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The energetics of activator–promoter recognition

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Abstract

Eukaryotes and bacteria have evolved entirely different mechanisms to cope with the problem of how to reconcile regulatory specificity in transcription, the recognition of specific DNA sequences by transcriptional activators, with speed, the ability to quickly respond to environmental change. It is argued here that eukaryotes enhance the specificity of activator–promoter recognition via ATP-dependent chromatin remodeling, whereas bacteria employ allosteric effectors to control specific activator–DNA binding reactions.

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Transcriptional activator, Stochastic thermodynamics, Entropy production, Irreversibility, Hopfield barrier.

Introduction

Regulated transcription depends on the recognition of specific DNA sequences by transcriptional activators, which promote the transcription of genes that bear their recognition motifs, but not other genes. The principle appears to be straightforward. Both bacteria and eukaryotes make use of it to regulate transcription in a gene-specific manner; yet differences abound.

In the absence of it allosteric effector, cyclic-AMP (cAMP), the bacterial transcriptional activator CRP binds DNA nonspecifically with a standard free energy change of $\Delta F^\circ = -9\,k_BT$ per molecule, corresponding to an equilibrium association constant of approximately $10^4\,M^{-1}$ [1]. [Throughout we assume that molecules exchange energy with a heat reservoir of absolute temperature T. At 25°C, $k_BT = 4.1 \times 10^{-21}$ J, where k_B is the Boltzmann constant. The standard free energy change of the activator-promoter binding reaction is related to the ratio of on-rate and off-rate constants, κ_+/k_-, *via* $\Delta F^\circ = -k_BT\ln(\kappa_+/k_-)$.]

In the presence of micromolar concentrations of cAMP, ΔF° decreases to $-23\,k_BT$ per molecule for high affinity target sequences of CRP [1]. Thus, the energetic difference between specific and nonspecific DNA binding, $\Delta\Delta F^\circ$, may be as large as $-14\,k_BT$ per molecule, corresponding to a 10^6-fold increase in DNA affinity upon cAMP binding. The affinity increase accounts for CRP's ability to specifically select its target genes, approximately 3% of *E. coli's* genes.

Surprisingly, for many eukaryotic transcriptional activators, $\Delta\Delta F^\circ$ is comparatively small, between -2.5 and $-5\,k_BT$ per molecule, corresponding to a 10^1-fold to 10^2-fold difference in affinity between correct and incorrect sequences [2]. Differences in affinity between different sequences and the same activator are largely attributable to differences in DNA residence time, *i.e.* different dissociation rate constant [3]. In vitro, average residence times fall into the range of 3 to 100 seconds at specific DNA sequences, and 0.1 to 1 second at nonspecific sequences [3].

In principle, short activator dwell times on target sequences are desirable, for frequent random dissociation of the activator provides the opportunity for reevaluation, for hypothesis testing: The activator may rebind its target sequence, if need be, or else is exported into the cytosol, if transcription of the target gene is no longer required—many eukaryotic activators are regulated in this way. This however begs the question of how cells reconcile the dual requirements of speed, *i.e.*, openness to change, and specificity.

Unlike bacterial activators, which make specific contacts—*i.e.*, contacts that rely on structural complementarity—with RNA polymerase or its associated sigma factor [4], eukaryotic activators are thought to activate transcription by nonspecific "recruitment" of a host of proteins [5], among them general transcription factors, the mediator complex, chromatin remodelers and histone modifying enzymes [6–11], but not RNA polymerase. Recruitment occurs by virtue of multivalent "fuzzy" contacts: variable interactions between a randomly coiled activation domain and hydrophobic patches of the target protein that are devoid of structural complementarity [6]. The mechanism of recruitment

may have evolved to allow for both high affinity binding and the quick exchange of binding partners [6]. Evidently, unlike bacterial activators, individual eukaryotic activators must be able to facilitate multiple promoter transitions. What explains the need for this "activator multitasking" in eukaryotic transcription?

Perhaps the most conspicuous difference between bacteria and eukaryotes with immediate impact on transcription has been the invention of the nucleosome, which imposed a transcriptionally repressive default state upon nuclear genes [12–14]. Transcriptionally induced eukaryotic promoters are thought to randomly transition between alternative nucleosome configurations, including the nucleosome-free and fully nucleosomal promoter, the prevailing configuration in repressing conditions [15,16]. While the purpose of nucleosome removal for transcription is evident, the goal of nucleosome reformation in activating conditions is not. Both formation and removal of nucleosomes are catalyzed by ATP-dependent chromatin remodelers [17]. In steady state, the net result appears to be no other than ATP hydrolysis. Do ATP-fueled promoter nucleosome dynamics serve a purpose, or do they represent a "futile cycle" [18], the expenditure of free energy to no effect?

Results

To explore the implications of activator-mediated recruitment of ATP-dependent chromatin remodelers on transcriptional error—*i.e.*, initiation of transcription by an incorrect activator—we consider the following model (Figure 1): a promoter with one nucleosome and one site for activator binding, which may either be occupied by a correct (*i.e.*, specific) or incorrect (nonspecific) activator. Thus, we may distinguish six promoter "microstates": The nucleosomal activator-free promoter (State 0), the nucleosomal promoter bound to the correct activator (State 1) or incorrect activator (State 2), and the nucleosome-free promoter bound to the correct activator (State 3) or incorrect activator (State 4), and finally the naked promoter (State 5). Representation of allowed transitions between microstates by directed edges (arrows) generates a reaction graph with two cyclic subgraphs, one each for the correct and incorrect activator, that is mirror symmetrical with regard to nodes and edges, but not labels [19,20]. The two subgraphs share one edge and two nodes (Figure 1). Only activator-bound states without nucleosome (States 3 and 4) are assumed to be transcriptionally active.

On the assumption of a time-homogeneous Markov process [21], the probability current from state n to j, c_{jn}, is a linear function of the probability mass at state n, $p_n(t)$—*i.e.*, $c_{jn}(t) = w_{jn}p_n(t)$, where w_{jn} is a (pseudo first-order) rate constant. The *net* current between two adjoining nodes (states), thus, is given by

$$J_{jn}(t) = c_{jn}(t) - c_{nj}(t) \tag{1}$$

The system dynamics, then, are described by the Markovian master equation:

$$\frac{dp_j(t)}{dt} = \sum_n J_{jn}(t) \tag{2}$$

for all j. The system is in steady state when $dp_j(t)/dt = 0$ for all j. Steady state probabilities, p_j, are uniquely defined since the reaction graph is strongly connected, *i.e.*, every node can be reached from any other by one or more transitions [22].

For simplicity, we assume that the activator on-rates are equal for correct and incorrect activator and independent of the presence of the nucleosome (*i.e.*, $w_{10}, w_{20}, w_{45}, w_{35} \equiv k_+$), and that the rate of transcription in States 3 and 4 is the same (*i.e.*, both activators are equally "strong"). Correct and incorrect activators, thus, are distinguished only by their off-rate constants, $k_{-1} < k_{-2}$ (*cf.* Figure 1). Furthermore, we put

$$f \equiv p_3/p_4 \tag{3}$$

and call f the fidelity of the process; f is the ratio of transcription events per time initiated by the correct versus incorrect activator in steady state [23].

By solving the set of linear equations $\sum_n J_{jn} = 0$ for all j (*cf.* Eq. (2)), the steady state probabilities p_j may be calculated as rational functions of the rate constants, which gives

$$f = \frac{k_{-2}}{k_{-1}}\left(\frac{(k_{-2}+M)(r_-m_+ + r_+m_+ + r_+k_{-1}) + k_+m_+(k_{-1}+k_{-2}+2M)}{(k_{-1}+M)(r_-m_+ + r_+m_+ + r_+k_{-2}) + k_+m_+(k_{-1}+k_{-2}+2M)}\right) \tag{4}$$

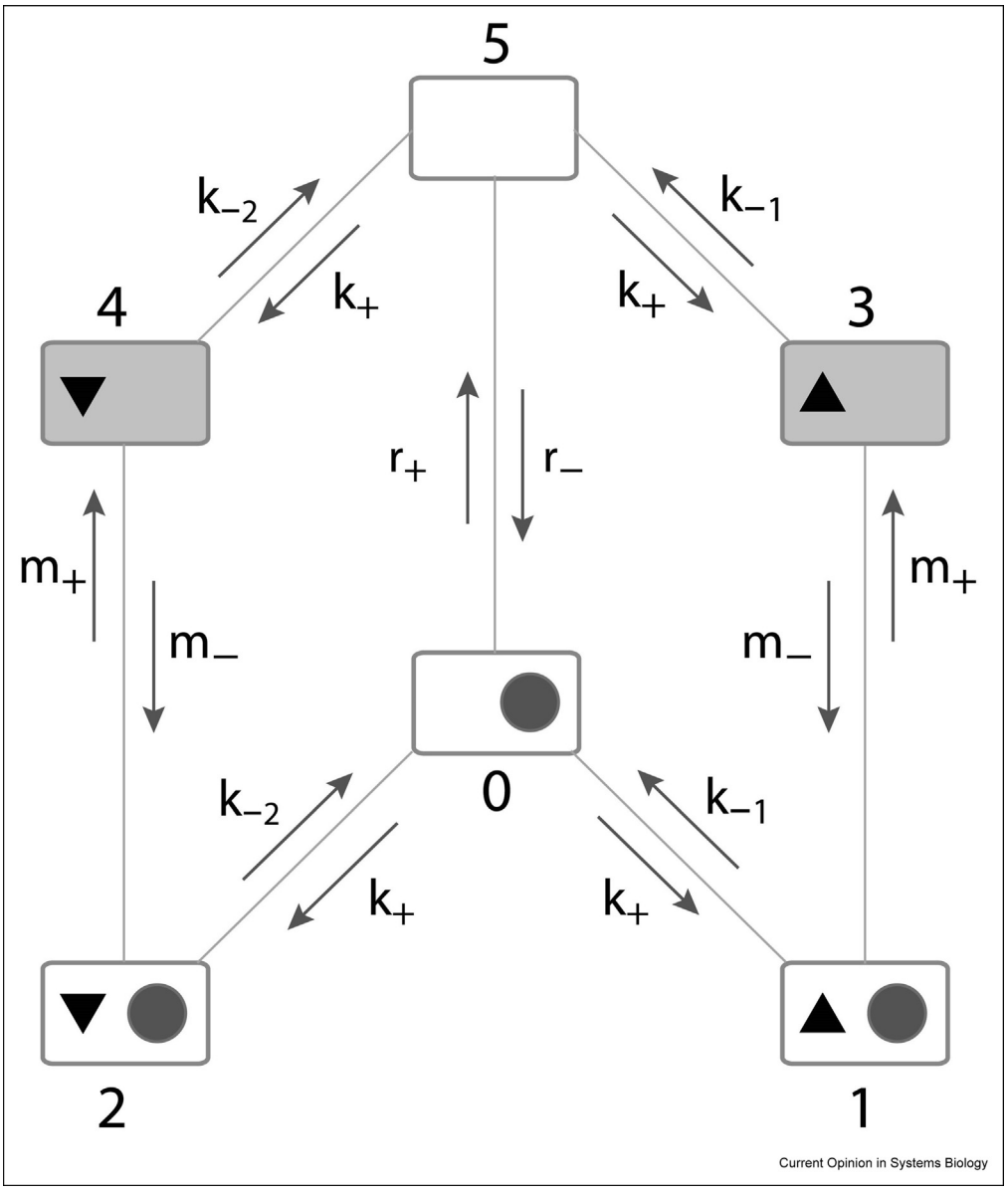
where, $M \equiv m_+ + m_-$.

In thermodynamic equilibrium, the detailed balance conditions are fulfilled [24], *i.e.*,

$$J_{jn} = 0 \tag{5}$$

for all j,n. Application of Eq. (5) to any closed loop (cycle) shows that the product of all rate constants that are

Figure 1



Reaction graph for nucleosomal proofreading of activator–promoter recognition. The promoter is depicted as a box, correct and incorrect activators by a triangle and inverted triangle, respectively. The gray dot represents promoter nucleosomes. Gray shading indicates transcriptionally active states. Labels on directed edges are (pseudo first-order) rate constants.

encountered as the loop is traversed clockwise equals the product of the rate constants encountered in the counterclockwise direction (the cycle condition for detailed balance) [22]. Applied to the cycle for the correct activator (*cf.* Figure 1), the cycle condition for detailed balance yields $r_+k_+m_-k_{-1} = k_+m_+k_{-1}r_-$, and after cancelation of common terms:

$$r_-m_+ = r_+m_- \qquad 6$$

(The same result is obtained for the incorrect activator cycle.) Eq. (6). is both necessary and sufficient for equilibrium [25]. Replacement of r_-m_+ in Eq. (4). with r_+m_- gives

$$f = \frac{k_{-2}}{k_{-1}} \equiv f_0 \qquad 7$$

The ratio on the right side of Eq. (7) may be expressed in energetic terms. A simple calculation shows that

$$f_0 = e^{-\beta\Delta\Delta F^\circ}, \quad 8$$

where, $\beta = (k_B T)^{-1}$.

Unlike many bacterial transcription factors (*e.g.*, CRP at class II promoters), eukaryotic activators don't have to bind DNA at a fixed position relative to core promoter sequences to be effective. This, in principle, allows for simultaneous activation by both correct and incorrect activators. The model of Figure 1 ignores this possibility, an assumption that is valid only in the limit of infinite activator dilution, $k_+ \to 0$ Actual fidelities are expected to fall well below f_0, which therefore marks the upper bound to fidelity in equilibrium [23]. This upper limit is called the "Hopfield barrier" [26] to activator fidelity.

However, away from equilibrium the process may surpass the Hopfield barrier: For $r_+ \to 0$ (violating Eq. (6)), followed by $M \to 0$ (while keeping all other constants fixed), we find with Eq. (4) and Eq. (8). that

$$f \to f_0^2 = e^{-2\beta\Delta\Delta F^\circ}. \quad 9$$

This remarkable result is analogous to the results of Hopfield and Ninio for the suppression of enzymatic error by kinetic proofreading [27,28].

The graph of Figure 1 focuses on the state of the promoter; it is equivalent to the reaction topology suggested by Nino. Focus on the activator state instead yields a graph equivalent to Hopfield's [29]. The conclusion is the same in either case: At the expense of breaking the time-reversal symmetry of detailed balance, the process fidelity may surpass its Hopfield barrier.

The steady-state rate of entropy production (change in total entropy) in the subgraph for the correct activator is given by

$$\frac{dS_{tot}}{dt} = J_{31}\Delta S_+^{res}, \quad 10$$

where, $J_{31} = J_{53} = J_{10}$ (in steady state) is the probability net current in the subgraph and

$$\Delta S_+^{res} = k_B \ln\left(\frac{r_- m_+}{m_- r_+}\right) \quad 11$$

is the entropy change in the coupled heat reservoir per counterclockwise cycle [29]. Notably, it can be shown that the logarithmic argument in Eq. (11), $r_- m_+/(m_- r_+)$, is equal to the ratio of forward and reverse cycle probabilities [30,31], which according to Eq. (11) exponentially increases with ΔS_+^{res}.

For detailed balance, $\Delta S_+^{res} = 0$ and $J_{31} = 0$, since $r_- m_+ = r_+ m_-$ (cf. Eq. (6)). For $r_+ < r_- m_+/m_-$, detailed balance is broken, $\Delta S_+^{res} > 0$ and $J_{31} > 0$ (the net probability current runs counterclockwise), for $dS_{tot}/dt \geq 0$ by virtue of the second law of thermodynamics (the analogous argument holds for clockwise cycles in the incorrect-activator subgraph). The reduction of error beyond its equilibrium value requires entropy production [32].

$T\Delta S_+^{res} > 0$ is the energy that the system dissipates into the heat bath per cycle ($0 \to 1 \to 3 \to 5 \to 0$ for the correct activator, and $0 \to 2 \to 4 \to 5 \to 0$ for the incorrect activator, *cf.* Figure 1). This energy must be supplied by a coupled work reservoir, since the change in system energy for a circular path is zero (first law of thermodynamics). The coupling occurs in the transition from State 1 to 3 (2 to 4 for the incorrect activator) on the assumption that the activator recruits ATP-dependent chromatin remodelers for nucleosome removal. The coupling allows for violation of detailed balance:

$$\frac{m_+}{m_-} > \frac{r_+}{r_-} \quad 12$$

(*cf.* Eq. (6)) [31]. The free energy of ATP hydrolysis, in part, is transferred to the system — nucleosome removal drives the system away from equilibrium (work is done on the system) — and fully dissipated only upon nucleosome reformation. As a consequence, both activator binding reactions, with the repressed and derepressed promoter, are out of equilibrium too: the activator is more likely to bind to, rather than dissociate from, the repressed promoter, and more likely to dissociate from, rather than bind to, the derepressed promoter (*cf.* Figure 1).

In vivo, the free energy change of ATP hydrolysis may be as large as $-23\, k_B T$ per molecule [33]. Thus, hydrolysis of a single ATP per "forward" cycle ($0 \to 1 \to 3 \to 5 \to 0$ for the correct activator) reduces the chance of the reverse cycle by a factor of $e^{-23} \approx 10^{-10}$ (*cf.* Eq. (11)). Yet, effective kinetic proofreading of activator identity requires that the binding reaction between incorrect activator and the repressed promoter remains close to equilibrium (almost all binding events, then, are rejected, i.e., reversed), which is achieved via small M and r_+. The detailed fluctuation theorem of stochastic thermodynamic shows that "violations" of the second law—here, dissociation (rejection) of the incorrect activator from the repressed promoter—are probable only if the forward reaction, activator binding to the repressed promoter, produces little entropy, on the order of k_B [30,31]. On the other hand, binding events between correct activator and the repressed promoter

should not be rejected too often, or else transcription becomes exceedingly noisy [23]. In general, biochemical processes evolved not toward maximum specificity but the right balance between specificity, speed, and energy cost (*i.e.*, free energy dissipation, entropy production) [19,34–36].

The above analysis suggests that the ATP-consuming removal and reformation of promoter nucleosomes is not futile at all; rather, free energy is expended to "distill" activator-promoter interactions [23]. The result of this distillation—improved regulatory specificity—is achieved without affecting the energetics of activator–DNA binding.

Not surprisingly, gains in fidelity due to free energy expenditure disappear unless initiation of transcription remains dependent on the presence of the activator on the promoter after nucleosome removal [23], *i.e.*, State 5 must be transcriptionally inactive. The requirement for activator multitasking is thus explained. Beyond recruitment of chromatin remodelers for nucleosome removal, activators recruit mediator and general transcription factors for transcription [8,37], which may render subsequent transcription events dependent on the persistent interaction between activator and promoter.

Activation of σ70-dependent bacterial promoters, the predominant promoter class in bacteria, are activated by stabilization of the closed complex through direct physical contact between activator and polymerase. The mechanism requires activator–DNA binding in close proximity and fixed rotational orientation relative to RNA polymerase [4]. In contrast, σ54-dependent promoters are controlled by activators whose activation domain is an AAA+ ATPase that utilizes the free energy of ATP hydrolysis to reconfigure σ54 for open complex formation [4]. Like eukaryotic activators, activators of σ54-dependent promoters may bind DNA at variable rotational positions far from the core promoter and come in contact with the transcription apparatus via looping of the intervening DNA. However, the free energy consumed in the activation process does not increase regulatory fidelity, since the activator is not required for any other step than open complex formation [38].

How, then, do bacterial activators reconcile the dual requirements for speed (openness to change) and specificity? For one, bacterial transcription factors are effective at only a small number of specific positions relative to the core promoter sequences [4] for which correct and incorrect transcription factors must compete.

The possibility of activation by incorrect activators is further limited by the fact that different promoter classes entail different mechanisms of activation—activators that stimulate σ54-dependent promoters don't activate σ70-dependent promoters and *vice versa*. In contrast, the activation mechanism of eukaryotic activators appears to be universal: enhancers and core promoters may be arbitrarily mixed and matched [39], further underscoring the specificity problem of transcriptional activation in the eukaryotic cell.

Finally, the DNA residence time of bacterial transcription factors is subject to allosteric regulation [40]. This allows for the active removal of transcription factors from their specific DNA-binding sites. For example, the lac repressor resides at the lac O_1 operator for tens of minutes in the absence of its allosteric effector [41], but dissociates from DNA within seconds in its presence [42]. The energy required for removal is provided by the interaction between repressor and its allosteric effector [40].

The cAMP-CRP complex dwells longer on its high-affinity target sequence than most eukaryotic activators—nearly 300 s, on average [43]—but less long than would be expected if the on-rate constant for DNA binding were similar to eukaryotic activators. This, however, is not the case. On-rate constants are close to the diffusion limit for bacterial transcription factors [40], but not eukaryotic activators [3]. The reason for this disparity is not known [44]—DNA binding may constrain the conformation of eukaryotic activators to a greater extent than their bacterial counterparts, perhaps because allosteric regulation of DNA binding requires greater conformational rigidity. Whatever the reason, high on-rate constants allow for large ΔF° without risking unduly long DNA-residence times.

Conclusion

Eukaryotes and bacteria arguably employ fundamentally different strategies to resolve the conflict between regulatory specificity and speed. The conjectured eukaryotic mechanism distills activator–promoter interactions by kinetic proofreading. The distillation requires the expenditure of free energy from a coupled work reservoir, possibly by virtue of ATP-dependent chromatin remodeling enzymes. The bacterial mechanism, instead, relies on equilibrium thermodynamics with fast activator on-rates and allosteric regulation of DNA residence times. Although kinetic proofreading explains many experimental observations, and the eukaryotic cell, no doubt, expends free energy in transcriptional regulation, a demonstration that the regulatory process is irreversible, *i.e.*, out of equilibrium, remains a major experimental challenge [45,46].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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