BIOTECHNOLOGY LETTERS
Volume 17 No.7 (July 1995) pp.669-674
Received as revised 31st May

A MATHEMATICAL MODEL FOR THE G1/S TRANSITION OF THE MAMMALIAN CELL CYCLE

Vassily Hatzimanikatis, Kelvin H. Lee, Wolfgang A. Renner
and James E. Bailey *

Institute of Biotechnology, ETH Zurich
CH-8093 Zurich, Switzerland

SUMMARY

Genetic intervention in cell-cycle regulation is a promising strategy to obtain mammalian cell culture proliferation in the absence of exogenous growth factors. In order to gain insights into this approach, known interactions among the four proteins cyclin E, cdk2, the retinoblastoma gene product (RB), and the transcription factor E2F, all centrally involved in control of the G1/S transition of the eucaryotic cell cycle, guided the formulation of kinetics in intracellular mass balances on these components. Stable oscillatory solutions of these equations, which include the diluting effects of cell volume increase and a resulting special boundary condition, correspond to cell proliferation. The model simulates the qualitative consequences on cell cycle regulation of overexpression of cyclin E, E2F, and of RB deregulation in agreement with experiment. Bifurcation analysis of the model suggests strategies for rational manipulation of the cell cycle.

INTRODUCTION

Motivated primarily by concerns about adventitious infectious agents, elimination of all exogenous animal proteins from mammalian cell culture is a current high-priority objective of the biotechnology industry. Recently a new genetic strategy for rapidly converting Chinese hamster ovary (CHO) cells to protein-independent growth has been demonstrated (Renner *et al.*, 1995, Lee, 1995). This approach is based on genetic manipulation of the expression of proteins critically involved in regulating the cell cycle. In order to aid understanding of these initial experiments and to guide further refinement in this genetic approach, a molecular-level mathematical model of a key cell-cycle transition has been formulated and analyzed.

Mammalian cells in culture often arrest growth in the G1 phase of the cell cycle. Better understanding of the factors which control this arrest, or which can avoid it by activating progression to S phase, is therefore of central importance in biotechnology. Although the exact molecular mechanism controlling the G1/S transition is not fully understood, recent research has elucidated many of its features (see Peeper *et al.*, 1994 for a review). The most widely accepted hypothesis

considers a set of proteins (the G1 cyclins) which form phosphorylated complexes with kinases (the cyclin dependent kinases or CDKs) that subsequently phosphorylate other proteins, such as the retinoblastoma gene product, RB (Bin and Ying, 1992). The RB protein is phosphorylated in multiple sites and can bind to various transcription factors (Cooper and Whyte, 1989; Lees *et al.*, 1992) which activate genes encoding proteins involved in DNA replication. Hyperphosphorylation of RB by the cyclin E-cdk2 phosphorylated complex occurs in the late G1-phase and results in the release of the transcription factor E2F.

There are few mathematical models which describe biochemical oscillations of key proteins involved in cell-cycle regulation (Norel and Agur, 1991; Tyson, 1993; Novak and Tyson, 1993; Goldbeter, 1991); these models focus on mitotic regulation by maturation promotion factor (MPF) (Nurse, 1990; Murray and Kirschner, 1989). All reported models neglect the effect of cell growth which causes dilution of intracellular species (Fredrickson, 1976). The importance of this dilution effect and the mathematical consequences of its inclusion will be discussed later.

SUMMARY OF THE MODEL

Based on a number of simplifying assumptions, we have constructed a mathematical model to simulate the interactions of the key components involved in controlling the G1/S transition and progression of the system through the cell cycle. This model includes seven components (those depicted in Figure 1).

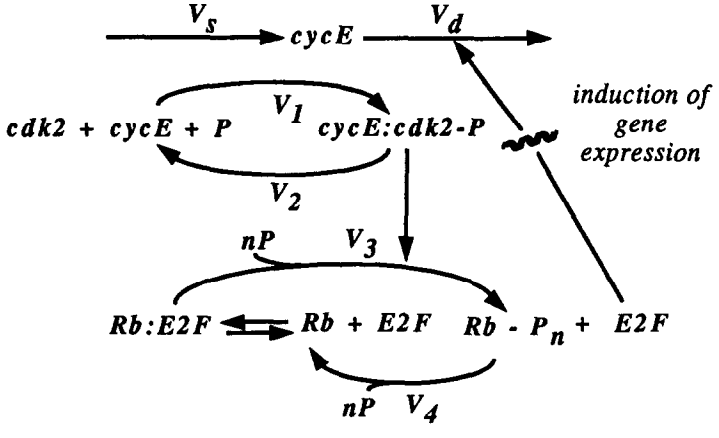

Figure 1: Schematic representation of the mathematical relations used in this model.

Cyclin E (cycE) is assumed to be constitutively produced at a rate V_S and to be degraded at a rate V_d. In the absence of sufficient information for a mechanistic description, this model assumes that the degradation rate of cyclin E increases during S phase, and implements this hypothesis mathematically by increasing the cyclin E degradation rate with increasing E2F concentration. The latter quantity is used here as an index of cell cycle position because it is observed that the level of free E2F peaks at the G1/S transition. Cyclin E forms a phosphorylated complex with the inactive form of the cyclin-dependent kinase cdk2 at a rate V_1, and this complex dissociates at a rate V_2. The cycE-cdk2 phosphorylated complex activates the reaction with rate V_3 which represents results in the release of E2F. V_4 represents the rate of the set of reactions which dephosphorylates the

hyperphosphorylated form of RB (RB-P) leading to the formation of the hypophosphorylated form of RB (RB). RB and E2F are assumed to be in equilibrium with the RB-E2F complex.

Little quantitative information is available on the kinetics of the processes and interactions shown in Figure 1. Therefore, the present modeling study does not attempt quantitative simulation of concentrations and generation times. However, a set of important qualitative questions can be addressed by this model which is based on the present state of qualitative knowledge about this network for cell-cycle control. It is qualitatively reasonable to assume Michaelis-Menten functionalities (Goldbeter, 1991) in the absence of contrary information, and likewise to describe activation and inhibition using standard expressions from enzyme kinetics. By scaling time and concentrations in the model by combinations of model parameters, the number of independent parameters can be minimized. This leads to the following set of conservation equations:

$$[K_T]=[K]+[K_p] \qquad (1)$$

$$[R_T]=[R]+[R_p]+[R_e] \qquad (2)$$

$$[E_T]=[E]+[R_e] \qquad (3)$$

$$q=\frac{[E][R]}{[R_e]} \qquad (4)$$

$$\frac{d[C]}{dt}=v_s-v_{d,m}[E]\frac{[C]}{K_d+[C]}-K_{d'}[C] - v_{1,m}\frac{[C]}{(K_c+[C])}\frac{[K]}{(K_1+[K])}+v_{2,m}\frac{[K_p]}{K_2+[K_p]}-\mu[C] \qquad (5)$$

$$\frac{d[K_{c,p}]}{dt}=v_{1,m}\frac{[C]}{K_c+[C]}\frac{[K]}{(K_1+[K])}-v_{2,m}\frac{[K_{c,p}]}{K_2+[K_{c,p}]}-\mu[K_{c,p}] \qquad (6)$$

$$\frac{d[R_p]}{dt}=v_{3,m}[K_p]\frac{[R_e]}{K_3+[R_e]}-v_{4,m}\frac{[R_p]}{K_4+[R_p]}-\mu[R_p] \qquad (7)$$

where [C] is the cyclin E concentration, [E] is the E2F concentration, [K] is the cdk2 concentration, $[K_{c,p}]$ is the phosphorylated cyclin E-cdk2 complex concentration, [R] is the concentration of the hypophosphorylated form of RB, $[R_p]$ is the concentration of the hyperphopsphorylated form of RB, and $[R_e]$ is the concentration of the hypophosphorylated form of RB that binds to E2F.

Equation (1) is the conservation relation for all forms of cdk2, the sum of which remains invariant throughout the cell cycle (Pagano *et al.*, 1992). Equations (2) and (3) are the conservation relations for all forms of RB and E2F, the sums of which are also assumed to be invariant. Equilibrium between the hypophosphorylated form of RB and free E2F with the RB-E2F complex is described by Equation (4).

Differential equations (5) through (7) are dynamic mass balances on the intracellular concentrations of free cyclin E, phosphorylated cyclin E-cdk2 complex, and the hyperphosphorylated form of RB, respectively. These equations follow from the kinetic representations already described for the reactions depicted in Figure 2, following standard methods (Bailey and Ollis, 1986). In Equations (5)-(7) the final terms on the right-hand-sides represent dilution caused by increase in cell volume at a specific rate μ = dln(cell volume)/dt (Fredrickson, 1976). The presence of the dilution term imposes an additional constraint on any cell-cycle model (in contrast to a solely biochemical model, Hatzimanikatis *et al.*, 1995). The period of a limit cycle calculated by the model, which is the same as the cell generation time, T_d, must be related to the volumetric specific growth rate μ (which appears as a parameter in several model equations) by the relationship:

$$\mu T_d = \ln 2 \qquad (8)$$

Equation (8), which is based on the requirement that the cell volume must double in one generation time, applies only for the special case, which is assumed here, that μ is time-invariant.

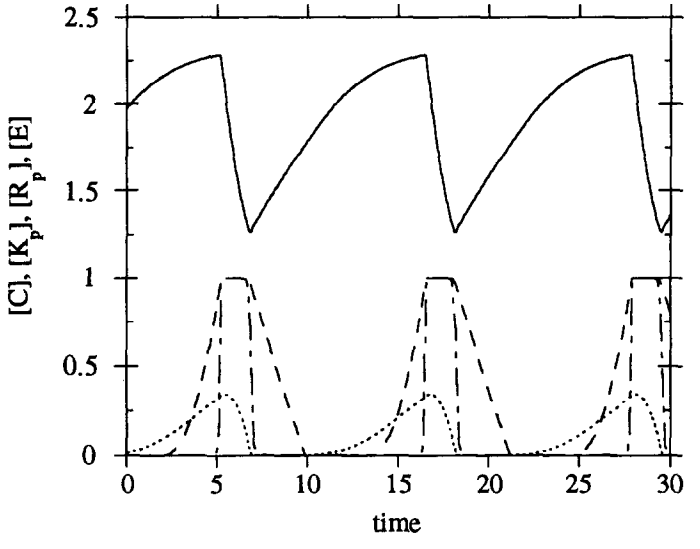

Figure 2: Periodic oscillations of cyclin E ([E]; solid line), cyclin E-cdk2 phosphorylated complex ($[K_p]$; dotted line), hyperphosphorylated form of RB ($[R_p]$; dashed line), and E2F ([E]; dashed-dotted line). Dimensionless parameter values used: V_S=0.25; $V_{d,m}$=1; K_d=0.0001; k_d=0.05; $V_{1,m}$=5; $V_{2,m}$=4; $V_{3,m}$=3; $V_{4,m}$=0.3; K_c=0.5; K_1=K_2=K_3=K_4=0.0001; $[K_T]$=$[E_T]$=1; $[R_T]$=10; q=0.01.

RESULTS

This model was used to study the effects of the cyclin E synthesis rate and the total E2F concentration on the appearance, or not, of oscillatory solutions to Equations (1) through (7) which satisfy Equation (8). These situations correspond to proliferating cells or quiescent (arrested) cells, respectively. After extensive computational analysis, we constructed an operating diagram (Figure 3) which shows the following features:

1. Downregulation of cyclin E synthesis results in cell-cycle arrest. Such a downregulation could result from growth factor withdrawal from the medium, as is observed experimentally (Matsushime *et al.*, 1991). Conversely, cell proliferation can be sustained in the absence of growth factors by overexpression of cyclin E, as has also recently been observed experimentally for CHO cells (Renner *et al.*, 1995).

2. Overexpression of cyclin E beyond a threshold value can inhibit cell growth. This threshold depends on the total E2F concentration which is cell-line dependent.

3. Quiescent cells can be activated to proliferate by overexpression of E2F only if they have a sufficiently high cyclin E synthesis rate. Proliferation of CHO K1 cells in the absence of growth factors following overexpression of E2F-1 has been observed experimentally (Lee, 1995).

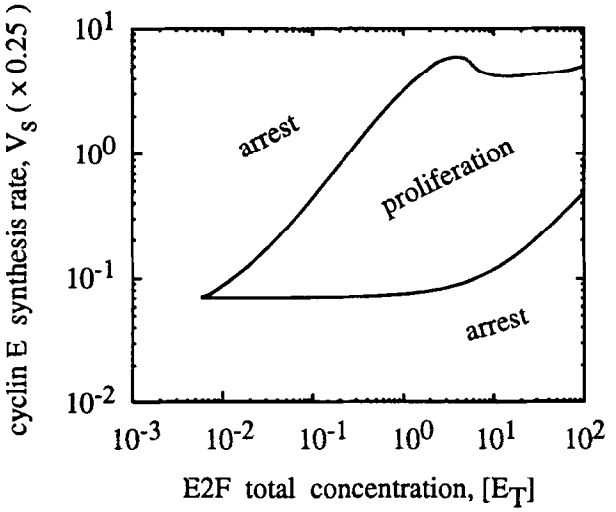

Figure 3: Operating diagram indicating the parametric areas for which proliferation and cell cycle arrest are possible. All other parameters as in Figure 2.

We also studied the dependence of the doubling time on the two parameters mentioned above (Figure 4). The results suggest that overexpression of cyclin E will result in shorter doubling times (i.e., faster growth), whereas an increase in total E2F concentration will give slower growth rates. The doubling time appears to be more sensitive to cyclin E expression level. Moreover, simulation studies reveal that the doubling time sensitivity to cyclin E synthesis rate decreases at higher total E2F concentration (data not shown). This correlation helps explain the different observed responses to cyclin E expression among various cell lines and tissue cells (Ohtsubo and Roberts, 1993; Renner *et al.*, 1995; Knoblich *et al.*, 1994).

DISCUSSION

Current understanding of the molecular events involved in regulation of the G1/S transition in eucaryotic cells suffices to formulate a mathematical model which reproduces many experimental observations and which makes new predictions (see point 2 above). This model, and others of its type, can be applied to aid rational manipulation of the cell cycle, whether to activate growth for

biopharmaceutical productions by cell culture or to identify specific targets for blocking proliferation of malignant cells.

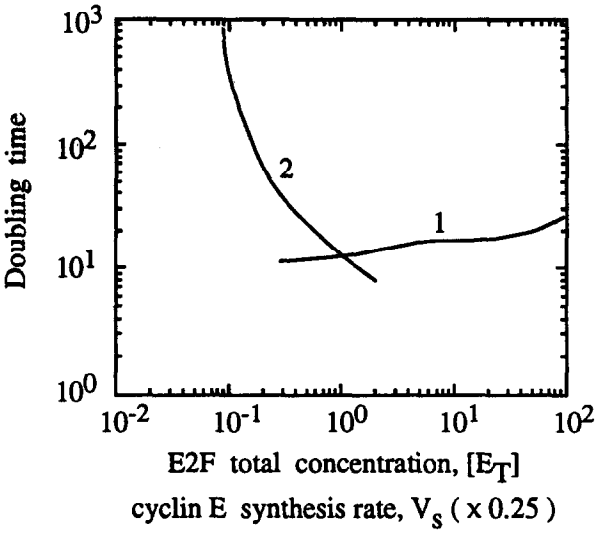

Figure 4: Dependence of doubling time on E2F total concentration ($[E_T]$; line 1) and cyclin E synthesis rate (V_S; line 2). For lines 1 and 2 all parameters, except $[E_T]$ and V_S, respectively, are as in Figure 2.

ACKNOWLEDGEMENTS

This work was supported by the Swiss Priority Program in Biotechnology (SPP Biotech).

REFERENCES

Bailey, J.E. and Ollis, D. (1986). *Biochemical Engineering Fundamentals*, 2 ed., New York McGraw-Hill.

Cooper, J. and Whyte, P. (1989). *Cell* 58, 1009.

Fredrickson, A. (1976). *Biotech. Bioeng.* 28, 1481.

Goldbeter, A. (1991). *Proc. Natl. Acad. Sci. USA* 88, 9107.

Hatzimanikatis, V., Lee, K.H., and Bailey, J.E. (1995). Manuscript in preparation.

Knoblich, J.A., Sauer, K., Jones, L., Richardson, Saint, R. and Lehner, C.F. (1994). *Cell* 77, 107.

Lee, K.H. (1995). Ph.D. thesis, California Institute of Technology.

Lees, E., Faha, B., Dulic, V., Reed, S., and Harlow, E. (1992). *Genes Dev.* 6, 1874.

Lin, B. and Wang, Y. (1992). In: *Regulation of the Eukaryotic Cell Cycle*, J. Marsh, ed. vol 170. pp. 227-243, New York: Wiley.

Matsushime, H., Roussel, M.F., Ashmun, R.A., and Sherr, C. (1991). *Cell* 65, 701.

Murray, A. and Kirschner, M. (1989). *Science* 246, 614.

Norel, R. and Agur, Z. (1991). *Science* 251, 1076.

Novak, B. and Tyson, J. (1993). *J. Cell Sci.* 106, 1153.

Nurse, P. (1990). *Nature* 344, 503.

Pagano, M., Draetta, G., Jansen-Dürr, P. (1992). *Science* 255, 1144 .

Peeper, D.S., van der Eb, A.J. and Zantema, A. (1994). *Biochim. Biophys. Acta* 1198, 215

Renner, W. A., Lee, K.H., Hatzimanikatis, V., Bailey, J.E., and Eppenberger, H.M. (1995). *Biotech . Bioeng.*, in press.

Tyson, J. (1993). *J. Theor. Biol.* 165, 101.