REVIEW ARTICLE

Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer

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INTRODUCTION

It is increasingly proposed that reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a key role in human cancer development [1–6], especially as evidence is growing that antioxidants may prevent or delay the onset of some types of cancer (reviewed in [7,8]). ROS is a collective term often used by biologists to include oxygen radicals [superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$) and alkoxyl ($RO^{\bullet}$)] and certain non-radicals that are either oxidizing agents and/or are easily converted into radicals, such as HOCl, ozone (O_3), peroxynitrite ($ONOO^-$), singlet oxygen ($^1O_2$) and H_2O_2. RNS is a similar collective term that includes nitric oxide radical ($NO^{\bullet}$), $ONOO^-$, nitrogen dioxide radical ($NO_2^{\bullet}$), other oxides of nitrogen and products arising when $NO^{\bullet}$ reacts with $O_2^{\bullet -}$, $RO^{\bullet}$ and $RO_2^{\bullet}$. 'Reactive' is not always an appropriate term; H_2O_2, $NO^{\bullet}$ and $O_2^{\bullet -}$ react quickly with very few molecules, whereas $OH^{\bullet}$ reacts quickly with almost anything. $RO_2^{\bullet}$, $RO^{\bullet}$, HOCl, $NO_2^{\bullet}$, $ONOO^-$ and O_3 have intermediate reactivities. ROS and RNS have been shown to possess many characteristics of carcinogens [4] (Figure 1). Mutagenesis by ROS/RNS could contribute to the initiation of cancer, in addition to being important in the promotion and progression phases. For example, ROS/RNS can have the following effects.

(1) Cause structural alterations in DNA, e.g. base pair mutations, rearrangements, deletions, insertions and sequence amplification. $OH^{\bullet}$ is especially damaging, but $^1O_2$, $RO_2^{\bullet}$, $RO^{\bullet}$, HNO_2, O_3, $ONOO^-$ and the decomposition products of $ONOO^-$ are also effective [9–13]. ROS can produce gross chromosomal alterations in addition to point mutations and thus could be involved in the inactivation or loss of the second wild-type allele of a mutated proto-oncogene or tumour-suppressor gene that can occur during tumour promotion and progression, allowing expression of the mutated phenotype [4].

(2) Affect cytoplasmic and nuclear signal transduction pathways [14,15]. For example, H_2O_2 (which crosses cell and organelle membranes easily) can lead to displacement of the inhibitory subunit from the cytoplasmic transcription factor nuclear factor κB, allowing the activated factor to migrate to the nucleus [14]. Nitration of tyrosine residues by $ONOO^-$ may block phosphorylation.

(3) Modulate the activity of the proteins and genes that respond to stress and which act to regulate the genes that are related to cell proliferation, differentiation and apoptosis [4,14–17]. For example, H_2O_2 can stimulate transcription of c-*jun* [18] and can activate mitogen-activated protein kinase in NIH 313 cells [19].

CHEMISTRY OF DNA DAMAGE

The endogenous reactions that are likely to contribute to ongoing DNA damage are oxidation, methylation, depurination and deamination [1,2]. Nitric oxide or, more likely, reactive products derived from it, such as $NO_2^{\bullet}$, $ONOO^-$, N_2O_3 and HNO_2, are mutagenic agents, with the potential to produce nitration, nitrosation and deamination reactions on DNA bases [3,6]. Methylation of cytosines in DNA is important for the regulation of gene expression, and normal methylation patterns can be altered during carcinogenesis [20]. Conversion of guanine to 8-hydroxyguanine (Figure 2), a frequent result of ROS attack [9,10,21], has been found to alter the enzyme-catalysed methylation of adjacent cytosines [20], thus providing a link between oxidative DNA damage and altered methylation patterns.

The chemistry of DNA damage by several ROS has been well characterized *in vitro* [9,11–13,21], although more information is needed about the changes produced by $RO_2^{\bullet}$, $RO^{\bullet}$, O_3, $ONOO^-$ and several of the RNS. Different ROS affect DNA in different ways, e.g. $O_2^{\bullet -}$ and H_2O_2 do not react with DNA bases at all [9,10]. $OH^{\bullet}$ generates a multiplicity of products from all four DNA bases and this pattern appears to be a diagnostic 'fingerprint' of $OH^{\bullet}$ attack [10]. By contrast $^1O_2$ selectively attacks guanine [13,22]. The most commonly produced base lesion, and the one most often measured as an index of oxidative DNA damage, is 8-hydroxyguanine (8-OHG). It is sometimes measured as the nucleoside, 8-hydroxydeoxyguanosine (8-OHdG) [2,23]. These assay methods have been reviewed in detail [9,10,23,24]. Figure 2 shows the structures of the products of ROS attack on DNA.

Damage to DNA by ROS/RNS appears to occur naturally, in that low steady-state levels of base damage products have been detected in nuclear DNA from human cells and tissues [2,23–26]. The pattern of damage to the purine and pyrimidine bases (Figure 2) suggests that at least some of the damage occurs by $OH^{\bullet}$ attack, suggesting that $OH^{\bullet}$ is formed in the nucleus *in vivo* [24].

MITOCHONDRIAL DNA DAMAGE

ROS/RNS can also damage mitochondrial DNA, and such damage has been suggested to be important in several human

Abbreviations used: ROS, reactive oxygen species; RNS, reactive nitrogen species; $O_2^{\bullet -}$, superoxide radical; $OH^{\bullet}$, hydroxyl radical; $RO_2^{\bullet}$, peroxyl radical; $RO^{\bullet}$, alkoxyl radical; O_3, ozone; $ONOO^-$, peroxynitrite; $^1O_2$, singlet oxygen; $NO^{\bullet}$, nitric oxide radical; $NO_2^{\bullet}$, nitrogen dioxide radical; GC-MS, gas chromatography/MS; 8-OHG, 8-hydroxyguanine; 8-OHdG, 8-hydroxydeoxyguanosine; PARP, poly(ADP-ribose) polymerase; AP, apurinic/apyrimidinic; XP, xeroderma pigmentosum; SOD, superoxide dismutase; MnSOD, manganese-containing SOD; IBD, inflammatory bowel disease; PMA, phorbol 12-myristate 13-acetate; iNOS, inducible form of nitric oxide synthase; 5-ASA, 5-aminosalicylic acid.

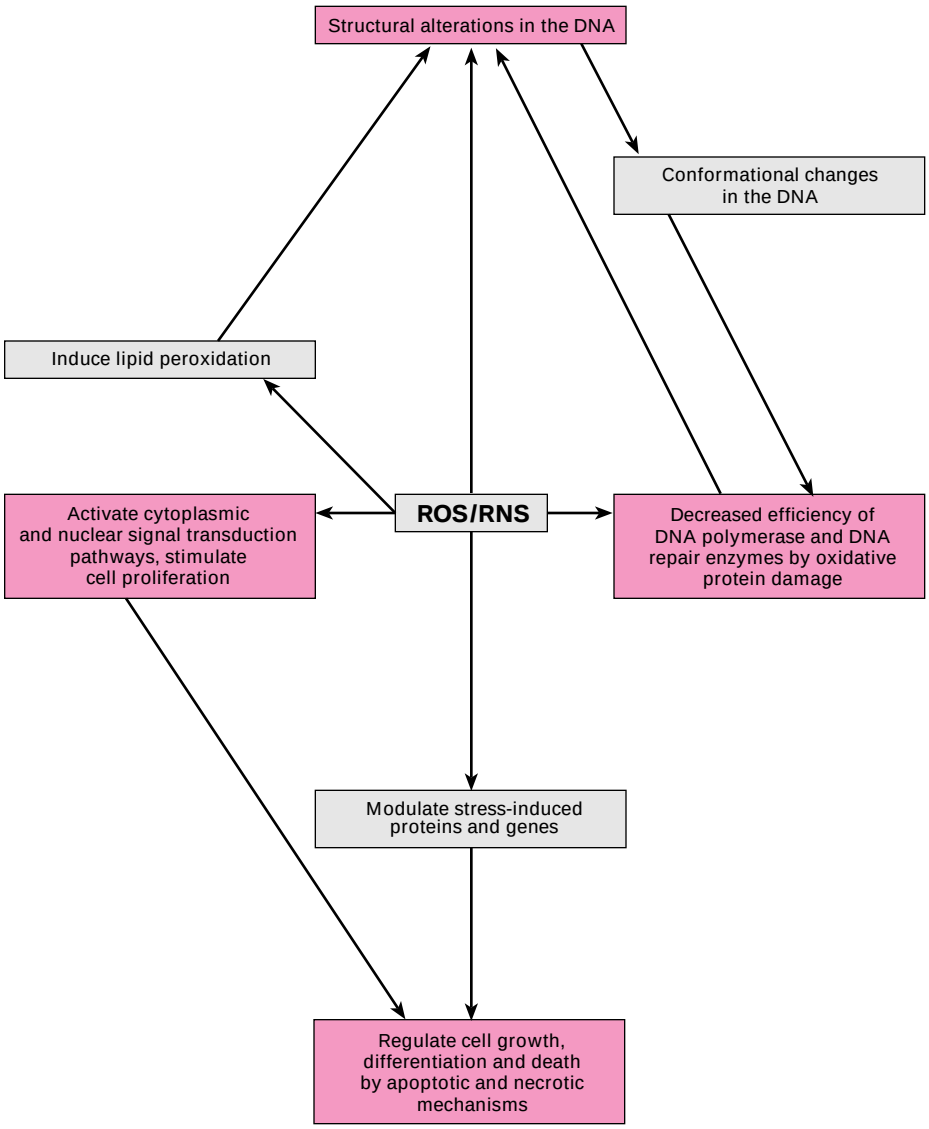


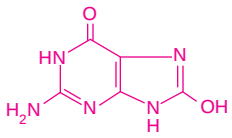
Figure 1 Potential carcinogenic characteristics of ROS/RNS

Key processes are shown in red. Aldehyde end-products of lipid peroxidation can bind to DNA and are potentially mutagenic [213].

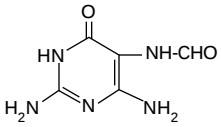
diseases and in the aging process [27,28]. The free-radical theory of aging postulates that aging is caused by free-radical reactions and that life expectancy can be increased by nutritious low calorific diets supplemented by free-radical inhibitors [27]. Indeed, it has been claimed that mechanisms of aging and life span shortening by enhanced calorific intake are associated with increased oxidative damage resulting from associated changes in mitochondrial ROS production [29].

Mitochondria are often said to be the most important intracellular source of ROS, but it is hard to be sure of this [30]. However, it seems very likely that the mitochondrial electron transport chain generates ROS *in vivo* [31,32] and that mitochondrial DNA is damaged by them. Indeed, oxidative DNA base damage (measured as 8-OHdG) has been detected in mitochondrial DNA at steady-state levels several-fold higher than in nuclear DNA [26,28,33]. Which ROS or RNS are responsible has not yet been elucidated. This apparent increased net oxidative damage in mitochondrial DNA compared with nuclear DNA could be because of the proximity of mitochondrial DNA to ROS generated during electron transport, the lack of histone proteins to protect the DNA against attack, or inefficient repair, so that base damage accumulates to higher levels. Intermediate radicals formed during lipid peroxidation, as well as end-products of peroxidation (Figure 1), can also attack DNA [34] and have been suggested to damage mitochondrial DNA, which is in close proximity to the mitochondrial inner membrane [35].

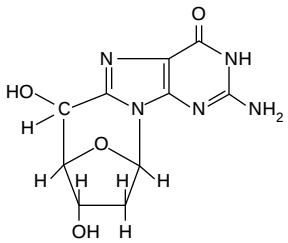
Oxidative damage by all the above mechanisms could contribute to the deletions and mutations in mitochondrial DNA that accumulate with age at a higher rate than in nuclear DNA [36]. Damage to mitochondrial DNA could play a role in neurodegenerative diseases: mitochondrial deletions and increased steady-state mitochondrial oxidative DNA damage (measured as elevations in 8-OHdG) have been reported in Alzheimer's disease [37]. Increased mitochondrial DNA damage in tissue from atherosclerotic hearts has also been reported [38].



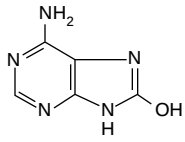
8OHG



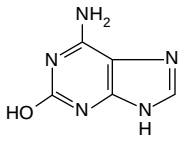
2,6-Diamino-4-hydroxy-5-formamidopyrimidine



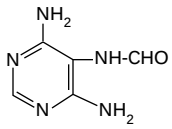
8,5′-Cyclo-2′-deoxyguanosine (5′ *R*- and 5′ *S*-)



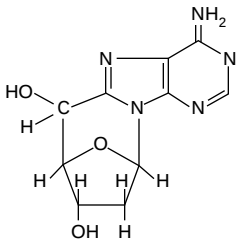
8-Hydroxyadenine



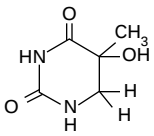
2-Hydroxyadenine



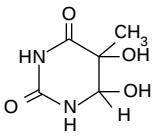
4,6-Diamino-5-formamidopyrimidine



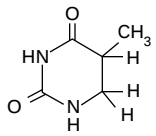
8,5′-Cyclo-2′-deoxyadenosine (5′ *R*- and 5′ *S*-)



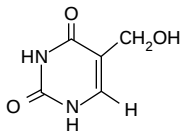
5-Hydroxy-6-hydrothymine



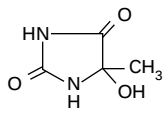
Thymine glycol (*cis*- and *trans*-)



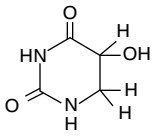
5,6-Dihydrothymine



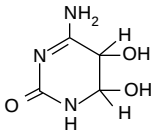
5-Hydroxymethyluracil



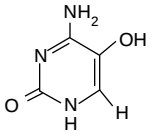
5-Hydroxy-5-methylhydantoin



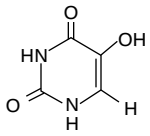
5-Hydroxy-6-hydrouracil



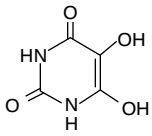
Cytosine glycol



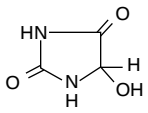
5-Hydroxycytosine



5-Hydroxyuracil



5,6-Dihydroxyuracil



5-Hydroxyhydantoin

Figure 2 Structures of modified DNA bases

8-OHG is shown in red.

SOURCES OF OH$^{\bullet}$

The pattern of damage to DNA bases in nuclear DNA suggests that OH$^{\bullet}$ attack occurs *in vivo* (no data are available as yet on whether the same is true for mitochondrial DNA). How could OH$^{\bullet}$ be formed to attack DNA in the nucleus and mitochondria? If OH$^{\bullet}$ is attacking DNA, it must be produced very close to the DNA since it is so reactive that it cannot diffuse from its site of formation [11,30]. Background radiation may be one source [11], but radiation-generated OH$^{\bullet}$ is formed over the whole cell and only a small fraction would be likely to hit DNA [39]. Other sources of OH$^{\bullet}$ include reaction of O_2^- with HOCl [40] and reaction of nitric oxide radical (NO$^{\bullet}$) with $O_2^{\bullet -}$. NO$^{\bullet}$ reacts very quickly with $O_2^{\bullet -}$ to give $ONOO^-$ [41] and with $RO_2^{\bullet}$ and RO$^{\bullet}$ to give organic peroxynitrites, which appear much more stable than $ONOO^-$ [42]. $ONOO^-$ itself is probably directly damaging to DNA bases (e.g. by deamination and nitration of guanine residues) and, at physiological pH, it decomposes into a range of toxic products, including species identical with (or closely resembling) $NO_2^{\bullet}$, OH$^{\bullet}$ and NO_2^+ [43–45].

By far the greatest interest has been, however, in Fenton chemistry as a source of OH$^{\bullet}$ [30,46]. The question of whether transition metal ions that can convert H_2O_2 into OH$^{\bullet}$ (e.g. iron and copper ions) really are in close proximity to DNA *in vivo* is clearly a critical one. Although iron and copper appear to be present in the nucleus [10,46] and may easily be released from non-haem iron proteins in mitochondria (perhaps as a result of attack by ROS [47]), it remains to be established how they could reach the DNA. Iron and copper ions are normally carefully sequestered by the body, into proteins such as ferritin, transferrin, caeruloplasmin and metallothionein [48]. DNA is a powerful chelating agent for transition metal ions, however, and oxidative stress may cause the release of intracellular iron and/or copper ions into forms that could then bind to DNA [10]. Thus $O_2^{\bullet -}$ releases some iron from ferritin [49], H_2O_2 can release iron from haem proteins [50] and $ONOO^-$ releases copper from caeruloplasmin [51].

DNA-associated copper ions in cells might also react with phenolic compounds to produce ROS and electrophilic phenolic intermediates [52–54]. This interaction could cause a range of DNA lesions including base modifications, strand breaks and phenol adducts to the DNA bases, all of which might contribute to the carcinogenicity of certain phenolic compounds. Phenolic compounds that cause DNA damage in the presence of copper ions include 2-hydroxyoestradiol, 2-methoxyoestradiol, diethylstilboestrol, butylated hydroxytoluene, butylated hydroxyanisole, L-DOPA, dopamine, ferulic acid and caffeic acid [52–54]. However, phenols have complex pro- and anti-oxidant effects *in vitro*, depending on the assay system used, and it is often hard to predict their net effect *in vivo* [55]. For example, many synthetic and dietary polyphenols (including quercetin, catechin, gallic acid ester and caffeic acid ester) can protect mammalian and bacterial cells from the cytotoxicity induced by peroxides such as H_2O_2 [56].

DNA REPAIR

DNA damage can be repaired by the action of a series of enzymes (reviewed in [57]). However, DNA from human cells and tissues contains low levels of DNA base damage products [2,24,25,58–61], suggesting that these enzymes do not achieve complete removal of modified bases, perhaps because they operate at close to maximum capacity *in vivo*. In agreement with this, the steady-state levels of one or more base damage products have been observed to increase in a number of chronic inflammatory diseases accompanied by increased ROS/RNS production, including hepatitis [62] and rheumatoid arthritis [58]. They are also increased in DNA isolated from cancerous tumour biopsies of human lung, colon, kidney, breast, liver and bladder (e.g. see [59–62]) and from benign prostatic hyperplasia tissues [63]. Of course, a rise in DNA base damage products could be due either to increased oxidative damage and/or to decreased repair activity; the increased damage to DNA in inflammation is presumably due to increased ROS/RNS production, often by the activation of phagocytes [10,17].

DNA glycosylases exist for the repair of several DNA base lesions, including oxidized, methylated and deaminated bases. A repair system for the abasic [apurinic/apyrimidinic (AP)] sites produced by spontaneous depurination also exists. Areas of current interest include the role of poly(ADP-ribose) polymerase (PARP) in the rejoining of DNA strand breaks, including those induced by ROS [64,65], and the fact that repair of oxidative DNA damage is defective in xeroderma pigmentosum (XP) cells [65,66]. Human XP is a genetic disorder with an autosomal recessive mode of inheritance, and there are seven genetic complementation groups (A–G) [67]. The defective gene products in these groups are involved in nucleotide excision repair, particularly in damage recognition and incision processes. Thus the XPA protein recognizes and binds to damaged DNA, whereas XPB and XPD are involved in DNA unwinding to facilitate the removal of the faulty base [67]. Normal repair can be restored by mixing two XP cell extracts derived from different complementation groups [65]. The XPG protein is an endonuclease that plays a direct role in making one of the incisions required to excise a damaged base [67]. It seems likely that the products of RNS- and ROS-induced damage may accumulate in XP cells; this could contribute to the neurological deterioration and increased occurrence of cancer observed in XP patients [65,66], an illustration of the importance of DNA repair processes. Defective DNA repair is also responsible for one of the most common cancers, hereditary non-polyposis colon cancer [67].

DNA damage by ROS/RNS can cause multiple lesions, including single and double strand breaks, AP sites and modified pyrimidines and purines. Repair of these lesions occurs primarily by base excision repair, although nucleotide excision repair may also be involved. Recognition of 'spontaneous' [hydrolysis of the base–sugar (glycosylic) bond] and ROS/RNS-generated AP sites may be carried out by various AP endonucleases with different specificities, and this can be used to differentiate between different types of AP site [68].

DNA repair enzymes have usually been purified by assaying their ability to act upon a single specific base lesion. However, recent studies using gas chromatography/MS (GC-MS) have investigated the ability of enzymes to repair DNA containing a wide range of lesions, and have shown that they can sometimes have a broader specificity than expected. For example, *Escherichia coli* endonuclease III can excise several thymine- and cytosine-derived lesions, e.g. 5-hydroxy-2′-deoxycytidine, from DNA [57,69,70].

MEASUREMENT OF OXIDATIVE DNA DAMAGE

In principle, there are two types of measurement of oxidative DNA damage. Steady-state damage is measured when DNA isolated from human cells and tissues is analysed: it is the balance between damage and repair. It is worth mentioning that the measurement of baseline levels of modified DNA bases, although very important, does not provide information as to whether this damage is in active genes or in quiescent DNA. It does, however, seem likely that 'exposed' DNA could be more

sensitive to damage by ROS/RNS than that packaged into condensed chromatin.

It is also useful to have an index of total DNA damage (i.e. that which has occurred but has been repaired) and this has been attempted in humans by analysis of urine. Several base damage products are excreted in urine [2,71–73] but the one most exploited is 8-OHdG because it can be assayed using HPLC with electrochemical detection [2,23]. 8-OHG is sometimes measured, but the 8-OHG content of urine is affected by the diet (cooking foods oxidizes their DNA and proteins, just as it oxidizes their lipids) and it can also arise by oxidative damage to RNA. By contrast, the 8-OHdG content of urine is thought not to be affected by the diet since nucleosides are not absorbed from the gut. However, some or all of the 8-OHdG measured in urine may come not from DNA, but from the deoxyGTP precursor pool [74]. An enzyme is believed to hydrolyse dGTP containing oxidized guanine to prevent its incorporation into DNA [74,75]. The activity of this enzyme may vary between different human cells, perhaps contributing to an explanation of variable mutation rates in different tissues exposed to oxidative stress [76]. However, if some or all of the 8-OHdG in urine comes from oxidized dGTP, it follows that we urgently need better markers of total body oxidative DNA damage.

8-OHG and 8-OHdG are the products most frequently measured in isolated DNA as an indicator of oxidative DNA damage. The former can be released from DNA by acid hydrolysis, whereas enzymic hydrolysis liberates 8-OHdG. The methods commonly used for their analysis also raise some questions. Measurement of 8-OHdG by HPLC with electrochemical detection is a highly sensitive method [23]. One alternative is GC-MS with selected ion monitoring, which can measure a wide spectrum of modified (methylated, oxidized, deaminated, etc.) DNA bases [9,10,71]. Both methods are sufficiently sensitive to measure steady-state levels of oxidative base damage in human cells and tissues, but more comparisons of these methods are needed. HPLC can underestimate the amount of 8-OHdG in DNA if the enzymic hydrolysis is not completely efficient; the efficiency of the exonucleases and endonucleases used to hydrolyse the DNA may be diminished by oxidative modification of the bases [24,77]. By contrast, GC-MS might overestimate base damage products if they are generated artifactually during derivatization procedures [78]. Dizdaroglu (e.g. [79]) has used stable isotope dilution MS to quantify DNA damage.

Many additional methods have been described, e.g. GC-MS has been used to analyse thymine glycol residues [80], uracil [81] and malondialdehyde–guanine adducts [82]. Analysis of uracil in DNA by GC-MS following its removal by uracil DNA glycosylase has been used to demonstrate that inhibition of folic acid metabolism induces uracil accumulation in DNA [81]. We have used HPLC after acid hydrolysis of DNA to measure several base damage products, including 8-OHG [83]. HPLC coupled to ^{32}P post-labelling has been used to measure 8-OHdG adducts in the peripheral blood of human subjects exposed to ionizing radiation [84]. Indeed, ^{32}P post-labelling has been used to measure several DNA adducts [85,86].

Different approaches to measuring oxidative base damage have been reported [87,88]. One of these [87] utilizes the ability of endonuclease III to make breaks in DNA at sites of base damage; the breaks are then measured by single-cell gel electrophoresis. For example, normal human lymphocytes *in vitro* were found to contain several hundred endonuclease III-sensitive sites per cell. By contrast, no endonuclease III-sensitive sites were found in HeLa cells, perhaps reflecting less oxidative damage and/or more efficient repair processes in these cells as compared with lymphocytes.

A rigorous comparison and standardization of these various methods is clearly needed (discussed in [24]). Another problem to be considered is the possibility that DNA is oxidatively damaged during its isolation, particularly if phenol-based methods are used [89]. However, the rigorous control of isolation procedures and avoidance of phenol (e.g. by isolation of chromatin for GC-MS analysis [90]) in many laboratories does not decrease oxidative DNA base damage to zero [24,58,59,87,91], strongly supporting the view that there is a low steady-state DNA damage level *in vivo*. Indeed, the fact that an extensive system of repair enzymes exists (see above) supports this view.

HOW COULD DNA DAMAGE BY ROS/RNS CAUSE MUTATION AND CANCER?

ROS/RNS can cause DNA base changes, strand breaks, damage to tumour-suppressor genes and enhanced expression of proto-oncogenes [4,17], and oxidative stress has been shown to induce malignant transformation of cells in culture [92,93]. However, the development of human cancer depends on many other factors, including the extent of DNA damage (excessive DNA damage can cause cell suicide by activation of PARP; see below), antioxidant defences, DNA repair systems, the efficiency of removal of oxidized nucleosides (e.g. oxo-dGTP) before they are incorporated into DNA, and the cytotoxic effects of ROS in large amounts (a dead cell will not lead to cancer) as well as their growth-promoting effects in small amounts [15,94]. For example, the proliferative responses of Syrian hamster embryo fibroblasts to $O_2^{\cdot -}$ have been found to depend on tumour-suppressor gene function, and low levels of $O_2^{\cdot -}$ can enhance cell growth [95]. Persistent excessive oxidative DNA damage may transmit signals to other cellular components (including the tumour-suppressor protein p53) and may trigger apoptosis. In addition, DNA damage (including ROS/RNS-induced DNA strand breaks) can result in p53 accumulation in the nucleus, which may arrest cell growth at the G_1/S border in an attempt to allow repair of DNA lesions before replication. Roles for ROS in apoptosis are frequently suggested (although fiercely contested, e.g. see [96]); the anti-apoptotic gene *bcl*-2 decreases the overall cellular production of ROS [16] and ROS can trigger apoptosis [16,97,98]. This does not mean that ROS are essential mediators of apoptosis, of course. The *bcl*-2 gene is the prototype of a family of genes that inhibit apoptosis and was originally cloned from the t(14;18) translocation breakpoint found in follicular B-cell lymphomas. Unfortunately, the *bcl-2* gene is also an oncogene and the oncogenic properties of the *bcl*-2 protein could relate to its ability to prevent 'useful' apoptotic cell death. The *bcl*-2 protein has a C-terminal membrane anchor and is localized to the nuclear, endoplasmic reticulum and mitochondrial outer membranes; it may protect cells by inhibiting ROS-induced lipid peroxidation [16,98,99]. Reasoning from first principles, this could be a direct antioxidant effect of *bcl-2*, and/or it could occur if *bcl-2* up-regulates endogenous antioxidant defences. Thus, while a high cellular antioxidant capacity tends to protect DNA from oxidative damage and related mutagenesis, antioxidant activity may also (ironically) protect 'initiated' cells from ROS-mediated killing. Down-regulation of the *bcl-2* gene by the p53 tumour-suppressor protein in human breast cancer cells has been reported [100]. However, *bcl-2* can rescue cells from apoptosis caused by non-oxidative events, so it does not simply act by antioxidant mechanisms [96,98,99].

Which of the multiple different types of chemical alteration in DNA caused by ROS/RNS are relevant to cancer development? Recent studies have attempted to identify mutations that are

caused by ROS/RNS damage to DNA, with the aim of ascertaining their association (if any) with cancer [4].

Damage to DNA by ROS, as measured in a single-stranded DNA, *E.coli*-based, forward mutation assay, was found to induce a wide spectrum of mutations, which depended not only on the ROS used but also on the DNA replication apparatus that encountered the lesion [5]. The most frequent mutations found in this system were C to T transitions. However, mutations arising from C to T transitions are not diagnostic for mutagenesis by ROS because they can be caused by DNA polymerase errors and by DNA damage arising from the action of other genotoxic agents [5]. Incubation of DNA with ROS-generating systems such as $FeSO_4/O_2$ or $CuCl_2/H_2O_2$ resulted in the formation of mainly C to T, G to T and G to C substitutions in the iron system; and C to T and G to T substitutions and CC to TT tandem double transitions in the copper ion system [101]. Each of these mutation patterns was clustered in 'hot spots' that are characteristic for each system; some of this effect might relate to different binding of the two metal ions to DNA, so that ROS are directed to particular DNA base sequences [5]. For example, copper ions bind preferentially to GC-rich sequences [102].

There are several different pathways leading from initial DNA base damage by ROS/RNS to subsequent mutation (Figure 3). The first (and probably the simplest) is the chemical modification of DNA bases causing a change in their hydrogen bonding specificity, e.g. 8-OHG, thymine glycol and 2-hydroxyadenine [101,103,104]. In addition, 8-hydroxyadenine, ring-opened purines and a number of pyrimidine fragmentation products can block replication in *E. coli* and could thus be mutagenic [105,106]. Singlet-oxygen-induced DNA damage is targeted selectively at guanine residues [22,107] and the G to T transversion mutations induced may be generated by an error-prone bypass of damaged G [108]. Oxidation products of cytosine (5-hydroxycytosine and 5-hydroxyuracil) exhibit sequence-context-dependent mispairing *in vitro* resulting in C to T transitions and C to G transversions [109]. Although it is not obvious which modified DNA bases are responsible for the mutations that can be introduced by DNA polymerases α and β, it seems likely that the C to T and C to A

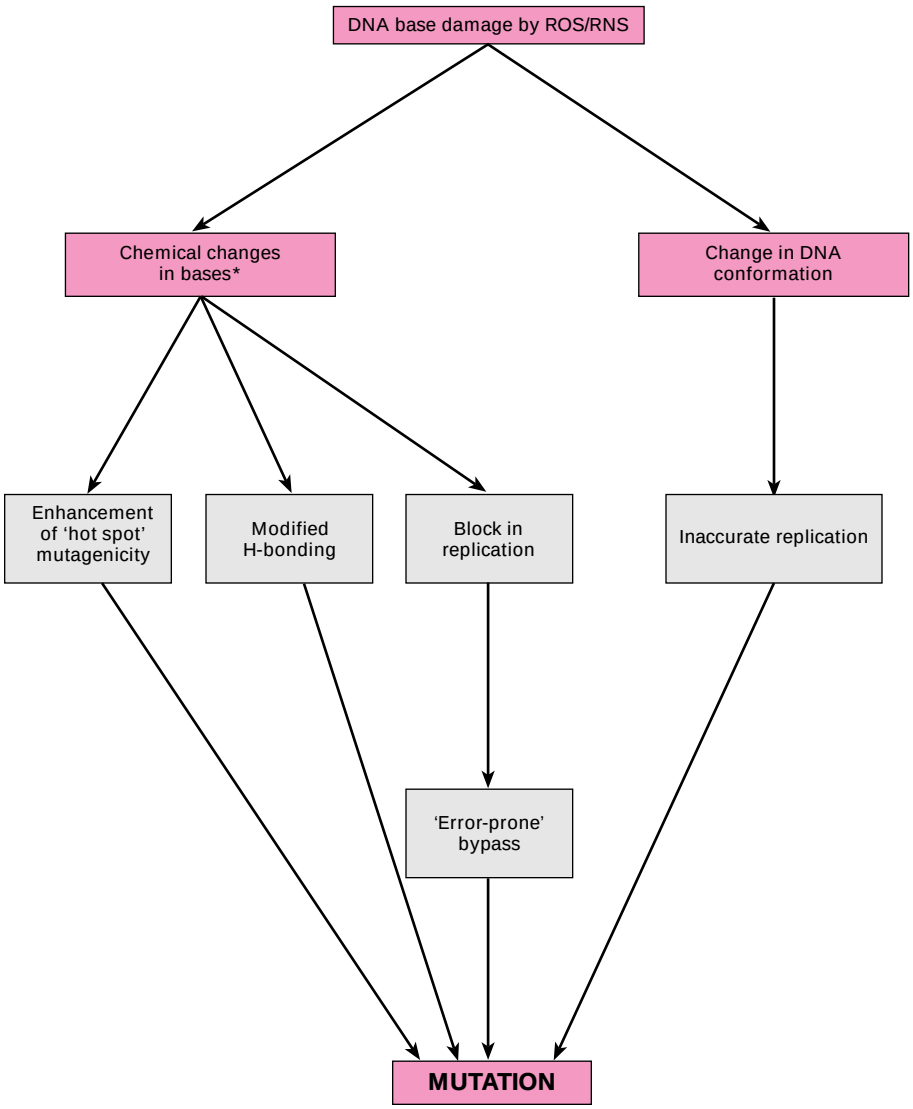


Figure 3 Pathways leading from initial base damage to subsequent mutation

Key processes are shown in red. * See Figure 2.

substitutions observed in the *E. coli* system result from the oxidative modification of cytosines [5]. DNA polymerase is known to be sensitive to damage-induced errors at guanines.

The contribution of oxidative damage to polymerase-specific 'hot spots', which is a likely major contributor to DNA polymerase-mediated mutagenesis, is a second possible mechanism. In studies on the *lacZ* gene, T to G transversions at positions +70 and +103 were observed when polymerase was used to copy an undamaged DNA template [110]. A 6–10-fold increase in the frequency of transversions at both sites was caused by iron-ion-mediated oxidative damage, and an increased frequency of mutation at position +103 only was produced by copper-ion-dependent damage [110]. The 'background' mutations might also be caused by endogenous oxidative damage. Perhaps also the sequences used were inherently mutagenic for polymerase and could thus enhance the miscoding potential of oxidatively induced adjacent lesions [5].

A third mechanism is linked to a conformational change in the DNA template that diminishes the accuracy of replication by DNA polymerases [110]. Evidence for this mechanism has been provided by the finding that iron-ion-dependent damage to M13mp2 double-stranded circular DNA produces a high frequency of mutants that possess substitutions at positions +95 and +103 on the *lacZ* gene. The frequency of this double mutation in the polymerase spectrum is four orders of magnitude higher than would be expected from two independent mutational events, and it has been suggested [110] that oxidative damage to the DNA template alters the pausing pattern for DNA polymerase at the two sites of mutation by changing the DNA structure. The frequency of mutation is also greatly increased by exposure of the DNA to ROS, probably by mechanisms involving rearrangements of the nascent strand-template. Conformational changes that cause an increase in the pausing of the DNA polymerase or a decrease in processivity could enhance such rearrangements. Although direct studies of the effects of base modifications on DNA conformation are just beginning, it is known that many oxidized bases are non-planar and could change local DNA structure [5].

Human relevance of bacterial studies

Studies of bacterial mutation depend on the so-called SOS response (which includes DNA repair enzymes) and the nature of the bacterial DNA replication system. Such studies can only provide indications about the mutations likely to be caused in human cells by ROS/RNS. Indeed, studies with mammalian DNA polymerases have shown that DNA damage by ROS can yield mutations different from those observed in *E. coli* [110]. Nevertheless, studies using bacterial systems have provided useful information, including the suggestion that tandem CC to TT double substitutions, induced by ROS from many different sources including iron(II), copper(I) and nickel(II) ions and γ radiation [111,112], may be specific markers for oxidative DNA damage, at least in cells not exposed to UV light (which can also induce this mutation [113]). Nickel(II) is a known human carcinogen which has been shown to increase oxidative DNA base damage in rats [114] and to induce the above 'signature mutation' for ROS damage in an *E. coli* forward mutation assay [112].

The *p53* tumour-suppressor gene and the *ras* family of proto-oncogenes are known to be important cancer-related genes. It is possible that ROS may be the mutagens involved in some of the mutations observed in these genes [4,17,115], e.g. the G to T transversion often found in Ki-*ras* and H-*ras* in non-melanoma skin tumours could be produced by misreplication of the 8-OHdG lesions induced by ROS (although GT tranversions may be generated by other mutagenic mechanisms). G to T transversions have also been observed in *p53* codons in hepatocellular carcinoma and smoking-related lung carcinoma, and again they might arise by the actions of ROS. In colorectal cancer ROS may yet again be involved, as around 95% of the mutations at *p53* hot spot codons in these tumours are C to T and G to A transitions, and these base-pair changes are often produced by the deamination of 5-methylcytosine, which is enhanced by ROS and RNS [4].

Indirect mutagenicity by ROS/RNS: damage to proteins and lipids

ROS/RNS-induced mutations could arise not only from DNA damage, but also from protein damage. Protein damage is a major consequence of excess ROS generation *in vivo* [116] and damage to DNA polymerases could alter their fidelity. It has been suggested that an alteration in the conformation of DNA polymerase could explain the frequency of close-proximity double mutations that occur secondarily to a wide range of genetic stresses [5,117]. Several RNS, e.g. $ONOO^-$ and $NO_2^{\bullet}$, can attack proteins, nitrating aromatic amino acid residues and possibly affecting their ability to participate in signal transduction mechanisms [43,44,118]. Oxidative protein damage could also affect the activity of DNA repair enzymes.

Another possible mutagenic effect of ROS involves their attack on lipids, to initiate lipid peroxidation (Figure 2). Peroxides can decompose to a range of mutagenic carbonyl products [34]. For example, 4-hydroxynonenal is genotoxic to lymphocytes and hepatocytes and also disrupts gap-junction communications in cultured endothelial cells [119]. In a study on a baby hamster kidney cell line (BHK-21/C13) and its polyoma-virus-transformed malignant counterpart (BHK-21/PyY cells) the level of lipid peroxidation (as measured by HPLC of malondialdehyde) was higher in transformed cells than in non-transformed cells, suggesting that the level of lipid peroxidation is increased in the malignant state [120]. By contrast, earlier work claimed that susceptibility to lipid peroxidation is decreased in malignant hepatoma cells (e.g. Novikoff and Yoshida ascites hepatoma cells) (reviewed in [34]) and this was found to be directly related to their extent of dedifferentiation [121]. It is of related interest that regenerating rat liver shows increased steady-state levels of 8-OHdG in nuclear DNA [122] and that partially hepatectomized rats show increased urinary excretion of thymine glycols [123].

These findings illustrate the well-known observation that cell transformation alters cell responsiveness to oxidative stress. Changes in antioxidant defence enzymes such as superoxide dismutase (SOD) have been widely described in cancerous cells, although the most consistent reports seem to be of falls in Mn-containing SOD (MnSOD) (the mitochondrial enzyme) activity [124]. This change may be significant in relation to the malignant phenotype, since radiation-induced neoplastic transformation in mouse C3H 10T1/2 cells (a cell line susceptible to transformation by ROS [93]) was decreased by transfection of the cells with a gene encoding human MnSOD [125]. Similar results have been reported in human melanoma cells [126] and a mouse fibrosarcoma cell line [127].

'Slippery' DNA

Another area worthy of consideration in the ROS/RNS context is that of misalignment mutagenesis ('slippery DNA'). The human genome contains many sequences in which 1–6-nucleotide motifs are tandemly repeated a number of times. The contraction and expansion of these 'microsatellites' is associated with cancer (e.g. mono-, di- and tri-nucleotide repeats are unstable in colon

cancer cells and this instability has been linked to a gene on chromosome 2) and several genetic diseases, including myotonic dystrophy and Huntington's disease [128]. The gain or loss of repetitive DNA sequences may be caused by errors that result from strand slippage during DNA replication remaining uncorrected because of defective post-replication heteroduplex repair [129]. The instability of these repeats is associated with DNA polymerases slipping during replication, and some types of colorectal cancer may reflect mutations in genes involved in DNA mismatch repair [129]. Theoretically, ROS/RNS should be capable of accelerating such changes [129].

PARP

The nuclear enzyme PARP modifies proteins (including itself; automodification) by the attachment of poly(ADP-ribose) polymers. PARP is involved in base excision repair but not in nucleotide excision repair, which requires the formation of a multiprotein repair complex before DNA incision that may prevent the binding of PARP to DNA breaks [64,65]. The repair of DNA single strand breaks induced by the antibiotic bleomycin [65] is totally dependent on the activation of PARP, and the repair of modified bases generated by alkylating agents is also promoted by PARP activation. In base excision repair, damaged bases are eliminated by DNA glycosylases, breaks are then induced at the AP sites by AP endonuclease and this is followed by the excision of deoxyribose phosphate, DNA synthesis and finally ligation. The process of DNA break rejoining initiated by AP endonuclease is aided by PARP, which may have a structural role in chromatin conformation and may provide temporary protection for the DNA breaks during the initial stage of the recombination and repair processes. Excess activation of PARP can kill cells by depleting NAD^+ and preventing energy production [130], and this may be one mechanism by which cells with excess DNA damage are eliminated (see above).

ROLE OF INFLAMMATION IN INCREASED DNA DAMAGE, MUTATION AND CANCER

The cumulative risk of cancer increases with approximately the fourth power of age [2]. This is true for both short-lived species such as mice, where about 30% have cancer by the end of their 2–3-year life span, and in long-lived species such as humans, where approx. 30% have cancer by age 85. Much cancer can thus be considered as a degenerative disease of old age and it is frequently suggested that this is related to the effects of continuous damage over a life span by ROS and RNS [1,2]. For example, prostate cancer is most prevalent among elderly males and has been suggested to be associated with endogenous cellular processes, in particular ROS generation [5].

Inflammation can accelerate the development of cancer [3,131]. Many sources of inflammation are effective, including that caused by viral, bacterial and parasitic infections. In colon cancer, predisposing sources of chronic inflammation include ulcerative colitis and infection with the parasite *Schistosoma japonicum* [28,62,92].

However, the link between cancer and inflammation is by no means a simple one. One chronic inflammatory disease in which patients suffer oxidative stress is rheumatoid arthritis. There is increased damage by ROS [132,133] (and probably also by RNS [134]) to lipids, proteins and DNA. Rheumatoid arthritis patients also show increased urinary excretion of 8-OHdG [135]. Increased DNA damage, and increased susceptibility to killing by H_2O_2, have also been reported in lymphocytes from patients with autoimmune diseases: lymphocyte DNA from patients with rheumatoid arthritis, systemic lupus erythematosus, vasculitis and Behcet's disease contained significantly more 8-OHdG than that from healthy controls [58].

Despite all this, there is no clear evidence that rheumatoid arthritis patients develop cancers at an increased rate, certainly not at the most intense site of oxidative stress, the inflamed joint. Perhaps this is linked to the nature of the cells of the synovium. Synovium seems to be a hostile environment for neoplastic cells [136], although one might argue that the excessive synovial cell proliferation in rheumatoid arthritis patients might be related to the growth-promoting properties of ROS.

Despite the apparent anomaly of rheumatoid arthritis, there is considerable evidence that ROS/RNS are somehow involved in the link between chronic inflammation and cancer [3,28,62,92,131]. A notable activity of tumour promoters is their ability to recruit inflammatory cells and to stimulate them to generate ROS/RNS. Indeed, there is a strong relationship between the capacity of tumour promoters to stimulate inflammatory cells to release ROS/RNS and their capacity to promote tumours [131,137,138]. Genetic damage and neoplastic transformation have been demonstrated in cells co-cultured *in vitro* with activated phagocytes [92]. The genotoxic effects observed in these cells include the formation of DNA strand breaks [138], sister chromatid exchange [139] and mutations [140]. The DNA base modifications observed in cells co-cultured with phorbol 12-myristate 13-acetate (PMA)-activated inflammatory cells were characteristic of attack by $OH^{\cdot}$ [141]. Presumably phagocyte products such as H_2O_2 penetrated to the nucleus and were converted into $OH^{\cdot}$ by reaction with localized transition metal ions.

Tumour promotion can be inhibited in animal models by the use of agents that can inhibit the phagocyte respiratory burst, including certain antioxidants, as well as steroids and retinoids [131,137]. Increased levels of oxidatively modified DNA bases (thymidine glycol, 5-hydroxymethyl-2′-deoxyuridine and 8-OHdG [142]) have been induced in the skin of mice by topical PMA exposure. 8-OHdG has also been identified in the epidermis of nude mice exposed to near-UV [143]. The production of ROS *in vivo* following the application of phorbol esters to mouse skin requires two applications; each triggers a separate biochemical event, priming and activation [144]. Both of these events can be triggered by tumour-promoting phorbol esters but not by non-promoting ones. Priming events include the recruitment of inflammatory cells: PMA recruits neutrophils and activates them to produce ROS/RNS. The inhibitor of phorbol ester tumour promotion, retinoic acid, inhibits activation but not priming and similar results are found for several phenolic antioxidants [142,144]. This suggests that retinoic acid, usually considered to inhibit promotion by altering gene expression in transformed cells, might act additionally to exert indirect antioxidant effects under certain circumstances, at least when applied topically to mouse epidermis. Similarly, 13-*cis*-retinoic acid inhibits X-ray-induced skin cancer in XP patients (see above), where it has been proposed to exert antioxidant effects [145].

Inflammatory cells may also increase DNA damage by activating pro-carcinogens to DNA-damaging species, e.g. neutrophils can activate aromatic amines, aflatoxins, oestrogens, phenols and polycyclic aromatic hydrocarbons by ROS-dependent mechanisms [131,146]. RNS can generate carcinogenic nitrosamines [3,147,148], e.g. nitrosation of morpholine has been reported in immunostimulated rats [149].

Some examples

Schistosomiasis

The schistosomiasis model has been used to study the interrelationship between inflammation, oxidative DNA damage,

chromosomal instability and dysregulated cell proliferation [131]. Infection with *Schistosoma haematobium* produces chronic bladder inflammation and is associated with increased cancer at this site. Indeed, infected individuals show elevated levels of genetic damage in their bladders, as measured by the exfoliated cell micronucleus test [131,150], and micronucleus frequency is decreased by treatments that kill the parasite. It is possible that clones of cells in these patients develop an inherited altered ability to repair oxidative DNA damage and thus an increased sensitivity to the ROS/RNS produced by activated inflammatory cells. Alterations in chromosome 11 are common in bladder cancer [151] and loci on this chromosome may be involved in controlling the level of chromosomal breakage caused by oxidative DNA damage. This sensitivity to oxidative stress is apparently not due to a difference in single-strand DNA breakage or repair [152]. In addition, an abnormally high frequency of chromatid breaks and gaps has been reported when human tumours (of many different tissue origins and/or histopathology) are X-irradiated during the G_2 phase of the cell cycle; insertion of a normal chromosome 11 decreased radiation-induced damage, possibly by restoration of a defective repair process (see [153]). Bladder carcinoma cells have been shown to be sensitive to micronucleus induction by tumour-promoter-activated neutrophils and protection is possible via the insertion of a normal chromosome 11 [131]. Again, this is thought to restore the defective DNA repair process [131]. It is of related interest that infection with the pro-inflammatory bacterium *Helicobacter pylori* appears to be an important risk factor for stomach ulcers, gastritis and possibly stomach cancer [154].

Lung cancer

Lung cancer is a frequent cause of death, and most cases are linked to smoking. Cessation of smoking leads to a rapid decrease in the risk of lung cancer, suggesting that a series of smoking-related events is required for cancer development. Cigarette smoke is rich in carcinogens such as nitrosamines, acrolein and carcinogenic hydrocarbons, but ROS/RNS may also contribute to cancer development, since smoke is rich in ROS and oxides of nitrogen [118,155]. Higher levels of oxidative DNA base damage have been reported in lung cancer tissue compared with surrounding normal tissue [156] and in cells exposed to cigarette smoke [157]. Additionally, a 4–10-fold elevation of urinary 8-OHdG excretion has been found in smokers [72,73].

Exposure to asbestos is a major risk factor for mesothelioma; asbestos-induced chronic inflammation and resulting DNA damage may contribute [158–160]. Crocidolite (one of the most carcinogenic types of asbestos) induces release of ROS from neutrophils and macrophages, and increased 8-OHdG levels in the DNA of a human promyelocytic leukaemia cell line (HL60) [159]. Furthermore, exposure of rat pleural mesothelial cells to crocidolite and chrysotile fibres resulted in DNA damage and cell toxicity that was partly due to ROS [160].

Liver cancer

In both Asia and Africa, hepatocellular carcinoma is a major cause of mortality. Primary hepatoma in these countries is often associated with chronic infection with hepatitis viruses B or C [161], or ingestion of aflatoxin [162]. In primary hepatocellular carcinoma, aflatoxin exposure often results in mutations involving codon 249 of the *p53* gene [163]. Indeed, aflatoxin frequently produces G to T transversions, and this is the predominant substitution at codon 249 in *p53* found in aflatoxin-associated tumours. This transversion can also be produced by oxidative damage [115]. Chronic hepatitis [164] is associated with the presence of inflammatory cells, presumably generating ROS and RNS. Indeed, increased levels of 8-OHdG have been detected in DNA from livers with chronic hepatitis [62,165].

Inflammatory bowel disease (IBD)

IBD is the general name given to a series of chronic inflammatory diseases of the gastrointestinal tract, including ulcerative colitis and Crohn's disease. ROS are formed in excess in IBD and are likely to play an important role not only in the pathogenesis of IBD [166,167] but also in the increased risk of cancer seen in certain IBDs. An elevated production of ROS has been shown using colorectal biopsy specimens [168]. Further evidence consistent with damage by ROS is provided by the increase in lipid peroxides in rectal biopsy specimens from patients with active ulcerative colitis, and the reports of low levels of GSH, SOD and glutathione peroxidase in patients with active IBD [166,169,170]. The main sources of ROS in the gut are probably phagocytes, which accumulate in the mucosa of patients with IBD and generate ROS (and presumably RNS) upon activation. A marked increase in the activity of the inducible form of nitric oxide synthase (iNOS) in the inflamed colonic mucosa from patients with ulcerative colitis has been reported [171,172]. By contrast, there was no increase in iNOS activity in the inflamed colonic mucosa from patients with active Crohn's disease, even though the extent of inflammation was similar [171]. In addition, nitrotyrosine (a putative chemical marker for the formation of $ONOO^-$ [43,173]) has been detected by an immunohistochemical stain in an animal model of chronic gut inflammation and found to co-localize with NOS [174]. This suggests that both $NO^{\bullet}$ and $O_2^{\bullet -}$ are formed *in vivo*, and undergo reaction to give $ONOO^-$. Furthermore, intracolonic instillation of $ONOO^-$ is pro-inflammatory in a rat colitis model [175].

Another important ROS in IBD may be HOCl. HOCl, produced by the enzyme myeloperoxidase from activated neutrophils, may attack membrane proteins directly (e.g. by oxidizing -SH groups, destroying methionine and chlorinating aromatic amino acid residues), or indirectly by the formation of chloramines. Both HOCl and chloramines can stimulate colonic secretion [176]. A role for ROS has also been described in the stimulation of colonic mucosal proliferation by bile salts [94]. It has been proposed that some of the drugs effective in the treatment of IBD may act by scavenging HOCl and other ROS [167,177].

What is the evidence for increased oxidative DNA damage in IBD? DNA from colon biopsies from patients with ulcerative colitis had significantly increased levels of 8-OHG, 2-hydroxyadenine, 8-hydroxyadenine and 2,6-diamino-5-formamidopyrimidine as measured by GC-MS/selected ion monitoring (H. Wiseman, M. Dizdaroglu and B. Halliwell, unpublished work). These lesions, suggestive of $OH^{\bullet}$ attack, could signify increased DNA damage and/or decreased repair. The constitutive and ROS-induced activity of PARP has been shown to be decreased in patients with IBD and colon cancer [178].

Breast cancer

Oxidative DNA damage may be involved in the development of breast cancer. Increased steady-state levels of DNA base damage, with a pattern characteristic of $OH^{\bullet}$ attack, have been reported in DNA from invasive ductal carcinoma [60,179]. One study found a 9-fold increase in 8-OHG, 8-hydroxyadenine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine in DNA from invasive ductal carcinoma compared with control tissue [60,179].

Whether this is due to decreased DNA repair and/or increased oxidative DNA damage is uncertain. DNA damage by ROS is also implicated in inflammatory breast disease [180], where malignant progression can occur.

PROSPECTS FOR DIETARY AND DRUG ANTIOXIDANT INTERVENTION IN THE PREVENTION OF OXIDATIVE DNA DAMAGE AND HUMAN DISEASE

Dietary antioxidants

Cells have multiple antioxidant defences to protect themselves against ROS. These protective mechanisms are not present in excess; if they were, oxidative damage would not occur and repair mechanisms would not be required. Instead, oxidative damage occurs continuously in the human body (Table 1). Fortunately, enzymic and some other (e.g. metallothionein, caeruloplasmin and haptoglobin) antioxidant defences are often inducible in response to oxidative damage [30].

We also obtain several antioxidants from the diet. Indeed, the consumption of fruit, grains and vegetables, which are the main sources of these antioxidants, is of importance in protecting against oxidative damage and resulting disease [7,8,181–184]. Intake of fresh fruit and vegetables appears to be inversely correlated with cancer of the stomach, pancreas, oral cavity and oesophagus, and to a lesser extent of the breast, cervix, rectum and lung [183,184], and emphasis has been placed on the protective role of ascorbate. Indeed, there is evidence that ascorbate can react with, and/or inhibit the formation of, carcinogenic *N*-nitroso compounds such as *N*-nitrosamines [185,186]. Vitamin C supplementation has been reported to decrease mucosal DNA damage, as measured by a ^{32}P post-labelling assay, in 28 of 43 patients attending a gastric follow-up clinic [187]. In patients with normal gastric mucosa, treatment with vitamin C resulted in an elevation of intragastric ascorbate in all cases, whereas in patients with chronic atrophic gastritis the effect was variable [187]. These data support epidemiological evidence suggesting that vitamin C may exert a protective effect against the development of gastric cancer. Very low vitamin C intakes have been associated with elevated levels of 8-OHdG in sperm DNA [188]. However, ascorbate supplementation did not affect tumour development in chemically induced bladder, mammary or colon cancers in rodents (reviewed in [7]). Therefore the extent of the benefits of ascorbate in human cancer prevention remain to be ascertained.

A sufficient level of dietary antioxidants has been suggested to be achievable by the intake of a minimum of three servings of vegetables and two of fruit per day. Furthermore, dietary supplementation of this daily intake of fruit and vegetables with moderate amounts of the relatively inexpensive and non-toxic vitamins ascorbate and α-tocopherol may also be desirable in some population groups, such as smokers [8,181,183,184,189]. In addition to antioxidants, fruit and vegetables contain many vital micronutrients that may be protective. These include folic acid, which is required for the synthesis of DNA precursors, and niacin, which is required for the NAD^+ used by PARP. Many carotenoids, including β-carotene, can be metabolized to vitamin A (retinol), and the antioxidant activity of carotenoids is thought to be particularly directed against $^1O_2$ [190–193].

However, it would be naive in the extreme to assume that the protective effects of fruits and vegetables are related only to their antioxidant content; among the many other potentially protective substances present are anti-angiogenesis factors, inducers of carcinogen-removing enzymes, fibre and phytates [194]. Diets rich in fruits and vegetables are often low in fat, which could contribute to their anti-cancer effect [195]. Such diets are also often low in iron; high body iron levels are (controversially) associated with increased risk of cancer [196–199]. Furthermore, in a prospective study of the intake of vitamins C, E and A and the risk of breast cancer, it was found that large intakes of vitamins C or E were not protective to women [200]. There is an urgent need to investigate to what extent dietary changes can decrease steady-state and total-body oxidative DNA damage in humans (which relates to the methodological questions discussed earlier) and, if so, what is the optimal intake of, for example, fruit, vegetables or antioxidant supplements. The rapid development of accurate assays for measuring oxidative damage to DNA, lipids and proteins in the human body should help to make this possible (reviewed in [181]).

Table 1 Evidence for ROS/RNS-mediated damage *in vivo*

Target of damage	Evidence
DNA	Urinary excretion of damaged DNA bases; low baseline levels of damaged DNA bases in DNA isolated from human cells
Lipid	Lipid peroxidation in atherosclerotic lesions; presence of peroxidation end-products in plasma and urine
Uric acid	Damaged by ROS to form products (including allantoin) found in human body fluids; levels increase during oxidative stress
Protein	Protein carbonyls and *o*-tyrosine formed from ROS attack and nitrosothiols/nitro-aromatics from RNS attack; low levels of some of these products can be detected in human tissues and body fluids and may increase during oxidative stress

Drug antioxidants

Several drugs in current clinical use may exert some antioxidant effects *in vivo* [201]. Tamoxifen, which is widely used in the treatment of breast cancer and is being investigated as a prophylactic treatment for this disease, may exert antioxidant effects additional to its anti-oestrogenic properties [202]. Thus it has been reported to suppress H_2O_2 production by human neutrophils [203–205]. The tamoxifen metabolite 4-hydroxytamoxifen is a more powerful inhibitor of lipid peroxidation than tamoxifen in lipoproteins [206] and in the nuclear membrane [207].

Another drug that may act as a free-radical scavenger *in vivo* is sulphasalazine and its metabolites, used in the treatment of IBD. Sulphasalazine is converted by colonic bacteria into 5-aminosalicylic acid (5-ASA), which has a number of antioxidant properties. It is an excellent scavenger of several ROS, especially HOCl [177,208,209]. The finding, in IBD patients treated with sulphasalazine, of 5-ASA-derived products identical to those formed when this compound reacts with $OH^{\bullet}$ *in vitro*, suggests that ROS scavenging by 5-ASA is a significant mechanism of action *in vivo* [210,211].

CONCLUSION AND FUTURE PROSPECTS

We need oxygen in order to survive, but the constant assault on our DNA by ROS/RNS may lead to cancer development [1,2]. Even phagocyte ROS/RNS production, useful in the short term as a defence against infection, may harm us in the long term, and certainly harms patients with chronic inflammatory diseases. Understanding the mechanisms by which these chemical changes relate to alterations in gene expression and the development of cancer, and how they can be affected by drug treatment and/or dietary changes, requires a combination of expertise in molecular

biology and analytical biochemistry. As an example we have little information on how oxidative base modification affects the PCR. Thus amplification of oxidized DNA (e.g. ancient DNA [212]) may produce misleading results. Correlations of structural chemistry and analytical methodology with changes in gene expression should lead to valuable new concepts and, hopefully, novel ways of preventing cancer.

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