MITOCHONDRIAL DNA DEFICIENT PETITE MUTANTS OF YEAST

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SUMMARY - Ethidium bromide induced neutral petite mutants of Saccharomyces cerevisiae have been isolated, which are shown to lack mitochondrial DNA. This lack of DNA accounts for the genetic properties of the mutants. The true neutral petites are contrasted with petites of very low suppressiveness, by using a 2% suppressive petite which retains cytoplasmic information and contains mitochondrial DNA of normal buoyant density. The neutral petites offer a convenient approach for determining the contribution of nuclear coded function to mitochondriogenesis.

Cytoplasmic mutant petite strains of yeast are respiratory incompetent and unable to synthesize the mitochondrial cytochromes a, a_3, b and c_1. It was at first reported that the petite mutation was a consequence of the loss of mitochondrial DNA (1, 2). However since the report of Mounolou et al. (3)that a variety of petite yeast strains contain mitochondrial DNA of normal or changed buoyant density, there has been general agreement that petites contain mitochondrial DNA in normal or slightly reduced quantities (4) albeit sometimes with a grossly abnormal base composition (5, 6). It has long been known that different petites may be distinguished from one another by virtue of their degree of suppressiveness (7) which is a measure of the proportion of petites in the diploid progeny resulting from a cross with a respiratory competent grande strain. In a cross using a petite of 100% suppressiveness all the diploid progeny are petites, whilst using a petite of zero suppressiveness (neutral), theoretically no petites will be present in the progeny (7).

It was recently demonstrated in this laboratory that

some petites may retain certain defined cytoplasmic genetic markers such as the erythromycin resistance determinant (ERY^r) (8, 9) and using this marker as an example, the genetic information retained could be correlated with the degree of suppressiveness of petites (10). The petites of very low and high suppressiveness usually failed to retain ERY^r (10).

In this communication we report studies on ethidium bromide induced neutral petites with minimal information retention, which are shown within the limits of our methods to contain no mitochondrial DNA.

STRAINS

Saccharomyces cerevisiae L411 (αur hi ERY^r) (ref. 8) is the grande parent of the petites studied here. K5272 is derived from a spontaneously arising cytoplasmic petite mutant of L411, purified through several steps of subcloning to stabilise its cytoplasmic genetic traits (10). It has very low suppressiveness (2-3%) and shows a high retention of the cytoplasmic determinant ERY^r, such that 75-80% of the grande diploids resulting from a cross with the erythromycin sensitive haploid strain L2200 are resistant to erythromycin (9, 10). E 1-5 are neutral petites (i.e. have no detectable suppressiveness), and have lost the erythromycin resistance determinant (10). They were induced by treatment of L411 with ethidium bromide (20 μg/ml) overnight.

RESULTS AND DISCUSSION

The results of preparative CsCl gradient centrifugation of lysed protoplasts of strains L411 and E5 are shown in Fig. 1. The mitochondrial DNA of the grande strain L411 as shown by analytical CsCl gradient centrifugation corresponds to a buoyant density of 1.683 g. cm^{-3}, and comprises about 15% of

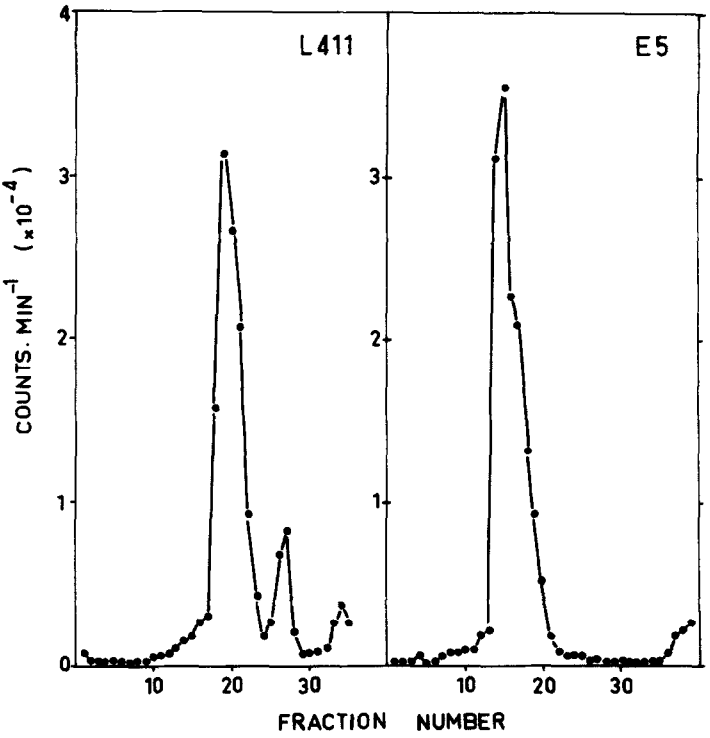

Figure 1. Preparative CsCl gradient centrifugation of lysed protoplasts of strains L411 and E5.

Cells were grown aerobically in a 1% yeast extract, 0.1% peptone, salts medium supplemented with uracil and histidine, with glucose (5%) as the energy source, and 2, 8 (^{3}H) adenine (Radiochemical Centre, Amersham, U.K.) included at 5 µC/ml to label nucleic acids. Protoplasts, prepared by snail gut enzyme treatment, were lysed in 0.01 M tris - 0.01 M EDTA, pH8, - 1.3% sarkosyl detergent (Geigy) and added directly to solid CsCl (Analar grade, British Drug Houses Ltd.) to a final density of 1.690 g. cm^{-3} in 5.0 ml volume, and centrifuged in an MSE Superspeed 65 ultracentrifuge at 34,000 rev. min^{-1} for 60 - 70 hours at 25^{o} in a 10 x 10 ml titanium rotor. The 0.13 ml fractions obtained by dripping from the bottom of the tube were treated overnight with 0.5 N NaOH, then precipitated with ice-cold TCA in the presence of 100 µg bovine serum albumin. Precipitates were collected on nitrocellulose membranes (Sartorius Membranfilter, 0.45µ pore size), dried and counted in toluene based scintillant in a Packard Tricarb Scintillation counter.

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the total cellular DNA. On the other hand the neutral petite E5 shows no visible mitochondrial DNA peak (Fig. 1).

Analytical centrifugation of E5 protoplast lysates also fails to

reveal any DNA band other than that of nuclear density (1.698 g. cm^{-3}) and its satellite band (1.704 g. cm^{-3}). The 2% suppressive petite K5272 protoplast lysates in contrast to E5, showed identical CsCl gradient profiles to those of the grande L411.

Mitochondria from K5272 were isolated and gradient purified, and the analytical DNA profile shown is in Fig. 2A. While the mitochondrial DNA band is considerably enriched, the preparation is still appreciably contaminated with the two nuclear components. In confirmation of the results of Borst et al. (14) for chick liver mitochondria, following DNAase I digestion of our yeast mitochondria, the two nuclear components are almost completely removed, while mitochondrial DNA is found to be resistant to hydrolysis (Fig. 2B). From K5272, 1 mg mitochondrial protein yields 0.7 µg mitochondrial DNA.

Mitochondria from the neutral petite E5 were similarly prepared and examined. The results (Fig. 2C) show that the only DNA species clearly recognisable, even from 5mg mitochondrial protein, are the two nuclear components, which are almost completely removed by DNAase I digestion of the mitochondria (Fig. 2D). The density of the gradients used ranges upwards from 1.635 g. cm^{-3} which is well below 1.647 g. cm^{-3}, the density of the polymer dA-dT (15) which has the lowest known density of a deoxyribopolymer containing the usually occurring bases. The lowest detectable amount of DNA banding in the centrifuge cell under our conditions has been determined to be 0.1 µg, and such DNA would need a molecular weight of at least 1×10^6 daltons to form a sharp band. The shortest pieces of DNA observed in petite mitochondrial DNA preparations, circular or linear, so far reported have been about 0.3µ long (5, 16)

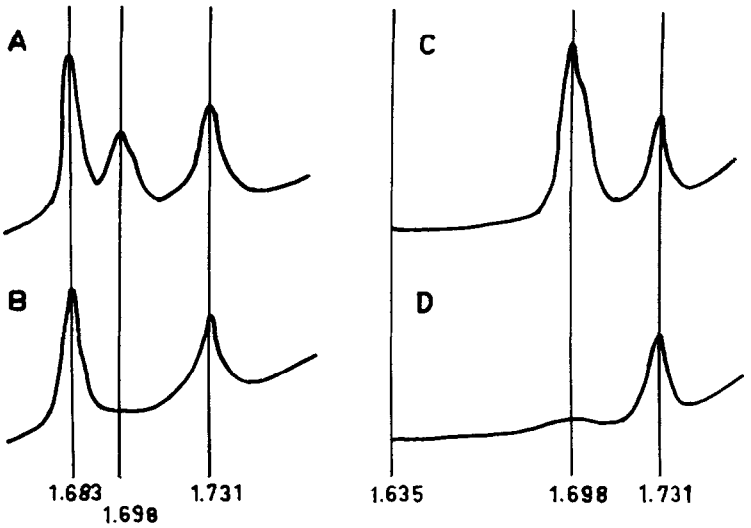

Figure 2. Analytical CsCl gradient centrifugation of DNA from isolated mitochondria of strains K5272 and E5.

Cells were grown as described for Fig. 1, except that (^{3}H) adenine was omitted. Mitochondria were isolated from protoplasts and purified on a sorbitol gradient (11). Where required, DNAase digestion of mitochondria was carried out in 0.35 M sucrose - 5 mM $MgCl_2$ using DNAase I (Sigma) at 100 µg/ml for 20 min at 0° and the mitochondria freed of enzyme by two washes in 0.35 M sucrose - 0.1 M EDTA, pH8. Untreated mitochondria were washed twice in sucrose-EDTA. To isolate DNA mitochondria were homogenised in 0.15 M NaCl - 0.1M EDTA, pH 8, - 1.3% sarkosyl, containing 0.1 vol. diethylpyrocarbonate to destroy nucleases (12), followed by chloroform deproteinisation, gentle pronase treatment, RNAase and ∝ - amylase digestion, a further deproteinisation and exhaustive dialysis against 0.15 M NaCl - 0.015 M sodium citrate, pH7, over 4-5 days at 4°. Samples were added to solid CsCl (Optical grade, Stanley H.Cohen, New York), and centrifuged using 2° Kel-F centrepieces in a 4-place rotor at 44,770 rev. min^{-1} and 25° in a model E analytical ultracentrifuge. Micrococcus lysodeikticus DNA (density 1.731 g. cm^{-3}) was used as a marker, quantities of DNA calculated from the areas of the peaks on the Beckman Analytrol scan of the film, and densities calculated according to Schildkraut et al. (13).

Profiles shown are from K5272 mitochondria (1 mg protein), untreated (A), or DNAase pretreated (B), and E5 mitochondria (5 mg protein) untreated (C) or DNAase pretreated (D). Buoyant densities (in g.cm^{-3}) are indicated at the bottom of figure.

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corresponding to 0.6 x 10^6 daltons. DNA of molecular weight less than 1 x 10^6 daltons, down to about 0.2 x 10^6 daltons, would however still be concentrated in the region of its buoyant

density, and although not forming sharp bands, would still be detectable as a region of absorption superimposed on the base-line of the scan. Such broad peaks were not obtained with mitochondria from E5 (Fig. 2).

It is therefore unlikely that E5 contains mitochondrial DNA. The absolute absence of mitochondrial DNA from E5 of especially low molecular weight cannot be eliminated, but its occurrence is considered unlikely. On a protein basis, strain E5 contains no more than 3% of the mitochondrial DNA present in the 2% suppressive petite strain K5272.

Direct comparison between E5 and K5272 mitochondria appears valid to us, as under the conditions of glucose growth used herein both strains contain a similar number of mitochondrial profiles per cell, as evidenced from electron microscopical studies (unpublished observations).

The neutral petites E1-4, yield similar results to those obtained with strain E5.

The existence of mitochondrial DNA-less mutants of yeast provides a simple explanation for the phenomenon of the neutral petite. If suppressiveness is regarded at its simplest level as a competition between the two types of mitochondrial DNA (grande and petite) to establish themselves in diploids resulting from a cross (even though control phenomena and recombination as well as simple reassortment undoubtedly play a part in the process (17)), the true neutral is defined as having no competing ability whatsoever, viz. no mitochondrial DNA. Comparison of the results obtained with strains K5272 and E5 emphasize that care must be taken to distinguish between a petite of very low suppressiveness and the absolute neutral. In the crosses, the suppressiveness is measured against the

background of "spontaneously" arising petites from the grande partner (1-3% in the strain L2200 used here). This makes the measurement of very low suppressiveness exacting; strain K5272, in crosses with L2200, has consistently given about 2% more petites above the background, whereas E5 has not.

The availability of a petite mitochondrion devoid of DNA has important biochemical implications. As noted above, the E5 petite shows definite mitochondrial profiles in general similar in development to the minimal deviant petite K5272. This observation implicates the nucleus to a very great extent in mitochondriogenesis, and any gene product found in DNA-less mitochondria must be of nuclear origin. Detailed comparisons are at present being made between E5 and K5272 under conditions of minimal mitochondrial glucose repression, in an attempt to clarify the roles of nuclear and mitochondrial genomes in mitochondriogenesis.

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