

Mathematics of Cellular Control Processes

I. Negative Feedback to One Gene

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(Received 24 January 1968)

Repression of mRNA synthesis is discussed for situations in which the repressor is either the protein encoded by the mRNA or a metabolite formed under the catalytic control of that protein. Following Goodwin (1965), plausible physicochemical equations are set up. They contain a non-linear element. Standard methods of the theory of non-linear equations are used to determine the properties of these equations for general values of the parameters contained therein. Undamped oscillations in the concentrations of the components can never occur for any values of the parameters when the repression is accomplished by the protein. Such oscillations are possible when repression is due to the metabolite, but only when there is a co-operative repression of such a high order as to be unlikely in practice.

1. Introduction

Considerable interest exists in the control equations involving protein synthesis and the regulation of mRNA production. Two questions particularly have arisen. One asks in what circumstances stable oscillations in the concentrations of the components can occur? (Goodwin, 1963, 1965). The other asks when a particular arrangement of control interactions can lead to more than one possible stable, i.e. enduring, set of concentrations. The second question is especially important in relation to the mechanism of differentiation, proposed by Monod & Jacob (1961), and subsequently used as a basis for explanatory hypotheses about various biological problems (Bonner, 1965; Roberts & Flexner, 1966; Griffith, 1967*a,b*).

One theoretical method of tackling these questions is by means of computer simulations. However, simulations can only be run for certain chosen values of the parameters in the control equations and, although these may range over many choices, they obviously cannot work through all of them. Hence it is desirable, as far as this is possible, to complement these simulations with general mathematical proofs of the existence or non-existence of oscillations

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or other features for wide classes of equations. The equations concerned are non-linear and this fact increases the mathematical difficulties enormously. Nevertheless we shall find that useful information can be obtained by applying standard methods of the theory of non-linear equations. We shall consider several simple and representative examples and start, in this paper, with the simplest kind of self-repressing, or negative feedback, system. In it, a single gene G produces a mRNA, M , which codes for a protein E . Either this protein, or a metabolite, P , formed in a reaction catalysed by E , acts as a repressor for the same gene G . This situation has been discussed previously by Goodwin (1963, 1965) and Maynard Smith (1965), and very similar equations, in more variables, to the ones we shall use have appeared in the work of Walter (1968) on enzyme-catalysed sequences of reactions.

2. The Equations

Following Goodwin (1963, 1965) we shall suppose the repressor, R , combines with the gene, G , according to the equation

$$G + mR = GR_m \quad (1)$$

and that G , but not GR_m , is active for the production of mRNA. R may be either E or P . Current experimental evidence on repression suggests that the case $m = 1$ will prove the most important (Koch, 1967), but we shall make our analysis for other values of m also. It follows from equation (1) that the proportion of time G is active is given by

$$p = \frac{1}{1 + Kx^m} \quad (2)$$

where x is the concentration of R , and K is the equilibrium constant of equation (1). Equation (2) becomes inaccurate near $x = 0$ (see Koch, 1967), but we shall be interested primarily in the behaviour of the control equations away from $x = 0$ and so shall neglect that fact here.

If we assume we can write ordinary macroscopic equations for the concentrations of mRNA and protein then, in the case that E is the repressor, we shall take them as

$$\begin{aligned} \dot{M} &= \frac{dM}{dt} = \frac{a}{1 + KE^m} - bM \\ \dot{E} &= \frac{dE}{dt} = cM - dE, \end{aligned} \quad (3)$$

where a, b, c, d are positive constants. Equations (3) are of the type discussed by Goodwin (1965) and assume that the rate of messenger production is proportional to the fraction of time G is active, and that the protein is synthesized at a rate proportional to the amount of messenger present.

M and E decay in proportion to their concentrations, which must surely often happen, even if it is not always so. It follows at once from equations (3) that if, initially, $M \geq 0$ and $E \geq 0$, then they both remain non-negative subsequently. This is obviously necessary, because of the physical significance of M and E , and in the mathematical analysis which follows, we shall not concern ourselves with negative values for them.

Equations (3) contain a large number of constants and in a mathematical analysis it is desirable to simplify them as much as we can do without thereby reducing the generality of the problem. We do this by a change of units for each of M , E and t . After a little simple algebra, it is apparent that the units can be chosen so that

$$\begin{aligned}\dot{M} &= \frac{1}{1+E^m} - \alpha M \\ \dot{E} &= M - \beta E\end{aligned}\tag{4}$$

without loss of generality. α and β are both positive constants. The analogous equations to (3), which arise when the metabolite P acts as the repressor, can similarly be shown to be reducible to the form

$$\begin{aligned}\dot{M} &= \frac{1}{1+P^m} - \alpha M \\ \dot{E} &= M - \beta E \\ \dot{P} &= E - \gamma P\end{aligned}\tag{5}$$

with α , β and γ positive.

Goodwin (1963) has remarked that a two-variable equation may be regarded as a limiting case of a three-variable one. Suppose γ is very large in equation (5). Then P will change much more rapidly than M or E and will usually be close to its "equilibrium" value for fixed E , i.e. $P = \gamma^{-1}E$. This enables us to simplify equations (5) by eliminating P and thus reducing them to the form (3) which can then, by a change of units, be transformed to the form (4). Evidently we can go further and, in (4), let β be large. This suggests considering, together with (4) and (5), the one-variable equation

$$\dot{M} = \frac{1}{1+M^m} - \alpha M.\tag{6}$$

In this equation, as M runs from 0 to ∞ so $(1+M^m)^{-1}$ runs monotonically from 1 to 0. Hence $\dot{M} = 0$ for a unique value $M = M_0$ satisfying

$$\alpha M_0(1+M_0^m) = 1.\tag{7}$$

When $M < M_0$, $\dot{M} > 0$ and when $M > M_0$, $\dot{M} < 0$. Hence $M \rightarrow M_0$, whatever the initial value of M .

3. The Two-variable Case

Here we use standard techniques of the theory of non-linear equations (see, e.g. Leimanis & Minorsky (1958); Andronov, Vitt & Khaikin (1966). First we set $\dot{M} = \dot{E} = 0$ in equations (4) to obtain the stationary points. They occur when M and E satisfy

$$\begin{aligned} M &= \beta E, \\ \alpha \beta E(1 + E^m) &= 1. \end{aligned} \quad (8)$$

As with equation (7) there is just one solution and so just one stationary point, (M_0, E_0) say. We expand near this point by writing $M = M_0 + X$, $E = E_0 + Y$ and obtain

$$\begin{aligned} \dot{X} &= -m\alpha^2\beta^2E_0^{m+1}Y - \alpha X + O(Y^2), \\ \dot{Y} &= X - \beta Y. \end{aligned} \quad (9)$$

E_0 , α and β are all positive and therefore the point satisfies the conditions for stability for all values of the parameters (for these conditions see Leimanis & Minorsky (1958, p. 121).

We conclude the analysis by showing that there are no limit cycles and that the motion in the phase plane (the M, E plane) contains no trajectories which go off towards infinity. Both these are easy. The quantity

$$\frac{\partial}{\partial M}(\dot{M}) + \frac{\partial}{\partial E}(\dot{E}) = -\alpha - \beta,$$

has constant sign everywhere which allows us to apply the Bendixson criterion (Andronov *et al.*, 1966, p. 305) which says that it follows that no motion can describe a closed trajectory in the phase plane. So there are no limit cycles and hence no stable oscillations for any values of α , β and m . The behaviour towards infinity is treated by observing that the motion is entirely inwards across the boundaries of the rectangle having vertices $(0, 0)$, $(0, A)$, $(\beta A, 0)$, $(\beta A, A)$ for any A satisfying $\alpha\beta A > 1$.

It is interesting to refer here to the equations discussed by Goodwin (1963), which are of the form

$$\begin{aligned} \dot{M} &= \frac{1}{1 + E^m} - \alpha, \\ \dot{E} &= M - \beta. \end{aligned} \quad (10)$$

He showed that these give oscillatory behaviour of arbitrary amplitude. These oscillations are of indifferent stability (Leimanis & Minorsky, 1958, p. 129) rather than limit cycles. However, equations (10) are not satisfactory when E and M are small because if we start the system with $M = E = 0$ we find $\dot{M} = 1 - \alpha$, $\dot{E} = -\beta$ and so E subsequently becomes negative, which

is physically unacceptable. Hence the best we can do is to replace β with some function $f(E)$ such that $f(E) \approx \beta$ for E large, but $\lim_{E \rightarrow 0} f(E) = 0$. If $f(E)$ is also a monotonic function of E with $f'(E) > 0$, for all E , we may apply Bendixson's criterion to show there can be no stable oscillations. However, there may still be damped oscillations with very slow decay of amplitude.

4. The Three-variable Case

Again, there is a unique stationary point (M_0, E_0, P_0) , where

$$\begin{aligned} M_0 &= \beta E_0, \\ E_0 &= \gamma P_0, \\ \alpha \beta \gamma P_0 (1 + P_0^m) &= 1. \end{aligned} \quad (11)$$

The stability of this point is discussed by expanding about it by writing $M = M_0 + X$, $E = E_0 + Y$, $P = P_0 + Z$. Let us write R for the column vector (X, Y, Z) and retain only the first order terms in X, Y and Z . We readily find $\dot{R} = QR$, where Q is the matrix

$$Q = \begin{bmatrix} -\alpha & 0 & \phi m(\phi P_0 - 1) \\ 1 & -\beta & 0 \\ 0 & 1 & -\gamma \end{bmatrix}$$

and we have set $\phi = \alpha\beta\gamma$. The point is stable if and only if the real parts of all the eigenvalues of the matrix Q are negative. To test this, we use Hurwitz's criteria (Uspenski, 1948) applied to the characteristic equation, which is

$$(E + \alpha)(E + \beta)(E + \gamma) + \phi m(1 - \phi P_0) = 0. \quad (12)$$

Hurwitz's criteria applied to a cubic equation

$$E^3 + p_2 E^2 + p_1 E + p_0 = 0$$

are satisfied if and only if $p_1 > 0$ and $p_1 p_2 - p_0 > 0$. As $p_1 = \alpha\beta + \beta\gamma + \gamma\alpha$, it is evidently positive. The other condition reads

$$D \equiv (\sum \alpha)(\sum \alpha\beta) - \phi - \phi m(1 - \phi P_0) > 0 \quad (13)$$

where

$$\sum \alpha = \alpha + \beta + \gamma \quad \text{and} \quad \sum \alpha\beta = \alpha\beta + \beta\gamma + \gamma\alpha.$$

We now use the well-known result (Hardy, Littlewood & Pólya, 1952) that for all positive α, β, γ

$$\frac{1}{3} \sum \alpha \geq (\frac{1}{3} \sum \alpha\beta)^{\frac{1}{2}} \geq (\alpha\beta\gamma)^{\frac{1}{3}},$$

and equality holds only if $\alpha = \beta = \gamma$. Hence

$$(\sum \alpha)(\sum \alpha\beta) \geq \sqrt{3}(\sum \alpha\beta)^{3/2} \geq 9\alpha\beta\gamma. \quad (14)$$

So we have

$$D \geq 8\phi - \phi m(1 - \phi P_0) = \phi(8 - m) + m\phi^2 P_0 \quad (15)$$

and therefore $D > 0$, and thus the point is stable, for $m \leq 8$ and any values of α, β, γ .

On the other hand, when $m > 8$, we can always find values of α, β and γ which make D negative and the stationary point unstable. For if we let $\alpha = \beta = \gamma$, equations (14) and (15) become equalities, and we then require

$$\phi P_0 < 1 - 8m^{-1}, \phi P_0(1 + P_0^m) = 1. \quad (16)$$

Now as

$$\phi \rightarrow 0, \text{ so } P_0 \rightarrow \infty \text{ and } \phi P_0 = (1 + P_0^m)^{-1} \rightarrow 0,$$

so this can always be done by taking ϕ small enough.

No trajectories can go off to infinity. This is easily seen by using equations (5) to show that all motion is inwards across the faces of the rectangular box which has its sides parallel to the axes of co-ordinates and two opposite vertices at $(0, 0, 0)$ and $(\beta\gamma A, \gamma A, A)$, where $\alpha\beta\gamma A > 1$.

The foregoing results indicate strongly that there will be one limit cycle whenever $m > 8$ and $D < 0$, and none in any other circumstances. I have been unable to establish rigorously that there are none under the latter conditions. However, we have run a large number of simulations on a digital computer, with various choices for the parameters, in complete agreement with the predictions. In these simulations, whenever an oscillation has been found, it has been carefully checked that it is really a limit cycle and it has been observed to be unique. The present results are inconsistent with the report of Goodwin (1965), who claimed to have found a limit cycle for $m = 1$ and certain values of α, β and γ . He informs me, however, that he now considers his result to have arisen erroneously out of errors in the analogue simulation which he employed.

Our mathematical formulation has depended upon equations (1) and (2), which do not represent the only ways in which repression could occur. The inclusion of an aporepressor in the scheme, or replacing equation (2) with the more complicated one suggested in Koch's (1967) work would greatly complicate the analysis. However, the absence of limit cycles in the two-variable case should still hold, whilst in the three-variable case it would be surprising if limit cycles appeared for very low values of m . Thus the present work must be regarded as casting serious doubt on the possibility that negative feedback from a product of a single gene can ever give rise in practice to undamped oscillations in the concentrations of cellular constituents. It has not, however, discussed at all the question of whether such oscillations might arise in systems in which two or more genes are inductively or repressively coupled.

The author wishes to thank Mrs C. J. Mufti for writing the computer programs used for the simulations referred to in this and the following paper. They were run on the Atlas Computer of the University of London.

The work was supported by a grant from the Science Research Council.

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