CHAPTER 9

# Summary and Perspective

The efficiency of DNA-mediated charge transport (CT) is influenced by the structure and dynamics of the base-pair π-stack. Specifically, the presence of mismatched or damaged bases in the duplex can dramatically hinder charge migration through the base-pair stack (*1, 2*). This property of DNA CT has been exploited to develop electrochemical devices for biosensing applications (*1*). In addition, we have proposed that the natural machinery for sensing DNA damage inside the cell, DNA repair enzymes, may employ DNA CT as an expedient method for the detection of lesions (*3*).

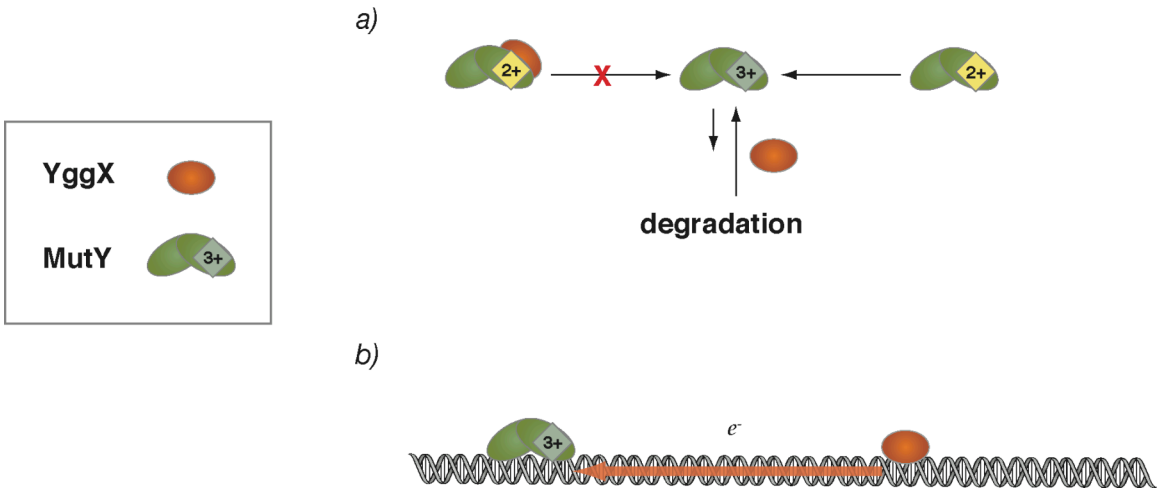
In exploring the role of DNA CT in DNA repair, we have focused on a class of base-excision repair (BER) glycosylases that contain [4Fe4S] clusters. In these enzymes, the function of the cluster is not well understood. Experimental methods relying on DNA-mediated CT reveal that the [4Fe4S] cluster in these enzymes is redox-active when the protein is DNA-bound (*3*). Indeed, DNA-mediated oxidation of these enzymes is a more favorable process and results in a more stable product than oxidation in the absence of DNA (*4*). A ~ 280 mV potential shift is observed for the 2+/3+ redox couple of the [4Fe4S] cluster upon DNA binding and this difference could translate into a differential DNA binding affinity for the oxidized and reduced forms of the enzyme. These experiments, and others (*5, 6*), demonstrate that methods employing DNA-mediated CT are a valuable tool when studying the properties of redox-active proteins that bind DNA.

Might redox activity in [4Fe4S] cluster BER glycosylases allow them to use DNA CT to search for damage? We have put forth a model describing how this process might happen (*3, 7*). A protein, the acceptor, in the $[4Fe4S]^{3+}$ state is bound to DNA. If this protein is surrounded by undamaged DNA, other [4Fe4S] cluster DNA-binding enzymes may bind nearby and reduce the acceptor protein in a DNA-mediated electron transfer reaction. If, instead, the acceptor protein is in the vicinity of damaged DNA, it is less

likely to be accessible for reduction *via* the π-stack of DNA. Thus the protein would remain bound near the lesion site. These reactions, when considered in the highly complex and dynamic environment present inside a cell, provide a viable explanation for how repair enzymes might swiftly locate damage. Furthermore, DNA-mediated CT as a damage detection mechanism also allows for the possibility of cooperative searching among different [4Fe4S] DNA repair enzymes, as long as they are of the appropriate redox potential.

MutY and Endonuclease III (EndoIII) are the most widespread [4Fe4S] cluster DNA repair enzymes; both are found in organisms ranging from *Escherichia coli* to humans and both repair oxidatively damaged bases in DNA (*8*). The recent discovery of [4Fe4S] clusters in several DNA repair enzymes from archaeal organisms may expand the scope of our model (*9, 10*). The discovery of a [4Fe4S] uracil DNA glycosylase (UDG) in a number of hyperthermophilic organisms, where the rate of uracil production in DNA is greatly enhanced, may indicate that the presence of an [4Fe4S] center could help fulfill a need for greater DNA repair in extreme environments. Perhaps the [4Fe4S] cluster could allow these UDGs to exploit a fast and efficient lesion detection mechanism based on DNA-mediated CT. An iron-sulfur cluster in an archaeal nucleotide excision repair (NER) helicase (XPD) is of great interest because sequence analyses suggest that it may be a universal cofactor present in helicases from a broad range of organisms. Though these helicases have a very different enzymatic function than [4Fe4S] BER glycosylases, they do face a common challenge in that they must locate lesions or forked structures in DNA and, subsequently, catalyze strand separation at these sites. As proposed for BER glycosylases, these helicases could use DNA-mediated CT to locate these unusual DNA structures cooperatively (Figure 9.1).

**Figure 9.1.** Proposed mechanisms of interaction between MutY and YggX. YggX may interact directly with MutY and prevent oxidation and degradation (*a*). Or YggX could bind DNA and transfer electrons to MutY in a DNA-mediated fashion (*b*).

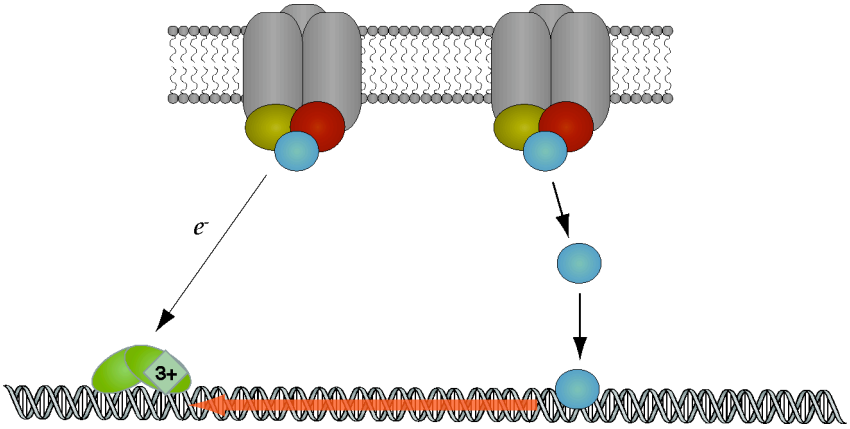


MutY and EndoIII are expressed as part of multigene operons in *E. coli* and other bacterial species (*11, 12*). MutY is expressed along with YggX, a protein proposed to be involved in iron trafficking and oxidative stress protection. The interaction between these proteins is currently uncharacterized, though there is some evidence that they might functionally interact inside the cell (*13*). YggX may serve as a signaling partner for MutY or as a protective or restorative element for the [4Fe4S] cluster in MutY (Figure 9.2). EndoIII is expressed as the final gene in an eight gene operon in *E. coli*. Several of the other genes in this operon are postulated to contain multiple iron-sulfur clusters, while others are predicted to be transmembrane proteins (*14*). These proteins have not been biochemically characterized in *E. coli* but are homologous to a set of genes, termed *rnf*, that are required for nitrogen fixation in *Rhodobacter capsulatus* and other diazotrophs. In *R. capsulatus*, the *rnf* gene products are proposed to form a membrane bound complex that may deliver reducing equivalents to nitrogenase or nitrogenase reductase. It is also postulated that they may be involved in iron-sulfur cluster maturation (*15*). It is possible that the *E. coli rnf* homologs may play a similar role, but instead, providing reducing equivalents to DNA-binding iron-sulfur cluster proteins. The *E. coli rnf* proteins might (either as the membrane-bound complex or in concert with soluble redox shuttle proteins) reduce EndoIII and other DNA-binding proteins (perhaps in a DNA-mediated fashion) or they could play a role in general iron-sulfur cluster assembly or repair (Figure 9.2). In fact, it has already been suggested that the *E. coli rnf* homologs may be involved in reduction of SoxR, a redox-sensitive transcription factor. In any case, further investigation of YggX and the *rnf* proteins in *E. coli* may allow us to learn more about the role of iron-sulfur clusters in DNA repair enzymes, since genes that are transcribed together as operons in prokaryotes often perform similar or related functions.

In the course of investigating the cooperative nature of DNA-mediated CT among DNA repair enzymes MutY and EndoIII we have discovered an EndoIII mutant (Y82A) that hinders the efficiency of DNA-mediated CT as measured at DNA-modified electrodes (*7*). This result could provide an important clue about the requirements for effective charge transport across a protein-DNA interface, a reaction that is not well understood. Experimental studies of DNA-mediated CT with small molecules indicate that strong coupling of the donor and acceptor entities into the base-pair stack is required for rapid and efficient reactions. The [4Fe4S] cluster in EndoIII is located relatively close (~ 15–20 Å) to the DNA (*16*) making it possible that protein-DNA CT could occur in a simple tunneling reaction (*17*). Many aspects of this reaction remain elusive, though. We do not know if the protein must be in a particular conformation for the reaction to occur or if there are amino acids residues that serve as intermediates in the CT process. We also do not understand which elements of the protein are required for coupling into the DNA $\pi$-stack or appropriate positioning of the protein relative to the DNA base-pair stack for efficient CT. Furthermore, it is not yet known if protein-DNA CT has universal features or if this reaction occurs by a different mechanism for different proteins. Our observation that Y82A EndoIII displays a diminished CT efficiency implies that aromatic residues may be important for effective protein-DNA CT, but further experimentation will be required to understand these reactions in full.

The human homolog of MutY has been recently implicated in inherited colorectal cancer (*18*). The mutational spectrum identified in cancer patients includes frameshift, truncating, splice-site, and missense variants. Of these, the over 50 different missense, or single amino acid, mutations that have been identified reveal some interesting

**Figure 9.2.** Proposed role for *rnf* gene products in *E. coli*. The membrane bound complex may deliver electrons to redox-active DNA-binding proteins. This could happen through a direct interaction between the complex and the DNA-binding protein (left) or *via* soluble shuttle proteins (right).



patterns. One of the most common disease-associated variants found is Y165C, which results in substitution of an intercalated tyrosine for a cysteine residue. It will be interesting to examine the redox properties of this variant. Does eradication of the intercalated tyrosine affect the efficiency of protein-DNA CT? Will introduction of a cysteine in its place result in increased DNA-protein crosslinking as a result of DNA CT? Another interesting pattern is the large number of mutations in positively charged residues that map to the DNA-binding interface. Many of these residues are mutated to bulky aromatic residues. While these mutations could result in disrupted protein structure, they might also result in misalignment of the protein for efficient protein-DNA CT, formation of inappropriate radical intermediates, or increased DNA-protein crosslinking. In general, investigation of cancer-associated MutY variants may offer an important opportunity for our laboratory to learn more about the relationship between the metal center in these enzymes and impaired function *in vivo*.

Clearly, base-excision repair by glycosylase enzymes within the complex environment of a cell is a complicated process and the first step of damage detection by these enzymes within the genome is not well understood. The importance of this step in the repair pathway is highlighted by the growing body of evidence indicating that damage detection is likely the rate-limiting step for repair inside the cell (*19*). Thus, a better understanding of lesion recognition by DNA repair enzymes will not only allow us to understand how nature solves an exceedingly complex molecular recognition problem but it could also inspire more creative and effective therapeutic solutions for the problems that arise when DNA repair goes awry.

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