Histone exchange program

Histone assembly into chromatin is often studied in vivo by using fluorescently tagged proteins. But these studies rely on making guesses about what machinery might be involved. In vitro systems are less biased but generally lack the structural complexity and heterogeneity of natural chromatin.

Kimura’s group got the best of both worlds by using permeabilized cells, which maintain their natural chromatin structure. Yet the system allowed the authors to purify unanticipated histone assembly and exchange factors, including protein phosphatase 2Cγ (PP2Cγ), which inserted histone H2A and H2B into preassembled nucleosomes.

PP2Cγ also dephosphorylated H2A and H2B. As phosphates mark histones at DNA-damaged sites, they must be removed from histones that will be inserted into undamaged sites. Cells lacking PP2Cγ were more sensitive to DNA damage only if they lacked the delay imposed by replication and damage checkpoints. Most dephosphorylation is thus probably done by PP2A, while PP2Cγ picks up the slack when cells have very little time before the next mitosis.

Chromatin from permeabilized cells remains competent for replication and transcription, so the authors now hope to characterize the nucleosome changes that accompany these events. JCB

An integrin’s gut instincts

Epithelial β1 integrins are known for their anchoring abilities. But their talents are different in the gut, say Jones et al. (page 505), where cells without β1 integrin stick just fine but overproliferate.

By anchoring cells to the matrix, β1 integrin probably keeps cells alive and cycling. Indeed, proliferation is promoted, not halted, by this integrin in skin and mammary epithelia. Indeed, proliferation is promoted, not halted, by this integrin in skin and mammary epithelia. So Jones and colleagues were surprised to find that mouse gut epithelial stem cells were hyperproliferative upon loss of β1 integrin.

Too busy dividing, these stem cells did not differentiate into a proper epithelium. As a result, the mutant mice died soon after birth from a lack of nutrient absorption.

Different receptors must stick these stem cells to their matrix, as the authors found no anchorage or survival problems. Here, β1 integrin appears to be more concerned with signaling; its loss led to a PI3K-dependent decrease in Hedgehog (Hh) expression. The loss of intestinal Hh is known to increase cell proliferation.

Gut epithelial cells lack Hh receptors, so the authors suspect that Hh acts on the surrounding stromal cells, which respond by making molecules such as BMPs that downregulate epithelial proliferation. The situation is different in the skin and breast, where Hh is both expressed and detected by epithelial cells. JCB

Cortex construction

On page 477, blebbing cells open a window into the formation of a contractile actin cortex. Charras et al. use the blebs to order the events of cortex assembly.

Blebs form (usually during apoptosis or cytokinesis) as a result of strong actomyosin forces that separate a section of the plasma membrane from its underlying cortex and inflate it with cytosol. This free membrane is then a platform for new cortex assembly, which the authors watched by tracking potentially relevant proteins.

Cortex assembly occurred via three independent steps. First, ezrin and moesin—proteins that establish links between the membrane and cytoskeleton—were recruited to the blebs.

Actin polymerization and the membrane recruitment of several actin-binding proteins followed. The authors suspect that new actin is nucleated by a formin, based on filament morphology and the lack of Arp2/3 at the membrane, although the Dia1 formin was also missing. The relevant formin may be activated—either constitutively or in response to the loss of membrane–cortex contact—by RhoA, which had an unusually strong membrane localization in blebs.

The final step included the generation of force on the cortex, which followed myosin recruitment into foci at the membrane. That only a few foci exert force on the network suggests that cells might lock in tension by cross-linking actin filaments. The foci and the unpolarized actin network also distinguish the contractile cortex of blebbing cells from the ordered actomyosin network of muscle cells. JCB