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Genetic networks with tens to hundreds of genes are difficult to analyze with currently available techniques. Because of the many parallels in the function of these biochemically based genetic circuits and electrical circuits, a hybrid modeling approach is proposed that integrates conventional biochemical kinetic modeling with the framework of a circuit simulation. The circuit diagram of the bacteriophage lambda lysis-lysogeny decision circuit represents connectivity in signal paths of the biochemical components. A key feature of the lambda genetic circuit is that it operates as an active integrated logic component and introduces signal time delays essential for the in vivo behavior of phage lambda.

Genetic networks that include many genes and many signal pathways are rapidly becoming defined in prokaryotes and eukaryotes. As network size increases, intuitive analysis of feedback effects is increasingly difficult and error prone. Electrical engineers routinely analyze circuits with thousands of interconnected complex components. Electromechanical devices switch in small fractions of a second, and common transistor circuits can operate at more than $10^8$ cycles per second. In contrast, the protein signal-controlled switching rate in genetic circuits is around $10^{-2}$ per second. Although there is a great disparity in time scales between genetic switching circuits and electrical switching circuits, there are many parallels in their function. These similarities lead to the question: Which electrical engineering circuit analysis techniques are applicable to genetic circuits that comprise tens to hundreds of genes?

The conducting pathways between components determine the connectivity of electrical circuits. The connectivity of genetic circuits is determined by the connection between the source of a protein signal and its site of action established by its site-specific biochemical address. Site-specific biochemical addressing permits many genetic circuits to operate in parallel within the same small cell volume. Thus, the cell can achieve high computational density in terms of operations per second per cubic centimeter and the instantaneous amount of genetic computation within any living organism is enormous, in spite of slow switching rates.

Electrical circuits are typically described by circuit diagrams and characterized by simulation models. The simulation provides a calculating tool for predicting time behavior of the interconnected system. The circuit diagram shows the overall organization of the circuit and the detailed interconnectivity between components. We now apply this perspective to the genetic circuit used by bacteriophage lambda (λ) to choose between lysis and lysogeny (1-5). After infecting a host Escherichia coli cell, phage λ either propagates as a prophage integrated into the host DNA (lysogeny) or becomes an actively replicating virus (lysis). The λ decision circuit, which controls one phase of a single phage life cycle and operates synergistically within a single E. coli cell, is perhaps the most completely characterized complex genetic network. Many genetic circuits have broader scope. For example, elements of several cells may be components of a circuit that controls the life cycle of the different cell types (6) and cell generations (7). Symbiotic relations between bacteria and higher organisms involve genetic circuits that cross species boundaries (8).

Biochemical feedback plays an essential role in cellular regulation (9), and algebraic formalisms for analysis of biological networks represented as asynchronous automata have been proposed (10). As with electrical networks, however, the algebraic approach quickly becomes obtuse for all but the simplest networks. Short-term dynamics of small-scale biochemical reaction networks that control physiological mechanisms in bacteria have been simulated (11), and modeling of metabolic biochemical reaction networks is well advanced (12). A hybrid approach is needed to integrate conventional biochemical kinetic modeling with models of control and delay mechanisms in large genetic circuits.

**Signal Timing in Genetic Networks**

Electrical switching circuits are frequently characterized as networks of idealized switching devices; that is, devices with instantaneous transitions between states at precise times. However, practical electrical devices exhibit finite transition times and transient responses. The idealization of practical electronic devices permits simplified characterization of switching circuits based on Boolean logic while retaining the observed behavior of the system. In a parallel manner, a Boolean gate representation can characterize biochemical repression or activation of transcriptional promoter elements when the switching action is definitive and relatively fast. (The term “gate” refers to a circuit element that outputs a signal when its required input conditions are satisfied. Boolean algebra treats relations between logical variables with the values TRUE and FALSE.) Transcription elongation and translation control mechanisms augment promoter-based logic to determine expression of specific genes. Biochemical mechanisms described below that determine the dynamic balance between protein production and decay, and thus determine signal levels, are important parameters in genetic circuit logic. Time delay mechanisms, especially transcription delays and signal accumulation delays, are central to the correct function of the circuits.

Figure 1A shows the interplay of these mechanisms in a hypothetical genetic circuit with two promoters, three genes, and a termination site. The time evolution of signals in this circuit (Fig. 1B) is determined.

Fig. 1. (A) Circuit diagram of a hypothetical genetic network. Bold line indicates the RNAP path on the DNA after binding at $P_A$. Promoter $P_C$ is ON when protein signal A is ON; that is, at an effective concentration at its site of action. Switch T, which is usually open but closes to permit RNAP passage in the presence of the protein signal C, models terminator site function. (B) Timing diagram of signals A, B, and C. Time lags depend on the gene spacing on the DNA, transcription rates, the time required for accumulation of an effective signal concentration, and protein decay rate constants.
by transcription time delays resulting from the rate of transcription, from delays while transcription is blocked at the terminator site, and from protein signal accumulation delays. Transcription of the operon containing genes a and b is initiated at time zero when RNA polymerase (RNAP) binds at promoter P_{A}, an open complex is formed, and RNAP transcribes to and through the gene (a) encoding protein A. Time delays in this operon result from (i) the rise and fall times of the protein signals A and C, and (ii) the time required for RNAP to transcribe the operons initiated at P_{A} and P_{C}. The time from P_{A} ON to P_{C} ON, for example, is the sum of these two times. The initial rate of signal protein production is proportional to the product of the transcript production rate under the prevailing repression or activation condition of the promoter, the number of proteins translated from each transcript, and the multiplicity of infection (MOI). After open complex formation at the promoter site, the rate and extent of subsequent transcription are actively controlled by pause sites and by termination sites. Transcription delays can range from a few seconds to several minutes, depending on the distance and the average transcription rate (13).

After appearance of the first gene a transcript, an additional delay is required to achieve a concentration of A sufficient to turn on P_{C} at an effective rate. Signal protein C controls terminator switch T. When T closes (is antiterminated), transcription can continue through b to produce signal protein B. The time from P_{C} ON to the closing of T is the sum of the delay time attributable to RNAP movement plus the rise time of the C protein signal. Determinants of the time from initiation of binding at P_{A} to initiation of B production are more complex. Both the RNAP travel time along the DNA from P_{A} to the end of b and the delaying effect of events that influence terminator switch T must be included.

When the controlling signal concentrations change and a promoter turns off, a pipeline of RNAPs must be cleared before transcription ceases. This latent signal capacity, which continues until transcript translation ends, must be considered when modeling the circuit.

Steady-state signal protein concentrations are determined by the dynamic balance between protein production and degradation (Fig. 2A, Eq. 1). [Equation numbers refer to equations for signal dynamics in Fig. 2A (14).] A short signal protein half-life results in low steady-state signal levels and a short time to steady state (Fig. 2A, Eqs. 7, 9, and 10). Cells actively control protein signal degradation rates and thus steady-state signal levels. For example, active control of degradation is central to the control of protein CII.

**Feedback Circuit Dynamics**

Two feedback circuits involving proteins CII and Cro, with differing configurations and dynamics, are critical design elements of the λ decision circuit. The CI feedback loop shown in Fig. 3 is a self-regulating circuit. When conditions are favorable for the lyticogenic path, CII increases to a level that turns on promoter P_{RE} (Fig. 3A). There is a transcription delay before production of the first CI transcript. Translation of this and successive transcripts adds to the cumulative CI signal; simultaneously, protein degradation reduces the signal. The instantaneous signal change rate, ΔCI(t), is determined by these processes, and the current signal level, CI(t), is the time integral of ΔCI(t). Initially, when the CI signal level is low, P_{RM} does not initiate transcripts, but there is a high level of transcription from P_{RE}. The CI concentration rapidly increases to a level sufficient for P_{RM} to initiate transcription (Fig. 3C) and to sustain CI production after P_{RE} becomes inactive when the CI signal decreases. At the steady-state CI concentration (~140 to 200 molecules per cell (15, 16)), the rate of transcription from P_{RM} results in a rate of CI production equal to that of CI degradation. This steady-state CI concentration represses P_{R} and P_{L} (Fig. 3B) to prevent induction of the lysogen and to prevent transcription from additional λ phages that may infect the host promoter. Promoter P_{R} is repressed at a CI concentration much less than the steady-state concentration and, consequently, is switched off rapidly. P_{L} dynamics are similar. The switching of P_{L} and P_{R} is definitive and rapid, and, hence, Boolean in character.

When the lytic path is favored, negative feedback loop mediated by Cro repression of P_{R} controls signal levels and timing. The sustaining level of transcription in the CI loop described above produces only CI molecules. However, when the Cro loop is active, the sustaining transcripts initiated at P_{R} extend beyond σ70 to other genes (Fig. 4). The dynamics of the Cro feedback loop as calculated from Eqs. 5, 7, 10, and 11 in Fig. 2A are shown in Fig. 2B. B to E. The steady-state signal level is lower with feedback than without (Fig. 2B). The steady-state signal protein level and transcript initiation rate increase with MOI and there is an initial transient burst of transcription for higher MOI (Fig. 2, C to E). The steady-
state protein concentration from each downstream gene in the $P_R$ operon will be proportional to the product of the characteristic proteins/transcript ratio of each gene and the half-life of the resulting protein (Fig. 2A, Eq. 9). This product can vary widely from gene to gene in the same operon. Promoters $P_I$ and $P_{RM}$ are switched rapidly with a design similar to that of $P_R$ and $P_{I}$ switching by the CI loop. These two feedback circuits support both rapid Boolean switching (of $P_R$, $P_I$, and $P_{RM}$) and controlled, sustained synthesis from several genes (from $P_R$ on the lytic path) when needed.

**Time Delays**

The transcription delay between a promoter and each gene is $NT_{PC}/(R_T)$, where $NT_{PC}$ is the nucleotide count from the promoter to the end of the gene and $(R_T)$ is the average rate of transcript elongation. The initial rate of signal protein increase is linear and proportional to MOI (Fig. 2A, Eqs. 3 and 5; Fig. 2, B and C). Thus, the total time delay ($T_{delay}$) from promoter ON to effectiveness at the site of action of a gene is approximately given by

$$T_{delay} = \frac{NT_{PC}}{(R_T)} + \left(\frac{K_s V_{cell}}{(MOI A_{GENE} \times C_{MAX})}\right),$$

where $K_s$ is the equilibrium constant for the signal protein–site of action interaction (see Fig. 2A for the definition of other terms). This equation applies when $K_s$ is much smaller than the steady-state signal concentration. The second term can be relatively small at higher MOIs (Fig. 2C) or for switching configurations designed for speed (for example, $P_R$ and $P_I$ control by CI in Fig. 3). A transcription delay mechanism assures time delays necessary for correct circuit function even at high MOI. For example, gene Q is $\sim 6500$ nucleotides from $P_R$, requiring several minutes for transcription (6). In the second term, the $K_s V_{cell}$ ratio can differ widely for genes in the same operon, contributing to the flexibility of the operon structure as a genetic circuit control element.

**Lambda Decision Circuit**

The $\lambda$ genetic circuit that determines the course of the phage infection is shown in Fig. 4 (17). A switch selects between the two stable configurations determined by the CI- or Cro-based negative feedback loops (4). The physiological state of the bacterial host and the MOI together bias the switch toward latching in one state if conditions favor lysogeny and the other if conditions favor lysis (Fig. 4, gate G1).

Electrical engineers avoid "races" (simultaneously changing signals along two different, but interacting, signal paths) in switching circuit designs; they especially avoid "critical" races (a race condition in which the outcome differs depending on which path completes first). A central element of the $\lambda$ decision circuit is the critical race created by the competitive buildup of CI and Cro in which the outcome determines whether the phage will integrate into the host DNA or begin replication, resulting in lysis. The phage circuit design creates a "fuzzy logic" mechanism by integrating dependence on internal health (state of nutrition) and extracellular environment (MOI) into the stability, rate of growth, and steady-state concentration of CI and CII that determines the logical outcome at gate G8.

(G1) $P_R$ and $P_{RM}$ promoters control two-state switch. After $\lambda$ infection of E. coli, transcription initiates at $P_R$ and $P_I$ (1, 2, 4). Translation of the $P_R$ transcript produces Cro. Promoter $P_{RM}$ is initially OFF. Transcripts initiated at $P_R$ and $P_I$ induce a cascade of events that result in rapid production of CI if environmental conditions favor CI stability, and low or no CI production if not (gate G8). Cro and CI bind competitively and in sequence, but in opposing order, to three sites (OR1, OR2, and OR3) on the $\lambda$ DNA. As the CI concentration increases, $P_R$ is repressed by CI at OR1, after which $P_{RM}$ is stimulated by CI at OR2 and repressed by CI at OR3 (Fig. 2C). As the Cro concentration increases, $P_{RM}$ is repressed by Cro at OR3, and $P_R$ is then repressed by Cro at OR2 or OR1. Eventually, either the CI or the Cro feedback loop is locked on to determine the lytic or lysogenic path choice. The overall effect is of an integrated logic component providing a bistable two-state switch.
The control of Int and Xis is mediated in part by a topologically determined mechanism that depends on the state of the phage DNA (prophage or not). In the unintegrated phage DNA, the sib region located downstream of int is transcribed by the N-antiterminated RNAP initiated at PT. The sib portion of this mRNA facilitates nucleases attack followed by sequential destruction of int progressing back toward N (reexpression), so that Xis is preferentially produced by the PT-initiated transcript. In contrast, the P1-initiated transcript does not transcribe a complete xis mRNA and terminates before sib, thus producing only Int. The attachment point (AttP) of the phage is between int and sib, so that, during λ induction, P1-initiated RNAP transcribes through int, but the resulting mRNA does not contain the sib region. The location of xis, well separated from P1 on the DNA, assures a time delay to permit execution of the lysogenic logic when conditions are correct for that decision.

The genetic logic produced by these biochemical mechanisms that govern Int and Xis production is

"Int is produced" if "CII* is above threshold" OR ("state is prophage" AND "P1-initiated RNAP is present").

"Xis is produced" if "P1-initiated RNAP is present" AND NOT "CII* is present."

(G12) State-dependent logic based on Int and Xis controls integration and excision. Relative concentrations of Xis and Int control phage integration and excision (Fig. 5) (29–31). Integration requires only the Int protein among the phage-specified proteins; Xis inhibits integration. Excision requires both Int and Xis; less Int is required for excision than for integration. As in gate G11, the control logic depends on the state of the phage DNA (prophage or not). Integration initiates in the unintegrated (NOT prophage) phase when Int is present above threshold and in significant excess over Xis. Excision initiates in the integrated phase (prophage) when both Int and Xis are present above threshold. Integration and excision are accomplished by separate pathways.

The genetic logic governing initiation of integration or excision is

"Next state is prophage" if ("current state is prophage" AND NOT "Int above threshold") OR ("Int above threshold" AND NOT "Xis above threshold").

Genetic Circuits as Sequential Logic Circuits

The logic circuit diagram in Fig. 5A is an equivalent symbolic representation of the genetic logic statements for gates G11 and
G12 given above. The logic controlling integration and excision depends on the state of the phage (prophage or not). Thus, the λ prophage state functions as a long-term memory mechanism in the logic circuit. Time delays in the circuit provide short-term memory.

A sequential circuit is defined as a circuit in which the output of the circuit depends not only on current inputs, but also on the stored state of the circuit consistent with the model in Fig. 5B. Comparison of Figs. 5A and 5B shows that the logic configuration of G11 and G12 fits the sequential circuit model. Sequential circuits in which the memory results from time delays are asynchronous sequential circuits. The analysis and design of a large fraction of all electrical logic circuits, including virtually all digital computation circuits, are based on elaboration of the sequential circuit paradigm.

The checkpoint phenomena observed in cell cycles (32) represent another parallel between well-known electrical logic circuit phenomena and genetic circuits. When the external input to a sequential circuit changes, the combined input vector (stored plus external input values) to the combinational logic results in a new output vector (new external outputs plus next-state values). The next-state values are stored and, after a time delay, the output values from the memory representing the present state change to the next-state values (Fig. 5B). The new combined input to the logic may result in another change in the outputs; this cycle will continue until the circuit settles into a stable state in which the current state equals the next state. A sequential circuit may pass through several of these transitional states before reaching a stable state. Initiation of a transition out of a stable state depends on receipt of changed inputs. When the changed inputs reflect completion of necessary precursor actions, this sequential circuit mechanism of operation is analogous to the checkpoint control mechanism.

Across the λ phage, different proteins, with different mechanisms of action, perform analogous circuit functions (31, 33). In these instances, genome organization and the pattern of gene grouping into modules with similar function is highly preserved (34). For electrical circuits, there are always many equivalent alternative implementations of any logic function based on alternative choices of components or design details. Engineers select among these implementations on the basis of criteria such as cost, reliability, or power consumption. Similarly, evolution selects among alternative biochemical implementations of logic functions, but the selection criteria relate to survival value. We conjecture that the phage logic circuit design is the most conserved element that defines the λ phage species, with each specific phage representing an alternative implementation of the λ logic design—the λ algorithm. The biochemical design of any individual λ phage then represents optimization of the implementation of the common λ logic design for a specific host environment.

Genetic circuits exhibit hierarchical organization: Regulators control operons, which control gene groupings. Electronic circuit designers structure complex systems as hierarchical structures to facilitate reuse of modular functions and simplified control by a few signals. The multigene genetic subfunctions in the hierarchy are points of high leverage for evolutionary adaptability because a single mutation in circuit logic can change the control of a large genetic cascade, thereby amplifying evolutionary consequences. The evolutionary consequences of rearrangement of modular functions in the genome by homologous recombination are well documented (34).

Relocation of a single gene that encodes a controlling signal protein can change connectivity and, hence, the circuit logic, resulting in a radical effect on timing, duration of effect, or sequencing of the controlled subcircuits. Identification of the circuit-level organization of genetic circuits, as in Figs. 4 and 5, together with established methods for logic circuit analysis, will provide a functional framework for analysis of such large-scale reorganization of genetic logic.

**Verification of Decision Circuit Logic**

Conventionally, biochemical simulations have emphasized modeling coupled kinetic equations. Electrical circuit simulations emphasize the circuit connectivity and the functionality of the circuit components, such as resistors, capacitors, and transistors. Consideration of electrical circuit simulations suggests a hybrid approach to genetic circuit modeling that integrates the following ideas with kinetic models: (i) identify the circuit connectivity and model point-to-point signal paths, (ii) simplify transcription control logic by treating it as Boolean logic when justified, and (iii) model the functionality of complex or nonlinear control elements in specialized subcircuits.

In many instances, the signal path dynamics can be modeled (Fig. 2) with four parameters: (i) rate of signal protein transcription production, (ii) average protein transcript, (iii) signal protein half-life, and (iv) the equilibrium constant for the signal protein–site of action interaction. Past experimental effort has not been directed toward characterizing the point-to-point links, per se, under controlled conditions, because these parameters were not viewed in the circuit context presented here. As a result, we find solid data for only two of the four needed parameters for most links in Fig. 4, and three of four for a few (35). We expect that this situation will improve as the importance of understanding the signal paths and timing in complex genetic networks is widely realized. Because overall genetic circuit functions are highly interdependent, an integrated simulation will allow inference of missing protein parameters from a combined consideration of the circuit design, the known parameters, and the overall timing and outcome of the circuit logic under varying conditions. This capability to exploit the interdependence of circuit elements will be a major benefit from simulating the circuits.

Operons function as key signal-generating and control components in genetic circuits and are candidates for modeling with specialized subcircuits. The operon model and associated routines must treat (i) promoter control logic, (ii) promoter activity (transcript initiation rate), reflecting repressor or activator kinetics, (iii) operon layout (gene location), (iv) any elongation control (for example, antitermination mechanisms), (iv) posttranscriptional controls, and (v) translation efficiency (for example, average proteins/transcript) for each gene. Common control of one or more genes by several promoters (for example, common control of cd by $P_{RE}$ and $P_{RM}$ (Fig. 5A)
Fig. 6. Timing diagrams of promoter and protein signal sequencing for (A) lysoygeny decision at an MOI of 10 and (B) lysis decision at an MOI of 1. Signals are normalized from 0 (no signal) to 1 (maximum signal). Prom and light shading identify promoters. Prot and dark shading identify signal molecules. The curve labeled Prophage? is 1 if the phage DNA is integrated as a prophage and 0 otherwise.

3A] should be incorporated into one operon model. Our approach is to define a software operon "object." This object is comprised of a data structure that specifies the operon configuration plus software procedures for operating on the structure. The operon is viewed as a sequence of multinucleotide segments represented by array elements in the data structure. A parameter representing the instantaneous distribution of RNAP transcription complexes in each DNA segment is stored in each array element. Two other parallel arrays reflect any termination sites and antitermination; one contains the fraction of RNAP molecules that moves forward along the DNA at each segment (for example, 0, if blocked; 1, if not; intermediate, if partially blocked), the other carries a flag along indicating whether the RNAP in that segment has been antiterminated. A procedure shifts the RNAP distribution along the array at each time step (taking account of effects at termination sites) to model transcript elongation. This operon object construct models transcription time delays to different genes on the operon and the pipelines of RNAP molecules that continue transcription after the promoter is off. It also provides a simple technique for modeling RNAP flow control by transcription termination sites. Within a circuit simulation, when the promoter control logic determines the promoter is ON, RNAP molecules are injected into one end of the array and then shifted along the array automatically at each time step. At any time, the program can determine the level of transcription of a gene on an operon object by querying the object regarding the rate of RNAP molecules traversing the location of that gene.

In the limits of high MOI (10, favoring high CII and, hence, lysogeny) and low MOI (1, low CII and, thus, lysis), the action of the λ switch (36) and of the CII-CIII interaction is definitive and control of the other promoters fits the Boolean approximation. Thus, we can check the circuit logic in Figs. 4 and 5 by assessing whether correct outcomes are achieved in these limiting cases. The logic validation program incorporates the signal path connectivity and the promoter control logic in Figs. 4 and 5. Transcription time delays, the first term in the T_delay equation above, can be modeled by operon objects defined as described above for the five operons headed by P_R, P_L, P_KE, P_AQ, and P_A (4). Because data to model signal path dynamics are not available, signal accumulation delays were estimated for the high and low MOI cases with the second term in the T_delay equation and plausible parameters. Signal time delays (not full signal dynamics) were modeled with a signal proxy constrained to the range 0 to 1.0 and using linear growth when the signal gene is being transcribed and translated, and linear decay otherwise. Growth and decay slopes were chosen to produce the required delays.

Figure 6A (high MOI) shows the correct sequencing of promoters, the delays during signal protein buildup, the onset of regulation by CI, and initiation of integration of the lysogen. After integration, the CI feedback loop decreases y_RM activity to the low level sufficient to maintain the CI repressor protein concentration necessary to prevent excision. CI concentration falls to its steady-state value, production of other λ proteins ceases, and the proteins are degraded. As predicted, the repression of Cro production (gate G3) and anti-Q mRNA regulation (gate G7) stabilize the lysogenic path. Figure 6B (low MOI) shows establishment of the lytic path. For the MOI = 1 case, CII was set to zero because it never rises above threshold (gate G8). Then, promoters P_RMP, P_L, and P_AQ remain OFF and the circuit proceeds to events that result in production of S and products of other late genes on the lytic path. The time delays are essential to correct sequencing of circuit functions. If the controlling signal paths in the circuit are eliminated or connected incorrectly, the circuit does not operate correctly. In summary, the order of observed promoter activation, gene expression, and decision outcomes validates the circuit logic shown in Figs. 4 and 5.

We conclude from experience with the λ decision circuit that construction of a simulation model of a genetic circuit that is hypothesized to explain experimental observation provides a powerful test of the hypothesis. The simulation forces identification of connectivity and explicit accounting for timing and sequencing of events. Because intuitive analysis of systems with time lags and feedback is notoriously difficult and error prone, the simulation calculations provide a check on the intuitive understanding. If the simulation does not replicate observed behavior, then the hypothesized circuit is incorrect unless the deviation can be explained by modeling approximations. For example, we initially had a simpler conception of the λ circuit. We were led to detailed examination of transcription time delays and the MOI dependence of logic involving CII and CIII by simulation results. We are optimistic that libraries of generic object-oriented software models of common genetic mechanisms can be developed to provide geneticists the type of user-friendly simulation tools that electrical circuit analysts now take for granted.

REFERENCES AND NOTES
13. The in vivo transcription rate in E. coli has been directly observed at 42 nucleotides per second [S. L. Gotta, O. L. Miller Jr., S. L. French, J. Bacteriol. 173, 6647 (1991)]. The overall in vivo rate is 12 to 19 nt/s [A. Kornberg and T. A. Baker, DNA Replication (W. H. Freeman, New York, 1992), p. 248]. Higher rates, in the range of 50 to 60 nt/s, are also observed, particularly in vitro. See also U. Vogel and K. F. Jensen, J. Bacteriol. 176, 2807 (1994).
14. Steady-state concentrations of all cellular proteins are determined by the dynamic balance between production and losses. Assumptions for the equations in Fig. 2A include (i) transcription initiation is the rate-limiting step; (ii) the repressing protein-promoter interaction has first-order kinetics (higher order kinetics enter through the repression term, Yf), in Eq. 5; (iii) transcription and translation are coupled so that the number of proteins produced per transcript is a constant; (iv) independent of transcription rate but differing from gene to gene [16] C. Yarchuk, N. Jacques, J. Gullenn, M. Dreyfus, J. Mol. Biol. 226, 581 (1992); (v) for low (<10%) MOI, protein production is proportional to MOI (RNP, ribosome, or substrate availability is limiting at high MOI) K. L. Luuk and K. Mark, J. Virol. 64, 183 (1983); G. P. Zambetti and R. C. Shuster, Mol. Gen. Genet. 193, 322 (1984). In vivo experiments at an average MOI result in a Poisson distribution of MOIs within the experimental cell population, with the exception of induced monocytes for which the initial MOI is 1. We neglect (i) additional concentration reduction attributable to dilution in growing cells and (ii) additional production from newly replicated viral DNA.
17. The circuit in Fig. 4 includes the functions essential to the path choice decision. Functions such as the OCP antisense RNA whose role in λ regulation appears to be nonessential for the path choice are not included [1] L. Kranke and D. L. Wulf, Genes Dev. 1, 1005 (1987); ibid. 4, 2223 (1990).
35. Measurements of the signal protein half-lives are available. The rate of signal protein transcript production can be estimated for the critical operons, but with diminishing certainty for downstream genes on P2 and P1 operons as a result of polarity effects. The kinetics of the P2R, P1, P1R, and P1R promoters have been extensively studied. The average proteins/ transcript parameter is not available.
36. For a kinetic model of the switch, see (16).
38. We thank L. Hershkowitz, D. Botstein, D. Kaiser, S. Kustu, and A. Campbell for valuable suggestions.