

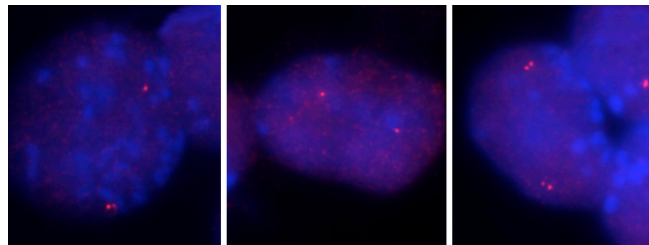
A chromatin twist to silencing choice

Although cells typically transcribe both copies of a particular gene, they sometimes flip off one copy and rely on the other. A histone-modifying protein might help determine which copies are turned on or off by tweaking the structure of chromatin, as Alexander et al. show.

This silencing—called monoallelic expression—reaches an extreme in female mammals, in which one X chromosome almost completely shuts down. Which copy a cell chooses to switch off appears to be random, and the selection mechanism remains unexplained.

Last year, the researchers showed that, in embryonic stem cells, would-be active and inactive X chromosomes differ even before one gets silenced. When the scientists tagged specific genes on the chromosomes using fluorescence in situ hybridization (FISH), one X chromosome typically carried two glowing spots (usually a sign that it will be shut down), whereas its counterpart had one (a sign of future expression). What structural differences between chromosomes this pattern reveals is unknown. Autosomal genes that don't need to be silenced tend to show up as either two single dots or two double dots.

This single dot–double dot (SD) pattern also marked monoallelic genes on autosomes, Alexander et al. found when they examined embryonic stem cells, which haven't yet picked which allele to close down. Before a stem cell made that choice, however, the

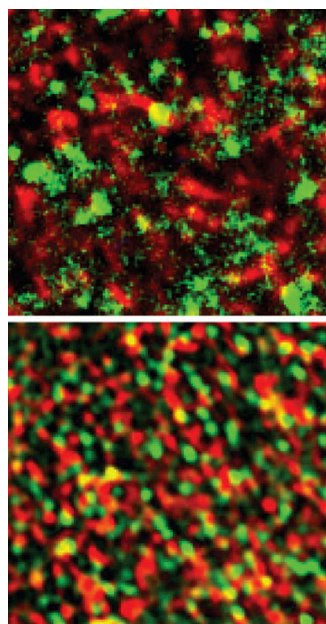


The single dot–double dot pattern (left) characterizes genes with monoallelic expression, versus two single dots (center) and two double dots (right).

alleles often flipped between single and double states, indicating that the cell is sometimes undecided about which allele to quiet. Switching also occurred on X chromosomes.

Suspecting that the SD arrangement might reflect a difference in chromatin structure, the researchers tested the effects of deleting the protein Eed, which helps tighten chromatin by methylating histone H3. Loss of Eed reduced the prevalence of SD cells and resulted in more double dot states. A single spot might indicate scrunched together sister chromatids, while a double spot might reveal standoffish sisters. But how Eed chooses which allele to target is unknown. The researchers now want to determine whether the single-spot-on–double-spot-off pattern shown by X chromosomes holds true for autosomes. **JCB**

Reference: Alexander, M.K., et al. 2007. *J. Cell Biol.* 179:269–276.



EGFR (red) finds its way to inhibitory Cav1 domains (green) when galectin lattices are missing (bottom).

EGFR molecules interlink glycoproteins on the cell surface. The enzyme Mgat5 promotes these connections by modifying the ends of the glycoproteins. The researchers previously showed that the lattice

A receptor with divided loyalties

The epidermal growth factor receptor (EGFR) is caught in a tug-of-war between two membrane domains, as Lajoie et al. reveal. Which domain wins the competition for this division-promoting protein helps determine whether a cell becomes cancerous.

Creating one of the domains is Caveolin1 (Cav1), which congregates in plasma membrane indentations called caveolae. Cav1 clusters pen in EGFR molecules and block them from relaying progrowth signals into the cell. Cav1 is faulty or absent in many tumors. Another domain, the galectin lattice, forms when galectin molecules interlink glycoproteins on the cell surface. The enzyme Mgat5 promotes these connections by modifying the ends of the glycoproteins. The researchers previously showed that the lattice

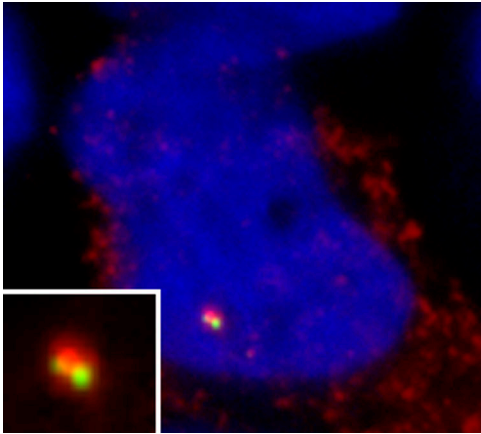
holds EGFR at the membrane and increases cells' sensitivity to growth stimulators such as epidermal growth factor (EGF).

In the current study, Lajoie et al. identified interactions between the two types of domains. The researchers found that in tumor cell lines that lack Mgat5, adding a little Cav1 squelched EGF signaling—and thus tumor growth—by trapping EGFR. Eliminating Cav1 from these cells restored their sensitivity to EGF. Tumor cells that make Mgat5 were also responsive to EGF. The results suggest that galectin lattices block Cav1's inhibition of EGFR.

The lattices interfered by restricting EGFR movement. The authors tracked the receptor's movements by photobleaching part of the membrane and following labeled EGFR molecules. If tumor cells had normal levels of Mgat5, EGFR was less likely to cozy up to Cav1 clusters than in cells lacking the enzyme.

Overall, the study indicates that the galectin lattice speeds tumor growth by capturing EGFR and preventing Cav1 domains from ensnaring the receptor and shutting it down. Because the lattice gets first dibs on the receptor, Cav1 serves as a tumor suppressor only when the lattice is down. The work also explains the mysterious observation that some of the most dangerous cancers produce copious Cav1: other molecules can override its ability to block growth. **JCB**

Reference: Lajoie, P., et al. 2007. *J. Cell Biol.* 179:341–356.



A glow marking an HIV transcription site allows RNA polymerase's speed to be determined.

RNA polymerase on the clock

Is RNA polymerase II slacking off while it's supposed to be on the job? Probably not. But as Boireau et al. report, the first measurements of RNA kinetics in individual cells show an unaccounted for two and a half minutes in the protein's schedule.

Using groups of cells, researchers have previously determined averages for how many nucleotides RNA polymerase II can add in a minute (900–1,800), its initiation rate, and other variables. To refine these figures, Boireau et al. gauged how fast single cells transcribe a segment of the HIV genome. The researchers altered the segment to contain multiple binding sites for the protein MS2. By adding GFP-labeled MS2, which binds to the transcript, the researchers could track the growth of the new RNA strand. Mathematical models then allowed the team to calculate the timing of different steps.

RNA polymerase II added more than 1,800 bases/min—above the average gleaned from in vitro studies, suggesting that an HIV protein might boost its speed. The polymerase spent just over five and a half minutes at the transcription site. Of this time, making the RNA strand took 114 seconds, and processing the 3' end and other housekeeping tasks required another 63 seconds. That left 156 seconds unexplained.

What the protein does during this gap is unclear. The extra time might indicate that the protein pauses on the promoter before it starts transcribing or that it performs some other undiscovered function. The next step, the researchers say, is making the same measurements for cellular genes. **JCB**

Reference: Boireau, S., et al. 2007. *J. Cell Biol.* 179:291–304.

New mRNA modification?

Like the rough draft of a novel, a newly transcribed pre-mRNA molecule undergoes plenty of polishing before it's fit to be read. Custódio et al. now report evidence for a previously undiscovered editing step in the production of mRNA.

Cells are fussy about mRNA. They detain a would-be strand in the nucleus until enzymes cleave the 3' end, excise introns, stick a cap on the 5' end, and affix a tail of multiple adenines. The carboxyl end of RNA polymerase II, the enzyme that transcribes RNA, orchestrates processing by latching onto editorial proteins. This end normally carries 52 copies of a 7-amino acid sequence. By deleting different combinations of these duplications, researchers previously determined that certain repeats attract proteins that perform specific mRNA alterations.

Custódio et al. engineered mouse cells to make RNA polymerase molecules with untested combinations of deletions. The protein carrying five repeats was nonfunctional. But the one with 31 repeats could transcribe a human β -globin gene and complete the four processing steps. Nevertheless, the RNA strand remained stuck at the transcription site. Its retention suggests that the pre-mRNA must pass through an as-yet undefined editing round before the cell will release it into the cytoplasm.

RNA polymerase presumably draws in proteins that perform this new alteration. The researchers hope to pin down these proteins by comparing the binding partners of RNA polymerases with truncated and full-length carboxyl ends. Once the team knows the proteins' identities, they can work out their functions. **JCB**

Reference: Custódio, N., et al. 2007. *J. Cell Biol.* 179:199–207.

Filopodia motor ahead

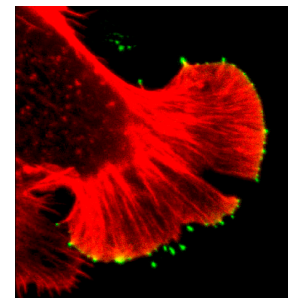
The motor protein MyoX is more than a cellular U-Haul, as Tokuo et al. now show. The protein also helps a cell crawl by muscling actin filaments into position at the front edge of the membrane.

As a cell slithers, it sends out skinny extensions called filopodia that help guide its movements. Previous work has shown that the cargo-hauling protein MyoX spurs formation of these structures. The molecule's head grips and slides along actin filaments, while its tail holds cargo. MyoX travels to the tips of filopodia, and researchers assumed that the cargos it takes there stimulate the extensions to sprout and grow.

That explanation was only half right, as Tokuo et al. found when they tested tailless MyoX molecules that can't ferry anything. Dimers of the trimmed molecules still triggered filopodia, but the extensions were stumpy and short lived. Bundles of actin filaments normally line up along the leading edge of a crawling cell. This orderly arrangement vanished when MyoX was eliminated using RNAi.

The results indicate that MyoX has two jobs during filopodium formation. First, the motor portion bunches up actin filaments at the base of the incipient filopodium, prompting it to bulge out. Then MyoX can slide along the actin fibers into the protrusion, where it deposits its cargos that cause further elongation. The team now wants to determine how MyoX gets to the cell's leading edge and how it wrenches the actin filaments into place. **JCB**

Reference: Tokuo, H., et al. 2007. *J. Cell Biol.* 179:229–238.



Filopodia sprout from a cell after a cargo-free MyoX (green) dimerizes.