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Drosophila blastoderm patterning

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The *Drosophila* blastoderm embryo is a classic model for the study of the genetics of pattern formation. In recent years, quantitative empirical approaches have been employed extensively in the study of blastoderm pattern formation. This quantitative work has enabled the development of a number of data-driven computational models. More than in other systems, these models have been experimentally validated, and have informed new empirical work. They have led to insights into the establishment of morphogen gradients, the interpretation and transduction of positional information by downstream transcriptional networks, and the mechanisms by which spatial scaling and robustness of gene expression are achieved. Here we review the latest developments in the field.

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Introduction

The blastoderm embryo of *Drosophila melanogaster* is one of the most thoroughly and intensively studied morphogenetic fields. In the blastoderm, most of the nuclei are arranged as a monolayer at the cortex (or periplasm) of the embryo. This stage starts 1 min after completion of the ninth cleavage division when the nuclei have arrived at the cortex, lasts approximately 1.5 hours until the onset of gastrulation, and includes cleavage cycles 10–14A (Figure 1a) [1].

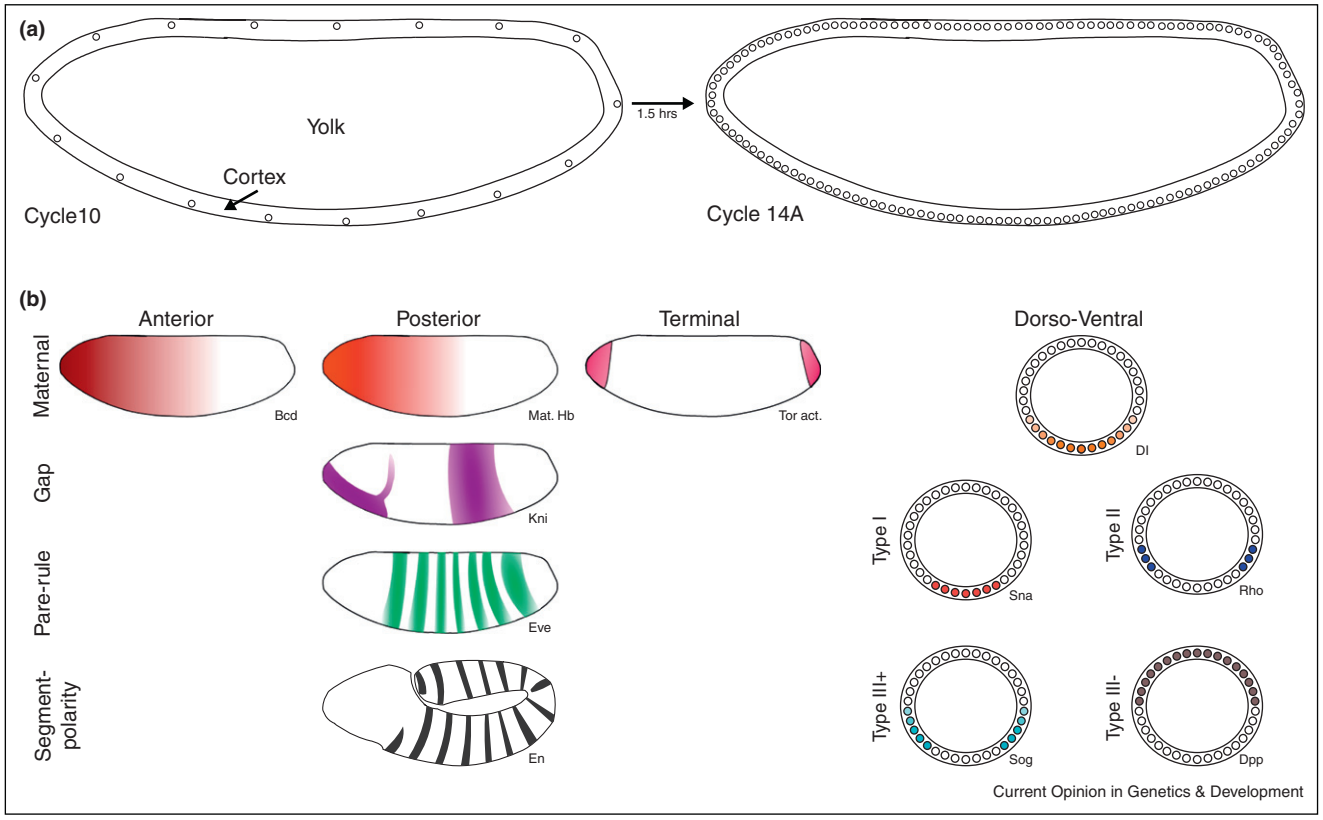
The basic body plan of *Drosophila* is determined during the blastoderm stage. Four systems of maternal protein gradients specify polarity along the main embryonic axes (Figure 1b) [2–4]. The anterior system, centered around the Bicoid (Bcd) gradient, the posterior system, including the maternal Hunchback (Hb) gradient, and the terminal system, consisting of graded signals of the Torso (Tor) MAP-kinase pathway, specify the antero-posterior (A–P) axis of the embryo. Graded nuclear localization of the Dorsal (Dl) morphogen specifies the dorso-ventral (D–V) axis. All of these maternal gradients act by regulating zygotic downstream gene expression (Figure 1b). The A–P systems activate gap, pair-rule, and segment-polarity genes, which constitute the segmentation gene network, as well as homeotic genes that specify segment identity [5–7]. The D–V system interacts with the Decapentaplegic (Dpp) morphogen, an ortholog of BMP signaling ligands, and activates targets that are involved in specification of the mesoderm, as well as the neural and dorsal ectoderm [8–10].

All of these systems use graded signals to subdivide the embryo into discrete territories along the main embryonic axes. This agrees with a classic paradigm of pattern formation first described by the French Flag model [11,12]. Since then, the blastoderm embryo has been used by many pioneering modeling studies, which have established that the situation is a lot more complex than initially thought. Complex regulatory interactions among target genes lead to a dynamic view of positional information, encoded by expression domain boundaries that change location over time [13,14]. Models have also been used to elaborate on the mechanisms of maternal gradient formation, to distinguish between competing hypotheses for spatial scaling and robustness, and to predict gene expression from DNA sequence. Here, we provide a brief critical review of modeling efforts in the blastoderm system over the past two or three years. A more detailed historical review of earlier models is provided elsewhere [15••].

Steady or not: modeling maternal gradients

A lot of the modeling work on morphogen gradients in the *Drosophila* blastoderm is focused on Bcd, which forms an exponential gradient with a scale of ~100 μm along the A–P axis (Figure 2a) [16•,17•]. Over the past few years, great progress has been made in measuring parameters required to constrain and distinguish different models of Bcd gradient formation. First, the half-life of Bcd protein has been determined to be between 20 and 50 min [18•–20•]. Second, the diffusion coefficient for cytoplasmic Bcd has been measured to be approximately 7.4 $\mu m^2/s$ [21•,22•], an order of magnitude higher than previously estimated [23]. Intriguingly, although gradient scale [24] and precision [25] were predicted to depend on nuclear absorption, these properties are not altered in embryos that have impaired nuclear association of Bcd protein

Figure 1



Pattern formation systems in the *Drosophila* blastoderm. **(a)** The blastoderm in cleavage cycle 10 after completion of nuclear migration to the cortex (left) and in cycle 14A (right). Longitudinal section. Anterior is to the left, dorsal is up. **(b)** Antero-posterior (A–P), and dorso-ventral (D–V) maternal systems. Maternal morphogen gradients patterning each system are depicted in the top row. A–P patterns shown as lateral views, D–V patterns in cross section. Tor (Torso) activity is based on [43]. Below, example expression patterns are shown for each class of downstream genes: gap, pair-rule, and segment-polarity for A–P, and types I, II, III+, and III− for D–V. En (Engrailed) expression is shown in an extended germ-band stage embryo. Bcd: Bicoid, Hb: Hunchback, Kni: Knirps, Eve: Even-skipped, Dl: Dorsal, Sna: Snail, Rho: Rhomboid, Sog: Short-gastrulation, Dpp: Decapentaplegic.

[26•]. Finally, the exact shape and extent of the bcd mRNA gradient has been determined [27•], and it has been shown that Bcd translation increases over time with maximum production coinciding with a peak in the length of poly-A tails of *bcd* mRNA in early cycle 14A [20•]. Models based on these measured parameters unambiguously establish that Bcd protein diffusion from an anteriorly localized source of mRNA is required for gradient formation [20•,27•,28] disproving earlier models postulating a gradient based on mRNA transport alone [29,30].

Another question is whether the Bcd gradient is at steady state when exerting its regulatory influence. This issue has raised some controversy in the past [16•,17•]. A recent study supports pre-steady state decoding of the Bcd gradient based on measurements of positional precision in downstream target domains [31]. However, the interpretation of these results has been disputed [32,33]. They are further challenged by more recent quantitative evidence. Although overall nuclear Bcd levels increase slightly over time during cycles 10–12 [20•,27•], the gradient is close to exponential, with a length scale that is invariant over time, and hence cannot provide a basis for differential target domain shifts [34] or precision [31] along the A–P axis (Figure 2b).

In contrast to Bcd, the nuclear Dl gradient exhibits a very dynamic pattern. Its ventral peak amplitude rises significantly, while dorsal basal levels decrease during the blastoderm stage [35–38]. A modeling study suggests that this process depends on nuclear export (as well as import) of Dl protein [38]. There is some controversy over the spatial extent of the Dl gradient [36•–39•,40,41]. Despite this, it is clear that the gradient retains its shape as it matures [36•–38•]. Dynamic changes in Dl are reflected in its target genes. A simple threshold model shows that the expansion of ventral, and the compaction of dorsal target gene domains roughly follow the changing concentration thresholds of nuclear Dl concentration, although Dl is not sufficient to account for the precise shape and placement of dorsal boundaries [38•].

In addition, most Dl targets depend on the ubiquitous co-regulator Zelda (Zld), which acts by modulating Dl threshold responses [42].

Another maternal system that has been studied using quantitative modeling is the terminal Tor MAP-kinase signaling cascade. Here, models have been used to investigate the gradual sharpening of the signaling gradient over time, which can be explained by nuclear trapping of downstream signaling factors [43,44]. Furthermore, kinetic models have been used to gain interesting new insights into the role of MAP-kinase substrate competition in gene regulation and the establishment of asymmetry along the A–P axis [45••–47••].

Pattern formation: more than a French Flag

Maternal gradients alone are not sufficient to position target gene expression domains in the blastoderm embryo. The trunk gap genes *hb*, *Krüppel* (*Kr*), *knirps* (*kni*), and *giant* (*gt*), for example, rely on cross-repressive interactions among each other for sharpening, maintenance, and positioning of their expression domain boundaries (Figure 2c) [7]. Dynamic anterior shifts in boundary positions are caused by asymmetric repressive feedback among overlapping gap domains [48,49,50••]. A number of recent studies show that regulation of head gap genes also relies on combinatorial regulation [51–53]. In this case, Bcd is activating its target proximally (close to the gradient source), while activating a repressor in more distal regions. Unlike stated in [53] this does not constitute evidence for diffusion-driven (Turing) patterning. Instead, this mechanism is reaction-driven (just as for trunk gap genes) depending on regulatory interactions among morphogen targets. Finally, D–V target domain boundaries also depend on regulation among factors downstream of Dl, especially in the dorsal region of the embryo [37•,38•].

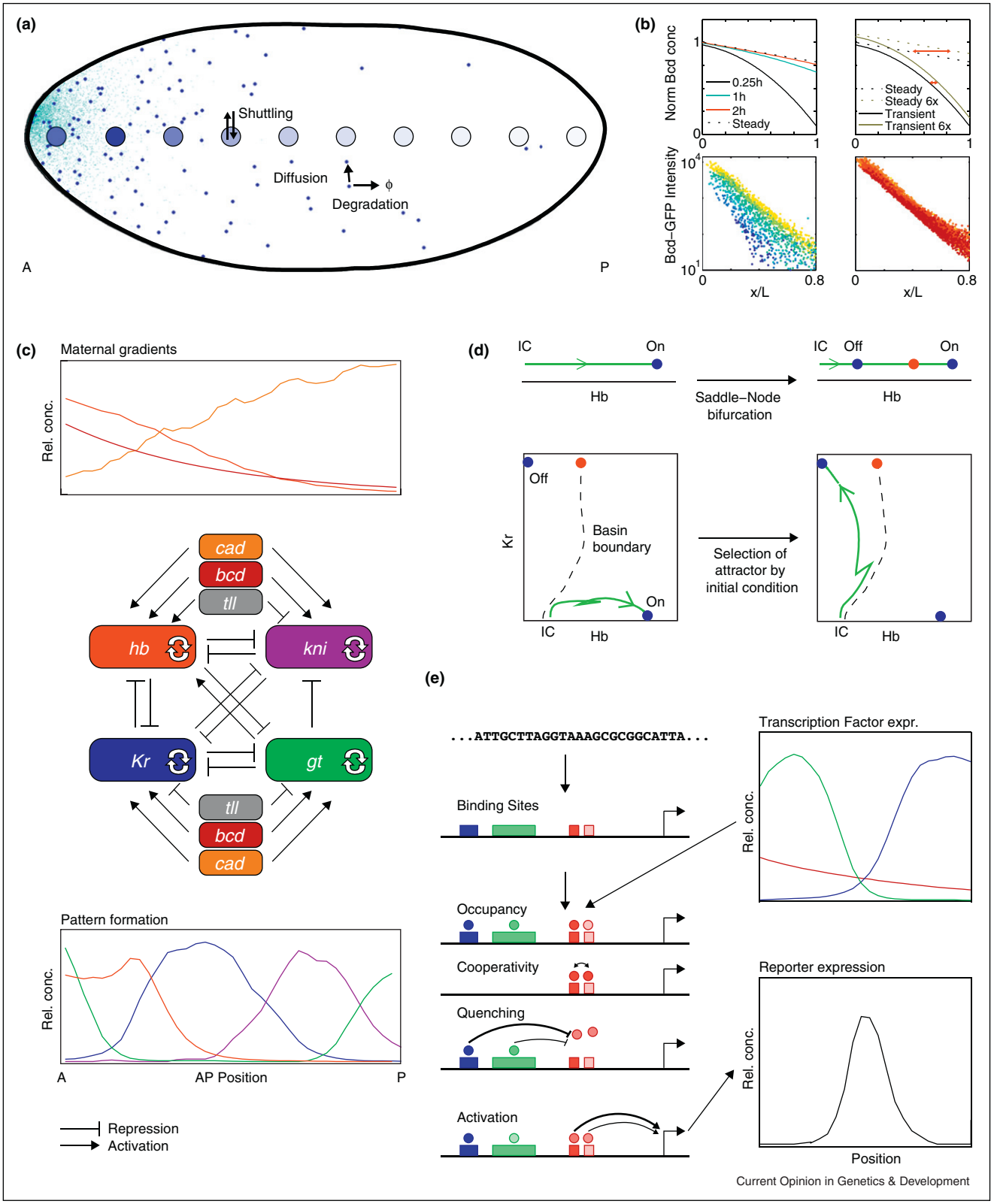
These interactions give rise to complex gene regulatory networks, whose function can be studied using the theory of non-linear dynamical systems [54••,55••]. This theory describes dynamical behavior in terms of state trajectories that converge to attractors. The set of attractors represents the dynamical repertoire of a system. A system with two alternative point attractors, for example, is called bistable. Attractors are more or less insensitive to small changes in the values of system parameters. The extent of this resilience delineates the structural stability (or robustness) of the system. Structural stability breaks down at critical values of parameters, called bifurcation points.

Investigations of non-linear dynamics can generate specific and distinct hypotheses that are amenable to empirical tests. We illustrate this with the following example. The sharp boundaries of gap and pair-rule domains, together with evidence for auto-regulation and mutual repression has led to proposals that these genes operate as bistable switches [56–58]. In the simplest model [57], the posterior hb boundary forms owing to bistability arising from hb auto-activation. As Bcd concentration decreases from anterior to posterior, a bifurcation creates a 'Hb off' state, repressing *hb* in the posterior of the embryo. However, a boundary formed by this mechanism is extremely sensitive to fluctuations in Bcd concentration. More generally, creating a series of boundaries along the A–P axis in this manner will not be structurally stable since it would require bifurcations to occur every few nuclei.

While the models described above remain largely conceptual, the non-linear dynamics of morphogen target interactions can also be studied using regulatory networks inferred from quantitative gene expression data [48,50••,59,60]. The key advantage of such an approach is that it does not prescribe any particular mechanism, such as bistability, but instead derives systems dynamics directly from data. This has led to important new insights into gap gene regulation: for instance, the establishment of seven gap gene boundaries, involving 24 regulatory interactions, can be understood in terms of just three dynamical mechanisms: (1) movement of attractor position, (2) selection of attractors by initial conditions, and (3) selection of states on a transient attracting trajectory. In contrast to the model described above [57], posterior hb boundary formation does not rely on the creation of a 'Hb off' state by a bifurcation – such a state coexists with 'Hb on' in both anterior and posterior nuclei – but on the selection of one of these two states by maternal Hb concentration (Figure 2d). Since the attractors and their basins of attraction are determined by Bcd and Cad concentrations and their selection is determined by maternal Hb concentration, these dynamics imply that *hb* integrates both anterior and posterior maternal information to form its border. The integration of regulatory input from both anterior and posterior maternal systems is supported by experimental evidence [21•,61]. It underlies the insensitivity of *hb* boundary position to Bcd variation [49,60]. There is only one bifurcation in the middle of the embryo, posterior to the hb boundary, and therefore, the dynamics in the two halves of the embryo are structurally stable.

Regulatory networks among morphogen targets are complex, and remain difficult to model. No models exist that accurately and systematically reproduce interactions involving pair-rule genes, or D–V target genes. Furthermore, little progress has been made in the past few years, beyond the models described above and in [15••], with regard to modeling gap or segment-polarity gene expression. One direction of research has been to extend models of gap and other segmentation genes to two or three dimensions using accurate embryo geometries. One such model predicts that the curvature (or splay) of segmenta-

Figure 2



Modeling the blastoderm embryo. **(a)** Models of morphogen gradient formation. Bcd protein (blue dots) is produced at the anterior pole from bcd mRNA (cyan dots), diffuses through the cytoplasm, and is degraded throughout the blastoderm. The protein is shuttled between nuclei and cytoplasm and accumulates within nuclei (circles). **(b)** The hypothesis of pre-steady-state decoding of Bcd in comparison to Bcd-GFP data. The top-left panel

tion gene expression patterns along the D–V axis is caused by asymmetries in the Bcd gradient owing to the bulging ventral contour of the embryo [62]. However, a full 3D model of the gap gene system indicates that this may only be true in the anterior part of the embryo, while Bcd asymmetry is insufficient to explain the splay of more posterior patterns [63]. Neither of two recent 3D models of gap gene expression [63,64] have led to new insights into gap gene regulation beyond those achieved with one-dimensional models, and a model-based attempt to dissect the gap gene system into functional modules [58] has not identified any regulatory principles beyond those described in earlier work [59].

Scaling, precision, and robustness

Development produces body proportions that are invariant with respect to egg size – a property referred to as scaling. Scaling between different species of flies has been shown to depend on the evolution of Bcd protein stability, which leads to larger length-scale gradients in big, and shorter length-scale gradients in small eggs [65]. Bcd and its target genes also scale, albeit partially, between and within *D. melanogaster* populations [66–68,69•]. This effect is inherited maternally [66], and relies on the level of *bcd* mRNA present in these embryos rather than direct adjustment of the length scale of the gradient [69•]. The hypothesis that nuclear degradation or trapping of Bcd could provide scaling if the number of nuclei is constant [23,31,70] has been invalidated by the observation that nuclear import does not affect the gradient [26•], and that the number of nuclei varies with embryo size [68]. These studies suggest that maternal gradients such as Bcd scale with egg size, although the mechanisms differ between evolutionary time scales.

The evidence reviewed above does not entirely exclude a role of target gene interactions in scaling. A model of the gap gene network [49,71] predicts size regulation in the absence of Bcd scaling owing to negative regulatory feedback within the network. This model implicitly depends on diffusion of maternal gradients, but not on diffusion of gap gene products. Although this mechanism remains to be tested empirically, it is a potential explanation for why pair-rule gene expression scales across 80% of the blastoderm [68] even though the Bcd gradient exhibits size regulation only in the middle of the embryo [69•].

Precision and robustness of patterning are achieved despite variability in initial conditions (maternal gradients) and stochastic fluctuations in gene expression. Insensitivity to initial conditions is reflected by the fact that positional error in target genes is lower than in maternal gradients [49,72] and reduces over time [73]. In addition, shifts in gap domain positions are much smaller than expected when *bcd* dose is varied [74–76]. Evidence for the presence of stochastic fluctuations is provided by the small number of Bcd molecules in nuclei [21•,22•,77], which, in the absence of averaging mechanisms, cannot reliably specify the sharp borders observed in cycle 14.

There are three main models for the reduction of initial variation. The first postulates an unknown posterior gradient, which is not (yet) supported by any experimental evidence (reviewed in [15••]). The second depends on pre-steady-state decoding of the Bcd gradient [31,34]. It is unlikely to apply for reasons discussed above. The third model predicts that reduction in variability occurs as a result of negative feedback loops within the gap gene network [49]. This mechanism was experimentally validated by measuring the variance of Hb boundary position in a mutant background lacking the relevant feedback regulation [49].

(Figure 2 Legend Continued) shows normalized transient and steady-state solutions of the one-dimensional source, diffusion, and degradation (SDD) equation for Bcd [34] on a semi-log scale. Solutions were computed using the values of the diffusion and degradation constants estimated by [21•] and [20•] respectively. The top-right panel shows the effect of increasing source strength six-fold. The positional shift induced by the source perturbation is much smaller in the transient solution in the posterior of the embryo. The attenuation of the shift relies upon the non-exponential shape of the transient solution. The bottom two panels are taken from Figure 7a,b of [27•]. They show the measurement of Bcd-GFP fluorescence in fixed tissue during cleavage cycles 6–11 (bottom left; blue-yellow) and 12–14 (bottom right; orange-maroon), in semi-log scale. As early as cycle 10 (green), the scatter approximates a straight line (exponential) and remains so thereafter. **(c)** Models of morphogen gradient interpretation. The top panel shows the anterior and posterior maternal gradients along the A–P axis in the middle third of the embryo. The middle panel is the gap gene network (simulated using coupled differential equations) that produces gap gene expression patterns shown in the bottom panel. *bcd: bicoid*, *cad: caudal*, *hb: hunchback*, *Kr: Krüppel*, *kni: knirps*, *gt: giant*, *tll: tailless*. **(d)** Comparison of a model of Hb bistability [57] with non-linear dynamics inferred from gene expression data [50••]. Left and right panels illustrate phase portraits of anterior and posterior nuclei with high and low concentrations of Hb respectively. The top two panels show Hb border formation owing to a saddle-node bifurcation in the model of Lopes *et al.* [57], which occurs because of lower Bcd concentration in the posterior. Blue points are attractors and red are saddle nodes. Hb initial condition (IC) has no role in the switch, which is exclusively driven by Bcd. The bottom two panels illustrate hb border formation in the model of Manu *et al.* [50••]. The 'Hb on' and 'Hb off' states coexist in the phase space of both anterior and posterior nuclei. The switch occurs because posterior nuclei have lower Hb initial concentration (specified by the concentration of maternal Hb). The boundary between the basins of 'Hb on' and 'Hb off' is determined by Bcd and Cad concentrations [50••]. These dynamics imply that *hb* integrates posterior and anterior positional information provided through Hb initial concentration and the basin boundary respectively. **(e)** Models of cis-regulation. Binding sites are identified in DNA using bioinformatic and/or experimental approaches. Transcription factor expression data are used to estimate occupancy of the binding sites. The occupancy at each site can change owing to protein–protein interactions such as co-operativity and quenching (short-range repression). The modified occupancy is used to compute the transcriptional activity of the CRM and predict reporter expression.

While this mechanism can reduce the effect of variability in maternal gradients, it is doubtful that it can also provide robustness against internal molecular fluctuations. A number of recent modeling studies have provided new insights into the sources of fluctuations in Bcd levels and their effect on patterning precision. The first of these studies shows that positional precision provided by the Bcd gradient is largely limited by internal fluctuations, rather than embryo-to-embryo variability in the amplitude of the gradient [78•]. The signature of these fluctuations is passed on to target gene expression patterns indicating a significant and lasting regulatory influence of Bcd on target gene expression during the blastoderm stage [79,80]. The effect of these fluctuations on target gene expression can be reduced, however, by temporal and spatial integration of regulatory input [77] and *hb* auto-activation by maternal Hb in cycles 11–12 [21•]. Temporal and spatial averaging effects were confirmed and analyzed in detail by two studies based on stochastic models of hb regulation by Bcd [80,81]. Another modeling study reached similar conclusions [82]. However, it is based on immunostaining on fixed tissue rather than live imaging which tends to mask intrinsic noise [83].

The devil is in the details: transcriptional regulation

Most models we have discussed so far coarse-grain the detailed structure of cis-regulatory elements, or the molecular mechanisms of transcriptional regulation. A number of models incorporating such details have been used to study the structure and function of regulatory sequences, and the mechanisms by which transcription factors act, or to predict expression patterns from sequence (Figure 2e; reviewed in [15••]). One recent study focused on the arrangement of activator versus repressor binding sites to investigate the mechanism of short-range repression, or quenching [84]. Another study also focused on the role of quenching, considering other transcriptional mechanisms such as co-operative and synergistic transcription factor binding as well [85]. Finally, a simple logistic regression model was used to show that the pattern-generating potential of different regulatory sequences could be predicted with an accuracy and success rate similar to previous (more mechanistically accurate) models [86•]. This indicates that such models are good as prediction tools, but must be used with caution when investigating mechanisms of transcriptional regulation.

There are further indications that transcriptional modeling in the blastoderm is still in its infancy. All of the studies described above suffer from the fact that they do not yet represent the dynamics of gene regulation correctly, since the data they are fit to are not temporally resolved. Furthermore, data fits are often somewhat suboptimal. Finally, many of these models suffer from problems concerning their predictive power: in many cases parameter values cannot be estimated with confidence from the data. This was demonstrated by a rigorous analysis of parameter identifiability in two previously published models [87]. The first model considered in this study was able to predict quenching coefficients from models fits [84], but the analysis showed that conclusions drawn about the importance of co-operative transcription factor binding in another study [88] were not statistically well founded. All of these problems will have to be resolved, if we are to gain a rigorous quantitative understanding of the role of dynamic transcriptional regulation in pattern formation.

Conclusions

Up until very recently, modeling efforts in the *Drosophila* blastoderm have focused on gene regulatory networks and their role in specifying positional information [15••]. The past few years, however, have seen an increasing shift of focus toward modeling the molecular mechanisms of transcriptional regulation and the biophysics of morphogen gradient formation. While the former efforts are still at an early stage, the latter have made impressive and rapid progress. In particular, the properties of the Bcd gradient have been described and measured in great detail. These results are encouraging and exciting. However, we must remind ourselves that they are not sufficient to completely understand blastoderm pattern formation. A more holistic approach will be required that includes the complex regulatory interactions among morphogen targets. This poses a grand challenge for data-driven modeling. We must develop new methods and learn to think in different conceptual frameworks – such as that of non-linear systems theory – if we are to meet this challenge in the future.

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