

Scientists discover dynamics of transcription in living mammalian cells

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Transcription — the transfer of DNA's genetic information through the synthesis of complementary molecules of messenger RNA — forms the basis of all cellular activities. Yet little is known about the dynamics of the process — how efficient it is or how long it takes. Now, researchers at the Albert Einstein College of Medicine of Yeshiva University have measured the stages of transcription in real time. Their unexpected and surprising findings have fundamentally changed the way transcription is understood.

The researchers used pioneering microscopy techniques developed by Dr. Robert Singer, co-chair of anatomy & structural biology at Einstein and senior author of the study, which appears in the August issue of *Nature Structural & Molecular Biology*.

The study focused on RNA polymerase II--the enzyme responsible for transcription. During transcription, growing numbers of RNA polymerase II molecules assemble on DNA and then synthesize RNA by sequentially recruiting complementary RNA nucleotides.

To visualize the transcription process, the researchers used living mammalian cells, each of which contained 200 copies of an artificial gene that they had inserted into one of the cell's chromosomes. Then, by attaching fluorescent tags to RNA polymerase II, they were able to closely monitor all three phases of the transcription process: binding of the enzyme molecules to DNA, initiation (when the enzyme links the first few RNA nucleotides together) and elongation (construction of the



rest of the RNA molecule). As they observed the RNA polymerase II molecules attaching to DNA and making new RNA, they saw many cases where enzyme molecules attached — and then promptly fell off.

"One surprising finding was how inefficient the transcription process really is, particularly during its first two stages," says Dr. Singer. "It turns out that only one percent of polymerases that bind to the gene actually remain on to help in synthesizing an RNA molecule. Transcription is probably inefficient for a reason. We're not sure why, but it may be because all the factors needed for transcription have to come together at the right time and the right place, so there's a lot of falling off and adding on of polymerases until everything is precisely coordinated."

The researchers observed that the binding phase of transcription lasted six seconds and initiation lasted 54 seconds. By contrast, the final stage of transcription — elongation of the RNA molecule — took a lengthy 517 seconds (about eight minutes). The possible reason: The "lead" polymerase on the growing polymerase II enzyme sometimes "paused" for long periods, retarding transcription in the same way that a Sunday driver on a narrow road slows down all traffic behind him. But in the absence of pausing, elongation proceeded much faster — about 70 nucleotides synthesized per second — than has previously been reported.

These two phenomena — pausing and rapid RNA synthesis during elongation — may be crucial for regulating gene expression. "With this sort of mechanism, you could have everything at the ready in case you suddenly needed to rev up transcription," says Dr. Singer. "Once the 'paused' polymerase starts up again, in a very short time you could synthesize a new batch of messenger RNA molecules that might suddenly be needed for making large amounts of a particular protein."

Source: Albert Einstein College of Medicine



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