Demand Theory of Gene Regulation. I. Quantitative Development of the Theory

Michael A. Savageau

Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109-0620

Manuscript received December 15, 1997
Accepted for publication May 6, 1998

ABSTRACT

The study of gene regulation has shown that a variety of molecular mechanisms are capable of performing this essential function. The physiological implications of these various designs and the conditions that might favor their natural selection are far from clear in most instances. Perhaps the most fundamental alternative is that involving negative or positive modes of control. Induction of gene expression can be accomplished either by removing a restraining element, which permits expression from a high-level promoter, or by providing a stimulatory element, which facilitates expression from a low-level promoter. This particular design feature is one of the few that is well understood. According to the demand theory of gene regulation, the negative mode will be selected for the control of a gene whose function is in low demand in the organism's natural environment, whereas the positive mode will be selected for the control of a gene whose function is in high demand. These qualitative predictions are well supported by experimental evidence. Here we develop the quantitative implications of this demand theory. We define two key parameters: the cycle time *C*, which is the average time for a gene to complete an ON/OFF cycle, and demand *D*, which is the fraction of the cycle time that the gene is ON. Mathematical analysis involving mutation rates and growth rates in different environments yields equations that characterize the extent and rate of selection. Further analysis of these equations reveals two thresholds in the *C vs. D* plot that create a well-defined region within which selection of wild-type regulatory mechanisms is realizable. The theory also predicts minimum and maximum values for the demand *D*, a maximum value for the cycle time *C*, as well as an inherent asymmetry between the regions for selection of the positive and negative modes of control.

DIFFERENTIAL regulation of gene expression is central to much of modern biology. Animal development can be thought of in terms of an early phase, which begins with an egg and ends with an embryo, and a late phase, which begins with an embryo and ends with the mature organism (Slack 1992). Some genes function only in the early phase while others only in the late phase. The inability to express a gene when it should be ON or the excess expression of a gene when it should be OFF is usually dysfunctional and often lethal. For any given gene, expression can be considered a roughly periodic function, which in the simplest case is OFF for a period and ON for another period with the total duration being the lifetime of the organism. The differential regulation of many such genes in time and space determines the pattern of cell-specific expression that underlies development of the organism.

The life cycle of a bacterial association with a host organism also can be thought of in terms of an early phase, which begins with entry into a host organism and ends with successful colonization, and a late phase, which begins with colonization and ends, after a period of stable association, with the entry of another host (Salyers 1994). Some bacterial genes function only in the early phase of initial colonization while others only in the late phase of stable association. Again, the inability to express a gene when it should be ON or the excess expression of a gene when it should be OFF is dysfunctional and in some cases lethal. Expression of any given gene is OFF for a period and ON for another period with the total duration in this case being the time for the bacteria to cycle from one host to another. Although the organisms in these two examples are quite different, in each case appropriate differential regulation of gene expression is clearly key to their survival.

A great deal is known about the molecular details of many gene systems, particularly in well-studied prokaryotic organisms. The wealth of studies in this area has revealed a variety of designs for the regulation of gene expression. However, we are just beginning to understand the functional implications of these various designs and to grasp the factors that have influenced their evolution.

One of the first variations in molecular design to be addressed was negative *vs.* positive modes for controlling gene expression. For example, the lactose (*lac*) operon in *Escherichia coli* is an inducible system with a negative mode of control by a repressor protein, the *lacI* gene product (Miller and Reznikoff 1980). In an appropriate environment, induction occurs in response

Address for correspondence: 5641 Medical Science Bldg. II, Department of Microbiology and Immunology, The University of Michigan Medical School, Ann Arbor, MI 48109-0620. E-mail: savageau@umich.edu

to addition of the specific inducer, which results in removal of repressor and initiation of transcription. In contrast, the maltose (*mal*) operon is an inducible system with a positive mode of control by an activator protein, the *malT* gene product (Schwartz 1987). Induction in this case involves the specific inducer binding to the activator protein, which is then able to interact with RNA polymerase and facilitate initiation of transcription. The same physiological function, induction, is being realized in each of these cases, but by alternative molecular mechanisms. Are these alternative designs historical accidents that are functionally equivalent, or have they been selected in nature because they exhibit functional differences?

An answer to this question was provided by demand theory (Savageau 1974, 1977, 1983a, 1989), which is based on selectionist arguments. In its simplest form, the theory can be understood in familiar qualitative terms and leads to the following predictions: a negative mode of control will be selected when there is a low demand for expression of the effector genes in the organism's natural environment; a positive mode will be selected when there is a high demand for their expression. These predictions, and a number of others that follow as natural extensions, have been tested in over 100 cases and there has been excellent agreement (Savageau 1979, 1983b, 1985).

Here I develop the quantitative implications of demand theory. Models that include consideration of the organism's life cycle, molecular mechanisms of gene control, and population dynamics are used to describe mutant and wild-type populations in two environments with different demands for expression of the genes in question. These models are analyzed mathematically to identify conditions that lead to either selection or loss of a given mode of control. It will be shown that this theory ties together a number of important variables, including growth rates, mutation rates, minimum and maximum demands for gene expression, and minimum and maximum durations for the life cycle of the organism. An application of the theory is provided in the accompanying article (Savageau 1998), where regulation of the *lac* and *mal* operons of *E. coli* is analyzed and the results are compared with independent experimental data.

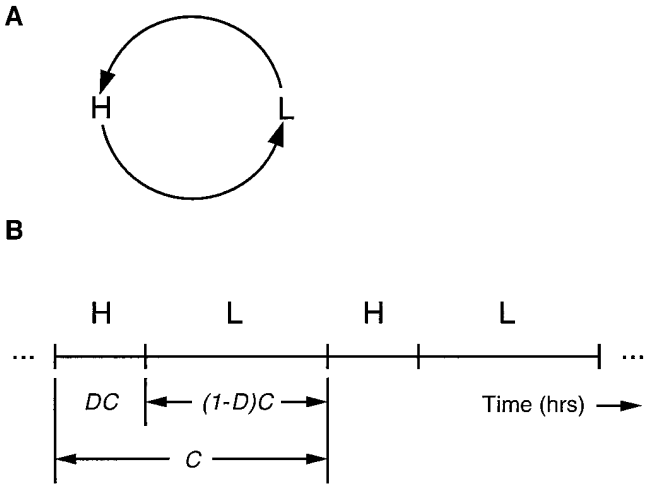

Figure 1.—The life cycle of an organism alternating between two different environments. (A) Expression of the genes that are specifically required for growth in the environment labeled H is in high demand, whereas in the alternative environment labeled L their expression is in low demand. (B) The average time required for the organism to complete its life cycle is denoted by *C*. The fraction of its cycle time spent in environment H is denoted by *D*, which also represents demand for expression of the H-specific genes.

MODELS

Life cycle: We shall consider a given effector gene in an organism that cycles between two alternative environments, a high-demand environment H, and a low-demand environment L, as shown in Figure 1. The average *cycle time* required for one complete passage through both H and L environments is denoted by *C*. The average fraction of time spent in the high-demand environment is denoted by *D*. Note that *D* also signifies *demand for expression* of the regulated effector gene. If $D = 0$, demand is minimal because the organism is always in the low-demand environment; if $D = 1$, demand is maximal because the organism is always in the high-demand environment.

Gene expression: The models of gene expression and mutation that will be treated are shown schematically in Figures 2 and 3. The effector genes in each case are normally expressed in environment H but not in environment L. To simplify the diagrams and the discussion, we shall consider mutations in the regulatory mechanism to be an alteration in the modulator site. Mutations in the structural gene for the regulator protein also can disrupt the normal interaction between the regulatory protein and the modulator site to which it binds, and these will be suitably accounted for even though they will not be represented diagrammatically or discussed in detail. Other types of mutations will be considered briefly in the discussion section.

In the negative mode of control (Figure 2), environment H involves expression of the effector gene in the wild-type organism. It also involves expression in the mutants with a defect in the modulator site to which the negative regulator binds. Normal expression is prevented in the mutants with a defect in the promoter site. Environment L involves the absence of expression of the effector gene in the wild-type organism and in the mutants with a defect in the promoter site. There is inappropriate expression in the mutant with a defect in the modulator site. The mutation rates between the different populations are as indicated.

In the positive mode of control (Figure 3), environ-

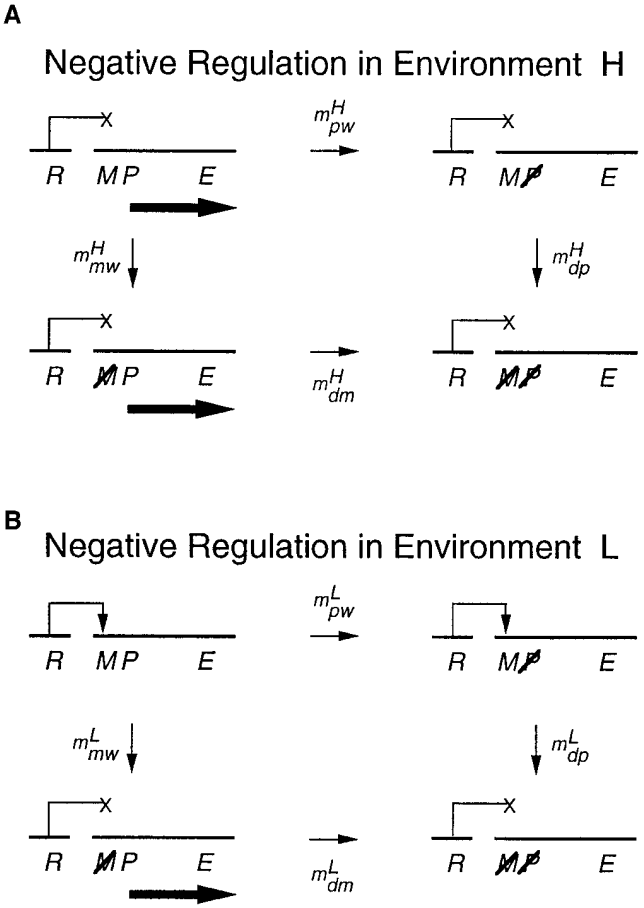

Figure 2.—Expression of genes governed by the negative mode of control in the high-demand (H) and low-demand (L) environments. The symbols are as follows: structural gene for the regulator protein, *R*; structural gene for the effector protein, *E*; nucleotide sequence for the promoter site, *P*; and nucleotide sequence for the modulator site, *M*. The wild-type promoter in the negative mode must be a high-level promoter to achieve full expression upon removal of repressor, and a functional modulator site (operator) is necessary for expression to be turned off in the presence of repressor. The heavy arrows indicate transcription of the effector gene. The four diagrams in A and B represent the genotypes of the wild-type (w), promoter mutant (p), modulator mutant (m), and double mutant (d). The mutation rates between the populations of organisms that harbor each of these genotypes are as indicated with the appropriate subscripts and superscripts; *e.g.*, m^{H}_{dm} represents the mutation rate in the high-demand environment for production of double mutants (d) from modulator mutants (m).

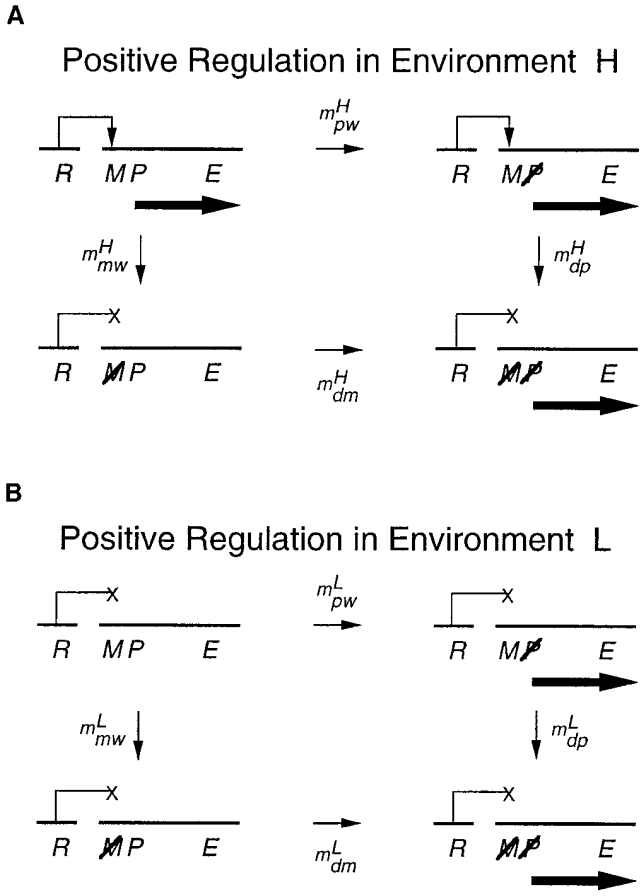

Figure 3.—Expression of genes governed by the positive mode of control in the high-demand (H) and low-demand (L) environments. The symbols are as follows: structural gene for the regulator protein, *R*; structural gene for the effector protein, *E*; nucleotide sequence for the promoter site, *P*; and nucleotide sequence for the modulator site, *M*. The wild-type promoter in the positive mode must be a low-level promoter for expression to be turned off upon removal of activator, and a functional modulator site (initiator) is necessary to achieve full expression in the presence of activator. The heavy arrows indicate transcription of the effector gene. The four diagrams in A and B represent the genotypes of the wild-type (w), promoter mutant (p), modulator mutant (m), and double mutant (d). The mutation rates between the populations of organisms that harbor each of these genotypes are as indicated with the appropriate subscripts and superscripts; *e.g.*, m^{L}_{pw} represents the mutation rate in the low-demand environment for production of promoter mutants (p) from wild-type organisms (w).

ment H involves expression of the effector gene in the wild-type organism. It also involves expression in the mutants with a mutationally enhanced promoter site. Normal expression is prevented in the mutants with a defect in the modulator site. Environment L involves the absence of expression of the effector gene in the wild-type organism and in the mutants with a defect in the modulator site. There is inappropriate expression in the mutants with a mutationally enhanced promoter site. The mutation rates between the different populations are as indicated, but it should be noted that the values for these parameters need not be the same for the two modes of control.

Populations: All of the relevant populations and conditions can be represented in a common abstract diagram in which the growth rates of the individual populations and the mutation rates between populations are explicitly depicted (Figure 4). There will be four sets of parameter values associated with this diagram, one each for the negative mode in high demand, the nega-

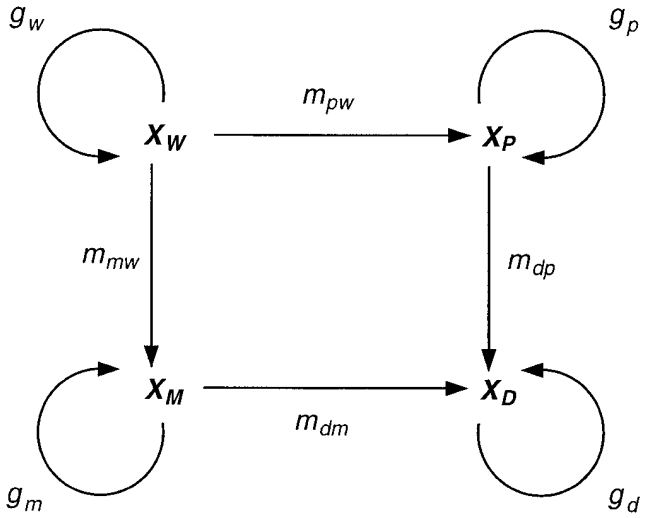

Figure 4.—Schematic diagram representing the populations of wild-type and mutant organisms. The symbols are as follows: number of wild-type organisms, X_W; number of promoter mutants, X_P; number of modulator mutants, X_M; and number of double mutants, X_D. The growth rates of each population are indicated by the symbol *g* with the relevant subscripts, and the mutation rates between populations are indicated by *m* with the appropriate subscripts. See text for further discussion.

tive mode in low demand, the positive mode in high demand, and the positive mode in low demand.

Assumptions: These models are based on a number of assumptions. First, the organisms harboring these gene systems are assumed to be otherwise isogenic. Second, because we are interested in the conditions for selection of the wild-type regulatory mechanism, we shall assume that the ratio of wild-type to mutant organisms is initially 1 / 10 its steady-state value and then examine the conditions that lead to enrichment of the wild type. Third, sites in the DNA consist of a number of critical bases, and mutation in any one of these leads to a loss of function in the modulator sites. The same is true of the high-level promoter in the negative mode. The low-level promoter in the positive mode consists of a smaller number of critical bases, and mutation in any of these leads to a mutationally enhanced promoter level. Fourth, the regulator gene consists of a number of critical bases, and mutation in any one of these leads to a loss of the regulator function. Fifth, we will be concerned only with the forward mutational events as indicated in Figures 2–4. The back mutational events can be neglected because the mutant populations will be small, according to our criterion for selection, and the probability of back mutation is lower than that in the forward direction. Sixth, although our models will account for the dynamics of the doubly mutant population, we will neglect this aspect because the singly mutant populations will be small and the probability of a second mutation will make the production rate of the doubly mutant population that much smaller. Finally, we shall assume that expression is fully ON or fully OFF and that both the positive and negative modes of control have the same capacity for gene regulation (Savageau 1989), which we take to be 100 for the ratio of full expression to basal expression.

PARAMETERS

The macroscopic parameters in our theory can be decomposed into constituent parameters that are defined in terms of reference values and relative values for mutation rates and growth rates.

Mutation rates: The reference mutation rate μ is given by the spontaneous mutation rate per base per DNA replication. The spontaneous mutation rate for various structures in our model can be determined from estimates of the spontaneous mutation rate per base and the relative mutation rate given by the number of critical bases that define the DNA targets for these structures. We will consider the following relative mutation rates in our model: π for loss of a high-level promoter site, υ for gain of a high-level promoter site, τ for loss of a regulator's functional target site, and ρ for loss of a functional regulator protein. We can also define a relative mutation rate ε and explore the effects of gene expression on mutation rate (Datta and Jinks-Robertson 1995; Francino *et al.* 1996).

Growth rates: The reference growth rate γ is defined as the growth rate of the wild-type organism in the nutritionally richer of the two environments. Its value is not critical because one can simply rescale time accordingly and none of our results would change. The growth rates in other circumstances can be expressed as the product of the reference growth rate and the appropriate relative growth rate. We will consider the following relative growth rates in our model: λ for mutants that have lost normal expression of the effector gene, σ for mutants that exhibit superfluous expression of the effector gene, and δ for the more nutritionally deficient of the two environments.

Criterion for selection: Our criterion for selection is that each mutant population shall be reduced to no more than θ of the wild-type population. A typical value for θ is 0.05% (Leclerc *et al.* 1996).

These relationships are summarized in Table 1. Numerical estimates for these parameters are given in the accompanying article (Savageau 1998), which provides a specific application of the theory.

QUANTITATIVE DEVELOPMENT OF THE THEORY

The mathematical analysis needed for this development can be significantly reduced by taking advantage of two fundamental symmetries in our model. First, there is a symmetry between the promoter-mutant and modulator-mutant populations that is evident in Figure 4. If the subscripts p and m are simply interchanged the model remains unchanged. This means that we need

TABLE 1

Decomposition of macroscopic parameters into constituent parameters

	Mode of control			
	Negative		Positive	
Parameter[a]	High demand	Low demand	High demand	Low demand
g_w	γ	$\gamma\delta$	$\gamma\delta$	γ
g_p	$\gamma\lambda$	$\gamma\delta$	$\gamma\delta$	$\gamma\sigma$
g_m	γ	$\gamma\delta\sigma$	$\gamma\delta\lambda$	γ
g_d	$\gamma\lambda$	$\gamma\delta$	$\gamma\delta$	$\gamma\sigma$
m_{pw}	$\mu\pi\varepsilon$	$\mu\pi$	$\mu\upsilon\varepsilon$	$\mu\upsilon$
m_{mw}	$\mu\ (\tau + \rho)\varepsilon$	$\mu\ (\tau + \rho)$	$\mu\ (\tau + \rho)\ \varepsilon$	$\mu\ (\tau + \rho)$
m_{dp}	$\mu\ (\tau + \rho)$	$\mu\ (\tau + \rho)$	$\mu\ (\tau + \rho)\ \varepsilon$	$\mu\ (\tau + \rho)\ \varepsilon$
m_{dm}	$\mu\pi\varepsilon$	$\mu\pi\varepsilon$	$\mu\upsilon$	$\mu\upsilon$

See Figures 2–4 for definition of parameters.

[a] The parameters for growth rates and mutation rates in turn determine the parameters for the rate constants in the dynamic Equations 1–4: $\alpha_{ww} = [1 - (m_{pw} + m_{mw})]g_w$, $\alpha_{pw} = m_{pw}g_w$, $\alpha_{pp} = (1 - m_{dp})g_p$, $\alpha_{mw} = m_{mw}g_w$, $\alpha_{mm} = (1 - m_{dm})g_m$, $\alpha_{dm} = m_{dm}g_m$, $\alpha_{dp} = m_{dp}g_p$, $\alpha_{dd} = g_d$.

only carry out the analysis for the promoter-mutant population; the corresponding results for the modulator-mutant population can then be obtained simply by interchanging the subscripts p and m. Second, there is a symmetry between the first and second phases of the cycle depicted in Figure 1. If the H and L phases are interchanged along with the symbols D and $(1 - D)$ the temporal pattern remains unchanged. This means that we need only carry out the analysis from the beginning of the H phase; the corresponding results from the beginning of the L phase can then be obtained by interchanging the superscripts H and L and the symbols D and $(1 - D)$.

Dynamics: The equations describing the dynamic behavior of the model in Figure 4 are

$$dX_w/dt = \alpha_{ww} X_w \tag{1}$$

$$dX_p/dt = \alpha_{pw} X_w + \alpha_{pp} X_p \tag{2}$$

$$dX_m/dt = \alpha_{mw} X_w + \alpha_{mm} X_m \tag{3}$$

$$dX_d/dt = \alpha_{dm} X_m + \alpha_{dp} X_p + \alpha_{dd} X_d, \tag{4}$$

where the numbers for each population as a function of time are given by the symbol X with appropriate subscripts and the first-order rate constants are given by the symbol α, again with appropriate subscripts. The rate constants are in turn related to the various mutation rates and growth rates, represented by the symbols m and g with suitable subscripts: $\alpha_{ww} = [1 - (m_{pw} + m_{mw})]g_w$, $\alpha_{pw} = m_{pw}g_w$, $\alpha_{pp} = (1 - m_{dp})g_p$, $\alpha_{mw} = m_{mw}g_w$, $\alpha_{mm} = (1 - m_{dm})g_m$, $\alpha_{dm} = m_{dm}g_m$, $\alpha_{dp} = m_{dp}g_p$, $\alpha_{dd} = g_d$.

Equations 1–4 are linear and easily solved to obtain numbers for the wild-type and mutant populations as a function of time. The numbers for the wild-type and promoter-mutant populations at the end of a full period in environment H are given in terms of the initial values at an arbitrary time t:

$$X_w(t + DC) = X_w(t)\ \exp[\alpha^H_{ww} DC] \tag{5}$$

$$\begin{aligned} X_p(t + DC) = {} & [\alpha^H_{pw}/(\alpha^H_{ww} - \alpha^H_{pp})]X_w(t)\ \exp[\alpha^H_{ww} DC] \\ & + \{X_p(t) - [\alpha^H_{pw}/(\alpha^H_{ww} - \alpha^H_{pp})]X_w(t)\} \\ & \times \exp[\alpha^H_{pp} DC]. \end{aligned} \tag{6}$$

These numbers then become the initial values for the solution in environment L, and the numbers at the end of the period in environment L are then

$$X_w(t + C) = X_w(t)\ \exp[\alpha^H_{ww} DC]\ \exp[\alpha^L_{ww}(1 - D)C] \tag{7}$$

$$\begin{aligned} X_p(t + C) = {} & X_w(t)\ \{[\alpha^L_{pw}/(\alpha^L_{ww} - \alpha^L_{pp})]\ \exp[\alpha^H_{ww} DC] \\ & \times \{\exp[\alpha^L_{ww}(1 - D)C] - \exp[\alpha^L_{pp}(1 - D)C]\} \\ & + [\alpha^H_{pw}/(\alpha^H_{ww} - \alpha^H_{pp})]\ \exp[\alpha^L_{pp}(1 - D)C] \\ & \times \{\exp[\alpha^H_{ww} DC] - \exp[\alpha^H_{pp} DC]\}\} \\ & + X_p(t)\ \exp[\alpha^H_{pp} DC]\ \exp[\alpha^L_{pp}(1 - D)C]. \end{aligned} \tag{8}$$

Thus, the temporal behavior is determined by four exponential functions with time constants that are independent of C.

The ratio of the promoter-mutant to the wild-type numbers, which is plotted in Figure 5, yields

$$\begin{aligned} X_p(t + C)/X_w(t + C) = {} & \{[\alpha^L_{pw}/(\alpha^L_{ww} - \alpha^L_{pp})] \\ & \times \{1 - \exp[(\alpha^L_{pp} - \alpha^L_{ww})(1 - D)C]\} \\ & + [\alpha^H_{pw}/(\alpha^H_{ww} - \alpha^H_{pp})] \\ & \times \{1 - \exp[(\alpha^H_{pp} - \alpha^H_{ww})DC]\} \\ & \times \exp[(\alpha^L_{pp} - \alpha^L_{ww})\ (1 - D)C]\} \\ & + \{\exp[(\alpha^H_{pp} - \alpha^H_{ww})DC] \\ & \times \exp[(\alpha^L_{pp} - \alpha^L_{ww})(1 - D)C]\} \\ & \times X_p(t)/X_w(t) \end{aligned}$$

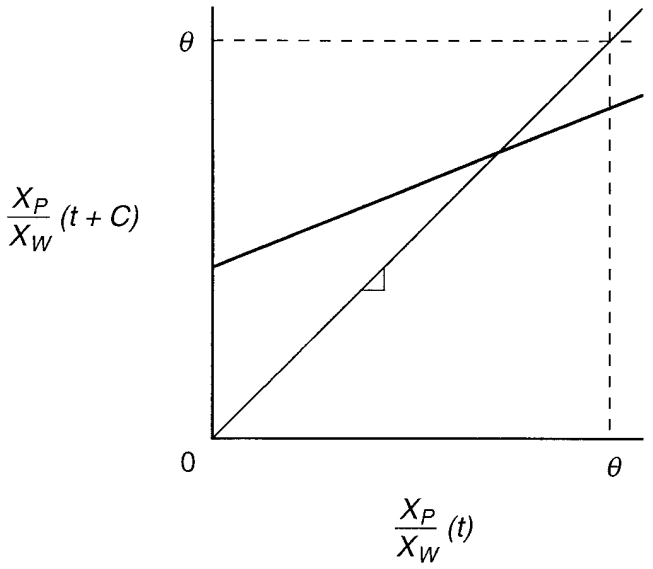

Figure 5.—Recursive relationship for the ratio of population sizes for promoter-mutant and wild-type organisms. The horizontal axis gives the value of the ratio at an arbitrary time t; the vertical axis gives the value at the subsequent time $t + C$, which is one complete cycle later. Selection for the wild-type organism is indicated when the recursive relationship, which is the straight line given by Equation 9, has a slope between 0 and 1 and an intercept between 0 and 0.0005. The intersection of this line with the 45° line determines a value for the ratio that represents a stable steady state.

or

$$X_p(t + C)/X_w(t + C) = (\text{intercept}) + (\text{slope})\ X_p(t)/X_w(t). \tag{9}$$

Note that the intercept and slope in this expression are both positive quantities. A slope greater than 1 implies that the ratio tends to infinity with time and thus that the wild-type promoter is lost. A slope between 0 and 1 implies that the ratio tends to a fixed value (given by the intersection with the 45° line) with time and, if this value is less than θ (the criterion for selection), that the wild-type promoter will be preserved. An intercept greater than θ implies loss of the wild-type promoter no matter what the value of the slope.

Starting with any set of values for the wild-type and promoter-mutant populations, Equations 7–9 can be applied recursively to calculate the subsequent population sizes and ratios as a function of time. From these results one can determine the rate of selection of the wild-type regulatory mechanism.

Steady-state pattern: The ratio of promoter-mutant and wild-type populations increases in one environment and decreases in the other to produce a sawtooth pattern. Once the initial transients have died away, a repeating pattern with two steady-state values is established. The first value of the ratio in steady state, when it exists, is calculated by equating the ratios on the two sides of Equation 9 and solving to obtain the following expression:

$$\begin{aligned} X_p/X_w = {} & \{[\alpha^L_{pw}/(\alpha^L_{ww} - \alpha^L_{pp})] \\ & \times \{1 - \exp[(\alpha^L_{pp} - \alpha^L_{ww})(1 - D)C]\} \\ & + [\alpha^H_{pw}/(\alpha^H_{ww} - \alpha^H_{pp})] \\ & \times \{1 - \exp[(\alpha^H_{pp} - \alpha^H_{ww})DC]\} \exp[(\alpha^L_{pp} - \alpha^L_{ww}) \\ & \times (1 - D)C]\}/\{1 - \exp[(\alpha^H_{pp} - \alpha^H_{ww})DC \\ & + (\alpha^L_{pp} - \alpha^L_{ww})(1 - D)C]\}\ . \end{aligned} \tag{10}$$

If, instead of starting the analysis at the beginning of the period in environment H, we were to start it at the beginning of the period in environment L, then the results would be equivalent to those in Equations 5–10 except for an exchange of the superscripts H and L and the symbols D and $(1 - D)$. The second value of the ratio in steady state, when it exists, is thus

$$\begin{aligned} X_p/X_w = {} & \{[\alpha^H_{pw}/(\alpha^H_{ww} - \alpha^H_{pp})]\{1 - \exp[(\alpha^H_{pp} - \alpha^H_{ww})DC]\} \\ & + [\alpha^L_{pw}/(\alpha^L_{ww} - \alpha^L_{pp})]\{1 - \exp[(\alpha^L_{pp} - \alpha^L_{ww}) \\ & \times (1 - D)C]\} \\ & \times \exp[(\alpha^H_{pp} - \alpha^H_{ww})DC]\}/\{1 - \exp[(\alpha^L_{pp} - \alpha^L_{ww}) \\ & \times (1 - D)C + (\alpha^H_{pp} - \alpha^H_{ww})DC]\}\ . \end{aligned} \tag{11}$$

Equations 10 and 11 represent different aspects of the same steady-state pattern. One of the two steady-state solutions for this ratio gives the maximum value whereas the other gives the minimum value. These values can be used to define the extent of selection. We shall always be interested in the maximum value of the ratio; if this is less than the criterion for selection, then the minimum value will certainly be less as well.

Definition of the threshold for selection: The threshold for selection of the wild-type promoter is obtained from the solution of Equation 10 or 11, whichever gives the maximum value for the ratio. The values for the growth rates and mutation rates in the high- and low-demand environments (for either the positive or the negative mode of control in Table 1) determine the values for the rate-constant parameters that appear in Equations 10 and 11. The ratio X_p/X_w is then fixed with a value equal to θ, which is the criterion for selection. The result of these parameter assignments is a nonlinear equation involving the cycle time C and the demand for gene expression D that defines the *threshold for selection*. There is no explicit solution for C as a function of D. However, the threshold for selection of the wild-type promoter can be obtained by bisection (Press *et al.* 1988) when numerical values are assumed for the parameters in Equation 10 or 11.

As noted at the beginning of this section, the corresponding results for the modulator-mutant population can be obtained from Equations 5–11 simply by inter-

changing the subscripts p and m. We will make use of these expressions below.

Although there is no analytical solution that gives the thresholds for selection, their asymptotic behavior can be determined analytically. As will be seen in the following sections, the analytical expressions allow one to draw general conclusions that are independent of particular numerical values for the parameters.

Threshold for selection of a promoter with the negative mode: The ratio of promoter-mutant and wild-type populations is decreasing in environment H and increasing in environment L. Thus, the maximum value in steady state is determined from the analysis that starts in H. The asymptotic character of the threshold for selection of the promoter can be determined from Equation 10. First, it should be noted from Table 1 that $(\alpha^{L}_{pp} - \alpha^{L}_{ww}) > 0$ and $\alpha^{L}_{pw}/(\alpha^{L}_{ww} - \alpha^{L}_{pp}) = -1$. Second, for typical values of the parameters, $(\alpha^{H}_{pp} - \alpha^{H}_{ww}) < 0$.

When $C \gg 1$, and $D > (\alpha^{L}_{pp} - \alpha^{L}_{ww})/[(\alpha^{L}_{pp} - \alpha^{L}_{ww}) - (\alpha^{H}_{pp} - \alpha^{H}_{ww})]$, Equation 10 can be approximated as

$$\theta = \exp[(\alpha^{L}_{pp} - \alpha^{L}_{ww})(1 - D)C] - 1 + [\alpha^{H}_{pw}/(\alpha^{H}_{ww} - \alpha^{H}_{pp})] \exp[(\alpha^{L}_{pp} - \alpha^{L}_{ww})(1 - D)C], \quad (12)$$

where θ is the criterion for selection of the promoter. Solving for C as a function of D yields

$$C = \frac{\log[1 + \theta] - \log[1 + \alpha^{H}_{pw}/(\alpha^{H}_{ww} - \alpha^{H}_{pp})]}{(\alpha^{L}_{pp} - \alpha^{L}_{ww})} \frac{1}{1 - D}. \quad (13)$$

The arguments of the logarithms are nearly unity, so that

$$C = \frac{\theta - \alpha^{H}_{pw}/(\alpha^{H}_{ww} - \alpha^{H}_{pp})}{(\alpha^{L}_{pp} - \alpha^{L}_{ww})} \frac{1}{1 - D} \quad (14)$$

or

$$C = \frac{\theta - \mu\pi\varepsilon/[1 - \lambda(1 - \mu\tau - \mu\rho) - \mu\varepsilon(\pi + \tau + \rho)]}{\mu\pi\gamma\delta} \times \frac{1}{1 - D} \approx \frac{\theta}{\mu\pi\gamma\delta} \frac{1}{1 - D}. \quad (15)$$

Thus, the high-C asymptote in a log C *vs.* log D plot is given by a line that is nearly horizontal for values of $D \ll 1$ and that approaches infinity as D goes to unity.

When $C \ll 1$, the exponential functions in Equation 10 can be approximated by the first three terms of their Taylor series and the resulting equation can be solved for C as a function of D. The value of $D = D_{min}$ that makes $C = 0$ is given by

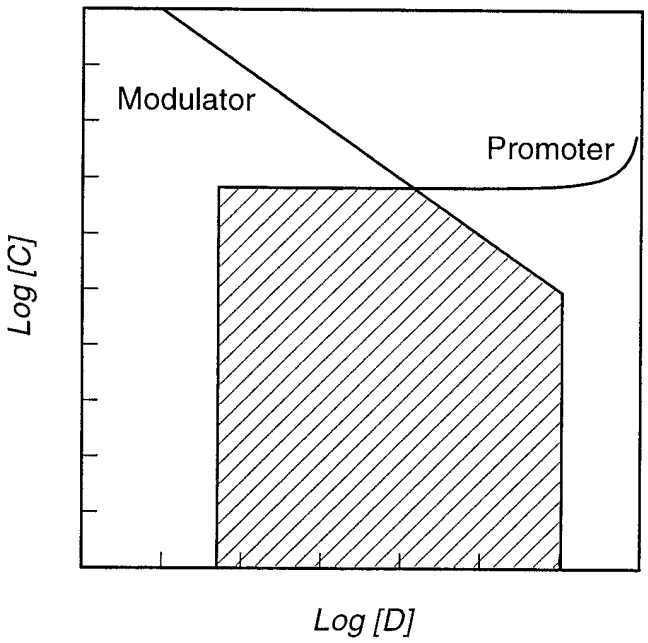

Figure 6.—Schematic representation of the thresholds for selection of the wild-type regulatory mechanism as functions of the cycle time and the demand for gene expression. The threshold for selection against the promoter mutants is obtained for a given set of parameter values by setting the ratio of $X_p/X_w = 0.0005$ in Equation 10 or 11 and then solving for the cycle time C as a function of the demand for gene expression D. The threshold for selection against the modulator mutants is obtained in a similar fashion (see text for discussion). In each case, selection is indicated by values for C and D that lie below the calculated threshold. Selection for the wild-type regulatory mechanism occurs for those values of C and D that lie below both threshold simultaneously. These thresholds define minimum and maximum values for demand.

$$D_{min} = \frac{(\alpha^{L}_{pp} - \alpha^{L}_{ww})(1 + \theta)}{(\alpha^{L}_{pp} - \alpha^{L}_{ww})(1 + \theta) - \alpha^{H}_{pw} - (\alpha^{H}_{pp} - \alpha^{H}_{ww})\theta} \quad (16)$$

or

$$D_{min} = \mu\pi\delta(1 + \theta)/\{\mu\pi\delta(1 + \theta) + [1 - \lambda(1 - \mu\tau - \mu\rho) - \mu\varepsilon(\pi + \tau + \rho)]\theta - \mu\pi\varepsilon\} \approx \mu\pi\delta/\theta(1 - \lambda). \quad (17)$$

Thus, the low-C asymptote is given by a vertical line located at $D = D_{min}$ in a log C *vs.* log D plot.

The threshold for selection of the promoter is characterized by the combination of these high- and low-C asymptotes as shown schematically in Figure 6.

Threshold for selection of a modulator (regulator) with the negative mode: The ratio of modulator-mutant and wild-type populations is decreasing in environment L and increasing in environment H. Thus, the maximum value in steady state is determined from the analysis that starts in L. The asymptotic character of the

threshold for selection of the modulator (regulator) can be determined from Equation 11 after interchanging the subscripts p and m. In this case, $(\alpha^{H}_{mm} - \alpha^{H}_{ww}) > 0$, $\alpha^{H}_{mw}/(\alpha^{H}_{ww} - \alpha^{H}_{mm}) = -1$ and, for typical values of the parameters, $(\alpha^{L}_{mm} - \alpha^{L}_{ww}) < 0$.

When $C \gg 1$, and $D < (\alpha^{L}_{mm} - \alpha^{L}_{ww})/[(\alpha^{L}_{mm} - \alpha^{L}_{ww}) - (\alpha^{H}_{mm} - \alpha^{H}_{ww})]$, Equation 11 can be approximated as

$$\theta = \exp[(\alpha^{H}_{mm} - \alpha^{H}_{ww})DC] - 1 + [\alpha^{L}_{mw}/(\alpha^{L}_{ww} - \alpha^{L}_{mm})] \exp[(\alpha^{H}_{mm} - \alpha^{H}_{ww})DC], \quad (18)$$

where θ is the criterion for selection of the modulator. Solving for C as a function of D yields

$$C = \frac{\log[1 + \theta] - \log[1 + \alpha^{L}_{mw}/(\alpha^{L}_{ww} - \alpha^{L}_{mm})]}{(\alpha^{H}_{mm} - \alpha^{H}_{ww})} \frac{1}{D}. \quad (19)$$

The arguments of the logarithms are nearly unity, so that

$$C = \frac{\theta - \alpha^{L}_{mw}/(\alpha^{L}_{ww} - \alpha^{L}_{mm})}{(\alpha^{H}_{mm} - \alpha^{H}_{ww})} \frac{1}{D} \quad (20)$$

or

$$C = \frac{\theta - \mu(\tau + \rho)/[1 - \sigma(1 - \mu\pi\varepsilon) - \mu(\pi + \tau + \rho)]}{\mu(\tau + \rho)\varepsilon\gamma} \frac{1}{D} \approx \frac{\theta}{\mu(\tau + \rho)\varepsilon\gamma} \frac{1}{D}. \quad (21)$$

Thus, the high-C asymptote is given by a straight line with slope equal to -1 in a log C *vs.* log D plot.

When $C \ll 1$, the exponential functions in the steady-state ratio can be approximated by the first three terms of their Taylor series and the resulting equation can be solved for C as a function of D. The value of $D = D_{max}$ that makes $C = 0$ is given by

$$D_{max} = \frac{[-\theta(\alpha^{L}_{mm} - \alpha^{L}_{ww}) - \alpha^{L}_{mw}]}{[-\theta(\alpha^{L}_{mm} - \alpha^{L}_{ww}) - \alpha^{L}_{mw}] + (\alpha^{H}_{mm} - \alpha^{H}_{ww})(1 + \theta)} \quad (22)$$

or

$$D_{max} = \delta\{\theta[1 - \sigma(1 - \mu\pi\varepsilon) - \mu(\pi + \tau + \rho)] - \mu(\tau + \rho)\}/\delta\{\theta[1 - \sigma(1 - \mu\pi\varepsilon) - \mu(\pi + \tau + \rho)] - \mu(\tau + \rho)\} + \mu(\tau + \rho)\varepsilon(1 + \theta) \approx 1/\{1 + \mu(\tau + \rho)\varepsilon/[\delta\theta(1 - \sigma)]\}. \quad (23)$$

Thus, the low-C asymptote is given by a vertical line located at $D = D_{max}$ in a log C *vs.* log D plot.

The threshold for selection of the modulator (regulator) is characterized by the combination of these high- and low-C asymptotes as shown schematically in Figure 6.

Region in which selection for the negative mode of control is realizable: Selection for both wild-type promoter and wild-type modulator (regulator) requires values of C and D that lie in the shaded region below the two thresholds shown schematically in Figure 6. The low-C asymptotes of these thresholds (Equations 17 and 23) define the minimum D_{min} and maximum D_{max} values of the demand for gene expression. The intersection of the two thresholds yields a prediction for maximum cycle time C_{max}. As shown elsewhere, with numerical estimates for the various parameters, the theory predicts other more relevant values not only for maximum cycle time, but also for minimum cycle time and optimal cycle time (Savageau 1998). Thus, the thresholds define a region of the C *vs.* D plot within which selection for the wild-type regulatory mechanism is realizable and outside of which it is not.

Existence of a region of realizable selection for the negative mode: Clearly, $D_{max} > D_{min}$ is required for a region of realizable selection to exist. These boundaries for selection are strongly influenced by the selection coefficients ($1 - \lambda$ and $1 - \sigma$), which are related to the differences in growth rates for wild-type and mutant organisms. This is seen most clearly for the simplified case in which all relative mutation rates are equal to unity and all mutants have the same reduction in growth rate. The inequality involving Equations 17 and 23 yields a critical value for the selection coefficients; selection of the wild-type regulatory mechanism is possible only when the selection coefficients exceed this critical value:

$$(1 - \lambda) = (1 - \sigma) > \frac{\mu(1 + \delta)}{2\theta}\left[1 + \sqrt{1 + \frac{4(1 - \delta)}{(1 + \delta)^2}}\right]. \quad (24)$$

This can be seen graphically in Figure 7 where the thresholds for selection are plotted for different values of the selection coefficients.

Discriminate selection for the negative mode of control: When the reduction in growth rate for the mutants is sufficiently small ($<\sim$0.0005% in this illustration) there is no overlap beneath the thresholds. No selection for the wild-type regulatory mechanism is possible when the selection pressure is too weak. When the reduction in growth rate has an intermediate value (between 0.0005 and 0.01% in this illustration) there is a significant and well-delineated overlap beneath the thresholds. Discriminate selection for the wild-type regulatory mechanism occurs within a range of relatively *low* values for demand, but not outside it. When the reduction in growth rate is sufficiently large ($>\sim$0.01% in this illustration) the overlap is so large that it encompasses almost the entire range of values for demand. Indiscriminate selection for the wild-type regulatory mechanism occurs under these conditions.

Threshold for selection of a promoter with the positive mode: The ratio of promoter-mutant and wild-type

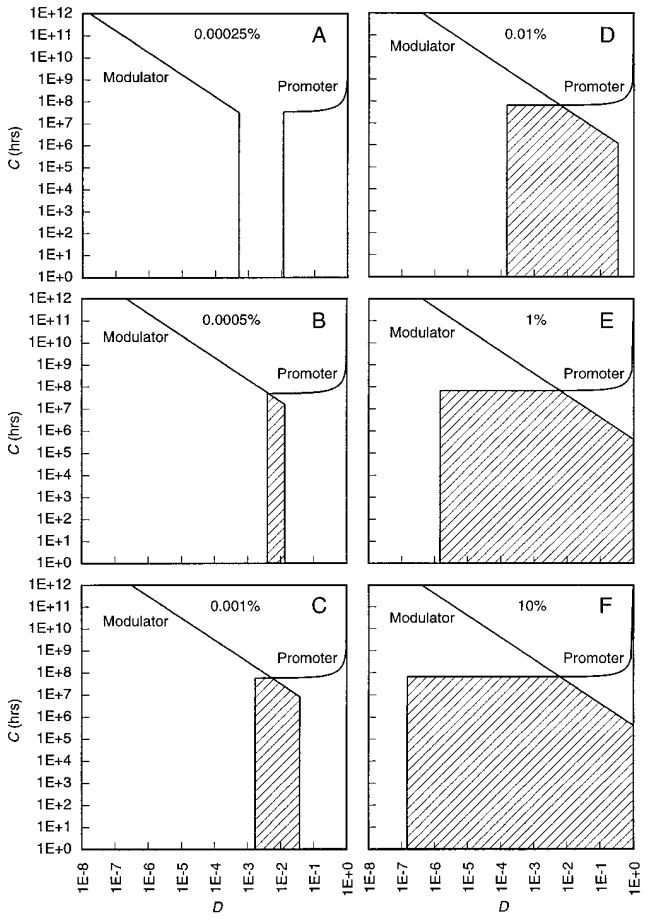

Figure 7.—Discriminate selection for wild-type regulatory mechanisms with alternative modes of control requires intermediate values for the selection coefficients. Results (A–F) are shown for the negative mode in a simplified case (see text for discussion). When selection coefficients are too low (<0.0005%), there is no selection for the wild type. At intermediate values (0.0005–0.01%), discriminate selection for the wild type occurs at relatively low values of demand. When selection coefficients are too high (>0.01%), selection for the wild-type regulatory mechanism occurs indiscriminately at nearly all values of demand. The results for the positive mode are similar, except that discriminate selection occurs at relatively high values of demand.

populations is decreasing in environment L and increasing in environment H. Thus, the maximum value in steady state is determined from the analysis that starts in L. The asymptotic character of the threshold for selection of the promoter can be determined from Equation 11. In this case, it can be seen from Table 1 that $(\alpha^{H}_{pp} - \alpha^{H}_{ww}) > 0$, $\alpha^{H}_{pw}/(\alpha^{H}_{ww} - \alpha^{H}_{pp}) = -1$ and, for typical values of the parameters, $(\alpha^{L}_{pp} - \alpha^{L}_{ww}) < 0$.

When $C \gg 1$ and $(1 - D) > (\alpha^{H}_{pp} - \alpha^{H}_{ww})/[(\alpha^{H}_{pp} - \alpha^{H}_{ww}) - (\alpha^{L}_{pp} - \alpha^{L}_{ww})]$, Equation 11 can be approximated as

$$\theta = \exp[(\alpha^{H}_{pp} - \alpha^{H}_{ww})DC] - 1 + [\alpha^{L}_{pw}/(\alpha^{L}_{ww} - \alpha^{L}_{pp})] \exp[(\alpha^{H}_{pp} - \alpha^{H}_{ww})DC]. \quad (25)$$

Solving for C as a function of $1 - D$ yields

$$C = \frac{\log[1 + \theta] - \log[1 + \alpha^{L}_{pw}/(\alpha^{L}_{ww} - \alpha^{L}_{pp})]}{(\alpha^{H}_{pp} - \alpha^{H}_{ww})} \times \frac{1}{1 - (1 - D)} \quad (26)$$

or

$$C = \frac{\theta - \mu\upsilon/[1 - \sigma(1 - \mu(\tau + \rho)\varepsilon) - \mu(\upsilon + \tau + \rho)]}{\mu\upsilon\varepsilon\gamma\delta} \times \frac{1}{1 - (1 - D)} \approx \frac{\theta}{\mu\upsilon\varepsilon\gamma\delta}\frac{1}{1 - (1 - D)}. \quad (27)$$

Thus, the high-C asymptote in a log C *vs.* log$(1 - D)$ plot is given by a line that is nearly horizontal for values of $(1 - D) \ll 1$ and that approaches infinity as $(1 - D)$ goes to unity.

When $C \ll 1$, the exponential functions in Equation 11 can be approximated by the first three terms of their Taylor series and the resulting equation can be solved for C as a function of $1 - D$. The value of $1 - D = 1 - D_{max}$ that makes $C = 0$ is given by

$$1 - D_{max} = \frac{(\alpha^{H}_{pp} - \alpha^{H}_{ww})(1 + \theta)}{(\alpha^{H}_{pp} - \alpha^{H}_{ww})(1 + \theta) - \alpha^{L}_{pw} - (\alpha^{L}_{pp} - \alpha^{L}_{ww})\theta} \quad (28)$$

or

$$1 - D_{max} = \mu\upsilon\varepsilon\delta(1 + \theta)/\{\mu\upsilon\varepsilon\delta(1 + \theta) + [1 - \sigma(1 - \mu(\tau + \rho)\varepsilon) - \mu(\upsilon + \tau + \rho)]\theta - \mu\upsilon\} \approx \mu\upsilon\varepsilon\delta/\theta(1 - \sigma) . \quad (29)$$

Thus, the low-C asymptote is given by a vertical line located at $1 - D = 1 - D_{max}$ in a log C *vs.* log$(1 - D)$ plot.

The threshold for selection of the promoter in this case is characterized by high- and low-C asymptotes that are similar to those for the negative mode shown schematically in Figure 6, except that the horizontal axis is given by log$(1 - D)$ rather than log D (data not shown).

Threshold for selection of a modulator (regulator) with the positive mode: The ratio of modulator-mutant and wild-type populations is decreasing in environment H and increasing in environment L. Thus, the maximum value in steady state is determined from the analysis that starts in H. The asymptotic character of this threshold can be determined from Equation 10 after interchanging the subscripts p and m. In this case, $(\alpha^{L}_{mm} - \alpha^{L}_{ww}) > 0$, $\alpha^{L}_{mw}/(\alpha^{L}_{ww} - \alpha^{L}_{mm}) = -1$ and, for typical values of the parameters, $(\alpha^{H}_{mm} - \alpha^{H}_{ww}) < 0$.

When $C \gg 1$, and $(1 - D) < (\alpha^{H}_{mm} - \alpha^{H}_{ww})/[(\alpha^{H}_{mm} -$

$\alpha^{H}_{ww}) - (\alpha^{L}_{mm} - \alpha^{L}_{ww})]$, Equation 10 can be approximated as

$$\theta = \exp[(\alpha^{L}_{mm} - \alpha^{L}_{ww})(1 - D)C] - 1 + [\alpha^{H}_{mw}/(\alpha^{H}_{ww} - \alpha^{H}_{mm})] \exp[(\alpha^{L}_{mm} - \alpha^{L}_{ww})(1 - D)C] . \quad (30)$$

Solving for C as a function of $1 - D$ yields

$$C = \frac{\log[1 + \theta] - \log[1 + \alpha^{H}_{mw}/(\alpha^{H}_{ww} - \alpha^{H}_{mm})]}{(\alpha^{L}_{mm} - \alpha^{L}_{ww})} \frac{1}{1 - D} \quad (31)$$

or

$$C = \frac{\theta - \mu(\tau + \rho)\varepsilon/[1 - \lambda(1 - \mu\upsilon) - \mu\varepsilon(\upsilon + \tau + \rho)]}{\mu(\tau + \rho)\gamma} \times \frac{1}{1 - D} \quad (32)$$

$$\approx \frac{\theta}{\mu(\tau + \rho)\gamma} \frac{1}{1 - D} .$$

Thus, the high-C asymptote is given by a straight line with slope equal to -1 in a log C *vs.* $\log(1 - D)$ plot.

When $C \ll 1$, the exponential functions in the steady-state ratio can be approximated by the first three terms of their Taylor series and the resulting equation can be solved for C as a function of $1 - D$. The value of $1 - D = 1 - D_{min}$ that makes $C = 0$ is given by

$$1 - D_{min} = \frac{-\alpha^{H}_{mw} - (\alpha^{H}_{mm} - \alpha^{H}_{ww})\theta}{-\alpha^{H}_{mw} - (\alpha^{H}_{mm} - \alpha^{H}_{ww})\theta + (\alpha^{L}_{mm} - \alpha^{L}_{ww})(1 + \theta)} \quad (33)$$

or

$$1 - D_{min} = \frac{\delta\{\theta[1 - \lambda(1 - \mu\upsilon) - \mu\varepsilon(\upsilon + \tau + \rho)] - \mu(\tau + \rho)\varepsilon\}}{\delta\{\theta[1-\lambda(1-\mu\upsilon)-\mu\varepsilon(\upsilon+\tau+\rho)]-\mu(\tau+\rho)\varepsilon\}+\mu(\tau+\rho)(1+\theta)}$$

$$\approx \frac{1}{1 + \mu(\tau + \rho)/[\delta\theta(1 - \lambda)]} . \quad (34)$$

Thus, the low-C asymptote is given by a vertical line located at $1 - D = 1 - D_{max}$ in a log C *vs.* $\log(1 - D)$ plot.

The threshold for selection of the modulator (regulator) in this case is characterized by high- and low-C asymptotes that are similar to those for the negative mode shown schematically in Figure 6, except that the horizontal axis is given by $\log(1 - D)$ rather than $\log D$ (data not shown).

Discriminate selection for the positive mode of control: The results for the positive mode of control are completely symmetrical to those obtained for the negative mode of control under the simplifying conditions in Figure 7; one need only replace D by $(1 - D)$. When the percentage reduction in growth rate for the mutants is small, no selection for the wild-type regulatory mechanism is possible. At intermediate percentages, discriminate selection for the positive mode of control occurs within a well-delineated range of relatively *high* values for demand, but not outside this range. At large percentages, selection occurs indiscriminately at nearly all values for demand, and, given the above results for the negative mode, one would expect positive and negative modes of control to arise at random with nearly equal probability. Such indiscriminate selection is inconsistent with the experimental evidence, which suggests discriminate selection of negative and positive modes of control based on demand for gene expression (Savageau 1989).

Asymmetric regions in which selection for the alternative modes is realizable: The simplified case examined in Figure 7 suggests completely symmetric regions in which selection for the alternative modes occurs. Alternatively, the region for the positive mode with $1 - D$ as the horizontal axis is identical to that for the negative mode with D as the horizontal axis. This implies that the value of D_{max} (Equation 23) for the negative mode is equal to the value of $1 - D_{min}$ (Equation 34) for the positive mode. This would be true if the following conditions were satisfied: $\theta_N = \theta_P$, $\mu_N = \mu_P$, $\tau_N = \tau_P$, $\rho_N = \rho_P$, $\varepsilon_N = \varepsilon_P = 1$, $\sigma_N = \lambda_P$, $\pi_N = \upsilon_P$. While it is reasonable to assume that the first four conditions are satisfied (criterion for selection θ, mutation rate μ, size of the modulator target τ, and size of the regulator ρ are the same for both the negative N and positive P mode), it is very unlikely that the last three would ever be satisfied. There is evidence that gene expression has an influence on mutation rate ($\varepsilon \neq 1$), that the reduction in growth rate due to superfluous gene expression is less than that due to the loss of normal gene expression ($\sigma_N < \lambda_P$), and that down-promoter mutations in the negative mode are more frequent than up-promoter mutations in the positive mode ($\pi_N > \upsilon_P$). From these considerations we can predict asymmetric regions in which selection for the alternative modes is realizable. Furthermore, because loss of normal expression typically causes a more significant reduction in growth rate than superfluous expression, we can predict that the realizable region for selection of the positive mode is greater than that for the negative mode.

Time course of selection: If we start with each mutant ratio (X_p/X_w and X_m/X_w) at some value larger than its steady-state value, then these mutant ratios will monotonically decrease with time, as can be seen from Figure 5. Alternatively, the wild-type regulatory mechanism is enriched with time, since the ratio of wild-type to mutant organisms $X_w/(X_m + X_p)$ is equal to the reciprocal of the *mutant fraction*, which we define as f_m. The temporal behavior of the populations is a function of the demand for gene expression D. However, the behavior is independent of the cycle time C in the following sense. The time scale is actually discrete, given by values of nC, where n is the number of cycles. Thus, within a fixed time period, the same degree of enrichment can be

achieved with either a large value for C and a small number n or a small value for C and a larger number n.

Extent of selection: While there is selection for the wild-type regulatory mechanism throughout the region of overlap beneath the thresholds (*e.g.*, Figure 6), the extent of the selection varies as a function of cycle time C and demand D. We define the *extent of selection* as the steady-state value of $X_w/(X_m + X_p)$, which is the inverse of the mutant fraction in the population $(1/f_m)$. For a given value of $C < C_{max}$, one mutant population increases as the corresponding threshold is approached; it dominates the mutant fraction and the extent of selection reaches its minimum $(1/\theta)$. Similarly, the second mutant population increases as the other threshold is approached; it dominates the mutant fraction and the extent of selection again reaches its minimum. Thus, the extent of selection reaches its maximum at a value of D that is intermediate between its threshold values.

Rate of selection: Equations 7–9 can be applied recursively to calculate population sizes and ratios as a function of time. The rate at which selection occurs is independent of cycle time, as noted above. We define *response time* as the time required for the ratio $X_w/(X_m + X_p)$ to reach 99% of its steady-state value starting from an initial state in which the numbers of the two types of mutants are equal and the ratio is equal to 1/10 of its steady-state value. Recall that the time points are given in units of nC, where C is the cycle time and n is the number of cycles. The same temporal behavior is obtained regardless of whether C is large (n small) or small (n large). However, the resolution is poorer for large values of C because the minimum value of n is 1. There is no analytical expression for response time, but it is readily determined by numerical means in specific cases, as can be seen in the following application (Savageau 1998).

DISCUSSION

Demand theory of gene regulation predicts that the molecular mode of control is correlated with the demand for gene expression in the organism's natural environment (Savageau 1989). The quantitative development presented in this article not only confirms and quantifies the previous qualitative predictions, but it also identifies critical factors and reveals new relationships.

The recursive equations that characterize the population dynamics of mutant and wild-type organisms (Equations 7–9) allow one to predict the time course for selection. The form of these equations also allows one to predict that the response time for selection is independent of the cycle time C, whereas it is strongly dependent upon the demand for gene expression D. The steady-state solution of the recursive equations provides estimates for the extent of selection (Equations 10 and 11). A threshold for selection is determined by the relationship between cycle time C and demand D that results when the extent of selection is set equal to the criterion for selection.

The thresholds for selection in the C *vs.* D plot define regions within which selection of the positive or negative mode of regulation is realizable (Figure 6). Their intersection defines a maximum value for the cycle time C_{max}, and their asymptotes define minimum D_{min} and maximum D_{max} values of the demand for gene expression. These regions also exhibit an inherent asymmetry that favors selection of the positive mode.

As can be seen from the asymptotic expressions for D_{min} and D_{max} (Equations 17, 23, 29, and 34), the ratio of mutation rate to selection coefficient is the most relevant determinant of the allowed region for selection. Indeed, if the target sizes for the various types of mutations and the selection coefficients are increased by the same order of magnitude, then the results are essentially unchanged.

These predictions, and others that are made possible by the assignment of specific values for the parameters, are examined further in the accompanying article (Savageau 1998), where we apply this theory to the regulation of the lactose and maltose operons of *Escherichia coli*.

The quantitative version of demand theory presented in this study provides a framework for further development. Other types of mutations can be incorporated in a relatively straightforward manner. Mutations that result in a phenotype similar to that of an existing mutation can be included by simply adding their target size, as was done here for mutations in the regulator gene and in the modulator site to which the regulator binds $(\tau + \rho)$. Mutations in the structural gene for the effector protein could be included by adding the appropriate target size to the target size of the promoter (π), in the case of the negative mode, or the modulator/regulator $(\tau + \rho)$, in the case of the positive mode. Similarly, in this study we have emphasized the predominant types of mutations that disrupt normal function. Those that might augment normal function can be considered by again adding their target size to the target size of another mutation that results in a similar phenotype. For example, a mutation in an operator site might result in tighter binding of the cognate repressor and failure to allow induction of gene expression in the high-demand environment. Such a mutant would exhibit the same phenotype as the promoter mutants we have considered. The target size for mutations that augment binding, which is presumably smaller than the target size for mutations that disrupt the normal operator, can be added to the target size for mutations in the promoter (π).

Mutants that result in phenotypes different from those considered here also can be added in a straightforward manner. In these cases, one first calculates the individual threshold for each class of mutation; this may involve entirely different sets of parameters and not just a different target size for mutation. Then one adds these

thresholds to obtain the region of allowable selection for the wild-type regulatory system. For the cases described in the previous paragraph, this method and the method of simply adding the appropriate target sizes produce the same results (data not shown).

In summary, the quantitative development of demand theory reveals unexpected relationships between the demand for gene expression D and the average ON/OFF cycle time for the gene C, which is a manifestation of the organism's life cycle. The theory provides equations for the rate and extent of selection, and these reveal well-defined regions of the C *vs.* D plot within which selection is realizable. The realizable regions for the positive and negative mode exhibit an inherent asymmetry with characteristic values for D_{min}, D_{max}, and C_{max}. The demand theory of gene regulation can be extended within the framework presented here to include organisms with life cycles that are more complex than the two phases illustrated in this article and regulatory systems that are more complex than a single mechanism of gene control.

I thank Drs. S. Cooper, R. G. Freter, D. E. Kirschner, J. V. Neel, and M. S. Swanson for critically reading the manuscript and two anonymous reviewers who made valuable suggestions for clarifying key concepts in the theory. This work was supported in part by U.S. Public Health Service grant RO1-GM30054 from the National Institutes of Health and U.S. Department of Defense grant N00014-97-1-0364 from the Office of Naval Research.

LITERATURE CITED

Datta, A., and S. Jinks-Robertson, 1995 Association of increased spontaneous mutation rates with high levels of transcription in yeast. Science **268:** 1616–1619.

Francino, M. P., L. Chao and M. A. Riley, 1996 Asymmetries generated by transcription-coupled repair in enterobacterial genes. Science **272:** 107–109.

Leclerc, J. E., B. Li, W. L. Payne and T. A. Cebula, 1996 High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. Science **274:** 1208–1211.

Miller, J. H., and W. S. Reznikoff, 1980 *The Operon*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Press, W. H., B. P. Flannery, S. A. Teukolsky and W. T. Vetterling, 1988 *Numerical Recipes in C*. Cambridge University Press, New York.

Salyers, A. A., 1994 *Bacterial Pathogenesis: A Molecular Approach*. ASM Press, Washington, DC.

Savageau, M. A., 1974 Genetic regulatory mechanisms and the ecological niche of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **71:** 2453–2455.

Savageau, M. A., 1977 Design of molecular control mechanisms and the demand for gene expression. Proc. Natl. Acad. Sci. USA **74:** 5647–5651.

Savageau, M. A., 1979 Autogenous and classical regulation of gene expression: a general theory and experimental evidence, pp. 57–108 in *Biological Regulation and Development*, Vol. 1, edited by R. F. Goldberger. Plenum, New York.

Savageau, M. A., 1983a Regulation of differentiated cell-specific functions. Proc. Natl. Acad. Sci. USA **80:** 1411–1415.

Savageau, M. A., 1983b Models of gene function: general methods of kinetic analysis and specific ecological correlates, pp. 3–25 in *Foundations of Biochemical Engineering: Kinetics and Thermodynamics in Biological Systems*, edited by H. W. Blanch, E. T. Papoutsakis and G. N. Stephanopoulos. American Chemical Society, Washington, DC.

Savageau, M. A., 1985 Coupled circuits of gene regulation, pp. 633–642 in *Sequence Specificity in Transcription and Translation*, edited by R. Calendar and L. Gold. Alan R. Liss, New York.

Savageau, M. A., 1989 Are there rules governing patterns of gene regulation?, pp. 42–66 in *Theoretical Biology—Epigenetic and Evolutionary Order*, edited by B. C. Goodwin and P. T. Saunders. Edinburgh University Press, Edinburgh.

Savageau, M. A., 1998 Demand theory of gene regulation. II. Quantitative application to the lactose and maltose operons of *Escherichia coli*. Genetics **149:** 1677–1691.

Schwartz, M., 1987 The maltose regulon, pp. 1482–1502 in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by F. C. Neidhardt. American Society for Microbiology, Washington, DC.

Slack, J. M. W., 1992 *From Egg to Embryo*, Ed. 2. Cambridge University Press, Cambridge.

Communicating editor: R. H. Davis