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Morphogen gradient formation and action

Insights from studying Bicoid protein degradation

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In a recent publication,[1] we identified a novel F-box protein, encoded by *fates-shifted* (*fsd*), that plays a role in targeting Bcd for ubiquitination and degradation. Our analysis of mutant Drosophila embryos suggests that Bcd protein degradation is important for proper gradient formation and developmental fate specification. Here we describe further experiments that lead to an estimate of Bcd half-life, <15 min, in embryos during the time of gradient formation. We use our findings to evaluate different models of Bcd gradient formation. With this new estimate, we simulate the Bcd gradient formation process in our own biologically realistic 2-D model. Finally, we discuss the role of Bcd-encoded positional information in controlling the positioning and precision of developmental decisions.

Key words: morphogen gradient, Bicoid, half-life, diffusion constant, steady state, positional information, robustness, Drosophila, *fates-shifted*, ubiquitination

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Models of Bcd Gradient Formation

Bcd is a morphogenetic protein that forms a concentration gradient along the anterior-posterior (A-P) axis in early Drosophila embryos.[2] It controls embryonic patterning by activating its target genes in a concentration-dependent manner.[3-7] Despite extensive studies, it currently remains controversial how the Bcd concentration gradient is formed. There are two broad, contrasting models. A simple diffusion model[8] has been widely used to explain the exponential gradient of Bcd,[9-11] where Bcd protein is synthesized at the anterior, diffuses and decays throughout the embryo (hence also referred to as the SDD model). The diffusion model produces a steady state exponential profile of the Bcd concentration: $B = Ae^{-x/\lambda}$, where A is the amplitude, x is distance from the anterior and λ is the length constant.[9] However, since *bcd* mRNA, the source for Bcd production, is not restricted to a single point in the actual embryo,[12,13] the idealized version of this model is inadequate for Bcd. In fact, based on the observed redistribution of *bcd* mRNA, a contrasting model was proposed recently in reference 14. In this model, the process of Bcd gradient formation and its final shape are dictated by the redistribution process of *bcd* mRNA.[14,15] We refer to this model as the mRNA-dictated-gradient model. Since the measured *bcd* mRNA profile differs from the exponential Bcd protein profile,[14] the pure form of the proposed mRNA-dictated-gradient model also appears inadequate for explaining fully how the Bcd protein gradient is formed.[12,16]

In addition to these two contrasting models, several other models have also been proposed for Bcd.[17-19] Since Bcd diffusion remains a component of all these models, they as a group are more related to the diffusion model than the mRNA-dictated-gradient model. Each of these models was proposed to explain specific properties of the Bcd gradient system. For example, an intriguing property of the Bcd gradient is the stability of its nuclear concentrations as a function of developmental time.[20] While the nuclear number undergoes an exponential increase after each nuclear division, the profiles of nuclear Bcd concentration remain relatively stable at the interphase of nuclear cycles 10–14. This observation led to the proposal of a nuclear trapping model,[17] where Bcd protein is

reversibly trapped by the nucleus to allow the formation of a stable nuclear Bcd concentration gradient. The nuclear trapping model proposes that Bcd does not decay during the period of gradient formation.[17] Another model, a pre-steady-state model,[18] was proposed to explain the precision of the expression patterns of Bcd target genes among different embryos. This model proposes that the positional information provided by the Bcd gradient is decoded before the gradient reaches its steady state. It also requires Bcd to be a stable protein with a half-life comparable to the proposed decoding time (60~90 min).[18]

An Estimate of Bcd Half-Life to Evaluate Different Models

An important finding reported in our recent study is that perturbed Bcd degradation, in embryos from *fsd* females (referred to as *fsd* embryos), led to an altered Bcd gradient profile.[1] These results suggest that Bcd degradation is important for the gradient formation process. To evaluate different models of Bcd gradient formation, it is critical to have an estimate of the Bcd half-life, $t_{1/2}$, in embryos at the time of Bcd gradient formation. Such a value is currently unavailable. Based on the kinetics of the disappearance of Bcd after cellularization,[2] it was estimated that Bcd $t_{1/2}$ in cellularized embryos is <30 min. This value is for embryos that are actively "clearing up" the Bcd gradient that is no longer needed. In our reported study in reference 1, we used 0–3 hr embryonic extracts to assay Bcd degradation. These extracts reflect, collectively, the properties of embryos that are both undergoing Bcd gradient formation and actively clearing up the Bcd gradient (i.e., before and after cellularization, respectively). To gain further insights into Bcd degradation properties in embryos as a function of developmental time, we generated extracts from staged 0–1, 1–2 and 2–3 hr embryos, with all experiments performed side-by-side to allow direct comparisons. Our Bcd degradation assays in these extracts revealed an estimated $t_{1/2}$ of 19.7, 18.9 and 43.6 min, respectively (**Fig. 1**). These results show that embryos undergoing the process of Bcd gradient formation (0–1 and 1–2 hr) actually have higher Bcd degradation activities than later embryos (2–3 hr). Since the 2–3 hr embryos contain those that are actively clearing up the Bcd gradient, we used the previous estimate obtained from such embryos[2] to calibrate our biochemical data. Using such calibration, we estimate that Bcd $t_{1/2}$ in embryos at the time of Bcd gradient formation is <15 min. While we are aware of the inherent limitations of biochemical assays for quantifying biophysical properties inside an embryo, this value represents the best estimate currently available.

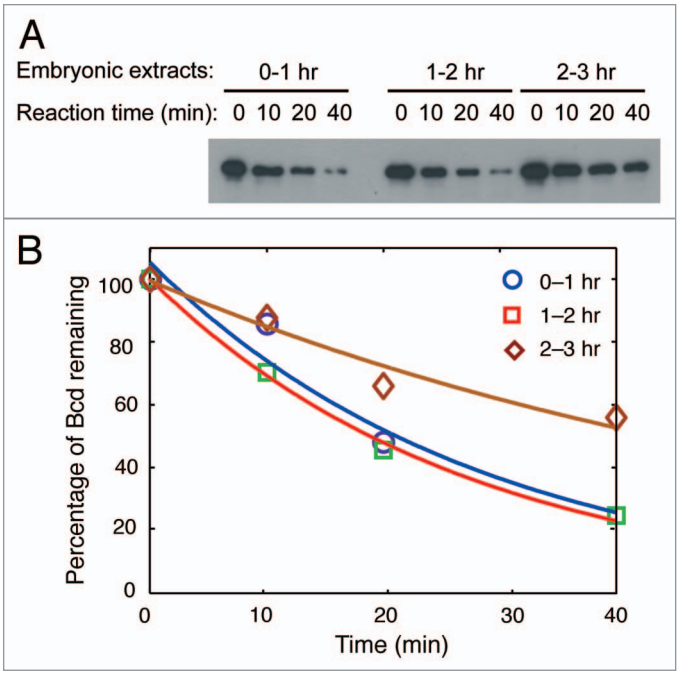

Figure 1. Bcd degradation and estimation of Bcd half-life. (A) Extracts generated from 0–1, 1–2 and 2–3 hr w^{1118} embryos were used to assay Bcd degradation.[1] As shown previously in reference 1, Bcd degradation in embryonic extracts is inhibited by MG132, indicating that proteasome-dependent activities, as opposed to some non-specific activities, are responsible for Bcd degradation. All experiments shown here were performed side-by-side. As discussed previously in reference 1, data from experiments that are not done side-by-side cannot, and should not, be compared with each other. (B) The plot shows the percentage of Bcd protein remaining at different time points of the degradation reaction. Solid lines represent exponential fitting to experimental data. (For further details, see text and ref. 1).

Our estimated Bcd $t_{1/2}$ of <15 min has important implications for the mechanisms of Bcd gradient formation. First, this value is not consistent with the assumptions made in the nuclear trapping and pre-steady-state models,[17,18] where Bcd is proposed not to decay or to decay slowly during the gradient formation process. Our reported finding that Bcd degradation is important for gradient formation[1] is also inconsistent with the mRNA-dictated-gradient model because, according to this model, the final shape of the protein gradient is determined exclusively by the *bcd* mRNA gradient, rather than Bcd protein degradation and diffusion.[12,14-16] Second, our estimated Bcd $t_{1/2}$ sheds light on an outstanding controversy over the diffusion constant D of Bcd in the cytoplasm of embryos. While an earlier study[20] suggested a D value of ~0.3 $\mu m^2 s^{-1}$, a more recent study in reference 21 revealed a value that is >20 times larger, ~7.4 $\mu m^2 s^{-1}$. In a simple diffusion model,[8,9] the length constant λ of the steady state exponential profile is a function of the diffusion constant D and decay rate ω, $\lambda^2 = D/\omega$. Under this framework, our estimated half-life of Bcd ($t_{1/2}$ = 15 min corresponds to ω = 0.0008 s^{-1}) and a consensus λ estimate of ~100 μm are more consistent with a large D value. We note that, since our estimated Bcd $t_{1/2}$ represents an upper bound, a formal possibility exists that the actual D value for Bcd could be even larger than the reported ~7.4 $\mu m^2 s^{-1}$.

Bcd Gradient Formation Simulated in a Biologically Realistic Model

We have developed recently a biologically realistic 2-D model for the Bcd gradient formation process.[12,22] This model is different from the simple diffusion model because it has incorporated several key biological features relevant to the Bcd gradient system, including the location and amount of *bcd* mRNA and the exponential increase in nuclear numbers after each nuclear division (reviewed in ref. 12). Using this model and our newly estimated Bcd $t_{1/2}$, we conducted simulation studies to evaluate Bcd gradient properties (for details, see **Fig. 2** legend). **Figure 2A** shows the simulated profiles of nuclear Bcd concentration, [B_n], at nuclear cycles 10–14, which exhibit an experimentally observed stability.[20] As further discussed in reference 12, an interaction between Bcd and the genome within the nucleus in our model plays an important role in maintaining the stability of nuclear Bcd concentrations during the time when the nuclear number is increasing exponentially (see also ref. 17). **Figure 2**B shows that our simulated [B_n] profile and experimentally measured Bcd profile,[23] both at early nuclear cycle 14, exhibit strong resemblance to each other. These and additional results (below) show that our biologically realistic model can recapitulate key properties of the Bcd gradient. For example, it also readily explains the differences in Bcd gradient profiles caused by the geometric asymmetry between the dorsal and ventral sides of the embryo.[22] In addition, it recapitulates the scaling properties of the Bcd gradient[23] by simply assuming a correlation between the Bcd production rate and embryo volume.[12] This hypothesized correlation was recently observed experimentally[13] through quantitative measurements of *bcd* mRNA in large and small embryos from selected Drosophila lines.[24] Since our model is based on Bcd diffusion and degradation while incorporating relevant, biologically realistic features of the embryo, our results suggest that the diffusion model—despite the inadequacies of its idealized form in fully capturing Bcd gradient properties—remains a useful framework in guiding our thinking and analyses of the Bcd gradient formation process.

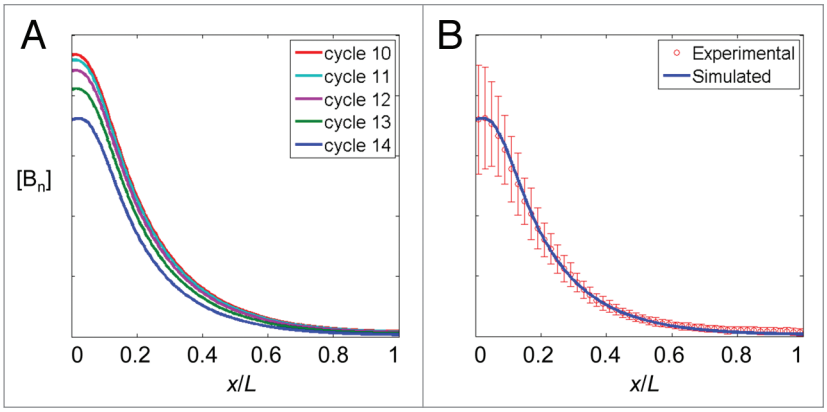

Figure 2. Simulated nuclear Bcd gradient profiles. (A) Nuclear Bcd concentration [B_n], in arbitrary units, within the cortical layer of a simulated embryo was obtained in a biologically realistic 2-D model.[12] Here the simulated [B_n] profiles as a function of fractional embryo length *x/L* are shown for nuclear cycles 10 to 14. These simulated [B_n] profiles, as seen experimentally,[20] differ by <10%, with a calculated *g* value of -0.09 (for details see ref. 12). This simulation was performed using $\omega = 0.0008\ s^{-1}$ and $D = 8\ \mu m^2 s^{-1}$. All other parameters used here were the same as in the main model described in reference 12, except the center coordinate of the *bcd* mRNA sphere (55 μm in current work). (B) Comparison between simulated [B_n] profile and the experimentally measured mean Bcd profile (with standard deviation shown).[23] Both profiles are from early nuclear cycle 14 and each has a length constant λ of ~100 μm.

Interpretation of Bcd-Encoded Positional Information

One of the fundamental questions regarding the actions of morphogens relates to the precision of the positional information provided by a morphogen gradient and the extent to which such information can influence the precision of developmental decisions.[25,26] For the Bcd gradient profile, embryo-to-embryo variations were initially reported to be very large, suggesting that the positional information provided by the Bcd gradient is imprecise.[9,27,28] These findings prompted the proposal of different models that can correct embryo-to-embryo fluctuations in Bcd-encoded positional information.[18,29-33] However, more recent studies revealed that the Bcd gradient profile is highly precise among different embryos.[23,34,35] But whether a precise Bcd gradient is important for precise patterning remains controversial. It was suggested that, based on thermal perturbations, a precise Bcd gradient is not required for precise patterning.[36] Our own studies based on genetic perturbations have led to a contrasting proposal.[23,35] In two separate cases where mutant embryos exhibit increased variability in the *hunchback* (*hb*) expression boundary, we were able to trace the origin of such variability directly to perturbed Bcd gradient properties.[23,35] To our knowledge, these results represent the only experimental evidence that a precise Bcd gradient is necessary for precise developmental decisions. Our finding[1] that the shift in Bcd-encoded positional information in *fsd* embryos matches the shift in *hb* expression boundary further underscores a direct and dominant role of Bcd in instructing *hb* expression (see also ref. 22). Exactly how the positional information provided by the Bcd gradient feeds into precise patterning decisions is a subject of intense theoretical investigations.[34,35,37-39] In a recent experimental study,[40] we investigated the role of Bcd in the actual transcriptional events of its target genes in developing embryos. Our results suggest that Bcd acts as a direct and sustained input for these transcriptional events. In addition, a comparison between the noise in Bcd-dependent transcriptional events and the noise in Bcd-dependent transcriptional products provides a first experimental demonstration of the effect of time/space averaging in reducing the output noise.[40]

Recent studies of the role of the terminal system in the expression of Bcd target genes have led to the proposal of a morphogen network model.[41-44] This model emphasizes the integration of maternal inputs of both the terminal system and the Bcd gradient; cross-regulation between the zygotic products of gap genes is also a hallmark feature of the proposed gene regulatory network models.[18,33,45,46] Understanding mechanistically how genes respond to distinct Bcd concentration thresholds remains an important and challenging problem.[41-43,47,48] Meanwhile, how *hb* is expressed in response to Bcd has attracted most extensive studies in the field.[9,22,23,33-35,46,49,50] Unlike genes that are expressed near the head region, the *hb* expression boundary does not appear to be influenced strongly by the terminal system.[41] Thus, the positioning and precision of the *hb* expression boundary provides a best readout of Bcd-encoded positional information and properties intrinsic to the Bcd gradient system. In addition, the *hb* expression boundary is located near the middle of the embryo, where the scaling properties can influence the patterning landscape along the entire A-P axis.[51] Importantly, the scaling properties of *hb* can also be traced directly to the scaling properties of the Bcd gradient.[12,13,23] These and other results have led us to propose in reference 23 that the Bcd gradient itself is a robust system (see also refs. 34 and 49). Understanding the precise mechanisms of Bcd-activated *hb* transcription will further expand our knowledge of not only how Bcd works in particular, but also how morphogens work in general.

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