Cell dynamics: a new look at the cytoskeleton

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focus on CYTOSKELETON

Cytoskeletal networks were once seen as predominantly static structures, but now, thanks to a battery of new techniques, are known to be highly dynamic, capable of rearranging themselves rapidly during processes such as cell movement. A recent symposium highlighted this view of the ever-changing cytoskeleton.

About two decades ago, the term 'cytoskeleton' was welcomed by cell biologists as a label that captures the idea of the pervasive filamentous networks that endow cells with structure, order and shape. But in the view of many, the term has mutated from a trademark to a misnomer that overemphasizes the static aspects of cellular organization and fails to encompass the rapid rearrangements of cytoplasmic networks that accompany motile processes. It took a series of stunning technical developments — including computer-enhanced light microscopy, ultrasensitive video microscopy, laser trapping, and labelling of macromolecules with green fluorescent protein (GFP) — before the dynamics that underlie processes at all levels of cellular organization could be fully appreciated. To highlight these advances, Michael Schleicher from the Adolf-Butenandt Institute at the Ludwig-Maximilians University recently organized a conference on the subject of 'Cell Dynamics — from Molecular Structure to Cellular Motility'[1]. It was the first in a proposed series of symposia on this topic, and it stood out not only for the excellence of the science discussed, but also for its attempt to kindle an awareness of political issues in the scientific community. For the first time in Europe, a scientific conference was combined with a discussion of important issues of basic research, the fragmentation of the science community, and the need for unity in issues of funding in Europe[2].

Of course, for scientists, the uniting principle is science itself. The meeting was launched with a discussion of molecular motors. The kinesin superfamily of microtubule-associated motors is structurally diverse; the kinesins form monomers or multimers of various shapes, with amino-terminal, carboxy-terminal or median motor domains. Determining which structural features confer directionality of movement is fundamental to an understanding of these proteins, as specific kinesin motors can move towards either the plus (quickly growing) or the minus (slowly growing) end of the microtubule. Recent studies point to the neck, a coiled-coil region adjacent to the motor domain, as a key determinant of motor polarity[3]. In a stunning extension of these findings, S. Endow (Duke Univ. Medical Center, Durham, NC) described two neck mutants of the minus-end-directed *Drosophila* kinesin motor Ncd that lack apparent directionality of movement. The first mutant has an altered asparagine residue in the neck, while the second mutation is of a lysine in the motor core. These two residues interact in the intact molecule, representing a crucial head–neck link. It is surprising that single amino-acid changes can control directionality, but this fact indicates that some motors might be reversible depending on a modification such as phosphorylation. Although it is becoming clear that the kinesin head–neck region is important for kinesin directionality, future studies will reveal whether these motors are indeed more versatile than we had thought.

A basic feature of at least the 'conventional' dimeric kinesins is their ability to move processively along microtubules, a property believed to be dependent on alternate microtubule binding and ATP hydrolysis by the two heads. Surprisingly, a monomeric motor, KIF1A, appears to be capable of processive movement as well. KIF1A-related motors have an insertion in the motor domain that is rich in lysine residues, termed the K-loop. This K-loop is thought to maintain the motor in close contact with the negatively charged surface of the microtubule during the working cycle of the motor. In support of this contention, N. Hirokawa (Univ. Tokyo) presented structural evidence that the K-loop forms an extra extension that makes contact with the microtubule surface, giving the motor the appearance of a koala bear (KIF1A) clinging to a tree trunk (the microtubule). So a sliding and gripping motion, similar to that of a subway train moving along a track, may be another way to achieve processivity.

Cytoplasmic dynein, another microtubule motor, is usually found associated with a large multisubunit complex called dynactin. T. Schroer's laboratory (Johns Hopkins Univ., Baltimore, MD) has recently discovered a new member of the actin-related protein (Arp) family, Arp11, and this protein is a subunit of the dynactin complex. Thus the complex contains two Arp proteins — it was shown previously to contain a short filament composed of Arp1. Schroer described a role for dynein/dynactin in

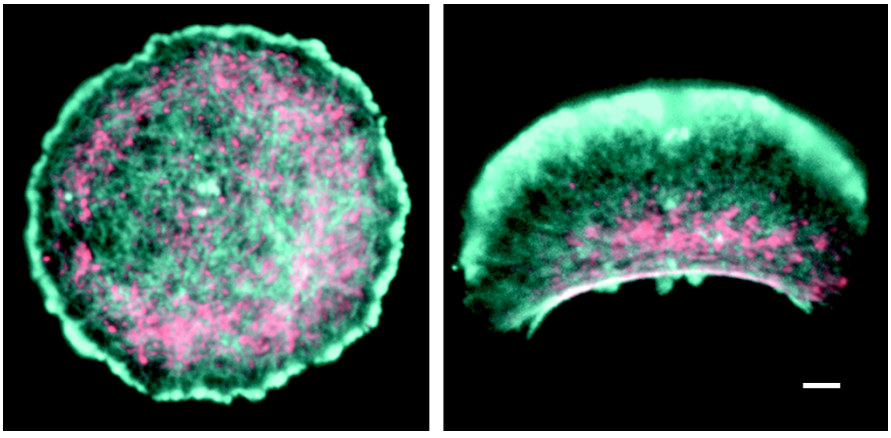

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Figure 1 **Cytoskeletal organization of goldfish keratocyte fragments. A stationary, symmetric fragment is shown at the left and a motile, polarized fragment is shown at the right. Polarization can be induced by simply nudging one side of a symmetric fragment with a micropipette. Actin is shown in cyan and myosin in red. Scale bar represents 2 μm.**

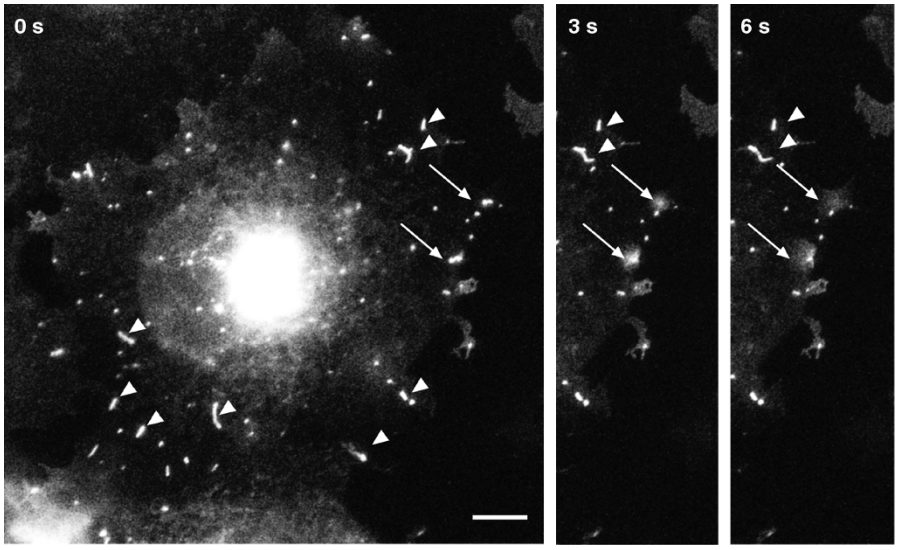

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Figure 2 **Golgi-to-plasma-membrane transport intermediates in a cell expressing GFP-tagged VSV G-protein 60 minutes after a shift to the permissive temperature. Arrowheads indicate post-Golgi carriers; arrows indicate post-Golgi carriers that are fusing with the plasma membrane. Scale bar represents 5 μm.**

anchoring microtubules at centrosomes and in the maintenance of a radial microtubule array. So dynein/dynactin joins a rapidly growing collection of proteins that are found at the centrosome, either as permanent residents or as temporary recruits from a cytoplasmic pool. E. Nigg (Univ. Geneva) is using a new centrosome-duplication assay, involving the use of hydroxyurea to block the cell cycle in S phase, to identify these centrosome components, his aim being to reveal the molecular basis for centrosome-based mitotic checkpoints.

Our view of the actin cytoskeleton has been one of the most changeable aspects of the study of cell biology in the past year. G. Borisy (Univ. Wisconsin, Madison, WI), L. Machesky (Univ. Birmingham, UK) and T. Pollard (Salk Institute, La Jolla, CA) put together a picture of signalling to actin dynamics that has emerged from the work of several groups, including those present at this meeting. The Arp2/3 complex appears to be a key regulator of actin assembly in lamellipodia, acting through *de novo* nucleation of new filaments as branches from pre-existing filaments. Working in concert with assembly of actin branches, cofilin/ADF acts to ensure that older filaments are recycled, by depolymerizing them. All of this activity is regulated by signalling molecules such as the small GTPases Rac and Cdc42. These GTPases connect to the Arp2/3 complex through WASP-family proteins and to cofilin/ADF through LIM-kinase, which phosphorylates cofilin/ADF to modulate its activity.

How these signals all work together to cause a cell to move in a polarized fashion will no doubt be the subject of future meetings, but Borisy's presentation, as well as a poster by I. Kaverina of V. Small's laboratory (Institute of Molecular Biology, Salzburg), challenged us all to think about mechanisms of cell polarity. Both groups have found that something as simple as a mechanical stimulus (Fig. 1) or the inhibition of the actin-associated motor protein myosin II on one side of a cell can induce polarity. Thus cells may be polarized by small instabilities, including mechanical stimuli, that favour one side over the other. New approaches — such as local laser-induced photoactivation of caged compounds (K. Jacobson, Univ. North Carolina, Chapel Hill, NC) — may help us to understand polarized cell movement better. All of these presentations emphasized the fact that the concept of cell polarity may need to be re-evaluated in terms of a more global picture than we had thought necessary.

Pathogens such as *Listeria monocytogenes*, vaccinia virus and enteropathogenic *Escherichia coli* use the host-cell actin cytoskeleton to become motile and evade the host immune response. We learned from M. Way (EMBL, Heidelberg) that vaccinia virus uses the same pathways that the host cell is suspected to use in inducing actin assembly[4]. The virus encodes a protein on its surface that appears to mimic a tyrosine-kinase receptor. Once phosphorylated by a host kinase, this protein recruits WASP-family proteins and so induces actin assembly through the WASP→Arp2/3-complex pathway. This system appears to be used by *Listeria*[5], *Shigella*[5] and enteropathogenic *E. coli*[6] as well, with each pathogen capitalizing on a unique point in the pathway to activate actin assembly (B. Finlay, Univ. British Columbia, Vancouver). The universal nature of this hijacking of the actin cytoskeleton by pathogens indicates that the pathway they are subverting is a very important mechanism for actin assembly in the cell, and indeed may be the primary way by which cells achieve actin-based motility.

Moving on to membrane transport, J. Lippincott-Schwartz (NICHD, Bethesda, MD) showed us that GFP can be used for much more than just pretty pictures, by applying surprisingly simple first-order linear kinetic analysis to describe the passage of proteins through the secretory pathway (Fig. 2)[7]. Lippincott-Schwartz tracked GFP-labelled VSV G-protein, a viral protein specifically engineered for use in studying the secretory pathway, in individual cells and quantitatively analysed this protein in space and time. The number of molecules passing from the endoplasmic reticulum through the Golgi apparatus to the plasma membrane could be quantified over time, resulting in a clear picture of the journey taken by each molecule through the cell. Perhaps the most surprising result obtained by this technique was that long, tubular, Golgi-derived intermediates appear to be key in transport to the plasma membrane, and that the fusion of these intermediates results in dramatic waves of VSVG–GFP that coincide with insertion of this protein into the membrane.

Webster's dictionary defines 'dynamics' as "that area of mechanics that deals with forces and their effects on bodies in motion or at rest and the patterns of change or growth in objects". Forces, motion and change were all dealt with during this meeting, at several levels of cellular organization: at the level of single molecules, where changes on a timescale of microseconds form the basis for rapid movements and large forces; at the level of supramolecular complexes, where rearrangements on a millisecond timescale govern cell shape and movement; and at the level of whole cells, where more long-term cellular interactions are controlled. The rigid cytoskeleton of the 1970s has been superseded by a dynamic matrix of interacting supramolecular complexes. □

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