# Heterodimer Autorepression Loop: A Robust and Flexible Pulse-Generating Genetic Module

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We investigate the dynamics of the heterodimer autorepression loop (HAL), a small genetic module in which a protein $A$ acts as an autorepressor and binds to a second protein $B$ to form an $AB$ dimer. For suitable values of the rate constants, the HAL produces pulses of $A$ alternating with pulses of $B$. By means of analytical and numerical calculations, we show that the duration of $A$ pulses is extremely robust against variation of the rate constants while the duration of the $B$ pulses can be flexibly adjusted. The HAL is thus a minimal genetic module generating robust pulses with a tunable duration, an interesting property for cellular signaling.

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Living cells regulate their response to stimuli through biochemical reaction networks where genes, messenger RNAs (mRNAs), and proteins interact with each other [1]. Genes control the synthesis of proteins via mRNAs, while their activities are regulated by specific DNA-binding proteins called transcription factors (TFs). Proteins bind to each other to regulate their properties. These multiple interactions are organized in entangled feedback loops, which generate a complex and collective dynamics. Despite the high complexity of biological networks, many specific dynamical mechanisms can be attributed to small genetic modules comprising a few genes, their mRNAs, and proteins [2,3]. Thus, many studies have aimed to uncover the dynamical design principles of such modules, viewed as building blocks for larger systems or as devices for synthetic biology. For example, the appearance of oscillations has been linked to negative feedback and time delays [2], and the importance of mechanisms such as complexation [4] or saturated degradation [5–7] for oscillations has been highlighted.

While much effort has been devoted to assessing the robustness of biochemical oscillations, it has generally been quantified only by the constancy of the total period. The latter is an important criterion for oscillations whose purpose is time keeping, as in circadian clocks, but it is not always relevant. Recent studies (see [8] for a review) revealed that also signaling proteins, which detect and deliver cellular signals, can display oscillating dynamics. In some systems oscillations appear as discrete pulses separated by constant time intervals [9], while in others the intensity of upstream signals determines the time interval between pulses [10,11], which may thus be used to encode information [7]. A natural question is then whether we can identify simple model systems that display similar behavior. In this Letter, we investigate the dynamical properties of such a minimal genetic module, the heterodimer autorepression loop (HAL). The HAL generates a periodic "pulsating" output in the concentrations of two different proteins where the pulses of one protein alternate with the pulses of the other one. We will use the term "pulses" rather than "oscillations" to emphasize that we think primarily of the model as a genetic device for cellular signaling rather than for time keeping. Remarkably, the duration of the pulses of one protein is robust against variations in the rate constants, while the time interval between two pulses, where the other protein is dominant, is tunable.

The HAL consists of a self-repressing TF protein $A$ that can bind to its own gene to inhibit mRNA synthesis, or to another protein $B$, then becoming inactive (Fig. 1). Self-repression is a pervasive motif in transcriptional networks [12–14], and protein-protein interactions modifying TF activity are also ubiquitous [15], making the HAL very plausible biologically. Accordingly, the HAL appeared with high frequency in evolutionary algorithm calculations searching for oscillating modules [16]. The HAL can be described by the following deterministic differential

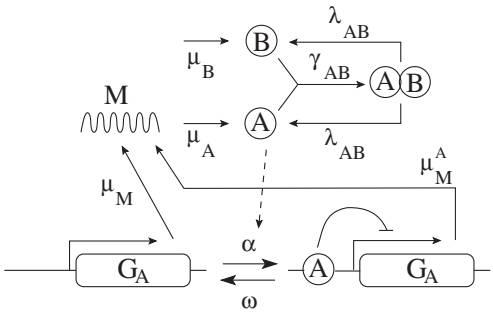


FIG. 1. A schematical representation of the HAL module. The gene $G_A$ is repressed by its own protein $A$, which forms an inactive dimer $AB$ with a second protein or molecule $B$. Proteins and mRNA degrade with rates $\delta_A$, $\delta_B$, $\delta_{AB}$, and $\delta_M$ (reactions not shown).

 

equations, obtained from the reactions in Supplemental Fig. 1 [17] using mass action kinetics:

$$\begin{aligned}
\frac{d[G]}{dt} &= \omega(1-[G]) - \alpha[G][A], \\
\frac{d[M]}{dt} &= \mu_M[G] + \mu_M^A(1-[G]) - \delta_M[M], \\
\frac{d[A]}{dt} &= \mu_A[M] - \delta_A[A] - \gamma_{AB}[A][B] \\
&\quad + \lambda_{AB}[AB] + \omega(1-[G]) - \alpha[G][A], \\
\frac{d[B]}{dt} &= \mu_B - \delta_B[B] - \gamma_{AB}[A][B] + \lambda_{AB}[AB], \\
\frac{d[AB]}{dt} &= \gamma_{AB}[A][B] - \lambda_{AB}[AB] - \delta_{AB}[AB], \qquad (1)
\end{aligned}$$

where $[A]$, $[B]$, $[AB]$, and $[M]$ are the concentrations of $A$, $B$, $AB$, and the mRNA produced by the gene $G_A$, respectively (since $G_B$ is unregulated, the concentration of its mRNA is not a variable). The first equation in (1) describes the dynamics of gene $G_A$ activity, which is a continuous variable $0 \le [G] \le 1$, with $[G] = 0$ (respectively, $[G] = 1$) when the gene is permanently protein-bound and repressed (respectively, unbound and active) [4,18]. Such an average activity appears naturally in rate equations derived from a moment expansion of the chemical master equation [19]. It takes into account that, due to transcriptional bursting [20–24], gene activity is out of equilibrium and lags variations in TF concentration. The equation used here is valid only when the gene response is not too slow compared to mRNA and protein lifetimes [19]; thus, the predictions of our deterministic approach will be carefully checked with stochastic simulations of the HAL.

To explore the dynamics of the HAL, the rate constant values were randomly sampled in typical biological ranges obtained from the literature [25–29], as shown in Table I. Robust pulses were found in a significant domain of parameter space (Supplemental Fig. 2 [17]). As a general rule, pulses are observed if $\gamma_{AB}$ is large while $\lambda_{AB}$ is small, so that the complex is irreversibly formed (large or small meaning close to the upper or lower bound, respectively, in Table I). Also, the protein production rates $\mu_A$ and $\mu_B$ need to be sufficiently large and to verify $\mu_B \lesssim \mu_A\mu_M/\delta_M$. The latter condition expresses that the productions of $A$ and $B$ should be balanced, with $A$ synthesized faster than $B$ for a fully active gene ($[G] = 1$, with mRNA concentration $[M] = \mu_M/\delta_M$) and more slowly for an inactive gene. The average period was $T_{\mathrm{tot}} \approx 100$ min.

Figure 2 shows a typical pulsating solution of (1). The mutual "sequestration" of $A$ and $B$ induced by the dimerization leads to an alternation of pulses where either $A$ or $B$ is predominant (referred to as the $A$ and $B$ phase, respectively), the other protein remaining at low levels. Inside each pulse, the dominant protein first accumulates as it is synthesized faster than the other, while complexation removes the two proteins in equal quantities. Then, it decreases to almost zero when the situation is reversed. During the $B$ phase, the gene is unrepressed, and the $A$ synthesis rate increases as mRNA builds up. During the $A$ phase, the gene is repressed and the $A$ synthesis rate decreases as mRNA is degraded. The key for cycling is thus that, during each phase, there is a time where $A$ and $B$ synthesis rates become equal, which is at the peak of the pulse.

Thus, the mRNA lifetime plays the role of a time delay, a crucial ingredient for oscillations [30]. The sequestration of the TF $A$ also plays an important role by inducing an ultrasensitive response in gene activity [31], a strong nonlinear effect [32] which favors oscillations like a high transcriptional cooperativity would do. This ultrasensitivity is presumably also important in other gene circuits where sequestration induces oscillations [4].

TABLE I. Typical biological ranges for rate constants in the model, as obtained from the literature. The last three parameters are guessed. $\delta$'s and $\mu$'s are the degradation and synthesis rates, respectively. $\gamma_{AB}$ and $\lambda_{AB}$ are the association and dissociation constants, respectively, of the $AB$ dimer. $\alpha$ and $\omega$ are the binding and unbinding rates, respectively, of the protein $A$ to the gene. The ratio $[A]_0 \equiv \omega/\alpha$ defines a regulation threshold: For $[A] \gg [A]_0$ the promotor region has a protein bound to it, while for $[A] \ll [A]_0$ the promotor is free. The system is considered to be enclosed in a cell of volume $V = 50\ \mu\mathrm{m}^3$. We take this as the volume unit. The concentration $[X]$ of a species $X$ then corresponds to the number of molecules $X$ in $V$. All values are expressed in minutes, except for $[A]_0$, which is a dimensionless number.

| Parameter | Value | Reference |
|---|---|---|
| $1/\mu_M$ | [0.1,100] | [25] |
| $1/\delta_M$ | [3,60] | [26] |
| $1/\mu_A$ | $[10^{-4}, 10]$ | [27] |
| $1/\mu_B$ | $[10^{-3}, 100]$ [a] | [27] |
| $1/\delta_A$, $1/\delta_B$, $1/\delta_{AB}$ | [4,2000] | [28] |
| $1/\omega$ | [1,60] | [29] |
| $1/\gamma_{AB}$ | [0.02,20] | [4] [b] |
| $1/\lambda_{AB}$ | 100 | ... [c] |
| $1/\mu_M^A$ | $10^3$ | ... [d] |
| $[A]_0$ | [1,100] | ... [e] |

[a]Obtained from the value of $\mu_A$ and assuming a typical number of 10 mRNAs in the cell.
[b]Assuming that the formation of the $AB$ complex is diffusion limited and $D = 1\ \mu\mathrm{m\,s}^{-1}$.
[c]This choice implies a small dissociation rate, so that the complex is irreversibly formed.
[d]This is the transcription rate from a gene with the protein bound. For an ideal repressor $\mu_M^A = 0$, we assume here that there is a weak transcription even with the protein bound. This rate is, however, at least 10 smaller than the transcription rate from a free gene (see the value of $1/\mu_M$ above).
[e]Here it is assumed that one needs from 1 to 100 proteins in the volume at the threshold to bind to the gene.

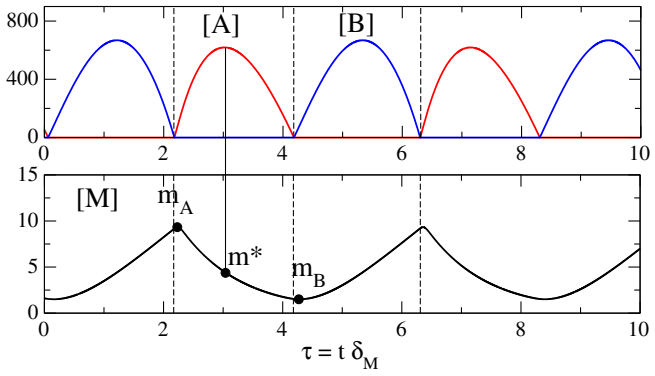


FIG. 2. Protein (top) and mRNA (bottom) concentrations vs time (in units of the characteristic mRNA degradation time $\delta_M^{-1}$) for the following parameter values: $\mu_M^{-1} = 0.5$, $\delta_M^{-1} = 20$, $\mu_A^{-1} = 0.067$, $\mu_B^{-1} = 0.015$, $\delta_A^{-1} = \delta_B^{-1} = 10^3$, $\delta_{AB}^{-1} = 10$, $\gamma_{AB}^{-1} = 0.02$, $\omega^{-1} = 100$, and $[A]_0 = 1$ ($\lambda_{AB}$ and $\mu_M^A$ are fixed as in Table I). $A$ (respectively, $B$) concentration is plotted in red (respectively, blue). Dashed lines indicate the beginning of the $A$ and $B$ phases. During the $A$ phase, the mRNA concentration decays as the $A$ protein strongly represses its own gene.

To get an estimate of the pulse period, we make some simplifications. We assume perfect repression ($\mu_M^A = 0$) and irreversible complex formation ($\lambda_{AB} = 0$). With the latter assumption, we do not need to track dimer $AB$, leading from Eqs. (1) to a system of four differential equations only. Considering that proteins dimerize before they degrade, we set $\delta_A = \delta_B = 0$. We neglect the variation of $[A]$ due to the binding or unbinding of one molecule, which removes the terms involving $[G]$ in the equation for $d[A]/dt$ in (1). Rescaling the time as $\tau \equiv t\delta_M$ and the concentrations as $a \equiv [A]\gamma_{AB}/\delta_M$, $b \equiv [B]\gamma_{AB}/\delta_M$, $m \equiv [m]\delta_M/\mu_M$, and $g = [G]$, one gets

$$\begin{aligned} \frac{dg}{d\tau} &= \Omega(1-g) - \sigma g a, \\ \frac{dm}{d\tau} &= g - m, \\ \frac{da}{d\tau} &= k_a m - ab, \\ \frac{db}{d\tau} &= k_b - ab, \end{aligned} \tag{2}$$

where the rescaled parameters are $\Omega \equiv \omega/\delta_M$, $\sigma \equiv \alpha/\gamma_{AB}$, $k_a \equiv \mu_A\mu_M\gamma_{AB}/\delta_M^3$, and $k_b \equiv \mu_B\gamma_{AB}/\delta_M^2$. There is no protein degradation in (2), but the irreversible complexation $A + B \to AB$ prevents unbounded growth.

Assuming total repression in the $A$ phase ($g = 0$) and slow unbinding of $A$ from the gene in the $B$ phase (small $\Omega$), we get the following two equations for $T_a$ and $T_b$, the durations of the $A$ and $B$ phase, respectively [17]:

$$\frac{T_a}{e^{T_a} - 1} = \beta \frac{-1 + T_b + e^{-T_b}}{e^{T_a} - e^{-T_b}}, \tag{3a}$$

$$\frac{T_a}{e^{T_a} - 1} = \frac{\beta(T_b - \frac{T_b^2}{2}) + T_b}{1 - e^{-T_b}} - \beta, \tag{3b}$$

which depend on a single parameter:

$$\beta \equiv \frac{k_a\Omega}{k_b} = \frac{\omega}{\delta_M}\frac{(\mu_A\mu_M)/\delta_M}{\mu_B}, \tag{4}$$

which is the ratio of the mRNA lifetime to the gene response time, multiplied by the ratio of the maximal $A$ synthesis rate to the $B$ synthesis rate.

Figure 3 displays $T_a$, $T_b$, and the total period $T_{\rm tot} = T_a + T_b$, obtained by numerically solving Eqs. (3). Remarkably, $T_a$ depends little on $\beta$, varying by about 30% ($1.67 \le T_a \le 2.13$) when $\beta$ changes over 3 orders of magnitude ($10^{-1} \le \beta \le 10^2$). On the contrary, $T_b$ is very sensitive to $\beta$ and ranges over 2 orders of magnitude. The pulses of $A$ are "robust", i.e., of almost constant duration, while the duration of the $B$ pulses can be tuned by changing $\beta$. Hence, any parameter which $\beta$ depends on [see Eq. (4)] can be used to regulate the separation between the pulses of $A$.

A detailed analysis of Eqs. (3) is presented in Supplemental Material [17]. Here we give simple arguments explaining the main features observed. During the $A$ phase, $m(\tau)$ decays exponentially [set $g = 0$ in Eqs. (2)]. Denoting by $m_A$ and $m_B$ the mRNA concentrations at the beginnings of the $A$ and $B$ phases, respectively (Fig. 2), we have $m_B = m_A e^{-T_a}$. To get pulses, $A$ synthesis must be faster than $B$ synthesis when the $A$ phase starts ($k_a m_A > k_b$) and slower when the $B$ phase starts ($k_a m_B < k_b$), which yields $m_B < k_b/k_a < m_A$. Assuming stationarity of the $B$ protein ($db/d\tau \sim 0$) in the $A$ phase, we get

$$\frac{da}{d\tau} = k_a m(\tau) - k_b = k_a m_A e^{-\tau} - k_b. \tag{5}$$

The solution of (5) is a pulse with a peak ($da/d\tau = 0$) at mRNA concentration $m^* = k_b/k_a$ (Fig. 2). The pulse duration $T_a$ is found by setting $a(T_a) = 0$:

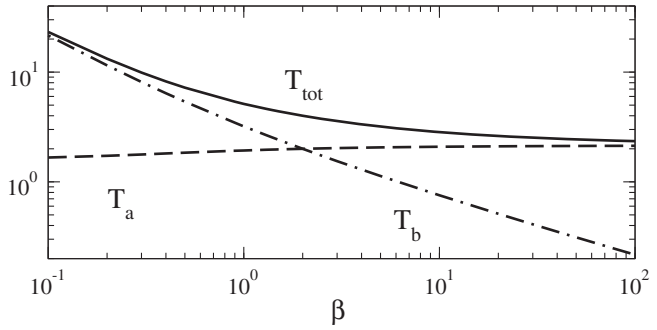


FIG. 3. Log-log plot of $T_a$ (dashed line) and $T_b$ (dot-dashed line), the solutions of Eqs. (3) as a function of $\beta$. The total period $T_{\rm tot} = T_a + T_b$ is shown as a solid line.

$$\frac{T_a}{1-e^{-T_a}} = \frac{k_a m_A}{k_b}. \quad (6)$$

Hence, $T_a$ depends only on the ratio $k_a m_A/k_b$. Since pulses require $k_a m_A/k_b > 1$, $T_a$ cannot become too small. Equation (6) might suggest that large values of $k_a/k_b$ lead to arbitrarily large $T_a$. However, this is not true, because the $B$ phase shrinks as $k_a/k_b$ gets larger, since $B$ synthesis is then faster than $A$ synthesis only for a short time. Hence, the variations of $m$ during the $B$ phase become smaller and smaller as $k_a/k_b$ increases, since the mRNA characteristic time is 1. Consequently, $m_B/m_A = e^{-T_a}$ remains close to 1, thus bounding $T_a$. In simple words, changes in the rate constants which could affect $T_a$ are compensated by an associated change in the mRNA maximum concentration $m_A$. Thus, there is a natural negative feedback loop stabilizing the $A$-pulse duration.

To corroborate these results, based on the reduced model (2) and further approximations, we numerically computed $T_a$ and $T_b$ using the full equations (1) for parameter sets $\{k_i\}$ centered around the set $\{k_i^0\}$ used in Fig. 2. Each $k_i$ was selected randomly and uniformly on a logarithmic scale in the interval $[\frac{1}{2}k_i^0, 2k_i^0]$. In total, $10^3$ sets were generated, of which 98% had a pulsating output, showing that the parameter set in Fig. 2 is well inside the pulsating domain in parameter space. Although the data span a wide range of values of $\beta$, the computed values of $T_a$, $T_b$, and $T_{\rm tot}$ are in close agreement with the analytical approximation (Fig. 4).

A legitimate question is then whether our findings still hold true when the stochastic nature of biochemical networks cannot be ignored, especially since a slow promoter dynamics may be needed to obtain long intervals between $A$ pulses. We therefore carried out stochastic simulations of the reaction network of Fig. 1, using the Gillespie algorithm [33]. Pulses are observed for both high and low values of $\beta$, with a stable time interval between $A$ pulses (Fig. 5 and Ref. [17]), which confirms the relevance of our analysis.

In summary, we have investigated the dynamics of the HAL, a pulse generator based on the competing effects of self-repression and complexation. Self-repression alone does not typically induce oscillations, unless time delays [34] or strong nonlinearities are introduced. Protein complexation generates an effective ultrasensitive response [31] which can induce oscillations as in other examples [35], including the mixed-feedback loop [4] or the monomer-dimer oscillator [16]. Since the only role of $B$ is to sequester $A$, $B$ does not need to be a protein but could be any inhibitor molecule binding to $A$ to block its transcriptional activity.

A striking feature of the HAL is that the duration of the $A$ pulses is robust against variation of the rate constants, whereas the duration of the $B$ pulses is tunable. It has been suggested that biological signals may be encoded in the time interval between pulses [7–11]. Since the HAL is a robust and flexible pulse generator, it would perfectly fit into this design.

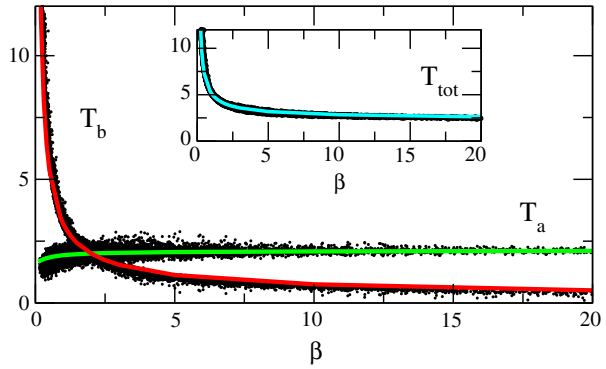


FIG. 4. Solid lines: Analytical estimates of $T_a$ and $T_b$ from Eqs. (3). Circles: Durations of the $A$ and $B$ phases as computed from the numerical integration of (1). Inset: Comparison for the total period $T_{\rm tot} = T_a + T_b$.

The self-repression motif is highly represented in genetic networks [3]. It would be interesting to see if the HAL, a simple extension of this motif, is also ubiquitous. Known examples of oscillations based on a self-repressing protein $A$ have been attributed to delay or high cooperativity, perhaps sometimes obscuring the implication of a binding partner $B$. A closely related oscillator is the mixed-feedback loop (MFL) [4], which is also based on an $AB$ dimer formation, but the protein $A$ activates the transcription of gene $G_B$ instead of repressing itself. Interestingly, an analysis of *E. coli* motifs involving both transcriptional and protein-protein interactions led to the discovery of the MFL but, since it excluded self-repression, was not able to detect the HAL [36]. The MFL network motif is over-represented in yeast cells [36] and is also at the core of circadian clocks in mammals, neurospora, or drosophila [4]. It is natural to expect that the HAL, being closely

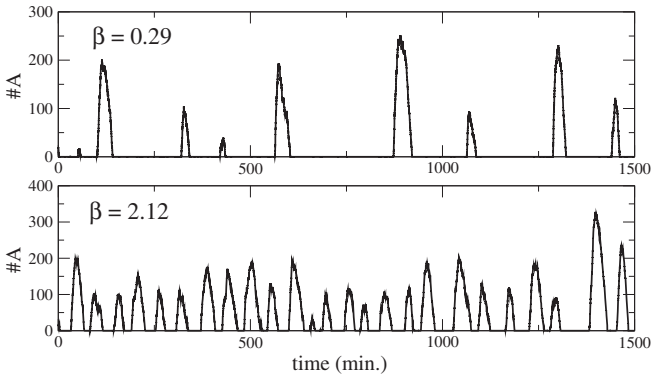


FIG. 5. Stochastic simulations of the HAL for low and high values of $\beta$, corresponding to short and long time intervals between $A$ pulses, respectively. Parameters of the top graph: $\mu_M^{-1} = 1.11$, $\delta_M^{-1} = 16.67$, $\mu_A^{-1} = 0.59$, $\mu_B^{-1} = 0.05$, $\delta_A^{-1} = \delta_B^{-1} = 10^3$, $\delta_{AB}^{-1} = 10$, $\gamma_{AB}^{-1} = 0.02$, $\omega^{-1} = 73.11$, and $[A]_0 = 1$. For the bottom graph, the parameters are the same except $\omega^{-1} = 10$.

related to the MFL, is also the core component of some natural biochemical oscillators. Its simplicity and interesting dynamical properties also make the HAL a promising module for synthetic biology.

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[1] B. Alberts, A. Johnson, J. Lewis, K. Roberts, and P. Walter, *Molecular Biology of the Cell* (Garland Science, New York, 2002).
[2] J. J. Tyson, K. C. Chen, and B. Novak, Curr. Opin. Cell Biol. **15**, 221 (2003).
[3] U. Alon, *An Introduction to Systems Biology: Design Principles of Biological Circuits* (Chapman and Hall, London, 2006).
[4] P. Francois and V. Hakim, Phys. Rev. E **72**, 031908 (2005).
[5] S. Krishna, M. H. Jensen, and K. Sneppen, Proc. Natl. Acad. Sci. U.S.A. **103**, 10840 (2006).
[6] W. Mather, M. R. Bennett, J. Hasty, and L. S. Tsimring, Phys. Rev. Lett. **102**, 068105 (2009).
[7] B. Mengel, A. Hunziker, L. Pedersen, A. Trusina, M. H. Jensen, and S. Krishna, Curr. Opin. Genet. Dev. **20**, 656 (2010).
[8] J. E. Purvis and G. Lahav, Cell **152**, 945 (2013).
[9] G. Lahav, Advances in Experimental Medicine and Biology **641**, 28 (2008).
[10] \N. Hao and E. K. O'Shea, Nat. Struct. Mol. Biol. **19**, 31 (2012).
[11] J. Locke, J. Young, M. Fontes, M. Hernàndez, and M. Elowitz, Science **334**, 366 (2011).
[12] R. Hermsen, B. Ursem, and P. R. ten Wolde, PLoS Comput. Biol. **6**, e1000813 (2010).
[13] H. Salgado, A. Santos-Zavaleta, S. Gama-Castro, D. Millan-Zarate, E. Diaz-Peredo, F. Sanchez-Solano, E. Perez-Rueda, C. Bonavides-Martinez, and J. Collado-Vides, Nucleic Acids Res. **29**, 72 (2001).
[14] I. M. Keseler, J. Collado-Vides, S. Gama-Castro, J. Ingraham, S. Paley, I. T. Paulsen, M. Peralta-Gil, and P. D. Karp, Nucleic Acids Res. **33**, D334 (2005).
[15] D. Szklarczyk, A. Franceschini, M. Kuhn, M. Simonovic, A. Roth, P. Minguez, T. Doerks, M. Stark, J. Muller, P. Bork, L. J. Jensen, and C. v. Mering, Nucleic Acids Res. **39**, D561 (2011).
[16] M. van Dorp, B. Lannoo, and E. Carlon, Phys. Rev. E **88**, 012722 (2013).
[17] See Supplemental Material at http://link.aps.org/supplemental/10.1103/PhysRevLett.117.018102 for additional details on the model, its phase behavior, for the analytical calculations of $T_a$ and $T_b$ and for further discussion of robustness and stochastic effects.
[18] P. E. Morant, Q. Thommen, F. Lemaire, C. Vandermoëre, B. Parent, and M. Lefranc, Phys. Rev. Lett. **102**, 068104 (2009).
[19] J. Wang, M. Lefranc, and Q. Thommen, Biophys. J. **107**, 2403 (2014).
[20] I. Golding, J. Paulsson, S. M. Zawilski, and E. C. Cox, Cell **123**, 1025 (2005).
[21] X. Darzacq, Y. Shav-Tal, V. de Turris, Y. Brody, S. M. Shenoy, R. D. Phair, and R. H. Singer, Nat. Struct. Mol. Biol. **14**, 796 (2007).
[22] J. R. Chubb, T. Trcek, S. M. Shenoy, and R. H. Singer, Curr. Biol. **16**, 1018 (2006).
[23] D. M. Suter, N. Molina, D. Gatfield, K. Schneider, U. Schibler, and F. Naef, Science **332**, 472 (2011).
[24] C. V. Harper, B. Finkenstädt, D. J. Woodcock, S. Friedrichsen, S. Semprini, L. Ashall, D. G. Spiller, J. J. Mullins, D. A. Rand, J. R. E. Davis, and M. R. H. White, PLoS Biol. **9**, e1000607 (2011).
[25] V. Pelechano, S. Chávez, and J. E. Pérez-Ortín, PLoS One **5**, e15442 (2010).
[26] Y. Wang, C. L. Liu, J. D. Storey, R. J. Tibshirani, D. Herschlag, and P. O. Brown, Proc. Natl. Acad. Sci. U.S.A. **99**, 5860 (2002).
[27] B. Schwanhäusser, D. Busse, N. Li, G. Dittmar, J. Schuchhardt, J. Wolf, W. Chen, and M. Selbach, Nature (London) **473**, 337 (2011).
[28] A. Belle, A. Tanay, L. Bitincka, R. Shamir, and E. K. O'Shea, Proc. Natl. Acad. Sci. U.S.A. **103**, 13004 (2006).
[29] K. Poorey, R. Viswanathan, M. N. Carver, T. S. Karpova, S. M. Cirimotich, J. G. McNally, S. Bekiranov, and D. T. Auble, Science **342**, 369 (2013).
[30] B. Novák and J. J. Tyson, Nat. Rev. Mol. Cell Biol. **9**, 981 (2008).
[31] N. E. Buchler and F. R. Cross, Mol. Syst. Biol. **5**, 272 (2009).
[32] A. Goldebeter and D. Koshland, Proc. Natl. Acad. Sci. U.S.A. **78**, 6840 (1981).
[33] D. T. Gillespie, J. Phys. Chem. **81**, 2340 (1977).
[34] J. Stricker, S. Cookson, M. R. Bennett, W. H. Mather, L. S. Tsimring, and J. Hasty, Nature (London) **456**, 516 (2008).
[35] P. Francois and V. Hakim, Proc. Natl. Acad. Sci. U.S.A. **101**, 580 (2004).
[36] E. Yeger-Lotem and H. Margalit, Nucleic Acids Res. **31**, 6053 (2003).