6. Bichet, A., Desnos, T., Turner, S., Grandjean, O., and Hofte, H. (2001). BOTERO1 is required for normal orientation of cortical microtubules and anisotropic cell expansion in Arabidopsis. Plant J. *25*, 137–148.
7. Roll-Mecak, A., and McNally, F.J. (2010). Microtubule-severing enzymes. Curr. Opin. Cell Biol. *22*, 96–103.
8. Qiang, L., Yu, W., Liu, M., Solowska, J.M., and Baas, P.W. (2010). Basic fibroblast growth factor elicits formation of interstitial axonal branches via enhanced severing of microtubules. Mol. Biol. Cell *21*, 334–344.
9. Zhang, D., Grode, K.D., Stewman, S.F., Diaz-Valencia, J.D., Liebling, E., Rath, U., Riera, T., Currie, J.D., Buster, D.W., Asenjo, A.B., *et al*. (2011). Drosophila katanin is a microtubule depolymerase that regulates cortical-microtubule plus-end interactions and cell migration. Nat. Cell Biol. *13*, 361–370.
10. Srayko, M., O'Toole, E.T., Hyman, A.A., and Muller-Reichert, T. (2006). Katanin disrupts the microtubule lattice and increases polymer number in C. elegans meiosis. Curr. Biol. *16*, 1944–1949.
11. Wightman, R., and Turner, S.R. (2007). Severing at sites of microtubule crossover contributes to microtubule alignment in cortical arrays. Plant J. *52*, 742–751.
12. Lin, D., Cao, L., Zhou, Z., Zhu, L., Ehrhardt, D., Yang, Z., and Fu, Y. (2013). Rho GTPase signaling activates microtubule severing to promote microtubule ordering in Arabidopsis. Curr. Biol. *23*, 290–297.
13. Loughlin, R., Wilbur, J.D., McNally, F.J., Nedelec, F.J., and Heald, R. (2011). Katanin contributes to interspecies spindle length scaling in Xenopus. Cell *147*, 1397–1407.
14. Whitehead, E., Heald, R., and Wilbur, J.D. (2013). N-terminal phosphorylation of p60 katanin directly regulates microtubule severing. J. Mol. Biol. *425*, 214–221.
15. Nakamura, M., Ehrhardt, D.W., and Hashimoto, T. (2010). Microtubule and katanin-dependent dynamics of microtubule nucleation complexes in the acentrosomal Arabidopsis cortical array. Nat. Cell Biol. *12*, 1064–1070.
16. Davis, L.J., Odde, D.J., Block, S.M., and Gross, S.P. (2002). The importance of lattice defects in katanin-mediated microtubule severing in vitro. Biophys. J. *82*, 2916–2927.
17. Diaz-Valencia, J.D., Morelli, M.M., Bailey, M., Zhang, D., Sharp, D.J., and Ross, J.L. (2011). Drosophila katanin-60 depolymerizes and severs at microtubule defects. Biophys. J. *100*, 2440–2449.
18. Sudo, H., and Baas, P.W. (2010). Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. J. Neurosci. *30*, 7215–7226.
19. Portran, D., Zoccoler, M., Gaillard, J., Stoppin-Mellet, V., Neumann, E., Arnal, I., Martiel, J.L., and Vantard, M. (2013). MAP65/Ase1 promote microtubule flexibility. Mol. Biol. Cell *24*, 1964–1973.
20. Odde, D.J., Ma, L., Briggs, A.H., DeMarco, A., and Kirschner, M.W. (1999). Microtubule bending and breaking in living fibroblast cells. J. Cell Sci. *112*, 3283–3288.

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Development: Lights, Camera, Action — The *Drosophila* Embryo Goes Live!

Live imaging of developmental gene expression in *Drosophila* embryos opens up exciting new prospects for understanding gene regulation during development.

Jacques Bothma and Michael Levine*

For the past 30 years, the early *Drosophila melanogaster* embryo has been used as a model to visualize differential gene activity in development [1]. It is ideally suited for such studies due to its rapid development, ease of collection, and the simple arrangement of nuclei in the syncitial blastoderm stage. During the maternal–zygotic transition, which marks the onset of transcription from the embryo's genome, there are two synchronous mitotic divisions, followed by an extended interphase period when the embryo is composed of ~6,000 nuclei arranged as a monolayer at the cortex of the syncitial blastoderm. These nuclei display fast and furious expression of key patterning genes during a period of less than one hour, resulting in localized stripes and bands of gene expression that establish the basic blueprint of the adult fly. The visualization of gene expression in this system has provided numerous insights into the spatial control of gene expression, such as the modular organization of enhancer DNAs, the importance of localized repressors in delineating restricted patterns of gene expression, and the regulation of enhancer–promoter interactions [1,2]. Recently developed quantitative methods allow exact measurements of the numbers of mRNAs and proteins encoded by critical patterning genes such as Bicoid, and highlight the remarkable precision in the regulation of gene expression during the maternal–zygotic transition, when broad maternal gradients give way to sharp on/off patterns of gene expression [3]. Despite the extensive information on the spatial control of gene expression, we know little about temporal dynamics. Time is a more abstract concept than space and much trickier to control experimentally. Most of our insights into gene regulation stem from the use of fixed preparations, whereby snapshots of many dead embryos are pieced together to get a sense of temporal dynamics. With the publication of two studies in this issue of *Current Biology*, by Garcia *et al.* [4] and Lucas *et al.* [5], this static view has given way to real-time dynamics of gene activation in living embryos. With the advent of new technologies comes the opportunity for new discoveries, and the dividends provided by live imaging are immediate and exciting.

Both papers investigate one of the classical paradigms of gene regulation in development: the activation of the gap gene Hunchback by the gradient of the Bicoid protein [6,7]. This gradient is distributed across the anterior–posterior axis of the embryo, with the highest levels present at the anterior pole. Both high and intermediate levels are sufficient to activate Hunchback expression in the anterior half of the embryo, corresponding to the future head and anterior thorax of the embryo. Low levels of Bicoid appear to be insufficient to activate Hunchback expression in the posterior thorax and abdomen. The formation of the sharp Hunchback border within the presumptive thorax has been the subject of extensive experimental and theoretical studies [8].

Both studies [4,5] examined the dynamic activation of gene expression by the proximal Hunchback enhancer

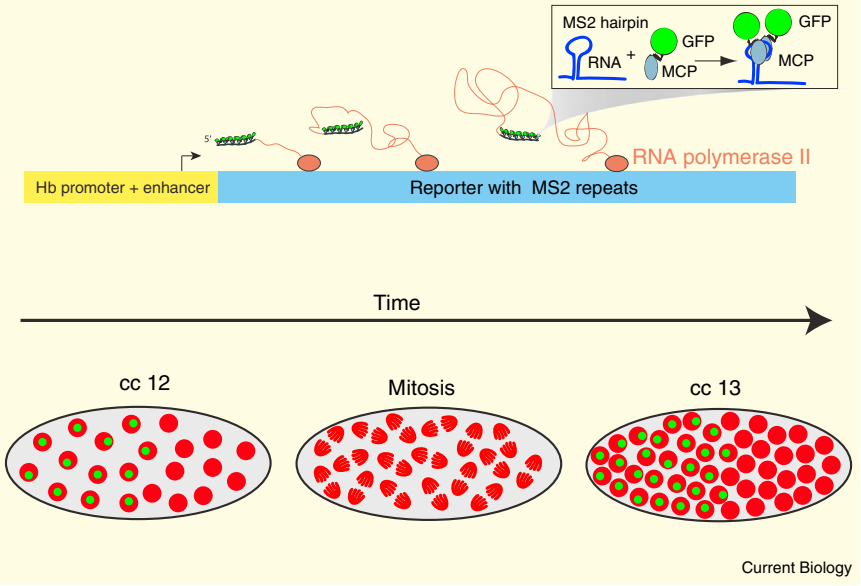

Figure 1. Live visualization of gene expression in *Drosophila* embryos.

Both Garcia *et al*. [4] and Lucas *et al*. [5] visualize transgenes containing the proximal Hunchback enhancer, P2 promoter, and reporter genes containing 24 tandem MS2 stem loop sequences. Each stem loop is approximately 50 nucleotides in length and recognized by a MCP–GFP fusion protein that is maternally expressed and uniformly distributed throughout the early embryo (upper panel). The fusion protein binds to the stem loops in nascent transcripts, resulting in the visualization of nuclear dots (green) at the site of RNA synthesis (bottom panels). These dots are restricted to nuclei (filled red circles) in the anterior half of early *Drosophila* embryos. Abbrevations: cc 12, nuclear cleavage cycle 12; cc 13, nuclear cleavage cycle 13.

The enhancer was attached to reporter genes containing a series of ~50 nucleotide stem loop sequences that bind with high affinity to the bacteriophage coat protein, MS2 (Figure 1). This interaction is responsible for the packaging of the viral RNA in the bacteriophage. It also permits detection of nascent RNAs in living cells [9,10]. A MS2–GFP fusion protein was expressed throughout early embryos, producing 'dots' in nuclei upon binding to MS2 stem loops contained in the tens of nascent RNAs synthesized at the site of transcription. Garcia *et al.* [4] inserted 24 tandem MS2 repeats in the 5' UTR as this placement produces repeats in all of the nascent transcripts spanning the reporter gene. Instead, Lucas *et al.* [5] inserted 24 tandem MS2 repeats in the 3' UTR, which reduces the number of nascent transcripts containing repeats, but has the potential advantage of being less likely to perturb RNA synthesis and processing than placement of the stem loops at the 5' end of the gene.

In both cases, the principle of detection rests with the increase in specific signals resulting from the concentrated binding of the MS2–GFP fusion protein to the many stem loop repeats present at the site of transcription. The visualization of the signals is facilitated by the superior detection systems of the two-photon and confocal microscopes that are currently available, and by the implementation of image segmentation algorithms that distinguish specific signals from background. Efforts are made to achieve adequate but not excessive expression of the MS2–GFP fusion protein. High levels will increase background signals, while low levels will fail to provide optimal occupancy of the MS2 stem loops in nascent transcripts.

The proximal Hunchback enhancer used for these studies was identified in 1989 [6,7]. What have these live-imaging studies revealed that was missed by the preceding 25 years of fixed-tissue analyses? A key insight provided by both studies is that the posterior Hunchback border, where there are diminishing levels of the Bicoid activator, exhibits stochastic expression of Hunchback/MS2 nascent transcripts [4,5]. Thus, at the onset of nuclear cycle 14, as the nuclei enter the protracted interphase when definitive stripes and bands of gene expression are first formed, individual nuclei at the posterior border exhibit essentially an all or none expression of the MS2 transgene. The authors of both studies intepret these observations to suggest that the levels of Bicoid determine the probability of gene activation, with low levels producing stochastic variability across the Hunchback border. This view of activation is evocative of the earliest models for enhancer function, which suggested that enhancers augment gene activity by increasing the probability of transcription events among the different cells of a population [11,12].

Lucas *et al.* [5] emphasize the ectopic activation of Hunchback/MS2 nascent transcripts in posterior regions of the embryo where there is little or no Bicoid activator present. Indeed, these transcripts persist in mutant embryos derived from *bicoid* mutant mothers. The authors suggest that there may be two modes of Hunchback regulation: activation by the Bicoid gradient, and general induction by one or more unknown factors. Evidence for ectopic expression of Hunchback transgenes was generally ignored in previous fixed-preparation studies, but the live-imaging analysis leaves little doubt that there is significant expression in posterior regions. It is possible that this expression is normally suppressed by additional regulatory elements in the Hunchback locus, such as the distal shadow enhancer that was not included in the reporter gene used for this analysis [8].

Garcia *et al.* [4] emphasize the opportunities for quantitative measurements and present evidence that the shape of the Hunchback border can be explained by averaging the mRNAs produced from stochastically-expressing nuclei. Moreover, the authors calculated an RNA polymerase II (Pol II) elongation rate of ~1.5 kilobases per minute, which is consistent with earlier, indirect measurements [13]. This calculated rate suggests that the binding of the MS2–GFP fusion protein to 5' stem loop repeats does not impede Pol II elongation. By quantifying the intensity of the nuclear

dots the authors were also able to estimate the number of elongating Pol II complexes along the length of the MS2/lacZ transgene. These calculations suggest that there are approximately 30 elongating Pol II complexes distributed along the length of the transgene at maximal induction. This corresponds to a density of one Pol II complex every 150 basepairs, or one Pol II released from the promoter every six seconds. This is quite a high rate of RNA synthesis, given that the theoretical limit is approximately one Pol II complex every 70–80 basepairs due to the large size of the Pol II footprint.

These studies are the harbingers of things to come: the visualization of time. We are getting the first glimpses into the dynamic activation of gene expression during development. There is no going back, and there is little doubt that these studies are ushering in a new era for the elucidation of temporal control, comparable to the insights gained into the spatial regulation of gene expression provided by the fixed *in situ* hybridization methods first introduced around 30 years ago [14–16]. At long last, the dynamic developing embryo is ready for its close-up.

References

1. Levine, M. (2010). Transcriptional enhancers in animal development and evolution. Curr. Biol. *20*, R754–R763.
2. Arnosti, D.N., and Kulkarni, M.M. (2005). Transcriptional enhancers: Intelligent enhanceosomes or flexible billboards? J. Cell Biocehm. *94*, 890–898.
3. Little, S.C., Tikhonov, M., and Gregor, T. (2013). Precise developmental gene expression arises from globally stochastic transcriptional activity. Cell *154*, 789–800.
4. Garcia, H.G., Tikhonov, M., Lin, A., and Gregor, T. (2013). Quantitative imaging of transcription in living Drosophila embryos links polymerase activity to patterning. Curr. Biol. *23*, 2140–2145.
5. Lucas, T., Ferraro, T., Roelens, B., De Las Heras Chanes, J., Walczak, A.M., Coppey, M., and Dostatni, N. (2013). Live imaging of Bicoid-dependent transcription in *Drosophila* embryos. Curr. Biol. *23*, 2135–2139.
6. Struhl, G., Struhl, K., and Macdonald, P.M. (1989). The gradient morphogen bicoid is a concentration-dependent transcriptional activator. Cell *57*, 1259–1273.
7. Driever, W., Thoma, G., and Nuesslein-Vohard, C. (1989). Determination of spatial domains of zygotic gene expression in the Drosophila embryo by the affinity of binding sites for the bicoid morphogen. Nature *340*, 363–367.
8. Perry, M.W., Bothma, J.P., Luu, R.D., and Levine, M. (2012). Precision of hunchback expression in the Drosophila embryo. Curr. Biol. *22*, 2247–2252.
9. Rodriguez, A.J., Condeelis, J., Singer, R.H., and Dictenberg, J.B. (2007). Imaging mRNA movement from transcription sites to translation sites. Semin Cell Dev. Biol. *18*, 202–208.
10. Jaramillo, A.M., Weil, T.T., Goodhouse, J., Gavis, E.R., and Schupbach, T. (2008). The dynamics of fluorescently labeled endogenous gurken mRNA in Drosophila. J. Cell Sci. *121*, 887–894.
11. Muller, M.M., Gerster, T., and Schaffner, W. (1988). Enhancer sequences and the regulation of gene transcription. Eur. J. Biochem. *176*, 485–495.
12. Sutherland, H., Martin, D.I.K., and Whitelaw, E. (1997). A globin enhancer acts by increasing the proportion of erythrocytes expressing a linked transgene. Mol. Cell Biol. *17*, 1607–1614.
13. Kwak, H., and Lis, J.T. (2013). Control of transcriptional elongation. Annu. Rev. Genet., in press.
14. Singer, R.H., and Ward, D.C. (1982). Actin gene expression visualized in chicken muscle tissue culture using in situ hybridization with a biotinylated nucleotide analog. Proc. Natl. Acad. Sci. USA *79*, 7331–7335.
15. Levine, M., Hafen, E., Garber, R.L., and Gehring, W.J. (1983). Spatial distribution of Antennapedia transcripts during Drosophila development. EMBO J. *2*, 2037–2046.
16. Akam, M.E. (1983). The location of Ultrabithorax transcripts in Drosophila tissue sections. EMBO J. *2*, 2075–2084.

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Animal Communication: Hidden Complexity

A hallmark of human communication is vocal turn taking. Until recently, turn taking was thought to be unique to humans but new data indicate that marmosets, a new world monkey, take turns when vocalizing too.

Jessica C. Flack

Communication allows individuals to coordinate their behavior and can therefore facilitate cooperation. This effect can be amplified when the communication itself is cooperative, with individuals working together to make sure their messages get transmitted and properly decoded. Despite its obvious advantages, cooperative communication is rare in nature. And, cooperative vocal exchange, in which individuals take turns signaling over an extended sequence of exchanges and control the properties of their utterances, like timing, has been observed only in humans [1]. Results of a new study by Takahashi, Narayanan, and Ghazanfar [2] reported in this issue of *Current Biology* suggest, however, that the common marmoset (*Callithrix jacchus*; Figure 1) also exhibits this kind of cooperative communication.

Takahashi *et al.* [2] found that pairs of marmosets coordinate their vocal exchanges over extended periods, such that the monkeys' call timing is periodically coupled, with the receiver waiting for approximately 5 seconds before responding to the call of its partner and both individuals speeding up or slowing down their calls as necessary to maintain the coupling. The study also suggests that the mechanism underlying the turn taking is mutual entrainment with dynamics characteristic of coupled oscillators.

This is an important study for a number of reasons. It demonstrates that interlocution — like many other traits, including tool making [3], mirror self-recognition [4], and naming of individuals as recently shown in dolphins [5] — can no longer be used as a trump card by proponents of human exceptionalism. More profoundly, the Takahashi *et al.* [2] study raises the question of whether the evolution of turn taking is a signaling innovation that paved the way for finely tuned coordination even when signals themselves are relatively simple. This possibility, as I discuss, injects new energy into the flagging debate in animal communication about how signal–channel design effects signal decoding and, ultimately, what can be communicated.