

Effect of Overall Feedback Inhibition in Unbranched Biosynthetic Pathways

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ABSTRACT We have determined the effects of control by overall feedback inhibition on the systemic behavior of unbranched metabolic pathways with an arbitrary pattern of other feedback inhibitions by using a recently developed numerical generalization of Mathematically Controlled Comparisons, a method for comparing the function of alternative molecular designs. This method allows the rigorous determination of the changes in systemic properties that can be exclusively attributed to overall feedback inhibition. Analytical results show that the unbranched pathway can achieve the same steady-state flux, concentrations, and logarithmic gains with respect to changes in substrate, with or without overall feedback inhibition. The analytical approach also shows that control by overall feedback inhibition amplifies the regulation of flux by the demand for end product while attenuating the sensitivity of the concentrations to the same demand. This approach does not provide a clear answer regarding the effect of overall feedback inhibition on the robustness, stability, and transient time of the pathway. However, the generalized numerical method we have used does clarify the answers to these questions. On average, an unbranched pathway with control by overall feedback inhibition is less sensitive to perturbations in the values of the parameters that define the system. The difference in robustness can range from a few percent to fifty percent or more, depending on the length of the pathway and on the metabolite one considers. On average, overall feedback inhibition decreases the stability margins by a minimal amount (typically less than 5%). Finally, and again on average, stable systems with overall feedback inhibition respond faster to fluctuations in the metabolite concentrations. Taken together, these results show that control by overall feedback inhibition confers several functional advantages upon unbranched pathways. These advantages provide a rationale for the prevalence of this control mechanism in unbranched metabolic pathways in vivo.

INTRODUCTION

Biochemical control systems have been studied for more than 45 years. The discovery of control by molecular feedback inhibition in biochemical pathways was initially made in unbranched biosynthetic pathways (Umbarger, 1956; Yates and Pardee, 1956). In these pathways, the most common pattern of control is inhibition of the initial reaction by the final product of the pathway (end-product inhibition or overall feedback inhibition).

There are several criteria for the functional effectiveness of control in such pathways that can be used to evaluate the biological significance of the overall feedback inhibition mechanism. A biochemical pathway should be robust, i.e., it should function reproducibly despite perturbations in the values of the parameters that define the structure of the system. The operating point (state) of the system should be stable so that the system returns to the steady state following small random fluctuations in the values of the dependent variables; if not, the system tends to be dysfunctional because spurious environmental fluctuations will lead to loss of the steady state. The flux through the pathway should be responsive to changes in the demand for the final product.

This ensures that the amount of material flowing through the pathway is intimately coupled to the metabolic needs of the cell. Finally, the system should be temporally responsive to changes, because, otherwise, the system is unlikely to be competitive in rapidly changing environments. [A more extensive discussion of these criteria and their quantification can be found in Savageau (1976) and Hlavacek and Savageau (1997).]

There have been several studies focused on the effect of control by overall feedback inhibition on the stability of unbranched pathways. In general, the first enzyme of the pathway is considered to be allosteric, whereas the others are considered to be Michaelian (e.g., Goodwin, 1963; Morales and McKay, 1967; Walter, 1969a,b, 1970; Viniegra-Gonzalez, 1973; Hunding, 1974; Rapp, 1976; Dibrov et al., 1981). The stability of an unbranched pathway with overall feedback inhibition and enzymes confined to one of two spatial compartments with diffusion between compartments has been studied by Costalat and Burger (1996). They found that stability can be increased by this type of compartmentation. These studies considered pathways with no internal feedback inhibitions.

Several other patterns involving control by inhibitory feedback can, in principle, perform the same qualitative functions as overall feedback inhibition. One such pattern is, for example, a sequence of feedback inhibitions in which each intermediate inhibits the reaction that immediately precedes it (Koch, 1967). Other patterns of internal feedback inhibition can be found by searching either the litera-

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ture or some of the databases for metabolism that are burgeoning on the world wide web (e.g., KEEG: <http://www.genome.ad.jp/kegg/>; ECOCYC: <http://ecocyc.PangeaSystems.com/ecocyc/server.html>; PUMA: http://www.unix.mcs.anl.gov/compbio/PUMA/Production/puma_graphics.html; EMP: <http://wit.mcs.anl.gov/EMP/>). However, even when intermediate feedback inhibition patterns exist, control by overall feedback inhibition remains a prevalent theme in biosynthetic pathways.

Savageau (1972, 1974, 1975, 1976) studied the function of various patterns of feedback inhibition and explained the prevalence of control by overall feedback inhibition by using arguments based on selection. He assumed that the design of a pathway is selected to optimize certain systemic characteristics, and then compared those systemic characteristics in unbranched pathways with overall feedback inhibition to the same characteristics in pathways with alternative inhibitory feedback designs. He showed that the pathway with control by overall feedback inhibition is more robust, i.e., less sensitive to perturbations in parameter values than the pathway with many alternative designs (Savageau, 1974).

The stability of cases with control by internal feedback inhibitions has also been examined (e.g., Savageau, 1976; Thron, 1991a,b; Demin and Kholodenko, 1993). These authors found that systems with internal feedback inhibitions have larger stability margins than systems without these interactions. They also determined that, for systems without internal feedback inhibition, control by overall feedback inhibition decreases the stability margins of the pathway.

In this paper, we consider unbranched pathways with all possible patterns of internal feedback inhibitions (the “fully-wired” case) and use all of the criteria mentioned above to determine the biological significance of control by overall feedback inhibition in such pathways. We use a technique called Mathematically Controlled Comparison that was originally developed to determine irreducible qualitative differences in systemic behavior of models with alternative regulatory designs for the same network of reactions (Savageau, 1972, 1976; Irvine and Savageau, 1985). This qualitative technique requires the existence of closed-form solutions for the steady state. Such solutions can be obtained by using the local S-system representation to characterize the pathway of interest. Important functional constraints are introduced by equating relevant steady-state properties of the alternative systems being compared. The limitations of this technique have been overcome by a recently developed generalization that uses numerical methods to obtain results that are general in a statistical sense (Alves and Savageau, 2000a).

METHODS

Alternative models and key systemic properties

Consider the unbranched pathways depicted in Fig. 1. The independent variable X_{n+1} represents the cell's demand for the end product X_n . If the cell requires large amounts of X_n , then the value of X_{n+1} will be high; if small amounts of X_n are required, then the value of X_{n+1} will be low. The dynamic behavior of such systems can be described in principle by a set of ordinary differential equations. There is no generic representation of these

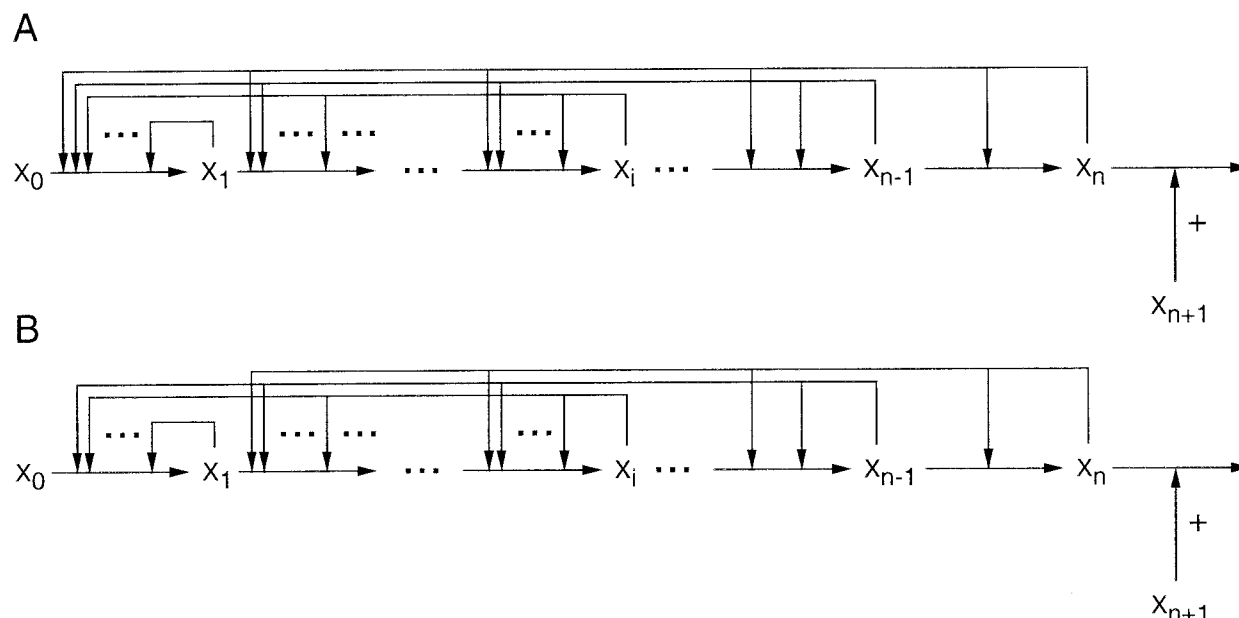


FIGURE 1 (A) Model of an unbranched pathway with all possible inhibitory feedback interactions (reference model). (B) Model of an unbranched pathway with all possible inhibitory feedback interactions except overall feedback inhibition (alternative model). The horizontal arrows represent biochemical reactions, whereas the vertical arrows represent inhibitory feedback interactions.

equations that can provide a globally accurate description of the behavior [see Appendix]. However, the set of equations can be approximated to the first order in logarithmic space (Savageau, 1969), yielding ordinary differential equations with the canonical form of an S-system (Savageau, 1996). This representation has a solid theoretical foundation based on Taylor's theorem. Thus, the validity of the results is guaranteed within some neighborhood of the nominal steady-state operating point. The size of this neighborhood cannot be specified in general, because it depends on the characteristics of each individual system.

For pathways with n intermediates, the general case in which all possible feedback inhibitions exist (Fig. 1 A) can be described in the local S-system representation as

$$\frac{dX_1}{dt} = \alpha_1 \prod_{j=0}^n X_j^{g_{1j}} - \alpha_2 \prod_{j=1}^n X_j^{g_{2j}}, \quad (1)$$

$$\frac{dX_i}{dt} = \alpha_i \prod_{j=i-1}^n X_j^{g_{ij}} - \alpha_{i+1} \prod_{j=i}^n X_j^{g_{i+1,j}}, \quad (2)$$

$$\frac{dX_n}{dt} = \alpha_n \prod_{j=n-1}^n X_j^{g_{nj}} - \alpha_{n+1} \prod_{i=n}^{n+1} X_j^{g_{n+1,i}}. \quad (3)$$

The corresponding case without overall feedback inhibition (Fig. 1 B) can be described by the same set of equations, except that Eq. 1 is replaced by

$$\frac{dX_1}{dt} = \alpha'_1 \prod_{j=0}^{n-1} X_j^{g'_{1j}} - \alpha_2 \prod_{j=1}^n X_j^{g_{2j}}. \quad (4)$$

The rate law for each reaction is represented by a simple product of power-law functions. The values for the parameters in this representation can be determined directly from conventional experimental measurements of initial rate as a function of reactant and modifier concentrations (Savageau, 1976). The range of values for the concentrations is chosen to sample the region about the nominal steady state of interest.

The parameters are defined according to Taylor's theorem as

$$g_{ij} = \left(\frac{\partial \log v_i}{\partial \log X_j} \right)_0 = \left(\frac{\partial v_i}{\partial X_j} \right)_0 \left(\frac{X_{j0}}{v_{i0}} \right), \quad (5)$$

$$\alpha_i = v_{i0} \prod_{j=i-1}^n X_{j0}^{-g_{ij}}, \quad (6)$$

where the additional subscript zero signifies that the variables and their derivatives are evaluated at the steady-state operating point. The definition of these parameters allows them to be directly related to the parameters in other representations such as the traditional Michaelis-Menten representation. In the simplest case of the Hill rate law,

$$v = \frac{V_m X^n}{K_M^n + X^n} \quad (7)$$

[and the irreversible Michaelis-Menten rate law ($n = 1$)], these relationships are well known (Savageau, 1971a),

$$g = n \frac{K_M^n}{K_M^n + X_0^n}, \quad (8)$$

$$\alpha = v_0 X_0^{-g}. \quad (9)$$

The multiplicative parameters, α , can be interpreted as rate constants that are always positive. The exponential parameters, g , can be interpreted as kinetic orders that represent the direct influence of each intermediate on each rate law. If X_i is directly involved in the rate law V_j , either as a substrate or a modulator, and if an increase in X_i causes an increase in the rate V_j , then the kinetic order will be positive. If an increase in X_i causes a decrease in V_j , then the kinetic order will be negative. If X_i is not directly involved in V_j , then the kinetic order will be zero. The positive kinetic orders in Eqs. 1–4 are $g_{i+1,i}$ ($0 \leq i \leq n$) and g'_{10} , because these are the kinetic orders for substrates of reactions, and $g_{n+1,n+1}$, which, together with X_{n+1} , represents the demand for the end product X_n . The remaining kinetic orders, which represent feedback inhibitions, are negative.

At a steady state, the rate of production and the rate of consumption will be equal for each intermediate, and Eqs. 1–3 reduce to the following matrix equation (Savageau, 1969), which can be solved analytically.

$$\begin{bmatrix} b_1 - g_{10}Y_0 \\ b_2 \\ \vdots \\ b_{n-1} \\ b_n + g_{n+1,n+1}Y_{n+1} \end{bmatrix} = \begin{bmatrix} a_{11} & \cdots & a_{1n} \\ a_{21} & \cdots & a_{2n} \\ \vdots & \vdots & \vdots \\ a_{n-1,1} & \cdots & a_{n-1,n} \\ a_{n1} & \cdots & a_{nn} \end{bmatrix} \begin{bmatrix} Y_1 \\ Y_2 \\ \vdots \\ Y_{n-1} \\ Y_n \end{bmatrix}, \quad (10)$$

where $b_1 = \log(\alpha_2/\alpha_1)$, $b_i = \log(\alpha_{i+1}/\alpha_i)$, $a_{ij} = g_{ij} - g_{i+1,j}$, and $Y_i = \log(X_i)$. Eq. 10 is linear and therefore easily solved to obtain the steady-state value for each Y_i , and then the corresponding value for each X_i is obtained by simple exponentiation. Eqs. 2–4 reduce to an identical matrix equation, except that the parameters of the first row are primed and $g'_{1n} = 0$.

Two types of systemic coefficients, logarithmic gains and parameter sensitivities, can be used to characterize the steady state of such models (Shiraishi and Savageau, 1992). Logarithmic gains measure the relative influence of each independent variable on each dependent variable of the integrated model. For example,

$$L(X_i, X_0) = \frac{d \log(X_i)}{d \log(X_0)} = \frac{dY_i}{dY_0} \quad (11)$$

measures the percent change in the concentration of intermediate X_i caused by a percentage change in the concentration of the initial substrate X_0 . Logarithmic gains provide important information concerning the amplification or attenuation of signals as they are propagated through the system. The experimental measurement of a logarithmic gain involves the determination of steady-state fluxes and concentrations at different values for a given independent variable (Savageau, 1971a).

Parameter sensitivities measure the relative influence of each parameter on each dependent variable of the model. For example,

$$S(X_i, p_j) = \frac{d \log(X_i)}{d \log(p_j)} = p_j \frac{dY_i}{dp_j} \quad (12)$$

measures the percent change in the concentration of intermediate X_i caused by a percentage change in the value of the parameter p_j . Parameter sensitivities provide important information about system robustness, i.e., how sensitive the system is to perturbations in the parameters that define the structure of the system. Because enzymes usually have a first-order influence on the process they catalyze, the logarithmic gain in flux and in each concentration with respect to change in the concentration of each enzyme is the same as the sensitivity in flux and in each concentration with respect to change in the rate constant of the corresponding enzyme. The experimental measurement of a parameter sensitivity involves the determination of steady-state fluxes and concentrations before and after changing the value of a parameter by mutation or other means (Savageau, 1971b).

Because we can calculate closed-form steady-state solutions for Eqs. 1–3 and 2–4, we can also calculate each of the two types of coefficients

simply by taking the appropriate derivatives of those solutions. Although the mathematical operations involved are the same in each case, it is important to keep in mind that the biological significance of the two types of coefficients is very different.

The local stability of the steady state can be determined by applying the Routh criteria (Dorf, 1992). The magnitude of the two critical Routh conditions can be used to quantify the margin of stability (e.g., Savageau, 1976).

The use of the S-System formalism allows for an analytical study of the dynamical systems at steady state. Comparisons of systems with only one feedback inhibition to systems without feedback regulation can be done and interpreted in a fully symbolic way. However, for comparisons involving many feedback inhibitions, numerical values must be introduced for the parameters to make the comparisons interpretable. The steady-state behavior of the alternative models is compared with respect to their flux, intermediate concentrations, logarithmic gains with respect to changes in initial substrate and demand for end product, robustness, and stability margins. The differential equations also are solved numerically to characterize the temporal responsiveness of the alternative designs. To evaluate this, we increase the steady-state concentration of each X_i by 20% and measure the time the system takes to relax back to within 1% of its original steady state.

Calculating constraints for the mathematically controlled comparison

Only the first step in the pathway is allowed to differ between the reference model (Fig. 1 A) and the alternative model (Fig. 1 B). Therefore, to establish "internal equivalence" (Savageau, 1972, 1976; Irvine, 1991) between the two designs, we require the values for the corresponding parameters of all other steps in the two models to be the same.

The first step of the pathway differs between the reference model and the alternative model, and the degrees of freedom associated with this difference must be eliminated to the extent possible. If we reason that loss or gain of an inhibitory site on the first enzyme comes about by mutation, and that this mutation can cause changes in all the parameters of the first reaction, then a mutation causing loss of overall feedback inhibition would change the parameters α_1 and g_{10} through g_{1n} in Eq. 1 to the corresponding primed parameters in Eq. 4. Clearly, the value of the parameter g'_{1n} , which equals zero, differs from that of g_{1n} , which is nonzero. The remaining primed parameters also will have values that, in general, are not equal to the values of the corresponding parameters in the reference model. Because we wish to determine those effects that are due solely to changes in the structure of the system and not simply to arbitrary changes in the values of parameters, we shall specify values for the primed parameters that minimize all other effects. This can be accomplished by deriving the mathematical expression for a given steady-state property in each of the two models, equating these expressions, and then solving the constraint equation for the value of a primed parameter. This process establishes an "external equivalence" between the two designs (Savageau, 1972, 1976; Irvine, 1991). After values for all the primed parameters have been specified in terms of the known values for the reference system, the extra degrees of freedom have been eliminated, and we can proceed with the comparison.

Three classes of constraint equations are used to fix the values for the $k + 2$ primed parameters when there are k interactions that feed back to the first step of the alternative pathway. These are obtained by equating steady-state logarithmic gains, concentrations, and parameter sensitivities as described below.

First, equating the logarithmic gains for any one of the metabolites with respect to change in the initial substrate,

$$L(X_i, X_0)_A = L(X_i, X_0)_B \quad i = 1, 2, \dots, n, \quad (13)$$

which causes each of the other corresponding intermediates to have the same logarithmic gain, specifies the value of the kinetic order g'_{10} in terms

of known values for the reference system. This condition also makes the corresponding logarithmic gain in flux the same for the two designs.

Second, equating the concentrations for any one of the metabolites in the pathway,

$$Y_{iA} = Y_{iB} \quad i = 1, 2, \dots, n, \quad (14)$$

which causes each of the corresponding intermediates to have the same concentration, specifies the value of the rate constant α'_1 . This condition also makes the flux the same for the two designs.

Finally, the remaining $k - 1$ primed parameters are fixed by equating the rate-constant parameter sensitivities,

$$S(X_i, \alpha_j)_A = S(X_i, \alpha_j)_B \quad i = 1, 2, \dots, n \quad j = 1, 2, \dots, n, \quad (15)$$

for any X_i and $k - 1$ different rate constants α_j . Different results will be obtained, depending upon which of the parameter sensitivities are not used in this procedure.

For example, consider the case in which all $n - 1$ intermediates feed back on the first step in the pathway. If the unconstrained sensitivity in Eq. 15 is $S(X_n, \alpha_n)$, then the values of the primed parameters are given by

$$\log(\alpha'_1) = \log(\alpha_1) + \frac{g_{1n}}{g_{n+1,n} - g_{nn}} \log(\alpha_{n+1}/\alpha_n), \quad (16)$$

$$g'_{1p} = g_{1p} \quad 0 \leq p < n - 1, \quad (17)$$

$$g'_{1,n-1} = g_{1,n-1} + \frac{g_{1n}}{g_{n+1,n} - g_{nn}} g_{n,n-1}. \quad (18)$$

If the unconstrained sensitivity in Eq. 15 is $S(X_i, \alpha_1)$, then the values of the primed parameters are

$$\log(\alpha'_1) = \frac{g_{n+1,n}}{g_{n+1,n} - g_{1n}} \log(\alpha_1) - \frac{g_{1n}}{g_{n+1,n} - g_{1n}} \log(\alpha_{n+1}), \quad (19)$$

$$g'_{1p} = \frac{g_{2n}}{g_{2n} - g_{1n}} g_{1p} \quad 0 \leq p \leq n - 1. \quad (20)$$

If the unconstrained sensitivity in Eq. 15 is $S(X_i, \alpha_j)$ where $1 < j < n$, then the values of the primed parameters are

$$\log(\alpha'_1) = \log(\alpha_1) - \frac{g_{1n}}{g_{jn} - g_{j+1,n}} \log(\alpha_{n+1}/\alpha_{j+1}), \quad (21)$$

$$g'_{1p} = g_{1p} \quad 0 \leq p \leq j - 1 \quad (22)$$

$$g'_{1p} = g_{1p} - \frac{g_{1n}}{g_{jn} - g_{j+1,n}} g_{2j} \quad j - 1 < p. \quad (23)$$

Because the objective of a controlled comparison is to minimize the differences between the systems being compared, we chose the unconstrained sensitivity that leads to the smallest number of systemic properties with values that differ between the reference system and the alternative system. The systemic differences are minimized when the unconstrained sensitivity is $S(X_i, \alpha_{n+1})$; any other choice leads to at least one additional systemic property that differs between the two systems.

If only a subset of the intermediates feed back on the first step of the pathway, and if we use the constraint set that causes the smallest number of properties to be different between systems A and B, then each kinetic order representing a feedback inhibition has the same value in both models, except for the kinetic order representing the last intermediate to feed back on the first step of the pathway. In general,

$$g'_{1k} = g_{1k} + \frac{g_{1n}}{\Delta_{nk}} \prod_{p=k}^{n-1} g_{p+1,p}, \quad (24)$$

where X_k is the last intermediate to feed back on the first step of the pathway, and Δ_{nk} is a positive subdeterminant of $[A]$ that depends on the actual X_k and on the length n of the pathway. The kinetic orders g_{1p} with $p < k$ are the same for both systems. As for the rate constant α'_1 , its general form is

$$\log(\alpha'_1) = \log(\alpha_1) + \sum_{p=k}^n \frac{\chi_{kp}}{\Delta_{np}} \log(\alpha_{n+1}/\alpha_{p+1}), \quad (25)$$

where χ_{kp} is either a function of the kinetic orders or zero.

For the special case in which the final product is the only metabolite to feed back on the initial step, the primed parameters are given by

$$\log(\alpha'_1) = \log(\alpha_1) \frac{g_{n+1,n}}{g_{n+1,n} - g_{1n}} - \log(\alpha_{n+1}) \frac{g_{1n}}{g_{n+1,n} - g_{1n}}, \quad (26)$$

$$g'_{10} = \frac{g_{n+1,n}}{g_{n+1,n} - g_{1n}} g_{10}. \quad (27)$$

This means that g'_{10} is always smaller than g_{10} . (To contrast these results with the analogous results expressed within the Michaelis–Menten formalism, see the Appendix.)

Numerical comparison

It is straightforward to compare analytically corresponding magnitudes from each of the two designs. For two- and three-step pathways, the comparisons are clearly interpretable for most systemic properties. The analytical results give qualitative information that characterizes the role of overall feedback inhibition for the system in Fig. 1A. As the length of the pathway increases, the analytical interpretation becomes problematic. To determine if a given magnitude is larger in the reference system or the alternative system requires knowledge of actual parameter values in these cases and a method, such as that found in Alves and Savageau (2000a), for making the numerical equivalent of a mathematically controlled comparison.

To obtain numerical information, one must introduce specific values for the parameters and compare systems. For this purpose, we have randomly generated a large ensemble of parameter sets and selected 5000 of these sets that define systems consistent with various physical and biochemical constraints. These constraints include mass balance, low concentrations of intermediates and small changes in their values to minimize utilization of the limited solvent capacity in the cell, small values for parameter sensitivities so as to desensitize the system to spurious fluctuations affecting its structure, and stability margins large enough to ensure local stability of the systems. A detailed description of these methods can be found in Alves and Savageau (2000c). Mathematica (Wolfram, 1997) was used for all the numerical procedures. Pathways of up to seven steps were studied using this numerical methodology.

To interpret the ratios that result from our analysis, we use density of ratios plots as defined in Alves and Savageau (2000b). The primary density plots of the raw data have the magnitude of some property for the reference system on the x -axis and the corresponding ratio of magnitudes (alternative system to reference system) on the y -axis. The primary plot can be viewed as a list of 5000 paired values that can be ordered with respect to the reference magnitude, thereby forming a list L_1 in which the first pair has the lowest measured value for property P in the reference model, the second has the second lowest, and so on.

Secondary density plots are constructed from the primary plots by the use of moving quantile techniques with a window size of 500. The procedure is as follows. One collects the first 500 ratios from the list L_1 , calculates the quantile of interest for this sample, and pairs this number $\langle R \rangle$ with the median value of the corresponding P values for the reference model, denoted $\langle P \rangle$. One advances the window by one position, collects ratios 2–501, calculates $\langle R \rangle$, and pairs it with the corresponding $\langle P \rangle$ value and continues in this manner until the last ratio from the list L_1 was used for the first time (for further explanation of moving median techniques see, e.g., Hamilton, 1994).

The slope in the secondary plot measures the degree of correlation between the quantities plotted on the x - and y -axes. This technique also is used to examine correlations between ratios of interest and other magnitudes shared by the two systems, e.g., the correlation between the ratio of stability margins and the magnitude of a rate constant common to the two systems (for traditional applications of correlation analysis, see Wherry, 1984).

RESULTS

Mathematically controlled comparison

Response to availability of substrate and demand for end product

The responsiveness of each system to changes in the independent concentration variables X_0 , which represents the availability of initial substrate, and X_{n+1} , which represents the demand for end product, is characterized by a set of logarithmic gains that provides a quantitative measure of signal propagation through the system.

The logarithmic gains of the two systems in response to changes in the initial substrate are identical at each step in the pathway [i.e., $L(V_i, X_0)_A = L(V_i, X_0)_B$ and $L(X_i, X_0)_A = L(X_i, X_0)_B$ for $1 \leq i \leq n$] because of the constraints for external equivalence described in the Methods section. Hence, the responsiveness of the two systems to changes in the availability of initial substrate is identical.

In contrast, the responsiveness of the two systems to changes in the demand for their end product is different. The ratio of the logarithmic gains in flux is given by

$$\left| \frac{L(V, X_{n+1})_A}{L(V, X_{n+1})_B} \right| = \left| 1 + \frac{g_{1n}}{\zeta} \prod_{j=1}^n g_{j+1,j} \right| > 1, \quad (28)$$

where ζ is always a negative sum of products of the kinetic orders, $g_{1n} < 0$, and $g_{j+1,j} > 0$ for $j = 1, 2, \dots, n-1$. These results demonstrate that the flux in the reference system is more responsive than that in the alternative system to changes in demand for end product.

The ratio of the logarithmic gains in concentration is given by

$$\left| \frac{L(X_i, X_{n+1})_A}{L(X_i, X_{n+1})_B} \right| = \left| 1 + \frac{g_{1n}}{\zeta} \prod_{j=1}^{n-1} g_{j+1,j} \right| \quad i = 1, 2, \dots, n, \quad (29)$$

where ζ is a sum of products of the kinetic orders that depends on i and the length of the pathway, $g_{1n} < 0$, and $g_{j+1j} > 0$ for $j = 1, 2, \dots, n-1$. When $i = 1$ or $i = n$, ζ is always positive and, thus, the reference model is always less sensitive to demand. When $1 < i < n$, ζ is positive in most cases. This shows that the concentrations are usually less sensitive to demand in the system with overall feedback inhibition.

Robustness of flux

The robustness of any systemic property with respect to perturbations in the values of the parameters that define the system is characterized by a set of parameter sensitivities. The steady-state flux of reference and alternative systems has different sensitivities with respect to the parameters α_n , α_{n+1} , $g_{1,n-1}$, $g_{n+1,n}$, g_{nn} , and $g_{n,n-1}$ that are common to the two systems. The sensitivities are the same with respect to all other parameters common to the two systems.

The sensitivities of the steady-state flux with respect to the parameters α_n , g_{nn} , and $g_{n,n-1}$ exhibit a common pattern. If we take the ratio of a sensitivity in the reference system to the corresponding sensitivity in the alternative system, we find that the ratio of the sensitivities is always less than 1. That is,

$$\left| \frac{S(V, p)_A}{S(V, p)_B} \right| = \left| 1 + \frac{g_{1n}}{\gamma} \prod_{j=1}^{n-1} g_{j+1j} \right| < 1, \quad (30)$$

where γ is a positive sum of products of the kinetic orders, $g_{1n} < 0$, $g_{j+1j} > 0$ for $j = 1, 2, \dots, n-1$, and

$$-1 \leq \frac{g_{1n}}{\gamma} \prod_{j=1}^{n-1} g_{j+1j} < 0. \quad (31)$$

Thus, the flux in the reference system is less sensitive to parameter variations, i.e., is more robust than that in the alternative system.

The sensitivities of the steady-state flux with respect to the parameter $g_{1,n-1}$ exhibit a similar pattern. The ratio of the sensitivities in this case is given by

$$\left| \frac{S(V, p)_A}{S(V, p)_B} \right| = \left| 1 - \frac{g_{1n}g_{n,n-1}}{g_{1n}g_{n,n-1} - g_{1,n-1}(g_{nn} - g_{n+1,n})} \right| < 1. \quad (32)$$

Although the function of the kinetic orders is different from that in Eq. 30, the flux in the reference system is again less sensitive to parameter variations, i.e., is more robust than that in the alternative system.

In contrast, the ratio of the sensitivities with respect to the parameters α_{n+1} and $g_{n+1,n}$ exhibits a different pattern,

$$\left| \frac{S(V, p)_A}{S(V, p)_B} \right| = \left| 1 + \frac{g_{1n}}{\zeta} \prod_{j=1}^{n-1} g_{j+1j} \right| > 1, \quad (33)$$

where ζ is a negative sum of products of the kinetic orders. These parameter sensitivities are related to the last enzyme and reflect the design for responsiveness to changes in demand for end product.

As the position of the last intermediate that provides feedback inhibition to the first reaction approaches the beginning of the pathway, the number of sensitivities that differ between reference and alternative systems increases. This is so because the number of primed parameters decreases and a smaller number of conditions for external equivalence are needed to eliminate the extra degrees of freedom. In general, if the last intermediate that provides an inhibitory feedback to the first reaction is X_k for $k < n-1$, then the sensitivities of the flux to the rate constants α_k to α_{n+1} and those to the kinetic orders g_{ij} ($k \leq i \leq n$ and $i \leq j \leq n$) will differ between the reference and the alternative systems. In most cases, the sensitivities will be less in the reference system. There are exceptions to this, depending on the length of the pathway and on the last intermediate that provides feedback inhibition to the first step, and, in the case of α_{n+1} and $g_{n+1,n}$, the sensitivities of the reference system will always be greater, for the reasons we have already mentioned.

Robustness of concentrations

The steady-state concentrations of reference and alternative systems have different sensitivities with respect to many parameters that define the systems. In some cases, the ratio of the corresponding sensitivities is always <1 or always >1 , but, in others, the ratio is <1 for some values of the parameters and >1 for other values. In the latter cases, an examination of actual numerical values for the parameters is critical.

The ratio of sensitivities for the concentration of each intermediate in the pathway with respect to changes in the kinetic order $g_{1,n-1}$ is identical to that given in Eq. 32. Similarly, the ratio of sensitivities for X_n with respect to changes in the rate constants α_n or α_{n+1} is always of the form

$$\left| \frac{S(X_n, \alpha_p)_A}{S(X_n, \alpha_p)_B} \right| = \left| 1 + \frac{g_{1n}}{\zeta_p} \prod_{j=1}^{n-1} g_{j+1j} \right| < 1 \quad p = n, n+1, \quad (34)$$

where ζ_p is a different positive sum of products of kinetic orders for each α_p , $p = n, n+1$, and

$$-1 \leq \frac{g_{1n}}{\zeta_p} \prod_{j=1}^{n-1} g_{j+1j} < 0 \quad p = n, n+1. \quad (35)$$

Thus, the reference system is always less sensitive to changes in these parameters.

In contrast, the ratio of sensitivities for X_n with respect to changes in the kinetic orders $g_{n+1,n}$, $g_{n,n-1}$, or g_{nn} is always of the form

$$\left| \frac{S(X_n, g_{pq})_A}{S(X_n, g_{pq})_B} \right| = \left| 1 + \frac{g_{1n}}{\zeta_{pq}} \prod_{j=1}^{n-1} g_{j+1,j} \right|, \quad (36)$$

where ζ_{pq} is a different positive sum of products of the kinetic orders for each g_{pq} . In this case, the ratio can be >1 or <1 . This means that the sensitivity of the reference system will be greater than the sensitivity of the alternative system for some values of the parameters and less for others. Similarly, the ratio of sensitivities for each intermediate X_i with respect to changes in each parameter can be ≥ 1 , depending on values of the parameters.

Again, as the position of the last intermediate that provides feedback inhibition to the first reaction approaches the beginning of the pathway, the number of sensitivities that differ between reference and alternative systems increases. In general, if the last intermediate that provides an inhibitory feedback to the first reaction is X_k , then the ratio of sensitivities for each metabolite with respect to changes in the kinetic order g_{1k} is given by

$$\left| \frac{S(X_i, g_{1k})_A}{S(X_i, g_{1k})_B} \right| = \left| 1 + \frac{g_{1n}}{\zeta_{1k}} \prod_{j=1}^{n-1} g_{j+1,j} \right| < 1 \quad i = 1, 2, \dots, n. \quad (37)$$

In this equation, ζ_{1k} is a positive subdeterminant of the $[A]$ matrix. The ratio of sensitivities for the end product with respect to changes in each of the parameters common to the two systems also is always ≤ 1 . Similarly, the ratio of sensitivities for the last intermediate that feeds back to the first reaction, X_k , with respect to the parameters α_k or g_{kj} ($k \leq j \leq n$) is always <1 . Thus, the reference system is always more robust than the alternative system in these cases. As for the remaining cases, the sensitivities of the reference system will be greater than the sensitivities of the alternative system for some values of the parameters and less for others.

Stability

The characteristic equation for Eqs. 1–3 operating near the steady state can be written as

$$\begin{vmatrix} F_1 a_{11} - \lambda & F_1 a_{12} & \cdots & F_1 a_{1n} \\ F_2 a_{21} & F_2 a_{22} - \lambda & \cdots & F_2 a_{2n} \\ 0 & F_3 a_{32} & \cdots & F_3 a_{3n} \\ \vdots & \vdots & \ddots & \vdots \\ 0 & \cdots & F_{n-1} a_{n-1,n-2} & F_{n-1} a_{n-1,n-1} - \lambda & F_{n-1} a_{n-1,n} \\ 0 & \cdots & 0 & F_n a_{nn} - \lambda \end{vmatrix} = 0, \quad (38)$$

where $F_i = V_{i0}/X_{i0}$ and $a_{ij} = g_{ij} - g_{i+1,j}$. Eq. 38 can be expanded into polynomial form and the Routh conditions for local stability determined. The last two Routh conditions

are critical for stability (Frazer and Duncan, 1929). The last condition is equivalent to the condition $(-1)^n \det(A) > 0$, which is always true for the systems we are considering (Savageau, 1976, Appendix B).

The two critical Routh conditions for a two-step pathway are

$$R_1 = F_1(g_{11} - g_{21}) + F_2(g_{22} - g_{32}) < 0 \quad (39)$$

and

$$R_2 = F_1 F_2 [g_{11}(g_{22} - g_{32}) + g_{21}(g_{32} - g_{12})] > 0. \quad (40)$$

Both these conditions are always satisfied for both system A ($g_{12} < 0$) and system B ($g'_{12} = 0$ and $g'_{11} = g_{11} + g_{12}g_{21}/(g_{32} - g_{22}) < g_{11} < 0$), so these systems are always stable. The ratio of the last Routh condition for the two systems is equal to unity, whereas that for the penultimate condition is given by

$$\frac{R_{1A}}{R_{1B}} = 1 - \frac{F_1 g_{12} g_{21}}{(F_1 g_{12} g_{21} - F_1 g_{11} g_{22} + F_1 g_{21} g_{22} - F_2 g_{22}^2 + F_1 g_{11} g_{32} - F_1 g_{21} g_{32} + 2F_2 g_{22} g_{32} - F_2 g_{32}^2)} < 1. \quad (41)$$

Thus, the stability margin is larger for the alternative system B.

The two critical Routh conditions for a three-step pathway are already considerably more complex. Whereas the last condition is always positive, the most critical condition is the penultimate one that can be positive or negative, depending upon the particular values for the parameters. The ratio of the last condition for the two systems is equal to 1; the ratio of the penultimate condition can be >1 or <1 , depending on the values for the parameters. These same conclusions are obtained for pathways of length four or greater: the ratios cannot be determined analytically to be >1 or <1 , and we must resort to numerical methods.

Transient time

There is no analytical way to accurately calculate the transient times of the pathway. This must be done numerically.

Numerical comparisons

Unlike the symbolic analysis performed in the previous section, using actual numbers for the values of the parameters limits the absolute generality of the results. However, it does allow us to obtain general conclusions in a statistical sense. The results described below have been obtained for pathways of up to seven intermediates. The trends in these results remain constant throughout all the tested lengths (i.e., pathways from 2 to 7 intermediates), which suggests that they will remain so for longer pathways. The use of these numerical methods allows us not only to study the

effects of overall feedback inhibition, but also to study correlations that exist between systemic properties and the different parameters of the system.

Response to availability of substrate and demand for end product

The logarithmic gains in concentrations of the two systems in response to changes in the initial substrate X_0 are identical at each step in the pathway because of the constraints for external equivalence described in the Methods section. The same is true for the logarithmic gains in flux. Hence, the responsiveness of concentrations and fluxes in the two systems to changes in the availability of initial substrate is identical numerically as well as analytically.

The logarithmic gain in flux for system A in response to changes in the demand for end product was shown analytically to be greater than that for system B. The graph of $L(V, X_{n+1})_A / L(V, X_{n+1})_B$ versus $L(V, X_{n+1})_A$ (Fig. 2 A), which is the moving median density of ratios plot introduced in Alves and Savageau (2000c), shows how much greater, on average, the response is for system A. It also shows a negative correlation between the ratio of responses and the response of the reference system. This means that, as $L(V, X_{n+1})_A$ increases, the ratio $L(V, X_{n+1})_A / L(V, X_{n+1})_B$ tends to decrease.

The logarithmic gain in end-product concentration for system A in response to changes in the demand for end product also was shown analytically to be smaller than that for system B. The graph of $L(X_n, X_{n+1})_A / L(X_n, X_{n+1})_B$ versus $L(X_n, X_{n+1})_A$ (Fig. 2 B) shows how much smaller, on average, the response is for system A. It also shows a positive correlation between the ratio of responses and the response of the reference system.

Robustness

Figure 2 shows typical moving median density of ratios plots for the aggregate parameter sensitivities of flux and concentrations. The aggregate parameter sensitivity of the flux V is smaller, on average, for system A (Fig. 2 C). Assume that X_k is the last intermediate to feed back on the first reaction of the pathway. The aggregate parameter sensitivity of X_k is smaller, on average, for system B (Fig. 2 D). The average difference in aggregate sensitivities for this metabolite is never larger than a few percent. With regard to the remaining intermediates, the graphs for X_i (Fig. 2 E) and X_j (Fig. 2 F) represent typical plots of aggregate parameter sensitivities. In these cases, we find that random reference systems are less sensitive than the equivalent alternative systems. The average differences can range from a few percent to fifty or more percent. The individual parameter sensitivities of X_n were analytically determined to be smaller in system A. In the example presented here, the difference is, on average, just a few percent (Fig. 2 G);

however, depending on the length of the pathway, this difference can increase to more significant values.

The flux (Fig. 2 C) and concentrations X_i , $i < n$, (Fig. 2, D, E, and F) show a positive correlation between the ratio of their aggregate sensitivities in the two systems and the aggregate sensitivity in the reference system when its value is low. For systems with low sensitivities, system A is, on average, much less sensitive than system B. For higher values of the aggregate sensitivities in the reference system, there is no correlation. In the case of X_k , the ratio is fairly independent of the values of the aggregate sensitivity in the reference system.

Stability

The last critical Routh criterion is always the same in the reference and alternative systems, as has been shown analytically. For a two-step pathway, the margin of stability determined by the penultimate criterion is always larger in system B. For longer pathways, the margin of stability can be larger in either the reference or the alternative system, depending on the numerical values of the parameters. The differences between the two systems with respect to this penultimate criterion are small (on average less than 2%, Fig. 2 H), which implies that systems with and without overall feedback inhibition will have comparable stability margins.

Transient time

Fig. 2 I shows a typical moving median density of ratio plot for transient time. This plot shows that the reference system usually responds to perturbations in the steady state more quickly than the alternative system. For reference systems with a fast response to changes, the transient times can be, on average, half that of the corresponding alternative systems. For reference systems that are sluggish, the difference is, on average, smaller, though it still exists.

Effects of parameter values on systemic properties

Rate-constant effects on aggregate sensitivities

Assume that X_k is the last intermediate to feed back on the first reaction. Plotting the aggregate sensitivities as a function of α_j , $n \leq j$, shows that there is a correlation between each rate constant α_j and each of the aggregate sensitivities (Fig. 3 A). For small α_j , the correlation is either nonexistent or slightly negative, whereas, for large values, this correlation is positive. As for the other rate constants, with $j < n$, there are no obvious correlations that are general for all the pathway lengths studied, although, for some lengths, specific correlations are observed.

Kinetic-order effects on aggregate sensitivities

For X_n , the aggregate sensitivity is correlated with several parameters. There is a positive correlation between this

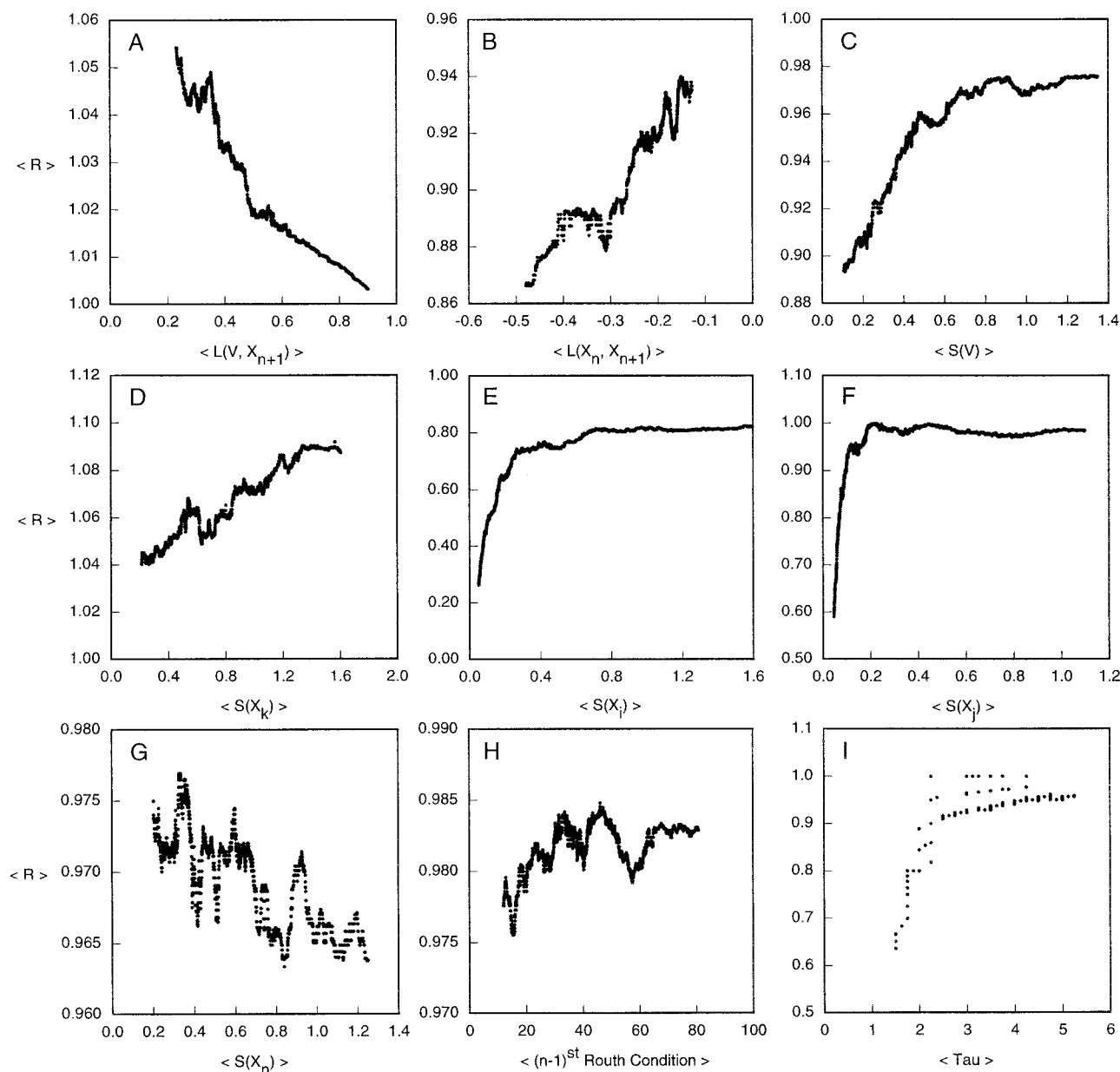


FIGURE 2 Typical moving median density of ratios plots for different magnitudes. The values on the X-axis represent the moving median of the relevant magnitude in the reference system. The values on the Y-axis represent the moving median of the ratio of that magnitude in the reference system to the corresponding magnitude in the alternative system. (A) Logarithmic gain in flux in response to changes in demand for the end product, $L(V, X_{n+1})$. (B) Logarithmic gain in end-product concentration in response to changes in demand for the end product, $L(X_n, X_{n+1})$. (C) Aggregate sensitivity of the pathway flux, $S(V)$. (D) Aggregate sensitivity of the concentration of the last intermediate to feed back on the first reaction, $S(X_k)$. (E) Aggregate sensitivity of the concentration of any intermediate in the pathway before X_k , $S(X_i)$. (F) Aggregate sensitivity of the concentration of any intermediate in the pathway after X_k , $S(X_j)$. (G) Aggregate sensitivity of the concentration of the end-product, $S(X_n)$. (H) The penultimate (i.e., $n - 1$ st) Routh criterion; this represents the margin of stability. (I) Transient time, τ in normalized units, is the time the pathway takes to return within 1% of its steady state following a 15% perturbation in the steady-state values. Each of these plots is for a specific pathway length; only the parameter values are changed randomly. However, because the trends observed for different pathway lengths are the same, we have only shown a representative case.

sensitivity and g_{1n} . Because g_{1n} is always negative, this means that the aggregate sensitivity of X_n , $S(X_n)$, is usually smaller for high values of overall feedback inhibition. The same is true for the correlation between $S(X_n)$ and g_{in} when $i < n$ (Fig. 3 B). If $i = n$, there is a negative correlation

between this aggregate sensitivity and g_{1n} . The correlation of the aggregate sensitivities of the other intermediates with g_{1n} is usually small or nonexistent. There is a negative correlation between the aggregate sensitivity of X_i and $g_{i+1,i}$ or $g_{n,n-1}$ (Fig. 3 C) and a positive correlation between that

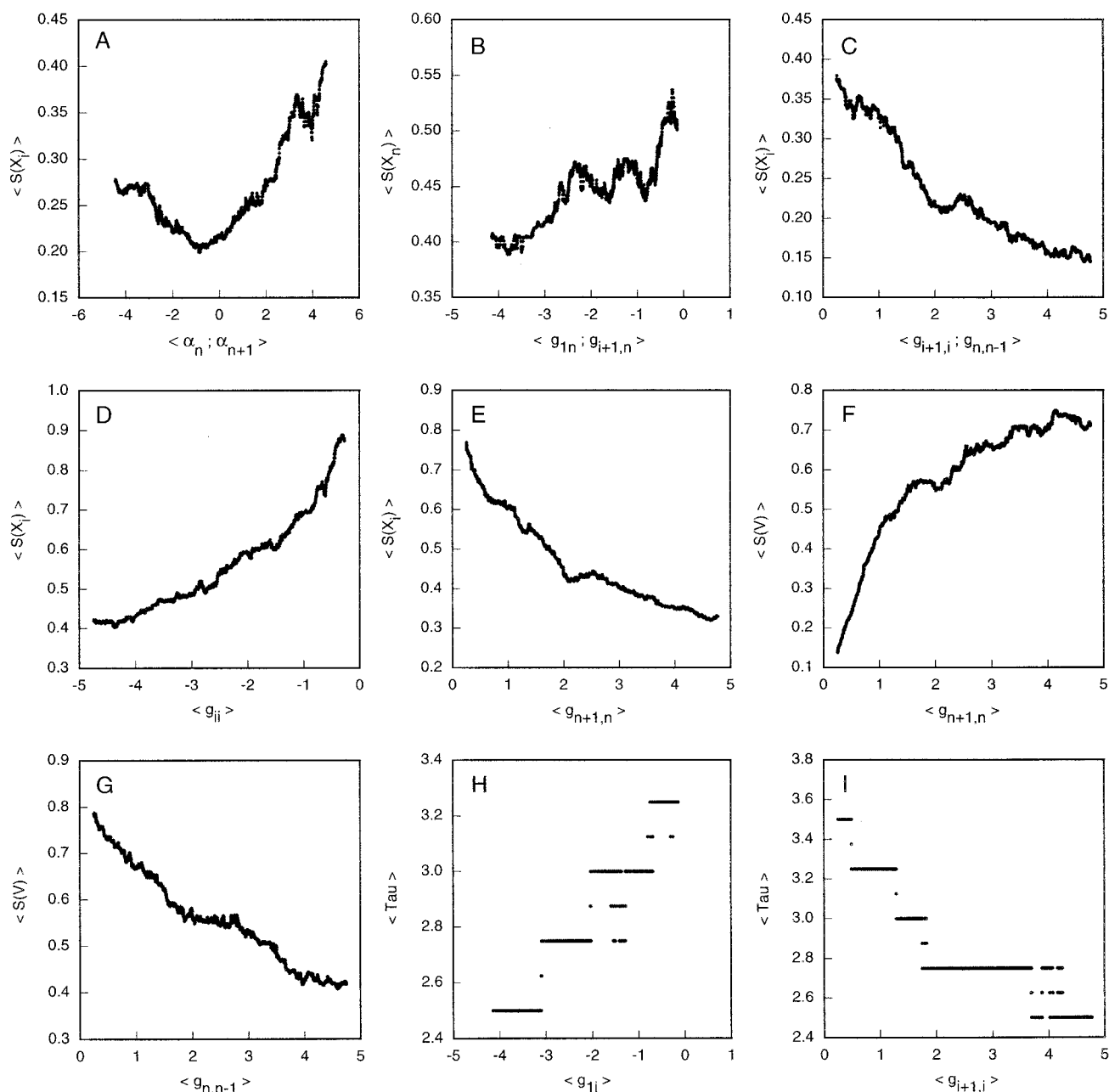


FIGURE 3 Typical moving median correlation plots between different systemic properties and different kinetic parameters of the reference system. The values on the X-axis represent the moving median of the relevant kinetic parameter. The values on the Y-axis represent the moving median of the relevant systemic property. (A) Aggregate sensitivity of the concentration of any pathway intermediate X_i versus the rate-constant parameters α_n or α_{n+1} . (B) Aggregate sensitivity of the concentration of the end product X_n versus the kinetic-order parameters g_{1n} or $g_{i+1,n}$. (C) Aggregate sensitivity of the concentration of any pathway intermediate X_i versus the kinetic-order parameters $g_{i+1,i}$ or $g_{n,n-1}$. (D) Aggregate sensitivity of the concentration of any pathway intermediate X_i versus the kinetic-order parameter g_{ii} . (E) Aggregate sensitivity of the concentration of the end product X_n versus the kinetic-order parameter $g_{n+1,n}$. (F) Aggregate sensitivity of the pathway flux V versus the kinetic-order parameter $g_{n+1,n}$. (G) Aggregate sensitivity of the pathway flux V versus the kinetic-order parameter $g_{n,n-1}$. (H) Transient time τ versus the kinetic-order parameter g_{ii} . (I) Transient time τ versus the kinetic-order parameter $g_{i+1,i}$. Each of these plots is for a specific pathway length; only the parameter values are changed randomly. However, because the trends observed for different pathway lengths are the same, we have only shown a representative case.

of X_i and g_{ii} (Fig. 3 D). Also, the aggregate sensitivity of each X is negatively correlated with $g_{n+1,n}$ (Fig. 3 E). These are the correlations that are generally observed for the aggregate sensitivities of concentrations, although other in-

dividual correlations can be found for specific intermediates and specific pathway lengths.

The correlations between aggregate sensitivities of flux and the various kinetic parameters are less clear. The

correlation with $g_{n+1,n}$ is positive for low values of $g_{n+1,n}$, but it disappears as the value of $g_{n+1,n}$ increases (Fig. 3 *F*). The only other general correlation observed is that between the aggregate sensitivity of the flux and the kinetic order $g_{n,n-1}$. This is a negative correlation that also vanishes as the value of $g_{n,n-1}$ increases. This can be seen in Fig. 3 *G*.

Rate-constant and kinetic-order effects on margin of stability

The correlations between a given Routh criterion and the various parameters depends on which criterion is considered. The results are pathway length-specific, and no general trend can be found.

Rate-constant and kinetic-order effects on transient time

There is no clear correlation between transient time and the various rate constants. There are, however, positive correlations between transient time and the kinetic orders $g_{1,i}$, for $i \geq 1$ (Fig. 3 *H*). There also are negative correlations between transient time and the kinetic orders $g_{i+1,i}$ for $i > 1$ (Fig. 3 *I*). These were the only observed correlations with transient time.

Effects of enzyme levels on systemic variables

We have determined the logarithmic gains in flux and concentrations in response to changes in the level of individual enzymes. When comparing logarithmic gains in flux and concentrations in the reference and alternative systems, the equivalence conditions will make all corresponding coefficients identical except the last two. We also have examined the correlations among the logarithmic gains.

The last two logarithmic gains in concentrations are, on average, lower in the system controlled by overall feedback inhibition (see also Eq. 34). However, there is no general pattern of correlation among the logarithmic gains in concentrations.

The penultimate logarithmic gain in flux is always larger in the alternative system (Fig. 4 *C*). The last logarithmic gains in flux, which is a measure of coupling between flux and the demand for final product, is always larger in the reference system (Fig. 4 *D*). The logarithmic gains in flux with respect to changes in each individual enzyme except the last are directly correlated (Fig. 4 *A*, *B*, and *C*). The last logarithmic gain in flux is inversely correlated with all the others (Fig. 4 *D*). This is a well-known effect of feedback inhibition, i.e., it decreases the sensitivity of the flux through the system to parameters (in this case enzyme levels) inside the feedback loop while increasing the sensitivity to parameters outside the loop.

DISCUSSION

In this paper, we are addressing a generic property characteristic of an entire class of biochemical systems: Why is the pattern of overall feedback inhibition in unbranched biosynthetic pathways so prevalent? Because there are innumerable specific cases that could be examined, most of which have never arisen or may no longer exist because of natural selection, one could never hope to answer this type of question with an experimental approach. However, on a more fundamental level (beyond the sheer number of possibilities that would have to be constructed and examined), one must face the difficulty of performing even a single experimental comparison under well-controlled conditions so that the results will not be confused by extraneous differences.

The method of mathematically controlled comparison was developed specifically to address these issues. It allows one to examine enormous numbers of alternatives in parallel, more than would ever be possible by experimental means; it also allows essentially ideal controlled comparisons, comparisons that could only be done with an enormous experimental effort. In short, this is the type of question that is more appropriately answered by means of a theoretical analysis than by the accumulation of experimental evidence for one specific system after another.

The experimental difficulty in doing the equivalent of a mathematically controlled comparison can be seen from the expressions in the Appendix. One would first have to generate a large number of feedback-resistant mutants. Each independent mutant would, in general, have different values for the resulting K'_M and V'_m parameters. One would have to measure the K'_M for each of the mutants until one was found that had the appropriate value, as determined by the constraints for external equivalence in Eqs. A4–A8. If one was lucky enough to find that this mutant also had the correct value for V'_m , as determined by the constraints for external equivalence in Eqs. A4–A8, then one could measure the systemic differences between the wild-type and mutant to experimentally verify the theoretical results. If the V'_m value was not appropriate, one might construct a mutant strain with the structural gene for the first enzyme under the control of a promoter whose activity can be independently varied. In such a construct, one might be able to adjust the promoter activity to provide the appropriate value for V'_m . Again, one could measure the systemic differences between the wild-type and mutant to experimentally verify the theoretical results. As can be seen from this discussion of what it would take to do the experiments properly, it is unlikely that anyone would undertake the task. This is especially so when the result will only be valid for one special system, and will not contribute significantly to the validation of the general principle.

This discussion is in no way a criticism of the experimental approach. It simply acknowledges the fact that only

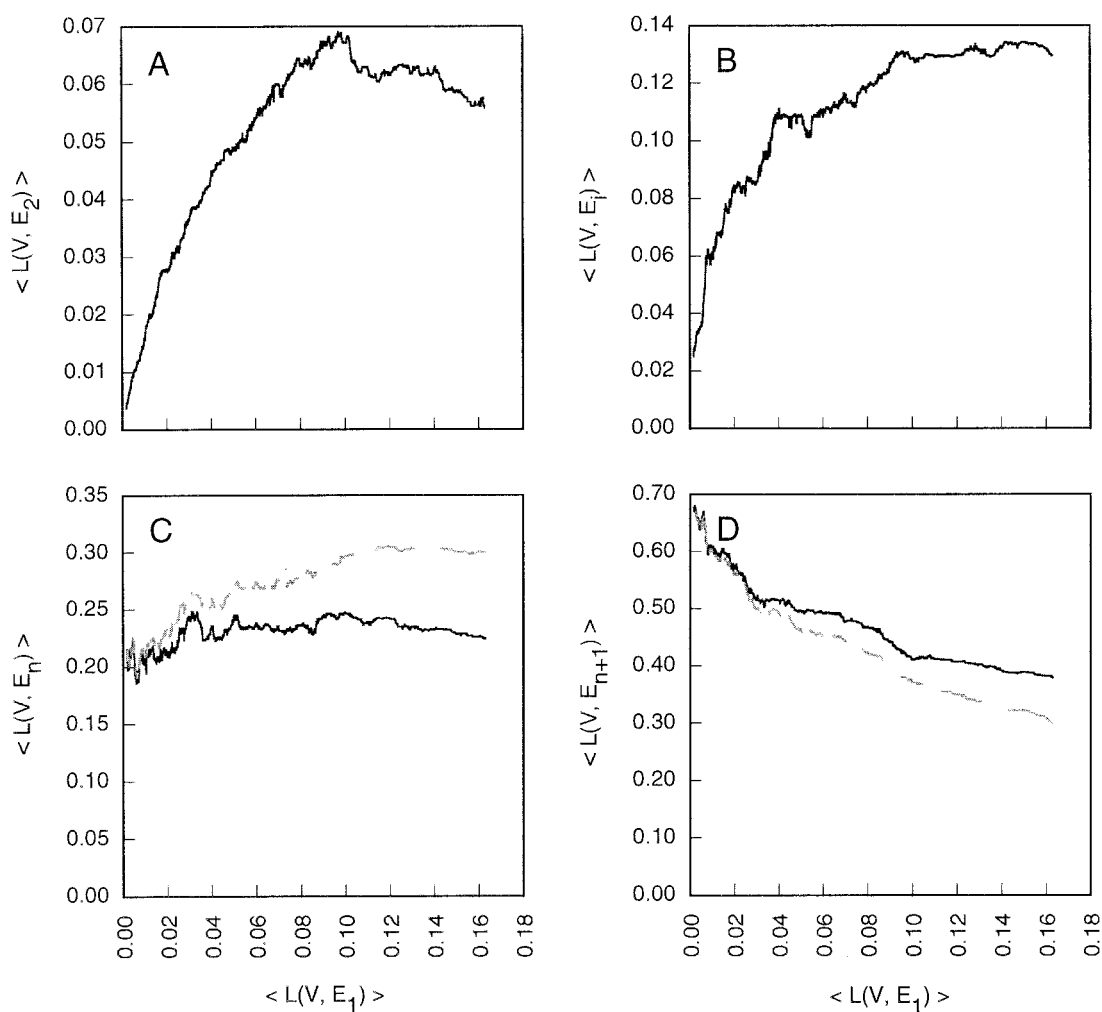


FIGURE 4 Typical moving median correlation plots between different logarithmic gains in flux with respect to changes in individual enzyme levels. The values on the X -axis represent the moving median of the logarithmic gain with respect to the first enzyme of a pathway. The values on the Y -axis represent the moving median of the logarithmic gains with respect to subsequent enzymes in the pathway. Full lines indicate curves for the reference system, and dashed lines indicate curves for the alternative system. (A) Logarithmic gain in flux with respect to the second enzyme of the pathway versus logarithmic gain in flux with respect to the first enzyme of the pathway. (B) Logarithmic gain in flux with respect to the i th enzyme of the pathway ($i \neq 1, 2, n, n + 1$) versus logarithmic gain in flux with respect to the first enzyme of the pathway. (C) Logarithmic gain in flux with respect to the penultimate enzyme of the pathway versus logarithmic gain in flux with respect to the first enzyme of the pathway. (D) Logarithmic gain in flux with respect to the last enzyme of the pathway versus logarithmic gain in flux with respect to the first enzyme of the pathway. Each of these plots is for a specific pathway length; only the parameter values are changed randomly. However, because the trends observed for different pathway lengths are the same, we have only shown a representative case.

specific theoretical predictions are amenable to direct experimental test. More general theoretical predictions that apply to an entire class of systems require experimental information for many members of the class. The experimental validation of the theory presented here is the fact that it can account for the prevalence of overall feedback inhibition in biosynthetic pathways.

In this work, we have used a numerical generalization of the method of mathematical controlled comparison to examine systemic properties of models with and without overall feedback inhibition in unbranched pathways that otherwise have an arbitrary pattern of feedback inhibitions. In

summarizing our findings, we shall interlace the results of the older analytical approach with those of the more recently developed numerical approach. This has the advantage of showing how the numerical approach goes beyond the analytical approach to broaden the scope of mathematical controlled comparison.

By using mathematically controlled comparisons, we have ensured that the systems achieve the same steady-state flux, metabolite concentrations, and logarithmic gains with respect to changes in the concentration of initial substrate, whether overall feedback inhibition is present or not. However, the alternative designs exhibit differences for many

other systemic properties. In the following seven types of results, the analytical approach yields unambiguous qualitative differences.

1. The logarithmic gain in flux resulting from an increase in demand for end product is always greater in the system with overall feedback inhibition. This ensures a tighter control of the material flowing through the pathway by the demand for such material.
2. The logarithmic gain in the concentration of the first and last metabolite resulting from an increase in demand for end product is always less in the system with overall feedback inhibition. This shows that these concentrations tend to be buffered against changes in demand for end product.
3. The sensitivities of the flux to changes in the parameters of the intermediate reactions for the system with overall feedback inhibition are less than or equal to those of the otherwise equivalent system without this inhibition. This shows that overall feedback inhibition increases the robustness of the flux.
4. The sensitivities of the flux to changes in the parameters of the last reaction for the system with overall feedback inhibition are greater than or equal to those of the otherwise equivalent system without this inhibition. This is related to the first point above.
5. The sensitivity of the end-product concentration to each rate-constant parameter of the system with overall feedback inhibition is always less than or equal to that of the otherwise equivalent system without this mechanism. This was shown to be analytically true independent of pathway length. The reference system is thus more effective in buffering the final product of the pathway against parameter fluctuations.
6. The sensitivity of each concentration to the parameter representing the last intermediate to feed back on the first reaction is always less in the system with overall feedback inhibition. Again, the reference system is better protected against fluctuations of this parameter.
7. For the special case of pathways with two intermediates, the alternative system has larger stability margins than the reference system with overall feedback inhibition. The more general case is discussed below.

From the above results, we conclude that pathway flux is more responsive to change in demand for the end product when overall feedback inhibition is present and that the concentration of final product, and the magnitude of pathway flux, is less sensitive to changes in the parameters of the system with overall feedback inhibition.

In each of the above results, the numerical method not only confirmed the qualitative differences, but also showed how large the differences were on average. In the following four types of results the analytical approach yields either no results or ambiguous qualitative differences, whereas the

numerical approach gives statistical regularities in either situation.

1. The logarithmic gain in the concentration of intermediates X_2 to X_{n-1} resulting from an increase in demand for end product may be either larger or smaller in the reference system depending on the intermediate, the pathway length, or the values of the parameters. The numerical results show that, on average, these logarithmic gains are smaller in the reference system.
2. For all concentrations, there are some sensitivities that may be either larger or smaller in the reference system. The numerical approach shows that, on average, these concentrations have smaller aggregate sensitivities in the reference system. The differences between the reference system and the alternative system can range anywhere between a few percent to fifty percent or more, depending on the length of the pathway and the concentration of interest.
3. The stability margins for pathways longer than two reactions can be larger in either the reference system or the alternative system, depending on the values of the parameters. Use of the statistical methodology shows that, on average, overall feedback inhibition decreases the margin of stability. However, the differences between systems with and without overall feedback inhibition are, on average, less than 3% and typically less than 5%.
4. The transient time of the pathways cannot be determined analytically. Numerical results show that transient times tend to be smaller in pathways with overall feedback inhibition. Although a small percentage of systems with overall feedback inhibition have higher transient times, on average, overall feedback inhibition decreases transient times in stable systems. Systems with overall feedback inhibition can be, on average, a few percent faster to twice as fast as systems without overall feedback inhibition, depending on the length of the pathway.

In addition to resolving ambiguities in the analytical comparisons, the numerical methods allowed us to identify some general effects of parameter values on systemic properties. We found that there is a correlation between the values of α_j ($j = n, n + 1$) and the values of the aggregate sensitivities for each metabolite as well as the flux. For very low values of α_j , the aggregate sensitivities will not be strongly affected by a change in those parameters. As these parameters become larger than 1, a correlation develops. As the value of α_j increases, so does the aggregate sensitivity on average. The rate constant α_{n+1} is a parameter that can be interpreted as the demand for X_n . This means that, as the demand increases, so do the aggregated sensitivities. Why this happens is not clear.

General correlations between systemic properties and kinetic-order parameters also were identified. For example, we found that the transient times of the pathway are inversely correlated with the kinetic orders $g_{i+1,i}$. This means

that, on average, a system will respond faster to perturbations if the kinetic orders for the substrates of the reactions are higher. The perturbations that were given to the systems were always positive, i.e., the substrates were increased above their nominal steady-state values. Higher kinetic orders with respect to substrate mean that the rate will have a sharper response to an increase in the substrate, thus causing it to return to the steady-state value faster. In addition to this, there is a positive correlation between transient times and feedback parameters. Lower magnitudes for the kinetic orders representing inhibitory feedback make the rate less sensitive to increases in the concentrations of its inhibitors. Thus, after an increase in inhibitor concentrations, systems with lower magnitudes for the feedback interaction will have faster rates than systems with high magnitudes. It is not clear why these correlations exist only with respect to the parameters representing feedback to the first reaction of the pathway.

In conclusion, it is important to note that the results presented here are also valid for simpler patterns of feedback inhibition, i.e., those that are not “fully-wired.” If a pathway with a smaller number of internal feedback interactions is considered, the qualitative results remain the same. To be more specific, the number of sensitivities that are different between pathways with and without overall feedback inhibition may be smaller for pathways with less internal wiring, but the ones that are different remain larger or smaller in the same model as in the fully-wired comparison. This demonstrates the generality of the fully-wired case and the results provide a rationale for the widespread occurrence of overall feedback inhibition in nature.

APPENDIX

One could address the generic questions in this paper because the power-law formalism is systematically structured and is thereby able to represent systems with essentially any type of mechanism, i.e., the representation is mechanism independent. This is in contrast to the Michaelis–Menten formalism, which does not have a well-defined structure [see Savageau (1996)]. One cannot address the generic questions examined in this paper if one insists on using the Michaelis–Menten formalism. The following is an example illustrating why this is the case.

Consider a special case in which one happens to know the specific mechanisms for each reaction in the pathway. For example, assume that all the reactions in common are governed by simple irreversible Michaelis–Menten kinetics, in particular, that the rate law for the degradation of the end product X_n is given by

$$v_n = \frac{V_{mn}X_n}{K_{Mn} + X_n}. \quad (A1)$$

Further assume that the first enzyme has a specific cooperative mechanism with the rate law,

$$v_1 = \frac{V_m X_0^2}{X_0^2 + K_M^2(1 + (X_n^2/K_I^2))}, \quad (A2)$$

and that a mutation-eliminating inhibition by the end product results in the following rate law for the alternative system:

$$v_1 = \frac{V'_m X_0^2}{X_0^2 + K'_M{}^2}. \quad (A3)$$

In general, the K_M and V_m values will be different in Eqs. A2 and A3, hence primes are used to indicate that the values will be different in the two systems.

If one now generates the conditions for external equivalence, one obtains the following constraint relationships after some differentiation and algebraic manipulation:

$$K'_M = K_M \left\{ \frac{1 + (X_{n0}^2/K_I^2)}{1 + 2 \frac{X_{n0}^2}{K_I^2} \frac{K_M^2}{X_0^2} \left(\frac{K_{Mn} + X_{n0}}{K_{Mn}} \right)} \right\}^{1/2} \quad (A4)$$

and

$$V'_m = V_m \left\{ \frac{X_0^2 + \frac{K_M^2(1 + (X_{n0}^2/K_I^2))}{1 + 2 \frac{X_{n0}^2}{K_I^2} \frac{K_M^2}{X_0^2} \left(\frac{K_{Mn} + X_{n0}}{K_{Mn}} \right)}}{X_0^2 + K_M^2(1 + X_{n0}^2/K_I^2)} \right\}. \quad (A5)$$

Note that X_{n0} in these expressions has a single positive real solution given by

$$X_{n0} = A + B, \quad (A6)$$

where

$$A = \sqrt[3]{\left[\frac{V_m X_0^2 K_{Mn} K_I^2}{2 V_{mn} K_M^2} + \frac{\sqrt{V_m^2 X_0^4 K_{Mn}^2 K_I^4} + \frac{K_I^6 [V_{mn} K_M^2 + (V_{mn} - V_m) X_0^2]^3}{27 V_{mn}^3 K_M^6}}{4 V_{mn}^2 K_M^4} \right]} \quad (A7)$$

and

$$B = \sqrt[3]{\left[\frac{V_m X_0^2 K_{Mn} K_I^2}{2 V_{mn} K_M^2} - \frac{\sqrt{V_m^2 X_0^4 K_{Mn}^2 K_I^4} + \frac{K_I^6 [V_{mn} K_M^2 + (V_{mn} - V_m) X_0^2]^3}{27 V_{mn}^3 K_M^6}}{4 V_{mn}^2 K_M^4} \right]}. \quad (A8)$$

If this solution is inserted into the constraint expressions for K'_M and V'_m , one sees that they become even more complex.

These are among the simplest of assumptions regarding the Michaelis–Menten formalism, and one can see how much more complicated this approach is compared to the approach in the power-law formalism [contrast Eqs. A4–A8 with Eqs. 26 and 27 in the text]. The above expressions would be different for different mechanisms, and, when the mechanisms are more complex, the process would become quite impractical. Yet, one obtains the same results for the local behavior.

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