Plasma Membrane Ultrastructural Differences Between the Exponential and Stationary Phases of *Saccharomyces cerevisiae* as Revealed by Freeze-Etching

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SUMMARY

Ultrastructural changes in the plasma membrane of *Saccharomyces cerevisiae* during the exponential and stationary growth phases were studied by freeze-etching. In the exponential phase, plasma membrane-intercalated particles were distributed randomly. In the stationary phase, several areas of the plasma membrane showed a hexagonal arrangement of particles; these areas appeared to increase with the age of the culture. The polarity of the particles also changed partially: the E-face of the plasma membrane was only sparsely embedded with particles in exponential phase cells, but relatively densely embedded in stationary phase cells. Invaginations of the plasma membrane on the P-face were devoid of particles during both growth phases. Invaginations of the E-face were sparsely embedded with particles in exponential phase cells, but densely embedded with particles in stationary phase cells.

INTRODUCTION

Use of the freeze-etching technique has revealed two structural components of plasma membranes: protein particles intercalated and randomly distributed in the lipid bilayer continuum (Moor & Mühlethaler, 1963; Branton, 1966, 1971). Little has been reported on the conformation and arrangement of the lipid molecules, but detailed knowledge of physiological importance has been accumulated through observation of the particles. (i) The density of the particles correlates qualitatively with the metabolic activities of certain types of cells (Scott, Carter & Kidwell, 1971; Scott & Marchesi, 1972). (ii) Specialized parts of the plasma membrane, for example ciliary membrane in *Tetrahymena*, may show specialized shapes and/or distribution pattern of the particles (Sattler & Staehelin, 1974). (iii) The distribution pattern of the particles may change with the physiological state of the cells, for example, vasopressin-induced changes in the bladder membrane (Kachadorian, Wade & DiScala, 1975), mucocyst secretion in *Tetrahymena* (Satir, Schooley & Satir, 1973), and contact-inhibition-induced aggregation of the particles in cultured mammalian cells (Scott, Furcht & Kersey, 1973; Furcht & Scott, 1974, 1975), though the last phenomenon was not confirmed (Pinto da Silva & Martinez-Palomo, 1975).

Ultrastructural features of the plasma membrane of yeasts have been studied in detail by means of freeze-etching (Moor & Mühlethaler, 1963; Streiblová, 1968; Matile, Moor & Robinow, 1969; Nečas, Kopecká & Brichta, 1969; Bauer, 1970; Takeo *et al.*, 1973). No ultrastructural changes have been reported in the plasma membrane lipids, or the particles,

during the different growth phases of yeasts, though the number of invaginations increases with the age of the cell.

This paper describes differences in the distribution patterns and polarities of the plasma membrane particles in rapidly growing and stationary phase *Saccharomyces cerevisiae*.

METHODS

Organism and growth conditions. *Saccharomyces cerevisiae* IFO1346 was used for all experiments except that shown in Fig. 4. The organism was grown, with shaking, in Sabouraud medium [4 % (w/v) glucose, 1 % (w/v) polypeptone] supplemented with 0.2 % yeast extract, usually at 28 °C but sometimes at 31 °C. Pre-culture medium (2 ml) which had been incubated overnight was inoculated into a culture flask containing 100 ml fresh medium. Growth was followed turbidimetrically at 610 nm. Rapidly growing cells ($E_{610} \sim 3$) were used as exponential phase cells. Cultures with an E_{610} of 6 to 9 were used as late exponential phase and early stationary phase cells. Stationary phase cells were obtained by incubating cultures for 1 to 9 days.

A mixed population of cells was prepared by mixing stationary phase cells, grown for 3 days, harvested, washed twice with fresh medium, and resuspended in fresh medium ($E_{610} = 2.9$), with an equal volume of culture medium in which cells were rapidly growing ($E_{610} = 2.8$). This mixture was incubated for 15 min.

In one experiment, the pH of the medium (initially at pH 6.3) in which stationary phase cells had been grown for 3.5 days was adjusted, by adding 1 M-NaOH at 15 min intervals during the last 1.5 h of incubation, to maintain the pH at 5.9 ± 0.1 , this being the pH of the medium during exponential growth.

Preparation of samples for electron microscopy. The yeast was collected by centrifuging (1000 g; 3 min; room temperature) and fixed with 2.5 % (w/v) glutaraldehyde in 0.07 M-phosphate buffer pH 7.2 for about 2 h at room temperature. Usually, the yeast was further fixed at 4 °C for a few days. Before examination, the cells were immersed overnight at 4 °C in 30 % (v/v) glycerol in 0.07 M-phosphate buffer pH 7.2. Freeze-etching was done as described previously (Takeo *et al.*, 1973). The electron micrographs were printed in reverse to facilitate interpretation.

RESULTS AND DISCUSSION

The P-face (convex fracture face) of the plasma membrane from *Saccharomyces cerevisiae* grown to the exponential phase was densely embedded with particles of about 15 nm diam. at 3×10^3 to $4 \times 10^3 \mu\text{m}^{-2}$ (Fig. 1). These particles were randomly distributed across the entire face. Hexagonal arrangements of the particles were only occasionally observed under our experimental conditions. The few rod-like invaginations of the plasma membrane were short, shallow and devoid of particles. There were only a small number of particles embedded in the E-face (concave fracture face) of the plasma membrane of exponential phase cells (Fig. 2); these were scattered randomly at about $500 \mu\text{m}^{-2}$. The depressions in the plasma membrane lipids left after the particles had been splintered off to the P-side were difficult to observe. Also the invaginations of this fracture face were only sparsely embedded with particles.

The ultrastructural features of plasma membranes from *S. cerevisiae* grown to the stationary phase showed distinct differences from those of the exponential phase cells. Although the P-face of the plasma membrane of yeast cultured for 2 days was also densely embedded with particles (Fig. 3), there were several areas in which particles assumed a hexagonal arrangement, previously reported by Moor & Mühlethaler (1963). In most cases the hexagonal areas

Bar markers represent 1 μm .

Fig. 1. The P-face of the plasma membrane of *S. cerevisiae* in the exponential phase of growth showing randomly distributed particles (arrows) and invaginations (I) which are devoid of particles.

Fig. 2. The E-face of the plasma membrane of *S. cerevisiae* in the exponential phase of growth showing small numbers of particles scattered randomly (arrows). Note also that invaginations (I) are sparsely embedded with particles.

were slightly depressed. In an extreme case (Fig. 4) the inner part of the wall adjacent to the depressed and hexagonal areas of the membrane appeared smooth, while the outer part of the wall had a particulate appearance. This separation of the membrane in the hexagonal areas from the wall suggests that the hexagonal areas do not have any special affinity with the wall, and that the direct factor or force which produces the hexagonal arrangement of the particles may be in the plasma membrane. In the rest of the plasma membrane the particles were still randomly distributed. The density of the particles was slightly greater (about 1.5-fold) in the hexagonal areas than in the other areas, and, as a whole, nearly equalled that in exponential phase cells. The number of invaginations increased and they were deep and somewhat irregular in shape; they were devoid of particles as was the case in exponential phase cells. In old cultures some invaginations were very long.

The E-face of the plasma membrane from stationary phase cells was more densely embedded with particles (about $1.3 \times 10^3 \mu\text{m}^{-2}$) than that from exponential phase cells (compare Figs 5 and 2), but less so than the P-face. Furthermore the hexagonal pattern made up by these particles was seen to have many particles absent from the matrix, indicating that more than half of them had split off to the P-side of the plasma membrane during fracturing. The hexagonal areas on the E-face were in most cases slightly raised, corresponding to the depression of these areas on the P-face. There was no significant increase in the density of particles found on the E-face of the plasma membrane in cells incubated for between 1 and 4 days, but after incubation for 9 days there was a distinct decrease in the density of particles on the E-face. However, the areas of hexagonal arrangement of the particles appeared to increase with culture age. Thus, the polarity of the particles seems to change independently from their distribution pattern. The viability of the cells was also checked: most were viable in cultures incubated for up to 9 days. The polarity of the plasma membrane particles of non-fixed endothelial cells has been reported to be opposite to that of the normal cells, but that it becomes normal on fixation (Dempsey & Bullivant, 1973). In *S. cerevisiae*, the change in polarity may be a response to the different growth phases, but it could also be due to other unknown factors.

The distribution pattern of the plasma membrane particles was also studied in late exponential and early stationary phase cells. Some cells scarcely showed the hexagonal arrangement of the particles; others revealed several areas where the particles took on the hexagonal arrangement, though each area was smaller than those seen in plasma membranes of stationary phase cells. This may suggest that the difference in the plasma membrane features between the exponential and stationary phases is not due to subtle differences in the preparation of samples.

The change in the distribution pattern of particles seems to resemble, from the physiological point of view, that of cultured mammalian cells; the plasma membrane particles are

Bar markers represent 1 μm .

Fig. 3. The P-face of the plasma membrane of *S. cerevisiae* in the stationary phase of growth (2-day culture) showing several areas where particles are in a hexagonal arrangement (arrows). The hexagonal areas are usually slightly depressed. Invaginations (I) are devoid of particles.

Fig. 4. *Saccharomycodes ludwigii* plasma membrane showing an extreme case of a separation of the plasma membrane from the wall (W). The separation is distinct where particles are in a hexagonal arrangement (arrows); this is also observed, but to a lesser extent, in *S. cerevisiae*.

Fig. 5. The E-face of the plasma membrane of *S. cerevisiae* in the stationary phase of growth (2-day culture). Particles are much denser than in the exponential phase cells (compare with Fig. 2). Note also several slightly raised areas where particles are in a hexagonal arrangement (arrows). Invaginations (I) are densely embedded with particles.

randomly distributed when the cells are growing but are aggregated when the cells are contact-inhibited. These phenomena were first reported by Scott *et al.* (1973) and again by Furcht & Scott (1974, 1975) but were not confirmed by Pinto da Silva & Martinez-Palomo (1975). Thus subtle experimental changes may cause distinct differences in results. To minimize such changes exponential and stationary phase cultures were prepared in pairs, and the cells were collected and fixed simultaneously. In addition, stationary phase cells were mixed with the exponential phase cells, and cultured for 15 min. In both cases, the same two types of membrane as hitherto described were recognized, although in the latter case the areas showing the hexagonal arrangement of the particles had decreased considerably.

Several treatments have been reported to cause aggregation or redistribution of plasma membrane particles. Some seem to be involved in a specific cell type, such as the particle redistribution in the bladder membrane elicited by vasopressin (Kachadorian *et al.*, 1975). Others are more physicochemical treatments: lowering the pH (Pinto da Silva, 1972); lowering the temperature (Haest *et al.*, 1974); cryoprotectant treatment (McIntyre, Gilula & Karnovsky, 1974); and incubation in phosphate-buffered saline (Pinto da Silva & Martinez-Palomo, 1975). In view of these observations, the fall in the pH of the stationary phase culture medium might possibly cause the change in distribution pattern observed in *S. cerevisiae* during the different growth phases. However, this possibility is considered unlikely because the typical hexagonal arrangement of particles in plasma membranes from stationary phase cells was still seen when the stationary phase cells were held in the same medium as the exponential phase cells (see above), and when the pH of the medium containing the stationary phase cells was adjusted to pH 5.9 (the pH of the medium during exponential growth). Thus the pH change of the external medium is probably not a principle cause of the hexagonal distribution of particles though the fall in the pH inside the stationary phase cells might be a cause.

A characteristic feature of the yeast plasma membrane is the existence of invaginations, but their physiological role is not clear. Nečas *et al.* (1969) showed that the invaginations on the E-face were densely particulate and suggested that the particles were preferentially bound to the P-face of the entire plasma membrane except in the invaginations where the polarity of the particles was reversed. Our results show that this is true only of cells which are in the stationary phase. In exponential phase cells, the invaginations are almost entirely devoid of particles, on both the P- and E-faces. As the plasma membrane lipids are a fluid in which the particles may move relatively freely (Singer & Nicolson, 1972), the invaginations probably have a special mechanism which inhibits the entrance of the particles, e.g. by accumulation of certain kinds of lipids which solidify at the growth temperature and squeeze out the protein particles. As protein in the membrane seems to exist predominantly in the form of particles, the metabolic activities in the invaginations in exponential phase cells are expected to be low.

The density of the plasma membrane-intercalated particles in stationary phase *S. cerevisiae* is no less than that in exponential phase cells; Bauer (1969) has reported similar results in *Wickerhamia fluorescens*. No correlation of particle density and metabolic activities was observed in these yeasts although it has been reported in phytohaemagglutinin-stimulated lymphocytes (Scott & Marchesi, 1972). Other methods have suggested that the activities of the plasma membrane in stationary phase *S. cerevisiae* are inhibited. The hexagonal arrangement of the particles may be a reflection of the inhibitory mechanism of the plasma membrane activities (see also Takeo, 1976).

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REFERENCES

- BAUER, H. (1970). A freeze-etch study of membranes in the yeast *Wickerhamia fluorescens*. *Canadian Journal of Microbiology* **16**, 219–222.
- BRANTON, D. (1966). Fracture faces of frozen membranes. *Proceedings of the National Academy of Sciences of the United States of America* **55**, 1048–1056.
- BRANTON, D. (1971). Freeze-etching studies of membrane structure. *Philosophical Transactions of the Royal Society of London B* **261**, 133–138.
- DEMPSEY, G. P. & BULLIVANT, S. (1973). Endothelial cell membrane: polarity of particles as seen by freeze-fracturing. *Science* **179**, 190–192.
- FURCHT, L. T. & SCOTT, R. E. (1974). Influence of cell cycle and cell movement on the distribution of inter-membranous particles in contact-inhibited and transformed cells. *Experimental Cell Research* **88**, 311–318.
- FURCHT, L. T. & SCOTT, R. E. (1975). Modulation of the distribution of plasma membrane intramembranous particles in contact-inhibited and transformed cells. *Biochimica et biophysica acta* **401**, 213–220.
- HAEST, C. W. M., VERKLEIJ, A. J., DE GIER, J., SCHEEK, R., VERVERGAERT, P. H. J. & VAN DEENEN, L. L. M. (1974). The effect of lipid phase transitions on the architecture of bacterial membranes. *Biochimica et biophysica acta* **356**, 17–26.
- KACHADORIAN, W. A., WADE, J. B. & DiSCALA, V. A. (1975). Vasopressin-induced structural change in toad bladder luminal membrane. *Science* **190**, 67–69.
- MCINTYRE, J. A., GILULA, N. B. & KARNOVSKY, M. J. (1974). Cryoprotectant-induced redistribution of intramembranous particles in mouse lymphocytes. *Journal of Cell Biology* **60**, 192–203.
- MATILE, PH., MOOR, H. & ROBINOW, C. F. (1969). Yeast cytology. In *The Yeasts*, vol. 1, pp. 219–302. Edited by A. H. Rose and J. S. Harrison. London and New York: Academic Press.
- MOOR, H. & MÜHLETHALER, K. (1963). Fine structure in frozen-etched yeast cells. *Journal of Cell Biology* **17**, 609–628.
- NEČAS, O., KOPECKÁ, M. & BRICHTA, J. (1969). Interpretation of surface structures in frozen-etched protoplasts of yeasts. *Experimental Cell Research* **58**, 411–419.
- PINTO DA SILVA, P. (1972). Translational mobility of the membrane intercalated particles of human erythrocyte ghosts. pH-dependent, reversible aggregation. *Journal of Cell Biology* **53**, 777–787.
- PINTO DA SILVA, P. & MARTINEZ-PALOMO, A. (1975). Distribution of membrane particles and gap junctions in normal and transformed 3T3 cells studied *in situ*, in suspension, and treated with concanavalin A. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 572–576.
- SATIR, B., SCHOOLEY, C. & SATIR, P. (1973). Membrane fusion in a model system. Mucocyst secretion in *Tetrahymena*. *Journal of Cell Biology* **56**, 153–176.
- SATTler, C. A. & STAEHELIN, L. A. (1974). Ciliary membrane differentiations in *Tetrahymena pyriformis*. *Tetrahymena* has four types of cilia. *Journal of Cell Biology* **62**, 473–490.
- SCOTT, R. E. & MARCHESI, V. T. (1972). Structural changes in membranes of transformed lymphocytes demonstrated by freeze-etching. *Cellular Immunology* **3**, 301–317.
- SCOTT, R. E., CARTER, R. L. & KIDWELL, W. R. (1971). Structural changes in membranes of synchronized cells demonstrated by freeze-cleavage. *Nature New Biology* **233**, 219–220.
- SCOTT, R. E., FURCHT, L. T. & KERSEY, J. H. (1973). Changes in membrane structure associated with cell contact. *Proceedings of the National Academy of Sciences of the United States of America* **70**, 3631–3635.
- SINGER, S. J. & NICOLSON, G. L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720–731.
- STREIBLOVÁ, E. (1968). Surface structure of yeast protoplasts. *Journal of Bacteriology* **95**, 700–707.
- TAKEO, K. (1976). Ultrastructural features underlying the hexagonal arrangement of plasma membrane-intercalated particles of *Saccharomyces cerevisiae*. *Journal of General Microbiology* **97**, 331–334.
- TAKEO, K., UESAKA, I., UEHIRA, K. & NISHIURA, M. (1973). Fine structure of *Cryptococcus neoformans* grown *in vitro* as observed by freeze-etching. *Journal of Bacteriology* **113**, 1442–1448.