Histones help clones forget their pasts

Amid the hype surrounding cloning by somatic cell nuclear transfer, the astonishingly low success rate of the process has received relatively little attention. Now, on page 37, Kim et al. show that histone deacetylation is an important feature of the nuclear reprogramming that occurs in oocytes, both during normal meiosis and in nuclear transfer experiments. The work suggests strategies that could improve the efficiency of cloning, and also helps to explain how somatic cells retain their identities during mitosis.

Using immunocytochemistry, the authors examined changes in histone acetylation in oocytes during meiosis, and compared these with mitotic acetylation patterns. In meiotic oocytes, histone acetylation levels drop markedly. A similar decrease occurs in somatic cell nuclei that are transferred into enucleated oocytes. During mitosis, however, the same sites on histones remain acetylated. The histone deacetylase enzyme HDAC1 colocalizes with chromosomes during meiosis, but not during mitosis.

Kim et al. suggest that the acetylation states of histones propagate "cell memory," ensuring that cells retain information about their lineages during mitosis. In meiotic oocytes or transferred somatic cell nuclei, histone deacetylases gain access to the chromosomes, erasing cell memory and reprogramming the nucleus so that it can give rise to all of the cell types in a new embryo.

Currently, the only way to determine whether a transferred somatic cell nucleus has been reprogrammed in a cloning experiment is to implant the oocyte and wait for the embryo to develop. The new work suggests that an assay of histone acetylation levels might sort the reprogrammed nuclei from the failures, potentially increasing the efficiency of cloning. Reagents that increase deacetylation or target histone deacetylases to the chromosomes might also make the process more reliable. The authors are now trying to identify the molecular mechanisms that regulate the localization of histone deacetylases during meiosis.

A fusion inhibitor on endosomes

Endocytosed proteins come together in the early endosome to be sorted to different locations. Some endosome fusion is needed to promote this mixing, but the process must be regulated both to stop the formation of one giant early endosome and to promote eventual sorting. Now, Sun et al. (page 125) have developed a clever assay for studying homotypic endosome fusion and used it to uncover a surprising new fusion inhibitor activity for an early endosome protein called Hrs.

The authors allowed different populations of HeLa cells to endocytose EGF linked to one of two different fluorophores, and then isolated pools of endosomes or lysosomes from the cells. When the isolated organelles were allowed to fuse in cell-free reactions, the mixing of their contents caused fluorescence resonance energy transfer between the two fluorophores, providing an easily quantified readout.

Using this technique, Sun et al. show that Hrs, a mammalian protein found primarily on early endosomes, specifically inhibits homotypic fusion of early endosomes, and that the coiled-coil domain of Hrs is necessary and sufficient for this activity. The Hrs coiled-coil domain binds to a SNARE complex consisting of SNAP-25 and syntaxin 13, thus preventing the binding of VAMP2, which is required for fusion.

The results are surprising: first, because Hrs was thought to bind to endosomes through a phosphorylated lipid rather than a specific protein receptor; and second, because SNAP-25 was thought to be involved only in exocytosis, whereas the new work shows a requirement for SNAP-25 in an endocytic pathway.

Sun et al. suggest that although the Hrs coiled-coil domain prevents fusion, other domains of Hrs might simultaneously direct cargo sorting or endosome movement. The combined activities of preventing an early endosome from fusing with its neighbors and moving it toward its destination would help Hrs direct the sorting and separation of endosomal cargo. The authors are now examining the regulation of the Hrs-containing SNARE complex and developing an automated, high-throughput version of their assay for future studies.