Horizons in Biochemistry and Biophysics

Volume 3

E. QUAGLIARIELLO, Editor-in-Chief **F. PALMIERI,** Managing Editor
Department of Biochemistry, University of Bari

Thomas P. Singer, Consulting Editor
University of California, San Francisco, School of Medicine
Veterans Administration Hospital, San Francisco

1977

Addison-Wesley Publishing Company
Advanced Book Program
Reading, Massachusetts
London · Amsterdam · Don Mills, Ontario · Sydney · Tokyo

CONTENTS

48. Hochman, J., and Perlman, R. L., *Biochim. Biophys. Acta 421*, 168 (1976).

49. Ladbrooke, B. D., and Chapman, D., *Chem. Phys. Lipids 3*, 304 (1969).

50. De Kruijff, B., Cullis, P. R., and Radda, G. K., *Biochim. Biophys. Acta 406*, 6 (1975).

51. Bashford, C. L., Johnson, L. N., Radda, G. K., and Ritchie, G. A., *Eur. J. Biochem. 67*, 105 (1976).

52. Marsh, D., Radda, G. K., and Ritchie, G. A., *Eur. J. Biochem. 71*, 53 (1976).

53. De Kruijff, B., Cullis, P. R., and Radda, G. K., *Biochim. Biophys. Acta 436*, 729 (1976).

54. Nikodijevic, O., Nikodijevic, B., Zinder, O., Mei-Ying, W. Y., Guroff, G., and Pollard, H. B., *Proc. Nat. Acad. Sci. 73*, 771 (1976).

MITOCHONDRIAL RIBOSOMES

Walter Neupert

INTRODUCTION

The formation of mitochondria capable of performing their essential functions competently requires the cooperation of two separate genetic systems. The first and more elaborate of these systems consists of the nuclear genetic information complement and the cytoplasmic translation apparatus. The other consists of mitochondrial DNA and the intramitochondrial transcription and translation apparatus. The contribution made by the intramitochondrial system is generally accepted to be quite small in terms of the number and quantity of proteins synthesized. However, formation and continuity of competent mitochondria inevitably depend on the formation of these proteins. It follows that the intramitochondrial protein-synthesizing system is indispensable to the existence of eukaryotic cells, at least for the

Horizons Biochem. Biophys. 3 [1977]. ISBN 0-201-02713-5; 0-201-02723-2 *pbk*

obligate aerobic cells, since these cells must live on the energy produced in the form of ATP in the mitochondrion.

The realization of these correlations has attracted much attention to the study of the intramitochondrial genetic system. Mitochondria have been shown to contain a unique complement of DNA, DNA-dependent DNA polymerase, DNA-dependent RNA polymerase, messenger RNA, ribosomes, ribosomal factors, transfer RNAs, and aminoacyl-transfer-RNA synthetases. These components are distinguishable from their counterparts in the nucleus and cytoplasm, but appear to be involved in quite similar reactions. The reader who is interested in these more general aspects of mitochondrial genetics and protein synthesis is referred to a number of excellent reviews, and to the proceedings of three recent conferences, all of which provide comprehensive documentation of current knowledge on many aspects of this subject (1-10).

This chapter will concentrate, instead, on the mitochondrial ribosomes, as central constituents of the intramitochondrial protein-synthesizing system. It is not possible to offer here a comprehensive survey of all the publications relating to this subject, a task that would entail some three to four hundred references. Nor have we attempted to discuss controversial issues at length. More extensive reviews are available in which contentious and technical details are considered (11-14).

IDENTIFICATION OF MITOCHONDRIAL RIBOSOMES BY SPECIFIC CHARACTERISTICS

The unequivocal identification of a ribosome as being of mitochondrial origin has presented some difficulties and not a little controversy. Several factors are responsi-

ble for this. First, mitochondrial ribosomes constitute only a small part of the total cellular ribosomes; for example, in rapidly growing cells of *Neurospora*, which have an appreciably high content of mitochondria (about 25% of total cellular protein), mitochondrial ribosomes make up only 3% of the total cellular ribosomes. Furthermore, few reliable criteria for unequivocal identification are available. The main criteria appear to be as follows: (a) The ribosome must be derived from a compartment that is confined by the inner mitochondrial membrane. (b) The ribosome must be involved in the synthesis of proteins of the inner mitochondrial membrane--namely, polypeptide subunits of the enzyme complexes, cytochrome oxidase, cytochrome bc_1, and the oligomycin-sensitive ATPase. (c) The ribosome must be sensitive to chloramphenicol, but not to cycloheximide.

The lack of further criteria of a generally applicable and reliable nature stems from the fact that mitochondrial ribosomes show a wide variation in their physical and chemical composition among different phylogenetic groups. This is in sharp contrast to cytoplasmic ribosomes, which are remarkably similar in different organisms.

For this reason, a survey of the properties and composition of mitochondrial ribosomes may most conveniently be arranged in such a way that the ribosomes are grouped according to phylogenetic affinity. Four broad categories are distinguished: (a) fungi, (b) protozoa, (c) plants, and (d) animals.

It should be stated at the very outset, however, that mitochondrial ribosomes operate on the same basic principles as prokaryotic or cytoplasmic and chloroplastic ribosomes from eukaryotes. They are composed of two distinct

subunits, containing at least two high-molecular-weight RNA species, and have basically the same mechanism for the formation of peptide bonds using aminoacyl transfer RNAs and messenger RNA.

ISOLATION AND PURIFICATION OF MITOCHONDRIAL RIBOSOMES

In view of the small proportion of mitochondrial ribosomes, it is generally impossible to distinguish them from cytoplasmic ribosomes without first isolating and purifying the mitochondria. The most critical step in this procedure is the removal of cytoplasmic ribosomes, which are normally found as contaminants of crude mitochondrial preparations. This contamination arises in several ways, such as (a) cosedimentation of rough endoplasmic reticulum; (b) nonspecific attachment of cytoplasmic ribosomes to mitochondrial membranes; (c) precipitation of cytoplasmic membranes and ribosomes when Mg^{++} ions are included in the isolation medium; and (d) possibly specific attachment of cytoplasmic ribosomes to the outer mitochondrial membranes.

Essentially three procedures have been used to remove contaminating cytoplasmic ribosomes: First, washing of mitochondria with EDTA to dissociate and remove ribosomes from membranes. Inclusion of EDTA in the isolation medium appears not to affect the structure and function of the intramitochondrial ribosomes, since EDTA does not penetrate the mitochondrial inner membrane. Second, extensive purification of mitochondria in Mg^{++}-containing media by means of gradient centrifugation. Third, washing of mitochondrial preparations with digitonin; this detergent is known to

solubilize outer mitochondrial membranes and endoplasmic membranes preferentially, thereby removing cytoplasmic ribosomes.

The purified mitochondria are detergent-lysed in the presence of Mg^{++} in concentrations of 5 to 10 m*M*, to release the mitochondrial ribosomes, which are then collected and purified according to procedures established for cytoplasmic or bacterial ribosomes.

STRUCTURE OF MITOCHONDRIAL RIBOSOMES

PHYSICAL PROPERTIES OF MITOCHONDRIAL RIBOSOMES.

Sedimentation Analysis. The mostly widely applied technique for characterizing a mitochondrial ribosome is the determination of the sedimentation coefficient by measuring the sedimentation velocity in sucrose density gradients upon ultracentrifugation. The sedimentation coefficient is a rather complex function of the molecular weight, the specific density, and the conformation of the ribosome. A sedimentation coefficient ($S_{20,w}$ value) of 70 has been attributed to the *Escherichia coli* monomeric ribosome, and sedimentation coefficients of 50 and 30 to its subunits. Generally, this ribosome is used as a standard to calibrate gradients. Cytoplasmic ribosomes from a wide range of organisms have been found to have *S* values of around 80, most values ranging between 77 and 84 (see Table I).

The *S* values of mitochondrial ribosomes from various organisms differ widely. Examples of these ribosomes from the four mentioned groups of organisms are listed in Table I.

In the first group, which consists of the various fungi, the monomeric ribosome is characterized by a sedimen-

TABLE I

Physical Characteristics of Mitochondrial and Cytoplasmic Ribosomes

	Mitochondrial ribosomes			Cytoplasmic ribosomes			Ref.
	Sed. coeff. ($S_{20,w}$)		Buoyant density	Sed. coeff. ($S_{20,w}$)		Buoyant density	
	Monomer	Subunits	(g/cm^3)	Monomer	Subunits	(g/cm^3)	
Fungi							
Neurospora	73	50 + 37		77	60 + 37		(1, 15)
Saccharomyces	72-74	50 + 37	1.64	80	60 + 38	1.55	(16, 17)
Candida	72	50 + 36	1.48	78	61 + 37	1.53	(18)
Protozoa							
Tetrahymena	80	55 + 55	1.46	80	60 + 40	1.56	(19)
Euglena	71	50 + 32		87	67 + 46		(13, 20)
Plants							
Turnip	77-78			80	60 + 40		(21)
Maize	78	60 + 44	1.56			1.56	(22)
Animal							
Locust	60	40 + 25		80	60 + 40		(23, 24)
Xenopus	60	40 + 26	1.45	87		1.59	(25)
Rat	55	39 + 29	1.45	83	60 + 40	1.55	(8, 26, 27)
Calf	56	39 + 28	1.43				(8)
HeLa cells	55-60	40 + 30	1.40				(28, 29)

tation coefficient of 72 to 74 *S*, as opposed to *S* values of 77 to 80 for the cytoplasmic ribosomes. The mitochondrial subunits have values of 50 and 37 *S*, compared with cytoplasmic values of 60 and 37 *S*. Dissociation of mitochondrial ribosomes is observed at relatively high concentrations of Mg^{++} (0.1 to 1 m*M*) at which cytoplasmic ribosomes remain undissociated. This property is not restricted to fungal mitochondrial ribosomes but appears to be a general feature of mitochondrial ribosomes (1, 11, 14).

The first characterization of fungal mitochondrial ribosomes was achieved with *Neurospora* (15), and very similar properties were later reported for yeast mitochondrial ribosomes. There are, however, quite a number of reports that have not reached the same conclusions. In several cases the mitochondrial ribosome of yeasts was reported to have sedimentation coefficients close to 80 *S*--that is, that of cytoplasmic ribosomes. In at least two cases such an *S* value was found to be a misinterpretation of experimental data. The first reason for such an occurrence was that the mitochondria were isolated in the presence of Mg^{++} ions (10 m*M*). Under these conditions cytoplasmic ribosomes were found to be present in mitochondrial preparations (16). A further source of error was found in an unexpected dimerization of large mitochondrial ribosomal subunits of yeast in the presence of high levels of Mg^{++} (18). In other reports, deviations from values of 72 to 74 *S* may be due to insufficient calibration of gradients. Cosedimentation of mitochondrial and cytoplasmic ribosomes from one organism, or of *E. coli* ribosomes, should be adopted as a routine procedure to establish the *S* value of a mitochondrial ribosome. However, in view of possible errors and technical difficulties,

an unequivocal statement to the effect that the mitochondrial ribosome of yeast is not 80 *S* cannot be made. Recent and more detailed studies have shown that a value of 72 to 74 *S* is more probable.

The concept of the 73 *S Neurospora* mitochondrial ribosome as the functional intact ribosome has been challenged recently (8, 10). It was claimed that isolating the mitochondria in the presence of EDTA damages the ribosome within the mitochondria. As a result, only the damaged ribosomes have a value of 73 *S*, whereas the "real" ribosome has a value of 80 *S*. Such ribosomes were obtained only when cells were broken in the presence of Mg^{++}. Rigorous proof that the putative mitochondrial 80 *S* ribosome is not a contaminating cytoplasmic ribosome has not been presented, however. In fact, several lines of evidence have proved incompatible with the idea of 73 *S* ribosomes resulting from the damaging action of EDTA. The first isolation of 73 *S* particles was achieved with mitochondria that were isolated and carefully purified in the presence of Mg^{++}(15). Ribosomes in EDTA-isolated mitochondria, on the other hand, were shown to be capable of synthesizing the same polypeptide components as ribosomes *in vivo* (30). Furthermore, *Neurospora* mitochondria isolated in the presence of Mg^{++} by differential centrifugation were described as being contaminated by large amounts of cytoplasmic ribosomes (10, 31). A prompt and definitive solution to this controversy is desirable, since the idea of damage to intramitochondrial ribosomes by treatment of mitochondria with EDTA presents a challenge to the published findings of many authors working with different organisms.

A mitochondrial ribosomal particle quite different from that of fungi was isolated from various protozoan organisms, such as *Tetrahymena* and *Paramecium*. The monomeric ribosome is not distinguishable from its cytoplasmic counterpart with respect to its sedimentation velocity (80 *S*). However, it dissociates into two subunits with the same *S* value--namely, 55 *S*. On the other hand, the cytoplasmic ribosome yields 60 and 40 *S* subunits. In view of the apparent novelty of this particle, the first reports were greeted with some reservations. In the meantime, other laboratories have confirmed the results and extended them to other species (10, 14, 32). Moreover, electron microscopic studies support the correctness of the sedimentation data.

Analysis of the mitochondrial ribosome from another protozoan, *Euglena gracilis*, produced a surprisingly different *S* value. This ribosome was found to have a sedimentation constant of 71 *S*, the cytoplasmic counterpart being 80 *S*. The 71 *S* particle dissociates into subunits of 50 and 32 *S* (see Table I).

The mitochondrial ribosomes from plants fall into a third category. Early reports on mung bean mitochondria suggested an *S* value of 70. The evidence in support of this suggestion is slight, however, and is in conflict with more recent studies in which the mitochondrial ribosome has been carefully characterized. In mung bean, as in some unrelated genera, the findings favor a value of 78 *S* rather than 70 *S*. The 78 *S* ribosomes could be dissociated into 60 and 44 *S* subunits. Thus, the sedimentation characteristics of mitochondrial ribosomes in plants do not seem to be very different from those of their cytoplasmic counterparts.

The mitochondrial ribosomes of the fourth category of organisms--those from animals, in species ranging from insects to man--appear to be quite uniform in structure. A large number of studies concerning this type of ribosome are available, and initially contradictory findings have been satisfactorily resolved in recent years. Following a period of confusion, the early claim that the mitochondrial monomer had an *S* value of about 55 to 60 (26) has been confirmed by numerous independent studies on a variety of organisms. All mitochondrial ribosomes from animals studied so far have *S* values between 55 and 60. They may be consistently dissociated into subunits of about 40 *S* and 25 to 30 *S*.

Buoyant Density. Determination of the buoyant density of ribosomal particles by isopycnic centrifugation in CsCl is an established procedure and may be used as a criterion for the purity and composition of ribosomes. Essentially, the buoyant density is considered to be an expression of the RNA/protein ratio of ribosomes (14). Cytoplasmic and bacterial ribosomes are characterized as having uniform buoyant densities of 1.54 to 1.58 g/cm^3 and 1.64 g/cm^3, respectively. Again, mitochondrial ribosomes appear to fall into different groups with respect to this property.

The values reported for the two different yeasts, *Saccharomyces* and *Candida*, differ appreciably (see Table I). It appears quite possible, however, that these variations are due to differing experimental conditions.

With animal mitochondrial ribosomes similar buoyant densities were reported for different species (see Table I). These values are very low compared with those of cytoplasmic and bacterial ribosomes, ranging between 1.40 and 1.45 g/cm^3.

They would indicate very low RNA/protein ratios, a finding that appears to be supported by chemical analysis of these particles. It is interesting to note that the buoyant density of the ribosomes from mitochondria of *Tetrahymena* was likewise found to be relatively low.

Electron Microscopy. Ribosome-like particles had been visualized in mitochondria *in situ* long before their function was known and long before they were isolated. The size of these particles in sections of animal tissues fixed with glutaraldehyde and osmium tetroxide and stained with uranylacetate was usually found to be smaller than that of the ribosomal particles in the cytoplasm (24, 33). A clear identification of the stained granules was not possible, however, without biochemical analysis.

In recent years electron microscopy has contributed much to the understanding of ribosomal structure through the use of the negative staining technique. This technique was shown to be capable of revealing remarkable details of the fine ribosomal structure, as in the case of cytoplasmic ribosomes from rat liver (34).

Studies on mitochondrial ribosomes from fungi, protozoa, and animals were carried out by using the technique of negative staining (see Table II). The results substantiate the view that mitochondrial ribosomes are morphologically similar in different organisms, and similar to bacterial and cytoplasmic ribosomes. Nevertheless, mitochondrial ribosomes from different sources do show individual characteristics which may be of help in recognizing them.

The dimensions of the mitochondrial ribosome in the yeast *Candida utilis* have been found to be similar to those

TABLE II

Dimensions of Negatively Stained Ribosomes

	Mitochondrial ribosomes	Cytoplasmic ribosomes	Ref.
Candida	266 x 214	262 x 222	(18)
Tetrahymena	369 x 260	274 x 227	(35)
Euglena	251 x 202	274 x 218	(10)
Locusta	271 x 210	294 x 257	(24)
Rat	259 x 201	312 x 257	(10, 34)
	Chloroplast ribosomes		
Spinach	198 x 164		(10)
	Bacterial ribosomes		
E. coli	213 x 178		(10)

The mean values of the two major axes of the ribosomes are given in Angstroms. The first axis is the total height of the ribosome (height of large + small subunit). The second axis represents the maximal width of the ribosome, which is coincident with the maximal width of the large subunit. The standard deviations of the mean values are in the range of 4 to 8%.

of their cytoplasmic counterparts, and larger than those of the *E. coli* ribosome. As with ribosomes from other sources, the most prominent projections displayed are the so-called frontal and lateral views (34).

Ribosomes from animal mitochondria were for some time thought to be "miniribosomes"--that is, a very small variety--as inferred from their extremely low *S* value. Electron microscopy of negatively stained particles, however, has revealed that these ribosomes are by no means "mini." They appear to be even larger than the bacterial ribosomes, so that the term "miniribosome" is a misnomer. They are, nonetheless, smaller than their cytoplasmic counterparts, but it should be borne in mind that such size determinations must be interpreted with caution, since volume changes due to fixation and staining artifacts cannot be excluded. Nevertheless, the measurements are in substantial agreement with the molecular weights of the particles as calculated from the buoyant density and from the sum of the protein and RNA components.

Special attention has been devoted to the electron microscopic analysis of protozoan mitochondrial ribosomes because of their unusual sedimentation behavior. It has been found that the *Tetrahymena* mitochondrial ribosome has in fact a rather unusual structure. Its overall dimensions are much greater than those of the protozoan cytoplasmic ribosome (see Table II). Moreover, this ribosome was found to be composed of two subunits of almost equal size. These observations strongly support the finding that the functional monomeric ribosome from protozoans, such as *Tetrahymena* and *Paramecium*, is an 80 *S* ribosome composed of two 55 *S* subunits.

The dimensions of the mitochondrial ribosome from *Euglena* are similar to those of fungal and animal mitochondrial ribosomes, and are clearly different from those of the other protists described above. Thus, the data from sedimentation analysis and from electron microscopy are in substantial agreement.

Gel Electrophoretic Analysis. A recently introduced method for the characterization of ribosomes is the analysis of their electrophoretic mobility in large-pore polyacrylamide gels (10). Even though the discriminating parameters appear to be quite complex--size, shape, and RNA/protein ratio--mitochondrial ribosomes have manifested a common feature, a relatively low mobility as compared with that of prokaryotic and eukaryotic cytoplasmic ribosomes. This apparently holds true for all the mitochondrial ribosomes from fungal, protozoan, and animal species so far studied.

COMPONENTS OF MITOCHONDRIAL RIBOSOMES. Ribosomal RNA. The variations observed in mitochondrial ribosomes from different species are inevitably reflected in the composition of their RNAs. It is therefore not possible to present a simple and unified account of the ribosomal RNA of mitochondria, as has been possible with cytoplasmic and bacterial ribosomal RNA. Fortunately, a measure of agreement has emerged in recent years concerning the mitochondrial RNAs from a broad range of organisms (see Table III).

A wide variety of techniques for the characterization of high-molecular-weight RNA components are available. These include determination of sedimentation velocity by density gradient centrifugation, sedimentation equilibrium

centrifugation, gel electrophoresis under denaturing and nondenaturing conditions, and electron microscopy of denatured RNAs. Very few studies, however, have offered a systematic comparison of these different techniques (for example, ref. 36). Even the most elaborate techniques provide different molecular weights. In most cases, however, they are reasonably close to the weighted average obtained from all the individual procedures. Highly precise determinations will probably be obtained only by fingerprinting and sequencing studies.

The main problem in determining reliable molecular weights for mitochondrial ribosomal RNAs stems from the fact that these RNA species are characterized by an unusually low content of guanosine and cytidine (G+C) (see Table III). Since the GC base pair has a considerably higher binding energy than the AU pair, unfolding of these RNAs occurs at ionic strengths and at temperatures where ribosomal RNAs of cytoplasm and of bacteria are still in a folded conformation. The unfolded molecules migrate more slowly upon electrophoresis in polyacrylamide gels. Accordingly, the rule that the log (molecular weight) is proportional to the electrophoretic mobility (37) does not necessarily hold for mitochondrial RNAs under the conditions in which it holds for cytoplasmic and bacterial RNAs. Reliable data may be obtained, however, if care is taken to ensure, first, that the temperature of electrophoresis is below the point where thermal unfolding starts, and, second, that the ionic strength of the electrophoresis buffer is high enough to preserve the secondary structure of the RNA. Another approach to this problem has been to denature the RNA and determine the characteristics of the unfolded RNA. Neverthe-

TABLE III

Ribosomal RNAs of Mitochondria and Cytoplasm

	Mitochondrial ribosomes		Cytoplasmic ribosomes		
	Molecular weights x 10^{-6} of RNA from large and small subunits	G+C content (%)	Molecular weights x 10^{-6} of RNA from large and small subunits	G+C content (%)	Ref.
Fungi					
Neurospora	1.28 + 0.72	27	1.28 + 0.67	51	(15, 38)
Aspergillus	1.27 + 0.66	32			(39)
Saccharomyces	1.30 + 0.70	30	1.21 + 0.72	53	(36, 40)
Candida	1.21 + 0.71	33	1.67 + 0.80	50	(18)
Protozoa					
Tetrahymena	0.90 + 0.47	29	1.18 + 0.52	47	(19, 41)
Euglena		30		56	(20)
Plants					
Turnip	1.15 + 0.70		1.36 + 0.70		(21)
Maize	1.25 + 0.76		1.19 + 0.67		(22)
Animals					
Locust	0.52 + 0.28	32	1.50 + 0.70		(10, 42)
Xenopus	0.53 + 0.30	41	1.52 + 0.70		(37, 43)
Rat	0.50 + 0.30	47	1.48 + 0.66	64	(11, 27)
HeLa cells	0.54 + 0.35	45	1.75 + 0.70	65	(37, 44)
	Chloroplast ribosomes				
Spinach	1.05 + 0.56	54			(45)

less, the data in Table III may not be taken as definitive in all cases.

The most reliable data for fungal mitochondrial ribosomal RNA point to molecular weights of 1.20 to 1.30 x 10^6 and 0.66 to 0.72 x 10^6 daltons for the species from large and small subunits, respectively. Thus, these RNAs appear to be appreciably larger than the corresponding RNAs from *E. coli* (1.10 and 0.56 x 10^6) and not notably different from the corresponding cytoplasmic RNAs.

The mitochondrial ribosome of *Tetrahymena*, despite its quite high *S* value, contains ribosomal RNAs that are distinctly smaller than bacterial RNAs--namely, 0.90 and 0.47 x 10^6 daltons.

There are variations in the reports on plant mitochondrial ribosomal RNAs. The most reliable data suggest relatively high molecular weights of 1.15 to 1.25 x 10^6 and 0.70 to 0.78 x 10^6 daltons, very similar to those of their cytoplasmic counterparts.

The ribosomal RNAs from mitochondria of animals are strikingly small. This has been verified for a wide variety of organisms by a number of independent methods. Bringing together all the findings from human beings to grasshoppers, we find the molecular weight of the large ribosomal RNA to be in the range of 0.50 to 0.58 x 10^6, and that of the small RNA to be between 0.28 and 0.35 x 10^6. Direct measurements by electron microscopy of the RNA from HeLa cells give lengths of 0.42 and 0.26 micron for large and small RNAs, respectively. The molecular weights calculated from these length determinations are in substantial agreement with the molecular weights determined by other techniques.

The size of these animal RNAs, therefore, is only one-half (or less) the size of the corresponding cytoplasmic ribosomes and of bacterial ribosomes. It is fascinating to note how a ribosome with such "mini-RNAs" retains a shape markedly similar to that of other ribosomes and performs apparently the same reactions in the synthesis of proteins. The RNA data suggest that it is possible for two of each of these RNAs to be present in a single subunit, but the protein analysis data and the buoyant density measurements seem to exclude this possibility.

Recent reports on the presence and nature of methylated nucleotides in mitochondrial ribosomal RNAs from yeast, *Neurospora*, and cultured animal cells appear to reveal a common feature of mitochondrial RNA (13, 14). This is due to their relatively low degree of methylation compared with that of cytoplasmic and bacterial RNAs. The significance of this low degree of methylation is unknown.

The 5 *S* RNA Component. The 5 *S* RNA species has been found to be an integral part of the large subunit of prokaryotic and eukaryotic cytoplasmic ribosomes. It is thought to play a role in the binding of transfer RNA, and its requirement for reconstitution of a functional large subunit from the individual RNA and protein components has been demonstrated (46). Accordingly, the question of whether mitochondrial ribosomes contain a 5 *S* RNA or a 5 *S* RNA equivalent has been investigated with much interest.

Surprisingly, no 5 *S* RNA component was detected in fungal 73 *S* and animal 55 to 60 *S* mitochondrial ribosomes. Proposals have been made as to how different structures might serve as substitutes for 5 *S* RNA. Since a functional

test for such a component is not available at the present time, however, and since reconstitution experiments appear to be beyond present experimental possibilities, the assignment of a 5 *S* RNA function to a molecule of different size would seem to be a complicated task.

In contrast to fungal and animal mitochondrial ribosomes, the presence of 5 *S* RNA has been reported for *Tetrahymena* and a number of plant mitochondrial ribosomes (19, 47).

The large subunit of cytoplasmic ribosomes has been shown to contain a further noncovalently linked RNA component to which an *S* value of 5.8 was attributed. This component has been found to be absent from the mitochondrial ribosome of all species so far studied (10, 13, 31, 47).

Ribosomal Proteins. It has been demonstrated for a variety of organisms that mitochondrial ribosomes contain a specific set of ribosomal proteins, different from that of their cytoplasmic counterparts. This conclusion was reached on the basis of results obtained with several different techniques such as gel electrophoresis, isoelectric focusing, ion exchange chromatography, and immunological methods (1, 13, 14). However, it must be borne in mind that these methods do not allow so fine a resolution as to state with certainty that cytoplasmic and mitochondrial ribosomes do not have a single protein in common, or that a certain degree of sequence similarities does not obtain between these two sets of ribosomal proteins.

Detailed studies on the number and properties of proteins from mitochondrial ribosomes are available only for a limited number of organisms. *Neurospora* mitochondrial

ribosomes have been reported to contain a total of fifty-three proteins (48). The results of protein analyses on animal mitochondrial ribosomes have been rather surprising: The protein content of these ribosomes was found to be unexpectedly high, ranging between 75 and 65% (10, 14, 26, 49). It was thought at first that these ribosomes were insufficiently purified and that some contaminating membrane material must have been present. It was then realized, however, that ribosomes from different sources such as *Xenopus* oocytes and calf and rat liver contain an unexpectedly high number of ribosomal proteins. Two-dimensional gel electrophoresis showed forty proteins in the large subunit and forty-four proteins in the small subunit of *Xenopus laevis* mitochondrial ribosomes (49). Bovine liver mitochondrial ribosomes were found to contain fifty-three and forty-one proteins in the large and small subunits, respectively (10, 14). Careful studies with these ribosomes have revealed that the protein content cannot be reduced to less than about 67% by treatment with high salt buffers without the loss of functional activity. The number of proteins is clearly greater than that of either bacterial or corresponding cytoplasmic ribosomes.

When protein and RNA components of animal mitochondrial ribosomes are summed, a molecular weight of about 2.8 to 3.2 x 10^6 is obtained. This value is very similar to that of bacterial ribosomes. It is in agreement with the morphological data which indicate a particle size at least as large as that of the bacterial ribosome. Furthermore, the high protein content of the animal mitochondrial ribosome would explain this low buoyant density.

It remains a mystery that two particles as different in their make-up as the *E. coli* and the animal mitochondrial ribosome should be so similar in shape and function. The plasticity of ribosomal structure and composition is clearly contrary to the rigidity of its functional characteristics. This presents an urgent challenge to those interested in the rules that govern evolution on a molecular basis.

BIOSYNTHESIS OF MITOCHONDRIAL RIBOSOMES

BIOSYNTHESIS OF RIBOSOMAL RNAs. There is now convincing evidence that mitochondrial ribosomal RNA is coded for by mitochondrial DNA and synthesized within the mitochondrion. Furthermore, in all organisms studied so far, only one cistron for each of the ribosomal RNAs has been detected per mitochondrial genome (that is, one circle of mitochondrial DNA). This is in sharp contrast to the nuclear and bacterial genome where large-scale redundancy of ribosomal RNA cistrons has been established.

Analysis of mitochondrial DNA has progressed so far that for several organisms the relative positions of the ribosomal RNA cistrons are known. In yeast, the cistrons for large and small ribosomal RNAs lie on approximately opposite sides of the circular DNA, separated on both sides by established mitochondrial genetic markers. In contrast, the two RNA cistrons in *Neurospora* were reported to lie close together (9, 10). This position of the cistrons is in agreement with the finding that the ribosomal RNAs are synthesized as a precursor with a molecular weight of 2.4×10^6, which is then cleaved to give the RNA species with molecular weights of 1.28 and 0.72×10^6 daltons (8). Several reports

indicate that in certain higher organisms (HeLa cells, *Xenopus, Drosophila*) the RNA genes are similarly located close to one another (9, 10). It is suggested, however, that the cistrons are separated by a sequence of about 160 nucleotides. With HeLa cells and *Xenopus* oocytes it has been found that one 4 *S* RNA gene is located in this gap. Most of the recent developments in this field (including one report describing a different arrangement in rat liver) are presented in the proceedings of two conferences (9, 10).

Little is known about the exact mode of synthesis of mitochondrial ribosomal RNAs. So far the search for precursor molecules analogous to those for the cytoplasmic ribosomal RNAs have proved positive only in one instance--*Neurospora*. In HeLa cells the sites for the ribosomal RNAs were detected on the heavy strand of the mitochondrial DNA. It was suggested that this strand was transcribed as a whole, but no information on possible processing of ribosomal RNA has been forthcoming (50).

BIOSYNTHESIS OF RIBOSOMAL PROTEINS. A number of experiments with *Neurospora* and yeast have provided conclusive evidence that the bulk of mitochondrial ribosomal proteins are synthesized outside the mitochondrion--that is, on cytoplasmic ribosomes--and are eventually transported into the mitochondrion. Although there is no direct genetic proof for the coding of these proteins on nuclear DNA, all the available evidence points in that direction (12).

Several studies have been devoted to establishing whether one or more of the mitochondrial ribosomal proteins are coded for by mitochondrial DNA and synthesized within the mitochondrion. The results of a study with *Neurospora*

led to the conclusion that all proteins are synthesized on cytoplasmic ribosomes (48), but recently it has been suggested that one protein of the small subunit may actually be synthesized within the mitochondrion (10). A similar situation has arisen in yeast, where a mitochondrially synthesized protein has been found to be associated with the small subunit (8). It has not been established whether this protein is an integral part of the ribosome or a contaminant of mitochondrial origin.

Studies similar to those with *Neurospora* and yeast have been carried out with protozoa. The results indicate that some proteins may be synthesized outside and some inside the mitochondrion (51). A detailed study is necessary, however, to establish the significance and nature of the mitochondrially synthesized proteins. The possible existence of mitochondrially synthesized ribosomal proteins could have far-reaching implications for mitochondrial genetics.

An interesting approach to the problem of genetic control of mitochondrial ribosomal proteins has been made with *Paramecium* (9). Hybrid species of *Paramecium aurelia* can be obtained by microinjection of the mitochondria of one species into the cells of another species. Hybrids so obtained contain the complete mitochondrial genome of the donor species. The results of the immunological analysis of mitochondrial ribosomal proteins in such species are compatible with the view that some of these proteins are coded for by the mitochondrial DNA. It may be pertinent that, in the case of chloroplast ribosomes, the involvement of both chloroplast and nuclear genes in the formation of chloroplast ribosomal proteins is documented by a large number of studies (10, 12).

Investigations on the site of synthesis of animal mitochondrial ribosomal proteins are not available. This is largely due to the experimental difficulties involved in such studies. A genetic approach has been made to this problem in *Xenopus* species, yielding interesting results (52). The two related species *X. laevis* and *X. mulleri* display differences in the proteins of the large mitochondrial ribosomal subunits. Protein patterns obtained in two-dimensional gel electrophoresis show a difference in some seven proteins. When hybrids are produced between these frogs, the nuclear genes are contributed equally by both species, whereas the mitochondrial DNA is derived only from the maternal species. Analysis of the mitochondrial ribosomal proteins in such hybrids showed that four of the species-specific proteins were present only when their corresponding species was the mother. The authors consider this result consistent with a mitochondrial coding of some of the ribosomal proteins, not excluding the possibility of a nuclear coding of these proteins.

In summary, there is convincing evidence that most of the ribosomal proteins are coded for by nuclear DNA and synthesized on cytoplasmic ribosomes. It might be that one or a few of the proteins of mitochondrial ribosomes are coded for and synthesized within the mitochondrion. It is possible that the situation is different among different organisms. Clear information in this area would contribute much to our understanding of mitochondrial biogenesis.

Practically nothing is known about the assembly of protein and RNA constituents and about the regulation of this process.

FUNCTIONAL ASPECTS OF MITOCHONDRIAL RIBOSOMES

Despite wide variations in physical and chemical characteristics among mitochondrial ribosomes in different evolutionary lines, there is a surprising unity of function: (a) Mitochondrial ribosomes are involved in the synthesis of a quite restricted number of polypeptides. (b) The function of these polypeptides appears to be the same in all eukaryotes. (c) The mechanism of translation seems to be the same in different eukaryotes and very similar to that in prokaryotes.

PROTEINS SYNTHESIZED BY MITOCHONDRIAL RIBOSOMES.

The observation that the number of proteins formed on mitochondrial ribosomes is quite restricted is inferred from several lines of experimental evidence.

The coding capacity of mitochondrial DNA from animals is quite low. The DNA has a length of about 5 microns, or a molecular weight of about 10×10^6 daltons. For the coding of mitochondrial ribosomal RNA and of mitochondrial transfer RNAs (some twenty different species), about 25 to 30% of the total coding capacity is consumed, not taking into consideration the possible presence of large sequences in the original transcripts, later lost through maturation processes. The remainder is sufficient to code for about 3500 amino acids, again not taking into account possible sequences of regulatory genes.

There is good reason, although no definite proof, to assume that mitochondrial ribosomes are committed solely to the translation of messages of mitochondrial origin (6, 53).

The most reliable information concerning the number, structure, and function of proteins translated derives from experiments with yeast and *Neurospora*. In these organisms, the mitochondrial DNA is considerably larger than in animals--about 25 microns. Accordingly, a larger number of gene products than in animal mitochondria seems possible. We do not, however, have any definite indication that this might be true. Mitochondrial DNA from fungi may contain a large number of "spacer sequences" which do not code for protein or RNA. The number of proteins translated in the fungal mitochondria known so far might well be coded for by a 5-micron DNA. We do not know very much about the translation products in animal mitochondria, but there is evidence that polypeptides that are structurally and functionally analogous to those in fungi are synthesized.

It appears on this basis that proteins totaling roughly 3500 amino acids may be synthesized on mitochondrial ribosomes. As mentioned above, we have, with regard to fungi, a considerable number of reports on the structure, function, and number of mitochondrially synthesized proteins (5-10):

1. Three subunits of the cytochrome oxidase complex with molecular weights of about 40,000, 30,000 and 20,000. These three are the largest subunits of the enzyme complex; the other four or five subunits are translated on cytoplasmic ribosomes.

2. One or two subunits of the cytochrome bc_1 complex, corresponding to cytochrome b (apparent molecular weight about 30,000).

3. Two to four subunits of the oligomycin-sensitive ATPase complex with molecular weights between 29,000 and 7500.

The number of amino acids present in these six to nine proteins would total about 2000. Thus, as an upper limit, information for some 1500 amino acids would remain. As already discussed, part of this coding capacity might be necessary for regulatory sequences and for one or more of the proteins of the mitochondrial ribosomes. In any case, the number of unknown polypeptides that might be translated on mitochondrial ribosomes, apart from those mentioned above, is probably quite small.

We can summarize the findings on the mitochondrially synthesized proteins as follows: Mitochondrial ribosomes are involved in the synthesis of a restricted number of polypeptides, at least six but perhaps not more than fifteen. The polypeptides so far identified are integrated into the inner mitochondrial membrane and constitute subunits of enzyme complexes involved in respiration and oxidative phosphorylation. They are quite high in their content of nonpolar amino acids; that is, they are hydrophobic and are not soluble in aqueous media in the absence of detergents.

MECHANISMS OF TRANSLATION BY MITOCHONDRIAL RIBOSOMES. A large body of evidence indicates that ribosomes in mitochondria operate on the same basic principles as ribosomes from prokaryotes and as eukaryotic cytoplasmic or chloroplastic ribosomes. Mitochondria from a variety of species have been shown to contain a full complement of transfer RNAs and of aminoacyl-transfer-RNA synthetases. Earlier reports that animal mitochondria may contain only eleven to fourteen transfer RNAs have been revised, and now a complete set of transfer RNAs (and even isoaccepting species) also seems probable in animal mitochondria (9, 10, 13). Likewise,

the participation of T and G factors in mitochondrial translation has been demonstrated (1, 54).

One interesting peculiarity of mitochondrial ribosomes is that they use formyl methionyl transfer RNA for polypeptide chain initiation (6, 13, 55). This is in contrast to cytoplasmic ribosomes, which initiate with methionyl transfer RNA, but similar to bacterial ribosomes, which also use this specific transfer RNA for initiation. In the latter case, however, the formyl group and the methionine residue are split off during completion of the chain. This appears not to be the case with mitochondrial translation products, as suggested by two lines of evidence: (a) Labeled formate applied to whole yeast cells appears preferentially in mitochondrial translation products in a position which is the N-terminal methionine residue (56). (b) Amino acid sequence analysis of subunit II (apparent molecular weight 28,000) from beef heart cytochrome oxidase, which presumably is synthesized on mitochondrial ribosomes, revealed the N-terminal amino acid to be *N*-formylmethionine (10).

Little is known about mitochondrial messenger RNA. Several reports indicate the presence of poly-A sequences, since RNA with messenger properties extracted from whole mitochondria or mitochondrial polymeric ribosomes might be bound to poly-dT-cellulose or to poly-U-sepharose columns (9, 10; for review, see ref. 13). Translation of fractions with messenger character from yeast in a bacterial cell-free system has recently been reported to yield immunologically detectable subunits of cytochrome oxidase (57). However, a careful study by another group using a similar system has

cast some doubt on the identity of the *in vitro* translation products (9).

Evidence for the existence of polysomes in mitochondria has been presented by a variety of observations. Polysome-like structures have been detected in the electron microscope (18). Polymeric ribosomes with the characteristics of messenger-RNA-ribosome complexes were isolated--from *Neurospora*, *Euglena*, and HeLa cells, for example. However, these preparations are distinguished by a low number of polymeric aggregates. They consist mainly of di-, tri-, and tetramers and in at least two cases have proved to be ribonuclease-resistant (13). It is not known whether these polyribosomes are free or are bound to the inner membrane, although some evidence has been presented in favor of the latter possibility (58). A clear account of the functional ribosome cycle in mitochondria is long overdue.

ANTIBIOTIC SENSITIVITY OF MITOCHONDRIAL RIBOSOMES.

The mitochondrial ribosome may be recognized by its specific sensitivity to certain antibiotics. As early as 1960 it was shown that amino acid incorporation into isolated mitochondria of animal cells is sensitive to chloramphenicol, an antibiotic that does not affect eukaryotic cytoplasmic ribosomes and that is known to specifically block peptidyl transferase of 70 *S* bacterial ribosomes (59). It was later shown that a variety of antibiotics known to be potent inhibitors of translation in bacteria also block translation in mitochondria. These antibiotics include the macrolides erythromycin, carbomycin, and spiramycin; lincomycin and clindamycin; and the aminoglycosides paromomycin and neomycin (3, 4, 11, 14).

It should be emphasized that these antibiotics have not been definitely proved to act on mitochondrial ribosomes of all organisms, nor is it known whether they affect different ribosomes to the same degree. It has been observed that erythromycin and lincomycin do not block translation in isolated mitochondria from animal cells, but do block translation in isolated yeast mitochondria. Two explanations have been offered for this observation: (1) Animal and fungal mitochondrial ribosomes differ in this antibiotic sensitivity, thus displaying a phylogenetic difference in their structures (60). (2) The resistance of animal mitochondria to some of these antibiotics, especially erythromycin and lincomycin, is simply an expression of the impermeability of the mitochondrial membranes to these substances (8, 61). Reports that isolated mitochondrial ribosomes from rat liver are sensitive to these antibiotics in partial reactions of protein synthesis, such as poly-U-dependent polyphenylalanine synthesis, and in the "fragment reaction," would support the latter explanation. Certainly, in intact animals permeability barriers to these antibiotics influence their pharmacological action.

There is a second group of antibiotics which do not inhibit mitochondrial ribosomes but do inhibit cytoplasmic ribosomes. This group, which is also known for its inability to inhibit bacterial translation, includes cycloheximide (actidione) and anisomycin.

The selective sensitivity of mitochondrial and cytoplasmic ribosomes has important implications for a variety of factors--functional, phylogenetic, and practical. These antibiotics provide an excellent tool for discovering translation products of the mitochondrial genetic system, for

analyzing the synthesis sites of defined mitochondrial protein components, and for studying the interplay between the two translation systems in the eukaryotic cell. Whole cells exposed to the first group of antibiotics (particularly chloramphenicol) incorporate radioactively labeled amino acids selectively into cytoplasmic translation products, whereas antibiotics of the second group (particularly cycloheximide) allow the selective radioactive labeling of mitochondrial translation products. Identification of the polypeptides translated on the mitochondrial ribosomes listed above was achieved by using essentially this technique. The experimental and theoretical benefits of this procedure can hardly be overemphasized. Nevertheless, it should be pointed out that this experimental approach also has its drawbacks, since the uncoupling of the two translation systems may give rise to a number of reactions that are not directly linked to translational events. Moreover, it should be borne in mind that these antibiotics have been reported to interfere with other cellular reactions, such as respiration and phosphorylation.

A third group of antibiotics was found to inhibit both mitochondrial and cytoplasmic translation. Puromycin and fusidic acid belong to this group (1, 11, 14).

MUTATIONS AFFECTING MITOCHONDRIAL RIBOSOMAL FUNCTIONS AND STRUCTURE. Alterations in ribosome structure and function on the basis of mutational events bear wide-ranging theoretical and practical consequences. This certainly holds true also for mutations affecting mitochondrial ribosomes.

Two types of mutation may be distinguished: (a) mutations causing resistance to antibiotics; (b) mutations causing structural defects in mitochondrial ribosomes.

Antibiotic-Resistant Mutations. Such mutants were first reported for yeast (62). Strains resistant to chloramphenicol and to erythromycin were selected, and the mutations were found to be inherited in a non-Mendelian (uniparental) fashion, indicating that they reside in the mitochondrial DNA. A large number of such mutants has since been obtained, and, at least in a few cases, the resistance was shown to originate in the mitochondrial ribosome. In addition, mutants resistant to paromomycin have been obtained.

The genes for these antibiotic resistances on mitochondrial DNA have been mapped by genetic and physical techniques. There is general agreement that the chloramphenicol and erythromycin resistance genes are closely linked. Hybridization techniques have shown the resistance loci to be at least partially identical to the cistron for the large ribosomal RNA. The paromomycin resistance gene is located close to, but does not overlap, the small ribosomal RNA cistron (9).

In the case of bacteria, resistance to antibiotics such as erythromycin, streptomycin, and spectinomycin has been found to be based on alterations of ribosomal proteins. It has been tempting to assume that in the case of mitochondrial antibiotic resistances in yeast the same type of alterations has taken place. However, despite intensive efforts, no altered ribosomal protein in any one of these mutants could be demonstrated. This is a negative result,

but it appears to be supported by some other observations. There is no clear evidence that any of the mitochondrial ribosomal proteins in fungi is translated within the mitochondrion (see above). Since the mutations resulting in antibiotic resistance to chloramphenicol and erythromycin in yeast are probably mapped within the large ribosomal RNA gene, the possibility exists that the antibiotic resistances arise from alterations of ribosomal RNA and not of ribosomal proteins. This explanation is especially appealing, since, as mentioned above, only one cistron for each of the ribosomal RNAs in the mitochondrial genome has been demonstrated. Accordingly, the possibility that a genetic alteration of a ribosomal RNA gene leads to an alteration in the ribosome has a much higher probability in mitochondria than in those systems where ribosomal RNA genes show redundancy (for example, bacteria). However, these two examples may certainly not be taken as a rule, since it is reported that paromomycin resistance is mapped at a position some 6000 base pairs apart from the small ribosomal RNA cistron (9).

The situation with regard to protozoan and animal mitochondria, for both of which antibiotic resistances that are inherited in a non-Mendelian fashion have been reported, is even more unclear. Here, also, no definite proof for an altered mitochondrial ribosomal protein has been presented (9, 10).

Mutations Affecting Ribosome Structure. Extreme cases of mutations affecting mitochondrial ribosomes are the "petite" mutants of *Saccharomyces cerevisiae*. These mutants have large deletions in the mitochondrial DNA and are unable to perform mitochondrial protein synthesis. This inability

is correlated with a deficiency in mitochondrial ribosomes, probably due to the loss of cistrons for the ribosomal RNAs and perhaps of other genes necessary for ribosome formation (4-7).

Less severe cases of mutations affecting mitochondrial ribosomes are the *mi-1* and the *cni-1* mutants of *Neurospora*. The first is a cytoplasmic mutant, the second a nuclear mutant. Each has a deficiency in the small ribosomal subunit, and in both cases the primary genetic alteration has not been identified (10, 12). Some highly interesting data are available for the *mi-1* mutant. Here, an alteration in the maturation of ribosomal RNA has been proposed. On the other hand, recent data suggest that the mutation may affect a mitochondrially synthesized protein of the small subunit (10, 12). However, this protein awaits further characterization before its integral role in the small subunit can be taken for granted.

EVOLUTION OF MITOCHONDRIAL RIBOSOMES

Since the discovery of mitochondria as separate cellular entities, their evolutionary origin has attracted much attention. With the realization that mitochondria house a genetic apparatus, speculations on their endosymbiotic origin have received considerable impetus. According to this speculation, mitochondria are derived from a prokaryotic and aerobic type of organism. Some one billion years ago, this organism invaded a nucleated anaerobically living cell and stayed there as an endosymbiont. Eventually, genetic information of the endosymbiont was transferred into the nuclear genome of the host.

It follows that mitochondrial ribosomes should be considered as originating from such an endosymbiotic organism. As a matter of fact, the characteristics of the mitochondrial ribosome lend support to the theory of evolution of mitochondria from prokaryotic ancestors:

1. As in the case of bacterial ribosomes, mitochondrial ribosomes are inhibited by antibiotics such as chloramphenicol, erythromycin, and lincomycin; and like bacterial ribosomes they are not affected by antibiotics that block cytoplasmic ribosomes, such as cycloheximide and anisomycin.

2. Mitochondrial ribosomes use formyl methionyl transfer RNA for polypeptide chain initiation as do bacterial ribosomes, in contrast to cytoplasmic ribosomes.

3. In submitochondrial protein-synthesizing systems which show poly-U-dependent polyphenylalanine synthesis, the G and T factors may be replaced by those from bacteria, but not by the cytoplasmic variety from the same organism (1, 14, 54).

These observations may suggest that bacterial and mitochondrial ribosomes are derived from a common ancestor. In bacteria this ancestor would have developed into a rather uniform particle with respect to size, shape, and properties of RNA and protein constituents. In mitochondria the development from the common ancestor was accompanied by large variations in these traits. Thus, during evolution of eukaryotic species, widely differing groups of mitochondrial ribosomes have arisen. Despite the variations shown in physical and chemical properties, the mechanism of protein synthesis mediated by these ribosomes has been conserved.

There is one more fascinating aspect of mitochondrial evolution which is open to speculation but not readily open to an experimental approach. The vast majority of mitochondrial proteins are synthesized on cytoplasmic ribosomes and eventually transported into the mitochondrion. On the other hand, the mitochondria have retained ribosomes and the rather complicated translation machinery attached to them, consisting of probably more than one hundred proteins, in order to produce a strikingly small number of proteins within the mitochondrion. The puzzling question then is, Why does the eukaryotic cell afford this "luxury" of a special genetic system in order to produce only these few proteins? The answer may lie in the very nature of the proteins synthesized by mitochondrial ribosomes, in a possible advantage for regulatory mechanisms in having two interdependent systems, or perhaps in a principle of evolution that we do not know.

REFERENCES

1. Küntzel, H., *Curr. Topics Microbiol. Immunol. 54*, 94-118 (1971).

2. Borst, P., *Ann. Rev. Biochem. 41*, 333-376 (1972).

3. Kroon, A. M., Agsteribbe, E., and De Vries, H., in *The Mechanism of Protein Synthesis and Its Regulation* (L. Bosch, ed.), pp. 539-582, North-Holland, Amsterdam (1972).

4. Linnane, A. W., Haslam, J. M., Lukins, H. B., and Nagley, P., *Ann. Rev. Microbiol. 26*, 153-198 (1972).

5. Mahler, H. R., *CRC Critical Rev. Biochem. 1*, 381-460 (1973).

6. Schatz, G., and Mason, T. L., *Ann. Rev. Biochem. 43*, 51-87 (1974).

7. Lloyd, D., *The Mitochondria of Microorganisms*, Academic Press, London (1974).

8. Kroon, A. M., and Saccone, C., eds., *The Biogenesis of Mitochondria*, Academic Press, New York (1974).

9. Saccone, C., and Kroon, A. M., eds., *The Genetic Function of Mitochondrial DNA*, North-Holland, Amsterdam (1976).

10. Bücher, Th., Neupert, W., Sebald, W., and Werner, S., eds., *Genetics and Biogenesis of Chloroplasts and Mitochondria*, North-Holland, Amsterdam (1976).

11. Borst, P., and Grivell, L. A., *FEBS Lett. 13*, 73-88 (1971).

12. Chua, N.-H., and Luck, D. J. L., in *Ribosomes* (M. Nomura, A. Tissières, and P. Lengyel, eds.), pp. 519-539, Cold Spring Harbor Laboratory, New York (1974).

13. Avadhani, N. G., Lewis, F. S., and Rutman, R. J., *Sub-Cell. Biochem. 4*, 93-145 (1975).

14. O'Brien, T. W., and Matthews, D. E., in *Handbook of Genetics* (R. C. King, ed.), Vol. 5, pp. 535-580, Plenum Press, New York (1976).

15. Küntzel, H., and Noll, H., *Nature 215*, 1340-1345 (1967).

16. Schmitt, H., *Eur. J. Biochem. 17*, 278-283 (1970).

17. Grivell, L. A., Reijnders, L., and Borst, P., *Biochim. Biophys. Acta 247*, 91-103 (1971).

18. Vignais, P. V., Stevens, B. J., Huet, J., and André, J., *J. Cell Biol. 54*, 468-492 (1972).

19. Chi, J. C. H., and Suyama, Y., *J. Mol. Biol. 53*, 531-556 (1970).

20. Avadhani, N. G., and Buetow, D. E., *Biochem. J. 128*, 353-365 (1972).

21. Leaver, C. J., and Harmey, M. A., *Biochem. Soc. Symp. 38*, 175-193 (1973).

22. Pring, D. R., and Thornbury, D. W., *Biochim. Biophys. Acta 383*, 140-146 (1975).

23. Kleinow, W., Neupert, W., and Bücher, Th., *FEBS Lett. 12*, 129-133 (1971); Kleinow, W., and Neupert, W., *ibid. 15*, 359-363 (1971).

24. Kleinow, W., Neupert, W., and Miller, F., *J. Cell Biol. 62*, 860-875 (1974).

25. Swanson, R. F., and Dawid, I. B., *Proc. Nat. Acad. Sci. U. S. 66*, 117-124 (1970).

26. O'Brien, T. W., and Kalf, G. F., *J. Biol. Chem. 242*, 2180-2185 (1967).

27. Sacchi, A., Cerbone, F., Cammarano, P., and Ferrini, U., *Biochim. Biophys. Acta 308*, 390-403 (1973).

28. Attardi, G., and Ojala, D., *Nature New Biol. 229*, 133-136 (1971); Ojala, D., and Attardi, G., *J. Mol. Biol. 65*, 273-289 (1972).

29. Brega, A., and Vesco, C., *Nature New Biol. 229*, 136-139 (1971).

30. Rücker, A. V., Werner, S., and Neupert, W., *FEBS Lett. 47*, 290-294 (1974).

31. Lizardi, P. M., and Luck, D. J. L., *Nature New Biol. 229*, 140-142 (1971).

32. Tait, A., *FEBS Lett. 24*, 117-120 (1972).

33. André, J., and Marinozzi, V., *J. Microsc. 4*, 615-626 (1965).

34. Nonomura, Y., Blobel, G., and Sabatini, D., *J. Mol. Biol. 60*, 303-324 (1971).

35. Curgy, J. J., Ledoigt, G., Stevens, B. J., and André, J., *J. Cell Biol. 60*, 628-640 (1974).

36. Reijnders, L., Sloof, P., and Borst, P., *Eur. J. Biochem. 35*, 266-269 (1973).

37. Loening, U. E., *Biochem. J. 113*, 131-138 (1969).

38. Neupert, W., Sebald, W., Schwab, A., Massinger, P., and Bücher, Th., *Eur. J. Biochem. 10*, 585-588, 589-591 (1969).

39. Edelman, M., Verma, I. M., and Littauer, U. Z., *J. Mol. Biol. 49*, 67-83 (1970); Edelman, M., Verma, I. M., Herzog, R., Galun, E., and Littauer, U. Z., *Eur. J. Biochem. 19*, 372-378 (1971).

40. Morimoto, H., and Halvorson, H. O., *Proc. Nat. Acad. Sci. U. S. 68*, 324-328 (1971).

41. Reijnders, L., Sloof, P., Sival, J., and Borst, P., *Biochim. Biophys. Acta* 324, 320-333 (1973).

42. Kleinow, W., and Neupert, W.,*Z. Physiol. Chem. 351*, 1205-1214 (1970).

43. Dawid, I. B., and Chase, J. W., *J. Mol. Biol. 63*, 217-231 (1972).

44. Robberson, D., Aloni, Y., Attardi, G., and Davidson, N., *J. Mol. Biol. 60*, 473-484 (1971).

45. Hartley, M. R., and Ellis, R. J., *Biochem. J. 134*, 249-262 (1973).

46. Erdmann, V. A., Sprinzl, M., and Pongs, O., *Biochem. Biophys. Res. Commun. 54*, 942-948 (1973).

47. Leaver, C. J., and Harmey, M. A., *Biochem. J. 157*, 275-277 (1976).

48. Lizardi, P. M., and Luck, D. J. L., *J. Cell Biol. 54*, 56-74 (1972).

49. Leister, D. E., and Dawid, I. B., *J. Biol. Chem. 249*, 5108-5118 (1974).

50. Aloni, Y., and Attardi, G., *J. Mol. Biol. 70*, 375-381 (1972).

51. Millis, A. J. T., and Suyama, Y., *J. Biol. Chem. 247*, 4063-4073 (1972).

52. Leister, D. E., and Dawid, I. B., *J. Mol. Biol. 96*, 119-123 (1975).

53. Mahler, H. R., and Dawidowicz, K., *Proc. Nat. Acad. Sci. U. S. 70*, 111-114 (1973).

54. Richter, D., and Lipmann, F., *Biochemistry 9*, 5065-5070 (1970).

55. Smith, A. E., and Marcker, K. A., *J. Mol. Biol. 38*, 241-243 (1968).

56. Mahler, H. R., Dawidowicz, K., and Feldman, F., *J. Biol. Chem. 247*, 7439-7442 (1972).

57. Padmanaban, G., Hendler, F., Patzer, J., Ryan, R., and Rabinowitz, M., *Proc. Nat. Acad. Sci. U. S. 72*, 4293-4297 (1975).

58. Kuriyama, Y., and Luck, D. J. L., *J. Cell Biol. 59*, 776-784 (1973).

59. Mager, J., *Biochim. Biophys. Acta 38*, 150-152 (1960).

60. Towers, N. R., Dixon, H., Kellerman, G. M., and Linnane, A. W., *Arch. Biochem. Biophys. 151*, 361-369 (1972).

61. Ibrahim, N. G., Burke, J. P., and Beattie, D. S., *J. Biol. Chem. 249*, 6806-6811 (1974).

62. Wilkie, D., Saunders, G., and Linnane, A. W., *Genet. Res., Cambridge 10*, 199 (1967).