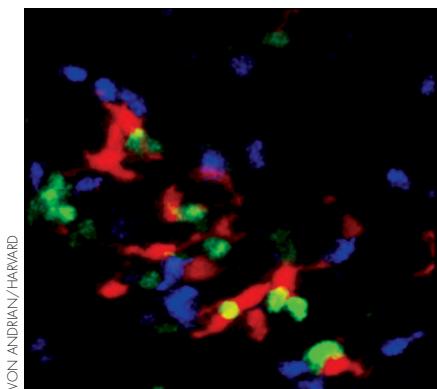
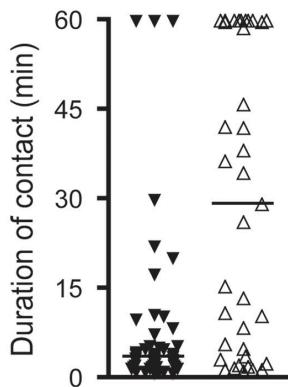


# Research Roundup



VON ANDRIAN/HARVARD

T cells (green) interact (left) with antigen-bearing DCs (red). T cells spend little time with DCs (filled triangles, right) that carry little antigen but linger longer (open triangles) when high antigen-density DCs are also present.



## T cells count and then decide

T cells tabulate the density of antigens in their surroundings before deciding whether to activate, say Sarah Henrickson, Ulrich von Andrian (Harvard Medical School, Boston, MA), and colleagues.

In a prior study, von Andrian says, his group had noticed T cells within lymph nodes flit "like bumblebees" from one antigen-presenting dendritic cell (DC) to another. Now they have found that when antigens are scarce, it takes many hours for them to settle down and connect with DCs, the prelude to full activation.

To determine the rules governing this transition to a stationary phase, the authors injected foreign T cells and observed them in a lymph node directly behind the knee in mice. When they also injected foreign DCs carrying labeled antigen into the footpad, they could watch as T cells and DCs met in the lymph node.

They found that the more antigen in the environment, the less time a T cell spent flitting around before latching onto a DC. Using DCs with differing antigen density, they showed that T cells would rapidly settle down with low-antigen density DCs, as long as they had previously encountered high amounts of antigen before.

"It was the total dose exposure, not the dose on any one dendritic cell, that triggered the response," von Andrian says. "The T cell has to make a binary decision, but antigen dose is an analogue signal, dependent on number and density. Somehow, the T cell is able to integrate that signal from its multiple encounters." This feature would allow the T cell to respond when there is a lot of low-affinity antigen, as might occur during an infection. "A T cell can make an informed decision only by remembering how much antigen it has been exposed to. Now we must understand how it does so." **JCB**

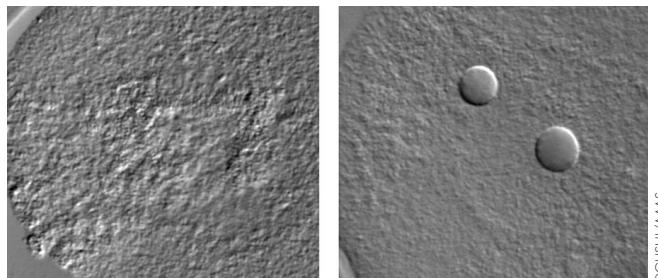
Henrickson, S.E., et al. 2008. *Nat. Immunol.* doi:10.1038/ni1559.

## Embryos need Mom's nucleolus

**T**hank Mom for your nucleolus, say Sugako Ogushi (Kobe University, Japan) and colleagues, who show that the earliest stages of mammalian embryogenesis require a nucleolus from the oocyte.

Unlike somatic cell nucleoli, those in fully developed but unfertilized oocytes are transcriptionally inactive and have an odd, compact form, which led Ogushi to ask what contribution they make to the embryo. Using a fine needle, she removed the nucleoli from mouse oocytes, taking the internal contents while leaving the surrounding heterochromatin. The seemingly unharmed enucleolated oocytes matured properly. But when they were fertilized, no new nucleoli formed in the embryos, which stopped developing after a few rounds of cleavage. If Ogushi put the nucleoli back in before maturation, functional nucleoli developed. She could even wait until after maturation to reinject and still obtain viable embryos and healthy mouse pups.

Whatever the essential material was, it was only present in oocyte nucleoli. When Ogushi injected somatic cell nuclei and their nucleoli into enucleolated oocytes, the reconstructed oocytes failed to develop normal nucleoli, and they too stopped dividing shortly after fertilization.



Injecting enucleolated oocytes (left) with isolated nucleolar materials causes nucleoli to reform (right).

Sperm have no nucleoli, so it makes sense that the zygote relies on the oocyte for its nucleolar beginnings. But what do they provide? "Little is known about nonmammalian embryonic nucleoli," says Ogushi. "But if this is a mammal-specific phenomenon, we might suspect that the maternal nucleolus organizes the three-dimensional chromatin structure in the nucleus of zygotes and very early embryos, influencing mammal-specific gene expression, such as for imprinted genes." The contents of the oocyte nucleolus are unknown, and identifying them will be the next step in the group's research. **JCB**

Ogushi, S., et al. 2008. *Science*. 319:613–616.

## Kinesin's parts pull together

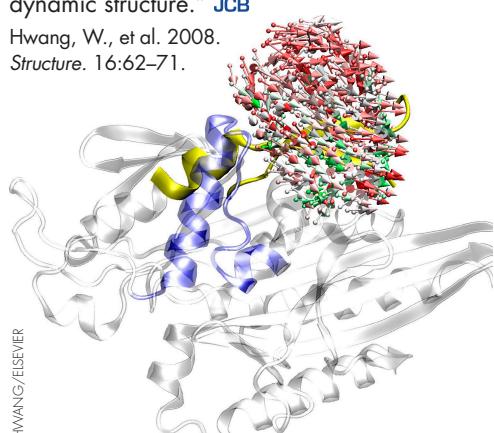
**K**inesin creates a new domain to generate its power, according to Wonmuk Hwang (Texas A&M University, College Station, TX), Matthew Lang (Massachusetts Institute of Technology, Cambridge, MA), and Martin Karplus (Harvard University, Cambridge, MA).

Kinesin's structure includes a cargo-binding domain, a long stalk, and a pair of motor heads, which move along microtubules mostly hand over hand. Each motor head is connected to the stalk by a short neck linker. The neck linker has been suggested to help kinesin motility by making a large, ATP-driven conformational change that docks it to the motor head and results in a forward-stepping motion. But an unbound neck linker is flexible and interacts only weakly with the motor head; thus, it alone cannot generate the required force. The authors now discover an additional mechanical element not previously implicated in kinesin motility—the cover strand, which dangles nearby.

When ATP binds to the motor head, the cover strand and the neck linker form a new  $\beta$ -sheet domain, which the authors named the cover neck bundle. The bundle pushes the neck linker into its binding pocket, where it then latches on, powering the free motor head forward. Force analysis indicated that forces generated by the cover neck bundle agreed with experimental measurements.

"The force is generated by the formation of this domain rather than by switching between well-defined conformations," Hwang says. "It was not possible to obtain these results from structural studies only, since it's a dynamic structure." **JCB**

Hwang, W., et al. 2008. *Structure*. 16:62–71.



Force analysis shows the formation of the cover neck bundle (yellow) is at the heart of kinesin's power stroke.

## Reach out and gRab something

**W**hen families cooperate, their effects can be far reaching, according to Suzanne Pfeffer (Stanford University, Stanford, CA) and colleagues, whose study of Rab6 and Arl1 shows that these members of different GTPase families work together to anchor a vesicle-tethering protein onto the Golgi.

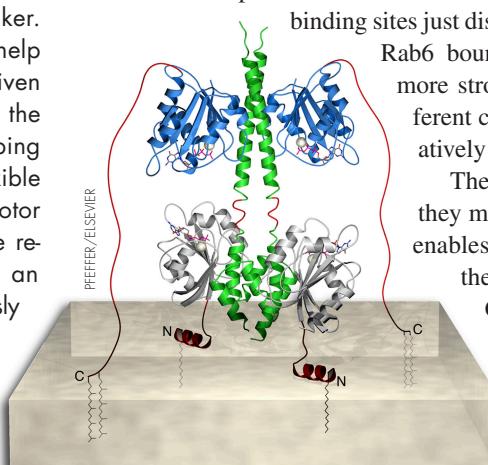
The tethering protein, GCC185, is thought to bind to transport vesicles destined for the Golgi. GCC185 contains a GRIP domain, which is known to bind to a possible anchor at the Golgi called Arl1. But previous work showed that Arl1 only bound weakly, if at all, to GCC185.

Since Rab6 is another tether-linking Golgi protein, the authors looked in detail at its possible interactions with GCC185. They found a pair of Rab6-binding sites just distal to those for Arl1 on GCC185. When

Rab6 bound the tether first, Arl1 bound much more strongly. "Nobody expected that two different classes of GTPases would work cooperatively to localize this protein," Pfeffer says.

The structure of the complex suggests how they may cooperate. Rab6 has a long tail that enables it to reach up to  $\sim 100$  Å away from the membrane to grab onto distant GCC185. Once Rab6 latches onto the tether, Arl1 binds it closer in to secure it in place. "It may be a molecular handoff," says Pfeffer. Once the protein passes into the "GRIP" of Arl1, Rab6 is free to reach out and grab some more. **JCB**

Burguete, A.S., et al. 2008. *Cell*. 132:286–298.



Rab6 (blue) and Arl1 (gray) cooperate to bind GCC185 (green), a vesicle-transporting tether.

## Self-assembly of the cell wall

**F**or plant cell walls, opposites attract, according to Maura Cannon (University of Massachusetts, Amherst, MA), Marcia Kieliszewski (Ohio University, Athens, OH), and colleagues. Their results suggest that a positively charged protein network forms the frame to which negatively charged carbohydrates attach to make the mature wall.

Previously, the authors characterized a mutation that prevents normal development of the new cell wall that separates cells at the end of plant mitosis. In the current work, they identified the mutated gene as *AtEXT3*, one of 20 cell wall extensins in *Arabidopsis*.

As a new cell wall forms at the end of mitosis, *AtEXT3*-carrying Golgi vesicles fuse along the plane between daughter cells. Using atomic force microscopy *in vitro*, the authors showed that *AtEXT3* formed a meshwork by cross-linking its multiple, repeating tyrosine residues. The authors propose that the highly periodic primary structure of *AtEXT3* helps align copies of itself for cross-linking within the plane between daughter cells.

The protein's many lysines give it a strong positive charge—perfect for attracting cell wall sugars such as pectin. "Since pectins are strongly negatively charged," Cannon says, "we propose that the network of the cell wall forms by extensin laying down a framework on which pectins can assemble." The pectins and extensins bond through an acid–base reaction, and pectins themselves can also form linkages with each other. **JCB**

Cannon, M.C. 2008. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.0711980105.