STRUCTURE OF MACRONUCLEAR ENVELOPES OF *TETRAHYMENA PYRIFORMIS* IN THE STATIONARY PHASE OF GROWTH

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INTRODUCTION

In a preceding publication a method was reported by which fragments of the nuclear envelopes of *Tetrahymena pyriformis* could be isolated (10). When macronuclear envelopes were isolated from exponentially growing cultures of the amicronucleate *Tetrahymena pyriformis* strain GL, two types of envelope pieces were observed which differed markedly in the structure of their pore complexes. Type A contained pores with a diameter in the range of 55–60 mμ and showed almost no annulus material; whereas, the pores of type B possessed an extremely broad annulus with an inner annulus diameter of about 20 mμ.

The nuclear pore complex, according to a general hypothesis, is thought of as a site of processes which regulate nucleocytoplasmic interaction (4–7, 21), e.g. those controlling the passage of macromolecules involved in cellular protein synthesis (11, 22, 26). Therefore, it is of particular interest to compare the structure of the macronuclear pores from exponentially growing cells with that of the macronuclear pores from cells in the stationary phase, i.e. under conditions in which the nuclear activity is minimal (27). Whereas the previous study (10) reported the structure of macronuclear membranes from log phase *Tetrahymena* cells, the present study deals with the pore complex of stationary phase cells.

MATERIAL AND METHODS

Stock cultures of *Tetrahymena pyriformis*, amicronucleate strain GL, were routinely kept at 18°C in a medium consisting of 2% proteose peptone (Difco Laboratories, Detroit, Mich.) and 0.4% liver extract (The Wilson Laboratories, Chicago, Ill.). Cells were inoculated in 600 ml of medium in Fernbach flasks and were cultivated for 48 hr at 28°C up to a final population density of about 250,000 cells per milliliter. Cell counting curves established that the populations at this time were in the early stationary phase of growth. Macronuclear envelopes were isolated and prepared for examination with the electron microscope (Siemens Elmiskop I and IA) as described previously (9–11). The ciliates were incubated in the isolation medium for 1–4 hr. For comparison, similar preparations were made from exponentially growing cultures of the same stock which were cultured either in the same medium or in a pure 2% proteose peptone solution.

RESULTS

The macronuclear envelope fragments obtained from stationary phase *Tetrahymena* populations were found to belong to type A; no remarkable annulus could be recognized in the pores (Fig. 1). B-type pore complexes with well-developed annuli, which occur in log phase cells (10; Fig. 3 *e*), were not observed. The general appearance of the negatively stained pores resembled somewhat that described by Gall (13–15) for nuclear pores in oocytes of various amphibia and of the starfish *Henricia*. In the *Tetrahymena* A-type envelopes, the outline of the pore shows up in the negative staining as a 4–6 mμ thick white line which, in agreement with Gall, we interpret as the perimeter of the pore. This perimeter is sometimes accentuated by small amounts of particulate material which is presumed to represent remnants of the well-developed annulus present in the pores of B-type envelopes from the log phase cells. Most of the pores appear circular in outline, but some polygonal ones may also be detected as was described for the nuclear envelopes of the oocyte material mentioned above (14, 15; see, however, references 10, 23, 32). The inner width of the pores ranges from 56 to 65 mμ in the total fraction of envelopes, but it is markedly constant within a particular envelope piece. Similar values for the sizes of the nuclear pores were obtained by measurements in ultrathin sections of whole *Tetrahymena* cells.

The pore frequency in different envelope pieces varies from 75 pores per square micron to 145 pores per square micron; again, the values are essentially constant in a given envelope piece. These values correspond to those reported for exponentially growing *Tetrahymena* (10).

In those envelopes which exhibit an extraordinarily high pore frequency, areas with a

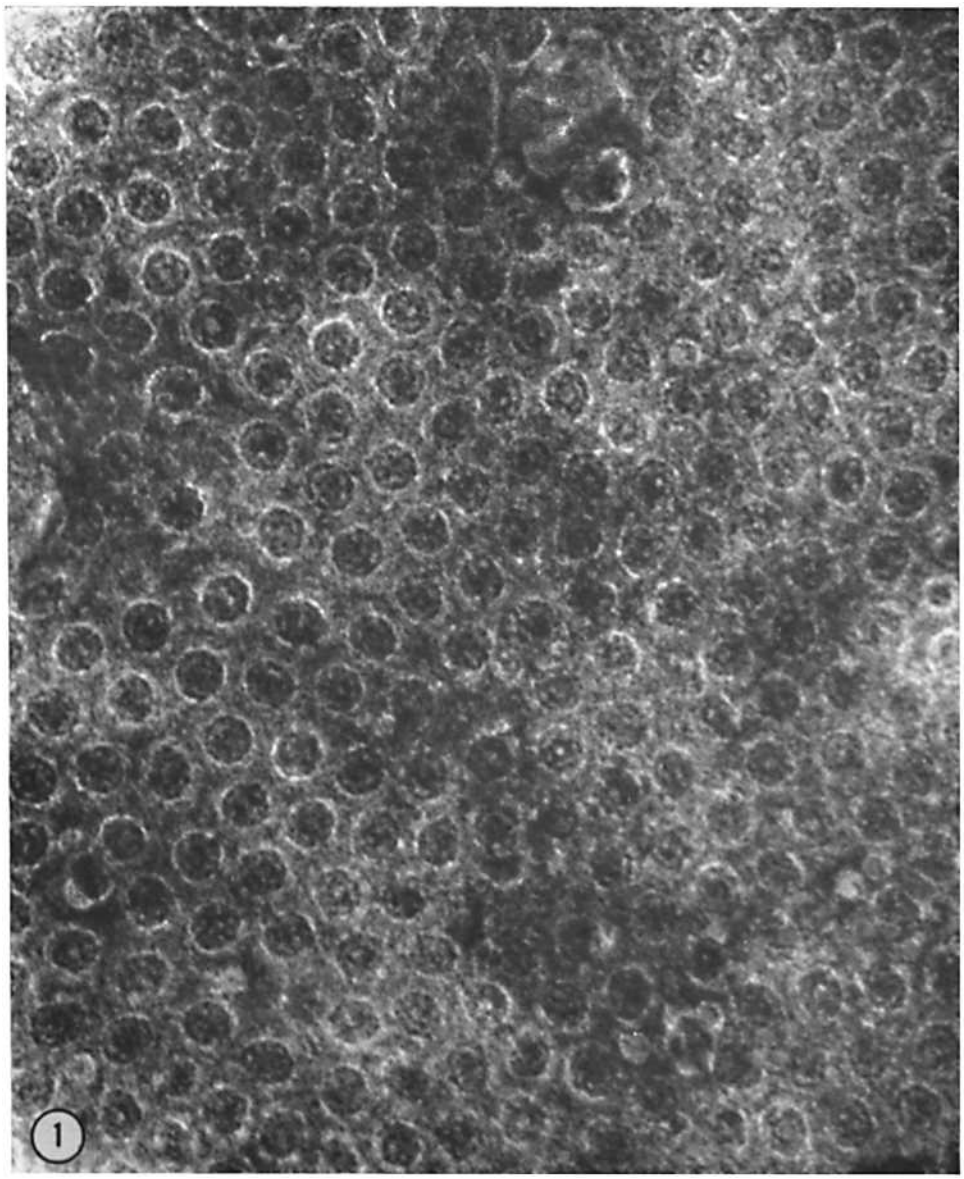

FIGURE 1 Survey micrograph of a macronuclear envelope isolated from *Tetrahymena pyriformis*, strain GL, in the stationary phase of growth, fixed in OsO_4, negatively stained with 2% phosphotungstic acid. No annulus can be seen in the nuclear pores. × 96,000.

hexagonal array of the pores can be observed (Fig. 2). The center-to-center spacing of the pores in this pattern is about 90 mμ.

The lumen of the pore is filled with the contrast-giving phosphotungstate which has been channeled around the central dot. This central granule (diameter, 8–18 mμ) has been described, by many authors, in sections tangential to the nuclear surface of whole cells (reviews in references 13, 29, 31) as well as in air-dried, shadowed, or negatively stained preparations of isolated nuclear envelopes (9–13, 18–20, 23, 35).

In a great many preparations, especially those in which the negative staining is faint, some fur-

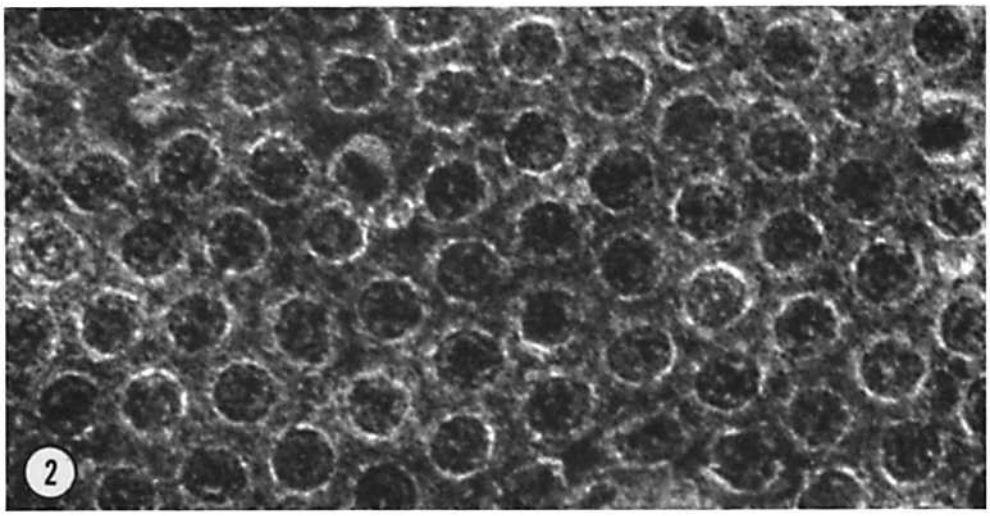

FIGURE 2 Isolated macronuclear envelope of *Tetrahymena pyriformis*, strain GL. Nuclear pores are arranged in a close hexagonal packing. × 140,000.

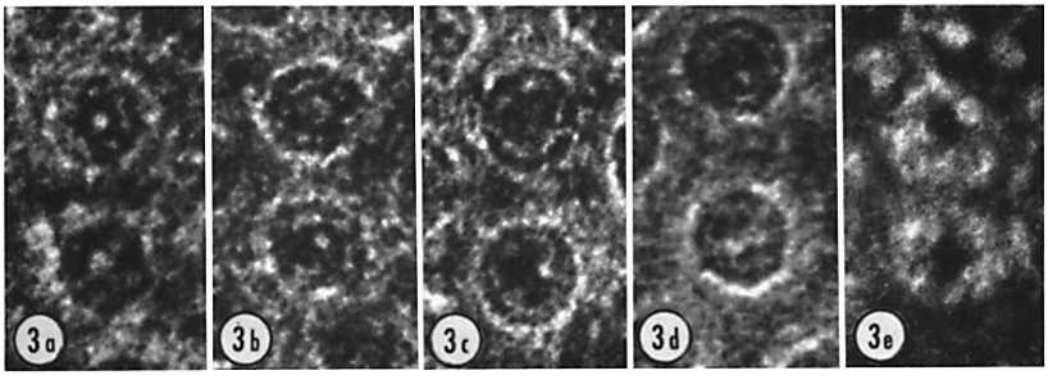

FIGURE 3 *a–e* Structural details of the negatively stained pore complex of the macronuclear envelope of *Tetrahymena pyriformis*, strain GL. × 230,000.

Figs. 3 *a–d*, pore complexes from stationary phase cells. *a*, Shows the particulate material accentuating the perimeter of the pore, the central dot, and tips of more diffuse material projecting from the perimeter. *b*, The "inner ring" can be revealed as consisting of distinct particles. *c*, In some pores the inner ring seems to be connected to the perimeter by thin strands. *d*, Strandlike material connecting the perimeter and the central dot.

Fig. 3 *e*, shows for comparison the characteristic B-type pore complexes from exponentially growing cultures. The well-developed annulus leaves a narrow inner pore diameter of about 20 mμ.

ther structural details of the inner part of the pore complex can be identified (Fig. 3). Between the pore perimeter and the central dot, another circular line of low contrast is distinguished and in some cases seems to be composed of regularly distributed granular particles with diameters below 5 mμ. This "inner ring," first described by Yoo and Bayley (35) for the nuclear pores of pea seedling tissue, can generally be detected in less intensely stained envelopes isolated from various plant and animal nuclei (Wunderlich, F., and W. Franke. Unpublished observations. See also Fig. 6 in reference 9). Besides this inner ring material, tips of diffuse or strandlike material are seen projecting from the perimeter towards the pore's center or towards the inner ring. Both the inner

ring and the tips are tentatively interpreted as being representative of a particular part of the material which is present in vivo in the pore's lumen and which remains attached to the wall of the pore during the isolation procedure.

DISCUSSION

When isolated according to the methods described, the macronuclear envelopes isolated from stationary phase *Tetrahymena* cells differ from those isolated from cells in the exponential phase of growth, in that they lack the characteristic annulate B-type pore complexes shown in Fig. 3 *e* (10). Other discernible structural properties of the nuclear pore complexes, such as pore frequency and pore diameter, are nearly the same in both the exponential and the stationary phases of cell growth.

Another conclusion can be drawn from the measurements of the pore diameters and pore frequencies. The percentage of the nuclear surface occupied by pores is 31 ± 4 in the whole fraction. This value of about 31% of nuclear surface occupied by pores also holds true for the nuclear envelopes from log phase *Tetrahymena* cells. Even in the case of a very infrequently found type of "log phase envelope" with an extremely high pore frequency of 180 ± 5 pores per square micron, the aforementioned value of 31% for the percentage of nuclear surface occupied by pores comes out as a result of an extremely small pore diameter of 47 ± 4 mμ. The nuclear pore frequencies for *Tetrahymena* are by far the highest heretofore reported. Other cells exhibit pore frequencies (pores per square micron of nuclear surface) of 7–12 (onion root tip, reference 2), 10–15 (yeast, reference 24), about 13 (HeLa cells, reference 8), 25–35 (frog oocytes, reference 20), 40–80 (sea urchin oocytes, reference 1), 35–55 (mouse liver, reference 10), 20–100 (different onion root tip nuclei, reference 9), 64 (pea seedling, reference 35), 65 (salivary glands of *Drosophila*, reference 30), and 80–100 (salivary glands of *Simulium*, reference 17).

That nuclear pores in certain cells may display a regular pattern has been mentioned by Merriam (19, 20) for limited areas of the nuclear envelopes of *Rana* oocytes (orthogonal square pattern) as well as for the "denser patches" on these envelopes (hexagonal pattern), and by Wiener et al. (30) for salivary gland cells of *Drosophila* and *Chironomus* and oocytes of *Triturus* and *Xenopus* (hexagonal patterns). Drawert and Mix (3) presented micrographs of the desmid green alga *Micrasterias* which revealed an orthogonal square pattern. Regular arrangements of nuclear pores can also be detected in micrographs of oocytes of the serpulid *Mercierella* published by Sichel (25). Hexagonal arrays of pores occur also in the annulate lamellae as was first described by Swift (28).

In view of the lack of comparative data on the development of the annular material in different states of cell metabolism, particularly of RNA and protein synthesis, it would be presumptuous to speculate about the possible causes of the changes in the pore complex structure, the functional role of the annulus, and the material within the pore. In this connection, however, one should remember the statement of Merriam (20) that in *Rana* eggs the annulus is significantly thicker in the earlier stages of oogenesis than in the mature eggs which synthesize less RNA and protein. Furthermore, of great importance in this problem is the finding of Yasuzumi et al. (34) that the ATPase activity in Leydig cells of young men is preferentially associated with the annulus and the central dot (see also references 16, 33) and that this activity is absent in the metabolically altered cells of ageing men.

Since *Tetrahymena* serves as an excellent organism for studies of cell physiology and morphogenesis, further attempts, including experiments with heat-synchronized cultures, will be made to examine the changes in the pore complex structure in different, well-defined states of cell metabolism.

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REFERENCES

1. AFZELIUS, B. A. 1955. *Exptl. Cell Res.* **8**:147.
2. BRANTON, D., and H. MOOR. 1964. *J. Ultrastruct. Res.* **11**:401.
3. DRAWERT, H., and M. MIX. 1961. *Z. Naturforsch.* **16b**:546.
4. FELDHERR, C. M. 1964. *J. Cell Biol.* **20**:188.
5. FELDHERR, C. M. 1965. *J. Cell Biol.* **25** (1, Pt. 1):43.
6. FELDHERR, C. M. 1966. *J. Cell Biol.* **31**:199.
7. FELDHERR, C. M., and C. V. HARDING. 1964.

In Protoplasmatologia. M. Alfert, H. Bauer, and C. V. Harding, editors. Springer-Verlag, Vienna. **5**:35.

8. FISHER, H. W., and T. W. COOPER. 1967. *J. Cell Biol.* **35**:40A.
9. FRANKE, W. W. 1966. *J. Cell Biol.* **31**:619.
10. FRANKE, W. W. 1967. *Z. Zellforsch. Mikroskop. Anat.* **80**:585.
11. FRANKE, W. W. 1967. Doctorate Thesis. University of Heidelberg, Heidelberg, Germany.
12. GALL, J. G. 1954. *Exptl. Cell Res.* **7**:197.
13. GALL, J. G. 1964. *In* Protoplasmatologia. M. Alfert, H. Bauer, and C. V. Harding, editors. Springer-Verlag, Vienna. **5**:4.
14. GALL, J. G. 1965. *J. Cell Biol.* **27**:121A.
15. GALL, J. G. 1967. *J. Cell Biol.* **32**:393.
16. KLEIN, R. L., and B. A. AFZELIUS. 1966. *Nature.* **212**:609.
17. MACGREGOR, H. C., and J. B. MACKIE. 1967. *J. Cell Sci.* **2**:137.
18. MENTRÉ, P. 1966. Proceedings of the 6th International Congress for Electron Microscopy held in Kyoto, Japan in 1966. Maruzen Co., Ltd., Tokyo. **2**:347.
19. MERRIAM, R. W. 1961. *J. Biophys. Biochem. Cytol.* **11**:559.
20. MERRIAM, R. W. 1962. *J. Cell Biol.* **12**:79.
21. MIRSKY, A. E. 1964. *In* Protoplasmatologia. M. Alfert, H. Bauer, and C. V. Harding, editors. Springer Verlag, Vienna. **5**:1
22. MITCHISON, J. M. 1966. *Intern. Rev. Cytol.* **19**:97.
23. MONROE, J. H., G. SCHIDLOVSKY, and S. CHANDRA. 1967. *J. Ultrastruct. Res.* **21**:134.
24. MOOR, H., and K. MÜHLETHALER. 1963. *J. Cell Biol.* **17**:609.
25. SICHEL, G. 1966. *La Cellule.* **66**:97.
26. STEVENS, B., and H. SWIFT. 1966. *J. Cell Biol.* **31**:55.
27. SUMMERS, L., E. BERNSTEIN, and T. W. JAMES. 1957. *Exptl. Cell Res.* **13**:436.
28. SWIFT, H. 1956. *J. Biophys. Biochem. Cytol.* **2** (Suppl. 4):425.
29. VIVIER, E. 1967. *J. Microscopie.* **6**:371.
30. WIENER, J., D. SPIRO, and W. R. LOEWENSTEIN. 1965. *J. Cell Biol.* **27**:107.
31. WISCHNITZER, S. 1960. *Intern. Rev. Cytol.* **10**:137.
32. WOOD, R. L. 1966. *J. Cell Biol.* **31**:125A.
33. YASUZUMI, G., and I. TSUBO. 1966. *Exptl. Cell Res.* **43**:281.
34. YASUZUMI, G., Y. NAKAI, I. TSUBO, M. YASUDA, and T. SUGIOKA. 1967. *Exptl. Cell Res.* **45**:261.
35. YOO, B. Y., and S. T. BAYLEY. 1967. *J. Ultrastruct. Res.* **18**:651.