DNA-Mediated Charge Transfer Between [4Fe-4S] Cluster Glycosylases

Thesis by

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it is!!!).

Later in graduate school, I joined **Prufrock Group**, a cooking group that also became a community of treasured friends. They provided many free meals and the opportunity to transfer my scientific frustrations into culinary endeavors. There's a reason I'm good at chopping potatoes! More importantly, though, most members of Prufrock were close to my year in graduate school and, as we grew up together, they also provided me with encouragement and perspective as I navigated its difficult moments. Many thanks to **Zuli Kurji, Theresa Emery, Matt Kelley, Heather McCaig**, and **Michael Mendenhall** for keeping my stomach full and your ears open. In addition to maintaining the Prufrock club's backyard garden, **Heather McCaig** was an outstanding roommate for the better part of five years. Heather is a fellow tea fanatic, an outstanding cook of often unusual cuisines, and a willing participant in late-night social gatherings. I am still amazed that she and I have managed to remain such good friends after living in the same apartment for so long. She has remained upbeat and undeniably quirky through all the difficulties of her Ph.D. project, and I admire her spirit. I am also constantly amazed when I watch her dance ballet. I wish Heather all the best in her post-Caltech endeavors.

In addition to providing a wonderful support network of friends and fellow scientists-in-training, Caltech maintains many student support services that have greatly enriched my Ph.D. experience. I cannot endorse the **Caltech Y** enough. The staff there is always positive and friendly, and they maintain constant smiles and calm demeanors amidst what must be a very demanding workload for such a small office. In addition to overseeing volunteer activities and cultural trips in which I have participated, the Caltech Y **Outdoor Committee** has dramatically changed my years in Pasadena. I came to Southern California

expecting to find Mickey Mouse, some palm trees, and lots of smog. The Caltech Y's many day hikes, camping trips, and backpacking trips helped me realize that California is a much more diverse, complex, and gorgeous place to live. I did not realize that so much natural beauty and dramatically varying landscapes were located so close to such populous urban centers. The Caltech Y taught me to love the great outdoors, which prompted my participation in the **Caltech Hiking Society**, and my desire to travel all over California, and even into Arizona's Grand Canyon, in search of wilderness adventure. These adventures inspired me to elect a post-doc that involves research in Yellowstone National Park.

Another student support office that was pivotal for my progress was the Dean of Graduate Student's' Office. The two desks in the front of this office are occupied by **Icy Ma** and **Natalie Gilmore**, who pleasantly saw me through the piles of paperwork that marked almost every milestone on the path towards a Ph.D. The wonderful **Dr. Felicia Hunt** occupies one of the back offices. I cannot say enough about Felicia. Her job requires constant oversight and organizing of campus events, recruitment weekends, orientation, and graduate student quality of life concerns, although she always makes the time to meet one-on-one with those students who need assistance. She is an academic advisor, emotional counselor, and professional advocate all rolled into one. She fights for all students, particularly women, to have the opportunity to complete their Ph.D. and pursue a career in science. Felicia helped me overcome my most severe obstacles in graduate school and celebrate the tiniest accomplishments. She is still, carefully, but proudly, watching me approach the finish line.

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Graduate school and all its struggles have actually strengthened my relationship with my parents, **Martha** and **Michael Romano**, and their continued support has meant the

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Abstract

The work performed herein describes three proteins: Uracil DNA glycosylase (UDG) from *Archaeoglobus fulgidus*, MutY, and Endonuclease III (EndoIII) from *Escherichia coli*. They are DNA repair glycosylases that contain [4Fe-4S] clusters. While the catalytic mechanisms of many BER enzymes have been studied in detail, questions remain about how these enzymes search the vast amount of cellular DNA to find their substrates, and why some require a [4Fe-4S] cluster. The iron-sulfur cluster is not necessary for catalysis, and it only displays a physiologically relevant midpoint potential when bound to DNA. We have proposed that UDG, MutY, and EndoIII use their [4Fe-4S] clusters to participate in DNA-mediated charge transport (CT), and that these proteins mediate long-range electrochemical signaling in order to detect DNA damage.

This scheme for DNA damage detection assumes that CT occurs efficiently between the DNA helix and the [4Fe-4S] cluster of the bound protein. In order for efficient CT to occur, a pathway of amino acids must be present that facilitates CT between the DNA and the iron-sulfur cluster. For each of the enzymes mentioned, this pathway was explored through mutagenesis. In UDG, MutY, and EndoIII, several amino acids thought to be important for CT were mutated and the resulting proteins were characterized biochemically. Their CT capabilities were analyzed by cyclic voltammetry on DNA-modified electrodes. In these experiments, the mutants' signal intensities were quantified and compared to those of wild-type enzyme. An attenuated signal relative to wild-type protein may indicate that the mutant is deficient in CT and that the targeted amino acid is part of the protein-DNA CT pathway in the native enzyme. Many mutants were also

screened by enzymatic assays and circular dichroism spectroscopy to further characterize their DNA-binding properties and structural stability.

The *A. fulgidus* UDG mutants examined, C17H, C85S, and C101S, all contained mutations in the cysteine residues that ligate the [4Fe-4S] cluster. These mutants were designed to determine how the iron-sulfur cluster coordination environment affects protein-DNA CT. The mutants exhibited varying signal strengths relative to WT UDG on DNA-modified electrodes. C85S produced a weaker signal, indicating a CT deficiency. The signal intensity from C101S was within error of that of WT, and the signal from C17H was larger than that of WT, possibly indicating that this mutant is less structurally stable than WT UDG.

In *E. coli* MutY, position Y82 aligns with Y165 in MUTYH, a residue in which mutations have been found in many colorectal cancer patients. To better understand the correlation between protein-DNA CT and colorectal cancer, the MutY mutants Y82C and Y82L were prepared and characterized. Y82C exhibited a CT deficiency relative to WT MutY, whereas Y82L did not. These data indicate that Y82 forms part of the CT pathway in native *E. coli* MutY, but that other long-chain amino acids, such as leucine, can also mediate CT efficiently at this position.

Several different mutants of *E. coli* EndoIII were examined. First, the Y82 position was targeted, since the aligning MUTYH residue has been found mutated in colorectal cancer patients and because this residue is located near the protein-DNA interface. Five mutations were made at or near the Y82 position, and their cyclic voltammetry signals demonstrated that aromatic amino acids best mediate CT at this position. Other residues towards the interior of the protein, Y75, Y55, and F30 were also mutated to alanines.

These mutants exhibited CT deficiencies, implicating the residues as part of a potential CT pathway. Residues W178 and Y185, located near the [4Fe-4S] cluster of EndoIII, were also mutated to alanines. The resulting mutants produced larger signals than that of WT EndoIII. These mutants were later shown by circular dichroism spectroscopy to be less stable structurally than WT EndoIII. All of the mutants mentioned exhibited enzymatic properties similar to those of WT, suggesting that they are able to bind DNA and excise damage nucleobases as well as the native enzyme. Several of these mutants were also used in a mutagenesis-based experiment to assay how EndoIII variants help MutY search for DNA lesions, although data from these experiments showed no significant differences in mutation rate between strains expressing different EndoIII variants.

In total, the mutagenesis studies performed here helped determine the characteristics of BER enzymes that enable them to mediate DNA-protein CT. All these enzymes must contain a stable, well-protected metallocluster that charge can access through a series of CT-facilitating amino acids. In discovering several residues important for protein-DNA CT in UDG, MutY, and EndoIII, we have strengthened support for the hypothesis that these enzymes facilitate DNA-mediated CT *in vivo*. These enzymes may in fact be part of a much larger array of redox-active DNA-binding proteins that communicate electrochemically to help each other detect and repair DNA lesions inside the cell.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Appendix II

Appendix III