Long-Range Photoinduced Electron Transfer Through a DNA Helix

C. J. Murphy, M. R. Arkin, Y. Jenkins, N. D. Ghatlia, S. H. Bossmann, N. J. Turro, J. K. Barton*

Rapid photoinduced electron transfer is demonstrated over a distance of greater than 40 angstroms between metallointercalators that are tethered to the 5′ termini of a 15–base pair DNA duplex. An oligomeric assembly was synthesized in which the donor is $Ru(phen)_2dppz^{2+}$ (phen, phenanthroline, and dppz, dipyridophenazine) and the acceptor is $Rh(phi)_2phen^{3+}$ (phi, phenanthrenequinone diimine). These metal complexes are intercalated either one or two base steps in from the helix termini. Although the ruthenium-modified oligonucleotide hybridized to an unmodified complement luminesces intensely, the ruthenium-modified oligomer hybridized to the rhodium-modified oligomer shows no detectable luminescence. Time-resolved studies point to a lower limit of 10^9 per second for the quenching rate. No quenching was observed upon metallation of two complementary octamers by $Ru(phen)_3^{2+}$ and $Rh(phen)_3^{3+}$ under conditions where the phen complexes do not intercalate. The stacked aromatic heterocycles of the DNA duplex therefore serve as an efficient medium for coupling electron donors and acceptors over very long distances.

Experiments in many laboratories have focused recently upon measurements of electron transfer rates between metal centers over long distances in proteins or protein pairs as a function of distance, driving force, and the intervening medium (*1*). Model complexes have also been prepared to explore systematically how different structural and electronic factors may mediate electron transfer reactions (*2*), and theories exploring optimal pathways for electron transfer have been devised to reconcile experimental studies (*3*). Among the many ideas put forth concerning how the medium may serve to modulate or direct electron transfer has been the notion that aromatic groups could serve as efficient "π-ways" over which electron transfer reactions might be promoted efficiently, yet few experimental measurements of electron transfer through π-stacks have been accomplished (*4*).

The DNA helix provides a novel medium through which to examine electron transfer processes (*5–8*) and, in particular, electron transfer through π-stacks. The DNA helix may be considered a polymer which contains a rigid, electronically coupled aromatic column of stacked base pairs within a water-soluble polyanion, the sugar-phosphate backbone. The DNA helix enhances rates of electron transfer between donors and acceptors which associate with DNA (*6–8*). Factors contributing to this enhancement include: (i) the effects of increased local concentration of the bound species; (ii) facilitated diffusion of the noncovalently bound species along the duplex; as well as (iii) the possibility of electron transfer at long range being mediated by the π-stack. In these systems, however, a multiplicity of binding sites of donors and acceptors on the helix makes it difficult to evaluate how each of these factors contribute to the overall rate.

In this report we demonstrate rapid photoinduced electron transfer over distances >40 Å between metallointercalators that are tethered to the 5′ termini of a 15–base pair (bp) DNA duplex. Intercalation of the donors and acceptors provides an interaction that can directly probe the nature of the purported π-way in DNA, and covalent attachment of one metal complex to each end of the helix permits measurements of quenching rates with discrete, well-defined species.

Both donor and acceptor bind to double-helical DNA by intercalation with affinities of $\geq 10^6\ M^{-1}$ (*9, 10*). The donor, photoexcited bis(phenanthroline)(dipyridophenazine)ruthenium(II), $Ru(phen)_2dppz^{2+}$, shows no luminescence in aqueous solution, but luminesces intensely in the presence of DNA because intercalation of the dppz ligand protects the phenazine nitrogen atoms from quenching by water (*9*). The acceptor is bis(9,10-phenanthrenequinone diimine)(phenanthroline)rhodium(III), $Rh(phi)_2phen^{3+}$. Rh(III) complexes containing phi also bind tightly to nucleic acids via intercalation of this ligand (*10–12*). Two-dimensional nuclear magnetic resonance experiments provide direct evidence for specific intercalation by $Rh(phen)_2phi^{3+}$ in an oligonucleotide (*11*). Phi complexes of Rh(III), furthermore, promote DNA and RNA strand cleavage upon photoactivation (*12–14*). The electronic characteristics of these complexes are also well suited to this study. The lowest excited state (*) of $Ru(phen)_2dppz^{2+}$ is a metal-to-ligand charge transfer transition centered on the dppz ligand, and the luminescence observed in nonaqueous solvents can be quenched by electron acceptors (*15*). Likewise, the lowest excited state of $Rh(phi)_2phen^{3+}$ contains ligand-to-metal charge transfer character from the phi ligand (*16*), permitting an arrangement where

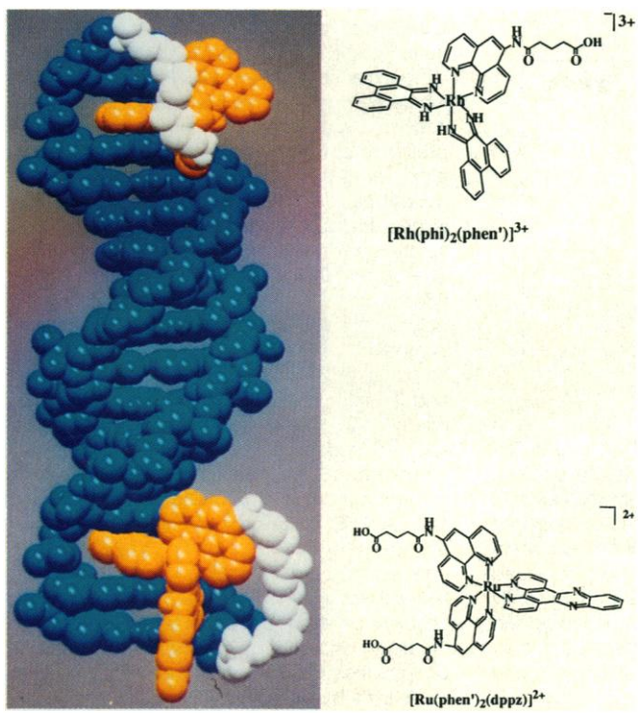

Fig. 1. Structures of the donor and acceptor complexes, $Ru(phen')_2dppz^{2+}$ and $Rh(phi)_2phen'^{3+}$, and schematic illustration of the doubly modified oligonucleotide showing the 3.4 Å interbase pair separation and the shortest donor-acceptor distance of 41 Å.

C. J. Murphy, M. R. Arkin, Y. Jenkins, J. K. Barton, Beckman Institute, California Institute of Technology, Pasadena, CA 91125.
N. D. Ghatlia, S. H. Bossmann, N. J. Turro, Department of Chemistry, Columbia University, New York, NY 10027.
*To whom correspondence should be addressed.

photoinduced electron transfer may be directed from the Ru(II) donor to the Rh(III) acceptor through the DNA helix (Fig. 1).

Luminescence quenching experiments with these metallointercalators in the presence of DNA indicate that $Rh(phi)_2phen^{3+}$ is an unusually efficient quencher of $Ru(phen)_2dppz^{2+*}$ emission (*17*). In experiments on the nanosecond time scale in the presence of DNA, static quenching of $Ru(phen)_2dppz^{2+*}$ emission by $Rh(phi)_2phen^{3+}$ contrasts the dynamic quenching of $Ru(phen)_2dppz^{2+*}$ by $Ru(NH_3)_6^{3+}$, which binds DNA through hydrogen bonding in the groove. These experiments with metallointercalators noncovalently associated with DNA have provided the impetus for synthesizing a well-defined electron transfer assembly with the donor and acceptor bound to the DNA at a discrete distance.

Oligonucleotides were metallated at their 5′ terminus by coupling of a 15-mer functionalized with a hexamethylene amine at its 5′ terminus to either $Ru(phen')_2dppz^{2+}$ (phen′ = 5-amido–glutaric acid–1,10-phenanthroline) (*18*) or $Rh(phi)_2(phen')^{3+}$ (*19–21*). Modeling studies suggested the ability of the tethered complexes to intercalate 2 bp in from the 5′ end of the metallated strand. The relative rigidity of a 15-bp double helix eliminates the possibility of collisions between metal complexes tethered to the same duplex even in the absence of intercalation.

Intercalation into the duplex by the covalently attached Ru complex may be monitored by observing the intense luminescence of the Ru-modified oligomer annealed to its complement. The Ru-modified oligonucleotide, without complement or in the presence of noncomplementary single-stranded DNA, displays little luminescence (*18*). Dilution studies have been consistent with intramolecular intercalation by the covalently attached Ru complex at concentrations ≤5 μM. Hybridization studies with mismatched complementary strands show that the covalently bound Ru complex preferentially stabilizes base mismatches near the site of covalent attachment, supplying additional evidence for intramolecular intercalation and underscoring the strong binding of the dppz ligand to DNA. All experiments reported here were accomplished at micromolar concentrations in the presence of 50 mM NaCl, conditions which strongly disfavor intermolecular aggregation.

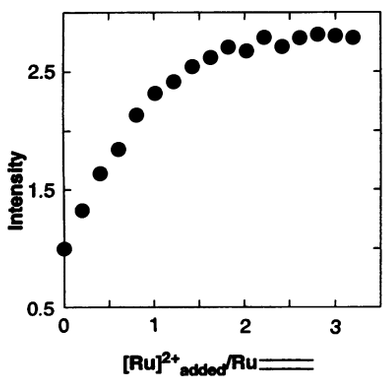

Fig. 2. Luminescence titration of 5 μM Ru-modified duplex with free $Ru(phen)_2dppz^{2+}$. Ru(II)* emission increases linearly, indicative of independent binding by the tethered and free metal complexes, until saturation. Luminescence intensity saturates at 1.8 eq of added $Ru(phen)_2dppz^{2+}$, showing that the covalently bound complex is not displaced by the added intercalators. The covalently ruthenated duplex behaves as an oligomer containing one bound intercalator.

Luminescence titrations of the ruthenated duplex with additional free $Ru(phen)_2\ dppz^{2+}$ demonstrate that the ruthenated duplex behaves as a 15-mer bearing one intercalator. As free $Ru(phen)_2dppz^{2+}$ is added to a solution of ruthenated 15-mer duplex (Fig. 2), the luminescence increases linearly until the emission reaches saturation at a ratio of 1.8 equivalents of free Ru(II) per duplex. Saturation of luminescence at this relative concentration is consistent with competitive binding of Ru complexes to the 15-mer duplex and an average binding site size of ~5 bp (*22*). When the analogous experiment is conducted with unmetallated duplex, saturation of luminescence occurs after 2.8 equivalents of $Ru(phen)_2dppz^{2+}$ are added. This comparison indicates that: (i) the metallated duplex is properly annealed, because an incompletely hybridized oligonucleotide would offer fewer binding sites for additional intercalators; and (ii) the covalently bound Ru complex is not ejected by the added intercalators. The DNA binding affinities of phi complexes of Rh(III) are somewhat lower than that of $Ru(phen)_2dppz^{2+}$ (*9*, *23*); consequently, $Rh(phi)_2phen^{3+}$ and its covalent analog are even less likely to displace the covalently bound Ru complex. Furthermore, photocleavage studies in which a 28-mer oligonucleotide duplex is cleaved by $Rh(phi)_2phen^{3+}$ in the presence and absence of $Ru(phen)_2dppz^{2+}$ indicate that the two complexes do not bind cooperatively (*17*); no change in the distribution of Rh(III) cleavage is observed in the presence of Ru complex.

Hybridization of the Rh-modified oligonucleotide to its unmodified complement permits the determination of the position of

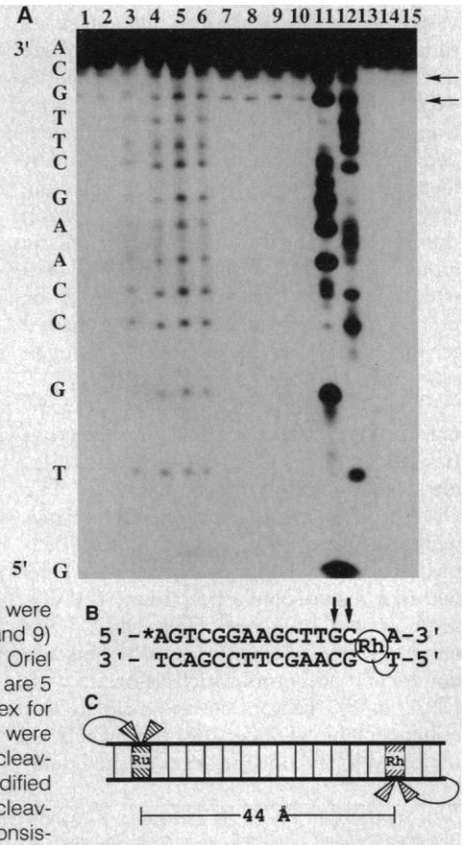

Fig. 3. Determination of the position of intercalators on the helix and separation distances between bound donors and acceptors. (**A**) Autoradiograph of a 20% polyacrylamide denaturing gel showing cleavage by covalently and noncovalently bound Rh. Lanes 1 and 2 and 7 to 10 show cleavage of *5′-AGTCGGAAGCTTGCA-3′ by $Rh(phi)_2phen'^{3+}$ covalently linked to the complementary strand. The intercalated Rh complex cleaves primarily 1 and 2 bp from the 5′ end to which it is covalently bound. Lanes 3 to 6 show the nonspecific cleavage by noncovalent $Rh(phi)_2phen^{3+}$ of the unmodified oligonucleotide. Lanes 13, 14, and 15 show labeled oligonucleotide irradiated without metal complex, the labeled oligonucleotide in the presence of $Rh(phi)_2phen^{3+}$ but without irradiation, and the labeled oligonucleotide after hybridization to the Rh-modified strand but without irradiation, respectively. Lanes 11 and 12 are Maxam-Gilbert sequencing reactions for G + A and C + T, respectively. Oligonucleotides were ^{32}P-labeled at the 5′ end with T4 polynucleotide kinase and annealed either to unmodified or Rh-modified complement. Samples were irradiated at 313 nm for 5 min (lanes 1, 3, 5, 7, and 9) and 7.5 min (lanes 2, 4, 6, 8, 10, and 13) with an Oriel model 6140, 1000W Hg-Xe lamp. Concentrations are 5 μM in duplex for lanes 1 to 4 and 1 μM in duplex for lanes 5 to 10. Varying amounts of radioactivity were loaded in order to emphasize the distribution of cleavage sites. (**B**) Schematic of duplex covalently modified at one end by $Rh(phi)_2phen'^{3+}$, showing sites of cleavage on the complementary strand. Cleavage is consistent with intramolecular intercalation of the Rh complex at interbase pair sites 1 or 2 bp in from the covalent linkage. (**C**) Schematic of the doubly modified duplex, showing a separation distance of the donor and acceptor. If we assume that both the Rh and the Ru complex can intercalate 1 to 2 bp from their linkage with equal probability, then 25% of the donor-acceptor pairs are separated by 41 Å, 50% by 44 Å, and 25% by 48 Å.

intercalation on the helix, because photoactivation of phi complexes of Rh(III) promotes strand cleavage directly at the intercalation site without a diffusing intermediate (*12*). Figure 3 shows the results of photoinduced strand cleavage of the Rh-modified duplex and, for comparison, photocleavage of the same oligomer by noncovalently bound $Rh(phi)_2phen^{3+}$. Covalent modification leads to cleavage on the unmodified ^{32}P end-labeled strand at positions 2 and 3 from the 3′ terminus with approximately equal efficiency. This result indicates the positioning of the intercalator with equal probability 1 or 2 bp in from the 5′ terminus of the metallated strand (*24, 25*). In contrast, a uniform distribution of cleavage across the strand is observed for the noncovalently bound Rh complex. Specific cleavage also confirms that the intercalation by the covalent species is primarily intramolecular at these concentrations and that the Rh-modified duplex is properly hybridized. Because the tether for the Ru intercalator is identical to that for the Rh complex, we deduce a comparable positioning of the bound Ru. Thus, as illustrated schematically in Fig. 3C, the photocleavage studies establish that the separation distance between bound intercalating ligands would be either 41, 44, or 48 Å in a duplex formed by annealing the Rh-modified strand to the Ru-modified oligomer (*26*).

Figure 4 displays the steady-state luminescence spectra of the Ru-modified oligomer annealed to its complementary strand and also annealed to the Rh-modified strand. Annealing the Ru-modified oligomer to its unmetallated complement yields intense luminescence. Annealing the two metallated strands together, however, leads to complete quenching of the Ru emission. Time-resolved luminescence decay experiments at 10-ns resolution were insufficient to determine the rate of electron transfer quenching in the fully metallated duplex (*27*). These measurements are consistent with the steady-state experiments that set an upper limit on the excited-state lifetime for the metallated duplex of 2.5 ns (*28*). Picosecond single-photon counting experiments were conducted and were also limited by the time resolution of the detector (300 ps), establishing a lower limit of $\sim 10^9\ s^{-1}$ for the quenching rate (*29*).

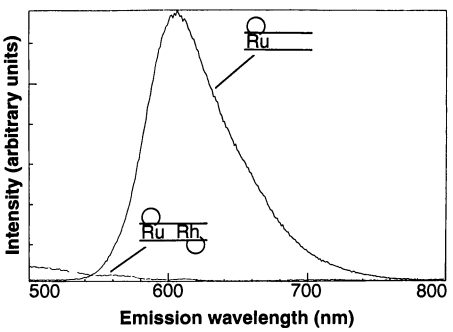

Fig. 4. Emission spectrum of 5 μM 5′-Ru(dppz)-$(phen')_2$-$(CH_2)_6$-5′-AGTGCCAAGCTTGCA-3′ annealed to its complement. The upper trace is for Ru-DNA + unmetallated complement, and the lower trace is for 5 μM Ru-DNA annealed to complementary Rh-DNA. Although intense emission is observed with the Ru-modified duplex hybridized to unmodified complement, complete quenching of the emission is apparent with complement modified to contain the Rh intercalator.

Table 1 compares the remarkable quenching seen when the Ru(III) donor and Rh(III) quencher are intercalated in the *same* helix to several companion experiments where intermolecular quenching may occur. A 5 μM solution of Ru covalently attached to the oligonucleotide and hybridized to an unmodified complement (B) shows comparable luminescent intensity to 5 μM $Ru(phen)_2dppz^{2+}$ noncovalently bound to the helix (A). Addition of the quenched, doubly modified duplex to the Ru-modified duplex (D) does not quench the luminescence from the Ru-modified duplex, demonstrating the absence of any adventitious quenchers in the Rh sample. The addition of 2.5 μM Rh-modified duplex to 2.5 μM Ru-modified duplex gives the intermolecular analog of the doubly metallated oligomer; in (E), 86% of the luminescence is retained. This experiment establishes that intermolecular quenching of a ruthenated duplex by one containing Rh(III) is not substantial and, therefore, quenching at these concentrations in the doubly metallated oligomer must be primarily intramolecular. From these studies as well as from the photocleavage experiments (Fig. 3), we estimate that at least 85% of this interaction is intramolecular at these concentrations. Because the Ru-bearing 15-mer can accommodate two additional intercalators (Fig. 2), it is not surprising that the addition of stoichiometric free $Rh(phi)_2phen^{3+}$ to the Ru-modified duplex (G) leads to substantial but not complete quenching of the Ru emission. As seen with the doubly metallated oligomer, free $Rh(phi)_2phen^{3+}$ efficiently quenches Ru luminescence. With noncovalent Rh complex, there is still residual emission because of the random distribution of Rh species on the Ru duplexes; a few duplexes accommodate two Rh complexes, and therefore some Ru duplexes remain unoccupied and unquenched. That the emission is not completely quenched further indicates that quenching in the doubly metallated duplex is intramolecular because the same concentration of $Rh(phi)_2phen^{3+}$ is present in both experiments.

Table 1. Luminescent properties of intramolecular metal-DNA intercalation complexes.

	Sample *	Relative intensity†
A	$Ru(phen)_2dppz^{2+}$ + ═	0.88
B	(Ru)═	1.00
C	(Ru)═(Rh)	0.00
D	(Ru)═(Rh) + (Ru)═	1.15
E	1/2 (Ru)═ + 1/2 ═(Rh)	0.43
F	(Ru)═ + 2 $Ru(phen)_2dppz^{2+}$	2.78
G	(Ru)═ + $Rh(phi)_2phen^{3+}$	0.08

*(Ru)═ refers to 5′-Ru(phen′)$_2$dppz-AGTGCCAAGCTTGCA-3′ annealed to its complement; (Ru)═(Rh) refers to 5′-Ru(phen′)$_2$dppz-AGTGCCAAGCTTGCA-3′ annealed to 5′-Rh(phi)$_2$phen′-TGCAAGCTTGGCACT-3′; and ═ refers to the duplex DNA. Samples were dissolved in buffer (5 mM tris, 50 mM NaCl, pH 7.2) to ≤5 μM. †All spectra were taken on an SLM Aminco 8000 spectrofluorimeter. Intensities were integrated from 500 to 800 nm and are relative to that of (Ru)═. The integrated intensities have an uncertainty of ±10%.

In principle, either electron or energy transfer quenching mechanisms may be operative over 40 Å distances (*30*). There are several reasons why the quenching in this system may most reasonably be attributed to a long-range electron transfer reaction rather than energy transfer. The large thermodynamic driving force for electron transfer of 0.75 eV (*31*) favors this mechanism. Precedence for an electron transfer quenching mechanism has been documented with other covalently linked Ru(II)-Rh(III) polypyridyl systems (*32*). Förster energy transfer is clearly ruled out because the donor excited state is not singlet in character and because there is no spectral overlap between the photoexcited Ru(II) donor and Rh(III) acceptor. Because transient intermediates have thus far not been detected on the nanosecond time scale, our experiments cannot rule out the possibility of triplet energy transfer as a component of the observed quenching. However, this interaction is itself a form of electron transfer. Moreover, that either mechanism should occur with such facility over this distance is unprecedented and appears to require a special participation of the DNA helix in the quenching step.

Electron transfer through the π-stack can be compared with the absence of quenching seen in a shorter metallated duplex under

conditions where no intercalation occurs. The complexes $Ru(phen)_2phen'^{2+}$ and $Rh(phen)_2phen'^{3+}$ have been linked to the smaller oligonucleotide 5′-CGATTAGC-3′ and its complement, respectively. $Ru(phen)_3^{2+}$ differs from $Ru(phen)_2\ dppz^{2+}$ with regard to the depth of its intercalation, with binding constants for intercalation three orders of magnitude lower than for $Ru(phen)_2dppz^{2+}$ (*9*, *33*). Unlike $Ru(phen)_2dppz^{2+}$, $Ru(phen)_3^{2+}$ does luminesce in aqueous solution in the absence of DNA and shows an increase in luminescent lifetime when intercalated (*33*). As can be seen in Table 2, no long-lived luminescent lifetime is observed upon hybridization of the $Ru(phen)_3^{2+}$-tethered oligomer to unmetallated complement, indicating the absence of intercalation by this tethered complex. The inability of tethered $Ru(phen)_2(phen')^{2+}$ to intercalate is consistent with the more shallow and weaker intercalation of phenanthroline versus the dppz ligand. Also, the rate of ferrocyanide quenching of $Ru(phen)_3^{2+}*$ is the same when the Ru complex is free in solution as when it is covalently bound to the oligonucleotide duplex. Quenching of Ru luminescence by $Fe(CN)_6^{4-}$ is known to be inhibited when the Ru complex is intimately associated with the DNA polyanion; the failure of the 8-mer duplex to protect the metal complex from quenching is another indication that the tethered tris(phenanthroline) metal complex does not intercalate. Therefore, the duplex derivatized with both $M(phen)_3^{n+}$ complexes provides a covalently bound analog in which the metal complexes are not coupled to the π-stack. In contrast to the fully intercalated doubly metallated assembly, here no luminescence quenching of the Ru center is observed upon hybridization of the $Ru(phen)_3^{2+}$-modified oligonucleotide to the Rh-modified oligomer (*34*).

These results demonstrate that intercalation is required for electron transfer to occur. The absence of luminescence quenching when the covalently tethered donor and acceptor are not intercalated is fully consistent with the comparison seen earlier between the emission quenching of $Ru(phen)_2dppz^{2+}*$ by $Rh(phi)_2phen^{3+}$ versus $Ru(NH_3)_6^{3+}$ (*17*); when $Rh(phi)_2phen^{3+}$ serves as the electron acceptor, the luminescence is quenched on a subnanosecond time scale, whereas $Ru(NH_3)_6^{3+}$ quenches $Ru(phen)_2dppz^{2+}*$ emission at diffusion-controlled rates. $Ru(NH_3)_6^{3+}$ binds to DNA by electrostatic attraction and hydrogen bonding and is therefore not bound within the DNA π-stack. Similarly, since the tethered phenanthroline complexes are not intercalated, the pathway joining the donor and acceptor involves only σ-bonds, with no direct π-stack interaction. Our results indicate that the rapid electron transfer requires the coupling of donor and acceptor to the π-stack. More generally, the different quenching efficiencies found with these different pathways suggest that electron transfer can proceed much more readily through stacked π-systems than either through a covalent σ-framework or by ionic interactions.

Based on these results, we conclude that photoinduced electron transfer between intercalators occurs very rapidly over >40 Å through the DNA helix over a pathway consisting of π-stacked base pairs. From Marcus theory (*35*), one may calculate the extent to which the DNA π-stack couples donor and acceptor, which is denoted by the quantity β (units of $Å^{-1}$):

$$k_{et} \propto \exp[-\beta(d - r_0)] \qquad (1)$$

where k_{et} is the rate of electron transfer (s^{-1}), d is the distance between donor and acceptor, and r_0 is the distance of closest approach of donor and acceptor. From our measurements of driving force and distance and assuming a lower limit in the rate of $3 \times 10^9\ s^{-1}$, we estimate β to be ≤0.2 $Å^{-1}$ (*36*). This value may reasonably be compared to a value of β of 0.14 $Å^{-1}$ found for Ru(II)/Ru(III) centers covalently linked by a dipyridyl polyene bridge (*37*).

The DNA double helix therefore serves as an efficient medium for coupling electron donors and acceptors over very long distances (>40 Å). We have constructed a duplex assembly bearing a donor and an acceptor intercalated at each end which promotes electron transfer over distances that are comparable to those found in biological systems (*38*). The efficiency of DNA in mediating long-range electron transfer gives credence to the notion that noncovalently stacked aromatic heterocycles can serve as rapid "π-ways." Finally, these results offer the possibility that nature takes advantage of the electron transport properties of nucleic acids in some context.

Table 2. Luminescent properties of tris(phenanthroline)metal-DNA complexes. Samples were dissolved in buffer (5 mM tris, 50 mM NaCl, pH 7.2) to ≤5 μM. Time-resolved emission experiments were performed as in (*28*) but at 10°C to ensure duplex stability.

Sample	Lifetime (ns)
$Ru(phen)_2phen'^{2}$	820
$Ru(phen)_2phen'^{2+}$ + 5′-CGATTAGC-3′	850 (90%)*
3′-GCTAATCG-5	3460 (10%)*
$Ru(phen)_2phen'$-5′-CGATTAGC-3′	820
$Ru(phen)_2phen'$-5′-CGATTAGC-3′	
3′-GCTAATCG-5′	830
$Ru(phen)_2phen'$-5′-CGATTAGC-3′	
3′-GCTAATCG-5′-$Rh(phen)_2phen'^{3+}$	830

*The values in parentheses represent the percentages of each decay component in the biexponential curve. The long-lived component makes only a small but significant contribution to the decay under the conditions required for this experiment.

REFERENCES AND NOTES

1. B. E. Bowler, A. L. Raphael, H. B. Gray, *Prog. Inorg. Chem.* **38**, 259 (1990); B. M. Hoffman, M. J. Natan, J. M. Nocek, S. A. Wallin, *Struct. Bonding* **75**, 85 (1991); G. McLendon, *Acc. Chem. Res.* **21**, 160 (1988).
2. A. D. Joran, B. A. Leland, G. G. Geller, J. J. Hopfield, P. B. Dervan, *J. Am. Chem. Soc.* **106**, 6090 (1984); G. L. Closs, L. T. Calcaterra, N. J. Green, K. W. Penfield, J. R. Miller, *J. Phys. Chem.* **90**, 3673 (1986); A. D. Joran *et al.*, *Nature* **327**, 508 (1987); G. L. Closs and J. R. Miller, *Science* **240**, 440 (1988).
3. D. N. Beratan and J. N. Onuchic, *Adv. Chem. Ser.* **228**, 71 (1991); M. A. Ratner, *J. Phys. Chem.* **94**, 4877 (1990); C. A. Naleway, L. A. Curtiss, J. R. Miller, *ibid.* **95**, 8434 (1991); S. Larsson, *Chem. Phys. Lett.* **90**, 136 (1982).
4. T. J. Marks, *Science* **227**, 881 (1985); M. Tanaka, H. Yoshida, M. Ogasawara, *J. Phys. Chem.* **95**, 955 (1991); P. G. Schouten, J. M. Warman, M. P. de-Haas, M. A. Fox, H.-L. Pan, *Nature* **353**, 736 (1991); M. R. Wasielewski, *Chem. Rev.* **92**, 435 (1992).
5. T. A. Hoffmann and J. Ladik, *Adv. Chem. Phys.* **7**, 84 (1964); E. Clementi and G. Corongiu, *Int. J. Quant. Chem. Quant. Biol. Symp.* **9**, 213 (1982).
6. P. Fromherz and B. Rieger, *J. Am. Chem. Soc.* **108**, 5361 (1986).
7. J. K. Barton, C. V. Kumar, N. J. Turro, *ibid.*, p. 6391; M. D. Purugganan, C. V. Kumar, N. J. Turro, J. K. Barton, *Science* **241**, 1645 (1988).
8. A. M. Brun and A. Harriman, *J. Am. Chem. Soc.* **114**, 3656 (1992).
9. A. E. Friedman, J.-C. Chambron, J.-P. Sauvage, N. J. Turro, J. K. Barton, *ibid.* **112**, 4960 (1990); R. Hartshorn and J. K. Barton, *ibid.* **114**, 5919 (1992); Y. Jenkins, A. E. Friedman, N. J. Turro, J. K. Barton, *Biochemistry* **31**, 10809 (1992).
10. A. M. Pyle, E. C. Long, J. K. Barton, *J. Am. Chem. Soc.* **111**, 4520 (1989).
11. S. S. David and J. K. Barton, *ibid.* **115**, 2984 (1993).
12. A. Sitlani, E. C. Long, A. M. Pyle, J. K. Barton, *ibid.* **114**, 2303 (1992).
13. A. M. Pyle, T. Morii, J. K. Barton, *ibid.* **112**, 9432 (1990); K. Uchida, A. M. Pyle, T. Morii, J. K. Barton, *Nucleic Acids Res.* **17**, 10259 (1989); A. Krotz, T. P. Shields, L. Y. Kuo, J. K. Barton, *J. Am. Chem. Soc.* **115**, 3877 (1993).
14. C. S. Chow and J. K. Barton, *J. Am. Chem. Soc.* **112**, 2839 (1990); C. S. Chow, L. S. Behlen, O. C. Uhlenbeck, J. K. Barton, *Biochemistry* **31**, 972 (1992).
15. E. Amouyal, A. Homsi, J.-C. Chambron, J.-P. Sauvage, *J. Chem. Soc. Dalton Trans.* **1990**, 1841 (1990); J.-C. Chambron, J.-P. Sauvage, E. Amouyal, P. Koffi, *New J. Chem.* **9**, 527 (1985).
16. A. M. Pyle, M. Y. Chiang, J. K. Barton, *Inorg. Chem.* **29**, 4487 (1990); A. H. Krotz, L. Y. Kuo, J. K. Barton, *ibid.*, in press.
17. C. J. Murphy *et al.*, unpublished results.

18. Y. Jenkins and J. K. Barton, *J. Am. Chem. Soc.* **114**, 8736 (1992). Association of the tethered metal complex with single-stranded DNA may lead to anomalous luminescent behavior; thus careful annealing is required.
19. $Rh(phi)_2phen'^{3+}$ can be prepared in a manner analogous to that for the parent phenanthroline complex (*16*).
20. 5′-d(AGTGCCAAGCTTGCA)-3′ and its complement were synthesized on an Applied Biosystems 394 DNA synthesizer and modified at the 5′ end with the reagent Aminolink2 (A.B.I.), which yields a 5′-NH_2-$(CH_2)_6$-OPO_3–terminus. Coupling the metal complexes to the amino-DNA has been accomplished with 1-hydroxybenzotriazole and dicyclohexylcarbodiimide in a 1:1 dimethylformamide:dioxane slurry (*18*). Products were separated by high-performance liquid chromatography on a Hewlett-Packard 1050 system with a C_{18} column (Vydac) and a triethylamine–acetic acid/acetonitrile gradient. Yields were poor (1% at best). Metal complex content was established by ultraviolet-visible spectroscopy, and DNA content was determined by an analytical test for phosphate [O. Lindberg and L. Ernsten, *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience: New York, 1954), vol. 3].
21. For other examples of the tethering of metal-phenanthroline complexes to oligonucleotides, see W. Bannwarth, D. Schmidt, R. L. Stallard, C. Hornung, and R. Knorr [*Helv. Chim. Acta* **71**, 2085 (1988)]; J. Telser, K. A. Cruickshank, K. S. Schanze, and T. L. Netzel [*J. Am. Chem. Soc.* **111**, 7221 (1989)]; and C. H. B. Chen and D. S. Sigman [*Proc. Natl. Acad. Sci. U.S.A.* **83**, 7147 (1986)].
22. $Ru(phen)_2dppz^{2+}$ intercalated itself spans 4 bp; the statistical distribution of complexes on the oligomer generates a larger average binding site.
23. N. Gupta *et al.*, *Angew. Chem. Int. Ed. Engl.* **31**, 1048 (1992).
24. Photocleavage at the intercalation site leads to strand scission with single base 5′ asymmetry. See (*12*).
25. Intimate binding of the phi ligand is essential for productive strand cleavage.
26. The distance between intercalators (the intercalating planes) is given by (the number of base pairs separating the intercalators) × 3.4 Å + 3.4 Å, where the 3.4 Å base pair stacking distance is for B-form DNAs. Thus, intercalation 2 bp in from either end of the 15-bp duplex results in the closest metal-metal distance of 40.8 Å; the most probable distance of 44 Å corresponds to an assembly with one intercalation site 2 bp in from the end of the helix and another intercalation site 1 bp in from the other end.
27. The experiments were performed with a Lambda-Physik excimer-pumped dye laser with excitation at 480 nm and detection at 610 nm. The signal was directed through a monochromator to a Hamamatsu R928 PMT, and decay traces were fit to a multiexponential program. Excitation energies ranged from 0.5 to 1.0 mJ; time resolution was ~10 ns.
28. Steady-state emission experiments were performed on a SLM-Aminco 8000 spectrofluorimeter.
29. The picosecond experiments were performed at the Center for Fast Kinetic Research at the University of Texas at Austin. Laser excitation at 420 nm was provided by a mode-locked Nd:YAG-pumped dye laser. The signal was directed through a 620-nm bandpass filter to a Hamamatsu R928 photomultiplier tube. Decay traces were acquired on a Tracor-Northern TN 7200 multichannel analyzer and deconvoluted from measured instrument response functions.
30. N. J. Turro, *Modern Molecular Photochemistry* (Benjamin-Cummings, Menlo Park, CA, 1978).
31. The driving force is calculated from cyclic voltammetry and spectroscopic measurements, see (*17*). The oxidation potential for $Ru(phen)_2dppz^{2+}$ is −1.56 V versus a standard hydrogen electrode (SHE) and the reduction potential for $Rh(phi)_2phen^{3+}$ is 0.01 V versus SHE.
32. M. Furue, M. Hirata, S. Kinoshita, T. Kushida, M. Kamachi, *Chem. Lett.* **1990**, 2065 (1990); K. Kalyanasundaram, M. Gratzel, Md. K. Nazeeruddin, *J. Phys. Chem.* **96**, 5865 (1992); K. Nozaki, T. Ohno, M. Haga, *ibid.*, p. 10880.
33. C. V. Kumar, J. K. Barton, N. J. Turro, *J. Am. Chem. Soc.* **107**, 5518 (1985); J. K. Barton, J. M. Goldberg, C. V. Kumar, N. J. Turro, *ibid.* **108**, 2081 (1986).
34. The driving force for the photoinduced electron-transfer reaction from $Ru(phen)_3^{2+*}$ to $Rh(phen)_3^{3+}$ is 0.25 V.
35. R. A. Marcus, *Annu. Rev. Phys. Chem.* **15**, 155 (1964); _____ and N. Sutin, *Biochim. Biophys. Acta* **811**, 265 (1985).
36. Electron transfer rates were calculated as in (*1*) and assume an exponential decay with distance. The dielectric constant of DNA is taken to be 78 at large intercharge separations [B. E. Hingerty, R. H. Ritchie, T. L. Ferrell, J. E. Turner, *Biopolymers* **24**, 427 (1985)]. The product of the nuclear frequency factor (ν_n) and the electronic coupling factor when donor and acceptor are in closest contact (κ_e^0) is assumed to be 10^{13} s^{-1} (*1*). The calculated reorganization energy λ is 0.4 eV, neglecting inner-sphere effects (*1*).
37. S. Woitellier, J. P. Launay, C. W. Spangler, *Inorg. Chem.* **28**, 758 (1989).
38. C. C. Moser, J. M. Keske, K. Warncke, R. S. Farid, P. L. Dutton, *Nature* **355**, 796 (1992).
39. We are grateful to C. V. Kumar for helpful discussions and to J. Winkler for expert technical assistance. In addition, J.K.B. thanks the National Institutes of Health (GM49216) and the National Foundation for Cancer Research for their financial support. N.J.T. thanks the National Science Foundation and Air Force Office of Scientific Research for their financial support. We are also grateful to the National Science Foundation for postdoctoral (C.J.M.) and predoctoral (M.R.A.) fellowships as well as the Deutsche Forschungsgemeinschaft (S.H.B.) and the Parsons Foundation (Y.J.). We also thank A. Harriman and S. Atherton of the Center for Fast Kinetic Research, which is supported jointly by NIH and the University of Texas at Austin, for assistance in the single-photon counting measurements.

4 June 1993; accepted 29 September 1993

Picosecond Resolution in Scanning Tunneling Microscopy

G. Nunes Jr.* and M. R. Freeman

A method has been developed for performing fast time-resolved experiments with a scanning tunneling microscope. The method uses the intrinsic nonlinearity in the microscope's current versus voltage characteristics to resolve optically generated transient signals on picosecond time scales. The ability to combine the spatial resolution of tunneling microscopy with the time resolution of ultrafast optics yields a powerful tool for the investigation of dynamic phenomena on the atomic scale.

The scanning tunneling microscope (STM) (*1*) first found wide use in the field of surface science as an atomic-scale probe of topography and electronic structure, but applications of tunneling microscopy now extend from the imaging of such complex molecules as DNA (*2*) to the fabrication of structures out of single atom building blocks (*3*). The success of the STM has also inspired a rapidly growing variety of new scanned probe microscopies based on atomic (van der Waals) forces, magnetic forces, capacitance, and near field optics, to name but a few (*4*). Among these powerful techniques, the STM alone relies on the extremely localized quantum mechanical tunneling of electrons between the sample and the tip and therefore offers the advantage of an extraordinarily high three-dimensional spatial resolution. Although the possibility has long been recognized (*5*), researchers have so far met with limited success in attempting to extend that resolution to the "fourth" dimension, that is, to allow the STM to probe phenomena on atomic *time* scales as well as atomic length scales.

Adding such time resolution to the capabilities of the STM requires that some important obstacles be overcome. Although the intrinsic time scale for tunneling across the junction between the sample and tip in an STM has been estimated to be of order 10 fs or less (*6*), stray capacitance in the wiring and the unavoidable trade-off between signal-to-noise ratio and speed in the external electronics limit the bandwidth in typical instruments to ~30 kHz or less. Similar difficulties arise in the field of ultrafast optics, where the speed of the electronics is no match for femtosecond optical pulses, and it is natural to adapt optical "pump and probe" methods for detecting fast transient signals to time-resolved STM experiments.

In optical experiments, these pump and probe methods often rely on some nonlinearity in the system under study so that the separate response to each of a pair of fast excitations (the "pump" and "probe" pulses) is different from the response when the pulses arrive simultaneously. To overcome the fact that the signal from a lone pump and probe pair will be both too small and too fast to record directly, a continuous train of pulses is applied so that the detec-

IBM Thomas J. Watson Research Center, P.O. Box 218, Yorktown Heights, NY 10598.

*Permanent address: Department of Physics and Astronomy, Dartmouth College, 6127 Wilder Laboratory, Hanover, NH 03755–3528.