CYTOPLASMIC TRANSFORMATION: MITOCHONDRIA OF WILD-TYPE BAKER'S YEAST RESTORING RESPIRATORY CAPACITY IN THE RESPIRATORY DEFICIENT "PETITE" MUTANT

H. Tuppy and G. Wildner
Institut für Biochemie, University of Vienna, Austria

Received August 16, 1965

One of the most thoroughly investigated examples of extrachromosomal inheritance is the respiratory deficiency of the acriflavin—induced "petite" mutant of Saccharomyces cerevisiae (Ephrussi, 1953). The failure of the mutant cells to respire is due to an irreversible hereditary alteration of the structure and function of their mitochondria, which involves a complete loss of cytochrome oxidase activity and a partial loss of other mitochondrial components (Ephrussi and Slonimski, 1955; Linnane and Still, 1956; Yotsuyanagi, 1962; Schatz et al., 1963; Mahler et al., 1964). Consequently, mitochondria have hypothetically been considered to be the carriers of an extrachromosomal genetic information responsible for respiratory capacity. This view has recently been strengthened by the detection in highly purified yeast mitochondria of a small but significant amount of DNA (Schatz et al., 1964; Tuppy et al., 1965) and by the demonstration of its involvement in mitochondrial RNA and protein synthesis (Wintersberger and Tuppy, 1965; Wintersberger, 1965).

In the present study spheroplasts of the respiratory deficient "petite" mutant were exposed to isolated yeast mitochondria. The treated cells were shown to acquire respiratory capacity and to undergo a heritable cytoplasmic transformation.

MATERIALS AND METHODS

The nomenclature used to designate yeast mutants has been adopted from the work of Sherman (1963). The symbols ρ^+ and ρ^-, resp., denote the wild-type and mutant character of the cytoplasmic factor which governs respiratory capacity; leu, ad, and th indicate nutritional requirements, and α the mating type.

The haploid yeast strains 276/3br α ad th ρ^+ and D273-10B $\rho^+\alpha$ were made available to us by Dr. H. Jakob and Dr. F. Sherman. The respiratory deficient vegetative mutant, 276/3br α ad th ρ^-, was prepared from the wild-type (ρ^+) strain by treatment with acriflavin according to the method of Nagai and Nagai (1958); it was unable to utilize glycerol or acetate for growth and failed to reduce triphenyltetrazolium chloride (Ogur, 1957). The leucine auxotroph, D273-10B α leu ρ^+, was selected from an ultraviolet irradiated sample of its leucine independent parent strain.

The yeasts were cultivated at 27°C in the medium described by Ephrussi and Slonimski (1950) which was slightly modified so as to contain only 0.3% yeast extract and 0.8 to 2.0% glucose. Minimal agar contained the following per liter: 18 g agar, 2 g $(NH_4)_2SO_4$, various salts and trace elements, 2 mg biotin, and either 20 g glucose or 68 g glycerol. The strain 276/3br α ad th was grown on minimal agar supplemented by the addition of 10 mg adenine and 80 µg thiamine hydrochloride per liter.

Spheroplasts were obtained from the "petite" mutant strain, 276/3br α ad th ρ^-, by treatment with digestive juice of Helix pomatia under conditions closely similar to those of Duell et al. (1964). Prior to digestion, the yeast cells were grown in a medium containing 2% glucose for a period of 7 to 9 hr. The yield of spheroplasts ranged from 75 to 60%.

For the preparation of mitochondria, D273-10B α leu ρ^+ cells

were harvested in the stationary phase, washed, suspended in a solution containing 0.25 M sucrose, 0.02 M Tris buffer pH 7.4, and 0.005 M EDTA, and disrupted by shaking with glass beads in a Merkenschlager disintegrator for 25 sec (Schatz et al., 1963). The homogenate was adjusted to pH 7.4 and centrifuged twice at 4600 x g in order to remove intact cells, cell debris, and nuclei. The mitochondrial fraction was sedimented by centrifugation at 73300 x g.

Spheroplasts obtained from 0.5 g of moist 276/3br α ad th ρ^- cells were combined with the mitochondrial fraction prepared from 7 g (wet weight) of D273-10B α leu ρ^+ cells and suspended in a mannitol solution containing 0.02 M tris buffer pH 7.4 and 0.005 M EDTA to give a total volume of 3.7 ml. Concentrations of mannitol in the medium of 0.3, 0.55, 0.8, and 1.1 M were tested for their effect on the yield of transformed cells. The mixtures were kept at 4°C. After various incubationstimes a sample was withdrawn, diluted with mannitol solution, and inoculated on minimal agar plates containing glycerol and, in addition, adenine and thymine.

RESULTS AND DISCUSSION

The mitochondria used for the present studies of mitochondrial transmissibility were prepared from a leucine auxotroph of a respiring haploid *S.cerevisiae* strain. This strain grew readily on media containing either glycerol or glucose as the source of carbon and energy, provided that leucine was supplied. On glycerol-containing agar it formed white colonies of normal size.

The respiratory deficient recipient strain was an acriflavin-induced "petite" mutant, haploid, auxotrophic for adenine and thymine, and of the same mating type as the mitochondrial

donor strain. It was unable to use glycerol for growth, and on agar containing glucose, adenine and thymine it formed small white colonies. The respiring strain, on the other hand, from which the "petite" mutant had been obtained by treatment with acriflavin, grew well on media containing glycerol, and the colonies formed on agar plates (in the presence of added adenine and thymine) were large and red-brown in color (Ephrussi et al., 1949). The acriflavin mutant was entirely stable, no spontaneous reversions to respiration, large-colony growth, or pigmentation being ever observed, in full agreement with the irreversibility of the "petite" mutation reported by Ephrussi et al.(1949).

When spheroplasts of the respiratory deficient (ρ^-) strain were treated under suitable conditions with mitochondria of the respiring (ρ^+) "donor" strain, a considerable number of them regained respiratory capacity and formed stable colonies consisting of respiring, haploid, non-sporulating cells. The transformation could readily be demonstrated by plating on agar containing glycerol as the carbon and energy source and adenine and thymine as supplementary nutrients, but lacking leucine. On such plates cells of the "donor" strain contaminating the mitochondrial fraction fail to grow because of their leucine deficiency, and cells and spheroplasts of the recipient "petite" strain fail to grow due to their inability of utilizing glycerol. Spheroplasts, however, which have been transformed to respiratory sufficiency, do grow out and form large red-colored colonies.

Two hours of treatment of spheroplasts with mitochondria, an incubation temperature of 4°C, and a mannitol concentration in the medium of 1.1 M have been found to be conditions suitable for mitochondrial transfer. 2.6% of the respiratory deficient spheroplasts could be converted into respiring cells forming

Table I. Conversion of respiratory deficient yeast spheroplasts into respiring cells

Effect of the duration of incubation with mitochondria and of the molarity of mannitol in the medium on the percentage of cells transformed at 4°C

Concentration of mannitol in the medium	Percentage of cells transformed after incubation with mitochondria for 2 hr	5 hr	2o hr
1.1 M	2.6	2.3	2.1
0.3 M, 0.55 M, 0.8 M } average:	0.03	0.03	0.02

pink colonies (Table I). An increase in the incubation time resulted in a decreased yield of transformed cells. When the molarity of mannitol was reduced from 1.1 M to 0.8, 0.55, or 0.3 M, the yield diminished by almost two orders of magnitude.

The successful transfer of respiratory capacity mediated by mitochondria may be considered as evidence for these organelles to be transmissible carriers of cytoplasmic genes. However, further experiments are required to explore the scope of these findings and to exploit the technique of mitochondrial transfer for more detailed investigations of cytoplasmic inheritance and of nuclear-cytoplasmic relations. It also remains to be established whether for such cytoplasmic transformations whole mitochondria can be replaced by modified or fragmented mitochondria or by isolated mitochondrial DNA. Such experiments are under way in this laboratory.

SUMMARY

Respiratory deficient cells of *S.cerevisiae*, which lack the extrachromosomal genetic determinant of respiratory capacity, were converted into their spheroplasts and the latter were treated with mitochondria of a respiring yeast strain. A size-

able portion of the treated cells acquired the ability to respire and to form normal-sized colonies, indicating that mitochondria had been incorporated into them. Mitochondria may thus be regarded as transmissible carriers of cytoplasmic genes.

We wish to thank Dr. H. Jakob, Gif-sur-Yvette, France, and Dr. P. Sherman, Rochester, N.Y., for providing us with two yeast strains.

This work was supported by grant GM 11225 from the U.S. Public Health Service.

REFERENCES

Duell, E.A., Inoue, S., and Utter, M.F., J.Bacteriol. 88, 1762 (1964).
Ephrussi, B., "Nucleo-cytoplasmic Relations in Microorganisms", Clarendon Press, Oxford, 1953.
Ephrussi, B., Hottinguer, H., and Tavlitzki, J., Ann.Inst.Pasteur 76, 419 (1949).
Ephrussi, B., and Slonimski, P.P., Biochim.Biophys.Acta 6, 256 (1950).
Ephrussi, B., and Slonimski, P.P., Nature 176, 1207 (1955).
Linnane, A.W., and Still, J.L., Austral.J.Sci. 18, 165 (1956).
Mahler, H.R., Mackler, B., Grandchamp, S., and Slonimski, P.P., Biochemistry 3, 668 (1964).
Nagai, S., and Nagai, H., Experientia 14, 321 (1958).
Ogur, M., Science 125, 928 (1957).
Schatz, G., Haslbrunner, E., and Tuppy, H., Biochem.Biophys. Res.Comm. 15, 127 (1964).
Schatz, G., Tuppy, H., and Klima, J., Z.Naturforsch. 18b, 145 (1963).
Sherman, F., Genetics 48, 375 (1963).
Tuppy, H., Haslbrunner, E., and Schatz, G., Mh.Chem., in press (1965).
Wintersberger, E., Biochem.Z. 341, 409 (1965).
Wintersberger, E., and Tuppy, H., Biochem.Z. 341, 399 (1965).
Yotsuyanagi, Y., J.Ultrastruct.Res. 7, 141 (1962).