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Long-Range DNA Charge Transport

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The stack of base pairs within double helical DNA has been shown to mediate charge transport reactions. Charge transport through DNA can result in chemistry at a distance, yielding oxidative DNA damage at a site remote from the bound oxidant. Since DNA charge transport chemistry depends on coupling within the stacked base pair array, this chemistry is remarkably sensitive to sequence-dependent DNA structure and dynamics. Here, we discuss different features of DNA charge transport chemistry, including applications as well as possible biological consequences and opportunities.

The inner core of double helical DNA is composed of a stacked array of aromatic, heterocyclic base pairs (Figure 1). This array of π-orbitals resembles a one-dimensional aromatic crystal, and it was suggested shortly after elucidation of the double helical structure of DNA that the base stack might provide a pathway for charge transport (CT) reactions.[1] Numerous solid-state π-stacked arrays have been identified, and these materials tend to exhibit semiconductive or conductive behavior, especially in the presence of dopants.[2] However, double-helical DNA, as a molecular π-stack in solution, presents a unique, well-defined system in which to explore CT. Critical to the characterization of DNA CT was the ability to construct, through chemical synthesis, well-defined DNA assemblies with pendant probes of the CT process. Through a variety of spectroscopic, biochemical, and biophysical studies, it is now established that the DNA π-stack can, indeed, provide a medium for CT.[3−6] Interestingly, the differences between DNA as a molecular π-stacked array and π-stacked solids may be as important as the chemical similarities in characterizing DNA CT chemistry.

This Perspective describes some of the experiments conducted in our laboratory to probe and exploit DNA-mediated CT chemistry. Efficient chemistry is observed when both the charge donor and acceptor are electronically coupled into the base stack. To this end, we have exploited various intercalating donors and acceptors, covalently tethered to the ends of a double helix and we have monitored the reaction spectroscopically.[7] We have also learned that DNA CT can yield chemistry at a distance.[8] Thus, the DNA itself can directly participate in the redox chemistry, functioning as the electron donor. Of the nucleic acid bases free in solution, guanine (G) is the easiest to oxidize (E_0 = 1.3, 1.4, 1.6, and 1.7 V vs NHE for G, A, C, and T, respectively),[9] and photooxidants bound to DNA can promote oxidative damage at a remote guanine site through DNA CT. Using a variety of DNA-bound oxidants, many laboratories have now probed the factors affecting DNA CT and the yield of resultant oxidative damage. As a result, scientists are now asking not *if* DNA can mediate CT but rather *how* this process occurs.

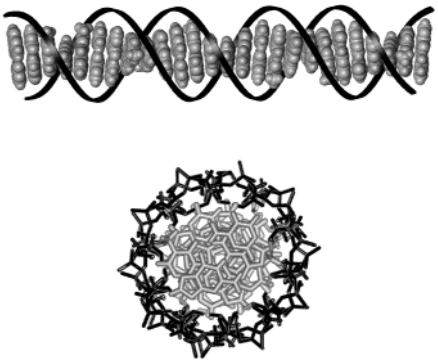

FIGURE 1. Schematic representation of double-helical DNA. The array of π-stacked bases is shown in gray, the sugar−phosphate backbone as a ribbon in black: above is a side-on view, below a view down the helical axis.

Given that DNA CT can be efficient and lead to chemistry over long molecular distances, we can also begin to ask what are the biological consequences and opportunities for DNA CT. Does DNA CT play some role in the oxidative damage of the genome? Are there regions of the genome to which damage is funneled through CT? How is long-range oxidative damage affected by packaging of the DNA within chromatin? Additionally, does DNA CT offer a means of long-range signaling between

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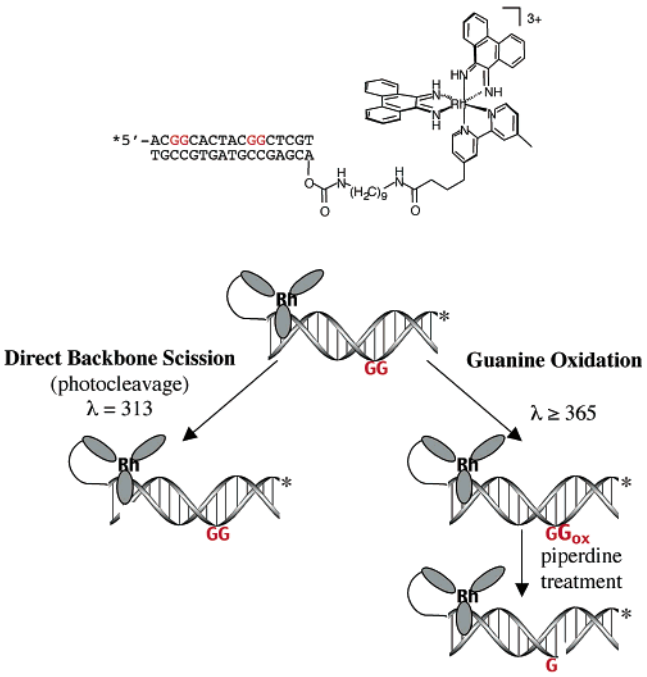

FIGURE 2. Shown above is the first DNA assembly in which long-range oxidative damage to guanine bases was observed using a tethered photooxidant. Damage to DNA by the photooxidant $[Rh(phi)_2(bpy')]^{3+}$ can occur by two distinct paths. After irradiation at high energy, a short-range reaction, which identifies the site of intercalation, occurs (left side). Long-range CT, which promotes oxidative damage (G_{ox}) at a distance, occurs after low energy excitation (right side). These two mechanisms allow for clear delineation of site of radical generation and site of CT damage enabling long-range chemistry to be identified.

proteins bound to DNA? Here, we also consider some of these possibilities.

1. Oxidative DNA Damage via Charge Transport

Oxidative DNA damage at a distance was demonstrated first in an oligonucleotide assembly containing two 5′-GG-3′ sites spatially separated from a tethered photooxidant, $[Rh(phi)_2bpy']^{3+}$ (phi = phenanthrenequinone diimine; bpy′ = 4′-methylbipyridine-4-butyric acid).[8] High-resolution NMR studies[10] and a recent 1.2 Å crystal structure[11] of a phi complex of rhodium bound to DNA reveal intercalative binding of the photooxidant from the major groove; importantly, these complexes bind with minimal perturbation of the surrounding bases. The phi ligand inserts deeply into the base stack and behaves essentially like an additional base pair. The rich photochemistry of phi complexes of rhodium allows not only for the initiation of long-range CT chemistry, but also identifies the exact binding site of the photooxidant (Figure 2).[12] When irradiated at high energy (λ = 313 nm), these complexes promote direct DNA strand cleavage by hydrogen atom abstraction from the sugar ring near the photoexcited intercalated phi ligand, marking the site of intercalation. Irradiation at lower energy ($\lambda \geq$ 365 nm) generates a potent photooxidant (E_0 ($Rh^{3+*/2+}$) $\sim$ 2 V vs NHE) that leads to damage of the guanine bases in DNA. Piperidine treatment results in strand breakage neighboring the damaged bases[13] and the yield of damage products can be analyzed by gel electrophoresis.

Remarkably, in the assemblies designed, oxidative DNA damage was observed at both 5′-GG-3′ sites, located 17 and 34 Å from the site of rhodium binding.[8] Specifically, damage was observed at the 5′-G of the 5′-GG-3′ guanine doublets. Ab initio molecular orbital calculations have revealed the HOMO for stacked guanines is localized on the 5′-G of guanine doublets.[14] This 5′-G reactivity is now considered the hallmark of long-range CT chemistry; nonspecific reaction at guanine bases suggests instead an alternate chemistry, such as reaction with reactive oxygen species.

Since these first studies with the rhodium intercalator, organic intercalators such as naphthalene diimide (NDI),[15] ethidium,[16] and modified anthraquinones[17] have been used to promote long-range oxidative DNA damage. Modified nucleotides such as 5-cyanobenzene deoxyuridine[18] and 4′-pivaloyldeoxythymine[19] have also been photolyzed to generate hot base and sugar radicals, respectively, that lead to oxidative guanine damage from a remote site. The ability to affect long-range chemistry with a family of such varied oxidants indicates that the ability to mediate CT is a characteristic of the DNA duplex, not the oxidant utilized. Use of the full family of oxidants results in damage patterns consistent with CT, oxidation of the 5′-G of 5′-GG-3′ sites.

2. Distance Dependence of DNA Charge Transport

To probe systematically the distance dependence of long-range oxidative damage, a series of 28 base pair duplexes containing tethered $[Rh(phi)_2bpy']^{3+}$ and both proximal and distal 5′-GG-3′ sites was constructed.[20] The proximal guanine doublet was fixed with respect to the rhodium intercalator, while the distal guanine doublet was marched out in two base pair increments relative to the photooxidant binding site. The ratio in yield of damage at the distal versus proximal guanine doublets provides a measure of the relative efficiency of the CT reaction. Over distances of 75 Å, the yield of oxidative damage was not significantly diminished, suggesting a very shallow distance dependence. Further evidence for a shallow distance dependence in DNA CT was observed using a 63 base pair duplex containing either tethered $[Rh(phi)_2bpy']^{3+}$ or $[Ru(bpy')(dppz)(phen)]^{3+}$ (dppz = dipyridophenazine; phen = 1,10-phenanthroline); the assemblies also contained six 5′-GG-3′ sites located 31 to 197 Å from the metallointercalator (Figure 3).[20] Extraordinarily, in both assemblies, oxidative damage was observed at all 5′-GG-3′ sites including that almost 200 Å from the site of charge injection. Damage over this distance regime has been confirmed in analogous experiments using a tethered anthraquinone moiety as photooxidant.[21] These experiments made clear that DNA CT can proceed over biologically significant distances.

While oxidative damage over a long range has been seen using a variety of oxidants, it has become increasingly clear that variations do occur in the efficiency of long-range reaction depending upon the oxidant employed. It had been proposed that differences seen with rhodium photooxidants versus anthraquinone photooxidants might reflect aggregation by the rhodium tethered species.[22] The possible clustering of metallointercalators on DNA was probed earlier using NMR and no evidence for such clustering was seen.[23] Moreover, in examinations

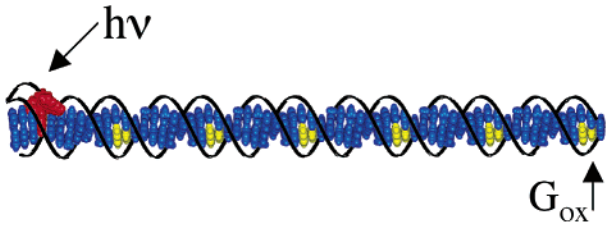

FIGURE 3. Schematic representation of a DNA duplex with a tethered rhodium photooxidant containing six 5′-GG-3′ guanine doublets up to 200 Å from the metallointercalator binding site. Oxidative damage at each of the guanine doublet sites, as a result of photoexcitation of the rhodium intercalator, has been demonstrated.

of tethered duplex assemblies under the conditions utilized for CT studies, no aggregation was observed. Nonetheless, recent work[24] in our laboratory to compare directly oxidative DNA damage by a phi complex of rhodium, a dipyridophenazine complex of ruthenium, and modified anthraquinones under identical conditions and using identical sequences has shown that the amount of damage seen at a 5′-GG-3′ site proximal versus distal to the tethered oxidant varies significantly with the photooxidant. The differences we observe likely arise from several factors that depend on the oxidant employed: energetics, the efficiency of back electron transfer, and the coupling,[25] or lack thereof, of the oxidant to the base stack. Understanding mechanistically the basis for these differences is something we need still to achieve.

3. Sensitivity of DNA Charge Transport to Base Stacking

While charge migration through DNA is possible over long molecular distances, it is nonetheless modulated by intervening DNA structure and stacking. DNA CT is exquisitely sensitive to static and dynamic perturbations in base stacking. An effectively coupled aromatic π-array is requisite for long-range CT and variations in stacking can lead to substantial changes in efficiency and yield (Figure 4). As an example, we found that the introduction of base bulges between 5′-GG-3′ sites located distal and proximal to a tethered rhodium intercalator resulted in significant diminutions in the distal/proximal ratios of oxidative damage.[26] Upon the insertion of a 5′-ATA-3′ bulge, the amount of charge reaching the distal site was attenuated by 75%, clearly reflecting the importance of an intact base stack.

Introducing a series of base-pair mismatches between 5′-GG-3′ sites located distal and proximal to a tethered, intercalated ruthenium(III) oxidant also produced decreased distal/proximal ratios of oxidative damage, in fact, to extents similar to those seen with base bulges.[27] A systematic examination of base mismatches revealed that some mismatches severely destabilize the helix, while others yield more subtle variations. The ratio of distal/proximal oxidative damage varies in the order GC ~ GG ~ GT ~ GA > AA > CC ~ TT ~ CA ~ CT. The purine−purine mismatches do not greatly diminish CT to the distal guanine doublet, while introduction of a pyrimidine-pyrimidine mismatch results in significantly attenuated yields of oxidative damage. The extent of long-range guanine oxidation was compared with the thermodynamic stability of the mismatch-containing duplexes and although a correlation exists, the trend most closely correlates with base-pair opening lifetimes derived from ^{1}H NMR measurements of imino proton exchange rates; this parameter reflects the dynamical motion of the mismatch and extent of stacking with the adjacent base pairs. In general, purine−purine mismatches do not greatly perturb the base stack; these mispairs are able to hydrogen bond and the larger aromatic surface area of the purines allows for significant coupling with the bases above and below the mismatch. However, in a pyrimidine−pyrimidine mismatch, lack of proper hydrogen bonding, and small stacking surface area make these mismatches particularly destabilizing to the helix. These results further implicate base stacking and dynamics in modulating long-range oxidative damage in DNA.

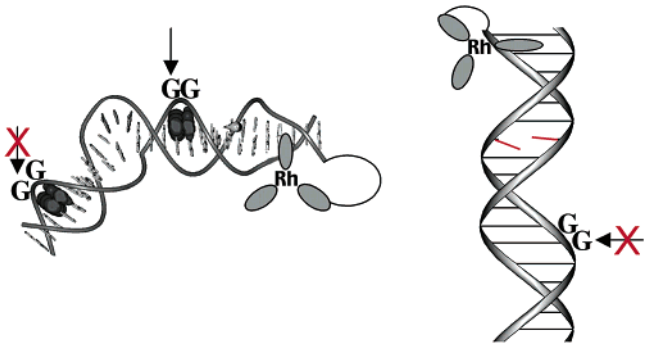

FIGURE 4. Schematic representations of some of the base stacking perturbations that have been examined using guanine oxidation ratios. A mismatch containing DNA duplex (right) and a duplex with a 5′-ATA-3′ base bulge (left). Both mismatches and base bulges attenuate the amount of CT through the duplex by disrupting the π-stacking array. After photoexcitation of the oxidant, no long-range guanine oxidation is observed in assemblies containing a perturbation in the π-stack.

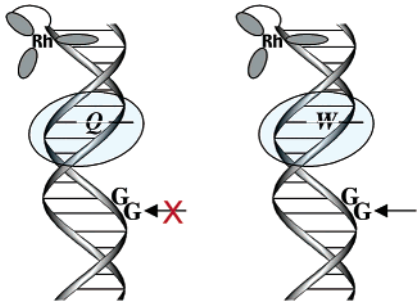

FIGURE 5. Schematic illustrations of a DNA-binding protein modulating CT, both positively and negatively. M.*Hha*I binds to DNA and inserts a hydrophobic glutamine residue into the base stack (left) which does not allow for efficient CT. The amino acid side chain acts as a hydrophobic "plug" in the aromatic base-stacking array which disrupts CT. On the other hand, a mutant M.*Hha*I which inserts instead an aromatic tryptophan residue (right) does not disrupt the π-array and allows for CT. Depending on the nature of the DNA/protein interaction, DNA-binding proteins can regulate CT both positively and negatively.

In addition to base bulges and mismatches, some DNA-binding proteins that perturb the DNA structure also can modulate long-range oxidative DNA damage (Figure 5).[28] Methyltransferase *Hha*I (M.*Hha*I) methylates a cytosine in 5′-G*CGC-3′ sequences by flipping out the cytosine into

its active site and inserting a glutamine side chain in its place.[29] This glutamine side chain creates a nonaromatic plug within the base stack, and as a result, when M.*Hha*I is bound between two 5′-GG-3′ sites on a rhodium tethered assembly, oxidative damage to the distal site is greatly diminished. Interestingly, when a mutant enzyme, that inserts a heterocyclic, aromatic tryptophan into the base stack was tested, charge transport to the distal guanine site was restored.[28] This result is consistent with the idea that tryptophan, resembling a DNA base when inserted in the π-stack, completes the π-stack and thus does not disrupt long-range CT. Hence, depending on the specific nature of the interaction, DNA-binding proteins can regulate long-range CT both positively and negatively.

It is important to emphasize that the sensitivity of DNA CT in metal-tethered duplexes to these perturbations in intervening base pair structure underscores that the path for charge transport is necessarily through the base pair stack. Oxidative damage cannot, for example, arise in these systems from aggregration or intermolecular reaction. Indeed a photophysical study of photoinduced electron transfer between two tethered intercalators provided the first indication of the sensitivity of CT to intervening mismatches and early compelling evidence that long-range CT through the DNA π-stack could occur.[30]

4. Charge Transport through Different DNA Structures

DNA duplexes modified to contain tethered photooxidants provide well-defined systems in which to explore long-range CT chemistry. However, other π-stacked arrays have demonstrated efficiency in mediating oxidative DNA damage (Figure 6). In DNA/RNA hybrids, containing both ribo- and deoxyribonucleotide strands, a pendant ethidium photooxidant can promote oxidative damage from a distance of 35 Å.[31] These hybrids adopt an A-like structure and possess a narrower major groove than B-DNA. For this reason, use of metallointercalators does not result in efficient CT; the oxidant cannot intercalate within the narrow groove and is therefore not well coupled to the π-stack. Ethidium, however, does intercalate in A-form helices and can promote efficient CT. These results underscore the importance of efficient coupling of the charge donor and acceptor with the base stack.

Another base-stacked array in which CT was explored is the triple helix. Triplex structures furthermore provide a means to introduce a photooxidant site-specifically to a long DNA fragment. An NDI intercalator attached to the center of a 16 base pair triplex-forming oligonucleotide (TFO) was selectively targeted to a single site on a ~250 base pair restriction fragment.[32] Oxidative DNA damage was observed over at least 85−130 Å in each direction from the site of binding. Notably, however, the CT reaction was significantly more efficient to the 3′ side of the triplex. Interestingly, when NDI or $[Rh(phi)_2bpy']^{3+}$ are covalently tethered to the 5′-end of the TFO, significant amounts of damage were observed only in the immediate vicinity of oxidant binding, suggesting the base stacking is distorted at the 5′-end of the triplex−duplex junction so as to interrupt CT. Triplex targeting

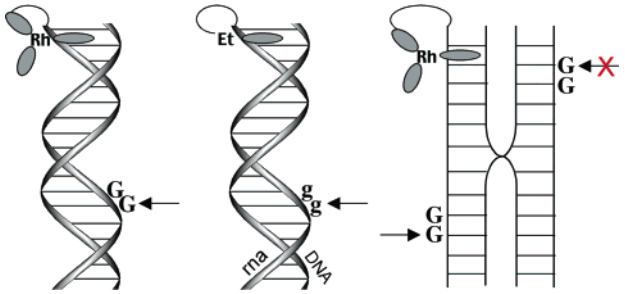

FIGURE 6. Schematic illustrations of some of the DNA structures studied for their ability to mediate CT. A DNA duplex (left), a DNA/RNA hybrid containing a tethered ethidium photooxidant (center), and a DNA four-way junction (right). All three DNA structures provide an intact base-stacking array, as is requisite for CT chemistry. Following excitation of the photooxidant, these DNA structures all efficiently mediate long-range oxidative damage.

to a restriction fragment to yield oxidative damage provided us with an estimate of the distance distribution of genomic charge transport of ~200 Å around the site of radical injection.

Multiple-stranded DNA assemblies, in addition to double and triple-stranded arrays, provide unique base stacks in which to explore CT. Four-way DNA junctions (also called Holliday junctions) are composed of four partially complementary DNA strands that form parallel base stacks which rapidly exchange between different stacking isomers. Photoactivation of a tethered rhodium complex, displaying photocleavage and therefore intercalation in only one arm of the assembly, results in oxidative damage in all arms of the four-way junction.[33] These assemblies are relatively mobile and interchange between different stacking isomers provides multiple π-stacked pathways, and hence, oxidative damage is possible in all arms of the four-way junction. In contrast, similar experiments utilizing a tethered anthraquinone moiety revealed CT in only two of the four arms of the four-way junction. While it was proposed[22] that the differences seen reflected aggregation by the rhodium tethered species, instead we consider that the variations among oxidants seen reflect variations in the time scale for CT, which may vary with the oxidant employed.

To restrain the flexibility of the four-way junction, DNA double-crossover (DX) assemblies were also constructed.[34] DX assemblies are composed of a collection of partially complementary strands annealed into one supermolecule which has two connected, but spatially separated, π-stacks. Unlike a four-way junction, DX assemblies are relatively rigid and when $[Rh(phi)_2bpy']^{3+}$ is tethered to one end of the DX, yet constrained so as to only allow intercalation into one base stack, oxidative G damage is observed selectively down the base stack bearing the metallointercalator. Remarkably, despite tight packing, no CT crossover to the adjacent base stack was observed; the two base stacks are effectively insulated from one another. These data underscore also the importance of the π-stacked array as the critical path for CT.

5. Gating of CT by Dynamical Motions

Biochemical experiments probing the distance dependence or effect of helix-destabilizing mismatches, bulges,

or nonaromatic side chains measure only a change in *yield* of CT products; might there also be a change in CT *rate*? To address this question, assemblies containing a tethered ethidium photooxidant and 7-deazaguanine as the electron donor, with donor−acceptor distances 6−24 Å, were constructed and their ability to support long-range CT examined spectroscopically.[35] Ultrafast transient absorption spectroscopy revealed biphasic kinetics for the CT with populations having two time constants, 5 and 75 ps. The 5 ps component was assigned to direct CT from 7-deazaguanine, while the 75 ps component corresponded to the orientation time of ethidium within its binding site to align in a conformation allowing CT. Interestingly, with increasing donor/acceptor separation, the two components decreased in yield but not significantly in their decay times. The *rate* of CT was independent of donor/acceptor separation while the *yield* decreased significantly. These data suggested to us first that dynamical motions within the π-stack gate long-range CT. Recent studies of base-base CT as a function of temperature, both time-resolved and steady state, highlight the role of base dynamics in modulating CT and provide additional support for the idea that intervening base motions serve to gate the CT process.

6. Toward a Mechanistic Understanding of Long-Range Charge Transport

Based upon many experiments that established long-range oxidative damage to DNA, the focus of research has shifted from questions of *whether* long-range CT occurred to *how* charge propagates through the base stack. Our first interest was in determining experimentally the scope and parameters governing DNA CT. As we do so, now we and others can begin to address mechanistically how DNA CT proceeds. There are two general mechanistic possibilities: tunneling through the DNA, forming a "virtual" bridge between the donor and acceptor, and charge hopping between discrete base orbitals (Figure 7).[36] In a tunneling mechanism, the DNA orbitals are energetically higher than the donor and acceptor and the charge tunnels through the bridge without formally occupying it. With only virtual occupation of the DNA bridge, the rate of CT would show exponential dependence on donor/acceptor separation. In contrast to tunneling, in a hopping mechanism, the donor and acceptor orbitals are close in energy to the bridge. Thus, in thermally induced hopping, charge transiently occupies the bridge orbitals, hopping from one low energy site to the next. So long as hopping to the next "stepping stone" is faster than radical trapping, charge would be able to propagate through the base stack with a very shallow distance dependence.

Bixon and Jortner proposed a theoretical model to explain the sequence-dependence associated with long-range oxidative damage and the shallow distance dependence. They first proposed sequential hopping between guanine bases with tunneling through A−T base pairs.[37] Utilizing yield measurements of oxidative DNA damage as a function of intervening sequence, Giese, Jortner, and co-workers offered experimental support for this guanine hopping model of CT through DNA.[38] They observed decreased yields of guanine oxidation with increasing separation of guanine "stepping stones" by TA steps.

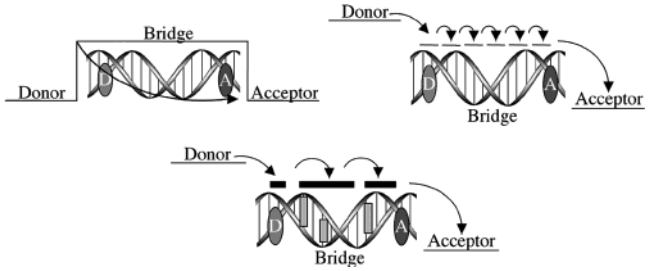

FIGURE 7. Schematic representations of three possible mechanisms for DNA CT. In superexchange (top left), the charge tunnels from the donor (D) to the acceptor (A) through the bridge. An exponential decrease in the rate of CT with increasing bridge length is expected. In a hopping mechanism (top right), charge occupies the bridge hopping between discrete molecular orbitals. If hopping is faster than radical trapping, the charge should be able to migrate over long molecular distances. In a domain hopping mechanism (bottom), charge occupies the bridge by delocalizing over several bases. This domain hops along the bridge to travel from donor to acceptor. As in a pure hopping mechanism, the charge should be able to travel long distances.

We considered whether base dynamics might also explain the sequence-dependence in efficiency of DNA CT observed. By inserting 5′-TA-3′ steps, which are known to be quite flexible, into the bridge, the base−base coupling is significantly altered; this could also explain the diminished yields of oxidative damage. To probe more directly the notion that charges tunnel through TA steps, we therefore varied the length of AA, TT, and AT tracts intervening two 5′-GG-3′ sites and monitored the yield of oxidative damage using a tethered rhodium intercalator.[39] Significant damage at the distal guanine doublet site was observed in all cases with up to 10 A's, T's, or alternating AT sequence intervening. The distal/proximal ratio of oxidative damage was consistently higher for assemblies containing all A's intervening; this we attributed to significant stacking overlap of the purine tract. Moreover, increasing the number of A's between the guanine doublets only slightly decreased the guanine oxidation ratio, and remarkably, an increase in oxidation ratios was observed with increasing from four to eight intervening T's or AT sequence. In fact, guanine oxidation was observed through up to five TA steps with no significant loss of yield over that distance. Furthermore, insertion of a GC pair in this TA tract actually decreased the oxidative damage yield, inconsistent with a guanine hopping model.

On the basis of our results and those of Schuster, the model of Giese and Jortner for sequence-dependent CT was recently revised to distinguish unistep superexchange tunneling over "short" $(A-T)_n$ bridges ($n < 3-4$) and thermally induced hopping over "long" $(A-T)_n$ bridges ($n > 3-4$).[37] We consider, however, that sequence-dependent DNA dynamics and flexibilities can gate CT. Rather than hopping from guanine to guanine, we have proposed that CT over long molecular distances might be best considered as *domain hopping*,[20,39] where charge is transiently delocalized over sequence-dependent domains defined by local structure. Variations in length and sequence contribute to the conformational dynamics of the helix, and these may define a delocalized domain.

Accordingly, inserting a GC pair into the TA tract may have disrupted a local domain and the resultant yield of CT.

Also based on oxidative yield determinations, Schuster and co-workers have proposed phonon-assisted polaron hopping between guanine bases.[40] In this model, transient formation of polarons in DNA allows for charge delocalization over regions of sequence; propagation of these polarons throughout the helix is aided by phonons. Counterion distribution may be a critical factor in considering phonon-assisted polaron hopping.[41] By simply varying the position of phosphate termini with respect to a tethered rhodium intercalator, we were able to modulate the amount of long-range guanine oxidation.[42] In one case, sequences containing two spatially separated 5′-GG-3′ sites, six intervening A's, and a tethered rhodium intercalator were examined for their ability to mediate long-range CT. With a 5′-^{32}P-end label (5′-OPO_3^{2-}, 3′-OH) a distal/proximal ratio of 5.2 was obtained. By simply 3′-end labeling the duplex (5-OH, 3′-PO_2^--OR) instead, the ratio dropped to 0.4. Thus, moving the negative charge to the end proximal to the rhodium intercalator dramatically decreased oxidative damage at the distal guanine doublet. Analogous fluorescence measurements of electron transfer between photoexcited 2-aminopurine and G did not show significant modulations in fluorescence as a function of charge distribution at the duplex termini. This led to a proposal of altered oxidation potentials at the distal relative to proximal guanine doublet sites as a function of charge at the termini. Assuming the results reflect a change in the thermodynamic potential at the 5′-GG-3′ sites, then one can roughly calculate the internal longitudinal dielectric constant of DNA based on the these data. High values ranging from 30 to 300, depending on extent of screening of the pendant charges by counterions, are obtained; thus these results pointed to the possibility that a high longitudinal polarizability of DNA may play a part in the mechanism for DNA CT.[43]

There is, however, much we still do not understand mechanistically. We cannot yet account for many variations seen with different oxidants; in some cases CT appears to be rate limiting, but in other cases, not. Clearly, a critical feature we have learned, irrespective of the methodology employed, is the sensitivity of DNA CT to nucleic acid structure, both statically and dynamically. To delineate further these differences depending upon oxidant and nucleic acid structure, we need to look not just at the irreversible oxidative damage found at long distance but also more directly to monitor the radicals formed and their rates of formation.

7. Spectroscopic Identification of Radical Intermediates in Long-Range CT

Following our biochemical experiments designed to establish oxidative chemistry from a distance, using the rhodium intercalator primarily, we therefore became interested in characterizing the time scales and radical intermediates in the long-range CT process. Dipyridophenazine complexes of ruthenium(II) possess remarkable photophysical properties and, given their avid intercalative binding to DNA, thus provide a unique spectroscopic handle. Luminescence of these dppz complexes is evident in organic solvents; however, in aqueous solution the luminescence is quenched by proton transfer to the phenazine nitrogens.[44] Upon intercalative binding to DNA, these phenazine nitrogens are protected from solvent and luminescence is restored; binding to DNA thus sensitively modulates the luminescent properties, and hence, these complexes have been dubbed "molecular light switches". These complexes furthermore provide a valuable spectroscopic handle for DNA CT. Photoexcitation of dppz complexes of Ru(II) yields a metal-to-ligand charge-transfer excited state which is localized on the dppz ligand. Quenching of this excited state by a non-intercalating, diffusible species (e.g., $[Ru(NH_3)_6]^{3+}$ or $[Co(NH_3)_5Cl]^{2+}$) generates in situ a powerful Ru(III) oxidant (E_0 ($Ru^{3+/2+}$) $\sim$ 1.5 V vs NHE) that is capable of oxidizing guanines from a distance.[45]

The flash quench technique, coupled with transient absorption spectroscopy, has been applied effectively in characterizing the resultant neutral guanine radical in duplex DNA; deprotonation of the cation radical must occur faster than the 10^{-7} s time scale of the experiment.[45] We have also utilized flash/quench experiments in characterizing radical products in peptide/DNA assemblies[46] and a protein/DNA complex.[47] In particular, CT and radical trapping were examined in DNA assemblies in the presence of a site-specifically bound methyltransferase *Hha*I mutant. The methyltransferase mutant, which can flip out a base and insert a tryptophan side chain within the DNA cavity, was found to activate long-range hole transfer through the base pair stack. Protein-dependent DNA charge transport was observed over 50 Å with guanine radicals formed $>10^6$ s^{-1}; hole transport through DNA over this distance was found not to be rate-limiting. Thus, the flash/quench technique, originally designed to study CT in proteins,[48] provides a method to generate powerful ground-state oxidants and to follow the formation of radical intermediates associated with long-range DNA CT chemistry.

Assemblies containing 4-methylindole (M) as the electron donor embedded between two G bases, for greater stability, as well as a tethered ruthenium intercalator were also constructed to explore long-range DNA CT spectroscopically.[49] The methylindole radical cation is particularly amenable as an artificial base in these studies because of its strong absorptivity at 600 nm and its relatively low oxidation potential (1 V vs NHE). To explore the distance dependence of radical formation, the separation between the ruthenium oxidant and M was varied over 17−37 Å with only intervening A−T base pairs composing the DNA bridge. Formation of the M radical at all distances was found to be coincident with quenching of the ruthenium excited state to form the Ru(III) oxidant. Thus the rate of formation of the radical at long range across a path of AT bases is $\geq 10^7$ s^{-1} and over this distance regime CT is not rate limiting.

Recently, additional assemblies containing a pendant ruthenium oxidant and M as the charge donor were constructed to examine spectroscopically the effects of intervening sequence on long-range CT.[50] In all cases, a Ru−M distance of 30.8 Å was maintained. Sequences contained either a G or inosine (I) at the hole injection site with the intervening sequence to the GMG oxidation site varied as all A's, all T's, or containing an intervening

AA mismatch. In the presence of the intervening mismatch, consistent with measurements of long-range oxidative damage, no indole cation radical was formed. In comparing assemblies containing inosine and guanine, inosine is harder to oxidize than G by ~200 mV, and the initial expectation might have been that hole injection into the bridge would be less efficient for sequences containing I at the injection site. In fact, rapid radical formation was observed with either G or I at the injection site, except for the mismatch-containing assembly. Remarkably, in sequences containing I at the injection site and no intervening guanines, formation of radical product was also observed at rates $\geq 10^7$ s^{-1}. Even more intriguing, the 600 nm signal is significantly larger for sequences containing I at the injection site, indicative of a higher yield of radical formation. Biochemical analysis of analogous assemblies where GGG was substituted for GMG, suggest these differences can be accounted for based upon the extent of radical localization at the injection site and subsequent reaction with Q^{red}. Using $[Ru(NH_3)_6]^{3+}$ as Q, more irreversible oxidative damage products were observed at the GGG site in duplexes containing I at the injection site, consistent with spectroscopic measurements of radical yield. However, using a sacrificial quencher such as $[Co(NH_3)_5Cl]^{2+}$, which is unstable in its reduced form, can minimize reaction of the DNA radical at the injection site with Q^{red}. Indeed, when the possibility of reaction with reduced quencher was eliminated by utilizing $[Co(NH_3)_5Cl]^{2+}$, damage yields at the GGG site were found to be comparable for sequences containing G or I at the injection site. Thus, the yield of oxidative damage at a site spatially separated from the oxidant can be modulated sensitively by reactivity at the injection site; the sequence determines the extent of hole localization and hence the probability of hole propagation. This sensitivity in long-range oxidative damage to the DNA sequence surrounding hole injection was seen also with capped anthraquinone moieties.[51]

8. Electrochemical Detection of Base Stacking Perturbations and Applications for DNA Sensing

A variety of experimental techniques has been shown to be useful in probing DNA mediated CT. Analysis of oxidative damage yields by biochemical means has provided invaluable insight into the effects of DNA sequence conformations and base stacking perturbations. Spectroscopy has allowed us to explore more vigorously rates of CT reactions and has revealed an exquisite sensitivity to dynamical base motions. Additionally, we have developed an electrochemical probe of DNA mediated CT, and this electrochemistry may lead to powerful diagnostic applications of DNA CT.

Exploiting molecular self-assembly of thiol-modified DNA duplexes on gold electrodes, we are able to monitor electrochemically the reduction of a redox active intercalator bound to the DNA at a site remote from the gold surface (Figure 8).[52] Reduction of the distantly bound intercalator is monitored by cyclic voltammetry or chronocoulometry and is a direct probe of the efficiency of CT through the intervening DNA bridge. It is notable that applying a negative potential to the DNA film and monitoring the reduction of a redox active intercalator exploits *electron* transport through the base stack; this

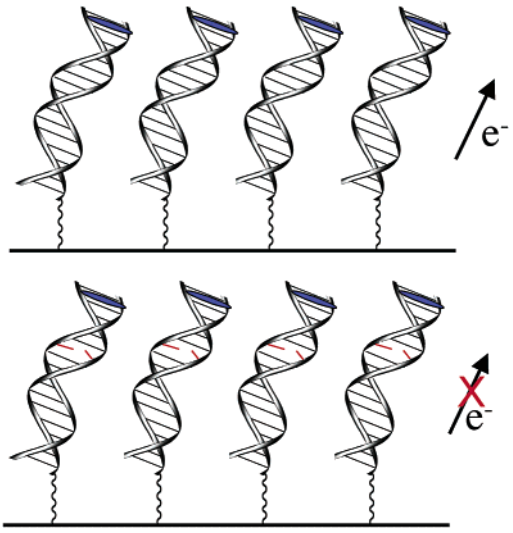

FIGURE 8. Schematic illustrations of electrochemistry experiments utilizing DNA films self-assembled on a gold surface. The reduction of a redox-active intercalator is monitored after electron transport through the DNA (top). The efficiency of reduction of the intercalator is a measure of the ability of the intervening π-stack to support CT and can be used to monitor for stacking perturbations. In the presence of a mismatch (bottom) the base stack is perturbed and electron transport to the redox probe is diminished.

is in contrast to biochemical and spectroscopic assays which rely on *electron hole* transport. Mechanistically, this process is not well understood, as the DNA bridge orbitals are thought to be significantly higher in energy than the applied potentials, although the energies may be altered in stacked DNA or the films described here. If not perturbed significantly in energy, thermally induced hopping would be hard to reconcile. Nonetheless, these electrochemical experiments have served to support results of the biochemical and spectroscopic studies and confirm the superb sensitivity of CT reactions to base stacking.

In fully hybridized DNA duplexes containing a single base mismatch, the electrocatalytic signal of methylene blue, a redox active intercalator, coupled to $[Fe(CN_6)]^{3-}$ distinguished all single base mismatch-containing DNA from perfectly matched duplexes.[53] Remarkably, even thermodynamically stable GT and GA mismatches, notoriously difficult to identify by means of differential hybridization, are detected and distinguished from well-matched sequences. Furthermore, physiologically relevant base lesions and "hot spot" mutations can be readily discerned.

In studies analogous to biochemical assays utilizing M.*Hha*I, the effect on CT of DNA-binding proteins has also been explored electrochemically.[54] In general, it is observed that CT yields correlate with protein-dependent alterations in DNA base stacking. Base-flipping enzymes such as uracil DNA glycosylase, TATA-binding protein which crystallography reveals kinks DNA 90° upon binding,[55] and M.*Hha*I drastically diminish CT out to the distant redox probe. In agreement with biochemical assays,[28] binding of a mutant M.*Hha*I, which inserts an aromatic, heterocyclic amino acid into the π-stack, does not disrupt CT out to the redox probe.[54] Similarly, proteins that bind DNA without perturbing the base

stack, such as restriction endonuclease R.*Pvu*II and the transcription factor Antennapedia homeodomain, do not significantly lessen the yield of CT. Hence, protein binding is able to modulate DNA CT both negatively and positively, depending on the specific nature of the DNA−protein interactions and the extent of helix perturbation.

9. Biological Consequences

Our observations concerning the sensitivity of DNA CT to structure, mismatches, lesions, and binding by proteins, as well as the fact that DNA CT can proceed over long molecular distances all beg the question of whether DNA CT is physiologically relevant and indeed important. Might some DNA-binding proteins utilize DNA-mediated CT for long-range signaling or activation? Perhaps DNA-binding proteins containing redox active cofactors or structural elements such as flavins or Fe−S clusters take advantage of DNA CT for communication in vivo. Additionally, CT chemistry provides an approach to sensing base-stacking perturbations and lesions; might Nature take advantage of this chemistry?

Before considering these possibilities, it is first necessary to demonstrate that long-range oxidative damage can occur in DNA as packaged within the cell. In vivo, DNA is not floating free in solution, but rather packaged and protected in nucleosome core particles (NCP). In eukaryotes double helical DNA is wrapped around a core of positively charged histone proteins. A crystal structure of a NCP has been determined for a histone octamer and a 146 base pair palindromic DNA sequence.[56] The DNA is highly bent as it wraps ~1.5 times around the outside of the histone octamer. Remarkably, using a rhodium intercalator tethered to the 5′-end of the DNA, guanine bases within the NCP were oxidized from a distance of over 80 Å (Figure 9).[57] Perhaps binding to the histone core stabilizes a base stacking conformation particularly suited to long-range oxidative damage. More importantly, these results concerning long-range damage have to be considered in the context of our thinking about DNA packaging within chromatin. Our intuition suggests that packaging DNA in NCP protects it from damage; in fact DNA within chromatin is well protected from solution-borne oxidants. Despite this protection from solution-borne radicals, however, this packaged DNA is quite susceptible to oxidative damage through long-range CT mediated by the base stack.

Further evidence suggesting the possibility of CT damage in vivo, comes from long-range oxidative damage demonstrated in whole nuclei.[58] Treatment of *HeLa* nuclei with a rhodium intercalator, followed by photoactivation, results in oxidative DNA damage. This damage is revealed by treatment with base excision repair enzymes and amplification of the genomic DNA by ligation-mediated PCR. Oxidative damage has been probed in exon 5 of the p53 gene and in the transcriptionally active PGK1 promotor; damage at the 5′-G of guanine doublets and triplets was observed, the hallmark of DNA mediated CT. Moreover, in the PGK1 promotor, oxidative damage occurs at protein-bound sites that are inaccessible to rhodium. Protein footprinting analyses allowed us to conclude CT damage occurs over distances of at least 34 Å, and potentially further. Thus, on transcriptionally active DNA within the nucleus, long-range CT can result in oxidative base lesions.

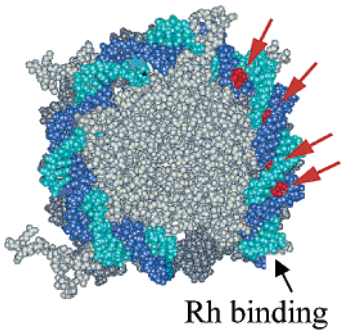

FIGURE 9. In a nucleosome core particle the DNA (blue and cyan) is wrapped ~1.5 times around an octamer of histone proteins (gray). The site of rhodium attachment and binding is indicated. Oxidative damage is observed at guanine doublets (red) located over 80 Å from the site of rhodium intercalation after photoactivation. (The picture was adapted from PDB coordinates 1aoi, ref 55).

The demonstration of long-range oxidative damage in NCP and nuclei extends DNA CT as a feasible mechanism for the generation of cellular base lesions. Perhaps organisms have evolved to protect the genetic code from CT damage. Certain regions of the genome may have evolved to be more or less susceptible to long-range oxidative DNA damage. After statistical analysis of the human genome, Heller has proposed a means of cathodic DNA protection, similar to the way in which Zn^{2+} protects steel.[59] The number of 5′-GGG-3′ triple guanine sites is elevated in the regions flanking protein-coding exons and it is suggested that charges injected into DNA might be funneled to these sacrificial G-rich introns. Telomeric DNA, located at the ends of all linear chromosomes is also G-rich and charges may be funneled to these noncoding regions. Understanding how damage may be funneled to certain sites and insulated at other sites could be an important element in biochemical mechanisms for DNA damage and its repair.[60]

10. Conclusions

Oxidative damage to DNA from a distance has now been demonstrated using a variety of photooxidants and establishes the effectiveness of DNA-mediated CT chemistry over long molecular distances. Indeed, damage has been observed 200 Å from the site of radical injection. The chemical synthesis of well-defined DNA assemblies with pendant probes has been critical in the characterization of DNA-mediated CT and the parameters that affect it. Using time-resolved spectroscopy, the time scale for the rate of CT to effect long-range damage has also been probed. With our ruthenium intercalators as tethered oxidant, CT proceeds at a rate $\geq 10^7$ s^{-1} over 30 Å, and over this distance regime no significant variations in rate are observed. CT is exquisitely sensitive to sequence-dependent base stacking and may be gated by the dynamical motions within DNA. DNA CT to yield oxidative DNA damage can best be explained in the context of a hopping model, but one where holes may migrate among delocalized domains rather from base to base, where these hopping domains are defined by sequence-dependent stacking and dynamics. DNA CT in

DNA films is less well understood mechanistically but, given the similar sensitivity to stacking, provides a powerful probe for base mismatches, lesions, and protein-dependent perturbations in DNA structure. Long-range oxidative damage to DNA is relevant physiologically since long-range CT damage to DNA has also been detected in cell nuclei and nucleosome core particles using rhodium intercalators.

The characteristics of DNA CT chemistry that have been delineated, however, prompt many more questions. Given that DNA CT can occur over long molecular distances, does this reaction play a role in oxidative damage within the genome? Can radicals generated at one site in the base stack migrate or be funneled through the helix to other regions? Perhaps even more intriguing, DNA-binding proteins can modulate CT both positively and negatively; is the base pair stack used for signaling between proteins, to scan for DNA damage and mismatches or possibly for transcriptional activation from a distance? Can we describe the DNA CT chemistry we have developed using DNA assemblies with pendant redox probes truly as biomimetic chemistry, modeling what can occur within the cell? Designing experiments to probe these questions, both in the test tube and within the cell, provides us with still more tantalizing challenges.

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