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Bicoid by the Numbers: Quantifying a Morphogen Gradient

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DOI 10.1016/j.cell.2007.06.036

Morphogen gradients are typically analyzed from static images of fixed embryonic tissues. Two papers in this issue of *Cell* now report live imaging of the Bicoid gradient in developing fruit fly embryos (Gregor et al., 2007a, 2007b). Their findings indicate that the gradient is highly reproducible from embryo to embryo and reveal that the nuclear dynamics of Bicoid are critical for maintaining precision within the gradient.

The existence of morphogens, diffusible substances that can induce differential gene expression in a concentration-dependent manner, was suspected well before any molecular basis for their action was identified (e.g., Crick, 1970). “One is acutely conscious of the absence of the physiological and molecular basis of positional information and polarity,” wrote Lewis Wolpert in the closing section of his seminal 1969 exposition on positional information. “But unless the correct questions are asked one has little hope of finding out how genetic information is interpreted in terms of spatial patterns” (Wolpert, 1969). In the intervening years, many of the correct questions have been asked and answered, primarily through the marriage of genetics with qualitative methods for the visualization of mRNA and proteins in situ. The analysis of spatial patterning is now moved another important step forward thanks to a pair of papers in this issue that employ quantitative live imaging to establish

the temporal and spatial dynamics of a morphogen gradient in embryos of the fruit fly *Drosophila melanogaster* (Gregor et al., 2007a, 2007b).

During early development of the *Drosophila* embryo, a maternally supplied anterior cache of mRNA encoding the homeodomain transcription factor Bicoid (Bcd) is translated and gives rise to a morphogen gradient of Bcd protein along the embryonic anterior-posterior (A/P) axis (Driever and Nüsslein-Volhard, 1988a, 1988b). This transcription factor gradient is interpreted with remarkable precision such that target genes like Hunchback (Hb) are switched on in highly reproducible domains and trigger a subsequent developmental cascade that varies minimally from embryo to embryo (Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989).

The spectacular initial discovery of the Bicoid gradient conferred molecular genetic respectability upon morphogens as critical regulators of embryonic pattern formation (see

Ephrussi and St. Johnston, 2004) and sparked a sustained period of intense interest in identifying other morphogens and understanding their roles in development and disease. Today, although genetics and imaging have certainly advanced our understanding of morphogen gradients, sizable gaps in knowledge still remain, even in the case of the venerable Bicoid. One fundamental limitation is that genetic analysis and qualitative imaging of fixed tissue do not always foster a quantitative understanding of the fine-scale dynamics and precise concentration thresholds central to the morphogen concept, leaving some critical questions unanswered. For example, given the rapid pace of early development and the heterogeneity of the embryonic milieu, how rapidly and how reproducibly is the Bcd gradient established? How can the Bcd profile accommodate both rapid proliferation (an increase in the number of nuclei) and a gen-

eral increase in nuclear size during successive rounds of division in the fly embryonic syncytium? How is the gradient scaled in embryos of differing size?

Addressing such quantitative issues head on, the two papers by Gregor and colleagues now critically reevaluate the Bcd morphogen, focusing on both spatio-temporal aspects of gradient formation as well as issues of precision and reproducibility in how cells interpret the gradient (Gregor et al., 2007a, 2007b). The experimental underpinning of both studies is the elegant use of a Bcd-GFP fusion gene (containing endogenous *bicoid* 5' and 3' regulatory sequences) to fully recapitulate the morphogen activity of Bicoid with a form of the protein that, with appropriate calibration against a fluorescent standard, can be quantified by scanning two-photon microscopy in living embryos.

In the first paper, "Stability and nuclear dynamics of the Bicoid morphogen gradient," the authors validate the Bcd-GFP transgene by rescuing *bcd* null mutant flies and demonstrating that Bcd-GFP-dependent positioning of the cephalic furrow is indistinguishable from that of wild-type embryos. They then use Bcd-GFP to make a series of quantitative observations from live imaging experiments. First, the authors show that the Bcd gradient is established rapidly, within 90 min of fertilization, and remains stable thereafter. The authors subsequently characterize dynamics of the Bcd gradient at the level of individual nuclei, reporting that maximal nuclear concentrations of Bcd-GFP occur during interphase and fall dramatically during mitosis as Bcd-GFP becomes free to flow into the cytoplasm. Perhaps the most striking observation made here is that peak interphase Bcd levels are precisely restored in a position-dependent manner in each successive nuclear division cycle with 10% accuracy—despite a doubling of the total number of nuclei and a general increase in nuclear volume during embryogenesis (Figure 1, top). The duality of complex spatial dynamics

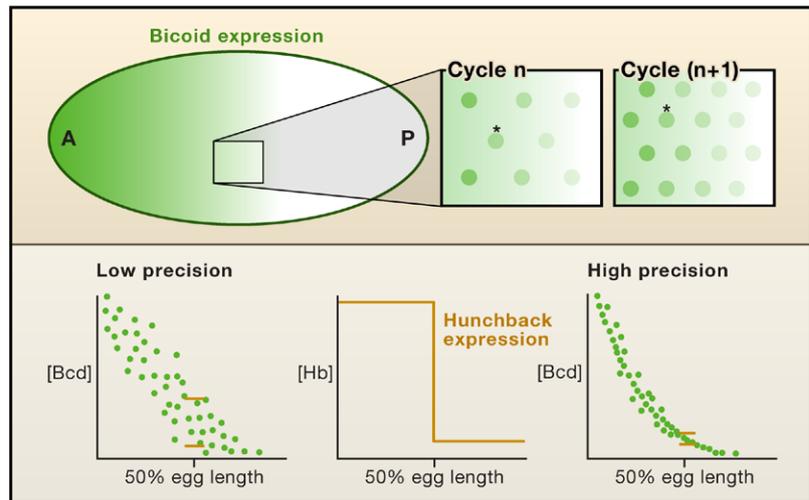


Figure 1. Spatial Dynamics and Biological Noise in the Bicoid Morphogen Gradient

In fly embryos where endogenous Bcd is replaced by Bcd-GFP, a functional morphogen gradient forms along the embryonic anterior-posterior (A/P) axis and directs a downstream developmental program indistinguishable from that of wild-type embryos. This system allows for direct quantification of protein levels during embryonic development. (Top) During rapid progression through the cell cycle in the syncytial fly embryo, nuclear Bcd levels fall dramatically during mitosis and rise again during interphase. Despite a doubling in the total number of nuclei during each cycle, for a given location along the A/P axis (asterisks), peak intranuclear levels of Bcd are restored with an accuracy of $\pm 10\%$ in each successive interphase (Gregor et al., 2007a). (Bottom) Two views of precision, reproducibility, and noise in the Bcd-dependent activation of the *Hunchback* (Hb) gene. Previous studies have generally regarded the Bcd gradient as imprecise (left), suggesting that additional noise-filtering mechanisms would be required to achieve precision control of Hunchback expression at 50% egg length (center). Gregor et al. (2007b) use quantitative imaging to demonstrate that the Bcd gradient is much more reproducible than previously believed, with 10% accuracy between embryos (right). Further evidence is presented that the Bcd gradient is sufficiently reproducible and its interpretation sufficiently precise to directly determine spatial position along the embryonic A/P axis.

and exacting resolution illustrates a previously unrecognized facet of the Bcd gradient and begs for a better understanding of the active mechanism underlying the equilibrium between nuclear and cytoplasmic pools of Bicoid. A further mystery raised by these findings is how Bicoid molecules move rapidly enough to establish the full embryonic gradient following fertilization and yet, according to photobleaching experiments, appear to diffuse quite slowly at the local level within the cortical cytoplasm. Future studies will undoubtedly address both of these questions.

The companion study by Gregor et al. (2007b) addresses the problem of noise in biological networks by analyzing precision and reproducibility in the Bcd gradient and its readout by expression of the target gene *Hunchback*. Here, the authors argue against the notion that the Bcd gradient is inherently imprecise

and that a corrective noise-filtering mechanism in the network of target genes is needed to generate a precision readout (Houchmandzadeh et al., 2002). Instead, the authors establish that theoretically, nuclei would need to sense the Bcd concentration with at least 10% accuracy in order to determine their correct spatial position. A series of direct measurements demonstrate that the Bcd gradient is indeed reproducible from embryo to embryo with an accuracy of 10%. Similarly, within single embryos, 10% precision is achieved in the readout of Bcd by *Hunchback*. Although this degree of accuracy and precision seems to contradict earlier evidence that the spatial profile of Bcd is highly variable from embryo to embryo (Houchmandzadeh et al., 2002), a revised normalization scheme by Gregor and colleagues reconciles the differences in image analysis between the two studies. Taken in

sum, this study provides compelling evidence that the Bcd gradient is sufficiently reproducible and its interpretation sufficiently precise that cells could determine their position based on Bcd alone (Figure 1, bottom). Indeed, given the small number of Bcd molecules in the nucleus and the stochastic nature of their interaction with the *Hunchback* control region, the authors contend that the Hunchback readout is likely to be close to the physical limits of the system. Looking forward, these results should focus future research on the biological mechanisms underlying precision and reproducibility. Is there a yet-undefined mechanism for spatial averaging/noise reduction at the level of Bcd gradient interpretation? What is the role of mRNA concentration

in determining initial establishment of the gradient, and how are mRNA levels controlled and scaled to the correct size of the embryo?

Several new lines of inquiry will undoubtedly emerge as developmental biologists, physicists, and theoreticians join forces to seek an ever more detailed understanding of morphogen gradients. Importantly, the two studies described here not only advance our understanding of *Drosophila* development, but they also address broader issues of precision and reproducibility in biological systems and suggest improved technical approaches for studying protein dynamics in vivo. Hopefully these methods will see duty elsewhere, providing further insights into the biological roles of Bicoid and other morphogens.

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RNA Polymerase II: Just Stopping By

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DOI 10.1016/j.cell.2007.06.040

In this issue of *Cell*, Guenther et al. (2007) analyze the presence of chromatin marks and RNA polymerase at transcription start sites in the human genome. Their results reveal that many “inactive” genes harbor histone marks associated with active transcription at their 5′ ends and that although these genes initiate transcription, they do not generate full-length transcripts.

Transcription of genes by RNA polymerase II (Pol II) is a multistep process that requires initiation of transcription followed by the transition to productive elongation to generate full-length mRNAs. Each step is quality controlled and involves a multitude of accessory factors (Saunders et al., 2006). Many of these factors are known to modify chromatin, and it has been proposed that establishment of an open chromatin structure is a key limiting event in transcription.

Consistent with this model, genome-wide studies in yeast and *Drosophila* have shown that covalent histone modifications around the transcription start site, including trimethylation of lysine 4 (H3K4me3) and acetylation of lysine 9 and 14 (H3K9,14Ac) of histone H3, are excellent predictors of active transcription (Pokholok et al., 2005; Schubeler et al., 2004).

A new report in this issue of *Cell* (Guenther et al., 2007) reveals that the picture is more complicated in human

cells. Using chromatin immunoprecipitation (ChIP) coupled to DNA microarray analysis (ChIP-chip), Young and coworkers surveyed the entire genome in human embryonic stem cells and found that the majority of all protein-coding genes are marked by H3K4me3 (a mark of active chromatin) in the regions surrounding their promoters. This is a perplexing result given that previous studies have indicated that only ~30%–45% of known genes have detectable transcripts.