Chapter 1

DNA-Mediated Charge Transport*

*Adapted from J. K. Barton, E. D. Olmon, and P. A. Sontz, *Coord. Chem. Rev.* **255**, 619–634 (2011).

1.1 Introduction

Oxidative DNA damage has been implicated in a host of adverse medical conditions including aging, heart disease, and various forms of cancer.[1,2] Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$) are a constant threat. Exogenous sources of ROS, such as cigarette smoke, air pollution, and ultraviolet radiation, have been linked to the formation of DNA strand breaks and lesions, which can lead to mutagenesis and carcinogenesis.[3,4] The danger of endogenous sources of ROS is also considerable: ROS are byproducts of oxidative respiration in mitochondria; they are produced by macrophages during immune response; and they are generated during P450 metabolism.[5,6] In order to develop diagnostics and therapeutics for the prevention of medical conditions associated with DNA damage, it is necessary to understand the chemical mechanisms which result in oxidative DNA lesions, as well as the biological pathways that exist to prevent and repair them.

Before discussing factors that affect the oxidation of DNA, it is prudent to review the chemical characteristics of the macromolecule itself. DNA consists of long polymeric strands of nucleic acid bases, specifically the planar, aromatic heterocycles adenine (A), guanine (G), thymine (T), and cytosine (C). Pairs of strands are held together by specific hydrogen bonds formed between the nucleobases: A pairs with T, and G pairs with C. Within the strands, the bases are joined by anionic deoxyribophosphate units, which, upon formation of the duplex, wrap the stack of nucleobases in a negatively charged double helix. Consecutive base pairs are stacked closely together, allowing the aromatic π system of one to interact with that of its neighbors. In this way, the stacked aromatic bases resemble stacked sheets of graphite, as illustrated in Figure 1.1 on page 5. In much the same way that electricity can be conducted perpendicular to stacked graphite sheets,[7] DNA can mediate the transmission of charge along its length.

In our laboratory, we have utilized the rich redox chemistry of transition metal complexes in conjunction with the ability of DNA to mediate charge transport (CT) reactions to generate and study oxidative damage in DNA. In the first chapter, we examine the properties of metal complexes that make them ideal probes for initiating and monitoring DNA

CT events. We also discuss the ability of DNA to mediate charge transfer reactions between a charge donor and a charge acceptor, as well as the ability of DNA bases themselves to participate in DNA-mediated redox chemistry. Several biological implications of DNA-mediated CT are also described, including the accumulation of oxidative damage at sites of high guanine content, and the mechanism by which DNA may mediate cellular signaling and transcriptional regulation. In the second chapter, the design, synthesis, and characterization of a new Re photooxidant is described. The properties that make this a useful probe for the study of DNA-mediated CT are discussed. In the third chapter, the use of this new probe in time-resolved infrared spectroscopy experiments is detailed. In these experiments, it was shown that the Re photosensitizer is capable of oxidizing guanine from a distance. The mechanism of this reaction and the factors affecting the yield of guanine oxidation are discussed. In the fourth chapter, the oxidation strength of three DNA-binding metal complex photooxidants is compared directly in biochemical and spectroscopic experiments. Differences in the oxidatizing ability of the three complexes are discussed in terms of their DNA binding strength and redox properties. The fifth chapter outlines the results of time-resolved spectroscopic experiments that have been conducted on a number of redox-active DNA-binding proteins. Successes, failures, and opportunities for future work are discussed.

1.2 Metal Complexes as Probes for DNA-Mediated CT

Metal complexes are powerful initiators and probes of DNA-mediated CT. By varying photophysical properties, redox potentials, and DNA-binding abilities of many metal complexes, as well as the DNA sequences through which charge transport occurs, we have been able to characterize the parameters that govern long range DNA-mediated oxidation and reduction.

1.2.1 Advantages of Metal Complexes in Studies of DNA-Mediated CT

Any study of DNA CT must involve some means of injecting charge onto the DNA bridge and some means of reporting the CT event. Although there is a wide array of molecular probes that can carry out these tasks, the most effective ones share many chemical and physical characteristics. First, in order to utilize the electronic system of the bases as a

conduit for charge, the probe should interact strongly with the DNA base stack. Such an interaction can be difficult to achieve, considering the geometry of DNA. In general, the only access a diffusing molecule has to the base stack is either at the ends of the DNA strand or within the relatively narrow major and minor grooves which run lengthwise along the sides of the DNA molecule. Probes which are too large, or which are strongly negatively charged and therefore are repelled by the phosphate backbone of DNA, do not easily interface with the DNA π-stack. Second, depending on the function of the probe, it must provide a straightforward means of either initiating or reporting on DNA CT, or both. Often, the photophysical or electrochemical properties of a molecule are utilized for these purposes. Some probes may also report CT events through chemical pathways such as degradation. Third, the probe should not degrade or interact chemically with the DNA strand or with other components of the sample unless this is by design. Not only must the probe be stable enough to persist in solution, but the excited state of the molecule must also be stable if photochemical means are used to initiate or report CT, and the various redox states of the molecule must be able to withstand the charge transfer process. Finally, the ideal probe would be synthetically versatile and easy to build or modify in order to control sensitively the parameters of the experiment. Metallointercalators, transition metal complexes which bind DNA primarily by intercalation, are one class of molecules that fulfill all of these requirements.

Intercalation, first reported by Lerman in 1961,[8] is a binding mode in which the ligand, usually a planar, aromatic moiety, slips between two adjacent bases in the DNA base stack. Structural changes in the DNA associated with intercalation include a slight unwinding of the helix at the intercalation site, an extension in length equal to the height of one base pair, and an increase in DNA stability, as indicated by a higher duplex melting temperature. The overall structure of the DNA is unperturbed: no bending or kinking of the helix is observed,[9] and the C2′-endo sugar pucker found at non-intercalation sites is retained.[10] The effect of intercalation on the structure of DNA is shown in Figure 1.2. Intercalators, often large, heterocyclic structures, physically and electronically resemble the DNA bases themselves, so intimate associations may form between the DNA base stack

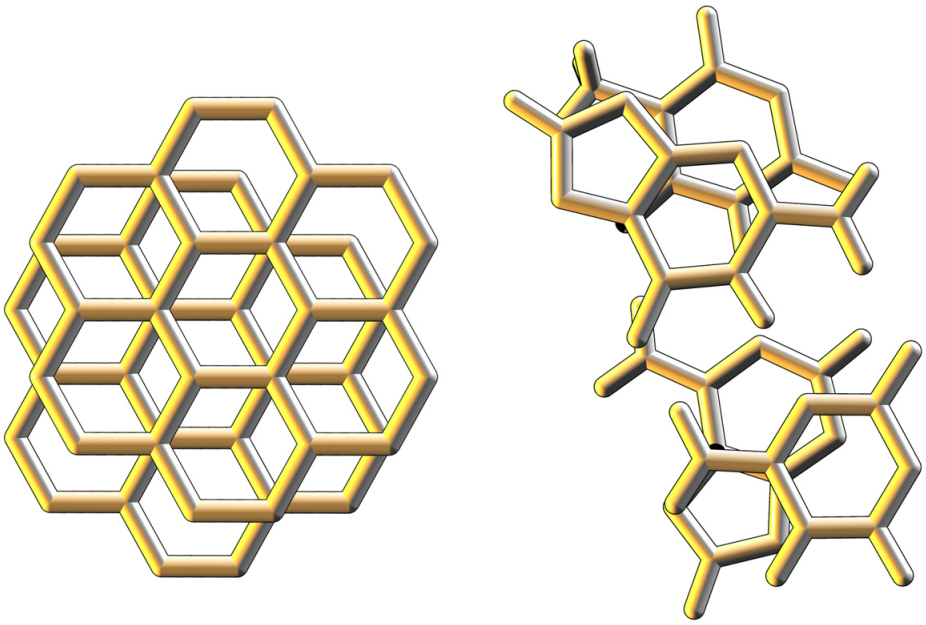


Figure 1.1: The structure and geometry of stacked graphene sheets (left) is similar to that of stacked DNA base pairs (right).

and the binding ligand. In a sense, the intercalating molecule acts as an additional base, enabling strong interactions with the electronic structures of the flanking bases.[10] Many molecules are known to bind DNA through this mode, including the organic intercalating drugs 9-aminoacridine, ethidium, and daunomycin,[9] among others. Many metal complexes, also, may bind DNA through intercalation if they bear one or more planar, aromatic ligands such as phen (1,10-phenanthroline), phi (9,10-phenanthrenequinone diimine), or dppz (dipyrido[3,2-a:2′,3′-c]phenazine). Interestingly, by incorporating a ligand that is slightly wider than DNA, it is possible to selectively target binding at thermodynamically destabilized mismatch sites through the insertion binding mode, where the bulky ligand enters the helix from the minor groove, pushing the mismatched base pair out into the major groove.[11–13] Because insertion involves substitution in the base stack of the inserting ligand for the mismatched base pair, insertion, like intercalation, should facilitate strong electronic interactions between the inserting metal complex and the DNA base stack. Although other DNA binding modes such as electrostatic binding and groove binding have been observed,[14,15] these do not offer the strong electronic coupling to the base stack that is characteristic of intercalation and insertion.

Not only do metallointercalators bind strongly to DNA, but they also possess rich and well understood photochemistry and photophysics, which make them advantageous for use as probes for DNA interactions, as injectors of charge onto the DNA bridge, and as reporters of DNA CT events. Particularly interesting and effective examples are the dppz complexes of ruthenium, which display the "light switch effect".[16] $[Ru(bpy)_2dppz]^{2+}$ and $[Ru(phen)_2dppz]^{2+}$ are not luminescent in aqueous solution due to deactivation of the luminescent state via hydrogen bonding of the dppz ligand with water. However, in solutions containing duplex DNA, the complexes intercalate, the dppz ligand is protected from solution, and luminescence is restored.[16–20] Although most metal complexes do not display this remarkable discrimination, many do luminesce. In addition, many complexes absorb strongly in the visible region due to their intense metal-to-ligand charge transfer (MLCT) and intraligand (IL) charge transfer transitions. These properties allow for manipulation and monitoring of the electronic and redox states of the metal complexes spectroscopically.

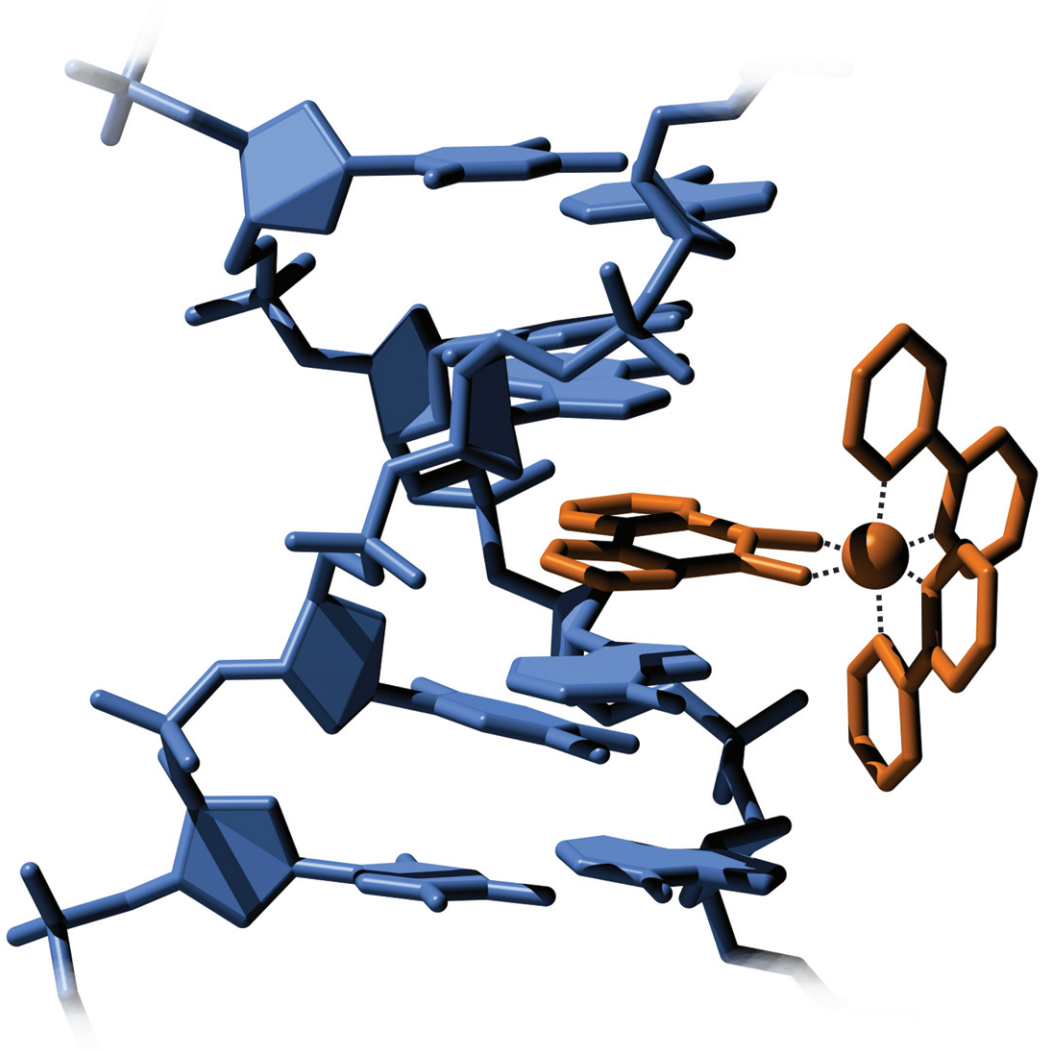


Figure 1.2: Intercalative binding to DNA results in an increase of the rise at the site of binding, as well as a slight unwinding of the helix. Shown is a model of $[Rh(phi)(bpy)_2]^{3+}$ (orange) bound to DNA (blue), adapted from the crystal structure of a similar construct.[11]

The MLCT transitions may also be exploited to initiate CT processes, since many metal complexes become strong oxidizing or reducing agents upon optical excitation. In circumstances under which the excited state of a metal complex cannot carry out the desired chemistry, it may be necessary to utilize the "flash-quench" technique.[21] This method involves the use of a diffusing molecule which is competent to oxidize or reduce the excited metal complex, thus creating a strong ground state oxidant or reductant.

Several other characteristics of metallointercalators make them suitable for studies of DNA-mediated CT. They are coordinatively saturated, substitutionally inert, and rigid, making them extremely stable in solution, and preventing coordination between the metal complexes and DNA. Metallointercalators are also modular. Unlike organic intercalators, the properties of metal complexes can be altered subtly and systematically by adding electron donating or withdrawing group to the constituent ligands, or by using different sets of ligands. In addition, the three-dimensional structure of metallointercalators enables them to interact with DNA in a stereospecific, and sometimes sequence-specific, manner, while organic intercalators, which are often planar, cannot. For example, many studies have shown that Δ complexes tend to bind more tightly to right-handed B-DNA, while Λ complexes have been useful in probing left-handed Z-DNA.[22–30] This result has mainly to do with the steric agreement between the intercalated metal complex and the DNA: the ancillary ligands of Δ complexes tend to lie along the major groove of the DNA helix, whereas those of Λ complexes collide with the phosphate backbone.

The versatility of metallointercalators also facilitates the sensitive tuning of their electronic and electrochemical properties. Complexes have been synthesized that absorb and emit across the visible spectrum and that sample a wide variety of redox potentials. The addition or elimination of a single functional group on either the intercalating ligand or the ancillary ligands can serve to alter the photophysical, electrochemical, or DNA binding properties of the complex. For example, addition of a carboxylic acid or benzyl group to the end of dppz, or introduction of an additional heterocyclic nitrogen, eliminates the light-switch effect and alters the absorption and emission maxima and luminescence lifetime of the complex.[17]

The modularity of metal complexes also makes it possible to extend their functionality by modifying their ancillary ligands. For example, it is possible to create a covalent linkage between a metal complex and DNA through the use of a carboxyalkyl chain.[31,32] Such linkages serve to ensure a binding ratio of unity between the metal complex and the DNA while precisely defining the binding site of the metal, without disrupting the mode of binding or DNA structure. Alkyl chains have also been used to append organic fluorophores to metallointercalators in an effort to develop luminescent reporters of mismatches.[33] Additionally, modification of a ruthenium complexes with octaarginine allows for the facile uptake of these complexes into the nuclei of cancer cells.[34,35] Functionalization of the ancillary ligands may also lead to sequence-selective recognition and cleavage by metallointercalators via hydrogen bonding or van der Waals interactions with modified ethylenediamine ligands,[36–40] peptide sequences,[41–43] or modified phen ligands.[44–47] Functionalization may also confer nuclease activity.[48]

Although many classes of molecules may serve as effective intercalators for the study of DNA-mediated CT, metallointercalators provide several advantages. The array of metal complexes described in this chapter is shown in Scheme 1.1 on page 11. In addition to their inherent stability in solution, they display strong coupling to the DNA base stack. Unlike organic intercalators, the photophysical, electrochemical, and DNA-binding properties of metallointercalators may be tuned in an efficient and systematic manner to modify their properties in sensitive and subtle ways. Finally, the modularity of metal complexes allows for external functionalities to be applied, expanding the utility of these probes.

1.2.2 Metal Complexes as Charge Donors and Acceptors in DNA CT

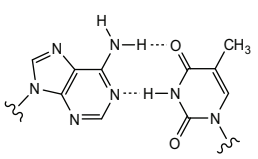
The first experiment that suggested the possibility of charge transport through the DNA base stack was an investigation of photoinduced electron transfer from $[Ru(phen)_3]^{2+}$ to either $[Co(bpy)_3]^{3+}$, $[Co(phen)_3]^{3+}$, or $[Co(dip)_3]^{3+}$ (dip = 4,7-diphenyl-1,10-phenanthroline).[30] It was found that quenching scaled with the DNA binding affinity of the quencher, and that Δ−$[Ru(phen)_3]^{2+}$ was quenched more efficiently than Λ−$[Ru(phen)_3]^{2+}$. Further, the estimated electron transfer rate was two orders of magnitude faster than the rate ob-

served in the absence of DNA. Although the increase in rate was primarily ascribed to the reduced dimensionality of diffusion at the DNA surface, it was suggested that electron transfer through the π-framework of DNA may play a role.

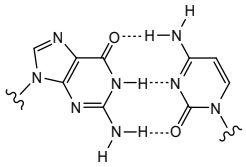
Evidence for DNA mediation of CT mounted in a study involving electron transfer from excited $[Ru(phen)_3]^{2+*}$ to either $[Co(phen)_3]^{3+}$, $[Rh(phen)_3]^{3+}$, $[Cr(phen)_3]^{3+}$, or $[Co(bpy)_3]^{3+}$.[49] These complexes are known to bind intercalatively in the major groove as well as electrostatically in the minor groove. Upon addition of DNA, luminescence quenching rates for each of these pairs increased. Interestingly, in 90% glycerol solutions at 253 K, where diffusion of all species is restricted, quenching rates were lower than in buffered aqueous solutions at ambient temperature, but they were still higher than the observed quenching rates in the absence of DNA. This result suggests that for these phen complexes, DNA-mediated electron transfer is a major quenching pathway. Nonetheless, with the use of freely diffusing charge donors and acceptors, it was difficult to discern the nature of DNA mediation due to rapid equilibration between binding modes and uncertainty in the distance between donor-acceptor pairs. Further experiments were necessary to establish DNA-mediated CT as an appreciable quenching mechanism.

Due to the larger hydrophobic surface area and further extension from the metal center, the incorporation of dppz allows for stronger DNA binding by intercalation than is allowed by phen. The use of $[Ru(phen)_2dppz]^{2+}$ in electron transfer experiments rather than $[Ru(phen)_3]^{3+}$ made it possible to probe ET events in which the donor was primarily bound by intercalation. Further, because non-intercalated $[Ru(phen)_2dppz]^{2+*}$ is quenched by water on an ultrafast timescale, any luminescence observed originates from the intercalated species. Steady-state and time-resolved emission quenching of $[Ru(phen)_2dppz]^{2+*}$ by either the strongly intercalating $[Rh(phi)_2phen]^{3+}$ or the groove binding $[Ru(NH_3)_6]^{3+}$ were examined.[50] In experiments involving the intercalated quencher, no change in emission rate was observed with increasing amounts of quencher; however, the initial luminescence intensity decreased. This result meant that quenching between the two intercalated species was occurring at rates faster than the instrument could detect. When $[Ru(NH_3)_6]^{3+}$ was used instead as quencher, increasing its concentration yielded an increase in the rate of

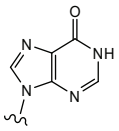
DNA Bases



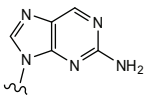
Adenine Thymine



Guanine Cytosine

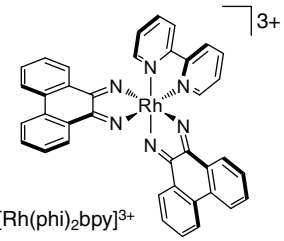


inosine

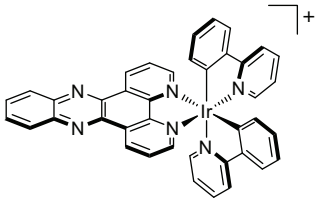


2-aminopurine

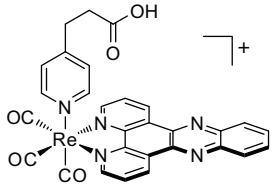
Metal Complexes



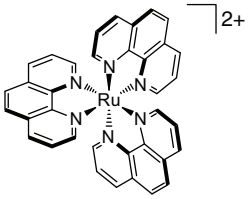
$[Rh(phi)_2bpy]^{3+}$



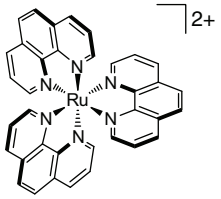
$[Ir(ppy)_2(dppz)]^{+}$



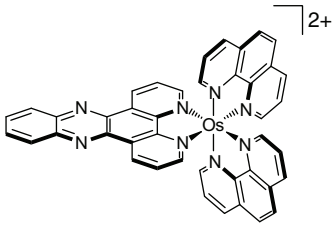
$[Re(CO)_3(dppz)(py')]^{+}$



Δ-$[Ru(phen)_3]^{2+}$

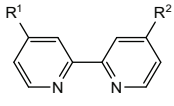


Λ-$[Ru(phen)_3]^{2+}$



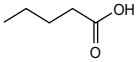
$[Os(phen)_2dppz]^{2+}$

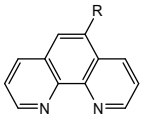
Ligands



bpy: $R^1 = R^2 = H$

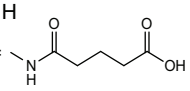
bpy′: $R^1 = CH_3$; R^2 =

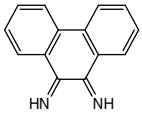




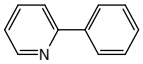
phen: R = H

phen′: R =

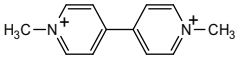




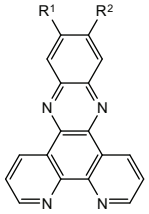
phi



ppy



Methyl Viologen

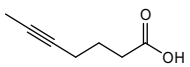


dppz: $R^1 = R^2 = H$

dmp: $R^1 = R^2 = CH_3$

DIP: $R^1 = R^2$ = phenyl

dppz′: $R^1 = H$; R^2 =



Scheme 1.1: Structures of DNA bases and representative metal complexes used in DNA-CT experiments

luminescence decay but did not alter the initial luminescence yield. These results, in addition to comparisons with results of steady-state emission quenching experiments, showed that quenching by $[Ru(NH_3)_6]^{3+}$ is a dynamic process, while quenching by the intercalated $[Rh(phi)_2phen]^{3+}$ is a static process.

Further mechanistic insight was gained by covalently tethering $[Ru(phen')_2dppz]^{2+}$ as an electron donor and $[Rh(phi)_2(phen')]^{3+}$ as an acceptor (phen′ = 5-amido-glutaric acid-1,10-phenanthroline) to complementary strands of a DNA oligomer,[51] as shown in Figure 1.3 on page 14 (top). The covalent tether was long enough to allow intercalation of the complexes, but short enough to prevent direct contact between them. By covalently attaching the donor and acceptor to opposite ends of the DNA duplex, the possibility for quenching through a diffusive mechanism was abolished, and the donor-acceptor distance was well defined. Excitation of assemblies in which the Ru-tethered strand was hybridized to its unmetallated complement resulted in strong luminescence. Addition of the covalently-tethered Rh complex to the complementary strand, however, resulted in complete quenching. Appropriate controls ensured that the quenching was intraduplex, and the imposed separation between the donor and acceptor precluded quenching by diffusion. These results meant that quenching of $[Ru(phen)_2dppz]^{2+}$ luminescence was occurring from over 35 Å away.

That the mechanism of quenching was in fact electron transfer and not energy transfer was irrefutably established by experiments involving charge donors other than $[Ru(phen)_2dppz]^{2+}$. In one study, the transient absorption of systems containing varying amounts of non-covalent $[Ru(dmp)_2dppz]^{2+}$ (dmp = 4,7-dimetheyl-1,10-phenanthroline) and $[Rh(phi)_2bpy]^{3+}$ with DNA were investigated and compared with the transient spectrum obtained upon oxidative $[Ru(dmp)_2dppz]^{2+*}$ quenching by $[Ru(NH_3)_6]^{3+}$.[52] With increasing amounts of Rh, the luminescence decay lifetimes did not change, but the initial luminescence yield did, again signifying that the quenching in this system involves a static mechanism. The transient spectrum obtained by using the Rh complex as the quencher matched that obtained using $[Ru(NH_3)_6]^{3+}$ as the quencher, positively identifying the transient intermediate in the Rh experiment as the oxidation product, $[Ru(dmp)_2dppz]^{3+}$, and the mechanism of luminescence quenching as electron transfer. In another study,

$[Os(phen)_2dppz]^{2+}$, rather than $[Ru(phen)_2dppz]^{2+}$, was used as the electron donor.[53] The Os complex emits at a higher wavelength, and its emission lifetime (< 10 ns) is several orders of magnitude shorter than that of $[Ru(phen)_2dppz]^{2+}$.[54] Despite these photophysical differences, $[Os(phen)_2dppz]^{2+}$ behaves similarly: it is also a light switch, it binds DNA primarily through intercalation, and quenching by $[Rh(phi)_2bpy]^{3+}$ in the presence of DNA takes place through a static mechanism. Interestingly, the dependence of the quenching yield on the concentration of $[Rh(phi)_2bpy]^{3+}$ is the same between $[Os(phen)_2dppz]^{2+}$ and $[Ru(phen)_2dppz]^{2+}$, so the quenching mechanism is the same despite photophysical and electronic differences. Also, transient spectra obtained upon photoexcitation of $[Os(phen)_2dppz]^{2+}$ in the presence of DNA and $[Rh(phi)_2bpy]^{3+}$ match spectra obtained through oxidative quenching of DNA-bound $[Os(phen)_2dppz]^{2+*}$ by $[Ru(NH_3)_6]^{3+}$ and through direct ground state oxidation of $[Os(phen)_2dppz]^{2+}$ by $[Ce(NO_3)_6]^{2-}$. The agreement between these three spectra indicates that the same oxidized Os species is being formed in each case. In addition, because the emission band of $[Os(phen)_2dppz]^{2+}$ does not overlap with the absorption band of the Rh complex, energy transfer is not a viable quenching pathway. These results together mean that $[Os(phen)_2dppz]^{2+*}$ and $[Ru(phen)_2dppz]^{2+*}$ are both quenched almost exclusively by $[Rh(phi)_2bpy]^{3+}$ through DNA-mediated electron transfer.

Incidentally, $[Ru(phen)_2dppz]^{2+}$ was not the complex used to identify the intermediate involved in DNA-mediated electron transfer because no long-lived transient that could be ascribed to Ru(III) was ever observed spectroscopically in mixed-sequence DNA. As was speculated and later confirmed, this was because the Ru(III) intermediate was a strong enough oxidant to oxidize the guanine bases within the DNA strand and was depleted as soon as it formed. This property was later utilized to great effect to gain a better understanding of the DNA CT process by oxidizing the bases of DNA directly.

1.2.3 Long-Range Oxidation of DNA

1.2.3.1 Characteristics of bases and base analogues

For metallointercalators of sufficiently high redox potential, the DNA bases themselves may serve as partners in charge transfer reactions. The redox potentials of the base nucleosides

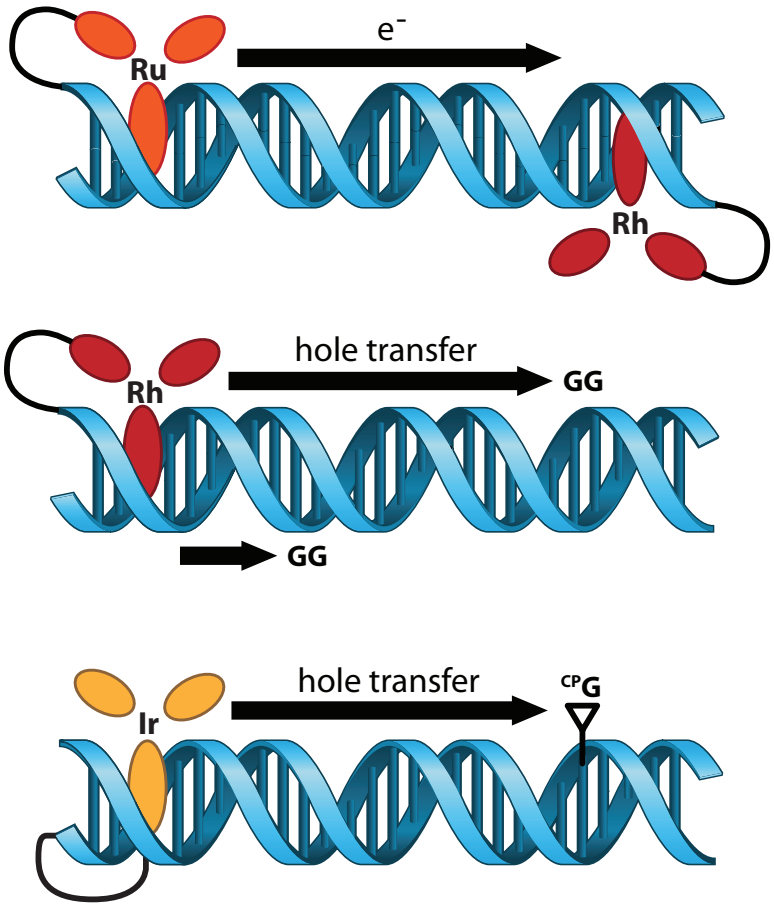


Figure 1.3: Metal complex-DNA conjugates used to study DNA-mediated CT. Top: covalent tethering of $[Ru(phen')_2(dppz)]^{2+}$ and $[Rh(phi)_2(phen')]^{3+}$ to complementary DNA strands enables the study of DNA-mediated CT over large distances. Middle: DNA-bound $[Rh(phi)_2(bpy')]^{3+}$ is competent to oxidize 5′-GG-3′ sites from the excited state. Bottom: cyclopropylamine traps enable the fast capture of a charge as it travels along the DNA bridge following excitation of tethered $[Ir(ppy)_2(dppz')]^{+}$.

increase in the order: G (1.29 V vs. NHE) < A (1.42 V) < T (1.6 V) < C (1.7 V).[55] Therefore, a metal complex such as $[Ru(phen)_2dppz]^{3+}$ [$E^{\circ}(3+/2+) = 1.63$ V vs. NHE] or excited $[Rh(phi)_2bpy]^{3+*}$ [$E^{\circ}(3+^*/2+) \approx 2.0$ V vs. NHE][50] should be competent to oxidize some or all of the bases. Interestingly, within the DNA base stack, the propensity for electron transfer to occur from a particular base is influenced by electronic interactions with its neighbors. For example, *ab initio* molecular orbital calculations have predicted that the electron donating ability of guanine should increase as: 5′-GT-3′, 5′-GC-3′ ≪ 5′-GA-3′ < 5′-GG-3′ < 5′-GGG-3′.[56] Further, the HOMO of the 5′-GG-3′ doublet is calculated to lie primarily on the 5′-G, indicating that the 5′-G site should be preferentially oxidized at guanine doublets, as has been observed experimentally. The relative ease with which guanine, guanine doublets, and guanine triplets are oxidized leads to biological implications: given a random sequence of bases, regions of high guanine content are the most likely places to find large amounts of oxidative damage.

The use of non-natural base analogues further extends the ability to exploit the intimate interactions between bases in the study of DNA CT. Many base analogues only slightly perturb the geometry and energetic structure of the base stack and interact in a natural way with the other bases, becoming part of the base stack and sometimes forming hydrogen bonds with natural bases. Base analogues provide advantageous functions for the study of DNA CT. For example, 2-aminopurine is fluorescent and pairs with thymine; and inosine, which shares a strong resemblance with guanine, nevertheless has a significantly higher oxidation potential (1.5 V vs. NHE).[57] Bases that are modified by a cyclopropylamino group in the major groove serve as sensitive indicators of charge occupation. The properties of natural bases, non-natural base analogues, and cyclopropylamine-modified bases, can be exploited for the study of DNA CT.

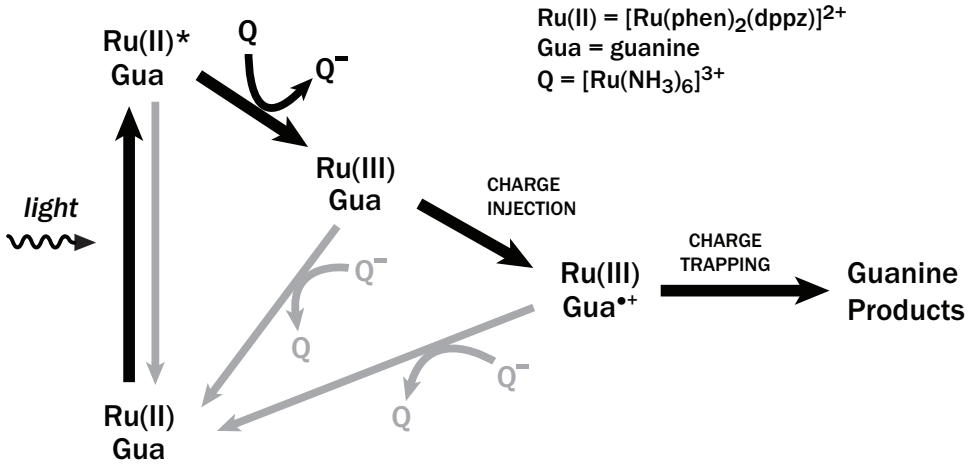
1.2.3.2 Oxidation of Guanine by a Metallointercalator

Direct proof of guanine oxidation by a ruthenium intercalator was obtained in a study involving $[Ru(phen)_2dppz]^{2+}$, DNA, and a variety of oxidative luminescence quenchers.[58] The quenchers used in the study, $[Ru(NH_3)_6]^{3+}$, methyl viologen (MV^{2+}), and $[Co(NH_3)_5Cl]^{2+}$,

associate with DNA through groove binding and quench $[Ru(phen)_2dppz]^{2+*}$ dynamically on the nanosecond timescale.[50] The study was an application of the flash-quench technique,[21] shown in Scheme 1.2 on page 17: following photoexcitation of the intercalated complex, oxidative quenching by a diffusible molecule creates the strong ground-state oxidant $[Ru(phen)_2dppz]^{3+}$ *in situ*, which then proceeds to oxidize guanine. The reaction may be interrupted by any of several processes, including depopulation of the $[Ru(phen)_2dppz]^{2+*}$ excited state through luminescence, reduction of the Ru(III) oxidized species by back electron transfer (BET) from the reduced quencher, or guanine cation radical neutralization by the reduced quencher. In the absence of these deactivation pathways, the guanine radical may react with O_2 or H_2O, forming permanent oxidation products.

In transient absorption experiments, the microsecond decay of a long-lived transient indicated formation of the oxidized ruthenium species in the presence of poly(dA-dT). In poly(dG-dC), no long-lived intermediate attributable to Ru(III) was observed; instead, a new transient species appeared on the timescale of Ru(II)* emission decay. This new transient was assigned to the neutral guanine radical, and its spectrum matched that previously observed by pulse radiolysis.[59]

The yield of oxidized guanine product formation was then studied by gel electrophoresis. $[Ru(phen)_2dppz]^{2+}$ was irradiated at 436 nm in the presence of 18 base pair DNA duplexes containing guanine doublets or triplets and a quencher. Following radiolabeling and treatment with aqueous piperidine, which cleaves DNA at sites of guanine damage, the cleaved strands were separated by polyacrylamide gel electrophoresis and imaged by phosphorimagery. Damage occurred primarily at the 5′-G in duplexes containing 5′-GG-3′ doublets, although small amounts of damage also occurred at single G sites, while strands incorporating both a 5′-GG-3′ and a 5′-GGG-3′ triplet exhibited damage mainly at the 5′-G of the triplet. Damage products were analyzed by enzymatic digestion followed by HPLC. Comparison with an authentic sample identified the major product as 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG), the primary oxidative base lesion found within the cell.[60]



Scheme 1.2: The flash-quench technique. Following photoexcitation, Ru(II)* is oxidized by a diffusing quencher to form the powerful ground state oxidant Ru(III). Charge injection results on charge localization at guanine (Gua). Trapping by reaction of this radical with H_2O or O_2 results in charge trapping and the formation of permanent products. Several BET pathways ($Q^- \rightarrow Q$) lower the efficiency of formation of guanine damage products.

1.2.3.3 Guanine Oxidation Over Long Distances

Studies of guanine oxidation were also carried out in systems containing metal-DNA conjugates. In one notable experiment, $[Rh(phi)_2(bpy')]^{3+}$ [bpy′ = 4-methyl-4′-(butyric acid)-2,2′-bipyridine] was tethered to the end of a DNA 15-mer containing two 5′-GG-3′ doublets: one 17 Å away from the Rh binding site (proximal), and one 34 Å away from the binding site (distal).[61] Such a construct is shown in Figure 1.3 on page 14 (center). Rhodium complexes such as these serve as potent photooxidants when irradiated by 365 nm light, but promote direct strand cleavage at the site of intercalation when irradiated at 313 nm. When the conjugates were irradiated with 313 nm light, damage was only observed at the expected Rh binding site, three bases in from the end of the duplex. Upon excitation of the tethered complex with 365 nm light, guanine oxidation was observed primarily at the 5′-G of both 5′-GG-3′ doublets. While the irradiation experiment at 313 nm supported an intraduplex reaction, confirmation that the reaction was intraduplex was obtained in a mixed labeling experiment (Figure 1.4). Rhodium-DNA conjugates that were not radioactively tagged were mixed with DNA oligomers of the same sequence that were labeled but did not contain tethered Rh. Irradiation at 360 nm and subsequent piperidine treatment showed no damage to the DNA. Thus, in the Rh-tethered and labeled samples, oxidative damage was seen at distances of 17 Å and 34 Å from the bound Rh. This long-range damage was mediated by DNA.

Interestingly, very little difference was observed in the damage yields between distal and proximal 5′-GG-3′ sites in these experiments, meaning that radical delocalization and equilibration occurs more quickly than radical trapping and formation of permanent oxidation products. This suggests that the distance dependence of DNA CT is quite low. In addition, guanine oxidation yields in conjugates containing the Δ isomer were higher than in those containing the Λ isomer, indicating that the efficiency of guanine damage is dependent on the interaction of the photooxidant with the base stack. Incorporation of a 5′-GGG-3′ far from the binding site led to oxidation primarily of the 5′-G of the triplet, 37 Å away from the intercalated Rh complex. Similar damage patterns were observed with the use of $[Ru(phen)(bpy')(Me_2dppz)]^{2+}$ (Me_2dppz = 9,10-dimethyl-dipyrido[3,2-a:2′,3′-c]phenazine)

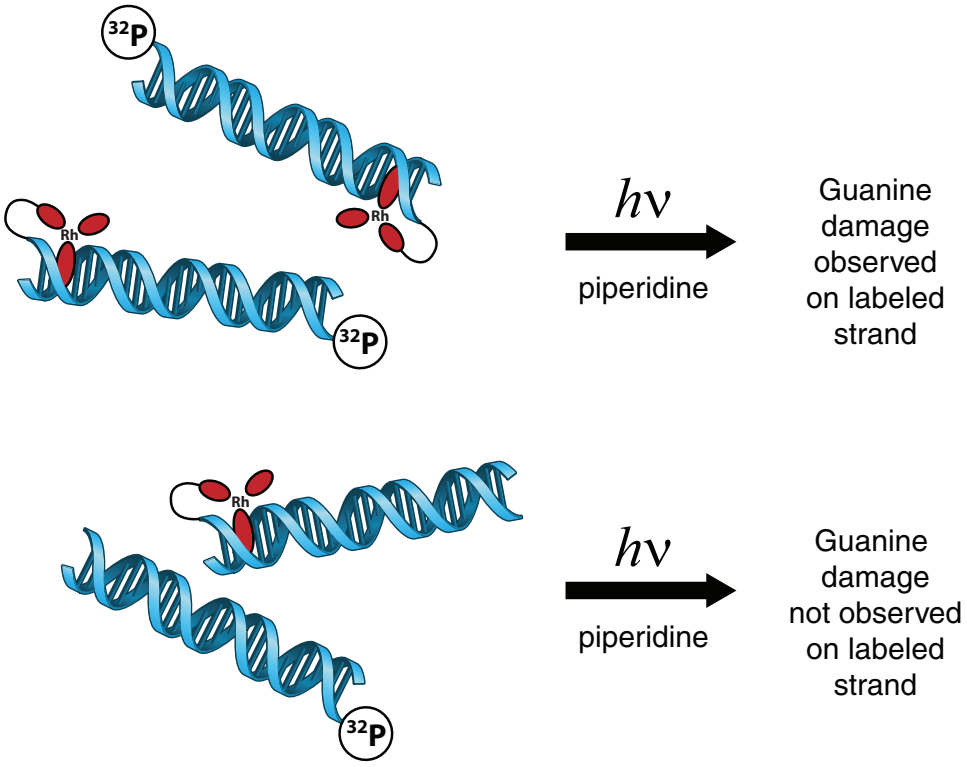


Figure 1.4: DNA-mediated oxidation is an intraduplex process. Top: guanine damage is observed by PAGE following irradiation and piperidine treatment of photooxidant-DNA conjugates that contain a ^{32}P label. Bottom: no guanine damage is observed following the irradiation and piperidine treatment of mixtures which contain unlabeled photooxidant-DNA conjugates and labeled DNA that has no photooxidant bound.

and the flash-quench reaction.[62] Interestingly, when only guanine singlets (no 5′-GG-3′ doublets) are incorporated into the base sequence, equal damage is observed at each guanine site, again suggesting that in the absence of a unique low energy site, charge migration and equilibration to sites of low oxidation potential occur at a faster rate than hole trapping.

Because oxidation yields at 5′-GG-3′ sites showed little variation with charge transfer distance over 11 base pairs, it was necessary to extend the length of the DNA to gain a better understanding of the distance dependence. To this end, a series of 28 base-pair duplexes were prepared with tethered $[Rh(phi)_2(bpy')]^{3+}$.[63] Each duplex in the series contained two 5′-GG-3′ sites that were separated from one another by increments of two base pairs, so that the distance between 5′-GG-3′ sites spanned a range from 41 to 75 Å. Upon irradiation, damage occurred at both sites, but the distal site consistently showed more damage than the proximal site. The ratio of damage between the distal and proximal sites decreased only slightly and fairly linearly over the distances measured. Because the 5′-GG-3′ sites were separated by increments of only two base pairs (6.8 Å, or one-fifth of a turn in the helix), any helical phasing effects on the relative damage yields could be ruled out. In order to test the effects of CT over even greater distances, 63 base-pair DNA duplexes containing six well-separated 5′-GG-3′ sites along their length and a tethered photooxidant (either $[Ru(phen)(bpy')dppz]^{2+}$ or $[Rh(phi)_2(bpy')]^{3+}$) were constructed by ligating smaller strands together. Irradiation of the ruthenated duplex by 436 nm light in the presence of MV^{2+} resulted in damage at the 5′ guanine of each doublet with a small diminution in oxidation with distance, showing that facile DNA-mediated oxidation can occur over 197 Å. The same experiment, carried out using the Rh-tethered duplex, yielded similar results. In these longer duplexes, damage yields decreased somewhat at longer distances, and this effect was more severe for ruthenium than for rhodium. The differences in damage yield at long distances were attributed to the ability of the flash-quench system to promote BET, differences in the extent of electronic coupling between the donor and the base stack in the two systems, and differences in the redox potentials of the donors. Interestingly, the damage yield ratio between distal and proximal sites increased dramatically with temperature, suggesting that higher temperatures facilitate charge equilibration along the length of the duplex.

In the 28 base-pair duplexes, replacement of a G·C base pair by a T·A base pair in the base sequence intervening between the two guanine doublets decreased the ratio of distal to proximal guanine damage by 38%.[63] This effect was more rigorously examined in subsequent work. Duplexes were constructed in which two guanine doublets were separated by increasing lengths of A- and T-containing sequences.[64] Photoexcitation of a tethered $[Rh(phi)_2(bpy')]^{3+}$ complex resulted in large differences in the ratio of distal to proximal oxidative damage. Sequences that showed the lowest ratio contained 5′-TATA-3′ sequences intervening between the guanine doublets, while those showing the highest ratio contained only adenine. Interestingly, when the number of thymine bases intervening between guanine doublets was increased from two to ten by increments of two, damage ratios were 0.9, 1.2, 2.2, and 0.4, respectively. These results illustrate that factors such as DNA conformation, energetics, and base dynamics, in addition to distance, affect the efficiency of CT.

Mismatches intervening between two guanine doublets also affect the distal-to-proximal damage ratio, although in a manner that is not intuitive. When each of the sixteen possible combinations of matched and mismatched base pairs were incorporated between two guanine doublets, the highest distal/proximal damage ratio was observed for the C·G matched pair (2.05), while the A·T matched pair showed the third lowest ratio (0.23), after the T·C (0.15) and T·T (0.19) mismatches.[65] The observed differences in damage ratios did not correlate with the duplex stability, the thermodynamic stability of the mismatches, or the redox potential of the mismatched base. While there was a reasonable correlation with the free energies of helix destabilization of the mismatches, the best qualitative agreement was with base pair lifetimes based on imino proton exchange rates between mismatched bases, as measured by ^{1}H NMR.

From these studies, it is apparent that many factors affect the yield of oxidative damage in DNA. Although shorter strands show little dependence on distance, damage yields are lower at longer distances in longer strands. Changes in the sequence intervening between two guanine doublets have a strong effect on the relative damage observed at the two sites, indicating that small changes in local conformation may disrupt the base stack locally, and that dynamic destacking at mismatch sites is sufficient to decrease severely

the amount of damage further down the strand. The observed temperature dependence in long strands is also an indication of the major role that dynamic motions in DNA play in facilitating CT, since higher temperatures allow the DNA to sample more conformational states within the lifetime of the radical. Finally, differences in damage yields depending on the oxidant used indicate that the ability of the oxidant to couple electronically to the base stack and the propensity for BET strongly affect the efficiency of long-range DNA CT. These experiments involving metal complexes, as well as experiments involving organic oxidants such as ethidium,[66–69] anthraquinone,[70] or thionine[71] and base analogues such as 1,N^6-ethenoadenine[57] and 2-aminopurine[72–74] have shown that long-range DNA oxidation is a general phenomenon.

1.2.4 Fast Charge Trapping to Monitor Charge Occupancy on the DNA Bridge

Traditionally, models for DNA CT (see Genereux and Barton[75] for a recent review) have fallen into two basic categories. The first is superexchange, in which the charge moves from the donor to the acceptor in a single coherent step, tunneling through an intermediating bridge. The second is localized hopping, in which the charge moves from base to base along the bridge, briefly occupying each site. These two models were refined as more sophisticated measurements of DNA-mediate CT were conceived and conducted. For example, during hole transport, simple hopping models predict hopping to occur between guanine sites, since they are lowest in energy. The observed charge occupation on bridging adenine led to the development of thermally assisted hopping models that resolve this inconsistency. Similarly, the influences of other bases and the solvation environment were included in even more complex polaron hopping models.

The guanine base, however, is a poor radical trap. The lifetime of a neutral guanine radical in DNA is greater than one millisecond,[58] and on that timescale, the electron can migrate extensively and equilibrate throughout the DNA duplex. In order to gain mechanistic insight into the process of DNA-mediated CT, cyclopropylamine-modified bases, which report on short-lived charge occupancy at specific sites in DNA, were incor-

porated into various sequence contexts. As illustrated in Scheme 1.3 on page 28, these modified bases, N^2-cyclopropylguanine (^{CP}G),[76] N^6-cyclopropylcytosine (^{CP}C),[77] and N^6-cyclopropyladenine (^{CP}A),[78] contain cyclopropyl groups that undergo a rapid ring-opening reaction upon oxidation. The rates of ring-opening are on the order of 10^{11} s^{-1}, as suggested by comparison with similar molecules,[79,80] making this reaction competitive with BET in most contexts. Further, the oxidation potentials, base pairing characteristics, and stacking properties of cyclopropyl-substituted bases are expected to be similar to those of the unmodified bases.[76,77,81]

Our first studies of DNA CT to ^{CP}G involved the use of photoexcited 2-aminopurine (Ap*) as the oxidant.[82] This analogue base-pairs with thymine and is well stacked in the DNA duplex. In addition, the CT process can be followed by monitoring quenching of Ap* fluorescence by guanine. In duplexes containing ^{CP}G, increasing temperatures caused an increase in the yield of ring-opened product until the melting temperature of the duplex was reached, at which point duplex stacking was lost and almost no product was formed. The same experiment, using free Ap* rather than Ap incorporated into the base stack, showed no temperature dependence, indicating that temperature only affects the CT process, not the trapping process. This increase in ring-opening yield with increasing temperature suggests that DNA CT is a dynamic process that is facilitated by the motion of the bases. In order to study the distance dependence of ^{CP}G ring-opening yield, several strands were synthesized in which adenine bridges of increasing length were incorporated between Ap and ^{CP}G. Surprisingly, the quenching data showed a reproducible nonmonotonic periodicity in the distance dependence. In addition, little damage was observed for sequences in which the Ap and ^{CP}G were neighbors, or were separated by one intervening base pair.[81] These observations suggest that charge delocalization among small, transient, well-stacked groups of bases facilitates charge transfer, and that at short distances, BET is kinetically favored over ring-opening. To accommodate these observations, a new model for DNA CT was proposed that involves conformationally gated hopping between well-stacked domains of delocalized charge.

This model was verified in further studies involving ^{CP}C oxidized by $[Rh(phi)_2(bpy')]^{3+}$.

When ^{CP}C was incorporated into strands 4–7 base pairs away from the tethered Rh complex, efficient ring-opening was observed upon photoexcitation, signifying that there must be some hole occupancy on cytosine during DNA CT, despite its high oxidation potential.[77] Interestingly, when ^{CP}G was incorporated at the site neighboring ^{CP}C, damage yields between the two traps were comparable, but when the distance between the ^{CP}C and the ^{CP}G traps was increased, the decomposition yield of the distal ^{CP}G decreased by a factor of two.[83] By examining ^{CP}C damage yields in various sequence contexts, the effects of neighboring bases were investigated further. In these studies, ^{CP}C decomposition depended not only on the sequence of bases intervening between the photooxidant and the hole trap, but also on the sequence distal to the hole trap. These results suggest that dynamic hole distribution on the DNA bridge is not just a function of the energies of the individual bases, and that some charge delocalization among the orbitals of neighboring bases must occur. Interestingly, while non-covalent $[Rh(phi)_2bpy]^{3+}$ is competent to oxidize both traps, non-covalent $[Ru(phen)(dppz)(bpy')]^{2+}$ in the presence of $[Ru(NH_3)_6]^{3+}$ does not show appreciable oxidation of ^{CP}C. This difference is consistent with the redox potentials of the two metal complexes.

The distance dependence of DNA CT was further studied by analyzing the decomposition yields of ^{CP}A and ^{CP}G within A tracts. Interestingly, when ^{CP}A was incorporated serially at each position along a 14 base pair A tract, very little change in decomposition was observed with distance following irradiation of the tethered $[Rh(phi)_2(bpy')]^{3+}$ photooxidant.[84] When ^{CP}G was incorporated at each position, however, the distance-dependent periodicity previously observed in 2-aminopurine studies was reproduced with the same apparent period, regardless of whether a $[Rh(phi)_2(bpy')]^{3+}$, anthraquinone, or Ap photooxidant was used.[85] Although this periodicity was similar to that observed earlier using an Ap* fluorescence quenching assay, the plots of damage yield versus distance obtained from the fluorescence quenching assay and the ^{CP}G assay were slightly different. These differences were explained recently: due to the nature of the assay, fluorescence quenching informs on the yield of single-step CT, while the ring-opening assay informs on total CT; therefore, any difference between the two is the yield of multistep CT.[86] At a distance of

8–9 bp, the yields obtained by Ap* fluorescence quenching and ^{CP}G ring-opening are equal, signifying that at this distance (27–30 Å), coherent transport takes place.

The ability of cyclopropyl traps to report on charge occupancy at various positions on the DNA bridge has allowed us to determine the relative influence of the various factors affecting the efficiency of DNA CT. Consistently and within a range of experiments, the ring-opening yield of the traps was observed to vary with distance, temperature, sequence context, and the redox potential of the donor. These observations support a model for DNA CT that consists of conformationally gated hopping of delocalized charge.

1.2.5 Comparing Long-Range DNA-Mediated Hole and Electron Transport with a Single Probe

Although the body of literature concerning DNA-mediated hole transport (HT) is quite extensive, complementary studies of DNA-mediated electron transport (ET) are relatively sparse. Our laboratory has extensively studied DNA-mediated ET using DNA-modified electrodes on gold.[87–93] While these experiments are interesting for many reasons, perhaps the most important question regarding DNA-mediated ET is whether the mechanism of this process differs in any way from that of DNA-mediated HT. Unfortunately, ET rates in these electrochemical constructs are limited by slow transfer through the thiol linker that connects the DNA to the gold surface.[94] Complexes such as $[(mes)_2Pt(dppz)]^{2+}$, which have been used both to oxidize ^{CP}G and to reduce ^{CP}C, are promising probes for solution state studies of DNA HT and ET, but these complexes are difficult to tether to DNA, making comparative studies of the distance dependence of HT and ET untenable.[95]

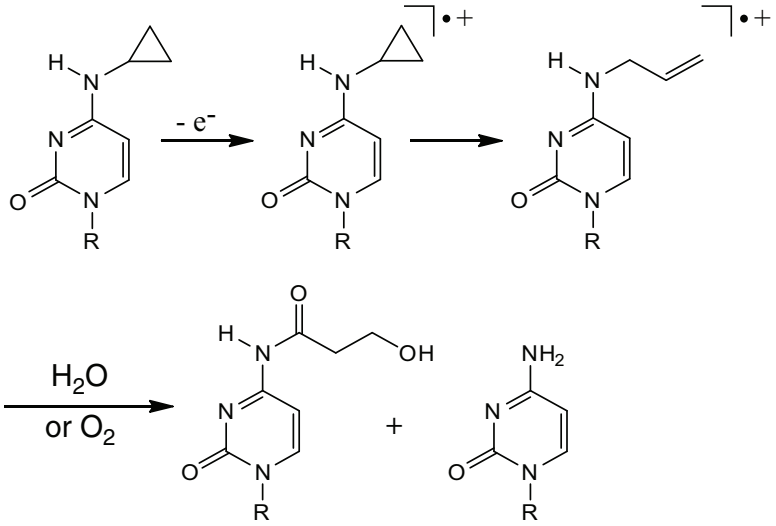
To this end, our laboratory has developed an iridium complex that is amenable to functionalization and acts as both a photooxidant and a photoreductant in the presence of DNA.[96] The complex, $[Ir(ppy)_2(dppz')]^+$ (ppy = 2-phenylpyridine), contains a dppz ligand modified with a carboxylic acid functionality that enables covalent tethering of the complex to the 5′ end of a DNA single strand via a C_6 alkyl chain. We envisage the intercalation of this complex as though the dppz ligand were threaded through the DNA, with the tether on one side of the duplex and the metal center and ancillary ligands on the other. Such

a binding mode could easily be achieved during annealing of the DNA single strands to create the duplex. The excited state oxidation and reduction potentials of the complex are estimated to be 1.7 and -0.9 V vs. NHE, respectively, indicating that it is competent for both oxidative HT to guanine and reductive ET to thymine or cytosine. Because this single complex can be used to probe both DNA HT and DNA ET, the mechanisms and efficiencies of these processes can be directly compared in the same duplex.

We conducted these studies by taking advantage of the fast ring-opening kinetics of cyclopropylamine-modified nucleobases. When non-covalent Ir complex was added to duplexes containing ^{CP}G, only ten minutes of irradiation were needed to achieve complete degradation of the CP rings. The reaction was less efficient in the case of ^{CP}C: after twenty minutes of irradiation, the yield of ring opening was 86%.[96] The mechanism of ^{CP}G ring-opening in this construct is oxidative, while that of ^{CP}C ring-opening is reductive. If the ^{CP}C ring-opening reaction were to occur by an oxidative mechanism, substitution of inosine for guanine opposite ^{CP}C should result in more efficient damage due to the decreased competition for holes. This effect was not observed. Further experiments involving covalently tethered Ir-DNA conjugates support these observations.[97] When ^{CP}G was incorporated into an adenine tract several bases away from the Ir complex intercalation site, the ^{CP}G ring-opening yield after one hour of irradiation was 46%. When ^{CP}C was incorporated into the duplex at the same site, the ring-opening yields were 31% when ^{CP}C was base paired with inosine and only 10% when ^{CP}C was base paired with guanine. These results suggest that within an adenine tract, ^{CP}C decomposition is an oxidative process, and that HT through an adenine tract is preferred over ET. When the modified bases were incorporated into thymine tracts instead, the results turned out differently. In these duplexes, the ^{CP}G ring-opening yield was very similar: 55% after one hour of irradiation. However, the ring-opening yields for ^{CP}C embedded within thymine tracts were much lower. The yields were 5% when ^{CP}C was paired with guanine and only 2% when ^{CP}C was paired with inosine. In this case, the yield was lower for the inosine-containing duplex, suggesting that ^{CP}C had been reduced and that ET is the preferred mechanism of charge transport through pyrimidines. Thus, the mechanism of ^{CP}C ring-opening depends strongly on the sequence context.

By systematically varying the distance between the intercalation site of the tethered Ir complex and the electron or hole acceptor in these assemblies, it is possible to compare the distance dependences of hole and electron transport directly, from the same probe and within the same sequence context. Experiments of this type were carried out utilizing ^{CP}A as a kinetically fast hole trap and 5-bromouridine (^{Br}U) as a fast, irreversible electron trap.[98] In order to reduce ^{Br}U, excited Ir was first reduced by ascorbate via the flash-quench technique. The distance dependence of each process was characterized by the parameter β, which serves as a proxy for the resistivity of the sequence and is a measure of the exponential decay in CT yield with distance. For HT, $\beta = 0.05$, while for ET, $\beta = 0.10$ or 0.12, depending on whether ^{Br}U was embedded within an adenine tract or a thymine tract, respectively. The shallow distance dependence observed in both cases suggests that HT and ET occur by similar mechanisms. Importantly, the amount of attenuation in CT yield upon the incorporation of a mismatch or abasic site at the position in the bridge neighboring the CT trap was identical for the ^{CP}A and ^{Br}U strands, indicating that successful charge migration along the duplex is less strongly affected by a change in the redox potential of the bridge than it is by perturbations in base stacking.

The ability of $[Ir(ppy)_2(dppz')]^+$ to participate in both electron and hole transfer within DNA allows for a two-step CT process, dubbed the "ping-pong" reaction, shown in Figure 1.5 on page 31. In this reaction, the complex is first reduced by DNA-mediated HT, then subsequently reoxidized by DNA-mediated ET, following a single photoexcitation event. The ping-pong reaction was utilized in a series of experiments involving HT to ^{CP}A followed by ET to either ^{Br}U or ^{CP}C, in order to understand more fully the similarities and differences between DNA HT and ET.[99] In one experiment, the distance between the ^{CP}A and the Ir binding site was increased while the distance between the ^{Br}U and the Ir binding site remained the same. As the ^{CP}A was moved further from the Ir binding site, both the ^{CP}A ring-opening yield and the ^{Br}U decomposition yield decreased, but the ratio of the decomposition yield to the ring-opening yield remained at about 40%. Importantly, very little ^{Br}U decomposition was observed in the absence of either ^{CP}A or the Ir complex. Strikingly, when ^{CP}C was substituted for ^{Br}U, ^{CP}C ring-opening was stoichiometric with



Scheme 1.3: The ^{CP}C ring-opening mechanism

^{CP}A ring-opening even though ^{CP}A and ^{CP}C ring-opening yields decreased as the ^{CP}A distance increased. Base pairing ^{CP}C with inosine rather than guanine had no effect, suggesting that ^{CP}C is opened reductively in this sequence context. These results show that the ping-pong reaction is generalizable and very efficient. Importantly, by using a single probe to trigger both HT and ET under the same experimental conditions, we have shown that the two mechanisms have similar characteristics, and that DNA CT, whether reductive or oxidative, is a general reporter for the integrity of the DNA base stack.

1.3 DNA Charge Transport in a Biological Context

Given the remarkable ability of DNA to mediate CT reactions over long molecular distances and the exquisite sensitivity of this process to perturbations in the base stack, we have begun to explore the biological implications of this chemistry. Below, we consider several cellular processes in which DNA-mediated CT may be utilized, including funneling of damage to particular sites of the genome and the participation of metalloproteins in DNA-mediated redox chemistry.

1.3.1 Generation of Mitochondrial DNA Mutations

DNA-mediated CT experiments have revealed that one-electron oxidation reactions, initiated using covalently tethered or intercalative metal complexes, can occur over distances as large as 200 Å.[63] This observation suggests that DNA CT can act in a physiological context, where CT over such long molecular distances may serve as a strategy to protect the genome from incessant oxidative stress. Experimental observations are in line with this proposal. Using ligation-mediated PCR to identify lesions, we have utilized $[Rh(phi)_2(bpy)]^{3+}$ to probe DNA CT in nuclei isolated from HeLa cells. The patterns of oxidative damage observed in this system are the same as those observed for guanine oxidation by $[Rh(phi)_2(bpy)]^{3+}$ *in vitro*; damage occurs at the 5′-G of 5′-GG-3′ sites. This result suggests that guanine oxidation occurs via DNA-mediated CT even in the presence of constitutively bound proteins.[56,100]

Hallmarks of DNA-mediated oxidation are observed in other cellular environments

such as mitochondria. These organelles contain their own DNA (mtDNA), making them particularly interesting systems in which to examine the possibility of DNA-mediated CT *in vivo*. Such experiments were carried out using mitochonria from HeLa cells. Oxidative damage was introduced by incubating HeLa cells with $[Rh(phi)_2(bpy)]^{3+}$, which is readily absorbed, and then irradiating them.[101] Sites of DNA-mediated oxidation were revealed by a primer extension assay. Base oxidation resulting from DNA-mediated hole migration was observed primarily in conserved sequence block II of mtDNA, a critical regulatory element involved in DNA replication.[102–104] Mutations within this region of mtDNA are associated with tumor formation and other disease conditions.[105]

Remarkably, sites of oxidation within the mitochondria occur as far as 70 bases from the intercalated metal complex, suggesting that DNA-mediated CT plays a role in the regulation of oxidative damage in mtDNA.[102] Conserved sequence block II, which contains seven consecutive guanines, is a site of very low oxidation potential, and could therefore act as a sink for lesions such as 8-oxo-dG.[100] Presumably, funneling damage to this region via DNA-mediated CT halts replication of a damaged mitochondrial genome, preventing the propagation of genetic errors (Figure 1.6). Nevertheless, replication of DNA containing lesions ultimately leads to mutations within conserved sequence block II such as G-to-T transversions. These mutations ultimately reduce the amount of guanine in the regulatory region, eliminating the checkpoint and funneling functions of the site, and decreasing mitochondrial efficiency. Tumor cells, which do not necessarily depend on respiration, could survive by utilizing alternative energy pathways despite mitochondrial malfunction.[101]

1.3.2 DNA-Mediated CT with Metalloproteins: Establishing DNA-Bound Redox Potentials

1.3.2.1 Base Excision Repair Enzymes

Base excision repair (BER) proteins identify and remove oxidized bases from DNA. Several of these enzymes, such as MutY and endonuclease III (EndoIII) from *E. coli*, are known to contain redox-active $[4Fe\text{-}4S]^{2+}$ clusters. However, a distinct role for these cofactors has been investigated only recently. Interestingly, although MutY is capable of folding in the absence

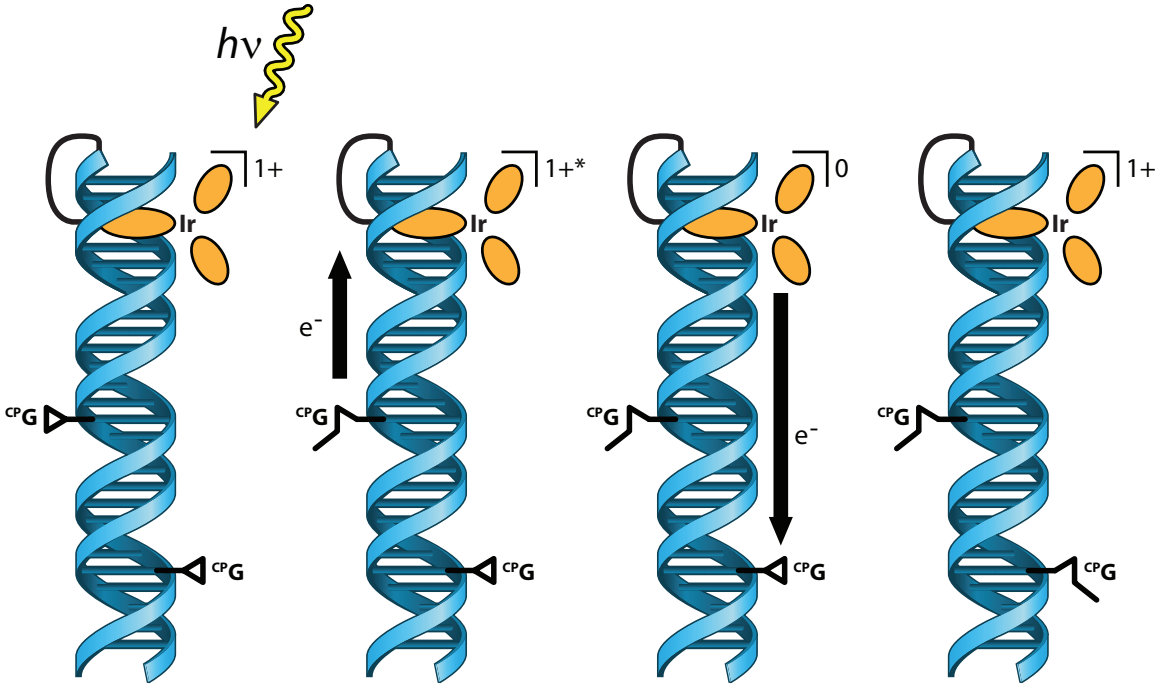


Figure 1.5: Ping-pong electron transfer. From left to right: photoexcitation of the Ir complex results in DNA-mediated ET from the ^{CP}G base. Subsequent ET from the Ir complex reduces the ^{CP}C base.

of the cluster, the cluster is required for DNA binding.[106] Experiments with EndoIII showed that the solvent-accessible cluster undergoes decomposition when treated with oxidizing ferricyanide. The protein is resistant to reduction, having an estimated $[4Fe{-}4S]^{2+/1+}$ couple midpoint potential of less than -600 mV.[107,108] Notably, initial measurements of the redox potentials of these proteins were performed in the absence of DNA.

Experiments on DNA-modified electrodes were conducted to determine whether DNA binding might alter the redox properties of the [4Fe-4S] cluster (Figure 1.7). Cyclic voltammetry of MutY at the DNA-modified surface yielded a midpoint potential of +90 mV vs. NHE, an appropriate potential for a physiologically active redox switch.[109] In the absence of DNA, or when the electrode was modified with duplex DNA containing an abasic site, no signal was observed, proving that electron transfer occurs through the DNA base stack to the redox cofactor of bound MutY. In similar experiments, the redox potentials of EndoIII and the *Archeoglobus fulgidus* uracil DNA glycosylase (AfUDG) were measured as 59 mV vs. NHE and 95 mV vs. NHE, respectively.[109]

In order to compare the redox potential of EndoIII in the absence and presence of DNA directly, electrochemical experiments were conducted using highly oriented pyrolytic graphite (HOPG) electrodes.[110] On a bare electrode, oxidative scans of EndoIII yielded an irreversible anodic peak at ~ 250 mV and loss of the yellow solution color, indicating degradation of the $[4Fe\text{-}4S]^{2+}$ cluster to form the $[3Fe\text{-}4S]^{+}$ cluster. Consecutive positive scans showed broad, irregular signals at -80 and -710 mV vs. NHE, consistent with degradation. In contrast, on electrodes modified with pyrenated DNA, a reversible midpoint potential of ~ 20 mV vs. NHE was observed. DNA is thus necessary for the stable oxidation of EndoIII. By comparing the oxidation potential of EndoIII in the absence and presence of DNA, it was determined that EndoIII in the 3+ oxidation state binds DNA at least 1000 times more tightly than the reduced 2+ form, suggesting that iron-sulfur cluster-containing proteins become activated upon oxidation.

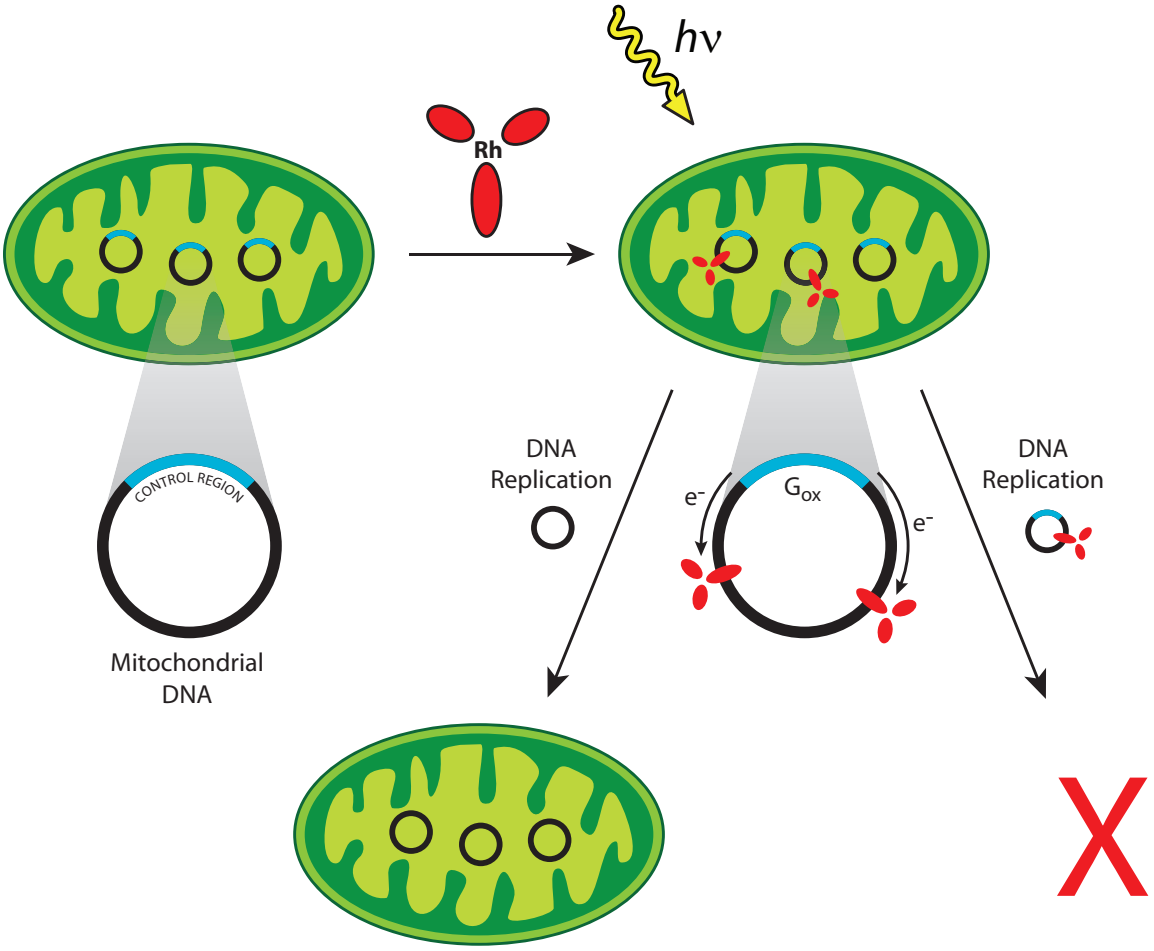


Figure 1.6: Damage of mitochondrial DNA via DNA-mediated CT. Multiple copies of mitochondrial DNA (black) are found in mitochondria (green) within the cell. Following incubation with $[Rh(phi)_2(bpy)]^{3+}$, irradiation of the cells results in oxidation at low potential guanine sites (G_{ox}). Damage in the genome is funneled (curved arrows) via DNA-mediated CT to the control region (blue), preventing replication of the lesion-filled plasmid (bottom right). In the absence of Rh, mitochondrial replication occurs as normal (bottom left).

1.3.2.2 The SoxR Transcription Factor

Other redox-active proteins may also become activated upon oxidation. The SoxR transcription factor regulates the response to superoxide within the cell. SoxR binds DNA as a dimer and contains two [2Fe-2S] clusters (one in each monomer) that are not required for protein folding.[111–114] The oxidation of SoxR leads to the expression of the SoxS transcription factor, which controls expression of genes involved in protecting the cell from stress. Interestingly, the oxidized and reduced forms of the protein show equal affinities for the SoxR promoter.[115] The redox potential of SoxR in the absence of DNA, however, is approximately -290 mV.[113,116,117] This value, much lower than the potential within the cell, is inconsistent with the proposed mechanism of SoxR; a redox sensor for oxidation cannot function if it is always switched on. Determination of the DNA-bound redox potential of SoxR provides insight into the activation mechanism of the protein. On HOPG surfaces modified with pyrenated DNA, a quasi-reversible electrochemical signal was observed for the [2Fe-2S] cluster of SoxR at +200 mV vs. NHE.[115] Similar potentials were observed for SoxR extracted from several different organisms. Thus, DNA binding shifts the redox potential of SoxR by $\sim +500$ mV. It is likely that this shift in potential provides the energy for the torquing of DNA by oxidized SoxR, activating transcription. Critically, we see the importance of performing redox measurements of DNA binding proteins in the presence of DNA, as the DNA polyanion alters the protein environment and the potential. MutY, EndoIII, and SoxR show redox activity in a physiologically relevant regime only when bound to DNA.

1.3.3 DNA-Mediated Cross-Linking and Oxidation of MutY

The methods used to probe long-range DNA-mediated oxidation of 5′-GG-3′ have also aided in the study of DNA/protein interactions, allowing us to gain insight into how these metalloproteins might take advantage of the unique property of DNA to conduct charge. It has been shown that photoactivation of metallointercalators in the presence of DNA-bound protein can lead to the formation of protein-DNA cross-links. Experiments harnessing DNA-mediated CT to generate such cross-links have provided a more detailed look at the

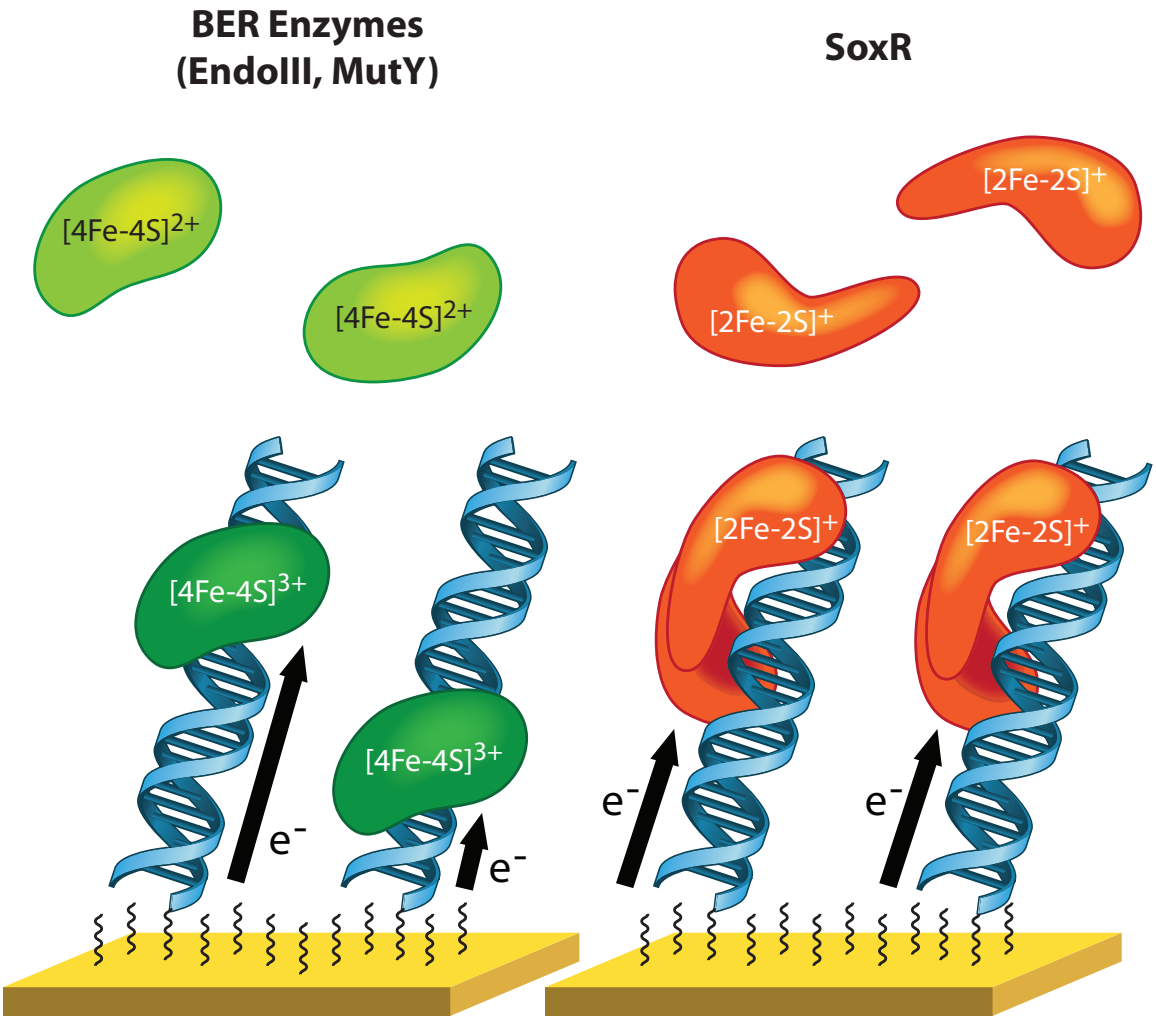


Figure 1.7: Illustration of surfaces used for DNA-protein electrochemistry of BER proteins (left) and SoxR (right). DNA duplexes are attached to the gold surface via a 5′ thiol linker. Mercaptohexanol (curved lines) is used as a backfilling agent, preventing direct contact between the redox probe and the electrochemical surface. Electrons travel (arrow) from the gold surface to the bound protein. DNA binding activates EndoIII and MutY toward oxidation to the 3+ state, and SoxR binds as a dimer.

amino acid/DNA interface.[118–126]

DNA photooxidation and protein cross-linking experiments were performed in the presence of MutY to better understand the factors affecting the detection of its target lesion, adenine mispaired with 8-oxo-dG. In these experiments, $[Rh(phi)_2(bpy')]^{3+}$, covalently tethered to the DNA strand, was used to generate oxidative damage.[126] Early models suggested that MutY searches for damage by flipping bases out of the DNA duplex one by one for interrogation. In our experiments, protein binding decreased the oxidation yield, but it did not affect the oxidation pattern, even at high concentrations.[127] This result suggests that MutY binding does not perturb the DNA structure, but that MutY may act as a hole sink in addition to guanine. It also indicates that the protein might use a mechanism other than base flipping to search for damage. In cross-linking experiments, adducts were observed to form between 8-oxo-dG and lysine 142 of MutY, even though the protein binding site was separated from the Rh photooxidant binding site by more than 20 Å. It should be noted that lysine 142 in MutY was thought to play a large mechanistic role during the repair of 8-oxo-dG:A lesions based on early cross-linking and NMR investigations.[50,119,128] This result indicates that protein/DNA cross-links can be formed by long-range DNA-mediated CT.[126]

Several experiments have shown that the strategies used to oxidize guanine can also be used to oxidize DNA-bound MutY. For example, low-temperature EPR measurements of samples including $[Ru(phen)_2dppz]^{2+}$, the quencher $[Co(NH_3)_5Cl]^{2+}$, and poly(dG-dC) or poly(dA-dT) in the absence or presence of MutY revealed a primary g value of 2.02. This signal was attributed to the $[3Fe\text{-}4S]^{+}$ cluster, formed upon oxidative degradation of the $[4Fe\text{-}4S]^{2+}$ cluster. Smaller signals at g values of 2.08 and 2.06 were assigned to the stable oxidized cluster, $[4Fe\text{-}4S]^{3+}$.[129,130] Interestingly, these latter signals were of significantly lower intensity in the poly(dA-dT) sample than in the poly(dG-dC) sample. This result suggests that the oxidation of MutY occurs more efficiently when transient charge occupation on guanine is possible. This reaction is described in Scheme 1.4.

The DNA-mediated oxidation of MutY was also investigated by time-resolved spectroscopy using the flash-quench technique.[129] In the absence of protein, the transient ab-

sorption decay corresponded to decay of the guanine radical. In the presence of protein, the transient absorption decays showed two phases: a fast phase due to decay of the guanine radical and a slow phase with a spectral profile characteristic of the $[4Fe\text{-}4S]^{3+/2+}$ difference spectrum. Thus, the DNA-mediated oxidation of MutY can be observed directly. Importantly, these experiments established that guanine radical formation can directly lead to the oxidation of bound protein.

Collectively, biochemical experiments, EPR, and transient absorption spectroscopy indicate that MutY can be oxidized by a DNA-mediated mechanism. Considering the function of this enzyme, it is reasonable that, within the cell, DNA-mediated CT initiated by oxidative stress may serve as a means to activate repair. Further, considering that the redox potentials of BER proteins lie in a physiologically relevant regime only when the proteins are bound to DNA, and that mismatches and lesions block DNA-mediated CT,[88,131,132] it is even possible that DNA-binding redox active proteins may scan large stretches of the genome for damage by passing charge between them.[109,110,115,127,133]

Our model for DNA-mediated signaling between redox-active proteins is described for MutY as follows.[127] Binding to DNA shifts the potential of the MutY $[4Fe\text{-}4S]^{2+}$ cluster toward oxidation. In the absence of oxidative stress, the cluster remains in the 2+ state. Guanine cation radicals, formed endogenously under conditions of oxidative stress, can oxidize the cluster from the 2+ to the 3+ state. This reaction neutralizes the guanine radical and increases the affinity of MutY for the duplex by three orders of magnitude. If a second MutY protein is bound in the 2+ state at a distant site, charge can be transferred through the duplex from the first to the second protein. The first protein, now in the 2+ state, loses affinity for DNA and is free to relocate to another site. This process comprises a scan of the region of DNA between the proteins for damage. However, in the event that a lesion known to attenuate charge transfer, such as a base pair mismatch or an oxidized base, intervenes between the bound enzymes, DNA-mediated CT cannot proceed. If this occurs, the proteins remain in the vicinity of the damage, slowly processing to the site of damage.

Interestingly, this process is not limited to pairs of the same protein, or even to iron-

sulfur cluster proteins; any redox-active protein with a potential in the vicinity of 100 mV is expected to participate in the search process. The ability of MutY to help EndoIII find its target lesions has already been reported.[133] In addition, experiments are currently being conducted in our laboratory to determine the extent to which iron-sulfur cluster-containing helicase enzymes, such as XPD and DinG, can assist in the search for damage.

1.3.4 Transcriptional Activation in SoxR by DNA-Mediated Oxidation

In light of our findings regarding the role that DNA-mediated CT may play in the BER pathway, we have examined the DNA-mediated oxidation of SoxR, an iron-sulfur cluster-containing transcription factor. SoxR is activated in the presence of oxidative stress; however, the identity of the specific oxidant is unknown. *In vivo* studies using redox-cyclers such as paraquat to induce oxidative stress show that superoxide is not the direct activator of SoxR. Rather, the redox-cyclers deplete cellular NADPH, which is normally required to keep SoxR in a reduced form. The redox-cyclers then undergo autooxidation, losing an electron to dioxygen and producing superoxide. In laboratory experiments, the protein can be reversibly reduced with dithionite, or it can be reversibly oxidized with plumbagin and phenazine methosulfate.[113] Electrochemistry of SoxR shows a redox signal for the [2Fe-2S] cluster at +200 mV vs. NHE, indicating that the protein undergoes one-electron oxidation when bound to DNA.[115] Due to the redox shift observed upon DNA binding, it is reasonable that the DNA-bound form of SoxR might be the missing oxidative switch. In a process similar to that observed for MutY, guanine radicals generated by ROS may activate SoxR. Once oxidized, SoxR can then promote transcription to combat oxidative stress.

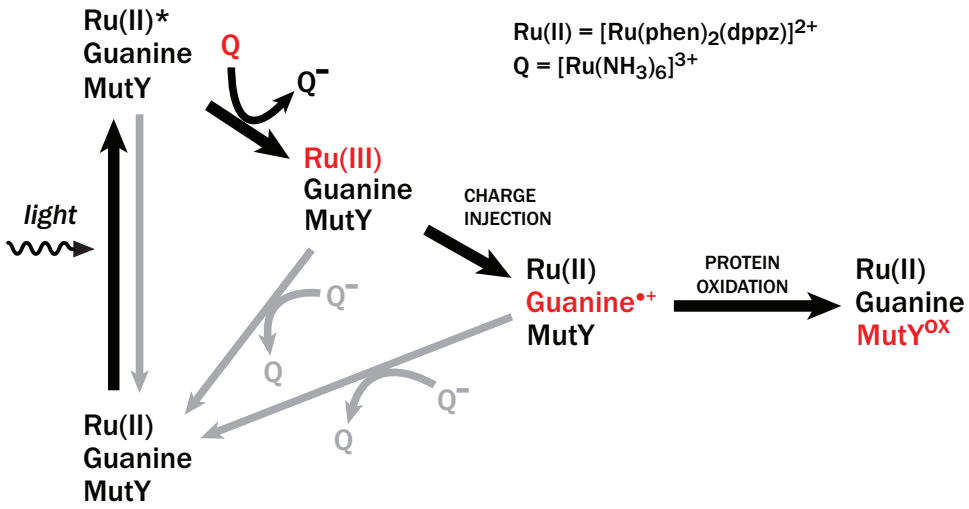
To test this theory, SoxR oxidation experiments were recently conducted in our laboratory using $[Ru(phen)(dppz)(bpy')]^{2+}$ and the flash-quench technique.[134] In these studies, $[Co(NH_3)_5Cl]^{2+}$ was used as the quencher, eliminating back electron transfer. Similarly to the MutY oxidation experiments described earlier, the yield of 5′-GG-3′ oxidation products decreased in the presence of reduced (activatable) SoxR. This indicates that SoxR is able to donate an electron to the oxidized guanine, filling the radical hole with its own lost electron, and being oxidized itself. Conversely, in the absence of SoxR, or when fully

oxidized SoxR was included in solution, no attenuation in damage was observed. These results clearly show that SoxR is able to interact with the DNA base stack and participate in DNA-mediated CT. Further, the SoxR response to guanine radicals generated in DNA suggests that oxidative damage can initiate protein activation.

In order to examine the SoxR response to DNA damage within the cell, *E. coli* cultures were treated with $[Rh(phi)_2bpy]^{3+}$. This intercalator, upon photoactivation, was previously shown to generate guanine damage in the mitochondria of HeLa cells.[101,102,135] In our experiment, transcription of the *soxS* RNA product, observed using reverse transcription PCR, indicated activation of SoxR. Bacteria irradiated in the presence of the Rh photooxidant showed much higher *soxS* product levels than non-irradiated bacteria. Additionally, expression levels of *soxS* in the presence of $[Rh(phi)_2bpy]^{3+}$ were similar to those measured when methyl viologen, a redox-cycler, was used instead. Since the complex cannot oxidize SoxR in the absence of DNA, it is assumed that SoxR oxidation takes place through a guanine radical intermediate. To determine whether SoxR can be activated over a distance by long-range DNA-mediated CT, $[Rh(phi)_2bpy]^{3+}$ was tethered to a 180-mer duplex of DNA containing the SoxR binding site and the -10 and -35 promoter regions of *soxS* (Figure 1.8).[61,134] Following irradiation, gene products were monitored with an anaerobic abortive transcription assay. The abortive transcription product, a 4-mer, was only observed in samples containing reduced SoxR and Rh-tethered DNA. Importantly, there was no direct photooxidation of SoxR by $[Rh(phi)_2bpy]^{3+}$ in the absence of DNA. These results indicate that DNA-mediated transcriptional activation of SoxR can occur over a distance of 270 Å. DNA CT has therefore emerged not only as an indicator of oxidative damage, but also as a signal to metalloproteins in the genome to initiate repair.

1.4 Conclusions

Initially used as general probes to understand the nature of DNA-mediated CT, the utility of metal complexes increased greatly. Precise tuning of reactivity and specificity has resulted in complexes that are capable of generating damage *in vivo*. Probe design has become more nuanced, taking into account characteristics including ligand sterics, DNA binding modes,



Scheme 1.4: The flash-quench technique can be used to generate Ru(III) and subsequently oxidize DNA-bound MutY. Following photoexcitation, Ru(II)* is quenched, forming the powerful ground state oxidant Ru(III). This species proceeds to oxidize guanine within the base stack. The guanine radical can form oxidation products or it can oxidize a bound protein such as MutY. Back electron transfer reactions, which decrease the yield of oxidized protein, are shown in gray. The species that holds the moving charge at each step is shown in red.

the extent of electronic coupling to DNA, and stereospecificity. Metallointercalators such as $[Rh(phi)_2bpy]^{3+}$ initiate electron transfer in DNA upon photoactivation, resulting in observable damage to guanine, which is the most easily oxidized nucleobase. Charge transport through DNA can occur over extremely long molecular distances. The rich photophysical properties of these complexes in particular have facilitated spectroscopic characterization of the mechanism of DNA-mediated CT, and experiments with cyclopropyl-modified bases have allowed for direct comparison between hole and electron transfer.

Experiments conducted *in vivo* also depend on our understanding of the photophysical mechanics of metal complexes since these photooxidants are used to trigger DNA damage within cells. Utilizing metal complexes as photooxidants, the funneling of damage to specific regulatory sites in the mitochondrial genome has been observed. Metal complexes have also been used to activate repair proteins and to initiate transcription. In these latter applications, we have also observed DNA-mediated redox chemistry to metal centers, but here the coordination scaffolds are the amino acid backbones of metalloproteins. Using early experiments with simple coordination complexes to guide our investigations, our goal is now shifting from the understanding of DNA-mediated CT as a fundamental physical process to the recognition of this fascinating chemistry in cellular sensing and signalling.

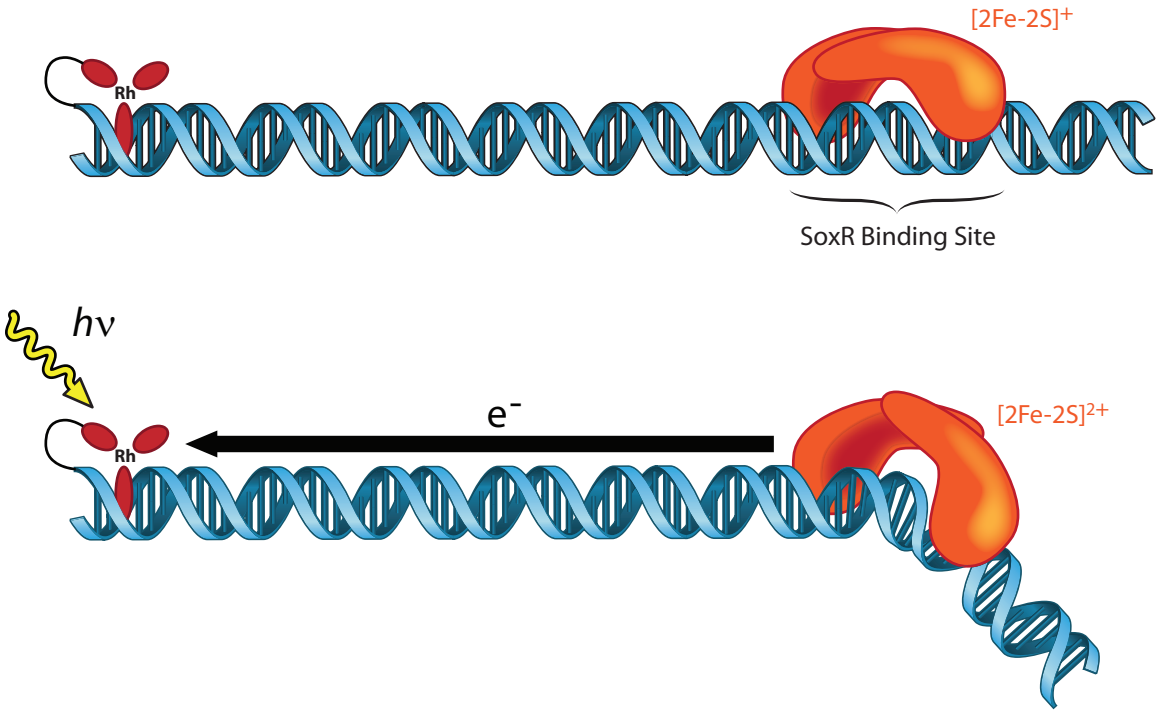


Figure 1.8: A model for the transcriptional activation of SoxR via DNA-mediated oxidation. Top: SoxR is bound to a DNA duplex at a position well removed from the covalently bound photooxidant $[Rh(phi_2)(bpy')]^{3+}$. SoxR initially binds in the reduced (+1) state. Bottom: Photoactivation of the metal complex triggers electron transfer, resulting in the oxidation of SoxR to the (2+) state and a structural change in the protein. The structural change induces kinking of DNA at the SoxR binding site, which signals recruitment of transcription machinery such as RNA polymerase.

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