Re-examining the Stability of the Bicoid Morphogen Gradient

In a recent pair of papers published in *Cell*, Gregor et al. provide an unprecedented glimpse into the dynamics of Bicoid (Bcd), a classical morphogen whose graded distribution along the anterior-posterior axis of the *Drosophila* embryo provides a key polarity cue guiding embryonic patterning (Gregor et al., 2007a, 2007b). With their data, the authors challenge the prevailing view of how the Bicoid gradient is established and interpreted. However, our reanalysis of their data demonstrates that their findings are consistent with the well-accepted paradigm of diffusion-based patterning and provides further support for the notion that the Bicoid profile is decoded prior to reaching its steady state (Bergmann et al., 2007).

The patterning process in the fly embryo mediated by Bcd is arguably the best-studied example of positional information encoded by molecular gradients. Bcd distribution was the first graded profile to be visualized in a developing system, and its ability to regulate the transcription of different zygotic gap genes (which control the early stages of anterior-posterior pattern formation in the fly embryo) in a concentration-dependent manner has been characterized in great detail. The data reported by Gregor and colleagues (Gregor et al., 2007a, 2007b) thus provide long sought dynamic insights into fly development but also raise two apparent paradoxes. First, the authors argue that the Bcd gradient cannot be formed by simple diffusion. Second, they claim that the available knowledge cannot explain the high degree of reproducibility of the Bcd gradient output. Here, we discuss both claims and conclude that they are not supported by the reported data. Rather, we find the data to be fully consistent with the traditional diffusion-based model for gradient establishment, with the Bcd profile continuing to evolve at least until cycle 13 of nuclear cleavage. We further show that the reproducibility of the Bcd gradient output can be readily explained if the expression domains of Bcd target genes are defined early in development during division cycles 9–10 (Bergmann et al., 2007).

It is widely believed that the Bcd gradient arises by diffusion. The dynamic behavior characterized by Gregor et al. is indeed consistent with Bcd movement being governed by diffusion, with a characteristic diffusion coefficient of D = 0.37 $\mu m^2/s$. Considering the spread of the Bcd gradient (l ~ 100 μm), this diffusion coefficient is too small to establish a steady-state profile by the reported measurement time (cleavage cycle 9, ~100 min after egg laying). This would not have raised a problem if the Bcd profile at that time had not yet reached its steady state but continued to evolve. The concentration of nuclear Bcd, however, does appear to be stable between consecutive nuclear division cycles, prompting Gregor et al. to question the relevance of the standard diffusion-based model for gradient establishment and to invoke alternative explanations. We believe that constant nuclear Bcd levels are not indicative of a stable gradient. Furthermore, various parameters measured by Gregor et al. clearly indicate that the Bcd profile continues to evolve up to division cycle 13, as expected by the low diffusion coefficient measured.

Given that the long-range movement of Bcd occurs outside the nuclei, the stability of its profile cannot be judged based on nuclear Bcd but should be based on the levels of extranuclear Bcd. It is intuitively appealing that the stability of nuclear Bcd reflects the stability of the overall profile. This intuition breaks down, however, when one considers the temporal changes in nuclear diameter. In fact, Gregor et al. have shown analytically, and verified experimentally, that the relation between the concentration of nuclear (c_{in}) and cytoplasmic (c_{out}) Bcd depends on the nuclear diameter r_n (c_{in}/c_{out} ~$1/r_n^2$; see Equation 4 in Gregor et al., 2007b and explanations therein). As nuclear diameter changes significantly between consecutive division cycles (from 10.5 μm at cycle 11 to 6.5 μm at cycle 14), a stable concentration profile of Bcd in the cytoplasm would imply increasing (rather than stable) levels of nuclear Bcd, and it is not immediately clear how a stable nuclear concentration is in fact maintained. One possibility is that the lifetime of nuclear Bcd (the second parameter controlling the c_{in}/c_{out} ratio) changes between consecutive cell cycles in a fine-tuned manner, but it is difficult to envisage mechanistically such a tuning mechanism. Moreover, such tuning could obviously also account for possible temporal changes in extranuclear Bcd. We conclude that the temporal stability of nuclear Bcd cannot be taken as an indication for the stability of the Bcd profile itself.

By definition, Bcd continues to accumulate prior to reaching a steady state but remains fixed once a steady state has been reached. To more rigorously assess the stability of the Bcd profile, we calculated the overall level of Bcd at consecutive nuclear division cycles. Bcd is distributed between the nuclei and the internal and cortical cytoplasm of the fly embryo. Our goal was to sum all of these contributions. We used four pieces of evidence reported by Gregor et al. First, the maximal concentration of Bicoid within a single nucleus is approximately constant (within 10% variation). Second, the number of nuclei increases by a factor of two following each division cycle. Third, the maximal nuclear diameter (measured at the end of interphase) r_n changes between consecutive division cycles (with r_n = 10, 10.5, 9.2, 8.2, and 6.5 μm at cell cycles n = 10–14, respectively, n denoting the division cycle number). Fourth, the fraction of the total protein f_n contributed by nuclear Bcd is relatively large (estimated as 33%, 39%, 41%, and 41% during division cycles 11–14, respectively). These values correspond to an increase in both

the number of Bcd molecules that are localized to the nuclei (with NucBcd (n) ~$r_n^3 2^n$ increasing by 130%, 35%, and 40% between division cycles 10–11, 11–12 and 12–13, respectively) and the total number of Bcd molecules (with TotBcd (n) = NucBcd (n)/f_n increasing by 14% and 35% between cell cycles 11 and 12 and 12 and 13, respectively). An apparent stabilization occurs only at nuclear division cycle 14. Thus, the measurements of Gregor et al. indicate that the Bcd profile continues to develop and has not yet reached its steady state during cleavage cycles 10–13. Notably, in fertilized eggs, Bcd mRNA starts to degrade at stage 12 (Surdej and Jacobs-Lorena, 1998), perhaps explaining the apparent stabilization of Bcd protein levels between stages 13 and 14.

Accumulating evidence suggests that the expression domains of the Bcd target genes are defined prior to cycle 14. Zygotic gene expression is first observed at division cycles 9–10 (Nasiadka et al., 2002), and this is also the time when the expression domain of the *hunchback* (*hb*) gene, a primary Bcd target, is most sensitive to environmental perturbations (Lucchetta et al., 2005). Such an early readout of the Bcd profile may resolve an additional quandary described by Gregor et al. concerning the apparently unrealistic averaging times required for accurate interpretation of the Bcd gradient. At division cycle 14, nuclei are closely spaced (positioned ~8 μm apart), and the Bcd concentration at two adjacent nuclei that reside on the border of the *hb* expression domain differs by only 10% (Gregor et al., 2007a). Accordingly, proper establishment of the *hb* expression domain requires a transcriptional readout that is precise to within 10%. The precision of this readout, however, is limited by the random arrival of the individual Bcd molecules to the small *hb* promoter region (with the maximal precision e given by $e = (Dac_{in}T_{ave})^{-1/2}$, with $a = 3_{\mathrm{nm}}$ the length of the Bcd binding site, $c_{in} = 8_{nM}$ the concentration of nuclear Bcd, and T_{ave} the averaging time). Approximating the diffusion coefficient D~1 $\mu m^2/s$, Gregor et al. estimated that 10% accuracy requires nearly 2 hr averaging time (Gregor et al., 2007a). Obviously, this is well beyond the relevant developmental time, raising the question of how such precision is achieved. This apparent paradox is readily resolved, however, if the *hb* expression domains are defined at earlier division cycles, when adjacent nuclei are far more distant from each other. For example, at division cycle 9, adjacent nuclei are separated by ~50 μm (assuming an even distribution of nuclei during all nuclear cycles) and accordingly subjected to a Bcd profile that differs by more than 50% rather than 10%. The averaging time required for achieving 50% accuracy is reduced by a factor of 5^2 to ~5 min, well within the available range. Moreover, this averaging time is probably shorter due to the sliding of Bcd on DNA. For example, DNA residence time of t_{res} ~5_{ms} (as measured for the Lac repressor, see Elf et al., 2007) will extend the effective binding region to $a = \sqrt{2Dt_{res}} \sim 100_{nm}$, predicting an averaging time of less than half a minute. Notably, once the initial pattern of a downstream gap gene is defined, it is stabilized and further refined by interactions within the gap gene network itself (see Bergmann et al., 2007 and references therein).

An additional issue discussed by Gregor et al. is the scaling of the Bcd profile with the widely varying sizes of embryos from different fly species. They suggest that nuclear-mediated degradation of Bcd may account for such size-dependent scaling. In apparent contradiction of this model, however, the Bcd profile (as measured at late cycle 14) does not scale with embryo size within the same species (Houchmandzadeh et al., 2002). Notably, under the same conditions, the *hb* expression domain does in fact scale with embryo size (Houchmandzadeh et al., 2002). Once again, this apparent paradox may be resolved if Bcd is decoded early, before its steady state has been reached. Indeed, as we have recently shown, under a wide range of parameters, rapid equilibrium between cytoplasmic and nuclear Bcd amounts to an effective scaling of time with the nuclear density ($t_{eff} \sim t/\rho_N \sim t\,L^2/\mathrm{N}$, where ρ_N denotes the two-dimensional nuclei density in the embryo, and L the size of the embryo; Bergmann et al., 2007; Gregor et al., 2007b). At early times, the Bcd profile spreads over a distance $l_{Bcd} = \sqrt{2Dt_{eff}} \sim L$ that indeed scales with embryo size. Accordingly, if the *hb* expression domain is defined by this pre-steady-state profile, it will exhibit size-dependent scaling, as is indeed found experimentally. By contrast, while the exchange of Bcd between nuclei and cytoplasm changes the time of approaching steady state, it does not affect the steady state itself. Thus, the long-term Bcd profile is not expected to scale, again consistent with experimental observations.

In conclusion, the data reported by Gregor et al. are fully consistent with the well-accepted model of diffusion-controlled establishment of morphogen gradients. Furthermore, these data provide support for the proposal that the Bcd profile is decoded before reaching its steady state. As we have demonstrated recently, pre-steady-state decoding enhances the ability to buffer fluctuations in the Bcd production rate and explains the long-standing enigma of surprisingly small fate-map shifts upon changes in *bcd* gene dosage (Bergmann et al., 2007). Such pre-steady-state decoding may also account for the ability of the *hb* expression domain (but not the long-term Bcd profile) to properly scale with natural variations in embryo size. However, proving this assessment will require further experimentation. Importantly, these properties of pre-steady-state decoding depend only on the shape of the profile outside the nucleus and are insensitive to possible time-dependent (linear) modulation of the rate by which nuclear Bcd accumulates or is degraded.

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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