DEREPRESSION OF MITOCHONDRIA IN YEAST SPHEROPLASTS

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SUMMARY: Spheroplasts of yeast (*Saccharomyces cerevisiae*) which had been grown in 10% w/v glucose showed respiratory derepression when placed in a non-fermenting medium. The derepressing spheroplasts were analyzed by electron microscopy and mitochondria obtained from the spheroplasts by gentle lysis were analyzed by rate-zonal and isopycnic centrifugation. As the respiratory rate of the spheroplasts increased, a new population of mitochondria appeared with the ultrastructure and sedimentation behavior characteristic of derepressed yeast. These smaller, less dense mitochondria appeared to replace the large, dense mitochondria characteristic of repressed yeast.

The ability of yeast to grow equally well by fermentation or oxidation has made it exceptionally suitable for the study of mitochondrial development (cf. Work et al., 1968; Roodyn and Wilkie, 1968). Much has been learned by following the appearance of mitochondrial enzymes as yeast changes from "repressed," largely fermentative metabolism, to "derepressed" oxidative metabolism. Intact mitochondria can be obtained from spheroplasts, but the long intervals required to digest

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away the tough cell walls of yeast have made it difficult to obtain intact mitochondria during repression or derepression.

The discovery by Hutchinson and Hartwell (1967) of a technique for producing spheroplasts that were still capable of synthesizing RNA led us to seek spheroplasts capable of respiratory derepression. The advantages of spheroplasts are that intact mitochondria can be obtained from them by rapid and gentle lysis.

We started with *Saccharomyces cerevisiae*, strain *Iso-N* (Avers et al., 1965) subcultured on a semi-synthetic medium (Nagai, 1959) containing 10% w/v glucose to repress respiration. The cells were harvested, suspended in M sorbitol, and spheroplasted with an extract of snail gut according to Fogel and Hurst (1963), and Hutchinson and Hartwell (1967).

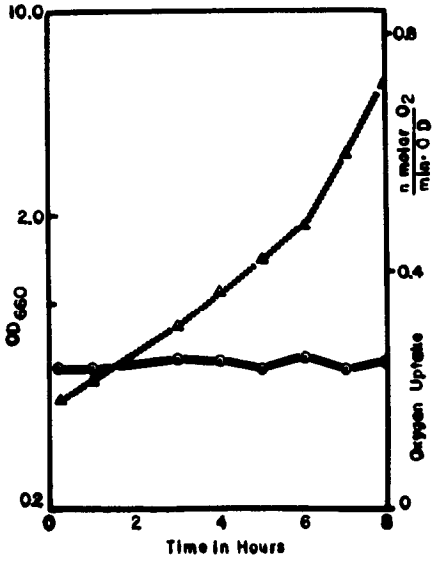

Figure 1. Respiration of spheroplasts from repressed cells placed in derepressing medium.

Specific respiratory rate (dotted line) rises continuously from initially repressed rate, while cell density (solid line) remains unchanged.

The spheroplasts were then resuspended in the growth medium, but with 2% v/v ethanol as a carbon source plus M sorbitol to prevent lysis. The spheroplasts were incubated with rotary shaking at 25° as during the initial culture of the cells. The respiration of aliquants of the spheroplast suspension was tested at intervals polarographically. That the spheroplasts do derepress is shown in Figure 1. In other experiments in our laboratory derepression of spheroplast reached 66% of that of fully derepressed cells.

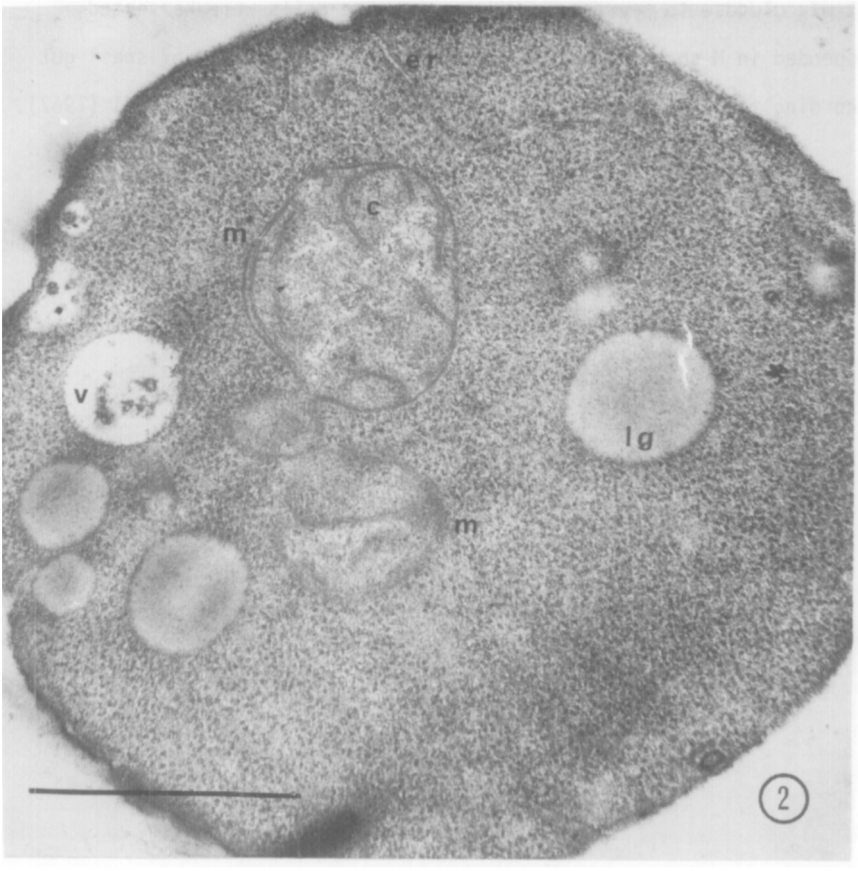

Figure 2. Ultrastructure of spheroplast of repressed yeast.

The spheroplasts were fixed in veronal acetate-buffered glutaraldehyde (pH 9), post-fixed with Palade's veronal acetate-buffered osmium tetraoxide (pH 7) and embedded in Epon. The few, large mitochondria (m) have

external double membranes, cristae (c), and contain a few particles of ribosomal size and short DNA-like fibrils. Other structures are endoplasmic reticulum (er), vacuoles (v), and lipoid granules (lg).

The reverse process, repression of derepressed spheroplasts, can also be demonstrated, but because derepressed yeast cells are more resistant to enzymatic digestion of their cell walls, this system is less convenient to use.

The morphological changes in derepressing spheroplasts can be seen by electron microscopy (Figs. 2 and 3). The repressed spheroplasts contain a few large mitochondria and little endoplasmic reticulum. The spheroplasts themselves are small. Derepressed spheroplasts are larger, and contain numerous mitochondria and more endoplasmic reticulum. These same morphological characteristics are seen in derepressed whole cells (Neal, 1969; Neal et al., in preparation).

We expected that the different sizes of mitochondria should be resolvable *in vitro* by differences in their sedimentation behavior.

Mitochondria were liberated from spheroplasts by lysis in 0.25 M sorbitol, 0.1 mM EDTA, and 0.65 mM potassium phosphate, pH 6.5, followed by passage through a Logeman hand mill. A crude mitochondrial suspension was obtained from the cell brei by clarification for 5 minutes at 2000 g followed by sedimentation for 9 minutes at 5000 g. The resuspended particles were then taken for zonal centrifugation (Price, 1970). For isopycnic separations, 400 ml of a gradient of 40-60% w/w sorbitol and 0.1 mM EDTA, pH 6.5, were led into a B-XIV rotor; 20 ml of sample and 200 ml of overlay were added and spun at 30,000 rpm for 30 minutes in the IEC B-35. Identical results were obtained after centrifugation at 35,000 rpm for 60 minutes. For rate zonal centrifugations, the gradient was 600 ml of 0-55% w/w sorbitol and 0.1 mM EDTA, pH 6.5, in a Z-15 rotor; 20 ml of sample were used and the sample was pumped to a starting radius of 6 cm. The rotor was spun at 8000 rpm for 15 minutes in the IEC B-20.

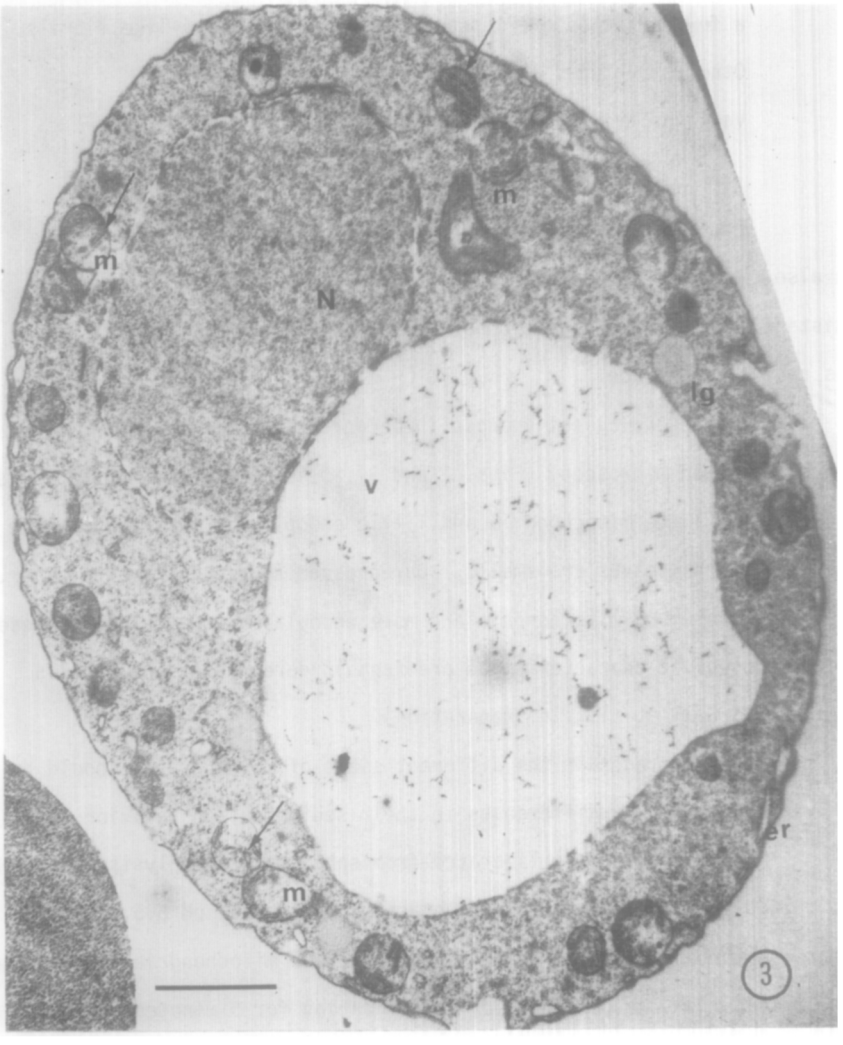

Figure 3. Ultrastructure of spheroplast during derepression.
The spheroplasts were treated and the structures marked as in Fig. 2. Note that the mitochondria are more numerous, smaller, and are distributed near the periphery. (m) mitochondria; cristae (at arrows); (N) nucleus; (v) vacuole.

Sedimentation profiles of mitochondria taken at several stages during derepression are shown in Figures 4 and 5. Mitochondria from

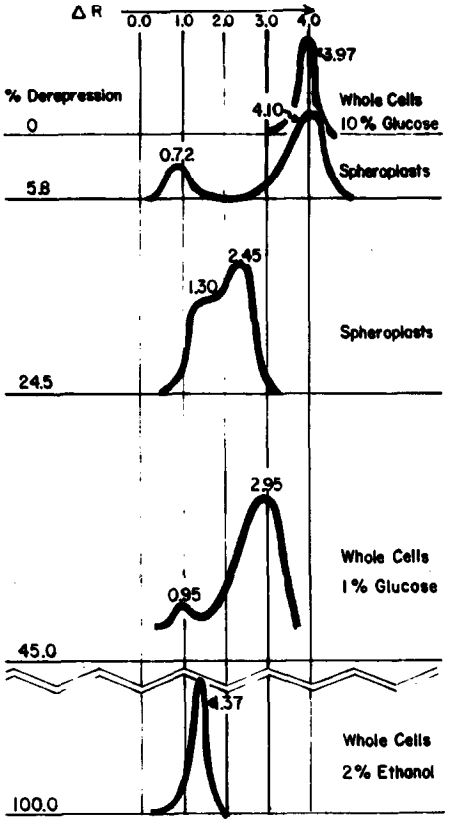

Figure 4. Rate-sedimentation profiles of mitochondria from yeast spheroplasts harvested during derepression. The distance migrated by the center of the mitochondrial band is shown in cm. Sedimentation was under standard conditions in the Z-15 zonal rotor (see text).

Note that repressed mitochondria sediment statistically farther into the gradient than derepressed mitochondria. Intermediate stages show two classes of mitochondria corresponding to repressed and derepressed particles. Variations within classes were not significantly different. The bottom curve shows for comparison the sedimentation of mitochondria from fully derepressed cells.

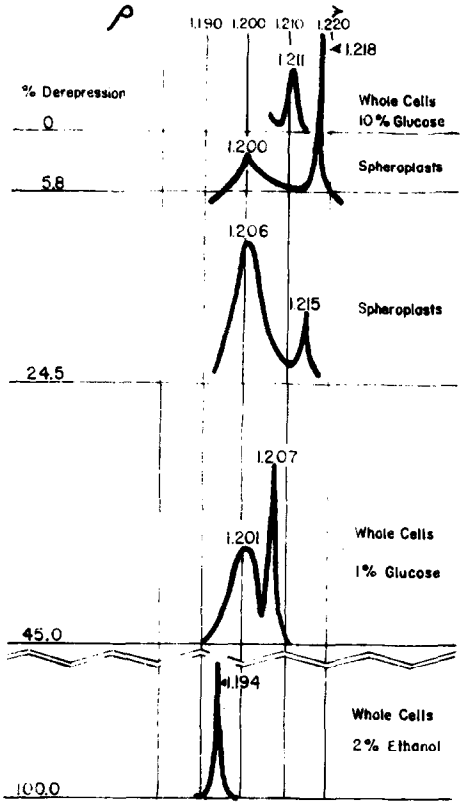

Figure 5. Isopycnic-sedimentation profiles of mitochondria from yeast spheroplasts harvested during derepression.

Repressed mitochondria have greater ρ_{eq} values than derepressed mitochondria; spheroplasts at intermediate stages show two density classes corresponding to fully repressed and fully derepressed mitochondria. The bottom curve shows for comparison the sedimentation of mitochondria from fully derepressed cells.

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initially repressed spheroplasts migrated a mean distance of 3.97 cm under the standard conditions of rate-zonal centrifugation and to a density of 1.218 in sorbitol after isopycnic centrifugation. By comparison, mitochondria from spheroplasts of derepressed cells moved

1.37 cm and to a density of 1.194 in sorbitol. During intermediate stages of spheroplast derepression, a band of mitochondria characteristic of derepressed mitochondria appeared (1.30 cm, 1.206 $gm \cdot cm^{-3}$) while the band of repressed mitochondria seemed to decrease in quantity. We also found patterns of mixed mitochondrial populations from partially derepressed yeast grown in 1% w/w glucose (Figs. 4 and 5).

In conclusion, we find derepression of spheroplasts by the criteria of respiration, ultrastructure, and the sedimentation behavior of their mitochondria. Taken at face value, the sedimentation profiles are consistent with a model in which repressed mitochondria serve as precursors of derepressed mitochondria.

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