CHARGE TRANSFER THROUGH DNA

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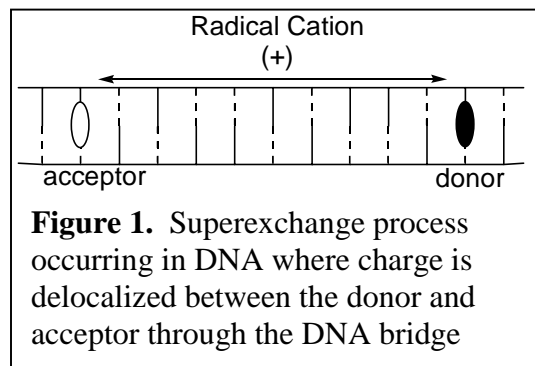
INTRODUCTION

From quantum computing to photosynthesis, charge transfer occurs in a variety of chemical and biological systems. Recently, the properties of charge transfer in DNA has come under scrutiny. From these studies, three possible mechanisms of charge transfer have emerged: (1) DNA as a molecular wire, (2) charge transfer by hole hopping, and (3) phonon-assisted polaron hopping (PAPH). Presented here is a critical survey of research on charge transfer in DNA, highlighting the stengths and inadeqacies of the different mechanistic models.

MECHANISTIC MODELS

The initial portion of this review will introduce the mechanistic models that have been proposed. The experiments performed to elucidate these models will be discussed, followed by a brief conclusion.

DNA as a Molecular Wire

Double stranded DNA contains two polyanionic strands that associate by base pair formation. The base pairs stack one atop another and, it is speculated that this stack may conduct charge along the

Figure 1. Superexchange process occurring in DNA where charge is delocalized between the donor and acceptor through the DNA bridge

axis of the double helix.[1] Charge can then be transferred through a superexchange process, in which an excited state acceptor and donor are separated through a conjugated base pair bridge (Figure 1).[2-4] The superexchange process can be described by the simplified Marcus-Levich-Jortner equation

$$k_{cs} = k_o \ e^{R\beta} \qquad (1)$$

(Equation 1) for nonadiabatic electron transfer, where the rate constant for charge separation, k_{cs}, in a donor-bridge-acceptor system is dependent upon a preexponential factor k_o, the donor-acceptor center-to-center distance R, and β, which is dependent upon the nature of the bridge and its coupling with the donor and acceptor.[5] The rates of charge transfer (CT) processes are dependent upon the integrity of the aromatic base stack, with small imperfections affecting the value of β. Because of the fluid-like motion of DNA,[6] random imperfections within the stack tend

to occur, and, as a consequence, superexchange becomes inefficient over long distances. For DNA to act as a molecular wire, a system of highly ordered, stacked aromatic base pairs must be present.

Hole Hopping

The hole hopping model requires that DNA not exist as a conjugated aromatic stack, but as a collection of discrete base pairs.[2,7,8] Because of the lack of conjugation by stacking, charge will be localized at the base with the lowest oxidation potential, which is guanine (G).[9] Charge will not be able to migrate to adjacent bases with higher oxidation potential, therefore, charge transfer is proposed to occur through a process of tunneling from low energy base to low energy base (Figure 2). In this fashion, a radical cation hole can "hop" from guanine to guanine, in an individual superexchange. The modified Marcus equation indicates that the hop with the largest R (G to G distance) will be the rate-determining step. Charge transfer efficiency (E) is described by the equation ln $E \bullet$ ln N, where N is equal to the number of hopping steps that take place.[10] A high efficiency of charge transfer and low β can be expected from a hole hopping model.
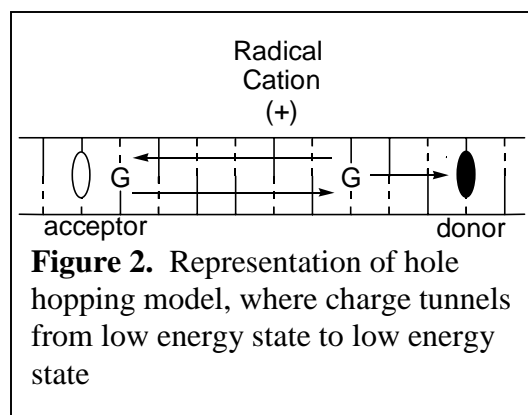
Figure 2. Representation of hole hopping model, where charge tunnels from low energy state to low energy state

Phonon Assisted Polaron Hopping

Where the molecular wire and hole hopping models are the two extremes, phonon assisted polaron hopping (PAPH) is a hybrid of the two models. A DNA polaron is a radical cation or anion that is extended over 5-7 base pairs. The DNA unwinds, increasing molecular orbital overlap between bases while decreasing the base-to-base distance. The distortion results in a minimization of the radical cation energy in the DNA. Phonons are internal motions such as changes in winding or inclination angle. These motions may promote hopping of the polaron.[11] When charge is introduced into DNA in the PAPH model, a polaron is formed in the helix, stabilizing the charge. By interaction with a phonon, the polaron will undergo a hopping mechanism where small groups of base pairs with similar energy will enter and leave, thereby moving the polaron (Figure 3).[3] A superexchange occurs inside of the polaron transferring charge as described by the Marcus equation for electron transfer, eq 1, giving almost instantaneous exchange of an electron through the polaron.
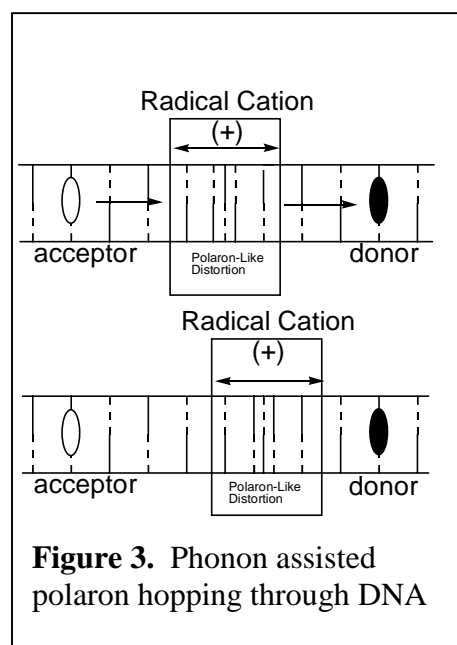
Figure 3. Phonon assisted polaron hopping through DNA

However, long-range migration of the polaron itself must occur through PAPH, as a long-range superexchange would be unlikely.[3] By incorporating polarons and phonons into the mechanistic model, PAPH takes into account the dynamic nature of DNA.

MECHANISTIC ELUCIDATION

Aromatic Base Pair Stacking Dependence

The molecular wire model assumes a conjugated base pair stack is necessary to mediate charge transfer. To determine the validity of this assumption Barton and coworkers introduced the tethered acceptor $Rh(phi)_2DMB^{3+}$ (phi, di-9,10-phenanthrene-quinone diimine; DMB, 4,4'-dimethyl-2-2'-bipyridine) (**2**) and G-G doublet donors to a DNA duplex containing an N-N-N bulge (N = base pair) which disrupts aromatic stacking (Figure 4a).[13] The irradiation of **2** causes an excited state, an electron from a base pair adjacent to the intercalation point thereby, leaving a radical cation in the duplex.[13] The radical cation then migrated through the base pairs of the DNA bridge to the lowest oxidation point of the D-B-A system. Guanines neighboring other guanine bases are more reactive than other base pairs toward oxidation,[9] therefore a large population of radical cations will congregate at the G-G doublet. When the radical cation is localized at the guanine doublet, there is a higher probability of trapping by H_2O or O_2 will occurring, causing DNA oxidation.[14] Reaction of oxidized DNA with piperidine results in cleavage at the point of oxidation. The cleavage products, which are analyzed by gel electrophoresis followed by autoradiography, can be used to determine the length over which charge transfer occurred.[8]

Barton and coworkers, observed a higher percentage of total DNA cleavage products at the proximal G-G doublet versus the distal doublet in the presence of an N-N-N bulge when compared with an unmodified duplex control.[13] The bulge disrupts the CT to the distal G-G doublet without affecting the proximal doublet, indicating disruption of the aromatic stacking by the N-N-N bulge is sufficient to hinder CT beyond the bulge.


Figure 4a. DNA duplex with a bulge disrupting base pair stacking.
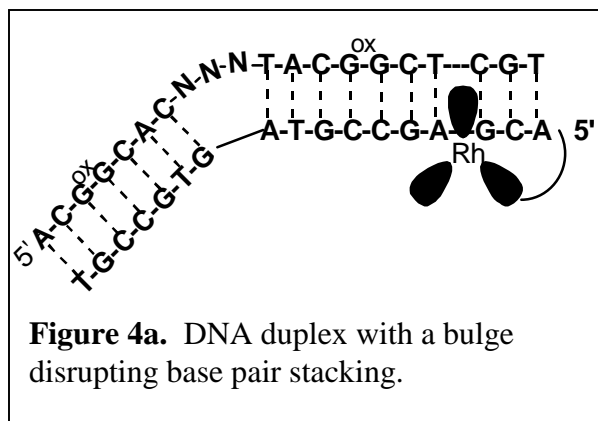
Barton and coworkers designed and synthesized a DNA duplex which contains a 3' tethered ethidium donor (**1**) and a 5' tethered (**2**) (Figure 4b).[15] Steady state fluorescence spectroscopy was used to measure the charge transfer (CT) through the DNA, as quenching of fluorescence is observed in charge transfer systems.[15] When the duplex was heated above its T_m, a loss of base pair stacking

occurs, as measured by fluorescence quenching.[15] Upon cooling, base stacking was reestablished and quenching was restored,[15] demonstrating that fluorescence, and therefore charge transfer, is dependent upon base stacking.

Figure 4b. Orientation of tethered ethidium donor and rhodium acceptor intercalating in DNA

In a separate study, Barton and coworkers varied the sequence of base pairs, introducing G-A and C-A mismatches into a DNA duplex.[15] NMR and crystallographic studies indicate that C-A mismatches significantly perturb the aromatic stacking of the base pairs, while G-A mismatches stacked favorably.[15] Fluorescence studies showed a decreased quenching in duplexes containing C-A base pair mismatches, with no quenching observed in the G-A containing duplexes. These result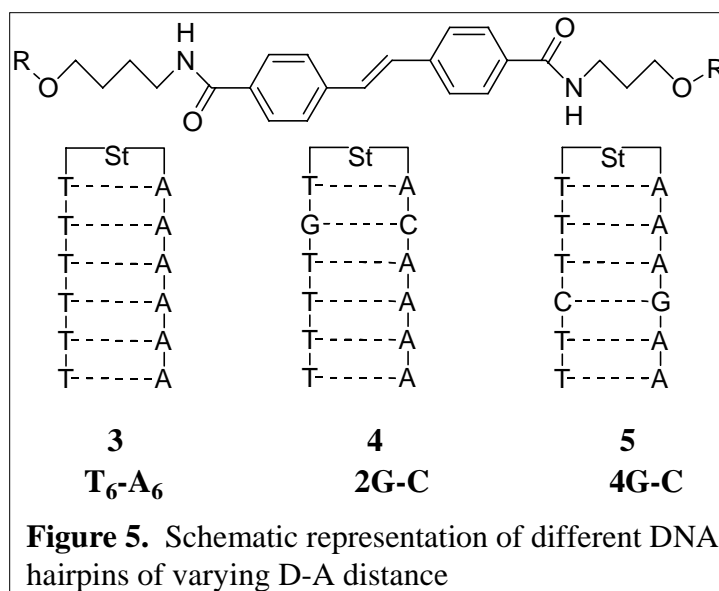s suggest that charge transfer is dependent on the aromatic stacking of the base pairs. The previous three examples all concur that highly ordered aromatic stacking of DNA base pairs is necessary for CT, best supporting the models of a molecular wire and PAPH. A criterion for the hole hopping model was the discrete molecular orbitals of the base pairs. The results collected this far do not support the model of hole hopping.
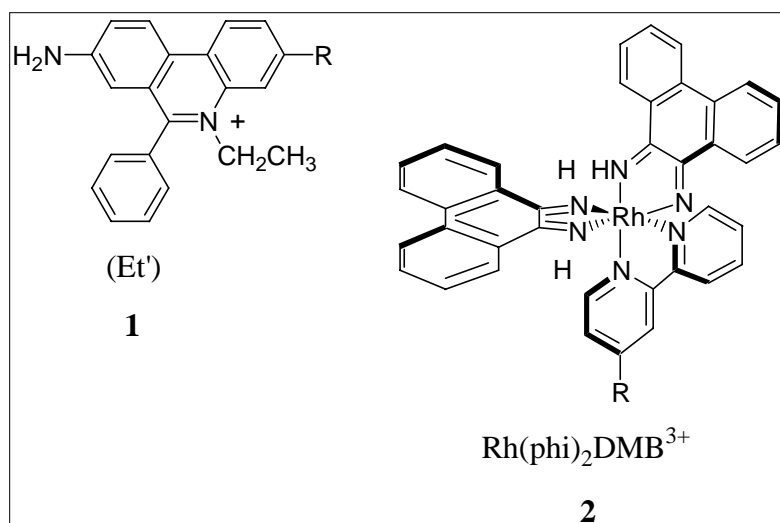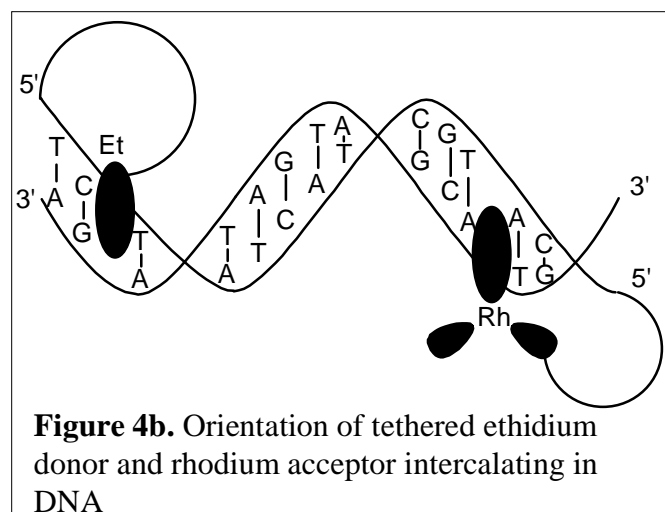

Distance Dependence of Charge Transfer

To investigate the origin of the discrepancies between values of β in different charge transfer systems, Lewis and coworkers studied synthetic DNA hairpins with a stilbene tether. The stilbene unit, which links two nucleotide arms possessing variable lengths and sequences (Figure 5).[5] Initial studies on the hairpin system, T_6-St-A_6 (**3**), showed no quenching of fluorescence, however, upon subsequent substitution of G-C base pairs for A-T base pairs, quenching was observed, demonstrating charge transfer between acceptor the stilbene and a guanine donor.[5,16] By moving the guanine residues farther

from the stilbene moiety (i.e. **4** and **5**), the magnitude of quenching decreases. The values for β were calculated to be from 0.66 to 0.71 Å^{-1}.[5] When guanine was present on a poly A arm versus a poly T arm, slightly higher rates were observed, demonstrating the preference for CT through purine bases A and I.[5] The work of Lewis and coworkers demonstrates fundamental concepts of CT in DNA, a distance dependence present giving values of $\beta \cdot 0.7$ Å^{-1} and preference for purine bases in CT.

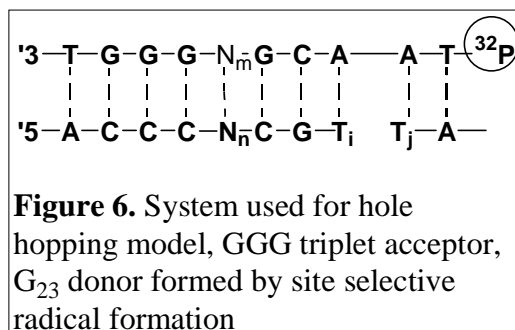**Figure 5.** Schematic representation of different DNA hairpins of varying D-A distance

Barton and coworkers studied the effect of distance between the donor and acceptor of a DNA duplex with a 2-aminopurine acceptor (Ap) **(6)** and a guanine or inosine (I) **(7)** donor.[4] Transient absorption spectroscopy, a femtosecond spectroscopic process that allows characterization of the rate of decay from an excited state to the ground state, was used to measure the rate of charge transfer from Ap to an adjacent base (G, A, I, C, T).[4] These bases were all able to transport charge. Addition of adenine units between Ap and G/I increased the distance between the donor and acceptor, which resulted in a decreased rate until a constant rate was reached at a distance of 14 Å. The rates of CT were greateer for G than I, as would be expected for the lower oxidation potential of guanine. These results indicate that the rates of CT are highly dependent on the nature and number of the bridging bases. The addition of adenine units increases the distance between the donor acceptor, which is consistent with decreasing rates of CT in a superexchange mechanism. However, the distance of 14 Å only constitutes 3 base pairs, which is not long enough to classify DNA as a molecular wire.
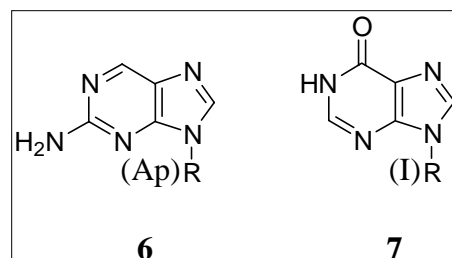
Elucidation of a Hole Hopping Mechanism

The hole hopping model was proposed by Giese and coworkers.[10] based on the effects of A-T base pairs on CT.[2,7,8,10] Site-selective radical cation generation was used to investigate CT in duplex DNA containing a G-G-G triplet acceptor and a guanine radical cation (Figure 6).[2] A series of DNA duplexes were created varying the number of A-T base pairs between the putative G^{+} and the G-G-G triplet (N_m and N_n). A site specific radical cation was formed, which migrated to the G-G-G triplet

where irreversible oxidation occurred.[2] The charge transfer was characterized by cleavage of oxidized DNA by piperidine. The cleavage products were analyzed by gel electrophoresis followed by autoradiography.[8] As more A-T base pairs are added between the donor and acceptor, the rate of CT decreases, following the typical superexchange trend, eq 1. However, upon the addition of a G-C base pair between
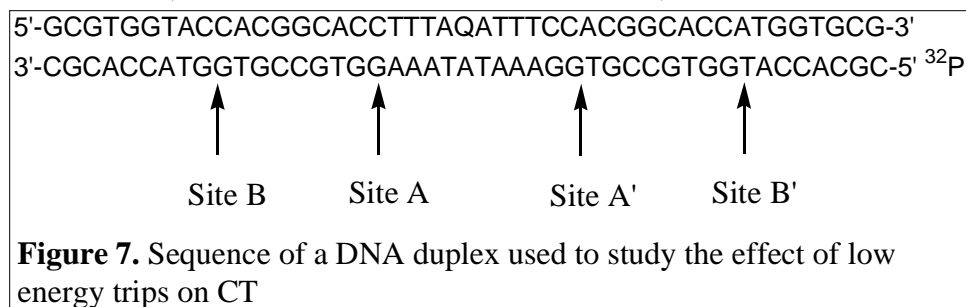

Figure 6. System used for hole hopping model, GGG triplet acceptor, G_{23} donor formed by site selective radical formation

A-T pairs (N_m = A-A-G-A-A), an increase in the rate is observed.[8] This increase is rationalized as a tunnelling process between two low energy sinks.[8] The tunnelling process is evidence for a hole hopping mechanism, however, the hopping could also be interpreted as PAPH.

Phonon-Assisted Polaron Hopping

Schuster and coworkers employed 7,8-dihydro-8-oxoguanine (8-OxoG) (**8**), a molecule useful because of its low oxidation potential, which acts as a trap for charge.[17] For the molecular wire model to be valid, the base stack would have to be in complete conjugation,[13,18] if so, in
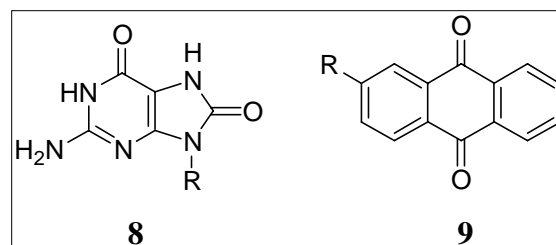
this state any areas of low energy will be "sensed" upon removal of an electron in the DNA bridge. To test this hypothesis, Schuster and coworkers attached an anthraquinone acceptor (AQ) (**9**) to uridine, and then synthesized a DNA duplex incorporating this nucleotide. The AQ unit is positioned so that the duplex is symmetrical about the attached uridine residue, placing GG doublets approximately equal distance from AQ (Figure 7).[3] Charge transfer through DNA was detected by piperidine-mediated causing strand cleavage at previously damaged base pairs. The products of the strand cleavage were then studied using gel electrophoresis, and audioradiography.[3] Initial irradiation of AQ gave similar oxidative damage, and therefore CT, to sites A, A', B, and B'. A duplex was synthesized, substi-tuting 8-OxoG was substituted for the 5' G of site A. Upon irradiation, CT was observed. Compared to a control system, CT to site B was halted, sites A' and B' showed no effects, while site A' showed increased CT. Similarly, the 5' G of Site A' was substituted with 8-OxoG, then irradiated.[11] CT increased dramatically at

Figure 7. Sequence of a DNA duplex used to study the effect of low energy trips on CT

A', was nearly non-existent at B', and remained the same for sites B and A.[11] As 8-OxoG has the lowest oxidation potential of the bases in the duplex, if it were in conjuation, the charge would migrate towards it. The lack of diminished CT to the side of the duplex opposite 8-OxoG indicates there is no conjugation, as charge would preferentially migrate towards 8-OxoG. This experiment demonstrates a completely conjugated stack of aromatic base pairs is not existent, therefore the molecular wire model is not reasonable.

Theoretical studies have been undertaken in an attempt to more fully understand the mechanism of CT in DNA. Conwell studied the feasibility of polarons existing and moving through DNA.[12] The Schreiffer-Heeger (SS) Hamiltonian, modified to allow the inclusion of an electric field, was used to study random base sequences. Indise the region of the polaron wave function, five to six base pairs, hopping of the polaron was observed from G/C to either G/C or A/T.[12] This process is similar to that described by Giese and coworkers,[2,7,8,10] but occurs faster, on a nanosecond timescale.[12] Over longer distances, the rate of polaron hopping will be much slower, and will not occur without phonon assistance, indicating a PAPH process.[12]

Temperature dependent electron spin resonance (ESR) was used by Sevilla and coworkers to determine the mode of CT in DNA[19]. A strict tunnelling mechanism will not show any temperature dependence in its rate, while a hole hopping mechanism will depend on the energy of the system to facilitate hopping. Variable temperature ESR of a DNA duplex shows no change in rate from 4 to 77 K, indicating a tunnelling mechanism. However, at temperatures of 150 K and higher, recombination of radical cation species is observed. The recombination clearly involves electron movement, and possibly the activation of elecron hopping.[19] This study indicates that CT in DNA can occur by both hole hopping (tunnelling) and a superexchange type system, however, at a physiological temperatures a superexchange system will dominate.[19]

CONCLUSION

The two extreme mechanistic models proposed for charge transfer in DNA are not realistic for charge transfer through DNA. DNA as a molecular wire assumes complete conjugation of the base pairs, highly unlikely over any reasonable distance. Charge transfer demonstates a distance dependence that would not be expected in the molucular wire model. Hole hopping, on the other hand, relies upon electrons hopping through discrete molecular orbitals of low oxidation guanine base pairs. Schuster and coworkers propose phonon-assisted polaron hopping. The phonon-assisted polaron hopping model

describes delocalization of charge through a polaron, while movement of the charge occurs by hopping. Phonon-assisted polaron hopping currently best describes charge transfer in DNA.

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