Why does the positive regulation in bacteriophage λ affect isomerization of the RNAP-DNA complex?

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We use a model due to Santillán and Mackey, which is based on the Ackers chemical equilibrium description of the promoter binding by the regulatory factors, to show that the stability of the phage λ lysogen will be severely compromised if CI had a 10-fold effect on K_B and no effect on k.

1 Introduction

The initiation of transcription involves three steps: binding, opening, and escape of RNA polymerase. To model these steps in the simplest way we will treat opening and escape as a single reaction with forward reaction rate k determined by the regulatory proteins and their interaction with the DNA. Binding will be treated as a reversible reaction with an equilibrium constant K_B. Using mathematical models, it is demonstrated that in the phage λ induction the binding constant K_B plays a fundamentally different role from the opening and clearing constant k. In particular modifications in K_B cannot be directly compensated for by modifications in k and vice versa.

2 The Mathematical Model

After an invasion of *E.coli*, the phage λ either lysis the cell and multiplies (*lytic pathway*), or inscribes its DNA into the host DNA, which propagates with the cell (*lysogen*). Upon UV exposure, the phage is induced, leaves the lysogen and re-enters lytic pathway. The lysogen is maintained by competition between two proteins CI and Cro, and their binding to six binding sites on two operator regions O_R and O_L. Interaction of these proteins regulates RNA polymerase (RNAP) binding to promoters P_R and P_{RM}, which initiate transcription of cro mRNA and cI mRNA, respectively. For a more complete description see [2].

Following Ackers *et. al.* [1] the state s of the promoter is a description of which of the eight sites above are empty or occupied by which of the three possible molecules CI_2, Cro_2, or RNAP. Then the transcription initiation rate is a function of state

$g_s([CI_2], [Cro_2]) = k(s)\dfrac{K_B(s)[Cro_2]^{\alpha_s}[CI_2]^{\beta_s}[RNAP]^{\gamma_s}}{\sum_i K_B(s_i)[Cro_2]^{\alpha_i}[CI_2]^{\beta_i}[RNAP]^{\gamma_i}}$, where $K_B(s) = e^{\frac{-\Delta G_s}{RT}}$ determines the equilibrium

constant for the binding of the regulatory proteins and/or RNAP to the DNA. The probability of a given state is multiplied by a constant, $k(s)$, which captures forward reaction rates of the opening and escape steps to get a rate of transcription initiation. We assume that the rate constants $k(s)$ take on three values: k_{cro} when RNAP is bound to P_R, k_{cI}^c when RNAP is bound to P_{RM} and CI_2 is bound to O_R2, and k_{cI} when RNAP is bound to P_{RM} and CI_2 is not bound to O_R2. Correspondingly, we let $f_R([CI_2], [Cro_2]) = k_{cro}g_R([CI_2], [Cro_2])$ be the sum of all combinations of terms g_s with the restriction that each state s has a RNAP bound to P_R, with O_R1 and O_R2 unbound; let $f_{RM}^c([CI_2], [Cro_2]) := k_{cI}^c g_{cI}([CI_2], , [Cro_2])$ and $f_{RM}([CI_2], [Cro_2]) := k_{cI}g_{cI}([CI_2]_{\tau_M})$ be the sums of all states, where CI_2 is bound to O_R2 and the second when it is not. The functions f_R and $f_{RM}^c + f_{RM}$ describe the transcription initiation rate of gene cro and the gene cI.

$$\frac{d[M_{cI}]}{dt} = [O_R]f_{RM}^c([CI_2]_{\tau_M}, [Cro_2]_{\tau_M}) + [O_R]f_{RM}([CI_2]_{\tau_M}, [Cro_2]_{\tau_M}) - (\gamma_M + \mu)[M_{cI}] \tag{1}$$

$$\frac{d[M_{cro}]}{dt} = [O_R]f_R([CI_2]_{\tau_M}, [Cro_2]_{\tau_M}) - (\gamma_M + \mu)[M_{cro}] \tag{2}$$

$$\frac{d[CI]}{dt} = \nu_{cI}[M_{cI}]_{\tau_{cI}} - (\gamma_{cI} + \mu)[CI] \tag{3}$$

$$\frac{d[Cro]}{dt} = \nu_{cro}[M_{cro}]_{\tau_{cro}} - (\gamma_{cro} + \mu)[Cro]. \tag{4}$$

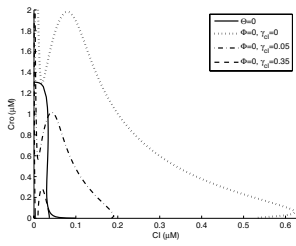* Corresponding author E-mail: rardin@math.montana.edu, Phone: +1 406 994 5360, Fax: +1 406 994 1789

Fig. 1 Nullclines for $\Theta = 0$ (solid) and $\Phi = 0$ with $\gamma_{cI} = 0$ min^{-1} (dots), $\gamma_{cI} = 0.05$ min^{-1} (dash-dot) and $\gamma_{cI} = 0.35$ min^{-1} (dash).
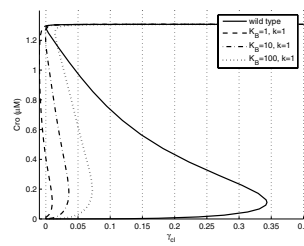

Fig. 2 Bifurcation diagram of γ_{cI} versus [Cro]. Solid is the wild type, dots is for K$_B$=100, k=1, dot-dash K$_B$=10, k=1, and dash K$_B$=1,k=1

Table 1 Estimated parameter values from [5] (with the addition of ϕ) for equations (1)-(4).

$\mu \simeq 2.0 \times 10^{-2}$ min^{-1}	$k_{cro} \simeq 2.76$ min^{-1}
$k_{cI}^c \simeq 4.29$ min^{-1}	$k_{cI} \simeq 0.35$ min^{-1}
$\gamma_M \simeq 0.12$ min^{-1}	$\gamma_{cI} \simeq 0.0$ min^{-1}
$\gamma_{cro} \simeq 1.6 \times 10^{-2}$ min^{-1}	$\nu_{cI} \simeq 0.09$ min^{-1}
$\nu_{cro} \simeq 3.2$ min^{-1}	$\tau_{cI} \simeq 0.24$ min
$\tau_{cro} \simeq 6.6 \times 10^{-2}$ min	$\tau_M \simeq 5.1 \times 10^{-3}$ min
$K_D^{cI} \simeq 5.56 \times 10^{-3} \mu$M	$K_D^{cro} \simeq 3.26 \times 10^{-1} \mu$M
$[O_R] \simeq 5.0 \times 10^{-3} \mu$M	[RNAP] $\simeq 3.0 \mu$M
$\Delta G_{RL} \simeq -3.1$ kcal/mol	$\phi \simeq 4.29/.35 = 12.26$

Square brackets denote concentration of molecules, $*_2$ denotes a dimer, and M_* is mRNA of $*$. The subscript notation $[M_{cro}]_{\tau_{cro}}$ indicates that the concentration of cro mRNA is evaluated at $t - \tau_{cro}$ where t is the present time.

The effect of UV light irradiation, which lowers the effective concentration of CI dimers, is modeled by an increase in the degradation rate γ_{cI}. We study the equilibria as a function of γ_{cI}. Setting left hand side to zero and combining equations (1) and (3) we get equation $\Phi([CI], [Cro], \gamma_{cI}) = 0$; combining (2) and (4) we get $\Theta([CI], [Cro]) = 0$. The intersection of these two curves in the [CI], [Cro] plane determines two protein concentrations at equilibrium. In Figure 1 we graph $\Theta([CI], [Cro]) = 0$ (black) and $\Phi([CI], [Cro], \gamma_{cI}) = 0$ (dash, dash-dot, dot) for three different values of γ_{cI}.

Clearly, the set of equilibria changes as a function of γ_{cI}. This is indicated in the bifurcation diagram of Figure 2, where the equilibrium values of $[Cro]$ are plotted on the vertical axis as a function of γ_{cI}. This graph allows us to describe the induction process. When no UV radiation is applied to bacterial population, $\gamma_{cI} = 0$ min^{-1} and the phage occupies lysogenic equilibrium. As γ_{cI} is slowly increased, the lysogenic equilibrium moves and the phage state tracks this slowly moving equilibrium. Immediately after γ_{cI} crosses the value of 0.343 the lysogenic equilibrium disappears and the state rapidly approaches the lytic equilibrium.

The constant $\phi := k_{cI}^c/k_{cI} = 12.26$ measures k-cooperativity. The constant $\beta := exp(-\frac{1}{RT}(\Delta G_{O_R2P_{RM}}^{CI_2RNAP}))$, where $\Delta G_{O_R2P_{RM}}^{CI_2RNAP}$ is the cooperative binding energy between O_R2 bound CI and RNAP, represents cooperative binding. In summary, the k-cooperativity is manifested by the constant $\phi > 1$ and K$_B$-cooperativity by $\beta > 1$.

3 Results

We verified our model on phage mutants described by Little *et. al.* [3], Michalowski and Little [4] and a *pc*-mutant described in Ptashne [2]. In all these cases the model captured accurately their qualitative observations. Being able to match these scenarios, we investigate how changes in K$_B$ and k affect the system.

The bifurcation diagrams in Figure 2 compare the wild type phage ($\phi = 12.26, \beta = 1$) with "zero cooperativity" mutant ($\phi = 1, \beta = 1$), and two binding compensated mutants ($\phi = 1, \beta = 10$) vs ($\phi = 1, \beta = 100$). Since the stability of the lysogen in this diagram marked by the γ_{cI} coordinate of the right knee, we see that the lysogenic state of wild type phage is significantly more stable then the mutants.

Even in the case of unrealistically strong K$_B$-cooperativity, $\beta = 100$, the induction value is only $\gamma_{\beta=100}^* = 0.07$ min^{-1}. We conclude that K$_B$- and k-cooperativity are not equivalent.

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