Cytoskeleton

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The cytoskeleton is a fibrous meshwork of three major components – actin microfilaments, microtubules and intermediate filaments. These are assembled from soluble precursors, under precise control by many different cellular processes. Other proteins interlink filaments or travel along with them. The cytoskeleton is found in all eukaryotic cells, and is required both for the maintenance of cell structure and for the organization of many aspects of cell movement.

Introductory article

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Shape, Polarity and Movement are Generated by the Cytoskeleton

Eukaryotic cells are generally far larger and more flexible than prokaryotes. Viewed under an appropriately powerful microscope, eukaryotic cells seem extraordinarily dynamic. A wide range of different structures is visible, which move with respect to one another, and the shape and proportions of the cell may be constantly remodelled. Bacteria, by contrast, are rigid in shape and appear homogeneous in content.

The flexibility and complexity of eukaryotes depend entirely upon a network of different types of filaments, collectively called the cytoskeleton. The cytoskeleton maintains the shape and polarity of cells, and anchors and transports different components within the cell. In one sense, the term 'skeleton' is misleading, for while the fibres of the cytoskeleton can frequently provide rigidity, in other cases the entire structure may be disassembled and remodelled within minutes. Even plant cells and yeasts, whose shapes are usually defined by a rigid cell wall, depend completely on the presence of a complete cytoskeleton.

The cytoskeleton and disease

Since the cytoskeleton is central to the lives of cells, it is hardly surprising to find that it is highly relevant to modern medicine. Several pathogens (for example, the bacterium *Shigella* and *Vaccinia virus*) make use of the host cytoskeleton to gain entry into and to infect neighbouring cells. Other organisms (e.g. the death cap toadstool *Amanita phalloides* and the meadow saffron *Colchicum*) make toxins that interfere with particular aspects of cytoskeletal function. Conversely, an increasing number of medicines work by attacking the cytoskeleton of disease-causing cells, for example paclitaxel for cancer and fluconazole for yeast infections. Several examples are discussed in detail below.

Actin Filaments, Microtubules and Intermediate Filaments

A transmission electron micrograph of a typical eukaryotic cell shows three distinct families of cytoskeletal filaments (**Figure 1**). Microfilaments, the smallest, are about 8 nm in diameter. The largest fibres are microtubules, with a diameter of about 25 nm, which are so called because they

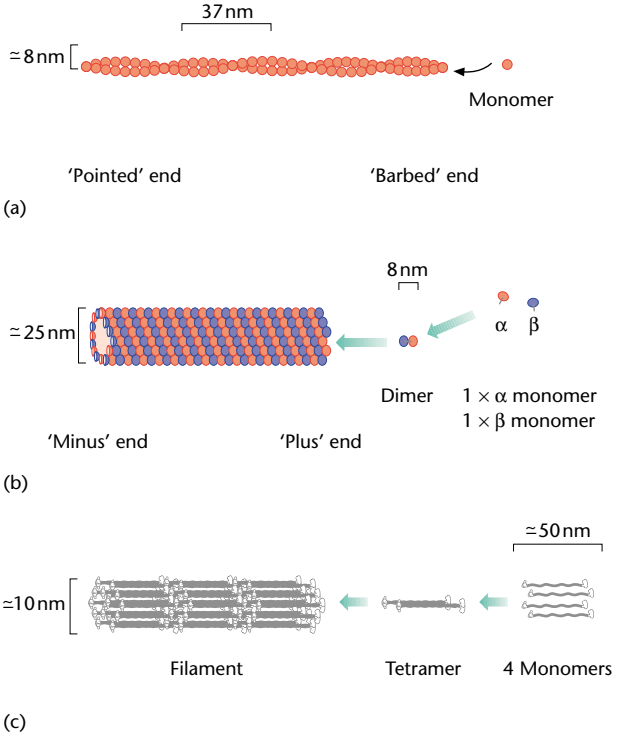

Figure 1 The three major classes of cytoskeletal filaments: (a) microfilaments (composed of actin monomers); (b) microtubules (composed of α- and β-tubulin); (c) intermediate filaments (various different types of monomer).

clearly possess a hollow core in electron micrographs. Intermediate filaments have a diameter between that of microfilaments and microtubules, about 10 nm.

The localization of these structures gives clear clues about their functions. Microfilaments are most prevalent at the edges of cells, especially in areas where the cell shape is rapidly changing, for example in pseudopodia and ruffles. These structures are associated with crawling movements, which suggests a role for microfilaments in cell crawling and motility. Pseudopodia are often so rich in microfilaments that other, granular, components of the cytoplasm are excluded. This gives them a hyaline (i.e. glass-like) appearance under the microscope.

Microtubules, on the other hand, radiate from a point in the centre of the cell, near the nucleus, and only occasionally extend as far as the edge of the cell. During mitosis, the structure of the nucleus breaks down and is replaced by a cage-like spindle, which pulls the chromosomes apart after they have been duplicated. The spindle is largely composed of microtubules, suggesting that they have a role in moving and redistributing components within the cell.

Most intermediate filaments are long, and form a meshwork which appears to span the whole width of cells. They are most prevalent in static cells, such as skin and heart muscle which have to resist externally applied forces. It therefore seems that their role is to strengthen the cell, in particular against pulling and shearing stresses. In addition, intermediate filaments make up the nuclear lamina, the meshwork that surrounds the nucleus during interphase.

Protein Filaments are Produced by the Polymerization of Subunits

Despite the considerable differences in size, location and function, the components of the cytoskeleton are assembled in a strikingly similar fashion. Each type of fibre is assembled from relatively small units, which are polymerized to form long linear chains. The individual properties of microfilaments, microtubules and intermediate filaments derive from differences in the monomers and the way in which they are assembled (see **Figure 1**).

Structure of cytoskeletal filaments

Microfilaments and actin

Microfilaments are constructed from a single type of subunit, named actin. In its monomeric form, actin is usually referred to as G-actin (for globular actin). Each protein unit is about 375 amino acids long and 42 kDa in size, and contains an adenine nucleotide (either adenosine triphosphate (ATP) or adenosine diphosphate (ADP)). When these monomers polymerize, which can be caused experimentally by increasing the concentrations of K^+ and Mg^{2+}, they associate head-to-tail to yield F-actin (filamentous actin). This process is usually accompanied by hydrolysis of bound ATP to ADP; ATP hydrolysis is not necessary for polymerization, but modulates it in an important way (see below). F-actin is a tightly packed, simple helix containing just under two subunits per turn, resulting in a rather misleading resemblance to a double helix. It is also polar (because the monomers are all aligned in the same direction, the ends of the filament are chemically different), which is central to its behaviour within cells.

Microtubules

Microtubules, on the other hand, are built from dimers of two 50-kDa proteins, α- and β-tubulin, each about 450 amino acids and 50 kDa in size. As in actin, each monomer contains a bound nucleotide, but tubulins use the guanine nucleotides guanosine triphosphate (GTP) and guanosine diphosphate (GDP) instead of ATP and ADP. Also as in actin, the dimers polymerize end to end, giving a polar filament. However, microtubules are composed of 13 filaments (protofilaments) encircling the hollow core, giving them far greater rigidity than actin microfilaments.

The flagella of protozoa and the cilia of vertebrates use a specialized assembly of tubulin and associated proteins to produce bending, whip-like movements. Viewed in cross-section, these show a characteristic '9 + 2' structure. An array of nine double-width microtubules, each formed like a figure of eight, is arranged radially around a core of two single microtubules.

Intermediate filaments

Unlike the other two classes of cytoskeletal fibres, intermediate filaments are not homogeneous in composition. The monomers that make up intermediate filaments come from a large family of related genes, and have a range of sizes from about 40 kDa to more than 100 kDa. Most prominent are the keratins (members of this family make up tissues such as hair and nails); the family also includes a nerve-specific group called the neurofilaments, as well as desmin and vimentin. Each of these proteins is found in a subset of differentiated cells in higher eukaryotes, whereas lower eukaryotes often possess none of them. The assembly of intermediate filaments is again different from that of microfilaments and microtubules: the monomers assemble to form a rope-like structure, with several monomers intertwined together to make a structure with considerable tensile strength. It is not yet clear whether the monomers assemble with any polarity, but if the filaments are polar it seems not to be important for their function.

The lamins, which make up the nuclear lamina during interphase, behave differently again. Unlike the other intermediate filament proteins, they polymerize to form a

latticework sheet. During mitosis they abruptly depolymerize, presumably in response to phosphorylation, and become soluble; this event is a key part in the breakdown of the nucleus. Once nuclear division is complete, the lamins are dephosphorylated and nuclear envelopes reassemble around the daughter nuclei.

The behaviour of intermediate filaments is completely different from that of other cytoskeletal components. They do not bind nucleotides and do not use motors (see below). Most are very stable, unlike microfilaments and microtubules which are constantly remodelled. Above all, individual cells can live happily without cytoplasmic intermediate filaments, although organisms with deficiencies in intermediate filament genes experience a range of difficulties, most of which derive from the inability of particular cells types to resist stress.

Actin and tubulin form highly dynamic structures

Actin microfilaments and microtubules are constantly being remodelled and redistributed within the cell. This continual change is fundamentally important to the working of the cytoskeleton, but our understanding of the underlying mechanisms is at best incomplete. The control of actin and tubulin polymerization is therefore the subject of intense study. In particular, researchers would like to know what controls (a) where in the cell new filaments are formed, (b) the rate at which they are extended, and (c) whether they remain stable or are broken down.

Nucleation is the key to new filament formation

The underlying behaviour of actin and tubulin is surprisingly similar considering their different cellular roles. If a pure solution of actin monomers under appropriate salt conditions is concentrated sufficiently, they will polymerize *in vitro* without needing any additional factors. The critical concentration defines the amount of monomer that is needed to start polymer formation. However, the initial rate of polymerization is very slow; if the fraction of protein included in filaments is measured over time, a relatively long lag phase is seen, followed by a second phase of rapid polymerization (**Figure 2**).

This complex behaviour reflects a property that is shared by actin and tubulin. Actin monomers or tubulin dimers are easily added to existing filaments. However, the formation of new filaments is very slow by comparison. The smallest functional filaments (called 'seeds' or 'nuclei') contain three monomers in the case of actin, or a ring of 13 tubulin dimers. Thus the lag phase is caused by the slow appearance of new nuclei, and the rapid phase reflects the extension of the newly formed filaments.

In cells, as opposed to pure solutions, nucleation is thought not to occur by this random association of monomers. Instead, specific structures have the job of forming new 'nuclei' at appropriate times. The cell can therefore dictate when and where new filaments are formed by controlling the activation and location of these structures.

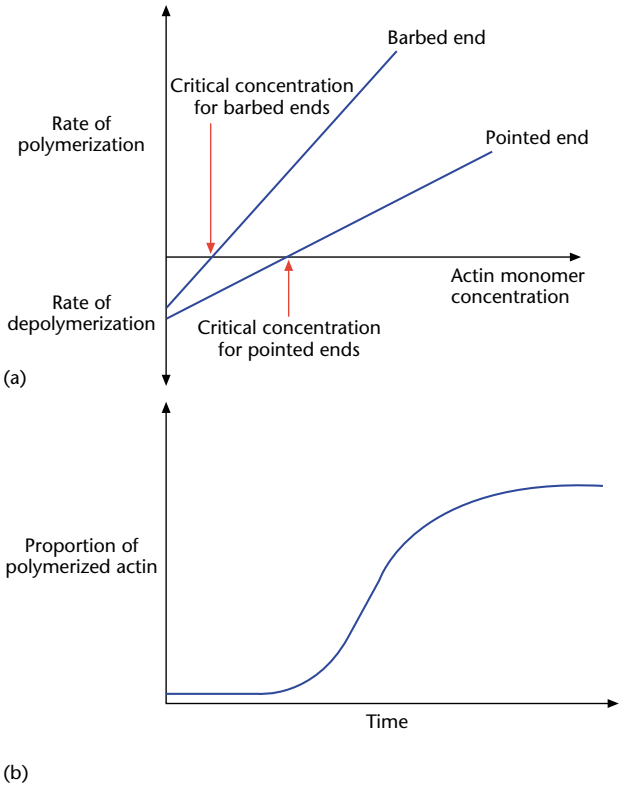

Figure 2 Polymerization of actin. (a) The rate of polymerization or depolymerization depends on the actin monomer concentration. Note that each end behaves differently. The critical concentration for each end is the concentration of actin monomers at which there is no net polymerization or depolymerization. (b) Kinetics of polymerization of an actin solution above the critical concentration for barbed ends. The long delay is caused by the slow formation of three-subunit nuclei.

Mammalian cells in interphase contain only one microtubule organizing centre (MTOC), a structure that includes two centrioles fixed at right angles to each other. The centriole, like cilia and flagella, is made of nine fused 'double' microtubules forming a small cylinder. Surrounding the centrioles is a cloud of amorphous pericentriolar material. This entire structure is the centrosome. Higher land plants and some animal cells do not possess centrioles and so their MTOCs may be diffuse. Staining the tubulin of interphase cells reveals an array of cytoplasmic microtubules all converging on the centrosome. Microtubules are initiated in the pericentriolar material (not from the centrioles) from a protein complex containing a third member of the tubulin family, γ-tubulin. γ-Tubulin is not incorporated into the length of the microtubule but as part

of a complex found at the so-called minus ends (see below) of microtubules embedded in the centrosome.

When the cell undergoes mitosis and starts to divide, a second centrosome must be constructed to provide one for each daughter cell. The new centrosome is not assembled independently; instead the original centrosome is somehow divided into two new daughters, which move apart towards the dividing halves of the cell. The mitotic spindle, the microtubule structure that actually separates the chromosomes, is then formed between the two new centrosomes.

Actin microfilaments, unlike microtubules, are nucleated by a mechanism that remains unknown despite decades of investigation.

Polarity and treadmilling

The biochemistry of both actin and tubulin polymerization holds a surprise. Since the subunits of both are added in the same direction, head-to-tail, it might be expected that they would polymerize and depolymerize similarly at each end of the filament. In fact, measurements of the polymerization at each end reveal different critical concentrations. For actin, typical critical concentrations are $0.6\,\mu mol\,L^{-1}$ (pointed) and $0.15\,\mu mol\,L^{-1}$ (barbed), although these values depend on the conditions and the source of the actin. This means, in practice, that polymerization is favoured at the barbed end; indeed, nearly all actin polymerization within the cell is thought to occur at the barbed ends of filaments. Since the actin filaments near the plasma membrane are all oriented with their barbed ends pointing outwards, this means that actin monomers usually appear to be added between the membrane and the end of the filaments.

The 'barbed' and 'pointed' ends of actin filaments

To distinguish the ends of an actin filament, it is necessary to be able to discern its direction. This is impractical with pure microfilaments; even the best transmission electron microscope does not reveal sufficient molecular detail. However, the S1 fragment of myosin II (see below) binds to actin filaments at a constant angle. Incubation of actin filaments with S1 before electron microscopy gives 'decorated' filaments, in which the myosin fragments are oriented like an arrow, with a point at one end and a barbed tail at the other. The end whose S1 fragments point outwards is therefore called the 'barbed' end, as opposed to the 'pointed' end in which the S1 points back along the filament.

The ends of microtubules behave similarly. The 'plus' or growing end, which points outwards from the MTOC, has a higher affinity for free tubulin than the 'minus' end, which is measured as a lower critical concentration for tubulin dimers. In a tubulin solution with a concentration between the critical concentrations for the two ends, dimers will tend to dissociate from the minus ends of all filaments, while at the same time adding to their plus ends. This simultaneous assembly of one end of a filament while disassembling the other has been called 'treadmilling'. Treadmilling can probably also occur in actin filaments, but thus far has been observed only in microtubules.

Energy is required for treadmilling

The conditions that underpin treadmilling – a higher affinity for monomers at one end of the filament – require a source of energy. Each actin and tubulin monomer contains energy in the form of a nucleotide triphosphate, ATP for actin and GTP for tubulin. Following polymerization, the ATP or GTP molecules are hydrolysed to ADP or GDP, which drives a change in the binding affinity between subunits. If the concentrations of subunits are sufficiently high, polymerization may occur faster than ATP or GTP hydrolysis, creating a cap of ATP- or GTP-bound subunits at the polymerizing end. Conversely, if polymerization occurs slowly, the subunit at the growing end of the filament may hydrolyse its ATP/GTP, leaving a filament with relatively low affinity for new subunits at either end.

Behaviour of actin and tubulin in live cells

Treadmilling is not believed to occur significantly in live cells, because the low-affinity ends of filaments are capped by other proteins. The minus ends of microtubules are associated with MTOCs; the pointed ends of actin filaments are usually not free, although the proteins that cap them *in vivo* are not known.

Although microtubules appear not to treadmill in cells, they show another dynamic behaviour, which has been called dynamic instability. Microtubules with an intact GTP cap tend to grow at rates of anything up to $0.5\,\mu m\,min^{-1}$. Should the GTP cap be hydrolysed, however, leaving GDP-bound monomers at the plus end, the equilibrium changes in favour of depolymerization and the microtubule shortens at a catastrophic rate. When the microtubules within living interphase cells are followed, using either enhanced video-microscopy or fluorescent labels, they can be clearly seen undergoing successive random cycles of slow elongation and rapid shortening.

Severing

Actin does not appear to shorten by catastrophic depolymerization. Instead, actin is disassembled by a family of proteins that bind the middle of the microfilaments and sever them. Two families of proteins are known to sever actin filaments, although radically different functions have been ascribed to them. Actin-depolymerizing factor and its relatives cofilin and actophorin are particularly associated with the dismantling of assembled actin microfilaments. Gelsolin and its relatives, on the other hand, are believed to have a more complex role.

When gelsolin cuts an actin filament, it creates a new barbed end, to which it remains bound. Certain intracellular signals cause the release of bound gelsolin, which leaves free barbed ends to act as nuclei for further actin polymerization. Gelsolin is therefore associated with both disassembly of existing actin filaments and nucleation of fresh ones.

Cytoskeletal Connections

The elements of the cytoskeleton are not independent. Different filaments are connected to one another, and to the surface of the cell. Motor proteins, which not only connect to the cytoskeleton but drive up and down filaments, are a special case and are discussed in the next section.

Crawling cells need to attach to surfaces, tissues in multicellular organisms must be attached to one another, and it is obviously important to connect the cytoskeleton to sites of attachment. A discussion of cell attachment may be found in articles on integrins, adherens junctions, tight junctions, adhesion proteins and laminae.

Filament bundles and arrays

Because actin filaments are narrow and flexible, they can accomplish relatively little independently of one another. In living cells this is overcome by actin-binding proteins (ABPs). This is a large family, in which different proteins possess slightly different functions; the cell uses different combinations to make actin structures with different properties. For example, epithelial cells of the small intestine are covered by finger-like projections called microvilli, which increase absorption by maximizing the cell's surface area. Microvilli are built from long actin microfilaments which are aligned in bundles (**Figure 3a**). This structure is maintained by several actin-binding proteins, in particular villin, which bundle microfilaments into parallel arrays. Inappropriate expression of villin can cause formation of microvilli, even in cells that would not normally possess them. The lamellipodia (the active, ruffling, leading edge) of crawling cells, on the other hand, contain a mesh of actin in which fibres are joined to one another at an angle (**Figure 3b**), which gives a resilient, deformable but elastic structure instead of the rigid fingers of microvilli. This type of crosslinking, where the join is flexible and hinge-like, is mediated by proteins such as filamin.

The number of known F-actin-binding proteins is in the hundreds, each of which presumably joins actin filaments in a different geometry, with a different strength, or in response to different stimuli. Most cells contain significant amounts of a number of different ABPs, which must make the structure of the actin in living cells highly complex.

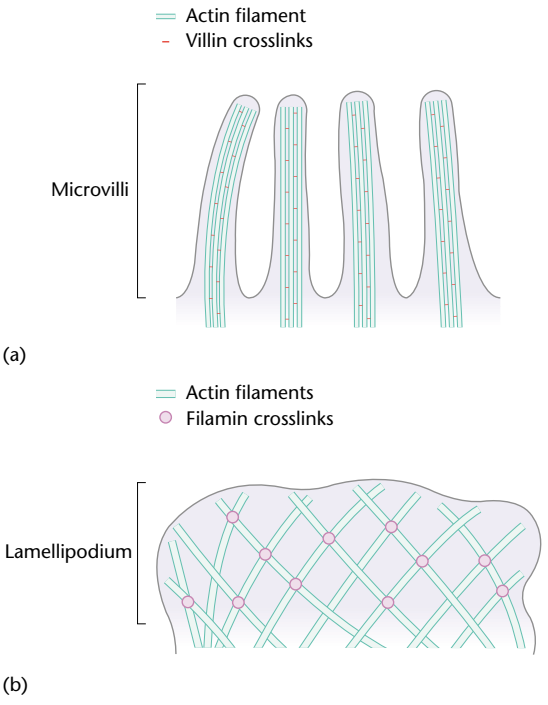

Figure 3 Contrasting actin-rich structures. (a) Microvilli. In these long finger-like structures on the surfaces of absorptive epithelia, the actin filaments are bundled in the same direction (isotropically). (b) Lamellipodium. The actin-rich edges at the leading edges of motile cells contain actin filaments crosslinked at an angle to one another.

Several mutants have been made which lack one or more ABPs; these tend to show subtle phenotypes, however, which implies that the functions of the different proteins overlap, so other ABPs can compensate for loss of any one.

Connections between the cytoskeleton and membranes

A number of proteins are responsible for connecting the actin cytoskeleton to the plasma membrane. The best characterized system is in red blood cells (erythrocytes), which need to maintain a large surface area for maximal gas exchange but remain flexible enough to travel through capillaries. The characteristic biconcave shape is generated using tension from a network of F-actin, attached to the membrane through large complexes of several proteins. Two proteins, band III and glycophorin, are integral membrane-spanning proteins, and join the complexes to the membrane itself. Actin is bound by a large protein called spectrin, which is in turn connected to band III through ankyrin and to glycophorin through protein 4.1. The net result is a compact crosslinked array of protein connecting multiple actin filaments to the whole area of the membrane.

Most eukaryotic cells need to be less rigid than erythrocytes. They therefore use more flexible and controllable connections between membrane and cytoskeleton, but similar proteins are used (including a large family of spectrin-like proteins) and the underlying mechanisms are thought to be similar.

Motors: Proteins that Move Along Actin Filaments and Microtubules

Actin microfilaments form a tangled crosslinked mesh in most cells. In striated muscle, however, they form part of a highly ordered structure, in which F-actin 'thin filaments' are interdigitated between 'thick filaments' made of myosin II. The muscle contracts when the thick and thin filaments pull against one another, which in turn compacts the muscle. Myosin II was the first such motor to be discovered. Its structure is based around head groups, which use energy from ATP to pull against actin filaments, and a long coiled-coil tail, which forms a filament by binding to other myosin II molecules. Although most clearly visible in muscle, myosin II is present in essentially all eukaryotic cells.

More recently, a remarkably large family of related myosins has been found, and named unconventional myosins to distinguish them from myosin II. All myosins have similar head groups, which bind to actin and hydrolyse ATP, but the range of tails varies enormously. As a general rule, however, the tails of unconventional myosins do not form filaments, so they probably act individually, binding to the plasma membrane or other cargoes. They perform a range of functions *in vivo* such as reinforcing actin protrusions, relocating organelles and rearranging the cytoskeleton during endocytosis and phagocytosis.

Microtubule motors: kinesins and dyneins

Microtubules have their own individual motors, which resemble unconventional myosins in acting alone rather than as part of organized filaments. The major role of these motors is in carrying vesicles and organelles along microtubules. In nerve cells of the brain, the long extensions, or axons, may be as much as 1 m in length and microtubule motors perform the vital task of shuttling material between the cell body and the axon termini. Since all microtubules point in the same direction, with their minus ends attached to the centrosomes and plus ends at the cell periphery, motors that move from the minus towards the plus end will carry cargo out from the cell body. The major such 'plus-end directed' motor is kinesin, a fairly large tetramer comprising two 120-kDa and two 62-kDa peptides. Kinesin-coated polystyrene beads move smoothly along microtubules *in vitro*, demonstrating that nothing more than ATP is required for motor activity.

An unrelated motor is responsible for the return path. Dynein, an enormous molecule which contains two heavy chains of more than 470 kDa and several smaller subunits, carries vesicles *in vivo* or beads *in vitro* towards the minus ends of microtubules. The directionality of the motors does not appear to be determined by their family alone, however: a *Drosophila* protein called Ncd moves towards minus ends, despite being a member of the kinesin family. Ncd is required for correct movement of the chromosomes during mitosis, another principal role for microtubule motors. The anaphase mitotic spindle is composed of two claw-like arrays of microtubules, radiating from the two centrosomes, with the chromosomes caught between the fingers. Motors like Ncd, localized at the kinetochores where the chromosome and microtubule meet, separate the chromosomes by pulling them towards the centrosomes.

Cellular Signalling and the Cytoskeleton

Extracellular signals – from growth factors, chemotactic factors, extracellular matrix and a range of other cues – directly control the cytoskeleton's form and movement. The mechanisms by which signals and cytoskeleton are connected are one of the fastest-moving areas of current research, so there is room here to discuss only a few selected points.

Calcium and muscle

Calcium, the ubiquitous intracellular messenger, controls the decision about whether or not a muscle fibre should contract. The concentration of Ca^{2+} ions in resting muscles is about 10^{-7} mol L^{-1}. When the muscle is stimulated by nerve impulses, intracellular calcium channels open and the concentration rapidly rises to 10^{-5} mol L^{-1}. The change in calcium ion concentration causes a change in the actin-binding proteins troponin and tropomyosin, which in resting muscle inhibit the ability of myosin II to move along the actin filaments. Calcium binds directly to a subunit of troponin, and sufficient concentrations prevents it from inhibiting myosin II activity. Thus the rise in intracellular Ca^{2+} concentration activates the myosin II motor and allows the muscle to contract.

The behaviour of muscle fibres is relatively simple. Intracellular calcium regulates multiple aspects of the movement of other cell types, including contraction, adhesion and polarization. The complexity of these responses makes it impossible to provide a coherent picture of the effects of calcium on cell movement.

Rac, Rho and cdc42

The Rho family of small GTP-binding proteins, relatives of the proto-oncogene *Ras*, have a fundamental role in controlling the shape and movement of cells. Small GTP-binding proteins are molecular switches; when they are turned on, they bind to GTP, and after a time they hydrolyse the GTP to GDP and turn themselves off.

In mammalian cells, the Rho protein is involved in contraction and adhesion. When cells are microinjected or transfected with mutant Rho which is constitutively GTP bound, they change rapidly. They may round up into a more rigid, contracted morphology. Small plaques called focal adhesions form, which provide firm, stable anchorage to rigid surfaces. The focal adhesions may be connected by stress fibres, which contain large numbers of actin filaments bundled together into a cable-like structure. The signals that cause Rho activation in living organisms are not well understood, but Rho is known to be vital for the response to known hormones such as lysophosphatidic acid.

Other family members have contrasting effects. Rac, when introduced into cells in an activated form, causes a variety of responses that suggest increased cell movement. Cells appear to turn over their actin cytoskeletons more rapidly, making large actin-rich ruffles and lamellipodia, and may migrate at faster speeds. Again, Rac has been shown to be important for normal responses to growth factors such as platelet-derived growth factor. These messengers normally stimulate cell motility as well as several other processes, so Rac appears to be a central connection between cell surface receptors and the cytoskeleton. The small GTP-binding protein cdc42 appears to have a related role, in particular in the regulation of cell polarity; activation of cdc42 causes cells to elongate in a particular direction, even cells that are usually almost round. The function of cdc42 appears to have been conserved all the way from budding yeast to mammals: *Saccharomyces* cells containing certain mutations in the *CDC42* gene are unable to localize new daughter buds correctly, despite relative normality in growth.

Chemotaxis and actin polymerization

A range of cells, from mammalian white blood cells to amoebae, have the ability to crawl towards sources of attractive chemicals. This process is called chemotaxis, and the chemicals are referred to as chemoattractants. Chemotaxis is essential for processes as different as nervous system development (growing nerves elongate towards peptides secreted by their targets), immunity (macrophages and neutrophils locate sites of infection by chemotaxis towards immune factors and bacterial metabolites) and feeding of amoebae (which locate their prey by chemotaxis).

Most chemotaxis is mediated by serpentine receptors and heterotrimeric G-proteins, the larger relatives of small GTP-binding proteins. When the receptors bind an appropriate ligand, the actin cytoskeleton is rapidly reorganized; in resting cells around 30–40% of actin monomers are assembled into filaments, but this amount can double within a few seconds of a chemoattractant being added. One particularly interesting feature of this process is that cells can somehow detect the location of chemoattractants as soon as they arrive. When cells are challenged with a micropipette filled with chemoattractant, they frequently extend long pseudopods directly towards the mouth of the pipette, and almost never in the wrong direction. This implies that the whole signalling pathway is locally activated, and that cells can somehow compare chemoattractant concentrations from all over their surfaces in order to choose the right direction. How they achieve this is intriguing, but still unknown.

Further Reading

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