Cytoskeletons by the sea

Margaret A. Titus

The ESF-EMBO meeting, 'Emergent Properties of the Cytoskeleton: Molecules to Cells', took place in October 2010 in San Feliu de Guíxols on the eastern coast of Spain. It brought together a diverse group of international cytoskeletal researchers who gave presentations on topics from structural biology and biophysical analyses of the cytoskeleton and its motors, to studies of the role of cytoskeletal proteins in multicellular development.

This stimulating meeting was organized by Michelle Peckam (U. Leeds, UK) and Claudia Viegel (U. Munich, Germany), it took place in a beautiful hotel overlooking the sea, which contributed to the convivial atmosphere and many stimulating interactions between participants. The excellent range of talks led to many discussions throughout the meeting and the variety of interesting posters also fostered many lively exchanges. Here is a summary of a few of the talks that were of particular interest to this attendee.

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Motor proteins couple the hydrolysis of ATP and subsequent release of its products—phosphate (P_i) and ADP—to conformational changes that ultimately result in force generation. Understanding the molecular details of the way in which hydrolysis drives structural changes in the motor to produce movement is an important goal for the field. For myosin, it has been accepted that P_i release is an important first step, but its escape route—the so-called 'back door'—has remained unclear. It was postulated that one of the two key structural elements that coordinate nucleotide binding and hydrolysis—switches I and II—would be required to move out of the way, to allow P_i to exit. Analyses of crystal structures had led to suggestions that switch I must move, as a change in switch II must be

coupled to a change in the position of the lever arm, which would result in a power-stroke. As P_i release is believed to precede a change in lever-arm position, it seemed likely that a shift in the position of switch I was necessary for P_i release. However, Anne Houdusse (Institute Curie, Paris, France) presented results from new crystal structures that reveal an unexpected position for switch II that does not correspond with a change in the lever-arm angle, but would allow for the release of P_i . An analysis of the enzymatic activity of mutants that are predicted to affect P_i release is consistent with the proposed model. This view of the structural rearrangement of switch II is exciting, and provides us with a new view of the way in which the myosin motor might function.

Tropomyosin is most often thought of for its role in the regulation of skeletal muscle contraction, but non-muscle cells also contain tropomyosin, which can affect myosin activity and work in opposition to ADF/cofilin to stabilize actin filaments. However, the existence of several potential tropomyosin isoforms—over 40, which arise from extensive alternative splicing of four genes in mammals—has meant that understanding the function of any individual tropomyosin is a daunting task. Work presented by Peter Gunning (U. New South Wales, Australia) revealed that progress has been made recently in tackling this problem. Targeted deletion of the γ -tropomyosin gene in mice (*TPM3*), which potentially encodes 11 isoforms, is embryonically lethal in many genetic backgrounds, indicating that the remaining tropomyosins cannot compensate for its loss. Two splice forms—TM5 NM1 and TM5 NM2—were found to have a role in regulating cell proliferation; cells and

mice overexpressing these tropomyosins have higher levels of actin filaments and increased rates of cell growth. Conversely, loss of TM5 NM1/2 resulted in slower cell proliferation, most notably in cancer cells. This provided Gunning and colleagues with a successful screen for a new class of anti-cancer drug that selectively targets specific tropomyosin isoforms in the cytoskeleton; transforming what had seemed to be a problem for understanding the function of this ubiquitous and varied protein into a virtue.

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The mechanosensory hair cells of the cochlea detect sound through the movement of their actin-rich stereocilia. Hair cells do not divide during the lifetime of most vertebrates, which means they cannot regenerate if they are damaged (bad news for those who love turning up the volume on their iPods while working in the lab!), but it also means that these cells must steadily renew their cytoskeleton and other organelles over the lifetime of the animal. A longstanding question in the field is whether the actin-filled stereocilia are relatively static structures, or whether there is constant and rapid turnover of the molecules from which they are made, similar to the turnover of actin and myosin molecules that occurs in sarcomeres. There is evidence of significant actin treadmilling in stereocilia (Rzadzinska *et al.*, 2004), consistent with the rapid turnover hypothesis.

This also suggests that the sensory cells have a mechanism for regulating the exchange of components, such that the length of each stereocilium is unchanged.

Valeria Piazza (Harvard U., USA) and colleagues addressed the question of hair-cell protein turnover by using a completely different approach—multi-isotope imaging mass spectrometry—that allowed them to determine the lifetime of proteins in sensory hair cells from mice and frogs. Turnover was observed in the cytoplasm of these cells as one might expect, but surprisingly little turnover was found in the stereocilia themselves; the average lifetime of the cells was weeks to months. This suggests that stereocilia are stable structures, and challenges the results of the earlier study. It seems difficult to reconcile these findings at first, but emerging imaging technologies for looking at live cells and new genetic control of protein expression will probably resolve this issue.

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The filamentous fungus *Ustilago maydis*—better known as corn smut fungus—is a key plant pathogen and a good model system for studying intracellular transport and cell polarization. Gero Steinberg (U. Exeter, UK) presented a talk on the long-distance transport and targeting of a chitin synthase that contributes to the synthesis of the cell wall and is essential for virulence (see Treitschke *et al* (2010) for more details). This chitin synthase—Mcs1—is fascinating as it is a membrane-anchored fusion protein of an unconventional myosin motor domain (class XVII) and chitin synthase (class V). Mcs1 is associated with vesicles that are transported along the length of the hyphal microtubules by a kinesin and targeted to the growing hyphal tip of *Ustilago*. Deletion of the myosin motor domain results in the loss of tip-targeting. As expected, vesicles still move along hyphal microtubules towards the tip, but once there, they are not retained. Elimination of microtubules by drug treatment abolishes long-distance transport of Mcs1. These results suggest similarities with the coordination of motor function in melanocytes, in which, for example, the myosin motor—myosin V—is not necessarily responsible for cargo transport, but

is used for retention at the periphery of the cell. This is an intriguing example of the way in which two different types of myosin might have similar molecular functions.

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The plus ends of microtubules are extremely busy places. Anna Akhmanova (Erasmus Medical Centre, the Netherlands) presented the efforts of her group to identify the proteins that reside there, and the way in which they interact with each other and the tip itself. This is an intriguing problem for the plus end because of its dynamic nature—it is constantly growing and shrinking—and the comet-tail localization of many proteins; they are concentrated at the end, and are progressively less concentrated further from the tip. How do proteins remain specifically associated, and what determines their localization not only at the tip, but also along the end of the microtubule? A search for the interaction partners of EB1 and EB3—well-known microtubule end-binding proteins—uncovered many proteins and led to the identification of several tip-binding complexes. Most interestingly, some of these might stabilize the tip whereas others—such as the complex that includes the kinesin MCAK—depolymerize microtubules from the tip (see Montenegro Gouveia *et al*, 2010). Thus, EB1 and EB3 can act to concentrate both positive and negative microtubule regulators, and the microtubule end can be stabilized or shortened, depending on which complex is present at the tip. The way in which these competing activities are regulated and coordinated is not yet known, but it seems that progress is being made.

Cytoplasmic dyneins have a range of cellular roles including organelle transport and interaction with microtubules at kinetochores and the cell cortex. Erika Holzbaur (U. Pennsylvania, USA) discussed recent experiments on dynein function in axonal transport where it is responsible for antero-grade transport, working in opposition to kinesin. Dynein and kinesin are both present on motile vesicles, which are often observed to switch direction. This raises the question of what the basis is for such reversals.

By using transgenic mice that express low levels of GFP-dynactin—a dynein-binding protein—Holzbaur and colleagues were able to isolate motor-bound vesicles, quantify the number of bound dynein–dynactin complexes and determine the ratio of kinesins to dyneins (Hendricks *et al*, 2010). There are significantly more dyneins than kinesins (about 4:1), but the two motors have different stall forces (the force at which the motor no longer moves): dyneins stall at 1 pN, whereas the stall force of kinesin is 4–6 pN. Thus, the excess dyneins balance the kinesins; a finding that is consistent with directional switching resulting from a ‘tug-of-war’ between the two motors, rather than simple turning on or off of the motors. The balance in forces is also consistent with the rapid changes in direction that are observed *in vivo*.

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A lively controversy has recently erupted in the cytoskeleton field about whether the actin network in the lamellipodia of motile cells is branched, as it was originally described to be by Svitkina & Borisy (1999). Branched networks have been observed in many contexts and *in vitro* experiments provide evidence that the Arp2/3 complex nucleates new actin filaments from pre-existing ones at a 70° angle. However, a new study of the lamellipodial network in several cell types reveals long, unbranched filaments, presenting a strong rebuttal to the textbook dendritic branching model (Urban *et al*, 2010). John Heuser (Washington U., USA) led a spirited discussion of this issue, outlining the merits of the techniques used in both studies, as well as methods for interpreting the resulting images. The main arguments are around the effects of harsh fixation methods used in the original study on actin-filament networks, compared with the large computational demands of the tomographical method used in the more recent work. Heuser made a strong case in support of the original dendritic-branching model, but it became clear that there is now a sense in the field that this needs more testing with additional approaches, perhaps even using different imaging techniques that might

someday allow visualization of actin-filament network formation in live cells.

It is well known that cells sense and respond to mechanical forces. The identity of the effectors and the alterations in cell behaviour that are caused by applied forces is of great interest in the field. Alexander Bershadsky (Weizman Institute, Israel) discussed his recent collaborative work with Benjamin Geiger's group at the same institute, to understand the role of focal-adhesion mechanosensing in cell polarization. Cells plated on surfaces of different stiffness exhibit focal adhesions of varying size and turnover rate (the softer the substrate, the smaller and more mobile the focal adhesions). Formation of the array of focal adhesions aligned with parallel actomyosin-filament bundles ('stress fibres') is a prerequisite for cellular elongation

and polarization. On the soft substrate (Young modulus 5 kPa), the array of parallel focal adhesion/stress fibres cannot be formed and, hence, cells cannot polarize. With this knowledge, the Bershadsky group developed a screen for identifying proteins with roles in focal-adhesion mechanosensitivity and surface-rigidity sensing. The early results suggest that this powerful screen will lead to the discovery of new and unexpected regulators of mechanosensing, as well as to better understanding of the mechanisms of cell polarization.

The meeting was a great success and it achieved its stated goal of showcasing the many exciting advances in cytoskeletal research and highlighting future directions for the field. A huge thanks is owed to the organizers for putting together such a stimulating programme, and to all of the

participants; both for sharing their terrific science and for contributing enthusiastically to many thought-provoking discussions.

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Margaret A. Titus is at the Department of Genetics, Cell Biology and Development at the University of Minnesota, Minneapolis, USA.

E-mail: titus004@umn.edu

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