

Nuclear Actin and Myosin As Control Elements in Nucleocytoplasmic Transport

Melvin Schindler and Lian-Wei Jiang

Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824. Dr. Jiang's permanent address is Department of Medical Physics, Beijing Medical University, Beijing, China.

Abstract. Fluorescence redistribution after photobleaching (FRAP) was used to examine the role of actin and myosin in the transport of dextrans through the nuclear pore complex. Anti-actin antibodies added to isolated rat liver nuclei significantly reduced the flux rate of fluorescently labeled 64-kD dextrans. The addition of 3 mM ATP to nuclei, which enhances the flux rate in control nuclei by ~250%, had no enhancement effect in the presence of either anti-

actin or anti-myosin antibody. Phalloidin (10 μ M) and cytochalasin D (1 μ g/ml) individually inhibited the ATP stimulation of transport. Rabbit serum, anti-fibronectin, and anti-lamins A and C antibodies had no effect on transport. These results suggest a model for nuclear transport in which actin/myosin are involved in an ATP-dependent process that alters the effective transport rate across the nuclear pore complex.

THE accompanying paper (10) demonstrated that the rate of flux of fluorescently labeled dextrans (64 kD apparent molecular weight) across nuclear pore complexes in isolated rat liver nuclei could be quantitated using the technique of fluorescence redistribution after photobleaching (FRAP).¹ Dextran transport was greatly enhanced in the presence of ATP, phosphoinositides, RNA, and insulin. In the context of early observations by DuPraw (7), which detail mechanical stretching properties of the nuclear surface, and others that describe pore diameter fluctuations (8, 21, 32), our results suggest that the nuclear pore may function in the manner of a diaphragm capable of altering its transport properties in response to effector substances. As demonstrated by Paine et al. (21), a variation of only 10 Å in radius of the pore could enhance transport rates by a factor of 10. To explain our induced dextran flux rate enhancements, we suggested that the pore may contain a diaphragm composed of ATP-dependent contractile proteins, e.g., actin and myosin that mediate changes in pore state. The existence of actin in nuclei has been a matter of interest since Ohnishi et al. (19, 20) identified actin in extracts of isolated calf thymus nuclei. Because of the ubiquitousness of actin in the cell and its ability to translocate across the nuclear pore complex (26), a true nuclear localization or role has been difficult to establish. LeSturgeon et al. (16) and Jockusch et al. (11) demonstrated nuclear localization of actin in *Physarum polycephalum* nuclei, Douvas et al. (6) provided evidence that actin existed in rat liver nuclei, while Clark and Rosenbaum characterized an actin filament matrix in *Xenopus laevis* oocyte nuclei (5). Perhaps the most direct example that intranuclear actin has a nuclear localization and function was presented by Rungger

et al. (25). Injection of anti-actin antibodies into the germinal vesicles of *Xenopus laevis* oocyte severely affected chromosome condensation. Injection into the cytoplasm had no effect. The equally ubiquitous myosin has also been characterized as an endogenous nuclear protein (6, 16, 17, 20). LeSturgeon points out: "If a protein appears to exist in more than one subcellular compartment after cell fractionation, then an organelle consistent function must actually be elucidated before cytoplasmic contamination can be eliminated as the explanation for its presence" (17). This communication provides evidence that actin and myosin are components of nuclear transport mechanisms in isolated rat liver nuclei, and may be structural elements of the pore complex.

Materials and Methods

Nuclear Isolation

Rat liver nuclei were isolated as described (13) with minor modifications (27). These nuclei were characterized (27) and stored at 4°C in 0.25 M sucrose-10 mM Hepes-1 mM Mg⁺⁺-pH 7.4 buffer. All measurements were done on nuclei within 3 d of preparation.

Reagents

Fluorescein-labeled dextrans, ATP, and phalloidin were products of Sigma Chemical Co. (St. Louis, MO). Cytochalasin D was a product of Aldrich Chemical Co. (Milwaukee, WI). Rabbit anti-actin (lot No. R863) and anti-myosin (lot No. R067) were products of Miles Laboratories Inc. (Naperville, IL), whereas rabbit anti-fibronectin (lot No. 002B) was obtained from Transformation Research Inc. (Farmingham, MA). Rabbit serum was obtained from GIBCO (Grand Island, NY), whereas purified calf thymus actin and rabbit muscle myosin was a gift of T. Metcalf III, Department of Biochemistry, Michigan State University. The rabbit muscle myosin was maintained in myosin buffer, which consists of 50 mM potassium phosphate, pH 6.8, 0.5 M KCl, 1 mM dithiothreitol, 50% glycerol, 0.05% sodium azide. Approximately 210 μ g/ml of myosin was used in the various assays. The myosin buffer at the dilution used had no effect on control flux rates. Actin was stored in 3 mM

¹ Abbreviation used in this paper: FRAP, fluorescence redistribution after photobleaching.

Imidazole buffer, pH 7.2, 0.5 mM ATP, 0.1 mM CaCl₂, 0.75 mM β-mercaptoethanol. The actin buffer, at the dilutions used, had no effect on control flux rates. Dr. Larry Gerace, Department of Cell Biology, The Johns Hopkins University, graciously supplied guinea pig anti-lamins A and C antibodies.

Fluorescent Dextran Influx Assay

The FRAP experiments were done, and results calculated as described (10). Antibodies, actin, and myosin were added to a 1-ml nuclear suspension after the addition of fluorescein-labeled dextrans. When antibodies were added to the nuclear suspension with their appropriate ligands, a preincubation step was included before addition to the dextran-nuclei suspension.

Results

The method of fluorescence photobleaching was used in conjunction with fluorescent-labeled dextrans (64 and 150 kD in apparent molecular weight) to examine nuclear transport/translocation in isolated rat liver nuclei. The technique and calculations are described in the accompanying paper (10). The addition of 3 mM ATP to isolated nuclei enhances the transport rate for 64-kD dextrans from $2.2 \pm 0.8 \times 10^{-3} \text{ s}^{-1}$ to $7.2 \pm 1.6 \times 10^{-3} \text{ s}^{-1}$ (10). Fig. 1A represents the fluorescence recovery curve for nuclei in the presence of anti-actin + actin (100 μg/ml) + 3 mM ATP, and Fig. 1B is the

recovery curve for nuclei in the presence of anti-actin antibody. Whereas recovery is observed under the incubation conditions presented in Fig. 1A, slow or no recovery is observed in the presence of only anti-actin (Fig. 1B, Table I). Fluorescence recovery is indicative of fluorescent dextran flux (10, 22–24). Table I summarizes the data obtained by the FRAP/nuclear transport technique in the presence of agents that bind and affect actin and myosin.

As the control results indicate, rabbit serum and anti-actin and anti-myosin in the presence of saturating concentrations of appropriate ligand do not prevent ATP-stimulated transport of 64-kD dextran. Anti-fibronectin, a nonnuclear antibody control, and anti-lamins (A and C) (lamins comprise a submembranous nuclear matrix [9]), also do not decrease the ATP-stimulated flux rate. The addition of anti-actin antibody either significantly reduces transport (~50% nuclei examined are essentially blocked for dextran transport) or greatly diminishes the ATP stimulatory effect (~61 to ~90% from ATP-stimulated control). Phalloidin, an F-actin stabilizer and F-actin ATPase inhibitor (31), and cytochalasin D, an F-actin disrupter (28), both greatly decrease ATP-promoted stimulation (~64% and ~49% from ATP-stimulated control). Anti-

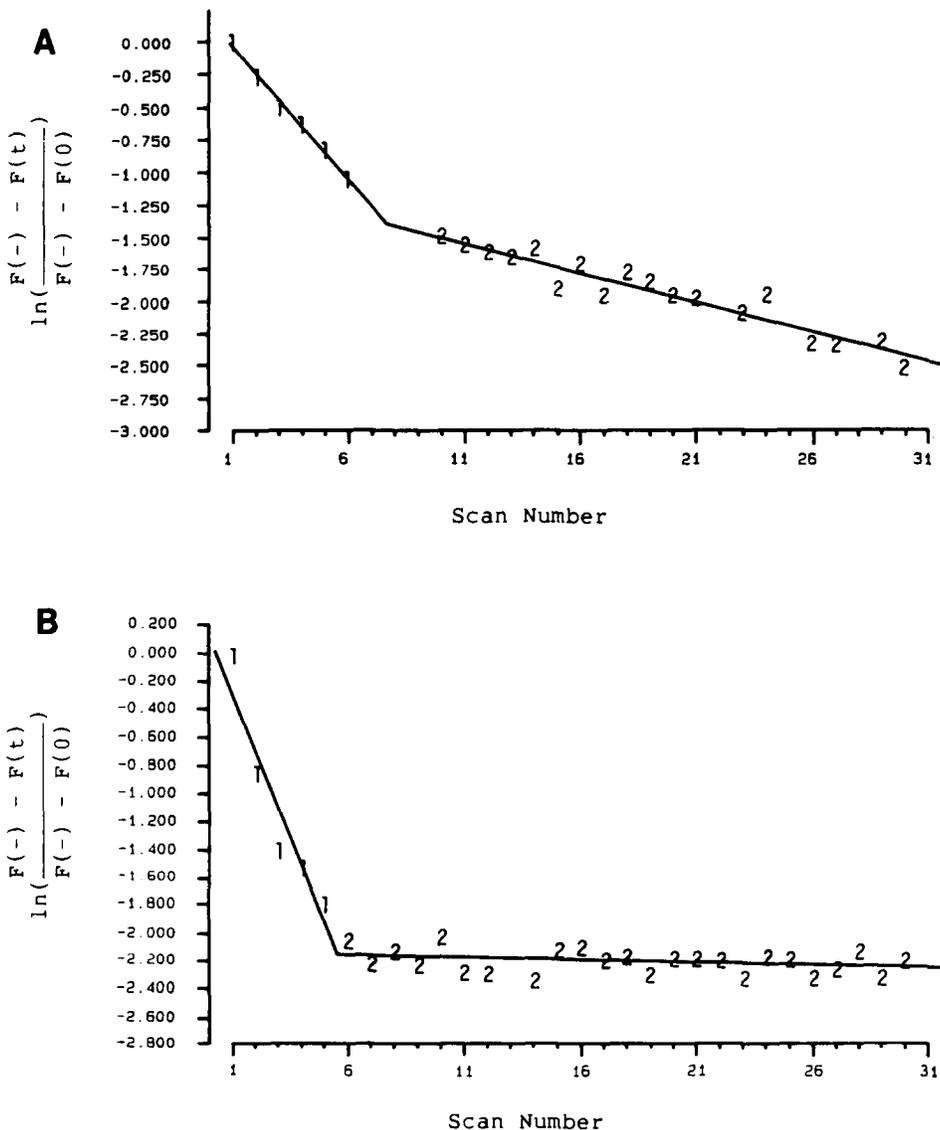


Figure 1. Fluorescence recovery of labeled dextrans in isolated rat liver nuclei in the presence of anti-actin. Anti-actin (20 μl of an anti-actin solution of 640 μg/ml total protein) was preincubated with 100 μg/ml of purified calf thymus actin and then added to a nuclear suspension that contained fluorescein-labeled 64-kD dextrans and 3 mM ATP (A), whereas B represents measurements without the presence of actin. Photobleaching measurements on single nuclei were done as described (10). The results are presented as a semilogarithmic plot of $\ln [F(-) - F(t)]$ vs. scan number. $F(-)$, $F(0)$, and $F(t)$ are the fluorescence before the photobleach, after, and at time t after the bleach (22). Component 1 is ascribed to surface adsorption (10), whereas component 2 corresponds to the recovery term used by Peters (22) to calculate dextran flux. Each scan with delay is 5 s.

Table I. Control of Nuclear Transport by Actin and Myosin

Treatment	Transport rate ($\times 10^3 \text{ s}^{-1}$)	% Change from ATP- stimulated control
	<i>mean \pm SD</i>	
Controls		
ATP (3 mM)	7.2 \pm 1.6 (12)*	—
Rabbit serum (20 μ l) + ATP (3 mM)	6.8 \pm 0.7 (8)	-6
Anti-actin (20 μ l) + actin (100 μ g/ml) + ATP (3 mM)	7.1 \pm 1.6 (5) [‡]	-1
Anti-myosin (20 μ l) + myosin (210 μ g/ml) + ATP (3 mM)	7.2 \pm 1.6 (5) [§]	0
Actin (100 μ g/ml) + ATP (3 mM)	6.7 \pm 0.9 (6)	-7
Myosin (210 μ g/ml) + ATP (3 mM)	6.9 \pm 1.8 (5)	-4
Anti-fibronectin (20 μ l)	3.0 \pm 0.7 (5)	—
Anti-fibronectin (20 μ l) + ATP (3 mM)	7.8 \pm 0.8 (4)	+8
1% Triton	4.4 \pm 0.4 (7)	—
1% Triton + ATP (3 mM)	9.4 \pm 2.4 (5)	+31
Actin effectors		
Anti-actin (20 μ l)	1.5 \pm 0.2 (4)	—
	\sim 0.1 (3)	—
Anti-actin (20 μ l) + ATP (3 mM)	2.8 \pm 0.6 (7)	-61
	0.7 \pm 0.1 (2)	-90
1% Triton + anti-actin (20 μ l)	1.7 \pm 0.3 (4)	—
1% Triton + anti-actin (20 μ l) + ATP (3 mM)	3.7 \pm 1.9 (6)	-49
Phalloidin (10 μ M) + ATP (3 mM)	2.6 \pm 0.9 (7)	-64
Cytochalasin D (1 μ g/ml) + ATP (3 mM)	3.7 \pm 1.0 (5)	-49
Myosin effectors		
Anti-myosin (20 μ l)	2.6 \pm 0.9 (4)	—
Anti-myosin (20 μ l) + ATP (3 mM)	3.7 \pm 1.5 (9)	-49

* Number of experiments (in parentheses).

[‡] 20 μ l of an anti-actin solution of 640 μ g/ml total protein.

[§] 20 μ l of an anti-myosin solution of 600 μ g/ml total protein.

^{||} 20 μ l of an anti-fibronectin solution of 1,000 μ g/ml total protein.

myosin appears not to block dextran transport, but significantly decreases ATP stimulation of transport (\sim 49% from ATP-stimulated control, Table I). Inhibition of ATP-enhanced transport does not occur when myosin and anti-myosin are added to the nuclei.

Detergent Treatment and the Effect on Anti-actin Inhibition

Nuclei were treated with 1% Triton X-100 (1 ml) for 20 min at 4°C, and then washed three times with 1 ml nuclear buffer (1 mM Hepes-1 mM Mg^{++} pH 7.4). These nuclei have been characterized in reference 27 and have both membranes removed. Triton X-100 treatment enhances transport over whole nuclear controls by +100%, $2.2 \times 10^{-3} \text{ s}^{-1}$ to $4.4 \times 10^{-3} \text{ s}^{-1}$ (Table I). Addition of 3 mM ATP to Triton X-100-treated nuclei further enhances dextran flux +31% compared

to whole nuclei stimulated by 3 mM ATP and +114% compared to Triton-treated nuclei. The latter comparison, however, is considerably less than the enhancement of transport observed when nondetergent-treated nuclei are incubated with ATP (3 mM) (enhancement of +227%). This may suggest the possibility that Triton X-100 unmasks additional transport channels that maintain the molecular sieve properties (1% Triton X-100-treated nuclei exclude 150-kD dextrans), but these channels are not affected by ATP (Jiang, L., and M. Schindler, manuscript submitted for publication). Addition of anti-actin to Triton X-100-treated nuclei inhibits transport by \sim 61% when compared with Triton X-100-treated nuclei and blocks ATP-mediated dextran transport stimulation in Triton X-100-treated nuclei also by \sim 61% (Table I).

Discussion

The variations in nuclear pore diameter (8, 14, 32) and the observed mechanical stretching of these structures (7) have led to models of a nuclear pore whose accessibility and transport function may be controlled by contractile proteins, most prominently, actin and myosin (7, 17). Considering the general features of nuclear transport and the observation that ATP effects transport (10), it is not surprising that an actin/myosin complex, actomyosin, may play a role in the translocation activity of the nuclear pore complex. Nonmuscle myosin (an ATPase that forms an enzymatically active complex with actin) has been demonstrated to provide energy for cell and organelle movement and maintain the intracellular structural organization of the cytoskeleton (15). In the context of our results, note that an actomyosin-based pore model presented by LeStourgeon (17) incorporates myosin and actin as transport components. He suggests that myosin molecules are radially positioned around the pore with their tails embedded or absorbed against the outside nuclear membrane, while the globular heads protrude into the lumen of the pore, free to bind actin in thin filaments and pull the filaments through the pore as a function of ATP hydrolysis. We can use this model with a number of modifications. The results with anti-actin and anti-myosin antibodies, both of which do not have access to the nucleoplasm, provide evidence that either actin and myosin have some antigens exposed on the cytoplasmic surface of the nuclear pore complex, or they are located on the outer nuclear membrane. Removal of both membranes by Triton X-100 (Table I) did not effect the anti-actin inhibition of transport, which suggests a nuclear pore complex localization for this contractile element. The direct blocking effect of anti-actin antibody would also argue in favor of a pore-associated actin with bound antibody that occludes a transport channel. Since alterations of actin structure may interfere with its ability to initiate ATP hydrolysis alone (15, 31) or in a complex with myosin (15), the antibody, cytochalasin, and phalloidin results suggest a direct role for actin and myosin in nuclear pore-mediated transport. Because dextrans appear not to have potential nuclear transport signals (12), the variation in rate of dextran flux observed can most simply be explained by a variation in pore structure that we suggest is controlled by energy-dependent mechanochemical proteins, e.g., nonmuscle actomyosin. We would alter the LeStourgeon model (17) to suggest that myosin assemblies at the cytoplasmic face of the nuclear pore interact with actin to form an ATP-dependent variable diaphragm in the pore

whose opening may be controlled by agents that affect actin/myosin interactions, particularly ATPase activity. The ATPase observed by Smith and Wells in the nuclear envelope fraction of rat liver nuclei (29) and the ATPase observed by Berrios et al. (2) in *Drosophila* nuclear matrix-pore complex-lamina fraction, if not identical to myosin or perhaps myosin-like spectrin (3, 15), may indeed turn out to be components of this mechanochemical assembly. Considering the connections of the cytoplasmic actin-based cytoskeleton with the nuclear surface, particularly the nuclear pore complex (4), a plasma membrane-intracellular-nuclear communication system that relies on actin and associated protein interactions may be entertained.

Caveat Investigator—Are Transport Studies in Isolated Rat Liver Nuclei Valid?

In a comparison study, Peters examined the transport rate of dextrans microinjected into cultured liver cells (24) and in isolated nuclei suspensions that contained dextrans (23). In the cell, the rate constant for 62-kD dextrans was reported to be $0.7 \pm 2.1 \times 10^{-3}$, whereas in isolated nuclei $2.6 \pm 0.6 \times 10^{-3}$. Although a rate constant was calculated for 62-kD dextrans in vivo, Peters points out that the dextran did not or very slowly permeated into the nucleus. The results were interpreted to demonstrate that in vivo, the functional pore radius is $\sim 5 \text{ \AA}$ smaller than in the isolated nucleus. Paine et al. point out: "There is evidence that the patent pore radius is different in different cell types and varies within a single cell type as a function of the cell cycle and nutritional state" (21). Differences in transport observed between the in vivo and in vitro system we believe reflect the sensitivity of the transport mechanism to activating ligands concentrations and cytoskeletal elements as demonstrated (10). Peters, in fact, suggests the possibility that isolated nuclei may have a much larger area density of functional pores. Thus, the comparison of an in vitro and in vivo nuclear transport system results in the hypothesis that in resting cells (primary hepatocyte culture) the majority of nuclear pores may be closed! Based on all the electron microscopic comparisons of isolated nuclei with those in whole cells (14, 18), the demonstration that pore complex integrity is maintained even under harsh purification schemes (30), and the large body of transport literature for rat liver nuclei that has now been reconciled by Agutter (1), which demonstrates the reasons for laboratory variability, we believe that our results in isolated rat liver nuclei are significant and may be extended to the cell. A comparison of in vivo and in vitro nuclear transport using FRAP may serve to further illuminate additional regulating and controlling influences.

This work was supported by National Institutes of Health grant GM 30158.

Received for publication 28 May 1985, and in revised form 14 November 1985.

References

1. Agutter, P. S. 1984. Nucleocytoplasmic RNA transport. *Subcell. Biochem.* 10:231-357.

2. Berrios, M., G. Blobel, and P. A. Fisher. 1983. Characterization of an ATPase/dATPase activity associated with the *Drosophila* nuclear matrix-pore complex-lamina fraction. *J. Biol. Chem.* 258:4548-4555.
3. Burrige, K., T. Kelly, and P. Mangeat. 1982. Nonerythrocyte spectrins: actin-membrane attachment proteins occurring in many cell types. *J. Cell Biol.* 95:478-486.
4. Capco, P. G., K. M. Wang, and S. Penman. 1982. The nuclear matrix: three-dimensional architecture and protein composition. *Cell.* 29:847-858.
5. Clark, T. G., and J. L. Rosenbaum. 1979. An actin filament matrix in hand-isolated nuclei of *X. laevis* oocytes. *Cell.* 18:1101-1108.
6. Douvas, A. S., C. A. Harrington, and J. Bonner. 1975. Major nonhistone proteins of rat liver chromatin: preliminary identification of myosin, actin, tubulin, tropomyosin. *Proc. Natl. Acad. Sci. USA.* 72:3902-3906.
7. DuPraw, E. J. 1970. In *DNA and Chromosomes*. Holt, Rinehart, and Winston, New York. 170-180.
8. Gall, J. G. 1967. Octagonal nuclear pores. *J. Cell Biol.* 32:391-399.
9. Gerace, L., and G. Blobel. 1982. Nuclear lamina and the structural organization of the nuclear envelope. In *Cold Spring Harbor Symposia on Quantitative Biology*, XVI. 967-978.
10. Jiang, L.-W., and M. Schindler. 1985. Chemical factors that influence nucleocytoplasmic transport: a fluorescence photobleaching study. *J. Cell Biol.* 102:853-858.
11. Jockusch, B., D. F. Brown, and H. P. Rusch. 1971. Synthesis and some properties of an actin-like nuclear protein in the slime mold *Physarum polycephalum*. *J. Bacteriol.* 108:705-714.
12. Kalderon, D., W. D. Richardson, A. F. Markham, and A. E. Smith. 1984. Sequence requirements for nuclear location of Simian virus 40 large-T antigen. *Nature (Lond.)* 311:33-38.
13. Kay, R. R., D. Fraser, and I. R. Johnston. 1972. Method for the rapid isolation of nuclear membranes from rat liver. Characterization of the membrane preparation and its associated DNA polymerase. *Eur. J. Biochem.* 30:145-154.
14. Kessel, R. G. 1973. Structure and function of the nuclear envelope and related cytomembranes. *Prog. Surf. Membr. Sci.* Academic Press, Inc., New York. 243-329.
15. Korn, E. D. 1978. Biochemistry of actomyosin-dependent cell motility (a review). *Proc. Natl. Acad. Sci. USA.* 75:588-599.
16. LeStourgeon, W. M., A. Forer, Y.-Z. Yang, J. S. Betram, and H. P. Rusch. 1975. Major components of nuclear and chromosome non-histone proteins. *Biochim. Biophys. Acta.* 379:529-552.
17. LeStourgeon, W. M. 1978. The occurrence of contractile proteins in nuclei and their possible functions. In *The Cell Nucleus Chromatin*, Part C. Volume VI. H. Busch, editor. Academic Press, Inc., New York. 305-326.
18. Maul, G. G. 1977. The nuclear and the cytoplasmic pore complex structure, dynamics, distribution, and evolution. *Int. Rev. Cytol.* G. H. Bourne and J. F. Danielli, editors. Academic Press, Inc., New York, San Francisco, London. 75-186.
19. Ohnishi, T., H. Kawamura, and T. Yamamoto. 1963. Extraktion eines dem aktin ähnlichen proteins aus DEM zellkern des kalbsthymus. *J. Biochem.* 54:298-300.
20. Ohnishi, T., H. Kawamura, and Y. Tanaka. 1964. Die aktin und myosin ähnliche proteine in kalbsthymus zellkern. *J. Biochem.* 56:6-15.
21. Paine, P. L., L. C. Moore, and S. B. Horowitz. 1975. Nuclear envelope permeability. *Nature (Lond.)* 254:109-114.
22. Peters, R. 1983. Nuclear envelope permeability measured by fluorescence microphotolysis of single liver cell nuclei. *J. Biol. Chem.* 258:11427-11429.
23. Peters, R. 1984. Flux measurement in single cells by fluorescence microphotolysis. *Eur. Biophys. J.* 11:43-50.
24. Peters, R. 1984. Nucleo-cytoplasmic flux and intracellular mobility in single hepatocytes measured by fluorescence microphotolysis. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1831-1836.
25. Rungger, D., E. Rungger-Brandle, C. Chaponnier, and G. Gabbiani. 1979. Intracellular injection of anti-actin antibodies into *Xenopus* oocytes blocks chromosome condensation. *Nature (Lond.)* 282:320-321.
26. Sanger, J. W., J. M. Sanger, T. E. Kreis, and B. M. Jockusch. 1980. Reversible translocation of cytoplasmic actin into the nucleus caused by dimethylsulfoxide. *Proc. Natl. Acad. Sci. USA* 77:5268-5272.
27. Schindler, M. 1984. Alterations in nuclear anatomy by chemical modification of proteins in isolated rat liver nuclei. *Exp. Cell Res.* 150:84-96.
28. Schliwa, M. 1982. Action of cytochalasin D on cytoskeletal networks. *J. Cell Biol.* 92:79-91.
29. Smith, C. D., and W. W. Wells. 1984. Solubilization and reconstitution of a nuclear envelope-associate ATPase. *J. Biol. Chem.* 259:11890-11894.
30. Unwin, P. N. T., and R. A. Milligan. 1982. A large particle associated with the perimeter of the nuclear pore complex. *J. Cell Biol.* 93:63-75.
31. Wieland, Th. 1977. Interaction of phallotoxins with actin. *Adv. Enzyme Regul.* 15:285-300.
32. Wunderlich, F., and W. W. Franke. 1968. Structure of macronuclear envelopes of *Tetrahymena pyriformis* in the stationary phase of growth. *J. Cell Biol.* 38:458-462.