In This Issue

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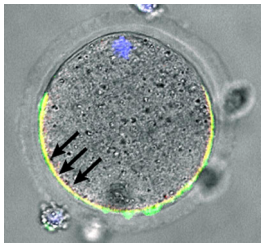
Ovastacin cuts off sperm binding



Ovastacin (red) localizes to secretory granules (green) in the cortex of an unfertilized egg.

Burkart et al. describe how a secreted protease helps egg cells avoid being fertilized by more than one sperm.

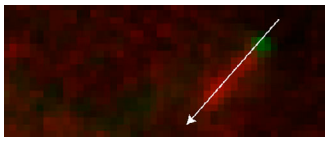
Because polyspermy disrupts embryonic development, oocytes take several steps to ensure they only fuse with a single sperm. One key step is to prevent additional sperm from binding to the surface of an already-fertilized egg, a blockade that involves the release of secretory granules and cleavage of the glycoprotein ZP2, a component of the zona pellucida matrix that surrounds eggs. ZP2 is cleaved upstream of two acidic amino acids, a cleavage site recognized by the astacin family of metalloendoproteases. Burkart et al. therefore investigated the function of ovastacin, an astacin family member expressed in oocytes.

Ovastacin localized to cortical granules that were exocytosed after fertilization, and recombinant ovastacin cleaved ZP2 when added to zonae pellucidae. Mice lacking ovastacin failed to cleave ZP2 after fertilization, allowing sperm to continue to bind to the surface of early embryos. Ovastacin-null female mice had slightly fewer offspring than wild-type animals but otherwise appeared normal.

The researchers found that ovastacin targeted several sites in ZP2's N terminus. Senior author Jurrien Dean now wants to investigate how this proteolysis blocks sperm binding, a critical question because the molecular interactions between sperm and egg cells remain unknown. He also wants to examine how ovastacin is packaged into oocyte cortical granules and to identify other components of these secretory organelles.

Burkart, A.D., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201112094.

γ-Tubulin stands up to be counted



γ-Tubulin (green) localizes to the minus end of a single microtubule (red) that has been detached from the spindle pole body.

Erlemann et al. count the number of γ-tubulin complexes that assemble together to nucleate microtubules in *S. cerevisiae*.

Budding yeast microtubules are nucleated from spindle pole bodies (SPBs) by γ-tubulin small complexes (γ-TuSCs), which consist of two γ-tubulin subunits and one molecule each of Spc97 and Spc98. In vitro, γ-TuSCs form a spiral with 13 γ-tubulin molecules per turn, which could serve as a template for the 13 tubulin protofilaments of a microtubule. But whether similar numbers of γ-TuSCs nucleate microtubules in vivo was unknown.

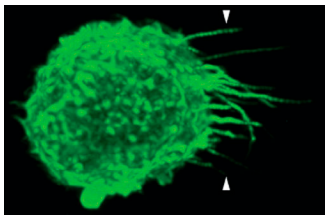
By comparing the fluorescence of GFP-tagged γ-TuSC proteins to known fluorescent standards, Erlemann et al. counted the number of γ-TuSC components at yeast SPBs and at the minus ends of individual microtubules, which had been released into the cytoplasm by a genetic trick. The quantification revealed that approximately seven γ-TuSCs nucleate each microtubule in vivo, with an additional three γ-tubulin and two Spc98 molecules also incorporating into each nucleation site.

Budding yeast probably only nucleate microtubules during G1, but γ-TuSC components remained tightly associated with SPBs throughout the cell cycle and changed very little in abundance, suggesting that similar numbers of γ-TuSCs nucleate microtubules in G1 and anchor them to SPBs during the rest of the cell cycle.

Erlemann et al.'s results support the idea that γ-TuSCs nucleate microtubules in vivo by forming a spiral-shaped template with 13 γ-tubulin monomers per turn. The authors think that the extra γ-tubulin and Spc98 molecules may stabilize the spiral, fulfilling roles played in higher eukaryotes by specialized proteins that are absent in budding yeast.

Erlemann, S., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201111123.

Leukocytes drop anchor



To avoid being displaced by fluid flow, monocytes accumulate F-actin (green) in upstream structures (arrowheads), which keep the cells firmly anchored to their attachment site.

Rullo et al. describe how actin polymerization helps leukocytes maintain their attachments to blood vessel walls.

A key step during the recruitment of white blood cells into inflamed tissues is chemokine-triggered activation of integrins, such as VLA-4, that attach leukocytes to the endothelial cells lining the blood vessel. These adhesions are quickly stabilized to prevent the leukocytes from being swept away in the flow of blood. Rullo et al. treated monocytes with inhibitors of actin polymerization and found that, although the drugs had no effect on the activation of VLA-4's affinity for its ligand VCAM-1, they reduced the cells' ability to form flow-resistant adhesions in vitro.

Monocytes exposed to fluid flow quickly formed actin-rich upstream structures, which contained VLA-4 and kept the cells anchored to VCAM-1. But when actin polymerization was blocked, these anchors became longer and more fragile, stretching out behind the leukocytes as they detached from the surface. The accumulation of actin within the leukocyte anchors was dependent on the small GTPases Rap1 and Rac, as well as on activation of the lipid kinase PI3K. Inhibiting any of these proteins blocked actin polymerization and reduced stable leukocyte attachment.

Rap1 and PI3K can both be activated by mechanical stress, suggesting that these two proteins sense when leukocyte adhesions are under tension and trigger a rapid reinforcement of the cell's attachments. The authors now want to investigate whether integrin-based signaling pathways contribute to tension sensing and to examine the role of myosin in stabilizing leukocyte adhesions.

Rullo, J., et al. 2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201107140.