Irreversibility in Unbranched Pathways: Preferred Positions Based on Regulatory Considerations

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ABSTRACT It has been observed experimentally that most unbranched biosynthetic pathways have irreversible reactions near their beginning, many times at the first step. If there were no functional reasons for this fact, then one would expect irreversible reactions to be equally distributed among all positions in such pathways. Since this is not the case, we have attempted to identify functional consequences of having an irreversible reaction early in the pathway. We systematically varied the position of the irreversible reaction in model pathways and compared the resulting systemic behavior according to several criteria for functional effectiveness, using the method of mathematically controlled comparisons. This technique minimizes extraneous differences in systemic behavior and identifies those that are fundamental. Our results show that a pathway with an irreversible reaction located at the first step, and with all other reactions reversible, is on average better than an otherwise equivalent pathway with all reactions reversible, which in turn is on average better than an otherwise equivalent pathway with an irreversible reaction located at any step other than the first. Pathways with an irreversible first reaction and low concentrations of intermediates (one of the primary criteria for functional effectiveness) exhibit the following profile when compared to fully reversible pathways: changes in the concentration of intermediates in response to changes in the level of initial substrate are equally low, the robustness of the intermediate concentrations and of the flux is similar, the margins of stability are similar, flux is more responsive to changes in demand for end product, intermediate concentrations are less responsive to changes in demand for end product, and transient times are shorter. These results provide a functional rationale for the positioning of irreversible reactions at the beginning of unbranched biosynthetic pathways.

INTRODUCTION

Several types of theoretical studies have reported properties of enzymes that could account for their selection during the evolution of metabolic pathways. The simplest type involves determining the distribution of parameter values that produces the maximal catalytic efficiency of an isolated enzyme (Fersht, 1974; Crowley, 1975; Albery and Knowles, 1976; Cornish-Bowden, 1976; Mavrovouniotis et al., 1990; Heinrich and Hoffman, 1991; Peterson, 1992; 1996; Wilhelm et al., 1994; Bish and Mavrovouniotis, 1998; Heinrich and Schuster, 1998). Waley (1964) considered a three-step pathway with reactions described by Michaelis-Menten rate laws and determined the distribution of enzyme concentrations that maximizes flux through the pathway. Similar studies were performed for *n*-step pathways (Schuster and Heinrich, 1987; Klipp and Heinrich, 1994; Heinrich and Klipp, 1996). Other theoretical studies have dealt with the design of regulatory patterns that, according to multiple criteria, optimize the local behavior of unbranched biosynthetic pathways with *n* steps and arbitrary mechanisms (Savageau, 1972, 1974, 1975, 1976; Savageau and Jacknow, 1979).

An aspect that has been less thoroughly studied is the distribution of irreversible reactions in unbranched biosynthetic pathways and how this distribution might be related to the optimization of various systemic properties. Although each reaction is in principle reversible, in practice some reactions in a pathway operate far from thermodynamic equilibrium and are effectively irreversible. It has been observed experimentally that, in most cases, unbranched biosynthetic pathways have irreversible reactions near the beginning, many times at the first step, of the pathway (see, e.g., EMP:http://wit.mcs.anl.gov//EMP/).

If there were no functional reasons for irreversible reactions to be at the beginning of a pathway, then one would expect irreversible reactions to be equally distributed among all positions in the pathway. Since this is not the case, we have attempted to identify the functional consequences of having an irreversible reaction early in the pathway. We systematically varied the position of the irreversible reaction in model pathways and compared the resulting systemic behavior according to several criteria for functional effectiveness. The model pathways were represented by a power-law formalism that faithfully captures their nonlinear behavior, independent of mechanistic detail, within a local neighborhood of an arbitrary steady-state operating point. We used the method of mathematically controlled comparison to minimize extraneous differences and to identify fundamental differences. With this approach, we have been able to find a rationale for irreversible reactions at the beginning of unbranched biosynthetic pathways.

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METHODS

Alternative models and their systemic description

Consider the unbranched biosynthetic pathways depicted in Fig. 1. The initial substrate X_0 is an independent variable with fixed value. 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 end product inhibits the first reaction, as has been experimentally observed (Umbarger, 1956; Yates and Pardee, 1956; Monod et al., 1963) and theoretically rationalized (Alves and Savageau, 2000d). The dynamic behavior of such systems can be described by a set of ordinary differential equations.

Assume that the net flux through the pathway is positive (i.e., material is coming into the system from X_0, which is held constant, and exiting the system through X_n). The net positive flux through the reaction immediately before the intermediate X_i (considered the net influx to the pool of X_i) can be accounted for by a single aggregate rate law, representing either the difference between the rate laws for the constituent forward and reverse reactions when the overall reaction is reversible or the rate law for the forward reaction alone when the overall reaction is irreversible. Similarly, the net positive flux through the reaction immediately after the intermediate X_i (considered the net efflux from the pool of X_i) can be represented by a single aggregate rate law.

The dynamical behavior of the models in Fig. 1 can be accurately described in a region about their nominal steady state by using a local S-system representation within the power-law formalism (Savageau, 1969, 1971a, 1976, 1996). For details about different ways to aggregate rate laws and approximate them as S-systems, see Sorribas and Savageau (1989). The resulting equations are the following:

$$
\begin{aligned}
\frac{dX_1}{dt} &= \alpha_1 X_0^{g_{10}} X_1^{g_{11}} X_n^{g_{1n}} - \alpha_2 X_1^{g_{21}} X_2^{g_{22}} \\
&\vdots \\
\frac{dX_i}{dt} &= \alpha_i X_{i-1}^{g_{i,i-1}} X_i^{g_{i,i}} - \alpha_{i+1} X_i^{g_{i+1,i}} X_{i+1}^{g_{i+1,i+1}} \qquad 0 < i < n \qquad (1) \\
&\vdots \\
\frac{dX_n}{dt} &= \alpha_n X_{n-1}^{g_{n,n-1}} X_n^{g_{nn}} - \alpha_{n+1} X_n^{g_{n+1,n}} X_{n+1}^{g_{n+1,n+1}}
\end{aligned}
$$

The aggregate rate law V_i for the influx of X_i is characterized by a multiplicative parameter (rate constant), α_i, which influences the time scale of the reaction and is always positive, and a set of exponential parameters (kinetic orders), g_{ij}, which represents the influence of metabolite X_j on aggregate rate law V_i. If X_j influences the aggregate rate law V_i, either as a reactant or a modulator, and if an increase in the concentration of X_j causes an increase in the rate V_i, then the kinetic order will be positive. If an increase in the concentration of X_j causes a decrease in the rate V_i, then the kinetic order will be negative. If an increase in the concentration of X_j causes neither an increase nor a decrease in the rate V_i, then the kinetic order will be zero. Thus, the positive kinetic orders in Eq. 1 are $g_{i,i-1}$ ($1 \leq i \leq n + 1$), since these are the kinetic orders for substrates of reactions. All other exponents are negative or zero, depending on whether X_i is the product of a reversible ($g_{ii} < 0$) or an irreversible ($g_{ii} = 0$) reaction. The fact that g_{ii} is negative if the reaction is reversible is evident from thermodynamic considerations. If the concentration of the product is increased, the thermodynamic potential across the reversible reaction is reduced and the net flux must decrease. Hence, the kinetic order g_{ii} must be negative to represent this decrease.

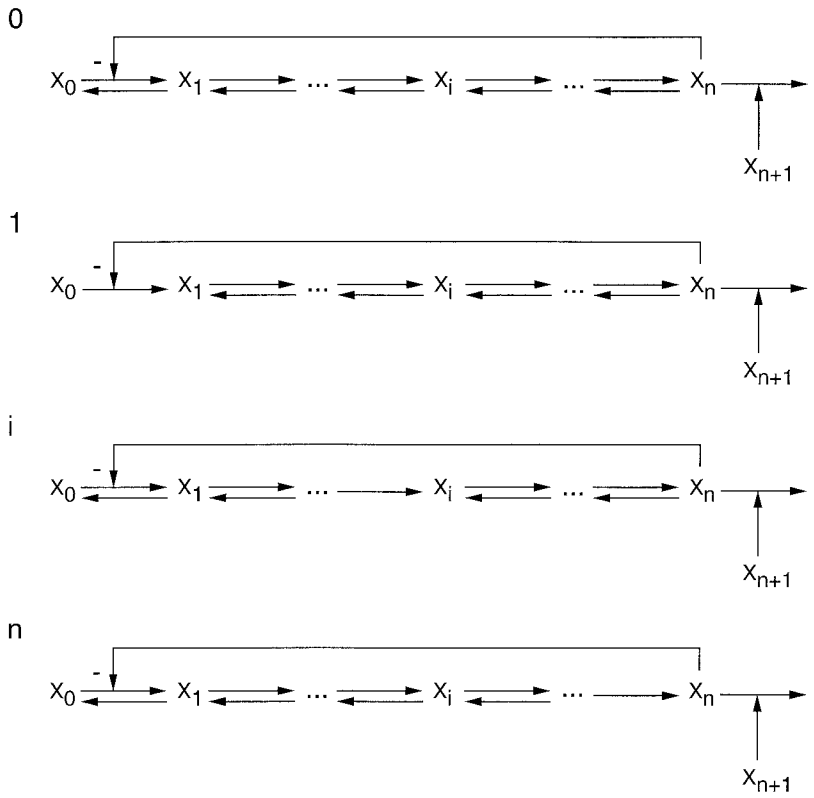

FIGURE 1 Schematic representation of an unbranched biosynthetic pathway subject to control by end-product inhibition. The concentration of the initial substrate X_0 is an independent variable with fixed value; the demand for the end product X_n is represented by X_{n+1}, which also is an independent variable. The reference System 0 has n fully reversible reactions. The alternative systems have one irreversible reaction and the other reactions are identical to the corresponding reactions in the reference system; System 1 has an irreversible reaction at the first position; System i has an irreversible reaction at the ith position; System n has an irreversible reaction at the nth position.

Steady-state solution and key systemic properties

The S-systems describing the dynamic behavior of the models in Fig. 1 can be solved analytically for the steady state (Savageau, 1969, 1971a), where the rates of production and consumption for each metabolite are the same. By equating these rates and taking logarithms of both sides of the resulting equations, one can write the following matrix equation:

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

where $Y_i = \log(X_i)$, $b_i = \log(\alpha_{i+1}/\alpha_i)$, and $a_{ij} = g_{ij} - g_{i+1,j}$ for $1 \leq (i, j) \leq n$.

Two types of coefficients, logarithmic gains and parameter sensitivities, can be used to characterize the steady state of such models. Logarithmic gains measure the relative influence of each independent variable on each dependent variable of the model (Savageau, 1971a; Shiraishi and Savageau, 1992). For example,

$$L(X_i, X_0) = \frac{d\log(X_i)}{d\log(X_0)} = \frac{dY_i}{dY_0} \quad (3)$$

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. Parameter sensitivities measure the relative influence of each parameter on each dependent variable of the model (Savageau, 1971b; Shiraishi and Savageau, 1992). For example,

$$S(X_i, p_j) = \frac{d\log(X_i)}{d\log(p_j)} = p_j \frac{dY_i}{dp_j} \quad (4)$$

measures the percentage 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 structural determinants of the system. Because steady-state solutions exist in closed form, we can calculate each of the two types of coefficients simply by taking the appropriate derivatives. 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 (Savageau, 1976).

Systems should respond quickly to changes in their environment (Savageau, 1975). Thus, another key property of the systems is their temporal response, which was determined as follows. At time zero, each intermediate concentration was set to a value 20% less than its steady-state value. The dynamics were then followed from this initial condition, and the time for all the concentrations to settle to within 1% of their final steady-state value was calculated.

Mathematically controlled comparison

The method of Mathematically Controlled Comparison was specifically developed to make rigorous comparisons of alternative regulatory designs (Savageau, 1972, 1996; Irvine and Savageau, 1985; Alves and Savageau, 2000c, d). This method compares alternative designs for a system that performs a given function and, by using mathematical equivalence constraints to reduce their extraneous differences, determines the irreducible differences between their systemic behaviors. This method requires closed-form solutions for the steady state, which, as noted above, can be obtained with the local S-system representation. Important functional constraints are introduced by equating relevant steady-state properties of the alternative systems being compared. Further analysis (dynamic as well as steady-state) is performed and a profile of ratios is constructed for corresponding results from the alternative systems. In some cases, a ratio can be determined analytically to be less than, equal to, or greater than unity. For example, if the ratio of values for some property P in a reference system to the same property in an alternative system is larger than unity, then the reference system can always be made to have a larger value for P, no matter how large the value for P in the alternative system.

However, if one wishes to know how much greater than unity a given ratio is, then one needs to know actual parameter values. These parameter values are not always available; if they are available, they are not always accurate. Moreover, there are cases in which the ratio can be less than or greater than unity, depending on the specific values for the parameters, so Mathematically Controlled Comparisons that use actual parameter values may lack analytical generality.

In this work we use our method (Alves and Savageau, 2000c), which is a generalization of the original analytical method for making mathematically controlled comparisons; it includes numerical comparisons in which statistical techniques (Alves and Savageau, 2000a) yield results that are general in a statistical sense. We compare the systemic performance of a fully reversible pathway (Fig. 1, System 0) with that of pathways in which only one of the reactions is irreversible (Fig. 1, System 1—System n). We consider all possible positions for the irreversible reaction in pathways with 2 to 7 reactions. The system in which each reaction of the pathway is reversible will be referred to as the reference system or System 0, and the otherwise equivalent system in which the ith reaction of the pathway is irreversible will be referred to as an alternative system or System i. This method also allows direct comparison of System i and System j, each of which has an irreversible reaction but in different positions.

Internal and external equivalence

We are concerned with the irreducible differences in systemic behavior between two pathways of reversible reactions that differ only by the existence of one irreversible reaction in a pathway where the other has a reversible reaction. By irreducible differences we mean differences that persist no matter what the values are for the parameters that define the systems. It is therefore important to ensure that all other changes in systemic behavior are eliminated to the extent possible. To achieve this aim, we shall require that the reference and alternative systems be equivalent from both an internal and external perspective (Savageau, 1972, 1976; Irvine and Savageau, 1985).

By internal equivalence we mean that the values of the corresponding parameters for all the unchanged reactions are the same in both the reference and alternative systems. By external equivalence we mean that systemic behaviors of the reference and alternative systems are made identical, which leads to constraints upon the values for the parameters of the changed reaction. For example, consider the reference system (Fig. 1, System 0) and an alternative system in which the first reaction is irreversible (Fig. 1, System 1). The parameters that characterize the first reaction of the pathway will differ in general between these two systems. The parameters α_1, g_{10}, g_{11}, and g_{1n} of System 0 become the parameters α'_1, g'_{10}, $g'_{11} = 0$, and g'_{1n} of System 1. Since we wish to determine the necessary systemic effects that are due to the change from reversibility to irreversibility, we shall specify values for the parameters α'_1, g'_{10}, and g'_{1n} that eliminate as many extraneous systemic effects as possible. This is accomplished by deriving mathematical expressions for a given steady-state property in each of the two models, equating these expressions to produce a constraint equation, and then solving the constraint equation for one of the primed parameters in terms of the unprimed parameters. When all

primed parameters have been specified in this fashion, there will be no more degrees of freedom with which to make systemic properties equivalent between the two models, and the two systems will be maximally equivalent from an external perspective.

Calculating the constraints for external equivalence

We require the reference and alternative systems in Fig. 1 to have the same steady-state logarithmic gains with respect to the initial substrate of the pathway and the same concentrations (and thus flux). These two types of constraints are sufficient to fix the two primed parameters of the irreversible reaction when its position is beyond the first step.

When the position of the irreversible reaction is at the first step, there are three primed parameters that need to be fixed (see previous section). For the third constraint we require the reference and alternative systems in Fig. 1 to have the same sensitivity of the concentrations with respect to changes in the parameter α_1. This constraint is preferred over other possibilities because the reference system and alternative system will then exhibit the smallest number of systemic differences, which is the objective in a mathematically controlled comparison. One could choose a different systemic property to form the third constraint. However, the reference system and alternative system would then exhibit a larger number of systemic differences, some of which could be eliminated by the choice of the preferred constraint.

Thus, the following system of algebraic equations is solved to obtain the analytic constraints for the primed parameters of the irreversible reaction at the ith step:

$$L(X_i, X_0)_{\text{Reference}} = L(X_i, X_0)_{\text{Alternative}} \quad 1 \le i \le n \tag{5a}$$

$$S(X_i, \alpha_1)_{\text{Reference}} = S(X_i, \alpha_1)_{\text{Alternative}} \quad i = 1 \tag{5b}$$

$$\log[X_i]_{\text{Reference}} = \log[X_i]_{\text{Alternative}} \quad 1 \le i \le n \tag{5c}$$

By constraining one of the logarithmic gains (Eq. 5a), all of them are constrained. This allows us to fix the kinetic order $g'_{i,i-1}$. When the irreversible reaction occurs at the first step, the additional constraint (Eq. 5b) allows us to fix the kinetic order g'_{1n}. By constraining one of the concentrations (Eq. 5c), all of them, as well as the steady-state flux, are constrained. This allows us to fix the rate constant α'_i.

The parametric constraints obtained by solving Eq. 5 have the following form:

$$\begin{aligned} g'_{i,i-1} &= g_{i,i-1} f_i(g, n) \\ g'_{1n} &= g_{1n} + f_n(g, n) \\ \log(\alpha'_i) &= f_\alpha(\alpha, g, n) \end{aligned} \tag{6}$$

where the parameters α and g in the functions f are intended to represent a set of rate constants and kinetic orders that depend both on the length of the pathway and on the systems being considered. The specific forms of these constraints are presented in the Appendix for $n = 2$ to $n = 7$.

Numerical analysis

The analytical results give qualitative information that characterizes the effect of irreversibility in the systems of Fig. 1. To obtain quantitative 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 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 (2000b). Mathematica (Wolfram, 1997) was used for all the numerical procedures.

Density of ratios plot

To interpret the ratios that result from our analysis, we use Density of Ratios plots as defined in Alves and Savageau (2000a). The primary density plots from the raw data have the magnitude for some property of the reference system on the x-axis and the corresponding ratio of magnitudes (reference system to alternative 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 to form 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 of the reference model, denoted $\langle P \rangle$. One advances the window by one position, collects ratios 2 through 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 is used for the first time. This procedure generates a second list L_2 and the corresponding secondary plot. The slope in the secondary plot measures the degree of correlation between the quantities plotted on the x- and y-axes.

Mathematically controlled comparison

Several criteria are considered to determine the functional effectiveness of unbranched biosynthetic pathways (Savageau, 1976; Alves and Savageau, 2000d). The systems being compared will be equal on the bases of the first two criteria because of external equivalence constraints, whereas they will differ with respect to the remaining five criteria.

1. The concentration of intermediates should be low, because otherwise it would tax the limited solvent capacity of the cell and potentially interfere in a nonspecific way with unrelated reactions (e.g., Atkinson, 1969; Savageau, 1972; Srere, 1987; see Levine and Ginsburg, 1985, for a general discussion of the subject from different perspectives). Due to the conditions for external equivalence that we shall impose, the concentrations of the corresponding intermediates will be the same for all comparable systems being examined.
2. The changes in concentration of intermediates caused by changes in the initial substrate should be small. This also will ensure that the solvent capacity is not exceeded when the concentration of intermediates changes. Again, due to the conditions for external equivalence, the corresponding logarithmic gains will be the same for all the systems being examined. These changes are quantified by means of the logarithmic-gain factors $L(X_i, X_0)$ as defined in Eq. 3.
3. The systems should be robust, i.e., the concentrations and flux should be insensitive to changes in the parameters that define the structure of the system (Savageau, 1971b; Shiraishi and Savageau, 1992). If these sensitivities are high, then small fluctuations in parameter values (e.g., due to physical changes such as temperature or to errors in replication, transcription, or translation) would lead to large deviations from the normal behavior of the system. These changes are quantified by means of the parameter sensitivities $S(X_i, p_j)$ and $S(V, p_j)$ as defined in Eq. 4. Aggregate sensitivities for intermediate concentrations and flux are defined as follows: $S(X_i) = \sqrt{\Sigma_j S(X_i\ p_j)^2}$ and $S(V) = \sqrt{\Sigma_j S(V, p_j)^2}$.
4. The systems should have a steady state that is dynamically stable following small perturbations in the concentration variables, otherwise they would be dysfunctional, i.e., unable to maintain homeostasis in the

face of spurious perturbations. Furthermore, the margins of stability should be sufficiently large that changes in parameter values will not produce an unstable steady state. There are n Routh conditions that determine whether the steady state of a system with n variables will be stable. The margins of stability are quantified by the size of the critical Routh conditions, which are the last two (Savageau, 1976; Hlavacek and Savageau, 1997).

5. The flux through the pathway should be highly responsive to changes in the demand for end product. This ensures that the amount of material flowing through the pathway is tightly coupled to the needs of cellular metabolism. This criterion is quantified by the logarithmic gain $L(V, X_{n+1})$, as defined in Eq. 3.
6. The changes in concentration of the intermediates caused by changes in demand for the end product should be small. This ensures that the depletion of end product is minimized when there is an increase in demand. It also ensures that the solvent capacity is not exceeded by the intermediates when demand for the end product changes. These changes are quantified by means of the logarithmic-gain factors $L(X_n, X_{n+1})$ and $L(X_i, X_{n+1})$ as defined in Eq. 3.
7. The systems should respond quickly to changes in their environment, i.e., they should have short transient times (Savageau, 1975). Organisms harboring systems with a sluggish response to change will be at a disadvantage when competing with other organisms in a rapidly changing environment. Transient time will be measured as the time it takes the system to return to its steady state after a small perturbation in concentrations.

RESULTS

In all the results described below, the reference and alternative systems have the same steady-state values for the flux through the pathway, the same concentrations of the corresponding metabolites, and the same logarithmic gains for pathway flux and for metabolite concentrations in response to changes in the initial substrate. These equivalent behaviors are a direct consequence of the constraints for internal and external equivalence, as described above in Methods. The reference and alternative systems differ on the basis of their robustness, margin of stability, response to demand for end product, and transient time.

Robustness

We compare the robustness of the reference system having all reversible reactions with that of an otherwise equivalent alternative system having one irreversible reaction in all possible positions. In most cases, symbolic analysis is sufficient to determine whether the ratio of a given parameter sensitivity in the reference system to the corresponding sensitivity in the alternative system is larger or smaller than 1; in the remaining cases, symbolic analysis is incapable of determining the value for the ratio because it depends on the specific values of the parameters. Results of the symbolic analysis are summarized in Table 1 for pathways of length 2 to 7. The following patterns can be observed in the data.

The reference system is always more robust than the alternative system with an irreversible synthesis of the end product, because the ratios of parameter sensitivities are all less than or equal to 1. As the position of the irreversible reaction approaches the beginning of the pathway, the number of sensitivities that are equal in the systems being compared decreases. The concentration of the product of the irreversible reaction is always more sensitive to parameter changes than the product of the corresponding reversible reaction in the reference system.

In general, numerical methods are needed to decide which systems are more robust because this cannot be done by examining just the symbolic sensitivities. The numerical results in Fig. 2 *A* show that the aggregate sensitivity of X_i to parameters is on average the same in the reference and alternative systems if X_i is surrounded by reversible reactions. If either the reaction that produces or the reaction that consumes X_i is irreversible, then that concentration is on average more robust in the reference system. Fig. 2 *B* shows that, on average, the reference System 0 has smaller aggregate sensitivities for flux than alternative Systems i. However, these differences are only significant for alternative Systems 1 and n.

Margin of stability

Comparing System 0 with System i shows that the stability margins for systems with 2 reactions are always larger in a reference System 0. For systems with 3 to 7 reactions, these margins can be larger in either system. Direct comparison of System i with System j shows that the stability margins can be larger in either system, depending on the parameter values.

Numerical results show that, on average, the reference System 0 has larger margins of stability than alternative Systems i ($i > 1$). Numerical results also show that, on average, the reference System 0 has larger margins of stability than System 1, although the differences are insignificant (Fig. 2 *C*).

Response to demand for end product

Symbolic comparisons with the reference system show that the flux through System 1 is more responsive to changes in the demand for end product than is the flux through System 0. However, for $i > 1$, the flux through System 0 is more responsive to changes in the demand for end product than is the flux through System i. This demonstrates that, with respect to this systemic property, System 1 is better than System 0 and better than any of the other alternatives. Direct comparison of Systems i and j with respect to this systemic property reveals additional information. If $i, j > 1$, then the flux through Systems i and j is equally responsive to changes in the demand for end product.

Numerical results (Fig. 2 *D*) show that average differences between the reference System 0 and alternative System 1 are about 120%, whereas the differences between the

TABLE 1 Comparison of parameter sensitivities for the reference and alternative systems as a function of pathway length and of position for the irreversible step in the pathway

	$n = 2$				$n = 3$				$n = 4$				$n = 5$				$n = 6$				$n = 7$			
	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?
1st reaction irreversible ($i = 1$)																								
V	3	3	2	0	6	3	2	0	6	6	2	0	12	0	2	3	1	17	2	0	0	21	2	0
X_1	0	4	2	2	0	7	2	2	0	6	2	5	1	12	2	2	16	0	2	2	19	0	2	2
X_2	5	1	2	0	3	4	2	2	3	2	2	7	4	11	2	0	12	4	2	2	15	4	2	2
X_3	—	—	—	—	8	1	2	0	11	1	2	0	8	7	2	0	9	7	2	2	12	7	2	2
X_4	—	—	—	—	—	—	—	—	8	4	2	0	11	2	2	2	7	9	2	2	10	9	2	2
X_5	—	—	—	—	—	—	—	—	—	—	—	—	13	2	2	0	3	13	2	2	6	13	2	2
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	17	2	0	4	15	2	2
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	21	2	0
2nd reaction irreversible ($i = 2$)																								
V	2	1	5	0	5	1	5	0	8	1	5	0	11	1	5	0	3	12	5	0	3	15	5	0
X_1	0	3	5	0	3	3	5	0	3	6	5	0	9	3	5	0	3	12	5	0	3	15	5	0
X_2	0	3	5	0	0	4	5	2	0	2	5	7	0	10	5	2	13	0	5	2	16	0	5	2
X_3	—	—	—	—	3	3	5	0	3	6	5	0	3	7	5	2	10	3	5	2	13	3	5	2
X_4	—	—	—	—	—	—	—	—	6	3	5	0	6	4	5	2	7	6	5	2	10	6	5	2
X_5	—	—	—	—	—	—	—	—	—	—	—	—	9	3	5	0	3	10	5	2	6	10	5	2
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	12	5	0	3	13	5	2
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	15	5	0
3rd reaction irreversible ($i = 3$)																								
V	—	—	—	—	2	1	8	0	5	1	8	0	8	1	8	0	3	9	8	0	2	13	8	0
X_1	—	—	—	—	0	3	8	0	0	6	8	0	6	3	8	0	3	9	8	0	3	12	8	0
X_2	—	—	—	—	0	3	8	0	0	6	8	0	6	3	8	0	3	9	8	0	3	12	8	0
X_3	—	—	—	—	0	3	8	0	0	6	8	0	0	7	8	2	10	0	8	2	13	0	8	2
X_4	—	—	—	—	—	—	—	—	3	3	8	0	3	4	8	2	7	3	8	2	10	3	8	2
X_5	—	—	—	—	—	—	—	—	—	—	—	—	6	3	8	0	4	6	8	2	7	6	8	2
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	9	8	0	3	10	8	2
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	13	8	0
4th reaction irreversible ($i = 4$)																								
V	—	—	—	—	—	—	—	—	2	1	11	0	5	1	11	0	7	2	11	0	2	10	11	0
X_1	—	—	—	—	—	—	—	—	0	3	11	0	4	3	11	0	5	4	11	0	7	5	11	0
X_2	—	—	—	—	—	—	—	—	0	3	11	0	4	3	11	0	4	5	11	0	6	6	11	0
X_3	—	—	—	—	—	—	—	—	0	3	11	0	4	3	11	0	6	3	11	0	9	3	11	0
X_4	—	—	—	—	—	—	—	—	0	3	11	0	0	4	11	2	7	0	11	2	10	0	11	2
X_5	—	—	—	—	—	—	—	—	—	—	—	—	4	3	11	0	4	3	11	2	7	3	11	2
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	7	11	0	3	7	11	2
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	10	11	0
5th reaction irreversible ($i = 5$)																								
V	—	—	—	—	—	—	—	—	—	—	—	—	2	1	14	0	2	4	14	0	2	7	14	0
X_1	—	—	—	—	—	—	—	—	—	—	—	—	0	3	14	0	2	4	14	0	6	1	14	2
X_2	—	—	—	—	—	—	—	—	—	—	—	—	0	3	14	0	1	3	14	1	5	2	14	2
X_3	—	—	—	—	—	—	—	—	—	—	—	—	0	3	14	0	1	3	14	2	5	2	14	2
X_4	—	—	—	—	—	—	—	—	—	—	—	—	0	3	14	0	2	2	14	2	5	2	14	2
X_5	—	—	—	—	—	—	—	—	—	—	—	—	0	3	14	0	2	2	14	2	5	2	14	2
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	4	14	0	0	7	14	2
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	10	14	0
6th reaction irreversible ($i = 6$)																								
V	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	17	0	5	1	17	0
X_1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	17	0	2	2	17	2
X_2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	17	0	2	2	17	2
X_3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	17	0	3	1	17	2
X_4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	17	0	2	2	17	2
X_5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	17	0	4	0	17	2
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	17	0	4	0	17	2
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	1	17	

TABLE 1 Continued

	$n = 2$				$n = 3$				$n = 4$				$n = 5$				$n = 6$				$n = 7$			
	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?	>1	<1	=1	?
7th reaction irreversible ($i = 7$)																								
V	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	20	0
X_1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0
X_2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0
X_3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0
X_4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0
X_5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0
X_6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0
X_7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	3	20	0

The sensitivities of the steady-state flux (V) through the pathway and of the steady-state concentrations (X_i) are calculated with respect to each of the parameters in both the reference system and the alternative system. The ratio of a given sensitivity in the reference system relative to the corresponding sensitivity in the alternative system is determined to be greater than one, less than one, equal to one, or indeterminate. The number of reactions in the pathway, n, varies from 2 to 7. The position of the irreversible reaction in the pathway, i, varies from 1 to n. The ratios are the values of the parameter sensitivities for reference System 0 relative to those for alternative Systems i (see Fig. 1). Column legend: >1, number of sensitivities that are larger in reference System 0; <1, number of sensitivities that are smaller in reference System 0; =1, number of sensitivities that are the same in both systems under comparison; ?, number of sensitivities that can be larger in either system, depending on parameter values. For example, the number 5 at the 3rd row, 1st column position of the $i = 1$, $n = 2$ section of the table means that there are five different parameters in a two-step pathway for which the sensitivities of X_2 are larger in System 0 than in System 1.

reference System 0 and alternative Systems i ($i > 1$) are, on average, less than 2%.

The end-product concentration in System 1 is less responsive to changes in the demand for end product than is the end product in System 0. However, for $i > 1$, the end product concentration in System 0 is less responsive to changes in the demand for end product than is the end product in System i. Again, System 1 is better than System 0 and better than any of the other alternatives.

Numerical results (Fig. 2 *E*) show that average differences between the reference System 0 and alternative System 1 can be between 50 and 100%, whereas the differences between the reference System 0 and alternative Systems i ($i > 1$) are, on average, much smaller (2–8%).

Transient time

There is no explicit solution for the dynamic equations given in Eq. 1 that would allow one to determine symbolically the transient responses of the various systems in Fig. 1. The numerical results in Fig. 2 *F* show that the transient time for alternative Systems i ($i < n$) is, on average, smaller than that for the reference System 0, whereas the transient time for alternative System n is larger than that for the reference System 0. A direct comparison of System i and System j ($i, j \neq n$) shows that the transient time can be larger in either system, depending on the length of the pathway (data not shown).

Correlations between ratios and systemic properties

The aggregate sensitivities of the concentrations in System i on average approach those in System 0 as the concentrations of intermediates decrease, i.e., the ratio of aggregate sensitivities approaches 1 (Fig. 2 *A*). The ratio for aggregate sensitivities of flux in System 0 and System 1 also approaches 1, whereas the same ratio in System 0 and Systems i ($i > 1$) decreases away from 1 (Fig. 2 *B*). Thus, the differences in robustness (criterion 3) in System 0 and System 1 become less significant, whereas the differences in System 0 and Systems i ($i > 1$) become more significant at low concentrations of intermediates, which is our first criterion for functional effectiveness.

The ratios involving the critical margins of stability can be positively or negatively correlated with the concentrations of intermediates, depending on the particular comparison (Fig. 2 *C*). There is no general pattern apparent in this panel, so these correlations provide no further information regarding criterion 4.

The ratios for System 0 relative to System 1 of logarithmic gains in flux with respect to changes in the demand for end product are positively correlated with low concentrations of intermediates, although the slope for this correlation is small. The same ratios, but for System 0 relative to System i ($i > 1$), are negatively correlated with low concentrations of intermediates, although the slope for this correlation is also small (Fig. 2 *D*). Thus, the differences in responsiveness of flux to changes in demand for end product (criterion 5) in System 0 and System 1, and in System 0 and System i ($i > 1$), become more significant at low concentrations of intermediates.

The ratios involving logarithmic gains in end product concentration with respect to changes in the demand for end product are positively correlated with the concentrations of intermediates (Fig. 2 *E*). Thus, the differences in depletion of end product following an increase in demand for end product (criterion 6) in System 0 and System 1 become less significant at low concentrations of intermediates.

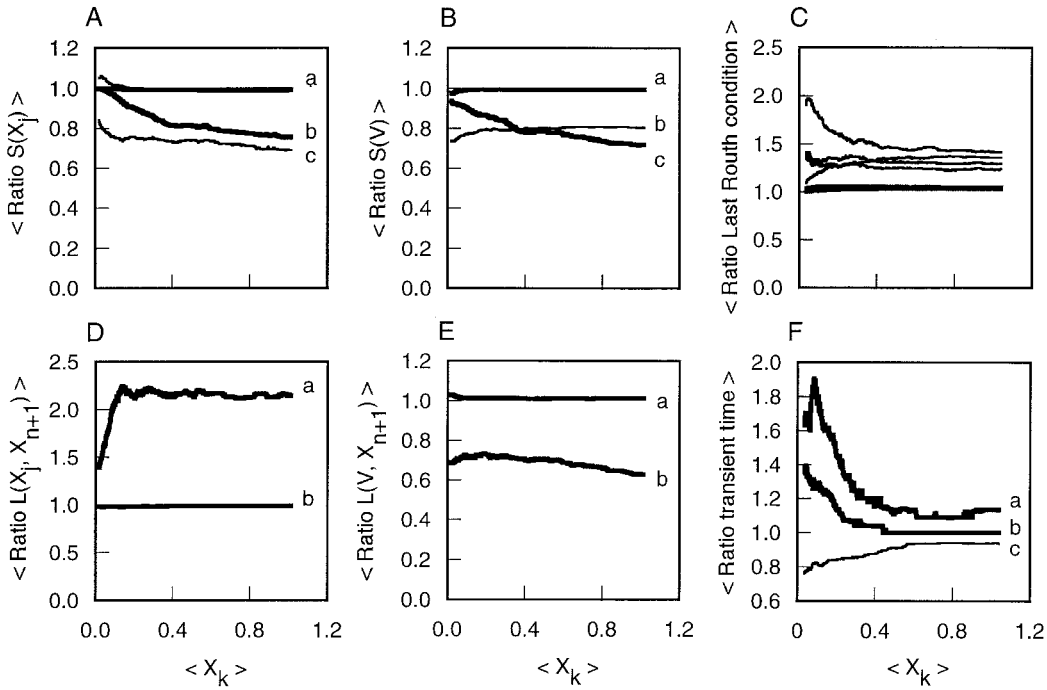

FIGURE 2 Typical correlation curves between ratios of magnitudes in reference System 0 relative to those in alternative Systems i versus concentrations of intermediates. The data, which are generated by changing all of the parameter values randomly within the constraints described in the Methods section, are displayed in a density of ratios plot (Alves and Savageau, 2000a). The y-axis indicates which of two systems on average has the larger magnitude; the x-axis indicates how this difference changes as a function of the concentration of intermediates (see criterion 1 in the text). The subscripts j and k refer to arbitrary pathway intermediates, which have different concentrations in general. We have made individual plots for each pathway length and combination of intermediates. However, since the trends observed for different pathway lengths and intermediates are the same, we show only representative examples. (*A*) Ratios of aggregate sensitivities of concentrations: *a*, aggregate sensitivities of metabolites that have both their production and consumption catalyzed by reversible reactions; *b* and *c*, aggregate sensitivities of metabolites that have either their production or consumption catalyzed by an irreversible reaction. (*B*) Ratios of aggregate sensitivities of flux: *a*, ratio for reference System 0 relative to alternative Systems i ($1 < i < n$); *b,* ratio for reference System 0 relative to alternative System n; *c*, ratio for reference System 0 relative to alternative System 1. (*C*) Ratios of critical criteria for local stability. (*D*) Ratios of logarithmic gains in concentration with respect to changes in demand for the end product: *a*, ratio for reference System 0 relative to alternative System 1; *b*, ratio for reference System 0 relative to alternative System i ($i > 1$). (*E*) Ratios of logarithmic gains in flux with respect to changes in demand for the end product: *a*, ratio for reference System 0 relative to alternative Systems i ($i > 1$); *b*, ratio for reference System 0 relative to alternative System 1. (*F*) Ratios of transient times: *a* and *b*, ratio for reference System 0 relative to two different alternative Systems i ($i < n$); *c*, ratio for reference System 0 relative to alternative System n.

The ratios involving transient times are inversely correlated with the concentrations of intermediates (Fig. 2 *F*). Thus, the difference in transient times (criterion 7) in System 0 and Systems i ($i < n$) increases as the concentration of intermediates decreases, whereas this difference in System 0 and System n decreases.

DISCUSSION

We analyzed the effect of having an irreversible reaction at different positions in an unbranched biosynthetic pathway with all other reactions being reversible. We also analyzed the effect of having a reversible reaction at different positions in pathways with all other reactions being irreversible (data not shown). The results are qualitatively similar; namely, the best position for the single irreversible reaction is at the beginning of the pathway, whereas the best position for the single reversible reaction is at the end of the pathway. The method used for our analysis, mathematically controlled comparisons, often allows one to obtain symbolic (and thus general) results when comparing systemic properties of alternative models. When this is not possible, the method also can be used numerically to obtain results that are general in a statistical sense. Comparisons were made based on functional effectiveness, as judged by the seven quantitative criteria described in detail in the Methods section.

In this work we have found a limited number of symbolic comparisons whose conclusions do not depend on the specific values of the parameters. The reference pathway with all reactions fully reversible (System 0) is more robust to perturbations in the values of the parameters (criterion 3) than is an otherwise equivalent alternative pathway with an irreversible synthesis of end product. Also, when comparing reference System 0 with alternative Systems i ($i \neq 1$), where reaction i is irreversible, the flux through System 0 is more responsive to changes in the demand for end product (cri-

terion 5), whereas the concentrations of its intermediates are less responsive (criterion 6). On the other hand, the flux is more responsive (criterion 5) and the concentrations are less responsive (criterion 6) to the demand for end product in System 1 than in System 0. Taken together, these results imply that reference System 0 is superior to alternative System n on the bases of criteria 3, 5, and 6, superior to alternative Systems i ($i \neq 1, n$) on the bases of criteria 5 and 6, but inferior to alternative System 1 on the bases of criteria 5 and 6. Not much can be said analytically about the comparison of these systems based on other criteria.

Additional conclusions that are general in a statistical sense can be obtained by means of numerical comparisons. These indicate that the reference System 0 is, on average, better than or similar to the alternative Systems i ($i > 1$) on the bases of all the criteria except transient time (criterion 7). These numerical comparisons also indicate that the alternative System 1 is, on average, better than or similar to the reference System 0 on the bases of all the criteria except some components of robustness (criterion 3). The differences in value for those components that favor reference System 0 over alternative System 1 are less significant when the systems are optimized according to criterion 1 than when these systems are not so optimized. Thus, alternative System 1 is, on average, better than or similar to all other systems under the following conditions: The concentrations of intermediates are equally low (criterion 1). The logarithmic gains in concentration with respect to change in the level of initial substrate also are equally low (criterion 2). The robustness of all the intermediates, with one exception, is similar. Although, as noted above, the first intermediate and the flux are less robust in System 1, these differences are less significant when criterion 1 is satisfied (criterion 3). The margins of stability are similar (criterion 4). Flux is more responsive to changes in demand for end product (criterion 5). Concentrations of intermediates are less responsive to changes in demand for end product (criterion 6). Transient times are shorter (criterion 7).

The combination of analytical and numerical results presented in this paper provides a functional rationale for why irreversible reactions are found predominantly at the beginnings of unbranched biosynthetic pathways.

APPENDIX

Parametric constraints for external equivalence. The number of reactions in the pathway is n, where n varies from 2 to 7. The position of the irreversible reaction in the pathway is i, where i varies from 1 to n. An extra constraint, $g'_{10} = g_{10}$, is common to all cases when the irreversible reaction is in the first position, i.e., when i = 1.

$n = 2$

$$i = 1\text{: } \log[\alpha'_1] = \log[\alpha_1] - g_{11} \log[\alpha_2/\alpha_3]/g_{21}; \quad g'_{12} = g_{12} + g_{11}(g_{32} - g_{22})/g_{21}$$

$$i = 2\text{: } \log[\alpha'_2] = (g_{32} \log[\alpha_2] - g_{22} \log[\alpha_3])/(g_{32} - g_{22}); \quad g'_{21} = g_{21}g_{32}/(g_{32} - g_{22})$$

$n = 3$

$$i = 1\text{: } \log[\alpha'_1] = \log[\alpha_1] - g_{11}(g_{32} \log[\alpha_2/\alpha_4] - g_{22} \log[\alpha_3/\alpha_4])/(g_{21}g_{32});$$

$$g'_{13} = g_{13} + g_{11}(g_{32}g_{43} - g_{22}(g_{43} - g_{33}))/(g_{21}g_{32})$$

$$i = 2\text{: } \log[\alpha'_2] = (g_{43}(g_{32} \log[\alpha_2] - g_{22} \log[\alpha_3]) + g_{22}g_{33} \log[\alpha_4])/(g_{32}g_{43} - g_{22}(g_{43} - g_{33}));$$

$$g'_{21} = g_{21}g_{32}g_{43}/(g_{32}g_{43} - g_{22}(g_{43} - g_{33}))$$

$$i = 3\text{: } \log[\alpha'_3] = (g_{43} \log[\alpha_3] - g_{33} \log[\alpha_4])/(g_{43} - g_{33}); \quad g'_{32} = g_{32}g_{43}/(g_{43} - g_{33})$$

$n = 4$

$$i = 1\text{: } \log[\alpha'_1] = \log[\alpha_1] - g_{11}\frac{g_{32}g_{43} \log[\alpha_2/\alpha_5] - g_{22}(g_{43} \log[\alpha_3/\alpha_5] - g_{33} \log[\alpha_4/\alpha_5])}{g_{21}g_{32}g_{43}};$$

$$g'_{14} = g_{14} + g_{11}(g_{22}g_{33}(g_{54} - g_{44}) + g_{43}g_{54}(g_{32} - g_{22}))/(g_{21}g_{32}g_{43})$$

$$i = 2\text{: } \log[\alpha'_2] = \frac{g_{43}g_{54}(g_{32} \log[\alpha_2] - g_{22} \log[\alpha_3]) + g_{22}g_{33}(g_{54} \log[\alpha_4] - g_{44} \log[\alpha_5])}{g_{32}g_{43}g_{54} - g_{22}(g_{43}g_{54} - g_{33}(g_{54} - g_{44}))};$$

$$g'_{21} = g_{21}g_{32}g_{43}g_{54}/(g_{32}g_{43}g_{54} - g_{22}(g_{43}g_{54} - g_{33}(g_{54} - g_{44})))$$

$$i = 3\text{: } \log[\alpha'_3] = (g_{54}(g_{43}\log[\alpha_3] - g_{33}\log[\alpha_4]) + g_{33}g_{44}\log[\alpha_5])/(g_{43}g_{54} - g_{33}(g_{54} - g_{44}));$$

$$g'_{32} = g_{32}g_{43}g_{54}/(g_{43}g_{54} - g_{33}(g_{54} - g_{44}))$$

$$i = 4\text{: } \log[\alpha'_4] = (g_{54}\log[\alpha_4] - g_{44}\log[\alpha_5])/(g_{54} - g_{44}); \quad g'_{43} = g_{43}g_{54}/(g_{54} - g_{44})$$

$n = 5$

$$i = 1\text{: } \log[\alpha'_1] = \log[\alpha_1] - g_{11}\frac{(g_{32}g_{43}g_{54}\log[\alpha_2/\alpha_6] - g_{22}g_{43}g_{54}\log[\alpha_3/\alpha_6] + g_{22}g_{33}g_{54}\log[\alpha_4/\alpha_6] - g_{22}g_{33}g_{44}\log[\alpha_5/\alpha_6])}{g_{21}g_{32}g_{43}g_{54}};$$

$$g'_{15} = g_{15} + g_{11}\frac{g_{22}g_{33}(g_{54}g_{65} - g_{44}(g_{65} - g_{55})) + g_{43}g_{54}g_{65}(g_{32} - g_{22})}{g_{21}g_{32}g_{43}g_{54}}$$

$$i = 2\text{: } \log[\alpha'_2] = \frac{(g_{32}g_{43}g_{54}g_{65}\log[\alpha_2] - g_{22}g_{43}g_{54}g_{65}\log[\alpha_3] + g_{22}g_{33}g_{54}g_{65}\log[\alpha_4] - g_{22}g_{33}g_{44}g_{65}\log[\alpha_5] + g_{22}g_{33}g_{44}g_{55}\log[\alpha_6])}{g_{32}g_{43}g_{54}g_{65} - g_{22}(g_{43}g_{54}g_{65} - g_{33}(g_{54}g_{65} - g_{44}(g_{65} - g_{55})))};$$

$$g'_{21} = g_{21}g_{32}g_{43}g_{54}g_{65}/(g_{32}g_{43}g_{54}g_{65} - g_{22}(g_{43}g_{54}g_{65} - g_{33}(g_{54}g_{65} - g_{44}(g_{65} - g_{55}))))$$

$$i = 3\text{: } \log[\alpha'_3] = \frac{g_{43}g_{54}g_{65}\log[\alpha_3] - g_{33}g_{54}g_{65}\log[\alpha_4] + g_{33}g_{44}(g_{65}\log[\alpha_5] - g_{55}\log[\alpha_6])}{g_{43}g_{54}g_{65} - g_{33}(g_{54}g_{65} - g_{44}(g_{65} - g_{55}))};$$

$$g'_{32} = g_{32}g_{43}g_{54}g_{65}/(g_{43}g_{54}g_{65} - g_{33}(g_{54}g_{65} - g_{44}(g_{65} - g_{55})))$$

$$i = 4\text{: } \log[\alpha'_4] = (g_{54}g_{65}\log[\alpha_4] - g_{44}(g_{65}\log[\alpha_5] - g_{55}\log[\alpha_6]))/(g_{54}g_{65} - g_{44}(g_{65} - g_{55}));$$

$$g'_{43} = g_{43}g_{54}g_{65}/(g_{54}g_{65} - g_{44}(g_{65} - g_{55}))$$

$$i = 5\text{: } \log[\alpha'_5] = (g_{65}\log[\alpha_5] - g_{55}\log[\alpha_6])/(g_{65} - g_{55}); \quad g'_{54} = g_{54}g_{65}/(g_{65} - g_{55})$$

$n = 6$

$$i = 1\text{: } \log[\alpha'_1] = \log[\alpha_1] - g_{11}\left(\frac{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}\log[\alpha_2/\alpha_7] - g_{22}g_{43}g_{54}g_{65}\log[\alpha_3/\alpha_7] \\ + g_{22}g_{33}g_{54}g_{65}\log[\alpha_4/\alpha_7] - g_{22}g_{33}g_{44}g_{65}\log[\alpha_5/\alpha_7] \\ + g_{22}g_{33}g_{44}g_{55}\log[\alpha_6/\alpha_7] \end{pmatrix}}{g_{21}g_{32}g_{43}g_{54}g_{65}}\right);$$

$$g'_{16} = g_{16} + g_{11}\frac{g_{32}g_{43}g_{54}g_{65} - g_{22}(g_{43}g_{54}g_{65} - g_{33}(g_{54}g_{65} - g_{44}(g_{65}g_{76} - g_{55}(g_{76} - g_{66}))))}{g_{21}g_{32}g_{43}g_{54}g_{65}}$$

$$i = 2\text{: } \log[\alpha'_2] = \frac{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}g_{76}\log[\alpha_2] - g_{22}g_{43}g_{54}g_{65}g_{76}\log[\alpha_3] + g_{22}g_{33}g_{54}g_{65}g_{76}\log[\alpha_4] \\ - g_{22}g_{33}g_{44}g_{65}g_{76}\log[\alpha_5] + g_{22}g_{33}g_{44}g_{55}g_{76}\log[\alpha_6] - g_{22}g_{33}g_{44}g_{55}g_{66}\log[\alpha_7] \end{pmatrix}}{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}g_{76} - g_{22}g_{43}g_{54}g_{65}g_{76} + g_{22}g_{33}g_{54}g_{65}g_{76} \\ - g_{22}g_{33}g_{44}g_{65}g_{76} + g_{22}g_{33}g_{44}g_{55}g_{76} - g_{22}g_{33}g_{44}g_{55}g_{66} \end{pmatrix}};$$

$$g'_{21} = \frac{g_{21}g_{32}g_{43}g_{54}g_{65}g_{76}}{(g_{32}g_{43}g_{54}g_{65}g_{76} - g_{22}g_{43}g_{54}g_{65}g_{76} + g_{22}g_{33}g_{54}g_{65}g_{76} - g_{22}g_{33}g_{44}g_{65}g_{76} + g_{22}g_{33}g_{44}g_{55}g_{76} - g_{22}g_{33}g_{44}g_{55}g_{66})}$$

$$i = 3\text{: } \log[\alpha'_3] = \frac{(g_{43}g_{54}g_{65}g_{76}\log[\alpha_3] - g_{33}g_{54}g_{65}g_{76}\log[\alpha_4] + g_{33}g_{44}g_{65}g_{76}\log[\alpha_5] - g_{33}g_{44}g_{55}g_{76}\log[\alpha_6] + g_{33}g_{44}g_{55}g_{66}\log[\alpha_7])}{g_{43}g_{54}g_{65}g_{76} - g_{33}g_{54}g_{65}g_{76} + g_{33}g_{44}g_{65}g_{76} - g_{33}g_{44}g_{55}g_{76} + g_{33}g_{44}g_{55}g_{66}};$$

$$g'_{32} = \frac{g_{32}g_{43}g_{54}g_{65}g_{76}}{g_{43}g_{54}g_{65}g_{76} - g_{33}g_{54}g_{65}g_{76} + g_{33}g_{44}g_{65}g_{76} - g_{33}g_{44}g_{55}g_{76} + g_{33}g_{44}g_{55}g_{66}}$$

$$i = 4\text{: } \log[\alpha_4'] = \frac{g_{54}g_{65}g_{76}\log[\alpha_4] - g_{44}g_{65}g_{76}\log[\alpha_5] + g_{44}g_{55}g_{76}\log[\alpha_6] - g_{44}g_{55}g_{66}\log[\alpha_7]}{g_{54}g_{65}g_{76} - g_{44}g_{65}g_{76} + g_{44}g_{55}g_{76} - g_{44}g_{55}g_{66}};$$

$$g_{43}' = \frac{g_{43}g_{54}g_{65}g_{76}}{g_{54}g_{65}g_{76} - g_{44}g_{65}g_{76} + g_{44}g_{55}g_{76} - g_{44}g_{55}g_{66}}$$

$$i = 5\text{: } \log[\alpha_5'] = (g_{65}g_{76}\log[\alpha_5] - g_{55}g_{76}\log[\alpha_6] + g_{55}g_{66}\log[\alpha_7])/(g_{65}g_{76} - g_{55}g_{76} + g_{55}g_{66});$$

$$g_{54}' = g_{54}g_{65}g_{76}/(g_{65}g_{76} - g_{55}g_{76} + g_{55}g_{66})$$

$$i = 6\text{: } \log[\alpha_6'] = (g_{76}\log[\alpha_6] - g_{66}\log[\alpha_7])/(g_{76} - g_{66}); \quad g_{65}' = g_{65}g_{76}/(g_{76} - g_{66})$$

$n = 7$

$$i = 1\text{: } \log[\alpha_1'] = \log[\alpha_1] - g_{11}\frac{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}g_{76}\log[\alpha_2/\alpha_8] - g_{22}g_{43}g_{54}g_{65}g_{76}\log[\alpha_3/\alpha_8] + g_{22}g_{33}g_{54}g_{65}g_{76}\log[\alpha_4/\alpha_8] \\ - g_{22}g_{33}g_{44}g_{65}g_{76}\log[\alpha_5/\alpha_8] + g_{22}g_{33}g_{44}g_{55}g_{76}\log[\alpha_6/\alpha_8] - g_{22}g_{33}g_{44}g_{55}g_{66}\log[\alpha_7/\alpha_8] \end{pmatrix}}{g_{21}g_{32}g_{43}g_{54}g_{65}g_{76}};$$

$$g_{17}' = g_{17} + g_{11}\frac{(g_{32}g_{43}g_{54}g_{65}g_{76} - g_{22}g_{43}g_{54}g_{65}g_{76} + g_{22}g_{33}g_{54}g_{65}g_{76} - g_{22}g_{33}g_{44}g_{65}g_{76} + g_{22}g_{33}g_{44}g_{55}g_{76} - g_{22}g_{33}g_{44}g_{55}g_{66}}{g_{21}g_{32}g_{43}g_{54}g_{65}g_{76}}$$

$$i = 2\text{: } \log[\alpha_2'] = \frac{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}g_{76}g_{87}\log[\alpha_2] - g_{22}g_{43}g_{54}g_{65}g_{76}g_{87}\log[\alpha_3] \\ + g_{22}g_{33}g_{54}g_{65}g_{76}g_{87}\log[\alpha_4] - g_{22}g_{33}g_{44}g_{65}g_{76}g_{87}\log[\alpha_5] \\ + g_{22}g_{33}g_{44}g_{55}g_{76}g_{87}\log[\alpha_6] - g_{22}g_{33}g_{44}g_{55}g_{66}g_{87}\log[\alpha_7] + g_{22}g_{33}g_{44}g_{55}g_{66}g_{77}\log[\alpha_8] \end{pmatrix}}{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}g_{76}g_{87} - g_{22}g_{43}g_{54}g_{65}g_{76}g_{87} + g_{22}g_{33}g_{54}g_{65}g_{76}g_{87} \\ - g_{22}g_{33}g_{44}g_{65}g_{76}g_{87} + g_{22}g_{33}g_{44}g_{55}g_{76}g_{87} - g_{22}g_{33}g_{44}g_{55}g_{66}g_{87} \\ + g_{22}g_{33}g_{44}g_{55}g_{66}g_{77} \end{pmatrix}};$$

$$g_{21}' = \frac{g_{21}g_{32}g_{43}g_{54}g_{65}g_{76}g_{87}}{\begin{pmatrix} g_{32}g_{43}g_{54}g_{65}g_{76}g_{87} - g_{22}g_{43}g_{54}g_{65}g_{76}g_{87} + g_{22}g_{33}g_{54}g_{65}g_{76}g_{87} \\ - g_{22}g_{33}g_{44}g_{65}g_{76}g_{87} + g_{22}g_{33}g_{44}g_{55}g_{76}g_{87} - g_{22}g_{33}g_{44}g_{55}g_{66}g_{87} \\ + g_{22}g_{33}g_{44}g_{55}g_{66}g_{77} \end{pmatrix}}$$

$$i = 3\text{: } \log[\alpha_3'] = \frac{\begin{pmatrix} g_{43}g_{54}g_{65}g_{76}g_{87}\log[\alpha_3] - g_{33}g_{54}g_{65}g_{76}g_{87}\log[\alpha_4] \\ + g_{33}g_{44}g_{65}g_{76}g_{87}\log[\alpha_5] - g_{33}g_{44}g_{55}g_{76}g_{87}\log[\alpha_6] \\ + g_{33}g_{44}g_{55}g_{66}g_{87}\log[\alpha_7] - g_{33}g_{44}g_{55}g_{66}g_{77}\log[\alpha_8] \end{pmatrix}}{(g_{43}g_{54}g_{65}g_{76}g_{87} - g_{33}g_{54}g_{65}g_{76}g_{87} + g_{33}g_{44}g_{65}g_{76}g_{87} - g_{33}g_{44}g_{55}g_{76}g_{87} + g_{33}g_{44}g_{55}g_{66}g_{87} - g_{33}g_{44}g_{55}g_{66}g_{77})};$$

$$g_{32}' = \frac{g_{32}g_{43}g_{54}g_{65}g_{76}g_{87}}{(g_{43}g_{54}g_{65}g_{76}g_{87} - g_{33}g_{54}g_{65}g_{76}g_{87} + g_{33}g_{44}g_{65}g_{76}g_{87} - g_{33}g_{44}g_{55}g_{76}g_{87} + g_{33}g_{44}g_{55}g_{66}g_{87} - g_{33}g_{44}g_{55}g_{66}g_{77})}$$

$$i = 4\text{: } \log[\alpha_4']$$

$$= \frac{(g_{54}g_{65}g_{76}g_{87}\log[\alpha_4] - g_{44}g_{65}g_{76}g_{87}\log[\alpha_5] + g_{44}g_{55}g_{76}g_{87}\log[\alpha_6] - g_{44}g_{55}g_{66}g_{87}\log[\alpha_7] + g_{44}g_{55}g_{66}g_{77}\log[\alpha_8])}{g_{54}g_{65}g_{76}g_{87} - g_{44}g_{65}g_{76}g_{87} + g_{44}g_{55}g_{76}g_{87} - g_{44}g_{55}g_{66}g_{87} + g_{44}g_{55}g_{66}g_{77}};$$

$$g_{43}' = \frac{g_{43}g_{54}g_{65}g_{76}g_{87}}{g_{54}g_{65}g_{76}g_{87} - g_{44}g_{65}g_{76}g_{87} + g_{44}g_{55}g_{76}g_{87} - g_{44}g_{55}g_{66}g_{87} + g_{44}g_{55}g_{66}g_{77}}$$

$$i = 5\text{: } \log[\alpha_5'] = \frac{g_{65}g_{76}g_{87}\log[\alpha_5] - g_{55}g_{76}g_{87}\log[\alpha_6] + g_{55}g_{66}g_{87}\log[\alpha_7] - g_{55}g_{66}g_{77}\log[\alpha_8]}{g_{65}g_{76}g_{87} - g_{55}g_{76}g_{87} + g_{55}g_{66}g_{87} - g_{55}g_{66}g_{77}};$$

$$g_{54}' = g_{54}g_{65}g_{76}g_{87}/(g_{65}g_{76}g_{87} - g_{55}g_{76}g_{87} + g_{55}g_{66}g_{87} - g_{55}g_{66}g_{77})$$

$$i = 6\text{: } \log[\alpha_6'] = (g_{76}g_{87}\log[\alpha_6] - g_{66}g_{87}\log[\alpha_7] + g_{66}g_{77}\log[\alpha_8])/(g_{76}g_{87} - g_{66}g_{87} + g_{66}g_{77});$$

$$g_{65}' = g_{65}g_{76}g_{87}/(g_{76}g_{87} - g_{66}g_{87} + g_{66}g_{77})$$

$$i = 7\text{: } \log[\alpha_7'] = (g_{87}\log[\alpha_7] - g_{77}\log[\alpha_8])/(g_{87} - g_{77}); \quad g_{76}' = g_{76}g_{87}/(g_{87} - g_{77})$$

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