J. theor. Biol. (1988) **132**, 307-318

Complex Behaviour of the Repressible Operon

SOMDATTA SINHA†

Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India

AND

RAMAKRISHNA RAMASWAMY

School of Physical Sciences, Jawaharlal Nehru University, New Delhi 110 067, India

(*Received 12 June 1985, and in revised form 15 January 1988*)

The repressor-mediated repression process in bacteria is modelled using a gene-enzyme-endproduct control unit. A combined analytical-numerical study shows that the system, though stable normally, becomes unstable for super-repressing strains even at low values of the cooperativity of repression, provided demand for the endproduct saturates at large endproduct concentrations. In addition the system also shows bistability, i.e., the co-existence of a stable steady-state and a stable limit cycle. The tryptophan operon is used as a model system and the results are discussed in the light of differential regulation of gene expression in lower organisms, especially in mutant strains.

Introduction

The process of regulation of gene expression in prokaryotic systems was put in a coherent form through the operon hypothesis (Jacob & Monod, 1961). This was originally put forward for the *lac* operon, but was also found suitable for both classical inducible and repressible systems, such as, *lac*, *trp* and *arg*, where the endproduct of a particular metabolic pathway controlled by the operon induces or represses the activity of the operon in turn. This hypothesis was subsequently given mathematical form (Goodwin, 1965, 1966) using a genetic control circuit describing a gene-enzyme-endproduct unit. Much work has been done since on this unit, involving both positive and negative feedback loops (representing inducible and repressible systems), from the point of view of its stability. Modifications have been made to introduce oscillations or instability into the system (Griffith, 1968; Rapp, 1975; Sanglier & Nicolis 1976; Goldbeter & Nicolis, 1976; Allwright, 1977; MacDonald, 1977; Tyson & Othmer, 1978). The equations have also been used to model a number of periodic phenomena observed in cellular systems (Goodwin, 1976; Bliss *et al.*, 1982; Tyson, 1983).

Both induction and repression are in many cases mediated by a small molecule (usually a protein) called the "inducer" or the "repressor". The gene for this molecule does not reside in the operon, but is controlled by the respective endproduct. These molecules have two binding sites—one for the endproduct of the pathway and the

† To whom all correspondence should be addressed.

0022-5193/88/110307+12 $03.00/0 © 1988 Academic Press Limited

other for the operator DNA. Binding of the endproduct changes the conformation of the molecule thereby influencing its binding to the operator DNA. The binding constants of these two interactions differ considerably. The kinetics of these interactions between ligands and macromolecules decide the behaviour of the system, i.e., the synthetic profile of the endproduct. It is clear that mutations at the operon or the repressor or inducer genes can influence the dynamics of the system considerably through structural changes in the molecules of interest, which then lead to changes in the kinetics.

In this paper we formulate a model for the repressible operon by considering a repressor-mediated repression process. We assume that the endproduct–repressor binding is cooperative, obeying Hill's equation, and also consider the factor of utilization of the endproduct in cellular processes, such as protein synthesis. The model system considered for these equations is the tryptophan operon for which parameter values are known (Bliss *et al.* 1982; Tyson, 1983; Schevitz *et al.*, 1985).

Our model differs from previous treatment of the repressible operon in that we use a more accurate functional representation of the repression process. This then does not permit us to assume simplifications introduced by earlier investigators (Tyson & Othmer 1978; Tyson 1983), and makes comparison with earlier work on similar systems difficult.

We have carried out a detailed analysis of this model to determine the changes in behaviour this system can show under the different conditions that arise due to mutations. Our principal findings are: (a) the wild-type operon is stable; (b) the operon is always stable if the endproduct is not utilized (i.e., $g = 0$); (c) when the endproduct is utilized, the operon can lose stability (through a Hopf bifurcation) for super-repressing strains; and (d) the system shows bistability for realistic parameter values.

The aim of the present study is to see how a typical operon that follows the given kinetic scheme behaves under changing circumstances. Kelley & Yanofsky (1985) have isolated a large number of tryptophan repressor gene (trpR) mutations in *E. coli* which have varied effect on tryptophan biosynthesis in the cell. They obtained "super-repressor" and "loose-binding" mutants in which the L-tryptophan and free repressor binding is unchanged but the holorepressor–operator binding is affected. Such results, where the two steps of the repression process are delineated, opens up the possibility of testing predictions based on our present model.

The Model

In the case of an operon concerned with the regulated biosynthesis of a particular endproduct, the temporal evolution of the system consisting of messenger RNA (M), protein or enzyme (E) and the endproduct (P) of the pathway can be represented by:

$$\frac{dM}{dt} = K_m D F(P) - K_1 M$$

$$\frac{dE}{dt} = K_e M - K_2 E$$

$$\frac{dP}{dt} = K_p E - K_d P - V_{\max} \tag{1}$$

where K_m, K_e, K_p, K_1, and K_2 are rate constants, D is the copy number of the operon (gene dosage), K_d is growth rate, and $V_{\max}$ is the constant rate of utilization of the endproduct (in protein synthesis). $F(P)$ is a function describing the transcription of the operon as regulated by the endproduct. In a system where repression of the operon is through a repressor protein, the process occurs in two steps: first, the endproduct P (i.e. co-repressor) binds to the free repressor (aporepressor R_0), and then this complex (called holorepressor R^1) binds to the operator DNA of the operon and inhibits transcription by preventing the RNA polymerase from binding or proceeding. Assuming that n molecules of endproduct bind to each repressor molecule and the interaction is allosteric in nature, the fraction of holorepressor molecules (R^1) can be obtained from the saturation function:

$$\frac{R^1}{R_t} = \frac{P^n}{K_R^n + P^n} \tag{2}$$

where R_t is the total concentration of repressor molecules in the cell ($= R_0 + R^1$) and K_R denotes the pseudo-Michaelis constant for the interaction.

This holorepressor (R^1) now can interact with the free operator (O^-) and repress the operon. The rate of transcription is directly proportional to the fraction of free operators, that is to

$$\frac{O^-}{O_t} = \frac{1}{1 + KR^1} = \frac{1}{1 + \frac{R^1}{K_0}} \tag{3}$$

where O_t is the total number of operators (free and bound) and K (K_0) is the association (dissociation) constant for the operator–holorepressor interaction. Using eqn (2), we find that:

$$F(P) = \frac{O^-}{O_t} = \frac{K_0(K_R^n + P^n)}{K_0 K_R^n + (K_0 + R_t)P^n} = \left(\frac{\gamma}{1+\gamma}\right)\frac{K_R^n}{K_R^n + (1+\gamma)P^n} + \frac{1}{1+\gamma} \tag{4}$$

where $\gamma = R_t / K_0$, a dimensionless number.

This repression function $F(P)$ differs from those used earlier (Goodwin, 1965; Tyson & Othmer, 1978; Bliss *et al.*, 1982; Tyson, 1983) owing to the kinetics of the two distinct steps of the repression process. The form of the repression function (equation (4)) does not permit any further simplifying assumption such as $R_t \approx R_0$ (Tyson & Othmer, 1978).

The first equation in (1) can now be written as:

$$\frac{dM}{dt} = \left(\frac{DK_m \gamma K_R^n}{1+\gamma}\right)\frac{1}{K_R^n + (1+\gamma)P^n} + \frac{DK_m}{1+\gamma} - K_1 M. \tag{5a}$$

The enzyme and endproduct equations are:

$$\frac{dE}{dt} = K_e M - K_2 E \tag{5b}$$

$$\frac{dP}{dt} = K_p E - K_d P - V_{max}. \tag{5c}$$

It is convenient for analytical purposes to write the above equations in dimensionless form. Changing variables as:

$$x = \frac{M}{M_0}, \quad y = \frac{E}{E_0}, \quad z = \frac{P}{P_0}, \quad T = \frac{t}{t_0}$$

where

$$M_0 = \left(\frac{K_R}{K_e K_p t_0^2}\right), \quad E_0 = \frac{K_R}{K_p t_0}, \quad P_0 = K_R, \quad t_0 = \left(\frac{K_R}{D K_m K_e K_p}\right)^{1/3}$$

$$\alpha_1 = K_1 t_0, \quad \alpha_2 = K_2 t_0, \quad \alpha_3 = K_d t_0 \quad \text{and} \quad g = V_{max} t_0 / K_R,$$

the equations (5a, b, c) then become:

$$\frac{dx}{dT} = \left(\frac{\gamma}{1+\gamma}\right)\frac{1}{1+(1+\gamma)z^n} - \alpha_1 x + \frac{1}{1+\gamma}$$

$$\frac{dy}{dT} = x - \alpha_2 y \tag{6}$$

$$\frac{dz}{dT} = y - \alpha_3 z - g.$$

Our choice of scaling leaves all the γ (or K_0) dependence complete and explicit in the repression factor alone. This not only allows us to study the behaviour of the system under variation of γ, but also helps us to use the same values for the dimensionless parameters α_1, α_2, α_3 and g used earlier which were obtained experimentally for the tryptophan operon (Tyson, 1983). Non-dimensionalizing the set of equations reduces the number of independent parameters to 5.

The steady-states and the eigenvalue equation in the linear approximation are given by:

$$\bar{x} = \alpha_2 \alpha_3 \bar{z} + \alpha_2 g, \qquad \bar{y} = \alpha_3 \bar{z} + g \qquad \text{(7a) and (7b)}$$

$$\alpha_1 \alpha_2 \alpha_3 (1+\gamma)\bar{z}^{n+1} + \{\alpha_1 \alpha_2 (1+\gamma) g - 1\}\bar{z}^n + \alpha_1 \alpha_2 \alpha_3 \bar{z} + (\alpha_1 \alpha_2 g - 1) = 0 \tag{7c}$$

and

$$P(\lambda) = \lambda^3 + (\alpha_1 + \alpha_2 + \alpha_3)\lambda^2 + (\alpha_1 \alpha_2 + \alpha_2 \alpha_3 + \alpha_3 \alpha_1)\lambda + (\alpha_1 \alpha_2 \alpha_3 + B) = 0 \tag{8}$$

where

$$B = \frac{\gamma n \bar{z}^{n-1}}{\{1+(1+\gamma)\bar{z}^n\}^2} > 0.$$

To get an idea of the conditions at which instability can occur we choose a combined analytical-numerical procedure. For a Hopf bifurcation to occur, a pair

of eigenvalues must cross the imaginary axis with the third eigenvalue being real and negative (Marsden & McCracken, 1976). Hence, we assume that at critical values of the parameters, the set of three zeroes of eqn (8) consists of a pair of pure imaginary conjugate and one real negative root. Thus $P(\lambda)$ in eqn (8) must be of the form (with r and η positive real numbers).

$$P(\lambda)=(\lambda-r)(\lambda-i\eta)(\lambda+i\eta)$$

or

$$\lambda^3+r\lambda^2+\eta^2\lambda+r\eta^2=0. \tag{9}$$

Equations (8) and (9) are identical only when

$$r=(\alpha_1+\alpha_2+\alpha_3), \qquad \eta^2=\alpha_1\alpha_2+\alpha_2\alpha_3+\alpha_3\alpha_1>0, \quad \textit{and} \quad r\eta^2=\alpha_1\alpha_2\alpha_3+B$$

or

$$\eta^2=\alpha_1\alpha_2+\alpha_2\alpha_3+\alpha_3\alpha_1=\frac{\alpha_1\alpha_2\alpha_3+B}{\alpha_1+\alpha_2+\alpha_3}>0. \tag{10}$$

The necessary and sufficient condition for there to be two pure imaginary and one real eigenvalue is:

$$(\alpha_1\alpha_2+\alpha_2\alpha_3+\alpha_3\alpha_1)(\alpha_1+\alpha_2+\alpha_3)-\alpha_1\alpha_2\alpha_3=(\gamma n\bar{z}^{n-1})/\{1+(1+\gamma)\bar{z}^n\}^2. \tag{11}$$

We consider only positive z roots.

We use expressions (7c) and (11) to find values of γ (for different values of g) at which bifurcation may occur. Reasonable values of the parameters are chosen from the tryptophan operon system. The tryptophan repressor protein is a dimer and binds two molecules of L-tryptophan per dimer (Schevitz *et al.*, 1985); so $n=2$. The value of γ for a wild-type strain is 10 (K_0 and R_t are about 10^{-10} mol/litre and 10^{-9} mol/litre) (Platt, 1978). Since the scaling used by us and Tyson (1983) are the same (K_R = pseudo-Michaelis constant describing the binding of co-repressor P to aporepressor $R_0=6\times10^{-5}$ mol/litre), we use $\alpha_1=1{\cdot}0$, $\alpha_2=\alpha_3=0{\cdot}01$, and $g=4$. The system was studied for a large range of parameter values around the basal values.

Results

It is clear from eqn (4) that as P increases from zero to infinity, the free operator regions decrease from 1 to $1/(1+\gamma)$. Transcription of the wild-type strain is 90% inhibited at large values of endproduct concentration. "Super-repressing" strains (for which $\gamma>10$) will show even more inhibition, and "loose-binding" strains (for which $\gamma<10$) will show noticeably incomplete inhibition even at large endproduct concentrations. We now discuss results of the variation of different parameters on the system.

(A) BEHAVIOUR OF THE SYSTEM FOR $g=0$

When $g=0$ the system never loses its stability, even under large variations in the parameter values. This is in accord with earlier observations. It was shown by Bliss *et al.* (1982) and Tyson (1983) that introduction of g destabilizes the system in these ranges of parameter values. In our model, the search was done for $0{\cdot}001<\alpha_1, \alpha_2, \alpha_3<10$, $1<n<10$ for γ up to 10^6 varying one parameter at a time and keeping the others at basal values. Equation (11) was not satisfied in this range. It is thus clear that for an operon to be unstable for realistic parameter values, g must be greater than zero. Note that Tyson's model (1983) had stable steady-state for $n>208$ (!) for $g=0$ for same parameter values. Tyson & Othmer (1978) in their model (with different kinetics of the repression process) find instability for low values of n (i.e., $n\geq 8$), but under the stringent condition of $\alpha_1=\alpha_2=\alpha_3$. They also note that for unequal parameters, the minimum value of n necessary for instability increases dramatically.

(B) BEHAVIOUR OF THE SYSTEM FOR $g>0$

(i) Normal operon

For normal values of the parameters, condition (11) is never satisfied, so the normal operon is always stable in nature. Table 1 gives results of the study of the strain having wild-type γ value (i.e., $\gamma=10$), but under different values of α_1, α_2 and α_3. For both lower and higher values of g ($g=1$ and 5) the operon is mostly stable, except when $0{\cdot}01<\alpha_2<0{\cdot}05$ for $g=5$. This shows that increasing α_2 can destabilize the normal operon for reasonable values of g.

It was also shown by Tyson (1983) that in his model the operon corresponding to our wild-type operon ($n=2$, $g=4$) is stable. Direct comparison with earlier results is not straightforward since they differ in their underlying mechanisms.

TABLE 1

Effect of n, α_1, α_2 and α_3 on the stability of the normal and super-repressor strains. The basal values of the parameters are $n=2$, $\alpha_1=1$, $\alpha_2=0{\cdot}01$, $\alpha_3=0{\cdot}01$

Parameter changed	γ	g	Bifurcation value (range)
$1<n<5$	10	1, 5	nil
$0{\cdot}001<\alpha_1<20$	10	1, 5	nil
	100	5	$0{\cdot}1<\alpha_1<0{\cdot}5$
$0{\cdot}001<\alpha_2<10$	10	1	nil
		5	$0{\cdot}01<\alpha_2<0{\cdot}05$
	100	3	$0{\cdot}001<\alpha_3<0{\cdot}01$
$0{\cdot}001<\alpha_3<10$	10	1, 5	nil
	100	3	$0{\cdot}01<\alpha_3<0{\cdot}1$

(ii) Strains with altered repressor–operator binding

In the first part of this section we saw that the normal (or wild-type) operon is generally stable. Under similar conditions the loose-binding strains ($\gamma < 10$) also remained stable. But the system showed the possibility of losing stability through a Hopf bifurcation for the super-repressing strains. Figure 1 shows the bifurcation loci (in g–γ parameter space) for normal and high values of co-operativity of repression. The other parameters were kept at the basal values. This locus is drawn using eqns (11) and (7c). Systems residing above the loci are unstable and the ones below are stable. It is clear that changing n does not make a significant difference in the shape of the locus. It is also clear that for the bifurcation to occur at normal values of g (i.e., $g = 4$), the strain should be super-repressing ($\gamma \approx 29$). In short, instability can take place for wild-type and loose-binding strains as well, if they possess high demand for endproduct utilization. Figure 2a–c shows the temporal behaviour of the normal strain ($\gamma = 10$) for normal and higher values of endproduct utilization (g). The system evolves to the stable steady-state exponentially (Fig. 2a) and with damped oscillation (Fig. 2b) when $g = 4$ and $g = 10$. But for $g = 12$, the system shows a stable periodic pattern of synthesis (Fig. 2c). Table 1 also shows that instability can be obtained for lower than normal values of g and altered values of α's for super-repressing strains.

(iii) Bistability

Having determined the bifurcation locus in the g–γ plane, we simulated the system around the critical parameter set to observe the temporal pattern of the system. The system was integrated close to the steady-state and the stability of the

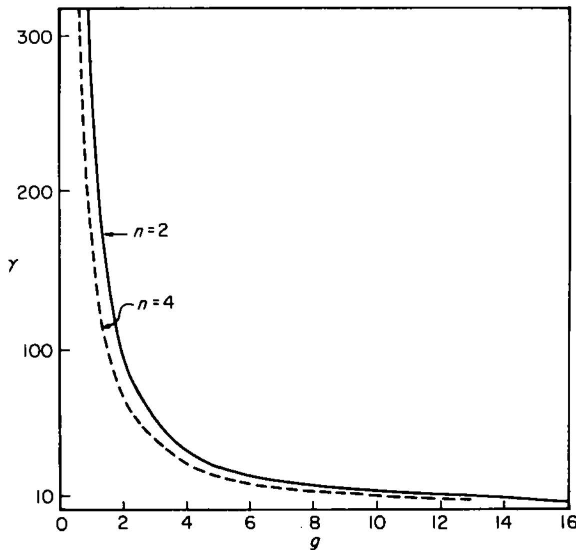

FIG. 1. Bifurcation locus in $g - \gamma$ parameter space for $n = 2$ and 4. The other parameters were kept at the basal values, i.e., $\alpha_1 = 1$, $\alpha_2 = 0{\cdot}01$, $\alpha_3 = 0{\cdot}01$.

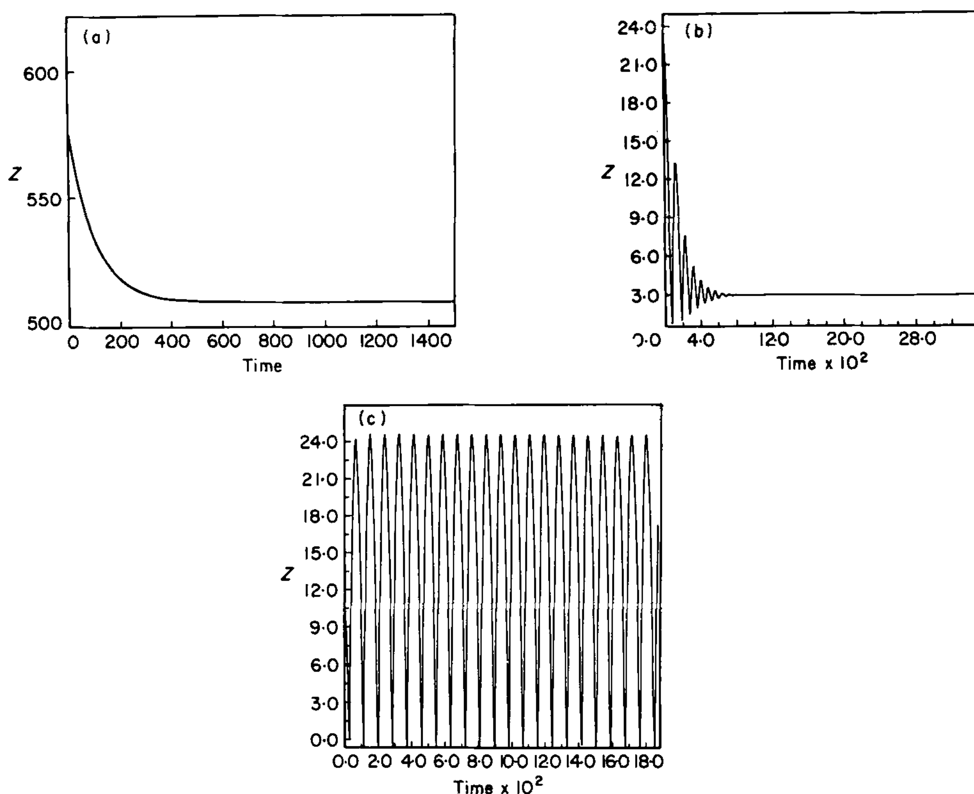

FIG. 2. Behaviour of the normal ($\gamma = 10$) operon at $g = 4$ (a), $g = 10$ (b), $g = 12$ (c) showing stable, damped and periodic pattern of endproduct synthesis on perturbation. Here $n = 2$.

steady-state was obtained from the corresponding eigenvalues of the linearized system. It was observed that this system shows bistability close to the bifurcation value at stable steady-states. There is coexistence of a stable steady-state and a limit cycle for the same parameter values.

It is known from Fig. 1 that the bifurcation value of γ for $g = 3$ is between 55 and 60. We made an exhaustive numerical search of the system behaviour around the bifurcation value to describe the phenomena of bistability. The steady-states for the values of γ studied were almost constant, except for $\bar{z}$ being marginally dependent on γ ($\bar{z}$ decreased from 2·02 to 1·08, $\bar{x}$ being 0·03, and $\bar{y}$ changing from 3·01 to 3·02 when γ increased from 40 to 60). The eigenvalues consisted of one real negative and pair of complex conjugate roots whose real part decreased when γ was changed from 40 to 55 and became positive when $\gamma = 60$. The imaginary part was finite. Figure 3a shows the time evolution of z at $\gamma = 40$ when perturbed. The steady-state was globally attracting here. A pair of stable and unstable limit cycles appeared as γ was increased. $\gamma = 45$ in Fig. 3b and c shows the bistable behaviour where although the system is attracted towards the stable steady-state in an oscillatory manner (Fig. 3b) when perturbed close, it also evolved to a limit cycle (Fig. 3c) when the

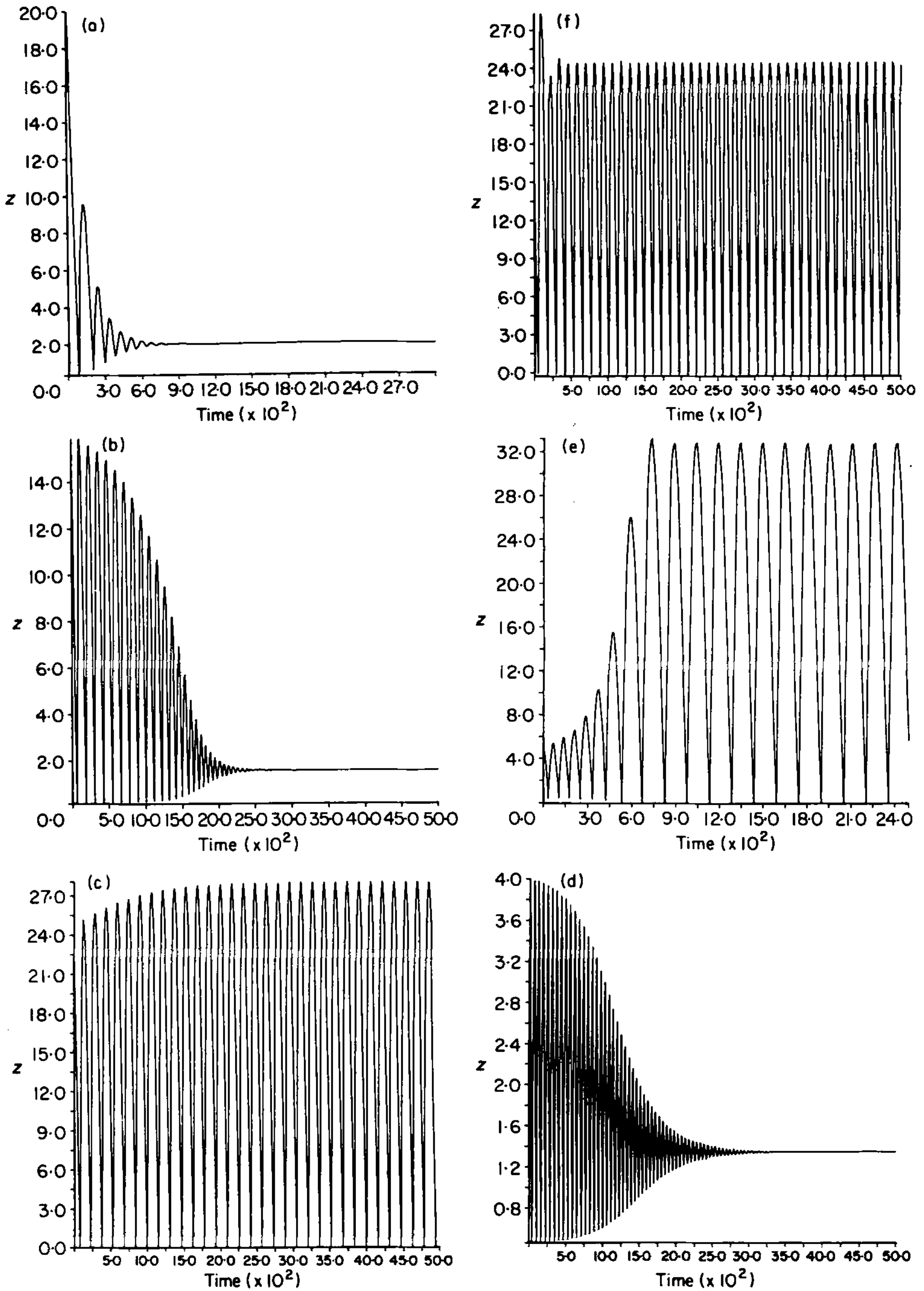

FIG. 3. Bistability studies in a super-repressing strain. (a) $\gamma = 40$, (b) $\gamma = 45$, $z = 15$, (c) $\gamma = 45$, $z = 22$, (d) $\gamma = 50$, $z = 4$, (e) $\gamma = 50$, $z = 5$, (f) $\gamma = 60$. The other parameters were kept at the basal value and the respective steady state values of x and y were used.

perturbation was stronger. On increasing the value of γ ($\gamma=50$), the region of attraction became smaller, i.e., the size of the unstable limit cycle reduced with small reduction in the time period and amplitude of the stable oscillation (see Fig. 3d and e). At $\gamma=55$, the unstable limit cycle had almost collapsed to the stable steady-state; real λ is a very small negative number ($\approx 10^{-4}$ here), keeping the stable limit cycle intact. When perturbed very close to the steady-state, it oscillated with an amplitude of 0·014 (in z) for more than 5000 time units without showing any indication of asymptoting to the stable limit cycle. For $\gamma=60$ the steady-state is unstable and the system evolves to the limit cycle of period, approximately 119 units, and an amplitude of 24·4 units (Fig. 3f). The above behaviour of the system around the bifurcation value, clearly shows that the system loses its stability through a Hopf bifurcation of subcritical type, since the system loses its stability through the disappearance of an unstable limit cycle. On increasing γ further, the limit cycle disappears and the system spirals out of the steady-state. In fact, from eqn (6) it is clear that, as $z(T)\to 0$, the system becomes unbounded. To ensure the existence of a limit cycle, whenever the steady-state is unstable, "g" may be replaced with a hyperbolic (Michaelis-Menten) function of z.

Discussion

In this study we have formulated a general model for the bacterial repressible operon system which can describe the behaviour of super-repressing and loose-binding strains. We have shown that even a simple genetic control circuit with a single negative feedback loop is capable of showing bistable behaviour under realistic parameter values. We have shown that though the wild-type strain is always stable, it can be made unstable by changing other parameters, for example by increasing the rate of utilization of the end-product or by increasing the stability of the endproduct.

As stated before, the earlier models of the repressible operon (Goodwin, 1966; Tyson & Othmer, 1978) simplified the repression mechanism and also did not consider the endproduct utilization as an important parameter. Later (Bliss *et al.*, 1982; Tyson, 1983) similar models were used to describe the behaviour of the *trp* operon where the repression process is actually repressor-mediated. Though these models used the simplified repression mechanism, they took into account the feedback inhibition of enzyme activity in the tryptophan metabolic pathway and time delays in the transcription and translation processes. They showed (both theoretically and experimentally) that the stable normal operon loses stability if the feedback inhibition of the enzyme activity is reduced. Later, Tyson (1983) simplified the model and showed that instability can occur at normal parameter values without considering the time delays and enzyme inhibition—the endproduct utilization term is sufficient to induce oscillations.

The existence of bistability in our model is interesting from the point of view of its functional implications. Analogous situations have been observed in chemical reactions in continuously-stirred-flow-reactors (Epstein *et al.*, 1981; Papsin *et al.*

1981; Dateo *et al.*, 1982, Alamgir *et al.*, 1983). Its advantage in a living cell may have some consideration of regulating its behaviour against changes in the internal pool size due to external stimuli. The fact that a variety of behaviour such as stable, bistable and periodic pattern of synthesis of the endproduct can be achieved by varying a control parameter, such as the degradation rate of the endproduct, points towards the possibility that if such a parameter is developmentally regulated or if the parameter changes with the life cycle of the organism, then the operon expression can also change its pattern. This could be one of the possible mechanisms which regulate differential expression of genes; however, such a pattern has not been seen experimentally yet.

Our model can also be used to study the unlinked operon systems in a cell controlled by the same repressor and endproduct such as the *trp* repressor of *E. coli* (Kelley & Yanofsky, 1982). In such cases, the same active repressor (i.e., K_R being the same) binds to different operons with different K_0 leading to different functional responses of those operons to the same concentration of endproduct (Kelley & Yanofsky, 1982).

In the last few years, isolation of many mutant strains possessing the required properties have given the possibility of testing the results of our model. Biochemical and mutational studies of tryptophan repressor-operator interaction (Kelley & Yanofsky, 1985) have yielded a number of *trp* repressor gene mutants giving rise to both super-repressing and loose-binding properties. They report of strains where the *trp* repressor protein has been altered in such a way that only the repressor-operator interaction is altered without affecting the endproduct-free repressor interactions. Tryptophanase (−) mutants (altered α_3) are also available (Aiba *et al.*, 1982). To offer a measure of translational control, mRNAs of different stabilities (different α_1) are known (Britz & Demain, 1985). The parameter "g" can be altered by changing the level of charged t-RNA for the respective amino acids in the cell. Construction of strains incorporating the necessary mutations can allow us to examine the mathematical results of this model.

We thank the referees for suggestions which have substantially improved this paper. We also thank Mr. Mohan Kumar and Mr. Giridharan for their secretarial help.

REFERENCES

AIBA, S., TSUNEKAWA, H. & IMANAKA, T. (1982). *Appl. Environ. Microbiol.* **43**, 289.

ALAMGIR, M., DE KEPPER, P., ORBAN, M. & EPSTEIN, I. R. (1983). *J. Am. Chem. Soc.* **105**, 2641.

ALLWRIGHT, D. J. (1977) *J. Math. Biol.* **4**, 363.

BLISS, R. D., PAINTER, P. R. & MARR, A. G. (1982). *J. theor. Biol.* **97**, 177.

BRITZ, M. L. & DEMAIN, A. L. (1985). In: *Comprehensive Biotechnology*, Vol. 1. (Moo-Young, M., ed.) p. 617. Oxford: Pergamon.

DATEO, C. E., ORBAN, M., DE KEPPER, P. & EPSTEIN, I. R. (1982). *J. Am. Chem. Soc.* **104**, 504.

EPSTEIN, I. R., DATEO, C. E., DE KEPPER, P., KUSTIN, K. & ORBAN, M. (1981). In: *Nonlinear Phenomena in Chemical Dynamics* (Vidal, C. & Pacault, A., eds) Berlin: Springer-Verlag.

GOLDBETER, A. & NICOLIS, G. (1976). *Progr. Theor. Biol.* **4**, 65.

GOODWIN, B. C. (1965). *Adv. Enz. Regul.* **3**, 425.

GOODWIN, B. C. (1966). *Nature* **209**, 479.

GOODWIN, B. C. (1976). *Analytical Physiology of Cells and Developing Organisms.* London: Academic Press.

GRIFFITH, J. S. (1968). *J. theor. Biol.* **20**, 202.
KELLEY, R. D. & YANOFSKY, C. (1982). *Proc. Natl. Acad. Sci., U.S.A.* **79**, 3120.
KELLEY, R. D. & YANOFSKY, C. (1985). *Proc. Natl. Acad. Sci., U.S.A.* **82**, 483.
JACOB, F. & MONOD, J. (1961). *J. Mol. Biol.* **3**, 318.
MACDONALD, N. (1977). *J. theor. Biol.* **67**, 549.
MARSDEN, J. & MCCRACKEN, M. (1976). *The Hopf Bifurcation and its Applications.* New York: Springer-Verlag.
PAPSIN, G. A., HANNA, A. & SHOWALTER, K. (1981). *J. Phys. Chem.* **85**, 2575.
PLATT, T. (1978). In: *The Operon* (Miller, J. H. & Reznikoff, W. S., eds) Cold Spring Harbor.
RAPP, P. E. (1975). *Math. Biosci.* **25**, 165.
SANGLIER, M. & NICOLIS, G. (1976). *Biophys. Chem.* **4**, 113.
SCHEVITZ, R. W., OTWINOWSKI, Z., JOACHIMIAK, A., LAWSON, C. L. & SIGLER, P. B. (1985). *Nature* **317**, 782.
TYSON, J. J. & OTHMER, H. G. (1978). *Prog. Theor. Biol.* **5**, 1.
TYSON, J. J. (1983). *J. theor. Biol.* **103**, 313.