Measuring Transcription to Follow Embryo Development

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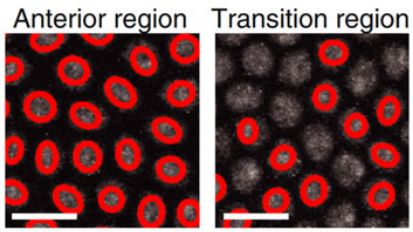
Sarah C.P. Williams

By watching the rate and positioning of gene transcription in real time, researchers get a closer look at how embryos establish gene expression patterns. Learn more...

A team of scientists has adapted techniques originally designed for use in single-celled organisms to track real-time transcription in developing *Drosophila* embryos. Their measurements have already provided new insight into how embryos establish spatial transcription profiles that are key to the body plan of the developing organism. “With this method, we’ve brought the embryo up to the task of answering basic quantitative questions about transcription,” said Thomas Gregor of Princeton University, senior author of a new paper in the journal *Current Biology* (1).



The anterior region of the developing fly embryo had many more actively transcribing nuclei than the center of the embryo

Early in development, embryos establish gradients that give the growing mass of cells a sense of direction. One gene might be expressed at higher levels on one side of the embryo, while another has the reverse pattern of expression. Such patterns are vital for ensuring the correct differentiation of the cells forming the body.

At a macroscopic level, these gradients are well-established. But how they are set up at a molecular level has been less clear. Many hypotheses revolve around transcription thresholds where a cell switches from one state to another when it reaches a particular level of gene expression. Other theories involve bursts of transcription that vary throughout the cell cycle, rather than smooth increases and decreases.

Gregor’s team wanted to get more information on exactly how and when such gene expression patterns appear and how the boundary between the “on” and “off” states of a gene is established. So they turned to a method that uses fluorescence to light up newly synthesized mRNA transcripts. The gene of interest is engineered so that its RNA transcript forms stem loops during transcription. These loops are then bound by specially designed molecules fused to green fluorescent protein (GFP), which can be visualized with microscopy.

The scientists used the approach to quantify the rate of transcription of the developmentally relevant gene *hunchback* in growing *Drosophila* embryos. “With that initial data on rate of transcription and timing of transcription, we could come up with a model that partially explained the transcription boundary we saw,” said Gregor. “But it was still off by a factor of two.”

The team noticed that transcription was either on or off; it wasn’t a matter of reaching a threshold.“The nuclei can adopt an active or an inactive state that is basically random,” said Gregor. “And in the transition region is where we saw nuclei randomly adopting these different states.” When the scientists added this new observation into their model, it fully explained the transcription boundary.

Earlier this year, Gregor’s lab optimized a different fluorescence method—a variation of fluorescence in situ hybridization (FISH)—to count individual mRNA transcripts in an embryo (2). By combining the two approaches, his team plans to move towards a fully quantitative picture of transcription and its dynamic regulation in living embryos.

“What these two methods have brought us is that we can now use the embryo to study transcription in the way that other scientists use single-celled organisms, but exploiting all the advantages that come with multicellular organisms,” said Gregor.

References

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2) Little S.C., Tikhonov M., Gregor T. (2013) Precise developmental gene expression arises from globally stochastic transcriptional activity. Cell 154, 789-800.

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