

# A Mathematical Model of Cyclin B1 Dynamics at the Single Cell Level in Osteosarcoma Cells

J. Pérez-Velázquez, N. D. Evans, M. J. Chappell\*  
R. J. Errington, P. J. Smith, I. Khan\*\*

\* School of Engineering, University of Warwick, Coventry CV4 7AL,  
UK (e-mail: Judith.Perez-Velazquez@warwick.ac.uk;  
Neil.Evans@warwick.ac.uk; M.J.Chappell@warwick.ac.uk)  
\*\* School of Medicine, Cardiff University, Heath Park, Cardiff CF14  
4XN, UK (e-mail: erringtonrj@cardiff.ac.uk; SmithPJ2@cardiff.ac.uk;  
khan1a1@cardiff.ac.uk)

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**Abstract:** Cyclin B1 tracking provides information on cell cycle progression and cell-cycle regulator dynamics. We have developed a mathematical model which describes the continuous tracking of cyclin B1 through the cell cycle at the single cell level, including interactions with the cyclin B1 inhibitor, p21. The cell line used is a cancer cell line, human osteosarcoma (U-2 OS). An examination of the sensitivity of the model is presented, where the aim is to identify those parameters which have most influence on the cyclin B1 profile and its changes through the cell cycle. High temporal resolution cyclin B1 data involving non-invasive techniques (green fluorescent protein, GFP) were used to validate the model.

Keywords: Biomedical Systems; Biomedical control; Life Cycle; Modelling.

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## 1. INTRODUCTION

We have developed a mathematical model of cyclin B1 dynamics through the cell cycle. Data arising from individual human osteosarcoma U-2 OS cells, produced by the Department of Medical Biochemistry and Immunology at Cardiff University, were used in the development of the model and subsequently for model validation. The model is an extended version of a transition state cell cycle model by Tyson and Novak [2001] and has been linked with a model accounting for the inhibition dynamics of p21 on cyclin B1 [Pomerening et al., 2003, 2005].

Populations of cells regulate proliferation through the cell cycle. The cell cycle is a sophisticated mechanism which ensures that cellular division will lead to two identical daughter cells. The cell cycle is usually divided into four phases: Gap 1 (G1), Synthesis (S), Gap 2 (G2) and Mitosis (M) [Thomas and Goodyer, 2003]. See Fig. 1 (A) to (C). The cycle is tightly regulated and includes checkpoints at which it could stop if the correct signals are not satisfied [Thomas and Goodyer, 2003]. Central molecules in the regulation of the cell cycle are cyclins and cyclin-dependent kinases (Cdks). There are several different cyclins which are active in different parts of the cell cycle, cyclin B1 in particular is a mitotic cyclin. The amount of cyclin B1 (which binds to its cyclin-dependent kinase Cdk1) and the activity of the cyclin B1-Cdk1 complex rises through the cell cycle until mitosis and later decreases (see Fig. 1 (D)). The action of the Cyclin and cyclin/Cdks complexes are, in turn, regulated by inhibitors (such as p21) and activators.

Novel technologies like time lapse microscopy [Feeney et al., 2003, White and Errington, 2005] allow high resolution live-cell protein-tracking providing, in particular, continuous read-out of cyclin B1 through labelling with green fluorescent protein (GFP). High resolution monitoring of cyclin B1 levels through the mammalian cell cycle has proved to be useful at different levels, from testing research hypotheses to developing experimental therapeutics [Thomas, 2003]. In particular, it potentially offers the opportunity to study the cell cycle under stress conditions (DNA damage) [Khan et al., 2007]. For example, DNA damage activates a signalling cascade that can block cell cycle progression, which directly affects the cyclin B1 profile.

The model reproduces basic features of cyclin B1 tracking through the cell cycle, for example cyclin B1 concentration at the start of tracking (this is equivalent to the cell cycle phase in which the cell was at the time of the start of the tracking) will affect the time of the first mitotic event. If the tracking starts when a cell is in G1-phase the first mitotic event will take longer than for a cell in G2, we have verified this with the model simulations. More importantly, the model has also generated predictions that may be testable experimentally. For example our model predicts that cells will not enter mitosis under some circumstances, for example if levels of p21 are changed. As part of the analysis performed to our model, we study the sensitivity of the parameters involved. This allows us to infer information from the model concerning components of the system that cannot be measured directly experimentally. We also successfully fit our results to the experimental data. We are able to investigate how the parameters affect factors

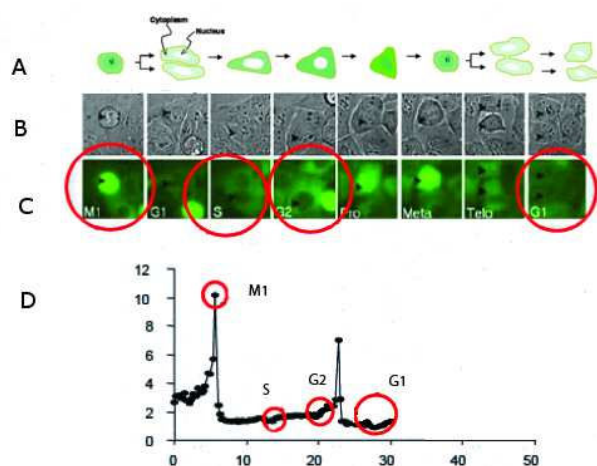


Fig. 1. Continuous cell cycle tracking: (A) Schematic diagram of a cell expressing the cyclin B1-GFP reporter as it progresses through the cell cycle to mitosis. (B) Snapshots from a time lapse phase contrast sequence. (C) Corresponding fluorescence channel, cell is tracked through G1, S and G2 and mitosis. (D) Single cell tracking of cyclin B1-GFP intensity throughout the cell cycle phases. Time scale is in hours.

such as time between mitotic events, time of first mitotic event, number of mitotic events and cyclin levels.

## 2. DYNAMICS OF CYCLIN B1

Cell cycle events are controlled by a network of molecular signals, whose central components are cyclins, Cdks and a group of proteins, making up the anaphase-promoting complex (APC), activated at the end of the cell cycle. The concentration of cyclins varies in a cyclical fashion during the cell cycle. A cyclin forms a complex with its partner, a cyclin-dependent-kinase. There are several types of cyclins. As an example, Cyclin B1 is a mitotic cyclin which has an important role in the G2-M phase transition of the cell cycle. Cyclin B1 binds to the cyclin-dependent kinase Cdk1. Cyclin B1/Cdk1 inhibitors can increase or decrease the frequency of cell division or stop division all together.

### 2.1 p21, a Cyclin Inhibitor

The cyclin-dependent kinase inhibitor p21 is a human gene that encodes a cyclin-dependent kinase inhibitor that directly inhibits the activity of cyclin-Cdk complexes. The p21-activated kinases signal through a number of cellular pathways is fundamental to growth [Anupama et al., 2005], for example p21 could directly prevent mitosis by inactivating and maintaining the inactive state of mitotic cyclin-Cdks complexes [Charrier-Savournin et al., 2004]. The expression of p21 is controlled by the tumour suppressor protein p53.

### 2.2 Cdc25, a Cyclin Activator

Cdc25 proteins control entry into and progression through various phases of the cell cycle, including mitosis and S

phase. “Cdc” refers to cell division cycle. The central role of Cdc25 in the cell cycle makes them potential targets for novel anti-cancer agents.

### 2.3 Experimental Data

The parental cell line used was a human osteosarcoma cell line U-2 OS derived from a 15 year old Caucasian female. High temporal resolution monitoring of cell cycle progression enabled the tracking of single cells in a non-invasive manner, using green fluorescent protein (GFP) (see Figs. 2(a) and 2(b)). The cells were transfected with G2M Cell Cycle Phase Marker (GE Healthcare, UK). The expressing cells were enriched using high speed FACS and sorted into well plates (1 green fluorescent cell/well). Colonies were expanded and clones whose green fluorescence varied with the cell cycle as predicted and as determined by flow cytometry were selected for the current study. The cultured dishes were placed on to a time-lapse instrument designed to capture bright-field phase images and GFP fluorescence. Sequences were captured every 20 minutes for 48 hours, ordinarily at least three fields per treatment regime. At the end of the experiment the images were stacked, saved and transformed into a parametrised database using MetaMorph (Molecular Devices, California) and FluroTRAK (Cardiff in-house software). These data attribute a unique encryption tag for each starting cell within the field (termed progenitor cell), so that every subsequent event within the lineage can be rooted to the progenitor cell including tagged parameters.

## 3. CELL CYCLE MODELS

During the cell cycle the cell passes through two irreversible transitions. The first of these transitions occurs at the end of G1 and can be called *Start*. If conditions are suitable, the cell commits itself to DNA synthesis and division. This transition is irreversible and can be called *Finish*, it occurs when DNA replication is completed. In parallel, as mentioned earlier, progression through the cell cycle is controlled by a series of molecular signals whose central components are the proteins: cyclins, their associated Cdks and APC. Their role is highly complex, but for modelling purposes Tyson and Novak [2001] suggested to take into account only their essential features. During G1, Cdk activity is low because the relevant cyclin partners are missing. At *Start*, Cdks are activated and remains high during S, G2 and M. At *Finish*, the anaphase protein complex (APC) is activated. Tyson and Novak developed a model that reproduces these antagonistic interactions between cyclin/Cdks and APC to show how the progress through the cell cycle can be thought of as the irreversible transitions *Start* and *Finish* between two stable states G1 and S-G2-M.

Tyson and Novak’s model is just one example of a huge range of more general cell cycle models proposed by their research group, see the review by Clyde et al. [2006]. Although a great deal of knowledge of the biochemistry and the physical processes of the proteins that regulate the cell cycle is fairly recent, mathematical models of the cell cycle can be traced back to as early as the 1970s [Hastings et al., 1977, Tyson and Sachsenmaier, 1978, Tyson, 1974/75]. The focus of such theoretical studies ranges from phase

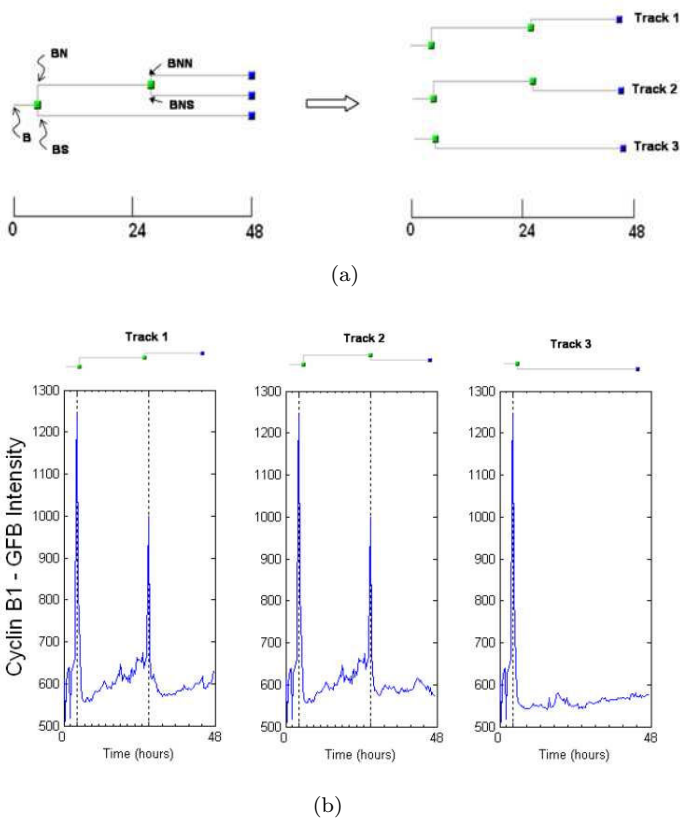


Fig. 2. (a) An example of encoded cell lineage from a progenitor cell (B) where the cell divides into two daughter cells. The north daughter (BN) divides again into BNN and BNS, while the south daughter BS failed to divide within the duration of the experiment. Three living cells (*BNN*, *BNS* and *BS*) at the end of the experiment yielded three tracks labelled as track 1, 2, and 3, respectively. (b) Single cell tracking of intensity for the three tracks derived from the encoded lineage, the plot depicts the cyclin B1-GFP intensity (nucleus) profile along each track.

transitions in the cell cycle [Alarcón and Tindall, 2007, Novák et al., 1999] to the response of the cell cycle under special conditions, such as for cancer cells and the use of anticancer agents [Alarcón et al., 2004, 2006], including some cyclin B-based early models [Goldbeter, 1991, Tyson, 1991]. There are instances where the predictions of mathematical models have been tested. Pomerening et al. [2003] carried out experiments and concluded that a bistable positive feedback system together with a negative feedback loop produces self-sustaining oscillations (spikes). Their model is for the cell cycle of *Xenopus laevis* embryos, which they used to investigate whether Cdc2-cyclin B functions as an autonomous oscillator. They also examined Cdc2 activation and showed this to be bistable, exhibiting hysteresis.

Negative feedback and oscillations have been consistently discussed in cell cycle models [Baguley and Marshall, 2005]. Negative feedback occurs where the products of a process can act at an earlier stage in the process to inhibit their own formation. We have used an adaptation of the approach of Pomerening *et al* [Pomerening et al., 2003, 2005] to regulate Cyclin B1 dynamics. Pomerening *et al*

studied the changes of Cdc2/cyclin B through the cell cycle as an autonomous oscillator. Their model crucially requires a negative feedback loop: cyclin accumulates and produces active Cdc2/cyclin B2; Cdc2 activates APC; APC then promotes cyclin degradation and inactivates Cdc2. Cdc2 regulation also requires positive feedback loops, in which active Cdc2/cyclin B stimulates its activator Cdc25 and inactivates its inhibitors Wee1 and Myt1.

#### 4. THE MODEL

A model is formulated here in which the essential components are cyclin B1 dynamics and p21 activity. The model is a modified version of the approach taken by Tyson and Novak [2001] where the governing equations account for the regulation of Cdk/Cyclin B and APC, which constitute the cell cycle engine. Additionally, we account for Cyclin B1 regulation through the dynamics of the cyclin B1 activator and inhibitors. The equations are for Cyclin B1/Cdk dimers  $Y$ ; active Cdh1/APC complexes  $X$  (average concentrations, grams of protein per gram of total cell mass);  $P_{21}$  a cyclin inhibitor; an equation for cell growth (mass,  $M$ ); and  $Z$  a cyclin activator. Cdh1 is activated by a generic APC activator  $A$ . The model equations are given below:

$$\frac{dY}{dt} = k'_1 - (k'_2 + k'_3 X + k'_4 A)Y + k'_5 P_{21}, \quad (1a)$$

$$\frac{dX}{dt} = \frac{(k''_1 + k''_2 A)(1 - X)}{(k''_3 + 1 - X)} - \frac{(k''_4 MXY)}{(k''_5 + X)} \quad (1b)$$

$$\frac{dM}{dt} = gM \left(1 - \frac{M}{m_s}\right) \quad (1c)$$

$$\frac{dA}{dt} = a_1 A - a_2 X \quad (1d)$$

$$\frac{dZ}{dt} = \frac{z_1 Y^4 Z}{z_2^4 + Y^4} - z_3 Z \quad (1e)$$

$$\frac{dP_{21}}{dt} = -\frac{p_1 Y^4 P_{21}}{p_2^4 + Y^4} + p_3 P_{21} \quad (1f)$$

Equations (1a)-(1d) are an adaptation of Tyson and Novak's equations for the antagonistic interactions between cyclin/Cdks and APC [Tyson and Novak, 2001]. Equations (1e)-(1f) account for cyclin B inhibition and activation, being an adaptation of Pomerening's mechanism [Pomerening et al., 2003]. The term  $-a_2 X$  in equation (1d) is a negative feedback by  $X$  on the production of  $A$  since an increase in  $X$  decreases the production of  $A$ . Similar negative feedback mechanisms in cell cycle models have been considered previously [Pomerening et al., 2005]. Note that  $A$  is an activator of  $x$  if  $\frac{\partial f}{\partial A} > 0$  [Murray, 2001], where  $f$  is the right-hand-side of equation (1b). In our case  $\frac{\partial f}{\partial A} = \frac{k''_2(1-X)}{k''_3+1-X}$  which is positive for our chosen parameter values (see Table 1), as  $X \leq 1$ . It is assumed that  $p21/CycB/Cdk$  trimers are always in equilibrium with p21 monomers and  $CycB/Cdk$  dimers; an equation for the trimer can be written in terms of  $y$  and the cyclin inhibitor (see [Tyson and Novak, 2001]).

We solved system (1) numerically using the commercial simulation software package FACSIMILE (MCPA Software, UK), which uses a robust (implicit prediction-correction)

	Original	Fitted	Component
$k_1'$	0.04*	0.04	cyclin synthesis
$k_2'$	0.04*	0.041	cyclin degradation
$k_3$	1*	1	cyclin degradation by Cdh1/APC
$k_4'$	1*	1	cyclin degradation by Cdh1 Activator
$k_5'$	0.000154	0.00015821	p21
$k_6''$	1*	1	Cdh1/APC production
$k_7''$	12	12	Cdh1/APC activation
$k_8''$	35*	35	Cdh1/APC degradation
$g$	0.01*	0.01	growth rate
$m_s$	10*	10	maximum size to which a cell may grow if it does not divide
$a_1$	0.001336	0.0013	Cdh1 activator production
$a_2$	0.001336	0.001	Degradation of Cdh1 activator by Cdh1
$z_1$	0.0016	0.0016	Rate of cyclin B1 activator synthesis
$z_2$	0.001336	0.0013	Hill coefficient
$z_3$	0.001336	0.0013	Rate of cyclin B1 activator degradation
$p_1$	0.0000008	0.0000008	p21 activation rate
$p_2$	0.001336	0.0013	Hill coefficient
$p_3$	0.000144	0.0001443	p21 deactivation rate

Table 1. Model constants ( $min^{-1}$ ). Dimensionless constants  $k_3'' = 0.04, k_5'' = 0.04$ . Source for \*: Tyson and Novak [2001].

numerical integrator. The model parameters are listed in Table 1. We use initial conditions  $X(0) = 0.05; Y(0) = 1.05; M(0) = 5; A(0) = 0.9; P_{21}(0) = 1.3; Z(0) = 0.001$ . Typical simulations are presented in Fig. 4.

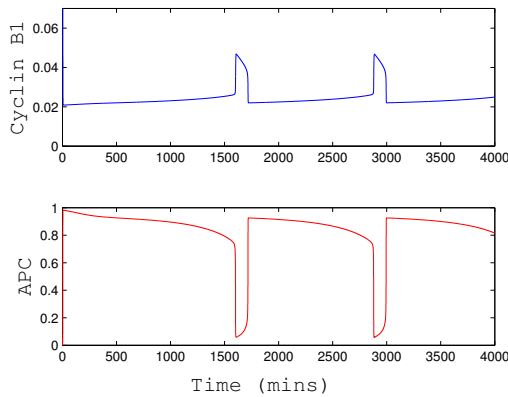


Fig. 3. Simulation of system (1), with parameter values in Table 1. Cyclin B1 and APC profiles. The spike in the Cyclin B1 profile is only an indication (time) of mitosis (cell division).

#### 4.1 First Mitosis and Initial Cyclin B Concentration

For a single cell, Cyclin B1 concentration at the start of the tracking is equivalent to the cell cycle phase in which the cell lies. If the tracking starts when a cell is in G1-phase, and therefore the Cyclin B1 concentration is low, the first mitotic event will take longer than for a cell that was in G2 (and for which Cyclin B1 concentration is higher) at the start of the tracking. The model reproduces this feature (see Fig. 5).

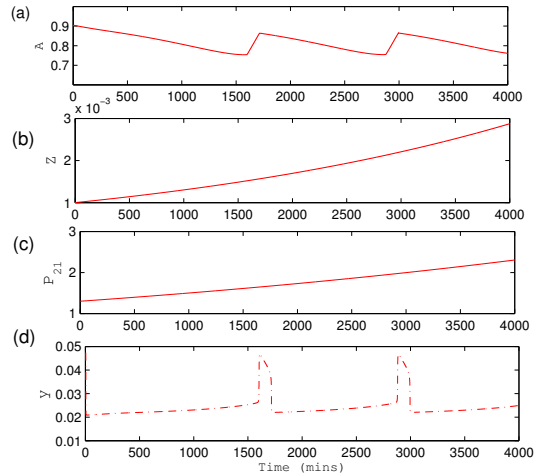


Fig. 4. Simulation of system (1), with parameter values in Table 1. From top to bottom: (a) APC activator, (b)  $Z$  and (c)  $P_{21}$  profiles (d) Cyclin B1. Spikes are an indication of cell division.

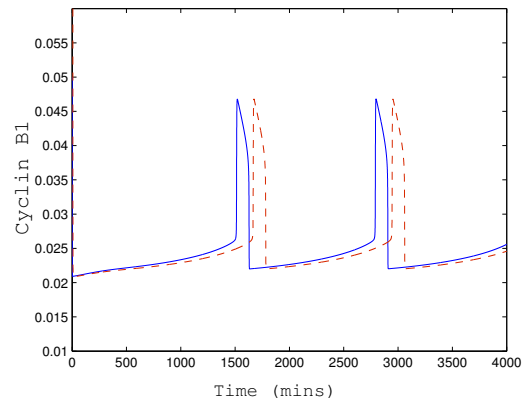


Fig. 5. Changing the initial cyclin concentration  $Y(0)$  affects the time of the first mitotic event, note however that the time between mitotic events (intermitotic time) remains relatively unchanged. Simulations of system (1) and parameter values from Table 1, for the solid curve  $Y(0) = 0.05$  and for the dashed curve  $Y(0) = 10.05$ .

#### 4.2 Parameter Sensitivity

We note that not all variables in the model are observable experimentally, in which case our model can still produce information concerning parts of the system that are not directly measured. We now analyze the effects of varying certain chosen parameter values. To this end, we first vary  $k_1''$ ; this parameter controls Cdh1/APC production. In Fig. 6 we show how the behaviour of the model variables changes as  $k_1''$  is increased. The main feature is that, for  $k_1'' = 1.2$ , the first mitotic event happens earlier than when  $k_1'' = 1$  (see Fig. 6(b)), which is to be expected, if there is a higher concentration of APC than usual this seems to speed up the cycle. This, in turn, affects the time of the second mitotic event and gives enough time to produce a third mitosis. Note that the intermitotic time is also affected, in particular it decreases when  $k_1''$



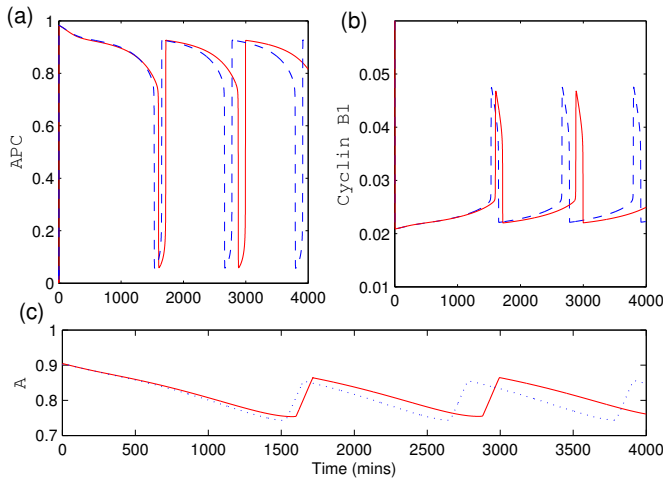


Fig. 6. Varying the parameter  $k_1''$ . The solid line corresponds to  $k_1'' = 1$  and the dashed line  $k_1'' = 1.2$ . (a) APC (b) Cyclin B1 (c) A. The parameter values used for these simulations are shown in Table 1.

increases. Indeed for values of  $k_1'' < 1$  we are not able to see any mitosis whatsoever (results not shown), which is in qualitative agreement with the role of APC, if there is no or little effect of APC, the cell cycle will not be completed. This change (as one may expect) does not affect the cyclin regulators, but it does produce a slight effect on the APC activator Z (see Fig. 6 (c)).

#### 4.3 Comparison to Experiments

Figure 7 shows model fits with the parameters taking values from Table 1. These fits show that there is close reproduction of the data by simulated output from the model, which confirms Pomerening's results regarding the need for positive feedback together with a negative feedback loop. Note that the comparison to experiments considers one full mitosis (from the first mitotic event to the second), once the cell has divided and produced two daughters they will each have their own cyclin B1 track (see Fig. 2(b)) and therefore parameter values (volume for example) would need to be adjusted.

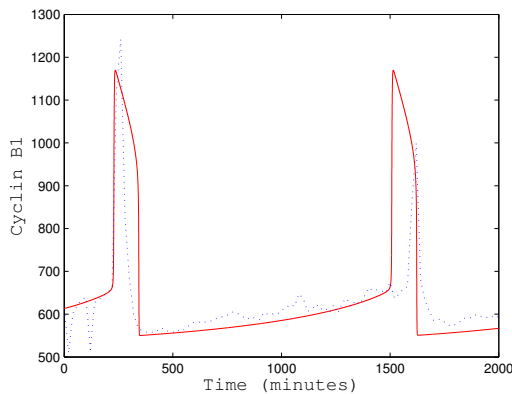


Fig. 7. Simulated output from model (1) with parameters taking values from Table 1 plotted (solid) against the experimental data (dotted).

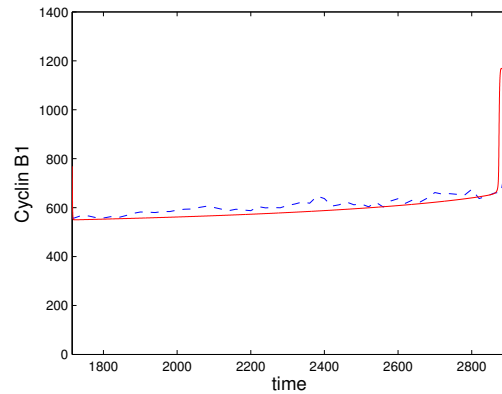


Fig. 8. Simulated output from model (1) (parameter fitting was performed on  $a_2$  and  $k_5'$ ) plotted (solid) against the experimental data (dotted). One mitotic event. Parameter Values from Table 1.

#### 4.4 Parameter Estimation

Model fits in Fig. 7 comprise one full mitotic event. After mitosis, the size of the cell changes and therefore it is expected this will have an effect on the rest of the system conditions and parameters would need to be estimated for each mitotic event. FACSIMILE (MCPA Software, UK), which uses a robust (implicit) numerical integrator, is used to perform the parameter estimation. Figure 8 shows the model fits using the fitted parameters over one mitotic event for a typical cell.

## 5. CONCLUSION

We have developed a mathematical model of cyclin B1 progression through the cell cycle. We have used cyclin B1 data arising from individual human osteosarcoma U-2 OS cells to validate the model. Our model is an extended version of the Tyson and Novak [2001] transition state cell cycle model and has been linked with a model accounting for the inhibition dynamics of p21 on cyclin B1 [Pomerening et al., 2005, 2003]. We have shown that the model reproduces basic features of cyclin B1 tracking through the cell cycle, but also offers the possibility to infer information from the model concerning parts of the system that are not directly measured. We successfully fit our results to the experimental data provided for certain sets of parameter values. More importantly, the model has also generated predictions that may be testable experimentally. Through the study of the sensitivity of the parameters we have been able to identify which (and how) parameters affect important features of the cell cycle, like time between mitotic events, time of first mitotic event, number of mitotic events and cyclin levels.

Tyson and Novak's Cell Cycle model allows us to account for cyclin B1 dynamics. However, our model confirms Pomerening's results on the need for the inclusion of both a negative and a positive feedback loop and it becomes unnecessary to artificially introduce periodic-like behaviour. While our model is consistent with experimental results and is able to generate predictions, there are some additional features that could improve the current version of the model, in particular we need to define how the cell

volume will be divided after mitosis. In addition we could incorporate a stochastic approach to deal with the issue of asymmetric division. At present we have only accounted for the Cyclin B1 present in the nucleus but this is a simplification. Experimental data are available for other cell regions and this will be the subject of future work. One of the main things to point out is the need of the APC activator profile to be cyclic, introduced by the negative feedback.

Having successfully reproduced cyclin B1 dynamics in normal conditions, our aim is to extend the current model to introduce the cell cycle response to perturbations, such as anti-cancer agents. In such cases our model would correspond to describing cells in control conditions and we can add the effect of drug by incorporating its dynamics. This could be done by considering the coupling of our cell cycle model with a pharmacokinetic (PK) model [Evans et al., 2005, 2004]. This approach allows the dynamic and temporal interactions of the drug with its target to be examined and would enable us to investigate how the cell cycle can be affected by the delivery of the agent and its impact on cell growth and death. Our aim will be to study the drug dynamics as a perturbation of the mammalian cell cycle using the same protein-tracking technologies as the control case. There are already available data for this purpose produced by the same research group at Cardiff.

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