DNA Base Excision Repair Nanosystem Engineering: Model Development

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Abstract—DNA base damage results from a combination of endogenous sources, (normal metabolism, increased metabolism due to obesity, stress from diseases such as arthritis and diabetes, and ischemia) and the environment (ingested toxins, ionizing radiation, etc.). If unrepaired DNA base damage can lead to diminished cell function, and potentially diseases and eventually mutations that lead to cancer. Sophisticated DNA repair mechanisms have evolved in all living cells to preserve the integrity of inherited genetic information and transcriptional control. Understanding a system like DNA repair is greatly enhanced by using engineering methods, in particular modeling interactions and using predictive simulation to analyze the impact of perturbations. We describe the use of such a “nanosystem engineering” approach to analyze the DNA base excision repair pathway in human cells, and use simulation to predict the impact of varying enzyme concentration on DNA repair capacity.

Keywords—DNA repair, nanobioengineering, systems biology, modeling and simulation

I. INTRODUCTION

DNA damage causes changes in protein-coding and regulatory sequences, leading to diminished cell function, disease and cancer. DNA repair is a complex system of interacting biomolecular processes [1]. DNA repair mechanisms have evolved and are partially conserved in all life, bacteria through mammals. Operation of the DNA repair system is determined by the number of repair proteins (regulated in response to DNA damaging stress) and their binding and enzyme kinetics (defined by protein structure and subject to interactions with other proteins and damaged DNA). Defects in the regulation, structure, and interactions of DNA repair proteins can result in accelerated aging phenotypes, increased cancer susceptibility, and other significant health effects.

Understanding how defects in individual components of DNA repair affect overall cellular tolerance for DNA damage requires a system-level nano-scale understanding. A nanoscale system model of DNA repair will allow us to design therapeutic control strategies that target the DNA repair system efficiently and effectively – either to enhance DNA repair activity or destabilize it (i.e., complementing anti-tumor treatment).

Chemical modification of individual bases can lead to base loss and base modification, leading to potential mutations and transcriptional defects. Relatively large amounts of DNA base damage result from oxidation due to reactive oxygen species (ROS), produced by endogenous (metabolism) and exogenous sources (e.g., secondary effects of ionizing radiation, chemical toxins). DNA base excision repair (BER) is the key system for repairing single-base damage, and inability to adequately repair oxidative (as well as alkylation, etc.) [2]. DNA base damage has been connected to aging, cell death, genome instability and disease. We are developing a mathematical model of human DNA base excision repair to solve the following problems:

1. Identify and determine the significance of protein interactions in BER reactions (active displacement of DNA glycosylases, potential “scaffolding” or recruitment proteins).
2. Determine the biological role of “backup” sub-pathways (“short-patch” repair of single bases versus “long-patch” repair of 5-7 base regions, dual-function DNA glycosylases).
3. Predict the impact of subtle (non-lethal) mutations in BER proteins on overall pathway kinetics (specifically considering polymorphisms identified in population studies).
4. Predict the effect of perturbing cellular protein levels either to enhance or destabilize the BER pathway (with potential application to cancer therapeutics [3], or to use enzyme levels as a diagnostic tool for individualized medicine).

In this paper, we demonstrate the application of the human BER model to the last question, predicting the impact of varying enzyme concentration on cellular DNA base excision repair capacity and physiological DNA base damage levels.

II. METHODOLOGY

We have developed a first-principles chemical kinetics model based on reactions, kinetic parameters, and enzyme levels found entirely in the literature. Figure 1 shows a schematic of the current version of the BER model [4]. We compare and validate our model against published experimental data from in vitro pathway reconstitution, assays using whole cell protein extracts, and direct measurement of DNA damage levels in tissue. To date, our BER system model:

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• Supports predictions in the literature for cooperativity between AP endonuclease (Ape1) and Polymerase β (Polβ) and the role of Ape1 displacing 8-oxoguanine (8-oxoG) DNA glycosylases (Ogg1). [5]
• Predicts that Polβ gap-filling and 5’-dRp lyase activities are consecutive and coordinated (performed by the same enzyme). [5]
• Predicts that short-patch repair dominates over long-patch repair in mammalian cells (supported by other experimental studies). [5]
• Estimates that the “normal” background level of oxidative DNA base damage resulting from metabolism is up to 100 lesions/cell. This is a robust prediction that agrees with the low end of controversial experimental data ranging across 4-5 orders of magnitude. [4]

Table 1 summarizes the parameters used in this model (see [Sokhansanj 2004a], Supplementary Material, for references). Equations are generated by mass balance from the reaction schematic of Figure 1, using a Michaelis-Menten enzyme kinetic model (the full set of equations is given in Supplementary Material of [5]). Assumptions of the Michaelis-Menten model (well-mixed reactants in the mammalian cell nucleus, no product inhibition, etc.) are discussed in detail in [4].

Table 1

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$k_{cat}$ (s⁻¹)</th>
<th>$K_M$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogg1 8-oxoG excision</td>
<td>1.39x10⁻³</td>
<td>1836</td>
</tr>
<tr>
<td>Ogg1 glycosylase (&amp; Ape1)</td>
<td>7.55x10⁻³</td>
<td>8.9</td>
</tr>
<tr>
<td>Ogg1 AP lyase</td>
<td>8.9x10⁺⁴</td>
<td>13.7</td>
</tr>
<tr>
<td>Ogg1 AP lyase (&amp; Ape1)</td>
<td>1.335x10⁴</td>
<td>13.7</td>
</tr>
<tr>
<td>Ape1 AP endonuclease</td>
<td>0.362 - 10 (3.08 average)²</td>
<td>9.2 - 230 (32.5)</td>
</tr>
<tr>
<td>Ape1 3’-phosphodiesterase</td>
<td>0.05</td>
<td>$k_8$ 130</td>
</tr>
<tr>
<td>Polβ gap-filling</td>
<td>0.45 – 0.8 (0.625)</td>
<td>$k_9$ 300</td>
</tr>
<tr>
<td>Polβ dRp lyase</td>
<td>0.075</td>
<td>$k_9$ 500</td>
</tr>
<tr>
<td>Lig1/3 ligation</td>
<td>0.04</td>
<td>$k_9$ 100</td>
</tr>
<tr>
<td>Polδ gap-filling (&amp; PCNA)</td>
<td>0.0022</td>
<td>$k_9$ 67</td>
</tr>
<tr>
<td>Fen1 5’-flap endonuclease</td>
<td>0.39</td>
<td>39</td>
</tr>
<tr>
<td>Fen1 5’-endo (&amp; Ape1)</td>
<td>0.78</td>
<td>$k_7$ 39</td>
</tr>
</tbody>
</table>

Figure 2 shows the effect of varying enzyme concentration (Ogg1, solid; Polβ, dashed) on the steady state level of 8-oxoguanine lesions and unrepaired intermediates for a constant damage formation rate of 2,000 8-oxoG/cell/day and 10,000 AP sites/cell/day. Figure 3 shows the effect of varying enzyme concentration (Ape1, dashed; Polβ, solid) from the baseline level of Table 1 on AP sites and unrepaired intermediates, given constant damage formation of 10,000 AP sites/cell/day.

The rates of constant damage formation correspond to those estimated for oxidative DNA damage and base loss by spontaneous hydrolysis through experimental measurements [4]. These are normal levels, corresponding to those expected to result from endogenous damage, i.e., due to normal metabolic activities in cells that produce ROS. Our previous work in [4] validated our model for normal endogenous damage formation, based on supporting lower experimental estimates of steady state DNA damage.
Comparing the proportional ability of a given set of operational characteristics (enzyme kinetic parameters and concentrations) based on a set damage formation rate gives us a measure for relative DNA repair capacity. As previous work ([4] and [5]) shows, for physiologically relevant DNA damage rates, the pathway shows a close to linear response. Consequently, we expect that the sensitivity of the pathway to enzyme concentration to be independent of realistic variation in damage formation rate (results not shown).

### IV. DISCUSSION

As Figure 2 shows, there is no “rate-limiting” step in 8-oxoguanine repair. Varying either enzyme, the polymerase or the DNA glycosylase, results in a change in steady-state damage level that is almost equivalent. Without complex system modeling, it would be almost impossible to intuitively predict this result. In addition, both Figures 2 and 3 demonstrate that like perhaps many human pathways, increasing enzyme concentration does little to improve pathway efficiency. This is expected, since the ubiquity of DNA base damage is such that evolutionary processes have “optimized” pathway performance in human cells. Thus, little is to be gained by increasing enzyme concentrations as a therapeutic strategy. However, inhibiting BER enzymes, such as polymerases and DNA glycosylases, may be effect as a “co-therapy” with chemotherapy to help selectively kill tumor cells.

### V. CONCLUSION

We are now establishing an experimental capability to measure cellular BER kinetics (in vitro and in vivo using fluorescently labeled damaged DNA; [6], [7]). This technology will aid in validating the prediction of the impact of enzyme concentration variation on BER kinetics in living cells and provide a tool to develop improved pathway models.

Our work on the human BER pathway exemplifies the “systems biology” paradigm: studying and eventually design control strategies for a biological pathway, taking advantage of advancing quantitative biology tools to work towards developing a quantitative, predictive system model of a critical human cellular process.

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### REFERENCES