Long-range oxidative damage to cytosines in duplex DNA

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Charge transport (CT) through DNA has been found to occur over long molecular distances in a reaction that is sensitive to intervening structure. The process has been described mechanistically as involving diffusive charge-hopping among low-energy guanine sites. Using a kinetically fast electron hole trap, N_4-cyclopropylcytosine (^{CP}C), here we show that hole migration must involve also the higher-energy pyrimidine bases. In DNA assemblies containing either $[Rh(phi)_2(bpy')]^{3+}$ or an anthraquinone derivative, two high-energy photooxidants, appreciable oxidative damage at a distant ^{CP}C is observed. The damage yield is modulated by lower-energy guanine sites on the same or complementary strand. Significantly, the efficiency in trapping at ^{CP}C is equivalent to that at N_2-cyclopropylguanosine (^{CP}G). Indeed, even when ^{CP}G and ^{CP}C are incorporated as neighboring bases on the same strand, their efficiency of photodecomposition is comparable. Thus, CT is not simply a function of the relative energies of the isolated bases but instead may require orbital mixing among the bases. We propose that charge migration through DNA involves occupation of all of the DNA bases with radical delocalization within transient structure-dependent domains. These delocalized domains may form and break up transiently, facilitating and limiting CT. This dynamic delocalized model for DNA CT accounts for the sensitivity of the process to sequence-dependent DNA structure and provides a basis to reconcile and exploit DNA CT chemistry and physics.

charge transport | delocalized domain | radical trap | base dynamics

Oxidative damage to DNA from a distance through long-range migration of charge has now been established in many DNA assemblies by using different pendant photooxidants through both biochemical and spectroscopic assays (1–8). The DNA base pair stack can mediate charge transport (CT) over at least 200 Å (2, 3), and the reaction is exquisitely sensitive to the dynamic structure and stacking within the DNA duplex (9, 10). This sensitivity to perturbations in base pair stacking has been advantageous in the development of DNA-based sensors for mutational analysis (11) and may provide a role for DNA-mediated CT within the cell (12), but it has limited the application of physical techniques to explore CT mechanistically.

Although not a robust molecular wire, the DNA duplex has in some experiments been characterized as a wide band gap semiconductor (13, 14). More prevalent have been models of incoherent CT involving a mixture of localized charge-hopping among low-energy sites, guanines and sometimes adenines, and tunneling through higher-energy pyrimidine bases (15–18). These mechanisms do not provide a rationale, however, for the sensitivity of CT to DNA structure. We have observed that DNA CT is gated by the dynamical motions of the DNA bases (9, 19) and have described DNA CT as conformationally gated hopping through transient, well stacked DNA domains (20).

Our experimental strategy to probe for hole density on pyrimidines exploits the rapid (nanoseconds to picoseconds) ring opening of cyclopropylamine radical cations (21). Saito and coworkers have developed kinetic traps for holes residing on G (22) and A (23) based on the rapid ring opening of N_2-cyclopropylguanosine (^{CP}G) and N_6-cyclopropyladenine upon oxidation through DNA-mediated CT. These kinetic traps have been useful also in establishing CT for those photooxidants that produce limited oxidative DNA damage owing to rapid back CT (24, 25). Guanine, although a low-energy site, provides a relatively slow (milliseconds) (26) hole trap through irreversible reaction with water and/or oxygen (27). Significantly, in experiments using N_6-cyclopropyladenine, CT involving adenines has been documented (23).

Here, using the kinetically fast electron hole trap, N_4-cyclopropylcytosine (^{CP}C), we demonstrate that hole migration involves not only low-energy purine sites but also the higher-energy pyrimidine bases. We examine long-range CT in DNA assemblies containing two different high-energy photooxidants, and we find appreciable oxidative damage at a distant ^{CP}C. The yield of ^{CP}C decomposition is modulated by lower-energy guanine sites within the assembly and is comparable in magnitude to that for a neighboring ^{CP}G. Thus, during the course of CT through DNA, appreciable hole density must reside also on high-energy pyrimidine sites. CT through DNA involves all of the DNA bases.

Materials and Methods

Modified Bases. N_4-cyclopropylcytidine (r^{CP}C) and ^{CP}C were synthesized by stirring 4-thiouridine or 4-thiouracil, respectively, in a 1:2 cyclopropylamine:ethanol solution at 55°C for 2 days. After purification by reverse-phase HPLC, the product was confirmed by ^{1}H NMR and electrospray ionization–time-of-flight MS. Cyclic voltammetry was carried out in aqueous solution (pH 7) by using a glassy carbon electrode with Ag/AgCl as a reference.

Oligonucleotides. All DNA oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer (trityl on), twice purified by HPLC, and analyzed by electrospray ionization–time-of-flight MS. ^{CP}C- or ^{CP}G-containing strands were obtained by incubating oligonucleotides possessing 4-thiouridine or 2-fluoroinosine, respectively, in 6 M aqueous cyclopropylamine at 60°C for 16 h before HPLC purification. The photooxidants, $Rh(phi)_2(bpy')^{3+}$ [phi, 9,10-phenanthrenequinone diimine; bpy′, 4-methyl-4′(butyric acid)-2,2′-bipyridine] (Rh) and an anthraquinone derivative [anthraquinone-2-carboxylic acid (2-hydroxyethyl) amide] (AQ), were synthesized and tethered to the complementary strands as described in ref. 25; for Rh-tethered assemblies, only the Δ-isomers (determined from CD spectroscopy) were used.

DNA oligonucleotides were quantitated by UV-visible spectroscopy. Duplex solutions (5 μM in 10 mM sodium phosphate and 50 mM NaCl, pH 7) were prepared by combining equimolar amounts of the desired DNA complements and annealing with regulated cooling from 90°C to ambient temperature over a period of 2 h. Under our experimental conditions the duplexes melt between 53 and 60°C (2 μM duplexes in 10 mM sodium phosphate and 50 mM NaCl, pH 7), depending on the DNA

Abbreviations: AQ, anthraquinone; ^{CP}C, N_4-cyclopropylcytosine; ^{CP}G, N_2-cyclopropylguanosine; CT, charge transport; I, inosine; r^{CP}C, N_4-cyclopropylcytidine; Rh, $[Rh(phi)_2(bpy')]^{3+}$.

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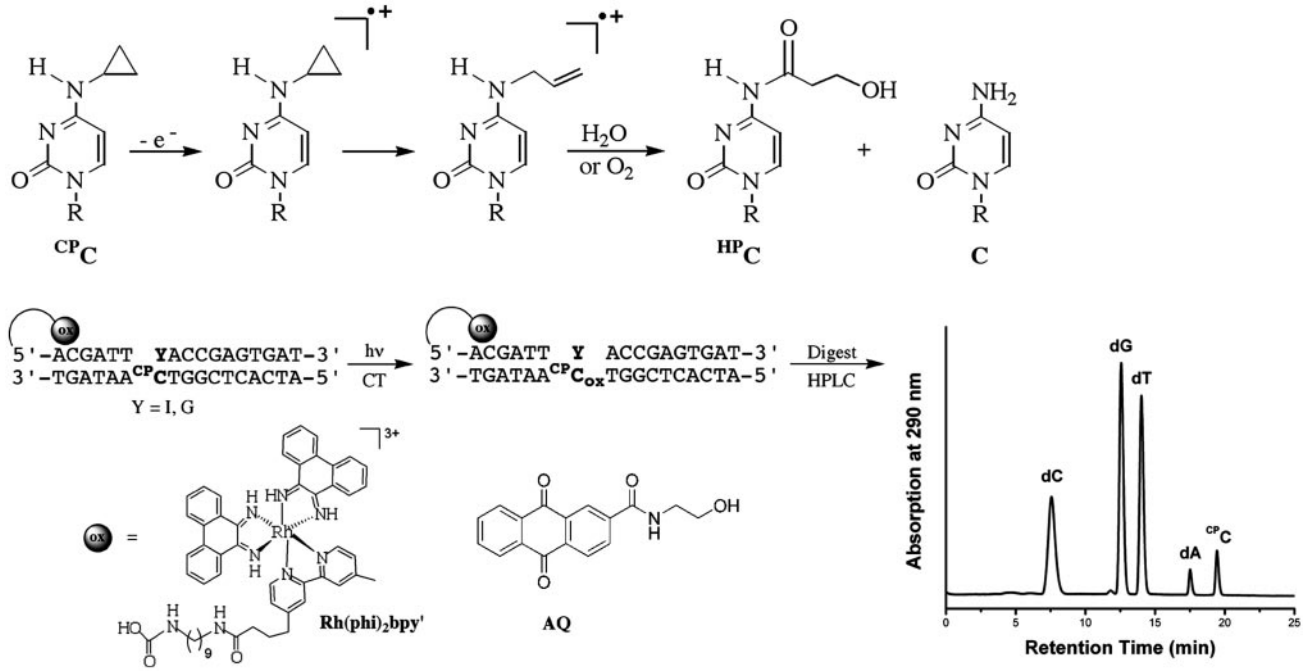

Fig. 1. Experimental assay to test for hole occupation of pyrimidines during CT through duplex DNA. (*Upper*) Oxidation of ^{CP}C leads to rapid decomposition through ring scission and subsequent generation of two characteristic products. (*Lower*) Two potent photooxidants, Rh and AQ, covalently positioned remote from ^{CP}C [for Rh through the 5′-sugar and for AQ through the 5′-phosphate (25)] initiate hole injection into DNA. Photooxidation of ^{CP}C in DNA, detected by HPLC analysis of the enzymatically digested duplexes, requires that the hole first migrate through the lower-energy intervening bases.

sequence. The presence of ^{CP}C depresses the duplex melting temperature by ≤1°C.

Photooxidation. Aliquots of Rh-tethered duplexes were irradiated at 365 nm with a 1,000-W Hg/Xe lamp equipped with a 320-nm long pass filter and a monochromator. Aliquots of AQ-tethered duplexes were irradiated at 350 nm by using the same apparatus. Light controls were conducted by irradiating duplexes in the absence of tethered photooxidant. Interduplex controls were conducted by irradiating a mixture of duplexes containing both photooxidant without ^{CP}C and ^{CP}C without photooxidant. Experiments reported here were carried out aerobically; however, similar results were obtained in the absence of oxygen. After irradiation (0–40 min), duplex samples were digested by 37°C incubation with phosphodiesterase, S1 endonuclease, and alkaline phosphatase for 4 h to yield the free nucleosides, and the samples were analyzed by reverse-phase HPLC (Chemcobond 5-ODS-H, 4.6 × 100 mm). Oxidation of r^{CP}C (250 μM) by Rh or AQ (25 μM) and subsequent HPLC analyses were carried out under the same conditions described for the DNA samples. The oxidation products of r^{CP}C nucleoside were characterized by electrospray ionization–time-of-flight MS (Figs. 5 and 6, which are published as supporting information on the PNAS web site).

Results

The Pyrimidine Hole Trap and Its Oxidation. We have prepared ^{CP}C by reaction of cyclopropylamine with 4-thiouracil. Just as the oxidation potential of ^{CP}G does not differ significantly from that of G (22, 28), the oxidation potential of ^{CP}C is expected to be very similar to unmodified C. Cyclic voltammetry reveals no oxidation of ^{CP}C at potentials <1.6 V vs. NHE in aqueous solution (pH 7), whereas oxidation of G was observed at ≈1.3 V vs. NHE. Thus, the cyclopropyl moiety does not significantly reduce the oxidation potential of C. For the isolated nucleosides, then, ^{CP}C, like C, is significantly harder to oxidize than G.

Two potent photooxidants, Rh and AQ, are used to initiate the chemistry. Both have sufficient excited-state reduction potentials (2.0 and 1.9 V vs. NHE, respectively) (29, 30) to oxidize each of the four natural DNA bases, and neither photoreduces DNA bases (31, 32). Photoinduced electron transfer from r^{CP}C to each of these oxidants is observed in solution. The oxidation products are completely analogous to those found upon oxidation of ^{CP}G (22). Irradiation of either Rh or AQ in the presence of r^{CP}C induces efficient decomposition of the nucleoside and leads exclusively to the products of one electron oxidation followed by ring scission and trapping with water and/or oxygen (Figs. 1, 5, and 6).

Assay for Pyrimidine Radical Formation. Our experimental assay to test for hole occupation of pyrimidines is also shown schematically in Fig. 1. This assay employs duplex DNA assemblies containing ^{CP}C positioned remotely from a covalently tethered photooxidant. Irradiation initiates hole injection and migration through the duplex; the presence of hole density on ^{CP}C can be detected by its decomposition monitored by HPLC after enzymatic digestion. As with N_6-cyclopropyladenine and ^{CP}G (22, 23), substitution of the cyclopropyl moiety onto C causes little structural perturbation in the duplex. It is readily accommodated in B-DNA, with little change in duplex melting temperature (≤1°C) and no change in hypochromism.

In the DNA sequences used (Table 1), ^{CP}C is positioned 5′ to either an AA or a GG site and is base-paired with G or inosine (I). Importantly, ^{CP}C is 4–7 bp away from the site of the tethered photooxidant; to oxidize ^{CP}C, a hole must first traverse this intervening low-energy bridge. Based on current mechanistic models involving hopping among low-energy bases, one would expect essentially no reaction at ^{CP}C in these duplexes (28).

Hole-Trapping on ^{CP}C with CT Through DNA. Fig. 2 shows evidence of the long-range oxidative damage to cyclopropylamine-substituted cytosines in the DNA duplex. Despite the significant energetic differences among the intervening DNA bases, decomposition of ^{CP}C is clearly observed upon irradiation of the Rh-tethered DNA

Table 1. Assemblies to test long-range oxidative damage to ^{CP}C in duplex DNA

Duplex*	Sequence†	% Decomposition of ^{CP}C‡
Rh-G-1	Rh: 5′-ACGATT *G*ACCGAGTCAT	
Cp-1	3′-TGCTAA**^{CP}C**TGGCTCAGTA	36
Rh-I-1	Rh: 5′-ACGATT *I*ACCGAGTCAT	
Cp-1	3′-TGCTAA**^{CP}C**TGGCTCAGTA	92
Rh-G-2	Rh: 5′-ACGACC *G*ATTGAGTCAT	
Cp-2	3′-TGCTGG**^{CP}C**TAACTCAGTA	5
Rh-I-2	Rh: 5′-ACGACC *I*ATTGAGTCAT	
Cp-2	3′-TGCTGG**^{CP}C**TAACTCAGTA	23
AQ-G-1	AQ: 5′-ACGATT *G*ACCGAGTCAT	
Cp-1	3′-TGCTAA**^{CP}C**TGGCTCAGTA	10§
AQ-I-1	AQ: 5′-ACGATT *I*ACCGAGTCAT	
Cp-1	3′-TGCTAA**^{CP}C**TGGCTCAGTA	36§
AQ-A-1	AQ: 5′-ACGATT *A*ACCGAGTCAT	
Cp-1	3′-TGCTAA**^{CP}C**TGGCTCAGTA	15§
AQ-I-3	AQ: 5′-ACIATT *I*TTACCIAITCAT	
Cp-3	3′-TICTAA**^{CP}C**AATGGCTCAITA	86
AQ-I-4	AQ: 5′-ACIATT *IC*TTACCIAITCAT	
Cp-4	3′-TICTAA**^{CP}CG**AATGGCTCAITA	93
AQ-I-4	AQ: 5′-ACIATT*I* *C*TTACCIAITCAT	
Cp-5	3′-TICTAA**C^{CP}G**AATGGCTCAITA	100¶
AQ-7	AQ: 5′-ACIATT *I* *C*TTACCIAITCAT	
Cp-7	3′-TICTAA**^{CP}C^{CP}G**AATGGCTCAITA	90‖/100**
AQ-8	AQ: 5′-ACIATT *C* *I*TTACCIAITCAT	
Cp-8	3′-TICTAA**^{CP}G^{CP}C**AATGGCTCAITA	86/98

*See Fig. 1.

†The ^{CP}C or ^{CP}G substitutions alter the melting temperatures (T_m) by ≤1°C, e.g., the T_ms of 2 μM G-1/Cp-1 and G-1/C-1 are both 58°C in 10 mM sodium phosphate and 50 mM sodium chloride, pH 7.

‡After 10 min of irradiation unless indicated (see *Materials and Methods*). Relative uncertainties are ±5%.

§After 40 min of irradiation.

¶Percent decomposition of ^{CP}G.

‖Decomposition of ^{CP}C after 10 min of irradiation.

**Decomposition of ^{CP}G after 10 min of irradiation.

assemblies (Table 1). This observation provides direct chemical evidence for the existence of hole density on the pyrimidine.

The extent of hole occupation on the pyrimidine, moreover, is modulated by the energetics of the full duplex. When ^{CP}C is base-paired with G, limited oxidation of ^{CP}C (≈5%) is observed in the sequence context 3′-GG^{CP}CT-5′ (Rh-G-2) after 10 min of irradiation at 365 nm. However, the yield of ^{CP}C photodecomposition after 10 min of irradiation increases by 7-fold when the sequence context around ^{CP}C is altered to 3′-AA^{CP}CT-5′ (Rh-G-1). This difference in yield of ^{CP}C decomposition is attributed to the sequence dependence of hole distribution; in 3′-GG^{CP}CT-5′, the hole density is likely more localized on the GG doublet, whereas in 3′-AA^{CP}CT-5′ the hole density is more diffuse, and there is thus a greater distribution on the ^{CP}C.

A similar but even more dramatic modulation is apparent when we replace the G base-paired to ^{CP}C with an I. For the 5′-AA^{CP}CT-3′ assembly (Rh-I-1), we observe rapid photodecomposition (>90% after 10 min) of ^{CP}C. As seen with the Rh-G duplexes, the efficiency of photodecomposition is attenuated somewhat when the sequence context is changed to 5′-GG^{CP}CT-3′ (Rh-I-2). However, in both cases, when ^{CP}C is base paired with I, the efficiency of decomposition is significantly greater than when ^{CP}C is based-paired with G. This again can be reconciled based on different distributions of hole density; owing to the higher oxidation potential of I (≈1.5 V vs. NHE) (33), the hole distribution is altered with greater hole density on ^{CP}C. Thus, the modulation by base sequence is apparent both with intra- and interstrand substitutions. Importantly, this modulation also establishes that the ring opening of the cyclopropylamine radical cation does not drive the reaction; variations in ring opening are instead dependent on the sequence of the DNA assembly and its energetics.

Note that control experiments have also been conducted under parallel conditions where ^{CP}C substituted assemblies lacking Rh are mixed with Rh-tethered assemblies lacking ^{CP}C. This control provides a test of whether any interduplex reaction occurs, as might be expected if a diffusible species were involved rather than long-range DNA-mediated CT. In these control experiments, no photodecomposition of ^{CP}C is observed (Figs. 5 and 6). Hence, the oxidative reaction is intraduplex.

Sequence-dependent oxidation of ^{CP}C is an inherent feature of DNA CT, irrespective of oxidant. Covalently tethered AQ is therefore also found to be effective at photooxidation of ^{CP}C from a distance (Table 1 and Fig. 2), albeit at a somewhat lower efficiency. Noteworthy here is the fact that in these duplexes AQ is restricted to capping the duplex terminus, whereas the Rh photooxidant intercalates predominantly between the third and fourth bases. Photooxidation of ^{CP}C by AQ is also found to be an intraduplex reaction.

As with Rh, photooxidation of ^{CP}C by AQ is more efficient when ^{CP}C is based-paired with I than it is when base-paired with G (Table 1). In assemblies AQ-I-3/Cp-3 and AQ-I-4/Cp-4, containing only I between ^{CP}C and AQ, extensive photodecomposition of ^{CP}C is observed. Again, this can be attributed to the modulation of hole density distribution through base-pairing. This consistent modulation by sequence shows that the fast *trapping reaction probes hole density rather than driving a redistribution.* The cyclopropylamine kinetic trap does not determine the hole density but simply reports it.

It is also noteworthy that H-bonding to ^{CP}C is not required for long-range oxidation of ^{CP}C. We still find significant oxidation of ^{CP}C in assemblies where ^{CP}C opposes A, creating a non-hydrogen-bonded mismatch (Table 1; AQ-A-1/Cp-1). The extent of reaction at ^{CP}C is therefore governed by intrinsic sequence-dependent variations in the distribution of hole density in double-stranded DNA.

Comparisons in Photooxidation of ^{CP}C and ^{CP}G, Modified Bases with Equal Trapping Rates but Different Energetics. To compare further the hole occupancy on purines and pyrimidines, we examined two duplexes of equivalent sequence, one containing ^{CP}C and the other containing ^{CP}G at the neighboring site (^{CP}G in AQ-I-4/Cp-5 and ^{CP}C in AQ-I-4/Cp-4 in Table 1). Based on a thermal equilibration of charge, given a conservative difference in potential between C and G of ≈0.3–0.5 V, the relative hole occupancy on ^{CP}C vs. ^{CP}G should be ≈1:10^5 to 1:10^8. As is evident in Table 1, however, ^{CP}G and ^{CP}C in these two duplexes are seen to decompose with similar efficiency.

As shown in Fig. 3, we also examined photooxidation of duplexes containing both ^{CP}G and ^{CP}C in the same assembly. Here, then, trapping times for the two modified bases should be comparable, and one would expect the relative energetics of the fast traps to determine their relative efficiencies in photodecomposition. Remarkably, however, in these duplexes (AQ-7/Cp-7 and AQ-8/Cp-8), when ^{CP}G and ^{CP}C are incorporated as neighboring bases on the same strand, their efficiency of photodecomposition is comparable, although not identical (Fig. 3 and Table 1).

These data provide unequivocal evidence that hole density is transported onto the pyrimidines, and that hole density is appreciable. The hole distribution on the DNA bridge does not reflect the relative energies of the *isolated* bases.

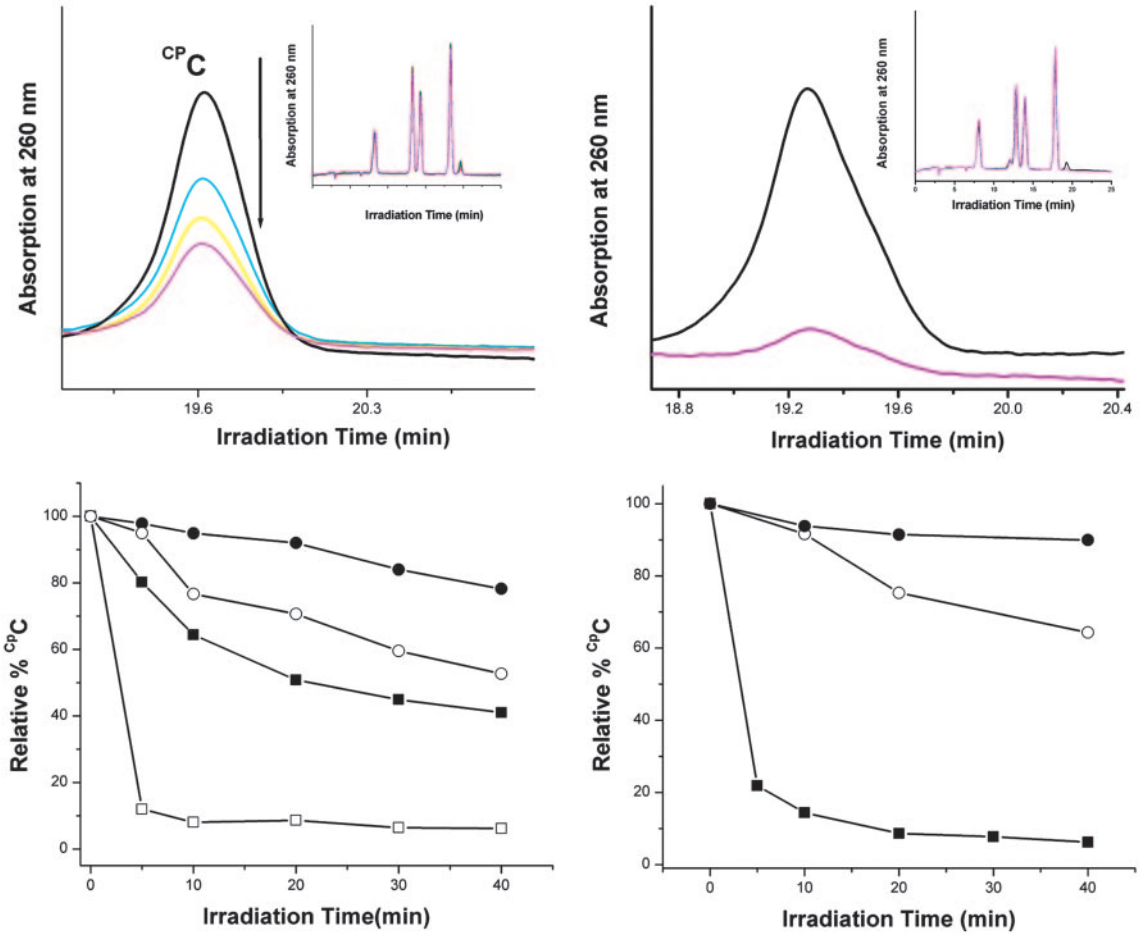

Fig. 2. Long-range oxidative damage to cytosines in duplex DNA. HPLC traces show the oxidative decomposition of ^{CP}C with increasing irradiation, 0–40 min for Rh-G-1 (*Upper Left*) and 0 (black) vs. 5 (purple) min for Rh-I-1 (*Upper Right*). (*Insets*) Full HPLC traces. Plots of the amount of ^{CP}C as a function of irradiation time reveals the sequence dependence of ^{CP}C decomposition with Rh (*Lower Left*) and AQ (*Lower Right*). In *Lower Left*: filled circles, Rh-G-2; open circles, Rh-I-2; filled squares, Rh-G-1; open squares, Rh-I-1. In *Lower Right*: filled circles, AQ-G-1; open circles, AQ-I-1; filled squares, AQ-I-3. For AQ, note the dramatic increase in efficiency of ^{CP}C photooxidation when the intervening guanines (AQ-I-1) are replaced with inosines (AQ-I-3). Decomposition of ^{CP}C is an intraduplex reaction that requires light and photooxidant.

Discussion

Mechanistic Models for Long-Range DNA CT. In many experiments characterizing DNA CT, a shallow distance dependence in the reaction has been demonstrated (1–8), and this shallow distance dependence has been explained through models primarily involving long-range diffusive charge-hopping (15, 16, 34). Models involving a mixture of hopping among low-energy guanine sites and tunneling through AT tracts provided a useful starting point for reconciling many experiments. Once experiments demonstrated rapid, long-range DNA CT across assemblies containing adenine tracts (5, 7, 34, 35), the model was modified to include also hopping on low-energy adenines (18). These models, however, consistently explain DNA CT in the context solely of base energetics. Yet, many experiments have underscored the sensitivity of DNA CT to DNA structure and dynamics (9–11, 19, 20). Indeed, it is now apparent that conformational motions of the DNA bases are required for effective CT; increased rigidity of DNA assemblies limits CT (36).

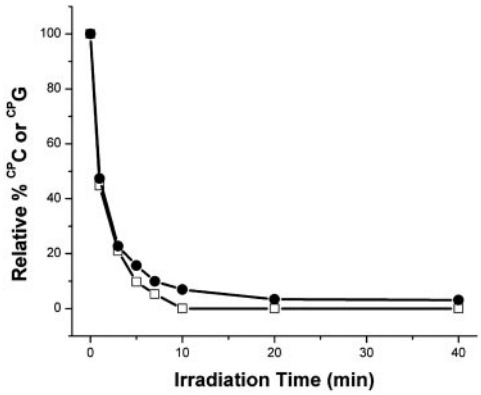

Fig. 3. Hole distribution on the DNA bridge does not reflect the relative energies of the individual bases. Shown is a plot of the amount of ^{CP}C (filled circles) or ^{CP}G (open squares) as a function of irradiation time in AQ-7/Cp-7. These duplexes contain ^{CP}C and ^{CP}G at neighboring sites, 5′-$^{CP}C^{CP}G$-3′. Similar results were observed for AQ-8/Cp-8 possessing a 5′-$^{CP}G^{CP}C$-3′ sequence within an otherwise identical duplex.

The results shown here cannot be understood through descriptions for DNA CT that are defined primarily by the energetics of individual bases. We find that, during the course of DNA CT, holes must occupy both the pyrimidine and purine bases, despite the fact that the highest occupied molecular orbitals of isolated pyrimidines are ≈0.5 eV higher in energy than purines. Clearly, then, the current descriptions of DNA-mediated CT in terms of incoherent G or A hopping cannot account for these data.

Certainly base energetics do affect the reaction. Hole density

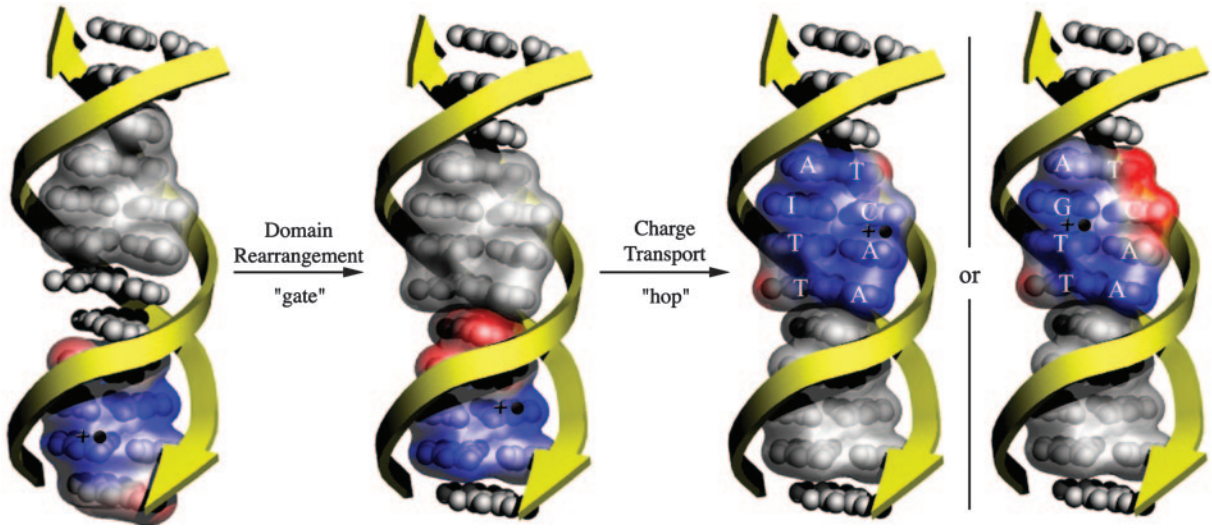

Fig. 4. Schematized delocalized domain model for DNA CT. Charge migrates through the DNA bridge by hopping among domains: Extended π-orbitals formed transiently in a manner governed by DNA sequence and dynamics. DNA domains form transiently, irrespective of charge. Base motion causes domains to form and break up, and consequently, migration of charge among domains is conformationally gated. In duplex DNA, hole density is distributed through the domain (high–low, blue–white–red) in a sequence-dependent fashion, as shown, for instance, when an I–C base pair is replaced by a G–C base pair. Here, hole density distribution in the domains is shown schematically by static snapshots, but it will fluctuate in time with the dynamic motion of the DNA bases.

on the pyrimidines is found to be modulated by relative energies of bases on the same and opposite strand. Theoretical predictions have been made indicating the modulation of base ionization potentials in DNA by sequence-dependent structure (37) and dynamics (38). In this context, it is also interesting to consider the asymmetrical distribution of oxidative damage at the 5′-G of 5′-GG-3′ doublets (39), observed after relatively slow secondary reactions leading to trapping of the G radical and ultimately DNA strand cleavage. Yet these modulations do not provide sufficient energetic fluctuations to account for differences in oxidation potential between C and G. Here, experimentally, once the rate of trapping at C and G are made comparable by cyclopropyl substitution, the data indicate that the radical is delocalized *comparably* over the two neighboring bases. Thus, not only is there modulation, in fact there must necessarily be orbital mixing among the purines and pyrimidines to generate extended π-orbitals.

Models involving superexchange tunneling through pyrimidines are also unsatisfactory, because these data establish that the hole occupies the full DNA bridge, both pyrimidines and purines (15, 40). Models derived solely on base energetics, furthermore, do not provide a reasonable rational for the sensitivity of CT to sequence-dependent structure and dynamics. An alternative mechanistic description is required.

Charge Transport Through Transient, Delocalized Domains. As illustrated in Fig. 4, we propose a model where charge migrates through the DNA bridge among delocalized domains. Our data require occupation of all bases of the DNA bridge, although not necessarily simultaneously. These DNA domains can be described as extended π-orbitals formed transiently, depending on DNA sequence and dynamics. Spectroscopic investigations of base–base CT with 2-aminopurine as a function of bridge length and temperature have provided evidence for a domain size of ≈4 bp (20) and distinguish a domain from a polaron (3, 41). In the polaron, a structurally distorted domain forms in response to the injected charge, trapping the charge, but the delocalized DNA domain described here forms transiently, irrespective of the charge. Recently, a variable-range hopping model was developed and it was found that delocalized bridge states are required to account for experimental yields of DNA hole transport (42). Data here, where comparable efficiency of reaction at ^{CP}C and a neighboring ^{CP}G are observed, provide direct experimental support for delocalization of charge.

However, delocalization clearly does not occur over the entire duplex. The transport is partly incoherent because we have found also that CT is gated by base motions (9). Thus, these delocalized domains form and break up transiently, facilitating and limiting CT. This model accounts well for the attenuation in CT seen in AT-rich sequences, owing to their inherent flexibility, and tunneling through AT sequences need not be invoked (15, 16). It also explains oxidative repair of thymine dimers at a distance, another fast trap (43, 44). Although this model is more challenging to test experimentally, and to describe theoretically, than superexchange or localized hopping, these and other data (45, 46) show that DNA-mediated CT requires a more complete treatment.

Importantly, delocalized DNA domains reflect the rich sequence-dependent structure and dynamics of nucleic acids, known to be essential for DNA function. It is the sequence, with its inherent structure and dynamics, that defines a domain. Moreover, it is precisely this dependence on nucleic acid structure and dynamics that is required for DNA-mediated CT to be exploited in molecular electronics and sensors, as well as to discover how it may be used in a biological context.

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1. Hall, D. B., Holmlin, R. E. & Barton, J. K. (1996) *Nature* **382,** 731–735.
2. Núñez, M. E., Hall, D. B. & Barton, J. K. (1999) *Chem. Biol.* **6,** 85–97.
3. Henderson, P. T., Jones, D., Hampikian, G., Kan, Y. Z. & Schuster, G. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 8353–8358.
4. Giese, B. (2000) *Accounts Chem. Res.* **33,** 631–636.
5. Takada, T., Kawai, K., Cai, X., Sugimoto, A., Fujitsuka, M. & Majima, T. (2004) *J. Am. Chem. Soc.* **126,** 1125–1129.
6. Nakatani, K., Dohno, C. & Saito, I. (2000) *J. Am. Chem. Soc.* **122,** 5893–5894.
7. Yoo, J., Delaney, S., Stemp, E. & Barton, J. K. (2003) *J. Am. Chem. Soc.* **125,** 6640–6641.

8. Delaney, S., Yoo, J., Stemp, E. D. A. & Barton, J. K. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 10511–10516.
9. O'Neill, M. A., Becker, H. C., Wan, C., Barton, J. K. & Zewail, A. H. (2003) *Angew. Chem. Int. Ed.* **42,** 5896–5900.
10. Bhattacharya, P. & Barton, J. K. (2001) *J. Am. Chem. Soc.* **123,** 8649–8656.
11. Boon, E. M., Ceres, D. M., Drummond, T. G., Hill, M. G. & Barton J. K. (2000) *Nat. Biotechnol.* **18,** 1096–1100.
12. Boon, E. M., Livingston, A. L., Chmiel, N. H., David, S. S. & Barton, J. K. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 12543–12546, and correction (2004) **101,** 4718.
13. Porath, D., Bezryadin, A., Vries, S. & Dekker, C. (2000) *Nature* **403,** 635–638.
14. Fink, H. & Schonenberger, C. (1999) *Nature* **398,** 407–410.
15. Bixon, M., Giese, B., Wessely, S., Langenbacher, T., Michel-Beyerle, M. E. & Jortner, J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 11713–11716.
16. Berlin, Y. A., Burin, A. L. & Ratner, M. A. (2002) *Chem. Phys.* **275,** 61–74.
17. Lewis, F. D., Liu, J. Q., Zuo, X. B., Hayes, R. T. & Wasielewski, M. R. (2003) *J. Am. Chem. Soc.* **125,** 4850–4861.
18. Giese, B., Amaudrut, J., Kohler, A. K., Spormann, M. & Wessely, S. (2001) *Nature*, **412,** 318–320.
19. Wan, C. Z., Fiebig, T., Kelley, S. O., Treadway, C. R., Barton, J. K. & Zewail, A. H. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 6014–6019.
20. O'Neill, M. A. & Barton, J. K. (2004) *J. Am. Chem. Soc.* **126,** 11471–11483.
21. Musa, O. M., Horner, J. H., Shahin, H. & Newcomb, M. (1996) *J. Am. Chem. Soc.* **118,** 3862–3868.
22. Nakatani, K., Dohno, C. & Saito, I. (2001) *J. Am. Chem. Soc.* **123,** 9681–9682.
23. Dohno, C., Ogawa, A., Nakatani, K. & Saito, I. (2003) *J. Am. Chem. Soc.* **125,** 10154–10155.
24. O'Neill, M. A., Dohno, C. & Barton, J. K. (2004) *J. Am. Chem. Soc.* **126,** 1316–1317.
25. Williams, T. T., Dohno, C., Stemp, E. D. A. & Barton, J. K. (2004) *J. Am. Chem. Soc.* **126,** 8148–8158.
26. Stemp, E. D. A., Arkin, M. & Barton, J. K. (1997) *J. Am. Chem. Soc.* **119,** 2921–2925.
27. Burrows, C. J. & Muller, J. G. (1998) *Chem. Rev.* **93,** 1109–1151.
28. Steenken, S. & Jovanovic, S. V. (1997) *J. Am. Chem. Soc.* **119,** 617–618.
29. Turro, C., Evenzahav, A., Bossmann, S. H., Barton, J. K. & Turro, N. J. (1996) *Inorg. Chim. Acta* **243,** 101–108.
30. Armitage, B., Yu, C., Devadoss, C. & Schuster, G. B. (1994) *J. Am. Chem. Soc.* **116,** 9847–9859.
31. Turro, C., Hall, D. B., Chen, W., Zuilhof, H., Barton, J. K. & Turro, N. J. (1998) *J. Phys. Chem. A* **102,** 5708–5715.
32. Ma, J. H., Lin, W. Z., Wang, W. F., Han, Z. H., Yao, S. D. & Lin, N. Y. (1999) *Radiat. Phys. Chem.* **54,** 491–497.
33. Kelley, S. O. & Barton, J. K. (1999) *Science* **283,** 375–381.
34. Schuster, G. B. (2000) *Acc. Chem. Res.* **33,** 253–260.
35. Williams, T. T., Odom, D. T. & Barton, J. K. (2000) *J. Am. Chem. Soc.* **122,** 9048–9049.
36. O'Neill, M. A. & Barton, J. K. (2004) *J. Am. Chem. Soc.* **126,** 13234–13235.
37. Voityuk, A. A., Jortner, J., Bixon, M. & Rösch, N. (2000) *Chem. Phys. Lett.* **324,** 430–434.
38. Voityuk, A. A., Siriwong, K. & Rösch, N. (2004) *Angew. Chem. Int. Ed.* **43,** 624–627.
39. Sugiyama, H. & Saito, I. (1996) *J. Am. Chem. Soc.* **118,** 7063–7068.
40. Lewis, F. D., Letsinger, R. L. & Wasielewski, M. R. (2001) *Acc. Chem. Res.* **34,** 159–170.
41. Barnett, R. N., Cleveland, C. L., Joy, A., Landman, U. & Schuster, G. B. (2001) *Science* **294,** 567–571.
42. Renger, T. & Marcus, R. A. (2003) *J. Phys. Chem. A* **107,** 8404–8419.
43. Dandliker, P. J., Holmlin, R. E. & Barton, J. K. (1997) *Science* **275,** 1465–1467.
44. Dandliker, P. J., Núñez, M. E. & Barton, J. K. (1998) *Biochemistry* **37,** 6491–6502.
45. Ceres, D. M. & Barton, J. K. (2003) *J. Am. Chem. Soc.* **125,** 14964–14965.
46. Drummond, T. D., Hill, M. G. & Barton, J. K. (2004) *J. Am. Chem. Soc.* **126,** 15010–15011.