

Meeting report

Beyond a 'skeleton': understanding cellular functions of the cytoskeleton

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A report on the cytoskeleton sessions of the 40th Annual Meeting of the American Society for Cell Biology, San Francisco, 9-13 December 2000.

The American Society for Cell Biology Annual Meeting covered topics from immunology to neurobiology, at the cellular level. Here, we focus on a number of exciting presentations concerning the function and regulation of the cytoskeleton.

Cytoskeletal motors

The minisymposium on Regulation of Cytoskeleton Motors had several interesting talks covering three closely related areas of motor research: regulation, interaction with cargo and cellular function.

Regulation

Peter Hollenbeck (Purdue University, Indiana, USA) presented a time-lapse microscopy study of the transport of mitochondria in neurons. The thought-provoking result he obtained is that anterograde transport - towards the neuronal cell body - seems to respond to the dynamics of the growth cone tens of micrometers away. Transport is highly stimulated when the growth cone is rapidly growing but is repressed in axons with resting growing cones. One clue to the nature of the signal that regulates mitochondrial motility came from an experiment in which a bead coated with nerve growth factor (NGF) leads to an accumulation of mitochondria at the site of contact by the bead, suggesting that the motility responds directly to NGF signaling.

Organelle motility may be regulated in several ways: by modification of cytoskeleton 'tracks', along which organelles travel;

by controlling motor activity; and/or by controlling the cargo-motor interaction. An example of the last type of regulation was shown in elegant experiments presented by Vladimir Gelfand (University of Illinois at Urbana-Champaign, USA). His lab studies the transport of pigment-containing melanosomes, which depends on the motor protein myosin V. This motility appears to be inhibited during mitosis. Gelfand showed that it is the interaction of the cargo, in this case melanosomes, that provides the site of regulation. Phosphorylation of a site on the tail of myosin V in an extract from mitotic cells results in release of the motor from the melanosome-containing cell fraction. This site is phosphorylated by Ca²⁺/calmodulin-dependent (CaM) kinase II, and inhibitors of this kinase prevent the release of myosin V in mitotic extracts.

Motor-cargo interaction

Motor-cargo interaction is currently a hot topic because it sheds light on the diverse functions of the increasing number of cytoskeleton motors that are being identified. Two talks presented exciting new data on how motors might be linked to their cargoes. Kristen Verhey (Harvard Medical School, Boston, USA) used a yeast two-hybrid protein-protein interaction screen to look for proteins that bind to the cargo-binding domain of conventional kinesin. Three proteins were found, the JNK-interacting proteins JIP-1, JIP-2 and JIP-3, which are scaffold proteins for the JNK (Jun kinase) signaling pathway. She further showed that the concentration of JIPs into nerve terminals requires kinesin, a finding that supports a role for motor proteins in the spatial regulation of signal transduction pathways. James Goldenring (Medical College of Georgia, Augusta, USA) found that the tail of myosin Vb binds directly and specifically to GTPases of the Rab11 family, which are associated with plasma-membrane recycling systems. Rab11a and transfected myosin Vb-tail colocalize, and overexpression of myosin Vb-tail blocks the recycling of transferrin in HeLa

cells. These results indicate that the Rab11 family may provide a key link between myosin Vb and the vesicles involved in recycling membrane receptors.

Non-vesicular functions of motors

There were two talks on functions of motor proteins other than in vesicular transport. Gregory Pazour (University of Massachusetts Medical School, Worcester, USA) presented work that received the 'Molecular Biology of the Cell paper of the year' award. The work started from an analysis of the role of the LC2 outer-arm dynein light chain in *Chlamydomonas* flagellar motility, and went on to explain how a mutation in an LC2 homolog in the mouse gives rise to ratio distortion in chromosome transmission. Terry Lechler (Harvard Medical School, Boston, USA) talked about the involvement of a type I myosin in actin polymerization at the plasma membrane. This work was first carried out in yeast, where he showed that myosin I controls actin polymerization through an interaction of the myosin tail domain with the Arp2/3 complex and also through a functional motor domain. Preliminary results now suggest that this function may also be conserved in animal cells. Specifically, the data support a model in which the motor activity of type I myosin is required for maintaining an active actin-nucleation complex at the leading edge of the plasma membrane in migrating cells.

Actin nucleation

Major effort has been invested in recent years to identify the cellular components required for actin nucleation. The Arp2/3 complex has emerged as a key player in this process, acting downstream of a signaling pathway that involves the GTPase Cdc42 and the Wiskott-Aldrich syndrome protein (WASP). A special interest group session on 'Actin nucleation through Arp2/3 complex and WASP/Scar proteins', and several symposia on this topic, were held during the meeting. The emphasis was on the elucidation of the structural basis of the nucleation process mediated by the Arp2/3 complex, its regulation by WASP family members, and the cellular roles of these proteins.

Tom Pollard (The Salk Institute, La Jolla, USA) and collaborators provided evidence for the generation of a dendritic actin network, by following actin nucleation *in vitro* using a novel fluorescence assay. They find that activation of the Arp2/3 complex by WASPs induces Arp2/3 binding to the sides of preexisting actin filaments, as a route to generating new, branched filaments. In contrast to this view, Marie-France Carlier and collaborators recently reported that the Arp2/3 complex binds to the barbed ends of actin filaments and not to their sides, and that it does not actually function as a nucleator. Further work will be required to clarify this apparent discrepancy.

Functional dissection of the human and the yeast Arp2/3 complexes has allowed characterization of the roles of the

Arc subunits in both the topology and the biochemical properties of the complex. Helene Gournier and Matt Welch (University of California, Berkeley, USA) have obtained recombinant full and partial human Arp2/3 complex following expression in baculovirus. Their data indicate that the human complex has a similar topology to the yeast complex. Our biochemical analysis of yeast partial complexes (C.E. and R.L.) indicates that, in contrast to what has been reported earlier, p21-Arc and its yeast homolog Arc18 are not required for WASP to interact with the Arp2/3 complex but are essential for the activation process.

Jonathan Zalvesky and Dyché Mullins (University of California, San Francisco, USA) provided the first results indicating a topological reorganization of the Arp2/3 complex after activation of N-WASP. Arp2/3 complex activation by the activation domain of N-WASP, the WA domain, brings p18-Arc and p19-Arc together, leading to a potent activation of the complex. The WA domain of Scar, another member of the WASP family, is a less potent activator than N-WASP because it is unable to induce similar reorganization.

Several presentations focused on the functions of the Arp2/3 complex and WASP *in vivo*. The Arp2/3 complex is required for phagocytosis in macrophages (Robin May and Laura Machesky, University of Birmingham, UK). Robert Insall (University of Birmingham, UK) presented the identification of the Arp/Arc genes in *Dictyostelium*, and Charles Saxe (Emory University, Georgia, USA) discussed the role of Scar during cell locomotion, chemotaxis and endocytosis in *Dictyostelium*. Current work is focused on identification of the signaling components upstream of the Arp2/3 complex.

Elegant microscopic work presented by Tatyana Svitkina (Northwestern University, Illinois, USA) on the structure of actin filaments induced by beads coated with ActA (the *Listeria monocytogenes* activator of the Arp2/3 complex) indicates that ActA also induces formation of a dendritic network of actin filaments via the Arp2/3 complex. In contrast, the cellular protein VASP appears to inhibit dendritic network formation, explaining the recently described negative effect of VASP on cell locomotion (Svitkina and Frank Gertler, MIT, Cambridge, USA).

A large number of posters presented the identification and characterization of non-WASP-family activators of the Arp2/3 complex. As it is quite probable that WASP proteins are not responsible for the recruitment of the Arp2/3 complex to lamellipodia and are not the sole activators of the complex, several labs have focused on the identification of the components that activate the complex in this context. Pollard's lab reported that fission yeast myosin I binds to and activates the Arp2/3 complex. Bruce Good, David Drubin and collaborators (University of California, Berkeley, USA) have identified three yeast proteins that bind the Arp2/3 complex - coronin, Abp1 and Pan1. As Abp1 and Pan1

interact with proteins involved in endocytosis in yeast, these results provide a potential function of the Arp2/3 complex during endocytosis. Cortactin, a protein enriched in lamellipodia, was identified as another non-WASP Arp2/3 complex-binding protein and activator (Xi Zhan and collaborators, American Red Cross, Rockville, USA).

The cytoskeleton and disease

While the fundamental properties of cytoskeletal dynamics and motor proteins are still yielding new insights and posing new questions, the larger role of the cytoskeleton in physiological and pathological processes is also becoming more apparent. A special interest sub-group focusing on the cytoskeleton and diseases also met at the meeting.

Mutations in the microtubule-binding protein Tau cause a diverse array of neurodegenerative diseases characterized by aberrant localization and/or aggregation of Tau. These mutations tend to cluster around the microtubule-binding region of the protein, prompting Ken Kosik (Harvard Medical School, Boston, USA) and colleagues to examine the intracellular localization of the mutant proteins. Surprisingly, their findings challenge the notion that Tau inclusions are necessarily formed from the free pool of Tau. Instead, some Tau splice forms that are specifically found in inclusions seem to out-compete other splice forms for microtubule binding. Additional work is needed to clarify this situation and to determine the mechanism by which mutant Tau forms inclusions.

A large number of genetic studies in mice and humans has demonstrated the importance of intermediate filament structure in both neuronal and epithelial cell structure and function. Now, additional findings have implicated cytoskeletal linker proteins, the plakins, as important mediators of cell structure and disease. Elaine Fuchs (University of Chicago, USA) and colleagues have demonstrated that differentially spliced isoforms of these genes can encode microtubule-, actin-filament-, and intermediate-filament-binding properties, as well as regions that bind to sites of cell adhesion. Plakins appear to stabilize cytoskeletal structures at cortical areas of epidermal cells and microtubule bundles throughout nerve axons. In fact, the mouse condition dystonia musculorum, which results in neurodegeneration a few weeks after birth, is due to mutations in a plakin gene.

The session ended with a discussion of cytoskeletal proteins as targets for drug design. Tim Mitchison (Institute for Chemistry and Cell Biology, Harvard Medical School, Boston, USA) discussed work using high-throughput screens to identify inhibitors of cytoskeletal components. This approach offers the possibility of discovering research tools that can be used quickly and reversibly to inhibit cellular factors; it has already led to the discovery of monastrol, an inhibitor of the kinesin Eg5, and several inhibitors of cell

migration with unknown targets. Chemical inhibitors should prove especially useful for cytoskeletal studies, as the highly dynamic nature of the cytoskeleton often makes the prolonged perturbation caused by genetic ablation insufficient to understand function. We can expect the combination of genetic, biochemical, and chemical approaches described at the meeting to shed significant light on cytoskeletal function in the year ahead.