

Research Roundup

Is *Botryllus* a natural killer?

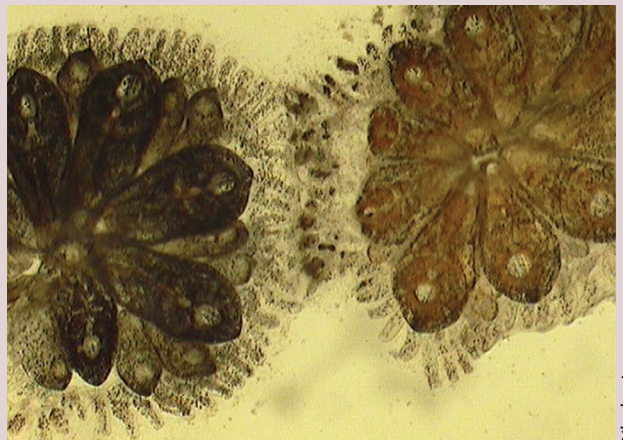
Natural killer (NK) cells in mammals attack virus-infected and tumor cells that stop making major histocompatibility complex (MHC) proteins. But the discovery by Konstantin Khalturin, Thomas Bosch (University Kiel, Kiel, Germany), and colleagues of a protein in 1050 squirts related to a key NK cell receptor suggests that the evolutionary predecessors of NK cells may have targeted genetic interlopers of their own species.

The sea squirt in question, *Botryllus schlosseri*, exists as a flower-shaped accumulation of petals, or zooids. When two clusters of zooids meet one another they either fuse or reject one another. For fusion to occur, the two must share at least one allele of the Fu/HC locus.

The molecular identity of Fu/HC remains unknown, and the search for direct relatives of MHC proteins, which determine transplant rejection in humans, has not produced any candidates. The recent genome sequencing of the sea squirt *Ciona intestinalis* also failed to turn up any MHC proteins, although *Ciona* is solitary and thus lacks the rejection reaction of *Botryllus*.

Khalturin and Bosch searched for genes whose expression changed during the rejection process. One of the down-regulated genes encodes BsCD94–1, a protein on the surface of *Botryllus* blood cells that is very similar to the vertebrate NK cell receptor CD94. The group does not yet have functional evidence tying the protein to rejection, but Bosch says “the structural similarity is absolutely convincing.”

The pattern of recognition in the two systems is also a match. In *Botryllus*, an *A/B* genotype rejects *C/D* but fuses with *B/C*. Likewise, NK cells kill only if CD94 fails to recognize self (in the form of



Khalturin

NK-like cells may have arisen to help sea squirt colonies reject each other (center).

class I MHC), whereas the B and T cells of the vertebrate adaptive immune system attack as long as they see any trace of nonself.

Adaptive immunity has been traced back only as far as jawed vertebrates. The new work suggests that a type of NK cell existed much earlier, but it may have been specialized not for immunity but for adjudicating land grabs.

And even those land grabs have a twist. Others have found that, although one of the colonies often dies off after fusion, its germ cells can parasitize the survivor. Hence, the sea squirt chooses to fuse only with genetic relatives, so that after fusion its energy is devoted to propagating infiltrating germ cells that are at least related. Natural killing, it seems, began with family loyalty. ■

Reference: Khalturin, K., et al. 2003. *Proc. Natl. Acad. Sci. USA*. 10.1073/pnas.0234104100.

Bacterial aging

The discovery of a novel substrate for CobB, a bacterial Sir2 protein, has provided a link between a cell's energy status and carbon usage. The finding, described by Vincent Starai, Jorge Escalante-Semerena (University of Wisconsin, Madison, WI), and colleagues, may also help explain how Sir2 slows aging in yeast, worms, and, perhaps, mammals.

The team started out with a mutant, *cobB*⁻, that could not grow on low levels of acetate. They found that the acetyl coenzyme A (CoA) synthetase (Acs) that initially derivatizes acetate to form acetyl CoA was inactive because of acetylation of a specific lysine residue of Acs. CobB, the bacterial Sir2, removed this acetylation and thus activated Acs.

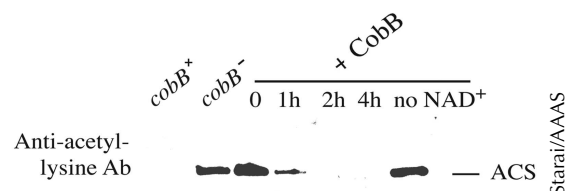
The link to energy status and redox comes about through NAD⁺. A bacterial cell that is low in energy will use up most of its NADH to generate ATP. The resulting

high levels of NAD⁺ provide the necessary cosubstrate for Sir2 proteins like CobB. Active CobB activates Acs, which generates more acetyl CoA, thus shunting more carbon into the energy- and NADH-generating TCA cycle.

Active Sir2 is now known both to generate more acetyl CoA and to extend lifespan. How are these two phenomena linked? More acetyl CoA for the TCA cycle means more respiration, which has been associated with yeast lifespan extension when caloric intake is restricted. And perhaps Sir2 activation allows for better scavenging of acetate—a molecule that is generated by lipid breakdown and can be easily lost to excretion. How an increase in carbon utilization efficiency leads to reduced aging is anyone's guess.

A standard aging argument is that aging involves a hunkering down—when animals are short of food they alter their metabolism so that both aging and reproduction are postponed until better times. The new findings are consistent with this metabolism-centric view. “People have taken for granted that we know everything about metabolism,” says Escalante-Semerena, but aging research may be proving them wrong. ■

Reference: Starai, V.J., et al. 2002. *Science*. 298:2390–2392.



A Sir2 homologue can deacetylate a metabolic enzyme.

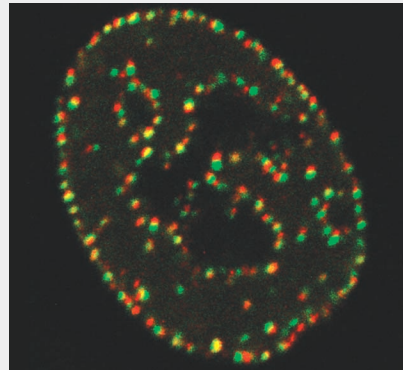
Replicating dominos

The choreography of DNA replication has been a mysterious black box. How is replication targeted to different, reproducibly localized DNA domains at the early, middle, and late stages of S phase? Rather than invoking a homunculus, Anje Sporbert, Cristina Cardoso (Max Delbrück Center for Molecular Medicine, Berlin, Germany), and colleagues have come up with a domino model that runs itself once the replication program gets started.

Cardoso's group focused on the dynamics of PCNA, a sliding clamp that aids in DNA polymerase processivity. Recovery of fluorescence after bleaching of PCNA was slow, suggesting that PCNA from within a given replication focus is recycled to load at each new Okazaki fragment.

But the picture over the longer term is

very different. Based on pulse labeling of DNA replication and PCNA, bulk PCNA is released from the sites that were replicated earlier and keeps up with the sites of ongoing replication. The recycling at this timescale is indirect—it is unbleached PCNA from



Early (green) and later (red) replication foci are next to each other.

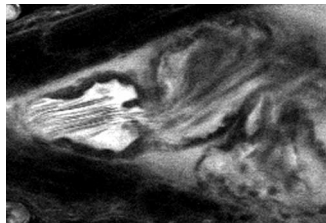
the nucleoplasm that assembles at the new replication foci. Importantly, these foci are found preferentially right next to the recently bleached foci.

Earlier workers mapping DNA replication sites had noted, but had not dwelt upon, this close apposition of sequential replication foci. Cardoso believes that the pattern is crucial. "We think the major DNA replication program could be explained by this," she says. Activity at early replication foci may transmit a signal down the DNA strand to activate neighboring foci, either by pulling physically on the DNA (like undoing a shoelace) or by an unknown chromatin-modifying mechanism. "The program would be fixed," says Cardoso, "as long as the first sites to fire were fixed." ■

Reference: Sporbert, A., et al. 2002. *Mol. Cell.* 10:1355–1365.

Hearts grow with the flow

A properly formed heart comes only after effort—the pumping of cells through a primitive heart to generate gene-activating shear forces. That is the conclusion of Jay Hove, Reinhard Köster, Scott Fraser, Morteza Gharib, and colleagues (California Institute of Technology, Pasadena, CA), who have come up with a method for watching heart development by culturing developing zebrafish on microscope stages.



Blood flow (visible as streaks) directs heart development in zebrafish.

Köster/Macmillan

The researchers tracked the movement of blood cells against the background of fluorescently stained blood serum. High-speed imaging (1,000 frames/s) and digital particle-tracking yielded movement vectors for the blood cells. The cells moved at speeds of up to 1.5 mm/s in a primitive, valveless heart and 0.5 cm/s in a more developed heart. The resultant churning forces should be more than enough to activate the many endothelial genes known to respond to shear stress.

The high speeds surprised the group. The small scale of the developing heart was thought to slow down blood cells because of frequent collisions of cells with vessel walls. But apparently the zebrafish has a mighty heart, even at the age of 37 h.

If flow was blocked with a bead, the juvenile hearts continued to beat but failed both to form one heart chamber and to loop correctly. Thus, says Köster, "it's not just a genetic program that directs heart development." He hopes to determine which developmental genes are responding to flow forces to shape the development of the heart. ■

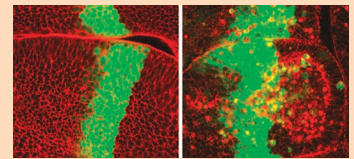
Reference: Hove, J.R., et al. 2003. *Nature.* 421:172–177.

Poised to migrate

Epithelial cells are ready to run off at any moment, at least based on the results of Olga Speck, Richard Fehon (Duke University, Durham, NC), and colleagues. They have found that moesin, previously thought to be a stolid structural component, is actually a signaling protein that maintains epithelial cell identity by suppressing Rho activity.

Ezrin, radixin, and moesin (ERM)—represented only by Moesin in flies—are proteins that link actin to various transmembrane proteins at the apical surface of epithelial cells. The link was thought to help maintain epithelial structures such as microvilli, but Fehon's group found that flies lacking Moesin had epithelial cells that lost polarity, dropped out of monolayers, and migrated invasively.

Moesin appears to act by suppressing Rho. Interfering with ERM proteins in mammalian epithelial cells boosted



Epithelial cells (green) go for a walk when Moesin is missing (right).

Fehon/Macmillan

Rho activity, and fly *Moesin* mutants improved when Rho1 dose was halved. Rho1 overexpression in wild type, however, phenocopied Moesin loss.

Thus epithelial cells must be actively maintained in their polarized state, lest they slip off into the motile night. This instability may reflect the transitions from structured epithelium to motile founder cells that occur frequently during development—a transition that carcinoma cells apparently recapitulate when they become metastatic. ■

Reference: Speck, O., et al. 2003. *Nature.* 421:83–87.