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The effects of time-varying temperature on delayed gene networks

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Delays in gene networks result from the sequential nature of protein assembly. However, it is unclear how models of gene networks that use delays should be modified when considering time-dependent changes in temperature. This is important, as delay is often used in models of genetic oscillators, such as circadian oscillators, that can be entrained by periodic fluctuations in temperature. Here, we analytically derive the time dependence of delay distributions in response to time-varying temperature changes and use these results to examine a model of a synthetic gene oscillator. We demonstrate that, under a common Arrhenius scaling alone, the frequency of the oscillator is sensitive to changes in the mean temperature but robust to changes in the frequency of a periodically time-varying temperature. When a mechanism for temperature compensation is included in the model, however, we show that the oscillator is entrained by periodically varying temperature even when maintaing insensitivity to the mean temperature.

Circadian oscillators have an intricate relationship with temperature. For instance, circadian oscillators exhibit temperature compensation [1, 2], *i.e.* their periods do not vary with changes in the average temperature. Theorists have investigated methods of temperature compensation in models of circadian oscillators, often minimizing the effects of Arrhenius-scaled rate constants [3–8]. Periodic changes in temperature have also been implicated in the entrainment of circadian oscillators to the day/night cycle [9–13]. Entrainment of circadian oscillators is most commonly associated with periodic changes in light, and mathematical models have been developed explaining this phenomenon [14–23], but less theoretical work has investigated temperature-mediated entrainment.

A common mathematical technique for modeling genetic oscillators is the use of dynamical delay. Delay in gene networks is the result of the sequential assembly of first mRNA [24] and then protein [25] (see Fig. 1(a)). Delay-based models play a central role in understanding the origin of oscillations in genetic networks [26, 27] and other nonlinear systems [28, 29]. Importantly, the incorporation of delay greatly simplifies models of genetic oscillators while simultaneously maintaining qualitative similarities to experimental data [30–33]. However, it has yet to be established how delay is affected by time-varying changes in temperature. Since changes in temperature influence each biochemical step in the sequence that constitute the delay, the value of the delay time will change.

Here, we investigate how time-varying temperatures affect mathematical models of biochemical oscillators with delays. Motivated by circadian oscillators, we derive a method for incorporating delays in an Arrhenius-scaled biochemical network with periodically time-varying temperature. To do this, we assume that delays arise from a sequence of first-order reactions that can be modeled as an aggregate delay and that each reaction in the sequence is scaled by a common time-dependent Arrhenius factor. From these assumptions we derive an expression for the time-dependent distribution of delay times. We incorporate our findings into a model of a synthetic gene oscillator with temperature compensation presented by Hussain *et al.* [30]. We find that, when the temperature varies sinusoidally in time, the oscillator can be entrained by temperature, but that this entrainment does not occur in the absence of the temperature compensation mechanism. These results lead to the seemingly paradoxical conclusion that a temperature compensated oscillator that is insensitive to changes in the mean temperature can also be entrained by periodically varying temperatures.

Characterization of time-varying delays. We begin by approximating protein production with a linear sequence of reactions, the dynamics of which can be modeled by

$$\dot{x}_j = -a(t)(x_j(t) - x_{j-1}(t)) \quad \text{for} \quad j = 1, ..., N, \quad (1)$$

where $x_i(t)$ is the concentration of the ith species at time t, $x_0(t)$ is the time varying initial complex, $a(t)$ is the time-varying rate coefficient, and the overdot represents differentiation with respect to time. The effects of time-varying temperatures can be reflected in the time-varying rate coefficients. From this we deduce the effects of time-varying temperatures on the delay distribution, *i.e.* the time it takes to go from the initial complex, x_0, to mature protein, x_N.

To find the distribution function, we first rewrite system (1) as

$$\dot{x}(t) = \mathbf{A}(t)\, x(t) + y(t), \quad (2)$$

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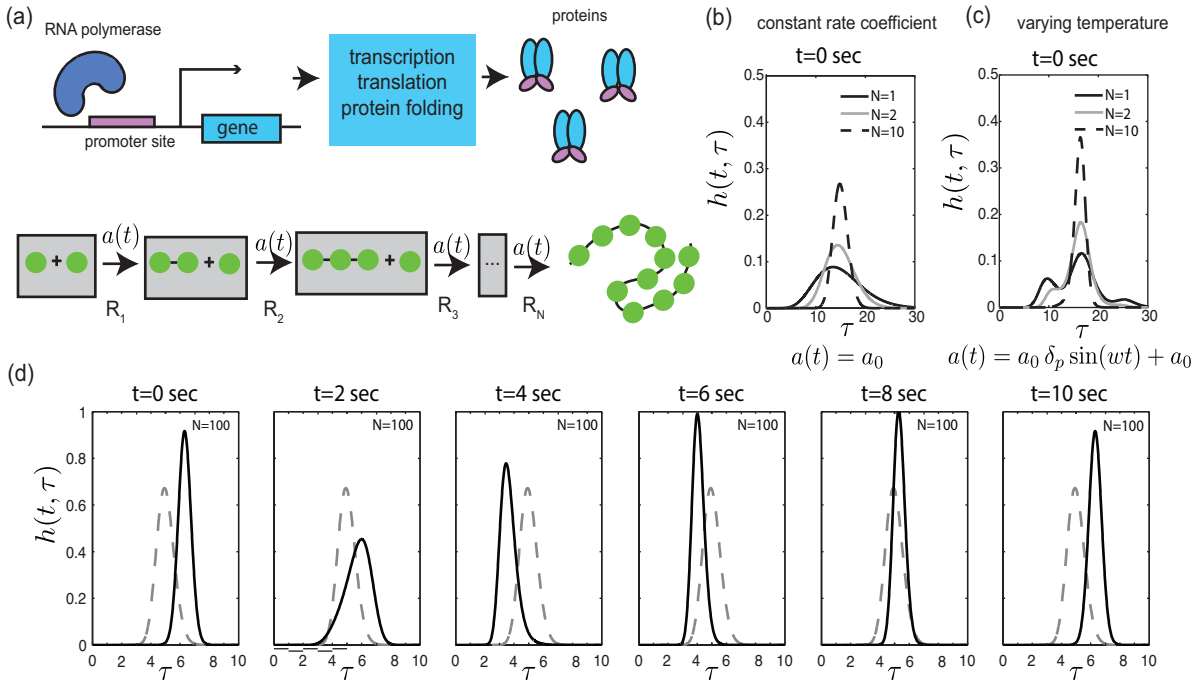

FIG. 1. (a) We are modeling transcriptional delays (top) by a sequence of chemical reactions (bottom) with common reaction rate $a(t)$ for each reaction $\mathrm{R}_1, \ldots, \mathrm{R}_N$. (b) Delay distribution for different values of N with $E = 15$ for a constant rate coefficient a. (c) Delay distribution for different values of N with $E = 15$ for a time-varying rate coefficient $a(t) = .5\, a_0\, \sin(\omega\, t) + a_0$ at time $t = 0$. (d) Distribution as a function of time with $a(t) = a_0\, \delta_p \sin(\omega\, t) + a_0$, $N = 100$, $(N+1)/a_0 = 15$, $\delta_p = .5$, and $\omega = 2\pi/20$. The dashed line indicates the nominal time-invariant distribution with $a(t) = a_0$.

where $x(t) = [x_N, x_{N-1}, \ldots, x_1]^T$, $y(t) = [0, \ldots, 0, a(t)x_0(t)]$ and $\mathbf{A}(t) = a(t)\,\mathbf{J}_{-1,N}$. Here $\mathbf{J}_{-1,N}$ is the N-dimensional Jordan matrix with eigenvalues -1. Because $\mathbf{A}(t_1)$ commutes with $\mathbf{A}(t_2)$ for all (t_1, t_2), we can write the general solution to Eq. (2) as

$$x(t) = \boldsymbol{\phi}(t, t_0)\, x(t_0) + \int_{t_0}^{t} \boldsymbol{\phi}(t, \sigma)\, y(\sigma)\, \mathrm{d}\sigma, \qquad (3)$$

where

$$\boldsymbol{\phi}(t, t') = \exp\left(\int_{t'}^{t} \mathbf{A}(s)\, \mathrm{d}s\right). \qquad (4)$$

Without loss of generality we set $t_0 = 0$ and substitute $\sigma = t - \tau$. If we assume $x_j(t_0) = 0$ for $j = 1..N$, the solution reduces to

$$x(t) = \int_0^t e^{\alpha(t,\tau)\mathbf{J}_{-1,N}}\, y(t-\tau)\mathrm{d}\tau, \qquad (5)$$

where $\alpha(t, \tau) \equiv \int_{t-\tau}^{t} a(s)\mathrm{d}s$. We can now extract the expression relating the input, $x_0(t)$, to the measured output, $x_N(t)$, with the result

$$x_N(t) = \int_0^t h(t, \tau) x_0(t-\tau)\mathrm{d}\tau, \qquad (6)$$

where the function

$$h(t, \tau) = a(t-\tau)\frac{\alpha(t,\tau)^{N-1}}{(N-1)!} e^{-\alpha(t,\tau)} \qquad (7)$$

is the impulse response function relating the output to the input of the system, which represents the delay distribution given the constraint $\int_0^\infty h(t, \tau)\mathrm{d}\tau = 1$, for any time t. This condition holds true if $a(t-\tau) > 0$ (which is satisfied by the physics of the problem) with $\alpha(t, 0) = 0$ and $\lim_{\tau\to\infty} \alpha(t, \tau) = \infty$. When $a(t) \equiv$ const., Eq. (7) is the *Erlang* distribution [?]. If $a(t)$ is not constant, the delay distribution will be a function of time. Figure 1(b)-(c) shows the delay distribution $h(t, \tau)$ for different values of N, comparing the time-invariant case to the time-varying case. Note that, unlike the time-invariant case (Fig. 1(b)), the distribution in the time-varying case (Fig. 1(c)) need not be unimodal, especially for small N. Figure 1(d) shows how the time-varying distribution changes with time for fixed N and a time-varying rate coefficient $a(t)$.

Next, consider the limit as the number of reactions within the sequence tends to infinity. In the time-invariant case, one would consider the limit as $N \to \infty$ such that $N/a = E$ remains constant, which reduces to the distribution function to the Dirac delta function $\delta(\tau - E)$, which agrees with results shown by Bel *et al.* [34]. To investigate the time-varying case, we assume that $a(t) = a_0 f(t)$, where $a_0 > 0$ and $f(t)$ is a positive definite, bounded function of time. In this case, if we take the limit $N \to \infty$ with the constraint $N/a_0 = E$, the ratio $N/\alpha(t, \tau)$ remains finite for finite τ and we find that there ex-

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ists a unique delay τ_{eff} such that

$$\lim_{N\to\infty} h(t,\tau) = \begin{cases} 0 & \tau \neq \tau_{\text{eff}} \\ \infty & \tau = \tau_{\text{eff}}. \end{cases} \tag{8}$$

Therefore, in the above limit, $h(t,\tau)$ can be approximated by a delta function centered at τ_{eff}. The delay $\tau_{\text{eff}}(t)$ can be found, at least numerically, by solving

$$\int_{t-\tau_{\text{eff}}}^{t} f(s)ds = E \tag{9}$$

for τ_{eff} (see Supplementary Information). Note that $\tau_{\text{eff}}(t)$ is a function of the mean delay and the time-varying function $f(t)$.

Temperature Entrainment of a Dual-Feedback Oscillator. We now consider the entrainment properties of a temperature compensated dual-feedback oscillator presented by Hussain *et al.* [30]. The oscillator, as depicted in Fig. 3(a), can be modeled as [30]:

$$\frac{1}{A(T)}\cdot\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{\alpha_x\left(\eta + \frac{y(t-\tau_y)}{C_y}\right)}{\left(1+\frac{y(t-\tau_y)}{C_y}\right)\left(1+\frac{x(t-\tau_x)}{C_x(T)}\right)} - \beta\, x(t) - \frac{\gamma_x\, x(t)}{R_0 + x(t) + y(t)} \tag{10}$$

$$\frac{1}{A(T)}\cdot\frac{\mathrm{d}y}{\mathrm{d}t} = \frac{\alpha_y\left(\eta + \frac{y(t-\tau_y)}{C_y}\right)}{\left(1+\frac{y(t-\tau_y)}{C_y}\right)\left(1+\frac{x(t-\tau_x)}{C_x(T)}\right)} - \beta\, y(t) - \frac{\gamma_y\, y(t)}{R_0 + x(t) + y(t)}, \tag{11}$$

where x and y are the concentrations of the repressor (LacI) the activator (AraC); α_x and α_y are the maximal transcription initiation rates for x and y, respectively; C_x and C_y are the binding affinities of LacI and AraC to the promoter, respectively; β is the dilution rate due to cellular growth; η is a measure of the strength of the positive feedback loop; R_0, γ_x, and γ_y are Michaelis-Menten constants for enzymatic decay of the proteins; τ_x an τ_y are the delay times for the production of LacI and AraC, respectively; and $A(T)$ is the common Arrhenius scaling of all reaction rates.

Note that the binding affinity of LacI, $C_x(T)$ is a function of the temperature

$$C_x(T) = (C_{x,\max} - C_{x,\min})\frac{(T/T_{\text{lac}})^b}{1+(T/T_{\text{lac}})^b} + C_{x,\min}, \tag{12}$$

and provides the mechanism for temperature compensation in the oscillator [30]. $C_{x,\min}$ and $C_{x,\max}$ are the minimum and maximum biding affinities of LacI to its promoter. Additionally, the Arrhenius scaling term has the form $A(T) = A_0\, e^{-\theta/T}$, where θ is the temperature scale. Note that increasing temperature increases the scaling coefficient $A(T)$ and hence speeds up the dynamics of the system.

We now apply the method derived in this paper to investigate entrainment properties of the oscillator under periodically time-varying temperatures. We begin by assuming

$$T(t) = \Delta T \sin(\omega t) + T_0. \tag{13}$$

If $\Delta T \ll T_0$, we can write

$$A(t) \approx \delta_p \sin(\omega t + \phi) + 1, \tag{14}$$

where $\delta_p \approx \frac{\theta \Delta T}{T_0^2}$ and A_0 is chosen such that $A(T_0) = 1$, *i.e.* $A_0 = \exp(\theta/T_0)$. The time-varying rate coefficient for the reaction rates implicit in the delay are given by $a(t) = a_0 \cdot A(t)$. From Eq. (9), the effective delay reduces to solving

$$\int_{t-\tau_{\text{eff}}}^{t} [\delta_p \sin(\omega s + \phi) + 1]ds = E, \tag{15}$$

where E is the expected delay for $a(t) = a_0$ corresponding to the constant temperature $T(t) = T_0$. Note that

$$\tau_{\text{eff}} \approx \begin{cases} E & \text{for} \quad \omega \to \infty \\ \frac{E}{\delta_p \sin\phi + 1} & \text{for} \quad \omega \to 0. \end{cases}$$

Fig. 2 shows the delay as a function of time for a periodically varying temperature. The solution is found numerically for a discretized range of time. We choose the expected delay values E for τ_x and τ_y based on parameters chosen in Hussain *et al.* [30]. In Fig. 2 we consider the expected delay $E = 13.5$ min., which is what is chosen for τ_x.

In Hussain *et al.* [30], the period of the oscillator is shown to remain largely unaffected by changes in temperature due to a temperature sensitive LacI mutant, which is modeled by a temperature dependent binding affinity $C_x(T)$. In order to compare entrainment properties to a system without such a temperature dependent mechanism, we consider a similar

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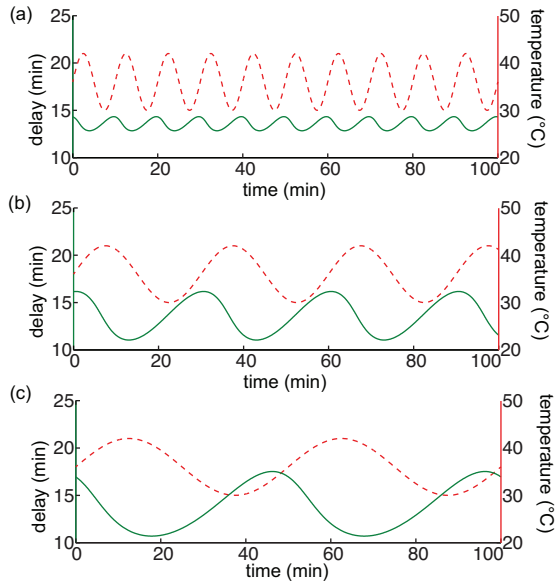

FIG. 2. Time-varying delays (green solid lines) corresponding to various time-varying temperature $T(t) = \Delta T \sin(\omega t) + T_0$ (red dashed lines). Parameter values here are $\theta = 4500K$, $E = 13.5$ min., $\Delta T = 6K$, $T_0 = 309K$, and $\delta_p = .28$. (a) $\delta_p = .27$ and $\omega = \frac{2\pi}{10}$. (b) $\delta_p = .28$ and $\omega = \frac{2\pi}{30}$. (c) $\delta_p = .28$ and $\omega = \frac{2\pi}{50}$.

model where the binding affinity $C_x(T) = C_x(T_0)$ remains constant. In this case temperature affects are introduced solely through an Arrhenius scaling. Given Eq. (14), we drive the system with a time-varying temperature described by Eq. (13) with $\theta = 4500K$ and $\Delta T = 2K$. In Fig. 3(b) we fix the frequency at $\omega = 2\pi/50\,\mathrm{min}^{-1}$ and vary the mean temperature T_0 in order to verify the temperature compensating property achieved through the temperature dependent LacI mutant. Without the temperature compensating mechanism the frequency of oscillations changes linearly with the mean temperature but remains constant with the LacI mutant. Figure 3(c) we fix the mean temperature at $T_0 = 309K$ and vary the frequency ω to study frequency entrainment for the same system with and without the temperature compensating mechanism. It is clear that the system entrains only under the influence of the temperature sensitive promoter. A common Arrhenius scaling alone does not allow for frequency or phase entrainment. The same mechanism that provides temperature compensation (insensitivity to changes in mean temperature) also makes the system sensitive to temperature dynamics, achieving entrainment.

Concluding remarks. It was found that periodic temperature fluctuations induce periodically time-varying delays. With this, we investigated properties of a delay-based model of a temperature sensitive oscillator. We focused on properties important in circadian oscillators, namely, temperature compensation and temperature entrainment. Ideally, a circadian oscillator should demonstrate conflicting properties of entrainment with insensitivity to changes in mean temperatures [7]. Here we highlight a case where the entrainment is a byproduct of the same mechanism which makes the system insensitive to changes in mean temperature. There is an inherent tradeoff between robustness to unwanted temperature fluctuations and robustness to changes in mean temperatures, with the latter admitting temperature entrainment. Similar results have been achieved with asymmetric Arrhenius scaling. Here, we ease such analysis through the use of a delay-based model of a circuit with circadian clock-like properties and understanding of the effects of temperature on delays.

Acknowledgements. The authors thank Jae Kyoung Kim for insightful discussions. This work was funded by the National Institutes of Health, through the joint NSF/NIGMS Mathematical Biology Program grant R01GM104974 (M.R.B.) and the Robert A. Welch Foundation grant number C-1729 (M.R.B.).

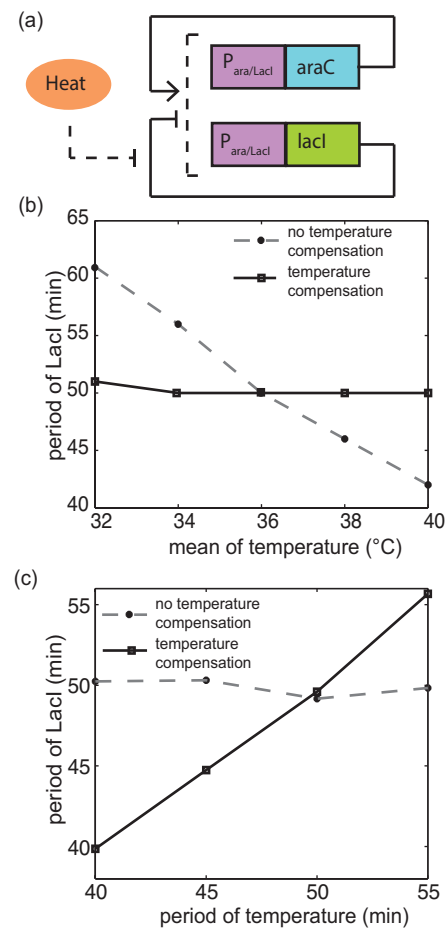

FIG. 3. (a) Schematic of the temperature compensating oscillator [30]. (b) Period of the circuit with and without temperature compensation for $\omega = 2\pi/50\,\mathrm{min}^{-1}$, $\Delta T = 2K$, and different mean temperatures T_0 in Eq. (13). (c) Frequency entrainment of the circuit with and without temperature compensation for $\Delta T = 2K$ and $T_0 = 309K$.

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