Coupling oscillations and switches in genetic networks

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1. Introduction

Switches and oscillations are found in many biological systems (Tyson et al., 2008). Oscillatory behaviors have been described at various levels of organism organization, ranging from neuronal rhythms to biochemical oscillations and circadian clocks (Goldbeter, 1996). These oscillations often originate from negative regulatory feedbacks and, usually, take the form of limit cycles in the phase plane. For example, the core molecular mechanism of circadian clocks is based on the repression exerted by a clock protein on the expression of its own gene (Dunlap, 1999; Young and Kay, 2001). In parallel, since the work of Jacob and Monod (1961) the switch phenomenon has become more and more popular because it provides a rational basis to explain the condition-specific activation of some genes. Bistability is a particular mode of switch in which two stable steady states coexist. Such a situation was described in detail for the lactose operon (Novick and Weiner, 1957; Ozbudak et al., 2004) but is likely to occur in many genetics or other molecular systems.

With the recent availability of large scale data on genetic regulations, much attention has been given to unravel the regulatory motifs in genetic regulatory networks (Shen-Orr et al., 2002; Milo et al., 2002; Alon, 2003, 2007). Over-represented motifs in those networks include positive and negative feedback loops, feedforward loops, etc. (Alon, 2007). These motifs constitute the building blocks of large gene regulatory networks. Similar motifs are also found in other biological networks, including signaling cascades (Kholodenko, 2006) and neuronal networks (Sporns and Kotter, 2004). The dynamical properties of these motifs have been extensively studied, mainly by means of mathematical models (Tyson et al., 2003; Alon, 2006). These approaches are indeed commonly used nowadays to unravel the design principles of large genetic networks. It should nevertheless be stressed that the dynamics of regulatory motifs has already been the object of numerous investigations in the past (Griffith, 1968a,b; Glass and Kauffman, 1973; Tyson and Othmer, 1978; Thomas and D’Ari, 1990). These pioneer works already established general properties of genetic networks and have shown, for instance, that a negative circuit is required to produce oscillations whereas a positive circuit is required to generate multistability.

Complementary to theoretical modeling and motivated by these models, synthetic switches and oscillators have been designed, analysed mathematically, and implemented in real biological systems.
systems. The Repressilator (Elowitz and Leibler, 2000) and the Toggle switch (Gardner et al., 2000) constitute two prototypes of such types of systems. The Repressilator is composed of three genes coding for repressor proteins. Their promoters are genetically modified in such a way that the expression of each gene is repressed by the next protein of this three-gene cyclical network. Because it is based on a negative circuit, under some assumptions, this system exhibits self-sustained oscillations. The Toggle switch is composed of two genes which mutually repress each other. Under appropriate conditions, this positive circuit leads to bistability.

The dynamical properties of the Repressilator and the Toggle switch have been the subject of several theoretical investigations. Previous works include stochastic simulations of the Toggle switch (Tian and Burrage, 2006; Wang et al., 2007), stochastic simulations of the Repressilator (Loinger and Biham, 2007) and synchronization of coupled Repressilators (Garcia-Ojalvo et al., 2004; Wang et al., 2006). Each model is based either on the Repressilator alone or the Toggle switch alone. However, biological systems are composed of interconnected positive and negative circuits (Tsai et al., 2008).

The aim of the present study is to unravel the compositional rules that govern the dynamics of systems combining simple modules. While systems of coupled biological oscillators have been intensively studied (Zhou et al., 2008), the coupling between biological switches and clocks was not systematically investigated yet. Here, we study the dynamical properties resulting from the coupling between the Repressilator and the Toggle switch model. This coupled model differs from the models proposed by Tsai et al. (2008) and by Kim et al. (2008) in the way the two circuits are connected. In the latter models, one variable of the oscillator is directly involved in a positive circuit. The coupling is thus obtained by a common variable between the two circuits. The coupling considered here is indirect. Two types of coupling are considered. In the first case, the expression of one gene of the Toggle switch is under the control of one protein of the Repressilator. In the second type of coupling, the expression of one gene of the Repressilator is controlled by the Toggle switch. These two models can thus be regarded as master/slave systems in which one system is under the control of the other. Such type of unidirectional coupling, which should be distinguished from mutual coupling, is likely to be present at multiple stages of genetic regulatory networks which were shown to be hierarchical.

The paper is organized as follows. In Section 2, we recall the equations of the Repressilator and of the Toggle switch models and illustrate the main dynamical properties of these two systems. In Section 3, we describe the dynamics resulting from the two kinds of coupling described above. In Section 4, we discuss possible applications of the results in biological systems.

2. Model

2.1. Repressilator

The Repressilator is a model in which three genes are cyclically organized in such a way that the protein coded by each gene acts as a repressor of the transcription of the next gene in the cycle (Elowitz and Leibler, 2000). The dynamics of this model is described by six ordinary differential equations:

\[
\frac{dM_i}{d\tau} = \tau \left( -M_i + \frac{\alpha_i}{1 + \rho_i^{\text{mod}(i+1,3)}} \right) \quad \text{with } i = 1, 2, 3  
\]

\[
\frac{dP_i}{d\tau} = \tau \left( \beta_i M_i - \gamma_i P_i \right) \quad \text{with } i = 1, 2, 3  
\]

(1)

(2)

In these equations, \(M_i\) and \(P_i\) stand for the concentration of mRNA and protein corresponding to gene \(i\) (with \(i=1,\ldots,3\)). The inhibition is described by the Hill function \(\alpha_i/(1 + \rho_i^{\text{mod}(i+1,3)})\) where \(\text{mod}\) is the modulo function. Parameters \(\alpha_i\) represent the maximum rate of mRNA synthesis of gene \(i\). Parameter \(\tau\) has been introduced to allow us to control the time scale of the dynamics (and thereby the period of the oscillations generated by this model). Variables and time have been rescaled and adimensionalized.

The dynamics of the Repressilator is shown in Fig. 1. For the default parameter values (see Elowitz and Leibler, 2000, and legend of Fig. 1), the model displays limit-cycle oscillations. Because of the symmetry in the model and in the parameters values chosen, each mRNA (protein) oscillates with the same amplitude, but the oscillations are out-of-phase (Fig. 1A). For each gene, the protein level directly follows the mRNA level. This explains why the limit cycle, in the plane mRNA/protein is close to the diagonal (Fig. 1B). The bifurcation diagram shown in Fig. 1C shows that the amplitude of the oscillations increases when parameter \(\alpha_1\) is increased and that the oscillations are lost when this control parameter goes below a critical value. This value, called a Hopf bifurcation, is located at \(\alpha_{1\text{crit}}=6.3\). Fig. 1D shows how the period is affected when parameter \(\alpha_1\) is changed within the oscillatory domain. The period of the oscillations slightly increases as \(\alpha_1\) increases.

2.2. Toggle Switch

The Toggle switch system is constituted by two genes which mutually inhibit each other (Gardner et al., 2000). The dynamics of this model is described by two differential equations:

\[
\frac{dX}{d\tau} = \frac{a_1}{1 + Y^n} - d_1 X + b_1  
\]

\[
\frac{dY}{d\tau} = \frac{a_2}{1 + X^n} - d_2 Y + b_2  
\]

(3)

(4)

In this model, no distinction is made between the gene and the protein. Adding evolution equations analogous to Eq. (2) to distinguish protein from mRNA would not affect the results qualitatively. The inhibition is described by the Hill functions \(a_i/(1 + Y^n)\) and \(a_j/(1 + X^n)\) where \(a_i\) and \(a_j\) denote the maximum rate of \(X\) and \(Y\) mRNA synthesis, respectively, and \(n\) is the cooperativity. Parameters \(b_1\) and \(b_2\) describe an independent synthesis source of \(X\) and \(Y\), resulting for example from another promoter, which is not subject to the inhibitory effect of \(X\) and \(Y\), but can be controlled by other, external factors. Here again, variables and time have been rescaled and adimensionalized.

The dynamics of the Toggle switch is illustrated in Fig. 2. For the default parameters values (see Gardner et al., 2000, and legend of Fig. 2), the model displays bistability; i.e. coexistence between two stable steady states. As shown in Fig. 2A, bistability occurs in a range of values of \(\alpha_1\) delimited by two bifurcation points, called “saddle nodes”. These bifurcations, characteristic of a hysteretic behavior, are located at \(\alpha_{SN1}=1.4\) and \(\alpha_{SN2}=6.8\). Analysis in the phase space highlights the bistability: the two nullclines cross each other in three points, the middle one being the unstable steady state, while the two others correspond to stable steady states (Fig. 2C). Depending on the initial conditions, the system will converge to either one of the other stable steady state (Fig. 2E).

When the value of \(\alpha_1\) is larger than the value of the second saddle node \(\alpha_{SN2}\), there is no bistability (Fig. 2B): the two nullclines cross each other at a single point, corresponding to the unique stable steady state (Fig. 2D). However, starting from an initial condition corresponding to the lower steady state observed for a smaller value of \(\alpha_1\), the trajectory does not jump immediately to the upper steady state, but stays transiently at a low value (Fig. 2F).
Fig. 1. Dynamics of the Repressilator. For the following (default) parameter values, limit-cycle oscillations occur: $\alpha_1 = \alpha_2 = \alpha_3 = 100, m = 2, \beta = 5$. Because of the symmetry of the model, each mRNA level (shown on panel (A)) and associated protein level (not shown) oscillate out-of-phase. The period of the oscillations is about 8.5. The limit cycle represented in panel (B) shows that the protein level closely follows its mRNA level. Panel (C) displays the bifurcation diagram as a function of parameter $\alpha_1$. A Hopf bifurcation (denoted by “HB” on the figure) is located at $\alpha_1 = \alpha_{HB} = 6.3$. At $\alpha_1 < \alpha_{HB}$ the solid line denotes the stable steady state of $M_1$. At $\alpha_1 > \alpha_{HB}$, the dashed line represents the unstable steady state. In this region limit-cycle oscillations occur and the two solid lines correspond to the maximum and minimum levels of $M_1$ during these oscillations. The corresponding period of the oscillations is given in panel (D). These results have been obtained by numerical simulation of Eqs. (1) and (2). Bifurcation diagrams shown here and in the following figures have been obtained with XPP-AUTO (Doedel, 1981; Ermentrout, 2002).

2.3. Coupling

In this paper, we will consider two types of coupling between the Repressilator and the Toggle switch. In the first type of coupling, we will assume that one protein of the Repressilator, $P_1$, induces the expression of gene $X$ of the Toggle switch (see Fig. 3):

$$a_1 = AP_1 + B$$

(5)

In other words, if protein $P_1$ is absent ($P_1 = 0$), the maximum rate of transcription $a_1$ stays at a basal value $B$, but as soon as $P_1$ is present ($P_1 > 0$), the maximum rate of transcription is (linearly) increased.

In the second part of the paper, we will assume that gene $X$ of the Toggle switch activates the transcription of gene 1 (i.e. the synthesis of its mRNA, $M_1$) of the Repressilator (see Fig. 7):

$$a_1 = AX + B$$

(6)

For these two kinds of coupling, we chose the most simple, linear, functions. Our goal is to describe qualitatively some dynamical behaviors that can occur in the coupled system. These behaviors do not depend on the exact form of the coupling. Other, possibly non-linear, functions, or a more elaborate coupling involving several intermediary steps could have been used as well. For instance, we could have replaced Eq. (5) by a Michaelian kinetic equation of the form:

$$a_1 = A \frac{P_1}{K + P_1} + B$$

(7)

Such change in the coupling function leads to similar qualitative results, upon appropriate tuning of parameters $A, B,$ and $K$.

3. Results

3.1. Periodic Switch Induced by the Oscillator

In this section, we treat the first type of coupling. We assume that one protein of the Repressilator, $P_1$, activates the expression of one gene of the Toggle switch, $X$. The scheme of the model is illustrated in Fig. 3A. The kinetics of this model is described by Eqs. (1)–(4) with the coupling defined by Eq. (5). Our aim is to determine the conditions in which the oscillator induces a periodic switch of variables $X$ and $Y$ (Fig. 3B).

Numerical simulation of the model shows that oscillation-induced switches can be observed if the amplitude of the forcing is sufficient (Fig. 4A and B). The coupling parameters $A$ and $B$ have been chosen in such a way that the forced parameter $a_1$ undergoes oscillations from a value below the value of the first saddle node (i.e. $a_{min} < a_{SN1}$) to a value above the value of the second saddle node bifurcation of the Toggle switch (i.e. $a_{max} > a_{SN2}$). The limit-cycle oscillation then induced in the Toggle switch has the same period as the driving oscillator (here the period is fixed to 42.5 by setting $\tau = 0.2$).

It is necessary but not sufficient that $a_1$ reaches the value above the value of the second saddle node bifurcation of the Toggle switch. Indeed, as shown in Fig. 4C and D, when the amplitude of the forcing
Fig. 2. Dynamics of the Toggle switch. For the following (default) parameter values, bistability occurs: $a_1 = a_2 = 2$, $d_1 = d_2 = 1$, $n = 4$. Panels (A) and (B) show the bifurcation diagram as a function of parameter $a_1$. Two saddle node bifurcations (denoted by “SN”) delimit the region of bistability: $a_{SN1} = 1.4$ and $a_{SN2} = 6.8$. In panel (C) the nullclines obtained for the default parameter values are shown. Panel (E) displays the time evolution of $X$ for the default parameter values, but for two different initial conditions: $x(0) = 0.9$, $y(0) = 1$ (“CI1”) and $x(0) = 1.1$, $y(0) = 1$ (“CI2”). These initial conditions are marked by the black dots in panel (A). In panel (D) are given the nullclines for the same parameter values as in panel (C), except $a_1 = 8$. For this value, no bistability occurs. We observe however that the transition from an initial condition close to the proximal lower steady state ($x(0) = 0.38$, $y(0) = 1.98$, corresponding to CI0 in panel (B)) to the steady state displays some transients: the system tends to stay close to this initial value before jumping rapidly to the upper steady state (panel F). These results have been obtained by numerical simulation of Eqs. (3) and (4).

is not high enough, and despite the fact that $a_{max} > a_{SN2}$, no switches are induced. Variable $X$ remains close to the bottom branch of the hysteresis, and variable $Y$ close to its upper branch. Only small-amplitude oscillations of $X$ (and $Y$) are observed. This limit cycle is unique and reached regardless the initial conditions. Its period is the same as the period of the Repressilator.

When the forcing amplitude is further reduced, with $a_{min} > a_{SN1}$ and $a_{max} < a_{SN2}$, it is possible to induce birhythmicity. Depending on the initial conditions, variables $X$ and $Y$ can undergo small-amplitude oscillations around the upper or lower steady state, corresponding to the upper and lower branch of the hysteresis obtained in the uncoupled Toggle switch module (Fig. 4E and F). Two stable limit cycles thus coexist and their periods are identical and equal to the period of the driving oscillator.

There are thus conditions on the values of the coupling parameters $A$ and $B$ to obtain oscillation-induced switches. Fig. 5 summarizes the different behaviors that can be observed as a function of the values of $A$ and $B$. A periodic switch can be obtained only if $B$ is sufficiently low (i.e. $B < a_{SN1}$, to allow the trajectory to jump from the top branch to the bottom branch) and $A$ sufficiently high (to allow the trajectory to jump from the bottom branch to the top branch). The region where these conditions are fulfilled is denoted by (a) on Fig. 5. When both $A$ and $B$ are small, the system cannot jump from the bottom branch to the top branch and hence only low-amplitude oscillations around the bottom branch are observed (region (b)). When $B$ is large (i.e. $B > a_{SN1}$), the system cannot jump from the top branch to the bottom branch. In this case either $A$ is large and the system only displays low-amplitude oscillations around the top branch (region (c)), or $A$ is small enough to prevent the system to jump from the bottom branch to the top branch and, depending on the initial conditions, the systems will be trapped around the bottom or the top branch, and birhythmicity occurs (region (d)). Finally, when $B$ is very large ($B > a_{SN2} = 6.8$), only low-amplitude oscillations around the top branch are observed, regardless of the value of $A$ (not shown).

Oscillation-induced switches depend also on the relative time scale of the oscillations and the switch dynamics. In the results described above, the period of the Repressilator was around 42.5. With the same coupling parameter values as case in Fig. 4A and B, if the period of the oscillations is shortened ($\tau = 1$, corresponding to a period of about 8.5), no switch can be induced because the system does not have the time to reach the upper branch (Fig. 6A
and B). On the contrary, for the same coupling parameter values than in Fig. 4C and D, if the period of the oscillations is increased (\( \tau = 0.02 \), corresponding to a period of about 425), the system has enough time to jump to the upper branch of the hysteresis. In this case, the limit cycle induced in the Toggle switch follows closely the hysteresis (Fig. 6C and D). We can nevertheless notice that the switch from the lower to the upper steady state is achieved after a small delay after the parameter \( a_1 \) has overtaken the second saddle node. This delay effect was already observed in the dynamics of the uncoupled Toggle switch (Fig. 2F).

In Figs. 4–6, we considered a linear coupling function (Eq. (5)). As mentioned in the above section, similar results can be obtained with other coupling functions. If for example we consider the nonlinear coupling function defined by Eq. (7), oscillations-induced periodic switches can be observed with \( A = 20, B = 0, K = 20, \) and \( \tau = 0.2 \). This shows that the exact form of the coupling function is not crucial, but the parameter values should be appropriately tuned.

In practice, it might be difficult to control the values of the coupling parameters \( A \) and \( B \), and of the period \( \tau \). Instead of changing these parameters, one can alter one parameter of the oscillator itself, which affects its amplitude and period. As shown in Fig. 1, increasing the value of \( \alpha_1 \) in the Repressilator results in an increase of the amplitude and of the period. In this case, however, it remains difficult to control the amplitude and the period independently, unless several control parameters are modified at the same time.

### 3.2. Limit-Cycle Oscillations Induced by a Switch

In this second part of the paper, we investigate the dynamics generated when the Repressilator is under the control of the bistable Toggle switch (Fig. 7A). The scheme of the model is illustrated in Fig. 7A. The idea is to connect the Toggle switch and the oscillator in such a way that a transition from one steady state to the second steady state in the Toggle switch can, via the coupling, induce oscillations in the Repressilator. We constructed this coupling by assuming that gene \( X \) induces the expression of gene 1 of the Repressilator, and thus increases the production of its mRNA, \( M_1 \). The kinetics of this model is described by Eqs. (1)–(4) with the coupling defined by Eq. (6). We then wished to investigate if a transient perturbation inducing a permanent switch in the bistable system can induce self-sustained oscillations in the Repressilator. The scheme represented in Fig. 7B illustrates this idea.

Numerical simulation of the Repressilator coupled to the Toggle switch shows that this system is able to generate self-sustained oscillations through a switch induced by a transient perturbation (Fig. 8A and C). In this simulation, parameter \( b_1 \) was set to 1 during \( t = 100 \) and \( t = 110 \) (see region highlighted in grey on the figure) and remains 0 otherwise. This transient activation of the gene \( X \) induced a switch: \( X \) jumped to its upper steady state while \( Y \) dropped to its lower steady state. After the end of the perturbation, \( X \) stays at a high level, while \( Y \) stays at a low level. Because the values of the coupling parameters \( A \) and \( B \) have been chosen in such a way that \( \alpha_1 \) goes from a value below the Hopf bifurcation (\( \alpha_{\text{min}}(\text{at } X) < \alpha_{\text{HB}} \)) to a value located above the Hopf bifurcation (\( \alpha_{\text{max}}(\text{at large } X) > \alpha_{\text{HB}} \)), the switch induced permanent oscillations in the Repressilator.

By inducing the switch in the opposite direction, it is possible to obtain the opposite effect: a transient perturbation can stop permanently the oscillations (Fig. 8B and D). In this simulation, the perturbation is applied on parameter \( b_2 \). The transient increase of \( b_2 \) from 0 to 1 during \( t = 100 \) and \( t = 110 \) induced a switch where \( X \) dropped to its lower steady state while \( Y \) jumped to its upper steady state, this state being maintained even after the end of the perturbation. This transition caused the parameter \( \alpha_1 \) to decrease from a value above the Hopf bifurcation (\( \alpha_{\text{max}}(\text{at high } X) > \alpha_{\text{HB}} \)) to a value below the Hopf bifurcation (\( \alpha_{\text{min}}(\text{at low } X) < \alpha_{\text{HB}} \)) and the oscillations are then permanently arrested.

Finally, if \( \alpha_{\text{min}}(\text{at low } X) \) and \( \alpha_{\text{max}}(\text{at large } X) \) are both located inside the oscillatory domain, we observe that the switch alters the period and amplitude of the oscillations (Fig. 9). More specifically, here \( \alpha_{\text{min}}(\text{at low } X) = 15 \) and \( \alpha_{\text{max}}(\text{at large } X) = 110 \). As predicted from the bifurcation diagram shown in Fig. 1, this transition induces an increase of the amplitude and of the period of the oscillations.

As for the case of the first type of coupling, in practice, it might be difficult to control the coupling parameters \( A \) and \( B \). Instead, we could alter one parameter of the Toggle switch (such as \( a_1 \)) that will affect the level of the steady states. It might also be challenging to find the appropriate external perturbation that will induce the switch in the Toggle switch. Here we have seen that an increase of...
Fig. 4. Periodic switch induced by the oscillations. (A) When the amplitude of the coupling is sufficient, oscillations of the Repressilator are able to induce a periodic switch in the Toggle switch. The figure shows the bifurcation diagram of the variable $X$ as a function of parameter $a_1$. This parameter is a function of variable $P_1$ of the Repressilator and therefore oscillates. The vertical dashed lines correspond to the lower and upper limit of the $a_1$ oscillations. The solid close curve corresponds to the trajectory of variable $X$ and shows that this variable switches between the lower and the upper steady states. The corresponding time evolutions of $X$ and $Y$ are shown in panel (B). (C) When the amplitude of the coupling is insufficient, oscillations of the Repressilator are not able to induce a periodic switch in the Toggle switch. Despite the fact that $a_1$ goes beyond the second saddle node point, variable $X$ does not switch to the upper steady state. Instead, it oscillates with a very low amplitude around the lower steady state. The corresponding time evolutions of $X$ and $Y$ are shown in panel (D). (E) When the amplitude of the coupling is very low, oscillations of the Repressilator can induce a phenomenon of birhythmicity: depending on the initial conditions, variable $X$ can undergo small-amplitude oscillations around the upper or lower steady state. The corresponding time evolutions of $X$ and $Y$ are shown in panel (F). Data have been obtained with the same parameter values than those given in legend of Figs. 1 and 2, except $r = 0.2$. Coupling parameters are $A = 0.21$ and $B = 0$ (A and B), $A = 0.19$ and $B = 0$ (C and D), and $A = 0.08$ and $B = 2$ (E and F). For the latter case, initial conditions are $x(0) = 0.1$ and $y(0) = 0.9$ (denoted by CI1), leading to the lower limit cycle or $x(0) = 0.9$ and $y(0) = 0.1$ (denoted by CI2), leading to the upper limit cycle.

Parameter $b_1$ (or $b_2$) from 0 to 1 during 10 time units was sufficient. In other systems, finding the appropriate conditions on the perturbation might be more complicated. This issue was recently addressed in detail by Millat et al. (2008).

4. Discussion

Genome-scale gene regulatory networks are now available. Topological analyses of these networks show that they are modular and allow us to detect over-represented motifs (Alon, 2007). To decipher the function of these networks, it is essential to understand their dynamical properties. To achieve this goal, mathematical modeling is very helpful (Alon, 2006; Thieffry and Romero, 1999; Thieffry, 2007). Recently, synthetic biology, which is based on a combined theoretical and experimental approach (Judd et al., 2000; Hasty et al., 2001; Kobayashi et al., 2004), has been proven useful to characterize dynamical properties of small gene networks displaying oscillations or bistability. The Repressilator (Elowitz and Leibler, 2000) and the Toggle switch models (Gardner et al., 2000), originally used as guide models to construct artificial genetic systems displaying oscillations and bistability, are now used as prototypic models to investigate various properties of genetic switches and clocks, including their robustness to noise and synchronization abilities.

In real biological networks, clocks and switches are often interconnected. It is therefore necessary to analyse the type of behavior resulting from such coupled systems. Many models are already based on interlocked positive and negative feedback loops, the two circuits being embedded in each other. In these models there is usually one (or a few) variable(s) which is (are) involved in both circuits (Tsai et al., 2008; Kim et al., 2008). These models have highlighted many features of these complex networks including signal amplification, tunable frequency, robustness to noise, and reliable decision-making.
Fig. 5. Effect of the coupling parameters $A$ and $B$ on the oscillation-induced periodic switch. Other parameter values are as in Fig. 4. The $(A, B)$ parameter space can be divided in four different regions: (a) only when $B$ is small enough and $A$ sufficiently large, a periodic switch can be observed; (b) when both $A$ and $B$ are small, low-amplitude oscillations around the bottom branch are observed; (c) when both $A$ and $B$ are large low-amplitude oscillations around the top branch are observed; (d) when $B$ is large and $A$ small, birhythmicity is observed. In each region a schematic inset summarizes these behaviors.

In this paper, we have studied the dynamical properties resulting from the “control coupling” between a bistable system (the Toggle switch) and an oscillator (the Repressilator). By “control coupling”, we mean that one system is thus under the control of the other; there is no variable that belongs to both circuits. One system is thus under the control of the other. Such system can thus be seen as a master/slave system. We considered here two types of coupling. In the first type, the bistable switch is under the control of the oscillator. By means of numerical simulations, we show that self-sustained oscillations corresponding to a periodic switch between the two stable steady states can be induced in the Toggle switch. Our results also underline the importance of the amplitude of the coupling and the relative time scales of the clock and the switch dynamics. In addition we show how to generate birhythmicity in this coupled system. In the second type of coupling, the oscillator is placed under the control of the genetic Toggle switch. Analysis of the dynamics of this system shows that this construction could be exploited to generate a permanent transition from a stable steady state to self-sustained oscillations (and vice versa) or to alter permanently the period and amplitude of the oscillations after a transient perturbation.

Many genetic systems combine oscillatory and bistable mechanisms and may display some of the properties described here. The cell cycle is a typical example whose molecular mechanism involves multiple regulatory feedback loops. Theoretical models (Chen et al., 2004; Csikász-Nagy et al., 2006) were useful to elucidate the role of the various feedback loops and to understand how the dynamics of the full network results from the dynamics of elementary modules. In developmental biology, many models for somitogenesis are based on the “clock and wavefront” mechanism proposed by Cooke and Zeeman (1976). This conceptual model postulates the interplay between a clock and a bistable mechanism. Recently, molecular models describing these two aspects have been proposed (Goldbeter and Pourquié, 2008; Goldbeter et al., 2007) and attempts to couple the two mechanisms have been suggested (Baker et al., 2006; Santillán and Mackey, 2008). Besides genetic systems, signaling cascades have also been shown to contain positive and negative feedback loops, and theoretical studies have predicted that both bistability and oscillations could occur in these systems (Kholodenko, 2006). Although theoretical and abstract, the models analysed in the present study could guide further research on these (and other) complex regulatory networks.

Fig. 6. Effect of the period of the Repressilator on the oscillation-induced periodic switch. (A) When the period of the oscillations is too small, no switch is observed (unless the amplitude of the forcing is very large – not shown). Here, the coupling parameter values are as in Fig. 4A ($A=0.21$, $B=0$), but the period of the oscillations is not rescaled: $\tau = 1$ and the period is about 8.5. The corresponding time evolution is shown in panel (B). (C) When the period is very large, the switch can be induced as soon as parameter $a_1$ goes beyond the SN2 bifurcation. Here, the coupling parameter values are as in Fig. 4C ($A=0.19$, $B=0$), but the period is much longer: $\tau = 0.02$ and the period is around 450. The corresponding time evolution is shown in panel (D).
Fig. 7. Illustration of the second type of coupling: The Toggle switch controls the Repressilator. More specifically, gene X of the Toggle switch enhances the expression of gene 1 of the Repressilator (panel A). If the lower steady state of X corresponds to a non-oscillatory (i.e. steady) state of the Repressilator while the upper steady state is associated to oscillations, we should be able to induce (or stop) oscillations by a simple switch in the Toggle switch. If a permanent switch could be induced by a transient perturbation, we would thus be able to induce permanent oscillations by a single transient external signal (panel B). This mechanism is explored in the second part of the paper.

Fig. 8. A transient perturbation can either induce or stop permanently the oscillations. (A) A transient increase of $b_1$ induces a switch in the Toggle switch model: X is activated and stays at a high level even after the end of the perturbation. (C) Since X controls the parameter $\alpha_1$ of Repressilator in such a way that at low level of $X$, $\alpha_1$ stays below the Hopf bifurcation and at a high value of $X$, $\alpha_1$ is above the Hopf bifurcation, the activation of $X$ leads to limit-cycle oscillations. (B) A transient increase of $b_2$ induces the opposite switch in the Toggle switch model: X is deactivated and stays at a low level even after the end of the perturbation. (D) Consequently, once X is inactivated, limit-cycle oscillations are permanently stopped. On each panel, the grey shadow indicates the period of the perturbation. Data have been obtained with the same parameter values than those given in legend of Figs. 1 and 2, except $r=0.2$. Note that the latter parameter only affects the period of the oscillations but has no influence on the qualitative behavior of the system. The coupling parameter values are $A=50$ and $B=0$. In panels (A) and (C), the perturbation corresponds to an increase of $b_1$ from 0 to 1 during 10 (arbitrary) time units, between $t=100$ and $t=110$. In panels (B) and (D), the same perturbation is applied on $b_2$. 
The perturbation corresponds to an increase of \( X \) such a way that at both levels of model as in Fig. 8A. Here \( \beta \) has been obtained with the same parameter values than those given in legend of Figs. 1 and 2, except \( t = 110 \). The data of Fig. 9. A transient perturbation can alter permanently the amplitude and period of the oscillations. (A) A transient increase of \( b_1 \) induces a switch in the Toggle switch model as in Fig. 8A. Here \( X \) controls the parameter \( \alpha_1 \) of Repressilator in such a way that at both levels of \( \beta \), \( \alpha_1 \) is located inside the oscillatory domain. (B) Once \( X \) is activated, \( \alpha_1 \) is increased and the limit-cycle oscillations are permanently altered: both the period and amplitude of the oscillations are changed. Data have been obtained with the same parameter values than those given in legend of Figs. 1 and 2, except \( t = 0.2 \). The coupling parameter values are \( \lambda = 50 \) and \( \beta = 10 \). The perturbation corresponds to an increase of \( b_1 \) from 0 to 1, between \( t = 100 \) and \( t = 110 \).

The present model provides also a mechanistic explanation to a phenomenon encountered in circadian biology. Experiments in plants have shown the possibility to permanently arrest the oscillations by a pulse of light (Engelmann et al., 1978). Several mathematical models providing a possible explanation have already been studied. Winfree (1970) proposed that the perturbation brings the system close to a singularity point, while Leloup and Goldbeter (2001) proposed that the system presents a phenomenon of hard excitation (coexistence between a stable limit cycle and a stable steady state) and that the perturbation moves the system from the limit cycle to the steady state. Our results suggest an alternative mechanism for such an observation: through the coupling between an oscillator and bistable system, a transient perturbation could induce a permanent switch in the bistable system and through the coupling this transition could stop permanently the oscillations. This mechanism does not require a complex regulatory network producing hard excitation.

Finally, the present model could be used as a guide model for synthetic biology. The results described in this paper indeed provide suggestions to design a new synthetic biological system. Using controllable genes and assuming that we find a way to control the coupling between an oscillatory and a bistable system, we might be able to reproduce the dynamical properties described here in an artificial, yet experimental, system.

Acknowledgments

I would like to thank José Halloy for fruitful discussions and Karoline Faust for helpful comments on the manuscript. This work was supported by grant #3.4636.04 from the Fonds de la Recherche Scientifique Médicale (F.R.S.M., Belgium), by the European Union through the Network of Excellence BioSim (Contract No. LSHB-CT-2004-005137), and by the Belgian Program on Interuniversity Attraction Poles, initiated by the Belgian Federal Science Policy Office, project P6/25 (BioMaGNet).

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