Editorial overview
Cytoskeleton

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Abbreviation
arp actin-related protein

Regardless of one's research focus in cell biology, an encounter with the cytoskeleton is becoming inevitable. The cytoskeleton field is progressing in several ways. First, the known inventory of cellular filaments and interacting proteins is still in an exponential phase of growth. At the same time that new proteins are being discovered, the level of characterization of some of the key components of the cytoskeleton (e.g. motor proteins) has proceeded to the level of atomic resolution structures and detailed biophysical characterization, which are leading to a true understanding of how these proteins work. Furthermore, although the areas of actin, microtubules and intermediate filaments were once very much tackled as separate problems, cross-talk both among cytoskeletal components and between cytoskeletal components and molecules that control regulatory pathways is beginning to emerge. The above themes, and the overall breadth of activities of the cytoskeleton, are reflected in this series of reviews.

A blossoming area of research concerns interactions between signal transduction pathways and the cytoskeleton. The cytoskeleton is very dynamic and, in response to extracellular cues, must rapidly reorganize, often in only localized regions of the cell (e.g. to insure chemotactic migration of leukocytes and neurons). As discussed by Bray in a recent review [1], many proteins of the cytoskeleton are modified by second messengers and can collectively behave as a complex biological 'circuit' that integrates information from signal transduction pathways and responds by changing cell morphology. As is necessary for deciphering any complex circuit, understanding such behavior involves determining the nature of the molecular switches that control the activities of individual cytoskeletal proteins, and then adopting a more global understanding of how several proteins can act together to change cytoskeleton dynamics and structure.

Research along these lines is most advanced in the actin cytoskeleton, where many actin-binding proteins are already known and the effects of calcium, phosphatidylinositol 4,5-bisphosphate or phosphorylation on these proteins have been identified. Recently, considerable attention has focused upon the Rho family of G proteins, as microinjection of constitutively active forms of these proteins selectively elicits membrane ruffles, stress fibers or filopodia extension. The manner in which these G proteins could affect actin networks, in addition to the mechanisms by which phosphorylation can modulate actin polymerization and focal adhesion assembly, are covered in two complementary articles by Zigmond (pp 66–73) and Craig and Johnson (pp 74–85). Another intriguing link between signaling and the cytoskeleton is being uncovered in the Wnt-1/wingless signal transduction pathway, described in the review by Cowin and Burke (pp 56–65). In addition, Cowin and Burke describe recent evidence that plakoglobin and β catenin (which are proteins found in adherens junctions) can shuttle between adherens junctions and the nucleus. This work raises the possibility that key signal transduction molecules may be sequestered by being tethered to the cytoskeleton and then released in response to extracellular signals, upon which release interactions with new targets become possible. Mediators of signal transduction pathways are also appearing in the unexpected form of motor proteins. Bähler (pp 18–22) describes the recently discovered rat Myr 5 myosin and the *Drosophila ninaC* gene product, both of which contain a putative Rho GTPase activating protein (GAP) and a kinase domain fused to the carboxyl and amino termini, respectively, of the myosin motor domain. The motor may serve to localize these regulatory enzyme activities to particular regions of the cell.

Connections between the outside of the cell and the interior cytoskeleton are discussed in the articles by Craig and Johnson (pp 74–85) and Cowin and Burke (pp 56–65). Considerable attention has focused on protein–protein interactions occurring at focal adhesions, adherens junctions and desmosomes. New evidence for discrete, ordered steps in focal adhesion assembly is discussed by Craig and Johnson (pp 74–85) in the context of two *in vivo* systems for focal adhesion assembly: growth factor stimulation or mitogen stimulation of serum-starved Swiss 3T3 fibroblasts, and integrin-mediated recruitment of focal adhesion components in primary human fibroblasts, under serum-free conditions. Excitingly, it appears that development of cell-free systems for analysis of focal adhesion assembly is not far off; this conclusion is made on the basis of progress in reproducing some of the steps of focal adhesion assembly in permeabilized cell systems. At cell–cell adhesion sites (discussed by Cowin and Burke [pp 56–65]), direct interactions have been

demonstrated between cadherins and linker proteins, such as plakoglobin and catenins, that in turn appear to anchor the membrane complex to the actin cytoskeleton. Similarly, desmoplakins act as linkers between intermediate filaments and cell adhesion proteins at desmosomes. Again, as in focal adhesion assembly, much recent research has been focused on how signaling events regulate cadherin association with these linker molecules, and on the functional consequences of these interactions in development. The full spectrum of components and the interplay between molecules at these junctional sites is, however, still emerging.

Elucidation of the mechanisms by which cytoskeletal proteins affect membrane functions is also progressing rapidly. A plethora of new lamin-associated proteins has been identified in the inner nuclear membrane; these proteins may play roles in anchoring the lamin filaments to the inner surface of the nuclear envelope, in addition to regulating lamina functions (described by Cowin and Burke [pp 56–65]). Even old, established players are producing new and unexpected results. Spectrin is one of the first proteins described that self-assembles at the plasma membrane and presumably modulates membrane mechanical stability. Although the initial studies of erythrocyte spectrin painted a rather static portrait of the protein, recent work on spectrins from other cell types indicates that it is a multifunctional protein that engages in variety of protein–protein interactions (described by Viel and Branton [pp 49–55]).

New insights into the molecular mechanisms involved in regulating actin filament lengths in complex supramolecular structures come from recent research on two classic model systems, the erythrocyte membrane skeleton and the sarcomeres of striated muscle (discussed by Fowler [pp 86–96]). These systems continue to generate surprises, and serve as instructive paradigms. For example, tropomodulin, which was originally described as a tropomyosin-binding protein in erythrocytes, has now been shown to be a capping protein for the slow growing (pointed) ends of actin filaments both *in vitro* and in cardiac muscle cells *in vivo*. A new function for the erythrocyte spectrin–actin-binding protein adducin in capping the fast growing (barbed) filament ends has been discovered, while in muscle, the barbed end capping protein, capZ, has been shown to nucleate actin filament assembly during myofibril formation. Mechanisms for specifying the precise actin filament lengths in these cells remain speculative; possibilities discussed include the use of templates and verniers.

The explosive discovery phase of motor proteins is also continuing, and it appears likely that mammals contain >25 different genes encoding members of the myosin and kinesin superfamilies. Although discovery of new motor-encoding genes is becoming more routine through polymerase chain reaction and genome sequencing efforts, uncovering the biological activities of the motors constitutes a considerably greater challenge. However, recent successes in this area are underscored in the articles by Bähler (pp 18–22) on myosins, by Vernos and Karsenti (pp 4–9) on kinesins, and by Porter (pp 10–17) on dyneins. Vernos and Karsenti (pp 4–9) highlight a recently discovered class of kinesins that bind directly to chromatin and may contribute to a 'polar wind' that drives chromosomes away from the poles to the metaphase plate. Other kinesins organize spindle assembly and modulate microtubule dynamics at the pole, the kinetochore and in the cytoplasm (see also McNally [pp 23–29]). A DNA-binding region of the tail domain of 'chromokinesins', in addition to regions responsible for attaching dynein to axonemal microtubules (see Porter [pp 10–17]), has been identified in the past year. In general, however, the mechanisms by which motors become specifically localized in cells are poorly understood and no doubt will be a topic of considerable research in the next few years.

Although the functions and regulation of motor 'tail', or cargo-binding, domains are mysterious, the motor domain that generates force and unidirectional movement has been the subject of intense study in both myosin and kinesin. The notion that myosin can undergo a lever arm power stroke is receiving stronger support, and details of how this power stroke may be generated are being deciphered at the level of atomic resolution [2,3]. Furthermore, mutations in myosin genes obtained from patients with hypertrophic cardiomyopathies have identified key regions and residues that may be involved in mechanochemical transduction (see article by Vikstrom and Leinwand [pp 97–105]). Whether myosin and kinesin share a similar strategy for force generation will probably become clearer when atomic resolution structures for kinesin superfamily members become available in the next year.

The mechanism of motility of kinesin and myosin can be approached by studying the interaction of two components, namely the motor and the polymer. On the other hand, understanding the beating of eukaryotic cilia and flagella or the rotation of bacterial flagella presents a problem of significantly greater complexity, as interactions and cooperation of a myriad of different proteins are required. As discussed for eukaryotic flagella by Porter (pp 10–17), this problem demands a marriage of genetics, biochemistry and structural approaches. The inventory of eukaryotic flagellar proteins, and their location in the axoneme, is also coming into place, largely due to the combination of genetic and structural techniques used to study *Chlamydomonas*.

The inventory of proteins that modulate microtubule function is sparse in comparison with the larger numbers of such proteins that have been documented in the actin field. However, as described by McNally (pp 23–29),

this situation is beginning to change. Reorganization of microtubules in response either to transitions in the cell cycle or to signal transduction pathways is being better characterized, and some of the proteins involved in these changes have been identified in the past couple of years. Moreover, a structural understanding of microtubule nucleation has been advanced by the isolation of a ringlike template of γ tubulin, which has been genetically shown to be required for microtubule assembly in cells. Arrangements of microtubules into spindlelike arrays can now be studied in cell extracts, as described by Vernos and Karsenti (pp 4–9). These cell extracts also provide an enormous opportunity for dissecting the roles of DNA, centrosomes, motors and kinetochores in the process of spindle morphogenesis.

For many years, textbooks described three cytoskeletal systems: actin filaments, microtubules and intermediate filaments. It therefore comes as a surprise to many people that new filamentous systems are being uncovered. In last year's issue of *Current Opinion in Cell Biology*, Roberts and Stewart [4] reviewed a filamentous protein involved in nematode sperm localization (major sperm protein). Despite efforts to find it elsewhere, this protein has thus far been uncovered only in worms. In this edition, Longtine *et al.* (pp 106–119) describe the septin family of filamentous proteins, which have features distinct from those of the classical intermediate filaments. Although these proteins were first discovered in yeast and thought to be a curiosity of these lower eukaryotes, it is now clear that they are universal and play an essential, but as yet unknown, function in cytokinesis. The actin-related proteins (arps) described by Frankel and Mooseker (pp 30–37) also fall in the category of recently discovered cytoskeletal components. Although they share sequence homology, and probably a similar three-dimensional fold, with actin, they differ from actin in that they either form short polymers (in the case of arp1) or possibly do not polymerize at all (in the case of arps 2 and 3). Furthermore, many arps are not associated with the classical locations and activities of actin, but instead are involved in microtubule functions (e.g. arp1 of dynactin, a complex which regulates dynein function in mitosis and organelle transport) or in unknown functions within the nucleus. Frankel and Mooseker even raise the interesting speculation that the roles of many arps may not be as filamentous supports, but rather as molecular switches which undergo transitions from ATP-bound to ADP-bound states in order to form new protein–protein interactions (a mode of action reminiscent of that of the G-protein family). Many surprises are in store for us regarding the arp and septin families in the coming years!

Although biological research is dominated by mammalian or genetic (e.g. yeast, fly and worm) systems, it is also important to appreciate that other systems can provide unique vantage points from which to obtain an understanding of the cytoskeleton. Good examples are newt lung cells, which have provided a classical preparation for viewing events in mitosis for nearly a century, and the bacterium *Listeria*, which provides an exquisite model for understanding actin dynamics (discussed in last year's issue by Cossart [5]). In this issue, Menzel (pp 38–42) describes the role that the cytoskeleton plays in the generation of cell polarity in algal cells. The general mechanisms by which the marine brown algae *Fucus* establishes axis formation after fertilization, via linkages between the extracellular matrix and the actin cytoskeleton, are possibly common to a variety of organisms. Tilney and Tilney (pp 43–38) also describe the unique properties of cytoskeletal organization in protozoan parasites and the relevance of this information to understanding the life cycles of these organisms.

Finally, connections between the cytoskeleton and medical disease are continuing to emerge. In the past year, center stage belonged to the myosin motors. Bähler (pp 18–22) describes how myosin VII mutants were found to give rise to Usher syndrome, which involves hearing impairment and progressive retinitis pigmentosa. Vikstrom and Leinwand (pp 97–105) also describe the extensive recent clinical and genetic advances in understanding familial hypertrophic cardiomyopathies, a collection of diseases which often involve mutations in cardiac myosin genes. Mutations in other cytoskeletal genes that give rise to medically important diseases will undoubtedly continue to be identified. Furthermore, due to their divergent cytoskeletal functions, the cytoskeletal and motor proteins of protozoal parasites (see article by Tilney and Tilney [pp 43–48]) may represent a promising target for drug therapy.

Because of the many functions of the cytoskeleton, this set of reviews does not represent a comprehensive coverage of the field. Instead, we have selected subjects that have not been covered recently in *Current Opinion in Cell Biology*. For overviews of other topics on the cytoskeleton (e.g. intermediate filament organization), the reader may also wish to peruse last year's collection of reviews in this journal [6].

References

1. Bray D: **Protein molecules as computational elements in living cells.** *Nature* 1995, **376**:307–312.
2. Whittaker M, Wilson-Kubalek EM, Smith JE, Faust L, Milligan RA, Sweeney HL: **A 35-Å movement of smooth muscle myosin upon ADP release.** *Nature* 1995, **378**:748–751.
3. Jontes JD, Wilson-Kubalek EM, Milligan RA: **A 32° tail swing in brush border myosin I on ADP release.** *Nature* 1995, **378**:751–753.
4. Roberts TM, Stewart M: **Nematode sperm locomotion.** *Curr Opin Cell Biol* 1995, **7**:13–17.
5. Cossart P: **Actin-based bacterial motility.** *Curr Opin Cell Biol* 1995, **7**:94–101.
6. Cooper JA, Mitchison TJ: **Cytoskeleton.** In *Curr Opin Cell Biol* 1995, **7**:1–144.