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Biophysical Chemistry 114 (2005) 229–234

Biophysical Chemistry

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Transcriptional activation mechanisms of the P_{RM} promoter of λ phage

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Received 11 October 2004; received in revised form 10 December 2004; accepted 10 December 2004
Available online 24 December 2004

Abstract

We investigate the transcriptional activity associated with the P_{RM} promoter of λ phage. The probability for formation of a transcriptionally active (open) RNA polymerase–DNA complex is calculated by means of an equilibrium statistical–mechanical model. In particular, we study two different models of the transcriptional activation mechanism when the O_R2 site is occupied by a CI dimer (typical for a lysogen) compared to the situation when O_R2 is vacant: (1) transcription rate increases (wild-type mechanism) or (2) RNA polymerase becomes stronger (or weaker) bound to DNA (mutant mechanism). By applying experimental determined protein–DNA binding energies we show that these two mechanisms exhibit different characteristics when we study the activity versus CI concentration. We also show that our model may be fitted to in vivo activity data satisfactorily despite that the activated transcription rate is significantly reduced compared to the wild-type value. The model is consistent with experimental determined activities based upon mutations of CI and RNA polymerase.

Keywords: Lambda phage; Nonspecific binding; Statistical mechanics; Transcriptional activation

1. Introduction

The last decade has provided a lot of data on DNA sequences, e.g., the sequences of the *Escherichia coli* (*E. coli*) K-12 genome [1] and the human genome [2] are now sequenced. Today, we know the genes and to some extent the protein products of a long list of organisms. However, in order to understand how life is organized one needs to study how the cellular and organismal constituents form functional units [3]. One important thing in this circumstance is to understand which proteins in a cell that actually become transcribed and later on translated. In many cases promoter binding of RNA polymerase depends upon other proteins, called transcription factors, in order to initiate the transcription process [4]. The mechanism for activating an otherwise "silent" gene, occasionally called recruitment [5], implies that RNA polymerase binds to the promoter and transcribes the associated gene(s) with a high probability in the presence of transcription factor(s). One should here note that recruitment is usually associated with a binding energy stimulation mechanism (BES, explained in detail below).

About 50 years ago, Lwoff and colleagues observed that a colony of *E. coli* cells infected by bacteriophage lambda (λ phage) lysed when irradiated by UV–light [6]. Since then many details of the system have been revealed. The fate of the λ-infected cell seems to be closely connected to the right operator (O_R) of λ phage [7]. Upon λ phage infection of the *E. coli* bacterium the system chooses one of two directions: (1) the λ genome is integrated into the host genome (prophage), whereupon it silently becomes replicated for generations of *E. coli* life cycles, called the lysogenic pathway, or (2) the phage becomes massively reproduced within the *E. coli* cell, on the time-scale of a cell generation (~0.5–1 h), until the bacterium bursts (lyses) such that around 100 new phages are released, called the lytic pathway (see [8] for details).

Abbreviations: λ phage, bacteriophage lambda; O_R, right operator; TRS, transcription rate stimulation; BES, binding energy stimulation; SSD, standard sample deviation; GFE, Gibbs free energy.

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doi:10.1016/j.bpc.2004.12.008

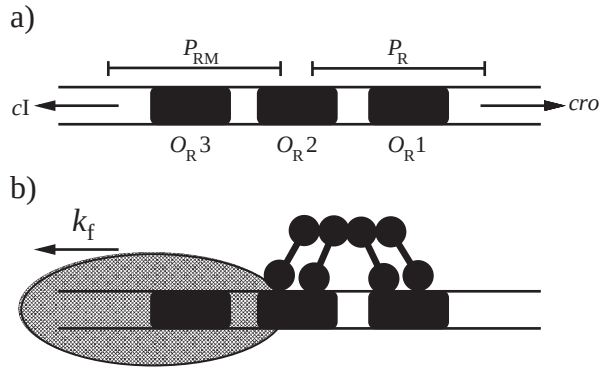

Fig. 1. (a) Schematic illustration of the O_R of the phage λ genome. Three operator (binding) sites are shown, O_R1, O_R2, and O_R3, where CI and Cro dimers are able to bind. P_{RM} and P_R indicate the promoter regions (−35 to −10 bases upstream) where RNA polymerase binds to initiate transcription of the *c*I and *cro* genes. The arrows associated with *c*I and *cro* indicate the transcription direction of these genes, respectively. (b) The normal situation for a lysogen (CI dominates) when the left operator is excluded [27]. O_R1 and O_R2 are occupied by one CI dimer each and P_{RM} is occupied by RNA polymerase. In the wild-type system, this situation leads to an increased (activated) P_{RM} transcription rate by a factor $k_f \approx 11$, compared to the unstimulated situation when O_R2 is vacant.

Two regulatory proteins (transcription factors), CI and Cro,[1] are able to bind as dimers to three different binding sites of O_R (Fig. 1). Depending on the specific binding pattern of these proteins, RNA polymerase may bind and transcribe in one of two directions from O_R such that either *c*I or *cro* is transcribed (divergent transcription). In a lysogen where the Cro concentration is assumed to be approximately zero, O_R1 and O_R2 are usually occupied by one CI dimer each, exhibiting a cooperative interaction, and P_{RM} is occupied by RNA polymerase such that CI is continuously expressed and thereby maintaining repression of *cro*. This is an example of an auto-regulating system, i.e., CI regulates its own synthesis. Auto-regulation is also the case when Cro is abundant, e.g., after an induction event, that will block for RNA polymerase association to P_{RM} such that only *cro* transcription is possible.

Inspired by experiments, we investigate two possible mechanisms, and combinations of these, for activation of P_{RM} transcriptional activity within the framework of a statistical–mechanical model. The activation when O_R2 is occupied by a CI dimer (Fig. 1b) is transcriptional rate stimulation (TRS) and/or DNA binding energy stimulation (BES) of RNA polymerase. By applying the common literature values of protein–DNA Gibbs free binding energies, we find that TRS and BES exhibit qualitatively different characteristics when the activity is plotted versus the CI concentration. However, we show that our model may be fitted to in vivo activity data satisfactorily when the activated transcription rate is significantly reduced compared to the wild-type value. Interestingly, when the TRS mechanism is excluded we are still able within the BES mechanism to fit our model very well to the in vivo activity data provided that the basal RNA polymerase binding strength at P_{RM} is reduced at least 3 kcal/mol compared to the common applied value in the literature (−11.5 kcal/mol). We are also able to reproduce satisfactorily experimental activity data for CI and RNA polymerase mutants.

[1] Nomenclature: genes are denoted with italicized letters and their protein products with Roman letters where the first letter is capitalized.

2. Thermodynamical model

To describe the specific protein binding to O_R of λ phage we apply an extension of the approach of [9]. It is possible to show that the bindings of CI dimers, Cro dimers, and RNA polymerase to O_R of λ phage occur in 40 different combinations s [10,11]. In this work we put Cro concentration to zero. The associated normalized probability f_s for finding the system in one particular state s is then

$$f_s = \frac{\exp(-\Delta G(s)/(RT))[\mathrm{CI}_2]^{i_s}[\mathrm{RNAP}]^{k_s}}{\sum_s \exp(-\Delta G(s)/(RT))[\mathrm{CI}_2]^{i_s}[\mathrm{RNAP}]^{k_s}}, \quad (1)$$

where R=8.31 J/(mol K) is the gas constant, T is the absolute temperature, and $\Delta G(s)$ is the Gibbs free energy difference (binding energy) between state s and the unoccupied state (s=1). $[\mathrm{CI}_2]$ and [RNAP] are the free (unbound) concentrations of CI dimers and RNA polymerase, respectively. $i_s \in \{0,1,2,3\}$ and $k_s \in \{0,1,2\}$ are the number of CI dimers and RNA polymerase bound to O_R in the state s. Both individual binding affinities and cooperative interactions contribute to the different $\Delta G(s)$ (listed in Table 1). Free monomers and dimers of CI are supposed to be in equilibrium with standard Gibbs free energy of association of −11.1 kcal/mol [12]. One should note that Eq. (1) will be modified in the case when the left operator (O_L) is present in the λ-genome, because this leads to a typical situation in a lysogen where O_R and O_L are linked via a stabilizing octamer [13]. However, in the experiments

Table 1
Gibbs free energies (GFEs) upon protein–DNA associations with reference to the unbound state (units of kcal/mol)

CI[a]	ΔG_1	−12.5
	ΔG_2	−10.5
	ΔG_3	−9.5
	ΔG_{12}	−2.7
	ΔG_{23}	−2.9
RNAP[b]	ΔG_{RM}	−11.5
	ΔG_R	−12.5

[a] CI binding data from [28]. ΔG_1 is the GFE associated with the binding between CI and operator site O_R1, etc., and ΔG_{12} is the GFE associated with cooperative binding between CI dimers at O_R1 and O_R2, etc.

[b] RNA polymerase binding data from [10]. ΔG_{RM} and ΔG_R are the GFEs associated with the binding between RNA polymerase and P_{RM} and P_R, respectively.

we compare our model to this DNA-loop is not present because activity data are obtained for alleles without O_L.

The total concentration of CI molecules in the cell, in monomeric equivalents, yields

$$[\mathrm{CI}_t] = [\mathrm{CI}_1] + 2[\mathrm{CI}_2] + 2N_{\mathrm{DNA}}[V^{-1}]\Big(\sum_s i_s f_s + N_b[\mathrm{CI}_2]\exp(-\Delta G_{\mathrm{NSB}}/(RT))\Big) \quad (2)$$

where the first and second term on the right hand side count the free monomeric and dimeric concentrations, respectively, and the last term accounts for both the average concentration of specifically bound CI dimers at or (first part of the last term) and the average concentration of nonspecifically bound CI dimers (second part of the last term). The reason for the nonspecific term is that the *E. coli* genome has N_b=4.6×10^6 bases [14] that all are possible binding sites for CI proteins. In a recent work we quantify the value of ΔG_{NSB} to −4.1 kcal/mol [15,16], based upon a fit of the model presented here to experimental data of P_{RM} activity by [17]. In the following we choose a doubling time of 40 min that corresponds to a cellular volume V=1.13 μm^3 [18]. [V^{-1}]=1.47 nM is then the molar concentration of one particle in these cells and the corresponding average number of DNA molecules (N_{DNA}) present in the *E. coli* cell is 2.3 [19]. The free RNA polymerase concentration is assumed to be 30 nM [9,10,20–22]. This value was also applied when [10] estimated the RNA polymerase affinities we apply in this work (ΔG_{RM} and ΔG_{R} in Table 1).

The activity at P_{RM} is

$$\mathrm{Activity}_{\mathrm{RM}} = a\big(k_f \mathrm{Prob}_{\mathrm{RM1}}(\Delta G_{\mathrm{RM+}}) + \mathrm{Prob}_{\mathrm{RM2}}\big), \quad (3)$$

where $\mathrm{Prob}_{\mathrm{RM1}}(\Delta G_{\mathrm{RM+}})$ is the normalized probability for binding of RNA polymerase at P_{RM} when $O_{\mathrm{R}}2$ is occupied by a CI dimer (see Fig. 1b). k_{f} is the corresponding activated relative transcription rate (dimensionless). $k_{\mathrm{f}}\approx 11$ in a wild-type lysogen [23], but, acts a tunable parameter in this work. The k_{f} value was obtained from in vitro experiments where a CI concentration of 60 nM was assumed to fully occupy $O_{\mathrm{R}}1$ and $O_{\mathrm{R}}2$, while $O_{\mathrm{R}}3$ was assumed to be vacant. Additionally, $\mathrm{Prob}_{\mathrm{RM1}}$ also depends upon $\Delta G_{\mathrm{RM+}}$ that is a parameter taking into account an additional Gibbs free energy for RNA polymerase–DNA binding when a CI dimer occupies $O_{\mathrm{R}}2$, i.e., ΔG_{RM} in Table 1 is added the value of $\Delta G_{\mathrm{RM+}}$ due to the BES mechanism when $O_{\mathrm{R}}2$ is occupied by a CI dimer. Furthermore, $\mathrm{Prob}_{\mathrm{RM2}}$ is the normalized probability for RNA polymerase binding at P_{RM} when $O_{\mathrm{R}}2$ is vacant, thus, $\mathrm{Prob}_{\mathrm{RM2}}$ does not depend upon the specific value of $\Delta G_{\mathrm{RM+}}$. We note that $k_{\mathrm{f}}>1$ or $\Delta G_{\mathrm{RM+}}\neq 0$ corresponds to the two different activation mechanisms as outlined in the last paragraph of Introduction, i.e., TRS and BES, respectively. a is a proportionality constant that scales the activity (unit: s^{-1}). The model in Eq. (3) has three free parameters: a, k_{f}, and $\Delta G_{\mathrm{RM+}}$. However, at one time we only consider one or two of these three parameters as free.

3. Results and discussion

First we investigate the difference between the two activation mechanisms, TRS and BES. In Fig. 2 we plot experimental P_{RM} activity data from [17] together with the best fit (least squares fit) of our model with the wild-type value k_{f}=11, where a acts as a free parameter.

The quality of the fit is very good, where the standard sample deviation between the experimental and theoretical data points (SSD)[2] is less than 6% of the average activity. When we exclude the TRS mechanism, i.e., put k_{f}=1, we are not able to fit the experimental data for any value of $\Delta G_{\mathrm{RM+}}$. With k_{f}=1 the best fit activity ($\Delta G_{\mathrm{RM+}}$=−1.2 kcal/mol) versus increasing CI concentration increases slightly, compared to the associated curve for k_{f}=11, up to a total CI concentration of ≈20 nM that is the concentration where CI dimers occupy $O_{\mathrm{R}}2$ most of the time. [23] considered a hypothetical activation mechanism, where RNA poymerase binding proceeds through two intermediates preceding the isomerization step, and concluded from this that P_{RM} could be activated more than tenfold for a BES-like mechanism.

We note that there exists a CI mutant (CI-pc2) that destroys the TRS mechanism without changing the RNA polymerase binding affinity much [24]. To obtain insight in the tendency when k_{f} is decreased, we systematically reduce k_{f} from 11 (wild-type rate) to 1 (unstimulated rate; Fig. 3). The effect is significant, with k_{f}=11 the activity–concentration curve has a pronounced peak around 1 μM that is the total CI concentration for which the combination of RNA polymerase association to P_{RM} and CI occupancy of $O_{\mathrm{R}}2$ is most favorable. For total concentrations >1 μM the activity gradually decreases versus increasing concentration because CI binds to $O_{\mathrm{R}}3$ with increasing probability and thereby prevents RNA polymerase to bind to P_{RM}, i.e., CI acts as a (self-)repressor in this concentration regime. When k_{f} decreases the peak of the activity–concentration curve gradually vanishes. For k_{f}=1 the activity is monotonously decreasing, because any [CI$_{\mathrm{tot}}$]>0 will hamper RNA polymerase in P_{RM} association as no rate stimulating mechanism exists, i.e., for k_{f}=1 CI acts as a repressor for P_{RM} at all concentrations.

We will now study the second activation mechanism, namely BES, which means that $\Delta G_{\mathrm{RM+}}\neq 0$ in Eq. (3) and, thus, systematically investigate our model for both positive and negative values of $\Delta G_{\mathrm{RM+}}$ with regards to the activity (Fig. 4). Negative values of $\Delta G_{\mathrm{RM+}}$, i.e., a stronger DNA binding of RNA polymerase in the presence of a CI dimer at

[2] $\mathrm{SSD}\equiv\sqrt{\frac{1}{N}\sum_{i=1}^{N}[X_1(\mathrm{exp})-X_i(\mathrm{theory})]^2}$, where X_i(exp) and X_i(theory) are the ith data points of the experimental determined activity and theoretical calculated activity, respectively, and N is the number of data points. The minimum of SSD corresponds to the best fit of the model with respect to the experimental data.

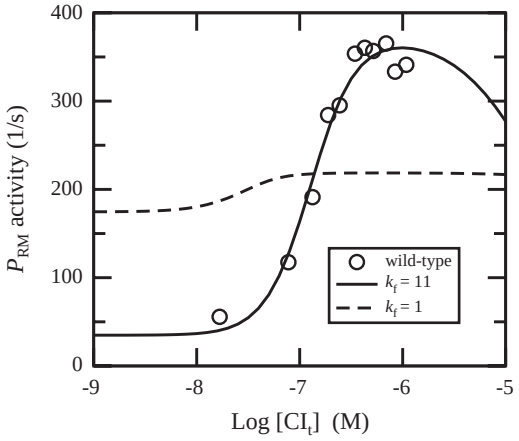

Fig. 2. P_{RM} activity versus total CI concentration (37 °C). Experimental P_{RM} activity data from [17] together with the best fit of our model (Eqs. (2) and (3)). k_f=11 corresponds to the TRS model (ΔG_{RM+}=0 and a=43.9 s^{-1}) and k_f=1 corresponds to the BES model (ΔG_{RM+}=−1.2 kcal/mol and a=301 s^{-1}). We note that the experimental data of [17] are obtained in absence of the left operator (O_L) that excludes the possibility of a long range loop formation between O_R and O_L [13] and thereby violating Eq. (1). Abscissa is drawn on logarithmic (decadic) scale.

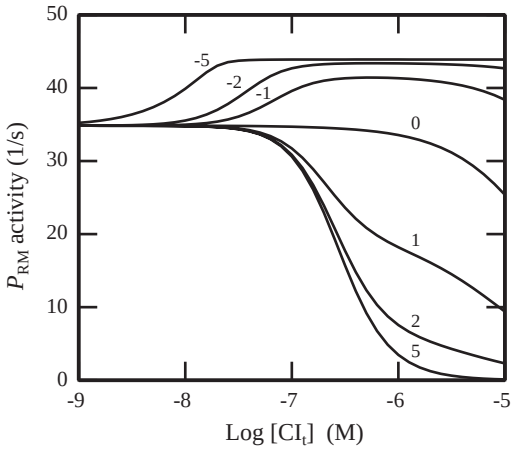

Fig. 4. P_{RM} activity versus total CI concentration (37 °C), where ΔG_{RM+} is systematically changed (k_f=1 and a=43.9 s^{-1}, i.e., no TRS is assumed). The associated value of ΔG_{RM+} for each individual curve is indicated by a number just above the curve (unit in kcal/mol). Abscissa is drawn on logarithmic (decadic) scale. The graph corresponding to ΔG_{RM+}=−5 kcal/mol indicates that no further improvement of the fit of the experimental data in Fig. 2 can be obtained by very low ΔG_{RM+} values.

O_R2 compared to a vacant O_R2, leads to an increased activity above a total CI concentration around 20 nM. This corresponds to CI concentrations where O_R2 with increasing likelihood (versus concentration) becomes occupied by a CI dimer such that a favorable interaction between CI and RNA polymerase can be formed. As we also see in Fig. 4 for ΔG_{RM+}<0, the activity gradually decreases for total concentrations >1 μM due to the P_{RM} repression mediated by O_R3-bound CI dimers, i.e., a negative feedback [8]. If ΔG_{RM+}>0, in contrast to the concentrational dependence of the activity when ΔG_{RM+}<0, the activity versus concentration will at around 20 nM start to decrease significantly due to the unfavorable interaction between CI at O_R2 and RNA polymerase at P_{RM}, i.e., a kind of anti-cooperativity.

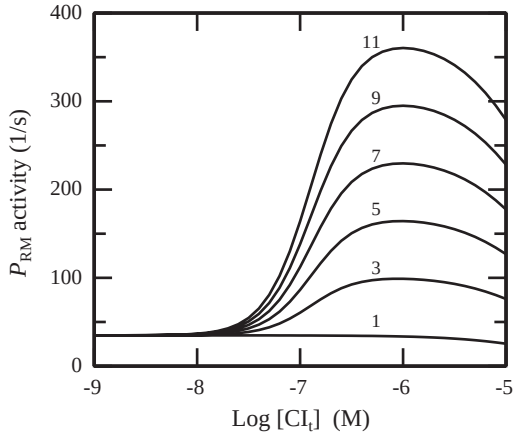

Fig. 3. P_{RM} activity versus total CI concentration (37 °C), where k_f is systematically reduced from the wild-type rate factor (k_f=11 and a=43.9 s^{-1}, i.e., same curve as the fully drawn curve in Fig. 2) to the unstimulated rate factor (k_f=1; see Eq. (3)). The associated value of k_f for each individual curve is indicated by a number just above the curve (k_f is dimensionless). Abscissa is drawn on logarithmic (decadic) scale.

The free RNA polymerase concentration ([RNAP]) is set to 30 nM, as mentioned above. However, [19] estimate the free RNA polymerase concentration in *E. coli* to lie between 400 nM and 1200 nM corresponding to 1.0 and 2.5 cellular doublings/hour, respectively. In the present work we compare to experimental data where the culture had 1.5 doublings/hour. By simply interpolating the data of [19] yield a [RNAP] around 600–700 nM for the culture we compare our model to. Thus, in order to take into account the uncertainty of the free [RNAP] we systematically fit our model to the activity data of [17] in the BES regime (k_f=1) for [RNAP] between 1 nM and 2000 nM. The quality of the fit of the model to the experimental data gradually becomes better versus decreasing concentration. These fits correspond to optimal values of ΔG_{RM+} between −1.2 and −2.2 kcal/mol. This means that the BES mechanism, for our particular choice of parameter values, works better at low [RNAP] (at [RNAP]=1 nM, i.e., less than one RNAP holoenzyme per cell, SSD is around 25%).

If we look at the statistical properties of Eq. (1), O_R is for most CI concentrations occupied by one (at P_{RM}) or zero RNA polymerase holoenzymes. This means that [RNAP] and ΔG_{RM} may to some extent substitute each other. Having this in mind and the findings in the previous paragraph, we alternatively investigate the BES mechanism (k_f=1) in light of a weaker ΔG_{RM} value, compared to the in vivo estimated value (−11.5 kcal/mol), and find that when ΔG_{RM}=−10.5 kcal/mol the SSD is reduced by a factor two compared to the corresponding value for ΔG_{RM}=−12.5 kcal/mol. For ΔG_{RM}=−8.5 kcal/mol the quality of the fit of the model to experimental data is almost equal to the best fit within the TRS mechanism. The reason for why the model

within the BES mechanism for ΔG_{RM}=−11.5 kcal/mol fails to reproduce the experimental data is that this relatively strong RNA polymerase binding energy saturates (more or less) P_{RM} with RNA polymerase, such that a negative ΔG_{RM+} does not assist to further recruit RNA polymerase significantly.

To test the uniqueness of the wild-type rate stimulating factor ($k_f \approx 11$) we fit the in vivo P_{RM} activity data of [17], with a gradually decreasing factor k_f. Interestingly, we find that for k_f as low as 3 that the SSD between experimental data points and model based data points is only increased by a factor two compared to the fit with k_f=11. Note that for all $k_f \geq 3$ the optimal fits are obtained for ΔG_{RM+}=0, i.e., our model with values $k_f \geq 3$ fits experimental data satisfactorily. However, for $k_f < 3$ the quality of the fit of the experimental data clearly aggravates, and for k_f=2 and k_f=1 the SSD between experiment and theory has increased by a factor 3 and 6, respectively, compared to the fit when k_f=11. For k_f=2 and k_f=1 the SSDs are only improved 3% and 13%, respectively, by applying the ΔG_{RM+} values −0.3 kcal/mol and −1.2 kcal/mol compared to the best fit for ΔG_{RM+}=0.

In order to further investigate the model, we implement the in vitro measured values from [25] of k_f and RNA polymerase binding affinities that correspond to the above-mentioned CI-pc2 mutant and an RNA polymerase mutant (*rpoD-RH596*; see Table 2), we are qualitatively, and to some extent quantitatively, able to reproduce their in vivo data (see Table 3). In particular, as also found in their experiment, we calculate that the CI-pc2 mutant combined with the RNA polymerase mutant fully restores the lysogenic wild-type activity. Interestingly, when we change [RNAP] to 640 nM the theoretical predictions of the activities fit the data of [25] much better, compared to the originally assumed [RNAP] of 30 nM (see Table 3). As previously mentioned, [19] estimates in vivo [RNAP] to lie between 400 nM and 1200 nM, depending upon the cellular doubling times.

Finally, we note that other procaryotic operons utilize a wide range of activation mechanisms. In contrast to the wild-type λ P_{RM} promoter, the CAP-cAMP activator at the $lacP_1$ promoter acts as a pure binding energy stimulator [26]. Other promoters as λ P_{RE}, λ P_I, and λ P_{aQ} are activated by the λcII protein that all seem to include both the TRS and BES mechanisms of activation. Although the state-space (i.e., pattern of protein-operator associations), binding affinities, and rate constants are different for these operons compared to λ O_R, the model presented in this work can in principle be valid for these systems, as we show for the λ P_{RM} operator that both TRS and BES can be accomplished within the present statistical model, however, as we also show here the effect of the two activation mechanisms crucially depends upon the specific values of, e.g., the RNA polymerase concentration and/or promoter affinity.

Table 2
In vitro data of [25] at 37 °C

Activator	RNAP wild-type[a]		RNAP mutant[b]	
	ΔG_{RM+}[c]	k_f[d]	ΔG_{RM+}	k_f
None	0	1	0	1.1
CI wild-type	0.1	17.2	−1.3	2.6
CI-pc2	−0.4	1	0.3	30

[a] RNA polymerase wild-type.
[b] ΔG_{RM}=0.4 kcal/mol compared to wild-type.
[c] Unit: kcal/mol.
[d] Dimensionless, measured relative to the unactivated (basal) activity for wild-type RNA polymerase.

Table 3
In vivo P_{RM} activity data at 30 °C

Activator	Relative β-gal activity (%)			
	RNAP wild-type		RNAP mutant	
	Exp	Theory[a]	Exp	Theory
None	15.0	6.7(15.0)	15.6	6.4 (15.5)
CI wild-type	100[b]	100(100)	79.1	19.5 (35.6)
CI-pc2	15.5	7.2(15.0)	133	133 (137)

Comparison between experimental data of [25] (exp) and theoretical predictions based upon our model in this work (theory).
[a] Theoretical predictions of the activities are based upon our model in Eq. (3), where in vitro data of [25] are applied (see Table 2). Other parameter values correspond to those in Table 1. Theoretically estimated activities in parentheses correspond to [RNAP]=640 nM, otherwise [RNAP]=30 nM.
[b] Reference value.

4. Summary and conclusion

We apply an expanded version of the statistical–mechanical model of [9] to calculate the probabilities of protein associations to the operator or of λ phage [21,16]. The transcriptional activity of the associated gene of the transcription factor CI is assumed to be proportional to the probability for binding of RNA polymerase to the promoter P_{RM} [10]. Experiments show that the wild-type transcriptional rate increases relatively by a factor $k_f \approx 11$ when the operator O_R2 is occupied by a CI dimer (see Fig. 1b) compared to a vacant O_R2 site [23]. However, other experiments show that a CI mutant destroys this transcriptional rate stimulating mechanism, leading to $k_f \approx 1$ [24,25]. In light of this, we study by means of the statistical–mechanical model the activity versus CI concentration for different k_f values and find that the pronounced peak appearing in an activity–concentration plot vanishes for decreasing k_f (Fig. 3).

We also systematically study the impact on the activity when we introduce an additional cooperativity between CI at O_R2 and RNA polymerase at P_{RM} as an alternative to the wild-type activation mechanism. For an attractive cooperativity (stronger binding) we obtain a flat maximum in the activity curve (Fig. 4). With this allele specific cooperativity (for k_f=1) we are not able to fit our model to in vivo activity data of [17]. Thus, the two activity stimulating mechanisms (rate versus binding energy) are qualitatively as well as

quantitatively different. But, we show that by reducing the basal RNA polymerase binding strength at P_{RM}, compared to the commonly applied value in the literature (−11.5 kcal/mol), we are by means of the binding energy stimulation mechanism (alone) also able to reproduce the in vivo activity data. Interestingly, by implementing in our model in vitro kinetic data from [25] on mutants of CI and RNA polymerase we are satisfactorily able to reproduce their in vivo activity data.

For the first time, to the authors knowledge, we have systematically studied theoretically different activity stimulating mechanisms of the P_{RM} promoter of λ phage in the context of in vivo experimental data. This opens up possibilities for new experiments and for further theoretical modeling; for example, in light of this work one could study the impact on the induction of λ phage, i.e., turnover from lysogenic state to lytic state, with respect to the transcription activation mechanism.

Acknowledgments

The author greatly acknowledges discussions with I. B. Dodd, R. Metzler, and K. Sneppen. This study was performed at Nordic Instituite for Theoretical Physics (NORDITA), Copenhagen.

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