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MITOCHONDRIAL GENES AND TRANSLATION PRODUCTS

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INTRODUCTION

As a sequel to the discovery that mitochondria contain an independent system of protein synthesis (1–3), many efforts have been devoted toward identifying the products formed and understanding their role in the morphogenesis of the organelle. Since mitochondria synthesize relatively few proteins, this work has progressed rapidly and there is presently a substantial body of knowledge about the synthesis, genetic origin, and, in some instances, the functions of this interesting class of proteins. This review summarizes some of the information that has emerged from studies of the past ten years and speculates on the future course of research in this area.

MITOCHONDRIAL TRANSLATION PRODUCTS

In Vivo Studies

Some of the earliest evidence, particularly from work done with yeast, indicated that the proteins synthesized in mitochondria were likely to be components of the terminal respiratory pathway. For example, it was known that in yeast, chloramphenicol and other selective inhibitors of mitochondrial ribosomes, blocked the development of a functional cytochrome system but had no pronounced effect on most of the other enzymatic machinery of the organelle (4, 5). A similar phenotype was found in cytoplasmic "petite" mutants of yeast (6) which are deficient in mitochondrial protein synthesis due to the loss of genetic information coding for mitochondrial ribosomal and transfer RNAs (7–9).

Based on these observations, a number of laboratories began to systematically study the relationship of mitochondrial translation products to the enzymes that were known to catalyze electron transport (10). These included the NADH- and succinate-coenzyme QH_2-reductase (10–12), coenzyme QH_2-cytochrome *c* reductase (13), and cytochrome oxidase (14). In addition to the four respiratory complexes, the oligomycin-sensitive ATPase which normally functions as an ATP synthetase (15, 16) was also suspected to contain mitochondrially derived polypeptides since only one part of this important inner membrane complex was synthesized in petite mutants or in yeast grown in the presence of chloramphenicol (17, 18).

The biosynthesis of the respiratory and ATPase complexes was first examined in the yeast *Saccharomyces cerevisiae* and in *Neurospora crassa,* each organism offering distinct experimental advantages for such studies. Several circumstances facilitated the translational sites of the subunit polypeptides to be established by rather straightforward in vivo labeling experiments.

1. Total cellular proteins could be labeled with high specific activity when cells were incubated in the presence of a radioactive precursor.
2. Antibiotics were available which selectively inhibited either mitochondrial or cytoplasmic protein synthesis.
3. The successful purification of the ATPase and some of the respiratory complexes of yeast and *Neurospora* made it possible to characterize their subunit compositions and at the same time provided the antibodies necessary for rapid isolation of the enzymes from small samples of cells.

In most studies, the protocol used to identify the mitochondrially translated subunits consisted of labeling cells with a radioactive amino acid in the presence of either an inhibitor of mitochondrial or cytoplasmic protein synthesis. Subsequent purification of the enzymes and analysis of the distribution of radioactivity in the various subunit polypeptides under each set of labeling conditions allowed their translation to be ascribed to either one of the two protein synthesizing systems.

When *S. cerevisiae, N. crassa,* or mammalian cells are labeled in the presence of cycloheximide, 8–10 prominant radioactive protein bands are seen in polyacrylamide gels of mitochondria solubilized with sodium dodecyl sulfate (19, 20). Minor bands are also occasionally observed but they most likely arise from incomplete inhibition of cytoplasmic protein synthesis. Similar results have been obtained in the absence of inhibitors by using a conditional mutant of yeast in which protein synthesis on cytoplasmic ribosomes is suppressed at the nonpermissive temperature (21) or by labeling wild type cells with fMet. Mitochondrial products can be selectively labeled with fMet since it is used as the initiator and the formyl group is retained in the completed polypeptide chain due to the absence of a deformylase (22). Most of the major mitochondrial translation products of yeast and *Neurospora* have been ascertained to be subunit polypeptides of the respiratory and ATPase complexes. The one exception is the protein designated as var 1. This mitochondrial product exhibits molecular weight variant forms (40,000–43,000 daltons) in different strains of yeast (23). The function of var 1 is still obscure but there are some indications that it might be a component of mitochondrial ribosomes (24).

Identity of Mitochondrial Products

The information currently available on the synthesis of the subunits of coenzyme QH_2-cytochrome *c* reductase, cytochrome oxidase, and ATPase is summarized below. Since both NADH- and succinate-coenzyme Q-reductases are synthesized exclusively on cytoplasmic ribosomes (25–28), relatively few studies have been devoted to these complexes and they are not further considered here.

CYTOCHROME OXIDASE: Cytochrome oxidase is composed of seven nonidentical subunit polypeptides with molecular weights ranging from 40,000–9,000 (29–32). The functional groups of the native enzyme are heme *a* (cytochromes *a* and a_3) and copper. Following depolymerization of cytochrome oxidase with denaturing agents, heme *a* has been found to copurify with subunit 1 (40,000 daltons) (33) and subunit 7 (9,000 daltons) (34) which suggests that they are the hemoprotein components of the complex. In general, however, the precise relationship of the redox carriers to the enzyme subunits is still not agreed upon.

The first demonstration that cytochrome oxidase is synthesized jointly on mitochondrial and cytoplasmic ribosomes came from studies on *N. crassa* (29). It was conclusively established from in vivo labeling experiments that the three largest polypeptides (subunits 1–3) of the enzyme are translated in mitochondria and that the low molecular weight polypeptides (subunits 4–7) are made externally on cytoplasmic ribosomes (35, 36). This was later confirmed in *S. cerevisiae* (37, 38) and *Xenopus laevis* (39) and appears to be an invariant feature of the mechanism of biosynthesis of cytochrome oxidase.

COENZYME QH_2-CYTOCHROME REDUCTASE This intermediary respiratory complex was first purified and characterized from bovine heart mitochondria (13). Homogeneous preparations of coenzyme QH_2-cytochrome *c* reductase have recently also been obtained from yeast (40) and *N. crassa* (41). It is composed of seven or eight different subunits not all of whose functions are known. The best understood components of the enzyme are cytochromes *b*, c_1, and a nonheme iron protein. Each has been shown to be an obligatory electron carrier in the catalysis of cytochrome *c* reduction (13, 42). Weiss (43, 44) found that in *N. crassa*, cytochrome *b* is synthesized in mitochondria. Although it was originally thought that there are two different cytochrome *b* apoproteins (45), more recent genetic and biochemical evidence suggests the presence of only one chemical species of this cytochrome (46, 47). Cytochrome *b* has also been shown to be synthesized by yeast mitochondria (48) and in fact appears to be the only subunit of coenzyme QH_2-cytochrome *c* reductase that has a mitochondrial origin—all the other subunits, including cytochrome c_1 (49), are synthesized in the cytoplasm. There are no reports at present that deal with the biosynthesis of this complex in higher animal and plant cells.

OLIGOMYCIN-SENSITIVE ATPase This is probably the most intricate enzyme of the mitochondrial inner membrane. It is concerned not only with the coupling of the energy of oxidation to ATP synthesis but also with the utilization of the energy of hydrolysis of ATP for various energy-dependent functions such as ion transport, pyridine nucleotide transhydrogenation,

and reverse electron flow (15, 16, 50). This multiplicity of functions is reflected in the large number of polypeptides that have been attributed to the ATPase. The yeast complex consists of at least ten different protein subunits (51) none of whose functions can be clearly stated at present.

The oligomycin-sensitive ATPase complexes of bovine heart and yeast mitochondria have been dissected into three fractions that can spontaneously reassociate to form the native enzyme (52, 53). The three reconstitutively active fractions are F_1 (54), OSCP (55), and a set of hydrophobic proteins referred to as the membrane factor (52). F_1 is a water-soluble polymeric protein with a molecular weight of 340,000 (56). It is comprised of five distinct subunit polypeptides and catalyzes the hydrolysis of ATP—this hydrolytic reaction, however, is not inhibited by oligomycin and other potent inhibitors of the native complex (16, 54). In yeast (18), *N. crassa* (57, 58), and *X. laevis* (39), the five subunits of F_1 are synthesized in the cytoplasm. It is interesting that chloroplast F_1 which is structurally and functionally related to the mitochondrial F_1, contains some subunits that are made on chloroplast ribosomes (59).

OSCP is a water-soluble protein (17,000 daltons) that has been postulated to act as a link between F_1 and the membrane factor (60, 61). This protein has been shown to be synthesized on cytoplasmic ribosomes in yeast (61). A cytoplasmic origin may also be assumed for the OSCP component of other mitochondrial ATPases, although this has not been experimentally verified.

The membrane factor is composed of hydrophobic proteins that are highly insoluble in water. Unlike F_1 and OSCP which are peripherally bound to the inner membrane, the polypeptides of the membrane factor are intrinsic proteins that are lodged in the lipid bilayer (62). The biosynthesis of the membrane factor has been studied in yeast, *N. crassa,* and *X. laevis* and intriguing differences have been noted. The ATPase of *S. cerevisiae* contains four mitochondrially translated subunits which correspond to the membrane factor (63). In *N. crassa,* only two of the ATPase subunits have been found to originate in mitochondria (57, 58). The best evidence concerns the DCCD-binding or proteolipid subunit of the membrane factor. This is a low molecular weight protein (7,800 daltons) that has been clearly demonstrated to be a mitochondrial product in yeast (64, 65) and a cytoplasmic product in *N. crassa* (66). The ATPase of *X. laevis* has been reported to have three mitochondrially synthesized subunits (39). The relationship of the mitochondrial products of this enzyme to those of *S. cerevisiae* and *N. crassa,* however, has not been established (58).

Regulation of Synthesis of Mitochondrial Products

Normally mitochondrial and cytoplasmic products are continuously synthesized and used for the assembly of the larger enzyme entities. In order

for this process to be efficient, the two sets of proteins must be produced in a stoichiometric fashion, which implies the existence of some sort of coordinate regulation of the two translational systems (for review see 20). In fact there is good evidence that the rate and extent of mitochondrial protein synthesis are influenced by cytoplasmic translation products. When yeast is inhibited with cycloheximide, mitochondrial protein synthesis proceeds at low rates and for only short duration (67). The rate of synthesis in the presence of cycloheximide is substantially increased if the cells are first incubated in chloramphenicol (67, 68). Presumably, the preincubation leads to an accumulation of cytoribosomal products which stimulate mitochondrial translation upon shifting to cycloheximide.

Cytoplasmic factors have also been observed to affect mitochondrial protein synthesis in vitro. Poyton & Kavanagh (69) have reported that the synthesis of cytochrome oxidase subunits in isolated mitochondria is stimulated by a crude postribosomal fraction of yeast and that the stimulation is proportional to the amount of supernatant added. More significantly, the effect was abolished when antibodies were used to adsorb cytochrome oxidase specific proteins from the cytosolic fraction (69). These experiments suggest that the synthesis of mitochondrial subunits of cytochrome oxidase depends on the presence of their cytoplasmically made counterparts. It is not unreasonable that the synthesis of mitochondrial translation products is tightly coupled to their integration with cytoplasmic subunits and that interruption of the assembly sequence due to the absence of one or more proteins may suppress further synthesis on mitochondrial ribosomes. The trivial explanation, namely that unassembled mitochondrial subunits are more susceptible to degradative enzymes, cannot be excluded however.

In addition to the biochemical evidence there are indications from genetic data of the involvement of cytoplasmic products in the regulation of mitochondrial protein synthesis. Ebner et al (70) have isolated nuclear mutants of *S. cerevisiae* in which the synthesis of just one of the three mitochondrial products of cytochrome oxidase is shut off. Similarly, other nuclear mutations have been reported to specifically block the synthesis of cytochrome *b* or of the ATPase proteolipid without affecting the synthesis of the other mitochondrial products (71).

Although there is abundant evidence that mitochondrial translation and/or transcription are controlled by the nucleocytoplasmic system of the cell (20, 72), the reverse does not appear to hold true. Most of the cytoplasmic products, including those that are destined to be integrated into enzymes containing mitochondrially derived subunits, are synthesized at near normal levels even when mitochondrial synthesis is inhibited. This is best seen in petite mutants of yeast that are totally unable to elaborate any mitochondrial products. Such mutants, nonetheless, have respiratory-deficient mito-

chondrial organelles which, with the exception of the mitochondrial products, have a protein composition identical to those of wild-type yeast (73, 74).

Properties of Mitochondrial Products

Mitochondrial translation products represent some of the most hydrophobic proteins of the inner membrane. They are highly insoluble in water and tend to form large aggregates that can only be dispersed by means of powerful ionic detergents. These properties have been attributed to their high content of nonpolar amino acids (32, 33, 64, 75, 76). The mitochondrially synthesized subunits of cytochrome oxidase, for example, average 10–15% more nonpolar residues than do the other four subunits of the enzyme (32, 33, 75, 76). The ATPase proteolipid is an even more extreme case, having only 23% polar amino acids (64)—this is to be contrasted with values of 50–60% observed in most water soluble proteins (77).

It has been speculated that the hydrophobic character of certain mitochondrial components necessitated an intraorganellar site of synthesis, thereby avoiding the logistic problem of transporting highly insoluble proteins through the aqueous cytosolic phase. This interpretation, however, is not entirely satisfactory in view of the fact that many other hydrophobic proteins of mitochondria are known to be synthesized on cytoplasmic ribosomes and subsequently transported by still undefined mechanisms to the interior of the organelle. The ATPase proteolipid of *N. crassa* is a case in point. Even though the amino acid compositions and primary structures of the yeast and *N. crassa* proteolipids are very similar (78), they have been shown to have different synthetic origins (58). The yeast proteolipid is a well established mitochondrial product (64)—in *N. crassa,* the same protein has been shown to be synthesized in the cytoplasm (57, 65). The hydrophobic properties of this component, therefore, did not interfere with the evolutionary process of transfer of a mitochondrial gene to the nucleus and concomitantly of its transcription and translation in the cytoplasm.

The notion that mitochondria are concerned with the synthesis of proteins with structural functions has not been borne out by the existent evidence. Both cytochrome oxidase (33) and coenzyme QH_2-cytochrome *c* reductase (43, 44) contain at least one hemoprotein carrier synthesized in mitochondria. Our inability to assign functions to the other mitochondrial products arises from a general lack of knowledge about the functions of the different subunit polypeptides of the respiratory and ATPase complexes.

In Vitro Synthesis of Mitochondrial Products

Initial attempts to identify the proteins synthesized by isolated mitochondria were hampered by poor incorporation of radioactive precursors

and technical difficulties in purifying the labeled products from small quantities of mitochondria. These problems have been largely overcome and it is now generally recognized that mitochondria synthesize completed proteins that are identical to the in vivo products. Poyton & Groot (79) have shown that isolated yeast mitochondria are capable of synthesizing the three large subunits of cytochrome oxidase. The in vitro products were integrated into a larger complex which could be precipitated with antisera to some of the smaller cytoplasmically made subunits. Presumably there is a sufficient endogenous pool of the cytoplasmic subunits to allow post-translational assembly of cytochrome oxidase. Although such experiments have not been extended to coenzyme QH_2-cytochrome *c* reductase and the ATPase complex, there is substantial evidence from studies with yeast and mammalian cells (80, 81) that the relative proportions and electrophoretic mobilities of the major mitochondrial products formed in vitro and in vivo are very similar.

Mitochondrial gene products have also been synthesized using poly(A)-RNA purified from yeast mitochondria and translated in an *E. coli* or wheat germ ribosomal system. Padmanaban et al (82) found that total mitochondrial poly(A) containing messenger RNA gave a fourfold stimulation of [^{3}H] leucine incorporation in a cell-free ribosomal system. Some of the proteins made under these in vitro conditions cross-reacted with cytochrome oxidase antibodies and comigrated with the authentic mitochondrially synthesized subunits of the enzyme (82).

A number of laboratories have tried to develop coupled transcription-translation systems to study the gene products of mitochondrial DNA. While there have been some reports of the synthesis of immunochemically reactive cytochrome oxidase gene products when mitochondrial DNA was transcribed and translated in an *E. coli* system (83), in other studies the products formed were of low molecular weight and could not be related to any bona fide mitochondrial proteins (84). In view of recent advances in mitochondrial genetics and the tentative identification of many of the genes on mitochondrial DNA, the usefulness of cell free transcription-translation studies has been somewhat lessened.

MITOCHONDRIAL GENES

Mitochondria contain circular duplex DNA with a molecular weight ranging from $1 \times 10^7 - 5 \times 10^7$, depending on the organism. The larger genome size is more prevalent among fungi, protists, and higher plants. Mammalian mitochondria tend to have smaller DNAs that are generally 1×10^7 daltons (9).

Mit and Syn Genes

Most of our current knowledge of mitochondrial genes has come from studies of *S. cerevisiae* which has proven to be especially suitable for genetic analysis (85). The DNA of this yeast has a molecular weight of 5×10^7 which is equivalent to about 75 kilobases (9). The first genes to be recognized in the yeast genome coded for components of the mitochondrial protein synthetic machinery (9, 86). These have been designated as *syn* genes and they include the transfer and the ribosomal RNAs of mitochondria (9, 86, 87). At least 30 different mitochondrial tRNAs have been detected in *S. cerevisiae* (88). The major tRNA species of this yeast, representing the twenty common amino acids, all have been shown by hybridization to be gene products of mitochondrial DNA (88–91). The total number of isoaccepting species is compatible with the Wobble hypothesis which suggests that in yeast there is no need for importation of cytoplasmic tRNAs into mitochondria. This is probably also true of *N. crassa* (92, 93) but not of animal mitochondria (94–95). For example, most of the tRNAs present in *Tetrahymena* mitochondria are transcribed from nuclear DNA; the mitochondrially encoded species are confined to the four amino acids, leucine, phenylalanine, tryptophan, and tyrosine (96). In at least some organisms, therefore, mitochondria make use of cytoplasmic tRNAs.

In contrast to the RNA components, aminoacyl synthetases, protein synthesis initiation factors, and most of the ribosomal proteins have been shown to be nuclear gene products that are synthesized on cytoplasmic ribosomes (9). There is some evidence, however, that at least one ribosomal protein may be a mitochondrial product. The classical "poky" mutation in *N. crassa* has been correlated with the absence of a specific protein of the small ribosomal subunit (97). In *S. cerevisiae,* the mitochondrial gene product, var 1, has also been found to be a component of the small ribosomal subunit (24).

The second important class of mitochondrial genes are the mit genes which code for proteins that function in electron transport and oxidative phosphorylation. The existence of this class of genes has been suspected for a long time but was only recently established as the result of the isolation of new antibiotic-resistant mutants (98–100) and mit$^-$ mutants (101–104) of *S. cerevisiae.* The mit$^-$ mutants in particular have been instrumental in showing that many of the proteins synthesized by mitochondria are also gene products of mitochondrial DNA. To date, mit$^-$ mutations have been found to affect the three inner membrane complexes, cytochrome oxidase (101–104), coenzyme QH_2-cytochrome *c* reductase (101–104), and the oligomycin-sensitive ATPase (105, 106). Based on genetic analyses of a large number of different mit$^-$ strains, six complementation groups have

been found (107) (Table 1). Three of the complementation groups (*oxi* 1, *oxi* 2, and *oxi* 3) code for products that have been tentatively identified to be the three mitochondrially synthesized subunits of cytochrome oxidases (108–109). Mutations that map in the *cob* complementation group are deficient in cytochrome *b* and have been convincingly shown to be in the structural gene of this respiratory carrier (46, 110). One group of ATPase-deficient mutants (*pho* 2) is now known to have lesions in the proteolipid component of the ATPase (106, 111). This gene also contains the *oil* 1 and *oil* 3 resistance loci (111). The gene products of the other ATPase mutants (*pho* 1) has not yet been identified. Another mitochondrial translation product known to be encoded in mitochondrial DNA is the var 1 protein (23, 112).

Although our information concerning mitochondrial genes is still fragmentary, it is nonetheless evident that of the nine proteins known to be synthesized in mitochondria, six have been documented to be specified by mitochondrial DNA.

Map of Mitochondrial DNA

All of the known mit$^-$ and antibiotic resistance markers of the mitochondrial genome of *S. cerevisiae* have now been localized on the circular map by a combination of genetic and physical mapping methods. Two approaches have been used in genetic mapping. 1. Co-retention and co-deletion of the mutated alleles in the DNA of ρ^- mutants (102, 113, 114). This type of analysis allows mutations to be positioned relative to each other on the wild-type genome and at the same time places limits on the retained segments of DNA in the ρ^- mutants. 2. In some instance mutations have been mapped relative to each other and map distances obtained by recombinational analysis in two and three factorial crosses (46, 102).

Various methods have been devised to locate mitochondrial genes on the physical map. Ribosomal and tRNA genes have been mapped by hybridiza-

Table 1 Complementation groups and gene products of the yeast genome

Enzyme deficiency	Complementation group	Gene product
Cytochrome oxidase	*oxi* 1	subunit 2
Cytochrome oxidase	*oxi* 2	subunit 3
Cytochrome oxidase	*oxi* 3	subunit 1
Coenzyme QH_2-cytochrome *c* reductase	*cob*	cytochrome *b*
ATPase	*pho* 1	?
ATPase	*pho* 2	subunit 9 (proteolipid)

tion to restriction fragments generated from wild-type (115, 116) or ρ^- DNAs (90). A number of mit$^-$ and antibiotic resistance markers have been localized by restriction analysis of genetically marked mit$^-$ and ρ^- clones (117–119). In general, the results obtained by genetic and physical methods have been in good agreement and have provided a fairly unambiguous map of the mitochondrial genome of *S. cerevisiae.* The map presented in Figure 1 shows the positions assigned to the known syn and mit genes as well as the antibiotic resistance markers, most of which have now been related to specific gene products. The map of the yeast genome reveals a considerable dispersity of genes that code for related functions. The three structural genes of cytochrome oxidase are separated by intervening regions containing syn and mit genes. The ribosomal RNA, tRNA, and ATPase genes are also scattered in different regions of the genome. This organizational feature argues against a coordinately controlled expression of genes by means of polycistronic messengers. By analogy with eucaryotic genes, those of the mitochondrion appear to be transcribed individually.

Saturation of the Genome

How inclusive is our present information on the genetic content of the *S. cerevisiae* mitochondrial genome? The evidence that most of the mit type of genes are now known is quite compelling. This is attested to by the fact that the more than a thousand mit$^-$ strains isolated and studied in different laboratories all tend to fall into a limited number of genetic loci that compromise some six complementation groups. It must be kept in mind, however, that the mit$^-$ class of mutants is selected on the basis of its inability to grow on nonfermentable substrates (101, 103, 104). Since this phenotype is most likely to result from mutations in gene products directly involved in respiratory functions (namely electron transport carriers), the selection procedure may exclude mutations that alter mitochondrial functions in more subtle ways. Mutations in regulatory genes that might be expected to reduce or enhance the transcription of a structural gene without necessarily abolishing the capacity for respiration entirely, would not necessarily be recognized by the selection procedures used.

There are also gaps that need to be filled in regard to syn genes. While it is true that in *S. cerevisiae* the major tRNAs have been shown to be transcribed from mitochondrial DNA, there are many isoaccepting species present in mitochondria whose genetic origin and function have not been clarified. Nor is it known whether mitochondria code for protein factors that participate in or regulate mitochondrial protein synthesis. For example, the paramomycin resistance marker, formerly thought to be in the 16S ribosomal RNA gene, has recently been shown to map in a restriction fragment that is adjacent to, but distinct from, the RNA gene (116). This

raises the possibility that paramomycin acts on some yet unknown component of the translation machinery. These and related questions are most likely to be answered when alternative selection methods are devised for the isolation of new types of mitochondrial mutants.

Constancy of the Mitochondrial Genome

The map of the *S. cerevisiae* genome shown in Figure 1 is based on studies of different laboratory strains of this yeast. All the strains examined appear to have the same composition and relative position of genes. This is not to say, however, that intra-species differences do not exist. Both genetic and physical mapping data indicate considerable heterogeneity of the DNA among yeast strains. Detailed physical maps of mitochondrial DNA have now been obtained for at least four different strains. Based on the analysis of HpaII and HaeIII digests, Prunell & Bernardi (120) have concluded that there are frequent strain-specific insertions of deletions of DNA in different parts of the genome. Insertions of up to 3,000 base pairs have been noted in the segment of DNA included between the *oli* 1 and *par* 1 resistance loci (121). This region of the genome seems to be the most variable in the strains studied so far (121).

Genetic data also point to considerable differences in gene spacer regions and even in the internal organization of genes. This is particularly evident in the cytochrome *b* (or *cob* region). In some strains mutations in cytochrome *b* are genetically linked to the *oli* 1 resistance marker—in other

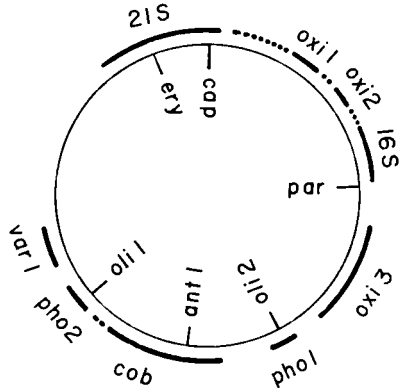

Figure 1 Map of the mitochondrial genome of *S. cerevisiae.* The following designations are used for the antibiotic resistance loci: *cap,* chloramphenicol; *ery,* erythromycin; *par,* paromomycin; *ant,* antimycin; *oli,* oligomycin. The *mit* complementation groups of Table 1 are indicated by the heavy lines. The 16S and 21S refer to the two ribosomal RNA genes and var 1 to the 40,000–43,000 dalton mitochondrial products studied by Butow and co-workers (23, 112). The approximate positions of the tRNA genes are indicated by dots.

strains they are completely unlinked, which suggests that the spacer between the ATPase proteolipid and cytochrome *b* genes varies in length depending on the strain (46). Even more intriguing discrepancies have been found in the properties of cytochrome *b* mutants of different strains. Thus, in some strains of *S. cerevisiae,* cytochrome *b* mutations fall into two genetically unlinked clusters or loci (*cob* 1 and *cob* 2) which behave as a single complementation group (46, 107). In other strains, the cytochrome *b* complementation group has been found to consist of six unlinked loci (*box* 1–6) (122)—many of the *cob* 1 and *cob* 2 mutations are nonetheless allelic to mutations in the various *box* loci (100). These observations are most simply explained by assuming the existence of insertions within the structural gene of cytochrome *b.* The genetic data indicate that the number and length of inserted sequences in the cytochrome *b* gene may be strain-dependent.

When mitochondrial DNA of *S. cerevisiae* is compared to other organisms, the divergences are much more profound. Aside from differences in physical size, buoyant density, and restriction maps, there is also considerable variation in the genetic content of mitochondrial DNA. At present such information is limited almost entirely to the ribosomal and transfer RNA genes. In *N. crassa* the ribosomal RNAs are transcribed as a single 32S precursor (123) and hybridization of the mature RNAs to restriction fragments indicates that they have a proximal location on the genome (93). This also appears to be true of most animal mitochondrial genomes (124, 125). There are equally significant differences in the number of tRNA genes. In *S. cerevisiae* and *N. crassa,* tRNA species corresponding to the twenty amino acids have been shown to be transcribed from mitochondrial DNA (88–91, 93). Animal mitochondria, however, have fewer tRNA genes (86, 87, 95). It was already pointed out that in *Tetrahymena* isoacceptors for only four amino acids are capable of hybridizing to mitochondrial DNA (96). In mammalian mitochondria, the number of hybridizable species is larger but again some of the organellar tRNAs appear to be imported from the cytoplasm. Attardi and co-workers have reported that in HeLa cells the tRNAs for twelve amino acids hybridize to the heavy strand of mitochondrial DNA, five other amino acids hybridize to the light strand, and four fail to hybridize altogether. Both strands of this genome are therefore transcribed (126). It is not known if this is also true for mitochondrial DNA of *S. cerevisiae.*

ATPase PROTEOLIPID

The proteolipid of the yeast ATPase is presently the best understood mitochondrial gene and translation product. This protein is particularly interest-

ing since there is now very good evidence that in *N. crassa* it is coded by a nuclear gene (127) and is translated on cytoplasmic ribosomes (58). The transfer of a gene from the mitochondrion to the nucleus (or vice versa) represents an important change in the cell which might be experimentally exploited to test some of the current theories of mitochondrial evolution (128, 129).

DCCD-Binding Properties

Dicyclohexylcarbodiimide (DCCD) is a potent inhibitor of oxidative phosphorylation and of the mitochondrial ATPase (130). Cattell et al (131) showed that DCCD reacts covalently with a low molecular weight proteolipid of beef heart mitochondria which could be extracted with chloroform: methanol and purified on silicic acid. The proteolipid was named the DCCD-binding protein and was later shown to be a component of the oligomycin-sensitive ATPase (58, 132). The DCCD-binding protein has also been found to be present in the yeast ATPase (65). The yeast protein is one of the mitochondrially synthesized subunits (subunit 9) of the ATPase (63, 64). It is interesting that there are approximately six molecules of the protein per enzyme and these form a hexameric complex that resists depolymerization by SDS and other detergents (58, 63). The hexamer, however, is dissociated by organic solvents and by strong alkali or base (64). Although the DCCD-binding protein has also been demonstrated to be a component of the *N. crassa* ATPase, it is synthesized in this organism on cytoplasmic rather than mitochondrial ribosomes. Jackl & Sebald (57) have shown that the *N. crassa* proteolipid becomes labeled with a radioactive amino acid in the presence of chloramphenicol but not cycloheximide. Similar to the yeast enzyme, the *N. crassa* ATPase appears to have six proteolipid subunits but a stable hexameric complex has not been detected (57, 58).

Function

In addition to binding DCCD, the proteolipid probably also contains the oligomycin binding site of the ATPase. Criddle et al (133) have postulated that one of the keto groups of oligomycin may form a Schiff base with an amino group on the protein. When the yeast ATPase was reacted with oligomycin and reductively alkylated with [^{3}H] sodium borohydride, the radioactive label was associated exclusively with a protein that was soluble in chloroform: methanol and whose electrophoretic migration on SDS polyacrylamide gels was identical to the proteolipid. Since ATPase preparations obtained from oligomycin-resistant mutants failed to incorporate the radioactive label into the proteolipid, these authors concluded that the oligomycin-binding site of the ATPase resides in the proteolipid (133).

Although it is reasonable to conclude that the binding sites for DCCD, oligomycin, and perhaps other inhibitors of the ATPase are present in the proteolipid, the precise function of this protein is still obscure. There is some recent evidence, however, suggesting that the proteolipid may act as a protonophore. It has been reported that the yeast proteolipid increases the permeability of artificial lipid membranes to protons (134). These experiments were especially interesting in view of the fact that proton transport in this model system was inhibited by oligomycin when the source of proteolipid was a wild-type oligomycin-sensitive strain of yeast. When the protein was obtained from an oligomycin-resistant mutant, the sensitivity to the inhibitor was decreased. Whether the proteolipid acts as a proton carrier or channel needs to be studied further but the above results are consistent with the earlier suggestion that oligomycin inhibits oxidative phosphorylation by blocking the discharge of protons generated during electron transport across the inner membrane (135).

Amino Acid Sequence

It is possible to obtain high yields of pure ATPase proteolipid directly from mitochondria by a relatively simple procedure involving extraction with chloroform : methanol and thin layer chromatography. This fact, combined with its small size, have made it possible to sequence the ATPase proteolipids of yeast and other types of mitochondria (111, 136). The primary structure of the various proteolipids was established by solid phase sequencing of the total polypeptide and of cyanogen bromide fragments.

The primary structures of the proteolipids from *S. cerevisiae* and *N. crassa* are shown in Figure 2. Both proteins are extremely hydrophobic as was already evident from their amino acid compositions. Even though the yeast and *N. crassa* proteins are synthesized in two different compartments of the cell, they show a great deal of sequence homology—this is especially evident in the amino acid sequences spanning residues 17–41 and 51–68. It is also significant that DCCD has been found to bind covalently to the glutamic acid residues that occur at positions 59 and 64 in the yeast and *N. crassa* proteolipids, respectively. The sequence homologies and identity of the DCCD-binding residues argue strongly in favor of a common genetic derivation of the two proteins. It is also interesting to note that the *N. crassa* proteolipid contains five extra amino acids at the amino terminus and has a tyrosine instead of a formyl methionine as the amino terminal residue. The biosynthetic evidence on the sites of translation of the two proteins is thus nicely complemented by the sequence data. Since mitochondria utilize formyl methione as the initiator (22), the occurrence of this amino acid in the yeast but not the *N. crassa* protein is consistent with their proposed synthetic origins (58).

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                                   5                  10                  15
                  f -Met-Gln-Leu-Val-Leu-Ala-Ala-Lys-Tyr-Ile-Gly-Ala-Gly-Ile-Ser-Thr-Ile-Gly-Leu-

                 5                  10                  15                  20                  25
Tyr-Ser-Ser-Glu-Ile-Ala-Gln-Ala-Met-Val-Glu-Val-Ser-Lys-Asn-Leu-Gly-Met-Gly-Ser-Ala-Ala-Ile-Gly-Leu-

 20                  25                  30                  35                  40
Leu-Gly-Ala-Gly-Ile-Gly-Ile-Ala-Ile-Val-Phe-Ala-Ala-Leu-Ile-Asn-Gly-Val-Ser-Arg-Asn-Pro-Ser-Ile-Lys-

                 30                  35                  40                  45                  50
Thr-Gly-Ala-Gly-Ile-Gly-Ile-Gly-Leu-Val-Phe-Ala-Ala-Leu-Leu-Asn-Gly-Val-Ala-Arg-Asn-Pro-Ala-Leu-Arg-

 45                  50                  55                  60                  65
Asp-Thr-Val-Phe-Pro-Met-Ala-Ile-Leu-Gly-Phe-Ala-Leu-Ser-Glu-Ala-Thr-Gly-Leu-Phe-Cys-Leu-Met-Val-Ser-

                 55                  60                  65                  70                  75
Gly-Glu-Leu-Phe-Ser-Tyr-Ala-Ile-Leu-Gly-Phe-Ala-Phe-Val-Glu-Ala-Ile-Gly-Leu-Phe-Asp-Leu-Met-Val-Ala-

 70                  75
Phe-Leu-Leu-Leu-Phe-Gly-Val

                 80
Leu-Met-Ala-Lys-Phe-Thr
```

Figure 2 Primary structures of the ATPase proteolipids (DCCD-binding protein) of *S. cerevisiae* and *N. crassa.* The yeast protein (upper sequence) starts with an fMet at the amino terminus and the *Neurospora* protein (lower sequence) with a tyrosine.

Genetic Specification of the Proteolipid

There was some earlier evidence that the *oli* 1 resistance locus is in the structural gene of the proteolipid (137, 138). Mutations in *oli* 1 were found to modify certain properties of the yeast proteolipid (137). The localization of alleles conferring the resistance phenotype in the structural gene of the proteolipid has been fully confirmed by protein sequence data (111). At present two different amino acid substitutions are known to occur in *oli* 1 resistant mutants. In one group of mutants a serine replaces cysteine at position 65 (111); in another, a phenylalanine replaces leucine at position 53 (78, 111). *Oli* 3 (*oli* 3 and *oli* 1 resistant mutants are distinguished by the cross-resistance of the former but not the latter to venturicidin) and *pho* 2 mutants also have amino acid substitutions in the proteolipid (111). These data established conclusively that in yeast, the ATPase proteolipid is a product of a mitochondrial gene.

Sebald et al (127) have isolated oligomycin-resistant mutants of *N. crassa.* The growth of these strains is inhibited by oligomycin and as in yeast this property results from a decreased sensitivity of the mitochondrial ATPase

to the antibiotic. In contrast to yeast, however, all the oligomycin-resistant strains of *N. crassa* were determined to have nuclear mutations that were linked to markers on chromosome VII (127). The amino acid sequence of the proteolipid from one such strain indicated a substitution of a serine for a phenylalanine at residue 61 (127). These results indicate that the structural gene of the proteolipid of *N. crassa* is in nuclear DNA and explains the extramitochondrial site of translation of the protein in this organism (47).

The biosynthesis and genetics of the proteolipid of animal cells have not been studied. There is some evidence that in *X. laevis* the proteolipid is synthesized in the cytoplasm and is probably a nuclear gene product (39). If this can be confirmed it would suggest that there may have been an early evolutionary transfer of this mitochondrial gene to the nucleus and that in more highly evolved eucaryotic cells it is a nuclear gene product whose messenger is translated in the cytoplasm.

Sequence of the Proteolipid Gene of Saccharomyces cerevisiae

Mitochondrial DNA is an especially attractive material for sequencing since it is present only in eucaryotic organisms, but in view of its postulated procaryotic origin, it may preserve some features of both types of genomes. In addition, it is of sufficiently small size that one may hope to obtain its complete nucleotide sequence. This information would be useful in understanding how mitochondrial genes are organized and perhaps how their expression may be regulated. Since DNA sequence data can be most meaningfully interpreted if the primary structure of the gene products is known, the ATPase proteolipid gene has been an obvious choice for sequencing.

As a result of recent studies, the complete nucleotide sequence of the yeast proteolipid gene has been obtained (Figure 3). These data were obtained from the analysis of mitochondrial DNA of a ρ^- mutant that contained the genetic markers *oli* 1 and *pho* 2 but had lost all the other currently known markers of the yeast genome (140). The retained segment of mitochondrial DNA in the ρ^- mutant was ascertained to be 1.8 kilobases long or approximately 2.5% of the original genome. Since *oli* 1 and *pho* 2 were known to be within the structural gene of the proteolipid, the 1.8-kilobase piece of DNA could safely be assumed to have the structural gene sequence.

The DNA sequence shows that there is an almost complete agreement between the primary structure of the proteolipid and the nucleotide sequence of its gene. The one exception is the amino acid residue at position 46 which, from the DNA data, should be a leucine rather than a threonine. The DNA sequence also confirms the utilization of the codons of the

```
                  1                   5                  10                  15
                fMet-Gln-Leu-Val-Leu-Ala-Ala-Lys-Tyr-Ile-Gly-Ala-Gly-Ile-Ser-Thr-Ile-
TAATAAATAAATATT ATG CAA TTA GTA TTA GCA GCT AAA TAT ATT GGA GCA GGT ATC TCA ACA ATT

         20                  25                  30                  35
Gly-Leu-Leu-Gly-Ala-Gly-Ile-Gly-Ile-Ala-Ile-Val-Phe-Ala-Ala-Leu-Ile-Asn-Gly-Val-Ser-
GGT TTA TTA GGA GCA GGT ATT GGT ATT GCT ATC GTA TTC GCA GCT TTA ATT AAT GGT GTA TCA

     40                  45                  50                  55
Arg-Asn-Pro-Ser-Ile-Lys-Asp-Leu-Val-Phe-Pro-Met-Ala-Ile-Phe-Gly-Phe-Ala-Leu-Ser-Glu-
AGA AAC CCA TCA ATT AAA GAC CTA GTA TTC CCT ATG GCT ATT TTT GGT TTC GCC TTA TCA GAA

 60                  65                  70                  75
Ala-Thr-Gly-Leu-Phe-Cys-Leu-Met-Val-Ser-Phe-Leu-Leu-Leu-Phe-Gly-Val-Ochre        Ochre
GCT ACA GGT TTA TTC TGT TTA ATG GTT TCA TTC TTA TTA TTA TTC GGT GTA TAA TATATA TAA
```

Figure 3 Nucleotide sequence of the ATPase proteolipid gene of *S. cerevisiae.* The DNA used for sequencing was obtained from a strain that was resistant to oligomycin (*oli* 1 locus) and was shown to have a substitution of a phenylalanine for a leucine at residue 53 of the proteolipid (142).

universal code by mitochondria and of one of the standard termination codons (ochre). Despite the fact that the universal codons are used, there appears to be little degeneracy in the code. This is seen in the preferential utilization of the UUA codon for leucine and UUC for phenylalanine. Whether this is true for other mitochondrial genes will have to await further sequence data. Another feature of the DNA that is not shown in Figure 3, is a long sequence rich in A+T that follows the gene. Some 1,000 nucleotides following the structural gene have been sequenced and, with the exception of two short regions that are rich in G+C, the DNA consists almost exclusively of A+T. The function of the A+T- and G+C- rich sequences which occur throughout the entire genome (141) is not yet known but may be important in the regulation of gene expression.

FUTURE PROBLEMS

Much of the recent work in mitochondrial biogenesis has revolved around the following three questions. What are the mechanisms of assembly of inner membrane complexes that contain both cytoplasmically and mitochondrially made subunits? What are the constituent genes of mitochondrial DNA? How are mitochondrial genes organized and what regulates their expression?

The progress made on all three fronts has been impressive and, as a consequence, many young investigators have been attracted to an already

fast growing field. There are still outstanding problems that need to be resolved and it is possible to make some predictions as to what approaches will be taken during the next few years.

The assembly of multisubunit enzymes such as cytochrome oxidase and the ATPase is still a poorly understood process. There are several related questions that can best be studied with mutants that have lesions in either structural or regulatory genes that control the biosynthesis of these enzymes. Fortunately there are now a large number of mitochondrial as well as nuclear mutants of *S. cerevisiae* that can be put to use in exploring the sequence in which the different subunits are integrated during the course of enzyme assembly. Many steps in bacteriophage genesis have been deduced from the intermediates found in assembly-deficient mutants and this basic approach may be equally successful in probing the assembly of mitochondrial enzymes. In addition, mutants can be used to study possible regulatory proteins that modulate the expression of mitochondrial structural genes. It was pointed out that in *S. cerevisiae,* there are nuclear mutations that selectively shut off the synthesis of single mitochondrial translation products. Such strains are likely to have mutations in regulatory proteins that may repress or activate the transcription or translation of specific mitochondrial genes. This interesting class of mutants has received little attention up to now and may provide clues as to how cytoplasmic factors regulate mitochondrial gene expression.

Even though most of the structural genes of mitochondrial DNA are now known, it is not excluded that other genes may be present that have not yet been identified. It is also still questionable whether the minor isoaccepting species of tRNAs present in mitochondria are separate gene products of mitochondrial DNA or post-transcriptional modification products. The complete saturation of the mitochondrial genome requires more extensive searches for mutants employing new selection procedures. It is difficult to understand, for example, why only a few tRNA and ribosomal RNA mutants have been found up to now. Equally strange is that no single mutant in the var 1 protein has been isolated despite the fact that it is the largest gene product of mitochondrial DNA. These observations tend to indicate that mutations in certain mitochondrial genes may not be clearly expressed and may not necessarily lead to total absence of growth on nonfermentable substrates, the standard test currently used to obtain mitochondrial mutants.

The third and probably most promising line of research is the sequence analysis of mitochondrial DNA. The first efforts in this direction have yielded promising results in the sense that most of the techniques that have been developed in recent years for the sequencing of procaryotic and eu-

caryotic DNAs can be applied without major complications to mitochondrial DNA (139). Since the mitochondrial genome is many orders of magnitude smaller than the smallest nuclear DNA, it is safe to assume that this will be the first eucaryotic DNA to be completely sequenced. Knowledge of the DNA sequence should answer the already mentioned question of whether the organization of mitochondrial genes follows the general outlines of eucaryotic or procaryotic genomes. Furthermore, it will tell us something about the regulation of the genome. Finally, the DNA sequence may turn out to be the least cumbersone way of obtaining the primary structure of mitochondrial translation products that have proven to be difficult to sequence by conventional methods.

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Literature Cited

1. McLean, J. R., Cohn, G. L., Brandt, I. K., Simpson, M. V. 1958. *J. Biol. Chem.* 233:657–63
2. Roodyn, D. B., Reis, P. J., Work, T. S. 1961. *Biochem. J.* 80:9–21
3. Beattie, D. S., Basford, R. E., Koritz, S. B. 1967. *J. Biol. Chem.* 242:3366–68
4. Lamb, A. J., Clark-Walker, G. D., Linnane, A. W. 1968. *Biochim. Biophys. Acta* 161:415–27
5. Mahler, H. R., Perlman, P. S. 1971. *Biochemistry.* 10:2977–90
6. Slonimski, P. P. 1953. *La Formation des Enzymes Respiratoire Chez la Levure.* Paris: Masson
7. Mounolou, J. C., Jakob, H., Slonimski, P. P. 1966. *Biochem. Biophys. Res. Commun.* 24:281–24
8. Nagley, P., Linnane, A. W. 1970. *Biochem. Biophys. Res. Commun.* 39: 989–96
9. Borst, P. 1972. *Ann. Rev. Biochem.* 41:333–76
10. Hatefi, Y. 1963. *Enzymes* 7:495–515
11. Hatefi, Y., Haavik, A. G., Griffiths, D. E. 1962. *J. Biol. Chem.* 237:1676–80
12. Ziegler, D. M., Doeg, K. A. 1962. *Arch. Biochem. Biophys.* 97:41–50
13. Hatefi, Y., Haavik, A. G., Griffiths, D. E. 1962. *J. Biol. Chem.* 237:1681–85
14. Fowler, L. R., Richardson, S. H., Hatefi, Y. 1962. *Biochim. Biophys. Acta* 64:170–73
15. Racker, E. 1977. *Ann. Rev. Biochem.* 46:1006–14
16. Pedersen, P. L. 1975. *Bioenergetics* 6: 243–75
17. Schatz, G. 1968. *J. Biol. Chem.* 243: 2192–99
18. Tzagoloff, A. 1969. *J. Biol. Chem.* 244: 5027–33
19. Ashwell, M., Work, T. S. 1970. *Ann. Rev. Biochem.* 39:251–90
20. Schatz, G., Mason, T. L. 1974. *Ann. Rev. Biochem.* 43:51–87
21. Bandlow, W. 1976. In *Genetics and Biogenesis of Chloroplasts and Mitochondria,* ed. Th. Bücher, W. Neupert, W. Sebald, S. Werner, pp. 819–26. Amsterdam: North-Holland
22. Feldman, F., Mahler, H. R. 1974. *J. Biol. Chem.* 249:3202–9
23. Douglas, M. G., Kendrick, E., Boulikas, P., Perlman, P., Butow, R. A. 1976. In *The Genetic Function of Mitochondrial DNA,* ed. C. Saccone, A. M. Kroon, pp. 199–207. Amsterdam: North-Holland
24. Groot, G. S. P., Grivell, L. A., van Harten-Loosbroek, N., Kreike, J., Moorman, A. F. M., van Ommen, G. J. B. 1979. In *Structure and Function of Energy Transducing Membranes,* ed. K. Van Dam, B. F. Van Gelder. Amsterdam: North-Holland. In press
25. Mahler, H. R., Perlman, P., Henson, C., Weber, C. 1968. *Biochem. Biophys. Res. Commun.* 31:474–80
26. Gorts, C. P. M., Hasilik, A. 1972. *Eur. J. Biochem.* 29:282–87

27. Rubin, M. S., Tzagoloff, A. 1973. *Fed. Proc.* 32:641 (Abstr.)
28. Lin, L. F. H., Kim, I. C., Beattie, D. S. 1974. *Arch. Biochem. Biophys.* 160: 458–64
29. Weiss, H., Sebald, W., Bücher, Th. 1971. *Eur. J. Biochem.* 22:19–26
30. Mason, T. L., Poyton, R. O., Wharton, D. C., Schatz, G. 1973. *J. Biol. Chem.* 248:1346–54
31. Rubin, M. S., Tzagoloff, A. 1973. *J. Biol. Chem.* 248:4269–74
32. Capaldi, R. A. 1978. In *Molecular Biology of Membranes,* ed. S. Fleischer, Y. Hatefi, D. H. MacLennan, A. Tzagoloff, pp. 103–19. New York: Plenum
33. Tzagoloff, A., Akai, A., Rubin, M. S. 1974. In *The Biogenesis of Mitochondria,* ed. A. M. Kroon, C. Saccone, pp. 405–21. New York: Academic
34. Schatz, G., Groot, G. S. P., Mason, T. L., Rouslin, W., Wharton, D. C., Saltzgaber, J. 1972. *Fed. Proc.* 31:21–29
35. Sebald, W., Weiss, H., Jackl, G. 1972. *Eur. J. Biochem.* 30:413–17
36. Schwab, A. J., Sebald, W., Weiss, H. 1972. *Eur. J. Biochem.* 30:511–16
37. Mason, T. L., Schatz, G. 1973. *J. Biol. Chem.* 248:1355–60
38. Rubin, M. S., Tzagoloff, A. 1973. *J. Biol. Chem.* 248:4275–79
39. Koch, G. 1976. *J. Biol. Chem.* 251: 6097–6107
40. Katan, M. B., Pool, L., Groot, G. S. P. 1967. *Eur. J. Biochem.* 65:95–105
41. Weiss, H., Juchs, B. 1978. *Eur. J. Biochem.* 88:17–28
42. Rieske, J. S. 1976. *Biochim. Biophys. Acta* 456:195–247
43. Weiss, H. 1972. *Eur. J. Biochem.* 30: 469–78
44. Weiss, H., Ziganke, B. 1974. *Eur. J. Biochem.* 41:63–71
45. Weiss, H., Ziganke, B. 1976. See Ref. 21, pp. 259–66
46. Tzagoloff, A., Foury, F., Akai, A. 1976. *Mol. Gen. Genet.* 149:33–42
47. Weiss, H., Ziganke, B. 1977. In *Mitochondria 1977, Genetics and Biogenesis of Mitochondria,* ed. W. Bandlow, R. J. Schweyen, K. Wolf, F. Kaudewitz, pp. 441–49. Berlin: Walter de Gruyter
48. Katan, M. B., Van Harten-Loosbroek, N., Groot, G. S. P. 1976. *Eur. J. Biochem.* 70:409–17
49. Ross, E., Schatz, G. 1976. *J. Biol. Chem.* 251:1997–2004
50. Tzagoloff, A. 1977. In *The Enzymes of Biological Membranes,* ed. A. Martonosi, 2:103–24. New York: Plenum
51. Tzagoloff, A., Meagher, P. 1971. *J. Biol. Chem.* 246:7328–36
52. Kagawa, Y., Racker, E. 1966. *J. Biol. Chem.* 241:2467–74
53. Tzagoloff, A., MacLennan, D. H., Byington, K. H. 1968. *Biochemistry* 7: 1596–602
54. Pullman, M. E., Penefsky, H. S., Datta, A., Racker, E. 1960. *J. Biol. Chem.* 235:3322–29
55. MacLennan, D. H., Tzagoloff, A. 1968. *Biochemistry.* 7:1603–10
56. Senior, A. E., Brooks, J. C. 1970. *Arch. Biochem. Biophys.* 140:257–66
57. Jackl, G., Sebald, W. 1975. *Eur. J. Biochem.* 54:97–106
58. Sebald, W. 1977. *Biochim. Biophys. Acta* 463:1–27
59. Mendiola-Morgenthaler, L. R., Morgenthaler, J. J., Price, C. A. 1976. *FEBS Lett.* 62:96–100
60. MacLennan, D. H., Akai, J. 1968. *Biochem. Biophys. Res. Commun.* 33: 441–47
61. Tzagoloff, A. 1970. *J. Biol. Chem.* 245:1545–51
62. Kagawa, Y., Racker, E. 1966. *J. Biol. Chem.* 247:2475–82
63. Tzagoloff, A., Meagher, P. 1971. *J. Biol. Chem.* 247:594–603
64. Sierra, M. F., Tzagoloff, A. 1973. *Proc. Natl. Acad. Sci. USA* 70:3155–59
65. Sebald, W. 1979. *Eur. J. Biochem.* In press
66. Sebald, W., Graf, T., Wild, G. 1976. See Ref. 21, pp. 167–74
67. Tzagoloff, A. 1971. *J. Biol. Chem.* 246:3050–56
68. Beattie, D. S., Lin, L.-F., Stuchell, R. N. 1974. See Ref. 33, pp. 465–75
69. Poyton, R. O., Kavanagh, J. 1976. *Proc. Natl. Acad. Sci. USA* 73:3947–51
70. Ebner, E., Mason, T. L., Schatz, G. 1973. *J. Biol. Chem.* 248:5369–78
71. Tzagoloff, A., Akai, A., Needleman, R. B. 1975. *J. Biol. Chem.* 250:8228–35
72. Mahler, H., Bastos, R. N., Feldman, F., Flury, U., Lin, C. C., Perlman, P. S., Pham, S. H. 1976. In *Membrane Biogenesis,* ed. A. Tzagoloff, pp. 15–61. New York: Plenum
73. Groot, G. S. P., Rouslin, W., Schatz, G. 1972. *J. Biol. Chem.* 247:1735–42
74. Weislogel, P. O., Butow, R. A. 1971. *J. Biol. Chem.* 246:5113–19
75. Sebald, W., Machleidt, W., Otto, J. 1973. *Eur. J. Biochem.* 38:311–24
76. Poyton, R. O., Schatz, G. 1975. *J. Biol. Chem.* 250:752–61
77. Capaldi, R. A., Vanderkooi, G. 1972. *Proc. Natl. Acad. Sci. USA* 69:930–32
78. Wachter, E., Sebald, W., Tzagoloff, A. 1977. See Ref. 47, pp. 441–49

79. Poyton, R. O., Groot, G. S. P. 1975. *Proc. Natl. Acad. Sci. USA* 72:172–76
80. Ibrahim, N. G., Burke, J. P., Beattie, D. S. 1973. *FEBS Lett.* 29:73–76
81. Lederman, M., Attardi, G. 1973. *J. Mol. Biol.* 78:275–83
82. Padmanaban, G., Hendler, F., Patzer, J., Ryan, R., Rabinowitz, M. 1975. *Proc. Natl. Acad. Sci. USA* 72:4293–97
83. Scragg, A. H., Thomas, D. Y. 1975. *Eur. J. Biochem.* 56:183–192
84. Moorman, A. F. M., Grivell, L. A. 1976. See Ref. 23, pp. 281–89
85. Coen, D., Deutsch, J., Netter, P., Petrochilo, E., Slonimski, P. P. 1970. In *Control of Organelle Development,* pp. 449–96. London: Cambridge Univ. Press
86. Nass, M. M. K., Buck, C. A. 1970. *J. Mol. Biol.* 54:187–98
87. Suyama, Y. 1976. In *Cell Biology,* ed. P. L. Altmann, D. D. Katz, pp. 228–30. Bethesda: FASEB
88. Martin, N. C., Rabinowitz, M. 1968. *Biochemistry.* 17:1628–34
89. Casey, J. W., Hsu, H. J., Getz, G. S., Rabinowitz, M., Fukuhara, H. 1974. *J. Mol. Biol.* 88:735–47
90. Martin, N. C., Rabinowitz, M., Fukuhara, H. 1977. *Biochemistry.* 21: 4672–77
91. Martin, R., Schneller, J. M., Stahl, A. J. C., Dirheimer, G. 1976. See Ref. 21, pp. 755–58
92. Barnett, W. E., Brown, D. H. 1967. *Proc. Natl. Acad. Sci. USA* 57:452–58
93. Kroon, A. M., Terpstra, P., Holtrop, M., deVries, H., van der Bogert, C., de Jonge, J., Agsteribbe, E. 1976. See Ref. 21, pp. 685–96
94. Dawid, I. B. 1972. *J. Mol. Biol.* 63: 201–16
95. Lynch, D. C., Attardi, G. 1976. *J. Mol. Biol.* 102:125–41
96. Suyama, Y., Hamada, J. 1976. See Ref. 21, pp. 763–70
97. Lambowitz, A. M., Chua, N.-H., Luck, D. J. L. 1976. *J. Mol. Biol.* 107:223–53
98. Avner, P. R., Coen, D., Dujon, B., Slonimski, P. P. 1973. *Mol. Gen. Genet.* 125:9–52
99. Pratje, E., Michaelis, G. 1977. *Mol. Gen. Genet.* 152:167–74
100. Colson, A. M., Slonimski, P. P. 1978. See Ref. 47, pp. 185–98
101. Tzagoloff, A., Akai, A., Needleman, R. B., Zulch, G. 1975. *J. Biol. Chem.* 250:8236–42
102. Slonimski, P. P., Tzagoloff, A. 1976. *Eur. J. Biochem.* 61:27–41
103. Rytka, J., English, K. J., Hall, R. M., Linnane, A. W., Lukins, H. B. 1976. See Ref. 21, pp. 427–34
104. Mahler, H. R., Bilinski, T., Miller, D., Hanson, D. 1976. See Ref. 21, pp. 857–63
105. Foury, F., Tzagoloff, A. 1976. *Eur. J. Biochem.* 68:113–19
106. Coruzzi, G., Trembath, M. K., Tzagoloff, A. 1979. *Eur. J. Biochem.* In press
107. Foury, F., Tzagoloff, A. 1978. *J. Biol. Chem.* 253:3792–97
108. Cabral, F., Rudin, Y., Solioz, M., Schatz, G., Clavilier, L., Slonimski, P. P. 1978. *J. Biol. Chem.* 243:297–304
109. Tzagoloff, A., Foury, F., Macino, G. 1978. In *Biochemistry and Genetics of Yeast,* ed. M. Bacila, B. L. Horecker, A. Stoppani, pp. 477–88. New York: Academic
110. Claisse, M. L., Spyridakis, A., Slonimski, P. P. 1977. See Ref. 47, pp. 337–44
111. Sebald, W., Wachter, E., Tzagoloff, A. 1979. *Eur. J. Biochem.* Submitted for publication
112. Perlman, P. S., Douglas, M. G., Strausberg, R. L., Butow, R. A. 1977. *J. Mol. Biol.* 115:675–94
113. Nagley, P., Spriprakash, K. S., Rytka, J., Choo, K. B., Trembath, M. K., Lukins, H. B., Linnane, A. W. 1976. See Ref. 23, pp. 231–42
114. Schweyen, R. J., Kaudewitz, F. 1976. *Mol. Gen. Genet.* 149:311–22
115. Van Ommen, G. J. B., Groot, G. S. P., Borst, P. 1977. *Mol. Gen. Genet.* 154: 255–62
116. Borst, P., Bos, J. L., Grivell, L. A., Groot, G. S. P., Heyting, C., Moorman, A. F. M., Sanders, J. P. M., Talen, J. L., Van Kreiyl, C. F., Van Ommen, G. J. B. 1977. See Ref. 47, pp. 255–70
117. Lewin, A., Morimoto, R., Merten, S., Martin, N., Berg, P., Christianson, T., Levens, D., Rabinowitz, M. 1977. See Ref. 47, pp. 271–89
118. Grivell, L. A., Moorman, A. F. M. 1977. See Ref. 47, pp. 371–84
119. Morimoto, R., Merten, S., Lewin, A., Martin, N., Rabinowitz, M. 1978. *Mol. Gen. Genet.* 163:241–55
120. Prunell, A., Bernardi, G. 1977. *J. Mol. Biol.* 110:53–74
121. Sanders, J. P. M., Heyting, C., Borst, P. 1976. See Ref. 21, pp. 511–17
122. Pajot, P., Wambire-Kluppel, M. L., Kotylak, Z., Slonimski, P. P. 1976. See Ref. 21, pp. 443–51
123. Kuriyama, Y., Luck, D. J. L. 1973. *J. Mol. Biol.* 73:425–37
124. Wellauer, P. K., Dawid, I. B. 1973. *Proc. Natl. Acad. Sci. USA* 70:2827–31
125. Wu, M., Davidson, N., Attardi, G., Aloni, Y. 1972. *J. Mol. Biol.* 71:81–83
126. Attardi, G., Albring, M., Amalric, F.,

Gelfand, R., Griffith, J., Lynch, D., Merkel, C., Murphy, W., Ojala, D. 1977. See Ref. 21, pp. 573–85
127. Sebald, W., Sebald-Althaus, M., Wachter, E. 1977. See Ref. 47, pp. 433–40
128. Margulis, L. 1970. *Origin of Eukaryotic Cells.* New Haven: Yale Univ. Press
129. Bogorad, L. 1975. See Ref. 72, pp. 201–45
130. Beechey, R. B., Roberton, A. M., Holloway, T., Knight, I. G. 1967. *Biochemistry* 6:3867–79
131. Cattell, K. J., Lindop, C. R., Knight, I. G., Beechey, R. B. 1971. *Biochem. J.* 125:169–77
132. Stekhoven, F. S., Waitkus, R. F., Van Moerkerk, T. B. 1972. *Biochemistry* 11: 1144–50
133. Criddle, R. S., Arulanadan, C., Edwards, T., Johnston, R., Scharf, S., Enns, R. 1977. See Ref. 21, pp. 151–57
134. Criddle, R. S., Packer, L., Shieh, P. 1977. *Proc. Natl. Acad. Sci. USA* 74: 4306–10
135. Mitchell, P. 1973. *FEBS Lett.* 33: 267–74
136. Wachter, E., Sebald, W. 1979. *Eur. J. Biochem.* Submitted for publication
137. Tzagoloff, A., Akai, A., Foury, F. 1976. *FEBS Lett.* 65:391–96
138. Groot Obbink, D. J., Hall, R. M., Linnane, A. W., Lukins, H. B., Monk, B. C., Spithill, T. W., Trembath, M. K. 1976. See Ref. 23, pp. 163–73
139. Macino, G., Tzagoloff, A. 1979. *Proc. Natl. Acad. Sci. USA.* 76:131–35
140. Dujon, B., Colson, A. M., Slonimski, P. P. 1977. See Ref. 47, pp. 579–669
141. Bernardi, G. 1977. See Ref. 21, pp. 503–10
142. Macino, G., Tzagoloff, A. 1979. *J. Biol. Chem.* In press