# Temperature-compensated chemical reactions

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Circadian rhythms are daily oscillations in behaviors that persist in constant light/dark conditions with periods close to 24 h. A striking feature of these rhythms is that their periods remain fairly constant over a wide range of physiological temperatures, a feature called temperature compensation. Although circadian rhythms have been associated with periodic oscillations in mRNA and protein levels, the question of how to construct a network of chemical reactions that is temperature compensated remains unanswered. We discuss a general framework for building such a network.

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## I. INTRODUCTION

The rates of most chemical reactions are sensitive to temperature with, in simple cases, an exponential dependence given by the Arrhenius equation. In contrast, certain chemical processes in biological systems, most notably circadian rhythms [1–3], are temperature compensated, making them quite insensitive to temperature changes [4–15]. Genetic mutations that disrupt temperature compensation have been identified [7,8,10,11], raising the hope that the underlying molecular mechanisms may one day be identified. At present, however, temperature compensation remains a mystery.

It has been suggested that temperature compensation can arise if the temperature dependence of one set of reactions is exactly opposite to that of another [8]. Models of temperature compensation have been constructed by producing quantities (typically oscillation frequencies) that depend on a number of reactions within the underlying model in such a way that their first derivatives with respect to temperature vanish [16–23]. This requires a cancelation between factors that increase and decrease as a function of temperature.

Because reactions are typically accelerated by increases of temperature, it is not immediately clear how the temperature-dependences of different reactions can cancel each other. If we restrict our attention to cases when reaction rates at temperature $T$ are described by an Arrhenius relation and thus are proportional to $\exp(-E/k_BT)$, where $E$ is the activation energy and $k_B$ Boltzmann's constant, the possibility of simple cancellation between the temperature dependences of different reactions is confounded by the absence of negative activation energies. Instead, cancellation in such systems amounts to finding mechanisms that generate ratios of rate constants. For example, if one reaction proceeds at a rate $\alpha \propto \exp(-E^\alpha/k_BT)$ and another at a rate $\beta \propto \exp(-E^\beta/k_BT)$, then a process that depends on the ratio $\alpha/\beta$ will be insensitive to temperature if $E^\alpha = E^\beta$. Our purpose is to place this idea on a firmer footing by showing how this form of cancellation can be generated in a precise and robust manner on the basis of general principles of equilibrium chemistry.

## II. TEMPERATURE COMPENSATION BY QUASIEQUILIBRIUM CHEMICAL CASCADES

The basic idea we exploit is that reaction-rate ratios arise naturally at chemical equilibria. Consider, for example, a reaction in which $A_1 \rightarrow A_2$ at a rate $\alpha$ and $A_1 \leftarrow A_2$ at a rate $\beta$ [Fig. 1(a)]. Initially, we simply assume that the concentration of $A_1$ is independent of temperature, but later on we will show how such a constant concentration can be obtained. Under this assumption, the concentration of $A_2$ is

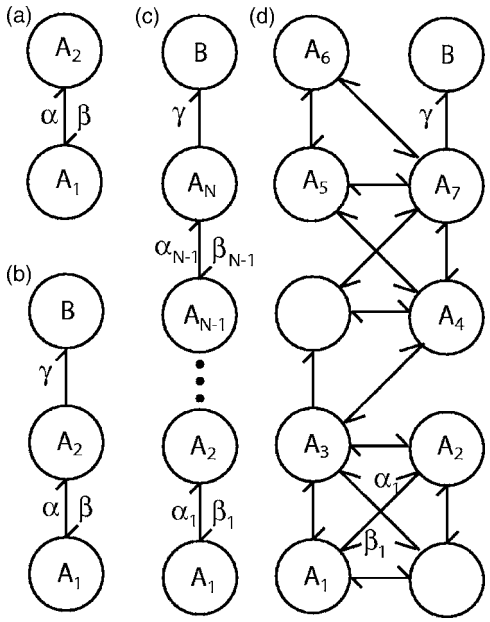


FIG. 1. Chemical reaction schematics. Each circle represents a reactant. The concentration of $A_1$ is assumed to be temperature independent, and the rate of production of $B$ is being temperature compensated. (a) A simple two-way reaction between reactants $A_1$ and $A_2$. The forward and reverse reaction rates are $\alpha$ and $\beta$, respectively. (b) Coupling of the reaction to the production of a reactant $B$ through a slow reaction with rate $\gamma$. (c) A chain of $N$ fast reactions coupled through a slow rate to $B$. The rate of production of $B$ in this scheme can be temperature compensated if Eq. (1) is satisfied. (d) Temperature compensation in a general, arbitrarily complex network of reactions. The rate of production of $B$ will be independent of temperature if Eq. (1) is satisfied along any pathway leading from the temperature-independent concentration $A_1$ to the slow reaction that produces $B$ as long as the remaining rate constants are much greater than $\gamma$. One such pathway is indicated by the labeled circles.

 

$$[A_1]\left(\frac{\alpha}{\beta}\right) \propto \exp\left(\frac{E^\beta - E^\alpha}{k_B T}\right).$$

This introduces the inverse dependence on temperature needed for compensation. However, to make use of this result we must extend it to a nonequilibrium situation.

We consider situations such as circadian rhythms, where the overall reaction rate is extremely slow relative to typical reaction rates. In such cases, subsets of the full set of reactions can come to equilibrium over time scales much shorter than the period of the overall rhythm. In the case shown in Fig. 1(b), imagine that the reaction $A_2 \rightarrow B$ proceeds at a rate $\gamma \propto \exp(-E^\gamma/k_B T)$ that is much slower than either $\alpha$ or $\beta$. Then, we can treat the reaction between $A_1$ and $A_2$ as approximately at equilibrium, and the rate at which $B$ is produced is

$$\gamma[A_2] = \gamma[A_1]\left(\frac{\alpha}{\beta}\right) \propto \exp\left(\frac{E^\beta - E^\alpha - E^\gamma}{k_B T}\right).$$

This is independent of temperature if $E^\beta - E^\alpha - E^\gamma = 0$.

There is an inconsistency with the above scheme. If $E^\beta - E^\alpha - E^\gamma = 0$, then $E^\beta$ must be at least as large as $E^\gamma$; but if this is so, we would not expect the reactions between $A_1$ and $A_2$ to be much faster than that between $A_2$ and $B$, as was assumed in order to approximate the $A_1 - A_2$ system as being at equilibrium. We can address this problem by considering a cascade of reactions as in Fig. 1(c). In this case, a whole sequence of reactants $A_i$ for $i = 1, 2, \ldots N$, are described by rate constants $\alpha_i$ and $\beta_i$ satisfying $\alpha_i \gg \gamma$ and $\beta_i \gg \gamma$ for all $i$. As a result, the entire string of reactions in Fig. 1(c), except for the last [the topmost reaction in Fig. 1(c)], come to approximate equilibrium and the rate at which $B$ is produced is

$$\gamma[A_N] = \gamma[A_1]\prod_{i=1}^{N-1}\left(\frac{\alpha_i}{\beta_i}\right) \propto \exp\left[\frac{1}{k_B T}\left(\sum_{i=1}^{N-1}(E_i^\beta - E_i^\alpha) - E^\gamma\right)\right].$$

This is independent of temperature if

$$\sum_{i=1}^{N-1}(E_i^\beta - E_i^\alpha) - E^\gamma = 0. \quad (1)$$

In this case, we must only require that $E_i^\beta$ be of order $E^\gamma/N$, thereby allowing the reactions between the $A_i$'s to be much faster than that from $A_N$ to $B$.

The linear chain of reactions shown in Fig. 1(c) might appear rather contrived, and one might wonder if the existence of additional reactions would spoil the scheme. In fact, due to the restrictions implied by chemical equilibrium, in particular the principle of detailed balance, the result generalizes to arbitrary networks of reactions. Consider a general scheme such as that shown in Fig. 1(d) in which all the reactions are fast other than the one with rate constant $\gamma$ that produces $B$. In this case, all the reactions except the single slow one will come to approximate equilibrium.

Detailed balance states that if a full system of chemical reactions is at equilibrium, so is any subset of those reactions, treated as if it was in isolation. We have already made use of this implicitly when we wrote the concentration of $A_N$ for Fig. 1(c) as $[A_1]\Pi(\alpha_i/\beta_i)$. This follows from the fact that each step in this cascade is separately at equilibrium, satisfying $[A_{i+1}] = [A_i]\alpha_i/\beta_i$ for $i = 1, 2, \ldots, N-1$. For an arbitrary scheme such as that shown in Fig. 1(d), we can follow any pathway from $A_1$ to $B$, such as the one labeled by the $A_i$'s in Fig. 1(d), and apply the above argument to it. The rate of production of $B$ will be temperature compensated provided that Eq. (1) is satisfied for the reaction rates along any such path. The other reactions in the network have no effect on this result.

The biochemical processes involved in biological circadian timekeeping are extremely complex, and building a detailed model of a circadian oscillator [22–28] is beyond the scope of this paper. A critical timing element in the Drosophila clock, however, is the slow buildup in the level of phosphorylation of PER protein that ultimately results in a dimerization with TIM, leading to nuclear entry and suppression of gene transcription [1–3,26]. Our mechanism can be applied in a straightforward manner to temperature compensate the rate of this phosphorylation process that is so vital to clock timing. To see how this can be done, it is important to appreciate that the key to the mechanism we propose is not the slow reaction from state $A_N$ to state $B$ seen at the top of Fig. 1, but rather the fact that the concentration of $A_N$ varies with temperature in a manner that can compensate for the Arrhenius factor in a typical chemical reaction rate constant.

To apply the mechanism we have proposed to the phosphorylation of PER, we assume that the reactant $A_N$ is a kinase that acts on PER, and that the concentration of $A_N$ is the rate limiting factor in this phosphorylation. In this case, the graph of quasiequilibrium reactions shown, for example, in Fig. 1(d) represents a regulatory network modulating the concentration of activated kinase (the transition from $A_N$ to $B$ should be left out in this interpretation). The key point is that this regulatory network, if it is near equilibrium, can modify the concentration of active kinase to compensate for the temperature dependence of the phosphorylation process, producing a temperature-compensated rate of phosphorylation. In other words, we consider the transformation from PER to a phosphorylated form $\mathrm{PER}^+$ to be a two step process: the binding of PER to the kinase, $\mathrm{PER} + A_N \rightarrow (\mathrm{PER}A_N)$, proceeds with rate constant $k_f$ while the reverse reaction takes place with rate constant $k_b$. Finally, the catalyzed phosphorylation, $(\mathrm{PER}A_N) \rightarrow \mathrm{PER}^+ + A_N$ proceeds with rate constant $\gamma$. The Michaelis-Menton rate for the phosphorylation is then

$$\frac{\gamma k_f [\mathrm{PER}][A_N]}{k_b + k_f[\mathrm{PER}]} \rightarrow \gamma[A_N],$$

where the limit follows when [PER] is large. This combination, $\gamma[A_N]$, is precisely what is temperature independent if Eq. (1) is satisfied.

It is also possible that the slow buildup in the level of phosphorylation is due to the counteracting effects of a kinase and a phosphatase. The rates of phosphorylation and dephosphorylation produced by this pair can both be temperature compensated if each is regulated by its own quasiequilibrium regulatory network in the manner we have discussed. In this way, a small difference in the rate of

phosphorylation and dephosphorylation could be maintained independent of temperature.

We have been assuming that the reactant $A_1$ has a concentration that is temperature independent, and we now address how this can be achieved. One way of doing this is to assume that $A_1$ is a complex consisting of a substrate $A_0$ bound to a catalyst $C$, and that this catalyst is released by the conversion of $A_1$ to $A_2$. If the rate of binding of $C$ to $A_1$ is much more rapid than the unbinding rate and the binding of $A_1$ and $C$ is limited by the availability of $C$, the concentration of $A_1$ will be very close to the total concentration of catalyst. More precisely, if $\alpha_0$ and $\beta_0$ are the binding and unbinding rates for the reaction between $A_0$ and $C$, the equilibrium concentration of $A_1$, the bound complex of these two reactants, is

$$[A_1] = \frac{\alpha_0[A_0]C_{\mathrm{T}}}{\alpha_0[A_0] + \alpha_1},$$

where $C_T$ is the total (bound and unbound) concentration of $C$. If $\alpha_0$ is sufficiently large and $A_0$ sufficiently plentiful, this reduces to $[A_1]=C_T$, which is constant because the catalyst is cycled between states but not consumed.

## III. CONCLUSIONS

The generality and robustness of the temperature compensation mechanism we have presented have implications for the evolution of a temperature compensated system such as the mechanism that produces circadian rhythms. Suppose that a biochemical network such as that in Fig. 1(b) and 1(c) evolves but does not yet have accurate temperature compensation. Additional reactions can then be added to this network, as in Fig. 1(d), until a better pathway develops, leading to better compensation.

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