Chapter 5

Discussion

In a developing organism, cells require information about their relative position in order to function and differentiate appropriately. Despite the discovery that key signaling pathways act as organizers of pattern formation in several systems, the details of how this positional information is distributed, processed, and interpreted by cells in a developing field remain little understood. In this thesis, we use a combination of theoretical tools and experimental work in *Drosophila* to investigate the origin and interpretation of positional information, the role of temporal changes in signaling activity on patterning, and the relationship between the location of patterns and the size of the system. Our contributions to these fundamental questions can be briefly summarized as follows (see details below):

- In Chapter 2, we challenge the prevailing idea that the Hh morphogen establishes positional information in a dose-dependent manner and proposed a model in which gradient dynamics, resulting from the Hh gene network architecture, determines pattern formation in the *Drosophila* wing disc.

- Chapter 3 introduces a general theoretical framework to design genetic experiments that isolate the effects of transient vs. steady-state signals on developmental patterning. This formalism is not limited to the study of signal dynamics and may be generally applicable to other problems.

- In Chapter 4, we investigate spatial scaling of gene expression patterns due to natural variations in the length of the DV axis in the *Drosophila* embryo. In contrast to

recent studies on scaling along the AP axis (de Lachapelle and Bergmann, 2010), we show that scaling in this system is a gene-dependent, rather than a position-dependent property. We propose that gene-specific scaling mechanisms depend on factors downstream of the Toll signaling pathway.

Mathematical Modeling as a Hypotheses-Generating Tool[*]

Mathematical modeling and theoretical biology have led efforts to investigate the question of how patterns emerge during development (Turing, 1952; Wolpert, 1968), and today, there is no doubt that the interplay between theory and experiment has significantly advanced our current understanding of developmental processes (Green, 2002; Ibañes and Izpisúa-Belmonte, 2006). A common approach has been to use available experimental data to devise mathematical models that can be employed as predictors of experimental results (Fig. 5.1A). Typically, these models are then used to explore properties of the system that are not easily exploited by experimentation. Alternatively, mathematical models can be formulated to discriminate between different interpretations of an experiment (Fig. 5.1B). Although the use of mathematical models as *predictors* (Fig. 5.1A**)** or *interpreters* (Fig. 5.1B) of experimental data have often resulted in important contributions to developmental biology, they have also been subject of skepticism from experimentalists. A major criticism to these approaches is that conclusions arise from the models themselves, and as such, depend on the details of their

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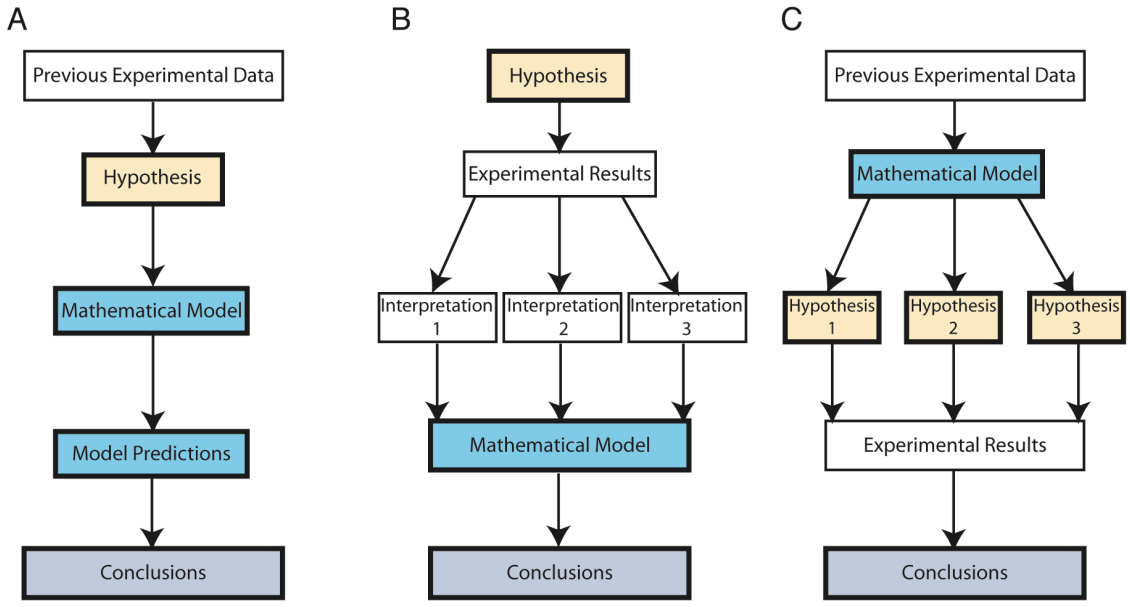

Figure 5.1. Different methodologies for using mathematical modeling in biological research.

(A) The mathematical model is used as a *predictor* of new experimental results. As new experimental data become available, the model is validated or refined. This approach is typically used to build general mathematical models that explain the phenomenon of interest. (B) The mathematical model as an *interpreter* of experimental data. In this case, the mathematical model is used to test the feasibility of different interpretations of an experiment. (C) Mathematical modeling as a *hypotheses-generating tool*. In this approach, the mathematical model is used to propose different hypotheses, but does not favor any particular one. Unlike the methodologies depicted in (A) or (B), in (C) conclusions are only derived from experimental data.

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mathematical formulation and their accuracy to represent the biological phenomenon.

In our study of the interpretation of Hh signaling in the *Drosophila* wing disc (Nahmad and Stathopoulos, 2009; see Chapter 2), we used mathematical modeling as a tool to formulate hypotheses that could be tested through direct experimentation (Fig. 5.1C). Importantly, these hypotheses would not be straightforward to propose without the mathematical model. Specifically, we modeled the Hh signaling pathway using a

system of partial differential equations and found that under certain assumptions (i.e., the value of a critical parameter), interpretation of the Hh gradient in a concentration-dependent manner was not possible. Furthermore, the model suggested that the formation of the gradient follows some unusual dynamics due to a property of the gene network architecture associated with the Hh signaling pathway, namely, that the Hh receptor and antagonist, *ptc*, was transcriptionally upregulated in response to Hh signaling. The gradient initially expands due to low Ptc levels, but then retracts as a result of Ptc accumulation, which leads to the sequestration and degradation of free extracellular Hh (Fig. 2.2E). Moreover, we showed experimentally that if Hh-dependent *ptc* upregulation is impaired, then the signal fails to establish different domains of gene expression (Fig. 2.3F). Thus, the model did not predict that the dynamics of the gradient were required for the interpretation of the signal, but rather prompted us to investigate it. In contrast to other modeling approaches that have utilized mathematical models as predictors of unknown data (Fig. 5.1A), or to interpret unclear experiments (Fig. 5.1B), in our study the model was used as a motor to propose non-trivial hypotheses (Fig. 5.1C). Our approach is somewhat similar to model-based experimental design strategies in which mathematical models are used to define possible experiments that can be performed. Although these approaches have become widely used in systems biology in the post-genomic era (Kitano, 2002; Kreutz and Timmer, 2009), our approach - to employ mathematical modeling as a tool to guide experimental research - is not common in the context of developmental biology.

A Network Architecture-Based Model of Hh-Dependent Patterning[*]

The most important conceptual contribution of our work on Hh signaling (Chapter 2), in our opinion, is the idea that the shape of the gradient is not the major factor contributing to pattern formation in this system. It is widely recognized that developmental patterning is tightly controlled by feedback components inherent within the gene regulatory network of the system (Kutejova et al., 2009). These feedback interactions have been shown to be essential for generating sharp boundaries of gene expression and to ensure reproducibility and precision under genetic or environmental perturbations. However, most models of morphogen-mediated developmental patterning are built under the main hypothesis that pattern formation is a function of the morphogen concentration profiles. In particular, changes in patterning are usually directly associated with changes in the morphogen distribution, and properties such as precision, robustness, or size-dependent scaling are generally studied assuming that the shape of the morphogen gradient is the predominant factor (Houchmandzadeh et al., 2002; Eldar et al., 2003; Bollenbach et al., 2008; Ben-Zvi et al., 2008). Our study suggests that Hh-dependent patterning in the *Drosophila* wing disc depends on temporal changes of the morphogen profile but, unlike the classical morphogen model, it does not primarily depend on concentration thresholds defined by the distribution of the gradient; instead, patterning is controlled directly by the architecture associated with the Hh gene network, particularly by the feedback that results from Hh-dependent *ptc* upregulation and Ptc-dependent ligand sequestration. Therefore, our model backs up a recent study (Smith et al., 2007) that supports the idea

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that pattern formation is inherent within the gene regulatory network of the system and implicates that the shape of the Hh concentration profile is not the primary source of positional information.

Steady-State Invariant Perturbations as a Tool to Study Morphogen Dynamics

The major challenge in modelling-based experimental design is not just that mathematical models can only capture a simplified view of the natural phenomenon, but that they are tightly constrained by the experiments that can actually be performed. The hope of the theoretical approach presented in Chapter 3 is to use it as a tool to design genetic experiments that separate transient from equilibrium effects in developmental patterning. The problem of computing invariant subsets that correspond to experimentally testable genetic perturbations is in general very difficult; even in the cases in which the steady-state invariant set can be obtained either exactly or approximately, it is unclear if the design of such a genetic experiment is possible (i.e., one in which the affected kinetic parameters remain on the steady-state invariant set). Experimentally, genetic perturbations that are expected to satisfy many constraints on independent kinetic parameters are difficult to realize. For instance, in our example of a free morphogen established by diffusion and linear degradation [Equation (3.1)], only those perturbations in which all three parameters are altered in the same proportion remain on the steady-state invariant set [Equation (3.4)]. Despite the simplicity of this example, a genetic perturbation subject to these constrains (i.e., such that Equation (3.4) holds) is very difficult to achieve experimentally because kinetic rates, in general, may not be precisely tuned using standard genetic techniques. Although this example is not of biological

interest, it illustrates a general conceptual problem of modeling-based experimental design: models that are simple enough so that the steady-state invariant set can be fully computed do not necessarily predict simple genetic experiments. On the other hand, in our model of Hh signaling, the steady-state invariant set cannot be computed exactly and yet, an approximate steady-state invariant subset that involves only one "freely" tuneable parameter can be obtained [Equation (3.11)] and the genetic design of steady-state invariant perturbations is plausible.

The theoretical approach presented in Chapter 3 faces some challenges that may limit its applicability. For example, the geometry of steady-state invariant sets depends on the details of the mathematical model. As these models are based on simplified representations of the developing system, a steady-state invariant perturbation predicted by the theory may not be so experimentally. Therefore, these tools should be used as a predictor of steady-state invariant genetic perturbations but appropriate control experiments should be performed to show that the steady-state distribution of the signal is in fact unchanged by the proposed perturbation. Another assumption of this theoretical approach is that the steady state is reached within a relevant developmental timescale, but this may not necessarily be the case. For example, the nuclear concentration of the transcription factor, Dorsal, in the early *Drosophila* embryo does not appear to reach a steady-state distribution by the time that gene expression patterns are specified (Liberman et al., 2009). However, in the cases when equilibrium is not reached within the patterning timescale, it is clear that positional information is established by signaling dynamics and that additional mechanisms are required to explain patterning in these systems.

Spatial Scaling along the DV Axis of the *Drosophila* Embryo is a Gene-Specific Property

Our finding in Chapter 4 that scaling of dl-target genes with respect to the embryo circumference depends on specific genes, rather than on particular positions along the DV axis, implies that scaling in this system does not result from the interpretation of position from a global system of scaled coordinates like in the French Flag problem (c.f. Wolpert, 1968). Instead, our results suggest that different genes may use different coordinate systems to establish their positional information in the embryo. An intriguing example is the comparison of the dorsal border of *vnd* vs. the ventral border of *ind* because previous studies suggest that *vnd* sets this border of *ind* (von Ohlen and Doe, 2000; Cowden and Levine, 2003). However, our data indicate that despite strict scaling of the dorsal border of *vnd* with the length of the DV axis (Fig. 4.1C), the ventral border of *ind* displays overcompensation and thus does not scale (Fig. 4.1H). One explanation for this result is that *vnd* and *ind* use different coordinate systems to specify the locations of their borders. For example, *vnd* may use feedback interactions downstream of dl to ensure precise scaling, whereas *ind* may employ another system of coordinates; for example, EGFR signaling has been suggested to play a role in *ind* expression (as *egfr* mutant embryos do not express *ind*) (von Ohlen and Doe, 2000).

Similarly, while more data are required to verify whether or not the dl gradient scales in wild-type embryos, our result that the dorsal border of *vnd* scales in embryos that ectopically express dl along the AP axis (in which it is clear that scaling of the dl gradient along the AP axis *does not* occur; see Fig. 4.3B,C,E) shows that dl scaling is not required to ensure scaling of the *vnd* pattern. In contrast, the dorsal border of *sog*, which conclusively scales in wild-type embryos (Fig. 4.1D), fails to scale in this genetic

background (Fig. 4.3F). This suggests that gene-specific coordinates are used to determine positional information with respect to the size of the embryo.

A Gene-Specific Model to Explain Scaling of the Ventral Border of *sog*

Although our experiments in Chapter 4 do not uncover any particular mechanism of scaling, we can speculate about gene-specific mechanisms of scaling in this system. For example, the ventral border of *sog* cannot be explained directly by concentration thresholds of the dl gradient in wild-type embryos (Fig. 4.2D), suggesting that additional factors contribute to scaling of this pattern. We suggest that scaling of the *sog* ventral border is an indirect consequence of scaling of the *snail* gene, which encodes the mesoderm-specific transcription factor implicated in sharpening borders of gene expression in ventrolateral patterns. However, the generation of sharp boundaries does not imply scaling (or viceversa). If scaling of the dl gradient width results in scaling of the *snail* border, then *snail* scaling would be a direct consequence of scaling of the nuclear dl gradient (Fig. 4.4A), while *snail* would work as gene-specific intermediary in scaling of the ventral border of *sog* (see Fig. 4.4B with X=*snail*).