

## Choreography of the Mammalian Cytoskeleton

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### What is the Cytoskeleton?

The cytoskeleton is made up of three major components: the microfilaments, microtubules and intermediate filaments. The microfilaments and microtubules are polymer structures that orchestrate cellular movement, cell division, intracellular transport and signalling via an intricate cross-talking network that involves interaction with a diverse range of proteins and signalling molecules (1). Microfilaments are composed of actin polymers and a large array of actin-binding proteins (ABPs). In cells, actin exists either in monomeric (G-actin) or polymeric forms (F-actin). Each actin subunit is able to bind ATP, which is hydrolysed to ADP shortly after incorporation of the actin into a growing filament. Polymers of actin assemble spontaneously from the pool of monomeric subunits by non-covalent bonds, using the energy derived from the intrinsic ATPase activity of actin. Actin filaments consist of double helical polymers that are arranged head to tail. Most filaments also contain a tropomyosin polymer which runs along the major groove in the microfilament (2). The tropomyosin does not have any van der Waals interactions with actin and hence, 'floats' over the surface of the actin filament (3). The actin cytoskeleton plays an important role in cell events such as motility, neuronal path-finding, differentiation, division and membrane organisation, all of which require the coordinated turnover and remodelling of actin filaments (4). The ability of actin filaments to contribute to this remarkable variety of activities derives from its selective interactions with actin binding proteins, which in turn are regulated in part by the actin and tropomyosin isoform composition of the filament (2).

Microtubules are composed of repeating  $\alpha/\beta$ -tubulin heterodimers that self-associate into polymers (1). Tubulin exists in different isotypic forms, and multiple  $\alpha$ - and  $\beta$ -tubulin isotypes display tissue- and developmental-specific expression (5). The various  $\beta$ -tubulin isotypes are evolutionarily conserved across species and differ from each other predominantly in their carboxy-terminal region (6). This region binds distinct microtubule-associated proteins (MAPs) and is therefore thought to influence microtubule stability and functionality.

Microtubules are highly dynamic structures that play an important role in cellular growth, vesicular transport and mitosis. The ability of microtubules to polymerise and depolymerise in a regulated manner is essential for the segregation of chromosomes during mitosis. The assembly and disassembly of microtubules occurs via GTP hydrolysis on the  $\beta$ -tubulin subunit of the  $\alpha/\beta$ -tubulin heterodimer. In an interphase cell, microtubules initiate from the centrosome forming a hub and spoke type network. This microtubule

network is responsible for vesicular transport. During cell division, this network is completely remodelled to form the mitotic spindles across which duplicate sets of chromosomes line up and are divided equally into two daughter cells (Fig. 1,2). Upon completion of mitosis, the spindle is disassembled and the interphase microtubule network reforms (1).

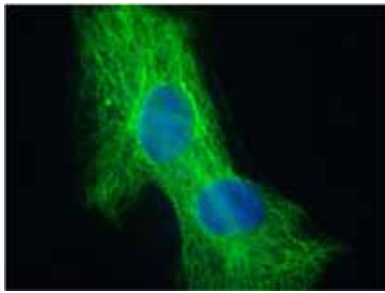
Intermediate filaments (IFs) are the principal structural determinants within cells. Unlike the filament structures of F-actin and microtubules, which are composed of highly conserved globular proteins, IFs can be formed from 40 different subunit proteins. They can be subdivided into five classes: keratins, neurofilaments, desmin, laminin and vimentin. The different types of intermediate filaments can be distinguished according to their localisation and protein composition. IFs are linked to the extracellular matrix (ECM) and extend to the cytoplasmic interior that surrounds the nucleus. This extensive network allows IFs to coordinate cytoskeletal activities by relaying information from the cell surface to the inner compartments of the cell (7).

### The Cytoskeleton is Important for Many Cellular Functions

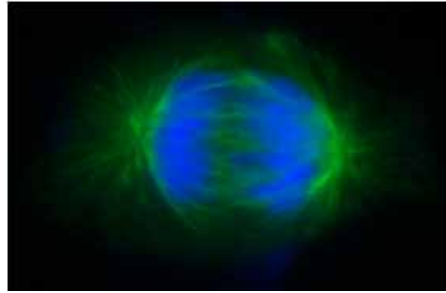
It is becoming increasingly clear that the great majority of cellular functions involve, either directly or indirectly, the cytoskeleton (1). This largely derives from the role of the cytoskeleton in the generation of contractile force, both long and short range transport, the provision of scaffolds for the binding of regulatory and signalling proteins, structural support for membrane proteins, and on and on. This may, to an extent, reflect opportunism on the part of the cell to what have become ubiquitous structural systems which have been adapted and/or recruited to a range of cellular demands. Whatever the evolutionary basis for this widespread involvement in most cellular functions, the challenge has been to understand how the two polymer systems, microfilaments and microtubules, are choreographed in both time and space.

### Organising Intracellular Space

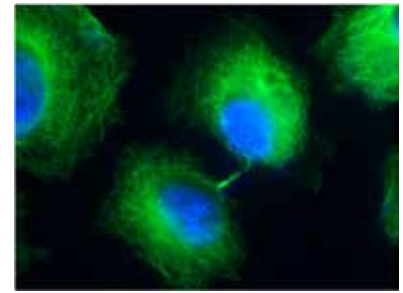
The building blocks of functional diversity of microtubules and actin filaments are the core constituents of each polymer system. In the case of microtubules, there are multiple  $\alpha$ - and  $\beta$ -tubulins that provide intrinsic diversity to the composition of the  $\alpha\beta$  core building block. In addition, there are multiple microtubule-associated proteins that provide further diversity and cell-specific function. The early observation that microtubule-associated proteins and specific  $\alpha\beta$  tubulin isotypes can be spatially segregated in neurons provided a potential mechanism to explain the spatial specialisation of microtubule function (8). Whereas this remains an attractive mechanism, it has not been easy to test.



Interphase



Anaphase



Two daughter cells

**Fig. 1. Backyard blitz of the microtubule cytoskeleton.**

Remodelling of the network during different phases of cell division. Microtubules (green), chromosomes (blue). Image by P. Gan (Children's Cancer Institute Australia for Medical Research).

In contrast, the actin cytoskeleton has provided striking examples of this principle. The different actins have been known for some time to provide the backbone for functionally distinct populations of actin filaments in a range of cells (Fig. 3). This is particularly dramatic in skeletal muscle, where the skeletal and cardiac  $\alpha$ -actins form the repeating arrays of thin filaments in the contractile apparatus, whereas cytoskeletal  $\beta$ -actin is located at the neuromuscular junction (9) and cytoskeletal  $\gamma$ -actin provides the cytoskeletal scaffold throughout the rest of the muscle fibre (10). In addition, the majority of actin filaments contain a second polymer, tropomyosin, running along the major groove in the actin filament. The different tropomyosins are subject to extensive intracellular sorting which is regulated in both time and space (2). It has therefore become clear that functionally distinct actin filaments are marked by both different actins and tropomyosins.

### Mechanisms of Functional Specificity

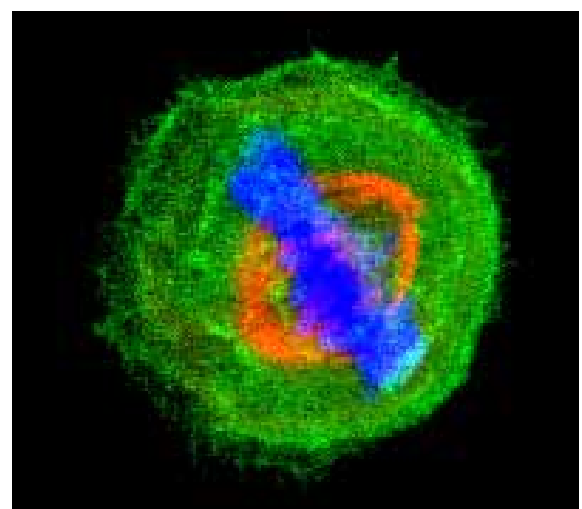
Do the different actins and tropomyosins dictate the functional specificity of the actin filaments they form? It has become increasingly clear that the answer to this question is yes. This has been most definitively demonstrated for the tropomyosins and the mechanism is based on the ability of the tropomyosins to dictate which actin-binding proteins can interact with a specific actin filament (2,11). Tropomyosins differ in their ability to compete/collaborate with cofilin and their facilitation of the interaction with different myosin motors leading to functionally distinct outcomes in terms of actin filament organisation and cell morphology (12). Protein chemistry studies indicate that this extends to most, if not all, actin-binding proteins (13). Thus, the spatial and temporal regulation of actin filament tropomyosin composition provides a simple mechanism to regulate functional specificity of the actin cytoskeleton.

### Microfilaments and Microtubules as Collaborators

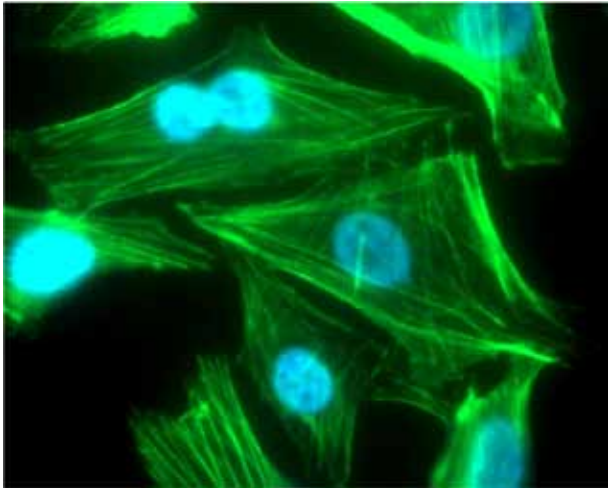
Nowhere is the collaboration of microtubules and microfilaments better defined than in the process of intracellular transport. It is notable that in yeast, the actin filaments, rather than microtubules, are responsible for intracellular vesicle transport (14). With the increasing sophistication of eukaryotic cells came the increased reliance on microtubules for long range trafficking. This is particularly evident in neurons where transport along the

axons and dendrites is primarily driven by the microtubules, whereas actin filaments participate in short range movement (15). It is now clear that in higher eukaryotes microtubules are responsible for the transport of vesicles from the Golgi to the cell periphery, where they hand over to the actin filaments for short range movement to the membrane. There is substantial evidence that at both the Golgi end and at the membrane destination actin filaments are contributing contractile force to facilitate vesicle budding and membrane fusion (16). Hence, intracellular trafficking has recruited these two polymer systems to specialise and diversify the different stages of transport.

The generation of these two polymer types and the further specialisation of function within each system have provided a remarkable flexibility to the eukaryotic cell to choreograph intracellular space. The intrinsic dynamics of both systems provides both the ability to create a remarkable variety of functional outcomes and also the ability to remodel rapidly in response to changes in intra- and extra-cellular signals. The challenge before us is to understand the mechanisms that underpin this flexibility and specificity which ultimately touches almost all cellular functions.

**Fig. 2. Spatial organisation of microtubules and microfilaments during cell division.**

F-actin stained with fluorophore-conjugated phalloidin, green; tubulin, red; chromosomes, blue.



**Fig. 3. Actin filaments (stress fibres) in neuroblastoma SHEP cells.**  
F-actin stained with fluorophore-conjugated phalloidin, green; chromosomes, blue.

### The Cytoskeleton and Disease

The various components of the cytoskeleton are highly integrated and their functions well orchestrated. Clinical problems can arise when mutations or deregulated expression of cytoskeletal proteins occurs. Mutations in the human skeletal muscle alpha-actin gene (*ACTA1*) are associated with structural abnormalities of muscle fibres (17). Mutations in *ACTA1* can lead to muscle weakness and disease states such as congenital myopathy or nemaline myopathy. As discussed in the preceding section, the various cytoskeletal filament structures display specialist functions. Indeed, alpha- and gamma-actin share a high degree of structural homology, yet mutations in the gamma-actin gene (*ACTG1*) are associated with a dominant progressive form of deafness (18). In a different context, mutations in *ACTG1* were found to be functionally mediating resistance to anticancer agents that target microtubules (19). In addition, decreased expression of gamma-actin could also mediate resistance to anticancer agents that target microtubules, while lower expression of this actin isoform was associated with relapsed disease in childhood acute lymphoblastic leukaemia (19). These studies further highlight the intricate co-ordination and influence of the cytoskeletal filament systems. Neurodegeneration-associated diseases, such as Alzheimer's, have been associated with mutations and aggregates of the microtubule-associated protein Tau (20). Anticancer agents that target the tubulin/microtubule system have been highly successful in the treatment of cancer. It is now clear that the composition of the beta-tubulin isotype repertoire within a cell can influence the behaviour and drug response of cancer cells (21,22). Mutations in class I beta-tubulin are frequently associated with resistance to tubulin-targeted agents due to changes in the polymer levels of microtubules or decreased binding to the drug target (23,24).

The actin cytoskeleton is important in the development of cancer metastasis. Changes in the polymerisation state of actin are important for cell motility, as actin polymerisation is essential for cell movement. Metastatic cancer cells acquire increased motility because of increased F-actin at the leading edge of cells, allowing them to leave the original tumour and

invade other organs (25, 26). Understanding how changes in cytoskeletal proteins mediate disease or influence therapies will aid in the development of novel treatment strategies.

### Conclusion

The complexity of the cytoskeleton is only beginning to be unraveled. Advances in live cell imaging that allow the visualisation of individual molecules and protein-protein interactions inside cells in real time are a new highly valuable complement to the molecular and cellular tools for understanding the inner workings and interactions of the cytoskeleton. Deeper understanding of the involvement of the cytoskeleton in diseases, and in particular cancer and cancer metastasis, may lead to the discovery of much needed new therapies.

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