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DEVELOPMENT

Seeing the pattern

> *in vivo* imaging of gene regulation can provide new insights into the basis of developmental processes

Two studies provide new insights into the spatiotemporal patterns of transcriptional regulation of gene expression in *Drosophila melanogaster* by imaging RNA in live embryos. The *in vivo* method used by these studies allows dynamic transcriptional events to be followed at resolutions that are not possible using static methods.

To fluorescently label RNA *in vivo* both studies used the MS2–MCP (MS2 coat protein) system, which is based on the RNA-binding MCP from the bacteriophage MS2 and its target — an RNA stem–loop repeat. The system comprises two components: a transgene that consists of a reporter gene driven by a promoter of interest and that contains MS2 stem–loop repeats, and a fusion protein consisting of MCP and green fluorescent protein (MCP–GFP). When the transgene is transcribed, MCP–GFP can bind to the stem–loops, thus allowing mRNA to be visualized.

Both studies investigated the Bicoid (Bcd) transcription factor and one of its downstream targets, the *hunchback* (*hb*) gene. Bcd functions at the beginning of a cascade that specifies anterior patterning in *D. melanogaster* embryos, which can be visualized by examining the expression of *hb*. However, the specific transcriptional events that underlie patterning by Bcd have been difficult to study using fixed cell methods, such as RNA-fluorescence *in situ* hybridization (RNA-FISH).

Garcia and colleagues investigated the expression of Bcd-activated *hb* to determine whether boundaries of gene expression are determined by the rate of mRNA production in each cell. Specifically, they determined the absolute number of actively transcribing polymerases in individual nuclei by measuring the fluorescence signal and connecting it to single mRNA molecule counts using RNA-FISH. They found that the formation of patterning boundaries cannot be solely explained by the rate of RNA production in each nucleus. Moreover, nuclei adopt either active or inactive transcriptional states, and it is the combination of these two effects that leads to the formation of a pattern.

Lucas and colleagues looked at how the length of transcriptional activity periods is important in establishing the *hb* boundary. They defined activity periods by several parameters, including the intensity of the fluorescence signal from MCP–GFP and the time that this signal persists. They propose that the establishment of the *hb* boundary involves at least three processes: Bcd causes strong and persistent *hb* expression in the anterior half of the embryo by lengthening transcriptional activity periods; repression progressively overcomes sporadic transcriptional activity in the posterior half of the embryo; and finally, comparison with endogenous expression suggests that posterior expression is also silenced very early on.

These studies show how *in vivo* imaging of gene regulation can provide new insights into the basis of developmental processes.

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ORIGINAL RESEARCH PAPERS Garcia, H. G. *et al.* Quantitative imaging of transcription in living *Drosophila* embryos links polymerase activity to patterning. *Curr. Biol.* http://dx.doi.org/10.1016/j.cub.2013.08.054 (2013) | Lucas, T. *et al.* Live imaging of Bicoid-dependent transcription in *Drosophila* embryos. *Curr. Biol.* http://dx.doi.org/10.1016/j.cub.2013.08.053 (2013)