REVIEW LETTER

MITOCHONDRIAL RIBOSOMES

P. BORST and L.A. GRIVELL
*Department of Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Amsterdam (The Netherlands)**

Received 8 January 1971

1. Introduction

Eleven years ago Mager [1] and Rendi [2] independently observed that mitochondrial protein synthesis is inhibited by chloramphenicol, an antibiotic that does not interfere with protein synthesis on the cell-sap ribosomes of eukaryotic cells. In the years that followed this fundamental observation was extended in three directions:

1) Sensitivity of protein synthesis to inhibition by D-chloramphenicol (but not by L-chloramphenicol [3]) is a characteristic of all mitochondria. For some time brain mitochondria appeared to be an exception to this rule, since their protein synthesis was only partly sensitive to inhibition by chloramphenicol [4]. Recent experiments indicate, however, that this chloramphenicol resistant protein synthesis takes place in non-mitochondrial structures contaminating the mitochondrial preparations [5, 6].

2) Mitochondrial protein synthesis is also inhibited by other antibiotics that specifically interfere with bacterial protein synthesis at the ribosome level, like tetracycline, lincomycin, macrolides like erythromycin, aminoglycosides like neomycin, and others [1,7–20].

3) Mitochondrial protein synthesis is insensitive to inhibitors of cell-sap protein synthesis that do not interfere with bacterial protein synthesis, like cycloheximide [9,21–23], emetin [24] or anisomycin [25].

Since all the antibiotics mentioned act on ribosomes [see 26, 27], the suggestion was made that mitochondria contain "bacterial-type" ribosomes [21, 28]. The search for these bacterial-type ribosomes has led to conflicting results, especially in the case of animal mitochondria. In the last year most of these controversies have been settled and a coherent picture is emerging. It is the purpose of this brief review to present this picture.

2. Mitochondrial ribosomes of ascomycetes

Perhaps on account of the absence of a well-developed endoplasmic membrane system in the cells of the ascomycetes, it has been relatively easy to demonstrate a characteristic mitochondrial ribosome merely on the basis of its predominance in mitochondrial fractions (see table 1). In the three ascomycetes so far studied, the mitochondrial ribosome has been isolated after lysis of mitochondria in either Triton X-100 or deoxycholate and identified as a particle which sediments through sucrose gradients at a rate intermediate between its cell-sap counterpart and ribosomes of *Escherichia coli*. The particles readily split into subunits (table 1) when the Mg^{2+} concentration is lowered to 0.1 mM, in contrast to the cell-sap ribosomes. Mitochondrial and cell-sap ribosomes contain very different RNA species and probably they do not have any proteins in common [40].

In early experiments the activity of these ribosomes in a submitochondrial poly-U directed poly-phenylalanine-synthesizing system was low (table 2), but recently one of us (L.A.G.) has isolated mitochondrial ribosomes from yeast with a catalytic activity of the order of that of *E. coli* ribosomes (table 2). No doubt remains, therefore, that these mitochondrial ribonucleoprotein particles are indeed ribosomes.

The mitochondrial rRNAs of the three ascomycetes

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

Abbreviation: M-DNA, mitochondrial DNA.

Table 1
Mitochondrial ribosomes of ascomycetes.

	Yeast	*Neurospora*	*Aspergillus*
Sedimentation coefficient (S)			
Ribosome	74 ± 1 (80)	73 (77)	67 (78)?*
Large subunit	53–58 (60)	50 (60)	50 (62)?*
Small subunit	35–40 (38)	37 (37)	32 (47)?*
Nascent protein pulse-labelled in intact mitochondria associated with ribosome after lysis	+	+	
Isolated ribosome active in protein synthesis	+	+	
22–23 S RNA extracted from large subunit; 15–16 S RNA from small subunit		+	
Reference	29–31; contrast 32, 33	34–37; contrast 38	39

Figures in brackets are sedimentation coefficients of corresponding cell-sap particles. Sedimentation coefficients are relative to *E. coli* ribosomes = 70 S, except for the *Aspergillus* values where *E. coli* ribosomes = 67 S.

* Sucrose gradients, in which *E. coli* ribosomes were present as internal marker, show that these sediment distinctly slower than the mitochondrial ribosomes (I.M. Verma, personal communication). The corrected sedimentation coefficients of yeast, *Neurospora and Aspergillus* mitochondrial ribosomes are, therefore, probably identical.

studied have three features in common to which we wish to direct attention (see table 3):

1) The mole percent G + C is extraordinarily low.

2) The electrophoretic mobility in acrylamide gels is lower than would be expected from the sedimentation behaviour in sucrose gradients.

3) The electrophoretic mobility relative to a cell-sap or *E. coli* marker RNA is strongly dependent on ionic strength and temperature.

The phenomena listed under (2) and (3) can be explained by assuming that these mitochondrial RNAs have a more loosely folded structure than *E. coli* rRNA under the conditions commonly used for sedimentation analysis or electrophoresis and that they unfold more readily when the temperature is raised or the ionic strength is lowered [44, 46, 52]. The low G + C content of the mitochondrial RNAs may be in part responsible for this, but other factors must be involved as well [see 44]. There is no evidence that this loosely folded RNA structure is also present in the intact ribosome.

A consequence of the unusual structure of these RNAs is that their molecular weights cannot be reliably inferred from their sedimentation behaviour or electrophoretic mobility [contrast 52]. In an attempt to overcome this problem Verma et al. [53] have determined the molecular length of *Aspergillus* mitochondrial RNA by electron microscopy. The values found correspond to molecular weights of 1.27 and 0.66×10^6, if one assumes that the internucleotide distance in the RNA spread in urea is 2.45Å, the average distance found for a number of other ribosomal RNAs [54]. Unfortunately, this distance is not constant for all RNAs and the internucleotide spacing found for messenger-type RNA is nearly 30% higher [54]. Since it is doubtful whether mitochondrial rRNA will spread in urea like other rRNAs in view of its unusual structure, we are not convinced that electron microscopy of this RNA will yield molecular weights that are more reliable than those calcalculated from rate sedimentation analysis or gel electrophoresis.

Table 2
Activity of mitochondrial ribosomes in poly-U directed polyphenylalanine synthesis.

Supernatant factors	Mitochondrial ribosome	Phenylalanine incorporated (pmoles/mg RNA/30–40 min)		Reference
		− Poly U	+ Poly U	
N. crassa mit	*N. crassa*	?	4.1*	41
S. cerevisiae mit.	*S. cerevisiae*	2.7	14.4	32
X. laevis mit	*X. laevis*	15	300**	42
E. coli	*N. crassa*	?	110	41
E. coli	*N. crassa*	?	9.2*	41
E. coli	*S. carlsbergensis*	8	765	29
E. coli	*E. coli*	26	1420	29

* Incorporated from ^{14}C-phenylalanine-tRNA.
** Recalculated from authors' data. Activity of crude mitochondrial extract four times higher.

Table 3
Mitochondrial ribosomal RNA of ascomycetes.

	Yeast (*S. cerevisiae*, *S. carlsbergensis*, *C. utilis*)	*Neurospora*	*Aspergillus*
Sedimentation coefficient (S)	21–22+14–15 (26+17)	23+16 (26+17)	23.5 + 15.5 (26.5 + 17.0)
Electrophoretic mobility			
2–5° S_E	25.0 + 17.1 (25.0 + 17.7)	25.5 + 18.4 (25.5 + 17.7)	
App. mol. wt. ($\times 10^{-6}$)	1.23 + 0.63 (1.23 + 0.67)	1.28 + 0.72 (1.28 + 0.67)	
15–25° S_E	28.9 + 20.5 (25.2 + 19.5)		26.5 + 18.4
App. mol. wt. ($\times 10^{-6}$)	1.60 + 0.87 (1.25 + 0.79)		1.29 + 0.72
Approximate molar ratio of the 2 components	1:1	1:1	1:1
Isolated from ribosomes	+	+	+
Mole percent GC	26 (46)	35–38 (49–50)	32 (51)
Degree of methylation		1:35 (1:87)	
Hybridization with M-DNA	+	+	
Reference	43–49; contrast 30, 32, 50	34, 35, 37, 38, 51	39

Figures in brackets give values for cell-sap components, when these differ from those quoted for mitochondrial components. The sedimentation coefficients are relative to *E. coli* rRNA (= 23 + 16 S); for *Aspergillus* the $s_{20,w}$ is given, determined in the analytical ultracentrifuge (*E. coli* rRNA = 24 + 16 S). The apparent molecular weight (app. mol. wt.) in gel electrophoresis experiments was calculated relative to *E. coli* rRNA ($1.10 + 0.56 \times 10^6$) by assuming that a linear relation exists between the electrophoretic mobility of an RNA and the reciprocal of the logarithm of its molecular weight. S_E is calculated in the same way using $(\log S)^{-1}$ instead of $(\log M)^{-1}$.

A number of observations on ribosomes of ascomycetes have been reported that differ from those reported in tables 1 and 2. We have omitted these results because we attribute them to technical problems in the isolation and characterization of mitochondrial ribosomes and their RNAs, e.g. poor resolution of the gradients used [32, 33, 38], contamination with cell-sap ribosomes [32, 33, 50], contamination with membrane fragments (which might explain the difficulties in obtaining dissociation of ribosomes into subunits [32, 33]) and partial degradation of the RNA [30].

3. Mitochondrial ribosomes of animal tissues

Mitochondrial ribosomes in animal tissues have been the subject of controversies that must be bewildering to the interested outsider, who will admire the agility with which some of the workers in this field change their views, often without retracting previous conclusions. The question presently dominating the field is whether the mini-ribosome is real or not.

The mini-ribosome entered the scene when O'Brien and Kalf [55] reported in 1967 that the nascent protein in a lysate of highly purified rat liver mitochondria sedimented with a ribonucleoprotein particle at 55 S. Although O'Brien [56] later retracted the conclusion that this 55 S particle was a complete ribosome, his results were confirmed (after an initial miscalibration of sucrose gradients [57]) by Ashwell and Work, who also opted for the idea that the rat liver mitochondrial ribosome is a 55 S particle [58].

The strongest case for the existence of such a mini-ribosome in animal tissues has been presented by Swanson and Dawid [42, 59]. They obtained a submitochondrial system from *Xenopus* egg mitochondria that incorporated phenylalanine at a high rate into acid-insoluble material in the presence of poly U (table 2). The extract contained 60 S, 43 S and 32 S particles, in addition to a trace of 87 S cell-sap (?) ribosomes. The bulk of the polyphe-synthesizing activity was associated with the 60 S particles (the heavier particles and the gradient pellet were not tested, however). The 60 S particles contained 17–18 S and 14 S RNA, both specifically hybridizing with mitochondrial DNA, the 43 S particle contained predominantly 18 S RNA, the 32 S particle 14 S RNA. The concentration of cell-sap ribosomal RNA in the purified 60 and 43 S particle was less than 1%. The authors conclude that the 60 S particle is the complete mitochondrial ribosome. They further suggest that this ribosome consists of subunits sedimenting at 43 and 32 S, containing 17–18 S and 14 S RNA, respectively.

Xenopus egg cells are convenient for the study of mitochondrial ribosomes because they contain little endoplasmic reticulum. This allows the isolation of mitochondrial preparations with only minor contamination with cell-sap ribosomes. With other cell types the situation is less favourable and mitochondrial ribosomes and their RNA must be detected among an excess of cell-sap ribosomes. This was accomplished by blocking the incorporation of precursors into cell-sap rRNA with actinomycin D in low concentrations that apparently do not affect mitochondrial RNA synthesis, or by using the ability of mitochondrial RNA to specifically hybridize with M-DNA. In addition, the specific inhibition of mitochondrial RNA synthesis by ethidium was found to be a useful tool to recognize RNA species synthesized on mitochondrial DNA [60]. Using these procedures ribonucleoprotein particles and RNA components similar to those of *Xenopus* mitochondria have been detected in mitochondria of several other animals (tables 4 and 5).

The striking diversity in sedimentation coefficients and electrophoretic mobilities of these mitochondrial RNAs requires some comment. We attribute the diversity in S values to technical problems in the calibration of gradients, possibly also to differences in the ionic conditions used. The divergent electrophoretic mobilities observed for the mitochondrial RNAs from related animal species are probably due to two factors: As with mitochondrial RNA from ascomycetes (see previous section), the electrophoretic mobility of the mitochondrial ribosomal-type RNA from animal tissues is very sensitive to ionic conditions and temperature used, as shown by the results of Groot et al. [71] with rat liver mitochondria (table 5). A second factor is the method used to calculate S_E. Some authors assume that a linear relation exists between the reciprocal of the electrophoretic mobility of the RNA and its sedimentation coefficient, rather than the log of its sedimentation coefficient, as would be pre-

Table 4
Properties of ribosomes from animal mitochondria.

	Toad (*Xenopus laevis* eggs)	Hamster (BHK-21 cells)	Rat (liver)	Man (HeLa cells)	Locust (*Locusta migratoria* muscle)
Sedimentation coefficient (S)*					
Ribosome	60	45–50	50–55	60	60
Large subunit	43	33		45	
Small subunit	32	25		35	
Nascent protein pulse-labelled in intact mitochondria associated with ribosome after lysis	+		+	+	+
Isolated ribosome active in protein synthesis	+				
"16–17 S" and "12–14 S" RNA isolated from large and small subunits, respectively	+	+		+	
References	42,59	61,62	55, 58; contrast 63–65	66, 67; contrast 68	69,70

* Relative to *E. coli* ribosomes = 70 S with the exception of the toad values for which the cell-sap ribosomes (87 S) were used as reference.

dicted on theoretical grounds*. As a consequence the S_E of the smaller ribosomal-type RNA is underestimated in some papers.

Although a definite assignment of molecular weight to the mitochondrial ribosomal-type RNAs will require conformation-independent methods of analysis, it is likely that the apparent molecular weights determined by electrophoresis at 2° are closer to the true molecular weight than those determined at room temperature, when the RNA is apparently much more unfolded than the cell-sap ribosomal RNAs used as reference RNAs. It follows that the "mini-ribosome" indeed contains mini-RNAs. Whether the mini-RNAs from different animals differ significantly in size cannot be decided from the data available.

The results presented in tables 4 and 5 are fully compatible with the speculation [42] that mitochondrial mini-ribosomes will be found in all animal cells that contain 5 μm M-DNA circles [80]. A number of experimental observations do not seem to fit into this simple picture, however.

80 S (and in one case also 70 S) ribosomes have been obtained from mitochondrial preparations of rodent tissues [56, 63–65]. Saccone and Gadaleta [65] recently reported that these 80 S ribosomes contain high-molecular weight mitochondrial RNA, hybridizable with mitochondrial DNA, whereas the 55 S particles also present in their mitochondrial lysates only contained degraded RNA. In contrast to these authors, we do not think that this observation disproves that the 55 S particle is a complete ribosome. The 80 S region may contain small polysomes, 55 S ribosomes still attached to membrane fragments, aggregated ribosomes or subunits, etc. The critical experiment will be to strip the 80 S material of tRNA, mRNA, membrane and other extraneous proteins and see whether there are still 80 S particles containing mitochondrial RNA left following this treatment.

Penman and coworkers [68, 76] have obtained results with HeLa cell mitochondria that differ some-

* $s = a M^b$, so log s is a linear function of log M; the electrophoretic mobility of a RNA is a linear function of $(\log M)^{-1}$ [78, 79] and therefore also of $(\log s)^{-1}$.

Table 5
Characteristics of ribosomal-type RNA from animal mitochondria.

	Animal studied					
	Toad (*Xenopus laevis* eggs)	Rat (liver)	Mouse (L-cells)	Hamster (BHK-21 cells)	Man (HeLa cells)	Locust (*Locusta migratoria* muscle)
Sedimentation coefficient (S)*	18–19+13	16+13	16+13	17+13	16+12	
Electrophoretic mobility** (see text)						
S_E	21+13	17+13 (2°) 21+15 (29°)	21+12			
Apparent mol. wt.($\times 10^{-6}$)	0.95+0.4	0.65+0.36 (2°) 0.95+0.50 (29°)	0.96+0.3 0.79+0.45	0.75+0.42	0.7+0.4	0.5+0.25
Approximate molar ratio of the two RNA components	1:1	1:1	1:1	1:1	1:1?	?
Base composition (mole % GC)***	41?	46.5??	37?	38?	45?	
Degree of methylation				absent	1:100?	
RNA components identified by:						
Extraction from biologically active ribosomes	+					
Extraction from ribosome-like particles and/or their subunits	+			+	+	
Incorporation of radioactive precursors in presence of actinomycin			+	+	+	
U.V. profile or total radioactivity after long-term labelling	+	+			+	+
Hybridization with M-DNA	+	+			+	
References	42, 59	71–73	62, 72	61, 62, 74, 75	66; contrast 76	69, 77

* Values calculated in relation to cell-sap ribosomal RNA components.

** At room temperature (conditions not always specified, however) with the exception of rat liver RNA that was run at the temperatures specified in brackets.

*** In no case were purified ribosomes used as a source of the RNA. The ribosomal-type RNA could, therefore, be contaminated by stable mRNA and by some cell-sap ribosomal RNA.

what from those presented in tables 4 and 5:

a) After pulse-labelling mitochondria with radioactive amino acids *in vivo*, the nascent protein in the mitochondrial lysate was associated with a structure sedimenting around 95 S and with a buoyant density in CsCl of 1.40 g/ml (cell-sap ribosomes 1.55 g/ml). Mild treatment with pancreatic ribonuclease converted the 95 S structure into 55 S particles, whereas treatment with EDTA caused it to sediment at 35 S, without releasing the nascent protein, however. The authors [68] point out that the 95 S structure may represent a 55 S polysome and we may add that the tenacious binding of nascent protein even in the presence of EDTA and the low buoyant density of the 95 S structure could be due to the association of the 55 S ribosome with membrane fragments. Therefore, these results are not necessarily in contradiction with those presented in table 4.

b) After labelling HeLa cells with ^{3}H-uridine for periods up to 4 hr the two ribosomal-type RNAs were equally labelled indicating the synthesis of 2–3 times more of the smaller than of the larger component [76]. The recent results of Dubin and Czaplicki [75] and of Attardi et al. [66] indicate, however, that this is apparently due to a faster turnover of the smaller component. In cultures labelled continuously for longer periods the larger component contains about twice the radioactivity of the smaller component.

c) Whereas Attardi et al. find significant methylation of mitochondrial ribosomal-type RNA in HeLa cells (table 5), virtually no methylation was found by Vesco and Penman [76] in the same cells using similar techniques and by Dubin and coworkers (table 5) in hamster cells. We cannot provide an explanation for this discrepancy, but we would like to stress [cf. 42] that there is no a priori reason why rRNA should be methylated.

In summary then, we think that the available evidence strongly supports the idea that animal mitochondria contain 55 S ribosomes. Alternative interpretations would require far-fetched assumptions, e.g. that the 55 S particles consist of a mixture of two types of subunits of a larger ribosome and that the two small RNA components are reproducible and specific partial degradation products of larger RNAs [cf. 81]. To completely exclude this alternative it will be necessary to obtain mitochondrial protein synthesis with highly purified "43" and "32" S subunits, each containing only one type of RNA.

The expression mini-ribosome implies that the low sedimentation coefficient of these ribosomes is due to their small size and not to an unusual shape or a high protein to RNA ratio. Although this interpretation is reasonable in view of the small RNA components obtained from these ribosomes it remains to be formally proven.

4. Mitochondrial ribosomes from *Tetrahymena* and *Euglena*

An 80 S ribosome was identified in mitochondrial lysates of *Tetrahymena pyriformis* by Chi and Suyama [82–84]. These ribosomes could be distinguished from their cell-sap counterparts on three accounts:

a) Lowering the Mg^{2+} concentration to 10^{-4} M led to a dissociation of cell-sap ribosomes into 60 and 40 S subunits. Mitochondrial ribosomes did not dissociate at this Mg^{2+} concentration; EDTA was required to convert them into particles sedimenting at 55 S.

b) After fixation with formaldehyde mitochondrial ribosomes had an equilibrium density in CsCl of 1.45 g/cm^3 against a density of 1.56 for cell-sap ribosomes.

c) Whereas cell-sap ribosomes yielded 26 and 17 S RNA hybridizable with nuclear DNA, mitochondrial ribosomes and the 55 S "subunit" yielded major peaks at 21 and 14 S, both specifically hybridizing with mitochondrial DNA. The ratio of the radioactivity in the 21 and 14 S components approached 2:1 in some experiments (table 6).

The results obtained by Suyama with cell-sap ribosomes of *Tetrahymena* are in good agreement with those of others [87]. We hesitate, however, to accept his conclusion that the 80 S particles isolated from *Tetrahymena* represent the mitochondrial ribosomes of this organism. The high absorbancy ratios 230/260 nm and 280/260 nm of these ribosomes [83] suggest to us that they were heavily contaminated with membrane fragments and that this contamination could be responsible for the anomalously low density in CsCl. Membrane attachment might also lead to the anomalous resistance of these ribosomes to dissociation at low Mg^{2+} concentrations and to an abnormally high sedimentation coefficient. It seems therefore possible that the true sedimentation coefficient of *Tetrahymena* mitochondrial ribosomes is 70 S or lower and that

Table 6
Characteristics of mitochondrial ribosomal-type RNA from *Tetrahymena* and *Euglena*.

	Tetrahymena pyriformis	*Euglena gracilis*
Sedimentation coefficient (S)*	21+14 (26+17)	14+11 (25+19)
Electrophoretic mobility (at 6°)*		
S_E	20+15 (27+18)	
Apparent mol. wt. ($\times 10^{-6}$)	0.82+0.52 (1.41+0.66)	
Approximate molar ratio of the two components	1:1	?
Isolated from ribosomes	+	
Mole percent GC		
Large component	27.9 (43.2)	27.4 (54.4)
Small component	30.6 (49.2)	
Hybridization with M-DNA	+	
Reference	82–85	86

* Calculated as in table 3; with *Euglena* the *E. coli* reference rRNA was taken as 23 and 18 S.

these ribosomes may even represent fore-runners of the mini-ribosome of animal tissues. This hypothesis would account both for the low molecular weight of the RNA components derived from these ribosomes and for the fact that thin sections of *Tetrahymena* reveal smaller ribosomes in the mitochondria than in the cell sap [88].

Two major RNA species, with a low GC content, and unusually low S values, were extracted from *Euglena* mitochondria by Krawiec and Eisenstadt [86]. It remains to be shown that these RNA species were derived from mitochondrial ribosomes and that they represent undegraded RNAs.

5. Mitochondrial 5 S RNA

Ribosomes from bacteria and eukaryotic cell-sap contain a 5 S RNA in addition to the two high-molecular weight components. The molecular weight of this RNA is about 40,000; it is not methylated; it is part of the large subunit and its function is unknown [see 89].

RNA components of low molecular weight have been found in a variety of mitochondria [59, 60, 66, 69, 74, 77, 83, 84, 90–93]. Generally, two major species have been distinguished by acrylamide gel electrophoresis.

1) "5 S" RNA; not methylated, synthesis inhibited by actinomycin but not by ethidium; not hybridizable with M-DNA. This component is lacking in mitochondrial preparations that are not significantly contaminated by cell-sap ribosomes and it is probably completely derived from these ribosomes.

2) "4 S" RNA*. The degree of methylation of this RNA is less than 50% of that of cell-sap "4 S" RNA; its synthesis is inhibited 50–80% by ethidium and is insensitive to actinomycin; it hybridizes with M-DNA. Probably this "4 S" RNA represents, at least in part, mitochondrial tRNA species.

In addition to these major components Knight [90, 91] has detected an unmethylated RNA component in HeLa cell mitochondria, that runs between the "4 S" and "5 S" RNA peaks in acrylamide gels. Since a large fraction of this RNA was not associated with ribosomes [90] it cannot be the mitochondrial equivalent of "5 S" RNA.

Inability to demonstrate a 5 S RNA in mitochondria may have one of two explanations:

* The "4 S" RNA of locust mitochondria runs significantly faster in acrylamide gels than cell-sap or *E. coli* 4 S RNA. The reason for this is not known. Mitochondrial "4 S" RNA from all other organisms studied co-migrates with cell-sap 4 S RNA.

1) Mitochondrial ribosomes do not contain a low-molecular weight RNA.

2) 5 S RNA does exist in mitochondria, but it escapes detection because it is smaller than other 5 S RNAs and therefore co-sediments and co-electrophoreses with mitochondrial tRNA. This could also explain the apparent undermethylation of mitochondrial "4 S" RNA [66, 74].

A careful analysis of the RNA components of the highly purified ribosomes from *Neurospora* or yeast that are now available, should make it possible to decide between these two alternatives.

6. Are mitochondrial ribosomes part of the inner membrane?

Linnane and coworkers [46, 94, 95] have recently raised the possibility that mitochondrial ribosomes are attached to the mitochondrial membrane and might even be an integral part of the membrane. Three arguments were brought forward to support this idea:

1) The ribosomes of yeast mitochondria are intimately associated with the organelle membranes; the difficulty of their separation by the use of detergents suggests a strong interaction [94].

2) The antibiotic-resistant mutants of yeast in which the resistance shows cytoplasmic inheritance, are of (at least) two types: In the first type involving erythromycin resistance (sometimes with cross-resistance to lincomycin and other macrolides), resistance is also observed in isolated mitochondria even after freezing and thawing [10]. The second type is exemplified by mutant L-3000 which shows resistance to an unusual series of antibiotics, mikamycin, chloramphenicol, lincomycin and carbomycin (but not erythromycin). Amino acid incorporation by mitochondria isolated from this type of mutant does not show resistance to any of these antibiotics. Moreover, when the mutant is grown anaerobically on a limited supply of unsaturated fatty acids and ergosterol, a procedure that strongly modifies the permeability properties of the mitochondrial membranes, induction of mitochondrial differentiation by O_2 has become sensitive to inhibition by mikamycin etc. Bunn et al. [94] suggest that resistance to antibiotics in the mikamycin-type mutants is due to a permeability barrier in the mitochondrial membrane which is lost when the cells lack unsaturated fatty acids and ergosterol, or when the membrane is damaged during isolation of the mitochondria, whereas mutation to erythromycin resistance is due to a change in a ribosomal protein. The authors further suggest that changes in the mitochondrial inner membrane and in mitochondrial ribosomal proteins could co-exist and "that interaction between them may occur" [94], the link apparently being that the ribosome is part of the membrane.

3) Some antibiotics that affect mitochondrial protein synthesis also affect oxidative phosphorylation. This might be due to a close spatial relation between ribosome and respiratory chain [95].

The proposal that mitochondrial ribosomes are attached to the membrane could indeed explain why it has been so difficult in many cases to isolate ribosomes from mitochondria. Attachment to membrane fragments could also explain the very low density of the mitochondrial ribosomes isolated from *Tetrahymena* and HeLa cells (see section 4). It should be stressed, however, that these results can also be explained by a non-specific aggregation of ribosomes with the mitochondrial inner membrane and that none of the arguments presented by Linnane for his proposal is conclusive. Electron micrographs of thin sections of mitochondria show free ribosomes but no ribosomes attached to or partly in membranes [88]. Even if the interpretation that resistance to antibiotics can arise either by a change in the mitochondrial inner membrane or by a change in a ribosomal protein proves to be correct, there is no reason to suspect that inner membrane and ribosome have any connection as long as no mutations have been found in which ribosome and membrane are changed simultaneously by one point mutation. Finally, the correlation between effects on protein synthesis and oxidative phosphorylation is not very compelling. In the case of chloramphenicol the inhibition of protein synthesis is seen at low (50 μg/ml) concentrations and completely specific for the D-isomer [3]; inhibition of respiration requires mg/ml concentrations and is not D-specific [see 96]. This clearly shows that the inhibition of protein synthesis and the inhibition of respiration are completely unrelated in this case.

Although we are not convinced by Linnane's arguments, none of the objections raised by us above disproves that the ribosomes are in the membrane or attached to it. The matter must be considered open.

7. The biosynthesis of mitochondrial ribosomes

Hybridization experiments indicate that mitochondrial ribosomal RNA is transcribed from mitochondrial DNA in all mitochondria analyzed (see tables 3, 5 and 6). Whether the RNA components are synthesized as such or in the form of precursor molecules is not yet known. In HeLa cells, however, brief pulses of radioactive uridine have revealed a large RNA that hybridizes with mitochondrial DNA. This RNA has the size expected for a complete transcript of mitochondrial DNA [66, 97]. It is possible that the ribosomal RNAs of animal cells are derived from such a gigantic precursor. It remains to be demonstrated, however, that the large RNA is a continuous polynucleotide chain containing the two ribosomal RNAs.

Three lines of evidence indicate that the proteins of mitochondrial ribosomes are synthesized outside the mitochondria on cell-sap ribosomes: When yeast is grown in the presence of chloramphenicol the synthesis of all cytochromes except cytochrome *c* stops, apparently because mitochondrial protein synthesis is blocked; nevertheless, complete ribosomes continue to be synthesized [12]. Similarly, incorporation of amino acids into *Neurospora* mitochondrial ribosomes is completely inhibited by cycloheximide but not affected by chloramphenicol [37, 40]. Finally, Linnane and coworkers [98] have recently observed that mitochondrial ribosomes are lost when yeast is grown anaerobically in the presence of a limiting supply of unsaturated fatty acids and ergosterol. This loss is reversible, again indicating that the proteins of mitochondrial ribosomes can be made in the absence of a functional mitochondrial system for protein synthesis.

The discovery that resistance to erythromycin in yeast shows cytoplasmic inheritance [10, 11, 15, 16, 99] and that the mutation to resistance appears to involve a change in the ribosome itself [10] complicates the simple picture presented in the preceding paragraph. Since resistance to erythromycin in bacteria is associated with a change in a ribosomal protein [100], it is not unreasonable to think that a similar explanation holds in the case of mitochondria. The cytoplasmic inheritance of the resistance would then imply that the structural gene for this ribosomal protein is located on mitochondrial DNA. If the ribosomal proteins are synthesized on cell-sap ribosomes one is forced to assume that the mRNA for these proteins is exported into the cell-sap and translated on cell-sap ribosomes and that the proteins made are reimported into mitochondria [12]. It is hard to believe that nature would stoop to such a clumsy arrangement and fortunately other explanations are still open. It is possible that the protein involved in erythromycin resistance is synthesized on mitochondrial ribosomes in contrast to the bulk of the mitochondrial ribosomal proteins and that even in the presence of chloramphenicol [cf. 101] or in cells grown anaerobically on limiting fatty acid supply (see above) sufficient protein-synthesizing capacity remains in mitochondria to make the proteins essential for the continuity of the mitochondrial genetic system. A second possibility is that even the erythromycin mutants are membrane mutants and not due to a change in the ribosome itself (see preceding section). This should be tested in the submitochondrial protein-synthesizing system now available (see table 2). Finally, the erythromycin resistance of these mutants might be caused by a change in the ribosomal RNA (which *is* coded for by the mitochondrial DNA) rather than to a change in a ribosomal protein [102], since it is not inconceivable that a change in the structure of rRNA could also lead to a change in the binding of an antibiotic to the ribosome.

The last two possibilities, which we prefer for reason of simplicity, imply that it should be possible to obtain nuclear mutations that result in the synthesis of antibiotic-resistant mitochondrial ribosomes. These have not been identified as yet.

8. How bacterial are mitochondrial ribosomes?

8.1. *Size and composition*

Neither in the ascomycetes nor in the animal tissues so far studied has the mitochondrial ribosome turned out to be a particle sedimenting at about 70 S. Mitochondrial ribosomes from ascomycetes sediment significantly faster than *E. coli* ribosomes (table 1), whereas animal ribosomes sediment much slower (table 4). The meagre indirect evidence available (sections 2 and 3) suggests that these differences in sedimentation coefficient reflect differences in size. Mitochondrial rRNAs differ from host bacterial rRNAs in the degree of methylation, G + C content, compactness after isolation and probably size; 5 S RNA, if present at all in mitochondrial ribosomes,

must be smaller than its bacterial counterpart. Hence, as far as size and composition is concerned, mitochondrial ribosomes cannot be considered true "bacterial-type" ribosomes.

8.2. *Response to antibiotics*

As mentioned in the Introduction, mitochondrial protein synthesis is inhibited by the major classes of antibiotics that interfere with bacterial protein synthesis at the ribosomal level without affecting cell-sap ribosomes of eukaryotes. Conversely, mitochondrial protein synthesis is insensitive to the specific inhibitors of cell-sap ribosomes, cycloheximide, emetin and anisomycin.

Two questions remains to be discussed. Are there antibiotics that inhibit mitochondrial protein synthesis without affecting bacterial protein synthesis; and, are there phylogenetic differences in the response of mitochondrial protein synthesis to antibiotics?

In regard to the first question, Haslam et al. [8] have reported that the phenanthrene alkaloids, cryptopleurine, tylocrebrine and tylophorine can be used to distinguish the ribosomes from yeast cell sap, yeast mitochondria and *E. coli.* Low concentrations of the alkaloids inhibit cell-sap protein synthesis, higher concentrations mitochondrial protein synthesis, whereas the *E. coli* ribosomes are relatively insensitive to these drugs. Although this differential effect could be due to differences in the ribosomes, as Haslam et al. suggest, alternative explanations were not excluded. First, it remains to be shown that these drugs do not interfere in an indirect way with mitochondrial protein synthesis, e.g. by affecting ATP production or amino acid transport; and, second, the apparently irreversible binding of these antibiotics to ribosomes raises the question whether the degree of inhibition is affected by the ribosome concentration in the assay. This might have been much higher in the analysis of *E. coli* ribosomes than of yeast mitochondria.

The question whether phylogenetic differences exist in the response to antibiotics was also raised by Linnane and coworkers [13, 103]. They found that intact and sonicated rat liver mitochondria were not inhibited by erythromycin, lincomycin, paromomycin and the neomycins B and C, antibiotics that readily inhibit protein synthesis by isolated yeast mitochondria. In contrast to these results Kroon and De Vries [18, 20] have clearly shown that mild swelling of rat liver mitochondria renders them susceptible to both erythromycin and lincomycin inhibition. There is little doubt, therefore, that the resistance of intact rat liver mitochondria to these antibiotics is due to a permeability barrier and not to a change in mitochondrial ribosomes in the course of evolution, as postulated by Firkin and Linnane [103]. A similar permeability barrier could explain the resistance of liver mitochondria to paromomycin and the neomycins.

In summary, no single difference has yet been found in the response of mitochondrial and bacterial ribosomes to a wide variety of antibiotics.

8.3. *Partial reaction of protein synthesis*

a. Initiation. It seems likely that bacteria and mitochondria share a common type of initiation process in protein synthesis. Formylmethionyl-tRNA has been found in mitochondria from yeast, *Neurospora,* rat liver and HeLa cells [104–106]; transformylase activity has been detected in *Neurospora* mitochondria [105]. Mitochondrial ribosomes from *Neurospora* are able to recognize, bind and translocate *E. coli* formylmethionyl-tRNA in response to the codon AUG [107]. The translocation step is sensitive to chloramphenicol and sparsomycin. Formylmethionyl-tRNA binding activity is lost after washing of the mitochondrial ribosomes with 1 M ammonium chloride, a procedure which removes initiation factors from *E. coli* ribosomes. Activity is restored by the addition of *E. coli* initiation factors. In control incubations cell-sap ribosomes from *Neurospora* bound only low amounts of formylmethionyl-tRNA and *E. coli* initiation factors produced negligible stimulation. Mitochondrial ribosomes can thus interact specifically with the bacterial initiation factors F_1 and F_2. It is not yet known whether the similarity between mitochondrial and bacterial ribosomes is high enough to allow the factor F_3-mediated translation of a natural bacterial mRNA by mitochondrial ribosomes.

b. Peptide chain elongation. Mitochondrial ribosomes from yeast and *Neurospora* exhibit activity in poly-U directed phenylalanine incorporation when combined with a bacterial supernatant fraction (see table 2); eukaryotic 80 S ribosomes do not [see 108]. Bacterial peptide-chain elongation factors are thus apparently interchangeable with mitochondrial but not

cell-sap factors. Similar conclusions were implied by Ciferri and Parisi to account for the presence of 70 S ribosome-specific Tu and G activities in whole cell extracts of yeast and rat liver [108]. Recent work has partially confirmed this [32, 109]. T and G factors, purified from yeast and *Neurospora* mitochondria by methods used in the preparation of bacterial factors, were tested for their ability to support polymerisation of phenylalanine on *E. coli* ribosomes when combined with the complementary *E. coli* factors. The combination Tu (yeast) + G (*E. coli*) is active [32], as is T (*E. coli*) + G (*Neurospora*) [109]; T (*Neurospora*) + G (*E. coli*) is not active, apparently because of the lability of the mitochondrial T factors [109]. For complete proof of factor interchangeability it is therefore still necessary to show that mitochondrial ribosomes from which T and G factors have been completely removed are still active in phenylalanine incorporation when combined with bacterial factors.

Incomplete removal of elongation factors from ribosomes may account for observations that yeast cell-sap ribosomes are active in protein synthesis when supplemented with *E. coli* supernatant enzymes [e.g. 8, 110]. The same explanation may hold for the apparent interchangeability of mitochondrial and cell-sap components in *Neurospora* when synthesis of polyphenylalanine is measured with phenylalanyl-tRNA rather than with free phenylalanine [41].

The results presented in this section, show that the functional similarities between mitochondrial ribosomes from ascomycetes and bacterial ribosomes are very striking, as expected already on the basis of the similar response to antibiotics interfering with various phases of protein synthesis. Similar experiments on the interchangeability of bacterial and mitochondrial components in protein synthesis remain to be done with animal ribosomes.

9. Concluding remarks

Although knowledge of mitochondrial ribosomes has rapidly increased in the last 3 years, it is clear from this review that firm facts are still few in this field. The following points, however, appear reasonably established:

1) Mitochondria from three ascomycetes contain "73 S" ribosomes sedimenting slightly faster than ribosomes from *E. coli*.

2) Animals, varying from man to locust, contain 55–60 S mitochondrial mini-ribosomes with mini-rRNAs.

3) "5 S" RNA is either lacking in mitochondrial ribosomes or it is smaller than the "5 S" RNA found in all other ribosomes.

4) Mitochondrial ribosomes are functionally similar to bacterial ribosomes. This follows not only from the close similarity between mitochondrial and bacterial protein synthesis in its response to antibiotics, which can be considered as probes of different phases of ribosome function, but also from the interchangeability between initiation and elongation factors from *E. coli* and fungal mitochondria.

5) Mitochondrial rRNA is a gene product of mitochondrial DNA. Probably the proteins of mitochondrial ribosomes are gene products of nuclear DNA and synthesized on cell-sap ribosomes. There is no evidence that cell-sap and mitochondrial ribosomes have any protein in common.

6) The catalytic activity of mitochondrial ribosomes in the poly-U directed synthesis of polyphenylalanine is in the same order of magnitude as that of *E. coli* ribosomes.

This meagre list already shows how much remains to be done. Apart from the many problems discussed earlier in this review, three major problems can be mentioned:

1) The large difference in size between mitochondrial ribosomes from animals and ascomycetes – if real – is intriguing. It could be due to a separate evolutionary origin or to a rapid evolution of the mitochondrial ribosome, imposed by the same evolutionary pressure that has also reduced the size of mitochondrial DNA from 25 μm in yeast to 5 μm in animal cells [see 102]. In either case it would be of interest to examine the mitochondria of a much wider range of eukaryotes, especially higher plants and other classes of unicellular organisms. It is possible that even smaller ribosomes than those of animal mitochondria would be found. Detailed analysis of these mini-ribosomes might establish the minimal requirements for ribosomal function and might provide clues to the advantages – in catalytic efficiency or regulatory flexibility – of making larger ribosomes.

2) The similarity in response of mitochondrial and bacterial ribosomes to antibiotics that affect ribosomal function raises two questions. The first question is whether the inhibition of mitochondrial protein synthesis could explain some of the toxic side effects of these antibiotics [18, 21, 28]. Since chloramphenicol can readily be shown to interfere with mitochondrial biogenesis in intact cells, both in yeast [28] and mammalian cells [18, 111, 112], it is conceivable that long-term chloramphenicol treatment of patients could lead to a shortage of mitochondria in rapidly growing cells. Important in this context is Kroon's discovery that the resistance of rat mitochondrial protein synthesis to inhibition by erythromycin is due to the impermeability of the mitochondrial membrane to this drug [18, 20]. It might be possible to develop chloramphenicol analogues that cannot penetrate the mitochondrial matrix space and these might be less toxic than the parent compound. This line of investigation is actively pursued by Kroon's group.

As second question, raised by the antibiotic results, one can ask why mitochondrial ribosomes are functionally so similar to bacterial ribosomes that they are inhibited by all main classes of antibiotics that interfere with bacterial ribosomal function. Why should mitochondrial ribosomes have maintained this sensitivity to antibiotics, while cell-sap ribosomes have either never possessed it or have lost it during evolution? The question is all the more puzzling because, as we have already pointed out, there appear to be no phylogenetic differences between mitochondrial ribosomes from various sources in their response to many antibiotics, despite differences in size and composition. We cannot see how sensitivity to chloramphenicol etc. would convey a special evolutionary advantage to the ribosome that possesses it. Hence, sensitivity to antibiotics must reflect a fundamental property of the molecular architecture of the ribosome and severe restrictions, dictated by the functional requirements of a protein-synthetic system employing tRNA and mRNA, prevent it from changing. More specifically, this implies that antibiotic-resistant ribosomes are inferior ribosomes, unless a drastic change in the over-all construction is made, as in all cell-sap ribosomes. Although in some cases antibiotic-resistant ribosomes have been shown to be inferior catalysts [cf. 26, 100, 113] or assembly defective at low temperature [114], a more detailed analysis of ribosome function would be necessary to verify whether this is a general phenomenon.

3) In spite of intensive study the proteins synthesized on the mitochondrial ribosomes discussed in this review have eluded identification. There is no doubt that these proteins are essential for mitochondrial biosynthesis and that they represent only a small fraction of all mitochondrial proteins, because only about 10% of the amino acid incorporation into mitochondrial proteins is insensitive to cycloheximide and inhibited by chloramphenicol [23, 115–118]. All attempts, however, to demonstrate incorporation of amino acids into a well-defined mitochondrial enzyme by isolated mitochondria or intact cells in the presence of cyclohemixide have failed [see 119–121]. Only the insoluble "structural (?)" proteins of the inner membrane become labelled. At present it seems likely, therefore, that only a limited number of inner membrane proteins, possibly including some of the mitochondrial inner membrane translocators or permeases, are synthesized on mitochondrial ribosomes. We expect that the discovery of cytoplasmic mutations affecting permeability to antibiotics in yeast, may provide a way to further characterize these proteins.

Acknowledgements

We thank Mr. C. Aaij and Dr. A.M. Kroon for helpful comments. Dr. L.A. Grivell is grateful to the Royal Society of Great Britain for the award of a European Programme Fellowship. Unpublished work by the authors, quoted in this paper, was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research and (in part) by The Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from The Netherlands Organisation for the Advancement of Pure Research (Z.W.O.).

Note added in proof

Electron microscopy of RNA spread under strongly denaturing conditions, has recently been used to estimate the molecular weights of HeLa cell mitochondrial ribosomal RNA species (D.L. Robberson, N. Davidson, Y. Aloni and G. Attardi, personal communication).

Values obtained with rat liver 18 S rRNA as standard (0.71×10^6) were 0.56 and 0.36×10^6.

References

[1] J. Mager, Biochim. Biophys. Acta 38 (1960) 150.
[2] R.Rendi, Exptl. Cell Res. 17 (1959) 585.
[3] A.M. Kroon, in: Atti del Seminario di Studi Biologici, Vol. 4, ed. E. Quagliariello (Adriatica Editrice, Bari, Italy, 1969) p. 9.
[4] M.W. Gordon and G.G. Deanin, J. Biol. Chem. 243 (1968) 4222.
[5] I.G. Morgan, FEBS Letters 10 (1970) 273.
[6] D. Haldar, Biochem. Biophys. Res. Commun. 40 (1970) 129.
[7] G.D. Clark-Walker and A.W. Linnane, Biochem. Biophys. Res. Commun. 25 (1966) 8.
[8] J.M. Haslam, P.J. Davey, A.W. Linnane and M.R. Atkinson, Biochem. Biophys. Res. Commun. 33 (1968) 368.
[9] A.J. Lamb, G.D. Clark-Walker and A.W. Linnane, Biochim. Biophys. Acta 161 (1968) 415.
[10] A.W. Linnane, A.J. Lamb, C. Christodoulou and H.B. Lukins, Proc. Natl.Acad. Sci. U.S. 59 (1968) 1288.
[11] A.W. Linnane, G.W. Saunders, E.B. Gingold and H.B. Lukins, Proc. Natl. Acad. Sci. U.S. 59 (1968) 903.
[12] P.J. Davey, R.Yu and A.W. Linnane, Biochem. Biophys. Res. Commun. 36 (1969) 30.
[13] P.J. Davey, J.M. Haslam and A.W. Linnane, Arch. Biochem. Biophys. 136 (1970) 54.
[14] D.Wilkie, G.W. Saunders and A.W. Linnane, Genet. Res. Camb. 10 (1967) 199.
[15] D.Y. Thomas and D. Wilkie, Biochem. Biophys. Res. Commun. 30 (1968) 368.
[16] D. Wilkie, in: Control of Organelle Development, ed. P.L. Miller (Cambridge University Press, Cambridge, 1970) p. 71.
[17] A.M. Kroon and H. De Vries, FEBS Letters 3 (1969) 208.
[18] A.M. Kroon and H. De Vries, in: Control of Organelle Development, ed. P.L. Miller (Cambridge University Press, Cambridge, 1970) p. 181.
[19] H. De Vries and A.M. Kroon, Biochim. Biophys. Acta 204 (1970) 531.
[20] A.M. Kroon and H. De Vries, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam, 1971) in press.
[21] P. Borst, A.M. Kroon and G.J.C.M. Ruttenberg, in: Genetic Elements: Properties and Function, ed. D. Shugar (Academic Press and Polish Scientific Publishers, London and Warsaw, 1967) p. 81.
[22] D.S. Beattie, R.E. Basford and S.B. Koritz, Biochemistry 6 (1967) 3099.
[23] W. Sebald, Th. Hofstötter, D. Hacker and Th. Bücher, FEBS Letters 2 (1969) 177.
[24] S. Perlman and S. Penman, Biochem. Biophys. Res. Commun. 40 (1970) 941.
[25] H. De Vries, E. Agsteribbe and A.M. Kroon, personal communication.
[26] B. Weisblum and J. Davies, Bacteriol. Rev. 32 (1968) 493.
[27] D. Vazquez, Biochim. Biophys. Acta 114 (1966) 289.
[28] M. Huang, D.R. Biggs, G.D. Clark-Walker and A.W. Linnane, Biochim. Biophys. Acta 114 (1966) 434.
[29] L.A. Grivell, unpublished observations.
[30] W.J. Stegeman, C.S. Cooper and C.J. Avers, Biochem. Biophys. Res. Commun. 39 (1970) 69.
[31] R. Kaempfer, Nature 222 (1969) 950.
[32] H. Morimoto, A.H. Scragg, J. Nekhorocheff, V. Villa and H.O. Halvorson, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam, 1971) in press.
[33] H. Schmitt, FEBS Letters 4 (1969) 234.
[34] H. Küntzel and H. Noll, Nature 215 (1967) 1340.
[35] H. Noll, in : Control of Organelle Development, ed. P.L. Miller (Cambridge University Press, Cambridge, 1970) p. 419.
[36] H. Küntzel, J. Mol. Biol. 40 (1969) 315.
[37] W. Neupert, W. Sebald, A.J. Schwab, A.Pfaller, P. Massinger and Th. Bücher, European J. Biochem. 10 (1969) 585; 589.
[38] M.R. Rifkin, D.D. Wood and D.J.L. Luck, Proc. Natl. Acad. Sci. U.S. 58 (1967) 1025.
[39] M. Edelman, I.M. Verma and U.Z. Littauer, J. Mol. Biol. 49 (1970) 67.
[40] H. Küntzel, Nature 222 (1969) 142.
[41] H. Küntzel, FEBS Letters 4 (1969) 140.
[42] R.F. Swanson and I.B. Dawid, Proc. Natl. Acad. Sci. U.S. 66 (1970) 117.
[43] M. Fauman, M. Rabinowitz and G.S. Getz, Biochim. Biophys. Acta 182 (1969) 355.
[44] L.A. Grivell, L. Reijnders and P. Borst, European J. Biochem. (1971) in press.
[45] P.J. Rogers, B.N. Preston, E.B. Titchener and A.W. Linnane, Biochem. Biophys. Res. Commun. 27 (1967) 405.
[46] I.T. Forrester, P. Nagley and A.W. Linnane, FEBS Letters 11 (1970) 59.
[47] S.A. Leon and H.R. Mahler, Arch. Biochem. Biophys. 126 (1968) 305.
[48] E. Wintersberger, Z. Physiol. Chem. 348 (1967) 1701.
[49] P.V. Vignais and J. Huet, Biochem. J. 116 (1970) 26P.
[50] A. Steinschneider, Biochim. Biophys. Acta 186 (1969) 405.
[51] L.S. Dure, J.L. Epler and W.E. Barnett, Proc. Natl. Acad. Sci. U.S. 58 (1969) 1883.
[52] R. Herzog, M. Edelman, E. Galun, I.M. Verma and U.Z. Littauer, Israel J. Chem. 8 (1970) 123 p.
[53] I.M. Verma, M. Edelman, M. Herzberg and U.Z. Littauer, J. Mol. Biol. 52 (1970) 137.
[54] N. Granboulan and K. Scherrer, European, J. Biochem. 9 (1969) 1.

[55] T.W.O'Brien and G.F. Kalf, J. Biol. Chem. 242 (1967) 2172; 2180.
[56] T.W.O'Brien, Federation Proc. 28 (1969) 885.
[57] M.A. Ashwell and T.S. Work, Biochem. J. 116 (1970) 25P.
[58] M.A. Ashwell and T.S. Work, Biochem. Biophys. Res. Commun. 39 (1970) 204.
[59] I.B. Dawid, in: Control of Organelle Development, ed. P.L. Miller (Cambridge University Press, Cambridge, 1970) p. 227.
[60] E. Zylber, C. Vesco and S. Penman, J. Mol. Biol. 44 (1969) 195.
[61] B.S. Montenecourt and D.T. Dubin, Biochem. Biophys. Res. Commun. 41 (1970) 458.
[62] B.S. Montenecourt, M.E. Langsam and D.T. Dubin, J. Cell Biol. 46 (1970) 245.
[63] D.E.S. Truman, Exptl. Cell. Res. 31 (1963) 313.
[64] I.R. Elaev, Biokhimiya 31 (1966) 234.
[65] C. Saccone and M.N. Gadaleta, Abstr. 8th Intern. Congr. Biochem., Switzerland, 1970, p. 185.
[66] G. Attardi, Y. Aloni, B. Attardi, D. Ojala, L. Pica-Mattoccia, D.L. Robberson and B. Storrie, Cold Spring Harbor Symp. Quant. Biol. 35 (1970) in press.
[67] G. Attardi and D. Ojala, Nature, submitted for publication.
[68] S. Perlman and S. Penman, Nature 227 (1970) 133.
[69] W. Kleinow, W. Sebald, W. Neupert and Th. Bücher, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam, 1971) in press.
[70] W. Kleinow, W. Neupert and Th. Bücher, FEBS Letters 12 (1971) 129.
[71] P.H.E. Groot, C. Aaij and P. Borst, Biochem. Biophys. Res. Commun. 41 (1970) 1321.
[72] B. Bartoov, R.S. Mitra and K.B. Freeman, Biochem. J. 120 (1970) 455.
[73] C. Aaij, personal communication.
[74] D.T. Dubin and B.S. Montenecourt, J. Mol. Biol. 48 (1970) 279.
[75] D.T. Dubin and S.M. Czaplicki, Biochim. Biophys. Acta 224 (1971) 663.
[76] C. Vesco and S. Penman, Proc. Natl. Acad. Sci. U.S. 62 (1969) 218.
[77] W. Kleinow and W. Neupert, Z. Physiol. Chem. 351 (1970) 1205.
[78] D.H.L. Bishop, J.R. Claybrook and S. Spiegelman, J. Mol. Biol. 26 (1967) 373.
[79] U.E. Loening, Biochem. J. 113 (1969) 131.
[80] P. Borst and A.M. Kroon, Intern. Rev. Cytol. 26 (1969) 108.
[81] B. Marrs and S. Kaplan, J. Mol. Biol. 49 (1970) 297.
[82] J.C.H. Chi and Y. Suyama, Bacteriol. Proc. (1968) 129.
[83] Y. Suyama, in: Atti del Seminario di Studi Biologici, Vol. 4, ed. E. Quagliariello (Adriatica Editrice, Bari, Italy, 1969) p. 83.
[84] J.C.H. Chi and Y. Suyama, J. Mol. Biol. 53 (1970) 531.
[85] L. Reijnders, personal communication.
[86] S. Krawiec and J.R. Eisenstadt, Biochim. Biophys. Acta 217 (1970) 120; 132.
[87] V. Leick and P. Plesner, Biochim. Biophys. Acta 169 (1968) 398.
[88] H. Swift, M. Rabinowitz and G. Getz, in: Biochemical Aspects of the Biogenesis of Mitochondria, eds. E.C. Slater, J.M. Tager, S. Papa and E. Quagliariello (Adriatica Editrice, Bari, Italy, 1968) p. 3.
[89] D.G. Comb and T. Zehavi-Willner, J. Mol. Biol. 23 (1967) 441.
[90] E. Knight Jr., Biochemistry 8 (1969) 5089.
[91] E. Knight Jr. and T. Sugiyama, Proc. Natl. Acad. Sci. U.S. 63 (1969) 1383.
[92] W. Neupert, P. Massinger and A. Pfaller, in: Autonomy and Biosynthesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam, 1971) in press.
[93] C.Aaij and P. Borst, Biochim. Biophys. Acta 217 (1970) 560.
[94] E.L. Bunn, C.H. Mitchell, H.B. Lukins and A.W. Linnane, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1233.
[95] A.W. Linnane and G.M. Kellerman, Reported at Proc. 8th Intern. Congr. Biochem., Switzerland, 1970. (Available only on video tape).
[96] K.B. Freeman, Can. J. Biochem. 48 (1970) 469.
[97] G. Attardi, G.Y. Aloni, B. Attardi, M. Lederman, D. Ojala, L. Pica-Mattoccia and B. Storrie, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland Amsterdam, 1971) in press.
[98] A.W. Linnane, personal communication.
[99] D. Coen, J. Deutsch, P. Netter, E. Petrochilo and P.P. Slonimski, in: Control of Organelle Development, ed. P.L. Miller (Cambridge University Press, Cambridge, 1970) p. 449.
[100] E. Otaka, H. Teraoka, M.J. Tamaki, K. Tanaka and S. Osawa, J. Mol. Biol. 48 (1970) 499.
[101] P.O. Weislogel and R.A. Butow, Proc. Natl. Acad. Sci. U.S. 67 (1970) 52.
[102] P. Borst, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam, 1971) in press.
[103] F.C. Firkin and A.W. Linnane, FEBS Letters 2 (1969) 330.
[104] A.E. Smith and K.A. Marcker, J. Mol. Biol. 38 (1969) 241.
[105] J.L. Epler, L.R. Shugart and W.E. Barnett, Biochemistry 9 (1970) 3575.
[106] J.B. Galper and J.E. Darnell, Biochem. Biophys. Res. Commun. 34 (1969) 205.
[107] F. Sala and H. Küntzel, European J. Biochem. 15 (1970) 280.
[108] O. Ciferri and B. Parisi, in: Progress in Nucleic Acid Research and Molecular Biology, Vol. 10, eds. J.N. Davidson and W.E. Cohn (Academic Press, New York, 1970) p. 121.
[109] M. Grandi and H. Küntzel, FEBS Letters 10 (1970) 25.
[110] A.G. So and E.W. Davie, Biochemistry 2 (1963) 132.

[111] F.C. Firkin and A.W. Linnane, Biochem. Biophys. Res. Commun. 32 (1968) 398.
[112] A.M. Kroon and R.J. Jansen, Biochim. Biophys. Acta 155 (1968) 629.
[113] D. Schlessinger and D. Apirion, Ann. Rev. Microbiol. 23 (1969) 387.
[114] H. Nashimoto and M. Nomura, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1440.
[115] W. Sebald, A.J. Schwab and Th. Bücher, FEBS Letters 4 (1969) 243.
[116] W. Sebald, G.D. Birkmayer, A.J. Schwab and H. Weisz, in: Autonomy and Biogenesis of Mitochondria and Chloroplasts, eds. N.K. Boardman, A.W. Linnane and R.M. Smillie (North-Holland, Amsterdam, 1971) in press.
[117] R. Schweyen and F. Kaudewitz, Biochem. Biophys. Res. Commun. 38 (1970) 728.
[118] E.S. Hawley and J.W. Greenawalt, J. Biol. Chem. 245 (1970) 3574.
[119] M. Rabinowitz and H. Swift, Physiol. Rev. 50 (1970) 376.
[120] M. Ashwell and T.S. Work, Ann. Rev. Biochem. 39 (1970) 251.
[121] D.S. Beattie, G.M. Patton and R.N. Stuchell, J. Biol. Chem. 245 (1970) 2177.