# Research Roundup

## Spitz on growth control

Self reliance is not enough. Some organs rely on the kindness of others to control their size, according to Joseph Parker (Columbia University, New York, NY).

Correct organ size is measured in mass, not cell numbers—forcing or blocking cell division generally does not change an organ's overall dimensions. Parker found the same is true in the fly embryonic P compartment, which forms part of the larval epidermis. Increases in P compartment cell numbers were countered by more apoptosis and smaller cells. With fewer numbers, on the other hand, each cell grew larger to accommodate for their missing comrades.

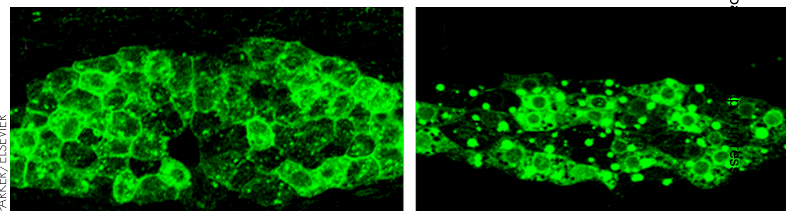
In looking for a molecular explanation, Parker figured it would make sense to "have size information encoded right there in the patterning system" that also controls cell fate and positioning. For the P compartment, these patterning molecules are extracellular ligands called Spitz and Wingless. The new results show that more or less Spitz signaling creates larger and smaller P compartments, respectively.

At the individual cell level, Spitz suppressed apoptosis and encouraged cell growth by activating the EGF receptor and downstream MAP kinase pathways. To explain how overgrowth is prevented, Parker reasoned that "the simplest model is that the level of Spitz is fixed in the compartment. More proliferation means less Spitz per cell." Those cells thus grow less and are more susceptible to apoptosis.

Organs were previously thought to have autonomous control over their size. But the new model only works if Spitz is provided by an external source—otherwise, a bigger P compartment would make more Spitz and grow even larger. Although Spitz is expressed everywhere, it is activated solely in the neighboring A compartment, from where it apparently diffuses into the P compartment.

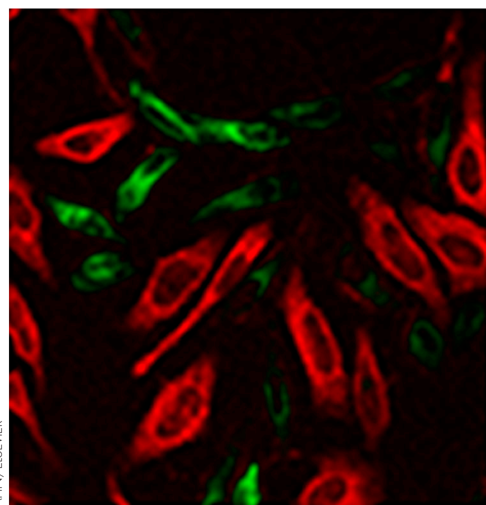
Because ligands such as Spitz are short-range diffusers, the model probably only applies to small or embryonic structures in the range of tens to hundreds of cells. "For larger organs like the liver," Parker says, "diffusion is a rickety thing" that might require backup size control mechanisms. JCB

Reference: Parker, J. 2006. *Curr. Biol.* 16:2058–2065.


PARKER/ELSEVIER

**Too much EGF receptor signaling increases P compartment size (left). Too little reduces it (right).**

## Fight infection with oxidation


AHN/ELSEVIER

**Viral US3 (green) degrades PDI (red) to thwart antigen presentation.**

Redox regulation and immunology collide in a new report by Boyoun Park, Kwangseog Ahn (Seoul National University, South Korea), and colleagues.

When scientists think of the immune system's antigen selection procedure, oxidation/reduction is just about the last thing to come to mind. Yet the group now shows that an oxidation step by protein disulfide isomerase (PDI) helps load antigenic peptides into MHC class I molecules, which then present the antigen on the cell surface.

PDI is best known as a chaperone, but the authors found it associated with ER proteins that pull antigens into the ER from the cytosol. Its chaperone activity seems to be intact here: it binds strongly to antigens and probably protects them from the abundant ER proteases. But PDI's role goes beyond chaperone duties.

PDI also regulates a disulfide bond in the peptide-binding sites of MHC class I molecules. Only in its oxidized form, the authors find, does an MHC class I molecule accept antigenic peptides. This oxidation is performed by PDI when optimal, high-affinity antigens are abundant. In their absence, however, PDI instead reduces MHC (cells contain an equilibrium of oxidized and reduced forms of PDI). The big mystery that remains is whether and how PDI distinguishes between optimal and suboptimal peptides, particularly since each type of MHC class I molecule has its own antigen preferences.

This selective sampling is important because cells may have only about four hours to present antigens before certain virus replicate and escape. In this time, MHC molecules have to sample lots of possible antigens before finding the right one. According to Ahn, "the PDI delivery function is what might help MHC find a substrate quickly enough to mount an immune response." In the absence of PDI's peptide-binding activity, most surface MHC class I molecules were empty, probably because they left the ER with low-affinity peptides that quickly fell off.

If the authors reduced PDI levels, virus-infected cells were unable to activate T cell responses. Cytomegalovirus brings about this defenseless state on its own by using its US3 protein to degrade PDI. JCB

Reference: Park, B., et al. 2006. *Cell.* 127:369–382.

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## A minus end checkpoint

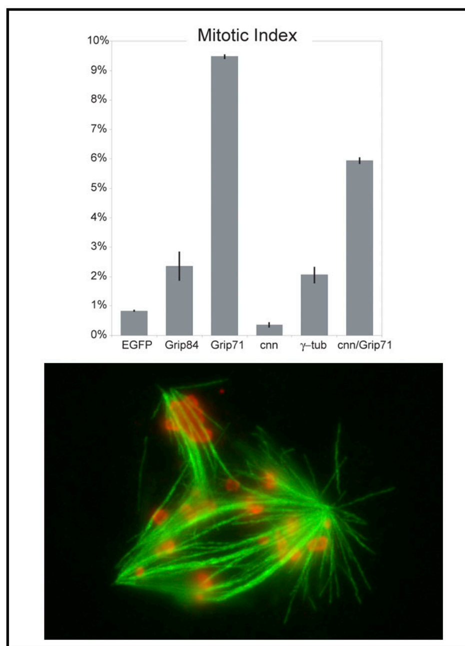
Both ends of microtubules get in on spindle checkpoint signaling. From kinetochores, proteins at microtubule plus ends relay the results of proper spindle-to-chromosome attachments. Now, Hannah Müller, Bodo Lange (Max Planck Institute for Molecular Genetics, Berlin, Germany), and colleagues find that, at minus ends, γ-tubulin signals that all is well with microtubule nucleation.

Spindle microtubule minus ends are focused at the centrosome, where the microtubule-nucleating γ-tubulin ring complex (γ-TuRC) resides. Müller et al. found that the loss of γ-TuRC proteins, including γ-tubulin, activates the spindle checkpoint. The problem does not seem to stem from fewer spindle-to-kinetochore attachments, as spindle microtubule density was not strongly reduced.

The group instead finds a connection between γ-TuRC and known spindle assembly checkpoint proteins. Both Cdc20 and BubR1 copurified with γ-tubulin in human and fly cell extracts. Loss of a functional checkpoint, via knockdown of BubR1 for example, overcame the mitotic stall caused by γ-tubulin loss.

The arrest could not, however, be overcome by disrupting centrosomes. Thus the checkpoint relies on γ-TuRC for microtubule nucleation, but the centrosome as a "molecular hub" is not required for this particular process. JCB

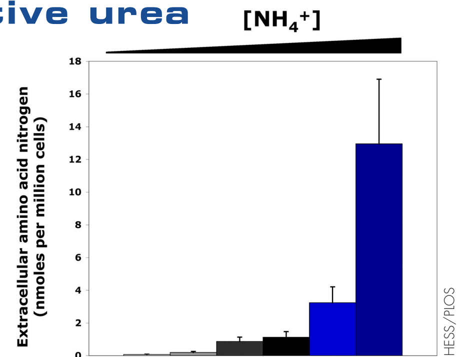
Reference: Müller, H., et al. 2006. *Science*. 314:654–657.




LANGE/AAAS

**Abormal spindles and mitotic stalls occur upon depletion of the γ-TuRC proteins Grip71 and γ-tubulin, but not the centrosomal structural protein cnn.**

## Yeast's primitive urea




HESS/PLOS

**Ammonium induces yeast to excrete amino acids.**

Yeast cells pee out amino acids to avoid ammonium toxicity, say David Hess (Princeton University, Princeton, NJ) and colleagues.

The group was initially interested in the effect of potassium, not ammonium. But their microarray data suggested that low potassium is harmful to yeast because ammonium—a similarly sized and charged ion—seeps in through the battery of induced potassium channels.

The yeast protect themselves from the intracellular ammonium by incorporating it into amino acids that they then secrete into the medium. Export seems to be through passive channels that also take up amino acids. The export may help natural yeast strains survive on ammonium-rich rotting vegetation (in which the external source of amino acids is probably also handy later on). Standard laboratory media have very high potassium levels that apparently masked ammonium's toxic effects on yeast before now.

The initial cellular response to high ammonium—converting it to glutamine or glutamate—is evolutionarily conserved. Since glutamine is a neurotoxin, however, mammals must further convert the excess amino acids to urea. "The root cause of ammonium toxicity is not understood in mammalian systems," says Hess. At least now, he says, "we can use yeast as a model." JCB

Reference: Hess, D.C., et al. 2006. *PLoS Biol.* doi:10.1371/journal.pbio.0040351.

## Myosin's need for speed

The fastest muscles are powered by a motor that is comparatively averse to energy-providing ATP, as shown by Douglas Swank (Rensselaer Polytechnic Institute, Troy, NY), Vivek Vishnudas, and David Maughan (University of Vermont, Burlington, VT).

The muscles that power *Drosophila* flight contract over 200 times per second. To determine what makes their myosin motor work so quickly, Swank et al. compared contraction speeds of flight myosins with a slower embryonic myosin while varying levels of ATP, whose hydrolysis fuels contraction, and its byproducts, $P_i$ and ADP.

Previous studies established that the speeds of slower myosins are limited by how quickly they releases ADP. But Swank found that the flight myosin is instead held back by $P_i$ release. "To increase speed," Swank reasons, "the ADP release step must be faster. Here, it's gotten so fast that $P_i$ release becomes limiting."

As ATP is needed for every contraction cycle, "you might think [fast myosin] would want to bind ATP [even tighter]," says Swank. "But that's not the case." The slow myosin had the higher affinity for ATP. Flight myosin's lower ATP affinity might be a side effect of its faster release of structurally similar ADP.

Flies probably compensate by maintaining very high ATP concentrations. Their flight muscles seem to have enough mitochondria for the task, but Swank plans to measure in vivo ATP levels directly to be sure. By swapping domains of fast and slow myosins, he also hopes to find the structural differences that evolved to power such hustle. JCB

Reference: Swank, D.M., et al. 2006. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.0604972103.

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