Text by Ben Short
bshort@rockefeller.edu

Stem cells get a cholesterol test

Study reveals that dietary cholesterol regulates Hedgehog signaling and stem cell proliferation in flies.

In addition to responding to changes in their local microenvironment, stem cells are regulated by systemic changes, such as the availability of nutrients in an organism's diet. In *Drosophila*, for example, egg production—which relies on the proliferation of both germline stem cells (GSCs) and follicle stem cells (FSCs)—is switched off when food is scarce and reactivated when conditions improve (1). Hartman et al. reveal that dietary cholesterol levels regulate FSC proliferation by controlling the release of Hedgehog (Hh) protein (2).

GSCs, which give rise to the nurse cells and oocyte of *Drosophila* ovaries, are regulated by insulin signaling. On the other hand, FSCs, which proliferate to form an outer layer of epithelial follicle cells, are stimulated by Hh and other proteins released from nearby cells in the ovary called apical cells. Alana O'Reilly, Tiffiney Hartman, and colleagues at the Fox Chase Cancer Center in Philadelphia have previously shown that the Hh receptor Boi limits Hh release and FSC proliferation by sequestering Hh on the surface of apical cells (3). "We were interested in identifying the pathway that triggers Hh's release from Boi," O'Reilly says. "And we thought that changes in nutrient levels might alter the equilibrium between sequestration and release to control stem cell proliferation."

When Hartman et al. placed female flies on a restricted diet of water and a few simple sugars, Hh remained on the surface of apical cells, and FSC proliferation and egg production were shut down (2). Refeeding the flies with nutrient-rich yeast, however, quickly prompted Hh's release from apical cells and its accumulation in proliferating FSCs. "We started fractionating yeast to find out what the critical nutrient was, [and we discovered that] it had to be a lipid," O'Reilly explains. "There's only one lipid required in the diet of flies, and that's cholesterol."

Sure enough, feeding nutrient-restricted flies with lipid-free yeast extract plus cholesterol stimulated Hh release and FSC proliferation. "One of the main roles of follicle cells is to produce yolk," O'Reilly says. "So cholesterol may be the trigger because these cells depend on it to generate yolk and produce a viable egg."

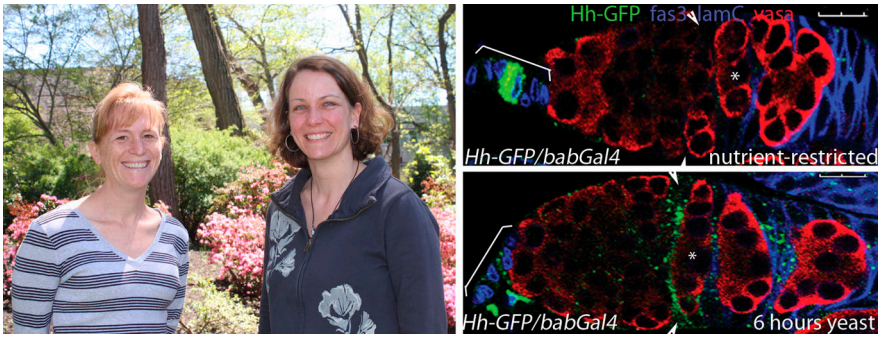
"As soon as we knew that cholesterol was the key nutrient," O'Reilly continues, "we looked at its receptor, DHR96." Starving flies whose apical cells lacked DHR96 failed to reinitiate FSC proliferation upon refeeding. Flies lacking Boi, on the other hand, failed to restrict FSC proliferation under starvation conditions, as Hh was constitutively released from apical cells. Boi mutants lacking the extracellular Hh-binding domain failed to rescue this phenotype, whereas mutants lacking the cytoplasmic domain successfully shut down FSCs upon starvation but failed to induce FSC proliferation upon refeeding. Boi's intracellular domain is therefore required for cholesterol-induced Hh release.

> "[Flies] can quickly drive egg production before the food runs out."

Hartman et al. narrowed down the critical region to a serine residue in Boi's cytoplasmic domain that was phosphorylated by S6 kinase. Flies that lacked S6 kinase or that expressed a nonphosphorylatable version of Boi failed to release Hh or stimulate FSC proliferation upon refeeding. S6 kinase is regulated by DHR96 and its vertebrate orthologues (4, 5) and can therefore link cholesterol levels to the release of Hh from apical cells. "We're now looking at how DHR96 controls S6 kinase," O'Reilly says. "DHR96 is best known as a transcription factor, but the feeding response happens so quickly—within 15 minutes—that a transcriptional response would probably be too slow. We're also trying to figure out how phosphorylation triggers Hh's release from Boi."

Hartman et al. think that the speed of the response to nutrient availability is critical. "It makes sense for flies to have an extremely rapid response so they can quickly drive egg production before the food runs out," O'Reilly says. "We're investigating whether the pathway is conserved in mammalian tissues or in Hh-dependent cancers. Promoting Hh sequestration could improve our ability to treat those types of tumors."

Focal Point



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Tiffiney Hartman (left), Alana O'Reilly (right), and colleagues (not pictured) reveal how dietary cholesterol regulates Hedgehog signaling to control follicle stem cell proliferation and egg production in *Drosophila*. In the ovaries of starving flies (right, top), Hedgehog protein (green) is sequestered on the surface of apical cells (bracket) by the membrane receptor Boi. Upon feeding (right, bottom), S6 kinase phosphorylates Boi, inducing Hedgehog's release and subsequent accumulation in follicle stem cells (arrowheads), where it stimulates proliferation and egg production.

1. Drummond-Barbosa, D., and A.C. Spradling. 2001. *Dev. Biol.* 231:265–278.
2. Hartman, T.R., et al. 2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201212094.
3. Hartman, T.R., et al. 2010. *J. Cell Biol.* 191:943–952.
4. Lindquist, R.A., et al. 2011. *Genome Res.* 21:433–446.
5. Bettoun, D.J., et al. 2002. *J. Biol. Chem.* 277:24847–24850.