

A Model for the Adjustment of the Mitotic Clock by Cyclin and MPF Levels

R. NOREL AND Z. AGUR*

A Model for the Adjustment of the Mitotic Clock by Cyclin and MPF Levels

R. NOREL AND Z. AGUR*

A mathematical model of cell cycle progression is presented, which integrates recent biochemical information on the interaction of the maturation promotion factor (MPF) and cyclin. The model retrieves the dynamics observed in early embryos and explains how multiple cycles of MPF activity can be produced and how the internal clock that determines durations and number of cycles can be adjusted by modulating the rate of change in MPF or cyclin concentrations. Experiments are suggested for verifying the role of MPF activity in determining the length of the somatic cell cycle.

RECENTLY IT HAS BEEN SUGGESTED that a single biochemical mechanism underlies the cell cycle progression in all eukaryotic organisms (1, 2). The basic mechanism involves activation and inactivation of the maturation promoting factor (MPF) by a protein called cyclin. During the interphase, cyclin accumulates until the rate of MPF activation by cyclin exceeds the rate of inactivation of MPF by MPF-inactivase, the concentration of which is assumed to be constant. As a consequence, active MPF accumulates, which leads to a series of modifications of other mitotic substrates. The activation of MPF also induces

the activation of cyclin degradation, and cyclin disappearance results in MPF destabilization by MPF-inactivase. The interphase structures are then reestablished, and cyclin begins reaccumulating to initiate the next cycle. This model is generally consistent with experimental data (1).

In early embryonic development, MPF activity serves as an internal clock that suffices to send the cell into mitosis (1, 3). In the present work we examine the regulation of this clock using a mathematical model for the MPF and cyclin reactions. Underlying our model is the principle of parsimony: by elucidating the minimal assumptions needed to retrieve the observed dynamics, we determine whether thresholds, such as the one postulated for signaling cyclin degradation (4), or time delays, thought to be missing in the present biochemical model (1), are in-

Department of Applied Mathematics and Computer Science, The Weizmann Institute of Science, Rehovot 76100, Israel.

*To whom correspondence should be addressed.

deed essential. Subsequently, we use the model to determine how the length of the cell cycle can be adjusted and suggest experiments that may indicate the relative roles of cyclin and MPF in regulating the more tightly controlled somatic cell cycle (3).

Let C and M denote the concentrations of cyclin and active MPF at any given moment, and \dot{C} and \dot{M} the rate of change in these concentrations. For formally describing MPF activation, we use the assumptions that (i) in the early embryos, cyclin synthesis suffices for the activation of MPF and for the induction of mitosis (1), with each cyclin molecule activating more than one pre-MPF molecule ($p34^{cdc2}$) (5), and that (ii) MPF activity is autocatalytic (2, 6). These assumptions are taken account of in the first two terms in Eq. 1. The third term in this equation describes the inactivation of MPF by a putative inactivase (7) [which may be, for example, a phosphatase (6)], whose activity remains constant throughout the cell cycle (1, 3). In Eq. 2 the rate of change in cyclin concentration, \dot{C} , is given by the difference between its constant rate of accumulation (4) and its rate of degradation. Because cyclin is known to be an essential component of active MPF (8), and its rapid degradation occurs immediately after the maximum in MPF activity, we assume that the degradation rate of cyclin depends on the cellular concentration of active MPF. For simplicity, we also assume that no constraints exist with respect to space and nutrients. Using the above assumptions, we obtain the following dimensionless equations (9):

$$\dot{M} = eC + fCM^2 - \frac{gM}{M+1} \quad (1)$$

$$\dot{C} = i - M \quad (2)$$

Equations 1 and 2 yield limit cycle behavior, that is, oscillatory changes in MPF and cyclin concentrations, with their amplitude and relative phase remaining invariant under small perturbations in initial conditions. This system represents a cell lineage with a constant cell cycle duration, which is resilient to small changes in initial concentrations of MPF and cyclin (Fig. 1A). If moderate amounts of cyclin were added before the maximum in MPF activity, the next cycle was shortened, as indeed was shown experimentally (1, 3, 8), but the system rapidly returned to its normal period in subsequent cycles (Fig. 1B). In contrast, addition of moderate amounts of cyclin after the maximum of MPF activity increased cycle duration, whereas addition of too much cyclin resulted in the complete disappearance of cyclin, later in the same cycle, which we interpret as cell cycle arrest. The stable os-

cillatory behavior of Eqs. 1 and 2 remained unaltered if we allowed for a saturating effect of cyclin on its own degradation, so that Eq. 2 became

$$\dot{C} = i - M \frac{C}{C+k} \quad (3)$$

where k , the reaction constant, was smaller than C . This modification had no qualitative effect on any of the results presented below. In contrast, when the cyclin degradation term was proportional to $-CM$, we did not observe oscillatory behavior in this system.

It has been suggested that somatic and embryonic cell cycles must be fundamentally similar (1). Using our model for embryonic cell cycle, we investigated how increasingly long cell cycles, such as those observed in senescent somatic cells (10), and erratic cycle durations, characteristic of cancer cells (11), could be obtained by manipulating the cyclin and MPF concentrations.

The changes in active MPF and cyclin concentrations are accelerated by the activity of the gene *cdc25* and retarded by that of the gene *wee1* (1). In fission yeast, these genes

Fig. 1. Periodic oscillations of cyclin and MPF [see (9) for units] obtained by a numerical solution of Eqs. 1 and 2, using the Euler method (13). Parameter values, chosen to be in the ranges that yield oscillations, are $e = 3.5$, $f = 1.0$, $g = 10.0$, and $i = 1.2$. Sensitivity analysis shows that, with other parameters unchanged, g can vary in the range 6.0 to 12.1. Other parameters must vary in a much narrower range. (A) Cell cycles with a constant duration. (B) Addition of cyclin early in the cell cycle (0.125 unit of cyclin at a time indicated by the arrow) shortens the duration of that cycle. Subsequent cycles return to a normal duration; _____ as in (A); ... with the addition of cyclin.

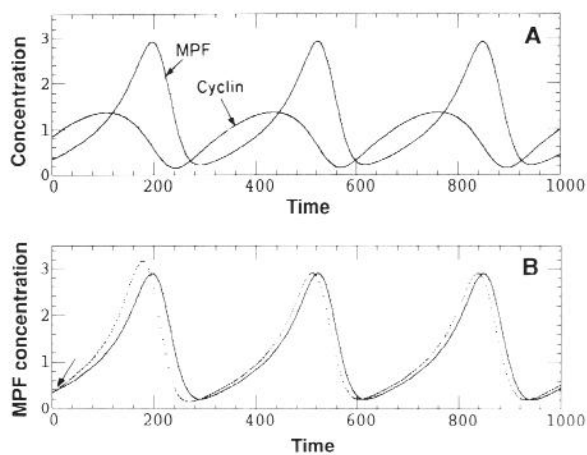


Fig. 2. Simulations of increasingly long cell cycles. Numerical solution of Eqs. 1 and 2 by the Euler method (13), with the right side of Eq. 1 (A) or the right side of Eqs. 1 and 2 (B) multiplied by F , whose value in successive cycles was 1.0, 0.9, 0.8, ... 0.1. Other parameters are as in Fig. 1A.

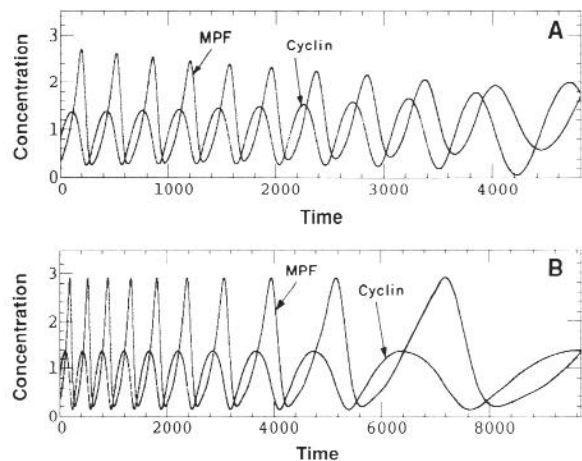
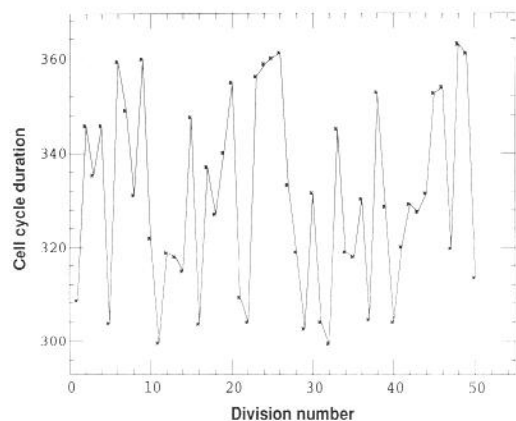


Fig. 3. Erratic cycle durations are drawn as a function of the division number. Numerical solution of Eqs. 1 and 2 by the Euler method (13), with the right side of Eqs. 1 and 2 multiplied by F . Here, F is a random function, uniformly distributed in the range 0.9 to 1.1. Other parameters are as in Fig. 1A.



control the entry into mitosis, and it seems likely that the ratio of their activity is altered by signals that influence the entry into mitosis (1). We described this control by a function F , which changed once per cycle, and checked the sensitivity of the period and amplitude of the oscillations to the modulation by F . To allow for a decreasing activity ratio of *cdc25* to *wee1*, we assumed that F decreased, once per cycle, starting from a value of one. Numerical computations, that included multiplying the right side of Eq. 1 alone, or Eqs. 1 and 2, by F , showed a gradual increase in phase, or cell cycle length. However, the pattern of change in MPF and cyclin phases and amplitudes depended on the modulated reaction. When only the rate of change in MPF concentration was modulated through the function F , the amplitudes of MPF oscillations progressively decreased and the amplitudes of cyclin oscillations increased (Fig. 2A), and the minima rapidly reached a value close to zero. The latter effect may be interpreted as a cell cycle arrest. When both processes were modulated, the amplitudes did not change (12), but the phase increased and tended to infinity when F tended to zero (Fig. 2B). In both cases the number of cell divisions until a cell cycle arrest was reached depended on the step size of the function F . On the basis of these results, we suggest that the modulation of the MPF and cyclin reaction rates may be responsible for the roughly constant number of cell divisions observed in some cell lineages. When the modulation was in the right side of Eq. 2 alone, or in each one of the accumulation terms alone, or in the degradation terms of each reaction alone, the effect on the increase in cell cycle duration appeared only marginal.

Erratic changes in cell cycle length were obtained when the function F was allowed to vary randomly, once per cycle, staying close to unity. Here too, if F modulated both reactions, the amplitudes of concentrations did not change, and the simulated cell line appeared "immortal" (Fig. 3). In contrast, if only the MPF reaction was modulated, cyclin concentrations occasionally reached zero.

The work presented here shows that the process in which cyclin accumulation activates MPF, which leads to cyclin degradation and hence in turn to MPF inactivation, suffices to explain multiple cell cycles. Our model relies on limited assumptions to yield robust oscillations in MPF and cyclin amounts. Time delays or thresholds postulated earlier were not required. The model mimics various aspects of the observed dynamics, such as cell cycle shortening after cyclin addition, or abrupt MPF inactivation. However, the possibility that other assump-

tions can generate a similar behavior should not be excluded. More information about the temporal pattern of change in the concentrations of these proteins may be instructive for verifying the role of MPF and cyclin in normal and neoplastic cell cycles.

Further experiments, in young, senescent, and neoplastic cell lines are warranted for quantifying the ratio of *cdc25* to *wee1* expression in subsequent cell divisions. These experiments may verify our prediction that senescence may be associated with a progressive decrease in the activity ratio of the genes that control the rate of change of the MPF and cyclin reactions, and that cancer cells are characterized by erratic changes in this ratio.

REFERENCES AND NOTES

1. A. W. Murray and M. W. Kirschner, *Science* **246**, 614 (1989).
2. P. Nurse, *Nature* **344**, 503 (1990).
3. A. W. Murray and M. W. Kirschner, *ibid.* **339**, 275 (1989).
4. P. H. O'Farrell, B. A. Edgar, D. Lakich, C. F. Lehner, *Science* **246**, 635 (1989).
5. A. W. Murray, M. J. Solomon, M. W. Kirschner, *Nature* **339**, 280 (1989).
6. A. W. Murray, *ibid.* **342**, 14 (1989).
7. This term is a classical description of enzymatic decay [C. C. Lin and L. A. Segel, *Mathematics Applied to Deterministic Problems in the Natural Sciences* (Society for Industrial and Applied Mathematics, Philadelphia, 1988)].
8. G. North, *Nature* **339**, 97 (1989).

9. The dimensional equations that describe the MPF and cyclin reactions are

$$\frac{dM'}{dt'} = e'C' + f'C'M'^2 - \frac{g'M'}{M' + h'} \quad (\text{A1})$$

$$\frac{dC'}{dt'} = i' - j'M' \quad (\text{A2})$$

It is convenient to introduce dimensionless variables. Thus we measure the MPF and cyclin concentrations as multiples of the half saturation concentration h' and measure time as a multiple of the inverse rate constant $1/j'$:

$$M = \frac{M'}{h'}, C = \frac{C'}{h'}, t = j't' \quad (\text{A3})$$

Replacing Eq. A3 in Eqs. A1 and A2 and dividing by $j'h'$, we obtain Eqs. 1 and 2, where the dimensionless reaction coefficients become

$$e = \frac{e'}{j'}, f = \frac{f'h'^2}{j'}, g = \frac{g'}{j'h'}, i = \frac{i'}{j'h'} \quad (\text{A4})$$

($e, f, g, i, > 0$).

10. S. W. Sherwood, D. Rush, J. L. Ellsworth, R. T. Schimke, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9086 (1988).
11. A. B. Pardee, *Science* **246**, 603 (1989).
12. The multiplication of both equations by the function F may account for a gradual increase in cell volume in senescent cell lines (10), which may slow the reactions. It can be shown analytically that multiplications of both equations by the same factor F essentially just modifies the time scale of events but does not alter the amplitudes of the oscillations.
13. S. D. Conte and C. de Boor, *Elementary Numerical Analysis* (McGraw-Hill, New York, ed. 2, 1965).
14. We thank A. Kimhi and L. A. Segel for comments on the manuscript.

28 August 1990; accepted 12 December 1990