Histones help clones forget their pasts

Among 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.
Controlling flagellar strokes

The movements of cilia and flagella are driven by axonemal dynein ATPases, whose activity must be coordinated in space and time. On page 47, Rupp and Porter provide the first molecular characterization of a component of the dynein regulatory complex (DRC), a crucial but poorly understood regulator of flagellar movement. Besides suggesting a model for DRC assembly, the work identifies a possible connection between motility regulation and a theoretical mechanism of cell growth arrest.

Using insertional mutagenesis in Chlamydomonas, the authors identified a new mutation in the PF2 locus that causes motility defects. Further analysis showed that PF2 encodes subunit 4 of the DRC. The PF2 protein is uniformly distributed along the length of the axoneme and also associates with the basal body region, and its predicted structure has several coiled-coil domains that could mediate protein–protein interactions. PF2 mutants fail to assemble five of the seven known DRC subunits.

The results suggest that PF2 acts as a molecular scaffold, stabilizing the DRC by interacting with other components of the complex. Close homologues of PF2 occur in a wide range of cell types, and include a trypanosome gene product required for directional motility, and mouse and human gene products enriched in growth-arrested cells. One exciting possibility is that primary cilia containing PF2 homologues may transmit a signal to the cell to initiate growth arrest. As a first step in confirming this idea, the localization of PF2-related products must be determined in cells lacking flagella.

Hierarchical condensation

As cells progress from interphase through metaphase, their chromosomes undergo structural changes that are easy to observe but difficult to understand. Strukov et al. (page 23) attacked this problem by engineering chromosome regions that can be labeled selectively, allowing the authors to analyze chromosome condensation at high resolution. Their initial results provide strong support for one model of chromosome folding while contradicting predictions of another.

Previous work has supported two different models of chromosome condensation. In the radial loop model, scaffold/matrix-associated region (SAR/MAR) sequences in DNA anchor portions of the chromosome to a central scaffold, producing loops of 30-nm fibers. In the hierarchical folding model, however, there is a continuum of folding steps, independent of the SAR/MAR sequences, that produces more complicated structures than the 30-nm loops.

In the new work, the authors created chromosome regions containing large copy numbers of a vector containing SAR/MAR sequences flanking lac operator repeats. Staining with lac repressor showed that the SAR sequences are not specifically targeted to the chromatid axis, contradicting predictions of the radial loop model. Closer analysis revealed a 250-nm diameter coiling subunit within native metaphase chromosomes, strongly supporting the hierarchical folding model. The authors are now trying to improve the resolution of the system even further, and hope to analyze the entire process of chromosome condensation.

Splicing fibronectin’s functions

On page 149, Muro et al. describe an elegant strategy for studying the functions of alternatively spliced isoforms of fibronectin. The work reveals surprisingly subtle phenotypes in mice that cannot carry out one form of alternative splicing, and demonstrates a general approach that could be applied to virtually any alternatively spliced gene.

Disruption of the fibronectin gene in mice is lethal to embryos, making it difficult to analyze the developmental functions of the protein’s isoforms. The authors got around this obstacle by inserting optimized splice sites and loxP recombination sites in the introns adjacent to the EDA exon of fibronectin. In mice homozygous for this allele, the EDA exon is constitutively included in fibronectin. Crossing this strain with a CRE recombinase–expressing “deleter” mouse produced animals lacking the EDA exon.

In wild-type mice, most of the embryonic fibronectin includes the EDA exon, but most of the adult fibronectin lacks it. Despite this pattern, the new work shows that mice that constitutively include or exclude EDA develop normally. Constitutive inclusion of EDA, however, causes a substantial decrease in total fibronectin levels in adult tissues, whereas a lack of EDA causes abnormal cutaneous wound healing. Both mutant strains have significantly shorter lifespans than wild-type mice, possibly because of defects in tissue repair and regeneration.

Healing gets messy without a fibronectin isofrom.