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Response

Can We Fit All of the Data?

Recently, we have reported what we consider to be a surprising combination of dynamics and stability in the Bicoid (Bcd) morphogen gradient in fly embryos (Gregor et al., 2007a). We found that the Bcd gradient is highly reproducible from embryo to embryo, and that its readout by the Bcd target gene *hunchback* (*hb*) is strikingly precise (Gregor et al., 2007b). In their Correspondence, Bergmann et al. suggest that these results are not surprising but rather are consistent with a model that they have proposed previously (Bergmann et al., 2007). This consistency is achieved only by selecting a subset of our observations.

Bcd is a transcription factor whose spatial profile provides a major source of information for anterior-posterior patterning in the *Drosophila* embryo. As a transcription factor, the functional molecules are those in the nuclei. We have found that this nuclear concentration at any particular location in the embryo is constant from cycle to cycle, to within 10% accuracy. Further, the entire profile of nuclear concentration versus position is reproducible from embryo to embryo, and the expression level of the Bcd target gene *hb* provides a readout of Bcd concentration, which also is accurate at the ~10% level. This reproducibility and precision is sufficient to reliably distinguish neighboring cells along the anterior-posterior axis during cycle 14. Here, we discuss these results in relation to the comments by Bergmann et al.

Is There a Steady State?

Bergmann et al. emphasize that the constancy of Bcd concentrations in the nucleus from cycle to cycle does not imply that the dynamics of the Bcd gradient are in a true steady state. This is correct, and this is why we refer to both stability and dynamics in the title of our paper. But since it is the Bcd in the nucleus that is functional, it is significant that this concentration is stable. The model suggested by Bergmann et al. has dynamics but does not explain this stability.

Reproducibility versus Robustness

A major motivation for the model proposed by Bergmann et al. (2007) is its enhanced robustness to variations in the strength of the Bcd source, which leads to variations in the absolute Bcd concentration from embryo to embryo. Since we have shown that the absolute concentration of Bcd is reproducible from embryo to embryo at the ~10% level, there is no evidence that this form of robustness is relevant for the organism.

Readout Precision

Our discussion of precision began with the observation that, in nuclear cycle 14, neighboring nuclei experience differences in Bcd concentration that differ by only 10%, but that these cells nonetheless can adopt distinguishable patterns of gene expression. Bergmann et al. suggest that cells could make decisions at cycle 9, where differences between neighbors are larger. If their model were literally correct, then by cycle 14 the domains of distinguishable expression would have a minimum width of $\sqrt{2^{14}/2^{9}} \sim 5$ cells along the anterior-posterior axis, whereas in fact many patterns have a width of exactly one cell. We took the observation of 10% differences as motivation to measure the precision with which *hb* responds to Bcd, and we found that this precision indeed reaches the 10% level.

Physical Limits

We tried to place the precision of the Bcd/Hb system on an absolute scale by considering the physical limits set by random arrival of the Bcd molecules at their target on the *hb* enhancer. We emphasized that there are uncertainties in estimating these limits—Bergmann et al. have taken one of these and suggested that sliding of the Bcd molecule along the DNA can effectively make the target region larger. To estimate the impact of this effect, they make three assumptions: the residence time is that measured for the *lac* repressor in the bacterium *Escherichia coli*, sliding along the DNA occurs with a diffusion constant comparable to the observed diffusion of Bcd in solution, and the only effect of sliding is to change the size of the target. Given the huge differences in the structure of the eukaryotic and prokaryotic chromosomes, we know of no basis for the first two assumptions, and the third assumption overlooks the fact that the statistics of diffusive fluctuations depend strongly on dimensionality (Tkačik and Bialek, 2007). Subsequent work also has shown that the quantitative relationship between the mean and variance of Hb expression levels is consistent with a model

in which the dominant source of noise is the random diffusive noise that sets the physical limit (Tkačik et al., 2007).

Spatial Correlations

Our comparison of precision to the physical limit led us to suggest that spatial averaging was needed to achieve noise reduction, and we found a signature of this averaging in spatial correlations of the noise in Hb expression. The scale of these correlations matches the scale needed to bring our estimate of the physical limit into accord with the observed precision of the system

Diffusion and Dynamics

Bergmann et al. emphasize that our measurement of the diffusion constant for Bcd is not consistent with the establishment of a steady concentration gradient within the early hours of embryonic development, and they take this as prima facie evidence for their model. But the problem is much more serious: if this diffusion constant (which we measured in experiments on the scale of minutes and microns) really governs the motion of Bcd on the time scale of hours and hundreds of microns, then it is difficult to understand why we see any Bcd molecules in the middle of the embryo. Thus, we take this conflict as evidence that the diffusion we measure on small scales cannot be the process that leads to gradient formation on the scale of the whole embryo.

Scaling

Bergmann et al. claim that their model is consistent with the observed scaling of Bcd profiles across *Drosophila* species with embryos of different sizes, as well as with the known absence of scaling across individual size variations within a species. In fact we stated explicitly that our results on reproducibility of the Bcd concentration as a function of normalized position along the embryo are significantly better than would be expected if there were no scaling across individuals. In addition, the experiments cited as evidence against scaling suffer from a substantial problem in data analysis, as explained at length in our work (Gregor et al., 2007b). Scaling across individual variations is an important problem that, as we emphasized, will require a new generation of very accurate quantitative experiments.

Summary

Bergmann et al. (2007) propose an interesting model that emphasizes the lack of evidence for the conventional assumption of a steady state in the Bcd morphogen gradient. The small values of the diffusion constant for Bcd that we reported (Gregor et al., 2007a) are superficially consistent with this model, but the model provides no basis for understanding any of our other observations.

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