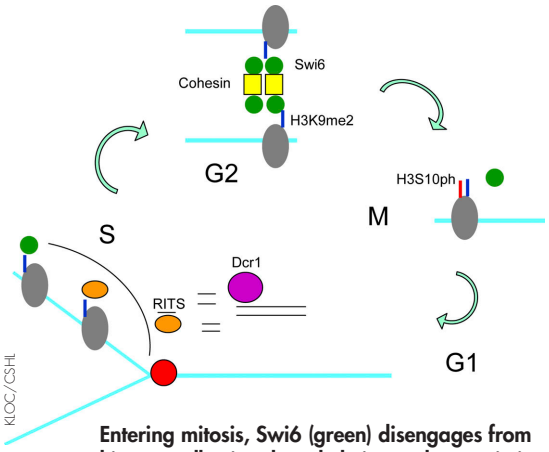
Research Roundup



KLOC/CSHL

Entering mitosis, Swi6 (green) disengages from histones, allowing demethylation and transcription in S phase. RNAi remethylates the histones to complete the cycle.

Transcribing "silent" DNA resilences it

Transcription of heterochromatin during S phase is the key to the faithful inheritance of silenced DNA at the centromere, according to Anna Kloc, Rob Martienssen (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), and colleagues.

Histone methylation keeps heterochromatin condensed and the genes within silent. Yet the same methylation patterns are passed on to daughter cells. This inheritence requires RNA interference—and thus transcription. "That's a paradox we've been looking at for several years," says Martienssen. To resolve the paradox, the authors examined yeast centromeres for changes in RNA transcripts, histone modifications, and RNAi activity throughout the cell cycle.

As they enter G2, methylated histones at the centromere link to a major heterchromatin structural protein called Swi6, which in turn binds to cohesin, thereby tying the replicated chromosomes together. But during mitosis, histone phosphorylation knocks off Swi6; its removal is a prerequisite for transcription. The authors showed that this loss of Swi6 was accompanied by histone demethylation, which is also required for transcription. After passing through G1, the exposed centromeric sequences were transcribed during very early S phase. The transcripts were quickly processed by the RNAi machinery into siRNAs, which the authors had previously shown directs the methylation machinery back to the same demethylated histones at the transcribed centromeric sequences. So by the end of S phase, those sequences were remethylated and beginning to rebind Swi6 and cohesin, forming true heterochromatin again.

The timing makes sense. "S phase is exactly when you would need to modify histones to inherit epigenetic modifications," says Martienssen, since that is when both copies can be targeted at once.

The authors also showed that temperature elevation inhibited the whole process. If the same is true in other systems, it might explain why some plants require a cold period before they can flower. This necessity depends on RNAi silencing and heterochromatin and requires cell division, suggesting that cold-driven gene modifications inherited during the winter trigger flowering in the spring. JCB

Kloc, A., et al. 2008. *Curr. Biol.* doi:10.1016/j.cub.2008.03.016.

Embryos experience tension

Tension helps organize tissue layers in early embryos, according to Michael Krieg, Carl-Philipp Heisenberg (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany), and colleagues.

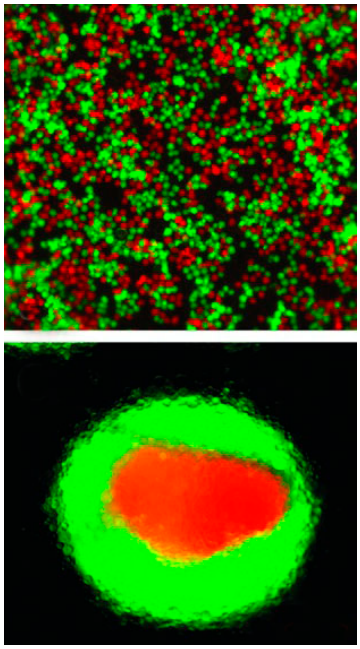
For Heisenberg, "The big question is what factors are important in cell sorting and tissue organization during development." Differential adhesion among cells has been the most prominent hypothesis. Differences in cell cortex tension, which is produced by actomyosin contraction, have never been carefully measured before.

Using an atomic force microscopy probe, the authors measured the resistance to deformation in cells from each layer of the embryo. They found that tension was highest in the outermost layer, the ectoderm, followed by the endoderm and finally the mesoderm. By inhibiting myosin, the authors reduced ectoderm tension to match that of the other cell types. Ectoderm tension was also reduced when cells were treated with a growth factor that diverted them to a nonectodermal fate.

Cells sorted according to their tension rather than adhesion levels. When mixed in vitro, ectoderm cells partitioned to the inside, surrounded by meso- and endoderm cells. This inside-out arrangement is probably due to the absence of yolk cells and other in vivo factors that interact preferentially with each layer. Treatment with actomyosin inhibitors, which did not change adhesion, prevented ectoderm cells from burrowing inwards, indicating that cells rely on tension differences for sorting.

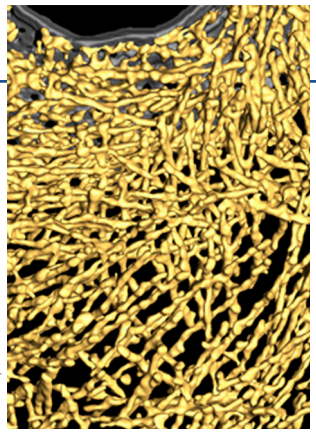
"In textbooks, you will read that differential intercellular adhesion of cells is sufficient to explain their sorting behavior," says Heisenberg. "But it's not just the adhesion that's doing it. It's a combination of several factors, including adhesion and tension." JCB

Krieg, M., et al. 2008. *Nat. Cell Biol.* doi:10.1038/ncb1705.



HEISENBERG/MACMILLAN

Tension helps sort (top to bottom) ectoderm (red) and mesoderm (green) cells into ordered layers in vitro.



ROBERTS/NAS

MSP filaments pack more loosely as they elongate away from the site of polymerization (top).

Text by Richard Robinson
rrobinson@nasw.org

Pack loose, grow long

Longer cytoskeletal rods have more empty space between them, and that space helps drive amoeboid cell motility, say Thomas Roberts (Florida State University, Tallahassee, FL) and colleagues.

Theories of amoeboid-like cell movement have largely relied on cytoskeleton polymerization to explain membrane protrusion. But in studying filaments of the worm major sperm protein (MSP) using electron tomography, the authors noted that filaments became more loosely packed as they elongated.

"Polymer physicists have known for a long time that shorter rods pack more tightly," says Roberts. By contrast, longer rods, regardless of composition, enclose more empty space. Measurements from the new images were consistent with this effect, and simulations confirmed that growing rods exhibited the same length-dependent space constraints as static ones. An MSP mutant that polymerized 2.4 times more slowly reduced the protrusion rate by 3.4-fold, indicating that both polymerization and packing-based expansion contribute to protrusion.

"It's plausible that this effect may occur in actin systems as well," says Roberts, although actin-based protrusion differs in its molecular details, including the use of cross-linking proteins. JCB

Miao, L., et al. 2008. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.0708416105.

MukBs cooperate to loop DNA

When it comes to corralling DNA, MukB is the little condensin that could, say Yuanbo Cui, Aoya Petrushenko, and Valentin Rybenkov (University of Oklahoma, Norman, OK).

Small in size but with a big effect, condensins are believed to stabilize large DNA loops, thereby giving order to an otherwise tangled mess of chromatin. To investigate the mechanics of the *E. coli* condensin MukB, the authors stretched DNA between a glass capillary and a magnetic bead and monitored MukB-induced DNA condensation.

The DNA compacted in a series of discrete steps, indicating that multiple MukBs are at work. The process could be reversed by applying excess force in the opposite direction, but the longer a condensed complex was allowed to sit, the more force was required, suggesting that MukBs link up over time.

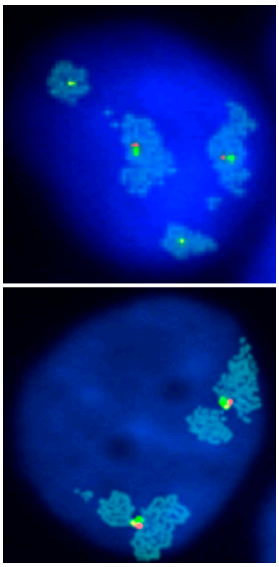
A small decrease in MukB concentration led to a dramatic decline in condensation rate. The slowed rate was primarily due to a longer lag before condensation began, which indicates that multiple MukBs must first come together in a nucleation step. ATP stimulated this nucleation but had no subsequent effect, and faster hydrolysis did not lead to faster nucleation. ATP thus seems to be a structural, rather than energetic, component.

According to Rybenkov, MukBs are acting as clamps to hold loops together. "I think we now have a biochemical explanation for how stable loops appear in such a big unruly molecule as DNA," he says. JCB

Cui, Y., et al. 2008. *Nat. Struct. Mol. Biol.* doi:10.1038/nsmb.1410.

Motors bring genes together

Nuclear motors rearrange chromosomes to enhance estrogen-driven transcription, according to Esperanza Nunez, Michael Rosenfeld, Xiang-Dong Fu (University of California at San Diego, CA), and colleagues.



ROSENFELD/ELSEVIER

Genes on chromosome 2 (red) and 21 (green) come together in the presence of estrogen (bottom).

Estrogen-responsive enhancers occur throughout the genome, often long distances from the genes whose transcription they enhance. To determine how these enhancers and their bound estrogen receptors are brought to their gene targets, the authors used FISH to follow their movements. Under the influence of estrogen, two genes on chromosomes 2 and 21 formed 2–21 pairs or tetrads of all four alleles. The team never observed 2–2 or 21–21 pairings. The factors that cause these specific pairings, and prevent others, are unknown.

Chromosomes usually came together within five minutes of estrogen exposure. The movements required cytoskeletal elements and their motors; blocking myosin or the polymerization of nuclear actin abolished the interactions and reduced gene expression. So did blocking dynein light chain 1, which is known to bind to the estrogen receptor. The authors suggest that dynein links the actin to the DNA-bound estrogen receptor.

Other estrogen-regulated gene sets converged in other nuclear locations, each the site of an RNA-processing nuclear speckle. When the authors knocked down a demethylase that is required for estrogen-dependent gene activation, genes came together but did not bind to the speckle. Gene expression from interacting alleles was much higher than for noninteracting ones, indicating that their linkages increase transcription rates. JCB

Nunez, E., et al. 2008. *Cell.* 132:996–1010.