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MITOCHONDRIAL CONTROL OF CELL SURFACE CHARACTERISTICS IN *SACCHAROMYCES CEREVISIAE*

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Summary

Defects in the inner mitochondrial membrane of *petite* mutants of yeast resulted not only in respiratory deficiency, but also in changes in cell surface characteristics. These were (1) concanavalin A agglutinability, (2) cell movement in a biphasic polymer system, (3) cell adhesion. Genetic analysis indicated that the control exerted by the mitochondria was on nuclear genes or on the products of these genes which were presumably specifying cell surface components. These findings ascribe a new role to mitochondria but also have implications for neoplastic transformation.

The mitochondria of yeast cells appear to have a controlling influence on cell surface characteristics, inasmuch as genetic lesions in the mitochondrion (*petite* mutations, ρ^-) lead to changes in cellular tolerance to drugs [1], inability of cells to take up and utilize certain sugars [2, 3], and in cell adhesiveness in flocculent strains [4]. Further evidence of a mitochondrial influence on cell surface properties is presented here, the characteristics studied being agglutination of cells with concanavalin A and cell partitioning in a biphasic aqueous polymer system. New results on flocculation changes are also presented. Once again, mitochondrial *petite* mutants are compared with wild-type cells. These mutants result from extensive deletions in mitochondrial DNA leading to a non-functional protein-synthesizing system in the organelle [5]. The inner membrane assembly thus lacks a number of components including cytochromes aa_3 and b of the respiratory chain. The muta-

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tion arises with a high spontaneous frequency, about 1% in most strains, so that *petites* are readily identified as small colonies on fermentable medium (see Fig. 2).

Concanavalin A was found to agglutinate yeast cells. This result was not unexpected since the lectin binds to glucopyranosides, *N*-acetylglucosamines and mannopyranosides (mannan, a mannose polymer, is located at the outer region of the yeast cell wall) [6]. Preliminary experiments with a number of laboratory (haploid) strains estimating agglutination by microscopic examination of clump size and frequency showed that agglutinability was strain dependent. In the most sensitive strain, 10 μg/ml concanavalin A caused detectable agglutinability while as much as 1000 μg/ml was necessary in some strains. In order to compare the agglutinability of isogenic ρ^+ and ρ^- strains, a more objective quantitative procedure was developed based on separation of cells from clumps by differential sedimentation through a mannitol cushion.

When concanavalin A agglutination of several *petite* mutants of strain D6 was compared with the parental strain, considerable differences in agglutinability were seen across a wide concentration range of concanavalin A, the

Fig. 1. Effect of the *petite* mutation on agglutination by concanavalin A (Con. A). Freshly grown cells (24 h in 1% yeast extract, 2% glucose medium of strain D6 (o) and of two *petite* mutants (D6ρ^{-1}, O, D6ρ^{-2}, □) were washed twice in distilled water and resuspended in 50 mM Hepes buffer, pH 7.0, to give suspensions of approximately the same absorbance (A500). 100-μl aliquots of these suspensions were mixed with 100-μl volumes of stock concanavalin A (Sigma Ltd.) solutions in Hepes buffer; agglutination being performed in triplicate. Each sample (200 μl total volume) was then layered onto a 3 ml cushion of 0.5 M mannitol. Cell aggregates were allowed to sediment through the mannitol cushion for 20 min after which the top 1.0 ml was removed and absorbance read at 500 nm in a Pye-Unicam SP-1800 recording spectrophotometer, against a Hepes buffer blank. Ordinate, absorbance at 500 nm of post-sedimentation samples; abscissa, concanavalin A concnetrations in presedimentation samples. Each point is the average of the three determinations, the bar representing the standard deviation.

mutants being more readily agglutinated at low concanavalin A concentrations. Data on two of the *petite* mutants are presented in Fig. 1 and compared with the parental strain; the contrast in agglutinability is evident. Also, to the naked eye, *petite* cells agglutinated much more rapidly than normal cells at various concentrations of concanavalin A. Other strains and their *petite* mutants showed similar changes in concanavalin A agglutination, the mitochondrial mutants being more readily agglutinable than their respective parental forms at appropriate concentrations of concanavalin A.

The conclusion may be drawn that cell surface changes detectable as alterations in concanavalin A agglutination result from mitochondrial defects such as occur in *petite* mutants.

Immiscible aqueous solutions of the polymers, poly(ethylene glycol) and Dextran, have recently been found suitable for separating cells with different surface properties such as normal and malignant leukocytes and in detecting subtle changes during differentiation and maturation [7]. Using such a biphasic system (see Table I), it was found that mitochondrial mutants distributed differently between the phases from parental cells, the relative affinities being strain dependent. *Petite* mutants of strains D6 and D4 showed a relative preference for the Dextran phase while *petite* cells of strains B21 and B/B tended to move into the poly(ethylene glycol) phase. These were stable features of these various cell lines and an attempt was made to determine the genetic basis of strain differences with respect to the effect of the *petite* condition on partitioning behaviour.

One complete tetrad from the cross D4 × B/B was analysed by isolating a *petite* mutant from each of the four spore cultures (following acriflavine treatment) [5] and testing both wild-type and mutant cells in the poly(ethylene glycol)/Dextran partition system. The results (Table II) indicated that

TABLE I

SEPARATION OF RESPIRATORY DEFICIENT (ρ^-) AND NORMAL CELLS OF YEAST STRAINS IN AN AQUEOUS BIPHASIC SYSTEM OF POLY(ETHYLENE GLYCOL) (PEG) AND DEXTRAN (D)

Solutions of poly(ethylene glycol) (M_r 6000, Koch-Light) and Dextran (T 500, Pharmacia Fine Chemicals) 9.2 and 10%, respectively, were made up in phosphate buffer (equimolar amounts of Na_2HPO_4 and NaH_2PO_4, total conc. 0.01 M). 4 ml of each was pipetted into a test tube to which cells (about 10^6 in each case) of parental and ρ^- mutant were added from 1-day-old cultures in 1% yeast extract, 2% glucose medium. All ρ^- mutants were induced by acriflavine. The mixture was shaken, aliquots (about 0.1 ml) removed and dilution plated on 1% yeast extract, 2% glucose medium (time zero). After standing for 20 min to allow poly(ethylene glycol) and Dextran phases to separate, aliquots were removed from each phase and dilution plated as for time zero. After 3 days incubation, *petite* colonies were scored on the basis of size, colour and inability to grow when transferred by velvet pad to non-fermentable (yeast extract, glycerol) medium. In tests with the D6/D6ρ^- mixture, the distribution of normal and ρ^- cells was unchanged when samples were taken from poly(ethylene glycol) and Dextran phases after standing for 1.5 h, showing that sedimentation of cells was not a significant factor. All experiments were repeated at least once and later results were not significantly different from those listed.

Cell mixture	% ρ^- colonies		
	Time zero	PEG phase	D phase
D6/D6ρ^{-1}	53 (90)*	4 (193)	89 (206)
B21/B21ρ^-	32 (198)	67 (258)	22 (393)
B-B/B-Bρ^-	48 (296)	77 (211)	21 (228)
D4/D4ρ^-	48 (113)	16 (108)	84 (105)

*Total colonies scored.

TABLE II

GENETIC ANALYSIS OF DIFFERENCES IN THE POLY(ETHYLENE GLYCOL)/DEXTRAN PARTITIONING PATTERNS OF *PETITE* MUTANTS OF STRAINS B-B AND D4 (SEE TABLE I)

A, B, C, D, cultures from four spores of a meiotic tetrad from the cross D4 (*a, arg*) × B-B (*α, his, try*). Aρ^- etc., respective *petite* mutants induced with acriflavine. All strains were pre-grown in 1% yeast extract, 2% glucose medium for 24 h and approx. $3 \cdot 10^6$ cells were inoculated into the poly(ethylene glycol)/Dextran mixture (see Table I). An aliquot of 0.1 ml was taken from the shaken mixture (time zero) and suspended in 9.8 ml water from which three aliquots of 0.02 ml were taken and plated on 1% yeast extract, 2% glucose medium. After phase separation (20 min), this procedure was repeated with aliquots from the poly(ethylene glycol) and Dextran phases. The aggregate of colonies from the three platings in each case gave 'Total colonies' PEG, poly(ethylene glycol); D, Dextran.

Culture	Total colonies		
	Time zero	PEG phase	D phase
A	181	171	190
B	123	98	99
C	203	214	182
D	156	112	141
Aρ^-	121	186	34
Bρ^-	92	49	153
Cρ^-	89	51	142
Dρ^-	105	140	73
D4	220	184	238
B-B	173	157	190

there was a 2:2 segregation for poly(ethylene glycol):Dextran preference among the four *petite* mutants, leading to the tentative conclusion that a nuclear gene difference in strains D4 and B/B was responsible for the partition patterns of their respective *petite* mutants. It may be noted (Table II) that normal cells of the tetrad showed little or no phase preference in the biphasic system.

The genetic evidence implicating nuclear genes parallels our previous findings that the extent of the mitochondrial control of sugar utilization also depends on the presence of certain genes in the nucleus [2, 3]. This point can also be made with regard to flocculation. The flocculent condition (tendency of cells to stick together and form clumps) is controlled by one or other of at least four nuclear genes [8]. We have reported that flocculation in the haploid laboratory strain B41 was overcome and cells grew normally following mitochondrial mutation to *petite* [4]. In an extension of these flocculation studies, a spontaneous flocculent mutant from strain B/A (B/A *flo*) was investigated. When crossed to the normal strain D6, tetrads segregated 2:2, flocculent:normal as expected, indicating control of flocculation by a nuclear gene. *Petite* mutants of B/A *flo* were non-flocculent in liquid culture and this altered phenotype was readily detectable in *petite* colonies on plates where they showed smooth morphology compared to the rough colony characteristic of the flocculent condition in B/A *flo* (Fig. 2). These findings support the previous conclusion that the activity of a nuclear gene specifying a cell surface characteristic (in this case cell adhesiveness) can be affected by the state of the mitochondrion.

In summary, the evidence clearly indicates that defective mitochondria can affect cell surface characteristics of yeast cells. In the case of concanavalin A agglutination, it is likely that mannoproteins are affected while phase

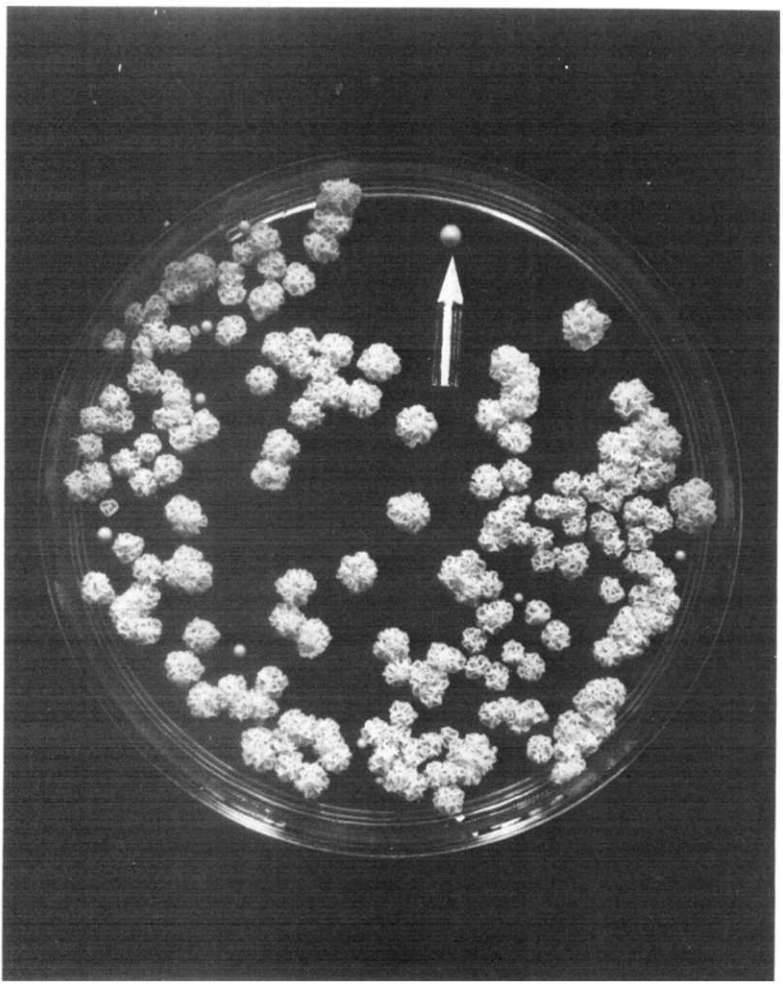

Fig. 2. Effect of *petite* mutation on flocculence. Colonies of strain B/A *flo* showing characteristic rough morphology due to cell clumping. *Petite* colonies (nine in number, arrows) are smooth and cells are not clumped. Cells were pretreated with benzidine which increased the *petite* mutation about 10-fold [4] and approx. 100 cells were plated on 1% yeast extract, 2% glucose medium. Since *Saccharomyces cerevisiae* is a facultative anaerobe, growth of *petite*, respiratory-deficient mutants takes place using ATP from glycolysis on the glucose medium. When glucose is depleted, *petite* cells stop growing (hence small colony) while respiratory normal colonies continue to grow by respiration.

partition behaviour relates to electrokinetic properties, as may also flocculence. The mechanism of the mitochondrial effects is unknown but it seems unlikely to be due to lack of mitochondrially synthesized ATP: respiratory-deficient cells were cultured in a glucose medium during testing providing adequate energy at least for growth and division, by glycolysis. Also *petites*, being all equally respiratory deficient, would be expected to show similar cell surface changes and be insensitive to nucelar genetic background if ATP supply alone were the crucial factor. The situation is reminiscent of our findings on mitochondrial involvement in the uptake and utilization of certain fermentable sugars where *petites* varied in their impairment in sugar utilization. Interestingly, inability of a *petite* to be induced for a sugar-degrading enzyme could be overcome by Me_2SO treatment [3] which makes the plas-

ma membrane permeable. This suggests mitochondrial involvement at a level other than mere provision of ATP, and that the organelle regulates, at least to some extent, the permeation characteristics of the cell.

We propose that mitochondria function in controlling the activity of certain genes or their products. Presumably, in the case of cell surface effects, these nuclear genes function directly or indirectly in plasma membrane/cell wall biogenesis. Differentiation of the inner membrane would be a prerequisite for normal gene function and cell membrane synthesis. Aberrations of the inner mitochondrial membrane which affect cell surface characteristics need not concomitantly affect respiration but would be expected to do so in most cases. Since mitochondrial damage appears to be the primary effect of many chemical carcinogens in yeast cells [4], extrapolation to mammalian cells would have implications for oncogenesis in which cell surface changes of the type described here play a crucial role. In view of the striking similarities of all mitochondria, yeast and human, even extending to idiosyncracies of the genetic code, extrapolation might seem justified. It is noteworthy that Soslau et al. [9] found alteration in plasma membrane glycoprotein in hamster cells following treatment in vitro with ethidium bromide, a highly selective inhibitor and mutagen of mitochondria.

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