

Dynamical Analysis of Gene Networks Requires Both mRNA and Protein Expression Information

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One of the important goals of biology is to understand the relationship between DNA sequence information and nonlinear cellular responses. This relationship is central to the ability to effectively engineer cellular phenotypes, pathways, and characteristics. Expression arrays for monitoring total gene expression based on mRNA can provide quantitative insight into which gene or genes are on or off; but this information is insufficient to fully predict dynamic biological phenomena. Using nonlinear stability analysis we show that a combination of gene expression information at the message level and at the protein level is required to describe even simple models of gene networks. To help illustrate the need for such information we consider a mechanistic model for circadian rhythmicity which shows agreement with experimental observations when protein and mRNA information are included and we propose a framework for acquiring and analyzing experimental and mathematically derived information about gene networks. © 1999 Academic Press

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INTRODUCTION

A common challenge in the life sciences is to relate genome sequence information to nonlinear cellular phenotypes. The sequencing of complete genomes has enabled the use of new technologies to simultaneously identify and quantify the expression of every gene in the genome or sets of genes by hybridization on a solid surface (Winzler *et al.*, 1998; Lockhart *et al.*, 1996; Schena *et al.*, 1995). An important motivation for the development of microarray technology to quantify mRNA levels is the expectation that by comparing complex quantitative gene expression patterns, one will be able to decipher the regulatory wiring of the genetic networks responsible for various observed phenotypes including developmental stages and disease (Loomis and Sternberg, 1995). Implicit in this assertion is that

responses at the mRNA level reflect the response at the protein level, i.e., that there is a one-to-one correspondence of protein to mRNA. The enormous amount of data generated from these studies requires mathematical treatment and processing to cull important information and to draw meaningful conclusions. For example, statistical analysis of gene expression information is currently used to cluster genes that share regulatory properties (Michaels *et al.*, 1998). However, the use of these methods to identify “coregulated” genes does not lead directly to conclusions about the regulatory wiring. Further, it does not make full use of the laboriously derived information about the quantitative levels of the mRNA expression (Schena *et al.*, 1995).

In this paper we test the proposition whether gene expression data (mRNA or protein) is sufficient to elucidate the relationship between genome sequence, gene regulation and cellular dynamics. Two different mathematical modeling methods for genetic networks (Boolean and continuous) are studied and compared for their ability to predict physiological phenomena. In contrast to the continuous approach, the Boolean approach (Kauffman, 1993) involves assumptions that ignore observed biological processes such as different rates of transcription of different genes and variability in mRNA stability (Carrier and Keasling, 1997). Therefore, we conclude that a continuous approach is more appropriate for understanding how the genes of the genome interact. Nonlinear stability analysis of continuous models proves that gene expression information at both the message and protein levels is required to understand gene networks. Therefore, gene regulation studies based on expression information obtained using either microarray technology (for mRNA levels) or proteomics technology alone involves incomplete information which can lead to incorrect conclusions about which genes are important to a particular

phenotype. However, the dynamic profiling of gene expression patterns is clearly critical to understanding a wide variety of biological phenomenon such as stress responses, pharmacologic responses, and metabolic shifts based on growth on different substrates.

TWO MODELING APPROACHES: BOOLEAN AND CONTINUOUS

Consider a simple genetic network consisting of two genes, X and Y . We assume that the product of gene X (an mRNA or a protein made in direct proportion to that mRNA) induces the expression of gene Y , whose product (mRNA or corresponding protein) represses the expression of gene X (Fig. 1A). Such a system has recently been hypothesized to describe the induction/repression wiring of two classes of genes as the genetic origin of circadian rhythms (Crosthwaite *et al.*, 1997; Somers *et al.*, 1998; Thresher *et al.*, 1998). Boolean modeling of this type of system suggests that the unique steady state of the system will exhibit oscillatory behavior (Fig. 1B) which corresponds to biologically relevant situations (e.g., circadian rhythms). Moreover, such dynamic behavior is consistent with the conceptual understanding of the system.

This simple example highlights the main advantage of Boolean modeling. Boolean models are based on logical rules and this framework is consistent with human conceptual

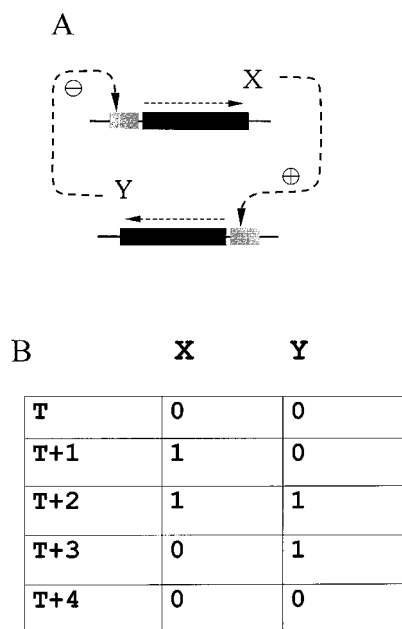


FIG. 1. A genetic network consisting of two genes, X and Y . (A) The molecular model where the product of gene X induces the expression of gene Y , and the product of gene Y represses the expression of gene X . (B) State-transition table for the Boolean model of the genetic network.

modeling which is also based on logic (Popper, 1992). Moreover, the low computational complexity of Boolean models makes them attractive for large genetic networks. For these reasons, the Boolean modeling approach is used to describe a number of different phenomena including the metazoan genome, the lactose operon, and predator–prey systems (Thomas, 1979, pp. 30–60, 352–402, and 502–507). A hybrid approach that is based on Boolean logic and includes time delays has been developed for lambda phage infection (McAdams and Shapiro, 1995).

Unfortunately, the Boolean modeling approach suffers from a number of limitations. Information about gene expression levels is not included when genes are modeled as simply on or off. Thus, Boolean modeling fails to capture the physics of the problem. For example, it does not consider mRNA degradation; thus, phenomena such as RNA stability are not included. Furthermore, the discrete, equally spaced time-steps ignore differences in transcription rates expected from differing gene sequence lengths or differences in transcription efficiency. The availability of a Boolean model for a genetic network does provide an approximate *in silico* system for hypothesis testing, re-designing genetic regulatory networks, knock-out studies, and steady-state analyses. However, the lack of quantification and of continuity in time does not permit the interpretation of, and guidance for, effective studies on dynamic systems including exogenously controlled gene expression, drug dosage studies, and gene therapy strategies. Finally, it has been shown that the application of reverse engineering algorithms based on a Boolean modeling framework (Liang *et al.*, 1998) could potentially lead to the identification of ambiguous multiple candidate regulatory structures governing a genetic circuit. For example, the same gene might be identified as an inducer in one structure and as a repressor in another.

An alternate approach, that circumvents some of the limitations inherent in Boolean modeling, is the formulation of continuous models (Neidhart and Savageau, 1996). Using the same physical description given in Fig. 1A, and given that available information is typically in the form of gene expression, a first attempt to formulate a continuous model will include two dynamic mass balance equations—one for each gene

$$\begin{aligned} \frac{dX}{dt} &= V_s^X(Y) - V_d^X(X) \\ \frac{dY}{dt} &= V_s^Y(X) - V_d^Y(Y), \end{aligned} \tag{1}$$

where t is time and V_s and V_d indicate synthesis and degradation rates, respectively, of the corresponding gene

expression products (mRNA) indicated in the superscript. We make three further assumptions:

(AI) the degradation rates are monotonically increasing functions of the concentration of the corresponding mRNAs ($\partial V_d^X(X)/\partial X > 0$, $\partial V_d^Y(Y)/\partial Y > 0$) with $V_d^X(X=0) = 0$ and $V_d^Y(Y=0) = 0$,

(AII) the synthesis rate of X is a monotonically decreasing function of Y ($\partial V_s^X(Y)/\partial Y < 0$) with $V_s^X(Y) \geq 0$ for $Y \geq 0$ due to repression, and

(AIII) the synthesis rate of Y is a monotonically increasing function of X ($\partial V_s^Y(X)/\partial X > 0$) with $V_s^Y(X=0) = 0$ due to induction.

From the above assumptions and from null-cline analysis (Murray, 1993), the system (Eq. (1)) possesses a unique steady state. For the Jacobian matrix of system (1), the trace ($\text{Tr} = -(\partial V_d^X(X)/\partial X) - (\partial V_d^Y(Y)/\partial Y)$) will always be negative and the determinant ($\text{Det} = (\partial V_d^X(X)/\partial X)(\partial V_d^Y(Y)/\partial Y) - (\partial V_s^X(Y)/\partial Y)(\partial V_s^Y(X)/\partial X)$) will always be positive. Therefore, from linear stability analysis (Wiggins, 1990), the unique steady state of the system will always be a stable node or it will be a stable focus. Moreover, the existence of a unique steady-state solution in the two-dimensional domain of nonnegative values for X and Y means, according to the Poincaré–Bendixson theorem (Wiggins, 1990), that there will be no stable or unstable limit cycle solution. The above mathematical considerations imply that, under the assumptions (AI)–(AIII), any steady-state solution of system (1), independent of the rate expressions, will always be asymptotically stable. Therefore, this system cannot describe oscillatory behavior in contrast to the corresponding Boolean model (Fig. 1B) and to an intuitive understanding of the system. Thus, this approach appears to fail to describe real biological phenomena.

The only conditions under which the continuous model will always display oscillatory behavior are the ones that assume an absence of degradation or that the degradation is independent of the concentration of mRNAs. Under these assumptions the trace of the Jacobian matrix of system (1) is zero and the determinant is positive. Therefore the unique steady state is unstable and, according to the Poincaré–Bendixson theorem, a limit cycle (periodic solution) exists. However, as discussed above, degradation is an important biochemical phenomenon associated with mRNA stability and cannot be ignored. The inactivation and degradation of mRNAs are enzymatically catalyzed processes where mRNAs act as substrates for exonucleases and endonucleases (Belasco and Brawerman, 1993). Therefore, these processes depend on the concentration of mRNAs. Moreover, the observation that mRNA stability is inversely correlated with the RNA

polymerase elongation rate further supports the assumption of a monotonic decrease of degradation rate as mRNA concentration increases (Chow and Dennis, 1994; Iost and Dreyfus, 1995; Makarova *et al.*, 1995).

The above considerations suggest that the Boolean model predictions are inconsistent with the continuous model predictions. On the other hand it has been shown (Tyson and Othmer, 1978) for the continuous model of the network of N species presented in Fig. 2A, where each species i induces the formation of species $i + 1$ and species N represses the formation of species 1, and where each species is degraded with a rate that depends on its concentration, that there exist kinetic parameters that can result in oscillatory behavior only when $N \geq 3$. This proof is relevant because in the simple genetic network depicted in Fig. 1A, we have ignored the process of translation that is responsible for gene product synthesis. The molecular model that includes translation and is associated with the 2-gene system is shown in Fig. 2B. The system is exactly the same as the one in Fig. 2A with $N = 4$. For this system there exist kinetic parameters that can lead to oscillations without the need to omit degradation processes. This observation suggests that to study and analyze genetic networks using continuous models, that information about *both* mRNA and protein expression profiles is required.

The reason that the first continuous model (1) fails to capture the potential oscillatory behavior of the system is the silent assumption about the time scales governing protein synthesis and transcription. Consider the dynamic mass balances for the system depicted in Fig. 2B written as follows

$$\begin{aligned} \frac{dX_{\text{mRNA}}}{dt} &= V_s^X(Y_{\text{protein}}) - V_d^X(X_{\text{mRNA}}) \\ \frac{dX_{\text{protein}}}{dt} &= U_s^X(X_{\text{mRNA}}) - U_d^X(X_{\text{protein}}) \\ \frac{dY_{\text{mRNA}}}{dt} &= V_s^Y(X_{\text{protein}}) - V_d^Y(Y_{\text{mRNA}}) \\ \frac{dY_{\text{protein}}}{dt} &= U_s^Y(Y_{\text{mRNA}}) - U_d^Y(Y_{\text{protein}}), \end{aligned} \quad (2)$$

where the subscripts “s” and “d” denote synthesis and degradation rates respectively, and V and U denote rates associated with mRNAs and proteins, respectively. All the rate expressions are monotonically increasing functions of the species indicated in the parentheses, except the synthesis rate of X_{mRNA} which is monotonically decreasing function of Y_{protein} which acts as repressor. It has been shown that

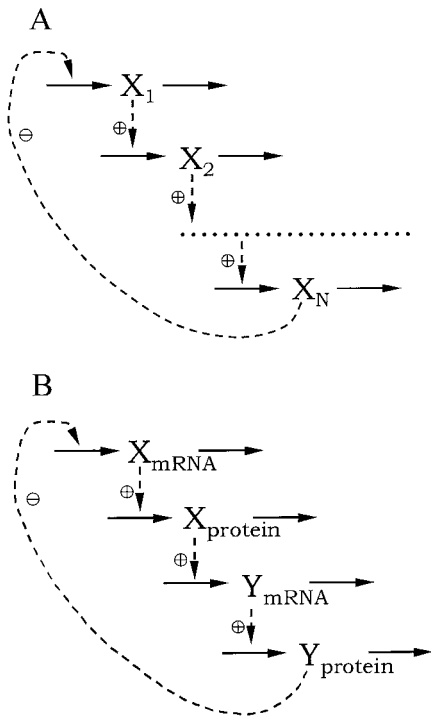


FIG. 2. (A) A network consisting of N chemical species where the i th species is activating the synthesis of the $(i+1)$ th species, and the N th species inhibits production of the first species. (B) A network consisting of two mRNAs and their corresponding proteins with the same interactions as in the system shown in (A).

a system with this type of stoichiometry and regulatory structure yields oscillatory solutions (Tyson and Othmer, 1978).

If we assume that the time scale for the protein net-synthesis rate (including translocation and post-translational modifications) is much smaller than the time scale for the mRNA net-synthesis rate, the protein concentrations are monotonically increasing functions of their corresponding mRNA concentrations at any time. Mathematically,

$$\begin{aligned} \frac{dX_{\text{protein}}}{dt} &= U_s^X(X_{\text{mRNA}}) - U_d^X(X_{\text{protein}}) \cong 0 \Rightarrow \\ X_{\text{protein}} &= \varphi(X_{\text{mRNA}}) \\ \frac{dY_{\text{protein}}}{dt} &= U_s^Y(Y_{\text{mRNA}}) - U_d^Y(Y_{\text{protein}}) \cong 0 \Rightarrow \\ Y_{\text{protein}} &= \psi(Y_{\text{mRNA}}), \end{aligned} \quad (3)$$

where φ and ψ are monotonically increasing functions due to the monotonicity assumed for the corresponding rates. These assumptions leads to the following dynamic model

$$\begin{aligned} \frac{dX_{\text{mRNA}}}{dt} &= V_s^X(Y_{\text{protein}}) - V_d^X(X_{\text{mRNA}}) \Rightarrow \\ \frac{dX_{\text{mRNA}}}{dt} &= V_s^X(\psi(Y_{\text{mRNA}})) - V_d^X(X_{\text{mRNA}}) \\ \frac{dY_{\text{mRNA}}}{dt} &= V_s^Y(X_{\text{protein}}) - V_d^Y(Y_{\text{mRNA}}) \Rightarrow \\ \frac{dY_{\text{mRNA}}}{dt} &= V_s^Y(\varphi(X_{\text{mRNA}})) - V_d^Y(Y_{\text{mRNA}}) \end{aligned} \quad (4)$$

which is exactly equivalent to system (1). Therefore, system (4) cannot predict oscillatory behavior, contrary to system (2). Thus one cannot assume that the net rate for protein synthesis is much smaller than that for mRNA translation. In *E. coli* for example, transcription occurs at 45 nt/s and translation occurs at 16 codons per second (48 nt/s) (Bremer and Dennis, 1987) which means that the rates are of the same order of magnitude. Therefore, genome-wide models that attempt to study the dynamic responses of genetic networks must include the description of the translation processes.

MODELS FOR CIRCADIAN RHYTHMICITY

Consider the proposed basis for circadian rhythms (reviewed in Dunlap, 1998). Experimental evidence suggests that circadian rhythmicity is based in the action of two types of genes and gene products (Antoch *et al.*, 1997; Barinaga, 1997; Crosthwaite *et al.*, 1997; So and Rosbash, 1997; Suri *et al.*, 1999). The first type of gene (proposed examples include WC-2 in *Neurospora* and CLOCK in *Drosophila*) is an activator which is able to “turn on” genes that make proteins required for proper rhythmic function. The second type of gene (examples include FRQ in *Neurospora* and period and timeless in *Drosophila*) acts as a negative feedback step. These interactions fit the mechanistic depiction given in Fig. 2B (see, Dunlap, 1998) where the X species is the positive regulator and the Y species is the negative regulator. The relative roles of the molecules have been elucidated primarily based on genetic screens, mRNA data or qualitative protein expression information. It is well-accepted that the amount of mRNA (Y_{mRNA}) and protein (Y_{protein}), corresponding to the negative-regulating component, oscillates in time such that the level of mRNA peaks well before the level of expressed protein (So and Rosbash, 1997). Experimental observations in *Drosophila* also suggest light acts to reset the clock and shorten the period by destabilizing PER (Dunlap, 1998). Other studies (Hardin *et al.* 1992) have shown that *per* mRNA fluctuations parallel *per* gene transcription and that the stabilization of *per* mRNA levels (in addition to transcriptional control) is an important contributor to observed fluctuations in *per* mRNA

levels (Suri *et al.*, 1999). Indeed, peak values of *per* mRNA are significantly lower in mutant *per* strains than in wild-type strains (So and Rosbash, 1997).

A mathematical description corresponding to Fig. 2B has been developed using standard kinetics to describe the circadian oscillator of *Drosophila* (Hill *et al.*, 1999). Parameter values were obtained which result in oscillations for the above system. The timing of transcription and translation are consistent with the experimentally observed circadian events—we observe a peak in Y_{mRNA} prior to a peak in Y_{protein} concentration (So and Rosbash, 1997). The time distribution of the various events, the mRNA and protein levels, and the period of the oscillations can be adjusted by appropriate changes of the parameters. This is one of the features of a continuous model which is not available in Boolean models.

The development of this mechanistic model permits the identification of which parameter changes simulate experimentally observed phenomena. Accordingly, we have simulated light-induction of the mechanism by decreasing the transcriptional efficiency of gene X (X_{mRNA}) by a factor of two (Fig. 3B). This change results in a decrease in circadian period (as measured by peaks in Y_{protein}) from 31.9 to 19.5—a decrease of 64%. These observations are consistent with experimentally observed phenomenon (Dunlap, 1998). Furthermore, we show that a decrease in X_{mRNA} transcription results in a decrease in peak levels of X_{mRNA} , also consistent with experimentally observed phenomena (So and Rosbash, 1997).

The predictions of this simple model also agree with an earlier model for circadian rhythmicity proposed by Scheper *et al.* (1999). The earlier model predicts a decrease in period as the X_{mRNA} degradation rate is increased or as the X_{mRNA} transcription rate is increased. In contrast to the earlier model, we do not include “time delays” to achieve observations consistent with experimental observations. The main difference between these models, and the reason time delays are not needed to observe oscillations in this work, is our use of a four component system that includes information on mRNA and protein for both gene products (the activator and the negative regulator). Building on this work, we can include quantitative and dynamic information on both mRNA and protein expression levels. Information about transcriptional control, mRNA stability and protein–protein interactions have proven to be critical in the proper functioning of the circadian rhythm generator (Antoch *et al.*, 1997; Crosthwaite *et al.*, 1997; Dunlap, 1998). Indeed, it has been suggested (Suri *et al.*, 1999) that part of the underlying biochemical mechanism may even involve control over the rate of Y_{mRNA} degradation rate; therefore, dynamical analysis of mRNA profiles would provide necessary information for complete elucidation of this gene network.

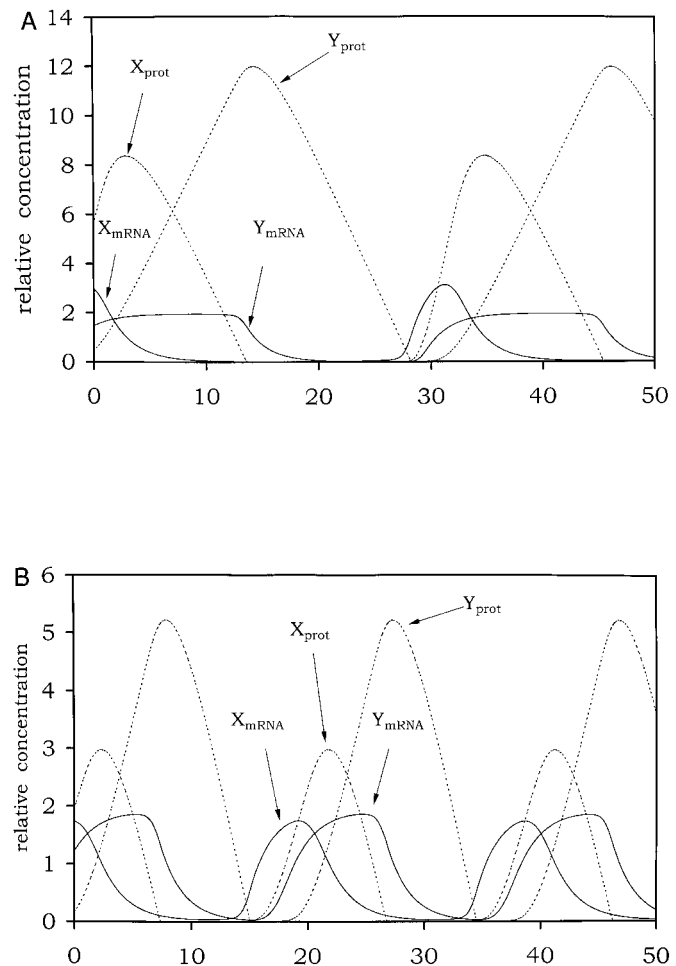


FIG. 3. (A) Oscillations in relative concentrations of components from the mechanism in Fig. 2B for circadian rhythms. (B) Oscillations in relative concentrations where the transcriptional efficiency X_{mRNA} has been doubled relative to (A). Other values were kept the same.

DISCUSSION

The statistical analysis of mRNA profiles and protein profiles provides information for tentative clustering of related genes and gene products via database mining techniques. DNA sequence information promotes further ordering of genes according to similar upstream regulatory sequences. It should be noted that the ability to efficiently process information from existing databases already poses limitations on the throughput for analyses and new algorithms and technologies are required for full genome-scale analysis. The formulation and qualitative analysis of Boolean networks can suggest multiple candidate gene network structures based on possible steady-states; however, thermodynamic and kinetic data together with quantitative mRNA and protein information is required to construct nonlinear

mechanistic kinetic models of gene and protein networks (Hatzimanikatis *et al.*, 1999). Despite the onslaught of basic biological information, a foreboding problem is the lack of detailed experimental information which can be caused by: limited system knowledge (e.g., what are all of the relevant components?), limiting technology (e.g., inability to access information about insoluble protein fractions), poor experimental design, or human error. Thus, a detailed analysis of gene networks and emergent biological properties of such networks (Bhalla and Iyengar, 1999) awaits complete information about mRNA and protein information.

The implications of these results are critical to the ongoing transformation of biology from a qualitative descriptive science into a quantitative mechanistic science (Koshland, 1998; Maddox, 1994). These results prove in a rigorous way, that a paradigm shift (from qualitative to quantitative) is not only a possibility, but a requirement for biology to evolve. Efforts to develop mathematical descriptions which investigate the regulation of genetic circuits based on quantitative mRNA expression levels alone (DeRisi *et al.*, 1997), neglect critical information. The combination of data from mRNA expression levels and their protein counterparts for the study of gene expression are required to develop continuous mathematical description of gene networks and to apply these descriptions toward the identification of the regulatory wiring and the understanding of biological phenomena. The need for such a combination is highlighted by observations with yeast, in particular, where the quantitative expression level of many genes as measured by mRNA analysis is significantly different than that measured with a proteomics strategy (Gygi *et al.*, 1999). Thus, a greater emphasis should be placed on developing quantitative proteome analytical technology consistent with ongoing efforts in microarray technology.

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REFERENCES

- Antoch, M. P., Song, E. J., Chang, A. M., Vitaterna, M. H., Zhao, Y., Wilsbacher, L. D., Sangoram, A. M., King, D. P., Pinto, L. H., and Takahashi, J. S. (1997). Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. *Cell* **89**, 655–667.
- Barinaga, M. (1997). New clues found to circadian clocks—including mammals. *Science* **276**, 1030–1031.
- Belasco, J., and Brawerman, G., Eds. (1993). “Control of Messenger RNA Stability,” Academic Press, New York.
- Bhalla, U. S., and Iyengar, R. (1999). Emergent properties of networks of biological signaling pathways. *Science* **283**, 381–387.
- Bremer, H., and Dennis, P. (1987). Modulation of chemical composition and other parameters of the cell by growth rate. In “*Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology,” Vol. 2 (F. C. Neidhardt, J. C. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger, Eds.), pp. 1527–1542, Am. Soc. Microbiol., Washington, DC.
- Carrier, T. A., and Keasling, J. D. (1997). Controlling messenger RNA stability in bacteria: strategies for engineering gene expression. *Biotech. Progr.* **13**, 699–708.
- Chow, J., and Dennis, P. (1994). Coupling between mRNA synthesis and mRNA stability in *E. coli*. *Mol. Microbiol.* **11**, 919–931.
- Crosthwaite, S. K., Dunlap, J. C., and Loros, J. J. (1997). *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. *Science* **276**, 763–769.
- DeRisi, J. L., Iyer, V. R., and Brown, P. O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 608–686.
- Dunlap, J. C. (1998). Common threads in eukaryotic circadian systems. *Curr. Op. Gene. Dev.* **8**, 400–406.
- Gygi, S. P., Rochon, Y., Franza, B. R., and Aebersold, R. (1999). Correlation between protein and mRNA abundance in yeast. *Mol. Cell. Biol.* **19**, 1720–1730.
- Hatzimanikatis, V., Choe, L. H., and Lee, K. H. (1999). Proteomics: theoretical and experimental considerations. *Biotech. Prog.* **15**, in press.
- Hardin, P. E., Hall, J. C., and Rosbash, M. (1992). Circadian oscillations in period gene mRNA levels are transcriptionally regulated. *Proc. Natl. Acad. Sci. USA* **89**, 11711–11715.
- Hill, C. C., Hatzimanikatis, V., and Lee, K. H. (1999). Manuscript in preparation.
- Iost, I., and Dreyfus, M. (1995). The stability of *E. coli lacZ* mRNA depends upon the simultaneity of its synthesis and translation. *EMBO J.* **14**, 3252–3261.
- Kauffman, S. A. (1993). “The Origins of Order,” Oxford Univ. Press, New York.
- Koshland, D. E. (1998). The era of pathway quantification. *Science* **280**, 852–853.
- Liang, S., Fuhrman, S., and Somogyi, R. (1998). REVEAL: a general reverse engineering algorithm for inference of genetic network architectures. *Pacific Symp. Biocomput.* **3**, 18–29.
- Lockhart, D. J., Dong, H., Byrne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H., and Brown, E. L. (1996). Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotech.* **14**, 1675–1680.
- Loomis, W. F., and Sternberg, P. W. (1995). Genetic networks. *Science* **269**, 649.
- Maddox, J. (1994). Cell-cycle regulation by numbers. *Nature* **369**, 6480.
- Makarova, O., Makarov, E., Sousa, R., and Dreyfus, M. (1995). Transcribing of *E. coli* genes with mutant T7 RNA polymerases: stability of *lacZ* mRNA inversely correlates with polymerase speed. *Proc. Natl. Acad. Sci. USA* **92**, 12250–12254.
- McAdams, H. H., and Shapiro, L. (1995). Circuit simulation of genetic networks. *Science* **269**, 650–656.
- Michaels, G. S., Carr, D. B., Askenazi, M., Fuhrman, S., Wen, X., and Somogyi, R. (1998). Cluster analysis and data visualization of large-scale gene expression data. *Pacific Symp. Biocomput.* **3**, 42–53.
- Murray, J. D. (1993). “Mathematical Biology,” 2nd ed., Springer-Verlag, New York.
- Neidhardt, F. C., and Savageau, M. A. (1996). Regulation beyond the operon. In “*Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology,” Vol. 1 (F. C. Neidhardt, Ed.), pp. 1310–1324, Am. Soc. Microbiol., Washington DC.

- Popper, K. R. (1992). “The Logic of Scientific Discovery,” pp. 78–92, 326–362, Routledge, London.
- Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* **270**, 467–470.
- Scheper, T., Dlinkenberg, D., Pennartz, C., and van Pelt, J. (1999). A mathematical model for the intracellular circadian rhythm generator. *J. Neurosci.* **19**, 40–47.
- So, W. V., and Rosbash, M. (1997). Post-transcriptional regulation contributes to *Drosophila* clock gene mRNA cycling. *EMBO J.* **16**, 7146–7155.
- Somers, D. E., Devlin, P. F., and Kay, S. A. (1998). Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* clock. *Science* **282**, 1488–1490.
- Suri, V., Lanjuin, A., and Rosbash, M. (1999). TIMELESS-dependent positive and negative autoregulation in the *Drosophila* circadian clock. *EMBO J.* **18**, 675–686.
- Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P., Dawut, L., Smithies, O., Takahashi, J. S., and Sancar, A. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* **282**, 1490–1494.
- Thomas, R., Ed. (1979). “Kinetic Logic: A Boolean Approach to the Analysis of Complex Regulatory Systems,” pp. 30–60, 352–402, 502–507, Springer-Verlag, New York.
- Tyson, J. J., and Othmer, H. G. (1978). The dynamics of feedback control circuits in biochemical pathways. *Prog. Theor. Biol.* **5**, 1–62.
- Wiggins, S. (1990). “Introduction to Applied Nonlinear Dynamical Systems and Chaos,” Springer-Verlag, New York.
- Winzler, E. A., Richards, D. R., Conway, A. R., Goldstein, A. L., Kalman, S., McCullough, M. J., McCusker, J. H., Stevens, D. A., Wodicka, L., Lockhart, D. J., and Davis, R. W. (1998). Direct allelic variation scanning of the yeast genome. *Science* **281**, 1194–1197.