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DNA Charge Transport within the Cell

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Abstract

The unique characteristics of DNA charge transport (CT) have prompted an examination of roles for this chemistry within a biological context. Not only can DNA CT facilitate long range oxidative damage of DNA, but redox-active proteins can couple to the DNA base stack and participate in long range redox reactions using DNA CT. DNA transcription factors with redox-active moieties such as SoxR and p53 can use DNA CT as a form of redox sensing. DNA CT chemistry also provides a means to monitor the integrity of the DNA, given the sensitivity of DNA CT to perturbations in base stacking as arise with mismatches and lesions. Enzymes that utilize this chemistry include an interesting and ever-growing class of DNA-processing enzymes involved in DNA repair, replication, and transcription that have been found to contain 4Fe-4S clusters. DNA repair enzymes containing 4Fe-4S clusters, that include Endonuclease III (EndoIII), MutY, and DinG from bacteria, as well as XPD from archaea, have been shown to be redox-active when bound to DNA, share a DNA-bound redox potential, and can be reduced and oxidized at long range via DNA CT. Interactions between DNA and these proteins in solution, in addition to genetics experiments within *E. coli*, suggest that DNA-mediated CT can be used as a means of cooperative signaling among DNA repair proteins that contain 4Fe-4S clusters as a first step in finding DNA damage, even within cells. Based on these data, we can consider also how DNA-mediated CT may be used as a means of signaling to coordinate DNA processing across the genome.

Our laboratory has focused on studies of DNA-mediated charge transport (CT). This chemistry offers a means to carry out redox chemistry at a distance and provides a sensitive reporter on the integrity of the intervening DNA. In photophysical, biochemical, and electrochemical experiments we have learned general features of this chemistry. Perhaps uniquely, DNA CT can occur by transport through the base pair stack over long molecular distances. But importantly, this long distance redox chemistry can only occur if the DNA helix is well stacked; anything that interrupts that stacking turns off CT. Thus the chemistry offers a means to effect long range redox signaling as long as the integrity of the DNA duplex is intact. The uniqueness of this chemistry thus begs the question: Is DNA CT utilized within the cell?

Here we describe experiments to begin to probe how this chemistry may be utilized within the cell. DNA CT chemistry is important to consider in the context of how DNA may be

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damaged under conditions of oxidative stress and how that damage is sensed and repaired. Increasingly, proteins involved in DNA processing have been found to contain 4Fe-4S clusters, cofactors generally thought to carry out redox reactions within the cell. Moreover DNA CT can promote redox chemistry over long molecular distances, potentially providing a means for signaling across the genome. Furthermore, because DNA CT reports on the integrity of the DNA duplex, this signaling can reflect whether the intervening DNA is damaged and in need of repair, whether DNA processing needs to begin, to be stalled or to be increased.

We begin by describing different experiments we have used to elucidate DNA CT chemistry. What are the characteristic features of DNA CT? Within that framework, we next describe experiments carried out on DNA-binding proteins to explore how this chemistry may be utilized. The work presented is not meant to be an exhaustive survey of the literature.[1–4] Instead, the experiments described are intended to illustrate DNA CT chemistry and particularly a role for proteins containing 4Fe-4S clusters in carrying out signaling using this chemistry. These studies thus present a framework for considering how DNA CT may be used for genomic signaling.

Platforms to Study the Characteristic Features of DNA CT

The conductivity of DNA arises most fundamentally from its continuous, π-stacked core of aromatic bases that extends down the helical axis. In fact, this conductivity was first predicted from early DNA structural studies which revealed striking similarities to sheets of graphite, both in π-stacking and interplanar spacing.[5] However, unlike graphite and other π-stacked solids, DNA is a dynamic, macromolecular array that functions in solution. This critical difference gives rise to several defining characteristics of DNA CT, and thus studies to probe this chemistry must be performed using platforms with aqueous conditions in which the dynamic motions of the DNA bases are unrestricted. In our laboratory we have established three such general platforms to study DNA CT in DNA duplexes from different vantage points: free in solution, tethered to electrode surfaces, and as single molecules.[2] Across these platforms we have observed several essential, conserved features of this chemistry, including (i) electronic coupling of the donor and acceptor to the DNA π-stack is required; (ii) even slight disruptions to the DNA π-stack between the donor and acceptor inhibit DNA CT; and (iii) the distance dependence of DNA CT is very shallow. Here we briefly summarize the platforms and fundamental studies that revealed these characteristics and that are critical for DNA CT in biological systems.

Given the need for aqueous conditions to make biologically relevant measurements of DNA CT, the first experiments to probe this chemistry were performed with free DNA duplexes in solution containing pendant redox donors and acceptors. In one such construct, the metallointercalators $Ru(phen)_2dppz^{2+}$ (phen = 1,10-phenanthroline, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) and $Rh(phi)_2phen^{3+}$ (phi = 9,10-phenanthrenequinone diimine) were covalently attached to either ends of a 15-mer DNA duplex as donor and acceptor handles, respectively (Figure 1).[6] Photoexcitation of the donor results in luminescence that is rapidly quenched by the acceptor via DNA CT. Importantly, coupling of the donor and acceptor to the DNA π-stack is essential for this chemistry to proceed; substitution of $Ru(phen)_2$(phen

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′)$^{2+}$ (phen′ = 5-amido-glutaric-acid-1,10-phenanthroline), a poor DNA intercalator, inhibits quenching.

Similar results were obtained with a more native construct in which fluorescence quenching of adenine base analogs by guanine allows for direct, base-to-base measurements of DNA CT.[7] In these experiments, for which the base analogs 2-aminopurine (A_2) or 1,N^6-ethenoadenine (A_ε) were incorporated into 12-mer DNA duplexes, quenching of A_2 occurs far more rapidly and over longer distances than quenching of A_ε. This result is consistent with the different structures of these analogs that allow for well integrated stacking of A_2 into the DNA helix and poor stacking of A_ε. Beyond the importance of electronic coupling to the π-stack to participate in DNA CT, solution-based platforms also provided the first indication of the exquisite sensitivity of this chemistry to perturbations along the π-stacked path; the introduction of even a single base mismatch between the donor and acceptor severely decreases the quenching yield.[8] Studies of base-base CT also taught us important lessons about the timescales and dynamics of DNA CT. DNA CT occurs on the picosecond timescale but is gated by the motions of the bases.[9]

In order to relate these observations more directly to DNA CT in biological systems, it was necessary to design platforms that allow ground state measurements of DNA CT, while still maintaining the DNA in an aqueous, buffered environment. In one such platform, a single DNA duplex is made to covalently span an etched gap in a carbon nanotube circuit.[10] Current flow through this device reports directly on DNA CT efficiency (Figure 2). By cycling the type of DNA incorporated into the device from well matched DNA to DNA with a single base mismatch to well matched DNA again, current flow is turned on, then off, then on again, respectively. This ground state, single molecule platform provides more direct measurements of DNA CT and confirms the high sensitivity of this chemistry to even minor structural disruptions of the π-stack.

In a second ground state platform, DNA functionalized with an alkanethiol linker is allowed to self-assemble vertically as a film on a gold electrode surface (Figure 3). These DNA-modified electrodes can then be used to monitor DNA CT electrochemically between the electrode and a redox-active probe molecule bound at the distal end of the DNA.[11,12] Taking advantage of the inherent capacity of electrochemistry for multiplexing, we developed 16-electrode chips to facilitate side-by-side analysis of multiple samples and controls, thereby opening the door for even more complex investigations of DNA CT (Figure 3). Importantly, the same characteristics of DNA CT that are observed with free DNA in solution are observed with DNA-modified electrodes; electronic coupling of the redox probe to the DNA π-stack is essential to observe a DNA-mediated redox signal[13] and DNA CT is disrupted by a variety of biologically significant perturbations to the DNA π-stack, including intervening base mismatches,[13–16] base lesions,[17] and structural distortions caused by protein binding and activity.[18,19]

Critical for studying DNA CT in biological systems, DNA-modified electrodes also make it possible to measure the DNA-bound potentials of proteins with redox-active cofactors such as iron-sulfur clusters (Figure 3).[20–25] Like synthetic redox probes, DNA CT to DNA-bound proteins show the same coupling requirement to the π-stack[22,23] and the same sensitivity to

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intervening structural disturbances, such as base mismatches and lesions.[20,24,25] Thus DNA-modified electrodes allow for the identification and characterization of proteins that have the capacity to participate in DNA CT chemistry in living organisms.

In addition to illustrating these first two characteristics of DNA CT chemistry, these diverse platforms were also used to probe a third critical parameter for understanding the role of DNA CT in biology: *how far can DNA effectively conduct charge?* This distance dependence was first studied with free DNA in solution, using a covalently attached metallointercalator photoxidant that, upon irradiation, induces DNA-mediated, long-range guanine oxidation.[26,27] By biochemical sequencing, this damage is observed at the 5′-G of guanine doublets, the site of lowest oxidation potential. Importantly, and consistent with a DNA CT mechanism, the degree of coupling of the photoxidant to the π-stack and the integrity of the intervening π-stack were found to be far greater determinants of oxidation yield than the distance of the oxidation site from the photoxidant. In fact, over the longest distance measured, 20 nm or 60 bp, the oxidation yield was unaffected by the separation distance.[27]

Using multiplexed, DNA-modified electrodes, ground state DNA CT to a distal, covalent redox probe was measured over an even longer distance of 34 nm or 100 bp (Figure 3).[16] For these experiments, the multiplexed chip platform was crucial for enabling the side-by-side comparison of DNA CT in short and long DNA duplexes. Remarkably, DNA CT over 34 nm yields the same redox signal size and the same signal attenuation from the incorporation of a single base mismatch as DNA CT in much shorter, 6 nm or 17 bp duplexes. Over both 34 nm and 6 nm, the rate of DNA CT is limited by the electron tunneling rate through the alkanethiol linker that attaches the DNA to the gold surface.[16,28] Thus, the distance dependence of DNA CT is very shallow, allowing efficient charge conduction by DNA over distances that challenge the physical limits of these *in vitro* platforms for measuring this chemistry. Indeed, we have not yet determined an upper limit in distance for DNA CT, only that over these 100 base pair distances, CT is efficient and no decay in yield is observed. After establishing these conserved, structurally derived characteristics of DNA CT, (i) required electronic coupling to the π-stack, (ii) high sensitivity to intervening structural perturbations of the π-stack, and (iii) a capacity to transport charge over very long distances, the clear next step was to utilize this foundation to consider a role for this chemistry in living cells.

General Observations of DNA CT in Biological Systems

Initial experiments probed broadly the possibility that DNA CT might play a role in biological systems and sought to identify the cellular players that have the capacity to engage in this chemistry. *Can cellular DNA participate in DNA CT? Can DNA-binding proteins participate in DNA CT?* From this beginning point, the ways in which DNA CT may be exploited by these players were then probed including the funneling of charge through cellular DNA to concentrate damage at distant sites throughout the genome and the sending and receiving of DNA-mediated charge by DNA-binding proteins as a means to sense and respond to oxidative stress. These general observations set the stage for our current, extensive studies on whether DNA-bound proteins may use DNA CT to signal to

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each other across the genome in order to achieve more efficiently a variety of logistically challenging biological tasks.

The measurement of long-range oxidative damage to DNA in vitro[26,27] set the stage to investigate this phenomenon in cellular DNA (Figure 4). Reactive oxygen species pose a constant threat to the integrity of the genome, making it critical for cells to reduce the net impact of inevitable damage. One strategy is to promote the accumulation of holes at specific regions of low oxidation potential, namely tracts of multiple guanine bases, in order to concentrate damage and spare the majority of the DNA.[29] The possibility that DNA can facilitate such long-range funneling of damage via DNA CT was investigated in several native cellular environments including isolated HeLa cell nuclei,[30] nucleosome core particles,[31] and mitochondria.[32,33] In these studies, oxidative damage was induced by photoexcited $[Rh(phi)_2(bpy)]^{3+}$ (bpy = 2,2′-bipyridine), a DNA intercalator and potent photooxidant (Figure 4). A comparison of the binding sites of the metal complex with the locations of guanine damage showed significant separation, necessitating some form of controlled charge migration. Additionally, damage was observed specifically at the 5′-guanine of guanine repeat sites, a hallmark of DNA CT. Thus, these studies verified that long-range DNA CT can indeed take place in a complex and congested native organelle environment and can divert DNA damage to distant reservoirs in the genome.[34]

Given the confirmation that cellular DNA can facilitate DNA CT across long distances, the next step was to determine whether DNA-binding proteins can access, and potentially exploit, this chemistry. A logical starting point was to consider redox-active proteins that protect the cell against oxidative stress and thus must be able to sense and respond to oxidative threats. The first such protein investigated was SoxR, a bacterial transcription factor that, when activated, induces the transcription of a battery of genes involved in the oxidative stress response (Figure 4).[35] SoxR generally remains bound to DNA as a dimer in the cell and each monomer contains a [2Fe-2S] cluster that critically allows it to sense and respond to oxidative stress; oxidation of the cluster results in an up to 100-fold increase in transcription of its stress response genes.[36] But how is the cluster oxidized? Electrochemistry of SoxR bound to DNA-modified electrodes revealed that redox activity of the [2Fe-2S] clusters can be accessed by DNA CT. Additionally, the potential of the cluster shifts by positive ~500 mV upon DNA-binding, thereby modulating the capacity of SoxR to function as an oxidative stress sensor.[37] To investigate whether SoxR can be activated by oxidative DNA damage from a distance, the DNA intercalating photooxidant $[Rh(phi)_2(bpy)]^{3+}$ was covalently attached to a DNA duplex, 80 bp from the SoxR binding site (Figure 4). Upon photoexcitation of the metal complex, which injects electron holes into the DNA and generates distant guanine radicals, transcription of SoxR-regulated genes is activated.[38] This result indicates that SoxR can utilize DNA CT to sense oxidative damage efficiently across long molecular distances in the genome and activate the appropriate protective response.

Recent studies were conducted on bacterial Dps and human p53, other DNA-binding redox-active proteins that are also involved in responding to oxidative stress. Dps, a bacterial mini-ferritin which uses ferroxidase activity to protect DNA in pathogenic bacteria from reactive oxygen species, was studied to determine if it can wield this capability from a distance, via

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DNA CT (Figure 4). DNA-bound, ferrous iron-loaded Dps, but not apo-Dps or ferric iron-loaded Dps, was observed to neutralize distant guanine radicals formed by a distally bound, covalent photoxidant, confirming its potential to use DNA CT for cellular protection.[39] Similarly, human p53, a transcription factor that decides the fate of human cells under stressful cellular conditions, was found to respond to distal hole injection into DNA by a covalent anthraquinone photoxidant.[40,41] Specifically, oxidation of multiple cysteine residues to disulfide bonds within p53 causes dissociation from specific DNA promoter sequences. Importantly, p53 binding sites with lower oxidation potential (ie. sites with guanine doublets and triplets) show more dissociation of p53 in response to the oxidative insult.[41] Thus, the redox sensitivities of p53-binding sites combined with long-range DNA CT provide an efficient mechanism for p53 to regulate the expression of specific genes in response to a genome-wide report of oxidative stress.

Collectively, these examples demonstrate that diverse DNA-binding proteins, with a variety of redox-active cofactors, have the ability to take advantage of DNA CT chemistry in order to achieve more efficiently a variety of challenging cellular tasks. This work has fueled more in-depth studies into perhaps the most intriguing and powerful question that arises from these initial observations of DNA CT in biological systems: *Do DNA-bound proteins use DNA CT to signal to each other?*

Signaling among DNA repair glycosylases containing 4Fe-4S clusters

A striking number of DNA-processing enzymes in both prokaryotes and eukaryotes have been found to contain 4Fe-4S clusters. This iron co-factor was first associated with DNA repair enzymes when a 4Fe-4S cluster was discovered in *E. coli* Endonuclease III (EndoIII), a DNA glycosylase from the base excision repair (BER) pathway.[42–44] Other BER enzymes, such as *E. coli* MutY and homologous BER enzymes from other organisms, were also shown to contain 4Fe-4S clusters.[45,46] The 4Fe-4S cluster in these enzymes resides near the DNA binding interface and the clusters do not play a redox role in catalysis. Originally, the role of these iron-sulfur clusters was thought to be solely structural. While iron-sulfur clusters often serve a redox role in other classes of enzymes, a redox role for the clusters in the DNA glycosylases was first rejected, since for EndoIII the reduction potential of the $[4Fe\text{-}4S]^{2+/1+}$ couple was found to be < -400 mV vs. NHE based on measurements using redox mediators.[43,44] Additionally, while the cluster in MutY is required for DNA binding, the overall structure of MutY is not affected by the absence of the cluster.[46]

Our laboratory hypothesized instead that the 4Fe-4S clusters in these proteins are used for DNA-mediated redox signaling.[20,22,23,47] Importantly, measurements of redox characteristics of the cluster were required with the protein bound to the DNA polyanion, where the repair protein carries out its function. DNA-modified electrodes were thus used to probe the redox characteristics of the 4Fe-4S cluster in DNA glycosylases. The first important observations made were that the 4Fe-4S cofactors in DNA glycosylases can be reduced or oxidized via long range DNA CT and binding to DNA shifts the reduction potential of the 4Fe-4S cluster.[20,21] Cyclic voltammetry on DNA-modified gold electrodes shows a reversible redox signal at a midpoint potential of ~80 mV vs. NHE (Figure 5). This electrochemical signal is dependent on the DNA bases being well stacked. By introducing a

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single abasic site in the DNA substrate, the electrochemical signal is significantly attenuated, consistent with the redox pathway from the electrode to the cluster being through the DNA π-stack. Thus, the 4Fe-4S cluster of the protein appears to be well-coupled electronically into the DNA base stack.

An array of EndoIII mutants have been studied electrochemically on DNA-modified electrodes.[22,23,48] Interestingly, all of the mutants examined so far have the same DNA-bound redox potential, though for many of the mutant enzymes, an increased or decreased signal intensity as measured by cyclic voltammetry is observed, indicating the electronic coupling of the mutant enzymes to the DNA base stack has changed (Figure 5) Of particular interest is the CT-deficient EndoIII Y82A mutant, which has a significantly lower electrochemical signal on DNA-modified electrodes compared to wild-type (WT) EndoIII, though it maintains enzymatic activity comparable to that of the WT enzyme.[22,48]

Direct electrochemical measurements of EndoIII on highly oriented pyrolytic graphite (HOPG) with and without DNA revealed that upon binding to DNA, the redox potential of the $[4Fe\text{-}4S]^{3+/2+}$ couple shifts negatively by ~200 mV.[21] The protein is thus activated towards oxidation upon binding to DNA. The earlier studies suggesting Endo III to be redox-inactive under physiological conditions still hold true, but not if the protein is bound to DNA. This shift in potential moreover corresponds to a three orders of magnitude higher affinity for DNA when the 4Fe-4S cluster is in the 3+ oxidation state relative to the 2+ oxidation state.[21] The increase in the binding affinity of the protein for DNA in the oxidized form versus the reduced form is understandable given that higher oxidation states should be preferentially stabilized when the 4Fe-4S cluster is in the vicinity of the polyanionic DNA backbone.

The role of MutY and EndoIII is to prevent oxidized bases from causing mutagenesis, by MutY excising bases that are mispaired with oxidized bases, or EndoIII directly excising oxidized bases, prior to replication. A question that has yet to be definitively answered is how these enzymes, thought to be at low copy number within cells, can locate and fix such subtle damage scattered across the 4.6 megabase genome of *E. coli* before the organism divides. The copy number of MutY is estimated to be ~30.[49] We have proposed that DNA CT may be used by MutY, EndoIII, and other DNA-processing enzymes containing 4Fe-4S clusters as a means to both scan the genome for damage and locate the damage as a first step in DNA repair.[22,23,47,50]

Our model for how DNA repair proteins with 4Fe-4S clusters use DNA-mediated CT as a means of signaling to locate and repair damage, illustrated in Figure 6, is based upon many of the redox characteristics discussed above.[22] When a DNA glycosylase is freely diffusing in solution, its 4Fe-4S cluster is expected to be in the 2+ oxidation state. Upon binding to DNA, the redox potential of the protein shifts and the 4Fe-4S cluster may be oxidized by reactive oxygen species, other endogenous chemicals, or guanine radicals from a distance.[51,52] If a second DNA glycosylase binds at a sufficient distance relative to the first glycosylase to participate in a DNA-mediated redox reaction and the DNA between the two proteins is undamaged, then the second glycosylase could transfer an electron through the DNA base stack to reduce the distally bound protein. Once in the reduced form, this protein

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would have a lower binding affinity, dissociate from DNA, and take advantage of 3-D diffusion to search the genome elsewhere. Importantly, this electron transfer event between the two glycosylases would represent an effective scanning of the DNA integrity between the two proteins. This scanning process would continue until the proteins bind near sites of damage. Since DNA CT through the damaged DNA would be attenuated, any oxidized protein would stay bound in the vicinity of the lesion, diffuse one-dimensionally to the lesion, and process the lesion according to previously described mechanisms.[53–56] DNA CT thus offers a mechanism for DNA glycosylases that contain 4Fe-4S clusters to use DNA-mediated signaling *as a first step* in the search for damage, using DNA CT to scan the genome and concentrate the repair proteins in the vicinity of damage.

An experiment using atomic force microscopy (AFM) was used to visualize protein-DNA complexes in order to test directly if EndoIII redistributes onto DNA that contains damage.[22,23] In this experiment, EndoIII was incubated with mixtures of DNA, one DNA containing a single C:A mismatch, which attenuates DNA CT but is not a substrate for EndoIII, the other DNA being fully matched and undamaged (Figure 7). The well-matched DNA and mismatched DNA can be distinguished in the AFM given their different lengths. The short strands (~1.9 kbps) are well-matched, while the long strands (~3.8 kbps) contain a single C:A mismatch near the center of the strand. After incubation, what is found is that the proteins do in fact redistribute to the long mismatched strands of DNA. Even though there is only a single base mismatch in 3800 base pairs, the binding density of the protein is found to be higher on the mismatched strand; as a control, where there is no mismatch in the long strands of DNA, the protein density is the same on both the well-matched and mismatched strands of DNA. Interestingly, we also tested the role of DNA CT in this experiment. As discussed previously, EndoIII Y82A is a mutant of EndoIII that is CT-deficient as assessed electrochemically, but has enzymatic activity comparable to that of WT EndoIII[22,48]; when EndoIII Y82A is incubated with the mixtures of mismatched and well-matched DNA, there is no observed redistribution. Adding hydrogen peroxide to the protein-DNA mixtures during incubation increases the extent of redistribution suggesting that oxidative stress may further drive redistribution to sites of damage.[23] Indeed, in subsequent experiments using various mutants of EndoIII, we found a direct correlation between the proficiency in carrying out DNA CT and the ability to redistribute onto the damaged strand. These results strongly indicate that DNA-mediated redox signaling can be used by DNA glycosylases to drive redistribution to the vicinity of DNA damage. But does this cooperative signaling occur within a cell?

As predicted by the model, which is supported by the AFM results, if EndoIII and MutY use DNA-mediated redox signaling as a first step to locate damage, then the activity within cells of one of the glycosylases should be affected by the presence or absence of the other. Genetics experiments were used to show that this indeed appears to be the case. A lac^+ forward reversion assay using the CC104 strain of bacteria was used to measure the activity of MutY upon silencing the gene encoding EndoIII, *nth*.[22,57,58] Within the CC104 strain of bacteria, MutY prevents GC:TA transversions within the *lacZ* gene by removing adenines placed opposite the oxidized lesion, 8-oxoG. Cells in which the GC:TA transversion has occurred, termed lac^+ revertants, can grow on media that contains lactose as the sole carbon

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source, providing a readout of MutY activity. When the *nth* gene was inactivated within CC104, the number of *lac*$^+$ revertants increased 1.5 to 2-fold reflecting a decrease in MutY activity.[22] This result is consistent with signaling between EndoIII and MutY. Moreover, mutants of EndoIII were expressed off of complementation plasmids in CC104 Δ*nth* to provide evidence that this signaling occurs via DNA-mediated CT. When the CC104 Δ*nth* cells are complemented with EndoIII Y82A, the CT-deficient mutant, there is no rescue of MutY activity. However, when the cells are complemented with EndoIII D138A, which is catalytically inactive but CT-proficient, MutY activity is restored. These results in combination with the results from AFM suggest that DNA glycosylases utilize DNA-mediated redox signaling as a first step in locating DNA damage efficiently within cells.

Signaling enzymatic activity of DNA Helicases with 4Fe-4S Clusters

The evidence that DNA glycosylases may use 4Fe-4S clusters to participate in DNA CT in order to localize to sites of damage in cells is intriguing, but importantly, other DNA-processing enzymes have also been shown to contain 4Fe-4S clusters. For example, XPD is an ATP-dependent helicase from the nucleotide excision repair pathway that is also part of transcription-coupled repair.[59,60] In eukaryotes XPD is part of the TFIIH complex, which is vital for both nucleotide excision repair and transcription. Mutations in XPD in humans can lead to Xeroderma Pigmentosum and Cockayne's syndrome. Recently, XPD along with an entire family of helicases were predicted to contain 4Fe-4S clusters.[60,61] In 2008, three separate crystal structures were published for XPD from three different species, confirming the presence of the 4Fe-4S cluster in XPD. One structure was for XPD from *S. acidocaldirus*[59], one from *S. tokodaii*[62], and one from *T. acidophilum*[63]. Two of the crystal structures contained the intact 4Fe-4S cluster when crystallized, while the third did not. Of particular note is the observation that the global structure of XPD from *S. tokadaii* was not perturbed even though it did not contain the 4Fe-4S cluster.

The XPD protein from *S. acidocaldarius* (SaXPD) was electrochemically characterized on DNA-modified electrodes. Here, unlike in previous studies where a DNA duplex was utilized, the substrate on the self-assembled DNA monolayers was a 20-mer double-stranded duplex with a 9-mer 5′ to 3′ single-stranded overhang, which is a substrate that can be unwound by helicases. Strikingly, the DNA-bound midpoint redox potential of SaXPD was found to be ~80 mV vs. NHE, the same DNA-bound potential measured for the glycosylases MutY and EndoIII from *E. coli*.[20,25] Moreover, since the substrate on the surface of the electrode is a substrate that SaXPD can unwind, the effect of adding ATP to the solution was studied. Upon adding ATP, the current intensity rises by 10–20%, and at a rate comparable to the rate of ATP hydrolysis for SaXPD (Figure 8). Adding ATPγS led to no increase in the signal, indicating the effect is driven by the hydrolysis of ATP. This DNA-modified electrochemical assay therefore reports on the enzymatic activity of SaXPD and likely reflects better coupling of the 4Fe-4S cluster to the electrode during a helicase reaction. This could have important implications for the function of XPD and coordination of nucleotide excision repair within the cell. If during enzymatic activity, the efficiency of DNA-mediated electronic signaling is increased, this could be used as a means for XPD to communicate its activity to proteins downstream of XPD that contain 4Fe-4S clusters.

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Since SaXPD shares a midpoint redox potential with MutY and EndoIII, chemically, XPD could shuttle electrons to and from MutY and EndoIII *in vitro*. AFM was used to test directly if SaXPD can thus use DNA CT to signal to EndoIII and to localize to sites of DNA damage (Figure 7). When SaXPD is incubated with samples of well-matched DNA and DNA containing a mismatch, the protein preferentially binds to strands of DNA with damage[64], analogous to the effect observed for *E. coli* EndoIII.[22] Again, a mismatch is not a substrate for XPD. SaXPD L325V is a mutant that was found to be deficient in DNA CT electrochemically, and here too, as found with EndoIII mutants, deficient in CT signaling, the L325V mutant of SaXPD is unable to redistribute to sites of damage. Significantly, when mixtures of EndoIII and SaXPD are mixed with the DNAs, the proteins redistribute to sites of damage, but when the mutants SaXPD L325V or EndoIII Y82A are swapped for their WT counterparts in the mixtures, redistribution is no longer observed. These results provide evidence that EndoIII and SaXPD can signal one another via DNA CT to localize to sites of damage.[64] Indeed, even though EndoIII and SaXPD are from different organisms, even different kingdoms, the fact that they contain 4Fe-4S clusters that share a similar redox potential and can electrochemically couple to the DNA π-stack, allows them to use DNA CT as a means of signaling, cooperating from a distance to find sites of DNA damage.

Cross-talk between repair pathways via DNA-mediated signaling in cells

DinG is a damage-inducible, ATP-dependent helicase from *E. coli* that also contains a 4Fe-4S cluster and shares significant homology to SaXPD.[65–67] DinG, like XPD, unwinds DNA with a 5′ to 3′ polarity. Most substrates that contain a 5′ to 3′ overhang of at least 15 base pairs can be unwound by DinG, though DinG cannot unwind double-stranded DNA (dsDNA). Uniquely, DinG can, however, unwind R-loops, which are RNA-DNA hybrids that form within a bubble. R-loops have been shown to be one of the target substrates of DinG *in vivo*.[68] Similar to EndoIII, the redox potential of the $[4Fe\text{-}4S]^{2+/1+}$ couple when not bound to DNA was found to be ~−400 mV vs. NHE using titrations with redox mediators.[66]

The electrochemical assay used to test the DNA-bound redox characteristics of EndoIII, MutY, and SaXPD was used to examine DinG. It was shown that the DNA-bound potential of DinG is ~ 80 mV vs. NHE.[50] Again, this is the same DNA-bound redox potential observed for the glycosylases EndoIII and MutY, and the helicase SaXPD. It appears that the emerging class of DNA-processing containing enzymes 4Fe-4S clusters all share a DNA-bound redox potential, which could facilitate self-exchange electron transfer reactions among the proteins. Based on the results for EndoIII,[20,21] we considered that the couple observed when bound to DNA is the $[4Fe\text{-}4S]^{3+/2+}$ couple, shifted towards oxidation upon binding to DNA. The substrate used to characterize DinG electrochemically was a 20-mer duplex with a 15-mer 5′ to 3′ single-stranded overhang, a substrate that can be unwound by DinG. When ATP is added to DinG on DNA-modified electrodes, a significant increase in current is observed, similar to the effect observed for SaXPD (Figure 8). The increase in the electrochemical signal over time for DinG is ~5–10 fold higher than that seen for SaXPD, which has a lower rate of ATP hydrolysis, establishing a correlation between the rates of ATP hydrolysis and increase in signal.[25] The AFM redistribution assay testing cooperative DNA-mediated signaling between DNA-binding proteins containing 4Fe-4S clusters also showed results analogous to that seen for SaXPD. DinG is found to redistribute to DNA

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containing a mismatch site that attenuates DNA CT. Moreover, DNA-mediated signaling between EndoIII and DinG was also established, since redistribution to long strands of DNA containing a mismatch are observed for mixtures of DinG and EndoIII, but not when the CT-deficient EndoIII Y82A mutant is mixed with DinG. These *in vitro* experiments indicate that DinG, EndoIII, and MutY are equipped to and may utilize DNA-mediated CT to cooperatively and efficiently find damage.

The important question of whether or not DNA-mediated CT signaling occurs between repair enzymes containing 4Fe-4S clusters from different pathways *in vivo* was next examined. Since DinG is from *E. coli*, genetics experiments can be conducted to explore whether cooperative signaling occurs among EndoIII, MutY, and DinG *in vivo*. Based upon the AFM results for DinG and the proposed model for redistribution, one would expect that the absence of DinG would lead to a decrease in MutY activity within cells, which was measured using a *lac*+ forward reversion assay in the CC104 strain of bacteria, as described earlier.[57,58] When DinG is knocked out of CC104, an increase in the number of lac^+ revertants is observed, reflecting the predicted decrease in MutY activity.[50] If it is the 4Fe-4S cluster of DinG and its participation in DNA-mediated signaling that is required for MutY to fully function, then EndoIII, with its analogous 4Fe-4S cluster could be used to rescue MutY activity. Complementation plasmids expressing various mutants of EndoIII were used to test if the observed signaling between MutY and DinG was due to DNA-mediated CT signaling. Indeed, expression of EndoIII D138A, which is catalytically inactive but can bind DNA and is CT-proficient, restores MutY activity. Moreover, expression of the CT-deficient EndoIII Y82A mutant does not rescue MutY activity.[50] Thus it appears that DinG is assisting MutY in preventing GC:TA transversions via DNA-mediated CT, assisting MutY in finding its target lesion, and substitution of EndoIII or MutY accomplishes the same goal.

In a second genetic study, using a sensitive assay for DinG activity, we then asked the question another way: If DinG can assist MutY or EndoIII, can EndoIII similarly assist DinG? A strain of bacteria designated InvA, constructed so that the activity of DinG is crucial, was used to show that EndoIII cooperates with DinG to dismantle R-loops. In conditions that promote stalled replication forks resulting from the collision of the fork with the transcription machinery, DinG is required for normal cell growth.[68] This requirement has been attributed to DinG being required to unwind buildups of R-loops at these stalled replication forks. A significant growth defect is observed when the gene encoding DinG is knocked out of the InvA strain[68], and overexpression of RNaseH, which degrades R-loops, rescues the growth defect. To test if EndoIII cooperates with DinG, assisting DinG in targeting and dismantling R-loops, the gene encoding EndoIII was silenced in InvA. When EndoIII is knocked out of the InvA strain, a significant growth defect is observed (Figure 9).[50] This growth defect appears to be due to signaling between EndoIII and DinG, since overexpression of RNaseH rescues the growth defect. But is this cooperation due to DNA-mediated CT signaling? Complementation with a plasmid encoding EndoIII D138A, which is catalytically inactive but CT-proficient, also rescues the growth defect, while, strikingly, expression of the CT-deficient mutant EndoIII Y82A does not (Figure 9). These results in combination with the results from the AFM redistribution assay and lac^+ forward reversion

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assay strongly point to MutY, EndoIII, and DinG all utilizing DNA CT as a means of cooperative signaling within cells to more efficiently repair DNA.

4Fe-4S clusters in Eukaryotes

While the work presented here has focused on DNA-processing enzymes that contain 4Fe-4S clusters in prokaryotes and archaea, 4Fe-4S clusters have also now been found in a wide array of enzymes in eukaryotic organisms (Table 1). It is interesting to consider that only a decade ago, few DNA-processing enzymes were known to contain 4Fe-4S clusters. One reason the presence of iron-sulfur clusters in these enzymes is now being uncovered is that isolating the cluster-loaded protein appears to depend greatly on the expression and purification conditions. Less typical expression conditions, using low concentrations of inducers of gene expression and carrying out the expression at low temperatures, have been needed to obtain high yields of cluster-loaded proteins.[23,50,59,63,69] In other examples, such as for yeast polymerase δ, the proteins must be purified from the native organism in order to be loaded with a 4Fe-4S cluster.[70] Using these new techniques and upon closer examination, many 4Fe-4S clusters are being found in an array of eukaryotic enzymes. The 4Fe-4S clusters have been found in members of the nucleotide excision and base excision repair families such as XPD, Ntg2, and OGG1, as well as in a host of helicases[59–61,71,72]. Enzymes functioning in recombinatorial repair such as FancJ and DNA2 also contain 4Fe-4S clusters in eukaryotes.[61,71–74] Even enzymes involved in the vital processes of transcription and replication such as RNA polymerase, DNA polymerases α/δ/ε, and primase have now been shown to contain 4Fe-4S clusters.[70, 71, 75–78] The role of the 4Fe-4S clusters in all of these enzymes remains to be established Yet it is clear that evolution has preserved and even expanded the presence of the 4Fe-4S cluster across the phylogeny. Mutations in enzymes containing 4Fe-4S clusters are, moreover, linked to human disease such as photosensitivity syndromes and early onset breast cancer.[59,61] Furthermore, disruption of the iron-sulfur cluster biogenesis machinery in yeast has been directly linked to genomic instability.[79–81] It is clear, then, that these clusters play an important role within these enzymes. Based upon the data we have presented, one may consider that one role for these clusters may be to help coordinate DNA repair.

One issue that has not been extensively studied is whether the oxidation state of the 4Fe-4S cluster serves to modulate the catalytic activity of a given protein in this family of proteins. Experiments to test activity of DNA-processing enzymes containing 4Fe-4S clusters in their oxidized versus reduced forms are now required. It is tempting to propose that these clusters may serve to facilitate DNA-mediated CT signaling in order to coordinate not only DNA repair, but also transcription and replication in eukaryotes. Can redox signaling from a distance serve to turn on replication, or perhaps more functionally, turn off replication by oxidizing the 4Fe-4S cluster of DNA polymerases under conditions of oxidative stress? Replication and transcription requires an extensive degree of coordination is at the replication fork or site of transcription, and involves many steps carried out by elaborate complexes of numerous enzymes. Utilizing DNA as a means of communication would serve as an attractive mechanism to help facilitate this critical process.

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Conclusion

DNA CT chemistry thus offers a means to carry out redox chemistry at a distance and in a fashion that depends upon the integrity of the intervening base pair stack. We have seen how this chemistry may be utilized as a sensor of oxidative stress, both in funneling damage and in oxidizing DNA-bound protein sensors. We have also seen how proteins containing 4Fe-4S clusters may signal one another using DNA CT chemistry and how specifically this signaling may be used as a first step in efficiently redistributing repair proteins to sites of DNA lesions for repair. The association of 4Fe-4S clusters, common redox cofactors in biology, with proteins involved in DNA repair is being found with increasing frequency and has apparently been preserved in higher organisms that carry out still more complex modes of repair. Utilizing DNA CT with these redox cofactors provides a role for these clusters in the repair proteins along with a mechanism to understand how the cell achieves the high level of efficient repair we require. Indeed, given findings of 4Fe-4S clusters now in proteins involved in all aspects of DNA processing, it is tempting to suggest that here too these redox cofactors may be present to carry out DNA CT. Thus DNA CT could provide a general means of signaling among DNA-bound proteins across the genome. Certainly a range of experiments to explore the many ways this redox chemistry may be utilized within the cell is now required.

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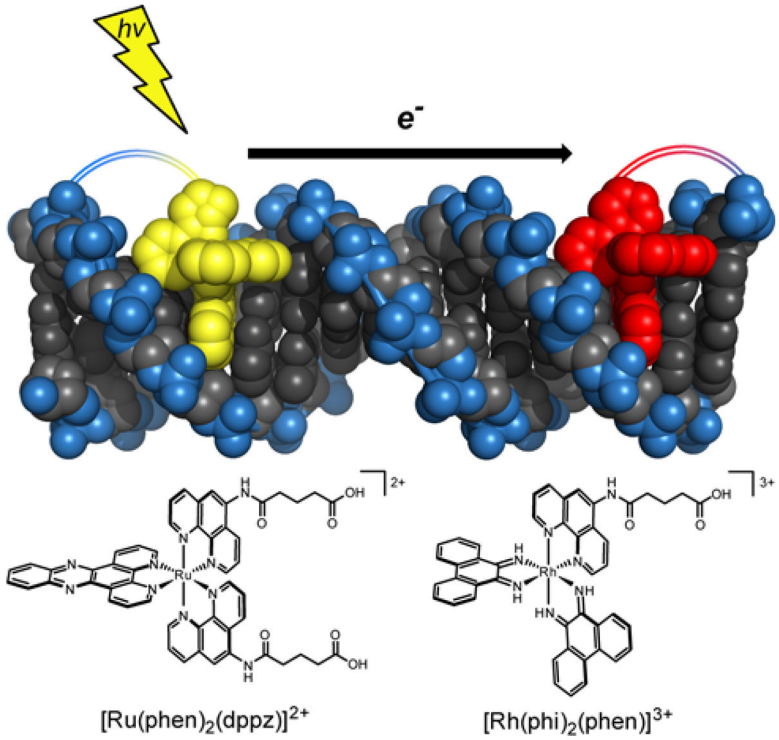

Figure 1.

Measuring DNA CT with metal complexes. Shown is a characteristic DNA assembly used to monitor DNA-mediated redox chemistry in solution, using metallointercalators to monitor luminescence quenching by electron transfer through the DNA base stack.[6] The chemical structures of the covalent donor and acceptor, $Ru(phen)_2dppz^{2+}$ (phen = 1,10-phenanthroline, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) (left) and $Rh(phi)_2phen^{3+}$ (phi = 9,10-phenanthrenequinone diimine) (right), respectively, are shown.

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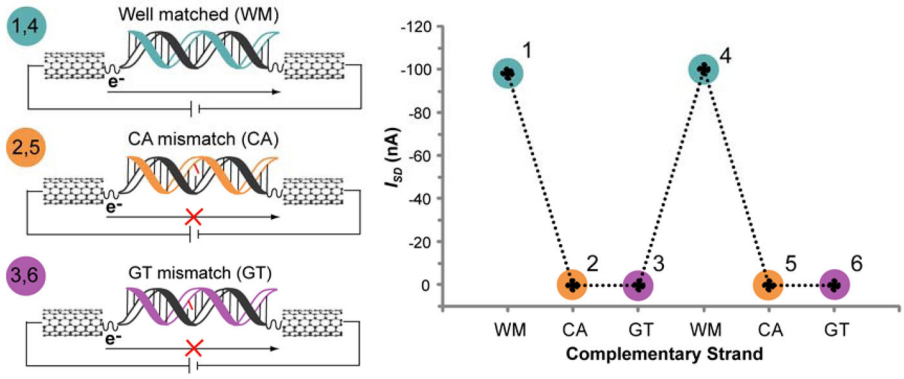

Figure 2.

Measuring DNA CT in single molecules. A single DNA duplex is made to covalently bridge a gap in an electronically wired, carbon nanotube device such that the measured current flow through the device reflects DNA CT efficiency.[10] To confirm that charge flow through the device is DNA-mediated and to illustrate the sensitivity of DNA CT to single base mismatches, an experiment was designed to allow the introduction of a single base mismatch through thermal dehybridization and rehybridization of the bridging duplex (left). One strand of the DNA duplex is covalently attached at either side of the gap (black), while the other, noncovalent strand is cycled between a well matched strand (turquoise) and strands with a single base mismatch (orange, purple) by sequential dehybridization, rinsing, and rehybridization. During this cycling between well matched and mismatched duplexes, the source-drain current (I_{SD}) for the device was measured at a constant gating voltage (V_G = −3V) and plotted for each bridging duplex (right plot, where the colors and numbers of the duplexes in the left illustration correspond to those on the plot). This experiment clearly illustrates the high sensitivity of DNA CT to single base mismatches; DNA CT is inhibited when the device is bridged with a mismatched duplex and restored when the device is rehybridized with a well matched duplex.

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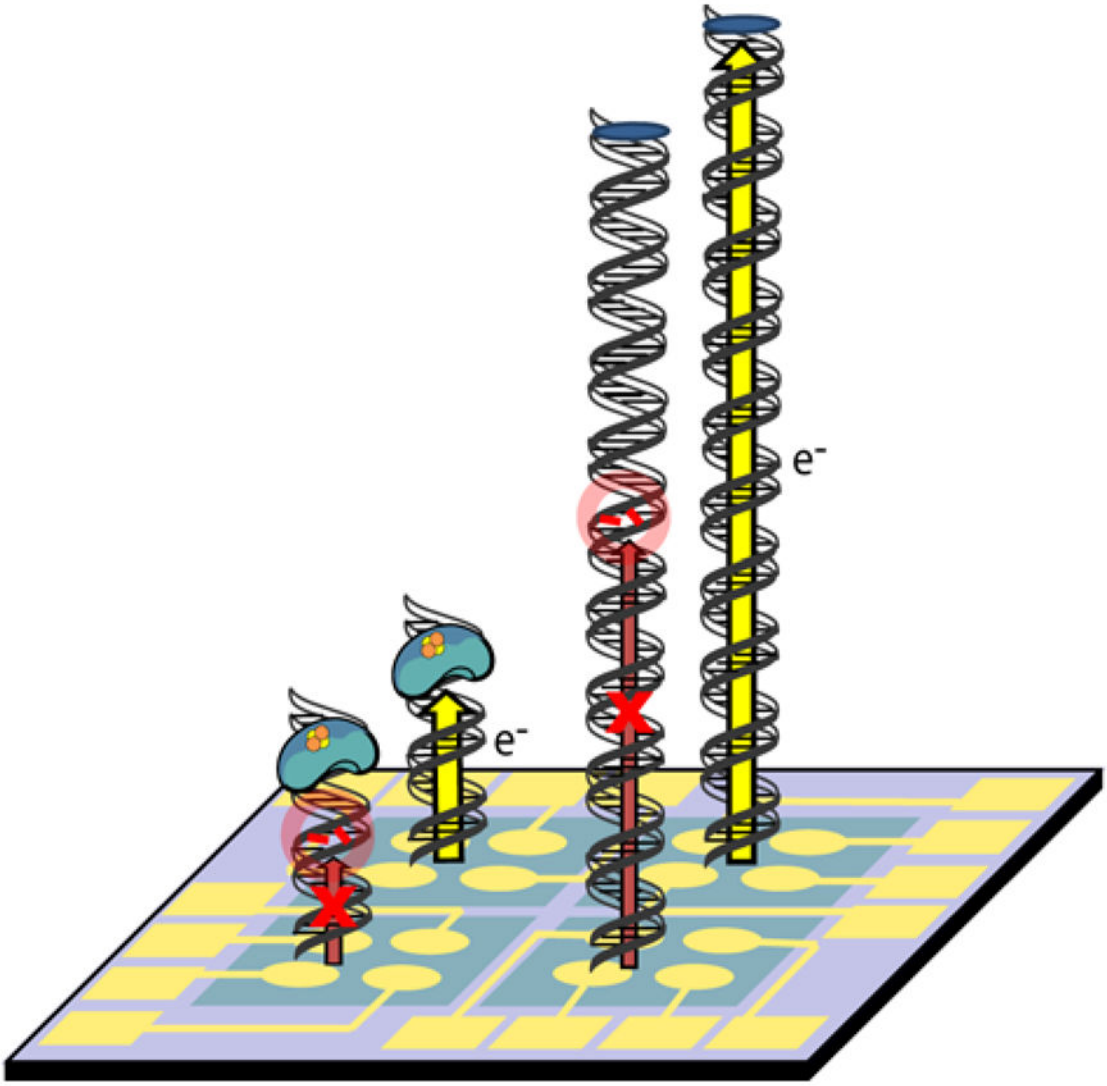

Figure 3.

Measuring DNA CT with DNA-modified electrodes. Schematic of a multiplex chip with four different DNAs in the four quadrants of the chip. Left side: mismatched (front) and well matched (back) 17-mers with DNA-bound, redox-active protein containing a [4Fe-4S] cluster signified by the cluster of two orange and two yellow spheres. The protein binds the DNA, which is covalently attached by one end to the gold surface, and reduction of the cluster proceeds via DNA CT. Right side: mismatched (front) and well matched (back) 100-mers with a covalent, small molecule redox probe. The location of the mismatch in the 17-mer and 100-mer is circled in red. DNA CT to the redox-active protein or small molecule probe is significantly attenuated (red X) in the presence of a single base mismatch for both the 17-mer and 100-mer.[15,16]

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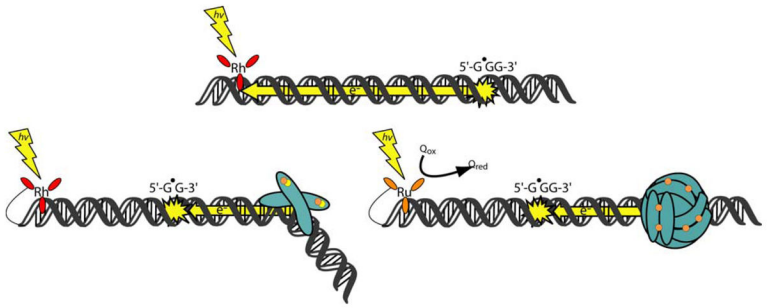

Figure 4.

Initial experiments to probe DNA CT in biology. Illustrated are some examples of experimental constructs used to evaluate the capabilities of different cellular players to participate in DNA CT including cellular DNA (top) and proteins with redox-active cofactors (bottom left and right). Top: the capacity of cellular DNA to funnel damage over long distances in the genome via DNA CT was studied within its native environment inside a variety of organelles.[29–33] Damage is induced by a photoexcited, intercalated metal complex and the appearance of damage at the 5′-guanine of distant guanine repeat sites supports that DNA CT facilitates this transport of electrons over such long distances. Bottom Left: SoxR, an oxidative stress response transcription factor binds DNA as a dimer, with each monomer containing a [2Fe-2S] cluster signified by a cluster of one orange and one yellow sphere. SoxR, is activated by oxidative DNA damage from a distance, induced by a covalent, photoexcited metal complex.[38] Bottom Right: Dps, a 12-subunit protein that binds DNA as a spherical dodecamer, contains 12 intersubunit ferroxidase sites that, in the depicted experiment, are occupied by 1 Fe^{2+} each (12 Fe^{2+}/Dps; each bound Fe^{2+} is represented by a single orange sphere). Dps uses ferroxidase activity to protect DNA from reactive oxygen species and can neutralize guanine radicals from a distance when they are formed by a photoexcited metal complex.[39]

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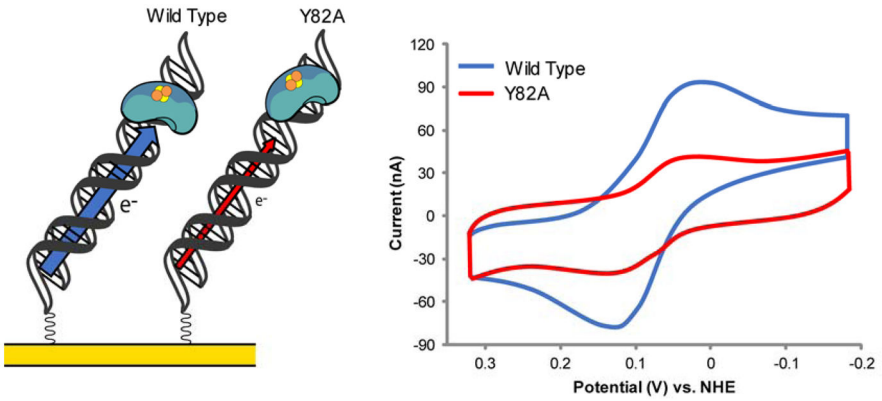

Figure 5.

Electrochemistry of DNA glycosylases on DNA-modified electrodes. Both single and multiplexed DNA-modified gold electrodes have been used to study the electrochemical characteristics of DNA glycosylases containing [4Fe-4S] clusters. In these experiments, the protein is bound to the DNA, which is attached to the gold electrode surface, and the [4Fe-4S] cluster is reduced via DNA CT (left). Cyclic voltammetry has been used to determine that the midpoint redox potential of these enzymes is around 80 mV vs. NHE.[20,22,48] and to establish that EndoIII Y82A is deficient in its ability to perform DNA-mediated CT (right). The intensity of the electrochemical signal of EndoIII Y82A is much lower than that of WT EndoIII, depicted by the red and blue arrows, respectively, in the illustration (left). The redox-active [4Fe-4S] clusters in the proteins are signified here by clusters of two orange and two yellow spheres.

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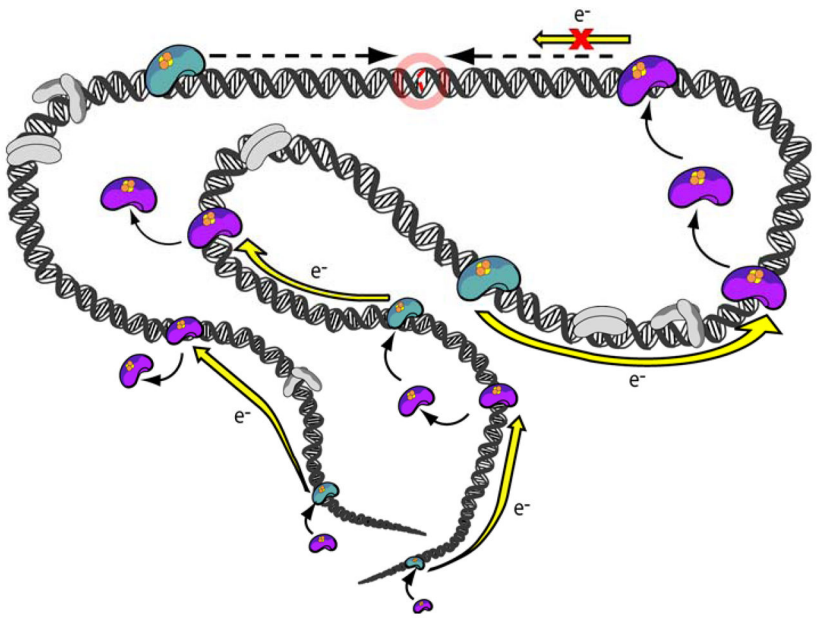

Figure 6.

Model for the enhanced DNA lesion search efficiency of DNA repair proteins with 4Fe-4S clusters by DNA CT signaling. Repair proteins with 4Fe-4S clusters, such as MutY and EndoIII, may use DNA CT to effectively scan long stretches of genomic DNA for damage as illustrated (from bottom to top).[22,23,47,50] In the cytoplasm, the 4Fe-4S cluster of repair proteins is in the 2+ oxidation state (purple), but the cluster is oxidized to the 3+ state (turquoise) upon DNA binding if an electron can be transferred to a distally bound protein (recepient) via DNA CT (yellow arrows). Importantly, the intervening DNA must be free of damage for this transfer to occur. Successful electron transfer reduces the cluster of the recepient protein from the 3+ to 2+ oxidation state which decreases its binding affinity for DNA and promotes its dissociation. This protein, diffusing freely in the cytoplasm, can then bind at a different DNA location and repeat this use of DNA CT to scan another segment of the genome. If, however, the protein binds a location where there is damage intervening it and a potential recepient protein, electron transfer cannot occur and the potential recepient protein remains tightly bound to the DNA with its cluster in the 3+ oxidation state. The bound proteins (either in the 3+ or 2+ state) which are now localized in the vicinity of the damage, can then process along the DNA (dashed black arrow) to efficiently find and repair the damage. The redox-active [4Fe-4S] clusters in the proteins are signified here by clusters of two orange and two yellow spheres.

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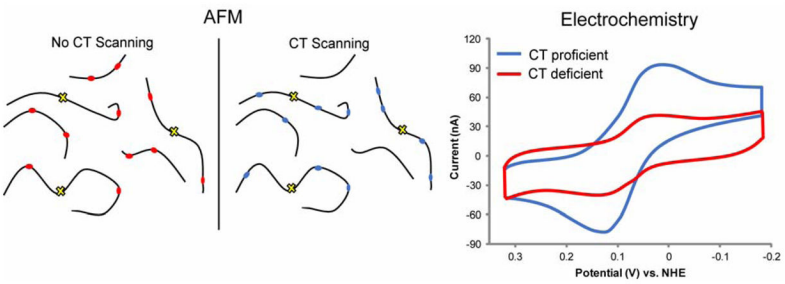

Figure 7.

AFM reveals that CT proficiency determines whether proteins localize near DNA mismatches. When wild-type, CT-proficient EndoIII, SaXPD, or DinG (blue in left diagram and cyclic voltammogram (CV) on right) are independently incubated with mixtures of long strands of DNA (3.8 kbps) containing a mismatch (yellow X) and well-matched short strands of DNA (1.9 kbps), a redistribution of the enzymes to the mismatched strand of DNA is observed (right).[22,47,50] This is consistent with DNA-mediated charge transport promoting redistribution to the site that attenuates DNA CT. This redistribution to the damaged strand is not observed for the CT-deficient EndoIII Y82A or SaXPD L325V mutants (red in diagram and in CV on right), nor is it observed when CT-deficient mutants are mixed with either SaXPD or DinG.

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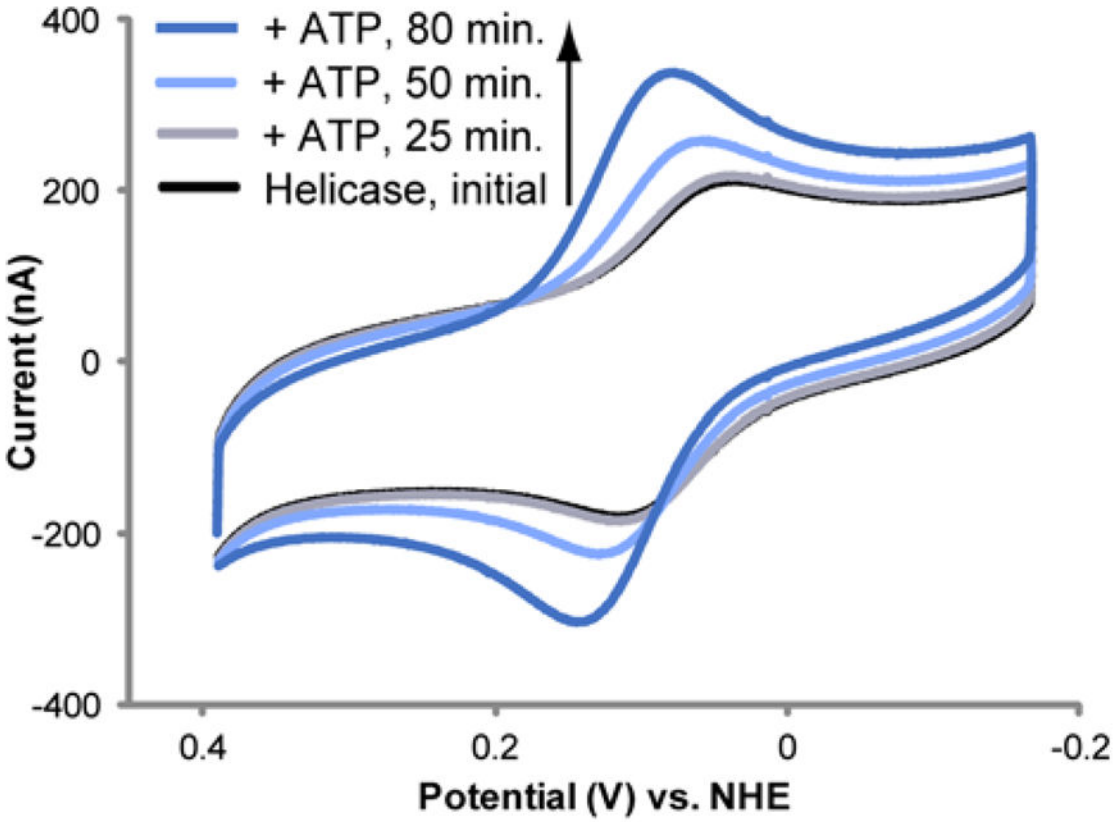

Figure 8.

ATP hydrolysis by helicases with 4Fe-4S clusters measured by CV. The helicases SaXPD and DinG both have a redox potential of ~80 mV vs. NHE.[25,50] When ATP is added to each enzyme on DNA-modified electrodes with 5′ to 3′ single-stranded overhangs, substrates that each enzyme is competent to unwind, an increase in the intensity of the electrochemical signal is observed. As the protein incubates on the electrode, the intensity of the signal slowly grows in over time. After the addition of ATP to the solution, the change in the growth of the signal increases by 10–20% for XPD and 40–50% for DinG. Shown above, 5 mM ATP was added to DinG that been allowed to equilibrate on a DNA-modified electrode overnight (black). The overlaid traces show the signal at several time points during the incubation of ATP including 25 minutes (grey), 50 minutes (light blue), and 80 minutes (blue). ATP hydrolysis leads to a significant increase in the intensity of the electrochemical signal.

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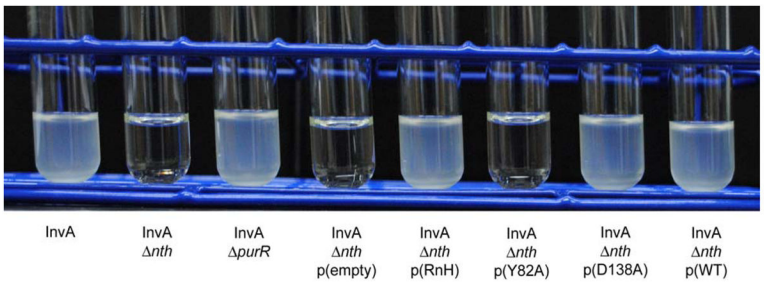

Figure 9.

Cultures of InvA strains transformed with complementation and rescue plasmids. Shown is a photograph of cultures of InvA, InvA Δ*nth*, and InvA Δ*purR* in addition to InvA Δ*nth* transformed with plasmids that express RNaseH, EndoIII Y82A, EndoIII D138A, or WT EndoIII, all grown in minimal media to stationary phaseIn minimal media, it is clear that silencing the *nth* gene in InvA leads to a severe growth defect that cannot be rescued by expression of the CT-deficient EndoIII Y82A but can be rescued by expression of WT EndoIII, EndoIII D138A, or RNaseH. InvA Δ*purR* was included as a control to show that the mutation in *purR* that was linked to the Δ*nth* deletion when constructing the InvA Δ*nth* strain did not cause the observed growth defect. An empty plasmid was used as a control to show that the presence of a plasmid does not affect growth.[50]

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Table 1

DNA-processing enzymes containing 4Fe-4S clusters

Enzyme[a]	Kingdom[b]	Enzymatic Activity	References[d]
MutY, EndoIII, UDG	A, B, E	glycosylase, BER	(42,44–46,82)
AddAB	B	helicase-nuclease, RR[c]	(69,83)
Dna2	A, E	helicase-nuclease, replication, RR	(73,83)
Exonuclease V	E	5′-exonuclease, replication and repair	(84)
Primase	A, E	RNA polymerase, replication	(75,76)
RNA polymerase	A, E	RNA polymerase, transcription	(77,78,85)
Rad3/XPD	A, E	helicase, NER transcription	(59–63)
FancJ, ChlR1, DOG-1, RTEL	E	helicases, multiple pathways	(60,61,72)
DinG	B	helicase, R-loop maturation	(66)
DNA polymerases α, δ, ε	E	DNA polymerase, replication	(70,86)

[a] This list is not comprehensive, but represents a sampling of the many enzymes found recently to contain 4Fe-4S clusters.[60,61,71,72]

[b] For kingdoms B = bacteria, A = archaea, E = eukarya

[c] RR = recombination repair

[d] References include predictions, spectroscopic, or crystallographic evidence for the presence of the 4Fe-4S clusters.