FEBS

Mitochondria:
Biogenesis and Bioenergetics

Biomembranes:
Molecular Arrangements and Transport Mechanisms

Volume 28

Organized by:
S. G. VAN DEN BERGH
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E. C. SLATER
J. M. TAGER

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MITOCHONDRIA: biogenesis and bioenergetics

BIOMEMBRANES: molecular arrangements and transport mechanisms

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S. G. VAN DEN BERGH, *Utrecht*
P. BORST, *Amsterdam*
L. L. M. VAN DEENEN, *Utrecht*
J. C. RIEMERSMA, *Leiden*
E. C. SLATER, *Amsterdam*
J. M. TAGER, *Amsterdam*

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INTRODUCTION

The scientific topics of the 8th FEBS Meeting were selected in the period between December 1969 and February 1970. It required some foresight, though not too much, to decide more than 2½ years in advance what parts of biochemistry would arouse most interest in August 1972. For the two symposia brought together in this volume, the choice seemed self-evident and without any risk.

The study of *mitochondria* has fascinated biochemists ever since these particles were first isolated in 1948 and were found to contain the multienzyme systems that catalyze oxidation of fatty acids, oxidation of all the Krebs citric acid cycle intermediates, electron transport and oxidative phosphorylation. From the major lines of investigation on mitochondria that have developed during the 1950s and 1960s, three were selected for presentation and discussion during the 8th FEBS Meeting (a) The *biogenesis* of mitochondria, comprising the synthesis of their macromolecular components and their assembly, is the most recent addition to mitochondriology; developments in this field have been most spectacular and significant. (b) Mitochondrial *bioenergetics* is a much older field of investigation. To the outsider, progress in this field seems slower. However, today many bioenergetic questions are largely resolved and even the central problem of attaining a molecular description of the ATP-producing machinery might have been solved in August 1972. (c) *Ion translocation* through mitochondrial membranes occurs both by active transport and by exchange diffusion mechanisms. Its importance in the regulation of mitochondrial metabolism has become increasingly clear in recent years.

The interest of biochemists in the architecture and function of *biomembranes* has increased greatly in recent years. Application of a great variety of physical techniques has revealed the complexity of membrane organization and led to a better recognition of the nature of the interactions of proteins and lipids in biological interfaces. Several dynamic features of biomembranes can now be described at the molecular level. Considerable progress has been made in the simulation of a number of properties of biomembranes in model systems. Various transport systems have been well-characterized and an understanding of the molecular mechanism of ion transport now appears to be within reach.

Although many topics were brought into focus during the symposia, many aspects could not be dealt with. Fortunately, the symposia were accompanied by some 200 free communications on biomembranes and well over 100 on mitochondria, demonstrating again the great interest of today's biochemist in these subjects.

In order to cover the field in such a way that neither large gaps nor overlaps would occur, we have taken the liberty of suggesting the topic to be dealt with by each speaker. We asked, moreover, to prepare essentially review papers with – hopefully – some original work, some general discussion and a personal view of thing. We are greatly indebted to all authors for their generous cooperation in this respect.

Finally, we wish to express our sincere gratitude to North-Holland Publishing Company for its invaluable help in producing this volume.

The organizers.

LIST OF CONTENTS

Mitochondria

LIST OF CONTENTS

Biomembranes

MITOCHONDRIAL DNA: STRUCTURE, GENES, REPLICATION

P.BORST AND R.A.FLAVELL
Section for Medical Enzymology*, Laboratory of Biochemistry, University of Amsterdam, Amsterdam, The Netherlands

CONTENTS

1. INTRODUCTION

In 1965 Margit Nass and her co-workers concluded from a cytochemical study of a wide variety of organisms that mtDNA is "an integral part of most and probably all mitochondria". This conclusion has been amply confirmed in the ensuing seven years and many of the properties of mtDNA are now known in detail, while knowledge about its replication and genetic role is rapidly accumulating. Since one of us has recently reviewed this subject in detail (Borst, 1972), we shall limit this brief review to recent developments and aspects not previously covered.

2. GENERAL PROPERTIES

All mtDNAs found in nature are small duplex DNAs, varying in size between 4.45 μm in sea urchin to 30 μm in pea (Tables 1 and 2). No unusual bases have been found in mtDNA at a 1% detection level, with the possible exception of 5-methyl-C in Physarum polycephalum mtDNA (Evans and Evans, 1970).

Circularity is the rule for mtDNA, the only well-documented exception being Tetrahymena mtDNA (Table 2). Although usually only one type of circle (and its oligomers) is found in one organism a more complex situation may be present in Ascomycetes (Table 2). In two strains of Neurospora only 20-μm circles were found, whereas in a third strain Agsteribbe et al. (1972) find predominantly a very heterogeneous collection of circles

* Postal address: Jan Swammerdam Institute, Eerste Constantijn Huygensstraat 20, Amsterdam (The Netherlands).

Table 1
Size and structure of animal mtDNAs

Species	Structure	Size (μ)
Chordata		
Mammals	Circular	4.7 - 5.6
Birds	Circular	5.1 - 5.4
Amphibia	Circular	4.9 - 5.8
Fish	Circular	5.4
Echinodermata		
Echinoidea	Circular	4.6 - 4.9
Arthropoda		
House fly	Circular	5.2
Annelida		
Urechis caupo	Circular	5.9
Nematoda		
Ascaris lumbricoides	Circular	4.8
Platyhelminthes		
Hymenolepis diminuta	Circular	4.8

Compiled from data reviewed in Borst and Kroon (1969) and Borst (1972).

varying in length between 0.5 and 7 μm with an occasional 19-μm circle. This recalls previous reports of such heterogeneous small circles in some yeast strains (see Hollenberg et al., 1970). It is possible that these small circles represent intermediates in recombination or selective amplification of certain sections of mtDNA. Proof that they are homologous to the large

Table 2
Size and structure of mtDNAs from protists and plants

Species	Structure	Size (μ)	Refs.
Protozoa			
Tetrahymena pyriformis	Linear	15	a)
Fungi			
Ascomycetes			
Saccharomyces	Circular	25	b)
Neurospora crassa:			
strain 1118A	Circular	20	c)
strain 5256	Circular	19	d)
strain 5297	Circular	0.5 - 19	d)
strain Em 5256	Linear	26	e)
Higher plants			
Pisum sativum	Circular	30	f)

a) Suyama and Miura (1968); Schutgens (1971); Flavell and Follett (1970); Charret (1970); Arnberg et al. (1972).
b) Hollenberg et al. (1970); Blamire et al. (1972).
c) Clayton and Brambl (1972).
d) Agsteribbe et al. (1972).
e) Schäfer et al. (1971).
f) Kolodner and Tewari (1972).

circles is still lacking, however, and the possibility that they are derived from other cell organelles (cf. Clark-Walker, 1972), contaminating the mitochondria, remains to be excluded. An intriguing point is further that Schäfer et al. (1971) extracted Neurospora mtDNA as a homogeneous population of 26-μm linear molecules. Assuming that the difference between 19 and 26 is due to calibration problems, it seems likely that under some conditions the circles sustain a single double-stranded cut during extraction. The enzyme responsible for this cut may either be part of the attachment site of mtDNA to the mitochondrial membrane or an enzyme involved in replication (see also Borst, 1972).

It should be noted that all mtDNA populations studied contain intermediates in the replication (and possibly recombination or repair) of mtDNA (see Table 3). Some of these intermediates

Table 3

Approximate composition of total mtDNA in adult rodent and chick liver

	Weight percent of total
Closed circular DNA (clean)	48
D-loop closed circular DNA	30
Catenated oligomer DNA	10
Open circular DNA	10
Open circles with D-loops or ss-tails	1 - 2
Unusual molecules (Cairns-type forked circles, gapped molecules, etc.)	1

Based on Clayton et al. (1968); Arnberg et al. (1971); Arnberg, A.C. and Flavell, R.A., unpublished expts.

like Cairns-type circles are rare (Kirschner et al., 1968), others like catenated oligomers may already form 10-20% of the mtDNA (Clayton et al., 1968), whereas D-loop DNA molecules make up at least 30-50% of the DNA from tissues like adult rat and chick liver, adult beef thyroid glands and mouse tissue culture cells (see Borst, 1972). These powerful minorities should be kept in mind when interpreting experiments with mtDNA.

The complementary strands of mammalian mtDNAs can be conveniently separated in alkaline CsCl equilibrium gradients (see Table 4). Little or no strand separation is obtained in these gradients with mtDNA from yeast or Tetrahymena, but with the latter DNA this was recently achieved with U-rich polyribonucleotides.

3. INFORMATION CONTENT

From extensive quantitative renaturation studies it appears that large-scale heterogeneity or redundancy are absent in mtDNAs (Table 5). The situation with mtDNA from yeast and Neurospora was at first unclear because it was found (Wood and Luck, 1969; Christiansen et al., 1971) that in 1 M NaCl the renaturation of these mtDNAs did not show the usual linear dependence on the square root of the DNA fragment size. Recent experiments

Table 4

Strand separation of mtDNAs in alkaline CsCl or in the presence of polyribonucleotides

	Δrho of complementary strands (mg/ml)		
	in alkaline CsCl	with G-rich polymers	with U-rich polymers
Sea urchin	5		
Crab	13		
Toad	13		
Chick	42	>40	>40
Rat	31 - 32	>40	20
Rabbit	27		
Sheep	27		
Monkey	40		
Man	39 - 41		
Tetrahymena	6	0[a)]	30[a)]

Compiled from data reviewed in Borst (1972).

a) Schutgens et al. (1972).

by C.Christiansen and co-workers (personal communication) with yeast have shown, however, that this is due to an unusually stable secondary structure of single-stranded yeast mtDNA. If renaturation of yeast mtDNA is carried out at a temperature only 10° below the T_m, it shows the usual dependence on fragment size and its kinetic complexity agrees with the expected molecular weight (see Table 2). Independent experiments by J.J. Van Berkel in our laboratory have shown that no anomalies are encountered with yeast mtDNA when renaturation is carried out at 0.2 M Na^+ even 25° under the T_m. A similar explanation may hold for the anomalous results with Neurospora mtDNA in 1 M NaCl, obtained by Wood and Luck (1969).

In a recent note Corneo et al. (1971) conclude that human

Table 5

Genome size of mtDNAs calculated from renaturation rates

mtDNA from	Mol. wt. from EM length	Mol. wt. from renaturation rate	Refs.
	dalton x 10^{-6}		
Rat	9.6	9.9	a)
Guinea pig	11	11	a)
Tetrahymena	29	30	b)
Saccharomyces	49	63	c)
		about 50	d)
Pea	66	74	e)

a) Borst (1971).
b) Flavell and Jones (1970).
c) Hollenberg et al. (1970) (corrected for low G.C according to Seidler and Mandel (1971)).
d) Christiansen, C., personal communication.
e) Kolodner and Tewari (1972).

mtDNA has a kinetic complexity only 1/5th of its size. The experiments supporting this conclusion are most unconvincing to us, the conclusion is incompatible with the results of DNA-RNA hybridization experiments with (human) HeLa-cell mtDNA (see Table 6) and incompatible with the data on the kinetic complexity of other mammalian mtDNAs presented in Table 5.

Table 6

The number of genes for rRNA and tRNA on mtDNAs

	Genes/mtDNA		Molecular weight ($\times 10^{-6}$)			Refs.
	rRNA	tRNA	rRNAs	tRNAs	mtDNA	
Xenopus	1.0	15	0.30 + 0.53	0.028	11.7	a)
HeLa cells	1.0	11	0.36 + 0.56	0.025	11	b)
Yeast	0.64	20	0.6 + 1.2 ?	0.025	49	c)
Neurospora	0.71		0.6 + 1.2 ??		51	d)
Tetrahymena	1.36		0.52 + 0.82 ?[e]		29	f)

a) Dawid (1972).
b) Aloni and Attardi (1971); Robberson et al. (1972).
c) Reijnders et al. (1972); Reijnders and Borst (1972).
d) Schäfer and Küntzel (1972).
e) Reijnders, L., unpublished expts.
f) Suyama (1967).

4. GENES

All mtDNAs studied hybridize with mitochondrial rRNAs and tRNAs. The hybridization plateaus obtained are given in Table 6. It is clear that in animal tissues each mtDNA circle contains one gene for each of the two rRNAs and it is likely that the same situation holds in the uni-cellular organisms studied. This confirms the conclusion drawn from renaturation experiments that the 5-fold difference in size of animal and yeast mtDNA is not merely due to a 5-fold redundancy of the latter.

The results in Table 6 show that like mycoplasmas, mitochondria have a genetic system which operates without multiple rRNA cistrons. An important consequence is that mutations affecting ribosomal function or assembly, caused by a mutation in the rRNA genes, might be quite frequent in mitochondria, although they have not been observed in other systems (see Borst, 1972 for discussion). In fact, all antibiotic-resistant yeast mutants analysed up till now lack detectable changes in ribosomal proteins and they could still all be rRNA mutants (L.A. Grivell, personal communication).

As Table 6 shows, the number of tRNA genes found on mtDNA is far less than the 33, minimally necessary to read 61 codons with maximal wobble. It is difficult to believe that any genetic system that utilizes only a small part of the 64 possible codons could survive and we prefer the idea, therefore, that an adequate number of different tRNAs is generated somehow, for instance by import of tRNAs coded for by nuclear genes or by secondary modification of a limited set of primary gene products. The possibility of a heterogeneity of tRNA cistrons in mtDNA is also not yet rigorously excluded.

Besides rRNA and tRNA mtDNA appears to specify a number of hydrophobic proteins that form part of the inner mitochondrial membrane. The nature of these proteins is discussed in other papers in this Symposium and in Borst (1972).

5. EVOLUTION

There are several reasons why the evolution of mtDNA is of interest. First, mtDNA is the only small DNA present in virtually all eukaryotes and its evolution can be traced over 400 million years. Second, mtDNA is small enough to allow a complete study of base sequence homologies, deletions and insertions and eventually of changes in individual base pairs. Third, mtDNA contains genes for rRNAs, tRNAs and mRNAs and the relative restrictions that these genes put on evolutionary change can, therefore, be studied in one molecule. Finally, the study of base sequence homologies in closely related DNAs should provide information about the possible presence of rapidly evolving "spacer regions" in mtDNA.

Comparisons of closely related mtDNAs have been made by three groups. Dawid (1972a) made a detailed comparison of the mtDNAs of two Xenopus species, X. laevis and X. mülleri, that are related enough to yield viable hybrid progeny. He found that 30% of the sequence has no homology, about 50% gives a hybrid with an average mismatching of 27% of the bases and the remaining 20% of the sequence, corresponding to the genes for rRNA and tRNA, formed a heteroduplex with 6% mismatching. Dawid concluded from these data that 80% of the sequence of these animal mtDNAs has evolved so rapidly that it may be equivalent to the "spacer" sequences, which have been found within certain nuclear DNAs.

Different results have been obtained in analyses of other related mtDNA couples. Brown and Hallberg (1972) analysed heteroduplexes constructed from the H-strand of one mtDNA and the L-strand of another. The melting curve of the chimpanzee-man heteroduplex was indistinguishable from that of the corresponding homoduplexes showing that mismatching must be less than 1%. On the other hand, 30% mismatching was observed in the human-green monkey heteroduplex. A high degree of homology was also found between mtDNAs of rat and mouse (F.G.Grosveld, R.W.J.Thuring and P.Borst, unpublished expts.). In DNA-DNA hybridization experiments a cross-hybridization of 80% was observed (Fig. 1) and the T_m of the hybrid was only 8° below that of the homologous duplex, indicating a higher degree of homology for the mtDNAs than for the nuclear DNAs of rat and mouse (Kohne, 1970). These results do not support the idea that most of the sequences of mtDNA are "spacers" not restricted in sequence evolution.

When mtDNAs from more distantly related organisms are compared, not much sequence homology remains. Although sequence homologies between chick and rat (Fig. 1) or chick, X. laevis and the Echiuroid worm Urechis caupo (Dawid and Brown, 1970; Dawid, 1972a) are detectable, the degree of mismatching is very large. No homology at all was detected by co-annealing X. laevis and yeast mtDNAs (Dawid and Wolstenholme, 1968) or mtDNAs from Paramecium and Tetrahymena (Flavell and Jones, 1971) and no cross-hybridization was found in DNA-DNA hybridization experiments between chick, yeast and Tetrahymena mtDNAs (R.A. Flavell and J.P.M.Sanders, unpublished expts.). An interesting aspect of these data is that all mtDNAs contain cistrons for

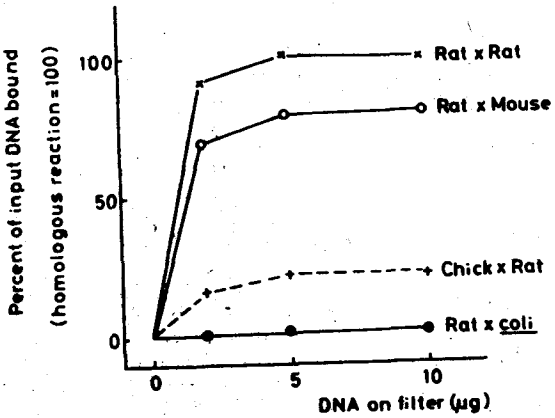

Fig. 1. Analysis of sequence homology between mtDNAs by DNA-DNA hybridization. 0.16 ng of radioactive, sonicated, denatured mtDNA was hybridized to different amounts of cold mtDNA or E. coli DNA fixed on nitrocellulose filters, as indicated. Rat x Mouse means rat DNA in solution, mouse DNA on filter. DNA-DNA hybridization was carried out as described by Ter Schegget et al. (1971), with a reaction volume of 0.9 ml instead of 5.0 ml.

rRNA, which are highly resistant to change in other systems studied. Considerable sequence homology is present between the cell-sap rRNAs of all eukaryotes and appreciable homology can even be detected between the rRNAs of Escherichia coli and pea (Bendich and McCarthy, 1970), although this remains disputed (Sinclair and Brown, 1971). This makes the absence remarkable of any cross-hybridization between animal, yeast and Tetrahymena mtDNAs, which contain rRNA cistrons. A more detailed analysis using rRNAs is required, however, to confirm that homology is completely absent.

A reason why the average sequence divergence of mtDNAs could be rather high, notwithstanding the presence of rRNA and tRNA genes, could lie in the fact that mtDNA turns over in resting cells (see Borst and Kroon, 1969; and Borst, 1972). If this also occurs in germ-line cells mtDNA might undergo many more divisions in these cells than the nuclear DNA, a difference amplified by the fact that there is only one nucleus but many mitochondria. Since sequence divergence is related to generation time rather than absolute time (Kohne, 1970), this would result in a greater sequence divergence for mtDNAs, especially in organisms with a long generation time. The fact that a 6% sequence divergence is found for the mitochondrial rRNAs of X. laevis and X. mülleri, whereas none was detected for the cell-sap rRNAs (Brown et al., 1972) would fit this idea unless one assumes that it is easier to tamper with a mitochondrial than with a cell-sap ribosome. This argument does not hold, however, for protists where turn-over of mtDNA is probably insignificant.

Finally, the question may be raised how heterogeneity of mtDNA in one species or even in one animal is prevented. Error correction or elimination mechanisms involving the master-slave

hypothesis, a nuclear master copy or a reduction of mtDNA to one copy in germ-line cells could be postulated, but there is no evidence that any of these mechanisms operate, or even that micro-heterogeneity is absent. In our opinion the following two mechanisms would suffice to prevent gross heterogeneity:

a) The possibility for mutations in mtDNA that are completely neutral could be severely restricted for three reasons:

1. Many of the known mitochondrial gene products show complex interactions, i.e. rRNA with ribosomal proteins, the cytochrome oxidase subunits with the other subunits and their attachment sites in the membrane. This will restrict the number of permissible alterations.

2. The mitochondrial genetic system is subordinated to the nucleo-cell-sap system and, because of the close cooperation of mitochondrial and nuclear gene products in all phases of mitochondrial biogenesis, most changes in mtDNA may only be compatible with survival if concomitant changes in nuclear genes occur. This links the evolution of mtDNA to that of nuclear DNA and forms a strong force counter-acting intra-species heterogeneity. Support for this notion comes from the observation that in mouse-human hybrid cells only mouse mtDNA survives, even if a large fraction of the human chromosomes is still present (Attardi and Attardi, 1972; Clayton et al., 1971).

3. Changes of protein-specifying sequences could be restricted at the tRNA level, because the possibility still exists that many codons cannot be read at all by the mitochondrial protein-synthesizing system (see Section 4).

b) The rare neutral variant mtDNAs that may arise, may have a low probability of establishing themselves, like the supposedly neutral variant genes in nuclear DNA (cf. Harris, 1970; and Ohno, 1970). In yeast such minority mtDNA could easily be lost, because the bud gets much less than 50% of the cytoplasm. In higher organisms turn-over of mtDNA in germ-line cells would tend to eliminate most minority variants.

6. ORIGIN

Two hypotheses have been brought forward to account for the presence in mitochondria of a separate genetic system with properties that resemble those of prokaryotic systems. According to the endosymbiont hypothesis, mitochondria are domesticated bacteria, that have retained a bacterial-type protein-synthesizing system. According to the episome hypothesis, mtDNA started out as an episome that enwrapped itself in membranes that contained the respiratory chain, at a time that the eukaryotic ancestor still had a prokaryotic character. This character was preserved in the episomal system during evolution. The ability to continue DNA replication in the absence of protein synthesis and the sensitivity of DNA synthesis to acridines echo this plasmid origin. Some of the problems involved in deciding between these alternatives are discussed in Borst (1972). Here we only present a summary of two types of experiments that bear on this question.

A. Master copies in the nucleus

The question whether the base sequence of mtDNA is represented in the nucleus can be assessed by straightforward hybridization

experiments. Tabak (1972) found about 2 mtDNA copies per haploid nuclear genome in chick erythrocyte nuclei, whereas R.A.Flavell and P.O.Trampé (unpublished expts.) found less than 0.2 copy of mtDNA integrated in Tetrahymena nuclear DNA. These results show that previous reports of wide-spread base sequence homology between mitochondrial and nuclear DNA are incorrect and they exclude the presence of an integrated nuclear master copy in Tetrahymena, although an episomal nuclear master copy is not excluded. Whether the 2 master copies found in chick nuclei are real or the consequence of a slight contamination of nuclear DNA with mtDNA could not be decided. Support for the latter alternative was obtained in recent experiments by Dawid and Blackler (1972). They used the large sequence difference between the mtDNAs from X. mülleri and X. laevis (see Section 5) to establish that the inheritance of mtDNA in the laevis-mülleri hybrid is purely maternal. They then showed that the 14-16 copies of mtDNA found in nuclear DNA preparations of the hybrid were exclusively derived from the maternal parent. From this they concluded that all mtDNA in these nuclear DNA preparations must result from cytoplasmic contamination and that "in the frog the nucleus does not contain a 'master copy' of the mitochondrial sequences". This may be correct, but the experiments only exclude the presence of a master copy contributed by the paternal parent and one might argue that maternal inheritance of mtDNA necessitates the destruction of the paternal master copies, if any, during gametogenesis. On balance, however, the existence of nuclear master copies of mtDNA now seems unlikely, even though proof for their absence is still incomplete.

B. Nearest-neighbour analysis

A comparison of doublet patterns of mtDNAs with those of bacterial DNAs might be instructive in two respects: First it might provide information about unitary versus multiple origins of mitochondria. Second, it might reveal a relation of the mitochondrial doublet pattern either to the characteristic pattern common to all eukaryotic nuclear DNAs or to one of the bacterial patterns found (see Subak-Sharpe, 1967). Three groups have analysed the doublet patterns of transcripts of mtDNAs, made with a bacterial polymerase (Cummins et al., 1967; Grossman et al., 1971; Antonoglou and Georgatsos, 1972). The assumption underlying this approach is that the transcript is essentially random. Unfortunately, there is now good evidence that this assumption is incorrect (Tabak and Borst, 1970; Schäfer et al., 1971; Dawid and Blackler, 1972) and the results obtained are, therefore, uninterpretable. A classical analysis of doublets with DNA polymerase was carried out by Russell et al. (1972). For mtDNAs from mouse, guinea-pig and Tetrahymena they find a similar and highly characteristic pattern, high in GpG and CpC and low in CpG. This pattern differs clearly from that of DNAs from eukaryotic nuclei, animal viruses, bacteria or bacteriophages (Subak-Sharpe, 1967). These results are compatible with a common origin of animal and Tetrahymena mtDNAs, even though they do not show detectable sequence homology (see Section 5). The fact that the doublet patterns of mtDNA are different from those of nuclear DNAs, does not really argue against the episomal hypothesis. There is strong indirect evidence (see Subak-

Sharpe, 1967) that deviations from random expectation in doublet patterns are due to restrictions imposed by the translational apparatus that reads the message transcribed from a DNA. Since the translational apparatuses of mitochondria and cell sap are so different, different restrictions on doublet patterns are to be expected.

7. ODD mtDNAs

A. mtDNA in yeast petite mutants (see Borst, 1972; and Linnane et al., 1972)

Saccharomyces strains have the unusual property that they are able to multiply without functional mitochondria. If supplied with ample glucose they do not require mtDNA and mutants that have lost part or all of their mtDNA are perfectly viable although they will not be able to synthesize functional mitochondria. Such "petite" mutants are readily obtained by exposing yeast to intercalating dyes, like acridines and ethidium, which block mtDNA synthesis and lead to degradation of pre-existing mtDNA. Long exposure to high drug concentrations results in cytoplasmic petites that contain no mtDNA. When the drug treatment is stopped before all mtDNA has been degraded, remaining DNA fragments may resume replication. At first such early petite clones are heterogeneous in mtDNA (see Nagley and Linnane, 1972; and Hollenberg et al., 1972), but eventually clones emerge that contain only one type of altered mtDNA. It is now clear that this alteration is a deletion with a compensatory amplification of remaining sequences to keep the total cellular content of mtDNA constant. The deletion shows up in the decrease of genetic complexity of mtDNA in renaturation reactions. It may also show up in the loss of mitochondrial genes that can be rescued in genetic crosses and of the mitochondrial genes for rRNA and tRNA. The compensatory amplification may also affect the rRNA and tRNA genes, however, leading to markedly elevated hybridization plateaus for these RNAs. The alteration of mtDNA is often large enough to change its base composition, in extreme cases from the wild-type 17 mole percent GC to 4-6% GC, the so-called "low-density" petites.

It was at first thought that the mtDNA of low-density petites was largely poly[d(A-T)·(A-T)] or nonsense DNA. A detailed analysis of one such petite has shown that this idea is incorrect. Renaturation of the petite mtDNA was completely second-order, albeit nearly 300-fold faster (Borst, 1971) than of wild-type mtDNA. This suggested that the petite mtDNA was constructed from a repeating unit of about 100-300 nucleotides and since the melting profile of the renatured DNA was identical to that of the native helix, repetition is apparently perfect. This was confirmed by a pyrimidine tract analysis (Van Kreijl et al., 1972), which has yielded a strikingly simple pattern, with more than 95% of all ^{32}P accounted for by 5 tracts, T_1, T_2, T_3, T_6 and T_5C_2. The entire repeating unit hybridizes to wild-type DNA (Hollenberg et al., 1972a; and R.A.Flavell and J.P.M.Sanders, unpublished expts.). This proves that this extremely altered mtDNA lacks "new" sequences not present in wild-type mtDNA. Evidence for the same conclusion was recently obtained by Michaelis et al. (1972) for another petite mtDNA that still contained about 50% of the sequence of wild-type mtDNA. These

results imply that wild-type mtDNA contains sequences with exceptionally low GC content, that can be amplified to yield "low-density" petite mtDNA. This implication is in good agreement with the results of a detailed investigation of wild-type yeast mtDNA by Bernardi and co-workers (Bernardi et al., 1972; Piperno et al., 1972; Ehrlich et al., 1972).

B. mtDNA in trypanosomes (see Simpson, 1972)

Flagellated protozoa belonging to the genera Trypanosoma, Crithidia and Leishmania contain a mass of cytoplasmic DNA that is large enough to be detectable by light microscopy after staining with Feulgen stain or acridines. The available evidence now indicates that this kinetoplast DNA (K-DNA) is present within the single, giant mitochondrion of these hemoflagellates and that it is equivalent to the mtDNA of other organisms. In situ K-DNA is organised into a highly-ordered comb-like structure, with tightly-packed rows of fibers oriented parallel to the longitudinal axis of the cell. This structure can be isolated as one complex, resistant to ribonuclease, pronase or 7 M CsCl and so tightly packed that it is highly resistant to shear (Laurent et al., 1971). In Crithidia luciliae the complex represents 25% of the total cellular DNA and its molecular weight is about 2.2×10^{10}, i.e. 10-fold higher than that of E. coli DNA (Laurent et al., 1971). The complex contains both long linear DNA and mini-circles varying in size from 0.29 μm in C. tarentolae to 0.79 μm in C. fasciculata and mostly present as catenated oligomers. Quantitative renaturation studies indicate that the approximately 10 000 mini-circles present in one kinetoplast are all identical; the remainder of the DNA renatures with a kinetic complexity of about 70 000 nucleotide pairs (M.Steinert, personal communication).

The nature of the forces that keep the K-DNA complex together is still poorly understood. It has been suggested that the mini-circle catenanes are linked like rings on a chain by the long linear molecules which may in fact be large circles when intact. Replication of such a complex should pose formidable segregation problems, however.

Although no cytoplasmic DNA other than K-DNA has been found in hemoflagellates, positive evidence that K-DNA provides genetic information essential for the biosynthesis of the mitochondrial membrane is still lacking. We have started an investigation to see if K-DNA is transcribed into RNA and if it codes for the mitochondrial rRNA and tRNA, like the mtDNA of other organisms. The possible function of the mini-circles, which are too small to code for rRNAs, is especially intriguing.

C. Comparison of the biogenesis of mitochondria in yeast and trypanosomes

Yeast and some of the kinetoplastidae share the unique ability to selectively suppress the biogenesis of their mitochondria and multiply in the absence of functional mitochondria or even mtDNA. In yeast this is exemplified by the "petite" mutation discussed under A), with the kinetoplastidae by the dyskinetoplastic bloodstream forms of pathogenic trypanosomes (see Simpson, 1972). Further exploration of this analogy might help in defining the control mechanisms that lead in trypanosomes to a switch from oxidative to glycolytic metabolism.