REVERSIBLE, THERMOTROPIC ALTERATION OF NUCLEAR MEMBRANE STRUCTURE AND NUCLEOCYTOPLASMIC RNA TRANSPORT IN *TETRAHYMENA*

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ABSTRACT

We examine the effect of cooling upon the freeze-etch ultrastructure of nuclear membranes, as well as upon nucleocytoplasmic RNA transport in the unicellular eukaryote *Tetrahymena pyriformis*. Chilling produces smooth, particle-free areas on both faces of the two freeze-fractured macronuclear membranes. Upon return to optimum growth temperature the membrane-associated particles revert to their normal uniform distribution and the smooth areas disappear. Chilling lowers the incorporation of [^{14}C]uridine into whole cells and their cytoplasmic RNA. Cooling from the optimum growth temperature of 28° to 18°C (or above) decreases [^{14}C]uridine incorporation into cells more than into their cytoplasmic RNA; chilling to below 18°C but above 10°C causes the reverse.

[^{14}C]Uridine incorporation into whole cells and their cytoplasmic RNA reflects overall RNA synthesis and nucleocytoplasmic RNA transport, respectively. RNA transport decreases strongly between 20° and 16°C, which is also the temperature range where morphologically detectable nuclear membrane transitions occur. This suggests that the nuclear envelope limits the rate of nucleocytoplasmic RNA transport at low temperatures. We hypothesize that a thermotropic lipid phase transition switches nuclear pore complexes from an "open" to a "closed" state with respect to nucleocytoplasmic RNA transport.

INTRODUCTION

Many studies indicate that membrane organization and function varies with temperature (e.g., 8, 12, 20, 21, 50, 62). Membrane lipids, in particular, are well known to be temperature sensitive and can exist as liquid crystalline domains at normal growth temperatures (4, 7, 11, 13, 15, 19, 39, 40, 44, 47, 59). However, lipids change from a liquid to a solid state when the temperature is dropped below a critical level; this transition point depends upon the proportions of saturated fatty acids and

cholesterol in the lipid mixture. Such thermotropic state transitions have been extensively studied by differential calorimetry, nuclear magnetic resonance, fluorescent and spin label probes, and X-ray diffraction (2, 5, 16, 44, 51, 56), and we here show that they can also be monitored by electron microscopy.

Our study concerns the nuclear envelope, which is reasonably suspect of regulating some nucleocytoplasmic interactions (for review see 18, 52, 53). The nuclear pore complexes, in particular, appear to participate in the nucleocytoplasmic exchange of macromolecules, such as RNA (e.g. 17, 18, 52, 54, 60, 61). Furthermore, it is known that nucleocytoplasmic RNA transport is thermotropic, i.e., it declines when temperature is lowered, even to levels where RNA synthesis continues (3, 23). As a consequence, RNA accumulates in the nuclei. This phenomenon can logically be attributed to structural alterations of the nuclear envelope (1, 23).

Despite such suggestive data, we know of no micromorphologic evidence that temperature changes can alter the structure of nuclear envelopes. We have accordingly investigated this matter in the macronuclei of the ciliate protozoan *Tetrahymena pyriformis*, using freeze-etch electron microscopy. In this approach, one fractures frozen membranes tangentially, exposing two faces of the apolar membrane interior (41, 55); these are then visualized by high resolution replication techniques. By this method, we find that nuclear membranes indeed undergo reversible, temperature-dependent structural alterations. We also find that major changes in nucleocytoplasmic RNA transport take place in the temperature range where the structural transitions occur.

MATERIALS AND METHODS

Freeze-Etch Electron Microscopy

We propagate *Tetrahymena* cells (amicronucleate strain GL) at 28°C in 450 ml 2% proteose peptone and 0.4% liver extract (PPL medium). At log phase, we cool the cell-containing flasks in an ice-salt bath to 3°C within about 5 min, leave them at 3°C for 10 min, and then reheat to 28°C within about 30 s. We take samples for freeze-etch electron microscopy during the chilling process at medium temperatures of 28°, 20°, 15°, and 3°C. We take two additional samples after 10 min at 3°C and one after reheating to 28°C.

To monitor the effect of rapid temperature changes on nuclear membrane structure, we pellet the cells by centrifugation at 2,000 g_{av} for 6 min, resuspend, and keep them at 28°C for 10 min. We then add 250 ml ice-cold PPL medium, to give a final temperature of about 3°C within about 5 s. We maintain the cells at 3°C for 10 min before reheating to 28°C within 30 s. We take samples at 28°C just before cooling, immediately after reheating, as well as at the beginning and the end of the low temperature period at 3°C.

We run each experiment in triplicate and keep the incubation period constant in each series (2–3 h). In each series, we fix the cell samples with the same concentration of glutaraldehyde (1–3%) in 0.05 M Na cacodylate, pH 7.2, for 10 min at the withdrawal temperatures, before stepwise transfer to 25% glycerol. We then pellet the cells at 1,000 g_{av} for 3 min and freeze them on cardboard disks in Freon 22. We perform fracturing, etching (1 min at −100°C), and replicating with a Balzers model 360 M (37). We examine the replicas in a Siemens Elmiskop Ia. We determine the microscope magnification by standard grating replica. We evaluate nuclear membrane areas and pore numbers on cut-outs of calibrated positives, and provide means with standard errors (SE) of the means.

Incorporation of [^{14}C]Uridine

We incubate log phase *Tetrahymena* with [2-^{14}C]uridine (56.7 mCi/mmol; NEN chemicals; The Radiochemicals Centre, Amersham, England) at 28°C for 20 min and bubble air through the culture medium 2 h before and during this period. We then divide the culture into equal portions and incubate these for an additional 40 min at 28°, 24°, 20°, 16°, 13°, 10°, and 7°C. During this period the diameters of macronuclei, measured by phase-contrast microscopy in 50 glutaraldehyde-fixed cells per sample, do not change significantly. We monitor incorporation of [^{14}C]uridine into whole cells, transferring three 3-ml samples from each portion to 1.5 ml ice-cold 15% trichloroacetic acid (TCA), thereafter washing the resulting precipitates three times with cold 5% TCA and once with cold 70% ethanol. After drying we add 1 ml solubilizer (Soluene, Packard Instrument Co., Inc., Downers Grove, Ill.) to each sample, heat at 60°C for at least several hours, or overnight, and then add 15 ml scintillation fluid containing 5 g 2,5-diphenyloxazole (PPO) and 5.0 g 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) per 1 liter toluene. Counting is done in a Packard liquid scintillation counter (model 3380). We isolate the cytoplasmic fraction of the *Tetrahymena* cells according to the method of Leick and Plesner (34). Here we freeze the cells and thaw them in a water bath at about 40°C with vigorous shaking. We then centrifuge at 1,500 g_{av} for 5 min, resuspend in 0.2 M sucrose, 2 mM $MgSO_4$, 2.5 mM KCl, 5 mM Tris-HCl, pH 6.8 (TKM buffer), and homogenize in a Teflon Potter-Elvejhem grinder (about 12 strokes). We centrifuge the homogenate at 12,000 g_{av} for 15 min at 4°C (angle rotor, Weinkauf

ultracentrifuge, Brandau, Darmstadt, West Germany) and add ice-cold TCA to the supernate (crude cytoplasmic faction) to a final concentration of 5%. After washing the precipitates five times with cold 5% TCA, we determine the amount of RNA accumulated (38) and assay aliquots for radioactivity, as described above.

RESULTS

Freeze-Etch Electron Microscopy

As in most other eukaryotic cells, the nuclear envelope of *Tetrahymena* macronuclei consists of an inner (i.e., nucleoplasmic) and outer (i.e., cytoplasmic) nuclear membrane. Upon freeze fracturing at −100°C, both inner and outer membranes reveal an outer fracture face (OFF; cf. reference 6) oriented either toward the cytoplasm or toward the nucleoplasm, and an inner fracture face (IFF) oriented toward the cisterna of the nuclear envelope. In appearance, the outer and inner faces of fractured *outer* nuclear membranes closely resemble those of fractured *inner* nuclear membranes. At 28°C, the outer faces are generally studded with numerous uniformly distributed particles. However, the inner faces always reveal uniformly distributed depressions (with about the same dimensions as the particles) but few particles (cf. Figs. 1, 2).

Fracturing exposes not only the faces of the nuclear membranes, but also yields face-on views of the nonmembranous nuclear pore complexes. At 28°C, we normally find 38.7 ± 1.4 pores/μm^2 (Tables I, II; cf. also reference 49). The freeze-etch pattern of the *Tetrahymena* macronuclear envelope does not change with different glutaraldehyde concentrations, or with different periods of incubation of glycerol.

Effects of Cooling

SLOW COOLING: Fractured nuclear membranes of *Tetrahymena* cells fixed at 20°C resemble those of cells fixed at 28°C, and their pore distribution lies in the same range (Table I). However, at 15°C small, smooth areas appear on the faces of the fractured inner and outer nuclear membranes (Fig. 2). These become more prevalent at 10°C, where they occupy 7.8 ± 2.1% of the total nuclear envelope area. At 3°C the smooth areas expand farther, occupying 18.2 ± 2.9% of the nuclear surface (Table I). Also, at this low temperature, one observes occasional nuclear pore complexes surrounded by smooth regions. This contrasts with the usual case where the nuclear pores lie within "rough" areas. At this temperature we find only 34.3 ± 0.9 pores/μm^2, while at 10° and at 15°C the pore number is not significantly altered (Table I). Storage for 10 min at 3°C changes neither the pore number (35.2 ± 2.3) nor the proportion of smooth areas (17.6 ± 3.1%). However, when we return the cells to 28°C the smooth areas disappear and the pore frequency of the macronuclei returns to the range (Table I) found in cells grown at 28°C, i.e., 39.0 ± 1.4.

QUICK CHILLING: Quick transfer of the cells from 28° to 3°C also induces the appearance of smooth areas on the faces of the fractured inner and outer nuclear membranes (Fig. 2; Table I). However, here the proportion of smooth areas reaches only 5.1 ± 1.8%, and the pore density stays at 38.8 ± 1.1 μm^2 immediately upon chilling. After 10 min at 3°C, the pore number decreases slightly (37.1 ± 2.1) and the smooth areas increase to 7.3 ± 1.4%. Returning cells to 28°C again leads to complete disappearance of smooth areas, as the pore number returns to control values (37.8 ± 1.8; Table II).

[^{14}C]*Uridine Incorporation*

We prelabel *Tetrahymena* with [^{14}C]uridine for 20 min at optimum growth temperature, we find 1,074, 936, and 1,123 cpm, respectively, incorporated per 3-ml TCA-precipitated cells, and 377, 198, and 489 cpm, respectively, in their cytoplasmic RNA. During the subsequent 40-min incubation at 28°C, we find an additional accumulation of 651, 2,945, and 2,041 cpm per 3-ml cells, and 279, 779, and 528 cpm in the corresponding "cytoplasmic" RNA. However, the lower the incubation temperature, the less the radioactivity incorporated into the cells and their cytoplasmic RNA (Fig. 3). At 24°C and 20°C the [^{14}C]uridine specific activity of whole cells is less than that of the cytoplasmic RNA. However, at 16° and 13°C the specific activity of [^{14}C]uridine in whole cells exceeds that in their cytoplasmic RNA. At 10° and 7°C there is no significant incorporation into cytoplasmic RNA.

DISCUSSION

Nuclear Membrane Transitions

Freeze-etch electron microscopy shows that exposure of *Tetrahymena* to low temperature induces structural alterations in the membrane cores of the

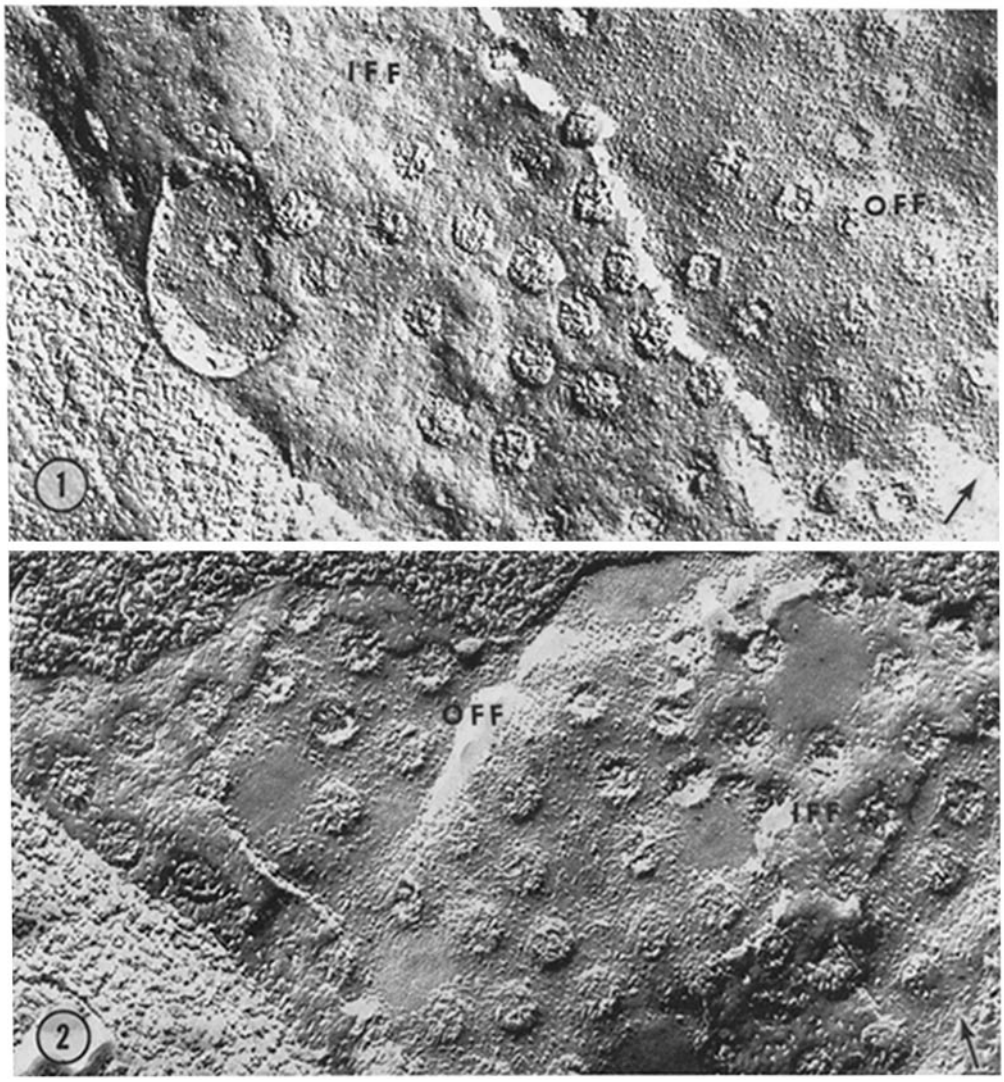

FIGURE 1 Freeze-fractured nuclear envelope of a *Tetrahymena* cell, slowly chilled to 20°C. Particles are uniformly distributed on the outer fracture face (*OFF*) of the fractured outer nuclear membrane. In contrast, the inner fractured face (*IFF*) of the inner nuclear membrane reveals many uniformly distributed "depressions" and only few particles. Arrow indicates shadowing direction. × 80,000.

FIGURE 2 Freeze-fractured nuclear envelope of a *Tetrahymena* cell quickly chilled to 3°C. Smooth areas are seen on the inner (*IFF*) and outer face (*OFF*) of the fractured inner and outer nuclear membranes, respectively. Arrow indicates shadowing direction. × 80,000.

macronuclear envelopes of these cells. Specifically, smooth areas segregate laterally from rough regions on the inner and outer faces of the two fractured nuclear membranes upon cooling. Similar lateral separation of membrane core components has been recently reported also for freeze-fractured plasma membranes of *Acholeplasma* and *Escherichia coli* upon chilling below the thermotropic phase transition points of their membrane lipids (58; cf., however, reference 25). We suggest the following mechanism for the emergence of smooth regions upon chilling. Below their transition temperatures, membrane lipids pack into crystalline arrays. This process starts at

TABLE I

Nuclear Pores Per Square Micrometer and Percent Smooth Areas in the Macronuclear Envelope of Slowly Chilled Tetrahymena Cells*·‡*

Fixation temperature§	Nuclear Pores/μm^2	Percent smooth areas	Total nuclear envelope area evaluated	Number of tangentially fractured nuclear envelopes evaluated
			μm^2	
28°C (Control) ‖	38.7 ± 1.4	—	49.7	9
20°C (During chilling)	38.3 ± 2.1	—	28.6	5
15°C (During chilling)	39.1 ± 1.9	1.9 ± 1.7	27.2	6
10°C (During chilling)	37.6 ± 2.2	7.8 ± 2.1	18.6	6
3°C (During chilling)	34.3 ± 0.9	18.2 ± 2.9	24.9	7
10 min at 3°C	35.2 ± 2.3	17.6 ± 3.1	19.1	6
28°C (Reheated)¶	39.0 ± 1.4	—	23.2	6

* "Smooth areas" are fracture areas largely devoid of particles or depressions, with average diameters of at least 100 nm.

‡ cf. Materials and Methods.

§ Cells fixed at stated temperatures and frozen for freeze cleaving at those same temperatures.

‖ Unchilled.

¶ After 10 min at 3°C, heated to 28°C within 30 s, fixed, and processed.

TABLE II

Nuclear Pores Per Square Micrometer and Percent Smooth Areas in the Macronuclear Envelope of Quickly Chilled Tetrahymena Cells*·‡*

Fixation temperature§	Nuclear pores/μm^2	Percent smooth areas	Total nuclear envelope area evaluated	Number of tangentially fractured nuclear envelopes evaluated
			μm^2	
28°C (Control) ‖	38.7 ± 1.4	—	49.7	9
3°C	38.8 ± 1.1	5.1 ± 1.8	20.8	6
10 min at 3°C	37.1 ± 2.1	7.3 ± 1.4	29.1	6
28°C (Reheated)¶	37.8 ± 1.8	—	34.3	7

* Smooth areas are fracture areas largely devoid of particles or depressions, with average diameters of at least 100 mn.

‡ cf. Materials and Methods.

§ Cells fixed at stated temperatures and frozen for freeze cleaving at those same temperatures.

‖ Unchilled.

¶ After 10 min at 3°C, heated to 28°C within 30 s, fixed, and processed.

nucleation centers and extends two-dimensionally in the membrane plane (50, 62). As a consequence, intercalated particles (proteins, glycoproteins; [35, 48]) become displaced in two directions, i.e., both parallel and normal to the membrane plane. When the cells are returned to their optimum growth temperature, the crystalline regions melt out and the particles again assume their normal distribution and orientation (50). Concordant with this interpretation, membranes whose lipids show no thermotropic disorder-order transition, e.g., erythrocyte ghosts, reveal no reversible thermotropic lateral separations of membrane core components by freeze etching (39).

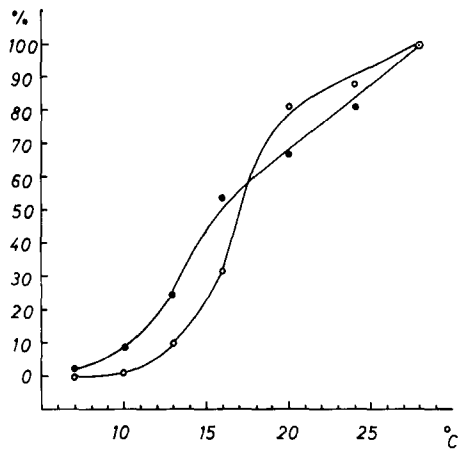

FIGURE 3 Proportion of [^{14}C]uridine incorporated during a 40-min period into TCA precipitates of whole cells (closed circles) and total RNA of their cytoplasmic fractions (open circles) as a function of temperature. Points represent means of three experiments normalized to 28°C, as given in Results.

Some data suggest that thermotropic crystallization of membrane lipids can occur without apparent "demixing" of membrane core components, i.e., displacement of the membrane-intercalated particles in freeze-fractured membranes. For instance, *Acholeplasma* grown on oleate show an identical particle distribution on the fractured plasma membrane faces above and below the phase transition temperatures of the membrane lipids (determined by scanning calorimetry [58]). Moreover, lateral demixing of membrane core components may be hindered by cholesterol, since increasing proportions of cholesterol in cholesterol-lecithin liposomes abolish the freeze-fracture pattern characteristic of the crystalline state of these phospholipids (58). Moreover, we find that the extent of the lateral separation of membrane core components decreases with increasing cooling rates (cf. also reference 50). Thus, when we chill *Tetrahymena* quickly to 3°C, we find only 7% of the membrane areas particle free. In contrast, upon slow chilling the cells to the same temperature, the value lies closer to 20% and the smooth areas are larger. Very likely, lipids in the crystalline state are not restricted to the smooth membrane regions, but occur also in the rough areas. In other words, the freeze-etch method can demonstrate the occurrence of thermotropic lipid transitions qualitatively but cannot define the total proportion of lipid in the crystalline state nuclear membranes.

The reversible temperature-dependent appearance of smooth patches on the fracture faces of nuclear membranes is not restricted to *Tetrahymena*. Thus, when we cool lymphocytes of various species to below 20°C, smooth patches appear on both fracture faces of both nuclear membranes. These disappear after heating cooled cells back to above 20°C (manuscript in preparation). Maul et al. (36) also report smooth areas on the nuclear membrane faces and suggest that these represent prospective nucleopore sites. However, our data suggest that this conclusion is not correct, since cold glycerinization, as used by these authors, produces smooth area artifactually.

Synthesis and Transport of RNA

According to Leick (30), incorporation of the RNA precursor [^{14}C]uridine into TCA precipitates of whole cells measures overall RNA synthesis. Since we propagate the cells in fully nutrient medium and use very short [^{14}C]uridine labeling periods, it is unlikely that the [^{14}C]uridine is incorporated significantly into compounds other than RNA (for review see reference 22). Leick (29) finds that at high growth rates, ribosomal RNA (rRNA) accounts for 90% of the total cellular RNA of the *Tetrahymena* strain we use. At very low growth rates, rRNA still accounts for 86% of the total RNA. rRNA is synthesized in the macronuclei as a large precursor molecule; this is converted to 17S and 25S soluble RNA, which finally appear in the cytoplasm (9, 10, 27, 28, 30-34, 42). Formation, processing, and the final appearance of these rRNA species in the cytoplasm occur within 3 min or less at the optimum growth temperature of 28°C (10, 27, 32, 42). We, therefore, conclude that our data, i.e., incorporation of [^{14}C]uridine into whole cells and their cytoplasmic fraction, reflect mainly the synthesis of rRNA and its transport from nucleus to the cytoplasm.

Accordingly, our data suggest that RNA synthesis is the rate-limiting step above 18°C, while RNA transport from nucleus to cytoplasm is rate limiting below 18°C. This fits the findings of other authors, showing that low temperatures block transport of RNA from nucleus to cytoplasm, even though RNA synthesis continues (1, 3, 23, 24; cf. also references 45, 46). It thus appears that there is a rate-limiting, posttranscriptional step for the

transfer of RNA to the cytoplasm. We suspect that this step involves the nuclear envelope since the temperature range (20° 16°C) where RNA transport from nucleus to cytoplasm drops sharply is identical with that where the micromorphologic nuclear membrane transitions occur.

In *Tetrahymena*, RNA transport probably proceeds through the nuclear pores only. Since RNA transport decreases in a temperature range where *pore number* does not change significantly (between 18° and 10°C during slow cooling), chilling may limit RNA transfer through *existing nuclear pores;* i.e., the pores change from a more "open" into a more "closed" state with respect to RNA transfer (61). We suggest that liquid crystalline lipids in the vicinity of the pores solidify upon chilling and thereby modify the state of permanent, nonmembranous material located within the pores and/or closely associated with the nuclear membranes near the pore rim. This material is widely believed to be responsible for the opening and closing of the nuclear pore complexes (18, 53, 61). Our reasoning rests on the circumstance that the activities of membrane-bound enzymes, including the protein-synthesizing machinery on membrane-bound ribosomes, also decrease with temperature, observations reasonably related to the thermotropic transitions of the membrane lipids (e.g. 42, 26, 57, 63). We are expanding the above experiments by gel electrophoretic analyses of the RNA synthesized in the nuclei and transported to the cytoplasm in *Tetrahymena* adapted to low temperatures. These organisms contain higher proportions of unsaturated fatty acids in their membrane phospholipids, which shifts the demixing points of membrane core components to lower temperatures (62).

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REFERENCES

1. AMOS, H., R. F. HOYT, and M. HORISBERGER. 1970. *In Vitro.* **6**:190.
2. ASHE, G. B., and I. M. STEIM. 1971. *Biochim. Biophys. Acta.* **233**:810.
3. BIER, K. 1965. *Chromosoma.* **16**:58.
4. BLASIE, I. K., and C. R. WORTHINGTON. 1969. *J. Mol. Biol.* **39**:417.
5. BLAZYK, I. F., and I. M. STEIM. 1972. *Biochim. Biophys. Acta.* **266**:737.
6. BRANTON, D. 1969. *Annu. Rev. Plant Physiol.* **20**:209.
7. BROWN, P. K. 1972. *Nat. New Biol.* **236**:35.
8. CHANG, S. B., and R. S. MATSON. 1972. *Biochem. Biophys. Res. Commun.* **45**:1529.
9. CHRISTENSSON, E. G. 1971. *Z. Biol.* **6**:411.
10. CHRISTENSSON, E. G. 1971. PhD. Thesis, University Lund, Sweden.
11. CONE, R. A. 1972. *Nat. New Biol. 236*:39.
12. DEMEL, R. A., S. C. KINSKY, C. B. KINSKY, and L. L. M. VAN DEENEN. 1968. *Biochem. Biophys. Acta.* **150**:655.
13. DEPETRIS, S., and M. C. RAFF. 1972. *Eur. J. Immunol.* **2**:523.
14. ELETR, S., and G. INESI. 1972. *Biochim. Biophys. Acta.* **290**:178.
15. ELETR, S., and D. KEITH. 1972. *Proc. Natl. Acad. Sci. U. S. A.* **69**:1353.
16. ENGELMAN, D. M. 1970. *J. Mol. Biol.* **47**:115.
17. FELDHERR, C. M. 1965. *J. Cell Biol.* **25**:43.
18. FELDHERR, C. M. 1972 *In* Advances in Cell and Molecular Biology. E. J. DuPraw, editor. Academic Press, Inc., New York. **2**:273.
19. FRYE, L. D., and M. EDIDIN. 1970. *J. Cell Sci.* **7**:319.
20. GITLER, L. 1971. *In* Biomembranes. L. A. Manson, editor. Plenum Publishing Corporation, New York. II:41.
21. GUTTMANN, R. 1968. *J. Gen. Physiol.* **51**:759.
22. HILL, D. L. 1972. *In* The Biochemistry and Physiology of *Tetrahymena*. D. E. Buetow, I. L. Cameron, and G. M. Padilla, editors. Academic Press, Inc., New York. 125.
23. HORISBERGER, M., and H. AMOS. 1970. *Biochem. J.* **117**:347.
24. ISCHIKAWA, K., C. KURODA, and K. OGATA. 1969. *Biochim. Biophys. Acta.* **179**:316.
25. JAMES, R., A. KEITH, and D. BRANTON. 1972. *J. Cell Biol.* **55**(2, Pt. 2):123 a. (Abstr.).
26. KUMAMOTO, J., J. K. RAISON, and J. M. LYONS. 1971. *J. Theor. Biol.* **31**:47.
27. KUMAR, A. 1970. *J. Cell Biol.* **45**:623.
28. LEE, Y. C., and J. E. BYFIELD. 1970. *Biochemistry.* **9**:3947.
29. LEICK, V. 1967. *C. R. Trav. Lab. Carlsberg.* **36**:113.
30. LEICK, V. 1969. *Eur. J. Biochem.* **8**:215.
31. LEICK, V. 1969. *Eur. J. Biochem.* **8**:221.
32. LEICK, V., and S. B. ANDERSEN. 1970. *Eur. J. Biochem.* **14**:460.
33. LEICK, V., J. ENGBERG, and J. EMMERSEN. 1970. *Eur. J. Biochem.* **13**:238.
34. LEICK, V., and P. PLESNER, 1968. *Biochim. Biophys. Acta.* **169**:398.
35. MARCHESI, V. T., T. W. TILLACK, R. L. JACKSON, I.

P. SEGREST, and R. E. SCOTT. 1972. *Proc. Natl. Acad. Sci. U. S. A.* **69**:1445.
36. MAUL, G. G., J. W. PRICE, and M. W. LIEBERMANN. 1971. *J. Cell Biol.* **51**:405.
37. MOOR, H., and K. MÜHLETHALER. 1963. *J. Cell Biol.* **17**:609.
38. OGUR, M., and G. ROSEN. 1950. *Arch. Biochem. Biophys.* **25**:262.
39. OVERATH, P., H. U. SCHAIRER, and W. STOFFEL. 1970. *Proc. Natl. Acad. Sci. U. S. A.* **67**:606.
40. PINTO DA SILVA, P. 1972. *J. Cell Biol.* **53**:777.
41. PINTO DA SILVA, P., and D. BRANTON. 1970. *J. Cell Biol.* **45**:598.
42. PRESCOTT, D. M., C. BOSTOCK, E. GAMOW, and M. LAUTH. 1971. *Exp. Cell Res.* **67**:124.
43. RAISON, J. K., J. M. LYONS, and W. W. THOMASON. 1970. *Arch. Biochem. Biophys.* **142**:83.
44. REINERT, J. C., and R. M. STEIM. 1970. *Science (Wash. D. C.).* **168**:1580.
45. SCHNEIDER, J. H. 1959. *J. Biol. Chem.* **234**:2728.
46. SCHUMM, D. E., and T. E. WEBB. 1972. *Biochem. Biophys. Res. Commun.* **48**:1259.
47. SINGER, S. J., and G. L. NICHOLSON. 1972. *Science (Wash. D. C.).* **175**:720.
48. SPETH, V., D. F. H. WALLACH, E. WEIDEKAMM, and H. KNÜFERMANN. 1972. *Biochim. Biophys. Acta.* **255**:386.
49. SPETH, V., and F. WUNDERLICH. 1970. *J. Cell Biol.* **47**:772.
50. SPETH, V., and F. WUNDERLICH, 1973. *Biochim. Biophys. Acta.* **291**:621.
51. STEIM, I. M., M. E. REINERT, M. E. TOURTELOTTE, R. N. MCELHANEY, AND R. L. RADER. 1969. *Proc. Natl. Acad. Sci. U. S. A.* **63**:104.
52. STEVENS, A. R. 1967. *In* The Control of Nuclear Activity. L. Goldstein, editor. Prentice-Hall International Inc., London. 189.
53. STEVENS, B. J., and J. ANDRE. 1969. *In* Handbook of Molecular Cytology. A. Lima-de-Faria, editor. North-Holland Publishing Co., Amsterdam. 837.
54. STEVENS, B. J., and H. SWIFT. 1966. *J. Cell Biol.* **31**:55.
55. TILLACK, T. W., and V. T. MARCHESI. 1970. *J. Cell Biol.* **45**:649.
56. TOURTELOTTE, M. E., D. BRANTON, and A. KEITH. 1970. *Proc. Natl. Acad. Sci. U. S. A.* **66**:909.
57. TOWERS, N. R., J. K. RAISON, G. M. KELLERMANN, and A. W. LINNARE. 1972. *Biochim. Biophys. Acta.* **287**:301.
58. VERKLEIJ, A. J., P. H. J. VERVERGAERT, L. L. M. VAN DEENEN, and E. ELBERS. 1972. *Biochim. Biophys. Acta.* **288**:326.
59. WILKINS, M. H. F., A. E. BLAUROCK, and D. M. ENGELMAN. 1971. *Nat. New Biol.* **230**:72.
60. WISE, G. E., A. R. STEVENS, and D. M. PRESCOTT. 1972. *Exp. Cell Res.* **75**:347.
61. WUNDERLICH, F. 1972. *J. Membrane Biol.* **7**:220.
62. WUNDERLICH, F., V. SPETH, W. BATZ, and H. KLEINIG. 1973. *Biochim. Biophys. Acta.* **242**:14.
63. ZEYLEMAKER, W. P., H. JANSON, C. VEEGER, and E. C. SLATER. 1971. *Biochim. Biophys. Acta.* **242**:14.