**Ab initio** phenomenological simulation of the growth of large tumor cell populations

Roberto Chignola$^{1,2}$, Alessio Del Fabbro$^{2,3}$, Chiara Dalla Pellegrina$^1$ and Edoardo Milotti$^{2,4}$

1 Dipartimento Scientifico e Tecnologico, Università di Verona, Strada le Grazie 15 – CV1, I-37134 Verona, Italy
2 Istituto Nazionale di Fisica Nucleare, Sezione di Trieste, Via Valerio 2, I-34127 Trieste, Italy
3 Facoltà di Scienze Motorie, Università di Verona, Via Casorati 43, I-37100 Verona, Italy
4 Dipartimento di Fisica, Università di Trieste, Via Valerio 2, I-34127 Trieste, Italy

E-mail: roberto.chignola@univr.it

Received 13 February 2007
Accepted for publication 22 May 2007
Published 12 June 2007
Online at stacks.iop.org/PhysBio/4/114

**Abstract**

In a previous paper we have introduced a phenomenological model of cell metabolism and of the cell cycle to simulate the behavior of large tumor cell populations (Chignola and Milotti 2005 Phys. Biol. 2 8). Here we describe a refined and extended version of the model that includes some of the complex interactions between cells and their surrounding environment. The present version takes into consideration several additional energy-consuming biochemical pathways such as protein and DNA synthesis, the tuning of extracellular pH and of the cell membrane potential. The control of the cell cycle, which was previously modeled by means of *ad hoc* thresholds, has been directly addressed here by considering checkpoints from proteins that act as targets for phosphorylation on multiple sites. As simulated cells grow, they can now modify the chemical composition of the surrounding environment which in turn acts as a feedback mechanism to tune cell metabolism and hence cell proliferation: in this way we obtain growth curves that match quite well those observed in vitro with human leukemia cell lines. The model is strongly constrained and returns results that can be directly compared with actual experiments, because it uses parameter values in narrow ranges estimated from experimental data, and in perspective we hope to utilize it to develop *in silico* studies of the growth of very large tumor cell populations ($10^6$ cells or more) and to support experimental research. In particular, the program is used here to make predictions on the behavior of cells grown in a glucose-poor medium: these predictions are confirmed by experimental observation.

1. Introduction

Individual tumors are complex biological systems and, in spite of great therapeutic advances, many tumors still escape treatment and lead to death. Part of the complexity of the problem is a sheer consequence of tumor size: clinicians deal with the macroscopic properties of tumors, i.e. masses that may eventually weigh a few kilograms, and thus with a number of cells that ranges between $10^9$ and $10^{12}$, and that may grow for months or years, with a corresponding number of cell cycles somewhere in the range between 100 and 10,000. Microscopically, the malignant transformation of single cells is a multistep process that involves the modification of several molecular circuits which, in turn, modify the cells’ behavior and the relationships between cells and the environment [1]. In addition, epigenetic and environmental factors, which include cell–cell interactions, also conspire with the bare genetic information to make tumor growth a highly variable process with very strong feedbacks [1].

The highly nonlinear character of the cells’ internal molecular machinery, combined with the cell–cell and environmental interactions, with the large number of cells in a tumor, and with the extended tumor lifespan, make predictions based on the behavior of a single molecular circuit quite
haphazard. We are thus in a frustrating situation, in which
the huge body of detailed knowledge that has been collected
in basic research areas, such as molecular biology, often seems
to be of little or no consequence in the clinical management of
the disease.

The availability of powerful computers has already helped
bridge the gap between observations and predictions in many
complex problems, and a few attempts have already been made
to attack the problem of tumor growth with numerical models
(see, e.g., [2–4] and references cited therein). Recently we
have proposed a numerical simulator of tumor growth [5, 6]:
the simulator should eventually reproduce the growth of
solid tumors in the prevascular phase and allow an in silico
investigation of the biophysical laws that govern tumor growth
dynamics and the response of cell clusters to anti-tumor
treatments. The program simulates both tumor cells and
the complex and changing environment where molecules,
such as nutrients and drugs, diffuse and where cells interact
mechanically with one another. The simulator deals with
very different spatiotemporal scales and eventually with a
large number of cells; using the prototype software we have
been able to achieve a linearization of time and memory
requirements using techniques and methods borrowed from
molecular dynamics and computational geometry [5]. An
essential part of the simulation software includes the code
for metabolism and cell cycle, and in a previous paper we
have shown and discussed the basic aspects of the underlying
biophysical model [6]. Here we refine and extend that code
and show that it can be used to simulate the proliferation
of tumor cells that grow in a closed environment with good
quantitative agreement with experimental data. We also use
the model to predict the behavior of cells that grow in non-
standard environments and compare these predictions with
actual experimental data.

The next section briefly describes the materials and
methods used in the experiments that have been carried out
to fix some of the necessary parameters and in the numerical
simulations. Section 3 contains a detailed description of the
simplified metabolic network included in the simulator, and
section 4 explains the implementation of the cell proliferation
dynamics. The results of the simulations and comparisons
with experimental data are given in section 5, while section 6
contains our conclusions and our outlook on future
developments.

2. Materials and methods

2.1. Cell lines and proliferation assays

MOLT3 (human T cell leukemia) and Raji (human B cell
leukemia) were obtained from the ATCC and maintained in
RPMI-1640 medium supplemented with 10% fetal bovine
serum. Cells were cultured at 37 °C in a 5% CO2 humidified
atmosphere and passaged weekly. For proliferation assays,
15 × 10^6 cells from exponentially growing cultures were
seeded into T25 culture flasks in 7 ml of the following
media: complete RPMI-1640 + 10% FBS or glucose-deprived
RPMI-1640 + 10% FBS. Fifty µl samples were drawn from
culture flasks at the time point indicated in the figures to
carry out measurements of cell survival. Alive and dead cells
were identified using the vital dye Trypan-blue and were
counted at the microscope using a Neubauer chamber. Four
measurements for each well were carried out and the values
were then averaged. Each data point in the growth curves
is the mean ± standard deviation (SD) of three independent
replicates. The amount of ATP in growing cell populations was
measured using the luciferine/luciferase method (Promega
kit) following the manufacturer’s procedures. Measurements
expressed in luminescence arbitrary units were converted to
concentration units by means of a calibration curve obtained
with known amounts of ATP. Luminescence was measured
using a microplate luminometer reader (Bio-Tek instruments).
Each data point in the figures is the mean ± SD of eight
independent replicates.

Cell cycle analysis was carried out by flow cytometry.
Cells were washed with PBS, and cell pellets produced from
1.5 × 10^6 cells where resuspended under mild vortexing with
1 ml of cold Tris/HCl-EDTA buffer (0.1 M Tris-
hydroxymethyl-aminooxetane, 1 mM EDTA, pH 7.4). Cells
were centrifuged at 4 °C and supernatants discarded. Pellets
were labeled with 1 ml of a cold ethidium bromide solution
(25 µM ethidium bromide, 0.3 mM Na-citrate, 1 mM NaCl,
0.1% v/v Triton X-100, 25 µg ml^-1 RNAseA) under mild
vortexing, and cells incubated overnight at 4 °C. The nuclei
were analyzed by flow cytometry on an EPICS-X (Coulter,
Hialeah, FL) flow cytometer using a preparation from human
peripheral blood mononuclear cells (blood drawn from healthy
donors) as the standard.

2.2. Simulations

The simulation program has been written in ANSI/ISO-C++
and has been run on Apple computers (both PowerPC- and
Intel-based). The program has been compiled with the GCC
4 compiler in a Mac OS X environment. The differential
equations that make up the model have been integrated with
a fixed time step Δt = 1 s; this choice is dictated by the
conflicting needs of reducing the total computation time as
much as possible and by the computational request, needed
for algorithmic stability, that Δt ≪ τk for all the Michaelis–
Menten (MM) reactions, where τk is the reaction time constant
for low concentrations for the kth MM reaction. The model
is partly stochastic, and pseudorandom numbers have been
generated by the linear congruent generator RAN2, taken from
the Numerical Recipes library [7].

3. A minimal model of the biochemical network of
tumor cells

The biochemical network implemented in the simulation
program is shown in figure 1: it is a really minimal network in
comparison to the complexity of the actual metabolic system
of tumor cells. The rationale behind the choice of the reactions
in this minimal set has been discussed extensively in our
previous paper [6], and we summarize again here the main
arguments: (1) many parameters of the metabolic network are
Figure 1. Schematic layout of the metabolic network that models cell metabolism and its relationship with the extracellular environment. Variables within circles represent molecular species and are expressed in units of concentration or mass. Symbols are as follows: \( G_{ex} \) = external glucose concentration, \( G_{in} \) = intracellular glucose concentration, \( G_6P \) = glucose-6-phosphate concentration, \( \text{STORE} \) = mass of glucose stored in the form of glycogen, \( \text{Ac.Lat.ex} \) = environmental lactic acid concentration, \( \text{Ac.Lat.in} \) = intracellular lactic acid concentration, \( \text{Aex} \) = environmental glutamine concentration, \( \text{Ain} \) = intracellular glutamine concentration, \( \text{ATP} \) = pool of ATP molecules concentration, \( \text{O}_2 \) = oxygen concentration, \( \text{H}^+ \) = mass of intracellular protons, \( \text{Proteins} \) = mass of intracellular proteins, \( \text{DNA} \) = relative mass of DNA (normalized to 1 for the whole genome). Rates are represented by squares. The dashed and dotted circuits represent the ATP and oxygen sensors, respectively, and have been described previously [6]. The dashed-and-dotted circuit named ‘Cell Cycle Checkpoints’ represents the molecular circuit of cell cycle control that has been modeled on the basis of previous studies on the dynamics of the allosteric effect (see [33, 34] and equations (12)–(14) for details). \( \Delta V \) is the cell membrane electric potential that has been used to calculate the energy costs for intracellular proton dissipation (see equations (9) and (10) for details). The calculation of the global energy cost also requires the rates \( v_{\text{Mit}}, v_{\text{P}} \) and \( v_{\text{DNA}} \) that model ATP consumption for mitochondria maintenance, protein synthesis and DNA synthesis, respectively.

actually unknown; (2) even if we had a detailed knowledge of the actual metabolic network, a numerical model of a single cell’s metabolic network would be computationally heavy, and the simulation of large cellular ensembles would be extremely impractical [5]; (3) many metabolic pathways are redundant and, in addition, it is now clear that the whole system has a hierarchical topology and that its dynamical behavior is dominated by the network’s hubs [8]. We have thus decided to keep only those metabolic pathways that in our opinion do determine the cell’s behavior: obviously this choice is somewhat arbitrary but it is eventually validated by the comparison with the experimental data.

The core of the metabolic network in figure 1 contains the uptake and conversion of glucose and of other molecules into energy (in the form of ATP), storage molecules (e.g. glycogen) and waste products (e.g. lactate) and hence this part of the model is a global representation of glycolysis, oxidative phosphorylation through the TCA cycle and gluconeogenesis. Certain pathways are controlled by sensors, and we have considered both ATP and oxygen sensors. These sensors depend on the biophysical state of the virtual cell and tune its behavior when ATP and/or oxygen become limiting factors because of harsh environmental conditions. All these aspects and their mathematical modeling have been described and discussed in our previous paper [6]. In the following subsections we concentrate on the new pathways that we have included to model growth in a closed environment. The values of model parameters for these pathways are listed in table 1. Since the environment is now closed it follows that we have to consider a conservation equation for the whole system’s volume and a detailed description of the transport kinetics of some chemical species (such as nutrient and waste products) inside and outside cells. But this, in turn, implies that the fate of the certain chemical species in cells must also be described and hence biosynthetic pathways that were previously neglected [6] must now be taken into consideration. We include also protein and DNA synthesis and since these paths consume energy the global energy balance of the cell must also be extensively revised as well.
### Table 1. Model parameters for the standard simulation that are either modified or new with respect to those listed in [6].

| Symbol  | Value | Units               | Meaning                                                                 | References |
|---------|-------|---------------------|-------------------------------------------------------------------------|------------|
| $V_{\text{max}, A}$ | $1 \times 10^{-9}$ | kg s$^{-1}$ m$^{-2}$ | Maximum rate of glutamine transport                                      | [12]       |
| $K_{\text{m}, A}$ | 0.0238 | kg m$^{-3}$         | Michaelis–Menten constant of glutamine transport                         | [58]       |
| $V_{\text{max}, \text{AL}}$ | $9.58 \times 10^{-8}$ | kg s$^{-1}$ m$^{-2}$ | Maximum rate of lactic acid transport                                   | [15, 59, 60] |
| $K_{\text{m}, \text{AL}}$ | 0.4053 | kg m$^{-3}$         | Michaelis–Menten constant of lactic acid transport                       | [15, 59, 60] |
| $V_{\text{ef}}$ | $-0.021$ | V                  | Cell membrane potential in standard conditions                           | This paper (see the text for details) |
| $H_{\text{Peff}}$ | 0.1    |                     | Phenomenological constant describing the inverse of the efficiency of proton pumps | This paper (by data fitting) |
| $D_{H}$ | $1.5 \times 10^{-18}$ | m$^3$s$^{-1}$ | Effective proton diffusion constant (assuming a plasma membrane thickness of 10 nm) | [61]       |
| $\beta_{\text{tot}}$ | 0.19953 | kg m$^{-3}$ | Buffering capacity of the environment                                    | [21]       |
| $p_s$ | $7 \times 10^{-20}$ | kg s$^{-1}$ | Rate of glutamine consumption for protein and DNA synthesis             | This paper (by data fitting) |
| $K_{\text{m}, \text{P}}$ | $9 \times 10^{-17}$ | kg | Michaelis–Menten constant of ATP utilization in protein synthesis        | This paper (by data fitting) |
| $K_{\text{m}, \text{DNA}}$ | $8 \times 10^{-18}$ | kg | Michaelis–Menten constant of ATP utilization in DNA synthesis            | This paper (by data fitting) |
| $\lambda_{\text{DNA}}$ | 0.008 |                     | Fraction of the total ATP production rate utilized for DNA synthesis | This paper (by data fitting) |
| DNA\_MAX\_SPREAD | 0.1 |                     | Maximum spread of the fluctuations in the DNA synthesis duration        | This paper (by data fitting) |
| $N$ | 16 | | Maximum number of phosphorylation sites on pRb | [38]       |
| $\alpha_{\text{th}}$ | 10 | | Number of sites that must be phosphorylated for pRb activation | [38]       |
| $K_{\text{rb}}$ | $10^{-6}$ | M$^{-1}$ | Equilibrium constant for phosphorylation/depolymerization process on pRb | [62$^a$] |
| $\alpha_{\text{d}, \text{pRb}}$ | $1.5 \times 10^{-2}$ | | Fraction of proteins which is pRb | This paper (by data fitting) |
| $\alpha_{\text{CycD}}$ | $7 \times 10^{-3}$ | | Fraction of proteins which is cyclin D | This paper (by data fitting) |
| $\alpha_{\text{CycE}}$ | $10^{-3}$ | | Fraction of proteins which is cyclin E | This paper (by data fitting) |
| $\alpha_{\text{CycX}}$ | $8 \times 10^{-3}$ | | Fraction of proteins which is cyclin X | This paper (by data fitting) |
| $S$ | $10^{-3}$ | M | Substrate concentration for the downstream Michaelis–Menten reaction controlled by pRb | This paper (by data fitting) |
| $K_{\text{SS}}$ | $10^4$ | s$^{-1}$ | Rate of S molecules consumption | [33, 34] |
| $K_{\text{MM}}$ | $10^{-3}$ | M | Michaelis–Menten constant of S molecules consumption | [33, 34] |
| $S_{\text{th}}$ | 0.8 | | Fraction of the S concentration that defines the threshold for the G1m/G1p transition | This paper (by data fitting) |
| $S_{\text{th}}$ | 0.05 | | Fraction of the S concentration that defines the threshold for the G1p/S transition | This paper (by data fitting) |
| $\text{CycX}_{\text{th}}$ | $8 \times 10^{-17}$ | kg | Amount of cyclin X that defines the threshold for the G2/M transition | This paper (by data fitting) |
| $C_1$ | $3 \times 10^{16}$ | kg$^{-1}$ | Phenomenological constant for the growth of mitochondria | This paper (by data fitting) |
| $C_2$ | $2 \times 10^{-18}$ | m$^3$ | Phenomenological constant for the increase of the cell volume | This paper (by data fitting) |
| $V_{\text{ATP}}$ | $4 \times 10^{-22}$ | kg s$^{-1}$ | Rate of ATP consumption for mitochondria maintenance | This paper (by data fitting) |
| $\text{ATP}_{\text{min}}$ | $1.5 \times 10^{-15}$ | kg | Minimum ATP amount for cell survival | This paper (by data fitting) |
| $S, T$ | 55 000 | s | Maximum duration of the S phase | [6] |
| $G2, T$ | 25 200 | s | Maximum duration of the G2 phase | [6] |
| $M, T_m$ | 1800 | s | Mean duration of the M phase | [6] |
| PHASE\_SPREAD | 0.5 | | Maximum spread for the fluctuations in the M phase duration | This paper (by data fitting) |

$^a$ Value measured for the equilibrium between ATP and cyclin-dependent kinases.

### 3.1. Closed environment

The first significant biophysical difference with respect to our previous model is the definition of the closed environment where cells grow. In the previous version, in fact, cells were assumed to grow in an open volume where nutrients were always available and waste products immediately removed [6]. Now the environment is closed—as it happens to be in most experimental settings and in real tumors embedded in animal tissues—and environmental concentrations of nutrients, waste products and other molecules become important variables. Because of the negligible compressibility of water solutions the environmental volume is effectively constrained by the equation

$$V_{\text{tot}} = V_{\text{env}} + \sum_{k=1}^{N} V_{\text{vol}} = \text{constant}$$

(1)
where $V_{	ext{ol}_{\text{env}}}$ is the volume of the extracellular environment and $V_{\text{ol}_k}$ is the volume of the $k$th cell.

3.2. The pool of ‘other nutrients’ in compartment A: glutamine as an essential component

In our previous work [6], we introduced compartment A to include nutrients other than glucose that can nonetheless be catabolized through the oxidative phosphorylation pathways or converted into storage molecules through gluconeogenesis, and indeed it is known that tumor cell metabolism does not rely on glucose only. The present version of the model takes into account the environment where cells grow, and thus the compartment has been split here into compartments $A_{\text{in}}$ and $A_{\text{ex}}$ of intracellular and extracellular A molecules, respectively (see figure 1). Since numerous but different molecules—each one involving different enzymatic mechanisms—can participate in cell metabolism, energy production and storage (in the form of glycogen, through the gluconeogenesis pathway that is described here phenomenologically by means of rate p11 (see figure 1)), we initially considered compartment A as a generic pool of ‘other nutrients’ (e.g. lactate, glutamine and other amino acids). However, in the present version of the model we also want to include biosynthetic pathways such as protein and DNA synthesis that are intimately linked to cell cycle kinetics (see the next paragraphs), and thus we need a better definition of the nutrients in the A compartment. We have decided to narrow the scope of the A compartment and focus on glutamine alone for the following reasons:

1. It is well known that glutamine is essential (together with glucose) to sustain tumor cell growth in vitro [9, 10], and it has also been shown that glutamine utilization for ATP production through the oxidative phosphorylation in tumor cells may actually overcome that of glucose [11].

2. It has been reported that most, if not all, tumor cells express the glycolytic isozyme pyruvate kinase type M2 (M2-PK) and that this enzyme is not expressed by normal cells [12]. The enzyme occurs in a highly active tetrameric form and in a dimeric form with low affinity for phosphoenolpyruvate. The switch between the two forms regulates glycolytic phosphometabolite pools and the interaction between glycolysis and glutaminolysis, the latter resulting in pyruvate and lactate production from glutamine [12]. Thus, these pathways do depend on glutamine (although the correlation depends on a series of enzymatic reactions) and this justifies the phenomenological description represented by rates p22 and p11, respectively (see figure 1).

3. Finally, glutamine is an essential building block both of proteins and of DNA (see below) [13, 14].

3.3. Transport of nutrients and of waste products across the cell membrane

Except for oxygen which diffuses freely across the cell membrane, transport of glucose, glutamine and lactate is mediated by saturable transporters whose activity is well known to follow a Michaelis–Menten type kinetics. Thus, if $X_{\text{in}}$ and $X_{\text{out}}$ represent the concentrations of a given molecule in the cell and in the environment, then transport across cell membrane is modeled by the equations

\[
\begin{align*}
\frac{dX_{\text{in}}}{dt} &= - \frac{dX_{\text{out}}}{dt} = v_{\text{in} \rightarrow \text{out}} - v_{\text{out} \rightarrow \text{in}} \\
v_{\text{out} \rightarrow \text{in}} &= \frac{v_{\text{max}} X_{\text{out}}}{K_m + X_{\text{out}}} \\
v_{\text{in} \rightarrow \text{out}} &= \frac{v_{\text{max}} X_{\text{in}}}{K_m + X_{\text{in}}} \\
v_{\text{max}} &= v_{\text{in}} \cdot \text{Surf}
\end{align*}
\]

where $v_{\text{in}}$ and $K_m$ are the Michaelis–Menten constants for the molecular transport and $\text{Surf}$ is the cell surface area. The transport of glucose depends on the oxygen concentration as well, and this dependence, along with the value of model parameters for glucose transport, has already been described [6].

3.4. Intracellular and extracellular pH. Dependence of various transport and metabolic rates on environmental acidity

The intracellular and the extracellular pH are assumed to vary primarily because of lactate production. Lactic acid is produced by cells during the conversion of glucose through the anaerobic glycolysis. During this conversion ATP is also produced, and from the stoichiometry of ATP and lactic acid production we know that 2 moles of both species are produced per mole of glucose. Thus, the production rates of both species (see ATP,noX in figure 1) also match and have been parameterized by means of the rates $g_1$ and $r_1$ (see figure 1) that describe the conversion of glucose-6-phosphate (G6P) and of glycogen storage molecules (STORE) through glycolysis (see [6] for further details).

Within the range of acidity that is physiologic for living cells, lactic acid is completely dissociated into lactate$^-$ and H$^+$ ions and these are co-transported through the cell membrane by means of MCT transporters. The MCT transporters do not require energy and transport proceeds bidirectionally along the lactate and proton gradients [15]. In a cell, protons participate to a complex series of chemical equilibria that regulate several important processes such as the cell osmotic pressure, the potential of the cell membrane and of the membranes of intracellular organelles (e.g. mitochondria), the transport of various ions across the cell membrane and the production of energy under the form of ATP. The regulation of the intracellular pH involves the action of energy-driven proton pumps (H$^+$ ATPase), proton channels and ion transporters that drive H$^+$ or equivalent H$^+$ and HCO$_3^-$ ions into and out of the cell and that are all expressed at the cell membrane [16]. The latter include an Na$^+$/H$^+$ antiport, Na$^+$-dependent HCO$_3^-$ transporters, an Na$^+$-independent Cl$^-$/HCO$_3^-$ exchanger, a Cl$^-$/OH$^-$ exchanger and other transporters such as the lactate-proton cotransporter [16]. In addition, a number of chemical reactions among which the set of reactions describing the buffering effect of the carbonate/bicarbonate system takes place both in the cellular cytosol and in the environment. Taken
as a whole this system is rather complex (see, e.g., [17, 18]) and includes fast chemical reactions, faster than the time interval of 1 s that we have taken as a compromise between algorithmic stability, computation time and computational load.

However, some simplifications are possible: firstly, the experimental evidence shows that the buffering capacity of cytosol in tumor cells is much higher than that of the surrounding environment. Although the intracellular buffering capacity has been shown to vary slightly during the cell cycle, the intracellular pH does not vary much during the cell’s life and this is due to the number of proton and ion pumps and of buffering systems that a tumor cell expresses and exploits for this purpose [19]. In addition, using a parameterization of the observed buffering capacity of cytosol [20], we carried out numerical simulations that demonstrate that intracellular pH in simulated cells falls below physiologic limits only under severe starvation, that would anyway lead to cell death, e.g., because of insufficient ATP or glycogen storage levels. Thus, as a first approximation, we have assumed intracellular pH to be constant and we have fixed it at 7.2, which is the observed value in tumor cells [16, 19, 20]. Another simplifying consideration is that the buffering capacity of the environment can be measured quite easily, and for standard culture media the pH has been shown experimentally to be directly proportional to environmental lactic acid concentration [21]. In the simulator we take the following phenomenological model for the acidity of the extracellular environment:

\[
\begin{align*}
\text{pH}_{\text{out}} &= \text{pH}_{\text{st}} - \frac{\text{AcL}_{\text{out}}}{\text{Vol}_{\text{out}} \cdot \beta_{\text{out}}} \tag{3a} \\
\beta_{\text{out}} &= -\frac{\text{dH}_{\text{out}}}{\text{dpH}_{\text{out}}} \tag{3b}
\end{align*}
\]

where \text{pH}_{\text{st}} is the standard pH of a clean medium, \text{Vol}_{\text{out}} is the volume of the environment, \beta_{\text{out}} is the buffering capacity of the environment estimated from experiments [21] and \text{AcL}_{\text{out}} is the mass of lactate secreted by the cells in the extracellular environment (and thus \text{AcL}_{\text{out}}/\text{Vol}_{\text{out}} is the lactate concentration in the environment). Lactate is produced intracellularly as a consequence of glucose metabolism as described in [6] (see \text{AcL}_{\text{in}} in figure 1) and it is transported across the cell membrane as described by the set of equations (2a)–(2d).

The Michaelis– Menten parameter \( v_{\text{max}} \) for the transport of glucose, lactate and of other molecules (see below) is known to depend on pH [22–25]. To the best of our knowledge, however, this dependence has not been studied in detail and no firm biophysical conclusions are available. Once again we resort to a phenomenological description that nonetheless takes into account the results of experimental observations. This description assumes that the \( v_{\text{max}} \) of the various transporters is weighed by a function \( w(\text{pH}) \) which is roughly a sigmoid curve as a function of pH. In practice, we take the piecewise linear approximation for the weight function:

\[
w(\text{pH}) = \begin{cases} 
  w_1 + \frac{w_2 - w_1}{\text{pH}_1 - \text{pH}_2}, & \text{pH} < \text{pH}_1 \\
  w_1 + \frac{w_2 - w_1}{\text{pH}_1 - \text{pH}_2}, & \text{pH}_1 \leq \text{pH} \leq \text{pH}_2 \\
  w_2, & \text{pH} > \text{pH}_2 
\end{cases}
\]

where \( w_1, w_2, \text{pH}_1, \text{pH}_2 \) are the parameters that we specify to approximate the few available data.

In particular, for the transport of glucose and of glutamine we take \( w_1 = 0, w_2 = 1, \text{pH}_1 = 6.3, \text{pH}_2 = \text{pH}_{\text{st}} = 7.54 \) (see [22, 25]), while for lactate transport, we take \( w_1 = 3, w_2 = 1, \text{pH}_1 = 6.0, \text{pH}_2 = 8.0 \) [23, 24].

It should be noted that since the intracellular pH is kept at a fixed value, then the flow of glucose, glutamine and lactate is not balanced if the environmental pH differs from the standard value, and in the closed environment described by the simulator this does indeed happen because of lactate production and secretion by cells. As the environment is gradually more and more acidic, the uptake of nutrients is also reduced and can eventually switch off completely, thereby leading to a depletion of the energy reserves and ultimately to cell death. This mechanism, which is entirely mediated by the weight function described above, represents an important feedback regulatory circuit between cells and their environment, and can be tested experimentally because it defines the carrying capacity of the environment where cells grow (see the results section).

We take a fixed intracellular pH; however, the increase of the concentration of H+ ions in the cells cannot be neglected. The increase is originated by two main processes: as the cell population grows the lactic acid secretion also rises and the environmental pH lowers below the standard intracellular level: in this case a cell can import lactic acid through the MCT transporters. Secondly, the continuous intracellular production of lactic acid may eventually overcome the maximal rate of secretion of MCT transporters. We have already remarked that H+ ions participate in a number of cellular processes in tumor cells, which collectively allow cells to maintain an almost fixed pH [16] and that these processes can be subdivided into two categories on the basis of their energy requirements. Since the energy consuming pathways are important in our model, we must take into account the consumption of ATP used to eliminate intracellular H+ when the MCT transporters alone cannot sustain the whole load. It should be noted that we are not interested in the fate of the H+ ions, because of the phenomenological modeling of the intracellular and extracellular pH, and we only need to focus our attention on the energy balance of H+ transport.

To model the energy consumption due to H+ transport, we recall that in the model H+ ions originate from the complete dissociation of lactic acid, and that lactic acid is synthesized by cells through glycolysis and this path is described by rates \( g_1 \) and \( r_1 \) (see figure 1; see also [6] for details). In addition, lactic acid is transported out and in the cells by means of the MCT transporters with a transport kinetics that follows the set of equations (2a)–(2d). H+ can finally move across cell membranes by passive diffusion. The mass variation of H+ ions in the cell therefore writes

\[
\frac{\text{dH}^+}{\text{dt}} = \left( 2g_1 + 2r_1 + v_{\text{out} \rightarrow \text{in}, \text{AL}} - v_{\text{in} \rightarrow \text{out}, \text{AL}} \right) \cdot \frac{\text{MW}_{\text{H}^+}}{\text{MW}_{\text{AL}}} + D_{\text{H}^+} \cdot \left( [\text{H}^+]_{\text{out}} - [\text{H}^+]_{\text{in}} \right) \tag{5}
\]

where the factor 2 takes into accounts the stoichiometric result that 2 moles of lactic acid are produced per mole of glucose,
\( v_{\text{gAL}} \) and \( v_{\text{eAL}} \) are the rates of lactic acid transport in and out of the cells through MCT transporters, \( MW_{H^+} \) and \( MW_{\text{AL}} \) are the molecular weights of \( H^+ \) and lactic acid, respectively, \( D_{\text{ij}}^+ \) is the effective diffusion constant (i.e. the diffusion coefficient multiplied by the thickness of the cell membrane) of \( H^+ \) in the cell membrane and \( [H^+]_{\text{out, in}} \) are the extracellular and intracellular \( H^+ \) concentrations, respectively. Equation (4) is then used to calculate the energy consumption required to move the charged ions in the electric field associated with the Nernst membrane potential when this field opposes \( H^+ \) secretion (see below).

### 3.5. Protein and DNA synthesis

Protein synthesis and DNA synthesis are two important new blocks in the present simulation program; however, the very high complexity of the actual cellular processes means that once again we have to resort to some form of approximate phenomenological description. In the case of protein synthesis we compute the number of glutamine and ATP molecules required to build an ‘average protein’. We take human serum albumin as a representative of the ‘average protein’; albumin contains 585 amino acids and its molecular weight is \( \approx 66.4 \text{kDa} \) [26]. Glutamine is incorporated in the protein as it is or after it has been changed into another amino acid by enzymatic modification of its side chain. Experimentally, it has been shown that in the proteins of HeLa tumor cells the amino acids Ala, Asp, Glu, Gly, Pro, Ser derive from glutamine. Thus, to synthesize one molecule of an average protein of 585 amino acids 584 amino acids are glutamine or are derived from glutamine. Therefore, on average, 5% of albumin monoacids are glutamine or are derived from glutamine. Thus, to synthesize one molecule of an average protein of 585 amino acids the cell needs on average 29.25 glutamine molecules as building blocks.

An average protein of 585 amino acids contains 584 peptide bonds that require the hydrolysis of 2 molecules of ATP and 2 of GTP per bond for formation [27], and hence 1168 ATP molecules are utilized on the whole to synthesize one molecule of the average protein (note that in the present model the GTP molecular pathways are not yet taken into account).

Protein synthesis requires both glutamine and ATP as building blocks, and since either of them can vary in time and become a limiting factor, the rate of protein synthesis must depend on the building block which, at any given time, is less abundant (at the moment we neglect recovery of amino acids due to protein degradation). From these considerations we find the equations that model protein synthesis:

\[
\begin{align*}
\frac{dP_S}{dt} &= \frac{P_{33}}{29.25} \cdot \frac{MW_{p_S}}{MW_{\text{Al}}} \quad \text{if} \quad \frac{N_{\text{ATP}}}{1168} > \frac{N_A}{29.52} \\
\frac{dP_S}{dt} &= \frac{v_p}{1168} \cdot \frac{MW_{\text{ATP}}}{MW_{p_S}} \quad \text{elsewhere} \tag{6a}
\end{align*}
\]

\[
\begin{align*}
 v_{p_S} &= p_{33} \cdot d \\
 v_p &= \frac{\text{ATP}_\text{TOT} \cdot \text{ATP} p}{K_{\text{M} + \text{ATP}}} \tag{6b}
\end{align*}
\]

where \( P_S \) is the protein mass, \( N_{\text{ATP}} \) and \( N_A \) are the numbers of available ATP and glutamine molecules, respectively, \( MW_{p_S} \), \( MW_{\text{ATP}} \), \( MW_A \) are the molecular weights of the ‘average protein’, ATP and glutamine, respectively, \( v_p \) is the rate of ATP pool consumption for protein synthesis (this is assumed to follow a Michaelis–Menten kinetics because of the activity of the enzymes that utilize ATP for protein synthesis), \( \text{ATP}_\text{TOT} \) is the total ATP production (see [6] for details), \( K_{\text{M}} \) is the Michaelis–Menten constant for ATP consumption, \( p_{33} \) is the rate of glutamine molecules channeled to protein synthesis and \( d \) is a homeostatic control function that assumes real values in the interval [0, 1] and that modulates the rate \( p_{33} \) as a function of the size of the glutamine compartment \( A \) (see [6] for details).

Initial guesses for \( p_{33} \) and \( v_p \) were obtained by considering that the protein content of one cell is approximately 10–20% of the cell’s mass [27], that a cell has a relative density \( \approx 1 \) and that at the end of one cell cycle the protein mass must be approximately twice as much as in a newborn cell.

We have modeled DNA synthesis much like protein synthesis. However, in this case we note that the atoms \( N_1 \) and \( N_9 \) of purines and \( N_3 \) and \( C_2 \) of pyrimidines derive from the side chain of glutamine after some biochemical processing [14, 27]. Thus, on average the stoichiometric relationship between glutamine and the bases of the DNA is one molecule of glutamine for each base. We also note that the number of bases in the human genome is \( 6 \times 10^9 \), and that the incorporation of nucleotides into the DNA sequence is sustained energetically by the hydrolysis of the high-energy phosphoril groups present in the nucleotides themselves. The formation of the phosphodiester bonds \( (\Delta G = 5.3 \text{ Kcal} \text{mol}^{-1}) \), which join one base to the other, requires energy that is provided by ATP; then if we assume that during the duplication of the genome approximately \( 6 \times 10^9 \) phosphodiester bonds are formed and that the hydrolysis of ATP releases \( \approx 11.94 \text{ Kcal mol}^{-1} \) [27], we can estimate that DNA duplication requires \( \approx 2.67 \times 10^9 \) ATP molecules. Thus both glutamine and ATP are required for DNA synthesis, and both may vary during the cell’s life and limit the DNA synthesis rate, which we model as follows:

\[
\begin{align*}
\frac{d\text{DNA}}{dt} &= \frac{\lambda_{\text{DNA}}}{\text{DNA}_\text{TOT} \cdot \text{ATP} p} \cdot \frac{N_{av}}{2.67 \cdot 10^9 \cdot \frac{MW_A}{\text{DNA}_\text{m}}} \quad \text{if} \quad \frac{N_{\text{ATP}}}{2.67 \cdot 10^9} > \frac{N_A}{6 \cdot 10^9} \\
\frac{d\text{DNA}}{dt} &= \frac{\lambda_{\text{DNA}}}{\text{DNA}_\text{TOT} \cdot \text{ATP} p} \cdot \frac{N_{av}}{2.67 \cdot 10^9 \cdot \frac{MW_{\text{ATP}}}{\text{DNA}_\text{m}}} \quad \text{elsewhere} \tag{7a}
\end{align*}
\]

\[
\begin{align*}
\lambda_{\text{DNA}} &= \frac{\text{DNA} \cdot (1 + \text{DNA}_\text{MAX}\cdot \text{SPREAD} \cdot \xi)}{K_{\text{mDNA}} + \text{ATP} p} \tag{7b}
\end{align*}
\]

where \( \lambda_{\text{DNA}} \) is the rate of ATP consumption for DNA synthesis, \( N_{av} \) is the Avogadro constant, \( \lambda_{\text{DNA}} \) is the fraction of the total ATP production rate \( \text{ATP}_\text{TOT} \) that is channeled to DNA synthesis in the S phase of the cell cycle, \( K_{\text{mDNA}} \) is the Michaelis–Menten constant of the enzymatic process of ATP utilization for DNA synthesis, \( \text{DNA}_\text{MAX}\cdot \text{SPREAD} \) is a constant, and \( \xi \) is a random variable drawn from a uniform distribution on the interval \((-1, 1)\). The term \( \text{DNA}_\text{MAX}\cdot \text{SPREAD} \cdot \xi \) is small cell-dependent random
spread which is determined at cell birth and it has been introduced to parameterize the fluctuations assumed to occur in certain mechanical aspects of DNA synthesis that involve processes such as the unidirectional translocation of the motor protein helicases to unwind and separate the two DNA strands [27]. Finally, it should be noted that we consider only one rate term for glutamine utilization for both protein and DNA synthesis. In this way the glutamine pool (compartment A in figure 1) is subdivided into three pools that sustain the STORE synthesis. In this way the glutamine pool (compartment \( p_1 \)) energy production (\( p_{22} \)) and biosynthesis (\( p_{23} \)) and where \( p_1 \) and \( p_{22} \) are dynamically interconnected through the ATP sensor (see also [6] for details).

### 3.6. The energy balance: summary of the energy producing and consuming processes

The model of cell metabolism takes into account diverse energy producing and consuming pathways. Energy, stored in the form of ATP, is produced by anaerobic glycolysis (rate ATP, pOX in figure 1) and oxidative phosphorylation of glucose taken up from the environment (rate ATP, OX in figure 1). In addition, if ATP, OX falls below a standard threshold (\( \text{p} \text{TOT} \)), energy production (\( \text{p}_{22} \)) and biosynthesis (\( \text{p}_{23} \)) and where \( \text{p}_{22} \) and \( \text{p}_{23} \) are dynamically interconnected through the ATP sensor (see also [6] for details).

\[
\text{ATP, TOT} = \text{ATP, pOX} + \text{ATP, OX} + \text{ATP2} + \text{ATP3} - \text{Cons ATP}
\]

\[
\frac{\text{dATP}}{\text{dt}} = \text{ATP, TOT} - r_c
\]

where ATPp is the ATP pool and \( r_c \) is a phenomenological ATP consumption rate. One of the main differences between the previous model and the present one is that now we define some of the energy consuming paths that were previously modeled by means of the single phenomenological rate \( r_c \). In this context, we have already described above the use of ATP for protein and DNA synthesis. In addition, we also consider a phenomenological rate of ATP consumption for mitochondria maintenance (i.e., maintenance of the mitochondria membrane potential which is essential for mitochondria to work properly [28], see \( v_{\text{mit}} \) in figure 1) which is proportional to the number \( M \) of mitochondria in the cell. Finally, we include in the model the consumption of ATP used to pump \( H^+ \) ions outside the cell when the environmental \( \text{pH} \) becomes lower than the intracellular \( \text{pH} \). The latter has been calculated as follows: we assume that, under normal environmental conditions, the cell has a membrane potential \( \text{V}_{\text{mit}} = -21 \text{ mV} \) (derived from calculations using the Nernst equation and assuming that the standard extracellular \( \text{pH} \) is 7.54 and the intracellular \( \text{pH} \) is 7.2, see below and [16]). When the concentration of environmental \( \text{H}^+ \) increases because of lactic acid production and secretion the membrane potential changes according to the Nernst equation:

\[
\text{V}_{\text{Nernst}} = \frac{R \cdot T}{z \cdot F} \cdot \ln \left( \frac{[\text{H}^+ \text{int}]}{[\text{H}^+ \text{out}]} \right)
\]

where \( R \) is the gas constant, \( T \) is the temperature (K), \( z \) is the proton charge and \( F \) the Faraday constant. Under normal conditions, the environmental \( \text{pH} \) is set to 7.54 and the intracellular \( \text{pH} \) to 7.2, so that \( \text{V}_{\text{Nernst}} = \text{V}_{\text{mit}} = -21 \text{ mV} \). As long as the environmental \( \text{pH} \) is greater than the intracellular \( \text{pH} \), \( \text{H}^+ \) ions diffuse passively through the cell membrane. However, with a higher extracellular acidity, \( \text{H}^+ \) ions can no longer diffuse and must be actively pumped outside the cell: the energy required to pump \( \text{H}^+ \) ions outside the cells can be calculated from the Gibbs energy of ATP hydrolysis (\( \Delta G_{\text{ATP}} = 3.1 \times 10^4 \text{ J/mole} \)) and the energy required to move a charged particle across the electric potential difference \( \Delta V = \text{V}_{\text{mit}} - \text{V}_{\text{Nernst}} \). The rate of ATP consumption is

\[
\frac{\text{dATP}}{\text{dt}} = \text{H}_{\text{Peff}} \cdot \frac{\text{dH}^+}{\text{dt}} \cdot \frac{F \cdot \Delta V}{\Delta G_{\text{ATP}}} \cdot \text{MW}_{\text{ATP}} \cdot (10^3 \text{ mole kg}^{-1})
\]

where \( \text{H}_{\text{Peff}} \) is a constant that parameterizes the efficiency of the \( \text{H}^+ \) pumps, the rate \( \frac{\text{dH}^+}{\text{dt}} \) has been defined in equation (4), \( \text{MW}_{\text{ATP}} \) is the molecular weight of ATP and the multiplicative factor \( 10^3 \text{ mole kg}^{-1} \) is the conversion factor that is needed to express the result in SI units (i.e., one mole of \( \text{H}^+ \) weighs 0.001 kg), so that \( \text{v}_{\text{ATP, H}} \) the rate of ATP consumption for proton secretion against the electric potential, is expressed in \( \text{kg s}^{-1} \) as all the other rates.

Overall, the balance between energy production and consumption can be written as

\[
\frac{\text{dATP}}{\text{dt}} = \text{ATP, TOT} - (\text{v}_{\text{p}} + \text{v}_{\text{DNA}} + \text{v}_{\text{mit}} \cdot M + \text{v}_{\text{ATP, H}})
\]

### 4. Cell proliferation dynamics

In this section we describe how the model of cell metabolism is integrated with the biochemical network that drives the cell cycle and cell growth, and in particular we describe how we link the metabolic network to growth and division, i.e. the mechanism of molecular threshold implemented in the simulator. Next, we discuss the sources of randomness in the dynamics of cell division that are important in the simulator, and finally we list criteria for cell death.

The proliferation dynamics presented here differs in many ways from the dynamics in the previous version described in [6] and hence some preliminary comments are in order. First of all, the control of cell progression along the cell cycle was previously modeled by means of phenomenological thresholds. The cell cycle is conventionally represented by a series of phases characterized by specific molecular events and there are four major phases that cells traverse from birth until duplication: the initial growth factor-dependent phase G1, the S phase where DNA duplication takes place, the G2 phase during which a cell prepares its genetic material for proper sorting into the two daughter cells and the M phase...
were duplication (mitosis) occurs. Passage from one phase to
the other is a unidirectional and highly coordinated process
which is regulated in particular by a class of proteins called
cyclins: the different members of the class are expressed at
specific points along the cell cycle and activate kinases, the
cyclin-dependent kinases (CDKs) [29]. Active CDKs, in turn,
phosphorylate specific protein substrates often on multiple
sites, and these phosphorylation events are tuned by a network
of proteins, inhibitors and enzymes that phosphorylate or
dephosphorylate the proteins involved, among which are the
CDKs themselves [29]. The result is a complex enzymatic
network with feedback regulatory circuits that regulates the
progression of a cell from one phase to the next at specific
points called cell-cycle checkpoints [29]. This network has a
fundamental relevance for both normal and tumor cell growth,
and various models of its inner workings have been proposed
[30].

The control of the progression along the cell cycle
ultimately consists in the activation of kinases which
phosphorylate substrates using ATP as the donor of high-
energy phosphoryl groups [29]; therefore ATP depletion can
be expected to block cell growth, and indeed the existence of
two energetic thresholds at the G1/S and G2/M transitions
has been demonstrated for tumor cells [31, 32]. In our
previous model we included only a rough description of these
thresholds [6]. However, in a recent study of the dynamic
properties of the multisite modification of proteins we have
shown how this mechanism may apply to proteins that are
central to the cell cycle checkpoint mechanism, and how it
can set a biochemical threshold [33, 34]. The threshold
obtained from multisite modification is robust and depends
on the concentrations of all the molecules involved in the
reaction, i.e., the checkpoints depend not only on the ATP
concentration, but also on the concentrations of enzyme and
substrate, and may better account for the observed variabilities
of the cell cycle [33, 34].

A second major difference is the link between energy
production and cell growth: the increase of cell volume was
previously assumed to be proportional to the net uptake of
glucose and to the overall rate of energy consumption [6]. The
idea was that glucose uptake and storage provide a source of
carbon atoms and that at the same time the cell mass increase
can only proceed with a corresponding energy expense. Thus
we considered a net balance of glucose uptake and storage
given by a linear combination of the rates $v_{1p}$, $v_{1m}$, $g_1$
and $g_2$ [6], while energy consumption was modeled by means of
the phenomenological rate $r_c$ discussed above. The rate $r_c$
described the global ATP consumption in processes such us
protein and DNA synthesis, tuning and maintenance of the
membrane potential and so forth, which, however, were not
explicitly included in our previous model [6]. Unfortunately
this description has a logical fault: ATP consumption depends
on the amount of available ATP that in turn depends on
the cell metabolism where molecules such as glucose itself
are converted into energy, and thus glucose uptake and ATP
consumption, and hence energy consumption, are directly
correlated, while they need not be. In addition, the hypothesis
that cell volume increases as a function of energy consumption
is biologically untenable since this does not consider possible
dissipative pathways that are not related to cell growth, such as
active secretion of newly synthesized proteins. Since we make
explicit several energy consumption paths we must revise the
link between cell metabolism and cell growth.

4.1. Linking the metabolic network to the cell cycle

In this subsection we summarize the conditions that each
cell must satisfy to step from one phase to the next in the
simulator, and we start with the most complex mechanism,
which is responsible for the G1/S phase transition. The G1/S
checkpoint is perhaps the best known and the most important
one, because as soon as cells pass the checkpoint they become
committed to progress along the remaining part of the cell
cycle [35, 36]. The molecular details have been reviewed in
several papers (see, e.g., [35–40]).

Central to the underlying molecular network is the
Rb/E2F complex. The retinoblastoma protein Rb (pRb)
has 16 putative phosphorylation sites and may exist in
various forms depending on the level of phosphorylation
[37–40]. In its hyperphosphorylated form, when at least
ten sites are phosphorylated [37–40], the Rb/E2F complex
is fully dissociated, but partial dissociation may occur for
intermediate phosphorylation levels of the Rb protein. E2F is
a transcription factor that once dissociated from the pRb starts
the transcription of genes involved in a series of important
pathways that ultimately bring about DNA synthesis [35–40].
This checkpoint, therefore, marks the end of the G1 phase
and the beginning of the S phase. The phosphorylation of
the Rb protein takes place thanks to the coordinated action
of at least two different cyclin-dependent kinases (CDKs)
that, in turn, are activated by cyclin D and cyclin E. These
cyclins are expressed at appropriate phases of the cell cycle—
in particular the expression of cyclin E is controlled by E2F
after it is released by pRb upon partial phosphorylation—
and form complexes with the specific CDKs. The CDKs’
concentration in the cell is rather constant throughout the cell
cycle, thus the timing of the CDKs’ action is determined by
the expression of the cyclins [29]. The enzymatic activity
of the CDKs is also tuned by phosphatases and inhibitors
that collectively form a complex biochemical network with
feedback regulatory circuits [29, 30]. The network has already
been modeled, and it has been shown that important dynamical
behaviors emerge: thresholds, hypersensitive response and
hysteresis [41]. In other words, the network reacts as an on–
off irreversible switch [41].

Here we apply once again the approach that we followed
in the case of cell metabolism: just as the metabolic network,
the checkpoint control network has a hierarchical structure,
and we assume its dynamics to be dominated by the system’s
hubs. Given its demonstrated importance [35–40], we have
selected the multiple phosphorylation process of the Rb protein
as the central hub of the checkpoint control network. We have
isolated this process from the network (figure 2) and studied
the general dynamical properties of the modifications of proteins
on multiple sites (multisite protein modification, MPM)
[33, 34]. The results have been published recently and here
we summarize the main conclusions:
Figure 2. Scheme of the molecular interactions that have been considered in the model of the G1/S transition of the cell cycle. The retinoblastoma protein (pRB) has been supposed to form a complex with an enzyme E and carries 16 putative phosphorylation sites. The pRB-E complex is synthesized in the G2 phase and is partitioned at random between the daughter cells at mitosis. At the beginning of the G1m phase the cyclin D protein (CyclD) is expressed and it phosphorylates the pRB-E complex upon rapid association with specific cyclin-dependent kinases [35–40]. Phosphoryl groups added to the pRB-E complex are represented graphically by means of black circles. The phosphorylation event is assumed to occur following the reversible bimolecular interaction between cyclin D and the pRB complex: a detailed study has shown that the precise mechanism of pRB phosphorylation is irrelevant with respect to the dynamics of the system’s response [34]. Upon partial phosphorylation of the pRB, a fraction of E molecules are released and catalyze a reaction whereby a substrate S is converted into a phosphorylation network.

1. MPM naturally produces a threshold in the system’s response.
2. The threshold is robust to noise perturbation, and this is important when dealing with low protein concentrations, that is to say when fluctuations are not negligible.
3. If the protein that carries multiple modification sites controls a downstream Michaelis–Menten reaction (figure 2), then MPM delays the downstream reaction and the delay may be several orders of magnitude larger than the characteristic times of enzyme kinetics. Thus MPM drives the information transfer from the fast kinetics of enzymes action to the slow kinetics of cellular response.
4. MPM dynamics does not depend on the attachment/detachment mechanism of the chemical groups (i.e. phosphoryl groups) that modify the protein with multiple modification sites and these may be represented by enzymatic processes or simplified as bimolecular interactions. It follows that MPM is equivalent to the classical allosteric effect [33, 34] and that, to model its dynamics, one can neglect the specific processes of protein modification.

The dynamical properties of MPM are rather attractive because they allow great simplification of the model of the G1/S checkpoint. In particular, from property 3 we can represent the duration of the G1 phase in terms of an enzymatic reaction controlled by a protein with multiple modification sites and this is reminiscent of the way E2F drives the transcription of genes once released by the Rb control protein. Moreover, properties 1 and 2 assure that the dynamical response behaves like a robust on–off switch, and property 4 justifies an approximate description.

The basic model for the control of the G1/S transition in our virtual cells is shown in figure 2 and is based on the following assumptions:

1. The amount of ATP required to phosphorylate the Rb protein is negligible with respect to the overall amount of available ATP in the cell that is described in our model by the variable ATPp.
2. CyclD and CyclE instantaneously form complexes with specific CDKs whose concentration is constant during the cell cycle. As soon as the complex is formed the phosphorylation of the Rb protein starts.
3. The phosphorylation/dephosphorylation events of the individual available sites on the Rb protein follow a stochastic dynamics and are fast with respect to the observation time (i.e. with respect to CyclD and CyclE expression rates).

With these assumptions we can describe the process of pRB phosphorylation by means of a probabilistic chain of phosphorylation/dephosphorylation events (transition chain) that occurs as a consequence of classical bimolecular reactions between cyclins and pRB [33, 34]. In particular, using assumption 3 we can neglect the dynamics of the transition chain and even the chain itself and concentrate instead on the equilibrium probabilities. The analysis in [33, 34] shows that if the E2F is released when at least \( n_{\text{th}} \) sites on pRB are phosphorylated, then we find the number of free E2F molecules:

\[
P_{\text{Rb}} = N_{\text{Rb}} \cdot \sum_{n=n_{\text{th}}}^{N} \binom{N}{n} \cdot p^n \cdot (1 - p)^{N-n} \tag{12a}
\]

\[
p = \frac{1}{2N[Rb]} \cdot \left\{ (N[Rb] + [Cyc] + K_{\text{Rb}}) - \sqrt{(N[Rb] - [Cyc])^2 + 2K_{\text{Rb}}(N[Rb] + [Cyc]) + K_{\text{Rb}}^2} \right\} \tag{12b}
\]

where \( P_{\text{Rb}} \) is the number of activated pRB molecules with at least \( n_{\text{th}} \) phosphorylated sites out of a total of \( N \) sites, \( N_{\text{Rb}} \) is the total number of pRB molecules, \( K_{\text{Rb}} \) is the ratio between backward and forward rate constant of phosphorylation supposed to occur in a bimolecular reaction between the cyclin/CDKs complexes (CyclD and CyclE) and pRB, \([Cyc] = \)
Figure 3. Simulation of cell cycle kinetics and its relationships with the expression of key molecular components of cell cycle control. A life cycle of a cell taken at random from a population growing exponentially and simulated with the parameters listed in table 1 has been chosen to show the time-dependent variations in the mass of key molecular components controlling the cell cycle. (A): kinetics of the cell cycle transitions from one phase to the other for the simulated cell. Cell cycle phases are as follows: 1 = G1m, 2 = G1p, 3 = S, 4 = G2 and 5 = M. (B)–(E): these graphs show the variation of cyclin X, D and E and of the pRb during the same time span as panel (A) (see text). Finally, panel (F) shows the fraction of hyperphosphorylated pRb that has been calculated using equations (12). Note that in the present model the oscillations in cyclin expression (and of other molecular components of the cyclin network), which have indeed been observed experimentally, are the result of protein expression and degradation in successive cell cycles rather than the result of complex nonlinear interactions between molecules. As far as we know, no models of cyclin oscillations have taken into account the intermittent protein expression and degradation through successive cell cycles as the driving force of their oscillatory behavior. More realistically, the two aspects are probably integrated in real cells.

\[ [\text{CycD}] + [\text{CycE}] \] is the total cyclin concentration, and where the square brackets denote concentrations.

A recent, more detailed analysis of the process dynamics has confirmed that it is safe to use the quasi steady-state assumption \[ [34] \].

The number of activated pRb molecules depends on the concentrations of pRb, CycD and CycE. Using data in the current literature we assumed a phenomenological model for the kinetics of the three proteins as a function of the cell cycle phase \[ [29, 35–40] \], and the results are shown in figure 3. In this scheme, the Rb protein has been hypothesized to be synthesized in the G2 phase and then to be partitioned randomly at mitosis between the two daughter cells (see the next paragraph for a further discussion on this point). As far
as we know, experimental evidence on the precise timing of the pRb expression is not available. Existing data indicate that the protein maintains an almost constant concentration in the cell but varies both its phosphorylation state and intracellular localization [29, 35–40]. Since cell division would result in a dilution of the pRb concentration the protein must be synthesized de novo sometime during the cell cycle of the mother cell. For the model described by the set of equations (12a) and (12b) it is only important that pRb molecules are available for a new phosphorylation cycle in the daughter cells at the beginning of their life; hence the precise timing of pRb expression is irrelevant. On the other hand, CycD is synthesized at the beginning of the cell’s life in the G1 phase and is destroyed immediately after the cell has overcome the G1m/G1p transition (see also figure 2); CycE is synthesized in the G1p phase and is destroyed as soon as a cell enters the following S phase. The synthesis rate of these proteins is also assumed to be proportional to the overall protein synthesis rate defined by the set of equations (5). Thus, in general if Xp is the mass of either pRb, CycD or CycE:

$$\frac{dX_P}{dt} = \alpha_{X_P} \cdot \frac{dP_S}{dt}, \quad (13)$$

The mechanism implemented in the simulator, whereby pRb releases E2F as soon as pRb is phosphorylated and subsequently E2F catalyzes the conversion of a substrate S into a product R, models the whole set of enzymatic reactions that occur upon the release of the E2F in the dynamics of the G1m/G1p/S transitions. The pRb/E complex is assumed to follow a 1:1 stoichiometry, thus the concentration of the enzyme E equals the concentration of active pRb molecules. This dynamics is described by the equations:

$$[E] = P_{rb} \cdot [Rb] \quad (14a)$$

$$\frac{d[S]}{dt} = \frac{k_{MM} \cdot [E] \cdot [S]}{K_{MM} + [S]} \quad (14b)$$

$$G1m \rightarrow G1p \text{ if } [S] < S_{th,1} \quad (14c)$$

$$G1p \rightarrow S \text{ if } [S] < S_{th,2}. \quad (14d)$$

where $[S]_0$ denotes the initial substrate concentration in the simulator, and $S_{th,1}$ and $S_{th,2}$ are two critical S concentrations. The initial substrate concentration $[S]_0$ is assumed to be the same for all cells. We remark here that the subdivision of the G1 phase into two subphases G1m and G1p has already been considered in our previous model [6] and is also a known experimental fact [42]. Here, however, this subdivision has a precise molecular meaning as it defines the transition to CycD destruction and CycE synthesis, and moreover both cyclin/CDKs complexes act on the same pRb substrate and this reflects realistically the present knowledge on the G1/S checkpoint [35–40].

At present, the molecular machinery of the G2/M checkpoint is more obscure than that of the G1/S checkpoint, and in that case we assume a much rougher threshold model, i.e., threshold crossing happens as soon as the concentration of the proper cyclin/CDK complex reaches a critical concentration. For this purpose, we consider a generic cyclin X that is synthesized at the beginning of the G2 phase and is destroyed after mitosis.

The M phase duration has small individual fluctuations associated with the mechanical aspects of chromosome condensation and sorting that take place during the M phase. Thus,

$$M_{T_M} = M_{T_M}(1 + \text{PHASE_SPREAD} \cdot \xi) \quad (15)$$

where $M_{T_M}$ is the mean duration of the M phase, PHASE_SPREAD is a constant and $\xi$ is a random variable drawn from a uniform distribution in the interval $(-1, 1)$. In the simulator, the fluctuation of the M phase is determined at cell birth.

The S phase is regulated differently: its duration is not determined by a checkpoint mechanism, but rather by DNA synthesis as described by the set of equations (6). The S phase ends when DNA completes the duplication process.

4.2. Cell proliferation dynamics and stochastic aspects

As a cell proceeds along the cell cycle its volume increases and all the cell material is roughly doubled in mass, so that after mitosis each daughter cell looks like the mother cell at birth. This also applies to mitochondria, and an approximately linear correlation between cell volume and the number of mitochondria has been demonstrated experimentally [43]. Mitochondria possess their own DNA that includes the genes for some, but not all, mitochondrial proteins. The other mitochondrial proteins are coded into the cell’s DNA and imported from the cell cytosol after their expression. Mitochondria proliferate by fission, a process that is reminiscent of bacterial proliferation, and their number also varies because of mitochondrial fusion and death [44, 45]. The proliferation of mitochondria is highly coordinated with cell growth and the molecular signals at the basis of the cell/mitochondria synchrony are presently under investigation [44, 45]. The regulation of cell volume is not well understood at present; however, in the simulator we decided to link the cell’s volume to the number of mitochondria, i.e., we assume that each cell increases its size to adapt its volume to the number of mitochondria in the cytosol (and this part of the model has been completely revised with respect to the previous version [6]).

Mitochondria contain the molecular circuits where the oxidative phosphorylation of glucose takes place and produce ATP. On the other hand, the proliferation of mitochondria requires energy and hence it depends on the overall energy balance of the cell. This establishes an interesting interplay: a cell takes up nutrients from the environment for energy production which is the primary task of mitochondria; mitochondria utilize part of the energy for their maintenance and proliferation; finally, mitochondria give back energy to the cell to accomplish its various tasks.

These arguments lead to the following equations:

$$\frac{dM}{dt} = C_1 \cdot \frac{dATP}{dP} \quad (16a)$$

$$\frac{d\text{Vol}}{dt} = C_2 \cdot \frac{dM}{dt} \quad (16b)$$

where $M$ is the number of mitochondria, Vol is the cell volume and $C_1$ and $C_2$ are two positive constants. It should be noted
that unlike the previous version, the simulation program has no upper bound for cell volume and number of mitochondria, so that, at least in principle, they can now increase without limit. This means that the volume and number of mitochondria are controlled by the metabolic network and by the growth dynamics, and the eventual stability hints at the correctness of the whole model.

At mitosis mitochondria are partitioned between the two daughter cells: the partitioning follows a binomial distribution, and the reasons for this have already been discussed [6]. The volume of the daughter cells is then calculated in accordance with equation (15b):
\[
\text{Vol} = \text{Vol}_{\text{min}} + C \cdot 2 \cdot M_0
\]
where \(\text{Vol}_{\text{min}}\) is the volume of the nucleus [6] and \(M_0\) is the number of mitochondria inherited from the mother cell.

The unequal sharing of cytosol in the daughter cells shows up as an additional source of randomness in the partitioning of pRb. The synthesis of pRb has been hypothesized here to occur in the nucleus during the G2 phase of the mother cell. In its hypophosphorylated form the protein is not released in the cytosol but is retained in the nucleus, bound to the nuclear matrix up to the early G1 phase [46], and thus the amount of pRb that the daughter cells receive depends on the distribution of the pRb molecules in the nucleus and on the dynamics of the nuclear division. In the simulator we implement this mechanism assuming that the nuclear matrix, and hence the associated pRb or any other such protein, is split according to a binomial distribution.

All the other molecules in the cytosol are partitioned proportionally to cell volume, and thus their concentration does not change at mitosis, and the randomness of their mass splitting depends on the fluctuation of the number of mitochondria.

4.3. Criteria for cell death

In the present version the model assumes the following criteria for cell death:

1. The ATP pool falls below a given threshold value \(\text{ATP}_{\text{min}}\). This may occur because of environmental nutrient deprivation.

2. The length of the S phase exceeds a given time span \(S_T\). The length of the S phase in the simulator depends on the availability of ATP and glutamine, and thus on nutrient uptake and utilization. Experiments show that a long-lasting blockade of DNA synthesis is not compatible with cell life and the molecular mechanisms leading to cell death involve the biochemical networks that form the so-called intra S checkpoints [47–50].

3. The length of the G2 phase exceeds a given time span \(G2_T\). This may occur in our model because of the G2/M checkpoint when \([\text{CycX}]\) does not overcome the threshold \(\text{CycX}_{\text{th}}\). Since \([\text{CycX}]\) is a function of protein synthesis and cell volume, this process depends on nutrient availability and utilization as well. And indeed, experimental observations show that a long-lasting blockade of G2 phase progression leads to cell death [47–50].

5. Simulations versus experimental results

5.1. Growth kinetics

In its present configuration, the program simulates the growth of proliferating cells dispersed in a closed environment. This is equivalent to the growth of tumor blood cells in a tissue-culture plate and hence the results of simulations can be directly compared to observations. This comparison is all the more direct because the simulator uses parameter values estimated from actual experimental data.

On the whole, the model presented in [6] and extended here is quite complex because it considers many biochemical and cellular paths, and a true fit of the model parameters cannot be carried out, given the high number of parameters and the scarcity of some key experimental data. Thus the model is not optimized, although the parameter estimates all lie within reasonable biophysical extremes.

And yet, even in the absence of a true optimization, the model seems to be quite robust and is able to reproduce the observed patterns, e.g., figures 4 and 5 show some simulation outputs and experimental data on the growth of two human leukemia cell lines. The simulation covers the same time span as the observations and there is good agreement between the simulated and the observed population dynamics, and the total computed ATP mass also fits quite well the experimental data. The only noticeable difference between simulations and experimental data is the fast decrease of the number of alive cells after the peak, and this probably means that the definition of cell death still lacks some relevant detail. However, cellular properties calculated for simulated cells using the parameters listed in table 1 and in table 1 in [6]—to which we refer as the ‘standard’ parameters and that give the ‘standard’ growth curve in figure 1—match fairly well those observed for real cells (table 2). Among them we find the cell size and the number of mitochondria per cell that, as mentioned earlier, have been modeled without built-in upper and lower bounds. On this basis, we conclude that the model is stable, and we discuss this topic further in the next section.

5.2. Cells grow and modify the surrounding environment

Growth curves such as those shown in figures 4 and 5 can be subdivided into two phases: an initial exponential growth phase followed by a decline in the cell number due to cell death. Both phases are well known to be related to the interplay between cells and their surrounding environment (see [51] and references cited therein). Initially, nutrients and space are abundant and we observe an exponential growth phase; however, as cells grow they produce toxic waste products that accumulate in the environment ultimately leading to cell death in spite of nutrient availability. In ecological terms the size of the environment is related to the so-called carrying capacity [51, 52]. The interplay between cells and the environment has been studied both experimentally and theoretically by Tracqui et al [52] using tumor cells that grow attached at the plastic surface of culture plates. The environmental accumulation of waste products, mainly lactic acid, has been monitored by these authors by means of pH measurements, and it has been
Ab initio simulation of cell proliferation

Figure 4. Simulation of cell growth and death in a limited environment. In both panels, data are expressed as cell density versus time. Upper panel: example of simulation outputs for different values of the parameters described in this paper. The figure reports 4 out of approximately 130 runs obtained during the simulation campaign carried out to check the model and to fine tune its free parameters and are meant to show the range of possible observed behaviors (see also the conclusions and outlook section for a discussion on the robustness of the model to parameter change). The curves marked as run 120 show the simulation outputs obtained with the parameter values listed in table 1 and in table 1 of [6] and which we call ‘standard’. Lower panel: experimental growth curves measured for the two human leukemia cell lines MOLT3 and Raji following the procedures described in the experimental section.

shown that a peak in the growth curves corresponds to a rapid change in environmental pH towards acidic values which are no longer compatible with cell survival [52].

The data shown by Tracqui et al can be simulated by the simulation program when we assume that cells die because of the decrease of environmental pH, and that this process does not depend on whether cells grow attached at the plastic surface of culture wells or in suspension. Figure 6 compares the result of a simulation with experimental data reported in [53] and redrawn here. To compare experiments with simulated data, time in figure 6 has been rescaled in units of doubling time both for real and simulated cells. Doubling time is obtained by fitting the exponential growth phase with the equation

$$N(t) = N(0) \cdot e^{k_p \cdot t}$$  \hspace{1cm} (18)

where \(N(0)\) is the initial cell number and \(k_p\) is the growth rate. Then doubling time \(\tau\) is calculated as follows:

$$\tau = \frac{\ln 2}{k_p}.$$  \hspace{1cm} (19)

Figure 5. Size of the ATP pool in simulated and real cells in growth assays. Upper panel: simulation outputs. Symbols are the same as in figure 4 for proper comparison. The gray dashes represent the SD calculated around mean values for the simulated populations. Lower panel: experimental results obtained with the human leukemia cell lines MOLT3 and Raji following the procedures described in the experimental section.
Figure 6 shows that our model is in good agreement with experimental data as far as cell behavior and environmental changes are concerned.

5.3. Simulations carried out under abnormal growth conditions: predictive capabilities of the model

The model parameters listed in Table 1 have been tuned with a lengthy procedure to simulate the growth of proliferating cells in an environment with standard nutrient concentration. Experimental growth media are supplemented with fetal bovine serum that provides hormones and various growth factors and contain a number of molecules that are required for cell growth such as vitamins, proteins, amino acids, lipids and so forth. However, it is well known that tumor cells can grow even in poorer media, though they are sensitive to the deprivation of some key molecules that include glucose, glutamine and oxygen.

To further test our model we have simulated the growth of proliferating cells in an environment characterized by low glucose concentrations using the standard parameters listed in Table 1. We developed the model without ever considering such limiting growth conditions and hence these simulations are truly predictive. Figure 7 shows some results of these simulations. When the environmental glucose concentration is kept at the standard value of 0.9 kg m\(^{-3}\), ATP production (per cell) remains roughly constant during growth. However, when the environmental glucose concentration is decreased, the model predicts a higher ATP production. Simulated cells in the model transiently produce more ATP in low glucose media because of the ATP sensor that has been fully described in our previous paper. The ATP sensor was introduced to tune the rates \( r_3 \), \( p_{22} \) and \( p_{11} \) shown in Figure 1 and was meant to model phenomenologically both the Pasteur and the Crabtree effects [6]. At low glucose concentrations, the ATP production rate falls below the standard assumed value (ATP\(_{\text{St.}} \) [6]) and turns on the ATP sensor. Additional STORE molecules and glutamine molecules are then catabolized resulting in a transient overshoot of ATP production (and oxygen consumption) [6].

As far as we know, this behavior has never been explored experimentally and therefore we carried out experiments on tumor cell growth in glucose-poor media (see also the materials and methods section). RPMI glucose-free medium has been supplemented with 10% FBS that contains approximately 0.9 kg m\(^{-3}\) glucose. Thus the glucose concentration in the whole medium is approximately 0.09 kg m\(^{-3}\). Under these experimental conditions tumor cells can grow and the ATP production can be measured. Experimental data show that in standard medium the ATP per cell remains almost constant throughout the growth process whereas in low glucose medium the ATP production is significantly higher, in good agreement with the model predictions.

Next, we studied the effects of fast modulations of the environmental glucose concentrations on cell growth (Figure 8). Both simulations and experiments show populations that alternate periods of cell proliferation and death which are out of phase with glucose fluctuations, although the first peak in population size is higher for simulated cells. At the end of the first period of glucose deprivation, the model predicts an accumulation of cells in the S phase: this prediction is confirmed by the experimental observations.

The simulations shown in both figures 7 and 8 agree with the experiments, although the quantitative agreement is not perfect. We have carried out a rather extensive exploration of the parameter space and we believe that the origin is not due to some bad parameter but rather to the missing definition of some biochemical path that finely tunes the cells’ behavior. Nevertheless, our model captures the major features of the cells’ behavior in these unusual conditions.

| Parameter          | Simulated  | Experimental | Reference |
|--------------------|------------|--------------|-----------|
| Growth rate\(^a\) (h\(^{-1}\)) | 0.035      | 0.0304\(^b\) | This work |
| Doubling time\(^a\) (h)    | 19.8       | 22.8\(^b\)   | This work |
| Cell cycle distribution (%) | Average Min Max Mean ±SD | | |
| G1                  | 52.5       | 48.4 59.3    | 54.4 ± 2.2\(^a\) [6] |
| S                   | 34.5       | 30.5 40.5    | 27.5 ± 5.8\(^a\) |
| G2/M                | 12.9       | 7.3 17.7     | 16.4 ± 1.7\(^a\) |
| ATP/Cell (10\(^{-15}\) g) | 5.47       | 5.37 5.55    | 5.76 ± 0.73\(^a\) This work |
| Radius (µm)         | 5.02       | 4.82 5.30    | ~7.5 [63] |
| Volume (µm\(^3\))  | 530        | 471 623     | 700-1500 [63, 64] |
| Mitochondria/Cell   | 220.4      | 190.6 266.9 | 83–677\(^d\) [65] |

\(^a\) The growth rate for both simulated and experimental cell populations was calculated by exponential fitting of growth curves. The doubling time was then calculated as log 2/(growth rate).

\(^b\) Data measured for the MOLT3 human T lymphoblastoid cell line.

\(^c\) Data measured for the Raji human B lymphoblastoid cell line.

\(^d\) Range of the number of mitochondria observed in different cell types.
While cells grow they modify the surrounding environment. This picture shows the results of simulations compared to actual data described in [52], and the relationships between cell concentration and environmental pH at different times during the growth assay. In both panels, the cell density has been normalized with respect to the initial cell concentration C(0). Time has also been rescaled in units of cells’ doubling time to compare the data obtained with fast growing simulated cells and slow growing real tumor cells (see the text for details). Upper panel: simulation outputs. Lower panel: experimental results redrawn from [52].

6. Conclusions and outlook

When dealing with real complex systems, such as animal cells, with a large number of parts and a lot of redundancy, it is difficult to decide which parts of the system are really necessary and must be kept when developing a viable computational model. Several approaches can be followed—and indeed have been followed over the years—to model the growth of proliferating cells, and all have advantages and disadvantages. At one extreme are the analytical models that use coupled differential equations, and the major drawback of these approaches is that the discrete events, which mark a cell’s life (such as the duplication of the genome and cell division), cannot be described because they lack the required analytical continuity. At the other extreme we find the models that try to approach numerically every single known molecular pathway, and still cannot describe all the cell’s details because of the huge amount of computational resources required for this task [53, 54]. Our approach lies somewhere in between these extremes, and we have already shown that it can be used to model quantitatively some key aspects of cell metabolism and of cell cycle kinetics [6]. The approach is based on the fact that biochemical networks in the cell possess a hierarchical structure [8], and if a network has a hierarchical topology then the system dynamics is known to be dominated by the network’s hubs [8]. Thus by modeling the hubs of the cell’s biochemical networks one should, at least in principle, be able to capture most of the information of the cell dynamics. In general, identifying the hubs is not an easy task, as a lot of information is being rapidly accumulated thanks to the integration of methods from classical biochemistry, computational biology, mathematics, engineering and physics (see, e.g., [8] and references cited therein). There are some well-established approaches, namely sensitivity analysis and robustness analysis (see, e.g., [55, 56] and references cited therein), which help in this selection. However, the model described here, although already quite complex, is still rather crude when compared to actual cells, and we model just those paths that are known to be essential for the cell’s life, such as glucose and glutamine uptake and the conversion of these two molecules—whose absence leads to cell death in vitro and in vivo [9, 10]—into energy, storage molecules and important biopolymers such as proteins and DNA. Future developments of the model shall certainly rely on more refined selection strategies.

A comparison between our previous model [6] and the model described here shows how model refinements lead to a better description especially when abnormal conditions require the turning on of seldom used molecular pathways. Indeed our previous model compared very favorably with many existing data in three related areas of cell biology: metabolism, growth and proliferation. However, that model was unable to account for deviations from standard growth conditions because of the incomplete or missing description of certain important paths. The refined model presented here tries to overcome several previous limitations, and includes important new details:

(1) the definition of a closed extracellular environment;
(2) the description of biosynthetic pathways such as protein and DNA synthesis;
(3) the description of a minimal biochemical network for the control of the cell cycle;
(4) the description of additional energy-consuming paths;
(5) the inclusion of stochastic aspects that are required to explain the observed fluctuations in tumor cell proliferation.

We believe that the introduction of a network that controls the cell cycle (point 3 above) is a major improvement in the model. Indeed, it is well known that the control of cell cycle is rather complex as it is formed by a large number of
Figure 7. Predictive potential of the model: ATP production by cells growing in low glucose environments. (A): amount of ATP calculated for cells in simulations carried out with the parameters’ values listed in table 1 for different concentrations of external glucose. The symbol +G refers to the standard glucose concentration of 0.9 kg m\(^{-3}\), which is the typical glucose concentration in complete culture media supplemented with FBS. After the startup phase of the program, which allows the concentrations of all the considered molecular species to settle at equilibrium values, the environmental glucose concentration has been reduced to 10% or to 7.5% of the standard value. (B): experimental results obtained with MOLT3 cells in complete medium (+G) and in two independent experiments where cells have been grown in glucose-free RPMI medium supplemented with 10% fetal bovine serum (FBS). Glucose is present in FBS with an approximate concentration of 0.9 kg m\(^{-3}\), but this may vary in different FBS batches. Thus in these two experiments the final environmental glucose concentration is approximately 0.09 kg m\(^{-3}\). (C), (D): cumulative ATP for simulated cells (panel (C)) and real cells (panel (D)) (the cumulative value has been calculated from the curves in panels (A) and (D) with a simple integration: \(\int_0^\infty ATP(t) \cdot dt\)).

proteins that act as enzymes and/or substrate with complex interactions that lead to a markedly nonlinear behavior [29, 30, 35–41]. These aspects have already been studied in detail both experimentally and theoretically and it has been shown that the network’s dynamics includes emergent properties such as limit cycle oscillations and chaos [29, 30, 35–41]. However, these results have been obtained mostly in cell-free systems using protein cell extracts and it is not clear whether cells share the same dynamics. In particular, the chemical oscillations of some proteins of the cyclin network, although evident in the test tube, cannot be directly connected to cell cycle kinetics where proteins are shared by the daughter cells and where cyclins are destroyed and regenerated after gene expression: processes such as protein degradation and expression are sure to influence the dynamics of the network.

The mechanism that we have included in the simulation program, i.e., the multisite protein modification, and in particular multisite phosphorylation of the Rb protein, has been shown to account for at least three important, observed, dynamic behaviors:

1. generation of a biochemical threshold, required to allow a cell to proceed from one cell cycle phase to the other;
2. generation of a time delay in the downstream reactions that span several orders of magnitude, that is required to transfer biochemical information from the fast enzymatic kinetics to the slow cell response;
3. stability of the dynamics even in the presence of environmental fluctuations.

The last remark means that Rb multisite phosphorylation opposes fluctuations, and tends to synchronize cells. Thus,
Figure 8. Predictive potential of the model: growth of tumor cells in environments subjected to cycles of glucose deprivation. (A) and (B): time-dependent variations of glucose concentrations in the environment of simulated cells (A) and of real cells (B). The concentration of environmental glucose for real cells has been estimated as described in the caption of figure 7. (C), (D): growth curves (cell density versus time) for simulated (panel (C)) and real (panel (D)) cells. (E): cell cycle phase distribution for simulated cells during the first cycle of environmental glucose deprivation. Simulation outputs show an accumulation of cells in the S phase and a strong reduction of cells in the G2/M phases. (F): raw flow cytometry data of cell cycle distribution of real cells during the first cycle of environmental glucose deprivation. Data have been collected as described in the experimental section. The accumulation of the cells in the early S phase is evident (arrow) as well as the reduction of the cells in the G2/M phases, although both data sets do not match quantitatively the simulation outputs. However, the qualitative pattern is well predicted by the model.
the desynchronization of cell cycle kinetics, which is experimentally evidenced [57] and has been addressed in the previous model [6], is greatly reduced and must be restored with the introduction of some additional source of randomness. Our description of such processes is still at the phenomenological level, and yet we obtain realistic population growth curves (see figures 4 and 5 as an example).

We have already remarked that the simulation program uses parameters that are estimated from actual data and that for this reason the results of simulations can be directly compared with observations. Here we add that during the simulation campaign that we have carried out to fine tune some parameter values, we have realized that most parameters are correlated and cannot assume arbitrary values. For example, if cells grow quickly because they express great amounts of cyclins or because they take up large amounts of nutrients, then they produce more waste products that lead to a fast increase of the toxicity of the environment. Thus the actual dimension of the parameter space is smaller than the total number of parameters, and parameter tuning is less complex than it appears to be at first sight.

Here we have shown that the model can reproduce quantitatively several aspects of the growth of large tumor populations of tumor blood cells and can be used to make testable predictions. We also wish to stress that the simulation program has already been able to simulate populations of more than a million cells: using a non-optimized version of the program we were able to simulate the growth of a cluster of cells that started from a single cell and exceeded 1.25 million cells after 5 days of processing using an Apple PowerMac G5. We believe that this marks an important milestone in the advancement of the simulation program and we hope that our model will eventually help the study of developmental features of tumor cell populations.

Acknowledgment

We wish to thank Professor Giancarlo Andrighetto for many useful discussions and for his enthusiastic support and encouragement.

References

[1] Hanahan D and Weinberg R-A 2000 The hallmarks of cancer Cell 100 57–70
[2] Kansal A-R, Torquato S, Harsh G-R, Chiocca E-A and Deisboeck T-S 2000 Simulated brain tumor growth dynamics using three-dimensional cellular automaton J. Theor. Biol. 203 367–82
[3] Mansury Y and Deisboeck T-S 2004 Simulating structure-function patterns of malignant brain tumors Physica A 331 219–32
[4] Peter J and Semmler W 2004 Integrating kinetic models for simulating tumor growth in Monte Carlo simulation of ECT systems IEEE Trans. Nucl. Sci. 51 2535–9
[5] Chignola R and Milotti E 2004 Numerical simulation of tumor spheroid dynamics Physica A 338 261–66
[6] Chignola R and Milotti E 2005 A phenomenological approach to the simulation of metabolism and proliferation dynamics of large tumor cell populations Phys. Biol. 2 8–22
[7] Press W-H, Teukolsky S-A, Vetterling W-T and Flannery B-P 1992 Numerical Recipes in C—The Art of Scientific Computing 2nd edn (New York: Cambridge University Press)
[8] Barabási A L and Oltvai Z-N 2004 Network biology: understanding the cell’s functional organization Nat. Rev. Genet. 5 101–13
[9] Souba W W 1993 Glutamine and cancer Ann. Surg. 218 715–28
[10] Medina M-A 2001 Glutamine and cancer J. Nutr. 131 12539S–2542S
[11] Guppy M, Leedman P, Zu X-L and Russell V 2002 Contribution by different fuels and metabolic pathways to the total ATP turnover of proliferating MCF-7 breast cancer cells Biochem. J. 364 309–15
[12] Mazurek S, Zwerschke W, Jansen-Dürr P and Eigenbrodt E 2001 Effects of the human papilloma virus type 16 E7 oncoprotein on glycolysis and glutaminolysis: role of pyruvate kinase type M2 and the glycolytic-enzyme complex Biochem. J. 356 247–56
[13] Levintow L, Eagle H and Piez K-A 1955 The role of glutamine in protein biosynthesis in tissue culture J. Biol. Chem. 215 441–60
[14] Salzman N-P, Eagle H and Sebring E-D 1958 The utilization of glutamine, glutamic acid, and ammonia for the biosynthesis of nucleic acid bases in mammalian cell cultures J. Biol. Chem. 230 1001–12
[15] Hales N A-P and Price N-T 1999 The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation Biochem. J. 343 281–99
[16] Pucéat M 1999 pH-linked regulatory ion transporters: an update on structure, regulation and cell function Cell. Mol. Life Sci. 55 1216–29
[17] Ho C and Sturtevant J-M 1963 The kinetics of the hydration of carbon dioxide at 25° J. Biol. Chem. 238 3499–501
[18] Burbea Z-H, Gullans S-R and Ben-Yaakov S 1987 Delta alkalinity: a simple method to measure cellular net acid-base fluxes Am. J. Physiol. Cell. Physiol. 253 C525–34
[19] Karumanchi S-A, Jiang L, Knebelmann B, Stuart-Tilley A-K, Alper S-L and Sukhatme V-P 2001 VHL tumor suppressor regulates Cl−/HCO3− exchange and Na+/H+ exchange activities in renal carcinoma cells Physiol. Genomics 5 119–28
[20] Quintart J, Leroy-Hoyuet M-A, Trouet A and Baudhuin P 1979 Endocytosis and chloroquine accumulation during the cell cycle of hepatoma cells in culture J. Cell. Biol. 82 644–53
[21] Newell K, Franchi A, Poussegur J and Tannock I 1993 Studies with glycolysis-deficient cells suggest that production of lactic acid is not the only cause of tumor acidity Proc. Natl Acad. Sci. USA 90 1127–31
[22] Kaminskas E 1978 The pH-dependence of sugar transport and of glycolysis in cultured Ehrlich ascites- tumor cells Biochem. J. 172 453–9
[23] Tildon J-T, McKenna M-C, Stevenson J and Couto R 1993 Transport of L-lactate by cultured rat brain astrocytes Neurochem. Res. 18 177–84
[24] McKenna M-C, Tildon J-T, Stevenson J-H, Hopkins I-B, Huang X and Couto R 1998 Lactate transport by cortical synaptosomes from adult rat brain: characterization of kinetics and inhibitor specificity Dev. Neurosci. 20 300–9
[25] Bröer A, Albers A, Setiaiwan I, Edwards R-H, Chaudhry F A, Lang F, Wagner C-A and Bröer S 2002 Regulation of the glutamine transporter SN1 by extracellular pH and intracellular sodium ions J. Physiol. 539.1 1–14
[26] Meloun B, Moravek L and Kostka V 1975 Complete amino acid sequence of human serum albumin FEBS Lett. 58 134–7
[27] Nelson D-L and Cox M-M 2005 Lehninger Principles of Biochemistry 4th edn (New York: Freeman)
Ab initio simulation of cell proliferation

[28] Drachev L-A, Jasaitis A-A, Mikelsaar H, Nemecek I-B, Semenov A-Y, Semenova E-G, Severina I-I and Skulachev V-P 1976 Reconstitution of biological molecular generators of electric current J. Biol. Chem. 251 7077–82
[29] Vermeulen K, Van Bockstaele D-K and Berneman Z-N 2003 The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer Cell Prolif. 36 131–49
[30] Tyson J-J, Csikasz-Nagy A and Novak B 2002 The dynamics of cell cycle regulation BioEssays 24 1095–109
[31] Sweet S and Singh G 1995 Accumulation of human promyelocytic leukemia (HL-60) cells at two energetic cell cycle checkpoints Cancer Res. 55 5164–7
[32] Martin D-S, Bertino J-R and Koutcher J-A 2000 ATP depletion-pyrimidine depletion markedly enhance cancer therapy: fresh insight for a new approach Cancer Res. 60 6776–83
[33] Chignola R, Dalla Pellegrina C, Del Fabbro A and Milotti E 2006 Thresholds, long delays and stability from generalized allosteric effect in protein networks Physica A 371 463–72
[34] Milotti E, Del Fabbro A, Dalla Pellegrina C and Chignola R 2007 Dynamics of allosteric action in multisite protein modification Physica A 379 133–50
[35] Weinberg R-A 1995 The retinoblastoma protein and cell cycle control Cell 81 323–30
[36] Blagosklonny M-V and Pardee A-B 2003 Therapeutic targets in cancer Adv. Cancer Res. 82 1–16
[37] DeGregori J 2004 The Rb network Modulation of pRb/ E2F functions in the regulation of cell cycle-dependent nuclear matrix-associated protein Proc. Natl Acad. Sci. USA 91 418–22
[38] Ezhevsky S-A, Ho A, Becker-Hapak M, Davis P-K and Dowdy S-E 2001 Differential regulation of retinoblastoma tumor suppressor protein by G1 cyclin-dependent kinase complexes in vivo Mol. Cell. Biol. 21 4773–84
[39] DeGregori J 2004 The Rb network J. Cell. Sci. 117 3411–13
[40] Seville L-L, Shah N, Westwell A-D and Chan W-C 2005 Modulation of pRb/E2F functions in the regulation of cell cycle and in cancer Curr. Cancer Drug Target 5 159–70
[41] Tyson J-J and Novak B 2001 Regulation of the eukaryotic cell cycle: molecular antagonism, hysteresis, and irreversible transitions J. Theor. Biol. 210 249–63
[42] Zetterberg A and Larsson O 1985 Kinetic analysis of regulatory events in G1 leading to proliferation or quiescence of 3T3 cells Proc. Natl Acad. Sci. USA 82 5365–69
[43] James T-W and Bohman R 1981 Proliferation of mitochondria during the cell cycle of the human cell line HL-60 J. Cell Biol. 89 256–60
[44] Garesse R and Vallejo C-G 2001 Animal mitochondrial biogenesis and function: a regulatory cross-talk between two genomes Gene 263 1–16
[45] Frank S 2006 Dysregulation of mitochondrial fusion and fission: an emerging concept in neurodegeneration Acta Neuropathol. 111 93–100
[46] Mancini M-A, Shan B, Nickerson J-A, Penman S and Lee W-H 1994 The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein Proc. Natl Acad. Sci. USA 91 418–22
[47] O’Connor P-M and Fan S 1996 DNA damage checkpoints: implication for cancer therapy Prog. Cell Cycle Res. 2 165–73
[48] Morgan S-E and Kastan M-B 1997 p53 and ATM: cell cycle, cell death, and cancer Adv. Cancer Res. 71 1–25
[49] Meek D-W 1998 Multisite phosphorylation and the integration of stress signals at p53 Cell Signal 10 159–66
[50] Taylor W-R and Stark G-R 2001 Regulation of the G2/M transition by p53 Oncogene 20 1803–15
[51] Chignola R, Dai Pra P, Morato L-M and Siri P 2006 Proliferation and death in a binary environment: a stochastic model of cellular ecosystems Bull. Math. Biol. 68 1661–80
[52] Tracqui P, Liu J-W, Collin O, Clement-Lacroix J and Planas E 2005 Global analysis of endothelial cell line proliferation patterns based on nutrient-depletion models: implications for a standardization of cell proliferation assays Cell Prolif. 38 119–35
[53] Bishop T-C, Skeel R-D and Schulten K 1997 Difficulties with multiple time stepping and fast multipole algorithm in molecular dynamics J. Comput. Chem. 18 1785–91
[54] Ayton G S D, Bardenhagen S, McMurty P, Sulsky D and Voit G-A 2001 Interfacing molecular dynamics with continuum dynamics in computer simulation: toward an application to biological membranes IBM J. Res. Dev. 45 417–26
[55] Fell D-A 1992 Metabolic control analysis: a survey of its theoretical and experimental development Biochem. J. 286 313–30
[56] Kim J, Bates D-G, Postlewaite I, Ma L and Iglesias P-A 2006 Robustness analysis of biochemical network models IEEE Proc. Syst. Biol. 153 96–104
[57] Chiorino G, Metz J A J, Tomasoni D and Ubezio P 2001 Desynchronization rate in cell populations: mathematical modelling and experimental data J. Theor. Biol. 208 185–99
[58] Masafumi W, Wang H-S and Okada A 2002 Characterization of L-glutamine transport by human retinoblastoma cell line Am. J. Physiol. Cell. Physiol. 282 C1246–53
[59] Spencer T-L and Lehninger A-L 1976 L-lactate transport in Erlich ascites-tumour cells Biochem. J. 154 405–14
[60] von Grumbckow L, Elnser P, Hellsten Y, Quistorff B and Juel C 1999 Kinetics of lactate and pyruvate transport in cultured rat myotubes Biochim. Biophys. Acta 1417 267–75
[61] al-Baldawi N-F and Abercrombie 1992 Cytoplasmic hydrogen ion diffusion coefficient Biophys. J. 61 1470–9
[62] Clare P-M, Poorman R-A, Kelley L-C, Watenpaugh K-D, Banow C-A and Leach K-L 2001 The cyclin-dependent kinases cdk2 and cdk5 act by a random, anticooperative kinetic mechanism J. Biol. Chem. 276 48292–95
[63] Freyer J-P and Sutherland R-M 1980 Selective dissociation and characterization of cells from different regions of multicell tumor spheroids Cancer Res. 40 3956–65
[64] Kunz-Schughart L-A, Grobe K and Mueller-Klieser W 1996 Three-dimensional cell culture induces novel proliferative and metabolic alterations associated with oncogenic transformation Int. J. Cancer 66 578–86
[65] Robin E-D and Wong R 1988 Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells J. Cell. Physiol. 136 507–13