Chapter 1

# [4Fe-4S] Cluster Base Excision Repair Glycosylases

## Introduction

Cells constantly undergo an onslaught of potentially mutagenic processes that include exposure to ultraviolet light, reactive oxygen species, and intracellular alkylating agents [1–3]. If cells cannot detect and repair the DNA damage that these processes create, then the genomic mutations that ensue could have a profound effect on the cells' ability to faithfully replicate and survive [4]. Consequently, most organisms have evolved a stunning array of enzymes that detect and repair damaged DNA [3], including the *E. coli* glycosylases endonuclease III (EndoIII) and MutY and *Archaeaglobus fulgidus* Uracil DNA glycosylase (UDG) (Figure 1.1). These enzymes are part of the base excision repair (BER) pathway, meaning that they repair damaged DNA by cleaving the *N*-glycosidic bond of a damaged nucleotide and excising it from the helix [5–8]. Endonuclease III repairs damaged pyrimidines [9, 10], MutY removes adenine from mismatches between adenine and 7,8-dihydro-8-oxo-2'-deoxyguanosine (8–oxo-G) [8, 11, 12], and UDG excises uracil present in DNA [13]. The molecular mechanisms by which these proteins excise their substrates are fairly well-understood, but less attention has been devoted to understanding how BER glycosylases detect DNA damage in the first place [2].

We have proposed that BER enzymes may detect DNA damage through DNA-mediated charge transfer [14, 15]. The base pair π-stack of duplex DNA is capable of mediating charge transport (CT) through distances of over 200 Å [16–18]. Importantly, alterations in the π-stack, such as those produced by DNA

lesions, can disrupt the flow of CT through the helix [19–22]. BER enzymes may sense these CT disruptions, and are thus alerted to the presence of a DNA lesion. In this model for lesion detection, the [4Fe-4S] clusters of BER enzymes are crucial to their *in vivo* function because they are the redox-active moieties that mediate CT interactions with the DNA helix. Several lines of evidence suggest that the [4Fe-4S] clusters of BER enzymes function as CT mediators. The clusters do not catalyze the base excision reaction [5], and they are not readily oxidized or reduced within a physiologically relevant range of potentials [9]. This apparent lack of activity has led some authors to suggest that the [4Fe-4S] clusters have a structural role [5, 23, 24]. However, there is some biochemical evidence that MutY can fold properly without the [4Fe-4S] cluster [25]. Furthermore, physiologically relevant midpoint potentials can be measured when the proteins are bound to DNA [14, 26], arguing that the iron-sulfur clusters confer redox capabilities rather than structural stability. These data have led to the development of the following model for DNA damage detection: 1) The [4Fe-4S] cluster of a BER enzyme typically resides in the $2^+$ state [9]. Oxidative stress activates the enzyme to the $3^+$ state, causing it to and bind DNA more tightly [27]. This oxidative stress may be funneled to the protein through DNA-mediated CT after having formed at guanine residues [28]. 2) The newly oxidized protein loses an electron that gets transmitted through the DNA helix and ultimately reduces a second, distally located DNA-bound repair protein. 3) Reduction reduces the DNA binding affinity of this second enzyme, which then dissociates from the DNA and re-binds to a different portion of the genome [27]. If part of the DNA helix is damaged, then this damage will impair CT in step 2 so that

the BER enzymes will remain bound to DNA and continue to search for the damaged nucleotide.

We have tested this model for DNA damage detection through DNA-mediated CT though a variety of experiments. These include *in vivo* assays of cooperativity between BER enzymes [29], AFM assays of whether BER enzymes redistribute onto mismatch-containing DNA strands [29], and experiments that detected an increase in DNA binding affinity upon oxidation of EndoIII [27]. However, one important component of this model remains to be examined: the mechanism by which charge is transported between the DNA helix and the metallocluster of the bound repair enzyme. In other charge-transfer active proteins, CT between redox active moieties often occurs through a pathway of amino acids that can act as "stepping stones" though which electrons or holes pass as they traverse the protein [30–33]. These CT-active residues can be discovered through mutagenesis experiments in which CT candidate residues are mutated, and the resulting protein sample is assayed for a deficiency in CT activity. Here, assays of electrochemical activity were performed on site-directed mutants of three different redox-active BER glycosylases: Uracil DNA Glycosylase (UDG), MutY, and Endonuclease III (EndoIII). These individual proteins are described in detail below.

**Uracil DNA Glycosylase**

Uracil DNA Glycosylases (UDG) remove uracil and related compounds from DNA [2], (Figure 1.2). Uracil can be misincorporated into DNA by synthesis

enzymes [34]. However, more commonly, it is formed by hydrolytic deamination of cytosine [2]. If not repaired, G:U base pairs will result in G:C → A:T transversion mutations [35]. Indeed, *E. coli* cells in which UDG has been inactivated show an increase in G:C → A:T transversion rates [35]. UDG enzymes are found in all three domains of life, although differences in substrate, structure, and active site residues have caused them to be divided into six different families [36]. Family I, II, and III UDG enzymes are found in eukaryotes and some bacteria [36]. UDG proteins from families IV, V, and VI have, thus far, only been isolated from extremophilic organisms. They are distinguished from other UDG families in that they contain a [4Fe-4S] cluster. Given its distance from the putative substrate binding site, this [4Fe-4S] cluster is unlikely to be catalytic in UDG. We have proposed that extremophilic UDG enzymes use their [4Fe-4S] clusters to participate in electrochemical long-range signaling for DNA damage detection. The ability to detect lesions electrochemically may prove particularly useful for extremophilic organisms because rates of cytosine deamination increase with temperature [37]. Many family IV, V, and VI UDGs have been isolated from thermophilic prokaryotes [38–40]. In order to better understand the role of the [4Fe-4S] clusters in thermophilic UDGs, C17H, C85S, and C101S mutants of *Archaeoglobus fulgidus* UDG were prepared. The mutations target residues that ligate the [4Fe-4S] cluster. *A. fulgidus* is a thermophilic archaeon, and the mutants that are examined here were tested to determine how changes in the iron-sulfur cluster coordination environment affect the charge-transfer properties of the protein.

**MutY**

MutY homologues are found in all three domains of life and are part of a repair system that helps cells respond to oxidative stress. When excess reactive oxygen species form *in vivo*, these species can damage DNA, particularly at guanine residues since guanine has the lowest oxidation potential of all the nucleobases [41, 42]. When guanine is oxidized, it forms 7,8-dihydro-8-oxoguanine (8-oxo-G, Figure 1.2) [43]. Excess intracellular 8-oxo-G is removed by the enzyme MutT [11]. If it gets incorporated into DNA, it is excised by MutM [11, 12]. However, if 8-oxo-G remains misincorporated, then subsequent rounds of DNA replication will mistakenly pair an adenine molecule with it [44]. The next round of DNA replication will then place a thymine across from this adenine, resulting in a G:C → T:A transversion. MutY is the "final defense" against these transversions, as it removes adenine mispaired with 8-oxo-G [11, 44, 45]. The enzymes that repair 8-oxo-G-based lesions were first discovered by genetic mapping of cells with a strong G:C → T:A mutator phenotype. The resulting experiments identified the *mutY* locus as being among those responsible [45].

In human cells, MutY also plays an important role in maintaining genomic integrity. The human homologue of MutY, MUTYH, has recently drawn the attention of colorectal cancer researchers. Patients with mutations in MUTYH are predisposed towards developing colorectal cancer [47]. These patients tend to acquire mutations in the Adenomatous Polyposis Coli (APC) gene, which governs the proliferation of colonic cells [47, 48]. If APC gene mutations accumulate, and MUTYH is unable to detect and repair them, then colorectal tissue may become cancerous. Several different cancer-relevant MUTYH mutations have been

discovered [47], and two of their *E. coli* counterparts Y82C and Y82L were prepared and examined for electrochemical activity herein.

**Endonuclease III**

Endonuclease III (EndoIII) acts upon a variety of substrates, excising several oxidative damage products of cytosine and thymine from the DNA backbone [2] (figure 2). Cells lacking EndoIII exhibit only a weak mutator phenotype, suggesting that many of its substrates may also be repaired by other enzymes. EndoIII contains a [4Fe-4S] cluster which, we argue, allows it to communicate electrochemically with other DNA-bound proteins such as MutY [14] and SoxR [49, 50] in order to detect DNA lesions. For the experiments discussed in this report, eleven mutants of EndoIII were prepared targeting residues suspected to form part of the CT pathway between the DNA and the [4Fe-4S] cluster in EndoIII. These mutants were then characterized biochemically to determine whether their CT capabilities were decreased relative to those of WT EndoIII. Because EndoIII bears structural and amino acid sequence homology to MUTYH, several of the EndoIII mutants examined are also relevant to colorectal cancer research.

In total, the research performed in this thesis uses three proteins as model systems to better understand the biological relevance of DNA-mediated charge transfer. For each of these three proteins, UDG, MutY, and EndoIII, several site-directed mutants were prepared with four questions in mind: 1) How do perturbations near the [4Fe-4S] cluster affect the CT properties of redox active DNA repair enzymes? 2) Which types of amino acid residues best mediate protein-

to-DNA CT? 3) What possible amino acid pathways may exist through which charge flows? 4) What are the biological consequences of having an impaired CT pathway? These questions were addressed though a variety of biochemical techniques including enzymatic activity assays, cyclic voltammetry on DNA-modified electrodes, circular dichroism spectroscopy, and *in vivo* assays of cooperativity between select BER proteins. In total, these experiments present how biological systems could exploit DNA CT *in vivo* in order to detect and repair damaged DNA.

**Figure 1.1: Crystal structures of the base excision repair proteins to be investigated**. Each contains a [4Fe-4S] cluster. EndoIII (*E. coli*), MutY (*B. stearothermophilus*), and UDG (*T. thermophilus*) structures were adapted from references [5, 23, 51, 52], respectively, and formatted in PyMol [53].

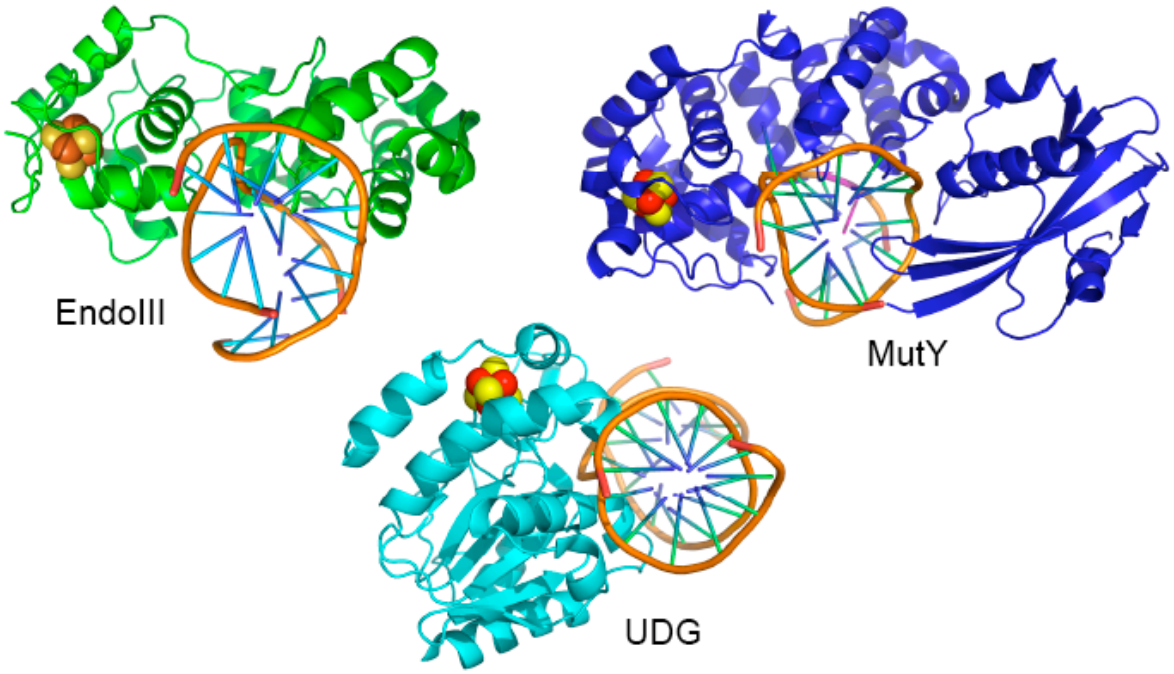
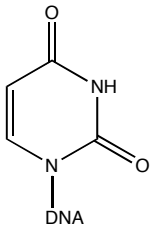
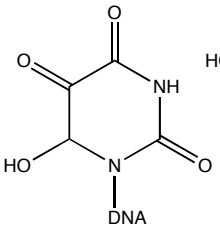



**Figure 1.2: Substrates of BER enzymes**. UDG excises uracil and related compounds from DNA, MutY excises adenine mispaired with 7,8-dihydro-8-oxoguanine, and EndoIII targets oxidized pyrimidines.

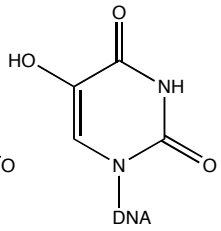
## UDG Substrates



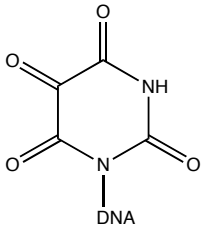

uracil




isodialuric acid

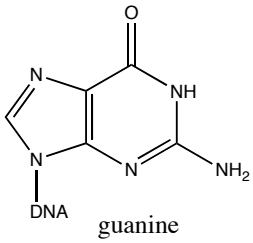



5-hydroxyuracil

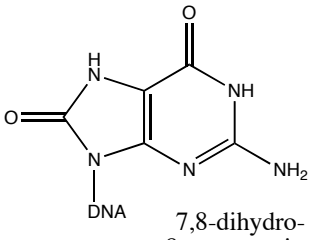



alloxan

## MutY Substrates

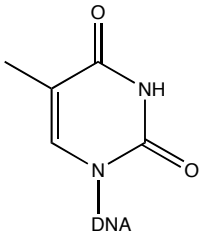



guanine

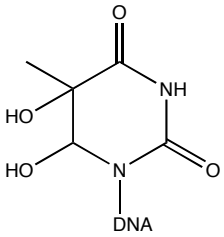



7,8-dihydro-
8-oxo-guanine

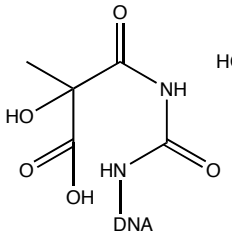
## EndoIII Substrates



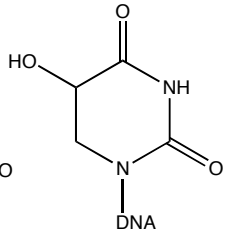

thymine



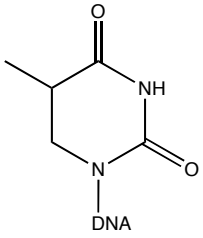

thymine glycol



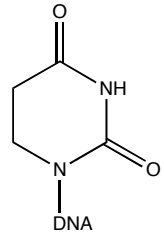

methyl tartonyl urea



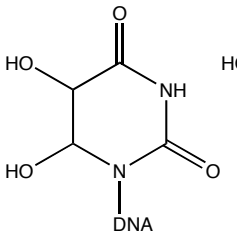

5-hydroxyuracil



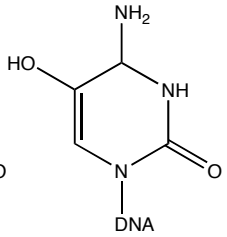

5,6-dihydro-
thymine




5,6-dihydro-
uracil




uracil glycol




5-hydroxycytidine

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