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Growth-Induced Instability in Metabolic Networks

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Abstract

Product-feedback inhibition is a ubiquitous regulatory scheme for maintaining homeostasis in living cells. Individual metabolic pathways with product-feedback inhibition are stable as long as one pathway step is rate limiting. However, pathways are often coupled both by the use of a common substrate and by stoichiometric utilization of their products for cell growth. We show that such a coupled network with product-feedback inhibition may exhibit limit-cycle oscillations which arise via a Hopf bifurcation. Our results highlight novel evolutionary constraints on the architecture of metabolism.

Networks of molecular interactions are essential for mass, energy, and information transport into and within cells. Thus, understanding the emergent physical properties of various network architectures is of fundamental interest in biology [1,2]. In general, networks with as few as three species can exhibit complicated dynamical behavior ranging from multiple steady states, to oscillations, to chaos. On the other hand, considerations of homeostasis require that intracellular networks remain dynamically stable over a wide range of inputs and parameters. One ubiquitous network architecture is product-feedback inhibition [3,4], a metabolic regulatory scheme in which an end product inhibits the first dedicated step of the chain of reactions leading to its own synthesis (Fig. 1). Product-feedback inhibition implements negative feedback and is thus homeostatic. However, a pathway regulated by product feedback may become unstable due to time delays as the number of intermediates between substrate and product increases [5,6]. In real biosynthetic pathways, intermediate reactions are typically fast, avoiding such time-delay-induced instabilities.

In a cell, biosynthetic pathways are coupled both by the use of common substrates and by the stoichiometric utilization of their products for cell growth. In this work, we show that such a coupled network, regulated by product-feedback inhibition, can surprisingly become unstable even if the branches are individually stable. In the unstable region, the coupled network exhibits limit-cycle oscillations which arise via a Hopf bifurcation. However, we find that stability is guaranteed if the branches are sufficiently symmetric in their affinity for substrates or in their stoichiometry of product utilization. This finding has implications for stable, growth-coupled synthesis of proteins, nucleic acids, and cell-surface polymers in growing cells. Our results highlight novel evolutionary constraints on the overall architecture of metabolism.

We consider networks with the product-feedback architecture shown in Fig. 1, but, for simplicity, with no intermediates. For our purpose the lack of intermediates is equivalent to the first step of each pathway being rate limiting for product formation. The three essential features of the network are that products are synthesized from a common substrate, each product inhibits its own synthesis, and all products are essential for growth. As an example, the substrate might be the nitrogen carrying amino acid glutamine with the products including other amino acids, purines (A,G), pyrimidines (C,T,U), etc. A schematic of a two-branch network is shown in the upper inset of Fig. 2. The total rate of conversion α_i from substrate s to product p_i is governed by Michaelis-Menten kinetics [7] with allosteric inhibition by the product p_i ,

$$\alpha_i = \alpha_i^{\max} \frac{s}{s + K_{mi}} \frac{K_{di}}{p_i + K_{di}}, \quad (1)$$

Where $\alpha_i^{\max}$ is the maximal rate of conversion, K_{mi} is the Michaelis-Menten constant for the enzyme-substrate complex, and K_{di} is the dissociation constant for the (allosteric) enzyme-product complex.

Any model for growth rate g should satisfy the following plausible constraints: g is a monotonically increasing function of each product pool, g approaches zero if any product pool approaches zero, and g becomes asymptotically independent of each product pool p_i above a saturating pool size p_i^* . The functional form for g used here is

$$g = \frac{g_{\max}}{\left(\frac{1}{N} \sum_{i=1}^N \frac{p_i + p_i^*}{p_i} \right)}, \quad (2)$$

where $g_{\max}$ is the maximal rate of growth.

The kinetic equations describing an N -branch product-feedback network with no intermediates are

$$\frac{ds}{dt} = \alpha_0 - \sum_{i=1}^N \alpha_i \quad (3)$$

$$\frac{dp_i}{dt} = \alpha_i - g c_i \quad (4)$$

where α_0 is the constant input flux of the substrate s , c_i is the stoichiometry factor for the usage of product p_i , and N is the number of branches in the network. For simplicity, we neglect the dynamics of the input flux α_0 , assuming a constant supply of nutrients.

Possible steady states of the network are obtained by setting the above time derivatives to zero, yielding

$$\alpha_i(p_i, s) = g c_i \quad (5)$$

$$g(\{p_i\}) = \alpha_0 / \sum_{i=1}^N c_i \quad (6)$$

Next we show that there exists at most one solution of these $N + 1$ steady-state equations in $N + 1$ variables. Considering the substrate pool s as an independent variable, the p_i are monotonically increasing functions of s ,

$$p_i = K_{di} \left(\alpha_i^{\max} \frac{s}{s + K_{mi}} \frac{\sum_{j=1}^N c_j}{\alpha_0 c_i} - 1 \right), \quad (7)$$

$$p_i^{\max} = p_i(s \rightarrow \infty) = K_{di} \left(\alpha_i^{\max} \frac{\sum_{j=1}^N c_j}{\alpha_0 c_i} - 1 \right). \quad (8)$$

These equations for the p_i s as functions of s determine a one-dimensional curve in the p_i coordinate system, and this curve intersects the constant-growth surface Eq. (6) to give the steady-state solution(s) of the network. If there is no intersection, then the network has no steady-state solution; this could happen if the maximum of any of the product pools from Eq. (8) is negative and/or the growth rate g evaluated at the maximum pool sizes is smaller than the value of g in Eq. (6), set by the input flux. These conditions can be translated to the following inequalities that ensure at least one steady state

$$p_i^{\max} > 0, \quad (9)$$

$$g(\{p_i^{\max}\}) \geq \alpha_0 / \sum_{i=1}^N c_i \quad (10)$$

Uniqueness of the steady state follows because g increases monotonically with the p_i , which in turn increase monotonically with s , so the one-dimensional curve can intersect the constant-growth surface at most once. The existence of at most one steady state also applies to a product-feedback network with intermediates.

However, coupling via growth could make the steady state unstable. For the case of N symmetric branches, at the steady state, the Jacobian matrix is given by

$$J = \begin{pmatrix} -Nx & y & y & \dots \\ x & -y-z & -z & \dots \\ x & -z & -y-z & \dots \\ \vdots & \vdots & \vdots & \ddots \end{pmatrix}, \quad (11)$$

where

$$x = \frac{\partial \alpha_i}{\partial s}, \quad y = -\frac{\partial \alpha_i}{\partial p_i}, \quad z = c_i \frac{\partial g}{\partial p_i}.$$

The eigenvalues of this $(N+1) \times (N+1)$ Jacobian matrix are

$$\lambda_1 = \lambda_2 = \dots = \lambda_{N-1} = -y, \\ \lambda_{N,N+1} = \frac{-(Nx + y + Nz) \pm \sqrt{(Nx + y + Nz)^2 - 4xzN^2}}{2}.$$

Therefore, since all the eigenvalues are negative, the steady state is locally stable for the N -branch symmetric network.

But in a biological system the branches may be asymmetric. We find numerically that for a two-branch case as the network becomes asymmetric, a pair of complex conjugate eigenvalues cross the imaginary axis such that their real part becomes positive (Fig. 2), meaning the steady state is locally unstable. In fact, the two-branch network exhibits limit-cycle oscillations in the unstable region (lower inset of Fig. 2).

The regime of instability requires parameters such that product pool p_2 inhibits its own production ($p_2 \gg K_{d2}$, $s \gg K_{m2}$) but does not limit growth ($p_2 \gg p_2^*$), while product pool p_1 limits growth ($p_1 \ll p_1^*$) but does not limit its own production ($p_1 \ll K_{d1}$, $s \ll K_{m1}$), or vice versa. The former situation is shown schematically in the upper left inset of Fig. 3; the kinetic equations describing this limiting asymmetric network are

$$\frac{ds}{dt} = \alpha_0 - \frac{\alpha_1^{\max}}{K_{m1}}s - \alpha_2^{\max}K_{d2}\frac{1}{p_2}, \quad (12)$$

$$\frac{dp_1}{dt} = \frac{\alpha_1^{\max}}{K_{m1}}s - \frac{2g_{\max}c_1}{p_1^*}p_1, \quad (13)$$

$$\frac{dp_2}{dt} = \alpha_2^{\max}K_{d2}\frac{1}{p_2} - \frac{2g_{\max}c_2}{p_1^*}p_1. \quad (14)$$

Qualitatively, the equations for the limiting asymmetric network suggest a mapping to a three-species linear chain (upper right inset of Fig. 3): pool p_2 positively affects pool s as increasing p_2 decreases the depletion of s , pool s positively affects pool p_1 via increased synthesis of p_1 , and pool p_1 negatively affects pool p_2 as larger p_1 increases the consumption rate of pool p_2 via faster growth. The three-species chain is known to undergo limit-cycle oscillations via a Hopf bifurcation [1].

In Fig. 3, we show that the substrate pool takes its steady-state value from Eqs. (5) and (6) except in the unstable region, where oscillations arise via a Hopf bifurcation [8]. Analytical calculation of the Hopf bifurcation [11] for the limiting asymmetric network, Eqs. (12)–(14), accurately predicts the onset of the bifurcation (lower right inset of Fig. 3) and the time period T of oscillations near the bifurcation point (lower left inset of Fig. 3).

It is worth remarking that there are metabolic pathways with an alternative feedback scheme where the product p changes the effective Michaelis-Menten constant K_m of the enzyme-substrate complex while leaving the maximal velocity unchanged; one example is the inhibition of aspartate transcarbamoylase by cytidine triphosphate (CTP) [12]. Such pathways are immune to the growth dependent instability we have identified.

The two-branch case is instructive in identifying the existence of a growth-induced instability and analyzing its nature. However, in biology, the number of branches coming from a given substrate can be more than two. A multibranch network composed of only two distinct types of branches can be readily transformed into an equivalent two-branch network. If the two types of the branches are described by the asymmetric limit, Eqs. (12)–(14), then, as the number of substrate-limited branches increases the system becomes more stable, while the system becomes more unstable as the number of product-limited branches increases.

To put our results in a biological context we review the typical stoichiometry ratios of various metabolites in microbes. A typical microbial cell synthesizes a variety of essential metabolic products: nucleotides (A,T,G,C,U) for the nucleic acids DNA and RNA, amino acids for proteins, lipids for cell membranes, and more. However, the stoichiometry of the usage of these essential products can be very different [13]. For example in bacterium *E. coli*, amino acids, mostly found in proteins, are 7 times more abundant than nucleotides, 40 times more abundant than phospholipids, and 300 times more abundant than glucosamine (a component of the cell wall and cell-surface lipopolysaccharide). Even among the amino acids the abundance of the most used amino acid (glycine) is almost 11 times that of the least used (tryptophan). One of the biggest stoichiometry ratios among the various monomeric products is almost 35 (between glycine and glucosamine). These numbers show that actual cells do have large enough stoichiometry ratios in principle for the metabolic networks to become unstable. However, stability can be guaranteed in cells by modest restrictions on the ratios of the Michaelis-Menten constants K_m for utilization of the common substrate. The biggest ratio of these constants in the glutamine utilization network [14] is almost 50, which would still make this network quite

stable. The modest range of stoichiometries and K_m values found in nature may in part reflect on constraint to avoid growth-coupled instability. The difficulty of rewiring metabolic networks *in vivo* suggests that a more practical way to realize a growth-coupled instability experimentally would be via *in vitro* polymer synthesis [15].

As argued above, the two-branch growth-coupled network in the oscillating regime can be understood as a three-species system with frustration—a network motif implicated in various oscillatory biological systems. More recently, a synthetic gene-expression network made from three coupled repressors has been shown to produce oscillations in bacteria [16]. Another example in higher organisms is the observed oscillation of the tumor suppressor protein, p53 [17]. There are other biological systems for which this motif could be relevant, for example, regulation by small RNAs (sRNAs) [18,19]. An sRNA molecule binds to its target messenger RNA (mRNA) and in some cases facilitates its degradation with the sRNA also getting degraded in the process. So, a network in which the mRNA for a transcription factor that activates the transcription of an sRNA that antagonizes the mRNA could lead to oscillations.

In this Letter, we have shown that the product-feedback architecture has at most one steady state, but may become unstable due to coupling of biosynthetic pathways by growth. Oscillations arise in the two-branch case via a Hopf bifurcation. The qualitative mapping of the oscillating network to a three-species system with frustration suggests that the existence of the instability is not dependent on the functional form used for growth [2], though we find somewhat enhanced stability for a polymerization-based growth function. Our analysis of an N -branch symmetric network highlights the requirement for asymmetry to generate the instability. The analysis also shows that the product-feedback architecture is typically stable since large ratios in both stoichiometry and Michaelis-Menten constants are required for the instability to arise.

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15. Assume the rate of attachment of one monomer of pool p_i to a polymer is governed by Michaelis-

Menten kinetics $\tau_i^{-1} = g_{\max} \frac{p_i}{p_i + p_i^*}$. If the polymer is made of monomers with relative

stoichiometries c_i and respective pool sizes p_i , then the average time to increase the length by one monomer is $\sum_{i=1}^N \tau_i c_i / \sum_{i=1}^N c_i$. Thus, the function describing the rate of growth of the polymer is

$$g_{\text{polymer}} = \left(\frac{\sum_{i=1}^N \tau_i c_i}{\sum_{i=1}^N c_i} \right)^{-1} = \frac{g_{\max} \sum_{i=1}^N c_i}{\left(\sum_{i=1}^N c_i \frac{p_i + p_i^*}{p_i} \right)}, \text{ which is similar to Eq. (2), except for the}$$

appearances of the stoichiometry factors. In this case, for growth to be limited by the product with lower stoichiometry (say p_1) requires $p_1 \ll p_1^* / c$, where $c = c_2/c_1$ is the stoichiometry ratio. Since $c > 1$, for the polymer-synthesis system to be asymmetric enough for the instability to arise requires the saturating pool p_1^* to be larger than for a system whose growth is given by Eq. (2).

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FIG 1.

Schematic of the metabolic product-feedback architecture. The essential features of this architecture are: (1) all branches start from a common substrate, (2) the branches are dedicated to the synthesis of their respective products, and (3) each product inhibits the first step dedicated to its synthesis. Dashed lines represent allosteric feedback, where the bar is used to represent inhibition. Key: substrate (square), metabolic intermediates (hashed ovals), and products (ovals).

FIG 2.

Phase diagram for the stability of the two-product network (shown in upper inset). The network becomes unstable as it becomes more asymmetric; the asymmetry of the network is measured by two variables, the ratio of the Michaelis-Menten constants, K_{m1}/K_{m2} and the stoichiometry ratio, c_2/c_1 . In the unstable region the network exhibits limit-cycle oscillations and the maximum eigenvalue from the linear-stability analysis is positive. Upper inset: Schematic of product-feedback architecture with two products and fast (or no) intermediate reactions. Lower inset: Limit-cycle oscillations observed for the parameters indicated by the black dot (0.5,1) in the unstable region. The parameter values used for the numerical integration are:

$\alpha_1^{\max} = 10$, $\alpha_2^{\max} = 5$, $K_{d1} = 1$, $K_{d2} = 0.5$, $c_1 = 0.5$, $c_2 = 12$, $p_1^* = 0.8$, $p_2^* = 10$, and $g_{\max} = 2$.

FIG 3.

Plot of substrate pool s as a function of the stoichiometry ratio c_2/c_1 . The solid line shows the steady-state value of s ; the network is stable in the range shown by a thick line but exhibits limit-cycle oscillations in the range shown by a thin line. The dashed lines represent the maximum and minimum values of the oscillating s . Same parameters are used as Fig. 2 with $K_{m1} = 10$ and $K_{m2} = 0.001$. Upper left inset: Schematic of the product-feedback architecture in the regime of oscillations for the two-product network. In this regime, the synthesis of p_1 is limited by s , p_1 limits growth, and p_2 limits its own synthesis. Dashed lines indicate fluxes that are limited by the upstream pool. Upper right inset: The network of substrate-product interactions in the oscillation regime is equivalent to a three-species linear chain with negative feedback; arrowheads indicate positive influence and the bar indicates a negative influence. Lower left inset: Time period (T) of oscillations for the asymmetric system. The solid line shows the predicted time period and the dots represent simulation results. Lower right inset: Behavior of $s_{\max}$ near the bifurcation point for the asymmetric system. The solid line shows the steady-state analytical solution, the dashed line shows analytical results for the amplitude of the oscillations, and the dots are simulation results. The parameters for the asymmetric system are: $(\alpha_1^{\max}/c_1 g_{\max})(p_1^*/K_{m1}) = 0.8$, $(\alpha_2^{\max}\alpha_1^{\max}/\alpha_0^2) \times (K_{d2}/K_{m1}) = 1$, and $c_1 = 0.5$.