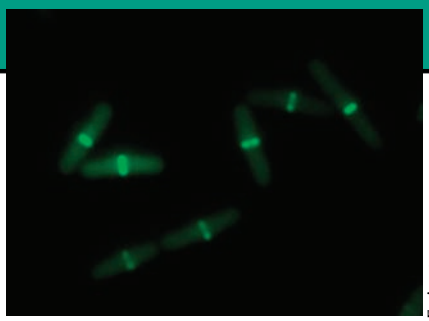
Noc-ing out division



Errington

Nucleoids keep rings of FtsZ (green), and thus division, in the right place.

Bacterial cells polymerize a tubulin homologue called FtsZ at their midpoint to drive cell division. FtsZ inhibitors called Min proteins prevent division near cell ends. But a second inhibition is thought to be mediated by the DNA mass in the center of the cell, which prevents division there until the two DNA masses are segregated away from the cell center. Now, Jeff Errington has announced the discovery of a candidate for this second inhibition event—a nucleoid occlusion protein called Noc.

"We stumbled across this gene completely serendipitously," said Errington in his talk. His team started with bacteria lacking a *min* gene, and combined it with a deletion in another cell division gene. But the second gene deletion happened to take out yet a third gene, now named *noc*. Although bacteria lacking Min proteins divide near their ends, the bacteria lacking both the Min system and Noc did not divide at all.

The double deletion discovery was a stroke of luck, because cells lacking only Noc appear wild type. Errington believes that in these Noc$^-$ cells the Min system still ensures that division happens approximately in the middle of the cell. Division does not occur until the cell has grown to a sufficient length for the middle of the cell to escape end-localized Min inhibition. But when both Min and Noc are missing there is no division, because FtsZ can polymerize anywhere, and the resulting dilution of FtsZ polymer means that at no point in the cell does it reach high enough levels to effect division.

After this first, rather puzzling result came the more direct experiments. Overexpressing Noc, a putative division inhibitor, increased cell length as predicted. Consistent with its DNA binding domain, Noc colocalized with the nucleoid. And in cells lacking Noc, FtsZ patches sometimes overlay the nucleoid, which never happens in wild-type cells.

To clinch his argument, Errington inhibited DNA replication. Wild-type cells elongated for a while and then put their FtsZ rings to either side of the nucleoid. But cells lacking Noc divided right through the DNA. "This was really one of the most exciting events in my scientific career," said Errington.

The putative DNA binding of Noc remains to be demonstrated. It is also possible that DNA binding is required for Noc to inhibit FtsZ polymerization, perhaps by arranging multiple copies of Noc into a form that can interact with FtsZ protofilaments. WW

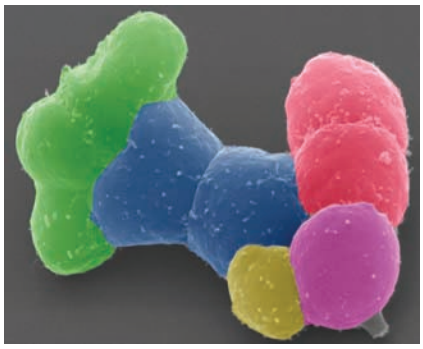
Reference: Sun, Q., and W. Margolin. 2001. *J. Bacteriol.* 183:1413–1422.

Late-dividing cells feel the pinch

Gastrulation—the internalization of the future endoderm—is an intricate dance of embryonic cells. In worms, this dance is choreographed by two endodermal precursor cells that use a repositioning mechanism driven by constriction, as presented by Jen-Yi Lee and Bob Goldstein (University of North Carolina, Chapel Hill, NC).

Historically, morphogenesis studies were done in frog or fly embryos, but each has had its limitations. "*Xenopus* is useful for in vitro culture of embryonic tissues but isn't ideal for genetics," says Goldstein. "Most of the genetics has been done in *Drosophila*. We wanted to sort of cross the two systems, so we tried using *C. elegans* to get the best of both."

During worm gastrulation, the endodermal precursors, Ea and Ep, move away from the egg envelope (which is on their apical side) and are then enclosed by their neighbors. These



Goldstein

Endodermal precursors (blue) make it to the middle during worm gastrulation.

movements are not conventionally explained by cell crawling, as Lee and Goldstein found. Although actin and myosin were necessary, no filopodia or lamellipodia were found. Rather, the authors noted a constriction of the apical side of the cells that forced them inwards. Goldstein likens the process to a gymnast on the rings: from a cross position, if the athlete pulls his arms downward (constriction), his head and body press upwards (ingression). "We were surprised to learn that apical constriction could position cells so early in development, even before anchoring cell–cell junctions have formed," says Goldstein.

Now the group wants to determine how Ea and Ep are selected. Early evidence suggests that Ea/Ep move because they divide later than the other cells. Gastrulation mutants in which this lag is missing can be rescued by delaying Ea/Ep division with a laser. Delaying division in mesodermal precursors causes these cells to ingress instead. Why delaying division causes constriction is unclear, but may be as simple as providing enough time for myosin to accumulate at the apical side, where it has been shown to lie in Ea and Ep. NL

Reference: Lee, J.-Y., et al. 2003. *Development.* 130:307–320.

The Journal of Cell Biology

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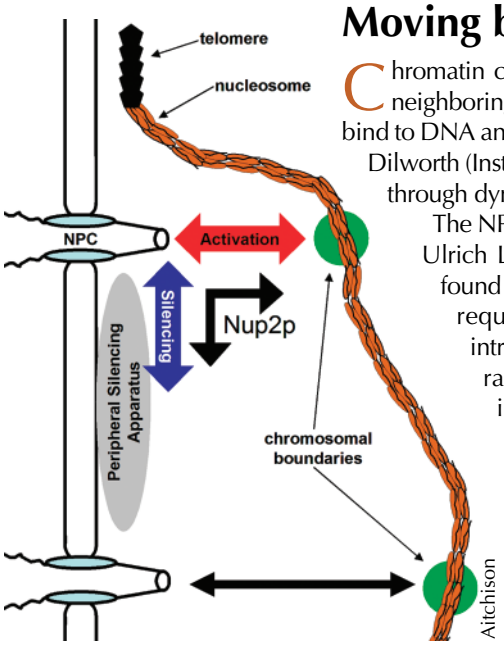
Moving boundaries with Nup2p



Aitchison

Nup2p takes boundary domains to and from the silencing apparatus.

Chromatin can be partitioned to insulate one section from the repressive or enhancing effects of neighboring domains. This separation is achieved by proteins with boundary activity (BA), which bind to DNA and protect the intervening sequence from outside influences. Results presented by David Dilworth (Institute for Systems Biology, Seattle, WA) now suggest that boundaries might come and go through dynamic interactions of a nuclear pore protein with the nuclear pore complex (NPC).

The NPC–boundary connection was made over a year ago in a screen for yeast BA proteins by Ulrich Laemmli's laboratory (University of Geneva, Switzerland). Many of the proteins they found were also involved in nuclear trafficking, such as cargo-binding karyopherins. BA also required the nuclear pore protein Nup2p. Dilworth, along with John Aitchison, was intrigued by the involvement of Nup2p, a protein they had previously shown exchanges rapidly between the NPC and the nucleoplasm. "It's not just being at the pore that's important, because not all nups worked [as BAs]," says Aitchison. "Nup2 is unique."

Aitchison's group is now establishing Nup2p's importance in insulating endogenous genes using microarray analyses. Genes that are misexpressed when Nup2p is missing are indeed clustered in chromosomal regions, particularly near normally silenced telomeres. Within these regions are genes that are associated with stress or environmental responses. Aitchison hypothesizes that the mobile Nup2p helps sections of chromatin move to and from the NPC, thus switching on or off groups of genes in response to rapidly changing environmental conditions. NL

Reference: Dilworth, D.J., et al. 2001. *J. Cell Biol.* 153:1465–1478.
Ishii, K., et al. 2002. *Cell.* 109:551–562.

Hooking together nucleus and centrosome

A worm protein called ZYG-12 glues together centrosomes and nuclei, say Christian Malone, John White (University of Wisconsin, Madison, WI), and colleagues.

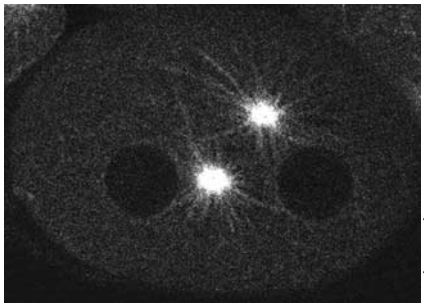
Malone deduced ZYG-12's function based on the wandering centrosomes of a mutant lacking the protein function. The consequences of such wandering are particularly severe in the large cells of the young worm embryo, with DNA often getting lost on the wrong side of a spindle or on a monopolar spindle.

ZYG-12 localizes to both the centrosomes and the nuclear envelope. Centrosome localization requires microtubules, possibly via ZYG-12's postulated microtubule-binding domain. Binding appears to be specific, however, to either microtubule minus ends or microtubules in the context of the centrosome.

Nuclear envelope localization requires SUN-1, a protein in the nuclear envelope whose presence is also required for attachment of centrosome and nuclear envelope. ZYG-12, as with other proteins in this "Hook" class, can bind itself, so simple dimerization may bring together nucleus and centrosome.

Previous studies had pointed to the microtubule motor dynein as a culprit for bringing the two organelles together. The Wisconsin group showed that dynein light chain also binds ZYG-12, so ZYG-12–anchored dynein on nuclei could be used to reel in centrosomes. But earlier equivocal results with dynein depletion imply that, by themselves, the ZYG-12 on centrosomes and nuclear envelope can often find each other, especially in smaller cells.

Both SUN-1 and ZYG-12 spread all around the nuclear envelope. Centrosomes, meanwhile, are attached only at two focused and aligned spots. This may have more to do with astral microtubule behavior than anything on the nuclear envelope. Astral microtubules push duplicated centrosomes apart, and the microtubule interactions with the cell cortex pull and align the centrosomes in the correct orientation for division.



Malone/Elsevier

Cells lacking ZYG-12 cannot attach centrosomes to nuclei.

That's not the end of the localization problems. Somehow the cell keeps ZYG-12 and SUN-1 localized in the nuclear envelope, and not in the endoplasmic reticulum (ER) membrane that is continuous with it. Perhaps one of the proteins reaches across the ~40-nm gap between the two nuclear membranes to anchor the proteins, so that centrosomes don't start attaching to the ER. WW

Reference: Malone, C.J., et al. 2003. *Cell.* 115:825–836.

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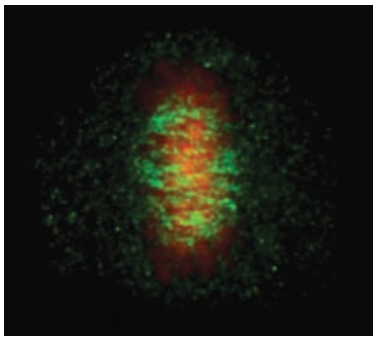
Chromosomes make a spindle

A chromatin-associated protein called NuSAP can nucleate microtubule polymerization, according to Katharina Ribbeck and Iain Mattaj (EMBL, Heidelberg, Germany). Thus, NuSAP may help chromatin to sculpt the growing spindle around it, rather than the chromatin just acting as a passive passenger of the fully formed spindle.

Mattaj and Isabelle Vernos have previously shown that a protein called TPX2 is necessary for microtubule assembly around chromosomes. By itself TPX2 can promote the formation of short microtubule fragments in solution. But now Ribbeck shows that the recently identified NuSAP has properties that TPX2 does not share: it can nucleate the centrosome-independent formation of long microtubule fibers, either from NuSAP-loaded beads or around isolated chromosomes. And, compared with TPX2, NuSAP is found closer to chromatin.



Carmeliet/RUP

NuSAP (green, at metaphase) may nucleate stable microtubules near DNA (red).

The microtubule fibers formed by NuSAP grow two to threefold slower than the centrosome-nucleated variety and are unusually stable. Ribbeck does not know whether this is caused by either binding of NuSAP along the fibers or polymerization of the tubulin into an unusual polymer arrangement.

The NuSAP-generated fibers are certainly important, as without NuSAP the cell forms a defective spindle that cannot efficiently capture, congress, or segregate chromosomes. And the microtubule fibers may provide a means by which the DNA can communicate with the rest of the spindle. As Ribbeck put it in her talk, "the chromosomes could actively contribute to spindle assembly, and thereby help the spindle adapt to the increased size of vertebrate chromosomes." ww

Reference: Raemaekers, T., et al. 2003. *J. Cell Biol.* 162:1017–1029.

Old, pesky, and weird

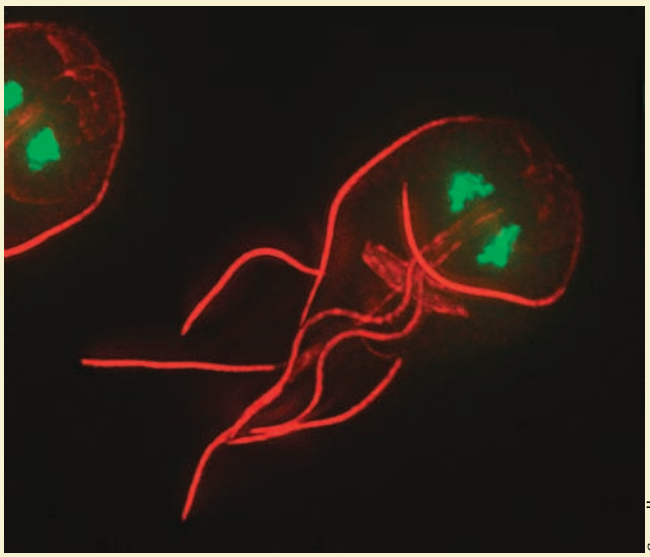
Biologist W. Zacheus Cande has all the right reasons for studying *Giardia lamblia*. It is an important human parasite. It is one of the most basal extant organisms in eukaryotic evolution. But the third reason might be most compelling of all. "I love it," he says, "because it's such a weird organism."

Cande and Meredith Johnson Sagolla (University of California, Berkeley, CA) have tantalizing clues that one of those weirdnesses—the two complete nuclei found in every cell—might give rise to a strange mitosis lacking a conventional spindle. "It's easier for us to think [the spindle] is really there and shy," says Cande, "but I could be wrong."

The evidence for *Giardia*'s early origins are several-fold. It has archaea-like transcription factors, extremely divergent (though numerous) kinesins, a single intron, and no mitochondria. Some of these features may have been lost rather than never gained, but sequencing is also consistent with an ancient origin for *Giardia*. The common ancestor of *Giardia* and the rest of us may have lived two or three times further in the past than more popular common ancestors, such as those between animals, plants, and yeasts. So *Giardia* might give Cande a glimpse of commonalities early in eukaryotic evolution.

But all that evolutionary time also leaves plenty of opportunities for weirdness to accumulate. The microtubule cytoskeleton may be a good place to start looking. *Giardia*'s infectious form has a spiral array of microtubules, the ventral disk, which mediates attachment to the gut microvilli of unfortunate backpackers. Large centrosomes and multiple basal bodies also hover near the nucleus.

But Cande and Sagolla have found that the nuclear envelope breaks down little if at all during mitosis. This means that a spindle, if it exists, would have to be built by microtubules that have to invade through the nuclear envelope to do their job.



Sagolla

Giardia (shown in interphase) may use their microtubules (red) in strange ways to segregate their multiple nuclei (green).

A spindle has not been apparent so far, although live experiments are still to come. What Sagolla has seen, however, is a strange cross-shaped microtubule structure that appears between the nuclei as soon as two nuclei become four. If there is no spindle, early DNA separation could operate via telomere attachment to the nuclear envelope (a process that has been visualized). Then the cross-shaped microtubules could do some sort of pushing to segregate nuclei into daughter cells.

But all that conjecture hangs on more observation. Live microscopy is proving difficult, as is synchronization. "Working on a non-model system," says Cande, "small victories are big." ww

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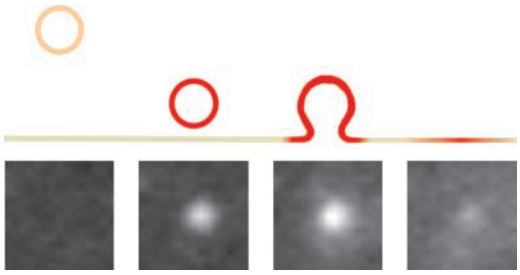
Swiftly SNARE'd vesicles



Simon

Using TIR-FM, a single vesicle is seen approaching and fusing with a target membrane (left to right).

A look at individual events reveals the simplicity and efficiency of membrane fusion reactions, as shown by groups led by James Rothman (Columbia University, New York, NY) and Sanford Simon (Rockefeller University, New York, NY).

Fusion is a function of SNAREs, those helical proteins that, in the right combinations, will zipper up to fuse two membranes. Rothman's laboratory has shown that SNAREs are sufficient for fusion: liposomes containing nothing but SNAREs fuse. However, fusion is not as rapid as expected based on rates of in vivo fusion events.

"Just because something does happen in vitro doesn't necessarily mean it happens at a rate that's fast enough to be meaningful for a cell," says Simon. To be sure SNAREs were enough for meaningful fusion, his group tested their sufficiency using TIR-FM—a microscopy technique that allows them to watch individual vesicles fuse as they encounter a flat membrane. Previous liposome fusion events were analyzed by fluorescent dye quenching, meaning the dynamics of fusion were gleaned through averaging, and the speed of individual events may have been underestimated due to a minority of slower events. But with TIR-FM, "rather than looking at the total, we are looking at a single event," says Simon. These results show that most of the fusions events occur within seconds, fast enough to account for fusion in cells.

Geometry may also contribute to the increased velocity. In a cell, vesicles are more likely to fuse to elongated structures such as the plasma membrane than to another vesicle. The TIR-FM arrangement more closely resembles these in vivo geometries. But as yet the groups have no biochemical explanation for why a flatter surface would support faster fusion.

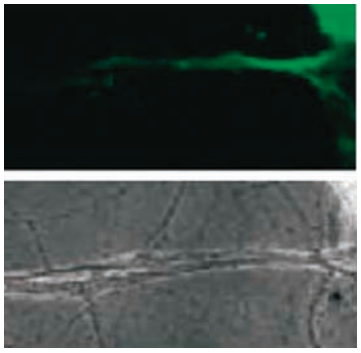
Although SNAREs were enough to get quick fusion, many vesicles docked to the membrane but never fused. This block was overcome, however, if the NH_2 terminus of syntaxin—the SNARE attached to the flat membrane—was removed. Rothman expects that this part of syntaxin may regulate fusion in vivo, and Simon plans to add potential regulatory proteins that might bind to the syntaxin NH_2 terminus to assess their effects on liposome docking and fusion. NL

Reference: Hu, C., et al. 2003. *Science.* 300:1745–1748.

Prions: a method in the madness

At least some prions have a positive purpose in the brain. Although prions of mad cow fame are associated with toxic holes in the brain, Kausik Si, Eric Kandel (Columbia University, New York, NY), and colleagues show that another prion-like protein is needed to retain lasting memories.

Long-term memory is established when a particular synapse undergoes enduring structural changes that require gene expression and local protein synthesis. Kandel and Si wanted to understand how individual synapses are selected to participate in this process. Using a snail neuronal model system, he and Si examined transcripts that are locally translated near synapses that receive impulses to make long-term memory-inducing changes. One such transcript encodes CPEB, a polyadenylation-element binding protein that the group shows has the unusual ability to function like a prion. And this ability may account for the maintenance of memory.



Kandel/Elsevier

CPEB (green) made locally at synapses makes memories last.

When expressed in yeast, CPEB existed in two conformational states. One state was a prion-like structure that formed small aggregates. As with prions, this dominant state could induce the other, globular conformation to switch to the prion-like form. Unlike all other known prions, however, it was the aggregated form that was active. In its active state, CPEB induces the polyadenylation and thus translation of otherwise dormant transcripts (such as cytoskeletal and translation-related mRNAs at synapses).

In snail neurons, CPEB protein levels increased at synapses receiving a neurotransmitter pulse through a translation- and PI3 kinase–dependent pathway. CPEB remained at the stimulated synapse, perhaps via attachment to the actin cytoskeleton, thus ensuring that alterations in protein synthesis are spatially restricted. When CPEB was inhibited, only transient synaptic changes like those that produce short-term memory could be made.

"Now we want to see if the prion characteristics [of CPEB] are found in the intact animal," says Kandel. If so, it would help explain the self-maintenance of memory. As long as a synapse is stimulated to produce enough CPEB such that some attains the prion-like state, the stimulation can be maintained indefinitely even if the synapse is no longer stimulated. "In principal," Kandel says, "you could even have a transcription factor that is self-perpetuating. Anything that cells need for the long term could be controlled by prion-like proteins." NL

References: Si, K., et al. 2003. *Cell.* 115:879–891.
Si, K., et al. 2003. *Cell.* 115:893–904.

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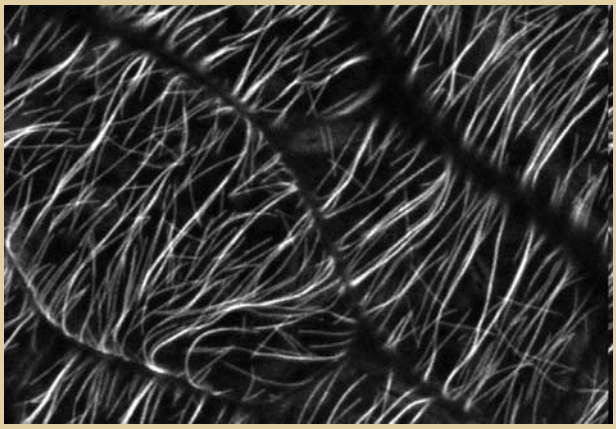
Stability in the bundle

Microtubules are central to shaping a plant cell as it expands. In tubular epidermal cells, for example, the microtubules resemble hoops around a barrel, with parallel filaments aligned with the short axis of the cell. How these arrays organize without centrosomes, which are lacking from higher plants, is unknown. New work from Sidney L. Shaw (Stanford University, Stanford, CA) and David Ehrhardt (Carnegie Institution, Stanford, CA) is helping to explain how microtubules attain organization through bundling.

Shaw and Ehrhardt use GFP-tubulin to show that new microtubules initiate randomly throughout the cell cortex and must somehow reorient into the aligned array. Transport of microtubules through microtubule motor activity was not found. However, nearly all of the microtubules in the array were moving via treadmilling—growing at the plus end and depolymerizing at the minus end. (In contrast, treadmilling is rare in animal interphase cells due to fixed minus ends.) Thus, says Shaw, "our hypothesis is that there might be a function for treadmilling in organizing the interphase arrays."

They show that treadmilling allows microtubules to form bundles—and there is strength in numbers. Bundling occurred when a single microtubule attempted to cross another, but coaligned instead. Once integrated, the microtubule was restricted from changing direction. "It's not the singles [that are the organized part of the array]," says Shaw. "It's the bundles. Long-term bleaching experiments show that the positional half-life of the bundles is at least three to four times what it is for singles, long enough to possibly influence cell shape," says Shaw.



Shaw

Bundles are more positionally stable than individual plant microtubules (white).

The angle at which intersecting microtubules begin to form bundles, from 0° to ~30°, is considerably wider than that expected based on microtubule rigidity. The high acceptance angle may contribute to microtubule self-organization into an ordered array—having a few, coarsely aligned bundles lowers the odds that new single microtubules can create a sustained bundle in an orientation other than parallel. The group is now modeling to what degree the bundling angle might affect organization. NL

Reference: Shaw, S.L., et al. 2003. *Science.* 300:1715–1718.

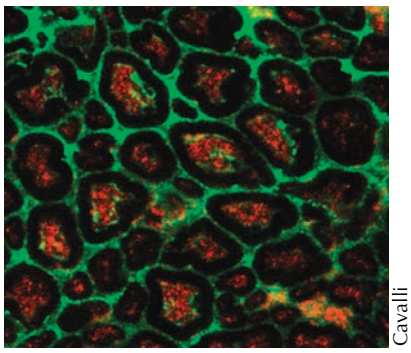
Nerve damage goes for a drive

Mouse nerves have a damage surveillance system that motors out along axons, say Valeria Cavalli and Larry Goldstein (University of California, San Diego, CA). But when the axon is damaged, the complex appears to switch directions and take its message back toward the cell body.

The messenger under study is Jun kinase (JNK) and its scaffold protein JIP-3, known as Sunday Driver (Syd) from its discovery in flies. Syd came to Goldstein's attention as a protein that links the microtubule motor kinesin to vesicles that move out into axons. Cavalli wanted to know if JNK activity was affecting the direction or extent of vesicle transport, so she looked at Syd/JNK movement under different conditions. Nerve damage was a good place to start, as accumulation of activated JNK had been seen in neuron cell bodies after axotomy.

In healthy neurons, most Syd was bound to kinesin and moved away from the cell body. A small subset was bound to a dynein motor regulatory complex and moved toward the cell body. This is consistent with dynein's minus end–directed movement along microtubules.

After nerve injury, however, JNK was activated and the complex switched allegiance. Most Syd and JNK now bound to the dynein complex and moved back toward the cell body.



Cavalli

Sciatic nerves (in cross section) use a surveillance system to detect damage.

Nerve injury probably prompts a series of signals, starting with disruption of action potentials, and then the lack of growth factors normally transported back from intact synapses. Activated JNK may be the next signal to arrive, followed by proteins with nuclear localization sequences (NLSs). Mike Fainzilber (Weizmann Institute, Rehovot, Israel) and colleagues recently showed that nerve injury prompts translation of axon-localized mRNA into importin β protein. The importin then drags NLS-containing proteins back to the cell body.

How those signals interact to help repair, regenerate, or kill off a damaged nerve is not clear. Cavalli is also interested in whether the cell uses tricks to maintain signals such as JNK during their long journey. WW

Reference: Hanz, S., et al. 2003. *Neuron.* 40: 1095–1104.

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Flying wounded

A fly wounding regimen shows distinct stages, including a curious cell fusion step, and has great promise as a model for vertebrate wounding, according to a poster presented by Michael Galko (Stanford University, Stanford, CA).

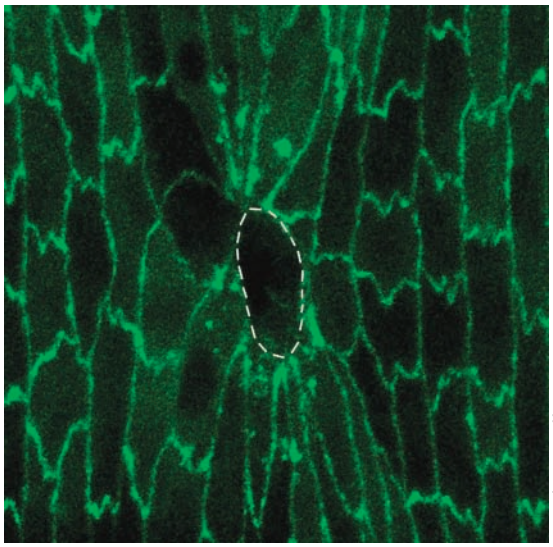
Vertebrate wounding studies have featured complicated tissues and confusing knockouts. Mice lacking supposedly critical components such as debris-scavenging macrophages or the clotting protein fibrin have shown little or no wounding defects.

Thus, researchers have turned to flies. Paul Martin (University College, London, UK) has shown that wound healing in fly embryos proceeds via "purse-string" constriction of an actin cable. But now Galko, working with Mark Krasnow, finds that healing in fly larvae, like that in vertebrates, lacks the cable. Fly larvae and vertebrates are also similar in that both form a clot that becomes a stabilizing scab—a feature missing in fly embryo wound healing.

As the scab is forming, cells near the wound orient toward the wound site and fuse to form a syncytium. The wound gap is closed via lamellipodial extension and cell migration. These later steps of cell migration and reepithelialization, but not the earlier steps of cell orientation and fusion, require activation of Jun kinase (JNK). JNK is known to be activated during vertebrate healing; it may respond to either cytokines or a change in mechanical stresses.

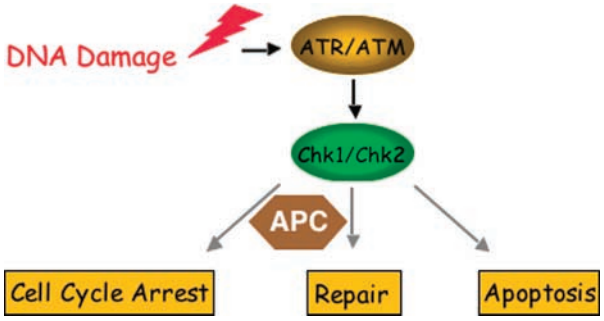
Epithelial cell fusion has not been seen during vertebrate wound healing, although it may have been missed because the vertebrate cells are small and pseudostratified. In the fly, the fusion may be a side-product of the phagocytic action of the epidermal cells, or it may be the fly's way of sealing otherwise leaky and compromised cell–cell junctions. Galko wants to address ambiguities such as these and conduct a genetic screen that will allow him, in this simpler system, to tease out the signals and responses involved in healing. WW

Reference: Wood, W., et al. 2002. *Nat. Cell Biol.* 4:907–912.



Wood/Macmillan

Fly embryo cells orient toward a wound.



Fang

APC lends a hand in both delaying mitosis and repairing DNA.

Stall and repair with APC

The anaphase-promoting complex/cyclosome (APC) is needed to exit mitosis. But new results from Wei-meng Zhao and Guowei Fang (Stanford University, Stanford, CA) suggest that APC can also delay entry into mitosis in DNA-damaged cells and possibly help these cells to do their repair.

Previous genetic evidence suggested that vertebrate cells lacking an APC activator called Cdh1 are deficient in the G_2 damage checkpoint. Fang and Zhao now have evidence that APC is needed at a late stage of this G_2 delay. They saw that, initially, cells were transiently delayed even without APC activity. Later, however, cells needed active APC to maintain a stable p53-dependent arrest. The second delay might be needed for cells to correct extensive damage.

In addition to making time for repair, APC might also help to provide the right tools. During a G_2 delay, activated APC degraded R2, a ribonucleotide reductase that generates dNTP pools. Active p53 then cooperated in stimulating the synthesis of a second form of R2, called p53R2. Although differences in R2 and p53R2 activities have not been shown, Fang hypothesizes that p53R2 might provide low-fidelity polymerases with the high concentrations of dNTP they need to polymerize through damaged DNA templates by incorporating mismatches. As yet, this is still speculation, but Fang plans to test whether p53R2 is less sensitive than R2 to inhibition by high cellular dNTP levels, thereby producing more dNTPs in response to damage. NL

Reference: Sudo, T., et al. 2001. *EMBO J.* 20:6499–6508.

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ES cells: one step forward, one step back

Human embryonic stem (hES) cell researchers battle on, said John Gearhart in his talk, despite both the genomic and political instability in the field.

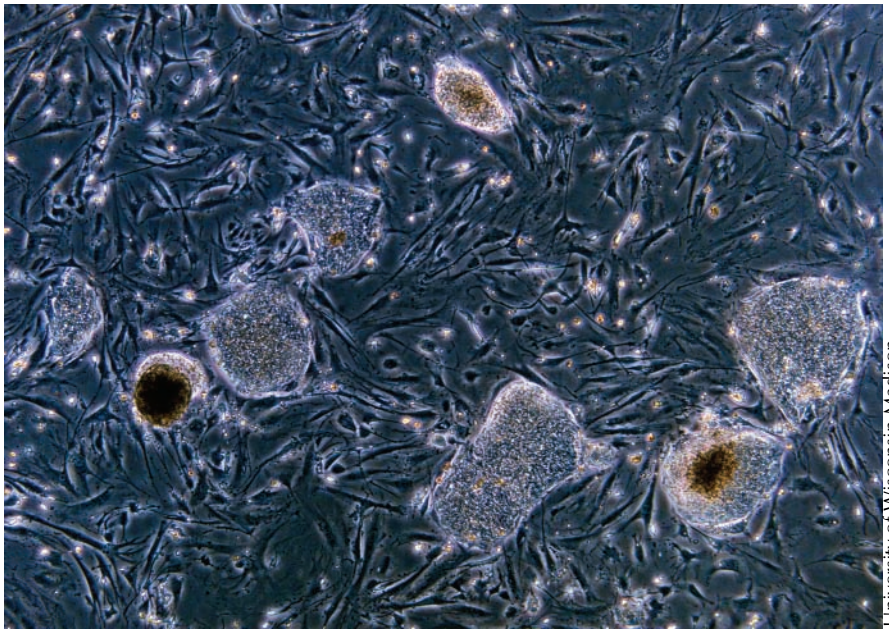
November 1988 was the banner month for human pluripotent stem cells. In that month, Gearhart (Johns Hopkins University, Baltimore, MD) announced his isolation of human embryonic germ (hEG) cells—which have much of the pluripotent promise of hES cells—and James Thomson (University of Wisconsin, Madison, WI) announced the isolation of hES cells.

But progress has been harder to come by since then. US President George W. Bush put a chill on the research when he dictated that only hES cell lines created before 9:00 pm EST on August 9, 2001, could be used in federally funded research. And reports from various sources have stated that hES cells are unstable both in terms of imprinting and chromosome segregation. Thomson recently published a paper describing gains of all or parts of chromosomes 12 and 17 in multiple different hES cell lines.

Gearhart has seen the chromosomal instability himself, and is not entirely surprised. "You can argue [that hES cells] are artifacts of culture to begin with," he says. "You've taken these [normally transient] cells out and you're culturing them long-term." Perhaps, he says, the body has mechanisms that break down or try to get rid of hES-like cells that mistakenly persist for too long in the body, and the same program is being switched on in culture.

Culturing protocols may not help matters. The culturing of the slow growing hES cells "is very difficult to do," says Gearhart. "It's still a bit of an art." And novices may make things worse by picking the cultures that are the least sluggish, and thus most likely to harbor errors and mutations.

The solution is simple. Cultures must be frequently karyotyped and tested for growth rate changes. "You have to be vigilant," says Gearhart. "We're now aware of it, and we need to monitor it."



University of Wisconsin-Madison

Human embryonic stem cells are genomically and politically unstable.

Meanwhile, Gearhart has been assessing the implications of the Bush policy on any potential hES therapeutic trials. A multidisciplinary panel convened at Johns Hopkins concluded recently that "it is unethical to expose human subjects to the stem cell lines that are currently approved for use in federally funded research" because these lines were derived in the presence of mouse feeder cells that may have harbored viruses. Isolation of hES cell lines with human cells as feeders has now been reported.

If hES cells are to be used in a transplant they will need to be a close immunogical match to the recipient. A group headed by Gearhart recently calculated the number of hES cell lines needed to match different percentages of the US population at three important immunological loci. They concluded that the first priority should be a research hES cell line bank that covers as many people as possible. For example, 14 homozygyous lines could match ~25% of all Americans including 5–10% of those in minority racial groups. A longer term necessity is a therapy bank that would cover a similar percentage of individuals from all racial groups, even though this would require many more cell lines for groups (such as African-Americans) that are immunologically more diverse.

Currently, such efforts could not be supported by US federal funds, but construction of a bank of hES cells is underway in the more permissive UK research environment. Meanwhile, there are other encouraging developments. Ali Brivanlou (Rockefeller University, New York, NY) recently found that inhibition of the Wnt pathway maintains hES pluripotency. And Gearhart says that interest in hES cells continues to grow. Attendance at meetings and workshops is up, as are grant requests. "These are all good signs that the interest is there," says Gearhart, "and it's building." WW

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