

DNA Repair by Non-Homologous End-Joining: Mathematical Model and Applications

Andrew Alamban, Steven Fried, & Jericho Lawson
The University of Arizona
Mentor: J. Rubin Abrams
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ABSTRACT. DNA damage from either chemical or radiation sources poses critical challenges to a cell's survival and may increase mutation rate. Hence, understanding cellular repair mechanisms to damaged DNA is a key step towards elucidating certain causes of cancer as well as effective treatment of cancer by radiotherapy. We analyze a proposed mathematical model describing the method of non-homologous end-joining (NHEJ) for repair of double-stranded DNA breaks induced by ionizing radiation. The model accounts for enzyme-mediated and mass action kinetics of steps involved in NHEJ, producing a system of coupled ordinary differential equations whose behavior is simulated using a stiff-ODE solver in MATLAB. Preliminary work is focused on verifying the model's applicability toward measuring DSB repair kinetics against experimental data for DSB repair kinetics in cell lines. Moreover, we propose to use this model to predict effectiveness of specific radiotherapy-coupled chemotherapies towards eliminating cancer cells, and to more accurately place NHEJ in the context of total DSB repair.

Key Words: DNA repair, Double-stranded break (DSB), Non-homologous end-joining (NHEJ), Mathematical modeling, Radiotherapy, Ionizing radiation (IR).

1. Introduction & Background

Information for life is encoded in polymers called deoxyribonucleic acid (DNA). Proper maintenance and replication of DNA allow for its propagation into the next generation, while dysfunction can lead to generally debilitating effects such as cell death and mutation. A number of sources may be responsible for DNA damage, including chemical reactivity and electromagnetic radiation of varying linear energy transfers (LETs). Each of these sources can lead to varying forms of damage, ranging from adduct formation and base excision for some types of chemical damage, pyrimidine dimerization in the case of lower-energy ultraviolet radiation damage, and double-stranded breaks (DSBs) in the DNA in the case of higher-LET ionizing radiation (IR) [5].

Due to the debilitating effects of DNA damage to cell function and survival, cells have developed a number of ways of repairing damage. Understanding these DNA repair pathways are a key front in cancer biology, where DNA damage can be both an instigator of cancer (through improper repair leading to mutation), and an advantageous means of eradicating cancer cells such as through radiotherapy. Numerous research over the last century has hence sought to understand diverse mechanisms of DNA damage and repair.

The 1930s-1940s gave rise to early studies on the deleterious effects of ultraviolet and ionizing radiation (IR) on organisms, and cellular responses to these effects. This time period witnessed the discovery of the photoreactivation pathway for the repair of thymine dimers, the very first known DNA-repair mechanism [5]. Following Watson and Crick's structural characterization of the DNA double helix in 1953, the DNA-repair field advanced lightyears by introducing new biochemical sophistication to studying and clarifying DNA repair mechanisms. New mechanisms discovered in

the 1960s and 1970s included base-excision repair (BER) and double-stranded break (DSB) repair, the latter of which is a key cellular strategy toward combating programmed cell death or genomic instability [5].

In order to carry out DSB-repair, organisms have fortunately evolved non-homologous end-joining (NHEJ), the most prominent DNA-repair mechanism in humans, to reconnect broken ends of DNA independently of a homologous template. Alternate mechanisms of DSB-repair include homologous recombination (HR) and microhomology-mediated end joining (MMEJ) [4]. Studies on NHEJ have yielded a mechanistic description regarding the proteins responsible for DSB repair. These mechanisms can then be used alongside mass action and enzyme-mediated kinetics to model and study their dynamics. We begin by reviewing current mathematical models of DNA repair, followed by a reiteration of one particular model of NHEJ, which involves monitoring the dynamics of 11 species. We then report a summary of current progress and present our plans of 1) incorporating enzyme inhibition 2) testing alternate hypotheses regarding Artemis, an enzyme of interest in NHEJ and 3) integrating the updated NHEJ model into a course-grained DSB-repair model for use in evaluating other outcomes.

2. Literature Review

The field of radiation biology has long been interested in how organisms, particularly single cells, respond to radiation-induced DNA damage. An outcome of interest to most researchers is the relationship between surviving cells and the dose of radiation inflicted. The linear-quadratic model, which contains the empirically determined parameters α and β , has been used to model cell survival S as a function of radiation dose D [8]:

$$S = e^{-\alpha D - \beta D^2} \quad (2.1)$$

Due to growing information regarding genetic makeup of species as well as molecular pathways regarding DNA repair, the linear-quadratic model has become outdated because it employs parameter sets that do not translate well to pathological cases, such as cancer.

McMahon, *et al.*, propose a new model giving rise to a parameter set that can be fitted using experimental data constants. This provides robust parameters that lead to a high correlation between simulated and experimental data. Previous models often use different parameter sets for a specific cellular response (e.g. number of DSB, mutation rates, cell survival, etc.) but the proposed model allows prediction for different cellular response using one parameter set. In addition, this parameter set can be applied across different cell lines and different conditions [8].

Double-stranded break (DSB) repair depends on the distance between the free ends; i.e., the further two ends are from each other, the more difficult it will be for them to rejoin. Similarly, the fidelity of DSB repair also depends on which mechanism the cell employs. Their model considers three repair pathways: 1) non-homologous end joining (NHEJ) 2) homologous recombination (HR) and 3) microhomology-mediated end joining (MMEJ). Each pathway has its own respective repair rate as well as repair fidelity. Depending on the genetics, some pathways may also vary in use or, in some cases, not be usable because of mutation. Lastly, the complexity of the DSB also depends on the linear energy transfer (LET) of the radiation [8].

McMahon, *et al.* constructed their parameter set consisting of a characteristic repair distance, pathway-dependent repair rate, and fidelity, the probability of a complex DSB-repair given geometric considerations that are cell-dependent. They then produced analytical solutions of exponential decay to model the dynamics of DSB repair as well as create probabilistic models to account for

the use of different repair pathways. Each of these models correlated well with experimental data, including the observations of repair kinetics, DSB misrepair, mutation rate, chromosomal aberrations (which differ from DSB misrepair in terms of scope), and cell survival [8].

The benefits of McMahon's model is that its application is bidirectional. On the input side, one can fit experimental data with a simulated curve from the model. This will give information that the experiment did not investigate. For example, the authors cite how kinetics of H2AX, a DSB-response protein, and aberration data can inform how DSB repair processes fail. On the output side, one can obtain mutation or survival response predictions using fitted parameters from other experiments [8].

In conclusion, McMahon *et al.* have presented a model which allows for the prediction of various cellular outcomes from a single set of parameters that can be applied to different cell types under various conditions [8]. The authors, however, commented that most of their assumptions were done on the scale of *in vitro* experiments. Additionally, their model did not take advantage of molecular mechanisms to radiation response. By incorporating well-studied molecular mechanisms, one can come up with a more granular model which will be desirable in treating tumors, as tumors are genetically heterogeneous.

Such an approach was proposed in 2018 by Rouhani, who modeled DSB repair by NHEJ. Several known empirical qualities of NHEJ are used in the construction of a biochemical mechanism that is kinetically modeled by a system of 11 coupled, ordinary differential equations. These models make use of either standard mass-action kinetics (in the case of binding/dissociation reactions) or Michaelis-Menten kinetics (in the case of enzymatic reactions), to model the interconversions between 11 different species appearing in the biochemical pathway. Parameters for these reactions (14 in total) were gathered from sources reported in the literature [11].

In Rouhani's model, the bifurcation of DSB repair, occurring by either a fast or a slow mechanism, is explained by the formation of ligatable (85%) and unligatable (15%) complexes from the broken DNA and recruited Ku proteins. Ligatable complexes may be immediately rejoined by an enzymatic XXL complex composed of ligase IV, XRCC4, and XLF proteins. Unligatable complexes, on the other hand, must first bind DNA-dependent protein kinase catalytic subunits (DNA-PKcs), and be rejoined into a ligatable complex by a hyperphosphorylated Artemis enzyme, whose phosphorylation levels are controlled by both the DNA-PKcs-DSB complexes and ATM enzyme. The ligatable complex is then rejoined enzymatically by an XXL complex, as in the fast mechanism. Generally, the fast and slow mechanisms are delegated to simple and more complex DSBs, respectively [11].

The system of stiff ordinary differential equations mechanistically modeling NHEJ was simulated using the `ode15s` solver in MATLAB and compared against experimentally measured kinetics of DSB repair in various cell lines. These include normal, ATM-deficient, and Artemis-deficient cell lines (both human fibroblast and mouse embryonic), subjected to varying dosages of ionizing radiation between 0.02 and 2 Gy. Prior to the publication of this paper, it was unknown whether the ligatable complexes of DSB with Ku produced in the fast reaction mechanism could still bind DNA-PKcs or not. By evaluating two different models, one allowing this step and one not, and comparing simulation results to experimental data, it was determined that the inclusion of this step significantly increases model accuracy. The model including this DNA-PKcs binding step demonstrated P-values above 0.72 for all radiation levels and correlation coefficients above 0.97 for the normal human cells and above 0.88 for the normal mouse cells. ATM-deficient human cells demonstrated a strong correspondence to the model, with P-values above 0.75 and correlation coefficients

above 0.93 for all radiation levels tested, while Artemis-deficient human cells corresponded to the model with P-values and correlation coefficients more than 0.87 and 0.95, respectively [11].

In summary, Rouhani's theorized biochemical pathway describing DSB repair by NHEJ results in a mathematical model that is highly accurate at predicting the kinetics of DSB repair in experimental systems. The model results indicate that the most important parameters controlling NHEJ rate are the Ku protein initial concentration, followed by XXL complex initial concentration and enzymatic activity, and then DNA-PKcs initial concentration and Artemis initial concentration and enzymatic activity. By prioritizing the pharmacological inhibition of NHEJ directors in this order, one takes a closer step towards effectively hindering this process in cancer cells subjected to radiotherapy, thereby increasing the success rate in radiotherapeutic treatment of cancer patients [11].

In addition to DSB repair, alternate types of DNA damage and their repair mechanisms have been explored and analyzed mathematically. For instance, a model for the formation of DNA adducts from oxidative estrogen metabolism and base-excision repair (BER) of the adducts was proposed by Crooke and Parl (2010) in their paper "A Mathematical Model for DNA Damage and Repair." The model is separated into two different stages: 1) the formation of adducts leading to apurinic (AP) sites (i.e. single-nucleotide DNA damage) and 2) the repair of these adducts using BER.

In the first stage of the model, mass action kinetics was used where the estrogen quinone forms adducts spontaneously without any enzyme involvement [3]. Michaelis-Menten kinetics was then used for enzymatic reactions that included parameters such as K_{m_1} and k_{cat_1} . Crooke and Parl also found direct experimental evidence that oxidative metabolism of hormone E_2 leads to the formation of deoxyribonucleoside adducts [3].

The second stage of the model is based on the BER pathway. BER is modeled by a system of nonlinear differential equations, once again invoking Michaelis-Menten kinetics. All k constants found in the system are based on experimental values. After testing these models, they found several ideas. They noted that doubling the concentration of E_2 doubles the number of AP sites, or empty places along a DNA strand with no nucleotide [3]. Additionally, they showed that commonly occurring variants of CYP1B1 differ in activity of producing 4-OHE₂ from the parent hormone. Not only that, but the more active CYP1B1 variant increased DNA damage [3].

Ultimately, Crooke and Parl's model shows dynamic interaction between the estrogen-mediated DNA damage and the DNA base-excision repair pathway in order to show the overall impact on carcinogenesis on DNA. The model predicts a larger increase in the number of AP sites, which indicates the involvement of other factors including enzymatic factors.

In total, approaches in mathematically modelling a range of DNA repair mechanisms have been reported. McMahon, *et. al* have proposed a model for DSB repair that incorporated three repair pathways, leading to simulations that can predict different outcomes in addition DSB repair [8]. Rouhani proposed a mechanistic model specifically for NHEJ which accurately predicts DSB repair dynamics and expands upon the role that DNA-PKcs plays in NHEJ [11]. Lastly, Crooke and Parl's mechanistic model of DNA adduct formation was compared with experimental data and their BER repair model incorporated parameters derived from experimental data [3].

Together, the literature show significant progress in simulating DNA repair. While McMahon, *et al.* created a model that can account for different repair pathways, their model failed to account for the mechanistic pathways reported in literature. Integration of mechanistic models such as one proposed by Rouhani would increase the prediction power of generalized models of NHEJ. To take a step further, integration of other types of DNA damage and repair, as discussed by Crooke and

Parl, would describe a more realistic scenario as organisms are constantly exposed to more than one type of DNA damage. Formulation of more accurate models could be used to further basic science as well as improve applications of radiotherapy in healthcare.

3. Model & Progress

3.1. Summary of Model

The model presented by Rouhani showcases the process of non-homologous end joining [11]. Figure 3.1 visualizes that very step-by-step process. As mentioned earlier, the slow and fast phases of the DSB repair are included in this model.

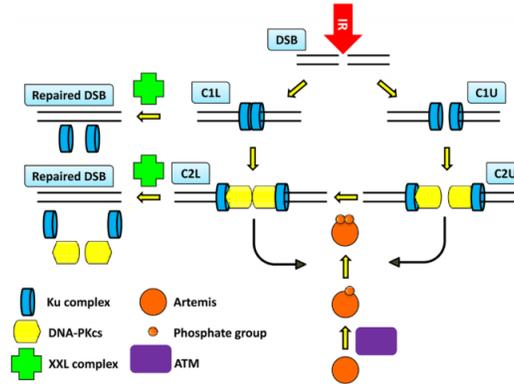


FIGURE 3.1. NHEJ Model [11]

Rouhani discovered that the model can be of the form of a system of 11 ODEs, each with very specific components. To better organize the ODEs, nine dummy variables were used. Appendix A highlights those choices.

Each of these nine dummy variables represents a certain part of the diagram in Figure 3.1. v_1 represents the use of IR dosage to develop double strand breaks. v_2 represents the chemical reaction of the double strand breaks and two sets of the Ku complexes to form C1L and C1U. v_3 and v_4 each represent chemical reactions of one ligatable or unligatable C1 complex and two molecules of DNA-PKcs to form the ligatable or unligatable C2 complex respectively [11]. The rest of the dummy variables are a result of reaction rates that can be described by the Michaelis-Menten equation, which can be represented as the following:

$$r = \frac{k_{cat} \times [E] \times [S]}{K_m + [S]}, \quad (3.1)$$

where r is the reaction rate, $[E]$ and $[S]$ are the enzyme and substrate concentrations, k_{cat} is the maximum rate of product formation, and K_m is the substrate concentration in which the reaction rate reaches half of the maximum rate. As such, v_5 is equivalent to the use of C1L complexes to produce repaired double strand breaks and two Ku complexes. Likewise, v_6 involves the use of C2L complexes to repair double strand breaks and produce two Ku complexes and two DNA-PKcs proteins. v_7 involves the usage of ArtemisPP to convert C2U complexes to C2L complexes. v_8 represents the use of ATM to convert Artemis to phosphorylated Artemis. Likewise, v_9 encapsulates the transformation of phosphorylated Artemis to hyperphosphorylated Artemis using DNA-PKcs proteins [11].

With these dummy variables in place to simplify the ongoing processes in the model, we can now write the system of 11 ODEs as the following:

$$\begin{aligned}
\frac{d[DSB]}{dt} &= v_1 - v_2 \\
\frac{d[Ku]}{dt} &= -2v_2 + 2v_5 + 2v_6 \\
\frac{d[C1L]}{dt} &= 0.85v_2 - v_3 - v_5 \\
\frac{d[C1U]}{dt} &= 0.15v_2 - v_4 \\
\frac{d[DNA - PKcs]}{dt} &= -2v_3 - 2v_4 + 2v_6 \\
\frac{d[C2L]}{dt} &= v_3 - v_6 + v_7 \\
\frac{d[C2U]}{dt} &= v_4 - v_7 \\
\frac{d[Repaired DSB]}{dt} &= v_5 + v_6 \\
\frac{d[Artemis]}{dt} &= -v_8 \\
\frac{d[ArtemisP]}{dt} &= v_8 - v_9 \\
\frac{d[ArtemisPP]}{dt} &= v_9
\end{aligned} \tag{3.2}$$

Terms that are either in blue or green will be discussed in the next subsection.

3.2. Approach & Results

Our first objective is to replicate the results that were found in Rouhani's paper, particularly the model that is proposed. Within MATLAB, there is a script called ODE15s, which is described as a stiff differential equation solver. Considering the complexity of the model, using a script like this is crucial to our replication status. For this model, we will use the initial concentrations that Rouhani used in her model and if stated, we will use the model 1 versions of the concentrations (e.g. $K_m ArtemisPP = 240$).

A MATLAB script with two various functions is used. The first function includes all dummy v variables and the 11 ODES that will need to be solved using the ODE15s functionality; the second function computes the result of Michealis-Menten equations, given the enzyme and substrate concentrations, the maximum rate of product formation, and the substrate concentration at half the maximum rate. The main MATLAB script contains several initial conditions, the ODE15s function call, and any plots. For any initial concentrations aside from the initial number of double strand breaks and the ones mentioned in Rouhani's paper, we set these values to zero. In our model, we set the initial number of double strand breaks to $c \times IRdose$ and used a time span from 0 to 100 hours, similar to what is seen in Rouhani's work. Two different models were compared as a means to replicate work done in our mentioned paper.

Figure 3.2 shows the remaining double strand breaks over time following radiation for models 1 and 2 [11]. To find the amount of remaining double strand breaks at time t , we took the amount of repaired double strand breaks at time t and subtracted the amount from the initial amount of DSBs (i.e. when $t = 0$). Both graphs have negative exponential behavior similar to those in Rouhani's research. Additionally, all values relatively match the values that Rouhani had in her paper when there is an IR dosage of 2 grays of X-rays. While there is strong replication of the models, there were slight modifications that were made to match the graphs in Rouhani's paper. Primarily, the $\frac{d[DSB]}{dt}$ term had to be changed to just $-v_2$. More work needs to be done to determine the reason for this fix.

Additionally, terms associated with dummy variable v_3 were removed from the system of ODEs for model 2. This is the result of the step associated with v_3 , which involves the conversion of the C1L complex to the C2L complex, not taking place in model 2. This removal differentiates model 1 from model 2.

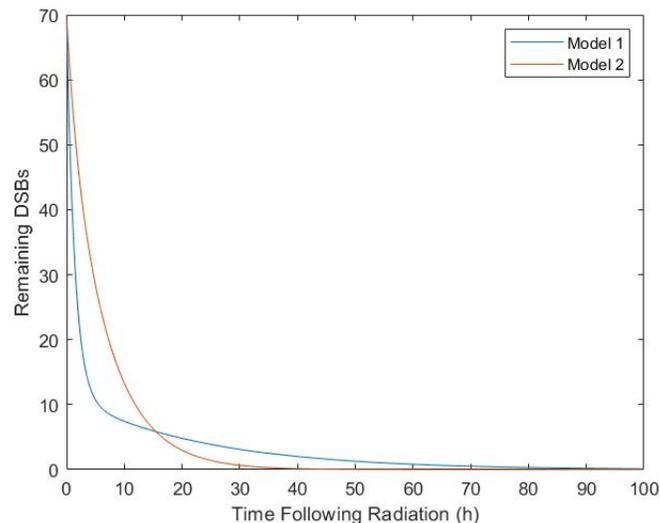


FIGURE 3.2. Replicated results of models 1 and 2 from Rouhani's paper using 2 Gy X-rays.

The successful reproduction of Rouhani's simulated data may be tested by using statistical tests to compare the mathematical model to experimental data. Rouhani reports a variety of experimental data describing DSB repair kinetics due to NHEJ in both human and mouse fibroblast embryonic cell lines [11]. These data include cellular responses to varying radiation doses (0.02 Gy, 0.2 Gy, 1.3 Gy, and 2 Gy) for wild-type, Artemis-deficient, and ATM-deficient cell lines. We propose to recreate the conditions simulating each of these conditions using the described model, and to compare the simulated curves to reported experimental data by calculating coefficients of determination R^2 and regression p-values, the latter of which can be calculated using polynomial terms to account for curvature.

4. Future Plans & Conclusions

4.1. NHEJ Inhibition Modeling

Having verified the accuracy of our model, we propose to next investigate medical applications of our model, specifically toward radiotherapy. The principle of radiotherapy hinges upon the destruction of cancer cells through targeted damage to cancer cell DNA by ionizing radiation (IR). Cancer cells, in response, will attempt to repair damaged DNA or undergo apoptosis. Hindering the repair of IR-induced DSBs is thus advantageous in promoting cancer cell death. The model proposed herein suggests that several proteins and enzymes involved in the NHEJ mechanism could be chemically targeted for inhibition in conjunction with radiotherapy, making the radiotherapy more potent against cancer. Specifically, reduction of available Ku proteins and inhibition of the enzymatic XXL complex are expected to have the greatest effect on NHEJ inhibition, since these proteins are universally involved in NHEJ no matter the specific pathway taken (fast or slow). The enzymatic activities of DNA-PKcs and Artemis should matter to a lesser extent, as these proteins are only involved in select routes of the NHEJ pathway.

Enzyme inhibition can occur by varying mechanisms that are classified by the type of complex the inhibitor binds. Competitive inhibitors bind the free enzyme, precluding some enzyme from binding substrate; uncompetitive inhibitors bind enzyme-substrate complexes to preclude the release of reacted substrate as product. Also possible are mixed inhibitors, which bind both free enzyme and enzyme-substrate complexes. We shall consider each type of inhibition by their impacts on Michaelis-Menten enzyme parameters. Competitive inhibitors increase the value of K_M to decrease substrate binding affinity as follows, $[I]$ represents inhibitor concentration and K_I , the dissociation constant of the enzyme to its inhibitor:

$$K_M^{+I} = K_M \left(1 + \frac{[I]}{K_I}\right). \quad (4.1)$$

Uncompetitive inhibitors reduce both k_{cat} and K_M by the same constant value:

$$K_M^{+I} = \frac{K_M}{\left(1 + \frac{[I]}{K_I}\right)} \quad (4.2)$$

and

$$k_{cat}^{+I} = \frac{k_{cat}}{\left(1 + \frac{[I]}{K_I}\right)}. \quad (4.3)$$

One common type of mixed inhibitor is the noncompetitive inhibitor, which decreases the parameter k_{cat} according to equation 4.3 without altering K_I .

We will predict the impact of real and hypothetical inhibitors to these enzymes by scaling the parameters appropriately and evaluating the impact on the NHEJ timescale. Among the enzymes involved in NHEJ, DNA-PK is perhaps the best characterized. We will explore five potential inhibitors to DNA-PK: the noncompetitive inhibitor Wortmannin, and the competitive inhibitors LY294002, Rutin, Quercetin, and Quercitrin. These inhibitors have experimentally verified K_I values of 120 nM, 6.0 μ M, 26 μ M, 110 μ M, and 208 μ M, respectively [7]. We will additionally consider the hypothetical case of a 6.0 μ M uncompetitive inhibitor and 6.0 μ M noncompetitive inhibitor as points of direct comparison between the three different inhibition modes.

Likewise, we consider the inhibition of ligase IV in the XXL enzymatic complex. One known inhibitor, discovered as Compound 189 by Chen *et al.*, is a known competitive inhibitor of ligase IV with $K_I = 5\mu$ M [2]. Similarly as for the DNA-PK inhibitors, we shall simulate the effect of

introducing this inhibitor on NHEJ kinetics. As a point of comparison, we shall also consider 6.0 μM competitive, uncompetitive, and noncompetitive inhibitors as hypothetical cases.

Few inhibitors of Artemis are known at present, with little quantitative data existing for those thought to be inhibitors [9]. However, as a point of comparison, we shall consider hypothetical cases wherein Artemis is inhibited by 6.0 μM competitive, uncompetitive, and noncompetitive inhibitors as before. Finally, we may consider the pharmaceutical targeting of Ku proteins. While Ku is not an enzyme and cannot have its activity inhibited by a similar mechanism, we can consider a depletion of available Ku proteins by gene therapy. Ayene *et al.* have described success in targeting Ku transcription with small-interfering RNAs (siRNAs), depleting the Ku concentration within cells by 70% [1]. We will consider this scenario by appropriately adjusting the initial concentration of Ku within the cell prior to DSB repair. Having considered pharmaceutical targeting of several different proteins within the NHEJ pathway and simulating the consequences of each in conjunction with radiotherapy, we anticipate being able to predict which protein will be the most influential target towards decreasing NHEJ kinetics.

4.2. Alternate Proposed NHEJ Mechanisms

Some controversy exists yet in the literature on the role of hyperphosphorylation of Artemis in NHEJ [10][6]. We hence propose to investigate additional situations where singly phosphorylated Artemis, or even unphosphorylated Artemis, perform the same catalytic ligation step in the NHEJ mechanism. As an initial alternate scenario, we plan to investigate the case where ArtemisP and ArtemisPP have the same catalytic activity, which is simply addressed by changing the form of equation

$$v_7 = \frac{k_{cat}ArtemisPP \times ([ArtemisPP] + [ArtemisP]) \times 2 \times [C2U]}{K_mArtemisPP + 2 \times [C2U]}. \quad (4.4)$$

Likewise, we can consider a case where all forms of Artemis, phosphorylated or not, have the same enzymatic activity:

$$v_7 = \frac{k_{cat}ArtemisPP \times ([ArtemisPP] + [ArtemisP] + [Artemis]) \times 2 \times [C2U]}{K_mArtemisPP + 2 \times [C2U]}. \quad (4.5)$$

As a more sophisticated method of addressing the role of Artemis phosphorylation in NHEJ, we may consider Artemis, ArtemisP, and ArtemisPP has three different enzymes, each with their own Michaelis-Menten parameters:

$$v_7 = \frac{k_{cat}ArtemisPP \times ([ArtemisPP]) \times 2 \times [C2U]}{K_mArtemisPP + 2 \times [C2U]} + \frac{k_{cat}ArtemisP \times ([ArtemisP]) \times 2 \times [C2U]}{K_mArtemisP + 2 \times [C2U]} + \frac{k_{cat}Artemis \times ([Artemis]) \times 2 \times [C2U]}{K_mArtemis + 2 \times [C2U]}. \quad (4.6)$$

Significantly, this model introduces four new parameters. In the event that experimental values for these parameters cannot be determined, we may consider a range of hypothetical values as a means of estimating the impact of these alternate situations on NHEJ kinetics. Specifically, we hypothesize successively smaller values of k_{cat} and/or larger values of K_M for decreasing phosphorylation levels, highlighting an enzymatic activity that is phosphorylation-dependent, yet more complex than a simple on-off switch.

4.3. NHEJ in the Broader Context of DSB Repair

It is important to recall that NHEJ is not the sole method of DSB repair in the cell. Additional means include homologous recombination (HR) and microhomology-mediated end-joining, which are typically slower and employed for more complex breaks that involve damage to nucleotides in the vicinity of the DSB, or multiple breaks on each strand [12]. To this end, McMahon *et al.* has proposed a simplified kinetic model describing all three methods of DSB repair together as a linear combination of exponential functions:

$$N(t) = N_0(p_f e^{-\lambda_f t} + p_s e^{-\lambda_s t} + p_m e^{-\lambda_m t}), \quad (4.7)$$

where $N(t)$ is the total number of DSBs after a time t , N_0 is the initial DSB number, λ_x are the three repair rate constants (NHEJ - fast, HR - slow, and microhomology, respectively), and p_x is the probability of a break being repaired by each process [8]. While advantageous in that macroscopic model accounts for each of the three repair processes, the model falls short in its accuracy of how each process is individually modeled. We can see, for instance, that the NHEJ model is more complex than an exponential function, and a next-level more accurate DSB repair model will thus incorporate the NHEJ model of Rouhani into the linear combination of exponentials proposed by McMahon *et al.*. Since the Rouhani model is described by a system of ordinary differential equations rather than an algebraic function, its behavior may be computationally simplified by fitting a smoothing spline to the data in MATLAB and incorporating this spline into the McMahon *et al.* linear combination of exponentials.

In addition to non-homologous end-joining, we propose to consider existing mathematical models of homologous recombination (HR) and microhomology-mediated end-joining (MMEJ), such as the coupled ODE-system for MMEJ proposed by Taleei and Nikjoo for DSB-repair in the G1 and early S phase of the cell cycle [13]. Successful simulation of one or more of these alternative pathways to DSB repair will enable incorporation into the combined McMahon model, giving information on the synergistic cooperation between three different DSB repair methods.

4.4. Conclusions

In summary, we evaluate here the applicability and accuracy of an eleven-coupled-ODE model for DNA repair by NHEJ towards simulating the kinetics of DSB repair in response to ionizing radiation. We propose to statistically compare the simulation to experimental results to verify accuracy. We also aim to perform new analyses with the model, such as considering alternate scenarios on the role of phosphorylation in Artemis activity, and placing the more accurate NHEJ model in the context of other methods of DSB repair. We additionally propose to analyze this model toward determining effective drug targets within NHEJ repair pathways. Effective inhibition of the NHEJ pathway in cancer cells through the targeting of these proteins should increase cancer cell destruction rate; hence we anticipate that this knowledge will be advantageous toward enhancing the efficacy of radiotherapy against cancer.

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Appendix A. Table of Dummy Variables

v	Expression	Parameters & Meaning
v_1	$c \times IR\ dose$	c - DSB induction
v_2	$Ku_DSB\ binding\ rate$ $\times [Ku]^2 \times [DSB]$	Ku - Ku70/80 complex DSB - Double-strand break
v_3	$DNA - PKcs_C1L\ binding\ rate$ $\times [C1L] \times [DNA - PKcs]^2$	$C1L$ - Ligatable complexes of main proteins $DNA - PKcs$ - DNA-dep. prot. kinase cat. subunit
v_4	$DNA - PKcs_C1U\ binding\ rate$ $\times [C1U] \times [DNA - PKcs]^2$	$C1U$ - Unligatable proteins of main proteins $DNA - PKcs$ - DNA-dep. prot. kinase cat. subunit
v_5	$\frac{k_{cat}XXLforC1L \times [XXL] \times [C1L]}{K_mXXLforC1L + [C1L]}$	XXL - XLF/XRCC4/ligase IV complex $C1L$ - Ligatable complexes of main proteins
v_6	$\frac{k_{cat}XXLforC2L \times [XXL] \times [C2L]}{K_mXXLforC2L + [C2L]}$	XXL - XLF/XRCC4/ligase IV complex $C2L$ - Ligatable from $C1L$
v_7	$\frac{k_{cat}ArtemisPP \times [ArtemisPP] \times 2 \times [C2U]}{K_mArtemisPP + 2 \times [C2U]}$	$ArtemisPP$ - Hyperphosphorylated form of Artemis $C2U$ - Unligatable from $C1U$
v_8	$\frac{k_{cat}ATM \times [ATM] \times [Artemis]}{K_mATM + [Artemis]}$	ATM - Ataxia telangiectasia mutated $Artemis$ - Artemis protein
v_9	$\frac{k_{cat}DNA - PKcs \times 2 \times ([C2L] \times [C2U]) \times [ArtemisP]}{K_mDNA - PKcs + [ArtemisP]}$	$C2L$ - Ligatable from $C1L$ $C2U$ - Unligatable from $C1U$ $ArtemisP$ - Phosphorylated form of Artemis

TABLE A.1. Nine dummy variables with all non-obvious parameters explained

DEPARTMENT OF MATHEMATICS, THE UNIVERSITY OF ARIZONA, TUCSON, AZ 85721, USA

Email address, A. Alamban: alamban@email.arizona.edu

Email address, S. Fried: sdefried@email.arizona.edu

Email address, J. Lawson: jerlawson13@email.arizona.edu