Meeting Report

Nerves made to order


Odde

Pulling neurites with needles was the old, inefficient way.

By tugging on neurons, Joseph Fass, David Odde, and colleagues (University of Minnesota, Minneapolis, MN) have taken the first step in their bioengineering quest. The eventual goal, said Odde, is "to create engineered neural networks with defined connections."

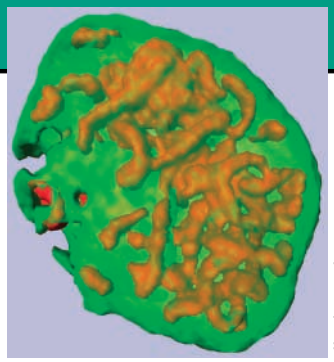
Neuron tugging is not a new sport, but the old experiments were done with glass needles that resulted in inconsistent forces and variable results. Fass switched to using an electromagnet that tugged on magnetic beads coated with integrin antibodies. This resulted in much more stable force production, allowing Fass and Odde to discover two novel features of induced neurite growth: a sharp transition between no outgrowth and relatively speedy outgrowth based on small increases in force, and transitions between neurite elongation and contraction at a fixed level of force.

Force-induced axon elongation occurs in vivo during growth of animals, but it can also be used to make in vitro networks. In a first step in this direction, the researchers dropped another nerve cell on top of an induced neurite, and saw long-lasting adhesions form between the cells. They have yet to show that this represents a functional synapse.

If synapse formation is succesful, Odde plans to use the system to test models of neural processing and the effects of disease symptoms on this processing. For now, he said, he will be content if he can "connect cell A to cell B." Eventually he hopes to start using a microfabricated version of his manipulator, which should allow him to manipulate thousands of neurons at once. WW

Reference: Odde, D.J., and M.J. Renn. 2000. *Biotechnol. Bioeng.* 67:312–318.

Tearing it up


Ellenberg/Elsevier

A large hole (left) initiates nuclear lamina breakdown.

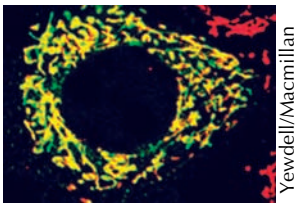
The initiation of mitosis is a violent event. Joël Beaudouin, Jan Ellenberg (EMBL, Heidelberg, Germany), and colleagues have found that microtubules tug on the nuclear envelope, creating folds and invaginations on the side nearest them, and rips on the far side. The result is a catastrophic beginning to nuclear envelope breakdown (NEBD) that allows rapid clearing of the nuclear lamina away from chromosomes and the forming spindle.

Beaudouin's new model, as he presented it at the meeting, is a departure from the two models previously put forward to explain NEBD. The mechanism underlying one of those models—phosphorylation-dependent depolymerization of nuclear lamins—may take over once the nucleus is breached, but Beaudouin did not see significant release of lamins before the large hole opened up. Others have proposed that microtubules poke holes in the nuclear envelope, but the newly observed holes appear opposite the site where microtubules interact with the nuclear envelope.

The nature of the microtubule–nuclear-envelope interaction remains a mystery. Beaudoin suggests that a motor, perhaps dynein, might interact with nuclear pore complexes. The complexes could then provide a link to the more rigid nuclear lamina. Pulling on the pores would produce the 10-μm invaginations seen in the nearby sections of the envelope, and the stretching observed on the opposite side. The same motors could then power the movement of envelope fragments toward the centrosomes, which the researchers saw after the opening of the hole. These movements clear the nuclear region to allow the formation of a spindle. WW

Reference: Beaudoin, J., et al. 2002. *Cell.* 108:83–96.

Flu's hidden virulence factor


Yewdell/Macmillan

A flu protein localizes to mitochondria.

The influenza genome was first characterized 20 years ago. Now a previously undetected 87 amino acid ORF, PB1-F2, has been discovered in frame 2 of the PB1 gene by Jonathan Yewdell and colleagues (National Institute of Allergy and Infectious Disease, Bethesda, MD). "We believe this is a groundbreaking finding," said Yewdell, "although we're not yet sure how deep the ground is."

Although the PB1-F2 peptide lacks any recognizable mitochondrial localization signals, it rapidly associates with both the inner and outer mitochondrial membranes. Minutes after the peptide has been added to cells—either by microinjection or direct addition to the culture medium—the cells show dramatic morphological changes. Mitochondria vesiculate, nuclei shrink, and cells round up.

All these activities have consequences: infection with wild-type virus induces apoptosis in 50% more cells than a comparable infection with mutant virus lacking PB1-F2. Daniela Malide, who presented the research, suggested one way in which PB1-F2 may have a proapoptotic effect. She said that the group has very preliminary data suggesting that PB1-F2 may induce the release of cytochrome *c*, a regulator of apoptotic cell death. RT

Reference: Chen, W., et al. 2001. *Nat. Med.* 7:1306–1312.

Rabiya Tuma and William A. Wells
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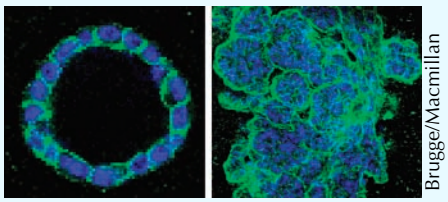
Death in the middle

A new three-dimensional (3-D) cell culture system is providing substantial clues about the differences between well-mannered, noninvasive breast epithelial cells and their malignant cousins, according to presentations from Joan Brugge's research group at Harvard Medical School in Boston, MA.

Brugge used this culture system to show that activation of ErbB2, a member of the EGF family of growth factor receptors, releases epithelial cells from a growth arrest, but activation of a closely related receptor, ErbB1, does not. These data were surprising because both receptors stimulate proliferation in a standard 2-D culture dish.



Brugge/Macmillan

ErbB2 makes mammary acini (left) go haywire (right).

A significant difference between the two culture systems appears to be the formation of proper basement membrane attachments. In the 3-D system, MCF10A breast epithelial cells form acinus-like structures typical of healthy glandular tissue. The acini have a single-layered ring of well-ordered epithelial cells surrounding a clear lumen. After activation of ErbB1, the basement membrane in this structure may provide some protection from abnormal proliferation, said Kenna Mills, a graduate student with Brugge.

But proliferation is not the whole story. ErbB2-expressing cells fill the lumen, a signature of early breast cancers, but cells expressing the strong oncogene HPV16 E7 proliferate but then die once they enter the lumen. Only with both E7 and an inhibitor of apoptosis does the lumen fill with cells.

Jayanta Debnath, who presented much of the work, concluded that "multiple processes contribute to the formation of the lumen during the development of mammary acini," and that several of these processes must go awry during tumor formation. RT

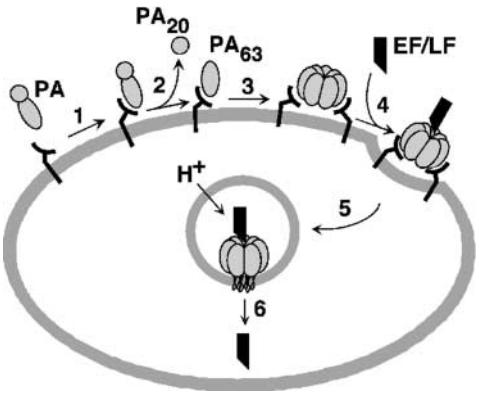
Reference: Muthuswamy, S.K., et al. 2001. *Nat. Cell Biol.* 3:785–792.

Closing the door on anthrax

Antitoxins may become the newest weapon in the arsenal against anthrax, reported R. John Collier (Harvard Medical School, Boston, MA) during a special session on the role of cell biologists in the battle against bioterrorism.

Bacillus anthracis uses a three-component toxin system, wherein the protective antigen (PA)—the main component in anthrax vaccines—helps transport two toxin proteins into the host cell: edema factor (EF), a calmodulin-dependent adenylate cyclase, and lethal factor (LF), a metalloprotease that can clip MAP-kinase kinase. By obstructing this transport mechanism, Collier and his colleagues hope to prevent anthrax fatalities.

Researchers are currently working on three antitoxin strategies, but one is significantly ahead of the others. Thus far, it seems that introducing a dominant-negative form of the PA protein completely disrupts toxin entry into host cytoplasm.



Collier/AAAS

Anthrax toxins make their own doorway into the cell.

Under normal circumstances, wild-type PA binds to a host receptor protein on the cell surface, initiating assembly of seven PA molecules into a ring. LF and EF bind to this heptamer and the whole complex enters the host cell by endocytosis. At this point, the previously disorganized loops in domain two of each of the PA proteins form a pore, allowing EF and LF to enter into the cytoplasm, where they begin to wreak havoc.

However, when Collier's group changes two amino acid residues on the lumenal face of PA's domain two, the final transport step is arrested, trapping the toxin proteins in the endosome. From there the cell transports them to a lysosome where they are destroyed. The strategy works even when the mutant form of the protein only accounts for 20% of the total amount of PA in the infected animal, and Collier found that the mutant protein can gain access to the surface of infected cells after a simple intravenous injection.

"I still find it amazing that changing two amino acids completely blocks translocation," said Collier. He cautioned, however, that further animal studies have to be completed before the dominant-negative antitoxin can be tested for safety in humans.

Researchers also have proof-of-principle evidence that two other strategies can work in vitro to block toxin entry. They can inhibit PA interaction with the cell receptor protein, called anthrax toxin receptor (ATR), by adding soluble receptor fragments to the system in vitro; this blocks assembly of the heptamer ring and toxin binding. Collier and colleagues also find that if they attach peptide mimics of the EF and LF recognition sequences to a flexible polyacrylamide backbone they can saturate PA binding sites and significantly reduce toxin entry into the cell.

But Collier is most excited about the dominant-negative approach. Admitting that it may be "a pie-in-the-sky idea," he pointed out that in the best situation the dominant-negative antitoxin could have two benefits for people exposed to anthrax: if they have systemic disease it will help to protect the macrophages—the cells primarily attacked by the toxins—and if they are not currently infected it could act as an immunizing agent to boost immune response in case of subsequent exposures. RT

Reference: Sellman, B.R., et al. 2001. *Science.* 292:695–697.

Getting off to a great Arp

Actin filaments start from seeds, and those seeds are formed by complexes containing the actin-related Arp2/3 proteins. Rows and rows of Arp2/3-related posters, and several talks, proved that these proteins are still commanding plenty of attention. Some of the studies suggested how Arp2/3's actions might be initiated and then restricted to the front of moving cells.

Bringing it all together

When it comes to building actin filaments, seeding is the hardest part. Mark Dayel, working with Dyche Mullins at the University of California, San Francisco, CA, suggests that seeding is accomplished using the energy of ATP hydrolysis.

He found that Arp2 and Arp3 bind ATP with micromolar affinity, and that subsequent hydrolysis of the ATP is necessary for nucleation of actin filaments. Thus hydrolysis may bring the two proteins together so they can form a nucleus for polymerization of the conventional actin monomer. The findings were a surprise, as ATP hydrolysis is not thought to be important for actin polymerization once the Arp nucleus is formed.

After nucleation, the ADP-bound Arp2/3 complex has a reduced affinity for its activators. This may release the complex, allowing the activators to be recycled.

Reference: Dayel, M.J., et al. 2001. *Proc. Natl. Acad. Sci. USA.* 98:14871–14876.

Side or barb?

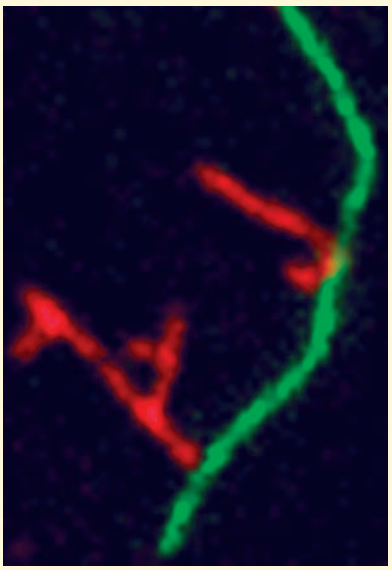
Moving cells need to direct actin polymerization forward. Understanding how they do that requires an understanding of where and how the Arp2/3 complex gets its start. This topic has been a source of controversy, with some researchers convinced that Arp2/3 binds to the side of actin filaments, and others equally sure that all the action is at filament ends. Two related studies presented at the conference may have resolved this issue with an answer that lies halfway in between.

Tom Pollard (Yale University, New Haven, CT) and colleagues reported that they can directly visualize Arp2/3-dependent polymerization from the sides of existing actin filaments by using total internal reflection fluorescence microscopy. Such a mechanism is consistent with earlier findings that nucleation is not prevented by proteins that cap the ends of existing actin filaments.

But other workers, such as Marie-France Carlier (Centre National de la Recherche Scientifique, Gif-sur Yvette, France), have noted that parent and daughter actin filaments are generally of the same length, suggesting that new filaments branch from the ends of old filaments, followed by equal growth of new and old filaments. A reconciliation of the two points of view may have arrived with the findings of John Condeelis (Albert Einstein College of Medicine, Bronx, NY) and colleagues, who suggest that nucleation is dependent on an ATP cap that extends a limited distance from the end of actin filaments.

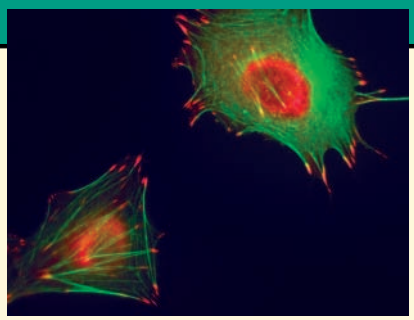
Actin binds to filaments in an ATP-bound form, but this ATP is soon hydrolyzed, leaving ADP-bound actin in the main body of the filament. Condeelis found that branching occurred preferentially from newly polymerized actin that should be rich in ATP. Studies with filaments uniformly loaded with particular nucleotides confirmed that ATP-bound subunits were needed for further branching nucleation.

"It's great," said Pollard. "[Condeelis] did a different experiment but ended up with the same result." Pollard noted only a slight bias of nucleation toward filament ends in his assays, but Condeelis believes that the bias would be stronger if Pollard used lower actin concentrations that would favor slower polymerization and thus a shorter ATP-bound cap.



Condeelis/Elsevier

Actin branches prefer new (red) not old (green) filaments.



deMali

Vinculin (red) may help anchor Arp2/3.

The size of that cap is still a matter of some disagreement, however. Pollard's new results include a much faster half-time for actin-dependent ATP hydrolysis, measured using new, more accurate methods. This would result in a vanishingly small ATP cap that in the light microscope would be indistinguishable from the polymer's end. Condeelis notes, however, that ATP hydrolysis is stochastic, and so a mixture of ATP- and ADP-bound actin monomers may extend for some distance downstream of the filament's end.

For Condeelis, the biochemistry reflects back on the biology, and the emphasis on ends makes perfect sense. "If you have a bias of the branching to the barbed ends," he says, "it would be a perfect way to create a structure that would push."

References: Amman, K.J., and T.D. Pollard. 2001. *Proc. Natl. Acad. Sci. USA.* 98:15009–15013.
Ichetovkin, I., et al. 2002. *Curr. Biol.* 12:79–84.

Anchoring Arp

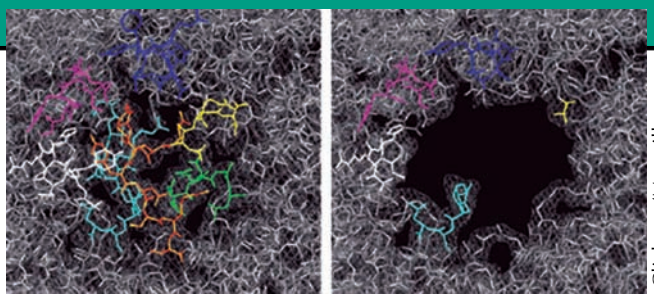
Even if Arp2/3 activity biases actin polymerization forward, the cell may need a way to link the resultant protrusion to cell adhesion. Kris deMali and Keith Burridge (University of North Carolina, Chapel Hill, NC) are suggesting that vinculin may supply that link. Vinculin is better known as a component of focal adhesions, but deMali and Burridge detected a direct, transient binding of vinculin to the Arp2/3 complex at the beginning of membrane protrusion. The link could give the Arp2/3 complex a site from which it can push off, thus rendering actin polymerization more effective. ww

Chewing themselves to death

Michael Glickman (Technion-Israel Institute of Technology, Haifa, Israel) has found a yeast proteasome mutant that chews up proteins more rapidly than normal. The phenotype of the mutant suggests that yeast stationary phase represents a true quiescence.

Glickman has been studying the regulation of proteasomes by lopping off the NH_2-terminal domains of the α subunit proteins, thereby creating an open-gate proteasome. These mutant proteasomes degrade proteins ~10-fold faster than wild-type proteasomes, but the mutant yeast don't show any growth defects—at least not until they are exposed to starvation conditions.

If yeast cultures are starved for more than about a week, the viability of the proteasome mutants begins to fall off rapidly, whereas the wild-type cells are unaffected. Glickman found that wild-type cells react to starvation by shutting down all protein degradation, whereas the open-gate mutants continue to break down their proteins, a trait that eventually kills them. This suggests that stationary phase involves a slowing of all metabolism: both the known reduction in synthesis and the newly demonstrated reduction in degradation. RT

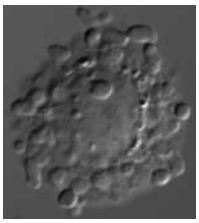


Glickman/Macmillan

The mouth of a mutant proteasome (right) opens too wide.

Reference: Groll, M., et al. 2000. *Nat. Struct. Biol.* 7:1062–1067.

Stop your blebbing



Stossel

Blebbing in cells without filamin (above) can be stopped with blebbistatin.

Small molecule inhibitors are beginning to reveal the secrets of myosin action. In a recent publication and at the meeting, Aaron Straight (Harvard Medical School, Boston, MA) and colleagues announced the discovery of two different myosin inhibitors with high specificity.

The first inhibitor, *N*-benzyl-*p*-toluene sulphonamide (BTS), hits fast muscle myosin II in preference to myosin found in slow or cardiac muscle or platelets. BTS or its relatives may find applications as a muscle relaxant. The second inhibitor, called blebbistatin, is specific for nonmuscle myosin II.

The two inhibitors were detected using similar screens. An activated form of the target myosin was incubated with ATP and one of 16,000 test chemicals. Most chemicals were inactive, allowing myosin to chew through the ATP. But a few chemicals inhibited ATP hydrolysis. The left-over ATP provided energy for luciferase, whose fluorescence identified the positive wells.

The screen yielding BTS went off without a hitch, but the pathway to blebbisatin was a little rockier. Resynthesis of what Straight thought was the active compound yielded an inactive chemical. But when the chemical was left in solvent for a day or two, an active breakdown product began to appear. That breakdown product was blebbistatin.

Straight has used blebbistatin not only to inhibit the blebbing of cells lacking filamin A, but also to inhibit cell motility and cytokinesis. Both studies have yielded new insights. When added to moving cells, blebbistatin interferes with the cell's polarity but not with protrusion: the confused cell now sends out membrane at both its front and back. In dividing cells, blebbistatin prevents contraction of the contractile ring, suggesting that a myosin-independent back-up pathway for cytokinesis seen in *Dictyostelium* does not exist in vertebrate cells. WW

Reference: Cheung, A., et al. 2002. *Nat. Cell Biol.* 4:83–88.

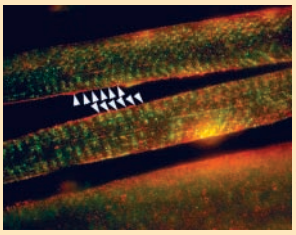
Clathrin: from basket weaving to muscle contraction

With a new clathrin gene on the scene, get ready to revise those dog-eared notions about clathrin-coated pits and endocytosis. The new protein, called CHC22—for clathrin heavy chain on chromosome 22—shares 85% amino acid identity, but little else, with the more familiar form. "It apparently has a completely different function [compared to] what we normally think of for clathrin and clathrin-coated pits," said Frances Brodsky (University of California, San Francisco, CA).

The new clathrin is predominantly expressed in muscle cells, in a pattern that suggests it could be involved in Z-disk formation. These disks separate sarcomeres in the myofibrils and serve as attachment sites for the thin actin filaments required for muscle contraction.

The structural basis for this muscle function is unclear. CHC22 forms timeric structures that superficially resemble those formed by the previously known clathrin, but these structures don't appear to include clathrin light chains. In fact, CHC22 has only weak affinity for the clathrin light chain in two-hybrid assays, and Brodsky's group has yet to find the two proteins associated in vivo.

But in a subset of muscular dystrophy patients, the presence of the light chain may be critical. Brodsky has hypothesized that in these cases the clathrin light chain may be overexpressed, enough so that CHC22 may overcome its weak affinity for the light chain and inappropriately bind to it. Her group is still working on the theory, but for now she says "I'm quite excited about having studied the biochemistry of clathrin all these years and now being able to use the information in the study of human disease." RT



Brodsky/EMBO

CHC22 (red) and clathrin light chain (green) do not colocalize (arrows).

Reference: Liu, S.-H., et al. 2001. *EMBO J.* 20:272–284.

Magical shrinking rafts

Biochemistry begat rafts, but imaging is belittling them. New work presented by Anne Kenworthy (Vanderbilt University, Nashville, TN) and colleagues, and Akihiro Kusumi (Nagoya University, Nagoya, Japan) and colleagues suggests that rafts are far smaller and more short-lived than was originally thought.

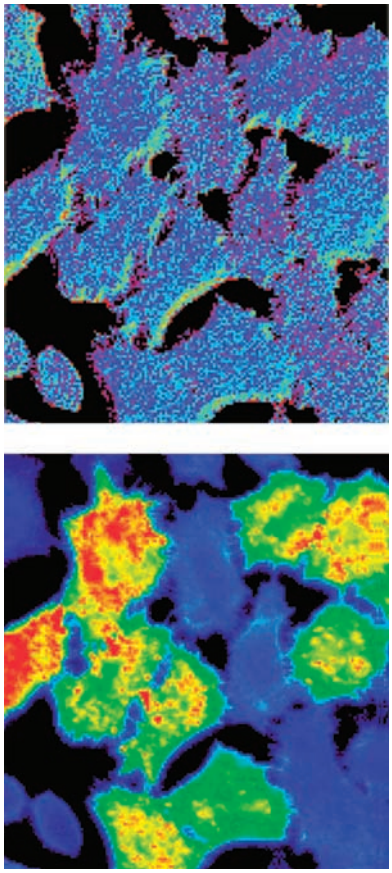
Rafts are collections of cholesterol, sphingolipids, and signaling proteins that were initially isolated based on their insolubility in certain detergents. Deborah Brown (State University of New York, Stonybrook, NY) and others used that detergent insolubility to determine which types of proteins partition into rafts. But, says Brown, the biochemistry "doesn't give you any real idea of what the rafts look like. In vitro people saw 1-μm sheets, and jumped to the conclusion that this is what rafts look like. It's completely clear now that that is not true."

Rafts began to shrink with the work of Rajat Varma and Satyajit Mayor (National Centre for Biological Sciences, Bangalore, India). They used fluorescence properties to demonstrate that diluted raft proteins remained close together, suggesting clusters. But the clusters appeared to be less than 70 nm in diameter.

Kenworthy tackled the problem by photobleaching large sections of the cell surface and measuring how rapidly different proteins diffused back into the bleached area. Diffusion was extremely rapid, and different raft proteins diffused at different rates. Kenworthy concludes that raft proteins must exist in a variety of dynamically partitioning, rather than stable, structures.

Kusumi is attempting to put more exact limits on the size and behavior of rafts by tracking beads that are attached to individual raft proteins. According to his measurements, both raft and nonraft proteins are constrained primarily by cytoskeleton-based fences. The proteins wander around 100-nm–diameter compartments for ~25 ms before hopping to an adjacent compartment, and this rate of hopping is slowed only if raft proteins are linked together by using a bivalent antibody. This suggests that rafts must be very small—containing perhaps only a few lipid molecules—or have half-lives of much less than 25 ms. Kusumi notes, however, that these properties may change in stimulated cells when signaling complexes are more active. WW

Reference: Simons, K., and D. Toomre. 2001. *Nat. Rev. Mol. Cell Biol.* 1:31–39.



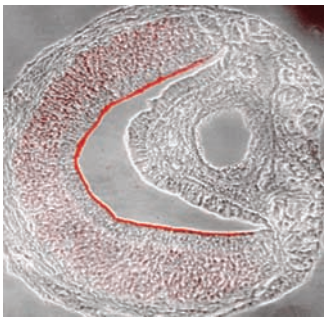
Mayor/Macmillan

Raft proteins stay uniformly close (top) despite overall changes in concentration (bottom).

Make love, not war

Mice lacking a particular ion channel involved in pheromone sensing retain a mating response but not an aggression response, according to Catherine Dulac (Harvard University, Cambridge, MA).

Dulac began by identifying an ion channel, TRP2, that is expressed in the lumen of the vomeronasal organ (VNO)—the organ responsible for pheromone detection in rodents. They realized that the location of the channel, right at the tip of the microvilli, made it a prime candidate for processing the pheromone response.



Dulac/NAS

TRP2 (red) in the VNO sniffs out pheromones.

The nature of that response is simple enough. "There may be as many as 400 pheromone receptor genes, but only two main responses: mating and aggression," said Dulac. When a female mouse is put into a cage that already contains a male mouse, the male immediately initiates mating behavior. But if the introduced mouse is also male, the resident male attacks, as it has already come to recognize the space as its personal territory.

If the resident male mouse lacks TRP2, however, the mutant male tries to mate with an introduced mouse regardless of whether it is a male or a female.

Based on these data, Dulac concluded in her talk that "mating behavior is elicited by multiple systems—the VNO and other systems—and seems to be the default pathway. But aggression behavior relies on the VNO." RT

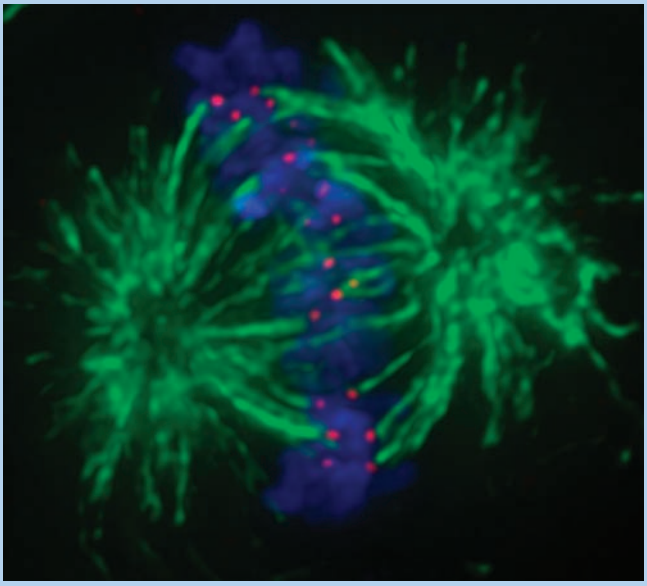
Reference: Liman, E.R., et al. 1999. *Proc. Natl. Acad. Sci. USA.* 96: 5791–5796.

Kinetochore construction

Fly and human kinetochores appear to be constructed in a modular, repeating fashion, according to Mike Blower and Gary Karpen (The Salk Institute, La Jolla, CA). They propose that the repeats either loop or spiral to form the mitotic kinetochore.

Hints of a repeating structure emerged from earlier studies using stretched metaphase chromosomes, but these studies relied on the use of autoimmune sera whose protein targets were unknown. Blower and Karpen undertook similar studies with antibodies to both histone H3 and the H3-like, centromere specific protein CENP-A (called CID in flies). In earlier work they found that CID localized at fly kinetochores and was required for their function.

Looking more closely, they now find that large domains of H3 and CENP-A/CID alternate along interphase chromatin when it is isolated using harsh conditions. But gently isolated mitotic kinetochores have a single, elliptical ball of CENP-A/CID that excludes H3. These two results can be reconciled by a model in which one half of each loop or spiral of centromere DNA is covered with CID. This organization may present the centromere DNA so that it is more accessible to kinetochore proteins and microtubules. ww



Karpen/Macmillan

CID (red) helps organize kinetochores.

Reference: Blower, M.D., and G.H. Karpen. 2001. *Nat. Cell Biol.* 3:730–739.

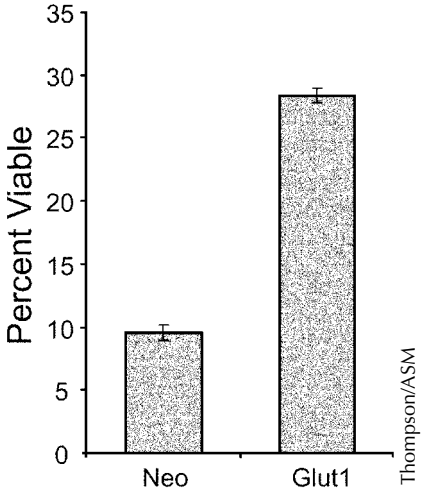
Cellular starvation

While most of us are obsessed with apoptotic master regulators, pan-tumor suppressors, and cell cycle kinases, Craig Thompson (University of Pennsylvania, Philadelphia, PA) wishes we would remember something a little more basic: the intermediary metabolism that we left safely back in Biochemistry 101. When it comes to controlling whether cells live or die, the Glut1 transporter, hexokinase 2, and phosphofructokinase 1 are where it's all at, said Thompson. And how did he get involved in this glycolytic nightmare? "Not by any choice, trust me," he said.

Thompson is addressing why mammalian cells die when a growth factor such as interleukin-3 (IL-3) is taken away. He believes that the growth factors are controlling access to nutrients. In the absence of the factors, cells may be starving themselves to death even in the midst of extracellular plenty.

Initially, Thompson studied how the antiapoptotic Bcl-x_L could rescue cells denied IL-3. To his surprise, he found that Bcl-x_L was restoring exchange of ADP into the mitochondria, thus providing the mitochondria with a substrate for continued ATP production. Thus Bcl-x_L must be correcting the situation in cells lacking IL-3, in which cells were deliberately sabotaging the bioenergetics of their own mitochondrion. "Why is the mitochondria the site of all these forms of apoptosis?," he asked. "It's because cells are reacting to starving to death."

Further evidence of deliberate starvation came when Thompson found that both IL-3 and glucose withdrawal induced similar drops in glycolytic rate, and similar movements of apoptotic regulators. IL-3 withdrawal leads to a rapid decline in the expression of three major glycolytic regulators, and restoring expression of one of these (the Glut1 transporter) increases survival from 10 to 30%. Thompson said that adding more glycolytic proteins back into the cell improves the situation even further.



Glucose transport helps cells survive without growth factors.

The focus on metabolic control has raised doubts in some. Those who study growth control, for example, have their sights set on regulation of translation, not metabolism. But in Thompson's group, he said, "we are arguing that protein synthesis is a slave, secondary to energy uptake." Thompson will need more data to prove that, but for now he believes his model is useful. "At some level we realize this will be an over-simplification," he said. "But this does seem to inform an awful lot of biological phenomena." ww

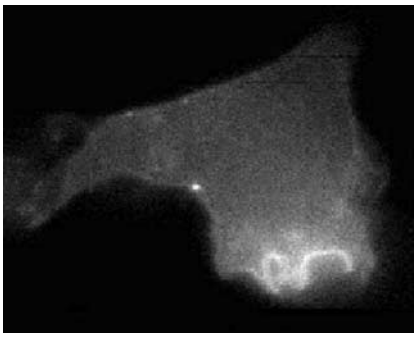
Reference: Vander Heiden, M.G., et al. 2001. *Mol. Cell. Biol.* 21:5899–5912.

Neutrophils on the move

In the 1950's, David Rogers at Vanderbilt University (Nashville, TN) filmed a neutrophil in hot pursuit of a *Staphylococcus* bacterium (see http://zk.bwh.harvard.edu/projects/motility/neutrophil.html). With each twist and turn, the cell reshaped its leading edge and detached its rear. Now, forty years later, Henry Bourne (University of California, San Francisco, CA) is mapping out the signaling pathways that control such movement in neutrophils. His results closely parallel those of Peter Devreotes (National Cancer Institute, Bethesda, MD) and Rick Firtel (University of California, San Diego, CA) in their motility studies of the soil amoeba *Dictyostelium discoideum*.

In both systems, the chemoattractant receptors are uniformly distributed on the cell surface but generate an asymmetric response that enables the cell to move in an organized manner. The first molecule in the response pathway to reflect this asymmetry is a membrane lipid, phosphatidylinositol-3,4,5-trisphosphate (PIP3), which can be detected with a protein probe. In the absence of a chemoattractant, the probe is uniformly distributed in the cytoplasm, but, seconds after addition of a chemoattractant, the probe concentrates at the leading edge of the cell and actin assembly begins in the same region.

In neutrophils, this leading edge appears to be stabilized and reinforced by feedback mechanisms downstream of PIP3. Two of the three Rho-GTPase family members—Rac and Cdc42, but not Rho—are involved. Rac and Cdc42 stimulate actin polymerization, and inhibition of their activity causes the cells to round up, losing their leading edges and polarized actin assembly, and flattening their PIP3 gradients. Thus, these GTPases not only respond to PIP3, but also mediate a positive feedback loop that allows PIP3 to stimulate its own accumulation, suggested Bourne. And, he pointed out, when Rho is inhibited, the back end of the cell is slow to detach and move forward. Thus, the GTPases work together to convert a single external signal into an elaborate cascade of activity—and eventually cell movement. RT

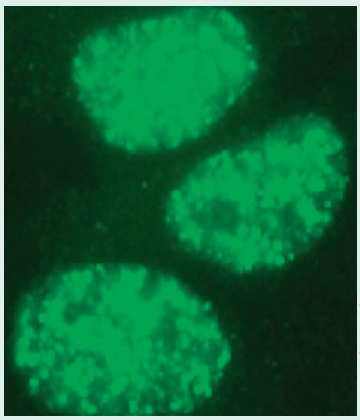


Bourne/AAAS

PIP3 is formed at the leading edge of neutrophils.

Reference: Servant, G., et al. 2000. *Science.* 287:1037–1040.

A double-agent in DNA repair



D'Andrea/Elsevier

FANCD2 is recruited to DNA repair sites.

Fanconi anemia (FA) and Ataxia Telangiectasia (AT) are two cancer-susceptibility disorders caused by a breakdown of DNA repair systems. Until now the two syndromes shared some basic similarities but no components. Now, however, Alan D'Andrea and colleagues at the Dana-Farber Cancer Center in Boston, MA, find that one protein is directly involved in both diseases.

When wild-type cells are exposed to DNA cross-linking agents, a DNA repair pathway is initiated. The FA complex, consisting of protein subunits encoded by five FA-linked genes, becomes active and monoubiquitinates the FANCD2 protein. Once monoubiquitinated, FANCD2 is recruited to a BRCA1 complex and DNA repair begins.

Mutations in any one of eight FA-linked genes can block this process, leading to an increased sensitivity to DNA cross-linking and an overall increased rate of chromosome breakage. In earlier work, D'Andrea's team found that if monoubiquitination of FANCD2 is prevented, then the protein doesn't aggregate with BRCA1 and DNA repair is stymied, thus producing FA symptoms.

D'Andrea has now found that mutation of FANCD2 phosphorylation sites has a very different effect. Lack of phosphorylation does not cause FA symptoms or increase sensitivity to cross-linking agents, but does block repair by AT-related proteins and increase sensitivity to ionizing radiation. Thus the FANCD2 protein may integrate inputs from the two DNA repair pathways. RT

Reference: Garcia-Higuera, I., et al. 2001. *Mol. Cell.* 7:249–262.