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## Spiers Memorial Lecture

### On the hypothesis of cathodic protection of genes

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Holes (electron vacancies) injected by oxidants are transferred across < 50 base pair (bp) long dissolved DNA duplexes in homogeneous solutions by hopping between CG base pairs.<sup>1–5</sup> The injected holes oxidize remote G-sites, particularly sites comprising sequences of multiple GC base pairs.<sup>1–22</sup> When not aligned in parallel, condensed-phase DNA duplexes are electrically insulating, but when aligned their one-dimensional conductivity in the direction of the aligned long axes increases at least  $10^3$  fold.<sup>23,24</sup> The conductivity of 600 nm long DNA “ropes” exceeds  $10^3 \Omega^{-1} \text{cm}^{-1}$ ,<sup>25</sup> a value typical of degenerate semiconductors and only two orders of magnitude below that of conventional metals like iron. The increase is attributed to the high unidirectional polarizability of DNA, which has been theoretically estimated to be  $1.5 \times 10^{-27} \text{F m}^{-2}$ , 13 orders of magnitude greater than that of water.<sup>26</sup> It is proposed that the cause of the high unidirectional polarizability is the rapid and concerted shift of protons between primary amines of G-, C- and A-bases of neighboring pairs of the DNA stack. Even though the buildup of high concentrations of oxidants like  $\text{H}_2\text{O}_2$  and NO is usually avoided in cells, their concentrations can exceed 1 nM,<sup>27</sup> a value translating to  $> 10^9$  oxidant-molecules per copy of the genome. Thus, oxidative attack of a cell’s genome, which can be several centimeters long, is not unlikely. Some species may have evolved so that their essential chromosomal domains, including their transcribed genes, are cathodically protected against oxidative damage: When an essential element of the genome is attacked by an oxidizing agent, a less essential element of their genome is sacrificially oxidized. The sacrificially oxidized domain may have only a protective function, or it may have an essential function the transient loss of which does not lead to cellular damage within the characteristic period required for damage recognition, excision and repair.<sup>28,29</sup> For cathodic protection of the essential parts of the genome, the sacrificially oxidized domain must be (a) in electronic contact with the protected domain and (b) more reducing and more rapidly oxidized than the protected domain. The redox potentials of the four bases of DNA vs. NHE at pH 7 are G, 1.04 V; T, 1.29 V; A, 1.32 V; and C,  $1.44 \pm 0.02 \text{V}$ .<sup>30–32</sup> Not only is G the most reducing of bases, but its catalytic one-electron oxidation kinetics in poly-GC sequences is particularly rapid.<sup>33</sup> The redox potentials of the GC and AT base pairs, estimated by averaging the potentials of their constituent bases are, respectively, 1.24 and 1.31 V. There can be three types of sacrificially oxidized domains, differing in their electronic conductivity: (a) Long, highly polarizable, electronically conducting GC-rich sequences within the chromosomes, exemplified by the 1–2 kilobase (kb) long double-stranded CpG islands found at the 5'-end of genes in chromosomes of animals; (b) very short, single-stranded G-rich sequences, that are neither particularly polarizable nor conductive, but are proximal to, or terminating,

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the protected conductive double-stranded sequence. These are exemplified by the 12–20 base long G-rich telomeric overhang; and (c) organized, moderately polarizable and somewhat electronically conductive arrays of aligned G-rich single strands, exemplified by the  $K^+$ -complexes of G-tetrads, the existence of which has been verified so far only *in vitro*. In all three cases the protective process resembles that of steel by zinc, where the more reducing zinc is sacrificially oxidized while protecting the steel against corrosion, a process described by Humphrey Davy, who was assisted by Michael Faraday.<sup>34</sup> If the hypothesis of cathodic protection of essential chromosomal domains is valid, then aging and genomic instability of cells may reflect the loss of protection against oxidative damage.

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## Introduction

Bioelectrochemical research has contributed significantly in the past two decades to the alleviation of human suffering. Self-monitoring diabetics use annually more than a billion electrochemical cells<sup>35</sup> for assaying their blood glucose concentration. The volume of blood required for the glucose assays has been reduced recently to 300 nL, a volume so small that its acquisition is no longer painful.<sup>36</sup> Patient-implanted miniature glucose electrodes, continuously monitoring the glycemia in the subcutaneous interstitial fluid will be available soon.<sup>37</sup> I shall, however, not dwell on past results but will discuss instead the basis for my expectation that bioelectrochemical research will contribute to the understanding of the aging of cells, mutagenesis, principles of radiation therapy and DNA-oxidation catalyzing chemotherapeutic drugs.

This expectation is based on recent results supporting, but not yet proving, the validity of two hypotheses: *First*, that in some organisms sacrificial G-rich DNA sequences cathodically protect genes and other essential chromosomal domains against oxidative damage; and *Second*, that loss of cathodic protection is a cause of aging and death of non-proliferating cells; of increased likelihood of mutation of cells; and of the particular sensitivity to oxidative damage in some proliferating cancer cells.

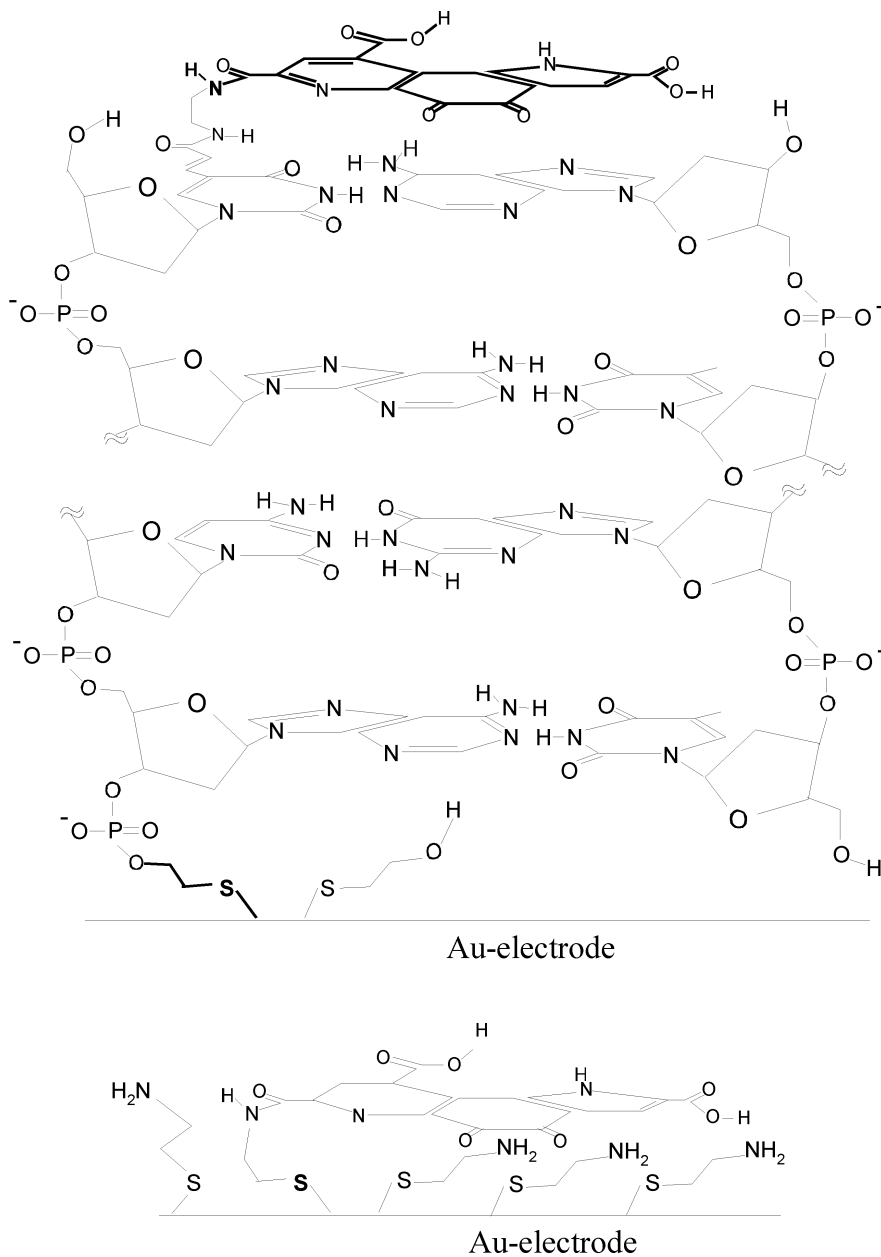
I shall not advocate the truth of these hypotheses, but will cite studies suggesting the value of their validation or refutation.

## Cathodic protection

When two electron conductors are in an oxidizing environment, are immersed in the same electrolytic solution and are in electrical contact with each other, then the nobler one is cathodically protected against oxidative damage and the less noble one is sacrificially oxidized. For example, the standard potential for the anodic oxidation of iron,  $Fe \rightarrow Fe^{2+} + 2e^-$ , is  $-0.41$  V (SHE) and for that of zinc,  $Zn \rightarrow Zn^{2+} + 2e^-$ ,  $-0.76$  V (SHE). Zinc cathodically protects steel against oxidation, being itself sacrificially oxidized ( $Fe^{2+} + Zn \rightarrow Fe + Zn^{2+}$ ). Cathodic protection of steel hulls of ships by zinc, now widely used, was introduced by Humphrey Davy, who was assisted at the time by Michael Faraday.<sup>34</sup> According to the hypothesis, some species evolved to cathodically protect their essential genomic domains, exemplified by their transcribed genes, against oxidative damage. The hypothesis can only be valid if dense and aligned chromosomal DNA conducts electrons or holes across thousands of base pairs, and if genes and other essential components of a genome are electrochemically noble relative to G-rich, less essential domains, which are sacrificially oxidized. The sacrificially oxidized protective domains may be subsequently excised and repaired.<sup>28,29</sup>

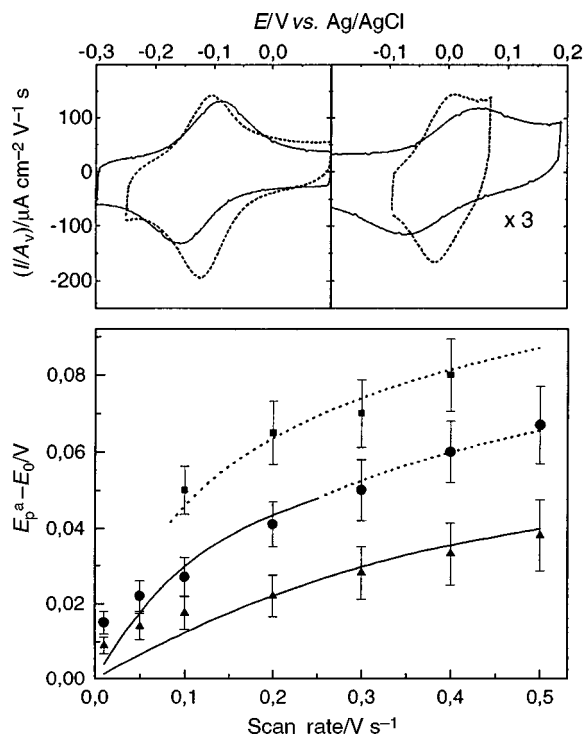
## The evidence for electronic conduction in DNA

One-dimensional electronic conductivity of DNA, of a magnitude similar to that of degenerate semiconductors and only 100 smaller than the conductivity of a metal like iron was reported by Fink and Schönberger.<sup>25</sup> They probed  $\sim 600$  nm long DNA “ropes” *in vacuo* with a tungsten tip and found that the current–voltage characteristics were simple ohmic, resembling that of a metal or a degenerate semiconductor. The resistance of a single rope was  $\sim 2.5$  M $\Omega$  and the upper



**Fig. 1** *Top*: Schematic drawing of Au-S-(CH<sub>2</sub>)<sub>2</sub>-ds-oligo-NH-PQQ, the 12-base PQQ-bound hybrid of the oligonucleotide (3'-ACGAAGGCTGAT-5') on gold. The PQQ redox function is attached to the 5'-thymine via a C-5-CH<sub>2</sub>-CH=CH-CO-NH-CH<sub>2</sub>-CH<sub>2</sub>-NH<sub>2</sub> spacer arm. The length of the unit is about 49 ± 2 Å. *Bottom*: Schematic drawing of the structure of Au-S-(CH<sub>2</sub>)<sub>2</sub>-NH-PQQ, the related thiol-monolayer coated electrode, which has no oligonucleotide. The remaining available gold surface is occupied by cystamine (from ref. 24).

limit of the resistivity was ~ 1 mΩ cm. Porath and co-workers found that individual 10.4 nm long poly-GC duplexes, or small clusters of duplexes, trapped electrostatically between a pair of Pt nano-electrodes, also conducted electrons or holes in air and *in vacuo* at both ambient and cryogenic temperatures.<sup>38</sup>



**Fig. 2** *Top*: Cyclic voltammograms, normalized for the scan rate, for the electrooxidation/reduction of PQQ/PQQ<sup>2-</sup> functions bound to the termini of monolayers on gold. Au-S-(CH<sub>2</sub>)<sub>2</sub>-NH-PQQ (left) and Au-S-(CH<sub>2</sub>)<sub>2</sub>-ds-oligo-NH-PQQ/Au-S-CH<sub>2</sub>-CH<sub>2</sub>-OH (right); scan rates (···) 10 mV s<sup>-1</sup> and (—) 500 mV s<sup>-1</sup>. *Bottom*: Dependence of the peak separation  $E_p^a - E_0$  on the scan rate for monolayers on gold: Au-S-(CH<sub>2</sub>)<sub>2</sub>-NH-PQQ (▲); 12 base pair duplex Au-S-(CH<sub>2</sub>)<sub>2</sub>-ds-oligo-NH-PQQ (●); 12 base pair duplex Au-S-(CH<sub>2</sub>)<sub>2</sub>-ds-oligo-NH-PQQ with 2 base pairs mismatched (■). Fit to the two domains of the theoretical model of Laviron<sup>43</sup> (—,···) (from ref. 24).

### The evidence for increased electronic conductivity upon alignment of the duplexes

Two studies show that the diffusivity of carriers in aggregates of DNA helices increases dramatically when the helices are aligned in parallel. Okahata and co-workers cast salmon testes DNA films on a slide, lifted these off and aligned the films by unidirectional mechanical stressing. The films were then reactively bound to interdigitated comb electrodes, spaced at micron distances. Alignment increased the steady-state DC conductivity more than one thousand fold in the direction of the applied stress, which is the direction of the long axes of the helices.<sup>23</sup> The steady-state DC current that they measured could have only resulted of electron or hole transport, because the interdigitated comb-electrodes that they used had  $\sim 5 \mu\text{m}$  spaces between the teeth of the combs and the mobile cations and anions were exhausted from the inter-electrode space by migrating to their respective electrodes within  $< 1$  min. After decline of the ionic current, only the electronic DC current could have persisted.<sup>39</sup>

The occurrence, or absence, of a faradaic reaction proves or disproves transport of electrons or holes in a film. Although passage of an electrical current through a film can result from transport of ions, of electrons, or of holes, faradaic reactions do not take place unless electrons or holes are transported. Study of the occurrence of faradaic reactions on electrodes coated with thin, solid, randomly-oriented, calf-thymus DNA films<sup>24</sup> showed that the films did not conduct electrons or holes: DNA did not connect reaction centers of soybean peroxidase to electrodes, even though this peroxidase is thermostable and particularly easy to connect ("wire").<sup>40,41</sup> The peroxidase-containing DNA films did not catalyze the electroreduction of hydrogen peroxide to water at the potential of the Ag/AgCl electrode. The diffusivity of electrons or holes in DNA was less than

$10^{-11} \text{ cm}^2 \text{ s}^{-1}$ .<sup>24</sup> Neither did thiol-terminated 50 Å thick monolayers of single-stranded DNA on gold conduct electrons or holes.  $\text{Fe}(\text{CN})_6^{3-/4-}$  was not electroreduced/oxidized on the monolayer-covered electrodes. Furthermore, when the anionic redox mediator PQQ was covalently bound to the remote side of the strand it was not electroactive. At the same time,  $\sim 50$  Å thick thiol-terminated double-stranded DNA monolayers on gold, in which the duplexes were aligned in parallel and tilted by about  $30^\circ$  vs. the normal to the surface, did conduct electrons or holes. The electrostatically excluded  $\text{Fe}(\text{CN})_6^{3-/4-}$  anion was electroreduced/oxidized on the surfaces. Similarly, PQQ covalently bound to the monolayers' solution side (Fig. 1) was also electroreduced/oxidized. The rates of electrooxidation/reduction decreased when 2 bases in the 12-base pair hybrid were mismatched.<sup>24</sup>

The rate constants for the electrooxidation/reduction of  $\text{Fe}(\text{CN})_6^{3-/4-}$ , calculated from the separation of the peaks of the cyclic voltammograms,<sup>42</sup> were nil for the single stranded 12-base DNA monolayer and  $2 \times 10^{-3} \text{ cm s}^{-1}$  for the hybrid. Similarly, the rates of electrooxidation/reduction of the covalently solution-side bound PQQ-centers of the DNA monolayers, determined by measuring the scan rate dependence of the separation of their voltammetric peaks,<sup>43</sup> were nil for the single stranded DNA film;  $1.5 \pm 0.2 \text{ s}^{-1}$  for the hybrid monolayer; and  $0.6 \pm 0.2 \text{ s}^{-1}$  for the hybrid with 2 of the 12 base pairs by substituting AT for CC (Fig. 2).<sup>24</sup>

### The electrochemical series of the DNA bases

The redox potentials of the four bases of DNA vs. NHE at pH 7 were determined by Faraggi and co-workers. They reported one-electron oxidation potentials of  $1.04 \pm 0.02 \text{ V}$  for G;  $1.29 \pm 0.02 \text{ V}$  for T;  $1.32 \pm 0.02 \text{ V}$  for A; and  $1.44 \pm 0.02 \text{ V}$  for C.<sup>30</sup> These values, particularly with respect to G being the most reducing of the four nucleotides, were confirmed by Oliveira-Brett and co-workers<sup>31</sup> and by Tomschik and co-workers.<sup>32</sup> Not only is G the most reducing base, but its catalytic one-electron oxidation kinetics in poly-GC sequences is particularly rapid.<sup>33</sup> The redox potentials of the GC and AT base pairs, estimated by averaging the potentials of their constituent bases, are, respectively, 1.24 and 1.31 V. However, according to Hutter and Clark the redox potential of the GC base pair is further downshifted relative to that of the non-hybridized G-base.<sup>44</sup>

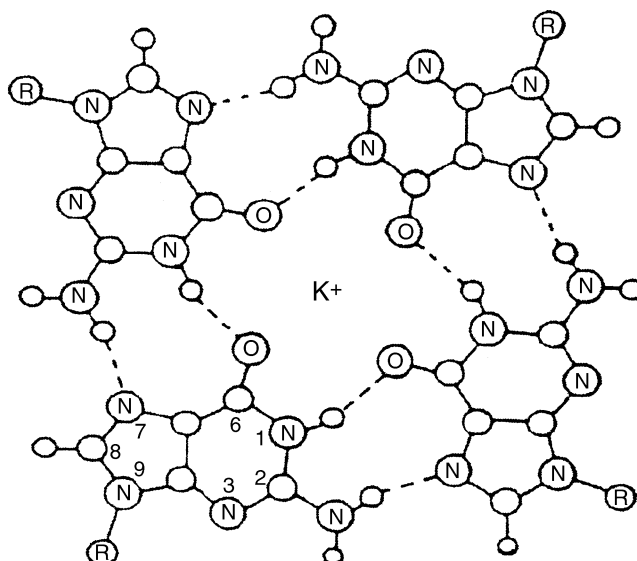
### The evidence for remote oxidation of G in poly-CG sequences

Migration of holes to and oxidation of remote poly-GC sequences of oligonucleotides has been reported by Barton and co-workers,<sup>6–10</sup> Schuster and co-workers,<sup>11–18</sup> Giese, Michel-Beyerle and co-workers,<sup>1–5</sup> Fukui and Tanaka<sup>19</sup> and Saito and co-workers.<sup>20–22</sup> Their photochemical, spectroscopic and theoretical studies establish firmly that G-bases in di- and poly-CG sequences are selectively oxidized upon oxidative attack of any position of up to  $\sim 50$  base-pair long dissolved oligonucleotides. The photochemical studies also show that oxidation of a G-base to 8-oxo-7,8-dihydro-2'-deoxyguanosine stops the long-range transport of the carrier;<sup>12</sup> that the oxidative damage can extend from the duplex to a nearby single strand;<sup>13</sup> and that GC base pairs retard the transport of carriers far less than AT base pairs.<sup>1–5</sup>

### G-rich regions of chromosomes: CpG islands and the overhang of telomeres. The hypothesis of cathodic protection of genes

Viewing the carrier-conducting gene from the perspective of a corrosion chemist, one sees a conducting rod composed of similar mole fractions of A, T, G and C in electronic contact with an end-piece that is particularly rich in G. According to the hypothesis, this end piece cathodically protects the main part of the rod against corrosion. The G-rich segment is sacrificially oxidized, just as a zinc-rich end-piece of a copper-zinc alloy protects the low-zinc part against corrosion. The sacrificially oxidized domain may have only a protective function, or it may have an essential function the transient loss of which does not lead to cellular damage within the characteristic period required for damage recognition, excision and repair.<sup>28,29</sup>

Cathodic protection of essential chromosomal domains, like genes and possibly some operon components, requires carrier (electron or hole) conduction through the thousands of base pairs of the protected domain and electrical contact between the protected domain and a domain that is



**Fig. 3** Structure of the square planar G-quartet formed by Hoogsten-pairings of guanines of the 3'-overhang of telomeres. The complexed  $K^+$  complex ions form the central axis of the quartets.<sup>45,46</sup> The  $K^+$  complexing oxygen atoms are circled.

much richer in G and is, therefore, reducing with respect to the protected one. Because of the requirement of electronic contact between the protected and the protective domains, the nature and the length of the protective domain is expected to depend on its conductivity. Protection can be provided by an insulating, but very short G-rich ( $\sim 10$ – $18$  base long,  $\sim 0.5$  mole fraction G) single strand, exemplified by the telomeric 3'-overhang located at termini of chromosomes;<sup>45,46</sup> or by a highly conductive, long double strand, of  $> 10^3$  base pair length, exemplified by the 1–2 kb long CpG islands<sup>47</sup> found at the 5'-ends of genes in animal chromosomes. These islands consist mostly, or exclusively, as their name indicates, of CG base pairs. Neither the CpG islands nor the telomeric overhangs are transcribed. Effective protection would also be provided by potassium complexes of tetrads of the telomere overhangs in which four G-rich segments are aligned (Fig. 3). Because their central  $K^+$  ions may shift concertedly, the tetrads may be polarizable and somewhat electronically conductive.

### **Biological implications of the hypothesis of cathodic protection of genes: possible relevance to the understanding of aging, mutagenesis, radiation therapy and chemotherapy**

If the concept of cathodic protection and the associated shift of the oxidative chromosomal burden to G-rich domains, like the CpG islands or the telomeric overhangs is indeed valid in some species, then the extent of protection provided by the G-rich region and the accumulated oxidative damage to the protecting G-rich domain define the likelihood of death or mutation under oxidative stress. The cellular processes of senescence, mutation, and sensitivity to damage by oxidizing agents produced by ionizing radiation, or by DNA-binding oxidation catalyzing chemotherapeutic drugs, like cisplatin and doxorubicin, will depend on the cathodic protection of the essential parts of the chromosomes. Upon oxidation of an unprotected CG base pair in a gene, its G-base is converted to 8-oxo-7,8-dihydro-2'-deoxyguanosine which, unlike G itself, does not hybridize exclusively with C, but hybridizes also with A. This results in substitution of the attacked G by T in the replicating gene. The mutation may cause death or disease.

Some carcinogens are metabolized to form DNA-binding oxidation catalysts. For example, the carcinogenic aromatic hydrocarbons are hydroxylated to DNA-binding *ortho*-diphenols which react rapidly and efficiently with molecular  $O_2$  to form quinones and  $H_2O_2$ . The reduction of the

quinones by NADH or NADPH, is rapid. Thus, the carcinogen-derived phenols/quinones catalyze the reaction  $\text{NADH} + \text{O}_2 + \text{H}^+ \rightarrow \text{NAD}^+ + \text{H}_2\text{O}_2$ . Reaction of  $\text{H}_2\text{O}_2$  with a reduced transition metal ion produces  $\cdot\text{OH}$  radicals in the well known Fenton reaction.

The molecular biology of G-rich domains has been subject to extensive studies. For example, the hairpin G-quartet formed by  $(\text{TTAGGG})_n$  at the 3' telomere overhang is specifically recognized and stabilized by the human repair protein, Ku70/Ku80 hetero-dimer, which is important to the stability of the telomere,<sup>48</sup> which, in turn, controls aging and the likelihood of mutation. Oxidative scission of the G-rich domain or its insulation from the proximal RNA-transcribed gene (by oxidation of the 5'-G of a poly-G sequence) is expected to lead to loss of cathodic protection of the gene and increase thereby the likelihood of mutation or inactivation under oxidative stress.

Until now, the assumed basis for radiation therapy has been the greater susceptibility of rapidly dividing cells to damage by oxidizing radicals. In the framework of the present hypothesis, some cells mutate because of poor cathodic protection of one of their genes. The mutated but poorly cathodically protected gene of the proliferating cells is expected to be particularly sensitive to oxidants produced by ionizing radiation in water or reactive oxidants produced in reactions catalyzed by DNA-binding chemical oxidation catalysts, like cisplatin or doxorubicin. The more likely it is that a gene will mutate under oxidative stress, the greater will be its sensitivity to ionizing radiation and to oxidation catalysts increasing the susceptibility of the mutated cells more to oxidizing species produced by ionizing radiation or through the chemotherapeutic drug-catalyzed reaction.

### DNA alignment in living organisms: biocrystallization

If alignment of the DNA does increase its conductivity and thereby its cathodic protection, one would expect alignment of the DNA, while not transcribed, in the organisms that evolved with cathodic protection. Structural analyses by X-ray diffraction, circular dichroism and electron and polarizing microscopies show that the DNA is liquid crystalline in the chromatin of sperm nuclei of mammals, fish and arachnids, of bacteria and of protozoa. The duplexes are aligned in parallel in the observed helical pre-cholesteric, cholesteric, smectic and columnar hexagonal liquid crystalline phases.<sup>49,50</sup> Upon reaching its weight fraction in the chromatin of nuclei, DNA spontaneously aggregates also *in vitro* to form liquid crystals, in which the duplexes are aligned in parallel.

Do some organisms enhance the conductivity of their chromosomal DNA and thereby its cathodic protection through aligning their DNA in parallel at times of stress? Stress-induced crystallization of the DNA of *E. Coli* was reported by Minsky and co-workers.<sup>51</sup> The crystallization protects the DNA against strong oxidants, such as  $\cdot\text{OH}$  radicals. Under oxidative or nutritional stress *E. coli* rapidly expresses a non-specific DNA-binding protein, *Dps*, which causes the crystallization of DNA. *Dps* and DNA form two-dimensionally ordered hexagonal arrays, the ordered planes separated by  $78 \pm 1 \text{ \AA}$ . Layers of pure crystalline DNA are interleaved between layers of *Dps* in the arrays.

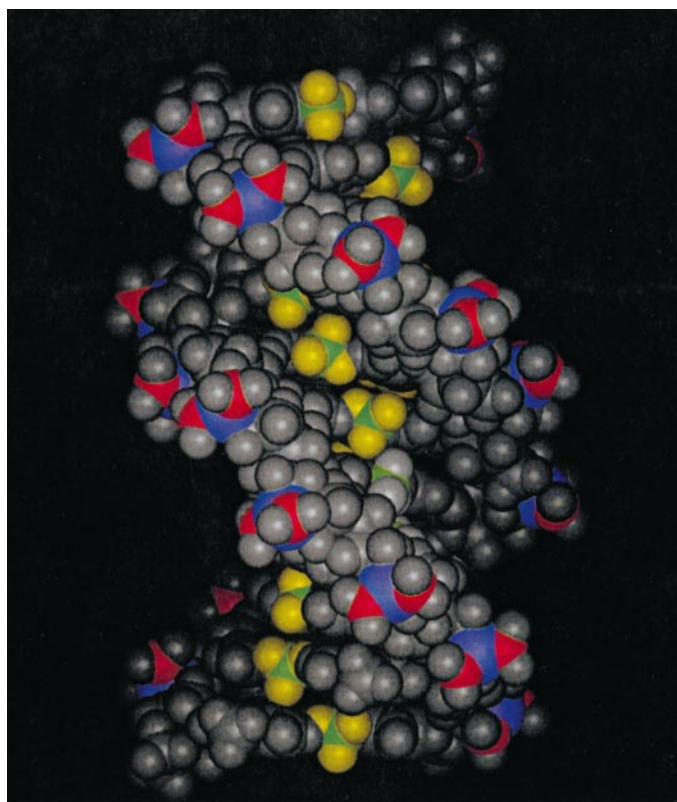
### Cause of the increase in the electronic conductivity upon alignment

Metals and intrinsic small band-gap semiconductors are black. DNA is colorless. The only known colorless conductors are highly doped large band-gap semiconductors. Among these,  $\text{TiO}_2$  doped with hydrogen, with oxygen vacancies or with  $\text{Ti}^{3+}$  is conductive. Other carrier-conducting large band-gap semiconductors include doped  $\text{SrTiO}_3$ ;  $\text{SnO}_2$  doped with indium or antimony; and  $\text{In}_2\text{O}_3$  doped with tin. These colorless solids are particularly well known to electrochemists, who use them as photoanodes in photoelectrochemical cells and as transparent electrodes in spectroelectrochemical cells. The band gaps of all four semiconductors exceed 3 eV. They conduct because their dielectric constants are high.

Classical solid state physics teaches that Bohr radii of donors or acceptors increase linearly with the high-frequency dielectric constant (eqn. (1)).<sup>52</sup> The radii,  $r_0$ , scale with the dielectric constant according to

$$r_0 = \epsilon \hbar^2 / (e^2 m_e) \quad (1)$$

where  $\epsilon$  is the dielectric constant,  $\hbar$  is Heisenberg's constant,  $e$  is the charge of the electron, and  $m_e$



**Fig. 4** Ball model showing the possible channels in which protons may shift concertedly in an electric field paralleling the main axis. The primary amines of C, G and A are shown as protonated  $sp^3$  hybrids. Colors: Phosphorus—violet; anionic and partially anionic phosphorus-bound oxygen—red; primary amine—ammonium nitrogen—green; primary amine—ammonium nitrogen-bound hydrogen/proton—yellow. Courtesy of Dr Jonathan Heller, Exelixis Pharmaceuticals, South San Francisco.

is the effective electron mass. The Bohr radii are typically of 30–100 Å when the dielectric constants are between 10 and 20. The ionization energies of donors scale with the inverse of the square of the dielectric constant,<sup>52</sup> according to eqn. (2):

$$E_g - E_d = e^4 m_e / (2\epsilon^2 \hbar^2) \quad (2)$$

When the high frequency dielectric constant is in the 10–20 range, the ionization energies shrink to tens of meV, on the order of  $kT$  at ambient temperature. Thus the donors are ionized.<sup>52</sup>

The polarization ( $\mathbf{P}$ ) and the dielectric constant  $\epsilon$  are related through  $\mathbf{P} = \mathbf{E}(\epsilon - \epsilon_0)$ , where  $\mathbf{E}$  is the electric field strength and  $\epsilon_0$  is the value of  $\epsilon$  *in vacuo*. The dielectric constant of DNA is unusually high. At 100 kHz its value was estimated to be 86.<sup>53</sup> Even though the dielectric constant of water is also high (78.4 at low frequencies at 298 K) *water differs profoundly from DNA in its high frequency polarizability and dielectric constant*. The  $7.4 \times 10^5$  D<sup>†</sup> theoretically estimated mean longitudinal thermal fluctuating dipole moment of DNA at 298 K is  $4 \times 10^5$  times greater than the 1.84 D permanent dipole moment of water; and its estimated static longitudinal polarizability is  $1.5 \times 10^{-27}$  F m<sup>-2</sup>,  $9 \times 10^{12}$  times greater than that of water.<sup>26</sup>

Any of the bases can be ionized in the highly polarizable medium of aligned DNA. When the bases are not ionized, their orbitals in DNA resemble long sausages. When ionized, the electrons they donate are unidirectionally mobile along the major axis of the duplex.

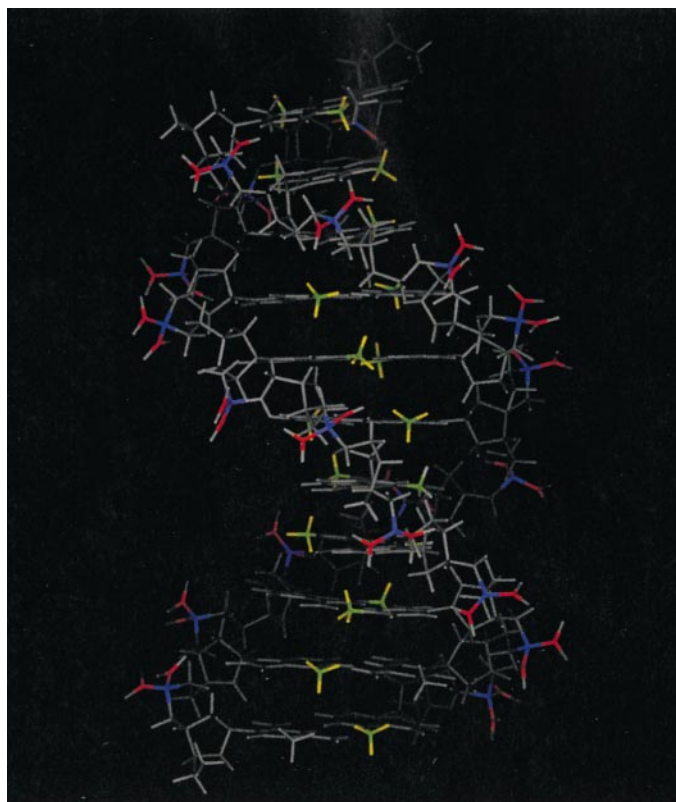
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<sup>†</sup> 1 D (debye)  $\approx 3.33564 \times 10^{-30}$  C m.

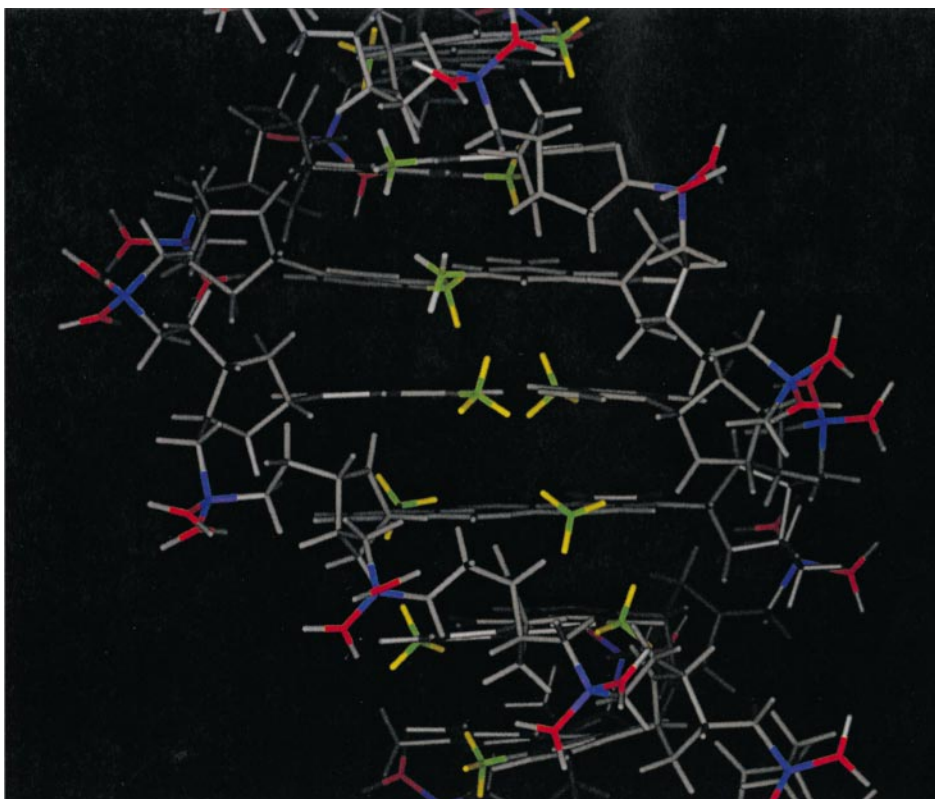
## The cause of the high polarizability of DNA: proton transport

An array of parallel rod-like DNA duplexes is polarized by protonation of one terminus and ionization of the opposite terminus. Concerted shift of protons (or alkali metal cations) between neighboring base-pairs can thus lead to polarization. DNA consists of multiple ion pairs; the phosphates are partially ionized and the primary amines are protonated. Figs. 4–6 show the existence of two channels through which protons can concertedly move. The phosphate channel spirals on the outside of the DNA rods. The amine channel, at the central axis of the rods, is formed of the primary amines of G, C and A. The proton-transfer distances depend on the specific base pair sequences. The N–N distances between the primary amines of neighboring base pairs are 4.2–5.5 Å. They are shorter for GC sequences than for AT sequences, because in GC both of the bases have primary amines, while in AT only A has an amine. The O–O distances of neighboring phosphates are longer, 5.6–7.6 Å. Because the protons shift concertedly, the polarization times are short. If one assumes that the diffusivity of protons,  $D$ , along the main axis is similar to that in water, about  $10^{-4} \text{ cm}^2 \text{ s}^{-1}$ , then for the  $l \approx 4.2\text{--}5.5 \text{ Å}$  amine spacings the inter-amine diffusion time,  $\tau \approx l^2/D$ , is about  $2 \times 10^{-11} \text{ s}$ .

The GC base pairs differ from AT base pairs in having twice as many proton-transferring amines, and therefore shorter average distances between the amines constituting the polarizable channel. Furthermore, ionized non-terminal GC base pairs do not carry a net charge, the charges of the phosphate anions being balanced by those of the ammonium ions. In contrast, the AT base



**Fig. 5** Stick model showing the possible channels in which protons may shift concertedly in an electric field paralleling the main axis. The primary amines of C, G and A are shown as protonated  $sp^3$  hybrids. Colors: Phosphorus—violet; anionic and partially anionic phosphorus-bound oxygen—red; primary amine-ammonium nitrogen—green; primary amine-ammonium nitrogen-bound hydrogen/proton—yellow. Courtesy of Dr Jonathan Heller, Exelixis Pharmaceuticals, South San Francisco.



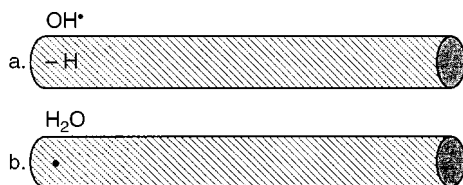
**Fig. 6** Magnified portion of the stick model showing the possible channels in which protons may shift concertedly in an electric field paralleling the main axis. The primary amines of C, G and A are shown as protonated  $sp^3$  hybrids. Colors: *Phosphorus*—violet; anionic and partially anionic phosphorus-bound *oxygen*—red; primary amine—ammonium *nitrogen*—green; primary amine—ammonium nitrogen-bound *hydrogen/proton*—yellow. Courtesy of Dr Jonathan Heller, Exelixis Pharmaceuticals, South San Francisco.

pairs are negatively charged because the ratio of primary amine to phosphate-functions is 1 : 2. The negative charge and the lesser polarizability of the AT pairs reduces the mobility of holes.

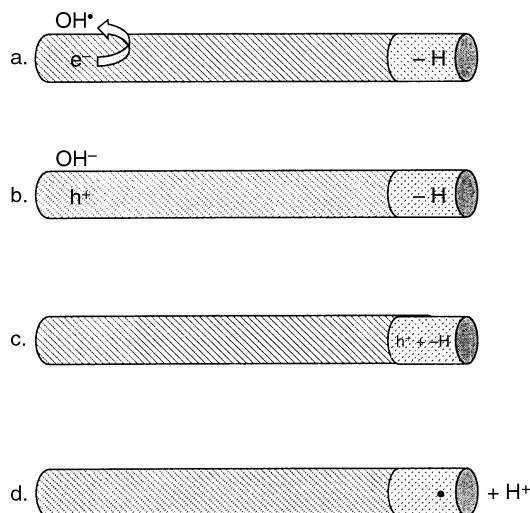
High unidirectional high frequency polarizability, dielectric constant and conductivity are unique to solid DNA arrays in which the molecules are aligned in parallel. The amine or phosphate channels that enhance the polarizability and thus the transfer of electrons are found however in all DNA hybrids. Transfer of electrons across  $\sim 50$  base pairs, for which the transfer-distance substantially exceeds the  $< 2$  nm maximal electron transfer distance in proteins, occurs in dissolved DNA.<sup>1–22</sup> These distances are much shorter than the massively larger electron transfer distances required for the cathodic protection of the multi kb long genes.

### **Maintenance of the intact genetic information in an oxidizing environment**

The fidelity of the chromosomal information is maintained by excision and replacement of damaged segments.<sup>28,29</sup> The genes are exposed, however, to oxidizers such as nitric oxide, singlet oxygen, hydrogen peroxide and  $\cdot\text{OH}$  radicals. The transient  $\sim 1$  nM concentration of nitric oxide is similar to that of other hormones; there are transiently  $\sim 10^6$  nitric oxide molecules in a cell, any of which may react with the single copy of a particular chromosome or gene. Although catalases abound in tissues, some of the continuously generated hydrogen peroxide will react with oxidizable transition metal ions, such as  $\text{Fe}^{2+}$  or  $\text{Cu}^+$ , to produce  $\cdot\text{OH}$  radicals. Cathodic protection may reduce the likelihood that these and other oxidants will alter a gene.



**Fig. 7** When the chromosome does not conduct electrons or holes, the site of the reaction of the oxidizing agent is the site approached by the agent. This is exemplified in the figure by an  $\text{OH}^\bullet$  radical abstracting a hydrogen atom and producing a reactive localized radical.



**Fig. 8** Cathodic protection of a gene through the sacrificial oxidation of a CpG island or of a telomere. The gene (lightly shaded) is attacked by an  $\text{OH}^\bullet$  radical (a); the radical captures an electron from the gene and is reduced to an  $\text{OH}^-$  anion; the capture of the electron leaves a mobile electron vacancy (hole,  $h^+$ ) in the gene (b); the hole diffuses to and is captured by the more reducing (lighter-shaded) CpG island or telomere (c), where it reacts by releasing a proton and forming a radical (d).

In an insulator, the attack by an oxidizer results in a local chemical change at the site of the attack (Fig. 7). In a conductor the reaction occurs at a remote reducing site to which the injected hole diffuses and in which it is trapped (Fig. 8). Thus, there is a fundamental change in the oxidation of a gene if it undergoes an insulator-to-semiconductor transition. *When in its insulating state, the site approached by the oxidizing agent is the site oxidized. When in its conducting state, the site from which the electron is captured or into which the hole is injected is not the reaction site.* The hole now diffuses to, and reacts at, a remote, more reducing G-rich domain, where it forms a remote radical, often by releasing a proton (Fig. 8).

### Dependence of the range of cathodic protection on the diffusivity of holes

The actual reaction site of the injected hole depends on the unidirectional diffusivity, which scales with the hole mobility and the conductivity. It also depends on the half-life of the hole. The higher the diffusivity and the half-life of the hole, the greater the protected chromosomal domain. The diffusivity  $D$  and the mobility  $\mu$  of carriers are related through the equation  $D = (kT/e)\mu$ ,  $e$  being the charge of the electron. The conductivity is the product of the diffusivity and the density of carriers. Fink and Schöenberger measured the longitudinal electron or hole conduction in solid DNA. They probed  $\sim 600$  nm long DNA “ropes” *in vacuo* with a tungsten tip. The  $i$ - $V$  characteristics were simple ohmic, resembling those of a metal or of a degenerate semiconductor. The resistance of a single rope was  $\sim 2.5$  M $\Omega$ , and the measured conductivity of DNA exceeded  $10^3$   $\Omega^{-1} \text{cm}^{-1}$ .<sup>25</sup>

## Conclusion

In solid arrays of duplexes, protons can concertedly and rapidly (in less than  $10^{-10}$  s) shift between the neighboring base pairs. As a result, their unidirectional bulk polarizability and dielectric constant are high. When the dielectric constant is high, the ionization energy of donor G-bases in DNA, viewed as a large band gap unidirectional semiconductor, approaches  $kT$  and the aligned DNA array behaves as a doped one-dimensional semiconductor at room temperature.

If genes conduct electrons or holes and are in electrical contact with reducing G-rich domains, exemplified by neighboring 1–2 kb CpG islands, where the mole fraction of G is  $\sim 0.5$  about twice that in the gene, they may be cathodically protected against oxidation. At the same time, the oxidative burden is also shifted to domains such as the telomeric overhang, that are of essence for the reproduction and repair processes of dividing cells. Their damage may contribute or lead to accelerated senescence and may increase both the likelihood of mutation and the sensitivity of the mutated cells to oxidants produced by ionizing radiation. Bioelectrochemical research can provide answers to the questions whether cathodic protection contributes to the maintenance of intact genes and whether sacrificial oxidation of G-rich domains does indeed lead to long-term damage.

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