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 pH in tissue culture cells, but a fellow group leader at UCSF, Todd Nystul, wondered whether pH 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 pH by transporting protons out of the cell. “Dne2-null flies are almost infertile,” Nystul says. “They lay very few eggs, suggesting that there must be defects in oogenesis.”

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 pH by overexpressing DNhe2 down-regulated the signaling protein Smoothed, 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 pH 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.

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