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REVIEW ARTICLE

Mechanisms of free radical-induced damage to DNA

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Abstract

Endogenous and exogenous sources cause free radical-induced DNA damage in living organisms by a variety of mechanisms. The highly reactive hydroxyl radical reacts with the heterocyclic DNA bases and the sugar moiety near or at diffusion-controlled rates. Hydrated electron and H atom also add to the heterocyclic bases. These reactions lead to adduct radicals, further reactions of which yield numerous products. These include DNA base and sugar products, single- and double-strand breaks, 8,5′-cyclopurine-2′-deoxynucleosides, tandem lesions, clustered sites and DNA-protein cross-links. Reaction conditions and the presence or absence of oxygen profoundly affect the types and yields of the products. There is mounting evidence for an important role of free radical-induced DNA damage in the etiology of numerous diseases including cancer. Further understanding of mechanisms of free radical-induced DNA damage, and cellular repair and biological consequences of DNA damage products will be of outmost importance for disease prevention and treatment.

Keywords: *free radicals, hydroxyl radical, hydrated electron, hydrogen atom, mechanisms of product formation, DNA base damage, DNA sugar damage, tandem lesions, clustered lesions, DNA-protein cross-links*

Abbreviations: *$^{\bullet}$OH, hydroxyl radical; $O_2^{\bullet-}$, superoxide radical; e_{aq}^-, hydrated electron; H$^{\bullet}$, H atom; k, reaction rate constant; Gua(–H)$^{\bullet}$, neutral guanine radical; Gua$^{\bullet+}$, guanine radical cation; 8-OH-Gua, 8-hydroxyguanine; Fapy Gua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; 2,5-FapyGua, 2,5-diamino-4-hydroxy-6-formamidopyrimidine; Guo, guanosine; 8-OH-dG, 8-hydroxy-2′-deoxyguanosine; 8-OH-Gua$^{\bullet+}$, radical cation of 8-OH-Gua; ESCODD, European Standards Committee on Oxidative DNA Damage; 2-OH-Ade, 2-hydroxyadenine; Ade(–H)$^{\bullet}$, neutral adenine radical; Ade$^{\bullet+}$, adenine radical cation; 8-OH-Ade, 8-hydroxyadenine; FapyAde, 4,6-diamino-5-formamidopyrimidine; dialuric acid, 5-hydroxy-2,4,6(1H,3H,5H)-pyrimidinetrione; alloxan, 2,4,5,6(1H,3H)-pyrimidinetetrone; R-cdG and S-cdG, R- and S-diastereomers of 8,5′-cyclopurine-2′-deoxyguanosine; R-cdA and S-cdA, R- and S-diastereomers of 8,5′-cyclopurine-2′-deoxyadenosine; 8-OH-Gua/Fo; 8-OH-Gua/formamido residue; Fo/8-OH-Gua; formamido residue/8-OH-Gua; Gua [8,5-Me]Thy and Thy[5-Me,8]Gua, intrastrand cross-link between the C8 of Gua and the CH_2 group of thymine; Ade [8,5-Me]Thy and Thy[5-Me,8]Ade, intrastrand cross-link between the C8 of adenine and the CH_2 group of thymine; Gua[8,5]Cyt, intrastrand cross-link between the C8 of Gua and the C5 of cytosine; Gua[8,5-Me]MeCyt, intrastrand cross-link between the C8 of Gua and the CH_2 group of 5-methylcytosine; Ade[6N,5-Me]Thy, interstrand cross-link between the 6NH of adenine on one strand and the CH_2 group of thymine on the opposing strand of DNA; DSBs, double-strand breaks; Thy-Tyr cross-link, 3-[(1,3-dihydro-2,4-dioxopyrimidin-5-yl)-methyl]-L-tyrosine.*

Introduction

Free radicals are continuously formed in aerobic living organisms by normal intracellular metabolism and by exogenous sources such as ionizing radiations, UV radiation, redox-cycling drugs, carcinogenic compounds, environmental pollutants, etc. [1]. Oxygen metabolism generates hydroxyl radical ($^{\bullet}$OH), superoxide radical ($O_2^{\bullet-}$) and non-radical H_2O_2. Hydroxyl

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radical is highly reactive and reacts with biological molecules such as DNA, proteins, lipids, etc. near or at diffusion-controlled rates, causing chemical modifications. Superoxide radical and H_2O_2 do not react with most biological molecules. No reaction occurs between these two species, either, at a considerable reaction rate, which is close to zero [1]. Transition metal ions such as iron and copper ions catalyse the reaction between $O_2^{\bullet -}$ and H_2O_2, generating $^{\bullet}OH$ (Haber-Weiss reaction) [1]. The interaction of ionizing radiations with cellular water produces $^{\bullet}OH$, $O_2^{\bullet -}$, H_2O_2, hydrated electron (e_{aq}^-) and H atom ($H^{\bullet}$), which is also a free radical [2]. Hydroxyl radical reacts with the constituents of DNA near or at diffusion-controlled rates, causing damage to the heterocyclic DNA bases and the sugar moiety by a variety of mechanisms. Addition of e_{aq}^- and $H^{\bullet}$ to double bonds of DNA bases also occur, causing damage [2]. In living organisms, DNA damage is repaired by a variety of mechanisms. If free radical damage to DNA is not repaired, it may lead to genetic instability, thus to disease processes such as carcinogenesis [3–7]. This article reviews the mechanisms of free radical-induced damage to DNA.

Mechanisms of DNA base damage

Guanine

Among the DNA bases, Gua has the lowest reduction potential (1.29 V). Therefore, it is the best electron donor and is preferentially oxidized [2,8–10]. Hydroxyl radical reacts with Gua at a diffusion-controlled rate with a rate constant (k) of 8×10^9 dm^3 mol^{-1} s^{-1} (measured using Guo at pH 7) [11]. A much later work reported a rate constant of ~5×10^9 dm^3 mol^{-1} s^{-1} for Guo and dG [12,13]. Hydroxyl radical adds to the C4-, C5- and C8-positions, and also to the C2-position of Gua to a much lesser extent [2,9,14] (Figure 1). An $H^{\bullet}$ abstraction by $^{\bullet}OH$ from the NH_2 group attached to C2 (2-NH_2 group) has also been reported [12,13,15,16] (Figure 1). Due to the electrophilic nature of $^{\bullet}OH$, additions preferentially occur at sites with high electron density. Addition reactions generate OH–adduct radicals (Figure 1), which possess different redox properties and are either reducing or oxidizing with the yields of both types being almost equal [9,14]. Thus, the C4-OH–adduct radical is oxidizing, whereas the C5-OH–and the C8-OH–adduct radicals are predominantly reducing. However, these radicals can also exist in different mesomeric forms that may be oxidizing or reducing representing a "redox ambivalence" [9]. The C4-OH–adduct radical and the C8-OH–adduct radical are formed with yields of 65–70% and 17% (relative to $^{\bullet}OH$), respectively [17]. The yield of the C5-OH–adduct radical appears to be less than 10%. Upon formation of the C4-OH– and C5-OH–adduct radicals, substantial conformational changes occur in the molecules [18]. The C4-OH–adduct radical eliminates water ($k = 6 \times 10^3$ s^{-1} at pH 7), generating a neutral Gua radical [Gua(−H)$^{\bullet}$], which subsequently protonates to give rise to the Gua radical cation (Gua$^{\bullet +}$), as shown in Figure 2 [9,17]. The C5-OH–adduct radical is also likely to undergo water elimination to yield Gua(−H)$^{\bullet}$, which would result in redox inversion [9] (Figure 2). The C2-OH–adduct radical may eliminate ammonia, the amount of which indicates that the yield of this radical must be no more than 1.5% [2]. The oxidation of this radical may result in the formation of xanthine.

In contrast to the findings by O'Neil, Steenken et al., a recent work reported that the main reaction of $^{\bullet}OH$ with Gua is not the addition to C4, but an $H^{\bullet}$ abstraction from the 2-NH_2 group of Gua to an extent of ~65%, as shown in Figure 1 [12,13]. According to this work, the thus-formed 2-N-centred radical (aminyl radical) subsequently undergoes tautomerization ($k = 2.3 \times 10^4$ s^{-1}) to yield a neutral Gua radical [Gua(–H)$^{\bullet}$] (Figure 2). This is the same radical that results from the water elimination of the C4-OH–adduct radical ($k = 6 \times 10^3$ s^{-1}), as Steenken et al. had reported more than two decades earlier [9,17] (Figure 2). Apparently, the end result is the same, whether $^{\bullet}OH$ adds to C4 or abstracts an $H^{\bullet}$ from the 2-NH_2 group. However, the proposed large extent of the $H^{\bullet}$ abstraction almost completely eliminates the addition of $^{\bullet}OH$ to C4 despite the well-known high electron affinity in purines [19], making the $^{\bullet}OH$ addition an energetically favoured reaction. The $H^{\bullet}$ abstraction from the 2-NH_2 group becomes the major reaction by the complete exclusion of the $^{\bullet}OH$ addition to C4, when one takes into account the $^{\bullet}OH$ addition to C8 that occurs to an extent of only 17% [9,12,13,17]. The reaction of $^{\bullet}OH$ with aromatic amines does not completely support this notion. In aniline for example, the $H^{\bullet}$ abstraction by $^{\bullet}OH$ takes place to a large extent (36%); nevertheless, the $^{\bullet}OH$ addition to double bonds is still the predominant pathway (64%) [20]. The rate constant for $^{\bullet}OH$ addition to the *ortho*-position of aniline is approximately 50% greater than that for $H^{\bullet}$ abstraction from the NH_2 group [20]. A Car-Parrinello molecular dynamics study of $^{\bullet}OH$ reactions with Gua found that the $H^{\bullet}$ abstraction from the 2-NH_2 group is an energetically favoured reaction in the gas phase; however, in the aqueous phase, this reaction is less favoured than the $H^{\bullet}$ abstraction from N9 and N2 [15,16]. Moreover, the same study showed that spontaneous hydroxylation at C8 and C4 occurs in accordance with experimental findings. A recent extensive review also stated that the $H^{\bullet}$ abstraction does not occur to any significant extent [2].

Just recently, Phadatare et al. reported spectral characterization of the C4-OH–adduct radical using quantum chemical calculations, pulse radiolysis and product analysis [21]. Their data contrasted the large extent of $H^{\bullet}$ abstraction by $^{\bullet}OH$ from the 2-NH_2 of

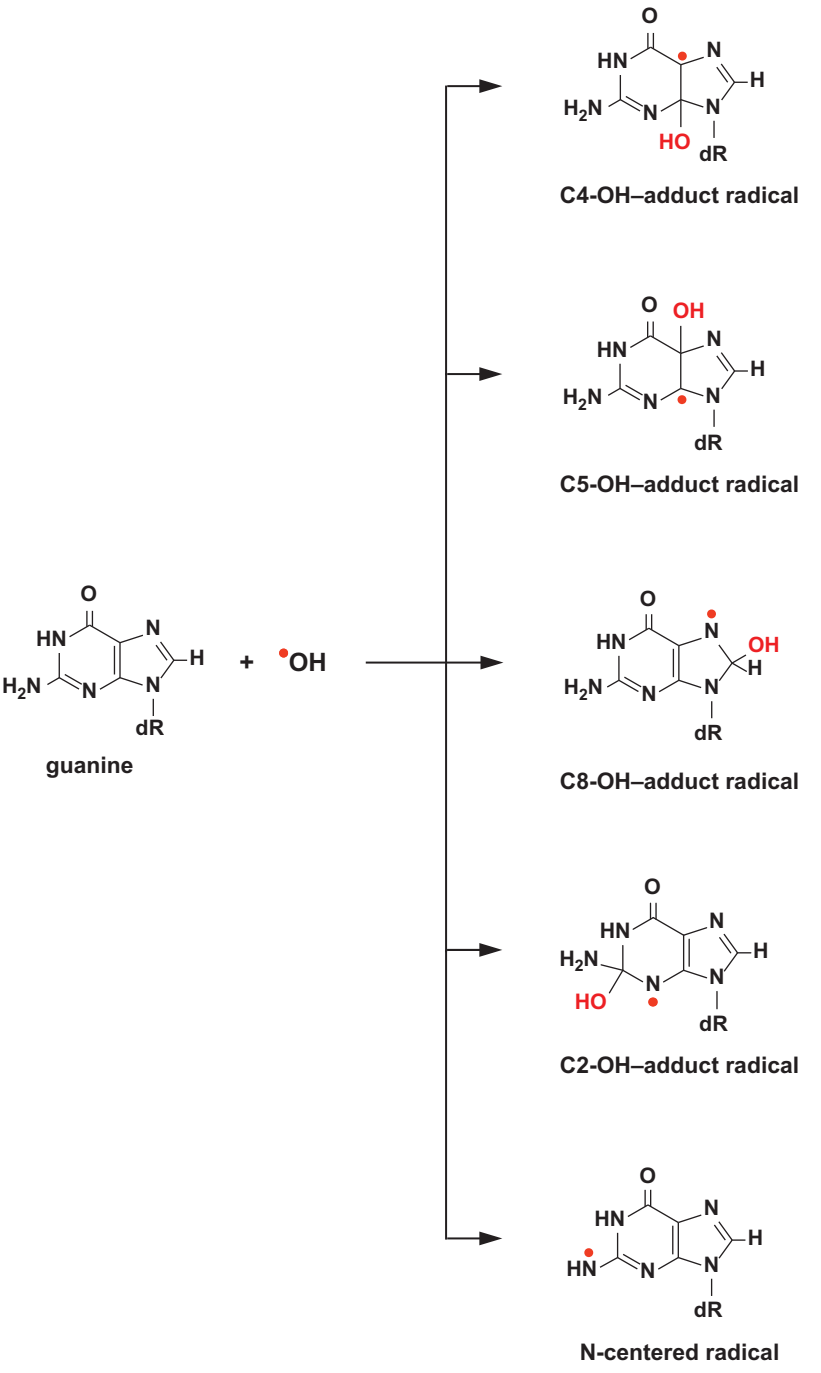

Figure 1. Reactions of •OH with Gua. dR denotes 2′-deoxyribose here and in all other relevant figure legends. (Adapted from [9, 14]).

Gua as reported by Chatgilialoglu et al. [12, 13], and showed that the addition of •OH to C4 is the preferred reaction pathway. Moreover, this work concluded that the H• abstraction from N1 and N9 of Gua were even energetically more favorable than that from 2-NH_2, in agreement with the findings by Mundy, Wu et al. [15, 16], but in contrast to the claim by Chatgilialoglu et al. [12, 13]. Furthermore, the diffusion-controlled reaction of •OH with Gua is a testament to addition reactions when compared to H• abstraction reactions, the rates of which are expected to be lower as in the case of aniline (see above). In the same context, one should point out that the N1-centered radical is a mesomeric form of 6-O–centered Gua(–H)•; however, the aminyl radical must undergo tautomerization to yield this radical (Figure 2) [21].

In H• abstraction reactions by •OH, one should also take into account the bond dissociation energies (bond enthalpies) of N–H and O–H bonds. The bond enthalpy of an N–H bond in the 2-NH_2 group should amount to ~452 kJ mol^{-1}, which is close to the bond enthalpy of the O–H bond in water (498 kJ mol^{-1}) [22]. Therefore, it is quite unlikely that •OH would

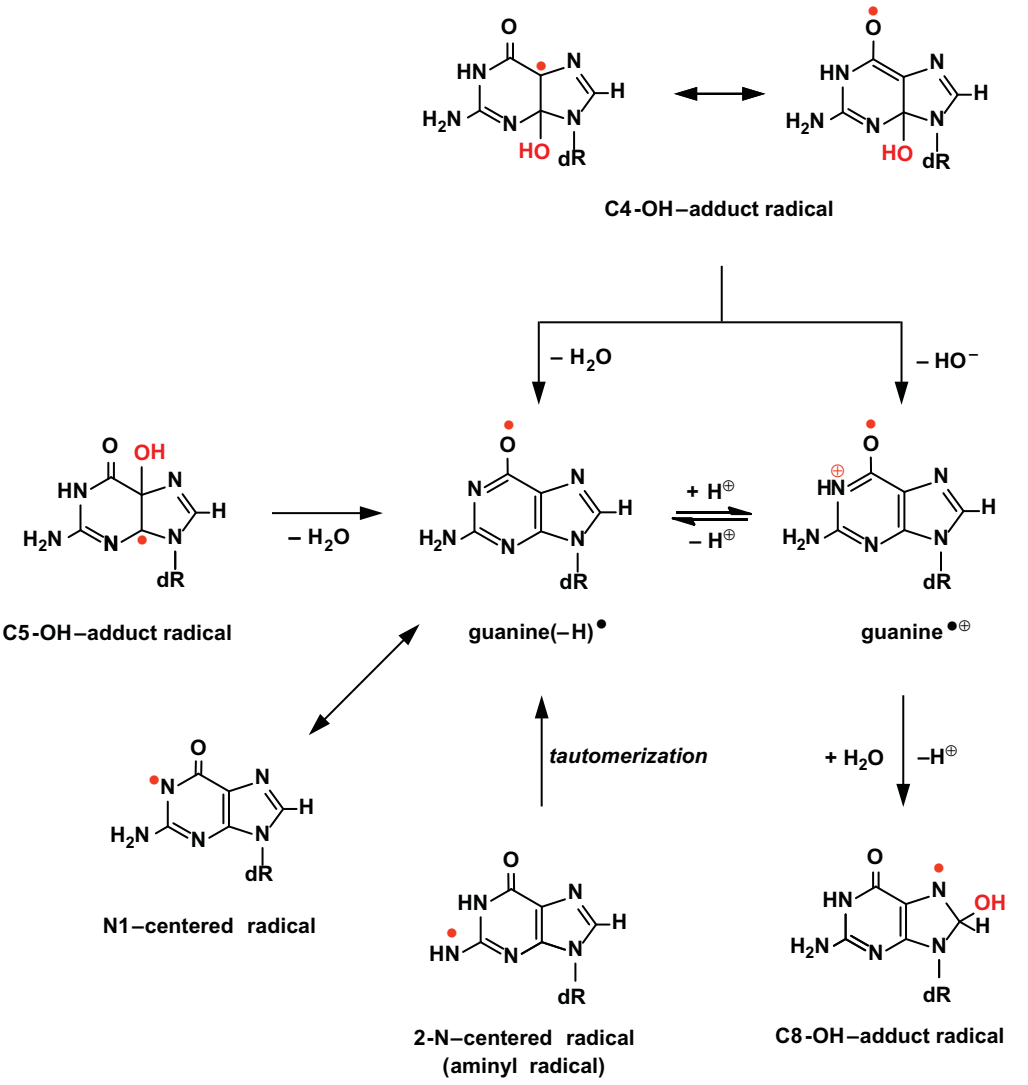

Figure 2. Reactions of C4-OH– and C5-OH–adduct radicals and N1-centred radical of Gua.

readily abstract an H• from the 2-NH_2 group rather than adding to C4 with the highest electron density in the molecule. This situation is similar to that in Thy, where •OH mainly adds to C5 with the highest electron density (60%) and to C6 (30%), and abstracts an H• from the CH_3 group (10%) (see below for more details). A comparative quantum chemical and Car-Parrinello molecular dynamics study supported these findings [16]. The bond enthalpy of a C–H bond in the CH_3 group amounts to ~460 kJ mol^{-1} [22], which is almost equal to that of the N–H bond in the 2-NH_2 group, and slightly less than the bond enthalpy of the O–H bond in water (498 kJ mol^{-1}). Thus, the H• abstraction from the CH_3 group should be energetically less favourable than •OH addition to the C5 = C6 double bond of Thy. Experimental results and final products unequivocally support this notion (see below). For the reasons outlined above, the so-called revised mechanism of the reaction of •OH with Gua [12,13] should be taken into consideration with caution, perhaps until the H• abstraction by •OH from the 2-NH_2 group with the complete exclusion of the •OH addition to C4 is confirmed by other laboratories using different techniques. In the same context, it should be pointed out that the same authors, in an earlier paper, described the •OH addition to C4 of Gua as the main reaction [23]. Thus far, available evidence suggests that the H• abstraction from the 2-NH_2 group of Gua is not the predominant reaction and that the •OH addition to C4 cannot be entirely excluded from •OH reactions with Gua. At best, both reactions may occur simultaneously, as the present data on other molecules with C = C double bonds, and 2-NH_2 and CH_3 groups suggest, albeit perhaps to different extents, leading to Gua(–H)• (Figure 2).

Gua(–H)• and $Gua^{\bullet+}$ are strong oxidants with a reduction potential of 1.29 V [10,17]. Gua(–H)• may be reduced reconstituting Gua, whereas the hydration of $Gua^{\bullet+}$ (addition of HO^-) may take place to generate the 8-OH–adduct radical as previously proposed

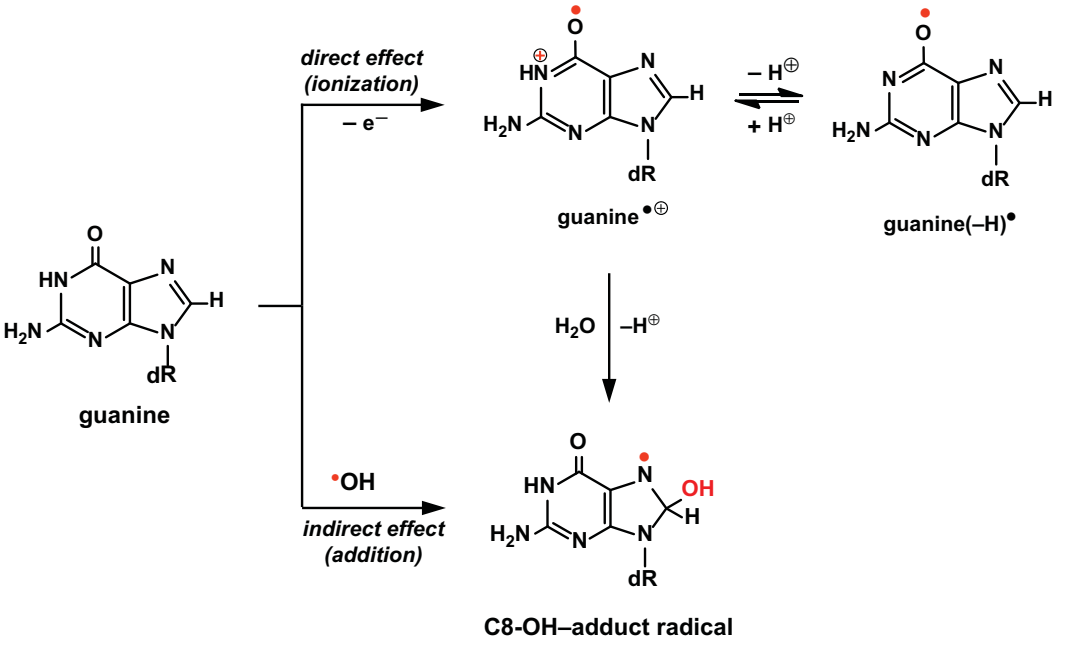

Figure 3. Direct and indirect effects of ionizing radiation on Gua.

[24–29] (Figure 2). Faster hydration of $Gua^{\bullet+}$ in ds-DNA than in monomeric $Gua^{\bullet+}$ has been suggested [17]. According to the density functional theory calculations, the addition of H_2O on the C8-site of $Gua^{\bullet+}$ is exothermic by –315.2 kJ mol^{-1}, whereas the energy of this reaction for $Gua(-H)^{\bullet}$ amounts to + 123.1 kJ mol^{-1}, which is endothermic [30]. The presence of the proton on the N1-site of $Gua^{\bullet+}$ appears to be crucial for H_2O addition. The positive charge density is higher on the C8 of $Gua^{\bullet+}$ than that on the C8 of $Gua(-H)^{\bullet}$; therefore, the nucleophilic attack of H_2O on the former is likely to have a lower activation energy than the attack on the latter. $Gua^{\bullet+}$ is also formed when ionization of Gua in DNA occurs, for example by direct effect of ionizing radiation (Figure 3). The positive charge generated by this ionization is able to migrate in DNA over a long distance until it is trapped probably at Gua [31–33]. Since $Gua^{\bullet+}$ can generate the 8-OH–adduct radical upon H_2O addition as discussed above, the direct effect and indirect effect of ionizing radiation may lead to the same products of Gua [34,35]. Furthermore, UV-radiation, photosensitization and singlet oxygen can generate $Gua^{\bullet+}$ (reviewed in [2]). The formation of 8-OH-Gua and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) in DNA by UV-irradiation and by photosensitization with visible light plus methylene blue or riboflavin supports this mechanism [25–27,36–38]. In contrast to $Gua^{\bullet+}$, $Gua(-H)^{\bullet}$ does not give rise to 8-OH-Gua; however, it is likely to react with 2′-deoxyribose in DNA by an $H^{\bullet}$ abstraction with an estimated $k \leq 4 \times 10^3$ dm^3 mol^{-1} s^{-1} (measured using ribose) [17]. This $H^{\bullet}$ abstraction can generate C-centred radicals of 2′-deoxyribose such as the C4′-radical, which is known give rise to strand breaks and formation of 2′-deoxyribose lesions (see below for more details) [39,40]. Indeed, there is evidence for the strand break formation in DNA originating from $H^{\bullet}$ abstraction at 2′-deoxyribose by $Gua(-H)^{\bullet}$ [41].

The OH–adduct radicals of Gua possess different reactivity toward O_2. Thus, the 4-OH–adduct radical practically does not react with O_2 ($k \leq 10^6$ dm^3 mol^{-1} s^{-1}), whereas the reaction between the 8-OH–adduct radical and O_2 is diffusion-controlled ($k = 4 \times 10^9$ dm^3 mol^{-1} s^{-1}) [17]. Cadet et al. proposed that the reaction of O_2 with $Gua(-H)^{\bullet}$ as the initial step for the formation of experimentally observed 2,5-diamino-4*H*-imidazol-4-one and 2,2,4-triamino-5(2*H*)-oxazolone as the final products of Gua oxidation [42,43]. However, this has not been confirmed by pulse radiolysis experiments and a kinetically more favoured mechanism has been put forward that includes the addition of $O_2^{\bullet-}$ to $Gua(-H)^{\bullet}$, followed by protonation to give rise to a Gua hydroperoxide (Figure 4). The addition of $O_2^{\bullet-}$ to $Gua(-H)^{\bullet}$ readily takes place with $k = 3 \times 10^9$ dm^3 mol^{-1} s^{-1} and $k = 4.7 \times 10^9$ dm^3 mol^{-1} s^{-1} for nucleosides and ds-DNA, respectively [17,44,45]. The addition of $O_2^{\bullet-}$ can occur at both the C5- and C8-positions. Subsequently, the Gua hydroperoxide undergoes elimination of CO_2, nucleophilic addition of water across the 7,8-double bond and loss of $HCONH_2$ generating 2,5-diamino-4*H*-imidazol-4-one. This is slowly hydrolysed with a half-life of about 10 hours, giving rise to 2,2,4-triamino-5(2*H*)-oxazolone [42, 45–47] (Figure 4). This compound has been detected in DNA *in vitro* and *in vivo* under various experimental conditions (reviewed in [47]).

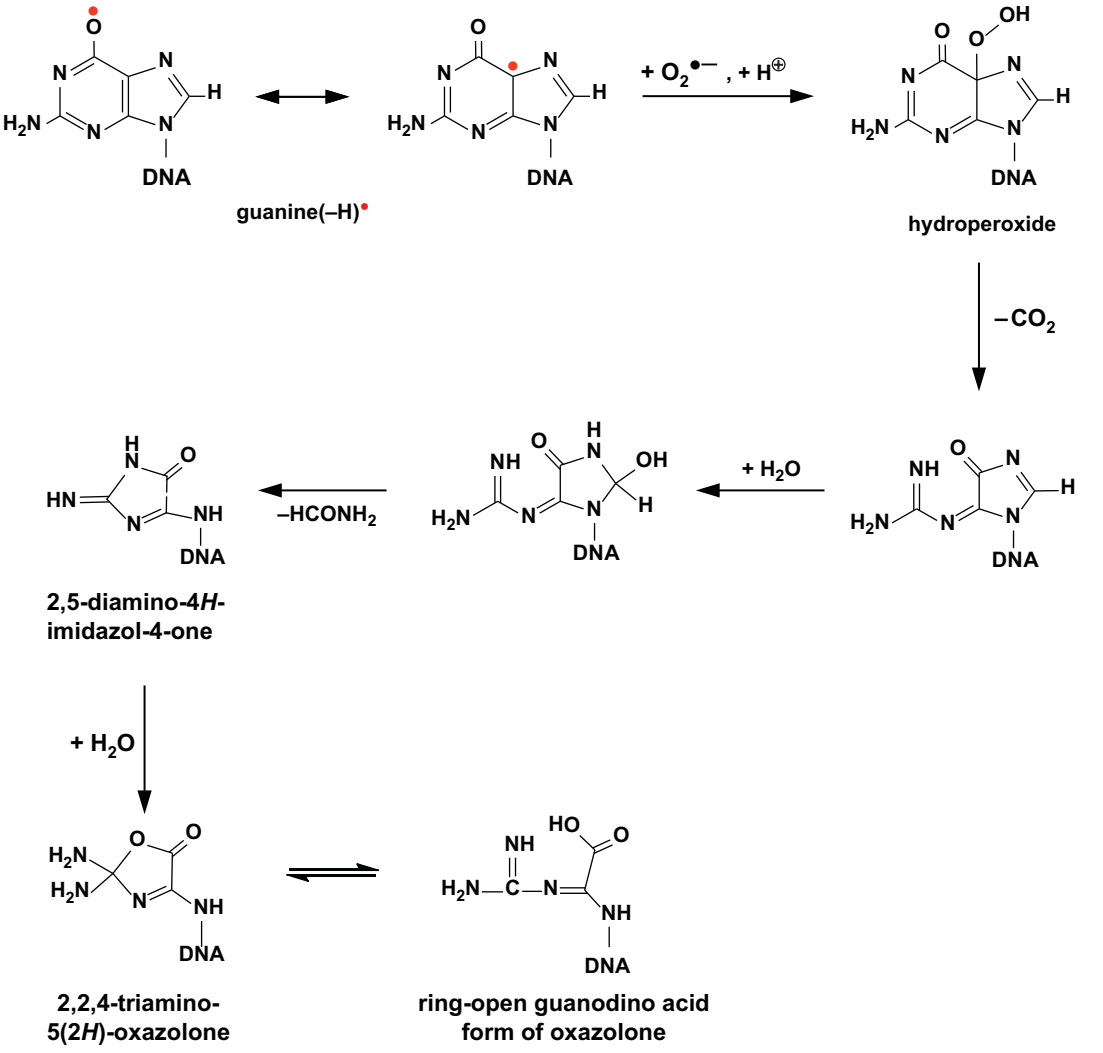

Figure 4. Mechanisms of product formation from reactions of Gua(–H)•.

The C8-OH–adduct radical produces the major products of Gua in DNA. Its one-electron oxidation leads to 8-hydroxyguanine (8-OH-Gua) (enol form) [9] (Figure 5). In a exothermic reaction by −60.3 kJ mol^{-1} [30,48], the tautomerization of the enol form leads to its keto form, which has been shown theoretically and experimentally to be the predominant form [49–51]. In the early 1980s, 8-OH-Gua has been identified in DNA damaged by different damaging agents including ionizing radiation [52–57]. Since then, there has been a vast amount of literature on the formation of 8-OH-Gua in DNA *in vitro* and *in vivo* under a large variety of experimental conditions. Because of its easy measurement and strong mutagenicity, this compound has been the mostly investigated DNA product, perhaps at the expense of other equally important DNA products in terms of understanding their mechanistic aspects and biological effects. For more detailed information, the reader is referred to extensive review articles on 8-OH-Gua (see e.g., [5,58,59]). In the absence of O_2, the C8-OH–adduct radical undergoes a reversible β-fragmentation leading to unimolecular ring opening with $k = 2 \times 10^5$ s^{-1} (Figure 5) [9,17]. The one-electron reduction of the ring-opened radical yields FapyGua. A 1,2-H–shift, which is typical of for heteroatom-centred radicals [2], may take place followed by one electron-reduction, leading to 7-hydro-8-hydroxyguanine. Being a hemiorthoamide, this compound is then readily converted into FapyGua (Figure 5). Since the ring opening is unimolecular, it can compete with the bimolecular oxidation or direct reduction. In a cellular environment, the ring opening in this competition may be favoured by the low O_2 concentration in the cell nucleus [60,61]. This notion is supported by the fact that FapyGua is formed in DNA with yields comparable to those of 8-OH-Gua under numerous *in vitro* or *in vivo* conditions (reviewed in [62]). It should be pointed out that formamidopyrimidines such as FapyGua and its Ade-derived counterpart (see below) differ from other pyrimidines such as Cyt and Thy in that they are attached to the sugar moiety of DNA

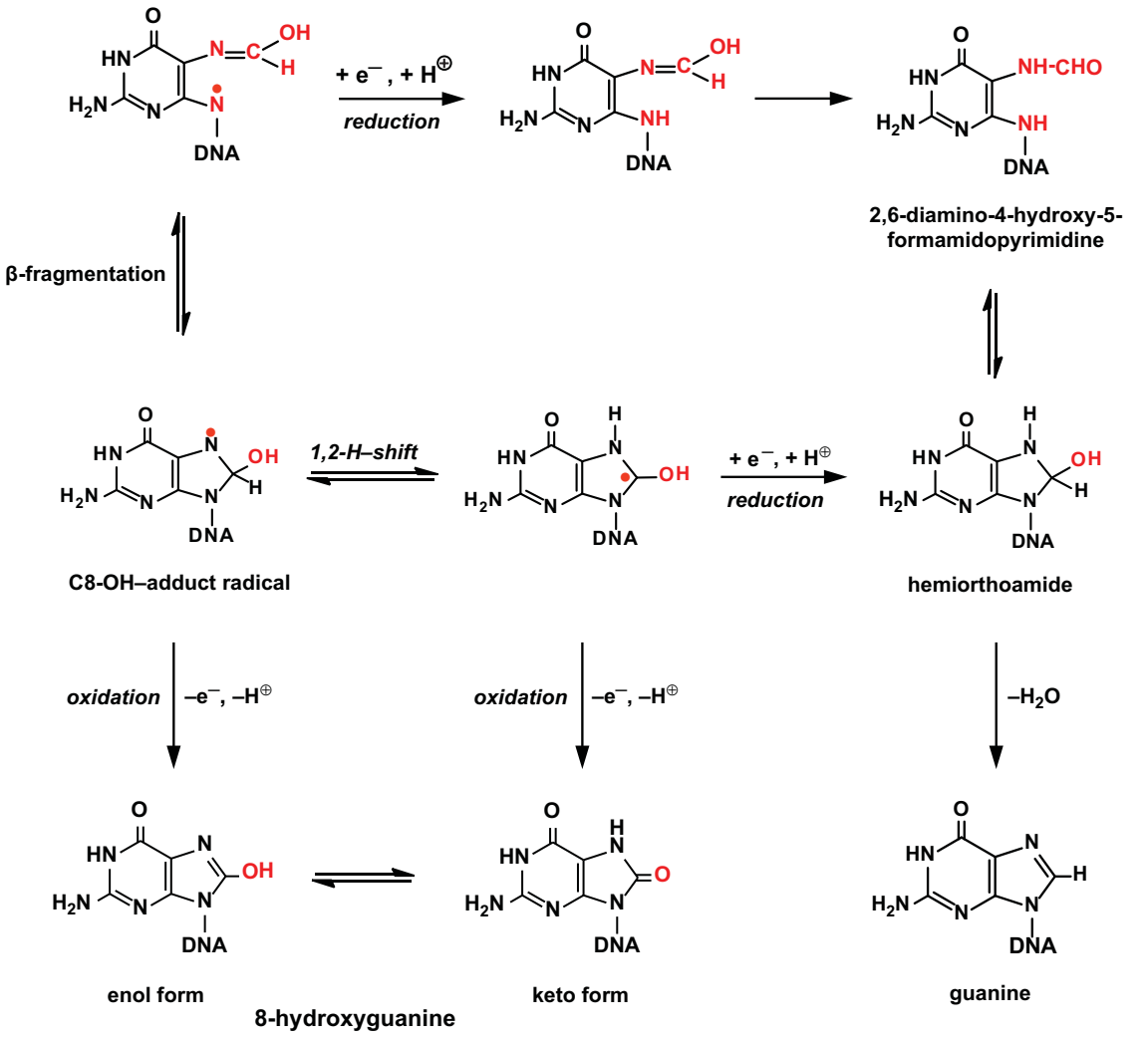

Figure 5. Mechanisms of product formation from oxidation and reduction reactions of C8-OH–adduct radical of Gua. (Adapted from [2]).

through the amino group at the C6-position of the pyrimidine ring. Furthermore, these compounds are chemically and mechanistically distinct from the methylation products of purines, which are formed under harsh experimental conditions by treatment with methylating agents followed by alkali treatment [63–65]. Moreover, biological effects of formamidopyrimidines are substantially different from those of their methylated counterparts (reviewed in [62]).

In nucleosides, 8-OH-Gua and FapyGua exist in both *anti* and *syn* conformations; however, both compounds retain the *anti*-conformation in ds-DNA [66,67]. In contrast, 8-OH-Gua assumes the *syn* conformation in ss-DNA. The rotation around C5–N7 and C8–N7 bonds indicates the possibility of four rotameric forms of FapyGua [68]. However, only two rotamers exist in solution, with the *cis*-conformation predominating over the *trans*-conformation, as found by NMR measurements [66,69,70]. This is supported by the ratio of the two rotameric ring-opened forms of N7-Me-FapyGua found in poly(dGdC) [63]. In DNA *in vivo*, the *cis*-confirmation has been suggested to dominate because of its stabilization by an hydrogen bond between the hydrogen atom at N9 and the oxygen atom of the formamido group [70]. Using density functional methods, the enol form of the ring-opened C8-OH adduct radical has been proposed to yield FapyGua by undergoing either one-electron reduction followed by tautomerization (as shown in Figure 5) or tautomerization followed by one-electron reduction with the former being favoured over the latter [71]. Two additional pathways have been proposed, leading to two formamidopyrimidine isomers, namely FapyGua and 2,5-diamino-4-hydroxy-6-formamidopyrimidine (2,5-FapyGua) [67]. In one pathway, the hemiorthoamide (Figure 5) undergoes ring opening and tautomerization to yield FapyGua and 2,5-FapyGua. In the other pathway, a proton transfer from the hydroxyl group to N7 of the C8-OH-adduct radical occurs. Subsequently, ring opening in two different directions takes place, followed by one-electron reduction of the two

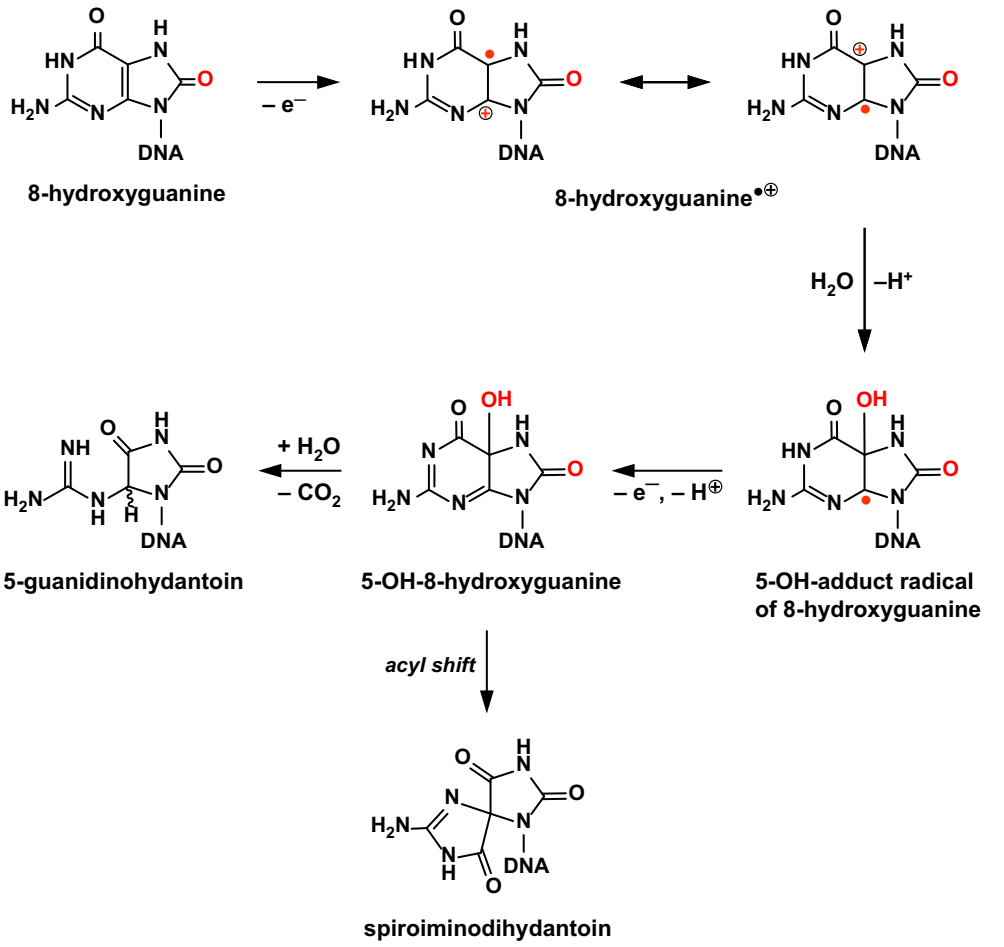

Figure 6. Mechanisms of product formation from oxidation of 8-OH-Gua.

ring-opened radicals to give rise to FapyGua in one case and to 2,5-FapyGua in the other case. The latter pathway having the lowest energy appears to be more favoured over the other three pathways. Thermodynamically, 2,5-FapyGua is less stable than FapyGua, although it may be formed initially and then converted into FapyGua *via* the hemi-orthoamide [67]. The formation of FapyGua in nucleosides and DNA *in vitro*, and in DNA *in vivo* under numerous experimental conditions has extensively been studied and reported in the past five decades. In most cases, the yields of FapyGua were comparable to, if not, greater than those of 8-OH-Gua. A recent extensive review of this field can be found elsewhere [62].

The reduction potential of 8-OH-Gua amounts to 0.74 V as compared to 1.29 V for Gua [72]. It is therefore prone to oxidation, giving rise to a radical cation (8-OH-Gua$^{\bullet+}$), which hydrates (addition of HO^-) producing the 5-OH–adduct radical of 8-OH-Gua as shown in Figure 6. The oxidation can be caused by a number of oxidizing agents such as ionizing radiations, singlet oxygen, metal ions, peroxynitrate, $IrCl_6^{2-}$, among others. Upon one-electron oxidation, this radical forms 5-OH-8-hydoxyguanine, the isomerization of which results in the formation of spiroiminodihydantoin and also in that of 5-guanidinohydantoin by loss of CO_2 depending on reaction conditions [73]. For almost 20 years, the structure of spiroiminodihydantoin has been misassigned by Cadet et al. as 4,8-dihydro-4-hydroxy-8-oxoguanine [74–78]. Moreover, this product has been routinely used for a marker of single oxygen-induced damage to Gua [77]. However, the use of various analytical techniques and the synthesis of the authentic material revealed the correct structure of this compound as spiroiminodihydantoin, which is a diastereomeric mixture [73,79–82]. The oxidation of 8-OH-Gua leading to spiroiminodihydantoin also occurs by triplet states [82–85]. Singlet oxygen reacts with 8-OH-Gua yielding oxaluric acid, parabanic acid and other products [86,87]. Moreover, 8-OH-Gua$^{\bullet+}$ [and also its the deprotonated form 8-OH-Gua(–H$^{\bullet}$)] reacts with $O_2^{\bullet-}$ ($k = 3 \times 10^9$ dm^3 mol^{-1} s^{-1}) to give rise to 5-hydroperoxide of 8-OH-Gua, which decomposes to form oxaluric acid and parabanic acid [88]. Spiroiminodihydantoin has also been identified in *E. coli* treated with potassium dichromate [89]. Sequence-dependent variation in the reactivity of 8-OH-Gua toward oxidation has also been reported [90]. Extensive reviews of this field can be

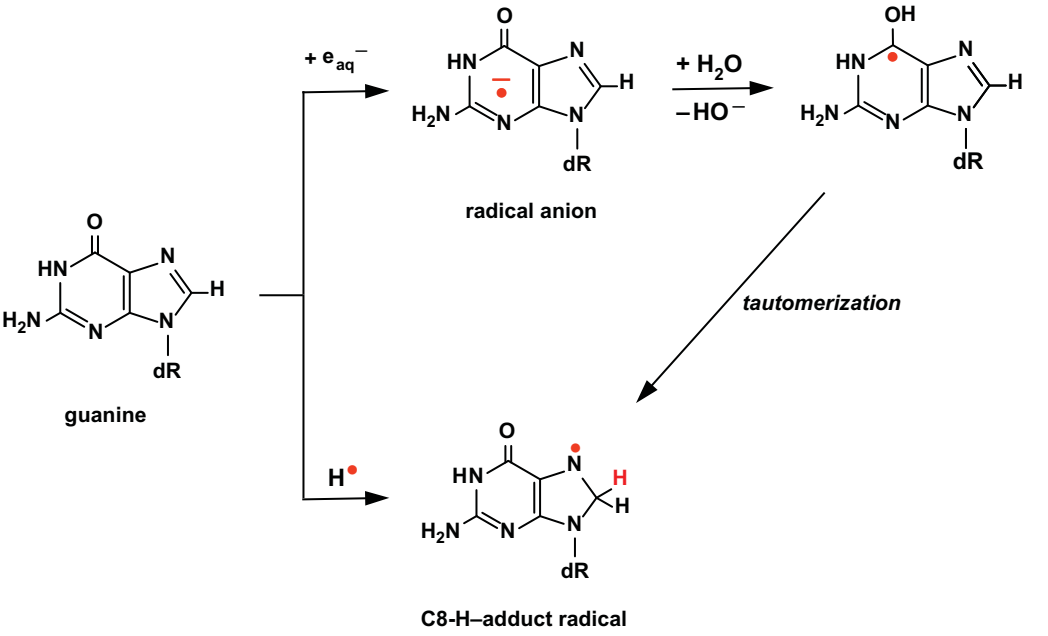

Figure 7. Reactions of Gua with e_{aq}^- and H•.

found elsewhere [91,92]. Obviously, there has been mounting evidence for the facile oxidation of 8-OH-Gua by a large number of oxidants to yield numerous products. All this evidence puts in doubt the validity of claims by the European Standards Committee on Oxidative DNA Damage (ESCODD) about the "correct" value of the background level of 8-OH-Gua in living cells, and the validity of its advice and recommendation to editors and reviewers of manuscripts not to accept reported values of the 8-OH-Gua level exceeding a certain range of level "established" by ESCODD [93–95]. Contrasting the claims by ESCODD, the so-called "established median value" has been obtained with an exceptionally wide range of 120× (by chromatographic methods) and 83× (by enzymatic methods) between the highest and lowest estimates of the 8-OH-Gua level among participating laboratories.

The reaction between e_{aq}^- and Gua nucleosides is diffusion-controlled ($k = 6 \times 10^9$ dm^3 mol^{-1} s^{-1}) [11,96]. Later, a similar rate constant of 3.3×10^9 dm^3 mol^{-1} s^{-1} has been reported [97]. The high rate of this reaction is likely due to many N atoms with high electron affinity in purines [19]. The addition of e_{aq}^- to Gua gives rise to a radical anion (Gua$^{\bullet-}$), which is readily protonated at a heteroatom (O6, N3 or N7) in reaction with H_2O ($k \geq 1 \times 10^7$ s^{-1}) followed by water-assisted tautomerization ($k = 1.2 \times 10^6$ s^{-1}) to yield a neutral C8-H–adduct radical (Figure 7). A subsequent work confirmed this mechanism and reported a similar rate for tautomerization ($k \approx 1.5 \times 10^{-6}$ s^{-1}) [97]. H• also reacts with guanine nucleosides ($k = 5 \times 10^8$ dm^3 mol^{-1} s^{-1}) by addition at C8 and generates the same adduct radical [96]. The C8-H–adduct radical of Gua is a weak oxidant. No products of this radical have been found so far in DNA.

Adenine

The reduction potential of Ade (1.56 V) is considerably greater than that of Gua (1.29 V) [2,8], and thus it is not as readily oxidized. As with Gua, •OH reacts with Ade by addition to its double bonds as shown in Figure 8. However, the distribution of additions is somewhat different. Thus, the addition at the C4 and C8 amounts to 50% and 37%, respectively, forming the C4-OH– and C8-OH–adduct radicals [9,98,99]. The tendency of •OH addition to C5 yielding the reducing C5-OH–adduct radical amounts to ≥5%, whereas the addition at C2 is likely to be no more than 2% due to the low electron density at this position [99]. The C4-OH–radical is weakly oxidizing (due to the unpaired spin density on N1 and N3) and undergoes H_2O elimination ($k = 1.9 \times 10^4$ s^{-1}) to give rise to a strongly oxidizing Ade(–H)• [98] (Figure 9). The reduction potential of this radical is ~1.6 V and may reconstitute Ade upon one-electron reduction [10]. Similar to Gua(–H)•, Ade(–H)• may protonate to give Ade$^{\bullet+}$, which would generate the C8-OH–radical upon hydration (Figure 9). Unlike its Gua-derived counterpart, the C4-OH–radical readily reacts with O_2 ($k = 1 \times 10^9$ dm^3 mol^{-1} s^{-1}; measured using 2′-deoxyadenosine) [99]. The C2-OH–adduct radical may give rise to 2-hydroxyadenine (2-OH-Ade) by one-electron oxidation (Figure 9). The identification of 2-OH-Ade in DNA *in vitro* and *in vivo* supports this notion [100,101].

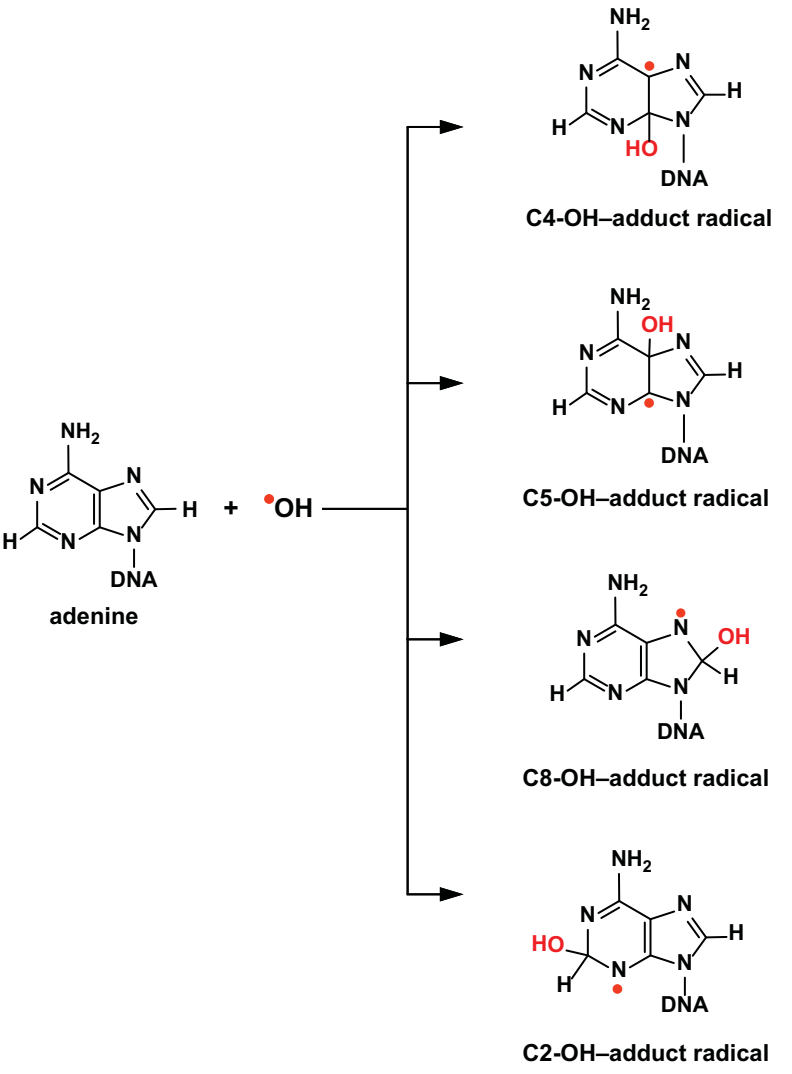

Figure 8. Reactions of •OH with Ade.

The one-electron oxidation of the C8-OH–adduct radical produces 8-hydroxyadenine (8-OH-Ade) (Figure 10). In competition with oxidation, this radical undergoes ring opening ($k = 1 \times 10^5$ s^{-1}), followed by one-electron reduction, producing 4,6-diamino-5-formamidopyrimidine (FapyAde). The reduction without ring opening can also occur, resulting in the formation of the hemiorthoamide (7-hydro-8-hydroxyadenine), which is sensitive to hydrolysis and is converted into FapyAde. 7-Hydro-8-hydroxyadenine may also dehydrate to reconstitute Ade (Figure 10). Oxygen reacts with the C8-OH–adduct radical more efficiently ($k \approx 4 \times 10^9$ dm^3 mol^{-1} s^{-1}) than with the C4-OH–adduct radical [99]. At low O_2 concentrations (20–30 μM), this reaction and ring opening may be equally efficient and thus competitive [99]. The abundant formation of FapyAde and 8-OH-Ade in DNA *in vitro* and *in vivo* confirms this notion (reviewed in [5,58,62,102]).

Adenine reacts with e_{aq}^- at a diffusion-controlled rate ($k = 6 \times 10^9$ dm^3 mol^{-1} s^{-1}; measured using adenosine) [11]. The thus-formed radical anion (Ade$^{\bullet -}$) is rapidly protonated by H_2O at a heteroatom (nitrogen) ($k \geq 1.4 \times 10^8$ s^{-1}; measured using adenosine) as shown in Figure 11 [9,103–105]. The neutral N-protonated radical [Ade(H)•] may exist in an equilibrium mixture with its mesomeric forms. These mesomers and Ade$^{\bullet -}$ possess strong reducing properties. Ade(H)• is converted into the carbon-protonated C8-H–adduct radical either spontaneously ($k = 1 \times 10^4$ s^{-1}) or by catalysts such as phosphate ($k = 2 \times 10^6$ s^{-1}) [105] (Figure 11). The reaction of Ade(H)• with H^+ ($k = 4 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) yields the carbon-protonated C2-H–adduct radical. Catalysis by phosphate converts this radical into the C8-H–adduct radical ($k = 6.1 \times 10^5$ dm^3 mol^{-1} s^{-1}). The C8-H–adduct radical is thermodynamically more stable than the C2-H–adduct radical, probably because the aromatic character of the pyrimidine ring is kept in the former. The addition of H• to the C8-position of Ade may also produce the C8-H–adduct radical (Figure 11). All these radicals of Ade may give rise to final products;

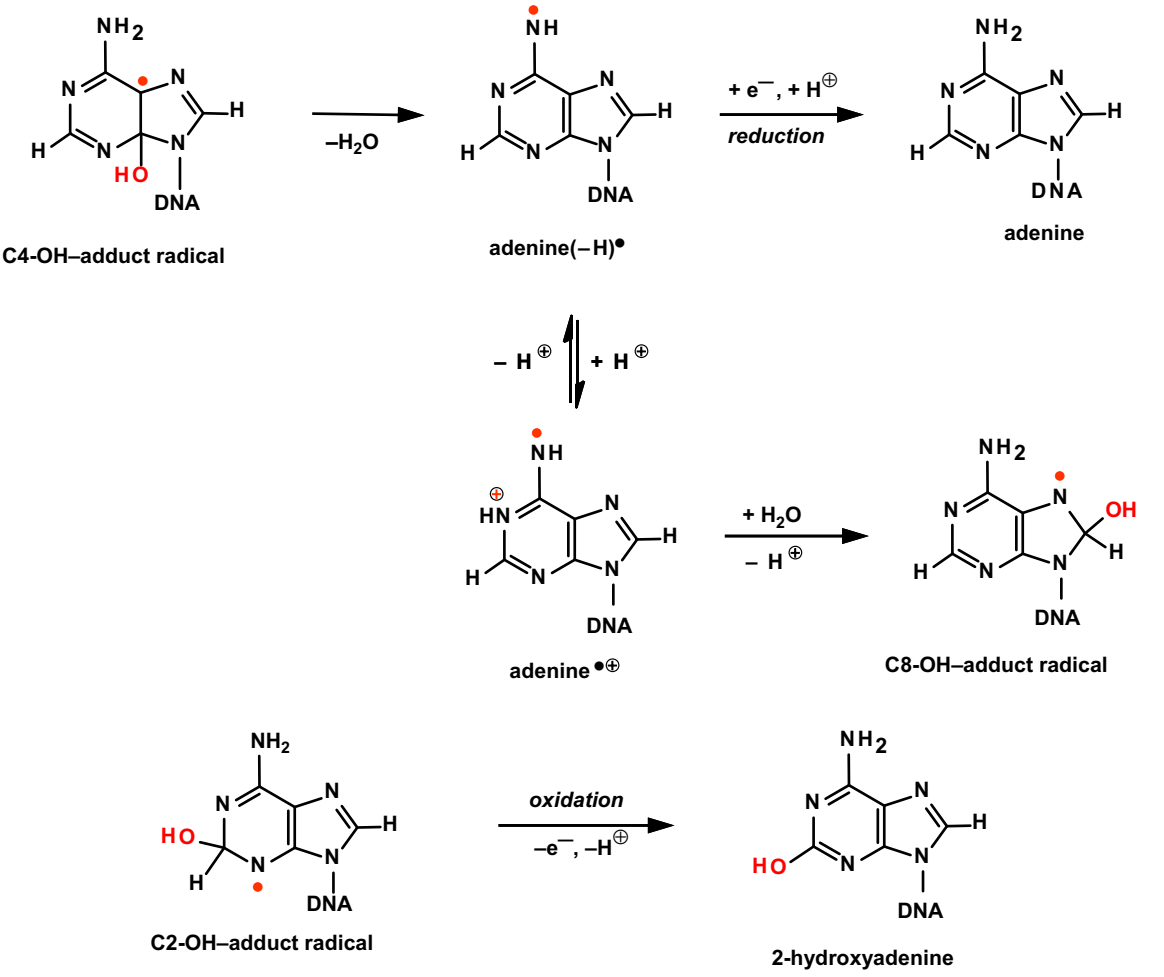

Figure 9. Reactions of the C4-OH–adduct radical of Ade (upper part). Oxidation of the C2-OH–adduct radical of Ade leading to 2-OH-Ade (lower part).

however, no such products have been identified in DNA. It may well be that the C2-H– and C8-H–adduct radicals rapidly transfer electron to other DNA bases such as Thy, thus disappearing before formation of final products [105].

Thymine

Thy reacts with •OH and e_{aq}^- at diffusion-controlled rates ($k = 6.4 \times 10^9$ dm^3 mol^{-1} s^{-1} and $k = 1.8 \times 10^{10}$ dm^3 mol^{-1} s^{-1}, respectively), and with H• at an order of magnitude slower rate ($k = 6.8 \times 10^8$ dm^3 mol^{-1} s^{-1}) [11]. Hydroxyl radical adds to the C5–C6-double bond of Thy to the extent of 60% at C5 and 30% at C6, and also abstracts an H• from the methyl group to a much lesser extent (10%) [106,107]. These reactions are exothermic to different extents according to a Car–Parrinello molecular dynamics study [16]. However, the calculated reaction energies of the C5–C6-additions and H• abstraction do not agree with the distribution of the •OH attack. The greater addition at C5 results from the higher electron density at C5 than at C6, and the ratio of the additions is on a par with that of the electron densities at these positions [2]. The reactions of •OH produce the C5-OH– and C6-OH–adduct radicals, and an allyl radical of Thy (Figure 12). The C5-OH–adduct radical has reducing properties, whereas the C6-OH–adduct radical is a strong oxidant. *Ab initio* molecular orbital calculations showed that the C6-OH–adduct radical is the most oxidizing among all OH–adduct radicals of DNA bases [18]. The allyl radical has no oxidizing or reducing properties. Thy radicals are oxidized or reduced depending on their redox properties, the presence or absence of oxygen and redox environment, producing a variety of products with different yields (reviewed in [2,5,58,102]). In the absence of O_2, the C5-OH– and C6-OH–adduct radicals undergo oxidation and H_2O addition (HO^- addition) to yield Thy glycol (*cis*– and *trans*–) (Figure 13). The C5-OH–adduct radical may also abstract an H• from the neighbouring 2′-deoxyribose, leading to DNA strand breaks [108,109]. The reduction of the C5-OH– and C6-OH–adduct radicals takes place, giving rise to 5-hydroxy-6-hydrothymine and 6-hydroxy-5-hydrothymine, respectively. The oxidation of the allyl radical followed by H_2O addition (HO^- addition) results in 5-(hydroxymethyl)uracil (Figure 13). The formation

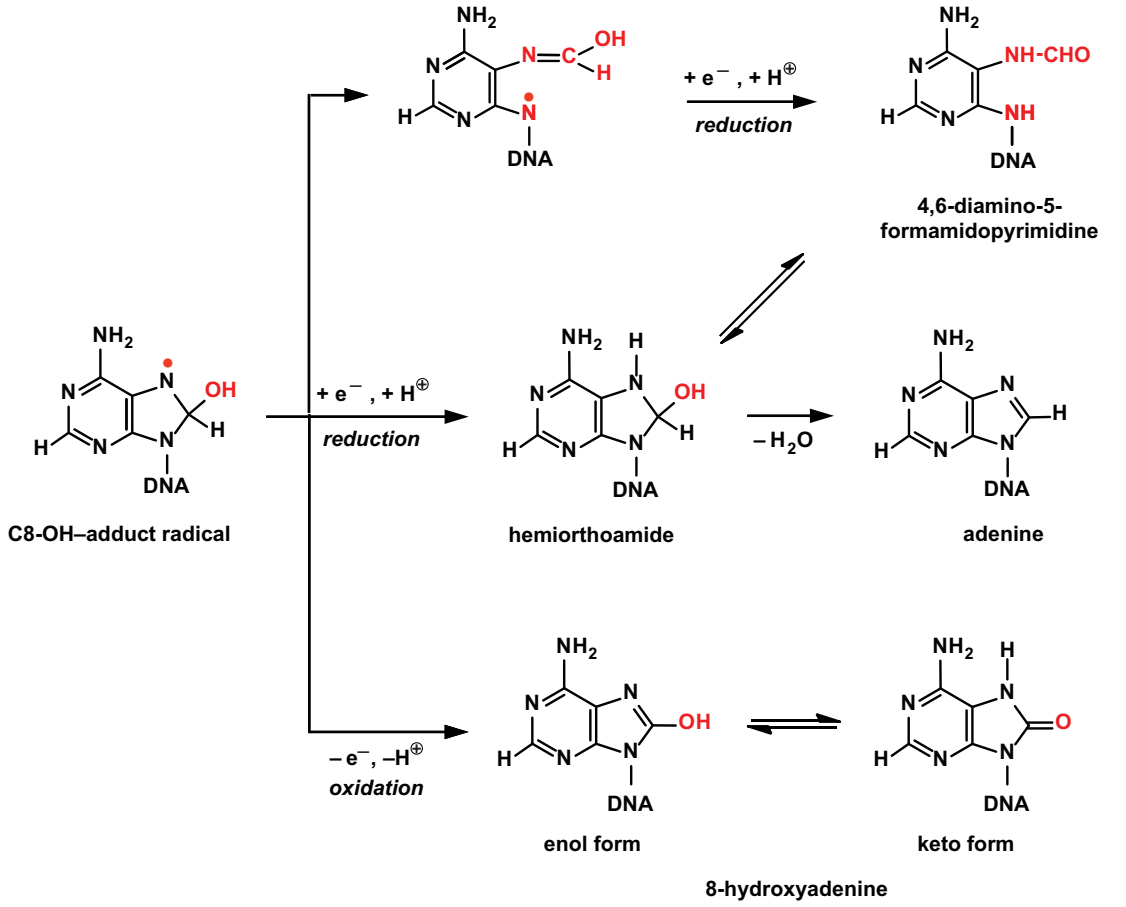

Figure 10. Mechanisms of product formation from oxidation and reduction reactions of the C8-OH–adduct radical of Ade.

of 5-hydroxy-6-hydrothymine and 6-hydroxy-5-hydrothymine is inhibited by O_2, because Thy radicals react with O_2 at diffusion-controlled rates with $k \approx 2 \times 10^9$ $dm^3\ mol^{-1}\ s^{-1}$, yielding peroxyl radicals (Figure 14) [110]. The C5-OH–peroxyl radical eliminates $O_2^{\bullet -}$ followed by H_2O addition and deprotonation to produce Thy glycol [2,106,111]. The C6-OH–peroxyl radical may undergo the same reactions. Peroxyl radicals are also reduced and protonated, yielding hydroxyhydroperoxides, which further decompose to give rise

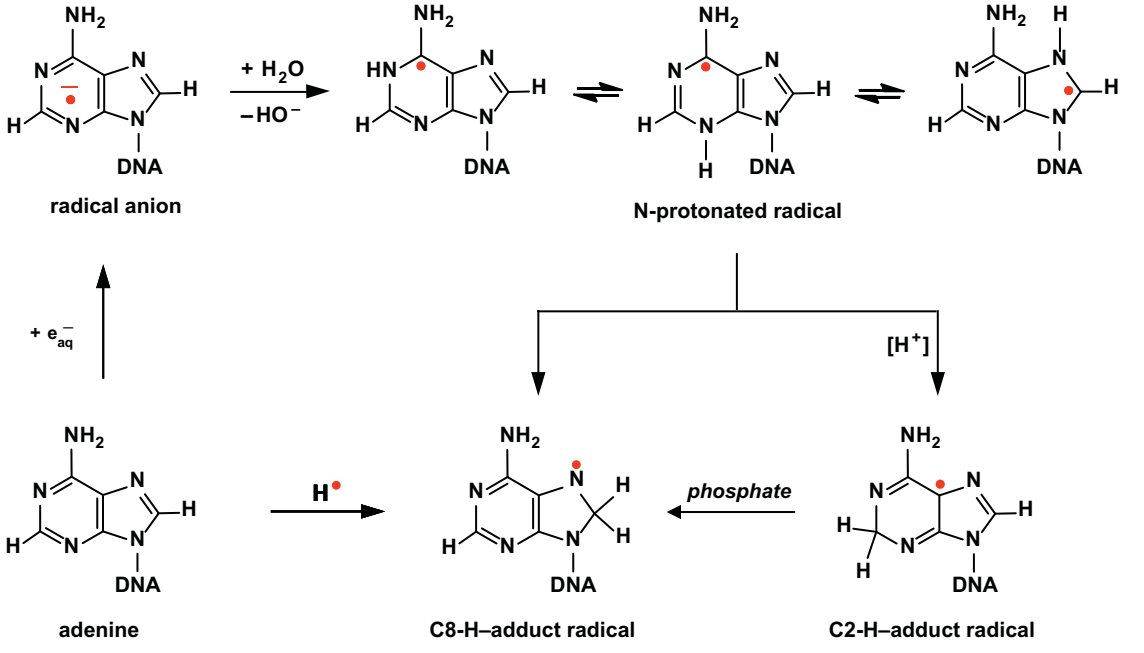

Figure 11. Reactions of Ade with e_{aq} and H•.

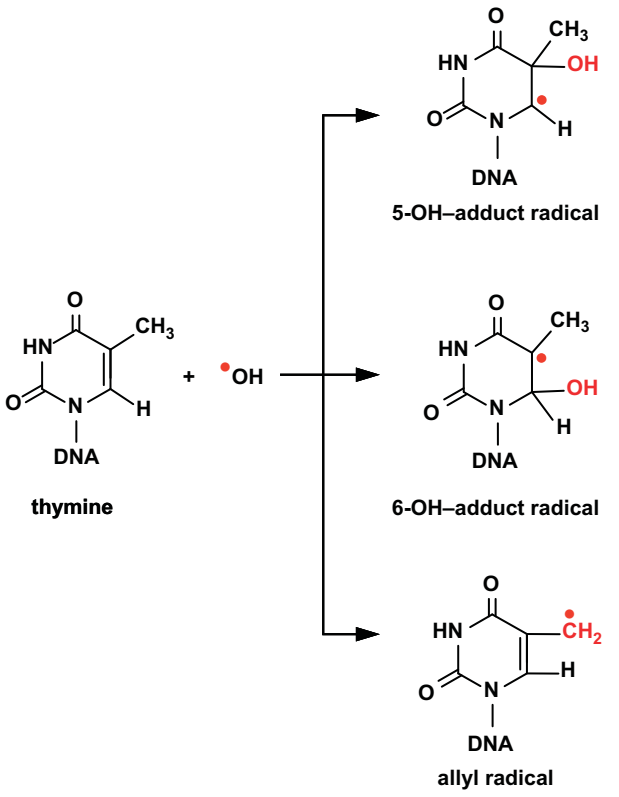

Figure 12. Reactions of •OH with Thy.

to Thy glycol, 5-(hydroxymethyl)uracil, 5-formyluracil and the ring reduction product 5-hydroxy-5-methylhydantoin (Figure 14) [111–113].

Ionizing radiation-generated e_{aq}^- reacts with Thy v addition at a diffusion-controlled rate ($k = 1.8 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) [11], generating an anion radical, which yields the 5-H–adduct radical upon protonation (Figure 15). The reaction between H• and Thy is slower; nevertheless, it has an appreciable rate ($k = 6.8 \times 10^8$ dm^3 mol^{-1} s^{-1}) [11], and also gives rise to the 5-H–adduct radical. Being an electrophilic radical, H• has a strong preference for addition at electron-rich sites, thus it preferentially adds to the C5-position [2,114]. The reduction of the 5-H–adduct radical results in the formation of 5,6-dihydrothymine (Figure 15). This product is not formed in the presence of O_2 because of the diffusion-controlled reaction of O_2 with both e_{aq}^- and H• ($k \approx 2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) [11]. However, a competition may take place between the reactions of these species with Thy and O_2 under the hypoxic conditions of the cell nucleus, allowing the formation of 5,6-dihydrothymine in DNA *in vivo*. Two diastereomers of 5,6-dihydrothymine have been identified in γ-irradiated HeLa cells [115].

Cytosine

Hydroxyl radical reacts with Cyt at a diffusion-controlled rate by addition to the C5–C6 double bond ($k = 6.8 \times 10^9$ dm^3 mol^{-1} s^{-1}) [11,116], generating the C5-OH– and C6-OH–adduct radicals (Figure 16). The distribution of •OH addition at Cyt substantially differs from that at Thy, with the addition taking place at C5 and C6 to the extent of 87% and ~10%, respectively, because of the exceptionally high electron density at C5 compared to that at C6 [2,117]. The addition at N3 has also been considered; however, it is much less likely to occur than the other additions. With Cyt nucleosides, more than 80% of hydroxyl radicals have been estimated to react with the base and the rest with the sugar moiety. This estimate roughly agrees with that obtained using the rate constants of •OH reactions with individual nucleoside components [11]. The C5-OH–radical has reducing properties, whereas the C6-OH–adduct radical is a weak oxidant. The former is a type of α-aminoalkyl radicals that are powerful one-electron donors [118,119]. The C4-OH–radical would be oxidizing, but its formation is uncertain [117]. In the absence of O_2, the oxidation of the C5-OH–adduct radical followed by hydration (HO^- addition) yields Cyt glycol (Figure 17) (reviewed in [2,5,58]). The reduction of this adduct radical leads to 5-hydroxy-6-hydrocytosine. Cyt products are unique in that they undergo dehydration and deamination. Thus, Cyt glycol produces 5-hydroxycytosine by dehydration, Ura glycol by deamination and 5-hydroxyuracil by deamination followed by dehydration (Figure 17) [111,120]. The deamination of 5-hydroxy-6-hydrothymine gives rise to 5-hydroxy-6-hydrouracil. However, these products may simultaneously exist in oxidatively damaged DNA as the evidence suggests [121,122].

Oxygen reacts with the Cyt radicals at diffusion-controlled rates ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}), leading to peroxyl radicals [110,117] (Figures 18 and 19). Unimolecular elimination of $O_2^{\bullet -}$ and subsequent hydration (HO^- addition) and deprotonation produces Cyt glycol [2,111,117,123], which then can dehydrate and deaminate leading to the products described above. However, 5-hydroxy-6-hydrocytosine and, consequently, 5-hydroxy-6-hydrouracil are not formed in the presence of O_2 because of the diffusion-controlled reaction of O_2 with their precursor (see above). Peroxyl radicals of Cyt are also reduced and protonated, yielding hydroxyhydroperoxides [123]. These compounds readily decompose to give rise to 4-amino-5-hydroxy-2,6(1H,5H)-pyrimidinedione from 5-OH-6-hydroperoxide (Figure 18) and 4-amino-6-hydroxy-2,5(1H,6H)-pyrimidinedione from 6-OH-5-hydroperoxide (Figure 19). The former dehydrates and deaminates to give rise to 5-hydroxy-2,4,6(1H,3H,5H)-pyrimidinetrione (dialuric acid), which is readily oxidized in aqueous solution to give rise to 2,4,5,6(1H,3H)-pyrimidinetetrone (alloxan) [124,125], and subsequently to the ring-reduced product 5-hydroxyhydantoin upon acidic treatment [126] (Figure 18). 5-OH-6-hydroperoxide undergoes intramolecular cyclization to give rise to *trans*-1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine as a major product

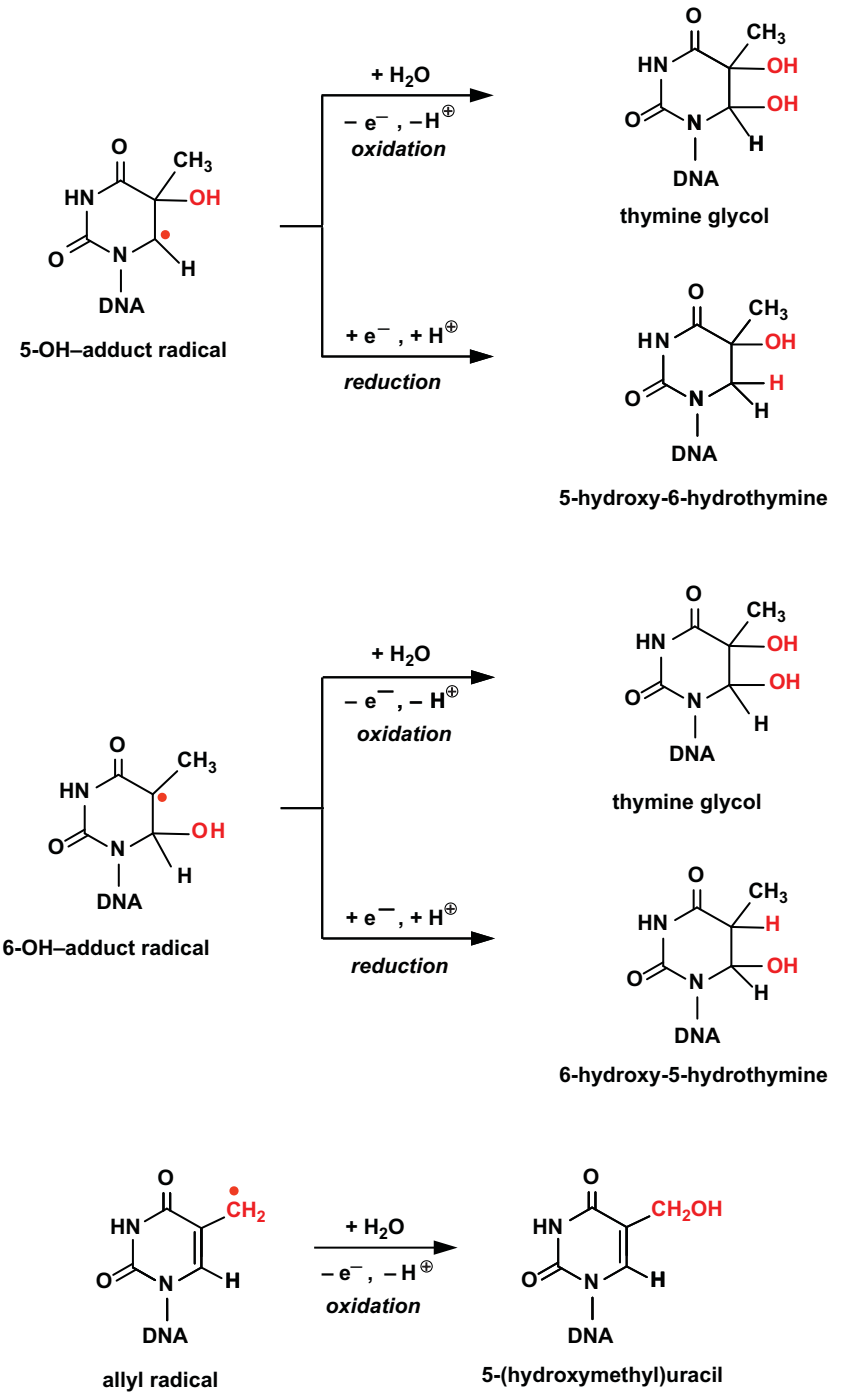

Figure 13. Mechanisms of product formation from reactions of the C5-OH– and C6-OH–adduct radicals, and the allyl radical of Thy.

of Cyt (Figure 18); however, this compound is formed in DNA as a minor product only [111,122,123,127]. 4-Amino-6-hydroxy-2,5(1H,6H)-pyrimidinedione deaminates to yield isodialuric acid. Both compounds may also exist in their enol forms 5,6-dihydroxycytosine and 5,6-dihydroxyuracil, respectively (Figure 19). The simultaneous existence of these two products has been shown in damaged DNA [121,122].

Hydrated electron reacts with Cyt by addition at a diffusion-controlled rate ($k = 1.3 \times 10^{10}$ dm^3 mol^{-1} s^{-1}), generating an electron adduct (anion radical), which gives rise to the 5-H–adduct radical upon rapid protonation by H_2O [104,128]. The reaction of H$^\bullet$ with Cyt has a slower rate ($k \approx 9.2 \times 10^7$ dm^3 mol^{-1} s^{-1}) and produces the same 5-H–adduct radical by addition to the electron-rich C5-position [11] (Figure 20). The one-electron reduction of the 5-H–adduct radical yields 5,6-dihydrocytosine, which is converted into 5,6-dihydrouracil upon deamination. In the presence of O_2, the formation of these products is

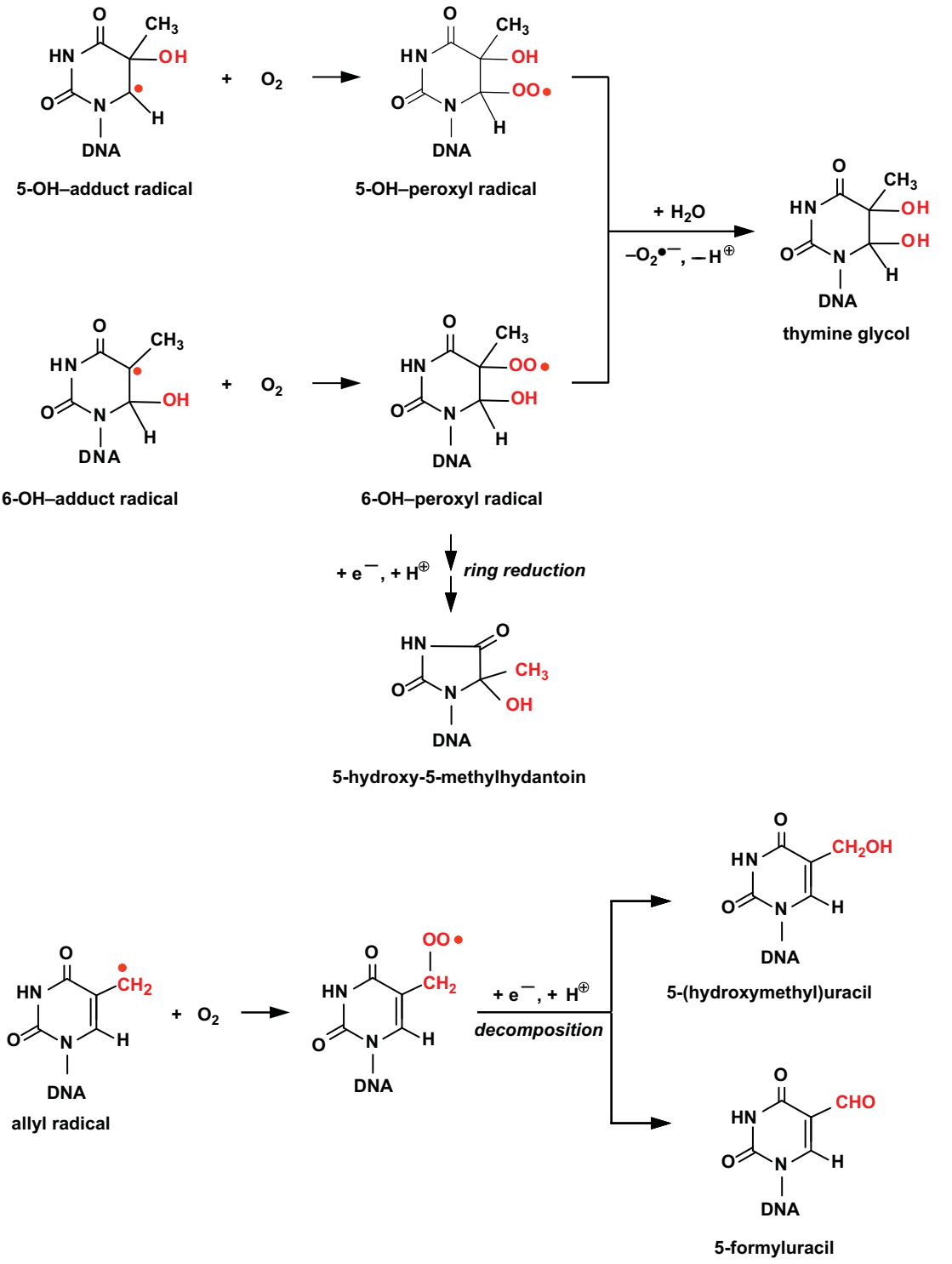

Figure 14. Mechanisms of product formation from reactions of the C5-OH– and C6-OH–adduct radicals, and the allyl radical of Thy with O_2.

inhibited because of diffusion-controlled reactions of O_2 with e_{aq}^- and H•. Nevertheless, hypoxic conditions of the cell nucleus may allow the formation of 5,6-dihydrocytosine and 5,6-dihydrouracil in DNA *in vivo*.

Final products

The reactions discussed above yield a plethora of products in DNA that have been identified *in vitro* and *in vivo* over the past five decades. It is important to note that the types and yields of these products depend on the reaction conditions. For example, some products are produced in the absence of O_2 only, and others under both oxygenated and deoxygenated conditions, but with different yields. The presence of reducing or oxidizing agents profoundly affects the product yields as well. In living cells and tissues, the product yields also depend on redox environment, the type of treatment, disease

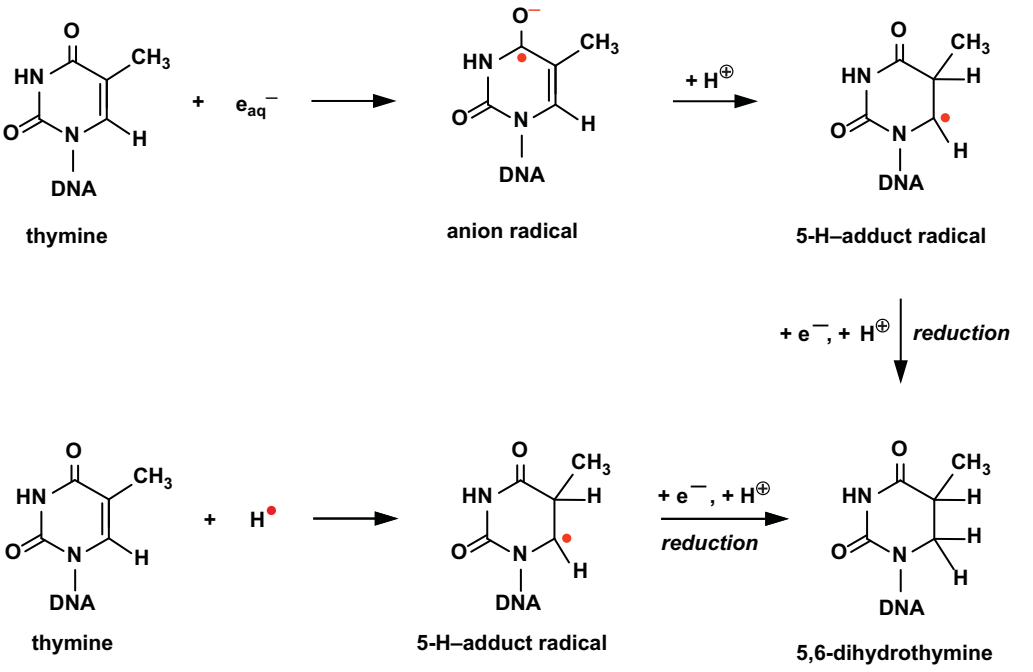

Figure 15. Reactions of Thy with e_{aq}^- and H•, leading to formation of 5,6-dihydrothymine.

conditions, DNA repair deficiency, availability of transition metal ions bound to DNA, radical-scavenger concentration, etc. Therefore, the yield of a given product is not a fixed value under all possible experimental or *in vivo* conditions. Unfortunately, this fact has often been ignored in many papers in the literature, and a certain product has been presented almost always as the most important or the most abundant product, no matter what conditions had been used. Figure 21 illustrates the main products of free radical damage to the heterocyclic bases in DNA identified *in vitro* and *in vivo* under numerous conditions. More details on these products can be found elsewhere (see e.g., [2,5,47,58, 102,129,130]).

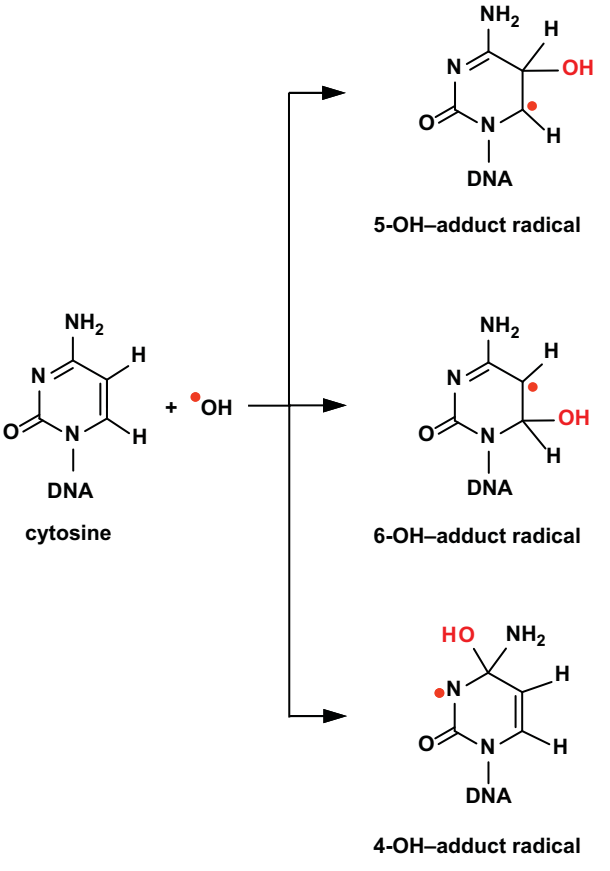

Figure 16. Reactions of •OH with Cyt.

Mechanisms of damage to the sugar moiety of DNA

Hydroxyl radical reacts with 2′-deoxyribose in DNA by H• abstraction from all its carbons leading to five C-centred radicals as shown in Figure 22. The overall rate constant of this reaction amounts to 2.5×10^9 $dm^3\ mol^{-1}\ s^{-1}$ [11]. However, the rate may depend on the C-atoms. The extent of •OH attack on 2′-deoxyribose in DNA generally amounts to less than 20% [2], although this amount may be different in the cell nucleus. In poly(U), for example, the amount of attack is 7% only [131]. However, the DNA strand breakage is greater than expected from the amount of •OH attack on 2′-deoxyribose, indicating a possible radical transfer from a base radical to 2′-deoxyribose. This in fact has been demonstrated using poly(U) [108,131–137]. Moreover, there is evidence for an intramolecular H• abstraction from 2′-deoxyribose by the Thy C5-OH–adduct radical in poly(dT) [109]. The calculated energies of H• abstractions by •OH from small molecules and 2-deoxyribose correlate with the strength of the C–H bonds [138,139]. However, the solvent accessibility plays a critical role when 2-deoxyribose

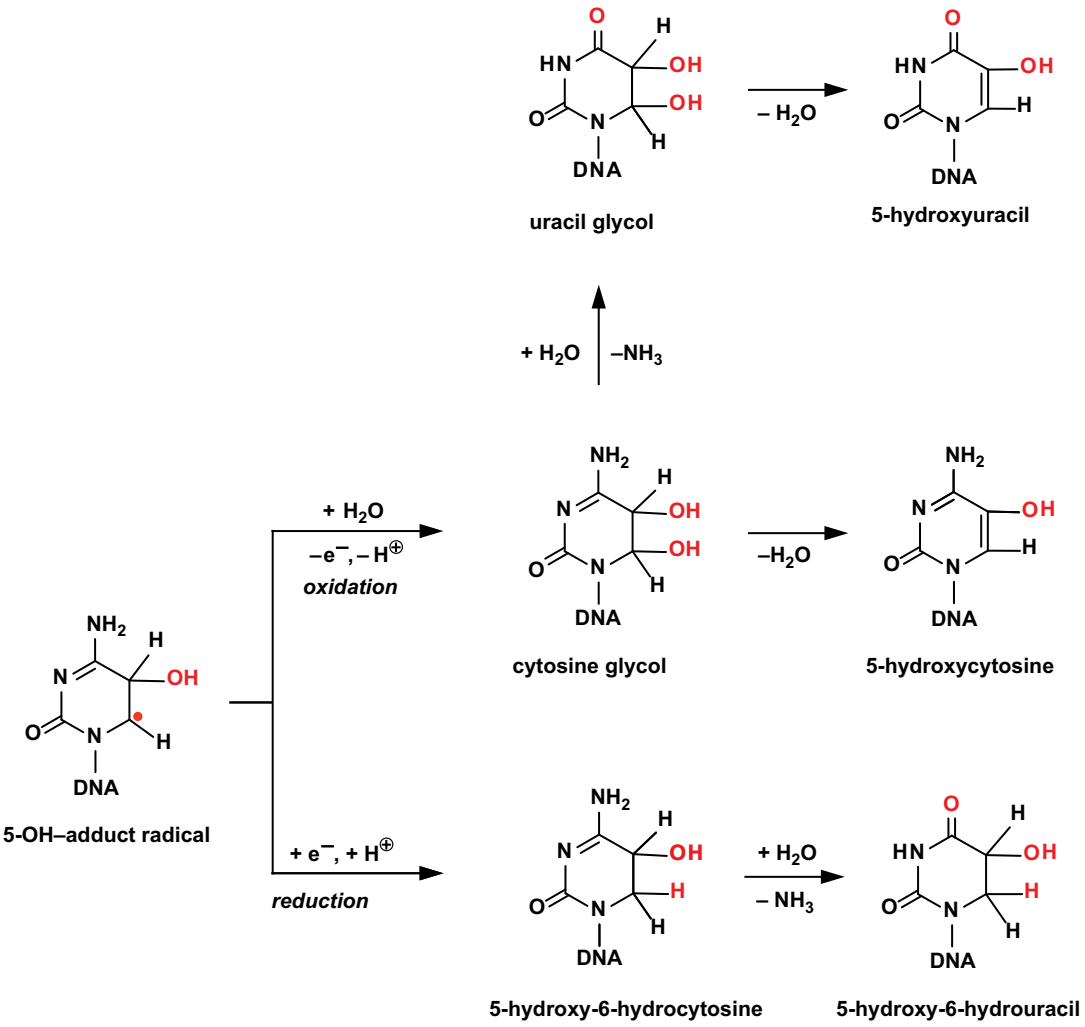

Figure 17. Mechanisms of product formation from oxidation and reduction reactions of the C5-OH–adduct radical of Cyt. Deamination and dehydration of the products.

is situated within DNA. H4′ and H5′ are more exposed to solvent and thus more accessible to H• abstraction by •OH than the other H atoms. The accessibility of H1′ is very low in the case of the double-stranded B-form of DNA. The C4′ radical appears to be the major radical produced by H• abstraction from 2′-deoxyribose in DNA [139]. The accessibility to •OH attack on the H atoms at the five carbons varies as calculated using a Monte-Carlo simulation and the RADACK procedure, and by an *ab initio* study [140–142]. According to these calculations, the H• abstraction is most probable from H4′ and H′5 in DNA. The calculated accessibility of the H4′ and H5′ atoms agreed well with experimentally determined DNA damage in terms of single-strand breaks. However, the accessibility to these sites exhibited a strong sequence dependency [140]. Reduced strand breakage occurred in sequences exhibiting low accessibility of H4′ and H5′2, leading to a low probability of abstraction by •OH due to a narrow, minor groove. Experimental results and calculations suggested that the C4′- and C5′-centred radicals cause strand breaks to roughly equal extents. In another context, deuterium kinetic isotope effects on the rate of various H• abstractions from 2′-deoxyribose by •OH have been measured [143,144]. These findings showed that •OH abstracts an H• from the five carbons in the order H5′ > H4′ > H3′≈ H2′≈ H1′ and that the C4′- and the C5′-positions are the most accessible to solvent and from the minor groove.

Damage to 2′-deoxyribose in DNA leads to products, strand breaks and abasic sites, and consequently to the release of unaltered DNA bases. The C-centred radicals undergo further reactions, yielding a variety of products of 2′-deoxyribose. Some products are released from DNA, whereas others remain within DNA or constitute end groups of broken DNA strands. The mechanisms understood first in detail have been those of the reactions of the C4′ radical in the absence of O_2, leading to strand breaks and products [39]. This radical is an alkoxyalkyl radical with a phosphate group at the β-position on both sites of the DNA chain. Such radicals readily lose the phosphate group as elucidated using model systems [145–148]. Heterolytic cleavage of the phosphate group at C3′

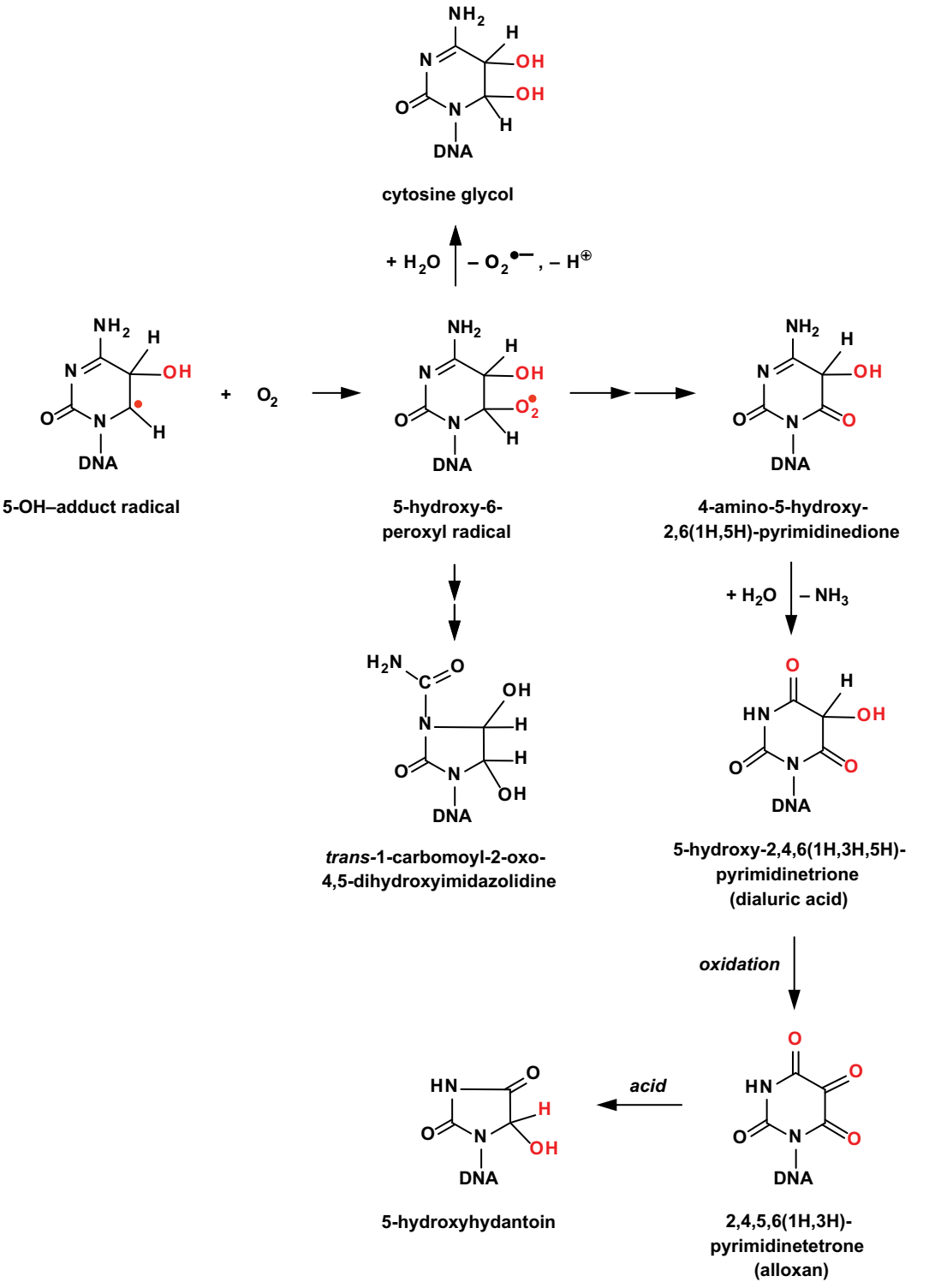

Figure 18. Mechanisms of product formation from reaction of the C5-OH–adduct radical of Cyt with O_2.

and C5′ with the former being predominant over the latter leads to strand breakage and formation of radical cations (Figure 23). Hydration (HO^- addition) of the radical cations followed by reduction and unaltered base release yields 2,3-dideoxypentos-4-ulose and 2,5-dideoxypentos-4-ulose as end groups in broken DNA chains [39,149]. The oxidation of the C4′-radical without phosphate elimination leads to a cation that gives rise to 2-deoxypentos-4-ulose within DNA upon hydration (HO^- addition) followed by unaltered base release (Figure 23). These three products are also released from DNA as free modified sugars [39,149]. The formation of 2,3-dideoxypentos-4-ulose and 2,5-dideoxypentos-4-ulose is inhibited in

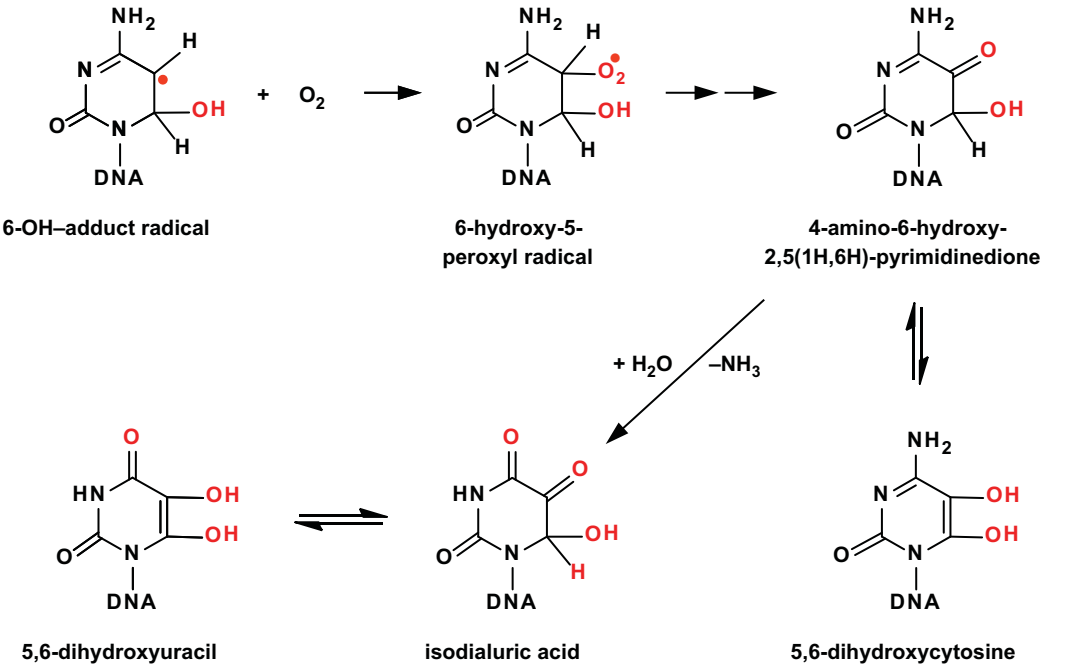

Figure 19. Mechanisms of product formation from reaction of the C6-OH–adduct radical of Cyt with O_2.

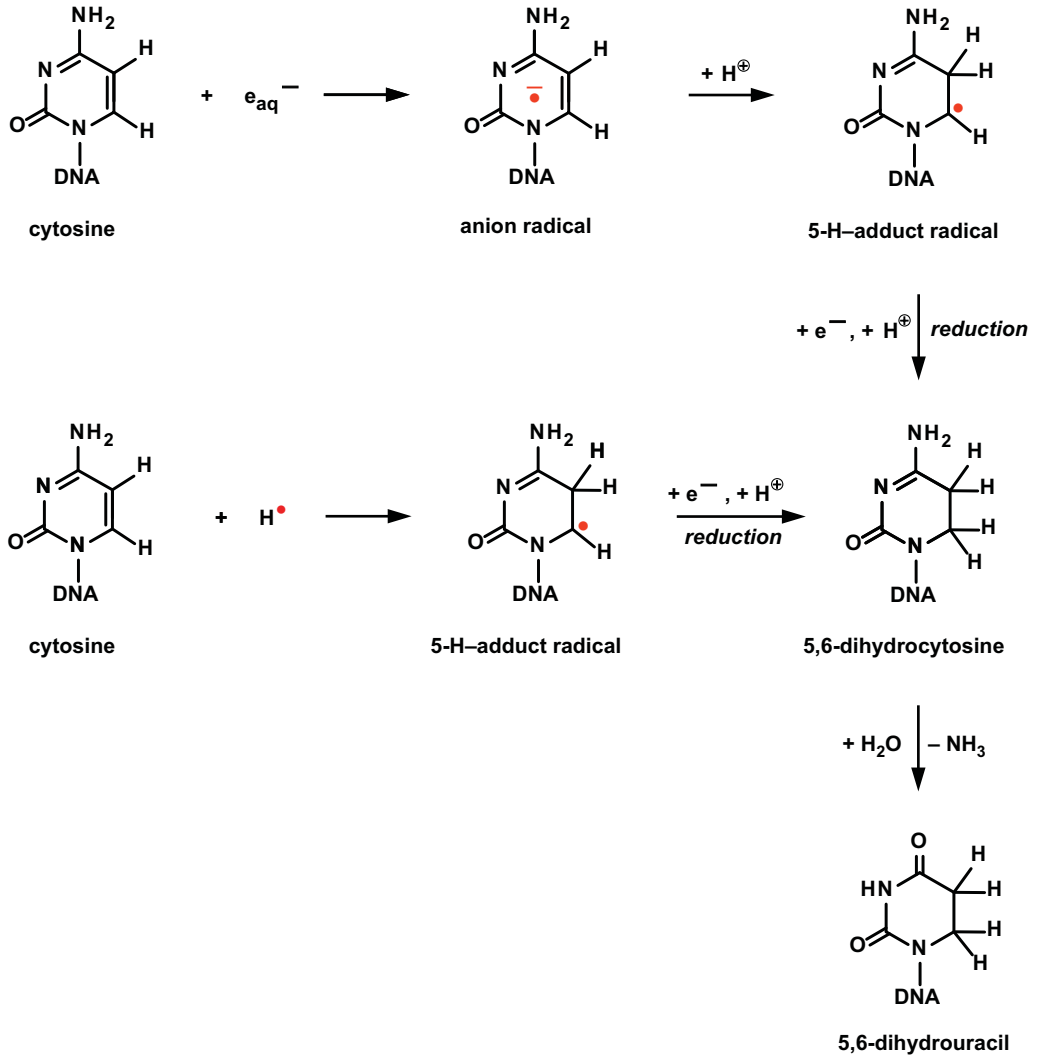

Figure 20. Reactions of Cyt with e_{aq}^- and H', leading to formation of 5,6-dihydrocytosine and 5,6-dihydrouracil.

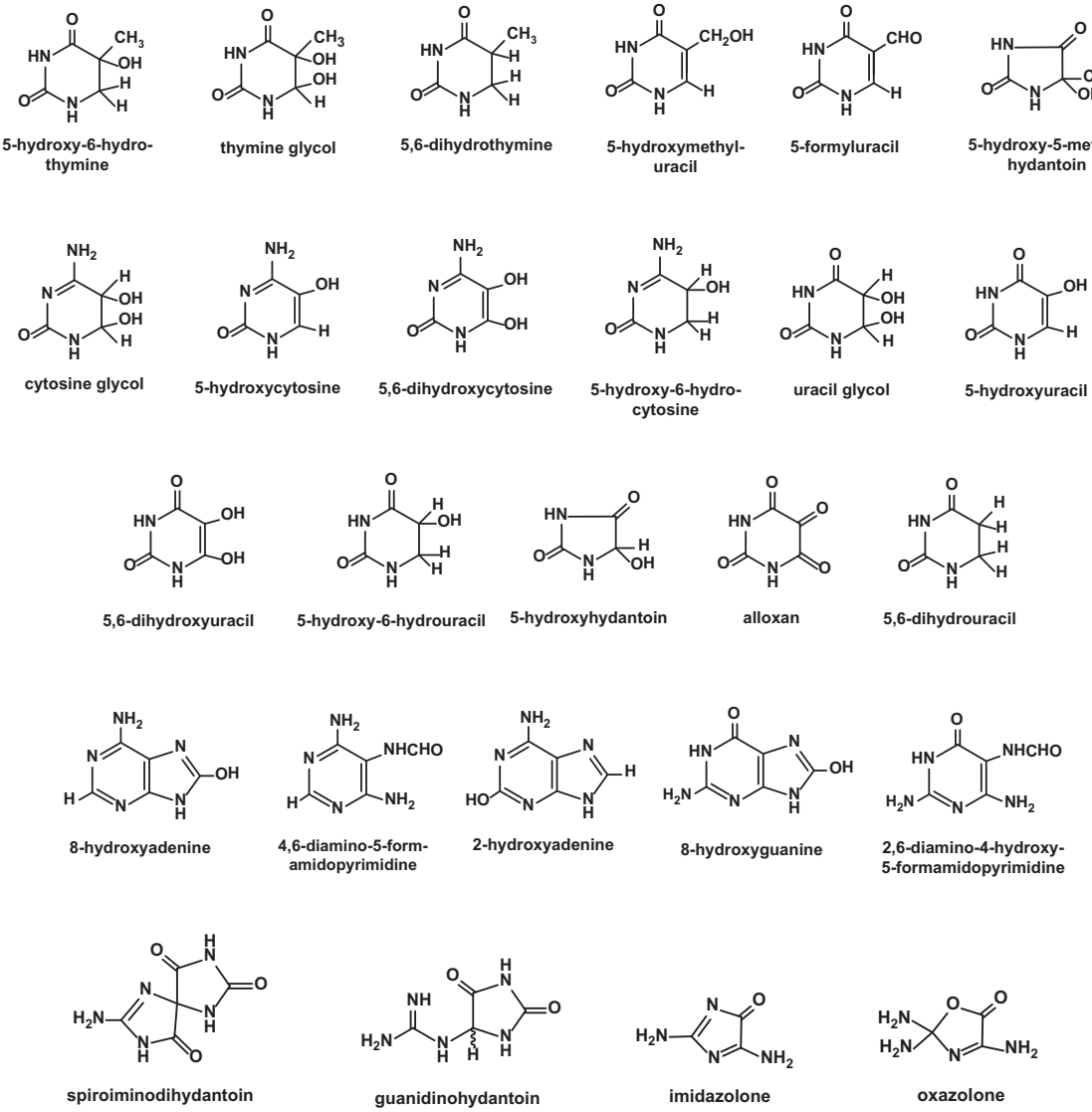

Figure 21. Structures of the major oxidatively induced products of DNA bases.

the presence of O_2, which rapidly reacts with the C4′-radical, leading to a peroxyl radical [40]. The C4′-peroxyl radical is also the precursor of 2-deoxypentos-4-ulose formed in the presence of O_2. In addition, the C4′-peroxyl radical is converted into an oxyl radical, which undergoes β-fragmentation and reaction with O_2, yielding a 3′-phosphoglycolate as an end group [2,144,150]. The C1′-radical yields 2-deoxypentonic acid within DNA upon oxidation followed by hydration (HO^- addition) and unaltered base release [151] (Figure 24). This compound is also formed in the presence of O_2. The C2′-radical reacts with O_2, generating a peroxyl radical, which is converted into an oxyl radical. The β-fragmentation of the latter followed by reaction with O_2 and by base + C1′ release, yields erythrose within DNA [152] (Figure 25). In a similar mechanism, the C5′-peroxyl radical generates 2-deoxytetradialdose as an end group of a broken DNA chain [39] (Figure 26). Moreover, the C5′-peroxyl radical leads to the formation of 5′-aldehyde as an end group with the unaltered base still attached to the altered sugar moiety [143,144]. Figure 27 illustrates the structures of the major products of 2′-deoxyribose of DNA.

Mechanisms of formation of tandem lesions

8,5′-Cyclopurine-2′-deoxynucleosides

One unique reaction of the C5′-centred 2′-deoxyribose radical in purine nucleosides is the highly stereospecific attack at C8 of the purine ring within the same purine nucleoside in the absence of O_2, leading to C5′–C8-intramolecular cyclization. The oxidation of the thus-formed N7-centred radical results in the formation of 8,5′-cyclopurine-2′-deoxynucleosides with a covalent bond between the C5′- and C8-positions. Both *R*- and *S*-diastereomers of these compounds are formed. This reaction has been first discovered by Keck to take place within adenosine-5′-monophosphate

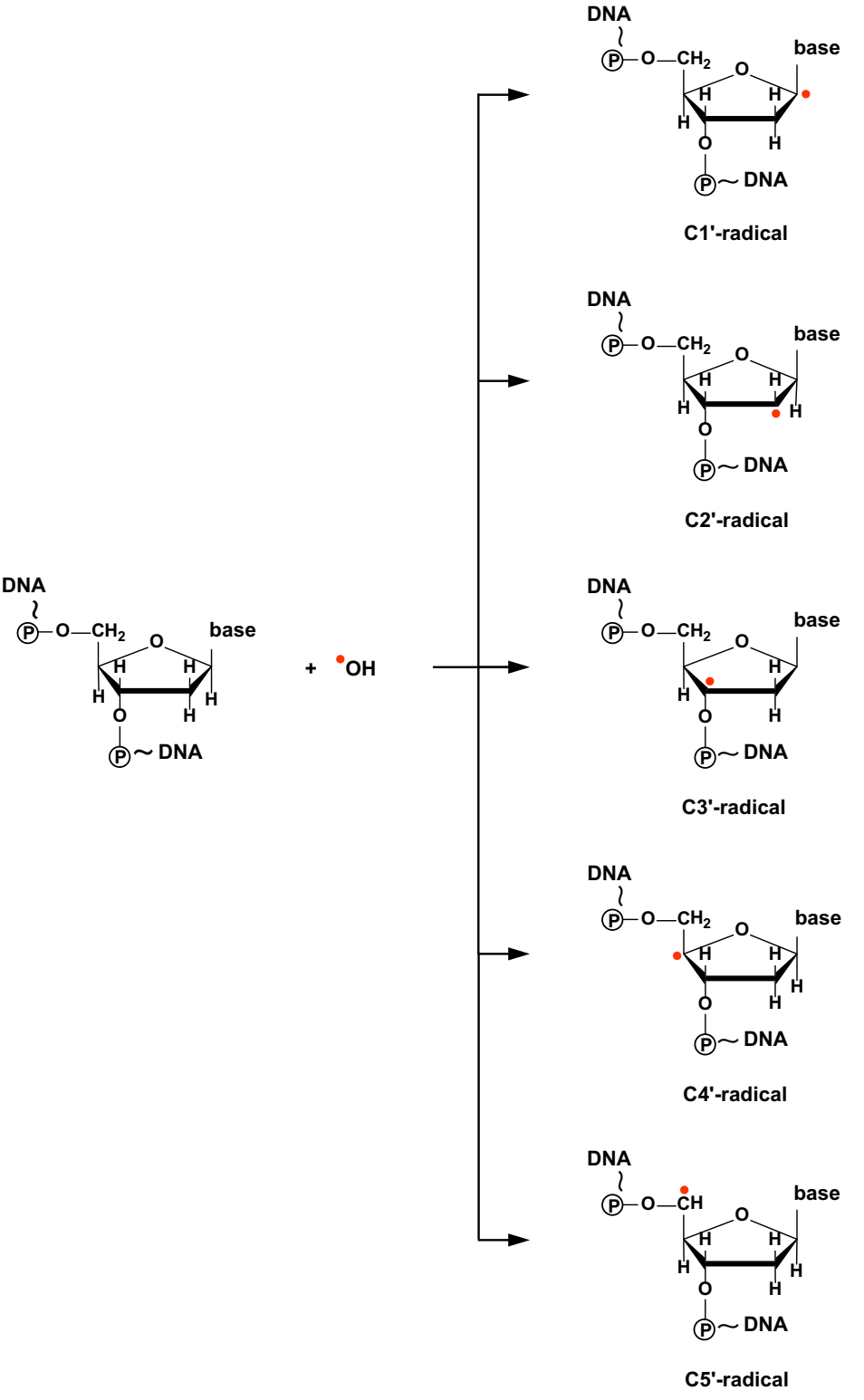

Figure 22. H•-abstraction by •OH from 2′-deoxyribose in DNA.

(AMP), giving rise to 8,5′-cyclo-AMP [153]. The intramolecular cyclization is supported by the fact that the C8 of purines are particularly reactive toward radical attack [154]. Subsequent studies showed the formation of 8,5′-cycloadenosine (cA) and 8,5′-cyclo-2′-deoxyadenosine (cdA) in polyadenylic acid (polyA) and in 2′-deoxyadenosine, respectively [155–164]. Hydroxyl radical has been shown to be the initiating radical species of C5′–C8-intramolecular cyclization. Both *R*- and *S*-diastereomers of 8,5′-cyclo-2′-deoxy guanosine (cdG) and cdA have subsequently been identified in DNA upon exposure to ionizing radiation in aqueous solution and to the antitumour agent 3-amino-1,2,4-benzotriazine 1,4-dioxide (Tirapazamine)

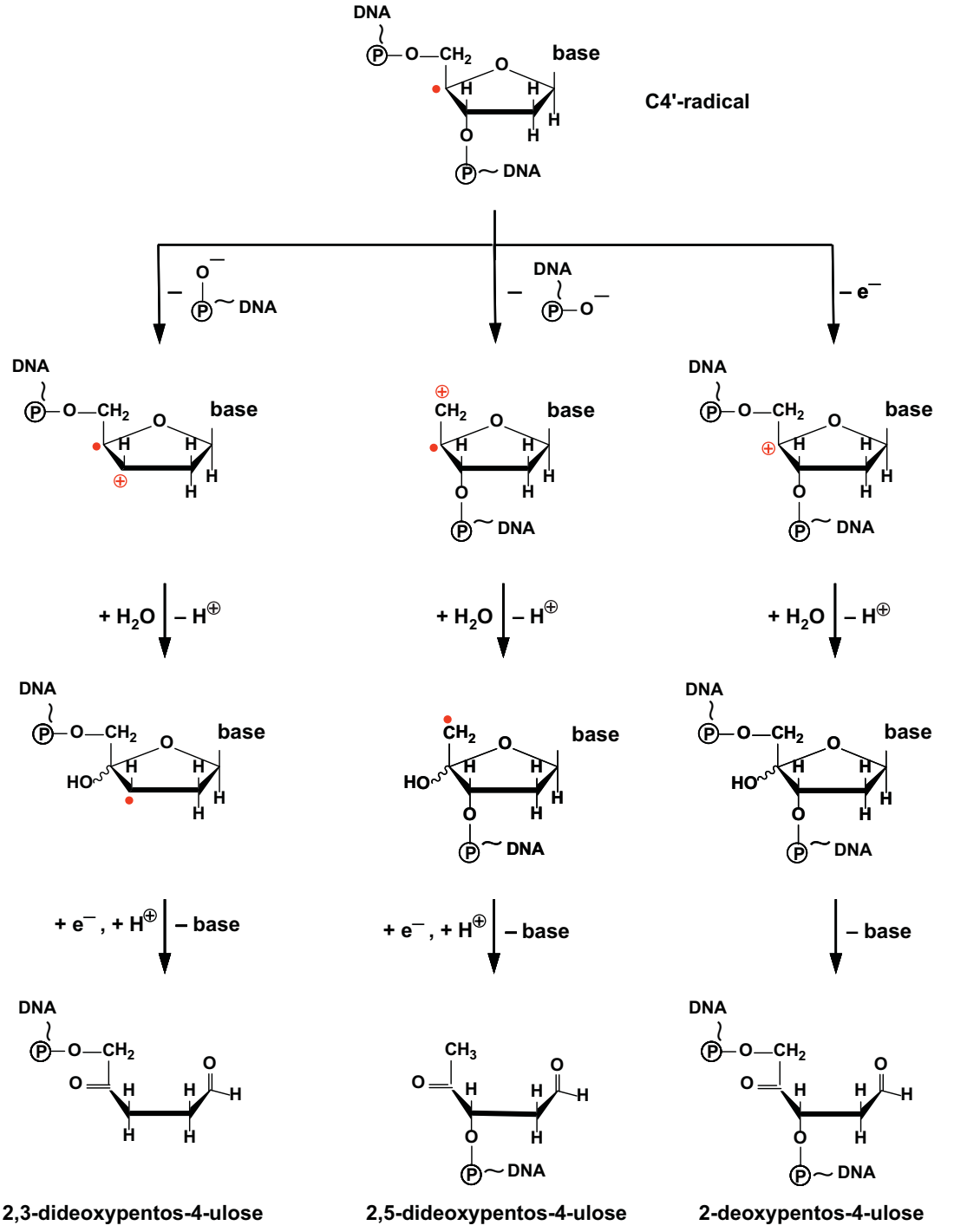

Figure 23. Mechanisms of product formation from reactions of the C4′-radical of 2′-deoxyribose, leading to 2-deoxypentose-4-ulose within DNA, and 2,3-dideoxypentose-4-ulose and 2,5-dideoxypentose-4-ulose as end groups of a broken DNA strand.

[165–167]. The formation of *R*-cdG and *S*-cdG has also been demonstrated in γ-irradiated human cells [168].

The mechanisms of formation of 8,5′-cyclopurine-2′-deoxynucleosides is shown in Figure 28 in the case of dA. This figure also illustrates the structures of the *R*- and *S*-diastereomers of cdG, which are produced by analogous reactions of dG. These compounds represent a concomitant damage to both the base and sugar moieties of the same nucleoside, and thus, are regarded as tandem lesions in DNA. The rate constants for the C5′–C8-intramolecular cyclization amount to 1.6×10^5 s^{-1} for dA and ~1×10^6 s^{-1} for dG, respectively [169–171]. This reaction is inhibited by O_2 because of its reaction with the C5′-centred radical at a near diffusion-controlled reaction rate ($k \approx 1.9 \times 10^9$ dm^3 mol^{-1} s^{-1}) [2,155,169]. However, the formation of both diastereomers has been observed at low liquid-phase O_2 concentrations [172–174].

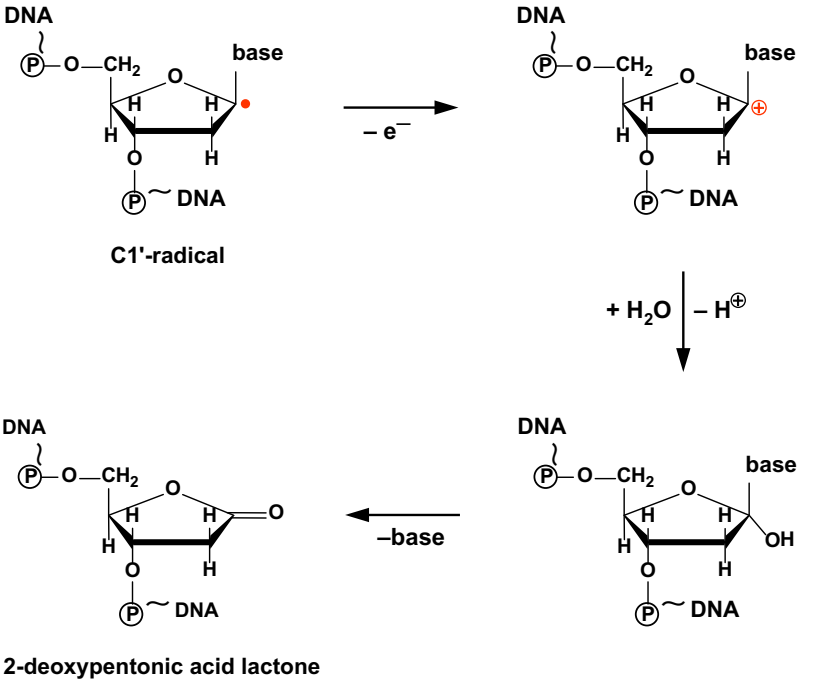

Figure 24. Mechanisms of product formation from oxidation of the C1′-radical of 2′-deoxyribose, leading to 2-deoxypentonic acid lactone within DNA.

This fact suggests that a competition may take place between the C5′–C8-cyclization and the reaction of O_2 with the C5′-centred radical depending on O_2 concentration. Because of hypoxic conditions of the cell nucleus [60,61], and possible steric hindrances, this competition may occur in living cells. Reactions of the C5′-radicals of dA and dG with glutathione by H• abstraction may also compete with the C5′–C8-cyclization because of the high intracellular concentration of glutathione [175], and because of the rapid reaction of the C5′-radical with glutathione ($k = 5 \times 10^7$ $dm^3\ mol^{-1}\ s^{-1}$; measured using dA) [173]. In general,

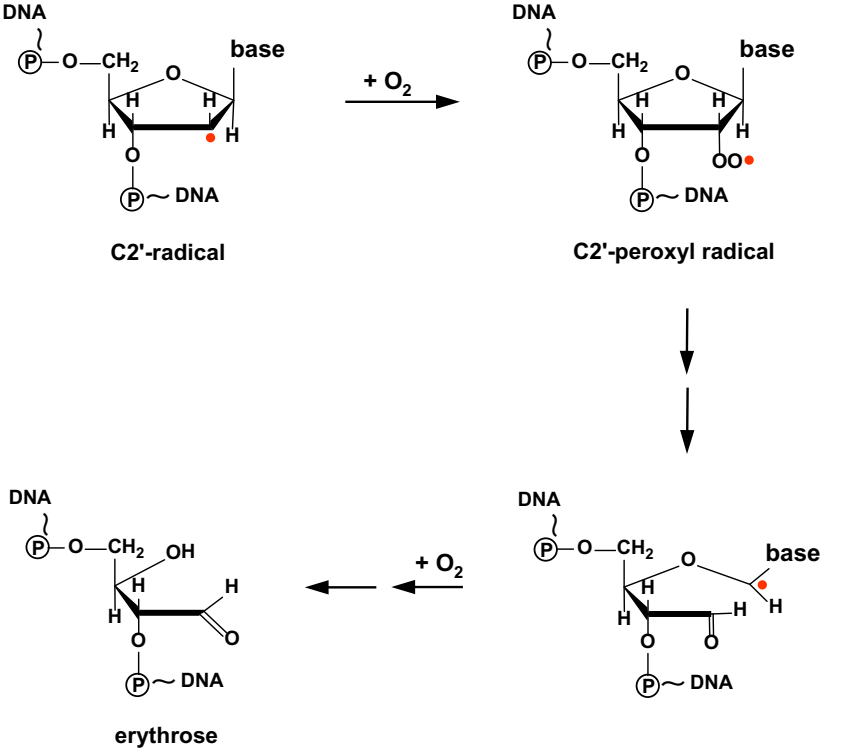

Figure 25. Mechanisms of product formation from reaction of the C2′-radical of 2′-deoxyribose with O_2, leading to erythrose within DNA.

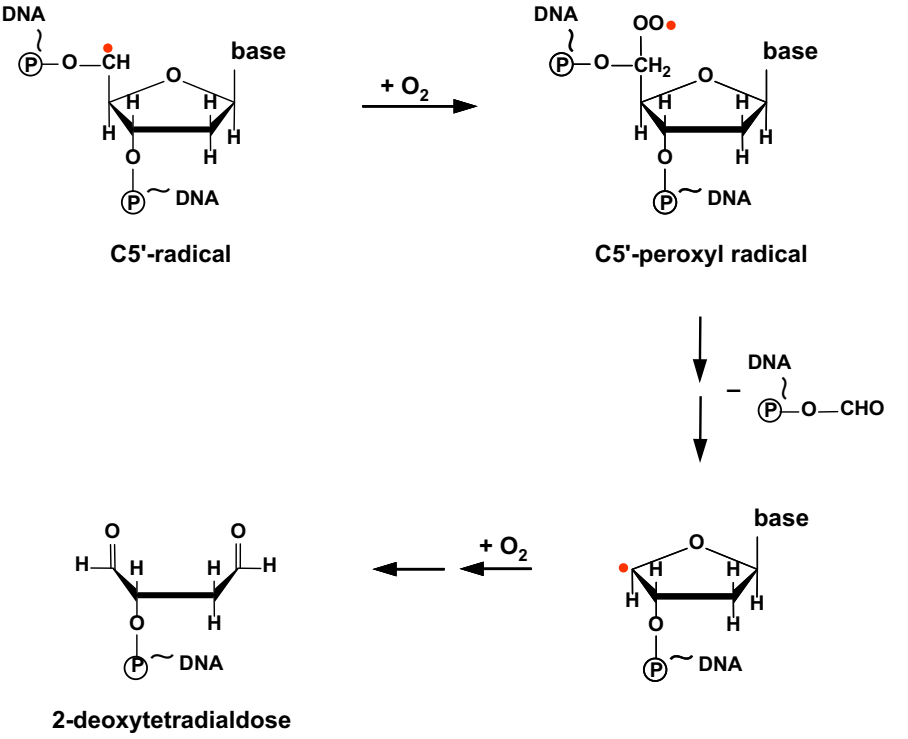

Figure 26. Mechanisms of product formation from reaction of the C5′-radical of 2′-deoxyribose with O_2, leading to 2-deoxytetradialdose as an end group of a broken DNA strand.

such radicals rapidly react with thiols ($k \approx 1 \times 10^8$ $dm^3\ mol^{-1}\ s^{-1}$) [2,11].

The substrate, experimental conditions and DNA conformation substantially affect the ratio of the *R*- and *S*-diastereomers of cA, cdA and cdG [156,160, 161,163,165,166,169,170,176–185]. The *R*-diastereomers predominate over the *S*-diastereomers in ss-DNA, whereas the formation of the *S*-diastereomers is favoured over that of the *R*-diastereomers in ds-DNA [166]. These data are on a par with the results of quantum chemical calculations [179]. The C5′–C8-cyclization causes an unusual puckering of the sugar moiety [158,171,177,179,186]. The length of the C2′–C3′-, C3′–C4′- and C4′–C5′-bonds become longer than those in the nucleoside and O4′ is located closer to the atoms of the purine ring when compared to normal nucleosides. The C5′–C8-cyclization requires the purine ring to rotate around the glycosidic bond, bringing C5′ and C8 close enough to form the C5′–C8-covalent bond. Moreover, it causes large changes in backbone torsion angles, resulting in weakening the hydrogen bonds and substantial perturbations of the double helix near the lesion [179]. The *R*- and *S*-diastereomers cause an equal degree of DNA distortion.

8,5′-Cyclopurine-2′-deoxynucleosides exist in living cells *in vivo* at background levels and are also formed under a variety of conditions. The formation and identification of *R*-cdG and *S*-cdG in cultured human exposed to ionizing radiation was first reported in 1987 [168]. Since then, these compounds have been identified in cultured mammalian cells, human and animal tissues *in vivo*, and in human urine in a variety of conditions such as disease states, gene knockouts and exposure to ionizing radiation or environmental pollutants [182,187–202]. A more extensive review of these findings can be found elsewhere [203].

Pyrimidine 2′-deoxynucleosides also undergo intramolecular cyclization upon exposure to ionizing radiation in frozen or liquid aqueous solution. 5′,6-Cyclo-5,6-dihydro-2′-deoxythymidine and 5′,6-cyclo-5,6-dihydro-2′-deoxyuridine have been identified in frozen aqueous solutions of dT and dC exposed to ionizing radiation [204]. The direct effect of ionizing radiation produces these products. The mechanism involves an H atom removal from C5′ by radiation followed by intramolecular attack of the thus-formed C5′-centred radical at C6 leading to C5′–C6-cyclization and a 5-yl radical, and subsequent electron transfer and protonation. In another instance, two diastereomers of 5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine have been identified in aerated aqueous solutions of 2′-deoxycytidine exposed to γ-radiation [205]. The proposed mechanism involves H$^{\bullet}$ abstraction by $^{\bullet}$OH from C5′ followed by C5′–C6-cyclization, reaction with O_2 and then deamination. Thus far, 5′,6-cyclo-5,6-dihydro-2′-deoxythymidine, 5′,6-cyclo-5,6-dihydro-2′-deoxyuridine and 5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine have

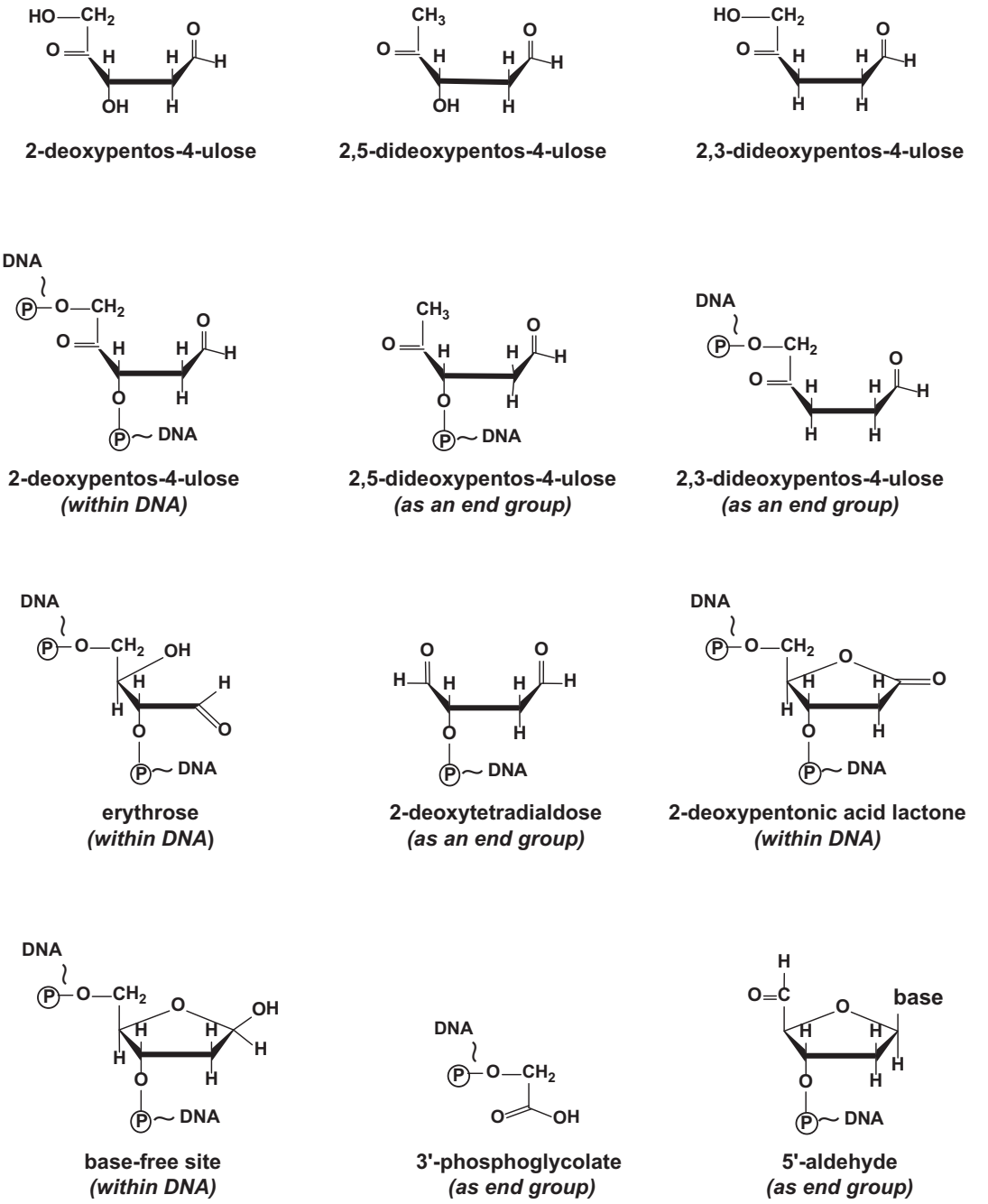

Figure 27. Structures of the major oxidatively induced products of the 2′-deoxyribose moiety of DNA.

not been identified in DNA exposed to ionizing radiation or any other DNA-damaging agents.

Adjacent, interstrand and intrastrand base-base tandem lesions

Besides the lesions discussed above, three other types of tandem lesions have been identified in oligodeoxynucleotides and DNA exposed to ionizing radiation or other •OH-generating systems: (1) Two adjacent damaged bases on the same strand; (2) An intrastrand cross-link between two adjacent DNA bases on the same strand; (3) An interstrand cross-link between two DNA bases on opposite strands. A tandem lesion consisting of an 8-OH-Gua and a formamido residue (8-OH-Gua/Fo) has been identified in d(GpT) exposed to •OH in the presence of O_2 [206]. Subsequent work observed the same type of reactions with d(GpC), d(TpG), d(CpG) and d(CpGpTpA), indicating Fo is also formed from Cyt next to 8-OH-Gua [178,206–210]. These lesions have been suggested to be formed from a single radical event initiated by ionizing radiation or other •OH-producing systems. The formation of 8-OH-Gua/Fo has also been shown in DNA exposed to ionizing radiation or to Fe^{2+}/H_2O_2, but as two types, namely 8-OH-Gua/Fo and

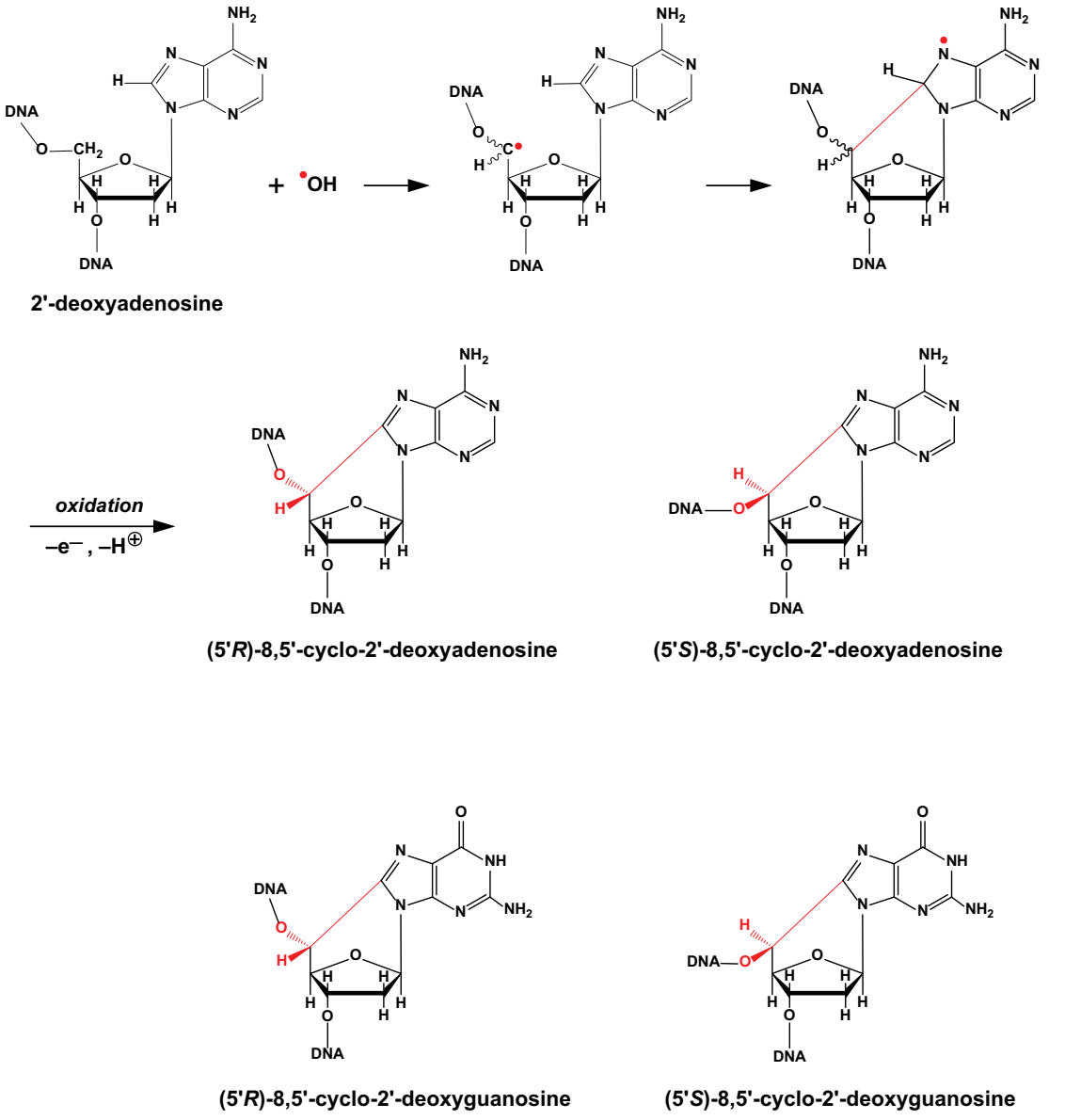

Figure 28. Mechanisms of formation of (5′*R*)- and (5′*S*)-8,5′-cyclopurine-2′-deoxyadenosines within DNA. Also shown are the structures of (5′*R*)- and (5′*S*)-8,5′-cyclopurine-2′-deoxyguanosines that result from analogous reactions of dG.

Fo/8-OH-Gua with the yield of the former being considerably greater than that of the latter [211–216]. Cadet et al. originally suggested a mechanism that involved the one-electron oxidation of a neighbouring Gua by the C5-OH-C6-peroxyl radical of Thy followed by hydration of $Gua^{\bullet+}$ and oxidation to form 8-OH-Gua and the decomposition of the C5-OH-C6-oxyl radical of Thy yielding Fo [214]. However, this mechanism has been dismissed as a very unlikely one because of the significantly lower reduction potential of a peroxyl radical than that of Gua rendering this reaction endothermic [2]. Subsequent work proposed a mechanism that involves $^{\bullet}OH$ addition to the C5 of Thy (or Cyt) followed by a peroxyl radical formation in reaction with O_2. The C5-OH-C6-peroxyl radical then attacks the C8 of Gua and gives rise to an N-centred radical, which does not react with O_2 and undergoes a 1,2-shift reaction yielding a C8-centred radical. A β-cleavage then takes place generating 8-OH-Gua and an oxyl radical at Thy or Cyt, which decomposes yielding Fo [215]. These studies also showed that these two lesions may contribute about 10% to the overall yield of 8-OH-Gua in DNA. In contrast, another study found an order of magnitude higher yield for Fo/8-OH-Gua, the formation of which has also been observed in the absence of O_2, albeit with a much lower yield [212]. However, such lesions have not yet been identified in cellular DNA [130].

In the absence of O_2, an intrastrand cross-link formation between the C8 of Gua and the CH_3 group

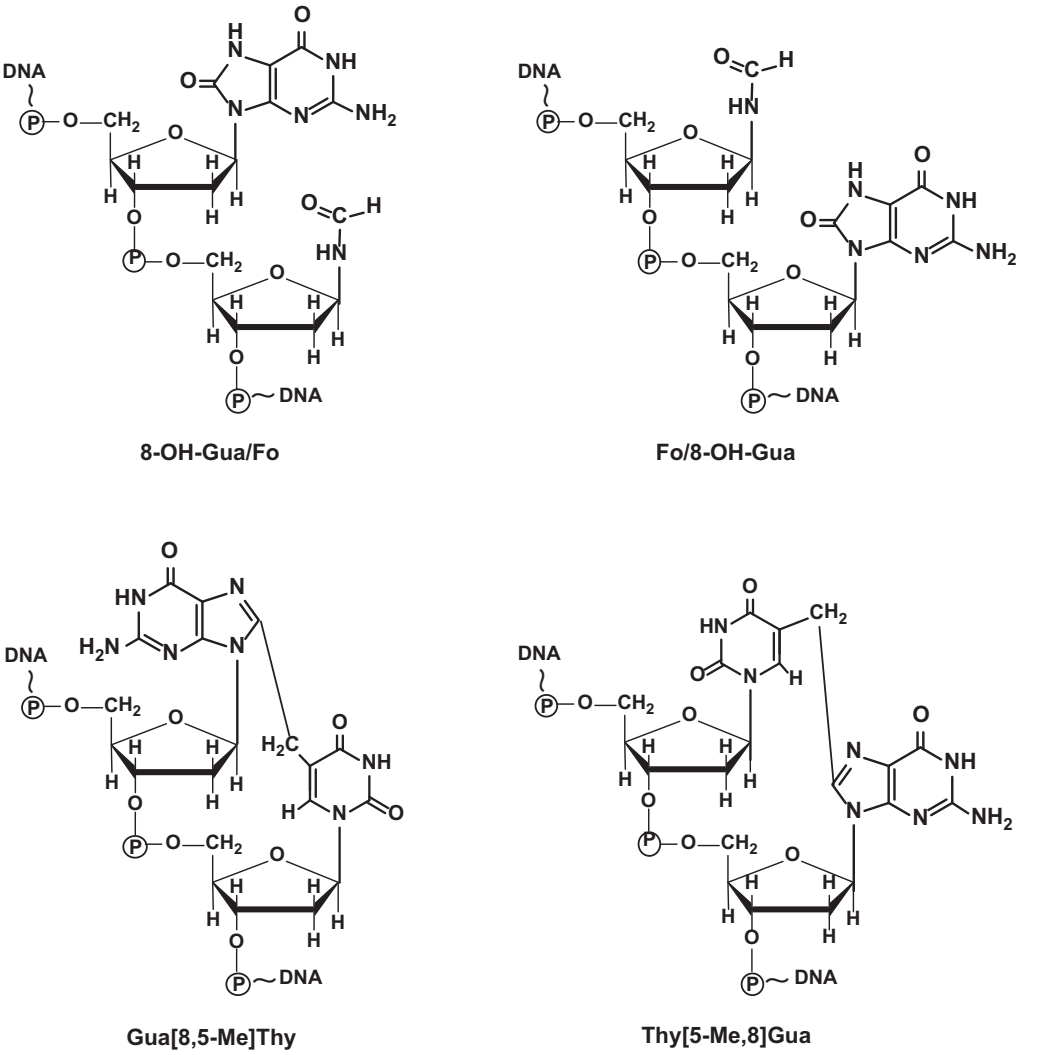

Figure 29. Structures of the tandem lesions 8-OH-Gua/Fo, Fo/8-OH-Gua, Gua[8,5-Me]Thy and Thy[5-Me,8]Gua.

of Thy (Gua[8,5-Me]Thy) has been detected in d(CpGpTpA) and similar oligomers exposed to ionizing radiation [210,217,218]. The proposed mechanism consists of the addition of the allyl radical of Thy to the C8 of Gua forming an N7-centred, followed by oxidation. Additional intrastrand covalent cross-links have been observed between the C5 of Thy and the C8 of Gua, and between the C5 of Cyt and the C8 of Gua [210,218]. Subsequent studies identified Gua[8,5-Me]Thy and an analogous Thy-Gua cross-link (Thy[5-Me,8]Gua) in DNA with the former generated at a much higher yield than the latter, indicating that cross-linking is favoured when the purine is located at the 5′-end of the pyrimidine 2′-deoxynucleoside [211,219–224]. In addition, Ade-Thy (Ade[8,5-Me]Thy) and Thy-Ade (Thy[5-Me,8]Ade) cross-links have been identified in γ-irradiated DNA [220,225]. As in the case of Gua-Thy cross-links, Ade[8,5-Me]Thy was generated in a greater yield than Thy[5-Me,8]Ade. Cross-links between the allyl radical of 5-Me-Cyt and the C8 of purines have also been observed [226–228]. Gua[8,5-Me]Thy and another cross-link between Gua and Cyt (Gua[8,5]Cyt) have been identified in γ-irradiated living cells [229,230]. Similar to the Cyt-Gua crosslink identified in oligomers [218], the proposed formation mechanism of Gua[8,5]Cyt involves the addition of the C6-OH–adduct radical of Cyt to the C8 of adjacent Gua on the 5′-end forming an N7-centred, followed by oxidation and dehydration. In addition, an interstrand cross-link has been reported to occur between the allyl radical of Thy on one strand and the amino group of Ade on the other strand of DNA exposed to $^{\bullet}OH$ [231–234]. The mechanism has been worked out using isotopic labelling and consists of the addition of the allyl radical to the N1-position of Ade followed by rearrangement leading to a covalent bond between the CH_2 of Thy and the 6-NH of Ade [234]. This interstrand cross-link has been observed in the presence and absence of O_2, although the yield was lower in the latter case. This is surprising, because

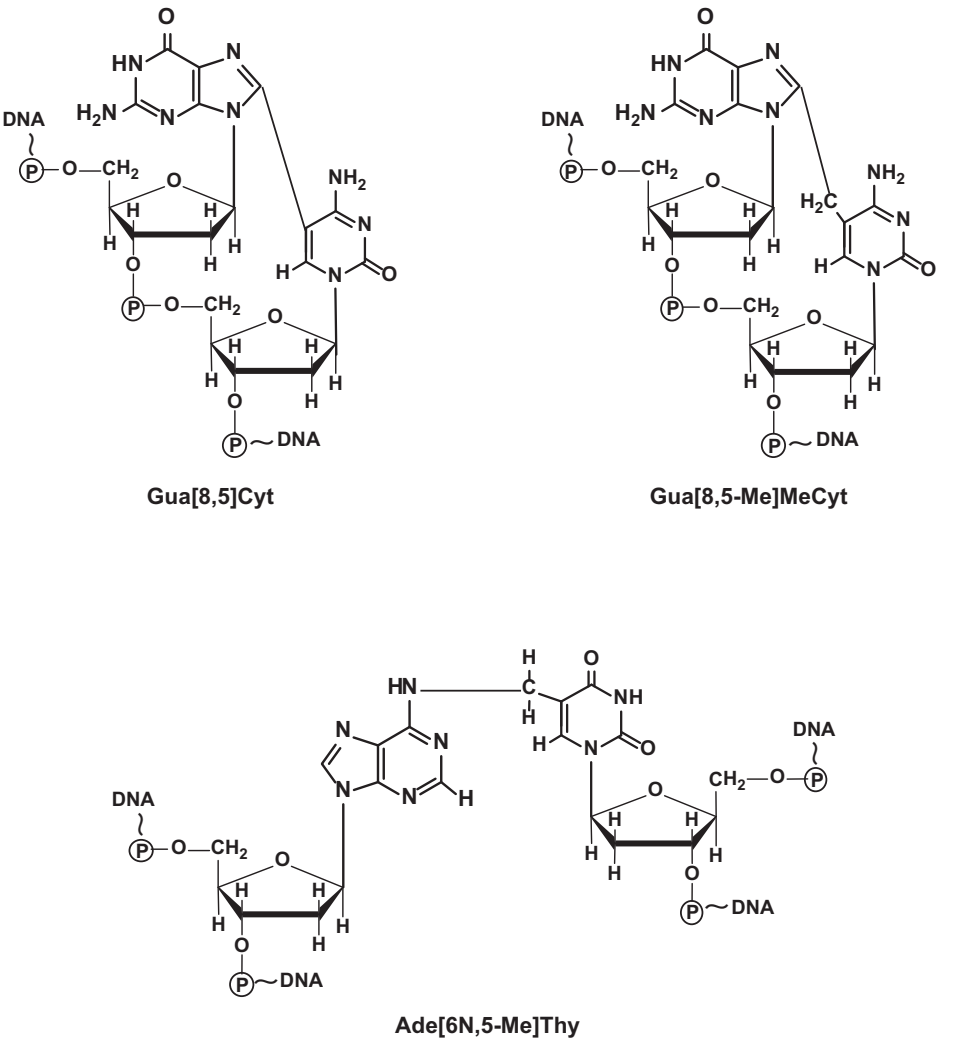

Figure 30. Structures of the intrastrand tandem lesions Gua[8,5]Cyt and Gua[8,5-Me]Cyt, and the interstrand tandem lesion Ade[6N,5-Me]Thy.

O_2 would react with the allyl radical at a diffusion-controlled rate. However, the close proximity of Ade and Thy in the double helix, which is required for cross-linking, and steric hindrances may prevent the reaction of O_2 with the allyl radical of Thy. Figures 29 and 30 illustrate the structures of the tandem lesions discussed above.

Clustered DNA damage

Another type of tandem lesions is the clustered damage in DNA produced by ionizing radiation. These lesions are also known as locally multiply damaged sites [235]. Clustered lesions can be tandem on the same strand or on opposite strands of DNA within one or two helical turns of DNA and are distinct from DNA double-strand breaks (DSBs) [235–247]. These lesions are produced almost exclusively by ionizing radiations [248,249]. Endogenously induced damage to DNA appears to be a quite unlikely source for them. Processing of diverse clustered lesions in living cells depends on the type of lesions, distance between lesions, presence of strand breaks, etc. For example, bistranded or tandem clusters may be resistant to repair by DNA glycosylases or endonucleases, and thus persist in cells for a significant time period [244]. Two closely spaced DNA lesions may generate DSBs during DNA repair processes. A greater accumulation of clustered lesions may occur depending on the mutation frequency, DNA repair capacity and genomic instability.

Mechanisms of DNA-protein cross-linking

Free radical reactions with chromatin cause formation of covalent DNA-protein cross-links in mammalian cells [250–255]. There is evidence for the involvement of ‘OH in the formation of DNA-protein cross-links induced by ionizing radiation or by H_2O_2/metal ions [253–256]. A Thy-Tyr cross-link has been found as a major product in γ-irradiated mixtures of Thy and Tyr in deoxygenated aqueous solution [257,258]. Subsequent work elucidated the structure

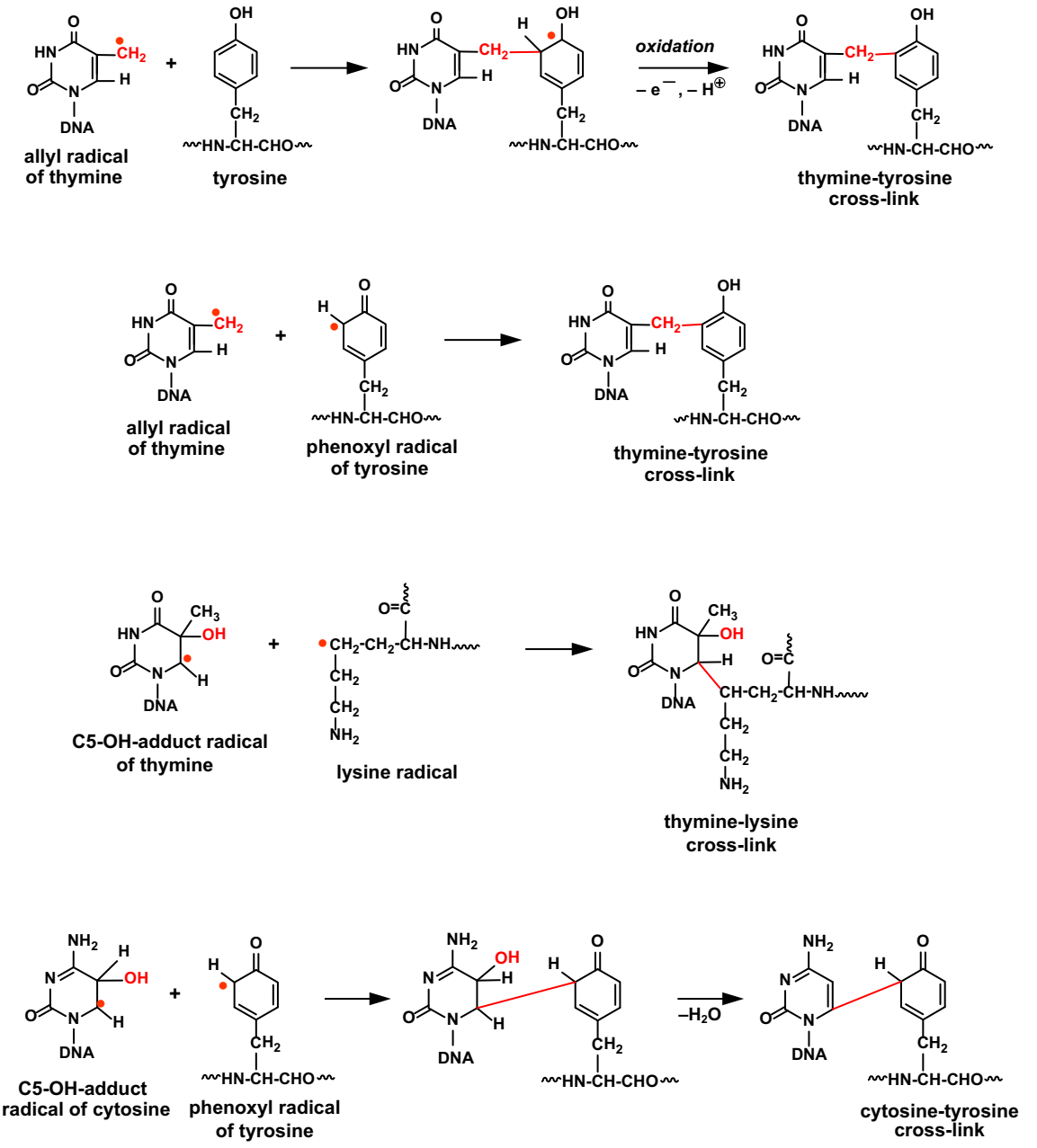

Figure 31. Mechanisms of DNA-protein cross-linking involving Thy, Cyt, Tyr and Lys.

of this cross-link and showed that the covalent cross-linking takes place between the allyl radical of Thy and the C3 of the Tyr ring [259–262]. The Thy-Tyr cross-link 3-[(1,3-dihydro-2,4-dioxopyrimidin-5-yl)-methyl]-L-tyrosine) has also been found in mammalian chromatin upon exposure to ionizing radiation in deoxygenated aqueous solution [263]. Two different mechanisms have been proposed for its formation: (1) The addition of the allyl radical of Thy to the C3 of the Tyr ring forming a C-centred radical, followed by oxidation; (2) The combination of the allyl radical of Thy with the phenoxyl radical of Tyr as shown in Figure 31. The latter radical is well known to be formed by addition of $^{\bullet}OH$ to the C3 of the Tyr ring followed by H_2O elimination [264]. The phenoxyl radical of Tyr is also formed by a reaction between $Gua^{\bullet+}$ and Tyr via charge transport leading to DNA-protein cross-links [265,266]. The first mechanism requires the close proximity of the allyl radical of Thy to a Tyr molecule in the DNA-protein complex. Hendry et al. reported the possible formation of a unique H-bond between the OH group of Tyr and the oxygen at the C4 of Thy [267]. This may permit the close proximity of the methyl group of Thy to the C3- of Tyr. The final product of both mechanisms is the same. Therefore, these two mechanisms cannot be distinguished from each other by the final product. However, the mechanism initiated by one radical (allyl radical) is more likely to be valid than the radical-radical combination mechanism, because the latter

requires the simultaneous formation of two adjacent radicals. The treatment of mammalian chromatin with H_2O_2 plus Fe^{3+}, Fe^{3+}-chelates, Cu^{2+} or Cu^{2+}-chelates also yielded the Thy-Tyr cross-link [268]. Oxygen did not inhibit the cross-linking. The reason may well be that the allyl radical of Thy adds to Tyr in close proximity immediately after its formation, before O_2 can react with it. The results obtained with the use of •OH scavengers supported this site-specific nature of cross-linking [268]. This notion is on a par with the site-specific generation of •OH upon reaction of chromatin-bound metal ions with H_2O_2 [269–271]. This means that •OH may be generated in the vicinity of Thy and Tyr in chromatin so that scavengers may not be able to completely scavenge •OH. Other DNA-protein cross-links between Thy and amino acids such as Gly, Ala, Val, Leu, Ileu, Thr and Lys, and between Cyt and Tyr have been observed in mammalian chromatin *in vitro* upon exposure to ionizing radiation in the absence of oxygen [272–275]. Figure 31 illustrates the proposed mechanisms for the formation of Thy-Lys and Cyt-Tyr cross-links. The addition of the phenoxyl radical to the C5-C6–double bond of Cyt is also a possible mechanism for the formation of Cyt-Tyr cross-links [276]. In subsequent studies, the formation of the Thy-Tyr cross-link has been observed in cultured mammalian cells exposed to ionizing radiation, H_2O_2, or Fe^{2+}-ions [277,278], and in renal chromatin of Wistar rats *in vivo* upon treatment with a renal carcinogen, ferric nitrilotriacetic acid [279].

Concluding remarks

This review shows that free radical-induced damage to DNA is rather complex and includes a large variety of different mechanisms and final products. The findings are the result of extensive investigations by many researchers and laboratories around the world that had been conducted for the past 50 years or so. Of course, this field of research also includes the measurement, cellular repair, biological consequences and role in disease processes of the final products. The present article deals with the mechanistic aspects only. The other articles in this series will no doubt deal with the mechanisms as well and the remaining aspects of free radical-induced damage to DNA. Evidence accumulated over many years point to an important role of free radical-induced DNA damage in the etiology of cancer and other diseases. There are still many unknowns in this field. More research will lead to our enhanced understanding of cellular repair mechanisms of DNA damage and disease processes, to the discovery of disease biomarkers of DNA damage for risk assessment, early detection and therapy monitoring, and to the development of drugs for DNA damage-based treatments.

Acknowledgement

Some of the figures in this article were adapted from original papers. Appropriate references related to those figures were given in the text.

Declaration of interest

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of the paper.

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