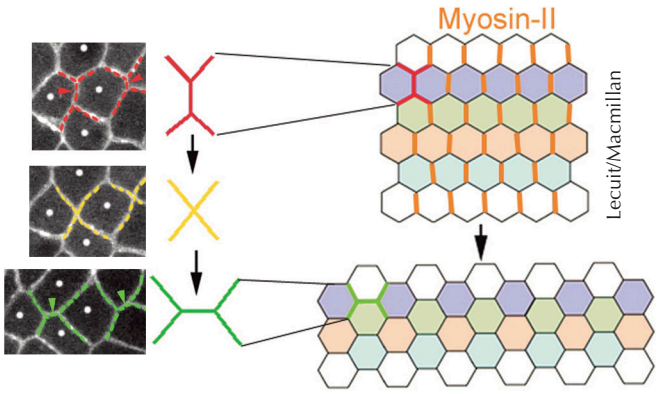
Research Roundup

Moving while sticking

Intercalation—the slotting of cells in between one another—is an established method for converting short and fat into long and slim. This is all very well in slippery, adhesion-sparse mesenchymal tissues. But many such extension events occur in epithelial tissues, where cells are glued together by adhesion complexes. Claire Bertet, Lawrence Sulak, and Thomas Lecuit (IBDM, Marseille, France) now report that epithelial intercalation relies on some strategic tugging by myosin that remodels junctions.

The French group expected that extension in fly embryos would involve a few individual motile cells nosing between other stationary cells. Instead they saw "a global and ordered reorganization," says Lecuit. Borders between cells that lay anterior and posterior to each other contracted to a point. Perpendicular expansion of this point generated a border between dorsal and ventral cells, thus pushing the anterior cell more anterior and the posterior cell more posterior. Extension was therefore achieved by a geometrical shuffling of the original hexagonal arrangement.

Myosin II was concentrated near the shrinking (anterior–posterior) membranes and reduced near the expanding (dorsal–ventral) membranes. In embryos with less myosin II, junctions froze and intercalation failed. Myosin need only destabilize adhesion proteins at anterior–posterior membranes, as junction proteins are naturally very dynamic.



Contraction and expansion of membranes reorganizes cells as the tissue extends (top to bottom). Anterior is left; posterior is right.

It is not known how myosin is concentrated preferentially near anterior and posterior membranes. Genes that define anterior–posterior polarity of the fly embryo are needed; they may exert this effect by either local (cell-to-cell) or global (gradient) messages.

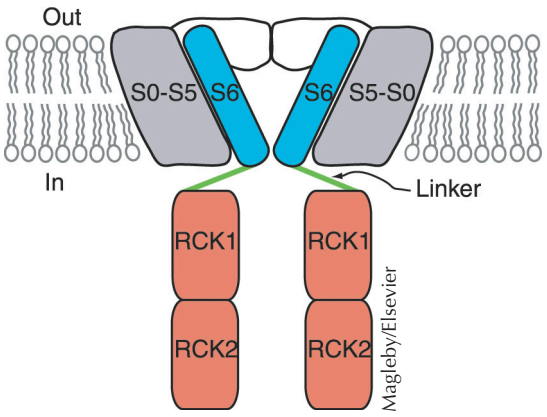
These polarity cues result in an intercalation method that, says Lecuit, maintains "the balance between stability and dynamics." It can account for a nearly twofold extension. If more extension is needed, for a gut tube or an arm, then cell shape changes and oriented cell division may come into play. ■

Reference: Bertet, C., et al. 2004. *Nature.* 429:667–671.

How a channel springs open

The gating mechanism of a K^+ channel includes a perfect spring, according to Xiaowei Niu, Xiang Qian, and Karl Magleby (University of Miami, Miami, FL).

The group studied the BK channel from mice. This channel is opened by changes in both voltage and intracellular Ca^{2+} concentration. Depolarization drives a positively charged section of the protein outwards and thus drags apart the S6 channel domains that form the channel's gate. Addition of Ca^{2+}, by contrast, widens the diameter of an intracellular gating ring, once again pulling apart the S6 gates.



The linker between the RCK gating ring and the S6 gate acts as a perfect spring.

The two control mechanisms converge on the same target—positioning of the S6 gates—so one control mechanism can be used to probe the mechanism used by the other. The authors changed the length of the linkers that run from gating ring to gates, and tested how much voltage was needed to open the new channel variants.

Shortening the linkers made it easier to open the channel with voltage changes, probably because the diameter of the gating ring is fixed, so shortening the linker pulls the S6 gates outwards toward the gating ring. Lengthening the linker made it more difficult to open the channel with a voltage change. There was a linear relationship between the changes in linker length and required voltage, indicating that the linker-gating ring complex is acting as a perfect, Hookean spring.

The same linear relationship did not hold up when channels with different linker lengths were probed with varying Ca^{2+} levels. "With calcium the gating ring becomes an active machine and changes in shape," says Magleby.

Magleby hopes to confirm the proposed movements with fluorescence proximity probes. But already, says Niu, "you don't have to look at the channels as blobs of protein. You can have mechanical models that really work." ■

Reference: Niu, X., et al. 2004. *Neuron.* 42:745–756.

Text By William A. Wells
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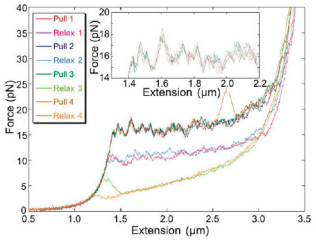


Bustamante/AAAS

DNA compacted with MukBEF extends in an ordered sequence as each complex releases its loop of DNA.

Caterpillar compaction

DNA compaction, say Ryan Case, Yun-Pei Chang, Nicholas Cozzarelli, Carlos Bustamante, and colleagues (University of California, Berkeley, CA), may work via a cooperative caterpillar-like mechanism.

The caterpillar is formed by multiple copies of condensin protein—in this case MukBEF—with each V-shaped condensin contributing two legs. Compaction occurs when the caterpillar's legs snap together.

"The main problem," says Bustamante, "is that we have not had any bulk assay for [the compaction activity of] this protein. We decided to do a rather risky search for a single molecule assay."

The assay involved binding MukBEF to DNA and then holding both ends of the compacted DNA strand using a dual beam optical trap. As the DNA strand was pulled, it gave way in a sawtooth pattern as individual copies of MukBEF stayed on the DNA but splayed apart to release their captured loops of DNA. The size of each peak depended on just how much DNA was released per event.

MukBEF binding was cooperative, so the proteins opened up in a set sequence starting at one end. Thus, after recondensing, restretching gave the exact same sawtooth pattern.

The open V of MukBEF may span 200bp or more. ATP binding (but not hydrolysis) may drive compaction by closing the V. In vivo, however, DNA is already compacted by other proteins and supercoiled, so each condensin would reach across many kilobases of DNA. Bustamante hopes to establish a bulk assay for condensin action so that he can see this process occur in real time. ■

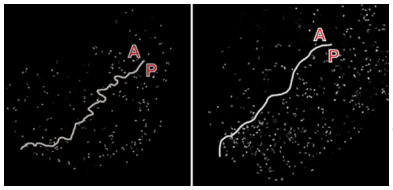
Reference: Case, R.B., et al. 2004. *Science*. 10.1126/science.1098225.

A message before dying

When a tissue is damaged, cells proliferate just enough to fill in the gap. Jun Huh, Bruce Hay (Caltech, Pasadena, CA), and colleagues now suggest that dying cells produce a signal promoting the multiplication of their surviving neighbors.

This type of compensatory proliferation has been recognized for almost 30 years. But the Caltech group stumbled upon the initiating signal while studying downstream mediators of the fly cell death activator Hid. To avoid the "confounding effects" of cell death in these studies they were coexpressing p35, which inhibits downstream caspase enzymes and thus prevents cell death. The result of this fake death regime was enlarged tissues thanks to a 2–4-fold increase in proliferation. "We wondered if it was these undead cells that were sending the [proliferation] signal," says Hay.

Hid kills cells by interfering with the stability and action of DIAP1, a caspase inhibitor. Reduction in DIAP1 levels did not, however, stimulate the compensatory proliferation effect. By contrast, expression of an upstream caspase called Dronc was both necessary and sufficient.

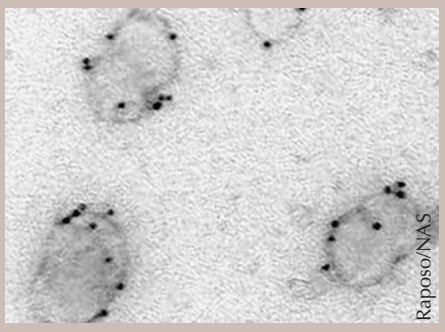


Hay/Elsevier

Death induction causes excess proliferation (white; right, posterior).

The response with Dronc but not low DIAP1 suggests that some of the upstream machinery remains obscure. Another uncertainty is the proliferation-related target of Dronc. Finding protease targets is not easy to do biochemically, so Hay will probably screen for genetic enhancers or suppressors of the compensatory proliferation phenotype. ■

Reference: Huh, J.R., et al. 2004. *Curr. Biol.* 10.1016/S0960982204004191.



Raposo/NAS

Extracellular PrPsc (gold particles) is on exosome-like vesicles.

Prions in packages

When neurons are dying left and right, the mechanism of cell-to-cell spread of infectious prion proteins would not appear to be a problem in need of a solution.

But prions originally enter their hosts via the gut and must somehow reach the brain. Now, Benoit Fevrier, Graça Raposo (Institut Curie, Paris, France), and colleagues suggest that prions might travel at least partly via tiny vesicles called exosomes.

Exosomes form when late endosomes invaginate to form small, internal vesicles. The bag of vesicles, or multivesicular body (MVB), can fuse with the plasma membrane to disgorge these vesicles, named exosomes, which then travel to other cells to transmit messages. In the immune system, for example, exosomes transfer peptide-laden MHC proteins.

When the French group looked at the supernatant of PrPsc-infected epithelial and neuroglial cell cultures they found PrPsc. The released PrPsc was in a fraction consisting largely of vesicles that had the size and protein make-up of exosomes. PrPc was also seen in association with MVBs.

PrPsc may be able to transfer between cells that contact each other, but exosomes provide a plausible means for prions to traffic over longer distances. How this ties in with the normal function of PrPc is not clear. But if exosomes turn out to be an important mechanism of PrPsc migration, then blocking exosome secretion may slow down the spread of prion diseases. ■

Reference: Fevrier, B., et al. 2004. *Proc. Natl. Acad. Sci. USA.* 10.1073 pnas.0308413101.