Tuning the cell cycle: a model based on averaging

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Abstract. The ability of intercellular communication and the basement membrane to revert the phenotypic behaviour of malignant cells suggests that such cells can be tuned to behave more benignly. In addition, the large variation in cell doubling times observed in tumour cells poses the question of whether or not cell doubling times, and hence, patient survival, can be lengthened by therapeutic intervention. In both cases, the understanding may be enhanced by obtaining a parsimonious and tractable model of the cell cycle which behaves appropriately and suggests a philosophical framework for addressing these complex issues. We introduce a simple two-dimensional model based on averaging cyclin and maturation promotion factor over a fast oscillating subsystem that exhibits the basic features of cellular division, and discuss the ramifications of the model.

INTRODUCTION

Several authors formulated the early models of the cell cycle oscillator based on the phosphorylation of Cdc2 or homologous gene products on the threonine site and dephosphorylation on the tyrosine location (Goldbetter 1991; Norel & Agur 1991; Tyson 1991). Since then many authors have extended the modelling, sometimes increasing the number of dimensions to as many as a dozen (Sveiczner *et al.* 2000). While these more complex models reflect the increasing knowledge of cell cycle biology, a two-dimensional system can still provide additional insight.

To build the two-dimensional model, it is no longer sufficient to suppose that only two species are interacting. Instead, we will take the perspective (Klevecz *et al.* 1992) that there is an underlying oscillation of a shorter period than that of the cell cycle that emerges from the transcriptome and various complex interactions. With a higher frequency oscillation, however, we can discuss the variables of our two-dimensional system as their average over the faster emergent oscillation (Frankel & Kiemel 1993). This simple assumption immediately suggests quantized cell division times (Klevecz 1976) a problem for earlier cell cycle models, as threshold crossings will most often occur near the peak or upswing of a fast oscillation.

We then model the cell cycle as determined by the average behaviour of two elements. It is not required, however, that the individual elements in this model correspond directly to chemicals involved in the cell-cycle. They could be ratios, sums, differences, or any other transformation that one may envision. For clarity, however, we will use the names of two essential species, a generalized cyclin variable and a maturation promotion factor (MPF) variable.

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Most of the early models exhibit a Hopf bifurcation, whereby a parameter goes beyond a threshold and produces a stable limit cycle of finite frequency. These earlier models assume a constant production of cyclin to offset the rapid degradation of cyclin due to phosphorylation involved in the cell cycle. As the production of cyclin is increased, each of these models will start to oscillate, representing cell division, with a finite division frequency. This precludes the possibility of extremely long intermitotic times.

In the early two-dimensional approximations of the cell cycle, the cyclin nullcline, the set of values for which the cyclin concentration would not change, is assumed to be a straight line. In fact, given the complex web of cyclin activity, it is possible to find pathways that allow for autocatalysis (Yee *et al.* 1996; Kohn 1999). By modelling such behaviour, we see that the nullclines of the system are changed in such a way as to permit a different type of onset to division that allows for long intermitotic times.

THE MODEL

For the two-dimensional model employed here, autocatalytic cyclin is combined with the simplicity of Norel and Agur's model and Tyson's non-linear MPF formation.

Let C = average cyclin concentration over T , the ultradian period, and M = average concentration of maturation promotion factor over T , to produce our two variable averaged model:

$$\dot{M} = \tau_M \left(eC + f_c CM^2 (1 - \alpha M) - \frac{gM}{M+1} \right) \quad (1)$$

$$\dot{C} = \tau_C (i + a\sigma(C) - M) \quad (2)$$

where

$$\sigma(C) = (1 + \tanh(\frac{c-\theta}{\delta})) \quad (3)$$

and represents the autocatalytic effects of cyclin. The first term in equation 1 represents that MPF is produced by cyclin, and the second term represents that MPF can cause the dephosphorylation of the inactive MPF, and autocatalyse itself. The term $1 - \alpha M$ represents the physiological limitations on the extent of this step, and the final part of the first equation represents (Norel & Agur 1991) the inactivation of MPF due, perhaps, to a protein kinase or simple degradation. The second equation has i representing the independent production of cyclin. The term $\sigma(C)$ we have already mentioned, and the final term represents the degradation of cyclin due to the MPF uptake and phosphorylation of cyclin that occurs after the degradation of MPF (Norel & Agur 1991; Tyson 1991).

The parameter values are in Fig. 1, where the basic structure of the nullclines are given. As Figs 1 and 2 show, as i is increased, the system can go from a steady state, to long-period oscillations.

The system can exhibit finite-period onset of cell division by decreasing a , reducing the autocatalytic effects of cyclin. Finally, when a is zero, there is no feedback of cyclin on itself and the system will behave like the early models. The parameters τ_C and τ_M represent, respectively, the time scale of build up (or decay) of cyclin and MPF.

Figure 1. Parameter values for the saddle-node system: $a = 1$, $\tau_M = 2$, $\tau_C = 1$, $f_c = 1.5$, $e = 3.5$, $\alpha = 0.2$, $g = 12$, $\theta = 0.8825$, $\delta = 0.05$. The value of i is specified in each panel. For an explanation of the dimensionless aspect of the time and concentrations see Norel & Agur (1991). Note that as i is increased from 0.2935 to 0.4 the cyclin nullcline (where $\dot{C} = 0$) is shifted to the right (the MPF nullcline corresponds to $\dot{M} = 0$). As this occurs, the intermitotic times go from about 50 time units, to five time units. In the time courses, the dashed line represents the cyclin concentration, and the solid line represents the MPF concentration. Figure 2 has a clearer picture of the saddle, and node equilibrium points which have already coalesced in this figure. At the point where the two equilibrium coalesce there is an infinite period intermitotic time representing a homoclinic orbit.

Note that the time scale of MPF or cyclin production is not changed in this process of altering the intermitotic time. Instead, the model creates a long intermitotic time by having dynamic behaviour associated with the coalescing of two steady-states, one unstable and the other stable. This is known as a saddle-node bifurcation. Previous models contained a Hopf bifurcation which relates to the change in character of one steady state. Note that excitability is easily explained as a perturbation across the separatrix near the unstable steady state. In Fig. 2, if we start as shown on the right of the unstable equilibrium point, one large excursion is made in MPF levels, before returning to the rest state. This phenomena is known as excitability as a perturbation could displace the system to such a starting point. Furthermore, a careful examination of the nullclines reveals that, if instead of varying i , the cyclin independent production of cyclin, we varied the degree of autocatalysis, we could go from a non-excitable state to an excitable

Figure 2. Excitability. Note the three equilibrium points. The far right equilibrium point is unstable and remains that way. A decrease in a , the amount of autocatalysis, would stabilize this point, and, via a Hopf bifurcation, the oscillations would cease. The left-most point is stable, and the middle equilibrium is unstable. If the system is perturbed, then the system goes through one division cycle, and settles into the far left equilibrium point demonstrating excitability.

one. See Edelstein-Keshet (1988) for a good introduction to nullcline analysis, and Guckenheimer and Holmes (1983) for mathematical discussion of bifurcation theory.

It is important to note that the equilibrium points in this averaged system correspond to relatively stable high-frequency excursions of both MPF and cyclin. Other models (Klevecz *et al.* 1992), based on chaotic rhythms continually cycling in the cells could also produce long intermitotic times by modelling the division process as a threshold on the behaviour of certain chemical constituents. The main advantage of the averaged system of equations proposed here is simply tractability. The quantization of cell division times created by considering these equations as resultant from averaging over a higher frequency process is obtained simply by considering that in the unaveraged model, the concentration of the constituents would cross any threshold on the upswing of the ultradian cycle. For an example of the role of averaging over subharmonics and a variety of proofs of convergence see Frankel & Kiemel (1993).

CONCLUSION

We have established a simple, yet plausible, model which could explain long intermitotic times, quantized cell cycle times and excitability. The simplicity of the model allows for the generalization

of the related phenomena. It shows that autocatalysis can induce long time scale behaviour in the cell cycle. Furthermore, the model is capable of exhibiting a Hopf bifurcation with finite dividing frequency at the decision point when the autocatalysis is tuned down.

From a modelling perspective, other systems with onset to periodic behaviour showing extremely long periods or excitability are also good candidates for saddle-node behaviour and this should be examined along with autocatalysis as a possible cause.

From a therapeutic viewpoint, altering the emergent ultradian rhythm is unlikely, but down-regulating the autocatalytic cyclin production or reducing the independent cyclin production, along with modifying the pharmacology related to MPF production, can theoretically lengthen survival by reducing the number of cells dividing or increasing the cell doubling time. More generally, we have now both experimental evidence (Mehta *et al.* 1991; Deng *et al.* 1996; Park *et al.* 2000) and models that suggest the importance of tuning genetically transformed cells into a more benign class, and by simply considering such possibilities, more avenues for therapeutics can arise. Directions range from indirect methods, such as altering gap junctional communication, or improving the basement membrane characteristics, to directly tuning the cellular chemistry. These steps are not too far off, as carotenoids and genetic insertion have already demonstrated their ability to modify gap junctional communication and normalize transformed cells, while certain experimental therapeutics can reconstitute basement membrane and normalize cells. Finally, substances such as retinoids can directly modify basic cellular metabolism such as polyamine synthesis (Persson *et al.* 1988) and alter tumour growth and development.

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