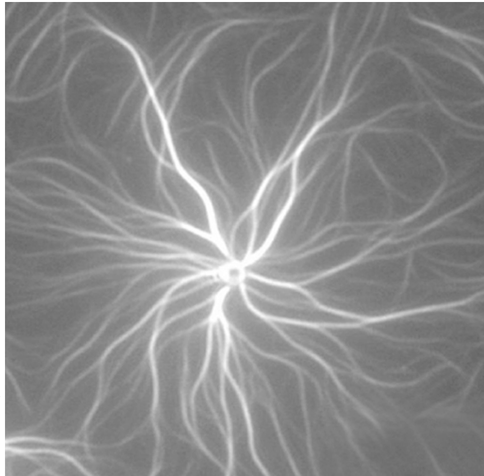
Research Roundup



Actin filaments growing out from a formin-coated bead can group together into stable cables.

Actin's stable cable

Individual actin filaments are built and broken randomly; but line them up side-by-side, and they form breakage-resistant cables, report Alphee Michelot, Laurent Blanchoin (CEA, Grenoble, France), Julien Berro, Jean-Louis Martiel (TIMC, Grenoble, France), and colleagues.

Actin filaments power multiple cellular processes such as motility, morphogenesis, and polarity, but the mechanisms controlling their dynamics is poorly understood. Michelot et al. investigated the dynamics of actin building, bundling, and breakage in vitro by covering polystyrene beads with an actin filament–promoting protein called formin and then adding fluorescently labeled actin monomers.

The formin and actin monomers were enough to induce continuous actin polymerization at the surface of the bead. When an actin-severing factor called cofilin was added to the mix, filaments began to switch rapidly between elongation and shortening. The shortening always occurred from the filament's "older" end (the one further from the bead). According to Blanchoin, this end preference results from the gradual conversion of each actin monomer's ATP to ADP after its incorporation into the filament, as only the ADP form is a suitable cofilin substrate.

As filaments grew out from the bead, neighboring filaments often "zipped" together to form thicker multifilament cables. These cables were considerably more resistant to cofilin severing than were individual filaments, probably because cofilin cuts one filament at a time and thus would only nick the cable rather than chop right through.

The in vitro system used by the authors involved a minimal set of proteins and yet show that actin filaments can generate their own stability simply by grouping together. The team now plans to add other actin associated factors, one by one, to observe how increasing complexity affects actin dynamics. JCB

Reference: Michelot, A., et al. 2007. *Curr. Biol.* doi:10.1016/j.cub.2007.04.037.

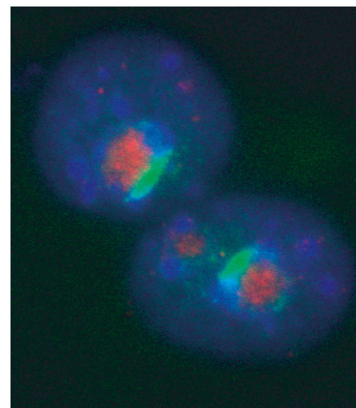
Positioning X for inactivity

In female mammalian cells, switching off the second X chromosome requires coating it in noncoding *Xist* RNA and converting it into heterochromatin. Li-Feng Zhang, Khanh Huynh, and Jeannie Lee (HHMI, Boston, MA) now suggest that this inactive state is maintained in each cell division by a visit to the periphery of the nucleolus.

A study in 1949 revealed that the inactive X (Xi) appeared as a nucleolar satellite in cat neurons. Despite this finding, the overwhelming impression in more recent decades has been that, in most cell types, the Xi is associated with the periphery of the nucleus, not nucleolus. The nuclear periphery, however, is also the preferred location for the active X (Xa). Using mouse embryonic stem cells, Zhang and colleagues now show that, although both Xs are often seen at the nuclear periphery, Xi takes a trip to the nucleolus at S phase, while Xa stays put.

Positioning of Xi at the nucleolus required *Xist* RNA, which is transcribed from within the X inactivation center (*Xic*). The *Xist* gene is only activated on Xi, and its noncoding product spreads along Xi, coating and silencing the entire chromosome. Chopping out the *Xic* led to an immediate loss of this *Xist* coating and of Xi's association with the nucleolus.

Previous studies implied that the *Xic* and *Xist* were necessary for the initiation of X inactivation but dispensable for its maintenance. Zhang et al. found that, immediately after the removal of the *XIC*, heterochromatin marks on Xi did indeed remain even after the *Xist* RNA had left. After several cell cycles, however, these marks began to be erased and Xi genes began to reactivate. Previous studies might have missed this maintenance role for Xist because they did not examine silencing long enough.



The inactive X chromosome (green) visits the nucleolus (red) to maintain its inactivity.

The authors believe that the *Xist*-dependent nucleolar recruitment of Xi is necessary for long-term maintenance of its inactivation. In support of this hypothesis, the team found that a ring around the nucleolus contained a chromatin-remodeling protein that is required to reestablish heterochromatin on newly replicated DNA. JCB

Reference: Zhang, L.-F., et al. 2007. *Cell.* 129:693–706.

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Cilia signaling

It takes well-built cilia to send developmental signals on their way, show Tamara Caspary, Kathryn Anderson (Sloan-Kettering Institute, New York, NY), and Christine Larkins (Emory University, Atlanta, GA). Cilia that lack the correct internal architecture fail to transduce Hedgehog (Hh) signals correctly.

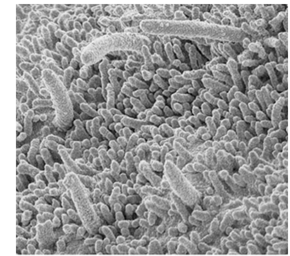
Hh signaling is particularly well-characterized in the developing nervous system. Depending on the amount of Hh activity, different types of neurons are produced. The particular type of neuron that forms is a result of the combination and amount of Hh-induced Gli transcription factors.

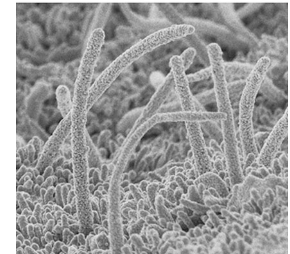
Caspary et al. now show that this neuronal choice is impaired in a mouse mutant called hennin, which has stumpy cilia. In these animals, different Hh levels were unable to increase or decrease Gli2 activity, which was stuck at a constitutively low level. As a result, one particular type of neuron was preferentially produced.

It's not the shortness of the cilia that's to blame, however—other mutants with short cilia have normal Hh signaling—it's the cilia's internal structure. The hennin mice lacked a cilial protein called Arl13b, which appeared to be required for correct cilial microtubule architecture.

Gli2 has also been found in cilia. Given the intermediate level of Gli2 activity in the mutant mouse cells, the authors suggest that correct cilial architecture might be required for both activating and restricting Gli2 activity. Cilial microtubules might, for instance, be needed for processing Gli2 into a highly active form and also for tethering Gli2 to prevent its action when it is not needed. **JCB**

Reference: Caspary, T., et al. 2007. *Dev. Cell.* 12:767–778.





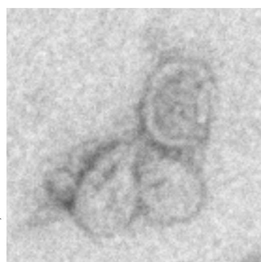
ANDERSON/ELSEVIER

Stumpy cilia with malformed microtubules (top) mess up hedgehog signaling.

The exosome exchange

Cells send RNA messages to each other by packing them into tiny membrane vesicles called exosomes, according to Hadi Valadi, Jan Lötvall (Göteborg University, Göteborg, Sweden), and colleagues. The study uncovers a new mechanism of genetic exchange between cells.

Exosomes form from the inward budding of vesicles into endosomes. If an endosome later fuses with the plasma membrane, its exosome packets can be released into the extracellular space. In this way, exosomes have been shown to send signals and present antigens to recipient cells. Proteins within the exosomes' bellies and on their surfaces have been the main focus of study, but nobody had looked to see whether exosomes contained nucleic acids, explains Lötvall.



LÖTVALL/MACMILLAN

Exosomes transport RNA between cells.

His team now finds that exosomes isolated from immune cells known as mast cells contain large amounts of RNA, including translatable mRNA. DNA, however, was not found in the packets.

Microarray analysis revealed the presence of approximately 1,300 different mRNAs within exosomes, which also contained miRNAs. Many of the exosomal RNAs were absent from the mast cell cytoplasm, suggesting that these might be ferried rapidly from the nucleus to their exosome transporters. Of the exosome-specific mRNAs, nearly one-fifth are implicated in pathways that regulate cellular development, protein synthesis, and posttranscriptional RNA modification.

Mast cells transferred exosomal RNA efficiently to other mast cells but not to T cells. Although it is not yet clear how dispatched exosomes interact with recipient cells, this preference suggests that exosomes have specific destinations as well as particular RNA contents. **JCB**

Reference: Valadi, H., et al. 2007. *Nat. Cell Biol.* doi:10.1038/ncb1596.

Peptides prompt prions

Short primary peptide structure drives the nucleation and self-perpetuation of prions, report Peter Tessier and Susan Lindquist (HHMI, Cambridge, MA).

Prions self-perpetuate by binding their normal protein counterparts and inducing them to fold into the same abnormal conformation. Previous studies of a yeast prion called Sup35 identified a broad region of the protein that is responsible for initiating this self-perpetuation. Part of the region has unusually low sequence complexity, suggesting that its overall structure, rather than its particular amino acid sequence, might be responsible for prion proliferation. Indeed many protein domains that promote folding are themselves organized into complex tertiary structures.

Single amino acid substitutions of the low-complexity region, however, can increase or decrease Sup35's prion-perpetuating ability. To see whether particular peptide elements might therefore be responsible, Tessier and Lindquist arrayed overlapping peptides from the broad suspect region of Sup35 onto glass slides. Only a very small set of overlapping peptides could bind and nucleate prions. And the longer the incubation continued, the more the Sup35 prion amyloids grew at these particular peptide spots.

Exactly how binding promotes conformational change is unclear. But whatever the mechanism, it does not appear to be unique to Sup35. Another distantly related yeast prion possessed a similarly short cluster of unrelated sequences capable of nucleating prions. Determining whether such sequences, which the authors call recognition elements, promote amyloid production in mammalian prions is the next step. **JCB**

Reference: Tessier, P.M., and S. Lindquist. 2007. *Nature.* doi:10.1038/nature05848.

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