RELATIONSHIPS BETWEEN CYTOSKELETAL PROTEINS AND THE TOXIC EFFECTS OF DRUGS

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ABSTRACT — Many cell functions and aspects of cell configuration are linked with changes in cytoskeletal proteins, by intracellular signal transduction. Histochemical and pathophysiological changes caused by toxins or drugs affect on dynamic aspects of cytoskeletal proteins. Here we review that biochemical and molecular biological properties of the microtubules and microfilaments and the relationships between these cytoskeletal proteins and the toxic effects of drugs and toxins. The intracellular signaling pathway makes use of cytoskeletal proteins.

Perspectives

Cytoskeletal proteins, microtubules, microfilaments, and intermediate filaments are abundant in all eukaryotic cells, where they may undergo many changes. They can be found at equilibrium between being polymerized and depolymerized while maintaining the cell configuration, and they can bind to other proteins including associated or regulatory proteins that regulate their polymerization or depolymerization. Through various changes, cytoskeletal proteins participate in multiple physiological functions, including maintenance of secretion, cell motility, cell division, cell configuration, and intracellular transport. Many books about cytoskeletal proteins have been published (Bershadsky and Vasiliev, 1988; Preston *et al.*, 1990; Amos and Amos, 1991; ros Remedios and Barden, 1993). We focus here on the effects of drugs on cytoskeletal proteins from the viewpoint of cell biology, and briefly describe the basic biochemical properties of microtubules and microfilaments.

Microtubules

Microtubules are composed of tubulin heterodimers and microtubule-associated proteins (MAPs). Tubulin (55 kDa) is a highly conserved protein, and the tubulin family includes alpha- and beta-tubulin, major subunits of mammalian brain microtubules. Vertebrate beta-tubulin genes have six isotypes (Wang *et al.*, 1986; Lopata and Cleveland, 1987). Their expression levels vary depending on the animal species and tissue. The major variable regions of beta-tubulin are at the carboxy terminus. Classes I, II, and IVa as classified by Sullivan (1988) are expressed in many tissues; class II, III, and IVa beta-tubulin are found mostly in brain neurons, and class IVb beta-tubulin is found only in the testes. Class VI is an important component of erythrocytes and platelets. Alpha-tubulin genes of vertebrates are similar to their beta-tubulin genes, although there is a difference between mammalian and avian tubulin genes. The mouse has at least seven alpha-tubulin genes encoding six polypeptide isotypes (Lewis *et al.*, 1985; Hecht *et al.*, 1988), but in the chicken, five or six functional alpha-tubulin genes encode many different polypeptides (Pratt and Cleveland, 1988). These isoforms of tubulin polymerize with each other. Probably there being

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multiple tubulin genes allows the synthesis of this ubiquitous cellular component to be regulated under multiple, independent programs of gene expression during differentiation. FtsZ, a prokaryotic homologue of tubulin, forms a ring at the leading edge of dividing bacterial cells, and is a prokaryotic cytoskeletal component (Erickson, 1995; Erickson *et al.*, 1996). This protein may be an evolutionary progenitor of tubulin (Erickson and Satoffler, 1996).

After alpha-tubulin is expressed, it undergoes lysine-epsilon-amino acetylation/deacetylation (L'Hernault and Rosenbaum, 1983, 1985) and carboxy-terminal tyrosination/detyrosination (Thompson, 1982). These modifications may be related to microtubule stability. The physiological meaning of these post-translational modifications is not understood.

Drugs affecting tubulin assembly

Microtubule proteins can be purified by two cycles of temperature-dependent polymerization and depolymerization in the presence of GTP (Vallee, 1986). Pure tubulin separated from microtubule proteins by phosphocellulose column chromatography can be polymerized more slowly in the absence of MAPs (Microtubule-associated proteins) than tubulin in the presence of MAPs, and aberrant forms of polymerized tubulin such as twisted ribbons and curved sheets of protofilaments can be obtained (Weingarten *et al.*, 1975). In studies of the effects of drugs on microtubule assembly, the changes in turbidity of microtubule proteins are usually monitored spectrophotometrically.

Colchicine, colcemid, and nocodazole are inhibitors of tubulin polymerization (Saltarelli and Pantaloni, 1982; Dustin, 1984). One molecule of the inhibitor can bind to each dimer, but these drugs block polymerization completely at much lower stoichiometric ratios. The presence of one or a few inhibitors bound to dimers is sufficient for blockage of tubulin polymerization at the microtubule end. Bromyl is a specific inhibitor of fungal tubulin polymerization and therefore useful as an antifungal agent (Maxwell and Brody, 1971). The herbicides aminoprophos-methyl [o-methyl-o-(4-methyl-6-nitrophenyl)-N-isopropylphosphorothioamidate], and Synonyms, Ryzelan, and Dirimal (derivatives of oryzalin) bind specifi-

cally to tubulin of higher plants in the same way as colchicine does to animal tubulin (Bajer and Mole-Bajer, 1986). Vinblastine and vincristine (from *Vinca Rosea*) bind to tubulin and prevent microtubule assembly (Luduena, 1979), and are used clinically to treat Hodgkin's disease and acute lymphocytic leukemia. Maytansine, from an African plant, also binds tubulin and blocks microtubule assembly (Luduena *et al.*, 1986) and is an anti-tumor agent against various cancers, but highly toxic. Taxol, from the yew tree, hyperstabilizes microtubules and is useful in microtubule research, especially for the isolation of MAPs such as kinesin and dynein. Recently, a method for taxol synthesis was developed, and synthetic taxol has been used in the treatment of breast cancer (Nicolaou *et al.*, 1994).

Microtubule-associated proteins

The molecular weights and physiological functions of MAPs are various (Table 1). The MAPs in mammalian brain have been well characterized, although details of their functions are still not known. MAP-1 is the name of a group of three polypeptides termed MAP-1A, -1B, and -1C, with molecular weights of 300,000 -350,000, as estimated by SDS-PAGE, and the group has distinctive immunological properties (Bloom *et al.*, 1984). The functions of MAP-1A and -1B are not known, but both of these proteins are present in high concentrations in neurons at early developmental stages (Cambray-Deakin *et al.*, 1987; Diaz-Nido *et al.*, 1988; Tucker *et al.*, 1989). MAP-1C is a dynein-like protein that acts as a motor for retrograde transport of intracellular organelles of axons (Vallee *et al.*, 1988). Consequently, MAP-1 may participate in neuronal development through phosphorylation, and

Table 1. Brain microtubule-associated proteins.

<i>Protein</i>	<i>Mr</i>	<i>Protein</i>	<i>Mr</i>
MAP-1A	350,000	MAP-3	180,000
MAP-1B(MAP-5)	325,000	Kinesin	120,000
dynein (MAP-1C)	300,000	Dynamin	100,000
Vesikin	295,000	Chartins	80,000
MAP-2A, B	270,000	MAP-2C	70,000
MAP-4	210,000	Tau	55,000
(200-KDa family)			

highly phosphorylated MAP-1 may cause teratological events in the brain.

MAP-2 is a major component of the neuronal cytoskeleton, and is found in neuronal cell bodies and dendrites. It is a high-molecular-weight polypeptide (270,000) that is thermostable and can be resolved by SDS-PAGE into two bands, corresponding to MAP-2A and -2B. The nucleotide sequences of the bands are very similar (Garner and Matus, 1988; Garner *et al.*, 1988). MAP-2A and -2B each have a KSP motif in the carboxyl terminal that is presumably a domain promoting microtubule assembly, and may have a phosphorylation site. Phosphorylated MAP-2 does not mediate microtubule assembly. Cyclic AMP-dependent protein kinase, calmodulin-dependent protein kinase, and C-kinase all phosphorylate MAP-2 (Theurkauf and Vallee, 1982; Schulman, 1984; Tsuyama *et al.*, 1986). Consequently, drugs or other compounds affecting the activities of these protein kinases should modulate microtubule assembly or disassembly. Phorbol ester and calmodulin regulate microtubule assembly, and these compounds and cAMP induce histochemical changes in cultured cells. The mechanism and specific enzymes of MAP-2 dephosphorylation have not been identified. MAP-2 contains 46 mol of phosphate per mole of MAP-2 *in vivo*, but 16 mol of phosphate per mole of MAP-2 may bind to the tubulin binding domain of MAP-2 (Tsuyama *et al.*, 1987).

The morphology of most cancer cells is abnormal. Therefore, the relationship between cancer and microtubule assembly is of interest. In the cell cycle, microtubule severing and reorganization occur in the M-phase. During this phase, phosphorylation and dephosphorylation of MAPs are controlled by an M-phase promoting factor and cyclin-dependent kinase (Vale, 1991; Shiina and Moriguchi, 1992; McNally and Vale, 1993). Microtubule reorganization in the cell cycle has been studied by many research groups and is reviewed elsewhere (Hunt, 1989; Forsburg and Nurse, 1991).

Tau is a heat-stable protein with has a molecular weight of 50,000 as estimated by SDS-PAGE, and it binds to microtubule lattices. The amino acid sequence of tau includes three or four repeats of a motif containing positively charged amino acid residues at the carboxy-terminal of the

tubulin-binding sites, which are similar to MAP-2 (Staden, 1982). Phosphorylated tau inhibits microtubule assembly. Hyperphosphorylated tau is present in paired helical filaments in human brains affected by Alzheimer's disease (Bramblett *et al.*, 1993). Protein phosphatase 2A in the human brain may control the tau phosphorylation level (Sontag *et al.*, 1996). Alcoholic hepatitis and the toxic influence of griseofulvin on the liver causes the appearance of hyalin cytoplasmic inclusions (Mallory bodies) containing entanglement elements with microtubule elements (Irie *et al.*, 1991). Abnormally phosphorylated tau has been found in a component of Mallory bodies in alcoholic hepatitis (Kenner *et al.*, 1994). However, the relationship between tau and protein kinases has not been identified.

All phosphorylated MAPs can be dephosphorylated by protein phosphatases, which may regulate the switching off of signal transduction for various stimulations. Cohen's group classified protein phosphatases according to various criteria including inhibitor sensitivity, regulatory cofactors, and substrate specificity (Ingebritsen *et al.*, 1983). Protein phosphatases 1, 2A, and 2B catalyze MAPs dephosphorylation *in vitro* and *in situ*. However, the specific protein phosphatases for phosphorylated MAP-1, MAP-2, and tau have not been found. Thus, MAPs promote microtubule assembly and disassembly via phosphorylation and dephosphorylation.

Drugs affecting microtubule assembly have been used in cancer treatment. Aryl chloroethyl urea, a member of a new class of antineoplastic agents, stimulates synthesis of two cytoskeletal proteins, tubulin and vimentin, in human breast cancer cells (Poyet *et al.*, 1993). Photodynamic therapy is a new approach useful for cancer treatment. After treatment of cancer cells with the carbocyanine dye 3,3'-dihexyloxacarbocyanine, illumination of cancer cells with a standard fluorescent light disrupts the microtubule network and completely inhibits CV-1 epithelial cells from being motile (Lee *et al.*, 1995). 3-Aminobenzamide, a poly-ADP-ribosyltransferase inhibitor, also is targeted to microtubules and microfilaments (Tiozzo *et al.*, 1996). A new class of anticancer drugs such as carbocyanine and 3-aminobenzamide that may be related to microtubule organization may be developed.

Microfilaments

Actin has a molecular weight of 42,000 as estimated by SDS-PAGE, and is globular. In eukaryotic cells, actin is the most abundant protein; about half of the actin is present as a monomer (globular actin, G-actin) and the other half is present as a polymer (filamentous actin, F-actin). Its amino acid sequence is highly conserved among various eukaryotic cells including human cells (Miwa *et al.*, 1991; Hennessey *et al.*, 1993). Alpha-actin in skeletal muscle is involved in muscle contraction and relaxation. On the other hand, beta- and gamma-actins in nonmuscle cells have the same function as microtubules. Therefore, the assembly mechanism of F-actin is important not only as related to the production of F-actin with the correct structure for interaction with other components of the cytoplasm but also as a cell function, because controlled assembly or disassembly of F-actin is needed for nonmuscle cell activities. Cytoskeletal actin is very active in most eukaryotic cells, with the biological half-life of F-actin being on the order of minutes. Thus, F-actin in cells undergoes continuous change, with assembly of new F-actin and disassembly of old F-actin. The polymerization of actin is reversible, and the extent of its polymerization *in vitro* in the absence of actin-binding proteins is governed by the temperature and ionic character of the incubation solution. The turnover of F-actins and the properties of particular actin-binding proteins that control the organization of cytoskeletal actin are modulated by environmental signals via hormones, growth factors, and extracellular matrix receptors. Therefore, F-actin assembly and disassembly are regulated by many actin-binding proteins through signal transduction. Cytoskeletal G-actins have been purified by column chromatography and cycles of polymerization and depolymerization *in vitro*. Actin polymerization is usually monitored by the measurement of their viscosity (Pollard and Cooper, 1982).

Toxins and drugs affecting actin assembly

The cytochalasins are a group of toxic metabolites produced by fungi of the genus *Ascomycotina*; they bind to the glucose transporter of eukaryotic cells and to actin (Rampal *et al.*, 1980). Experiments with *p*-mercuribenzenes

sulfonate have shown that cytochalasin binds to cysteine-374 of actin at one mol per F-actin molecule, inhibiting G-actin exchange at the barbed ends of F-actins (Lin *et al.*, 1984). The binding affinities of the members of this class of toxins for glucose transporters differ from those for F-actin (Brown and Spudich, 1981; Suzuki and Mihashi, 1991).

Phallotoxins, which can cause cyanosis, vomiting, convulsions, and blood stools, are cyclic peptides produced by the basidiomycetes *Amanita phalloides*; one phalloidin binds to F-actin at a stoichiometry of one mol to two mol of actin, but does not bind to G-actin (Miyamoto *et al.*, 1986). Derivatization studies have shown that the toxin binds to methionine-119 and -355 and may bridge two actin molecules in F-actin (Vandekerckhove *et al.*, 1985; Faulstich *et al.*, 1992). Fluorescence-labelled phalloidin is useful for investigation of the organization of F-actins in cells.

Cholera toxin and pertussis toxin mono-ADP-ribosylate the alpha-subunit of the heterotrimeric GTP-binding proteins. On the other hand, *Clostridium perfringens* iota toxin and botulinum C2 toxin from *Clostridium botulinum* cause muscular paralysis and diarrhea, respectively, and are specifically mono-ADP-ribosylated with arginine-177 of actin (Aktories *et al.*, 1986; Ohishi and Tsuyama, 1986; Vandekerckhove *et al.*, 1988). ADP-ribosylated actin does not bind to the barbed end of F-actins. Therefore, mono-ADP-ribosylation of actin by these toxins may cause abnormal cell configurations, through actin polymerization and depolymerization in muscle and nonmuscle cells.

Aminoglycoside causes hearing loss, which may result from interference with the formation of actin filaments in the cells in the outer or inner ear (Zenner and Gitter, 1989; Pettigrew *et al.*, 1988).

Latrunculin A and mycalolide B from a marine sponge cause actin depolymerization (Coue *et al.*, 1987; Saito *et al.*, 1994).

In the post-translational modification of actin, processing of the amino-terminus occurs in all actins examined so far, apparently shortly after translation. The main consequence of this processing is the addition of an acetyl group to the alpha-amino group of the terminal amino acid

(Hennessey *et al.*, 1991). Most actins contain a methylated histidine residue at histidine-73 of actin, but the functional effects of this modification are not known.

Other drugs, such as cannabitol, cyclosporin A, and reserpine, act on actin in nonmuscle cells.

Actin-binding proteins

In histochemical studies, actin filaments in nonmuscle cells were found in subplasma membrane sites where actin-binding proteins are present. When actin-binding proteins are added to actin *in vitro*, the properties of the actin are changed and F-actin or G-actin is formed. Many actin-binding proteins have been isolated and characterized (Table 2). Actin-binding proteins have been classified into two groups. One consists of Ca^{2+} -dependent proteins, with their activity controlled by Ca^{2+} . Therefore, it is appropriate to consider the known structure of a highly specific Ca^{2+} -binding site for control of actin polymerization, usually called an EF-hand (Nakayama *et al.*, 1992). The other group consists of proteins that control actin polymerization, originally called capping-proteins, monomer-sequestering proteins, and depolymerizing-factors.

Calmodulin has a total of four EF-hands in two globular domains separated by a long α -helix. This 17-kDa protein accounts for 1% of the total protein mass in eukaryotic cells, and was first discovered in skeletal muscle (Gerday, 1988). Calmodulin is a multipurpose intracellular Ca^{2+} receptor and mediates many Ca^{2+} -regulated processes. Ca^{2+} /calmodulin binding to actin can cause a large change in the conformation of cells and may stimulate phosphorylation of myosin light chains in smooth muscle and nonmuscle (Bayley *et al.*, 1988). Myosin light chains are somewhat similar in sequence to calmodulin, and in general Ca^{2+} binds to only one of the four EF-hands. Myosin is usually considered in terms of its mechanochemical activity in generating contractile forces and in its movement together with actin, but this molecule is also an actin cross-linking agent and acts on actomyosin-mediated platelet contractility and erythrocyte morphology (Bourguignon *et al.*, 1985; Fowler, 1986).

Troponin C, which has properties including crystal structure similar to those of calmodulin,

causes changes in conformation that allow myosin heads to interact with F-actin in muscle cells. Therefore, troponin C is closely related to calmodulin, which mediates Ca^{2+} -signaled responses in nonmuscle cells (Fine and Blitz 1975; Fattoum *et al.*, 1983).

Gelsolin (90 kDa) is both a barbed-end capping protein and a Ca^{2+} -activated filament-severing protein; it binds two mol of Ca^{2+} per mole with high affinity and can bind to both G- and F-actin. One mol of gelsolin binds to two mol of G-actin, and its binding activity depends on the Ca^{2+} concentration (Bryan and Kurth, 1984; Janmey *et al.*, 1985).

Villin (95 kDa) is found in vertebrate cells with microvilli (Alicia and Mooseker, 1988). The function of villin is similar to that of gelsolin.

Other Ca^{2+} -dependent actin-binding proteins such as fragmin and severin are present in slime molds.

The other actin-binding proteins affect actin polymerization and depolymerization via protein-protein interaction in the absence of Ca^{2+} . These proteins usually bind to F-actin barbed or pointed ends, and thereby prevent G-actin association with actin polymers. G-actin-sequestering protein might bind to G-actins at a site different from that at which the G-actin binds to F-actin *in vivo*. DNase I, a G-actin-sequestering protein in nonmuscle cells, forms a tight complex with G-actin at lysine residues in the amino terminus of actin (Sutoh, 1984). DNase I binding gel is useful for isolation of G-actins from crude cell extracts. A vitamin-D-binding protein in mammalian plasma depolymerizes F-actin, but the details of the mechanism are not known (Haddad *et al.*, 1992). Profilin (15 kDa), purified from various animal tissues, usually binds at cysteine-374 of G-actin, and such binding inhibits the ability of G-actin to self-assemble into F-actin (Malm, 1984). Thymosin (5 kDa), found in many kinds of cells, is also an actin-sequestering polypeptide, but it is not clear how this polypeptide inhibits actin polymerization (Heintz *et al.*, 1993).

There are many reports on actin-binding proteins, and therefore we only briefly describe here actin-binding proteins other than those already mentioned. Two F-actin bundling proteins that have been identified are fimblin (68 kDa) and α -actinin (2 X 100 kDa), both of which medi-

Table 2. Proteins that bind to actin.

<i>Protein</i>	<i>Mr</i>	<i>Sources</i>
Motor proteins		
Myosin II	175,000 - 220,000	Muscle, nonmuscle cells
Myosin I	125,000 - 135,000	<i>Acantamoeba</i> , <i>Dictinostelium</i>
Proteins controlling F-actin interactions		
Tropomyosin	35,000	Muscle, nonmuscle cells
	30,000	Nonmuscle cells
Troponins I, T, and C	30,000	Striated muscle
Caldesmon	70,000 - 90,000 and 140,000?	Smooth muscle and nonmuscle cells
Actin-membrane proteins		
Calpactins I and II	30,000 - 40,000	Vertebrate cells
Isotropic network-forming proteins (gelation factors)		
Spectrin family	220,000; 240,000	Red blood cells and vertebrate cells
Fordin	235,000; 240,000	Brain and avian lens
Filamin	250,000 - 270,000	Smooth muscle
Bipolar filament bundling proteins		
alpha Actinin family	95,000	Striated and smooth muscle
	105,000	Vertebrate and non- muscle cells
Polar filament bundling proteins		
Fascin	53,000	Porcine brain
	57,000	Sea-urchin eggs
Other bundling proteins		
Band 4.9	48,000; 52,000	Erythrocytes
Table 2. Continued.		
MAP-2	200,000	Neurons
Synapsin I	76,000	Presynaptic junctions
Pointed end-blocking proteins		
β -Actinin	36,000 - 37,000	Kidney and skeletal muscle
Simple barbed end-blocking proteins		
Capping proteins	28,000 - 31,000	<i>Acantamoeba</i>
cap Z	32,000; 34,000	Muscle
Barbed end-blocking and severing proteins		
Gelsolin	90,000	Mammalian cells
Monomer-sequestering proteins		
Profilin	12,000 - 15,000	Most cells
Monomer-sequestering and filament-severing proteins		
Cofilin/ADF/19 kD	19,000 - 21,000	Vertebrate brain
Destrin	19,000	Kidney
.....probably irrelevant to living cytoskeleton		
Vitamin-D-binding protein		Blood plasma
DNAse I		Bovine pancreas

Table 2 is cited from Amos and Amos, 1991.

ate cross-linking of F-actins into parallel arrays. Filamin (2 X 270 kDa) also mediates the cross-linking of F-actins and promotes the formation of a loose and highly viscous F-actin network by clamping together two F-actin molecules that cross each other in various cells. One important actin-binding protein is spectrin, which laminates the cytoplasmic side of the plasma membrane. Although spectrin was first identified in erythrocytes, spectrin isoforms have been purified from a wide variety of cells and tissues; these isoforms are fodrin, TW 260/240, calspectrin, and nonerythrocyte spectrin. Spectrin is a heterodimer of two large subunits (alpha-subunit, 240 kDa; beta-subunit, 220 kDa) that are structurally similar. The heterodimers self-associate head-to-head to form long tetramers 200 nm long. The tail ends of four or five tetramers are linked to short F-actins and to other cytoskeletal proteins in a junctional complex. Humans and mice with spectrin gene abnormalities are anemic and have erythrocytes that are spherical and abnormally fragile (Fowler, 1986). The binding of spectrin to F-actin depends on the presence of a large intracellular attachment protein named ankyrin (210 kDa), which attaches to both spectrin and the cytoplasmic domain of the transmembrane protein band 3, which is another major erythrocyte membrane protein (Weaver *et al.*, 1984). The cross-linking of F-actin and microtubules is interesting from the viewpoint of cell morphology. When F-actins are incubated with MAP-2 or tau, bundles of F-actin that can be seen by electron microscopy are formed (Griffith and Pollard, 1982). Phosphorylation of MAP-2 or tau decreases the ability of the bundles to interact with F-actins (Selden and Pollard, 1983).

There are various actin-binding proteins in animal cells, but a specific drug or toxin affecting actin-binding proteins has not been identified.

Intracellular signaling and actin

As mentioned above, the assembly and disassembly of cytoskeletal proteins are regulated by intracellular signaling. For example, Ca^{2+} influx into a cell causes actin assembly in the presence of calmodulin. In other cases, phosphorylation of MAPs and ADP-ribosylation of actin causes changes in cytoskeletal protein assemblies.

The Rho GTPases form a subgroup of the

Ras superfamily of small GTP-binding proteins, comprising Rho, Rac, and Cdc42 (Boguski and McCormick, 1993). Recently, evidence has been obtained that the Rho family regulates cell morphology and cell motility. Microinjection of botulinum C3 neurotoxin, which selectively ADP-ribosylates Rho and inactivates it, or of dominant negative Rho inhibits motility of Swiss 3T3 cells (Takaishi *et al.*, 1993), and phorbol-ester-induced cell motility of keratinocytes (Takaishi *et al.*, 1994). Further, microinjection of either material into eggs inhibits cytokinesis in *Xenopus* embryos (Kishi *et al.*, 1993), and no F-actins were detected in the contractile ring of sand dollar eggs (Mabuchi *et al.*, 1993). In fibroblasts, polymerized actin is assembled into a variety of different structures, and Rho controls the formation of stress fibers made of F-actin bundles that abut the plasma membrane at focal adhesions (Ridley and Hall, 1992). Rac controls the dynamics of lamellipodia (Ridley *et al.*, 1992), which are pleat-shaped protrusions at the cell periphery that might act in cell migration. Cdc42 controls the morphology of filopodia, which are thin finger-like cytoplasmic extensions thought to be involved in recognition of the extracellular environment (Nobes and Hall, 1995). Ras signaling is involved in 1-oleyl lysophosphatidic acid-induced invasion of hepatoma cells in a mesothelial cell monolayer *in vitro* (Imamura *et al.*, 1996). T-lymphoma cells expressing Rac become invasive, so a signaling pathway involving a Rho small GTPase family could be operating in the invasion and metastasis of tumor cells (Michiels *et al.*, 1995). Fig. 1 shows a diagram of a proposed signaling cascade of cytoskeletal actin via small GTP-binding proteins.

The mechanism by which Rho family members regulate actin polymerization was recently elucidated. Activated Rac causes uncapping of barbed ends of F-actin in platelets made permeable (Hartwig *et al.*, 1995). Rac also stimulates phosphatidylinositol (4,5)-bisphosphate synthesis, and Rac-induced uncapping of F-actins is inhibited by a peptide derived from gelsolin and binding to this product. This finding suggests that the control of capping protein activity by Rac is mediated by changes in the rate of polyphosphoinositide synthesis. Cdc 42 and Rho stimulate the production of polyphosphoinositide (Zhang *et al.*,

1993; Zheng *et al.*, 1994).

The role of the effectors of these small GTPases in cell proliferation, if any, remains to be elucidated, and the precise mode of coupling of these GTPases into cascades might depend on the kind of cell and experimental conditions.

Future directions

Cytoskeletal proteins seem to mediate dynamic cellular events through their assembly and disassembly. Cytoskeletal proteins may be involved in all cellular functions; therefore, toxicological events in humans and other animals might be related to cytoskeletal functions. Recently, many reports on investigations of the bacterial cytoskeleton at cell division have appeared. If the physicochemical and biochemical properties of the bacterial cytoskeleton differ from those of the animal cytoskeletons, a new class of antibiotics probably can be developed in the near future. Detailed knowledge of the molecular mechanism of the regulation of assembly and disassembly of cytoskeletons in cell division and the relationships between the extracellular matrix and cytoskeleton will facilitate develop-

ment of new anticancer drugs.

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Fig. 1.

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