

Core genetic module: The mixed feedback loop

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The so-called mixed feedback loop (MFL) is a small two-gene network where protein A regulates the transcription of protein B and the two proteins form a heterodimer. It has been found to be statistically over-represented in statistical analyses of gene and protein interaction databases and to lie at the core of several computer-generated genetic networks. Here, we propose and mathematically study a model of the MFL and show that, by itself, it can serve both as a bistable switch and as a clock (an oscillator) depending on kinetic parameters. The MFL phase diagram as well as a detailed description of the nonlinear oscillation regime are presented and some biological examples are discussed. The results emphasize the role of protein interactions in the function of genetic modules and the usefulness of modeling RNA dynamics explicitly.

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I. INTRODUCTION

Biological cells rely on complex networks of biochemical interactions. Large scale statistical analyses have revealed that the transcriptional regulation networks and the networks of protein-protein interaction for different organisms are far from random and contain significantly recurring patterns [1] or motifs. Mathematical modeling is useful to determine if these motifs can by themselves fulfill useful functions and it has, for instance, helped to show that the common “feed forward loop” motif in transcriptional regulation [1] can act as a persistence detector [2]. More recently, a combined analysis of protein-protein interactions and transcriptional networks in yeast (*Saccharomyces cerevisiae*) has pointed out several motifs of mixed interactions [3,4]. The simplest such motif composed of both transcriptional and protein-protein interactions is the two-protein mixed feedback loop (MFL) depicted in Fig. 1. It is composed of a transcription factor A , produced from gene g_a , and of another protein B , produced from gene g_b . A regulates the transcription of gene g_b and also directly interacts with protein B . The MFL has independently been obtained as the core motif of several networks produced in a computer evolutionary search aiming at designing small functional genetic modules performing specific functions [5]. Here, to better understand the possible functions of this basic module, we propose a model of the MFL based on the simplest biochemical interactions. This mathematical model is described in Sec. II. This is used to show that there exist wide ranges of kinetic parameters where the MFL behaves either as a bistable switch or as an oscillator. For the convenience of the reader, an overview of the different dynamical regimes is provided in Sec. III. They are delimited in parameter space and their main characteristics are summarized. We then give detailed descriptions of the bistable regime in Sec. IV and of the nonlinear oscillations in Sec. V. A comparison is also made with a simple autoinhibitory gene model with delay. In the concluding section, the important role played by protein dimerization and

the usefulness of explicitly modeling mRNA dynamics are underlined and biological examples of the two proposed functions of the MFL are discussed.

II. A MATHEMATICAL MODEL OF THE MFL

A. Model formulation

As previously described, the MFL consists of two proteins A and B and their genes g_a and g_b , such that A regulates the transcription of gene g_b and also directly interacts with B . Our aim is to analyze the dynamics of this small genetic module and see what can be achieved in the simplest setting. Therefore, different cellular compartments and separate concentrations for the nucleus and cytoplasm are not considered and biochemical reactions are modeled by simple rate equations.

The proposed MFL model is represented schematically in Fig. 2 and consists of four equations that are described and explained below. The concentration of a chemical species X is denoted by square brackets and the cell volume is taken as volume unit. So, $[X]$ represents the effective number of X molecules present in the cell.

The first two equations model the transcriptional regulation of gene g_b by protein A

$$\frac{d[g_b]}{dt} = \theta[g_b:A] - \alpha[g_b][A], \quad (1)$$

$$\frac{d[r_b]}{dt} = \rho_f[g_b] + \rho_b[g_b:A] - \delta_r[r_b], \quad (2)$$

where it is assumed that gene g_b exists under two forms, with A bound to its promoter with probability $[g_b:A]$ and without



FIG. 1. Schematic representation of the over-represented MFL motif [4]. The bold arrow represents a transcriptional regulation interaction and the dashed double arrow represents a protein-protein interaction.

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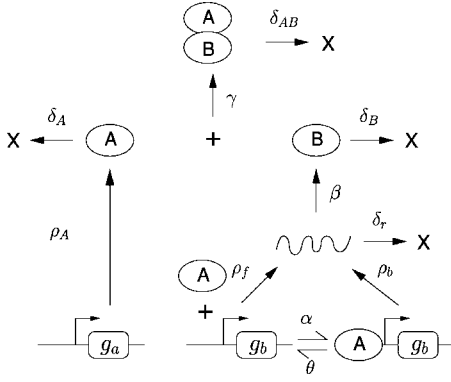


FIG. 2. The proposed model of the MFL motif. The Greek letters denote the model's different kinetic constants. The large crosses symbolize the degradation of the corresponding species.

A with probability $[g_b]$. Since $[g_b] + [g_b:A] = 1$, the single Eq. (1) is sufficient to describe the transition between the two forms. Specifically, A proteins bind to the g_b promoter at a rate α and when bound they are released at a rate θ . The regulation of transcription of gene g_b by protein A is described by Eq. (2) where r_b stands for g_b transcripts. When A is bound to the g_b promoter, the transcription is initiated at a rate ρ_b and, otherwise, it is initiated at a rate ρ_f . Thus, $\rho_b > \rho_f$ corresponds to transcriptional activation by A and $\rho_b < \rho_f$ to transcriptional repression. A first-order degradation for g_b mRNA at a constant rate δ_r , has also been assumed. As given, the description is strictly valid for a single copy gene. However, it also applies for a gene with g_0 copy (e.g., $g_0 = 2$) provided that ρ_f and ρ_b are understood to be g_0 -fold greater than the corresponding elementary rates.

The production of the A and B proteins and their complexation are described by the following two equations that complete our description of the MFL module:

$$\frac{d[A]}{dt} = \rho_A - \gamma[B][A] - \delta_A[A] + \theta[g_b:A] - \alpha[g_b][A], \quad (3)$$

$$\frac{d[B]}{dt} = \beta[r_b] - \gamma[B][A] - \delta_B[B], \quad (4)$$

where $[A]$ and $[B]$, respectively, denote the concentration of proteins A and B . Since regulation of gene g_a is not considered, separate descriptions of its transcription and translation are not needed and it is simply assumed in Eq. (3) that protein A is produced at a given basal rate ρ_A . The second crucial interaction of the MFL, the direct interaction between protein A and B is taken into account by assuming that A and B associate at a rate γ . It is assumed that B does not interact with a protein A that is bound to the g_b promoter. In addition, Eq. (3) assumes a first-order degradation of protein A at a constant rate δ_A , and its last two terms come from the (small) contribution to the concentration of A in solution of the binding (unbinding) of A to (from) the g_b promoter. Equation (4) assumes that B proteins are produced from the transcripts of gene g_b at a rate β and are degraded at a rate δ_B in addition to their association with A proteins.

As described by Eqs. (3) and (4), the complexation between A and B proteins proceeds at a rate γ . For simplicity, we suppose that the AB complex does not interact with genes g_a and g_b , we neglect its dissociation [6] and simply assume that it is fully degraded at a rate δ_{AB} after its formation,

$$\frac{d[AB]}{dt} = \gamma[B][A] - \delta_{AB}[AB]. \quad (5)$$

Since the complex AB does not feed back on the dynamics of the other species, its concentration does not need to be monitored and Eq. (5) is not explicitly considered in the following.

B. Values of kinetic parameters and a small dimensionless parameter

Even in this simple model, ten kinetic constants should be specified. It is useful to consider the possible range of their values both to assess the biological relevance of the different dynamical regimes and to orient the model analysis.

Half-lives of mRNA range from a few minutes to several hours and are peaked around 20 min in yeast [7]. Therefore, $\delta_r = 0.03 \text{ min}^{-1}$ can be taken as a typical value.

For the transcription factor-gene promoter interaction, typical values appear to be a critical concentration $\theta/\alpha = [A]_0$ in the nanomolar range, a bound state lifetime of several minutes and activated transcription rates of a few mRNAs per minute. Therefore, we assume $\alpha = \theta/40$, that θ is of the same order as δ_r and ρ_f and ρ_b range from 0.1 min^{-1} to 10 min^{-1} .

Protein half-lives vary from a few minutes to several days [8]. The hour appears as a typical value. We choose $\delta_A = \delta_B = 0.01 \text{ min}^{-1}$ and more generally consider that the A and B protein half-lives are of the same order or longer than that of g_b mRNA. For protein production, $\beta = 3$ protein molecules per mRNA molecule per minute appears a plausible value for an eucaryotic cell [9]. This gives $\rho_A \approx 100 \text{ min}^{-1}$ when combined with the previous values for mRNA production.

We assume that the protein interaction is essentially limited by diffusion. A diffusion constant $D \approx 2.5 \mu\text{m}^2 \text{ s}^{-1}$ [10] gives a time s^2/D of about a minute for diffusion across a cell of a size $s = 10 \mu\text{m}$. Therefore, we choose $\gamma \approx 1 \text{ min}^{-1}$.

It is convenient to introduce dimensionless variables [Eqs. (1)–(4)] to decrease as far as possible the number of independent parameters.

We first normalize the g_b mRNA concentration by the concentration that gives a production of B protein equal to that of A . Thus, we define the dimensionless concentration $r = \beta[r_b]/\rho_A$. We normalize the protein concentrations by the equilibrium concentration resulting from production at a rate ρ_A and dimerization at a rate γ and define $A = \sqrt{\gamma/\rho_A}[A]$, $B = \sqrt{\gamma/\rho_A}[B]$. We also take as the time unit the inverse of g_b mRNA degradation rate $1/\delta_r$. With these substitutions, Eqs. (1)–(4) read,

$$\frac{dg}{dt} = \tilde{\theta} \left[(1-g) - g \frac{A}{A_0} \right], \quad (6)$$

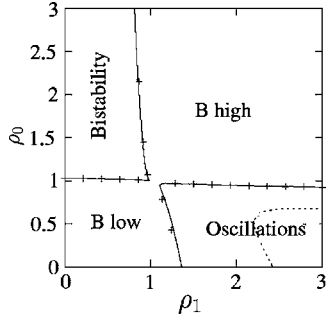


FIG. 3. Different dynamical regimes of the MFL as ρ_0 and ρ_1 are varied. The borders between different regimes are shown as computed from numerical solutions of Eqs. (18) and (31) for $\tilde{\theta} = 1.33$ (full lines) or $\tilde{\theta} = 26.6$ (dashed lines) as well as given by the asymptotics expansions [Eqs. (28), (30), (36), and (41)] for $\tilde{\theta} = 1.33$ (+ symbols). Here, and in all the following figures, except when explicitly specified, the other parameters are $[A]_0 = 40$ mol, $\beta = 3$ min $^{-1}$, $\delta_r = 0.03$ min $^{-1}$, $\delta_A = 0.01$ min $^{-1}$, $\delta_B = 0.01$ min $^{-1}$, $\rho_A = 100$ mol min $^{-1}$, $\gamma = 1$ mol $^{-1}$ min $^{-1}$ where mol stands for molecules and min for minutes. The corresponding dimensionless parameters are $\delta = 0.003$, $d_a = d_b = 0.33$, $A_0 = 4$, $\mu = 0.31$.

$$\frac{dr}{dt} = \rho_0 g + \rho_1 (1 - g) - r, \quad (7)$$

$$\frac{dB}{dt} = \frac{1}{\delta} (r - AB) - d_b B, \quad (8)$$

$$\frac{dA}{dt} = \frac{1}{\delta} (1 - AB) + \mu \tilde{\theta} \left[(1 - g) - g \frac{A}{A_0} \right] - d_a A, \quad (9)$$

where we have defined the following rescaled parameters $\delta = \delta_r / \sqrt{\rho_A \gamma}$, $\rho_1 = \beta \rho_b / (\rho_A \delta_r)$, $\rho_0 = \beta \rho_f / (\rho_A \delta_r)$, $\tilde{\theta} = \theta / \delta_r$, $\mu = \sqrt{\gamma} / \rho_A$, $d_a = \delta_A / \delta_r$ and $d_b = \delta_B / \delta_r$. We have also introduced the dimensionless critical binding concentration $A_0 = \sqrt{\gamma} / \rho_A \theta / \alpha$. The model still depends on seven parameters. In order to simplify its analysis, it is useful to note that δ is a small parameter (approximately equal to 3×10^{-3} with the previous estimations). The influence of three key parameters of order one in Eqs. (6)–(9) is particularly examined in the following. These are ρ_0 and ρ_1 which measure the strengths of the two possible states of B production (with or without A bound to gene g_b) as compared to that of A , and $\tilde{\theta}$ which compares the rates of A unbinding from DNA to that of mRNA degradation (α is supposed to vary with θ to maintain a fixed critical binding concentration A_0).

III. OVERVIEW OF THE DYNAMICS IN DIFFERENT PARAMETER REGIMES

We provide here an overview of the different dynamical regimes of the MFL, which are depicted in Fig. 3, and summarize their characteristics. The behavior of the MFL depends on whether protein A is a transcriptional activator or a transcriptional repressor and on the strengths of the two transcription rates of gene g_b (i.e., with A bound or not to its

promoter). More precisely, the key parameters are the strengths of B protein production, $\beta \rho_f / \delta_r$ and $\beta \rho_b / \delta_r$, as compared to the production rate ρ_A of protein A . Therefore, it is convenient to consider the previously introduced ratios $\rho_0 = \beta \rho_f / (\delta_r \rho_A)$ and $\rho_1 = \beta \rho_b / (\delta_r \rho_A)$.

We have observed that depending on the values of ρ_0 and ρ_1 the MFL can be monostable, exhibit bistability, or display oscillations. We qualitatively describe these three different cases in the following. Their biological relevance is further discussed in Sec. VI.

A. Monostable steady states

The simplest case occurs when the production rate of A is higher or lower than the production rate of B , irrespective of the state of the g_b promoter. That is when both $\beta \rho_f / \delta_r$ and $\beta \rho_b / \delta_r$ are either higher or lower than ρ_A . In this case, the MFL has a single stable state to which it relaxes starting from any initial conditions.

When both B production rates are higher than the production rate of A (i.e., $\rho_0 > 1$ and $\rho_1 > 1$), A proteins are quickly complexed by B and are unable to interact with the g_b promoter. The concentration $[A]$ of uncomplexed A proteins is, therefore, low and results from a simple balance between production and complexation. The high concentration of uncomplexed B proteins is the effective result from transcription at the free g_b promoter rate, complexation, and degradation,

$$[B] \approx \frac{1}{\delta_B} \left(\beta \frac{\rho_f}{\delta_r} - \rho_A \right), \quad [A] \approx \frac{\rho_A}{\gamma [B]}. \quad (10)$$

An equally simple but opposite result holds when both B production rates are smaller than the production rate of A ($\rho_0 < 1$ and $\rho_1 < 1$). Then, the concentration of uncomplexed A is high, the g_b promoter is occupied by A , and a low B concentration results from a balance between complexation and degradation,

$$[A] \approx \frac{1}{\delta_A} \left(\rho_A - \beta \frac{\rho_b}{\delta_r} \right), \quad [B] \approx \frac{\beta \rho_b}{\delta_r [A] \gamma}. \quad (11)$$

The dynamics of the MFL is richer when the production rate of A is intermediate between the two possible production rates of B , $\beta \rho_f / \delta_r$ and $\beta \rho_b / \delta_r$, i.e., when either $\rho_0 > 1 > \rho_1$ or $\rho_1 > 1 > \rho_0$. We consider these two cases in turn.

B. Transcriptional repression and bistability

We first discuss the case when A is a transcriptional repressor, $\rho_0 > 1 > \rho_1$. Then, two stable steady states can coexist. Let us suppose first that no A is bound to the g_b promoter. Then the production rate of B is larger than the production rate of A , and all produced A proteins are quickly complexed. This stably prevents the binding of A proteins to the g_b promoter and maintain a steady state with low A and high B concentrations approximately equal to

$$[B]_1 \approx \left(\beta \frac{\rho_f}{\delta_r} - \rho_A \right) \frac{1}{\delta_B}, \quad [A]_1 \approx \frac{\rho_A}{\gamma [B]_1}. \quad (12)$$

The second opposite possibility is that A is sufficiently abundant to repress the transcription of gene g_b . Then, since the

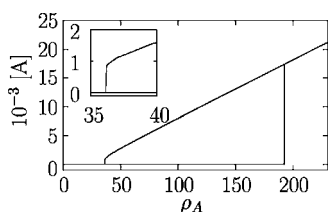


FIG. 4. In the bistable regime, a graded increase of ρ_A , the production of A , results in a jump in A concentration. The parameters are $\rho_f=0.2 \text{ mol min}^{-1}$, $\rho_b=2 \text{ mol min}^{-1}$, and $\theta=0.04 \text{ min}^{-1}$. The other parameters are as in Fig. 3.

production rate of A has been supposed to be higher than the production rate of B in the repressed state, B proteins are quickly complexed but uncomplexed A proteins are present to maintain the repression of the gene g_b transcription. This gives rise to a second stable state with high A and low B concentrations approximately equal to

$$[A]_2 \approx \left(\rho_A - \beta \frac{\rho_b}{\delta_r} \right) \frac{1}{\delta_A}, \quad [B]_2 \approx \frac{\beta \rho_b}{\gamma [A]_2 \delta_r}. \quad (13)$$

The bistability domain is only exactly given by the simple inequalities $\rho_0 > 1$ and $\rho_1 < 1$ when the ratio δ of g_b mRNA degradation rate over the effective protein dynamics rate is vanishingly small. As shown in Sec. IV and in Fig. 3, for small values of δ , it is more accurately given by

$$\begin{aligned} \rho_0 &> 1 + 2\sqrt{(1 - \rho_1)\delta_B/A_0}, & \rho_1 &\leq 1, \\ \rho_1 &< 1 - 2\sqrt{(\rho_0 - 1)A_0\delta_A}, & \rho_0 &\geq 1. \end{aligned} \quad (14)$$

In this intermediate range of production of A proteins, the network reaches one or the other fixed points, depending on its initial condition. The existence of this bistability domain can serve to convert a graded increase in A production in a much more abrupt switchlike response in A (and B) concentration as shown in Fig. 4. The usefulness of this general

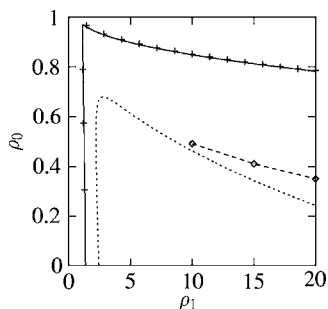


FIG. 5. The oscillation domain for the same parameters as in Fig. 3 but for a larger domain of ρ_1 , the ratio of activated production of B to that of A . As in Fig. 3, the short-dashed line marks the boundary of the domain where the steady state is unstable to oscillations for $\tilde{\theta}=26.6$. Numerical simulations (diamonds) show that the oscillating regime is stable up to the long-dashed line. Therefore, both the limit cycle and the fixed point are stable attractors for $\tilde{\theta}=26.6$ in the region between the short-dashed and long-dashed lines.

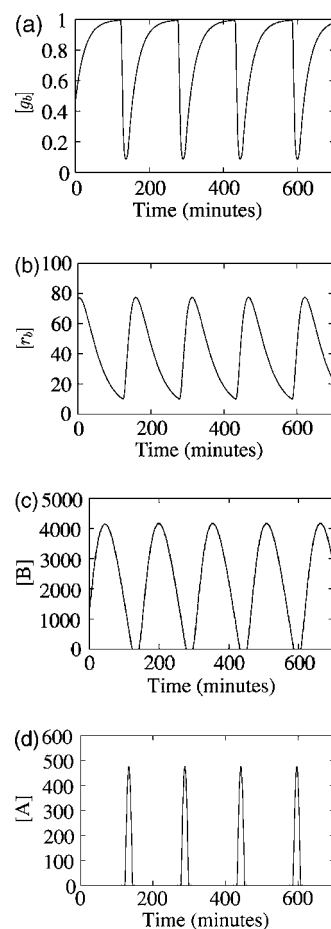


FIG. 6. MFL in the oscillatory regime. The concentrations of the different species are shown as a function of time and display sustained oscillations. Constants are the same as in Fig. 4 except that $\rho_A=100 \text{ mol min}^{-1}$, $\rho_f=0.1 \text{ mol min}^{-1}$, and $\rho_b=5 \text{ mol min}^{-1}$.

feature of multistability has been recently discussed in different contexts [11,12].

C. Transcriptional activation and oscillations

When A is a transcriptional activator, the complexation of B with A acts as a negative feedback and can serve to diminish the variation in B protein concentration when A varies. This leads also to oscillations when A production lies in the intermediate range $\rho_1 > 1 > \rho_0$. This oscillatory behavior mainly depends on the ratios of protein production ρ_0 , ρ_1 and exists for a large range of DNA-protein interaction kinetics. However, a faster kinetics leads to a smaller oscillatory domain as shown in Fig. 3. Oscillations cannot be sustained when $\tilde{\theta}$ becomes large and comparable to $1/\delta$ (with A_0 fixed) or equivalently when $\tilde{\theta} \sim \delta_r/\delta$. It can also be noted that for a sufficiently large activation of transcription by A , there exists a domain of coexistence between oscillations and steady behavior as shown in Fig. 5, i.e., depending on the initial conditions, concentrations will oscillate in time or reach steady levels.

For small δ , oscillations are nonlinear for most parameters. As can be seen in Fig. 6, an oscillation cycle comprises

two phases in succession, a first phase of duration T_1 when the concentration of protein A is high followed by a second phase of duration T_2 where the concentration of B is high. A full oscillation cycle of period $T=T_1+T_2$ proceeds as follows. Let us start with low concentrations of A and B proteins at the beginning of the first phase. When no A is bound to the g_b promoter, the B production rate is lower than the A production rate and complexation cannot prevent the increase of A concentration. When the concentration of A has reached a critical level, A starts to bind to the g_b promoter and activate transcription. This results in a higher production of B than A and the diminution of free A by complexation. Since the A concentration is high, the produced B s are quickly complexed and the concentration of uncomplexed B s remain low. Eventually, the A concentration drops below the binding level and no longer activates the g_b transcription. This marks the transition between the two parts of the oscillation cycle. B continues for a while to be produced from the transcripts, and since few A s are present, this now leads to a rise of the concentration of free B s. Finally, the concentrations of B transcripts and B proteins drop and phase II of the oscillation cycle terminates. A new cycle begins with low concentrations of A and B proteins.

One remarkable feature of the nonlinear oscillations coming from the smallness of the parameter δ is that the concentrations of A and B proteins in their respective high phase depend weakly on the complex association rate γ , as long as it is not too small for the oscillation to exist, and that the period of the oscillations is strikingly insensitive to the exact value of γ . For the parameters of Fig. 6, the oscillation period only changes by about 1% when γ is reduced from 1 min^{-1} to $2 \times 10^{-2} \text{ min}^{-1}$. This remains true even if the formation of the complex AB is not irreversible as is supposed in the present model. We have checked that the case when the complex AB forms with an association rate γ_a dissociates with a rate γ_d and is degraded with a rate δ_{AB} is well described by the present model when one takes the effective rate $\gamma = \gamma_a \delta_{AB} / (\gamma_d + \delta_{AB})$ for the irreversible formation of the complex (as obtained from a quasiequilibrium assumption).

In Secs. IV and V, we provide a more detailed analysis of the MFL different dynamical regimes and derivations of the results here summarized.

IV. THE SWITCH REGIME

We first determine the possible steady states for the MFL. The free gene, mRNA, and B protein concentrations are given in a steady state as a function of the A concentration by,

$$g = \frac{A_0}{A + A_0}, \quad (15)$$

$$r = \frac{\rho_1 A + \rho_0 A_0}{A + A_0}, \quad (16)$$

$$B = \frac{\rho_1 A + \rho_0 A_0}{(A + A_0)(A + \delta d_b)}. \quad (17)$$

The concentration of A itself satisfies the following equation:

$$1 = \delta d_a A + \frac{(\rho_1 A + \rho_0 A_0) A}{(A + A_0)(A + \delta d_b)}. \quad (18)$$

For $\delta=0$, the right-hand side (rhs) goes monotonically from ρ_0 at small A to ρ_1 at large A and a solution, $A_2 = A_0(\rho_0 - 1)/(1 - \rho_1)$ exists only when $\rho_0 < 1 < \rho_1$ or $\rho_1 < 1 < \rho_0$. For small δ , two other steady states are possible. A steady state with a small concentration of A , $A_1 \approx \delta d_b / (\rho_0 - 1)$, exists when $\rho_0 > 1$. Inversely a steady state with a large concentration of A , $A_3 \approx (1 - \rho_1) / (\delta d_a)$ exists when $\rho_1 < 1$. Therefore, Eq. (18) has multiple (i.e., three) fixed points only when A is a transcriptional repressor in the region $\rho_1 < 1 < \rho_0$. This is the parameter regime, which we examine in Secs. IV A–IV C.

A. The high A state

We show that the state with a high concentration of A proteins is stable. The forms of Eqs. (8) and (9) suggest simplifying the analysis by using the fact that the protein quickly reach a quasiequilibrium state. However, both proteins cannot be in quasiequilibrium at the same time. For instance, when proteins $A \gg B$, only B is in quasiequilibrium and A scales as $1/\delta$. When the A concentration is high, in the limit of small δ , we set $a = \delta A$. Substitution in Eqs. (6) and (8) shows that g and B reach on a fast time scale their quasiequilibrium concentration

$$g \approx \delta A_0 / a, \quad (19)$$

$$B \approx \delta r / a. \quad (20)$$

Therefore, the dynamics of the MFL reduces to the following two equations in the limit $\delta \rightarrow 0$:

$$\frac{dr}{dt} = \rho_1 - r, \quad (21)$$

$$\frac{da}{dt} = 1 - r - d_a a. \quad (22)$$

Equations (21) and (22) clearly show that the high A fixed point is stable and, as found above, satisfies $r \approx \rho_1$, $B \approx g \approx 0$, $a = (1 - \rho_1) / d_a$ at the lowest order. This steady state is possible only if $\rho_1 < 1$, that is when the B production rate is not high enough to titrate A and to prevent the repression by A .

B. The high B state

The high B state can be analyzed in a very similar way in the limit of small δ . When the B concentration is high, we define $b = \delta B$. In that case, A quickly reaches its quasiequilibrium concentration,

$$A \approx \delta b, \quad (23)$$

and the MFL dynamics reduces to the following three equations:

$$\frac{dg}{dt} = \tilde{\theta}(1 - g), \quad (24)$$

$$\frac{dr}{dt} = \rho_0 g + \rho_1(1-g) - r, \quad (25)$$

$$\frac{db}{dt} = r - 1 - d_b b. \quad (26)$$

The concentrations tend toward those of the high B fixed point $g \approx 1$, $r \approx \rho_0$, $b \approx (\rho_0 - 1)/d_b A \approx 0$. This steady state exists only if $\rho_0 > 1$, when the production of B proteins is high enough to titrate A proteins and to prevent transcriptional repression by A .

C. Bifurcation between the monostable and bistable regimes

The previous analysis has shown that bistability exists in the domain $\rho_0 > 1 > \rho_1$ when $\delta \ll 1$. We analyze more precisely the nature and position of the bifurcations between the monostable and bistable regimes.

We consider first the transition between the bistable region and the monostable region with a high concentration of A (bottom-left corner in Fig. 3). It follows from Eq. (18) that the low (A_1) and middle (A_2) A concentration fixed points coalesce and disappear when $\rho_0 - 1 = O(\sqrt{\delta})$ and both A_1 and A_2 are of order $\sqrt{\delta}$. For $A \sim \sqrt{\delta}$, Eq. (18) becomes at the dominant order after expansion,

$$\rho_0 - 1 = (1 - \rho_1) \frac{A}{A_0} + \delta \frac{d_b}{A}. \quad (27)$$

For a given repression strength $\rho_1 < 1$, the rhs has a minimum for $A = \sqrt{\delta d_b A_0 / (1 - \rho_1)}$ (that is of order $\sqrt{\delta}$ as assumed) such that

$$\rho_0 = 1 + 2 \sqrt{\frac{\delta d_b (1 - \rho_1)}{A_0}} + O(\delta). \quad (28)$$

Above this value of ρ_0 , Eq. (27) has two solutions and below it has none. Therefore, Eq. (28) marks the saddle-node bifurcation line that separates the bistable domain from the monostable domain with a high A concentration fixed point. For $\delta \rightarrow 0$, the zeroth order boundary $\rho_0 = 1$ is, of course, retrieved.

The approximate expression (28) agrees well with an exact numerical determination of the bifurcation line as shown in Fig. 3.

The transition between the bistable regime and the low A concentration monostable regime (top-right corner of Fig. 3) can be similarly analyzed. The high (A_3) and middle (A_2) A concentration fixed points can coalesce when for both the concentration A is of order $1/\sqrt{\delta}$. Expansion of Eq. (18) for $A \sim 1/\sqrt{\delta}$ gives

$$1 - \rho_1 = (\rho_0 - 1) \frac{A_0}{A} + \delta d_A A. \quad (29)$$

For a given $\rho_0 > 1$, the rhs of Eq. (29) is the minimum for $A = \sqrt{A_0(\rho_0 - 1)/(\delta d_A)}$ and at this minimum, ρ_1 is equal to

$$\rho_1 = 1 - 2\sqrt{d_A A_0 \delta (\rho_0 - 1)}. \quad (30)$$

For ρ_1 smaller than this value, Eq. (29) has two roots corresponding to A_2 and A_3 . The two roots merge and disappear

on the saddle-node bifurcation line (30) that marks the boundary between the bistable domain and the low A concentration monostable regime. Comparison between the approximate expression (30) and an exact numerical determination of the bifurcation line is shown in Fig. 3.

V. THE OSCILLATOR REGIME

We consider now the case when A is a transcriptional activator, that is when $\rho_0 < \rho_1$. In this case, the MFL has a single steady state since the rhs of Eq. (18) is a monotonically increasing function. However, as we show below, this steady state is unstable in a large domain of parameters and the MFL behaves as an oscillator.

A. The linear oscillatory instability

We begin by analyzing the linear stability of the fixed point (g^* , r^* , B^* , A^*) where A^* is the single solution of Eq. (18) and g^* , r^* , and B^* are given as functions of A^* by Eqs. (15)–(17). After linearization of the MFL dynamics [Eqs. (6)–(9)] around this fixed point, the complex growth rates σ of perturbations growing (or decreasing) exponentially in time like $\exp(\sigma t)$ are found to be the roots of the following characteristic polynomial:

$$\begin{aligned} & \left[\sigma + \tilde{\theta} \left(1 + \frac{A^*}{A_0} \right) \right] [\sigma + 1] \\ & \times \left[\left(\sigma + d_a + \frac{B^*}{\delta} \right) \left(\sigma + d_b + \frac{A^*}{\delta} \right) - \frac{A^* B^*}{\delta^2} \right] \\ & = \frac{g^* \tilde{\theta}}{A_0} \left[\frac{A^*}{\delta^2} (\rho_0 - \rho_1) - \mu \sigma (\sigma + 1) \left(\sigma + d_b + \frac{A^*}{\delta} \right) \right]. \end{aligned} \quad (31)$$

Again, the fact that δ is small simplifies the analysis. We consider first the case when the rescaled concentrations g^* , r^* , B^* , A^* are of order one. This corresponds to the fixed point A_2 of the previous part in the parameter regime $\rho_0 < 1 < \rho_1$, with $A^* \approx A_0(1 - \rho_0)/(\rho_1 - 1)$, $B^* \approx 1/A^*$, and $g^* \approx (\rho_1 - 1)/(\rho_1 - \rho_0)$. Let us assume that the roots σ diverge as δ tends to zero. Then, Eq. (31) reduces at dominant order to

$$\sigma^3 (\delta \sigma + A^* + B^*) = \frac{A^* \tilde{\theta} g^*}{A_0 \delta} (\rho_0 - \rho_1). \quad (32)$$

Therefore, three roots σ_k , $k=0, 1, 2$, are of order $\delta^{-1/3}$ and proportional to the three cubic roots j^k of unity

$$\sigma_k = -j^k \left[\frac{A^* g^* \tilde{\theta}}{A^* + B^* A_0 \delta} (\rho_1 - \rho_0) \right]^{1/3}, \quad k=0, 1, 2. \quad (33)$$

The fourth root σ_3 is of order $1/\delta$ and corresponds to a stable mode of real part $-(A^* + B^*)/\delta$. The two roots σ_1 and σ_2 are complex conjugates and have a positive real part. Thus, in this parameter domain, the MFL fixed point is oscillatory unstable. The dynamics tends toward an attractive limit cycle that we will analyze in Sec. V B. When A^* or B^* grows (i.e., when $\rho_1 \rightarrow 1$ or $\rho_0 \rightarrow 1$) the fixed point instability disappears

via a Hopf bifurcation. We analyze these two boundaries in turn.

1. A^* high

When $\rho_1 \rightarrow 1$, A^* becomes large and the real parts of the roots become of order one. Thus, Eq. (31) needs to be approximated in a different way. We neglect $\delta(\sigma + d_b)$ and B^* as compared to A^* in Eq. (31) and obtain

$$\left(\sigma + \frac{\tilde{\theta}(\rho_1 - \rho_0)}{\rho_1 - 1}\right)(\sigma + 1)(\sigma + d_a) = -\frac{\tilde{\theta}(\rho_1 - 1)}{\delta A_0}, \quad (34)$$

where we have replaced A^* and g^* by their expressions at the A_2 fixed point. When the rhs is the dominant constant in Eq. (34), its three roots are proportional as above to the three roots of (minus) unity and two of them have positive real parts. On the contrary, when ρ_1 becomes sufficiently close to 1, the rhs of Eq. (34) becomes negligible and Eq. (34) has obviously three real negative roots. Therefore, when $\rho_1 \rightarrow 1$, one traverses the boundary of the linearly unstable region. Two roots of Eq. (34) traverse the imaginary axis on this boundary in a Hopf bifurcation. Assuming (and checking afterward) that, on this boundary, these roots are small compared with $1/(\rho_1 - 1)$, their expressions are found perturbatively by expanding the first factor in Eq. (34),

$$\sigma_{\pm} = -\frac{1 + d_a}{2} + \frac{(\rho_1 - 1)^3}{2(\rho_1 - \rho_0)^2 A_0 \tilde{\theta} \delta} \pm \frac{i}{2} \sqrt{\frac{(\rho_1 - 1)^2}{A_0(\rho_1 - \rho_0) \delta}}. \quad (35)$$

This provides the location of the stability boundary,

$$\rho_1 = 1 + (\delta \tilde{\theta} A_0 (d_a + 1) (\rho_1 - \rho_0)^2)^{1/3}. \quad (36)$$

In the limit $\delta \rightarrow 0$ the zeroth order boundary $\rho_1 = 1$ is of course retrieved. It can be checked that the obtained result $\rho_1 - 1 \sim \delta^{1/3}$ justifies *a posteriori* the use of the A_2 δ -independent expression for the fixed point. This fixed point linear stability boundary (36) can also be directly obtained from the Routh-Hurwitz condition [14] on the third degree polynomial (34). Comparison of the small δ [Eq. (36)] with the exact linear stability boundary is provided in Fig. 3.

2. A^* small

We now consider the upper boundary ($\rho_0 \sim 1$) of the linearly unstable region. When ρ_0 tends toward 1, B^* grows as $1/(\rho_0 - 1)$ and becomes large compared to $\delta(\sigma + d_a)$ and A^* . Equation (31) then simplifies and reduces at leading order to

$$(\sigma + \tilde{\theta})(\sigma + 1)(\sigma + d_b) = -E \quad (37)$$

with

$$E = \frac{A^* \tilde{\theta}}{A_0 B^* \delta} (\rho_1 - 1). \quad (38)$$

Again when E is large the roots are proportional to the three roots of -1 and two have positive real parts. On the contrary,

the three roots are real and negative when E vanishes. There is a critical value E_c such that for $E < E_c$ the fixed point is linearly stable. This occurs when the real part of the two complex conjugate roots becomes negative at the value E_c given by the Routh-Hurwitz criterion [14],

$$E_c = (d_b + 1 + \tilde{\theta})(\tilde{\theta} + d_b + d_b \tilde{\theta}) - d_b \tilde{\theta}. \quad (39)$$

The allied value of A at the fixed point concentration is obtained from Eq. (38),

$$A^* = \sqrt{\frac{E_c A_0 \delta}{\tilde{\theta}(\rho_1 - 1)}}. \quad (40)$$

Using the fixed point Eq. (18), this in turn corresponds to the line in parameter space

$$\rho_0 = 1 - \sqrt{\frac{E_c \delta (\rho_1 - 1)}{A_0 \tilde{\theta}}} \left[1 - \frac{\tilde{\theta} d_b}{E_c} \right]. \quad (41)$$

Equation (41), of course, reduces to the zeroth order boundary $\rho_0 = 1$ when $\delta \rightarrow 0$. Comparison of the small- δ asymptotic expression (41) with the exact linear stability line is provided in Fig. 3.

B. A description of the nonlinear oscillations

The MFL oscillations quickly become strongly nonlinear away from threshold (or, of course, when the bifurcation is subcritical). As there are no autoregulatory direct or indirect positive feedback loops in the MFL, two-variable reductions have a negative divergence. It follows from the well-known Bendixson's criterion [13], that they cannot be used to describe the oscillatory regime. It is, for instance, not possible to properly describe the oscillations by only focusing on the protein dynamics. A specific analysis, therefore, is required and developed in this section.

A full period of the nonlinear oscillations can be split into two parts: a first phase with $A \gg 1$, and a second phase with $B \gg 1$ (see Fig. 7).

In the following, these two main phases of the limit cycle are described. We use simple continuity arguments to match the two phases and obtain a description of the whole limit cycle. The oscillation period is computed (at the lowest order in δ). A full justification of this simple matching procedure and a detailed study of the transition regimes between the two main phases are provided in the Appendix using matched asymptotic techniques.

1. Phase I: High A , Low B phase

This phase is defined as the fraction of the limit cycle where the concentration of protein A is larger than $[A]_0$, i.e., when $A \gg 1 \gg B$. Equation (9) leads us to assume that A is of the order $1/\delta$. So we define $a = \delta A$. In the limit of small δ , as A scales as $1/\delta$, both the binding to the g promoter and the dynamics of B resulting from its complexation with A are fast compared to that of mRNA and A . Consequently, g and B are in quasiequilibrium and obey at the lowest order in δ ,

$$g = \delta A_0 / a, \quad (42)$$

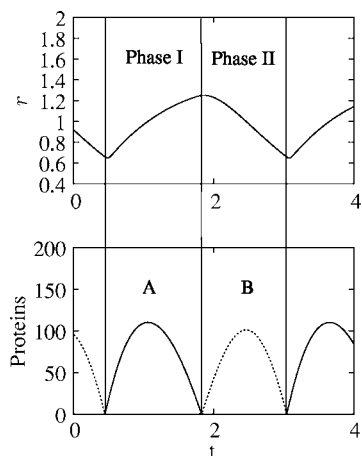


FIG. 7. Distinction between the two phases for the dimensionless equations [Eqs. (6)–(9)]. Top panel: oscillation of r . Bottom panel: oscillations for A (full line) and B (dotted line). In order to clearly depict phases I and II of an oscillation cycle, the parameters are here chosen so that the two phases have similar durations: $\delta = 0.001$, $\rho_1 = 1.5$, $\rho_0 = 0$, $\tilde{\theta} = 2$, $A_0 = 1$, $\mu = 1$, and $d_a = d_b = 0$.

$$B = \delta r/a. \quad (43)$$

The dynamics of the MFL, therefore, reduces to the following two equations at lowest order in δ :

$$\frac{dr}{dt} = \rho_1 - r, \quad (44)$$

$$\frac{da}{dt} = 1 - r - d_a a. \quad (45)$$

The beginning of phase I coincides with the end of phase II where, as explained below, A concentration is small. Therefore, continuity requires that a vanishes at the start of phase I (a detailed study of the transition region between the two phases is provided in the Appendix). Denoting by r_1 , the value of r at the start of phase I, an easy integration of the linear Eqs. (44) and (45) gives,

$$r_I(t) = \rho_1 + (r_1 - \rho_1)e^{-t}, \quad (46)$$

$$a_I(t) = \frac{1 - \rho_1}{d_a} [1 - e^{-d_a t}] + \frac{r_1 - \rho_1}{d_a - 1} [e^{-d_a t} - e^{-t}]. \quad (47)$$

Subscript I has been added to r and a in Eqs. (46) and (47) to emphasize that the corresponding expressions are valid during phase I only. Phase I ends with the fall of A concentration, after a time t_1 such that $a_I(t_1) = 0$. The mRNA concentration is then equal to $r_I(t_1) = r_2$.

One can note that the rise and fall of the concentration of protein A imposes restrictions on the parameters. The rise at the beginning of phase I is possible only if A production dominates over its complexation with B , that is, if $r_1 < 1$ [Eq. (45)]. The following fall requires the reverse which can only happen if B production becomes sufficiently important, namely $r > 1$. This requires $r_2 > 1$ and *a fortiori* $\rho_1 > 1$ [Eq. (44)].

2. Phase II: High B phase

This second phase is defined as the part of the limit cycle where the concentration of protein A falls below $[A]_0$, i.e., $B \gg 1 \gg A$. The form of Eq. (8) leads us to assume that B scales as $1/\delta$ and we define $b = \delta B$.

In the limit of small δ , the dynamics of A is fast compared to that of the other species and A is in quasiequilibrium. At the lowest order, its concentration reads,

$$A \approx \delta/b. \quad (48)$$

Thus, the dynamics of the MFL reduces, at the lowest order in δ , to the following three equations:

$$\frac{dg}{dt} = \tilde{\theta}(1 - g), \quad (49)$$

$$\frac{dr}{dt} = \rho_0 g + \rho_1(1 - g) - r, \quad (50)$$

$$\frac{db}{dt} = r - 1 - d_b b. \quad (51)$$

Continuity with the previous phase I leads us to require that at the beginning of phase II $b = 0$, $r = r_2$, and $g = 0$ (see the Appendix for a detailed justification). With these boundary conditions, the linear Eqs. (49)–(51) can readily be integrated to obtain

$$\begin{aligned} g_{II}(t) &= 1 - e^{-\tilde{\theta}t}, \\ r_{II}(t) &= \rho_0 + \left[r_2 - \rho_0 + \frac{\rho_1 - \rho_0}{\tilde{\theta} - 1} \right] e^{-t} - \frac{\rho_1 - \rho_0}{\tilde{\theta} - 1} e^{-\tilde{\theta}t}, \\ b_{II}(t) &= \frac{\rho_0 - 1}{d_b} [1 - e^{-d_b t}] - \frac{\rho_1 - \rho_0}{\tilde{\theta} - 1} \frac{e^{-d_b t} - e^{-\tilde{\theta}t}}{\tilde{\theta} - d_b} \\ &\quad + \left[r_2 - \rho_0 + \frac{\rho_1 - \rho_0}{\tilde{\theta} - 1} \right] \frac{e^{-d_b t} - e^{-t}}{1 - d_b}. \end{aligned} \quad (52)$$

Since b vanishes at the beginning of phase II, b should start by rising. This imposes that B production dominates over complexation and requires that the concentration r at the beginning of phase II is greater than 1, i.e., $r_2 > 1$ [see Eq. (51)]. Thus, r always remains larger than ρ_0 and would decrease toward ρ_0 for a long enough phase II [Eqs. (50) and (52)]. At the end of phase II, b should decrease to 0 to continuously match the beginning of phase I. This requires that the complexation then dominates over production of B , that is, $r < 1$ [Eq. (51)], and it is only possible when $\rho_0 < 1$. With these conditions met, phase II lasts a time t_2 and ends when $b_{II}(t_2) = 0$.

3. Matching of the two phases and period determination

In order to complete the description of the limit cycle, it remains to determine the four unknowns r_1 , r_2 , t_1 , and t_2 from the four conditions coming from the continuity of proteins and mRNA concentrations,

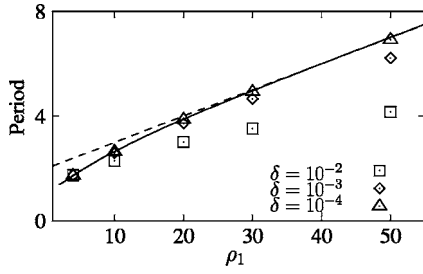


FIG. 8. Comparison between the lowest-order theoretical rescaled period T_r (bold line), the approximate expression for large ρ_1 [Eq. (61)] (dashed line) and numerically computed periods for different values of δ , as a function of parameter ρ_1 . Other parameters are $\tilde{\theta}=10$, $A_0=10$, $\mu=1$, and $d_a=d_b=0$.

$$r_I(t_1) = r_2, \quad a_I(t_1) = 0, \quad (53)$$

$$r_{II}(t_2) = r_1, \quad b_{II}(t_2) = 0. \quad (54)$$

One possibility to solve these equations is to use Eq. (53) to express r_1 and r_2 as a function of t_1 . It is then not difficult to see that the third equation [(54)] implicitly determines t_2 as a function of t_1 . Once r_1 , r_2 , and t_2 are obtained as functions of t_1 , the last equation $b(t_2)=0$ can be solved for t_1 by a one-dimensional root finding algorithm. In this way, we have determined r_1 , r_2 , t_1 , t_2 for different sets of kinetic constants and obtained the rescaled period of oscillation,

$$T_r = t_1 + t_2, \quad (55)$$

the dimensionful period being $T = T_r / \delta_r$. Results are shown in Fig. 8 and Fig. 9. The analytical period compares well to results obtained by direct numerical integration of Eqs. (6)–(9) for different values of δ with, of course, a closer agreement for smaller δ .

Equations (53) and (54) are difficult to solve analytically but analytic expressions can be obtained for various limiting cases. For instance, if the degradation of protein B is negligible ($d_b=0$), Eq. (52) simplifies to

$$b_{II}(t) = (\rho_0 - 1)t - \frac{\rho_1 - \rho_0}{(\tilde{\theta} - 1)\tilde{\theta}}(1 - e^{-\tilde{\theta}t}) + \left[r_2 - \rho_0 + \frac{\rho_1 - \rho_0}{\tilde{\theta} - 1} \right] (1 - e^{-t}). \quad (56)$$

If we further consider the limit of large ρ_1 , the condition $b_{II}(t_2)=0$ gives the following estimate for the duration of phase II, up to exponentially small terms,

$$t_2 \approx \frac{r_2 - \rho_0}{1 - \rho_0} + \frac{\rho_1 - \rho_0}{\tilde{\theta}(1 - \rho_0)}, \quad (57)$$

as well as the transcript concentration at the end of phase II

$$r_1 \approx \rho_0. \quad (58)$$

Similarly, the condition $a_I(t_1)=0$ together with Eq. (47) show that t_1 is small for large ρ_1 ,

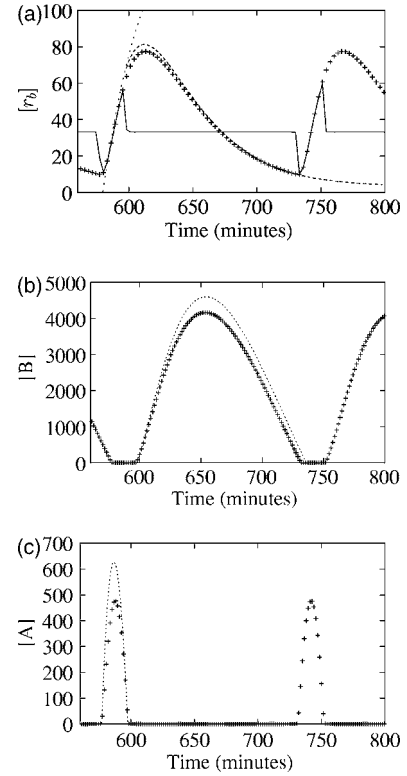


FIG. 9. Comparison between the full dynamics (+) symbols and the asymptotic description at order $\sqrt{\delta}$ (dashed and dotted lines) during one oscillation cycle. (a) Concentration of mRNA behavior. Phase I asymptotics (dotted lines) and phase II asymptotics (dashed lines) are shown separately. The product AB/β is also plotted (full line). The quasistatic assumption is seen to be well-satisfied away from the transition regions between the two phases. (b) Concentration of protein B . (c) Concentration of protein A . Parameters are as in Fig. 6.

$$t_1 \approx 2(1 - r_1)/\rho_1 \approx 2(1 - \rho_0)/\rho_1, \quad (59)$$

where expression (58) has been used in the second equality. Given the duration (59) of phase I, the concentration r_2 of the transcript at its end is directly obtained from Eqs. (46) and (58)

$$r_2 \approx 2 - \rho_0. \quad (60)$$

This finally provides the estimate of the period for large ρ_1 (with $d_b=0$),

$$T_r \approx t_2 \approx 2 + \frac{\rho_1 - \rho_0}{\tilde{\theta}(1 - \rho_0)}. \quad (61)$$

A comparison between Eq. (61) and numerically determined oscillation periods is provided in Fig. 8

C. Comparison with a simple delayed negative feedback

Popular models for genetic oscillators consist in a protein repressing its own production with a phenomenological delay [15–17]. Using a Hill function to model this repression, the simplest model of this kind reads,

$$\frac{dB(t)}{dt} = \frac{\rho}{1 + [B(t - \tau)/B_0]^n} - cB, \quad (62)$$

where ρ is the protein production rate, c the protein degradation rate, and τ the phenomenological delay for repression. The phase diagram of this simple oscillator can be computed [18]. In the limit of long delays ($c\tau \gg 1$), the oscillations are nonlinear. In an expansion in the small parameter $\epsilon = 1/c\tau$, their period T is

$$T/\tau = 2 + \frac{\kappa}{c\tau} + \dots, \quad (63)$$

where κ is a constant which is approximately equal to 2 for $n=2$ and ρ/c of order one [19].

It is interesting to note some analogies between this simple model with delay and the previously studied MFL. When $\tau \gg 1/c$, the period of the delayed model scales as τ while the period of the MFL scales as δ_r^{-1} . The mRNA half-life in the MFL thus plays the role of the phenomenological delay in Eq. (62). This is in line with the mRNA being the major pacemaker of the MFL oscillator. The rescaled parameter ϵ in the simple model with delay plays a role similar to the rescaled parameter δ in the MFL in the sense that, first, for high values of ϵ , oscillations disappear [18], and for small values of this parameter, the period of the oscillations is independent of $1/c\tau$ at dominant order. In the delayed model, ϵ represents the ratio between the typical lifetime of protein over the delay, while in the MFL, δ represents the ratio between the typical time scale of protein production and sequestration over the typical lifetime of RNA. These analogies show that for the MFL $\sqrt{\rho_A \gamma}$ plays the role of the protein degradation constant c in the delayed model. Thus protein sequestration by complexation is the MFL analog of protein degradation in the simple model with delay. The fastness of both these processes relative to a slow mechanism (delay τ , RNA dynamics) ensures the flipping of both oscillators between two states (two concentrations of B for the delayed model, A high and B high for the MFL).

VI. DISCUSSION

We have proposed a simple model of the mixed feedback loop, an over-represented motif in different genomes. We have shown that by itself this motif can serve as a bistable switch or can generate oscillations.

A. Importance of dimerization and of RNA dynamics

The MFL dynamics crucially depends on the post-transcriptional interaction between A and B proteins. The dynamics of dimerization is fast and allows a high concentration of only one of the two proteins, thus effectively creating a dynamical switch between the two species. If, for instance, A proteins are present at a higher concentration than B proteins, all B s are quickly titrated and there only remains free A and AB dimers.

A bistable system is obtained by coupling this post-transcriptional mechanism to a transcriptional repression. The created bistable switch is quite different from the clas-

sical ‘‘toggle switch’’ [20], which is based on reciprocal transcriptional repressions between two genes. In the MFL model, the single transcriptional repression of gene g_b is sufficient for a working switch. Moreover, dimerization bypasses the need for cooperativity in the toggle switch [21] and may render the present module simpler to implement in a biological system.

When the dimerization between A and B is coupled to a transcriptional activation, the system behaves as an oscillator if the production of A proteins is intermediate between the two possible for B . Then, the level of g_b transcripts controls A protein dimerization and, of course, B production rates. At the beginning of the derepression phase (phase I), the low level of transcripts leads to a small production of B proteins and to a rise of A concentration. This first phase ends when the subsequent high production and accumulation of g_b transcripts give rise to a production of B proteins sufficient for the titration of all free A s. During the ensuing repression phase (phase II), the g_b transcription rate is low and the transcripts degradation produces a continuous decrease of their concentration. The parallel decrease of the B production rate ultimately leads to the end of this second phase when the B production rate is no longer sufficient for the titration of all produced free A s.

In summary, the core mechanism of the clock is built by coupling the rapid switch at the protein level to the slow mRNA dynamics. The protein switch in turn controls mRNA dynamics via transcriptional activation.

It is interesting to note that the MFL model in the simple form analyzed here provides a genetic network that oscillates without the need for several specific features previously considered in the literature for related networks. As already mentioned, oscillations require neither an additional positive feedback loop [22], nor the self-activation of gene A [23], nor a highly cooperative binding [24] of transcription factors to gene promoters. Similarly, an explicit delay is not needed although delays of various origins have been considered in models of oscillatory genetic networks in different contexts, as is further discussed below.

Besides the motif topology, one feature of the present MFL model that renders this possible is the explicit description of transcription and translation. The condensed representation of protein production by a single effective process that is often used, would necessitate supplementary interactions to make oscillations possible. The description of mRNA was for instance omitted in the first version of a previously proposed evolutionary algorithm [5]. Oscillatory networks with core MFL motifs were nonetheless produced but much less easily than in a refined second version that incorporates mRNA dynamics. This role of mRNA dynamics may well be worth bearing in mind when considering the elaborate regulation of translation that is presently being uncovered [25].

B. Biological MFL oscillators and switches

Given the proposed functions for the MFL motif, it is of primary interest to know whether cases of its use as an oscillator or a switch are already documented. The oscillator function seems the clearest. Circadian clocks are genetic os-

TABLE I. Some biological examples of MFL motifs. The yeast motifs are reproduced from Ref. [3]. An annotation taken from the SGD database [32] has been added.

<i>A</i> species	<i>B</i> species	Main biological functions
Bistable systems		
Lac Repressor	Allolactose	Lactose metabolism
Oscillators		
WC-1, WC-2	FRQ	Neurospora circadian clock
dCLK	PER, TIM	Drosophila circadian clock
CLOCK, BMAL	PER, CRY	Mammals circadian clock
p53	Mdm2	Stress response oscillators
Detected Yeast MFLs		
Swi6	Swi4	G1/S transition
Gal4	Gal80	Galactose metabolism
Gal4	Gal3	Galactose metabolism
Gal4	Gal1	Galactose metabolism
Ime1	Rim11	Meiose activation
Ume6	Ime1	Meiose activation
Ste12	Fus3	Pseudohyphal growth
Ste12	Far1	Pseudohyphal growth
Cbf1	Met28	Sulfur metabolism
Met4	Met28	Sulfur metabolism
Swi4	Clb2	Cell cycle
Mbp1	Clb5	G1/S transition
Stb1	Cln1	
Stb1	Cln2	

oscillators which generate endogenous rhythms with a period close to 24 h and which are locked to an exact 24 h period by the alternation of day and night. A MFL motif is found at the core of all known eucaryotic circadian oscillators. Taking, for illustrative purposes, the fungus *Neurospora crassa*, one of the best studied model organisms, it has been established that a dimer of white-collar proteins (WCC) plays the role of *A* and activates the transcription of the *frequency* gene, the gene *g_b* in this example. In turn, the frequency (FRQ) protein interacts with WCC and prevents this activation [26]. Analogous motifs are found in the circadian genetic networks of flies and mammals, as shown in Table I. The p53/Mdm2 module [27] provides a different example. The p53 protein is a key tumor suppressor protein and an important role is played in its regulation by the Mdm2 protein. On the one hand, p53, similar to the *A* protein, binds to the Mdm2 gene and activates its transcription. On the other hand, the Mdm2 protein, as the *B* protein, binds to p53 and both blocks transcriptional activation by p53 and promotes its rapid proteolytic degradation. Cells exposed to stress have indeed been observed to present oscillations in both p53 and Mdm2 levels [27]. In both these examples, many other genetic interactions exist besides the above described MFL motifs and have been thought to provide delays necessary for oscillations. These have been postulated to come from chains of phosphorylations in circadian networks [28] or from a yet

unknown intermediate species [27] in the p53-Mdm2 system. The realization that the MFL can lead to oscillations without further interactions may be useful in suggesting alternative models of these particular genetic networks or in reassessing the role of known interactions. For instance, this has led one of us to formulate a model of the *Neurospora crassa* circadian clock [29] in which kinases and phosphorylation influence the cycle period by modifying protein degradation rates but in which phosphorylation is not needed to create key delays. The prevalence of the MFL motif probably arises from the usefulness of negative feedback, but it may also imply that oscillations in the genetic network are more common than is usually thought.

The evidence for uses of the MFL motif as a switch appears less clear cut at present. The classic case of the *E. coli* lactose operon [30] can be thought of as an effective version of a bistable MFL. The lac repressor represses the lac gene and plays the role of *A* in the MFL. The lac gene directs the production of a membrane permease, which itself drives the absorption of external lactose. Since allolactose binding to the lac repressor blocks its transcriptional activity, allolactose effectively plays the same role as *B* in the MFL. The search for a more direct switch example has led us to consider the different MFL motifs in yeast as reported in [3] and reproduced in Table I. It was noted in [3] that most of these MFL motifs were central modules in biochemical pathways. Most

of them take part in the cell cycle or in differentiation pathways, which certainly are processes where the cell switches from one function to another. However, we have found it difficult to disentangle the MFLs from numerous other known interactions and to confidently conclude that any of these motifs implements the proposed switch function. Similar difficulties have been recently emphasized for general motifs determined on purely statistical grounds and have led to the questioning of their functional significance [31]. In the present case, the difficulty of identifying biological cases of MFL switches, of course, arises from our own very partial knowledge of the networks listed in Table I, but it may also be due to the fact that the possible role of the MFL module as a switch was not fully realized in previous investigations. The present study will hopefully help and trigger direct experimental investigations of these questions.

APPENDIX: TRANSITIONS BETWEEN THE TWO PHASES OF AN OSCILLATION CYCLE AND $\sqrt{\delta}$ CORRECTION TO THE PERIOD

In the following, we analyze the transitions between phase I and phase II of an oscillation period and use matched asymptotics to precisely justify the assumptions made in Sec. V. This also provides the leading order ($\sqrt{\delta}$) correction to the zeroth order results of Sec. V.

1. From phase I to phase II

We consider the transition between the end of the high A /low B phase I and the start of the high B /low A phase II. This protein switch occurs on a time scale of order δ/δ_r . On this fast time scale the mRNA concentration does not have time to change. Thus, in Eqs. (6)–(9), we assume $r=r_2$ and introduce $\tau=t/\delta$. Equations (8) and (9) simply become at dominant order,

$$\frac{dB}{d\tau} = r_2 - AB, \quad (A1)$$

$$\frac{dA}{d\tau} = 1 - AB. \quad (A2)$$

The imposed boundary conditions are

$$A \rightarrow (1 - r_2)(\tau - \tau_1) + o(1), \quad B \rightarrow 0 \text{ at } \tau = -\infty, \quad (A3)$$

$$B \rightarrow (r_2 - 1)(\tau - \tau_2) + o(1), \quad A \rightarrow 0 \text{ at } \tau \rightarrow +\infty \quad (A4)$$

with the dimensionless transcript concentration $r_2 > 1$ (see Sec. V B 1) and τ_1 and τ_2 two constants to be determined. Equations (A1) and (A2) are autonomous in time and, therefore, invariant by time translation. For general nonintegrable equations, numerical integration would be required to obtain the difference $\tau_2 - \tau_1$. Here, however, the difference of protein concentrations, $A - B$, is easily integrated across the transition region. Fixing the time origin at the instant when $A = B$, one readily obtains the exact formula

$$A - B = (1 - r_2)\tau. \quad (A5)$$

Comparison with Eqs. (A3) and (A4) shows that with this choice of time origin $\tau_1 = \tau_2 = 0$ (and more generally $\tau_1 = \tau_2$).

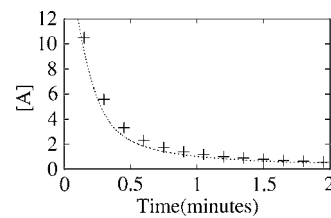


FIG. 10. The evolution of A given by the Riccati Eq. (A6) (+) is compared to that given by the complete MFL dynamics (dashed) during the transition from phase I to phase II. Parameters are as in Fig. 6.

The asymptotic conditions (A3) and (A4) coincide with the limiting behavior of $a_I(t)/\delta$ at the end of phase I near $t=t_1$, and with the limiting behavior of $b_{II}(t)$ at the beginning of phase II near $t=0$, provided that $a_I(t_1) = b_{II}(0) = 0$ as was required in the main text. Thus, Eqs. (A1) and (A2) provide a uniform approximation of $A(t)$ and $B(t)$ throughout the transition from phase I to phase II. Given the exact result (A5), the integration of Eqs. (A1) and (A2) can in fact be replaced to the integration of the single following Riccati equation:

$$\frac{dA}{d\tau} = 1 - [A + (r_2 - 1)\tau]A. \quad (A6)$$

A comparison between Eq. (A6) and full numerical evolution is shown in Fig. 10. There is one subtlety, however. The quasiequilibrium approximation for g [Eq. (42)] diverges at the end of phase I when $A \rightarrow 0$ and g becomes larger than 1 before the transition region (A1) and (A2) of order δ . This clearly signals the breakdown of the approximation (42) in a larger intermediate region at the end of phase I. In a region of size $t_1 - t \sim \sqrt{\delta}$, the evolution of g reduces to

$$\frac{dg}{dt} = \tilde{\theta} \left(1 - g \frac{a}{\delta A_0} \right). \quad (A7)$$

Indeed, taking $a = (1 - r_2)(t_1 - t) + o(\sqrt{\delta})$ shows that all three terms kept in Eq. (A7) are of the same magnitude when $g \sim t_1 - t \sim \sqrt{\delta}$ and that the additional term $\tilde{\theta}g$ in Eq. (6) is negligible. Explicit integration in this intermediate region gives

$$g(t_1) - g(t) \exp[-\kappa_2(t_1 - t)^2] = \tilde{\theta} \int_{t-t_1}^0 du \exp(\kappa_2 u^2) \quad (A8)$$

where $\kappa_2 = \tilde{\theta}(1 - r_2)/(2A_0\delta)$. In particular, the limit $t \rightarrow -\infty$ determines the value of g at the end of phase I and beginning of phase II

$$g(t_1) = g_2 = \sqrt{\frac{\pi A_0 \tilde{\theta} \delta}{2(r_2 - 1)}}. \quad (A9)$$

Equation (A9) is the source of a correction of order $\sqrt{\delta}$ to the asymptotic result (55) as will be shown below.

2. From phase II to phase I

The transition from phase II to phase I can be analyzed quite similarly to the transition from phase I to phase II. In a time of order δ/δ_r at the end of phase II, r has no time to change and the transition is described by Eqs. (A1) and (A2) with r_2 replaced by $r_1 < 1$ (see Sec. V B 1) and the boundary conditions

$$B = (r_1 - 1)(\tau - \tau'_1) + o(1), \quad A \rightarrow 0 \text{ at } \tau = -\infty, \quad (\text{A10})$$

$$A = (1 - r_1)(\tau - \tau'_2) + o(1), \quad B \rightarrow 0 \text{ at } \tau = +\infty. \quad (\text{A11})$$

Again, $\tau'_1 = \tau'_2 = 0$ if the time origin is taken when $A = B$. This transition regime is followed by a longer transition on a time scale $t - t_2 \sim \sqrt{\delta}$ where g decreases from a value of order one to values of order δ characteristic of phase I. This reflects the fact that the binding of protein A to the promoter of gene g_b is not instantaneous and requires some accumulation of protein A at the beginning of phase I. Taking $A = (1 - r_1)t/\delta$, g evolution is described in this intermediate regime by

$$\frac{dg}{dt} = \tilde{\theta} \left[1 - t(1 - r_1) \frac{g}{A_0 \delta} \right]. \quad (\text{A12})$$

The corresponding evolution of g is

$$g(t) = \exp(-\kappa_1 t^2) \left[g_1 + \tilde{\theta} \int_0^t du \exp(\kappa_1 u^2) \right], \quad (\text{A13})$$

where $\kappa_1 = \tilde{\theta}(1 - r_1)/(2A_0\delta)$. It indeed describes the transition from $g_1 = 1 - \exp(-\tilde{\theta}t_2)$, the value of g at the end of phase II, to the quasiequilibrium regime since the asymptotic behavior of Eq. (A13) for $t \gg \sqrt{\delta}$ is

$$g(t) \sim \frac{A_0 \delta}{(1 - r_1)t}. \quad (\text{A14})$$

3. Dominant correction to the oscillation period

The form of Eqs. (6)–(9) could lead one to think that the oscillation period has an expansion in powers of the small parameter δ . However, here as often, boundary layers lead to more complicated expansions. The two transition regimes of duration $\sqrt{\delta}$ give rise to a dominant correction of order $\sqrt{\delta}$ to the oscillation period.

As pointed out above, due to the transition regime at the end of phase I, the starting value of g in phase II is g_2 [Eq. (A9)] of order $\sqrt{\delta}$. Therefore, the first Eq. (52) is replaced at order $\sqrt{\delta}$ by the corrected evolution,

$$g_{II,c}(t) = 1 - (1 - g_2)\exp(-\tilde{\theta}t). \quad (\text{A15})$$

This leads in turn to corrected evolutions $r_{II,c}(t)$ and $b_{II,c}(t)$ for the mRNA and B protein concentrations that are obtained by simply replacing $(\rho_1 - \rho_0)$ by $(\rho_1 - \rho_0)(1 - g_2)$ in the expressions (52) of the zeroth-order expressions $r_{II}(t)$ and $b_{II}(t)$.

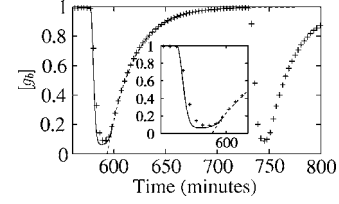


FIG. 11. Comparison of $[g_b]$ obtained from a full numerical integration (+) with the uniform approximation obtained by matching the different transition regimes (bold line). The exponential relaxation in phase II [Eq. (A15)] is also shown (dashed). An enlargement of phase I and the transition regimes is shown in the inset. Parameters are as in Fig. 6.

The other transition regime between phase II and phase I does not lead to modifications of the form of Eqs. (46) and (47) describing the evolutions of mRNA and A protein during the bulk of phase I. Instead, it results in a difference of order $\sqrt{\delta}$ between $r_{II,c}(t_2)$ and r_1 the effective concentrations of transcripts at the start of phase I. The transcript concentration depends on the promoter states at previous times. So, integrating Eq. (7) from the end of phase II, one obtains

$$r(t) = \rho_1 + [r_{II,c}(t_2) - \rho_1]\exp(-t) + (\rho_0 - \rho_1)\exp(-t) \int_0^t du \exp(u)g(u). \quad (\text{A16})$$

Replacing $g(t)$ by its expression (A12) during the transition regime at the start of phase I leads for $t \gg \sqrt{\delta}$ to

$$r(t) = \rho_1 + \left[r_{II,c}(t_2) - g_1 \sqrt{\frac{\pi}{4\kappa_1}}(\rho_1 - \rho_0) - \rho_1 \right] \exp(-t). \quad (\text{A17})$$

Namely, the previous Eq. (46) but with

$$r_1 = r_{II,c}(t_2) - g_1 \sqrt{\frac{\pi}{4\kappa_1}}(\rho_1 - \rho_0). \quad (\text{A18})$$

Note that only the g_1 term between the square brackets in Eq. (A12) contributes to Eq. (A17), the integral term giving a subdominant contribution.

Finally, the durations T_1 and T_2 of both phases and the total period $T_\gamma = T_1 + T_2$ are obtained to $\sqrt{\delta}$ accuracy by solving as before

$$r_I(t_1) = r_2, \quad a_I(t_1) = 0 \quad (\text{A19})$$

together with the corrected version of Eq. (54), namely Eq. (A18) and $b_{II,c}(t_2) = 0$.

The asymptotic description of g_b evolution agrees well with numerical simulations of the full MFL model as shown in Fig. 11. The $\sqrt{\delta}$ correction terms lengthen the zeroth-order asymptotic estimation. For a moderate value of δ , a numerically comparable contribution, however, is coming from higher-order terms that tend to lengthen the period [coming, for instance, from the breakdown of the quasiequilibrium approximation for g_b Eq. (42)] as can be seen in Fig. 8.

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