

# Dynamic behavior in mathematical models of the tryptophan operon

Moisés Santillán<sup>a)</sup>

*Escuela Superior de Física y Matemáticas, Instituto Politécnico Nacional, 07738, México D.F., Mexico*

Michael C. Mackey

*Department of Physiology and Department of Physics and Mathematics, Centre for Nonlinear Dynamics, McGill University, McIntyre Medical Sciences Building, 3655 Drummond Street, H3G 1Y6 Montreal, Quebec, Canada*

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This paper surveys the general theory of operon regulation as first formulated by Goodwin and Griffith, and then goes on to consider in detail models of regulation of tryptophan production by Bliss, Sinha, and Santillán and Mackey, and the interrelationships between them. We further give a linear stability analysis of the Santillán and Mackey model for wild type *E. coli* as well as three different mutant strains that have been previously studied in the literature. This stability analysis indicates that the tryptophan production systems should be stable, which is in accord with our numerical results. © 2001 American Institute of Physics. [DOI: 10.1063/1.1336806]

**The development of the operon concept of gene regulation 40 years ago was one of the seminal developments in molecular biology. In the intervening four decades experimentalists have intensively studied regulation in many different operons, but none so extensively as the *lac* (lactose) and *trp* (tryptophan) operons. Strangely enough, in spite of the extensive knowledge of the biology of these two systems and the wealth of experimental data available about their dynamics, there have been few attempts on the part of applied mathematicians to integrate this knowledge into coherent mathematical models. In this paper we review the earliest mathematical treatments of the general operon concept, and then consider three specific models for the tryptophan operon. The most recent of these is due to Santillán and Mackey, and we provide new local stability analysis of the steady state of that model which indicates that the steady state is locally stable. This stability is also found in numerical simulations that we present.**

repressor molecule binds the operator, blocking it and preventing the binding of *mRNA* polymerase.

The most extensively studied operon systems are those controlling the production of lactose and tryptophan, namely the *lac* and *trp* operons. Hand in hand with the acquisition of experimental knowledge about these systems, a few applied mathematicians have developed models to help understand the experimental data that has been collected (cf. Refs. 2–4 for modeling related to the *lac* operon, and Refs. 5–10 for the *trp* operon). Studies of the tryptophan operon have been especially fruitful, and offer prime examples of the way in which experimentalists in molecular biology and applied mathematicians can work cooperatively to untangle nature's secrets.

In this paper, we first briefly discuss the most general model of the operon developed 35 years ago by Goodwin<sup>11</sup> and Griffith<sup>12</sup> in Sec. II. Then in Sec. III we discuss specific models of the tryptophan operon developed by Bliss<sup>5</sup> and Sinha,<sup>9</sup> respectively. Section IV discusses a much more recent model for the tryptophan operon due to Santillán and Mackey,<sup>7</sup> and briefly comments on comparisons between this newer model and experimental data taken from *E. coli*. Section V points out the connections between these three models by taking appropriate limits in the Santillán and Mackey model. Finally, Sec. VI offers a partial local stability analysis of the Santillán and Mackey model under the conditions that these limiting procedures are fulfilled and shows that the dynamics of tryptophan production are locally stable (in accord with numerical results) in wild type *E. coli* as well as in three mutant strains that have been previously studied. The paper concludes with a brief discussion in Sec. VII.

## I. INTRODUCTION

This paper considers the development of models related to operon regulation. Perhaps one of the most important concepts in modern molecular biology, the notion of the operon has revolutionized the way in which molecular biologists think about the regulation of intracellular processes.

The general theory of the operon was introduced by Jacob *et al.* in 1960.<sup>1</sup> This theory postulated that all genes are controlled by means of operons subject to a single regulatory mechanism known as repression. An operon consists of a set of genes preceded by a small DNA segment (the operator), where repression takes place and *mRNA* polymerase binds to initiate transcription. An operon is repressed when an active

## II. THE GENERAL THEORY OF THE OPERON

In 1965 Goodwin<sup>11</sup> proposed a mathematical model of the operon based on the general theory. This model considers three independent variables: The operon-related *mRNA* concentration ( $M$ ), the concentration of the enzyme produced by the operon genes ( $E$ ), and the concentration  $T$  of the end

<sup>a)</sup> Author to whom correspondence should be addressed; electronic mail: moyo@esfm.ipn.mx

product of the reactions catalyzed by enzyme  $E$ . The equations describing the Goodwin model, as modified by Griffith,<sup>12</sup> can be written without loss of generality as

$$\frac{dM}{dt} = \frac{1}{1+T^n} - \alpha M, \quad (1)$$

$$\frac{dE}{dt} = M - \beta E, \quad (2)$$

and

$$\frac{dT}{dt} = E - \gamma T. \quad (3)$$

The first term on the right-hand side of Eq. (1) models repression, and the positive Hill constant  $n$  specifies the strength of the repression. The constants  $\alpha$ ,  $\beta$ , and  $\gamma$  are all positive and account for dilution by growth and enzymatic degradation. The parameter  $\gamma > 0$  includes both of these effects as well as end product consumption. The enzyme production rate is assumed proportional to the  $mRNA$  concentration, while the end product production rate is taken to be proportional to the enzymatic concentration.

Griffith<sup>12</sup> proved analytically that the equilibrium solution of this model for operon regulation is locally stable with  $n < 8$ . He further showed that for  $n > 8$ , the solution can always be made unstable by a proper choice of the parameters  $\alpha$ ,  $\beta$ , and  $\gamma$ . From these results, Griffith concluded that oscillations of operon activity could occur only if there is cooperative repression of such a high order as to be unlikely from a biochemical point of view. Nevertheless, the introduction of biologically significant time delays as well as a nonlinear demand for end product may modify the stability properties of the model. Tyson and Othmer<sup>13</sup> have given an early and very complete survey of dynamics in biochemical feedback pathways that stands as one of the most complete available.

### III. MODELING OF THE TRYPTOPHAN OPERON REGULATORY SYSTEM

With the development of more refined experimental techniques and the acquisition of more data, it became clear that in addition to repression there are many regulatory mechanisms involved in the control of operons, and that these vary from operon to operon. One of the most intensively studied of these systems from an experimental point of view is the tryptophan operon. An excellent review of this system and its regulatory mechanisms is to be found in Ref. 14. Considering the intensity of the experimental study of the tryptophan operon, there have been surprisingly few mathematical models.<sup>5,6,10,9</sup>

#### A. The Bliss model

One of the first mathematical models of the tryptophan operon was introduced by Bliss *et al.* in 1982.<sup>5</sup> The independent variables of this model are the  $trp$   $mRNA$  concentration ( $M$ ), the concentration of anthranilate synthase ( $E$ ), which, according to the authors, of all the enzymes formed with the polypeptides of the tryptophan operon is the most important

from a regulatory point of view, and the tryptophan concentration ( $T$ ). The equations governing the dynamic evolution of these three quantities are

$$\frac{dM}{dt} = K_m OR(t - \tau_m) - K_1 M, \quad (4)$$

$$\frac{dE}{dt} = K_p M(t - \tau_p) - K_2 E, \quad (5)$$

and

$$\frac{dT}{dt} = K_t EI(T) - G(T) - KT. \quad (6)$$

In Eq. (4),  $K_m$  is the intrinsic rate of transcription initiation of an operon that is not repressed,  $R(t)$  is the probability that an operon is not repressed at time  $t$ ,  $\tau_m$  is the time delay between initiation of transcription and initiation of translation,  $O$  is the total operon concentration, and  $K_1$  is a positive constant accounting for  $mRNA$  depletion due to dilution by growth and enzymatic degradation.

Bliss *et al.* assumed that  $R(t)$  can be modeled by a Hill function

$$\frac{K_r^m}{K_r^m + T^m}, \quad (7)$$

with  $m = 4$ . In Eq. (5),  $K_p$  denotes the rate of translation initiation per  $mRNA$  molecule,  $\tau_p$  is the time delay between initiation of translation and the appearance of functional enzyme, and  $K_2$  is a positive constant accounting for enzyme depletion due to dilution by growth and enzymatic degradation. Finally, in Eq. (6),  $K_t$  is the tryptophan production rate per enzyme,  $I(T)$  is the fraction of enzyme not inhibited by the end product,  $G(T)$  is the tryptophan consumption rate, and  $K$  is the growth rate of the bacterial culture.

This model clearly considers two different regulatory mechanisms, repression and enzymatic feedback inhibition by tryptophan.  $R(t)$  accounts for repression, while  $I(T)$  stands for feedback inhibition. Bliss *et al.* also assert that  $I(T)$  can be modeled by a Hill function

$$\frac{K_i^n}{K_i^n + T^n}, \quad (8)$$

with  $n = 2$ , and that the demand for tryptophan obeys a Michaelis–Menten type equation

$$G(T) = G_{\max} \frac{T}{T + K_g}. \quad (9)$$

The authors made a careful estimation of the parameters in this model based on the available experimental data. They solved the model equations numerically and also performed an analytical stability analysis of the steady states. They were able to reproduce the results of derepression experiments with cultures of wild and mutant *E. coli* strains reported in Ref. 15. From these experiments, the tryptophan operon loses stability in mutant *E. coli* cell cultures that have a partial loss of feedback inhibition. The previously stable steady state is replaced by an oscillatory production of

tryptophan.<sup>15</sup> This mutation is modeled by increasing  $K_i$  to ten times its wild type value. However, to our knowledge, these experiments have never been successfully repeated. Moreover, more recent experimental evidence has demonstrated that the function  $R(t)$ , which Bliss *et al.* used to model repression, is in disagreement with the experimental facts about the interaction between the *trp* operon and repressor molecules. The issue of oscillations in tryptophan control was revisited by Xiu *et al.*<sup>10</sup> in their investigation of the role of growth and dilution rates on stability.

## B. The Sinha model

In 1988 Sinha<sup>9</sup> introduced a different model for the tryptophan operon regulatory system in which the DNA–repressor interaction is modeled in a more detailed way. The independent variables of the Sinha model are the same as in the Bliss *et al.* model, i.e., the *trp* mRNA concentration ( $M$ ), the anthranilate synthase enzyme concentration ( $E$ ), and the tryptophan concentration ( $T$ ). The dynamic equations for these variables are

$$\frac{dM}{dt} = K_m \frac{K_o K_d + K_o T}{K_o K_d + K_o T + nRT} O - K_1 M, \quad (10)$$

$$\frac{dE}{dt} = K_p M - K_2 E, \quad (11)$$

and

$$\frac{dT}{dt} = K_t E - G_{\max} - KT. \quad (12)$$

The first term on the right-hand side of Eq. (10) is the mRNA production rate. It is assumed to be proportional to the concentration of unrepresed operons, with a proportionality constant  $K_m$ . The concentration of unrepresed operons is calculated by taking into account the fact that active repressor molecules reversibly bind operons to block transcription initiation, and assuming that this reaction takes place sufficiently rapidly that it is in a quasisteady state. From this, the concentration of unrepresed operons is  $OK_o/(K_o + R_A)$ , where  $K_o$  is the dissociation constant of the repressor–operon reaction,  $O$  is the total operon concentration, and  $R_A$  is the concentration of active repressor molecules.

When produced by the *trpR* operon, repressor molecules are unable to repress *trp* operons. For this repression to take place, they need to be activated by two tryptophan molecules which sequentially bind noncooperatively in two independent places. From this, and assuming that this reaction takes place in a quasisteady state, Sinha obtained the concentration of active repressor given by  $R_A = nTR/(T + K_d)$ , where  $n = 2$ ,  $R$  is the total repressor concentration, and  $K_d$  is the repressor activation dissociation constant.

Compared with the Bliss *et al.* model, the Sinha model considers the DNA–repressor and repressor–tryptophan interactions in a more detailed way. On the other hand, it also ignores the fact that anthranilate synthase is feedback inhibited by tryptophan as well ignoring the time delays inherent to the system. These are both important features of the tryptophan operon regulatory system, and their exclusion makes the Sinha model unable to reproduce some of the important dynamic system characteristics.

It is also important to note that Sinha assumes a constant tryptophan consumption rate  $G_{\max}$ . Noting this, Sen and Liu<sup>8</sup> modified the Sinha model to study the case of a nonlinear tryptophan consumption rate given by a Michaelis–Menten function  $G(T) = G_{\max} T / (T + K_g)$ . Sinha, as well as Sen and Liu, studied the stability of their models for various values of parameters  $K_o$  and  $K_2$  and found the corresponding stability regions in the  $K_o$ – $K_2$  parameter space. However, they neither compare their models with experimental data nor do they discuss whether the values for  $K_o$  and  $K_2$  where stability is lost are physiologically attainable in wild type or mutant bacterial strains.

## IV. A MORE DETAILED MODEL

Recently Santillán and Mackey<sup>7</sup> introduced a more detailed mathematical model of the tryptophan operon regulatory system which is shown schematically in Fig. 1. This model considers four independent variables: the concentration of *trp* operons with operators free to be bound by mRNA polymerase molecules ( $O_F$ ), the concentration of *trp* mRNA molecules with free *TrpE*-related ribosome binding sites ( $M_F$ ), the concentration of the enzyme anthranilate synthase ( $E$ ), and the tryptophan concentration ( $T$ ).

Although five different polypeptides are synthesized during the expression of the *trp* operon and they combine to form the enzymes that catalyze the reaction pathway which synthesizes tryptophan from chorismic acid, this model concentrates on anthranilate synthase. The reason for this focus is that this enzyme is the most important from a regulatory point of view since it is subject to feedback inhibition by tryptophan and is the first to play its catalytic role in the reaction pathway.

Anthranilate synthase is a complex of two *TrpE* and two *TrpD* polypeptides. From this, Santillán and Mackey assume that the anthranilate synthase production rate is twice that of the *TrpE* polypeptide and look only at the *TrpE*-related ribosome binding sites of the mRNA chain. The equations governing the dynamics of these variables are

$$\begin{aligned} \frac{dO_F}{dt} = & \frac{K_r}{K_r + R_A(T)} \{ \mu O - k_p P [O_F(t) - O_F(t - \tau_p) e^{-\mu \tau_p}] \} \\ & - \mu O_F, \end{aligned} \quad (13)$$

$$\begin{aligned} \frac{dM_F}{dt} = & k_p P O_F(t - \tau_m) e^{-\mu \tau_m} [1 - A(T)] \\ & - k_p \rho [M_F(t) - M_F(t - \tau_p) e^{-\mu \tau_p}] \\ & - (k_d D + \mu) M_F(t), \end{aligned} \quad (14)$$

$$\frac{dE}{dt} = \frac{1}{2} k_p \rho M_F(t - \tau_e) e^{-\mu \tau_e} - (\gamma + \mu) E(t), \quad (15)$$

and

$$\frac{dT}{dt} = KE_A(E, T) - G(T) - F(T, T_{\text{ext}}) - \mu T. \quad (16)$$

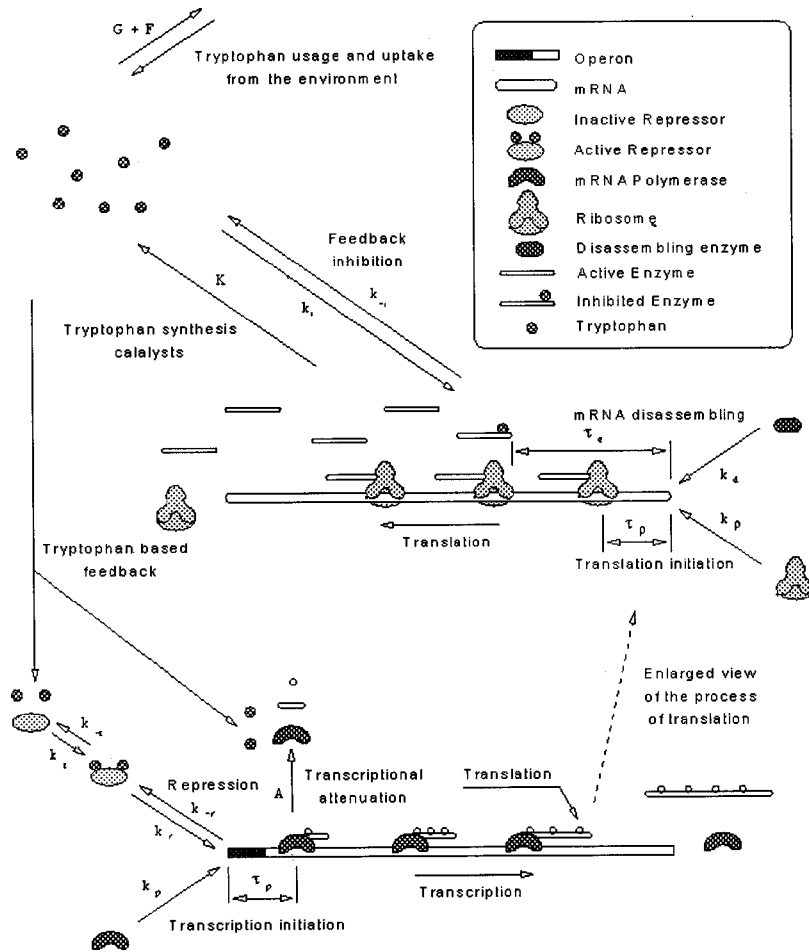


FIG. 1. Schematic representation of the tryptophan operon regulatory system. See the text for details.

All the parameters in this model were estimated based on reported experimental results, and the details of the parameter estimation process are in Ref. 7, which also contains a description of the model derivation process.

The factor

$$\frac{K_r}{K_r + R_A(T)} \tag{17}$$

in Eq. (13) is the fraction of unrepressed operons. This follows from the fact that when active repressor reversibly binds free operons the experimentally reported reaction rates allow one to make a quasisteady state assumption.  $K_r$  is the dissociation constant of this reaction, and  $R_A(T)$  is the active repressor concentration. The experimentally reported rates of the repressor activation reaction also support a quasisteady state assumption. Repressor molecules activate when two tryptophan molecules sequentially bind an inactive repressor in two independent sites noncooperatively. From this

$$R_A(T) = \frac{RT(t)}{T(t) + K_t}, \tag{18}$$

where  $R$  is the total repressor concentration and  $K_t$  is the dissociation constant of the repressor activation constant.

$\mu$  is the growth rate of the bacterial culture. The term  $\mu O$  stands for the operon production rate due to DNA replication,

which was assumed to be such that it keeps the total operon concentration at a constant value  $O$ , balancing dilution by growth.

The term

$$-k_p P [O_F(t) - O_F(t - \tau_p) \exp(-\mu \tau_p)] \tag{19}$$

accounts for the rate of free operons binding by  $mRNA$  polymerase molecules and later freed when the polymerases have traveled for a distance along the operon in a time  $\tau_p$ .  $k_p$  is the reaction rate of the DNA-polymerase binding reaction, assumed to be irreversible, and  $P$  is the  $mRNA$  polymerase concentration. The exponential factor  $\exp(-\mu \tau_p)$  takes into account the dilution due to exponential growth suffered by  $O_F$  during the time  $\tau_p$ .

In Eq. (14),  $\tau_m$  is the time it takes for a polymerase to produce a  $mRNA$  chain long enough to have an available *TrpE*-related ribosome binding site.  $A(T)$  is the probability for transcription to be prematurely terminated due to transcriptional attenuation. Santillán and Mackey<sup>7</sup> take  $A(T) = b[1 - \exp(-T/c)]$ .

$$-k_p \rho [M_F(t) - M_F(t - \tau_p) \exp(-\mu \tau_p)] \tag{20}$$

accounts for the rate of  $mRNA$ 's being bound by ribosomes and liberated after they travel for a time  $\tau_p$  along the  $mRNA$  chain.  $k_p$  is the  $mRNA$ -ribosome binding reaction rate and  $\rho$  is the ribosomal concentration.

The first term on the right-hand side of Eq. (15) is the anthranilate synthase production rate. The factor  $\frac{1}{2}$  is incorporated because, as mentioned earlier, the enzyme production rate is assumed to be one half that of *TrpE* polypeptide. The *TrpE* production rate equals the rate of ribosome binding free *TrpE*-related ribosome binding sites a time  $\tau_e$  ago.  $\tau_e$  is the time it takes for a ribosome to synthesize and release a *TrpE* polypeptide.

The tryptophan production rate, the first term on the right-hand side of Eq. (16) is assumed to be proportional to the concentration of active (uninhibited) enzymes ( $E_A$ ) with a proportionality constant  $K$ . Anthranilate synthase is inhibited when two tryptophan molecules bind the *TrpE* subunits in a sequential cooperative reaction with a Hill coefficient of  $n_H \approx 1.2$ . The enzyme feedback inhibition reaction rate constants support a quasisteady state assumption, from which

$$E_A(E, T) = E(T) \frac{K_i^{n_H}}{T^{n_H} + K_i^{n_H}}. \tag{21}$$

$K_i$  is the dissociation constant of this reaction. The tryptophan consumption rate constant is modeled as a Michaelis–Menten-type function

$$G(T) = G_{\max} \frac{T}{T + K_g}. \tag{22}$$

Finally, *E. coli* can uptake tryptophan from the environment. This can be managed by different tryptophan permeases. The rate of tryptophan uptake is modeled by Santillán and Mackey as

$$F(T, T_{\text{ext}}) = d \frac{T_{\text{ext}}}{e + T_{\text{ext}}[1 + T/f]}. \tag{23}$$

$T_{\text{ext}}$  is the external tryptophan concentration.

After estimating all the parameters from the available experimental data, this model was solved numerically, and compared with experiments performed by Yanofsky and Horn<sup>16</sup> on bacterial cultures of wild type as well as *trpL29* and *trpL75* mutant strains of *E. coli*. In these experiments, a bacterial culture is first grown in a medium with a high tryptophan concentration during a period of time long enough for the *trp* operon to reach a steady state. Then the bacteria are washed and shifted to a medium without tryptophan, and the response of the anthranilate synthase activity is measured as a function of time after the nutritional shift.

The *trpL29* mutant strain has a mutation *A* to *G* at bp 29 in the leader region of the *trp* operon. This change replaces the leader peptide start codon by *GUG*, and decreases operon expression in cells growing in the presence or absence of tryptophan. This mutation is simulated by Santillán and Mackey by decreasing the rate constant  $k_p$  to 0.04 times its normal value.

The strain *trpL75* of *E. coli* has a mutation of *G* to *A* at bp 75 in the leader region of the *trp* operon. This change decreases the stability of the transcription antiterminator structure, and increases transcription termination at the attenuator. Consequently, it decreases operon expression in cells growing in the presence or absence of tryptophan. This mutation was simulated by increasing the value of parameter

$b$ , which determines the probability of transcriptional attenuation at high tryptophan levels. The normal value of this parameter is  $b \approx 0.85$ , and to simulate the mutation it was increased to  $b \approx 0.9996$ .

The experiments of Yanofsky and Horn were simulated numerically. In all three cases (wild type and two mutants), the model results show a reasonable qualitative agreement with the experiments, given the simplifying assumptions inherent to the model.

## V. COMPARISON BETWEEN THE MODELS

To compare the model of Sec. IV with the Bliss and Sinha models, we need to simplify the Santillán and Mackey model.

The time a *mRNAP* takes to free an operator after binding is estimated to be as  $\tau_p \approx 0.1$  min. Experimental results and model simulations have shown that typical relaxation times of the *trp* regulatory system are of the order of a few dozen minutes for wild type as well as *trpL29* and *trpL75* mutant strains of *E. coli* strain *CY15000*. This means that the change in  $O_F$  is negligible on the time scale of  $\tau_p$ . The growth rate is estimated as  $\mu \approx 0.01$  min. From these two observations,  $\exp(-\mu\tau_p) \approx 1$  and this permits us to simplify Eq. (1) by neglecting the term  $-k_p P [O_F(t) - O_F(t - \tau_p)] \times \exp(-\mu\tau_p)$ .

Similar considerations allow us to simplify Eq. (2) by neglecting the term  $k_p \rho [M_F(t) - M_F(t - \tau_p) \exp(-\mu\tau_p)]$  ( $\tau_p$  is estimated to be  $\tau_p \approx 0.05$  min). These simplifying assumptions reduce the number of time delays in the model from four to two.

To compare with previous models, we consider a culture growing in a medium without tryptophan ( $T_{\text{ext}} = 0$ ). With all of these simplifications and lumping parameters, the model's system of governing equations becomes

$$\frac{dO_F}{dt} = \mu \left\{ \frac{T(t) + K_t}{(1 + 1/K_r)T(t) + K_t} O - O_F(t) \right\}, \tag{24}$$

$$\begin{aligned} \frac{dM_F}{dt} = & K_p O_F(t - \tau_m) e^{-\mu\tau_m} [(1 - b) + b e^{-T(t)/c}] \\ & - (K_D + \mu) M_F(t), \end{aligned} \tag{25}$$

$$\frac{dE}{dt} = \frac{1}{2} K_p M_F(t - \tau_e) e^{-\mu\tau_e} - (\gamma + \mu) E(t), \tag{26}$$

and

$$\frac{dT}{dt} = K \frac{K_i^{n_H}}{K_i^{n_H} + T^{n_H}(t)} E(t) - G_{\max} \frac{T(t)}{T(t) + K_g} - \mu T(t). \tag{27}$$

The values of all the parameters in Eqs. (24)–(27) were estimated from experimental results in Ref. 7, and these values are tabulated in Table I.

When we compared the numerical solutions of this simplified model and the original one, we found they are very similar for the wild as well as for the *trpL29* and *trpL75* mutant strains. This comparison was performed for many

TABLE I. The values of the parameters in the model equations (24)–(27) as previously estimated. See Santillán and Mackey (Ref. 7) for details.

$O \approx 3.32 \times 10^{-3} \mu\text{M}$	$R \approx 0.8 \mu\text{M}$
$\mu \approx 1.0 \times 10^{-2} \text{min}^{-1}$	$K_i \approx 4.09 \mu\text{M}$
$K_r \approx 2.61 \times 10^{-3} \mu\text{M}$	$K_d \approx 0.6 \text{min}^{-1}$
$K_\rho \approx 20.01 \text{min}^{-1}$	$K_g \approx 0.2 \mu\text{M}$
$K_p \approx 10.14 \text{min}^{-1}$	$n_H \approx 1.2$
$K \approx 118.84 \text{min}^{-1}$	$K_i \approx 60.34 \mu\text{M}$
$G_{\max} \approx 25 \mu\text{M min}^{-1}$	$\gamma \approx 0 \text{min}^{-1}$
$c \approx 2.0 \times 10^{-2} \mu\text{M}$	$b \approx 0.85$
$\tau_e \approx 0.1 \text{min}$	$\tau_m \approx 0.66 \text{min}$

different sets of initial conditions, and in all cases no significant difference was observed between the solutions of the two models.

Making a quasisteady state assumption in Eq. (5), setting the time delays  $\tau_m$  and  $\tau_e$  equal to zero, and ignoring transcriptional attenuation ( $b=0$ ), reduces this model to the Sinha model. If we only make the quasisteady state assumption in Eq. (5) and ignore transcriptional attenuation, we obtain a model similar to that of Bliss *et al.* However, the repression dynamics in the Bliss *et al.* model is different.

### VI. LOCAL STABILITY ANALYSIS AND NUMERICAL RESULTS

Given a model as embodied in Eqs. (24)–(27), one of the first questions a modeler is likely to ask is “What are the steady states of the system?” and second, “Are the steady states stable?”

Steady states in this circumstance are defined by the requirement that all of the temporal derivatives in Eqs. (24)–(27) are identically equal to zero. Without giving the explicit form for the steady states, a little algebra shown that there is a *unique* nonzero steady state that we denote by  $(\bar{O}_F, \bar{M}_F, \bar{E}, \bar{T})$ .

To answer the second question about the stability of this steady state in complete generality is usually impossible, and one must be content with an examination of the *local stability*. Briefly, in this case this involves only looking at very small deviations in the dependent variables away from their steady state values. If these deviations are very small, then it is appropriate to *linearize* all of the nonlinear terms in Eqs. (24)–(27). We then make the *ansatz* that the solutions of the linearized equations are of the form  $\exp(zt)$ , and from this obtaining the *characteristic equation* in  $z$  that must be analyzed to determine the nature of the roots  $z$ . If  $z$  is real, then we must have  $z < 0$  for linear stability, and in the case that  $z$  is a complex number,  $z = x + iy$ , then we must have  $\text{Re}(z) \equiv x < 0$ . In both cases a small deviation away from the steady state will decay back to the steady state—either smoothly, or in a damped oscillatory fashion.

In our case, the characteristic equation of the model given by Eqs. (24)–(27) is

$$(\Omega_{11} - z)(\Omega_{22} - z)(\Omega_{33} - z)(\Omega_{44} - z) + \Omega_{24}\Omega_{32}\Omega_{43} \times (\Omega_{11} - z)e^{-\tau_e z} - \Omega_{14}\Omega_{21}\Omega_{32}\Omega_{43}e^{-(\tau_e + \tau_m)z} = 0, \quad (28)$$

where the constants  $\Omega_{ij}$  are defined by

$$\Omega_{11} = -\mu < 0, \quad (29)$$

$$\Omega_{14} = -\frac{K_i R O \mu K_r}{[K_r \bar{T} + K_r K_i + \bar{T} R]^2} < 0, \quad (30)$$

$$\Omega_{21} = K_p e^{-\mu \tau_m} [(1 - b) + b e^{-\bar{T}/c}] > 0, \quad (31)$$

$$\Omega_{22} = -(K_D + \mu) < 0, \quad (32)$$

$$\Omega_{24} = -K_p \bar{O}_F e^{-\mu \tau_m} \frac{b}{c} e^{-\bar{T}/c} < 0, \quad (33)$$

$$\Omega_{32} = \frac{1}{2} K_\rho e^{-\mu \tau_e} > 0, \quad (34)$$

$$\Omega_{33} = -(\gamma + \mu) < 0, \quad (35)$$

$$\Omega_{43} = K \frac{K_i^{n_H}}{K_i^{n_H} + \bar{T}^{n_H}} > 0, \quad (36)$$

and

$$\Omega_{44} = -K \frac{K_i^{n_H} n_H \bar{T}^{(n_H-1)}}{(K_i^{n_H} + \bar{T}^{n_H})^2} E - G_{\max} \frac{K_g}{(\bar{T} + K_g)^2} - \mu < 0. \quad (37)$$

After normalizing, the characteristic equation becomes  $C(z) = 0$ , with

$$C(z) = (1 + z/\omega_1)(1 + z/\omega_2)(1 + z/\omega_3)(1 + z/\omega_4) + \beta_1(1 + z/\omega_1)\exp(-\tau_1 z) + \beta_2 \exp(-\tau_2 z), \quad (38)$$

with  $\omega_1, \omega_2, \omega_3, \omega_4, \beta_1, \beta_2 > 0$  and  $\tau_2 > \tau_1$ . The constants  $\omega_1, \omega_2, \omega_3, \omega_4, \beta_1, \beta_2, \tau_1$ , and  $\tau_2$  are defined as

$$\omega_1 = -\Omega_{11},$$

$$\omega_2 = -\Omega_{22},$$

$$\omega_3 = -\Omega_{33},$$

$$\omega_4 = -\Omega_{44},$$

$$\beta_1 = \frac{\Omega_{24}\Omega_{32}\Omega_{43}}{\Omega_{22}\Omega_{33}\Omega_{44}},$$

$$\beta_2 = -\frac{\Omega_{14}\Omega_{21}\Omega_{32}\Omega_{43}}{\Omega_{11}\Omega_{22}\Omega_{33}\Omega_{44}},$$

$$\tau_1 = \tau_e,$$

and

$$\tau_2 = \tau_m + \tau_e.$$

Once we have all of the parameters estimated and the steady state values of the independent variables computed, the values of the constants  $\omega_1$  through  $\omega_4, \beta_1, \beta_2, \tau_1$ , and  $\tau_2$  can be calculated from their definitions. This was done for the *E. coli* wild type, the *trpL29* and *trpL75* mutant strains studied by Yanofsky and Horn, and the feedback inhibition resistant mutant strain studied by Bliss *et al.*

From Santillán and Mackey,<sup>7</sup> the *trpL29* mutant strain can be modeled by decreasing  $K_p$  to 0.04 times its normal wild type value, while the *trpL75* mutant strain is modeled

TABLE II. Values of the characteristic equation constants for the wild and three different mutant strains. FBIR stands for feedback inhibition resistant.

	Wild strain	FBIR mutant strain	<i>trpL29</i> mutant strain	<i>trpL75</i> mutant strain
$\omega_1 \approx$	0.01 min <sup>-1</sup>	0.01 min <sup>-1</sup>	0.01 min <sup>-1</sup>	0.01 min <sup>-1</sup>
$\omega_2 \approx$	0.61 min <sup>-1</sup>	0.61 min <sup>-1</sup>	0.61 min <sup>-1</sup>	0.61 min <sup>-1</sup>
$\omega_3 \approx$	0.01 min <sup>-1</sup>	0.01 min <sup>-1</sup>	0.01 min <sup>-1</sup>	0.01 min <sup>-1</sup>
$\omega_4 \approx$	3.78 min <sup>-1</sup>	1.05 min <sup>-1</sup>	22.62 min <sup>-1</sup>	30.32 min <sup>-1</sup>
$\beta_1 \approx$	$3.5 \times 10^{-42}$	$3.5 \times 10^{-80}$	1.34	8.36
$\beta_2 \approx$	1.37	0.97	33.18	35.96
$\tau_1 \approx$	0.66 min	0.66 min	0.66 min	0.66 min
$\tau_2 \approx$	0.76 min	0.76 min	0.76 min	0.76 min

by increasing  $b$  to 0.9996 (the normal wild value is 0.85). Bliss *et al.* modeled a feedback inhibition mutant strain by increasing  $K_i$  to ten times its normal wild value. The values of all the characteristic equation constants calculated for the wild and mutant strains with these changes are tabulated in Table II. Note that constants  $\omega_1$  through  $\omega_3$ ,  $\tau_1$ , and  $\tau_2$  have the same value in all cases.

By definition, the system's steady state is locally stable for a given parameter set if all of the roots of the characteristic equation have negative real part. In the Appendix it is demonstrated for any of the parameter sets of Table II that if the characteristic equation has any root with a positive or zero real part, it must be in the region  $|z| < (\beta_1 + \beta_2 + 2)\omega_3$ . Thus, to prove that the system is locally stable, in every case it is sufficient to numerically show that  $C(z)$  has no root  $\tilde{z}$  satisfying the conditions  $\text{Re}(\tilde{z}) \geq 0$  and  $|\tilde{z}| < (\beta_1 + \beta_2 + 2)\omega_3$ .

In Fig. 2, three-dimensional plots of  $|C(z)|$  (where  $z = x + iy$ ) as a function of  $x$  and  $y$  in a range containing the region defined by  $|z| < \omega_3(\beta_1 + \beta_2 + 2)$  are shown for all the

wild and mutant strains. In all cases  $|C(z)| > 0$ , which means that  $C(z)$  has no root in the set  $|z| < \omega_3(\beta_1 + \beta_2 + 2)$ . Therefore, the characteristic equation does not have any root with real positive part either for the wild or for the mutant strain parameter values and thus the system steady states are locally stable in all cases.

The stability of this system was also tested numerically for the wild and three mutant strains. For this, the system equations (24)–(27) were numerically solved using a fourth order Runge–Kutta method implemented in FORTRAN. The parameter values were those of Table I for the wild strain, while for the feedback inhibition resistant, *trpL29*, and *trpL75* mutant strains, the parameters  $K_i$ ,  $K_p$ , and  $b$  were, respectively, modified as discussed previously. In each case, the initial value of all the model variables was set to their corresponding steady-state value, except for the *Trp* concentration initial value, which was set equal to 0.9 times its corresponding steady-state value. In all numerical simulations with these parameter values, the system was stable supporting the results of the local stability analysis.

### VII. DISCUSSION

Here we have shown the interrelationships between three major models for the regulation of tryptophan production. We have also been able to provide a local stability analysis of the steady states in the Santillán Mackey model that confirms the numerical results indicating that the steady states in this model are globally stable for wild type *E. coli* as well as for three different mutant strains.

Given the extensive knowledge about the molecular biology of regulation in the *trp* and *lac* operons, and the body of experimental data available on both systems, we find it surprising that there have been so few realistic modeling ef-

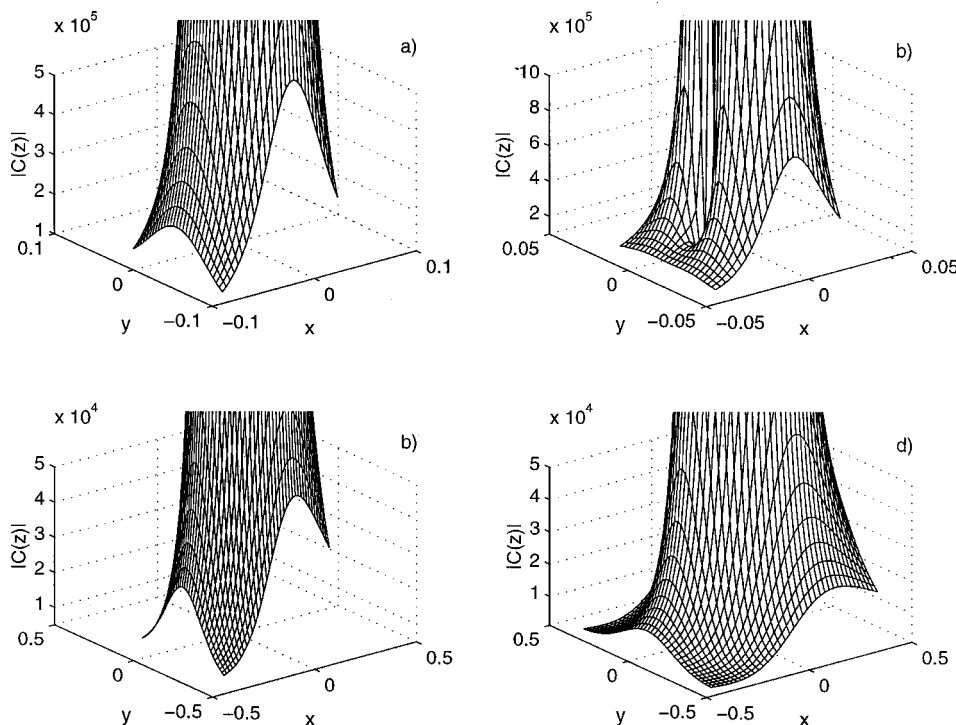


FIG. 2. Plots of  $|C(z)|$  (where  $z = x + iy$ ) vs  $x$  and  $y$  for the wild and three different mutant strains of *E. coli*: (a) wild strain, (b) feedback inhibition resistant strain, (c) *trpL29* mutant strain, and (d) *trpL75* mutant strain. In all cases, the plots are given in a range containing the region  $|z| < \omega_3(\beta_1 + \beta_2 + 2)$ . The parameter values corresponding to each strain are given in Table II. Notice that  $|C(z)| > 0$  for all the wild and mutant strains. This means that  $C(z)$  has no root in the set  $|z| < \omega_3(\beta_1 + \beta_2 + 2)$ .

forts on the part of theoretical biologists to understand the dynamics of these two systems. One can only hope that with the current intense interest in "molecular biology" this situation will change and experimentalists and theoreticians will join forces to try to understand these well characterized systems as prototypes of molecular regulatory systems.

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## APPENDIX: BOUNDING THE ROOTS OF THE CHARACTERISTIC EQUATION

Consider the function

$$C(z) = \left(1 + \frac{z}{\omega_1}\right) \left(1 + \frac{z}{\omega_2}\right) \left(1 + \frac{z}{\omega_3}\right) \left(1 + \frac{z}{\omega_4}\right) + \beta_1 \left(1 + \frac{z}{\omega_1}\right) e^{-\tau_1 z} + \beta_2 e^{-\tau_2 z},$$

where constants  $\omega_1$ - $\omega_4$ ,  $\beta_1$ ,  $\beta_2$ ,  $\tau_1$ , and  $\tau_2$  are all real and positive.

Rewrite equation  $C(z)=0$  as follows:

$$\left(1 + \frac{z}{\omega_2}\right) \left(1 + \frac{z}{\omega_3}\right) \left(1 + \frac{z}{\omega_4}\right) = -\beta_1 e^{-\tau_1 z} - \frac{\beta_2 e^{-\tau_2 z}}{1 + z/\omega_1}.$$

Notice that when the real part of  $z$  is greater than or equal to zero we have

$$\left|1 + \frac{z}{\omega_1}\right| \geq 1, \quad \left|1 + \frac{z}{\omega_2}\right| \geq 1, \quad \left|1 + \frac{z}{\omega_4}\right| \geq 1,$$

and

$$|e^{-\tau_1 z}| \leq 1, \quad |e^{-\tau_2 z}| \leq 1.$$

Moreover,  $|1 + z/\omega_3| \geq \beta_1 + \beta_2 + 1$  when  $|z| \geq \omega_3(\beta_1 + \beta_2 + 2)$ . Therefore:

$$\begin{aligned} \left| \left(1 + \frac{z}{\omega_2}\right) \left(1 + \frac{z}{\omega_3}\right) \left(1 + \frac{z}{\omega_4}\right) \right| &\geq \beta_1 + \beta_2 + 1 > \beta_1 + \beta_2 \\ &\geq \left| -\beta_1 e^{-\tau_1 z} - \frac{\beta_2 e^{-\tau_2 z}}{1 + z/a} \right| \end{aligned}$$

when  $\text{Re}(z) \geq 0$  and  $|z| \geq \omega_3(\beta_1 + \beta_2 + 2)$ , and so the equation  $C(z)=0$  does not have any solution under this condition.

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