*Structure and Function of Mitochondrial Nucleic Acids**

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Introduction

50 years ago many scientists believed that all eukaryotic cells contain inclusions which are able to propagate autonomously; that means independently from the nucleus. In the course of the last fifty years these socalled symbionts have been identified as the cell-organelles that we now designate mitochondria and chloroplasts. However, the idea of autonomy of these organelles has never been skipped completely. In a recent *Nature*-issue[1] we could still read: "chloroplast culture with replication is now a realistic possibility". Similar predictions with respect to mitochondria can be found in the literature. The question is now: are these predictions correct? and the answer is: No! *Not* because mitochondria are not completely equipped for autonomic propagation activities such as replication, transcription and translation (for they are), but because of the fact that the amount of genetic information present within mitochondria does not meet all the needs for a completely autonomous existence of mitochondria in the cell.

So at the one hand we are faced with the presence within mitochondria of nucleic acids and the machineries to multiply them and to express their genetic information, at the other hand the contribution of mitochondria to their own synthesis has to be limited and modest.

In this paper two main points are discussed:

I. The question what we know about the structure of mitochondrial nucleic acids and how we recognize them as real mitochondrial components.
II. Our present state of knowledge about the functions these nucleic acids fulfill and how their functions can be studied in biological systems.

Mitochondrial DNA

Mitochondria from all organisms studied so far contain DNA. The crucial data are summarized in Table I. For animal mitochondria it has been firmly established by physicochemical means and by electron-microscopy, that mitochondrial DNA consist of circular duplex molecules with a contour length of about 5 microns, corresponding to a molecular weight of $10 \cdot 10^6$ daltons. Apart from these

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Table I. Some Characteristics of Mitochondrial DNA (*m*-DNA) from different Organisms

Source of mitochondria	Conformation of *m*-DNA	Molecular weight calculated from E.M. length measurements	Genetic complexity, based on quantitative renaturation experiments	References
Animal tissues	circular	$10 \cdot 10^6$	$10 \cdot 10^6$	2
Tetrahymena pyriformis	linear	$30–40 \cdot 10^6$	$30 \cdot 10^6$	3–5
Higher plants	linear	$14–40 \cdot 10^6$	more than $100 \cdot 10^6$	2, 6
Neurospora crassa	linear	up to $50 \cdot 10^6$	$66 \cdot 10^6$	2, 7
Yeasts				
Sacch. cerevisiae	circular	$50 \cdot 10^6$	$50 \cdot 10^6$	8
Sacch. carlsbergensis	circular	$50 \cdot 10^6$	$50 \cdot 10^6$	8

circles mitochondrial DNA preparations may contain molecules which consist of 2 or more 5-micron circles interlocked as the links in a chain (catenanes) and circular molecules with a contourlength of a multiple of 5 microns (circular multimers)[9–11]. These aberrant molecules have been first observed at a very high percentage in mitochondrial DNA from human leukocytes of patients suffering of leukemia. It was of course tempting to speculate that these abnormal mitochondrial DNA's be specific for malignant cells. However, more recently catenated molecules have been found also in normal cells, albeit in a lower percentage[12]. Hitherto double and multiple length circles have never been observed in mitochondrial DNA preparations of normal cells. The mechanism by which these oligomeric forms of mitochondrial DNA are formed is not yet completely understood. Either errors during replication or recombination events can lead to their formation.

For higher plants and lower eukaryotes the situation is different. The length of the mitochondrial DNA of these organisms is consistently longer than 5 microns and, furthermore, its conformation is in most cases linear. This means that one is unable to decide whether even the longest molecule seen is the intact molecule or a fragment. The exception is yeast, for which the presence of 25 micron circles has recently been shown.

For animal mitochondrial DNA all the experimental data have been obtained with DNA first isolated from the mitochondria and then examined. Using the same techniques for lower eukaryotes such as yeast, it appeared impossible to gather convincing evidence for a circular conformation of the mitochondrial DNA, although linear fragments with lengthes up to 25 micron were observed. To avoid the laborious procedures of isolation and purification of DNA, NASS developped a method for direct examination of mitochondrial DNA with the electronmicroscope[13]. The method makes use of the fact that mitochondria are osmotically active. They will swell and burst under hypotonic conditions. HOLLENBERG, BORST and VAN BRUGGEN, using this technique of osmotic shock for yeast mitochondria, have been able to release circular molecules also from yeast mitochondria. The contourlength of these molecules was 25 microns. That means that yeast mitochondrial DNA is five times longer than animal mitochondrial DNA and one may assume, therefore, that the genetic information in one such a molecule is also five times higher.

In considering this, an important question that should be answered first is: are all molecules apart from their uniformity in length also homogeneous in their base-sequence? Or in other words: do different mitochondrial DNA molecules contain the same genetic information or not? This problem has been tackled in a number of laboratories with the method of quantitative renaturation. The method is based on the fact that complementary stretches of single-stranded DNA are able to reanneal in a way that the original duplex is reformed. The rate of reannealing or renaturation depends on the concentration of the complementary stretches and follows second order reaction kinetics. At a given DNA-concentration renaturation will occur at a faster rate as the complexity of the DNA, that means the difference in base-sequences is smaller. The genetic complexity can be calculated from the second order reaction rate constants. The principle advantage of the method is, that it is not necessary and for circular DNA even not allowed to start out with intact molecules. The reaction is carried out with fragments of DNA[2]. The genetic complexity for mitochondrial DNA from different organisms as calculated from renaturation experiments and expressed as daltons molecular weight are also given in Table I. The electron-microscopy and renaturation data are in reasonable accordance with each other. So we are forced to conclude that the total information content of animal and yeast mitochondria is restricted to the information content of *one* circular molecule, although more than one molecule may be present per mitochondrion.

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Mitochondrial RNA

For mitochondrial RNA the situation is far less clear-cut. This is above all due to the fact that both the localization and properties of extramitochondrial RNA's are more complex and less well defined than in the case of DNA. For mitochondrial RNA, moreover, we do not have such nice and distinctive criteria as we have for mitochondrial DNA. Possible criteria to be put are:

1. enrichment on isolation and purification of mitochondria;
2. restistance to digestion if ribonucleases are added to intact isolated mitochondria (impermeability of mitochondrial membranes);
3. sedimentation characteristics different from non-mitochondrial RNA's;
4. chromatographic and/or electrophoretic behaviour different from extramitochondrial RNA;
5. specific base composition and
6. base-sequence complementary to mitochondrial DNA.

Non of these criteria, however, offers unequivocal proof for a mitochondrial origin and/or localization.

Contamination of mitochondrial preparations with extra-mitochondrial RNA is quite a problem also because the RNA-content is very low, especially in animal tissues. Let us consider for instance the contamination with rough endoplasmic reticulum, fragments called microsomes and containing ribosomes. It is impossible

Table II. Sedimentation Characteristics of Mitochondrial Ribosomes and Their RNA-Components

Authors	Organism	*S* value, c.q. molecular weight		References
		mitochondrial ribosomes	mitochondrial ribosomal RNA's	
	Lower Eukaryotes			
SUYAMA	*Tetrahymena pyriformis*		18 S / 14 S	14
KÜNTZEL and NOLL	*Neurospora crassa*	73 S	23 S / 16 S	15
RIFKIN *et al.*	„	81 S	25 S / 19 S	16
DURE *et al.*	„	–	23 S / 16 S	17
EDELMAN *et al.*	*Aspergillus nidulans*	–	24 S / 16 S	18
WINTERSBERGER	*Saccharomyces cerevisiae*	–	23 S / 16 S	19
ROGERS *et al.*	„	–	22 S / 18 S / 13 S	20
STEINSCHNEIDER	„		25 S / 16 S	21
LEON and MAHLER	„		22 S / 15 S	22
SCHMITT	„	80 S	–	23
STEGEMAN *et al.*	„	75 S	25 S / 17 S	24
MORIMOTO *et al.*	„	80 S	–	25
VIGNAIS *et al.*	*Candida utilis*	77–80 S	–	26
	Higher Eukaryotes			
ELAEV	Rat heart / Ox heart	83 S / 63 S	–	27
O'BRIEN and KALF	Rat Liver	55 S	–	28
KROON and AAIJ	Rat Liver		23 S / 16 S	29
O'BRIEN	Rat Liver	70 S / (55 S)	–	30
ASHWELL and WORK	Rat Liver	55 S	–	31
AAIJ and BORST	Rat Liver	–	18 S / 12 S	32
SWANSON and DAWID	*Xenopus laevis*	60 S	21 S / 13 S	33
DUBIN and BROWN	Hamster cells BHK-21		27 S / 17 S	34
KNIGHT	HeLa cells	–	28 S / 18 S	35
PERLMAN and PENMAN	HeLa cells	95 S / 55 S	(21 S / 12 S)	36

The rapidly growing literature on mitochondrial ribosomes has recently been reviewed by BORST and GRIVELL[37].

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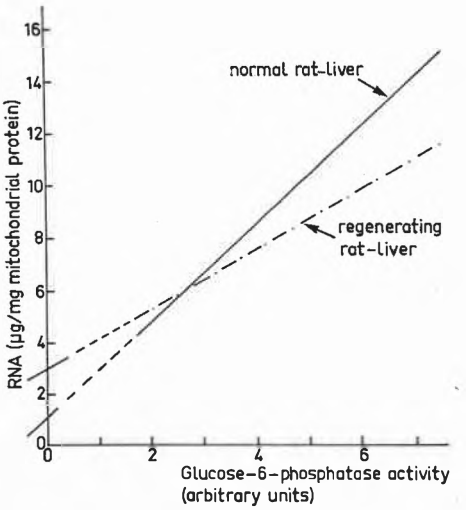

Figure 1. Relation between the RNA-content and glucose-6-phosphatase activity of mitochondrial preparations of normal and regenerating rat-liver. The curves are constructed from the experimental data of different experiments. Methodological details have been described elsewhere[38,39]

to isolate mitochondria in reasonable quantities completely free of these fragments without damaging the mitochondria in the meantime. To get a rough idea about the degree of contamination of rat-liver mitochondria we have measured the activity of an enzyme specific for the endoplasmic reticulum, glucose-6-phosphatase. If we plot the RNA-content of mitochondrial preparations as a function of the glucose-6-phosphatase activity in these preparations and calculate the content of "real" mitochondrial RNA from the regression-course we get a picture as shown in figure 1. The "real" RNA-content of mitochondria from normal and regenerating liver is very low, in the order of 1 an 3 μg RNA per mg protein respectively. The actual RNA-content of mitochondrial preparations from animal tissues is of the order of 10 μg per mg protein, even after a number of washings and in preparations, which by some authors are considered to be highly purified or pure[28].

In lower eukaryotes contamination is a less serious problem, because the mitochondria themselves contain more RNA than animal mitochondria and because in general they have a less well-developped reticular system with ribosomes bound to it. This is most likely one of the main reasons why the experimental data for mitochondrial RNA are much more reliable for lower than for higher organisms. In Table II literature data for ribosomes and their RNA's are collected. For the lower eukaryotes there are small discrepancies in sedimentation values, but in most studies additional criteria have been forwarded such as differences with the sedimentation characteristics of the corresponding components outside the mitochondria and differences in base-composition. The general idea evolving from these studies is that the mitochondria of lower eukaryotes contain ribosomes with characteristics similar to bacterial ribosomes. This similarity is further supported by the sensitivity of mitochondrial protein synthesis to inhibition by antibiotics specifically acting at the level of the bacterial ribosomes (*cf.* also Table V).

The situation in higher eukaryotes is much more confusing. The survey of literature data given in Table II clearly shows that there is very little agreement about the characteristics of the particles involved in mitochondrial protein synthesis in animals. This variation mirrors without any doubt the difficulties in obtaining intact ribosomes and ribosomal RNA from animal mitochondria. It should be noticed, however, that the idea that animal mitochondria contain a unique type of very small ribosomes, by some authors designated "miniribosomes",[37] is rapidly gaining ground. However, since the isolation of animal mitochondrial ribosomes which are chloramphenicol-sensitive and active in protein synthesis in a reconstituted system has not yet met with success, the "miniribosome" remains a contentious issue. The sensitivity to chloramphenicol should be put as a necessary condition because it offers the only indication for the mitochondrial origin of the particles involved.

With respect to transfer-RNA mitochondria from *Neurospora crassa* and from rat liver have been shown to contain a set of transfer-RNA's with physicochemical properties differing from the corresponding extra-mitochondrial transfer-RNA's. In *Neurospora crassa* unique transfer-RNA's have been identified for arginine, glutamic acid, histidine, isoleucine, leucine, methionine, phenylalanine, serine, tyrosine and valine; in rate liver for aspartic acid, leucine, serine, tyrosine and valine (see Table III). Concerning messenger-RNA the story can be very short. Rapid labeling of RNA in mitochondria has been described. However, it has never been shown that any mitochondrial RNA species can serve as a messenger in a reconstituted system for protein synthesis.

Functions of mitochondrial nucleic acids

The mere fact that mitochondria contain DNA and RNA is of course suggestive for the idea that mitochondria are equipped for replication, transcription and translation. In fact, not the nucleic acids first pointed to these synthetic capacity of the mitochondria, but the early observation by SIMPSON and coworkers[40,41] that isolated mitochondria are able to incorporate amino acids into protein. After it was firmly established that this incorporation process was a real and intrinsic mitochondrial phenomenon, a large number of studies have

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Table III. Factors within Mitochondria Involved in Replication, Transcription and Translation

Factors	Type of evidence for localization of these factors and their activities within mitochondria	Differences with corresponding factors and activities outside mitochondria	References
DNA	Electron microscopy: presence of DNAse sensitive fibrils within mitochondria	Structural similarities with bacterial nucleoid	2, 42–44
	Isolation of DNA with unique properties from isolated mitochondria	1) base composition (buoyant density, T_m, direct analysis) in many cases different from nuclear DNA 2) conformation 3) genetic complexity	
DNA polymerase	Incorporation of deoxyribonucleotides into DNA by isolated mitochondria or mitochondrial extracts	Sensitivity to inhibition by Ethidium bromide and acriflavin	44–49
	Identification of product of *in vitro* incorporation as mitochondrial DNA	1) base composition 2) conformation 3) genetic complexity	
	Purification and characterization of the mitochondrial enzyme	1) Specific affinity to homologous mitochondrial DNA as a template 2) Association with the mitochondrial membrane	
Polynucleotide ligase	Incorporation of deoxyribonucleotides into closed circular duplex molecules of mitochondrial DNA by isolated chick liver mitochondria	not relevant	44
RNA polymerase	Incorporation of ribonucleotides into RNA by isolated mitochondria or mitochondrial extracts	1) Sensitivity to inhibition by rifamycin and Ethidium bromide 2) RNA synthesized complementary to mitochondrial DNA	22, 44, 45, 50–53
Messenger RNA	Deduced from ability of isolated mitochondria to incorporate aminoacids into protein and ribonucleotides into RNA	RNA complementary to mitochondrial DNA	54, 55
Ribosomes	Electron microscopy: presence of RNAse sensitive particles within mitochondrial matrix	Dimensions of the particles inside mitochondria smaller than outside	Table II; 42, 56, 57
	Isolation of ribosomes or ribosomal components from purified mitochondria	1) Unique sedimentation characteristics 2) Unique pattern of ribosomal proteins	
	Incorporation of aminoacids into protein by isolated mitochondria	Sensitivity to inhibition by *e.g.* (oxy) tetracycline (*cf.* Table V)	
Peptidyl-transferase	Incorporation of aminoacids into protein by isolated mitochondria (puromycin- and sparsomycin sensitive)	Sensitivity to inhibition by *e.g.* chloramphenicol, insensitivity to inhibition by e.g. cycloheximide (*cf.* Table V)	Table V; 42, 58
Transfer-RNA	Isolation of transfer-RNA from extracts of highly purified mitochondria	1) formylmethionyl-*t*-RNA^F within mitochondria, methionyl-*t*-RNA^F outside mitochondria 2) Unique physicochemical characteristics shown for a) leut-*t*-RNA, tyr-*t*-RNA, asp-*t*-RNA, val-*t*-RNA and ser-*t*-RNA in rat liver b) arg-*t*-RNA, leut-*t*-RNA, met-*t*-RNA, phe-*t*-RNA, glu-*t*-RNA, ser-*t*-RNA, his-*t*-RNA, tyr-*t*-RNA, ile-*t*-RNA and val-*t*-RNA in *Neurospora crassa*	42, 59–66
	Deduced from independence of aminoacid incorporation by intact isolated mitochondria of added transfer-RNA	not relevant	
Aminoacyl-transfer-RNA synthetases	Purification of aminoacyl-*t*-RNA synthetases from extracts of highly purified mitochondria	Unique physicochemical properties shown for a) asp-*t*-RNA synthetase, phe-*t*-RNA synthetase and leu-*t*-RNA synthetase in *Neurospora crassa* b) leu-*t*-RNA synthetase in *Tetrahymena pyriformis*	42, 62, 66
	Deduced from aminoacid dependent exchange reactions in mitochondrial extracts and from the independence of aminoacid incorporation by intact, isolated mitochondria of added aminoacyl-*t*-RNA synthetases	not relevant	

been started as to characterize the different steps in the flow of mitochondrial genetic information more precisely, the wellknown dogma of molecular biology serving as the model. Within the scope of this paper it seemed advisable not to bore the reader with the details of all these studies. Instead Table III gives a compilation of the *status quo;* the balance sheet of all components and activities involved in the functioning of mitochondrial nucleic acids. The crucial evidence for the mitochondrial localization of all these factors and activities is summarized in the second column of Table III, whereas in the third column the main differences with the corresponding factors and activities in the cell compartments outside the mitochondria are given. Table III does not pretend to give a complete survey; however, it will be clear from this table that mitochondria are, indeed, actively involved in replication, transcription and translation.

The contribution of mitochondria to their own biogenesis

It has been outlined in the preceding paragraphs that mitochondria contain DNA and RNA and that these nucleic acids govern mitochondrial replication, transcription and translation activities. It can be concluded from Table III that these activities in mitochondria have many properties in common with the corresponding bacterial processes. For example, it is interesting to note that initiation of protein synthesis in mitochondria appears to proceed *via* formylmethionyl-*t*-RNA as has been shown by SMITH and MARCKER[59]. More recently these authors have shown that the difference of mitochondrial and non-mitochondrial initiation is most likely restricted to this formylation and due to the fact that there is no transformylase activity in the cellsap[67].

Since the genetic information content of mitochondria is limited (*cf.* Table I), the contribution of the mitochondria to their own biogenesis has to be limited too. The obvious questions then are: what is this contribution actually; which mitochondrial components are coded for by mitochondrial DNA and which by nuclear DNA; which mitochondrial constituents are synthesized within the mitochondria and which outside?

In principle one can use various experimental approaches to answer these questions. A number of these methods is summarized in Table IV. The genetic approaches (5–8) are for plain reasons applicable mainly in studies with lower eukaryotic organisms. The methods 2, 3 and 5 need the availability of well-defined and characterized mitochondrial proteins as references. The only mitochondrial protein completely fulfilling these criteria so far is cytochrome *c*. It has been shown that cytochrome *c* is *not* a product of mitochondrial synthetic activity. In yeast the structural gene for the apoprotein of cytochrome *c* is part of the nuclear genome[68]. For rat liver it has been shown by pulse-chase experiments that cytochrome *c* is synthesized in the cytoplasma and then transported into the mitochondria[69].

DNA-RNA hybridization has been used by several authors. The general conclusion to be drawn from these

Table IV. Experimental Approaches to the Intracellular Localization of the Genetic Information for Different Mitochondrial Components

1. DNA-RNA hybridization
2. Characterization of the products of *in vitro* mitochondrial protein synthesis
3. Pulse-chase experiments *in vivo*
4. Differential inhibition of the *in vivo* synthesis of mitochondrial proteins with antibacterial antibiotics
5. Correlation of aminoacid replacements in mitochondrial proteins with mutational changes in mitochondrial DNA
6. Correlation of mutations affecting mitochondrial processes with mutational changes in mitochondrial DNA
7. Localization of the structural genes of mitochondrial proteins on nuclear DNA
8. Search for mitochondrial enzymes in cells lacking functionally active mitochondrial DNA

For more detailed information and discussion see ref's 2 and 42.

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studies is that the mitochondrial ribosomal RNA's and at least a number of mitochondrial transfer-RNA's are coded for by mitochondrial DNA[2,32,33,44]. So far the hybridization method is a very useful tool for localizing genetic information. However, the method as such has its restrictions. For example it will be completely impossible to proof by this method that a RNA molecule of a base-sequence complementary to mitochondrial DNA is intact and not a fragment of a larger molecule. It is, therefore, not allowed to relate this base-complementarity to the physicochemical properties of the native molecule.

The method of differential inhibition of the *in vivo* synthesis of mitochondrial proteins with antibacterial antibiotics and other drugs lacks a number of the difficulties adhering to the other approaches and this method has been used quite succesfully with a variety of organisms. The possibilities and limitations of this method will be illustrated in the next paragraph.

Differential inhibition of RNA- and protein synthesis; studies with mammalian cells and tissues

It has been known for a long time already that mitochondrial protein synthesis is strongly inhibited by chloramphenicol. Chloramphenicol and a large number of other antibiotics and intercalating dyes have been used both *in vivo* and *in vitro* for the specific inhibition of the mitochondrial synthetic processes, leaving the extramitochondrial processes unaffected. By using these inhibitors in whole cell-systems or intact multicellular organisms one is able to look for inhibitions at the level of enzyme activity. Specially because purification and physicochemical characterisation of the products of mitochondrial protein synthesis gives great difficulties, the indirect method using inhibitors is advantageous. We have been working along this line for a long time already using a wide variety of inhibitors. A summary of our work is given in Table V. Only the principles and general implications of this work will be discussed here. The reader interested in more details may consult the references 42, 45, 58 and 70–74.

We have been working with isolated mitochondria, cultured rat-heart cells and regenerating liver. Inhibitors acting at the level of transcription have been tested as well as those acting at the level of translation. If we concentrate on the latter group in Table V, it is clear that the following conclusions can be drawn:

1. Antibiotics inhibiting bacterial growth by specifically interfering with the function of the bacterial ribo-

Table V. Effects of Various Inhibitors on the Biosynthetic Activities of Mitochondria of Rat Tissues *in vivo* and *in vitro*

All experimental details have been described elsewhere (see ref's 70–74)

Inhibitor	Mitochondrial Protein synthesis *in vitro*		Specific Inhibition of Cytochrome *c* oxidase formation	
	Intact Mitochondria	Swollen Mitochondria	Cultured Heart Cells	Regenerating Liver
Acting at the level of transcription				
Actinomycin D	−	+	0	0
Rifampicin	+	+	−	0
Ethidium Bromide	+ +	+ +	+ +	+
3,6-diamino-10-methylacridine (euflavin)	+ +	+ +	+ +	+ +
3,6-diamino acridine (proflavin)	+	+	cytotoxic	0
9-amino acridine	−	−	cytotoxic	0
Acting at the level of translation				
D(−)*threo* Chloramphenicol	+ +	+ +	+ +	+ +
L(+)*threo* Chloramphenicol	−	−	−	0
D(+)*threo* Thiamphenicol	+ +	+ +	+ +	+ +
Erythromycin	−	+ +	−	0
Oleandomycin	−	+ +	−	0
Carbomycin	+ +	+ +	+ +	0
Lincomycin	−	+	−	0
Streptomycin	+	+	+	0
Tetracycline	+ +	+ +	+ +	0
Oxytetracycline	+ +	+ +	+ +	+ +
Puromycin	+ +	+ +	0	0
Sparsomycin	+ +	+ +	0	0
Anisomycin	−	0	0	0
Cycloheximide	−	−	+	0

+ + = 50 to 100% inhibition; + = 20 to 50% inhibition; − = no inhibition; 0 = not tested

[70] A.M. KROON and R.J. JANSEN, *Biochim. Biophys. Acta 155* (1968) 629.

[71] A.M. KROON, in TH. BÜCHER and H. SIES (Eds.), *Inhibitors, Tools in Cell Research*, Springer-Verlag, Berlin 1969, p. 159.

[72] H. DE VRIES and A.M. KROON, *Biochim. Biophys. Acta 204* (1970) 531.

[73] H. DE VRIES and A.M. KROON, FEBS *Letters 7* (1970) 347.

[74] A.M. KROON, in E. QUAGLIARIELLO (Ed.), *Atti del Seminario di Studi Biologici*, vol. IV, Adriatica Editrice, Bari 1969, p. 9.

somes are also strong inhibitors of protein synthesis by isolated mitochondria (chloramphenicol, thiamphenicol, carbomycin, the tetracyclines).
2. In the case of chloramphenicol the inhibition is specific for the antibacterial isomer, L(+) *threo* chloramphenicol being inactive.
3. The apparent resistance of mitochondrial protein synthesis to erythromycin, oleandomycin and lincomycin is due to the impermeability of the mitochondrial membrane to these antibiotics. If the permeability is altered the mitochondria become sensitive (swollen mitochondria).
4. Mitochondrial protein synthesis is resistant to inhibition by antibiotics specifically acting at the level of the 80S cytoplasmic ribosomes (cycloheximide, anisomycin).
5. Using the formation of enzymatically active cytochrome *c* oxidase as a marker for the biogenesis of functionally active mitochondria it can be seen that those antibiotics inhibiting protein synthesis by intact isolated mitochondria also prevent the formation of this enzyme both in the cultures of beating heart-cells and (as far as tested) during liver regeneration.
6. From the inhibitions shown it *may only be concluded* that mitochondrial translation is necessary for the formation of active cytochrome *c* oxidase and *not* that the apoenzyme is synthesized within the mitochondria. This is even most likely not the case as can be concluded from the fact that heart-cell cultures, made deficient in cytochrome *c* oxidase by a chloramphenicol-treatment, remain deficient if the chloramphenicol in the medium is replaced by cycloheximide, whereas cytochrome *c* oxidase formation resumes if the chloramphenicol treatment is stopped after a certain time of culturing.

These experiments have a number of implications that, in the context of this paper, may well be formulated more or less as statements. They are, of course, still prone to and subject of careful investigations.

a) The biosynthesis of at least a number of mitochondrial enzymes is dependent on the activity of both the mitochondrial and the extramitochondrial protein synthetic machinery.
b) Inhibition of mitochondrial translation activity leading to the decrease or absence of a mitochondrial structural protein or enzyme does not justify the conclusion that the genetic information for this protein is present in mitochondrial DNA.
c) Antibiotics acting at the level of the bacterial ribosome will interfere with normal eukaryotic cell functions unless they are unable to enter the mitochondria.
d) Toxic effects in man of antibiotics interfering with bacterial protein synthesis should be related to the impaired biogenesis of mitochondria.
e) Newly developped antibiotics interfering with bacterial protein synthesis should be tested on their inhibitory effects on mitochondrial synthetic activities.

Perspectives

The aim of the present paper was to pinpoint the main problems in the search for the mechanism of mitochondrial biogenesis. It will be clear that many of these problems are not at all solved as yet, but it seems justified to expect that further work along the lines dilineated will lead to a better understanding in the next few years. One of the most intriguing and challenging problems will undoubtedly be to unravel why mitochondria in lower organisms and plants contain so much more genetic information to perform (as far as we can see now) the same main functions as animal mitochondria, namely the effective generation and conservation of energy.

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