

# Interaction of Actin Filaments with Microtubules

THOMAS D. POLLARD, S. CHARLES SELDEN, and PAMELA MAUPIN

*Department of Cell Biology and Anatomy, Johns Hopkins Medical School, Baltimore, Maryland 21205*

Several cytological observations support the idea that actin filaments interact with microtubules, but the strongest evidence comes from biochemical experiments. The cytological observations include the colocalization of microtubules and actin in the mitotic spindle of dividing cells by electron microscopy (1–3) and light microscopy with fluorescent probes for actin (4–6). There is, however, debate about the concentration of actin in the spindle relative to the cytoplasm (7). There is one report (8) that fluorescent phalloidin (a peptide that binds to actin filaments) does not bind to the spindle—an observation that is inconsistent with the other cytological studies. There also is a report (9) of fine, filamentous material, called microtrabeculae, associated with microtubules in interphase cells prepared by critical point drying. The chemical composition of these microtrabeculae has yet to be established, but it is conceivable that they are, in fact, actin filaments.

A second impetus for investigating actin-microtubule interactions at the molecular level has been the difficulty of identifying an energy-transducing enzyme associated directly with microtubules that might power the wide variety of microtubule-dependent movements observed in cells (10, 11). A number of investigators have sought a cytoplasmic homologue of the microtubule-interactive, energy-transducing enzyme, dynein, found in the axonemes of cilia and flagella. A small amount of a high molecular weight ATPase that could be related to dynein is associated with isolated brain microtubules (12), but most of the ATPase activity in these preparations is clearly a component of membrane-bounded vesicles (13). Sea urchin eggs also have a high molecular weight ATPase that shares some properties with axonemal dynein, but not enough material has been purified to make a definitive identification (14, 15).

Vanadate and ENHA (erythro-9-[3-(2-hydroxyonyl)]adenine, an ATP analog) inhibit both axonemal dynein and some types of microtubule-dependent, cytoplasmic movements (16–18), leading to speculation that these movements require a cytoplasmic dynein. These inhibitors are not specific for dynein, and in at least one cell, vanadate inhibits ciliary beating but not saltatory, microtubule-dependent particle movements (19).

Until there is stronger evidence for cytoplasmic dynein, we feel that it is wise to consider the possibility that microtubule-dependent movements are powered by another energy-transducing enzyme that is not bound directly to microtubules. At

present, myosin is the only well-documented alternative enzyme of this sort known to exist in the cytoplasm. This led us to investigate whether actin associates with microtubules and participates in microtubule-dependent movements indirectly through its ability to interact with myosin. Our work has also received impetus from two other lines of evidence: (a) actin filament-disrupting agents such as DNase I (20) and plasma gelsolin (brevin) (21) can inhibit fast axonal transport—a well-established microtubule-dependent movement; and (b) plastic beads coated with myosin can move along actin filaments at velocities comparable to those of rapid axonal transport of small vesicles (22).

Our approach to the problem has been primarily biochemical. We have studied the interactions of purified microtubules and microtubule-associated proteins (MAPs)<sup>1</sup> with purified actin filaments. We established that these two major cytoplasmic fiber systems can interact with each other. The evidence (23–25) shows that actin filaments and microtubules form three-dimensional networks, with the actin filaments crosslinked to the microtubules by MAPs. The crosslinking activity of the MAPs is inhibited when they are heavily phosphorylated (25).

At the cytological level, we have concentrated on improving methods to preserve actin filaments in intact cells for electron microscopy (26, 27). We find that there are no long actin filaments associated with microtubules in the mitotic spindles; however, the spindle contains a large number of short filaments of the same diameter as actin filaments (28). The work of others suggests that a similar situation may exist in neurites (29, 30). If these short filaments are composed of actin and are associated with the microtubules, the system may be composed of very long structural elements or “bones” (the microtubules) associated with very tiny “muscles” (the actin filaments).

## *Biochemical Evidence for Actin-Microtubule Interactions*

Our experiments have been carried out with microtubule protein purified from hog or beef brains by cycles of polymerization and depolymerization and with actin purified from rabbit skeletal muscle (23–25). Limited comparisons with

<sup>1</sup> *Abbreviation used in this paper:* MAPs, microtubule-associated proteins.

actins isolated from brain and from amebas have demonstrated that muscle and cytoplasmic actins behave similarly in our experimental system (25). Most of our work has been

with a very simple assay for network formation in solution. We measure the apparent viscosity of our samples using a miniature falling-ball viscometer (31). Because all of the solutions we study are non-Newtonian in their rheological behavior and because this viscometer amplifies differences in the viscosity (31), the measured apparent viscosities are only semiquantitative indicators of the extent of network formation in the samples. The assay does have the advantage that it is inexpensive, rapid, and uses little protein. Many of our experiments would not be possible if we relied exclusively on a more sophisticated and quantitative rheological assay.

When actin monomers and cold-dissociated microtubule protein are polymerized together, they form actin filaments and microtubules that interact to form a gel (23). The apparent viscosity of the sample depends on the concentration of microtubule protein, the concentration of actin, and the solution conditions. For example, ATP and some other nucleoside triphosphates inhibit the gelation of mixtures of actin filaments and microtubules. In the electron microscope (Fig. 1 *a*) the gels are found to consist of randomly arranged actin filaments and microtubules. These make contact with each other at a frequency high enough that most thin sections contain numerous examples of actin filaments near the surface of the microtubule. Characteristically, the actin filaments appear to be held approximately 10 nm away from the surfaces of the microtubules.

Three lines of evidence support the conclusion that the interaction of actin filaments with microtubules depends on the presence of MAPs. First, mixtures of pure actin and pure tubulin can polymerize to form mixtures of actin filaments and microtubules that do not have a high viscosity or form a gel (23). Second, both purified high molecular weight MAPs and purified tau are actin crosslinking proteins (24, 32). The apparent viscosity of mixtures of these MAPs with actin depends on the concentration of the MAPs and the solution conditions, much as the apparent viscosity of mixtures of microtubule protein and actin. The apparent viscosity of the actin-MAPs mixtures is inhibited by nucleoside triphosphates and by high salt concentrations and is promoted by an acidic pH. An important feature of this interaction is that the affinity of MAPs for actin filaments is so low that essentially no MAPs pellet with actin filaments under the conditions of our experiments. Third, we have been able to reconstitute a crosslinked

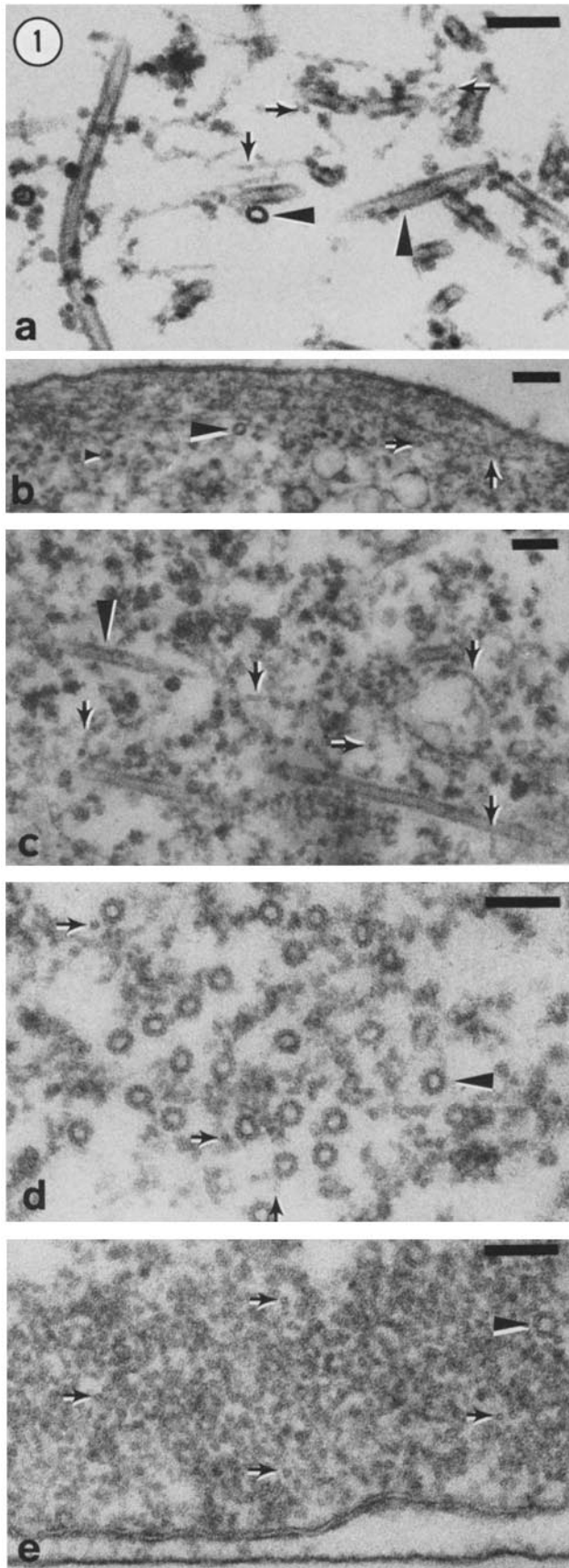


FIGURE 1 Electron micrographs of thin sections of (a) a gel of purified actin filaments and microtubules and (b-e) HeLa cells, all prepared by methods that maximize preservation and staining of actin filaments. (a) A gel composed of skeletal muscle actin filaments and brain microtubule protein that was fixed in situ with glutaraldehyde and tannic acid followed by a gentle osmium tetroxide treatment (described in reference 23). (b-e) HeLa cells fixed with glutaraldehyde-tannic acid-saponin followed by osmium tetroxide (described in references 27 and 28). The resulting intense staining provides enough contrast to visualize individual actin filaments in dark gray (~450 nm) sections, avoiding some of the superimposition in conventional silver-gray (~750 nm) sections. (b) Contractile ring sectioned perpendicular to the long axis of the mitotic spindle. (c) Section parallel to the spindle axis showing a microtubule bundle of anaphase spindle fibers. (d) Section perpendicular to the spindle axis showing cross sections of microtubules of anaphase half-spindle fibers. (e) Stress fiber of an interphase cell. Arrows: actin filaments. Small arrowheads: intermediate filaments. Large arrowheads: microtubules. Horizontal labels: fiber cross sections. Vertical labels: fiber longitudinal sections. (a, d, and e)  $\times 110,000$ . (b and c)  $\times 70,000$ . Bars, 0.1  $\mu\text{m}$ .

network of actin filaments and microtubules with purified components (33). Mixtures of purified actin, purified tubulin, and purified high molecular weight MAPs (principally MAP-2) form networks much like actin filaments mixed with crude microtubule protein (Table I). These reconstituted experiments were successful only after the solution conditions had been optimized to favor the polymerization of the actin and tubulin as well as the crosslinking of the microtubules to the actin. The most important variable turned out to be the ionic strength. In contrast to MAP-2, purified tau does not appear to crosslink purified actin filaments and pure tubulin microtubules, even though it can associate with each of these fibers individually.

Relatively little is known about how the cell regulates the interaction of actin filaments and microtubules, but one promising discovery is that the level of phosphorylation of the MAPs determines their ability to crosslink the actin filaments (25, 32). We and others (25, 34, 35) have determined that the high molecular weight MAP, called MAP-2, is a phosphoprotein. It has at least 12 and possibly as many as 22 phosphorylation sites. Tau is also a phosphoprotein and has at least two phosphorylation sites (25). The actin-crosslinking activity of both of these proteins is inversely related to the level of phosphorylation (reference 25 and Fig. 2). We have studied this most extensively in crude, heat-stable MAP preparations containing both MAP-2 and tau. When isolated directly from microtubule proteins, the heat-stable MAP fraction has about 7 mol of phosphate per 300,000 g of protein. By incubating the microtubule protein with ATP to promote phosphorylation or by treating the isolated MAPs with phosphatases, it is possible to vary the total phosphate content from about 4 phosphates to 10 phosphates per 300,000 g of protein. The low-phosphate MAPs have high actin-crosslinking activity and the heavily phosphorylated MAPs have low crosslinking activity. These effects of phosphorylation are completely reversible (25). In the reconstituted system with pure actin and tubulin, heavily phosphorylated MAP-2 does not crosslink as well as dephosphorylated MAP-2.

Workers in two other laboratories have confirmed our observations and added valuable new information. Nishida et al. (32, 36) first suggested that phosphorylation of MAPs might regulate their interaction with actin filaments. Sattilaro et al. (37, 38) showed that MAPs can aggregate actin filaments into bundles and that purified MAP-2 can bind weakly to actin filaments. Their preliminary experiments on proteolytic fragments of MAP-2 even suggest that it has two actin-binding sites. One of these sites may be on the long arm that projects from the surface of the microtubule and the second is on the

smaller, tubule-binding domain (Fig. 3). Presumably, both sites can bind actin filaments in the absence of microtubules. In the presence of microtubules, it is likely that the tubule-binding site binds with high affinity to the tubules, leaving the site on the projection to bind to actin filaments.

These biochemical experiments provide the evidence that actin filaments and microtubules can interact with each other.

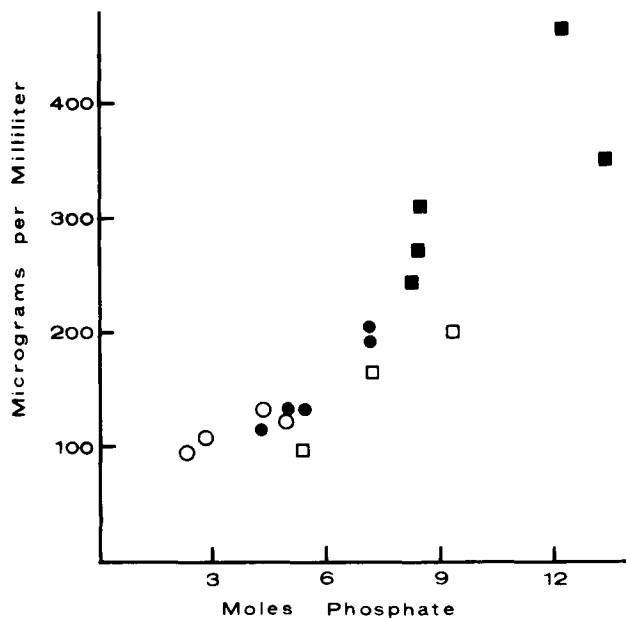


FIGURE 2 Phosphorylation of MAPs inhibits their actin filament crosslinking activity. The graph shows how the critical gelation concentration (the concentration of MAPs required to increase the apparent viscosity of the actin filaments from 35 to 1,000 cp) depends on the extent of phosphorylation (mol/300,000 g) of a crude fraction of heat-stable brain MAPs. (■) MAPs from microtubule protein incubated with EGTA, ATP, and cAMP as previously described (25) before heating to isolate the MAPs. (□) The same material incubated with acid or alkaline phosphatase. (●) MAPs from microtubule protein incubated as described (25) without adenine nucleotides. (○) the same material incubated with acid or alkaline phosphatase. Conditions: 400  $\mu$ g/ml actin, 100 mM KCl, 2.5 mM EGTA, 1 mM  $MgCl_2$ , 600  $\mu$ M 2-(*N*-morpholino)ethane sulfonic acid, 40  $\mu$ M TRIS-HCl, 200  $\mu$ M HEPES, 100  $\mu$ M dithiothreitol, 60  $\mu$ M ATP, 40  $\mu$ M  $CaCl_2$ , pH 6.35.

TABLE I  
Viscosity of Solutions of MAP-2 or Tau and Actin with or without Microtubules

	MAP fraction			
	None	3 $\mu$ M MAP-2	10 $\mu$ M MAP-2	16 $\mu$ M tau
	centipoise			
Actin	25	35	4,000	50,000
Actin and tubulin	350	80,000	>150,000	2,000

Conditions: 32  $\mu$ M actin,  $\pm$ 127  $\mu$ M tubulin, 60 mM PIPES, 40 mM KCl, 2 mM  $MgCl_2$ , 2 mM EGTA, 1 mM GTP, 500  $\mu$ M DTT, 200  $\mu$ M  $CaCl_2$ , 100  $\mu$ M ATP, 7% DMSO (v/v), pH 6.6; incubation at 37°C for 20 min. Low-shear falling-ball viscometry gives apparent viscosities.

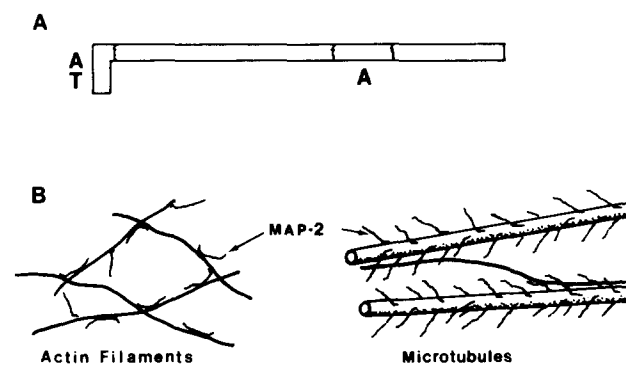


FIGURE 3 A drawing of one interpretation of the experiments on the interaction of actin filaments with microtubules. A model of MAP-2 based on proteolytic fragmentation experiments by Vallee (35) that identified the tubule-binding site (T) and with the two potential actin-binding sites (A) identified by Sattilaro and Dentler (38). (B) A highly schematic drawing of how MAPs might crosslink actin filaments to each other and to microtubules.

Given that the concentrations of both microtubules and actin filaments are considerably higher inside cells than in our experiments, it is likely that these two polymers also interact with each other inside the cell. Although the work is at an early stage, we predict that it will be found that the interaction of these two polymers is regulated, at least in part, by the level of phosphorylation of the MAPs. An important feature of the interaction is that the association of MAPs with microtubules is strong and association of MAPs with actin filaments is relatively weak. While weak associations between proteins are generally considered nonspecific, low-affinity associations of actin filaments and microtubules may be most appropriate for a dynamic system like the cytoplasmic matrix. (For comparison, the dissociation constant ( $K_d$ ) for the myosin-actin complex under contraction conditions is  $>10^{-5}$  M. This is probably the same range as the  $K_d$  for the actin-MAP complex.) One supposes that multiple weak bonds by the many MAP-2 molecules arrayed over the surface of a microtubule are sufficient to bind actin filaments and link the microtubules and actin filaments into a network.

### Cytological Observations

Associations of actin filaments with microtubules are often seen in cells prepared for electron microscopy, but it is nearly impossible to prove by microscopic observation alone that the polymers are physically bound to each other. First, all of the techniques used to preserve cells for electron microscopy have the potential of introducing artifacts. This is particularly true in the case of actin, where it is well known (26, 39) that the filaments are difficult to preserve in their natural state. Second, juxtaposition of polymers in a micrograph does not mean that the polymers are actually attached to each other.

Having accepted these limitations, we have tried to improve cell fixation techniques to the point where we can be confident that cytoplasmic actin filaments are preserved. In our hands, the best approach has been to fix cells initially with a mixture of glutaraldehyde, tannic acid, and the detergent saponin (27). The detergent is used in such low concentrations that it does not disrupt the microscopic appearance of most membranes, but it does make the plasma membrane permeable to tannic acid. The tannic acid binds to actin filaments and protects them during a subsequent fixation with osmium tetroxide. This results in improved preservation and staining of actin filaments in all parts of interphase cells. Tannic acid also stains most other cellular structures.

In dividing HeLa cells there are many long, straight actin filaments in the cell cortex but no similar long actin filaments in the mitotic spindle (Fig. 1 *b-e* and reference 28). Instead, the spindle contains numerous short filaments that have the same diameter and staining characteristics as the cortical actin filaments. These short filaments are scattered among the microtubules of the spindle, and some appear to be associated with the microtubules (Fig. 1 *c* and *d*). In cross sections the filaments are relatively easy to identify, but they are very difficult to follow from section to section, at least in comparison with the robust, easily identified microtubules. Their mean length is about 50 nm, based on our measurements made on micrographs of longitudinal sections and of serial cross sections through the mitotic apparatus (28). Microfilaments similar in size to actin have also been observed in the mitotic spindles of *Oedogonium* (40) and PtK cells (41). As is the case with the filaments with which we have been working,

the chemical nature of these spindle filaments is unknown.

Although these mitotic apparatus filaments have not yet been positively identified, it is likely that they are actin filaments. It is very interesting that these presumptive actin filaments are so short. Populations of stable, short filaments could be maintained by capping the filaments at one or both ends with specific proteins (42). A similar situation may exist in neurites where there is biochemical evidence for actin filaments but no morphological identification of long actin filaments. Rapid-freezing studies suggest that in neurites all of the filaments having the diameter of actin are short (29, 30).

### Conclusions

At the biochemical level there is little doubt that actin filaments and microtubules can interact via MAPs. The major question that remains open is whether these interactions are used by the cell. One can imagine that such interactions are purely structural, contributing to the integrity of the cytoplasmic matrix. It is also possible that the association of actin filaments with microtubules provides a link to myosin, a potential source of power for microtubule-dependent movements. In thinking about the physiological functions of networks of actin filaments and microtubules, it is now worth considering that the actin filaments in these networks may be very short, which, if true, would suggest to us that the actin filaments bound to microtubules are more important for motility than cellular structure.

On the molecular level there are a number of important questions that need to be resolved. We need to characterize in much more detail the molecular interactions of MAPs with actin filaments and microtubules to understand how the crosslinks are formed and how they might be regulated by phosphorylation and other factors. We need to learn why the apparent affinity of MAPs for actin filaments is low and what this has to do with physiological functions. The mechanical properties of networks of actin filaments and microtubules need to be established, and direct tests should be made to determine whether actin filaments associated with microtubules form an appropriate scaffolding for myosin-mediated particle movements.

This work was supported by National Institutes of Health (NIH) research grant GM-26132 to T. D. Pollard and National Research Service award 08580 to S. C. Selden from the NIH.

### REFERENCES

- Gawadi, N. 1974. Characterization and distribution of microfilaments in dividing locust testis cells. *Cytobios.* 10:17-35.
- Forer, A., and O. Behnke. 1972. An actin-like component in spermatocytes of a crane fly (*Nephrotoma suturalis* Loew). I. The spindle. *Chromosoma.* 39:146-173.
- Hinkley, R., and A. Telsner. 1974. Heavy meromyosin binding filaments in the mitotic apparatus of mammalian cells. *Exp. Cell Res.* 86:161-165.
- Sanger, J. W. 1975. Presence of actin during chromosomal movement. *Proc. Natl. Acad. Sci. USA.* 72: 2451-2455.
- Cande, W. Z., E. Lazarides, and J. R. McIntosh. 1977. A comparison of the distribution of actin and tubulin in the mammalian mitotic spindle as seen by indirect immunofluorescence. *J. Cell Biol.* 72:552-567.
- Herman, I. M., and T. D. Pollard. 1979. Comparison of purified anti-actin and fluorescent-heavy meromyosin staining patterns in dividing cells. *J. Cell Biol.* 80:509-520.
- Aubin, J. E., K. Weber, and M. Osborn. 1979. Analysis of actin and microfilament-associated proteins in the mitotic spindle and cleavage furrow of PTK2 cells by immunofluorescence microscopy. *Exp. Cell Res.* 124:93-109.
- Barak, L. S., E. A. Nothnagel, E. F. DeMarco, and W. W. Webb. 1981. Differential staining of actin in metaphase spindles with 7-nitrobenz-2-oxa-1,3-diazole-phalloidin and fluorescence DNase—is actin involved in chromosomal movement. *Proc. Natl. Acad. Sci. USA.* 78:3034-3038.
- Wolosewick, J. J., and K. R. Porter. 1979. Microtubular lattice of the cytoplasmic

- ground substance. Artifact or reality. *J. Cell Biol.* 82:114-139.
10. Gibbons, I. R., and E. Fronk. 1979. Latent adenosine-triphosphatase form of dynein-1 from sea-urchin sperm flagella. *J. Biol. Chem.* 254:187-196.
  11. Porter, M. E., and K. A. Johnson. 1983. Transient state kinetic analysis of the ATP-induced dissociation of the dynein-microtubule complex. *J. Biol. Chem.* 258:6582-6587.
  12. Burns, R. G., and T. D. Pollard. 1974. A dynein-like protein from brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 40:274-280.
  13. Murphy, D. B., K. T. Wallis, and R. R. Heibsch. 1983. Identity and origin of the ATPase activity associated with neuronal microtubules. II. Identification of a 50,000-dalton polypeptide with ATPase activity similar to F-1 ATPase from mitochondria. *J. Cell Biol.* 96:1306-1315.
  14. Pratt, M. M. 1980. The identification of a dynein ATPase in unfertilized sea urchin eggs. *Dev. Biol.* 74:364-378.
  15. Sakai, H., I. Mabuchi, S. Shimoda, R. Kuriyama, K. Ogawa, and H. Mohri. 1976. Induction of chromosome motion in the glycerol isolated mitotic apparatus: nucleotide specificity and effects of anti-dynein and anti-myosin sera on the motion. *Dev. Growth Differ.* 18:211-219.
  16. Gibbons, I. R., M. P. Cosson, J. A. Evans, B. H. Gibbons, B. Houck, K. H. Martinson, W. S. Sale, and W.-J. Y. Tang. 1978. Potent inhibition of dynein ATPase and of the motility of cilia and sperm flagella by vanadate. *Proc. Natl. Acad. Sci. USA.* 75:2220-2224.
  17. Cande, W. Z., and S. M. Wolniak. 1978. Chromosome movement in lysed mitotic cells is inhibited by vanadate. *J. Cell Biol.* 79:573-580.
  18. Forman, D. S., K. J. Brown, and M. E. Promersberger. 1983. Selective inhibition of retrograde axonal transport by ENHA. *Brain Res.* 272:194-197.
  19. Buckley, I., and M. Stewart. 1983. Ciliary but not saltatory movements are inhibited by vanadate microinjected into living cultured-cells. *Cell Motil.* 3:167-184.
  20. Isenberg, G., P. Schubert, and G. W. Kreutzberg. 1980. Experimental approach to test the role of actin in axonal transport. *Brain Res.* 194:588-593.
  21. Goldberg, D. J., D. A. Harris, B. W. Lubit, and J. H. Schwartz. 1980. Analysis of the mechanism of fast axonal transport by intracellular injection of potentially inhibitory macromolecules: evidence for a possible role of actin filaments. *Proc. Natl. Acad. Sci. USA.* 77:7448-7452.
  22. Sheetz, M. P., and J. A. Spudich. 1983. Movement of myosin-coated fluorescent beads on actin cables in vitro. *Nature (Lond.)* 303:31-35.
  23. Griffith, L. M., and T. D. Pollard. 1978. Evidence for actin filament-microtubule interaction mediated by microtubule-associated proteins. *J. Cell Biol.* 78:958-965.
  24. Griffith, L. M., and T. D. Pollard. 1982. The interaction of actin filaments with microtubules and microtubule associated proteins. *J. Biol. Chem.* 257:9143-9151.
  25. Selden, S. C., and T. D. Pollard. 1983. Phosphorylation of microtubule associated proteins regulates their interaction with actin filaments. *J. Biol. Chem.* 258:7064-7071.
  26. Maupin-Szamier, P., and T. D. Pollard. 1978. Actin filament destruction by osmium tetroxide. *J. Cell Biol.* 77:837-852.
  27. Maupin, P., and T. D. Pollard. 1983. Improved preservation and staining of HeLa cell actin filaments, clathrin-coated membranes, and other cytoplasmic structures by tannic acid-glutaraldehyde-saponin fixation. *J. Cell Biol.* 96:51-62.
  28. Maupin, P., and T. D. Pollard. 1982. Microfilament structure and distribution in mitotic HeLa cells. *J. Cell Biol.* 95(2, Pt. 2):309a. (Abstr.)
  29. Tsukita, S., J. Usukura, S. Tsukita, and H. Ishikawa. 1983. The cytoskeleton in myelinated axons: a freeze-etch replica study. *Neuroscience.* 7:2135-2147.
  30. Hirokawa, N., and D. Standaert. 1983. Structural basis for organelle transport in crayfish axon. *J. Cell Biol.* 97(2, Pt. 2):266a. (Abstr.)
  31. MacLean-Fletcher, S., and T. D. Pollard. 1980. Viscometric analysis of the gelation of *Acanthamoeba* extracts and purification of two gelation factors. *J. Cell Biol.* 85:414-428.
  32. Nishida, E., S. Kotani, T. Kuwaki, and H. Sakai. 1982. Phosphorylation of microtubule-associated proteins (MAPs) controls both microtubule assembly and MAPs-actin interaction. *In Biological Functions of Microtubules and Related Structures. Proceedings of the Oji International Seminar.* H. Sakai, H. Mohri, and G. G. Borisy, editors. Academic Press, Inc., Japan. pp. 285-295.
  33. Selden, S. C., and T. D. Pollard. 1983. Reconstitution of gelled networks from purified actin, tubulin and microtubule associated proteins. *J. Cell Biol.* 97(2, Pt. 2):287a. (Abstr.)
  34. Burns, R. G., and K. Islam. 1982. Characterization of the chick brain high molecular weight, multiply phosphorylated, microtubule associated protein. *Eur. J. Biochem.* 122:25-29.
  35. Vallee, R. 1980. Structure and phosphorylation of microtubule-associated protein 2. *Proc. Natl. Acad. Sci. USA.* 77:3206-3210.
  36. Nishida, E., T. Kuwaki, and H. Sakai. 1981. Phosphorylation of microtubule-associated proteins (MAPs) and pH of the medium control interaction between MAPs and actin filaments. *J. Biochem.* 90:575-578.
  37. Sattilaro, R. F., W. L. Dentler, and E. L. Lecluyse. 1981. Microtubule-associated proteins and the organization of actin filaments in vitro. *J. Cell Biol.* 90:467-473.
  38. Sattilaro, R. F., and W. L. Dentler. 1982. The association of MAP-2 with microtubules, actin filaments, and coated vesicles. *In Biological Functions of Microtubules and Related Structures. Proceedings of the Oji International Seminar.* H. Saki, H. Mohri, and G. G. Borisy, editors. Academic Press, Inc., Japan. pp. 297-309.
  39. Lehrer, S. S. 1981. Damage to actin filaments by glutaraldehyde: protection by tropomyosin. *J. Cell Biol.* 90:459-466.
  40. Schibler, M. J., and J. D. Pickett-Heaps. 1980. Mitosis in *Oedogonium*: spindle microfilaments and the origin of the kinetochore fiber. *Eur. J. Cell Biol.* 22:687-698.
  41. McDonald, K. L. 1982. Membrane and filament distribution in the mitotic spindles of two mammalian tissue culture lines. *J. Cell Biol.* 95:310.
  42. Craig, S. W., and T. D. Pollard. 1982. Actin-binding proteins. *Trends Biochem. Sci.* 7:88-92.