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# Checkpoint Orientated Cell Cycle Modeling Issues in Simulation of Synchronized Situation

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**Abstract**

In this paper, the strengths of the checkpoint-orientated modeling in synchronizing cell population are highlighted. Through different experiments, this work shows how to synchronize a population of asynchronous and heterogeneous cells with our proposed model of cell cycle. We will show that the probabilistic modeling undertaken accurately reproduces the dynamics of cell population under specific environmental conditions.

## Introduction

Living world daily reveals its complexity. Understanding and assimilating this complexity is of major relevance. With the latest computation capacity explosion, *in-silico* models are positioned to provide new means of studying and exploring complex living systems. Many questions could be tackled with these approaches, specifically when experiments are difficult to address *in-vitro*. System modeling may therefore use fitted methodologies and bottom-up approaches tend to be the general paradigm. They focus on each functional component of the systems and their interactions; and allow to tame the natural complexity and to represent it in model.

Cellular cultures are a set of experiments used by biologists to characterize *in-vitro* specific features of the cell behavior. For instance, in cancer research, the culture is used to evaluate the impact of pharmacological compounds on specific regulatory mechanisms of the cells. Increasing the understanding of the cell cycle is at the heart of cancer research and therefore, the high opportunities foreseen with *in-silico* simulations of cellular systems let think that prospective search of new therapies could be addressed *in-silico*.

In the different fields of computational and molecular biology, the focus on aspects of the cell cycle differs. Molecular biology models focus on the modeling and simulation of the molecular regulatory network of cycline-dependent kinase (CDK) (Novak and Tyson, 2004). These models can be classified into two kinds of models, the discrete model and the continuous ones. Continuous models basically describe the evolution in the concentration of proteins using a set of ordinary differential equations, whereas discrete models focus on the activation state of each regulatory protein thanks to a predefined genetic regulatory network (GRNs) (Kauffman, 1969; Chavoya and Duthen, 2008). These models have been commonly used to simulate the cell cycle in yeast (Chen et al., 2004; Novak et al., 2001), frog eggs (Novak and Tyson, 1993; Pomerening et al., 2005), fruit flies (Calzone et al., 2007) and different mammalians cells (Aguda and Tang, 1999; Singhania et al., 2011). These models are molecular-based models and do not account for behavioral considerations at a macro-level, their aims being to focus on the regulatory mechanisms.

The other family of models used to simulate cell proliferation is called Individual Cell-Based Models (ICBMs) (Loeffler and Roeder, 2004). These are a subset of the agent-based models. Agent-based models have mainly proved their relevance in the simulation of different complex systems from social networks to the social behavior of hive insects. Basically, individual cell based models come under two classes: cellular automaton (CA) models and off lattice models. On the one hand, CA models are described by a discretization of the proliferative environment in 2-D/3-D evolution grid, and the cell shape is reduced to a lattice site. In this case, cell behavior is composed of the different update rules set up (Moreira and Deutsch, 2002). On the other hand, off-lattice models have the advantages of leaving evolving cells in a continuous media with continuous shapes. They can introduce topological aspects based on *in-vitro* observation or knowledge. This involves high stakes for investigative considerations. The ICBMs have been successfully used to study the pattern formation in multicellular cultures (Galle et al., 2005; Gerlee and Anderson, 2007), avascular tumor growth (Hoehme and Drasdo, 2010) and the spatio-temporal organization of tissues (Drasdo and Loeffler, 2001). These models generally consider the cell cycle as a single time unit decision and the update frequency is the global scheduler of the cell cycle. Basically, this representation does not allow any consideration on the relevance of the major events occurring during progression in the cell cycle phases.

Moreover, ICBMs and hybrid representations with GRNs have been widely used in Artificial Life to study the mechanisms of morphogenesis (Cussat-Blanc et al., 2010; Doursat, 2006). In these studies the cell cycle has to be seen as the cellular behavior with a bio-inspired paradigm.

Whereas molecular-based models accurately express the dynamics of the advancement of cells in each phase of the cell cycle, the individual-based models often do not, due to their meta-description of the cell cycle. Expressing these dynamics reveals interest in simulating *in-vitro* cultures where external compounds are introduced to study their effects in the dynamics of advancement. Our goal is to simulate as closely as possible the population response to an external stress expressing the dynamics of cells progression at a population scale. For that purpose, we use the simplicity of ICBM representations to describe cellular behavior and to introduce temporal considerations thanks to an accurate description of the cell cycle. This approach led us building a hybrid representation of the cell cycle with a hand-coded regulation network and probabilistic-based cellular processes.

In this paper the problem of synchronizing a population of cells is addressed. This consists in activating a specific checkpoint thanks to environmental modifications. Through these experiments, each checkpoint of the model will be specifically activated and the dynamics of the population under these conditions will be analyzed.

Next section introduces the cell cycle model with its biological background. Section 3 shows the different experiments led *in-silico* to synchronize a population of cells. Finally, the last section will discuss the assumptions made for this work and addresses some questions for the next step which is the experimental validation.

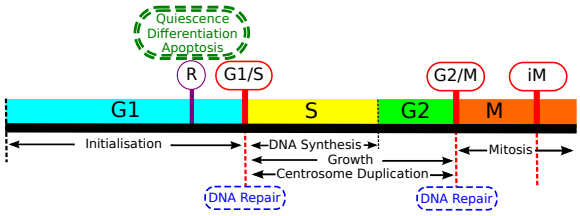


Figure 1: Localization of different cellular processes and checkpoints on the cell cycle timeline. Red simple-lined boxes represent checkpoints with iM being the intra-mitotic one; blue dotted boxes are processes that could be executed during the associated checkpoint; in black with arrows are represented the different processes executed during the cell cycle; the ringed R is the commitment point and the green double-dotted box represents the three exiting points

## Cell Cycle Modeling

### Biological Background

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). They are the warrants of the cell's genomic stability and their integrity ensures a good progression on the cell cycle timeline. 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 the cell cycle to a differentiation program. When a cell progresses in the cell cycle, it must accurately duplicate 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 DNA integrity checkpoint. Providing that DNA synthesis is fully 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 check for the integrity of its genetic material again. This is called the G2/M checkpoint. At mitosis, when cells are dividing, in order to ensure an even segregation of the genetic material into the two daughter cells, the mitotic checkpoint (iM) prevents 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 artifacts (or essential milestones) of our simulation model. Figure 1 shows cartography of the cell cycle with the localization of each cellular processes and checkpoints.

In this work the focus of our simulation is put on the temporal behavior of the cells. The checkpoints are the main regulatory mechanism of the cell cycle and are emphasized to study the influence of their activation over a population scale. The modeling process is driven by the temporal problematic. To accurately express the temporal specificities of the cell cycle, the different regulatory mechanisms are described and embedded in a close description of the cell cycle.

The functional and regulatory level of the cell cycle are disjointed. A weakness of traditional approaches in proliferation simulation is often to focus on only one of these aspects whereas the effective cell behavior depends on the interaction between these two levels. Particularly, from

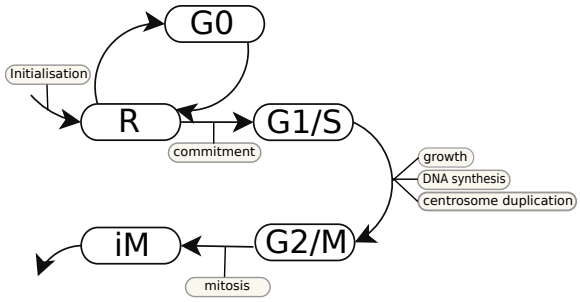


Figure 2: The defined regulators are connected to each other to build a finite state machine (FSM) which embeds the regulatory mechanisms of the cell cycle. The schema also indicates in which position of the FSM are executed each processes.

the cell's internal state depends the regulatory pathway followed. For instance a cell that has replicated its DNA will be allowed to continue its proliferative behavior. To represent these mechanisms and their interaction with the greatest accuracy, it is necessary to observe and describe both levels in accurate cell cycle modeling.

The next part will present how the cellular behavior is designed. This work is based on the model presented in Pascalie et al. (2011).

## Cell Cycle Instance

**Cellular Processes** The cellular behavior introduced leads to the splitting of the cell cycle into sub-behaviors. In this way, each cellular process could be expressed as an autonomous entity. Therefore modeling the checkpoints provides a control over the sequencing of the different cellular processes.

Figure 2 shows the finite state machine designed to schedule the cell behavior. The R $\Longrightarrow$ G1/S $\Longrightarrow$ G2/M $\Longrightarrow$ iM sequence of regulators represents the proliferative behavior of the cells. The cell starts its cycle trying to pass the restriction point (R) and ends with mitosis.

With this modeling approach, the generic cell cycle model designed allows the design of specific cell lineages by instantiating specific checkpoints and processes. The following list describes the different cellular processes. These processes have to be seen as the cell behavior during a transition between two nodes:

- **Initialization**: it represents the G1-phase of the classical cell cycle. During this process the cells have not yet been committed into proliferation, differentiation nor entry into quiescence. This process ends with the transition of the cell at the commitment point. This activity is more a scheduling activity than a functional process of the cell.

- **Commitment**: this action is the planning behavior of the cell. It occurs when the cell has ended its initialization and when it decides which behavior it will execute.

- **DNA Synthesis**: this activity represents the S-phase of the classical cell cycle. It starts at the end of DNA repair - if necessary - 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 mass doubling. 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 during the S- and G2-phases.

- **Mitosis**: This is the last action of the cell cycle. It requires prior checking of genomic activity at the G2/M transition. If all pre-conditions are met, mitosis occurs in the final stage of the cycle and ends with the beginning of the two new cycles of the daughter cells. Completion of mitosis requires chromosome alignment at the equatorial plan (mitotic checkpoint).

A cell is thus considered to be in G1-phase until it has passed the G1/S checkpoint (if it is executing *initialisation* or *commitment* activities to be precise). A cell is considered in the S-phase while executing *DNA synthesis* regardless of *growth* and *centrosome doubling*. Therefore the cell is considered in the G2-phase when it has ended its DNA synthesis and while it is ending its *growth* and its *centrosome doubling*.

**Cell Cycle States** The proliferation is not the only behavior observable in this model. The regulatory network presents alternative behavioral functions of the pathway followed by a cell:

- **Differentiation** represents one of the exit points of the cell cycle. If specific conditions are met, the cell will differentiate. This exiting point is available at the R-node (Restriction Point) of the regulatory network.

- **Quiescence**, also named G0-Phase, is an active survey loop used when environmental factors are insufficient for the cell proliferation. The quiescent cells are able to return to the cell cycle at any time if the growing conditions are met. This alternative behavior occurs when the cell is at the G0-node.

- **Apoptosis** 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 stationary situation of its cell cycle. Apoptosis can occur at any time of the cell cycle.

**Cell Regulators** The pathways previously introduced emerge from a particular sequence of activated checkpoints. The checkpoints are schedulers of the cell cycle in real cells, for that purpose cell regulators are designed and are the warrants of the good sequencing of the processes presented before. These regulators (*i.e* the nodes of the network) are composed of a list of activities along with the preconditions of their activation. They regulate the cell cycle and activate the different processes if their preconditions are fulfilled. If several activities are activated at the same time the cell executes them simultaneously. The preconditions are two sets of boolean flags, one representing the internal state of the cell and the other indicating which activities are done, under progress or planned.
The following list presents the different regulators we defined in our computational cell cycle model:

- The **R commitment point**: cell has to choose between commitment to the proliferation pathway, the quiescent stage, or the differentiation process.
- The **G1/S checkpoint**: here the cell checks its DNA for lesions. If lesions are found, the cell repairs them or die, 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, should not have detected any DNA damage, have 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 its centrosomes on the mitotic spindle poles.
- The **G0 regulator**: we chose to model the G0 state as a regulator because it represents an active survey loop of environmental factors for proliferation. In order to uncorrelate the cell functional level and its regulation, we consider this particular state as a regulatory element of our cell cycle model.

**Computational aspects** A natural population of cells presents heterogeneous features. Owing to 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 were equivalent. In this work, this heterogeneity is represented with a specific set of parameters for each cell. Therefore, the embedded parameters are generated according to a distribution law. The cell cycle model is thus able to produce a population of specific cells and not only a population of clones. If the cell population was composed of clones, the system would suffer from phasing and synchrony in the sequencing of the different phases, each sister cells going to division at the same time.

To represent the cellular activity in a temporal manner and remain at a macroscopic level of representation, we based the cellular process modeling on their scheduling. In this context, 3 parameters are used for each cellular process: the optimal time of realization, the maximum time before it eventually results in the cell's death, and the probability of success. Using these parameters, we generate a set of parameters which are used for the computation. Our processes are represented over time as Bernoulli processes. The average optimal time determines the number of successes needed to consider the process as achieved and the success rate is used to define the probability of success of one trial.

The simulations processed with this model are discrete-time simulations. The simulation time step is defined at the setup and is fixed to six minutes in the different experiments presented here. At each time step, the agents are randomly sorted and their behavior is processed. At each time step, a bernoulli experience with the parameters previously introduced is intended by each cell and the success of the different experiences lead the cell through its cycle. If a division occurs the divided cell is removed from the population and is replaced by two daughters cells. The parameters of the daughters cells are different. This ensures that the population will not converge to a population of clones. Nevertheless, the daughter cells inherit the DNA lesion of their mother if division could have occurred with it.

The multi-agent system built with the previous elements will be used to validate our cell cycle model with experimental data. The next part is dedicated to the simulation of cells population in synchronized situation.

## Experiments

In Pascalie et al. (2012), the qualitative aspects of the cell cycle model were presented. The results shown highlight the ability of the simulator to reproduce specific features of the cell proliferation. The simulation of the exponential growth phase was achieved using specific environmental features and the results presented here use the same specific conditions.

The aim of the work reported in this paper is to demonstrate the model ability to accurately reproduce an important feature of the regulation of cell proliferation that is the activation of specific cell cycle checkpoints. To reach that goal, four virtual synchronization experiments have been performed, each of them leading to the activation of a specific checkpoint. In *in-vitro* experiments, cell cycle synchronization is used to analyze the progress of a cell population through the different stages of their cycle. In this work, the first experiment aims at activating the restriction point (R) avoiding the cell commitment in the cell division cycle by suppressing growth factors from the the environment. The second experiment aims at activating the DNA-damage dependent G1/S and G2/M checkpoints. To achieve this, the deleterious consequences on the genetic material of ionizing

radiation exposure is simulated by defining DNA damage. Similarly, the third experiment aims at selectively activating the DNA-damage dependent G2/M checkpoint. The last experiment will evaluate the intra-mitotic checkpoint activation by simulating an alteration of the mitotic spindle assembly through a well-known procedure known as nocodazole block. Nocodazole is used to disrupt the reorganization of the microtubule network that is required to form a mitotic spindle and therefore leads to the activation of the mitotic checkpoint. This results in cell cycle arrest, thereby synchronizing the cell population at mitosis. All the results presented in this section are the average of 8 instances of simulation. This choice was made to minimize the artifact induced by the pseudo-random number generator used.

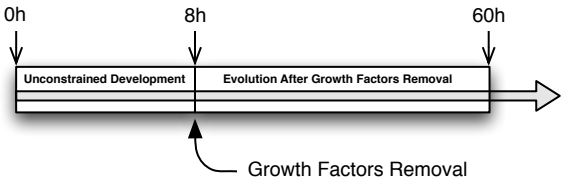


Figure 3: Timeline of the scenario dedicated to analyze the R checkpoint response to growth factors removal. The other experiments introduced in this paper use an equivalent timeline with a different compound introduced at $t = 8h$.

The first analyzed checkpoint is the restriction checkpoint (R). At this point, the cells have to decide whether they are prone or not to commit in the cell division cycle. This decision depends on the availability of various required environmental cues such as growth factors. With this aim a growth factors removal scenario was designed. Figure 3 shows the timeline of this scenario. After 8 hours of unconstrained development, the cells are submitted to a growth factor removal. This change in growth media condition is simulated thanks to a simulation event where the availability in growth factors is set to 0%. Figure 4 shows the result of the simulation. In (a) the evolution of population in each phase shows that after 8 hours of treatment, the cells start to accumulate in G1-phase. The RTmax parameter defines the time a cell can spend at the commitment point before entering into quiescence. This parameter is revealed by the results, it consists in the time elapsed between the growth factor removal and the cells entry into quiescence. The population dynamics shown in this experiment is consistent with the common results in this case.

We next analyzed the DNA-damage dependent checkpoints that are activated at G1/S and G2/M transition, when the integrity of the genetic material has been impaired, for instance after exposure to ionizing radiation. These checkpoints warrant the genomic stability in the proliferation of the cells. At these points, the cells have to test their DNA integrity before starting to duplicate it or to proceed into mitosis. To test this response, the cells are virtually exposed to ionizing radiations that lead to DNA lesions. This parameter is integrated in the model and DNA lesions are represented as a DNA injury rate. In this case, the value is set to 100% and the repairing ability of the cells is avoided. As all the parameters of the model, it represents an average value at the population scale. As in the previous experiments, cells are let proliferate for 8 hours in exponential growth phase. Once this time elapsed, the event simulating the UV exposition occurs and the cells receive DNA lesions. Figure 5 shows the result. As in the previous experiments, cells are in exponential growth phase during the first hours. On curves (a), it is noticeable that once the event representing the ionizing radiation occurs, the ratio of cells in S-Phase and Mitosis starts to decrease whereas it increases in the G1-Phase and in the G2-Phase. This is consistent because the cells can neither exit the G1-Phase nor the G2-Phase due to their DNA injuries on the one hand, and the cells exiting the S-Phase and the Mitosis respectively enter into G2-Phase and G1-Phase on the other hand. The decrease of the ratio of cells in G1-Phase and G2-Phase occurs when the cells start dying due to too much time spent trying to pass the activated checkpoint. Figure 5 curve (b) represents the evolution of the population size. The exponential growth phase is characterized by the increase of the population size at the beginning of the simulation. The population size starts decreasing when the cells start dying due to their DNA damages.

In order to refine this analysis, we next simulated the cells' response to the single activation of the G2/M DNA-damage dependent checkpoint. This is a classical situation that occurs in cancer cells that have lost, through the mutation of an essential suppressor gene called p53 , the ability to arrest at G1/S upon DNA injury. To perform this simulation we virtually exposed a p53 deficient population of cells to ionizing radiation and examined the consequences of this exposure. Actually, the model does not allow to represent directly this kind of cell lineage. To this purpose, the DNA integrity test that occurs at the G1/S transition is deactivated in an ad-hoc manner for this experiment. This problem will be addressed in the further works section.

Figure 6 shows the results. On these curves the G2 accumulation is observable and it fits with the expected behavior. On the second curve (b), representing the evolution of the population size, the results are consistent. The population stops increasing once entry into mitosis is inhibited by the activation of the G2/M checkpoint and starts decreasing once the cells have spent too much time at this stage and start dying.

The last checkpoint we attempted to simulate was the intra-mitotic one. Nocodazole is used to disrupt the reorganization of the microtubule network that is required to form a mitotic spindle and therefore leads to the activation of the mitotic checkpoint. This results in cell cycle arrest, thereby synchronizing the cell population at mitosis. To simulate the

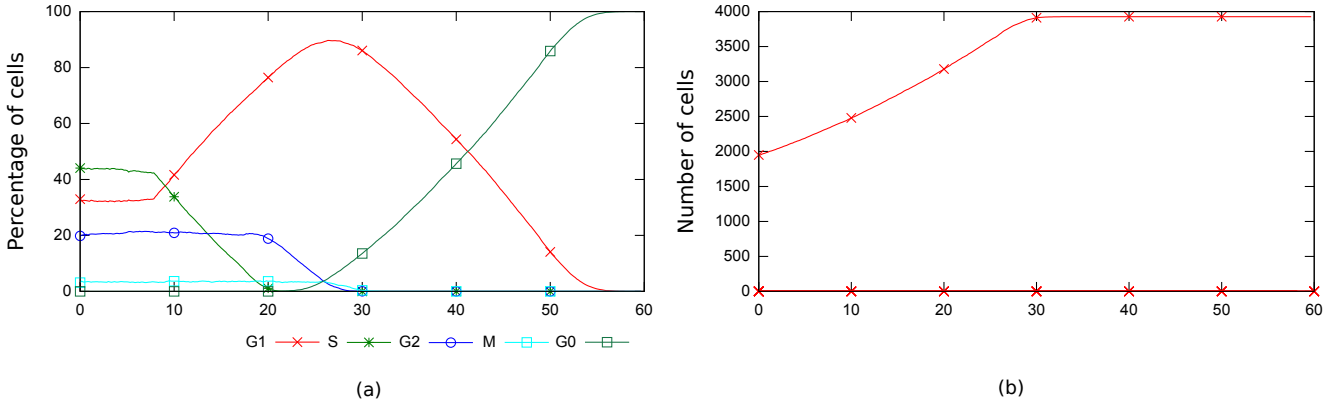


Figure 4: Results of the *in-vitro* experiment aiming the R checkpoint activation. (a) Evolution of the ratio of cells in each phase. Once the growth factors removed the cells start to accumulate in G1-Phase (b) Evolution of the population size. The population still increases after the growth factors removal until all the cells are arrested at the commitment point. The population size does not decrease because the cells enters into quiescence in this case.

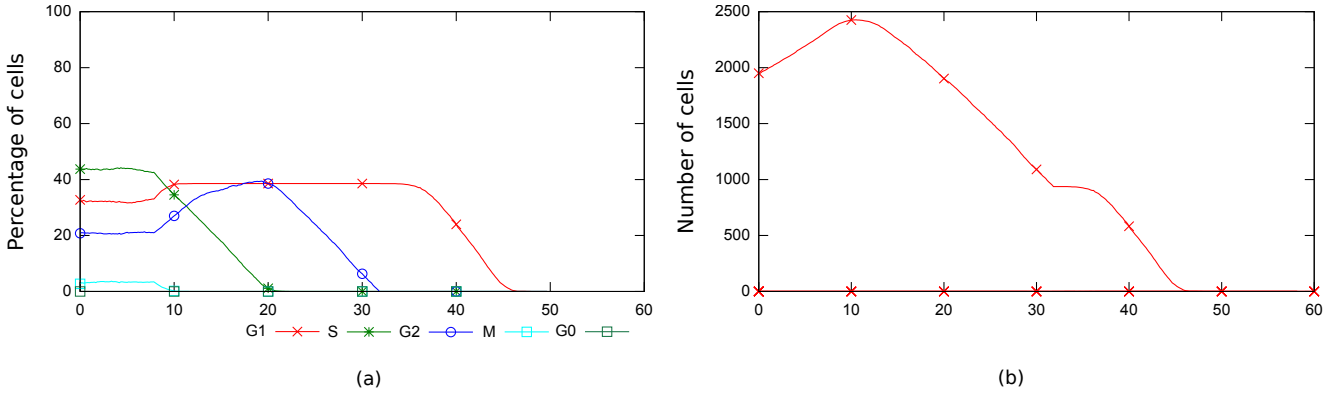


Figure 5: Results of the *in-silico* experiment aiming the simultaneous activation of G1/S and G2/M checkpoint. (a) Evolution of the ratio of cells in each phase. At $t = 8h$ ionizing radiation are induced and the cell proliferation stops. The cells are arrested in G1-Phase while they accumulate in G2-Phase by exiting the S-Phase. They start to die when they have spent too much time trying to repair their DNA damages. (b) Evolution of the population size. The population size stops to increase once the cells are exposed to ionizing radiation .

nocodazole adjunction, we set the mitosis rate of success to $\tau_M = 0\%$. With this parameter the cell will enter in mitosis but should not complete it due to a too small success rate. Figure 7 shows the results of this simulation. On the first stage of the simulation the evolution of the cells in each phase remains constant while the cells proliferate in exponential growth phase. When the event occurs, it is observable that the evolution of the cells in Mitosis starts increasing. This evolution is consistent with the nocodazole effect, which is to affect the mitosis machinery and thus avoid the mitotic spindle formation.

## Discussion and Further Works

The interest of the work presented here resides in the ability of the *in-silico* model to accurately reproduce the cells' response to environmental modification and to the activation of cell cycle checkpoints. The probabilistic modeling undertaken here allows to accurately reproduce the qualitative aspects of the cells dynamics. Nevertheless this approach undergoes a lack, which is the difficulty in parameters tuning due to the difficulty to map experimental biological values to probability. In some cases, it is conceivable that the search for the best values needs to carefully analyze the model response under different conditions in order to map biological observation to a particular setup. It is conceivable to automatize this search using evolutionary strategies. With a set of relevant *in-vitro* data, the best value, and moreover the best scenario fitting with these data, could be found.

The use of this model could be extended to research new compounds or to determine which cells interactions have to be highlighted to answer a particular need. It is conceivable that a scenario should be determined thanks to genetic

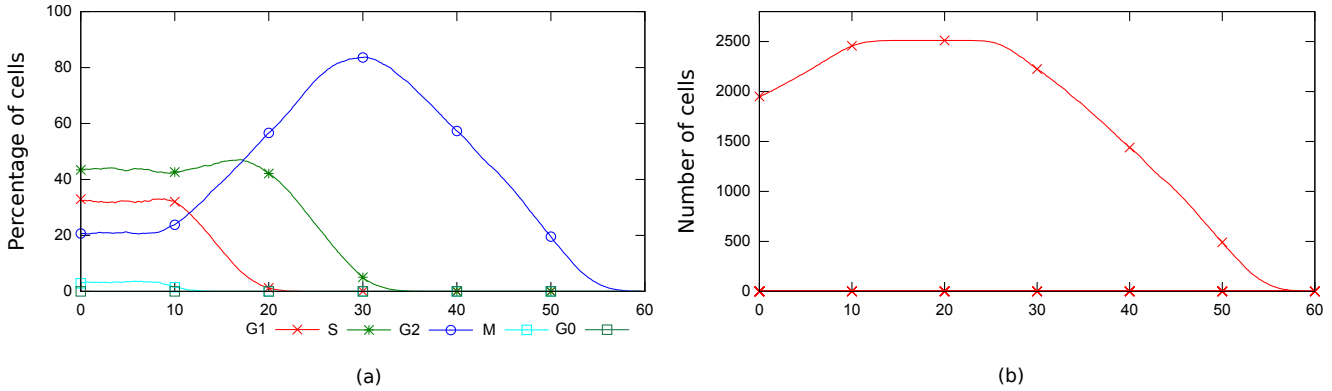


Figure 6: Results of the *in-silico* simulation aiming to solely activate the G2/M checkpoint. (a) Evolution of the population in each phase. The cells start to accumulate in G2-Phase once the ionizing radiation occurs. The G1-Phase accumulation is not observed because the G1/S DNA-integrity test has been deactivated. (b) Evolution of the population size. Once all the cells have divided the population size stop to increase and it starts to decrease when the cells start to die due to too much time spent trying to repair the DNA.

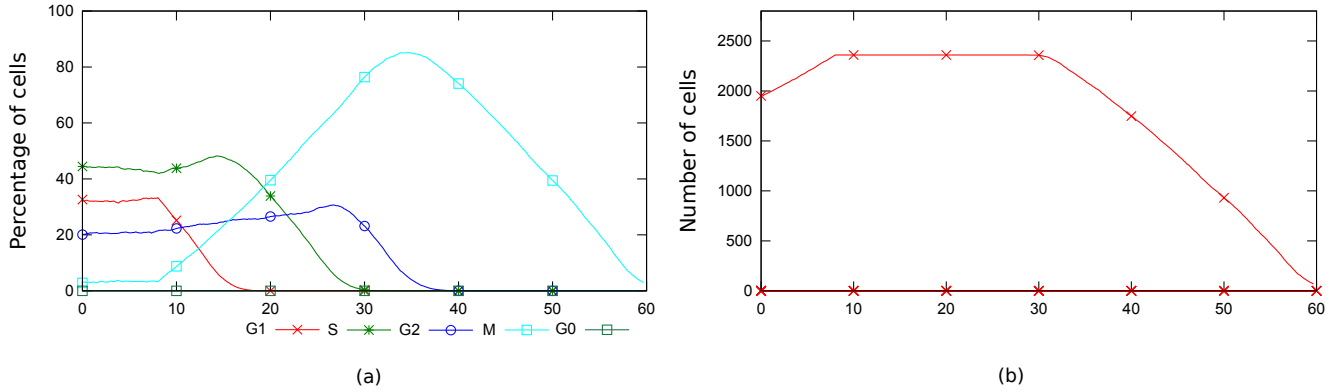


Figure 7: Results of the activation of the intra-mitotic checkpoint. (a) Evolution of the population in each phase. Cells start to accumulate in Mitosis once the nocodazole adjunction occurs. (b) Evolution of the population size. The cells stop to proliferate whereas they accumulate in Mitosis. They start to die when they have spent too much time trying to divide.

programming or other evolutionary strategy. The scenario could be represented as a sequence of parametric actions of the environment and therefore, in specific conditions, a goal could be specified and the best scenario, fitting with this goal, could emerge. The analysis of this scenario could help the biologists to determine qualitatively which regulatory pathway has to be targeted to avoid the uncontrolled proliferation.

The next step of this generic representation of cell cycle is to model the checkpoint permissiveness. If a checkpoint is permissive, cells will pass through the transition whereas the required preconditions are not fulfilled. For instance, the second experiment of this paper will be a test for this module. The G1/S checkpoint being permissive, the cells will pass through it rather than repairing their DNA. We want to express it as a cell belief, using a membership function, as in fuzzy logic, and therefore the combinations of the different conditions will be aggregated to let the checkpoint activate itself or not. This framework will allow to bring the internal and external perception of the cells to the same level. This representation will simplify the model giving a unique probability of transition for a given checkpoint, and therefore, the FSM presented in section 2 will be transformed in a Markov model.

The different modeling steps followed reduce the side-effect of the cell-environment interactions. The comparison between constrained and unconstrained *in-silico* simulations should allow the quantification of the impact of the environmental constraints. Therefore, the simplified environment will shortly be extended to a 2-D continuous environment and, finally, to a 3-D continuous environment. The final aim of simulating the spatial organization of multicellular tumor spheroids will thus be within reach. As an intermediate step, all the 2-D monolayer classical experiments done

*in-vitro* will be reproduced *in-silico*. This step will evaluate the response and the influence of the physical model by comparing the *in-vitro* experiments with the results of the simulation with the proposed simplified environment.

Precisely, this 2-D prototype is currently under validation by evaluating the convergence of *in-vitro* experiments and *in-silico* simulation with specific scenarii. For example, we will use the following validation experiments: cell cycle synchronization through a lack of environmental factors (arrest in G0); cell cycle synchronization using a procedure known as double thymidine block (arrest at G1/S) etc. All these experiments will be evaluated with different environments to quantify their impacts.

## Conclusion

In this paper the strengths of the checkpoint orientated modeling are highlighted. This approach allows to easily synchronize the cells thanks to environmental interactions. Our cell cycle model gives consistent results for each checkpoint that we try to analyze the response. The probabilistic modeling is an original approach to express specific features of the cell cycle. In this work, it has been shown how a whole population of cells could be synchronized. Nevertheless, this work actually needs to be compared with *in-vitro* experimental results. This multi-disciplinary approach will allow to map some experimental data to parameters value.

## References


Aguda, B. and Tang, Y. (1999). The kinetic origins of the restriction point in the mammalian cell cycle. *Cell proliferation*, 32(5):321–335.

Calzone, L., Thieffry, D., Tyson, J., and Novak, B. (2007). Dynamical modeling of syncytial mitotic cycles in Drosophila embryos. *Molecular systems biology*, 3(1).

Chavoya, A. and Duthen, Y. (2008). A cell pattern generation model based on an extended artificial regulatory network. *Biosystems*, 94(1-2):95–101.

Chen, K., Calzone, L., Csikasz-Nagy, A., Cross, F., Novak, B., and Tyson, J. (2004). Integrative analysis of cell cycle control in budding yeast. *Molecular Biology of the Cell*, 15(8):3841.

Cussat-Blanc, S., Pascalie, J., Luga, H., and Duthen, Y. (2010). Morphogen positioning by the means of a hydrodynamic engine. In *Artificial Life XII*. MIT Press, Cambridge, MA.

Doursat, R. (2006). The growing canvas of biological development: Multiscale pattern generation on an expanding lattice of gene regulatory networks. *InterJournal: Complex Systems*, 1809.

Drasdo, D. and Loeffler, M. (2001). Individual-based models to growth and folding in one-layered tissues: intestinal crypts and early development. *Nonlinear Analysis-Theory Methods and Applications*, 47(1):245–256.

Elledge, S. (1996). Cell cycle checkpoints: preventing an identity crisis. *Science*, 274(5293):1664.

Galle, J., Loeffler, M., and Drasdo, D. (2005). Modeling the effect of deregulated proliferation and apoptosis on the growth dynamics of epithelial cell populations in vitro. *Biophysical journal*, 88(1):62–75.

Gerlee, P. and Anderson, A. (2007). An evolutionary hybrid cellular automaton model of solid tumour growth. *Journal of theoretical biology*, 246(4):583–603.

Hoehme, S. and Drasdo, D. (2010). A cell-based simulation software for multicellular systems. *Bioinformatics*.

Kauffman, S. (1969). Metabolic stability and epigenesis in randomly constructed genetic nets. *Journal of theoretical biology*, 22(3):437–467.

Loeffler, M. and Roeder, I. (2004). Conceptual models to understand tissue stem cell organization. *Current opinion in hematology*, 11(2):81.

Moreira, J. and Deutsch, A. (2002). Cellular automation models of tumor development: a critical review. *Advances in Complex Systems*, 5(2/3):247–268.

Novak, B., Pataki, Z., Ciliberto, A., and Tyson, J. (2001). Mathematical model of the cell division cycle of fission yeast. *Chaos: An Interdisciplinary Journal of Nonlinear Science*, 11:277.

Novak, B. and Tyson, J. (1993). Numerical analysis of a comprehensive model of M-phase control in Xenopus oocyte extracts and intact embryos. *Journal of Cell Science*, 106(4):1153–1168.

Novak, B. and Tyson, J. (2004). A model for restriction point control of the mammalian cell cycle. *Journal of theoretical biology*, 230(4):563–579.

Pascalie, J., Lobjois, V., Luga, H., Ducommun, B., and Duthen, Y. (2011). A Checkpoint-Orientated Model to Simulate Unconstrained Proliferation of Cells (regular paper). In *European Conference on Artificial Life (ECAL), Paris, 09/08/2011-12/08/2011*, pages 630–637, http://www-mitpress.mit.edu/. The MIT Press.

Pascalie, J., Lobjois, V., Luga, H., Ducommun, B., and Duthen, Y. (2012). Checkpoint-Orientated Cell Cycle Modeling - Critical Role for cell age distribution (submitted). In *Gecco 2012*.

Pomerening, J., Kim, S., and Ferrell Jr, J. (2005). Systems-level dissection of the cell-cycle oscillator: bypassing positive feedback produces damped oscillations. *Cell*, 122(4):565–578.

Singhania, R., Sramkoski, R., Jacobberger, J., Tyson, J., and Beard, D. (2011). A Hybrid Model of Mammalian Cell Cycle Regulation. *PLoS Computational Biology*, 7(2):835–842.