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Dynamical roles of biological regulatory circuits[1]

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Abstract

Regulatory circuits are found at the basis of all non-trivial dynamical properties of biological networks. More specifically, positive circuits are involved in the generation of multiple differentiated states, whereas negative circuits can generate cyclic or homeostatic behaviours. These notions are briefly reviewed, from initial biological formulations to mathematical formalisations, further encompassing their application to the design of synthetic regulatory systems. Finally, current challenges for the analysis of increasingly complex regulatory networks are indicated, as well as prospects for our understanding of development and evolution.

Keywords: *Regulatory circuits; multistability; homeostasis; oscillations; synthetic biology; systems biology; dynamics*

BIOLOGICAL FEEDBACKS

Most remarkable properties of biological regulatory networks can be related to the occurrence of (generally non-linear) regulatory *feedbacks*. In biochemistry, the role of *feedback inhibition* of catalytic enzymes by biosynthetic products has been underlined already 50 years ago [1]. In parallel, *positive feedback* was postulated to account for enzymatic induction phenomena in bacteria [2]. Soon, the distinction between regulatory and structural genes, together with the specification of a concrete mechanism for gene regulation (*operon model*), led Monod and Jacob [3] to imagine different regulatory schemes accounting for the generation of multiple cell differentiation states by a single genotype (Figure 1).

During the 1970s, molecular analyses and modelling studies progressively enabled general characterisations of simple biochemical and genetic networks [4–6].

Notably, proper definition and classification of *regulatory circuits* (or *feedback loops*) were proposed by Thomas [7], together with general rules about circuit requirement to generate non-trivial dynamical behaviour. Indeed, defined as simple circular chains of oriented interactions, feedback circuits can be classified into positive or negative circuits, depending on the parity of the number of negative interactions (i.e. the sign of a circuit is given by the product of the signs of its constitutive interactions). *Positive circuits* are necessary to generate alternative cellular states (multiple *attractors*, using the terminology of *Dynamical Systems*), whereas *negative circuits* are needed to generate *homeostasis* or sustained oscillatory behaviour (Figure 2; cf. [8] for an extensive review).

FORMAL DEFINITIONS OF FEEDBACK CIRCUITS

The biological regulatory schemes found in the literature encompass arrows representing different types of molecular processes, e.g. metabolic reactions, transcriptional regulation, degradation, transport, etc. In order to investigate the dynamical roles of regulatory circuits, such regulatory schemes have to be translated into an homogenous mathematical formalism. In the present context, modellers often refer to a qualitative formalisation in terms of *graphs* or *logical equations*, or yet, to a quantitative representation in terms of *ordinary differential equations (ODEs)*.

In the case of a graph-based representation, the notion of regulatory circuit directly derives from the

[1]This article is dedicated to René Thomas, for his inspiring pioneering contributions to Systems Biology

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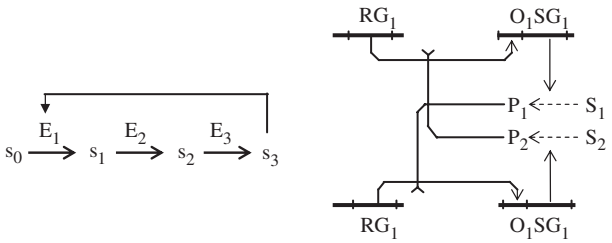

Figure 1: Examples of biochemical feedback (left) and genetic regulatory circuits (right, cf. [3]). S_i, E_i and P_i denote substrates, catalytic enzymes, and products, respectively; RG_i and SG_i denote regulatory and structural genes. Note that the enzymatic reaction on the left is considered as irreversible for sake of simplicity; in this metabolic pathway, the arrow from S_3 to E_1 represents a regulatory effect called *feedback*. The regulatory scheme on the right encompasses arrows with different meanings: regulatory interactions (solid arrows) and enzymatic reactions (dotted arrows); P_1 (resp. P_2) inhibits the activation of the expression of the structural gene SG_2 (resp. SG_1) by the regulatory gene RG_2 (resp. RG_1), thereby forming a cross-inhibitory, *positive regulatory circuit*.

	Positive circuits	**Negative circuits**
Dynamical properties	Maximal level / Bottom level	
Biological properties	Differentiation	Homeostasis/Oscillations
Number of negative interactions	Even	Odd
Examples	cI cro	tat rev

Figure 2: Classification of regulatory circuits. Depending on the parity of the number of negative interactions involved, regulatory circuits can be classified into positive versus negative circuits, endowed with remarkably different dynamical and biological properties. The last row provides simple examples of positive and negative circuits extracted from regulatory networks controlling the development of bacteriophage lambda and HIV virus, respectively. Normal arrows represent activations, blunt arrows inhibitions.

graph-theoretic *circuit* concept (interaction signs are then defined as arc labels). For given logical rules specifying the behaviour of each regulatory components, it is possible to induce the regulatory interactions, including their signs (positive, negative or yet dual, i.e. context-dependent sign) (cf. [9]; for another qualitative approach using piece-wise differential equations, see [10]).

In the differential case, regulatory circuits can be rigorously defined on the basis of the *Jacobian matrix* of the ODE system ($x_i' = F_i(\vec{x})$, stating that the evolution or the *derivative* of a component i depends on the levels of other components of the network, represented by a *state vector* $\vec{x}$). Indeed, the terms ($a_{ij} = \partial F_i / \partial x_j$) of this matrix provides information about pair-wise influences (from regulatory component j onto component i). Consequently, any set of non-zero elements a_{ij} of the Jacobian matrix of the differential system, such that the i (row) and j (column) indices form a circular permutation, defines a regulatory circuit, whose sign is given by the product of the signs of these elements (cf. example in Figure 3). Note that, for most biological models, the consideration of passive, linear degradation of molecular components gives rise to negative diagonal terms and thus to auto-inhibitory circuits.

The Jacobian matrix is most often defined at the level of a specific steady state to determine its dynamical properties (*linear stability analysis*; see e.g. [11]). In this respect, it is worth noting that the mathematical objects used for steady state characterization (*characteristic equation*, *eigenvalues*) exclusively rely on terms involved in feedback circuits. However, this does not impede other terms to play important roles in the location or the number of steady states (for a more detailed discussion on the relationship between the Jacobian matrix and steady state properties, see [12]).

FROM THOMAS' RULES TO MATHEMATICAL THEOREMS AND DEMONSTRATIONS

Thomas' rules [7] have attracted the interest of a number of mathematicians, who translated them into proper theorems. In the differential context, a series of increasingly general theorems have been proposed, stating the necessity of a positive circuit to generate multistationarity [13–17], or the necessity of a negative circuit to generate a cyclic attractor [14–16]. More recently, the same rules have been demonstrated in a biologically relevant, discrete, multilevel framework, considering transitions between discrete states affecting at most one component at a time, switching it to a neighbouring value (i.e. the *Hamming distance* between two following states is exactly 1) [9, 18].

$$\begin{cases} \dfrac{dx_1}{dt} = \kappa_{12} {x_2}^3 + \kappa_{13} {x_3}^3 - \gamma_1 x_1 \\ \dfrac{dx_2}{dt} = \kappa_{21} {x_1}^3 - \gamma_2 x_2 \\ \dfrac{dx_3}{dt} = -\kappa_{32} {x_2}^3 - \gamma_3 x_3 \end{cases}$$

$$A_{ij}\left[\frac{\partial F_i}{\partial x_j}\right] = \begin{bmatrix} -\gamma_1 & 3\kappa_{12}{x_2}^2 & 3\kappa_{13}{x_3}^2 \\ 3\kappa_{21}{x_1}^2 & -\gamma_2 & 0 \\ 0 & -3\kappa_{32}{x_2}^2 & -\gamma_3 \end{bmatrix}$$

Figure 3: Example of system of three ordinary differential equations (top), with its Jacobian matrix (bottom). The dynamical variable x_i denotes the concentration or activity level of a regulatory product, whereas κ_{ij} and γ_i denote (constant and strictly positive) kinetic parameters. According to these equations, the first gene (whose product level is given by x_1) activates the expression of the second gene (x_2), which in turn inhibits the third gene (x_3); finally, genes 2 and 3 both activate the first gene independently (sum). All regulatory products are further linearly degraded (i.e. proportionally to product concentration). In the bottom panel, the Jacobian matrix of this ODE system is displayed. Each term a_{ij} of this matrix gives the partial derivative of the evolution term i with respect to the variable j. As all these terms involve only positive parameters and variable squares, they have fixed signs. The system encompasses five circuits indicated by dotted circles and arrows on the matrix: three one-element negative circuits, one two-element positive circuit involving genes 1 and 2, and one three-element negative circuit.

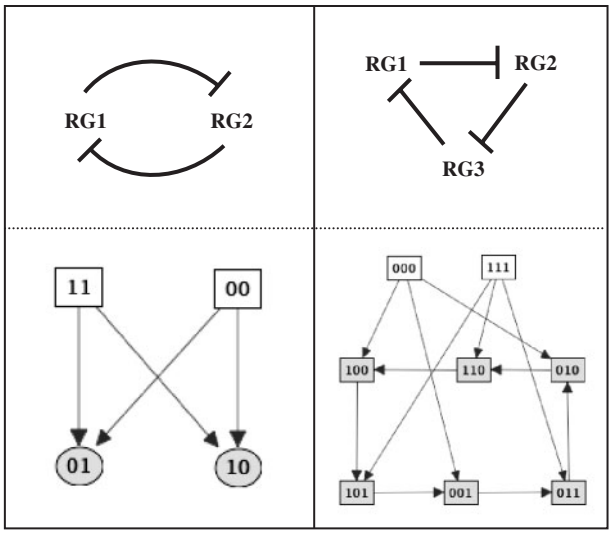

Figure 4: Typical dynamical behaviour dynamics of isolated feedback circuit dynamics in the simplest discrete (Boolean) case. Top: Examples of positive (left) and negative (right) regulatory circuits. Bottom: Corresponding state transition graphs. The two-element positive circuit, made of cross-inhibitions (upper left), gives rise to two alternative stable (circled grey states) with opposites gene-level configurations (01 denotes a state with gene 1 OFF, gene 2 ON). In contrast, the three-element negative circuit, made of consecutive negative regulations (upper right), gives rise to an attractive state transition cycle, composed of six successive states (grey states in the bottom left panel). These dynamical properties are typical of the corresponding (positive versus negative) circuit class, whatever the number of components or the precise regulatory sign configuration (only the circuit sign matters).

Lately, Thomas has proposed a third rule, stating that a negative circuit is necessary to have an attractor in the case of a differential system [12].

Altogether, these rules and theorems specify *necessary conditions*, which can be directly applied to specific networks. For example, whatever the complexity of the network considered, if it encompasses no positive circuit, one can directly conclude that the system can generate at most one attractor. Similarly, in the absence of negative circuit, one cannot expect a cyclic attractor.

However, the sole presence of a circuit in a regulatory graph (or in the Jacobian matrix of a differential system) does not necessarily imply the corresponding dynamical behaviour. Indeed, it is well known that at least some non-linearity is further necessary in continuous systems, as well as specific constraints on relevant parameters (kinetic parameters in the case of differential systems, logical rules directing component behaviours in the case of discrete systems). In the differential framework, these conditions can be established through the analysis of the sensitivity of specific state properties depending on crucial parameter values (*parameter bifurcation analysis*, cf. [19] and [20]). In the discrete case, it is possible to specify necessary conditions to obtain non-trivial dynamical behaviour with isolated (*functional*) feedback circuits, whatever the number and signs of involved interactions (see examples in Figure 4) [21].

ENGINEERING OF SYNTHETIC REGULATORY CIRCUITS

The appreciation of the crucial dynamical roles of regulatory circuits in real systems was at the root of the design of synthetic genetic circuits endowed with specific dynamical properties, including a cross-inhibitory positive circuit [22], a negative circuit

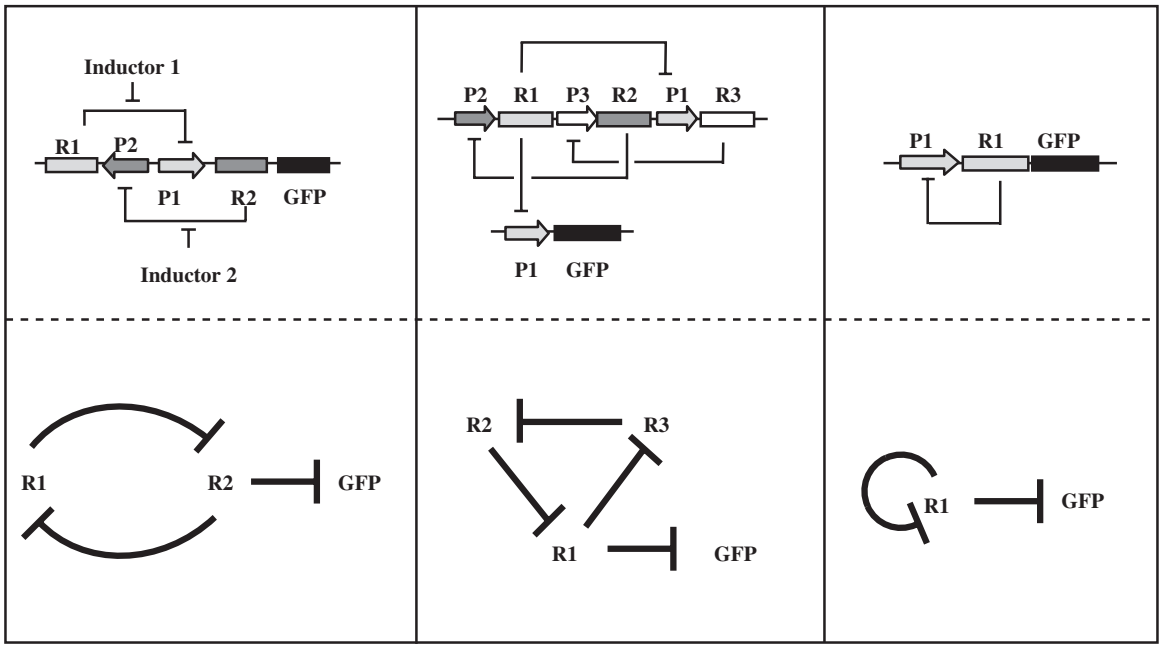

Figure 5: Design of simple regulatory circuit in bacteria. Using genetic components from bacteria or phages, several groups have built regulatory circuits with specific dynamical properties. Left: positive circuit involving two cross-inhibitory genes, giving rise to two alternative stable states and induction memorisation [22]. Middle: negative circuit involving three consecutive inhibitions, leading to oscillatory gene expression for proper degradation and synthesis coefficients [23]. Right: self-inhibitory circuit, leading to homeostatic expression of the auto-regulated gene (increased stability of gene expression in individual cells, decreased expression variability across cells, as well as buffering of copy number effects) [24]. The logical schemes corresponding to these genetic constructions (upper part) are given in terms of regulatory graphs (bottom part). The gene coding for the Green Fluorescent Protein (GFP) is used to reveal the gene expression state *in vivo*. RI, R2, R3 denote regulatory genes, whereas PI, P2, P3 denote the promoters sensitive to the corresponding regulatory genes; finally, blunt arrows represent inhibitory interactions.

made of the sequential inhibitions of three repressor genes [23], as well as a simple auto-inhibitory circuit [24] (see Figure 5; an example of circuit engineering in Yeast is found in [25]; for a review, see [26]).

A whole new field is presently emerging from these seminal studies, *Synthetic Biology*, which combines relatively standard molecular genetic protocols with mathematical modelling, under an engineering perspective.

In this respect, the MIT promotes a yearly international competition (*iGEM*) on biological circuit engineering, and organises the distribution of a growing collection of basic molecular genetic components or *modules*, which can be further combined with self-engineered regulatory modules *in vivo* to produce more sophisticated regulatory systems.

DISENTANGLING COMPLEX NETWORKS

As molecular data on regulatory components and interactions are quickly accumulating, biologists are facing increasingly complex networks, which are defying not only our intuition, but also standard modelling approaches.

In this context, an important challenge lies in the development of strategies to decompose large networks into functional *cross-regulatory modules*, thereby enabling stepwise dynamical modelling and analysis, while managing procedures to reassemble sub-models into a comprehensive model and to efficiently compute collective properties.

In this respect, a first instructive step would be to systematically identify the feedback circuits at the origin of remarkable dynamical properties of complex networks (*functional circuits*). Several groups are currently addressing this issue, either in terms of novel algorithmic developments, or through the formulation of mathematical theorems (*sufficient conditions* for circuit functionality). Preliminary results in the discrete framework suggest that only a relatively small fraction of (often short) regulatory circuits are functional in large networks, but that

intertwined circuits might collectively contribute to generate specific properties (e.g. multistability, cf. [27, 28]).

This last point leads to the question of whether other, higher level feedback structures should be systematically defined, which could be endowed with more sophisticated dynamical properties. Indeed, combinations of feedback circuits have already been evocated in the context of steady-state linear stability analyses for biological systems [29]. More recently, working on abstract dynamical systems, Thomas and Kaufman [12] have introduced the concept of *nucleus*, defined as a combination of disjoint circuits, which together involve all the components of a regulatory system. In differential systems, these nuclei appear to play a crucial role in the specification of steady-state properties. However, the significance of these nuclei and their potential practical application to systems biology remain to be properly assessed.

Finally, as our knowledge progresses and diversifies, it becomes possible to compare the design of regulatory networks controlling similar dynamical processes in different organisms, or yet similar processes at different time or place in the same organism. Such comparative approach is already underway in the case of networks controlling cell cycle [30]. Tentatively, comparative model analyses focusing on network design in relation with dynamical behaviour should ultimately contribute to our understanding of the essential molecular processes at the basis of biological development and evolution.

Key Points

- Regulatory circuits are at the basis of non-trivial dynamical properties of biological networks. They can be classified into positive versus negative circuits, depending on the sign of the product of the signs of their constitutive interactions.
- Two general rules relate the presence of signed circuits with specific dynamical properties.
- First, positive regulatory circuits are needed for multistability and cell differentiation.
- Second, negative circuits are necessary to generate sustained oscillations and homeostasis.
- Regulatory circuits can be formally defined in differential or discrete formalisms, leading to proper mathematical theorems and demonstrations.
- Knowledge of feedback circuit properties inspires the design of synthetic regulatory networks.
- Further (de)composition and analysis methods are needed to disentangle complex networks.
- Comparative topological and dynamical analyses of regulatory networks are expected to enlighten developmental and evolutionary processes.

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