DEOXYRIBONUCLEIC ACID ASSOCIATED WITH YEAST MITOCHONDRIA

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DNA is generally considered to be confined to the nucleus. Small amounts of DNA sometimes found in other cell fractions are usually attributed to contamination by nuclear material (Allfrey 1959). The presence of significant amounts of extranuclear DNA occurring in the cytoplasm of the amphibian oöcyte (Stich 1962), the kinetosomes (Clark and Wallace 1960), and in the chloroplasts of plant cells (Chun et al. 1963) is well documented but is regarded rather as an exception than as a rule, because in these cases DNA is associated with cellular components absent from higher cells.

This paper presents evidence that preparations of mitochondria from baker's yeast, purified by flotation in density gradients, contain a significant quantity of DNA. The amount found is far in excess of that accounted for by the polydeoxyribonucleotide component (Appleby and Morton, 1960) of mitochondrial cytochrome b_2.

Since the mitochondrion represents an organelle present in most cells, including those of mammals, the occurrence of DNA in yeast mitochondria suggests the possibility, that extranuclear DNA is much more common that hitherto suspected

Mitochondrial function in the yeast cell is in part controlled by extrachromosomal genetic factors (Ephrussi and Hottinguer 1951), whose exact chemical nature is unknown. The presence of DNA in yeast mitochondria is thus of special interest.

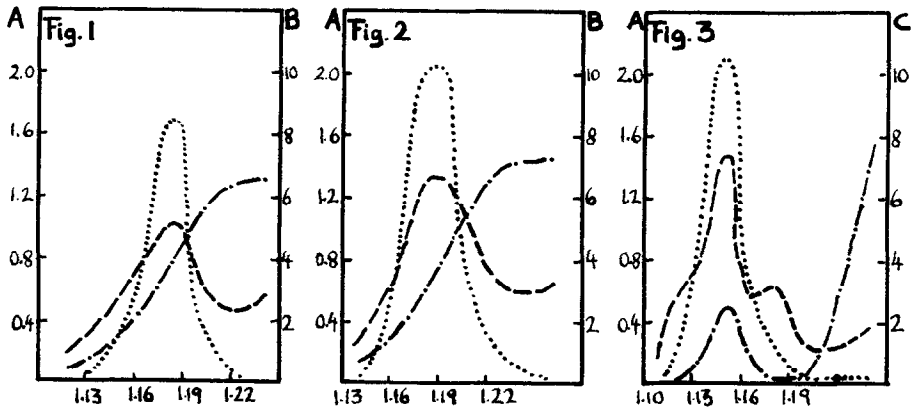
Experimental : The wild type strain W of Saccharomyces cerevisiae was grown as described previously (Schatz 1963). The concentration of glucose in the growth medium was 0.8 %. The preparation of the yeast homogenates and of the crude mitochondrial fractions was the same as in earlier work (Schatz et al. 1963), except that the medium employed for the homogenization and washing procedures contained 0.25 M mannitol, 20 mM tris-buffer pH 7.4 and 1 mM EDTA. For density gradient flotation, a linear continuous sucrose gradient (volume 25 ml; 0.98-1.66 M sucrose; additions as above)

was constructed (Bock and Ling 1954) on top of 4.5 ml of a suspension of a crude mitochondrial fraction in 1.8 M sucrose and centrifuged at 25 000 rpm for 5 hours in the rotor SW 25.1 of the Spinco ultracentrifuge. If sedimentation through the gradient was desired, 4.5 ml of a mitochondrial suspension in 0.5 M sucrose were layered on top of the preformed gradient and centrifuged as above. Experiments involving flotation in gradients of "Urografin" (N,N'-diacetyl-3,5-diamino-2,4,6-triiodo-benzoate, an X-ray contrasting agent manufactured by Fa. Schering)+) were conducted as follows : The crude mitochondrial fraction was dispersed in homogenization medium containing sufficient "Urografin" to adjust the density of the suspension to 1.25 $g.cm^{-3}$. 4.5 ml of this heavy suspension were overlayered by 25 ml of a linear "Urografin" gradient (1.10-1.20 $g.cm^{-3}$) and centrifuged as described above. After gradient centrifugation, fractions of 3.0 ml each were collected starting from the top of the gradient with the help of a specially designed syringe. They were then diluted with homogenization medium and the particles were washed twice by centrifuging at 30 000 rpm for 60 minutes in the Spinco no. 30 rotor. The pellets were resuspended in homogenization medium and an aliquot was withdrawn for the assay of protein, succinate-cytochrome c reductase activity and acid-soluble deoxyribose-containing compounds. The remainder of the particles was washed twice with 0.5 M perchloric acid in 35 % ethanol at 0^{o}. In some experiments lipid was also extracted with ethanol, followed by ethanol -ether 3 : 1 at room temperature. Finally, the DNA content of these washed particles was determined by the indole method (Ceriotti 1952). The results obtained with this procedure were frequently checked by use of the methods of Dische (1930) and Burton (1956). Agreement within the limits of experimental error was always observed. Digestion with pancreatic DNase (Fluka) was carried out at 26^{o} for 2 hours in 0.02 M acetate buffer pH 6.6 containing 150 µg/ml enzyme and 0.05 M $MgCl_2$. L-lactate-cytochrome c reductase activity was assayed under the conditions chosen by Nygaard (1961) for measurements of the turnover number of pure cytochrome b_2. The determination of succinate-cytochrome c reductase activity and that of protein were performed as described elsewhere (Schatz and Klima 1964). Specific activities are expressed as µmoles of acceptor reduced per minute per mg protein in a total volume of 3.0 ml at 20^{o}.

Results : An unambiguous demonstration of mitochondrial DNA requires the quantitative removal of nuclei and nuclear fragments from the mitochondrial fractions examined. This task poses difficult problems, as the sedimentation properties and the enzymic complement of the yeast nucleus and its fragments are not known. This lack of information precluded the adoption of differential centrifugation for the purification of the mitochondrial fractions as well as the assessment of their contamination with nuclear material by enzymatic tests. In view of the exceptionally high density of all isolated nuclei examined thus far (Allfrey 1959), however, it was hoped, that density gradient equilibrium centrifugation might overcome these difficulties. Thus, equilibrium centrifugation in sucrose gradients was initially tried for the preparation of pure yeast mitochondria. The resolution of

+) We are greatly indebted to Dr. Klose (Fa. Schering, Vienna) for the generous supply with this substance.

subcellular components effected by this procedure, however, proved to be unsatisfactory for the purpose of the present work. Whereas the mitochondria formed a well-defined band at a density of 1.18 $g.cm^{-3}$, DNA was found to be diffusely distributed within the gradient (figure 1). There was no clear separation between mitochondria and contaminating particles rich in DNA. The relatively large amounts of DNA (20-40µg/mg of protein) found in the band which contained the mitochondria could thus not be regarded as truly mitochondrial. Essentially the same results were obtained, if flotation (instead of sedimentation) through the gradient was employed in order to avoid complications due to slowly sedimenting nuclear fragments (figure 2). In an attempt to avoid the shortcomings of sucrose gradients, other types of gradients were investigated. After numerous trials, "Urografin" gradients were found to be well suited for the present work. The bands obtained by floating crude mitochondrial fractions in this type of gradient are schematically depicted in figure 4. The bulk of the protein was found in band B, which also contained most of the succinate-cytochrome c reductase activity of the crude mitochondrial fraction. If viewed in the electron microscope, the particles of this band appeared as a fairly homogeneous population of yeast mitochondria. In five experiments, they contained between 1.1 and 4.3 µg DNA per mg of protein (table I). When the different fractions of the gradient were analyzed for DNA, two well separated peaks were found (figure 3). One of the peaks was due to DNA associated with particles which were not floated under the experimental conditions employed and were thus clearly separated from the mitochondria. The DNA content of this dense material (probably including nuclear matter) varied widely from one experiment to the other and ranged from 20 - 250 µg/mg of protein. The other peak of DNA coincided exactly with the peak of mitochondrial protein and succinate-cytochrome c reductase activity and thus proved to be due to DNA associated with mitochondria. Upon digestion with pancreatic DNase, up to 75 % of this mitochondrial DNA was rendered acid-soluble. In addition to DNA, the purified mitochondria contained 3.5-4.8 µg/mg protein of deoxyribose-containing compounds not precipitable by acid, presumably, mono- or oligonucleotides (table I). The amount of DNA found in the purified mitochondrial fractions is not accounted for by the polydeoxyribonucleotide component (Appleby and Morton 1960) of mitochondrial cytochrome b_2. Table II gives the mitochondrial concentration of cytochrome b_2 as calculated from the specific activity of L-lactate-cytochrome c reductase measured in the purified mitochondrial fraction after detergent activation. (The extent of activation by the detergent as well as



Figures 1 -3 : Distribution of succinate-cytochrome c reductase activity (....), protein (------) and DNA (.-.-.-) after subjecting crude preparations of yeast mitochondria to sedimentation in a sucrose gradient (fig. 1), flotation in a sucrose gradient (fig. 2) and flotation in a "Urografin" gradient (fig. 3). Abscissa : density (g. cm^{-3}). Ordinate A : mg protein/ml fraction and ΔE_{550} per minute per 0.4 ml fraction; ordinate B : µg DNA/0.1 ml fraction; ordinate C : µg DNA/ml fraction.

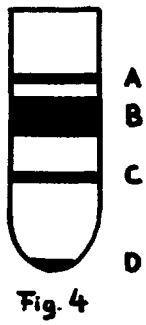


Fig. 4

Figure 4 : Banding of a crude mitochondrial fraction from yeast in a "Urografin" gradient.
A (1.12 g. cm^{-3}) : Mitochondrial precursors (Schatz 1963)
B (1.15 g. cm^{-3}) : Mitochondria
C (1.19 g. cm^{-3}) : Double Membranes rich in ATPase (Schatz et al. 1963)
D ($\geq$ 1.25 g. cm^{-3}) : Debris including nuclear material.

	Deoxyribose compounds not precipitable by acid ++) µg / mg protein	Deoxyribose compounds precipitable by acid (DNA) µg / mg protein
Experiment 1	3.48	1.55
Experiment 2	4.25	1.78
Experiment 3	4.83	1.06 §)
Experiment 4	-	1.51
4 a	-	3.60 +)
Experiment 5	-	4.30 &)
5 a	-	4.30 &) +)

Table I : Deoxyribose compounds in purified yeast mitochondria.
++) Calculated as nucleotides
§) Particles incubated at 26° for 30 min at pH 6.6 after acid precipitation
+) Particles incubated at 26° for 2 hours at pH 6.6 after acid precipitation
&) Lipid extracted as described under Experimental.

Enzyme tested	Specific activity		Calculated concentration of cytochrome b_2 (% of mitochondrial protein)
	without detergent	with 0.1 % Triton X 100	
L-lactate-cytochrome c reductase	0.054	0.12	0.17
succinate-cytochrome c reductase	0.39	nil	

Table II : Specific activities of L-lactate-cytochrome c reductase and of succinate-cytochrome c reductase in purified yeast mitochondria.

the activity of succinate-cytochrome c reductase are included for comparison). Taking into account, that cytochrome b_2 contains 5-6 % of polydeoxyribonucleotide (Appleby and Morton 1960) it can be calculated, that the amounts of DNA found in our mitochondrial preparations exceed, by a factor of 10 to 40, the amounts of DNA contributed by mitochondrial cytochrome b_2.

All of the DNA present in the yeast homogenates was found in the particulate pellet upon centrifugation for 2 hours at 40 000 rpm (Spinco no.40 rotor). The absence of DNA from the supernatants made it very unlikely that the DNA found in the purified mitochondrial fractions was an adsorbed contaminant.

Discussion : The investigations described here were prompted by the previous observations, that under certain conditions yeast mitochondria appear to arise from non-mitochondrial precursors (Linnane et al. 1962; Schatz 1963). The present work was undertaken to learn more about the factors controlling the organized assembly of mitochondrial structure. Structure and function of mitochondria in yeast are in part governed by extrachromosomal genetic factors (Ephrussi and Hottinguer 1951). The chemical nature of these factors is unknown, but available evidence suggests the involvement of nucleic acid (Raut and Simpson 1955; Moustacchi and Marcovich 1963). In view of the role of DNA as the primary carrier of genetic information the possibility was investigated that these factors regulating mitochondrial function might be extrachromosomal DNA present within the mitochondria themselves. This paper reports the detection of small, but significant amounts of DNA associated with yeast mitochondria purified by density gradient flotation. The material in question was identified as DNA by its acid-precipitability, the deoxyribose content (assayed with different methods) and its

digestion by pancreatic DNase. The possibility that the DNA associated with the mitochondrial fraction might be due to contamination by nuclear material can be excluded, since this DNA was found to occur in a discrete band well separated from the bulk of DNA and exactly coincided with the only peak of the mitochondrial marker enzyme, succinate-cytochrome c reductase. The other possibility to be regarded is that of adsorption artefacts. Because of the absence of DNA from the soluble portion of the yeast homogenates this is considered unlikely, but cannot be definitely ruled out. We are at present comparing the base ratio of mitochondrial DNA with that of DNA extracted from whole cells in order to corroborate the results obtained here. The detection of DNA in mitochondria lends support to the hypothesis, though not proving it, that the extrachromosomal factors controlling mitochondrial function in yeast might be mitochondrial DNA. Moreover, the presence of appreciable amounts of acid-soluble, deoxyribose-containing compounds in mitochondria suggests the occurrence of DNA metabolism in these organelles. Mitochondria have been found in virtually all plant and animal cells. The detection of DNA in the mitochondria of yeast may thus indicate, that the occurrence of small amounts of extranuclear DNA is a rather general phenomenon. This possibility is being investigated.

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Allfrey,V., in : "The Cell" (Brachet,J. and Mirsky,A., ed.). Academic Press 1959, vol. I, p. 193.
Appleby, C.A. and Morton,R.K., Biochem.J. 75, 258 (1960).
Bock,R.M. and Ling.N.S., Analyt.Chem.26, 1543 (1954).
Burton,K., Biochem.J. 62, 315 (1956).
Ceriotti,G., J.Biol.Chem. 198, 297 (1952).
Chun,E.H.L., Vaughan,M.H. and Rich.A., J.Mol.Biol.7, 130 (1963).
Clark,T.B. and Wallace,F.G., J.Protozool. 7, 115 (1960).
Dische,Z., Z.Mikrochem. 2, 26 (1930).
Ephrussi,B. and Hottinguer,H., Cold String Harbor Sympos.Quant.Biol. 16, 75 (1951).
Linnane,A.W., Vitols,E. and Nowland,P.G., J.Cell Biol. 13, 345 (1962).
Moustacchi,E. and Marcovich,H., Compt.rend. 256, 5646 (1963).
Nygaard,A.P., in : "Hematin Enzymes" (Falk,J.E., Lemberg.R. and Morton,R.K., ed.) Pergamon Press 1961, p. 547.
Raut,C.W. and Simpson,W.C., Arch.Biochem.Biophys. 57, 218 (1955).
Schatz,G., Biochem.Biophys.Res.Communic. 12, 448 (1963).
Schatz,G., Tuppy,H. and Klima,J., Z.Naturforsch. 18b, 145 (1963).
Schatz,G. and Klima,J., Biochim.Biophys.Acta, in press.
Stich,H.F., Exp.Cell Res. 26, 136 (1962).