Dynamics of maternal morphogen gradients in *Drosophila*

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The first direct studies of morphogen gradients were done in the end of 1980s, in the early *Drosophila* embryo, which is patterned under the action of four maternally determined morphogens. Since the early studies of maternal morphogens were done with fixed embryos, they were viewed as relatively static signals. Several recent studies analyze dynamics of the anterior, dorsoventral, and terminal patterning signals. The results of these quantitative studies provide critical tests of classical models and reveal new modes of morphogen regulation and readout in one of the most extensively studied patterning systems.

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Introduction

The fertilization of the *Drosophila* egg is followed by rapid nuclear divisions in a common cytoplasm (Figure 1a). The exponential increase in the number of syncytial nuclei is accompanied by progressive patterning of the embryo under the action of maternal morphogen gradients (Figure 1b and c). The regions of the embryo that give rise to the head and thorax of the future larva are patterned by the anteroposterior (AP) gradient of the Bicoid protein. The formation of the abdomen depends on a reciprocal gradient of a translational repressor Nanos. The dorsoventral (DV) axis is patterned by the ventral-to-dorsal gradient of the nuclear localization of transcription factor Dorsal. Finally, the termini of the embryo are patterned by the graded activation of the MAPK-signaling pathway.

Maternal morphogens were identified in genetic studies that used the larval cuticle as their main assay. This was followed by molecular studies that visualized the distribution of transcripts and proteins encoded by the identified genes [1]. Since most of these studies were done with fixed embryos, maternal gradients were viewed as relatively static signals. Several recent studies report quantitative measurements of the anterior, dorsoventral, and terminal gradients and propose biophysical models for their formation and interpretation. We review these studies and discuss how their conclusions affect our view of dynamics in one of the most extensively studied patterning systems.

Bicoid gradient: diffusion and reversible trapping of a stable protein?

In the end of 1980s, studies of the distribution and transcriptional effects of the Bicoid (Bcd) protein in the *Drosophila* embryo provided the first molecularly defined example of a morphogen gradient [2–4]. Bcd is a homeodomain transcription factor, which is translated from maternally deposited mRNA at the anterior of the embryo and patterns the AP embryonic axis by controlling the expression of multiple zygotic genes. Based on the quantitative analysis of the antibody staining of fixed embryos, the spatial distribution of Bcd appears to be an exponential function of the AP distance [5]. For more than two decades, this observation has been interpreted within the framework of the classical localized production, diffusion, and uniform degradation model [6••,7,8]. According to this model, degradation ensures the stability of the Bcd concentration profile, which would otherwise continue to spread throughout the embryo.

Last year, using the GFP-tagged Bcd constructs and two-photon confocal microscopy, Gregor *et al.* measured the Bcd gradient in live embryos (Figure 2) [9••]. This study provided the first information about the concentration of Bcd at the expression thresholds of its target genes and revealed a novel feature of the gradient dynamics. Specifically, it was shown that the gradient of the nuclear levels of Bcd disappears with every nuclear division and is then rapidly re-established by rapid nucleocytoplasmic shuttling of Bcd. Remarkably, both the spatial profile of nuclear Bcd along the AP axis and the levels of Bcd in individual nuclei were found to be invariant with respect to nuclear divisions (Figure 2b). Since Bcd is a transcription factor that must access its nuclear targets, it is not surprising that it shuttles in and out of the nuclei. It is also not surprising that the nuclear levels of Bcd are strongly affected by the dissolution and reformation of the nuclear envelope. At the same time it is not at all clear how these processes would affect the levels of Bcd at different nuclear cycles and the entire gradient.

Figure 1
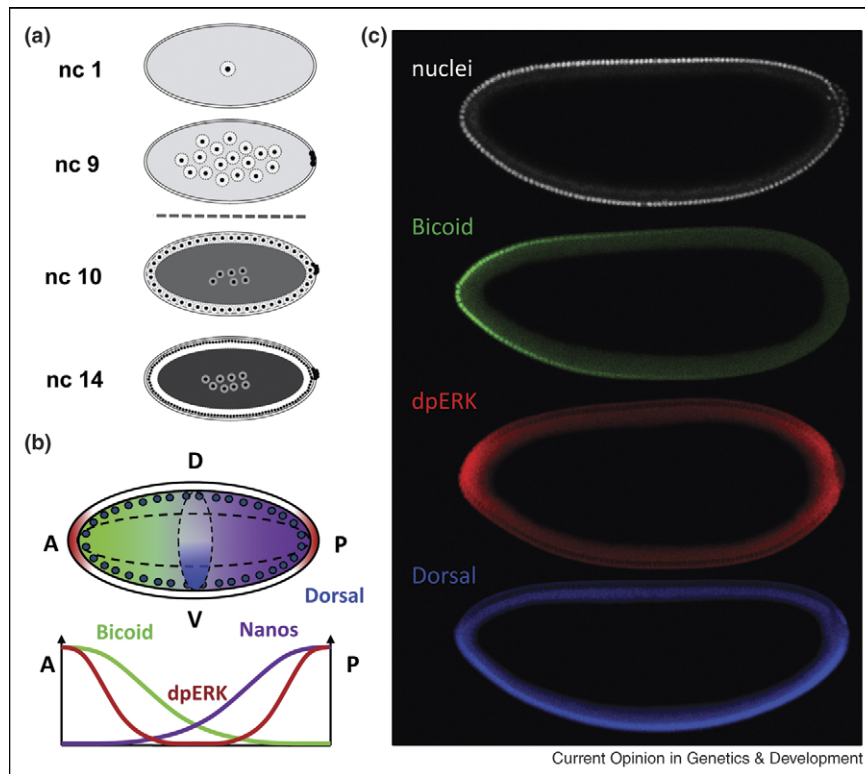
(a) Schematic representation of syncytial nuclear divisions in the early *Drosophila* embryo (nuclear cycles 1 to 14, where nuclei are black dots, cytoplasm is white, and yolk is gray). (b) Schematic representation of the concentration gradients of Bicoid (green) and Nanos (purple), the gradient of nuclear localization of Dorsal (blue), and the gradient of ERK/MAPK phosphorylation (red). (c) Midsagittal confocal images of syncytial nuclei and of the gradients of Bicoid (green), dpERK (red), and Dorsal (blue).

As a step toward answering this question, we formulated and analyzed a model that explicitly accounts for the dynamics of the nuclear density and the nucleocytoplasmic shuttling of Bcd (Figure 2a and c) [10•]. Since the time scale of Bcd degradation is unknown, we asked whether a gradient, which appears stable on the time scale of experimental observations, could be established without degradation at all. In our model Bcd is a stable molecule that rapidly equilibrates between the mobile (cytoplasmic) and trapped (nuclear) states, and nuclei act as reversible traps that slow down the Bcd diffusion. The model splits the entire process into two phases: during the first phase, with low nuclear density, syncytial divisions proceed in three dimensions (nuclear cycles 1–9). In the next phase, nuclei are distributed in a two-dimensional layer under the plasma membrane, and the nuclear density is high (Figure 2a).

Using our model and the previously measured durations of the different phases of syncytial nuclear divisions [11], we were able to show that dynamics of the gradient is controlled by only two dimensionless parameters [10•]. The first parameter, which characterizes the distance to which Bcd diffuses during the first phase

of the gradient formation, depends on the diffusivity of the free Bcd molecule, the size of the embryo, and the duration of the first phase in the model. The second dimensionless parameter, which characterizes the equilibrium between nuclear and cytoplasmic Bcd at the beginning of the second phase, depends on the rate constants of the nuclear import and export of Bcd, and on the nuclear density at the beginning of 10th nuclear division.

Analysis of the gradient dynamics requires a specific assumption about the time-dependence of the rate of the nuclear trapping of Bcd. We assumed that this rate is proportional to the number of nuclei per unit volume; therefore it is doubled after every nuclear division [10•]. Under this assumption, we identified a wide domain of the model parameters that is consistent with the experimentally determined length scale of the Bcd gradient and its temporal accuracy [6••,9••]. Based on this, we believe that the mechanism of diffusion and reversible trapping of a stable protein is a viable alternative to the commonly used diffusion and degradation model. In the future, the validity of this new mechanism must be tested by direct *in vivo* measurements of the lifetime of Bcd.

Figure 2
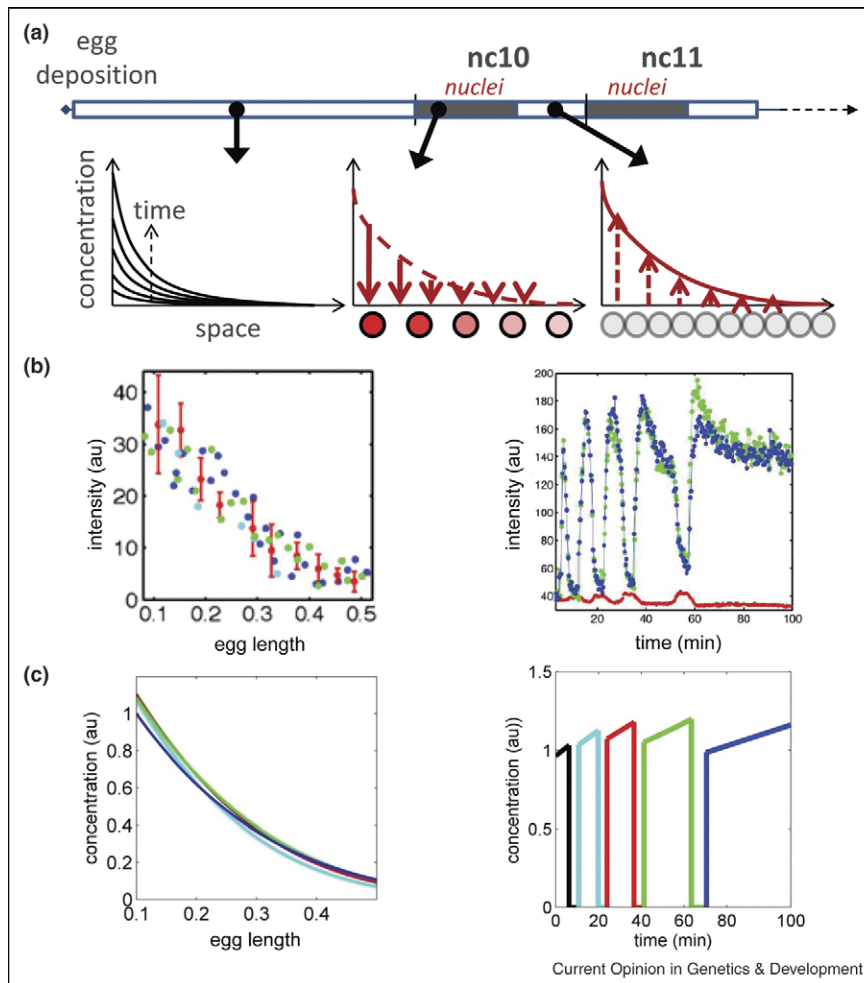
(a) Two phases of nuclear divisions in the biophysical model for the formation of the Bcd gradient. The first nine divisions occur in three dimensions; subsequent divisions happen with nuclei arranged as a two-dimensional layer under the plasma membrane. The durations of different phases of the nuclear division cycles were measured by Foe and Alberts [11] and provide direct input for the model by Coppey et al. [10•]. (b) Measurements of the dynamics of the Bcd gradient [9••]. Left: The spatial profiles of the nuclear levels of Bcd remain constant over the last four nuclear cycles. Color code: the cyan, red, green, and blue lines show nuclear cycles 11, 12, 13, and 14, respectively. Right: Dynamics of Bcd level within a single nucleus, followed over five nuclear division cycles. The blue and green lines correspond to two distinct nuclei and the red line corresponds to Bcd cytoplasmic level. (c) Computational predictions of the dynamics of the spatial profiles of nuclear Bcd (left) and the level of Bcd inside a single nucleus (right).

Our analysis suggests that firstly, the Bcd gradient is formed, essentially completely, by passive diffusion before nuclei migrate to the periphery of the embryo and secondly, that subsequent nuclear divisions do not affect the gradient. Thus, within the framework of our model, nuclei act as inert sensors of Bcd and do not contribute significantly to the formation of the AP patterning gradient. As we discuss below, nuclei play a very different role in shaping the signal that patterns the embryonic termini.

Terminal gradient: nuclear trapping shapes the spatial profile of MAPK phosphorylation

The terminal signal is initiated by the localized activation of Torso, a receptor tyrosine kinase, which is distributed uniformly throughout the membrane of the early embryo (Figure 3b). As a result of the localized processing of its extracellular ligand (encoded by the Trunk gene), Torso is activated only at the embryonic poles, where it signals through the canonical Ras signaling cascade, which culminates in the double phosphorylation and activation of the MAPK [12–16] (Figure 3a). The locally activated MAPK then phosphorylates the uniformly distributed repressors Capicua and Groucho [17–19]. This counteracts their repressive action at the poles and leads to the localized expression of *tailless* (*tll*) and *huckebein* (*hkb*), the two gap genes essential for the specification of the terminal structures [20–23]. Thus, unlike Bcd, which directly controls the expression of its targets, the terminal signal
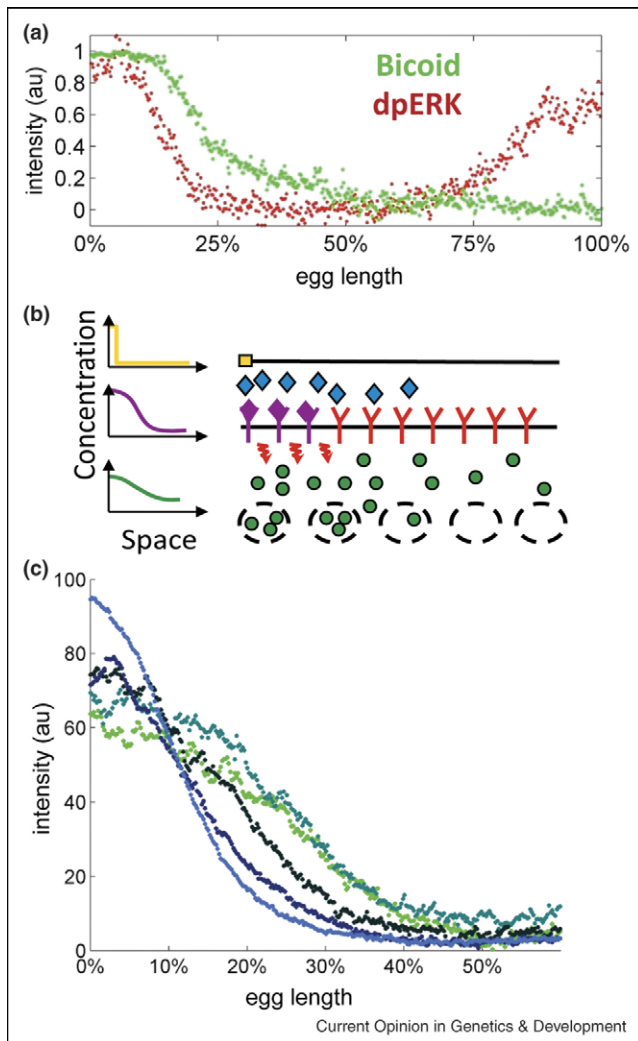
(**a**) Quantified gradients of Bcd (green) and phosphorylated MAPK (denoted by dpERK, red). (**b**) Schematic representation of the main processes responsible for the formation of the dpERK gradient. Left: spatial profiles of ligand release, Torso receptor occupancy and MAPK signaling. Localized ligand release gives rise to a spatial profile of Torso occupancy, which serves as a spatially distributed input for the MAPK signal transduction cascade. Phosphorylated MAPK diffuses within the syncytial cytoplasm and undergoes nucleocytoplasmic shuttling. (**c**) Dynamics of the MAPK phosphorylation gradient in the terminal patterning system from nuclear cycle 10 (green) to nuclear cycle 14 (blue).

acts indirectly, by the spatially restricted counteraction of the uniformly distributed transcriptional repressors [24].

Another distinction between the anterior and terminal systems is that, unlike the Bcd gradient, which is established by processes occurring entirely inside the embryo, the formation of the terminal signal also depends on the extracellular layer of regulation [24] (Figure 3b). The relative contributions of the intracellular and extracellular

processes to shaping of the terminal signal are unclear. In one possible scenario, the localized processing of the Torso ligand, its diffusion, and binding to uniformly distributed Torso establish a smoothly varying pattern of Torso occupancy that is interpreted by the localized signaling through the MAPK cascade [15]. Alternatively, the pattern of Torso occupancy could be sharply localized at the poles and the instructive gradient of MAPK signaling form mainly inside the embryo [25]. The two scenarios were proposed in the original studies of this system, but they could not be easily distinguished using the assays that relied on structures of the larva and the expression boundaries of the gap genes.

Recently, we have used a combination of imaging, genetic, and modeling approaches to revisit the mechanism of the formation of the MAPK activation gradient and to discriminate between the two scenarios [26••]. First, we developed a quantitative assay that allowed us to statistically compare the spatial patterns of MAPK phosphorylation (dpERK) across multiple genetic backgrounds. Using this assay, we established that both halving and doubling of the levels of Torso do not affect the spatial pattern of MAPK phosphorylation. Biophysical models of ligand diffusion, binding, and receptor-mediated internalization suggest that this is possible only when the wild-type pattern of receptor occupancy is sharply localized at the poles of the embryo, and essentially mirrors the spatial profile of ligand release [27]. Based on this, we propose that the smooth gradient or MAPK signaling is established inside the embryo.

Furthermore, we discovered that, over the time course of the last five nuclear cycles, the dpERK levels are amplified at the poles and attenuated in the rest of the embryo (Figure 3c). These dynamics could be explained by a model in which the syncytial nuclei act as traps for the dpERK molecules, preventing their diffusion from the poles toward the middle of embryo. The main ingredients of this model are supported by conclusions of biochemical and cellular studies that established that dpERK rapidly translocates to the nucleus, which can also serve as a compartment of its dephosphorylation [28•,29,30]. In combination with the progressive increase in the syncytial nuclear density, these processes can amplify the dpERK levels at the poles and attenuate them in the rest of the embryo. The active role of nuclei in shaping the dpERK gradient is directly supported by the analysis of the nucleocytoplasmic ratio of dpERK and by the analyses of the dpERK gradients in mutants with defects in the nuclear density [26••].

Thus, the pattern of MAPK phosphorylation appears to be controlled by two sequentially acting diffusion and trapping systems. In the extracellular compartment, the Torso receptors limit the spatial spread of the Trunk ligand (Figure 3b) [31]. Inside the embryo, nuclei limit

the spread of phosphorylated MAPK and play an active role in shaping the gradient of MAPK signaling in the terminal patterning system [26••]. Thus, quantitative studies of MAPK signaling in wild-type embryos identified a new layer of spatial regulation in the terminal system and quantified the relative contribution of the previously established ligand trapping effect [31]. In the future, our study that monitored only a single component within the MAPK cascade (dpERK), must be complemented by imaging other components within the cascade, as it was done in recent studies of MAPK dynamics in cultured cells [28•].

Conclusions and outlook

To summarize, recent studies of the dynamics of maternal morphogens provide critical tests of the classical mechanisms and reveal new modes of regulation. The new type of information provided by these studies (quantitative characteristics of the gradients versus qualitative data from experiments that monitor the structures of the embryo or transcriptional responses to the gradients) requires mathematical models for analyzing and conceptualizing the data. Analysis of these models leads to new questions related to the microscopic character of reaction and diffusion within the incredibly structured syncytial cytoplasm and plasma membrane [11,32,33]. Answering these questions requires the development of new genetic and imaging experimental approaches which should provide current, still predominantly phenomenological, models with the increasing amount of cellular and biochemical details. These details are essential for exploring both the precision of maternal gradients and the mechanisms of their transcriptional interpretation [34•,35,36•].

One of the exciting prospects for the near future is the development of a common biophysical framework for all maternal gradients in the *Drosophila* embryo. For instance, the MAPK activation gradient is sharpened over the last five nuclear cycles, whereas the Bcd gradient remains stable. Our models attribute this difference in the dynamics of the two gradients to the differences in the initial conditions and 'chemistries' of the two patterning systems: the Bcd gradient is initiated at egg deposition, whereas the formation of the terminal gradient starts only after the ninth nuclear division. In addition, we propose that Bcd is a stable molecule (on the time scale of the gradient formation), whereas the phosphorylated MAPK is not.

Recent live-imaging experiments have shown that Dorsal, the NF-κB transcription factor that patterns the DV axis, also undergoes rapid nucleocytoplasmic shuttling of Dorsal in syncytial embryos [37••]. Nuclear import of Dorsal requires its dissociation from Cactus, reviewed in [38]. Since Dorsal can bind to Cactus both in the cytoplasm and in the nucleus, it remains to be established whether the Dorsal gradient as a whole changes between

subsequent nuclear divisions. As a first step toward answering this question, it should be possible to model the Dorsal gradient, based on the imaging data in the syncytium and computational models of the NF-κB system [37••,39•].

Finally, we note a strong biophysical analogy between trapping of a diffusible ligand by cell surface receptors and trapping of a diffusible intracellular molecule by syncytial nuclei [40]. Thus we expect that, in addition to addressing specific issues in patterning of the early *Drosophila* embryo, quantitative studies of maternal morphogens will also provide insights into the morphogenetic patterning of cellular tissues.

Acknowledgements

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