Nonlinear Dynamics of Regulation of Bacterial *trp* Operon: Model Analysis of Integrated Effects of Repression, Feedback Inhibition, and Attenuation

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The *trp* operon encodes the five genes for the enzymes required to convert chorismate to tryptophan, and its switching on and off is controlled by both feedback repression and attenuation in response to different levels of tryptophan in the cell. Repression of the operon occurs when tryptophan concentration is high, and attenuation fine-tunes the transcription level at a lower cellular concentration of tryptophan. An extended mathematical model is established in this study to describe the switching on and off of the *trp* operon by considering the integrated effects of repression and attenuation. The influences of cell growth rate on the biosynthesis of tryptophan, stability and dynamic behavior of the *trp* operon are investigated. Sustained oscillations of tryptophan levels are predicted from the regulated turning on and off of the *trp* operon. It is interesting to note that during such oscillations the regulation of transcription displays a kind of "on" and "off" state in terms of gene expression, indicating the existence of a genetic circuit or switch in the regulation of the *trp* operon. Time lags between transcription and translation are also predicted and may explain the occurrence of such oscillation phenomenon.

Introduction

The present rapid development of genomics brings much attention to the regulation of gene expression and its products, mRNAs and proteins under physiological conditions and at a system level. The complex regulation mechanisms of genes may be inherent reasons for biodiversity and seemingly unusual nonlinear biological phenomena. For instances, life rhythm, cell cycle, pulsatile hormone release and other oscillatory behavior may depend primarily on the unique feature of gene expression as a kind of genetic switch (McAdams and Shapiro, 1995; McAdams and Arkin, 1998). The molecular mechanisms for the regulation of the *lac* and *trp* operons have been studied as model systems for a long time to understand gene regulation in prokaryotic cells, which may also provide certain insights in understanding the more complicated gene regulation system in eukaryotic cells. In addition, controlling elements of both the *lac* and the *trp* operons are used as to make efficient vectors for expressing recombinant proteins of pharmaceutical interest (Chevalet et al., 2000). However, the regulation mechanisms of the switching on and off of such operons are still not well described by mathematic models.

In this study the *trp* operon and L-tryptophan synthesis are chosen as a model system for understanding the nonlinear dynamics of gene regulation. L-Tryptophan is one of the human essential amino acids. Its biosynthetic pathway in *E. coli* is shown schematically in Figure 1. The synthesis of tryptophan from chorismate, a common precursor for aromatic amino acid, is catalyzed by four enzymes: anthranilate synthase (AS), anthranilate phosphoribosyl transferase (PRT), indole glycerol phosphate synthase (IGP) and tryptophan synthase (TS). The levels of these enzymes depend on five structural genes coded in the tryptophan operon (*trpEDCBA*). The tryptophan synthesis in bacteria such as *E. coli* and *C. glutamicum* is primarily controlled through feedback inhibitions of the end product tryptophan on the enzymes (mainly AS) at metabolic level and repression of the *trp* operon at transcriptional level. Previous studies revealed that termination of the operon transcription by attenuation also occurs at a lower intracellular concentration of tryptophan (Yanofsky and Crawford, 1987; Yanofsky and Horn, 1994). Attenuation is the conjunction of processes of transcription and translation and brings about a finer control for the regulation of transcription in the tryptophan biosynthetic pathway. It is a tryptophan-sensitive timing mechanism that determines whether transcription is attenuated or allowed to continue. The *trp* operon attenuation is defined by the concentrations of charged tryptophan tRNA (Trp-$tRNA^{Trp}$). When the tryptophan concentration is high, the concentration of Trp-$tRNA^{Trp}$ is also high. Translation will follow closely on the heels of transcription, proceeding rapidly past the Trp codons; the attenuator structure is formed and transcription is halted. When tryptophan concentrations are low, however, the ribosome stalls at the two Trp codons because charged $tRNA^{Trp}$ is unavailable; a base paired structure is formed and prevents attenuation; transcription continues to proceed but translation stops as a result of a

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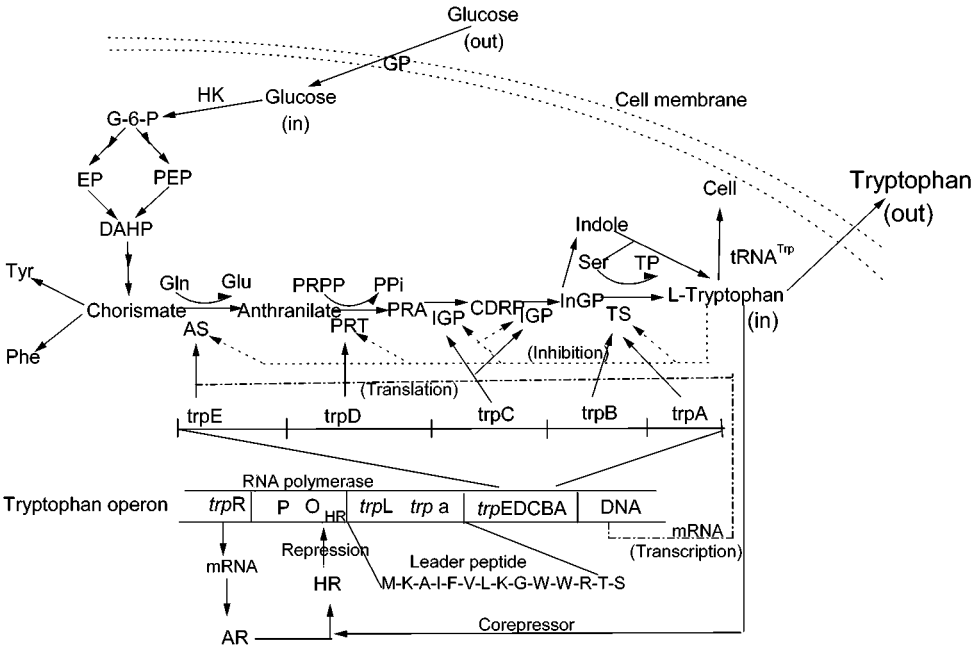

Figure 1. The main pathway of L-tryptophan biosynthesis and regulatory and structural gene regions of the trp operon of *E. coli*. Abbreviations: EP, D-erythrose-4-phosphate; PEP, phosphoenolpyruvate; DAHP, 3-deoxy-D-arabinoheptulosonate-7-phosphate; Tyr, tyrosine; Phe, phenylalanine; Gln, glutamine; Glu, glutamate; PRPP, 5-phosphoribosyl-1-pyrophosphate; Ppi, pyrophosphate; PRA, 5-phosphoribosyl anthranilate; CDRP, 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate; InGP, indole-3-glycerol phosphate; Ser, serine; TP, triose phosphate; AS, anthranilate synthase; PRT, phosphoribosyl anthranilate transferase; IGP, indole glycerol phosphate synthase; TS, tryptophan synthase; P, promotor; O, operator; AR, aporepressor; HR, holorepressor.

lack of tryptophan (Lehninger et al., 1993). The joint action of these three regulation mechanisms including feedback inhibition, repression and attenuation is believed to give rise to complicated dynamic behaviors such as oscillations (Bliss et al., 1982; Sinha, 1988; Sen and Liu, 1990; Xiu et al., 1997).

The proposed mathematical models describing the regulation of the *trp* operon and biosynthesis of tryptophan were based on a gene−enzyme−endproduct control unit model with a single negative feedback control loop postulated by Goodwin (1965) and improved by Griffith (1968). Most of them considered the corepression and feedback inhibition of tryptophan on enzyme (Bliss et al., 1982; Sinha, 1988; Sen and Liu, 1990; Xiu et al., 1997). However attenuation was not taken into account in the above models. Recently a structured model was postulated to describe attenuation in the *trp* operon (Koh et al., 1998). This model is relatively complex. It is desirable to set up a simple and mechanistically tractable model for dynamic analysis.

In this work, the dynamic behavior of the *trp* operon is studied in more detail by mathematically modeling the integrated effects of feedback inhibitions on enzymes level and repression and attenuation on transcriptional levels. This represents an extension of our previous work (Xiu et al., 1997) on the *trp* operon in which the attenuation was not considered. It is shown that a circuit-type on and off switch regulation of the *trp* operon can be predicted. Its role in the oscillatory behavior of the *trp* operon is discussed by using bifurcation theory.

Mathematic Model

An extended mathematical model is proposed on the basis of our previous work (Xiu et al., 1997) by taking into consideration repression, feedback inhibition and attenuation. Similar to our previous work, the formation of an endproduct−repressor complex P_nR (holorepressor) is assumed to be multiple-step equilibrium reactions, of which the first reaction is the limiting step and others are rapid. The formation of a holorepressor−operator complex P_nRO (bound operator) is considered to be a rapid equilibrium process. The reactions are expressed as follows:

$$nP + R \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} P_nR$$

$$P_nR + O \underset{k_2}{\overset{k_2}{\rightleftharpoons}} P_nRO$$

The holorepressor and free operator concentrations at equilibrium are as follows:

$$[P_nR] = R_tP/(P + K_d) \quad (1)$$

$$[O] = O_t(P + K_d)/(P + K_d + rP) \quad (2)$$

where R_t and O_t are the total repressor and operator concentrations, respectively. K_d and K_o are the dissociation constants of the holorepressor and the bound operator. K_d is equivalent to the equilibrium constant of the first reaction in the formation of holorepressor:

$$K_d = k_{-1}/k_1$$

$$K_o = k_{-2}/k_2$$

r in eq 2 is defined as a dimensionless parameter characterizing the strain level of the *trp* operon (repressor):

$$r = R_t/K_o$$

The trpR gene encodes a 108-residue polypeptide (aporepressor, R_t) containing tryptophan residues at positions 19 and 99 (Yanofsky and Crawford, 1987). The level of tryptophan would control the rate of the synthesis

of aporepressor given that the amount of the other amino acids were sufficient. In other words, at low tryptophan level, the synthesis of aporepressor would be limited. This synthesis behavior could be described by a similar Michaelis−Menten equation:

$$R_t = R_t^m P/(P + K_R) \quad (3)$$

where R_t^m is the maximum concentration of aporepressor and K_R is Michaelis constant. Thus r is a parameter dependent on the level of tryptophan, especially at low tryptophan levels. It is different from the previous works (Sinha, 1988; Sen and Liu, 1990; Xiu et al., 1997) in which r was considered to be a constant and depend only on the strain.

The *trp* operon attenuation is affected mainly by the level of tryptophanyl-tRNA^{Trp}. When tryptophan levels are low, the ribosome pauses at the Trp codons as a result of lack of Trp-tRNA^{Trp}. A base paired structure is formed and prevents attenuation. When tryptophan levels are high, the ribosome quickly translates the 14-residue leader peptide and blocks the formation of the paired structure. Continued transcription leads to attenuation at the terminator structure. The termination of transcription in the attenuator can be assumed to be determined only by the tryptophan concentration in the cell (Prokudina et al., 1991). In other words, the attenuation frequency (f) of transcription depends on the translation degree of leader peptide. The leader peptide contains 14 amino acid residues, among which two are tryptophan residues. For simplification, f can be expressed as an inhibition factor of tryptophan to the leader peptide:

$$f = K_A/(K_A + P) \quad (4)$$

Assuming that the biosynthesis of tryptophan is dependent on a rate-limiting biosynthetic enzyme, the equations governing the tryptophan synthesis are formulated as below. The whole process is composed of the transcription of the *trp* operon yielding mRNA (M), the translation of the mRNA to form the rate-limiting enzyme (E), and the action of the enzyme to produce the endproduct, tryptophan (P):

$$dM/dt = K_m D[O]f - (K_1 + \mu)M \quad (5)$$

$$dE/dt = K_e M - (K_2 + \mu)E \quad (6)$$

$$dP/dt = K_p E K_I^2/(K_I^2 + P^2) - (K_3 + \mu)P - nk_{-1}[P_nR] - (P_{pro}^m + \beta\mu)\mu CP/(P + K_s) \quad (7)$$

where M represents the mRNA concentration and E represents the rate-limiting enzyme in the synthetic pathway of tryptophan; D is the gene dosage; K_m, K_e, and K_p are rate constants for the formations of the species denoted by the appropriate subscripts, respectively; K_1, K_2, and K_3 are rate constants for the degradation of the mRNA, the key enzyme and tryptophan; and μ is the specific growth rate of cells. K_I is the concentration of tryptophan resulting in 50% inhibition of the enzymatic activity. K_s refers to a saturation constant of tryptophan for cellular protein synthesis. C represents the molar percentage of tryptophan in cellular proteins. P_{pro}^m is the maximum protein concentration. β represents a constant expressing the influence of growth rate on the protein content of the cells.

For convenience, the above equations can be rewritten in dimensionless forms by defining:

$$x = MK_eK_pT^2/K_d$$
$$y = EK_pT/K_d$$
$$z = P/K_d$$
$$\tau = t/T$$
$$u = T\mu$$
$$T = [K_d/(K_m DO_t K_e K_p)]^{1/3}$$

where x, y, z, τ and u are the dimensionless mRNA, enzyme, tryptophan concentration, time, and growth rate, respectively. Using these notations and substituting eqs 1−4 into eqs 5−7, the following dimensionless equations are obtained:

$$dx/d\tau = (1 + z)/[1 + (1 + r)z]k_a/(k_a + z) - (u + \alpha_1)x \quad (8)$$

$$dy/d\tau = x - (u + \alpha_2)y \quad (9)$$

$$dz/d\tau = yk_i^2/(k_i^2 + z^2) - (u + \alpha_3)z - \alpha_4 z/(1 + z)z/(z + k_r) - \alpha_5(1 + \alpha_6 u)uz/(z + k) \quad (10)$$

The dimensionless parameters (except for α_6) appearing in eqs 8−10 are all positive and have the following definitions:

$$\alpha_1 = TK_1$$
$$\alpha_2 = TK_2$$
$$\alpha_3 = TK_3$$
$$\alpha_4 = nk_{-1}R_tT/K_d = nk_1R_tT$$
$$\alpha_5 = CP_{pro}^m/K_d$$
$$\alpha_6 = \alpha/(TP_{pro}^m)$$
$$k = K_S/K_d$$
$$k_i = K_I/K_d$$
$$k_r = K_R/K_d$$
$$k_a = K_A/K_d$$
$$r_m = R_t^m/K_o$$
$$r = r_m z/(z + k_r)$$

For convenience, a relative growth rate (u_R) is defined here to replace the dimensionless cell growth rate (u):

$$u_R = \mu/\mu_m = u/u_m$$

Numerical Method and Choice of Parameters

By letting the left-hand sides of eqs 8−10 be zero, a steady-state solution (x_0, y_0, z_0) can be obtained by using a Gauss−Newton least-squares method supplied in the software MATLAB.

The method for stability analysis is similar to that reported before (Xiu et al., 1997, 1998). Briefly, local stability of a steady state can be analyzed by linearizing the differential equations of the system (eqs 8−10)

Table 1. Kinetic Parameters Chosen for Eqs 1−10 Associated with the *trp* Operon and Tryptophan Biosynthesis in Bacteria

parameter	reported value	value used in this study	reference
C	1.1%	1.1%	Neidhardt et al. (1990)
K_1	57.6 h^{-1}	57.6 h^{-1}	Bliss et al. (1982)
k_{-1}	7200 h^{-1}	7200 h^{-1}	Bliss et al. (1982)
K_d	4×10^{-5} mol/L	4×10^{-5} mol/L	Arvidson et al. (1986)
K_o	10^{-10}, 2×10^{-10} mol/L	10^{-10} mol/L	Sinha (1988), Hurlburt and Yanofsky (1992)
P_{pro}^m	2.64×10^6 mol/L	2.64×10^6 mol/L	Marr (1991)
T	0.0156 h	0.0156 h	Xiu et al. (1997)
β	-0.32×10^6 mol·h/L	-0.32×10^6 mol·h/L	Marr (1991)
n	2	2	Arvidson et al. (1986)
R_t^m	10^{-9} mol/L	7.5×10^{-9} mol/L	Sinha (1988)
r_m	5, 10, 20	75	Xiu et al. (1997)
α_1	1	0.9	Sinha (1988)
α_2	0.01	0.02	Sinha (1988)
α_3	0.01	0	Sinha (1988)
α_4	0.024	0.024	Xiu et al. (1997)
α_5	430	430	Xiu et al. (1997)
α_6	−7.5	−7.5	Xiu et al. (1997)
k	0.002−0.008	0.005	Sen and Liu (1990)
k_i	2283	100	Xiu et al. (1997)
μ_m	0.0312	0.0312	Xiu et al. (1997)

around the steady state and calculating the eigenvalues of the Jacobian matrix. The eigenvalues are usually in the form of conjugate pairs:

$$\lambda = p \pm q\mathrm{i}$$

where p and q are real numbers and i is the imaginary notation. If the real parts of all eigenvalues are negative ($q = 0$), the steady state is said to be locally asymptotially stable. In case some or one of the real parts of the eigenvalues are positive, the steady state is unstable. If $q \neq 0$ the system will display oscillation after perturbation. Depending on the sign of p the system may have damped oscillations or excited oscillations. In the particular case where the Jacobian matrix has a pair of purely imaginary eigenvalues ($p = 0$), a Hopf bifurcation can occur which results in a sustained periodic oscillation around the steady state.

On the other hand, dynamic behavior around a steady state can be also used to analyze the stability of the steady state. A steady state is said to be locally stable if the system returns to the pertinent steady state after a sufficiently small but otherwise arbitrary perturbation from the steady state. For dynamic simulation of the differential eqs 8−10 Runge−Kutta formulas with fourth and fifth orders in MATLAB are applied.

The kinetic parameters chosen from literature for eqs 1−10 are listed in Table 1. The degradation of tryptophan is omitted in this study. Thus $K_3 = 0$, and $\alpha_3 = 0$. In general, the enzyme catalyzing the first reaction in a metabolic pathway is regulatory and controls the rate of the whole pathway (Lehninger et al., 1993). In the biosynthetic pathway of tryptophan from chorismate, anthranilate synthase (AS) is the rate-limiting enzyme in the five enzymes encoded by *trp* operon, which is indicated by the fact that the inhibition constant (K_I) of tryptophan on AS is the lowest. For instance, K_I of tryptophan on TS in *E. coli* is 22.83 mM, and K_I of tryptophan on AS and PRT in *C. glutamicum* are 4.0 and 4.8 mM, respectively (Katsumata and Ikeda, 1993). So AS is determined to be the limiting enzyme in the biosynthesis of tryptophan (Prokudina et al., 1991). In our previous work K_I was chosen as 22.83 mM. The corresponding k_i was 2283. In this work K_I is chosen to be 4.0 mM because AS is the key enzyme on the biosynthesis pathway of tryptophan. So k_i is 100.

The first term in the right-hand side of eq 8 represents the relative concentration of mRNA. The first part ($(1 + z)/[1 + (1 + r)z]$) denotes the repression, and the second part ($k_a/(k_a + z)$) expresses the attenuation. If the concentration of tryptophan in the cell is very low, the repression, feedback inhibition and attenuation are lifted, resulting in a high transcriptional efficiency of RNA polymerase and a high mRNA level but a low translational level due to lack of tryptophan. When tryptophan is present, transcription termination is effective, and the attenuation allows only about 10% of the RNA polymerases to proceed. In the absence of tryptophan, attenuation allows virtually all of the polymerases to proceed. Together with the about 70-fold increase in initiation of transcription that results from the release of repression, this allows an about 700-fold range of regulation of the operon (Lewin, 1997). The intracellular tryptophan concentration varies in a range from 0.4 μM to 2 mM, corresponding to the dimensionless tryptophan concentration from 0.01 to 50. Experimentally, oscillation was found at tryptophan level between 0.1 and 1.2 mM (Bliss et al., 1982). On the basis of these facts, the following parameters are determined, as shown in Figure 2: $r_m = 75$, $k_r = 25$, $k_a = 5.5$.

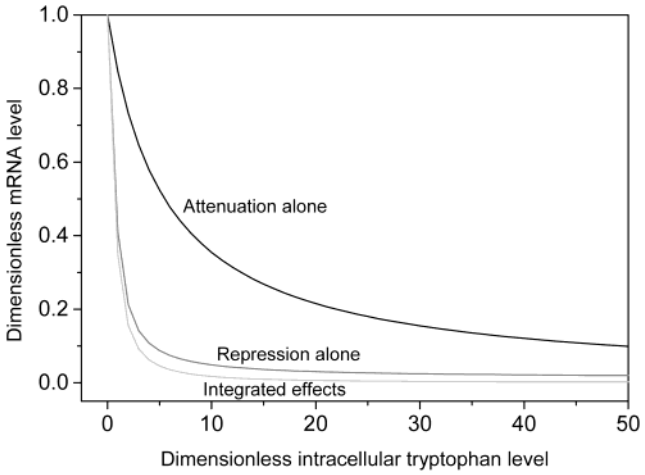

Figure 2. Effect of tryptophan level on the transcription of the *trp* operon regulated by attenuation, repression, and both, being denoted by the terms $k_a/(k_a + z)$, $(1 + z)/[1 + (1 + r)z]$ and their product (eq 8), respectively.

Results and Discussion

1. Influence of Growth Rate on Steady-State Solutions. The steady-state solutions of eqs 8−10 at different growth rates are depicted in Figure 3. The

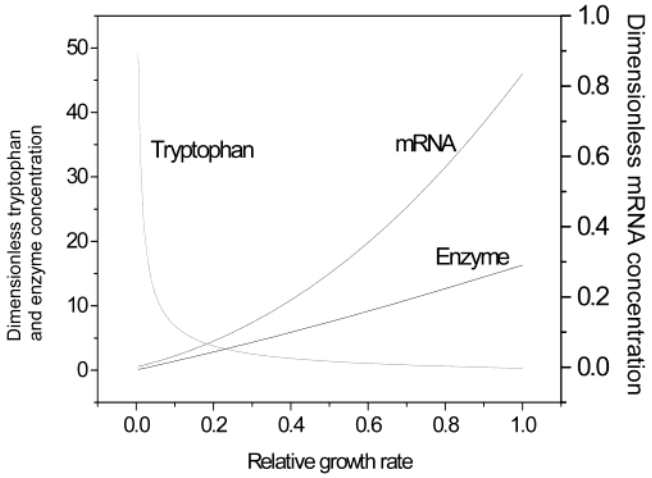

Figure 3. Effect of growth rate on the concentrations of intracellular tryptophan, enzyme, and mRNA. The parameters are those given in Table 1 and $k_r = 25$, $k_a = 5.5$.

tryptophan concentration in cells reduces as the growth rate increases, especially obviously at low growth rates. In contrast, the concentrations of enzyme and mRNA increase with the growth rate. The change tendencies of enzyme and mRNA are different from those calculated by a model that only considers feedback inhibition and repression (Xiu et al., 1997). The previous model revealed that the enzyme concentration decreases at first and then increases as the growth rate increases. The concentration of mRNA remains constant at low growth rate but begins to increase above a certain growth rate which corresponds the growth rate at which enzyme concentration increases. In addition, the tryptophan concentrations calculated from the previous model are much more than those obtained by the improved model at low growth rates, whereas the experimental findings for wild-type strains of *E. coli* showed that the ribosome content increased after the growth rate was shifted up (Yun et al., 1996). The improved model is distinctly more rational than the previous model. The main difference of the both models lies on whether the attenuation is taken into consideration or not. Thus it can be seen that the attenuation plays an important role in getting rational steady-state solutions at low growth rates.

The parameters k_r and k_a in eqs 8−10 are newly introduced to describe the biosynthesis of aporepressor and the attenuation process, respectively. The influences of the two parameters on the intracellular tryptophan concentration at different growth rates are investigated as shown in Figure 4. At the same growth rates, the smaller the k_a, the stronger the attenuation, and the less the k_r, the more the aporepressor. Both cases are unfavorable for the production of tryptophan. The influences of k_r and k_a on the intracellular tryptophan level are more profound at low growth rates, e.g., at relative growth rate $u_R < 0.2$. From Figures 3 and 4 it can be concluded that the growth rate should be controlled at low level for tryptophan production. The cells might also be immobilized or recycled when developing a fermentation process. The codons for Trp in the repressor and leader peptide might be knocked out by genetic engineering for tryptophan overproduction.

2. Stability Analysis. The operational plane of tryptophan concentration vs growth rate can be divided into four domains according to the eigenvalues of the Jacobian matrix and the Routh−Hurwitz criterion. In domain I as shown in Figure 5, the eigenvalues of Jacobian matrix are complex numbers with negative real parts and the

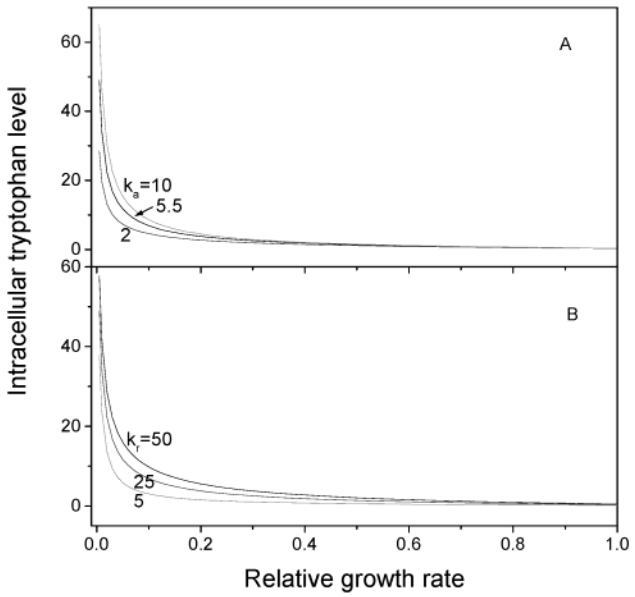

Figure 4. Influences of the parameters k_a (A) and k_r (B) on the intracellular tryptophan concentration at different growth rates. Other parameters except those denoted in the figures are constant: (A) $k_r = 25$; (B) $k_a = 5.5$.

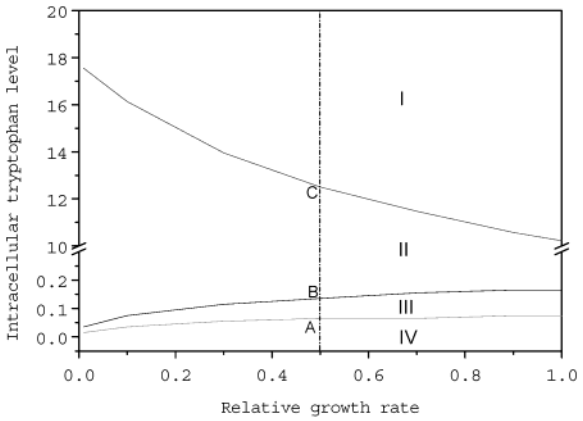

Figure 5. Division of the operational region on a growth rate and intracellular tryptophan plane according to the eigenvalues and dynamic behavior of the steady-state solutions. The eigenvalues are negative real parts in domain IV, complex number with negative real parts in domain III and I, and complex number with positive real parts in domain II, respectively.

steady states exhibit damped oscillatory transient behaviors. As the intracellular tryptophan concentration increases, the imaginary part of the eigenvalues disappears and the steady states are locally asymptotically stable (not shown in Figure 5). Domain II represents an unstable region in which the eigenvalues have positive real parts and the steady state solutions are unstable, leading to excited oscillatory behaviors. Domain III is similar to domain I, in which the eigenvalues are complex numbers with negative real parts and the steady states exhibit damped oscillatory transient behavior. In domain IV, the eigenvalues have only negative real parts and thus the steady states are locally asymptotically stable. The curves in Figure 5 represent critical lines between two regions, e.g., curve C is a disjunctive line between domain I and II, on which every eigenvalue has a pair of purely imaginary numbers. Sustained periodic oscillations (Hopf bifurcation) can occur around curve C. A similar case also appears around curve B, a critical line between domain II and III, which consists of Hopf bifurcation points. Curve A is a disjunctive line between

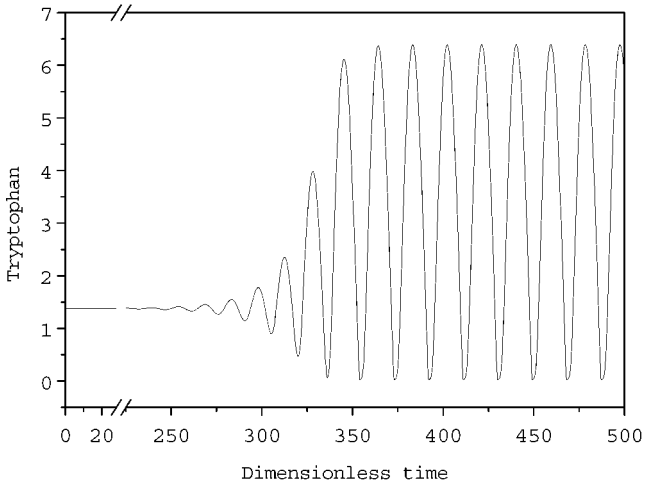

Figure 6. Transient behavior of the intracellular tryptophan concentration after the relative growth rate is shifted from 0.5 to 0.51 for 1 dimensionless time and then set back to 0.5 in the domain II of Figure 5.

a locally stable region (domain IV) and a damped oscillatory region (domain III), on which the imaginary parts of eigenvalues are just zero.

The growth rate has a less significant influence on the stability of the *trp* operon in comparison with its effect on tryptophan synthesis. Instead, the tryptophan concentration in the cells appears to be a determinant factor for the instability. Whereas the stable region in domains I and the stable domain IV encompass both high and very low intracellular tryptophan concentrations, the instability region is found at relatively low intermediate intracellular tryptophan concentrations. As the growth rate enhances, curve C tends to decline, but curve B slightly rises, leading to a decrease of the unstable domain II and an increase of domain I. Of course, the stability of the *trp* operon also depends on the strain level (r), the tryptophan consumption (α_5) and other factors. These factors have been discussed in detail in the previous publications (Sen and Liu, 1990; Xiu et al., 1997).

In comparison with the previous model's prediction, the oscillatory region (domain II) enlarges because the top critical line C shifts much up and the bottom critical line B shifts little down. This agrees much better with the experimental results for a feedback-resistant *E. coli* strain 1-42 that a sustained oscillation was found at tryptophan level between 0.2 and 0.5 mM (Bliss et al. 1982), corresponding to the dimensionless tryptophan concentration from 5 to 12.5.

3. Dynamic Behavior and Oscillation. The dynamic behavior of the steady-state solutions in the unstable domain II on the $z-u_R$ plane is investigated in this section. Figure 6 illustrates the transient behavior of the intracellular tryptophan concentration after the relative growth rate is shifted from 0.5 to 0.51 for 1 dimensionless time and then set back to 0.5 in the domain II of Figure 5. The steady-state solution at $u_R = 0.5$ in domain II has little response to a small perturbation for a long time. Until $\tau = 250$ the intracellular tryptophan concentration appears to change and exhibits the character of an excited oscillation. After $\tau > 350$ the oscillations finally become a sustained stable Hopf bifurcation. The corresponding phase planes are depicted in Figure 7. The limit cycles exhibit irregular shapes that are quite different from the previous results (Xiu et al., 1997).

The reason for the limit cycles in Figure 7 being irregular can be found by investigating the sustained

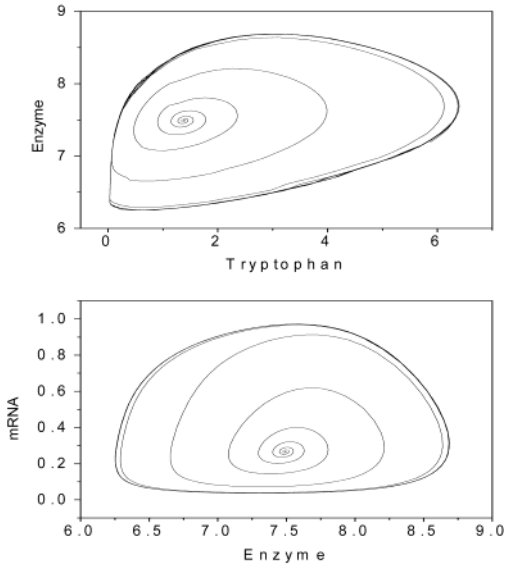

Figure 7. Phase-planes of intracellular concentration of tryptophan, mRNA and enzyme concentration under the condition as in Figure 6.

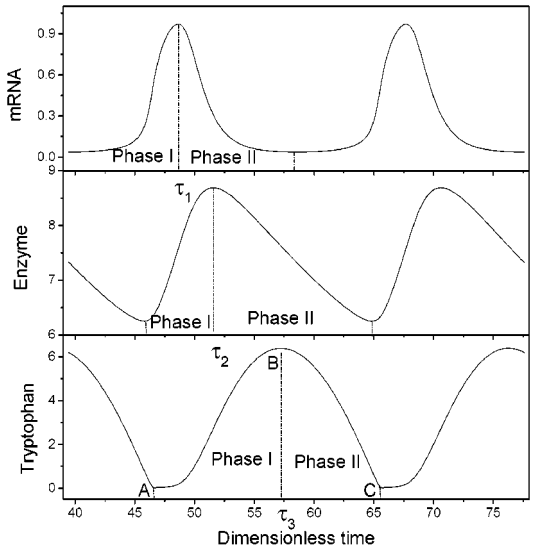

Figure 8. Switching on and off of transcription (mRNA) and sustained oscillations of enzyme and tryptophan concentrations under the condition as in Figure 6.

oscillations in Figure 6 in detail. As shown in Figure 8, the changing rules of mRNA, enzyme and tryptophan are completely different, although they possess the same periods ($\tau = 19$). It appears to be more obvious if one period is divided into two phases at the maximum corresponding time. In Figure 8, phase I is the AB section at the left side of the corresponding time τ_3 of the maximum tryptophan concentration in cells. The BC section at the right side of τ_3 is defined as phase II. The phase I of mRNA corresponds to the turning-on state of a gene and phase II to the turning-off state. Figure 8 shows that the two phases of mRNA seem to lie symmetrically at the two sides of τ_1. The phase I of enzyme is shorter than the phase II, revealing that the activity of enzyme reaches its maximum in a shorter time and the feedback inhibition of tryptophan on the activity of enzyme persists for a longer time. However it takes more time to reach the maximum tryptophan concentration (phase I) than to fall to the minimum value (phase II). In addition, the corresponding times of the occurrence of maximum mRNA, enzyme and tryptophan are different,

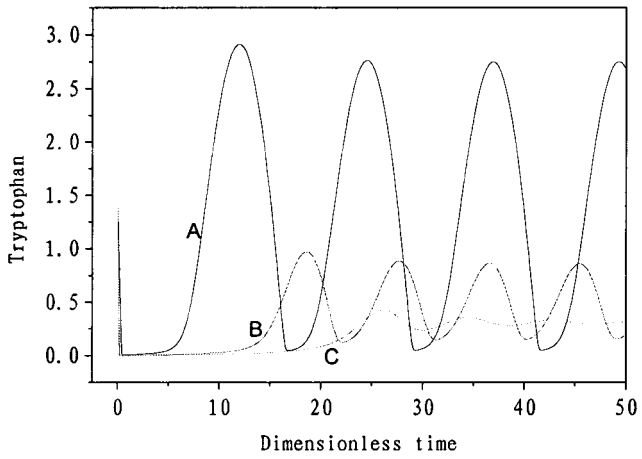

Figure 9. Transient behavior of the intracellular tryptophan concentration after the relative growth rate is shifted from 0.5 to 0.7 (A), 0.9 (B), and 1.0 (C), respectively, in the domain II of Figure 5.

i.e., $\tau_1 < \tau_2 < \tau_3$, existing a time lag between each other. This may be the reason of the formation of a gene circuit or switch (McAdams and Shapiro, 1995; McAdams and Arkin, 1998) and the occurrence of oscillation. When the intracellular tryptophan concentration is at a high level, the transcriptional efficiency is largely reduced by the integrated effects of repression and attenuation, leading to a decrease in both the formation of new mRNA and the biosynthesis of tryptophan. When the tryptophan concentration in cells declines to a certain threshold (not minimum) value, the structural gene gradually recoveries its transcriptional ability and produces new mRNA because of the gradual release of repression and attenuation. The newly translated and folded enzymes improve the biosynthesis of tryptophan and make the tryptophan concentration in cells rise out of the valley. If the tryptophan concentration rises to a certain threshold (not maximum) value again, the repression and attenuation close the gene once again. The mRNA concentration declines, and then the enzyme concentration also begins to reduce after reaching to the maximum, while the biosynthesis of tryptophan delays for the enzymes to be formed. The switching on and off of structural genes of the *trp* operon result in a fluctuation (oscillations) of tryptophan concentration. It is worth pointing out that the gene switch is different from both the physical switch and "1" and "0" in Boolean logical calculation. The switching on of a gene is a process of quick initiation of transcription and the switching off is a severe repression of the transcriptional process but not a complete termination of transcription.

From the above analysis it can be stated that the time lags between mRNA, enzyme and tryptophan biosynthesis play an important role in the formation of gene switch and oscillation. The time delay between transcription and translation ($\tau_2 - \tau_1$) is less than one between enzyme and tryptophan biosynthesis($\tau_3 - \tau_2$). In addition, the time lags vary as the operating conditions, e.g., growth rate (data not shown). This agrees with the experimental observations in gene expression and product biosynthesis.

The oscillations occurring in the domain II of Figure 5 are not entirely identical. The characteristics of oscillation, e.g., period and amplitude, depend not only on the inherent properties of a biological system but also on the operational conditions. For the same biological system, if the operational conditions are not alike, the characteristics of oscillation would also be different. For instance, the steady-state solution similar to Figure 6 exhibits different dynamic behaviors from Figure 6 when the relative growth rate u_R shifts up from 0.5 to 0.7, 0.9, and 1.0, respectively, as shown in Figure 9A, B, and C. Under these circumstances, the dynamic behaviors mainly depend on the final states after changing the relative growth rates. The intracellular concentration at a relative growth rate of 1.0 exhibits a damped oscillatory transient behavior (as shown in Figure 9C) because its eigenvalue is a complex number like in domain III in Figure 5. As the degree of change of growth rate increases, the period and amplitude of oscillation reduce until the oscillation disappears and a new stable steady state is obtained.

Conclusions

An expended mathematical model is established to describe the *trp* operon on the levels of transcription, translation, and enzyme catalysis by taking into consideration the integrated effects of repression and attenuation on transcription. The influences of growth rate are investigated on the biosynthesis of tryptophan, stability and dynamic behavior of the *trp* operon. The theoretical analysis indicates that a decrease of k_a or k_r is unfavorable for the biosynthesis of tryptophan, especially at low growth rates. The stability of the *trp* operon depends not only on the growth rate but also on the intracellular tryptophan concentration. Sustained oscillations of tryptophan levels are theoretically predicted from the integrated regulation of the *trp* operon. During such oscillations the regulation of transcription displays a kind of "on" and "off" state in terms of gene expression, indicating the existence of a genetic circuit or switch in the regulation of the *trp* operon. The time lags between mRNA, enzyme and tryptophan biosynthesis can be used to explain the formation of gene switch and oscillation.

Notation

C	molar percentage of tryptophan in cellular protein
D	gene dosage
E	intracellular enzyme concentration (mol/L)
f	frequency of attenuation
J	Jacobian matrix
K_1, K_2, K_3	degradation rate constants of mRNA, enzyme and tryptophan, respectively (h^{-1})
k_1, k_{-1}, k_2, k_{-2}	constants for the reactions of formation and dissociation of holorepressor and bound operator, respectively
K_A, K_R	inhibition constant of tryptophan on leader peptide and Michaelis−Menten constant of repressor synthesis(mol/L)
k, k_i	dimensionless inhibition constant of tryptophan on leader peptide and dimensionless Michaelis−Menten constant of repressor synthesis
K_d, K_o	dissociation constants of holorepressor and bound operator, respectively (mol/L)
K_e, K_m, K_p	rate constants of formations of enzyme, mRNA and tryptophan, respectively (h^{-1})
K_I, K_s	inhibition and saturation constants of tryptophan, respectively (mol/L)
k, k_i	dimensionless saturation and inhibition constants of tryptophan
M	intracellular mRNA concentration (mol/L)
n	number of binding sites of aporepressor
O, O_t	free and total operator, respectively
P	intracellular tryptophan concentration (mol/L)

P^{m}_{pro}	maximum cellular protein concentration based on intracellular cell volume (mol/L)
R, R_t, R_t^m	free, total and maximum aporepressor, respectively
r, r_m	dimensionless constant indicating strain level of the *trp* operon repressor and its maximum value
T	dimensionless parameter
t	time (h)
u, u_m, u_R	dimensionless growth rate, maximum and relative dimesionless growth rate
x, y, z	dimensionless concentrations of mRNA, enzyme, and tryptophan, respectively

Greek Symbols

α_1, α_2, α_3	dimensionless degradation rate constants of mRNA, enzyme, and tryptophan, respectively
α_4, α_5, α_6	dimensionless parameters
β	a constant indicating the effects of growth rate on protein content of cells
μ, μ_m	specific growth rate and maximum specific growth rate (h^{-1}), respectively
λ	eigenvalues of the system (eq 6)
τ	dimensionless time

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