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Quantitative Models of Developmental Pattern Formation

Review

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Pattern formation in developing organisms can be regulated at a variety of levels, from gene sequence to anatomy. At this level of complexity, mechanistic models of development become essential for integrating data, guiding future experiments, and predicting the effects of genetic and physical perturbations. However, the formulation and analysis of quantitative models of development are limited by high levels of uncertainty in experimental measurements, a large number of both known and unknown system components, and the multiscale nature of development. At the same time, an expanding arsenal of experimental tools can constrain models and directly test their predictions, making the modeling efforts not only necessary, but feasible. Using a number of problems in fruit fly development, we discuss how models can be used to test the feasibility of proposed patterning mechanisms and characterize their systems-level properties.

Introduction

One of the intellectual challenges in the analysis of developmental pattern formation is to synthesize the information from genetic, cellular, and biochemical studies into quantitative models that can be used to summarize existing results and guide future experiments. In addition to providing compact summaries of experimental data, models of patterning mechanisms are essential for exploring their systems-level properties, such as robustness and evolvability (Kirschner and Gerhart, 1998; Freeman and Gurdon, 2002; Eldar et al., 2004). Early models of development were based on the idea that complex patterns self-organize naturally when simple patterns lose their stability. Such a phenomenon can be realized in a variety of ways, e.g., by nonlinear interactions of diffusion and chemistry (Turing, 1952; Gierer and Meinhardt, 1972; Meinhardt, 1982; Murray, 1993; Pismen, 2006). The formulation of these models has long predated the molecular studies of development. As a result, they were phenomenological in nature, invoking hypothetical species and interactions rather than molecules and processes involved in specific patterning events. Phenomenological models can generate elaborate patterns with striking similarities to the ones observed in real embryos, but they are not well-suited for predicting the effects of specific genetic and biochemical perturbations. For example, mutant alleles of the gene *leopard* induce transitions from striped (wild-type) to spotted skin pattern in zebrafish (Johnson et al., 1995). All of the transitions can be reproduced by varying just a single parameter in a two-variable reaction-diffusion model, a clear success of a phenomenological description (Asai et al., 1999). At the same time, it is unclear how to use this model in order to understand how *leopard*, which encodes a connexin protein, controls the interactions between the pigment cells responsible for the striped skin pattern, or how these patterns evolve (Maderspacher and Nusslein-Volhard, 2003; Quigley et al., 2005; Watanabe et al., 2006). As molecular studies uncover increasingly detailed descriptions of development, mechanistic models will become both more feasible and preferable.

Any attempt to establish quantitative models of pattern formation is confronted by the high level of experimental uncertainty, a large number of components, and the multiscale nature of development (Longabaugh et al., 2005; Stathopoulos and Levine, 2005). As we learn more about developing systems, at least the structural uncertainty (i.e., uncertainty regarding the parts list of a patterning module and its connectivity) will be gradually reduced. Furthermore, while handling the size of a large modeled system and its associated parametric uncertainty (e.g., the lack of kinetic information about any given process) is nontrivial, there are systematic modeling and algorithmic approaches, such as sensitivity analysis and uncertainty propagation, to address these problems (Ghanem and Wojtkiewicz, 2004; Saltelli et al., 2005; El-Samad et al., 2006). On the other hand, the coupling between different processes and scales in the system—transcriptional regulation, signal transduction, and tissue-level patterning—will continue to present conceptual challenges for modeling, even after a "complete parts list" of pattern formation systems is compiled and their monitoring in space and time becomes commonplace.

By their structure and methods of analysis, current mathematical models of developmental patterning are similar to those of chemically reacting systems. Both the structural complexity of biological patterning networks and the current level of experimental uncertainty associated with their analysis are much greater than in purely chemical systems like combustion or heterogeneous catalysis. At the same time, as a consequence of their higher organizational complexity, biological systems can be manipulated and analyzed at a greater number of levels than their chemical analogs. Indeed, tools like site-directed mutagenesis, targeted expression systems, allelic series, and phylogenetic analysis do not have direct parallels in chemical systems. This higher manipulability of natural patterning systems makes it possible to design a very large number of tests that can be used to constrain and refine mechanistic models.

According to the 1994 perspective by Wolpert, the embryo should be computable "if a level of complexity of description of cell behavior can be chosen that is

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adequate to account for development but that does not require each cell's detailed behavior to be taken into account" (Wolpert, 1994). The choice of an adequate level of description can emerge only from iterations between the construction of abstract models, their computational analysis, and model-driven experiments. Here we discuss the current status of mechanistic modeling of developmental pattern formation. The review is organized as follows. In the next section, using long-range patterning of the fruit fly wing as an example, we discuss methodological issues and requirements for model formulation. Next, we discuss techniques for model analysis, emphasizing the power of dimensional analysis and simple scaling arguments. Finally, we provide a number of examples that illustrate how models can be used to test the feasibility of proposed pattern formation mechanisms and provide access to their systems-level properties.

Formulating Models

In this section, we discuss the prerequisites for model formulation. Before a developmental system can be modeled, one must first have at least a basic understanding of the interactions of the major players, as well as the tissue geometry. In addition to this, the multiscale nature of development, and biological systems in general, adds another requirement to basic model formulation, as a connection must be made between gene transcription, a highly stochastic process, and other cellular or tissue-level events. Here we focus on pattern formation by morphogen gradients (Gurdon and Bourillot, 2001; Tabata and Takei, 2004), an area where a number of experimental breakthroughs have recently been made and where the application of modeling approaches appears both necessary and feasible.

In the past years, convincing evidence has shown that ligands of the BMP, Hh, Wnt, and FGF families can act as morphogens in the development of a variety of tissues across species (Tabata and Takei, 2004). Thus, the question about the existence of morphogen gradients, which was one of the central questions of 20th century developmental biology, gave way to questions related to gradient formation, robustness, and interpretation (Wolpert, 1996; Gurdon and Bourillot, 2001; Eldar et al., 2004; Tabata and Takei, 2004; Ashe and Briscoe, 2006). Resolution of these questions requires quantitative measurements at a number of levels, including subcellular distributions of ligands along the trafficking pathway (Vincent and Dubois, 2002), receptor occupancy across the patterned field (Wang and Ferguson, 2005), and affinities of binding sites in gene regulatory sequences (Wharton et al., 2004).

To discuss just one example, Dpp (Decapentaplegic), a *Drosophila* homolog of mammalian Bone Morphogenetic Protein, acts as a morphogen in multiple stages of fruit fly development (Parker et al., 2004). In the larval wing imaginal disc, Dpp forms a long-range gradient of signaling activity that controls tissue patterning, growth, and morphogenesis (Figure 1A). This gradient is established by the combination of localized secretion of Dpp at the anterior-posterior compartment boundary, its endocytic degradation by cells in the wing disc, and ligand transport (Figure 1D). In three of the proposed transport mechanisms, Dpp moves either through the extracellular space; through the cell, by planar transcytosis; or along the cell surfaces, assisted by cell surface proteoglycans (Entchev et al., 2000; Lander et al., 2002; Belenkaya et al., 2004). Until the end of the 90s, these gradients could be monitored only indirectly, through their effects on target genes and resulting wing morphology (Lecuit et al., 1996; Nellen et al., 1996; Lecuit and Cohen, 1998). Recently, however, several groups have directly visualized Dpp gradients in the wing imaginal disc (Entchev et al., 2000; Teleman and Cohen, 2000; Belenkaya et al., 2004). As a result, it has been estimated that the diffusivity of Dpp is orders of magnitude lower than that of a similarly sized protein in water, that the time scale for the Dpp degradation in the wing disc is less than 3–4 hr, and that the gradient of Dpp can be considered to be in steady state (on the time scales of tissue growth and patterning).

These conclusions about the spatial pattern of Dpp in the wing are based on the observed changes in the ligand gradient (or the gradient of activity) in response to genetic perturbations of the system. For example, overexpression of receptor shrinks the activity gradient, suggesting that Dpp receptors not only transduce but also shape the spatial distribution of secreted Dpp (Lecuit and Cohen, 1998). Major advances in the description of the Dpp gradient in the wing have relied on experiments using GFP-tagged Dpp expressed from the AP compartment boundary and visualized in a number of genetic backgrounds (Entchev et al., 2000; Teleman and Cohen, 2000; Belenkaya et al., 2004). However, in most of these experiments, the GFP-tagged Dpp had been expressed in addition to the endogenous ligand, and indirect measurements indicate that the wing discs in these studies were exposed to approximately twice the level of ligand (Entchev et al., 2000). Thus, labeled ligand is hardly an inert tracer of the gradient in this system and may affect the wild-type concentration field of the ligand. This could pose a considerable problem in the quantitative measurements and should be carefully evaluated in each case.

Overall, the development and implementation of experimental assays to monitor Dpp and other morphogens is highly nontrivial at this time. As a result, the interpretation of results of such assays stimulated the formulation of the first generation of mechanistic models (Lander et al., 2002; Kruse et al., 2004). The first step in the formulation of such models is to provide a reasonable specification of tissue anatomy. In the case of the Wingless (and also likely Dpp) gradient in the wing imaginal disc, the ligand can be secreted from both apical and basolateral sides of columnar cells (Marois et al., 2006). Depending on the direction of secretion, the morphogen may be exposed to different extracellular environments. Since the apical and basolateral surfaces of columnar epithelial cells represent discrete membrane compartments, the apically secreted ligand may encounter different cell surface molecules than the basolaterally secreted ligand (Figures 1B and 1C). In addition, the extracellular space next to the apical and basolateral surfaces of columnar cells differ dramatically in morphology (Gibson and Schubiger, 2001; Pallavi and Shashidhara, 2005). The resulting differences in the geometries and boundary conditions for the apically and basolaterally secreted ligands can generate

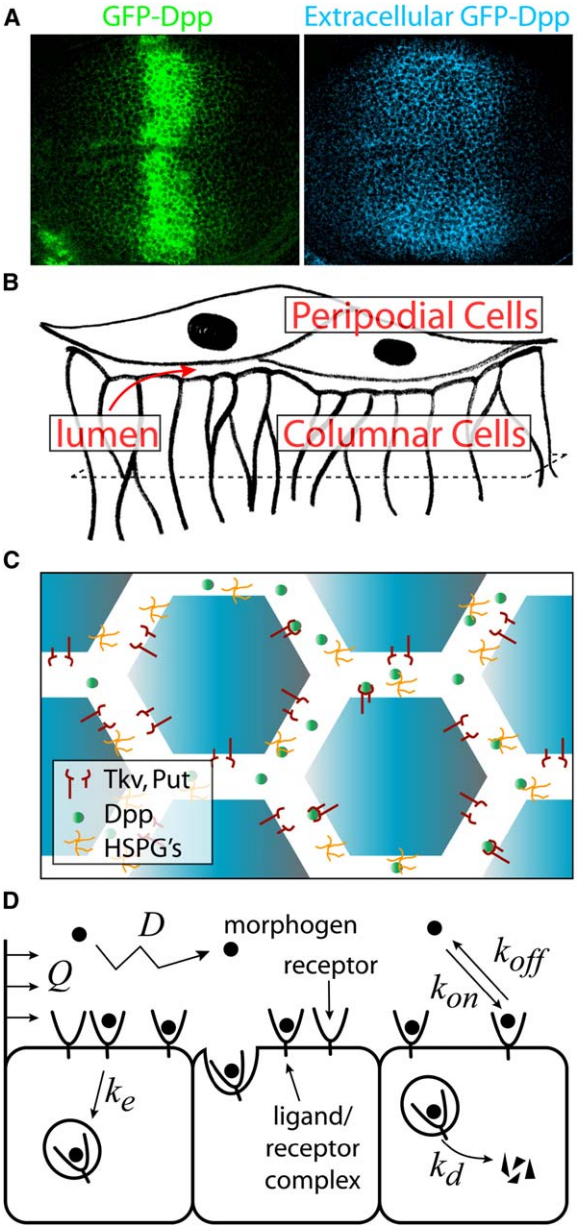

Figure 1. Dpp Gradient and Geometry in the Wing Disc

(A) Visualization of GFP-labeled Dpp in a third larval instar wing disc. The overall distribution of GFP-Dpp protein, as shown by the autofluoresence of the GFP tag (green), is highly peaked near the anterior-posterior compartment boundary. In contrast, the gradient of purely extracellular Dpp (blue) is broad across the entire wing disc. Reprinted from Belenkaya et al. (2004), copyright 2004, with permission from Elsevier.

(B and C) Schematics of cross-sections through a *Drosophila* wing disc. These schematics illustrate different cell types and different tissue compartments. In the vertical cross-section (B), large peripodial cells cover the surface of the imaginal disc proper, which consists of tall columnar cells in a tight epithelium. Between these two types of cells is the lumen of the imaginal disc. Ligand secreted from the apical side of the columnar cells enters the lumen and quickly forms a flat gradient across the imaginal disc. The dotted plane depicts the horizontal cross-section shown in (C). This cross-section depicts the extracellular space between the tall columnar cells. Ligands secreted from the lateral portion of the columnar cells encounter narrow, tortuous geometry, perhaps crowded by cell surface proteins to further impede transport. Due to tight junctions near the apical portion of the columnar cells, ligand secreted in the lumenal compartment cannot mix with that secreted in the columnar compartment. The schematic in (B) was reprinted with permission from Company of Biologists Ltd. (Pallavi and Shashidhara, 2005).

(D) Tissue-level description of BMP-mediated patterning in a one-dimensional system. Morphogen enters the tissue at the AP compartment boundary with a constant flux of Q. The ligand is free to diffuse, with effective diffusivity D. Binding to cell surface receptors is characterized by the rate constants k_{on} and k_{off}. The ligand/receptor complex undergoes endocytosis with rate constant k_e. The internalized ligand/receptor complex is degraded in a first order process with rate constant k_d.

pronounced differences in the length scales of secreted signals. This might reconcile the experimentally observed "flat" profile of Dpp in the lumen and a pronounced gradient of basolateral Dpp (Entchev et al., 2000; Gibson et al., 2002; Belenkaya et al., 2004).

Following the specification of tissue structure, models of morphogen gradients require quantitative descriptions of intracellular signaling. Indeed, most of the quantitative conclusions about the extracellular distribution and transport of Dpp are based on the observations of the pattern of phosphorylated SMAD (Entchev et al., 2000; Dorfman and Shilo, 2001; Lander et al., 2002; Belenkaya et al., 2004; Wang and Ferguson, 2005). Thus, models should explicitly describe the connection between the extracellular and intracellular compartments of the system. All of the published models of Dpp transport in the wing and in the embryo account for extracellular diffusion, ligand-receptor binding, and receptor-mediated internalization (Lander et al., 2002; Kruse et al., 2004; Umulis et al., 2006). In the future, these models can be interfaced with quantitative descriptions of receptor trafficking and nucleocytoplasmic SMAD shuttling (Schmierer and Hill, 2005; Dudu et al., 2006; Vilar et al., 2006). Eventually, quantitative understanding of morphogenetic patterning will require models of gradient interpretation (Ashe and Briscoe, 2006). For Dpp-mediated wing patterning, gradient interpretation relies on the Dpp-mediated expression of Brinker, a sequence-specific repressor of Dpp signaling (Saller and Bienz, 2001; Pyrowolakis et al., 2004; Gao et al., 2005; Moser and Campbell, 2005). The emerging model of transcriptional repression by Brinker and the visualization of the Brinker gradient in the wing can be linked to the kinetic models of nucleocytoplasmic SMAD dynamics, and through that to models of ligand/receptor dynamics and ligand transport.

Analyzing Models

The use of mechanistic models requires the analysis of their dynamics over a wide range of parameters, most of which have not been constrained by direct measurements. Indeed, quantitative biochemical measurements of ligand/receptor or transcription factors/binding site interactions are available for just a few developmental systems (Klein et al., 2004; Rentzsch et al., 2006), and cellular and tissue parameters, such as levels of protein expression, have not been characterized at all in vivo. In the absence of this information, one can start by using the data from studies with cultured cells and test how these data perform in a developmental setting (Lai et al., 2004; Saha and Schaffer, 2006). Alternatively, one can search the parameter space for parameter sets consistent with wild-type and mutant phenotypes

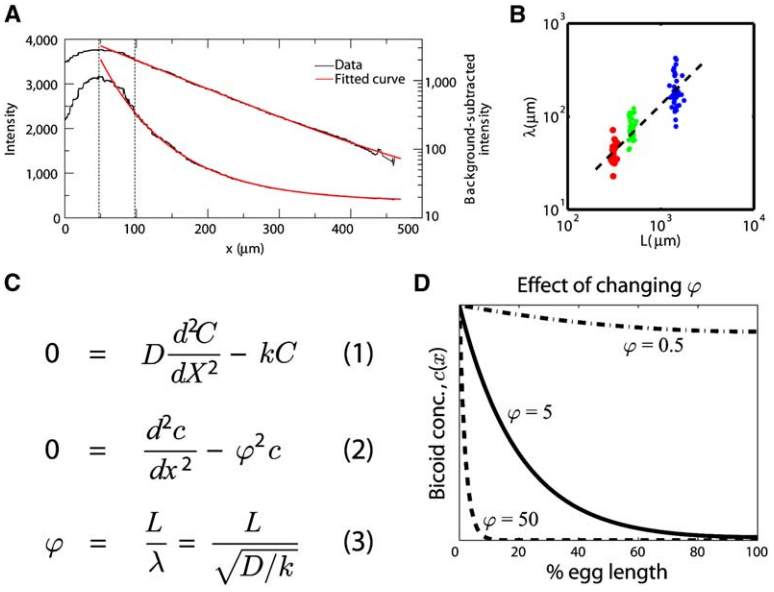

Figure 2. Quantitative Analysis of the Bicoid Morphogen in the *Drosophila* Embryo

(A) Plot of Bcd fluorescent immunostaining intensity versus egg antero-posterior coordinate, *x*. Note that the Bicoid gradient can be accurately approximated by an exponential profile. Reprinted by permission from Macmillan Publishers Ltd: *Nature* (Houchmandzadeh et al., 2002), copyright 2002.

(B) Plot of dynamic length scale, λ, versus egg length, *L*, for three different species. Note that L/λ, the ratio of the geometric and dynamic length scales for the Bicoid gradient, is conserved across these species. Red, *D. melanogaster*; green, *D. Busckii*; blue, *L. sericata*. Reprinted from Gregor et al. (2005), copyright 2005, National Academy of Sciences, USA.

(C) Simplified model of Bicoid spreading in the early *Drosophila* embryo. Equation 1 describes the diffusion, with diffusivity *D*, and first-order decay, with rate constant *k*, of Bicoid concentration, *C*. After scaling (Equation 2), the Thiele modulus, φ, naturally arises as the only free parameter, with $\varphi = L/\lambda$ (Equation 3). In Equation 2, *c* is the Bicoid concentration scaled by its maximum value, and *x* is anteroposterior coordinate scaled by egg length, *L*.

(D) L/λ can not be very different from unity for efficient patterning. In plots of *c* versus percentage egg length for different values of $\varphi = L/\lambda$, the gradient is significant across the whole egg only for $\varphi = 5$ (solid black curve). For $\varphi = 0.5$ (dot-dashed curve) and $\varphi = 50$ (dashed curve), the gradient is essentially flat for most of the egg. Neither too sharp a gradient ($\varphi = 50$), nor too shallow ($\varphi = 0.5$), can be responsible for efficient pattering.

(von Dassow et al., 2000; Eldar et al., 2002). Choice of parameter acceptance criteria and how densely to sample the parameter space are key questions in implementing such computational screens. Given the fact that, at least until now, the computational cost of developmental pattern formation models is quite modest, one can devise systematic computational approaches for the location and acceptance of suitable parameter sets (Flann et al., 2005). Two modeling studies of the planar cell polarity and sensory organ precursor systems in *Drosophila* show how the wild-type and mutant gene expression patterns can be used as constraints in the computational searches for the "right" parameter sets (Amonlirdviman et al., 2005; Hsu et al., 2006).

One way of dealing with multiple parameters in physicochemical problems relies on dimensional analysis and scaling. Dimensional analysis identifies competing processes and reduces the number of free parameters by combining the original parameters into new, dimensionless groups. Subsequently, scaling arguments can provide order of magnitude estimates for the values of the dimensionless groups even before the model equations are solved. As an example, consider a hypothetical morphogen with diffusivity *D* and first-order degradation rate constant *k*; the steady-state profile of the signal patterns a field of size *L*. This setup may be used to model the gradient of Bicoid, the first morphogen that has been experimentally observed, and one of the few morphogens for which accurate measurements of the gradient are available (Driever and Nusslein-Volhard, 1988a, 1988b). The Bicoid gradient, visualized by antibody staining, can be fit with high accuracy to the solution of the model with localized production, diffusion, and degradation (see Figure 2A). One can easily show that the shape of the steady-state gradient depends on a single dimensionless number: $\varphi = L/\sqrt{D/k}$ (Equations (2) and (3) in Figure 2C). This parameter, known as the Thiele modulus in the engineering literature, can be viewed as the ratio of the size of the system (*L*) and the spatial scale for the signal decay ($\lambda = \sqrt{D/k}$) (Weisz, 1973).

The L/λ ratio is of key importance for all morphogenetic patterning mechanisms. To constrain its value, we can use a simple scaling argument. When $L/\lambda \ll 1$, the morphogen will not vary appreciably across the system, and the whole field will be exposed to essentially the same value of the inductive signal. On the other hand, when $L/\lambda \gg 1$, the morphogen should decay very rapidly, and most of the field will again be exposed to the same (but now low) value of the patterning signal. From these arguments, it is clear that L/λ cannot be very different from unity for efficient patterning (Figure 2D). At this time, quantitative measurements of L/λ are available only for the Bicoid morphogen in the *Drosophila* embryo (Houchmandzadeh et al., 2002; Gregor et al., 2005), where it was established that $L/\lambda \approx 5$ (see Figures 2B and 2D). Recent measurements show that L/λ is conserved for Bicoid gradients across species (see Figure 2B), suggesting the importance of the quantitative control of the Thiele modulus for embryonic pattern formation (Gregor et al., 2005). In the future, it will be important to compare the magnitudes of this dimensionless group for other morphogenetic patterning systems. In the meantime, note that the use of dimensional analysis and a simple scaling argument not only reduced the number of free parameters by combining several biophysical parameters into a single dimensionless group, but also identified constraints on this group that would otherwise have been difficult to impose on the original parameters in isolation.

For morphogen gradients that are either difficult or impossible to visualize directly at this time, nondimensionalization of the mechanistic model can reduce the problem of characterizing the entire concentration field to the

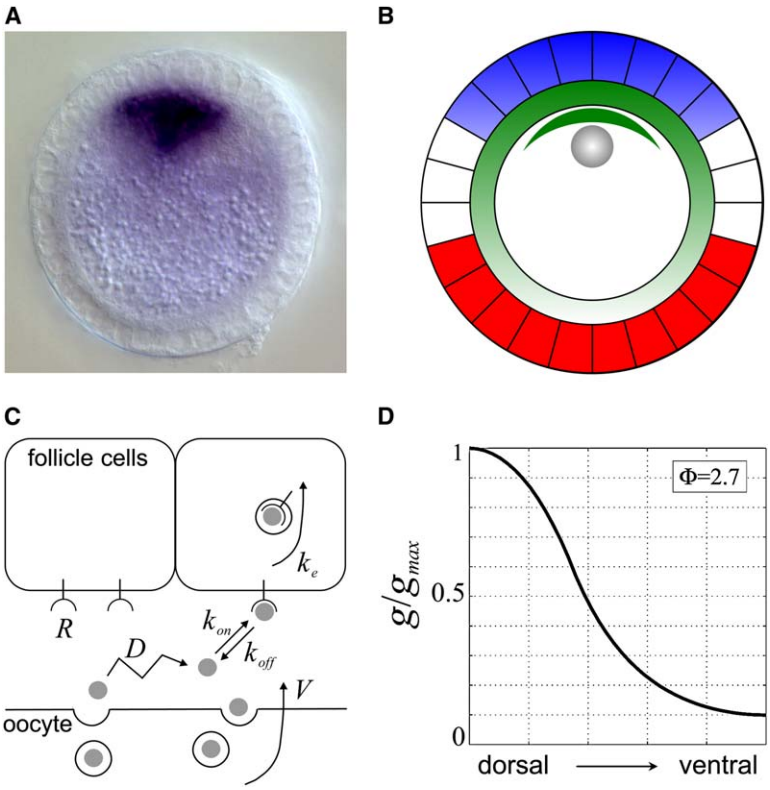

Figure 3. Model-Based Analysis of the Gurken Morphogen Gradient in *Drosophila* Oogenesis

(A) In situ hybridization of a stage 10 egg chamber against *gurken* mRNA. The transcript appears localized to the dorso-anterior cortex of the oocyte.

(B) Schematic of downstream targets of Gurken. Based on the results of genetic experiments, it was proposed that dorsally secreted Gurken acts as a long-ranged morphogen to pattern the follicular epithelium. Blue, *kekkon*; red, *pipe*.

(C) Illustration of biophysical model of the Gurken morphogen. This model accounts for its localized secretion from the oocyte (at rate *V*) and interactions with Egf receptors in the follicle cells (k_{on}, k_{off}, and k_e). Dimensional analysis of the model revealed that the shape of the Gurken gradient is controlled by a single dimensionless parameter, the Thiele modulus (Φ), which compares the relative strengths of Gurken diffusion and receptor-mediated degradation.

(D) Plot of anterior Gurken concentration along the dorsal-ventral coordinate of the egg chamber. A parameter estimation strategy provided a quantitative estimate for the Thiele modulus, enabling the inference of the wild-type Gurken gradient.

Illustrations in (B–D) reprinted from Goentoro et al. (2006), copyright 2006, with permission from Elsevier.

problem of estimating just a few parameters. Such an approach has been recently implemented for the Gurken morphogen gradient in *Drosophila* oogenesis (Figure 3). Gurken, a TGFα-like ligand of the fruit fly EGF receptor, is secreted from the dorsal anterior cortex of the oocyte and acts a morphogen that controls a large number of genes in the overlying follicular epithelium (Nilson and Schupbach, 1999). This morphogen model was put forward on the basis of genetic experiments, but all attempts to visualize the Gurken gradient directly have been so far unsuccessful (Pai et al., 2000; James et al., 2002; Peri et al., 2002). The tissue architecture and the tissue-level distribution of the key signaling components of the EGFR system in the ovary are relatively well understood. Based on this information, Goentoro et al. formulated a mechanistic model of the Gurken morphogen (Goentoro et al., 2006). According to their model, the profile of the Gurken gradient depends on the anatomical parameters of the egg, the cellular levels of expression of multiple components, and the strengths as well as the rates of their interaction (Figure 3C).

Out of all model parameters, only the geometry of the developing egg chamber can be characterized in a relatively straightforward way. However, analysis of the model shows that in order to characterize the shape of the Gurken gradient it is not important to know the values of all of these parameters independently. What matters is their dimensionless combination, which compares the relative strengths of ligand diffusion and degradation, again the Thiele modulus of the problem (Goentoro et al., 2006). Thus, within the framework of this model, the problem of quantifying the shape of the Gurken gradient was reduced to the problem of estimating the Thiele modulus. To extract this parameter, the authors have formulated a parameter estimation method that relies on quantitative measurements of the domain of the expression of *pipe*, one of the targets of Gurken/EGFR signaling in the follicle cells (Pai et al., 2000). By implementing this parameter approach, the authors found that, for the Gurken gradient, $L/\lambda \approx 3$ (Figure 3D). This work provides a direct quantitative characterization of the key patterning input in *Drosophila* oogenesis and demonstrates how parameter estimation techniques can be used to quantify morphogen gradients.

In more complex examples, analysis of the scaled equations can identify limiting cases in which key dimensionless groups are either very small or very large. This simplifies the equations, as terms containing the small dimensionless groups, or the reciprocals of large dimensionless groups, can be neglected. Often, the investigation of such asymptotic (i.e., limiting) regimes opens the model to a more direct analysis (Muratov and Shvartsman, 2003; Berezhkovskii et al., 2004; Reeves et al., 2005). For example, a nondimensionalized model of the Dpp gradient in wing imaginal disc patterning contains two parameters: the Thiele modulus and a dimensionless group that quantifies the extent of Dpp receptor saturation (Lander et al., 2002). In the ligand limited regime (where the second dimensionless group is close to zero), the problem becomes linear and can be solved analytically (Merkin and Sleeman, 2005). Solutions derived in various limiting cases are often valid in a reasonably wide domain of parameters and can thus provide an accurate approximation of the full problem.

Using Models

Modeling can be used to test the feasibility and sufficiency of patterning mechanisms (e.g., Lander et al.,

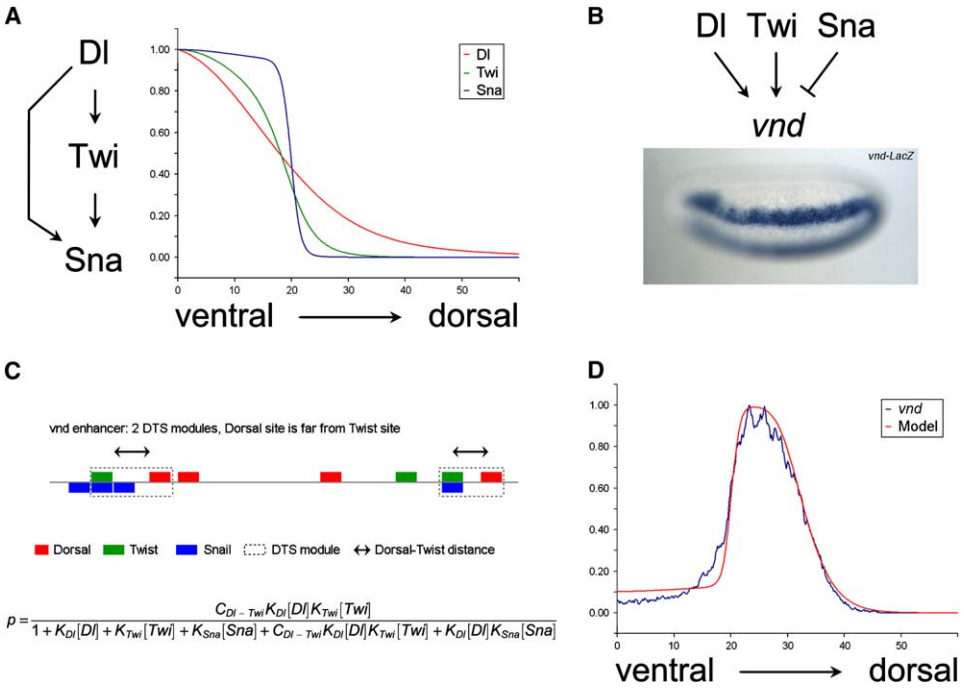

Figure 4. Modeling of Dorsoventral Pattern Formation in the Neurogenic Ectoderm

(A) Regulatory interactions between Dorsal (Dl), Snail (Sna), and Twist (Twi) expression in the neurogenic ectoderm (left) and quantified spatial distributions of Dl, Sna, and Twi during cycle 14 in the *Drosophila* embryo (right).

(B) Experimental dissection of the *vnd* enhancer established that *vnd* expression is controlled by Dl, Twi, and Sna.

(C) Dorsal, Twist, and Snail binding sites in the *vnd* enhancer (top). Equilibrium occupancy model links the probability (*p*) of achieving a successful transcriptional state as a function of local concentrations of Dl, Twi, and Sna (bottom).

(D) Model fit of *vnd* expression.

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2002; Esser et al., 2006). In a direct example of this approach, Zinzen et al. have recently shown that equilibrium models of transcription factor binding site occupancy are sufficient to quantitatively describe neurogenic pattern formation in the *Drosophila* blastoderm (Bintu et al., 2005a, 2005b; Siggia, 2005; Zinzen et al., 2006). By fitting the spatial expression patterns of three genes with previously dissected enhancers (*vnd*, *rho*, and *vn*) to the concentration profiles of three transcription factors (Dorsal, Snail, and Twist), this work demonstrated that simple thermodynamic models provide an adequate quantitative framework for one of the best studied pattern formation systems (Stathopoulos et al., 2002; Markstein et al., 2004). The authors have also shown that their approach has predictive power: computational predictions of the effects of mutagenesis of binding sites in the *vnd* enhancer were in quantitative agreement with the expression patterns obtained with genetically engineered *vnd*-LacZ reporter constructs (Figures 4B and 4D). In the near future, it should be possible to explore both the capabilities and the limitations of this approach in other patterning contexts. For instance, similar correlation between transcription factor binding affinity and expression was demonstrated for the dorsoventral embryonic patterning by Dpp, a system where both the quantitative measurements of the patterning input and the sequence-specific information about the regulatory sequences are available (Raftery and Sutherland, 2003; Wharton et al., 2004).

Computationally, the work of Zinzen et al. involves fitting of a single algebraic equation with a handful of parameters. With the rapid increase in the computational power, it should be possible to handle more complex mathematical structures, such as systems of nonlinear partial differential equations. This has been demonstrated by the recent computational analysis of planar cell polarity (PCP) (Amonlirdviman et al., 2005; Le Garrec et al., 2006). PCP is a phenomenon of long-range cell polarization within the plane of the epithelium (Klein and Mlodzik, 2005; Strutt and Strutt, 2005). In the fruit fly wing, PCP manifests itself in the long-range alignment of actin-rich hair spikes which are secreted from the distal side of the cells and point toward the distal side of the wing (see Figure 5B). The high fidelity of polarization of hundreds of cells is controlled by a mechanism that depends on both long-range positional information and short-range intercellular signaling (Klein and Mlodzik, 2005; Strutt and Strutt, 2005). In the emerging model of PCP patterning in the wing, the long-range signaling, which depends on genes *four-jointed* (*fj*), *dachsous* (*ds*), and *fat* (*ft*), provides an initial bias for proximodistal cell polarization. This initial polarization, which is believed to be transient, is amplified and maintained by the intercellular feedback circuit formed by *flamingo* (*fmi*), *frizzled* (*fz*), *disheveled* (*dsh*), *van gogh* (*vang*), *diego* (*dgo*), and *prickle-spiny-legs* (*pk*) (Figure 5A). The cellular basis of coupling between the biasing and feedback circuits is unclear, but it is established that both systems are essential for robust polarization of

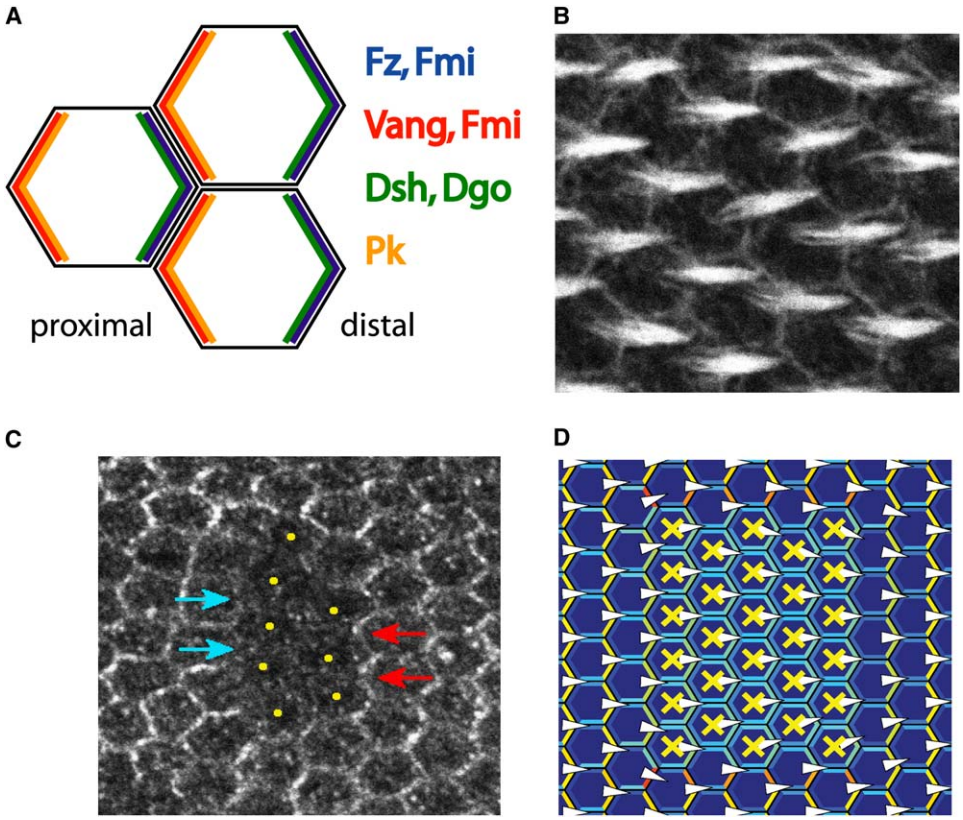

Figure 5. Quantitative Analysis of the Planar Cell Polarity System in the *Drosophila* Wing

(A and B) Pattern formation and cellular response. Asymmetric subcellular localization of proteins in the core PCP network (A) precedes the formation of actin rich prehair structures on the distal side of the cells (B). Illustration in (A) reprinted from Strutt and Strutt (2005) with permission from Wiley.

(C and D) Models of the PCP system are constrained by observations in clonal analysis experiments. (C) Experiments with pk^{-} cells (marked by yellow dots) show that the wild-type cells accumulate Pk on their proximal sides. Red arrows show enriched proximal signal, and blue arrows show lack of distal signal in the affected cells. pk^{-} cells (marked by yellow dots) do not affect Pk protein distribution in the neighboring cells. (D) Computational prediction of the effect of pk^{-} cells. The model-derived intracellular distribution of Dsh is used to determine the direction of the prehair. The illustration in (D) is from Amonlirdviman et al. (2005). Reprinted with permission from AAAS.

individual cells and of the entire epithelium (Ma et al., 2003).

Proposed on the basis of genetic interaction and protein colocalization studies, mathematical models of the intercellular feedback focus on the formation and transport of multiprotein complexes in the *fz/dsh/pk/dgo/vang* system (Amonlirdviman et al., 2005; Le Garrec et al., 2006). The genes in this network work together as a module: in the wild-type wing, the polarization of individual components is highly coordinated, e.g., Fz is localized to the distal boundary and Pk is localized to the proximal cell boundary. In addition, removal of any one component completely abolishes asymmetrical distribution of other components. Rigorous biochemical analysis of most of the interactions is yet to be performed, but this does not prevent the formulation of systems-level questions about the operation of the intercellular feedback loop (Strutt, 2005). For example, what is the time scale on which the feedback operates? What is the minimal amount of initial asymmetry that leads to robust intracellular polarization? What is the size of spatial imperfections in the input system that can still be corrected? Questions like these would be hard enough to answer even we had a complete parts list of molecules in the PCP patterning system. Nevertheless, under a well-defined set of assumptions, the feasibility of proposed pattern formation mechanisms can be tested computationally, paving the way for their systems-level analysis.

Leaving the analysis of the initial biasing system for future studies, mathematical models of PCP patterning focus on the feedback system and the experiments with mosaic wing discs (Strutt, 2005). In both studies, the feasibility of the mechanism was identified with the existence of a set of parameters, such as intracellular concentrations and rate constants, which lead to solutions that resemble wild-type asymmetric patterns in localization of Dsh and other components of the feedback circuit. Thus, analysis of mechanism feasibility is reduced to the problem of identifying parameters that lead to solutions consistent with the maximal set of experimental observations. Amonlirdviman et al. present a systematic computational approach for solving this problem (Amonlirdviman et al., 2005). Specifically, they define an objective function which quantifies the mismatch between model predictions and experimental observations in a number of genetic backgrounds and then minimize this function over the space of ~30 parameters

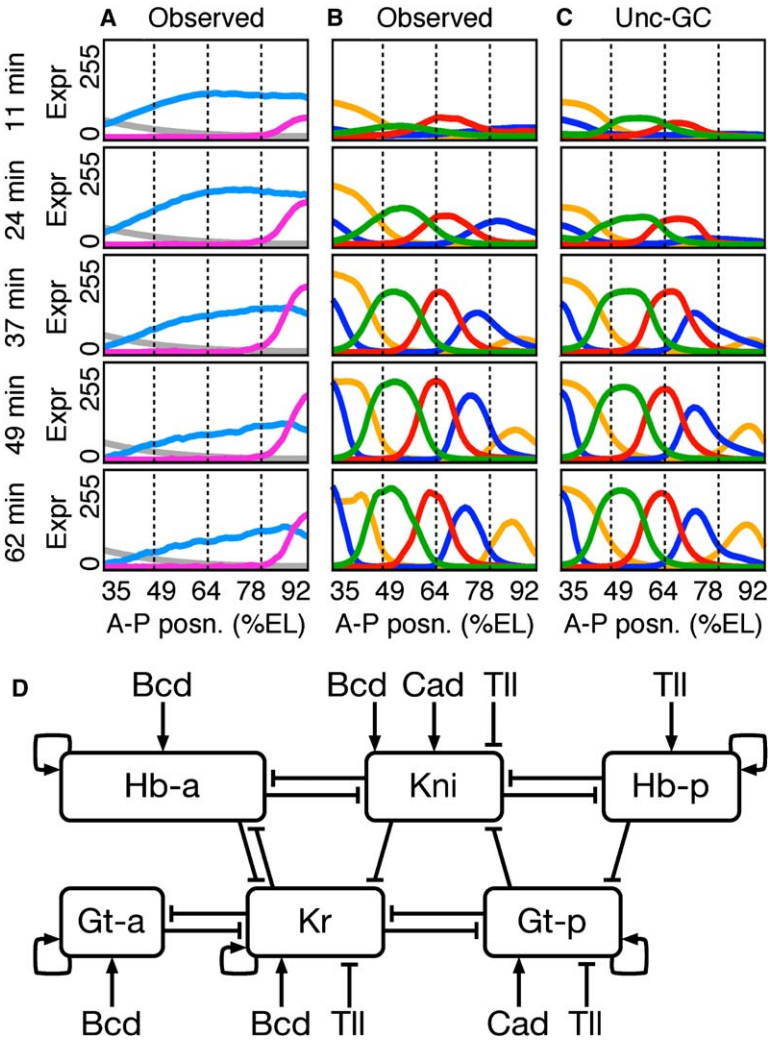

Figure 6. Model-Based Inference of the *Drosophila* Gap Gene Network

(A) Quantified spatiotemporal distributions of Bcd, Cad, and Tll, which serve as inputs for the expression of gap genes.

(B and C) Spatiotemporal patterns of Hb, Kr, Kni, and Gt: (B), observed; (C), predicted by the Gene Circuit reaction diffusion model that is based on the combination of linear diffusion and degradation terms and nonlinear production terms for Hb, Kr, Kni, and Gt.

(D) The updated summary of regulatory interactions is derived from the analysis of the production terms fitted on the basis of the wild-type expression patterns.

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of the model that consists of 10 coupled reaction-diffusion equations.

While locating parameter sets consistent with data completes the feasibility analysis, it is only the first step in the systems-level analysis of the patterning mechanism. Using a feasible set of model parameters as a base value, it is possible to examine the robustness of the underlying mechanism. In this way, Amonlirdviman et al. used their model to examine how their form of intercellular feedback copes with spatial imperfections in the biasing system, consistent with the elegant experiments by Ma et al. (Ma et al., 2003; Amonlirdviman et al., 2005). Similarly, Le Garrec et al. have shown that their form of intercellular feedback can convert transient biasing signals into persistent cell polarization patterns (Le Garrec et al., 2006). The underlying assumptions of both models are likely to be revised in the future. This is particularly true in light of the recent live imaging studies, which revealed the critical role of microtubules and vesicular transport in the proximodistal distribution of Fz and Fmi, an important effect which is yet to be incorporated into the mathematical models (Shimada et al., 2006).

In addition to testing the consistency of proposed mechanisms, models can provide access to properties that are either difficult or impossible to extract from direct measurements. For example, one approach to extracting the intracellular diffusivities relies on direct microscopic observations of molecular motion (Bacia and Schwille, 2003). Alternatively, one can derive intracellular diffusivities from data by fitting the macroscopic observations of intracellular concentration fields to models which contain diffusivities as their parameters; see (Gregor et al., 2005) for an example. A similar approach has been developed to infer regulatory interactions in the *Drosophila* gap gene network (Jaeger et al., 2004a, 2004b; Perkins et al., 2006).

Induced by the gradients of maternal transcription factors Bicoid (Bcd) and Caudal (Cad) and an early zygotic gene Tailless (*tll*), the four gap genes, *hunchback* (*hb*), *Krüppel* (*Kr*), *knirps* (*kni*), and *giant* (*gt*), engage a complex set of interactions that lead to their final expression patterns (Figure 6). Together with Bcd and Cad, the gap genes drive the expression of pair rule genes. Much of the knowledge about the architecture of the gap gene network was derived from experiments with mutants and the analysis of regulatory DNA of gap genes (Gaul and Jackle, 1990). The resulting picture represents an enormous advance in the understanding of gene regulation in development, but it must be viewed

as a working model for the following reasons. While proving the sufficiency of regulatory interactions, most of the experiments with reporters can not be quantitatively compared with wild-type expression patterns. At the same time, information derived from overexpression studies and mutants can be indirect. Finally, even in the gap gene network, which has "only" three inputs and four outputs, regulatory interactions were inferred on the basis of a relatively small number genetic perturbations (Gaul and Jackle, 1990).

To address these methodological issues, Reinitz and colleagues developed an approach that uses time series measurements of gene expression in the wild-type background and does not rely on genetic perturbations and reporter constructs (Jaeger et al., 2004a). The first step of their approach was to establish a database of spatiotemporal expression patterns of the three inputs to the gap gene network as well as the four gap genes during 1 hr before the onset of cellularization (Figures 6A and 6B). These data were then used to fit a reaction-diffusion model that describes the diffusion, degradation, and production of gap gene products (Figure 6C). Diffusion and degradation were modeled by simple linear terms, but production terms were highly nonlinear, allowing all inputs and every gap gene to potentially influence the production of any other gap gene. As with conventional approaches, the goal of the model-based approach is to derive the structure (i.e., the diagram) of the gap gene network (Figure 6D). At the end of the fitting procedure, the network structure is obtained from the fitted rates of production of gap genes as a function other gap genes and input factors.

The original implementation of this approach was quite expensive, with the results reported in 2004 requiring almost 2 years of computer time (Jaeger et al., 2004a). Recent algorithmic advances reduced run times to just a couple of hours, enabling the comparison of a number of different modeling assumptions (Perkins et al., 2006). The derived network architecture was largely consistent with the one that was established on the basis of experiments with mutants and reporters. However, the model-based approach had also identified a number of new regulatory links and suggested that some of the previous regulatory interactions are not essential. For example, among the additions for *hb* are the requirement of Tailless for posterior *hb* expression and repression of *hb* by Kni (in addition to the established repression by Kr). Ultimately, it should be possible to test the new regulatory interactions proposed by the model. One way of doing so can rely on mutational analysis of enhancers in the AP patterning network, similar to the tests of the binding site occupancy models of neurogeneic patterning (Zinzen et al., 2006). Given the advanced state of experimental and bionformatics studies of the embryonic AP patterning, this goal is within the reach of current experiments (Berman et al., 2002; Rajewsky et al., 2002; Sinha et al., 2004).

Conclusions

Models of embryonic pattern formation can summarize large amounts of experiments, compute properties that are difficult to measure, and evaluate the relative feasibility of competing patterning mechanisms. The development and validation of productive modeling frameworks and computational approaches will definitely take time. However, already now, it is reasonable to demand that quantitative descriptions of pattern formation describe mutant phenotypes and predict the effects of specific genetic and physical manipulations. For model genetic organisms, mechanistic modeling is enabled by a large arsenal of genetic tools that can be used to directly challenge model predictions and assumptions. The need to validate models experimentally emphasizes the need for experimental tools to perturb patterning events at multiple levels and to quantify both the responses and perturbations. For instance, a number of recent experiments motivated by the model-based analysis of robustness relied on targeted gene expression systems and heterozygous mutants (Eldar et al., 2002; Mizutani et al., 2005). However, the magnitude of perturbations, e.g., fold-change in the expression level of a particular molecule, was rarely quantified, making the interpretation of patterning processes in perturbed genetic backgrounds rather nontrivial. Along the same lines, model-based analysis of robustness requires quantitative information about the natural variations in the inputs and outputs in the patterning networks; again, such measurements are extremely rare (Houchmandzadeh et al., 2002; Crauk and Dostatni, 2005).

Most of the models developed to date focus only on a single level of description. For example, different models of the embryonic patterning by Dpp emphasized regulation by extracellular proteases, ligand dimerization, and positive feedback (Eldar et al., 2002; Mizutani et al., 2005; Shimmi et al., 2005; Umulis et al., 2006). In putting all of these pieces together for Dpp in the embryo and other patterning systems, one can use simple input/output maps to describe coupling between different parts of the system (Pribyl et al., 2003; Giurumescu et al., 2006). For example, a sharp boundary in the expression of a transcriptional target of a signaling pathway suggests that there is a clear threshold in its expression as a function of the activity of the upstream signaling event. This can be modeled using a sharp nonlinearity, with the only parameters being the location of the threshold and the maximal level of expression (Reeves et al., 2005; Goentoro et al., 2006). The elucidation of such input/output maps between various parts of signaling systems or between signaling levels and cellular responses, such as proliferation or migration, is one of most important activities for the formulation of realistic models of development (Janes et al., 2005; Melen et al., 2005; Shraiman, 2005).

While modeling helps to explore what *can* happen under a given set of assumptions about the cell biology and biochemistry, ruling something out is much more challenging. For instance, it has been suggested that transport by planar transcytosis is too slow to be consistent with the experimentally observed rates of ligand spreading (Lander et al., 2002). A different study argues that a model with purely diffusive extracellular transport of Dpp is inconsistent with clonal analysis experiments (Kruse et al., 2004). Neither study makes a completely convincing argument, since invalidating a model, i.e., showing that it is inconsistent with a particular set of data, is extremely difficult to achieve computationally at this time, in particular for nonlinear and spatially distributed models. In the future, new algorithms for model

invalidation which are being developed now for small systems of nonlinear differential equations can be used for the analysis of patterning mechanisms (El-Samad et al., 2006; Prajna, 2006).

In addition to advancing our understanding of development, models can be used to design new experiments aimed at testing the regulatory interactions. For example, optimization-based algorithms can suggest realizable genetic perturbations and assays that would be most informative in discriminating between alternative network diagrams. Similar approaches have been successful in the identification and discrimination of chemical reaction mechanisms (Marquardt, 2005; Ross et al., 2006). It will be interesting to try them in developmental contexts where multiple competing mechanisms have been proposed, e.g., for the gap gene network. In the future, models can guide the design of new patterns and morphologies. Recently, the first successes of synthetic pattern designs have been reported for in vitro and bacterial systems, and we expect that in the future it should be possible to extend this paradigm to multicellular organisms as well (Basu et al., 2005; Isalan et al., 2005).

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References

Amonlirdviman, K., Khare, N.A., Tree, D.R.P., Chen, W.S., Axelrod, J.D., and Tomlin, C.J. (2005). Mathematical modeling of planar cell polarity to understand domineering nonautonomy. Science *307*, 423–426.

Asai, R., Taguchi, E., Kume, Y., Saito, M., and Kondo, S. (1999). Zebrafish leopard gene as a component of the putative reaction-diffusion system. Mech. Dev. *89*, 87–92.

Ashe, H.L., and Briscoe, J. (2006). The interpretation of morphogen gradients. Development *133*, 385–394.

Bacia, K., and Schwille, P. (2003). A dynamic view of cellular processes by in vivo fluorescence auto- and cross-correlation spectroscopy. Methods *29*, 74–85.

Basu, S., Gerchman, Y., Collins, C.H., Arnold, F.H., and Weiss, R. (2005). A synthetic multicellular system for programmed pattern formation. Nature *434*, 1130–1134.

Belenkaya, T.Y., Han, C., Yan, D., Opoka, R.J., Khodoun, M., Liu, H., and Lin, X. (2004). *Drosophila* Dpp morphogen movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. Cell *119*, 231–244.

Berezhkovskii, A.M., Batsilas, L., and Shvartsman, S.Y. (2004). Ligand trapping in epithelial layers and cell cultures. Biophys. Chem. *107*, 221–227.

Berman, B.P., Nibu, Y., Pfeiffer, B.D., Tomancak, P., Celniker, S.E., Levine, M., Rubin, G.M., and Eisen, M.B. (2002). Exploiting transcription factor binding site clustering to identify cis-regulatory modules involved in pattern formation in the Drosophila genome. Proc. Natl. Acad. Sci. USA *99*, 757–762.

Bintu, L., Buchler, N.E., Garcia, H.G., Gerland, U., Hwa, T., Kondev, J., Kuhlman, T., and Phillips, R. (2005a). Transcriptional regulation by the numbers: applications. Curr. Opin. Genet. Dev. *15*, 125–135.

Bintu, L., Buchler, N.E., Garcia, H.G., Gerland, U., Hwa, T., Kondev, J., and Phillips, R. (2005b). Transcriptional regulation by the numbers: models. Curr. Opin. Genet. Dev. *15*, 116–124.

Crauk, O., and Dostatni, N. (2005). Bicoid determines sharp and precise target gene expression in the *Drosophila* embryo. Curr. Biol. *15*, 1888–1898.

Dorfman, R., and Shilo, B.Z. (2001). Biphasic activation of the BMP pathway patterns the *Drosophila* embryonic dorsal region. Development *128*, 965–972.

Driever, W., and Nusslein-Volhard, C. (1988a). The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell *54*, 95–104.

Driever, W., and Nusslein-Volhard, C. (1988b). A gradient of bicoid protein in *Drosophila* embryos. Cell *54*, 83–93.

Dudu, V., Bittig, T., Entchev, E., Kicheva, A., Julicher, F., and Gonzalez-Gaitan, M. (2006). Postsynaptic mad signaling at the *Drosophila* neuromuscular junction. Curr. Biol. *16*, 625–635.

El-Samad, H., Prajna, S., Papachristodoulou, A., Doyle, J., and Khammash, M. (2006). Advanced methods and algorithms for biological networks analysis. Proc. IEEE *94*, 832–853.

Eldar, A., Dorfman, R., Weiss, D., Ashe, H., Shilo, B.Z., and Barkai, N. (2002). Robustness of the BMP morphogen gradient in Drosophila embryonic patterning. Nature *419*, 304–308.

Eldar, A., Shilo, B.Z., and Barkai, N. (2004). Elucidating mechanisms underlying robustness of morphogen gradients. Curr. Opin. Genet. Dev. *14*, 435–439.

Entchev, E.V., Schwabedissen, A., and Gonzalez-Gaitan, M. (2000). Gradient formation of the TGF-beta homolog Dpp. Cell *103*, 981–991.

Esser, A.T., Smith, K.C., Weaver, J.C., and Levin, M. (2006). Mathematical model of morphogen electrophoresis through gap junctions. Dev. Dyn. *235*, 2144–2159.

Flann, N., Hu, J., Bansal, M., Patel, V., and Podgorski, G. (2005). Biological development of cell patterns: characterizing the space of cell chemistry genetic regulatory networks. Eighth European Conference on Artificial Life, Canterbury, Kent, United Kingdom, September 2005.

Freeman, M., and Gurdon, J.B. (2002). Regulatory principles of developmental signaling. Annu. Rev. Cell Dev. Biol. *18*, 515–539.

Gao, S., Steffen, J., and Laughon, A. (2005). DPP-responsive silencers are bound by a trimeric Mad-Medea complex. J. Biol. Chem. *280*, 36158–36164.

Gaul, U., and Jackle, H. (1990). Role of gap genes in early Drosophila development. Adv. Genet. *27*, 239–275.

Ghanem, R., and Wojtkiewicz, S. (2004). Special issue on uncertainty quantification. SIAM J. Sci. Comput. *26*, vii.

Gibson, M.C., and Schubiger, G. (2001). Drosophila peripodial cells, more than meets the eye? Bioessays *23*, 691–697.

Gibson, M.C., Lehman, D.A., and Schubiger, G. (2002). Lumenal transmission of decapentaplegic in *Drosophila* imaginal discs. Dev. Cell *3*, 451–460.

Gierer, A., and Meinhardt, H. (1972). A theory for biological pattern formation. Kybernetic *12*, 30–39.

Giurumescu, C.A., Sternberg, P.W., and Asthagiri, A.R. (2006). Intercellular coupling amplifies fate segregation during Caenorhabditis elegans vulval development. Proc. Natl. Acad. Sci. USA *103*, 1331–1336.

Goentoro, L.A., Kowal, C.P., Reeves, G.T., Martinelli, L., Schupbach, T., and Shvartsman, S.Y. (2006). Quantifying the Gurken morphogen gradient in *Drosophila* oogenesis. Dev. Cell. *11*, 263–272.

Gregor, T., Bialek, W., de Ruyter van Steveninck, R.R., Tank, D.W., and Wieschaus, E.F. (2005). Diffusion and scaling during early embryonic pattern formation. Proc. Natl. Acad. Sci. USA *102*, 18403–18407.

Gurdon, J.B., and Bourillot, P.Y. (2001). Morphogen gradient interpretation. Nature *413*, 797–803.

Houchmandzadeh, B., Wieschaus, E., and Leibler, S. (2002). Establishment of developmental precision and proportions in the early Drosophila embryo. Nature *415*, 798–802.

Hsu, C.P., Lee, P.H., Chang, C.W., and Lee, C.T. (2006). Constructing quantitative models from qualitative mutant phenotypes: preferences in selecting sensory organ precursors. Bioinformatics *22*, 1375–1382.

Isalan, M., Lemerle, C., and Serrano, L. (2005). Engineering gene networks to emulate Drosophila embryonic pattern formation. PLoS Biol. *3*, 488–496.

Jaeger, J., Blagov, M., Kosman, D., Kozlov, K.N., Manu, Myasnikova, E., Surkova, S., Vanario-Alonso, C.E., Samsonova, M., Sharp, D.H., and Reinitz, J. (2004a). Dynamical analysis of regulatory interactions in the gap gene system of Drosophila melanogaster. Genetics *167*, 1721–1737.

Jaeger, J., Surkova, S., Blagov, M., Janssens, H., Kosman, D., Kozlov, K.N., Manu, Myasnikova, E., Vanario-Alonso, C.E., Samsonova, M., et al. (2004b). Dynamic control of positional information in the early Drosophila embryo. Nature *430*, 368–371.

James, K.E., Dorman, J.B., and Berg, C.A. (2002). Mosaic analyses reveal the function of Drosophila Ras in embryonic dorsoventral patterning and dorsal follicle cell morphogenesis. Development *129*, 2209–2222.

Janes, K.A., Albeck, J.G., Gaudet, S., Sorger, P.K., Lauffenburger, D.A., and Yaffe, M.B. (2005). A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. Science *310*, 1646–1653.

Johnson, S.L., Africa, D., Walker, C., and Weston, J.A. (1995). Genetic control of adult pigment stripe development in zebrafish. Dev. Biol. *167*, 27–33.

Kirschner, M., and Gerhart, J. (1998). Evolvability. Proc. Natl. Acad. Sci. USA *95*, 8420–8427.

Klein, T.J., and Mlodzik, M. (2005). Planar cell polarization: an emerging model points in the right direction. Annu. Rev. Cell Dev. Biol. *21*, 155–176.

Klein, D., Nappi, V.M., Reeves, G.T., Shvartsman, S.Y., and Lemmon, M.A. (2004). Argos inhibits epidermal growth factor receptor signalling by ligand sequestration. Nature *430*, 1040–1044.

Kruse, K., Pantazis, P., Bollenbach, T., Julicher, F., and Gonzalez-Gaitan, M. (2004). Dpp gradient formation by dynamin-dependent endocytosis: receptor trafficking and the diffusion model. Development *131*, 4843–4856.

Lai, K., Robertson, M.J., and Schaffer, D.V. (2004). The sonic hedgehog signaling system as a bistable genetic switch. Biophys. J. *86*, 2748–2757.

Lander, A.D., Nie, W., and Wan, F.Y. (2002). Do morphogen gradients arise by diffusion? Dev. Cell *2*, 785–796.

Le Garrec, J.F., Lopez, P., and Kerszberg, M. (2006). Establishment and maintenance of planar epithelial cell polarity by asymmetric cadherin bridges: a computer model. Dev. Dyn. *235*, 235–246.

Lecuit, T., and Cohen, S.M. (1998). Dpp receptor levels contribute to shaping the Dpp morphogen gradient in the Drosophila wing imaginal disc. Development *125*, 4901–4907.

Lecuit, T., Brook, W.J., Ng, M., Calleja, M., Sun, H., and Cohen, S.M. (1996). Two distinct mechanisms for long-range patterning by Decapentaplegic in the Drosophila wing. Nature *381*, 387–393.

Longabaugh, W.J., Davidson, E.H., and Bolouri, H. (2005). Computational representation of developmental genetic regulatory networks. Dev. Biol. *283*, 1–16.

Ma, D., Yang, C.H., McNeill, H., Simon, M.A., and Axelrod, J.D. (2003). Fidelity in planar cell polarity signaling. Nature *421*, 543–546.

Maderspacher, F., and Nusslein-Volhard, C. (2003). Formation of the adult pigment pattern in zebrafish requires leopard and obelix dependent cell interactions. Development *130*, 3447–3457.

Markstein, M., Zinzen, R., Markstein, P., Yee, K.P., Erives, A., Stathopoulos, A., and Levine, M. (2004). A regulatory code for neurogenic gene expression in the Drosophila embryo. Development *131*, 2387–2394.

Marois, E., Mahmoud, A., and Eaton, S. (2006). The endocytic pathway and formation of the Wingless morphogen gradient. Development *133*, 307–317.

Marquardt, W. (2005). Model-based experimental analysis of kinetic phenomena in multi-phase reactive systems. Chem. Eng. Res. Des. *83*, 561–573.

Meinhardt, H. (1982). Models of Biological Pattern Formation (London: New York Academic Press).

Melen, G.J., Levy, S., Barkai, N., and Shilo, B.Z. (2005). Threshold responses to morphogen gradients by zero-order ultrasensitivity. Mol Syst Biol *1*, E1–E11. 10.1038/msb4100036.

Merkin, J.H., and Sleeman, B.D. (2005). On the spread of morphogens. J. Math. Biol. *51*, 1–17.

Mizutani, C.M., Nie, Q., Wan, F.Y., Zhang, Y.T., Vilmos, P., Sousa-Neves, R., Bier, E., Marsh, J.L., and Lander, A.D. (2005). Formation of the BMP activity gradient in the *Drosophila* embryo. Dev. Cell *8*, 915–924.

Moser, M., and Campbell, G. (2005). Generating and interpreting the Brinker gradient in the Drosophila wing. Dev. Biol. *286*, 647–658.

Muratov, C.B., and Shvartsman, S.Y. (2003). An asymptotic study of the inductive pattern formation mechanism in Drosophila egg development. Physica D. *186*, 93–108.

Murray, J.D. (1993). Mathematical Biology, Volume 19 (Berlin, New York: Springer-Verlag).

Nellen, D., Burke, R., Struhl, G., and Basler, K. (1996). Direct and long-range action of DPP morphogen gradient. Cell *85*, 357–368.

Nilson, L.A., and Schupbach, T. (1999). EGF receptor signaling in Drosophila oogenesis. Curr. Top. Dev. Biol. *44*, 203–243.

Pai, L., Barcelo, G., and Schupbach, T. (2000). D-cbl, negative regulator of the Egfr pathway, is required for dorsoventral patterning in *Drosophila* oogenesis. Cell *103*, 51–61.

Pallavi, S.K., and Shashidhara, L.S. (2005). Signaling interactions between squamous and columnar epithelia of the Drosophila wing disc. J. Cell Sci. *118*, 3363–3370.

Parker, L., Stathakis, D.G., and Arora, K. (2004). Regulation of BMP and activin signaling in Drosophila. Prog. Mol. Subcell. Biol. *34*, 73–101.

Peri, F., Technau, M., and Roth, S. (2002). Mechanisms of Gurken-dependent pipe regulation and the robustness of dorsoventral patterning in Drosophila. Development *129*, 2965–2975.

Perkins, T.J., Jaeger, J., Reinitz, J., and Glass, L. (2006). Reverse engineering the gap gene network of Drosophila melanogaster. PLoS Comput Biol *2*, e51.

Pismen, L.M. (2006). Patterns and Interfaces in Dissipative Dynamics (Berlin: Springer).

Prajna, S. (2006). Barrier certificates for nonlinear model validation. Automatica *42*, 117–126.

Pribyl, M., Muratov, C.B., and Shvartsman, S.Y. (2003). Discrete models of autocrine signaling in epithelial layers. Biophys. J. *84*, 3624–3635.

Pyrowolakis, G., Hartmann, B., Muller, B., Basler, K., and Affolter, M. (2004). A simple molecular complex mediates widespread BMP-induced repression during *Drosophila* development. Dev. Cell *7*, 229–240.

Quigley, I.K., Manuel, J.L., Roberts, R.A., Nuckels, R.J., Herrington, E.R., MacDonald, E.L., and Parichy, D.M. (2005). Evolutionary diversification of pigment pattern in Danio fishes: differential fms dependence and stripe loss in D. albolineatus. Development *132*, 89–104.

Raftery, L.A., and Sutherland, D.J. (2003). Gradients and thresholds: BMP response gradients unveiled in Drosophila embryos. Trends Genet. *19*, 701–708.

Rajewsky, N., Vergassola, M., Gaul, U., and Siggia, E.D. (2002). Computational detection of genomic cis-regulatory modules applied to body patterning in the early Drosophila embryo. BMC Bioinformatics *3*, 30.

Reeves, G.T., Kalifa, R., Klein, D., Lemmon, M.A., and Shvartsman, S.Y. (2005). Computational analysis of EGFR inhibition by Argos. Dev. Biol. *284*, 523–535.

Rentzsch, F., Zhang, J., Kramer, C., Sebald, W., and Hammerschmidt, M. (2006). Crossveinless 2 is an essential positive feedback regulator of Bmp signaling during zebrafish gastrulation. Development *133*, 801–811.

Ross, J., Schreiber, I., and Vlad, M.O. (2006). Determination of complex reaction mechanisms: analysis of chemical, biological, and genetic networks (New York: Oxford University Press).

Saha, K., and Schaffer, D.V. (2006). Signal dynamics in Sonic hedgehog tissue patterning. Development *133*, 889–900.

Saller, E., and Bienz, M. (2001). Direct competition between Brinker and Drosophila Mad in Dpp target gene transcription. EMBO Rep. *2*, 298–305.

Saltelli, A., Ratto, M., Tarantola, S., and Campolongo, F. (2005). Sensitivity analysis for chemical models. Chem. Rev. *105*, 2811–2828.

Schmierer, B., and Hill, C.S. (2005). Kinetic analysis of Smad nucleocytoplasmic shuttling reveals a mechanism for transforming growth factor beta-dependent nuclear accumulation of Smads. Mol. Cell. Biol. *25*, 9845–9858.

Shimada, Y., Yonemura, S., Ohkura, H., Strutt, D., and Uemura, T. (2006). Polarized transport of Frizzled along the planar microtubule arrays in *Drosophila* wing epithelium. Dev. Cell *10*, 209–222.

Shimmi, O., Umulis, D., Othmer, H., and O'Connor, M.B. (2005). Facilitated transport of a Dpp/Scw heterodimer by Sog/Tsg leads to robust patterning of the *Drosophila* blastoderm embryo. Cell *120*, 873–886.

Shraiman, B.I. (2005). Mechanical feedback as a possible regulator of tissue growth. Proc. Natl. Acad. Sci. USA *102*, 3318–3323.

Siggia, E.D. (2005). Computational methods for transcriptional regulation. Curr. Opin. Genet. Dev. *15*, 214–221.

Sinha, S., Schroeder, M.D., Unnerstall, U., Gaul, U., and Siggia, E.D. (2004). Cross-species comparison significantly improves genome-wide prediction of cis-regulatory modules in Drosophila. BMC Bioinformatics *5*, 129.

Stathopoulos, A., and Levine, M. (2005). Genomic regulatory networks and animal development. Dev. Cell *9*, 449–462.

Stathopoulos, A., Van Drenth, M., Erives, A., Markstein, M., and Levine, M. (2002). Whole-genome analysis of dorsal-ventral patterning in the *Drosophila* embryo. Cell *111*, 687–701.

Strutt, D. (2005). Mathematical modeling of planar polarity. Dev. Cell *8*, 134–136.

Strutt, H., and Strutt, D. (2005). Long-range coordination of planar polarity in Drosophila. Bioessays *27*, 1218–1227.

Tabata, T., and Takei, Y. (2004). Morphogens, their identification and regulation. Development *131*, 703–712.

Teleman, A.A., and Cohen, S.M. (2000). Dpp gradient formation in the *Drosophila* wing imaginal disc. Cell *103*, 971–980.

Turing, A.M. (1952). The chemical basis of morphogenesis. Phil. Trans. Roy. Soc. Lond. *B237*, 37–72.

Umulis, D.M., Serpe, M., O'Connor, M.B., and Othmer, H.G. (2006). Robust, bistable patterning of the dorsal surface of the Drosophila embryo. Proc. Natl. Acad. Sci. USA *103*, 11613–11618.

Vilar, J.M., Jansen, R., and Sander, C. (2006). Signal processing in the TGF-beta superfamily ligand-receptor network. PLoS Comput Biol *2*, e3.

Vincent, J.P., and Dubois, L. (2002). Morphogen transport along epithelia, an integrated trafficking problem. Dev. Cell *3*, 615–623.

von Dassow, G., Meir, E., Munro, E.M., and Odell, G.M. (2000). The segment polarity network is a robust developmental module. Nature *406*, 188–192.

Wang, Y.C., and Ferguson, E.L. (2005). Spatial bistability of Dpp-receptor interactions during Drosophila dorsal-ventral patterning. Nature *434*, 229–234.

Watanabe, M., Iwashita, M., Ishii, M., Kurachi, Y., Kawakami, A., Kondo, S., and Okada, N. (2006). Spot pattern of leopard Danio is caused by mutation in the zebrafish connexin41.8 gene. EMBO Rep., in press. Published online July 14, 2006. 10.1038/sj.embor.7400757.

Weisz, P.B. (1973). Diffusion and chemical transformation. Science *179*, 433–440.

Wharton, S.J., Basu, S.P., and Ashe, H.L. (2004). Smad affinity can direct distinct readouts of the embryonic extracellular Dpp gradient in *Drosophila*. Curr. Biol. *14*, 1550–1558.

Wolpert, L. (1994). Do we understand development? Science *266*, 571–572.

Wolpert, L. (1996). One hundred years of positional information. Trends Genet. *12*, 359–364.

Zinzen, R.P., Senger, K., Levine, M., and Papatsenko, D. (2006). Computational models for neurogenic gene expression in the *Drosophila* embryo. Curr. Biol. *16*, 1358–1365.