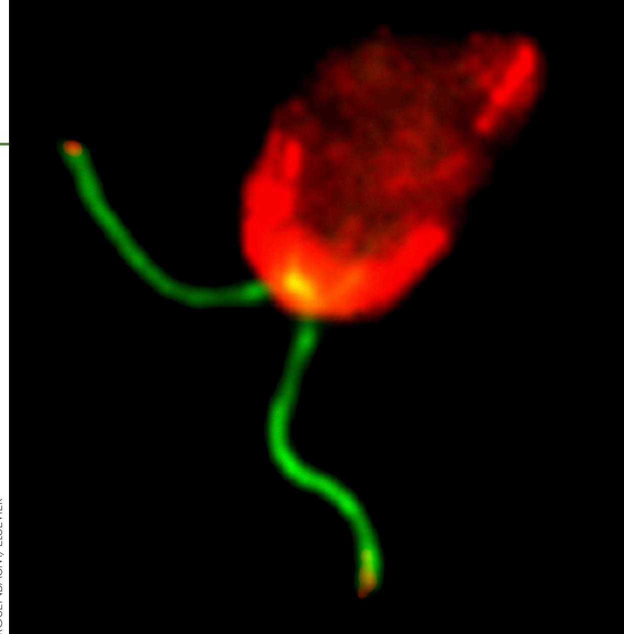
Meeting Report

IFT172 turns traffic around



ROSENBAUM/ELSEVIER

EB1 (red) is found at the tips of flagella.

Flagella and cilia are constantly turning over proteins at their tips. Assembly at the tip and removal of turnover products require the intraflagellar transport (IFT) system, which uses axonemal microtubules as tracks to drop off flagellar tip proteins and return turnover products to the cell body. The change in IFT direction at the tip was discussed by Lotte Pedersen (Yale University, New Haven, CT), whose work implicates IFT172 as an orchestrator of IFT traffic at flagellar tips.

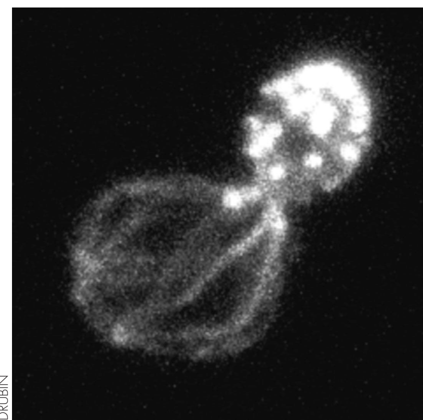
Along with different cargo, the two IFT directions come with different motors—kinesin-2 brings the IFT complex and its cargo out to the tip, and cytoplasmic dynein 1b/2 drives retrograde transport back to the cell body. Axonemal microtubules point their plus ends toward flagellar tips, so Pedersen thought perhaps plus-end tracking proteins might be involved in IFT turnaround at the tip.

Sure enough, she and coworkers found the algae version of the microtubule plus-end tracking protein EB1 out on flagellar tips. This localization was disturbed in a temperature-sensitive IFT mutant called *fla11^{ts}*. These mutant flagella accumulated IFT particles at the tips, as though the transport machinery was unable to head back into the cell body.

Pedersen and colleagues have now found that *FLA11* encodes an IFT complex protein called IFT172. They see that IFT172 and EB1 interact, but only when IFT172 is not bound to some of the other IFT proteins. Pedersen hypothesizes that IFT172 helps to link the IFT machinery to cytoplasmic dynein 1b/2. "At the tip," she says, "when IFT172 encounters EB1, we think it binds to EB1, and somehow that promotes the reorganization of the IFT particle." This reorganization may switch the particle from kinesin- to dynein-mediated transport. Since Pedersen still needs proof that EB1 is involved, she plans to use RNAi to get at its flagellar function. **NL**

Reference: Pedersen, L.B., et al. 2005. *Curr. Biol.* 15:262–266.

Actin-powered endocytosis in real-time



DRUBIN

Fluorescent phalloidin reveals yeast actin cortical patches and cables.

Although it is known that actin function and turnover are essential for endocytosis in budding yeast, the order of events remains elusive. David Drubin (University of California, Berkeley, CA) presented a sharper picture of actin's role using two-color, real-time fluorescence microscopy.

The beauty of the system is that one can light up endocytic proteins and actin and watch an endocytic patch being born, invaginating, and moving off into the cytoplasm. Additionally, yeast endocytic mutants can be screened using this system for any spatial or temporal problems caused by a protein's absence.

In a previous study, the group localized an endocytic receptor and its cargo to actin cortical patches, thus providing the first direct evidence for the patches as sites of endocytosis. By hooking GFP variants to actin, Arp2/3 activators, actin binding protein-1, and endocytic adaptor proteins Sla1 and Sla2, the group was able to order events roughly as follows: endocytic and actin-nucleating proteins are recruited to the site, actin is nucleated as the vesicle begins to form, scission occurs, and then an actin-powered vesicle moves into the cytoplasm.

Now the group, led by Marko Kaksonen and Chris Toret, is characterizing known endocytic mutants, especially those with mammalian homologues, to compare yeast and mammalian endocytosis. One yeast mutant, for example, gives a yo-yo phenotype in which the vesicle starts to move off the membrane before snapping back suddenly, perhaps an indication of the mutated protein's role in scission. Another mutant erupts volcano-like plumes of actin from the membrane.

The analysis should answer questions about actin's role in vesicle invagination, scission, and transport. "Until you do things in real-time," says Drubin, "you can't appreciate how intricate spatial and temporal regulation really are." **KP**

Reference: Kaksonen, M., et al. 2003. *Cell.* 115:475–487.

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Early sorting for membrane proteins

Soluble, cytoplasmic proteins can be localized by controlling the location of their mRNAs, and thus the ribosomes that translate them. Polarized secreted proteins are thought, in contrast, to achieve their deposition after sorting in the trans-Golgi, where they are directed into apical- or basolateral plasma membrane.

Secreted proteins, however, can also be localized via precise mRNA placement. Catherine Rabouille (University Medical Centre Utrecht, Netherlands) showed that, in fly oocytes, these proteins undergo a quick exit from the ER and local transport to the extracellular space through the local exocytic machinery.

Fly oocytes do not have the usual Golgi ribbon found in mammalian cells; rather, their Golgi is separated into 1000 individual stacks, each associated with an ER exit site (tER) to form a tER-Golgi unit. The new results show that only tER-Golgi units that are near the *gurken* mRNA, located at the dorsal/anterior corner, secrete the protein at the same corner. Changes in mRNA location thus changed where the protein was made and secreted.

The ER is one big lumen, so Gurken must be prevented from diffusing into distant tER-Golgi units. Rabouille's group found that this is achieved by rapid export from the ER. When this export was blocked, Gurken was seen throughout the ER. Next is to determine whether other polarized cells, such as neurons, use a similar strategy for locating polarized secreted proteins. **NL**

Reference: Herpers, B., and C. Rabouille. 2004. *Mol. Biol. Cell.* 15:5306–5317.

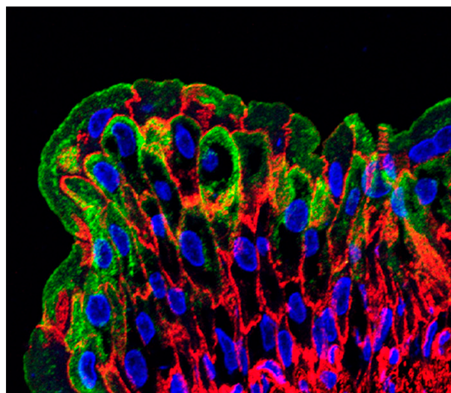
ATP-powered umbrellas

The bladder grows and shrinks thanks in part to umbrella cells—stretchy epithelial cells lining the inner surface of the organ. When the bladder expands, the umbrella cells increase their surface area by secreting lots of vesicles at their apical (or lumenal) membrane. Gerard Apodaca (University of Pittsburgh, Pittsburgh, PA) discussed his recent finding that ATP tells umbrella cells that it's time to expand.

ATP is released from umbrella and other epithelial cells in response to the pressure of a filling bladder, although the mechanosensory mechanism that lets out ATP is not known. Apodaca finds that this released ATP is the trigger for exocytosis in umbrella cells. "We can take away ATP and block exocytosis in stretched cells," he says, "or add ATP to stimulate exocytosis [without filling]."

ATP is sensed by ion channels in the umbrella cell plasma membrane. Umbrella cells from mutant mice lacking the P2X2 or P2X3 ATP-binding channels failed to undergo filling-induced exocytosis. Calcium was also needed, suggesting that ATP-bound receptors either channel calcium directly into the cell or let in other ions that then open calcium channels.

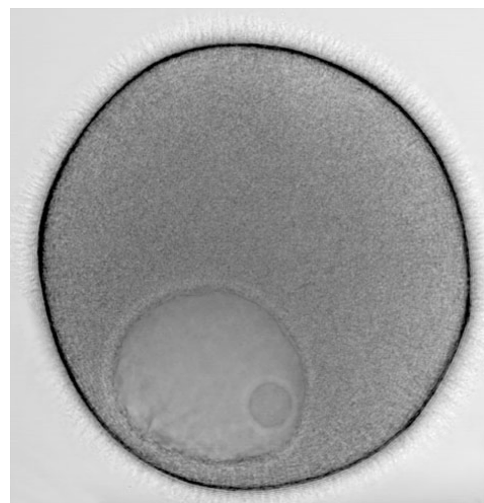
Nerves running to the bladder also have ATP receptors. ATP might thus serve two functions: to expand the umbrella cells, and to let the nerves know that the bladder is filling. **NL**



APODACA

Bladder umbrella cells (outermost layer) swell when ATP tells them to.

Reference: Apodaca, G. 2004. *Traffic.* 5:117–128.



ELLENBERG

The large size of the starfish oocyte means that actin has to help bring chromosomes to the spindle.

Actin herds DNA

Size matters for oocytes. After all, in animals, the oocyte must store most of the RNA and proteins that will drive early development after fertilization. According to Jan Ellenberg (EMBL, Heidelberg, Germany), the wide expanse of the egg nucleus poses "a fundamental problem of geometry [during meiosis]."

This geometric problem lies in the difficulty that spindle microtubules have reaching chromosomes in a large nucleus. Now, work from Ellenberg and Péter Lénárt suggests that actin helps to solve this problem in meiotic starfish oocytes.

Animal oocytes undergo an asymmetric division that sets the spindle near the surface of the animal pole. All chromosomes must therefore be pulled near the surface—some from the far side of the nucleus. But spindle microtubules are relatively short, due to their high dynamic instability. Lénárt and Ellenberg now find that microtubules are in fact too short to grab all the chromosomes in a starfish oocyte. "Over long distances," says Ellenberg, "it's the actin cytoskeleton that's doing the movement."

Actin seemed to form a meshwork with patches that attached to DNA. The net appeared at the time of nuclear envelope breakdown and then contracted toward the animal pole to bring the chromosomes within reach of spindle microtubules. Drugs that prevent actin polymerization caused chromosome losses, because the spindle captured only nearby DNA. Now, Ellenberg's group is looking for the polarity cues that control the direction of actin contraction. **NL**

Reference: Lénárt, P., et al. 2003. *Curr. Opin. Cell Biol.* 15:88–95.

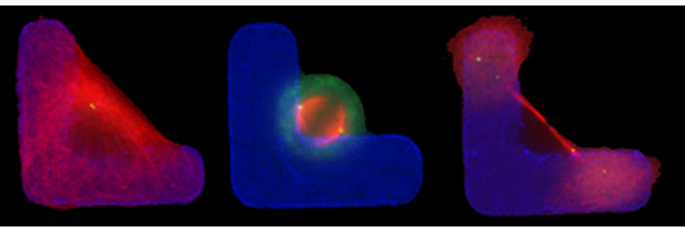
Spindles adhere to adhesion pattern

When an adherent cell divides, the cell rounds up for mitosis but the daughter cells spread back out to the original shape of the mother cell. Fine retraction fibers remain connected to the mother cell's adhesion pattern of secreted fibronectin and appear to control this phenomenon.

Now, Manuel Thery and Michel Bornens (Institut Curie, Paris, France) have presented an elegant experiment to show that the adhesion pattern directs much more than just daughter cell shape—it may also align the mitotic spindle.

By plating thousands of HeLa cells on coverslips coated with a fibronectin "stamp," the team could create cells in various shapes: rectangles, triangles, squares, and circles. After double-labeling cells for centrin and actin, the group used shape recognition software and video recording to measure automatically the orientation of division in relation to the long axis of actin cables.

It appeared that spindle orientation correlated with a cell's premitotic shape. But was this due to the overall cell shape or to the underlying adhesive pattern? Thery used a clever trick of "painting" fibronectin stamp patterns that would give similar shapes but with different adhesion patterns. For example, painted fibronectin in either a triangle or an "L" shape gives, in both cases, triangular cells, but with different adherence points and therefore different axes of actin cables.


BORNENS

Adhesion pattern (blue) directs spindle (red) orientation of a dividing HeLa cell. Green shows a rounded cell.

By analyzing these types of manipulations of the adhesion pattern, the group shows that adhesion, not cell shape, determines the spindle orientation. The spindle is parallel to the premitotic actin–vinculin network and the plane of cell division is perpendicular to it. "We are proposing," says Thery, "that it is really the internal forces of tension that drive spindle orientation," possibly through adhesion-dependent signaling during interphase. **KP**

Reference: O'Connell, C.B., and Y.L. Wang. 2000. *Mol. Biol. Cell.* 11:1765–1774.

Young blood revives old stem cells

Aging is marked by a decline in the ability of stem cells to regenerate tissues. But whether the decline is due to intrinsic properties of aged stem cells or extrinsic factors of an aged body remains unknown. Now, Irina Conboy (University of California, Berkeley, CA) presents evidence that, at least in the case of muscle, old stem cells just need some young blood to repair injured tissue effectively again.

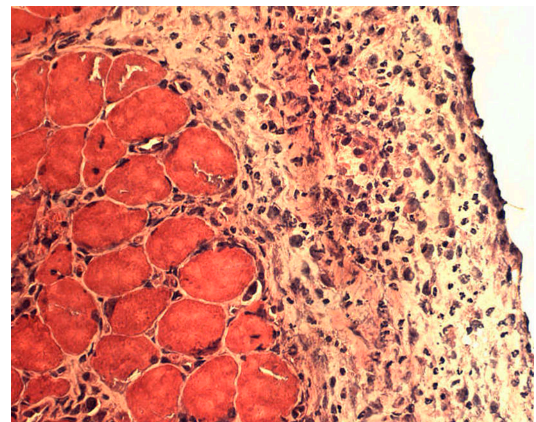
In work started at Stanford University (Palo Alto, CA) with Thomas Rando, Conboy had previously shown that mouse muscle stem cells, called satellite cells, are activated through the Notch signaling pathway. This pathway becomes inefficient with age but, by forced activation, Conboy could get aged satellite cells to repair tissue with the same enthusiasm as that shown by young satellite cells.

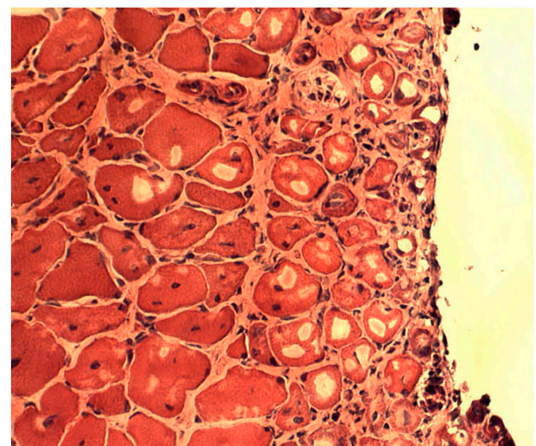
But were the stem cells losing their intrinsic ability to activate the Notch pathway, or was an extrinsic factor missing? Because satellite cells sit nestled in muscle tissue and respond to signals from nearby injured cells, the system is difficult to replicate in a cell culture or explant system.

In a collaborative effort, Conboy decided to use a parabiosis experiment—connecting the circulatory systems of a young mouse and an old mouse. "This made the system very clean," says Conboy. "The whole mouse is still old, and the only contribution from the young mouse is blood." In these pairs, an injury to the old mouse is repaired significantly better when young blood is supplied, and an in vitro test confirms that young blood serum restores Notch activation to old satellite cells.

Now Conboy has a head start on identifying the circulating molecules that change with age. And the parabiosis system, an experimental technique that has been around for hundreds of years, should prove useful for studying stem cell aging in other organs. **KP**

References: Conboy, I.M., et al. 2003. *Science.* 302:1575–1577.
Conboy, I.M., et al. 2005. *Nature.* In press.




CONBOY

Old muscle repairs poorly (top), but adding young blood ramps up its repair (bottom).

Traffic in the nanotube

The discovery in 2004 that PC12 rat neuroendocrine cells stay physically connected to each other by delicate membrane nanotubes raised a question: do the structures function in cell communication, or are they just a quirky biophysical property of membranes?

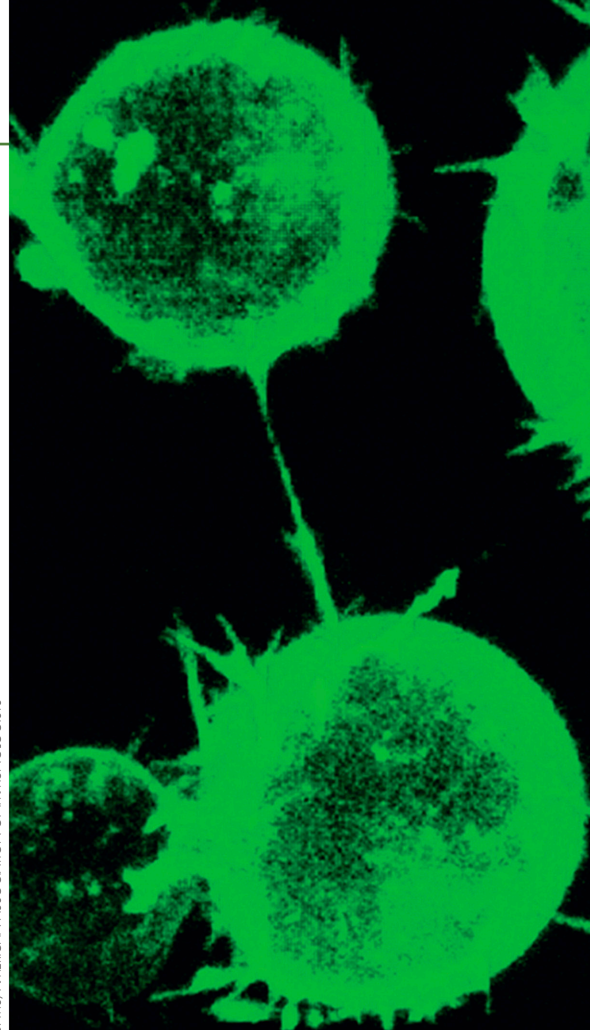
Daniel Davis (Imperial College London, UK) presented observations that argue that perhaps both viewpoints are correct. Davis's studies of the immune synapse often have him asking whether processes happening between neural cells also occur between immune cells. And so it did not take him long, scanning live human macrophages and lymphocytes, to find nanotube structures forming between cells after they had "kissed" at the immune synapse.

The structures can be up to 150 μm, or 10 cell diameters, long and can persist for hours. The Davis lab's most striking observation, however, was that both large and small bulges can be seen traveling along the tubes from one cell toward another. Some bulges are large clumps of cellular material that deform the nanotube membrane as they travel; others are small presumptive vesicles that move within the diameter of the nanotube.

By measuring the direction and speed of these smaller vesicles, the group shows that the vesicle moves stepwise and at a constant speed when in motion. And it appears that the tubes support bidirectional movement. This suggests that, rather than simple diffusion, perhaps traffic is directed along cytoskeletal components. However, no such fibers have yet been identified in the tubes connecting immune cells.

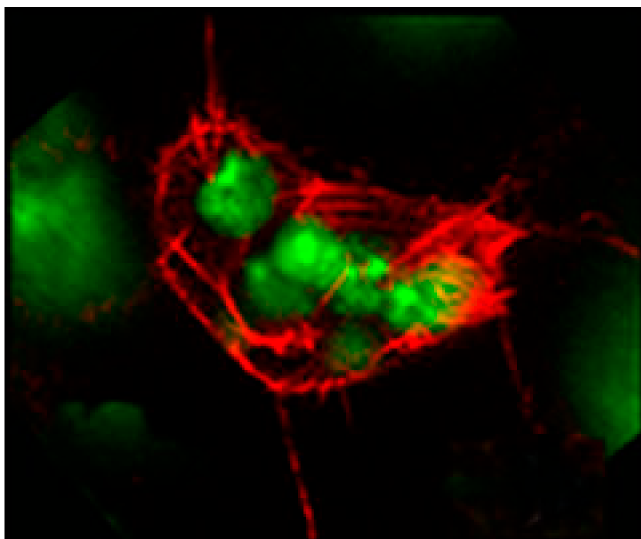
Indeed, Davis knows that much hypothesis-testing remains to be done. Skeptics are quick to point to the fact that nanotubes form between pure liposomes. But Davis notes that, even if the tubes are simply a result of membrane biophysics, that still leaves room for cells to have evolved ways to exploit this property for cell–cell communication. **KP**

Reference: Önfelt, B., et al. 2004. *J. Immunol.* 173:1511–1513.



DAVIS/AMERICAN ASSOCIATION OF IMMUNOLOGISTS

A nanotube stretches between human B cells.



RØRTH/ELSEVIER

Border cells (green) use endocytosis to keep RTK signaling localized.

Putting signals in their place

Active receptor tyrosine kinases (RTKs) are endocytosed and either recycled to the plasma membrane or sent to degradative pathways. Pulling the RTKs off the membrane is one way to turn down their signaling volume. But Pernille Rørth (EMBL, Heidelberg, Germany) is finding that endocytosis also contributes to localizing RTK signaling to direct cell migration.

The migration of border cells in the fly is controlled by two RTKs called PVR and EGFR. By sensing gradients of the PVF1 and Gurken ligands, respectively, the RTKs direct the border cells from somatic tissue to the oocyte. Rørth showed that overactivating or dampening RTK signaling had little effect on migration. Mutations that blocked RTK endocytosis, however, impaired migration.

In some systems, endocytosis brings receptors to an appropriate intracellular signaling compartment. But genetic experiments have shown that this is not the case for border cell guidance. Rørth finds that endocytosis affects the spatial extent, not the level, of signals downstream of the RTKs. "What is important here is not regulating receptor levels," suggests Rørth. "It is ensuring that the signals stay localized. [For guidance,] cells need to measure where the signals are to find the front of the cell. They are not interested in what the levels of the signal are."

Rørth hypothesizes that endocytosis brings active receptors to an RTK hot spot. The cell could thereby "exaggerate small differences in ligand concentration between its front and back, and make it into an all-or-none thing—in other words, polarize," she says. **NL**

Reference: Duchek, P., and P. Rørth. 2001. *Science.* 291:131–133.

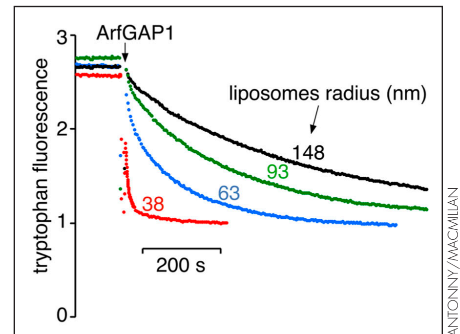
How to hug the curves

Membrane curvature can be sensed via BAR domains, whose banana-like shape and basic residues fit nicely over rounded acidic membranes. But Bruno Antonny (CNRS, Valbonne, France) showed that the vesicle budding protein ArfGAP1, which lacks a BAR domain, has found another way.

Antonny's group previously showed that ArfGAP1 is active only on membranes of high curvature. On budding COPI vesicles, ArfGAP1 helps Arf1 to hydrolyze its bound GTP, which in turn releases the COPI coat. ArfGAP1's ability to sense curvature is "a control in time and space for the budding vesicle," says Antonny. "If the bud is young, it is flat, and the coat has not finished its job," which is to induce more curvature. "As the buds get older, they are more curved, allowing access of the GAP."

A yeast homologue of ArfGAP1, Gcs1, is also sensitive to membrane curvature. Its activity ranges several fold in magnitude based on the size of the liposome presented to it. Some GAPs of this family are not sensitive to membrane curvature, so Antonny's group used sequence comparisons to help them find the region that imparts the curve-sensing ability.

Gcs1 and ArfGAP1 share a short, central region, which the group found was necessary and sufficient for preferential binding to highly curved membranes. The stretch contains a tryptophan that is critical to binding. Antonny supposes that curvature introduces spaces between membrane lipids that are wide enough to fit the tryptophan and other bulky, hydrophobic side chains in the region. **NL**

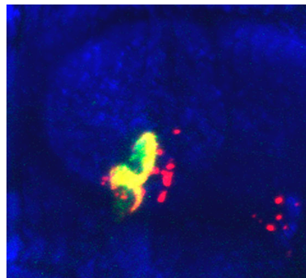


ArfGAP1 is more active on smaller liposomes.

Reference: Bigay, J., et al. 2003. *Nature*. 426:563–566.

MSP worms its way out of sperm

Worms waste no energy to mature oocytes if sperm are not around to fertilize them. The sperm themselves start the maturation process by sending out a signal to alert the oocyte to their presence. That signal is the cytoskeletal protein MSP—a sort of sperm version of actin that also helps mature sperm to move. David Greenstein (Vanderbilt University, Nashville, TN) presented new work describing the unusual delivery system worms use to send out MSP.



***C. elegans* sperm (yellow) send out MSP vesicles (red) to signal to oocytes.**

Eph receptors on the oocyte surface bind to MSP, which results in activation of a MAPK pathway that tells the oocyte to resume meiosis and ovulate. But how MSP gets out of the sperm and to the oocyte was rather a mystery. "Sperm lack ER, Golgi, and ribosomes [needed] for secreting proteins," says Greenstein. "MSP has no recognizable signal sequence, and there's no evidence that it's proteolytically processed." Instead, his lab is finding that MSP polymers may push their way out of the sperm.

Using high pressure–frozen samples for electron microscopy in collaboration with Kent McDonald (University of California, Berkeley, CA), the researchers spotted extracellular vesicles containing MSP sandwiched between two membranes (probably derived from the plasma membrane). Protrusions from which MSP escapes lacked a cofactor that is known to disassemble MSP filaments during motility.

The vesicles were labile, like time-release caplets of MSP. Immature sperm somehow made more stable vesicles, which makes sense for their time schedule—the young sperm conserve their MSP signaling and motility ability until the hermaphrodite matures. **NL**

Reference: Miller, M.A., et al. 2001. *Science*. 291:2144–2147.

Regulating sticky sisters

The anaphase promoting complex (APC) ubiquitin ligase, which regulates cell cycle progression, has two activators. Cdc20 turns on APC at the metaphase-to-anaphase transition so that it can degrade securin and promote sister chromatid separation. Then, after anaphase, Cdh1 activates APC to degrade Cdc20, setting up the cycle again for cohesion of the next sister chromatid pair. But for reasons unknown, Cdh1 stays active through late G1.

Susannah Rankin and Marc Kirschner (Harvard Medical School, Boston, MA) asked whether more Cdh1-dependent substrates of APC could be identified to elucidate other cell cycle mechanisms regulated by Cdh1.

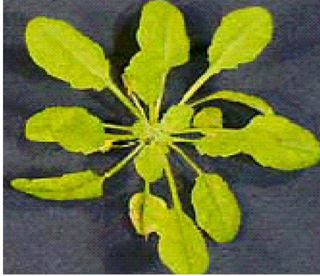
Using frog egg extracts, which lack endogenous Cdh1, Rankin screened ~60,000 in vitro expressed proteins for Cdh1-dependent degradation.

She pulled out an unknown protein that, on closer inspection, appears to regulate sister chromatid cohesion. When overexpressed, the protein, named sororin, leads to too much cohesion; when inhibited, the result is too little cohesion.

Rankin hypothesizes that sororin may be important for the establishment and/or maintenance of cohesion. If so, its degradation by Cdh1-activated APC during G1 would keep sororin levels low until after DNA synthesis, when chromatid cohesion would be established. If cohesion were around too soon, nonsister chromatids might be joined mistakenly. **KP**

Reference: Uhlmann, F. 2004. *Exp. Cell Res.* 296:80–85.

On guard in the plant cell



DANGL

Pseudomonas syringae, which causes leaf spot disease (top), can be thwarted by R genes (bottom).

About 125 A. thaliana R protein genes have been cloned based on their ability to confer resistance to pathogenic viruses, bacteria, fungi, nematodes, and aphids. The guard hypothesis of plant immunology holds that disease resistance proteins (R proteins) guard a limited number of key cellular machines to recognize when they are targeted by pathogen virulence factors. This recognition event activates the plant's defense response, resulting in restricted pathogen growth. Jeff Dangl (University of North Carolina, Chapel Hill, NC) presented progress toward identifying one such guarded protein complex in *Arabidopsis thaliana*.

By infecting plants with *Pseudomonas syringae*, Dangl's group has identified a complex of two R proteins, RPS2 and RPM1, and the protein they presumably monitor—the R protein interactor, RIN4. During infection, RIN4 can be modified by at least three bacterial proteins, including the cysteine protease AvrRpt2, which cleaves RIN4.

The Dangl lab now shows that the RIN4 cleavage product is detected by the plant to activate resistance, but is further manipulated by the pathogen so the pathogen can thrive in susceptible plants. Although this seems paradoxical, it actually fits the hypothesis—recognition of a modified host target alerts a resistant plant cell to the invader. A few R proteins can thereby recognize many different invaders who target the same cellular machinery.

Not surprisingly then, the RIN4 cleavage site domain is shared by other recognized proteins. In addition, all three bacterial effector proteins that target RIN4 are from different strains of *P. syringae*, showing convergence on the R protein complex.

As yet, it is unclear how RIN4 manipulations allow pathogens to survive in a susceptible plant. "Why does the cell have to guard RIN4?" asks Dangl. "It's guarded not once, but twice, and it is targeted [by pathogens] not once, but thrice." **KP**

Reference: Mackey, D., et al. 2003. *Cell.* 112: 379–389.

Unrest in the senescent cell

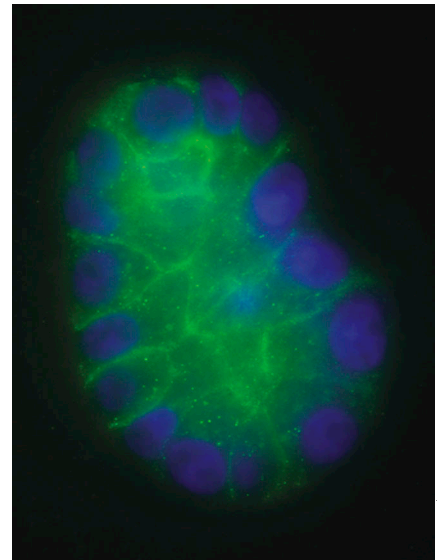
Senescence is one way for cells to be shut down, thereby avoiding the cancer risks that arise from continued mitosis in a multicellular organism. But Judith Campisi (Lawrence Berkeley National Laboratory, Berkeley, CA) now proposes that senescent cells are far from quiescent, secreting factors that alter their cellular neighborhood, which may be detrimental later in life.

Senescence is triggered by a multitude of cell damage or stress signals, and although senescent cells can no longer divide, many of them become resistant to apoptosis. The number of these cells thus increases, and in fact they have been found at sites of aging-related pathologies.

Campisi's group had previously shown that senescent cells could provoke nearby cells that had already initiated the cancer process into a full-blown malignancy. Senescent cells "are not functioning as normal cells in normal tissue," says Campisi. Now, the group has characterized the secretory profile of senescent human breast fibroblasts and found that the cells are pouring out chemokines, inflammatory cytokines, proteases, and growth factors. Why the cells do this is unknown, but one hypothesis is that they are locked in an activated wounding response.

The team has also shown that senescent fibroblasts mixed in vitro with breast epithelial cells from a pregnant mouse alter the epithelial cells, lowering their milk production and increasing their formation of branching structures. So senescent cells influence both normal and precancerous cells in their environment.

Perhaps as an organism ages, the higher percentage of senescent cells synergizes with mutation accumulation to drive late-onset cancers. "A balance exists between tumor suppression and aging," says Campisi. Early on, the tumor suppression mechanism predominates, but the later consequences of this may mean more tumors. "So the question," says Campisi, "is can we uncouple them?" **KP**



CAMPISI/COMPANY OF BIOLOGISTS

An alveolus of breast epithelial cells grown with senescent fibroblasts secretes less milk than one grown with presenescent cells.

References: Krtolica, A., et al. 2001. *Proc. Natl. Acad. Sci. USA.* 98:12072–12077.
Parrinello, S. et al. 2005. *J. Cell Sci.* doi:10.1242/jcs.01635.