Research Roundup

Rosettes for elongation

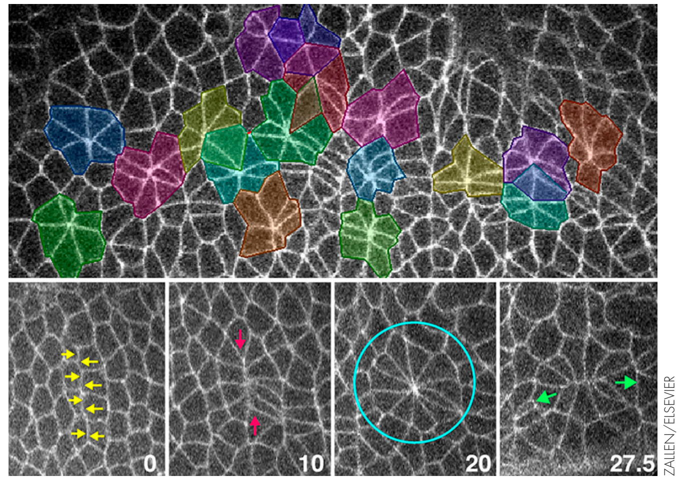
A blob becomes a body by becoming thinner and longer—a process that involves cell shuffling called intercalation. Flies do it by making and resolving rosettes of cells, say J. Todd Blankenship, Jennifer Zallen, and colleagues (Sloan-Kettering Institute, New York, NY). Up to 11 cells join these pinwheels, which squeeze together cells that were arrayed in the dorsal–ventral axis, before letting them relax back into a line running from anterior to posterior.

The rosettes are striking, but it has taken a long time for them to be identified. "I also spent a lot of time not seeing them," says Zallen. "It was making movies that made the difference—then you see they are directional. Now when I read papers I see them all the time."

In a previous model for elongation, called neighbor exchange, single-cell junctions running vertically were proposed to contract to a point, and then expand back out again horizontally. "These behaviors are happening, but we think they are only part of the story," says Zallen. "The starting order they require is not there."

But how to define "order"? Initially, says Zallen, "I didn't have a vocabulary to describe it." But with her physicist father she used quantitation methods familiar to those who study soap bubbles. Paradoxically, they found that disorder at the cellular level increased even as the tissue got closer to its elongated, more globally ordered state.

The increased disorder appears to be from rosette formation. Patterning genes drive actin then myosin accumulation at anterioposterior cell borders, and actomyosin tugging parallel to the membrane probably helps form the rosettes.

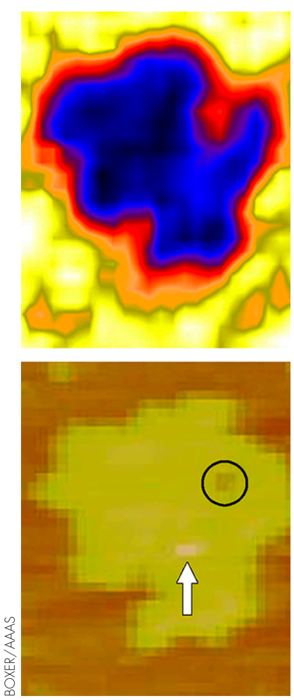


Rosettes (top) form via vertical contraction then relax horizontally (bottom, left to right).

When tracked for 25 minutes of so-called germband extension, 87% of cells are transiently incorporated into one or more rosettes. This amount of rearrangement, together with neighbor exchange, can account for most of the elongation seen. The mechanism for rosette resolution is unclear; clues should come from isolating components that lie downstream of patterning genes. **JCB**

Reference: Blankenship, J.T., et al. 2006. *Dev. Cell.* 11:459–470.

Lipid microscopy



Lipid domains chemically identified by SIMS (top) match images from AFM (bottom).

A chance meeting with a cosmochemist has led Steven Boxer to a new way to precisely image lipid locations. With Mary Kraft (Stanford University, Stanford, CA) and colleagues, he hopes to test ideas generated by the raft hypothesis. "A lot of this is cartoons," says Boxer. "We want to translate these cartoons of membrane molecules into reality."

The technique fills in a gap between FRET (operating over a maximum of a few nanometers) and optical microscopy (several hundreds of nanometers or more). Now, the NanoSIMS (secondary ion mass spectrometry) machine identifies lipid distributions with a lateral resolution of $\sim$100 nm.

The NanoSIMS sweeps a focused beam of cesium ions over the sample in around 10 minutes. The high-energy beam almost completely fragments proteins and lipids. Thus, molecules can be easily identified only if they are labeled with a particular isotope. The advantage, however, is that "we are reducing this thing to dust and we get a lot of dust per molecule," says Boxer. "So sensitivity is very high." In the future, different types of beams may allow identification without the need for labeling.

The next trick is to get access to the instrument. The 5 instruments in the US cost \$2–3 million each, and are tricky to run. They are not yet bio-friendly, as their original use was the analysis of specks of dust from comets. "These instruments were the private domain of that [cosmochemist] community," says Boxer.

But he thinks they complement the alternative: atomic force microscopy (AFM), which feels the shape of the protein and lipid landscape. "AFM has no chemical information," he says, "and is notorious for confusing real signals and debris. Those are the most expensive measurements of dirt you are ever going to see."

He remains skeptical of much of the raft concept, but asserts that "there must be organizing principles of some sort." Those principles should emerge once experiments are applied not only to the current lipid mixtures made in vitro but also to membrane samples isolated from cells. **JCB**

Reference: Kraft, M.L., et al. 2006. *Science.* 313:1948–1951.

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Innate tumorigenesis

A pathway required for innate immunity is also required to keep tumorigenic cells alive, report Yuchen Chien, Michael White (University of Texas Southwestern, Dallas, TX), and colleagues.

The RalB GTPase heads one of three pathways downstream of Ras that help drive tumorigenesis (the others are headed by MAP kinase and PI3K). This tumorigenic proliferation often triggers apoptosis. Preventing the apoptosis downstream of RalB, according to the Texas group, is Sec5. (Sec5's partners in secretion are not necessary, however.)

Also required was TBK1, an atypical IκB kinase that was found to bind Sec5. It is better known as part of the innate immune response. Sure enough, viruses induced RalB activity and transcription downstream of TBK1.

Why the connection? Virus-infected cells may stave off death long enough so that they can produce interferons, and this anti-death pathway may have been coopted by cancers. Or the pathway may be used for completely different purposes in the different locations. Either way, says White, the tumorigenesis "makes the [cells] more dependent on a pathway normally not required for survival." Such conditional dependencies "might be ideal therapeutic targets." JCB

Reference: Chien, Y., et al. 2006. *Cell*. 127:157–170.

Mopping up chemokines

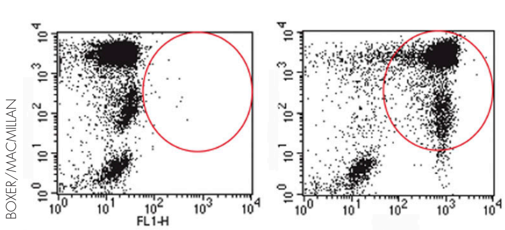
In both Iraq and the immune system, it's not the initial victory but the subsequent cleaning up that is the hardest part. Now, Amiram Ariel, Charles Serhan (Harvard Medical School, Boston, MA), and colleagues show that the mess after an infection is cleared up in part by dying cells.

This very act of cell death—notably of neutrophils and T cells—is one way of actively resolving an inflammatory event. But the signals that first drew immune cells to the site of inflammation also need to be destroyed: chemicals via enzymatic degradation and proteins and peptides by other means. Now, the Boston team shows that some of these chemokine proteins are mopped up by apoptotic cells. These dying cells turn up expression of their CCR5 chemokine receptors even as the cells are about to be engulfed by macrophages.

CCR5 is important because it binds several key chemokines—"the lion's share of the chemokines at a site of inflammation," says Serhan. "So you need a good mechanism to explain their clearance."

Late apoptotic cells had higher CCR5 staining; this was reduced by a proinflammatory cytokine but increased by proresolution mediators. The result was more chemokine binding to late apoptotic cells. If CCR5 was knocked out or antagonized, however, fluid from resolving infections had higher chemokine levels.

Thus it appears that the dying cells are mopping up chemokines and taking them to the grave. "It sounds almost too simple," says Serhan, but he doesn't think this is his "Mission Accomplished." Rather it is a reminder, he says, that "when cells go through apoptosis they don't stop signaling." JCB



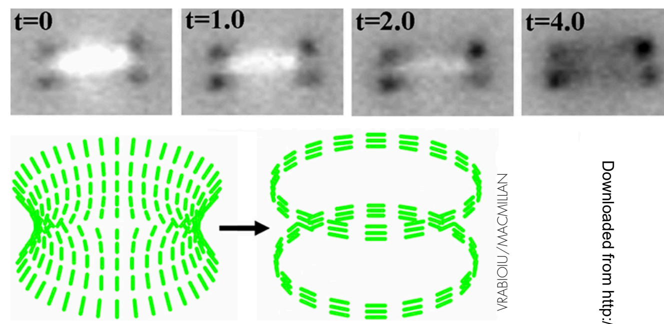
Apoptotic cells (right) accumulate CCR5 (top right).

Reference: Ariel, A., et al. 2006. *Nat. Immunol.* doi:10.1038/ni1392.

Spinning septins

Septin filaments in yeast do a right-angled turn in the middle of cell division, according to Alina Vrabioiu and Tim Mitchison (Harvard Medical School, Boston, MA). The filaments initially align parallel to the spindle axis but then rotate 90° to form two circumferential rings that flank the cytokinetic furrow.

Septin filaments help cells divide, but just how they do so has remained mysterious. The direction of septin-dependent striations varied between experiments, plus the striations may have been a pattern set up by proteins that bind septins, not the septins themselves.



Septins spin in a polarizing microscope (top) as septin rings mature (bottom).

Now, the Boston team has directly monitored the direction of septin filaments. They attached a green fluorescent protein (GFP) to septin by linking together two rigid α-helices. Polymerizing the septin–GFP molecules in a filament lined up all the GFPs. Polarized light would effectively excite these GFPs only when the light's electromagnetic oscillations were aligned with the GFPs' dipoles—the direction along which an excited electron preferentially moves to the higher energy state.

The team established in vitro what direction of polarized light best excited a filament of known orientation. Applying this to in vivo data, in which polarized light excited septin–GFP in cells whose orientation was carefully controlled, they could deduce the direction of septin filaments in vivo.

The septins are initially aligned parallel to the spindle axis, in an hourglass shape that spans the bud neck. Dephosphorylation has been implicated in reshaping the septins into two rings; if it acts selectively in the middle of the hourglass it might detach septins at one end with the other end providing a pivot point. The force driving turning might then come from membrane insertion between the two rings. JCB

Reference: Vrabioiu, A.M., and T.J. Mitchison. 2006. *Nature*. 443:466–469.