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ABSTRACT: Purification of yeast mitochondrial DNA has been studied and a method is presented whereby mtDNA can be isolated in a rapid simple manner from an unpurified yeast cell lysate. Mechanically disrupted cells are treated with RNase to degrade RNA and the DNA selectively extracted from the crude cell supernatant by mixing with poly(L-lysine)-coated kieselguhr (PLK). Nuclear DNA is selectively removed from the complex by washing with buffered saline of an appropriate molarity as determined by analytical PLK chromatography. The mtDNA is then eluted with a linear gradient of buffered saline. mtDNA may be obtained by this method with greater than 95% purity as assessed by analytical CsCl density gradients. The material obtained has the configuration of high molecular weight double-stranded DNA as shown by a sharp thermal denaturation profile and an $s_{20,w}$ value of 16.4 S.

The problem of purifying yeast mtDNA[1] has been approached by two general multistep procedures. As mtDNA is only a minor component of the total cellular DNA, one approach has been to isolate mtDNA from purified mitochondria (Corneo *et al.*, 1966), however, isolation of mitochondria is a lengthy procedure (Schatz *et al.*, 1964), and the yields of intact mitochondria are generally low. In addition this procedure is not useful for all strains of yeast. The mitochondria of petite mutants are usually fragile (Blamire *et al.*, 1972a) and low yields of mtDNA are the rule rather than the exception (Cohen *et al.*, 1972). To avoid the problems inherent in mitochondrial isolation, most workers have attempted to obtain yeast mtDNA by fractionating purified total cellular DNA. Fractionation of nuclear and mtDNA is based upon either buoyant density differences in CsCl or Hg^{2+}–Cs_2SO_4 (Schweizer *et al.*, 1969), or differential elution from hydroxylapatite columns (Bernardi *et al.*, 1970). Density gradient fractionation of DNA has a limited capacity, generally requires more than one step to obtain pure mtDNA, is time consuming and relatively expensive. While hydroxylapatite is not so limited in its capacity, it has until recently required repeated column steps to obtain pure mtDNA (Michaelis *et al.*, 1972). Bernardi *et al.* (1972) have recently described a procedure for the purification of mtDNA by a single elution from hydroxylapatite columns. This procedure, however, still requires prior DNA purification before the column chromatography.

In the preceding paper (Blamire *et al.*, 1972b) we have described a rapid procedure for the isolation and partial fractionation of yeast DNA from a crude cell supernatant by the use of poly(L-lysine) kieselguhr (PLK) chromatography. We were able to demonstrate that n- and mtDNA differ in their salt molarity of elution from PLK and have exploited this fact to develop a rapid procedure for the purification of mtDNA from crude cell supernatants. The method involves batch extracting total cellular DNA from a crude cell supernatant with PLK, selectively eluting nDNA and other contaminants from the PLK complex, and then eluting pure mtDNA with a gradient of buffered saline.

Materials and Methods

A. Strain. The haploid grande yeast strain *Saccharomyces cerevisiae* A664a/18A *ura*3 (to be referred to as 18A) was used in this work.

B. Growth of Cells. Cultures were grown at 30° in a New Brunswick gyrorotary shaker in a liquid medium containing

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[1] Abbreviations used are: n- and mtDNA, nuclear and mitochondrial DNA; PLK, poly(L-lysine)-coated kieselguhr.

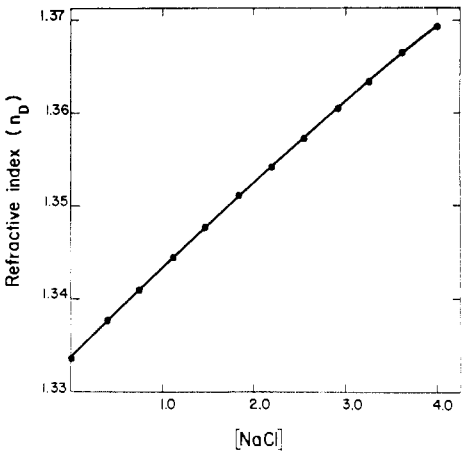

FIGURE 1: Relationship between refractive index and salt molarity of buffered saline. Volumetric solutions of 0.4 and 4.0 M buffered saline were mixed in varying proportions and the refractive index determined using an Abbé refractometer.

1% Bacto yeast extract, 2% Bacto peptone, and 5% dextrose. Where noted, 2% sodium lactate (Fisher lactic acid, neutralized with NaOH) replaced glucose as a carbon source.

C. Reagents. TG BUFFER was 0.5 M sodium thioglycollate–0.1 M Tris, adjusted to pH 8.8.

BUFFERED SALINE was sodium chloride buffered with 0.02 M KH_2PO_4, and adjusted to pH 6.8.

BUFFERED SALINE–EDTA was buffered saline containing 0.1 M EDTA.

POLY(LYSINE) was poly(L-lysine) hydrogen bromide (type 1 or 1B from Sigma, or New England Nuclear), stored at a concentration of 10 mg/ml in 0.4 M buffered saline at 4°. This solution is stable for at least 18 months.

KIESELGUHR Hyflo Super Cel (Fisher) was used as supplied without prior treatment.

POLY(LYSINE)-COATED KIESELGUHR (PLK). Kieselguhr is suspended in 0.4 M buffered saline (5 ml/g) and boiled briefly to expel trapped air. The suspension is allowed to cool to room temperature, and mixed with the poly(lysine) solution (0.05–1.0 ml/g of kieselguhr).

STANDARD PLK was PLK mixed in the proportion of 3 mg of poly(lysine)–2.5 of g of kieselguhr.

D. Isolation of mtDNA. PREPARATION OF A CRUDE CELL SUPERNATANT. Yeast cells are harvested by centrifugation, washed with water, resuspended in TG buffer[1,(2)] to a minimum concentration of 5 ml/g wet wt of cells, and incubated at 30° for 1 hr. The cells are then washed once with H_2O[2] and resuspended with 10 ml of 0.4 M buffered saline–EDTA for each 10 g of cells. An amount of liquid containing 10 g of cells is transferred to a 75-ml glass homogenizing flask (Braun MSK) containing 50 g of 0.45- to 0.50-mm glass beads and the cells are disrupted by shaking the flask at top speed for 1 min in a B. Braun MSK mechanical cell homogenizer cooled with CO_2[3]. The crude lysate is separated from the beads with a Pasteur pipet and the beads are washed with four 5-ml aliquots of 0.4 M buffered saline. The washes and lysate are combined and cellular debris is removed by centrifugation at 27,000*g* for 30 min at 4°. The cell supernatant is transferred to another centrifuge bottle[4], pancreatic RNase is added to a concentration of 50 μg/ml, and the mixture is incubated at 30° for 30 min. The cell supernatant is divided into two parts and immediately complexed with PLK as described in the next two sections.

II. CHARACTERIZATION OF nDNA ELUTION FROM PLK. An aliquot of the crude cell supernatant equivalent to 1.0 g of cells is diluted to 10 ml with 0.4 M buffered saline and loaded onto a standard PLK column[5,6]. The column is washed with 15 ml of 1.4 M buffered saline to elute unbound material and degraded RNA, and then eluted with a linear salt gradient of 1.4–3.0 M buffered saline (100 ml of each). Fractions of 1 ml are collected and assayed for absorbance at 260 nm. The molarity of the salt corresponding to the elution of the nDNA is determined by measuring the refractive index of the peak fraction[7–9].

III. PREPARATION OF mtDNA. A slurry of PLK is added to the remaining larger portion of the cell supernatant[5,10] and allowed to equilibrate for at least 15 min with occasional swirling. The PLK–nucleic acid complex is separated from unbound material by centrifugation at 4000*g* for 10 min. The supernatant is gently removed and discarded[11]. The PLK nucleic acid complex is gently resuspended with the aid of a glass rod in a minimum of 10 ml of 0.4 M buffered saline/g of kieselguhr and washed with occasional swirling for at least 10 min. The washing with 0.4 M buffered saline is repeated until the PLK is clean of unbound material as judged by a clear supernatant after centrifugation[12]. The complex is then washed at least two times with 1.4 M buffered saline.

Once the salt molarity of nDNA elution has been determined as described in section II above, the PLK complex is washed twice with buffered saline of this *exact* salt molarity[13]. At this point the PLK complex is resuspended with the same buffer (5 ml/g of column material) and transferred to a column over a plug of glass wool as described previously[14]. The column is then washed with more of the same buffered saline to remove any final traces of nDNA[15]. The mtDNA is then eluted from the column with a linear salt gradient of 1.4–3.0 M buffered saline (100 ml of each)[16,17].

Procedure Notes. (1) Treatment of yeast cells with TG buffer prior to disruption inactivates some unidentified cell constituent which reduces PLK capacity for DNA (unpublished observations). (2) The cell pellet may be frozen at this point if desired and stored for at least 2 months. (3) Smaller quantities of cells are disrupted for 1 min in a volume of 10 ml in a 50-ml homogenizing flask containing 35 g of glass beads. Larger quantities of cells are disrupted in 10-g aliquots using fresh glass beads for each aliquot. (4) The turbidity of the supernatant varies. Small amounts of loose debris which may be accidently transferred do not interfere with subsequent steps in the procedure. (5) PLK may conveniently be prepared during the centrifugation of the crude cell lysate. The standard PLK column is prepared as described in the preceding paper (Blamire *et al.*, 1972b). (6) Air pressure (4 psi) may be used to speed up the loading procedure. (7) Salt molarity is assayed by refractive index measurement and comparison to standard solutions. Figure 1 shows the relationship between refractive index and salt molarity, as determined using laboratory solutions. (8) DNA elutes as a single peak containing a shoulder of mitochondrial DNA on the high salt side. The salt molarity of elution of DNA varies slightly with different sources of poly(lysine). (Sigma poly(lysine) preparations usually release DNA at approximately 1.6 M NaCl while New England Nuclear poly(lysine) preparations release DNA at approximately 1.8 M NaCl.) (9) The total DNA recovered per gram wet weight of cells is approximately 200–250 μg for all strains of *S. cerevisiae* so far examined (both haploid and diploid). (10) The amount of PLK to use depends upon the *total amount*

[2] Numbers refer to the procedure notes, in the following section.

of DNA present in the crude supernatant. The ratio of poly(lysine) to DNA must be greater than 10:1 (w/w), however, the ratio of poly(lysine) to kieselguhr may be varied from 0.5 to 10.0 mg of poly(lysine)/g of kieselguhr. As an example, for 10 g of cells which contain 2.5 mg of DNA (see note 9), one should use 25 mg of poly(lysine) and a minimum of 2.5 g of kieselguhr to prepare the PLK. (11) PLK should not be allowed to dry out. If the complex is to be left for awhile it should be in suspension and not stored as pellet. (12) Generally two or three washes are sufficient. Occasionally small amounts of protein are seen in the PLK pellet. These may be ignored as they do not interfere with later elution. (13) *This is the most critical step of the isolation procedure.* The salt molarity of the washing buffer must be exactly determined by comparison to the refractive index as determined in the preceding section ($\Delta n_D \pm 0.0001$) as no other method is precise enough. Repeated washes are necessary due to the presence of occluded liquid in the PLK pellet. (14) No top layer of kieselguhr is used on this column. (15) The column is monitored until no more ultraviolet-absorbing material elutes and a stable base line has been obtained. The elution gradient starts at a lower salt molarity because of slight elution artifacts obtained at the beginning of gradients. (17) The elapsed time for the entire procedure, starting from the disruption of the cells is approximately 8–9 hr.

E. Preparation of Partially Purified DNA. Yeast cells were converted to spheroplasts as described by Blamire *et al.* (1972a), resuspended in 9 ml of 0.1 M NaCl–0.15 M EDTA (pH 9) per g wet wt of cells and lysed by the addition of one-tenth volume of 25% sodium dodecyl sulfate. Nucleic acids were isolated from this lysate as described by Bernardi *et al.* (1972). RNA was degraded by incubation with pancreatic ribonuclease (50 μg/ml for 30 min at 37°).

F. Analytical CsCl Density Gradients. Analytical CsCl density gradient centrifugation was carried out as previously described (Grossman *et al.*, 1969) using *Bacillus subtilis* phage PBS2 DNA ($\rho = 1.722$ g/cm^3) as a density marker. All densities are related to that of *Escherichia coli* DNA, taken to be 1.710 g/cm^3.

G. Sedimentation Velocity Analysis. Sedimentation velocity analysis was performed using a 30-mm centerpiece, in a Spinco AnE rotor of a Beckman Model E ultracentrifuge at 20° at a speed of 35,600 rpm. Nucleic acid concentrations were 10 μg/ml in 1.0 M buffered saline and the sedimentation was recorded using ultraviolet optics. The sedimentation coefficient was determined by measuring the movement of the midpoint of the boundary. Corrections were made for the ionic strength of the solution as described by Studier (1965).

H. Thermal Denaturation of DNA. The thermal denaturation profile of DNA was determined by following the increase in absorbance at 260 nm of a solution of DNA (15–25 μg/ml) in standard saline citrate (SSC, 0.15 M NaCl–0.015 M trisodium citrate, pH 7.0) with increasing temperature as described by Marmur and Doty (1962).

Results

A. Preliminary Considerations. In the previous paper it was shown that the release of nDNA as measured by its elution from PLK differs from that of mtDNA by about 0.1 M NaCl. This suggested to us that PLK, equilibrated at the salt molarity at which nuclear DNA was released, would selectively extract mtDNA from a mixture of the two components. The mtDNA thus bound could then be released in a pure form by simply raising the salt molarity of the PLK–DNA complex above that at which mitochondrial DNA would normally be released.

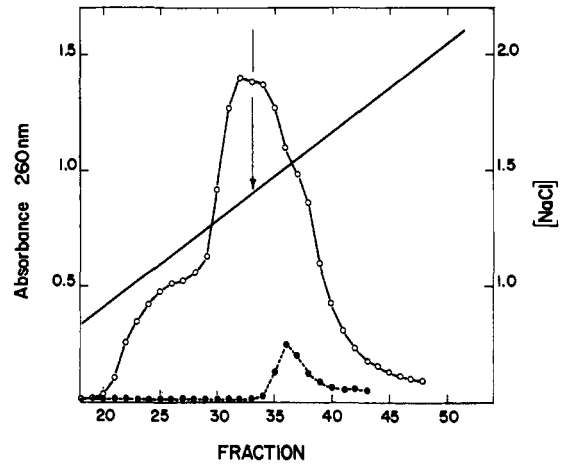

FIGURE 2: Selective loading and elution of mtDNA. Elution profile of 500 μg of DNA from a standard PLK column (○). Fractions were taken and the refractive index was determined (—). Elution profile of an identical sample of DNA loaded as described in the text at the salt molarity defined by the arrow (●). Both samples were eluted with linear salt gradients of 0.4–4.0 M buffered saline (100 ml of each). Thirty-drop fractions were collected and monitored for absorbance at 260 nm.

The prediction was tested using partially purified total yeast DNA. Total DNA (500 μg in 0.4 M buffered saline) was loaded onto a standard PLK column (2.5 g of kieselguhr and 3 mg of poly(L-lysine)) as described previously (Blamire *et al.*, 1972b) and the DNA eluted with a linear salt gradient of 0.4–4.0 M buffered saline (100 ml of each). The fractions where the DNA appeared were detected and quantitated by their absorbancy at 260 nm and assayed as well for their refractive index. The latter value accurately reflects the salt molarity of each fraction. These results are shown in Figure 2. It can be seen that the n- and mtDNA are once again released at different salt molarities and that in this case the nDNA is being eluted from the complex at 1.4 M NaCl. A second batch of PLK was prepared and equilibrated with 1.4 M buffered saline. This was then mixed with an identical aliquot of total yeast DNA in 1.4 M buffered saline. Complexing was allowed to take place by gently stirring in a stoppered flask. After washing with 1.4 M buffered saline, the entire complex was packed into column form. The complex was washed with additional 1.4 M buffered saline solution to remove any remaining unbound material and then the bound material eluted, using a linear salt gradient of 0.4–4.0 M buffered saline (100 ml of each). The results are also shown in Figure 2. A single small peak is observed eluting in the position of mitochondrial DNA. When samples of this material were taken and assayed in analytical CsCl gradients, only a single species was observed, with a buoyant density characteristic of mtDNA. It thus appeared as if this approach could indeed be used to selectively extract mtDNA from a total DNA preparation.

The results of the above experiment show that the elution difference between n- and mtDNA are slight, thus it is necessary to determine the elution molarity of nDNA very accurately for each preparation of mtDNA. Trial runs on small samples are first carried out to determine the elution molarity of nDNA. However, when experiments were performed to isolate large amounts of mtDNA, it was necessary to determine

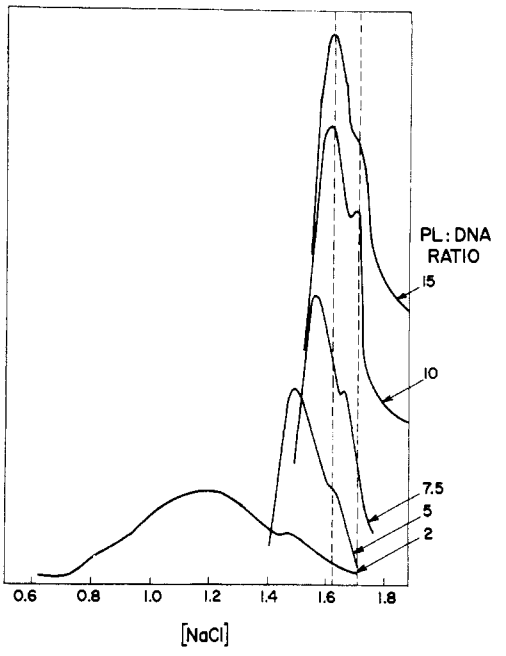

FIGURE 3: Effect of poly(lysine): DNA ratio on elution of total DNA from PLK. Aliquots (500 μg) of partially purified DNA were eluted from PLK columns containing 2.5 g of kieselguhr and increasing amounts of poly(lysine) as shown. The results are plotted so that the abscissa represents the increasing salt molarity of the eluent. The profiles obtained at varying ratios of poly(lysine): DNA (w/w) are offset for ease of identification. The two dotted lines represent the salt molarity of elution of n- and mtDNA at high poly(lysine): DNA ratios. Recovery of DNA was identical in all cases.

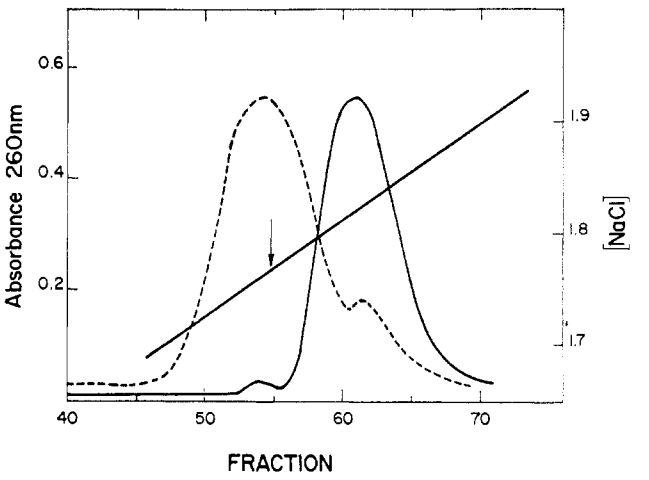

FIGURE 5: Isolation of mtDNA from 12 g of lactate-grown cells. Isolation of mtDNA was carried out using the procedure described in the Materials and Methods. Characterization of the critical salt molarity required for the extraction of mtDNA was carried out by determining the elution of the DNA extracted from 1 g wet wt of cells from a standard PLK column (– – –). Using the salt molarity as shown by the arrow the supernatant of the remaining 11 g of cell was treated as described and eluted from the PLK (30 ml of poly(lysine)–3 g of kieselguhr) complex (—). Salt molarity (—).

the practical capacity of PLK. Although DNA will bind to poly(lysine) on a PLK column in a 1:1 (w/w) ratio (unpublished results), at this ratio the column is overloaded and the elution profile of the DNA is a broad undefined peak. In order to characterize the practical capacity of PLK, an experiment was performed in which identical aliquots of partially purified DNA were eluted from PLK columns containing various amounts of poly(lysine). The results of such an experiment are shown in Figure 3. It may be seen that the salt molarity of elution of DNA is dependent upon the weight ratio of poly(lysine):DNA. At low ratios the DNA elutes at a lower salt molarity and the peak of DNA is broadened. At ratios of poly(lysine):DNA of 10 or greater, the elution molarity of DNA remains constant. This property of PLK becomes important in preparative mtDNA extraction experiments where very large amounts of starting material are used. Thus, for all such extractions, a ratio of poly(lysine):total DNA present of 10:1 (w/w) is recommended. The ratio of poly(lysine) to kieselguhr does not appear to be critical to column performance. No deleterious effects have been observed for amounts of poly(lysine) between 0.5 and 10 mg per g of kieselguhr.

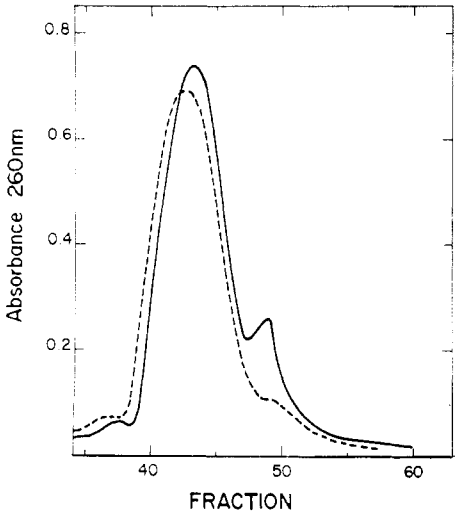

FIGURE 4: Effect of carbon source on the amount of mtDNA in strain 18A. Cells were grown using 2% lactate or 5% glucose as a carbon source and crude cell supernatants complexed and eluted from standard PLK columns using linear salt gradients of 1.4–3.0 M NaCl (100 ml of each). Fractions (1 ml) were collected and the absorbance at 260 nm was measured. Elution profile of DNA from 1 g of lactate-grown cells (—). Elution profile of DNA from 1 g of glucose-grown cells (– – –).

As a final point for characterizing the DNA of a given strain of yeast, it should be mentioned that the growth medium of cells has been found to be important for optimum yields of mtDNA with the strain of yeast used in these experiments. Figure 4 compares the elution profile of a crude cell supernatant obtained from cells grown using 2% lactate as a carbon source with one obtained from cells grown using 5% glucose as a carbon source. The mtDNA shoulder from the glucose grown cells is considerably smaller than that from the lactate grown cells. Thus, the carbon source is important in strain A664a/18A for optimum yields of mtDNA. This glucose repression may vary with different strains of yeast (Bleeg *et al.*, 1972).

B. Isolation of mtDNA. In the previous paper it was demonstrated that it is possible to fractionate total DNA from a crude cell supernatant by use of PLK. This fractionation is no less efficient than that using purified DNA as a starting material and would thus appear to make prior purification of DNA unnecessary for the isolation of mtDNA. However, the turbidity of a crude cell supernatant precludes the determination of salt molarity by refractive index measurements

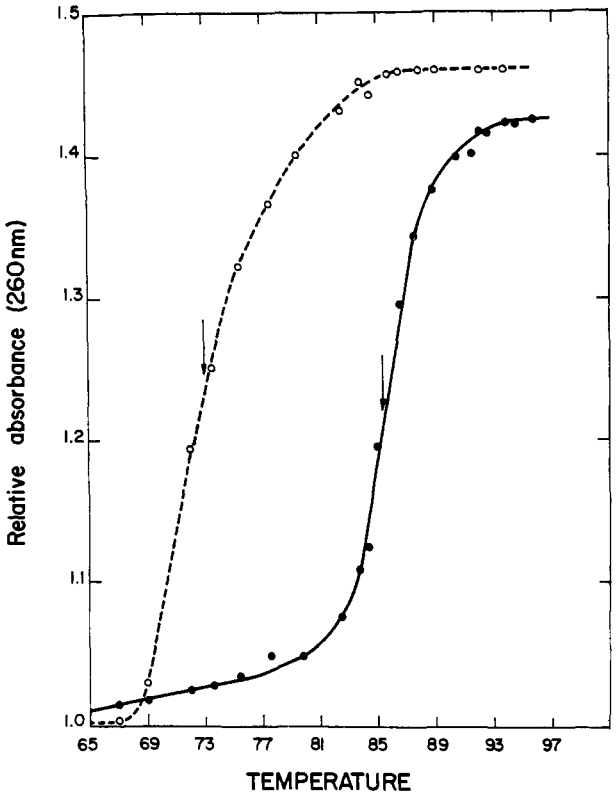

FIGURE 6: Thermal denaturation profile of n- and mtDNA. PLK-purified n- and mtDNA from the preparation described in Figure 5 were dialyzed for 12 hr against SSC at 4°. Thermal denaturation was carried out as described in Materials and Methods. The T_m is shown by the arrow. nDNA (●) and mtDNA (○).

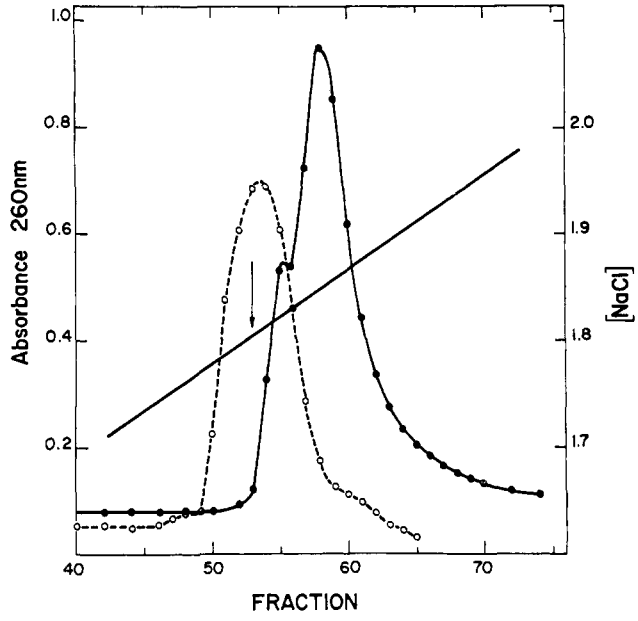

FIGURE 7: Isolation of mtDNA from 20 g of cells grown using 5% glucose as a carbon source. Conditions for isolation are as described in Figure 5. Characterization elution profile of the DNA from 1 g equiv of cell supernatant (○). The arrow indicates the critical salt molarity chosen for mtDNA extraction. Elution profile of mtDNA from PLK (50 mg of poly(lysine)-10 g of kieselguhr) complex following the extraction procedure described in Materials and Methods (●). NaCl molarity of eluents (—).

and so it is not possible to selectively complex mtDNA to PLK. This problem is overcome by using an alternate procedure which involves the batch loading of total DNA to PLK at a low salt concentration. nDNA and other contaminants are removed from the PLK complex by repeated washes with increasing salt concentrations as described in Materials and Methods. Finally, the PLK complex is loaded into a column and the mtDNA is eluted with a salt gradient.

Figure 5 demonstrates the isolation of mtDNA from a crude cell supernatant obtained from 11 g wet wt of lactate grown cells. The total time elapsed for this procedure, starting from harvesting of cells until the final elution of pure mtDNA, is approximately 12 hr. The recovery of mtDNA in this experiment is approximately 12% of the total DNA and would appear to be quantitative, although an absolute determination of the percentage of mtDNA in the starting material by analytical CsCl centrifugation is not possible due to the large amounts of ultraviolet-absorbing material in the crude cell supernatant.

The DNA from this preparation was characterized with respect to sedimentation coefficient and thermal denaturation profile. The results are summarized in Table I. The melting profiles of both n- and mtDNA are shown in Figure 6.

It is also possible to recover mtDNA from cells where its proportion is as little as 2–4% of the total DNA. This point is illustrated in Figure 7 where mtDNA was purified from 20 g of cells grown in 5% glucose. As noted above, cells grown under these conditions contain reduced levels of mtDNA. The small shoulder which precedes the mtDNA in this elution is nDNA. This is seen when the washing is carried out with a buffer whose molarity is too low to remove all of the nDNA bound to the PLK. The presence of such nDNA is indicative of quantitative recovery of mtDNA. Even under conditions where a small amount of nDNA is observed, the mtDNA eluting from such columns (fractions 56–65) is still greater than 90% pure as judged by analytical CsCl density gradient centrifugation.

Discussion

Previous methods for the purification of mitochondrial DNA started either with the isolation of purified mitochondria or the purification of total cellular DNA. The ability of PLK to selectively extract DNA from a crude cell supernatant has allowed us to develop simple rapid procedures for the isolation and fractionation of yeast DNA. The purification of

TABLE I: Physical Properties of Yeast DNAs.

Nucleic Acid Species	$s_{20,w}$ (S)[b]	T_m (°C)	% Hyperchromic Shift
nDNA[a]	20.0	85.3	42
mtDNA[a]	16.4	73.0	46

[a] The DNA obtained from the extractions shown in Figure 5 were used for these determinations. [b] Peak fractions taken from the elution profile in Figure 5 were diluted with phosphate buffer (0.02 M KH_2PO_4 pH 6.8) to a concentration of 1.0 M buffered saline. Sedimentation coefficients were determined as described in Materials and Methods.

mDNA by PLK chromatography is obtained by the selective elution of nDNA from the PLK complex. As this elution step is very critical, it is recommended that the elution molarity of nDNA be determined by the use of analytical PLK columns. These methods allow the purification of milligram quantities of both n- and mtDNA, free of contaminants, in a single working day. The yield of DNA by PLK chromatography is as high as the best isolation procedures reported to date. DNA (and RNA) prepared by PLK is suitable for hybridization experiments without further purification. The capacity, simplicity, and reproducibility of PLK should recommend it as a method for nucleic acid purification, especially from organisms such as yeast where the DNA concentration is low and where the DNA is readily degraded by host nucleases.

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Gel Electrophoresis of Deoxyribonucleic Acid†

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ABSTRACT: The electrophoretic mobility of duplex DNA from T-2, T-5, and T-7 bacteriophages of molecular weights ranging from 8.5×10^4 to 1.2×10^8 was investigated in 2.5–6% w/v polyacrylamide and 8.7–10% w/v starch gels. DNAs of all molecular weights investigated exhibit significant electrophoretic mobility in the above gel systems. These mobilities are strongly dependent on the field strength of the experiment; this is in contrast with proteins, whose mobilities are independent of field strength in gel electrophoresis. The electrophoretic mobility of DNAs we investigated was a function of molecular weight up to 10^6 in polyacrylamide gels and 5×10^6 in starch gels. Molecular weight resolution was achieved on a preparative scale with DNA of molecular weights between 8.5×10^4 and 3.5×10^5. If ethidium bromide is present during the polymerization of acrylamide gels, it is covalently incorporated into the gel. DNAs migrate through such gels with a greatly reduced mobility. This reduction in mobility of DNA in such gels almost certainly is due to interactions between the incorporated ethidium moiety and DNA since a change in absorbance of the incorporated ethidium is seen in the region of migrating DNA bands. The mobilities of all investigated DNAs in such gels at field strengths above 20 V cm^{-1} are dependent on the molecular weight of the DNAs, thus enabling molecular weight resolution to be achieved with DNAs of molecular weights up to at least 1.2×10^8.

Polyacrylamide gel electrophoresis and starch gel electrophoresis have been widely used for the fractionation of proteins (Davis, 1964; Ornstein, 1964; Smithies, 1955), RNA (Peakcock and Dingman, 1968; Loening, 1969), ribosomes (Dessev *et al.*, 1969), oligonucleotide length DNA (Elson and Jovin, 1969), and recently for circular and linear DNA of higher molecular weights (Fisher and Dingman, 1971; Dingman *et al.*, 1972). We also have investigated the usefulness of this technique for fractionating high molecular weight DNA.

Methods

Polyacrylamide Gels. Acrylamide, $CH_2CHCONH_2$, was recrystallized from hot acetone and dried *in vacuo*. *N,N'*-Methylenebisacrylamide, $(CH_2CHCONH)_2CH_2$, was used as the cross-linking reagent. The polymerization initiator system was ammonium peroxydisulfate, $(NH_4)_2S_2O_8$, and *N,N,N',N'*-tetramethylenediamine, $(CH_3)_2NCH_2CH_2N(CH_3)_2$, at concentrations of 0.07 and 0.4% w/v, respectively, in the final gel forming solution.

The buffer system used was discontinuous. The reservoir buffer was 0.01 M sodium aspartate (pH 8.8), and the gel buffer was 0.0157 M NaCl–0.006 M Tris, pH adjusted to 8.8

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