PHYSIOLOGICAL VARIATIONS IN SATELLITE COMPONENTS OF YEAST DNA DETECTED BY DENSITY GRADIENT CENTRIFUGATION*

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In order to study changes in the molecular properties of yeast DNA during both vegetative growth and meiosis, we have explored density gradient centrifugation of protoplast lysates as a means of isolating DNA in a relatively undegraded condition. Application of this approach to several strains of Saccharomyces revealed that the main nuclear component was usually accompanied by two minor satellites which varied in amount during growth. One of these corresponded to the recently described low density mitochondrial DNA (Tewari et al., 1965) and was absent from the two cytoplasmic "petite" mutants examined. The second minor component had a higher bouyant density than the main nuclear DNA and was present in the "petite" mutants.

MATERIALS AND METHODS

Strains. Diploid Saccharomyces carlsbergensis (NCYC 74) and an acriflavine-induced (Ephrussi et al., 1949) cytoplasmic "petite" mutant 74A; haploid Sacch. cerevisiae N123 and a derived radio-resistant mutant N123P4 (Moustacchi and Marcovich, 1962); haploid Sacch. cerevisiae DP1-1C (kindly provided by Prof. P. Slonimski), a segregational respiratory deficient mutant which carries the cytoplasmic genetic determinant "ρ" (Sherman, 1964). A derived "ρ^-" cytoplasmic mutant DP1-1CA was obtained by acriflavine treatment.

Preparation of Protoplasts. Routinely, stationary phase cells were used to inoculate YEPD (1% Bacto yeast extract, 2% Bacto peptone, 2% Glucose) with about 5×10^4 cells/ml, and the cultures were shaken at 30°C. Protoplasts were usually isolated (Eddy and Williamson, 1959) from log phase cultures using mannitol (15%,w/v) as osmotic stabilizer. However, good yields of protoplasts from the cytoplasmic mutants and from stationary phase cultures of strain 74 were obtainable only by using a higher stabilizing osmotic pressure, and sorbose (25%,w/v) satisfied this requirement.

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Isolation and Analysis of DNA. After thorough washing, 1-4 $x10^9$ protoplasts, containing 50-100 µg DNA, were suspended in chilled saline-EDTA (0.2M NaCl, 0.005M EDTA, pH 8.0) containing Duponol (ca. 0.5%, w/v). Lysis was complete in a few seconds. CsCl was then added to give a calculated density of 1.690 g/cm^3, and preparative ultracentrifugation performed using a Spinco model L centrifuge (Meselson et al., 1957). The tube was punctured and about 5 fractions collected per ml of suspension. Dilutions of the fractions were examined for UV-absorption at 260mµ, and their DNA contents estimated using diphenylamine (Burton, 1956) on extracts prepared by a modified Schmidt and Thannhauser (1945) technique. Fractions containing DNA were pooled and a suitable dilution of the resulting mixture analysed by CsCl density gradient centrifugation in a "Spinco" model E ultracentrifuge (Vinograd and Hearst, 1962). Densitometric tracings of the UV-absorption photographs were used to estimate the relative proportions of the different DNA components, and bouyant densities were calculated using the position of Pseudomonas aeruginosa DNA (density 1.727g/cm^3; kindly supplied by Dr. B. McCarthy) as reference (Schildkraut et al., 1962). The effect of heat on bouyant densities was investigated by heating diluted fractions for 10 min at 100°C.

RESULTS

1). DNA Components. Densitometer tracings depicting the three components we have observed are shown in Fig. 1. The major component, designated α, and presumed to be of nuclear origin, had a bouyant density of 1.699g/cm^3, which on heating increased by 0.015g/cm^3. A second and minor component (γ) had a bouyant density of 1.706g/cm^3. It has not so far been obtained free from contaminating α material, but has been observed to increase in density on heating and therefore seems to be a native DNA. A third component, (β) had a bouyant density of 1.683g/cm^3, which increased on heating by 0.015g/cm^3. It clearly corresponded to the low density mitochondrial constituent recently described by Tewari et al. (1965).

2). Variation During Growth. Cytological and enzymatic studies have shown that the mitochondrial content of yeast grown aerobically on media rich in glucose varies with the age of the culture; it is minimal during the log phase and maximal during the stationary phase (Ephrussi et al., 1956; Yotsuyanagi, 1962). It seemed of interest therefore to determine if similar fluctuations occurred in the relative proportion of the β (known to be mitochondrial) component of the cell's DNA. Moreover, it was hoped that such a study might throw light on the nature of the γ DNA. YEPD containing additional glucose (5.4%, w/v, total) was used as growth medium and adequate samples during the early growth stages were obtained

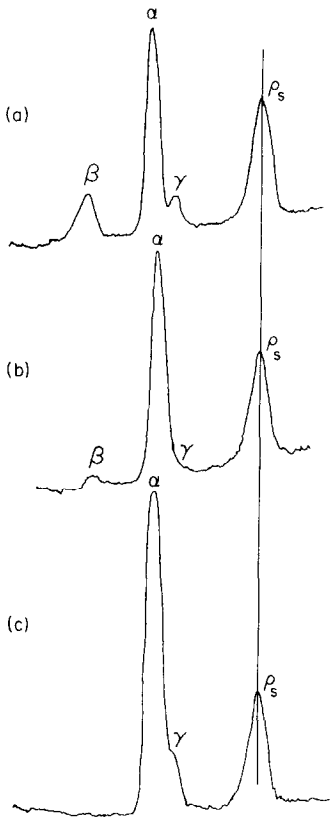

Figure 1. Microdensitometer tracings from density gradient preparations of yeast DNA. The peak on the right in each tracing is the reference DNA (Pseudomonas, density 1.727 g/cm^3). "α", "β" and "γ" had densities of 1.699 g/cm^3, 1.683 g/cm^3 and 1.706 g/cm^3 respectively. (a) Strain 74, 24 hrs culture. (b) Strain 74, 6 hrs culture. (c) Strain 74A, a cytoplasmic petite mutant.

by using a heavy inoculum (0.6x 10^6 cells/ml). From Fig. 2 it is seen that during the first 8 hrs of growth, the proportion of the β component fell to about 3% of the total DNA. Later on, it increased, reaching about 20% of the total DNA in the stationary phase (24 hrs). The γ component varied in a similar fashion, the minimal level observed (8 hrs) being less than 1% of the total DNA, while the stationary phase DNA contained as much as 11% of this component.

3). Comparison of normal strains and respiratory deficient mutants. Each of the three DNA components occurred in the haploid strains N123 and N123P4 in about the same proportions as in the diploid strain 74. This was also true for the haploid strain DP1-1C, a segregational respiratory de-

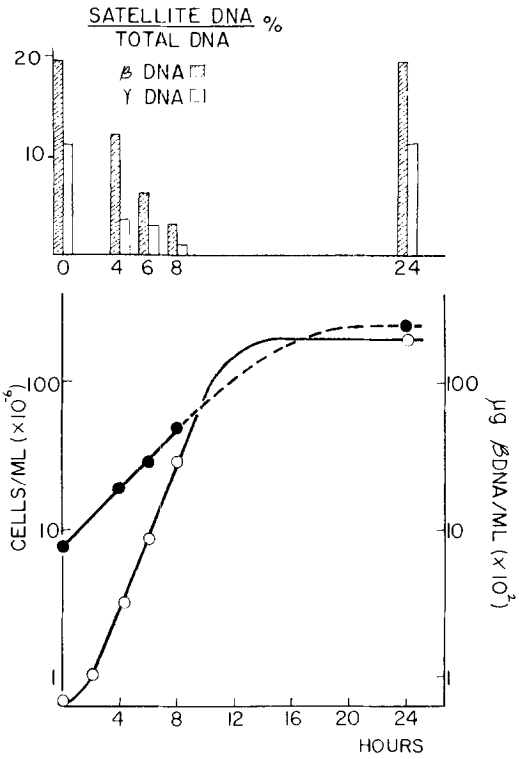

Figure 2. Growth curve and course of DNA synthesis in strain 74. (a) Changes in the proportion of satellite DNA during growth. (b) Growth curve (○) determined by haemocytometer counts. Total β DNA per ml of culture (●), calculated from data in (a) assuming a total DNA content/cell of 0.045 μg x 10^{-6}. The course of synthesis between 8 and 24 hrs has not been studied.

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ficient mutant which nevertheless carries the "ρ" cytoplasmic factor. In contrast, the two cytoplasmic "petite" mutants DP1-1CA and 74A contained no detectable amounts of the β component, though they both carried about as much of the γ component as their parental strains (Fig. 1).

DISCUSSION

The DNA of many types of cell contains minor density components which may comprise up to 10% of the cell's total DNA (Chun and Littlefield, 1963; Chun et al., 1963; Leff et al., 1963; Luck and Reich, 1964; Suyama and Preer, 1965). In many cases these are located in cytoplasmic organelles such as mitochondria, and the occurrence of DNA in the latter bodies seems to be universal (Nass et al., 1965). The conformity of yeast mitochondria to this rule was established by Tewari et al., (1965), who isolated from these organelles a discrete native DNA having a bouyant density of 1.685g/cm^3.

This material corresponds to our low density β component. Knowing that strain 74 in stationary phase contains about 0.045 $x10^{-6}$ μg DNA per cell (cf.Ogur et al., 1952), and assuming about 50 mitochondria/cell (Avers et al., 1965), our analysis of the stationary phase cells indicates an average β DNA content of about 1.6 $x10^{-10}$ μg (9.6×10^7 daltons) per mitochondrion. This figure is in good agreement with the estimate of Tewari et al.(1965) which was based on a different experimental approach. The function of this DNA is not certain. However, the accumulated evidence concerning cytoplasmic inheritance of mitochondrial function in yeast (Ephrussi, 1953; Slonimski, 1953) has been interpreted in terms of a cytoplasmic genetic determinant [referred to as "ρ" (Sherman, 1964)] whose behavior could be accounted for by the presence in mitochondria of a DNA which directs the synthesis of certain mitochondrial constituents. Our observation that the β component was absent from the cytoplasmic "petite" mutants, which are known to lack certain cytochromes and enzymes (Slonimski, 1953),is clearly consistent with this possibility. Certainly the amount of β DNA in the mitochondrion could code for at least 100 proteins of average size. Nevertheless, its detection in the segregational respiratory deficient mutant, and the ability of cytoplasmic mutants to form partial mitochondrial structures (Ephrussi et al., 1956; Yotsuyanagi, 1962; Avers et al., 1965) shows that the presence of this DNA is not the sole requirement for a fully functional mitochondrion.

Further support for a controlling function of the β component was provided by our observation of its variation in amount during growth. From Fig. 2 it appears that during the early stages of growth the β DNA was synthesized exponentially with a mean doubling time of about 3 hrs, i.e. at about one third of the rate of synthesis of nuclear DNA. If the β component were synthesizing respiratory enzymes during this period at a constant specific rate, the culture's total rate of oxygen consumption should increase exponentially, but more slowly than its mass, with the consequence that the Q_{O_2} would decrease. This in fact is precisely what happens (Ephrussi et al., 1956). The simplest hypothesis which would account for these findings would be that the β DNA directly controls synthesis of certain mitochondrial enzymes, and our observations therefore are entirely consistent with the possibility that this DNA is the cytoplasmic genetic determinant "ρ".

A further feature of our observations concerns the control of synthesis of β DNA. Although the exact course of its synthesis after 8 hrs is not clear, it is evident from its enhanced proportion in the stationary phase cells that in the final stages of growth, the rate of its synthesis relative to that of the nuclear DNA must have increased. Thus, during this period

as well as during the early stages of the culture's growth, the synthesis of the mitochondrial and nuclear DNA components seemed to be under independent control. It appears therefore that the glucose-induced depression of relative respiration rate observed by Ephrussi et al. (1956) may be explained in terms of a specific inhibition of synthesis of the mitochondrial DNA, rather than as a consequence of direct repression of enzyme synthesis.

The function of the γ component is not clear. Its presence in the cytoplasmic "petite" mutants makes a mitochondrial location unlikely. The fact that its amount relative to the α component varied during the growth of the culture suggested that it is not a chromosomal constituent, and a metabolically variable component such as the nucleolus (McLeish, 1964) might be considered as a possible candidate.

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Note. During the final preparation of this manuscript, Corneo et al.(Science 151:687, 1966) reported the presence of the γ component in whole yeast DNA preparations and its absence from isolated mitochondria, thus indirectly supporting our views about the possible nuclear location of this material in the cell.

REFERENCES

Avers, C., Pfeffer, C., Rancourt, M., J. Bact. 90, 481 (1965)
Burton, K., Biochem. J., 62, 315 (1956)
Chun, E., Vaughan, M., Rich, A., J. Mol. Biol. 7, 130 (1963)
Chun, E., Littlefield, J., J. Mol. Biol. 7, 245 (1963)
Eddy, A.A., and Williamson, D. H., Nature, 183, 1101 (1959)
Ephrussi, B., Hottinguer, H., Chimenes, A. N., Ann. Inst. Past. 76, 351 (1949)
Ephrussi, B., Nucleo-cytoplasmic relations in microorganisms. Clarendon Press Oxford, England. (1953)
Ephrussi, B., Slonimski, P., Yotsuyanagi, Y., Tavlitski, J., Compt. Rend. Lab. Carlsberg 26, 87 (1956)
Leff, J., Mandel, M., Epstein, H. T., Schiff, J. A., Bioch. Bioph. Res. Comm. 13, 126 (1963)
Luck, D. J., and Reich, E., Proc. Natl. Acad. Sc. U.S. 52, 931 (1964)
McLeish, J., Nature, 204, 36 (1964)
Meselson, M., Stahl, F. W., Vinograd, J., Proc. Natl. Acad. Sc. U.S., 43, 581 (1957)
Moustacchi, E., and Marcovich, H., Ann. Inst. Past. 103, 841 (1962)
Nass, M. M., Nass, S., Afzelius, B., Exptl. Cell. Res. 37, 516 (1965)
Ogur, M., Minckler, S., Lindegren, G., Lindegren, C. C., Arch. Bioch. Biophys., 40, 175 (1952)
Schildkraut, C. L., Marmur, J., Doty, P., J. Mol. Biol. 4, 430 (1962)
Schmidt, G., and Thannhauser, S. J., J. Biol. Chem., 161, 83 (1945)
Sherman, F., Genetics, 49, 39 (1964)
Slonimski, P., Formation des enzymes respiratoires chez la levure, Masson Paris (1953)
Suyama, Y., and Preer, J. R., Genetics 52, 1051 (1965)
Tewari, K. K., Jayaraman, J., Mahler, M. R., Bioch. Bioph. Res. Comm. 21, 141 (1965)
Vinograd, J., and Hearst, J. E. in Progress in the Chemistry of Organic Natural Products (Ed. L. Zeichmeister) Vol.xx,P.372, Springer-Verlag, Vienna (1962)
Yotsuyanagi, Y., J. Ultrastruct. Res. 7, 121 (1962) and 7, 141 (1962)