Text by Mitch Leslie
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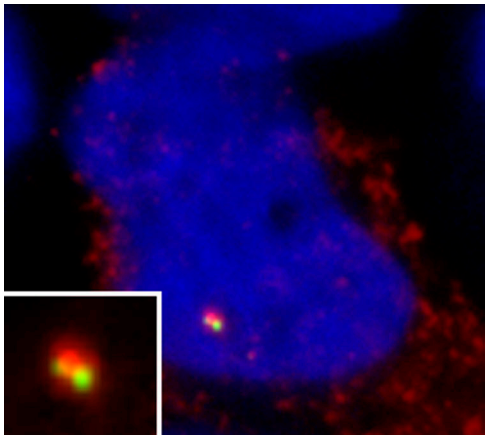


A glow marking an HIV transcription site allows RNA polymerase's speed to be determined.

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.

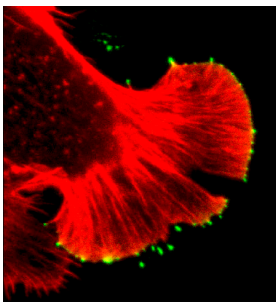
Filopodia motor ahead

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



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

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