

# GENETICS AND BIOGENESIS OF CHLOROPLASTS AND MITOCHONDRIA

---

Interdisciplinary Conference on The Genetics  
and Biogenesis of Chloroplasts and Mitochondria,  
Munich, Germany, August 2–7, 1976,  
held under the auspices of the  
Bayerische Akademie der Wissenschaften,  
Mathematisch-Naturwissenschaftliche Klasse.

## *Editors*

Th. Bücher  
W. Neupert  
W. Sebald  
S. Werner



1976

NORTH-HOLLAND PUBLISHING COMPANY  
AMSTERDAM · NEW YORK · OXFORD

## Contents

Preface	v
<b>ROLE OF CHLOROPLAST AND NUCLEAR GENES IN PRODUCTION OF CHLOROPLAST PROTEINS</b>	
Role of chloroplast and nuclear DNA genes during evolution of fraction I protein K. Chen, S. Johal and S.G. Wildman	3
Polypeptide chains of the large and small subunits of fraction I protein J.C. Gray, S.D. Kung and S.G. Wildman	13
Studies on the primary structure of the small subunit of ribulose-1,5-diphosphate carboxylase C. Poulsen, S. Strøbaek and B.G. Haslett	17
Ontogeny, insertion, and activation of two thylakoid peptides required for photosystem II activity in the nuclear temperature sensitive T4 mutant of <i>Chlamydomonas reinhardtii</i> F. Kretzer, I. Ohad and P. Bennoun	25
Methods for the detection and characterization of photosynthetic mutants in <i>Chlamydomonas reinhardtii</i> P. Bennoun and N.H. Chua	33
Biochemical studies on a plastid ribosome-deficient mutant of <i>Hordeum vulgare</i> T. Börner, B. Schumann and R. Hagemann	41
Sites of synthesis of chloroplast membrane proteins in <i>Vicia faba</i> W. Hachtel	49
Synthesis of chlorophyll-free thylakoids in <i>Chlorella</i> after clindamycin-treatment and in a temperature sensitive mutant of <i>Chlorella</i> G. Gallig	53
Genes affecting erythromycin resistance and sensitivity of <i>Chlamydomonas reinhardtii</i> chloroplast ribosomes L. Bogorad, J.N. Davidson and M.R. Hanson	61
Genetic control of chloroplast ribosome biogenesis in <i>Chlamydomonas</i> N.W. Gillham, J.E. Boynton, E.H. Harris, S.B. Fox and P.L. Bolen	69
<b>CONTROL OF FORMATION AND ASSEMBLY OF CHLOROPLAST CONSTITUENTS</b>	
Cellular origins of plastid membrane polypeptides in <i>Euglena</i> S. Bingham and J.A. Schiff	79
Synthesis of the major thylakoid polypeptides during greening of <i>Chlamydomonas reinhardtii</i> Y-1 J.K. Hooper	87
Relationship between chloroplastic metabolism and cytoplasmic translation G. Ledoigt and M. Lefort-Tran	95
Temperature-sensitivity of chloroplast ribosome formation in higher plants J. Feierabend	99
Temperature control of chloroplast development R.M. Smillie	103
Chlorophyll synthesis and the development of photosynthetic activity W.T. Griffiths, N.L. Morgan and R.E. Mapleston	111

Genetic regulation of chlorophyll synthesis analyzed with double mutants in barley A. Kahn, N. Avivi-Bleiser and D. von Wettstein	119
MITOCHONDRIAL ATPase COMPLEX	
Mutation in <i>Saccharomyces cerevisiae</i> mitochondrial F <sub>1</sub> leading to aurovertin resistance E. Agsteribbe, M. Douglas, E. Ebner, T.Y. Koh and G. Schatz	135
Mitochondrially encoded oligomycin-resistant mutants of <i>S. cerevisiae</i> : Structural integration of ATPase and phenotype M. Somlo and J. Cosson	143
Investigation of the oligomycin binding protein in yeast mitochondrial ATPase R.S. Criddle, C. Arulanandan, T. Edwards, R. Johnston, S. Scharf and R. Enns	151
Polypeptides encoded by mitochondrial genes in ascomycetes H. Küntzel, M.A. Marahiel, D.E. Leister and P. Nelson	159
Cytoplasmic synthesis of the dicyclohexylcarbodiimide-binding protein in <i>Neurospora crassa</i> W. Sebald, Th. Graf and G. Wild	167
Biochemical genetics of oxidative phosphorylation an approach to the reaction mechanism D.E. Griffiths	175
MITOCHONDRIAL RESPIRATORY COMPLEXES	
Analysis of the polypeptide chains of cytochrome oxidase from beef heart mitochondria C. Buse and G. Steffens	189
Partial sequence of a chloroform-methanol soluble polypeptide from <i>Neurospora</i> mitochondrial membranes W. Machleidt, R. Michel, W. Neupert and E. Wachter	195
Identification of an assembly intermediate of cytochrome oxidase in <i>Neurospora crassa</i> S. Werner and M. Neuner-Wild	199
The assembly of cytochrome <i>c</i> oxidase from <i>Saccharomyces cerevisiae</i> R.O. Poyton and E. McKemie	207
Structure and biosynthesis of cytochrome <i>c</i> oxidase F. Cabral, J. Saltzgaber, W. Birchmeier, D. Deters, T. Frey, C. Kohler and G. Schatz	215
Coordination of mitochondrial and cytoplasmic protein synthesis in <i>Neurospora crassa</i> W. Neupert and A. v. Rücker	231
The use of double mutant strains containing both heat- and cold-sensitive mutations in studies of mitochondrial biogenesis T. Mason, P. Boerner and C. Biron	239
Cold sensitivity of mitochondrial biogenesis in a nuclear mutant of <i>Neurospora crassa</i> R. Kientsch and S. Werner	247
Isolation and characterization of a cytochrome oxidase deficient mutant of <i>Neurospora crassa</i> K. Bruckmooser and S. Werner	253

Subunit structure and arrangement of mitochondrial cytochrome <i>b</i> H. Weiss and B. Ziganke	259
The <i>bc</i> <sub>1</sub> -complex from beef heart prepared by adsorption chromatography in Triton X-100 G. von Jagow, W.D. Engel, P. Riccio and H. Schägger	267
Complex III of yeast: Subunit composition and biosynthesis M.B. Katan and G.S.P. Groot	273
Purification and biogenesis of cytochrome <i>b</i> in bakers' yeast L.-F. Lin and D.S. Beattie	281
Biosynthesis of cytochrome <i>c</i> in the honey bee, <i>Apis mellifera</i> M. Osanai and H. Rembold	289
CHLOROPLAST DNA: GENES AND MOLECULES	
The circular diploid model of chloroplast DNA in <i>Chlamydomonas</i> R. Sager	295
On the search for a molecular mechanism of cytoplasmic inheritance: Past controversy, present progress and future outlook K.S. Chiang	305
Transmission, segregation and recombination of chloroplast genes in <i>Chlamydomonas</i> J.E. Boynton, N.W. Gillham, E.H. Harris, C.L. Tingle, K. Van Winkle- Swift and G.M.W. Adams	313
A uniparental mutant of <i>Chlamydomonas reinhardtii</i> with a variant thylakoid membrane polypeptide N.-H. Chua	323
Plastid distribution and plastid competition in higher plants and the induction of plastom mutations by nitroso-urea-compounds R. Hagemann	331
Structural and functional analysis of <i>Euglena gracilis</i> chloroplast DNA E. Stutz, E.J. Crouse, L. Graf, B. Jenni and H. Kopecka	339
Restriction endonuclease map of chloroplast DNA from <i>Euglena gracilis</i> P.W. Gray and R.B. Hallick	347
The location of rRNA genes on the restriction endonuclease map of the <i>Spinacia oleracea</i> chloroplast DNA R.G. Herrmann, H.-J. Bohnert, A. Driesel and G. Hobom	351
Analysis of the coding capacity of EcoRI restriction fragments of spinach chloroplast DNA P.R. Whitfeld, B.A. Atchison, W. Bottomley and C.J. Leaver	361
Physical and transcriptional mapping of <i>Zea mays</i> chloroplast DNA J.R. Bedbrook and L. Bogorad	369
Studies with chloroplast DNA-plasmid hybrids from <i>Chlamydomonas reinhardtii</i> J.-D. Rochaix	375
Replication of circular chloroplast DNA K.K. Tewari, R.D. Kolodner and W. Dobkin	379
Studies of the growth and replication of spinach chloroplasts and of the location and segregation of their DNA J.V. Possingham and R.J. Rose	387

## MITOCHONDRIAL DNA: GENES AND MOLECULES

Mechanisms and rules for transmission, recombination and segregation of mitochondrial genes in <i>Saccharomyces cerevisiae</i> B. Dujon and P.P. Slonimski	393
Confirmations and exceptions to the phage analogy model: Input bias, bud position effects, zygote heterogeneity, and uniparental inheritance P.S. Perlman, C.W. Birky, Jr., C.A. Demko and R.L. Strausberg	405
On homozygotization of mitochondrial mutations in <i>Saccharomyces cerevisiae</i> A. Putrament, R. Polakowska, H. Baranowska and A. Ejchart	415
Genetic determination of mitochondrial cytochrome <i>b</i> A. Tzagoloff, F. Foury and A. Akai	419
The isolation and simultaneous physical mapping of mitochondrial mutations affecting respiratory complexes J. Rytka, K.J. English, R.M. Hall, A.W. Linnane and H.B. Lukins	427
Genetic analysis of mitochondrial polymorphic proteins in yeast M.G. Douglas, R.L. Strausberg, P.S. Perlman and R.A. Butow	435
Regulation of cytochrome oxidase formation by mutations in a mitochondrial gene for cytochrome <i>b</i> P. Pajot, M.L. Wambier-Kluppel, Z. Kotylak and P.P. Slonimski	443
Mitochondrial genes determining cytochrome <i>b</i> (complex III) and cytochrome oxidase function G.S. Cobon, D.J. Groot Obbink, R.M. Hall, R. Maxwell, M. Murphy, J. Rytka and A.W. Linnane	453
Antimycin- and funiculosin-resistant mutants in <i>Saccharomyces cerevisiae</i> : New markers on the mitochondrial DNA B. Lang, G. Burger, W. Bandlow, F. Kaudewitz and R.J. Schweyen	461
Two mitochondrial antimycin A resistance loci in <i>Saccharomyces cerevisiae</i> E. Pratje and G. Michaelis	467
Mitochondrial inheritance of mucidin resistance in yeast J. Šubík	473
Behaviour of <i>Saccharomyces cerevisiae</i> mutant resistant to Janus Green A. Kruszewska and B. Szczesniak	479
Mitochondrial mutations conferring heat or cold sensitivity in <i>Saccharomyces cerevisiae</i> W.E. Lancashire	481
High spontaneous petite frequency strains of <i>Saccharomyces cerevisiae</i> generated in complementation tests G.D. Clark-Walker, K.M. Oakley, C.R. McArthur and G.L.G. Miklos	491
Extrachromosomal inheritance in a petite - negative yeast - <i>Schizosaccharomyces pombe</i> K. Wolf, G. Seitz, G. Lückemann, B. Lang, G. Burger, W. Bandlow and F. Kaudewitz	497
The mitochondrial genome of yeast: Organization and recombination G. Bernardi	503
The variability of the mitochondrial genome of <i>Saccharomyces</i> strains J.P.M. Sanders, C. Heyting and P. Borst	511
Restriction endonuclease mapping and analysis of grande and mutant yeast mitochondrial DNA R. Morimoto, A. Lewin, S. Merten and M. Rabinowitz	519
The control of mitochondrial DNA synthesis in yeast petite mutants P. Borst, C. Heyting and J.P.M. Sanders	525

A segment of mitochondrial DNA carrying oligomycin resistance K. Wakabayashi	535
Gene identification by coupled transcription-translation of yeast mitochondrial DNA A.F.M. Moorman and L.A. Grivell	539
Mitochondrial mutations that affect mitochondrial transfer ribonucleic acid in <i>Saccharomyces cerevisiae</i> G. Faye, M. Bolotin-Fukuhara and H. Fukuhara	547
Structure and genetics of the 2 $\mu$ m circular DNA in yeast M. Guerineau, C. Grandchamp and P.P. Slonimski	557
Electron microscopical analysis of native and cloned 2- $\mu$ m DNA from <i>Saccharomyces cerevisiae</i> C.P. Hollenberg and H.-D. Royer	565
The study of the genetic function of <i>Paramecium</i> mitochondrial DNA using species hybrids A. Tait, J.K.C. Knowles, J.C. Hardy and H. Lipps	569
Organization and expression of the mitochondrial genome in HeLa cells G. Attardi, M. Albring, F. Amalric, R. Gelfand, J. Griffith, D. Lynch C. Merkel, W. Murphy and D. Ojala	573
Functional organization and evolution of animal mitochondrial DNA W.B. Upholt and I.B. Dawid	587
Physical map and replication of rat mitochondrial DNA K. Koike, M. Kobayashi, S. Tanaka and H. Mizusawa	593
Measurement of the relative rate of mitochondrial DNA synthesis under experimentally varied conditions D. Bogenhagen and D.A. Clayton	597
Use of antibiotic inhibitors in studies of replication and repair of animal mitochondrial deoxyribonucleic acid G.G. Gause, Jr., V.S. Mikhailov, S.I. Tomarev and R.D. Zinovieva	605
Hormonal control of mitochondrial DNA replication in maturing oocytes M. Barat, C. Dufresne, H. Pinon, M. Tourte and J.-C. Mounolou	613
TRANSCRIPTION AND TRANSLATION APPARATUS OF CHLOROPLASTS	
<i>In vitro</i> transcription and translation of chloroplast DNA of <i>C. reinhardi</i> S.J. Surzycki, J.A. Surzycki and R. Lutz	621
Localization of the gene coding for the large subunit of ribulose bisphosphate carboxylase on the chloroplast genome of <i>Chlamydomonas</i> <i>reinhardi</i> S. Howell, P. Heizmann and S. Gelvin	625
Characterization of the RNA compounds synthesized by isolated chloroplasts H.J. Bohnert, A.J. Driesel and R.G. Herrmann	629
Incorporation of <sup>32</sup> P-orthophosphate into nucleoside 5'-triphosphates and RNA by isolated pea chloroplasts J. Bennett and Y. Milewska	637
Phylogenetic origin of chloroplast 16S ribosomal RNA D.E. Buetow, M.S. Kissil and L. Zablen	641
A sequence analysis of low-molecular-weight rRNA from chloroplasts of flowering plants T.A. Dyer and C.M. Bowman	645
Chloroplast ribosomal proteins of <i>Euglena gracilis</i> . Immunological studies G. Freyssinet, F. Morlé and V. Nigon	653

A chloroplast membrane fraction enriched in chloroplast ribosomes M.M. Margulies and J. Weistrop	657
The tRNAs and aminoacyl-tRNA synthetases of <i>Euglena</i> chloroplasts W.E. Barnett, S.D. Schwartzbach and L.I. Hecker	661
tRNAs and aminoacyl-tRNA synthetases in plant organelles J.H. Weil, G. Burkard, P. Guillemaut, G. Jeannin, R. Martin and A. Steinmetz	667
TRANSCRIPTION AND TRANSLATION APPARATUS OF MITOCHONDRIA	
Characterization and translation of yeast mitochondrial RNA F. Hendler, A. Halbreich, S. Jakovcic, J. Patzer, S. Merten and M. Rabinowitz	679
The mitochondrial RNAs of <i>Neurospora crassa</i> : Their function in translation and their relation to the mitochondrial genome A.M. Kroon, P. Terpstra, M. Holtrop, H. de Vries, C. van den Bogert, J. de Jonge and E. Agsteribbe	685
Dual origin of mRNA associated proteins in Ehrlich ascites mitochondria N.G. Avadhani, V.A. Aroskar, F.S. Lewis, G.J. Hansel and M.P. Wolf	697
Mitochondrial transcription in rat liver. Studies on the synthesis of poly(A)-containing RNA C. Saccone, P. Cantatore, G. Pepe, R. Gallerani, C. De Giorgi and C. De Benedetto	701
Properties and purification of poly(A) polymerase from rat liver mitochondria R. Gallerani, C. De Benedetto, C. De Giorgi and C. Saccone	709
The <i>poky</i> mutant of <i>Neurospora crassa</i> A.M. Lambowitz	713
The proteins of <i>Neurospora crassa</i> mitochondrial and cytoplasmic ribosomes H. de Vries and C. van den Bogert	721
Significance of 80-S ribosomes associated with <i>Neurospora crassa</i> mitochondria R. Michel, G. Hallermayer, M.A. Harmey, F. Miller and W. Neupert	725
Comparative studies of ribosomes from mitochondria, chloroplasts and cytoplasm. Morphology and electrophoretic behavior B.J. Stevens, J.-J. Cury, G. Ledoigt and J. André	731
Protein composition of the bovine mitochondrial ribosome T.W. O'Brien, D.E. Matthews and N.D. Denslow	741
Transfer RNAs of yeast mitochondria N.C. Martin and M. Rabinowitz	749
Isoacceptor tRNA species in yeast mitochondria. Methionine and formyl- methionine specific tRNAs coded by mitochondrial DNA R. Martin, J.M. Schneller, A.J.C. Stahl and G. Dirheimer	755
Isoaccepting tRNA <sup>Ser</sup> in mitochondria from <i>Saccharomyces cerevisiae</i> : Mitochondrially coded and cytoplasmic species G. Baldacci, C. Falcone, L. Frontali, G. Macino and C. Palleschi	759
Imported tRNA: Its synthetase as a probably transport protein Y. Suyama and J. Hamada	763
Characterization of rRNA and tRNA from mitochondria of <i>Locusta migratoria</i> H. Feldmann and W. Kleinow	771
Immunological study of yeast mitochondrial phenylalanyl-tRNA synthetase J.M. Schneller, C. Schneller and A.J.C. Stahl	775
Mitochondrial protein synthesis in higher plants C.J. Leaver	779

## GENERAL ASPECTS OF MITOCHONDRIAL BIOGENESIS

Mitochondrial phospholipid synthesis and the phospholipid exchange proteins K.W.A. Wirtz, R.H. Lumb, H.H. Kamp, G.M. Helmkamp, H. van den Bosch and L.L.M. van Deenen	785
Incorporation of mitochondrial membrane proteins into liposomes G.D. Eytan	793
The role of mitochondria-bound 80S ribosomes in mitochondrial biogenesis W.F. Bennett, A. Gutierrez-Hartmann and R.A. Butow	801
Studies on the synthesis of mitochondrial proteins in the cytoplasm and on their transport into the mitochondrion G. Hallermayer and W. Neupert	807
<i>In vitro</i> synthesis and transport into mitochondria of cytoplasmically translated proteins M.A. Harmey, G. Hallermayer and W. Neupert	813
Specific labelling of mitochondrially synthesized proteins in yeast cells in the absence of antibiotics W. Bandlow	819
Integration and disintegration of proteins synthesized in mitochondria H.-D. Hofmann, E. Hundt and B. Kadenbach	827
Synthesis of mitochondrial DNA, -proteins and -phospholipids in the young sea urchin embryo <i>Sphaerechinus granularis</i> H. Bresch	831
Inhibition of cytoplasmic protein synthesis by mitochondrial soluble factors in rat liver and Walker carcinosarcoma N. González-Cadavid, B. Dorta and A. Carmona	835
Mammalian embryos: A model for studying the dependence of growth and differentiation processes on mitochondrial biogenesis and function R. Bass	843
Unmasking of mitochondrial precursors stored in the yolk platelets of <i>Artemia salina</i> dormant gastrulae C.G. Vallejo and R. Marco	847
Screening tests for suppressors of respiratory deficient mutants in <i>Schizosaccharomyces pombe</i> and model for a mitochondrial partial suppression of nuclear pleiotropic strain A. Goffeau, F. Labaille, O. Mohar and A.-M. Colson	851
Respiration deficient mutants with intact mitochondrial genomes: Casting a wider net H.R. Mahler, T. Bilinski, D. Miller, D. Hanson, P.S. Perlman and C.A. Demko	857
Assembly of the cyanide-insensitive respiratory pathway in <i>Neurospora crassa</i> D.L. Edwards, J.H. Chalmers, Jr., H.J. Guzik and J.T. Warden	865
Physiological and genetical analysis of the respiratory chain of <i>Paramecium</i> J. Doussière, A. Adoutte, A. Sainsard, F. Ruiz, J. Beisson and P. Vignais	873
Genetic control of glycerol-3-phosphate dehydrogenase synthesis in <i>Neurospora</i> J.B. Courtright	881
Primary antimitochondrial activity of carcinogens in <i>Saccharomyces cerevisiae</i> V. Egilsson, I.H. Evans and D. Wilkie	885
Author index	893



SIGNIFICANCE OF 80-S RIBOSOMES ASSOCIATED WITH NEUROSPORA CRASSA MITOCHONDRIA

R. Michel, G. Hallermayer, M.A. Harmey<sup>†</sup>, F. Miller and W. Neupert  
Institut für Physiologische Chemie, Physikalische Biochemie und Zellbiologie  
der Universität München, Germany

The mitochondrial ribosome of *Neurospora crassa* was first described as having a sedimentation coefficient of 73-S (subunits 50-S and 37-S) compared to 77-S for the cytoplasmic ribosome (subunits 60-S and 37-S) (1). The results of KÜNTZEL and NOLL (1) were accepted for some years. Recently however, DATEMA et al. (2) have described the mitochondrial ribosome as having an S-value of 80. They further stated that the 73-S mitoribosome of *Neurospora* is an artefact of the isolation procedure. It has been suggested (2) that washing mitochondria with EDTA-containing media results in the loss of essential components giving 73-S ribosomes, whereas isolation of mitochondria in the presence of magnesium and heparin yields intact ribosomes with an S-value of 80. Here we attempt to evaluate the significance of 73-S and 80-S ribosomes occurring in mitochondria isolated by different procedures.

1. Ultracentrifugation of ribosomes from mitochondria isolated in the presence and absence of magnesium ions

Mitochondria were isolated according to four different procedures as outlined in the table. Ribosomes were prepared from each preparation in an identical manner. Ribosomes from SE<sub>1.0</sub>T mitochondria show a prominent 73-S monomer with 50-S and 37-S subunits and a small amount of dimers (103-S) and trimers (fig. 1). In the case of SAMT ribosomes obtained from mitochondria isolated as described by DATEMA et al. (2), the gradient in addition to the 73-S component obtained in

TABLE

Designation of mitochondrial preparations	SE <sub>1.0</sub> T	SAMT	SME	SE <sub>0.1</sub> T
Isolation medium	A	B	B	C
Wash medium	2 x A	3 x B	2 x A	2 x C

A: 0.44 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.6; B: 0.44 M sucrose, 100 mM NH<sub>4</sub>Cl, 10 mM MgCl<sub>2</sub>, 10 mM Tris-HCl, pH 7.6; C: 0.44 M sucrose, 0.1 mM EDTA, 10 mM Tris-HCl, pH 7.6.

SE<sub>1.0</sub>T preparations, shows a prominent peak at 79-S with a smaller peak at 109-S (fig. 1). These additional peaks are coincident with mono- and dimers of cytoplasmic ribosomes. In the case of SME mitochondria (isolated in SAMT but twice washed

<sup>†</sup>M.A.H. (present address: Department of Botany, University College, Dublin (Irish Republic) wishes to thank CIBA GEIGY for a visiting fellowship.

with  $SE_{1.0}T$ ), a profile qualitatively similar to that obtained from SAMT mitochondria is obtained. The amount of subunits is however greater and the content of 73-S, dimers and trimers is lower (not shown).

These data are in agreement with those of DATEMA et al. (2). We however, interpret them in a different manner. We conclude that the 79-S and the 109-S components can be attributed to contaminating cytoplasmic ribosomes.

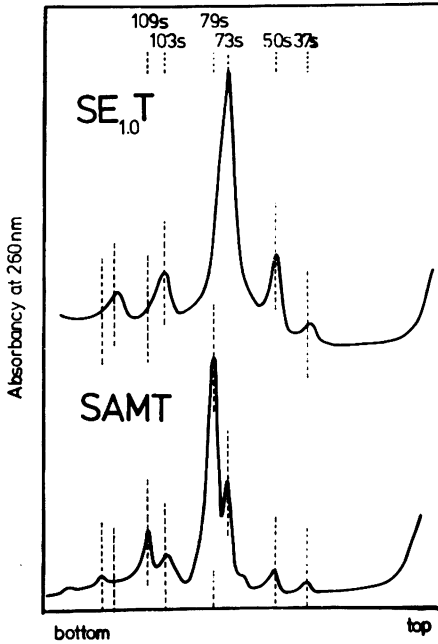
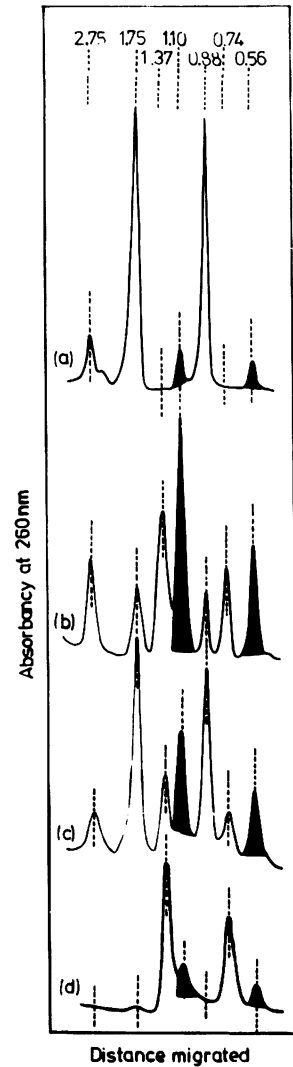


Fig. 1

Density gradient centrifugation of ribosomes isolated from mitochondria prepared in  $SE_{1.0}T$  and SAMT buffer.

Fig. 2

Polyacrylamide gel electrophoresis of high molecular weight RNA extracted from mitochondria, which were isolated in different buffers: (a)  $SE_{1.0}T$ , (b) SAMT, (c) SME, (d) total cytoplasmic RNA. Apparent molecular weights were determined by comparison with *E. coli* ribosomal RNA co-electrophoresed with the RNA extracts. The gels were run at  $25^{\circ}C$ . Apparent molecular weights in daltons by  $10^{-6}$  are shown at the top of the figure.



## 2. Ribosomal RNA from mitochondria isolated under different conditions

RNA was extracted from the different mitochondrial preparations and subjected to polyacrylamide gel electrophoresis. The  $SE_{1,0}T$  preparation (fig. 2a) shows two ribosomal components with apparent molecular weights of  $1.75$  and  $0.88 \times 10^6$  daltons and a small DNA peak. These components are clearly distinguishable from the cytoplasmic RNAs (apparent molecular weights  $1.37$  and  $0.74 \times 10^6$  daltons; cf. fig. 2d). SAMT and SME mitochondrial preparations (fig. 2b and 2c) show profiles, which are clearly composites of  $SE_{1,0}T$  profile (fig. 2a) and that of the cytoplasmic RNA (fig. 2d). The SAMT profile (fig. 2b) also shows an extremely high DNA content. The individual RNA species are clearly distinguishable by their mobilities relative to *E. coli* ribosomal RNA (apparent molecular weight  $1.1$  and  $0.56 \times 10^6$  daltons). The data in fig. 2 firmly support the conclusion that ribosomes from mitochondria isolated in the presence of magnesium consist of a mixture of cytoplasmic and mitochondrial ribosomes.

The presence of cytoplasmic ribosomes in SAMT preparations can be further shown by electrophoretic analysis of the low molecular weight RNAs.  $SE_{1,0}T$  mitochondria show only a 4-S component and heating and cooling fails to show any further low molecular weight components. RNA from cytoplasmic ribosomes on the other hand shows only a 5-S RNA and on heating and cooling shows an additional peak of 5.8-S RNA. SAMT low molecular weight RNA shows the presence of 5-S and 4-S peak and on heat treatment shows in addition a 5.8-S peak. The latter peak is characteristic of cytoplasmic ribosomes and its presence in SAMT-RNA we attribute to the presence of cytoplasmic ribosomal RNA (3).

## 3. Electron microscopic examination of mitochondrial preparations

In view on the heterogeneity of the ribosomes obtained from SAMT mitochondria we compared the SAMT and  $SE_{1,0}T$  mitochondrial preparations by electron microscopy.

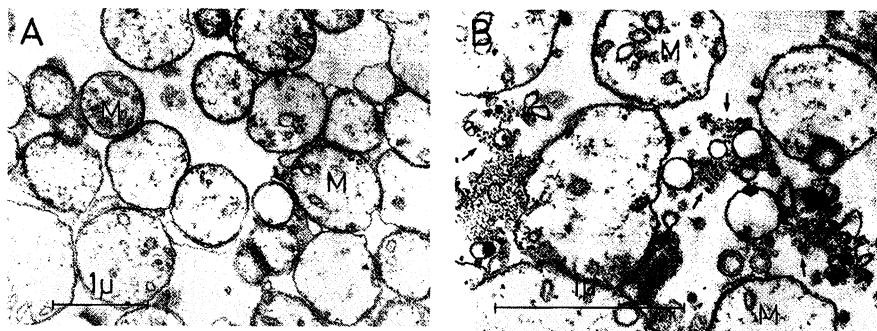


Fig. 3

Electron microscopy of mitochondrial preparations isolated in  $SE_{1,0}T$  (A) and SAMT (B). Both fractions were treated with antibodies specific for cytoplasmic ribosomes.

M: mitochondria, C: tentatively identified chromatin; Arrows indicate agglutination of cytoplasmic ribosomes.

SE<sub>1.0</sub>T mitochondria can be seen to consist of a homogeneous population of organelles with well preserved inner and outer membranes. In contrast, the SAMT preparations are clearly heterogeneous containing much mitochondrial material, with patches of material resembling chromatin, and membrane attached ribosomes. The treatment of SAMT ribosomes with antibodies specific for cytoplasmic ribosomes led to the aggregation of much rough membrane vesicles and to the formation of large clumps of aggregated particles, which have the appearance of cytoplasmic ribosomes (fig. 3B). SE<sub>1.0</sub>T mitochondria treated with the same antibody show no evidence of either chromatin, rough vesicles or ribosome-like aggregates (fig. 3A). We therefore attribute the ribosomal heterogeneity of SAMT preparations (cf. fig. 1 and fig. 2b) to the presence of contaminating cytoplasmic ribosomes.

#### 4. Effect of heparin on ribosomes from different mitochondrial preparations

DATEMA et al. (2) have stated that the isolation of the mitochondrial 80-S ribosomes requires the presence of magnesium ions and heparin during the lysis of the mitochondria to protect the ribosomes from degradation to the 73-S condition. The effect of heparin in the isolation media for ribosomes is shown in fig. 4

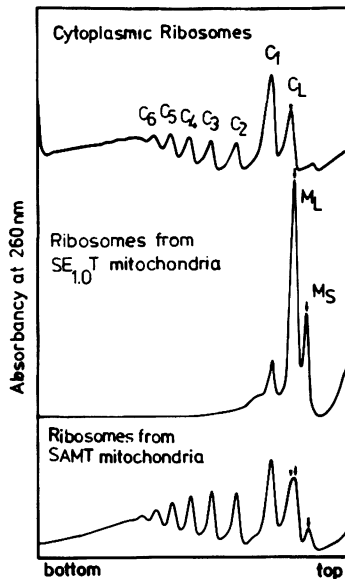


Fig. 4

Density gradient centrifugation of ribosomes isolated in the presence of heparin (0.5 mg/ml isolation medium)

Cytoplasmic ribosomes are effectively protected and only a small amount of 60-S subunit appears in heparin preparations. The effect of heparin on the SE<sub>1.0</sub>T preparations is quite striking. The ribosomes are almost completely dissociated and

mainly subunits are obtained. In SAMT preparations the inclusion of heparin causes the disappearance of the 73-S ribosome with concomitant appearance of 50-S and 37-S subunits leaving the 80-S ribosome intact so that the gradients show only one monomer-type, viz. the cytoplasmic.

#### 5. Immunoprecipitation of ribosomes from different mitochondrial preparations

We have previously described the preparation of specific antibodies against cytoplasmic and mitochondrial ribosomes (4). These antibodies were used to demonstrate in SAMT ribosomal preparations the presence of cytoplasmic and mitochondrial ribosomes. SAMT ribosomes were prepared from cells homogeneously labeled with  $^3\text{H}$ -leucine. The ribosomes were subjected to density gradient centrifugation. The gradient was divided into 30 fractions and total radioactivity was measured (fig. 5A). Each fraction was then divided into two equal portions. One portion was treated with antibodies against cytoplasmic ribosomes and cold carrier cytoplasmic ribosomes, the other with antibodies against mitochondrial ribosomes and cold carrier 73-S ribosomes. Radioactivities in the precipitates and in the supernatants (fig. 5B and 5C) were determined. The antibody against the cytoribosomes completely precipitates the 79-S monomer and the 109-S dimer and leaves the 73-S monomer, the 103-S dimer and the 50-S subunit in the supernatant. Conversely, the antibody against mitochondrial 73-S ribosomes precipitates the 73-S monomer, the 103-S dimer and the 50-S subunit, leaving the 79-S particle in the supernatant.

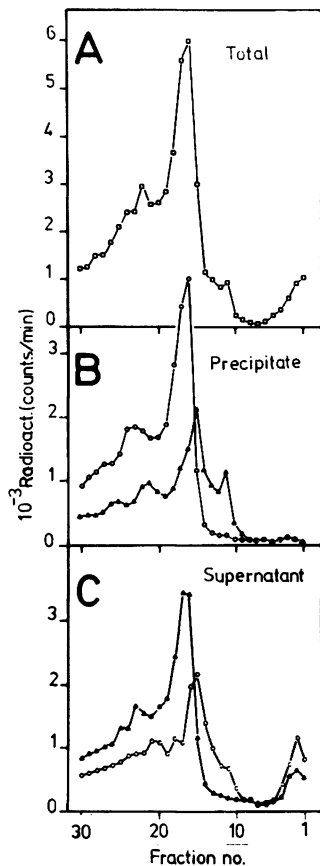


Fig. 5

Immunoprecipitation of mitochondrial and cytoplasmic ribosomes from density gradient fractions of ribosomes isolated from mitochondria prepared in SAMT buffer.

○—○—○ : Radioactivity precipitated with antiserum against cytoplasmic ribosomes;  
 ▲—▲—▲ : Radioactivity precipitated with antiserum against mitochondrial ribosomes.

#### 6. Functional properties of ribosomes of mitochondria prepared in the presence and absence of magnesium ions

We have shown in an earlier publication that mitochondria prepared in  $SE_{1,0}^T$  under appropriate conditions are able to form subunits 1, 2 and 3 of cytochrome oxidase (5). This shows clearly that the ribosomes in  $SE_{1,0}^T$  mitochondria are capable of carrying out the synthesis of defined mitochondrial proteins. Since only one type of monomer is found in the  $SE_{1,0}^T$  mitochondria, viz. the 73-S, we conclude that this is the functional mitochondrial monomer. The isolated 73-S ribosome is able to react with puromycin and to release the nascent chains. In the absence of a crude preparation of G-factor, which may also contain a dissociation factor, after the release the ribosomes remain as 73-S monomer, in its presence they fall apart into 50-S and 37-S subunits.

#### Conclusions

The data presented clearly show that mitochondrial preparations obtained in the presence of magnesium contain both cytoplasmic and mitochondrial ribosomes. In our view, as first suggested by KÜNTZEL and NOLL (1), the 73-S ribosome is the real functional mitochondrial ribosome.

#### References

1. KÜNTZEL, H. & NOLL, H. (1967) Nature 215, 1340-1345
2. DATEMA, R. et al. (1974) Biochem. Biophys. Acta 335, 386-395
3. LEAVER, C.J. & HARMEY, M.A. (1976) Biochem. J. 157, 275-278
4. HALLERMAYER, G. & NEUPERT, W. (1974) FEBS Letters 41, 264-268
5. RUECKER, A.v. et al. (1974) FEBS Letters 47, 290-294