PUTTING THE "CONTROL" IN METABOLIC CONTROL ANALYSIS [1]

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Abstract: Metabolic control analysis is a framework for characterizing the parametric sensitivity of metabolic pathways and genetic networks. We establish a connection between metabolic control analysis and control theory. The main result is that we can use classical control theory and the associated signal-flow (block) diagrams to analyze biochemical reaction networks and that many results in metabolic control analysis have direct counterparts in control. In the process, we illustrate how some problems in biochemical network analysis can be reformulated in a control-theoretic framework.

Keywords: Metabolic control analysis (MCA), sensitivity, biology, networks

1. INTRODUCTION

Metabolic control analysis (MCA) is a framework for analyzing metabolic pathways and genetic networks. The theory aims to link steady-state changes in the individual pathway components to steady-state changes in the systematic behavior of the network. The basic framework was proposed over the thirty years ago in a variety of forms by a number of biologists (Kacser and Burns, 1973; Burns, 1971; Heinrich and Rapoport, 1974*b*; Heinrich and Rapoport, 1974*a*; Savageau, 1971; Savageau, 1972). The theory was motivated by the realization that metabolic flux is not controlled by one rate-limiting enzyme. Rather, the control is shared by all of the enzymes in the pathway. This distributed control is particularly striking in regulated and branched pathways. The basic theory is now mature (Heinrich and Schuster, 1996; Fell, 1997), though many opportunities exist to extend MCA.

In addition to its name, there is striking similarity between the equations in MCA and those found in classical control theory. Intrigued by the cursory resemblance, we develop this connection and demonstrate that MCA can be entirely reformulated in control-theoretic terms. In the process we demonstrate that the MCA formulation, which is really just specialized sensitivity analysis, offers a promising foothold at the interface of biology and control theory.

2. BACKGROUND

We assume that the dynamics of the biochemical reaction network are described by the following differential equation

$$\dot{s}(t) = \mathbf{N}v(s(t), p), \tag{1}$$

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where the vector $s \in \mathbb{R}^n$ contains the concentrations for n chemical species, the matrix $\mathbf{N} \in \mathbb{I}^{n \times m}$ defines the stoichiometry of the reaction network, the function $v(\cdot,\cdot) : \mathbb{R}^n \times \mathbb{R}^p \mapsto \mathbb{R}^m$ describes the rates for m chemical reactions, and the vector $p \in \mathbb{R}^p$ contains the relevant kinetic parameters for these rates such as enzyme concentrations and catalytic efficiencies.

As the inclusion of some species in the model (1) leads to redundant equations because of mass conservation constraints, one commonly removes these species to create a reduced stoichiometric matrix $\mathbf{N_r}$ with linearly independent rows (Reder, 1988). We can recover the full matrix by introducing the link matrix $\mathbf{L}$ where

$$\mathbf{N} = \mathbf{L}\mathbf{N_r}.$$

Let $s_i(t)$ denote the concentration of the reduced set of species. Then, we can recover $s(t)$ from $s_i(t)$ using the relation

$$s(t) = \mathbf{L}s_i(t) + T$$

with the appropriate choice for the constant vector T. The vector T accounts typically for mass conservation. For example, the mass constraint $a(t)+b(t) = 1$ yields the link matrix $\mathbf{L} = \begin{bmatrix} 1 & -1 \end{bmatrix}^T$ and constant vector $T = \begin{bmatrix} 0 & 1 \end{bmatrix}^T$ with $s(t) = \begin{bmatrix} a(t) & b(t) \end{bmatrix}^T$ and $s_i(t) = a(t)$.

MCA is concerned with how the properties of the network change when the parameters p are perturbed. The sensitivity function or **control coefficient** is defined as

$$x(t) \triangleq \frac{ds_i(t)}{dp}.$$

Using the chain rule for differentiation, one can show that the control coefficient $x(t)$ satisfies the following differential equation

$$\dot{x}(t) = \left(\mathbf{N_r}\frac{\partial v(s(t),p)}{\partial s(t)}\mathbf{L}\right)x(t) + \mathbf{N_r}\frac{\partial v(s(t),p)}{\partial p}. \quad (2)$$

Note that equation (2) is linear: it is identical to the linearization of equation (1). If the reaction network (1) is at a steady state s_{ss}, then the sensitivity equation (2) for infinitesimal perturbations about the steady state satisfies the linear, time-independent, differential equation

$$\dot{x}(t) = (\mathbf{N_r}\varepsilon_s\mathbf{L})\,x(t) + \mathbf{N_r}\varepsilon_p(t), \quad (3)$$

where

$$\varepsilon_x \triangleq \frac{\partial v(s_{ss},p)}{\partial s}, \;\; \varepsilon_p \triangleq \frac{\partial v(s_{ss},p)}{\partial p}.$$

The matrices $\varepsilon_x \in \mathbb{R}^{m \times n}$ and $\varepsilon_p \in \mathbb{R}^{m \times p}$ are called the **elasticity coefficients**. They provide a measure for how strongly a single reaction in isolation is changed by infinitesimal perturbations either to the concentrations s or parameters p. It is possible to directly measure the elasticity coefficients from experiments without needing to know the rate laws $v(\cdot)$ explicitly. In fact, it is easier to determine experimentally the elasticity coefficients than the actual rate laws (Fell, 1997).

As equation (3) is linear and time invariant, we can take the Laplace transform and obtain the frequency response for the control coefficients:

$$X(j\omega) = (j\omega I - \mathbf{N_r}\varepsilon_x\mathbf{L})^{-1}\mathbf{N_r}\varepsilon_p, \quad (4)$$

where $X(j\omega)$ is the Laplace transform of $x(t)$ and j is the complex number $\sqrt{-1}$. While the perturbations are typically constant, it is also possible to consider time-varying perturbations $\Delta(t)$. To explore frequency variations, we replace ε_p with $\varepsilon_p\Delta(j\omega)$ in equation (4). At steady state ($\omega = 0$), the sensitivity equation (4) become

$$X(0) = -(\mathbf{N_r}\varepsilon_x\mathbf{L})^{-1}\mathbf{N_r}\varepsilon_p,$$

assuming the inverse exists. This equation is known as the **control equation**, because it relates the control coefficients $X(0)$ to the elasticity coefficients ε_p at steady state. It is a cornerstone of MCA (Burns, 1971; Heinrich and Rapoport, 1974*b*; Heinrich *et al.*, 1977; Reder, 1988).

In addition to the concentrations $s(t)$, MCA is concerned with perturbations to the flux through the network. We define the flux control coefficient as

$$y(t) \triangleq \frac{dv}{dp}.$$

The flux control coefficient $y(t)$ is related to the control coefficient $x(t)$ by the affine relation

$$\begin{aligned} y(t) &= \frac{\partial v}{\partial p} + \left(\frac{\partial v}{\partial s}\mathbf{L}\right)x(t), \\ &= \varepsilon_p + (\varepsilon_x\mathbf{L})\,x(t), \end{aligned}$$

where the second relation holds for perturbations about the steady state s_{ss}. Note the difference between y and ε_p.

If $\mathbf{A} \triangleq (\mathbf{N_r}\varepsilon_x\mathbf{L})$, $\mathbf{B} \triangleq \mathbf{N_r}$, $\mathbf{C} \triangleq \varepsilon_x\mathbf{L}$ and $\mathbf{D} \triangleq \mathbf{I}$, then we represent the control equations using the following state-space form:

$$\dot{x} = \mathbf{A}x + \mathbf{B}\varepsilon_p, \quad (5a)$$

$$y = \mathbf{C}x + \mathbf{D}\varepsilon_p, \quad (5b)$$

where ε_p is the input.

In the remainder of this article, we explore the control equation (5) and demonstrate how some

elementary tools from control analysis can be applied to this equation to explore regulation in biochemical networks.

3. SIGNAL-FLOW GRAPHS

Molecular biologists commonly draw complex diagrams describing intracellular pathways in *cartoon* format. While these diagrams successfully enumerate the players and interactions within the pathway, they are unable to convey any information regarding dynamic or regulatory properties.

When analyzing and designing control systems, engineers often employ signal-flow graphs. Signal-flow graphs diagram the feedback structure using unambiguous notation. Signal-flow diagrams are also able to convey dynamic information. While signal-flow graphs are less relevant for design now with numerous CAD procedures, they are still indispensable for analysis, especially for linear systems.

As we demonstrate, it is possible to diagram the (linear) regulatory structure of pathways characterized by the control and elasticity coefficients using signal-flow graphs. While we would be too optimistic if we expect molecular biologists will adopt a similar convention, our pragmatic goal is to bridge biological network analysis with control theory, and in the process leverage the tools, theories, and intuition from control for biology. What better way than to start with a control diagram!

3.1 Linear Chain

Consider the following linear chain of reactions

$$() \underset{\leftarrow}{\overset{v_1}{\rightleftharpoons}} s_1 \underset{\leftarrow}{\overset{v_2}{\rightleftharpoons}} s_2 \underset{\leftarrow}{\overset{v_3}{\rightleftharpoons}} () \tag{6}$$

We assume that the reaction mechanisms are elementary to the degree that

$$v_1 = v_1(s_1, p), \;\; v_2 = v_2(s_1, s_2, p), \;\; v_3 = v_3(s_2, p).$$

The kinetic equations for this mechanism are of the form

$$\begin{aligned} \dot{s}_1 &= v_1(s_1, p) - v_2(s_1, s_2, p), \\ \dot{s}_2 &= v_2(s_1, s_2, p) - v_3(s_2, p). \end{aligned}$$

Let

$$\varepsilon_j^i = \frac{\partial v_i(\cdot)}{\partial s_j},$$

where s_j denotes the j^{th} element of the n-dimensional vector s.

The control equations for this reaction mechanism are

$$\dot{x}_1(t) = \left(\varepsilon_1^1 - \varepsilon_1^2\right) x_1(t) - \varepsilon_2^2 x_2(t) + \varepsilon_p^1 - \varepsilon_p^2 \quad (7a)$$

$$\dot{x}_2(t) = \left(\varepsilon_2^2 - \varepsilon_2^3\right) x_2(t) + \varepsilon_1^2 x_1(t) + \varepsilon_p^2 - \varepsilon_p^3 \quad (7b)$$

If we take the Laplace transform, then we can recast the control equations (7) in the frequency domain

$$\begin{aligned} j\omega X_1(j\omega) &= \left(\varepsilon_1^1 - \varepsilon_1^2\right) X_1(j\omega) - \varepsilon_2^2 X_2(j\omega) \\ &\quad + \varepsilon_p^1(j\omega) - \varepsilon_p^2(j\omega), \\ j\omega X_2(j\omega) &= \left(\varepsilon_2^2 - \varepsilon_2^3\right) X_2(j\omega) + \varepsilon_1^2 X_1(j\omega) \\ &\quad + \varepsilon_p^2(j\omega) - \varepsilon_p^3(j\omega). \end{aligned}$$

We can represent the control equations for the single perturbation ε_p^1 using a signal-flow diagram (Figure 1). For aesthetic reasons, we prefer this graphical representation to the block-diagram representation.

Evident from the diagram (Figure 1), simple reactions are equivalent to local feedback loops. These loops result from the reaction dynamics. For example, a degradation reaction is equivalent to a negative feedback loop. Consider the kinetic equation

$$\dot{s} = p - ks$$

where p is the rate of production and k is the degradation rate constant. In this mechanism, the degradation rate ks functions as a negative feedback loop on the concentration s. In the linear chain example, the reactions v_2 and v_3 act as negative feedback loops on s_1 and s_2 respectively ($-\varepsilon_1^2$ and $-\varepsilon_2^3$). For reversible reactions, the product negatively regulates the reactant ($-\varepsilon_2^2$). Likewise, if the reactions v_1 and v_2 are reversible, then the product inhibits its synthesis. This inhibition is equivalent to a negative feedback loop on the substrates s_1 and s_2 respectively ($\varepsilon_1^1 < 0$ and $e_2^2 < 0$). If these reactions are autocatalytic, then the product enhances its synthesis and the process is equivalent to a positive feedback loop ($\varepsilon_1^1 > 0$ and $e_2^2 > 0$). In terms of control, the perturbations ε_p are equivalent to additive disturbances.

We do not need to know the specific rate equations in order to construct the signal flow diagram. All we need to know is the stoichiometry of the reaction network and variable dependencies for the rate laws. If we wish to numerically evaluate the network, then we need to know the elasticity coefficients or the kinetic parameters.

For a signal-flow graph, the transfer function T_{ij} between i^{th} and j^{th} node is given by Mason's rule (Mason, 1953; Mason, 1956):

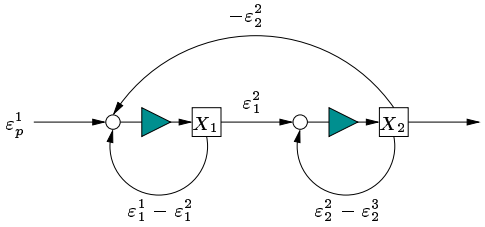


Fig. 1. A signal-flow representation of the dynamic control equations. The open circle denotes a summer and the shaded triangle denotes an integrator: $\frac{1}{j\omega}$. Evident from the diagram, reversible reactions introduce feedback loops as the concentration of the downstream species regulates the concentration of the upstream species.

$$T_{ij} = \frac{\sum_k P_{ijk}\Delta_{ijk}}{\Delta}$$

where the summation is taken over all possible paths from node i to j and

$$\begin{aligned} P_{ijk} &= k^{\text{th}} \text{ path from node } i \text{ to } j, \\ \Delta &= \text{ determinant of flow graph}, \\ \Delta_{ijk} &= \text{ cofactor of the path } P_{ijk}. \end{aligned}$$

Numerous algorithms exist for applying Mason's rule to generic signal-flow networks.

Applying Mason's rule we obtain the following transfer function relating ε_p^1 to the control coefficients $X_1(j\omega)$ and $X_2(j\omega)$:

$$X_1(j\omega) = \frac{\left(j\omega - (\varepsilon_2^2 - \varepsilon_2^3)\right)\varepsilon_p^1 + \varepsilon_2^2\varepsilon_p^2}{-\omega^2 - \left((\varepsilon_1^1 - \varepsilon_1^2) + (\varepsilon_2^2 - \varepsilon_2^3)\right)j\omega + \varepsilon_1^1\varepsilon_2^2 - \varepsilon_1^1\varepsilon_2^3 + \varepsilon_1^2\varepsilon_2^3}$$

$$X_2(j\omega) = \frac{\varepsilon_2^1\varepsilon_p^1 + \left(j\omega + (\varepsilon_1^1 - \varepsilon_1^2)\right)\varepsilon_p^2}{-\omega^2 - \left((\varepsilon_1^1 - \varepsilon_1^2) + (\varepsilon_2^2 - \varepsilon_2^3)\right)j\omega + \varepsilon_1^1\varepsilon_2^2 - \varepsilon_1^1\varepsilon_2^3 + \varepsilon_1^2\varepsilon_2^3}$$

Note that both equations have the same denominator and the perturbations ε_p arise only in the numerator. This fact is well known in control: the denominator is the characteristic equation for the loop.

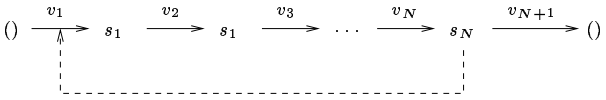
At steady state, we have the control equations

$$\begin{aligned} X_1(0) &= \frac{-(\varepsilon_2^2 - \varepsilon_2^3)\varepsilon_p^1 + \varepsilon_2^2\varepsilon_p^2}{\varepsilon_1^1\varepsilon_2^2 - \varepsilon_1^1\varepsilon_2^3 + \varepsilon_1^2\varepsilon_2^3}, \\ X_2(0) &= \frac{\varepsilon_2^1\varepsilon_p^1 + (\varepsilon_1^1 + \varepsilon_1^2)\varepsilon_p^2}{\varepsilon_1^1\varepsilon_2^2 - \varepsilon_1^1\varepsilon_2^3 + \varepsilon_1^2\varepsilon_2^3}. \end{aligned}$$

Both these equations describe how perturbing a single reaction effects the entire network.

3.2 Linear Chain With Feedback Inhibition

Consider the following set of reactions with endproduct inhibition



The kinetic equations for this mechanism are

$$\begin{aligned} \dot{s}_1 &= v_1(s_1, s_N, p) - v_2(s_1, s_2, p), \\ \dot{s}_2 &= v_2(s_1, s_2, p) - v_3(s_2, s_3, p), \\ &\vdots \\ \dot{s}_N &= v_N(s_{N-1}, s_N) - v_{N+1}(s_N, p). \end{aligned}$$

For this example, the control equations become

$$\begin{aligned} \dot{x}_1(t) &= \left(\varepsilon_1^1 - \varepsilon_1^2\right)x_1(t) - \varepsilon_2^2 x_2(t) + \varepsilon_N^1 x_N + \varepsilon_p^1 \\ \dot{x}_2(t) &= \left(\varepsilon_2^2 - \varepsilon_2^3\right)x_2(t) + \varepsilon_1^2 x_1(t) - \varepsilon_3^3 x_3(t) \\ &\vdots \\ x_N(t) &= (\varepsilon_N^N - \varepsilon_{N+1}^N)x_N + \varepsilon_{N-1}^N X_{N-1} \end{aligned}$$

Figure 2 diagrams the control equations as a signal-flow graph. As with the previous example, the reactions result in local feedback loops. However, there is also the global negative feedback loop resulting from endproduct inhibition ($-\varepsilon_5^1$). This example illustrates how it is often difficult to identify regulatory genes as the control may be embedded directly in the process (Savageau, 1972; Kacser and Burns, 1973).

3.3 Tryptophan Biosynthesis

The *trp* operon encodes five genes that synthesize tryptophan from chorismate, the common precursor for the aromatic amino acids phenylalanine, tyrosine, and tryptophan. As with many biosynthetic pathways, the process is subject to a hierarchy of regulatory feedback loops. At the level of the gene, expression of the operon is negatively regulated by tryptophan: tryptophan binds the *trp* repressor and inhibits transcription. Tryptophan, when bound with tRNAs, inhibits transcription through the process of translation attenuation. At the metabolic level, tryptophan allosterically inhibits the enzymes in the pathway.

A simple model (Xiu *et al.*, 1997) for tryptophan biosynthesis is described by the following differential equations

$$\dot{m} = \underbrace{v_1(p)}_{\text{transcription}} - \underbrace{v_2(m)}_{\text{mRNA deg.}}$$

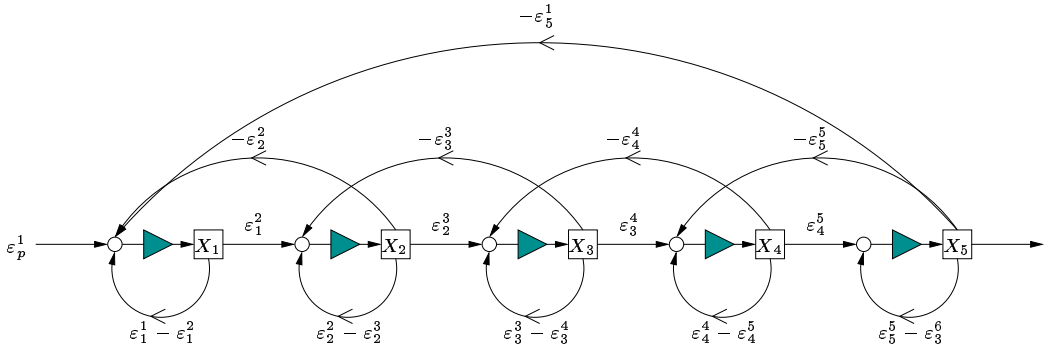


Fig. 2. Signal-flow diagram for a linear cascade with feedback inhibition ($N = 5$). The open circle denotes a summer and shaded triangle denotes the integrator $\frac{1}{j\omega}$.

$$\dot{e} = \underbrace{v_3(m)}_{\text{translation}} - \underbrace{v_4(e)}_{\text{prot. deg.}}$$

$$\dot{p} = \underbrace{v_5(e,p)}_{\text{trp synthesis}} - \underbrace{v_6(p)}_{\text{trp deg.}} - \underbrace{v_7(p)}_{\text{trp consump.}}$$

where m is the concentration of mRNA, e is the lumped concentration of the *trp* enzymes, and p is the concentration of tryptophan.

The control equations for this model take the following form

$$\begin{aligned}
\dot{x_1} &= \varepsilon_3^1 x_3 - \varepsilon_1^2 x_1 \\
\dot{x_2} &= \varepsilon_1^3 x_1 + \varepsilon_3^3 x_3 - \varepsilon_2^4 x_2 \\
\dot{x_3} &= \varepsilon_2^5 x_2 + (\varepsilon_3^5 - \varepsilon_3^6 - \varepsilon_3^7) x_3
\end{aligned}$$

where x_1 is the control coefficient for m, x_2 for e, and x_3 for p. The signal diagram (Figure 3) illustrates the signaling hierarchy in the tryptophan example. The global loop ($-\varepsilon_3^1$) results from the genetic regulatory mechanisms. The loops ($\varepsilon_3^5 < 0$, $-\varepsilon_3^6$, and $-\varepsilon_3^7$) result from allosteric inhibition by tryptophan, degradation, and consumption, respectively. In terms of the control equations and local dynamics, they are equivalent, though obviously they each have a unique role. One limitation of the MCA representation is that we see only a snapshot of the regulatory dynamics around the steady state. In the case of tryptophan, there are two feedback loops controlling gene expression. In terms of the control equations, these loops are equivalent. However, their relative strength depends on the concentration of tryptophan as they likely form a split-range controller. The control equations cannot capture this behavior as they are in reference to a single steady state.

3.4 Branched Pathway

Consider the branched pathway illustrated in Figure 4 where the goal is to produce substrate s_2 without effecting a key metabolic substrate s_3. The kinetic equations are

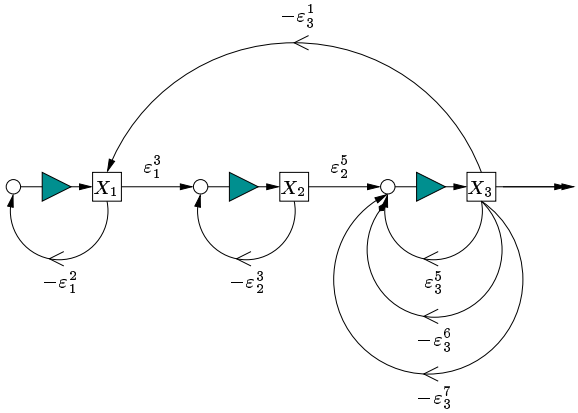


Fig. 3. Tryptophan Example: Signal-flow diagram for the tryptophan pathway. The open circle denotes a summer and the shaded triangle denotes the integrator $\frac{1}{j\omega}$.

$$\begin{aligned}
\dot{s}_1 &= v_1(p) - v_2(s_1,p) - v_3(s_1,p), \\
\dot{s}_2 &= v_2(s_1,p) - v_4(s_2,p), \\
\dot{s}_3 &= v_3(s_1,p) - v_5(s_2,p).
\end{aligned}$$

The steady-state control equations are

$$\begin{aligned}
x_1 &= \frac{\varepsilon_p^1 - \varepsilon_p^2 - \varepsilon_p^3}{\varepsilon_p^2 + \varepsilon_p^3} \\
x_2 &= \frac{1}{\varepsilon_2^4}\left[\frac{\varepsilon_1^2}{\varepsilon_1^2 + \varepsilon_1^3}\left(\varepsilon_p^1 - \varepsilon_p^2\right) + \varepsilon_p^2\right] \\
x_3 &= \frac{1}{\varepsilon_3^5}\left[\frac{\varepsilon_1^2}{\varepsilon_1^2 + \varepsilon_1^3}\left(\varepsilon_p^1 - \varepsilon_p^2\right)\right]
\end{aligned}$$

The control equations are diagrammed in Figure 5. Simple inspection of these equations indicates that we need to simultaneously perturb reactions v_1 and v_2 in order to satisfy the design objective. This is also evident from the signal-flow diagram (Figure 5). While perturbing reaction v_2 increases the concentration of s_2, it depletes the concentration of s_1 and s_3. To compensate for the substrate depletion, we need to increase the rate for reaction v_1 to satisfy the design objective.

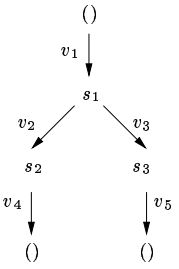


Fig. 4. Branched Pathway Example

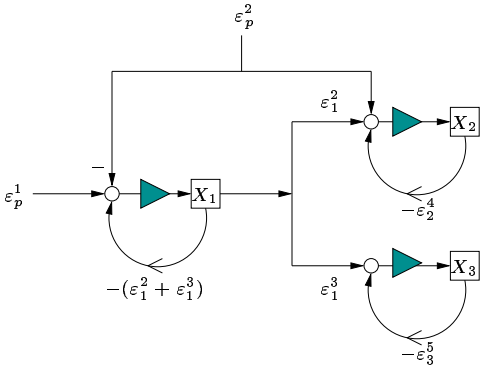


Fig. 5. Branched Pathway Example: Signal Flow Diagram. The open and shaded circles denote summer and integrators $\frac{1}{j\omega}$ respectively.

3.5 Comments

The aim in the preceding sections was to demonstrate that MCA is subsumed by control theory. In fact, the previous discussion extends MCA which until recently was limited to steady-state analysis (Ingalls, 2004). For brevity, we have avoided explicit rate expressions and numerical values. However, one can imagine how tools from control theory can be applied to the analysis of biochemical networks (at least to first order approximation). Possible questions include controllability, robustness, and the influence of noise using frequency domain analysis.

4. CONCLUDING REMARKS

While MCA is a popular and powerful tool for analyzing the regulatory properties of intracellular networks, there is still much to be desired. The analysis is linear and consequently can only characterize infinitesimal perturbations to the dynamic behavior in a small neighborhood about the steady state. Despite its limitations, the MCA formulation provides a convenient gateway between biology and control. As much of the complexity in biology results from the regulatory interactions between individual genes, there are many interesting and important problems at the interface between control and biology. Currently, there is no satisfactory theory or set of tools (MCA included) for unraveling these complex regulatory interactions. By illustrating the similarity between the problem formulations in MCA and control, our goal was to show that this interface does exist and suggest MCA as one possible route for exploration. In some respects MCA is a mature theory, but in other respects it is still young and uncharted. Many important problems such as multivariable interactions, nonlinearity, noise, robustness, and discrete events have not been addressed yet in MCA. As each of these topics has a rich history in control, perhaps control can be useful for the analysis of biological networks.

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