

Key early steps in gene expression captured in real time

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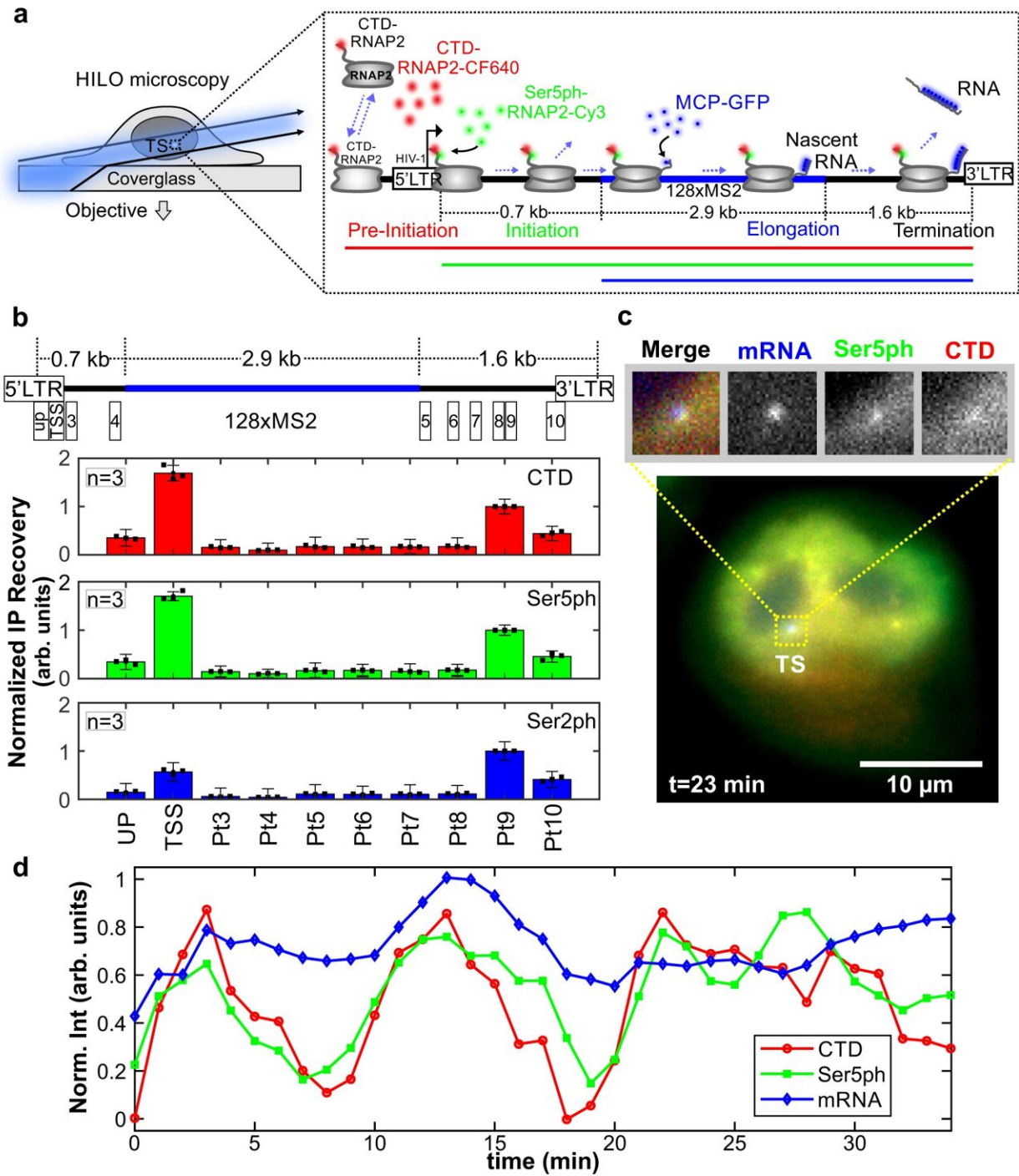


Fig. 1: A system for imaging the endogenous RNAP2 transcription cycle at single genes. From: Live-cell imaging reveals the spatiotemporal organization of endogenous RNA polymerase II phosphorylation at a single gene

On scales too small for our eyes to see, the business of life happens through the making of proteins, which impart to our cells both structure and function. Cellular proteins get their marching orders from genetic instructions encoded in DNA, whose sequences are first copied and made into RNA in a multi-step process called transcription.

A [research collaboration](#) at Colorado State University specializes in high-resolution fluorescence microscopy and computational modeling to visualize and describe such stuff-of-life processes in exquisite detail, in real time, at the level of single genes. Now, scientists led by postdoctoral researcher Linda Forero-Quintero have, for the first time, observed early RNA transcription dynamics by recording where, when and how RNA polymerase enzymes kick off transcription by binding to a DNA sequence.

The [breakthrough technology](#), detailed in the journal *Nature Communications*, has countless possible outshoots; these include sharpening understanding of basic biological processes, to unveiling the genetic underpinnings of certain diseases.

"This is the first time someone has looked at RNA polymerase phosphorylation dynamics in a single-copy gene," said Forero, who is a postdoctoral researcher co-advised by Tim Stasevich, Monfort Professor and associate professor in biochemistry, and Brian Munsky, associate professor in chemical and biological engineering. In the past, such early transcription activity could only be visualized using gene arrays, which are artificial structures composed of hundreds of copies of a gene and not commonly found in the cell nucleus.

Stasevich and Munsky lead a collaboration funded by the W.M. Keck Foundation and the National Institute of General Medical Sciences (through two Maximizing Investigators' Research Awards) that's seeking to unveil and quantify real-time genetic expression in living, single cells.

Forero, who works in both labs under the auspices of the collaboration, had previously studied proteins and transporters in cell membranes associated with neurological conditions.

Early transcription activity

As described in Nature Communications, Forero et al. designed a method using an established mammalian cell line, engineered fluorescent antibody fragments, and a custom super resolution microscope to capture the process of early transcription in vivid colors: blue, green and red. More specifically, they observed the start of the transcription cycle that happens when the RNA polymerase II (RNAP2) transcription enzyme becomes phosphorylated, or decorated with phosphate groups, on its amino acid tail.

"The interdisciplinary science here is a fantastic blending of new experimental capabilities and a new approach for mechanistic computational modeling of single-cell dynamics, both of which are very novel in their respective fields," said Munsky, who supervises the computational aspects of the collaboration.

In the lab, the researchers loaded their antibody fragments into an established mammalian cell line containing a reporter gene that when transcribed, is lit up by a fluorescently tagged protein. The antibody fragments, which Stasevich helped develop several years ago, are tagged with fluorescent molecules that light up their specific targets in the RNAP2 tail. Using these tagging technologies together, the researchers could distinguish three distinct steps in the transcription cycle, marked by different colors. The images obtained with this system translate into fluorescent intensity fluctuation. The researchers then used those signals to interpret the spatiotemporal organization of RNAP2 phosphorylation throughout the transcription cycle at a single-copy gene.

New information via computational model

Munsky's team led by graduate student William Raymond took Forero and Stasevich's microscopy data and translated it into a [computational model](#) based on stochastic differential equations. By fitting this statistical model to reproduce all the experimental results, the computational team then extended their analyses to glean new mechanistic and quantitative information about the different molecules and their states through the transcription process.

For example, they estimated how many individual RNA polymerase molecules collect to form transient clusters in the region of the DNA's promoter, how long these clusters persist, and how, when and where the polymerases distribute themselves along the DNA. They found, for example, that each burst of transcription activity produces a cluster of between five and 40 RNA polymerases to form around the promoter region of the gene, of which 46% eventually succeed to transcribe RNA. They also found that each RNA takes approximately five minutes to be fully transcribed and processed prior to release.

Forero says the technology has far-reaching potential, especially combined with newer technologies like CRISPR, in which specific genes can be singled out and manipulated. Choosing a certain gene of interest, say one implicated in a disease, and applying the CSU researchers' real-time readout of the transcription cycle, could then allow researchers to watch disease processes happening at the activity level of single [genes](#).

"The ability to resolve the spatial and temporal dynamics of the [transcription](#) cycle, in one gene, is the most exciting aspect of this work," Forero said.

More information: Linda S. Forero-Quintero et al, Live-cell imaging reveals the spatiotemporal organization of endogenous RNA polymerase

II phosphorylation at a single gene, *Nature Communications* (2021). [DOI: 10.1038/s41467-021-23417-0](https://doi.org/10.1038/s41467-021-23417-0)

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