capable of excluding large macromolecules, is assembled around condensed chromatin before expansion, and that, therefore, the telophase nucleus encloses a sealed and soon to be expanded aqueous volume. The small aqueous space enclosed by the forming nuclear envelope would contain few dextran molecules. Large dextrans trapped in this small space would, according to this model, remain in the nucleus after its expansion but would be further diluted by nuclear expansion. Smaller dextrans would freely redistribute into the nucleus through the pores in the envelope.

In conclusion, our data identify a rapid, efficient, and size-selective mechanism by which large molecules can be excluded from the reassembling nucleus during mitosis. Although not all sufficiently large macromolecules would be expected to distribute similarly to dextrans (*4*), our data suggest that large, freely diffusible proteins would be excluded. We therefore propose that such exclusion might be among the earliest steps in reestablishing the interphase macromolecular identities of nucleoplasm and cytoplasm. Periodic access of cytoplasmic proteins to the nucleus, or the gradual accumulation of proteins in the nucleus of the postmitotic cell, could provide a novel mechanism for cell cycle–dependent gene regulation. Finally, the mechanism described here could periodically dilute soluble cytoplasmic proteins, such as tubulin (*3*), when nuclear envelope breakdown admits them into the nucleus, and then concentrate them during postmitotic expansion of the resealed daughter nuclei. Such concentration changes could consequently alter cytoplasmic architecture or metabolism.

REFERENCES AND NOTES

1. P. L. Paine and C. M. Feldherr, *Exp. Cell Res.* **74**, 81 (1972).
2. W. M. Bonner, *J. Cell Biol.* **64**, 431 (1975).
3. E. M. De Robertis, R. F. Longthorne, J. B. Gurdon, *Nature (London)* **272**, 254 (1978).
4. K. Luby-Phelps, F. Lanni, D. L. Taylor, *J. Cell Biol.* **102**, 2015 (1986).
5. P. L. Paine *et al.*, *Nature (London)* **254**, 109 (1975); R. Peters, *J. Biol. Chem.* **258**, 11427 (1983).
6. P. L. McNeil, R. F. Murphy, F. Lanni, D. L. Taylor, *J. Cell Biol.* **98**, 1556 (1984).
7. Cover slips containing subconfluent monolayers of cells were washed with warm, divalent cation-free phosphate-buffered saline (PBS), *p*H 7.4, then incubated briefly in 25 μl of PBS containing TRDx10 (5 mg/ml; Molecular Probes) and FDx (10 mg/ml) of one of several sizes: average molecular weight of 4,100 (FDx4), 10,000 (FDx10), 40,000 (FDx40), 70,000 (FDx70), or 150,000 (FDx150); Sigma Chemical Co.). These values are, according to their manufacturer, the number average molecular weight ± 5%. Cover slips were then scratched with jeweler's forceps, thus creating many "wounds" in the monolayer. After gentle rinsing with warm medium to remove unincorporated dextrans, cells were incubated for 24 hours at 37°C, by which time many of the cells had undergone mitosis.
8. G. J. Todaro, G. Lazar, H. Green, *J. Cell. Comp. Physiol.* **66**, 325 (1965); R. Dulbecco, *Nature (London)* **227**, 802 (1970).
9. Most cells on scratched cover slips were not fluorescent, as only a small percentage lined denuded zones, and cells that were loaded exhibited a wide range of fluorescence intensities (three orders of magnitude). This heterogeneity of fluorescence simplified identification of daughter cells, which, when derived from a loaded cell, exhibited fluorescence intensities similar to one another (within 10%). Other distinguishing characteristics of daughter cells were their rounded or mirror-image morphologies and their occasional residual connection by a cytoplasmic bridge. Fluorescence excitation in the microscope inhibited cell progression through mitosis, making it technically unfeasible to follow a single cell through mitotic division.
10. Fluorescence from cells was measured quantitatively by means of a photomultiplier (9558B, Thorn EMI); the current from it was amplified (current amplifier 427, Keithley) and displayed as a deflection on a chart recorder. The photomultiplier was coupled optically to a Zeiss photomicroscope and a 40× phase Neofluar (Zeiss) lens by means of a Zeiss photometer head equipped with an aperture that limited the measured region of fluorescence to a 5-μm-diameter spot size. This aperture was positioned over cell cytoplasm or nucleus by movement of the microscope stage. Then FDx and TRDx10 fluorescence were measured from the 5-μm spot by manual alternation of rhodamine and fluorescein filter sets (Zeiss). Background fluorescence, measured from a cell-free area of the microscope slide, was subtracted from cellular fluorescence. Background was never more than 10% of cellular fluorescence.
11. In positioning the photometric aperture over the nucleus, we collected fluorescence from nucleoplasm plus the cytoplasm above and below the nucleus. Our measurements underestimate the true ratio of FDx_{nuc} to FDx_{cyt}, as this would require a negligible volume of cytoplasm above and below the measured region of the nucleus.
12. C. M. Feldherr, *J. Cell Biol.* **31**, 199 (1966).
13. D. J. Fry, in *The Diversity of Membranes*, vol. 2 of *Mammalian Cell Membranes*, G. A. Jamieson and D. M. Robinson, Eds. (Butterworth, London, 1977), pp. 197–265; S. Ito, K. Dan, D. Goodenough, *Chromosoma* **83**, 441 (1981).

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Technical Comments

Quick-Freeze Lipid Techniques: Correction

In my report "Lipid domains in fluid membranes: A quick-freeze differential scanning calorimetry study" (*1*), one of the lipids used, dipalmitoyl phosphatidylcholine (DPPC), was incorrectly labeled throughout the text as distearoyl phosphatidylcholine (DSPC) (*2*). Accordingly, figure 2A, a conventional scan of a mixture of pure dimyristoyl phosphatidylcholine (DMPC) bilayers and pure DPPC bilayers has two main endotherms with transition temperatures of 24°C (DMPC) and 42°C (DPPC). Figures 2B, 2D, and 2E are conventional scans of bilayers formed from a 1:1 molar mixture of DMPC and DPPC (DMPC-DPPC bilayers). These scans show, as expected, one major endotherm characteristic of bilayers formed from a mixture of these two lipids.

Unlike the conventional scans of DMPC-DPPC bilayers, the quick-freeze scan, figure 2C, shows two separate endotherms. Unlike conventional scans of pure DMPC bilayers or pure DPPC bilayers, no pretransitions are observed. The onset temperatures of the two endotherms seen in the quick-freeze scan are approximately the same (19° and 44°C) as those of the main transitions of pure DMPC and DPPC. The endotherms observed in the quick-freeze scan of the DMPC-DPPC bilayers are, however, substantially broader and differ in shape from those observed by conventional calorimetry on bilayers composed of the individual lipid species (figure 2A). Although the higher temperature endotherm seen in figure 2C begins at about 44°C, heat absorption is not complete until approximately 63°C. Since the transition temperature of pure DSPC bilayers is 55°C, the temperature range of the higher temperature endotherm seen in figure 2C makes the error in labeling DPPC as DSPC less obvious. I regret any confusion this may have caused.

It should be emphasized that for bilayers formed from a single lipid species, our quick-freeze techniques have never given samples whose calorimeteric behavior differed from that of conventional preparations. The major finding illustrated in figure 2 is, therefore, that quick-freeze methodology applied to DMPC-DPPC bilayers gives thermograms that differ from those obtained by conventional differential scanning calorimetry (DSC) on this mixture. The two peaks obtained for DMPC-DPPC bilayers by quick-freeze DSC also differ from those for pure bilayers of either DMPC or DPPC.

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REFERENCES AND NOTES

1. D. L. Melchior, *Science* **234**, 1577 (1986).

2. I would like to thank Michael A. Singer and Leonard Finegold for pointing out the labeling error.

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Quick-freeze lipid techniques: correction

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