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Isolation and Fractionation of Yeast Nucleic Acids. I. Characterization of Poly(L-lysine) Kieselguhr Chromatography Using Yeast Nucleic Acids†

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ABSTRACT: Yeast nucleic acids have been investigated for their fractionating properties on poly(L-lysine)-coated kieselguhr (PLK) columns. Nucleic acids have been shown to bind to these columns, and in turn may be eluted with an increasing gradient of buffered saline. As the salt molarity passing over the PLK–nucleic acid complex is increased, nucleic acid species are eluted in the following order; degraded RNA, rRNA, and DNA. This has been shown by assaying the alkali lability of the various fractions obtained when total nucleic acid mixtures were fractionated, and also by fractionating previously purified yeast DNAs and RNAs. Poly(lysine) kieselguhr has also been shown to be capable of removing nucleic acids selectively from the supernatant of mechanically disrupted yeast cells which have undergone no prior purification. This property can be used to extract and fractionate yeast nuclear and mitochondrial DNA from RNase-treated cell supernatants. It has been shown that some separation can be achieved between n- and mtDNA. DNA prepared in in this way is pure of protein and degraded RNA contaminants and may be used in DNA–RNA hybridization experiments directly.

Yeast contains protein RNA, nDNA,[1] and mtDNA[1] in the ratio of approximately 200:100:1:0.1 (Bhargava and Halvorson, 1971), which accounts for the historical designation of RNA as yeast nucleic acid. Such large excesses of RNA (and protein) makes the purification of DNA much more difficult from yeast than from bacteria. The standard methods of isolating DNA are multistep procedures which start with the deproteinization of cell lysates using either denaturing agents such as chloroform (Marmur, 1961), or detergents and salt precipitation (Kay *et al.*, 1952). The nucleic acids are precipitated from solution using ethanol and the RNA can then be removed from the redissolved nucleic acid mixture by digestion with RNase. Because of the large excess of RNA in yeast, several RNase treatments are usually necessary to degrade all the RNA, and if high purity of the DNA is required, preparative CsCl density gradient centrifugation must be used (Schweizer *et al.*, 1969). Recently hydroxylapatite chromatography has been used as the final stage of purification and fractionation of yeast DNA rather than CsCl centrifugation (Bernardi *et al.*, 1970).

These standard methods rely on the successive removal of contaminating components from crude cell lysates until one is left with DNA of the desired level of purity. This approach has been necessary up to now because of the lack of a truly selective means of extracting DNA from a crude cell lysate.

The method of PLK chromatography (Ayad and Blamire, 1968), although only used here on a small scale, has been shown to selectively remove nucleic acids from the supernatants of mechanically disrupted yeast cells. The nucleic acids may be fractionated by elution of the PLK complex with a salt gradient. This paper concerns the methodology of fractionation of yeast nucleic acids by PLK chromatography.

Materials and Methods

Strain. The haploid wild-type grande yeast strain *Saccharomyces cerevisiae* A664a/18A *a*, ur_3 (kindly donated by Dr. B. Dorfman) was used throughout this work.

Growth and Radioactive Labeling. The strain was maintained and routinely subcultured on agar slants containing 1% Bacto yeast extract, 2% Bacto peptone, 2% dextrose, and 2% agar. Liquid cultures were grown in the same media (but lacking

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[1] Abbreviations used are: nDNA and mtDNA, nuclear and mitochondrial DNA, respectively; TG buffer, 0.5 M sodium thioglycolate–0.1 M Tris (pH 8.8); 0.4 M buffered saline, 0.4 M NaCl–0.02 M KH_2PO_4 (pH 6.8); 4.0 M buffered saline, 4.0 M NaCl–0.02 M KH_2PO_4 (pH 6.8); YPB, 0.1 M Hepes (*N*-2-hydroxyethylpeperazine-*N*-2-ethanesulfonic acid)–0.1 M NaCl–0.03 M $MgCl_2$ (pH 7.4). PLK, poly(L-lysine)-coated kieselguhr.

the agar) at 30° in a New Brunswick gyrotory shaker for 16–18 hr.

Radioactive labeling was carried out by growth under similar conditions in liquid media consisting of 0.67% Bacto yeast nitrogen base (YNB) containing 2% dextrose and 20 μg/ml of uridine. [^{3}H]- or [^{14}C]adenine was used to label nucleic acids and [^{14}C]lysine was used to label protein. All radioisotopes were supplied by New England Nuclear Corp. and the specific conditions for each experiment are given in the figure legends.

Aliquots of the material under investigation were monitored for radioactivity by filtration of acid-precipitable material (10% trichloroacetic acid, 100 μg of bovine serum albumin/ml) onto Whatman GF/A glass fiber filters which were then washed with 5% trichloroacetic acid and ethanol (95%) before drying. Alkali-stable acid-precipitable material was assayed in a similar way after the aliquots had been treated with 0.3 N KOH for 18 hr at 37°. Each dry filter was placed in a counting vial, 5 ml of toluene–Liquifluor scintillant (New England Nuclear) was added, and all samples were then counted in an Intertechnique SL-40 liquid scintillation spectrometer. In experiments involving more than one radioisotope, the per cent spillover from the upper channel into the lower channel was determined and results corrected accordingly.

POLY(LYSINE) SOLUTION contained 10 mg/ml of poly(L-lysine) hydrogen bromide (40,000 molecular weight, Sigma) in 0.4 M buffered saline.

Preparation of Crude Cell Lysates. Cultures of yeast were grown to stationary phase and harvested by centrifugation at 8000*g* for 5 min. The cells (5–50 g) were washed once with water and incubated with TG buffer (5 ml/g wet weight of cells) for 1 hr at 30°. Cells were then washed twice with water and resuspended in 10 ml of 0.4 M buffered saline containing 0.1 M EDTA. The suspension was then added to 35 g of 0.45- to 0.50-mm glass beads in a 50-ml homogenization flask (MSK Braun) and the cells disrupted by homogenization in a a CO_2-cooled B. Braun MSK mechanical cell homogenizer.

The lysate was then removed from the glass beads using a Pasteur pipette and the beads washed with three 5-ml portions of 0.4 M buffered saline. The washings and the lysate were then combined and the suspension was then centrifuged at 27,000*g* for 30 min at 4° to remove cellular debris. The supernatant (about 20 ml) was used immediately for fractionation experiments.

Preparation of Ribosomes. Yeast cells were converted to spheroplasts as described previously (Blamire *et al.*, 1972) and suspended in 2 ml of YPB. These spheroplasts were lysed by the addition of 0.5% deoxycholate and vortexing. After cooling on ice for 5 min, Brij 58 was added to a concentration of 0.75%. The crude lysate was centrifuged at 27,000*g* for 15 min to remove debris. Ribosomes were pelleted from the supernatant by centrifugation at 105,000*g* for 2 hr through a layer of 15% sucrose (w/w in YPB buffer). The ribosomal pellet was resuspended in 0.4 M buffered saline by vortexing and then loaded onto a freshly prepared PLK column.

Preparation of Standard PLK Columns. Poly(L-lysine)-coated kieselguhr (PLK) columns were prepared by a modification of the method of Ayad and Blamire (1968).

The column materials were packed into a glass tube (1 × 20 cm) under air pressure of 4 psi in three layers, starting from the bottom. The bottom layer was 1 ml of a suspension of Whatman cellulose powder (0.2 g/ml in 0.4 M buffered saline), or a small amount of glass wool. Kieselguhr (2.5 g; Hyflo Super Cel, Fisher) was suspended in 12.5 ml of 0.4 M buffered saline and boiled briefly to remove trapped air. The cooled suspension was then mixed and 0.3 ml of the poly(lysine) solution was added with gentle stirring. This PLK compound was then packed over the glass wool (or cellulose powder) using air pressure. Finally, 1 ml of a suspension of untreated kieselguhr (0.2 g/ml in 0.4 M buffered saline) was added as the topmost layer. The complete column was then washed with two column volumes (about 14 ml) of 0.4 M buffered saline before use.

Fractionation Procedure. Samples containing nucleic acids under investigation were loaded onto standard PLK columns by dilution into 0.4 M buffered saline and passing them through the column using air pressure. This air pressure accelerates the loading. The loaded column was washed free of unbound material using 15 ml of 0.4 M buffered saline and then eluted using a linear gradient of 0.4–4.0 M buffered saline (100 ml of each). Ultraviolet-absorbing material was continuously monitored using an LKB Uvicord (254 nm) and 30-drop (1.8 ml) fractions were collected. Individual fractions were taken and the absorbance monitored at 260 nm. The salt concentration of individual fractions was determined by refractive index measurements, as compared to standard solutions, and reported as molar NaCl.

Analytical CsCl Density Determinations. The buoyant densities of nucleic acid species was determined analytically using a Beckman Model E ultracentrifuge as described previously (Grossman *et al.*, 1969). All densities were related to that of *Escherichia coli* DNA, taken to have a buoyant density of 1.710 g/cm^3.

Results

In order to characterize the fractionating properties of PLK columns with regard to yeast nucleic acids, cells were grown in the presence of [^{3}H]adenine to label the DNA and RNA specifically. DNA may be differentiated from RNA by its resistance to either alkali hydrolysis or RNase digestion. The supernatant of a crude yeast lysate, prepared from 100 ml of cells (0.1 g wet weight) grown in YNB in the presence of 50 μCi of [^{3}H]adenine, was loaded onto a standard PLK column washed with 0.4 M buffered saline, and eluted with a linear gradient of 0.4–4.0 M buffered saline (100 ml of each). Figure 1 shows the elution profile obtained. A large amount of ultraviolet-absorbing material was not retained by the column and either passed straight through or was washed off with the extra 0.4 M buffered saline. The salt gradient eluted several peaks of ultraviolet-absorbing material, the main one starting at about 1.2 M NaCl and tailing out to about 1.8 M NaCl. This was preceded by several smaller peaks and followed by a single peak eluting at about 1.6 M NaCl. In order to identify the material in all these regions, aliquots of each fraction were assayed for trichloroacetic acid precipitable ^{3}H counts as described in Materials and Methods, and the results plotted as shown (Figure 1). These findings indicate that greater than 90% of the precipitable counts (taken as belonging to nucleic acid) are bound to the PLK at 0.4 M NaCl and only eluted by the salt gradient. Identical aliquots of each fraction were treated with KOH as described (see Materials and Methods) and again assayed for trichloroacetic acid precipitable counts. The results (Figure 1) indicate that only one peak, the one eluting at about 1.6 M NaCl, is stable to alkali hydrolysis and this material was classified as DNA. Similar findings were also observed when a sample was treated with pancreatic RNase, therefore the remaining material was classified as RNA.

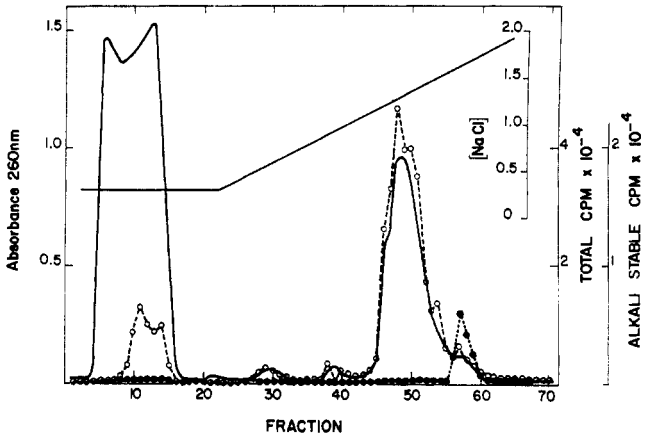

FIGURE 1: Elution profile of a crude yeast cell lysate (0.1 g wet weight, from 100 ml of culture labeled with 50 μCi of [^{3}H]adenine) prepared as described in Materials and Methods, from a standard PLK column using a linear gradient of 0.4–4.0 M buffered saline (100 ml of each). Thirty-drop fractions (1.8 ml) were collected. (—) A_{260nm}. (O) Total acid-precipitable cpm of 0.1-ml aliquot. (●) Alkali-stable acid-precipitable cpm of 0.1-ml aliquot.

The distribution of protein in such a PLK elution profile was monitoring by fractionating a crude yeast lysate prepared from a 50-ml culture of yeast grown in YNB in the presence of 1 μCi of [^{14}C]lysine and 20 μCi of [^{3}H]adenine. Virtually all the [^{14}C]acid-precipitable material is not retained by the column and passes straight through, whereas, once again the ^{3}H counts are retained to greater than 90% (Figure 2).

Thus, it would appear that yeast nucleic acids are fractionating in a similar way to that observed with bacterial nucleic acids on PLK columns (Ayad and Blamire, 1969). Little or none of the protein is being retained, and DNA and RNA elute at salt molarities higher than 0.4 M NaCl.

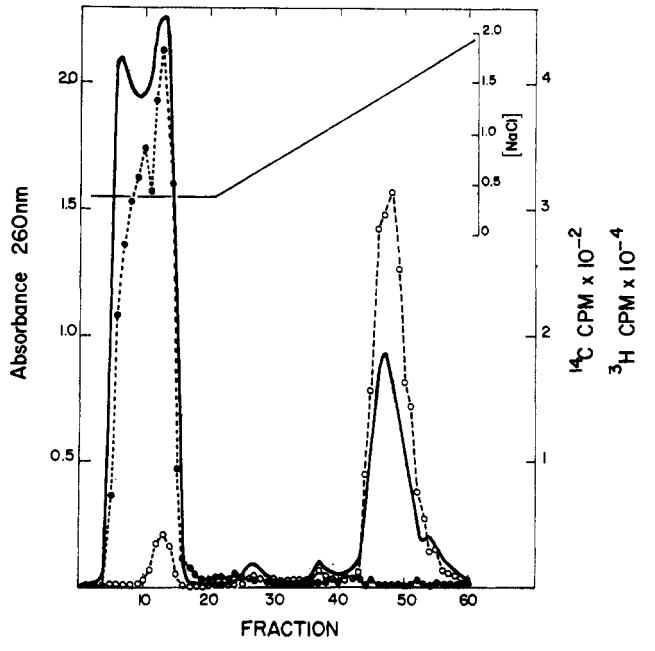

FIGURE 2: Elution profile of a crude yeast cell lysate prepared from 50 ml of culture (0.1-g cell wet weight) labeled with 1 μCi of [^{14}C]-lysine and 20 μCi of [^{3}H]adenine. Column and elution were as in Figure 1. (—) A_{260nm}. (O) Acid-precipitable ^{3}H cpm. (●) Acid-precipitable ^{14}C cpm.

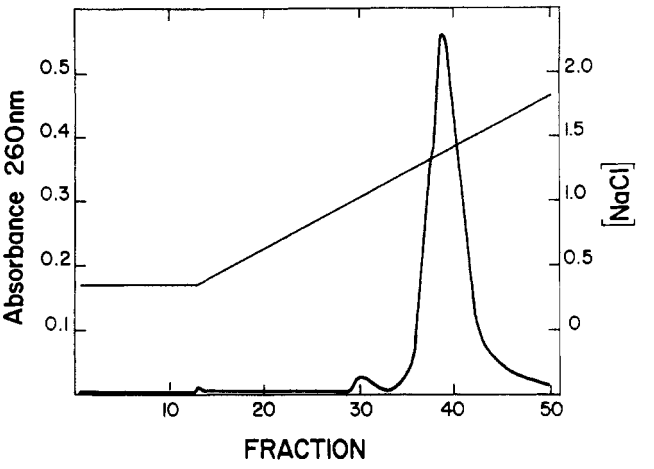

FIGURE 3: Elution profile of yeast ribosomes prepared from 20 ml of culture as described in Materials and Methods. Column and elution were as in Figure 1.

The RNA peak which starts eluting at about 1.2 M NaCl has been identified as rRNA by preparing yeast ribosomes (see Materials and Methods) and fractionating the ribonucleoprotein on standard PLK columns as before (Figure 3). Under these conditions it is clear that virtually all the RNA binds to the column and is eluted by the gradient. It would appear that the loading conditions are such that most of the protein is stripped from the ribosomes and only the nucleic acid is bound.

No attempt has been made in this study to locate and fractionate tRNA from yeast as this has been studied elsewhere (Ayad and Blamire, 1970).

Fractionation of Total Yeast DNA. It has been shown that PLK columns can fractionate bacterial transforming DNA into various activities (Ayad and Blamire, 1969). Yeast DNA consists of two components, n- and mtDNA (Corneo *et al.*, 1966), which can be identified on the basis of differences in their buoyant densities in CsCl. Investigations were therefore carried out to determine whether PLK columns could fractionate these two DNA components. Total DNA, purified from disrupted yeast cells using a modified Marmur (1961) procedure, was loaded (100 μg/ml) on a PLK column under standard conditions. The bound material was eluted with a linear salt gradient and a characteristic peak with a shoulder on its tailing edge was found between 1.5 and 1.7 M NaCl (Figure 4). Samples of each fraction were taken and monitored by analytical CsCl density gradient centrifugation, with some of the results shown in Figure 4 (insets A, B, and C).

It is clear from these tracings that two species of DNA are present in this preparation. All of these fractions contain DNA with a density of 1.699 g/cm^3, characteristic of nDNA. However, commencing at fraction 47 (inset B), a second band of DNA appears having a density of 1.683 g/cm^3 which is characteristic of mtDNA in this strain of yeast (Grossman *et al.*, 1969). It would appear, therefore, that some fractionation of n- and mtDNA is being achieved.

To follow the specific elution pattern of mtDNA from PLK columns in the presence of nDNA, the following experiments were performed. ^{14}C-Labeled mtDNA was obtained from purified mitochondria (Blamire *et al.*, 1972) by preparative CsCl density gradient centrifugation, and added (~7 μg) to purified total DNA. This DNA mixture was then frac-

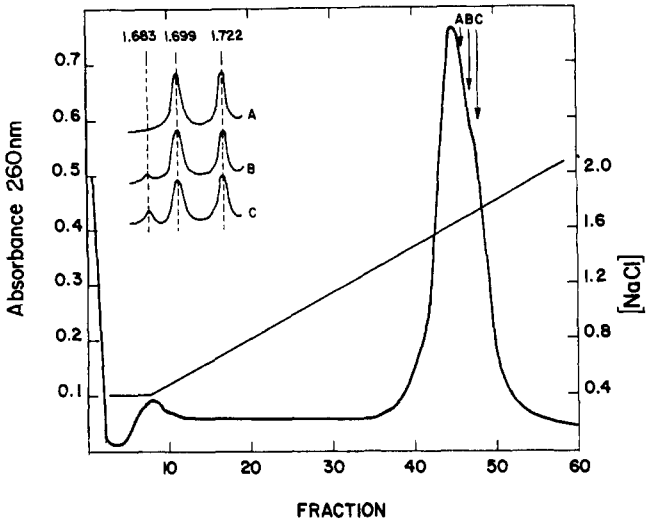

FIGURE 4: Elution profile of yeast DNA purified by a modification of the Marmur method (Marmur, 1961). Column and elution were as described in Figure 1. The insets show the results of analytical buoyant density CsCl gradients performed on selected fractions. *Bacillus subtilis* phage PBS2 DNA (ρ = 1.722 g/cm^3) was used as a density marker.

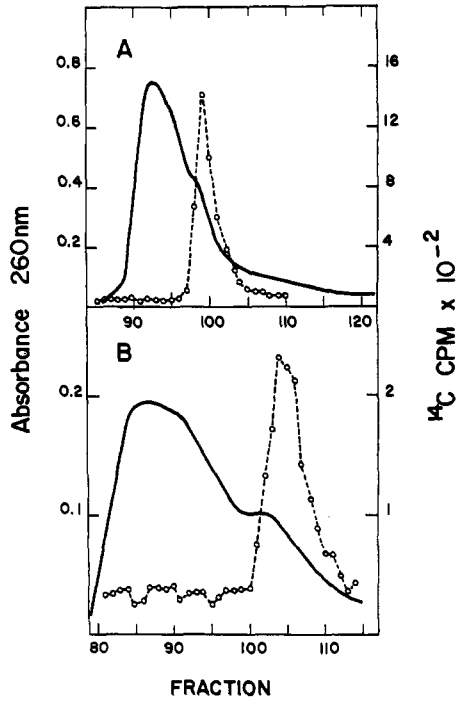

FIGURE 5: Elution profiles of purified DNA (prepared as in Figure 4) plus 7 μg of ^{14}C-labeled mDNA prepared from purified mitochondria. Column as described in Materials and Methods. Fifteen-drop fractions were collected. (A) Linear gradient of 0.4–4.0 M buffered saline (100 ml of each). (B) Linear gradient of 1.5–2.5 M buffered saline (100 ml of each). (—) A_{260nm} and (O) ^{14}C cpm.

tionated by PLK chromatography. The fraction size was reduced to 15 drops (about 0.95 ml), each fraction assayed for absorbance at 260 nm and trichloroacetic acid precipitable counts. The results are shown in Figure 5A. As can be seen, the mtDNA appears as a single sharp peak on the tailing edge of the nDNA under these conditions.

The resolution between the n- and mtDNA can be increased by altering the elution gradient. If the standard 0.4–4.0 M buffered saline gradient (100 ml of each) is replaced by a shallower gradient of 1.5–2.5 M buffered saline (100 ml of each) and an identical sample of DNA is eluted, the pattern shown in Figure 5B is obtained. The DNA peak is broader and once again the mtDNA is located on the tailing edge at higher salt molarities. Even the shallow gradient however does not completely resolve the two DNA components. In order to purify mtDNA free from the nDNA, other methods have to be used (Finkelstein *et al.*, 1972).

Purification of Yeast DNA. The above experiments have characterized the fractionation of yeast nucleic acids on PLK columns and demonstrated that it is possible to selectively extract nucleic acids from a crude cell supernatant. Experiments were performed in order to determine if DNA could be purified from a crude cell lysate by use of PLK chromatography. When DNA and RNA are present together in a crude cell supernatant, the DNA appears to have a selective advantage during loading, which is demonstrated in the following experiment. Figure 6 shows the elution profile of a crude yeast lysate prepared from 1.1 g wet weight of yeast grown in YNB and labeled with 100 μCi of [^{3}H]adenine. The fractions were assayed for absorbance at 260-nm, total trichloroacetic acid precipitable ^{3}H counts and trichloroacetic acid precipitable ^{3}H counts following KOH hydrolysis. Under these conditions it can be seen that greater than 90% of the DNA loaded and eluted exactly as before, however, the RNA did not completely load, and greater than 80% of this material was not retained by the column and came through with the 0.4 M buffered saline wash. The reason for the preferential binding of DNA has not been investigated any further at this stage; however Ayad and Blamire (1970) have demonstrated with bacterial nucleic acids that DNA has a selective advantage during loading when in competition with RNA. Intact rRNA and DNA are not fully separated by gradient elution from PLK, so that if this method was to be used for the preparation of DNA from a crude cell lysate, it was necessary to find a simple way of preventing rRNA from eluting close to the DNA. It was known from previous work on bacterial RNA fractionation (Ayad and Blamire, 1970) that degraded rRNA elutes at lower salt molarities than intact RNA thus experiments were performed to determine the effect that RNase treatment would have on the subsequent elution

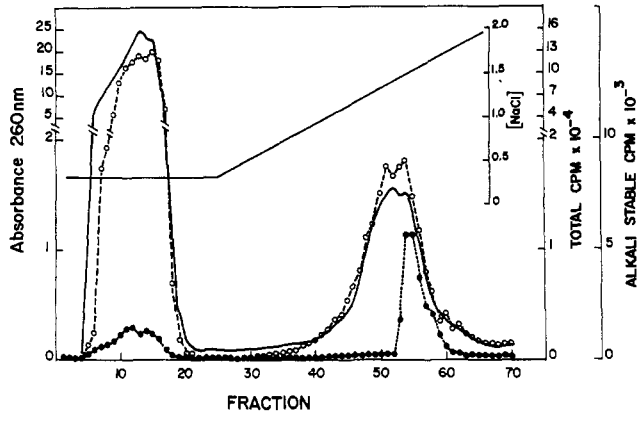

FIGURE 6: Elution profile of a crude yeast cell lysate prepared from 500 ml of culture labeled with 100 μCi of [^{3}H]adenine (1.1-g cells wet wt). Column and elution were as in Figure 1. (—) A_{260nm}. (O) Total acid-precipitable cpm of 0.1-ml aliquots. (●) Alkali-stable acid-precipitable cpm of 0.1-ml aliquots.

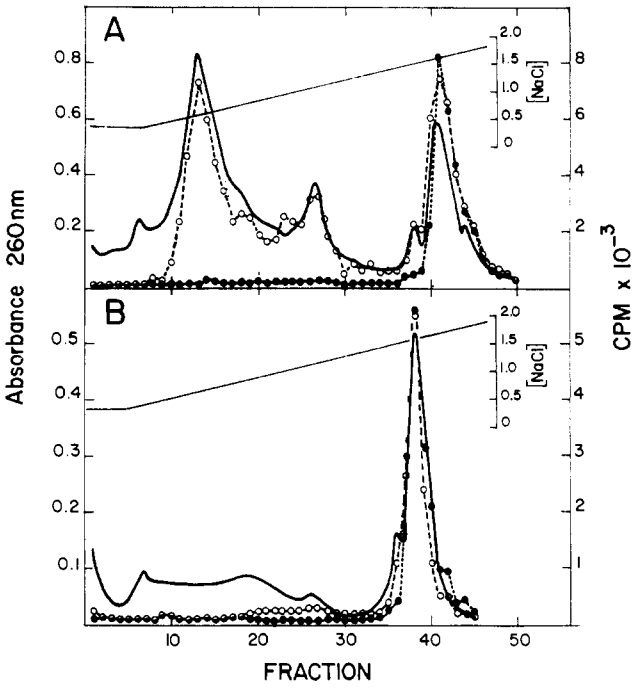

FIGURE 7: Elution profile of RNase-treated crude yeast cell lysates. (A) Lysate from 1 l. of culture labeled with 500 μCi of [^{3}H]adenine (1.5 g wet wt) prepared as before and treated with pancreatic RNase (20 μg/ml) for 15 min at 30° before loading. Column and elution as in Figure 1. (B) Lysate from 500-ml culture labeled with 150 μCi of [^{3}H]adenine (1.4 g wet wt) prepared as above and treated with pancreatic RNase (100 μg/ml) for 15 min at 30° before loading. Column and elution as above. (—) A_{260nm}. (○) Total acid-precipitable cpm. (●) Alkali-stable acid-precipitable cpm.

profiles of crude yeast cell lysates from PLK columns. Figure 7A shows the elution profile of a crude yeast supernatant, obtained from cells labeled with 500 μCi of [^{3}H]adenine (1.5 g wet weight) and treated with pancreatic RNase (20 μg/ml) for 15 min at 30° before loading on the PLK column. The fractions were assayed for absorbance at 260 nm, total trichloroacetic acid precipitable ^{3}H counts and KOH-stable trichloroacetic acid precipitable ^{3}H counts.

DNA is once again observed eluting at about 1.6 M NaCl. The RNA normally seen as a large peak eluting at 1.5 M NaCl has been reduced to a shoulder on the leading edge of the DNA peak, and degraded RNA now appears as a series of peaks at lower salt molarities. It should be noted that the DNA eluted from this column is as pure as material fractionated after extensive prior purification. The remaining RNA peaks can be completely eliminated by a more extensive RNase digestion of the crude lysate (100 μg/ml of RNase for 15 min at 30°). The result is shown in Figure 7B.

Discussion

Poly(L-lysine)kieselguhr chromatography, has been used in these studies to fractionate yeast nucleic acids. The results obtained show that the PLK column can be used to isolate nucleic acids rapidly and simply from crude cell lysates which have undergone no prior purification. The PLK–nucleic acid complex when washed free of unbound material can then be eluted by salt in a variety of ways. The standard method involves a linear gradient of 0.4–4.0 M buffered saline (100 ml of each) which elutes nucleic acids in the following order: partially degraded RNA, rRNA, nDNA, and mtDNA. The resolution between nucleic acid species can be increased by altering the shape of the salt gradient, and also (not shown here) by the use of stepwise increases in salt molarity. It is possible to load nucleic acids at higher salt molarities than 0.4 M NaCl, and thus eliminate certain classes of nucleic acid; *e.g.*, at 1.4 M NaCl partially degraded RNA does not load, and the subsequent elution of total DNA is considerably assisted. With any of the above methods the DNA is still eluted at its characteristic standard salt molarity. Flow rates from PLK columns have been as high as 40 ml/hr with no serious or deleterious changes, however when slower flow rates were observed it was generally attributed to either cellular debris trapped on the top of the column or to large amounts of DNA bound at the top of the PLK matrix. These difficulties may be overcome by binding the nucleic acids uniformily throughout the PLK. This is done by mixing the crude cell supernatant and the PLK together in a beaker before loading the complex into a column for further fractionation. In this way much larger amounts of DNA can be purified, and a fuller examination of these methods is given in the following paper (Finkelstein *et al.*, 1972).

The purity of the nucleic acids isolated and fractionated by PLK chromatography has been examined in several ways. All nucleic acids bound and subsequently eluted from PLK are free of protein (see Figure 2). DNA has no visible contamination of any uv-absorbing material (for example, RNA or its degradation products) when examined by the method of analytical CsCl density gradient centrifugation. Also, DNA and RNA prepared in this way have been routinely used in DNA–RNA hybridization studies, which is a good criterion of nucleic acid purity (Finkelstein *et al.*, 1972).

The ability of the PLK column to isolate DNA and RNA rapidly and simply from a crude cell lysate which has undergone the minimum of manipulations should not be overlooked as a reasonable alternative to other more tedious and complicated methods of isolation. A standard PLK column can be used to isolate quickly 300 μg of total DNA (enough for most hybridization experiments) in less than 8 hr, starting from a growing culture, with a high degree of purity.

The method can be also used as an analytical tool to investigate the amounts of DNA and RNA in small samples of yeast lysates or to check the purity of DNA prepared by other methods. It has been reported (Ayad and Blamire, 1970) however, that small traces of detergents eliminate the binding of nucleic acids to PLK, so efforts should be made to either avoid using detergents or to remove them before fractionating.

PLK chromatography has other advantages as well. All the ingredients are commercially available at low cost. Batches of poly(lysine) bought commercially (Sigma) have been found to be consistent over a period of greater than 4 years and are internally consistent from batch to batch with regard to the results obtained. Also, the poly(lysine) solution may be stored at 4° for many months without deterioration.

The column exhibits a high degree of reproducibility from one preparation to the next and is extremely simple to set up and use. These factors are important when experiments have to be monitored over extended periods of time and the experimenter needs confidence in the procedures being used. Because of its simplicity and cheapness, we have not routinely reused columns and fresh PLK was used for each experiment. It is possible, however, to regenerate and reuse the PLK columns if excessive protein contamination has been avoided.

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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.