Competition for Catalytic Resources Alters Biological Network Dynamics

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(Received 28 July 2011; published 5 January 2012)

Genetic regulation networks orchestrate many complex cellular behaviors. Dynamic operations that take place within cells are thus dependent on the gene expression machinery, enabled by powerful enzymes such as polymerases, ribosomes, or nucleases. These generalist enzymes typically process many different substrates, potentially leading to competitive situations: by saturating the common enzyme, one substrate may down-regulate its competitors. However, most theoretical or experimental models simply omit these effects, focusing on the pattern of genetic regulatory interactions as the main determinant of network function. We show here that competition effects have important outcomes, which can be spotted within the global dynamics of experimental systems. Further we demonstrate that enzyme saturation creates a layer of cross couplings that may foster, but also hamper, the expected behavior of synthetic biology constructs.

DOI: 10.1103/PhysRevLett.108.018102 PACS numbers: 87.18.Cf

Every cell contains a sophisticated catalytic platform dedicated to the production of its necessary components. For example, the biological production of proteins relies on the cell's genetic expression machinery, involving polymerases, ribosomes, nucleases, proteases, and many other modifying enzymes or cofactors.

It is, however, now well recognized that this gene transcription and translation apparatus is not only devoted to producing proteins, but can also enable the functioning of molecular networks, whose task is mostly of a computational nature [1]. Such information-processing molecular architectures orchestrate a variety of complex dynamic behaviors within cells: oscillating systems are used to clock the various biological rhythms [2]; multistable dynamic networks allow the switching between various states, providing both a memory of past events and a way to commit to a particular cell fate [3]; other systems, akin to Boolean circuits, compute molecular answers in response to complex combinations of environmental stimuli, etc. [4].

Synthetic biology, i.e., the harnessing of the cell's inner machinery to perform man-made reaction networks, has been instrumental in demonstrating this dual material or informational nature of gene expression. Indeed, some of the first demonstrations of this field were devoted to producing devices such as clocks [5], switches [6], or logic gates [7]. These studies have shown that it is possible to hijack the cell's catalytic platform to process non-natural computations, rationally programmed in DNA through the spatial arrangement of genes and promoters.

The reuse of a machinery primarily dedicated to producing matter for information-processing tasks implies some specific tuning. For example, the limited lifetime of proteins, which, from a productive point of view, may be seen only as a deleterious side effect (because it hampers efficient production), becomes a crucial cornerstone for the global functioning of, e.g., a genetically regulated oscillator [8]: in this case, the dynamic decay of proteins becomes compulsory in order to let the system cycle back to its initial state. The importance of controlling such destructive processes has been recognized in the early days of synthetic biology, and proteins were explicitly modified to accelerate their enzymatic degradation [5]. Another well-recognized subtlety arises from the limited load capacity of enzymatic processes. This saturation effect, known as Michaelis-Menten kinetics, has important nonlinear consequences on the system's dynamics, in some cases enabling interesting properties like zero-order ultrasensitivity [9].

On the contrary, it has gone mostly unnoticed that, given the modular nature [10] of genetic regulation, saturation of one enzyme by a given substrate will in general lead to a competitive situation, and globally affect the conversion of other substrates. Indeed, the architecture of genetic networks implies that the same catalytic resource (e.g., RNA polymerase) will typically be required simultaneously by different components (e.g., genes) of the network. When the enzyme saturates, the processing rate of one given substrate becomes dependent on the activity of the other compounds sharing the enzymatic pathway, even if they are not linked by obvious interactions. Although such competition for catalytic resources [11] may have potentially important consequences on the system dynamics of synthetic biology constructs, its outcomes have seldom been investigated [12–14]. Most synthetic biology systems, and their associated ordinary differential equation models, rather consider the host cell as an ''ideal chassis'' [15] with the underlying assumption that competitive rate-coupling effects should have only minor effects on the global dynamic of the system.

In this Letter, by reanalyzing the data of a previously published example, we show on the contrary that competition effects do have important dynamic consequences, affecting the global behavior of the system. Furthermore

we propose that these coupling terms do not necessarily hamper the rational engineering of artificial networks and that their consequences can be predicted—at least qualitatively—using simple rules.

All enzymes have a limited capacity, because there exists a maximum rate (V_i) at which they can process their substrates (x_i). Concentrations of substrates above a given limit (K_i) lead to a saturated situation, with the processing speed reaching its plateau. This well-known phenomenon is generally described in mass action kinetics by the Michaelis-Menten equation (assuming low enzyme concentration and validity of the quasisteady state approximation).

$$\dot{x}_i = \frac{V_i x_i}{K_i + x_i}. \tag{1}$$

When the substrate concentration stays well below the K_i, the situation simplifies to a first-order kinetic term ($\dot{x}_i = \frac{V_i}{K_i} x_i$). On the contrary, at high substrate concentration ($x_i \gg K_i$), the rate equation reduces to a constant term and the concentration of the substrate follows a linear evolution (zeroth-order kinetics; $\dot{x}_i = V_i$).

However, this approximation is only valid if a single substrate uses the enzymatic pathway. For a network of reactions within a cell, where a given catalytic resource is typically shared by more than one component, the situation becomes more complicated. The different substrates competitively inhibit each other and one must add an extra term to the denominator of the Michaelis-Menten equation (1), describing this competition.

$$\dot{x}_i = V_i \frac{x_i}{K_i(1 + \sum_j \frac{x_j}{K_j})}. \tag{2}$$

For concentrations of substrates well below their respective K_i, the previous first-order approximation remains valid, and each substrate reacts independently of the presence of the others: this situation is not affected by competition. However, when the enzyme gets closer to saturation, the zeroth-order approximation does not hold any more. Instead, the substrates start to compete and their degradation rates are directly dependent on each other. If one further assumes the same and small K_i for all substrates, (2) reduces to (3), meaning that the degradation rate of each substrate becomes simply proportional to its fraction in the pool. Therefore, saturation creates a supplementary layer of "hidden," noncanonical coupling between the species.

$$\dot{x}_i = V_i \frac{x_i}{\sum_j x_j}. \tag{3}$$

This phenomenon, and its potentially confusing nature, is nicely exemplified by a recent paper (Wong *et al.* [16]). The authors studied the degradation kinetics of the three proteins involved in a synthetic gene-metabolic oscillator called the metabolator [17][Fig. 1(a)]. They report that the enzyme responsible for this degradation saturates for very low substrate concentrations: hence each substrate, taken individually in the absence of the others, follows zeroth-order kinetics.

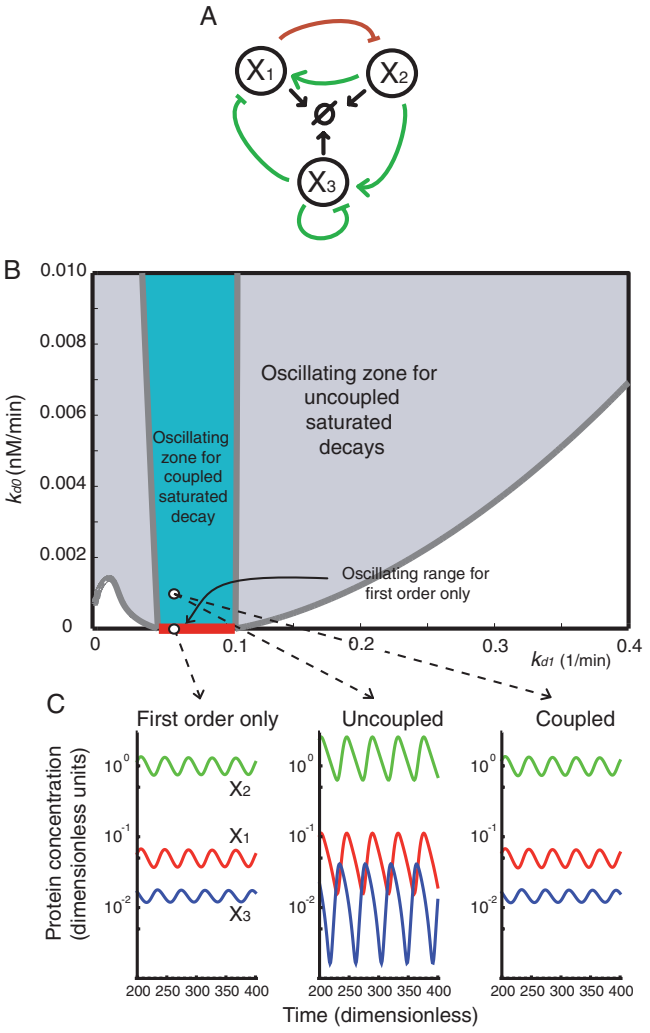

FIG. 1 (color). Oscillations in the metabolator. (A) simplified representation of the network. Only the 3 protein components are represented (X_1, LacI; X_2, Pta; X_3, Acs). Red (green) links show genetic (metabolic) regulations. Black arrows express the protein decay reactions, with the kinetic hypotheses given in Table I. (B) Bifurcation diagram showing the effect of saturated versus first-order degradation: in the pure first-order case (horizontal axis), oscillations are observed for a small range of k_{d1} values (in red). If one adds uncoupled saturated degradation, oscillations expand to the gray area (reported by Wong *et al.*). However, the correct description of the system (coupled saturated degradation) shrinks the oscillatory zone back to its original range (blue area). (C) Time evolution of the protein concentrations for the three decay hypothesis (parameter sets corresponding to the associated points in the bifurcation diagram). Note the log scale.

TABLE I. Kinetic hypotheses and models

Model	Dilution term	Enzymatic term	Global decay
First order only (Fung *et al.* [17])	$k_{d1}x_i$	$k_e x_i$	$k_d x_i = (k_{d1} + k_e)x_i$
Uncoupled saturated (Wong *et al.* [16])	$k_{d1}x_i$	$\frac{k_{d0}x_i}{K+x_i}$; $K \ll x_i$	$k_{d1}x_i + \frac{k_{d0}x_i}{K+x_i}$; $K \ll x_i$
Coupled saturated (this work)	$k_{d1}x_i$	$\frac{k_{d0}x_i}{\sum x_i}$	$k_{d1}x_i + \frac{k_{d0}x_i}{\sum x_i}$

In the metabolator, the three proteins of the network use the same degradation route: this is a typical case for competition. Nevertheless, Wong *et al.* dismiss the strong couplings that occur in their system and maintain the ideal chassis hypothesis, with zeroth-order kinetics. Under this assumption they theoretically predict that the oscillatory robustness of the system increases because of saturation: in Fig. 1(b), the initially restricted range of pure first-order decay leading to oscillations strongly expands when zeroth-order degradation terms are introduced. However, including the coupling effect in the mathematical model leads to a completely different conclusion: the oscillating range does not expand any more. The beneficial effect of zeroth- versus first-order degradation has been cancelled out by the coupling term arising from saturating the common degradation pathway. This is a clear example where an unintended overload of the catalytic machinery creates couplings and impacts the targeted dynamics.

A simple understanding of what happens in the metabolator can be gained from the time evolution of the system [Fig. 1(c)]. In the oscillating area of the parameter space, the protein pool is strongly dominated by X_2. Therefore, the uncoupled zeroth-order (concentration-independent) term would marginally affect the degradation rate of this high concentration species, but would boost the oscillating amplitude of low concentration X_1 and X_3, thereby stabilizing the oscillations. In the correct (coupled) case, however, the coupling term ($\sum x_i$) is always dominated by x_2 ($\sum x_i \approx x_2$), and the degradation of X_1 and X_3 reverts to a first-order case ($\frac{V_1 x_1}{\sum x_i} \approx \frac{V_1 x_1}{x_2} \approx V_1' x_1$), with no global effect on oscillatory robustness.

This example is not anecdotal since many other synthetic systems use the same saturated degradation route [5,18,19] and may be prone to similar competition effects [12]. Saturated degradation has also been observed in natural oscillatory networks [8] and other sources of nonlinear degradation have been proposed as well [20].

Furthermore, while we have focused here on the degradation process, we note that saturation can occur at any other stage of gene expression [2,21]—e.g., mRNA or protein polymerization and maturation—which would also lead to competitive couplings. Some reports on such effects *in vivo* have been published, describing these nonideal behaviors at various levels of protein expression [22]: high-copy-number plasmids may overload the cell transcription or translation machinery and lead to changes in its physiology [23,24]; sigma factor regulation builds on such limited availability of catalytic resources as a way to down-regulate a given set of genes, depending on the growth phase [25].

In these cases, the dynamics of the systems is controlled not only by the architecture of the genetic network, which is somehow the central precept of synthetic biology, but also by coupling effects due to the limits of the enzymatic resources themselves. In the metabolator, the outcome is obviously deleterious with regard to the targeted function. One may however wonder whether this negative impact is a general rule. If not, is it possible to rationalize and predict the effects of this hidden layer of interactions on the dynamics of the system? In order to qualitatively address these questions, we propose to consider that competitive saturation creates a supplemental parallel layer of interactions between the elements of the network. Below we exemplify this idea on two simple hypothetical models of oscillating systems (see also Supplemental Material [26]).

In the first example, three compounds X_1, X_2 and X_3 repress each other, forming a global negative feedback loop. The bifurcation diagram [Fig. 2(a)] shows that, while simple (uncoupled) zeroth-order degradations again promote oscillations, in the competitive case the oscillations are gradually suppressed. This observation may be rationalized as follows: when the substrates compete for degradation, their individual decay rates are decreased by the accumulation of their competitors. For example, X_1—which inhibits the production of X_2 through regulatory links—also decreases the X_2 decay rate, by saturating their common degradation pathway. Because decreasing the decay rate of a substrate amounts to positively regulating it, this hidden coupling has an effect opposite to that of the main regulatory link. The same arguments hold for X_2 and X_3. The resulting schizophrenic situation is intuitively not favorable and may explain why the increasing dominance of the competitive pathway leads to decreased robustness of the oscillator.

In the second example [Fig. 2(b)], X_1 negatively regulates its own production with a delay, but is also engaged in a positive feedback loop with X_2, in a topology that may lead to oscillations [18]. The bifurcation diagram of this system shows that oscillations—that are not observed for purely first-order degradation—emerge in both cases of independent or competitive saturated degradation of the proteins. In the latter case, one can argue that the

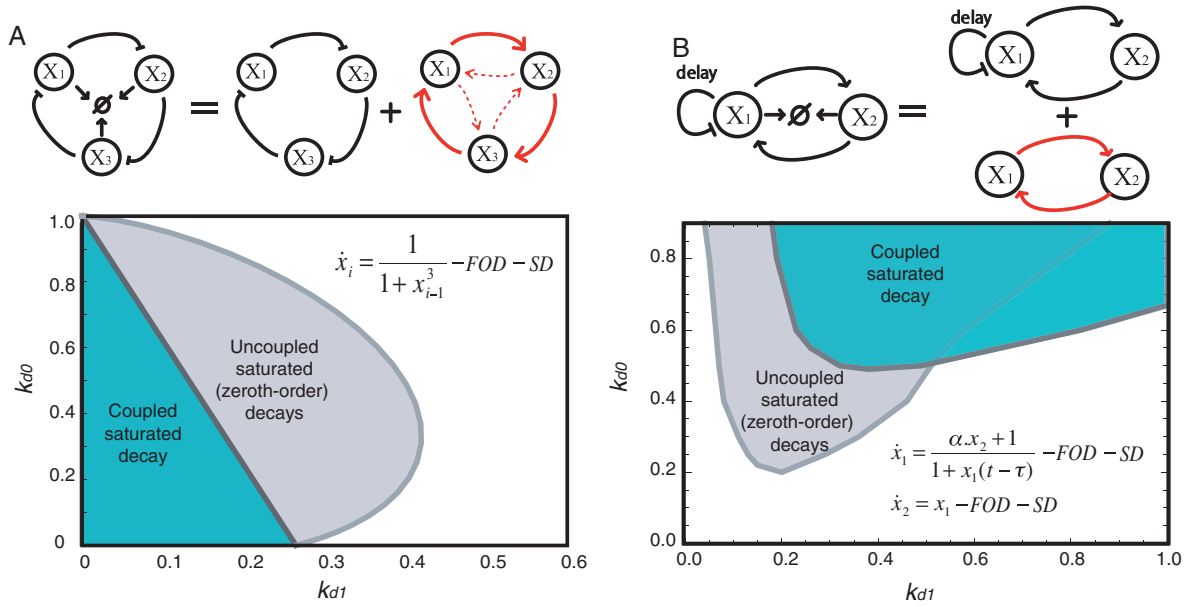

FIG. 2 (color). Bifurcation diagrams showing the effect of the saturated degradation kinetic on two nondimensional mathematical models of oscillatory networks. The first-order decay term (FOD $= k_{d1}.x_i$) represents, for example, the dilution due to growth. The saturated decay term [SD $= k_{d0}.x_i/(K + x_i + \xi\Sigma x_{j\neq i}$] with $K = 10^{-5}$) assumes $\xi = 0$ in the uncoupled case and $\xi = 1$ in the coupled (competitive) case. The network schematic above the plots shows how the various interdependencies between the proteins' evolution rates can be separated in two layers, one arising from genetic regulation and another from competitive degradation. (A) Three-node negative feedback loop. The oscillating range initially increases for small values of uncoupled zeroth-order degradation (in gray) but clearly decreases in the competitive case. (B) Two-node positive and delayed negative feedback system. With parameter $\alpha = 0.1$ and $\tau = 20$, both the coupled and uncoupled decays appear to allow oscillations (which cannot be observed with a pure first-order decay).

accumulation of X_1, which positively regulates the synthesis of X_2, also competitively inhibits X_2 destruction. Here, competition for the degradation pathway and regulatory links create similar patterns of interactions and reinforce each other to form a positive feedback loop, hence promoting the oscillations. Thus competitive couplings are not necessarily deleterious but can be harnessed to achieve the targeted behavior (e.g., [13]).

In conclusion, depending on which components share a given pathway, but also on the structure of the network, competitive degradation can either promote or suppress the targeted behavior. In simple cases, one can rationally understand and predict these consequences by using some qualitative rules of thumb: if the network of positive or negative interactions drawn by such competitive saturation effects tends to reinforce the structure of the main genetic regulatory links, then the intended behavior may be promoted. On the contrary, if both networks conflict on one or more vertices, the hidden coupling may hinder the expected dynamics. In more complex or realistic networks, such as the metabolator, these arguments need to be combined with some quantitative knowledge of the system: strong differences in the relative concentrations of the compounds may bring back the system to a simpler, pseudouncoupled case.

Here, we have mostly referred to *in vivo* artificial networks, but similar coupling effects may as well play an important role in natural systems [22,25] or synthetic *in vitro* networks [13,27]. We anticipate that the analysis of such effects at various stages and in various implementations of molecular reaction networks will lead to rational rules similar to those presented here, that will be integrated in the design toolbox and used to build smarter, more compact and functional systems [23].

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