A Checkpoint-Orientated Modelling for Cell Cycle Simulation

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Abstract. In this paper we propose a new model of cell cycle simulator which will be used to analyse checkpoint response in multicellular tumor spheroids. Whereas most of the models are phase orientated, our model integrates environmental parameters and checkpoint responses that are required to control cell cycle progression. We will present in this paper our work under progress and the different experiments to be performed in order to validate our cell cycle simulator.

Keywords: artificial life, cell simulation, oncogenesis, cell cycle.

1 Introduction

In silico simulations could provide attractive and fruitful new perspectives for the investigation in biological sciences. In complementarity with or when experiments are technically difficult to address in vivo, virtual environments might prove to be of tremendous interest [Sauro et al., 2006]. Such simulations are generally set up thanks to a high number of parameters. We focus here on cell cycle simulation and our goal is to provide the biologist with control tools on a set of representative and tunable parameters. These tools must give to the biologist a relevant view and understanding of the simulation results. The set of parameters must also offer a generic way of building different cell profiles with specific behaviors and of setting up biologically relevant scenario.

In addition to classical 2-D monolayer cell models, our current research on cell cycle control and regulation [Boutros et al., 2007] relies on the use of an in vivo model called Multicellular Tumor Spheroids (MCTS) [Kunz-Schughart et al., 2004]. This model recapitulates the main properties of growth found in a tumor and reproduces most of its characteristics. These include nutrient and hypoxia gradients that generate a regionalisation of cell proliferation, with actively dividing cells found in the outer layers, quiescent (or dormant cells) in the intermediate zones and a necrotic core in the center. This model is particularly relevant for pharmacological evaluation as well as for foundamental studies on cell proliferation thanks to the recent technical advances in molecular biology and in 3-D cell and tissue imaging [Frongia et al., 2009].

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The ultimate aim of our project is to develop a cell simulator which will reproduce *in silico* the spatial organisation and the proliferative behavior of cells in a MCTS 3-D model [Hoehme and Drasdo, 2010], taking into account the spatial and temporal organization of growth with a special emphasis on cell cycle checkpoints. To this end we have chosen to focus on the scheduling of the cell fate and its different states. The next section will present the concept of cell cycle and checkpoint control; section 3 will present our model based on two elements, activities and checkpoints. The fourth section presents the validation procedure we want to use and section 5 will present our perspectives for the use of this simulator and discuss some of the questions it will address.

2 Cell Cycle and Checkpoints

The cell cycle is often drawn as a circular timeline with different phases starting in G1 and ending at mitosis when a cell divides into two daughter cells. The study of the cell cycle by the biologists puts major emphasis on the essential role of the checkpoints [Elledge, 1996]. The integrity of the checkpoint is essential for cell cycle progression and for the maintenance of genomic stability. By the end of the G1-phase, at the commitment point (R), the cell integrates environmental signals before proceeding towards the G1/S transition. A lack of these signals will lead the cell to enter a quiescent (G0) state. If pro-apoptotic signals are detected the cell will undergo death, called apoptosis. Alternatively differentiation signals will drive the cell out of cell cycle to a differentiation program. If the cell progresses in the cell cycle, it must duplicate accurately all its internal material (DNA, centrosome etc) and double its mass before preparing for division. Before entering into S-Phase where DNA synthesis occurs, the cell must check for the integrity of its genetic material. This is called the G1/S checkpoint. Providing that DNA synthesis is completed the cell switches to G2-phase and it finishes doubling its mass. During S-phase and G2-phase, centrosome duplication and maturation occurs thus building the two platforms that will allow the assembly of the mitotic spindle required for mitosis to occur. However, before proceeding from G2 to mitosis, the cell must ensure again the integrity of its genetic material. This is called the G2/M checkpoint. At mitosis, when cells are dividing, in order to ensure an even segregation of the genetic material in the two daughter cells, the mitotic checkpoint will prevent division until the chromosomes are perfectly aligned on the equatorial plan. Any alteration in these checkpoint mechanisms (for instance a mutation in a key regulator) leads to a genetic instability often associated with transformation and cancer. For these reasons it is essential to integrate checkpoints as artefacts of our simulation model.

3 Modelling Cell Behaviour

3.1 Activities and Checkpoints

The proposed cell cycle simulation model is composed of several activities which represent specific cell behaviours and also checkpoints which are sets of pre-conditions of advancement on the timeline. Figure 1 shows a cartography of

the cell cycle with the localisation of each activity and checkpoint. Based on the experience of our previous work on evolution of developmental systems (Evo-Devo)[Chavoya and Duthen, 2008] where we used evolution strategies to compute the sequencing of the different actions, and on the Cell2Organ model [Cussat-Blanc et al., 2010] based on an artificial regulatory network, the control relies here on a well known seguencing of different actions for cells. The differences observed between cells are function of different schedulings for each action. For instance cancer cells that are found to be defective for a specific checkpoint, such as the mitotic one, will often undergo division with uneven chromosome segregation where normal cells would stop cycling.

Activities. The different activities are composed of an initialisation time $Tmin$, a time-out $Tmax$ and a probability of success between $Tmin$ and $Tmax$: $P(succes(A))$ where A is one of the designed activities . If an activity is not successful between $Tmin$ and $Tmax$ the cell goes to apoptosis. These parameters allow scientists to design cells with alternate behaviours.

- **Initialisation:** this action matches the G1-Phase of the cell cycle. All cells starting their cycle observe this phase which culminates at the R restriction point. During this phase, the cells have not yet been committed to proliferation, differentiation or entry into quiescence.
- **DNA Synthesis:** this activity matches the activity observed in the-S phase. This activity starts when DNA integrity has been verified at the G1/S transition. During this action the cell replicates its DNA.
- **Growth:** this action represents the cell's doubling of its mass. It starts at the beginning of the S-phase and ends during the G2-phase.
- **Centrosome Duplication:** this action represents the duplication of the centrosome. It occurs simultaneously with Growth accross the S and G2 phases.

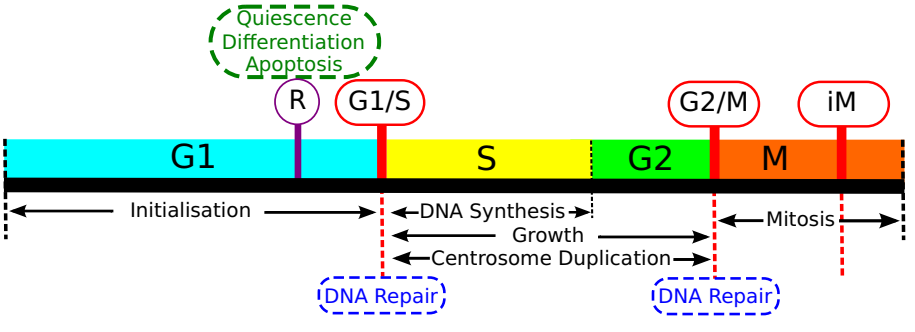

Fig. 1. Localisation of each activity and checkpoint on the cell cycle timeline. Red boxes represent checkpoints with iM being the intra-mitotic one; blue boxes are activities that could be executed during the associated checkpoint; in black with arrows are represented the different activities executed during the cell cycle; the purple circle is the commitment point and the green box represents the three exiting points.

- **Mitosis:** it is the last action of the cell cycle requiring prior checking of genomic activity at the G2/M transition. Mitosis occurs, if all pre-conditions are met, in the final stages of the cycle and ends with the beginning of the two new cycles of the daughter cells. Completion of mitosis requires chromosome alignement at the equatorial plan (mitotic checkpoint).
- **Differentiation:** it represents one of the exit points of the cell cycle. If specific conditions are available the cell will differentiate.
- **Quiescence:** also called G0-Phase, this state is an active survey loop for when environmental factors are insufficient for the cell to proliferate. The quiescent cells are able to return to the cell cycle at anytime if the conditions for growth are met.
- **Apoptosis:** it represents cellular death. Apoptosis happens if apoptotic factors or signals are delivered to the cell or if the cell spends too much time in a specific arrest situation during cell cycle. This activity has neither temporal flags nor probability of success. We assumed that apoptosis always succeeds instantaneously.
- **DNA Repair:** this action is executed if the cell detects lesions on its DNA before starting its replication or before mitosis. This activity is a recovery function allowing the cell to repair its DNA and to advance in its cycle instead of dying of damaged DNA.

Checkpoints. The other important elements of our model are the checkpoints. The checkpoints are composed of a list of activities along with the preconditions of their activation. If several activities are activated at the same time the cell executes them simultaneously. The different preconditions that we set at the checkpoints are boolean flags representing an internal state of the cell or the disponibility of environmental factors. The following list presents the different checkpoint of the cell cycle :

- The **G1/S checkpoint:** here the cell checks its DNA for lesions. If lesions are found, the cell repairs them else it starts DNA Synthesis, Growth and centrosome cycle.
- The **G2/M checkpoint:** to pass through this checkpoint the cell must have replicated its DNA, duplicated its centrosome and doubled its mass.
- The **intra-mitotic checkpoint:** to pass this checkpoint and to divide into two daughter cells, the cell needs to have aligned its chromosomes on the mitotic plan and placed centrosomes on the mitotic spindle poles.

The following list shows the different preconditions that we defined for the cell cycle and what they express when their value is true.

- $C(f)$ expresses that the environmental concentration of f factors is greater than the pre-defined threshold, f could represent glucose, oxygen, growth factors, differentiation factors or proapoptotic factors,
- F_c is the contact forces that inform the cell of its neighbouring environment,
- $Contact\_Inhibition$ indicates that the cell does not have enough place to divide,

- $Mass\_X2$ expresses that the cell has doubled its mass,
- $Centrosome\_X2$ expresses that the cell has duplicated its centrosome,
- DNA_X2 informs that the cell has replicated its DNA,
- DNA_Ok tells that the DNA does not present lesions,
- $Mitotic\_plan$ expresses that the chromosomes are correctly aligned on the equatorial plan and that the centrosomes are on opposit poles.

This is an open list and some preconditions could be added to design more specific behaviours. As an example we also use a $dNTP\_Carency$ flag which allows an inhibition of DNA replication. Cells can be blocked in a particular action if required conditions to pass through the checkpoint are not fulfilled. If a cell stays blocked at a checkpoint until the timeout of one or several actions it dies.

3.2 The Scheduling Policy

A naturally growing population of cells presents heterogeneous characteristics. Because of the variability of the duration of each cell cycle phase, two cells *born* at the same time will not divide simultaneously even if environmental conditions are equivalent. This property is also observed in *in vitro* cultures. In these cultures, the use of pharmacological compounds allows the synchronisation of cells, blocking them at a checkpoint. The need to understand temporal behaviour of cells is at the heart of cancer research and a simulator must offer a new way of investigating this problematic. For this purpose we wish to avoid using fixed handcoded parameters for the duration of the cell cycle. On the one hand, we want the evolution of the model to determine the duration of the cycle as function of the environment's evolution. On the other hand we need to fix some limits to avoid excessively abnormal behaviour. Keeping this goal in mind we propose the use of a set of temporal flags fixed by the designer.

The flags, as presented in the previous section, consist of a minimal time for each activity, a time out to avoid infinite processes and the probability of success between $Tmin$ and $Tmax$. This probability of success offers the simulation guarantees of heterogeneity throughout its cell population. This heterogeneity is one of the strengths of our model. It should normally prevent the emergence of computational artifacts like synchronisation or phasing. On a biological level it should offer us the means to build cellular synchronisation thanks to virtual molecular activity. In the same way this heterogeneity offers the simulation a biological relevance by not considering cells as homogeneous agents. To increase this heterogeneity and to stay as close as possible to what is observed *in vivo*, we also add noise to each temporal flag. Applied to the population these noises are expressed as a gaussian repartition centered on the flag value for each parameter.

4 Software Architecture

Our simulator is implemented using C++ language. Figure 2 shows a simplified class diagram of the simulator. GUI and functional core are executed on

two different threads which communicate thanks to Qt 4.4 signals/slots framework. This architecture allows to launch offscreen simulation. Future extension should need more computational ressources and offscreen simulations will be necessary to use parallelization on computer grid or supercomputer. Actually cells are executed sequentially with random sort between each top to simulate multithreaded execution. With its graphical interface our simulator offers to the user the possibilty to change simulation parameters in real time.

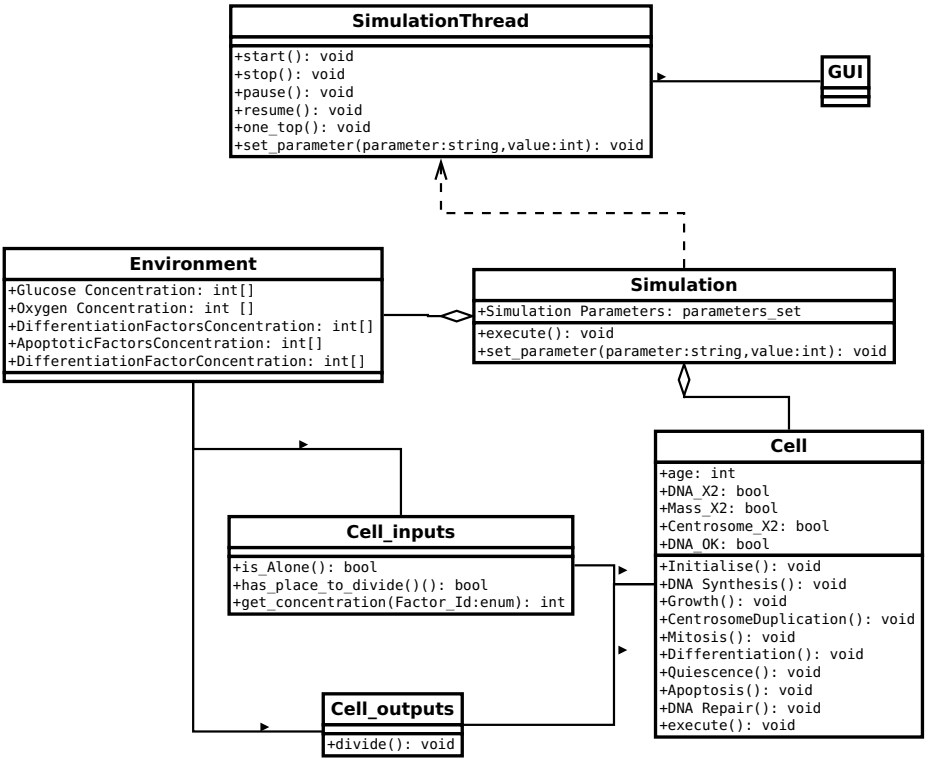

Fig. 2. Simplified class diagram of the simulator

5 Ongoing Validation Procedure

The validation of the model and of the scheduling policy will be done in a two steps process. First, we will implement a 2-D prototype to validate the cell cyle model with simple experiments. There are *in vivo* experiments in 2-D monolayer cultures that we could simulate to analyse our model response. This section shows what these experiments will be.

Our 2-D prototype will be validated by evaluating the convergence of simulation and well known biological results on different scenarii. We will validate individual and collective cell behaviour using in silico experiments that are reproduced *in vivo*. This validation will be done through proliferation experiments with and without environmental constraints. A good match between *in vivo* and *in silico* simulations would offer us the opportunity to build new simulations. These simulations would allow the investigation of biological theories presently unworkable in *in vivo* experimentation.

For example we will use the following validation experiments : cell cycle synchronisation through a lack of environmental factors (arrest in G0); cell cycle synchronisation using a procedure known as double thymidine block (arrest at G1/S); application of a compound targeting the assembly of the microtubules (arrest at mitosis); etc ...

6 Future Prospects

In this section we describe a set of experiments we will perform on the 3D simulator. All these experiments will be conducted at the same time on in vivo spheroid models to compare obtained results and increase the simulation's modalities. In a cross-talk logic between *in vivo* and *in silico* experiments, these simulations will be used to help the biologists to analyse experimental data along with the study of the efficiency of different mechanisms. The simulations may also lead us to formulate new hypothesis which would need the use of *in vivo* experiences to validate *in silico* results. To study checkpoint alteration we will introduce virtual mutation of the checkpoint machineries and analyse their consequences on the proliferative behaviour in unconstraints condition and under genotoxic stress or chimiotherapeutic treatment. Another kind of experiment that could be perform is a coevolution between two kinds of cells : a minority of mutant cells with damaged checkpoints and a population of normal cells.

These kind of experiments will then be extended to the study of the cells' response to different kinds of environmental signals. We will also study the proliferation, in basal conditions, of the occurence of a hypoxia gradient. One of the future extensions of our simulator will be a vascularisation module for the 3-D simulation by using the introduction of virtual neo-vessels. Finally, we will investigate the therapeutical response of cells exposed to external agent targetting the proliferation mechanisms implemented in the model. Many questions might be addressed such as the interest of a specific combination of therapeutical approaches. We will also address specific issues related to the association of a genotoxic agent and an abrogator of the G2/M checkpoint : are the consequences the same in 2-D and 3-D cultures ? Which cells are damaged ? What is their location ? What are the implications on the tumoral growth ?

7 Conclusion

We have briefly presented in this paper the design of a cell development simulator based on an accurate modeling of the cell cycle temporality. The interest of that

kind of numerical model is to provide the biologist with tools which permits to do cross-over between *in silico* and *in vivo* experiments.

Currently, our 2-D model is almost ready for the 2-D experimentation presented in section 4. We have already performed a number of experiments to test the good sequencing of the different activities and the checkpoints' responses in simple situations. These tests have been made on a single cell plugged into a virtual benchmark simulating its neighbourhood and its environmental conditions. Few elements are still under development but the 2-D simulator is near completion for proliferative simulation.

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