Gautam Prabhu

28 April 2017

Kinetic Modeling of Signal Transduction in the Intra-S Phase DNA Damage Checkpoint

Abstract

The cell cycle has recently received increased attention from biologists because of its importance in the progression of cancer. One important part of the cell cycle is the DNA damage checkpoint, which checks the DNA for damage, especially that caused by radiation, and stops replication to avoid forming a tumor.

Of the three major checkpoints in the cell cycle, the least understood is the intra-S phase checkpoint, which slows (but does not stop) DNA synthesis if there is a DNA issue. The checkpoint consists of two parallel paths of protein interaction, the ATM path and the ATR path.

Here ordinary differential equation (ODE) modeling was used to better understand the paths in the intra-S phase checkpoint. The model was created using reactions in the literature translated into ODEs through mass-action kinetics. Using the model, simulations were executed to learn more about which proteins are critical to the system and which are less important.

These simulations show that the ATM path is much more effective than the ATR path in slowing down DNA synthesis, but some reactions in the path are much more important than others.

The model was validated through comparison to experimental results by other scientists. Though there are some errors in the validation, the simulation follows the general trends.

Using this model, the intra-S phase checkpoint can be understood better. Researchers in the field can continue to use the model to run experiments in code before their lab experiments to get a quick estimate of the results.

In the future, to improve model's accuracy and similarity to real-world experiments, extra proteins and inputs/outputs can be added, constants can be further tuned, and biological experiments can be run alongside the model for better validation.
Though there is still much to learn about the intra-S phase checkpoint before we can cure cancer, this is the first step on a path to a better understanding.

Introduction

The cell cycle is the subject of a lot of research because of its importance in cancer – when the it breaks down and the cell undergoes mitosis when it should instead be fixing its DNA, a tumor can form and the progression of cancer can begin.

One very important part of the cell cycle is the DNA damage checkpoint (Figure 1A). There are several checkpoints within the cell cycle that check for DNA damage and halt or slow the cell cycle progression so that the DNA error can be fixed before it propagates to its daughter cells (Jackson 3). Another option for the cell is apoptosis: programmed cell death when DNA damage is too great to fix on its own. The three major checkpoints in the cell cycle are the G1/S checkpoint, which checks for DNA damage before the DNA is copied, the G2/M checkpoint, which stops mitosis if DNA damage has already been copied, and the intra-S checkpoint, which slows (but does not

stop) DNA synthesis if there is a DNA issue (Figure 1B). The intra-S checkpoint is the least understood of these checkpoints (Willis 2).

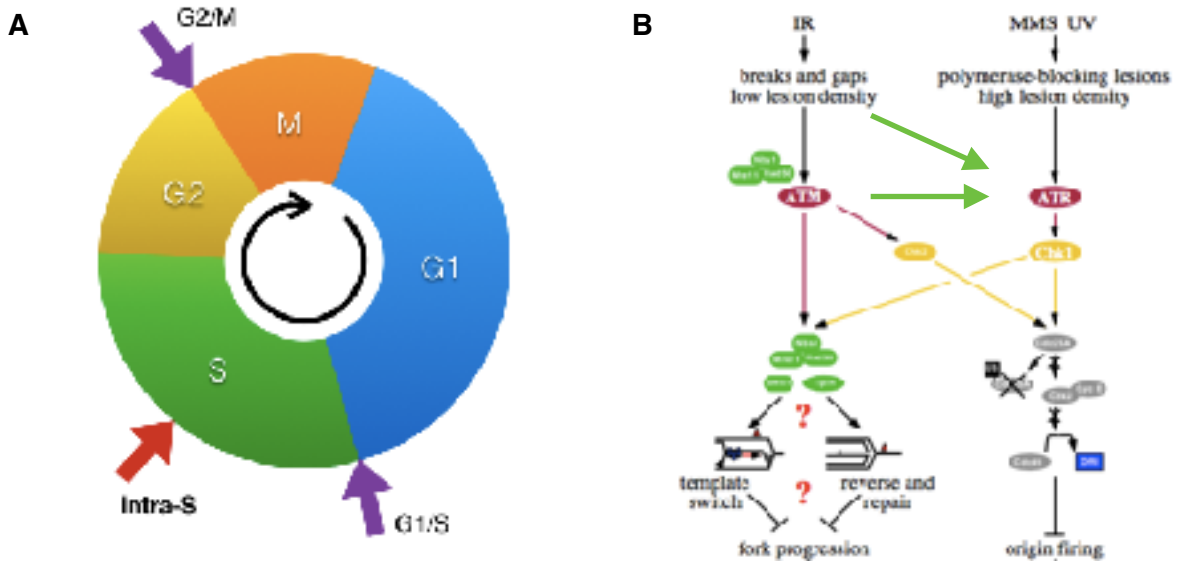

Figure 1. (A) Cell cycle. The cell cycle progresses from G1 (growth 1) to S (synthesis of new DNA) to G2 (growth 2) to M (mitosis). There are three main checkpoints - G1/S, G2/M, and intra-S. (B) The Intra-S Phase Checkpoint. Shows some of the reactions that make up the intra-S phase checkpoint. The signal is ionizing radiation (IR) and the output is either slowed fork progression or slowed origin firing. Source: Willis & Rhind 2009, green arrows added

Ordinary differential equation (ODE) modeling is a common way to understand chemical reactions through chemical kinetics (Fuß 1). Modeling signal transduction networks in the cell cycle using ODEs can be used to better understand the gene interaction network as a whole and can allow one to run experiments on the network *in silico* at a much faster rate than with *in vitro* studies.

ODE modeling is common for use to better understand DNA damage checkpoints – one of the earliest attempts focused on the oscillation in the concentration of the tumor suppressor protein *p53* and how that oscillation is affected by different types of DNA damage (Bar-Or 1). However, *p53* is not known to act in the intra-S phase

checkpoint (Jackson 1). Other analyses have been made to model the G1/S checkpoint (Iwamoto 1) as well as the G2/M checkpoint (Zhang 1). However, the intra-S phase checkpoint, which is the least understood of the three, has not yet been mathematically modeled.

As a result, the goal of this research is to mathematically model the intra-S phase checkpoint in order to better understand the checkpoint itself and the cell cycle as a whole.

Materials and Methods

The model (Figure 2) was created using ODEs based on the kinetics of protein kinase signal transduction (Heinrich 2). The order of each reaction was decided by mass action kinetics. The model itself incorporates three phosphorylation reactions –

$$\begin{aligned}
\frac{d}{dt}[ATM] &= -k_{signal\ deg}[ATM] \\
\frac{d}{dt}[ATR] &= -k_{signal\ deg}[ATR] + k_8[ATM] \\
\frac{d}{dt}[Chk2p] &= k_1[Chk2][ATM] - k_2[Chk2p] \\
\frac{d}{dt}[Chk2] &= k_2[Chk2p] - k_1[Chk2][ATM] \\
\frac{d}{dt}[Chk1p] &= k_3[Chk1][ATR] - k_4[Chk1p] \\
\frac{d}{dt}[Chk1] &= k_4[Chk1p] - k_3[Chk1][ATR] \\
\frac{d}{dt}[Cdc25ap] &= k_5[Cdc25a][Chk1p] + k_6[Cdc25a][Chk2p] - k_7[Cdc25ap] \\
\frac{d}{dt}[Cdc25a] &= k_7[Cdc25ap] - k_5[Cdc25a][Chk1p] - k_6[Cdc25a][Chk2p]
\end{aligned}$$

Figure 2. The model. These are the ODEs that can help determine the concentration of each chemical over time. Brackets [] represent concentration. Each k is a rate constant.

the phosphorylation of Chk1, activated by ATR, the phosphorylation of Chk2, activated by ATM, and the phosphorylation of Cdc25a, activated by Chk1p and Chk2p (Figure 3).

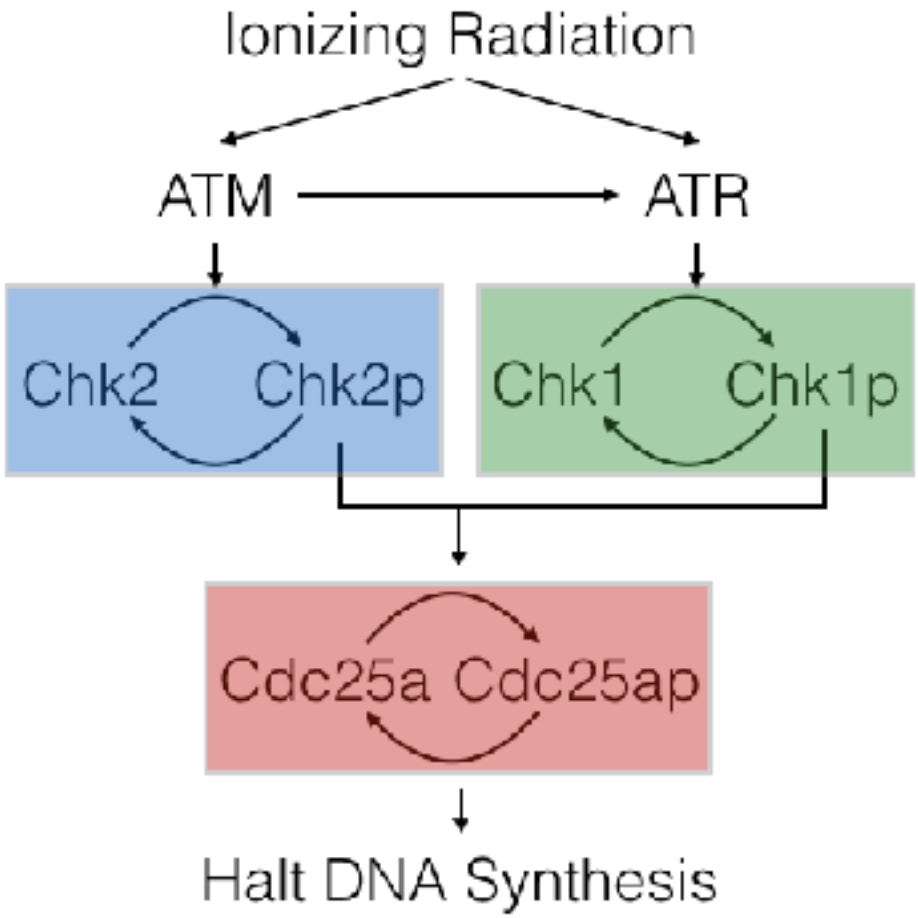

Figure 3. The reaction network in the model.
The model consists of three phosphorylation reactions (boxed), each catalyzed in turn by other chemicals in the network. The input to the network is ionizing radiation, measured in Grays (Gy) and the output is percent of total DNA synthesis (<100% when checkpoint active)

Each rate constant in the model was determined either by comparison to a similar mathematical model (Zhang 8) or arbitrarily (Figure 4). To improve the model, these constants can be further tuned to better fit experimental results.

The input of the model is ionizing radiation (IR), measured in Grays (Gy). IR causes the cell to recruit ATM and ATR for sensing and repair (Jackson 1). In the model,

$k_1 = 1.0$	assumed same as k3
$k_2 = 1.0$	assumed same as k4
$k_3 = 1.0$	derived from Zhang et al. 2013
$k_4 = 1.0$	derived from Zhang et al. 2013
$k_5 = 0.01$	assumed same as k6
$k_6 = 0.01$	derived from Zhang et al. 2013
$k_7 = 0.01$	derived from Zhang et al. 2013
$k_8 = 0.0004$	derived from info from Myers & Cortez 2006
$k_{signal\ deg} = 0.01$	arbitrary

Figure 4. The rate constants. These are the important constants found in the ODEs and how each was derived.

the input is a level of IR, and the model then generates the initial concentrations of ATM and ATR based on that data (Figure 5A). The functions for converting IR to [ATM] and [ATR] were derived from experimental data; [ATM] was interpolated from ATM foci data (Suzuki 4) and the [ATR] data was run through simulation based on data from A-T (ataxia-telangiectasia, or ATM deficient) cells (Painter 2).

The output of the model is the percent of regular DNA Synthesis, when compared to a healthy individual with no DNA damage. This is due to the fact that DNA Synthesis slows down in the intra-S phase checkpoint, so the amount of DNA Synthesis should decrease if there is DNA damage and the checkpoint is activated. The function to determine percent of DNA Damage based on Cdc25a concentration (the endpoint of the kinetic model) was interpolated from healthy cell data (Painter 2) (Figure 5B).

Using the completed model, simulations of the intra-S phase checkpoint can be run. A timecourse simulation was created in MATLAB that calculated concentrations from the rate laws over a series of 100 arbitrary-length “time steps”. The concentrations

at each time step was calculated using Runge-Kutta 4 approximation on each differential equation (Figure 6).

A

$$[ATM] = \frac{0.0429 + 45.4628r - 62.0548r^2 + 30.6344r^3}{200}$$

$$[ATR] = 0.02914286r + 0.02$$

B

$$\%\ DNA = 54.97(1 - e^{-0.0308[Cdc25a]}) + 48.08$$

Figure 5. Input and output. (A) Initial concentration of ATM and ATR based on amount of IR (represented r). (B) % DNA Synthesis based on concentration of last protein in network (Cdc25a).

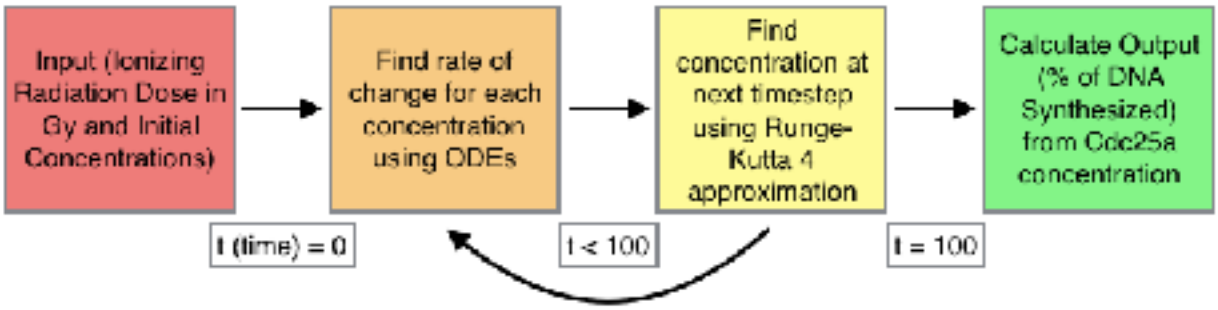

Figure 6. Flowchart of a timecourse simulation.

Results

Using the model, two simulations were run to help gain a better understanding of the intra-S phase checkpoint – a knockout simulation and a sensitivity analysis.

The knockout simulation computationally deactivates, or “knocks out” (KO), each protein in turn to see its effect on the results of the overall checkpoint. This can help to understand which proteins are most important to the workings of the checkpoint.

The results of the knockout simulation showed that knocking out ATR or CHK2 had relatively little overall effect on the result of the simulation (% DNA Synthesis), whereas knocking out ATM or CHK1 had relatively large effects on the overall system

(Figure 7). This implies that the ATM path (ATM→CHK2→CDC25A) is stronger than the ATR path (ATR→CHK1→CDC25A).

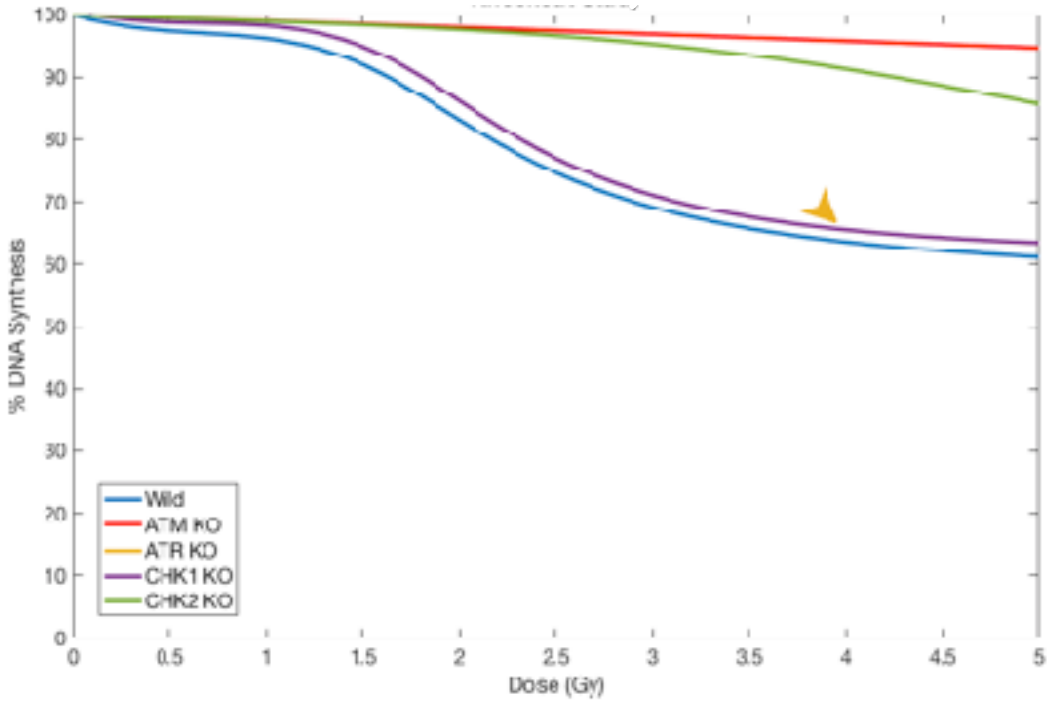

Figure 7. Knockout simulation. The simulation was run on each knockout, as well as a wild-type control, at a variety of levels of DNA Damage.

Secondly, a sensitivity analysis was completed on each kinetic parameter (rate constant) to determine how important each reaction is to the overall system and also to gauge how much changing the kinetic parameters (some of which were determined arbitrarily) will affect the results of the simulation (Figure 8).

The sensitivity analysis was completed by independently varying each kinetic parameter between 0 and double its current value to see how much that affects the % DNA Synthesis output of the simulation. If there is a major change to the simulation output at or around 0, that kinetic parameter is important to the overall workings of the

checkpoint. Those without a large change near 0 may be entirely unnecessary to the network.

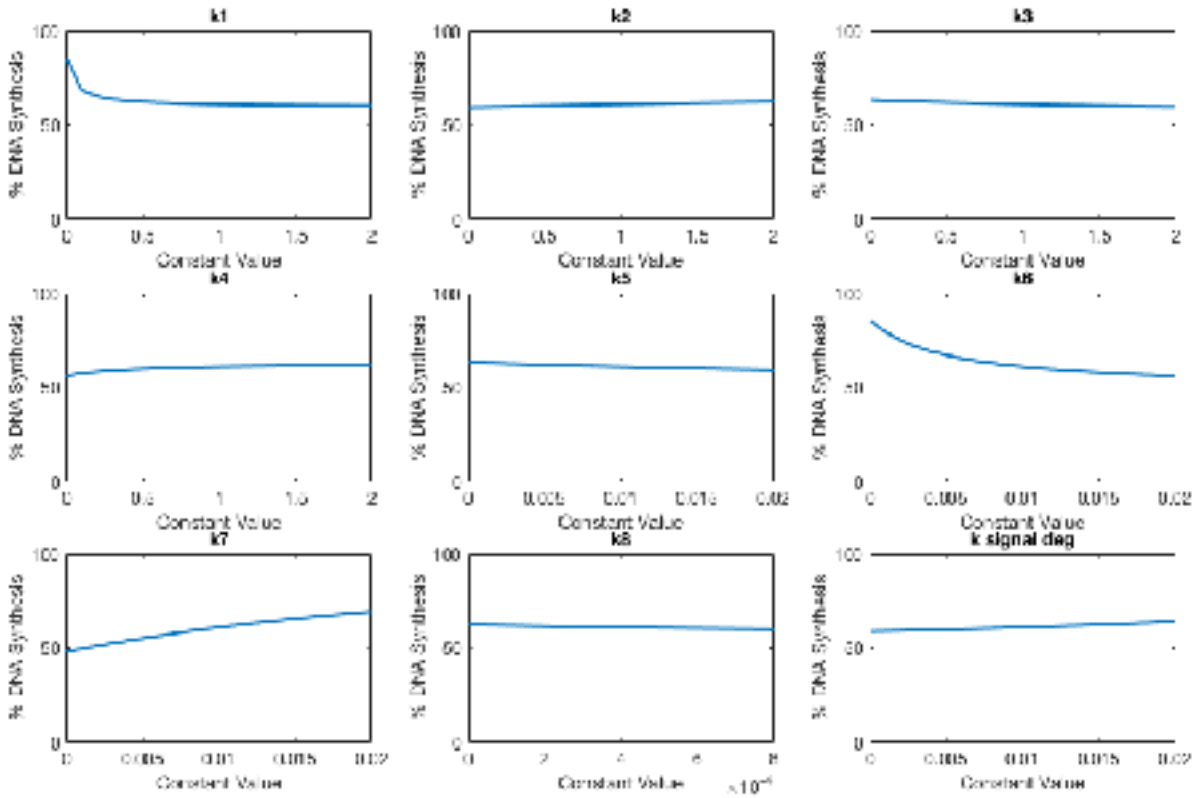

Figure 7. Sensitivity Analysis Simulation. This simulation shows how changes in the kinetic parameters affect the output.

Analysis and Conclusions

The simulation was validated through comparison to experimental data from other authors (Falck 1). The output of the simulation was compared in three phenotypes (wild-type, A-T, Nijmegen Breakage Syndrome (NBS)) to the experimental data. A-T was modeled through the knockout of ATM and NBS was modeled through the knockout of ATR (Figure 8). The simulation follows the general trends of the experimental data closely, especially at radiation doses under 10 Gy. The percent error between simulation and literature is 10% at lower levels of radiation.

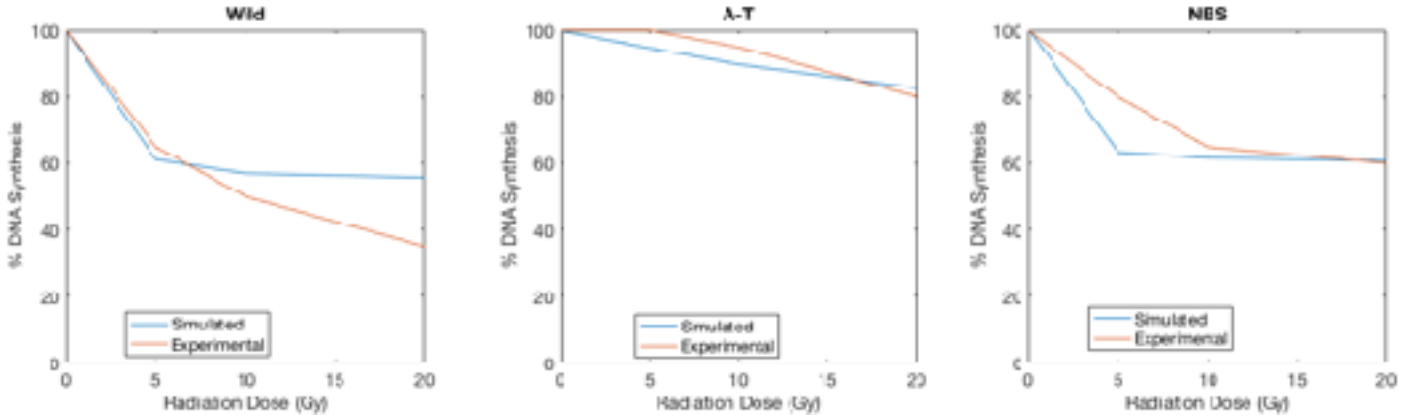

Figure 8. Validation from Falck et al. 2002. The simulation follows the general trends of the experimental data closely.

This is a valid simulation of this portion the intra-S phase DNA Damage checkpoint. Though there are some errors in the validation against Falck, the simulation follows the general trends of the experimental data, especially below 10 Gy of IR.

From the knockout analysis, we see that the ATM path is much more effective than the ATR path in slowing down DNA synthesis. Additionally, the ATM→ATR link strengthens the ATR path, but only at high radiation doses. At low doses, it does very little.

From the sensitivity analysis, we can see that some links in the series are much more important than others in a wild type cell. Note that the reverse reaction (k2) for phosphorylation of Chk2 has little effect on the overall, as does k8, the ATM->ATR connection. Constants that have a large change near 0 are very important to the network – especially k_1, k_6, and k_7. $k_{signal\ deg}$, which measures the rate at which [ATM] and [ATR] decrease naturally over time, has a large effect on the overall network and it was chosen arbitrarily (Figure 9).

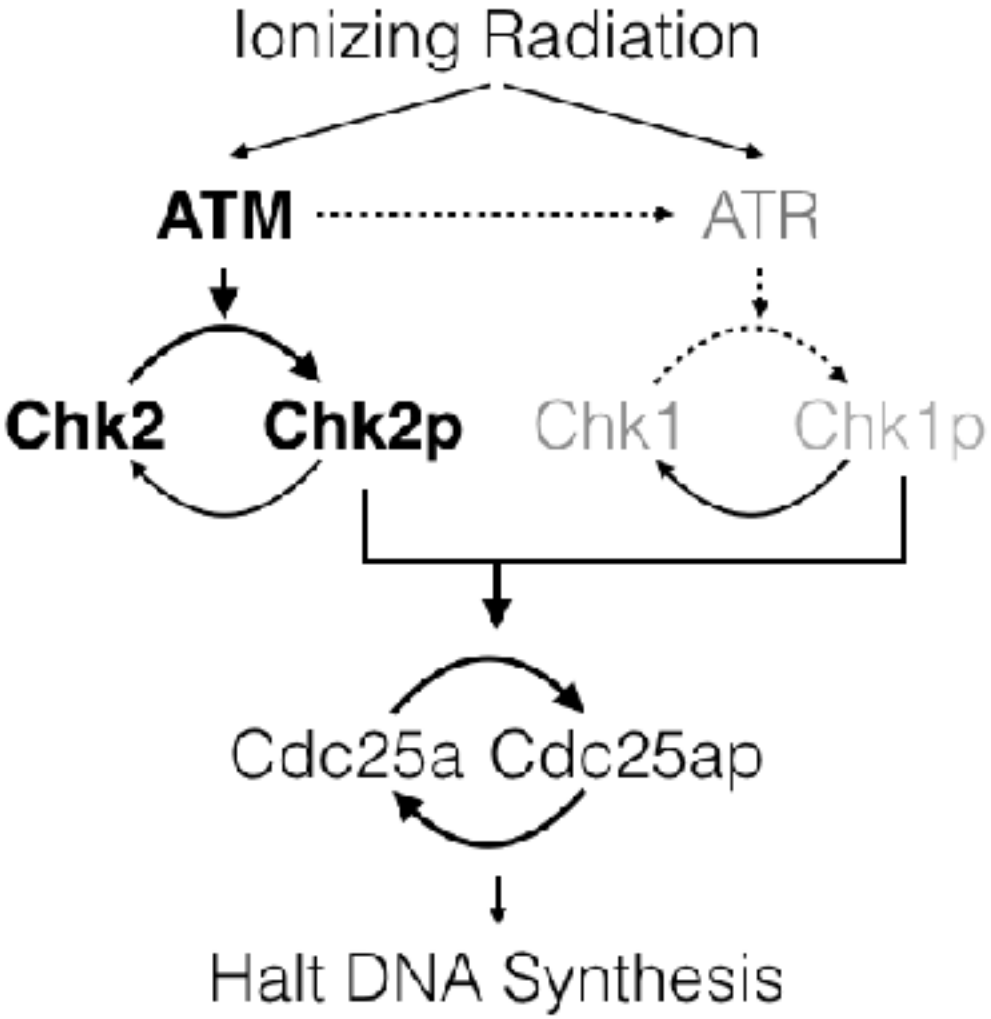

Figure 9. Visualization of the Simulation Results. Bold names and arrows are chemicals and reactions deemed to be crucially important by the KO study and the sensitivity analysis. Gray names and dashed arrows are least important to the network.

In the future, scientists can use and tune this model to better and better understand how the intra-S phase checkpoint functions. To improve the model's accuracy and similarity to real-world experiments, the rate constants should be further tuned and derived from experimental data, especially $k_{signal\ deg}$. Additionally, multiple extra proteins and inputs/outputs can be added, as this model is not exhaustive of mechanisms involved in the checkpoint. Working on this should improve the model's

accuracy, especially at higher levels of DNA damage, to better predict the results of *in vitro* experiments.

To further use the model to understand the intra-S phase checkpoint, researchers in the field can run their experiments in the model (*in silico*) before running *in vitro* experiments. Additional studies can be run using the model, including but not limited to steady-state simulations, knock-in studies, partial knock-outs, and double knock-outs. Additionally, the model can be used to simulate the symptoms of specific diseases, especially cancer, to better understand the checkpoint's role in disease.

Works Cited

Bar-Or, Ruth Lev, et al. “Generation of oscillations by the p53-Mdm2 feedback loop: a theoretical and experimental study.” *Proceedings of the National Academy of Sciences* 97.21 (2000): 11250-11255.

Falck, Jacob, et al. “The DNA damage-dependent intra–S phase checkpoint is regulated by parallel pathways.” *Nature Genetics* 30.3 (2002): 290-294.

Fuß, Hendrik, et al. “Mathematical models of cell cycle regulation.” *Briefings in Bioinformatics* 6.2 (2005): 163-177.

Hamada, Hiroyuki, et al. “Sophisticated framework between cell cycle arrest and apoptosis induction based on p53 dynamics.” *PLoS One* 4.3 (2009).

Heinrich, Reinhart, Benjamin G. Neel, and Tom A. Rapoport. “Mathematical models of protein kinase signal transduction.” *Molecular Cell* 9.5 (2002): 957-970.

Iwamoto, Kazunari, et al. “Mathematical modeling and sensitivity analysis of G1/S phase in the cell cycle including the DNA-damage signal transduction pathway.” *Biosystems* 94.1 (2008): 109-117.

Iwamoto, Kazunari, et al. “Mathematical modeling of cell cycle regulation in response to DNA damage: exploring mechanisms of cell-fate determination.” *Biosystems* 103.3 (2011): 384-391.

Jackson, Stephen P., and Jiri Bartek. “The DNA-damage response in human biology and disease.” *Nature* 461.7267 (2009): 1071-1078.

Jackson, Stephen P. “Sensing and repairing DNA double-strand breaks.” *Carcinogenesis* 23.5 (2002): 687-696.

Kitagawa, Risa, et al. “Phosphorylation of SMC1 is a critical downstream event in the ATM–NBS1–BRCA1 pathway.” *Genes & Development* 18.12 (2004): 1423-1438.

Myers, Jeremy S., and David Cortez. “Rapid activation of ATR by ionizing radiation requires ATM and Mre11.” *Journal of Biological Chemistry* 281.14 (2006): 9346-9350.

Othmer, Hans G. “An Introduction to Mathematical Modeling of Signal Transduction and Gene Control Networks.” Digital file.

Painter, R. B., and B. R. Young. “Radiosensitivity in ataxia-telangiectasia: a new explanation.” *Proceedings of the National Academy of Sciences* 77.12 (1980): 7315-7317.

Pandita, Tej K., et al. “Ionizing radiation activates the ATM kinase throughout the cell cycle.” *Oncogene* 19.11 (2000): 1386.

Petrini, John HJ. “The Mre11 complex and ATM: collaborating to navigate S phase.” *Current Opinion in Cell Biology* 12.3 (2000): 293-296.

Stiff, Thomas, et al. “ATR?dependent phosphorylation and activation of ATM in response to UV treatment or replication fork stalling.” *The EMBO Journal* 25.24 (2006): 5775-5782.

Su, Tin Tin. “Cellular responses to DNA damage: one signal, multiple choices.” *Annual Review Genetics* 40 (2006): 187-208.

Suzuki, Keiji, et al. “Qualitative and quantitative analysis of phosphorylated ATM foci induced by low-dose ionizing radiation.” *Radiation Research* 165.5 (2006): 499-504

Ünsal-Kaçmaz, Keziban, et al. “The human Tim/Tipin complex coordinates an Intra-S checkpoint response to UV that slows replication fork displacement.” *Molecular and Cellular Biology* 27.8 (2007): 3131-3142..

Willis, Nicholas, and Nicholas Rhind. “Regulation of DNA replication by the S-phase DNA damage checkpoint.” *Cell Division* 4.1 (2009): 1.

Zhang, L. W., Y. M. Cheng, and K. M. Liew. “A mathematical analysis of DNA damage induced G2 phase transition.” *Applied Mathematics and Computation* 225 (2013): 765-774.

Zou, Lee, and Stephen J. Elledge. “Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes.” *Science* 300.5625 (2003): 1542-1548.

Reflection

I did this project because I learned about kinetic modeling at my research internship over the summer and I became really interested. I searched for pathways that I could better understand with kinetic modeling (that had not been modeled before) and I found the DNA Damage checkpoints, which really interested me. The intra-S phase checkpoint was the least understood, so I decided it'd be my job to better understand it.

In this project I learned a lot about DNA damage, how the cell reacts to it, and about kinetic modeling's use in biology. Throughout both my projects, I think I've really helped myself to see how important computer science can be in the biological sciences and how varied its use is. In this project the main struggle was being able to translate data from a paper into an actual computational model; this required a lot of hit-or-miss reading papers looking for specific information about a specific protein or interaction.

I had a lot of frustrations throughout this project; a lot of it was reading papers and deciding when I had read enough and how to proceed when I didn't have enough data. The programming aspect wasn't too difficult, but the documentation process was certainly difficult. I felt I improved over last year, but I still have a lot of work to do.

If I were to redo the experiment, I would include more proteins and do a better analysis of the rate constants to get better validation of the model and hopefully have more interesting results.

Overall, I have really enjoyed my experience in ASR and both my projects have made me learn a lot about computational biology, the scientific process, documentation, and research in general. I really appreciated the opportunity to do work in this class and I look forward to working in research long into my future.