DNA Damage Detection and its Impact on the Cell Cycle

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1 STAGE OF THE RESEARCH

1.1 Biological Background

Thousands of DNA lesions are formed daily in each cell of the human body. They can be induced either endogenously, as well as exogenously - by physical and chemical agents from outside of the body. There are several types of DNA damage, from small chemical modifications of single-stranded DNA through the photoproducts and adducts caused by UV irradiation, to the potentially most dangerous double-strand breaks. The existence of such a large number of abnormalities in many cells may cause the death of the body after a very short time. During evolution number of mechanisms that protect cell from damages evolved to prevent cell death and lesions transformation to future generations. There are several pathways of DNA repair depending on nature of damage, their review can be found in (Ciccia and Elledge, 2010). For all of these complex mechanisms of repair, it is necessary to detect DNA damage just after it arises and spread the information about it to the proper regulatory units. This process takes place in a manner specific to the type of lesion. ATR (ataxia telangiectasia mutated and Rad3-related) module is activated by presence of single stranded DNA areas in the cell, which are caused by resection of various types of lesions or by stalled replication forks. Double strand breaks (DSBs) are detected indirectly by ataxia telangiectasia mutated (ATM). Stages of DNA damage detection by ATR and ATM and its further amplification are presented in fig. 1.

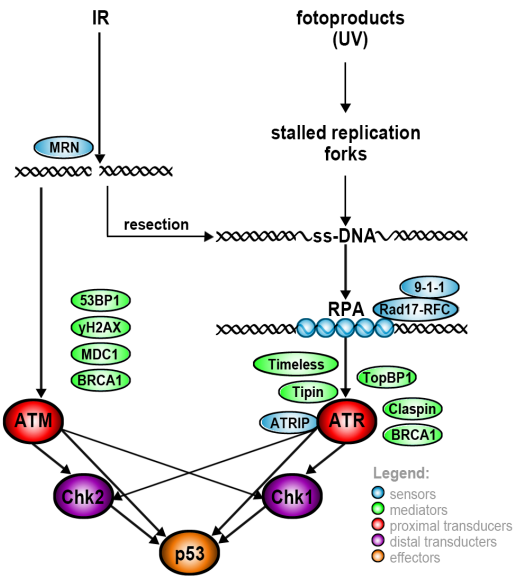

Figure 1: DNA damage detection and signal amplification.

In case of ATR subpathway, the signal strength of the checkpoint cascade is dependent on the length of RPA-ssDNA regions and possibility of ATR molecules located closely to each other. Other ATR phosphorylation targets are RPA subunits (RPA70 and RPA32), ATRIP, TopBP1, Chk2, p53 and histone H2AX. Double strand breaks are detected by repair complexes like MRN complex. MRN binds to DNA damage site and recruits ATM kinase, that after its autophosphorylation interacts with several protein in pathway (Chk1, Chk2, p53, Mdm, CREB, Wip1) and leads to p53 stabilization.

1.2 ATR-p53 Model

Our existing stochastic mathematical model of ATR signaling pathway is based on the Haseltine-Rawlings postulate (Haseltine and Rawlings, 2002) and is an extension of our previous model of the p53 signaling pathway (Puszynski et al., 2008). The model is activated by UV irradiation which results in SSBs lesions occurrence. The output of the model is the level of p53 protein which determines cell fate: DNA damage repair or cell apoptosis. Spontaneous DNA damage formation implemented in presented model results in basic ATR pathway activation. The core of the model are states of ATR: inactive protein, its phosphorylated state, and fully activated form. In this model, there are two feedback loops: positive, with the participation of PTEN protein, and negative, containing MDM - p53 suppressor (fig. 2). Details about

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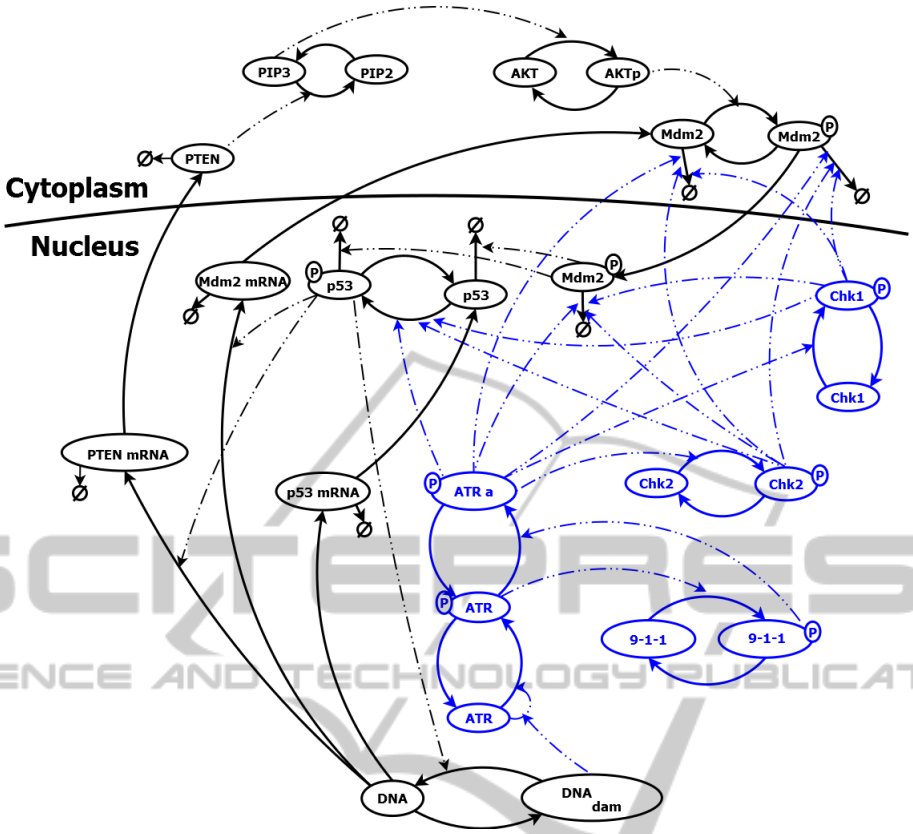

Figure 2: DNA damage detection model vizualization. Solid lines represent change of protein form; dashed lines describe the interactions that occur in the path. Components of ATR module are colored in blue.

p53 signaling pathway are available in (Puszynski et al., 2008). The model distinguish the nucleus and cytoplasm. It was assumed that each gene has two copies. None of them can be active, one of them or both can be active. For some proteins production and degradation was not modeled directly assuming that they are equal and protein only change the form (from active to inactive and vice versa). In presented model a simplified DNA repair was implementing depending on the number of p53 tetramers, repair rate and the amount of repair complexes, which is limited. Apoptosis condition is recognized as a permanently elevated level of the p53 protein (over 6 hours). Then the cell dies and all of its elements are degraded, thus further protein levels and the number of lesions are not taken into account.

1.2.1 Simulation Analysis of ATR Module

In the simulation analysis we examined cell response to different doses of radiation, we set the threshold of detection and apoptosis, as well as showed spontaneous activation of the ATR/p53 pathway. Deterministic and stochastic (for 100 cells) experiments were performed. At t=24 hours after start, simulated cells were irradiated by a specific dose of UVC, and then observed over the next 48 hours. The correctness of the model was verified based on the results of biological experiments from the literature.

3A. Basic Activation

According to (Kohn, 2002) in every cell of the human body in a day are formed approximately 55 000 single-strand breaks, which are responsible for base activation of the path (fig. 3A).

3B. Damage Detection Threshold

We examined response of the model (fig. 3B) to radiation dose causing one single strand break (0.0665 mJ/m^2).

4A. Apoptotic Death Threshold

Based on the observation of simulation results, the dose of 18 J/m^2 in which more than half of the cell becomes apoptotic cells was taken as the threshold for apoptosis (fig. 4A). . For comparison, the dose 17 J/m^2 causing death of 44/100 cells. Assumed that apoptosis occurs when the level of p53 is increased by more than 6 hours (in simulation exceeding the

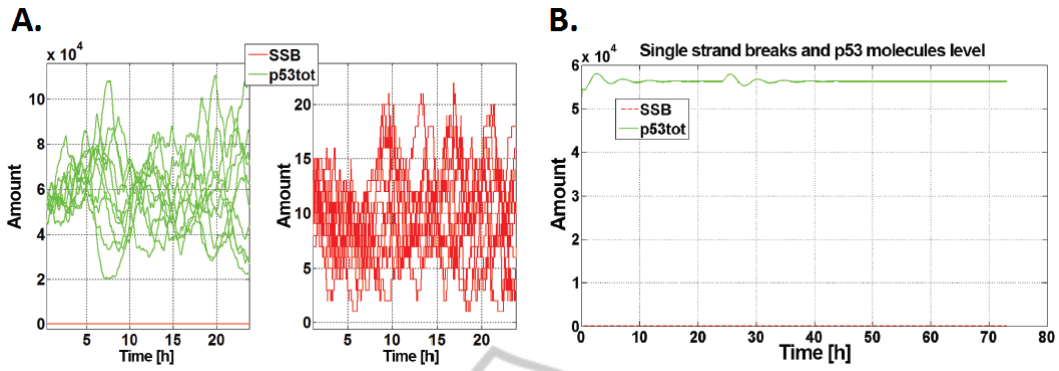

Figure 3: A. Spontaneous DNA damage formation and p53 activation. Result of 10 stochastic simulations; B. Response to one lesion occurrence. Red - number of SSBs, green - p53 total level.

threshold $2.1 \cdot 10^5$). Further experiments were run for the radiation dose of 18 J/m^2.

4B-D. Disabling Selected Effects

Inhibition of the p53 phosphorylation caused by Chk1 and Chk2 kinases results in the reduction of apoptotic fraction size (fig. 4B: 17/100 cells for the Chk1 and 14/100 cells to Chk2, for which plot appears almost identical), and a significant prolongation of DNA repair time. In the case of the inhibition of the ATR-dependent p53 activation, the apoptotic fraction decline was smaller (size of fraction was 46/100 cells).

A stronger effect was obtained excluding Chk1 (fig. 4C) and Chk2 dependent degradation of Mdm. None of the cells entered the state of apoptosis. As in the previous case, less system response change was observed for the ATR. Apoptotic fraction size was 20/100.

When total Chk1 (fig. 4D) or Chk2 protein kinase activity is blocked, none of the cells reach the state of apoptosis. Simulation analysis show the correct functioning of the model stress response which effect is known from the literature.

1.2.2 Conclusions from Recent Work

ATR module is able to detect a single strand break caused by UVC appearing in the cell and enhance the signal so as to cause an increase in p53 protein level. The threshold of apoptosis in the healthy cell is 18 J/m^2. However, if the pathway is defective, apoptotic threshold shifts. Despite extensive damage, the cells may not die, but transfer incorrect genetic material to daughter cells (because DNA damage repair takes a lot longer). This state could potentially be a cause of cancer and other genetic diseases. Developed model can be used to study the behavior of cells with specific mutations without the need for costly and time-consuming experiments in the laboratory.

1.3 ATM-p53 Model

Independently, the ATM model was created. ATM-p53 model is based on information from literature regarding DSBs detection pathways and role of Wip1 protein (Shimada and Nakanishi, 2013). ATM detection module regulates p53 phosphorylation via positive loop. Wip1 creates negative feedbacks for all of the proteins except Mdm2 in nucleus, where it activates inactive Mdm2 by dephosphorylation. Activation of the model takes place by the application of ionizing irradiation. The signaling pathway is also stimulated continuously by the small number of damages that occur spontaneously. Most of the assumptions about the structure of the model is the same as in ATR part. ATM model is also an extension of the p53 signaling pathway model (Puszynski et al., 2008). This model shows that cells with blocked transcription of Wip1 are more affected to apoptosis. The absence of PTEN and Chk2 in the model significantly affect the results of the simulation.

2 OUTLINE OF OBJECTIVES

First of all we planned to combine models ATR-p53 and ATM-p53. This approach is caused by interactions between these paths. However, a more interstsing goal is modelling dependence of described above path on cell cycle phase. We plan to investigate how the specific cell cycle phase affects the ATM-ATR-p53 path and the other hand, how the path influences the cell cycle.

3 RESEARCH PROBLEM

In our study we plan to examine how cell cycle progression influences DNA damage detection pathway.

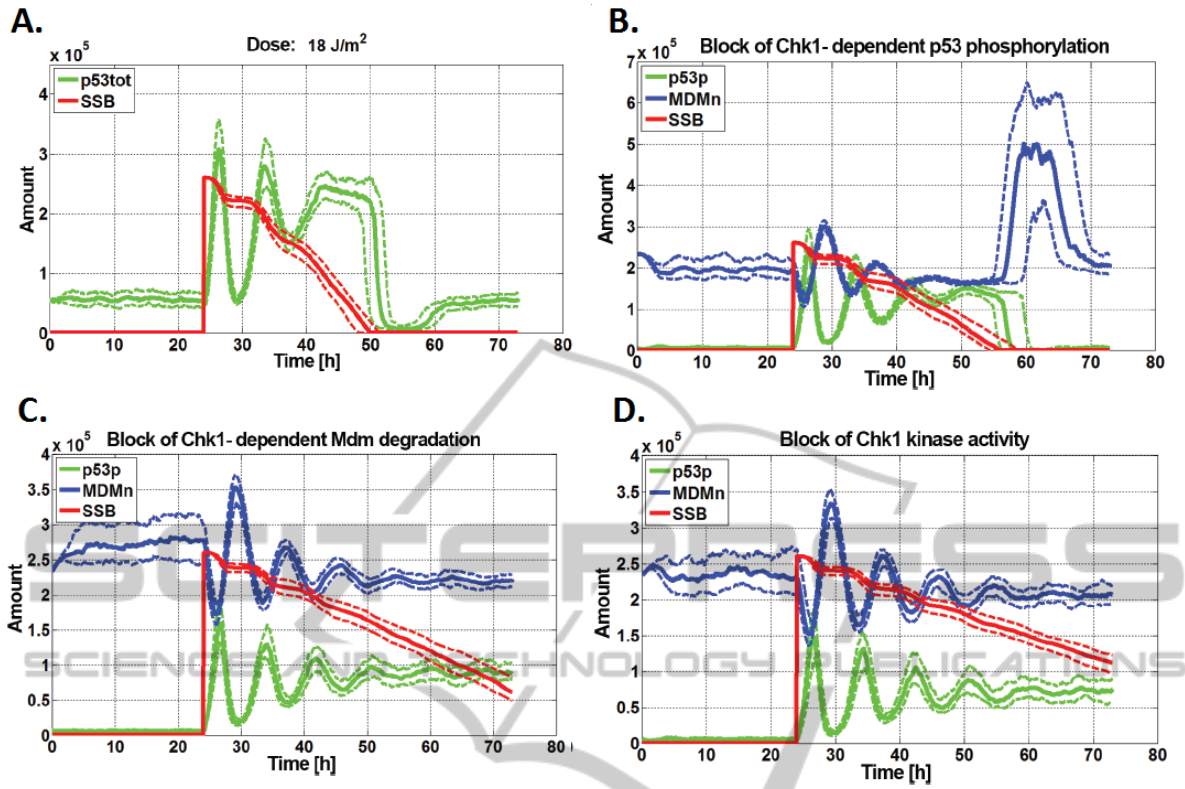

Figure 4: A. Apoptotic death threshold; B-D. Disabling selected effects. Results for 100 stochastic simulations. Solid line - median; dashed line - upper and lower quartile of results.

The speed of reactions occuring in the cell depends on the concentration of molecules participating in it. This concentration differs over time. It is caused e.g. by the variable volume of the cell depending on its cell cycle phase. It must be taken into account for modeling of the ATM-ATR-p53 pathway. Expression of some proteins and its nucleus or cytoplasm location may be different depending on the phase of the cycle, what will have to be reflected in the proposed model.

In another look at the model, we plan to examine how the damage formation caused by stressful factor in various forms (ionizing radiation, UV light) affects the cell cycle and for which radiation doses cell cycle will be stopped for DNA damage repair. We plan to determine which dose will cause apoptosis of cells. Another important issue is to check how the disabling of selected interactions in the pathway will affect the response of the cell.

The purpose of the construction of such a model is to illustrate the processes occurring in the cell after a lesion is detected in the different phases of the cycle, depending on the given force. With this model we can revise how mutations of the genes encoding the individual elements of the path and causing inhibition of their activity may influence the behavior of cells. The model can be used to verify the hypotheses without the need for costly and long lasting biological experiments.

4 STATE OF THE ART

4.1 Cell Cycle

The cell cycle consists of two main stages: interphase, which prepares the cell to the next division and the division (M phase): mitosis (somatic animal cells) or meiosis (generative animal cells). Mitosis (which we will deal more in our model) includes laryokinesis (division of the cell nucleus) and cytokinesis (division of cytoplasm). It results in the separation of the one cell into two daughter cells (Cooper, 2000). The major stages of interphase are:

- G_1 Phase - growth phase; biosynthetic processes in the cell, which were significantly slowed in the M phase, will be taken up again. In this phase the synthesis of various enzymes required for DNA replication in S phase takes place. Length of the

G_1 phase differs even between cells of the same species.

- S Phase - begins with DNA synthesis, and lasts for a similar period of time in all cells. The purpose of the processes taking place in the S phase is to double the amount of DNA present in the cell. Each chromosome has been replicated. RNA and protein synthesis in this phase of the cycle is very slow (with the exception of histone protein synthesis).
- G_2 phase - in this phase synthesis of proteins is increased again, mainly those responsible for the formation of the mitotic spindle (tubulin), which are necessary for the occurrence of a subsequent process of mitosis.
- G_0 Phase - eukaryotic cells (especially those fully differentiated) may move from the G1 phase to the G0 phase, where they do not undergo divisions and can remain for a long period of time. The aging of cells in response to damage is a process that prevents (without causing apoptosis) transfer of incorrect genetic material to progeny cells.

4.2 Cell Cycle Checkpoints

Cell cycle checkpoints (fig. 5) are mechanisms which verify correctness of the DNA. If genetic material is damaged or not all cellular processes specific for each phase have been completed, cell cycle progression is stopped until all will be finished and repaired. If the damage is too big that could have been repaired, cell is directed to apoptosis (Cooper, 2000; Shapiro and Harper, 1999). The main checkpoints in the eukaryotic cells are:

- G_1/S checkpoint - at the end of G_1 phase (before synthesis phase) decision whether cell should divide, delay division or enter a G_0 phase is taken.
- G_2/M checkpoint - occurs at the end of G_2 phase (before mitosis) and checks if cell is ready to mitosis (whether mitotic apparatus is fully formed and whether DNA lesions occur).

4.3 Cyclins, Cyclin-Dependent Kinases, Cyclin-Dependent Kinase Phosphatases and Cyclin-Dependent Kinase Inhibitors

To move to next phases of cell cycle is needed cooperation of two types of molecules: cyclin and cyclin-dependent kinases. Cyclins and the cyclin-dependent kinases (CDKs) form together the active

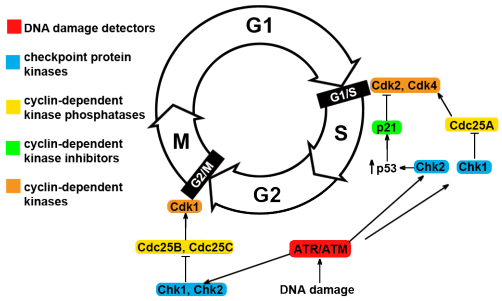

Figure 5: Cell cycle checkpoints and DNA damage detection.

heterodimer, where cyclins represent a regulatory unit and are synthesized in specific phases of the cell cycle in response to various molecular signals. CDKs play a catalytic function and their expression is independent of phase of cell cycle. CDKs upon binding to cyclins are activated and performs the target protein phosphorylation reactions, which thus become activated or inactivated, what coordinate entry into the next phase of the cell cycle. CDKs are often activated by cyclin-dependent kinase phosphatases (for example Cdc25) - tyrosine phosphatases, which acts by removing the blocking the CDKs activity phosphate residues (Orlando et al., 2008). Regulation of CDKs activity might be performed by CDKs inihibitors (like p21 encoded by a gene CDKN1) which nhibits the CDK-cyclin complexes activity. p21 binds to cyclin E/Cdk2 and cyclin D/Cdk4 complexes and inhibiting their activity acts as a regulator of the cell cycle in the G1 phase. p21 gene expression is tightly controlled by the p53 protein (Gartel and Radhakrishnan, 2005).

4.4 Role of Chk1 and Chk2 in Checkpoint Mechanism

Between G1 and S phase DNA damage results in the activation of ATM and ATR and following Chk2 and Chk1 phosphorylation and next phosphorylation of p53 and Mdm2, which results in activation and stabilization of p53. Active tetramers act as a transcription factor of (among others) p21 protein, which is a potent inhibitor of cyclin-dependent kinases and prevents cells before the entry to the S phase. In addition, Chk1 phosphorylates and inactivates Cdc25A phosphatase that is essential in CDKs activation.

Blocking of the G2-phase is performed with the signal transducers ATM or ATR (depending on the type of damage). They activate Chk1 and Chk2 kinases which phosphorylate Cdc25 phosphatase, causing its inactivation. This avoids the activation of Cdk1 kinase (encoded by the gene CDC2) necessary to initiate mitosis (Shapiro and Harper, 1999).

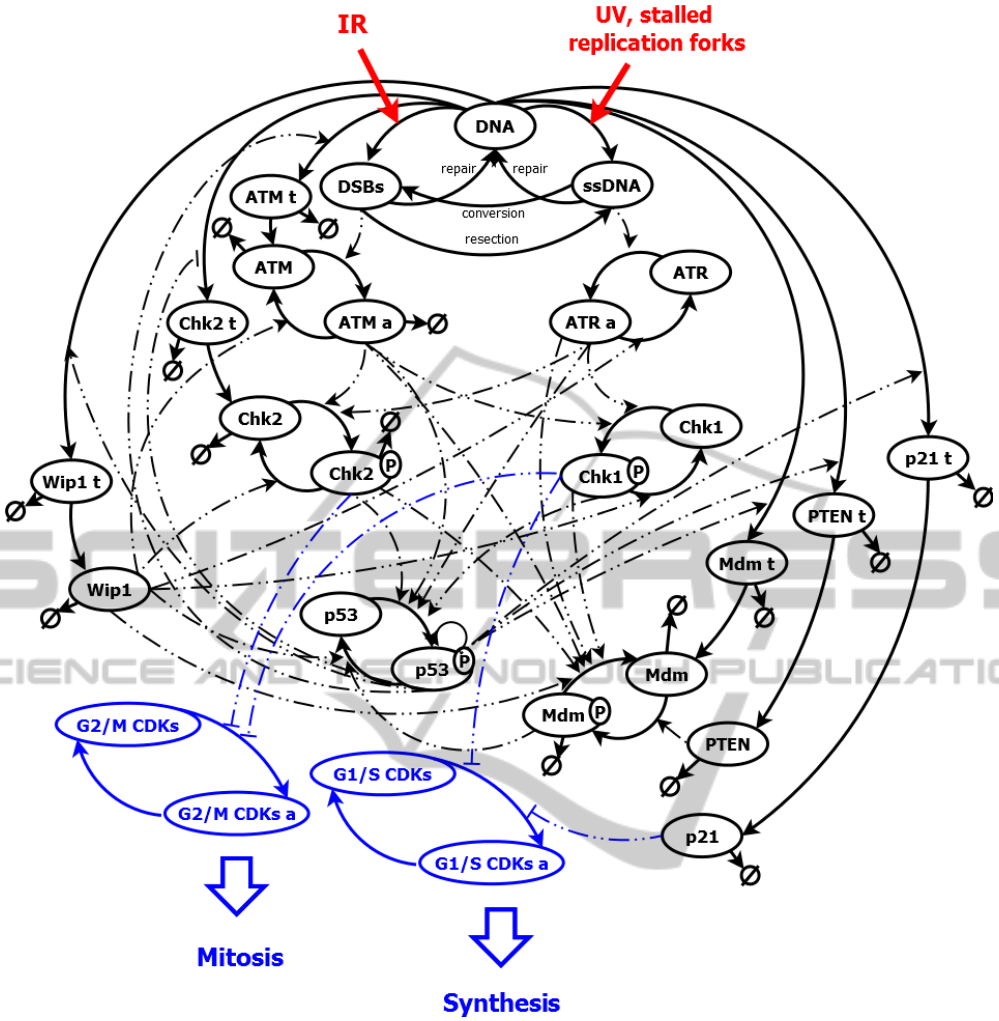

Figure 6: Schema of new ATM-ATR-p52 and cell cycle pathway.

5 METHODOLOGY

We created scheme od combined ATM-ATR-p53 pathways model with taking into account the key elements of cell cycle regulation in checkpoints (fig. 6). Both the scheme and the model are a simplification of reality what is necessary to enable the modeling of the pathway.

In signaling pathways modelling we use the basic laws known from biochemistry: the law of mass action and Michaelis-Menten kinetics. The kinetic parameters for our model we obtain from the results of biological experiments performed by us and from literature. Unknown parameters are estimated by fitting the model to the known data.

As described in caption 1 proposed model will be based on Haseltine-Rawlings postulate (Haseltine and Rawlings, 2002) which binds deterministic and stochastic approach. In our models we use ODE to simulate fast reactions (in example protein-protein interactions) and direct Gillespie method (Gillespie, 1977) to simulate slow reactions (enabling genes and DNA lesions number). In near future we will have computational system which enables modeling of variable terms for each of cell cycle phase. We plan to perform stochastic simulation for population of cells (in example 1000 cells).

6 EXPECTED OUTCOME

Expected outcome of this research will be to develop a model illustrating the processes occurring in the cell associated with the detection of damage and cell cycle progression. The resulting model will allow for the imaging of various kinds of extortions acting on

the cells (ioinizing irradiation, UV-light). The result of model action will be a system response which will be adequate to the applied force. For healthy cells at too high dose of damaging agents cells should be directed to apoptosis, whereas at lower doses cell cycle arrest and repair of damages should occur. For the population (in example, 1000) modeled stochastically, desynchronized cells after forcing will show a division into fractions of cells retained in the appropriate phase of the cycle. Constructed model will let investigate the effect of disable selected interactions at overall pathway answer. This allows to investigate how mutation emerged in a cell can influence the cell cycle and damage detection. Effect will show influence of abnormalities appearing for example in tumor cells which are known in the literature.

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