GROOVES IN THE PLASMALEMMA OF *SACCHAROMYCES CEREVISIAE* SEEN IN GLANCING SECTIONS OF DOUBLE ALDEHYDE-FIXED CELLS

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INTRODUCTION

Grooves on the surface of the plasmalemma-(plasma membrane) of frozen-etched yeast cells have been shown by many investigators (1, 10, 12). Nečas et al. (13) showed interconnected grooves on the surface of frozen-etched *Saccharomyces cerevisiae* protoplasts, but such structures could not be so effectively demonstrated in frozen-etched protoplasts prefixed in glutaraldehyde.

Structures comparable to these grooves have not been demonstrated in thin sections of chemically fixed whole cells of yeast. Darling et al. (3) reported cleft-like invaginations in tangential sections of *S. cerevisiae* protoplasts. Ludvik et al. (8) showed deep indentations in cross-sections of plasmalemma of *Candida lipolytica* grown on hydrocarbon.

In this note I am presenting my observation that by a double aldehyde fixation procedure after Karnovsky (6), numerous grooves can be demonstrated in the plasma membrane of the stationary phase cells of *S. cerevisiae*.

MATERIALS AND METHODS

Culture and Medium

SACCHAROMYCES CEREVISIAE strain 1829, obtained from W. J. Nickerson, was used in this investigation. Cells were maintained on a medium containing Difco yeast extract 1%, glucose 1%, and Bacto agar 2% (Bacto Bacteriological Products, Difco Labs, Detroit, Mich.), and were grown from a small inoculum at 28°C for 7 days with shaking in the same medium devoid of agar. At the end of this period the culture consisted primarily of nonbudding single cells.

Fixation and Embedding

Poor penetration of fixative into the yeast cell presents a problem regarding their chemical fixation. Thus adequate fixation with glutaraldehyde and osmium tetroxide could not be obtained (17) unless the cell wall is removed by mechanical treatment before (2), or by enzymatic treatment after, glutaraldehyde fixation (15). In the method described below, an attempt is made to overcome the problem of slow permeation of glutaraldehyde into the intact cell by the use of a mixture of formaldehyde and glutaraldehyde. The former is expected to enter rapidly into the cell and partially stabilize the structures which will later be further stabilized by extensive dialdehyde cross-linking.

Formaldehyde produced by the dissociation of paraformaldehyde is superior to commercially available formaldehyde since the latter generally contains methanol and formic acid. Sodium chloride was included in the 30–50% ethanolic rinsing solutions in order to minimize the extraction of protein from the cells (11). After double aldehyde prefixation, cells must be thoroughly washed before they are fixed with osmium tetroxide, in order to avoid reduction of the osmium tetroxide by the residual aldehyde.

The cells from the growth medium were harvested by centrifugation, washed twice in 0.1 M phosphate buffer pH 7.3 containing 0.09 M sodium chloride (phosphate-buffered saline [PBS] [5]), and fixed at room temperature (22°–24°C) in a double aldehyde fixative consisting of 2% (w/v) paraformaldehyde (Matheson, Coleman and Bell, East Rutherford, N.J.) and 2.5% (w/v) glutaraldehyde (Ladd Research Industries, Inc., Burlington, Vt.) in PBS. The fixed cells were washed twice with PBS, twice with Veronal-acetate buffer, pH 6.2–6.4 (7), and embedded in 2% (w/v) Noble agar. Small (*circa* 1 mm^2) blocks were treated with osmium tetroxide fixative (7) at room temperature in the dark (to reduce photodegradation of OsO_4). After 12–15 hr of fixation the blocks were treated for 2 hr with 0.5% uranyl acetate in Veronal-acetate buffer (7), rinsed with Veronal-acetate buffer once, with 30% ethanol containing 0.02 M sodium chloride (15 min, two changes), and with 50% ethanol containing 0.02 M sodium chloride (15 min, two changes). The rinsed blocks were dehydrated in 70% and 95% aqueous ethanol, absolute ethanol, and finally in propylene oxide. The dehydrated blocks were infiltrated with 1:1 (v/v) propylene oxide:Epon mixture (9) for 8 hr with shaking. Subsequently the blocks were washed with fresh Epon mixture, placed in BEEM capsules containing the same Epon mixture, and polymerized by heating at 37°C for 24 hr, at 45°C for 24 hr, and at 60°C for 48 hr.

Thin sections (gray to colorless) were made in a Porter-Blum Sorvall MT1 microtome (Ivan Sorvall, Inc., Norwalk, Conn.) and were stained in Reynolds'

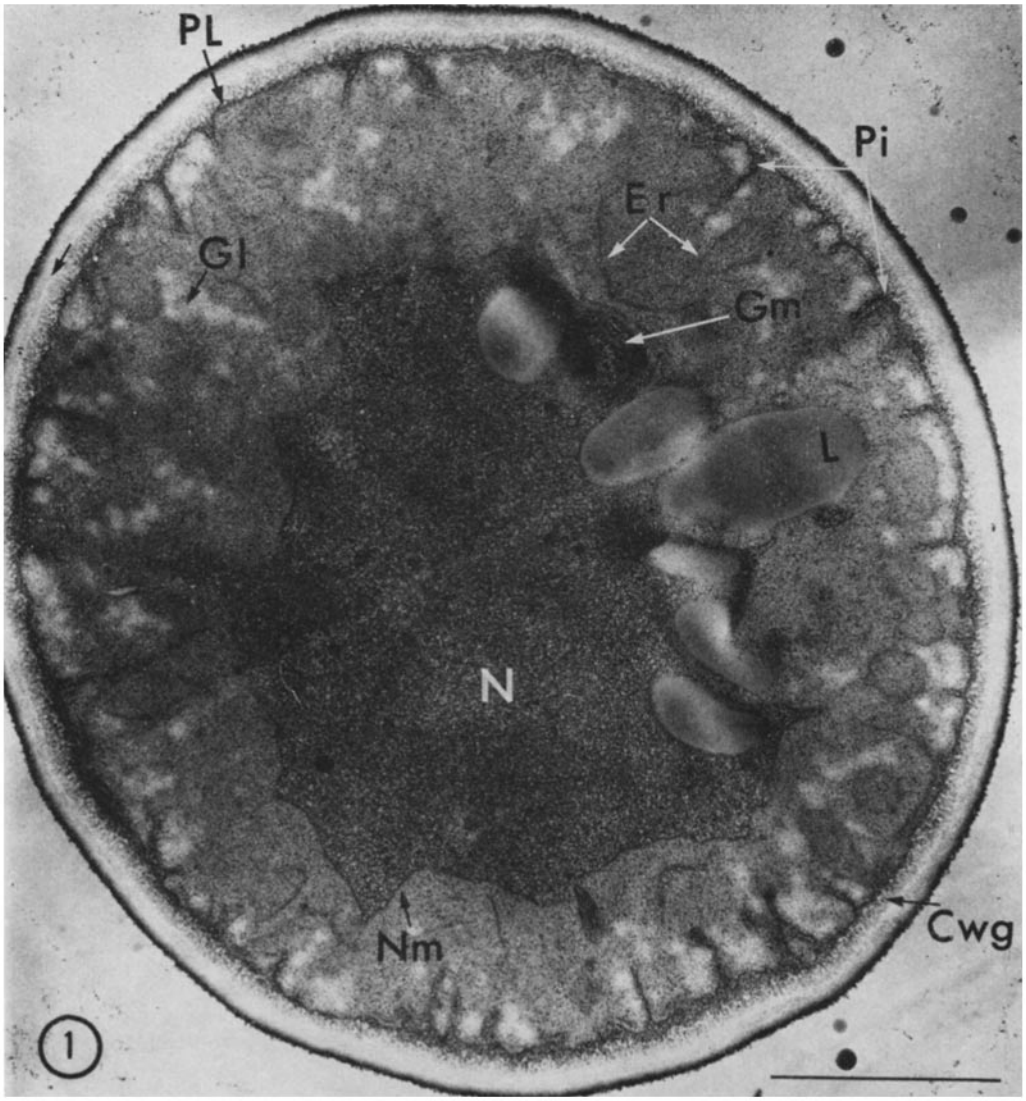

FIGURE 1 Cells fixed as described in the Methods section. The structures seen are plasmalemma (*PL*), plasmalemmal invagination (*Pi*), globular inner layer of cell wall (*CWg*); note the line of separation between inner and outer layer of cell wall (arrow), Golgi-like stack of membrane (*Gm*), endoplasmic reticulum (*Er*), glycogen deposit (*Gl*), lipid deposits (*L*), nucleus (*N*), and nuclear membrane (*Nm*). Marker Fig. 1, 1.0 μ; Figs. 2–4, 0.2 μ. × 30,000.

(14) lead citrate for 30 min. Thoroughly washed and air-dried grids were examined under JEM 120 electron microscope (JEOLCO U. S. A. Inc., Medford, Mass.) at 80 kv.

OBSERVATIONS AND DISCUSSION

Fig. 1 is a thin section showing the general morphology of a stationary phase cell. The cell has lipid granules (*L*) many, glycogen deposits (*Gl*), a well-defined nucleus (*N*), nuclear membrane (*Nm*), endoplasmic reticulum (*Er*), a Golgi-like stack of membranes (*Gm*), and globular inner layer of cell wall (*Cwg*). The membrane structure is well preserved. The plasmalemma (*PL*) has a generally expanded appearance (i.e., has very little wavy contour), but there are many narrow (200–

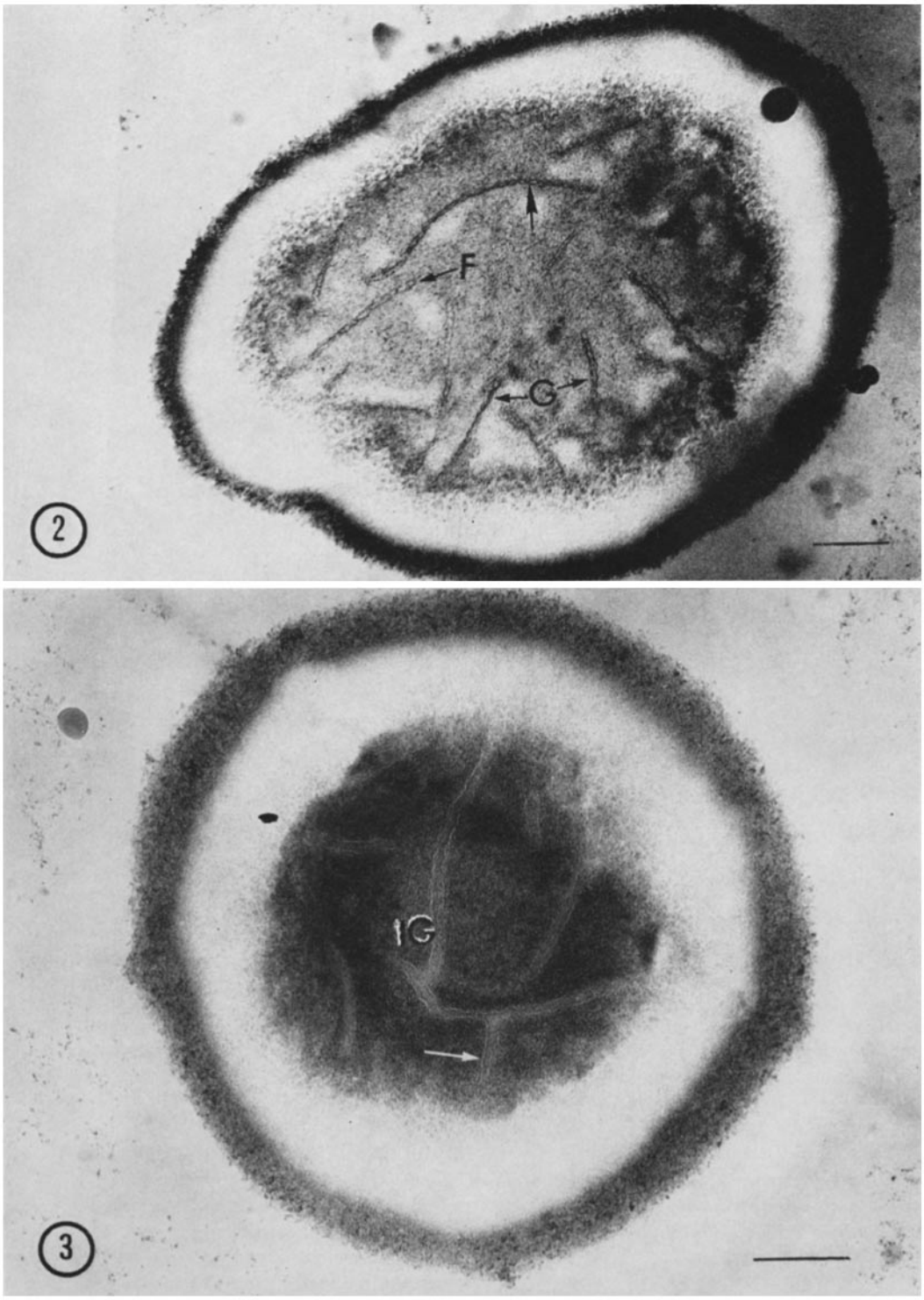

FIGURE 2 A Glancing section showing randomly distributed isolated free-end grooves (*G*) which contain many fibrils (*F*); note periodic constriction of grooves (arrows), wide cell wall and smeared appearance of plasmalemma. × 54,000.

FIGURE 3 A glancing section through a region close to the cell wall plasmalemmal interface showing interconnected grooves (*IG*); note clearly defined triple layered membrane of the grooves (arrow). Cell wall is very wide and plasmalemma has a smeared appearance. × 68,000.

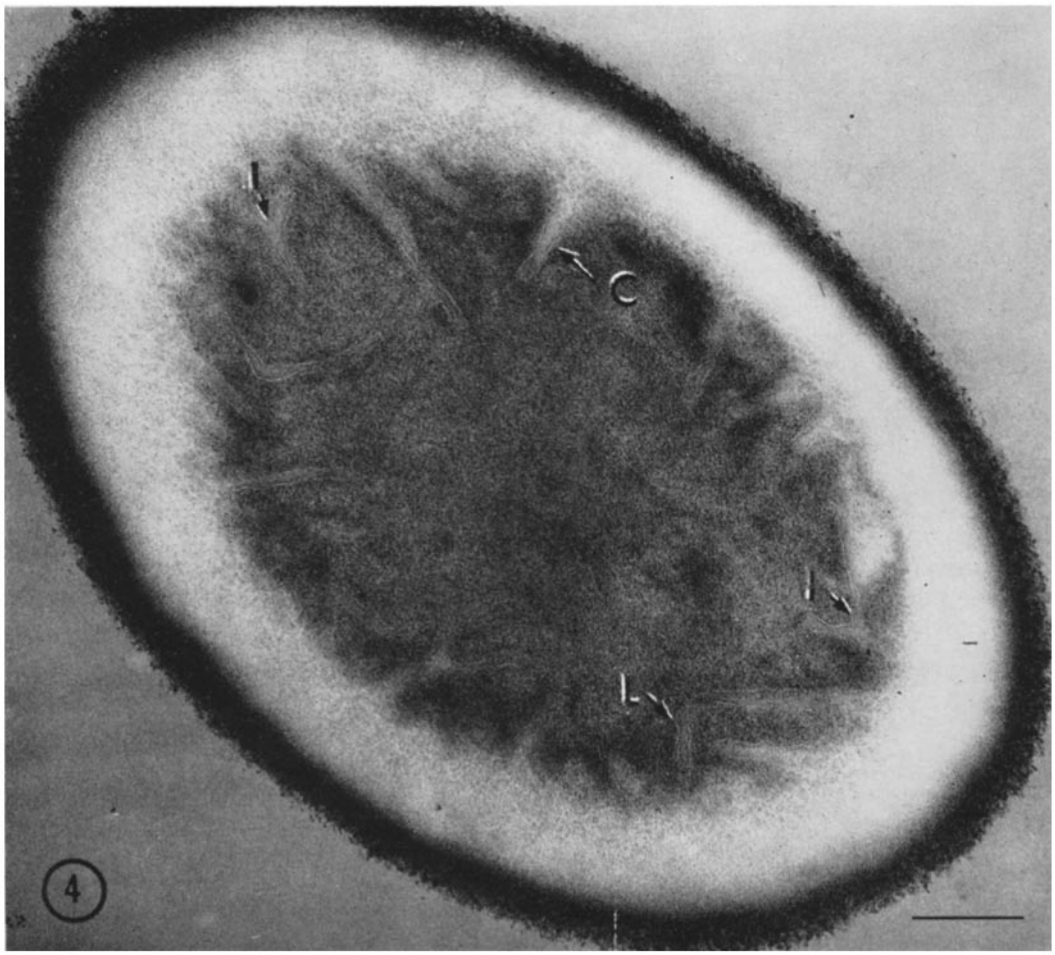

FIGURE 4 A glancing section (cutting plane passing through a deeper part of cytoplasm relative to Figs. 2 and 3) showing both cross (*C*)- and longitudinal-section (*L*) through grooves in the peripheral region of cytoplasm; note the interconnection of grooves (*I*) and absence of grooves in the central part of section. × 70,000.

300 A in diameter invaginations (*Pi*) extending maximally 1000 A into the cytoplasm. These invaginations seem to be different from the large ingrowths of plasmalemma seen in the thin sections of young cells of *S. cerevisiae* (reference 16; B. K. Ghosh, 1970, unpublished results) and *Cryptococcus neoformans* (4).

Figs. 2 and 3 are glancing sections showing the grooves of the plasmalemma. When the invaginations seen in Fig. 1 are sectioned in longitudinal directions in glancing sections they appear as the tubules seen in these figures. The three-dimensional reconstruction drawing shown in Fig. 5 clearly illustrates the identity of plasmalemmal grooves in glancing sections (Fig. 5, *A*, B_1, B_2) and in cross-sections (Fig. 5, *C*). The glancing sections can expose either a relatively deeper part of cytoplasm (Fig. 2 and Fig. 5, B_1 and B_2) or a superficial part of the cytoplasm close to plasmalemma cell wall interface (Fig. 3 and Fig. 5, *A*). This can be judged from the relative cytoplasmic and cell wall areas of the sections. The cell wall appears increasingly thicker as the sectioning plane approaches the plasmalemma–cell wall interface. The grooves are frequently interconnected in these superficial glancing sections (Fig. 3, *IG*), whereas

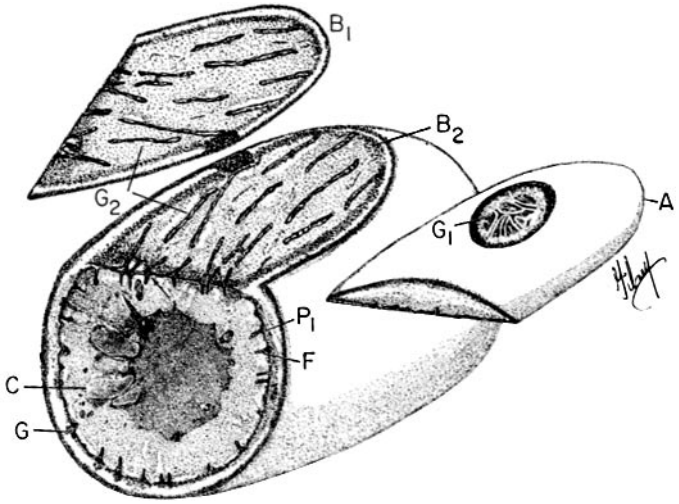

FIGURE 5 Three-dimensional reconstituted diagram; *A*, plane of section removing a small portion of cytoplasm showing interconnected grooves (G_1); B_1 and B_2 plane of section exposing deeper portion of cytoplasm relative to *A*; the turned-over bottom portion (B_1) and the exposed cytoplasm (B_2) show the narrow grooves cut along their longitudinal axis (G_2); *C*, plane of section pass through much deeper portion of cytoplasm and reveal cellular organelles; note the cross-section of invagination (P_1) and grooves are same structure (arrow); *G*, globular material of cell wall; *F*, fibrils in the invaginations.

sections through the relatively deeper cytoplasmic regions have grooves separated from each other (Fig. 2, *G*). These grooves sometimes show periodic constriction (Fig. 2, arrow) and fibrils inside (Fig. 2, *F*).

Fig. 4 is another glancing section, but here the plane of the section passes through a relatively deeper region of cytoplasm compared to Figs. 2 and 3. Thus the grooves escape sectioning in the central region of the section (Fig. 4). In these sections the grooves can be seen in the peripheral region of the cytoplasm; some of these grooves have been cut across and other lengthwise (Fig. 4, *C* and *L*). In some places the latter appear connected (Fig. 4, *I*).

Fig. 5 is a three-dimensional reconstruction prepared on the basis of a large number of yeast cell sections made according to the described procedure. In this model emphasis has been given to the appearance of plasmalemmal invagination as revealed in various sectioning planes. The invaginations generally penetrate up to 1000 A into the cytoplasm. It is clear from the diagram that these invaginations in cross-sections (Fig. 5, *C*) and the apparent tubules in glancing sections (Fig. 5, *A*, B_1, and B_2) are parts of the same structure. Thus it can be concluded that there are long, narrow interconnected grooves on the stationary phase yeast cell plasmalemmal surface; but the grooves lose their interconnection deeper into the cytoplasm forming long isolated pockets.

Nečas et al. (13) showed the presence of interconnected grooves on the frozen-etched protoplast surface; but such structures were largely destroyed after glutaraldehyde fixation. The close similarity of structures seen by Nečas et al. and in this paper suggests that double aldehyde fixation is of value in preserving delicate membranous structures.

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