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Role of Feedback Inhibition in Stabilizing the Classical Operon

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The Goodwin equations for a repressible operon (Goodwin, 1965) are modified (1) to describe a time lag between genetic regulation and appearance of functional enzyme, (2) to describe consumption of endproduct in protein synthesis, and (3) to describe feedback inhibition of enzyme activity. The stability of the modified equations is determined by a method outlined in the appendix which treats a class of negative feedback systems with time delays. With parameters estimated from experimental data on the tryptophan operon of *Escherichia coli*, we conclude that the operon becomes unstable as normal feedback inhibition is lost. Numerical solution of the modified equations shows that an example with a partial loss of feedback inhibition can have a period of oscillation less than the cell generation time, and the numerical solutions are shown to be in qualitative agreement with experiments showing oscillations in tryptophan operon expression.

Introduction

The Goodwin equations describe a gene–enzyme–endproduct control unit, the repressible operon, with a single negative feedback control loop acting on the first step in the process. These equations, as modified by Griffith

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(1968) without loss of generality, can be written

$$\dot{M} = \frac{1}{1+P^n} - \alpha M, \tag{1a}$$

$$\dot{E} = M - \beta E, \tag{1b}$$

$$\dot{P} = E - \gamma P, \tag{1c}$$

where M, E, and P denote, respectively, the concentrations of mRNA, of enzyme and of endproduct of the operon. Derivatives with respect to time are denoted by $\dot{M}$, $\dot{E}$, and $\dot{P}$; and α, β, and γ are positive constants. The fraction in (1a) describes endproduct repression, and the positive constant n, the Hill number, specifies the strength of repression. For the case $n = 1$, Goodwin (1965) published the results of analog computer simulations where M, E, and P exhibit undamped oscillations about an unstable equilibrium. However, Griffith (1968) proved analytically that the equilibrium is locally, asymptotically stable when $n < 8$, and he further showed that when $n > 8$ the equilibrium can always be made unstable by a proper choice of constants α, β, and γ. Later, Allwright (1977) and Banks & Mahaffy (1978) showed that the Goodwin equations are globally stable for $n = 1$. It therefore appears that the reported oscillations resulted from an artifact in the analog computer that introduced an error into the solution of equations (1).

From his results, Griffith concluded that oscillations of operon activity could occur only if there is co-operative repression of such a high order as to be unlikely. However, there are parameters other than co-operativity of repression that can, in some cases, prevent negative feedback systems from approaching a stable equilibrium. Introduction of time delays in the expression for repression has been shown by computer simulations to lead to stable oscillations in particular cases where the cooperativity is greater than 1 (Landahl, 1969; Rapp, 1976; Mackey & Glass, 1977). It has been shown analytically by MacDonald (1977), Allwright (1977), and Banks & Mahaffy (1978) that time delays cannot destabilize the system with $n = 1$, but the results of MacDonald (1977) and Tyson & Othmer (1978) show that some systems with $n \geq 2$ can be destabilized by time delays. In the 2-variable system with a time delay, the criterion for existence of periodic solutions has been given by an der Heiden (1979).

A second modification that can affect stability is introduction of a function into (1c) to describe non-linear demand for endproduct. Walter (1974) showed with simulations that hyperbolic demand for endproduct can destabilize a system that would be stable with linear, first-order demand as expressed in (1c). This observation is of potential biological significance

since most endproducts are metabolized by enzyme-catalyzed reactions with non-linear kinetics.

With the introduction of time delays and non-linear demand into (1), we can undertake a meaningful investigation of the effect of feedback inhibition in a system where the endproduct in a biosynthetic pathway is the small molecule effector for both genetic regulation and feedback inhibition. Such a system is modeled after classical repression where endproduct (corepressor) binds to protein aporepressor to form functional holorepressor as in the tryptophan operon of *Escherichia coli*. Our analysis does not apply to systems where attenuation, a regulatory mechanism that is not mediated by a repressor, is the dominant mode of genetic regulation. The mechanism of attenuation is not well understood. However, in the amino acid biosynthetic systems of bacteria, attenuation is apparently effected by the level of charged tRNA (Yanofsky, 1981). Attenuation does occur in the tryptophan operon (Bertrand, Squires & Yanofsky, 1976), but classical repression appears to be dominant. The effect of attenuation on stability will be discussed in a later paper.

Equations Describing the Repressible Operon

The rate of initiation of transcription of an operon at time t is assumed to be the product of k_m, the intrinsic rate of initiation of transcription of an operon that is not repressed, and $R(t)$, the probability that the operon is not repressed at time t. A molecule of mRNA is considered to be mature, i.e. it is counted as an mRNA molecule, as soon as it is already to be translated. The concentration of mRNA is assumed to decrease as a consequence of two first order processes, dilution by growth at constant specific rate k and enzymatic degradation at constant specific rate r. Therefore, the rate of change of mRNA concentration is

$$\dot{M} = gk_mR(t - t_m) - k_1M, \tag{2a}$$

where g is the concentration of the operon, t_m is the time delay between initiation of transcription and initiation of translation and where $k_1 = k + r$. It is reasonable to make the assumption that g is constant throughout the cell cycle even though it varies over a two-fold range as a consequence of DNA replication. Clearly, consideration of the DNA cycle would introduce oscillations in gene concentration that would confound our analysis.

The time delay between initiation of translation and appearance of functional enzyme is denoted t_p, and the rate of initiation of translation per mRNA molecule is denoted k_p. Depletion of enzyme is again the result of two processes, dilution by growth and enzymatic degradation, which we

assume can be described together by a single specific rate constant, k_2. Therefore the rate of change of enzyme concentration is

$$\dot{E} = k_p M(t - t_p) - k_2 E. \tag{2b}$$

Endproduct pool concentration is denoted P, and the rate of formation of endproduct is equal to the product of the enzyme concentration, E, the turnover number, k_t, and the inhibition ratio, I. The turnover number is the maximum rate of product formation per enzyme molecule (at a fixed temperature), and I is defined as the ratio of enzyme activity in the presence of endproduct to the maximum activity when $P = 0$. The endproduct can be depleted in two ways. It can be used in cell metabolism at rate described by a function $G(P)$ to be discussed in detail later, and it can be diluted by growth. Therefore, the rate of change in the concentration of endproduct is described by

$$\dot{P} = k_t EI - G(P) - kP. \tag{2c}$$

Stability Analysis

An equilibrium for equations (2) is defined by $\dot{M} = \dot{E} = \dot{P} = 0$. If equilibrium values are denoted M_0, E_0, and P_0, substitution into equations (2) leads to the equations;

$$M_0 = gk_m R_0 / k_1, \tag{3a}$$

$$E_0 = gk_m k_p R_0 / (k_1 k_2), \tag{3b}$$

$$P_0 = (k_t E_0 I_0 - G_0)/k, \tag{3c}$$

where I_0, R_0, and G_0 denote equilibrium values. We assume that R, I, and G are differentiable functions of the single variable P, and that R and I are strictly decreasing while G is a strictly increasing function of P with $G(0) = 0$. These assumptions are biologically reasonable and guarantee a unique solution of equations (3).

Stability of the above equilibrium can be determined by writing a first-order expansion of equations (2) in terms of the variables $m = M - M_0$, $e = E - E_0$, and $p = P - P_0$ to give the equations;

$$\dot{m} = gk_m R_p p(t - t_m) - k_1 m$$

$$\dot{e} = k_p m(t - t_p) - k_2 e,$$

$$\dot{p} = k_t I_0 e - (G_p - k_t E_0 I_p + k)p,$$

where the subscripts on R, I, and G denote differentiation with respect to P. Note that the autogeneous component of repression, $\partial R/\partial E$, has been

omitted. The methods developed here are not adequate for a rigorous treatment of this component of regulation.

Any solution of the above system of linear differential equations is of the form $m = m_0 \exp(zt)$, $e = e_0 \exp(zt)$, and $p = p_0 \exp(zt)$ where m_0, e_0, and p_0 are constants and z is a complex number satisfying the characteristic equation

$$\mathrm{DET}\begin{pmatrix} k_1+z & 0 & -gk_mR_p \exp(-zt_m) \\ -k_p \exp(-zt_p) & k_2+z & 0 \\ 0 & -k_tI_0 & k_3+z \end{pmatrix} = 0,$$

where $k_3 = G_p - k_tE_0I_p + k$. Expansion of the determinant gives

$$(1+z/k_1)(1+z/k_2)(1+z/k_3) = -L \exp(-zt_d), \tag{4}$$

where $t_d = t_m + t_p$ and $L = -gk_mk_pk_tR_pI_0/(k_1k_2k_3)$. Since R_p is negative or zero, L must be positive or zero. The expression for L can be simplified by substitution from equations (3):

$$L = -R_pk_tE_0I_0/(R_0k_3). \tag{5}$$

The equilibrium defined by equations (3) is locally stable if and only if all values of the complex variable $z = x + iy$ that satisfy the equation

$$(1+z/k_1)(1+z/k_2)(1+z/k_3) = -L \exp(-zt_d)$$

have a negative real part x. The issue of stability can be resolved using the method discussed in the appendix: a function $B(t_d)$ can be computed, where B is the smallest value of L that does not define a stable equilibrium for the given value of t_d and fixed constants k_1, k_2, and k_3. The graph of this function, which we call a $B - t_d$ diagram, separates the first quadrant of the $t_d - L$ plane into two regions: one consists of all points below the curve, and for these points (t_d, L) the equilibrium is stable. The second region consists of all points above the curve, and this corresponds to the unstable region. For an example of such a diagram, see Fig. 1.

Stability of a Repressible Operon

To assess stability of classical feedback regulatory systems, we choose parameters to describe the tryptophan operon of *Escherichia coli*. This is perhaps the best studied biosynthetic, repressible operon in the literature. Furthermore, this operon can be tested experimentally to see whether or not it is stable (Bliss, 1979*a*,*b*). Accordingly, we will estimate the constants in equation (4) for typical experimental conditions in minimal medium at 37° where the specific growth rate k is approximately 0·0002/sec. The

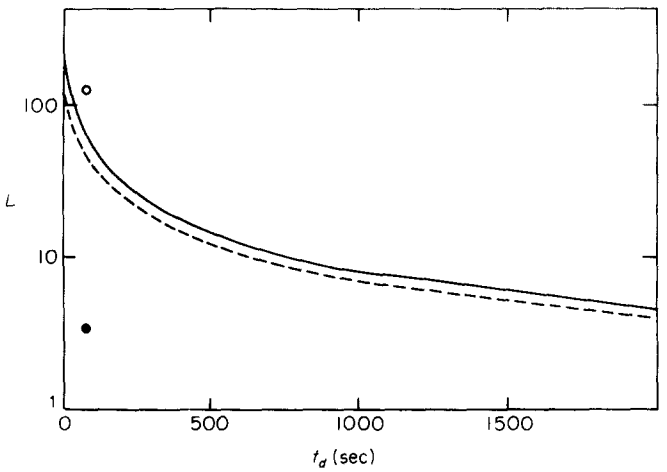

FIG. 1. $B - t_d$ diagrams for hypothetical examples of the tryptophan operon. The boundary of operon stability is plotted as a function of t_d, the transcription–translation time delay. The region below a curve corresponds to stability, and the region above corresponds to instability. The solid line is calculated from the example of the normal operon and the dashed line from the example of the operon with a partial loss of feedback inhibition. The closed circle is the point (t_d, L) for the normal operon indicating stability, and the open circle is the point for the case without normal feedback inhibition indicating instability. The cooperativity of repression is equal to 4 in each case.

specific rate of tryptophan mRNA degradation is approximately 0·016/sec from the experiments of Kennell & Talkad (1976), and degradation of enzyme is assumed to be negligible. Therefore we have $k_1 = 0{\cdot}016$/sec and $k_2 = 0{\cdot}0002$/sec.

The demand for tryptophan is normally determined by the rate of charging of tryptophan onto tRNA, and under most physiological conditions this rate must be nearly equal to the rate of incorporation of tryptophan into protein. The rate of charging is determined by the aminoacyl-tRNA synthetase which, at fixed ATP concentration, is described kinetically by

$$Q = Q_{\max} PU/((K_p + P)(K_U + U)),$$

where U is the concentration of uncharged $tRNA^{trp}$ and where $K_p = 10^{-5}$ M and $K_U = 5 \times 10^{-7}$ M (Joseph & Muench, 1971). Uncharged $tRNA^{trp}$ is regenerated in protein synthesis, and the rate of this process is denoted by the kinetic function W. The equilibrium tryptophan level, P_0, is estimated to be approximately 10^{-4} M from the experiments of Bliss (1979*a*,*b*). The concentration of $tRNA^{trp}$ is approximately $2{\cdot}5 \times 10^{-6}$ M (Joseph & Muench, 1971), and approximately 80% exists as the charged species. Therefore, denoting the concentration of the charged species by C, we have the

estimates $C_0 = 2 \times 10^{-6}$ M and $U_0 = 5 \times 10^{-7}$ M. At equilibrium, Q must equal W, which is approximately 4×10^{-6} M/sec. This demand is calculated to meet the requirements of a cell growing at the above growth rate with a protein content of 20–25% of the wet cell mass and where tryptophan comprises 1% of the amino acid residues in bulk protein (Lehninger, 1975).

The turnover time of a metabolite is defined as its pool level divided by its rate of metabolism and is the reciprocal of the specific rate of metabolism. The turnover time of tryptophan is 25 sec, that of charged tRNA is 0·5 sec, and that of uncharged tRNA is 0·12 sec. Clearly, the turnover time of uncharged tRNA is very short compared with that of tryptophan. We use this fact to justify the approximation $Q = W$: indeed it is readily calculated that the difference between Q and W cannot be as large as 1% of the equilibrium value of W for 1 sec. This approximation now defines U as a function of P. Therefore, writing $\mathrm{d}Q$ as a total differential leads to

$$\frac{\mathrm{d}Q}{\mathrm{d}P} = \frac{\partial Q}{\partial P} + \frac{\partial Q}{\partial U}\frac{\mathrm{d}U}{\mathrm{d}P}.$$

Since W is a function of the single variable C in this context, we have

$$\frac{\mathrm{d}Q}{\mathrm{d}P} = \frac{\mathrm{d}W}{\mathrm{d}C}\frac{\mathrm{d}C}{\mathrm{d}P}. \tag{6}$$

We assume that the total concentration of $\mathrm{tRNA}^{\mathrm{trp}}$ species is constant, so that $\mathrm{d}U = -\mathrm{d}C$. The two above equations can now be combined to give

$$\frac{\mathrm{d}U}{\mathrm{d}P} = -\frac{\partial Q}{\partial P}\Big/\left(\frac{\partial Q}{\partial U} + \frac{\mathrm{d}W}{\mathrm{d}C}\right).$$

Finally, substitution into (6) gives

$$\frac{\mathrm{d}Q}{\mathrm{d}P} = \frac{\mathrm{d}W}{\mathrm{d}C}\frac{\partial Q}{\partial P}\Big/\left(\frac{\partial Q}{\partial U} + \frac{\mathrm{d}W}{\mathrm{d}C}\right), \tag{7}$$

which, in the case where $\mathrm{d}W/\mathrm{d}C$ is much smaller than $\partial Q/\partial U$, leads to the approximation

$$\frac{\mathrm{d}Q}{\mathrm{d}P} = \frac{\partial Q}{\partial P}\frac{\mathrm{d}W}{\mathrm{d}C}\Big/\frac{\partial Q}{\partial U}. \tag{8}$$

To derive an expression for W, we note that the step time for protein synthesis, the time required to add an amino acid residue to a peptide chain on a ribosome, can be partitioned into two intervals: the time t_a required for adsorption of the appropriate charged tRNA to the ribosome and the time t_b required for peptide bond formation and translocation. The distribution of waiting times for adsorption is an exponential function with

a mean waiting time that is inversely proportional to the concentration of charged tRNA. In the case of tryptophan, we have $t_a = t_0 C_0/C$, where t_0 is the mean waiting time for adsorption of charged $tRNA^{trp}$ when $C = C_0$. Now assume that the mean time for adsorption of tRNA's other than $tRNA^{trp}$ is t_0, i.e. the adsorption kinetics of $tRNA^{trp}$ are the same as those of the average tRNA. Consequently, the average time for adsorption of tRNA, as a function of C, is the weighted average $0{\cdot}99t_0 + 0{\cdot}01t_0C_0/C$. We assume that t_b is the same for all amino acids, so that the mean step time for protein synthesis is $t_b + 0{\cdot}99t_0 + 0{\cdot}01t_0C_0/C$. The rate per ribosome is the reciprocal of this time. Therefore, the overall rate of protein synthesis, W, can be written

$$W = W_{max}C/(K_C + C),$$

where $K_C = 0{\cdot}01t_0C_0/(0{\cdot}99t_0 + t_b)$. Therefore, we have $K_C < C_0/99$, and this relationship approaches equality as the ratio t_b/t_0 approaches 0.

For functions of the Michaelis–Menten type, $V = V_{max}X/(K_M + X)$, the derivative $\partial V/\partial X$ is equal to $(1-s)V/X$, where s is the enzyme saturation, $X/(K_M + X)$, and V/X is the reciprocal of the turnover time calculated above. For the function Q, $1-s$ is 0·09 for tryptophan and 0·5 for $tRNA^{trp}$, so that we have the estimates $\partial Q/\partial P = 0{\cdot}0036$/sec and $\partial Q/\partial U = 4$/sec. For the function W, $1-s$ is less than 0·01 and dW/dC is less than 0·02. Therefore, dQ/dP is calculated from (7) or (8) to be less than 2×10^{-5}/sec. This value is used for G_p in the following. However, assuming a smaller value does not change any of our conclusions.

To estimate I_p, we assume that I is a Hill function $K_I^n/(K_I^n + X^n)$. Such functions have been widely used to describe negative feedback in the operon since the work of Griffith (1968). The enzyme anthranilate synthetase, which effects feedback inhibition, has two binding sites for tryptophan. Hence, we assume that n, the cooperativity of inhibition, is 2. From the experiments of Bliss (1979*b*), the inhibition ratio I is approximately 0·5 in strains with normal regulation. Therefore, we have the estimate $K_I = P_0$ which is approximately 10^{-4} M. The ratio I_P/I is computed to be $-n(1-I)/P$, and since k_tE_0I is 4×10^{-6} M/sec from equation (3c), we have the estimate $-k_tE_0I_p = 4 \times 10^{-2}$/sec in this case. Therefore, k_3 is estimated to be 0·04/sec for the normal tryptophan operon.

To estimate L, we assume that R can be described by a Hill function, $K_R^m/(K_R^m + P^m)$, so that $R_p = mR(1-R)/P$. Since the trp repressor is a tetramer (Gunsalus & Yanofsky, 1980), we estimate $m = 4$. This choice is consistent with the *in vitro* experiments of Rose *et al.* (1973). From these experiments, we also estimate that $R = 0{\cdot}1$ when $P = 10^{-4}$ M, but this

estimate may be biased high by non-specific initiation of transcription in such experiments. From equations (3c) and (5), we estimate $L = 3{\cdot}6$ for the normal tryptophan operon. Using the method outlined in the appendix, we have computed a $B - t_d$ diagram from this example (Fig. 1). The minimum transcription–translation time is estimated as 70 sec for the rate-controlling first enzyme on the pathway, anthranilate synthetase. This estimate is made from the time required to transcribe the leader RNA and trp *ED* mRNA, approximately 3500 nucleotides, at a rate of 50 nucleotides/sec. For this time delay, the value of B computed by the method in the appendix is 66 so that the normal operon appears to be stable.

Now consider a hypothetical case where K_I is increased ten-fold to 10^{-3} M as a consequence of mutation. The equilibrium pool level, P_0, in this case is approximately $1{\cdot}2 \times 10^{-4}$ M, and R_0 is approximately 0·05. Therefore, the equilibrium enzyme level is approximately one-half the level in the previous case, and the enzyme functions at more than 98% efficiency as opposed to 50% in the previous case. By the method outlined above, k_3 is estimated to be 0·0012 and B is 47. The value of L is calculated to be 125. Therefore, it appears that this example of classical repression becomes unstable as feedback inhibition is lost.

To illustrate the dynamic behavior of unstable operons, we solved equations (2) numerically using a digital computer. The initial conditions in these computations were chosen to simulate a derepression experiment where a bacterial culture grown in excess tryptophan is deprived of tryptophan by some procedure such as centrifugation and resuspension in medium lacking the amino acid. Accordingly, we assumed that the concentration of tryptophan within cells grown in the presence of exogenous tryptophan was 10^{-3} M. This relatively high pool level in turn defined the basal levels of mRNA and enzyme by equations (3a) and (3b), respectively. Our strategy in these simulations was to compute new values of the variables M, E, and P at 1 sec intervals using equations (2).

Parameters used in the first simulation were identical to those chosen above to describe the operon with a partial loss of feedback inhibition, i.e. with $K_I = 10^{-3}$ M. As predicted by stability analysis, the solutions in Fig. 2 appear to be unstable. Furthermore, they are oscillatory with a period of 47 min. Figure 2a shows that the operon in this example functions approximately as an on–off switch that is turned on for only a short period during each cycle. Consequently, enzyme (Fig. 2b) rises for a short time and then falls approximately exponentially during each cycle. Figure 2c shows that endproduct oscillates between a high level where feedback inhibition limits its rate of formation and a very low level when the concentration of enzyme can no longer meet the demands of growth.

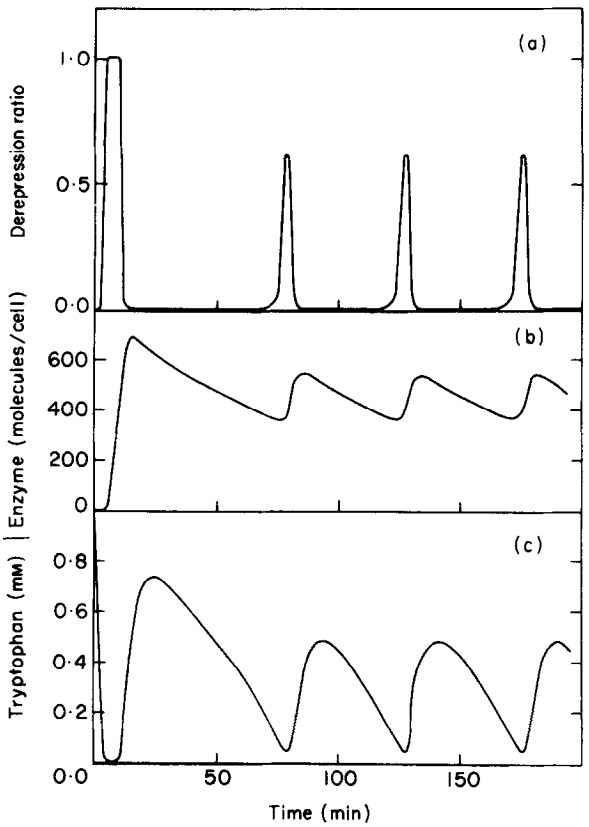

FIG. 2. Simulation of gene expression in the hypothetical example of a repressible operon with reduced feedback inhibition. Initial conditions were chosen to simulate derepression at $t = 0$ as described in the text. (a) Derepression ratio expressed as the fraction of maximal gene activity. (b) Intracellular enzyme concentration. (c) Intracellular endproduct concentration.

A second simulation, presented in Fig. 3, used parameters identical to those used in the above example with the single exception that K_I was 10^{-4} M, i.e. feedback inhibition was assumed to be normal. Consistent with stability analysis, this example is clearly stable with enzyme and endproduct rising asymptotically to their equilibrium values.

Results of derepression experiments redrawn from Bliss (1979*b*), are presented in Figs 4 and 5. In these experiments, cells were grown in medium containing 50 μg/ml L-tryptophan to repress the tryptophan operon. During exponential growth in this medium, they were harvested by centrifugation, washed in ice-cold medium lacking tryptophan and then resuspended in warm medium lacking tryptophan (at time designated 0). Figure 5 shows

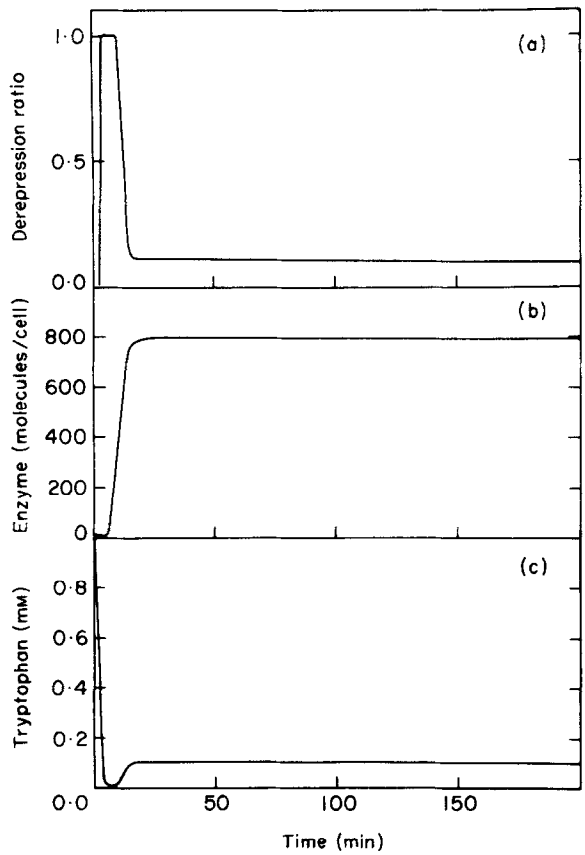

FIG. 3. Simulation of gene expression in the hypothetical example of a repressible operon with normal feedback inhibition. Initial conditions were chosen to simulate derepression at $t = 0$ as described in the text. (a) Derepression ratio expressed as the fraction of maximal gene activity. (b) Intracellular enzyme concentration. (c) Intracellular endproduct concentration.

the response of *Escherichia coli* strain W1485 tna$^-$, and Fig. 4 shows the response of this strain's feedback-resistant derivative, 1–42, which has a K_I for feedback inhibition that is much increased over that of the parent strain. As predicted by the foregoing equilibrium analysis and simulations, the tryptophan operon in the feedback resistant strain appears to be unstable.

Discussion

After the first period of derepression, simulations of the feedback-resistant case in Fig. 2 are in good qualitative agreement with the

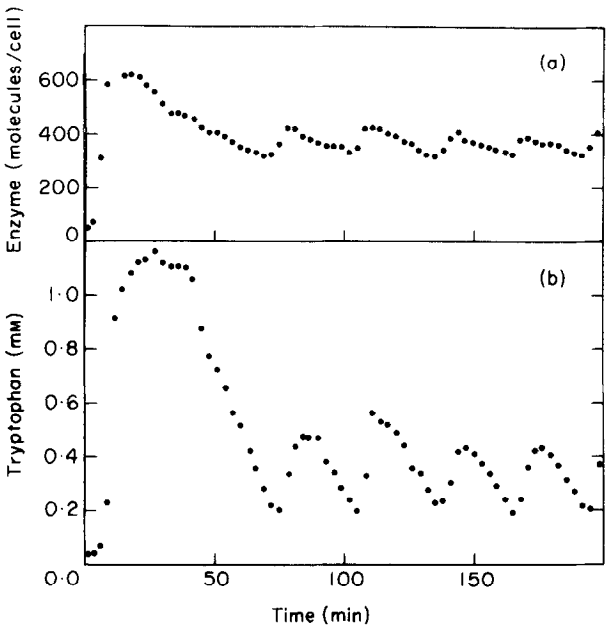

FIG. 4. Expression of the tryptophan operon of *Escherichia coli* in strain 1–42 which has reduced feedback inhibition. Cells were derepressed as described in the text. (a) Intracellular enzyme (anthranilate synthetase) concentration. (b) Intracellular endproduct (tryptophan) concentration. Data redrawn from Bliss (1979*b*).

experimental results in Fig. 4. However, there are several differences between the theoretical and the experimental results. First, the initial decline of endproduct in the simulations is absent in the experiments. It appears that the initial decline in the experiments occurred before sampling began. Second, the period of the derepressions following the initial one is longer in the simulation than in the experiment. Third, the minimum concentration of tryptophan is lower in the simulation than in the experiment.

An explanation for at least part of these discrepancies is that the numerical model did not account for the stringent response to deprivation of an amino acid (Cashel, 1970). When the concentration of a species of charged tRNA falls to low levels, transcription of tRNA, rRNA, and specific mRNA species including those for ribosomal proteins is reduced. As the concentration of these mRNA species falls, the demand for tryptophan should fall, preventing the pool from being severely depleted if there is a small mismatch between supply and demand as there is during the periods of derepression following the first cycle. However, there is a large mismatch between supply and demand during the initial derepression so that the stringent response

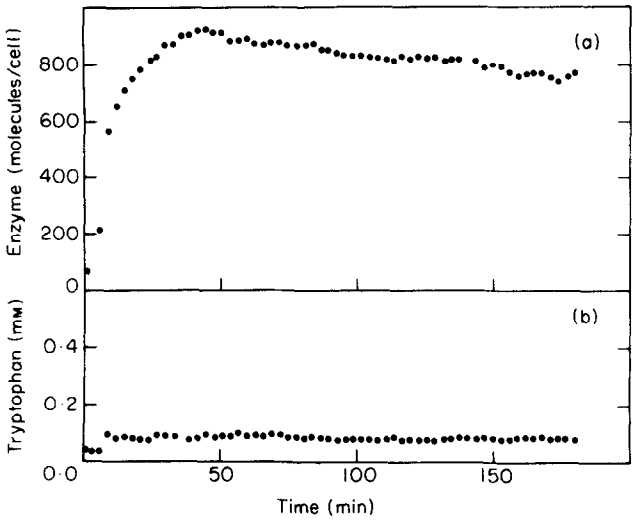

FIG. 5. Expression of the tryptophan operon of *Escherichia coli* in strain W1485 tna$^-$. Cells were derepressed as in Fig. 4. (a) Intracellular enzyme (anthranilate synthetase) concentration. (b) Intracellular endproduct (tryptophan) concentration. Data redrawn from Bliss (1979*b*).

would not be expected to prevent severe pool depletion. If the stringent response is largely responsible for buffering the pool during subsequent derepressions, then it may also account for the shortened period of the subsequent derepressions. Clearly, the higher minimum pool level implies that the operon is not as active during the subsequent cycles of derepression as it is during the initial period, and this reduced activity should lead to a shortened period for the subsequent cycles (Painter & Bliss, 1981).

In order to fit the experimental data of Figs 4 and 5 accurately, it may be necessary to incorporate factors other than the stringent response into our numerical model. Examples of such factors are (1) attenuation, (2) autogenous regulation of the tryptophan repressor (Gunsalus & Yanofsky, 1981), (3) leakage and transport of tryptophan through the cytoplasmic membrane, (4) the DNA replication cycle, (5) deviations of the regulatory functions R and I from Hill functions, and (6) the partial loss of synchrony in a population of cells with oscillating operon expression.

On the basis of simulations, stability analysis, and experiments, we conclude that the repressible operon, in the absence of feedback inhibition, can be unstable with reasonable values of the co-operativity of repression. This conclusion differs from that of Griffith (1968) as a consequence of two modifications introduced into the traditional description of the operon:

non-linear demand for the endproduct of the operon and a time delay in the transcription–translation process.

The standard description of demand since the work of Goodwin (1965) has been a first-order term γP. Such a term describes the demand for a cofactor such as biotin which is not consumed in metabolism: an appropriate demand term for the biotin operon is kP. First-order demand is also appropriate when endproduct is consumed in an enzyme-catalyzed reaction described by Michaelis–Menten kinetics with K_M greater than the endproduct pool level. However, the reactions that consume amino acids for protein synthesis appear to operate at pool levels where reaction rates are almost independent of concentrations (Maaløe & Kjeldgaard, 1967).

The effect of non-linear demand on stability can be illustrated by the case with reduced feedback inhibition (Fig. 2). If demand for tryptophan were made linear in this case, the value of L would be changed from 125 to 3·8. Since the value of B for this case is 47 we conclude that an unstable operon resulting from reduced feedback inhibition can be converted to a stable operon by a second change resulting in first-order demand. Examples of such a change include lowered affinity of aminoacyl-tRNA synthetase for tryptophan or production of large amounts of tryptophanase, an enzyme that catabolizes tryptophan with approximately first-order kinetics at low pool levels.

From Fig. 1, the normal tryptophan operon appears very stable: the operon would remain stable if the cooperativity of repression were increased five-fold or if the transcription–translation time delay were increased ten-fold. Other potentially destabilizing perturbations are increasing k_2 (by increasing the growth rate or increasing enzyme turnover) and decreasing the rate of mRNA degradation r. We calculate that the operon would be stable if k_2 were doubled or if r were halved. However, this stability is achieved at the cost of efficiency since the stabilizing mechanism, feedback inhibition, results in enzyme function at only half of maximal activity.

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APPENDIX

It is well known that stability of systems of differential equations depends on properties of roots of the characteristic equation. Such a system is asymptotically stable if and only if all roots of the characteristic equation have negative real parts. In the following, we analyze the equation

$$\prod_{j=1}^{j=n} (1 + z/k_j) = -Le^{-zT}, \tag{A1}$$

where z is a complex variable, where $k_1, k_2 k_n$ are positive constants and where T is a positive constant or 0. Now, define y_0 by the equation

$$\sum_{j=1}^{j=n} \tan^{-1}(y_0/k_j) + Ty_0 = \pi \tag{A2}$$

and define

$$B = \prod_{j=1}^{j=n} |(1 + iy_0/k_j)|.$$

The following result gives a criterion for asymptotic stability of the system derived in this paper.

Theorem. With the above definitions, equation (A1) has a solution z with $Re(z) > 0$ if and only if

$$B < L. \tag{A3}$$

Furthermore, if $B > L$ and if z is a solution of (A1), then $Re(z) < 0$.

Proof. First assume that $B < L$. Define the function $y(x)$ by the equation

$$\sum_{j=1}^{j=n} \tan^{-1}\left(\frac{y}{x+k_j}\right) + yT = \pi. \tag{A4}$$

Note that y is strictly increasing with $y(0) = y_0$. Now define the functions

$$\alpha(x) = \prod_{j=1}^{j=n} \left| 1 + \frac{x + iy(x)}{k_j} \right|$$

and

$$\beta(x) = |L \exp(-(x + iy(\bar{x}))T)|.$$

Note that both functions are continuous, that α is strictly increasing and unbounded, and that β is decreasing. Furthermore, $\alpha(0) = B$ and $\beta(0) = L$, so that $\alpha(0) < \beta(0)$. Consequently, the equation

$$\alpha(x) = \beta(x) \tag{A5}$$

has a solution x_1 where $x_1 > 0$. We define $y_1 = y(x_1)$ and $z_1 = x_i + iy_1$. By (A4), the complex numbers

$$\prod_{j=1}^{j=n} (1 + z_1/k_j), \tag{A6}$$

and $-L \exp(-z_1 T)$ have equal arguments, and (A5) states that their moduli are equal. Therefore, z_1 is a solution of (A1) and $Re(z_1) > 0$.

Now, assume that $z_1 = x_1 - iy_1$ satisfies (A1) and that $x_1 > 0$. Since the conjugate of z_1 is also a solution of (A1), we can assume that $y_1 > 0$. Equation (A1) states that the complex numbers, (A6) and $-L \exp(-z_1 T)$, have the same argument which implies that

$$\sum_{j=1}^{j=n} \tan^{-1}\left(\frac{y_1}{x_1 + k_j}\right) + y_1 T = (2N+1)\pi,$$

where N is a positive integer. It follows that

$$\sum_{j=1}^{j=n} \tan^{-1}\left(\frac{y_1}{x_1 + k_j}\right) + y_1 T > \pi.$$

Again define $y(x_1)$ by equation (A4). Clearly, $y(x_1) < y_1$, and since $y(x)$ is strictly increasing, we have $y_0 < y_1$. Consequently, we have

$$B < \prod_{j=1}^{j=n} \left(1 + \frac{x_1 + iy_1}{k_j}\right).$$

From (A1), we have

$$L = \exp(x_1) \prod_{j=1}^{j=n} \left| \left(1 + \frac{x_1 + iy_1}{k_j}\right) \right|,$$

so that $B < L$, and the proof of the first statement of the theorem is complete. The second statement follows from the observation that there is a solution with $Re(z) = 0$ only when $B = L$.

The above theorem is readily adapted to finding a computer approximation for B as a function of T. The procedure we have used is (1) to find a value for y_0 by successive approximations, and (2) to use this value of y_0 in order to calculate B. By repeating this procedure for a large number of values of T, we can plot a curve representing B as a function of T. Points above the curve correspond to an unstable equilibrium, and points below the curve correspond to an asymptotically, locally stable equilibrium.