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. . . But No Kinetic Details Needed

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With complete maps of the genome of a number of organisms in hand, researchers have turned to the next challenge: to determine the interactions between genes, the messenger RNAs and proteins they encode, and other cellular components. Signal transduction and gene regulatory networks control the conversion of extracellular signals into patterns of gene expression. Understanding how these networks function in the presence of fluctuations is a major problem that will undoubtedly require new mathematical approaches for its resolution.

The crucial role of inter-regulation amongst genes is especially evident during the development of a multicellular adult from a unicellular egg, for here what a cell becomes depends on where it is in a developing aggregate of cells [6]. Pattern formation in development refers to the spatially and temporally organized expression of genes, and this is controlled by the inputs and outputs of the gene control networks [2].

As for most networks, an understanding of gene control networks requires several ingredients: (i) the current state of the system, which here means the amounts of mRNAs, proteins, and other components needed, (ii) the wiring diagram, or topology, of the network, which encodes "who tickles whom," and (iii) the dependence of the strength of the interactions on the current state of the system. Given this information, it is possible to formulate an evolution equation for the state and to try to understand the asymptotic dynamics for various initial states.

One well-known way of describing the evolution is to use differential equations based on mass-action kinetics for the production and decay of all components; this is the approach used by Garrett Odell's group to model the segment polarity gene control network in Drosophila [5] (see accompanying article by Barry Cipra). The interactions between the mRNAs and proteins corresponding to 5 genes require 13 nonlinear differential equations with 48 kinetic parameters. After performing a systematic search in this 48-dimensional parameter space, Odell and colleagues found that biologically correct asymptotic states were achieved for a multitude of parameter combinations, if the correct initial conditions were used.

The robustness to changes in kinetic parameters suggests that it is the topology of the control network and the nature of an interaction, activation or inhibition of the target, and not the kinetic details, that determine the gene expression patterns. This led us to a model without any kinetic parameters, based solely on the net effect of the regulatory interactions between components [1]. We constructed the wiring diagram of the interactions among 7 segment polarity genes (a network topology similar to that considered by Odell's group), and formulated a Boolean description of this network in which the state of each mRNA and protein is either 1 (ON) or 0 (OFF). In our model, time is discretized into steps approximately the length of a transcription or translation event, and the next state of each node in the control network is determined by a Boolean function of its state and the state of nodes that influence it. This mapping defines a discrete dynamical system that is much easier to analyze than the differential equations.

The Boolean function for each node is determined from its state and the known activating and inhibiting interactions between nodes. For example, if an activator is present at time t, its target will be ON at time $t + 1$; in the presence of an inhibitor, the next state of a target will be OFF. When both activators and inhibitors act on a node, we assume that the inhibition is dominant; the node will therefore turn off. Examples of the Boolean rules governing the state of mRNAs (lower-case) and proteins (upper-case) in cell i are as follows [1]:

$$\begin{aligned}&\text{Translation of EN protein } EN_i^{t+1} = en_i^t\\ &\text{Transcription of } hh \text{ mRNA}\\ &\qquad hh_i^{t+1} = EN_i^t \text{ and not } CIR_i^t\\ &\text{Post-translational binding } PH_i^t = PTC_i^t\\ &\qquad \text{and } (HH_{i-1}^t \text{ or } HH_{i+1}^t) \qquad (1)\end{aligned}$$

The first step in validating a model like this is to determine whether it reproduces the "wild type," or normal behavior of the system. Starting from the known initial state of the segment polarity genes, which arises from prepatterning by genes expressed earlier (Figure 1a), we iterated the dynamical system and found that within six steps the gene expression pattern stabilizes into the state shown in Figure 1b, which is precisely the pattern observed experimentally [3, 4]. We also explored the effect of gene mutations and found that

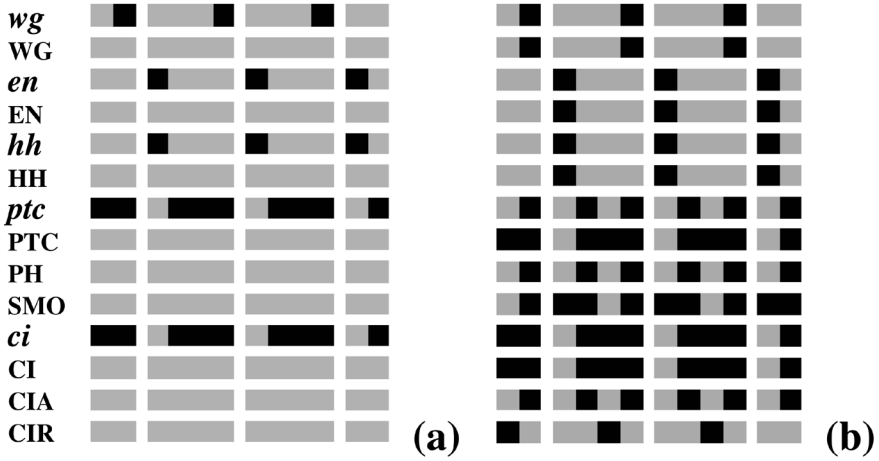

Figure 1. *The segment polarity genes have periodic patterns with a four-cell period. Horizontal rows correspond to the pattern of individual nodes specified at the left. Each shaded segment corresponds to four cells. A black (gray) box denotes a node that is ON (OFF) in the given cell. (a) The experimentally observed initial state. (b) The steady state given by the model when initialized with the pattern in (a). This pattern is in excellent agreement with the observed gene expression patterns. After [1].*

the spatial pattern of expression predicted by the model is in perfect agreement with experiments [1]. The agreement between observed and predicted patterns demonstrates that in this system a simple switching network suffices as long as the topology and character (activation or inhibition) of the interactions is correct: A detailed kinetic model is not needed at this level of description.

The model demonstrates that the combination of the wiring diagram and Boolean updating rules can successfully describe the gene regulatory network. The question now becomes whether we can integrate the two into a functional diagram that illustrates how the switching on of a node influences its neighbors and propagates through the network. The wiring diagram alone cannot do this, because a node is often regulated by two conflicting interactions.

We can, however, expand the network in such a way that repressing interactions are eliminated. For each repressing interaction, we add a new node whose state is the opposite of the repressor's; this new node will be an activator. Similarly, whenever several interactions have a combined effect, we introduce a node to express this combination [1]. One important insight we can gain from the expanded network is identifying which genes are coexpressed. In Figure 2 a path of directed edges connects *en* with *hh*, while the path starting with the complementary node nEN, expressing the absence of EN, reaches *ci*, *ptc*, and *wg*. Thus, we can conclude that the cells expressing *en* and *hh* never express *wg*, *ptc*, or *ci*, a well-known polarization that is responsible for the name "segment polarity genes" [6] but that was not evident from the wiring diagram.

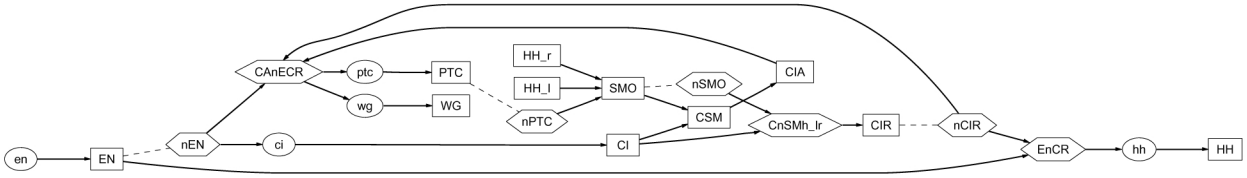

Figure 2. *The expanded network illustrates the effect of interactions. Ellipses correspond to mRNAs, rectangles to proteins; hexagons indicate pseudonodes added by the expansion process. Edges between nodes and their opposites are represented by dotted lines.*

Our experience suggests that a Boolean model which correctly integrates the topology and the nature of interactions in a gene control network can produce important insights into the dynamics of these networks. Our construction is not novel in itself; others have used Boolean models for gene control networks. What is novel is that we were able to compare the results very directly with experimental observations, and the analytical and computational effort with a more detailed kinetic model. The comparison suggests that Boolean models can play a significant role in understanding complex gene control networks.

We envision realistic topology-based Boolean modeling as an important first step in understanding the interplay between the topology and dynamics of gene control networks and in testing the completeness of available topological information. There are undoubtedly systems for which qualitative analysis must be complemented by more detailed kinetic models, but the segment polarity genes have proven that the robustness of cellular networks works in our favor.

References

[1] R. Albert and H.G. Othmer, *The topology of the regulatory interactions predicts the expression pattern of the Drosophila segment polarity genes*, J. Theor. Biol., 233 (2003), 1–18.

[2] E.H. Davidson, *Genomic Regulatory Systems: Development and Evolution*, Academic Press, San Diego, 2001.

[3] J.E. Hooper and M.P. Scott, "The Molecular Genetic Basis of Positional Information in Insect Segments," in *Early Embryonic Development of Animals*, W. Hennig, ed., Springer, Berlin, 1992, 1–49.

[4] B. Sanson, EMBO reports 2:1083 (2001).

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

[6] L. Wolpert, R. Beddington, J. Brockes, T. Jessell, P. Lawrence, and E. Meyerowitz, *Principles of Development*, Current Biology Ltd., London, 1998.

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