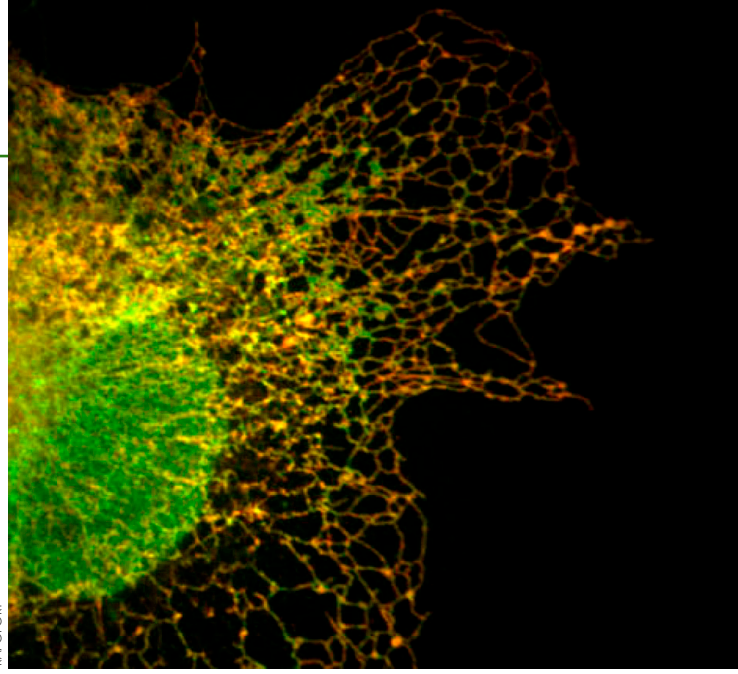
Meeting Report



RAPOPORT

Rtn4 (red) marks ER (green) tubules.

Shaping curved organelles

Tom Rapoport (Harvard University, Boston, MA) presented his group's efforts to understand how organelle shapes are formed and maintained. The findings identify two protein families that create tubules out of ER membrane.

A sphere is the most stable membrane shape; deformations that increase membrane curvature cost energy and must be actively stabilized. Rapoport and colleagues, including Gia Voeltz, used an in vitro system for ER network formation to identify factors that create curved membrane shapes. With just membranes, salt, and GTP, their system produces a network of ER tubules.

This simple, self-contained system was not ideal for identifying the shape-creating components. "When we first saw [that] everything was already in the membrane," said Rapoport in his talk, "we thought, 'what a bummer.'" But the group found a way around this difficulty by using small molecule inhibitors to block in vitro ER formation, and then identifying the inhibitors' targets.

One such target was an integral membrane protein called Reticulon4a (Rtn4a), previously named for its localization to ER membranes. All eukaryotes express at least one homologue of Rtn4a, and the proteins are the first known markers specifically localized to the tubular ER and absent from sheets.

Cells overexpressing Rtn proteins formed more tubules, but loss of the two yeast members did not prevent tubule formation under normal conditions. Only when mutant cells were subjected to osmotic stress were their tubules lost.

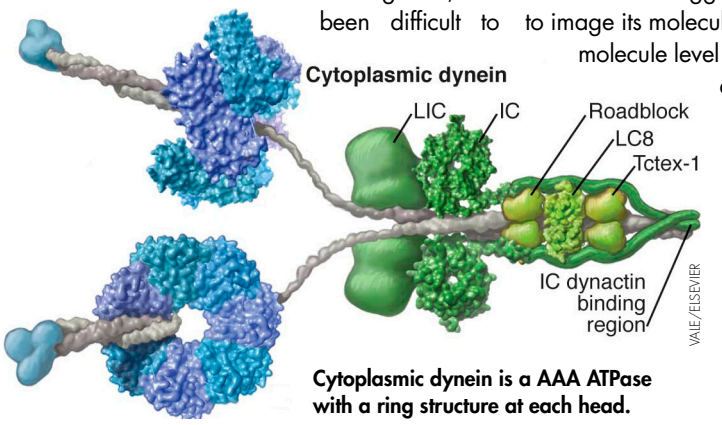
Rtn proteins form homo- and hetero-oligomers, so the group figured that another Rtn-interacting protein might be required for tubule formation. Indeed, they found that Rtn pulled down another ubiquitous integral membrane protein called DP-1. Loss of both the yeast DP-1 and the more abundant of its two Rtns now blocked tubule formation.

The group has proposed that Rtn and DP-1 might be wedge-shaped, with their wider sides in the outer membrane leaflet. The presence of these proteins would thus favor a highly curved membrane. They now plan to test whether purified Rtn and DP-1 can turn liposomes into tubules. **NL**

Reference: Voeltz, G. 2006. *Cell.* doi:10.1016/j.cell.2005.11.047.

Dynein steps in line

Transport within a cell occurs with the help of three classes of motor proteins: myosin, kinesin, and dynein, which carry their cargo along cytoskeletal tracks. Conventional kinesin and myosin V move processively—that is, they remain bound to their tracks for many steps. But the minus end–directed motor dynein, because of its large size and many ATP binding sites, has been difficult to study. Samara Reck-Peterson (University of California, San Francisco, CA) described recent experiments that demonstrate that, despite major structural differences, dynein's stepping mechanism has at least some similarities to that of kinesin and myosin.



VALE/ELSEVIER

Cytoplasmic dynein is a AAA ATPase with a ring structure at each head.

Using *S. cerevisiae,* Reck-Peterson and colleagues, led by Ronald Vale, engineered a recombinant version of dynein that could be tagged at various positions to image its molecular motion at the single molecule level in vitro. This technique allowed the researchers to visualize directly dynein's ability to move processively. As single monomers of the construct were not processive, Reck-Peterson showed that dynein achieves its processivity as a dimer.

Previous studies showed that both conventional kinesin and myosin V walk in a "hand-over-hand" manner, with step sizes that are based on the distance between binding sites on the microtubule (~8 nm) or the actin filament (~37 nm). Dynein, which is an AAA ATPase, differs both evolutionarily and structurally from kinesin and myosin. By measuring the change in position of a single-labeled head and of a central portion of the protein, Reck-Peterson and colleagues found that dynein, too, coordinates the action of its two motor domains and takes approximately 8-nm steps.

As rings on some AAA ATPases arrange in stacks, Reck-Peterson suggested that dynein might achieve these small steps by stacking and shuffling the position of the two rings of the dimerized protein. **AK**

Reference: Reck-Peterson, S.L., and R.D. Vale. 2004. *Proc. Natl. Acad. Sci. USA.* 101:1491–1495.

Text by Nicole LeBrasseur: lebrasn@rockefeller.edu
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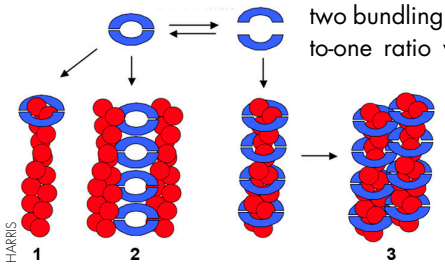
Variety in formin bundlers

Formins nucleate new actin filaments and accelerate polymerization rates. Elizabeth Harris (Dartmouth University, Hanover, NH) presented her studies from Henry Higgs lab that reveal a new function for mammalian formins—the ability to bundle existing filaments.

Formin's actin activities rely on its dimeric, donut-shaped FH2 domain. This domain, Harris showed, is sufficient for bundling for the mDia2 and FRL1 formins. mDia1, in contrast, had no bundling ability.

According to current models, the FH2 dimer sits on actin barbed ends as a washer would on the tip of a screw. This interaction did not seem like the sort that would lead to bundling, so Harris wondered whether formins might also bind further down actin filaments. Indeed, she found that dimers of the two bundling formins bind in a one-to-one ratio with actin subunits in the filament.



HARRIS

FH2 dimers might bundle actin filaments through either an interface (2) that is distinct from barbed-end binding (1) or a similar interface (3) that requires dimer dissociation.

FH2 dimers are thought to be very stable, based on structural and biochemical studies on the yeast formin Bni1p. Harris wondered how so many dimers could bind at the tip and slide down the filament quickly enough to account for their rapid bundling kinetics. She has now found that the FH2 domains of mDia2 and FRL1, unlike Bni1p (and mDia1), are able to dissociate and reassociate.

Harris imagines that the unstable FH2 domains are able to clamp an actin filament anywhere along its length as the monomers reassociate. Her in vitro experiments support this idea for FRL1, but suggest that mDia2 acts differently.

As her theory predicts that barbed-end binding and side binding should be competitive (because both should require interactions with the same residues), Harris increased the concentration of barbed ends. The bundling activity of FRL1, as predicted, decreased accordingly. But the same was not true of mDia2. And mutations in the inner ring of mDia2's donut—where barbed-end binding occurs—did not interfere with its bundling activity. Thus, mDia2 might use residues on the outside of the donut to bind to and bundle actin filaments.

Two other formins (yeast Bnri1p and an *Arabidopsis* formin) have also been shown to bundle in vitro. Still, the field has yet to prove that formins bundle in cells. But if the test tube studies hold true, it may be that formins rearrange actin networks even as they assemble the filaments (as in filopodia, for example). More conventional bundlers, such as fascin, might then be brought in to stabilize the networks. **NL**

Reference: Harris, E.S., et al. 2004. *J. Biol. Chem.* 279:20076–20087.

STAT6 escapes from cilia and harms kidneys

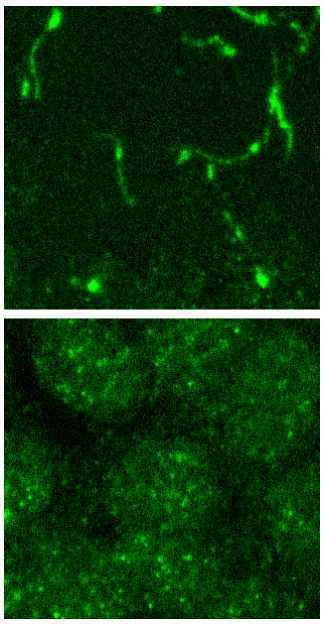
In the past few years, several mutations causing polycystic kidney disease (PKD) were shown to affect cilia proteins in both human cases and mouse models of the disease. But the functional link between epithelial cilia and the overgrowth of kidney tissue that marks this disorder was not clear. Thomas Weimbs (University of California, Santa Barbara, CA) reported that cellular overgrowth in PKD is driven by a transcription factor after it escapes from an unusual sequestration site—epithelial cilia.

Weimbs and colleagues were studying the cellular effects of dominant-negative versions of a PKD-associated protein called polycystin-1 (PC-1). Although PC-1 is a cilial integral membrane protein, one of the dominant-negative versions was found in the nucleus. A nuclear PC-1 fragment also occurs naturally—expression of full-length PC-1 in cell culture resulted in its proteolytic cleavage and the nuclear accumulation of the cytoplasmic tail.

In separate immunoprecipitation experiments, the group had discovered that PC-1 interacted with a transcriptional coactivator called P100. The interaction was also seen by colocalization at basal bodies and in cilia.

One of P100's well-known partners is the proliferation-inducing transcription factor STAT6. The group now finds that, under normal kidney apical flow conditions, STAT6 localizes to epithelial cilia along with PC-1. But when they disrupted flow, as might occur upon kidney injury, STAT6 and PC-1 moved to the nucleus.

Weimbs' model is that cleavage of PC-1 is flow-sensitive, such that the loss of flow frees the cytoplasmic domain. This portion then moves to the nucleus, bringing along its bound P100 and STAT6 and inducing the gene expression needed for wound healing. Indeed, overexpression of the cytoplasmic PC-1 fragment induced STAT6-dependent gene expression in cell culture and caused renal cysts to form in zebrafish embryos.

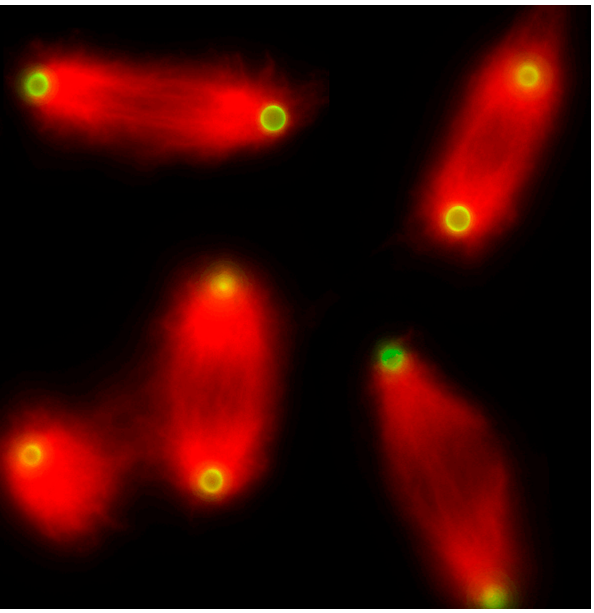

WEIMBS/ELSEVIER

STAT6 (green) is normally at cilia (top), but moves to the nucleus when fluid flow stops (bottom).

As the team found that PKD patients had high levels of nuclear PC-1, STAT6, and P100, the disease might be due to constitutive activation of this injury response pathway. Small molecule inhibitors, perhaps of the as-yet unidentified protease that cleaves PC-1, might therefore be promising PKD therapeutics. **NL**

Reference: Low, S.H., et al. 2006. *Dev. Cell.* 10:57–69.

Nuclear protein supports spindle



ZHENG/ELSEVIER

AurA magnetic beads (yellow) organize microtubules (red) into spindles in the presence of RanGTP.

The start of mitosis involves major architectural changes in the cell, culminating in the construction of a mitotic spindle. Yixian Zheng (Carnegie Institute of Washington and Howard Hughes Medical Institution, Baltimore, MD) proposed that an interphase nuclear lamina protein called Lamin B gives the spindle crucial structural support as it takes shape in mitosis.

To understand spindle assembly, Zheng's group turned to nuclear components, reasoning that they may have become fixed during the evolution of the eukaryotes because they helped the cell to divide. Indeed, her team and others have identified a handful of nuclear proteins that are important during mitosis.

In earlier work, Zheng's team and others found that Ran, a GTPase required for nuclear trafficking during interphase, forms a gradient along the chromosome that kick-starts mitosis by stimulating spindle microtubule assembly. In addition, Zheng's lab showed that Ran also activates a mitotic kinase, called Aurora A, which regulates microtubules to form the spindle with the help of other proteins.

Using a novel assay for spindle assembly involving the coupling of Aurora A antibodies to magnetic beads, the group's new experiments in *Xenopus* egg extracts show that Ran has another regulatory function in spindle assembly: it regulates Lamin B during mitosis. Lamin B localized to the spindle, and depleting it inhibited spindle formation. But Lamin B did not bind to microtubules, suggesting that it affects spindle assembly indirectly. In the nucleus, lamins form a filament system that is important for various nuclear functions. The researchers found that Lamin B may also have a structural role during mitosis, as it assembled around the spindles as a polymer.

"A mitotic scaffold has been hypothesized for decades, but molecular components have remained elusive," Zheng said in her talk. Lamin B may be just one of such components. It probably acts on spindle assembly factors, tethering them in place so that mitosis can proceed. **AK**

Reference: Tsai, M.Y., and Y. Zheng. 2005. *Curr. Biol.* 15:2156–2163.

Polarity from transport

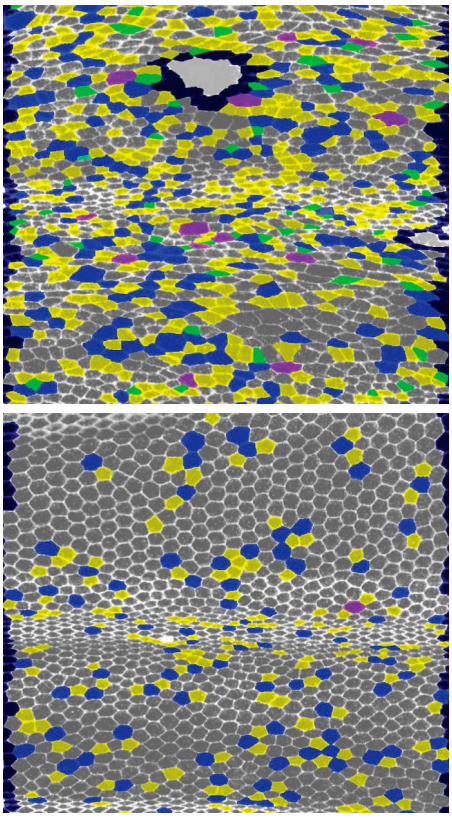
What do fly photoreceptor clusters and wing cells have in common with vertebrate inner ear hair cells and convergent extension? In all cases, tissue function depends on orderly cell packing and planar cell polarity (PCP). All four also require a highly conserved gene cassette encoding PCP proteins. Anne-Kathrin Classen (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany) suggested how PCP proteins control such diverse processes by directing vesicle transport.

The fly's epithelial wing cells reorganize into a perimeter-minimizing hexagonal packing arrangement at the pupal stage. Observing the process with confocal microscopy, Classen and colleagues, led by Suzanne Eaton, found that repacking occurred via E-cadherin–mediated remodeling of cell contacts at intercellular junctions.

Remodeling requires both E-cadherin internalization and its deposition via exocyst vesicles. PCP proteins, which are located near the cell membrane, attracted exocyst vesicles carrying E-cadherin and thus directed how the cell remodeled its contacts. Mutant cells lacking PCP proteins formed fewer new boundaries with neighboring cells and had impaired hexagonal repacking.

When cells orient, they must make certain connections and release others. "The unifying feature [of PCPs] may be to regulate the polarized trafficking of exocyst components," Eaton says. The cargo that exocysts carry could differ in each case. For example, whereas the cargo for convergent extension and wing cell packing should include cadherins, signaling proteins such as Delta may be the relevant cargo for orienting photoreceptor clusters. **AK**

Reference: Classen, A.-K., et al. 2006. *Dev. Cell.* 9:805–817.



EATON/ELSEVIER

During wing development, cells of varying shapes (top) remodel their connections to take on mostly hexagonal shapes (bottom).

Proteins turn cytokinesis sideways

It's old news that, during cytokinesis, actin and myosin accumulate at the cell's equator, forming a contractile ring that pinches the cell together like a purse string to divide it in two. What is less well-known is that these proteins localize and constrict asymmetrically. Ingression starts on one side of the division plane and progresses almost to its center before the other side starts to furrow in as well. Amy Maddox (University of California, San Diego, CA) reported that this asymmetry is generated by two conserved components of the contractile ring: anillin and the septins.

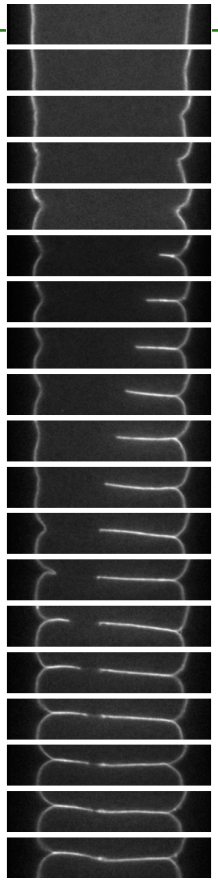
The group, led by Karen Oegema, had previously observed this asymmetry and surmised that it might be important for cell division. First, they imagined, one-sided ingression may rapidly create a barrier between the two masses of segregated chromosomes, ensuring that both do not end up in the same daughter cell. Second, in dividing epithelial cells, the upwards ingression of a cleavage furrow from the basal membrane might allow apical cell–cell junctions to remain intact.

In the present study, Maddox and colleagues imaged dividing cells and found that the septins, anillin, and actomyosin localize asymmetrically around the circumference of the contractile ring. Depleting cells of septins or anillin via RNAi left cytokinesis intact—but symmetrical. Without these proteins, actin and myosin were evenly distributed around the contractile ring, which contracted isometrically to cleave the cell.

Further experiments showed that, whereas actin and myosin are required for cytokinesis, anillin and the septins seem to make the process more robust. When the researchers weakened the contractile ring by partially compromising myosin function, the cell was still able to divide. However, when anillin was depleted from this sensitized background, cytokinesis failed and the cells became multinucleated.

Septins are GTP-binding, filament-forming proteins that were recently demonstrated to form a structural meshwork in the cell cortex. Anillin is a structural protein that appears to stabilize the cytoskeleton during cell division. Maddox suggested that, as anillin can bind to septins, actin filaments, and myosin, it may cross-link them, perhaps helping to arrange actin and myosin in relation to each other both to promote their uneven distribution around the contractile ring and to increase the efficiency of constriction. **AK**

Reference: Audhya, A., et al. 2005. *J. Cell Biol.* 171:167–179.



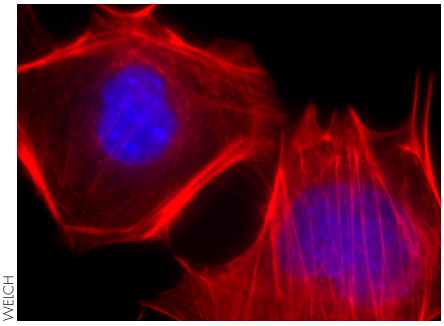
OEGEMA

Furrowing is asymmetric in *C. elegans* embryos.

An Arp2/3 activator for transport?

Membranes, microtubules, and actin might be connected via an Arp2/3 activator. Matt Welch (University of California, Berkeley, CA) described this newly identified actin nucleation activation factor, which he calls WHAMM, for WASP homology actin microtubules and membranes.

Known Arp2/3 activators include the WASP and WAVE/Scar families. In flies, mammals, and yeast, Arp2/3 and many of its known activators are essential proteins. Since they are so important to survival, Welch wondered if other regulators exist.



WELCH

WHAMM is one of several proteins that activate Arp2/3, which helps create actin (red) networks.

For his search, Welch looked to the Arp2/3-binding region (called the WCA domain) that is common to WASP and WAVE proteins. He then used a bioinformatics approach to identify other evolutionarily conserved proteins containing WCA sequences. Among others, his search turned up WHAMM.

As Welch had hoped, WHAMM promoted actin filament nucleation in vitro by activating Arp2/3. But he wondered what makes WHAMM necessary, as WASP and WAVE proteins are already doing this same job. The subcellular localization of the new protein gave him some ideas.

Although WAVE is located at the plasma membrane, where it regulates cell migration, WHAMM was found in a perinuclear compartment that might correspond to Golgi cisternae or late endosomes. It was also found on as-yet unidentified tubular membrane structures.

Domain-mapping experiments showed that, in addition to a domain for perinuclear membrane binding, WHAMM also contains a domain that interacts with microtubules. When expressed alone, this coiled-coil domain bundled and rearranged microtubules. Welch has yet to show that the full-length protein can also reorganize microtubules in vitro, but it does align with microtubules and with actin filaments in cells.

The binding of WHAMM to microtubules and to membranes might regulate its Arp2/3 activation function. If so, WHAMM might theoretically coordinate transport to and from the Golgi, for example, by synchronizing membrane movements with cytoskeletal rearrangements. **NL**

Reference: Goley, E.D., et al. 2004. *Mol. Cell.* 16:269–279.

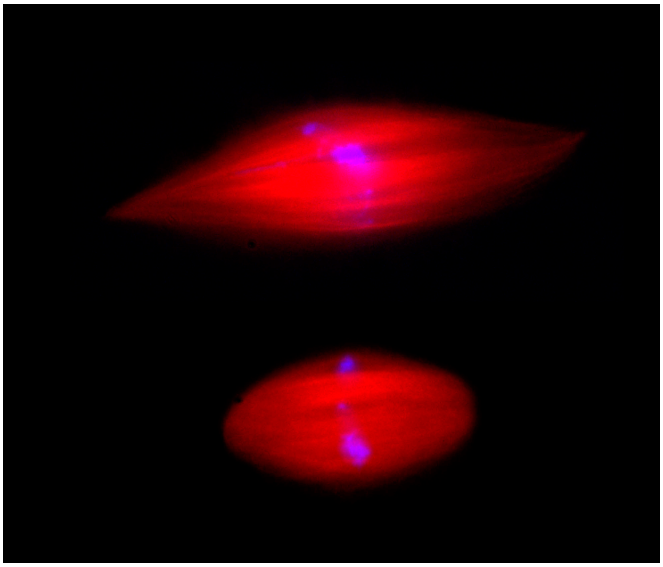
Motors help shape spindle

In many species, chromatin signals can induce spindle assembly. But whether features of spindle shape depend on the chromatin itself or on other components of the cytoplasm has remained under debate. Jedidiah Gaetz (Rockefeller University, New York, NY) described new results showing that several properties of spindle shape are indeed regulated independently of chromatin.

Gaetz and colleagues, headed by Tarun Kapoor, placed DNA-coated magnetic beads into a magnetic field to construct chromatin strands in cell extracts missing key organizing components such as centrosomes and kinetochores, and imaged spindle assembly in this simplified system. Spindle length and organization did not change, regardless of the length of the chromatin, suggesting that the chromatin signal merely induces assembly, leaving the details to other players.

Some of these players may be microtubule-associated proteins, such as dynein, which have been linked with spindle assembly. Previous work from the group showed that dynein, a microtubule minus end–directed motor, controls spindle length by regulating tubulin subunit depolymerization at the poles.

In the present study, Gaetz reported, inhibiting dynein led to spindle fibers extending as strands along the entire length of the chromatin, their minus ends failing to join up to form a pole at all. Dynein is known to grab microtubules and pull them along other microtubules to bring their minus ends together. The researchers thus speculate that dynein is depleting surrounding regions of microtubules or microtubule associated proteins to ensure that spindle organization occurs in just one place. **AK**



KAPOOR

Dynein inhibition increases spindle length (top) compared with control cells (bottom).

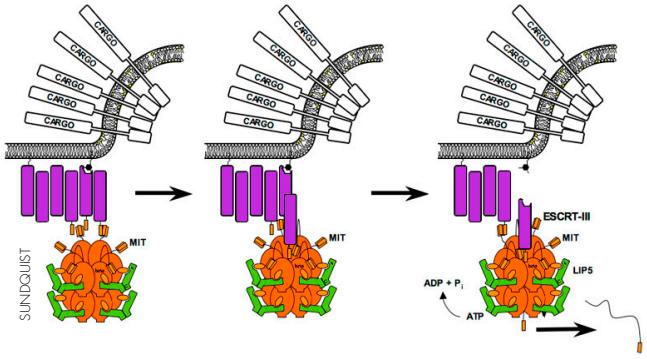
Reference: Gaetz, J., and T.M. Kapoor. 2004. *J. Cell Biol.* 166:465–471.

Pumping proteins for viral budding

With too few genes to set up its own export system, viruses deftly appropriate their host cell's endosomal machinery to get themselves repackaged in vesicles and shipped on to the next cell. Most viruses hook into the process via one of a series of protein complexes that form a membrane-bound lattice essential for multivesicular body formation, but the mechanism of subsequent viral budding is poorly understood.

Earlier research by Wes Sundquist (University of Utah, Salt Lake City, UT) showed that HIV packaged itself for export by recruiting an early-acting lattice component called ESCRT-I. In his talk, Sundquist described new research on a novel HIV budding pathway that hooks into later lattice steps that depend upon the ESCRT-III complex and an AAA ATPase protein called Vps4.

Structure and function studies of Vps4 suggested that, when the protein is activated by ATP, it forms hexameric rings that are recruited to the site of vesicle formation through interactions with ESCRT-III proteins. Sundquist's team then compared the structure of Vps4 to other proteins in the AAA ATPase family, including pore pumps that denature DNA and RNA, and bacterial chaperones that denature misfolded and aggregated proteins.



Vps4 (orange and green) may bind ESCRT-III proteins (purple) and denature them by pulling them through its central pore.

Based on this homology, Sundquist proposed that Vps4 similarly acts as a protein pump, binding to ESCRT-III proteins and pulling them up into the narrow central pore, where they are unfolded. When Vps4 was mutated by disabling a tag on its inner pore, HIV budding was strongly impaired, suggesting that ESCRT-III proteins must travel up into the Vps4 pore for viral escape.

The group is now studying how exactly ESCRT-III is coupled to vesicle formation. One possibility is that the release of ESCRT-III subunits may be necessary for membrane fission. "We are starting to understand what parts of Vps4 are important," says Sundquist. "But we can't say in mechanistic terms why ESCRT-III has to be unfolded for budding to take place, because we don't understand how you make a vesicle." **AK**

Reference: Scott, A., et al. 2005. *EMBO J.* 24:3685–3669.

Mismethylation in progeria

Premature aging and abnormal DNA methylation patterns both occur in cloned animals such as Dolly the sheep. In a talk by Dale Shumaker (Northwestern University, Chicago, IL), aging and histone methylation were linked by the disease progeria.

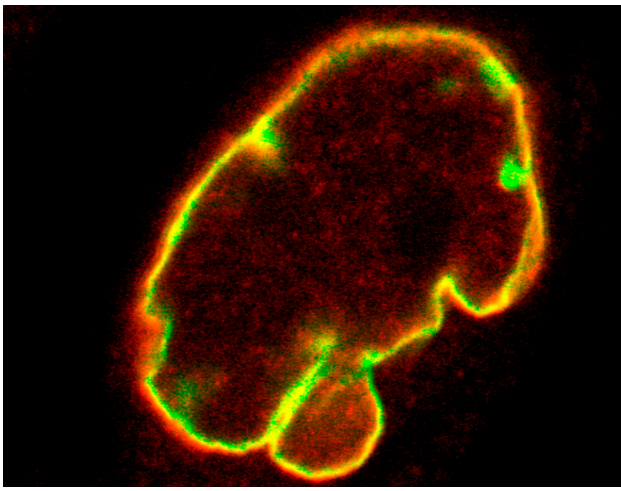
Progeria—the rare and mysterious disease of rapid childhood aging—is caused by dominant-negative mutant versions of the nuclear intermediate filament protein lamin A. Many nuclear morphological defects arise in cell lines that express this mutant lamin A: pore complexes cluster, the lamina thickens, lobulation occurs, and heterochromatin is lost. But exactly which consequence of the lamin mutation leads to disease is far from untangled. Shumaker and colleagues, working in Robert Goldman's laboratory, now find that histone methylation abnormalities appear long before these nuclear changes take place.

As heterochromatin is lost over time in progeria cells, the group examined whether specific chromatin modifications, such as histone methylation, might also be altered. Changes were measured by monitoring methylation on the inactive X chromosome. The group found that fibroblasts isolated from a progeria patient had fewer H3K27me3 methylation marks on the inactive X chromosome.

Transcript levels of the EZH2 transmethylase responsible for this epigenetic mark were strongly reduced in progeria cell lines. Lamins that comprise the lamina lining the inner nuclear membrane, as well as those dispersed throughout the nucleoplasm, act as a platform that supports and regulates DNA replication and Pol II–mediated transcription. Shumaker hypothesized that they might also support the multisubunit methylase complex.

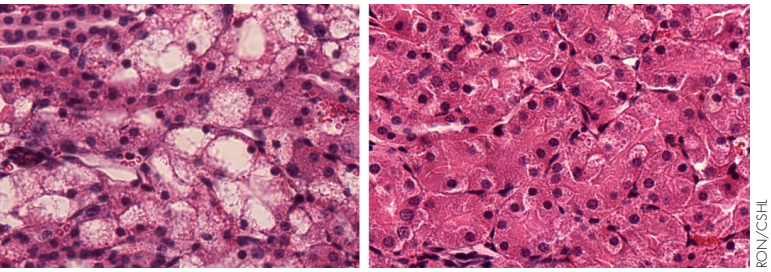
Mutant lamin A in progeria cells is known to remain inappropriately farnesylated, and thus has an unusually high affinity for the nuclear membrane (most likely causing the thickened lamina of progeria nuclei). If the lamina is indeed a large molecular scaffold, these physical changes might impair EZH2 function, possibly leading to negative feedback of EZH2 transcription. How methylation and heterochromatin influence aging, however, remains to be seen. **NL**

Reference: Goldman, R.D., et al. 2004. *Proc. Natl. Acad. Sci. USA.* 101:8963–8968.



GOLDMAN/NAS

Mutant lamin A (green) causes nuclear lobulation and progeria.



RON/CSHL

Cell death from ER stress is diminished by loss (right) of a CHOP-induced phosphatase that reactivates translation.

CHOPping down stress

David Ron (New York University, New York, NY) is dealing with stress. In his talk, Ron discussed two ways that cells cope with the stress of unfolded or misfolded proteins in the ER.

In the first part of his talk, Ron showed that mutant fibroblasts lacking the CHOP transcription factor are better able to survive ER stress that would kill normal cells. As CHOP activates apoptotic pathways, the group figured it killed normal stressed cells via its induction of death effectors such as Bax and Bak. But instead they found that CHOP counteracted the translational dampening that ER stress induces.

Normally, translation is down-regulated by stress to give the ER a bit of time to deal with the excess unfolded proteins. A negative feedback loop then leads to translational recovery. This loop, Ron showed, includes CHOP-mediated induction of a phosphatase that reactivates eIF2α.

The "lazy" CHOP knock-out cells, as Ron called them, keep translation down longer, and thus survive acute stressors such as tunicamycin because they have fewer proteins to fold. He speculated, however, that CHOP mutations might not be helpful under a more physiological stress such as wounding, which requires the translational up-regulation of collagen to help in healing.

Another protein that helps cells control the ER protein load, Ron showed, is $P58^{IPK}$. His group has found that $P58^{IPK}$ mutants turn on the unfolded protein response even in the absence of other stresses.

$P58^{IPK}$ is part of a family of cochaperones that work with Hsp70 proteins, and it is associated with the cytoplasmic side of the translocon. Ron proposed that $P58^{IPK}$ monitors the normal passage of ER proteins through the translocon. Those that stall as they pass through (due to unfavorable lumenal ER conditions, for instance) would be exposed to $P58^{IPK}$, which might lead to their extraction from the translocon and degradation. To this effect, Ron showed that the degradation of at least one stalled protein, $ApoB^{100}$, requires $P58^{IPK}$. **NL**

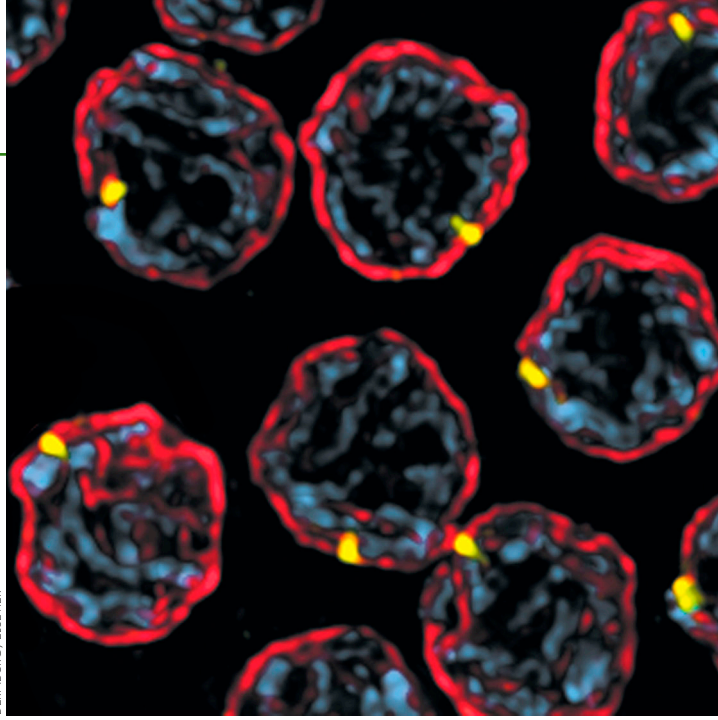
Reference: Marciniak, S.J., et al. 2004. *Genes Dev.* 18:3066–3077.

Her chromosomes paired by HIM

A family of zinc finger proteins plays matchmaker for worm chromosomes, as presented by Abby Dernburg (University of California, Berkeley, CA). Her work reveals site-specific DNA binding proteins that pair up homologues during meiosis.

Homologues must first come together before they can be equally distributed to daughter cells during meiosis. Pairing centers are the chromosomal sites that, in worms, are necessary for this coupling. Dernburg's group has now identified the trans-acting factors that recognize these sequences and make the correct matches.

The findings were led off by the group's studies of a mutation that results in the birth of mostly male worms. This *him-8* mutation created segregation defects due to ineffective meiotic pairing and synapsis. But unlike most pairing mutants, the *him-8* problems did not extend to the other chromosomes. Only X chromosome segregation was affected, which got Dernburg excited.



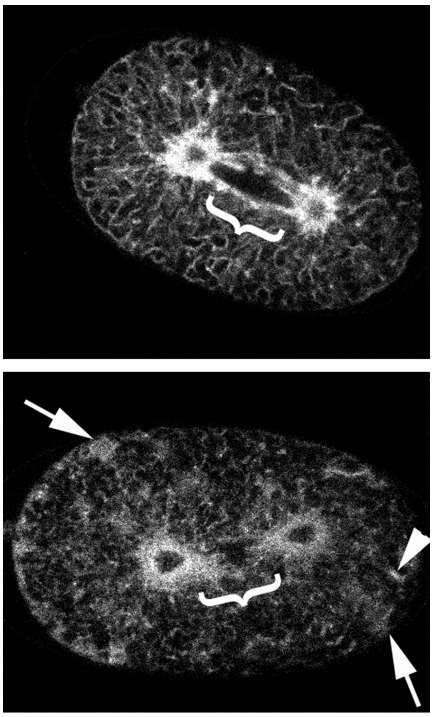
DERNBURG/ELSEVIER

Him8 (yellow) marks X chromosome pairs at the nuclear envelope (red).

Her group then cloned *him-8* and determined that it encodes a Zn-finger protein, perfect for interacting with DNA. As expected, HIM-8 concentrated at the X chromosome's pairing center. It also had an unexpected nuclear envelope localization. Dernburg imagines that the nuclear envelope might be a scaffold for pairing and synapsis.

Her more recent work has revealed that relatives of HIM-8 pair the other worm chromosomes. Some even control two different sets of homologues, which implies that initial pairing by HIM-8 relatives is not the final word. In her talk, Dernburg hypothesized that pairing centers and the Zn-finger proteins "create stable intermediate states where chromosomes assess their homology." But these pairs are readily reversible in case the wrong sets are brought together. **NL**

Reference: Phillips, C.M., et al. 2005. *Cell.* 123:1051–1063.



WHITE/ASCB

CAR-1 depletion (bottom) disrupts ER organization (arrows and arrow head) and reduces spindle-associated ER (bracket).

Membrane at the spindle

During cytokinesis, membrane must be quickly deposited at the scission site to close the gap between the daughter cells, often resulting in excess membrane accumulation at the site. Jayne Squirrell (University of Wisconsin, Madison, WI) presented new data suggesting that this membrane deposition involves proper ER organization by the protein CAR-1.

The sequence of *car-1* suggests that it encodes an RNA-associated protein, but CAR-1 has also been implicated in cytokinesis. Its homology to a suppressor of clathrin deficiency in yeast, along with its role in cytokinesis, suggested that this protein might be involved in membrane trafficking during cell division. When Squirrell and colleagues, led by John White, depleted CAR-1 in *C. elegans*, they found that embryos failed to complete the last stage of cytokinesis and that membrane did not accumulate at the scission site.

These CAR-1–depleted embryos had other problems, including defects in ER dynamics. Recent studies identified an arrangement of ER around the spindle and centrosomes during cytokinesis in worm and fly embryos. CAR-1 depletion led to the disruption of this structure. Further studies showed that CAR-1 localized to the spindle region with a similar distribution to that of the ER.

The group is further investigating CAR-1's association with the ER and the function of the ER at the spindle. Squirrell suggested that CAR-1 may be one of the organizing forces behind this ER arrangement, which might contribute to the maintenance of membrane at the scission site. Additionally, the two may work in tandem to stabilize the spindle structure. The ER is a regulator of calcium levels, and calcium causes spindle instability. "Perhaps the ER regulates calcium levels in a very local region for maintaining proper spindle structure at the right time," Squirrell said. **AK**

Reference: Squirrell, J.M., et al. 2006. *Mol. Biol. Cell.* 17:336–344.