The Cytoskeleton and Cell Signaling: Component Localization and Mechanical Coupling

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I. INTRODUCTION

The cytoskeleton is a filamentous network of F-actin, microtubules, and intermediate filaments (IFs) composed of one of three chemically distinct subunits, actin, tubulin, or one of several classes of IF protein (20, 191). Modulation of the cytoskeletal network changes the mechanical properties of the cell that are essential for functions such as locomotion (151, 209) and cytokinesis (61). The filamentous cytoskeletal network also provides a scaffolding on which motor proteins such as kinesin, dynein, and myosin can translocate to move organelles or generate internal stress. The transmembrane receptors and intracellular signals producing cytoskeletal changes in response to extracellular stimuli have been extensively studied and are the subject of several recent reviews (42, 44, 209, 214, 253).

The cytoskeleton undoubtedly regulates cellular mechanics, but there are probably other important functions of this ubiquitous and extensive organelle. Independent of its mechanical strength, the filaments of the cytoskeleton form a continuous, dynamic connection between nearly all cellular structures, and they present an enormous surface area on which proteins and other cytoplasmic components can dock. To put this feature in perspective, the surface area of the plasma membrane of a 20-μm-diameter cell is on the order of 700 μm², and the total surface area of all cytoplasmically disposed lipid bilayers is ~10 times this area. In contrast, the total surface area of a typical concentration of 10 mg/ml F-actin in such a 20-μm cell is 47,000 μm², and an approximately equal area is presented...
by microtubules and IFs. This large surface, along with the high negative electrostatic charge density of all cytoskeletal filaments (107, 212, 213), presents a strong potential for localization and immobilization of cytoplasmic components.

On one hand, some proteins bind to the cytoskeleton to alter its assembly in response to cellular signals. On the other hand, probably hundreds of proteins copurify with the cytoskeleton of detergent-permeabilized cells, often to an extent that depends on activation of specific cellular signals. Many such proteins have been shown to bind purified F-actin or other cytoskeletal filaments in vitro with affinities characterized by micromolar dissociation constants. Because the cytoplasmic concentration of actin and other cytoskeletal filaments is on the order of 10−100 μM, a major fraction of such proteins would be expected to bind the cytoskeleton in vivo.

With regard to the complexity of the cytoskeleton, the intricate association of one filament type with another, and the relatively high concentration of the cytoskeletal proteins, various criteria have been used to define instances where the cytoskeleton binds or otherwise alters elements of signal transduction pathways. In many cases, linkage of a signaling molecule to the cytoskeleton is detected by sedimentation from detergent-permeabilized cell extracts under conditions where it would, in the absence of the cytoskeleton, remain in the supernatant. Such data are often supplemented by finding that the coprecipitation with the cytoskeleton depends on whether the cell is activated by specific agonists, or that selective disruption of cytoskeletal elements alters its solubility. Whether coprecipitation depends only on proteins of the cytoskeleton is often difficult to ascertain because complexes such as focal adhesions (29), cell-cell junctions (142), and caveoli (53, 135, 136, 171, 246) appear to be sites involving both cytoskeletal proteins and membrane lipids where multiple signaling molecules concentrate.

The influence of cytoskeletal structures on signaling in vivo is often detected by the effects of specific agents, such as cytochalasin, colchicine, or acrylamide, that selectively disrupt actin filaments, microtubules, and IFs, respectively. Extending such relatively indirect evidence is an increasing body of data that identifies binding between elements of signaling pathways, including both receptors and cytoplasmic proteins, with specific cytoskeletal proteins using both biochemical and genetic methods. Such specifically defined interactions are emphasized throughout this text.

Among the components localized to the cytoskeleton are ribosomes and RNA. Except for a few examples of proteins involved in the translational machinery that interact specifically with cytoskeletal filaments, the reader is referred elsewhere for recent reviews of this important field (10, 99, 173). Transmembrane protein complexes that link cells to each other or to the extracellular matrix not only link the cell membrane to the actin or IF cytoskeleton but also localize and activate signaling molecules that ultimately lead to changes in cell structure and gene expression. Signaling events at these membrane sites have received several excellent recent reviews to which the reader is referred for detailed discussion (5, 10, 15, 38, 226, 245).

This review focuses on those aspects of signal transduction that depend on the unique capacity of the cytoskeleton to form a continuous three-dimensional network. One consequence of network formation is the establishment of an elastic structure by which mechanical stimuli can be sensed and transmitted. Such a mechanical continuity is likely to be required for cells such as endothelial cells to respond to fluid shear stress, as reviewed in References 48 and 49. Another consequence of polymerization and network formation is the potential for spatial segregation either to prevent or enhance the reaction of cytoskeleton-associated enzymes with their targets. The chemical and physical properties of the cytoskeleton suggest that it may have subtle and important effects on the way in which information is passed from points at the cell membrane to structures deep within the cytoplasm or the nucleus (62, 63).

II. LOCALIZATION OF ENZYMATIC ACTIVITIES TO THE CYTOSKELETON

A. Glycolytic Enzymes

Among the longest known and best-characterized cytoskeleton-binding proteins are glycolytic enzymes (4). Although these enzymes are not usually considered as central to signal transduction pathways, their localization to cytoskeletal filaments, along with some of their substrates (72), presents a model by which signal sequestration mechanisms may be interpreted. In some cases, glycolytic enzymes may themselves be important mediators of cell response and function. The fundamental idea (reviewed in Refs. 147, 165) is that some key glycolytic enzymes, in particular, aldolase, glyceraldehyde-3-phosphate dehydrogenase, lactate dehydrogenase (LDH), and phosphofructokinase-1, are sequestered near the hydrated surface of actin filaments (41) within a complex layer containing also some of their substrates (see Fig. 1). In some cases, binding to F-actin also affects enzymatic activity. The direct relevance of these interactions to glycolysis in vivo has recently been demonstrated by specific localization in vivo of aldolase with actin stress fibers (166), the colocalization of glucokinase with cellular actin (154), and the close correla-
in addition to the tight, highly ordered protein-protein bonds more commonly considered to provide specificity to biologically relevant binding events. These same concepts may likewise be crucial to placing the multiple complex associations of signaling factors with the cytoskeleton in a physiological context.

**B. Protein Kinases**

1. **Tyrosine kinases and Src homology domains**

   Nonreceptor tyrosine kinases related to pp60src were among the first protein kinases found to translocate to cytoskeletal fractions after activation of cells. Both v-Src and c-Src localize to the cytoskeleton in virus-infected cells, with the latter interaction proposed to be mediated by binding of the c-Src.polyoma middle-T antigen complex to the actin-binding protein vinculin (6). Differential cytoskeletal binding of c-Src and v-Src depends on differences in both SH2 and catalytic domains, but is independent of the SH3 domain (161). Release of c-Src may be induced by phosphorylation on Tyr-527 (95). Translocation of c-Src to the cytoskeleton of A172 glioblastoma cells is induced by both platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), in a process that also activates kinase activity (242), and stimulation of platelets by thrombin also recruits Src to the actin-based cytoskeleton (84).

   In fibroblasts, localization of both v-Src and c-Src to the cell periphery could be blocked by the actin-disrupting drug cytochalasin, but not by microtubule destabilization by nocodazole. Cytoskeleton-dependent translocation of Src could also be altered by microinjection of the small GTPase RhoA or by addition of PDGF to serum-starved cells, implicating small G proteins in relation between the changes in the actin filament network structure and metabolic rates during the cell cycle (17) and in cases of experimental manipulation of cytoskeletal structure (221).

   A striking feature of cytoskeletal localization of some glycolytic enzymes is that they are not entirely specific to actin filaments. Phosphofructokinase, for example, also binds to and is inhibited by tubulin and microtubules (124, 237), and glyceraldehyde-3-phosphate dehydrogenase binds all three filament systems. Although such promiscuous binding may suggest nonspecific, biologically irrelevant association, the accruing evidence that such associations occur in vivo and have metabolic consequences, along with recent demonstration that in some cases single point mutations (238) can ablate cytoskeletal binding, argue strongly for the importance of this mode of cytoskeletal localization. The concepts developed to explain enzyme sequestration and metabolic channeling emphasize the importance of multiple weak interactions at a surface,
cytoskeleton as well as phosphorylate its target proteins.

2. Protein kinase C and cAMP-dependent kinase-actin interactions

Some protein kinase C (PKC) isotypes bind to actin filaments, and this binding appears to be essential for the translocation of PKC-α to the nucleus of NIH 3T3 fibroblasts treated with phorbol ester (192). In other cases where the target of PKC is in the cytoplasm or in the plasma membrane, localization to actin filaments (250, 251) activates the enzymatic activity of PKC. Protein kinase C-βIII, an isotype presumably distinct from the PKC-β bound to vimentin (244), is specifically activated by binding F-actin (23) after stimulation by phorbol ester. Protein kinase C-ζ translocates to the actin system specifically after stimulation by interleukin-2 (83). In the latter case, association of PKC is needed to stabilize the actin network, and the function of PKC bound to the cytoskeleton is required for glutamate release in neuronal cells stimulated by phorbol (216). Protein kinase C-ε is also involved in regulation of synaptic function, and a specific binding site for F-actin is exposed when PKC-ε is activated. The anchoring of PKC-ε to actin filaments in the nerve ending appears to facilitate glutamate release in part by maintaining PKC in the active state (176).

The cAMP-dependent protein kinase IIb is also linked to the actin cytoskeleton in both neurons (131) and non-neuronal cells (130). Rather than a direct binding to F-actin, in this case the kinase is linked to the cytoskeleton by a kinase anchor protein AKAP75 (76). AKAP75 colocalizes with F-actin and the actin-membrane binding protein fodrin and may be directly bound not to actin but to actin-associated protein complexes (130). An especially interesting cytoskeletal association may occur with protein kinase N. This kinase is implicated as a downstream target of the small GTPase Rho, and associated with the cytoskeletal protein α-actinin, only in conjunction with phosphatidylinositol 4,5-bisphosphate (PIP₂), a lipid that activates the actin cross-linking function of α-actinin and has other effects on the actin system (153).

3. Binding of kinases to microtubules and intermediate filaments

In contrast to other tyrosine kinases such as Src and Abl, which bind the actin cytoskeleton, the tyrosine kinase Yes colocalizes with vimentin IFs, and in microglia, phorbol esters and dibutyryl cAMP stimulate its binding to the cellular vimentin network (40). The cGMP-dependent kinase that mediates relaxation of contracted vascular smooth muscle cells also binds to vimentin with high affinity (dissociation constant = 50 nM) (141). Protein kinase C binds vimentin in retinal cells (244), and the activated, phosphorylated PKC-δ isoform binds vimentin in phorbol-treated HL-60 cells (163). In rat basophilic leukemia cells, PKC-β specifically localizes to vimentin filaments, whereas PKC-α in these same cells localizes to the actin-rich cortex (206). Binding of PKC to vimentin is often associated with increased vimentin phosphorylation (163, 244). Similarly, a neurofilament-associated kinase is a major cause of neurofilament medium-molecular-weight subunit phosphorylation (98). In addition to an association with α-actinin, protein kinase N is identified by a yeast two-hybrid screen as a ligand for the neurofilament low-molecular-weight subunits of neurofilaments and may function in altering the neuronal cytoskeleton (153). In all these cases, localization of kinases to the IFs may either enhance phosphorylation efficiency or prevent inappropriate phosphorylation of soluble factors. In some cases, vimentin itself may be one of the main targets for the kinases, and localization may be part of the mechanism for selective phosphorylation.

Several distinct kinase activities copurify with microtubules, and there is evidence of age-related changes in this kinase activity (126). One such kinase activity derives from a subset of mitogen-activated protein (MAP) kinase that binds to microtubules (179). This bound pool, unlike the soluble pool, is constitutively active (152). In addition, the cAMP-dependent kinase regulatory subunit is linked to microtubules by its interaction with the microtubule-associated protein MAP2 (140, 159, 188).

C. Lipid Kinases and Phospholipases

Some of the same signals that promote cytoskeletal binding of protein kinases also recruit phospholipases and lipid kinases to the cytoskeleton. In platelets, thrombin stimulation causes the association of phospholipase A₂ (2), phosphatidylinositol (PI) 3-kinase, PI 4-kinase, diacyl-glycerol (DAG) kinase, and phospholipase C (PLC) to the cytoskeleton during the period when a massive polymerization of actin occurs (7, 84, 96, 156). Phosphatidylinositol 4-phosphate (PIP) 5-kinase and PLC appear to localize before the other enzymes, and the specific activities of both PI 3- and 4-kinases are higher in the cytoskeletal fraction (84). Much of the PLC activity localized to the cytoskeleton is the PLC-γ isoform that binds the actin cytoskeleton by its COOH-terminal SH2 domain (9, 169), whereas the isolated SH3 domain is not itself a direct link to filamentous actin (232). The membrane-bound G protein-coupled isoform (PLC-β) also depends on the actin cytoskeleton for its localization and coupling to the G protein (234). Phosphatidylinositol 3-kinase is reversibly recruited to the ADP-stimulated platelet cytoskeleton by a mechanism not dependent on Rho protein and probably distinct from the thrombin-signaling cascade (71). Phos-
phosphoinositide (PPI) kinase, DAG kinase, and PLC activities also localize to the cytoskeleton of A431 cells after stimulation by EGF (168).

Localization of PI3-kinase to the centrosome and other intracellular structures depends on its binding to tubulin (111, 112). In particular, there appears to be an insulin-stimulated binding of PI3-kinase to γ-tubulin that may be involved in regulating microtubule responses to insulin and other stimuli (112). A notable aspect of the recruitment of inositol lipid kinases and phospholipases is that the substrates for these enzymes also copurify with the cytoskeleton, especially after cell stimulation (96, 236).

Not only are lipid kinases and lipases localized to the cytoskeleton, but their activities are altered by a number of cytoskeletal proteins, especially those associated with actin. Phospholipase C-γ is strongly inhibited by profilin (81, 82) and gelsolin (8), and the inhibition by profilin is overcome when PLC-γ is tyrosine phosphorylated (81). Similarly, PLC-ε1 and PLC-β1 are inhibited by myristoylated, alanine-rich C-kinase substrate (MARCKS) protein by a mechanism involving redistribution of lipid packing by MARCKS that is reversed by PKC (77). In contrast, the microtubule binding protein tau acts as a cofactor with arachidonate to stimulate PLC-γ activity (101), and a peptide based on the sequence of PLC-β homologous to the PPI binding motif in gelsolin (249), strongly stimulates PLC-β activity (199–201). Opposing effects on phospholipase D (PLD) activity are produced by gelsolin (208), which stimulates, and fodrin (139), which inhibits, this enzyme. Lipid kinase activity is also altered by actin-binding proteins. Profilin and gelsolin, which inhibit PLC-γ, both stimulate PI3-kinase activity (202). A conjunction of these effects in vivo would lead to an upregulation of phosphorylated inositol lipids when these proteins are bound to PPIs at times when phosphoinositide turnover is stimulated.

The numerous effects of PPI-regulated cytoskeletal proteins on inositol lipid kinases suggest many possibilities for stimulation or inhibition of lipid-cytoskeletal complexes at sites such as focal adhesions. Several proteins that make up the complexes linking activated integrins to the cytoskeleton, including talin (113, 215), vinculin (75), and α-actinin (65, 66), bindPIP2 and in some cases are activated by this lipid to bind F-actin. At the same time, activated integrins recruit PIP5-kinase and strongly increase cellular PIP2 levels (149). A mechanism in which initial formation of PPIs by kinases activated at specific transmembrane receptors recruits cytoskeleton-membrane linkers that also enhance lipid kinase activity would lead to stimulation of these assemblies at highly localized sites. A similar mechanism may also affect binding of PIP2-bound ezrin/radixin/moesin-related proteins (158) to their transmembrane targets such as intracellular adhesion molecules (94) or CD44 (97).

D. GTPases

The regulation of cytoskeletal structure, in particular the various forms of actin-based cytoskeleton, has been shown in many cases to be directed by small GTPases of the rho family. This regulation has been the subject of recent reviews (211, 214). The focus of this section is not on how such proteins regulate the cytoskeleton, but on instances in which cytoskeletal localization of GTPases may function in transduction of their signals to other cellular targets.

1. Heterotrimeric G proteins

A number of heterotrimeric GTPases move onto or off the cytoskeleton of both mammalian and plant cells in response to cell activation (54). The $\gamma_{\text{i,}2}$ and $\gamma_{\text{i,}1}$ subunits distribute to the cytoskeletal fraction of platelets within 1 min after thrombin stimulation as platelets undergo an actin-dependent shape change, followed by binding of $\gamma_{\text{i,}}$ and $\beta_{\text{i}}$ during platelet aggregation (164). A similar recruitment of $\gamma_{\text{i,}2}$ to the cytoskeleton of HL-60 cells occurs after stimulation by formyl-methionyl-leucyl-phenylalanine (FMLP), which like thrombin signals through a seven-transmembrane loop receptor (243). $\gamma_{\text{i,}1}$ and $\gamma_{\text{i,}1}$ also bind the actin cytoskeleton of rat mammary tumor cells, where association of these G proteins with F-actin participates in vasopressin-dependent PL activity (102). In contrast to these examples of G proteins recruited to the cytoskeleton, the $\gamma_{\text{i,}1}$ subunit of neutrophils is concentrated on the cytoskeleton of unstimulated neutrophils and is released as actin polymerization and redistribution are stimulated by FMLP binding (189). In many cases, the activated form of the G protein is selectively bound to the cytoskeleton, and there is evidence that cytoskeletal interactions may directly participate in this activation. Specifically, a direct interaction between tubulin and the $\beta_{\gamma}$-subunits of Gs and G11a can transfer a GTP from tubulin to the $\alpha$-subunit of the G protein (186). In this way, the cytoskeleton may act not only to localize these signaling proteins but promote their activation at specific sites (187).

2. Small GTPases

Small G proteins also undergo reversible association with the cytoskeleton. In part, this activity is likely related to the regulation of the actin cytoskeleton by rho, rac, and cdc42 (reviewed in Ref. 214), but localization of these and other GTPases may also direct them to other targets or regulatory elements. Similar to the translocation of trimeric G proteins, the small GTPase rap2B binds to the cytoskeleton of platelets stimulated by thrombin, but in this case, recruitment also requires binding of fibrinogen to the GPIIb/IIa integrin (222). Two other unidentified
small G proteins copurify with the cytoskeleton of resting platelets and bind increasing amounts of GTP after thrombin stimulation (177). In at least some cases, binding of small GTPases to the cytoskeleton is mediated by linker proteins, including WASP (210), n-chimerin (117), and the ras GTPase-activating protein homolog IQGAP2, which contains a potential actin binding site homologous to that in calponin (27). The multiplicity of GTPases and associated proteins that undergo regulated binding to the cytoskeleton suggests that these events are not entirely related to signals directly leading to cytoskeletal reorganization but may serve other purposes to sequester and organize elements of signaling pathways.

### 3. Elongation factors

At least two eukaryotic elongation factors involved in protein synthesis in the cytoplasm are linked to cytoskeletal filaments, and evidence that this interaction affects the function of both the cytoskeleton and the protein translation machinery is beginning to accrue. In human skin fibroblasts, elongation factor (EF)-2 transits from the nucleus to all three cytoskeletal filament types as cells move from a proliferative state to G0 (195). Colocalization of EF-2 with actin filament bundles is evident by immunocytochemistry and is disrupted by cytochalasin (194, 196). Direct binding of EF-2 to F-actin assayed by sedimentation is stimulated by guanosine 5′-O-(3-thiotriphosphate), competitively inhibited by EF-1α, but unaffected by ADP-riboosylation of EF-2 that causes a block of protein synthesis (12). Elongation factor-1α also binds tightly and bundles F-actin in a pH- and calmodulin-dependent manner (55, 119, 137, 155), and the Dictyostelium isoform of EF-1α was identified as a protein mediating actin cytoskeletal bundling in response to cell stimulation (247). An important link between cytoskeletal regulation and control of protein synthesis is suggested by the finding that actin filament binding inhibits the interaction of EF-1α with aminoacyl-tRNA (137). A further link to signaling pathways controlling protein synthesis is a report that a cytoskeleton-bound activator of PIP 5-kinase in plants is highly homologous to EF-1α (248). Elongation factor-1α and EF-2 also bind microtubules, and EF-1α was identified as a factor capable of severing microtubules in vitro (198).

### III. THE CYTOSKELETON AND INTRACELLULAR CALCIUM

Several lines of evidence point to a direct link between cytoskeletal structure and intracellular Ca2+ concentration ([Ca2+]i) levels, and a number of different mechanisms have been proposed. Treatment of cells with cytochalasins that disrupt the actin cytoskeleton can lead to increased intracellular [Ca2+]. This effect may be mediated by increased PPI metabolism, consistent with the cytoskeletal dependence of phospholipases, cited above, that produce inositol 1,4,5-trisphosphate (IP3) and subsequent release of [Ca2+] from intracellular stores (233). On the other hand, some aspects of IP3-dependent Ca2+ release require an intact actin and microtubule system, as shown by the inhibitory effects of both cytochalasin and colchicine on IP3-dependent Ca2+ release in saponin-permeabilized platelets (25). The latter result is consistent with studies of Ca2+ release in permeabilized hepatocytes which suggest that the cytoskeleton is required for luminal communication between intracellular stores to allow maximal release of Ca2+ by IP3 (87). The assumption that intracellular Ca2+ is released entirely by independent IP3 receptor-containing internal organelles has been challenged by findings that the IP3 receptor of intact hepatocytes is primarily at the plasma membrane and that release from intracellular stores is achieved by a mechanical link from IP3-sensitive channels at the plasma membranes to ryanodine-sensitive channels in the endoplasmic reticulum mediated by the cytoskeleton (118).

A direct effect of the cytoskeleton, and F-actin in particular, on Ca2+ release is supported by the finding that intracellular release of Ca2+ in neutrophils stimulated by aggregated IgG binding to the Fc receptor is not mediated by IP3 but is inhibited by cytochalasin (181). In contrast, the IP3-dependent release stimulated by fMLP is unaffected by cytochalasin (181). A role for actin in signaling from the Fc receptor is further supported by the finding that the target of bromophenacyl bromide, a potent inhibitor of leukocyte activation and Fc receptor-mediated Ca2+ release, is L-plastin, an actin filament binding and bundling protein (182). How the function of plastin, itself a Ca2+-dependent protein, relates to Ca2+ release is not yet known. A very recent result suggests that the actin cytoskeleton may not only regulate Ca2+ release but may itself be part of the intracellular Ca2+ store (120). Despite the large differences in bulk concentrations of intracellular free Mg2+ and Ca2+, the probability that actin subunits near the cell membrane may bind Ca2+ and then incorporate into filaments would allow accumulation of several micromolar Ca2+ in the nearly millimolar pool of actin. This pool of Ca2+ would be released when the actin filaments depolymerize or treadmill, and even global changes of several hundred nanomolar appear to be realistically attainable. A similar release may also occur from microtubules and could possibly relate to observations that microtubule depolymerization can activate Ca2+-dependent enzymes such as myosin kinases leading to increased actomyosin contractility (115, 183).

### IV. ION CHANNELS AND TRANSPORTERS

Among the best-characterized roles for the cytoskeleton in cell signaling involves the regulation of transmem-
branes ion flux (47, 52). Most studies have employed drugs that selectively stabilize or destabilize either F-actin or microtubules, with resulting effects on specific ion channels measured either in whole cells or in single channels in a patch clamp. Although a direct binding of specific cytoskeletal proteins to individual channel proteins cannot be demonstrated by such studies, recent work in which exogenous purified actin has been added to the cytoplasmic side of channels excised from the cell membrane have in numerous cases directly confirmed an effect of cytoskeletal filaments.

One indirect way that cytoskeletal changes alter ion conductance is by altering the delivery, expression, or clustering of channel proteins at the plasma membrane (64, 128, 203). Lateral mobility of channels in the plasma membrane may be limited by interactions either with the three-dimensional cytoskeleton or the two-dimensional spectrin/fodrin-actin network underlying the plasma membrane lipid bilayer, as suggested by reports of channel proteins interacting with ankyrin (51, 207) (see Table 1). More direct effects on channel conductance, open probability, or inactivation have been found in several cases where either the actin or microtubule system has been altered. Some of these results are summarized in Table 1. Although it is probably premature to infer general patterns in these studies, there appears to be a trend to activate Na⁺ channels by actin-destabilizing drugs such as cytochalasins and to block this effect by the actin-stabilizing drug phalloidin or by adding F-actin itself. This is certainly not a strict rule, since short actin filaments activate rather than prevent Na⁺ channel opening in some cases (16), and a consensus for how such effects takes place has not been reached. In contrast, K⁺ channels are inactivated by a disrupted actin cytoskeleton caused either by cytochalasin or by the loss of expression of the major actin cross-linking protein ABP280 (33). Chloride channels, like Na⁺ channels, are more frequently activated by cytochalasin and inactivated by increasing actin cross linking, but again, there are exceptions to a simple interpretation, since F-actin itself can also activate some Cl⁻ channel activity.

In several cases where the effects of cytochalasin have been compared with those of nocodazole or colchicine, microtubule disruption has not been found to affect Na⁺, K⁺, or Cl⁻ channels. However, disruption of microtubules has been reported to enhance the activity of both Ca²⁺ (73, 218) and Cl⁻ (92, 109) channels in some cases.

An intriguing hypothesis for how disruption of actin may activate K⁺ channels is by release of protein kinase A (PKA) bound to the actin (175). In this model, the regulation depends not only on a link of actin to the receptor, but on changing localization of a cytoskeleton-bound signaling kinase, as discussed in section II B.

Some Ca²⁺ channels are inactivated by cytochalasin, and the effect is blocked by phalloidin. However, stretch-activated Ca²⁺ influx can be activated by cytochalasin and inhibited by F-actin. Moreover, there are several reports of microtubule disruption also altering Ca²⁺ channel activity (73, 218), with a strong enhancement in one case that was not achieved by cytochalasin. A scenario derived from Reference 78 for how actin filaments may affect Ca²⁺ influx resulting from mechanical stresses is shown in Figure 2. In this model, application of a mechanical stress at the plasma membrane of a resting cell causes deformation of channels in the plasma membrane that results in Ca²⁺ influx. In these cells, a sustained mechanical stress leads to assembly of two distinct structures: contractile elements containing actin filaments that create an opposing force and an elastic network that resists subsequent application of the external stress. As the stress is transmitted to the newly formed three-dimensional cytoskeleton, it is directed away from the plasma membrane, and the deformation of membrane elements near the site of force is now insufficient to activate Ca²⁺ influx.

One context in which mechanical effects on the cytoskeleton regulate ion fluxes is volume regulation after osmotic stress. Although the elastic modulus of the cytoskeleton is probably too low (1,000–10,000 Pa in some cell types and lower in others) to prevent volume changes caused by osmotic imbalance (10,000 Pa/mosM), its presence as an elastic element provides the cell with a countering tension and a memory of the undeformed state, at least for the time that the cross-linked cytoskeleton remains intact. It is not surprising, therefore, that the cytoskeleton affects ion transport in volume regulation (33, 43, 92, 129, 193). Among the clearest evidence for a mechanical function comes from the finding that melanoma cells containing approximately normal amounts of cytoskeletal filaments and ion channels, but lacking the cross-linking protein ABP280 that provides rigidity to the cell cortex, fail to volume regulate under hypotonic conditions. Transfection of ABP280 to these cells restores this capacity (33).

Whether the effects of the cytoskeleton on ion channel activity are a direct mechanical effect or an indirect effect due to such changes as regulation of kinases sequenced at the cytoskeleton remains a controversial issue. There are reports that the cytoskeleton has no effect on channel activity and reports that ion conductance through channels within a lipid bilayer can be altered by bilayer dilation in the absence of any internal network (162). On the other hand, there are intriguing hypotheses that actin filaments and microtubules could directly transmit ion fluxes because of their polyelectrolyte nature and the presence of a condensed counterion cloud (133). Although such effects appear plausible from a physical-chemical perspective, demonstrating their importance in
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<tr>
<td>K⁺</td>
<td>Rat cortical collecting duct</td>
<td>Inactivate</td>
<td>Cytochalasin B or D, blocked by phalloidin</td>
<td>240</td>
</tr>
<tr>
<td>K⁺</td>
<td>Melanoma</td>
<td>Lose volume regulation</td>
<td>Loss of ABP-280</td>
<td>33</td>
</tr>
<tr>
<td>CFTR (Cl⁻)</td>
<td>Mouse mammary adenocarcinoma</td>
<td>Activate/inactivate</td>
<td>Cytochalasin or F-actin/DNase or filamin</td>
<td>175</td>
</tr>
<tr>
<td>CFTR (Cl⁻)</td>
<td>Rat ventricular myocytes</td>
<td>Activate</td>
<td>Cytochalasin D or exogenous actin</td>
<td>32</td>
</tr>
<tr>
<td>CFTR (Cl⁻)</td>
<td>3T3 fibroblasts</td>
<td>Stimulate conductance</td>
<td>Cytochalasin D (actin) (releases PKA bound to F-actin)</td>
<td>60</td>
</tr>
<tr>
<td>Na⁺-K⁺-ATPase</td>
<td>PC-12</td>
<td>Binding to cytoskeleton</td>
<td>Cytochalasin D (colchicine, vinblastine no effect)</td>
<td>51</td>
</tr>
<tr>
<td>Volume regulation</td>
<td>Lymnaea neuron or hippocampal pyramidal neurons</td>
<td>Speed decline in ATP/stabilize in low ATP</td>
<td>Cytochalasin or colchicine/phalloidin or taxol</td>
<td>109, 110</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Carrot</td>
<td>Enhanced 6x</td>
<td>Colchicine or oryzalin (MTs) not cytochalasin</td>
<td>218</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Rat hippocampal neurons</td>
<td>Decreases Ca²⁺ influx cause by β-amyloid or kainate</td>
<td>Cytochalasin D and E, not colchicine</td>
<td>68, 69</td>
</tr>
<tr>
<td>Ca²⁺ (stretch activated)</td>
<td>Fibroblasts</td>
<td>Enhance/diminish</td>
<td>Cytochalasin D/increase intracellular actin assembly</td>
<td>79</td>
</tr>
<tr>
<td>NMDA (Na⁺, K⁺, Ca²⁺)</td>
<td>Hippocampal neurons</td>
<td>Enhance rundown/block rundown in Ca²⁺, low ATP</td>
<td>Cytochalasin, not colchicine/phalloidin. Not taxol</td>
<td>184</td>
</tr>
</tbody>
</table>

nAChR, nicotinic acetylcholine receptor; mAChR, muscarinic acetylcholine receptor; CFTR, cystic fibrosis transmembrane conductance regulator; NMDA, N-methyl-D-aspartate; ADH, antidiuretic hormone; PKA, protein kinase A; PPI, phosphoinositide; PIP₂, phosphatidylinositol 4,5-bisphosphate.
though molecules impinging on the cell surface that are later transduced to the biochemical reactions leading to acute cellular responses. In addition, acute or sustained application of forces such as the shear stress produced by vascular fluid flow often leads to remodeling of cell morphology or to expression of specific genes (45, 106, 220). The cytoskeleton is not the only structure capable of detecting and transducing such stimuli, and a number of membrane-bound molecules such as phospholipase A$_2$ (125) and alamethicin (162) can directly react to stresses within the lipid bilayer. However, for mechanical transduction to the cell interior, a three-dimensional elastic structure is required. In all these cases, signaling depends on elastic coupling between the site where the forces are applied and the potentially distant point at which the first biochemical change occurs (144). Recent elegant work has shown a direct effect of forces on the structure and function of individual molecules (14). Strong cases have been made for effects of tension in axonal development (93, 231) and for specific stress-induced acute responses (48, 49) and gene regulatory elements in endothelial cells (178). This section focuses on a few examples of such mechanical signaling and attempts to provide a context by which to evaluate how the cytoskeleton contributes to these signals.

One role of the cytoskeleton in mechanical stress-induced signaling is a direct elastic coupling from the site of the force applied at the cell membrane to the site of ultimate response, at a distant plasma membrane site, an internal organelle, or within the nucleus. The case for such a mechanical continuity has recently received strong support from findings of nuclear deformation caused by application of force at the plasma membrane (85, 144). In these studies, compressive (85) or elongational forces (144) applied to the plasma membrane of surface-attached cells led immediately to displacement of internal organelles (144) and deformation of the nucleus. Nuclear deformation was preserved in cells treated by both cytochalasin and nocodazole, suggesting that IFs alone are capable of linking the nucleus to the cell membrane, as has been proposed for many years (reviewed in Refs. 223, 224). Remarkably, although the elastic modulus of the nucleus is nearly 10 times higher than that of the cytoplasm in control cells, destabilization of the cytoskeleton by cytochalasin or acrylamide (an IF destabilizer) lowers the stiffness of the nucleus, suggesting that cytoskeletal proteins may have a direct effect on nuclear structure. The relation between forces and cell functions mediated by the cytoskeleton has recently been extensively reviewed in terms of a theory emphasizing the analogy between cell structure and constructs combining elastic continuity with internal stress called tensegrity elements (104, 105).

Alternative models for responses to mechanical sig-
nals arise from evidence that signaling enzymes such as protein kinases (18) or lipid kinases and hydrolases (21), which may or may not be bound to the cytoskeleton, alter their activation in response to mechanical stress. In the latter case, the initiating event may require the cytoskeleton, but the proximal signaling events are mediated by soluble messengers similar or identical to those generated by biochemical first messengers. There is no necessary contradiction in these results, and as in most cellular signaling pathways, a number of distinct, interrelated signals may achieve the same outcome or modulate each other’s functions.

One example of the interplay between mechanical and chemical messages is provided by studies of signaling by integrin-based focal adhesion sites (29, 39). Binding of integrins by appropriate extracellular ligands is a necessary part of establishing an adhesive contact, but occupation of the extracellular ligand binding site is not sufficient to construct a normal link between the membrane and the cytoskeleton. What is also apparently needed is mechanical tension (103). An early demonstration of this requirement was made by applying shear forces to beads bearing integrin binding peptides (239). Applying elongational stresses on collagen-coated beads bound to the dorsal cell surface actively promotes the assembly of actin stress fibers (Fig. 2) (78), and the strength of the membrane-cytoskeleton links depends on the rigidity of the extracellular material to which the cell is attached (37). These results support the remarkable idea that mechanical tension, possibly controlled by such factors as activation of myosin by rho and its downstream kinase targets, not only pulls the cytoskeleton against an already established adhesion site, but contributes to construct the focal adhesion site in the first place (29, 39).

How the cytoskeleton senses and responds to tension generated at adhesion sites is not known, and one hypothesis is that the structure of the cell in general and at these sites in particular is governed by the balance between load-bearing and tension-generating elements (105). In this case, altering one of these elements could produce large-scale structural changes independent of biochemical reactions or cytoskeletal disassembly. This concept is consistent with findings that depolymerization of microtubules increases contractility of cells and promotes focal adhesion formation (19), but other evidence suggests that this response is more complex than purely mechanical. Changes in stabilization of microtubules can affect the contraction of the actin-rich cortex of macrophages even at sites so far from the microtubules that a direct mechanical coupling seems unlikely, and the dependence of one filament system on the other may depend more on an as yet unidentified diffusible signal (183). The increased contractility that results when microtubules depolymerize involves increased myosin light-chain phosphorylation, suggesting that chemical changes leading to stronger contraction, in addition to or instead of release of the constraint against contraction, promote the shape change resulting from microtubule depolymerization (115).

VI. THE CYTOSKELETON AND APOPTOSIS

A hallmark of programmed cell death is an alteration of the cytoskeleton frequently resulting in membrane blebbing (122, 127, 229, 230). It is not yet clear whether these cytoskeletal changes are primarily a result of the activation of proteases and signaling molecules involved in apoptosis, or whether, instead, the reorganization of the cytoskeleton is a necessary event in order for the apoptotic program to proceed. A number of recent experiments suggest that the latter, more active role of the cytoskeleton takes place in at least some cases of apoptosis.

All three cytoskeletal filaments are reorganized or degraded in apoptosis. In lung cancer and neuroblastoma cell lines, cytokeratins and vimentin aggregate and degrade at early stages of apoptosis concomitant with exposure of phosphatidylycerine on the cell surface (230). Keratins can also become specifically phosphorylated during apoptosis (132), and experimental aggregation of cytokeratin or vimentin by overexpression of filagrin (46) or intoxication of Sertoli cells by mono-(2-ethylhexyl)phthalate can alter the course of apoptosis (180) without largescale alteration of either microtubules or F-actin.

A relation between the microtubule system and apoptosis has received particular attention in part because of the clinical importance of tubulin-directed drugs in treatment of human cancers (74, 225). Disruption of microtubule turnover by drugs such as taxol, vincristine, and vinblastine leads to phosphorylation of Raf1 and BCl2 associated with cell death (22, 88). Degradation of tubulin itself can occur during very early stages of apoptosis in neuronal cells treated with glutamate (3). How the disruption of microtubules leads to the later stages of apoptosis is not known, but evidence that diffusible factors may be released as the result of changes in microtubule polymerization (121) is consistent with the hypothesized role of microtubules in sequestering signals in other contexts (115, 183).

The disruption of the actin system during cell death has recently been related to specific changes in actin binding proteins, and a direct link between actin depolymerization and DNA degradation has been suggested. Actin is a prominent substrate for caspases in vitro and in vivo (28, 36, 114, 146), but in several cell types, actin resists cleavage during apoptosis, although it is readily cleaved by interleukin-1β-converting enzyme (ICE)-like proteases in vitro (205). Disruption of the actin system by cytochalasin D (190) or sphingolides (252) can induce apoptosis in
cultured cells, suggesting that depolymerization of actin may activate this process. The possibility that destabilizing actin is important for apoptosis is strengthened by the finding that overexpression of the actin monomer binding protein thymosin beta-10 can accelerate apoptosis (90). Furthermore, the actin filament-severing protein gelsolin is cleaved by caspase-3/32-kDa cysteine protease protein (CPP32) to generate a fragment no longer requiring activation by Ca^{2+} that leads to morphological changes in apoptotic cells (116). On the other hand, overexpression of intact gelsolin can inhibit apoptosis and block the activation of CPP32 (160). Cleavage of another actin-associated protein, Gas2, by ICE-like proteases can also activate it to disrupt the actin cytoskeleton (26).

The fact that actin filament pointed ends bind DNase 1 and that ATP-bound monomeric actin is a potent inhibitor of DNase 1 (123, 134, 172) has led to a specific model to relate actin cytoskeleton breakdown to the degradation of nuclear DNA (89, 145, 170). In this scenario, destruction of functional G-actin and loss of filament ends would liberate DNase 1 from the cytoskeleton and permit its entry into the nucleus. Proteins such as thymosins may augment this process (89), and gelsolin can activate actin-inhibited DNase 1 in vitro (50).

Although several lines of evidence show an important link between cytoskeletal integrity and cell viability, the molecular mechanisms for this link are likely to be numerous. In a broad sense, they may separate into two classes of events, either primarily mechanical or chemical. In the latter, signaling molecules or enzymes essential for nuclear or cytoplasmic breakdown are sequestered or inhibited by the cytoskeleton. Disruption of the cytoskeleton by any means would then liberate or activate these sequestered elements and allow them to reach their targets. A more directly mechanical role is suggested by the requirement for appropriately arranged extracellular contacts to prevent cell death (35). In this model, the mechanical tension produced at sites of cell-cell or cell-matrix contact is transmitted by an intact cytoskeleton throughout the cell. Loss of the mechanical integrity of the cytoskeleton would then alter the signals mediated by this tension.

VII. CYTOSKELETAL ROLE IN SIGNALING TO THE NUCLEUS

The intimate relation between cell shape and gene expression has generally been thought to be mediated in part by the cytoskeleton, since this is probably the only cellular structure directly linking the surface of the cell to the nucleus. Likewise, inhibition of cell growth by contacts is likely to require the cytoskeleton, since cytoskeletal connections enable a mechanical sensing of the number and strength of these adhesions. The particular involvement of the actin system is strongly suggested by the finding that overexpression of several actin-binding proteins that stabilize focal adhesions appears to have antiproliferative effects that are reviewed in Reference 15, and changes in actin binding protein expression are a common and dramatic feature of transformation by some oncogenes (34, 108, 228).

Three distinct ways in which elements of the cytoskeleton may be involved in nuclear signaling are illustrated in Figure 3. Although the particular examples focus on individual filament types and particular proteins that interact with them, it is important to stress that in vivo these filaments are often extensively intertwined, and perturbations of one system often perturb the structure of the other, usually by mechanisms that are not understood (80).

A direct cytoskeletal signal is possible because of the continuous link from the nuclear membrane or the nuclear matrix to sites of cell membrane/cytoskeletal anchors mediated most often by IFs (57, 58). This interconnection has been evident for many years, but whether a physical deformation, not mediated by the actions of soluble factors, can regulate gene expression in vivo is an unresolved matter. On the other hand, the physical evidence that such mechanical links exist continues to accumulate. The biochemical features of IF proteins, including their remarkably strong biochemical interactions with sequence-specific DNA, histones, and lipids suggests that such a link may have far-reaching consequences, and two extensive and thoughtful recent reviews make the case for such a possibility (223, 224). This hypothesis is strengthened by the findings that the elastic continuity of the cell persists even when the actin and microtubule systems are destabilized (144) and that there is a mechanical link between desmin IFs and chromatin in cardiac myocytes (24).

Other mechanisms by which cytoskeletal structure may control gene expression are illustrated by two other examples shown in Figure 3. In addition to the requirement for an intact actin cytoskeleton for nuclear targeting of PKC-alpha discussed in section IIb2 (192), a second example of how the actin system may mediate transcription regulation has recently been identified from studies of beta-catenin, a protein localized at cell-cell contacts by its association with cadherin (1, 86). Although beta-catenin does not directly bind actin, it is anchored to the cytoskeleton through cadherin and alpha-catenin. Displacement of beta-catenin from cell-cell contact sites due to mutations in cadherin or to posttranslational modifications of either protein can promote its association with the transcription factor lymphoid enhancer-binding factor (LEF-1) (11), resulting in transport of the beta-catenin/LEF-1 complex to the nucleus. This finding together with the demonstrations that beta-catenin binds c-erbB-2 (197) and adenomatous poly-
FIG. 3. Three distinct types of cytoskeletal effects in signaling to the nucleus. PKC, protein kinase C; IF, intermediate filament; MT, microtubule; LEF-1, lymphoid enhancer-binding factor.

VIII. CONCLUSIONS

One point that emerges from considering the multitude of factors bound to the cytoskeleton is that aside from matters of spatial sequestration, the functional consequences of this binding may not be evident from the binary reaction alone. Because the cytoskeleton is so large, and its surface area bears a high negative charge density, it can accommodate a large number of structurally diverse molecules and thus act as a catalytic surface as well as a protein cofactor. Membrane-cytoskeleton complexes such as focal adhesions, cell-cell junctions, and caveoli appear to be sites where signaling molecules concentrate to execute a coordinated function, and formation of such complexes may require both proteins and
specific lipids. Assembly or release of signaling molecules from cytoskeletal filaments can act to integrate spatially and temporally converging paths of signaling. These features reinforce the necessity to consider signaling as a network of interacting events rather than a linear chain of interactions leading from the plasma membrane to the ultimate target in the cell interior (30, 63, 204).

Perhaps the most intuitively evident potential of the cytoskeleton to control cell function is by direct mechanical coupling between distant points in the cell. There are compelling recent demonstrations that mechanical forces play a vital role in assembling such structures as focal adhesion sites, in regulating ion channels and calcium fluxes, and in controlling nuclear structure and perhaps function. As the methods to measure forces and displacements at the cellular level continue to develop, the relationship between physical effects and cellular function is likely to continue to provide exciting results.

Given the large number of publications in this field, important references have inevitably been omitted, and for this I express my regret. I am grateful to Lisa Flanagan for criticism and advice.

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