Oxidative Damage on RNA Nucleobases

Pascal A. Küpfer and Christian J. Leumann

Contents

1 Introduction 76
2 Definition of Reactive Oxygen and Nitrogen Species and Their Properties 77
3 Reactivity of ROS and RNS Versus Nucleic Acids 79
4 Oxidative Lesions in RNA 80
4.1 Base-Pairing Properties of Lesion Containing Duplexes 81
4.2 Mutagenicity of Oxidatively Damaged Nucleobases 84
5 Consequences of Oxidatively Damaged RNA 86
6 Fate of Oxidized RNA 87
7 Redox Chemistry of Oxidized Nucleosides 88
8 Conclusions and Perspective 89
References 89

Abstract Oxidatively damaged RNA has recently gathered more attention and has been closely related to different neurodegenerative diseases. The principles of oxidative stress and its influence on nucleic acids are reported. In contrast to DNA oxidative lesions of RNA have been scarcely described in the literature so far. These known stable RNA base modifications which arise under oxidative stress are reviewed here with regard to their biophysical properties and their potential mutagenicity. Furthermore the possible mechanisms of how cells deal with oxidized RNA are discussed. Posttranscriptional RNA modifications and the oxidation of RNA as an early event in several neurodegenerative diseases are not in the scope of this review.

Keywords RNA oxidation • Reactive oxygen species • Reverse transcription • 8-Oxo-guanosine • Modified RNA

P.A. Küpfer • C.J. Leumann (✉)
Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland
e-mail: kuepfer@dcb.unibe.ch; leumann@dcb.unibe.ch

V.A. Erdmann et al. (eds.), *Chemical Biology of Nucleic Acids*, RNA Technologies,
DOI 10.1007/978-3-642-54452-1_5, © Springer-Verlag Berlin Heidelberg 2014

1 Introduction

Oxidation of DNA has been known for over 40 years and considerable efforts have been made to describe the generation, metabolism, and repair of such lesions. The progress in this field is a recurrent topic in review articles (Cadet et al. 2010; Barciszewski et al. 1999). Unlike in DNA, RNA oxidative damage has not been in the focus of research until the past decade. This might be due to the assumed transient nature of RNA. Nonetheless it has been shown that most mRNAs have an average half-life of ~7 h in mouse cells and ~10 h in human cells, attributing the small percentages of mRNA with considerable lower half-lives to transcription factor mRNAs (Yang et al. 2003; Sharova et al. 2009). Moreover mRNA just accounts for a few percent of total RNA in cells, whereas the majority of RNA includes stable tRNA and rRNA which have considerably lower turnovers: the half-lives in vitro are estimated to be in the range of several hours to days for tRNA and several days for rRNA (Defoiche et al. 2009).

In the recent years the central dogma of RNA being "the messenger in the middle" between DNA and proteins had to be expanded by the disclosure of the activities of non-protein-coding RNAs (ncRNA): while over 85 % of our genome is transcribed to RNA, the known protein-coding gene exons only account for less than 3 % of the human genome (Hangauer et al. 2013). Such ncRNAs have been linked to diverse regulatory mechanisms (Nelson and Keller 2007).

Overall these data imply that oxidative damage to RNA could in principle have a strong and probably so far underestimated impact on different cell mechanisms. Recently, oxidatively damaged RNA has been closely linked to aging, a still growing number of neurodegenerative and other diseases (Poulsen et al. 2012). The data suggest that RNA oxidation is an early event in disease rather than the effect of cell decay (Shan et al. 2007; Chang et al. 2008). The progress in this field has been thoroughly reviewed elsewhere (Nunomura et al. 2009; Poulsen et al. 2012).

It is a long known fact that RNA is hydrolytically less stable than DNA due to the fact that the 2′-hydroxyl group of the ribose undergoes cyclophosphate formation with the 3′-*O*-phosphate under basic conditions which leads to strand cleavage. Compared to DNA, purified RNA is known to have a greater chemical oxidative stability (Thorp 2000). Nevertheless RNA is considered to be more prone to oxidation due to its higher abundance in cells, its extensive subcellular distribution (vicinity to mitochondria), and lesser association with protective proteins (Li et al. 2006). Indeed it has been found that in mitochondrial DNA there is a 16-fold higher abundance of 8-oxo-dG compared to nuclear DNA in rat liver. This has been explained by the spatial vicinity of the oxygen metabolism, the absence of histones in mitochondria, and the relatively inefficient DNA repair (Richter et al. 1988). Moreover it was shown recently that in H_2O_2-treated *E. coli* cells, levels of 8-oxo-rG in rRNA were the same or even higher than that for non-ribosomal RNA (Liu et al. 2012). According to the authors the results are

consistent with the observation of rRNA oxidation in vivo and suggest that complex structures and association with proteins do not protect rRNA from oxidation.

It has been found that oxidative stress causes greater damage to RNA than DNA in different mammalian cells: the basic levels of 8-oxo-rG in RNA increase up to the fivefold after oxidative stress and are 10–25 times higher than for 8-oxo-dG in DNA (Fiala et al. 1989; Shen et al. 2000; Hofer et al. 2005, 2006).

2 Definition of Reactive Oxygen and Nitrogen Species and Their Properties

Reactive oxygen and nitrogen species are collective terms for either oxygen or nitrogen containing molecules, radical or nonradical, that act as oxidizing agents or that can be easily converted into radicals.

Reactive oxygen and nitrogen species (ROS, RNS) are either produced endogenously as natural by-products of the normal cellular metabolism of oxygen and nitrogen or from exogenous sources like tobacco, drugs, xenobiotics, and ionizing radiation. Under oxidative stress, which implies an imbalance between the generation of ROS and the system's capability of neutralizing ROS or subsequent damage, these reactive intermediates may damage a wide spectrum of vital cell compounds like nucleic acids, amino acids, and unsaturated fatty acids.

With regard to nucleic acid damage the main reactive oxygen species include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and the radical species superoxide ($^{\bullet}O_2^-$) and hydroxyl radical ($^{\bullet}OH$). Reactive nitrogen species include peroxynitrite ($ONOO^-$), nitric oxide ($^{\bullet}NO$), and nitrogen dioxide ($^{\bullet}NO_2$). Endogenous sources of ROS mainly include the mitochondrial respiratory chain, NADPH oxidases (NOX), and 5-lipoxygenases (LOX) among others which mainly form the primary ROS $^{\bullet}O_2^-$ (Nathan and Cunningham-Bussel 2013). Mitochondria are among the most reactive organelles in the cell and consume nearly 90 % of the total oxygen content (Bolisetty and Jaimes 2013). Overall seven sites of superoxide production are known (Brand 2010). In the mitochondrial respiratory chain up to 5 % of the electrons flowing through the electron transport chain can be abstracted mainly at the levels of complex I (NADH/CoQ reductase) and complex III (ubiquinol/cytochrome c reductase) to form $^{\bullet}O_2^-$ (Novo and Parola 2008; Lenaz 2012). Superoxide, having a quite short half-life and low reactivity, is readily converted to hydrogen peroxide by superoxide dismutase (SOD), as depicted in Fig. 1. The further inactivation of H_2O_2 is catalyzed by a series of enzymes including glutathione peroxidase (GPX), peroxiredoxins (PRX), thioredoxin, or catalase in peroxisomes. Compared to $^{\bullet}O_2^-$, hydrogen peroxide is electrophobic and can easily diffuse across membranes. It was found that the concentration of H_2O_2 in mitochondria is about 100 times higher compared to $^{\bullet}O_2^-$ (Cadenas and Davies 2000). In the presence of divalent metal ions H_2O_2 can further react in Fenton-type reactions to yield the highly reactive hydroxyl radical.

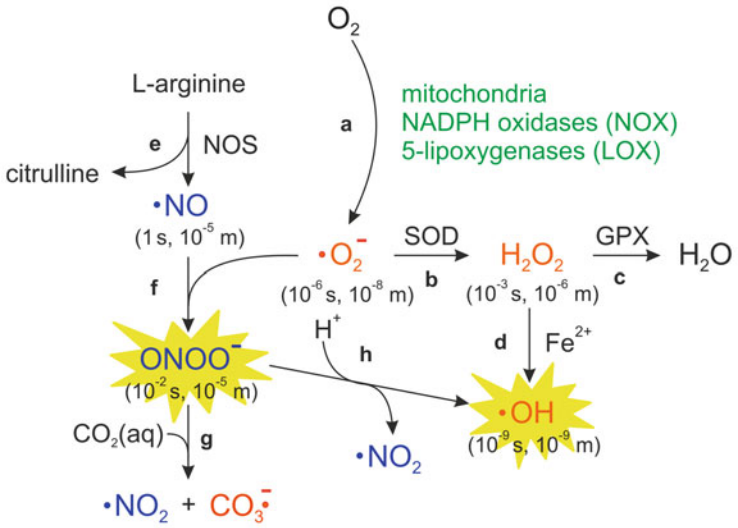

Fig. 1 Main ROS (*red*) and RNS (*blue*) formed in cells. Superoxide is generated mainly in mitochondria or by NOX and LOX (a) and is further dismuted by superoxide dismutase (SOD) to form hydrogen peroxide (b). H_2O_2 is then further metabolized to water by glutathione peroxidases (GPX) or other enzymes (c). Reactive hydroxyl radicals are produced by Fenton-type reactions of H_2O_2 in the presence of Fe^{2+} (d). Nitric oxide is formed in the conversion of L-arginine to citrulline by nitric oxide synthases (NOS) (e) and readily forms peroxynitrite upon reaction with superoxide (f). Reaction of peroxynitrite with carbon dioxide yields nitrite and carbonate radicals (g), while peroxynitrous acid generates hydroxyl and nitrite radicals (h). Magnitudes of half-life times and the respective mobilities of the different species are given in parentheses

Nitric oxide, which is formed through the conversion of L-arginine to citrulline by nitric oxide synthases (NOS), is a highly diffusible and relatively stable RNS. Upon reaction with $^{\bullet}O_2^-$ it forms the highly reactive peroxynitrite ($ONOO^-$) at diffusion-controlled rates (Beckman et al. 1990; Radi et al. 2001). Peroxynitrite is a short-lived species and it can as well in its protonated form participate directly in oxidation reactions. Peroxynitrous acid itself immediately undergoes homolytic fission and forms a hydroxyl radical and nitrogen dioxide (Fig. 1, h) or a nitrate anion. Upon reaction with carbon dioxide, peroxynitrite forms [$ONOOCO_2^-$] that decays to give either a carbonate radical and nitrogen dioxide (Fig. 1, g) or nitrate and carbon dioxide (Radi et al. 2001).

The magnitudes of half-life times and the respective mobilities of ROS (Pryor 1986; Karuppanapandian et al. 2011) and RNS (Pacher et al. 2007; Szabó et al. 2007) are depicted in Fig. 1.

3 Reactivity of ROS and RNS Versus Nucleic Acids

While H_2O_2 and $^{\bullet}O_2^-$ have been shown to be unreactive themselves with respect to nucleic acids (Lesko et al. 1980; Brawn and Fridovich 1981), $^{\bullet}OH$ efficiently reacts at diffusion-controlled rates near the site of its formation with all four nucleobases and the (deoxy-)ribose sugar. Hydroxyl radical footprinting has been used to cleave nucleic acids to the nucleotide level and for revealing RNA folding pathways (Tullius and Dombroski 1985; Tullius and Greenbaum 2005). Mechanisms for the oxidation on the sugar have been widely explored for DNA (Pogozelski and Tullius 1998; Evans et al. 2004; Dedon 2007), whereas for RNA the existing data are limited to a few examples. Hydroxyl radicals can in principle react with sugar units by the abstraction of H-atoms of any of the five carbon atoms present. It was found that oxidation at the C1′-center of the ribose sugar is slower than for the 2′-deoxyribose sugar, presumably due to the polar effect of the 2′-hydroxyl group in RNA as the authors suggest (Neyhart et al. 1995). A similar behavior was found for the generation of C4′ radicals in model nucleotides: fragmentation of the C3′–O3′ bond by formation of a C3′ radical was shown to be far slower in the presence of a 2′-*O* substituent on the sugar moiety (Crich and Mo 1997). It has been further shown that iron-bleomycin (FeBLM), which efficiently cleaves DNA by the generation of C4′ radicals, is far less reactive but more sequence selective with RNA as the substrate (Hecht 1994). The data suggest, although not entirely conclusive, that the ribose unit seems to be more stable against hydroxyl radical attack compared to deoxyribose.

Upon reaction with RNA, peroxynitrite was found to form 8-oxo-rG and 8-nitro-rG (Masuda et al. 2002). Peroxynitrite seems to react with RNA according to different mechanisms: it was found that bicarbonate caused a dose-dependent increase in the formation of 8-nitro-rG whereas it produced no apparent effect on 8-oxo-rG formation. While 8-nitro-dG in DNA was found to depurinate rapidly (Yermilov et al. 1995), in RNA it was shown that 8-nitro-rG was far more stable (Masuda et al. 2002). Data on reactions of peroxynitrite with DNA suggest two different mechanisms: an increasing bicarbonate concentration seems to favor the formation of $^{\bullet}NO_2$ (Fig. 1, g) and a low pH seems to induce the formation of hydroxyl radicals which can lead to strand breaks (Fig. 1, h) (Yermilov et al. 1996). Moreover it has been shown that peroxynitrite reacts mainly with dG nucleosides to form 8-oxo-dG and 8-nitro-dG while dA, dC, and dT showed only minimal reactivity (Burney et al. 1999).

Peroxynitrite has also been shown to induce strand cleavage in DNA (Salgo et al. 1995; Kennedy et al. 1997; Szabó and Ohshima 1997; Niles et al. 2006). According data for RNA cleavage is still missing, but it can be suggested that RNA cleavage happens to a lower extent. Overall the data suggest that the main oxidative damage seems to be coming from H_2O_2-generated $^{\bullet}OH$ and peroxynitrite itself, as well from $ONOO^-$-generated $^{\bullet}OH$ because of the high diffusibility of H_2O_2 and peroxynitrite. Other forms of ROS hardly get into the vicinity of nucleic acids because of their limited half-life and their lacking moving capability.

Singlet oxygen, another strong oxidant generated by light (photooxidative stress), has so far only been shown to be able to oxidize rG (Schneider et al. 1993). Overall the data show that the reactivity of RNA under the influence of ROS and RNS is comparable to DNA with respect to base modifications. On the other hand, RNA might be less vulnerable against sugar backbone oxidation and subsequent strand cleavage compared to DNA.

4 Oxidative Lesions in RNA

The four main RNA oxidative lesions (Fig. 2) have been isolated from *Torula* yeast RNA (Yanagawa et al. 1990, 1992). Two other base lesions that are generated indirectly by oxidative stress through lipid peroxidation products are 1,N^6-ethenoadenosine (ε-rA) and 3,N^4-ethenocytidine (ε-rC) (El Ghissassi et al. 1995). All of these lesions have subsequently been synthesized and chemically introduced into RNA: 8-oxo-7,8-dihydroguanosine (Kim et al. 1998; Koga et al. 2013), 8-oxo-7,8-dihydroadenosine (Kim et al. 2002), 5-hydroxyuridine (Cui et al. 2009), 5-hydroxycytidine (Küpfer and Leumann 2011), and ε-rA and ε-rC (Srivastava et al. 1994; Calabretta and Leumann 2013).

These oxidative modifications described for RNA only represent a small fraction of the lesions found in DNA (Cadet et al. 2010). For pyrimidines potentially interesting oxidative lesions include 5,6-dihydroxy-5,6-dihydropyrimidines (pyrimidine glycols) and 5-hydroxymethyl- and 5-formylpyrimidines (hm^5C, hm^5U, f^5C and f^5U). While pyrimidine glycols are known precursors for 5-HO-rC and 5-HO-rU, 5-hydroxymethyl- and 5-formylpyrimidines could be potential oxidation products of 5-methylcytidine and 5-methyluridine, which are found in tRNA and mRNA (for m^5C) or rRNA and tmRNA (for m^5U) (Cantara et al. 2011). For 8-oxo-purines, the low redox potentials make them especially vulnerable to undergo further oxidation which leads to 4,6-diamino-5-formamidopyrimidine (FapyA) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine nucleosides (FapyG) (Fig. 2) among a number of other derivatives for G including imidazolone, oxazolone, and spiroiminodihydantoin derivatives (Cadet et al. 2010). For FapyG it could be shown that the *N*-glycosidic bond of the isolated ribonucleoside is remarkably stable against anomerization and cleavage (Burgdorf and Carell 2002). Furthermore FapyG and FapyA were just recently quantified among other oxidative lesions in the analysis of RNA damage in Alzheimer's disease patients and it can therefore be assumed that they are also present in the pool of oxidatively damaged RNA lesions (Bradley-Whitman and Lovell 2013). Also it is known from DNA that lipid peroxidation products lead to a wide variety of substituted etheno-, propano-, as well as malondialdehyde adducts. So far there is no data available on the hybridization properties or possible mutagenicity of these lesions in RNA.

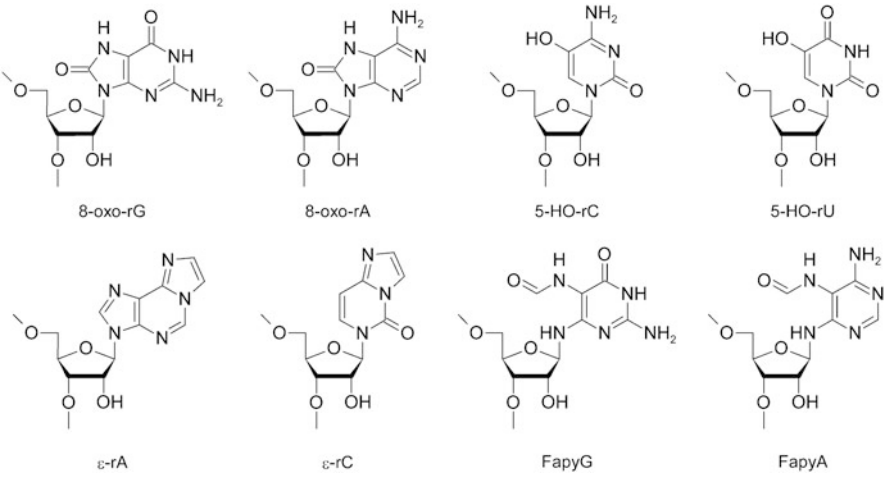

Fig. 2 Main stable oxidative lesions found in RNA: 8-oxo-7,8-dihydroguanosine (8-oxo-rG), 8-oxo-7,8-dihydroadenosine (8-oxo-rA), 5-hydroxycytidine (5-HO-rC), 5-hydroxyuridine (5-HO-rU), 1,N^6-ethenoadenosine (ε-rA), and 3,N^4-ethenocytidine (ε-rC). Further oxidation products of 8-oxo-purines: 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino-5-formamidopyrimidine (FapyA)

4.1 Base-Pairing Properties of Lesion Containing Duplexes

The influence of the oxidized bases on duplex stability was elucidated by UV melting experiments with RNA duplexes and RNA/DNA hybrids. The results of these T_m measurements in the same sequence context are summarized in Table 1.

5-Hydroxypyrimidines lead to no notable destabilization in neither of the duplexes compared to the natural cases and all formed base pairs are in accordance with Watson and Crick. An important fact to note is that in the case of 5-hydroxycytidine a biphasic melting behavior was observed (Küpfer and Leumann 2011).[1]

8-Substituted purine nucleosides have long been known to adopt the *syn* conformation (Uesugi and Ikehara 1977). For 8-oxo-rG it has been shown that it exists mainly in the neutral 6,8-diketo form with a pK_a of 8.5 for the 6-enolate-8-keto form and a pK_a of 11.2 for the 6,8-dienolate form (Cho et al. 1990). In contrast to 8-oxo-rG, the pK_a of 8.7 for the 8-enolate form of 8-oxo-rA is quite low (Cho and Evans 1991). The different tautomeric forms of the oxidized nucleosides with their respective pK_a values are depicted in Fig. 3.

[1] The melting curves for the matched case 5-HO-rC/G in RNA duplexes and RNA/DNA heteroduplexes show two distinct changes in hyperchromicity: the higher T_m is close to the matched case whereas the lower T_m is close to the mismatched case. This phenomenon is not yet fully understood and still under investigation.

Table 1 T_m data (°C, 260 nm) of duplexes of oligoribonucleotide 5′-AUGCU**X**AGUCGA-3′ with 5′-UCGACU**Y**AGCAU-3′ (RNA) or 5′-TCGACT**Y**AGCAT-3′ (DNA) as complement; values in parentheses indicate the difference in T_m compared to the corresponding natural duplex; duplex concentration: 2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0

	RNA				DNA			
Y=	rA	rC	rG	rU	dA	dC	dG	dT
X=								
8-oxo-rA	41.8 (−3.1)	37.8 (−8.8)	46.5 (−0.2)	50.5 (−7.1)	25.8 (−3.8)	20.8 (−13.8)	25.8 (−4.1)	35.2 (−10.1)
5-OH-rC	47.4 (−0.4)	44.0 (−1.6)	65.2, 47.7 (−0.5, −17.5)	44.6 (−1.4)	27.2 (−1.2)	25.4 (−1.3)	48.6, 25.9 (−1.3, −24)	25.4 (−1.2)
8-oxo-rG	54.9 (+9.9)	55.6 (−8.4)	52.0 (+5)	43.7 (−9.3)	37.6 (+6.6)	40.0 (−11)	31.3 (0)	29.0 (−9.6)
5-OH-rU	58.8 (−0.4)	43.8 (−0.4)	54.2 (−0.4)	45.9 (−0.4)	42.6 (+0.3)	23.6 (0)	32.6 (+0.6)	27.6 (−0.6)
ε-rA	43.9 (−1)	40.6 (−6)	44.9 (−1.8)	42.0 (−15.6)	28.6 (−1)	23.6 (−11)	29.3 (−0.6)	25.4 (−19.9)
ε-rC	43.9 (−3)	39.3 (−3.7)	46.6 (−18)	41.9 (−2)	25.2 (−2)	20.8 (−3.7)	25.3 (−23.3)	24.3 (−2.9)

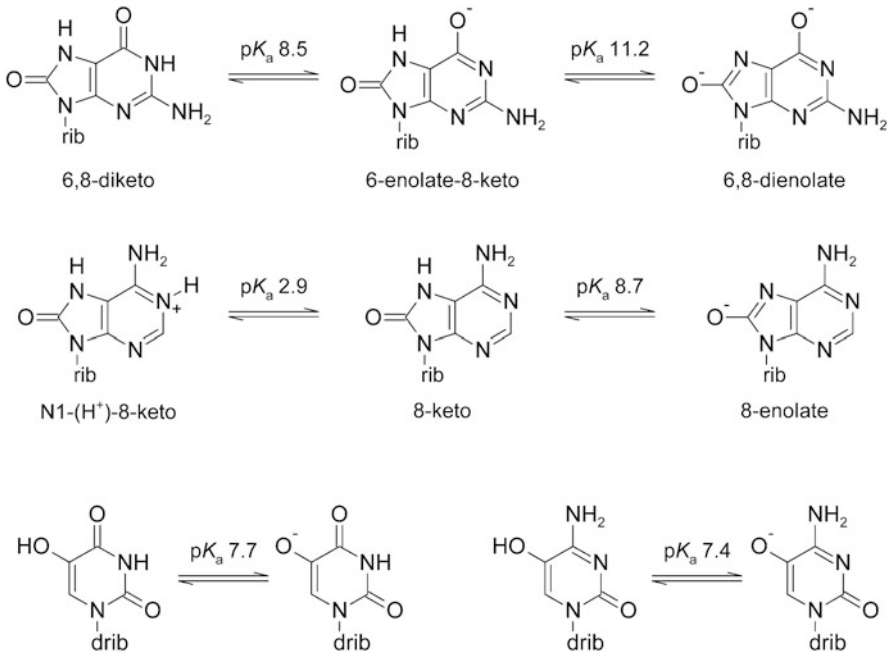

Fig. 3 pK_a values for the different tautomeric forms of 8-oxo-rG, 8-oxo-rA, 5-HO-rU, and 5-HO-rC. Values for the 8-oxo-purines are given for the ribonucleosides and values for the 5-hydroxypyrimidines are given for the deoxynucleosides

For 8-oxoguanosine the duplexes are destabilized in the case where 8-oxo-rG pairs to pyrimidine bases. When 8-oxo-rG is paired against purine bases the duplexes gain in stability compared to the natural mismatch duplexes: for 8-oxo-rG/dG the duplex has the same stability as the mismatch duplex with rG/dG; for 8-oxo-rG pairing against rG, rA, and dA the duplexes gain considerably in stability. Especially in the case of 8-oxo-rG/rA the duplex stability equals almost the stability of the 8-oxo-rG/rC duplex. These results have just recently been confirmed showing that the T_m of 8-oxorG/rA is even slightly higher than for the natural case (Koga et al. 2013).[2] This remarkable stabilization for 8-oxo-rG pairing rG and rA could be explained by data measured previously in DNA: it has been found that the Hoogsteen face of 8-oxo-dG pairs with dA forming a *syn*-8-oxo-dG/*anti*-dA base pair (Kouchakdjian et al. 1991; Gannett and Sura 1993). Such a *syn*-8-oxo-rG/*anti*-rA base pair would be expected to add to the overall duplex stability compared to an *anti*-rG/*anti*-rA mismatch. Furthermore it has been shown that rG can pair with the Hoogsteen face of rA forming an *anti*-rG/*syn*-rA base pair (Pan et al. 1999). It has been suggested that in a similar manner this could also give rise to an *anti*-8-oxo-rG/*syn*-rG base pair (Koga et al. 2013). The data suggest that 8-oxo-rG easily switches between *anti* and *syn* conformations to comfort the opposing base (Fig. 4).

For 8-oxoadenosine moderate to strong destabilization of the duplexes was found with the strongest decrease for the pairing to pyrimidine bases in both RNA/RNA and RNA/DNA duplexes. In DNA 8-oxo-dA was shown to pair dT according to Watson and Crick (Guschlbauer et al. 1991). In analogy to 8-oxo-dG it has also been found that 8-oxo-dA can pair to dG via its Hoogsteen face forming a *syn*-8-oxo-dA/*anti*-dG base pair (Leonard et al. 1992). Later on base pairs of *syn*-8-oxo-dA with dC and dA have been postulated (Kamiya et al. 1995). Nevertheless 8-oxoadenosine still discriminates the binding partners in favor of the matched base.

These findings suggest that all oxidized bases are capable of forming stable base pairs but with a lower discrimination of mismatches. This might subsequently lead to the synthesis of modified proteins via the interaction of oxidatively damaged mRNA with tRNA as was suggested earlier (Kong and Lin 2010).

Etheno-adenosine and -cytidine lesions show major destabilization in RNA duplexes and RNA/DNA heteroduplexes because of the loss of their ability to form standard Watson–Crick hydrogen bonds. Duplexes formed with a G opposite the lesion are slightly more stable than the others, most likely because of stronger base stacking interactions of the etheno bases (Calabretta and Leumann 2013).

[2] The published findings confirm our earlier observations that the previously used 6*O*,7*N*-bis (dimethylcarbamyl) protected phosphoramidite of 8-oxo-rG is not fully deprotectable once incorporated into RNA. The authors here used a 6*O*,7*N*-bis(diphenylcarbamyl)-protected while we used an 6*O*,7*N*-unprotected phosphoramidite of 8-oxo-rG (unpublished results). Both phosphoramidites were fully deprotectable as confirmed by mass spectrometry. The T_m values measured by Koga et al. correspond nicely to our findings (Table 1).

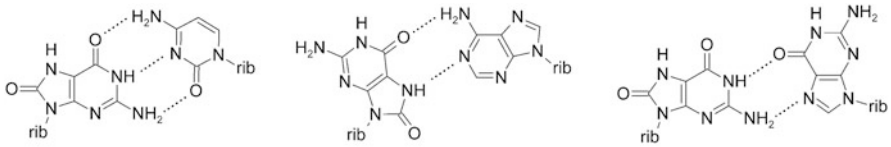

Fig. 4 Base-pairing modes for 8-oxo-rG: canonical 8-oxo-rG/rC base pair according to Watson and Crick (*left*), 8-oxo-rG/A base pair where 8-oxo-rG pairs with its Hoogsteen side (*middle*) and the 8-oxo-rG/G base pair where the Watson–Crick side of 8-oxo-rG pairs to the Hoogsteen side of rG

4.2 Mutagenicity of Oxidatively Damaged Nucleobases

The potential mutagenicity of oxidized RNA bases was elucidated by reverse transcription assays. In these assays a DNA primer is elongated on a modified RNA template using reverse transcriptases and the efficiency of the incorporation of natural 2′-*O*-deoxynucleoside monophosphates (dNMP) is determined.

4.2.1 5-Hydroxypyrimidines

For a 5-HO-rU containing RNA template it was found that reverse transcriptases[3] incorporated preferentially dAMP opposite the lesion. Notable misincorporation of dGMP was dependent on the reverse transcriptase used: MMLV-RT incorporated up to one-third, Superscript™II-RT up to one-fourth, and AMV only trace amounts of dGMP compared to incorporated dAMP (Cui et al. 2009).

In the case of a 5-HO-rC containing template there was found substantial misincorporation of dAMP besides the expected incorporation of dGMP: HIV1-RT incorporated dAMP almost as efficiently as dGMP while for the AMV-RT and MMLV-RT this process is two- to threefold less efficient. Interestingly, HIV1-RT also incorporated dTMP with about half the efficiency of dGMP (Küpfer and Leumann 2011). In DNA 5-HO-dU and 5-HO-dC have also been tested for potential mutagenicity during DNA replication and it was found that 5-HO-dU and 5-HO-dC cause C/T transition mutations (Kreutzer and Essigmann 1998; Suen et al. 1999). The mutagenicity has been associated with tautomeric variability of the oxidized base: while the amino form of 5-hydroxycytidine pairs to guanosine, the imino form would be able to pair to adenosine (Suen et al. 1999; La Francois et al. 2000).

[3] AMV-RT: avian myeloblastosis virus reverse transcriptase; MMLV-RT: moloney murine leukemia virus reverse transcriptase; HIV1-RT: human immunodeficiency virus type 1 reverse transcriptase; RAV2-RT: Rous-associated virus-2 reverse transcriptase.

4.2.2 8-Oxo-7,8-Dihydropurines

Reverse transcription assays with 8-oxo-rG containing templates were performed using MMLV-RT and AMV-RT (Kim et al. 1999) and HIV-RT and RAV2-RT (Kim et al. 2004). While MMLV-RT seemed to incorporate preferentially dTMP opposite 8-oxo-rG, AMV inserted the correct dCMP residue. Using HIV-1 RT and RAV2-RT, almost no dAMP and dGMP were incorporated opposite 8-oxo-rG. - HIV-1 RT incorporated almost exclusively dCMP opposite 8-oxo-rG. Using RAV2-RT, dCMP was only incorporated 1.5-fold of dTMP opposite 8-oxo-rG. These results have to be looked at carefully since there is evidence that the authors might have used the not fully deprotected 8-oxo-rG-DMC derivative (see Footnote 2). It was recently shown that duplexes of 8-oxo-rG-DMC containing RNA with DNA have considerably lower T_m values compared to 8-oxo-rG containing heteroduplexes and clearly discriminate in favor of dC opposite 8-oxo-rG-DMC (Koga et al. 2013).

Similar assays with 8-oxo-rA containing RNA were performed using AMV-RT, MMLV-RT, and RAV2-RT (Kim et al. 2002). All reverse transcriptases incorporated preferentially dTMP opposite the lesion. AMV-RT incorporated also dGMP and dAMP to a three- and fourfold lesser extent and MMLV- and RAV2-RT incorporated dGMP to a fivefold lesser extent compared to dTMP.

In DNA 8-oxo-dG is known to lead to G/T transversions in vitro (Wood et al. 1990) and in vivo (Moriya et al. 1991; Cheng et al. 1992). DNA polymerases incorporate dAMP and dCMP differently opposite 8-oxo-dG: DNA pol α, pol δ, and pol III preferentially incorporate dAMP, whereas DNA pol β and pol I incorporate dCMP (Wang et al. 1998). Replication of 8-oxo-dA containing DNA templates revealed that exclusively dTMP is inserted opposite the lesion by bacterial DNA polymerases pol I (Klenow fragment) and Taq DNA pol (Guschlbauer et al. 1991). Nonetheless, 8-oxo-dA is found to be mutagenic in mammalian cells where DNA pol α is found to misinsert dGMP and recombinant rat DNA pol β can misinsert dAMP and dGMP (Shibutani et al. 1993; Kamiya et al. 1995).

4.2.3 ε-Adenosine and ε-Cytidine

For reverse transcription assays it was found that only the error-prone HIV-1 RT was able to bypass the lesions. In case of ε-rA containing RNA templates HIV-1 RT incorporated deoxynucleoside triphosphates in the order dAMP > dGMP ≫ dCMP and dTMP. For ε-rC containing templates the incorporation order observed was dAMP > dTMP ≫ dCMP > dGMP. In both cases full-length cDNA was obtained in the presence of all deoxynucleoside triphosphates (Calabretta and Leumann 2013).

The mutagenicity of ε-dA and ε-dC has been shown to be strongly mutagenic when tested in mammalian cell lines, whereas in *E. coli* minor mutagenic properties were observed. In mammalian cells predominantly A/G transitions and A/T

transversions were found for ε-dA and C/T transitions for ε-dC (Pandya and Moriya 1996; Levine et al. 2000). In *E. coli* only minor mutagenic properties were found for ε-dA (Basu et al. 1993; Pandya and Moriya 1996) and only low frequent C/A transversions for ε-dC (Moriya et al. 1994).

4.2.4 Oxidative Damage to the Nucleotide Pool

Oxidative damage to the nucleotide pool can be induced by direct oxidation of ribonucleotides or by degradation of oxidatively damaged RNA. Leukocytes that were put under oxidative stress revealed increased levels of 8-oxo-rG in the nucleotide pool (Shen et al. 2000). Quality control of RNA synthesis is thus crucial and mechanisms have evolved to reduce incorporation of oxidatively damaged ribonucleotides. It was shown that 8-oxo-rGTP was incorporated into RNA by *E. coli* polymerase at a rate of ~10 % of that of rGTP (Taddei et al. 1997). To prevent the incorporation of 8-oxo-rGTP, MutT protein in *E. coli* degrades the di- and triphosphates of 8-oxo-rG to the monophosphate 8-oxo-rGMP (Ito et al. 2005). The reutilization of 8-oxo-rGMP is prevented since it was found that guanylate kinase does not phosphorylate 8-oxo-rGMP to 8-oxo-rGDP and therefore no 8-oxo-rGTP can be synthesized (Sekiguchi et al. 2013). A similar pathway has been disclosed for human cells. Here 8-oxo-rGTP is incorporated by human RNA polymerase II at a rate of 2 % of that of rGTP (Hayakawa et al. 1999). MTH1 protein has been shown to hydrolyze 8-oxo-rGTP to 8-oxo-rGMP. Additionally it could be shown that MTH5 and NUDT5 hydrolyze 8-oxo-rGDP to 8-oxo-rGMP (Ishibashi et al. 2005). Normally GTP is synthesized from GDP through phosphorylation by nucleotide diphosphate kinase (ND kinase) and GDP is itself produced through phosphorylation of GMP by guanylate kinase (GK). While ND kinase has been found to phosphorylate 8-oxo-rGDP as well, GK is not able to phosphorylate 8-oxo-rGMP, thus preventing the synthesis and incorporation of 8-oxo-rGTP into RNA (Hayakawa et al. 1999).

5 Consequences of Oxidatively Damaged RNA

During the last few years increasing evidence emerged that oxidatively damaged RNA causes severe effects on cellular function. Messenger RNA oxidation in vitro was shown to cause reduction in protein expression and ribosome stalling on the transcripts (Shan et al. 2007). In another study the authors found that oxidized mRNA induces translation errors (Tanaka et al. 2007). It was also shown that nitric oxide and peroxynitrite accelerate mutation of RNA viruses in vitro and in vivo, probably via the formation of 8-oxo-rG and 8-nitro-rG (Akaike et al. 2000).

Preliminary studies on oxidatively damaged ribosomes have shown that if the catalytic adenine, A2451 in the peptidyl transferase center (PTC) of the 23S ribosome subunit of *E. coli*, is replaced by an 8-oxo-adenine unit, peptide synthesis

is abolished (Polacek, unpublished results). This is quite remarkable since it has been shown earlier that several base modifications on A2451 only have a minor influence on the activity of the PTC as long as the 2′-hydroxyl group of the ribose is present (Erlacher and Polacek 2008).

Ongoing studies on the efficiency of translation on site specifically damaged mRNA show that in rabbit reticulocyte lysate ribosome stalling as well as read-through can be observed. Whether oxidized mRNA leads to mutations in the synthesized proteins is yet unclear (Leumann, unpublished results).

6 Fate of Oxidized RNA

High levels of oxidized RNA are not tolerated by cells. Several studies have shown that levels of oxidized RNA dropped after removal of oxidative stress inducing conditions (Shen et al. 2000; Kajitani et al. 2006). Recently it could be shown that H_2O_2-induced 8-oxo-rG levels in *E. coli* cells dropped rapidly after switching to a H_2O_2-free medium (Liu et al. 2012). These results suggest that oxidatively damaged RNA is actively removed from total RNA. In theory this might happen either by repair of the oxidized lesions or by degradation. So far there is no strong evidence supporting the existence of RNA repair mechanisms. The only repair mechanism reported for RNA is the dealkylation of alkylated RNA by DNA demethylases (Aas et al. 2003). Therefore it seems more likely that oxidatively damaged RNA is removed by degradation.

Removal of damaged RNA can be achieved by ribonucleases. Polynucleotide phosphorylase (PNPase) is a 3′–5′ exoribonuclease that is important for the degradation of RNA. In *E. coli* it has been shown that PNPase specifically binds 8-oxo-rG containing RNA with a higher affinity than undamaged RNA (Hayakawa et al. 2001). Undamaged RNA has been shown to be degraded efficiently while 8-oxo-rG containing RNA is tightly bound to PNPase and thus protected from nuclease attack. Human PNPase is located mainly in mitochondria and also specifically binds 8-oxo-rG containing RNA. Under induced oxidative stress, levels of hPNPase decreased rapidly while the levels of other proteins were left unchanged (Hayakawa and Sekiguchi 2006). The authors speculate that the PNP might thus play an important role in maintaining the high fidelity of translation by sequestering the oxidatively damaged RNA molecules and possibly direct them to degradation. In a further study it has been shown that PNPase-deficient cells are hypersensitive to H_2O_2 and less viable than wild-type cells (Wu et al. 2009). Introduction of a plasmid-borne PNP gene restores the viability against oxidative stress. Furthermore overexpression of hPNPase in HeLa cells reduces 8-oxo-rG in RNA and improves cell viability against H_2O_2 treatment (Wu and Li 2008).

Another protein that shows a similar binding capacity for 8-oxo-rG containing RNA is the mammalian Y box-binding protein 1 (YB-1 protein). YB-1 has multiple regulatory activities and was originally identified as a transcription factor which binds to the Y box. YB-1 is a major core protein of messenger ribonucleoprotein

(mRNP) and has a weak RNA binding capacity. It was found that YB-1 selectively binds 8-oxo-rG containing RNA to form stable complexes. Cells of *E. coli* expressing YB-1 were found to acquire resistance against oxidative stress. These data imply that YB-1 protein may be involved in sequestering oxidatively damaged RNA from normal cellular processes (Hayakawa et al. 2002).

Oxidized nucleobases are known to have low one-electron redox potentials and are therefore prone to undergo further oxidation processes (Yanagawa et al. 1992). In DNA 8-oxo-dG and 8-nitro-dG showed a higher reactivity with $ONOO^-$ than dG itself (Burney et al. 1999).

That such ongoing oxidation induces abasic sites could be shown by the reaction of RNA treated under Fenton conditions with the aldehyde reactive probe (ARP) (Tanaka et al. 2011a, b). The ARP has been shown earlier to react specifically with the aldehyde functions of abasic sites and formylcytidine but no other oxidative lesion, e.g., 8-oxo-rA, 8-oxo-rG, or pyrimidine glycols (Ide et al. 1993). RNA abasic sites have been shown to be remarkably more stable than DNA abasic sites (Küpfer and Leumann 2007). That such abasic sites can be substrates for enzymatic degradation has been shown by the disclosure of a new class of enzymes: the ribosomal RNA apurinic site-specific lyase (RALyase) (Ogasawara et al. 1999; Ito et al. 2002). These enzymes exhibited a very high specificity for the cleavage of the abasic site in the sarcin–ricin loop of intact ribosomes. On the contrary, RALyase failed to cleave DNA abasic sites as well as protein-free abasic rRNA. Thus, rigorous proof for broader RNA-specific lyase activity is still missing, as no other members of this class of enzymes could be identified so far.

7 Redox Chemistry of Oxidized Nucleosides

The one-electron redox potentials of the four main oxidative lesions have been reported to be far lower than for the standard nucleosides: 8-oxo-rG: 0.58 V (1.29 V for rG), 8-oxo-rA: 0.92 V (1.42 V for rA), 5-HO-rC: 0.62 V (1.6 V for dC), and 5-HO-rU: 0.64 V (1.7 V for dT) versus NHE (Yanagawa et al. 1992; Steenken and Jovanovic 1997); for 8-oxo-dG: 0.74 (Steenken et al. 2000). So far two examples of exploiting the low redox potentials of hydroxynucleosides have been published.

For oxidized RNA nucleosides it could be shown that they catalyze the oxidoreduction of NADH and $K_3Fe(CN)_6$ (Yanagawa et al. 1992). In this system 5-HO-rC showed by far the highest activity followed by 5-HO-rU and 8-oxo-rG which showed roughly half and a quarter of the reactivity compared to 5-HO-rC. The authors conclude that these hydroxyribonucleosides might also serve as redox cofactors at active sites of ribozymes with redox activity. So far this was not experimentally proven.

8-Oxo-dG, incorporated in proximity to a cyclobutane pyrimidine dimer (CPD), can mimic the function of a flavin in photorepair. The study supports a photolyase-type mechanism in which in a first step the excited state of 8-oxo-dG transfers an electron to the CPD effecting cleavage of the cyclobutane and in a second step the

back electron transfer regenerates 8-oxo-dG and the repaired pyrimidines (Nguyen and Burrows 2011, 2012b). The free tri-acetylated 8-oxo-rG and ribofuranosyluric acid nucleosides also showed enhanced repair of T=T and U=U CPDs (Nguyen and Burrows 2012a).

The possible role of oxidized RNA nucleosides as redox catalysts in a "RNA world" is not known. Their use in ribozymes might be a future prospect.

8 Conclusions and Perspective

For DNA a large number of oxidative lesions, their metabolism, and distinct repair mechanisms have been reported in the past. As reviewed here, only half a dozen lesions that arise under oxidative stress have been described, along with their biophysical properties for RNA. Nevertheless, in accordance with DNA it can be assumed that more lesions exist which are still lacking a detailed description.

While 85 % of the genomic DNA is transcribed into RNA, the function of 97 % of this RNA is still largely unknown. Thus, oxidatively damaged RNA cannot only interfere with the translation machinery but possibly also with a large number of mostly unknown regulation mechanisms of ncRNA. The number of such long intergenic noncoding RNAs (lincRNA) is still growing and their functions are yet elusive (Hangauer et al. 2013).

The data show that RNA is at least as prone to oxidation as DNA due to its spatial vicinity to the sources of ROS and RNS and the (so far) absence of a distinct repair machinery. Moreover the amount of RNA in a cell is considerably higher than that of DNA. Within this context, RNA cannot be viewed only as a transient messenger anymore: average lifetimes of RNA are probably long enough to have an impact on different cell mechanisms.

Oxidatively damaged RNA has been linked over the past decade to a still increasing number of neurodegenerative and other diseases (Poulsen et al. 2012). The data show that the oxidative damage is rather an early event in the development of such diseases than a consequence of cell decay. A deeper understanding of the biophysical properties of oxidative lesions in RNA would undoubtedly help to understand the more complex biological impact and mechanisms of such diseases.

Acknowledgments The authors wish to thank Dr. Alessandro Calabretta for the contribution of T_m data for Table 1.

References

Aas PA, Otterlei M, Falnes PO et al (2003) Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. Nature 421:859–863

Akaike T, Fujii S, Kato A et al (2000) Viral mutation accelerated by nitric oxide production during infection *in vivo*. FASEB J 14:1447–1454

Barciszewski J, Barciszewska M, Siboska G et al (1999) Some unusual nucleic acid bases are products of hydroxyl radical oxidation of DNA and RNA. Mol Biol Rep 26:231–238

Basu AK, Wood ML, Niedernhofer LJ et al (1993) Mutagenic and genotoxic effects of three vinyl chloride-induced DNA lesions: 1, N6-ethenoadenine, 3, N4-ethenocytosine, and 4-amino-5-(imidazol-2-yl)imidazole. Biochemistry 32:12793–12801

Beckman JS, Beckman TW, Chen J et al (1990) Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci U S A 87:1620–1624

Bolisetty S, Jaimes E (2013) Mitochondria and reactive oxygen species: physiology and pathophysiology. Int J Mol Sci 14:6306–6344

Bradley-Whitman M, Lovell M (2013) Increased oxidative damage in RNA in Alzheimer's disease progression. J Anal Bioanal Tech. doi:10.4172/2155-9872.s2-004

Brand MD (2010) The sites and topology of mitochondrial superoxide production. Exp Gerontol 45:466–472

Brawn K, Fridovich I (1981) DNA strand scission by enzymically generated oxygen radicals. Arch Biochem Biophys 206:414–419

Burgdorf LT, Carell T (2002) Synthesis, stability, and conformation of the formamidopyrimidine G DNA lesion. Chemistry 8:293–301

Burney S, Niles JC, Dedon PC et al (1999) DNA damage in deoxynucleosides and oligonucleotides treated with peroxynitrite. Chem Res Toxicol 12:513–520

Cadenas E, Davies KJA (2000) Mitochondrial free radical generation, oxidative stress, and aging. Free Radic Biol Med 29:222–230

Cadet J, Douki T, Ravanat J-L (2010) Oxidatively generated base damage to cellular DNA. Free Radic Biol Med 49:9–21

Calabretta A, Leumann CJ (2013) Base pairing and miscoding properties of 1, N6-ethenoadenine- and 3, N4-ethenocytosine-containing RNA oligonucleotides. Biochemistry 52:1990–1997

Cantara WA, Crain PF, Rozenski J et al (2011) The RNA modification database, RNAMDB: 2011 update. Nucleic Acids Res 39(suppl 1):D195–D201

Chang Y, Kong Q, Shan X et al (2008) Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. PLoS One 3:e2849

Cheng KC, Cahill DS, Kasai H et al (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. J Biol Chem 267:166–172

Cho BP, Evans FE (1991) Structure of oxidatively damaged nucleic acid adducts. 3. Tautomerism, ionization and protonation of 8-hydroxyadenosine studied by ^{15}N NMR spectroscopy. Nucleic Acids Res 19:1041–1046

Cho BP, Kadlubar FF, Culp SJ et al (1990) ^{15}N nuclear magnetic resonance studies on the tautomerism of 8-hydroxy-2′-deoxyguanosine, 8-hydroxyguanosine, and other C8-substituted guanine nucleosides. Chem Res Toxicol 3:445–452

Crich D, Mo X-S (1997) Nucleotide C3′,4′-radical cations and the effect of a 2′-oxygen substituent. The DNA/RNA paradox. J Am Chem Soc 119:249–250

Cui S, Kim Y-H, Jin C-H et al (2009) Synthesis and base pairing properties of DNA-RNA heteroduplex containing 5-hydroxyuridine. BMB Rep 42:373–379

Dedon PC (2007) The chemical toxicology of 2-deoxyribose oxidation in DNA. Chem Res Toxicol 21:206–219

Defoiche J, Zhang Y, Lagneaux L et al (2009) Measurement of ribosomal RNA turnover *in vivo* by use of deuterium-labeled glucose. Clin Chem 55:1824–1833

El Ghissassi F, Barbin A, Nair J et al (1995) Formation of 1, N6-ethenoadenine and 3, -N4-ethenocytosine by lipid peroxidation products and nucleic acid bases. Chem Res Toxicol 8:278–283

Erlacher MD, Polacek N (2008) Ribosomal catalysis: the evolution of mechanistic concepts for peptide bond formation and peptidyl-tRNA hydrolysis. RNA Biol 5:5–12

Evans MD, Dizdaroglu M, Cooke MS (2004) Oxidative DNA damage and disease: induction, repair and significance. Mutat Res 567:1–61

Fiala ES, Conaway CC, Mathis JE (1989) Oxidative DNA and RNA damage in the livers of Sprague-Dawley rats treated with the hepatocarcinogen 2-nitropropane. Cancer Res 49:5518–5522

Gannett PM, Sura TP (1993) Base pairing of 8-oxoguanosine and 8-oxo-2′-deoxyguanosine with 2′-deoxyadenosine, 2′-deoxycytosine, 2′-deoxyguanosine, and thymidine. Chem Res Toxicol 6:690–700

Guschlbauer W, Duplaa A-M, Guy A et al (1991) Structure and *in vitro* replication of DNA templates containing 7,8-dihydro-8-oxoadenine. Nucleic Acids Res 19:1753–1758

Hangauer MJ, Vaughn IW, McManus MT (2013) Pervasive transcription of the human genome produces thousands of previously unidentified long intergenic noncoding RNAs. PLoS Genet 9:e1003569

Hayakawa H, Sekiguchi M (2006) Human polynucleotide phosphorylase protein in response to oxidative stress. Biochemistry 45:6749–6755

Hayakawa H, Hofer A, Thelander L et al (1999) Metabolic fate of oxidized guanine ribonucleotides in mammalian cells. Biochemistry 38:3610–3614

Hayakawa H, Kuwano M, Sekiguchi M (2001) Specific binding of 8-oxoguanine-containing RNA to polynucleotide phosphorylase protein. Biochemistry 40:9977–9982

Hayakawa H, Uchiumi T, Fukuda T et al (2002) Binding capacity of human YB-1 protein for RNA containing 8-oxoguanine. Biochemistry 41:12739–12744

Hecht SM (1994) RNA degradation by bleomycin, a naturally occurring bioconjugate. Bioconjug Chem 5:513–526

Hofer T, Badouard C, Bajak E et al (2005) Hydrogen peroxide causes greater oxidation in cellular RNA than in DNA. Biol Chem 386:333

Hofer T, Seo Arnold Y, Prudencio M et al (2006) A method to determine RNA and DNA oxidation simultaneously by HPLC-ECD: greater RNA than DNA oxidation in rat liver after doxorubicin administration. Biol Chem 387:103–111

Ide H, Akamatsu K, Kimura Y et al (1993) Synthesis and damage specificity of a novel probe for the detection of abasic sites in DNA. Biochemistry 32:8276–8283

Ishibashi T, Hayakawa H, Ito R et al (2005) Mammalian enzymes for preventing transcriptional errors caused by oxidative damage. Nucleic Acids Res 33:3779–3784

Ito Y, Ozawa A, Sawasaki T et al (2002) OsRALyase1, a putative F-Box protein identified in rice, *Oryza sativa*, with enzyme activity identical to that of wheat RALyase. Biosci Biotechnol Biochem 66:2727–2731

Ito R, Hayakawa H, Sekiguchi M et al (2005) Multiple enzyme activities of *Escherichia coli* MutT protein for sanitization of DNA and RNA precursor pools. Biochemistry 44:6670–6674

Kajitani K, Yamaguchi H, Dan Y et al (2006) MTH1, an oxidized purine nucleoside triphosphatase, suppresses the accumulation of oxidative damage of nucleic acids in the hippocampal microglia during kainate-induced excitotoxicity. J Neurosci 26:1688–1698

Kamiya H, Miura H, Murata-Kamiya N et al (1995) 8-Hydroxyadenine (7, 8-dihydro-8-oxoadenine) induces misincorporation in *in vitro* DNA synthesis and mutations in NIH 3T3 cells. Nucleic Acids Res 23:2893–2899

Karuppanapandian T, Moon J-C, Kim C et al (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Aust J Crop Sci 5:709–725

Kennedy LJ, Moore K, Caulfield JL et al (1997) Quantitation of 8-oxoguanine and strand breaks produced by four oxidizing agents. Chem Res Toxicol 10:386–392

Kim SK, Yokoyama S, Takaku H et al (1998) Oligoribonucleotides containing 8-oxo-7,8-dihydroguanosine and 8-oxo-7,8-dihydro-2′-O-methylguanosine: synthesis and base pairing properties. Bioorg Med Chem Lett 8:939–944

Kim SK, Kim JY, Yokoyama S et al (1999) Misreading of RNA templates containing 8-oxo-7,8-dihydroguanosine or 8-oxo-2′-O-methylguanosine in cDNA synthesis by reverse transcriptases. Nucleosides Nucleotides 18:1335–1337

Kim SK, Kim JY, Baek AK et al (2002) Base pairing properties of 8-oxo-7,8-dihydroadenosine in cDNA synthesis by reverse transcriptases. Bioorg Med Chem Lett 12:1977–1980

Kim SK, Lee SH, Kwon O-S et al (2004) DNA-RNA heteroduplex containing 8-oxo-7,8-dihydroguanosine: base pairing, structures, and thermodynamic stability. J Biochem Mol Biol 37:657–662

Koga Y, Taniguchi Y, Sasaki S (2013) Synthesis of the oligoribonucleotides incorporating 8-oxo-guanosine and evaluation of their base pairing properties. Nucleosides Nucleotides Nucleic Acids 32:124–136

Kong Q, Lin C-L (2010) Oxidative damage to RNA: mechanisms, consequences, and diseases. Cell Mol Life Sci 67:1817–1829

Kouchakdjian M, Bodepudi V, Shibutani S et al (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex. 8-Oxo-7H-dG(syn):dA(anti) alignment at lesion site. Biochemistry 30:1403–1412

Kreutzer DA, Essigmann JM (1998) Oxidized, deaminated cytosines are a source of C→T transitions *in vivo*. Proc Natl Acad Sci U S A 95:3578–3582

Küpfer PA, Leumann CJ (2007) The chemical stability of abasic RNA compared to abasic DNA. Nucleic Acids Res 35:58–68

Küpfer PA, Leumann CJ (2011) Synthesis, base pairing properties and trans-lesion synthesis by reverse transcriptases of oligoribonucleotides containing the oxidatively damaged base 5-hydroxycytidine. Nucleic Acids Res 39:9422–9432

La Francois CJ, Jang YH, Cagin T et al (2000) Conformation and proton configuration of pyrimidine deoxynucleoside oxidation damage products in water. Chem Res Toxicol 13:462–470

Lenaz G (2012) Mitochondria and reactive oxygen species. Which role in physiology and pathology? In: Scatena R, Bottoni P, Giardina B (eds) Advances in mitochondrial medicine, vol 942, Advances in experimental medicine and biology. Springer, Dordrecht, pp 93–136

Leonard GA, Guy A, Brown T et al (1992) Conformation of guanine:8-oxoadenine base pairs in the crystal structure of d(CGCGAATT(O8A)GCG). Biochemistry 31:8415–8420

Lesko SA, Lorentzen RJ, Ts'o POP (1980) Role of superoxide in deoxyribonucleic acid strand scission. Biochemistry 19:3023–3028

Levine RL, Yang I-Y, Hossain M et al (2000) Mutagenesis induced by a single 1, -N6-ethenodeoxyadenosine adduct in human cells. Cancer Res 60:4098–4104

Li Z, Wu J, Deleo CJ (2006) RNA damage and surveillance under oxidative stress. IUBMB Life 58:581–588

Liu M, Gong X, Alluri Ravi K, Wu J, Sablo T, Li Z (2012) Characterization of RNA damage under oxidative stress in *Escherichia coli*. Biol Chem 393:123–132

Masuda M, Nishino H, Ohshima H (2002) Formation of 8-nitroguanosine in cellular RNA as a biomarker of exposure to reactive nitrogen species. Chem Biol Interact 139:187–197

Moriya M, Ou C, Bodepudi V et al (1991) Site-specific mutagenesis using a gapped duplex vector: a study of translesion synthesis past 8-oxodeoxyguanosine in *E. coli*. Mutat Res 254:281–288

Moriya M, Zhang W, Johnson F et al (1994) Mutagenic potency of exocyclic DNA adducts: marked differences between *Escherichia coli* and simian kidney cells. Proc Natl Acad Sci U S A 91:11899–11903

Nathan C, Cunningham-Bussel A (2013) Beyond oxidative stress: an immunologist's guide to reactive oxygen species. Nat Rev Immunol 13:349–361

Nelson PT, Keller JN (2007) RNA in brain disease: no longer just "the messenger in the middle". J Neuropathol Exp Neurol 66:461–468

Neyhart GA, Cheng C-C, Thorp HH (1995) Kinetics and mechanism of the oxidation of sugars and nucleotides by oxoruthenium(IV): model studies for predicting cleavage patterns in polymeric DNA and RNA. J Am Chem Soc 117:1463–1471

Nguyen KV, Burrows CJ (2011) A prebiotic role for 8-oxoguanosine as a flavin mimic in pyrimidine dimer photorepair. J Am Chem Soc 133:14586–14589

Nguyen KV, Burrows CJ (2012a) Photorepair of cyclobutane pyrimidine dimers by 8-oxopurine nucleosides. J Phys Org Chem 25:574–577
Nguyen KV, Burrows CJ (2012b) Whence flavins? Redox-active ribonucleotides link metabolism and genome repair to the RNA world. Acc Chem Res 45:2151–2159
Niles JC, Wishnok JS, Tannenbaum SR (2006) Peroxynitrite-induced oxidation and nitration products of guanine and 8-oxoguanine: structures and mechanisms of product formation. Nitric Oxide 14:109–121
Novo E, Parola M (2008) Redox mechanisms in hepatic chronic wound healing and fibrogenesis. Fibrogenesis Tissue Repair 1:5
Nunomura A, Hofer T, Moreira PI et al (2009) RNA oxidation in Alzheimer disease and related neurodegenerative disorders. Acta Neuropathol (Berl) 118:151–166
Ogasawara T, Sawasaki T, Morishita R et al (1999) A new class of enzyme acting on damaged ribosomes: ribosomal RNA apurinic site specific lyase found in wheat germ. EMBO J 18:6522–6531
Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315–424
Pan B, Mitra SN, Sundaralingam M (1999) Crystal structure of an RNA 16-mer duplex r $(GCAGAGUUAAAUCUGC)_2$ with nonadjacent G(syn):A^+(anti) mispairs. Biochemistry 38:2826–2831
Pandya GA, Moriya M (1996) 1, N6-ethenodeoxyadenosine, a DNA adduct highly mutagenic in mammalian cells. Biochemistry 35:11487–11492
Pogozelski WK, Tullius TD (1998) Oxidative strand scission of nucleic acids: routes initiated by hydrogen abstraction from the sugar moiety. Chem Rev 98:1089–1108
Poulsen HE, Specht E, Broedbaek K et al (2012) RNA modifications by oxidation: a novel disease mechanism? Free Radic Biol Med 52:1353–1361
Pryor WA (1986) Oxy-radicals and related species: their formation, lifetimes, and reactions. Annu Rev Physiol 48:657–667
Radi R, Peluffo G, Alvarez MN et al (2001) Unraveling peroxynitrite formation in biological systems. Free Radic Biol Med 30:463–488
Richter C, Park JW, Ames BN (1988) Normal oxidative damage to mitochondrial and nuclear DNA is extensive. Proc Natl Acad Sci U S A 85:6465–6467
Salgo MG, Bermudez E, Squadrito GL et al (1995) Peroxynitrite causes DNA damage and oxidation of thiols in rat thymocytes. Arch Biochem Biophys 322:500–505
Schneider JE, Phillips JR, Pye Q et al (1993) Methylene blue and rose bengal photoinactivation of RNA bacteriophages: comparative studies of 8-oxoguanine formation in isolated RNA. Arch Biochem Biophys 301:91–97
Sekiguchi T, Ito R, Hayakawa H et al (2013) Elimination and utilization of oxidized guanine nucleotides in the synthesis of RNA and its precursors. J Biol Chem 288:8128–8135
Shan X, Chang Y, Lin CL (2007) Messenger RNA oxidation is an early event preceding cell death and causes reduced protein expression. FASEB J 21:2753–2764
Sharova LV, Sharov AA, Nedorezov T et al (2009) Database for mRNA half-life of 19977 genes obtained by DNA microarray analysis of pluripotent and differentiating mouse embryonic stem cells. DNA Res 16:45–58
Shen Z, Wu W, Hazen SL (2000) Activated leukocytes oxidatively damage DNA, RNA, and the nucleotide pool through halide-dependent formation of hydroxyl radical. Biochemistry 39:5474–5482
Shibutani S, Bodepudi V, Johnson F et al (1993) Translesional synthesis on DNA templates containing 8-oxo-7,8-dihydrodeoxyadenosine. Biochemistry 32:4615–4621
Srivastava SC, Raza SK, Misra R (1994) 1, N6-etheno deoxy and ribo adenosine and 3, N4-etheno deoxy and ribo cytidine phosphoramidites. Strongly fluorescent structures for selective introduction in defined sequence DNA and RNA molecules. Nucleic Acids Res 22:1296–1304
Steenken S, Jovanovic SV (1997) How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. J Am Chem Soc 119:617–618

Steenken S, Jovanovic SV, Bietti M, Bernhard K (2000) The trap depth (in DNA) of 8-oxo-7,8-dihydro-2′-deoxyguanosine as derived from electron-transfer equilibria in aqueous solution. J Am Chem Soc 122:2373–2374

Suen W, Spiro TG, Sowers LC et al (1999) Identification by UV resonance Raman spectroscopy of an imino tautomer of 5-hydroxy-2′-deoxycytidine, a powerful base analog transition mutagen with a much higher unfavored tautomer frequency than that of the natural residue 2′-deoxycytidine. Proc Natl Acad Sci U S A 96:4500–4505

Szabó C, Ohshima H (1997) DNA damage induced by peroxynitrite: subsequent biological effects. Nitric Oxide 1:373–385

Szabó C, Ischiropoulos H, Radi R (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 6:662–680

Taddei F, Hayakawa H, Bouton M-F et al (1997) Counteraction by MutT protein of transcriptional errors caused by oxidative damage. Science 278:128–130

Tanaka M, Chock PB, Stadtman ER (2007) Oxidized messenger RNA induces translation errors. Proc Natl Acad Sci U S A 104:66–71

Tanaka M, Han S, Küpfer PA et al (2011a) An assay for RNA oxidation induced abasic sites using the aldehyde reactive probe. Free Radic Res 45:237–247

Tanaka M, Han S, Küpfer PA et al (2011b) Quantification of oxidized levels of specific RNA species using an aldehyde reactive probe. Anal Biochem 417:142–148

Thorp HH (2000) The importance of being r: greater oxidative stability of RNA compared with DNA. Chem Biol 7:R33–R36

Tullius T, Dombroski B (1985) Iron(II) EDTA used to measure the helical twist along any DNA molecule. Science 230:679–681

Tullius TD, Greenbaum JA (2005) Mapping nucleic acid structure by hydroxyl radical cleavage. Curr Opin Chem Biol 9:127–134

Uesugi S, Ikehara M (1977) Carbon-13 magnetic resonance spectra of 8-substituted purine nucleosides. Characteristic shifts for the syn conformation. J Am Chem Soc 99:3250–3253

Wang D, Kreutzer DA, Essigmann JM (1998) Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. Mutat Res 400:99–115

Wood ML, Dizdaroglu M, Gajewski E et al (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry 29:7024–7032

Wu J, Li Z (2008) Human polynucleotide phosphorylase reduces oxidative RNA damage and protects HeLa cell against oxidative stress. Biochem Biophys Res Commun 372:288–292

Wu J, Jiang Z, Liu M et al (2009) Polynucleotide phosphorylase protects *Escherichia coli* against oxidative stress. Biochemistry 48:2012–2020

Yanagawa H, Ogawa Y, Ueno M et al (1990) A novel minimum ribozyme with oxidoreduction activity. Biochemistry 29:10585–10589

Yanagawa H, Ogawa Y, Ueno M (1992) Redox ribonucleosides. Isolation and characterization of 5-hydroxyuridine, 8-hydroxyguanosine, and 8-hydroxyadenosine from *Torula* yeast RNA. J Biol Chem 267:13320–13326

Yang E, van Nimwegen E, Zavolan M et al (2003) Decay rates of human mRNAs: correlation with functional characteristics and sequence attributes. Genome Res 13:1863–1872

Yermilov V, Rubio J, Ohshima H (1995) Formation of 8-nitroguanine in DNA treated with peroxynitrite *in vitro* and its rapid removal from DNA by depurination. FEBS Lett 376:207–210

Yermilov V, Yoshie Y, Rubio J et al (1996) Effects of carbon dioxide/bicarbonate on induction of DNA single-strand breaks and formation of 8-nitroguanine, 8-oxoguanine and base-propenal mediated by peroxynitrite. FEBS Lett 399:67–70