Meeting Report

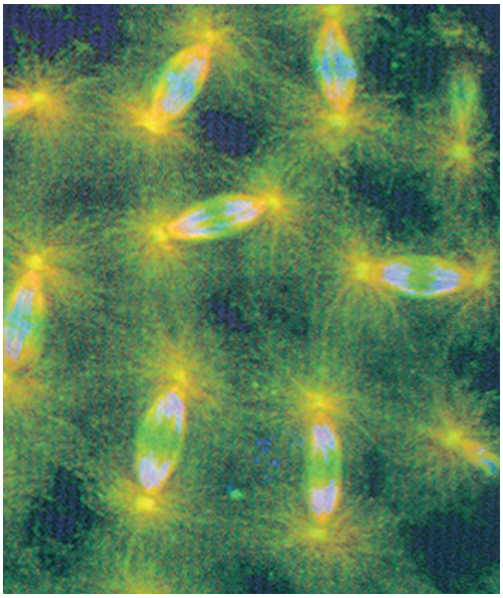
Kinesins for moving chromosomes

After all the years of searching, could the hunt for an anaphase motor be over? Greg Rogers did not make such a direct claim, but the possibility was the unspoken subtext of his talk about Kin I kinesins in flies.

These unusual kinesins can barely lay claim to being motors—their primary activity is to degrade microtubule ends rather than to walk over intact microtubules. But the mitotic spindle has been increasingly portrayed as a restless structure where tubulin subunits are constantly coming and going from microtubule ends. Now, Rogers and David Sharp (Albert Einstein College of Medicine, Bronx, NY) are proposing that the turnover of microtubules by Kin I kinesins is responsible for the majority of chromosome movement in anaphase.

The destruction in question occurs at both kinetochores and spindle poles. Chewing of microtubules at spindle poles pulls microtubules toward the pole in a movement termed flux. And chewing at the kinetochore results in a Pac-Man movement of chromosomes along microtubules.

Rogers used antibody injection to knock out the function of two Kin I kinesins in fly embryos. Flies lacking KLP10A no longer showed flux of marked microtubules from chromosome to pole, but still managed to get their chromosomes to move more slowly along the static microtubules to reach the pole. And flies lacking KLP59C appeared to lack the Pac-Man mode: they retained flux and moved to the pole at the flux rate, but no faster. The kinesins were found in the expected places, with the proposed flux motor KLP10A predominantly at the pole, and the Pac-Man motor KLP59C at the kinetochore.



Vale

Kinesins that chew up spindle microtubules might move chromosomes.

The Sharp group has previously claimed that dynein helps in anaphase chromosome movement—possibly by feeding microtubules into the Kin I destruction machines. But this and the newer Kin I claims are controversial because the motors also help to construct spindles, and defective spindles could explain the chromosome movement defects. The Sharp group feels, however, that they have uncovered a valid function of Kin I in chromosome movement. Their success may be based on the existence of two partially redundant mitotic Kin I's in flies rather than the single Kin I present in mammalian cells. WW

Reference: Walczak, C.E., et al. 2002. *Curr. Biol.* 12:1885–1889.

Microtubules nose around adhesions

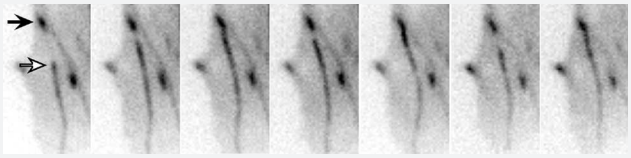
Irina Kaverina and Victor Small (Austrian Academy of Sciences, Austria) reveal that microtubules get up-close and personal with surface adhesions in migrating cells.

The importance of microtubules for cell polarity and migration has been previously demonstrated by Small's group. Using conventional fluorescence techniques, Kaverina and Small have shown that microtubules are targeted to actin-based substrate adhesion sites. This targeting is essential during cell migration because it somehow relaxes adhesions, in particular at the rear to promote tail retraction. In the absence of microtubules, strong cell contact sites form evenly around the cell edge, inhibiting directional migration. Migration can be restored by relaxing adhesions on one side of a cell using a myosin inhibitor.

The group has now taken a closer look at microtubule–adhesion interactions. Total internal reflection fluorescence microscopy shows that microtubules make frequent, repetitive trips to within nanometers of an adhesion, close enough to deliver proteins that could influence adhesion turnover.

The tips of multiple microtubules polymerized toward adhesions along what appeared to be tracks on the membrane. Kaverina has previously shown that tension promotes microtubule polymerization. Kaverina and Small currently hypothesize that the tracks represent tension-sensitive actin filaments that stimulate microtubule growth. They are currently looking for adaptor proteins, possibly a nonconventional myosin, that link microtubule tips and actin. NL

Reference: Kaverina, I., et al. 2002. *J. Cell Sci.* 115:2283–2291.



Small/Elsevier

Microtubules (white arrow) repeatedly approach (left to right) adhesion sites (black arrow).

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Magnetic bacteria

Bacteria that act like miniature bar magnets could shed light on prokaryotic organelle identity. This was the proposition of Arash Komeili, who, with Dianne Newman (California Institute of Technology, Pasadena, CA), is working on a strange bacterium called *Magnetospirillum* sp. AMB-1.

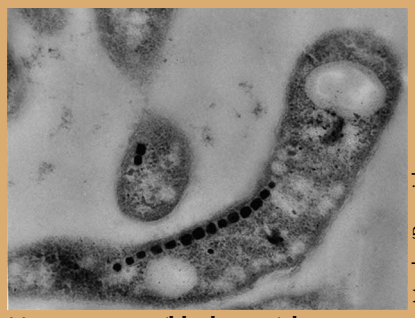
This aquatic bacterium lives near sediments, and thus its ideal environment (with just enough oxygen and nutrients) tends to be an entire layer rather than a single spot. This allows the bacterium to simplify its movements to up and down—a one-dimensional search problem—rather than wandering around in the confusion of three-dimensional space.

The bacteria can stick to up and down because of magnetism. After the bacteria take up iron, they pack it into specialized organelles called magnetosomes, and convert it into a naturally magnetic iron oxide crystal called magnetite. A chain of lipid-coated magnetosomes lines one side of the bacterium, and it aligns the bacterium in the earth's magnetic field.

Study of these bacteria has focused on the biomineralization, but Komeili is interested in the cell biological problems of establishing organelle identity, size, and number. "This thing has all the features of a eukaryotic organelle," he said.

The first magnetosome proteins to be identified have come from magnetic purification of magnetosomes. Komeili's approach is to use genetics. In his first screen he isolated bacteria that were no longer pulled to one side by a magnet. In future screens he may look for more specific phenotypes such as increased or decreased number or size of magnetosomes.

Early evidence suggests that a lipid structure can exist in the absence of magnetite. Affinity-based isolation of such structures may lead to in vitro reconstitution of iron import and magnetite synthesis. Meanwhile, Komeili's first batch of mutants has led him to a fascinating array of gene products, including an iron transporter, a two-component regulator, proteases, and a bacterial actin homologue that might line up along one side of the cell. ww



Moyles/Beveridge

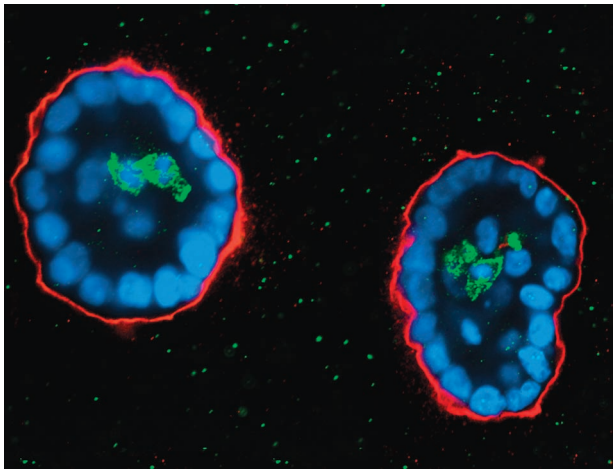
Magnetosomes (black spots) keep bacteria pointing in one direction.

Reference: Okamura, Y., et al. 2001. *J. Biol. Chem.* 276:48183–48188.

Deathly cavities

The creation of a hollow ball of cells, which may be a model for the formation of acini in breast tissue and alveoli in lungs, involves regulated cell death, according to a group including Jayanta Debnath, Kenna Mills, Mauricio Reginato, and Joan Brugge (Harvard Medical School, Boston, MA).

The group cultures breast-derived epithelial cells to generate the balls of cells. Debnath recently reported that the cultures show polarization of the outer cells followed by apoptosis of the inner cells. A combined boost in cell proliferation and block in apoptosis is necessary to prevent normal morphogenesis and fill the resulting lumen. Such a combination can only be provided by certain oncogenes, such as Erb2—the oncogene most often associated with an early form of breast cancer called ductal carcinoma in situ (DCIS).



Brugge

Caspase-3 staining (green) indicates that central cells are undergoing apoptosis.

But how, in the normal cultures, is apoptosis restricted to the central cells? Mills and Reginato reported at the meeting that early apoptosis requires the proapoptotic protein Bim. Bim was turned on at the time of cell death but was present in all cells, suggesting that some signal must render it inactive in the outer cells.

Reginato found a possible source of that regulation. He reported that cells in suspension, which now lacked contact between extracellular matrix (ECM) and cell surface integrins, failed to display growth factor receptors on their surface and thus did not shut off Bim. This death pathway may parallel the fate of the ECM-deprived inner cells in the acini. The outer cells, by contrast, have abundant contact with the ECM, which should allow them to turn off the death signal from Bim. ww

Reference: Debnath, J., et al. 2002. *Cell.* 111:29–40.

Pushing out the gut

Sally Horne-Badovinac and Didier Stainier (University of California, San Francisco, CA) have discovered an unexpectedly physical means by which the zebrafish gut loops, thus breaking the left–right symmetry in the early embryo.

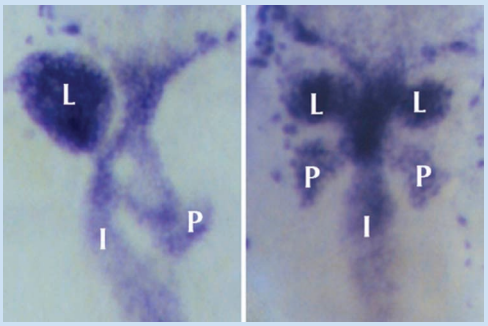
Their studies began with a zebrafish mutant, *heart and soul* (*has*), which was isolated in screens for heart and retinal defects. They found that the gene encoded atypical protein kinase C lambda (aPKCλ). This protein is, with Par-3 and Par-6, part of a polarity complex that is found at both adherens and tight junctions near the apical membranes of polarized epithelia. Thus, it was not surprising that various epithelia in the mutant showed incomplete polarity and problems with spindle orientation and adherens junctions.

But there was one phenotype that was unexpected. Instead of the normal organization of digestive tract organs—a gut that looped to the left, and a liver on the left and pancreas on the right—the mutant had a gut that remained on the midline and liver and pancreas in symmetrical positions.

The researchers speculated that the aPKCλ was turning on some unknown signaling program in the surrounding mesodermal tissue. The involvement of the entire Par complex seemed unlikely, as the mesodermal tissue is thought to be an unpolarized mesenchyme.

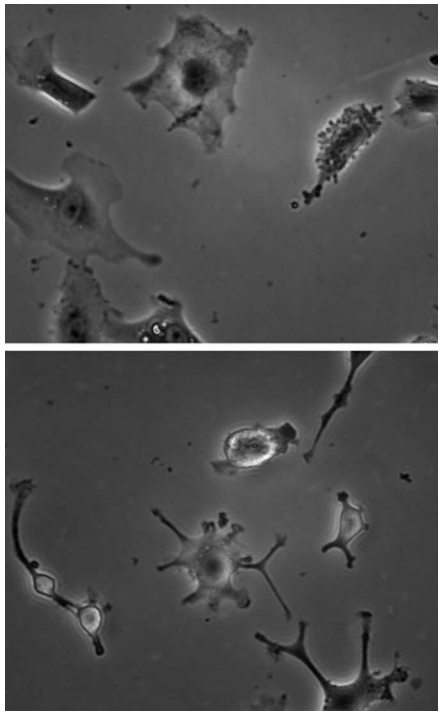
But now Horne-Badovinac has found that the aPKCλ is required for the correct behavior of two groups of columnar, polarized cells in the lateral plate mesoderm (LPM). These two groups of cells start off on either side of an endodermal rod, which will later become the gut. As the rod compacts and gets rounder, the LPM moves toward the middle, with one group of LPM cells migrating dorsally and the other ventro-laterally. The ventro-lateral migration pushes against the endodermal rod, thus displacing it to the left. In the *has* mutant, however, there may be a problem in establishing the polarity of the LPM, and both groups of LPM cells migrate dorsally, leaving the endodermal rod untouched. WW

Reference: Horne-Badovinac, S., et al. 2001. *Curr. Biol.* 11:1492–1502.



Stainier/Elsevier

Intestinal looping (I) and asymmetry of the liver (L) and pancreas (P) is lacking when aPKCλ is absent (right).



Huttenlocher/ASBMB

Chemotaxis is disturbed in neutrophils that lack calpain activity (bottom).

The source of the calpain

Two presentations highlighted the importance of calpain, a calcium-dependent protease thought to relax focal adhesions at the rear of the cell during cell motility.

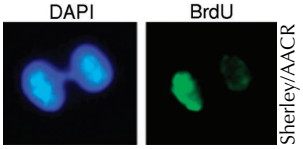
Kien Tran and Alan Wells (University of Pittsburgh, Pittsburgh, PA) suggested a simple method by which cells keep calpain turned off at the front of fibroblasts, where adhesion is necessary. They argue that cells localize calpain activity by limiting phospholipase C-gamma (PLCγ) to the front of the cell. PLCγ, which is activated at the front by Cdc42, depletes PIP2 in the lamellipod. PIPs have been shown to aid in calpain activation in vitro, and the PIPs and calpain are now shown to associate in vivo. Thus, the low levels of PIP2 in the lamellipod may limit calpain activity at the leading edge. The absence of activated PLCγ in the rest of the cell, however, means PIP2 is available for calpain activation to promote de-adhesion at the rear.

Mary Lokuta and Anna Huttenlocher (University of Wisconsin, Madison, WI) showed that neutrophils may also use gradients of calpain activity for directed migration. Lokuta showed that calpain is constitutively active in resting neutrophils. Inhibiting calpain throughout the cell initiated random migration patterns, but the cells ignored usually attractive chemotactic agents. Her results suggest that gradients of attractants such as IL-8 elicit directed migration because they locally down-regulate calpain where the cell contacts the attractant. IL-8 activates PI3K and could therefore inhibit calpain by depleting PIP2 at the leading edge. NL

Reference: Dourdin, N., et al. 2001. *J. Biol. Chem.* 276:48382–48388.

Immortal DNA

Stem cells have a lot of dividing to do—a burden that could lead to an unacceptable level of replication errors being introduced into and propagated by stem cell DNA. Stem cells respond, say Joshua Merok, James Sherley, and colleagues (Massachusetts Institute of Technology, Cambridge, MA), by retaining the same parental strand of DNA over many successive generations. This immortal DNA strand functions as an error-resistant master copy.



Immortal DNA marked with BrdU stays in stem cells.

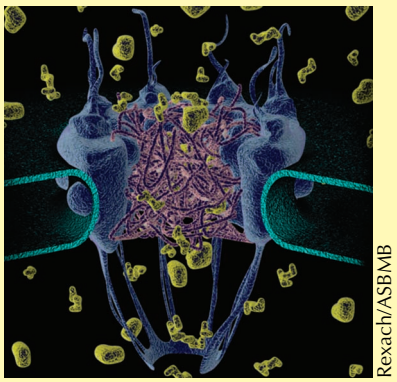
The idea of immortal DNA strands is not new, but in vivo efforts at proving the theory were equivocal—a supposedly immortal strand could simply be a slowly dividing stem cell. Sherley's group chanced upon an in vitro system to study the problem. Sherley's initial aim was to see the effects of restoring expression of the tumor suppressor p53 in an immortalized p53-null fibroblast cell line. Unexpectedly, the cells slowed from exponential growth because each division was now producing one stem cell and one differentiated cell.

Once this asymmetric division pattern was initiated, an immortal strand phenomenon became evident. One strand of DNA labeled with bromodeoxyuridine (BrdU) segregated with the stem cells, not the differentiated daughter cells, through multiple cell generations. As a template for conservation, the cell singled out the DNA strand that was replicated most recently before the switch to asymmetric divisions.

Sherley hypothesizes that immortal strand mechanisms may help to prevent stem cells from becoming cancerous. But eventually nonreplicative damage to the immortal DNA strand may catch up with the stem cell and help to drive it into senescence. Sherley's next focus is to isolate immortal strands based on the greater density of BrdU-labeled DNA, so that he can determine what marks a DNA strand as immortal. WW

Reference: Merok, J.R., et al. 2002. *Cancer Res.* 62:6791–6795.

No structure is good structure



Unstructured FG Nups (purple) form a flexible barrier to the nuclear pore.

The nuclear pore is guarded by a meshwork of flexible filamentous proteins, according to Dan Denning and Michael Rexach (Stanford University, Stanford, CA).

Denning characterized a class of nucleoporins distinguished by phenylalanine-glycine repeats (FG Nups). He found that at least one of these proteins in yeast, Nup2p, is a natively unfolded protein—a large, flexible, loosely arranged protein. Several lines of evidence support Denning's conclusion. Nup2p is rich in amino acids that predict a disordered secondary structure—charged, polar residues that tend to be on the surface of globular proteins. Nup2p has a large radius, as measured by in-gel filtration and sedimentation assays. Finally, it is highly sensitive to protease K digestion, even when in contact with its binding partners in isolated nuclei.

Recently, Denning has has found that several other yeast FG Nups are also natively unfolded proteins. He estimates that as much as 30% of the total mass of the yeast nuclear pore complex consists of unstructured proteins. It is not yet clear why the pore proteins need to be so flexible. However, Denning hypothesizes that the FG Nups may form a flexible mesh-like barrier that excludes large proteins (>30 kD) from diffusing through but can be easily pushed aside by cargo-carrier complexes as they are actively transported into or out of the nucleus. NL

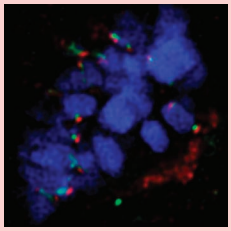
Reference: Denning, D., et al. 2002. *J. Biol. Chem.* 277:33447–33455.

The APCs of colon cancer

Inke Näthke (University of Dundee, UK) proposes that mutations in a human tumor suppressor may promote tumors in part by slowing down migration.

Over 80% of colon tumors are mutant for the multifunctional Adenomatous Polyposis Coli (APC) protein. One function of APC is to target β-catenin for degradation and thereby down-regulate cellular proliferation genes. Loss of this function has been thought to be critical for tumorigenesis.

However, APC is also a microtubule-binding protein found at kinetochores and has been associated with F-actin. New evidence indicates that NH_2-terminal fragments of APC similar to those expressed in colon cancer cells might promote cancer by affecting the actin cytoskeleton.



Mutations in the kinetochore (green) protein APC (red) are common in colon tumors.

Näthke's group is using an unusual system to study APC—*Dictyostelium*, a good model for cell migration studies. Although *Dictyostelium* does not appear to have an APC homologue, Näthke turned to the system because the slime mold has APC-binding proteins similar to those in human cells. Thus, it may turn out to be a good APC null system.

Sure enough, expression of NH_2-terminal APC fragments in *Dictyostelium* disrupted polarization and prevented directed migration. Expression of the fragments in chick embryos also interfered with developmmental cell migration.

The significance of these findings for colon cancer has yet to be established. But colon epithelial cells normally migrate from the crypt toward the gut lumen, so APC truncation mutations might cause cells to linger in toxic environments that they normally avoid. This may be a recipe for acquiring additional mutations that could lead to metastasis. NL

Reference: Mogensen, M., et al. 2002. *J. Cell Biol.* 157:1041–1048.

Expanding horizons

Andrew Murray has made his fair share of discoveries in chromosome segregation and mitosis, but he has no illusions about the progress of biological science. "We're constitutively optimistic," he said in his keynote address. "As a result of that, we overestimate how much we understand. We don't know nothing yet."

Murray (Harvard University, Cambridge, MA) was speaking about his hope that genomics will save us from the era of "one graduate student, one gene, one PhD." That era has supplied us with many gene names (an "alphabet soup" based on a "molecular fetish"), but often not much understanding of the overall process. "Underlying principles can be far removed from the components," he said. For example, the basis for mitotic spindle construction is the stabilization of a subset of initially random events—the outgrowth of microtubule polymers. That concept is not clear from the gene sequences of α and β tubulin.

Genomics allows researchers to study many components at the same time, but still this may not be enough. Murray cites evidence from artificially generated genetic networks that the output from a given network topology can differ depending on the individual rate constants used. "If that's true," he said, "then just working out the topology…is still not going to tell you the logical function of things."

Genome-scale approaches such as transcription profiling have been used by some as giant fishing expeditions. Murray agrees that "there's a role for exploratory rather than hypothesis-driven research. But to do interesting biology, by and large, you need a question you are trying to answer and you need to start at the top. Most of the things that we understand as concepts come from the top down."

He hopes that a top-down philosophy will encourage biologists to think big and tackle projects that have broad theoretical implications, rather than chipping away at one component after another. Top-down approaches also make modeling tenable. Many modelers start from the molecules. But this means that "usually there are fewer data points than there are unconstrained parameters," said Murray. A top-down approach replaces the molecules with a few phenomenological parameters—anything from voltage to flagellar length.

Murray

Andrew Murray is using cell biology and genomics to study evolution.

But in the end Murray wants to understand those complicated, molecule-rich webs. With 6,000 gene products, and up to 10 parameters (such as expression level, phosphorylation status, binding partners, and cellular location) controlling each, a given yeast cell is in 1 of 60,000 states. How does a cell make safe transitions between these states? Murray suspects that "there are not too many ways to hit the ball from the tee to the green without getting into the rough."

A cell also has to deal with multiple sensory systems detecting everything from nutrients and growth factors to adhesion molecules. The combinations and permutations of these inputs are numerous enough that "there is no time to explore all those combinations and adapt to them. So cells have probably evolved to respond to combinations they have never seen."

Evolution, says Murray, is the key to understanding these systems. He wants to connect cell biology and evolution, and to "bring the rigor of the best of genetics and molecular biology to experiments that address large, formal questions about evolution," such as what conditions allow sexual strains to out-evolve asexual strains. With any luck, his is not only a new way of studying evolution, but also one that will survive as the fittest. ww

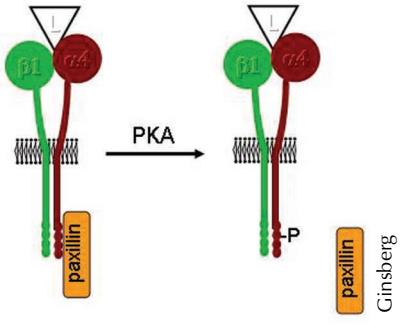
Reference: Hartwell, L.H., et al. 1999. *Nature.* 402:C47–C52.

A phosphate separates integrin from paxillin

Phosphorylation of an adhesion receptor must be spatially regulated to give directionality to a migrating cell, according to Lawrence Goldfinger and Mark Ginsberg (Scripps Research Institute, La Jolla, CA).

Ginsberg's group has been studying an apparent paradox of cellular migration—the effect of α4 integrin phosphorylation. Both constitutive phosphorylation and no phosphorylation of α4 block cell migration, even though phosphorylation has a clear effect in preventing the association of α4 and the adaptor protein paxillin.

Goldfinger now reports that α4 phosphorylation is restricted to the leading edge, where paxillin and integrin do not associate. Thus, paxillin may have a negative effect in the rest of the cell, rather than driving the cell forward at the leading edge. Indeed, forced association



Ginsberg

Phosphorylation of α4 integrin releases it from paxillin to allow formation of stable lamellipodia.

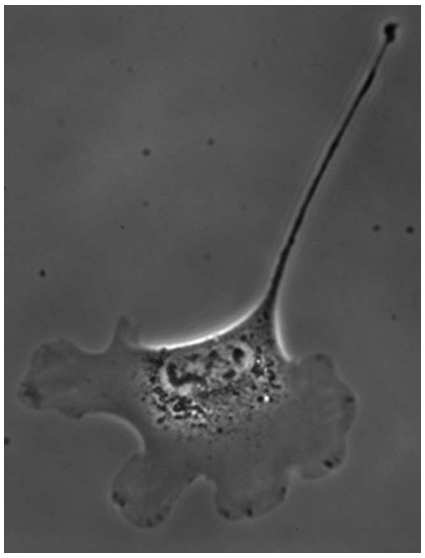
Lamellipodia lacking in 3D

The new way to look at migrating cells appears to be in three dimensions (3D). New studies, which are taking into account the fact that cells in vivo do not move in isolation across a flat plane, are hinting at differences that have been overlooked in the past.

Most in vitro studies of migrating fibroblasts are performed in two dimensions (2D), on matrix-coated glass plates. These studies have led to an emphasis on events occurring in the lamellipod, a prominent structure in cells migrating on slides, where actin stress fibers and focal adhesions form. But cells migrating in vivo probably rely on lamellipodia much less than has been suggested by in vitro experiments, based on results from Karen Beningo and Yu-Li Wang (University of Massachusetts, Worcester, MA) and from Gabriel Martins and John Kolega (State University of New York, Buffalo, NY).

Beningo sandwiched fibroblast cells between sheets of acrylamide, which allowed integrins on both top and bottom surfaces to bind. Like cells migrating in collagen gels, cells between acrylamide sheets had dramatically different shapes than cells migrating on only one surface. Most notably, cells with integrin receptors engaged on top and bottom surfaces lacked what are normally defined as lamellipodia. Instead, they stuck out long extensions and probed the environment much as a neuron does.

Fibroblasts growing in 3D have been shown to differ in the shape and constituents of their adhesions, lacking the distinct focal and fibrillar adhesions found in 2D. Beningo also noted differences in adhesions. In her system, cells grown in 3D were deficient in stress fibers, and the few focal adhesions that were seen were concentrated in small protrusions within the extensions. The acrylamide system is well suited to microscopy and should allow Beningo to evaluate how the differences in adhesions and cell shape affect migration.



Wang

Prominent lamellipodia like this may not be found on fibroblasts in vivo.

Martins has found that endothelial cells moving in three-dimensional collagen gels form long, tubular, branching protrusions, rather than a broad lamella. These cells relied more heavily on microtubules than did cells migrating on glass plates. When microtubules were disrupted, cells in the collagen gels were unable to pull themselves toward the protrusions that they extended, unlike cells cultured on slides.

Both studies are further warnings to biologists studying migration, and possibly other processes, that cells function in a three-dimensional world. Although simplified systems may be a convenient starting point, eventually more realistic systems are needed. NL

Reference: Cukierman, E., et al. 2002. *Curr. Opin. Cell Biol.* 14:633–639.

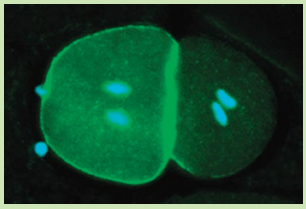
of α4 and paxillin throughout the cell—either by fusing the two proteins or by using a mutant α4 version that cannot be phosphorylated—inhibited migration by destabilizing lamellipodia. It is not clear how paxillin inhibits lamellipodial formation on the sides and back of cells. But, at the front of the cell, it may be protein kinase A that phosphorylates α4, allowing the front end to escape paxillin's negative effects. NL

Reference: Han, J., et al. 2001. *J. Biol. Chem.* 276:40903–40909.

Destroying symmetry

While most polarity researchers are busy finding mutants that destroy asymmetry, Shigeo Ohno has found a mutant that has an asymmetry not present in wild type. His results suggest that the worm proteasome destroys an early asymmetry-promoting factor.

Ohno, with Asuka Nishimura and Yuki Sugiyama (Yokohama City University, Yokohama, Japan), used RNAi to get rid of RPN-2, a component of the regulatory subunit of the proteasome. The second division of worm embryos is usually asymmetric in the posterior (thus eventually producing germ cells) and symmetric in the anterior. But the lack of RPN-2 resulted in two asymmetric divisions, and two sets of cells expressing markers of germ cell precursors.



Ohno

Worm embryos may use PKC-3 in the anterior (green, left) to regulate the proteasome.

Thus, it appears that the proteasome in the anterior usually destroys an asymmetry-promoting factor. Ohno also found that RPN-2 binds to PKC-3, which is part of a complex of polarity proteins in the early worm embryo. Thus PKC-3 may be affecting substrate selection by the proteasome, although this idea and the possible target of such action remain pure speculation. WW

Reference: Ohno, S. 2001. *Curr. Opin. Cell Biol.* 13:641–648.

Monopolar divisions

Mammalian cells with a single spindle pole can undergo a vigorous cleavage division, according to Julie Canman, Ted Salmon (University of North Carolina, Chapel Hill, NC), and colleagues.

Canman showed in earlier experiments that cells treated with nocodazole (to dissolve the spindle) and then injected with an antibody or mutant protein to disable the spindle assembly checkpoint were not capable of a normal division. The cells flailed around, contracting their cortex in random locations all around the cell. But they were not able to localize the contraction to a single site, as occurs in a normal cytokinesis.

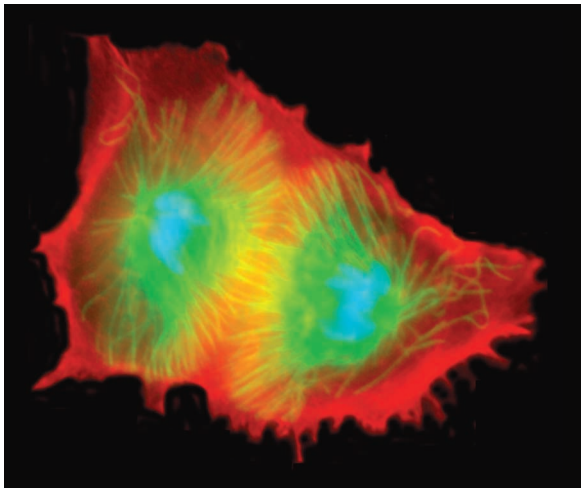
In the new experiments, Canman tricked the cells in two ways. First she treated them with the drug monastrol to prevent centrosome separation. The cells, which now had an asymmetric monopolar spindle, were then injected with a protein to disrupt the spindle assembly checkpoint, thus dislodging them from their prometaphase arrest.

The resulting cells cleaved on the DNA side of the spindle to produce one cell with and one cell without DNA. Thus, a bipolar spindle is not absolutely required for cytokinesis.

In Canman's system, it appears to be the DNA, via its stabilization of nearby microtubules, that directs the cytokinetic furrow to a single location. Others have shown, however, that DNA is also not absolutely required for cytokinesis.

The solution may be redundancy. Canman believes that furrow-forming factors may localize to equatorial microtubules either based on microtubule overlap (in bipolar spindles) or by transferring from DNA at the start of anaphase. Once on the microtubules, the factors would travel to the cortex to induce contraction. Canman now hopes to determine which factors are important by performing further antibody injections. WW

Reference: Canman, J.C., et al. 2000. *Curr. Biol.* 10:611–614.



Canman/Macmillan

Microtubules and DNA deliver a signal for cytokinesis.

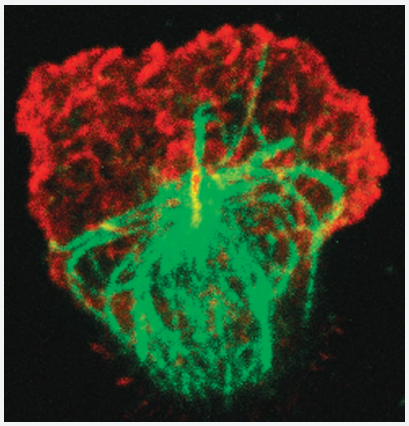
Microtubules point integrins in right direction

Moving neutrophils create a microtubule network to use for the recycling of integrins, according to results presented by Fred Maxfield (Cornell University, New York, NY).

Maxfield has previously demonstrated that integrins get recycled from the rear toward the front of migrating neutrophils. Blocking their release from attachment sites caused integrins to accumulate at the back of the cell. Integrin recycling is more important for neutrophils than for cells such as fibroblasts, which move slowly enough that they can leave integrins behind in the matrix and synthesize more as they crawl on.

Maxfield has now identified a regulated polarized microtubule network that neutrophils may use to move integrin-containing vesicles. Upon polarization, microtubules get swept toward the back of the cell, with their ends anchored at the MTOC, just behind the lamellipod. The final arrangement favors vesicle transport from the rear of the cell back toward the leading edge. Since microtubules do not first need to be disassembled, they apparently get swept back en masse. The intact microtubules are probably moved by actin–myosin, as the realignment was blocked by actin depolymerization and by myosin inhibitors.

Organization of the microtubule network probably depends on early establishment of lipid asymmetry, as has been shown in *Dictyostelium* and in neutrophils. Polarized neutrophils establish large-scale lipid asymmetry by using a myosin-dependent process to coalesce domains of detergent-resistant membrane toward the rear of the cell. Altering lipid organization by depleting cells of cholesterol prevented activation-induced polarity and migration by perturbing actin polymerization. NL



Maxfield/ASCB

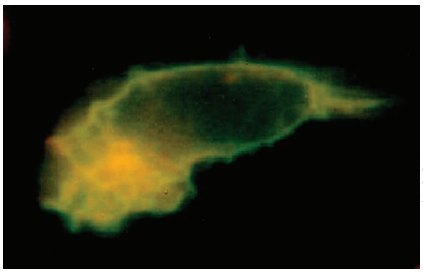
Microtubules (green) reorganize during migration to return integrins to the front of the cell.

References: Eddy, R., et al. 2002. *Mol. Biol. Cell.* 13:4470–4483.
Pierini, L., et al. 2003. *J. Biol. Chem.* 10.1074/jbc.M212386200.

Modeling movement

Neutrophil chemotaxis involves a zoo of proteins that influence actin polymerization. But Matt Onsum and Adam Arkin (University of California, Berkeley, CA) have come up with a way of modeling chemotactic movement without getting bogged down in the molecular details. Their results give a quantitative feel for how much cell shape and pathway cooperation contribute to directional movement.

Onsum was intrigued by an observation from Henry Bourne (University of California, San Francisco, CA) that neutrophil chemoattractant receptors are not actively concentrated at the front of the cell, but enriched there due to the accumulation of membrane in protrusions and ruffles. He derived a mathematical model for this process given the constraints of finite membrane area. The optimal shape for sensing a shallow chemical gradient turned out to be very close to the shape that neutrophils naturally assume—a kind of teardrop, with the fat end forward.



Bourne/ASCB

Chemotactic receptors (green) and the membrane (red) are equally localized to the front of a moving neutrophil.

This shape is not necessarily assumed by the cell to optimize chemotaxis, says Onsum, but it must be taken into account when calculating the amplification of a chemoattractant gradient. In addition to shape, another source of that amplification may be a positive feedback loop discovered by Bourne in which lipid mediators activate Rho GTPases that then increase the production of the lipids.

These forms of amplification should be site selective if the membrane generation and lipid diffusion remain local. Onsum therefore wants to know how local cooperation during pathway activation affects overall behavior. To simplify the system, he models the activation as occurring at the receptor level, so that an active receptor activates its immediate neighbors and inactivates those more distant. In the real world, however, the cooperation could occur at any level of the pathway.

In a regular chemotaxis assay, the neutrophils with cooperation fail at lower levels of noise, because they more often fixate on an erroneous direction that comes from the noise. But neutrophils normally operate in environments filled with obstacles (such as other cells), all of which shield the source of the chemotactic signal. When Onsum simulated such an obstacle-filled field, the neutrophils without cooperation oscillated as they lost signal due to a barrier, whereas those with cooperation plowed ahead with their original trajectory. Thus, for a neutrophil, blind determination might be the best strategy. WW

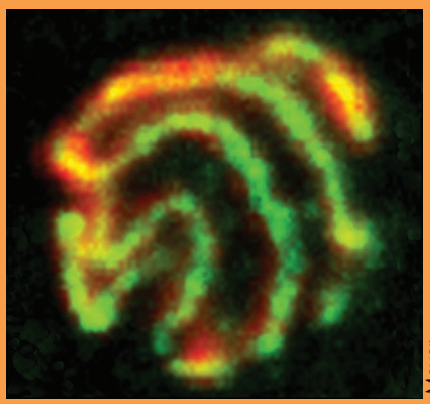
References: Servant, G., et al. 1999. *Mol. Biol. Cell.* 10:1163–1178.
Weiner, O.D., et al. 2002. *Nat. Cell Biol.* 4:509–513.

Circadian glue

Relatives of a protein known to regulate circadian timing may have a more ancient function during cell division, according to new results from Ray Chan and Barbara Meyer (University of California, Berkeley, CA). In both cases, the proteins seek the company of chromosomes.

The clock protein is Timeless (Tim), a regulator of circadian rhythm in flies. Tim binds to Period (Per) in the cytoplasm, and the Per–Tim complex is transported into the nucleus. There, the pair represses their own transcription as well as that of other clock genes.

Now, a close relative of Tim has been found in an unexpected place—the cohesin complex that glues chromosomes together before anaphase onset. Meyer's group was purifying cohesin-binding proteins and found the worm Tim homologue, TIM-1. Disruption of TIM-1 by RNAi or mutation interfered with meiosis and mitosis in a manner similar to cohesin defects. In the absence of TIM-1, the complete cohesin complex failed to localize to chromosomes.



Meyer

It looks like a clock gene, but TIM-1 is part of the cohesin complex (green) that holds chromosomes (red) together until anaphase.

Although a DNA-binding ability of the Tim family cannot be excluded, Timeless and its homologues have not been shown to bind to DNA. Possibly, Tim family members rather act as scaffolding proteins in DNA-binding complexes.

Mice have a TIM-1 homologue that may function in cohesion, but the Per-binding function is performed by cryptochromes. Thus, the cohesin function of the Timeless-like family appears to be more ancient, with only one of the two fly Timeless-like proteins (Timeless itself) containing a unique Per-interacting region to regulate circadian genes. But even the original Tim function is clock-like: it holds chromosomes together until the time is right for their separation. NL

References: Campbell, J., and O. Cohen-Fix. 2002. *Trends Biochem. Sci.* 27:492–495.
Young, M. 2000. *Trends Biochem. Sci.* 25:601–605.