Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 4678–4682, May 1992
Cell Biology

Thymosin β_4 (Fx peptide) is a potent regulator of actin polymerization in living cells

(cytoskeleton/assembly)

MITCHELL C. SANDERS*, ALLAN L. GOLDSTEIN†, AND YU-LI WANG*‡

*Cell Biology Group, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, MA 01545; and †Department of Biochemistry and Molecular Biology, The George Washington University School of Medicine and Health Services, Washington, DC 20037

Communicated by Paul C. Zamecnik, February 3, 1992

ABSTRACT **Thymosin β_4 (β_4) is a 5-kDa polypeptide originally identified in calf thymus. Although numerous activities have been attributed to β_4, its physiological role remains elusive. Recently, β_4 was found to bind actin in human platelet extracts and to inhibit actin polymerization *in vitro*, raising the possibility that it may be a physiological regulator of actin assembly. To examine this potential function, we have increased the cellular β_4 concentration by microinjecting synthetic β_4 into living epithelial cells and fibroblasts. The injection induced a diminution of stress fibers and a dose-dependent depolymerization of actin filaments as indicated by quantitative image analysis of phalloidin binding. Our results show that β_4 is a potent regulator of actin assembly in living cells.**

Actin is involved in many important nonmuscle cellular processes such as cell locomotion, chemotaxis, phagocytosis, and cytokinesis. Previous studies indicate that the regulation of these processes involves the dynamic assembly and disassembly of actin filaments and high-order structures, most likely through the interplay of a number of actin-binding factors (for reviews, see refs. 1–3). However, important questions remain concerning the actual mechanism for the regulation of actin assembly in living cells.

One important mechanism for the regulation of actin assembly involves the inhibition of spontaneous polymerization of the high concentration of actin subunits in nonmuscle cells. Previously we have demonstrated that such inhibition is probably achieved through the sequestration of actin monomers from addition onto active sites of assembly (4). However, although a number of monomer sequestering proteins, such as profilin, actin-depolymerizing factor/destrin, depactin, actobindin, and cofilin, have been identified (5–10), none of these factors appears to be present at a high enough concentration to account for the levels of unpolymerized actin in nonmuscle cells. In addition, despite their ability *in vitro* to inhibit the polymerization of purified actin subunits, the activity *in vivo* remains in question, since a number of factors are capable of stabilizing actin filaments or overcoming the sequestration effect (11).

The newest member to this family of potential actin regulators is thymosin β_4 (β_4), an ≈5-kDa polypeptide first isolated from an extract of calf thymus (12–14). β_4 was originally proposed solely as a thymic hormone, based on its ability to stimulate the T-lymphocyte terminal deoxynucleotidyltransferase after intraperitoneal injection into mice (13, 15) and to inhibit macrophage migration at a dosage of 5–500 pM (16). However, later studies indicated that β_4 is present at a relatively high abundance in several tissues (17–19) and cell lines (20, 21), raising the possibility that it may also serve a more ubiquitous role. Recently, β_4 was found to be identical to a small acidic peptide (Fx) in platelet extracts, which can bind a significant portion of monomeric actin and inhibit salt-induced polymerization of actin filaments *in vitro* (22, 23). Since β_4 appears to be present at a higher level than most other actin sequestration factors in platelets and cultured cells (20–23), it is possible that β_4 may represent the primary factor responsible for the regulation of actin polymerization (24).

One approach to test this putative physiological role of β_4 is to induce the "overexpression" in living cells and to examine the subsequent effects on specific structures or functions. In the present study, we have taken this approach by microinjecting excess synthetic β_4 into living epithelial cells and fibroblasts. The effects on actin were then determined by measuring the amount of binding of fluorescent phalloidin with quantitative image analysis. Our results indicate that β_4 is indeed capable of inducing extensive depolymerization of actin filaments in living cells.

MATERIALS AND METHODS

Preparation of Thymosins. β_4 and thymosin α_1 (α_1), provided by Alpha 1 Biomedicals (Washington), were synthesized as described (25, 26). The concentration of β_4 was determined by the bicinchoninic acid assay (BCA; Sigma) with bovine serum albumin as the reference and was calibrated with amino acid analysis of β_4.

Actin Polymerization Assay. The activity of synthetic β_4 *in vitro* was determined by the pyrene-conjugated actin assay. Pyrene-actin was prepared as described by Kouyama and Mihashi (27) with minor modifications (28). The pyrene-actin conjugate had a dye-to-protein molar ratio of 1.3. Labeled and unlabeled actin monomers were dialyzed for 2–3 days with three exchanges of G-buffer (0.5 mM ATP/0.2 mM $CaCl_2$/0.5 mM dithiothreitol/2 mM Tris·HCl, pH 7.0) and then centrifuged at 35,000 rpm in a 42.2 Ti rotor at 4°C for 2 hr. Pyrene-actin was mixed 1:20 (wt/wt) with unlabeled actin at a final actin concentration of 4.2 μM and incubated with various concentrations of β_4 for 10 min. At $t=0$, the solution was mixed with 0.5 volume of 3× polymerization buffer (6 mM $MgCl_2$/0.3 M KCl/3 mM ATP/75 mM imidazole, pH 7.0), and the fluorescence intensity was measured in a spectrofluorimeter (LS-3; Perkin–Elmer) at an excitation wavelength of 365 nm and an emission wavelength of 407 nm. For steady-state readings, the samples were allowed to polymerize for 15 hr.

Cell Culture and Microinjection. Normal rat kidney (NRK) epithelial cells (NRK-52E; American Type Culture Collection) were cultured in F12K medium (JRH Biosciences, Lenexa, KS) supplemented with 10% (vol/vol) fetal calf serum (JRH Biosciences), 50 μg of streptomycin per ml, and 50 units of penicillin per ml and were maintained at 37°C under 5% CO_2/95% air. Swiss 3T3 cells were cultured as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: β_4 and α_1, thymosins β_4 and α_1.

‡To whom reprint requests should be addressed.

described (4). The cells were plated on microinjection dishes (29) 36–40 hr prior to microinjection.

For most of the experiments, thymosin peptides were dissolved in 5 mM Tris acetate (pH 6.95) containing 3 mg of fixable fluorescein-conjugated dextran (Molecular Probes) per ml at a concentration of 0.48–1.63 mM. High concentrations (≈20 mg/ml) of either α_1 or β_4 were found to be cytotoxic for ≈30% of the cells (inducing an apparent disintegration of the cell as observed under phase optics) and therefore were not included in the analysis. Pressure microinjection was performed as described (4, 30). The average volume of microinjection was estimated to be 2–3% of the cell volume by the method of Lee (31) with minor modifications (32).

Lamellipodia extension was induced by scraping a confluent cultured monolayer with a Pasteur pipet. Cells along the edge were microinjected following a recovery for 15 min.

Fluorescent Staining. Cells were incubated for 1 hr at 37°C after microinjection, then fixed with 4% formaldehyde, permeabilized with acetone, and stained with rhodamine-conjugated phalloidin (Sigma) as described by Sanders and Wang (4). Lamellipodia were fixed and extracted with a special protocol that preserves the highly labile structure (see Fig. 3; ref. 33). Immunofluorescence staining with polyclonal antibodies against actin (Biomedical Technologies, Stoughton, MA) or myosin II (provided by Keigi Fujiwara, National Cardiology Research Center, Osaka) was performed essentially as described by Amato *et al.* (34). Rhodamine-conjugated secondary antibodies were obtained from Tago.

Microscopy and Image Processing. Fluorescence images were recorded with a Zeiss Axiovert 10 microscope (Zeiss) coupled to a cooled CCD camera (Star I; Photometrics, Tucson, AZ) using a 100-W quartz–halogen lamp and either a 25×/NA 0.8 Plan-Neofluar or a 100×/NA 1.3 Neofluar objective (Zeiss). Images recorded with the illumination lamp off were subtracted from integrated fluorescence images with a digital image processor. The boundary of injected and uninjected sister cell pairs was defined interactively by using a graphics tablet and computer software developed in this laboratory. The intensities of pixels within the boundary were then integrated with a computer. The percentage of actin depolymerization was calculated by dividing the integrated rhodamine-phalloidin fluorescence of β_4-injected cells with that of uninjected sister cells.

RESULTS

β_4 was prepared by solid-phase peptide synthesis. To ensure that synthetic β_4 has an inhibiting activity against actin polymerization similar to that of β_4 purified from platelets (23), we first examined its *in vitro* effect on actin polymerization, using pyrene-actin as a fluorescent probe (27, 28). Synthetic β_4 prolonged the nucleation phase of actin polymerization and decreased the initial rate of elongation (Fig. 1). Based on a critical concentration of actin of 0.2 μM (2) and the extent of actin polymerization at steady state in the presence and absence of β_4, we calculated the dissociation constant for synthetic β_4 to be 0.6 μM.

Synthetic β_4 was microinjected at a needle concentration of 0.48–1.63 mM into NRK cells to 2–3% of the cell volume (31, 32). Thus, the intracellular concentration of synthetic β_4 should be in the range of 9.6–49 μM. After recovery for 1 hr, injected cells showed little or no apparent retraction and were indistinguishable from uninjected neighboring cells in phase morphology. However, when cells were fixed and stained with rhodamine-phalloidin, which binds specifically to actin filaments but not to monomers, those injected with β_4 showed a significant decrease in fluorescence intensity (Fig. 2*a*), indicating that β_4 had induced the depolymerization of actin filaments. The effects persisted for at least 5 hr after injection

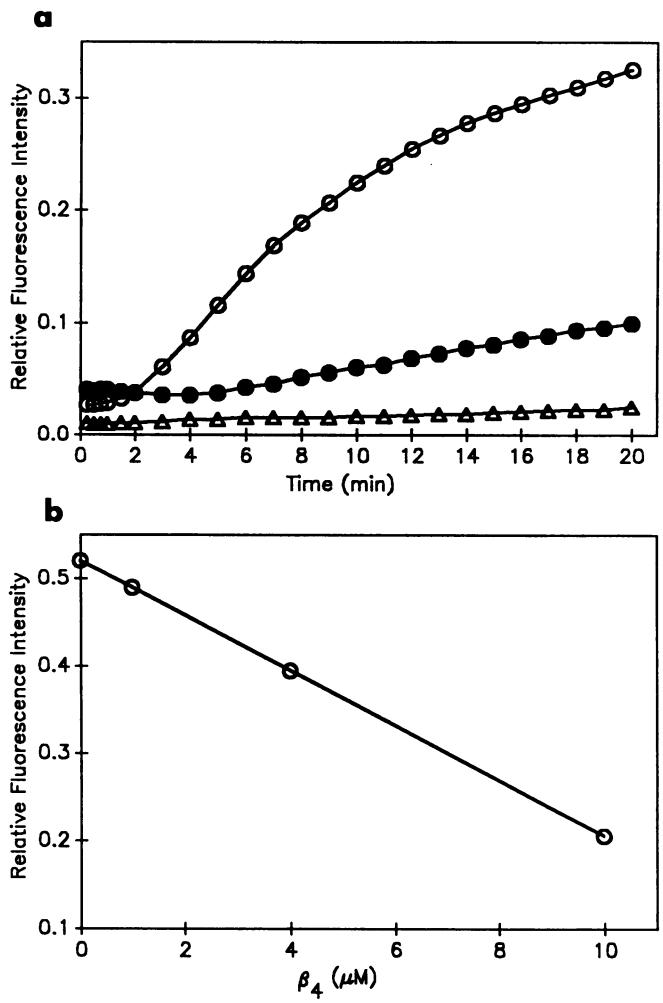

FIG. 1. Inhibition of actin polymerization by synthetic β_4 *in vitro*. Actin polymerization was measured by the increase of fluorescence intensity of pyrene-labeled actin. The assay mixture contains 4.2 μM total actin and 5% pyrene-actin. The lag period for nucleation was extended from 2 to 3 min for the control (○) to >5 min in the presence of 4 μM β_4 (●); the initial rate of filament elongation in the presence of 4 μM (●) and 6 μM (△) β_4 was also significantly reduced (*a*). In addition, the level of steady-state polymerization was reduced with a linear dependence on the concentration of β_4 (*b*).

and were most striking along stress fibers, although decreases could be discerned also along the circumferential bundles and lamellipodia in many cells (Figs. 2*a* and 3). In addition, immunofluorescence staining of 3T3 cells with a polyclonal antibody against actin revealed many prominent stress fibers in uninjected cells but few in injected cells (not shown). Control experiments were performed with either carrier solution alone or with α_1, an unrelated small peptide derived from the proteolysis of prothymosin (35). In neither case was there any apparent effect on filamentous actin structures as observed after fluorescent phalloidin staining (Fig. 2*b*). In addition, no effect on the level of filamentous actin was observed when β_4 was applied extracellularly through a microneedle (not shown).

The effects of β_4 on the lamellipodia were examined at high magnification after the induction of cell protrusion by wounding a confluent monolayer of NRK cells with a Pasteur pipet. Cells along the edge were injected with 1.28 mM β_4 15 min after the wound. A reduction in the intensity of phalloidin straining was observed both along the lamellipodia and in the more interior region (Fig. 3), indicating a decrease in the amount of actin filaments. However, most of the injected cells (87%, $n = 23$) spread out as well as uninjected neighbors,

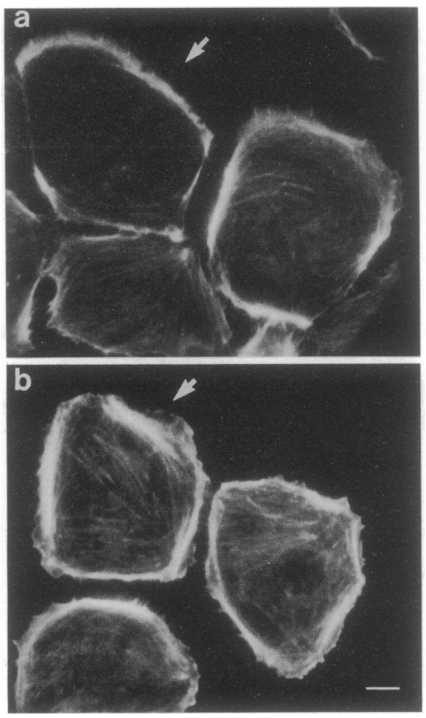

FIG. 2. Depolymerization of actin structures after microinjection of β_4. (*a*) Cell microinjected with 1.63 mM β_4 (arrow) showed a clear reduction in the intensity of rhodamine-phalloidin staining particularly along stress fibers. (*b*) Cell injected with α_1 (arrow) appeared identical to control cells. (Bar = 10 μm.)

similar to the response of cells injected with carrier solution alone (88.5%, n = 26). Furthermore, similar patterns of staining were observed in injected and uninjected cells.

To determine whether the injection of β_4 can affect the behavior of other actin binding proteins, injected 3T3 cells

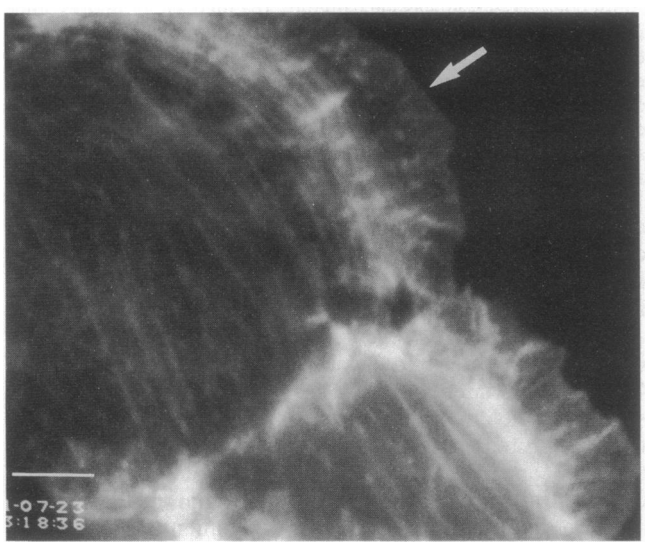

FIG. 3. Reduced amount of actin filaments in the apparently normal lamellipodia of β_4-injected cells. A confluent area of NRK cells was scraped with a Pasteur pipet and allowed to recover for 15 min. Cells along the edge were microinjected with 1.28 mM β_4 and incubated for 1 hr on the microscope stage. Cells were then fixed and stained with rhodamine-phalloidin. The injected (arrow) cell showed a decrease in the intensity of rhodamine-phalloidin staining in both the lamellipodia and other regions of the cytoplasm, although the pattern of staining appeared similar to that of the control. Lamellipodia extension appeared unaffected by this level of β_4 (arrow), as compared with a neighboring uninjected cell. (Bar = 5 μm.)

were stained with polyclonal antibodies against myosin II (Fig. 4). While myosin in control cells was organized as linear arrays of punctate structures (minifilaments) along stress fibers, cells injected with β_4 contained mainly disorganized myosin punctate structures. Thus, the organization of myosin appears to depend on the integrity of actin filament structures.

To quantify the effect of β_4 on actin assembly, we measured the relative amount of actin filaments in NRK cells by integrating the total fluorescence intensity of bound rhodamine-phalloidin within the cellular boundary. Cultures of NRK cells contained many pairs of sister cells that maintained a similar size and shape; thus, quantitative analysis of the effects of microinjection can be performed by using one of the sister cells as a reference. The extent of β_4-induced depolymerization was then calculated by dividing the intensity of injected cells with that of uninjected sister cells. A 40% depolymerization was observed upon the microinjection of 1.63 mM β_4 (needle concentration) (Fig. 5). The depolymerization effect appeared to be proportional to the concentration of β_4 within the range examined (0.48–1.63 mM), with a slope of 23.9% depolymerization per millimolar needle concentration. Control cells injected with carrier solution alone or α_1 showed no significant change in the intensity of phalloidin staining.

DISCUSSION

To determine the potential role of β_4 as a regulator of actin assembly, we have microinjected a synthetic β_4 into living cells and analyzed its effects on filamentous actin. As indicated by the significant reduction in the binding of fluorescent phalloidin to injected cells (Fig. 2), it is clear that β_4 can induce an extensive depolymerization of actin filaments.

The depolymerization effect of β_4 can be explained most easily by the on–off equilibrium of actin subunits that probably occurs at the ends of filaments *in vivo* (36, 37). Presumably subunits that come off the filaments would become associated with β_4 and unable to reincorporate into filaments. The effect of β_4 can be evaluated quantitatively based on the dose-dependence curve (Fig. 5) and the estimated volume of microinjection (3% cell volume). The slope of the curve indicates that each micromolar unit of injected β_4 can induce a depolymerization of 0.8% of actin filaments. However, without a definitive understanding of the state of cytoplasmic actin and the concentration of F-actin, it is difficult to obtain precise numbers for the extent of actin binding or the intracellular affinity. If one assumes an F-actin concentration of 100 μM (1), then each micromolar unit of injected β_4 should be able to bind 0.8 μM actin. Given the high abundance of β_4 in nonmuscle tissues (17–19) and cell lines (refs. 20 and 21; $\approx$59 μM in FS7 fibroblasts and 600 μM in platelets), one would then predict that β_4 can sequester a significant fraction of the unpolymerized actin in the cytoplasm.

While its effect was most prominent along stress fibers, β_4 appears to induce actin depolymerization in other structures such as lamellipodia and circumferential bundles. Surprisingly, even with a depolymerization of 31% of actin filaments, β_4 did not appear to affect the overall morphology of the cell or the extent of lamellipodia protrusion, indicating that many cellular functions can be achieved with a reduced amount of actin filaments. The apparently normal protrusion at the leading edge suggests that either the rate of protrusion is independent of the rate of actin polymerization (e.g., determined by interactions of actin filaments with myosin I), or β_4 may reduce the number and/or length of actin filaments without affecting the rate of subunit addition onto the ends of filaments in the lamellipodia.

Our results are in contrast with the effects of DNase I or vitamin D-binding protein (38), two extracellular proteins

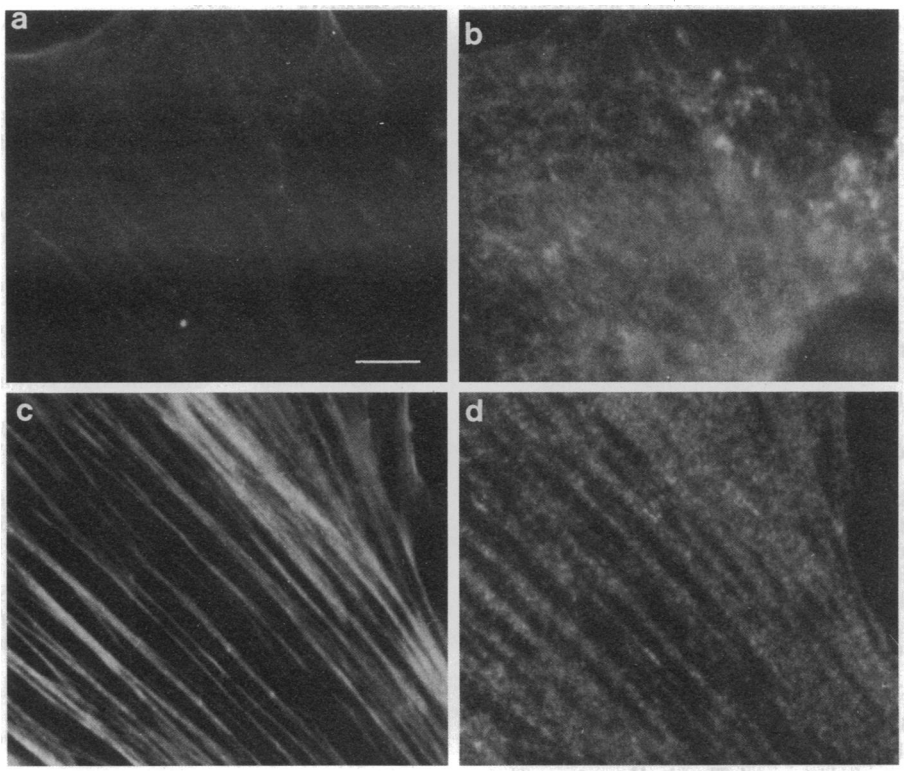

FIG. 4. Disruption of myosin organization by the injection of β_4. A Swiss 3T3 cell was microinjected with 2.3 mM β_4 and, after incubation for 1 hr, was fixed/permeabilized and stained with fluorescein-conjugated phalloidin (*a* and *c*) and anti-myosin polyclonal antibodies and rhodamine-conjugated secondary antibodies (*b* and *d*). Typical control cells show many actin stress fibers (*c*) colocalizing with well-organized punctate structures of myosin filaments (*d*), while injected cells show only faint stress fibers (*a*) and disorganized myosin filaments (*b*). (Bar = 5 μm.)

capable of sequestering actin monomers. The microinjection of these proteins can cause a dramatic cell retraction and arborization at a limited intracellular concentration of 6–12 μM. The difference may reflect other activities of DNase I and vitamin D-binding protein such as capping of filament ends (39–41).

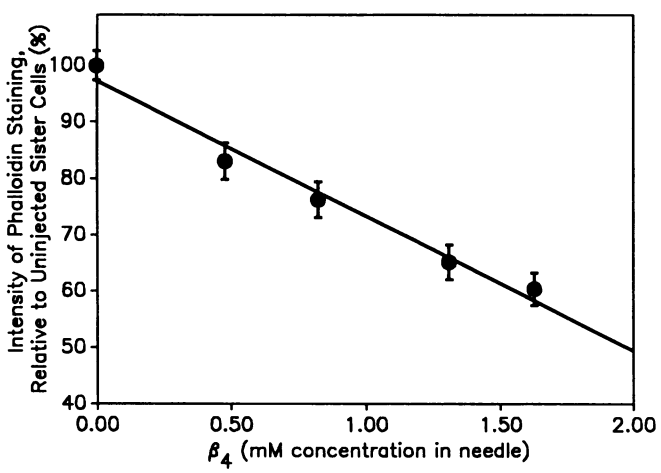

FIG. 5. Dependence of actin depolymerization on the concentration of microinjected β_4. Pairs of sister cells were identified based on their similar size and mirror-image shape. One member of the pair was microinjected with various concentrations of β_4 as indicated along the *x*-axis. The value for 0 mM β_4 was obtained by the microinjection of carrier solution containing dextran. Each point was obtained from 12–14 pairs of sister cells. The percentage of actin depolymerization was calculated by dividing the intensity of phalloidin staining of injected cells with that of uninjected sister cells. Filamentous actin was depleted in a dose-dependent fashion, showing a linear relationship with the concentration of β_4 injected. Vertical bars indicate the standard error of the mean.

Previous studies have identified a number of actin-sequestering proteins with molecular weights in the range of 10,000–23,000, such as actin-depolymerizing factor/destrin, cofilin, profilin, actophorin, actobindin, and depactin (5–10). However, until Safer *et al.* demonstrated the extensive β_4–actin interaction in platelet extracts (22, 23), the role of lower molecular weights peptides was largely overlooked. Subsequent sequence analysis has revealed homology of β_4 with actobindin and cofilin, two known monomer-sequestering proteins, within the region from Asp-13 through Thr-22 (Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr) (24, 42, 43). Our present results clearly demonstrate the physiological role of β_4 in living nucleated cells as a potent regulator of actin polymerization. Based on the available information, it is possible that β_4, together with other monomer-sequestering proteins, may be involved in parallel pathways for the regulation of actin assembly in response to different signals. This notion is supported by recent studies in this laboratory indicating that profilin, a 12- to 15-kDa protein capable of binding actin monomers, can induce similar depolymerization of actin filaments, with some differences in cellular response from those of β_4 (44). Thus, it is important to define the relationship among various sequestration factors and to determine whether the activity of β_4 itself can be modulated by any second messengers.

The authors thank Drs. D. Fishkind, E. Luna, and P. N. Naylor for helpful discussions and for reading the manuscript. The authors also thank Dr. J. Leszyk for determining the concentration of β_4 and Dr. Keigi Fujiwara for the anti-myosin II polyclonal antibody. This project was supported by National Institutes of Health Grant GM-41681 and a Muscular Dystrophy Association grant to Y.-L.W. and by a gift from the Institute for Advanced Studies in Immunology and Aging to A.L.G.

1. Cooper, J. A. (1991) *Annu. Rev. Physiol.* **53**, 585–605.

2. Pollard, T. D. & Cooper, J. A. (1986) *Annu. Rev. Biochem.* **55**, 987–1035.
3. Stossel, T. P., Chaponnier, C., Ezzell, R. M., Hartwig, J. H., Janmey, P. A., Kwiatkowski, D. J., Lind, S. E., Smith, D. B., Southwick, F. S., Yin, H. L. & Zaner, K. S. (1985) *Annu. Rev. Cell Biol.* **1**, 353–402.
4. Sanders, M. C. & Wang, Y.-L. (1990) *J. Cell Biol.* **110**, 359–365.
5. Nishida, E., Maekawa, S. & Sakai, H. (1984) *Biochemistry* **23**, 5307–5313.
6. Cooper, J. A., Blum, J. D., Williams, R. C., Jr., & Pollard, T. D. (1986) *J. Biol. Chem.* **261**, 477–485.
7. Lind, S. E., Janmey, P., Chaponnier, C., Herbert, T.-J. & Stossel, T. P. (1987) *J. Cell Biol.* **105**, 833–842.
8. Bamburg, J. R. & Bray, D. (1987) *J. Cell Biol.* **105**, 2817–2825.
9. Takagi, T., Konishi, K. & Mabuchi, I. (1988) *J. Biol. Chem.* **263**, 3097–3102.
10. Lambooy, P. K. & Korn, E. D. (1988) *J. Biol. Chem.* **261**, 17150–17155.
11. Stossel, T. P. (1989) *J. Biol. Chem.* **264**, 18261–18264.
12. Low, T. L. K., Thurman, G. B., McAdoo, M., McClure, J., Rossio, J., Naylor, P. H. & Goldstein, A. L. (1979) *J. Biol. Chem.* **254**, 981–986.
13. Low, T. L. K., Hu, S.-K. & Goldstein, A. L. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1162–1166.
14. Low, T. L. K. & Goldstein, A. L. (1982) *J. Biol. Chem.* **257**, 1000–1006.
15. Hu, S.-K., Low, T. L. K. & Goldstein, A. L. (1981) *Mol. Cell. Biochem.* **41**, 49–58.
16. Thurman, G. B., Low, T. L. K., Rossio, J. L. & Goldstein, A. L. (1981) in *Lymphokines and Thymic Hormones: Their Potential Utilization in Cancer Therapeutics*, eds. Goldstein, A. L. & Chirigos, M. A. (Raven, New York), pp. 145–157.
17. Hannappel, E., Xu, G.-J., Morgan, J., Hempstead, J. & Horecker, B. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2172–2175.
18. Erickson-Viitanen, S., Ruggieri, S., Natalini, P. & Horecker, B. L. (1983) *Arch. Biochem. Biophys.* **221**, 570–576.
19. Hannappel, E. (1986) *Anal. Biochem.* **156**, 390–396.
20. Goodall, G. J., Morgan, J. I. & Horecker, B. L. (1983) *Arch. Biochem. Biophys.* **221**, 598–601.
21. Hannappel, E. & Leibold, W. (1985) *Arch. Biochem. Biophys.* **240**, 236–241.
22. Safer, D., Golla, R. & Nachmias, V. T. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2536–2540.
23. Safer, D., Elzinga, M. & Nachmias, V. T. (1991) *J. Biol. Chem.* **266**, 4029–4032.
24. Weeds, A. & Way, M. (1991) *Curr. Biol.* **1**, 307–308.
25. Low, T. L. K., Wang, S.-S. & Goldstein, A. L. (1983) *Biochemistry* **22**, 733–740.
26. Wang, S.-S., Wang, B. S. H., Chang, J. K., Low, T. L. K. & Goldstein, A. L. (1981) *Int. J. Protein Res.* **18**, 413–416.
27. Kouyama, T. & Mihashi, K. (1981) *Eur. J. Biochem.* **114**, 33–38.
28. Cooper, J. A., Walker, S. B. & Pollard, T. D. (1983) *J. Muscle Res. Cell. Motil.* **4**, 253–262.
29. McKenna, N. M. & Wang, Y.-L. (1989) *Methods Cell Biol.* **29**, 195–205.
30. Graessman, A., Graessman, M. & Mueller, C. (1980) *Methods Enzymol.* **65**, 816–825.
31. Lee, G. M. (1989) *J. Cell Sci.* **94**, 443–447.
32. Fishkind, D. J., Cao, L.-G. & Wang, Y.-L. (1991) *J. Cell Biol.* **114**, 967–975.
33. Small, J. V., Rinnerthaler, G. & Hinssen, H. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **46**, 599–611.
34. Amato, P. A., Unanue, E. R. & Taylor, D. L. (1983) *J. Cell Biol.* **96**, 750–761.
35. Haritos, A. A., Blacher, R., Stein, S., Caldarella, J. & Horecker, B. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 343–346.
36. Kreis, T. E., Geiger, B. & Schlessinger, J. (1982) *Cell* **29**, 835–845.
37. Glacy, S. D. (1983) *J. Cell Biol.* **97**, 1207–1213.
38. Sanger, J. M., Dabiri, G., Mittal, B., Kowalski, M. A., Haddad, J. G. & Sanger, J. W. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5474–5478.
39. Pinder, J. C. & Gratzer, W. B. (1982) *Biochemistry* **21**, 4886–4890.
40. Podolski, J. L. & Steck, T. L. (1988) *J. Biol. Chem.* **263**, 638–645.
41. Holmes, K. C., Popp, D., Gebhard, W. & Kabsch, W. (1990) *Nature (London)* **347**, 44–49.
42. Vancompernolle, K., Vandekerckhove, J., Bubb, M. R. & Korn, E. D. (1991) *J. Biol. Chem.* **266**, 15427–15431.
43. Yonezawa, N., Nishida, E., Ohba, M., Seki, M., Kumagai, H. & Sakai, H. (1989) *Eur. J. Biochem.* **183**, 235–238.
44. Cao, L.-G., Babcock, G. G., Rubenstein, P. A. & Wang, Y.-L. (1992) *J. Cell Biol.*, in press.