1
Principles and Mechanisms of Photoinduced Charge Injection, Transport, and Trapping in DNA

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1.1
Introduction

The first observation that the π-stacked array of base pairs in B-form DNA might serve as a pathway for charge migration was published over 40 years ago [1]. Since then, the basic question of whether DNA serves as a wire or conducting biopolymer for long-range charge migration has been discussed in an intense and highly controversial scientific dispute. DNA was considered to be a molecular wire, a semiconductor, or an insulator [2]. Barton and coworkers pioneered this research through remarkable contributions about photoactivated charge transfer chemistry in DNA [3]. Motivated by the biological relevance of DNA damage and also by the controversy about charge transfer in DNA, interest in DNA-mediated charge migration grew enormously in the scientific community in the 1990s [4]. Research groups from different chemistry subdisciplines, such as organic chemistry, inorganic chemistry, physical chemistry, and biochemistry, as well as biologists, physicists, and material scientists have contributed significantly to this research topic. This interdisciplinary nature represents an important and exciting aspect of this subject.

Based on these experiments and results, a clear picture about charge transfer processes in DNA has emerged by now. The extreme controversy has been solved by the description of different mechanistic aspects, mainly the superexchange and the hopping mechanisms, which have been verified experimentally [5]. It has become clear that DNA-mediated charge transfer can occur on an ultrafast time scale and can result in reactions over long distances [4]. Hence, DNA-mediated charge transfer has been the subject of considerable interest, having biological relevance in the formation of oxidative damage to DNA that can result in severe consequences such as mutagenesis, apoptosis, or cancer [6]. Additionally, charge transfer plays a growing role in the recent development of DNA chips or microarrays detecting single-base mismatches or various DNA lesions by electrochemical readout methods [7]. Moreover, knowledge about charge transfer processes in DNA can be used for nanochemical applications, such as DNA-based devices [8].

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1.2 Synthetic DNA-Donor-Acceptor Systems

Due to the short lifetimes of the natural DNA bases, it is necessary to modify oligonucleotides with suitable chromophores as tools in order to photoinitiate and study charge transfer reactions in the DNA helix. The initial difficulties in the synthesis of oligonucleotides that are covalently modified with suitable charge donors and acceptors, as well as the high concentrations necessary for the measurements of charge transfer rates by laser spectroscopy, were reasons for the first charge transfer experiments to be performed with noncovalently bound intercalators [9]. Using these assays, it is difficult to determine how the base sequence influences the charge transfer efficiency. More importantly, these experiments provide only very limited information, since the distance between the unspecifically bound charge donor and acceptors is unknown. The major concern with such assays is that the cooperative binding of donor and acceptor could provide the structural basis for a shortcut yielding an efficient short-range charge transfer and fast electron transfer rates.

Thus, new DNA systems that bear the charge donor and the charge acceptor covalently bound in a distinct distance on the oligonucleotide strands were necessary. As a consequence, the whole spectrum of different methods for phosphoramidite syntheses and protocols for oligonucleotide modifications have been applied, developed, and improved dramatically during the last 10–15 years in order to prepare structurally well-defined DNA systems [9]. Significant experimental improvement came with the DNA assays bearing charge donors that have been attached covalently to oligonucleotides. Using these DNA systems, a systematic measurement of the distance dependence and the base sequence dependence of the charge transfer processes became possible.

Accordingly, most of the past studies of DNA-mediated charge transfer processes were performed with respect to the following strategies [10]:

1. Covalent labeling of the DNA with redox-active probes.
2. Photochemical initiation of the charge transfer process.
3. Spectroscopic or electrochemical detection of the charge transfer processes or analysis of irreversible DNA products yielded by the charge transfer reaction.

A broad variety of organic or inorganic intercalators, sugar modifications, and natural or modified DNA bases have been used as charge donors and charge acceptors in order to study charge transfer or transport phenomena in DNA. In principle, the existing DNA assays can be classified by their characteristic structural features (Figure 1.1) [9]:

A. DNA duplexes with unnatural or artificial DNA bases.
B. DNA duplexes with DNA base modifications pointing into the major or minor groove.
C. Capped DNA hairpins with a duplex stem.
D. DNA duplexes with organic or inorganic intercalators covalently attached via a flexible linker.

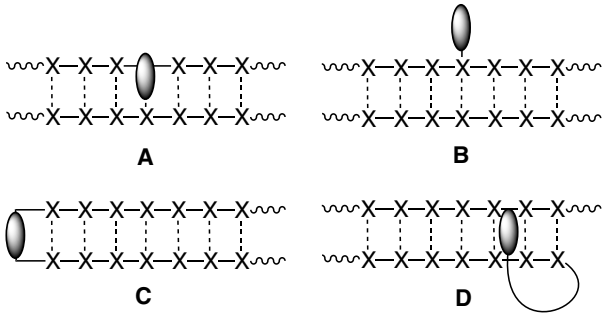


Fig. 1.1 Principal structures **A–D** of DNA donor-acceptor assays for the investigation of charge transfer processes in DNA.

In cases A, B, and C, the DNA base or sugar modifications can be introduced via automated solid-phase synthetic methods using suitable DNA building blocks. As an alternative route, DNA modifications can be introduced by solid-phase methods that are applied during or after the completed automatic solid-phase synthesis, as is the case for preparation of DNA assays B and D.

1.3
Photoinduced Oxidative Hole Transfer vs. Reductive Electron Transfer in DNA

In principle, DNA-mediated charge transfer processes can be categorized as either oxidative hole transfer or reductive electron transfer processes (Figure 1.2) [11, 12]. The phrase hole transfer is somewhat misleading since it describes an electron transfer in the opposite direction. Both processes, hole transfer and electron transfer, are in fact electron transfer reactions. However, with respect to orbital control it becomes clear that this categorization is not just a formalism about the different direction of the electron transfer. The oxidative hole transfer is a HOMO-controlled process, whereas the reductive electron transfer is LUMO-controlled.

With respect to biological motivation, most research groups have initially focused their work on the photochemically or photophysically induced oxidation of DNA and, furthermore, on the mobility of the created positively charged radical in the DNA. In this case, an electron is transferred from the DNA or the final acceptor (**A**) to the photoexcited charge donor (**D**). Such processes can be described as *oxidative hole transfer* or *hole transport*. On the other hand, charge transfer plays a growing role in the recent development of DNA-based nanowires and DNA microarrays detecting single-base mismatches or various DNA lesions by electrochemical readout methods [7]. For these purposes, the mobility of an excess electron in the DNA base stack has been applied. *Reductive electron transfer* or *excess electron transport* occurs if the photoexcited electron of **D** is injected into the DNA or transferred to the final electron acceptor **A**. In contrast to the broad and detailed knowledge about oxidative hole transfer and hole hopping in DNA, the mechanistic de-

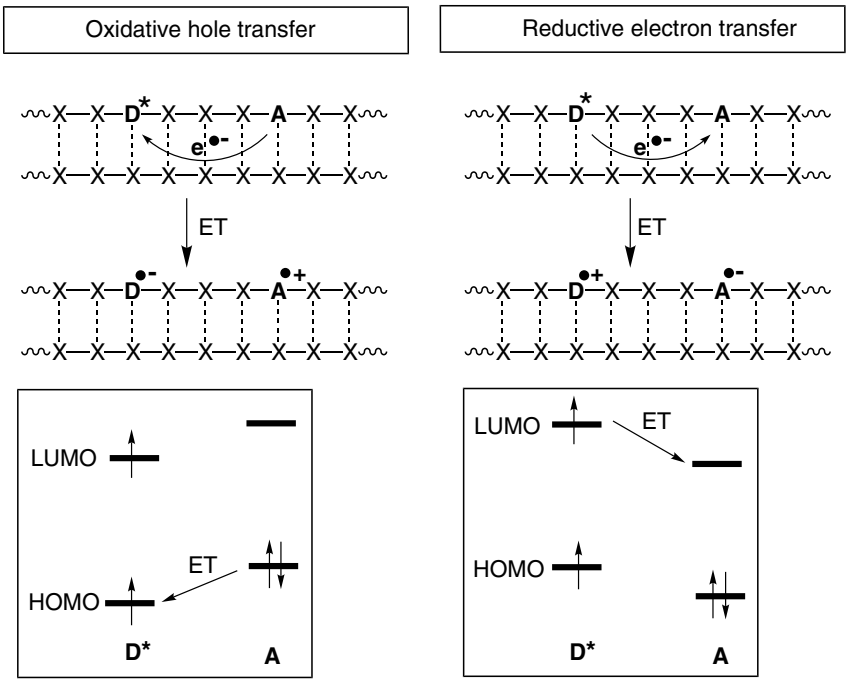


Fig. 1.2 Comparison of photoinduced oxidative hole transfer (HOMO control) and reductive electron transfer (LUMO control) in DNA (**D** = donor, **A** = acceptor, CT = charge transfer).

tails of excess electron transfer and migration remain unclear. Some lack of knowledge has been filled considerably during the last 2–3 years.

1.4 Hole Transfer and Hole Hopping in DNA

1.4.1 Spectroscopic Studies and Mechanisms of Hole Transfer in DNA

When the investigations of DNA-mediated charge transfer were started, most research groups interpreted their results according to the Marcus theory of nonadiabatic electron transfer [13]:

$$k_{ET} = \frac{4\pi^2 |V_{el}|^2}{h} \sqrt{\frac{1}{4\pi\lambda k_B T}} \exp\left(\frac{-\{\Delta G_{ET} + \lambda\}^2}{4\lambda k_B T}\right) \quad (1)$$

Three important quantities affect the rate of the electron transfer process (k_{ET}): the electronic coupling (V_{el}), the driving force of the electron transfer process

(ΔG_{ET}), and the reorganization energy (λ). A full understanding of DNA-mediated charge transfer requires knowledge of how these three variables are affected by the medium DNA as the bridge between donor **D** and acceptor **A**. In most synthetic DNA-donor-acceptor systems, this is not completely clear. In fact, especially the β-value has been highly over-interpreted, which was a major cause of the controversy. It is important to point out that the energetic level of the DNA bridge in relation to the energetic levels of **D** and **A** determines molecular wire-like behavior or a charge transfer via the superexchange mechanism (Figure 1.3). In the case of a molecular wire, the bridge states are energetically comparable to the level of **D** and the electron can be injected into the bridge. Upon injection, the electron is localized within the bridge and moves incoherently to **A**. However, in the case of the superexchange mechanism, the bridge states lie above the level of **D**. Consequently, the electron is transferred in one coherent jump and is never localized within the bridge. For the superexchange mechanism, the distance dependence behavior of k_{ET} is clearly exponential.

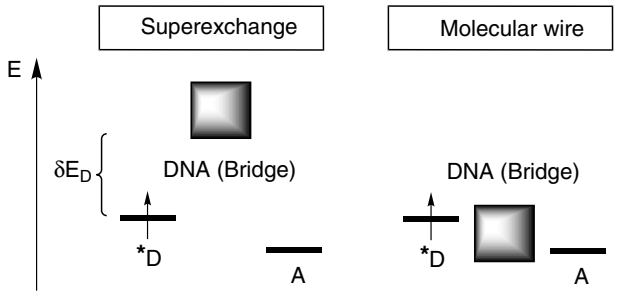


Fig. 1.3 Comparison of charge transfer via superexchange and via a molecular wire (**D** = donor, **A** = acceptor).

DNA represents a very special medium in terms of charge transfer processes. The planar heterocyclic aromatic systems of the DNA bases are stacked at a distance of 3.4 Å, which brought up the idea that DNA could conduct electrons like a molecular wire. Despite some initial controversies [2], it turned out that DNA as a medium for electron transfer is *not* a molecular wire. Accordingly, the DNA-mediated hole transfer processes were described in terms of a superexchange mechanism. The charge tunnels in one coherent step from **D** to **A** and never resides on the DNA bridge (**B**) between the two (Figure 1.4). This type of mechanism occurs if the bridge states are energetically higher than the photoexcited donor state (**D***). The rate k_{HT} of such a single-step process depends exponentially on the distance R between **D** and **A**, $k_{HT} \propto \exp(-\beta \cdot R)$. β represents the crucial parameter to describe the distance dependence of hole transfer in DNA which itself is dependent on the nature of the bridge **B** and its coupling with **D** and **A**. Values of β for charge transfer through proteins lie in the range of 1.0–1.4 $Å^{-1}$ [14]. In comparison, apparent β-values determined for hole transfer reactions in DNA can be found in a wide range from $\beta < 0.1$ $Å^{-1}$ to $\beta = 1.5$ $Å^{-1}$ (Table 1.1) [4].

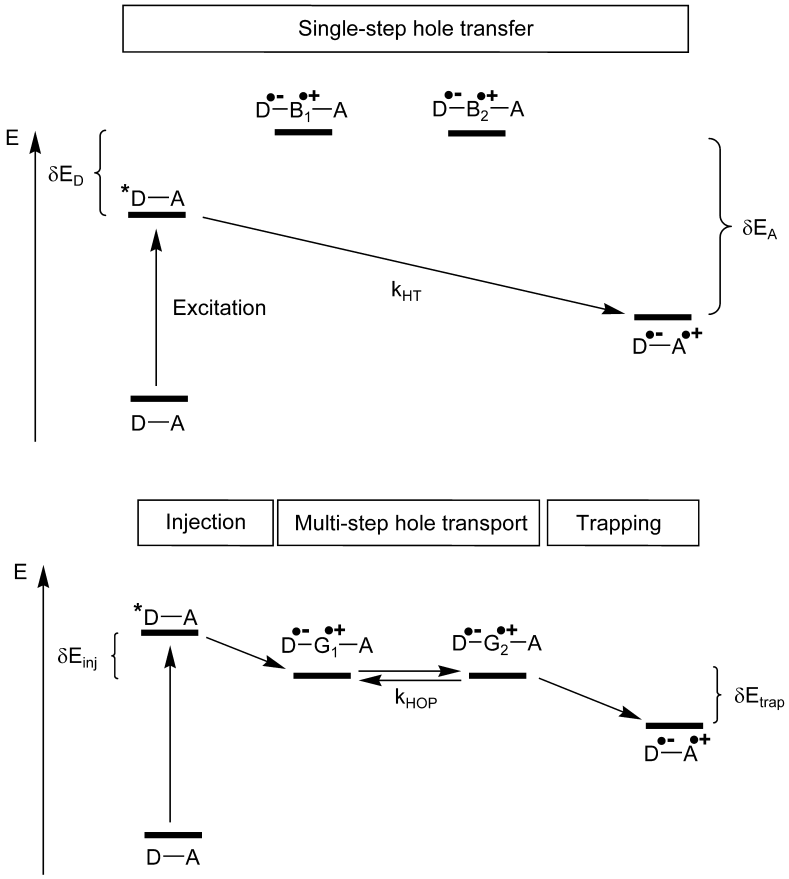


Fig. 1.4 Comparison of photoinduced DNA-mediated hole transfer via the superexchange mechanism and photoinduced hole transport via hopping (**D** = donor, **A** = acceptor, **B** = base, HT = hole transfer, inj = injection, HOP = hopping, trap = trapping).

Four important observations were drawn from this interpretation:

1. The hole transfer via the superexchange mechanism is limited to short distances (<10 Å).
2. Short-range hole transfer reactions occur on a very fast time scale ($k_{HT} = 10^9$–10^{12} s^{-1}).
3. The typical β-value of DNA-mediated hole transfer is 0.6–0.8 $Å^{-1}$.
4. The intercalation of the charge donor and acceptor is crucial for a fast and efficient hole transfer process.

The occurrence of very small β-values (≤ 0.1 $Å^{-1}$) exhibiting shallow distance dependence led to the description of an alternative mechanism, the hopping model (Figure 1.4) [5, 11]. Among the four different DNA bases, guanine (G) can

Table 1.1 Important spectroscopic studies of DNA-mediated hole transfer using DNA with covalently attached donor and acceptor.

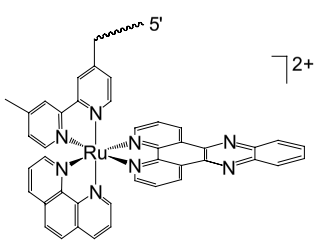
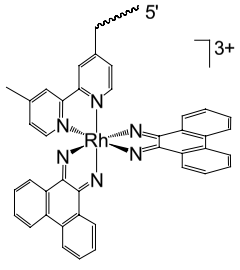
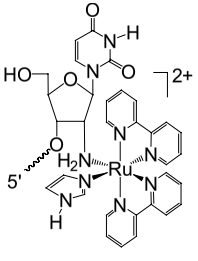
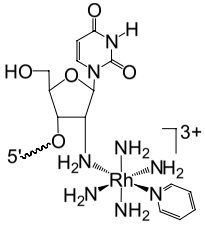
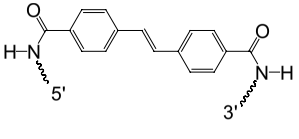
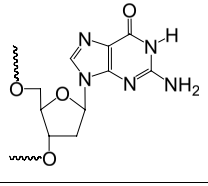
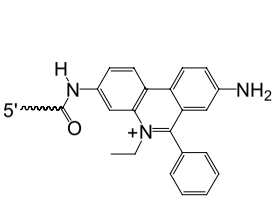
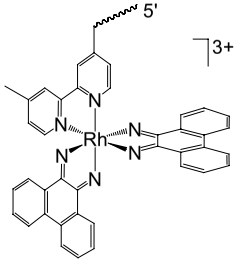
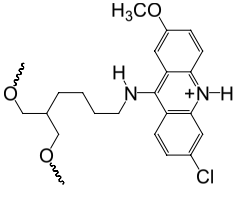
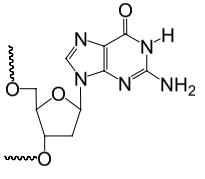
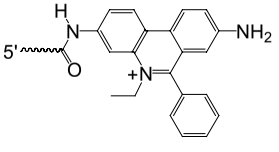
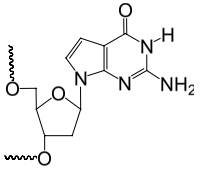
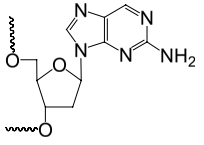
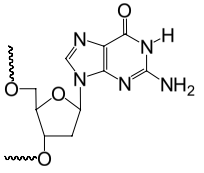
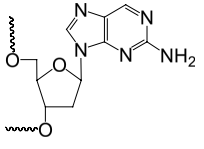
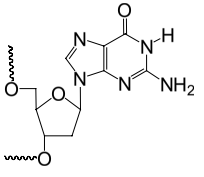
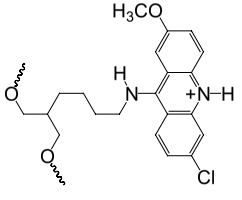
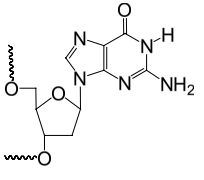
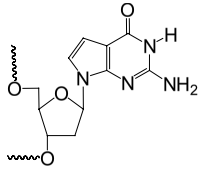
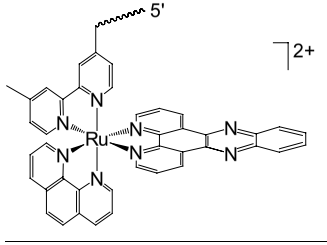
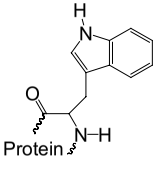
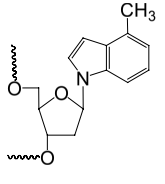
Charge donor	Charge acceptor	β (Å^{-1})	k_{CT} (s^{-1})	Research group	Year
		0.2	10^9	Barton et al. [3]	1993
		1.0–1.5	10^6	Meade et al. [15]	1995
		0.64	10^8–10^{12}	Lewis et al. [16]	1997

Table 1.1 (continued)

Charge donor	Charge acceptor	β (Å^{-1})	k_{CT} (s^{-1})	Research group	Year
		–	10^{10}	Barton et al. [17]	1997
		1.4	10^{5}–10^{10}	Tanaka et al. [18]	1998
		–	10^{12}	Barton/Zewail et al. [19]	1999

			0.1–1.0	10^9–10^{11}	Barton/Zewail et al. [20]	1999
			0.75	10^6–10^7	Shafirovich et al. [21]	2000
			2.0/0.8	10^7–10^{12}	Michel-Beyerle et al. [22]	2001
			–	$\geq 10^7$	Barton et al. [23, 24]	2002

be most easily oxidized [25, 26]. Hence, the G radical cation plays the role of the intermediate charge carrier during the hopping process. In contrast to the previously described superexchange mechanism, the bridge and donor levels have to be similar in order to inject a hole thermally into the DNA base stack. Subsequently, the positive charge hops from G to G and can finally be trapped at a suitable charge acceptor. If each single hopping step occurs over the same distance, then the dynamics of hopping displays a shallow distance dependence with respect to the number of hopping steps N [5]:

$$k_{ET} = N^{-\eta} \tag{2}$$

The value of η lies between 1 and 2 and represents the influence of the medium. Each hopping step itself is a superexchange process through the intervening adenine-thymine (A-T) base pairs, but only if the A-T stretch is not too long (see below). The rate for a single hopping step from G to GG was determined to be $k_{HOP} = 10^6 - 10^8\ s^{-1}$ [27]. Using the site-specific binding of methyltransferase *HhaI* to DNA, a lower limit for hole hopping in DNA $k_{HT} > 10^6\ s^{-1}$ was measured over 50 Å through the base stack [24]. Based on the absence of a significant distance dependence, it was concluded that hole hopping through the DNA is not a rate-limiting step.

Recently, it was proposed and underscored with experimental evidence that adenines can play the role of intermediate hole carriers (Figure 1.5) [28]. Such A-hopping can occur if G is not present within the sequential context, mainly in longer A-T stretches (at least four A-T base pairs) between the guanines (Figure 1.5). The oxidation of A by $G^{\bullet+}$ is endothermic. With respect to the low efficiency of this hole-hopping step, it was suggested that once $A^{\bullet+}$ has been generated, the A-hopping proceeds fast. In fact, the rate of A-hopping has been determined to be $k_{HT} = 10^{10}\ s^{-1}$ [29]. Moreover, it could be shown that hole transport over eight A-T base pairs is nearly as efficient as the hole transport over two A-T base pairs [30]. In comparison to G-hopping, A-hopping proceeds faster, more efficiently, and almost distance independently. Recent calculations suggest that hole transport through stacked A-T base pairs may be most favorable [31]. It is known from γ-radiolysis studies that the one-electron oxidation of DNA bases has drastic effects on their acidity. In theory, proton transfer processes could occur on time scales comparable to charge transfer reactions and can therefore dramatically influence the charge transport efficiency due to the separation of spin and charge [32]. The question of proton transfer in oxidized $G^{\bullet+}$-C base pairs is crucial for the understanding of hole hopping in DNA. The pK_a value of $G^{\bullet+}$ is ~3.9 [33]. The pK_a value of the complementary DNA base cytosine (C) is very similar (4.5) [33]. Hence, there is likely an equally distributed protonation equilibrium in a one-electron oxidized $G^{\bullet+}$-C base pair that is principally reversible but could interfere with the hole transport and potentially interrupt hole hopping in DNA. In fact, measurements of the kinetic isotope effect of hole transport in DNA have been performed and provide some evidence for a coupling between hole hopping and proton transfer processes [34]. The situation is different in the A-T pair. Oxidized adenine ($A^{\bullet+}$) represents a

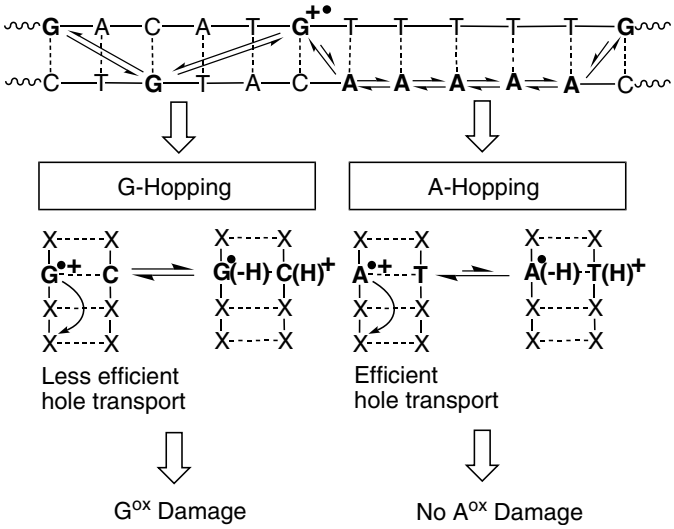


Fig. 1.5 DNA-mediated hole transport via guanine hopping and adenine hopping.

powerful acid with a pK_a of 1, and T shows an extremely low basicity (pK_a of $T(H)^+$ is –5) [33]. Taken together it becomes clear that charge and spin remain located on the A in an $A^{\bullet+}$-T base pair (Figure 1.5).

The discovery of A-hopping is an excellent example of how the mechanistic picture about hole transport and hole transfer processes through DNA becomes more complex and simultaneously more complete. For instance, a polaron-like model was suggested as a more precise mechanistic description for hole transport through DNA [35]. The polaron represents a structural distortion of the DNA that stabilizes and delocalizes the radical cation over several DNA bases. On the other hand, a formal relation between the charge transfer rate through a molecular bridge and the conductance of this bridge has been established [36]. This means that dephasing and relaxation effects influence the charge transfer rate. It is clear that the role of base motions in delocalization and propagation of charges through the DNA duplex must be taken into account. Most recently, an oscillatory component of the distance dependence with a period of 4–5 base pairs has been described experimentally and cannot be rationalized by the phonon-assisted polaron-hopping model or by a temperature-induced transition from superexchange to thermally induced hopping [37]. As a result, hole transport through DNA has been described as a conformationally gated hopping among stacked domains, the so-called charge transfer-active domains representing a conformation of a few well-coupled DNA bases that exists only in a distinct time frame [37].

1.4.2 Biochemical and Chemical Hole Trapping in DNA

Besides A-hopping, $G^{\bullet+}$ plays the major role of the intermediate charge carrier during the hole-hopping process. The G radical cation was identified as the precursor of a variety of different oxidative G lesions, which are normally described as G^{ox}. Some of these G oxidation products have been identified [6]. Bioanalytical experiments explore hole transport reactions through DNA by an indirect method. As described above, after the photochemical or photophysical oxidation of DNA using a suitable intercalator, G is preferentially oxidized. The resulting $G^{\bullet+}$ can react with H_2O and/or O_2, yielding G^{ox}. Such modified DNA strands can be cleaved at the site of G^{ox} by treatment with, e.g., piperidine at elevated temperature and then separated by gel electrophoresis and visualized by phosphorimagery using radioactive ^{32}P-labeling.

The most common photooxidants for DNA are metal complexes or organic intercalators such as Rh(III) complexes, Ru(II) complexes, ethidium derivatives, anthraquinone derivatives, uridine modified with cyanobenzoquinones, and modified 2′-deoxyribosides bearing a photoreactive group (Figure 1.6) [10]. These systems differ significantly in their structural properties, their redox potentials, and their absorbing wavelengths. It has been observed in all systems that the positive charge can be transported with high efficiency over very long distances (up to 200 Å) [38]. The observed efficiency of hole transport seems to be strongly dependent on the integrity of the conformation of the intervening DNA base pairs.

The hole injection system by Giese et al. represents an important exception in comparison to the others since it relies on a Norrish type I cleavage reaction of a 4'-modified uridine derivative yielding a sugar radical cation (Figure 1.7 a) [39]. This enol ether radical cation exhibits a higher oxidation potential compared to G and hence is able to inject a hole into the nearest G within the DNA duplex. This hole injection system works from the ground state, although it is photoinitiated, and thus has the advantage that fast back charge transfer processes do not occur. Additionally, this assay allows elucidation of some kinetic information about hole hopping in DNA, since the trapping reaction of the enol ether radical cation by water competes with the hole injection process and exhibits a rate of $10^8\ s^{-1}$.

The oxidation of G yielding the piperidine-labile G^{ox} represents probably the most prominent example of a thermodynamic hole trap for hole hopping in DNA. Additionally, chemically modified DNA bases have been presented as new and interesting kinetic hole traps that allow the site-specific trapping of holes in DNA and the chemical probing of hole hopping. Especially cyclopropyl-modified guanine (Cp-G) [40] and adenine (Cp-A) [41] have been applied, since the ring opening of the *N*-alkylcyclopropylaminyl radical [42] can be considered as a radical clock (Figure 1.7 b). Cp-A in particular represents an important tool for the elucidation of adenine hopping when it is incorporated into longer A-T bridges between two GG sites. As mentioned previously in this chapter, oxidative adenine damage (A^{ox}) has not yet been observed as a result of hole hopping over longer A-T bridges and involving $A^{\bullet+}$ as an intermediate charge carrier. In fact, using

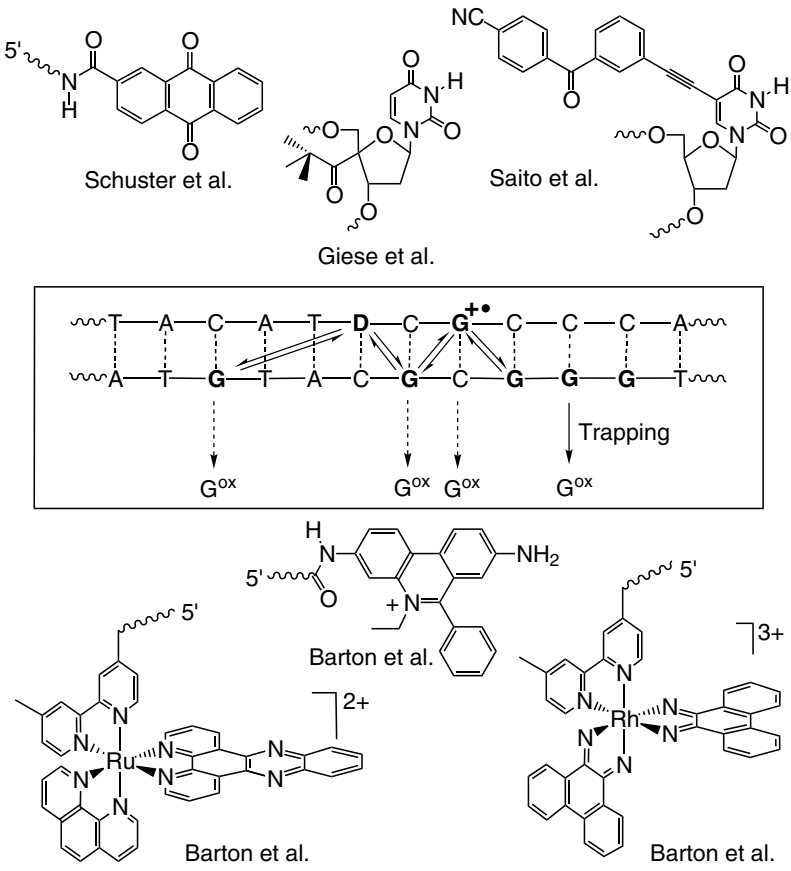


Fig. 1.6 Important examples of photooxidants (**D**) for the investigation of DNA-mediated hole transport by bioanalytical detection of oxidative guanine damage (G^{ox}).

Cp-A, the hole could be trapped within the A-T bridge, which proves the presence of a positive charge in this area [41]. Hence, cyclopropyl-substituted nucleosides are promising tools to prove the existence of the transient radical species in DNA. The corresponding chemical trapping is in agreement with the previous consideration that nucleobases possessing higher oxidation potentials than G, such as A, are also able to participate directly in the multi-step hopping mechanism.

1.4.3 Modulation of DNA-mediated Hole Transfer

DNA-mediated hole transfer exhibits an extremely high sensitivity to the π-stacking of the intervening DNA bases as well as to disruption and perturbation of the DNA structure or conformation. Hence, checking the DNA integrity and sensing

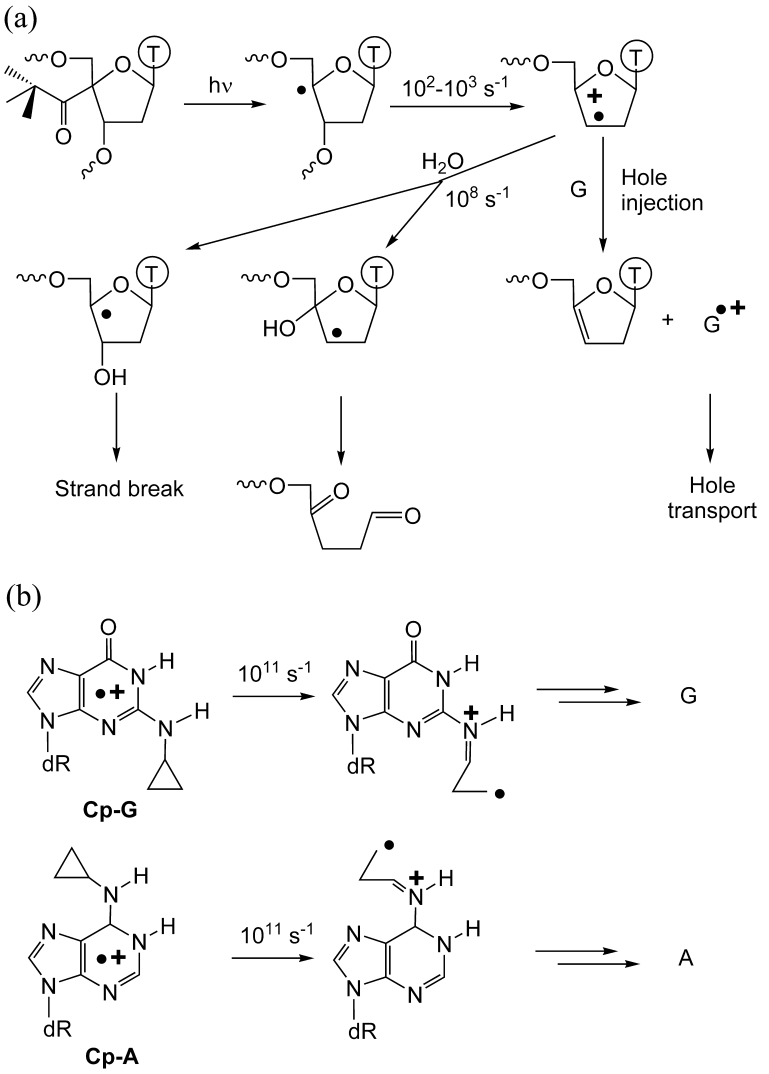


Fig. 1.7 Chemical hole-injection (a) and hole-trapping (b) assays.

of DNA damage at the various checkpoints during the cell cycle could be accomplished based upon charge transfer reactions. Recently, charge transfer in HeLa cell nuclei has been probed using a rhodium photooxidant [43]. After incubation and irradiation, the genomic DNA was isolated and analyzed, revealing that the base damage occurs preferentially at the 5′-G of GG sites. More importantly, oxidative G damage was found at protein-bound sites that were inaccessible to the rhodium photooxidant as examined by footprinting. This clearly indicates that hole transfer processes can occur in cells.

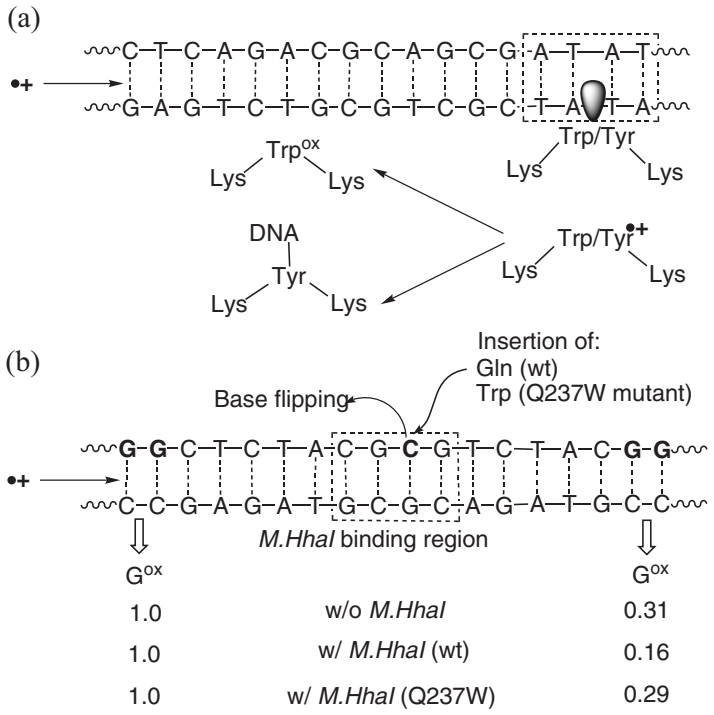


Fig. 1.8 (a) DNA-to-peptide hole transport. (b) Modulation of DNA-mediated hole transport by cytosine methyl transferase, *HhaI* wild-type, and Gln237 Trp mutant.

It is very reasonable to begin investigation of protein-modulated hole transport in DNA by using DNA-binding peptides. In principal, aromatic amino acids like tyrosine (Tyr) or tryptophan (Trp) have a lower oxidation potential than G and can therefore be used as peptidic traps for holes [44]. The smallest-possible DNA-binding peptides Lys-Trp-Lys and Lys-Tyr-Lys have been applied, and it was shown that the radicals of Trp and Tyr can be generated by DNA-mediated hole transport and can occur on the microsecond time scale in high yields (Figure 1.8 a) [45]. Hence, DNA-binding peptides as models for proteins play an important role in protecting the genome from radical damage. Interestingly, the generated peptide radicals exhibit a completely different reactivity. The DNA-bound Trp radical forms oxidized products in the presence of O_2, while the DNA-bound Tyr radical forms cross-links with the DNA bases at the peptide-binding site.

Specific DNA-protein interactions that either promote or inhibit hole transfer processes would be the most crucial part in biological charge transfer systems. The following experiments have shown clearly that DNA-mediated hole transfer processes are modulated both negatively and positively by DNA-binding proteins. Most importantly, each of the observed influences of the proteins can be explained by special structural features of the corresponding DNA-protein complexes.

Hence, specific DNA-protein interactions result in a characteristic modulation of the DNA-mediated hole transport.

One of the most interesting examples of how proteins can modulate charge transfer in DNA is the cytosine methyltransferase *HhaI* [23, 46]. This protein recognizes the sequence 5′-GCGC-3′, and during methylation a base-flipped complex is formed with the target C in an extrahelical position (Figure 1.8b). A glutamine (Gln) side chain fills the space in the DNA duplex. Not surprisingly, the hole transport in the DNA was attenuated as a result of the interruption of the base stack. The situation is different when using an M.*HhaI* mutant containing Trp rather than Gln237 (Q237W). Interestingly, long-range hole transport was restored upon binding with this Trp mutant of M.*HhaI*. This is the result of an electronic interaction of the flat aromatic indole heterocycle of Trp with the neighboring DNA bases. In conclusion, the indole side chain of Trp is able to replace a normal DNA base by electronic means [46]. The M.*HhaI*-DNA complex using the Q237W mutant was applied to time-resolved transient absorption spectroscopy [23]. The product radical was identified as a mixture of the Trp and G radicals occurring in the DNA-protein contact area. By laser experiments it was possible to establish a lower limit for hole transport in DNA of $k_{HT} > 10^6\ s^{-1}$ through 50 Å through the DNA base stack. Based on the absence of significant distance dependence, hole transport through the DNA is not rate limiting.

In contrast to wild-type M.*HhaI*, DNA-protein interactions could facilitate hole transport in DNA. Especially proteins that bind to the major groove but do not perturb the normal B-DNA structure can significantly enhance hole transport efficiency in DNA. This was demonstrated by using either the restriction endonuclease *PvuII* or the transcription factor ANTP (Figure 1.9) [47]. As a result of the binding of proteins, the DNA conformation is stiffened, the conformational movements are diminished, and, consequently, the hole transport is facilitated. In contrast to R.*PvuII* and ANTP, the TATA box-binding protein induces two 90-degree bends into the DNA duplex. Due to this strong conformational change in the helix structure, the hole transport efficiency decreases significantly [47]. More recently, the complex of endonuclease *BamHI* and the target DNA was investigated by guanine oxidation as the result of a DNA-mediated hole transport [48]. In this case, the direct contact of a positively charged guanidinium group of the protein to the recognition sequence of the DNA completely suppressed hole transport and dramatically lowered the guanine damage efficiency (Figure 1.9).

The counterions also play an important role in DNA-mediated hole transport. Interestingly, Schuster et al. elucidated that the migration of charges in DNA can be gated by ions [49]. However, a strong dependence of the efficiency of hole transport on the identity of the counterions is unlikely. Most recently, there have been attempts to modulate hole transport through DNA by artificial DNA bases that can tune the π-stacking properties within the DNA duplex. For instance, benzofused adenine bears a larger aromatic surface and enhanced stacking properties, thereby providing better hole transfer ability [50].

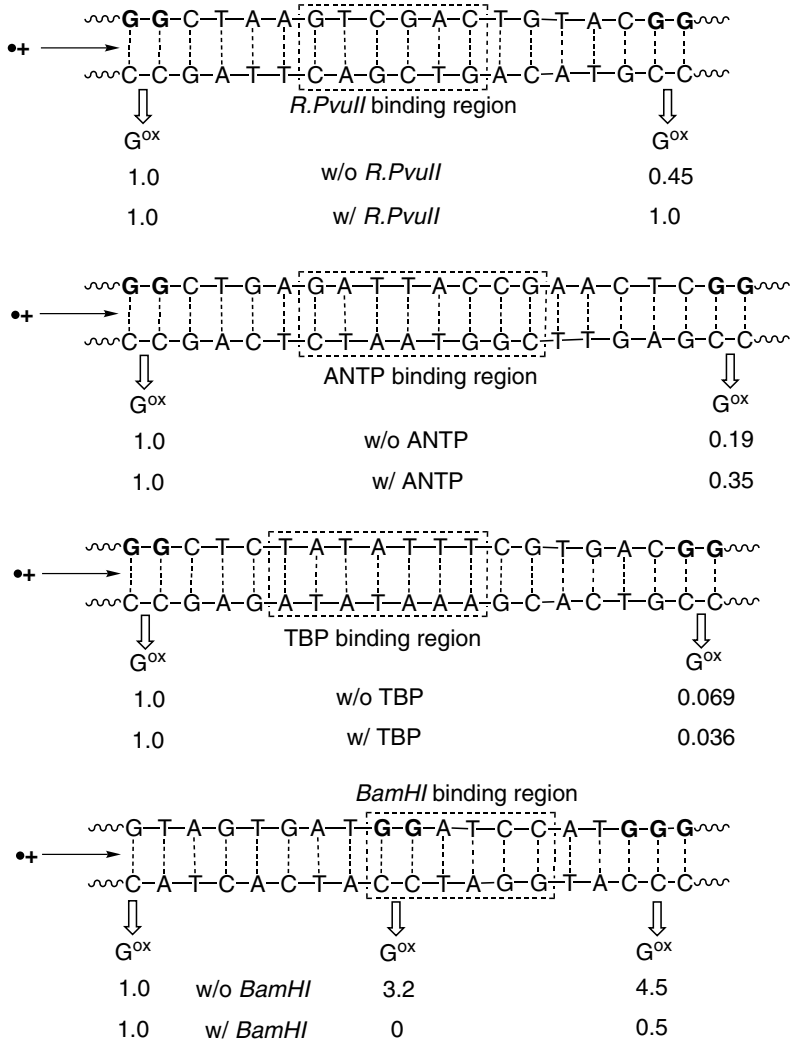


Fig. 1.9 Modulation of DNA-mediated hole transport by the following proteins: restriction endonucleases *PvuII* and *BamH1*, antennapedia homeodomain protein (ANTP), and TATA box-binding protein (TBP).

1.5
Reductive Electron Transfer in DNA

1.5.1
Mechanisms of Electron Transfer in DNA

In contrast to the broad knowledge available about oxidative hole transfer and hole hopping, as described in the previous sections, the mechanistic details of ex-

cess electron transfer and migration are not completely clear. This lack of knowledge has been filled at least partially during the last 2–3 years, but a well-defined and suitable donor-acceptor system for time-resolved spectroscopic measurements is still lacking [12]. Meanwhile, the mechanisms of the oxidative hole transfer and transport processes have been transferred to the problem of reductive electron transfer and excess electron migration. Accordingly, a hopping mechanism was proposed for the DNA-mediated transport of excess electrons over long distances (Figure 1.8) [11]. Furthermore, it was suggested that such electron hopping involves all base pairs (T-A and C-G) and the pyrimidine radical anions $C^{\bullet-}$ and $T^{\bullet-}$ as intermediate electron carriers [11]. This proposal is based on the trend of the reducibility of DNA bases, which is T, uridine (U) $\approx$ C $\gg$ A > G, making it clear that the pyrimidine bases C and T are reduced more easily than the purine bases A and G [26, 51]. In fact, the absolute values of the reduction potentials of DNA bases vary significantly depending on the solvent and the experimental method [26, 51] (Figure 1.10). Moreover, the situation within the DNA could be significantly different from the isolated monomer nucleosides. Calculations have shown that 5′-TTT-3′ and 5′-TCT-3′ probably serve as the strongest electron sinks [52]. Seidel et al. measured a complete set of polarographic potentials that are in the range between –2.04 V and –2.76 V [26]. In this context, the measured value $E(\mathrm{dC/dC^{\bullet-}})$ ~ $E(\mathrm{dC/dC^{\bullet-}})$ ~ –1.1 V provided by Steenken et al. [51] is difficult to understand and could reflect the result of a proton-coupled electron transfer. Thus, it is likely, that the –1.1-V potential corresponds to $E(\mathrm{dC/dC(H)^{\bullet}})$ and $E(\mathrm{dT/dC(T)^{\bullet}})$.

Until five years ago, most knowledge about reductive electron transfer and excess electron migration in DNA came from γ-pulse radiolysis studies [53]. The DNA samples were doped by intercalated and randomly spaced electron traps. The major disadvantage of this principal experimental setup is that the electron injection and the electron trapping do not occur site-selectively. Nevertheless, a few remarkable and principal conclusions and implications can be drawn from these studies. Below 77 K, electron transfer in DNA occurs via a superexchange mechanism with a distance dependence $\beta = 0.9\ \text{Å}^{-1}$. Above 170 K, the electron transfer mechanism changes completely to a thermally activated process.

The most recently developed photochemical studies of electron injection and transport in DNA follow the experimental design that was presented previously (Section 1.2). Flavin [54], naphthalene diamine [55], stilbenediether [56], phenothiazine [57], and pyrene [58] derivatives have been used as chromophores and photoexcitable electron donors that were covalently attached to oligonucleotides. They differ significantly in their structure and, more importantly, in their redox properties (Figure 1.10). In principle, the photoexcited flavin and naphthalene diamine nucleotide analogues could reduce all four DNA bases, whereas the stilbenediether, phenothiazine, and pyrene nucleoside analogues are able to selectively reduce the pyrimidine bases C and T in order to initiate an electron-hopping process within the DNA.

The major part of these recent photochemical assays focuses on the chemical trapping of the excess electron and the corresponding chemical analysis of the resulting DNA strand cleavages. Currently, two different chemical electron traps

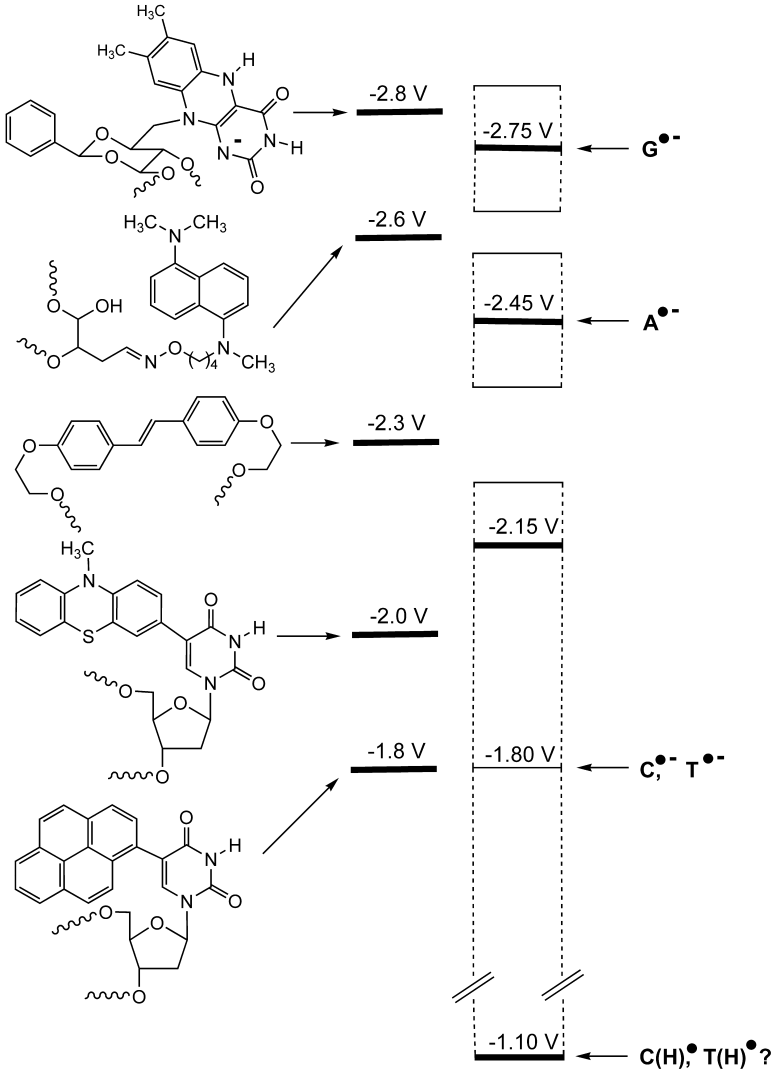


Fig. 1.10 Reduction potentials of photoexcited chromophores that have been applied for the investigation of excess electron transport, in relation to the reduction potentials of the DNA bases.

have been developed and applied: (1) a special T-T dimer lacking the phosphodiester bridge [54] and (2) 5-bromo-2′-deoxyuridine (Br-dU) [55, 57]. Both chemical probes yield strand cleavage at the site of electron trapping, Br-dU only after piperidine treatment at elevated temperature (Figure 1.11). The main difference between these two electron traps is the kinetic regime of the irreversible trapping reaction. Although the exact dynamic behavior has been examined only with the isolated nucleoside monomers, the rates are significantly different. The radical anion

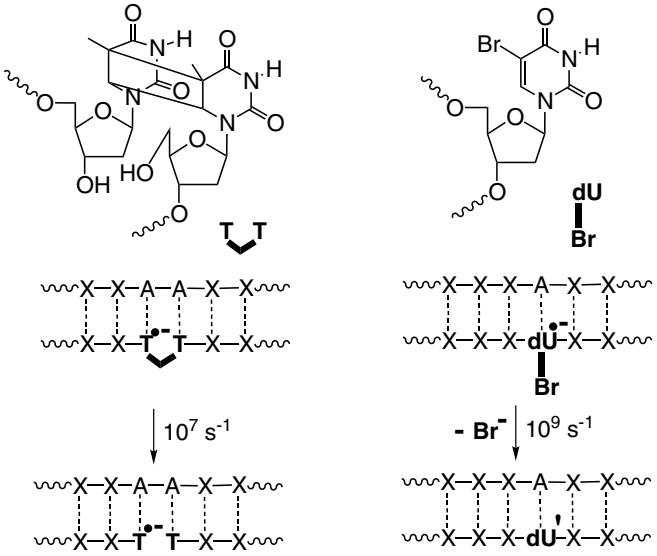


Fig. 1.11 Comparison between the T-T dimer and Br-dU as chemical electron traps.

of Br-dU loses its bromide with a rate of 7 ns^{-1} [59], whereas the radical anion of the T-T dimer splits with a much slower rate of 556 ns^{-1} [60]. This striking difference has important consequences for the elucidation of the distance dependence and DNA base sequence dependence of the excess electron transport efficiency. Hence it is not surprising that in the assay of Carell et al. the amount of T-T dimer cleavage depends rather weakly on the distance to the electron donor, which is a flavin derivative [54]. On the other hand, when using Br-dU as the electron trap, a significant dependence of the strand cleavage efficiency on the intervening DNA base sequence has been observed by the group of Rokita et al. [55] and our group [57]. Thus, Br-dU seems to be more suitable as a kinetic electron trap since the time resolution is better for the exploration of details of a presumably ultrafast electron transport process. It is important to point out that in contrast to Br-dU, where the trapped electron is consumed by the loss of the bromide anion, the cleavage of the T-T dimer is redox neutral. This means that subsequent to the T-T dimer cleavage, the excess electron could be transported further away. In fact, Giese and Carell et al. showed recently that a single injected electron could cleave more than one T-T dimer in the same DNA duplex [61].

By now, only the Lewis group [56] and our group [58, 62] are focusing on the study of the dynamics of DNA-mediated electron transfer processes using stilbenediether-capped DNA hairpins or pyrene-modified DNA duplexes, respectively. In both sets of time-resolved experiments, very fast electron injection rates (10^{11} s^{-1}) were detected. Until now, the measurements of electron transfer or electron transport rates have been elusive. Currently, these studies, as well as the previously mentioned studies using chemical electron traps, suggest conclusively a thermally acti-

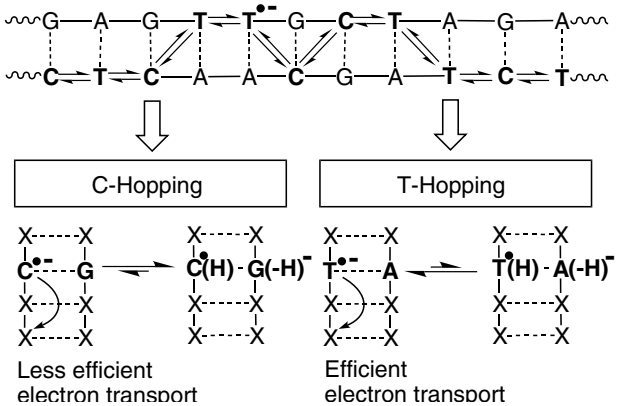


Fig. 1.12 DNA-mediated excess electron transport via cytosine hopping and thymine hopping.

vated electron-hopping mechanism over long distances. Both pyrimidine radical anions, $T^{\bullet-}$ and $C^{\bullet-}$, can play the role of intermediate charge carriers (Figure 1.12). The electron hopping via $T^{\bullet-}$ seems to be more favorable since the reduction potential of T is slightly lower than that of C in double-helical B-DNA (according to our studies). Moreover, $C^{\bullet-}$ and $T^{\bullet-}$ exhibit a large difference in terms of their basicity [62]. Thus, protonation of $C^{\bullet-}$ by the complementary DNA bases or the surrounding water molecules probably interferes with the electron hopping (Figure 1.12) [32, 33, 62]. As a result, it can be assumed that the protonation of $C^{\bullet-}$ in C-G base pairs decreases the efficiency and rate of electron transport but does not stop electron migration in DNA.

1.5.2
Outlook: Electron Transfer in DNA Chip Technology

In the last 10 years, genomic research has demanded highly parallel analytical approaches. Undoubtedly, the most powerful development has been the realization of DNA microarrays and DNA chips. In principal, DNA chips are segmented, planar arrays of immobilized DNA fragments that are used in a wide field of applications, from expression analysis to diagnostic chips [63]. In the latter case, a reliable detection of genomic sequence variations, mainly point mutations (single-nucleotide polymorphism), is critical for the study of population genetics, for the clinical diagnostics of cancer, for the diagnosis and treatment of genetic or viral diseases such as AIDS, and, most recently, for the concept of pharmacogenetics [64].

From the various studies of charge transfer in DNA, it has become clear that these processes show an extreme sensitivity towards perturbation and interruptions of base stacking that are caused by base mismatches or DNA lesions. Thus, charge transfer in DNA should be suitable to obtain a highly sensitive electrochemical readout on DNA chips. The basic idea is that the subsets of a critical gene

are immobilized as single-strand oligonucleotides on an electrode or chip and contain a redox-active probe that is intercalated and/or covalently attached (Figure 1.13). Intact DNA material added to the chip forms intact DNA duplexes leading to an efficient electron transfer between the chip surface and the distant redox-active probe. Base mismatches and DNA lesions significantly interrupt charge transfer in DNA, and as a result, the electrochemical response is missing.

One of the most convenient techniques for the depositing of biopolymers on solid-phase surfaces is the self-assembled monolayer [65]. According to this technique, DNA is attached to an alkyl thiolate linker via the 5′-terminal hydroxy group of the oligonucleotide, which then interacts with the gold electrode to form DNA films. Additionally, the DNA is labeled with redox-active probes, such as daunomycin, pyrrolo-quinoline-quinone, methylene blue, or ferrocene [7]. Using this methodology, a broad range of single-point mutations and DNA lesions can be detected without the context of certain base sequences. Hence, electron transfer through DNA films offers a new and suitable approach for the development of sensitive DNA sensors and chips. Normally, sensitive gene detection is accomplished by the amplification of the DNA material through the polymerase chain reaction (PCR). Inherent limitations of PCR often prohibit this application. Thus, research in the field of new DNA chips is currently focused on increasing sensitivity in such a way that PCR amplification becomes unnecessary.

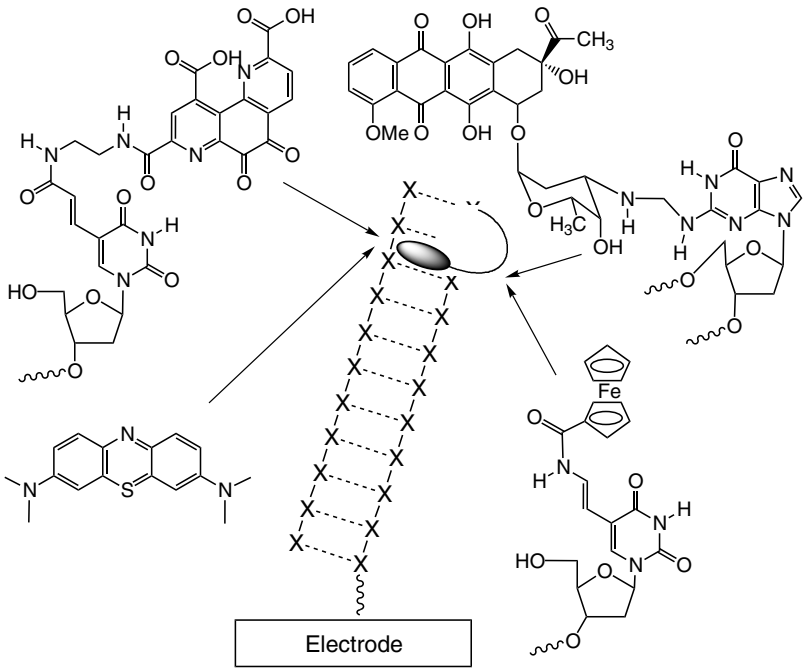


Fig. 1.13 Examples of redox-active probes for an electrochemical readout on DNA chips and microarrays.

1.6 Conclusions

This introductory chapter summarizes briefly all of the important and basic aspects related to charge transfer processes in DNA. This information should underscore (in addition to a variety of detailed questions) that a pretty clear picture about the phenomenon of "charge transfer processes in DNA" has emerged by now. The extreme controversy has been solved by very differential interpretations of the applied DNA systems and the description of alternative mechanisms.

In conclusion, it has turned out that excess electron transport occurs via a hopping mechanism over long distances (Figure 1.12), which is comparable to a certain extent with hole hopping in DNA (Figure 1.5). But it is important to note here that there are significant differences between both types of charge transport. First, hole transport occurs preferentially via guanine hopping and, only in stretches longer than 3–4 A-T base pairs, via adenine hopping. Hence, the hole-hopping process can be divided into distinct sequence regimes. In contrast, the electron transport occurs via mixed cytosine and thymine hopping, with some preference for the thymine radical anion as the intermediate electron carrier. Second, during hole hopping, the irreversible oxidation of the guanine radical cation yielding the G^{ox} damage competes with the hole transport. In contrast, no damage as a result of excess electron migration has been detected yet. This means that hole transport can never occur without causing damage, whereas electron transport potentially can.

The latter conclusion has important significance for the biotechnical application of DNA-mediated charge transfer as well as for the proposed biological role of charge transfer during DNA damage recognition. In both cases it can be assumed that it is better to use the transport of an excess electron rather than a hole during the sensing procedure in order to avoid DNA damage.

Despite this broad knowledge, DNA research is still far from a profound and clear understanding of the electronic properties and interactions in DNA. In the past, DNA-mediated charge transfer has been the subject of considerable interest, having biological relevance in the formation and repair of lesions and damage in DNA. The future will show the high potential for applications of these processes in the development of new DNA assays and microarrays as well as DNA-inspired devices for nanotechnology.

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