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Short Communication

The Size of Mitochondrial DNA from a Cytoplasmic *Petite* Mutant of *Saccharomyces cerevisiae* *

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Summary. Mitochondrial DNA has been isolated from a cytoplasmic *petite* mutant of *Saccharomyces cerevisiae* which has retained only about 2% of the mitochondrial *wild type* genome. The denatured DNA was analyzed by agarose gel electrophoresis and a homogeneous, single band of DNA was found. *Petite* and *wild type* mitochondrial DNAs exhibited similar gel electrophoretic mobilities. Using denatured DNA from the *E. coli* phages *T4* and *T3* for comparison a molecular weight of 55×10^6 daltons has been calculated for the double-stranded *petite* mitochondrial DNA. On the basis of this observation most of the mitochondrial DNA of this *petite* mutant appeared to consist of a polymer of about 50 repeats to account for a size similar to that of the *wild type* molecule. Thus a regulatory mechanism might exist which keeps constant the physical size of the mitochondrial DNA molecule in spite of the elimination of large fractions of the *wild type* genome.

Cytoplasmic *petite* mutants of *Saccharomyces cerevisiae* are the result of large deletions or the complete loss of *wild type* mitochondrial DNA (mit. DNA). *Petite* mutants which do contain mit. DNA apparently do so in amounts similar to that of the *wild type* strain (Nagley and Linnane, 1972). Considerable repetition of the non-deleted segment must occur in order to maintain a constant cellular level of mit. DNA. Since *petite* mit. DNAs have been reported to be of smaller size than *wild type* DNA (Carnevali *et al.*, 1969; Goldring *et al.*, 1971; Hollenberg *et al.*, 1972), the repetition of the non-deleted segment should be intermolecular, leading to an increased number of small molecules. Indeed small circular molecules have been found in *petite* mit. DNA (Faye *et al.*, 1973; Locker *et al.*, 1974a). In addition to the small circular molecules long linear molecules of *petite* mit. DNA, which are the result of intramolecular repetition, have been described. These long molecules contain linear repeats of the remaining segment as shown by electron microscopy (Faye *et al.*, 1973; Locker *et al.*, 1974b). Nothing is known about the size of these long repetitive DNA molecules in vivo. Attempts to isolate pure and intact mit. DNA from *wild type* strains have failed so far, although some few 25 μm circles have been visualized by Hollenberg *et al.* (1970) after lysis of *wild type* mitochondria on the electron microscopic grid. Pure mit. DNA preparations from *wild type* and *petite* strains contain linear fragments of heterogeneous size.

* Dedicated to Dr. Dr. h. c. Peter Michaelis on the occasion of his 75th birthday

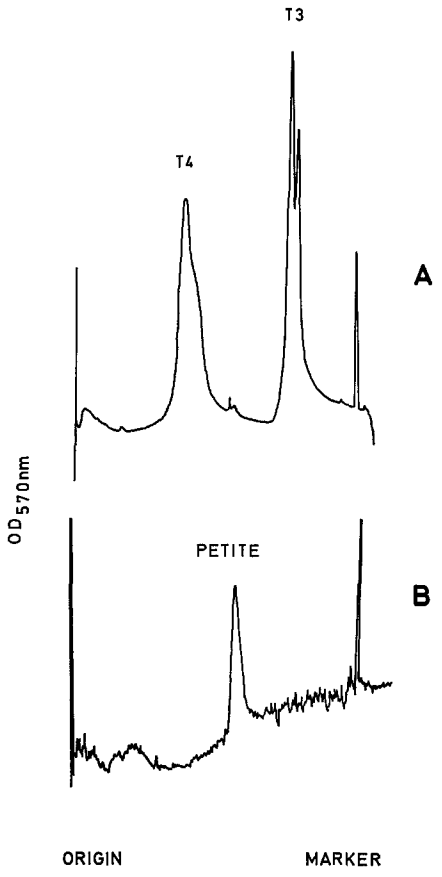

Fig. 1 A and B. Agarose gel electrophoresis of denatured DNA according to Hayward (1972). DNA samples (2–3 μg each) were denatured with 0.1 volume 1 M NaOH, followed by the addition of 0.1 volume 60% sucrose-0.05% bromophenol blue. Samples of 0.05 ml were layered onto cylindrical gels (5 mm × 11 cm) of 0.4% agarose (Bausch and Lomb GmbH, Seakem agarose) and electrophoresed for 5 hours at 4° and 2 mA/gel, in Tris/phosphate buffer (0.036M Tris, 0.03M NaH_2PO_4, 1 mM EDTA, pH 7.7). The gels were stained for three hours with 0.002% "Stains-All" (Serva, 19284) in 50% formamide and destained overnight with water. Staining and destaining were done in the dark. (A) Denatured DNA from *E. coli* phages *T4* and *T3*. (B) Denatured mit. DNA from the cytoplasmic *petite* mutant *IL8-8C/E41* (obtained from P. Slonimski)

For studying the size of long repetitive mit. DNA molecules we have chosen the well characterized cytoplasmic *petite* mutant *IL8-8C/E41*. This mutant has retained only about 2% of the *wild type* mitochondrial genome (Michel *et al.*, 1974; Locker *et al.*, 1974a, b). We have tried a variety of methods to isolate the pure mit. DNA of this mutant as intact as possible. In view of its sensitivity and high resolution the agarose gel electrophoretic technique for single-stranded DNA (Hayward, 1972) was used to characterize the DNA preparations. We have been able to obtain mit. DNA from the *petite* mutant, which after denaturation gave a single band in the agarose gels (Fig. 1 B). The gel electrophoretic mobilities of

single-stranded DNA species were shown to be inversely proportional to the logarithm of their molecular weights (Hayward and Smith, 1972). Denatured DNA from the *E. coli* phages *T4* and *T3* were used as references (Fig. 1 A). The following electrophoretic mobilities of single-stranded DNAs were obtained: *T4*=0.39, *T3*=0.77, *petite IL8-8C/E41*=0.57, *wild type IL8-8C*=0.55 (unpublished results). Assuming a molecular weight of $110/2\times10^6$ daltons for single-stranded *T4* DNA (Freifelder, 1970) and $25/2\times10^6$ daltons for single-stranded *T3* DNA (Bujard, 1969), the following values can be calculated for single-stranded mit. DNA: $55/2\times10^6$ daltons for *petite IL8-8C/E41* and $59/2\times10^6$ daltons for *wild type IL8-8C*. The latter value is in agreement with the molecular weight of 50×10^6 daltons (25 μm) for double-stranded mit. DNA from *wild type* yeast as determined by renaturation (Christiansen *et al.*, 1974) and electron microscopy (Hollenberg *et al.*, 1970). We have found that the large majority of mit. DNA from the *petite* mutant *IL8-8C/E41* is of high molecular weight and of homogeneous size. This is not necessarily contradictory to the result of Locker *et al.* (1974a). Even a high percentage of small circular molecules as seen by electron microscopy would not contribute very much to the total mass of mit. DNA. An apparent molecular weight of 55×10^6 daltons for the *petite* double-stranded DNA has been calculated from our data, a value which is practically identical with that for *wild type* DNA. This implicates that *petite* and *wild type* DNA have the same size and that the *petite* DNA should consist of about 50 repetitions of a 0.5 μm monomer sequence. Additional *petites* are under investigation by different methods to verify the interesting possibility that the yeast cell does not only regulate the amount of mit. DNA but also the length of these molecules to a standard size of 25 μm.

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References

Bujard, H.: Location of single-strand interruptions in the DNA of bacteriophage $T5^+$. Proc. nat. Acad. Sci. (Wash.) **62**, 1167–1174 (1969)

Carnevali, F., Morpurgo, G., Tecce, G.: Cytoplasmic DNA from petite colonies of *Saccharomyces cerevisiae*: a hypothesis on the nature of the mutation. Science **163**, 1331–1333 (1969)

Christiansen, C., Christiansen, G., Bak, A. L.: Heterogeneity of mitochondrial DNA from *Saccharomyces carlsbergensis*: renaturation and sedimentation studies. J. molec. Biol. **84**, 65–82 (1974)

Faye, G., Fukuhara, H., Grandchamp, C., Lazowska, J., Michel, F., Casey, J., Getz, G. S., Locker, J., Rabinowitz, M., Bolotin-Fukuhara, M., Coen, D., Deutsch, J., Dujon, B., Netter, P., Slonimski, P. P.: Mitochondrial nucleic acids in the petite colonie mutants: deletions and repetitions of genes. Biochimie **55**, 779–792 (1973)

Freifelder, D.: Molecular weights of coliphages and coliphage DNA IV. Molecular weights of DNA from bacteriophages *T4*, *T5* and *T7* and the general problem of determination of M. J. molec. Biol. **54**, 567–577 (1970)

Goldring, E. S., Grossman, L. I., Marmur, J.: Petite mutation in yeast II. Isolation of mutants containing mitochondrial deoxyribonucleic acid of reduced size. J. Bact. **107**, 377–381 (1971)

Hayward, G. S.: Gel electrophoretic separation of the complementary strands of bacteriophage DNA. Virology **49**, 342–344 (1972)

Hayward, G. S., Smith, M. G.: The chromosome of bacteriophage *T5* I. Analysis of the single-stranded DNA fragments by agarose gel electrophoresis. J. molec. Biol. **63**, 383–395 (1972)

Hollenberg, C. P., Borst, P., Van Bruggen, E. F. J.: Mitochondrial DNA V. A 25-μ closed circular duplex DNA molecule in wild-type yeast mitochondria. Structure and genetic complexity. Biochim. biophys. Acta (Amst.) **209**, 1–15 (1970)

Hollenberg, C. P., Borst, P., Van Bruggen, E. F. J.: Mitochondrial DNA from cytoplasmic petite mutants of yeast. Biochim. biophys. Acta (Amst.) **277**, 35–43 (1972)

Locker, J., Rabinowitz, M., Getz, G. S.: Electron microscopic and renaturation kinetic analysis of mitochondrial DNA of cytoplasmic petite mutants of *Saccharomyces cerevisiae*. J. molec. Biol. **88**, 489–507 (1974a)

Locker, J., Rabinowitz, M., Getz, G. S.: Tandem inverted repeats in mitochondrial DNA of petite mutants of *Saccharomyces cerevisiae*. Proc. nat. Acad. Sci. (Wash.) **71**, 1366–1370 (1974b)

Michel, F., Lazowska, J., Faye, G., Fukuhara, H., Slonimski, P. P.: Physical and genetic organization of petite and grande yeast mitochondrial DNA III. High resolution melting and reassociation studies. J. molec. Biol. **85**, 411–431 (1974)

Nagley, P., Linnane, A. W.: Biogenesis of mitochondria XXI. Studies on the nature of the mitochondrial genome in yeast: the degenerative effects of ethidium bromide on mitochondrial genetic information in a respiratory competent strain. J. molec. Biol. **66**, 181–193 (1972)

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