Unraveling the mystery of DNA transcription, one molecule at a time

December 2 2014, by Bendta Schroeder

Before DNA can be transcribed into RNA, an early step in turning the genetic template into protein, the nucleus must first assemble a molecular machine called the pre-initiation complex (PIC), capable of unzipping the double helix and loading the DNA onto the transcription enzyme.

The PIC's dozens of parts are scattered throughout a dense nucleus, packed with DNA, proteins, and other biomolecules. Transcription factors and enzymes must find their way to the transcription site, driven by weak and transient interactions, to be assembled into a living, working machine. The assembly can happen in a matter of seconds.

Weak and transient interactions are thought to propel, not just transcription, but the majority of vital cell processes. In these interactions, biomolecules join and disband easily, allowing them to act collectively and quickly in response to the needs of the cell. But exactly how these interactions work is a mystery.

Ibrahim Cissé, assistant professor of physics, wants to solve this mystery, molecule by molecule, in living cells, in real time.

"This is probably one of the most spectacular examples in nature where the interactions of individual biomolecules give rise to something we don't yet understand—the emergence of life," Cissé says.

Transcription, molecule by molecule

For Cissé to follow transcription as it unfolds, he would have to circumvent the limitations of conventional techniques for studying biomolecules. Biochemical techniques that isolate molecules in test tubes or label them in fixed cells destroy the conditions that make weak and transient interactions possible. Light microscopy can preserve those conditions, but most biomolecules are too small and interact too closely to be distinguished with the light diffraction limit of 200 nanometers.

Instead, Cissé uses tools from physics to illuminate the transcription process at high resolution. For example, he adapted a new fluorescent imaging technique called photoactivation localization microscopy (PALM). PALM activates fluorescent tagging proteins at random and then applies a statistical algorithm to determine the exact location of each protein with nanometer-accuracy within the pixel of light. When Cissé repeats the process at high speed and volume, he can map the precise location of tagged biomolecules as they cluster at a transcription site or trace the path of a single transcription factor as it moves across the nucleus. Furthermore, by developing a temporal correlation method coupled with PALM, called tcPALM, Cissé can get direct access to the clustering dynamics for the first time.

Recently, Cissé used tcPALM to show that the transcriptional enzyme RNA Polymerase II (Pol II) clusters for just a few seconds as transcription begins. The result is surprising, given that it takes several minutes for a full RNA sequence to be synthesized. When Cissé suppressed and then reactivated transcription just before imaging, he observed Pol II clustering at unusually high concentrations. When he blocked Pol II from escaping the promoter and transcribing the DNA, the cluster of Pol II around the promoter didn't dissipate.

Cissé theorizes that Pol II clusters are coupled with both transcription

initiation and promoter escape because of the way the cell uses weak and transient interactions to regulate transcription. In previous studies, scientists have shown that when Pol II interacts with the promoter region, it only ends up forming a pre-initiation complex that synthesize RNA about 1 percent of the time. Cissé thinks this inefficiency might be a good thing: Instead of every random collision of a polymerase and promoter resulting in an active gene, the cell may use clustering and other mechanisms to initiate transcription when and where it needs to.

Transient contacts

Cissé's journey from his native Niger to MIT was far from direct. His path would sometimes be shaped by happening to make the right contact at the right time—seemingly random collisions that produced big results.

Growing up, Cissé excelled at learning, particularly mathematics and physics. He was fascinated with the scientists he saw on television, recreating one of their pretend labs by emptying out his parents' storage room and putting "Cissé's Lab" on the door. But his dream for college was simply to learn English in the United States.

To get there, Cissé made a deal with his parents: If he passed the baccalaureat exam and finished school two years early, they'd have to let him go to college in the States. His parents agreed, hoping to encourage his academic ambitions, if not quite expecting him to pass an exam that had only a 30 percent pass-rate the previous year. Cissé, however, was determined and loved studying for the exam. His parents gladly lost their bet.

Cissé would spend two months in an English-as-a-second-language program in University of North Carolina at Wilmington before transferring colleges and eventually finding his way into a physics lab at North Carolina Central University. Cissé wasn't sure what he wanted to

major in, but when he visited NCCU, a physics professor showed him around his laboratory. He told Cissé that he would get his own space and do real research. It didn't matter to Cissé what he would be studying: the "Cisse Lab" would be real.

Cissé developed a real passion for physics in that laboratory, and he met both Nobel Laureate in physics Carl Wieman and his spouse, physicist Sarah Gilbert, when they visited NCCU. Wieman and Gilbert noticed Cissé's love for physics research and suggested he consider a summer program at a top-level research lab and, if he enjoyed his experience, graduate school.

Cissé ended up working at Princeton University with Paul Chaikin, a physicist studying the packing of particles poured at random into a vessel—an important but intractable problem in physics and chemistry. That summer, Cissé would use M&Ms as a model for showing how ellipsoids pack more efficiently than spheroids, counting the transient contacts between candies.

Cissé says that while he was experimenting with M&Ms, he thought of himself simply as an undergraduate "playing and having fun, asking a curious question." But when it was over, he realized that, at the same time, he could help solve one of the most fundamental questions in his field.

Cissé went on to graduate school in physics at the University of Illinois at Urbana-Champaign under his PhD mentor, HHMI investigator Taekjip Ha, a physicist who has been pioneering single-molecule imaging techniques. He then studied transcription as a postdoctoral fellow in the physics and biology departments at L'École Normale Supèrieure in Paris. Cissé then went on to spend several months at HHMI's Janelia Research Campus as a research specialist in the Transcription Imaging Consortium, before starting his position at MIT.

Cissé didn't know a lot about cell biology at first, but he was fascinated by the idea that some of the same principles he studied in Ha's and Chaikin's labs could be applied to unexplored areas in biology. Although tracking the transient contacts of M&Ms and the interactions among transcription factors require very different methods, Cissé says that, for him, they use the same thought processes.

"They're both complex systems," he explains, "and the transient contacts between the individual components can tell you more about the emergence of a global system."

This story is republished courtesy of MIT News (web.mit.edu/newsoffice/), a popular site that covers news about MIT research, innovation and teaching.

Provided by Massachusetts Institute of Technology

Citation: Unraveling the mystery of DNA transcription, one molecule at a time (2014, December 2) retrieved 6 July 2024 from https://phys.org/news/2014-12-unraveling-mystery-dna-transcription-molecule.html