EXPANSION AND APPARENT FLUIDITY DECREASE OF NUCLEAR MEMBRANES INDUCED BY LOW Ca/Mg

Modulation of Nuclear Membrane Lipid Fluidity by the Membrane-Associated Nuclear Matrix Proteins?

FRANK WUNDERLICH, GÜNTER GIESE, and CARMEN BUCHERER

From the Lehrstuhl für Zellbiologie, Institut für Biologie II, Universität Freiburg, 7800 Freiburg i. Br., West Germany

ABSTRACT

Macronuclei isolated from *Tetrahymena* are contracted in form (average diameter: 10.2 μm) at a final Ca/Mg (3:2)concentration of 5 mM. Lowering the ion concentration to 1 mM induces an expansion of the average nuclear diameter to 12.2 μm. Both contracted and expanded nuclei are surrounded by a largely intact nuclear envelope as revealed by thin-sectioning electron microscopy. Nuclear swelling is accompanied by an expansion of the nuclear envelope as indicated by the decrease in the frequency of nuclear pore complexes from 52.6 to 42.1 pores/μm^2 determined by freeze-etch electron microscopy. Contracted nuclear membranes reveal particle-devoid areas (average size: 0.21 μm^2) on 59% of their fracture faces at the optimal growth temperature of 28°C. About three-fifths of the number of these smooth areas disappear upon nuclear membrane expansion. Electron spin resonance using 5-doxylstearic acid as a spin label indicates a higher lipid fluidity in contracted than in expanded nuclear membranes. Moreover, a thermotropic lipid clustering occurs at ~17°C only in expanded nuclear membranes. In contrast to the nuclear membrane-bound lipids, free lipids extracted from the nuclei rigidify with increasing Ca/Mg concentrations. Our findings are compatible with the view that the peripheral layer of the fundamental nuclear protein-framework, the so-called nuclear matrix, can modulate, *inter alia,* the lipid distribution and fluidity, respectively, in nuclear membranes. We suggest that a contraction of the nuclear matrix's peripheral layer induces a contraction of the nuclear membranes which, in turn, leads to an isothermic lateral lipid segregation within nuclear membranes.

KEY WORDS nuclear envelope · nuclear membranes · nuclear matrix · membrane lipid fluidity · isothermic membrane lipid segregation

Biomembranes are widely considered as being 'fluid' entities, i.e., membrane integral proteins can diffuse laterally and/or rotationally within a fluid lipid bilayer continuum (41). Recently, evidence has accumulated that the mobility of membrane integral proteins can be controlled, *inter alia,* by membrane-associated skeletal proteins (e.g., references 11, 37, 42, 53).

Membrane integral proteins can be directly visualized as so-called membrane-intercalating particles (MIP) on the fracture faces of nearly all membrane types by freeze-etch electron microscopy. In erythrocytes, for example, the mobility of these MIPs has been shown to be controlled by membrane-associated skeletal proteins consisting of spectrin and actin-like molecules, which are envisaged as forming a dynamic mesh-work on the cytoplasmic surface of the plasma membranes (e.g., references 11, 12, 43; cf. also reference 22). Such a tight spectrin-MIP interaction implies that changes in the spectrin meshwork involve changes in the lateral MIP distribution within membranes. Indeed, a contraction of the spectrin mesh-work induced by Ca^{2+} leads to isothermic lipid-protein segregations which are manifested in the emergence of smooth areas on the fracture faces of the erythrocytes' plasma membranes (12, 43). Incidentally, such dramatic lipid-protein segregations could possibly also influence the virtual lipid fluidity of the membranes which, as far as we know, has never been explored to date under these conditions.

Membrane-skeletal proteins have been described not only in plasma membranes but also in intracellular membranes such as endoplasmic reticulum membranes, in which they obviously control the lateral mobility of ribosomes (28). Another intracellular membrane, biogenetically related to the rough endoplasmic reticulum, is the porous, double-membraned nuclear envelope separating the nucleus from the cytoplasm in eukaryotic cells (for review, see reference 48). Recent evidence demonstrates that the nuclear envelope is structurally linked to an intranuclear protein-skeleton superstructure, the so-called nuclear matrix (references 4–6, 8, 17–20; for review, see references 5, 45). This dynamic "nucleoskeleton" (18) is composed of acidic proteins and obviously determines the internal and overall organization of nuclei in a wide variety of cells. The peripheral layer of the nuclear matrix is structurally linked to the inner nuclear membrane (cf. also references 1, 10, 31, 36). Most recently, we have detected that the nuclear matrix is reversibly contractile (49). It is, therefore, reasonable to envisage that the expansion state of the nuclear matrix, specifically its peripheral layer, is critically involved in the modulation of both the distribution of MIP on the fracture faces and the virtual lipid fluidity of nuclear membranes.

This view prompted us to examine the MIP distribution and the lipid fluidity of nuclear membranes in both contracted and expanded macronuclei isolated from the unicellular eukaryote *Tetrahymena*. Under these conditions, we can reasonably expect that the nuclear matrix is in a more contracted or more expanded state. Nuclear contraction and expansion are induced by varying the Ca/Mg concentration. In contracted and expanded nuclei, we then investigated (*a*) the nuclear membranes' core structure by freeze-etch electron microscopy, and (*b*) the nuclear membranes' lipid fluidity by the direct labeling electron spin resonance technique.

MATERIALS AND METHODS

Cells

Static 10-l cultures of the ciliate protozoan *Tetrahymena pyriformis* amicronucleate strain GL were axenically grown at 28°C in proteose peptone-yeast extract (PPY) medium (for composition, see reference 33) up to the mid-logarithmic growth phase (40,000–60,000 cells/ml). Cells were centrifugally harvested as described previously (33).

Isolation of Macronuclei

To optimally preserve the structure of nuclear envelopes, we developed a new method for cell disruption. Cells suspended in PPY medium (~1–3 × 10^7 cells/ml) were mixed with a sevenfold volume of a PST-glycerin (4:1) solution; PST consisting of 0.2 M sucrose, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 1% polyvinylpyrrolidone K-90 (PVP), 0.1 mM ATP, and 20 mM Tris HCl (pH 7.4) was preequilibrated at 28°C. This suspension was gently shaken at ~25°C by hand. After 4.5–6 min, a 0.6-fold volume of ice-cold PST was added, and this suspension was centrifuged at 500 *g* for 3 min. The "deciliated" cells were resuspended in excess PST at 0°–4°C and centrifuged at 3,500 *g* for 5 min. This step induces disruption of nearly all cells as monitored by phase-contrast light microscopy. After washing the pellet twice in 280 ml of PST, highly purified macronuclei were isolated according to our two-step sucrose gradient technique developed previously (17) with the modification described in reference 49. The final nuclear fractions were always suspended in PST.

Lipid Extraction

Total nuclear lipids were extracted according to Folch et al. (13).

Electron Microscopy

A given nuclear fraction was divided into aliquots. These were centrifuged in the cold at 900 *g* for 5 min and then incubated at 28°C in either 5 ml of PST or 5 ml

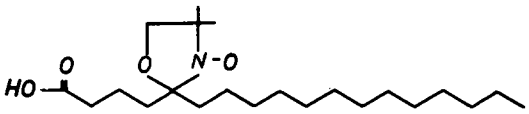
of LPST (0.2 M sucrose, 0.4 mM $MgCl_2$, 0.6 mM $CaCl_2$, 0.1 mM ATP, 20 mM Tris HCl [pH 7.4]). After 15 min, these suspensions were fixed with 2% glutaraldehyde buffered with 50 mM Na-cacodylate (pH 7.2) at 28°C for 15 min. After washing out the glutaraldehyde with the cacodylate buffer, these samples were processed as follows.

THIN SECTIONING: The nuclei were postfixed with 2% OsO_4 buffered with cacodylate at 0°C for 1 h, treated with 1% uranyl acetate for 2 h or overnight, dehydrated stepwise through ethanol and propylene oxide, and embedded in Epon. Thin sections were cut on a Reichert ultramicrotome (American Optical Corp., Buffalo, N. Y.) OmU_2 and double stained with uranyl acetate and lead citrate.

FREEZE-ETCHING: The nuclei were glycerinated stepwise up to a final concentration of 25% (51). They were frozen on cardboard disks in Freon 22 cooled by liquid nitrogen. Fracturing, etching (1 min at −100°C), and replicating were carried out on a Balzers model 360 M (Balzers High Vacuum Corp., Santa Ana, Calif.). The replicas and thin sections were examined in a Siemens Elmiskop I A.

Electron Spin Resonance

As spin label, we used 5-doxylstearic acid (doxyl refers to 4′,4′-dimethyloxazolidine-N-oxylring) synthesized according to Keana et al. (21).



5-doxylstearic acid

Spin labeling of the nuclei was performed as follows. The label dissolved in methanol was evaporated to dryness. To this, nuclei resuspended in either PST or LPST, which had been centrifugally pelleted shortly before, were added. The nuclear lipids were labeled by pipetting the methanol-dissolved label into the chloroform-dissolved lipids. After evaporation to dryness, PST or LPST was added. This suspension was mixed in an ultrasonic water bath for about 10 min. In different experiments, the nominal molar ratios of label to lipid ranged between 1:11 (maximally) and 1:28 (minimally).

~0.1 ml of the suspensions was sucked into the measuring capillary. The paramagnetic spectra were recorded at 9.37 GHz with a Varian E-9 spectrometer. (Varian Associates, Palo Alto, Calif.). Special care was taken that the electron spin resonance-measuring conditions remained the same in a given experiment, e.g., the same molar label/lipid ratio, avoidance of modulation broadening, etc. The temperature was measured with a thermistor above the measuring cavity.

RESULTS

Nuclear Membrane Expansion

The diameter of macronuclei isolated from *Tetrahymena* varies with different concentrations of Ca^{2+} and Mg^{2+} adjusted in a constant 3:2-ratio, as can be seen from Fig. 1. At a final Ca/Mg concentration of 5 mM, for example, the nuclei are contracted; their diameter amounts to 10.2 μm. Upon lowering the ion concentration to 1 mM, the nuclei expand, reaching an average diameter of 12.2 μm. Further lowering of the Ca/Mg concentration induces a further increase in the nuclear size. Concomitantly, however, an increasing number of nuclei burst.

Both contracted and expanded nuclei are surrounded by a largely intact nuclear envelope (Fig. 2*a* and *b*) which consists of (*a*) an inner nuclear membrane adjacent to the nucleoplasm, (*b*) an outer nuclear membrane still studded with ribosomes, and (*c*) the typical pore complexes (cf. also Fig. 3*a* and *b*). The nuclear envelope is not smooth, but rather ruffled, revealing in part large invaginations especially in the contracted nuclei. Thus, it would be meaningless to calculate the extent of nuclear membrane expansion upon nuclear swelling simply from the nuclear diameters mentioned above. The nuclear membrane area of

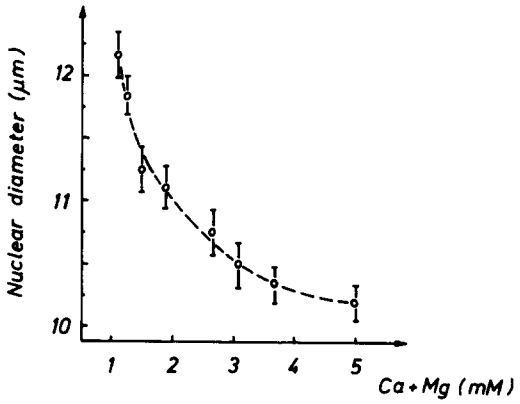


FIGURE 1 The size of *Tetrahymena* macronuclei in a given nuclear fraction as a function of the Ca/Mg concentration (adjusted to a constant ratio of 3:2). Nuclei in PST-buffer were pelleted at 900 *g* for 5 min, and then incubated in PST containing the indicated final Ca/Mg concentrations. For evaluating diameters, light micrographs were made from unfixed nuclei using differential interference contrast optics. On calibrated positives, 50 nuclei/sample were measured twice (the long diameter and its orthogonal). Means are given with standard deviations.

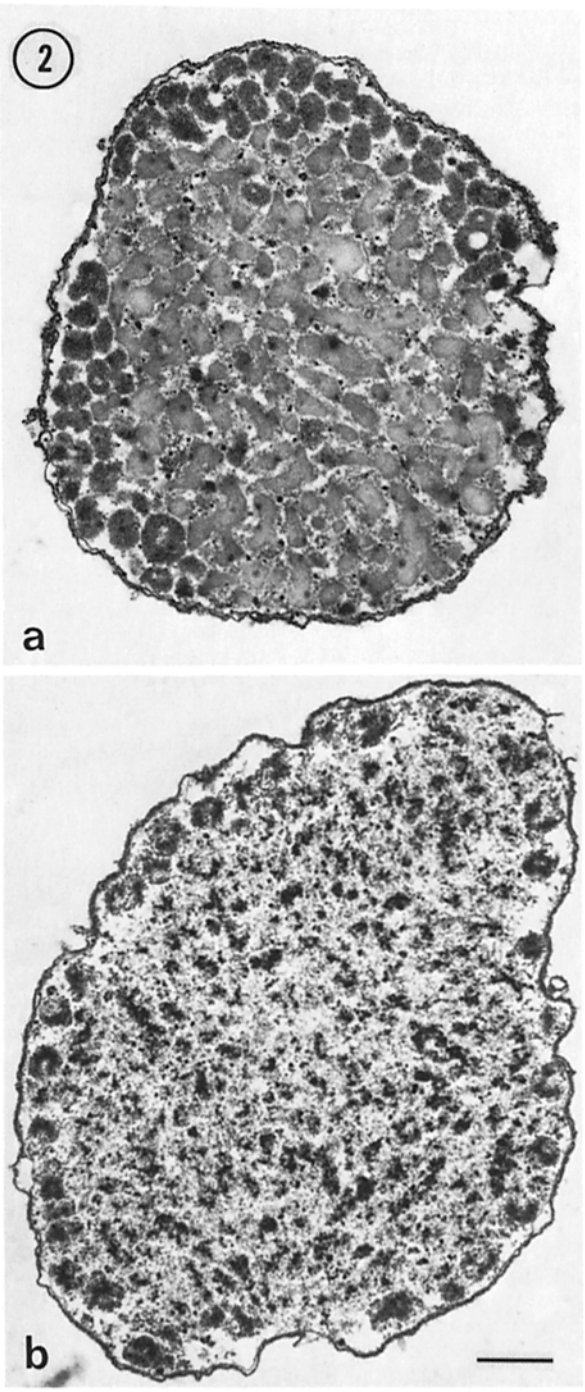


FIGURE 2 (*a* and *b*) Thin-section electron micrographs of a contracted (*a*) and expanded (*b*) macronucleus isolated from *Tetrahymena*. Macronuclei are surrounded by a largely intact nuclear envelope. The peripherally located nucleoli and the internal chromatin are very dispersed in expanded nuclei, while they reveal a clumped appearance in contracted nuclei. The clumped chromatin is always insufficiently stained, the reason for which is not understood. Bar, 1 μm. × 10,000.

contracted nuclei would be clearly an underestimation, resulting in a remarkable overestimation of the extent of nuclear membrane expansion.

Another more accurate approach to determine the extent of the nuclear membrane expansion is to use the pore complexes as a "natural marker." It is expected that nuclear membrane expansion is accompanied by a decrease in the number of pore complexes per unit area. The frequency of pore complexes can best be determined by freeze-etch electron microscopy, which allows the visualization of face-on views of large nuclear envelope areas containing up to 800 pore complexes (Fig. 3*a*). In contracted nuclei, the nuclear envelopes reveal 52.6 ± 4.1 pore complexes/μm^2 on average. This pore frequency diminishes, as expected, to 42.1 ± 4.3 pore complexes/μm^2 in expanded nuclei (Table I). The higher frequency of pore complexes in contracted as compared with expanded nuclei indicates an expansion of the nuclear membranes, the extent of which can be approximated to be 25%.

Differences between Contracted and Expanded Nuclear Membranes

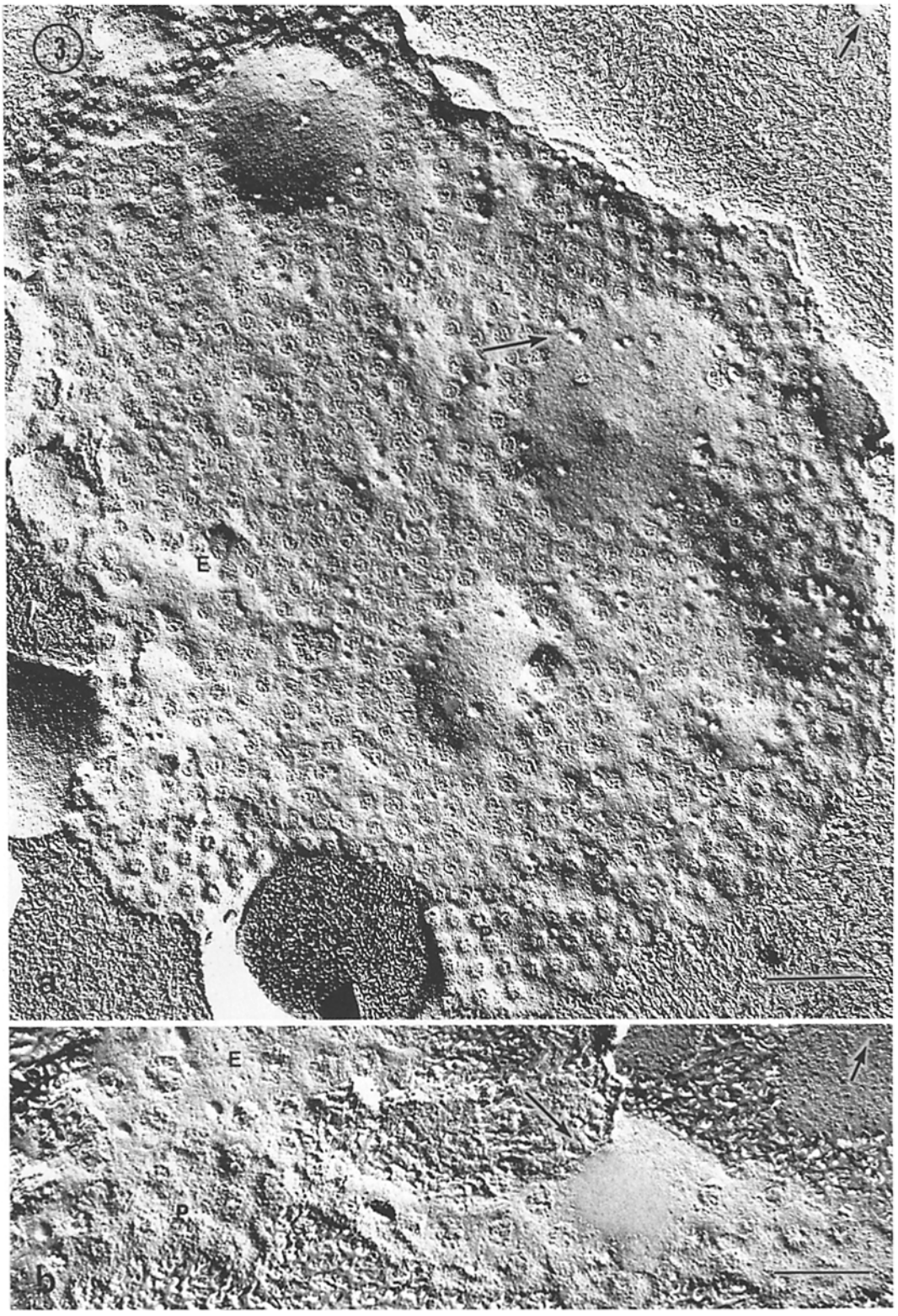
ISOTHERMIC LIPID-PROTEIN SEGREGATIONS: During fracturing, both the outer and inner nuclear membranes of the isolated *Tetrahymena* macronuclei are cleaved internally along their hydrophobic cores, thus exposing two fracture faces per membrane. At 28°C, the P faces of the outer and inner nuclear membranes are studded with numerous, uniformly distributed MIPs, whereas many pits and only few MIPs occupy the cisternal E faces of both outer and inner nuclear membranes (Figs. 3*a* and *b*). All nuclear membrane fracture faces reveal small bumps ~45 nm in diameter (Fig. 3*a* and *b*). Their number differs only slightly in expanded and contracted nuclear membranes (Table I).

At the optimal growth temperature of 28°C, MIP-devoid areas indicating domains of lipid-protein segregation can be visualized on nuclear membrane fracture faces. These smooth areas exhibit regularly a more or less circular shape, though occasionally a strong oval form can also be observed. The average size of these smooth areas amounts to ~0.22 μm^2 (Table I). The frequency of these smooth domains differs dramatically in expanded and contracted nuclear membranes. In the latter case, 59% of the fracture faces examined reveal such smooth areas (Table I). A considerable portion of these domains obviously disappears during expansion of the nuclear membranes, since they can be detected on only 25% of the fracture faces of the expanded nuclear membranes (Table I).

LIPID FLUIDITY: Fig. 4 shows representative first derivative electron spin resonance spectra obtained with the 5-doxylstearic acid label in contracted and expanded nuclear membranes at 28°C. These relatively sharp triplet spectra are characteristic of a rapid anisotropic motion of the spin label. Similar spectra are normally observed for spin labels in a lipid bilayer environment (38, 39). These spectra indicate that at least the major portion of the label molecules is dissolved in nuclear membrane regions.

The separation of the two extrema, $2T_{II}$, is an empirical indicator of the spin label mobility in the membrane. A widely accepted rule is that increasing $2T_{II}$ values signal a decrease in membrane fluidity and vice versa. In both contracted and expanded nuclei, $2T_{II}$ varies from experiment to experiment at ~5 G as shown in Table II. This variation is independent of the molar label/lipid ratio. Identical $2T_{II}$ values are normally measured in parallel samples of the same nuclear preparation in both contracted and expanded nuclei. Thus, we assume that the above $2T_{II}$ variation is mainly a result of unknown variations of the different nuclear preparations used.

Important, however, is the fact that, in all experiments, $2T_{II}$ is always ~1.1–1.8 G smaller in contracted than expanded nuclear membranes (Table II). Such a $2T_{II}$ decrease could possibly indicate an increased weak spin exchange in contracted nuclear membranes. In this case, one would expect a parallel line broadening, i.e., an increase in the line width (cf. references 34, 35). However, a decrease in line width of, e.g., the central band, H_0, seems to parallel the $2T_{II}$ decrease (Table II) even in these experiments with the maximal molar label/lipid ratios of 1:11 (Table II). In accord, spin exchange does not occur in human erythrocytes' plasma membranes at a label/lipid ratio of ~1:8, but only at 1:2, or higher ratios (7). Thus, the above slight $2T_{II}$ decrease appears to signal an increased lipid fluidity in contracted vs. expanded nuclear membranes. Remarkably, the reverse is found with total lipids extracted from the isolated nuclei. These are more fluid at a final Ca/Mg (3:2) concentration of 1 mM than at 5 mM at 28°C (Table III). A further difference between contracted and expanded nu-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E
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TABLE I

Quantitative Data of Nuclear Membrane Fracture Faces Obtained at 28°C from Contracted and Expanded Macronuclei of Tetrahymena

	Contracted nuclei	Expanded nuclei
Nuclear pores/μm^2	52.6 ± 4.1	42.1 ± 4.3
% of total fracture faces showing smooth areas	59 ± 7.3	25 ± 6.6
Size of smooth areas (μm^2)	0.21 ± 0.02	0.23 ± 0.06
Bumps/μm^2	6.4 ± 1.6	5.8 ± 1.3

Nuclei were incubated in either PST (= contracted nuclei) or LPST (= expanded nuclei). Values represent means ± SE from at least 10 examined replicas of at least four different experiments. Square micrometers were determined by weighing cut-outs of calibrated positives.

clear membranes can be observed in the temperature response of $2T_{II}$. In expanded nuclear membranes, a biphasic increase of $2T_{II}$ with an extrapolated "break" at ~17°C is found with falling temperatures (Fig. 5). Such a change in slope at ~17°C, however, is not found in contracted nuclear membranes, where $2T_{II}$ increases linearly upon temperature lowering. Occasionally, a slight bend can be seen at ~9°C.

DISCUSSION

Perturbed Lipid Distribution in Contracted Nuclear Membranes

Our data give satisfactory evidence for the conclusion that nuclear membranes of isolated *Tetrahymena* macronuclei can be expanded by

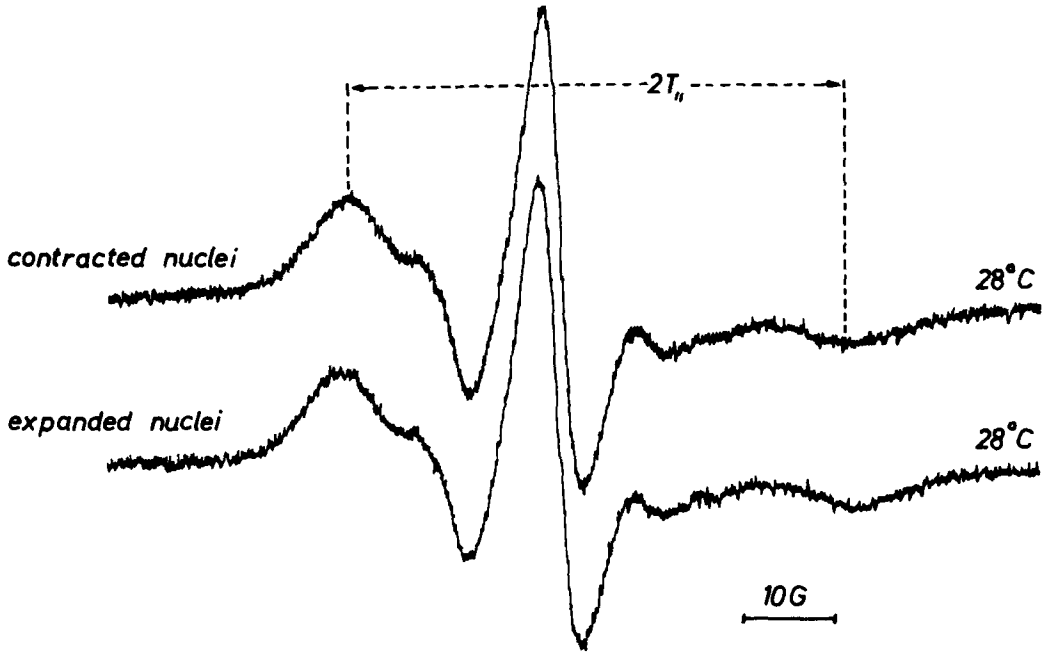

FIGURE 4 Representative electron spin resonance spectra obtained with 5-doxylstearic acid in contracted and expanded *Tetrahymena* nuclei at 28°C. $2T_{II}$ = the separation of the outer hyperfine extrema.

FIGURE 3 (*a* and *b*) Freeze-etch electron micrographs of nuclear envelopes of an expanded (*a*) and contracted (*b*) macronucleus isolated from *Tetrahymena*. In the expanded nuclear envelope, large areas free of the typical pore complexes can be seen. Arrow points to two of the numerous bumps ~45 nm in diameter. In contracted nuclear envelopes, particle-devoid domains can be regularly seen (arrow). *E* = cisternal fracture face of the inner nuclear membrane; *P* = outer fracture face of the outer nuclear membrane. Arrows in the top right corner indicate shadowing direction. (*a*) Bar, 0.5 μm; × 30,000. (*b*) Bar, 0.25 μm; × 60,000.

TABLE II

The Outer Hyperfine Splittings ($2T_{II}$) and the Line Width of the Central Band (H_o) Obtained with 5-Doxylstearic Acid at 28°C in Contracted and Expanded Macronuclei of Tetrahymena

Experiment number	Contracted nuclei		Expanded nuclei		Difference between contracted and expanded nuclei		Molar ratio label/lipid
	$2T_{II}$	H_o	$2T_{II}$	H_o	$\Delta 2T_{II}$	ΔH_o	
1	56.7	6.0	57.6	6.1	1.1	0.1	1:11
2	51.8	4.1	52.9	4.7	1.1	0.6	1:14
3	56.6	5.5	58.3	6.0	1.7	0.5	1:28
4	55.5	4.3	57.2	4.8	1.8	0.6	1:11
			57.3	5.0			
5	53.8	4.3	55.6	4.5	1.7	0.1	1:11
	53.9	4.5					

In each experiment, a given nuclear fraction was divided into two (exp nos. 1-3) or three aliquots (exp nos. 4, 5), which were incubated in either PST (= contracted nuclei) or LPST (= expanded nuclei) as described in Material and Methods. The values were taken from plots of $2T_{II}$ and H_o vs. temperature at 28°C.

TABLE III

The Outer Hyperfine Splittings ($2T_{II}$) Obtained with 5-Doxylstearic Acid at 28°C in Total Lipids Extracted from Isolated Tetrahymena Macronuclei at a Final Ca/Mg (3:2) Concentration of 5 mM and 1 mM

Experiment number	Total nuclear lipids at	
	5 mM Ca/Mg	1 mM Ca/mg
	$2T_{II}$ G	
1	49.8	47.5
2	49.1	46.5

swelling the nuclei. Moreover, our freeze-etch electron microscopical and electron spin resonance data show significant differences between contracted and expanded nuclear membranes. Several indications exist that expanded nuclear membranes resemble more the *in situ* situation than contracted nuclear membranes.

NUCLEAR DIAMETERS: The diameter of isolated *Tetrahymena* macronuclei increases from 10.2 μm to 12.2 μm upon lowering the final Ca/Mg concentration from 5 to 1 mM. Unfortunately, however, the nuclear diameter cannot be determined in living cells because of their swimming activity, but only in fixed cells. We have previously measured the nuclear diameter to be 10.9 μm in log-phase *Tetrahymena* cells fixed with 5% acetic acid supplemented with 0.5% methyl green (52). This value, however, is surely a clear underestimate of the true diameter, since this, as any, fixation procedure causes a shrinkage of the cells including the nuclei. Thus, it is reasonable to assume that the diameter of expanded nuclei is more comparable to the *in situ* situation than that of contracted nuclei.

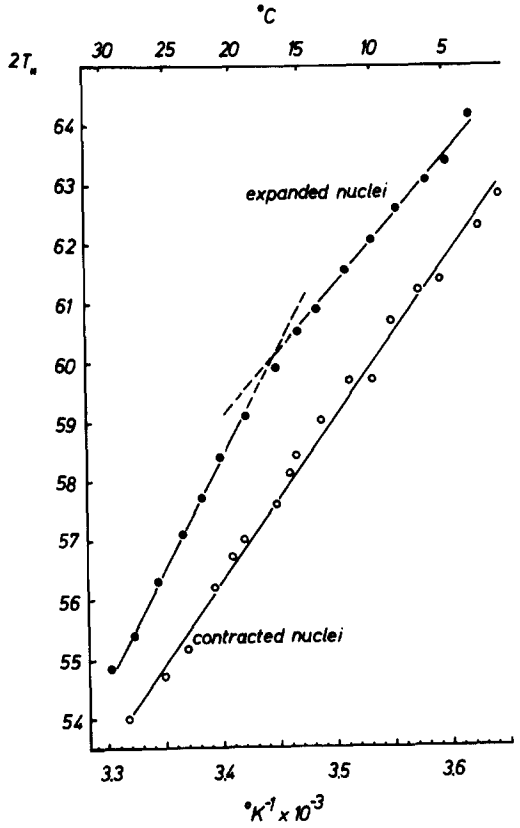

FIGURE 5 Separation of the outer hyperfine extrema, $2T_{II}$ (G), in contracted and expanded *Tetrahymena* nuclei as a function of temperature.

PORE COMPLEX FREQUENCY: Contracted and expanded nuclear membranes contain 52.6 nuclear pore complexes/μm^2 and 42.1 pores/μm^2, respectively. The latter pore complex frequency is more similar to *in situ* situation, where ~39 pore complexes/μm^2 have been previously found (reference 47; cf. also reference 25).

ISOTHERMIC LIPID-PROTEIN SEGREGATIONS: Contracted nuclear membranes reveal smooth domains on 59% of their total fracture faces at 28°C. About three-fifths of these smooth domains, however, disappear upon nuclear membrane expansion. ~75% of the fracture faces of expanded nuclear membranes exhibit a uniform MIP distribution, which is the typical *in situ* situation at 28°C (47).

THERMOTROPIC LIPID CLUSTERING: Only expanded and not contracted nuclear membranes show a "break" in their lipid fluidity in terms of $2T_{II}$ at ~17°C. In this temperature range, smooth domains emerge on the nuclear membrane fracture faces within whole *Tetrahymena* cells upon lowering the temperature from 28°C down to 3°C (reference 47; cf. also reference 25). These two phenomena are very probably induced by the same mechanism, namely a lateral clustering of ordered lipid domains. We have previously suggested that *Tetrahymena* cells adjust the lipid fluidity of their endomembranes within a broad fluid → ordered lipid phase separation (50, 51). This means that, at the physiological growth temperature, crystalline lipid domains are dispersed within fluid lipids. Upon lowering the temperature below a critical temperature range, these crystalline domains abruptly change their compositional quality which leads to a lateral clustering of these domains within the membrane plane.

In particular, the latter two findings that thermotropic lipid clustering is suppressed and that isothermic lipid-protein segregations are especially pronounced in contracted nuclear membranes suggest that the lipid distribution in contracted nuclear membranes is perturbed in comparison with expanded nuclear membranes. The latter in turn might be more similar to the *in situ* situation.

Apparent Fluidity Decrease in Nuclear Membranes upon Expansion

As far as we know, the lipid fluidity in terms of $2T_{II}$ has never been explored in any membrane type as a function of the membrane expansion state. On the basis of present membrane models, e.g., the fluid-mosaic model (41), one would expect that a membrane expansion induces an increase in the overall fluidity, i.e., disordering, of lipids within membranes and vice versa. Surprisingly, however, our present data appear to indicate the reverse at first sight: the fluidity is lower in expanded vs. contracted nuclear membranes.

On the other hand, one must be cautious with the interpretation of electron spin resonance data in terms of overall membrane fluidity. For instance, spin label molecules are known to be dissolved nonuniformity over the total membrane, that is, preferentially in "disordered or fluid" regions. Thus, after a more critical inspection, our data could also indicate that the portion of label molecules probing fluid membrane regions is larger in contracted than in expanded nuclear membranes.

Indeed, such a view of a different distribution of label molecules in contracted vs. expanded nuclear membranes is also supported by our freeze-etch electron microscopical data. Contracted nuclear membranes reveal smooth domains on 59% of their total fracture faces which is more than twice as much as in expanded nuclear membranes. Such smooth areas have been observed in a wide variety of membrane-types particularly at low temperatures by numerous investigators. In these cases, it is widely accepted that they contain ordered lipids (for *Tetrahymena*, e.g., references 42, 47, 50, 51). Thus, it would be plausible to assume that the label molecules are largely excluded from these smooth domains and are distributed predominantly in the MIP regions containing fluid lipids. In this case, a larger portion of label molecules would be localized in a smaller overall area of fluid regions in contracted vs. expanded nuclear membranes. On the other hand, we also have to keep in mind the reverse possibility, which cannot be excluded *a priori*, namely that the lipids in the smooth areas observed at physiological temperatures are in a fluid or even "super-fluid" state. In these regions, the label molecules would then be expected to be preferably distributed. Indeed, the smooth areas detected on plasma membrane fracture faces of spectrin-contracted erythrocytes are apparently enriched with lipids exerting a membrane "disordering" effect, such as lysophosphatidylcholine (reference 44; cf. reference 23). Moreover, smooth areas have been described, in many different membrane fusion processes (for review see, e.g., reference 46), as being the virtual sites of membrane fusion, in which lipids are postulated to be disordered (2, 9).

Possible Role of the Nuclear Matrix in the Modulation of Nuclear Membrane Lipid Fluidity

The major question is what is the cause of the higher lipid fluidity and perturbed lipid distribution, respectively, in contracted vs. expanded nuclear membranes. In particular, we have to envisage two principally different possibilities: Ca^{2+} and(or) Mg^{2+}, which we use for contraction and expansion of nuclear membranes, affects the lipids either directly or indirectly, i.e., via an expansion or contraction of membrane proteins. The first possibility would be in line with findings showing that Ca^{2+} is known to rigidify model membranes containing high concentrations of negatively charged phospholipids such as cardiolipin (16) or PS (30) or binary liposomes of PC-PS (27, 29). This Ca effect is generally ascribed to a Ca-induced isothermic lipid phase transition coupled with a separation of the fluid from the ordered lipids (27, 29, 30). Though *Tetrahymena* nuclear membranes contain only little, if any, negatively charged phospholipids (reference 26; Giese et al. Manuscript in preparation), we also find a rigidification of the extracted nuclear lipids of *Tetrahymena* with increasing Ca/Mg concentrations. Importantly, however, the reverse is found within nuclear membranes, in which Ca and(or) Mg induce an apparent increase in lipid fluidity. Though the label molecules may probe different regions in extracted nuclear lipids and nuclear membranes, it is nevertheless reasonable to assume that Ca and/or Mg obviously exert only a minor, if any, direct effect on the distribution of lipids within nuclear membranes.

At the moment, the more plausible explanation would be, therefore, that Ca and(or) Mg primarily interact predominantly with proteins which become changed in conformation and(or) distribution. These protein changes in turn secondarily influence the lipid distribution in nuclear membranes. Indeed, direct evidence for protein changes is provided by our freeze-etch electron microscope data which show isothermic lipid-protein segregations especially in contracted nuclear membranes, the contraction of which is induced by 5 mM Ca/Mg. This finding is in accord with other recent findings that Ca^{2+} induces isothermic lipid-protein segregations in erythrocytes' plasma membranes (12, 43, 44). Incidentally, in these membranes, Ca^{2+} aggregates the spectrin with the 88,000-mol-wt peptide, which is the major MIP constituent (22). Finally, our above explanation finds further support in a very recent in vitro investigation from Mombers et al. (24). Those authors found that the membrane-associated proteins spectrin and actin protect liposomes consisting of phosphatidylcholine and(or) phosphatidylglycerol against the action of Ca^{2+} and Mg^{2+}, which, in this case, normally induce lipid vesicle fusion.

Our suggestion implies that the nuclear membranes in *Tetrahymena* are asociated with Ca/Mg-sensitive "skeletal" proteins. Indeed, a large, and very probably integral portion of the inner nuclear membrane is obviously constituted by the peripheral layer of the nuclear matrix in *Tetrahymena* (17, 18, 45; cf., for a presumably slightly different situation in other cell-types, references 1, 10, 31, 36). This *Tetrahymena* nuclear matrix which is composed mainly of acidic proteins (reference 17; cf. also reference 5) can be reversibly contracted by Ca/Mg. For instance, it is maximally contracted at a final Ca/Mg (3:2) concentration of 5 mM. Under the same conditions, we also found here a contraction of the *Tetrahymena* macronuclei. Though this nuclear contraction must not necessarily be induced primarily by a nuclear matrix contraction, it is reasonable to conclude that, in contracted nuclei, the nuclear matrix including its peripheral layer is in a contracted state, manifested in the observed contraction of the nuclear membranes. Inversely, expanded nuclear membranes are associated with an expanded nuclear matrix. Incidentally, this view corresponds to a recent suggestion of Riley and Keller that the expansion state of the nuclear membranes in HeLa cells is under the control of an intranuclear macromolecular protein complex (32). Thus, the nuclear matrix's proteins can reasonably be regarded as being strongly involved in, even if not primarily responsible for, the expansion and contraction of nuclear membranes.

This view implies that the nuclear matrix is critically involved in the modulation of the fluidity and distribution of the lipids in nuclear membranes. It is totally unknown, however, which mechanism underlies this modulation. In this context, it is noteworthy that, for instance, in erythrocytes' plasma membranes, Ca^{2+} can apparently induce a contraction of the spectrin-actin meshwork which, in turn, is suggested to be accompanied by a lateral compression of the lipid bilayer in the membrane core. At the moment, a similar suggestion might be the most straightfor-

ward and illustrative interpretation for the situation in *Tetrahymena*. In contracted *Tetrahymena* nuclei, for example, the nuclear matrix's peripheral layer induces a contraction of the nuclear membranes, which is accompanied by a lateral compression of the lipid bilayer in the nuclear membranes' cores. This in turn entails an isothermic lateral lipid segregation within nuclear membranes which is manifested by a higher overall lipid fluidity and an increased number of fracture faces with smooth domains.

Biological Significance

The final question is whether our findings have biological relevance at all. For a long time it has been known that the shape and, especially, the volume of cell nuclei vary *in situ* depending on cell cycle, physiological state, circadian periodicity, etc. Particularly intriguing is that nuclei must obviously swell before DNA synthesis can start (e.g., references 3, 14). To our knowledge, however, it is unknown whether such volume variations in *in situ* nuclei are coupled with changes in intranuclear concentrations of Ca^{2+} and(or) Mg^{2+}. In any case, however, Ca^{2+} and Mg^{2+} are known to be critically involved in nuclear functions such as, e.g., transcription, i.e., RNA-polymerase activity. Moreover,these ions are enriched in the nuclei in comparison with cytoplasm in many cell types (for review see, e.g., reference 48) including *Tetrahymena* (15). In rat liver nuclei, for example, the absolute concentration of Ca^{2+} and Mg^{2+} is in the range of 25 mM (40), including very probably bound and free ions. Unfortunately, neither the absolute nor the free concentration of Ca^{2+} and(or) Mg^{2+} has been determined in *Tetrahymena* macronuclei to date. The fact, however, that *Tetrahymena* macronuclei burst upon lowering the Ca/Mg concentration below 1 mM indicates an essential role of Ca^{2+} and(or) Mg^{2+} for the structural integrity of these nuclei.

Finally, a physiological significance of our findings is also indicated by the fact that only expanded and not contracted nuclear membranes are capable of releasing RNA from isolated *Tetrahymena* macronuclei, which we will publish elsewhere.

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