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Coupling oscillations and switches in genetic networks

Didier Gonze

Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, CP 231, B-1050 Brussels, Belgium

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ABSTRACT

Switches (bistability) and oscillations (limit cycle) are omnipresent in biological networks. Synthetic genetic networks producing bistability and oscillations have been designed and constructed experimentally. However, in real biological systems, regulatory circuits are usually interconnected and the dynamics of those complex networks is often richer than the dynamics of simple modules. Here we couple the genetic *Toggle switch* and the *Repressilator*, two prototypic systems exhibiting bistability and oscillations, respectively. We study two types of coupling. In the first type, the bistable switch is under the control of the oscillator. Numerical simulation of this system allows us to determine the conditions under which a periodic switch between the two stable steady states of the *Toggle switch* occurs. In addition we show how birhythmicity characterized by the coexistence of two stable small-amplitude limit cycles, can easily be obtained in the system. In the second type of coupling, the oscillator is placed under the control of the *Toggle switch*. Numerical simulation of this system shows that this construction could for example be exploited to generate a permanent transition from a stable steady state to self-sustained oscillations (and *vice versa*) after a transient external perturbation. Those results thus describe qualitative dynamical behaviors that can be generated through the coupling of two simple network modules. These results differ from the dynamical properties resulting from interlocked feedback loops systems in which a given variable is involved at the same time in both positive and negative feedbacks. Finally the models described here may be of interest in synthetic biology, as they give hints on how the coupling should be designed to get the required properties.

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

E-mail address: dgonze@ulb.ac.be.

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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:

$$\frac{dM_i}{dt} = \tau\left(-M_i + \frac{\alpha_i}{1+P^m_{\mathrm{mod}(i+1,3)}}\right) \quad \text{with } i=1,2,3 \tag{1}$$

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

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 $\alpha_i/(1+P^m_{\mathrm{mod}(i+1,3)})$ where "mod" is the modulo function. Parameters α_i represent the maximum rate of mRNA synthesis of gene i. Parameter τ has been introduced to allow us to easily 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 α_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 $\alpha_{\mathrm{HB}} = 6.3$. Fig. 1D shows how the period is affected when parameter α_1 is changed within the oscillatory domain. The period of the oscillations slightly increases as α_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:

$$\frac{dX}{dt} = \frac{a_1}{1+Y^n} - d_1 X + b_1 \tag{3}$$

$$\frac{dY}{dt} = \frac{a_2}{1+X^n} - d_2 Y + b_2 \tag{4}$$

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 $a_1/(1+Y^n)$ and $a_2/(1+X^n)$ where a_1 and a_2 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 a_1 delimited by two bifurcation points, called "saddle nodes". These bifurcations, characteristic of a hysteretic behavior, are located at $a_{\mathrm{SN1}} = 1.4$ and $a_{\mathrm{SN2}} = 6.8$. 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 or the other stable steady sate (Fig. 2E).

When the value of a_1 is larger than the value of the second saddle node ($a_1 > a_{\mathrm{SN2}}$), 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 a_1, the trajectory does not jump immediately to the upper steady state, but stays transiently at a low value (Fig. 2F).