THE MACRONUCLEAR ENVELOPE OF *TETRAHYMENA PYRIFORMIS* GL IN DIFFERENT PHYSIOLOGICAL STATES

III. Appearance of Freeze-Etched Nuclear Pore Complexes

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The pore complexes of the nuclear envelope presumably regulate macromolecular exchange between the cytoplasm and the karyoplasm (7–9, 11, 14–16, 18, 22, 24–27, 30). We have previously presented quantitative data, obtained by negative staining (29, 32) and thin-sectioning, (31) on the number, size, and fine structure of nuclear pores in the macronuclear envelope of the ciliate protozoon *Tetrahymena pyriformis* GL in different physiological states. We now relate these results to those obtained by freeze-etch electron microscopy, a technique which allows observation of large areas of partially hydrated biomembranes. We also describe the influence of glutaraldehyde prefixation on the freeze-etch appearance of nuclear pores.

MATERIAL AND METHODS

Cultures

Stock cultures of *Tetrahymena pyriformis* (amicronucleate strain GL) were maintained axenically at 15°C in 2% proteose peptone containing 0.4% liver extract. We have examined cells of cultures in the logarithmic, as well as in the stationary, growth phase, and also those of heat-shock synchronized cultures at the end of the synchronization treatment (EHT) and at the division maximum (29, 30).

Freeze-Etching and Electron Microscopy

Cells in the four different physiological states were centrifugally concentrated into 2 ml of culture medium. Fixation, when performed, was done by addition of 2 ml of 3–4% glutaraldehyde, in 0.1 M cacodylate (pH 7.2).

After 15 min the cells were thoroughly washed with buffer, and transferred through buffered solutions of increasing glycerol concentration to a level of 20% glycerol. Unfixed cells were directly incubated in the glycerol solutions for 1–3 hr. Small samples of packed *Tetrahymena* cells were frozen on gold-alloy specimen holders in Freon 22 (E. I. duPont de Nemours & Co., Inc., Wilmington, Del.). The frozen specimens were replicated according to Moor and Mühlethaler (21) on a Balzers model BA 360 M freeze-etch device (Balzers A. G., Liechtenstein). The etching time was usually 1 min at −100°C. In some experiments, etching was minimized (see reference 28) by keeping the temperature of the specimen holder at −150°C and by replicating the freshly fractured surface in less than 5 sec after fracturing. Replicas were examined in a Siemens Elmiskop IA, the magnification of which was routinely calibrated by using a carbon grating replica.

Measurements

All measurements were made on calibrated positives. Pores per square micron were determined by direct counts on fracture faces with large areas of membrane. 100 pores of different macronuclei from

each of the four different physiological states of *Tetrahymena* cells were evaluated.

RESULTS AND DISCUSSION

Freeze-etch replicas show that the inner and outer nuclear membranes fuse together focally to form nuclear pores (Fig. 1). These pores are filled with granular and fibrillar material (Figs. 2, 4, 5, and 7–9). Without prefixation, the pore material is arranged more compactly, with fibrils often extending to adjacent areas of the nuclear membrane (Fig. 10, lower pore). However, occasional pores have a central globule and peripheral material arranged in a ring-like manner (Fig. 10, upper pore).

After fixation the pore material seems more granular (Figs. 4–9), and one often observes particles about 70–160 A in diameter arranged peripherally in a ring. They are occasionally arrayed in sets of eight within a distinct line surrounding the pore (Figs. 5, 6, and 9). This margin is often polygonal, and occasionally octagonal (Fig. 8) or circular. The "ground material" (21) in the interior of etched pores is made up of filamentous and granular structures about 30–70 A in diameter, often with a large globule 160 A in diameter at the very center (Figs. 6, 7, and 10). This structure is connected with the outer granules by filaments about 30 A in diameter (Figs. 7 and 9); in some cases the central globule appears caved in (Fig. 9).

The peripheral and central granules are seen infrequently in nonetched pores (Fig. 3), which often resemble Moor's "closed pores" (21) with their membrane-like surfaces. This indicates that etching is apparently necessary to reveal fine-structural details of nuclear pores.

The nuclear pore structures, described above as peripheral globules, central granules, and filaments, have been reported as annular subunits, central dots, and filaments, respectively, in air-dried (17, 18), freeze-substituted (13), shadowed (10, 14), and negatively stained, isolated nuclear envelopes (10, 14, 32, 33), as well as in thin-sectioned glutaraldehyde- and/or OsO_4-fixed material. No ordered pore structures have been described in the freeze-etch literature, although they are apparent in some published pictures (12, 23, 24). Since the granules are regularly observed in our freeze-etch preparations on the cytoplasmic as well as the nucleoplasmic faces of nuclear pores, particularly in prefixed material, we believe that these structures correspond to the annular material, central dot, and fibrils, revealed by other techniques of electron microscopy. Differences, however, in the appearance of annular material, i.e., pores with and without annular material covering the pore margin (32), as reported to occur in pores of negative stained, isolated nuclear envelopes, could not be revealed in pores of our freeze-etched preparations.

Different preparation techniques, however, reveal variations in pore dimensions, not only in various eukaryotic cell types, but also in the very same type. The mean diameters of freeze-etched pores of *Tetrahymena* cells in the four different physiological states studied differ less than those of negatively stained isolated macronuclear envelopes (29).

However, the diameters of freeze-etched pores (78 ± 5 nm; see Fig. 2) are significantly greater in all four physiological states than the diameters obtained by negative staining (66 ± 7 nm; see Fig. 12), and thin sectioning (67 ± 7 nm; see Fig. 11). A possible interpretation for this difference could be that the "true" pore margin is not exposed, i.e., the cleavage plane passes over that annular material which is located on and beyond the pore margin. In most cases, however, this seems to be unlikely because, according to recent pore models (1, 15, 24, 31), the annular material appears to lie largely within the pore in close association to the pore rim; that portion of annular material, extending over the pore rim on both sides of the nuclear envelope, is loosely attached to the nuclear envelope and should splinter away at cleavage.

An alternative interpretation might be that the pores contract during preparation for negative staining or thin sectioning. Thus, although the pore complex is reported to be a stable structure (3, 20), DuPraw found that hypotonic solutions induce a contraction of the outer annular diameter in isolated nuclear envelopes (5, 6). Shrinkage due to glutaraldehyde fixation may be ruled out, however, since prefixed and unfixed freeze-etched *Tetrahymena* cells have identical nuclear pore diameters.

Dehydration shrinkage (19) may also account for the different nuclear pore frequencies (number of pores per square micron) obtained with freeze-etched macronuclei (39 ± 9) and tangential sectioned ones (68 ± 14); see Table I). Much

higher pore frequencies are found with negative staining (119 ± 27).

Bajer and Mole-Bajer (2) recently reported the pore frequency in endosperm cells to be strikingly increased after breakage of the nuclear envelope during mitosis. It may thus be that the high pore frequencies of isolated nuclear envelope pieces from *Tetrahymena* (29, 32; see also Table I of this paper) are due to a contraction of the nuclear envelope from its in vivo state during mechanical disruption with associated shrinkage of the pore rim (see also references 5, 6). This would also explain why in isolated macronuclear envelopes higher pore frequencies are always correlated with smaller pore diameters and vice versa: this results in a constant value of 32% for the ratio of the pore area to the nuclear surface area (29). It was established that this correlation occurs in each of the four investigated physiological states of *Tetrahymena* cells. The same does not apply to thin-sectioned and freeze-etched preparations (Table I). Similar differences in nuclear pore frequencies between negatively stained, isolated nuclear envelopes, on the one hand, and freeze-etched and thin-sectioned envelopes on the other, have been noted in the cases of *Allium* root tip cells (4, 10), mouse liver cells, and HeLa cells, but not so strikingly in amphibian oocytes.[1]

[1] Speth, V. 1970. Manuscript in preparation.

Direction of shadowing from bottom left or right.

FIGURE 1 Stationary phase cell, prefixed with glutaraldehyde. Cross-fractured pores in the nuclear envelope, the inner membrane of which is double-layered (arrow; see reference 4), are visible. × 35,000.

FIGURE 2 Heat-shock synchronized cell at division maximum, prefixed with glutaraldehyde. This is a survey micrograph of the karyoplasmic face of the inner nuclear membrane, showing areas with pores in occasional hexagonal array, alternating with pore-free regions. Annular subunits and central granules are visible (arrows). ×39,000.

FIGURE 3 Logarithmic phase cell, prefixed with glutaraldehyde, and minimally etched. View of the outer nuclear membrane from the cisternal side. Central as well as annular granules are indistinct. × 44,000.

FIGURE 4 Logarithmic phase cell, prefixed with glutaraldehyde. The cytoplasmic face of the outer nuclear membrane (*OM*) appears to be smooth, with only a few particles and holes of about 90–150 A (4). In contrast, the cisternal face of the inner nuclear membrane (*IM*) is studded with numerous particles in the same size range. The fracture line is visible surrounding the pores (arrows). × 44,000.

FIGURE 5 Heat-shock synchronized cell at division maximum, prefixed with glutaraldehyde. The cisternal face of the inner nuclear membrane is apparent. Within the outline of the pore, eight holes arranged in a ring indicate the places where annular globules had been located before being cleaved away. × 130,000.

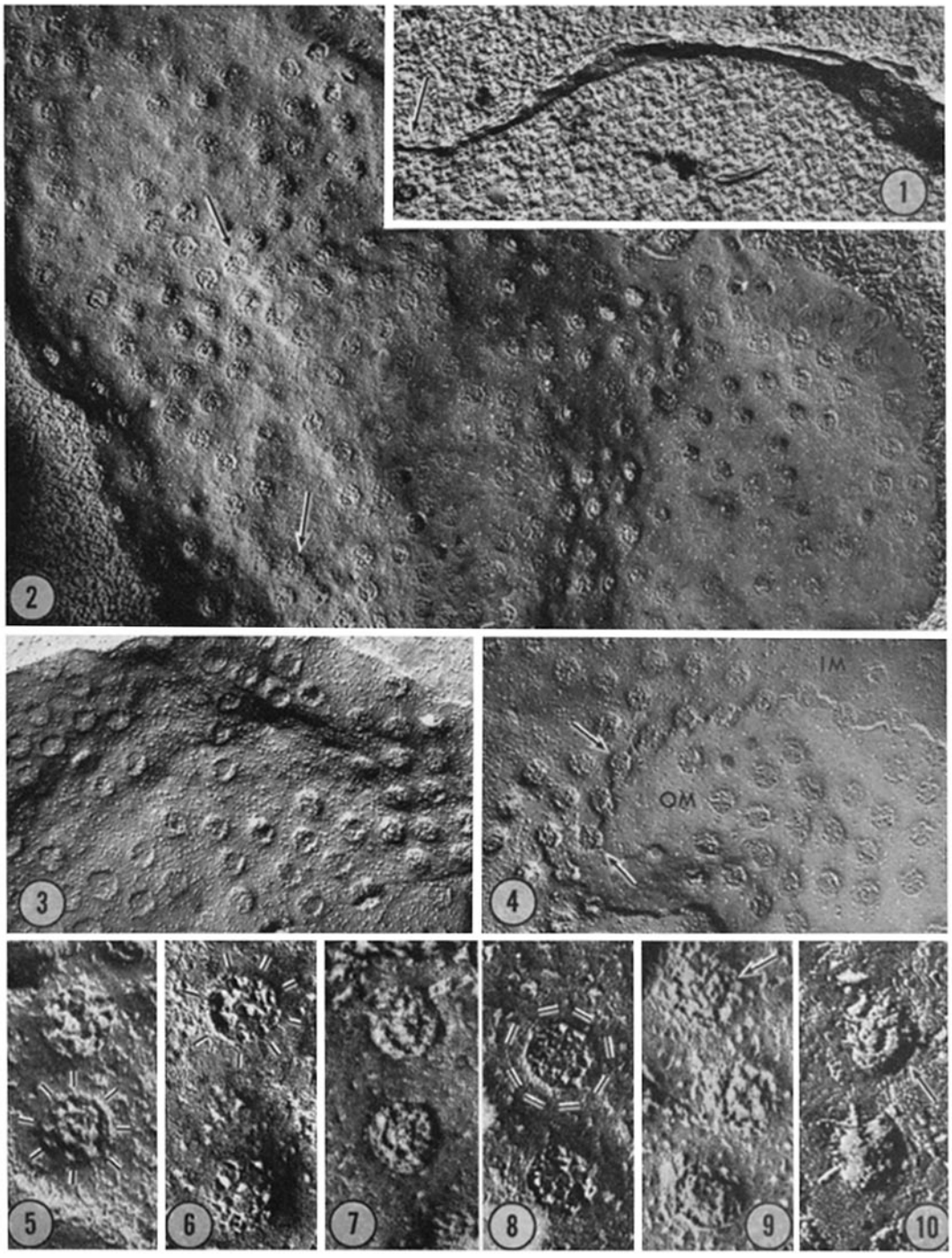
FIGURE 6 Stationary phase cell, prefixed with glutaraldehyde. Eight annular globules projecting into the karyoplasm are located within the outline of the pore. × 130,000.

FIGURE 7 Heat-shock synchronized *Tetrahymena* cell at division maximum, prefixed with glutaraldehyde. The karyoplasmic face of the inner nuclear membrane reveals pores, in which central granules are connected with annular material by filaments. × 130,000.

FIGURE 8 Stationary phase cell, prefixed with glutaraldehyde. The karyoplasmic face of the inner nuclear membrane reveals pores, the outline of which seems to be octagonal. × 130,000.

FIGURE 9 Logarithmic phase cell, prefixed with glutaraldehyde. The cytoplasmic face of the outer nuclear membrane reveals pores with central and peripheral granules. The upper pore shows filaments (arrow(connecting adjacent peripheral granules. The lower pore is surrounded by a polygonal outline. × 130,000.

FIGURE 10 Stationary phase cell without any prefixation. The cisternal face of the inner nucleas membrane reveals pores with central granules and peripheral annular material with a ring-like shape (arrow). Fibrils are visible extending over the outer annular rim of the lower pore. × 130,000.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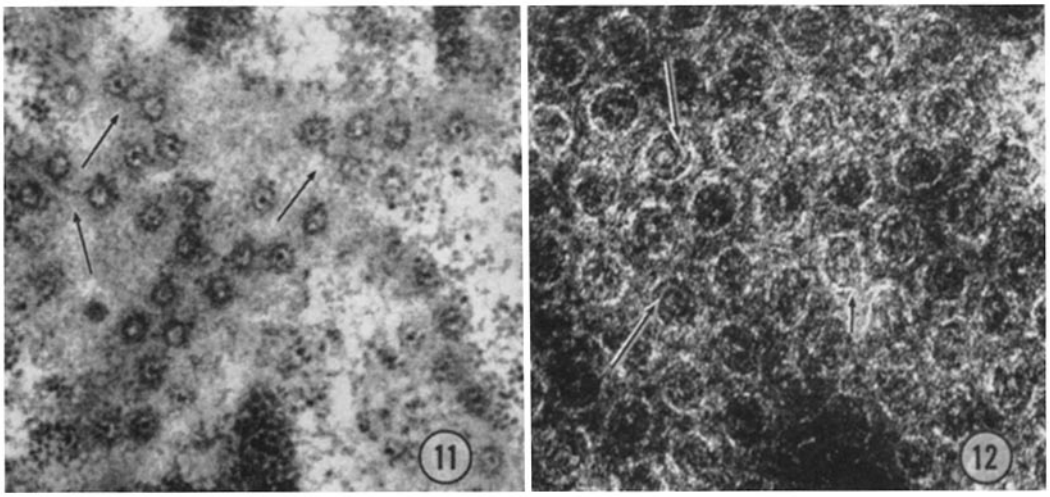

FIGURE 11 Survey micrograph of tangentially sectioned nuclear pores of a logarithmic phase *Tetrahymena* cell, simultaneously fixed with 3% glutaraldehyde and 1.3% OsO_4, and postfixed with 2% OsO_4. Nuclear pores reveal annular material with globules and central granules. Filaments connecting central granules and annular material are visible. Annular material of adjacent pores seems to be connected by strandlike material (arrows; see reference 15). × 56,000.

FIGURE 12 Survey micrograph of an isolated macronuclear envelope of a stationary phase *Tetrahymena* cell, fixed with 2% OsO_4 and negatively stained with 1% phosphotungstic acid. The pores reveal no annular material covering the pore margin (32). The "inner ring" (long arrows) and central granules within the pore can be observed. Filaments connecting central granules and the pore margin are present (short arrow). × 104,000.

TABLE I

*Quantitative Data of Nuclear Pore Complexes from Tetrahymena pyriformis GL Macronuclei after Different Preparation Techniques**

Preparation techniques	Outer annular diameter of nucleopores	Inner annular diameter of nucleopores	No. of nuclear pores per square micron	Diameter of central granules	Proportion of relative pore area to nuclear surface area
	nm	*nm*		*nm*	%
Thin sectioning‡	67 ± 7	35 ± 5	68 ± 14	18 ± 4	24 ± 5
Freeze-etching	78 ± 5	51 ± 4	39 ± 9	16 ± 4	19 ± 4
Negative staining§	66 ± 7‖	34 ± 3¶	119 ± 27	14 ± 6	32 ± 1.5

* Arithmetical mean values, with standard errors evaluated from the four different investigated physiological states.

‡ Data in reference 31.

§ Data in reference 29.

‖ This value is composed of the inner pore diameter of 59 nm (evaluation, reference 29) and of twice the width of the pore rim of 3.6 nm.

¶ Inner diameter of the "inner ring" (See references 29 and 31).

We conclude that pore frequencies and ratios of pore area to nuclear envelope area obtained from isolated nuclear envelopes may reflect shrinkage artifacts.

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