L. Reijnders

Section of Medical Enzymology, Laboratory of Biochemistry,
University of Amsterdam

Mitochondrial ribosomes and mitochondrial RNA from yeast

Centre for Agricultural Publishing and Documentation

Wageningen – 1973

2062317

ISBN 90 220 0451 1

The author graduated on 13 June 1973 as doctor in wiskunde en de natuurwetenschappen at the University of Amsterdam, the Netherlands, on a thesis with the same title and contents.

© Centre for Agricultural Publishing and Documentation, Wageningen, 1973.

No parts of this book may be reproduced and/or published in any form, by print, photoprint, microfilm or any other means without written permission from the publishers.

Abstract

REIJNDERS, L. (1973) Mitochondrial ribosomes and mitochondrial RNA from yeast. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 797, ISBN 9022004511, (vii) + 21 p., 7 tables, 1 fig., 90 refs, Dutch summary.
Also: Doctoral thesis, Amsterdam.

It is shown that MS2 RNA is active as mRNA in a system containing yeast mt ribosomes and *Escherichia coli* factors. This system displays a protein-synthesizing activity comparable with that of the homologous *E. coli* system, and appears to be a very suitable tool for the screening of antibiotic sensitivity of mt ribosomes. From our experiments it is unlikely, however, that MS2 RNA is translated correctly on yeast mt ribosomes. It is shown that several generally used methods for determination of RNA molecular weights (gel electrophoresis of native RNA, and gel electrophoresis or sedimentation velocity studies of formaldehyde-treated RNA) are too dependent on conformation to be reliable. It is found, that gel electrophoresis can be used for determination of RNA molecular weights in the range of $0.5 \times 10^6 - 1.5 \times 10^6$, when using completely denaturing conditions. Using these conditions molecular weights are determined for (i.a.) Q_β RNA (1.40×10^6) and *Tetrahymena* mt rRNAs (0.90×10^6; 0.47×10^6). The molecular weights of yeast mt rRNAs are determined by sedimentation-equilibrium centrifugation, gel electrophoresis and electron microscopy of denatured RNA. Values of 1.30×10^6 and 0.70×10^6 are found. It is shown that yeast mt DNA contains not more than one gene for each of the mt rRNAs and at least 20 genes for mt tRNAs. It is also shown that yeast mitochondria do not contain considerable amounts of stable nuclear transcripts. Finally it is found that yeast mt rRNA contains an extremely low percentage guanine + cytosine: 23%.

Contents

1 Introduction — 1

2 Protein synthesis on yeast mitochondrial ribosomes directed by MS2 RNA — 12

3 The effect of temperature and ionic strength on the electrophoretic mobility of yeast mitochondrial RNA (summary)
L. A. Grivell, L. Reijnders and P. Borst, Eur. J. Biochem. 19 (1971) 64-72 — 17

4 Gel electrophoresis of RNA under denaturing conditions (summary)
L. Reijnders, P. Sloof, J. Sival and P. Borst, Biochim. Biophys. Acta, submitted for publication — 17

5 The molecular weights of the mitochondrial ribosomal RNAs of *Saccharomyces carlsbergensis* (summary)
L. Reijnders, P. Sloof and P. Borst, Europ. J. Biochem., in press — 18

6 Hybridization studies with yeast mitochondrial RNAs (summary)
L. Reijnders, C. M. Kleisen, L. A. Grivell and P. Borst, Biochim. Biophys. Acta, 272 (1972) 396-407 — 18

7 The number of RNA genes on yeast mitochondrial DNA (summary)
L. Reijnders and P. Borst, Biochem. Biophys. Res. Comm. 47 (1972) 126-133 — 19

Samenvatting — 20

1 Introduction

During the preparation of this thesis several questions have dominated the field of mitochondrial molecular biology [see ref. 1-4]. The experimental work in this thesis is mainly concerned with two of these questions.
1. What are the contributions of the mitochondrial and nuclear genetic systems to the mitochondrial protein synthesizing system?
2. How 'bacterial' is mitochondrial protein synthesis?
Available data pertinent to these questions will be discussed below.

What are the contributions of the mitochondrial and nuclear genetic systems to the mitochondrial protein synthesizing system?

Choice of organism As suitable organisms for the study of aspects of mitochondrial biogenesis the yeasts *Saccharomyces cerevisiae* and *S. carlsbergensis* are often chosen. The main reasons for this choice are the poor development of the endoplasmatic reticulum (allowing easy isolation of relatively pure mitochondrial components) and the wide range of physiological and (in the case of *S. cerevisiae*) genetic manipulations possible in these organisms.

The main other target organisms or cells for the study of mitochondrial biogenesis are *Xenopus laevis* (especially the oocytes), HeLa cells, *Tetrahymena pyriformis* and the ascomycete *Neurospora crassa*.

Mitochondrial DNA specifies mitochondrial rRNA and tRNA From RNA -(mt)DNA hybridisation studies it can be concluded that in all organisms studied so far the mt DNA codes for the mt rRNAs and a number of tRNAs. The findings reported so far are summarized in Table 1. This table leaves us with two main questions:
- why is the number of rRNA genes per molecule of mt DNA different from 1 in the case of the ascomycetes and *Tetrahymena*?
- why is the number of tRNAs per molecule of mt DNA lower than 33, the minimum needed to code for a complete set of tRNAs?

The number of rRNA genes per molecule of mt DNA As is pointed out in ref. 9 the deviation from 1 for the number of rRNA cistrons per mt DNA in the case of *Tetrahymena pyriformis* is probably due to contaminating RNA species. The reason for the deviation from 1 in case of the ascomycetes however is less clear.

As will be pointed out in Chapter 5 the molecular weight values for the mt rRNAs

of yeast are well established; it also seems highly unlikely that the mt rRNAs of *Neurospora crassa,* which behave identically in sedimentation velocity [13] and gel-electrophoretic [14, 20] studies would differ significantly from these values. Also it is unlikely that the value of 0.6 is to a large extent due to self-complementarity of the rRNAs (see Chapter 6). Moreover: though it is in principle possible that trivial errors are the basis for the deviation from 1 (like faulty estimates of specific activities), it is unlikely in this case, because three different laboratories find the same plateau value.

It therefore seems probable, that the basis for the value of 0.6 is to a large extent to be found in the DNA used for hybridisation.

It could be that part of the single-stranded DNA, completely covered with RNA, is pulled from the filter, or that there is self-annealing of the DNA on the filter.

It could also be, that 40% of the 25 mikron circles found by Hollenberg *et al.* [15] and of the linear DNAs found by Schäfer and Küntzel [16] lack cistrons for rRNA (unlikely in view of the absence of indications for heterogeneity in the renaturation studies of Hollenberg *et al.* [15]).

Another possibility is that the DNA molecular weight is underestimated. This possibility is left open by published renaturation studies with yeast [15] and *Neurospora crassa* mt DNA [17]. (Recent renaturation studies of Christiansen (quoted in ref. 18) however seem to make the possibility of such an explanation for a large part of the deviation from 1 less likely.)

In my opinion the most likely reason for the low value of the hybridisation plateau is the cooperation of a number of factors mentioned before.

The number of tRNA genes per mt DNA The second question with which Table 1 leaves us is: why is the number of tRNA genes per molecule of mt DNA lower than 33, the number necessary for reading all possible codons with maximal wobble of the

Table 1. Number of genes for rRNAs and tRNAs on mt DNA.

Organism	Genes/mt DNA		Molecular weight ($\times 10^{-6}$)			Ref.
	rRNA	tRNA	rRNAs	tRNA	mt DNA	no.
HeLa cells	1.0	11	0.56;0.36	0.025(?)*	11	5,6,7
Xenopus laevis	1.0	15	0.53;0.30	0.028(?)	11.7	8
Tetrahymena	1.3		0.90;0.47		29	9,10
Neurospora	0.6**		1.30;0.70(?)		51	13
Yeast	0.6	20	1.30;0.70	0.025(?)	49	Chapter 6 and Chapter 7

** Recalculated for the molecular weights indicated in this table.

* (?) indicates that only rough estimates are available.

tRNAs [19]. Table 1 indicates that at least in the case of the mitochondria of *Xenopus laevis* and HeLa cells the number of tRNA genes per mt DNA is much smaller. It is possible, however, that the mt DNA of yeast codes for 33 tRNAs. Assuming for instance that the factors, that cause the deviation from 1 for the number of rRNA cistrons per mt DNA affect the number of tRNA genes to the same extent, the number of tRNA genes would go up from 20 to 33.

What could be the explanation of the phenomenon, that in several organisms the mt DNA codes for less than 33 tRNAs?

The first possible explanation is that mt protein synthesis uses only a limited number of triplets, and that the superfluous tRNA genes have been eliminated under the pressure towards a smaller mt DNA molecule. This possibility, however, is unattractive in view of the deleterious effects of mutations giving rise to untranslatable codons.

A second possibility is that the deficiency is made up by import of tRNAs coded for by the nucleus. This is not excluded by hybridisation experiments in the case of yeast (see Chapter 6). In this case a certain division of (tRNA) labour between the mitochondrial and nuclear DNAs seems highly arbitrary and unstable against evolutionary pressure. Reasoning along these lines, more data about the number of tRNA genes located on the mt DNAs in a variety of organisms could help to draw conclusions about the probability of tRNA import.

Other possibilities are, that there is heterogeneity in the mt DNA for the tRNAs, or that the primary gene products are modified. In this case, however, one has to assume highly intricate regulatory devices to keep order and one wonders why the cell would do it in a difficult (and 'DNA-consuming') way, when it could be done more easily. Also one can imagine that a larger wobble than the 'maximal' wobble postulated by Watson [19] is allowed. This would imply, that very low demands are made as to amino acid sequences of the products of mitochondrial protein synthesis, and this seems to be in contradiction with the probable function of these proteins (like being sub-units of enzymes).

Protein(s) involved in the mt protein synthesizing system specified by mt DNA The problem of the proteins specified by mt DNA which are involved in mt protein synthesis, has been attacked along two main lines:

- studies with inhibitors of mitochondrial and cell-sap protein synthesis
- the analysis of mutants, carrying mutations in the mt DNA affecting the antibiotic sensitivity of mitochondrial protein synthesis.

On the basis of inhibitor studies in *Neurospora* and yeast most authors agree, that at least a large majority of the mitochondrial ribosomal proteins are translated on cell-sap ribosomes and probably coded for by the nuclear DNA (see ref. 21-23 but contrast 24).

The possibility that mt messenger RNA is exported and translated on cell-sap ribosomes into mt ribosomal protein is, however, not rigorously excluded in these studies. Nevertheless it seems likely, that the contribution of the mt genome to the synthesis of mt ribosomal proteins is very small and possibly restricted to one protein,

strongly associated with the 37S ribosomal sub-unit (see ref. 23; also G. S. P. Groot, personal communication). This is somewhat unexpected in view of the frequent occurrence of cytoplasmically inherited ribosomal resistance in yeast against a number of antibiotics like paromomycin, erythromycin, spiramycin, D-chloramphenicol, lincomycin and others [25]. In bacteria resistance to several antibiotics result from changes in ribosomal proteins [27], with only two exceptions (due to defective RNA methylation [26, 26a]). Assuming that antibiotics, that affect the 30S subunits of bacterial ribosomes will affect the 37S yeast mitochondrial ribosomal subunits, and that the same holds for the respective 50S subunits, paromomycin resistance of yeast mt ribosomes can in principle still be explained in terms of a change in the protein found by Groot (personal communication), but no such explanation seems to be possible for the cytoplasmically inherited resistance of mt ribosomes against D-chloramphenicol, lincomycin, erythromycin and spiramycin (again: unless mitochondrial messengers are translated into ribosomal proteins on cell-sap ribosomes). This should mean, that in this case the resistances are possibly all due to changes in the mt ribosomal RNA. This is in line with the analysis of antibiotic resistant mutants of yeast by Grivell *et al.* [25], focussed on changes associated with the 50S ribosomal subunit. So far no change in ribosome-associated protein has been detected.

It seems likely that the difference in this respect between bacteria and yeast mitochondria can be explained in terms of the number of genes for rRNA per genome. As pointed out above, mt DNAs have probably one gene for rRNA per genome; and although there are many copies of mit DNA within the cell, they are probably free to segregate independently.

A mutation in the rRNA gene will therefore affect all rRNA made. In *E. coli*, however, there are many genes, and since resistance to the antibiotics is usually recessive [27a], resistance due to changes in the rRNA base sequence is very improbable.

Other mitochondrial proteins specified by mt DNA All or almost all of the products of mitochondrial protein synthesis seem to be closely associated with the mitochondrial membrane [28].

Components, the synthesis off which is blocked by specific inhibitors of mt protein synthesis and which are absent in cytoplasmic petite yeasts have been identified as specified by mt DNA. They include subunits of cytochrome aa_3 [29-31], cytochrome b [32] and some at the subunits of rutamycin-sensitive ATPase [33, 34].

The contribution of nuclear DNA to the biogenesis of mitochondria As can be concluded from the size of the mt DNAs and the number of stable RNA components and proteins associated with the mitochondria, the majority of the mitochondrial components must be coded for by nuclear DNA and be imported into the mitochondria. In principle there seem to be two ways open for mitochondria to make up for their genetic deficit:

- import of nuclear coded RNA, to be translated on mt ribosomes
- import of proteins, synthesized on cell-sap ribosomes

(The unlikely third possibility: the import of DNA-containing 'informosomes' [35] will not be discussed here.)

The existence of RNA-import into the mitochondria has been analysed in two ways:
- by trying to import polyribonucleotides into mitochondria in vitro
- by (mt) RNA- (nuclear) DNA hybridisation.

Measurement of incorporation of polyribonucleotides by mitochondria in vitro was performed by Swanson [36] and Metz (unpublished). Using mitochondria from a variety of sources only *Xenopus laevis* mitochondria were found by Metz to be able to perform poly(U)-directed, D-chloramphenicol-sensitive, RNAse-insensitive protein synthesis. However, uptake of more 'messenger-like' polyribonucleotides (having high secondary structure) was found by Swanson to be poor in these mitochondria.

The hybridisation approach was followed in the experiments described in Chapter 6. It could be concluded from these experiments that yeast mitochondria do not contain substantial amounts of imported nuclear transcripts. However it was not excluded that nuclear coded RNAs with a short half-life or RNAs complementary to a small section of the nuclear genome are present.

The import of protein into mitochondria has mainly been studied by:
- studies with inhibitors of mitochondrial and cell-sap protein synthesis
- the analysis of yeast petites lacking a functional mitochondrial protein synthesizing system
- the analysis of the biosynthesis of individual components of the mitochondrion.

The results of the study of petites or the inhibition of protein synthesis should of course be interpreted with caution, because of the possibility of 'coupling' between the mitochondrial and cell-sap protein synthesizing systems (i.e. the possibility, that a product of one of the systems regulates the activity of the other system). Nevertheless, from the lack of coupling at the synthesis of cytochrome aa_3 [29] in yeast it seems rather safe to estimate from published data about short-term inhibition studies, that cell-sap protein synthesis accounts for about 90% of the protein incorporated into mitochondria of animals, yeast and *Neurospora* [28, 37-39].

Inhibitor studies are also included in the study of the synthesis of individual mitochondrial components (see hereafter).

From the analysis of petites it can be concluded that a considerable number of mitochondrial enzymes are coded for by the nuclear DNA and synthesized on cell-sap ribosomes.

These enzymes include: DNA and RNA polymerizing enzymes [40], aconitate hydratase [41], lactate cytochrome c reductase [42, 43], succinate dehydrogenase [44], and mitochondrial peptide chain elongation factors [24].

Work along the line of study of the synthesis of individual mitochondrial components was done for cytochrome c, the results showing that this cytochrome is coded for by nuclear DNA and synthesized on cell-sap ribosomes [45-47].

There are also reports on cytochrome aa_3 [29], cytochrome b [32] and rutamycin-sensitive ATPase, showing that a large part of the proteins composing these enzymes is synthesized on cell-sap ribosomes and probably coded for by the nuclear DNA.

Surveying these data, it can be safely stated that the majority of the mitochondrial proteins are coded for by nuclear DNA and probably imported from the cytoplasm, and that the contribution of nuclear mRNA to the mt protein synthesizing system is probably small, if present at all.

How bacterial is mitochondrial protein synthesis?

A remarkable property of mitochondrial protein synthesis is its resemblance to bacterial protein synthesis (for recent reviews see refs. 2, 4 and 48). In fact, this property has provided the main support for the endosymbiont theory of the origin of mitochondria, which claims that mitochondria have evolved from a bacterial endosymbiont of the primordial eukariotic cell. On a more applied level this resemblance has led to a reinvestigation of the medical use of the antibiotic D-chloramphenicol [48].

It is well established that the mitochondrial ribosomes studied so far resemble all bacterial ribosomes in their sensitivity to D-chloramphenicol, whereas they are resistant to cycloheximide, an inhibitor of cell-sap protein synthesis.

Formylmethionyl-tRNA^{fmet} has been found in mitochondria from yeast [49], *Neurospora crassa* [50] and HeLa cells [51].

Formylmethionyl puromycin is formed in isolated mitochondria of yeast active in protein synthesis [52]. Sala and Küntzel [53] have shown that mitochondrial ribosomes from *Neurospora crassa* are able to recognize, bind and translocate *E. coli* formylmethionyl-tRNA in response to the codon AUG. This activity is lost after washing the mt ribosomes with 1M ammonium chloride (a procedure that removes *E. coli* initiation factors [54].) Activity is restored by the addition of *E. coli* initiation factors. Mitochondrial ribosomes from yeast, supplemented with *E. coli* tRNA and supernatant factors, show a high polyphenylalanine synthesizing activity in a poly (U) directed system, and dissociate under about the same conditions as *E. coli* ribosomes [55]. The cooperation of mitochondrial ribosomes with bacterial tRNAs and supernatant factors has also been reported for *Neurospora crassa* [4]. Furthermore, yeast mt polysomes show high protein synthesizing activity, when incubated with *E. coli* supernatant [56], and mitochondria contain 'bacterial type' aminoacyl-tRNA synthetases [57, 58]. The combination of elongation factors T(u) (yeast mt) and *E. coli* G [59, 60] and of T (*E. coli*) and G (*Neurospora* or yeast mt) [60, 61] are able to assist in polyphenylalanine synthesis on *E. coli* ribosomes (however: bacterial G factor seems to be poor in assistance of polyphenylalanine synthesis on *Neurospora* mt ribosomes [62]).

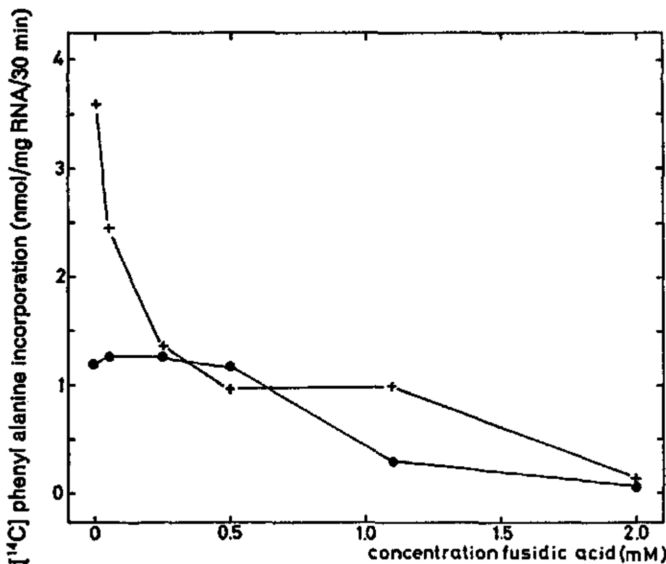
Whether animal mt ribosomes can synthesize polypeptides in a bacterial system or not is unknown. However, there have been reports [63, 64] about the possibility of substituting bacterial Tu and G factors by factors from rat liver. These factors could be mitochondrial in origin. Finally: Scragg *et al.* [65] have reported MS2 RNA-directed polypeptide synthesis on yeast mt ribosomes, and Richter *et al.* [66] have described T5 and T7 (*E. coli*) phage DNA directed enzyme synthesis in vitro, using a protein synthesizing system prepared from yeast mitochondria. In particular the

report of Richter *et al.* [66] is striking, because it seems to be a shortcut to all attempts to prove the 'bacteriality' of mitochondria. The fact, that yeast mitochondria do possess the property of proper starting on bacterial messengers is even somewhat astonishing in view of the failure of ribosomes of *B. stearothermophilus* to start properly on (*E. coli*) phage (Q_β) RNA [66a].

To get an independent confirmation of 'bacteriality' (and to get a better screening device for antibiotic resistance) I analysed MS2 RNA directed protein synthesis on yeast mt ribosomes. This is described in Chapter 2. Although as yet no firm conclusions can be drawn from these experiments, it seems likely that MS2 RNA is not translated correctly on mt ribosomes.

Another striking feature of the system described by Richter *et al.* [66] is that enzyme synthesis is strongly inhibited by fusidic acid, whereas mt protein synthesis in *Neurospora crassa* was found to be relatively resistant to fusidic acid [67]. The figure shows that we also find comparable resistance to fusidic acid with yeast mt ribosomes, supplemented with *E. coli* factors, catalyzing poly (U) directed polyphenylalanine synthesis. Assuming that the mechanism of fusidic acid inhibition is the same for mt ribosomes and *E. coli*, both this finding and the results of our experiments with MS2 RNA (Chapter 2) cast doubt on the criteria used to prove 'bacteriality' of mt ribosomes in the experiments of Richter *et al.* [66].

This brings us on a more general level to the question of differences between mitochondrial and bacterial protein synthesis. Indeed, important differences between



Inhibition by fusidic acid of *E. coli* ribosomal (+) and *S. cerevisiae* (strain PS 40) mitochondrial ribosomal (●) poly (U) directed incorporation of phenylalanine. Poly (U) directed protein synthesis in the presence of varying concentrations of fusidic acid was performed at 15 mM Mg^{2+} as described by Grivell *et al.* [55]. Specific activity of the [^{14}C]phenylalanine was 100 mC/mmol.

Table 2. Comparison of molecular weights of mt ribosomal RNAs with *E. coli* rRNAs.

Organism	Molecular weights ($\times 10^{-6}$)	Ref. no.
E. coli	1.10;0.56	80
S. carlsbergensis	1.30;0.70	Chapter 5
Tetrahymena	0.90;0.47	Chapter 4
Xenopus laevis	0.53;0.30	81
HeLa cells	0.56;0.36	6

bacterial and mitochondrial ribosomes have been found, both on a molecular and a functional level:

1. Ribosomal RNAs at the molecular level

a. The molecular weights of the mt rRNAs are very different from their bacterial counterparts. This is shown in Table 2, which also includes data from Chapter 4 and 5.

b. The 5S RNA is absent in yeast (R. van den Berg, unpublished, quoted in ref. 55), *Neurospora crassa* [14] and animal mitochondria [68, 69, 70] or does not have the same size as its bacterial counterpart. However, 5S RNA seems to be present in plant mitochondrial ribosomes. [71].

c. It seems probable that divergencies will be found with respect to methylation between bacterial and mitochondrial ribosomes. In HeLa cells 1% methylation was reported [72], the same percentage as in bacteria [73], but in hamster kidney cells 0.4% was found [74], whereas in yeast (C. Klootwijk, personal communication) preliminary experiments indicate a very low degree of methylation (0.2-0.3%; contrast ref. 75).

2. Ribosomal proteins at the molecular level

The behaviour of yeast (L. A. Grivell and G. S. P. Groot, unpublished) and *Neurospora crassa* [4] ribosomal proteins on polyacrylamide gels is significantly different from that of bacterial proteins [76], especially when the gels select on size.

3. Ribosomal subunits at the functional level

No sub-unit exchange can be found between yeast mitochondrial and *E. coli* ribosomes [77].

4. Ribosomes at the functional level

a. Whereas the activity of *E. coli* ribosomes in polyphenylalanine synthesis is increasingly stimulated by raising the NH_4^+ concentration to about 120 mM [78], yeast mitochondrial ribosomal polyphenylalanine synthesis is severely inhibited [55].

b. Mitochondrial protein synthesis in *Neurospora crassa* [67] and yeast (see Fig. 1) is found to be relatively resistant to fusidic acid, a potent inhibitor of bacterial (and cell-sap) protein synthesis, acting on the G factor.

The differences between bacterial and mt ribosomes have been invoked to support the episome theory about the origin of the mt DNA [79]. According to this theory, the mt DNA has developed from the nuclear DNA, and the resemblance between

bacterial and mitochondrial protein synthesis is not a matter of common origin but a matter of convergent evolution.

It should be stressed, however, that according to the endosymbiont theory differences between the bacterial and mitochondrial protein synthesizing systems are not unexpected, because obviously it is a long way from the bacterial genome (molecular weight 10^9) to the mitochondrial genome (molecular weight 10^7).

None of the data collected about mitochondrial protein synthesis so far can be considered as inconsistent with either the episome- or endosymbiont theory.

Firm conclusions with respect to the values of these theories can only be expected from analysis of homologies between the rRNA and tRNA, and of proteins involved in mitochondrial protein synthesis and the corresponding bacterial and cell-sap components in a variety of organisms.

References

1. P. Borst, Ann. Rev. Biochem. (1972) 333
2. P. Borst & L. A. Grivell, FEBS Letters 13 (1972) 73
3. A. W. Linnane, J. M. Haslam, H. B. Lukins & P. Nagley, Ann. Rev. Microbiology (1972) 26
4. H. Küntzel, Current topics in microbiology and immunology 54 (1971) 94
5. Y. Aloni & G. Attardi, Proc. Natl Acad. Sci. U.S.A. 68 (1971) 1757
6. D. Robberson, Y. Aloni, G. Attardi & N. Davidson, J. Mol. Biol. 60 (1971) 473
7. M. Wu, N. Davidson, G. Attardi & Y. Aloni, J. Mol. Biol. 71 (1972) 81
8. I. B. Dawid, J. Mol. Biol. 63 (1972) 201
9. R. H. B. Schutgens, L. Reijnders, S. P. Hoekstra & P. Borst, submitted for publication
10. Y. Suyama, Biochemistry 6 (1967) 2829
11. H. Morimoto, A. H. Scragg, J. Nekhorocheff, V. Villa & H. O. Halvorson, in: Autonomy and biogenesis of mitochondria and chloroplasts (1971). Eds. N. K. Boardman, A. W. Linnane & R. M. Smillie, North Holland Amsterdam, p. 282
12. K. P. Schaefer & H. Küntzel, Biochem. Biophys. Res. Commun 46 (1972) 1312
13. H. Küntzel & H. Noll, Nature (London) 215 (1967) 1340
14. W. Neupert, W. Sebald, A. J. Schwab, A. Pfaller & Th. Bücher, Eur. J. Biochem. 10 (1969) 585
15. C. P. Hollenberg, P. Borst & E. F. J. van Bruggen, Biochem. Biophys. Acta 209 (1970) 1
16. K. P. Schaefer, G. Bugge, M. Grandi & H. Küntzel, Eur. J. Biochem. 21 (1971) 478
17. M. L. Rifkin & D. J. L. Luck, Proc. Natl Acad. Sci. U.S.A. 66 (1971) 287
18. P. Borst & R. A. Flavell, Proc. 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, North Holland Amsterdam, in press
19. J. D. Watson, Molecular biology of the gene (1970). W. A. Benjamin Inc., New York, p. 411
20. P. M. Lizzardi & D. J. L. Luck, Nature (New Biol.) 229 (1971) 140
21. H. Schmitt, FEBS Letters, 15 (1971) 186
22. H. Küntzel, Nature (London) 212 (1969) 142
23. G. S. P. Groot, L. Reijnders & L. A. Grivell, Abstr. 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, 1972 North Holland, Amsterdam, no. 621.
24. J. C. H. Chi & Y. Suyama, Bacteriol. Proc. (1971) p. 125

24a. B. Parisi & R. Cella, FEBS Letters (14 (1971) 209

25. L. A. Grivell, P. Netter & P. P. Slonimski, Abstr. 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, 1972, North Holland Amsterdam, no. 623
26. T. L. Helser, J. E. Davies & J. E. Dahlberg, Nature (New Biol.) 233 (1971) 12

26a. C. J. Lai & B. Weisblum, Proc. Natl Acad. Sci. U.S.A. 68 (1971) 856

27. C. G. Kurland, Ann. Rev. Biochem. (1972) 402
27a. B. Weisblum & J. Davies, Bacteriol. Rev. 32 (1968) 493
28. W. Sebald, G. D. Birkmayer, A. J. Schwab & H. Weiss, in: Autonomy and biogenesis of mitochondria and chloroplasts (1971), Eds. N. K. Boardman, A. W. Linnane R. M. Smillie, North Holland, Amsterdam, p. 339.
29. G. Schatz *et al.*, Proc. 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, North Holland Amsterdam, in press
30. H. Weiss, Abstr. 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, 1972, North Holland Amsterdam, no. 656
31. W. Sebald & G. Jackl, ibid. no. 659
32. H. Weiss, Eur. J. Biochem. 30 (1972) 469
33. A. Tzagoloff & P. Meagher, J. Biol. Chem. 246 (1971) 1328
34. A. Tzagoloff & P. Meagher, J. Biol. Chem. 247 (1971) 546
35. E. Bell, Nature 224 (1969) 326
36. R. F. Swanson, Nature (London) 231 (1971) 31
37. R. Schweiger & F. Kaudewitz, Biochem. Biophys. Res. Commun. 38 (1970) 728
38. G. M. Kellerman, D. E. Griffith, J. E. Hansby, A. J. Lamb & A. W. Linnane, in: Autonomy and biogenesis of mitochondria and chloroplasts, Eds. N. K. Boarman, A. A. Linnane & R. M. Smillie, 1971. North Holland Amsterdam, p. 346
39. D. S. Beattie, FEBS Letters 9 (1970) 232
40. E. Wintersberger, Z. Physiol. Chem. 348 (1967) 1701
41. M. Ogur, L. Cohen & S. Ogur, Biochem. Biophys. Res. Commun. 19 (1964) 194
42. H. P. Mahler, B. Mackler, S. Grandschamp & P. P. Slonimski, Biochemistry 3 (1964) 668
43. B. Mackler, H. C. Douglas, S. Will, D. C. Hawthorne & H. R. Mahler, Biochemistry 4 (1965) 2016
44. A. W. Linnane & J. M. Haslam, Current topics in cellular regulation. Ed. B. L. Horecker, E. R. Stadtman, 1970, Academic Press, New York, 2:101
45. F. Sherman, J. W. Stewart, J. H. Parker, G. J. Putteman, R. B. L. Agrawal & E. Margoliash, Control of organelle development. Ed. P. Miller 1970. Cambridge University Press, Cambridge, p. 85.
46. N. F. Gonzalez-Cadavid & P. N. Campbell, Biochem. J. 105 (1967) 427-443
47. F. Sherman, J. W. Stewart, E. Margoliash, J. Parker & W. Campbell, Proc. Natl Acad. Sci. U.S.A. 55 (1968) 1098
48. A. M. Kroon & R. F. Arendzen, Proc. 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, in press
49. A. E. Smith & K. A. Marcker, J. Mol. Biol. 38 (1969) 241
50. J. L. Epler, R. Shugart & W. E. Barnett, Biochemistry 9 (1970) 3573
51. J. B. Galper & J. E. Darnell, Biochem. Biophys. Res. Commun. 34 (1969) 205
52. R. Bianchetti, G. Lucchini & M. L. Satirana, Biochem. Biophys. Res. Commun. 42 (1971) 97
53. F. Sala & H. Küntzel, Eur. J. Biochem. 15 (1970) 280
54. J. Albrecht, Ribosomal protein factors required for polypeptide chain initiation in E. coli, Ph. D. Thesis, 1971, Leiden.
55. L. A. Grivell, L. Reijnders & P. Borst, Biochem. Biophys. Acta 247 (1971) 91
56. D. E. Butow & N. G. Avadhani, Biochem. Biophys. Res. Commun. 46 (1972) 773
57. W. E. Barnett, D. Brown & J. L. Epler, Proc. Natl Acad. Sci. U.S.A. 51 (1967) 1775
58. C. A. Buck & M. M. K. Nass, J. Mol. Biol. 41 (1969) 67
59. H. Morimoto, A. H. Scragg, J. Nekhorocheff, V. Villa & H. O. Halvorson, in: Autonomy and biogenesis of mitochondria and chloroplasts (1971). Eds. N. K. Boardman, A. W. Linnane & R. M. Smillie, North Holland Amsterdam, p. 282
60. D. Richter & F. Lippman, Biochemistry 9 (1970) 5065
61. M. Grandi & H. Küntzel, FEBS Letters 10 (1970) 25

62. H. Küntzel, Nature, in press
63. L. Felicetti & G. F. di Matteo, J. Mol. Biol. 54 (1970) 611
64. D. Chiferri & B. Parisi, in: Progress in nucleic acid research and molecular biology, vol. 10, Ed. J. N. Davidson and W. E. Cohn, 1970, Academic Press, New York, p. 12
65. A. H. Scragg, H. Morimoto, V. Villa, J. Nechorocheff & H. O. Halvorson, Science 171 (1971) 908
66. D. Richter, P. Herrlich & M. Schweigert, Nature (New Biol.) 238 (1972) 74
67. M. Grandi, A. Helms & H. Küntzel, Biochem. Biophys. Res. Commun. 44 (1971) 864
68. I. B. Dawid & J. W. Chase, J. Mol. Biol. 63 (1972) 217
66a. J. A. Steitz, J. Mol. Biol. 73 (1973) 1
69. C. Aay & P. Borst, Biochem. Biophys. Acta 229 (1970) 140
70. B. Attardi & G. Attardi, J. Mol. Biol. 55 (1971) 231
71. C. J. Leaver, Abstracts 8th Meeting Fed. Europ. Biochem. Socs, Amsterdam, 1972, North Holland Amsterdam, no. 624
72. G. Attardi, Y. Aloni, B. Attardi, D. Ojala, L. Picca-Matoccia, D. L. Robberson & B. Storrie, Cold Spring Harbor Symp. Quant. Biol. 55 (1970) 599
73. M. L. Sogin, K. J. Dechman, L. Zabler, B. J. Lewis & C. R. Woese, J. Bact. 112 (1972) 13
74. D. T. Dubin & B. S. Montenecourt, J. Mol. 48 (1970) 279
75. H. Noll, in: Control of organelle development, Ed. P. Miller, Cambridge University Press, Cambridge, 1970, p. 419
76. T. A. Bickle & R. R. Traut, J. Biol. Chem. 246 (1971) 6828
77. L. A. Grivell & H. L. Walg, Biochem. Biophys. Res. Commun., submitted for publication
78. M. Lubin & H. L. Ennis, Biochem. Biophys. Acta 80 (1964) 614
79. R. A. Raff, H. R. Mahler, Science 177 (1972) 575
80. C. G. Kurland, J. Mol. Biol. 2 (1960) 83
81. I. B. Dawid & J. W. Chase, J. Mol. Biol. 63 (1972) 201

2 Protein synthesis on yeast mitochondrial ribosomes directed by MS2 RNA[1]

Introduction

The purpose of the set-up of a MS2 RNA directed protein synthesizing system using mt ribosomes from yeast was twofold. First, such a system might provide a better device for the screening of antibiotic resistance of yeast mt ribosomes (cf. ref. 1). Second, such a system might give more information about the 'bacteriality' of yeast mt ribosomes, especially in view of the report of Richter *et al.* [2], that *E. coli* T-phage messengers are translated correctly on yeast mt ribosomes (cf. Chapter 1).

Methods

– Yeast mt were prepared as described by Grivell *et al.* (Method II) [3], with the modification that the pellet formed after sedimentation of the mt ribosomes through the sucrose layer was extracted with 150 instead of 50 mM NH_4Cl.
– *E. coli* supernatant factors and ribosomes were prepared from *E. coli* Ka81, as described elsewhere [3].
– 'Washed *E. coli* ribosomes' were prepared from *E. coli* ribosomes according to Albrecht [4].
– *E. coli* [^{3}H]-formylmethionyl-tRNA was prepared as described by Voorma *et al.* [5].
– *E. coli* initiation factors were prepared according to Albrecht [4].
– MS2 RNA was prepared as described in Chapter 4 from MS2 phage prepared according to Nathans [6].
– Protein synthesis in vitro:

MS2 RNA directed protein synthesis was measured according to Grivell *et al.* [3], substituting MS2 RNA for poly (U) and [^{14}C]valine, [^{14}C]leucine, or [^{14}C]histidine for [^{14}C]phenylalanine. The magnesium concentration was 15 mM, unless otherwise indicated. Supernatant factors from *E. coli* and ribosomes were present in concentrations of 660μg/ml and $6A_{260}$ units/ml, respectively. MS2 RNA was present at a concentration of 600μg/ml. The specific activities of the [^{14}C]aminoacids used were: leucine 250-350 mC/mmol, histidine 57 mC/mmol and valine 52 mC/mmol.
– Binding of aminoacyl-tRNA to ribosomes was tested by the 'filter method' described by Voorma *et al.* [5], at a magnesium concentration of 7.5 mM, with the addi-

1. The experiments described in this chapter were performed in collaboration with Dr H. O. Voorma (Leiden) and Dr L. A. Grivell.

tions indicated in the table, in the absence or presence of MS2 RNA. Formation of formylmethionylpuromycin was measured under the conditions described above for the binding of aminoacyl-tRNAs to ribosomes. The concentration of puromycin was 1 mM [^{3}H]formylmethionylpuromycin was extracted as described by Grivell *et al.* [1].

Results

Mitochondrial ribosomes from yeast catalyse MS2 RNA directed incorporation of [^{14}C]leucine. Under standard conditions (see Table 1) values found were ranging from 60-300 pmol leucine incorporated/mg RNA/30min using 100-150 μg MS2 RNA/assay, with 3-15 fold stimulation by MS2 RNA. It is impossible to say how the activity of this system compares with that described by Scragg *et al.* [7], due to the absence of data about specific radioactivity of the incorporated aminoacids in their system. Addition of a number of inhibitors of bacterial protein synthesis gave the results presented in Table 1. This table shows that this MS2 RNA directed system is the best available tool for the screening of antibiotic sensitivity of yeast mt ribosomes (cf. ref. 1).

A considerable fraction of [^{14}C]leucine incorporation was preserved at lower Mg-concentration (Table 2). Though mitochondrial ribosomes do seem to translate MS2 RNA with a high efficiency the question remains whether they do so faithfully. To obtain more information about this we did two experiments worth mentioning here.

First we determined the valine/histidine ratio in the product of MS2 RNA directed

Table 1. MS2 RNA stimulated leucine incorporation catalysed by *S. carlsbergensis* mitochondrial ribosomes; inhibition by antibacterial drugs.

(mt) Ribosomes	Inhibitor	Concentration (μg/ml)	MS2 RNA stimulated incorporation of [^{14}C]leucine (pmol/mg RNA/30 min)	Inhibition (%)
E. coli	–	–	174	–
S. carlsbergensis	–	–	212	–
	erythomycin	10	60.5	72
	D-chloramphenicol	50	–2	101
	spiramycin	20	73	65
	spiramycin	50	66.5	69
	paromomycin	20	69.5	67
	streptomycin	20	42	80
	streptinomycin	3	45	79

MS2 RNA directed protein synthesis was performed as described in methods with ribosomes from *E. coli* and *S. carlsbergensis* mitochondrial ribosomes (strain NCYC 74). Inhibitors were added to the final concentrations indicated.

Table 2. MS2 RNA stimulated leucine incorporation catalysed by *S. carlsbergensis* mitochondrial ribosomes at 15 and 7.5 mM magnesium acetate.

Ribosomes	MS2 stimulated [^{14}C]leucine incorporation (pmol/mg RNA/30 min) at	
	7.5 mM magnesium acetate	15 mM magnesium acetate
E. coli	174	–
E. coli (washed)	55	–
S. carlsbergensis mt	121	212

MS2 RNA directed protein synthesis was performed as described in methods at 15 and 7.5 mM magnesium acetate, with *E. coli*, *E. coli* washed (with 1 M ammoniumchloride) and *S. carlsbergensis* (NCYC 74) mt ribosomes.

Table 3. Valine/histidine ratios in the product of MS2 RNA directed protein synthesis.

Ribosomes	Incorporation of		Ratio valine/histidine
	^{14}C valine (pmol/mg RNA/30 min)	^{14}C histidine (pmol/mg RNA/30 min)	
E. coli	323	16	20.2
S. carlsbergensis mt	108	27.1	4

Protein synthesis was performed as described in methods with ribosomes from *E. coli* and mitochondrial ribosomes from *S. carlsbergensis* strain NCYC 74.

protein synthesis on *E. coli* and yeast mt ribosomes (see Table 3). Because the coat protein of MS2 lacks histidine, the valine/histidine ratio can be used as an indicator of the frequency, with which the coat protein cistron is read relative to the other cistrons. [8]. As can be inferred from Table 3 the valine/histidine ratio is a factor 5 lower in the system with mt ribosomes than in the homologous *E. coli* system. This suggests that correct translation of the coat protein cistron is relatively less frequent in the system containing mitochondrial ribosomes, than in the system with *E. coli* ribosomes. This could be due to incorrect starting of the mt ribosomes on the MS2 RNA; however there is an alternative (but somewhat less likely) explanation. Fragmentation of MS2 RNA is known to increase the frequency of initiation at sites of the RNA other than the start of the coat protein cistron [5]. Therefore the low valine/histidine ratio could also be due to the fragmentation of MS2 RNA by nucleases introduced with the mt ribosomes.

Second, we tested yeast mt ribosomes, highly active in MS2 RNA directed protein synthesis, for their capacity to bind *E. coli* formylmethionyl-tRNA in response to MS2 RNA, and their ability to form formylmethionylpuromycin in the presence of MS2 RNA. Notwithstanding their high activity (0.3 nmol leucine incorporated/mg

Table 4. Formylmethionyl-tRNA binding to *E. coli* and *S. cerevisiae* ribosomes, stimulated by MS2 RNA.

Ribosomes	Additions	Formylmethionyl-tRNA bound (counts/min/assay)	
		− MS2	+ MS2 RNA
E. coli	–	1310	3579
	IF 1,2,3	3342	12187
S. cerevisae mt	–	354	439
	IF 1,2,3	502	598

Formylmethionyl-tRNA binding to ribosomes was performed as described in methods, using *E. coli* and *S. cerevisae* (strain PS 40); mitochondrial ribosomes *E. coli* initiation factors F_1, F_2, F_3 were added to final concentrations of 4 μg protein/ml.

Table 5. Formylmethionylpuromycin formation catalysed by *E. coli* and *S. cerevisiae* mt ribosomes, stimulated by MS2 RNA.

Ribosomes	Additions	Formylmethionylpuromycin (counts/min/assay)	
		− MS2 RNA	+ MS2 RNA
E. coli	–	4027	6072
	IF 1,2,3	5834	16922
S. cerevisiae mt	IF 1,2,3	382	422

Formylmethionylpuromycin formation was performed as described in methods, using *E. coli* and *S. cerevisae* (strain PS 40) mt ribosomes. Final concentrations of *E. coli* initiation factors were the same as in the experiment presented in Table 4.

RNA/30 min under standard conditions), only marginal stimulation of formylmethionyl-tRNA binding to mt ribosomes by MS2 RNA was found in the binding assay (Table 4). The addition of *E. coli* initiation factors (of which F_1 and F_2 were reported to be interchangeable with mt factors in the ascomycete *Neurospora crassa* [9] still resulted in marginal stimulation, both in the binding assay and in the formylmethionylpuromycin formation assay (Table 4 and Table 5). This experiment indicates either that mt ribosomes do not start on a formylmethionyl binding site on the MS2 RNA or that starting is correct but the complex between formylmethionyl-tRNA, mt ribosomes and MS2 RNA is very unstable under the conditions used. In view of the high efficiency of leucine incorporation directed by MS2 RNA the first alternative seems the most attractive.

In summary: mt ribosomes translate MS2 apparently with low fidelity. As yet however no firm conclusions can be drawn about the underlying causes for this.

References

1. L. A. Grivell, L. Reijnders & H. de Vries, FEBS Letters 16 (1971) 159
2. D. Richter, P. Herrlich & M. Schweigert, Nature (New Biol.) 238 (1972) 74
3. L. A. Grivell, L. Reijnders & P. Borst, Biochem. Biophys. Acta 247 (1971) 91
4. J. Albrecht, Ribosomal protein factors required for polypeptide chain initiation in *E. coli*, Ph. D. Thesis, Leiden (1971)
5. H. O. Voorma, R. Benne & T. J. A. den Hertog, Eur. J. Biochem 18 (1971) 451
6. D. Nathans, Methods in enzymology XII B (1968), Academic Press, New York, p. 787
7. A. H. Scragg, H. Morimoto, V. Villa, J. Nekhorocheff & H. O. Halvorson, Science 171 (1971) 908
8. K. Weber & W. Koningsberg, J. Biol. Chem. 242 (1967) 5563
9. F. Sala & H. Küntzel, Eur. J. Biochem. 15 (1970) 280

3 The effect of temperature and ionic strength on the electrophoretic mobility of yeast mitochondrial RNA (summary)

The electrophoretic mobilities of mitochondrial and cell-sap ribosomal RNAs from *Saccharomyces carlsbergensis* have been measured relative to the mobility of *Escherichia coli* rRNA in 2.4% polyacrylamide gels at various temperatures and ionic strengths. At 5°C and in 20mM sodium acetate, 40mM Tris-acetate, 2 mM EDTA (pH 7.8), mitochondrial and cell-sap rRNAs co-migrated and were only partially resolved from *E. coli* rRNA. When the temperature of electrophoresis was increased the mobility of the mitochondrial rRNAs (and to a lesser extent of the cell-sap rRNAs) decreased relative to the mobility of the *E. coli* rRNA species, reaching a minimum between 9 and 16°C. As a consequence the mitochondrial and cell-sap rRNAs were completely resolved in this buffer at temperatures above 9°C. Similar effects of temperature on relative electrophoretic mobility were observed in 90 mM Tris-borate buffer at pH 8.3 and the effect was even more pronounced at low ionic strength (10 mM NaCl, 5 mM Tris-HCl, 2 mM EDTA, pH 7.5).

At low ionic strength the decrease in the relative electrophoretic mobility of mitochondrial rRNA with an increase in temperature was accompanied by a decrease in sedimentation coefficient from 21-22S (large component) and 14S (small component) at 5°C to 16 and 11.5S at 20°C (calculated in relation to assumed S-values of 23 and 16S for the rRNA species of *E. coli*).

We conclude that the electrophoretic mobility of RNA in polyacrylamide gels is more dependent on secondary structure than previous work had suggested and that caution is needed to interpret the relative mobility of mitochondrial RNA on gels in terms of molecular weight. In addition, our results show that judicious variation of temperature and ionic strength may allow the separation on gels of RNA mixtures that cannot be resolved under standard conditions.

4 Gel electrophoresis of RNA under denaturing conditions (summary)

We have developed a new method for acrylamide gel electrophoresis of RNAs under completely denaturing conditions, using 8 M urea and low salt buffer at 60°C. In these 'urea gels' the mobilities of a number of RNAs in the molecular weight range of 0.5 to 1.5×10^6 are inversely related to their log molecular weights.

We have determined molecular weights in 'urea gels' of a number of RNAs for which controversial estimates were available. For phage Q_β RNA a value of 1.40×10^6 was found, for the mitochondrial rRNAs of *Tetrahymena pyriformis* values of 0.90 and 0.47×10^6, and for yeast mitochondrial rRNAs values of 1.30 and 0.70×10^6.

Determination of the molecular weights of nicked RNAs is also possible in 'urea gels', if nicks are at unique positions and give rise to a limited number of large RNA species on denaturation.

RNA molecular weights were determined by sedimentation equilibrium studies. Values different from those reported by others, using the same method, were found for the small cell-sap ('18-S') rRNA of yeast (0.70×10^6 instead of 0.62×10^6) and for the large cell-sap ('28-S') rRNA of rat liver (1.55×10^6 instead of 1.65×10^6).

For a number of viral and rRNAs relative gel electrophoretic mobilities were determined at a range of temperatures, in a standard neutral salt buffer. No simple relation was found between the variations in mobility and the gross characteristics of the RNAs.

Differences in relative gel electrophoretic mobilities of RNAs in the Tris-phosphate-EDTA buffer introduced by Loening, and the Tris-borate-EDTA buffer introduced by Peacock and Dingman, are due to differences in ionic concentrations.

5 The molecular weights of the mitochondrial ribosomal RNAs of Saccharomyces carlsbergensis (summary)

We have determined the molecular weights of the mitochondrial rRNAs from the yeast *Saccharomyces carlsbergensis* by methods not affected by the secondary structure of the RNA. Sedimentation equilibrium centrifugation gave 1.26 and 0.68×10^6; gel electrophoresis in 8 M urea at 60°C, 1.30 and 0.70×10^6; gel electrophoresis in 98% formamide at 30°C, 1.34 and 0.71×10^6; electron microscopy of dimethylsulfoxide-denatured RNA, 1.22 and 0.60×10^6. The weighted average molecular weights of 1.30 and 0.70×10^6 for these mitochondrial rRNAs are much higher than those of the rRNAs from *Escherichia coli* (1.10 and 0.56×10^6). This difference is not easily accounted for by the endosymbiont theory of mitochondrial origin.

6 Hybridization studies with yeast mitochondrial RNAs (summary)

RNA from highly purified mitochondrial ribosomes of *Saccharomyces carlsbergensis* was hybridized with homologous mtDNA, purified by a procedure that avoids equilibrium centrifugation in CsCl. Maximally 2.4% of the DNA could be converted into hybrid, showing that not more than one gene is present for each of the rRNAs.

Separate plateaus were observed for the rRNAs from purified ribosomal subunits, showing that substantial base sequence homologies between these RNAs are absent.

Co-sedimentation of mitochondrial rRNA with *Escherichia coli* rRNA through gradients containing 99% dimethyl sulphoxide, shows that both mitochondrial rRNAs are slightly larger than the corresponding *E. coli* rRNAs.

The base composition of mitochondrial rRNA in mole percent is 39% A, 39% U, 14% G and 9% C.

Total mtRNA hybridized to a large extent with nuclear DNA of *S. carlsbergensis*, but the hybridization was suppressed to near background (0.07% of DNA in hybrid)

by excess cold cell-sap rRNA. This indicates that yeast mitochondria do not contain substantial amounts of imported nuclear transcripts.

7 The number of 4-sRNA genes on yeast mitochondrial DNA (summary)

Hybridization of mitochondrial 4-S RNA from *Saccharomyces carlsbergensis* with mtDNA in the presence of excess unlabelled high-molecular-weight mtRNA gave a plateau of 0.9 μg RNA hybridized per 100 μg DNA. This indicates that yeast mtDNA contains at least 20 genes for tRNA.

Samenvatting

In de inleiding van het proefschrift wordt een overzicht gegeven over de stand van onderzoeks-zaken op het terrein van de twee hoofdvragen uit de moleculaire biologie van het mitochondrion, waarmee dit proefschrift raakvlakken heeft.
Deze vragen zijn: wat zijn de respectieve bijdragen van het mitochondriale DNA en het nucleaire DNA aan het mitochondriale eiwitsynthetiserende systeem, en: hoe 'bacterieel' is de mitochondriale eiwitsynthese?
Wat betreft de bijdrage van het mitochondriale DNA aan het mitochondriale eiwitsynthetiserende systeem kan worden vastgesteld, dat dit DNA codeert voor de mitochondriale ribosomale RNA's en een aantal transfer RNA's. Ook zijn er aanwijzingen, dat het mitochondriale DNA bij gist codeert voor één ribosomaal eiwit.
Het nucleaire DNA codeert voor het overgrote deel van de mitochondriale componenten. Deze componenten worden door het mitochondrion vanuit het celsap geïmporteerd. Alles wijst erop dat deze import voor het overgrote deel gebeurt in de vorm van eiwit en niet van RNA.
Het 'bacteriële karakter' van de mitochondriale eiwitsynthese is zowel van belang bezien vanuit het oogpunt van het medisch gebruik van antibiotica, zoals chloramphenicol, als gezien de theorievorming over het ontstaan van het mitochondrion.
De grote overeenkomsten, en niet-onaanzienlijke verschillen, tussen de bacteriële en mitochondriale eiwitsynthese worden besproken. Er wordt geconcludeerd, dat vanuit deze gegevens geen beslissing mogelijk is over de juistheid van de twee theoriën over het ontstaan van het mitochondrion (de endosymbiontheorie, die het mitochondriale DNA ziet als afkomstig van een bacteriële endosymbiont van de eukariote cel, en de episoom theorie, die dit DNA ziet als afkomstig van het kern DNA).
Hoofdstuk 2 van het proefschrift beschrijft MS2 RNA gestuurde eiwitsynthese op mitochondriale ribosomen uit gist. Er wordt geconcludeerd, dat dit eiwitsynthetiserende systeem goed bruikbaar is bij de analyse van gevoeligheid voor antibiotica van mitochondriale ribosomen uit gist. Tevens wordt geconcludeerd, dat MS2 RNA op mitochondriale ribosomen uit gist waarschijnlijk niet correct wordt vertaald.
In hoofdstuk 3 worden pogingen beschreven, om met een aantal gangbare technieken voor het meten van moleculairgewichten van RNA, de molecuulgewichten van de ribosomale RNA's van gist te meten. Deze methoden zijn gelelektroforese van al dan niet met formaldehyde behandeld RNA en sedimentatie van met formaldehyde behandeld RNA. Er wordt aangetoond, dat deze methoden niet deugen voor molecuulgewichtsbepaling van de mitochondriale RNAs, waarschijnlijk tengevolge van storende invloeden van de secundaire RNA-structuur.

In hoofdstuk 4 wordt de kritiek op de gangbare gelelektroforetische methoden gegeneraliseerd met behulp van de analyse van een aantal RNA's met sterk uiteenlopende karakteristieken. Tevens wordt in dit hoofdstuk een nieuwe methode gepresenteerd (gelelektroforese in 8 *M* ureum bij 60 °C) om gelelektroforetisch in het moleculairgewichts-gebied $0.5 \times 10^6 - 1.5 \times 10^6$ betrouwbare bepalingen van het molecuulgewicht te doen. Met behulp van deze techniek wordt dan een aantal moleculairgewichten van RNA gemeten, waarvoor tot op heden controversiële schattingen bestonden. Voor de mitochondriale RNA's van *Tetrahymena Pyriformis* worden waarden gevonden van 0.90×10^6 en 0.47×10^6 en voor (bacteriofaag) Q_β RNA een waarde van 1.40×10^6.

In hoofdstuk 5 wordt de bepaling beschreven van het moleculair gewicht van de mitochondriale ribosomale RNA's uit gist met behulp van een viertal methoden: electron-microscopie van met dimethylsulfoxide gedenatureerd RNA, sedimentatie-equilibrium-centrifugatie, elektroforese in formamide en elektroforese in 8 M ureum bij 60 °C. Het gewogen gemiddelde van de respectieve moleculairgewichten uit deze vier bepalingen is 1.30×10^6 resp. 0.70×10^6.

Hoofdstuk 6 en 7 beschrijven studies van RNA-DNA hybridisatie met mitochondriaal RNA uit gist.

Uit deze studies kan worden geconcludeerd, dat op het mitochondriale DNA uit gist niet meer dan één gen aanwezig is voor elk van de mitochondriale ribosomale RNA's, en dat aanzienlijke homologieën van basen tussen deze ribosomale RNA's onderling ontbreken. Verder volgt eruit dat er tenminste een twintigtal genen voor transfer RNA aanwezig is op het mitochondriale DNA uit gist, en tenslotte dat gist-mitochondriën geen aanzienlijke hoeveelheden geïmporteerde stabiele nucleaire transcripten bevatten. Tenslotte wordt vastgesteld, dat het gist-mitochondriale RNA een uitzonderlijk laag percentage guanine + cytosine bevat: 23%.