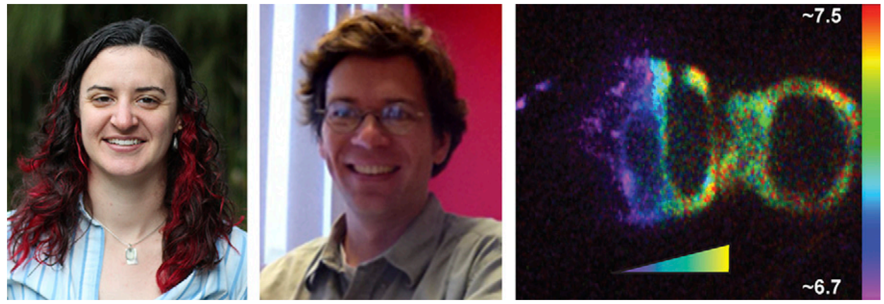
Text by Ben Short
bshort@rockefeller.edu

A basic guide to stem cell differentiation

Increasing intracellular pH promotes the differentiation of adult and embryonic stem cells.

FOCAL POINT



ULMSCHNEIDER PHOTO COURTESY OF THI NGUYEN; NYSTUL PHOTO COURTESY OF THE AUTHOR

Bryne Ulmschneider (left), Todd Nystul (center), and colleagues reveal that an increase in intracellular pH (pHi) promotes the differentiation of two different types of stem cells. A fluorescent pH biosensor shows that, in wild-type *Drosophila* (right), the pHi of follicle stem cells at the anterior end of the follicle epithelium increases as the cells differentiate to mature follicle cells in more posterior positions. This increase in pHi is smaller, and overall pH is lower, upon knockdown of the Na⁺–H⁺ exchanger *DNhe2*, resulting in impaired follicle cell maturation and oogenesis defects, at least in part because of a failure to down-regulate Hedgehog signaling at the appropriate stage of differentiation. An increase in pHi also promotes the differentiation of mouse embryonic stem cells, suggesting that pH dynamics may be a common regulatory mechanism in a variety of stem cell populations.

Many different signaling pathways combine to control the differentiation of stem cells by regulating cellular processes such as transcription and chromatin folding. Ulmschneider et al. now reveal that a more fundamental aspect of cellular physiology—intracellular pH—regulates the differentiation of both *Drosophila* follicle stem cells and mouse embryonic stem cells (1).

Cells use homeostatic mechanisms to maintain their cytoplasm at a pH close to neutral. Though this protects cells from the damaging effects of acids and alkalis, it is now known that small changes in intracellular pH (pHi) can also act as cytosolic signals that regulate a variety of processes, from cell cycle progression (2) to membrane trafficking (3). By altering the protonation and charge of amino acids such as histidine, changes in pHi can affect protein conformation and activity. In fact, Diane Barber and colleagues at the University of California, San Francisco (UCSF) view protonation as a reversible posttranslational modification akin to phosphorylation or ubiquitination (4).

Barber and colleagues have been studying the effects of pHi in tissue culture cells, but a fellow group leader at UCSF, Todd Nystul, wondered whether pHi might regulate the differentiation of stem cells in vivo. "I'm always interested in new ways for cells to control differentiation," Nystul explains. "So I thought we should take a look."

Nystul and colleagues, led by graduate student Bryne Ulmschneider, worked with Barber's group to analyze *Drosophila* lacking *DNhe2*, a Na^+–H^+ exchanger that increases pHi by transporting protons out of the cell. "*DheN2*-null flies are almost infertile," Nystul says. "They lay very few eggs, suggesting that there must be defects in oogenesis."

"pH dynamics may therefore be a more common component of cellular differentiation than we previously thought."

Ulmschneider et al. found that the ovaries of *DNhe2*-deficient flies showed a variety of morphological defects arising from the failure of follicle stem cells (FSCs) to properly differentiate into the various follicle cell types that support germ cell development (1). Using a genetically encoded pH biosensor, the researchers determined that, in wild-type flies, pHi rises from 6.8 to 7.3 as FSCs differentiate into mature follicle cells. This increase was smaller, and overall pH was lower, in flies expressing decreased amounts of *DNhe2*. "So the increase in pH promotes follicle cell maturation," Nystul says.

Hedgehog (Hh) signaling plays a key role in regulating follicle cell development; the pathway is activated early in the process to specify follicle cell fate, but must then be suppressed to allow follicle cell maturation. Ulmschneider et al. found that elevating pHi by overexpressing *DNhe2* down-regulated the signaling protein Smoothened, thereby attenuating the Hh pathway's activity. Indeed, overexpressing *DNhe2* partially rescued the oogenesis defects induced by Hh hyperactivation. "So at least one of the roles that pHi has during follicle cell differentiation is to down-regulate the Hh pathway at the right time," Nystul explains.

The researchers then examined whether pHi also affects the differentiation of another type of stem cell. When mouse embryonic stem cells (mESCs) were allowed to differentiate in vitro, they also showed an increase in pHi, albeit transiently, from 7.4 to a peak of 7.65. Treating the cells with an inhibitor of mammalian NHE1 blocked this increase in pHi and suppressed differentiation. Adult and embryonic stem cell differentiation are generally considered to be very different processes, but, says Nystul, "it's amazing that changes in pH are important in both cases. pH dynamics may therefore be a more common component of cellular differentiation than we previously thought."

Hh signaling isn't thought to be involved in mESC differentiation, but changes in pHi could affect any number of signaling pathways. Nystul and colleagues now want to investigate whether pH changes affect the differentiation of other stem cell populations in mice and flies. "We want to get a sense for how common this really is," Nystul says.

1. Ulmschneider, B., et al. 2016. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201606042
2. Putney, L.K., and D.L. Barber. 2003. *J. Biol. Chem.* 278:44645–44649.
3. Mukherjee, S., et al. 2006. *Biochem. J.* 398:97–105.
4. Schönichen, A., et al. 2013. *Annu. Rev. Biophys.* 42:289–314.