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Gene Regulation Models and Evolution

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Abstract— **We examine the dynamics of gene regulation for a system with *K* environments with each environment favoring a different pattern of gene transcription and regulation in a prokaryotic organism. We recast the model of the system as discrete-time *K*-periodic. This allows us to exploit the well known properties of periodic systems to characterize the steady-state behavior of wild type versus mutant populations of the organism: We also obtain conditions on the parameters of the state matrix using the properties of positive linear systems. We examine in more detail the example of the lac operon of E. Coli which has two environments: a high demand environment *H* and a low demand environment *L*. The gene regulation system alternates between the two environments as the organism completes one life cycle. We derive conditions under which the system reaches steady growth alternating between a state x_H and x_L as each life cycle is completed. Our results show the dependence of the mutant to wild type ratio on the ratio of high demand to low demand duration.**

I. Introduction

Gene regulation is necessary for the survival of prokaryotic enzymes. The regulatory mechanisms control transcription of messenger RNA (m-RNA) and translation through one of two types of regulation: positive or negative. In positive regulation, transcription begins when an activator is present in the organism's environment. In negative regulation, the regulator protein deactivates a transcription inhibitor to allow the initiation of protein synthesis.

The demand theory of gene regulation [4],[5], states that positive control is favored by a high demand environment while negative control is favored by a low demand environment. This controls genetic mutations of the organism and maintains the dominance of wild type organisms under normal environmental conditions.

E. Coli provides an excellent demonstration of the demand theory through its lac and mal operons [4][5]. In prokaryotes, an operon is a gene unit that functions in a coordinated manner by means of an operator, a promoter, and one or more structural genes that are transcribed together. We examine the mathematical model of gene regulation in E. Coli using concepts from system theory. In particular, we view the life cycle of an organism as a positive [2], periodic [1] system and examine the implications for the system parameters and for its mutations.

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We model the ratio of promoter mutant to wild type, both of which grow steadily after a transient period. The ratio of the two populations exhibits a sawtooth periodic form. We obtain constraints on the ratio of high demand to low demand duration. Our approximations use the assumption of a small mutation rate μ and a small selection coefficient $1-\sigma$. The results agree with the small cycle time results of [4], [5].

The paper begins with a discussion of a general gene regulation system and relevant properties of periodic systems and positive systems. We then apply the properties of periodic and positive systems to the model of [4], [5], to obtain conditions that govern the model parameter and characterize is steady-state behavior. We examine the implications for gene regulation as applied to E. Coli.

Nomenclature

- δ relative growth rate in more nutritionally deficient environment.
- ε relative mutation as a function of gene expression
- γ reference growth rate hr^{-1}
- λ relative mutation with loss of normal expression
- μ reference mutation rate per base per generation
- ν relative mutation rate for gain of high-level promoter site
- π relative mutation rate for loss of high-level promoter site
- ρ relative mutation rate for loss of a functional regulator protein
- σ relative growth rate with superfluous expression
- τ relative mutation rate for loss of a regulator's functional target site

II. System Dynamics for Gene Regulation

Consider a simple model of gene regulation where a given effector gene cycles between *K* alternative environments. Each environment requires a different mechanism of gene regulation to produce the enzymes that allow the organism to fully exploit available nutrients and ensure survival. We assume linear dynamic in each environment governed by

$$\dot{x} = A_i, i = 1, \cdots, K \qquad (1)$$

where *x* denotes an *n* by 1 state vector, and A_i is the state matrix in the domain *i*, $i = 1, \ldots, K$. Alternatively, the model may represent ratios of the numbers of mutant forms of the organism to the number of wild type organisms.

We assume that the system remains in *i* for a duration T_i and that the life cycle time is

$$C = \sum_{i=1}^{K} T_i \qquad (2)$$

After each life cycle, new organisms return to the first domain so that the state matrices are governed by

$$A_{i+K} = A_i \tag{3}$$

Hence the system can be considered periodic with period K [1].

If the initial state of the system is in domain i, then the state of the system at time t is given by

$$x(t) = e^{A_j \Delta t} \cdots e^{A_{i+1} T_{i+1}} e^{A_i T_i} x(t_0) \tag{4}$$

$$\Delta t = t - \sum_{k=i}^{j-1} T_i \tag{5}$$

For one complete life cycle starting at the beginning of the i^{th} phase, we have

$$x(t_0 + C) = \Phi(i+K, i) x(t_0)$$

$$\Phi(i+K, i) = \prod_{k=i}^{k=i+K-1} e^{A_i T_i} \tag{6}$$

We can treat the system as a discrete-time system with sampling period unity and analyze it using the model

$$x(k+1) = A(k)x(k)$$

$$A(k) = A(k+K) \tag{7}$$

$$A(k) = e^{A_k T_k}$$

The following result allows us to characterize the eigenvalues of the discrete-time periodic system.

Theorem 1 [3].
Given a periodic system with repetition period K

$$x(k+1) = A(k)x(k)$$

$$A(k) = A(k+K) \tag{8}$$

Then the state-transition matrix of the system is written as

$$\Phi(k, j) = P(k) R^{k-j} P^{-1}(j) \tag{9}$$

where $P(k)$ is K-periodic and R is a constant matrix. The response of the system is K-periodic if and only if at least one of the eigenvalues of R is unity. ∎

The state-transition matrix defined over the repetition period K is known as the **monodromy matrix**. Using the periodicity of the matrix $P(k)$, the monodromy matrix at time zero is given by

$$\Phi(j+K, j) = P(j) R^K P^{-1}(j) \tag{10}$$

Hence, the eigenvalues of R^K are also the eigenvalues of the monodromy matrix. The eigenvalues of the monodromy matrix, known as the characteristic values, determine the stability of the periodic system. The system is stable if and only if all its characteristic values are inside the unit circle.

We now examine the discrete-time system over a complete life cycle and discuss its steady-state behavior. We denote the monodromy matrix by Φ for brevity. We express the initial state $x(t_0)$ in terms of the eigenvectors v_i, i= 1, 2, …, n, of the monodromy matrix

$$x(t_0) = \sum_{i=1}^{n} \alpha_i v_i \tag{11}$$

If the eigenvalues are all inside the unit circle, then the state converges asymptotically to zero. If at least one eigenvalue is outside the unit circle, then the state will diverge (unless the initial state has zero coordinates in the direction of the unstable eigenvectors). The response of the discrete-time system (6) in terms of the eigenvectors is

$$x(t_0 + C) = \sum_{i=1}^{n} \alpha_i \Phi v_i = \sum_{i=1}^{n} \alpha_i \lambda_i v_i \tag{12}$$

Repeatedly updating the state over l cycles, we have

$$x(t_0 + lC) = \sum_{i=1}^{n} \alpha_i \Phi^l v_i = \sum_{i=1}^{n} \alpha_i \lambda_i^l v_i \tag{13}$$

The steady state reached depends on the locations of the characteristic values of the system. Any value whose magnitude is less than unity gives a term that decays to zero asymptotically. Hence, if one eigenvalue (or more) has magnitude unity while all others are inside the unit circle, then the system converges to a fixed state. For example, if only the i^{th} eigenvalues of the discrete system is unity, the system approaches the i^{th} eigenvector in the steady state.

III. Positive Systems

A positive system is one whose state trajectories with any nonnegative initial conditions correspond to state vectors whose entries are nonnegative. For an unforced system, we have the following condition for a positive system.

Theorem 2 [1]
An unforced state-space continuous-time system is positive if and only if all the off-diagonal entries of its state matrix are positive. An unforced state-space discrete-time system is positive if and only if all the entries of its state matrix are nonnegative. ∎

Since the gene regulation system governs variables that can only assume positive values, we conclude that all the off-diagonal terms in the model must be positive. This gives the following physical constraints

$$a_{ijH} \geq 0, a_{ijL} \geq 0$$

$$i, j = 1,2,\cdots,n, i \neq j \tag{14}$$

For the response of the system to remain bounded, all the diagonal terms must be negative or zero. For a system that switches between two state matrices, the response can remain bounded if one of the matrices has positive eigenvalues and the other has negative eigenvalues.

Combining the properties of periodic systems and positive systems, we have the following result.

Corollary 2.1
A discrete-time periodic system is positive if and only if all the entries of all its state and input matrices are nonnegative.

Proof

Sufficiency is trivial since the product of a nonnegative matrix and a nonnegative vector always yields a nonnegative vector. We prove necessity by contradiction. Let the state matrix of a positive system $A(k)$ have a negative entry a_{ij} and consider the zero-input response due to the input

$$\mathbf{x}(k) = \begin{bmatrix} \mathbf{0}_{1\times j-1} & 1 & \mathbf{0}_{1\times j+1} \end{bmatrix}^T$$

Then the state at time k+1 has the ij^{th} entry a_{ij}, which contradicts positivity. Similarly, if the input matrix of a positive matrix has a negative entry then there exists a

nonnegative input for which the response has a negative entry. The condition is therefore necessary. ■

IV. Application to Lac operon

Consider a simple model of E. Coli with four populations: wild type, promoter mutant, modulator mutant, and double (promoter and modulator) mutant. The organism cycles between two alternative environments: a high demand environment H and a low demand environment L

$$\dot{x} = \begin{cases} A_H x, x \in H \\ A_L x, x \in L \end{cases} \quad x = \begin{bmatrix} x_1 & x_2 & x_3 & x_4 \end{bmatrix}^T$$

where the state variables are the numbers of each population. Here, x_1 is the wild type, x_2 is the promoter mutant, x_3 is the modulator mutant, and x_4 is the double mutant. Both A_H and A_L are in the form

$$A_i = \begin{bmatrix} a_{11i} & 0 & 0 & 0 \\ a_{21i} & a_{22i} & 0 & 0 \\ a_{31i} & 0 & a_{33i} & 0 \\ 0 & a_{42i} & a_{43i} & a_{44i} \end{bmatrix}, i \in \{H, L\}$$

where "1" denotes wild type, "2" denotes promoter mutant, "3" denotes modulator mutant, and "4" denotes double mutation, i.e. both promoter and modulator mutation. The corresponding state-transition matrix (matrix exponential) is triangular and the product of two triangular matrices is triangular with the diagonal terms given by

$$e^{a_{jjH}T_H + a_{jjL}T_L}, j = 1,2,\cdots,n \tag{15}$$

Note that the transition from L to H while governed by a different state-transition matrix has the same diagonal terms. In both cases, the populations typically reach a state of steady growth after a transient period.

We are interested in the ratio of mutant to wild type populations which can exhibit periodic behavior. Since the probability of a double mutation is low, we consider the gene regulation system with only one mutation possible. We consider a two-state system with the state matrices

$$A_H = \begin{bmatrix} a_{11H} & 0 \\ a_{21H} & a_{22H} \end{bmatrix} \qquad A_L = \begin{bmatrix} a_{11L} & 0 \\ a_{21L} & a_{22L} \end{bmatrix} \tag{16}$$

where the subscript "1" denotes wild type and the subscript "2" denotes a "mutant" form. The entries of the matrices are derived from the mutation rates and are given by

$$\begin{aligned} a_{11i} &= \left[1 - \left(m_{21i} + m_{31i}\right)\right] g_{wi} \\ a_{22i} &= \left[1 - m_{42i}\right] g_{pi} \\ a_{21i} &= m_{21i} g_{wi} \\ i &\in \{H, L\} \end{aligned} \tag{17}$$

where g_{wi} (g_{pi}) denotes wild type (promoter mutant) growth rate, m_{kl} denotes the mutation rate from l to k.

The system parameters can be written in terms of more specific relative growth rates and mutation rates. The relative growth rates are: λ for mutants that have lost normal expression of the effector gene, σ for mutants that exhibit superfluous expression, δ for the more nutritionally deficient of the two environments. The relative mutation rates are: π for loss of a high-level promoter site, ν for gain of a high-level promoter site, τ for loss of a regulator target site, ρ for loss of a functional protein regulator. Using the definitions and numerical values provided in Table 1 [4], [5], gives the parameter values of Table 2.

Since the diagonal entries are typically unequal, the system starting in the state H has the state-transition matrices

$$\begin{aligned} e^{A_i t} &= \begin{bmatrix} e^{a_{11i}t} & 0 \\ \overline{a}_i \left(e^{a_{11i}t} - e^{a_{22i}t}\right) & e^{a_{22i}t} \end{bmatrix} \qquad i \in \{H, L\} \\ \overline{a}_i &= \frac{a_{21i}}{a_{11i} - a_{22i}} \end{aligned} \tag{18}$$

If the initial state of the system is in the domain H and lasts for the duration T_H, then the system switches to the domain L which lasts for the duration T_L. The state-transition matrix of the system after one complete cycle is given by

$$\begin{aligned} e^{A_L T_L} e^{A_H T_H} &= \begin{bmatrix} 1 & 0 \\ \overline{a}_L & 0 \end{bmatrix} e^{a_{11L}T_L + a_{11H}T_H} \\ &+ \begin{bmatrix} 0 & 0 \\ -\overline{a}_H & 1 \end{bmatrix} e^{a_{22L}T_L + a_{22H}T_H} \\ &+ \begin{bmatrix} 0 & 0 \\ \overline{a}_H - \overline{a}_L & 0 \end{bmatrix} e^{a_{22L}T_L + a_{11H}T_H} \end{aligned} \tag{19}$$

and the discrete-time state equation is

$$\begin{bmatrix} x_1(k+1) \\ x_2(k+1) \end{bmatrix} = \Phi(T_L, T_H) \begin{bmatrix} x_1(k) \\ x_2(k) \end{bmatrix} \tag{20}$$

$$\begin{aligned} \Phi(T_L, T_H) &= \begin{bmatrix} \phi_{11} & 0 \\ \phi_{21} & \phi_{22} \end{bmatrix} \\ \phi_{11} &= e^{a_{11L}T_L + a_{11H}T_H} \\ \phi_{22} &= e^{a_{22L}T_L + a_{22H}T_H} \\ \phi_{21} &= \overline{a}_L e^{a_{11L}T_L + a_{11H}T_H} - \overline{a}_H e^{a_{22L}T_L + a_{22H}T_H} \\ &+ \left[\overline{a}_H - \overline{a}_L\right] e^{a_{22L}T_L + a_{11H}T_H} \end{aligned} \tag{21}$$

For the modulator mutant, the peak occurs at the beginning of the low demand phase. If we start in the low demand region we have

$$\begin{aligned} \Phi(T_H, T_L) &= \begin{bmatrix} \phi_{11} & 0 \\ \phi_{21} & \phi_{22} \end{bmatrix} \\ \phi_{11} &= e^{a_{11L}T_L + a_{11H}T_H} \\ \phi_{22} &= e^{a_{33L}T_L + a_{33H}T_H} \\ \phi_{21} &= \overline{a}_H e^{a_{11H}T_H + a_{11L}T_L} - \overline{a}_L e^{a_{33H}T_H + a_{33L}T_L} \\ &+ \left[\overline{a}_L - \overline{a}_H\right] e^{a_{33H}T_H + a_{11L}T_L} \end{aligned} \tag{22}$$

For E. Coli, the typical high demand duration is 3 hours [4].

The form of the state-transition matrix is identical for the two mutant strains. We are particularly interested in the ratio of mutant to wild-type population, which we denote by r_p for the promoter mutant and r_m for the modulator mutant. Either ratio at the beginning of high demand is governed by

$$r(k+1) = a_r r(k) + b_r \tag{23}$$

$$
\begin{aligned}
a_r &= \phi_{22}/\phi_{11} = e^{(a_{22L}-a_{11L})T_L+(a_{22H}-a_{11H})T_H} \\
b_r &= \phi_{21}/\phi_{11} \\
&= \bar{a}_H\left(1-e^{(a_{22H}-a_{11H})T_H}\right)e^{(a_{22L}-a_{11L})T_L} \\
&\quad + \bar{a}_L\left[1-e^{(a_{22L}-a_{11L})T_L}\right]
\end{aligned} \tag{24}
$$

If we start in the low demand region, the ratio evolves with the dynamics (23) with the coefficients given by

$$
\begin{aligned}
a_r &= \frac{\phi_{22}}{\phi_{11}} = e^{(a_{22L}-a_{11L})T_L+(a_{22H}-a_{11H})T_H} \\
b_r &= \phi_{21}/\phi_{11} \\
&= \bar{a}_L\left(1-e^{(a_{22L}-a_{11L})T_L}\right)e^{(a_{22H}-a_{11H})T_H} \\
&\quad + \bar{a}_H\left[1-e^{(a_{22H}-a_{11H})T_H}\right]
\end{aligned} \tag{25}
$$

The solution of the difference equation (23) is given by

$$
\begin{aligned}
r(k) &= a_r^{k-k_0}r(0) + \sum_{i=0}^{k-1} a_r^{k-i-1}b_r \\
&= a_r^{k-k_0}r(0) + \frac{a_r^k - 1}{a_r - 1}b_r
\end{aligned} \tag{26}
$$

For a nonnegative response, we require both a_r and b_r to be positive. The ratio at the beginning of either the high or low demand phase approaches a fixed level. For a_r <1, the zero-input response due to $r(k_0)$ decays to zero and a steady-state is approached. For the promoter mutant, we have

$$
(a_{22L}-a_{11L})T_L + (a_{22H}-a_{11H})T_H < 0 \tag{27}
$$

which simplifies to

$$
T_H/T_L < (a_{11L}-a_{22L})/(a_{22H}-a_{11H}) \tag{28}
$$

Substituting for the parameters for different controls shows that that this condition is always met. For example, we have for negative control and the promoter mutant

$$
\begin{aligned}
T_H/T_L &< \frac{\{(1-\lambda)-\mu[(\tau+\rho)(\varepsilon-\lambda)+\pi\varepsilon]\}}{\mu\pi\delta} \\
&\approx (1-\lambda)/(\mu\pi\delta) = 4\times 10^8
\end{aligned} \tag{30}
$$

Similarly, for the modulator mutant we have

$$
\begin{aligned}
T_H/T_L &< (a_{11L}-a_{33L})/(a_{33H}-a_{11H}) \\
&\approx ((1-\sigma)\delta)/\mu(\tau+\rho) = 260.417
\end{aligned} \tag{31}
$$

The two conditions involve large values that are typically met by the ratio T_H/T_L. We conclude that the model is consistent with periodic steady-state behavior.

If a steady-state solution exists then we can rewrite the recursion for the population ratio as

$$
r = a_r r + b_r \tag{32}
$$

to obtain the steady-state solution

$$
r_{ss} = b_r/(1-a_r)
$$

The same result is obtained in the steady state from (26).

The number of cycles to reach 99% of the steady-state value with zero initial conditions is given by

$$
r(k_s) = \frac{1-a_r^{k_s}}{1-a_r}b_r = \frac{0.99b_r}{1-a_r} = r_{ss} \tag{33}
$$

Solving for the time to steady state gives

$$
k_s = \frac{\ln(0.01)}{\ln(a_r)} = \frac{\ln(0.01)}{(a_{22L}-a_{11L})T_L + (a_{22H}-a_{11H})T_H} \tag{34}
$$

Substituting from the values of Table 1 and Appendix I gives the approximation

$$
k_s \approx \begin{cases} \left\lceil \dfrac{-\ln(0.01)}{\gamma(1-\lambda)T_H} \right\rceil, & \text{negative control} \\ \left\lceil \dfrac{-\ln(0.01)}{\gamma(1-\sigma)T_L} \right\rceil, & \text{positive control} \end{cases} \tag{35}
$$

where $\lceil\ \rceil$ denotes the ceiling (round up). The numerical values based on the data in Appendix I are

$$
k_s \approx \begin{cases} \lceil 153.506/T_H \rceil, & \text{negative control} \\ \lceil 4605.170/T_L \rceil, & \text{positive control} \end{cases} \tag{36}
$$

Multiplying both sides of (36) by the cycle time C shows agreement with the results [5] over the linear range. The model predicts that for positive control the time to steady state decreases with T_L/C whereas for negative control it decreases with T_H/C.

For the promoter mutant we have the ratio

$$
r_p = \frac{\left\{\begin{aligned}&\bar{a}_H\left(1-e^{(a_{22H}-a_{11H})T_H}\right)e^{(a_{22L}-a_{11L})T_L} \\ &+\bar{a}_L\left[1-e^{(a_{22L}-a_{11L})T_L}\right]\end{aligned}\right\}}{1-e^{-a_{21}T_L+(a_{22H}-a_{11H})T_H}} \tag{37}
$$

and for the modulator mutant

$$
r_m = \frac{\left\{\begin{aligned}&\bar{a}_L\left(1-e^{(a_{22L}-a_{11L})T_L}\right)e^{(a_{22H}-a_{11H})T_H} \\ &+\bar{a}_H\left[1-e^{(a_{22H}-a_{11H})T_H}\right]\end{aligned}\right\}}{1-e^{-a_{21}T_L+(a_{22H}-a_{11H})T_H}} \tag{38}
$$

The parameter values of Tables 1-2 and the relations of Appendix I give the ratios summarized in Tables 3 and 4. Table 3 gives approximate expressions for the coefficient a_r whose magnitude determines the rate at which the steady state is reached. Table 4 gives the steady state ratio of mutant to wild type population. Table 5 gives the total mutant to wild type population ratio. Based on the tables, we make the following observations.

- *The steady-state level for the ratio of mutant to wild-type is proportional to the mutation rate and is therefore very small. While the mutants are always present, the wild type population remains much larger. The model predicts that, unless there is a major change in the mutation rate, wild type will always dominate.*
- *The steady state level for the promoter mutant increases with the ratio T_H/T_L for positive control but decreases for negative control.*
- *The steady state level for the modulator mutant increases with the ratio T_H/T_L for negative but decrease with the ratio for positive control.*
- *The mutant to wild type ratio for negative control and for positive control first decreases with T_H/T_L then increases, with inflexion point at 8×10^{-4} and 80 respectively (see Figure 1). The results agree approximately with the extent of selection curves of* [5].

V. Conclusion

We examined the equations governing gene regulation using well known concepts from system theory to characterize the behavior of mutant versus wild type populations. We used the properties of periodic systems and positive systems to obtain conditions for convergence to a steady-state. For values of the model parameter from the literature, the model predicts that wild type will dominate regardless of the initial population ratio. For a sudden increase in mutation rate, or for σ approximately equal to unity (relative growth rate with superfluous expression), mutant populations become a significant portion of the total population. The results also yield interesting relations between the steady-state population ratios and the ratio T_H/T_L. As in [4],[5], the results show that the high demand portion of the cycle T_H/C is a good indicator of demand. The results indicate that the time to steady-state is inversely proportional to T_H/C for negative control and to T_L/C for positive control. They also show that the mutant to wild type ratio goes through a minimum as a function of the ratio T_H/T_L.

Table 1 Numerical values

μ	τ	ρ	λ	δ	σ	π	γ, ε, ν
6E-10	20	60	0.97	1/80	0.999	10	1

Table 2 Parameter values for E. Coli.

$a_{11} = [1 - (m_{21} + m_{31})]\, g_w$

L		**H**	
N	P	N	P
$[1-\mu(\tau+\rho+\pi)]$ $\gamma\delta$	$[1-\mu(\tau+\rho+\nu)]$ γ	$[1-\mu(\tau+\rho+\pi)\varepsilon]$ γ	$[1-\mu(\tau+\rho+\nu)\varepsilon]$ $\gamma\delta$
0.0125	1	1	0.0125

$a_{22} = (1-m_{42})\, g_p$

L		H	
N	P	N	P
$[1-\mu(\tau+\rho)]$ $\gamma\delta$	$[1-\mu(\tau+\rho)\varepsilon]$ $\gamma\sigma$	$[1-\mu(\tau+\rho)]$ $\gamma\lambda$	$[1-\mu(\tau+\rho)\varepsilon]$ $\gamma\delta$
0.0125	0.0125	0.97	0.0125

$a_{33} = (1-m_{43})\, g_m$

L		**H**	
N	P	N	P
$[1-\mu\pi\varepsilon]$ $\gamma\delta\sigma$	$[1-\mu\nu]\gamma$	$[1-\mu\pi\varepsilon]\gamma$	$[1-\mu\nu\varepsilon]\gamma\delta$ λ
0.0125	1	1	0.97

$a_{21} = m_{21}\, g_w$

L		**H**	
N	P	N	P
$\mu\pi\gamma\delta$	$\mu\nu\gamma$	$\mu\pi\varepsilon\gamma$	$\mu\nu\varepsilon\gamma\delta$
7.5E-12	6E-10	6E-10	4.8E-8

$a_{31} = m_{31}\, g_w$

L		**H**	
N	P	N	P
$\mu(\tau+\rho)\gamma\delta$	$\mu(\tau+\rho)\gamma$	$\mu(\tau+\rho)\varepsilon\gamma$	$\mu(\tau+\rho)\varepsilon\gamma\delta$
6E-10	4.8E-8	6E-10	4.8E-8

Table 3 Coefficient a_r.

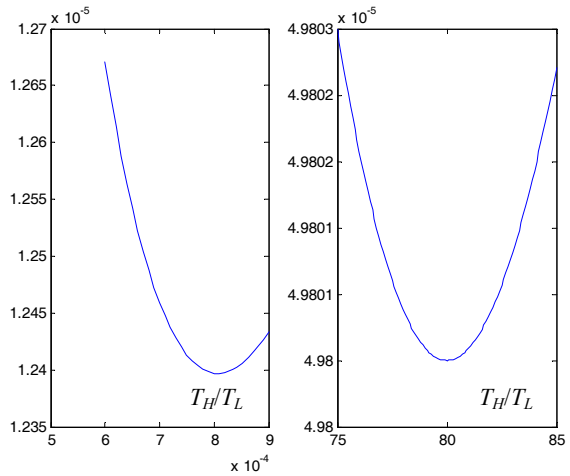
	Promoter mutant	Modulator mutant
P	$e^{-\gamma[(1-\sigma)T_L]}$	$e^{-(1-\sigma)\gamma\delta T_H}$
N	$e^{-\gamma(1-\lambda)T_H}$	$e^{-\gamma\delta(1-\sigma)T_L}$

Table 4 Population ratio r.

	Promoter mutant	Modulator mutant
P	$\frac{\mu}{1-\sigma}\left(\varepsilon\delta\frac{T_H}{T_L}+\nu\right)$	$\frac{\mu}{1-\sigma}\left[\left(\frac{\nu}{\delta}\right)\left(\frac{T_L}{T_H}\right)+\varepsilon(\tau+\rho)\right]$
N	$\frac{\mu\pi\delta}{1-\lambda}\left(\frac{\varepsilon}{\delta}+\frac{T_L}{T_H}\right)$	$\frac{\mu}{1-\sigma}\left[(\tau+\rho)\left(\frac{\varepsilon}{\delta}\right)\left(\frac{T_H}{T_L}\right)+\pi\right]$

Table 5 Mutant to Wild Type Ratio $r_{pss}+r_{mss}$

Negative Control	$\frac{\mu}{1-\sigma}\left\{\pi+\frac{\varepsilon}{\delta}(\tau+\rho)\frac{T_H}{T_L}\right\}+\frac{\mu\pi\delta}{1-\lambda}\left(\frac{\varepsilon}{\delta}+\frac{T_L}{T_H}\right)$
Positive Control	$\frac{\mu}{1-\sigma}\left\{\nu\left(\frac{T_L}{T_H\delta}+1\right)+\varepsilon\left(\tau+\rho+\nu\delta\frac{T_H}{T_L}\right)\right\}$



(a) Negative control. (b) Positive control.

Fig. 1 Mutant to wild type steady state ratios.

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Appendix

Computation and Approximation of Model Parameters

I- Negative control

a) Promoter mutant

$$a_{11H}-a_{22H}=\gamma\{(1-\lambda)-\mu[(\tau+\rho)(\varepsilon-\lambda)+\pi\varepsilon]\}$$
$$\approx\gamma(1-\lambda)$$
$$a_{22L}-a_{11L}=\mu\pi\gamma\delta$$
$$\bar{a}_H=a_{21H}/(a_{11H}-a_{22H})$$
$$=\mu\pi\varepsilon/\{(1-\lambda)-\mu[(\tau+\rho)(\varepsilon-\lambda)+\pi\varepsilon]\}$$
$$\approx\mu\pi\varepsilon/(1-\lambda)$$
$$\bar{a}_L=a_{21L}/(a_{11L}-a_{22L})=-1$$
$$a_r=e^{-a_{21L}T_L+(a_{22H}-a_{11H})T_H}$$
$$=e^{-\mu\pi\gamma\delta T_L-\gamma\{(1-\lambda)-\mu[(\tau+\rho)(\varepsilon-\lambda)+\pi\varepsilon]\}T_H}$$
$$\approx e^{-\gamma(1-\lambda)T_H}$$
$$\times\{1+\mu\gamma([(\tau+\rho)(\varepsilon-\lambda)-\pi\varepsilon]T_H+\pi\delta T_L)\}$$
$$\approx e^{-\gamma(1-\lambda)T_H}<1$$
$$b_r=\bar{a}_H\left(1-e^{(a_{22H}-a_{11H})T_H}\right)e^{(a_{22L}-a_{11L})T_L}$$
$$-\left(1-e^{(a_{22L}-a_{11L})T_L}\right)$$
$$\approx[\gamma(1-\lambda)T_H\ \mu\pi\varepsilon/(1-\lambda)](1+\mu\pi\gamma\delta T_L)+\mu\pi\gamma\delta T_L$$
$$\approx\gamma\mu\pi(\varepsilon T_H+\delta T_L)$$
$$r_{pss}=b_r/(1-a_r)$$
$$\approx\gamma\mu\pi(\varepsilon T_H+\delta T_L)/\left(1-e^{-\gamma(1-\lambda)T_H}\right)$$
$$\approx\mu\pi\delta(\varepsilon/\delta+T_L/T_H)/(1-\lambda)$$

b) Modulator mutant

$$a_{33H}-a_{11H}=\mu(\tau+\rho)\varepsilon\gamma$$
$$a_{33L}-a_{11L}=\gamma\delta(\sigma-1+\mu(\tau+\rho+\pi[1-\sigma\varepsilon]))$$
$$\approx\gamma\delta(\sigma-1)$$
$$\bar{a}_H=a_{31H}/(a_{11H}-a_{33H})$$
$$=-\mu(\tau+\rho)\varepsilon\gamma/\{\mu(\tau+\rho)\varepsilon\gamma\}$$
$$=-1$$
$$\bar{a}_L=a_{31L}/(a_{11L}-a_{33L})$$
$$=\mu\pi\gamma\delta/\{\gamma\delta(1-\sigma-\mu(\tau+\rho+\pi[1-\sigma\varepsilon]))\}$$
$$\approx\mu\pi/(1-\sigma)$$
$$a_r=e^{(a_{33L}-a_{11L})T_L+(a_{33H}-a_{11H})T_H}$$
$$=e^{\gamma\delta(\sigma-1+\mu(\tau+\rho+\pi[1-\sigma\varepsilon]))T_L+\mu(\tau+\rho)\varepsilon\gamma T_H}$$
$$\approx e^{-\gamma\delta(1-\sigma)T_L}<1$$
$$b_r=\bar{a}_L\left(1-e^{(a_{33L}-a_{11L})T_L}\right)e^{(a_{33H}-a_{11H})T_H}$$
$$+\bar{a}_H\left(1-e^{(a_{33H}-a_{11H})T_H}\right)$$
$$\approx e^{\mu(\tau+\rho)\varepsilon\gamma T_H}\left(1-e^{-\gamma\delta(1-\sigma)T_L}\right)\mu\pi/(1-\sigma)$$
$$-\left(1-e^{\mu(\tau+\rho)\varepsilon\gamma T_H}\right)$$
$$\approx\mu\gamma[\pi\delta T_L+(\tau+\rho)\varepsilon T_H]$$
$$r_{mss}=b_r/(1-a_r)$$
$$\approx\mu\gamma[\pi\delta T_L+(\tau+\rho)\varepsilon T_H]/\left(1-e^{-\gamma\delta(1-\sigma)T_L}\right)$$
$$\approx\mu[\pi+(\tau+\rho)(\varepsilon/\delta)(T_H/T_L)]/(1-\sigma)$$

II- Positive control

a) Promoter mutant

$$a_{22H}-a_{11H}=\gamma\delta\mu\nu\varepsilon$$
$$a_{22L}-a_{11L}=\gamma[(\sigma-1)+\mu((\tau+\rho)(1-\varepsilon\sigma)+\nu)]$$
$$\approx-\gamma(1-\sigma)$$
$$\bar{a}_H=a_{21H}/(a_{11H}-a_{22H})$$
$$=-1$$
$$\bar{a}_L=a_{21L}/(a_{11L}-a_{22L})$$
$$\approx\mu\nu/(1-\sigma)$$
$$a_r=e^{(a_{22L}-a_{11L})T_L+(a_{22H}-a_{11H})T_H}$$
$$\approx e^{-(1-\sigma)\gamma T_L+\gamma\delta\mu\nu\varepsilon T_H}$$
$$\approx e^{-\gamma[(1-\sigma)T_L]}<1$$
$$b_r=-\left(1-e^{(a_{22H}-a_{11H})T_H}\right)e^{(a_{22L}-a_{11L})T_L}$$
$$+\bar{a}_L\left[1-e^{(a_{22L}-a_{11L})T_L}\right]$$
$$\approx\gamma\delta\mu\nu\varepsilon T_H$$
$$r_{pss}=b_r/(1-a_r)$$
$$\approx\gamma\mu(\delta T_H-\nu T_L)/\left(1-e^{-\gamma(1-\sigma)T_L}\right)$$
$$\approx\mu\nu(\varepsilon\delta T_H/T_L+1)/(1-\sigma)$$

b) Modulator mutant

$$a_{33H}-a_{11H}=-(1-\sigma-\mu\varepsilon(\tau+\rho+\nu)-\mu\sigma\nu)\gamma\delta$$
$$\approx-(1-\sigma)\gamma\delta$$
$$a_{33L}-a_{11L}=\gamma\mu(\tau+\rho)$$
$$\bar{a}_H=a_{31H}/(a_{11H}-a_{33H})$$
$$=-\mu(\tau+\rho)\varepsilon/(1-\sigma-\mu\varepsilon(\tau+\rho+\nu)-\mu\sigma\nu)$$
$$\approx-\mu(\tau+\rho)\varepsilon/(1-\sigma)$$
$$\bar{a}_L=a_{31L}/(a_{11L}-a_{33L})$$
$$=-\nu/(\tau+\rho)$$
$$a_r=e^{(a_{33L}-a_{11L})T_L+(a_{33H}-a_{11H})T_H}$$
$$=e^{\gamma\mu(\tau+\rho)T_L-(1-\sigma)\gamma\delta T_H}$$
$$\approx e^{-(1-\sigma)\gamma\delta T_H}<1$$
$$b_r=\bar{a}_L\left(1-e^{(a_{33L}-a_{11L})T_L}\right)e^{(a_{33H}-a_{11H})T_H}$$
$$+\bar{a}_H\left(1-e^{(a_{33H}-a_{11H})T_H}\right)$$
$$\approx-\left(1-e^{\gamma\mu(\tau+\rho)T_L}\right)e^{-(1-\sigma)\gamma\delta T_H}\nu/(\tau+\rho)$$
$$-\left(1-e^{-(1-\sigma)\gamma\delta T_H}\right)\mu(\tau+\rho)\varepsilon/(1-\sigma)$$
$$\approx\gamma\mu\nu T_L[1-(1-\sigma)\gamma\delta T_H]+\mu(\tau+\rho)\varepsilon\gamma\delta T_H$$
$$\approx\mu\gamma[\nu T_L+\varepsilon\delta(\tau+\rho)T_H]$$
$$r_{mss}=b_r/(1-a_r)$$
$$\approx\mu\gamma[\nu T_L+\varepsilon\delta(\tau+\rho)T_H]/\left(1-e^{-\gamma(1-\sigma)\delta T_H}\right)$$
$$\approx\mu[(\nu/\delta)(T_L/T_H)+\varepsilon(\tau+\rho)]/(1-\sigma)$$