Chapter 6

Summary and Perspectives

Decades of research have elucidated many aspects of the phenomenon of DNA charge transport (CT) (*1*). Both electrons and electron holes are efficiently transported through the DNA π-stack over long molecular distances of at least 100 base-pairs. Despite this shallow distance dependence, DNA CT is sensitive to mismatches or lesions that disrupt π-stacking and is critically dependent on proper electronic coupling of the donor and acceptor moieties into the base stack. The mechanism of electron transfer through DNA is best described as incoherent hopping, consisting of multiple superexchange steps between delocalized (approximately 3 base pair) domains of well-coupled stacked bases that are created through conformational dynamics. Favorable DNA CT is very rapid, occurring on the picosecond timescale. Because of this speed, electron holes equilibrate along the DNA π-stack, forming a characteristic pattern of DNA damage at low oxidation potential guanine multiplets.

More recently, research in the Barton laboratory has focused on how DNA CT may be used within a biological context. These studies began with the base excision repair glycosylase enzymes MutY and EndoIII, which contain 4Fe4S clusters whose function was not well understood. While relatively insensitive to oxidation or reduction in solution, experiments on DNA-modified electrodes demonstrated different redox behavior once these proteins bound DNA. Specifically, the $[4Fe4s]^{3+/2+}$ couple of EndoIII negatively shifts by 200 mV upon binding to DNA, bringing this couple into the physiologically relevant range (*2*). A thermodynamic consequence this reduction potential shift is a significantly higher

DNA-binding affinity in the oxidized, $[4Fe4S]^{3+}$ state, rather than the reduced, $[4Fe4S]^{2+}$ state.

Intriguingly, more 4Fe4S cluster DNA processing enzymes, such as the helicases XPD and DinG, have been discovered to have DNA-bound $[4Fe4s]^{3+/2+}$ reduction potentials similar to those of EndoIII and MutY, of approximately 80 mV versus NHE (*3*,*4*). These findings prompted a model whereby DNA processing enzymes with 4Fe4S clusters can perform DNA-mediated electron transfer (ET) self-exchange reactions with other 4Fe4S cluster proteins, even if the proteins are quite dissimilar (from different repair pathways and even different organisms), as long as the DNA-bound $[4Fe4S]^{3+/2+}$ redox potentials are conserved. This mechanism would allow low copy number DNA repair proteins to find their lesions efficiently within the cell. Support for this model began with *in vitro* evidence, such as spectroscopic studies indicating the feasibility of MutY oxidation by guanine radicals (*5*). Grodick *et al.* have now obtained compelling *in vivo* evidence with an InvA *E. coli* strain for the necessity of DNA-mediated communication between EndoIII and DinG in order to maintain genomic stability (*4*).

DNA CT may also be used biologically for the long-range, selective activation of redox-active transcription factors. SoxR is a bacterial transcription factor with a $[2Fe2S]^{2+/+}$ cluster that responds to superoxide stress. Lee *et al.* demonstrated that SoxR can become oxidized, and transcriptionally active, by irradiating a construct with a metallointercalating photooxidant tethered 80 base-pairs from the SoxR promoter binding site (*6*). Thus SoxR can be transcriptionally activated from a distance via oxidation though DNA CT. Work has

also focused on p53, a vital tumor suppressor that contains a network of redox-active cysteine residues that modulate the DNA-binding affinity of p53. Schaefer *et al.* have revealed that sequence selective oxidation of these cysteine residues via DNA CT and subsequent dissociation of p53 is a viable mechanism by which p53 decides cellular fates through selective binding to different promoter sites within the genome (*7*). Overall, current work is now focused on discovering other proteins that may utilize DNA CT within the cell, as well as further elucidating aspects of the DNA-mediated ET self-exchange reaction of 4Fe4S cluster proteins.

Towards this end, we have investigated whether the DNA-binding bacterial ferritin Dps, which is known to protect DNA from oxidative stress, can utilize DNA CT to protect the genome from a distance. Dps proteins are implicated in the survival and virulence of pathogenic bacteria; thus understanding the specific mechanism by which Dps proteins protect the bacterial genome could inform the development of new antibiotics. One aspect of Dps protection involves ferroxidase activity, whereby ferrous iron is bound and oxidized selectively by hydrogen peroxide, thereby preventing formation of damaging hydroxyl radicals via Fenton chemistry; another aspect of this protection is non-specific DNA-binding. We explored the possibility of protection from a distance via DNA CT by using an intercalating ruthenium photooxidant to generate oxidative DNA damage via the flash-quench technique, which localizes to a low potential guanine triplet in mixed sequence DNA. We find that Dps loaded with ferrous iron, in contrast to Apo-Dps and ferric iron-loaded Dps, which lack available reducing equivalents, significantly attenuates the yield of

oxidative DNA damage at the guanine triplet (*8*). These data demonstrate that ferrous iron-loaded Dps is selectively oxidized to fill guanine radical holes, thereby restoring the integrity of the DNA. Luminescence studies indicate no direct interaction between the ruthenium photooxidant and Dps, supporting the DNA-mediated oxidation of ferrous iron-loaded Dps. Thus DNA CT may be a mechanism by which Dps efficiently protects the genome of pathogenic bacteria from a distance.

Further work has focused on spectroscopic characterization of the DNA-mediated oxidation of ferrous iron-loaded Dps. We have found that, because of the difference in affinity at the di-iron ferroxidase site, only the higher affinity iron site is occupied under anaerobic conditions. X-band EPR was used to monitor the oxidation of DNA-bound Dps after DNA photooxidation via the flash-quench technique. Upon irradiation with poly(dGdC)$_2$, a signal arises with $g = 4.3$, consistent with the formation of mononuclear high-spin Fe(III) sites of low symmetry, the expected oxidation product of Dps with one iron bound at each ferroxidase site. When poly(dGdC)$_2$ is substituted with poly(dAdT)$_2$, the yield of Dps oxidation is decreased significantly, indicating that guanine radicals facilitate Dps oxidation. The more favorable oxidation of Dps by guanine radicals supports the feasibility of a long-distance protection mechanism via DNA CT where Dps is oxidized to fill guanine radical holes in the bacterial genome produced by reactive oxygen species.

We have also explored possible electron transfer intermediates in the DNA-mediated oxidation of ferrous iron-loaded Dps. Dps proteins contain a conserved tryptophan residue in close proximity to the ferroxidase site (W52 in *E. coli* Dps) that it located between the

iron site and the protein surface. In comparison to WT Dps, in EPR studies of the oxidation of ferrous iron-loaded Dps following DNA photooxidation, W52Y and W52A mutants were deficient in forming the characteristic EPR signal at $g = 4.3$, with a larger deficiency for W52A compared to W52Y. In addition to EPR, we also probed the role of W52 Dps in cells using a hydrogen peroxide survival assay. Cells with W52Y Dps survived the hydrogen peroxide challenge more similarly to those containing WT Dps, whereas cells with W52A Dps died off as quickly as cells without Dps. Combined, these results suggest the possibility of W52 as a CT hopping intermediate.

Overall, we have discovered that the DNA-binding bacterial ferritin Dps can protect the bacterial genome from a distance via DNA CT, perhaps contributing to pathogen survival and virulence. We have spectroscopically confirmed the oxidation of *E. coli* Dps following DNA photooxidation with the flash-quench technique, suggested the possibility of an ET hopping intermediate, and moved towards understanding the role of DNA CT with Dps in inside cells. More work is required to fully elucidate the roles of Dps *in vivo*.

DNA-modified electrodes have become an essential tool for the study of the redox chemistry of DNA processing enzymes with 4Fe4S clusters. In many cases, it is necessary to investigate different complex samples and substrates in parallel in order to elucidate this chemistry. Therefore, we optimized and characterized a multiplexed electrochemical platform with the 4Fe4S cluster base excision repair glycosylase Endonuclease III (EndoIII) (*9*). Closely packed DNA films, where the protein has limited surface accessibility, produce EndoIII electrochemical signals sensitive to an intervening mismatch, indicating a DNA-

mediated process. Multiplexed analysis allowed more robust characterization of the CT-deficient Y82A EndoIII mutant, as well as comparison of a new family of mutations altering the electrostatics surrounding the [4Fe-4S] cluster in an effort to shift the reduction potential of the cluster. While little change in the DNA-bound midpoint potential was found for this family of mutants, likely indicating the dominant effect of DNA-binding on establishing the protein redox potential, significant variations in the efficiency of DNA-mediated electron transfer were apparent. On the basis of the stability of these proteins, examined by circular dichroism, we proposed that the electron transfer pathway in EndoIII can be perturbed not only by the removal of aromatic residues but also through changes in solvation near the cluster.

Other work has also focused on elucidating important factors in the DNA-mediated ET self-exchange reaction of 4Fe4S cluster proteins. While a reduction potential shift upon DNA binding has been demonstrated with electrochemistry experiments, these studies do not provide direct molecular evidence for the species being observed. In contrast, sulfur K-edge X-ray absorbance spectroscopy (XAS) can be used to probe directly the covalency of iron-sulfur clusters, which is correlated to their reduction potential (*10*). We have shown that the Fe-S covalency of the 4Fe4S cluster of EndoIII increases upon DNA binding, stabilizing the oxidized $[4Fe4S]^{3+}$ cluster, consistent with a negative shift in reduction potential. The 7% increase in Fe-S covalency corresponds to an approximately 150 mV shift, remarkably similar to DNA electrochemistry results. Therefore we have obtained direct molecular evidence for the shift in 4Fe4S reduction potential of EndoIII upon DNA

binding, supporting the feasibility of our model whereby these proteins can utilize DNA CT to cooperate in order to efficiently find DNA lesions inside cells.

These studies contribute to the understanding of possible DNA-mediated protein oxidation within cells. More work is necessary in the future to characterize the kinetics of DNA-protein electron transfer. Previous work has been limited by the time resolution of a diffusing quencher. Time-resolved infrared (TRIR) studies with proteins containing IR-active ligands appended to the iron-sulfur cluster may provide detailed kinetic information at fast timescales (picoseconds). Furthermore, research is ongoing regarding how other DNA processing enzymes with 4Fe4S clusters, such as primase and DNA polymerase, may use DNA CT in a cellular context. Finally, new families of proteins that may utilize this chemistry await discovery. For example, the MarR family of transcriptional repressors dissociate from DNA upon the oxidation of redox-active cysteine residues, allowing expression of various virulence factors and antibiotic resistance genes. These small proteins respond to a diverse array of structures under physiological conditions, prompting the question of secondary signals (*11*). Could these secondary signals be long distance oxidation via DNA CT?

References

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