identification of spacer tRNA genes in individual ribosomal RNA transcription units of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 74:2710–2714.
272. **Morowitz, H. J., and D. C. Wallace.** 1973. Genome size and life cycle of the mycoplasma. Ann. N.Y. Acad. Sci. 225:62–73.
273. **Muller, M.** 1975. Biochemistry of protozoan microbodies: peroxisomes, α-glycerophosphate oxidase bodies, hydrogenosomes. Annu. Rev. Microbiol. 29:467–483.
274. **Nass, M. M. K.** 1980. Analysis of the two heavy and light strand origins and the direction of replication of mitochondrial DNA within a detailed physical map of this genome in transformed hamster cells. J. Mol. Biol. 140:231–256.
275. **Nass, M. M. K., and S. Nass.** 1963. Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions. J. Cell Biol. 19:593–611.
276. **Nass, M. M. K., S. Nass, and B. A. Afzelius.** 1965. The general occurrence of mitochondrial DNA. Exp. Cell Res. 37:516–539.
277. **Nass, S., and M. M. K. Nass.** 1963. Intramitochondrial fibers with DNA characteristics. II. Enzymatic and other hydrolytic treatments. J. Cell Biol. 19:613–629.
278. **Nazar, R. N.** 1980. A 5.8S rRNA-like sequence in prokaryotic 23S rRNA. FEBS Lett. 119:212–214.
279. **Nechushtai, R., N. Nelson, A. K. Mattoo, and M. Edelman.** 1981. Site of synthesis of subunits to photosystem I reaction center and the proton-ATPase in *Spirodela*. FEBS Lett. 125:115–119.
280. **Nelson, N., H. Nelson, and G. Schatz.** 1980. Biosynthesis and assembly of the proton-translocating adenosine triphosphatase complex from chloroplasts. Proc. Natl. Acad. Sci. U.S.A. 77:1361–1364.
281. **Newman, D., H. D. Pham, K. Underbrink-Lyon, and N. C. Martin.** 1980. Characterization of tRNA genes in tRNA region II of yeast mitochondrial DNA. Nucleic Acids Res. 8:5007–5016.
282. **Nobrega, F. G., and A. Tzagloff.** 1980. Assembly of the mitochondrial membrane system. DNA sequence and organization of the cytochrome b gene in *Saccharomyces cerevisiae* D273-10B. J. Biol. Chem. 255:9828–9837.
283. **Nobrega, F. G., and A. Tzagoloff.** 1980. Assembly of the mitochondrial membrane system. Complete restriction map of the cytochrome b region of mitochondrial DNA in *Saccharomyces cerevisiae* D273-10B. J. Biol. Chem. 255:9821–9827.
284. **Nomiyama, H., Y. Sakaki, and T. Takagi.** 1981. Nucleotide sequence of a ribosomal RNA gene intron from slime mold *Physarum polycephalum*. Proc. Natl. Acad. Sci. U.S.A. 78:1376–1380.
285. **Nomura, M., E. A. Morgan, and S. R. Jaskunas.** 1977. Genetics of bacterial ribosomes. Annu. Rev. Genet. 11:297–347.
286. **O'Connor, R. M., C. R. McArthur, and G. D. Clark-Walker.** 1975. Closed circular DNA from mitochondrial-enriched fractions of four petite-negative yeasts. Eur. J. Biochem. 53:137–144.
287. **Ohi, S., J. L. Ramirez, W. B. Upholt, and I. B. Dawid.** 1978. Mapping of mitochondrial 4S RNA genes in *Xenopus laevis* by electron microscopy. J. Mol. Biol. 121:299–310.
288. **Ojala, D., and G. Attardi.** 1977. A detailed physical map of HeLa cell mitochondrial DNA and its alignment with the positions of known genetic markers. Plasmid 1:78–105.
289. **Ojala, D., C. Merkel, R. Gelfand, and G. Attardi.** 1980. The tRNA genes punctuate the reading of genetic information in human mitochondrial DNA. Cell 22:393–403.
290. **Ojala, D., J. Montoya, and G. Attardi.** 1980. The putative mRNA for subunit II of human cytochrome c oxidase starts directly at the translation initiator codon. Nature (London) 287:79–82.
291. **Ojala, D., J. Montoya, and G. Attardi.** 1981. tRNA punctuation model of RNA processing in human mitochondria. Nature (London) 290:470–474.
292. **Orozco, E. M., Jr., P. W. Gray, and R. B. Hallick.** 1980. *Euglena gracilis* chloroplast ribosomal RNA transcription units. I. The location of transfer RNA, 5S, 16S, and 23S ribosomal RNA genes. J. Biol. Chem. 255:10991–10996.
293. **Orozco, E. M., Jr., K. E. Rushlow, J. R. Dodd, and R. B. Hallick.** 1980. *Euglena gracilis* chloroplast ribosomal RNA transcription units. II. Nucleotide sequence homology between the 16S-23S ribosomal RNA spacer and the 16S ribosomal RNA leader regions. J. Biol. Chem. 255:10997–11003.
294. **Padmanabhan, U., and B. R. Green.** 1978. The kinetic complexity of *Acetabularia* chloroplast DNA. Biochim. Biophys. Acta 521:67–73.
295. **Palmer, J. D.** 1981. Chloroplast DNA evolution: molecular and phylogenetic studies. Ph.D. thesis, Stanford University, Stanford, Calif.
296. **Palmer, J. D., and W. F. Thompson.** 1981. Rearrangements in the chloroplast genomes of mung bean and pea. Proc. Natl. Acad. Sci. U.S.A. 78:5533–5537.
297. **Parker, R. C., and R. M. Watson.** 1977. Restriction endonuclease cleavage maps of rat and mouse mitochondrial DNAs. Nucleic Acids Res. 4:1291–1299.
298. **Perlman, S., and S. Penman.** 1970. Mitochondrial protein synthesis: resistance to emetine and response to RNA synthesis inhibitors. Biochem. Biophys. Res. Commun. 40:941–948.
299. **Powling, A.** 1981. Species of small DNA molecules found in mitochondria from sugarbeet with normal and male sterile cytoplasms. Mol. Gen. Genet. 183:82–84.
300. **Pritchard, A. E., L. M. Herron, and D. J. Cummings.** 1980. Cloning and characterization of *Paramecium* mitochondrial DNA replication initiation regions. Gene

11:43–52.
301. **Quetier, F., and F. Vedel.** 1977. Heterogeneous population of mitochondrial DNA molecules in higher plants. Nature (London) **268**:365–368.
302. **Quetier, F., and F. Vedel.** 1980. Physio-chemical and restriction endonuclease analysis of mitochondrial DNA from higher plants, p. 401–406. *In* C. J. Leaver (ed.), Genome organization and expression in plants. NATO Advanced Study Institute Series, vol. 29. Plenum Press, New York.
303. **Raff, R. A., and H. R. Mahler.** 1972. The non-symbiotic origin of mitochondria. Science **177**:575–582.
304. **Ramirez, J. L., and I. B. Dawid.** 1978. Mapping of mitochondrial DNA in *Xenopus laevis* and *X. borealis*: the positions of ribosomal genes and D-loops. J. Mol. Biol. **119**:133–146.
305. **Rastl, E., and I. B. Dawid.** 1979. Expression of the mitochondrial genome in *Xenopus laevis*: a map of transcripts. Cell **18**:501–510.
306. **Raven, P. H.** 1970. A multiple origin for plastids and mitochondria. Science **169**:641–646.
307. **Rawson, J. R. Y., S. R. Kushner, D. Vapnek, N. K. Alton, and C. L. Boerma.** 1978. Chloroplast ribosomal RNA genes in *Euglena gracilis* exist as three clustered tandem repeats. Gene **3**:191–209.
308. **Reijnders, L.** 1975. The origin of mitochondria. J. Mol. Evol. **5**:167–176.
309. **Ris, H., and W. Plaut.** 1962. Ultrastructure of DNA-containing areas in the chloroplast of *Chlamydomonas*. J. Cell Biol. **13**:383–391.
310. **Robberson, D. L., H. Kasamatsu, and J. Vinograd.** 1972. Replication of mitochondrial DNA. Circular replicative intermediates in mouse L cells. Proc. Natl. Acad. Sci. U.S.A. **69**:737–741.
311. **Rochaix, J.-D., and P. Malnoe.** 1978. Anatomy of the chloroplast ribosomal DNA of *Chlamydomonas reinhardii*. Cell **15**:661–670.
312. **Rochaix, J.-D., and P. Malnoë.** 1978. Gene localization on the chloroplast DNA of *Chlamydomonas reinhardii*, p. 581–586. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
313. **Roodyn, D. B., and D. Wilkie.** 1968. The biogenesis of mitochondria. Methuen and Co., London.
314. **Saccone, C., P. Cantatore, G. Gadaleta, R. Gallerani, C. Lanave, G. Pepe, and A. M. Kroon.** 1981. The nucleotide sequence of the large ribosomal RNA gene and the adjacent tRNA genes from rat mitochondria. Nucleic Acids Res. **9**:4139–4148.
315. **Sager, R.** 1972. Cytoplasmic genes and organelles. Academic Press, Inc., New York.
316. **Sala, F., M. G. Galli, M. Levi, D. Burroni, B. Parisi, G. Pedrali-Noy, and S. Spadari.** 1981. Functional roles of the plant α-like and γ-like DNA polymerases. FEBS Lett. **124**:112–118.
317. **Sanger, F., A. R. Coulson, B. G. Barrell, A. J. H. Smith, and B. A. Roe.** 1980. Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J. Mol. Biol. **143**:161–178.
318. **Sanger, F., A. R. Coulson, T. Friedmann, G. M. Air, B. G. Barrell, N. L. Brown, J. C. Fiddes, C. A. Hutchison III, P. M. Slocombe, and M. Smith.** 1978. The nucleotide sequence of bacteriophage ΦX174. J. Mol. Biol. **125**:225–246.
319. **Sawada, M., S. Osawa, H. Kobayashi, H. Hori, and A. Muto.** 1981. The number of ribosomal RNA genes in *Mycoplasma capricolum*. Mol. Gen. Genet. **182**:502–504.
320. **Schatz, G., and T. L. Mason.** 1974. The biogenesis of mitochondrial proteins. Annu. Rev. Biochem. **43**:51–87.
321. **Schmelzer, C., A. Haid, G. Grosch, R. J. Schweyen, and F. Kaudewitz.** 1981. Pathways of transcript splicing in yeast mitochondria. Mutations in intervening sequences of the split gene COB reveal a requirement for intervening sequence-encoded products. J. Biol. Chem. **256**:7610–7619.
322. **Schmitt, H.** 1972. Analysis and site of synthesis of ribosomal proteins from yeast mitochondria. FEBS Lett. **26**:215–219.
323. **Schwartz, R. M., and M. O. Dayhoff.** 1978. Origin of prokaryotes, eukaryotes, mitochondria, and chloroplasts. Science **199**:395–403.
324. **Schwartz, R. M., and M. O. Dayhoff.** 1978. Transfer RNA, p. 313–325. *In* M. O. Dayhoff (ed.), Atlas of protein sequence and structure, vol. 5, suppl. 3. National Biomedical Research Foundation, Georgetown University Medical School, Washington, D. C.
325. **Schwartz, R. M., and M. O. Dayhoff.** 1981. Chloroplast origins: inferences from protein and nucleic acid sequences. Ann. N.Y. Acad. Sci. **361**:260–269.
326. **Schwartzbach, S. D., L. I. Hecker, and W. E. Barnett.** 1976. Transcriptional origin of *Euglena* chloroplast tRNAs. Proc. Natl. Acad. Sci. U.S.A. **73**:1984–1988.
327. **Schwarz, Z., S. O. Jolly, A. A. Steinmetz, and L. Bogorad.** 1981. Overlapping divergent genes in the maize chloroplast chromosome and *in vitro* transcription of the gene for $tRNA^{His}$. Proc. Natl. Acad. Sci. U.S.A. **78**:3423–3427.
328. **Schwarz, Z., and H. Kossel.** 1980. The primary structure of 16S rDNA from *Zea mays* chloroplast is homologous to *E. coli* 16S rRNA. Nature (London) **283**:739–742.
329. **Schwarz, Z., H. Kossel, E. Schwarz, and L. Bogorad.** 1981. A gene coding for $tRNA^{Val}$ is located near 5′ terminus of 16S rRNA gene in *Zea mays* chloroplast genome. Proc. Natl. Acad. Sci. U.S.A. **78**:4748–4752.
330. **Searcy, D. G., D. B. Stein, and K. B. Searcy.** 1981. A mycoplasma-like archaebacterium possibly related to the nucleus and cytoplasm of eukaryotic cells. Ann. N.Y. Acad. Sci. **361**:312–323.
331. **Sebald, W., M. Sebald-Althaus, and E. Wachter.** 1977. Altered amino acid sequence of the DCCD-binding protein of the nuclear oligomycin-resistant mutant AP-2 from *Neurospora crassa*, p. 433–440. *In* W. Bandlow, R. J. Schweyer, K. Wolf, and F. Kaudewitz (ed.), Mitochondria 1977—genetics and biogenesis of mitochondria. W. de Gruyter, Berlin.
332. **Sekiya, T., M. Kobayashi, T. Seki, and K. Koike.** 1980. Nucleotide sequence of a cloned fragment of rat mitochondrial DNA containing the replication origin. Gene **11**:53–62.
333. **Shine, J., and L. Dalgarno.** 1975. Determinant of cistron specificity in bacterial ribosomes. Nature (London) **254**:34–38.
334. **Simpson, L., A. M. Simpson, G. Kidane, L. Livingston, and T. W. Spithill.** 1980. The kinetoplast DNA of the hemoflagellate protozoa. Am. J. Trop. Med. Hyg. **29**:1053–1063.
335. **Sparks, R. B., Jr., and R. M. K. Dale.** 1980. Characterization of $^3$H-labeled supercoiled mitochondrial DNA from tobacco suspension culture cells. Mol. Gen. Genet. **180**:351–355.
336. **Spremulli, L. L., C. A. Breitenberger, M. C. Graves, C. M. Beck, and D. A. Steege.** 1981. Comparison of the *Euglena* chloroplast protein synthesizing system with those of prokaryotes and eukaryotes. Ann. N.Y. Acad. Sci. **361**:461–470.
337. **Spruill, W. M., Jr., C. S. Levings III, and R. R. Sederoff.** 1980. Recombinant DNA analysis indicates that the multiple chromosomes of maize mitochondria contain different sequences. Dev. Genet. **1**:363–378.
338. **Sriprakash, K. S., and G. D. Clark-Walker.** 1980. The size of yeast mitochondrial ribosomal RNAs. Biochem. Biophys. Res. Commun. **93**:186–193.
339. **Stanier, R. Y.** 1970. Some aspects of the biology of cells and their possible evolutionary significance. Symp. Soc. Gen. Microbiol. **20**:1–38.
340. **Stanier, R. Y.** 1974. The origins of photosynthesis in eukaryotes. Symp. Soc. Gen. Microbiol. **24**:219–240.
341. **Stanier, R. Y., M. Doudoroff, and E. A. Adelberg.** 1970. The microbial world, 3rd ed. Prentice-Hall, Inc., Engle-

wood Cliffs, N.J.
342. **Steinmetz, A., M. Mubumbila, M. Keller, G. Burkard, J. H. Weil, A. J. Driesel, E. J. Crouse, K. Gordon, H. J. Bohnert, and R. G. Herrmann.** 1978. Mapping of tRNA genes on the circular DNA molecule of *Spinacia oleracea* chloroplasts, p. 573–580. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
343. **Stephenson, G., S. Marzuki, and A. W. Linnane.** 1980. Biogenesis of mitochondria: two-dimensional electrophoretic analysis of mitochondrial translation products in yeast. Biochim. Biophys. Acta **609**:329–341.
344. **Stiegler, P., P. Carbon, M. Zuker, J.-P. Ebel, and C. Ehresmann.** 1981. Structural organization of the 16S ribosomal RNA from *E. coli*: topography and secondary structure. Nucleic Acids Res. **9**:2153–2172.
345. **Synenski, R. M., C. S. Levings III, and D. M. Shah.** 1978. Physiochemical characterization of mitochondrial DNA from soybean. Plant Physiol. **61**:460–464.
346. **Tabak, H. F., J. van der Laan, K. A. Osinga, J. P. Schouten, J. H. van Boom, and G. H. Veeneman.** 1981. Use of a synthetic DNA oligonucleotide to probe the precision of RNA splicing in a yeast mitochondrial petite mutant. Nucleic Acids Res. **9**:4475–4483.
347. **Takaiwa, F., and M. Sugiura.** 1980. Cloning and characterization of 4.5S and 5S RNA genes in tobacco chloroplasts. Gene **10**:95–103.
348. **Takaiwa, F., and M. Sugiura.** 1980. Nucleotide sequences of the 4.5S and 5S ribosomal RNA genes from tobacco chloroplasts. Mol. Gen. Genet. **180**:1–4.
349. **Takaiwa, F., and M. Sugiura.** 1981. Heterogeneity of 5S RNA species in tobacco chloroplasts. Mol. Gen. Genet. **182**:385–389.
350. **Tapper, D. P., and D. A. Clayton.** 1981. Mechanism of replication of human mitochondrial DNA. Localization of the 5′ ends of nascent daughter strands. J. Biol. Chem. **256**:5109–5115.
351. **Taylor, D. L.** 1970. Chloroplasts as symbiotic organisms. Int. Rev. Cytol. **27**:29–64.
352. **Tewari, K. K.** 1979. Structure and replication of chloroplast DNA, p. 41–108. *In* T. C. Hall and J. W. Davies (ed.), Nucleic acids in plants, vol. 1. CRC Press, Cleveland, Ohio.
353. **Thalenfeld, B. E., and A. Tzagoloff.** 1980. Assembly of the mitochondrial membrane system. Sequence of the Oxi2 gene of yeast mitochondrial DNA. J. Biol. Chem. **255**:6173–6180.
354. **Thorne, S. W., E. H. Newcomb, and C. B. Osmond.** 1977. Identification of chlorophyll b in extracts of prokaryotic algae by fluorescence spectroscopy. Proc. Natl. Acad. Sci. U.S.A. **74**:575–578.
355. **Tiboni, O., G. Di Pasquale, and O. Ciferri.** 1978. Purification, characterization and site of synthesis of chloroplast elongation factors, p. 675–678. *In* G. Akoyunoglou and J. H. Argyroudi-Akoyunoglou (ed.), Chloroplast development. Elsevier/North-Holland Biomedical Press, Amsterdam.
356. **Tohdoh, N., K. Shinozaki, and M. Sugiura.** 1981. Sequence of a putative promoter region for the rRNA genes of tobacco chloroplast DNA. Nucleic Acids Res. **9**:5399–5406.
357. **Trager, W.** 1970. Symbiosis. Van Nostrand Reinhold Co., New York.
358. **Turner, G., G. Iman, and H. Kuntzel.** 1979. Mitochondrial ATPase complex of *Aspergillus nidulans* and the dicyclohexylcarbodiimide-binding protein. Eur. J. Biochem. **97**:565–571.
359. **Tzagoloff, A., G. Macino, and W. Sebald.** 1979. Mitochondrial genes and translation products. Annu. Rev. Biochem. **48**:419–441.
360. **Upholt, W. B., and I. B. Dawid.** 1977. Mapping of mitochondrial DNA of individual sheep and goats: rapid evolution in the D-loop region. Cell **11**:571–583.
361. **Uzzell, T., and C. Spolsky.** 1974. Mitochondria and plastids as endosymbionts: a revival of special creation? Am. Sci. **62**:334–343.
362. **Uzzell, T., and C. Spolsky.** 1981. Two data sets: alternative explanations and interpretations. Ann. N.Y. Acad. Sci. **361**:481–499.
363. **VanDemark, P. J.** 1969. Respiratory pathways in the Mycoplasma, p. 491–501. *In* L. Hayflick (ed.), The Mycoplasmatales and the L-phase of bacteria. Appleton-Century-Crofts, Educational Div., Meredith Corp., New York.
364. **VanDemark, P. J., and P. F. Smith.** 1964. Respiratory pathways in the Mycoplasma. II. Pathway of electron transport during oxidation of reduced nicotinamide adenine dinucleotide by *Mycoplasma hominis*. J. Bacteriol. **88**:122–129.
365. **VanDemark, P. J., and P. F. Smith.** 1964. Evidence for a tricarboxylic acid cycle in *Mycoplasma hominis*. J. Bacteriol. **88**:1602–1607.
366. **Van Ee, J. H., Y. J. Vos, and R. J. Planta.** 1980. Physical map of chloroplast DNA of *Spirodela oligorrhiza*; analysis by the restriction endonucleases PstI, XhoI and SacI. Gene **12**:191–200.
367. **Van Etten, R. A., M. W. Walberg, and D. A. Clayton.** 1980. Precise localization and nucleotide sequence of the two mouse mitochondrial rRNA genes and three immediately adjacent novel tRNA genes. Cell **22**:157–170.
368. **Van Ommen, G.-J. B., P. H. Boer, G. S. P. Groot, M. de Haan, E. Roosendaal, L. A. Grivell, A. Haid, and R. J. Schweyen.** 1980. Mutations affecting RNA splicing and the interaction of gene expression of the yeast mitochondrial loci cob and oxi-3. Cell **20**:173–183.
369. **Van'T Sant, P., J. F. C. Mak, and A. M. Kroon.** 1980. Regulation of the synthesis of mitochondrial proteins: is there a repressor?, p. 387–390. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
370. **Van Valen, L. M., and V. C. Maiorana.** 1980. The archaebacteria and eukaryotic origins. Nature (London) **287**:248–250.
371. **Vedel, F., and F. Quetier.** 1974. Physico-chemical characterization of mitochondrial DNA from potato tubers. Biochim. Biophys. Acta **340**:374–387.
372. **Vincent, R. D., P. S. Perlman, R. L. Strausberg, and R. A. Butow.** 1980. Physical mapping of genetic determinants on yeast mitochondrial DNA affecting the apparent size of the var1 polypeptide. Curr. Genet. **2**:27–38.
373. **Wachter, E., W. Sebald, and A. Tzagoloff.** 1977. Altered amino acid sequence of the DCCD-binding protein in the OLI1 resistant mutant D273-10B/A21 of *Saccharomyces cerevisiae*, p. 441–449. *In* W. Bandlow, R. J. Schweyen, K. Wolf, and F. Kaudewitz (ed.), Mitochondria 1977—genetics and biogenesis of mitochondria. W. de Gruyter, Berlin.
374. **Walker, W. F.** 1981. Proposed sequence homology between the 5′-end regions of prokaryotic 23S rRNA and eukaryotic 28S rRNA. FEBS Lett. **126**:150–151.
375. **Wallace, D. C.** 1981. Assignment of the chloramphenicol resistance gene to the mitochondrial DNA and analysis of its expression in cultured human cells. Mol. Cell. Biol. **1**:697–710.
376. **Wallace, D. C., C. L. Bunn, and J. M. Eisenstadt.** 1975. Cytoplasmic transfer of chloramphenicol resistance in human tissue culture cells. J. Cell Biol. **67**:174–188.
377. **Wallace, D. C., and H. J. Morowitz.** 1973. Genome size and evolution. Chromosoma **40**:121–126.
378. **Ward, B. L., R. S. Anderson, and A. J. Bendich.** 1981. The mitochondrial genome is large and variable in a family of plants (Cucurbitaceae). Cell **25**:793–803.
379. **Watson, J. D.** 1976. Molecular biology of the gene, 3rd ed. W. A. Benjamin, Inc., Menlo Park, Calif.
380. **Webster, K. A., N. A. Oliver, and D. C. Wallace.** 1982. Assignment of an oligomycin-resistance locus to human chromosome 10. Som. Cell Genet. **8**:223–244.
381. **Wells, R., and M. Birnstiel.** 1969. Kinetic complexity of

chloroplastal deoxyribonucleic acid and mitochondrial deoxyribonucleic acid from higher plants. Biochem. J. 112:777–786.
382. **Wesolowski, M., and H. Fukuhara.** 1979. The genetic map of transfer RNA genes of yeast mitochondria: correction and extension. Mol. Gen. Genet. 170:261–275.
383. **Wesolowski, M., and H. Fukuhara.** 1981. Linear mitochondrial deoxyribonucleic acid from the yeast *Hansenula mrakii*. Mol. Cell. Biol. 1:387–393.
384. **Westergaard, O., K. A. Marcker, and J. Keiding.** 1970. Induction of a mitochondrial DNA polymerase in *Tetrahymena*. Nature (London) 227:708–710.
385. **Whatley, F. R.** 1981. The establishment of mitochondria: *Paracoccus* and *Rhodopseudomonas*. Ann. N.Y. Acad. Sci. 361:330–340.
386. **Whatley, J. M.** 1981. Chloroplast evolution—ancient and modern. Ann. N.Y. Acad. Sci. 361:154–164.
387. **Whatley, J. M., P. John, and F. R. Whatley.** 1979. From extracellular to intracellular: the establishment of mitochondria and chloroplasts. Proc. R. Soc. London Ser. B. 204:165–187.
388. **Wildeman, A. G., and R. N. Nazer.** 1980. Nucleotide sequence of wheat chloroplastid 4.5S ribonucleic acid: sequence homologies in 4.5S RNA species. J. Biol. Chem. 255:11896–11900.
389. **Wilson, E. B.** 1925. The cell in development and heredity, 3rd ed. Macmillan, New York.
390. **Woese, C. R.** 1981. Archaebacteria. Sci. Am. 244:98–122.
391. **Woese, C. R., and G. E. Fox.** 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. Proc. Natl. Acad. Sci. U.S.A. 74:5088–5090.
392. **Woese, C. R., L. J. Magrum, and G. E. Fox.** 1978. Archaebacteria. J. Mol. Evol. 11:245–252.
393. **Wolstenholme, D. R., C. M.-R. Fauron, and J. M. Goddard.** 1980. The adenine and thymine-rich region of *Drosophila* mitochondrial DNA molecules, p. 241–250. *In* A. M. Kroon and C. Saccone (ed.), The organization and expression of the mitochondrial genome. Elsevier/North-Holland Biomedical Press, Amsterdam.
394. **Yatscoff, R. W., S. Goldstein, and K. B. Freeman.** 1978. Conservation of genes coding for proteins synthesized in human mitochondria. Som. Cell Genet. 4:633–645.
395. **Young, I. G., and S. Anderson.** 1980. The genetic code in bovine mitochondria: sequence of genes for the cytochrome oxidase subunit II and two tRNAs. Gene 12:257–265.
396. **Young, R. A., R. Macklis, and J. A. Steitz.** 1979. Sequence of the 16S-23S spacer region in two ribosomal RNA operons of *Escherichia coli*. J. Biol. Chem. 254:3265–3271.
397. **Zablen, L. B., M. S. Kissil, C. R. Woese, and D. E. Buetow.** 1975. Phylogenetic origin of the chloroplast and prokaryotic nature of its ribosomal RNA. Proc. Natl. Acad. Sci. U.S.A. 72:2418–2422.
398. **Zielinski, R. E., and C. A. Price.** 1980. Synthesis of thylakoid membrane proteins by chloroplasts isolated from spinach. Cytochrome b559 and P700-chlorophyll a-protein. J. Cell Biol. 85:435–445.
399. **Zillig, W., J. Tu, and I. Holz.** 1981. Thermoproteales—a third order of thermoacidophilic archaebacteria. Nature (London) 293:85–86.
400. **Zipkas, D., and M. Riley.** 1975. Proposal concerning mechanism of evolution of the genome of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 72:1354–1358.
401. **Zurawski, G., B. Perrot, W. Bottomley, and P. R. Whitfeld.** 1981. The structure of the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase from spinach chloroplast DNA. Nucleic Acids Res. 9:3251–3270.
402. **Zwieb, C., C. Glotz, and R. Brimacombe.** 1981. Secondary structure comparisons between small subunit ribosomal RNA molecules from six different species. Nucleic Acids Res. 9:3621–3640.