Mitotic Oscillators as MP Graphs

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Abstract. This paper proposes a model by Metabolic P graphs of a few important processes occurring during the biological phase where the choice is made to begin again mitosis or to arrest it. The cellular processes during this phase turn out to be especially interesting in the case of DNA damage, which triggers a specific destruction of Cdc25A phosphatase. It has important implications to understand the role of cell cycle checkpoints and the mechanism(s) guiding the proliferation of UV-resistant tumored cells. The formalism of metabolic P graphs highlights the relevant information of the biological network dynamics, and the individuation of few parameters rules the basic mechanisms of Cdc25A degradation, involving a couple of important mitotic oscillators. Work in progress is aimed at simulating a couple of guessed systems modelling the whole process.

Keywords: Cell Cycle Control, DNA Repair, Metabolic P Algorithm, Mitosis, Biological Oscillators.

1 Introduction

Membrane systems were proposed as a model to represent various aspects of molecular localization and compartmentalization, including the movement of molecules between compartments, the dynamic rearrangement of molecular reactions, and the interaction between molecules in a compartmentalized setting. They have been widely investigated as models and tools of interest for computer science [20], while recently the intent to employ membrane systems as a framework to study real biological systems is vividly pursued [4, 1, 3].

In particular, a novel perspective has been introduced by Manca and his group [4, 18] along with the idea of controlling the evolution of a (membrane) system by means of rules whose "strength" is identified with a numerical value (reactivity) which depends on the system state (that is the objects concentration). Every reactivity denotes the ability of the corresponding rule to compete against other rules in capturing part of a population, on which the reaction is performed [7]. By going ahead in this perspective, every reactivity is determined by the corresponding (reaction) map evaluated on the state of the system, and

a strategy for partitioning the objects in the system (at every transition) is given, which depends on the relative magnitude of every reactivity [5]. This new strategy of rule application was inspired by actual 'metabolic reactions', and it seems to lead membrane computing toward interesting simulations of biological processes, such as representations of signal transduction networks and complex oscillations [6, 8, 16].

The dynamics regulating the cyclic oscillation of some biochemicals is especially important to figure out the regulation mechanisms that provide the life of the cellular system. Namely mitotic oscillations are a mechanism exploited by nature to regulate the mitosis process, that is, the cell division aimed at producing two daughter cells identical to the single parent cell. Mitotic oscillations concern the fluctuation of activation state of the substances involved in the process. The context and the inspiration of this paper lies in [17], where the mitotic oscillator of Amphibian Embryos was studied by means of three models, differently inspired by the A. Goldbeter differential equations system and by the careful observation and the direct description of the biological oscillator itself. In particular, a formalism of Metabolic P Graph is introduced that represents all the information needed by the metabolic algorithm to calculate the dynamics of a biological network.

Here we analyze the interruption, after a DNA damage, of the cell division cycle due to the degradation of Cdc25A, which is a phosphatase crucial in the mitosis process [14, 15]. This is still more interesting if one considers that up to now the arrest induced by DNA damage has been ascribed only to the transcription factor and tumor suppressor protein p53. Surprisingly though, transient inhibition of Cdk2 (the kinase whose complex is activated by Cdc25A) in response to DNA damage occurs even in cells lacking p53 [11] or p21, [23] which is an inhibitory protein transcriptionally regulated by p53. Such a $p21^{WAF1/CIP1}$ is an important effector of the mitosis arrest [9] and plays a critical role in the well-documented p53 function. It has also important implications for understanding cell cycle checkpoints and the mechanism(s) through which p53 inhibits human neoplasia.

Given the importance of checkpoints for preventions of genetic diseases including cancer, we explore these alternatives mechanisms of mitosis arrest, by identify a signalling pathway p53-independent that causes the cell division arrest after DNA damage. We formalize such a biological reality as a metabolic P graph [17], with the aim to obtain a model to reproduce and observe the evolution of the system. The fluctuation of the key elements concentrations is crucial to capture typical healthy states of the system. The goal is to deflect whichever diseased path to healthy paths modulating, in a few times, the considered concentrations.

First we present a qualitative description of the main phenomena involving three mitotic oscillators, then the pathway in which we are interested, and finally the graph describing the corresponding process.

2 Checkpoints in Cell Cycle

The cell cycle consists of the four phases Gap 1, S, Gap 2, and M, that are displayed in Figure 1. Gap 1 (called **G1**) is the interval between mitosis and DNA replication, that is characterized by cell growth. G1 is a vital phase of cell growth because just in this cell cycle phase the choice to begin again mitosis is made.

The transition that occurs at the restriction point (called **R**) during the G1 phase commits the cell to the proliferative cycle. If the conditions that enforce this transition are not present, the cell exits the cell cycle and enters a non-proliferative phase (called **G0**) during which cell growth, segregation and apoptosis occur [12, 2]. Replication of DNA occurs during the synthesis phase (called **S**), which is followed by a second gap phase (called **G2**) during which growth and preparation for cell division occurs. Mitosis and production of two daughter cells occur in the phase called **M**. The switches from one phase to the next one are critical checkpoints of the basic cyclic mechanism of proliferating cells, and they are studied by wide interest [11, 13, 23].

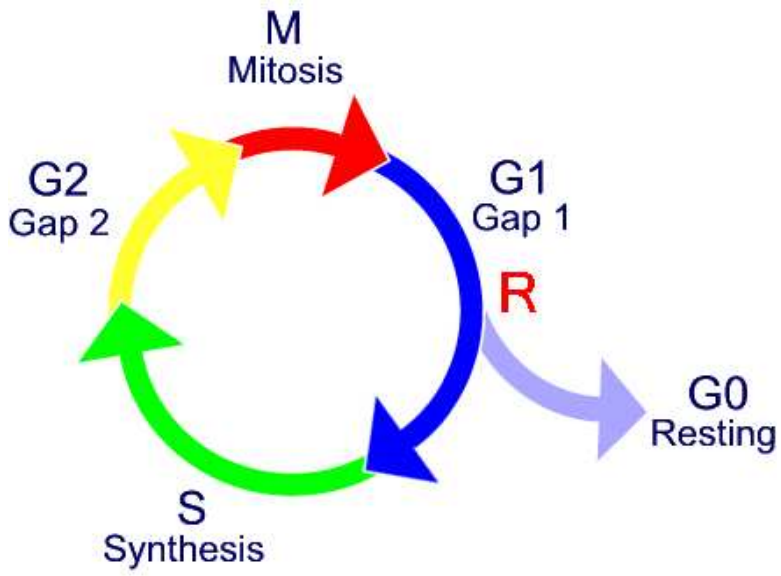

Fig. 1. Phases of the Cell Cycle. During the G1 phase progression or arrest (G0 phase) of the cycle is decided.

Passage through the four phases of the cell cycle is regulated by a family of *cyclins*[1] that act as regulatory subunits for *cyclin-dependent kinases cdks.*

Cyclins are a family of proteins involved in the progression of cells through the whole cell cycle. Cyclin forms a complex with the cyclin-dependent kinase, which activates the latter protein kinase function and promote the mitosis phase. When cyclin concentrations in the cell are low, it detaches from Cdk inhibiting the enzyme's activity (probably by producing a protein chain to block the enzymatic site). Therefore, the activity of the various *cyclin/cdk* complexes that regulate the progression through G1-S-G2 phases of the cell cycle is controlled by the synthesis of the appropriate cyclins during a specific phase of the cell cycle. In [17] one may find a few models for the minimal structure of such a mitotic oscillator, that is, the binding of the cyclin with the cdc2 which so passes from its inactive to its active state.

The cyclin/cdk complex is then activated by the sequential phosphorylation and dephosphorylation of the key residues of the complex located principally on the cdk subunits. In eukaryotes, protein phosphorylation is probably the most important regulatory event. Many enzymes and receptors are switched "on" or "off" by phosphorylation and dephosphorylation. Phosphorylation is catalyzed by various specific protein kinases, whereas phosphatases dephosphorylate. In the framework of the process we are going to describe in the next section, we have three oscillators: a DNA-damage induced activation of the kinases Chk1 and Chk2, and a consequent activation of Cdc25A (a serine/threonine phosphatase) due to its phosphorylation (phosphoCdc25A) made by the active Chk1 and Chk2. Finally, a massive amount of phosphoCdc25A dephosphorylates the complex cdk2-cyclinE by making it active and inducing the S-phase of cell division (see Figure 2).

We will examine the role of Cdc25 family members of which at least Cdc25A is essential both for the entry into S phase [14], at the checkpoint control of the G1-S transition, and for the cell cycle arrest in response to a DNA damage. In particular, we analyze the degradation of phosphorylated Cdc25A by ubiquitin mediation, which inhibits the activation of the complex cdk2-cyclinE and provokes the G1 arrest. Such a degradation takes place in the *cytosol* (which is the fluid portion of the cytoplasm, exclusive of organelles and membranes) and is mediated by the 'endopeptidase activity' of '26S proteasome', causing the dissociation of phosphoCdc25A in its constituting aminoacids (Figure 5). This mechanism is intriguing because we could learn how to module the quantity of Cdc25A in order to arrest the proliferation of tumored cells (that have DNA damage).

[1] Cyclins are so named because their concentration varies in a cyclical fashion during the cell cycle. They are produced or degraded as needed in order to drive the cell through the different phases of the cell cycle.

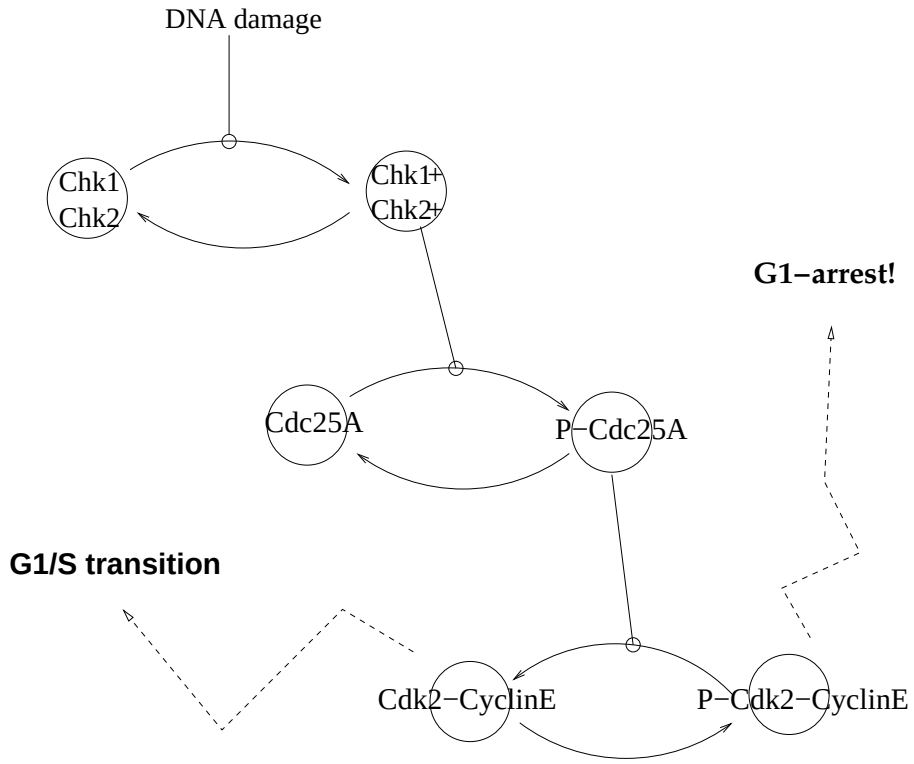

Fig. 2. Passages from the inactive states to the active ones in our process - oscillators are all reversible reactions.

3 Cycle Arrest in Response to Stress

Episodes of DNA damage during G1 pose a particular challenge, because replication of damaged DNA can be deleterious and because no other chromatid is present to provide a template for recombinational repair. Besides, by considering that cyclins operate as a promoting factor for the mitosis phase and that typical cancer evolutions act as a suppressor of certain components of the cyclins family, in case of DNA damage the desired (healthy) state is identified by the G0 phase. Thus, in this context, we are interested to figure out in particular the conditions under which G0 is reached.

There are several proteins that can inhibit the cell cycle in G1 but, when DNA damage has occurred, p53 is that protein which accumulates in the cell inducing the p21-mediated inhibition of cyclin D/cdk. In fact, DNA-damaging agents induce a p53-dependent G1 arrest that may be critical for p53-mediated tumor suppression [22]. There is an alternative way though, where inhibition of Cdk2 in response to DNA damage occurs even in cells lacking p53 or p21, and it is depicted in Figure 3.

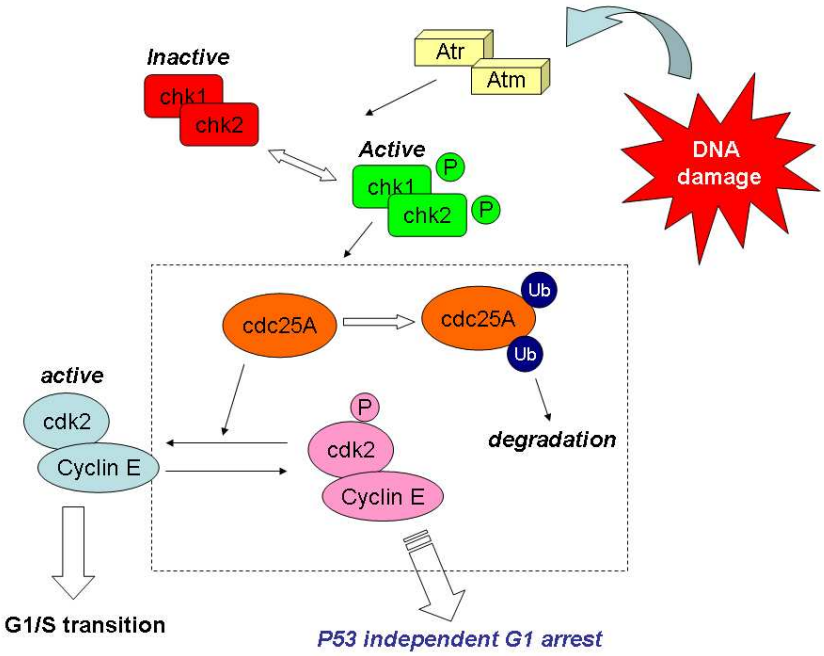

Fig. 3. p53 independent G1 arrest in response to stress.

Human cells respond to ultraviolet light or ionizing radiation by rapid, ubiquitin and proteosome-dependent protein degradation of Cdc25A[15, 13]. Namely

DNA damage triggers specific destruction of Cdc25A phosphatase. This event prevents entry of a cell into S-phase, by maintaining the cyclin E-Cdk2 complexes in phosphorylated form [19]. "Unfortunately", between 16 and 24 hours after exposure to UV, the cells resumed DNA replication and progression through the cell cycle, indicating that the UV-induced cell cycle arrest is reversible.

The overall phenomenon described here is not dependent on p53 and previous studies [12] have demonstrated that the abundance of Cdc25A appears to determine the extent of DNA synthesis upon UV-induced DNA damage. Thus, the elimination of Cdc25A evokes a cell cycle arrest promoting repair of the DNA crosslinks caused by UV and protects the cells from formation of the DNA strand breaks. Finally, a 3-hour period of expression of Cdc25A to prevent downregulation of the cellular Cdc25A activity by UV reduced the survival of the irradiated cells examined by colony formation assays. These results uncover a mechanism of cellular defense against genotoxic stress that can be rewritten in mathematical way and subsequently simulated.

The substances relevant to describe the process of degradation of Cdc25A in the cytosol, after its activation in the nucleus of the cell, are reported in Table 1.

Symbol	Name	Compartment
Chk1	Checkpoint kinase 1	nucleoplasm
Chk2	Serine/threonine-protein kinase Chk2	nucleoplasm
ATP	Adenosine Triphosphate	nucleoplasm
Cdc25A	M-phase inducer phosphatase 1	nucleoplasm
ADP	Adenosine diphosphate	nucleoplasm
phosphoCdc25A	M-phase inducer phosphatase 1	nucleoplasm
Ubiquitin	Ubiquitin	cytosol
UbiqutinLigase	E3 Ubiquitin Ligase	cytosol
Ubiquitinatedphospho25Cdc25A	M-phase inducer phosphatase 1	cytosol
proteasome26s	Proteosome complex	cytosol

Table 1. Actors of the model.

The relevant chemical reactions of the process (that represent the stoichiometric level of a metabolic P graph) are the following. First the phosphorylation of Cdc25A at ser123 in response to DNA damage occurs in the nucleoplasm.

Nucleoplasm

$$ATP + Cdc25A + Chk1 \rightarrow ADP + phosphoCdc25A \qquad (J_0)$$

$$ATP + Cdc25A + Chk2 \rightarrow ADP + phosphoCdc25A \qquad (J_1)$$

The rules J_0 and J_1 activate the phosphatase Cdc25A by means of active kinases. In such a way, the detection of DNA damage results in the phosphorylation of Cdc25A at Ser-123 by Chk1 and Chk2, by concurrently inhibiting Cdc25A. Then, phosphoCdc25A migrates from the nucleoplasm to the cytosol of the cell, and by notational convenience we call it phosphoCdc25A@Cytosol. Thus we have the following rule:

Migration to Cytosol

$$phosphoCdc25A \rightarrow phosphoCdc25A@Cytosol \qquad (J_2)$$

Finally, in the cytosol we have sequentially the degradation of phosphorylated Cdc25A by ubiquitin mediation and the degradation of Cdc25A by proteasome, that may be described respectively by the following J_3 and J_4 rules.

Cytosol

$$phosphoCdc25A@Cytosol + Ubiquitin + UbiquitinLigase \rightarrow UbiquitinatedphosphoCdc25A@Cytosol + UbiquitinLigase \qquad (J_3)$$

$$UbiquitinatedphosphoCdc25A@Cytosol + proteasome26s \rightarrow AminoAcid + proteasome26s. \qquad (J_4)$$

The J_3 reaction is mediated by the 'ubiquitin-protein ligase activity' of the catalyzing enzyme 'Ubiquitin ligase', and one molecule of 'Ubiquitinated PhosphoCdc25A' is produced by transforming one molecule of phosphoCdc25A@Cytosol. The reaction J_4 finally degrades UbiquitinatedphosphoCdc25A@Cytosol by transforming it into one molecule of its aminoacid by means of proteasome 26s activity.

The model of the above interactions may be depicted as in Figure 4 where reactants reside in compartment (nucleoplasm and cytosol) and interact between them (arrows). But no indication is given about the dynamics of the system, that is *how* the reactants interact each other. In order to point out the dynamics of the system, in the next section we describe the process of interest by means of a metabolic P graph, a formalism introduced in [17] which extends the *Stoichiometric Network Analysis* [10] developed in the context of complex reaction networks [21].

4 Metabolic P Graphs

The initial assumption is that the localization and the concentrations of any biochemical element at each instant determines all the relevant properties which underly the function that a biological system exhibits at that (observation) time.

By definition [17], a *MP graph* is a structure

$$G = (T, R, F, E, C)$$

where:

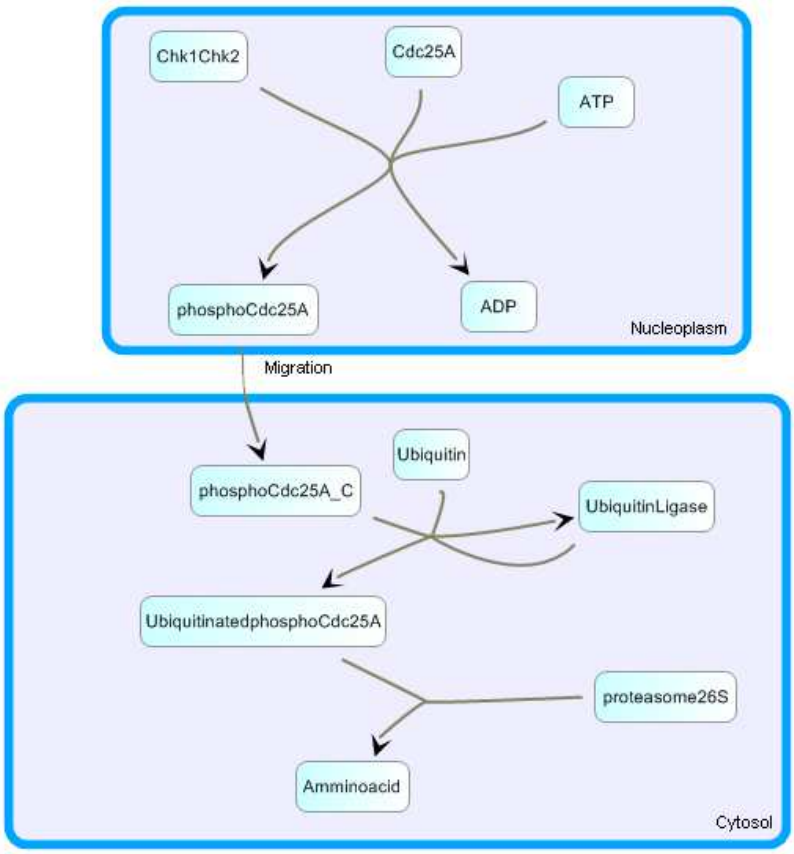

Fig. 4. Graph of Interactions.

- T is the set of nodes representing substances relevant for the process (for example those in Table 1). We may think of each element in T as the container of a certain amount of a peculiar kind of substance. We represent such a kind of nodes as circles with the type of objects contained in it (see Figure 5).
- R is the set of nodes representing biochemical reactions between substances. We represent each of the nodes in R as a full bullet and we label it with the name of the reaction represented by that node (see Figure 5).
- F is the set of nodes labelled by reaction maps represented in Figure 5 along with dotted arrows.
- E is a set of nodes presenting input or output gates. It usually contains two different kind of nodes: input gates and output gates. In our case, some non-definite substances induced by DNA damage are assumed from an input gate (denoted by a star in our graph), and the output gates are represented

by relevant effects of the process, such as mitosis, arrest, and degradation (see Figure 5).

- C is a set of edges (connections) between nodes. Edges are of two different kinds: plain edges or dashed edges.
  i) Plain edges connect types to biochemical reactions (circles and full bullets in the graph), in particular they specify *reactants* and *products* of the reactions. Arcs connecting reactants to reactions are depicted as lines while arcs connecting reactions to products appear as arrows (oriented arcs).
  ii) Dotted arrows connect a possibly empty set of substances to one full bullet (they represent the reaction map of the corresponding rule).

Two components are easily distinguishable in MP graphs: a *stoichiometric* component and a *regulation* component. The stoichiometric component is the subgraph obtained after removing from a MP graph $G = (T, R, F, E, C)$ the nodes F and the dotted arcs which connect them to the other nodes. This removed part is the *reaction regulation layout* of G.

In Figure 5 we abstract from the graph in Figure 4 the stoichiometric component of the system, and we insert the regulation component as a way to control the process. Note that in this model the rule J2 of the previous section is not present because it is not relevant for the dynamics of the substances variations, while J1 and J2 are assembled in the rule R2 because they act in parallel and, by experimental observations, their reactivities may be considered identical.

As one can easily see, the formalism of MP graphs highlights the information of the biological network dynamics by pointing out the crucial tuning points of the system. In Figure 5 for example, there is an important difference with respect to the graph in Figure 4: the dynamics of the process is expressed by the graph and few essential parameters regulating the pathways are identified in the reactivities of the rules. Namely, the reactivities f1,f2, f3 and f4 control the dynamics of the activation oscillators of the kinases and the complex CyclinE-cdk2 respectively. While the reactivities h2,h3, and h4 control the information flow of PhosphoCdc25A degradation process.

The dynamics of the graph is assumed (and will be simulated as) following the strategy of the metabolic P algorithm [17]:

- Reactants are distributed among all the rules step by step according to a "competition" strategy.
- A couple of rules need the same reactant in two cases (R2 and K2, R3 and J3), then each of these rules gets a portion of the available substance, in a percentage that is proportional to its reaction strength (*reactivity*) at that step (f2 and h2, f3 and h3).
- The reactivity of a rule at a given instant depends on the state of the system, defined as the concentration and localization of all substances.
- According to its stoichiometric "reading", any rule determines its own reaction unit and therefore the amount of substances which it consumes and produces.

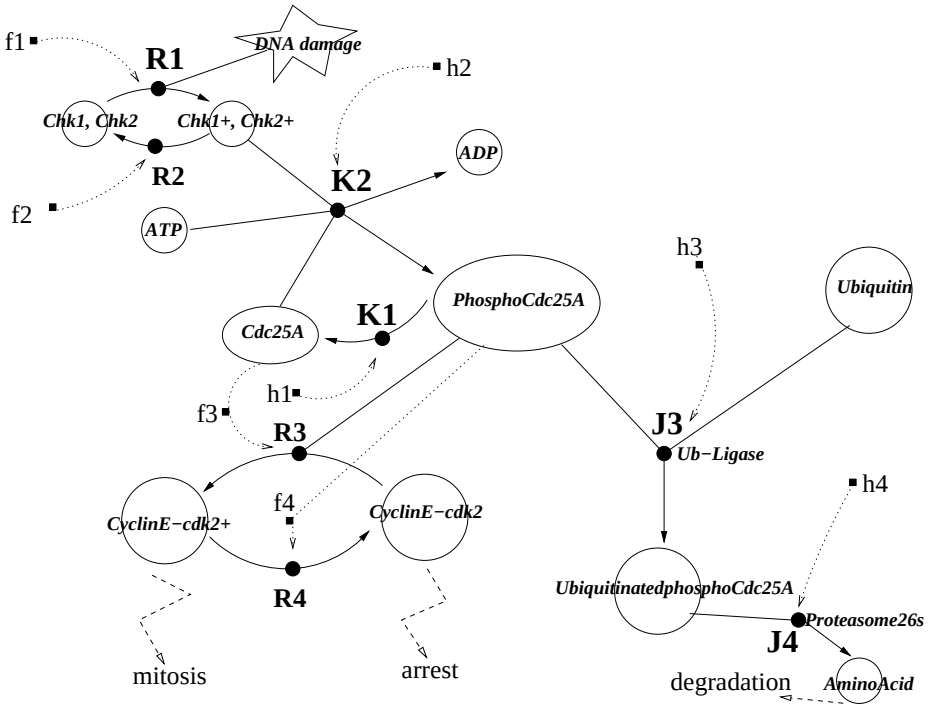

Fig. 5. Metabolic P graph of the process leading to a p53 independent G1 arrest. Rules K2,J3,J4, with their reactivities h2, h3, h4, are crucial for the Phospho-Cdc25A degradation process induced by UV radiation by means of DNA damage. The role of Ubiquitin Ligase and Proteasome26s that are catalyzing enzymes are identified with the reactions themselves (given the assumption that they are present in the cytosol in abundance for the process). Rules R1, R2, R3, R4 control the "back and fourth" of the oscillators.

In our case the reactivities are the unknown part of the system, and there is work in progress for their identification. Namely, the simulations of a couple of systems along with guessed values for the reactivities are been testing.

Our qualitative reasoning about the reactivities follows. The oscillator $(R1, R2)$ in a first approximation may be neglected: the reactions velocities are such that we can suppose to have enough amount of activated $Chk1$ and $Chk2$ kinases to trigger the process. $K2$ is the highest reactivity in the whole system (for instance, $h2 = 1$), while $K1$ being very slow (for instance, $h1 = 0.1$). We know that the amount of (inactive) $Cdc25A$ is from 0 to 40 reaction units, and that about one third of it is degraded. More precisely, the process performed by J3 and J4 has reactivity 0.3 times the amount of cdc25A, that is $h3 = h4 = 0.3|cdc25A|$. Finally, the oscillator $(R3, R4)$ has the most complex reactivities: $f3 = \alpha|Cdc25A|$ and $f4 = \beta|PhosphoCdc25A|$, with α switching from assuming values in [0,0.15] to assuming values in [0,1], and β switching from assuming values in [0,1] to

assuming values in [0,0.25]. The desired goal of the simulations is to show that both the amounts of cdc25A and of the complex CyclinE-cdk2 oscillate, and these oscillations depend on the reactivity of $K2$ which seems the crucial value of this system dynamics.

5 Future Work

We are further investigating two strategies to systematically compute the reactivities of MP metabolic graphs describing biological systems, along with genetic algorithms, which find "good" values in order to obtain a desired behaviour, and with the method of *MP Log-gain Regulation*, based on the computational progression of the MP algorithm starting by actual biological data.

The ultimate idea of this work is to insert this graph in a more general one which models also the pathways of p53 dependent G1 arrest, in such a way to figure out the relationships between the p53-mediated tumor suppression and the radiation-resistant DNA synthesis phase (and consequent cell mitosis).

References

1. N. Barbacari, A. Profir, and C. Zelinschi. Gene Regulatory Network Modelling by Means of Membrane Systems. In R. Freund, G. Lojka, M. Oswald, and Gh. Păun, editors, *Pre-proceedings of the 6th International Workshop on Membrane Computing, July 18-21 2005, Vienna, Austria*, pages 162–178, 2005.
2. J. Bartek and J. Lukas. Pathways governing g1/s transition and their response to dna damage. *FEBS Lett.*, 3(490):117–122, 2001.
3. F. Bernardini, M. Gheorghe, N. Krasnogor, R. C. Muniyandi, M. J. Pérez-Jiménez, and F. J. Romero-Campero. On P Systems as a Modelling Tool for Biological Systems. In R. Freund, G. Lojka, M. Oswald, and Gh. Păun, editors, *Pre-proceedings of the 6th International Workshop on Membrane Computing, July 18-21 2005, Vienna, Austria*, pages 193–213, 2005.
4. L. Bianco, F. Fontana, G. Franco, and V. Manca. P Systems for Biological Dynamics. In G. Ciobanu, Gh. Păun, and M. J. Perez-Jimenez, editors, *Applications of Membrane Computing*, Natural Computing Series, chapter 3, pages 81–126. Springer, 2006.
5. L. Bianco, F. Fontana, and V. Manca. Metabolic Algorithm with Time-Varying Reaction Maps. In *Proceedings of the Third Brainstorming Week on Membrane Computing, Sevilla, Spain*, pages 43–61, February 2005.
6. L. Bianco, F. Fontana, and V. Manca. P Systems and the Modeling of Biochemical Oscillations. In R. Freund, Gh. Păun, G. Rozenberg, M. Yung, and A. Salomaa, editors, *Membrane Computing, 6th International Workshop, WMC 2005*, volume 3850 of *Lecture Notes in Computer Science*, pages 200–209. Springer, January 2006.
7. L. Bianco, F. Fontana, and V. Manca. P Systems with Reaction Maps. *International Journal of Foundations of Computer Science*, to appear (2006).
8. L. Bianco and V. Manca. Symbolic Generation and Representation of Complex Oscillations. *International Journal of Computer Mathematics*, to appear (2006).
9. V. Chopin, R.A. Toillon, and N. Jouy. P21(waf1/cip1) is dispensable for g1 arrest, but indispensable for apoptosis induced by sodium butyrate in mcf-7 breast cancer cells. *Oncogene*, 23(1):21–29, 2004.

10. B. L. Clark. Stability of complex reaction networks. *Adv. Chem. Phys.*, 43:1–216, 1983.
11. J. D'Anna, J.G. Valdez, R.C. Habbersett, and H.A. Crissman. Association of g1/s-phase and late s-phase checkpoints with regulation of cyclin-dependent kinases in chinese hamster ovary cells. *Radiat. Res.*, 148:260–271, 1997.
12. K.I. Nakayama et al. Regulation of the cell cycle at the g1-s transition by proteolysis of cyclin e and p27kip1. *Biochem. Biophys. Res. Commun.*, 4(282):853–860, 2001.
13. J. Falck, N. Mailand, R.G. SyljuÃěsen, J. Bartek, and J. Lukas. The atm-chk2-cdc25a checkpoint pathway guards against radioresistant dna synthesis. *Nature*, 410(6830):842–847, 2001.
14. S. Jinno, K. Suto, A. Nagata, M. Igarashi, Y. Kanaoka, H. Nojima, and H. Okayama. Cdc25a is a novel phosphatase functioning early in the cell cycle. *EMBO J.*, 13:1549–1556, 1994.
15. N. Mailand, J. Falck, C. Lukas, R.G. SyljuÃěsen, M. Welcker, J. Bartek, and J. Lukas. Rapid destruction of human cdc25a in response to dna damage. *Science*, 288(5470):1425–1429, 2000.
16. V. Manca. Topics and problems in metabolic p systems. Technical report, Fourth Brainstorming on Membrane Computing, Sevilla, Spain, 2006.
17. V. Manca and L. Bianco. Biological networks in metabolic p systems. Submitted.
18. V. Manca, L. Bianco, and F. Fontana. Evolutions and Oscillations of P systems: Applications to Biological Phenomena. In G. Mauri, Gh. Păun, M. J. Pérez-Jiménez, G. Rozenberg, and A. Salomaa, editors, *Membrane Computing, 5th International Workshop, WMC 2004*, volume 3365 of *Lecture Notes in Computer Science*, pages 63–84. Springer, 2005.
19. E.A. Nilssen, M. Synnes, N. Kleckner, B. Grallert, and E. Boye. Intra-g1 arrest in response to uv irradiation in fission yeast. *Proc. Natl. Acad. Sci.*, 100(19):10758–63, 2003.
20. Gh. Păun. *Membrane Computing. An Introduction.* Springer, 2002.
21. L.A. Segel and I.R. Cohen. *Design Principles for the Immune System and Other Distributed Autonomous Systems.* Santa Fe Institute Studies in the Sciences of Complexity, Oxford University Press, 2001.
22. T. Waldman, K.W. Kinzler, and B. Vogelstein. p21 is necessary for the p53-mediated g1 arrest in human cancer cells. *Cancer Research*, 55(22):5187–5190, 1995.
23. H. Zhao, J.L. Watkins, and H. Piwnica-Worms. Disruption of the checkpoint kinase 1/cell division cycle 25a pathway abrogates ionizing radiation-induced s and g2 checkpoints. *Proc. Natl. Acad. Sci.*, 99:14795–800, 2002.