Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Francesca Carnevali, Gianni Piperno, Giorgio Tecce

Satellite components of DNA from a cytoplasmic "petite" mutant of Saccharomyces cerevisiae

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **41** (1966), n.3-4, p. 194–196.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1966_8_41_3-4_194_0>

Biofisica. — *Satellite components of* DNA *from a cytoplasmic "petite" mutant of Saccharomyces cerevisiae.* Nota (*) di FRANCESCA CARNEVALI (**), GIANNI PIPERNO (**) e GIORGIO TECCE (**), presentata dal Corrisp. M. AGENO.

RIASSUNTO. — È stato studiato il DNA di un mutante respiratorio citoplasmatico di *Saccharomyces cerevisiae*. Sono presenti tre componenti di cui due, tra i quali quello nucleare, hanno densità uguali a quelle dei componenti presenti nel wild type. Il terzo componente presenta una densità diversa (I, 670 g/cm³). Questa densità così bassa lascia supporre un'unione del DNA con un componente cellulare sconosciuto.

The presence of satellite DNAs is well established in DNA preparations from *Saccharomyces cerevisiae* [1–3].

The component with a buoyant density 1.685 g/cm³ has been identified as mitocondrial DNA by Corneo, Moore, Sanadi, Grossman and Marmur [4], but the component with a buoyant density approximately 1.704 g/cm³, which forms a shoulder of the major band of density 1.700 g/cm³ (nuclear component), has not been demonstrated to be associated with any particular cellular structure.

The function of mitocondrial DNA is still unknown, although it is hypothetically connected with the possible continuity of this organnelle and thus may be concerned with cytoplasmic heredity.

This letter reports a study concerning DNA extracted from a cytoplasmic mutant (DM_1), obtained from a diploid strain (DM) of *Saccharomyces cerevisiae* by acriflavine treatment.

This mutant was grown in enriched Czapek Dox medium ($NaNO_3$ 3.3 g; $MgSO_4 \cdot 7\,H_2O$ 1.0 g; KCl 0.5 g; $FeSO_4 \cdot 7\,H_2O$ 18 mg; KH_2PO_4 1.0 g; yeast extract 6.0 g; glucose 40 g; per liter of distilled water) and the absence of wild type cells was confirmed at the end of the growth period.

The cells were harvested by centrifugation and washed with saline–EDTA (0.2 M–NaCl plus 0.2 M–ethylenediaminetetra–acetate pH 8.0) and ground with celite. DNA was isolated by a modification of the method of Marmur [5]; because of the great amount of RNA in yeast cells, the samples of DNA were treated with pancreatic ribonuclease (Sigma, 100 μg/ml, two hours, 37° C).

The DNA samples were analysed by caesium chloride density–gradient centrifugation at 25° C at 44,770 rev/min in the Spinco model E analytical ultracentrifuge at the concentration of 10 μg/ml, using denaturated *Escherichia coli* DNA as the density marker (1.725 g/cm³).

(*) Pervenuta all'Accademia il 18 settembre 1966.

(**) Istituto di Fisiologia Generale, Università degli Studi, Roma, Italy.

Fig. 1 shows the microdensitometer tracing of cytoplasmic "petite" mutant DNA as compared with DNA from wild type cells.

The occurrence of a component having such a low density (1.670 g/cm³) led us to use, in these experiments, a gradient with a lower average density in order to shift this band from the meniscus.

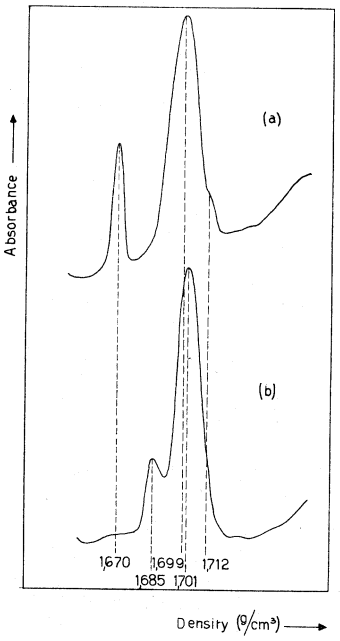

Fig. 1. – Microdensitometer tracings of DNA from a cytoplasmic "petite" mutant (a) and from wild type cells of *Saccharomyces cerevisiae* (b) at equilibrium in a CsCl gradient in the Spinco analytical ultracentrifuge model E.

This figure also demonstrates that, in all samples, there is a component having a higher density than nuclear DNA (1.699 g/cm³). The density value of this component is approximately 1.712 g/cm³, but it is difficult to establish this value precisely because of its position in the caesium chloride density-gradient.

All three components disappeared after incubating DNA with deoxyribonuclease (Worthington, 3 μg/ml, 4° C, 5 hours in 0.1 M–NaCl). When DNA from the cytoplasmic mutant was treated with pronase (Calbiochem, 20 μg/ml, 3 hours, 37° C) the densities of the main band as well as the satellite bands remained unaffected. Upon heat denaturation, all components, including the satellite band (1.670 g/cm³), increased in density approximately by 0.015 g/cm³.

DNA preparations from cells of the wild type strain grown in anaerobiosis show three components in caesium chloride density-gradient with densities the same as those of the components of DNA from aerobic cells.

The results reported here show that in the preparations of DNA from our cytoplasmic mutant the component of wild type cells with density 1.685 g/cm^3 is virtually absent.

On the other hand the component with a density higher than nuclear DNA (1.712 g/cm^3) is present in both.

The low density of satellite DNA in mutant cells is difficult to explain. It may be connected with some cellular component.

This work, supported by CNR research grants, has been presented during the 3th Convegno Nazionale di Biofisica e Biologia Molecolare of Rome, 9th July 1966.

References.

[1] G. Schatz, E. Haslbrunner and H. Tuppy, « Biochem. Biophys. Res. Commun. », *15*, 127 (1964).

[2] K. K. Tewary, J. Jayaraman and M.R. Mahler, « Biochem. Biophys. Res. Commun. », *21*, 141 (1965).

[3] F. Carnevali and G. Tecce, *Atti della XXXIV Assemblea Generale della Società Italiana di Biologia Sperimentale* (1965).

[4] G. Corneo, C. Moore, D. R. Sanadi, L. I. Grossman and J. Marmur, « Science », *151*, 687 (1966).

[5] J. Marmur, « J. Mol. Biol. », *3*, 208–218 (1961).