

Research Roundup

DNA spring in your spindle

DNA is a mechanical piece of the spindle, if results from Elaine Yeh, Kerry Bloom, and colleagues (University of North Carolina, Chapel Hill, NC) are any indication. The inherent springiness of DNA may be part of the spindle's tension-sensing mechanism.

During mitosis, tension between chromosomes indicates that each of two sister chromosomes is properly attached to its own spindle pole. The

creation of tension requires a protein called cohesin, which glues sisters together until its cleavage at anaphase. But lots of cohesin is seen even at sister centromeres that are widely separated.

Bloom and colleagues set out to understand what cohesin is doing at these locations, which seem too far apart for

cohesin to hook sisters together. They imaged dividing yeast cells containing labeled cohesin and found a cylinder of fluorescence encircling the entire central spindle, spanning sister centromeres.

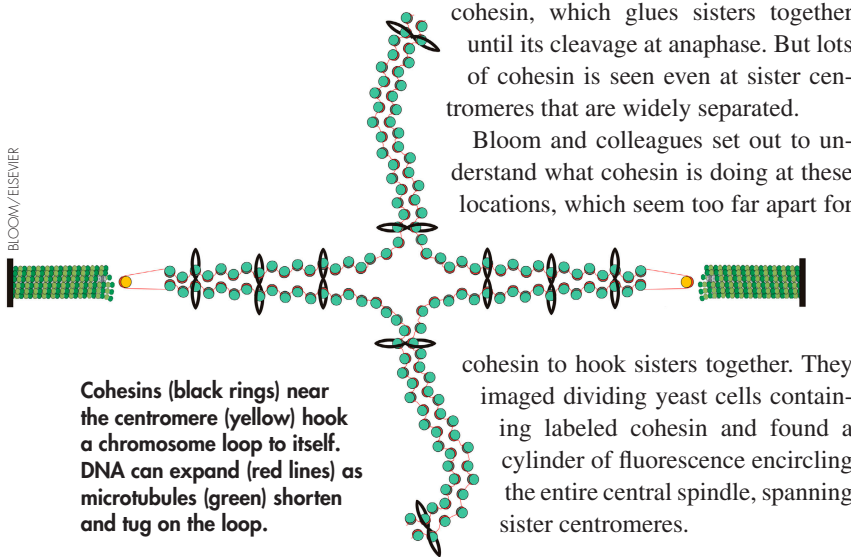
The arrangement suggested that cohesins near a centromere were hooking a chromosome to itself rather than to its distant sister. In this model, the links would cause the centromeric region to loop out from the rest of the chromosome. The group looked for such loops by cross-linking DNA in its *in vivo* conformation and performing inverse PCR. The resulting products indicated that the centromere is at the apex of a ~20-kb DNA loop.

The loops might act like a spring while microtubules at the centromeres grow and shorten during spindle assembly and bipolar attachment. "We are arguing that the centromere is dynamically unstable," says Bloom. "When microtubules shorten, the loop stretches; when they grow, the loop contracts."

Loops might be another place, along with kinetochores, where tension is sensed. "DNA is inherently an entropic spring, like a rubber band," says Bloom. "If you have a rubber band, and you need to build a tension-sensing mechanism, you might as well use the rubber band."

Yeast centromeres are only 120-bp long, but the entire loop might be a more accurate equivalent of the much larger mammalian centromeres, which have also been seen to form loops. **JCB**

Reference: Yeh, E., et al. 2008. *Curr. Biol.* 18:81–90.



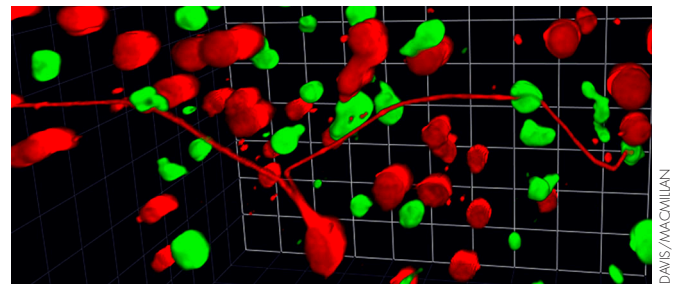
Nanotubes spread HIV

Transient contacts between T cells create nanotubes that may help spread HIV, based on findings from Stefanie Sowinski, Daniel Davis (Imperial College London, UK), and colleagues.

"T cells are particularly good at making transient contacts with other cells and then moving on," says Davis. "It's a specialized function of T cells, natural killer cells, and similar cell types." His group now shows that such short-lived greetings often leave in their wake membrane tethers known as nanotubes.

These delicate actin-filled tethers have been seen linking neuronal PC12 cells as well as macrophages, B cells, and other immune cell types. But the T cell nanotubes were distinct. Unlike the open-ended linkages of PC12 cells, these nanotubes maintained a membrane junction between T cells, either within the nanotube or at its tip. They also had the ability to curve around obstacles, as revealed when T cells were set in a 3D matrix.

The resolution of *in vivo* fluorescence microscopy is not yet sufficient to detect nanotubes directly. But circumstantial evidence suggests they exist *in vivo*, as cell surface proteins can be detected moving between cells somehow. Davis and colleagues are currently trying to identify immune cell materials that traffic within the tubes and the functional consequences of

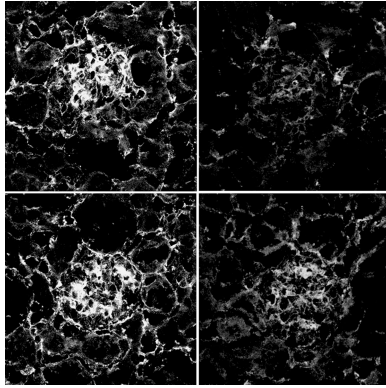


Nanotubes (red tubes) link cells and can bend around obstacles in a 3D matrix.

any transfer between T cells. Unlike myeloid cells, T cells were unable to transmit calcium signals via their nanotubes.

Whether nanotubes are used for T cell processes or are a simple byproduct of membrane physics, at least one virus has learned to exploit them. This opportunist is HIV, which invades and replicates within T cells. HIV antigens were found in nanotubes emanating from infected cells. And previously uninfected cells that connected to such nanotubes occasionally wound up with their own allotment of these antigens. Transfer required the viral receptor, CD4, suggesting that HIV transfer between cells by nanotubes requires membrane fusion mediated by HIV's Envelope protein. **JCB**

Reference: Sowinski, S., et al. 2008. *Nat. Cell Biol.* doi:10.1038/ncb1682.



The clearance (top; left to right) of IgG (white) from mouse kidney filters is slowed in the absence of FcRn (bottom).

Cleaning the kidney's filter

Filters in the mammalian kidney must be unclogged by a transport receptor, say Shreeram Akilesh, Andrey Shaw (Washington University, St. Louis, MO), and colleagues. The receptor moves obstructing proteins into the urine, where they can later be returned to the bloodstream.

The kidney filters the blood through a bed of capillaries known as the glomerulus. Large and highly charged proteins are mostly kept out of the urine filtrate first by a basement mem-

brane and then by finger-like projections of epithelial cells called podocytes. Shaw's group wondered why the gaps between podocyte projections do not get clogged by the proteins they prevent from passing.

The two dominant large proteins in the blood—those mostly likely to cause a clog—are albumin and IgG. Using microarray analyses, the team found that a receptor for these proteins, called FcRn, was expressed in podocytes. FcRn was first identified for its role in bringing IgG antibodies from breast milk across the

surface of an infant's gastrointestinal tract. The new findings suggest it similarly transports IgG and albumin across the podocyte surface into the urinary fluid.

In mice lacking FcRn, IgG clearance was slowed, and the antibody accumulated in the kidney filter. Clogged kidneys meant greater susceptibility to agents that would otherwise be quickly cleared; a normally harmless dose of a kidney-targeting antibody was toxic to mice whose kidneys were overloaded with IgG.

An overloaded filter might also explain why kidney failure is often the downfall of patients with lupus. "The autoantibodies in lupus patients aren't so uncommon," says Shaw. "But with clogging from all the excess antibodies, the kidneys probably cannot get cleared of them in time."

Because IgG and albumin are not normally found in urinary waste, the authors imagine that they are reabsorbed into the bloodstream in kidney tubules, where sugars and amino acids are also reclaimed. The expression of FcRn in tubules supports this idea. To prove their theory, the authors hope to put FcRn back into podocytes but not tubules of the knockout mice, to see whether IgG is sent out with the urine. **JCB**

Reference: Akilesh, S., et al. 2008. *Proc. Natl. Acad. Sci. USA*. 105:967–972.

Transcription factor translated in axons

A transcription factor is translated in axon tips, far away from its nuclear targets, as revealed by Llewellyn Cox, Samie Jaffrey (Cornell University, New York, NY), and colleagues. Its transport to the cell body helps keep the nucleus in touch with the distant axon's surroundings.

Throughout development, axons navigate long distances to find their ultimate synaptic partners. As it approaches its proper target, the axon often finds survival signals that stave off apoptosis. These signals must somehow be conveyed all the way back to the cell body. The new findings unveil a potential general mechanism for such survival signaling: local translation and retrograde transport of transcription factors.

Cox et al. were originally interested in identifying those mRNAs that are translated in axons, which contain ribosomal machinery that synthesizes signaling proteins and cytoskeletal regulators. In their search, says Jaffrey, "we found something strange, which in many ways had already

been discovered about ten years ago."

That oddity was mRNA for a transcription factor called CREB, which turns on antiapoptotic genes when neurons sense the NGF survival factor. The axonal *CREB* pool was translated when axons were given NGF, although how the local translation pathway is controlled is not known.

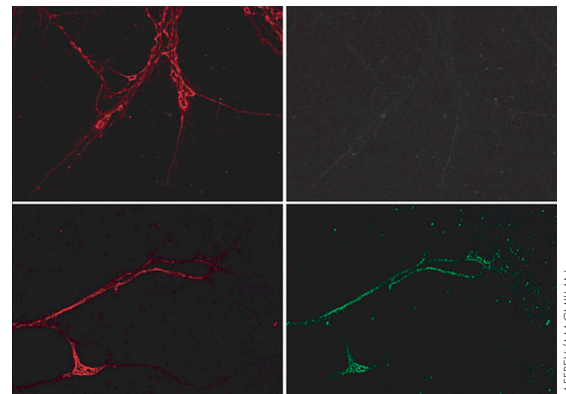
A decade ago, *CREB* was also found in dendrites. Getting at its function there was difficult, but the extra length of axons made it possible to examine its axonal role. The authors applied *CREB* siRNAs selectively to axons to inhibit its translation there. Without this pool of CREB, neurons no longer survived when their axons encountered NGF.

In untreated axons, NGF-induced CREB was seen traveling back toward the cell body along with motor-driven endosomes carrying NGF's receptor and the kinases it activates. The trans-

ported CREB sported a phosphate modification known to help it turn on pro-survival genes.

"CREB from axons is different from the nuclear pool," says Jaffrey. "It may undergo axon-specific modifications—phosphorylation, sumoylation, or association with binding proteins—that endow it with specific transcriptional abilities." **JCB**

Reference: Cox, L.J., et al. 2008. *Nat. Cell Biol.* doi:10.1038/ncb1677.



Axons (red) translate their own CREB (green) when given NGF (bottom row).