

Movement in Colorado: The Keystone Symposium on Cell Migration and Invasion Breckenridge, CO January 18–23, 2003

The use of complex and three-dimensional environments, plus the promise of collaborative work by a Migration Consortium, are heating up research into cell migration.

Stand a human on two pieces of wood and point them down a snowy hill and they will move: this much was clear at a recent meeting at Breckenridge, Colorado (Keystone Symposium on Cell Migration and Invasion, January 18–23, 2003). But conceiving just how cells might crank their gears into motion takes a little more imagination. Even as the decoding of individual components has continued, with researchers gaining a clearer picture of the function of many motility proteins, other workers are upping the stakes by putting cells into three-dimensional (3D) environments and tracking the different ways that cells move when they are confronted with more realistic substrates.

Pushing out the front

Actin polymerization drives cells forward (Fig. 1). The canonical version of this mechanism was put forward by Tom Pollard (Yale University, New Haven, CT) in his keynote address. Pollard outlined the dendritic nucleation model (Mullins et al., 1998; Svitkina and Borisy, 1999), in which the actin-nucleating Arp2/3 complex binds to the side of existing actin filaments to make branched structures that elongate toward the front of the cell. Further back, cofilin severs and debranches these filaments—in the latter case by promoting the dissociation of phosphate from actin, thus causing the branch to dissociate from the Arp2/3 complex (Blanchoin et al., 2000).

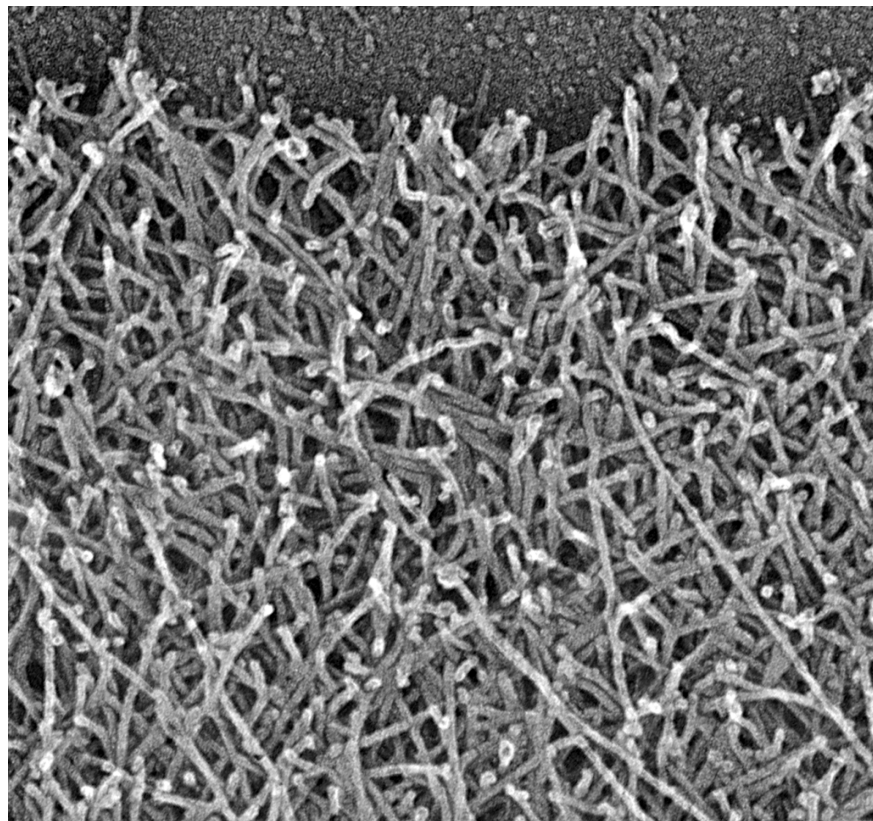


Figure 1. Polymerization of a web of actin drives forward movement of a fish keratocyte.

Actin capping proteins are one class of proteins that modify the shape and behavior of this dendritic network of actin. A seeming paradox of capping was explained by Frank Gertler (Massachusetts Institute of Technology, Cambridge, MA). Although Ena/VASP proteins can counteract capping activity, thus promoting extension at the barbed end of actin filaments, this results in slower cell movement. Gertler found the basis for this seeming contradiction: the extension rate of a protrusion is less important than its stability (Bear et al., 2002; Krause et al., 2002). The rapidly growing protrusions with high Ena/

VASP had longer, less branched actin filaments that readily collapsed, perhaps because the less branched structures cannot resist compression. They can, however, be clustered and turned into filopodia in neurons.

A leaky version of a cap was proposed by Sally Zigmond (University of Pennsylvania, Philadelphia, PA) as an activity mediated by the formin Bni1. Although the Arp2/3-containing branched networks are good at exerting protrusive force, the formins nucleate actin cables that may be more suited to bearing tension. Bni1 nucleates by actin dimer stabilization (Pring et al.,

Svitkina

2003), and Zigmond proposed that it competes directly with capping protein binding. Eventually, however, the more stable association of capping protein should win out.

Another association that may change over time is that involving cortactin and the Arp2/3 complex (Weaver et al., 2003). Both cortactin and N-WASP can bind to and activate the Arp2/3 complex (Weaver et al., 2002), although N-WASP is far more potent and is thought to be the important activator near the front of the cell. But according to Alissa Weaver and John Cooper (Washington University, St. Louis, MO) and J. Thomas Parsons (University of Virginia, Charlottesville, VA), this N-WASP function may be replaced by cortactin as the actin branch is pushed away from the membrane. Consistent with this idea, cortactin inhibits the debranching that can result from loss of Arp2/3.

Getting to the front

Somehow the protrusion and actin polymerization must be directed primarily to the front of the cell. Several investigators presented ideas for how this might be achieved. An idea of how signaling might make its way to the right general area of the cell—the cell membrane—was supplied by Martin Schwartz (University of Virginia, Charlottesville, VA). Growth factors activate Rac in both adherent and nonadherent cells, but only in the adherent cells does the Rac translocate to the membrane, thus leaving its inhibitor (RhoGDI) behind and taking it to a site where it can activate downstream targets such as Pak.

Schwartz found that Rac translocates to lipid rafts and caveolae, and its GTP-dependent binding to raft lipids is dependent on the presence of raft components such as cholesterol. Integrin engagement acts to prevent removal of rafts, and thus Rac, from the membrane. This removal can also be blocked in nonadherent cells if the cells lack caveolin—these suspended cells have active Rac on their membranes and continue to ruffle.

The importance of the uptake of Rac binding sites is not clear, as mice lacking

caveolin (and thus presumably lacking the uptake) are viable. A similar question mark hangs over an intriguing finding by Kris DeMali and Keith Burridge (University of North Carolina, Chapel Hill, NC), who found that Arp2/3 binds to the focal complex component vinculin (DeMali et al., 2002). Focal complexes are the first adhesive structures to form after the cell edge protrudes. The transient recruitment of Arp2/3 to vinculin after integrin engagement could help to localize Arp2/3 to the place where it is most needed. Then, by the time the focal complexes mature into focal adhesions, the Arp2/3 association is lost.

After this exciting localization and binding data, it comes as somewhat of a disappointment that the interaction may not be crucial for migration. Cells expressing only a version of vinculin that cannot bind Arp2/3 recover normal migration behavior (vinculin nulls migrate faster than wild type), although they have less spreading activity and fewer lamellae.

Getting integrins themselves to the front of the cell may be a task that falls to the newly isolated myosin X. Staffan Strömblad (Karolinska Institutet, Huddinge, Sweden) used the integrin $\beta 5$ tail as a bait to recover myosin X, and found that its overexpression caused the relocation of low levels of integrins to filopodial tips.

Finally, a special localizing mechanism may operate only when protrusion first begins. By the time protrusion has reached steady-state, monomeric actin (G-actin) subunits are limiting, and new G-actin for polymerization is supplied by cofilin. This protein chops up polymerized F-actin away from the front of the cell. But John Condeelis (Albert Einstein College of Medicine, Bronx, NY) pointed out that, when protrusion first starts, G-actin is abundant. Under these conditions cofilin can actually polymerize actin by severing filaments to form free barbed ends. Condeelis found that phospholipase C-mediated hydrolysis of PIP2 probably releases cofilin from the membrane causing severing and barbed end formation locally. As protrusion continues and G-actin levels fall, the

more established actin polymerization pathway (PI3K turning on Rac and thus Arp2/3) takes over.

Signaling the way

Once protrusion is underway, it can be amplified by a positive feedback loop. Orion Weiner (Harvard Medical School, Boston, MA) found that exogenously added PIP3 induces production of more PIP3 via a positive feedback loop that depends on endogenous Rac (Weiner et al., 2002). This circular pathway in neutrophils explains some confusing epistasis results from earlier experiments.

Pulling and adhesion then come into play. Yu-Li Wang (University of Massachusetts Medical School, Worcester, MA) suggested that these phenomena are united by actin-based traction. In his model, the traction forces cause the opening of stretch-activated channels as part of a feedback regulatory mechanism. The entry of calcium would activate myosin II and indirectly lead to strengthening of the adhesions, thus counteracting the traction forces. Wang showed that channel inhibition reduces motile forces, and that myosin IIB was necessary for the cell to tell the difference between soft and hard substrates, and to steer accordingly (Lo et al., 2000).

Localization can also be present at the level of signaling. Anne Ridley (Ludwig Institute for Cancer Research, London, UK) pointed out that migration signals arriving via Rho family proteins have a single known mode of operation: the Rho proteins bind to their targets and thus relieve auto-inhibition. This mechanism is used when Rac turns on WAVE and Pak, Cdc42 turns on Pak and WASP, and Rho turns on ROCK and mDia. But Ridley reported a new form of WASP regulation in macrophages, where a receptor (probably the CSF-1 receptor) can turn on the Src kinase Hck, which can then phosphorylate and thus activate the Arp2/3 activator WASP (Cory et al., 2002). The target residue is in a hydrophobic region of WASP, so introduction of a hydrophilic phosphate group may promote an unfolding event.

When migration goes wrong

Development and remodeling require frequent changes in the adhesion and motility of cells. For example, Ken Yamada (National Institute of Dental and Craniofacial Research, Bethesda, MD) described how epithelial branching (which takes place during processes such as salivary gland and lung development) occurs by replacing cell–cell cadherin interactions with cell–matrix interactions. The change is prompted by synthesis of fibronectin not by the usual suspect—the extracellular matrix (ECM)-rich mesenchyme surrounding the epithelial cells—but by the epithelial cells themselves. Ablation of fibronectin expression blocked branching and prevented the formation of the epithelial clefts.

A complete switch of the epithelial cells' properties is termed the epithelial–mesenchyme transition (EMT). It results in a gain of migratory capability and autonomous cell survival—processes that also underlie the development of invasive and metastatic cancer. Art Mercurio (Harvard Medical School, Boston, MA) presented evidence that activated macrophages in tumors could act as a source of TNF α that initiates an autocrine TNF α loop in tumor cells, thus accelerating the EMT. Mercurio also found that an increase in VEGF expression correlated with the EMT in carcinoma cells. The cause of this increase has not been determined, but the possibilities include the increase in TNF α signaling and a pathway downstream of the integrin $\alpha 6 \beta 4$ that is known to lead to increased VEGF production (Chung et al., 2002). Mercurio found that the VEGF functions in an autocrine loop that is essential for migration and survival. The loop involves the VEGF receptor neuropilin, which was originally characterized as a receptor for the semaphorin axon guidance molecules.

Once cancer cells have initiated movement they face two potential barriers. The first, encountered by cells that migrate early such as melanoma cells, is the ECM. The ability of matrix metalloproteases to break down such matrices has led many to develop drugs to inhibit this process. But Peter Friedl (University of Würzburg, Würzburg,

A migration consortium

Collaborative research is coming to the migration research community in a big way. At the Keystone meeting, Rick Horwitz (University of Virginia, Charlottesville, VA) outlined the efforts of the Cell Migration Consortium, which has brought together the efforts of 31 motility investigators at 15 institutions.

The results of the Consortium's work after the first 15 months have been inspiring, according to Horwitz. "Just being in the room is exhilarating," he says. "When these people are brainstorming en masse it is really something. The enthusiasm is substantial because most people haven't done interdisciplinary work before."

The group has an annual meeting and uses relatively inexpensive video conferencing hardware to communicate frequently during the year. Communication "is really the essence of it," says Horwitz, as finally the biochemists, structural biologists, physicists, chemists, engineers, and modelers can discuss with each other what approaches and data would be most useful.

The Consortium was designed to take on problems that no single laboratory could tackle alone. Horwitz and J. Thomas Parsons (University of Virginia, Charlottesville, VA) applied for one of the long-term "glue" grants provided by the National Institute of General and Medical Sciences (NIGMS). Other glue grants include the one used to found the Alliance for Cell Signaling, which is led by Alfred Gilman (University of Texas Southwestern, Dallas, TX). The Alliance has picked up additional money from industry, and therefore operates on a much larger scale.

The Cell Migration Consortium aims to develop reagents, technology, and information to be shared with the migration community. Several initiatives have different but related aims:

- The discovery initiative uses array analysis, localization of GFP fusions, screening for genes that promote migration in a slow-moving cell line, and tandem mass spectroscopy of complexes to identify and characterize migration-related proteins.
- The structure initiative is using correlative light–electron microscopy (EM), cryo- and tomographic EM, X ray crystallography, and NMR to determine the structure of motility complexes.
- The signaling initiative is developing better biosensors, including caged phosphoproteins.
- The photoimaging initiative is making high resolution force maps and developing methods for localized photoactivation/inactivation, image correlation microscopy, and in vivo imaging.
- The modeling initiative integrates many of the Consortium's activities by developing physico-chemical systems and correlative models of migration.

These groups are supported by other investigators generating knock-out and transgenic mice and designer biosubstrates, and by a team that unites all of the information generated in various databases.

Information about the Consortium is on a web site (www.cellmigration.org) that will be updated as the databases evolve. All investigators have signed agreements stating that data, information, and reagents will be shared promptly and freely. "I think there will be a lot of people who will be watching that site like a hawk," says Horwitz. He admits that, like any scientific endeavor, the Consortium will no doubt strike out on some projects. But the offer of free and timely information should spread the benefits of the successful projects well beyond those directly involved in the Consortium. After all, says Horwitz, "who wouldn't want to have a new gene to study"?

Germany) reported that inhibition of a large panel of extracellular proteases did not prevent migration through ECM. Instead, cells resorted to an amoeboid form of migration, allowing them to squeeze through gaps in the matrix (Wolf et al., 2003). In an even more impressive and puzzling maneuver, the cells, as observed by Friedl, can also migrate in clusters and large collectives of a few to several hundred cells (Hegerfeldt et al., 2002). Both the integrity of the clusters and the generation of the migration force are dependent on $\beta 1$ integrin function.

For cancer cells that migrate later, such as carcinoma cells, the primary barrier is the basement membrane. Condeelis reported that carcinoma cells can cross this barrier to move from tissues to bloodstream because of macrophages lining blood vessels. These macrophages make EGF that stimulates carcinoma cells both to migrate across the basement membrane and to produce a macrophage-stimulating factor.

Entering the third dimension

The cancer studies illustrate a growing trend toward using environments that are more realistic than a glass slide. Perhaps the most important change is a switch from two dimensions to three. There is plenty of evidence for differences between the two situations (Cukierman et al., 2002; Knight et al., 2000; Walpita and Hay, 2002). Yamada reported multiple differences in two-dimensional (2D) versus 3D culture, including changes in the organization of cell adhesion components and in signal transduction downstream of adhesion. In addition, cells in 3D culture had enhanced rates of attachment and

augmented proliferation and migration rates. Meanwhile, Wang used layers of polyacrylamide to construct his model 3D environments and found that migrating cells were elongated and needle-like with no visible lamellae but fine projections.

Wang suggested that much of the signaling in 2D cultures reflected attempts of the cells to spread as much as possible, thus reducing their exposed surface areas and unanchored integrins. But he is still uncertain whether the discrepancies seen between 2D and 3D cultures reflect fundamental differences in cellular characteristics or simply quantitative differences. That question, and the testing of a multitude of motility proteins in the new 3D conditions, will have to wait for future studies.

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