

CHAPTER 1

Biological Contexts for DNA Charge Transport Chemistry

Adapted from Merino, E. J., Boal, A. K., and Barton, J. K. (2008) *Current Opinion in Chemical Biology* (*in press*), and Boal, A. K., Yavin, E., and Barton, J. K. (2007) *Journal of Inorganic Biochemistry* 101, 1913.

INTRODUCTION

The double helical structure adopted by B-form DNA, where a negatively charged sugar phosphate backbone surrounds a π -stacked array of heterocyclic aromatic base-pairs, allows it to serve as an efficient medium for long-range charge transport (CT) (1). This chemistry has now been well established as a property of DNA. DNA CT can be rapid and it can occur over long molecular distances if the reaction is initiated by oxidants or reductants that are intercalated or otherwise well coupled into the base-pair stack. The observation that even very subtle changes to the structure of the base-pair stack, for instance, the presence of a single mismatched or damaged base, can drastically attenuate the efficiency of DNA-mediated CT further highlights the importance of the DNA base pair π -stack in these reactions. While many features of DNA CT under a variety of experimental conditions have now been elucidated, the role of DNA CT in biological processes requires more consideration.

DNA Damage over Long Range

It was first shown that DNA CT can promote damage to DNA from a distance in a DNA assembly containing a tethered rhodium intercalator, a potent photooxidant, spatially separated from two low energy guanine doublets (2). Guanines are the bases that are most easily oxidized in DNA, and the 5'-Gs of guanine doublets have a particularly low oxidation potential (3, 4). Since then, long range oxidative DNA damage has been extensively characterized using a variety of photooxidants. It has become clear that electron holes, oxidizing equivalents injected into the DNA through a host of damaging agents, formed at any site along the DNA duplex will migrate to low energy guanine sites. The distance range over which holes can migrate and whether guanine

radicals, once generated, provide a chemical signal for oxidative stress throughout the genome via DNA-mediated CT are questions that need to be addressed (Figure 1.1).

This long range migration of charge was explored in DNA oligonucleotides of defined length and sequence using covalently tethered photooxidants as initiators of oxidative damage. With $[\text{Rh}(\phi)_2\text{bpy}]^{3+}$ ($\phi = 9,10\text{-phenanthrenequinone diimine}$) as the photooxidant, guanine doublet sites throughout the duplex show intense levels of damage even when the oxidant is located 200 Å away (5). CT over similar distances has also been observed with other photooxidants (6). Longer duplexes have not been systematically examined, but, given the very shallow distance dependences observed thus far, efficient DNA CT over greater distance regimes is likely possible. Recently, in a Rh-tethered assembly containing an extended adenine tract, the distance dependence of DNA CT was shown to be essentially flat, with no change in damage over 5 nm (7) (Figure 1.2). Therefore, holes can migrate over long molecular distances to form permanent DNA lesions far from the oxidant binding site. In all of these experiments, strong damage is observed at the 5'-G of GG sites. Thus, this damage pattern has become the hallmark of one electron oxidative damage arising through DNA CT.

While DNA CT proceeds over long distances, the reaction is exquisitely sensitive to mismatches, base lesions, and other perturbations to the DNA base pair stack (7). This was evident first in the finding that DNA bulges can interfere with long range oxidative damage. Intervening mismatches, particularly those where local stacking is highly perturbed, also attenuate long range oxidative damage. Thus, while DNA CT can occur over remarkably long distances, it is a reaction that is modulated by the intervening sequence-dependent structure and dynamics of DNA.

Figure 1.1. DNA charge transport (CT) in a biological environment. DNA CT could play a role in many cellular processes ranging from funneling oxidative DNA damage to regulatory or noncoding regions in the mitochondrion and nucleus to mediating protein signaling in DNA repair and transcriptional regulation pathways.

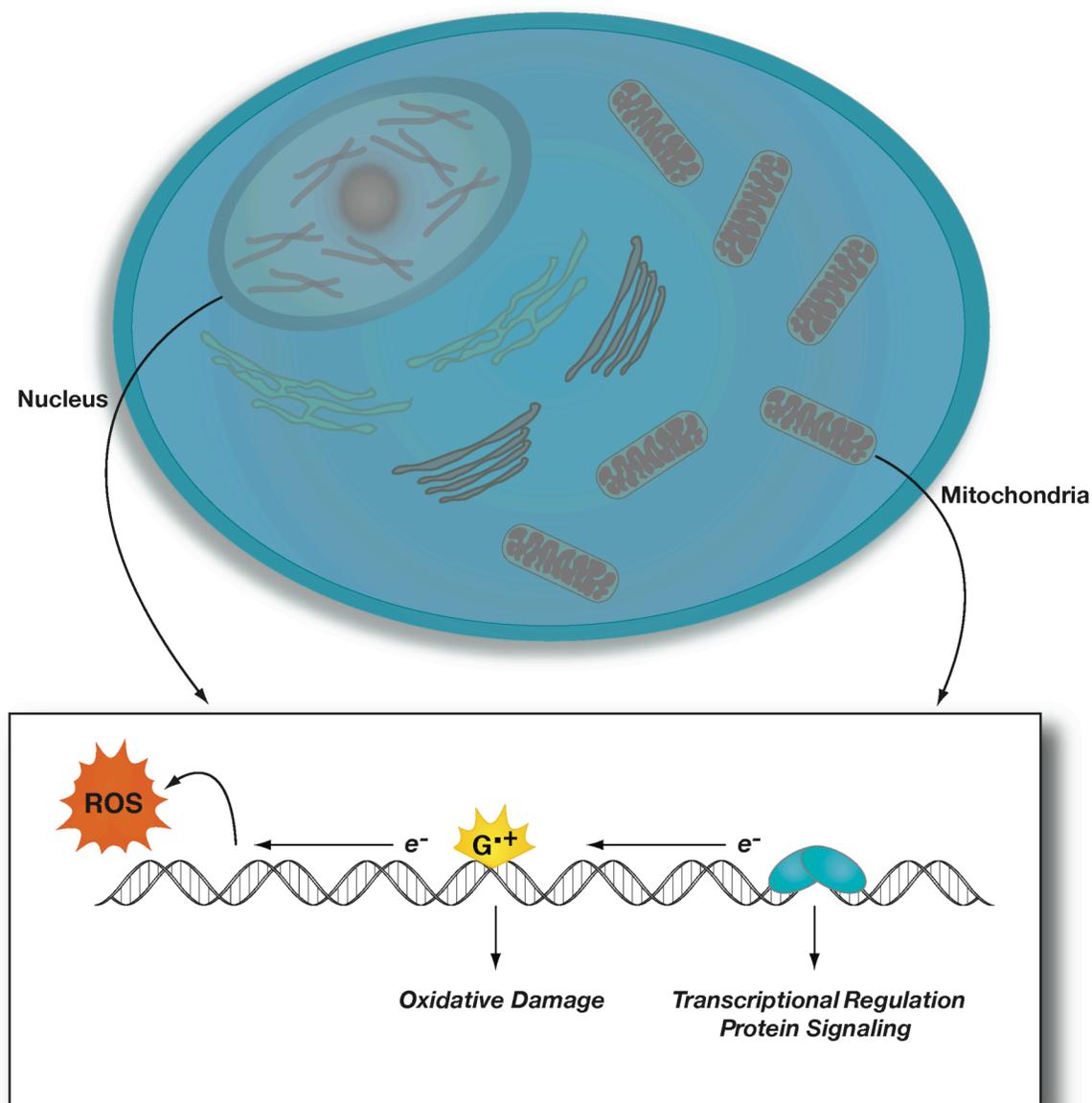


Figure 1.2. DNA CT in DNA damage. Upon irradiation, the intercalating Rh-oxidant accepts an electron (arrow) giving rise to an electron hole that is funneled to a low oxidation potential site, such as a guanine doublet, resulting in formation of a guanine radical (yellow). Guanine radicals can be quenched to generate oxidative DNA lesions.



Interestingly, fewer experiments have been carried out to explore electron transfer through DNA (8). DNA-mediated electrochemistry, involving ground state DNA-mediated reductions, exhibits a very shallow distance dependence with a remarkable sensitivity to intervening mismatches and lesions (9, 10). Recent solution experiments, where electron and hole transfer are compared using the same DNA and photoactivated group demonstrate that electron transfers through DNA are similarly characterized by these two important features: (i) a shallow distance dependence and (ii) a sensitivity to perturbations in the base pair stack (11).

The constant assault on DNA by endogenous and exogenous oxidizing agents often leads to covalent modification of DNA, and due to DNA-mediated CT, these modifications may not necessarily arise at the site of first collision (12). Oxidative reactions in DNA have important implications for mutation and subsequent pathogenesis inside cells. The most common biological oxidant, iron, undergoes Fenton chemistry to produce hydroxyl radicals and other species that can readily react with the DNA bases. Additionally, radicals generated on the sugar-phosphate backbone can lead to hole formation on the DNA bases (13). Thus, once a hole is produced in double stranded DNA, DNA CT can funnel the hole to low oxidation potential sites, where the hole reacts irreversibly with O₂ and H₂O. Oxidative reactions of DNA bases with O₂ and H₂O leads to mutagenic DNA lesions (12, 14). Further oxidation of DNA base lesions yields products that bypass the repair machinery and exacerbate DNA damage.

Funneling Oxidative Damage to Specific DNA Regions

The involvement of DNA CT in promoting the formation of oxidative lesions suggests that DNA damage products may not be uniformly distributed within a genome but may instead be funneled to specific sites. This hypothesis is supported by analysis

of genomic DNA showing that introns and exons contain differential amounts of low oxidation potential sites (15). Further examination of eight eukaryotic genomes illustrates that DNA CT may drive the ultimate distribution of oxidative DNA lesions (16). For instance, exons contain a 50-fold decrease in oxidation prone guanine. Therefore protection of protein coding regions from DNA lesions may be due to their lack of low oxidation potential sites such that DNA CT can funnel damage out of the exons and into introns. Telomeres, the ends of chromosomes, also represent hot spots for DNA damage as they are of particularly high guanine content. Moreover, the DNA telomeres may also adopt quadruplex structures, and it has been shown that holes are preferentially shuttled to guanines within these structures (17, 18).

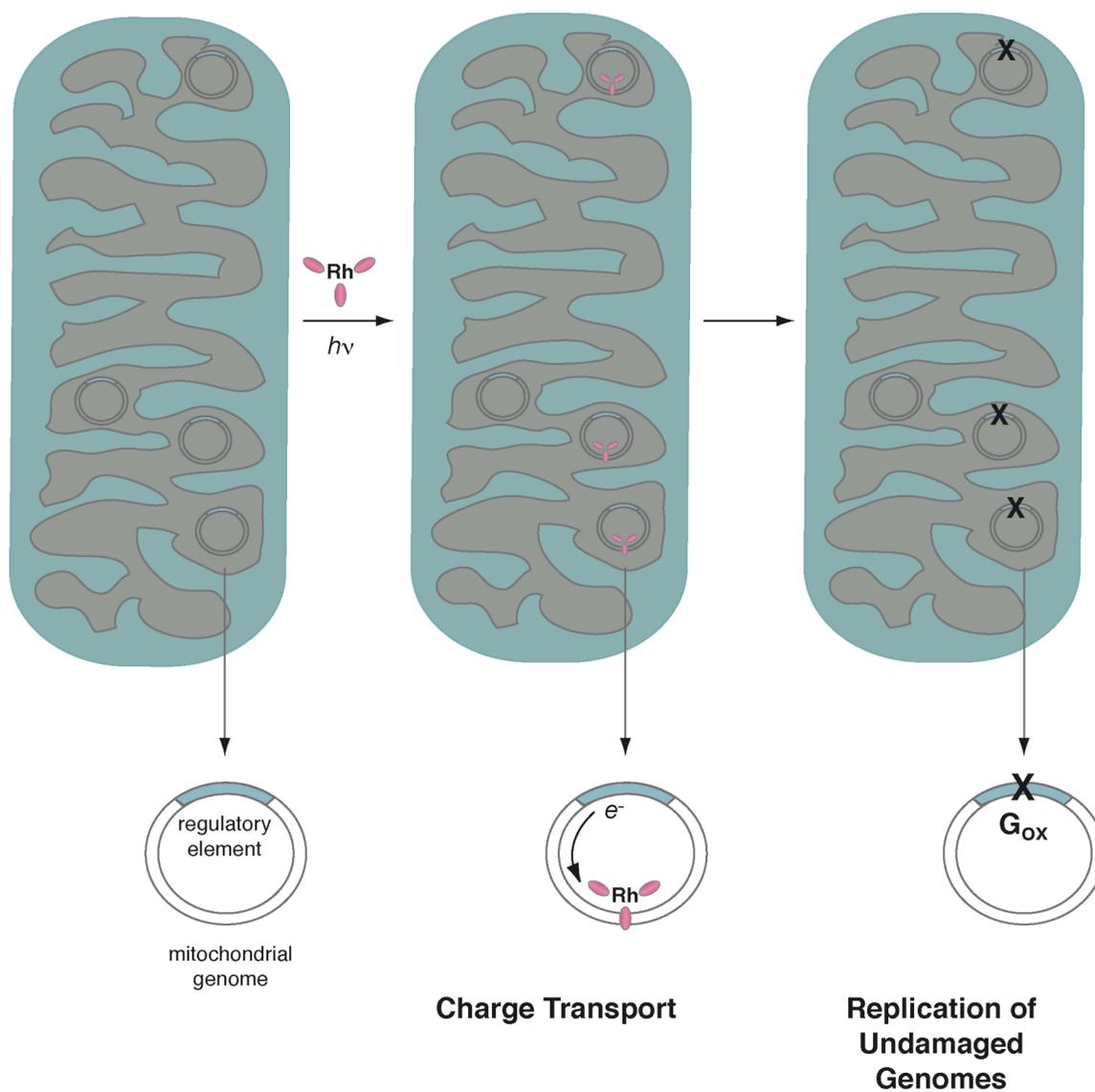
Whether DNA CT is important in funneling damage to discrete locations could be resolved by determining the location of oxidative lesions in a genome. Visualizing the sequence details of oxidative damage on a genome is difficult, however, due to their size and low copy number within the cell. Most methods only interrogate the total level of damaged DNA adducts by mass spectrometry as well as a variety of other techniques but do not yield the location in the sequence of the lesions produced. Ligation-mediated PCR has, however, been utilized to determine the sequence details of oxidative damage in DNA genomes (19). DNA CT was shown to occur in isolated nuclei from HeLa cells using ligation-mediated PCR in conjunction with $[\text{Rh}(\text{phi})_2\text{bpy}]^{3+}$; the complex binds to DNA without sequence specificity, and upon photoactivation, either promotes strand breaks directly at the oxidant site or induces one electron oxidative damage (20). The pattern of oxidative lesions reveals hallmarks of DNA CT, with damage occurring predominately at guanine-rich low oxidation potential sites, the 5'-G of guanine doublets and triplets. Moreover, the results showed that while oxidative damage was found

preferentially at guanine doublets, the rhodium photooxidant was bound primarily at distant sites. Hence, the damage must have occurred through DNA-mediated CT. This work established that CT can occur in DNA within the nucleus.

Another biologically important target for oxidative stress is the mitochondrion. Mitochondria contain their own DNA and also harbor an abundance of reactive oxygen species as a result of their function in oxidative phosphorylation (21). Mutations in mitochondrial DNA have been found in a variety of tumors and are associated with other diseases, while other DNA perturbations, like large scale rearrangements, are common in mitochondrial DNA (21). Oxidative damage to extracted mitochondrial DNA (22), as well as to mitochondrial DNA within functioning mitochondria (23), promoted by the rhodium photooxidant reveals that DNA lesions can arise from a distance using DNA CT. Again, this damage from a distance was demonstrated by comparing sites of Rh binding *versus* guanine oxidation. The spatial separation between the Rh binding sites and one electron guanine oxidation sites is striking; oxidation can occur more than 70 bases away from the nearest bound oxidant. Again these data support long range CT through DNA within a cellular organelle, here the mitochondrion (Figure 1.3).

Some interesting biological consequences of DNA CT emerged from these studies. First, sites of base oxidation by DNA CT in mitochondrial DNA overlap with known mutational hot spots associated with cancers. The correlation between mutation frequency (24) and lesions produced suggests that DNA CT may be a major contributor to mitochondrial oxidative lesions *in vivo*. Secondly, one highly damaged position found is a regulatory element known as conserved sequence block II that is vital for DNA replication. Conserved sequence block II contains a seven guanosine repeat, the largest guanosine repeat on the mitochondrial genome. Positioning such a low oxidation

Figure 1.3. Funneling oxidative DNA damage *via* DNA CT in mitochondria. Each mitochondrion (blue/grey) harbors several mitochondrial genomes. Replication is regulated through a critical regulatory element termed conserved sequence block II (cyan). Upon irradiation with a Rh photooxidant, CT funnels damage to the regulatory element. Oxidation of the regulatory element could decrease the ability of oxidized genomes to be copied, thereby favoring replication of undamaged genomes.



potential site as a regulatory element can be advantageous since each mitochondrion contains many copies of its genome. Funneling damage to a regulatory element, via DNA CT, could decrease the likelihood that damaged mitochondrial genomes will be replicated by the formation of an oxidative lesion that perturbs the replication machinery. These lesions might signal the level of damage in a particular genome, thus DNA CT may thus provide a protection mechanism to exclude damaged DNA from the replication cycle in mitochondria.

Long Range CT in the Presence of DNA-bound Proteins

Since it is apparent that DNA-mediated CT can take place in the crowded environment of a cell, it becomes important to ask systematically what are the effects of DNA-binding proteins on DNA CT? Moreover, within many organisms, DNA is packaged into chromatin or chromatin-like higher order structures via interactions with histone proteins. How does the nucleosome structure, containing DNA-bound histones, affect DNA CT?

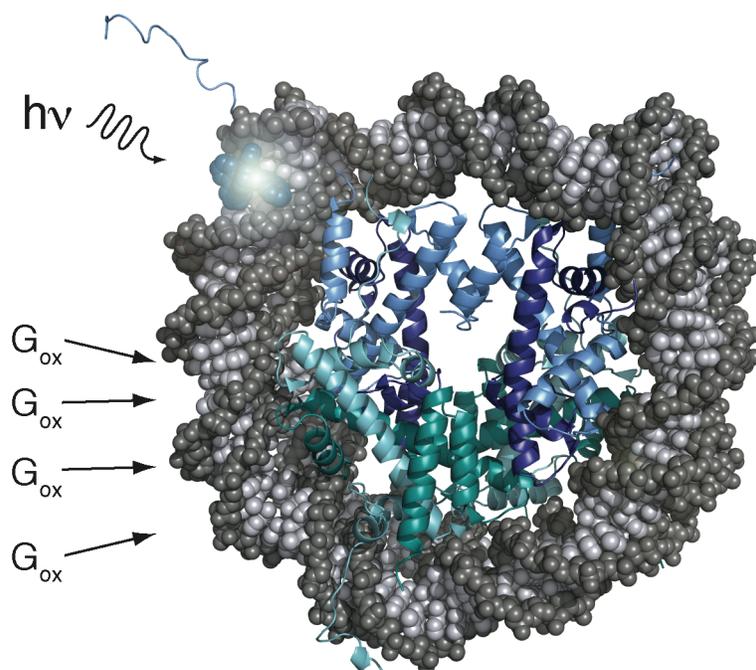
Several studies of DNA CT in the presence of specific DNA-binding proteins have been carried out. Experiments to monitor CT through the DNA base pair stack is unaltered when a protein, such as a helix-turn helix protein, is bound in such a way that it induces little structural change in the DNA (25). Proteins that perturb the structure of DNA, however, have a profound effect on the yield of CT (26). Uracil DNA glycosylase, a DNA repair enzyme that flips uracil residues out of the base-pair stack, does not allow CT to proceed beyond the protein binding site. TATA-binding protein, a transcription factor that kinks the DNA helix by > 90 degrees, also diminishes CT efficiency to guanine doublets. This sensitivity of DNA-mediated CT to protein binding has actually led to the

application of DNA electrochemistry as a sensitive probe for DNA binding by base-flipping proteins as well as proteins like TATA-binding protein (26, 27).

In studies of long range oxidation, DNA-binding proteins have also been found to tune the oxidation potential of possible damage sites in DNA. For example, the restriction enzyme BamHI, which binds the DNA sequence 5'-GGATCC-3' inhibits damage at the guanine doublet located within its binding site (28). BamHI makes extensive hydrogen bonding contacts to the guanines in its restriction site and these interactions are proposed to change the ionization potential, making the guanines less susceptible to oxidation. The mechanisms that proteins employ to perturb DNA CT, structural alteration of the π -stack or modification of the electronic properties of specific bases, are interesting to consider in a biological context. One could imagine DNA-binding proteins, through a specific interaction, could insulate a particular sequence or a region of the genome, disallowing the propagation of DNA CT. Whether such protection is actually utilized within the cell has not yet been established.

A question of significant interest has been whether DNA CT can proceed within the nucleosome core particle (Figure 1.4). Experiments were first carried out on DNA using the intercalating photooxidant, $[\text{Rh}(\text{phi})_2\text{bpy}]^{3+}$, in the presence and absence of bound histones (29). The 146 base pair DNA sequence employed in these studies was the same utilized for the crystal structure determination of the nucleosome core particle (30), which had a distinct kink in the DNA at its center in order to obtain consistent phasing of the DNA bound in the nucleosomes. We observed damage at all of the 5'-Gs of guanine doublets between the Rh, bound at the DNA terminus, and this central kink, both in the absence and presence of the histone proteins. Thus it appears that even

Figure 1.4. DNA CT in a nucleosome core particle. Photoactivation of a tethered Rh oxidant in histone-bound DNA generates oxidative damage at a distance in the nucleosome.



within the nucleosome, DNA CT may proceed. This long range CT within DNA in the nucleosome core particle was confirmed in similar experiments using tethered anthraquinone as the photooxidant (31). Some variations in relative intensities across the guanine doublets were observed for damage in the nucleosome versus that for the free DNA when comparing anthraquinone and the Rh intercalator. These variations may represent differences along the DNA in access to oxygen and water, required to make the irreversible damage products from the guanine radical, and possible tuning of local guanine oxidation potentials by the DNA-bound histones. Between Rh and anthraquinone as photooxidants, the small variations in guanine damage observed likely reflect differences in rates of back electron transfer for the two oxidants. Interestingly, anthraquinone-tethered nucleosomes were also recently utilized to show that DNA-protein crosslinking can result from long range DNA CT (32).

As indicated, DNA CT was found to occur in the mitochondrion, and here the DNA is also bound by its native suite of proteins (23). Mitochondrial DNA-protein interactions were found to be altered, perhaps also through crosslinking, as a result of oxidative damage arising via DNA CT. These results may resemble those seen in the nucleosome core particle. Importantly, in considering DNA being packaged in the nucleosome core particle, we generally consider that the DNA is being not only packaged but also protected from the assault of various damaging agents. Certainly these results show that within the nucleosome, the DNA is not protected from oxidative damage occurring *via* DNA-mediated CT.

Oxidation from a Distance of DNA-bound Proteins

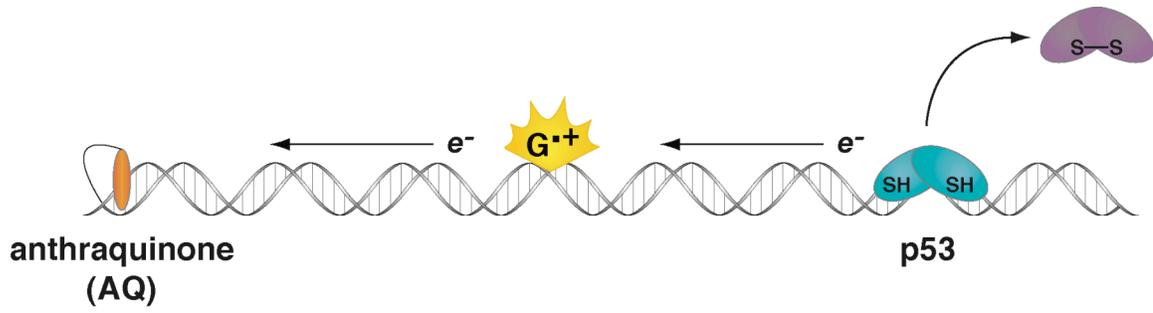
Not only can proteins serve to modulate DNA CT, DNA-binding proteins can also participate in reactions at a distance through DNA-mediated CT. DNA-binding proteins

contain a variety of functional motifs with oxidation potentials similar to or lower than that of guanine (33). Guanine radicals generated with a ruthenium photooxidant can be transferred to aromatic amino acid side chains (tyrosine and tryptophan) present in positively charged peptides (Lys-Tyr-Lys and Lys-Trp-Lys) (34). Photolyase, an enzyme that uses CT to repair thymine dimer lesions in DNA, contains a flavin cofactor that can also be oxidized and reduced via the DNA π -stack when probed electrochemically on DNA-modified electrodes (35). Additionally, appropriately positioned thiols incorporated into the sugar-phosphate backbone can be oxidized in a DNA-mediated reaction (36).

Similarly, many DNA-binding proteins contain cysteine residues that are redox-active, and these too may be oxidized at a distance through DNA CT (37). One example is p53, a redox-modulated transcription factor that contains ten conserved cysteine residues in its DNA-binding domain (38). We prepared a DNA assembly containing a pendant photooxidant, and the consensus sequence for binding p53 (37). As illustrated in Figure 1.5, we observe that photoactivation of the anthraquinone promotes oxidative dissociation of p53 from the DNA. The presence of an intervening mismatch, moreover, inhibits this DNA-mediated reaction. Analysis of the p53 crystal structure reveals several candidates for thiol oxidation close to the DNA, and mass spectrometry of trypsin digests of p53 after photolysis is consistent with disulfide bond formation in the DNA-bound protein. Hence DNA-bound p53 can be oxidized from a distance and induced to dissociate from its target site from a distance through DNA-mediated CT.

The oxidation of p53 through DNA CT was also probed within the cellular environment. Human HCT cells were treated with the Rh photooxidant and irradiated to generate high levels of guanine radicals. A new oxidized form of p53 was detected via western blot that could be reversed by addition of exogenous thiols, consistent with

Figure 1.5. DNA CT leads to the oxidative dissociation of p53 (a tetramer) from its promoter, triggered from a distance.



disulfide bond formation. In fact, the same oxidized p53 was produced upon addition of hydrogen peroxide. This oxidized p53 appears under conditions of oxidative stress.

The promoter sequences for p53 are diverse and can include those that control expression of important apoptotic or developmental genes. Biologically, p53 must distinguish between various promoters depending upon the cellular environment (39). Further investigation reveals that the DNA-mediated oxidation of p53 and subsequent dissociation is promoter specific (37). On a promoter involved in apoptosis, p21, p53 does not dissociate with photoactivation from a distance, although dissociation is observed on a promoter involved in DNA repair. We hypothesize that under high levels of oxidative stress, formation of guanine radicals via DNA CT occurs frequently, signaling that the DNA repair pathway is futile. When bound to DNA repair promoters, p53 oxidation followed by dissociation occurs, though p53 remains bound to promoters to activate cell cycle arrest under the high oxidative stress. Importantly, these results, taken together, provide a chemical rationale for the cellular response of p53 to oxidative stress through long range signaling using DNA-mediated CT.

The Possibility of DNA-mediated Signaling among Proteins

DNA repair proteins are another major class of DNA-binding proteins that could modulate or participate in DNA CT events. Given the well established sensitivity of DNA CT to a wide variety of damaged bases (10), it is interesting to consider that DNA repair proteins could harness CT to search DNA for damaged sites.

In base excision repair (BER), glycosylase enzymes are responsible for searching the genome for chemically modified bases and catalyzing their excision (40). These enzymes must first locate their substrate in a vast excess of undamaged DNA, flip the substrate into the active site of the protein, and catalyze scission of the N-glycosidic

bond between the errant base and the sugar-phosphate backbone. While much is known about the catalysis and substrate discrimination steps in this process, very little is known about the daunting initial search of the genome these enzymes must undertake. It has been demonstrated that many of these enzymes can move along the DNA helix in a processive manner (41, 42), but the *in vivo* relevance of this search mechanism as the primary mode of damage detection by DNA-binding proteins is disputed (43-45).

Many BER glycosylases contain a [4Fe4S] cluster (46-48), the function of which is unknown. Endonuclease III (EndoIII) was the first glycosylase discovered to contain this metal cofactor (46). EndoIII removes a wide variety of oxidized pyrimidines from DNA and contains the helix-hairpin-helix (HhH) recognition motif (49-57). MutY, structurally similar to EndoIII (56-59), is another BER glycosylase that contains a [4Fe4S] cluster (58). However, MutY instead removes adenine from 8-oxo-guanine:adenine mispairs (60-72).

The role of the [4Fe4S] cluster in these glycosylases is of great interest. Experiments were performed with EndoIII to determine the properties and function of the [4Fe4S] cluster (46, 73). Mossbauer and electron paramagnetic resonance (EPR) spectroscopy experiments confirmed that the protein contains the [4Fe4S]²⁺ cluster when the protein is not bound to DNA. The cluster was unable to be oxidized by ferricyanide without degradation to the [3Fe4S]¹⁺ species as observed by EPR at 4K. Photoreduction of EndoIII did give rise to the [4Fe4S]¹⁺ cluster but with an estimated reduction potential of less than -600 mV versus NHE. Since it appeared that stable oxidation of the [4Fe4S] cluster was not possible, nor was reduction feasible in a biological environment, the cofactor was relegated to a structural role.

The [4Fe4S] cluster was analogously assigned a structural role in MutY. However, the David laboratory has since performed several experiments to investigate

the role of the cluster in this protein (74-76). They have developed a method to remove reversibly the cluster from the protein and discovered that the cofactor is not necessary for protein folding nor does it contribute to the thermal stability of the protein.

Nonetheless, the [4Fe4S] cluster is necessary for DNA binding and enzyme activity. In addition, mutagenesis studies further highlight the necessity of the [4Fe4S] cluster for MutY repair. In these experiments, the cysteines that ligate the cluster are mutated to both coordinating (histidine and serine) and non-coordinating (alanine) residues leading to, in some cases, quite dramatic effects on the repair capacity of MutY.

Crystal structures are available for MutY and EndoIII both free and bound to DNA (56-59, 77, 78). These provide many clues about the environment of the cluster in both states. In each protein, the [4Fe4S] cluster is ligated by a unique cysteine motif (C-X₆-C-X₂-C-X₅-C). Some of these ligating residues are located in a loop termed the iron-sulfur cluster loop (FCL). This loop also contains many positively charged residues that interact with the DNA backbone. The overall structures of the free and DNA-bound proteins are similar (backbone RMSD = 1.3 – 2.1 Å); large conformational changes do not occur in the protein upon binding to DNA. In both MutY and EndoIII, the [4Fe4S] cluster is located ~ 13 Å from the nearest DNA backbone atom, and ~ 20 Å from both the center of the DNA helix and the glycosylase active site.

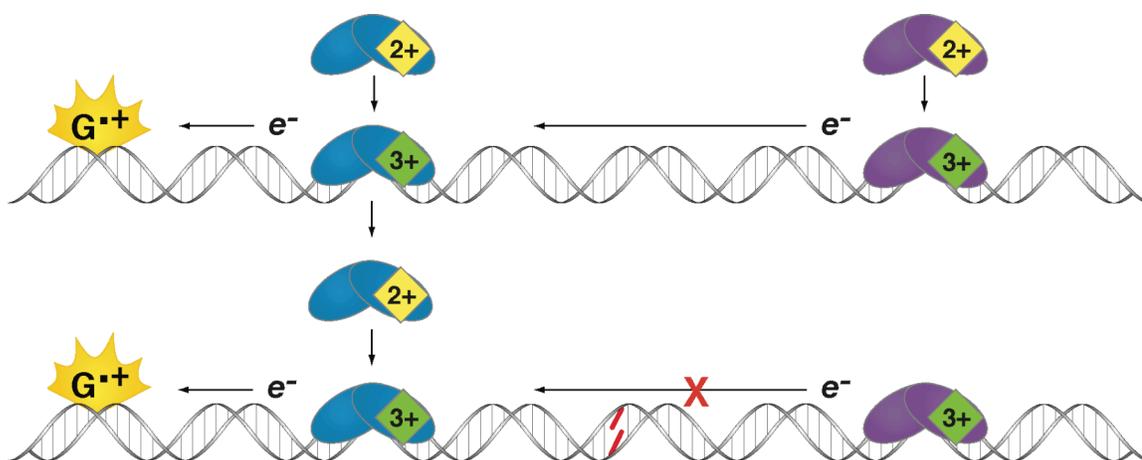
We have studied the DNA-mediated electron transfer properties of several repair proteins that contain [4Fe4S] clusters, a cofactor capable of being oxidized by guanine radicals (79, 80), using a variety of experimental techniques (81, 82). The electron lost upon oxidation of the [4Fe4S]²⁺ cluster can be trapped in DNA by a uridine base modified with a nitroxide spin label. The resulting nitroxide radical species is detected with EPR spectroscopy (81). Similarly, a guanine radical cation, generated with a ruthenium photooxidant and monitored spectroscopically or with gel electrophoresis, can be

quenched by MutY, resulting in formation of a $[4\text{Fe4S}]^{3+}$ cluster (82). Importantly, guanine radicals are the first products of oxidative DNA damage inside the cell, and these results indicate that base radicals could provide the driving force *in vivo* to activate DNA-mediated CT signaling among $[4\text{Fe4S}]$ BER glycosylases.

When investigated at DNA-modified electrodes, MutY and EndoIII are redox-active, displaying electrochemical signals with midpoint potentials (+50-100 mV versus NHE) typical of high-potential iron proteins, proteins that can adopt either the 2+ or 3+ cluster oxidation state (79, 80). These proteins exhibit dramatically smaller signals at electrodes containing an abasic site, indicating that CT to the $[4\text{Fe4S}]$ cluster is DNA-mediated and requires an intact π -stack. We have also electrochemically examined EndoIII in the absence of DNA at a graphite electrode (83). The signal associated with the 2+/3+ redox couple in this situation is much less reversible and has a much more positive potential (~ 280 mV positive shift) indicating that EndoIII is both less easily oxidized and more unstable in the $[4\text{Fe4S}]^{3+}$ form when the protein is not bound to DNA. Furthermore, the positive potential shift allows us to estimate that the protein containing the $[4\text{Fe4S}]^{3+}$ cluster binds DNA much more tightly than the reduced form of EndoIII; the difference in K_d when comparing the $[4\text{Fe4S}]^{2+}$ and $[4\text{Fe4S}]^{3+}$ forms of EndoIII is at least 3 orders of magnitude.

A new role for the $[4\text{Fe4S}]$ cluster in these glycosylase enzymes must now be considered. The presence of a redox-active $[4\text{Fe4S}]$ cluster could allow DNA repair proteins to use DNA-mediated CT as a way to search quickly and efficiently for damaged bases in DNA (79-83). Figure 1.6 illustrates a model for how this search process might transpire. Here we propose that DNA CT could help reduce the search problem faced by these enzymes, allowing glycosylases to rapidly eliminate a search through genomic regions devoid of lesions and instead spend most of their time bound in the vicinity of

Figure 1.6. A model for DNA CT in DNA repair. DNA-mediated redox activity in a class of DNA repair proteins that contain a [4Fe4S] cluster could allow these enzymes to use DNA CT as a damage detection strategy. Under conditions of oxidative stress, guanine radicals are generated and these can oxidize the [4Fe4S] cluster in the repair enzyme (top). A second protein, upon binding to DNA, becomes oxidized and transfers its lost electron, in a DNA-mediated CT reaction, to the first DNA-bound protein. The first protein becomes reduced, subsequently loses affinity for DNA, and binds elsewhere. If a lesion is present between the two proteins (bottom), the CT reaction occurs much less efficiently, thus the proteins remain in the oxidized state and bound near the lesion. As illustrated here, DNA CT therefore serves to redistribute DNA repair enzymes away from undamaged DNA and into the vicinity of lesion sites, facilitating fast and efficient damage detection.



damaged sites. Importantly, we have shown that guanine radicals can readily oxidize the [4Fe4S] cluster in these proteins, indicating that this event could trigger a DNA CT signaling cascade among these proteins initiating the search for lesions. Hence, DNA-mediated CT could play a simultaneous role in funneling DNA damage to sites of low oxidation potential and recruiting proteins to find and repair that damage.

It is interesting to note that other organisms also contain BER repair proteins with an iron-sulfur cofactor (47, 48). Most notably, in a set of thermophilic organisms, there are uracil DNA glycosylase (UDG) homologs (47) as well as XPD (a nucleotide excision repair helicase) homologs that contain iron-sulfur clusters. The presence of a [4Fe4S] cluster in a thermophilic UDG is especially noteworthy given that the primary process that leads to uracil in DNA, cytosine deamination, has an enhanced rate at high temperatures (84). Yet these organisms do not display a higher mutation rate (85). Might the presence of this cofactor help fulfill this greater requirement for repair? One of these, *Archaeoglobus fulgidus* UDG, has been evaluated at DNA-modified electrodes and also exhibits DNA-mediated redox activity with a midpoint potential of +95 mV versus NHE (80).

The recent discovery that mutations in the human gene for MutY (*MUTYH*) can cause predisposition to colorectal cancer (86) underscores the need to understand how repair enzymes effectively find and repair DNA damage. Over 50 different missense and in-frame deletion mutations in *MUTYH* have been observed in colorectal cancer patients. Two of the most common mutations implicated in *MUTYH*-associated polyposis (MAP), Y165C and G382D, involve highly conserved positions in the protein. In *E. coli* MutY, the corresponding mutations (Y82C and G253D) lead to modest decreases in substrate binding affinity and rate of excision (87). In addition, structural studies show that Y82 and G253 interact with the DNA near the 8-oxo-guanine lesion site (77, 78). It is likely

that Y82 and G253 are involved in substrate recognition, but it is still not completely understood how all of the mutations implicated in MAP give rise to cancer and it is clear that defects in the rate of excision and substrate binding affinity may not account for all of the deficiencies observed with these mutants *in vivo* (86, 87). Furthermore, an increasing body of evidence indicates that finding the lesion is likely the limiting step for effective BER inside the cell (89) and it is, therefore, of critical importance to understand all of the strategies employed by these proteins to detect damage.

Is the redox activity of the [4Fe-4S] cluster relevant *in vivo*? MutY and Endo III have similar redox potentials and could cooperatively search for damage using DNA CT inside the cell (80). In this instance, if Endo III were inactivated, a decrease in the *in vivo* activity of MutY should be observed. The CC104 *E. coli* strain, which uses a mutation in *lacZ* to report the frequency of G:C to T:A transversion mutations, is often used as an indicator of MutY activity *in vivo* (60, 90). When the Endo III gene (*nth*) is knocked out in the CC104 genetic background, a small increase in the G:C to T:A mutation rate is observed (91). While this observed effect appears at first to be attributed to overlapping substrate specificity with MutY, *in vitro* evidence to support this idea is lacking (55). Could this relationship instead have something to do with the iron-sulfur cofactor harbored by each protein?

SUMMARY

Guanine radicals are one the first signals of oxidative stress inside a cell and DNA CT could provide a mechanism to disseminate these radicals in genomic DNA. Given that certain sequences have markedly low oxidation potentials, the lesions that result from this process may be unevenly distributed throughout the genome. Thus, inside the cell, DNA CT may play a major role in the DNA damage process by funneling damage to specific sites. However, many fundamental characteristics of DNA CT *in vivo* still need to be addressed. In particular, it is not known which sequences are prone to oxidative damage via DNA CT, nor is it fully understood which distance regimes are possible for DNA CT in biological environments.

Guanine radicals may also be important in mediating protein signaling processes. These DNA-based radicals may transfer to low oxidation potential sites in proteins, including amino acid side chains or protein-bound cofactors, eliciting a functional change in the protein. Here, DNA CT could serve as an antenna for DNA damage, allowing proteins to monitor oxidation events that occur far away and respond to them quickly. DNA CT could also provide a mechanism for protein-protein communication and, to this end, we have proposed that DNA repair enzymes could use DNA CT to cooperatively search for damage. Understanding the full range of DNA-binding proteins that could participate in these signaling pathways, and their associated cofactors, is a major focus of investigation.

REFERENCES

1. O'Neill, M.A., and Barton, J.K. (2005) *Charge Transfer in DNA: from Mechanism to Application*. Wagenknecht, H.A. Wiley-VCH, Ed., 27.
2. Hall, D.B., Holmlin, R.E., and Barton, J.K. (1996) *Nature* 382, 731.
3. Steenken, S., and Jovanovich, S. (1997) *J. Am. Chem. Soc.* 119, 617.
4. Saito, I., Takayama, M., Sugiyama, H., Nakatani, K., Tsuchida, A., Yamamoto, M. (1995) *J. Am. Chem. Soc.* 117, 6406.
5. Nunez, M.E., Hall, D.B., and Barton, J.K. (1999) *Chem. Biol.* 6, 85.
6. Ly, D., Sanil, L., and Schuster, G.B. (1999) *J. Am. Chem. Soc.* 121, 9400.
7. Augustyn, K.E., Genereux, J.C., and Barton, J.K. (2007) *Angew. Chim. Int. Ed.* 46, 5731.
8. Wagenknecht, H.A. (2003) *Angew. Chim. Int. Ed.* 42, 2454.
9. Boon, E.M., Ceres, D.M., Drummond, T.G., Hill, M.G., and Barton, J.K. (2000) *Nat. Biotechnol.* 18, 1096.
10. Boal, A.K., and Barton, J.K. (2005) *Bioconjug Chem* 16, 312.
11. Elias, B., Shao, F., and Barton, J.K. (2008) *J. Am. Chem. Soc.* 130, 1152.
12. Burrows, C.J., and Muller, J.G. (1998) *Chem. Rev.* 98, 1109.
13. Giese, B. (2000) *Acc. Chem. Res.* 33, 631.
14. Valko, M., Izakovic, M., Mazur, M., Rhodes, C.J., and Telsler, J. (2004) *Mol. Cell. Biochem.* 266, 37.
15. Friedman, K.A., and Heller, A. (2001) *J. Phys. Chem. B* 105, 11859.
16. Friedman, K.A., and Heller, A. (2004) *J. Am. Chem. Soc.* 126, 2368.
17. Delaney, S., and Barton, J.K. (2003) *Biochemistry* 42, 14159.
18. Szalai, V.A., Singer, M.J., and Thorp, H.H. (2002) *J. Am. Chem. Soc.* 124, 1625.
19. Besaratinia, A., and Pfeifer, G.P. (2006) *Carcinogenesis* 27, 1526.
20. Nunez, M.E., Holmquist, G.P., and Barton, J.K. (2001) *Biochemistry* 40, 12465.
21. Wallace, D.C. (2005) *Annu. Rev. Biochem.* 39, 359.

22. Merino, E.J., and Barton, J.K. (2007) *Biochemistry* 46, 2805.
23. Merino, E.J., and Barton, J.K. (2008) *Biochemistry* 47, 1511.
24. Tan, D.J., Bai, R.K., and Wong, L.J.C. (2002) *Cancer Res.* 62, 972.
25. Rajski, S.R., and Barton, J.K. (2001) *Biochemistry* 40, 5556.
26. Boon, E.M., Salas, J.E., and Barton, J.K. (2002) *Nat. Biotechnol.* 20, 282.
27. Gorodetsky, A.A., Ebrahim, A., and Barton, J.K. (2008) *J. Am. Chem. Soc.* 130, 2924.
28. Nakatani, K., Dohno, C., Ogawa, A., and Saito, I. (2002) *Chem. Biol.* 9, 361.
29. Nunez, M.E., Noyes, K.T., and Barton, J.K. (2002) *Chem. Biol.* 9, 403.
30. Luger, K., Mader, A., Richmond, R., Sargent, D., and Richmond, T.J. (1997) *Nature* 389, 251.
31. Bjorklund, C.C., and David, W.B. (2006) *Nucleic Acids Res.* 34, 1836.
32. Bjorklund, C.C., and Davis, W.B. (2007) *Biochemistry* 46, 10745.
33. Stubbe, J., Nocera, D.G., Yee, C.S., and Chang, M.C. (2003) *Chem. Rev.* 103, 2167.
34. Wagenknecht, H.A., Stemp, E.D.A., and Barton, J.K. (2000) *Biochemistry* 39, 5482.
35. DeRosa, M.C., Sancar, A., and Barton, J.K. (2005) *Proc. Natl. Acad. Sci. USA* 102, 10788.
36. Takada, T., and Barton, J.K. (2005) *J. Am. Chem. Soc.* 127, 12204.
37. Augustyn, K.E., Merino, E.J., and Barton, J.K. (2007) *Proc. Natl. Acad. Sci. USA* 104, 18907.
38. Cho, Y., Gorina, S., Jeffrey, P.D., and Pavletich, N.P. (1994) *Science* 265, 346.
39. Prives, C., and Hall, P.A. (1999) *J. Pathol.* 187, 112.
40. David, S.S., and Williams, S.D. (1998) *Chem. Rev.* 98, 1221.
41. Blainey, P. C., van Oijen, A. M., Banerjee, A., Verdine, G. L., and Xie, X. S. (2006) *Proc. Natl. Acad. Sci. USA* 103, 5752.
42. Francis, A. W., and David, S. S. (2003) *Biochemistry* 42, 801.

43. Halford, S. E., and Szczelkun, M. D. (2002) *Eur. Biophys. J.* 31, 257.
44. Gowers, D. M., and Halford, S. E. (2003) *EMBO J.* 22, 1410.
45. Gerland, U., Moroz, J. D., and Hwa, T. (2002) *Proc. Natl. Acad. Sci. USA* 99, 12015.
46. Cunningham, R.P., Asahara, H., Bank, J.F., Scholes, C.P., Salerno, J.C., Surereus, K., Munck, E., McCracken, J., Peisach, J., and Emptage, M.H. (1989) *Biochemistry* 28, 4450.
47. Hinks, J. A., Evans, M. C. W., de Miguel, Y., Sartori, A. A., Jiricny, J., and Pearl, L. H. (2002) *J. Biol. Chem.* 277, 16936.
48. Rebeil, R., Sun, Y., Chooback, L., Pedraza-Reyes, M., Kinsland, C., Begley, T. P., and Nicholson, W. L. (1998) *J. Bacteriol.* 180, 4879.
49. Demple, B., and Linn, S. (1980) *Nature* 287, 203.
50. Katcher, H. L., and Wallace, S. S. (1983) *Biochemistry* 22, 4071.
51. Briemer, L. H., and Lindahl, T. (1984) *J. Biol. Chem.* 259, 5543.
52. Weiss, R. B., and Duker, N. J. (1986) *Nucleic Acids Res.* 14, 6621.
53. Boorstein, R. J., Hilbert, T. P., Cadet, J., Cunningham, R. P., and Teebor, G. W. (1989) *Biochemistry* 28, 6164.
54. Wagner, J. R., Blount, B. C., and Weinfeld, M. (1996) *Anal. Biochem.* 233, 76.
55. Dizdaroglu, M., Laval, J., and Boiteux, S. (1993) *Biochemistry* 32, 12105.
56. Kuo, C. F., McRee, D. E., Fisher, C. L., O'Handley, S. F., Cunningham, R. P., and Tainer, J. A. (1992) *Science* 258, 434.
57. Thayer, M. M., Ahern, H., Xing, D., Cunningham, R. P., and Tainer, J. A. (1995) *EMBO J.* 14, 4108.
58. Guan, Y., Manuel, R. C., Arvai, A. S., Parikh, S. S., Mol, C. D., Miller, J. H., Lloyd, S., and Tainer, J. A. (1998) *Nat. Struct. Biol.* 5, 1058.
59. Michaels, M. L., Pham, L., Nghiem, Y., Cruz, C., and Miller, J. H. (1990) *Nucleic Acids Res.* 18, 3841.
60. Nghiem, Y., Cabrera, M., Cupples, C. G., and Miller, J. H. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2709.
61. Micheals, M. L., Cruz, C., Grollman, A. P., and Miller, J. H. (1992) *Proc. Natl. Acad. Sci. USA* 89, 7022.

62. Michaels, M. L., and Miller, J. H. (1992) *J. Bacteriol.* 174, 6321.
63. Michaels, M. L., Tchou, J., Grollman, A. P., and Miller, J. H. (1992) *Biochemistry* 31, 10964.
64. Gogos, A. , Cillo, J., Clarke, N. D., and Lu, A. L. (1996) *Biochemistry* 35, 16665.
65. Lu, A. L., Tsai-Wu, J. J., and Cillo, J. (1995) *J. Biol. Chem.* 270, 23582.
66. Manuel, R. C., Czerwinski, E. W., and Lloyd, R. S. (1996) *J. Biol. Chem.* 271, 16218.
67. Francis, A. W., Helquist, S. A., Kool, E. T., and David, S. S. (2003) *J. Am. Chem. Soc.* 125, 16235.
68. Chepanoske, C. L., Porello, S. L., Fujiwara, T., Sugiyama, H., and David, S. S. (1999) *Nucleic Acids Res.* 27, 3197.
69. Vidmar, J. J., and Cupples, C. G. (1993) *Can. J. Microbiol.* 39, 892.
70. Bulychev, N. V., Varaprasad, C. V., Dorman, G., Miller, J. H., Eisenberg, M., Grollman, A. P., and Johnson, F. (1996) *Biochemistry* 35, 13147.
71. Porello, S. L., Leyes, A. E., and David, S. S. (1998) *Biochemistry* 37, 14756.
72. Radicella, J. P., Clark, E. A., and Fox, M. S. (1998) *Proc. Natl. Acad. Sci. USA* 85, 9674.
73. Fu, W., O'Handley, S., Cunningham, R. P., and Johnson, M. K. (1992) *J. Biol. Chem.* 267, 16135.
74. Porello, S. L., Cannon, M. J., and David, S. S. (1998) *Biochemistry* 37, 6465.
75. Messick, T. E., Chmiel, N. H., Golinelli, M. P., Langer, M. R., Joshua-Tor, L., and David, S. S. (2002) *Biochemistry* 41, 3931.
76. Golinelli, M. P., Chmiel, N. H., and David, S. S. (1999) *Biochemistry* 38, 6997.
77. Fromme, J. C., and Verdine, G. L. (2003) *EMBO J.* 22, 3461.
78. Fromme, J. C., Banerjee, A., Huang, S. J., and Verdine, G. L. (2004) *Nature* 427, 652.
79. Boon, E.M., Livingston, A.L., Chmiel, N.H., David, S.S., and Barton, J.K. (2003) *Proc. Natl. Acad. Sci. USA* 100, 12543.
80. Boal, A.K., Yavin, E., Lukianova, O.A., O'Shea, V.L., David, S.S., and Barton, J.K. (2005) *Biochemistry* 44, 8397.

81. Yavin, E., Stemp, E.D.A., O'Shea, V.L., David, S.S., and Barton, J.K. (2006) *Proc. Natl. Acad. Sci. USA* 103, 3610.
82. Yavin, E., Boal, A.K., Stemp, E.D.A., Boon, E.M., Livingston, A.L., O'Shea, V.L., David, S.S., and Barton, J.K. (2005) *Proc. Natl. Acad. Sci. USA* 102, 3546.
83. Gorodetsky, A.A., Boal, A.K., and Barton, J.K. (2006) *J. Am. Chem. Soc.* 128, 12082.
84. Lindahl, T., and Nyberg, B. (1974) *Biochemistry* 13, 3405.
85. Jacobs, K. L., and Grogan, D. W. (1997) *J. Bacteriol.* 179, 3298.
86. Cheadle, J.P., and Sampson, J.R. (2007) *DNA Repair* 6, 274.
87. Al-Tassan, N., Chmiel, N.H., Maynard, J., Fleming, N., Livingston, A.L., Williams, G.T., Hodges, A.K., Davies, D.R., David, S.S., Sampson, J.R., and Cheadle, J.P. (2002) *Nat. Genet.* 30, 227.
88. Pope, M.A., Chmiel, N.H., and David, S.S. (2005) *DNA Repair* 4, 315.
89. Livingston, A.L., O'Shea, V.L., Kim, T., Kool, E.T., and David, S.S. (2008) *Nat. Chem. Biol.* 4, 51.
90. Cupples, C. G., and Miller, J. H. (1989) *Proc. Natl. Acad. Sci. USA* 86, 5345.
91. Tano, K., Iwamatsu, Y., Yasuhira, S., Utsumi, H., and Takimoto, K. (2001) *J. Radiat. Res.* 42, 409.