SEPARATION AND CHARACTERIZATION OF MITOCHONDRIAL DNA FROM YEAST[1]

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The demonstration of the presence of DNA in chloroplasts and mitochondria by chemical, cytological and autoradiographic techniques (for review see Tewari et al, 1965), together with the production of certain cytoplasmically hereditable changes by treatment of cells with U.V. light and dyes capable of strong interaction with nucleic acids (Lyman et al, 1961; Gibor and Granick, 1962; Moustachi and Marcovich, 1963; Avers and Dryfuss, 1965) have led to the surmise that these organelles may be capable of at least partial self duplication and their structure and function controlled by extranuclear genetic determinants localized within the particle (Ephrussi, 1953; Michaelis, 1956; Sager, 1960; Gibor and Granick 1964; Jinks, 1964). A species of DNA particular to the chloroplast has been isolated free of nuclear DNA and shown to be a regular double helical molecule by its behaviour on melting and on banding in CsCl gradients (Edelman et al, 1964; Ray and Hanawalt, 1964). There have also appeared suggestive reports on the presence of satellite components in DNA isolated from mitochondria (Luck and Reich, 1964; duBuy et al, 1965). Rabinowitz et al (1965) have actually separated such a satellite from the DNA of chick embryo mitochondria by means of a CsCl gradient in which this component bands at a density higher than that of corresponding nuclear DNA.

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We have already reported the presence of DNA associated with mitochondria obtained either by mechanical or enzymatic disruption of yeast cells and in respiratory sub-particles (ETP) prepared by comminution of the mitochondria (Tewari et al, 1965). The DNA isolated from both particle types invariably contained a small proportion of a new component with a melting point (T_m) in 0.15 M NaCl--0.015 M Na-Citrate (SSC) of 74°, as compared to the bulk of mitochondrial or cellular DNA which melts at 84° in SSC. In confirmation of these observations, on analytical ultracentrifugation in CsCl gradients a component of lower density ($\rho = 1.685$)* invariably accompanied the predominant heavy, normal component ($\rho = 1.700$)* in all these DNA preparations. We now wish to assess the amount of this novel, mitochondrial DNA (m-DNA) actually present in commercially grown yeast (Fleischmann) and to report the isolation of this m-DNA completely free of associated whole cell (i.e. nuclear) DNA.

The amount of total DNA and RNA present in mitochondria and ETP as measured colorimetrically are shown in Table I.

The proportion of m-DNA was estimated from absorbance-temperature profiles similar to those of Fig. 1 after prior isolation of the DNA by phenol extraction in the presence of EDTA and sodium lauryl sulfate, purification by RNase treatment, and repeated ethanol precipitations. The overall recovery after this treatment is of the order of 50 percent. Total DNA in the particles varies between 3 and 10 μg per mg protein and the ratio RNA/DNA between 4 and 8. While the proportion of m-DNA in the total appears to fluctuate greatly between experiments, it is to be noted, however, that an inverse correlation appears to obtain between this proportion and the total DNA associated with the particles. For example one ETP preparation analyzing for a total of 3.2 μg DNA/mg protein, contained m-DNA and bulk DNA in a ratio of 25 : 75, while for another with a total of 10.4 μg the ratio was 4.3 : 95.7. These observations suggest that the variable component is the extent of contamination by nuclear DNA and not the true amount of m-DNA per respiratory

*relative to bacteriophage T_3DNA with $\rho = 1.709$.

Table I

NUCLEIC ACID CONTENT OF RESPIRATORY PARTICLES

PREPARATION		NUCLEIC ACID CONTENT			
A	B	Total DNA[a] μg/mg particle protein	RNA[b] μg/mg particle protein	RNA/DNA	Satellite DNA† (% of total)
1		8.2	40.6	4.9	5
2		7.4	37.2	5.0	8
3		6.8	36.4	5.3	7
4		8.3	40.8	4.9	5
5		5.2	18.6	3.6	12
6		4.8	25.4	5.3	15
	1	10.4	83.2	8.0	4.3
	2	4.2	29.2	6.9	14
	3	3.2	17.8	5.6	25
	4	3.6	25.7	7.1	20

A = mitochondria [prepared as described in Tewari et al, (1965)].
B = sub-mitochondrial electron transport particle (ETP) (Mahler et al, 1964).
† from T_m
a by diphenylamine reaction (Dische, 1930; Burton, 1956).
b by orcinol reaction (Dische, 1955).

particle. This is borne out in Table II, which presents pertinent data for four independent preparations of yeast mitochondria in which the proportion of m-DNA varied between 5 and 25% of the total.

We see that there is remarkable constancy of the amounts of total and m-DNA per cell (as measured by DNA/protein ratio), of the amount of m-DNA per mitochondrion and hence, of course, of the proportion of m-DNA in the total cellular DNA. The latter value (line 10) is of the order of 0.5%, while that

Table II

AMOUNTS OF m-DNA PRESENT IN YEAST

PREPARATION	I	II	III	IV
(1) Total yeast cell protein used (mg)	2130	1640	458	423
(2) Fraction of cells broken (from protein released into sup.)	0.45	0.40	0.60	0.60
(3) Mitochondrial protein isolated (mg)	91	67	31	33
(4) Total DNA in mitochondrial fraction (μg)	1200	1600	115	300
(5) m-DNA in (4), from T_m (μg)	96	88	28	30
(6) m-DNA originally present (μg) (5)/(2)	213	220	47	50
(7) μg m-DNA per mg mitochondrial protein (5)/(3)	1.05	1.31	0.90	0.91
(8) μg m-DNA per mg cell protein (6)/(1)	0.10	0.13	0.10	0.12
(9) μg total DNA per mg cell protein	21.0	20.5	24.0	23.0
(10) Proportion of m-DNA in total DNA (%) (8)/(9)	0.48	0.63	0.42	0.52

for the content of m-DNA of the particle (line 7) equals 1.0 ± 0.1 μg per mg mitochondrial protein. If we assume that one yeast mitochondrion contains approx. 1×10^{-13}g protein (Horowitz and Metzenberg, 1966) this would correspond to a DNA content of 1×10^{-16}g per mitochondrion. In any case these data provide the first reported measure of the actual amount of the characteristic m-DNA relative to mitochondrial protein. All previous reports have dwelled solely on the amount of total DNA observed in mitochondrial fractions (Schatz

et al, 1964; Kalf, 1964; Luck and Reich, 1964; Nass et al, 1965).

Crude m-DNA isolated from respiratory particles exhibits the biphasic melting profile shown in Fig. 1 with the two components melting at 74° and 84°, while the corresponding profile for DNA isolated from whole cells gives no evidence of a second component and melts sharply at 84°.

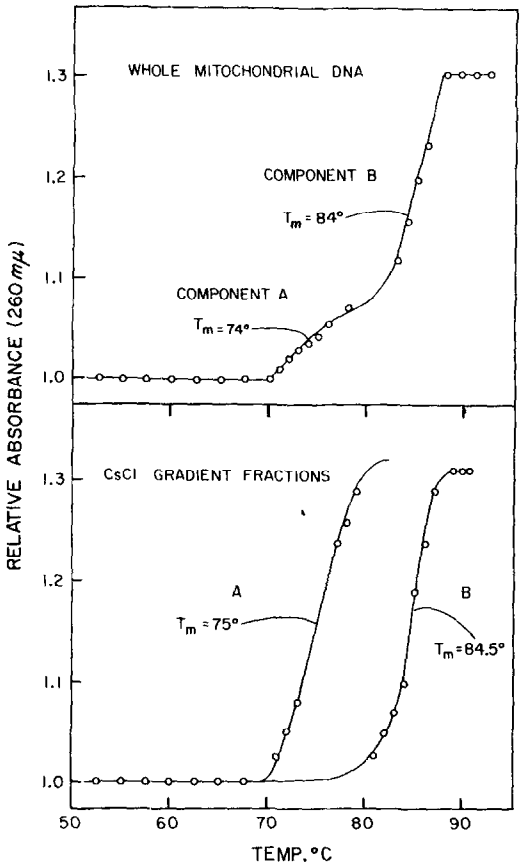

Fig. 1. Temperature-absorbance profile of DNA from mitochondria and its separated components. Fractions 82-85 (A) and 74-77 (B) were pooled from a number of preparative CsCl gradient runs (Fig. 2), dialyzed separately against SSC and T_m taken. Absorbance at the elevated temperature relative to the native material is plotted as function of temperature.

Purification was achieved by preparative gradient ultracentrifugation in CsCl solutions of density 1.710 using the SW 39 rotor of the Spinco L-2 ultracentrifuge operating at 20° for 60 hrs. at 37, 000 rpm. At the end of

the run the rotor was allowed to coast to a stop without braking, tubes were punctured and fractions containing 3 drops each collected. OD_{260} was measured after adding 0.5 ml of SSC to each fraction and plotted against fraction number as shown in Figure 2.

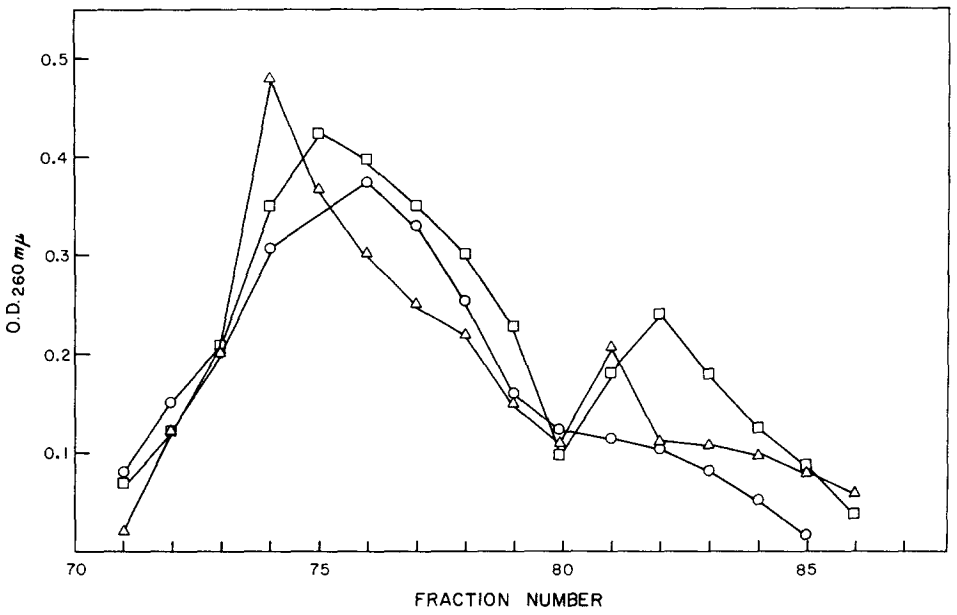

Fig. 2. Separation of the two components of mitochondrial DNA. Preparative density gradient centrifugation was carried out as described in the text. OD_{260} against fraction is plotted from three preparations containing 5 (O-O-O), 15 (Δ-Δ-Δ) and 25% (□-□-□) of m-DNA.

Two components of different buoyant densities have been separated. Although relative amounts of the lighter components varied from preparation to preparation, its equilibrium banding position on the gradient remained constant. The relative proportion of the lighter component in such CsCl runs calculated from relative OD values under the peaks corresponded closely to that calculated from melting point profiles. Fractions 82-85 were pooled from a number of runs and dialyzed against SSC. The melting profile of this component was taken and is shown in Fig. 1A. The behaviour is that of a

single, sharply melting component with a transition mid-point of 75°. Conversely the heavy component (Fractions 74-77) characteristically melted like whole cell DNA with a T_m = 84.5°. The dispersion ($\sigma_{2/3}$) and hyperchromicity ($OD_{max}/OD_{r.t.}$) for purified m-DNA were 6.6° and 1.32 respectively while the corresponding values for bulk DNA were 6.0° and 1.31.

A possible ambiguity in the characterization and study of extranuclear DNA in yeast is posed by the presence in these cells of an unusual single stranded DNA associated with L-lactate dehydrogenase (Mahler and Silva Pereira, 1962). However, this species of DNA (YLDH-DNA) in SSC has a T_m = 66.5° with $\sigma_{2/3}$ = 28.5°, an anomalous dispersion, reminiscent of that observed with certain RNAs and the DNA from ϕX 174, and considerably greater than that which characterizes either the bulk or m-DNA reported here. Furthermore the ionic strength dependence of these DNAs differ also: in going from SSC to SSC/100 the T_m of m-DNA and cellular yeast DNA are lowered by 27° and 25° respectively, that of YLDH-DNA by only 5°. Thus, on the basis of mid-point, dispersion, and ionic strength dependence of thermal transition profiles, m-DNA represents a new, mitochondrial entity, distinct from YLDH-DNA, possessing a highly ordered, double stranded structure. Full details concerning its properties are currently under investigation and will be reported elsewhere.

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