12 Exploring Modularity with Dynamical Models of Gene Networks

GEORGE VON DASSOW AND ELI MEIR

Introduction

If one is seeking a biochemical understanding of development then the language of dynamical systems theory seems a natural one to use.

If we are serious about attempting to understand the hierarchy of developmental decisions in molecular terms then we do not just need to identify the relevant genes and gene products but also to understand their dynamical behaviour. In the past this has proved to be necessary for understanding such things as the mechanism of nerve conduction or aggregation in slime moulds. In the future it seems probable that it will be through the mathematics of dynamical systems theory that embryological and molecular results can meaningfully be brought together.
—J. M. W. Slack, *From Egg to Embryo*

No one doubts the contribution of mathematical models to evolutionary theory, or the necessity of simulations and statistical modeling to ecology, or the role of kinetic models in enzymology, and yet the application of models to developmental biology seems always under question. The anticipations recorded by Jonathon Slack remain unfulfilled. For one thing, molecular biology has only recently begun to provide the kinds of facts from which empirically grounded models could be formulated. On the other hand, the reality of epigenetics is far more complex than envisioned by most earlier workers, although some, like Slack (1983) certainly appreciated the scope of the problem. Thus, the kinds of readily understood dynamical systems models reviewed by Slack for the most part fail to capture the complexity that lab-bench biologists confront. Consider a recent expression of the situation:

> 1980, the year that Christiane Nüsslein-Volhard and Eric Wieschaus embarked on their Nobel Prize–winning screen for embryonic lethal mutants in *Drosophila*, in some ways marked the end of the Age of Beautiful Theories in biology, and the dawn of the Age of Ugly Facts. . . . If Watson and Crick's double-helical model of the structure of DNA showed that imagination (with a sprinkling of data) could triumph over Nature, Nüsslein-Volhard and Wieschaus's saturation mutagenesis showed that evolution can produce biological mechanisms of such unimaginable complexity that it would be useless, if not laughable, to try to intuit them a priori. Nature's imagination, it showed, usually far outstrips that of the human brain . . . the baroque and counterintuitive biological mechanisms that evolution has produced so often mock the human imagination. (Anderson and Walter 1999, 557–558.)

Aside from implying that complex mechanisms are ugly, this passage highlights the fact that molecular biology has finally inverted the habit of biological inquiry. Instead of using phenomenology and perturbation experiments to deduce some mechanism, and then uncovering facts one by one to support that hypothesis, modern biologists increasingly turn to large-scale exploration (e.g., DNA microarrays, genome sequencing) to generate a mass of facts whose relevance is eventually established by phenomenology and from which mechanistic understanding might hopefully emerge.

How to accomplish that last step of making the mechanistic understanding emerge from the sum of the parts? When things get too complicated for human intuition and language, scientists turn to math and models. Our work on the segment polarity and neurogenic networks, reviewed below, is a preliminary exploration of how biologists might use dynamical models to come to grips with their ever-growing maps of epigenetic interactions. Elsewhere we have described our approach and the results of our first case studies (von Dassow et al. 2000; Meir et al. 2002b; von Dassow and Odell 2002; Meir et al. 2002a). To us, modularity is a working assumption: we are trying to build up some network that exhibits some lifelike behavior from parts that do not, by themselves, fully explain that behavior. This is the opposite of starting with a large-scale map, seeking to break it down into more-readily-understood bits. The two approaches will surely lead to different, but complementary, results. Here we address in general terms what we think are the prospects for our approach. We discuss several intertwined issues:

Plausibility: The most basic limit, presently, to making sense of the parts catalog of molecular biology is our own inability to tell in words whether or not a particular conspiracy of molecules *actually does* what we think it might do. When confronted with systems too complex to argue out in words, we need more rigorous methods than human language to sort out plausibility. Computer

models can tell us *whether it is plausible* that some phenomenon can be explained by some set of relevant facts.

Hole filling and inference: A converse of the plausibility issue is that the known facts are usually inadequate. A particular model's deficiencies often reflect gaps between the facts, as long as the model's assumptions cannot be trivially questioned. However, efforts to use models for inference will forever suffer from the inability of human imagination, as lamented by Anderson and Walter, to match the creativity of the evolutionary process.

Evolvability and variational tendencies: Assuming one constructs a realistic model that exhibits some lifelike behavior, the dependence of the model's behavior on its parameters constitutes a set of hypotheses about the evolutionary potential of the modeled mechanism. This will become an important use of dynamical models, since it is often difficult to deduce experimentally the variational tendencies of developmental processes.

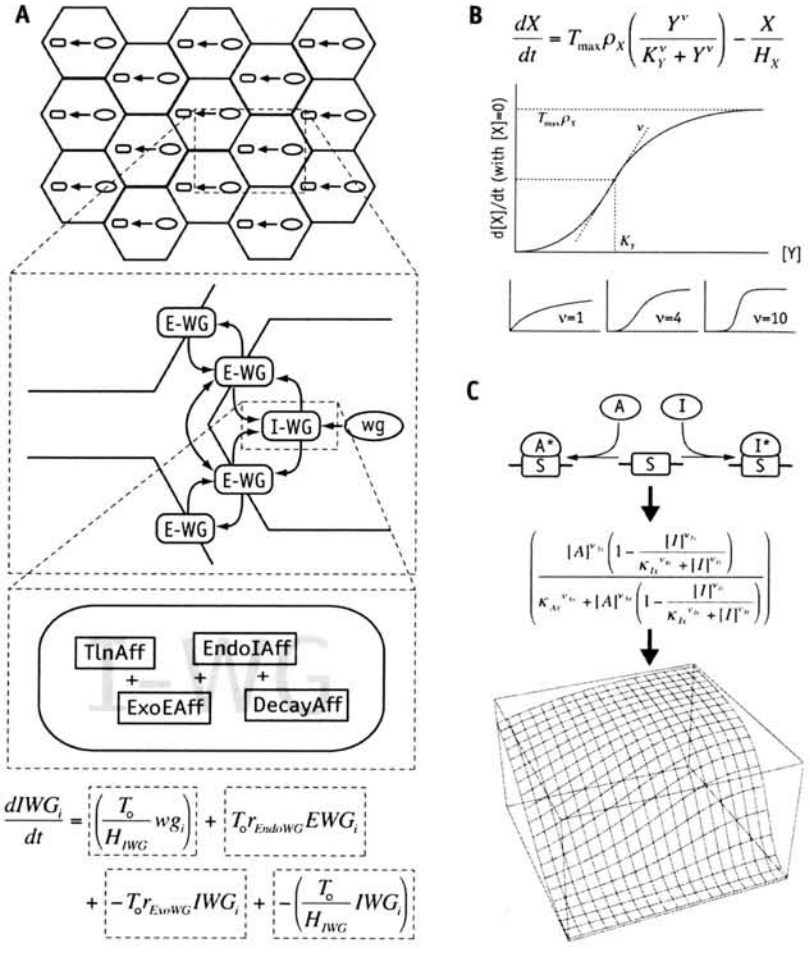
Functional design: Models allow us to explore whether the particular topology of an epigenetic process is merely contingent, that is, nature assembling mechanisms out of the junk heap of the genetic heritage, or whether in a particular case nature has hit upon a genuinely good way to solve a design problem. We can ask, How does a particular network achieve some systems-level property of functional value, such as robustness against perturbation, or modularity, and are there common mechanistic themes to such properties? Recalling once again Anderson and Walter's lament, are these mechanisms really so baroque?

Using mathematical or computer models to explore ideas about genetic and developmental mechanisms is hardly novel. Pioneers of several major threads include Glass and Kauffman (e.g., Glass and Kauffman 1972, 1973; Kauffman 1993), Turing (1952), and Meinhardt and colleagues (e.g., Gierer and Meinhardt 1972; Meinhardt 1977, 1984), and Waddington and Kacser (1957). These workers developed very different conceptual approaches to the problem of how to capture gene network dynamics in maths. However, until relatively recently most of these efforts have been abstract and phenomenological, rather than grounded in empirical facts, because the puzzle pieces have been mostly missing. Kauffman and his followers and (independently) Thomas and colleagues avoid the issue of missing pieces, while still confronting complex systems, by using randomly connected or reality-inspired networks of Boolean or thresholded interactors to explore the generic properties of complex networks (extensively reviewed by Kauffman 1993; examples in Thieffry et al. 1998; Thieffry and Romero 1999; Thomas et al. 1995). This approach is often intentionally divorced from the constraints of reality in order to get at what Kauffman calls the "statistical mechanics" of complex networks. Exploring Boolean models led Kauffman to a variety of conclusions, especially about the dependence of the existence of steady states on the density of connectivity in the model. Thus, Kauffman's and Thomas's schools have shown that analysis of these models in the ensemble provides insights into the general features to be expected of complex genetic circuits.

Meanwhile Meinhardt and his followers (among others) developed a variety of candidate models for hypothetical developmental mechanisms based on reaction-diffusion processes (Gierer and Meinhardt 1972; Meinhardt 1977, 1984). This approach was based on Turing's insight that coupled systems of diffusible reactants could, under certain conditions, elicit regular spatial patterns, and that developing embryos could employ such processes to differentiate initially homogenous cells in a tissue (Turing 1952). Indeed, Meinhardt (1984) anticipated many of the essential features of the segment polarity network before it was molecularly deduced. Despite widespread (and rather undeserved) contempt among modern molecular biologists for this approach, the Turing-style models deserve credit for showing that simple chemical processes could produce complex spatial patterns. The derision of working biologists comes from the fact that these models have typically been products of the modeler's skill, not derived from facts about the molecular processes causally involved in the phenomenon which the model proposes to explain.[1] In addition, simple reaction-diffusion models exhibit a variety of biologically unrealistic tendencies; Slack (1983) provides an excellent overview of the results and criticisms of reaction-diffusion models.

A third major thread, the use of dynamical systems models, is the direct lineage of our efforts. In his influential book, Slack (1983) justified the use of dynamical systems theory as the natural language for modeling developmental pattern formation and other epigenetic processes. He argued that one could readily capture measurable, and general, properties of biochemical reactions, and furthermore that the phenomenology of development parallels that of dynamical systems. Notably, Slack discussed the intimate connection between the stability of cell states and the attractors of dynamical systems, he pointed out the parallel between progressive determination and the time evolution of a dynamical system toward a steady state, and he highlighted the initial-condition dependence of cell fate specification and the choice of attractors by dynamical systems near the boundaries between basins of attraction. Slack acknowledged the inherent difficulty of working with nonlinear dynamics but recognized that this is a necessary cost of improved realism.

A handful of recent attempts use continuous nonlinear models to



$$\frac{dX}{dt} = T_{\max}\rho_X\left(\frac{Y^\nu}{K_Y^\nu + Y^\nu}\right) - \frac{X}{H_X}$$

$$\frac{dIWG_i}{dt} = \left[\left(\frac{T_o}{H_{IWG}} wg_i\right)\right] + \left[T_o r_{EndoWG} EWG_i\right] + \left[-T_o r_{ExoWG} IWG_i\right] + \left[-\left(\frac{T_o}{H_{IWG}} IWG_i\right)\right]$$

Fig. 12.1.—The Ingeneue modeling framework. *A*, Ingeneue does everything (in its current version) in a grid of hexagonal cells. The network topology is stamped out into every cell in a user-specified grid. The topology consists of nodes that interact according to formulas chosen by the user. Nodes may be intracellular or membrane bound; in the latter case Ingeneue tracks the concentration on each cell face. The equations governing interactions among nodes are built from "affectors" that encapsulate formulas for various dynamical processes; in the example illustrated, the node representing the intracellular form of the Wg protein, I-WG, is translated in proportion to the abundance of wg mRNA (governed by the TlnAff object), it experiences endo- and exocytosis (the affectors ExoEAff and EndoIAff govern equilibration with the extracellular form of Wg, E-WG), and it undergoes first-order decay (the DecayAff object). The actual formulas, in dimensional form, are shown at the bottom; dashed boxes correspond to the individual affector objects. At each time point, Ingeneue invites each node to compute its own time derivative simply by adding together its stable of affectors. This architecture makes it trivial to modify the network topology throughout the entire field; if we wanted to add a new interaction for I-WG, we simply add the appropriate tags to the input script, and Ingeneue handles sorting out all the neighbor relations within the cell grid. *B*, Most regulatory relationships in Ingeneue are represented by sigmoid dose-response curves. Shown here is a simple equation (in dimensional form) in which the first term endows transcriptional activation of X by Y, and the second confers first-order decay. The virtue of this approach is that it enforces several biologically realistic parameters: a saturation level (i.e., maximum transcriptional activity), a half-maximal level of regulator, and a shape parameter, which is equivalent to the Hill coefficient. Modulating the Hill coefficient ν changes the steepness of the dose-response curve. It is this parameter that we call "cooperativity," by analogy to classic allosteric systems; with $\nu = 1$ we say the response is noncooperative, and increasing cooperativity leads to a more and more steplike function. *C*, Individual nodes often must integrate multiple inputs. For example, an activator A and inhibitor I might compete for binding to an enhancer sequence S; this relationship can be captured by nesting dose-response curves to come up with an appropriate behavior, as judged by the graph. For this formula, as appropriate, the inhibitor can squelch the response to low concentrations of activator, but increasing activator concentration overwhelms any particular level of inhibitor.

explore real, well-understood epigenetic processes. Edgar and Odell (Edgar et al. 1989) developed one of the earliest realistic, nonlinear models of developmental pattern formation, showing that a subset of the *Drosophila* pair-rule genes can account, through mutual cross-repression, for how pair-rule gene products sharpen each other's expression boundaries. A variety of recent efforts similarly attempt to capture the behavior of entire (if as yet small) genetic circuits, deduced empirically, using continuous nonlinear models, the most emblematic of which is Barkai and Leibler's (1997) model of the core control circuit for bacterial chemotaxis. These authors not only used their model to predict that this mechanism would tolerate variation in the levels of gene expression, but also showed that the real biological circuit has this property as well (Alon et al. 1999).

Most such efforts borrow heavily from the well-developed body of formulations describing enzyme and binding kinetics, which has been under development for over a century and is deeply integrated with lab practice (Gutfreund 1995; Wyman and Gill 1990). We follow the same prescription because many interactions between gene products literally *are* binding reactions, enzyme-catalyzed transformation, or other straightforward chemical processes, so formulas for first- and second-order chemical reactions and so forth can get us pretty far as long as we assume that cells are well-stirred reaction vessels, and as long as we assume that molecular species are abundant enough in cells to use the continuum approximation. As described elsewhere (von Dassow et al. 2000; Meir et al. 2002a) we use a stereotyped formulation for dose-response relationships between regulators and targets, largely inspired by classical treatments of enzymatic processes and allosteric binding phenomena. Because many of the networks we are interested in mediate pattern formation in fields of cells, and because these networks are expressed by systems of differential equations too complex to be wielded comfortably by mere humans, we developed a gene network simulator program (Ingeneue) that weaves the equations together from a library of formulaic building blocks, guided by a text description of the network, and instantiates indexed copies of the network in each cell in a user-specified grid (see fig. 12.1). This program makes it easy to "rewire" network models, testing consequences within a common

framework, and makes it feasible to compare the results from models of entirely different circuits.

Lessons from the Segment Polarity Network

Our first task was to synthesize the known facts about the mechanism of segmentation and ask, simply, Do we know enough about this system to make a model that accounts for some aspect of the behavior and function of the real biological entity, and if not, what do we need to know better? The segment polarity network is the last tier in a cascade of ever-finer-scale patterning processes that start with maternally transcribed mRNAs localized to each end of the egg and end with a nearly cell-row-by-cell-row specification of positional information along the anterior-posterior axis of the embryo at about the time of cellularization (summarized in fig. 12.2, *A*, and reviewed by Martinez-Arias 1993; Pankratz and Jäckle 1993). The segment polarity network stabilizes and maintains the boundary between parasegments (the metameric units that patterning genes map out). The tier immediately above them in the segmentation cascade, the pair-rule genes, are expressed just long enough to map out the segments and activate patterned expression of segment polarity genes like *engrailed* (*en*), *wingless* (*wg*), *sloppy-paired* (*slp*). Thereafter the segment polarity genes have to "hold on to" the pattern imprinted by the pair-rule genes. This is accomplished, according to the canonical view, because the segment polarity genes mediate a codependence between cell states on either side of the parasegment boundary. Persistence of the *wg*-expressing cell state in the cells anterior to the compartment boundary depends on signaling by the product of the *hedgehog* (*hh*) gene under the control of En. In turn, the persistence of the *en*-expressing cell state *posterior* to the boundary depends on Wg signaling (fig. 12.2, *B*).[2]

Most of the core segment polarity genes are components of the Hh and Wg signal transduction pathways. Hh acts through the products

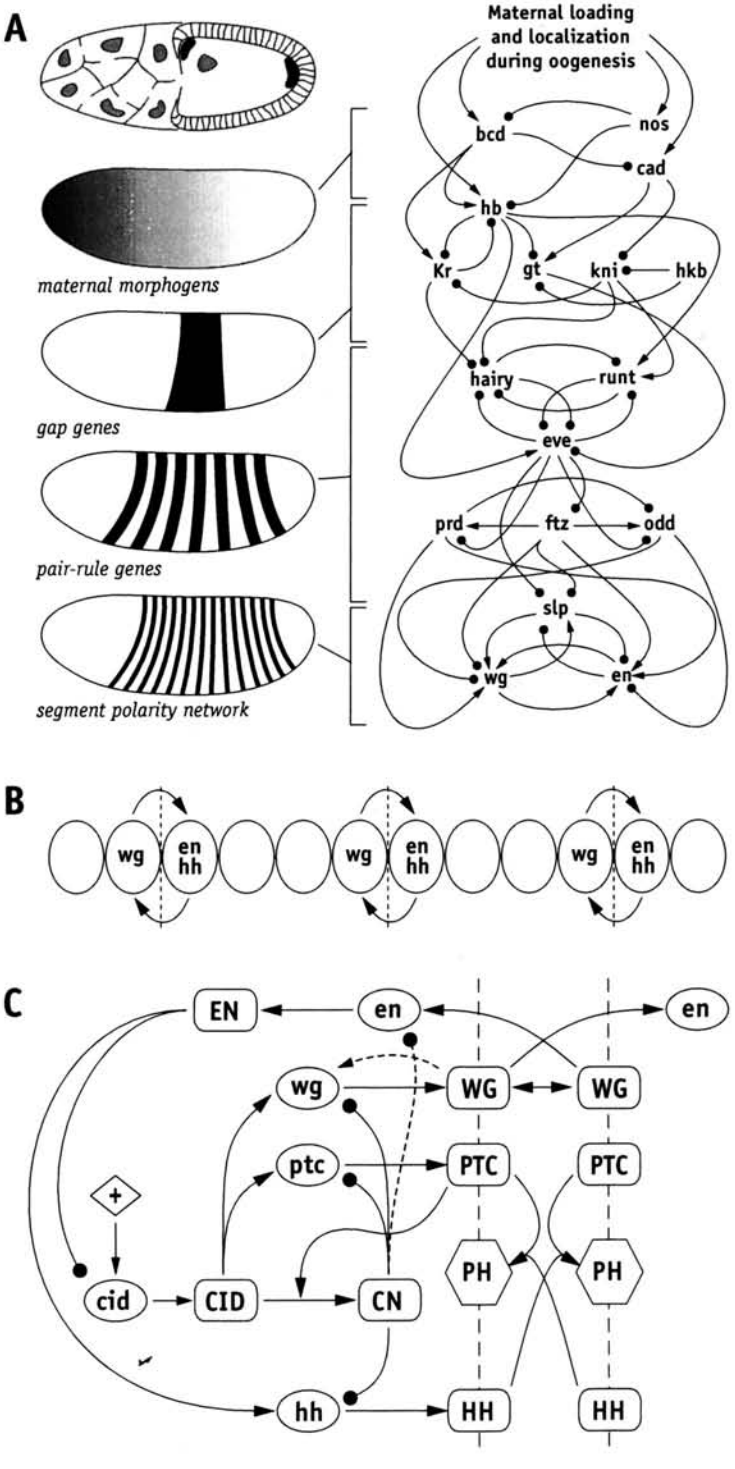


Fig. 12.2.—The segmentation cascade and the core segment polarity network. *A*, Cartoon-and-arrows summary of the segmentation cascade. This figure is meant to convey the flavor of the process, not every feature. Maternally expressed gene products such as *bicoid* (*bcd*) and *nanos* (*nos*) are localized within the oocyte; during early development localized synthesis leads to long-range gradient formation; gap genes, including *hunchback* (*hb*), *giant* (*gt*), *knirps* (*kni*) and *Krüppel* (*Kr*) respond to local Bicoid concentration and/or to each other, forming broad bands of expression; they in turn shape the emerging expression patterns of pair-rule genes, including *hairy* (*h*), *even-skipped* (*eve*), *runt*, *paired* (*prd*), and *fushi tarazu* (*ftz*) into finer-scale stripes; and the pair-rule genes shape the initial expression of the segment polarity genes, especially *wingless* (*wg*) and *engrailed* (*en*) and *sloppy-paired* (*slp*) *B*, A common textbook summary of the segment polarity cascade. *wg*-expressing cells on the anterior side of the boundary depend on *en*/*hh*-expressing cells on the posterior side, and vice versa. *C*, The wiring diagram for the simplest version of our segment polarity model. The diagram here was rationalized and analyzed in von Dassow et al. (2000); dashed links were added after an initial model without them failed to exhibit lifelike behavior. CID, Cubitus interruptus; CN, repressor fragment of Cubitus interruptus; PH, Patched-Hedgehog complex; PTC, Patched.

of the genes *patched* (*ptc*) (Hooper and Scott 1989; Marigo et al. 1996; Stone et al. 1996) and *smoothened* (*smo*) (Alcedo et al. 1996; van den Heuvel and Ingham 1996) and the transcriptional switch encoded by *cubitus interruptus* (*ci*) (Alexandre et al. 1996; Dominguez et al. 1996; Hepker et al. 1997; Von Ohlen et al. 1997). Ptc is the Hh-binding component of a complex that includes Smo, and in the absence of Hh, Ptc prevents Smo from sending an as-yet-poorly-understood signal (Chen and Struhl 1996; Alcedo and Noll 1997; Chen and Struhl 1998). The result of this signal (or signals), whatever it is, is to liberate Ci protein, the full-length form of which is a transcriptional activator, from a complex that includes the products of the genes *fused, Suppressor of fused* (*Su(fu)*), and *costal;* this complex both keeps Ci in the cytoplasm and directs it to be proteolyzed to yield a truncated protein (CN) that behaves as a repressor (Aza-Blanc et al. 1997; Ohlmeyer and Kalderon 1998; Wang and Holmgren 1999). Thus, in the absence of Hh, Ci is converted to a repressor that keeps Hh target genes off (including *ptc* and *wg*), and in the presence of Hh, due to removal of Ptc, Ci remains intact, mysteriously passes through various activation steps and enters the nucleus, and activates Hh target genes.

Wg signal transduction begins with products of *frizzled*-family genes (Bhanot et al. 1996; Bhat 1998; Bhanot et al. 1999). The details of Frizzled signaling remain mysterious, but, analogous to Hh signaling, the crucial switch involves a cytoplasmic complex that restrains a transcriptional regulator. In this case it is the product of the *armadillo* gene that is targeted for proteolysis; Wg signaling leads to the release of Arm from a cytoplasmic complex that includes the kinase encoded by *shaggy* and a *Drosophila* homologue of the oncogene APC (a very complex literature is reviewed by Cadigan and Nusse 1997). Free Arm binds to the product of *pangolin,* and Arm and Pan together act as a transcriptional activator (Brunner et al. 1997; van de Wetering et al. 1997).

There are many other segment polarity genes that participate in the process in flies, and that, when mutated, yield various phenotypes. We believed that the basic codependence outlined above would be sufficient to account for the basic function of the segment polarity network, namely, to maintain an asymmetric boundary with *wg* expressed on one side and *en* expressed on the other. Our goal was to start with the simplest dynamical representation of the network and build up piece by piece. Thus, to start with we abbreviated signal transduction pathways and refrained from including apparently redundant or "extra" components. We thought that perhaps the core network, the simplest network we could get to do the job required, would probably be relatively fragile, and that all the other segment polarity gene products—for instance, the transcription factor encoded by *gooseberry* (Li and Noll 1993), or the Wg-inducible signaling inhibitor encoded by *naked*

(Zeng et al. 2000)—might be required to make the network robust to various kinds of perturbations. Thus, we were not dismayed in the least when it turned out that our initial attempt to concoct a model was very hard to get to behave properly.

In fact it slowly dawned on us that it was completely impossible for our first attempt to work under any conditions. We had to add two specific links, one of which was more or less well demonstrated but ignored, and the other of which was, at the time, more or less a guess (fig. 12.2, *C*). Completely to our surprise, however, it turned out that with those two links in place, the core network was fabulously robust to variation in both governing parameter and initial conditions. As described in von Dassow et al. (2000), random sampling for parameter values throughout an enormous, high-dimensional parameter space allowed us to find "working" sets of values with unbelievably high frequency. Further explorations (von Dassow and Odell 2002) showed that the core network's boundary-maintaining function is also robust to architectural variations. In other words, once the right links are in place, there is no one single way to make the network function; once all the pieces are hooked up right, the lifelike behavior we sought to reproduce *in silico* became intrinsic to the topology of the network, rather than to any particular tuning of the connections and components within it.

This is a very satisfying finding given a certain evolutionary hypothesis that had originally been in our minds when we started the work. It appears that the upstream aspects of the segmentation cascade are not conserved among insects (see, for examples, Patel et al. 1992; Dawes et al. 1994; Dearden and Akam 1999), the furthest-upstream components not even beyond Diptera, but the segment polarity network *might* be involved in segmentation in everything from flies to beetles to grasshoppers and beyond (Patel et al. 1989; Nagy and Carroll 1994; Patel 1994). Although there is not as much evidence supporting this hypothesis as one might like, it remains appealing; the suggestion is that the upstream mechanism that lays out segment boundaries in other insects must be very different, despite the homology of all insect segments and the conservation of the gene network assigned to stabilizing those boundaries throughout development. Our results say that this hypothesis is plausible: intrinsic to the topology of this network is the ability to do the thing it does in embryogenesis, absent any extrinsic guidance, and if we could make an animal with only these genes, then practically any bias on their expression among the cells of that animal would result in at least one segmental boundary!

To summarize, our initial modeling effort resulted in at least six specific, empirically testable predictions:

1. Our model explicitly highlights a need for a repressor of *engrailed*

in the anterior compartment and suggests that the N-terminal fragment of Ci could fill this role. Another candidate, explored in later models (von Dassow and Odell 2002), is Sloppy-paired (Cadigan et al. 1994b; Grossniklaus et al. 1992); the model merely focuses attention on the missing link; obviously, it is an empirical problem to figure out what that link might be.

2. *wingless* autoactivation is functionally important and probably conserved, and must follow certain guidelines (described in von Dassow and Odell 2002) to fulfill its role. This phenomenon has received very little attention in the literature, and our model explains its importance.

3. Interactions among segment polarity genes should exhibit moderate to high cooperativity, except for interactions mediating negative feedback between *ci* and *ptc* (Meir et al. 2002a; von Dassow and Odell 2002).

4. Our model "prefers" the Wg diffusion rate to be low, suggesting that rapid diffusion makes pattern formation by this mechanism more difficult. Indeed, several findings show that Wg cell-cell transport is under fairly specific control (Dierick and Bejsovec 1998; Moline et al. 1999).

5. Since the model tolerates a variety of initial prepatterns, we would predict that the specific inputs to the segment polarity network from the pair-rule and gap genes will *not* be rigorously conserved even within long-germ insects.

6. We predict that the segment boundary maintenance mechanism is robust to quantitative variation in gene function. We are currently trying to test whether the real segment polarity network exhibits the same degree of robustness as our model.

Points 1 and 2 directly illustrate plausibility and inference applications for dynamical models. Although our initial model expressed a more detailed summary of the network topology than was at the time typical even of workers studying the segment polarity network empirically, we found that there were two specific defects that could not be overcome even by choosing kinetic parameters carefully. One of these defects was cured with the documented, but little-attended, phenomenon of *wg* autoregulation (Hooper 1994; Vincent and Lawrence 1994; Manoukian et al. 1995; Yoffe et al. 1995). The mechanism for this remains poorly understood to date, but our model showed that the *wg* autoregulation mechanism, whatever it is, may be central to the function of the segment polarity network even though it had heretofore figured barely at all in discussions of how segmentation works. The second defect also concerned a lack of attention by the community of experimental biologists to a detail of the mechanism, in this case to the regulation of *en*. Almost all the attention has gone to either the specification of the initial *en* expression pattern by pair-rule genes (DiNardo et al. 1988; Ingham et al. 1988), the subsequent dependence of *en* on Wg or En itself (the classic account is Heemskerk et al. 1991), or to the stabilization of the *en* activation state late in embryogenesis under the control of the Polycomb—and Trithorax—group genes (e.g., Moazed and O'Farrell 1992). The model forced us to notice what should have been obvious in the first place: something has to shut *en* off in the anterior compartment.[3]

In experimentally tractable model organisms like *Drosophila*, biologists are quite efficient enough to fill in these sorts of details sooner or later with or without the help of models like ours. Maybe work like ours can accelerate the process. Our approach is of far greater potential value if applied to less willing organisms where making transgenes and knockouts and the like is either a major technical challenge or otherwise out of the question. We have little intention of focusing our own efforts in such areas ourselves, but the point we want to underscore is the time has come that realistic enough computer models can make plausible suggestions about how to fill in the holes; computer models, unlike us, cannot be fooled by an arrow diagram backed by a rhetorically compelling word salad.

Points 3 and 4 represent inferences from the models about how the real mechanism might work, but also bespeak functional design. The segment polarity network model can be thought of as a set of spatially entrained switches in which the various stable states for each switch are mutually exclusive within an individual cell, but the network is structured such as to make these switches entrain each other to alternate states in neighboring cells. The switches are based on nonlinear responses, which could be due to cooperative binding effects; higher cooperativity increases the likelihood of choosing parameter values for which both switched states will be stable. In addition the negative feedback loop between *ci* and *ptc* keeps cells in the "ground" state responsive to Hh signaling; low cooperativity within this loop makes it more likely that it behaves as a homeostat, rather than generating oscillations. The mutual entrainment of neighboring cell states depends on signals' making it to neighboring cells but not much further; hence it is harder (though not fatally so) to tune up parameters to achieve the desired pattern the more rapidly the intercellular signals are allowed to diffuse. This contrasts with Gierer-Meinhardt-style reaction-diffusion models, in which the diffusion rates of intercellular signals determine the periodicity of the patterns they can make (Slack 1983 provides a critical review of this family of models).

Point 5 has some implications both for our understanding of functional design, and also for the evolvability of the segmentation mechanism. In describing the segment polarity network as a series of switches, it is the initial conditions that determine which switch gets thrown in

which cells. Again unlike the Turing-style models that have so often been suggested to explain pattern formation, the segment polarity network does not make patterns out of small perturbations in an undifferentiated field. Rather, the segment polarity network stably maintains (and can sharpen) a prepattern conferred upon it by anything which biases the initial conditions toward one or another switched state on a cell-by-cell basis. Whether or not the network can stably "make" a particular pattern thus depends not just on the kinetic parameters but also on the initial conditions. In the case of the target pattern we tried to get the simple, core model to make (von Dassow et al. 2000), the outcome is based on a race between *en* and *ci,* on the one hand, and on the other hand on making sure that *wg* gets a quick enough assist (from Ci) to keep itself on.[4] Thus, the model's demands on the initial conditions can be crudely stated like this: for any pattern of initial biases that swings these races in the right direction, there can be found some set of parameters that allows the model to lock on and hold that pattern. In other words, the blind watchmaker can fool around with the upstream regulators as long as certain guidelines are not violated, and as long as the kinetics can be tuned up at the same time.[5]

Point 6 surprised us most, and our lab is testing this prediction empirically. We tend to think of robustness as a design feature, and as something difficult to achieve. Certainly human-engineered devices do not exhibit the degree of insensitivity to control parameters that we found in the case of the segment polarity network; does nature need to evolve robust designs, or is this kind of property generic to genetic networks? Moreover it is not yet obvious to us *why* evolution should have made this mechanism so astonishingly robust, if indeed it is in reality. Even less transparent is *how* this module came to be (although we have an idea, discussed in Meir et al. 2002a, and touched on below); is its present state and employment in flies a highly derived, finely honed design, or a lasting legacy of a lucky co-option early in the evolutionary history of animal life? Only comparative data could answer this, and despite the misleading impression given by some authors (e.g., von Dassow et al. 2000), we know very little about whether the segment polarity module is evolutionarily static or whether details of its architecture adapt to different developmental modes, even within fruit flies.

Whence Robustness?

As recounted at the end of our first report (von Dassow et al. 2000), we originally hoped to explore the mechanistic origins of robustness in developmental mechanisms through *in silico* reconstitution. To reiterate, we expected that the simplest (but still realistic) models would require us to carefully select parameters (by intuition or optimization

strategies) to make it work, and that only carefully chosen initial conditions would lead to the desired behavior. Our hope was that by making progressively more complex models based on known interactions not incorporated into the simplest model we would reveal which design principles evolution had hit upon to make the process in question robust. There are a variety of flavors of robustness, such as tolerance of parameter variation, stochastic perturbations, or initial conditions, and it seems reasonable to expect that embryos, and cells everywhere, need special circuitry to tolerate all these insults. Much of the complexity we see in biological mechanisms might exist for the purpose of endowing some core process with robustness.

This remains an intuitively appealing general hypothesis, but with the segment polarity network it turned out that the core model is hard to improve upon with respect to the basic tests we can subject it to. Not only does it tolerate the kinds of variation enumerated above, but it also tolerates numerous different wiring choices, including whether or not certain secreted proteins diffuse, whether or not reactions are reversible, whether or not particular links and components are present, and so on.

In a forthcoming report (von Dassow and Odell 2002) we describe a test for stripe sharpening, in which the wiring really makes a difference in the performance of the network. The *wingless* and *engrailed* stripes are both reported to narrow as cells rearrange during germ-band extension; cells that move away from the parasegment boundary lose *wg* and *en* expression as they stray beyond the range of sustaining signals (see, e.g., Vincent and O'Farrell 1992; review in Martinez-Arias 1993). There are specific requirements for the model to mimic these behaviors: for *wg,* autoactivation must synergize with activation by Ci; for *en,* there must be stoichiometric balancing of certain components of the Wg signaling pathway. However, while those tests seem legitimate (especially in *Drosophila*), they are almost certainly not general to all uses of this network. The test in which we ask whether the network can restrict *en* stripes to one cell width in the face of cell rearrangement is probably irrelevant to segmentation in short-germ insects: in both grasshoppers and crayfish the En stripes widen as the segments develop (Patel 1994). Similarly, although we tested the ability of network variants to develop the target pattern from a very crude prepattern, in all cases we are aware of, En first appears in crisp stripes. Thus, we focus the discussion below on the robustness of the boundary-maintenance function of the segment polarity network. This may not be the only biologically relevant behavior, but it is the one we have the best handle on.

We consistently found three determinants of robustness. First, the higher the "cooperativity"[6] of most connections, the more variation the network tolerates (Meir et al. 2002a). Second, the right mix of pos-

itive feedback with both positive and negative cross-talk is essential to confer broad domains of parameter space and initial conditions in which the model functions. Third, intermediate steps tend to damp out temporal oscillations. While the second issue is a design concern specific to this network, the others are generic. The role of intermediate steps seems to be a byproduct of cooperative interactions. Because it seems to be such an important generic way to make gene networks robust, we discuss how cooperativity confers robustness.

Consider, for illustration, a trivial signal transduction cascade in which a signal activates a responder (say, a transcriptional activator), which then activates transcription of a secondary target gene. If all the responses are linear-saturating curves, then the output tracks the input: end of story. The more positively cooperative the response (we stretch the notion of cooperativity a little), the more a small change in the input *around the threshold* will result in a large change in output. Since the responses must saturate in the physical world, the higher the cooperativity at each step, the closer the whole chain will be to an all-or-none switch. This may not immediately strike one as the basis for robust behavior, since to call something robust roughly means it behaves the same for a variety of conditions. However, all-or-none responses mean that the behavior of a complex system becomes *less* sensitive to the exact value of off-to-on thresholds. In this pedagogical example, if the signal is moderately above the threshold, then we get a full response from the responder, which means that, as long as "full on" for this gene is also above its activity threshold, we get a full response from the target. This also explains (partly) why intermediate steps increase the robustness of the model; oscillations in the level of some regulator are damped by sharply thresholded dose-response curves as long as the regulator concentration never gets too close to the threshold level. As an aside, a corollary is that introducing delays would not be likely to improve robustness, and indeed might promote oscillatory behavior, in a network composed of more or less linear interactions.[7]

This is fine as long as the behavior we are interested in is one that can be described solely in terms of whether genes and enzymes are "on" or "off." So far, that is all we have demanded of the segment polarity network. But surely there are downstream effects of these genes that are differentially sensitive to quantitative levels of segment polarity gene expression. In imaginal discs Strigini demonstrated the expression patterns of various Hh and Wg targets are sensitive to local differences in the availability of these signals (Strigini and Cohen 1997, 2000). We have not yet explored how these phenomena could work, but these targets may simply be tuned to respond at threshold signal concentrations near the maximum level that that signal (or its effectors) could achieve.

If we modify the signal transduction example slightly, it reveals that

cooperativity does not beget *all* kinds of robustness. What if the responder has two targets, the output and an inhibitor? Imagine that this inhibitor both negatively feeds back on the responder and also feedforward inhibits the output (fig. 12.3, *A*). It turns out that this device has a variety of behaviors if high cooperativity is allowed in all the intermediate connections. The output can have a threshold response to a signal, as before (fig. 12.3, *B*). However, it can also exhibit an upper threshold above which it is inactivated, much like the response of gap genes to maternal morphogens in *Drosophila* (fig. 12.3, *C*). There are a variety of oscillatory regimes, including ones in which the period is tuned by the signal. However, if connections are all constrained to have low cooperativity (<2) then an entirely different kind of robust behavior emerges: the output can respond at intermediate levels over an enormous range of signal concentrations (fig. 12.3, *D*); in other words, this simple system buffers the input. Thus, cooperativity makes all-or-none switch-based mechanisms more robust but makes it difficult to obtain buffered responses.[8]

Furthermore, we have found that the segment polarity model suggests that some interactions, specifically those between *ci* and *ptc,* should have low cooperativity. Our core model tends to exhibit strong oscillations in the levels of full-length Ci and its derivative, CN. This behavior does not necessarily prevent the model from adopting stable patterns for other components, presumably (again) because cooperativity provides buffering as long as the input well exceeds its activity threshold. Nevertheless, forcing high cooperativity for Ci-*ptc* interactions leads to apparently inaccurate predictions about the relative strengths of Ci and CN and leads to oscillatory behaviors that seem unrealistic. Why? Ci and *ptc* form the only strictly negative feedback loop in the model. Full-length Ci activates *ptc,* but free Ptc causes Ci to be cleaved into CN, which represses *ptc.* If these interactions are governed by linear-saturating curves, then they can easily find a steady state, whose position in state space is tuned by the availability of Hedgehog. Equilibration depends on steady responses to changes in concentration of each component. However, sharp thresholds mean targets in effect fail to respond to changes in regulator levels in a certain range and then respond abruptly near the threshold. As in the toy model above, the result is oscillations.

Slack (1983) and Edgar and colleagues (Edgar et al. 1989) both demonstrated the requirement for threshold responses in the mechanism of cell state switches, so our findings merely pin that architectural principle to another specific case. Furthermore, again reminiscent of Edgar and Odell's model of a subset of the pair-rule genes, in our model the spatial regime of mutually entrained cell states depends on negative cross-talk among the active genes in each state. On the basis of experiments

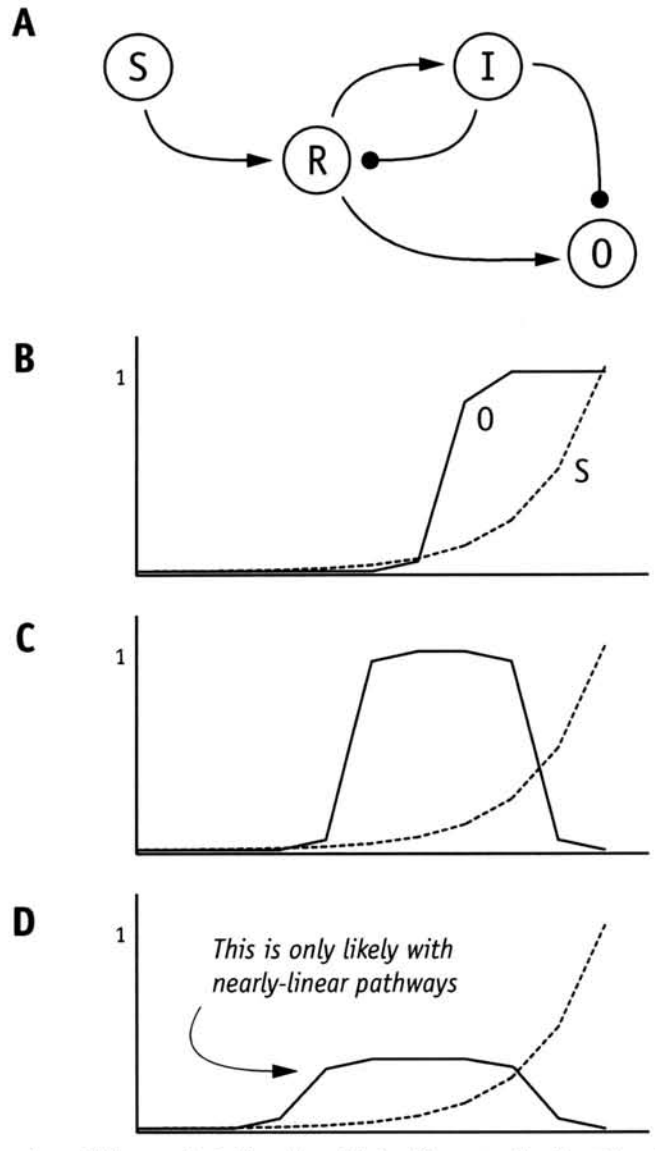


Fig. 12.3.—A potentially homeostatic signaling pathway. *A*, A signal, S, promotes either the activity or the synthesis of a responder, R, which in turn promotes the activity of an output, O. Simultaneously, high levels of R increase the activity of an inhibitor, I, which suppresses both the output and the responder. Each of the links represents a potentially cooperative regulatory effect. *B–D*, Charts showing steady-state output level (*solid lines*) in response to a gradation of input signal (*dashed lines*). When most links are cooperative, the most common responses are a simple threshold response (*B*) and separate thresholds for activation and inhibition (*C*); in both cases the output is either full on or full off. High cooperativity also fosters various oscillatory behaviors (not shown). If most interactions are linear or nearly so, then it becomes possible to find conditions under which the output responds at a fixed level over a large range of input signal strengths (*D*).

with protein synthesis inhibitors, Edgar and Odell modeled the pair-rule genes as if they were basally active. In the case of our segment polarity model, only *ci* is basally activated, so the cell state regime depends also on positive feedback both within some of the cell states and between the different cell states *in neighboring cells*. To repeat, the mechanism is a race between *en* and *ci;* En, via *hh*, enlists the help of *wg* expressed by neighboring cells to keep *en* active, and Wg maintains itself through a still-vague mechanism that probably involves both *slp* and *ci*. Steep thresholds help ensure that any edge in the races pushes the leader toward the attractor in the dynamical system's phase space, the stable cell state, characterized by the leader's expression. Meanwhile, thresholds help make sure that one cell state entrains its neighbors to adopt a different one, and vice versa, thus reinforcing the original choice. This argument at least partially explains the robustness of the segment polarity model's boundary-maintenance function to parameter variation. Further, any spatially varying biases in the initial conditions swing the cell state choice in one or the other direction, and since any such bias will do for some choices of parameters, this explains the robustness to initial conditions.

A final note: in the context of the segment polarity model, there is a direct relationship between (crudely speaking) the average cooperativity of all interactions, and the degree to which the network tolerates variation in either parameter values or initial conditions or architecture. In other words the model predicts that *canalization of this gene network is a direct effect of nonlinear, threshold dose-response functions,* as anticipated by Gibson (1996). The question often arises, How could canalization evolve? Teleologically, it seems that of course canalization should be selectively advantageous in certain circumstances. However, on the basis of population genetic models Gibson and Wagner (2000) suggest that it is actually rather difficult to find conditions under which canalization will arise through positive selection on some "canalizing" allele. These authors express the concern that such results may reflect only the inadequacy of canonical population genetic models.

We admit to such ignorance of population genetics that we could not even begin to agree or disagree, but our models *do* make two interesting suggestions: first, that in the segment polarity network (and the neurogenic network; Meir et al. 2002b), canalizing mutations might arise readily. For example, we found that there are mild to strong variational biases[9] on parameters as diverse as the Wg diffusion rate, the avidity with which En represses *ci,* and the maximum cleavage rate of Ci protein (von Dassow and Odell 2002). These things should be baby steps by mutation, point mutations adjusting the match of enhancer site to regulator, affinity of ligand and receptor, and so on. In other

words, the robustness of the model can be "tuned" via quantitative changes in the kinetics of intrinsic components, without the presumably more involved evolutionary step of changing the topology of the network (i.e., by adding new links or components).

Second, on the basis of the arguments above, it is clear that the higher the cooperativity embodied within the model's many positive feedback loops, the more it could tolerate (1) variability in individual parameters (i.e., mimicking genetic mutation), (2) coordinate, simultaneous changes in many parameters (e.g., to mimic variation in temperature or oxygen supply), or (3) stochastic fluctuations over the time course of pattern formation (due to inherent noisiness of gene expression or cell division or whatnot). Thus, for networks that work like these (i.e., coupled cell state switches) canalization against several sources of variability (mutation, environmental perturbation, developmental noise) may be coordinated. Ancel and Fontana (2000) point out that the reduction of phenotypic plasticity (which we think equates with canalization against either environmental variability or developmental noise) "requires a genotype-phenotype map in which plasticity mirrors variability" with respect to genetic mutation. They call this situation "plastogenetic congruence" and show that it is a generic feature of RNA folding, and that therefore "genetic canalization will ensue as a byproduct of selection for environmental canalization." We think something similar holds for gene networks, and we expect that by comparing the level of developmental noise in wild-type versus sensitized mutants of the segment polarity and neurogenic pathways we will be able to test whether such a congruence exists in reality.

Hierarchical Structure of Genetic Modules

We have often wondered how we can define the boundaries of genetic modules. What criteria define a module, as opposed to just another tangle in the genetic web? No one doubts that life as we know it involves gene networks with intrinsic behaviors; the genome of any organism is such a network, as is the genome of any virus. Similarly, no one doubts that such things are organized into modules; genes themselves, after all, are modules of a certain kind, as are genomes, at a very different level of organization. The question is to what extent genomes break down into, or genes and their products conspire to form, *logically separable guilds of the metabolic milieu*—that is, intermediate entities, made of genes and parts of genomes, that do something we can comprehend in isolation. There are a few cases that seem intuitively obvious: the *lac* operon, the yeast mating-type switch, bacteriophage, the cell cycle clock, and the segment polarity network. What do they all have in common? What exactly is it that our intuition tells us about these mechanisms? What criteria can we extract from our intuition that we can generalize? We do not have an answer yet because it turns out that none of the straightforward criteria (like connectivity) or simple analogies (e.g., to object-oriented programming; see below) seem useful.

It happens that the way biologists investigate genetics disposes the discovery process to reveal knots of locally relevant genes whose products all participate in some way in the production of a certain phenotype or characteristic. Perhaps genomics will change this, but presently it is the case that one finds such local tangles and has no way of knowing whether the membership in the tangle represents just the extent of exploration to date or the core membership of a genuine subunit of the genome. One can make a credible argument that developmental genetics is only possible to the extent that such local subunits are realistic; after all, pleiotropic genes are more difficult for geneticists to analyze than are those genes that specifically regulate particular characters (think of the *Drosophila ras* homologue, which seems to be involved in practically everything, versus the *bicoid* gene, which has a fairly specific function).

This hints at some kind of a criterion based on connectivity. It is tempting to suggest that what distinguishes module from not-module is a degree of interconnectivity, or in the strengths of connections. The suggestion is commonplace that modules are composed of "strong" (or "dense") connections, but have only "weak" (or "sparse") connections to other things. Such a notion turns out, for our purposes, to be largely fruitless. Consider an example: Ras has a starring role in EGF signaling, but only among its other roles; EGF signaling, in turn, one might say, is a module unto itself, a part of diverse morphogenetic control processes, appearing in various developmental mechanisms, and not just in a cameo, either. Do we say that EGF signaling does not count as a module unto itself because Ras participates as an essential step in other pathways? Do we say that in *Drosophila* ventral ectoderm patterning and dorsal eggshell patterning are logically inseparable because they share the EGF pathway? We think not. Consider an analogy: The futures markets for various agricultural products are each governed by various causal factors, some unique but many not. No one would claim that the dynamics of the market in pork bellies was inseparable from that in soybeans simply because they share some causal factors (like the weather). And no one would claim that the various futures markets were not separable from treasuries and stocks simply because they are all influenced, and strongly, by the price of crude oil. Instead, we think that the crucial thing our intuition tells us is to look for *things which have their own intrinsic dynamics.*

Furthermore, this suggests that rather than a how-to-break-it-down

problem, we really have a how-to-build-it-up issue. And gene networks, like economics, are richly hierarchical. Thus, we see the problem of defining a module in terms of the following thought experiment: Given some behavior of interest, which known facts account for that behavior? And, if there are more known facts than we need to account for the behavior of interest, what do they contribute? Hence, we have often described our approach to modularity by analogy to test-tube biochemical reconstitution, in which the procedure is to add purified components until some desired complex function emerges from the conspiracy of core parts and then try to add more purified components to see how they affect the performance or other aspects of the system of interest. No one would claim that cellular life can proceed without translation of mRNA to proteins, or that translation of the cell's protein complement can proceed without the rest of the cell's activities. No single molecular species can do it. Rather, we consider translation a unified phenomenon among the cell's activities because *biochemists can reconstitute that function from purified extracts.* So, our approach is to use computers to do the thought experiment "What if we had an animal with only these genes?" we add gene products and molecular interactions to a simulation until we get some behavior that seems lifelike.

Figure 12.4 illustrates the hierarchical nature of gene networks using the neurogenic network. At the heart of this network is a bistable switch consisting of the proneural genes, which encode basic helix-loop-helix (bHLH) transcriptional regulators, here represented by *achaete* (*ac*) and *scute* (*sc*). The products of these genes not only feed back positively on their own production; they also cross-activate each other (Martinez and Modolell 1991; Skeath and Carroll 1991; Van Doren et al. 1992). It is better to say the proneural circuit *could* make a bistable switch: there exist sets of parameter values such that there is a stable "off" state in which none of these genes is expressed, and a stable "on" state in which both are. If one pushes the system toward one or the other steady state, beyond some threshold determined by the governing parameters (most significantly those governing the potency of Ac and Sc proteins as regulators), the system will evolve toward and remain at that state until perturbed across the threshold again. For example, a sufficient pulse of *ac* transcription might, under certain conditions, be sufficient to flip the switch on. Thus, we claim this little circuit is a switch module; that is its intrinsic behavior (although it must be kept in mind that the behavior depends on parameter values, etc.).

As it happens, among the direct regulators of the proneural genes are some of the bHLH proteins encoded by genes of the *Enhancer-of-split* complex (E(spl)-C) genes (Oellers et al. 1994; hereafter we discuss these genes for simplicity as if there were a single one, say, *E(spl)*-m8

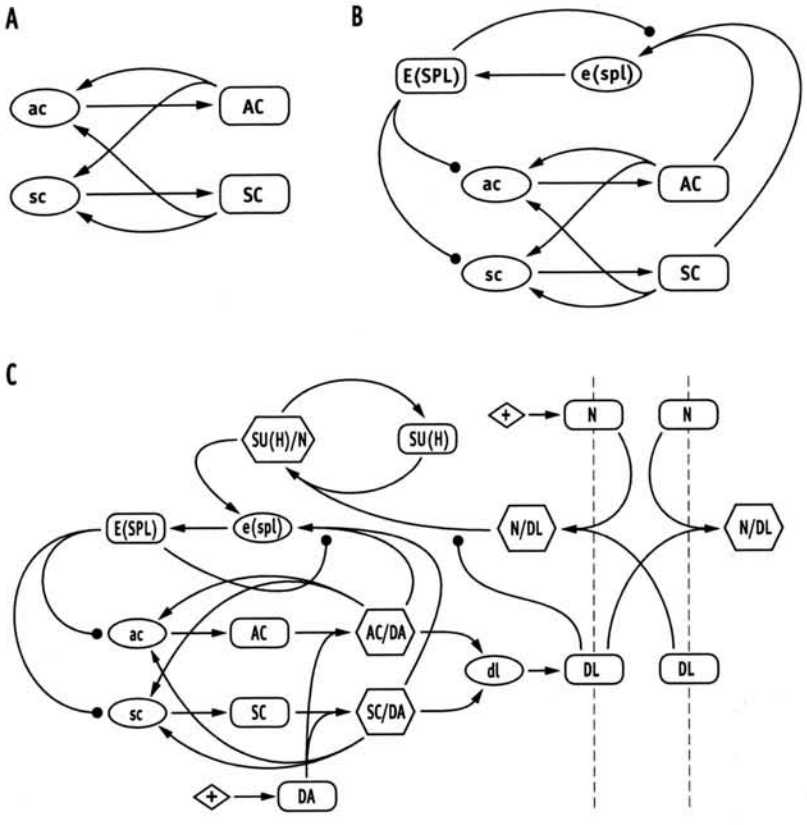


Fig. 12.4.—A hierarchy of modules. The kernel of the neurogenic network is a switch packaged into a homeostat. *A*, The proneural genes *achaete* and *scute* encode transcription factors that stimulate their own and each other's production. As long as these interactions are cooperative, this positive feedback loop can be a bistable switch. If the synthesis rate of Ac and Sc is sufficient to overwhelm degradation, their concentration increases until the synthesis rates saturate, degradation catches up, and the system remains at a stable "on" state; otherwise, degradation turns the switch off. *B*, *Enhancer of split* is a direct target of Ac and Sc activation, but its product shuts them down. At the same time it interferes with its own activation by the Ac and Sc. This circuit can still function as a switch but can also hold an intermediate steady state or oscillate around some middle expression level. *C*, The Delta/Notch signaling pathway couples the proneural/E(spl) homeostat in one cell to the same circuit in neighboring cells, because high-level Ac and Sc (as the proneural switch heads toward "on") activates *Dl*, and activated N leads to activation of *E(spl)*, which shuts the proneural switch off. DA, Daughterless; SU(H), Suppressor of Hairless.

itself). *E(spl)* not only represses the proneural genes, but is also a direct target of them; Ac and Sc activate *E(spl)* transcription (Kramatschek and Campos-Ortega 1994; Singson et al. 1994). Thus, layered around the proneural switch is a negative feedback loop. In addition, E(spl) interferes with its own activation by Ac and Sc. The larger circuit retains the ability to make a bistable switch, albeit the volume fraction of parameter space in which it does so is, while quite large, still much smaller than the analogous fraction for the proneural switch

without *E(spl)*. The negative feedback loop adds interesting new behaviors: under some conditions the circuit oscillates; under other conditions it achieves a stable intermediate state, neither on nor off. In either case it is obvious that if E(spl) were suddenly unplugged, we would be left with the proneural switch; that is, the new loop enables the circuit to sit still or wobble around between on and off, undecided, until some extrinsic influence comes along and defeats E(spl).

These clever switches-within-homeostats, one in every cell of some field, are coupled to each other through cell-cell signaling via *Delta* (*Dl*) and *Notch* (*N*). The proneural genes promote *Dl* expression (Kunisch et al. 1994); *Dl* encodes the ligand for a receptor encoded by *N* (Fehon et al. 1990); Notch, upon binding Dl, gets cleaved, and the intracellular portion forms a complex with the transcription factor encoded by *Suppressor of Hairless* (*Su(H)*); together they activate *E(spl)*, which represses the proneural genes (Bailey and Posakony 1995; Lecourtois and Schweisguth 1995). In a cluster of equipotent cells, in which some influence has gotten the proneural switch started (perhaps to the intermediate "deciding" state), the idea is that, because of stochastic differences or initial prepatterns or even specific localized signals, one cell might get a little bit ahead of the others in Dl production, or behind in N activity, such that it experiences less N signaling than the others, and thus flips the proneural switch on, consequently entraining neighbors to switch the same switch *off* (reviewed by Simpson 1997). We have shown that this mechanism is plausible; that is, a model encompassing the facts diagrammed in figure 12.4, *C*, succeeds in picking out a lucky neuroblast and shutting its neighbors off, if there is some initial difference to go on (Meir et al. 2002a).

Now, when we contrast parts *A*, *B*, and *C* of figure 12.4, which one is the module? We say all of them. The proneural switch is no less a switch because of the presence of *E(spl)*, even if *E(spl)*, when unmolested by extrinsic factors, completely abolishes switching. Similarly, the mutual entrainment of switches in a cluster of cells by Dl-N signaling in no way negates the fact that the *Ac-Sc-E(spl)* circuit has certain intrinsic behaviors. Modularity criteria based on connection density or strength would have a hard time putting a pair of scissors into figure 12.4, *B*, or even 12.4, *C*. That is why we prefer to think of genetic modularity in terms of the intrinsic functional behavior of some network.

It was only recently that we fully appreciated the hierarchical organization of the segment polarity network. That network, if we consider a version that incorporates *sloppy-paired* (fig 12.5, *A*), consists of two subnetworks: one based on the organization of the Hh signaling pathway, and the other, we think, based on interactions between targets of Wg signaling. The former (fig. 12.5, *C*) makes center-surround patterns (a Hh-producing center surrounded by cells expressing genes

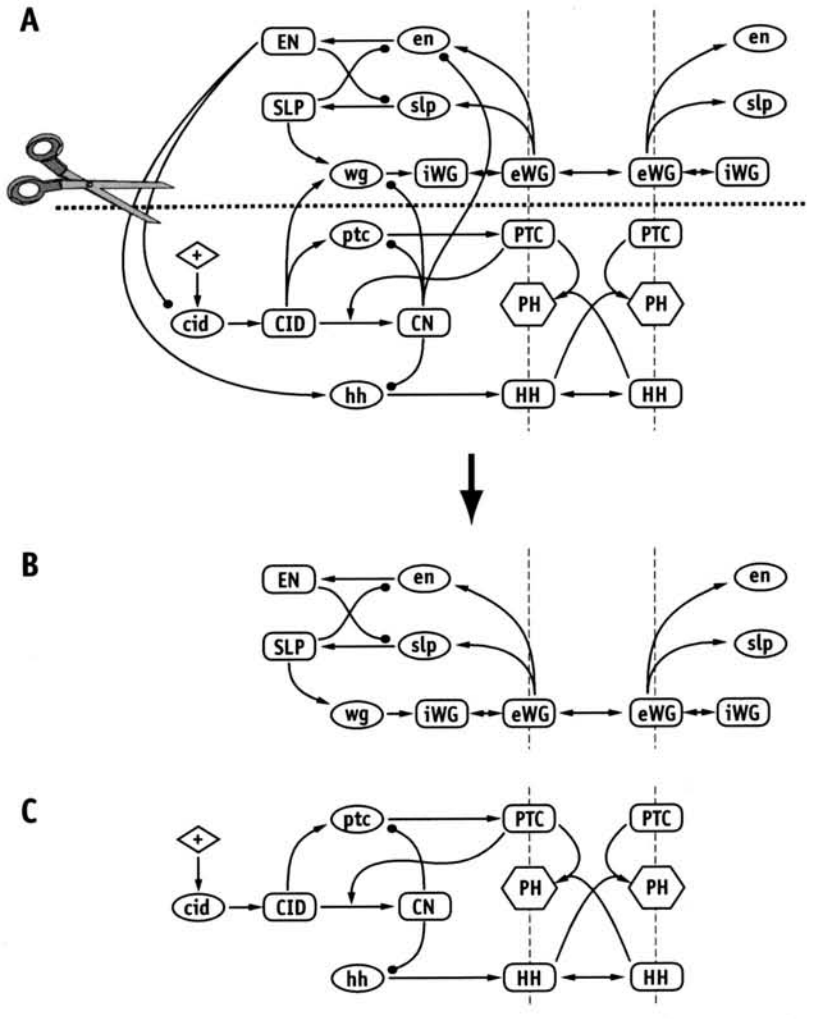


Fig. 12.5.—The segment polarity network is two subnetworks patched together. *A*, A version of the segment polarity network that shows how *sloppy-paired* might mediate *wingless* autoregulation. This network could be snipped in the middle to yield two subnets that each make center-surround patterns on their own: a coupled positive and negative feedback loop consisting of *wg*, *en*, and *slp* (*B*), and the Hh-Ptc-Ci signaling pathway (*C*).

regulated by Ci, the transcription factor that mediates the response to Hh); the Hh-binding component of the Hh receptor is encoded by the gene *ptc*, which is also activated by full-length Ci and repressed by the N-terminal fragment of Ci. Hh signaling inhibits cleavage of Ci to form the repressor. Thus, Hh signaling leads to increased Ptc expression at the cell surface, which sequesters Hh and limits the range of signaling. This subnetwork is unable to make asymmetric boundaries, the way the complete segment polarity network does.

The other subnetwork may consist of *en*, *wg*, and *slp*. *slp* encodes a transcriptional regulator that represses *en*, activates *wg*, and is activated by Wg and repressed by En (fig. 12.5, *B*) (Bhat et al. 2000; Cadigan et al. 1994b; Grossniklaus et al. 1992; Lee and Frasch 2000). This circuit, too, makes center-surround patterns, but recently we realized that under certain conditions this little subnetwork is able to do the same task as the whole segment polarity network. However, it does that task *much less robustly* than the complete network. Thus, by patching together two center-surround makers, one of which could have been the ancestral asymmetric-boundary module, we get a larger network that does the task of maintaining an asymmetric boundary very robustly. In no sense does the larger network invalidate the existence of the building blocks it is made out of; indeed, it is the *hh-ptc-ci* circuit that seems to have been co-opted most readily over the course of evolution for new roles (see, e.g., Goodrich et al. 1996).

Gene Networks and the Adaptive Landscape

Sewall Wright's metaphor of the "adaptive landscape" (see Futuyma 1998) conceives of a high-dimensional topography in which each phenotype (or genotype) is a point in the space of character states and associated with some fitness value (the independent variable that is the "height"). Thus, fitness is a function of phenotype (or genotype, if one prefers to think in terms of fitness as a function of continuously varying genetic traits), and the surface defined by *that* function is the landscape which evolving organisms populate, driven across it by mutation and winnowed by selection. The topography of the adaptive landscape constrains the evolvability of the traits that determine the landscape itself. Figure 12.6 diagrams some simplistic stereotypes in which fitness is a function of a single quantitatively varying trait. Intuitively, some of these possibilities will be easier to navigate using mutation, selection, and recombination. Should a population find itself on the sloping, low hill in figure 12.6, *A*, there is a trivial path by mutations of small effect (assuming that mutations can quantitatively affect this trait along the entire axis shown) that leads through selectively favored intermediates to the top. Not so for figure 12.6, *E*, although it is hard to imagine such a function for a simple quantitative trait. Even so, the point is that there may be no path (or even one that is easy to find by random mutation) that allows a population to move from local, but suboptimal, peaks to regions of the trait space associated with higher fitness values. Ruggedness in the fitness function may thus seriously constrain the rate of adaptation. Different shapes of the fitness function lead one to expect different levels of within-population diversity; the plateau of

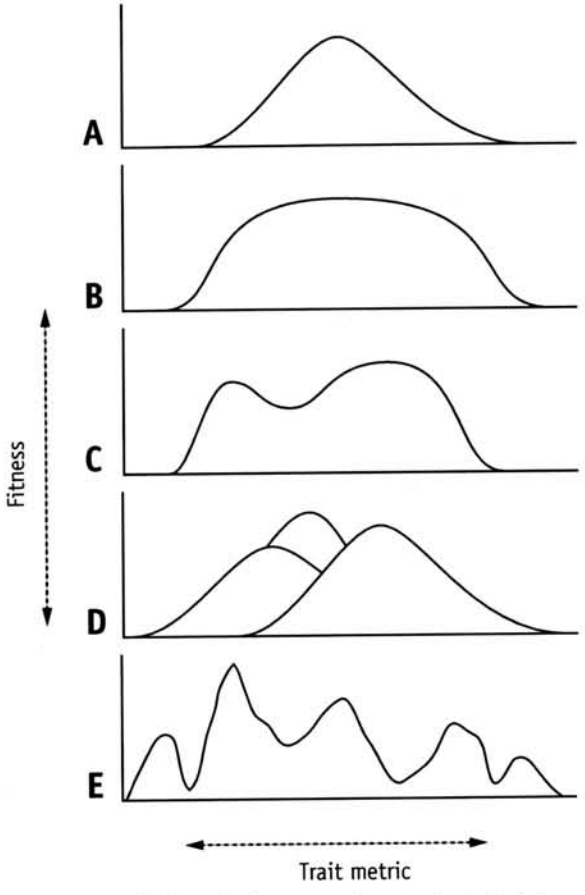


Fig. 12.6.—Possible adaptive landscapes in one dimension. An adaptive landscape is the surface defined by fitness as a function of traits, trait metrics, or character states. For simplicity this figure assumes that the traits of interest are continuous variables of which fitness is a continuous function. Traits of interest might be either phenotypic characters, such as the length of a limb, or genotypic or physiological characters, such as the affinity of an enzyme for its substrate. Mutation causes populations to diffuse across the adaptive landscape; selection causes populations to climb. In *A* there is a Fuji-like smooth-sided peak. If selection is strong enough, then the entire population should eventually cluster around the peak, because starting at any trait value there is a monotonic path to the trait value with the highest fitness. *B* shows a mesa. Again, monotonic paths lead to the top, but in this case a wide range of trait values are virtually indistinguishable. In *C* two peaks are divided by an alpine valley; both peaks are nearly equivalent in fitness, but unless mutation is very strong relative to selection, a population will probably not travel between peaks and instead will cluster around whichever peak it arrives at first. In *D* there is a mountain range, illustrating the possibility that the most fit value for a particular trait might depend on the environment, the genetic background, or even the makeup of the population. Finally, *E* illustrates that it is conceivable that adaptive landscapes could be very rugged indeed. A population trying to navigate *E* faces an adaptive Catch-22: if mutation is weak relative to selection, the population will become trapped on local, but seriously suboptimal, peaks, but if mutation is strong, then the population is very unlikely to be able to remain on any peak that it does find. If ruggedness were very common, we might expect to find that mutation rates, the rate of recombination, and the degree to which the effects of mutations are buffered from phenotypic effects are facultatively variable properties of individual organisms.

figure 12.6, *B*, would allow mutation to disperse populations across the most fit domain with a spectrum of neutral phenotypic variation.

By analogy with the adaptive landscape, consider the surface mapped out by the goodness-of-fit function we employ to evaluate the behavior of gene network models. If we were to pretend that only a single behavior of the gene network was functional, or rather that our function captures everything significant about that network's behavior, independent of ecology, then the surface determined by the objective function would be a *proxy* for the adaptive landscape. We can ask how easy this landscape would be to navigate by a local search in parameter space (analogous to what evolution accomplishes by selecting upon heritable variation in populations). We can ask how structural or architectural features of the network determine the topography of this landscape (for which we do not have a catchy name). We might be able to ask what kinds of mutations are likely to be neutral, and which might result in what kinds of phenotypic variation. Obviously no one can yet answer these questions, but the ever-improving knowledge of how limbs, fins, eyes, eyespots, teeth, toes, and so on are actually made during development is surely opening up this line of exploration. If biologists can develop a picture of the genetic module underlying any of these phenotypic modules, perhaps it will become possible to draw parallels between evolutionary trends manifest in nature and the behavioral repertoires of the genetic networks that shape development.

We have used several rudimentary approaches to come up with a caricature of the terrain in which the segment polarity and neurogenic networks live (von Dassow et al. 2000; von Dassow and Odell 2002; Meir et al. 2002b). Again, the parameter space combined with the goodness-of-fit function we use to judge the pattern produced by the model maps out a topography analogous to the adaptive landscape,[10] but we invert it for mathematical convenience. This function monitors the model's dynamics for measurable qualities that could possibly correspond to adaptive qualities of the segmentation mechanism (see supplement to von Dassow et al. 2000 for details). For example, in the case of the segment polarity network, the model gets better scores the earlier it achieves the desired pattern, the more stable that pattern is, and the sharper the definition of the pattern is; maybe there is evolutionary pressure to develop faster, perhaps oscillations lead to unreliability in quantitative control of downstream modules, and maybe sharply differentiated cell states are more stable than poorly differentiated ones. We can use sampling strategies, or various nonlinear optimization methods, to ask how easily we can navigate the parameter space of the model. The most straightforward approach is to cut transects across the parameter space: starting from a point at which the model gets a good score, we can hold all but one parameter fixed and then

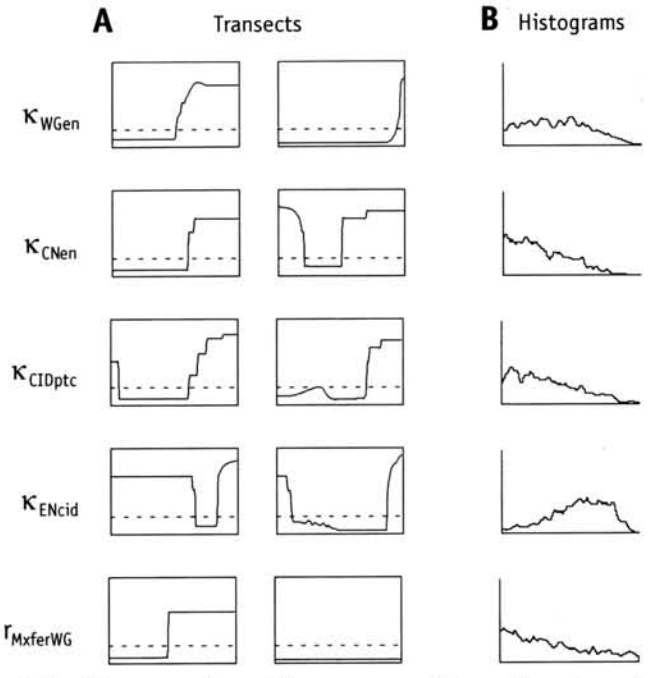


Fig. 12.7.—Profiles of the segment polarity model's parameter space. *A*, Transects along each named parameter's entire allowed range. Each box represents a case in which we started with a parameter set that worked, then varied a single parameter while holding the others fixed, and monitored the behavior of the model. The horizontal axis is the range of variation, which is three orders of magnitude, on a log scale. The vertical axis is the score the model received at that point in parameter space. The dashed line represents the cutoff *above* which we judge the model to have failed. Although the scoring function is designed to respond linearly around this region, note the predominance of sharp thresholds rather than slopes. Two cases are shown for each of five parameters which, from top to bottom, govern how potently Wg activates *en*, how effective CN is at repressing *en*, how effective Ci is at activating *ptc*, how potently En represses *ci*, and how fast Wg equilibrates between apposite faces of neighboring cells (the Wg "diffusion" rate). For the first four, the left side represents potent regulation, the right side weak regulation; for the Wg diffusion rate, the left side represents slow transport, the right side fast. The narrow gap in the fourth row of the first column represents approximately fourfold variation in that particular parameter. At the other extreme, the fifth row of the second column is a case in which the model is completely insensitive to this parameter. *B*, Histograms showing the frequency of working parameter sets as a function of the same parameters as in *A*. The horizontal axis is the same as in *A*. Approximately 1,200 working parameter sets, found in a random sample, are represented here. The interpretation is that working parameter sets are most dense wherever there are peaks in the distribution. For example, the fourth row means that the model is most likely to work when En is a moderately weak, but not *too* weak, repressor of *ci*. The plots in *A* are taken from von Dassow et al. 2000, and those in *B* are taken from von Dassow and Odell 2002.

vary the remaining parameter over several orders of magnitude while tracing the goodness-of-fit function. Several such transects are depicted in figure 12.7, *A*, and they are quite typical: a wide, flat-bottomed canyon, bounded by steep-walled cliffs (compare to fig. 12.6).

One can look for regions of the parameter space in which working sets are especially frequent. Given enough randomly sampled working parameter sets, we can do this by looking at histograms showing the

distribution of values for each parameter among all the working sets found within defined boundaries. Some parameter distributions exhibit biases toward some neighborhood within their allowed range, and others do not (fig. 12.7, *B*). We can bracket the peaks and thus narrow the boundaries of the parameter space. For the segment polarity network, we found (von Dassow and Odell 2002) that bracketing the modes with a tenfold range, instead of the thousandfold range in the original sampling, yielded a hit rate of 4 in 5, rather than the 1 in 200 reported for the original search. Thus, for this model there is a vast central canyon in which it is hard to find parameter sets that do *not* work.

In addition, we have tried optimization strategies to test whether one can get from outlying regions of parameter space into the central basin, and it seems that one can. However, the difficulty is that the typical sample point lands either above the canyon rim or on a flat, or at best gently sloping, canyon floor (see transects in fig. 12.7, *A*), and most nonlinear optimization strategies cannot tell where to go from either starting point. One can perturb the parameter set, trying to get the optimizer to ride down the ridgelines instead, and often this enables the optimizer to stumble its way into the central basin (G. von Dassow, unpublished observations). However, so far the most useful strategy has been to mimic what populations do: mutate and recombine (described in Meir et al. 2002b). It turns out that both models have a Grand Canyon in the middle of parameter space, within which the network tolerates essentially neutral variation in every parameter, and many tributaries feed into this canyon from its edges.

The discussion above pretends that the segment polarity network has a single functional behavior, and that we know exactly how to characterize it. This is because we have focused on its role in segmentation, which is relatively well understood, and on the question of whether it is plausible that this circuit could be dissociated from upstream developmental pattern-forming processes. Because of our original motivations in making this model, we have so far explored much less about how the same network could itself generate phenotypic variation, but it is certain that the real segment polarity network has been a major player in the evolution of the insects. In *Drosophila* and other insects, the segment polarity network or a variant thereof provides the basic plan for all the appendage primordia; it is involved in the patterning of the *Drosophila* gut; it lays out the pattern of cuticle structures in the larva; and so on. This module has found re-use in a wide variety of contexts in *Drosophila* alone, and in each case it is used to do something slightly different. Along the anterior-posterior compartment boundary in the imaginal discs, this module establishes a system of morphogens with complex responses by neurogenic patterning circuits (Mullor et al. 1997), vein-producing mechanisms in the wing (Gomez-Skarmeta and Modolell 1996), the proximo-distal patterning process in the leg (Diaz-Benjumea et al. 1994), and more. In the butterfly wing the segment polarity genes are re-deployed to position the eyespot (Keys et al. 1999). In each case one might hypothesize that "developmental context," whatever that is, selects among the various behaviors in the repertoire of the segment polarity module.

However, the modularity notion cannot go too far: it is not at all clear, in each of these cases, that the "module" is really the same. For instance, in imaginal discs it is impossible to imagine that *engrailed* depends on Wingless signaling since *engrailed* is expressed throughout the entire posterior compartment and Wingless only in a narrow stripe along the anterior-posterior boundary in leg discs, and in an even less suggestive pattern in wing discs (Baker 1988). However, in the context of embryonic segment specification, both in reality (Heemskerk et al. 1991) and in the more detailed versions of our model, Wg signaling is required only to get *engrailed* through an initial phase. Perhaps thereafter *en* expression is clonally inherited (or, in effect, autoactivated), even in all the posterior compartment cells of the disc. Realistically, the picture is somewhat more complicated, because in discs (but perhaps not in embryos) ectopic Hh expression can induce *en* and establish a novel posterior compartment (Gibson and Schubiger 1999; Guillen et al. 1995). Surely, aside from nice, pat ideas about re-deployment of modules and so forth, the evolutionary process will adapt every instance in which the segment polarity gene network is used according to the particular pressures on the trait in question and according to the opportunities the network affords for modifying its behavior.

In our future work we will attempt to account for how this network has been re-deployed in so many contexts, and what is different about the way it works in each one. In which cases has the network been restructured to perform different tasks (i.e., to make variant modules), and in which cases do extrinsic factors (such as the initial prepattern) merely select among the behaviors of the module? We have some preliminary ideas about what the module *could do* given different initial conditions or parameters or even topologies. For example, figure 12.8 shows a few alternate patterns produced by the simplest version of the model. This repertoire (which is incomplete, consisting of just a few patterns that are easy to find and are stable) emerges from variation in parameters but identical initial conditions (referred to as the "crisp" stripe prepattern in von Dassow et al. 2000). Parameter variation is analogous to quantitative changes in gene function but also could represent modulation by extrinsic control. Thus, figure 12.8 shows that the same topology could "do something else" if either mutations or extrinsic factors could tune it up appropriately. For a hypothetical example, some transcription factor, extrinsic to the segment polarity mod-

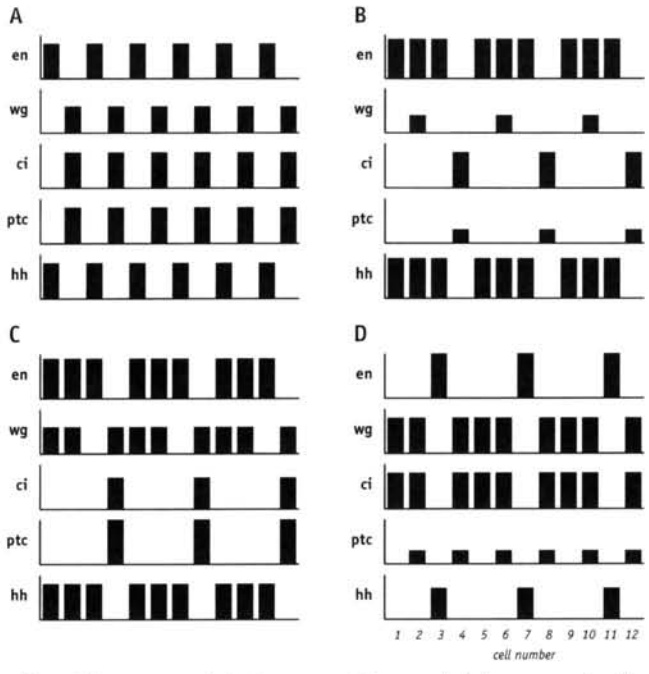


Fig. 12.8.—Near-neighbor patterns made by the segment polarity network. Each pattern results with one in every few hundred or thousand random parameter sets. *A*, A degenerate pattern, the only striped pattern the model could make without *wg* autoregulation and repression of *en* by CN. *B*, A pattern resulting from weak or no repression of *en*. *C*, Broad, overlapping stripes of *wg* and *en*. *D*, The pattern resulting if *wg* is strongly activated by Ci but weakly repressed by CN. For all of these, parameter sets that make these patterns are easy to find in the neighborhood of parameter sets that make the standard pattern.

ule, could "tune" the module's behavior by modulating the effect of, say, CN on *engrailed*, thus causing the model to make a different pattern (such as fig. 12.8, *B*) in some developmental context where that extrinsic factor is expressed. This scenario corresponds to the notion of selector genes which locally modulate developmental processes; the Hox complex genes, *engrailed*, and *vestigial* are just a few of the genes known to behave as selectors with respect to processes as diverse as denticle patterns, neuroblast formation, and adult appendage development. Another repertoire (not shown) results from variation in the initial conditions. Both sets represent a sample of the near neighbors in a pattern morphospace that can be explored by tuning the control of the segment polarity network. In other words, they're what's just up the canyon rim. While we cannot yet pin any one of them to a specific, real instance in a living organism, surely this "tuning" analogy captures something analogous to the way in which the evolutionary process tinkers with its tools.

Analogies for Genetic Architecture

He his fabric of the heav'ns hath left to their disputes, perhaps to move his laughter at their quaint opinions wide hereafter, when they come to model heaven and calculate the stars . . . how build, unbuild, contrive, to save appearances.
—Raphael to Adam, in John Milton's *Paradise Lost*

It seems tempting (to us and others) to compare genetic networks to other more familiar or man-made networks: electronic circuits, neural networks (the modeler's kind or the real thing), computer code, the internet, and so on. Analogies are at least as useful for the distinctions as for the similarities. Here we want to critique a common analogy with computer programming code.

One could possibly argue that the prevalence of the "developmental pathway," the "genetic program," or related metaphors owes something, historically, to the development of serial-instruction-chain computing machines. It would be interesting to know in a scholarly way, but our impression is that the embryology literature prior to the invention of digital, instruction-chain computers does not emphasize the notion of a chain of instructions, with the possible exception of the literature on induction phenomena. Even in the case of induction, most of the discussion took place in the context of the dynamical notion of the "morphogenetic field." Since the elaboration of the Central Dogma of Molecular Biology, pathway and program metaphors seem much more common, and developmental biologists do not seem to have used the notion of a morphogenetic field as if it had, any longer, the same explanatory power that it had been invested with in an earlier era.

So it is tempting to draw a parallel between the hypothesis of genetic modularity and object-oriented computer programming. Object-oriented programming means dividing up a computer program into building blocks that each encapsulate certain procedures, functions, and data. Each object is an instance of a "class" definition; the class defines a set of behaviors for all objects of that type and defines which data those objects store. The class also defines the "interface" of objects of that type: what messages they know how to interpret, what data they allow other objects access to, and so on. Once the programmer has defined all the classes that make an entire program, running the program means populating the computer with instances of those classes (the objects), letting them communicate and do whatever they need to do to interact with inputs (like the user) or generate outputs. One of the chief advantages of object-oriented programming is that, if done right, the building blocks not only can be used to build other structures, but also can be swapped with new versions as long as the interface remains the same. Our gene network simulation program, In-

geneue, was written in the object-oriented language Java. It is highly modular: everything from the user interface to the numerical routines to the terms in the differential equations is a class definition that, at runtime, gets instantiated into a bunch of objects as needed. When we need a new piece of a certain type, we need only make sure that it has the right interface. If we need to improve, say, the numerical integration routine, the rest of the program is none the wiser, because we simply replace the internal methods of the integrator module, without changing the interface.

Analogously, it is tempting to think of gene networks as building blocks for the larger programs of development. The segment polarity network has its own behaviors, states, and inputs (the pair-rule genes) and outputs (signals like Wingless and Hedgehog, transcription factors like Sloppy-paired, Gooseberry, and Engrailed), and a lot of "internal" machinery (the mediators of Wg and Hh signaling) that seem, when we look at a network diagram, neither input nor output. We like to think of the evolutionary process co-opting gene networks to use them in new contexts. Objects, like gene networks, are hierarchical; larger-scale building blocks contain smaller ones, and so on. But this analogy is deceptive in several ways: first of all, in the case of gene networks, the distinction between inputs, outputs, and internal methods is an artifact of how we choose to think about things, whereas in the case of software objects, it is a fact of life enforced by syntax.

Probably the most important of all is the issue of data hiding. Good object-oriented code requires data hiding, meaning that objects cannot interfere with the methods and data stored by other objects, unless granted a specific right to do so through defined methods for access. Nature cannot do data hiding. The closest thing to data hiding in gene networks is cellular compartmentalization. At the level of the networks we work with, data hiding is almost a meaningless notion: *nothing whatsoever* prevents some other network from fooling around with the "internal" workings of the neurogenic or segment polarity networks.[11] In fact, this is one of the things about genetic architecture that makes it so creative a substrate for evolution: nature can rewire it in all sorts of ways. For example, the neurogenic network may, in some cases, choose the winning neuroblast because local expression of Wingless, which just happens to bind to Notch and prevent it from functioning as a Delta receptor, reduces the amount of Notch available for signaling (see Wesley 1999; Wesley and Saez 2000 for evidence that Wg interacts with N; we do not know of direct evidence that Wg biases proneural cluster selection by such a mechanism). The closest that object-oriented code can come to *that* sort of thing is through the mechanism of inheritance, in which the programmer can define a subclass of a class she wants to modify, and add methods to it.

Another analogy, which we also find instructive, is to think of gene networks the same way that biochemists think about protein folding. Each amino acid has certain properties, such as physical size, and polarity. Within each small stretch of peptide chain, those properties determine the tendency of that stretch to adopt a particular secondary structure. The tertiary structure emerges from the secondary, and the quaternary from the tertiary. As with gene networks, each of the fundamental components is at once a potential input and a potential output (as long as it ends up on the surface of something such that it can interact with something else, even another domain of the protein, as when buried in the center). But what we find most appealing about this analogy is that in both cases, gene networks and protein folding, the emergence of a coherent higher-level structure subsumes the role of the lower-level building blocks as units of function or selection. In most cases we do not think of amino acid residues as units of function unless we are talking about how secondary structure arises; increasingly, we talk about the function of most individual genes only in the context of how they participate in networks. There are exceptions: the three crucial residues, positioned just so, in a serine protease, the crucial phosphorylatable tyrosines in a signaling protein, or those genes like superoxide dismutase whose function is their own apart from any network. But for most genes, they are not the units of interest once we get to the network level: it is the whole conspiracy we care about.

Limitations

WE DON'T RENT PIGS
—Augustus McCrae's sign, in Larry McMurtry's *Lonesome Dove*

To close we highlight four considerations that limit the usefulness of the approach we have developed over the past several years. Other issues specific to mathematical formulation are treated elsewhere (Meir et al. 2002b).

First, the models we have constructed required a wealth of information about the detailed circuitry of gene networks that is unlikely to be available in more than a few paradigm cases for the near future. This immediately raises certain questions about whether our conclusions should generalize to gene networks in the abstract. Both the segment polarity and neurogenic networks were (and still are being) worked out by armies of diligent molecular biologists, over the course of perhaps as much as a dozen man-millennia for each case, and it is highly unlikely that most developmental mechanisms will ever receive quite as much effort. We do not know how typical these mechanisms really are. R. Strathmann (personal communication) has suggested that

developmental mechanisms and developmental biologists coevolve: the nice, tidy modular mechanisms are more easily understood, leading to more fame and money for the biologists who choose to study them, in turn attracting more talent to the now-paradigmatic cases, and so on. Thus, we must keep in mind that our findings may document interesting properties only of the particular networks we have worked with, and we need further work (possibly involving randomly wired network models) to know whether our conclusions have general import.

A second major limitation is a methodological one: our focus on the parameter space as the central problem in gene networks imposes both a computational and conceptual burden. This burden may be so great as to prevent our methods from being useful for problems much more complex than the models we have treated here, especially if robustness turns out *not* to be a general feature of gene networks. While the mathematical formulation we use may be applicable to most gene network problems, it may be impossible to confront most problems using such a blunt tool as a random search in a wide rectangle in parameter space. We continue to research more sophisticated strategies to extend our methods, but from our experience the major challenges appear to be versatile methods for pattern recognition, rational approaches to defining the "reasonable" ranges of parameters, and developing a comprehensive library of formulas to deal with diverse and complex regulatory relationships between macromolecules. We expect to solve these problems as we develop experience with more and more case studies. Meanwhile, we expect to lessen the computational burden through numerical techniques such as the integration recipe described by Meir et al. (2002a), and a related fixed-point iteration method developed by E. Munro (unpublished), but there may be nothing for it in the case of a truly complex, yet not terribly robust, network.

Third is a tactical limit: we doubt seriously that biologists will be able to use models like ours to infer network topologies from DNA microarray (or similar) data on correlated patterns of gene expression. Even if it becomes feasible to obtain large-scale gene expression data from embryos, we fear it will be very difficult to deduce the wiring of such tightly looped networks as we have dealt with from such data sets. This may not be true in every case; "networks" that consist only of simple cascades with little feedback and cross-talk will surely be straightforward to deduce. But how do we know what we are dealing with a priori? Imagine if we tried to understand the segment polarity network from DNA microarray data alone, using models as a deductive aid. Say we gathered data on wild-type versus *engrailed* embryos and discovered that the expression of *patched* depends on *en* function; of course it does, because En causes *hedgehog* to be expressed, and *hedgehog*'s product ultimately causes *ptc* to be transcribed. But En itself represses

ptc (Hooper and Scott 1989; Hidalgo and Ingham 1990). To be sure, we might notice this if we also tried to measure responses to *en* overexpression, but we would be hard pressed to guess which effects were indirect and which direct and how each worked for even this simple fragment of the network. Perhaps one would try to compose a series of possible topologies that might account for the observed data and then choose specific experiments to decide among them. Ultimately, the combinatorics required to use network models to distinguish the possibilities would be overwhelming, and a sensible biologist would turn to experiments to figure things out. Thus, we worry that attempts to use compute-intensive nonlinear models to deduce network topologies from microarray data are doomed.[12]

Finally, the biggest problem is ascribing functions to gene networks. How, given some conspiracy of genes, are we to know what dynamical behavior they are "meant" to do in the living organism? For the segment polarity network, we used the notion, present in the literature for over a decade, that this network's job is to maintain parasegmental boundaries. For the neurogenic network, we used the notion, also present in the literature for over a decade, that its task is to mediate lateral inhibition within a cluster of equipotent cells. These ideas come *not* from the great mass of molecular data; they come *not* from synthesizing those data into mathematical models; they come, prior to the molecular facts, from careful perturbation experiments and developmental genetics—experiment, not the parts list, reveals the nature of the mechanism. For most networks, notions of function still end with statements like "genes X, Y, and Z are necessary for such-and-such an aspect of the phenotype," or "gene R is a master control gene for such-and-such a process, since activating it ectopically leads to the expression of X, Y, and Z." Sensible models demand a shift from assigning function based on phenotypic effects to assigning function in terms of intrinsic behavior. We suspect this is a matter of waiting for someone clever to do the right set of experiments and have that one critical intuition about what the process is all about. Genomics, microarrays, and all the other avatars of the Age of Ugly Facts hopefully make that process easier (rather than simply overwhelming it).

Lawrence and Sampedro (1993) opened a critique of early efforts to concoct a molecular "explanation" of the segment polarity network with a quote that bears repeating, Banquo's reaction to Macbeth's prophetic witches:

> The instruments of darkness tell us truths;
> Win us with honest trifles, to betray's
> In deepest consequence.—
> (Banquo, in William Shakespeare's *Macbeth*, act 1, scene 3)

Whether Lawrence and Sampedro had in mind the molecular biologists or the theorists in the role of the witches, or both, we do not presume to guess. The point is that the crucial step is an insight into the nature of the mechanism and the bounds of the device, and the Parts Catalog of Life will not tell anyone what all those parts are supposed to add up to. In every historical case we can think of, it has been perturbation experiments (physical, molecular, or genetic), analysis of phenotypes, and comparative studies that have brought about that crucial insight, whether the parts list follows or not.

Acknowledgments

We thank Garry Odell and Richard Strathmann for comments on an early draft of this paper, Gerhard Schlosser and Günter Wagner for constructive and stimulating critique as well as the opportunity to write it, and Virginia Rich for proofreading the manuscript. Our work on gene network models is supported by NSF grants MCB-9732702, MCB-9817081, and MCB-0090835 to G. M. Odell.

Notes

1. A little historical perspective is in order when evaluating the role of earlier theoretical efforts. Turing, Meinhardt, and their followers were trying to imagine how development could work at a time when very little was known about how development does work. The same is true of other theoretical attempts of the same era. All those efforts, given the history of ideas in developmental biology, ought to be seen as remarkable successes: they managed to show that developmental pattern formation could be due to relatively straightforward chemical processes, a conclusion that many classical embryologists, most famously Hans Driesch, had trouble accepting. Thus, such models gave direct encouragement to nascent attempts at developmental genetics: if simple chemical processes could explain development, then there was real reason to hope to discover the developmental control genes and understand their function. Is it possible that *Drosophila* developmental genetics, and the Age of Ugly Facts, was actually inspired by the Beautiful Theories? We suspect that what those theories did is convince a critical mass of people that is was worth looking for the molecular basis of morphogenesis because it might be a simple thing after all. At the same time we suspect that if developmental geneticists of the 1960s and 1970s had stuck with the sentiments of classical embryologists, they might have given up hope. A passing investigation shows that a significant fraction of the embryological literature of the first half of the 20th century reads like a hymn to vital forces; even the nonvitalists among the embryological community were unlikely to be committed reductionists (see the excellent volume edited by Scott Gilbert [Gilbert 1994], and also his recent review [Gilbert et al. 1996]). A relatively small number of workers, especially Waddington, Weiss, and Needham, appreciated the potential of genetics and biochemistry to reveal mechanistic explanations of complex developmental phenomena (Needham 1942; Waddington and Kacser 1957; Weiss 1968). Gierer and Meinhardt, Wolpert, Slack, Kauffman, and other contributors to theoretical developmental biology of the 1970s, constitute an important part of the intellectual weave that connects the morass of classical embryological phenomenology to the equally bewildering morass of modern molecular developmental genetics.

2. This codependence lasts, literally, for a couple of hours; it is a bridge between the transient input provided by the pair-rule genes and longer-term mechanisms for stabilizing cell states within the segment. By the end of germ-band extension, *en* expression no longer depends on *wg* (Heemskerk et al. 1991). Perhaps this is because En represses factors that would otherwise turn En off, and because perhaps, after the initial phase, there is enough free Arm in the absence of Wg signaling to allow *en* transcription in the absence of repressors. Most versions of the segment polarity model, except the simplest, have the potential for this kind of "En autoregulation."

3. To our knowledge, only Cadigan and Grossniklaus and colleagues (Cadigan et al. 1994a; Cadigan et al. 1994b; Grossniklaus et al. 1992), who characterized *sloppy-paired*, highlighted the need for something to keep *en* off in the anterior compartment.

4. These remarks apply to the version of the segment polarity network shown in figure 1. Other versions that incorporate additional components and interactions (von Dassow and Odell 2002) alleviate some of the problems described in this scenario. For example, versions that include *sloppy-paired* do not depend so much on Ci providing an early assist to *wg* expression, because *slp* is expressed early enough to fulfill that role. Nevertheless, the overall description, of a race between mutually exclusive but codependent cell states, remains valid.

5. The "blind watchmaker" is Dawkins's metaphor for the evolutionary process, in which natural design is the outcome of mutation and selection. The metaphor originates with William Paley's famous argument for the existence of divinity, since to Paley a complex device like a watch implies a watchmaker.

6. What we call "cooperativity" may be due to true allosteric cooperativity, or may not; we use it as a convenient, evocative term for the steepness of nonlinear, sigmoid dose-response curves.

7. Intermediate steps provide opportunities to damp out oscillations just by sluggishness. Given a chain of responders, if one step responds on a longer time scale than the step preceding (i.e., a protein with a longer half-life than its mRNA), it will respond slowly to variations in its inputs, thus converting high-amplitude oscillations to lower-amplitude oscillations of the same frequency.

8. This passage should also serve as a reminder that "robustness" is not a unified phenomenon; instead of saying, "this device is robust," we need to say, "such-and-such a functional behavior of this device is robust to such-and-such perturbations." The first statement makes no sense without a context to specify the behavior and perturbation in question.

9. By this term we mean the tendency for randomly sampled "working" parameter sets to include values, for a particular parameter, that cluster in some neighborhood, even when there is no absolute restriction. For example, working parameter sets are about three times as dense in the "slow" third of the range we allow for the Wg diffusion rate as they are in the range as a whole.

10. We emphasize that we are taking this as a proxy for an adaptive landscape. We do not mean to conflate the two. The adaptive landscape refers to a fitness function on a manifold whose axes are either phenotypic traits or genotypic characteristics. We haven't a clue how to relate the dynamical behavior of a gene network directly to survival and reproduction; all we can do is characterize how the dynamics of the model match observed gene expression patterns, which are but a manifestation of some machinery that creates the phenotype, whose fitness is determined by the ecology the organism finds itself in. For the sake of the metaphor we are pretending that the network has to do more or less what it does in a wild-type animal, and that the better it mimics the wild-type gene expression regime, the higher its fitness.

11. But we note that at higher levels of organization, there are barriers that to some extent isolate different developmental modules from one another. For example, the spa-

tial layout and morphogenesis of embryos can either prevent or enforce cross talk between different morphogenetic fields.

12. A more suitable approach to this challenge might be the neural-network-inspired method developed by Reinitz and colleagues, in which the weights governing all possible connections within the network are tuned to achieve the best possible fit to real data (Reinitz et al. 1998; Reinitz and Sharp 1995).

References

Alcedo, J., M. Ayzenzon, T. Von Ohlen, M. Noll, and J. E. Hooper. 1996. The *Drosophila* smoothened gene encodes a seven-pass membrane protein, a putative receptor for the hedgehog signal. *Cell* 86:221–232.

Alcedo, J., and M. Noll. 1997. Hedgehog and its patched-smoothened receptor complex: a novel signalling mechanism at the cell surface. *Biol. Chem.* 378:583–590.

Alexandre, C., A. Jacinto, and P. W. Ingham. 1996. Transcriptional activation of hedgehog target genes in *Drosophila* is mediated directly by the cubitus interruptus protein, a member of the GLI family of zinc finger DNA-binding proteins. *Genes Dev.* 10:2003–2013.

Alon, U., M. G. Surette, N. Barkai, and S. Leibler. 1999. Robustness in bacterial chemotaxis. *Nature* 397:168–171.

Ancel, L., and W. Fontana. 2000. Plasticity, evolvability, and modularity in RNA. *J. Exp. Zool. (Mol. Dev. Evol.)* 288:242–283.

Anderson, and Walter. 1999. Blobel's Nobel. *Cell* 99:557–558.

Aza-Blanc, P., F. A. Ramirez-Weber, M. P. Laget, C. Schwartz, and T. B. Kornberg. 1997. Proteolysis that is inhibited by hedgehog targets Cubitus interruptus protein to the nucleus and converts it to a repressor. *Cell* 89:1043–1053.

Bailey, A. M., and J. W. Posakony. 1995. Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to Notch receptor activity. *Genes Dev.* 9:2609–2622.

Baker, N. E. 1988. Transcription of the segment-polarity gene wingless in the imaginal discs of *Drosophila*, and the phenotype of a pupal-lethal wg mutation. *Development* 102:489–497.

Barkai, N., and S. Leibler. 1997. Robustness in simple biochemical networks. *Nature* 387:913–917.

Bhanot, P., M. Brink, C. H. Samos, J. C. Hsieh, Y. Wang, J. P. Macke, D. Andrew, J. Nathans, and R. Nusse. 1996. A new member of the frizzled family from *Drosophila* functions as a Wingless receptor. *Nature* 382:225–230.

Bhanot, P., M. Fish, J. A. Jemison, R. Nusse, J. Nathans, and K. M. Cadigan. 1999. Frizzled and DFrizzled-2 function as redundant receptors for Wingless during *Drosophila* embryonic development. *Development* 126:4175–4186.

Bhat, K. M. 1998. frizzled and frizzled 2 play a partially redundant role in wingless signaling and have similar requirements to wingless in neurogenesis. *Cell* 95:1027–1036.

Bhat, K. M., E. H. van Beers, and P. Bhat. 2000. Sloppy paired acts as the downstream target of wingless in the *Drosophila* CNS and interaction between sloppy paired and gooseberry inhibits sloppy paired during neurogenesis. *Development* 127:655–665.

Brunner, E., O. Peter, L. Schweizer, and K. Basler. 1997. pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in *Drosophila*. *Nature* 385:829–833.

Cadigan, K. M., U. Grossniklaus, and W. J. Gehring. 1994a. Functional redundancy: the respective roles of the two sloppy paired genes in *Drosophila* segmentation. *Proc. Natl. Acad. Sci. U.S.A.* 91:6324–6328.

Cadigan, K. M., U. Grossniklaus, and W. J. Gehring. 1994b. Localized expression of sloppy paired protein maintains the polarity of *Drosophila* parasegments. *Genes Dev.* 8:899–913.

Cadigan, K. M., and R. Nusse. 1997. Wnt signaling: a common theme in animal development. *Genes Dev.* 11:3286–3305.

Chen, Y., and G. Struhl. 1996. Dual roles for patched in sequestering and transducing Hedgehog. *Cell* 87:553–563.

Chen, Y., and G. Struhl. 1998. In vivo evidence that Patched and Smoothened constitute distinct binding and transducing components of a Hedgehog receptor complex. *Development* 125:4943–4948.

Dawes, R., I. Dawson, F. Falciani, G. Tear, and M. Akam. 1994. Dax, a locust Hox gene related to fushi-tarazu but showing no pair-rule expression. *Development* 120:1561–1572.

Dearden, P., and M. Akam. 1999. Developmental evolution: axial patterning in insects. *Curr. Biol.* 9:R591–*R*594.

Diaz-Benjumea, F. J., B. Cohen, and S. M. Cohen. 1994. Cell interaction between compartments establishes the proximal-distal axis of *Drosophila* legs. *Nature* 372:175–179.

Dierick, H. A., and A. Bejsovec. 1998. Functional analysis of Wingless reveals a link between intercellular ligand transport and dorsal-cell-specific signaling. *Development* 125:4729–4738.

DiNardo, S., E. Sher, J. Heemskerk-Jongens, J. A. Kassis, and P. H. O'Farrell. 1988. Two-tiered regulation of spatially patterned engrailed gene expression during *Drosophila* embryogenesis. *Nature* 332:604–609.

Dominguez, M., M. Brunner, E. Hafen, and K. Basler. 1996. Sending and receiving the hedgehog signal: control by the *Drosophila* Gli protein Cubitus interruptus. *Science* 272:1621–1625.

Edgar, B. A., G. M. Odell, and G. Schubiger. 1989. A genetic switch, based on negative regulation, sharpens stripes in *Drosophila* embryos. *Dev. Genet.* 10:124–142.

Fehon, R. G., P. J. Kooh, I. Rebay, C. L. Regan, T. Xu, M. A. Muskavitch, and S. Artavanis-Tsakonas. 1990. Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in *Drosophila*. *Cell* 61:523–534.

Futuyma, D. J. 1998. Evolutionary biology. Sunderland, Mass.: Sinauer Associates.

Gibson, G. 1996. Epistasis and pleiotropy as natural properties of transcriptional regulation. *Theor. Popul. Biol.* 49:58–89.

Gibson, G., and G. Wagner. 2000. Canalization in evolutionary genetics: a stabilizing theory? *BioEssays* 22:372–380.

Gibson, M. C., and G. Schubiger. 1999. Hedgehog is required for activation of engrailed during regeneration of fragmented *Drosophila* imaginal discs. *Development* 126:1591–1599.

Gierer, A., and H. Meinhardt. 1972. A theory of biological pattern formation. *Kybernetik* 12:30–39.

Gilbert, S. F. 1994. A Conceptual history of modern embryology. Baltimore: Johns Hopkins University Press.

Gilbert, S. F., J. M. Opitz, and R. A. Raff. 1996. Resynthesizing evolutionary and developmental biology. *Dev. Biol.* 173:357–372.

Glass, L., and S. A. Kauffman. 1972. Co-operative components, spatial localization and oscillatory cellular dynamics. *J. Theor. Biol.* 34:219–237.

Glass, L., and S. A. Kauffman. 1973. The logical analysis of continuous, non-linear biochemical control networks. *J. Theor. Biol.* 39:103–129.

Gomez-Skarmeta, J. L., and J. Modolell. 1996. araucan and caupolican provide a link

between compartment subdivisions and patterning of sensory organs and veins in the *Drosophila* wing. *Genes Dev.* 10:2935–2945.

Goodrich, L. V., R. L. Johnson, L. Milenkovic, J. A. McMahon, and M. P. Scott. 1996. Conservation of the hedgehog/patched signaling pathway from flies to mice: induction of a mouse patched gene by Hedgehog. *Genes Dev.* 10:301–312.

Grossniklaus, U., R. K. Pearson, and W. J. Gehring. 1992. The *Drosophila* sloppy paired locus encodes two proteins involved in segmentation that show homology to mammalian transcription factors. *Genes Dev.* 6:1030–1051.

Guillen, I., J. L. Mullor, J. Capdevila, E. Sanchez-Herrero, G. Morata, and I. Guerrero. 1995. The function of engrailed and the specification of *Drosophila* wing pattern. *Development* 121:3447–3456.

Gutfreund, H. 1995. Kinetics for the life sciences: receptors, transmitters, and catalysts. Cambridge: Cambridge University Press.

Heemskerk, J., S. DiNardo, R. Kostriken, and P. H. O'Farrell. 1991. Multiple modes of engrailed regulation in the progression towards cell fate determination. *Nature* 352: 404–410.

Hepker, J., Q. T. Wang, C. K. Motzny, R. Holmgren, and T. V. Orenic. 1997. *Drosophila* cubitus interruptus forms a negative feedback loop with patched and regulates expression of Hedgehog target genes. *Development* 124:549–558.

Hidalgo, A., and P. Ingham. 1990. Cell patterning in the *Drosophila* segment: spatial regulation of the segment polarity gene patched. *Development* 110:291–301.

Hooper, J. E. 1994. Distinct pathways for autocrine and paracrine Wingless signalling in *Drosophila* embryos. *Nature* 372:461–464.

Hooper, J. E., and M. P. Scott. 1989. The *Drosophila* patched gene encodes a putative membrane protein required for segmental patterning. *Cell* 59:751–765.

Ingham, P. W., N. E. Baker, and A. Martinez-Arias. 1988. Regulation of segment polarity genes in the *Drosophila* blastoderm by fushi tarazu and even skipped. *Nature* 331:73–75.

Kauffman, S. A. 1993. The origins of order: self organization and selection in evolution. New York: Oxford University Press.

Keys, D. N., D. L. Lewis, J. E. Selegue, B. J. Pearson, L. V. Goodrich, R. L. Johnson, J. Gates, M. P. Scott, and S. B. Carroll. 1999. Recruitment of a hedgehog regulatory circuit in butterfly eyespot evolution. *Science* 283:532–534.

Kramatschek, B., and J. A. Campos-Ortega. 1994. Neuroectodermal transcription of the *Drosophila* neurogenic genes E(spl) and HLH-m5 is regulated by proneural genes. *Development* 120:815–826.

Kunisch, M., M. Haenlin, and J. A. Campos-Ortega. 1994. Lateral inhibition mediated by the *Drosophila* neurogenic gene delta is enhanced by proneural proteins. *Proc. Natl. Acad. Sci. U.S.A.* 91:10139–10143.

Lawrence, P. A., and J. Sampedro. 1993. *Drosophila* segmentation: after the first three hours. *Development* 119:971–976.

Lecourtois, M., and F. Schweisguth. 1995. The neurogenic suppressor of hairless DNA-binding protein mediates the transcriptional activation of the enhancer of split complex genes triggered by Notch signaling. *Genes Dev.* 9:2598–2608.

Lee, H. H., and M. Frasch. 2000. Wingless effects mesoderm patterning and ectoderm segmentation events via induction of its downstream target sloppy paired. *Development* 127:5497–5508.

Li, X., and M. Noll. 1993. Role of the gooseberry gene in *Drosophila* embryos: maintenance of wingless expression by a wingless-gooseberry autoregulatory loop. *EMBO J.* 12:4499–4509.

Manoukian, A. S., K. B. Yoffe, E. L. Wilder, and N. Perrimon. 1995. The porcupine gene is required for wingless autoregulation in *Drosophila*. *Development* 121:4037–4044.

Marigo, V., R. A. Davey, Y. Zuo, J. M. Cunningham, and C. J. Tabin. 1996. Biochemical evidence that patched is the Hedgehog receptor. *Nature* 384:176–179.

Martinez, C., and J. Modolell. 1991. Cross-regulatory interactions between the proneural achaete and scute genes of *Drosophila*. *Science* 251:1485–1487.

Martinez-Arias, A. 1993. Development and patterning of the larval epidermis of *Drosophila*. In *The development of Drosophila melanogaster*, ed. M. Bate and A. Martinez-Arias, 517–608. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

Meinhardt, H. 1977. A model of pattern formation in insect embryogenesis. *J. Cell Sci.* 23:117–139.

Meinhardt, H. 1984. Models for positional signalling, the threefold subdivision of segments and the pigmentation pattern of molluscs. *J. Embryol. Exp. Morphol.* 83 (suppl): 289–311.

Meir, E., E. M. Munro, G. M. Odell, and G. von Dassow. 2002a. Ingeneue: a versatile tool for reconstituting genetic networks, with examples from the segment polarity network. *J. Exp. Zool.* 294:216–251.

Meir, E., G. von Dassow, E. Munro E, and G. M. Odell. 2002b. Robustness, flexibility, and the role of lateral inhibition in the neurogenic network. *Curr. Biol.* 12: 778–786.

Moazed, D., and P. H. O'Farrell. 1992. Maintenance of the engrailed expression pattern by Polycomb group genes in *Drosophila*. *Development* 116:805–810.

Moline, M. M., C. Southern, and A. Bejsovec. 1999. Directionality of wingless protein transport influences epidermal patterning in the *Drosophila* embryo. *Development* 126:4375–4384.

Mullor, J. L., M. Calleja, J. Capdevila, and I. Guerrero. 1997. Hedgehog activity, independent of decapentaplegic, participates in wing disc patterning. *Development* 124: 1227–1237.

Nagy, L. M., and S. Carroll. 1994. Conservation of wingless patterning functions in the short-germ embryos of *Tribolium castaneum*. *Nature* 367:460–463.

Needham, J. 1942. Biochemistry and morphogenesis. Cambridge: Cambridge University Press.

Oellers, N., M. Dehio, and E. Knust. 1994. bHLH proteins encoded by the Enhancer of split complex of *Drosophila* negatively interfere with transcriptional activation mediated by proneural genes. *Mol. Gen. Genet.* 244:465–473.

Ohlmeyer, J. T., and D. Kalderon. 1998. Hedgehog stimulates maturation of Cubitus interruptus into a labile transcriptional activator. *Nature* 396:749–753.

Pankratz, M. J., and H. Jäckle. 1993. Blastoderm segmentation. In *The development of Drosophila melanogaster*, ed. M. Bate and A. Martinez-Arias, 467–516. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press.

Patel, N. H. 1994. The evolution of arthropod segmentation: insights from comparisons of gene expression patterns. *Development Suppl*, pp. 201–207.

Patel, N. H., E. E. Ball, and C. S. Goodman. 1992. Changing role of even-skipped during the evolution of insect pattern formation. *Nature* 357:339–342.

Patel, N. H., E. Martin-Blanco, K. G. Coleman, S. J. Poole, M. C. Ellis, T. B. Kornberg, and C. S. Goodman. 1989. Expression of engrailed proteins in arthropods, annelids, and chordates. *Cell* 58:955–968.

Reinitz J., D. Kosman C. E. Vanario-Alonso and D. H. Sharp. 1998. Stripe forming architecture of the gap gene system. *Dev. Genet.* 23:11–27.

Reinitz J., and D. H. Sharp. 1995. Mechanism of eve stripe formation. *Mech. Dev.* 49: 133–158.

Simpson, P. 1997. Notch signalling in development: on equivalence groups and asymmetric developmental potential. *Curr. Opin. Genet. Dev.* 7:537–542.

Singson, A., M. W. Leviten, A. G. Bang, X. H. Hua, and J. W. Posakony. 1994. Direct

downstream targets of proneural activators in the imaginal disc include genes involved in lateral inhibitory signaling. *Genes Dev.* 8:2058–2071.

Skeath, J. B., and S. B. Carroll. 1991. Regulation of achaete-scute gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes Dev.* 5:984–995.

Slack, J. M. W. 1983. From egg to embryo: determinative events in early development. New York: Cambridge University Press.

Stone, D. M., M. Hynes, M. Armanini, T. A. Swanson, Q. Gu, R. L. Johnson, M. P. Scott, D. Pennica, A. Goddard, H. Phillips, M. Noll, J. E. Hooper, F. de Sauvage, and A. Rosenthal. 1996. The tumour-suppressor gene patched encodes a candidate receptor for Sonic hedgehog. *Nature* 384:129–134.

Strigini, M., and S. M. Cohen. 1997. A Hedgehog activity gradient contributes to AP axial patterning of the *Drosophila* wing. *Development* 124:4697–4705.

Strigini, M., and S. M. Cohen. 2000. Wingless gradient formation in the *Drosophila* wing. *Curr. Biol.* 10:293–300.

Thieffry, D., A. M. Huerta, E. Perez-Rueda, and J. Collado-Vides. 1998. From specific gene regulation to genomic networks: a global analysis of transcriptional regulation in *Escherichia coli*. *BioEssays* 20:433–440.

Thieffry, D., and D. Romero. 1999. The modularity of biological regulatory networks. *Biosystems* 50:49–59.

Thomas, R., D. Thieffry, and M. Kaufman. 1995. Dynamical behaviour of biological regulatory networks. 1. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bull. Math. Biol.* 57:247–276.

Turing, A. M. 1952. The chemical basis of morphogenesis. *Philos. Trans. R. Soc. B Biol. Sci.* 237:37–72.

van den Heuvel, M., and P. W. Ingham. 1996. smoothened encodes a receptor-like serpentine protein required for hedgehog signalling. *Nature* 382:547–551.

van de Wetering, M., R. Cavallo, D. Dooijes, M. van Beest, J. van Es, J. Loureiro, A. Ypma, D. Hursh, T. Jones, A. Bejsovec, M. Peifer, M. Mortin, and H. Clevers. 1997. Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* 88:789–799.

Van Doren, M., P. A. Powell, D. Pasternak, A. Singson, and J. W. Posakony. 1992. Spatial regulation of proneural gene activity: auto- and cross-activation of achaete is antagonized by extramacrochaetae. *Genes Dev.* 6:2592–2605.

Vincent, J. P., and P. A. Lawrence. 1994. *Drosophila* wingless sustains engrailed expression only in adjoining cells: evidence from mosaic embryos. *Cell* 77:909–915.

Vincent, J. P., and P. H. O'Farrell. 1992. The state of engrailed expression is not clonally transmitted during early *Drosophila* development. *Cell* 68:923–931.

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

von Dassow, G., and G. M. Odell GM. 2002. Design and constraints of the *Drosophila* segment polarity module: robust spatial patterning emerges from intertwined cell state switches. *J. Exp. Zool.* 294:179–215.

Von Ohlen, T., D. Lessing, R. Nusse, and J. E. Hooper. 1997. Hedgehog signaling regulates transcription through cubitus interruptus, a sequence-specific DNA binding protein. *Proc. Natl. Acad. Sci. U.S.A.* 94:2404–2409.

Waddington, C. H., and H. Kacser. 1957. The strategy of the genes; a discussion of some aspects of theoretical biology. London: Allen and Unwin.

Wang, Q. T., and R. A. Holmgren. 1999. The subcellular localization and activity of *Drosophila* cubitus interruptus are regulated at multiple levels. *Development* 126: 5097–5106.

Weiss, P. A. 1968. Dynamics of development: experiments and inferences: selected papers on developmental biology. New York: Academic Press.

Wesley, C. S. 1999. Notch and wingless regulate expression of cuticle patterning genes. *Mol. Cell. Biol.* 19:5743–5758.

Wesley, C. S., and L. Saez. 2000. Notch responds differently to Delta and Wingless in cultured *Drosophila* cells. *J. Biol. Chem.* 275:9099–9101.

Wyman, J., and S. J. Gill. 1990. Binding and linkage: functional chemistry of biological macromolecules. Mill Valley, Calif.: University Science Books.

Yoffe, K. B., A. S. Manoukian, E. L. Wilder, A. H. Brand, and N. Perrimon. 1995. Evidence for engrailed-independent wingless autoregulation in *Drosophila*. *Dev. Biol.* 170:636–650.

Zeng, W., K. A. Wharton, Jr., J. A. Mack, K. Wang, M. Gadbaw, K. Suyama, P. S. Klein, and M. P. Scott. 2000. naked cuticle encodes an inducible antagonist of Wnt signalling. *Nature* 403:789–795.