Sankhyā : The Indian Journal of Statistics
1987, Volume 49, Series B, Pt. 1, pp. 51-67

A STOCHASTIC MODEL OF GENE ACTIVATION AND RNA SYNTHESIS DURING EMBRYOGENESIS

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SUMMARY. A stochastic formulation for gene activation and transcription during embryonic development is presented and analysed. The model is based on the axiomatic fundamentals of the physiological characteristics and leads to a non-linear bivariate Markov process. The time-dependent approximate solutions of the moments are obtained from the master equation describing the biological process. Conditions for a stable periodic solution which is biologically significant have been worked out and fluctuations have been measured. In the absence of the product term in the model it is shown that one of the two variates exhibits normal distribution. This indicates that both the variables have approximately normal distributions. The investigation explores the advantages of stochastic modelling of a nonlinear biological system which is deterministically intractable, as well as less informative.

1. Introduction

Rhythmic biosynthesis of various macromolecules during mitotic cycles is an important biological phenomenon. Cyclic RNA synthesis in slime mould cells has been observed by Cummins and Rusch (1968) and a rhythmic protein biosynthesis in early cell cycles in sea urchins has been reported by Mano (1968). Mazia (1961) and Brodsky (1968) have presented excellent reviews on these rhythmic biosynthesis of RNA, protein and other macromolecules. Brahmachary *et al.* (1971) have also observed cyclic RNA synthesis during early mitotic cycles in *Limnaea*.

A number of mathematical models of cellular development have been contributed by several workers such as Weiss and Kavanau (1957), Goodwin (1963), Simon (1973), Wheldon (1973), Cummings (1975), Alberghina (1975), Thames and Elster (1976) and many others. A simple model showing oscillatroy biosynthesis of RNA and protein has been presented by Tapaswi and Roy (1978). By constructing an extended mathematical model of RNA and protein synthesis during embryonic development Tapaswi and Bhattacharya (1981) have demonstrated stable periodic (limit cycle) solutions for the entire biological system of transcription (i.e., RNA synthesis) and translation (i.e., protein synthesis). The general model of RNA and protein

AMS (1980) *subject classification :* 78A70

Key words and phrases : Stochastic model, Gene activation, Transcription and translation, Limit cycle oscillations, Normal approximation, Monte Carlo simulation.

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synthesis using a feedback mechanism in which the end product acts as a repressor molecule was discussed by Goodwin (1963, 1965), Griffith (1968), Walter (1970), Rapp (1957a, b, 1976), Tyson and Othmer (1977), Murray (1977) and others. Griffith observed that the model system possesses a stable periodic behaviour provided the cooperativity of the repressor metabolite as measured by the Hill coefficient ρ exceeds a high value, namely $\rho > 8$, which is biologically not realistic. The model developed by Tapaswi and Bhattacharya (1981) exhibited a stable periodic solution for $\rho > 4$ which was much nearer to a realistic value. In a further modified model including time delay, Tapaswi (1982) showed that the system exhibited a stable oscillation for a much lower value of ρ ($\rho = 1$), which is biologically realistic. The object of the present work is to develop stochastic formulations for a fundamental biological process like gene activation and RNA synthesis, a subject which has been little studied.

Application of Markov processes on biological growth has been dealt with by Bharucha-Reid (1960), Bartholomay (1962a) and Bailey (1964). Bartholomay constructed a stochastic model for enzyme-substrate reaction and by assuming $E[(e)(s)] = E[(e)]E[(s)]$ at the early part of the reaction, showed that the stochastic mean agreed with the deterministic mean. Goel and Richter-Dyn (1974) have made a condensed review of such studies.

The present work deals with a much more complicated biological mechanism which is not so easily accessible as the ordinary chemical reaction processes. Gene activation and RNA synthesis are discrete random processes, continuous in time, involving probabilities. Stochastic analysis will therefore be more appropriate than deterministic analysis in dealing with such a topic. The mathematical methods used here are similar to those followed by workers in other systems. One of the major difficulties that arises in the analytical work in deterministic models is the nonlinearity problem involved in most of the biological systems. The approximation method adopted here successfully overcomes this difficulty.

The formulation and analysis of the stochastic model are given in Section 2. Section 3 presents the numerical analysis and computer simulation to establish the validity of the normal approximation made in Section 2. Discussion and Conclusion appear in Section 4.

2. The stochastic model

For a probabilistic analysis of the reactions, we consider that the molecules of the activating substance (A) and DNA (D) genomes initiate a chain of random events which ultimately produces (or more correctly, stimulates

the formation of) the RNA molecules. These molecules are subject to random collisions because of their Brownian-like motions in the system. Thus, the question of certain probability is associated with the event that any pair of different molecules of A and D will collide, resulting in the formation of a complex molecule (AD) i.e., an activation of a DNA genome. Once formed, such a molecule (AD) has the probability of two eventual outcomes: (1) either AD stimulates the formation of one RNA (R) molecule, remaining itself unchanged, or (2) AD decomposes into one A and one D molecule (i.e., one unit of genome) by the inhibitory action of RNA molecules on it. These, added with the probability of degradation of one RNA molecule, give rise to the following transition probabilities which deal with all sorts of probabilities associated with the above reactions.

If n_1 is the constant number of the molecules of A, which is continuously replenished from a constant source inside or outside the cell and (n_2, n_3, n_4, t) is the state of the system at time t, where n_2, n_3 and n_4 are the numbers of genomes D, complex AD and molecules of RNA respectively, then the system may undergo the transitions given below.

transition	probability per unit time
$(n_2, n_3, n_4) \rightarrow (n_2-1, n_3+1, n_4)$	$\lambda_1^+ n_1 n_2$
$\rightarrow (n_2+1, n_3-1, n_4)$	$\lambda_2 n_3 n_4 + \lambda_1^- n_3$
$\rightarrow (n_2, n_3, n_4+1)$	$\lambda_3 n_3$
$\rightarrow (n_2, n_3, n_4-1)$	$\lambda_4 n_4$

$n_2+n_3 = n_{20}$ where n_{20} is the initial number of DNA (D) genomes and $\lambda_1^+, \lambda_1^-, \lambda_j$ $(j = 2, 3, \ldots, 6)$ are the constant probability parameters of the respective reactions.

Hence, the time derivative $p'(n_3, n_4, t)$ of the probability function $p(n_3, n_4, t)$ is

$$\begin{aligned}\frac{dp(n_3, n_4, t)}{dt} &= p(n_3, n_4-1, t)\lambda_3 n_3 + p(n_3-1, n_4, t)(n_{20}-n_3+1)\lambda_1^+ n_1 \\ &+ p(n_3+1, n_4, t)(n_3+1)(\lambda_1^- + \lambda_2 n_4) + p(n_3, n_4+1)(n_4+1)\lambda_4 \\ &- p(n_3, n_4, t)[n_1(n_{20}-n_3)\lambda_1^+ + n_3(\lambda_1^- + \lambda_2 n_4) + \lambda_3 n_3 + \lambda_4 n_4]. \quad \ldots \quad (1)\end{aligned}$$

From equation (1) we can easily calculate the time derivatives of the mean value functions for the variables n_3, n_4. These are given by

$$\frac{dE[n_3(t)]}{dt} = \sum_{n_3=0}^{n_{20}} \sum_{n_4=0}^{\infty} n_3 p'(n_3, n_4, t) \qquad \ldots (2)$$

$$\frac{dE[n_4(t)]}{dt} = \sum_{n_3=0}^{\infty} \sum_{n_4=0}^{\infty} n_4 p'(n_3, n_4, t).$$

Substituting the value for $p'(n_3, n_4, t)$ given by equation (1) into equation (2), we get

$$\frac{dE[n_3]}{dt} = \lambda_1^+ n_1 E[n_{20} - n_3)] - \lambda_1^- E[n_3] - \lambda_2 E[n_3 n_4]$$

$$\frac{dE[n_4]}{dt} = \lambda_3 E[n_3] - \lambda_4 E[n_4]. \qquad \ldots (3)$$

We must remember that $E[n_3 n_4] \neq E[n_3]E[n_4]$ here, since n_3 and n_4 are not independent variables.

Since the biological process under investigation is a stable oscillatory process as observed experimentally (Brahmachary *et al.*, 1971a, b), we have to examine whether the model can predict any stable periodic solution. In order to do so we require the solution of the master equation (1) which is similar to the forward Kolmogorov equation and due to its nonlinear nature, seems to be analytically intractable. However, we shall attempt to obtain the time-depent approximate solutions of the moments. Since, in the absence of the product terms in equation (1), one of the variables, n_3, tends to a normal distribution for sufficiently large n_{20} (see Appendix) and for small λ_4, n_4 is practically a function of n_3, we shall follow the normal approximation method (Whittle, 1957) to obtain analytically the moments of n_3 and n_4.

Writing $n_3 = m$, $n_4 = n$ and $p(n_3, n_4) = p_{mn}$, the master equation (1) can be written as

$$\frac{dp_{mn}}{dt} = p_{mn} \sum_{j,k} f_{jk}(m, n) + \sum_{j,k} p_{m-j, n-k} f_{jk}(m-j, n-k)$$

where

$$f_{0\,1} = m\lambda_3$$

$$f_{0\,-1} = n\lambda_4$$

$$f_{-1\,0} = m\lambda_1^- + mn\lambda_2$$

$$f_{1\,0} = \lambda_1^+ n_1 (n_{20} - m) \qquad \ldots (4)$$

and all other $f_{j,k}$ are zero.

Hence, the moment generating function defined by

$$M = \sum_u \sum_v \frac{\Sigma \mu'(t)_{uv} \theta^u \phi^v}{u!\, v!} \qquad \ldots (4)$$

satisfies the differential equation (Bailey, 1964)

$$\frac{\partial M}{\partial t} = \sum_j \sum_k (e^{j\theta + k\phi} - 1) f_{jk} \left(\frac{\partial}{\partial \theta}, \frac{\partial}{\partial \phi} \right) M. \qquad \ldots (5)$$

Using equation (4) and writing $K = \log M$ where K is the cumulant generating function, we find that K satisfies the following equation (using equation (5)) :

$$\frac{\partial K}{dt} = (e^{\theta} - 1)\lambda_1^+ n_1 \left(n_{20} - \frac{\partial K}{\partial \theta} \right) + \lambda_1^-(e^{-\theta} - 1) \frac{\partial K}{\partial \theta} + \lambda_2(e^{-\theta} - 1) \frac{\partial^2 K}{\partial \theta \partial \phi}$$

$$+ \lambda_3(e^{\phi} - 1) \frac{\partial K}{\partial \theta} + \lambda_4(e^{-\phi} - 1) \frac{\partial K}{\partial \phi} . \qquad \ldots (7)$$

Writing

$$K = \Sigma\Sigma k_{uv} \frac{\theta^u \phi^v}{u!\, v!} \qquad \ldots (8)$$

and equating coefficients of θ, ϕ, θ^2, ϕ^2 and $\theta\phi$, we get the following differential equations :

$$\frac{dk_{10}}{dt} = \lambda_1^+ n_1 n_{20} - (\lambda_1^+ n_1 + \lambda_1^-) k_{10} - \lambda_2 k_{11}$$

$$\frac{dk_{10}}{dt} = \lambda_3 k_{10} - \lambda_4 k_{01}$$

$$\frac{dk_{20}}{dt} = \lambda_1^+ n_1 n_{20} - (\lambda_1^+ n_1 - \lambda_1^-) k_{10} - 2(\lambda_1^+ n_1 + \lambda_1^-) k_{20} + \lambda_2 k_{11} - 2\lambda_2 k_{21}$$

$$\frac{dk_{02}}{dt} = \lambda_3 k_{10} + \lambda_4 k_{01} - 2\lambda_4 k_{02} + 2\lambda_3 k_{11}$$

$$\frac{dk_{11}}{dt} = \lambda_3 k_{20} - (\lambda_1^+ n_1 + \lambda_1^- + \lambda_4) k_{11} - \lambda_2 k_{12}. \qquad \ldots (9)$$

Since the above system is nonhomogeneous, we apply the transformation $k_{ij} = k'_{ij} + C_{ij}$ where C_{ij} is given by

$$\begin{aligned}
&\lambda_1^+ n_1 n_{20} - (\lambda_1^+ n_1 + \lambda_1^-) C_{10} - \lambda_2 C_{11} = 0 \\
&\lambda_3 C_{10} - \lambda_4 C_{01} = 0 \\
&\lambda_1^+ n_1 n_{20} - (\lambda_1^+ n_1 - \lambda_1^-) C_{10} - 2(\lambda_1^+ n_1 + \lambda_1^-) C_{20} + \lambda_2 C_{11} - 2\lambda_2 C_{21} = 0 \qquad \ldots (10) \\
&\lambda_3 C_{10} + \lambda_4 C_{01} - 2\lambda_4 C_{02} + 2\lambda_3 C_{11} = 0 \\
&\lambda_3 C_{20} - (\lambda_1^+ n_1 + \lambda_1^- + \lambda_4) C_{11} - \lambda_2 C_{12} = 0
\end{aligned}$$

i.e., C_{ij} is the equilibrium point of the system (the existence of a stable equilibrium is equivalent to the ergodicity of the Markov chain $(n_3(t), n_4(t))$ for which property see the last part of the Appendix).

Writing $\lambda_1^+ n_1 + \lambda_1^- = \alpha$ and $\lambda_1^+ n_1 - \lambda_1^- = \beta$, the transformed homogeneous system becomes

$$\begin{aligned}
\frac{dk'_{10}}{dt} &= -\alpha k'_{10} - \lambda_2 k'_{11} \\
\frac{dk'_{01}}{dt} &= \lambda_3 k'_{10} - \lambda_4 k'_{01} \\
\frac{dk'_{20}}{dt} &= -\beta k'_{10} - 2\alpha k'_{20} + \lambda_2 k'_{11} - 2\lambda_2 k'_{21} \qquad \ldots (11) \\
\frac{dk'_{02}}{dt} &= \lambda_3 k'_{10} - \lambda_4 k'_{01} - 2\lambda_4 k'_{02} + 2\lambda_3 k'_{11} \\
\frac{dk'_{11}}{dt} &= \lambda_3 k'_{20} - (\alpha + \lambda_4) k'_{11} - \lambda_2 k'_{12}.
\end{aligned}$$

We now assume that the distribution of each of n_3 and n_4 is approximately normal since in the absence of the nonlinear terms in the master equation (1) the distributions of n_3 tend to exact normal distribution shown in the Appendix and $n_4 = f(n_3)$ when λ_4 is small. Hence, keeping terms involving up to second order cumulants, i.e., neglecting k'_{21} and k'_{12} in equation (11) and assuming a solution of the form $k'_{ij} = A_{ij} e^{pt}$, the secular equation for k'_{ij} is given by

$$\begin{aligned}
&(\rho + \lambda_4)(\rho + 2\lambda_4) \\
&\times [\rho^3 + \rho^2(4\alpha + \lambda_4) + \rho(5\alpha^2 + 3\alpha\lambda_4 - \lambda_2\lambda_3) + 2\alpha^2(\alpha + \lambda_4) - \lambda_2\lambda_3(\alpha + \beta)] = 0 \qquad \ldots (12)
\end{aligned}$$

Applying Routh-Hurwitz criterion (Uspensky, 1974), we can show that this equation has a pair of complex conjugate roots with real part positive or zero if

$$\lambda_2\lambda_3 \geqslant \frac{3\alpha(6\alpha^2 + 5\alpha\lambda_4 + \lambda_4^2)}{(4\alpha + \lambda_4 - \gamma)} \qquad \ldots (13)$$

where

$$\gamma = \alpha + \beta < \frac{2\alpha(\alpha + \lambda_4)}{5\alpha + 3\lambda_4}, \quad \beta < 0.$$

Thus, for

$$\lambda_2\lambda_3 = \frac{3\alpha(6\alpha^2 + 5\alpha\lambda_4 + \lambda_4^2)}{(4\alpha + \lambda_4 - \gamma)}, \qquad \ldots (14)$$

equation (12) has a pair of purely imaginary roots $\pm i\omega$, and for a higher value of λ_2 than this the oscillation is unstable and growing in nature, whereas for a lower value there will be a decaying oscillation approachnging the stable equilibrium point. By Hopf bifurcation theorem it can be shown that in the

neighbourhood of the bifurcation point given by (14), there exists a stable limit cycle around the equilibrium point (Murray, 1977).

Thus, there exist some values of λ_2 for which the stochastic approximation envisages a stable periodic solution of the system under consideration, which is biologically significant.

The dominating limit cycle solution is given by

$$k_{ij} = C_{ij} + A_{ij} \cos \omega (t - t_{ij}) \qquad \ldots \quad (15)$$

where C_{ij} is given by (10) and A_{ij} and t_{ij} are determined from equation (11) and the initial conditions $k_{10} = a$, $k_{02} = b$, $k_{20} = k_{02} = k_{11} = 0$ at $t = 0$.

As an example, let $n_{20} = 200$, $n_1 = 10$, $\lambda_1^+ = 0{\cdot}1$ and $\lambda_1^- = 0{\cdot}9$ so that $\beta = -0{\cdot}8$ and $\gamma = \alpha + \beta = 0{\cdot}2$. Also, let $\lambda_3 = \lambda_4 = 1$. Then, to get a limit cycle solution we must require $\lambda_2 = 7{\cdot}5$ according to the condition (14). Thus, the purely imaginary roots (for $\lambda_2 = 7{\cdot}5$) of the secular equation (12) will be

$$\rho = \pm \sqrt{0{\cdot}5}\, i \quad \text{i.e.,} \quad \omega = \pm \sqrt{0{\cdot}5}.$$

The typical limit cycle solutions of k_{ij} for the above values of the parameters are shown in Figure 1.

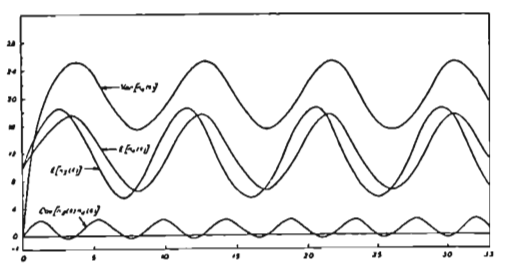

Fig. 1. Limit cycle (stable periodic) solutions of the first and second order moments of n_1 and n_2 as obtained analytically by the normal approximation methods.

It should also be noted that the oscillation observed in the present system is entirely due to the nonlinearity present, and if $\lambda_2 = 0$, i.e., when the system turns into a linear one, there will be no oscillation, since all the roots of the secular equation (12) will be real and negative so that the system will be ergodic as shown in the Appendix and will asymptotically approach a stable equilibrium.

3. Numerical analysis

Monte Carlo methods were used to check the usefulness and validity of the normal approximation. The constants chosen for the given stochastic model were the same as assumed in the example of the analytical approximate solutions in Section 2, satisfying the condition (14) for stable limit cycle solution (for the approximated case).

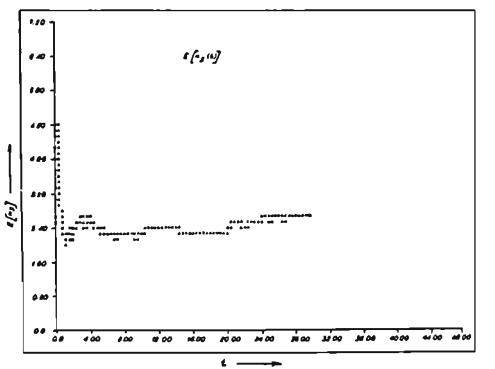

Fig. 2

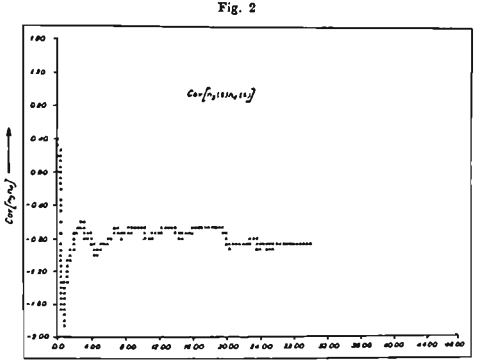

Fig. 3

An artificial realization was started with $n_3 = 1$ and $n_4 = 0$ and developed by the standard 'Monte Carlo' technique (cf., for example, Bartlett (1955, 1957), and Leslie (1958)) and continued for 3,000 instants, each time interval being 0·01. The series so obtained using a simplotter is graphed in Figs. 2–6. At first, each graph shows some decaying and chaotic oscillations due to the

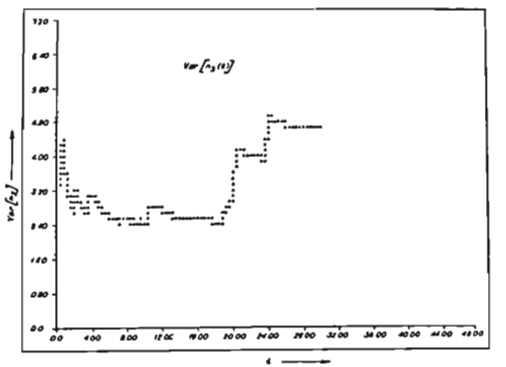

Fig. 4

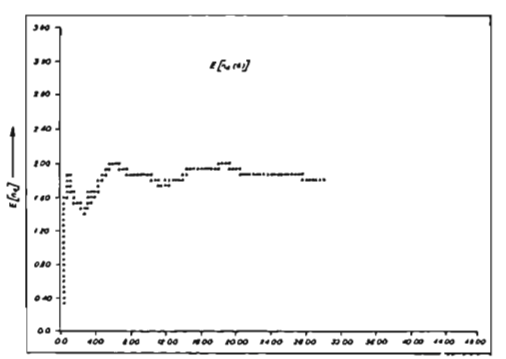

Fig. 5

presence of negative eigenvalues of the characteristic equation (12), as well as some initial 'noises' : later on they show nearly stable oscillations, mimicking the limit cycle solutions of the approximated analytical case.

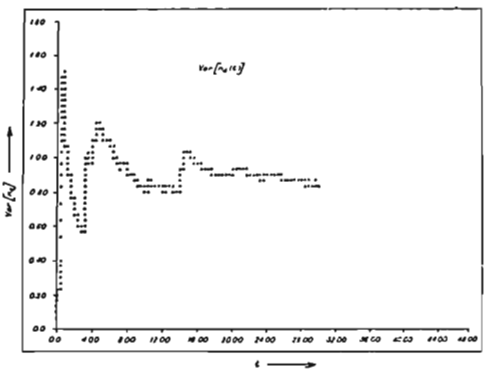

Fig. 6

The results obtained above indicate that the method of normal approximation should play a key role in the study of complex nonlinear biological situations which seem to defy a complete stochastic treatment analytically.

4. Discussion and conclusion

The utility of stochastic models for the investigations of biological processes has been established by various workers [Singer (1953), Bharucha-Reid (1953), Bartholomay (1962a, b), Kendall (1949) and many others]. Biological processes consist of discrete events at the molecular levels and are subject to the laws of chance both individually and collectively at every moment.

In the present work the nonlinearity problem associated with the biological process under investigation has been tackled efficiently by the stochastic approximation method. Emphasis has been given on the time-dependent properties of the stochastic model. The approximate solutions of the moments of n_3 (activated genomes) and of n_4 (RNA molecules) envisage a stable periodic solution for the system, which is biologically significant.

It has been assumed that the distribution of the variables n_3 and n_4 are approximately normal, since in the absence of the product terms in the transition probability table, one of the variables, n_3, is normally distributed

as shown in the Appendix and the other variable, n_4, is directly a function of n_3 when λ_4, the degradation rate, is too low. The validity of this normal approximation may still be questioned and a convincing answer to this is not possible because the effect of the product terms in the master equation (1) on the distribution of n_3 and n_4 cannot be ascertained analytically. However, since the condition of stable oscillation (14) depends upon the product $\lambda_2\lambda_3$ and not on λ_2 alone, for large λ_3 and sufficiently small λ_2 so that $\lambda_2\lambda_3$ satisfies (14) and at the same time, the effect of the product terms in (1) becomes sufficiently small, the assumption of normal approximation may be perfectly valid. Also, the computer simulation presented here establishes the validity of the normal approximation.

A rigorous justification of the results in this article can only come from a proper scaling of time and state space or, more precisely, scaling the parameter n_{20} as a function of time and letting time go to infinity. Although a precise analysis is very complex, the article provides adequate justification to indicate that if n_{20} is large and so is t, the first two moments of $n_3(t)$, $n_4(t)$ show a stable periodic oscillation if λ_2 is near a critical value. For lower values of λ_2 these moments converge to steady state values, while for higher values of λ_2 the moments grow exhibiting oscillations along the way. This paper also provides a calculation of the critical value.

The stochastic model constructed here does not claim to depict the perfect biological picture of the transcription process, since this is a most simplified bivariate expression of a complicated multivariate process. This work only gives a procedure and methodology for utilization of stochastic treatment for studying a real biological process.

5. Acknowledgement

The authors wish to thank Dr. R. Wette, Washington University Medical School, St. Louis, U.S.A. and Dr. Abdalla S. A. Mohamed of the Department of Systems and Biomedical Engineering, Cairo University, Egypt for their cooperation in the Monte Carlo Simulation, the results of which are reported in this paper. The first author would like to acknowledge visiting assignments in the Washington University Medical School, St. Louis, U.S.A. and Waterloo University, Waterloo, Canada. The research was partially supported by the NSERC (Natural Sciences and Engineering Research Council) of Canada, which is also gratefully acknowledged. Thanks are also due to Mr. S. K. Dey, Artist of the Indian Statistical Institute, for preparation of Figures 1-6. The authors would like to express their indebtedness to the referee for his critical comments and valuable suggestions to improve the standard of the paper.

Appendix

To solve equation (7) we require some knowledge of the approximate distribution of n_3 and n_4.

Distribution of $n_3(t)$ (*when* $\lambda_2 = 0$) : In the absence of the product terms in the master equation (1) the marginal distribution of n_3 is obtained from the following linear differential equation (taking $p(n_3, t) = \sum_{n_4=0}^{\infty} p(n_3, n_4, t)$):

$$\frac{dp(n_3,t)}{dt} = \lambda_1^+ n_1(n_{20}-n_3+1)p(n_3-1,t)+\lambda_1^-(n_3+1)p(n_3+1,t) -[\lambda_1^+ n_1(n_{20}-n_3)+\lambda_1^- n_3]p(n_3,t). \quad \ldots \text{(A.1)}$$

Let $P(x,t) = \sum_{n_3=0}^{n_{20}} p(n_3,t)x^{n_3}$ be the probability generating function.

Then

$$\frac{\partial P}{\partial t} = (x-1)\lambda_1^+ n_1 n_{20} P-[\lambda_1^+ n_1 x^2-(\lambda_1^+ n_1-\lambda_1^-)x-\lambda_1^-]\frac{\partial P}{\partial x} \quad \ldots \text{(A.2)}$$

with initial condition $P(x,0) = x^a$.

The subsidiary equations are

$$\frac{dt}{1} = \frac{dP}{(x-1)\lambda_1^+ n_1 n_{20} P} = \frac{dx}{\lambda_1^+ n_1 x^2-(\lambda_1^+ n_1-\lambda_1^-)x-\lambda_1^-}. \quad \ldots \text{(A.3)}$$

The first and second expressions in (A.3) gives

$$\frac{dt}{1} = \frac{dx}{\lambda_1^+ n_1(x+\psi)(x-\phi)}. \quad \ldots \text{(A.4)}$$

where $\psi\phi = \frac{\lambda_1^-}{\lambda_1^+ n_1}$ and $\psi-\phi = \frac{\lambda_1^-}{\lambda_1^+ n_1}-1$

i.e., $\psi = \frac{\lambda_1^-}{\lambda_1^+ n_1}$ and $\phi = 1$

Hence, integrating (A.4) gives

$$\lambda_1^+ n_1 t = \frac{1}{-(\psi+\phi)} \log\frac{x+\psi}{x-\phi}$$

or $$\frac{x-\phi}{x+\psi}\, e^{-\lambda_1^+ n_1(\psi+\phi)t} = \text{constant}$$

or, putting the values of ϕ and ψ

$$\frac{\lambda_1^+ n_1(x-1)}{\lambda_1^+ n_1 x+\lambda_1^-}\, e^{-(\lambda_1^-+\lambda_1^+ n_1)t} = \text{constant}. \quad \ldots \text{(A.5)}$$

From the second and third expression in (A.3) we have

$$\frac{dP}{\lambda_1^+ n_1 n_{20} P} = \frac{(x-1)dx}{\lambda_1^- n_1 (x+\Psi)(x-\phi)}$$

i.e.
$$\frac{dP}{n_{20}P} = \frac{dx}{(x+\Psi)} \text{ (since } \phi = 1). \qquad \ldots \text{ (A.6)}$$

Integrating (A.6) gives

$$P(x+\Psi)^{-n_{20}} = \text{constant}$$

i.e.
$$P\left(x+\frac{\lambda_1^-}{\lambda_1^+ n_1}\right)^{-n_{20}} = \text{constant}. \qquad \ldots \text{ (A.7)}$$

Hence the general solution is

$$P\left(x+\frac{\lambda_1^-}{\lambda_1^+ n_1}\right)^{-n_{20}} = \xi\left\{\frac{\lambda_1^+ n_1 (x-1)}{\lambda_1^+ n_1 x+\lambda_1^-} e^{-(\lambda_1^- + \lambda_1^+ n_1)t}\right\} \qquad \ldots \text{ (A.8)}$$

Using the initial conditions $P(x, 0) = x^a$ gives

$$x^a\left(x+\frac{\lambda_1^-}{\lambda_1^+ n_1}\right)^{-n_{20}} = \xi\left\{\frac{\lambda_1^+ n_1 (x-1)}{\lambda_1^+ n_1 x+\lambda_1^-}\right\}. \qquad \ldots \text{ (A.9)}$$

We now put

$$\frac{\lambda_1^+ n_1 (x-1)}{\lambda_1^+ n_1 x+\lambda_1^-} = W, \text{ i.e., } \quad x = \frac{\lambda_1^- W+\lambda_1^+ n_1}{\lambda_1^+ n_1-\lambda_1^+ n_1 W} \qquad \ldots \text{ (A.10)}$$

and substitute in (A.9) to give the explicit functional form

$$\xi(W) = \frac{(\lambda_1^-+\lambda_1^+ n_1)^{-n_{20}}(\lambda_1^- W+\lambda_1^+ n_1)^a}{\{\lambda_1^+ n_1(1-W)\}^{a-n_{20}}}. \qquad \ldots \text{ (A.11)}$$

Using this in (A.8) finally yields after some computation

$$P(x, t) = (\lambda_1^-+\lambda_1^+ n_1)^{-n_{20}} \frac{\lambda_1^+ n_1 x+\lambda_1^-+\lambda_1^-(x-1)q}{\lambda_1^+ n_1 x+\lambda_1^--\lambda_1^+ n_1(x-1)q} \qquad \ldots \text{ (A.12)}$$

where $q = e^{(\lambda_1^- + \lambda_1^+ n_1)t}$.

Now we can write (A.12) as

$$P(x, t) = (\lambda_1^-+\lambda_1^+ n_1)^{-n_{20}} \frac{\{x(\lambda_1^- q+\lambda_1^+ n_1)+\lambda_1^-(1-q)\}^a}{\{\lambda_1^+ n_1 x(1-q)+\lambda_1^-+\lambda_1^+ n_1 q\}^{a-n_{20}}}$$

$$= (\lambda_1^-+\lambda_1^+ n_1)^{-n_{20}} \frac{(\lambda_1^- q+\lambda_1^+ n_1)^a}{\{\lambda_1^+ n_1(1-q)\}^{a-n_{20}}} (x+u)^a (x+v)^{n_{20}-a} \qquad \ldots \text{ (A.13)}$$

where $u = \dfrac{\lambda_1^-(1-q)}{\lambda_1^- q + \lambda_1^+ n_1}$ and $v = \dfrac{\lambda_1^- + \lambda_1^+ n_1 q}{\lambda_1^+ n_1 (1-q)}$.

For convenience we choose $a = 0$.

Hence (A.13) can be written as

$$(\lambda_1^- + \lambda_1^+ n_1)^{-n_{20}} (\lambda_1^+ n_1 (1-q))^{n_{20}} \sum_{n_3=0}^{n_{20}} \binom{n_{20}}{n_3} v^{n_{20}-n_3} x^{n_3}. \quad \ldots \text{(A.14)}$$

Then

$$P(n_3, t) = (\lambda_1^- + \lambda_1^+ n_1)^{-n_{20}} (\lambda_1^+ n_1 (1-q))^{n_{20}} \binom{n_{20}}{n_3} \left\{ \frac{\lambda_1^- + \lambda_1^+ n_1 q}{\lambda_1^+ n_1 (1-q)} \right\}^{n_{20}-n_3}$$

which after some computation can be written in the suitable form

$$P(n_3, t) = \binom{n_{20}}{n_3} \left\{ \frac{\lambda_1^+ n_1 (1-q)}{\lambda_1^- + \lambda_1^+ n_1} \right\}^{n_3} \left\{ \frac{\lambda_1^- + \lambda_1^+ n_1 q}{\lambda_1^- + \lambda_1^+ n_1} \right\}^{n_{20}-n_3} \quad \ldots \text{(A.15)}$$

This is a binomial distribution with mean

$$E[n_3(t)] = \frac{n_{20} \lambda_1^+ n_1 (1-q)}{\lambda_1^- + \lambda_1^+ n_1} \quad \ldots \text{(A.16)}$$

and variance

$$\text{Var } E[n_3(t)] = n_{20} \frac{\lambda_1^+ n_1 (1-q)(\lambda_1^- + \lambda_1^+ n_1 q)}{(\lambda_1^- + \lambda_1^+ n_1)^2} \quad \ldots \text{(A.17)}$$

At $t \to \infty$, $q = e^{-(\lambda_1^- + \lambda_1^+ n_1)t} \to 0$.

Hence,

$$E[n_3(\infty)] = \frac{\lambda_1^+ n_1 n_{20}}{\lambda_1^+ n_1 + \lambda_1^-} \quad \ldots \text{(A.18)}$$

which absolutely agrees with the steady state value of $E[n_3(t)]$ obtained from equation (3) in the absence of the nonlinear terms.

Also we have

$$\text{Var}[n_3(\infty)] = \frac{n_{20} \lambda_1^+ \lambda_1^- n_1}{(\lambda_1^- + \lambda_1^+ n_1)^2} \quad \ldots \text{(A.19)}$$

Since the distribution of n_3 when there is no nonlinear effect ($\lambda_2 = 0$), is, as evident from (A.15), a binomial distribution, for sufficiently large n_{20} this tends to a normal distribution.

Ergodicity of the process when $\lambda_2 = 0$. The limiting distributions of $n_3(t)$ and $n_4(t)$ in the absence of the product terms in the master equation (1) are given by :

$$\lim_{t\to\infty} p(n_3, t) = \binom{n_{20}}{n_3} \left(\frac{\lambda_1^+ n_1}{\lambda_1^- + \lambda_1^+ n_1}\right)^{n_3} \left(\frac{\lambda_1^-}{\lambda_1^- + \lambda_1^+ n_1}\right)^{n_{20}-n_3} = p(n_3) \quad \ldots \quad (A.20)$$

and $$\lim_{t\to\infty} p(n_4, t) = \binom{b}{n_4} \left(\frac{\lambda_1^+ \lambda_3 n_1 n_{20}}{\lambda_4 br}\right)^{n_4} \left(1 - \frac{\lambda_1^+ \lambda_3 n_1 n_{20}}{\lambda_4 br}\right)^{b-n_4} = p(n_4) \quad \ldots \quad (A.21)$$

both of which are independent of the initial states. Hence the process is ergodic when $\lambda_2 = 0$, i.e., linear.

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Paper received : October, 1985.

Revised : November, 1986.