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BATTLE AGAINST RNA OXIDATION: Molecular mechanisms for reducing oxidized RNA to protect cells

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Abstract

Oxidation is probably the most common type of damage that occurs in cellular RNA. Oxidized RNA may be dysfunctional and is implicated in the pathogenesis of age-related human diseases. Cellular mechanisms controlling oxidized RNA have begun to be revealed. Currently, a number of ribonucleases and RNA binding proteins have been shown to reduce oxidized RNA and to protect cells under oxidative stress. Although information about how these factors work is still very limited, we suggest several mechanisms that can be used to minimize oxidized RNA in various organisms.

RNA is vital to all living cells; in addition to protein synthesis, it carries out a variety of other functions. In contrast to DNA damage research, RNA damage has received little attention until recently[1,2]. Although a majority of the total cellular RNA is encoded by minute portions of the genome of high organisms, recent evidence showing that most of the human genome is transcribed suggests there is a large collection of RNA species whose function is yet to be revealed. RNA is vastly more abundant than DNA in a cell, accounting for 80% to 90% of total cellular nucleic acids; therefore, RNA can be the major target of nucleic acid-damaging agents. Such RNA damage may affect cells due to alteration of any RNA function. Various insults such as UV light and reactive oxygen and nitrogen species (ROS and RNS) can damage RNA[2]. RNA damage could have serious deleterious effects on the multifaceted functions of RNA and the viability of the cell/organism.

Oxidative damage by ROS or RNS is a common insult in the cell that can affect all macromolecules under both physiological and pathological conditions. ROS are generated through the Fenton reaction[3] (iron-catalyzed oxidation) and are promoted by mitochondrial dysfunction[4,5]. The level of oxidative damage depends on the production of oxidants and the activity of the enzymatic and non-enzymatic antioxidant mechanisms. Inflammation, environmental hazards, and genetic conditions may cause oxidative stress in the organism, producing oxidants and hence oxidized macromolecules in excess[6]. Accumulation of oxidized macromolecules may render the cell dysfunctional and facilitate disease progression. In the case of DNA and proteins, repair and degradation of oxidized macromolecules provide further defenses for the cells against any deleterious effects.

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Although it has been recently recognized that RNA oxidation is high in cells, little is known about the mechanisms dealing with oxidized RNA.

Oxidation of RNA can result in strand breaks, abasic sites, and modified nucleobases and sugar [1,2,7,8]. The formation of the oxidized nucleobase 8-hydroxyguanine (8-oxo-G) in RNA has been the focus of studies because it appears to be particularly mutagenic and abundant[1]. It should be noted that RNA is oxidized in many forms, but the level of RNA oxidation is represented by 8-oxo-G in most studies, so the true amount of total oxidative damage must be higher. Table 1 shows an estimation of RNA oxidation levels from a study using *Escherichia coli*.

Several techniques have been developed to measure the level of RNA oxidation. However, due to the lag in RNA oxidation studies, the variety and breadth of detection methods for RNA do not match those for DNA, demonstrating the need for new development. Currently, oxidized RNA and its digested products can be detected using methods based on the chemical or antigenic properties of oxidized nucleotides. Nucleosides and nucleobases are commonly separated by HPLC, followed by detection of normal G and 8-oxo-G using an electrochemical detector (ECD) or mass spectrometer (MS) in combination with a UV detector[9,10,11,12]. Another development is based on the implementation of anti-8-oxo-G antibody in dot blot[13], ELISA[14], Northwestern blot[13,15,16], immunoprecipitation[15,17,18], and immunohistochemistry[19]. ELISA has been used to detect 8-oxo-G in the form of nucleosides or nucleobases in body fluids. However, compared to chromatography based ECD and MS detections, ELISA tends to output high values with high variability and low sensitivity[1,20,21]. Recently, a sensitive method has been developed to detect abasic sites in RNA using aldehyde-reactive probes[22]. Additionally, because it was found that RNA oxidation impedes reverse transcription, the rate of reverse transcription can be used to report the level of combined oxidative damage in RNA[23,24]. The appropriate method for a particular study depends on the form and availability of the biological samples as well as the desired scale of analysis[1,2,5].

Under both normal and oxidative stress conditions, RNA oxidation levels are much higher than DNA oxidation levels. For instance, the normalized 8-oxo-G content in RNA was found to be up to 20 fold of the 8-oxo-dG level in DNA when both were measured in the same work[9,10,11,12,25]. As RNA is more abundant than DNA in cells, RNA probably carries most of the oxidized nucleotides under any condition. This presents a great challenge to RNA function; however, information about the potential effects of RNA oxidation is scarce. A major reason is the misconception that normal turnover should take care of oxidized RNA, but because oxidation takes minutes to happen and the half-life of human mRNA is in the order of hours[1], there would be ample chance for oxidized RNA to have deleterious effects.

Recent studies have suggested a correlation between high levels of RNA oxidation and age-related disorders, most of them being neurodegenerative[26]. These neurodegenerative diseases have neuronal death in common, and include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD); many have shown high levels of RNA oxidation in the vulnerable regions of the brain

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(see Poulsen *et al*., 2012 for a recent compilation of related studies)[5]. It is important to note that when the oxidation levels of RNA and DNA were both analyzed, only increases in RNA oxidation were observed in tissues of AD patients[19]. The high levels of oxidized RNA found in these studies are likely related to the high abundance of mitochondria in the CNS. Dysfunctional mitochondria generate and release hydrogen peroxide and superoxide during the development of neurodegenerative diseases[27]. In a recent study of type 2 diabetes patients who were examined at 6 years post-diagnosis follow-up, urinary 8-hydroxy-guanosine (8-oxo-Guo) level has been shown to be a potential biomarker for mortality prediction[28,29]. Increased risk of mortality is significantly correlated with increased urinary 8-oxo-Guo and vice-versa, but not with 8-oxo-dGuo[29] *in vivo*. This work is among the very few human studies which clearlyEvi demonstrate that RNA oxidation is indeed relevant for death from diseases.

Evidence demonstrating RNA dysfunction due to oxidation is mounting, and is being linked to age-related diseases. In a study of 8-oxo-G-containing RNA isolated from the vulnerable neurons in the frontal cortices of mild AD patients, up to 50% of mRNAs was found to be oxidatively damaged, and only 2% was found in age-matched controls[16]. Proteins encoded by many of the oxidized mRNAs are related to the function of the brain, including free radical modulation, detoxification, cell death pathway, and long term potentiation among others[15]. Using a mouse model of ALS, Chang *et al*.[30] described an increase of mRNA oxidation in motor neurons and oligodendrocytes in pre-symptomatic stages and concluded that oxidation of mRNA species was implicated in ALS pathogenesis. Importantly, *in vitro* oxidation of mRNA led to a sharp drop in both protein level and activity when the mRNA was translated *in vitro* or in a cultured cell and produced abnormal proteins that aggregate[15]. Furthermore, oxidation did not affect the RNA's ability to associate with polysomes, but caused a reduction in the level and activity of the encoded protein and increased amount of truncated protein products[17,31].

There is also evidence that ribosomal RNA is affected by oxidative damage. A significant decline in protein synthesis was found in areas of the brain experiencing oxidative damage due to ribosomal dysfunction, featured by increased oxidation of rRNA[32]. Another study showed the high oxidation potential of ribosomes from vulnerable hippocampal neurons in AD patients is related to the rRNA's high affinity for redox iron[13]. When oxidized ribosomes were used for translation, protein synthesis was significantly reduced[13].

In patients with AD, PD, ALS, and other neurodegenerative diseases, mRNA and rRNA are highly oxidized in the early stages of the disease preceding cell death, with non-random, selective damage affecting the translational process[31,33,34]. All this evidence suggests that RNA oxidation can be a causative factor, or at least a preceding event in the development of the diseases. Once RNA is oxidized, and the protective mechanisms that reduce oxidized RNA are overwhelmed or non-functional, accumulation of oxidized RNA can cause the production of aberrant proteins, which may result in pathogenesis of neurodegenerative diseases[35].

It is important for living organisms to survive RNA oxidation and to reduce the risk of related diseases. Cells must have invested in mechanisms that reduce RNA oxidation levels

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in order to maintain normal function and to survive stress conditions. Such RNA surveillance and control mechanisms may prevent the deleterious effects of RNA oxidation by destroying or repairing oxidized molecules, or by preventing their formation. When these mechanisms are compromised or overrun, oxidized RNA may accumulate and the resulting pathogenesis may occur. Currently, little is known about any of the mechanisms. In this article, we will discuss recent progress and postulate potential activities that may be involved in controlling oxidized RNA.

1. Degradation of oxidized RNA

Degradation can play a major role in eliminating oxidized RNA. Under oxidative stress conditions where ~90% of *E. coli* cells survive, ~10% of RNA molecules may be damaged (Table 1). After the removal of the oxidant, oxidized RNA is reduced to almost a normal level within one generation time[25]. Selective degradation of oxidized RNA can account for the quick reduction of RNA oxidation levels in the surviving cells, although other mechanisms may also be involved (see below). Unlike when dealing with DNA damage, most cells should be able to afford degrading relatively low amounts of oxidized RNA molecules in order to keep a functional RNA repertoire.

1.1. Activities that potentially degrade oxidized RNA

Prokaryotic mRNA turnover is generally initiated by endonucleolytic cleavages followed by exonucleolytic digestion of the resulting RNA fragments into single nucleotides. Exonucleolytic digestion of structured RNA is usually difficult and is aided by polyadenylation and RNA helicase activity[36]. The RNA degradosome, a complex of enzymes found in many bacteria, is believed to degrade RNA with high efficiency[37,38,39]. In eukaryotes, decay of the majority of cytoplasmic mRNA is controlled by the dynamic length of the poly(A) tails. Once the poly(A) tail is shorter than a critical length, the mRNA is decapped and quickly degraded exonucleolytically in the 5′->3′ direction. RNA can also be degraded in the 3′->5′ direction by the exosome, a ring-shaped complex of exoribonucleases evolutionarily conserved in eukaryotes. Alternatively, eukaryotic mRNA can be cleaved endonucleolytically and the resulting intermediates are digested exonucleolytically from both ends. Importantly, inactivated mRNAs are found in large cytoplasmic foci named the RNA processing body (P-body) in which multiple RNA degrading activities are present[40].

Under stress conditions, bacteria can degrade RNA, thereby shutting down protein synthesis and growth. In *E. coli*, ribosomal RNA is broken down during starvation in a way similar to mRNA decay[36]. Massive mRNA degradation in *E. coli* can be carried out by ribotoxins activated by stress[41]. Stress may induce RNA inactivation or decay in eukaryotic cells by the formation of stress granules and P-bodies which contain inactivated RNA and many enzymes for RNA degradation[40,42]. Eukaryotic tRNA undergoes cleavage under various environmental stress conditions[43]. These cleaved tRNAs can be degraded or repaired (see below), providing a mechanism for temporarily or permanently shutting down cell growth.

Importantly, it is now known that RNA quality control activities are universally present to specifically eliminate aberrant RNAs. For instance, the bacterial trans-translation system

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rescues ribosomes during translation of aberrant mRNAs, causing degradation of the mRNA[44]. In *E. coli*, defective tRNA and rRNA are selectively degraded in a process involving specific exoribonucleases and polyadenylation[45]. In eukaryotes, specific mechanisms have been shown to degrade mRNA containing a premature termination codon, lacking a stop codon, or containing a structure that blocks translation. More recently, activities for degrading abnormal rRNA, hypomodified tRNAs or tRNA with a 3′-CCACCA sequence have been reported in yeast and humans[46,47,48,49,50]. It should be noted that RNA degradation enzymes may be shared between mRNA decay, degradation of stable RNAs, and RNA quality control, however, their activities on different RNA substrates may vary greatly[36].

Although degradation of oxidized RNA may share many activities and pathways described above, the reduction rate of oxidized RNA is faster than what can be caused by normal RNA turnover, suggesting a selective mechanism that targets oxidized RNA for efficient degradation[25]. Therefore, it is likely that oxidized RNA is recognized as aberrant RNA which can be sequestered for degradation. A number of activities have been proposed to participate in degradation of oxidized RNA[1,2,43]. The search for these activities has started, although little is known about their involvement and how they function to degrade oxidized RNA. Table 2 shows the type of activities that could be involved in reducing oxidized RNA within cells.

1.2. Polynucleotide phosphorylase plays a pivotal role in reducing RNA oxidation and protecting cells against oxidative challenge

PNPase plays an important role in many aspects of RNA metabolism in *E. coli* including mRNA turnover, degradation of rRNA under starvation, degradation of defective tRNA and rRNA, etc[51,52]. This exoribonuclease forms a trimeric ring-shaped structure similar to the eukaryotic exosome[53,54]. It degrades RNA processively in the 3′ to 5′ direction by phosphorolysis, producing nucleoside diphosphates. Importantly, it has been shown that PNPase specifically binds 8-oxo-G oligoribonucleotide[55] and oxidized RNA[56]. A role for PNPase to protect cells from oxidative stress was suggested by the observation that *Deinococcus radiodurans* strain lacking PNPase is hypersensitive to UV and H_2O_2[57], Moreover, Wu et al. reported that *E. coli* cells lacking PNPase are more sensitive to H_2O_2 and other oxidants than the wild type[56]. This work also demonstrated that 8-oxo-G increases to much higher levels in RNA in *E. coli* cells lacking PNPase than in the wild type under oxidative stress, while no difference was found in these strains under normal conditions. Expression of PNPase on a plasmid corrects both the growth defect and 8-oxo-G levels under oxidative stress[56], suggesting a direct role of PNPase in controlling RNA oxidative damage. In addition, other ribonucleases in *E. coli* also play a similar role in protecting cells against oxidative stress and reducing 8-oxo-G levels in RNA (unpublished observation), suggesting they function by degrading oxidized RNA. Based on this, a model for oxidized RNA elimination by degradation is proposed in Fig. 1.

PNPase is an important component of the *E. coli* degradosome that is composed of the endoribonuclease RNase E, the RNA helicase RhlB and enolase[58]. In addition, PNPase also exists in a degradosome-free form, and potentially interacts with RhlB independent of the

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degradosome. Interestingly, the association with RNase E in the degradosome or with RhlB is dispensable for PNPase to protect *E. coli* against oxidants[56]. Similarly, the *Yersinia pestis* PNPase also protects this bacterium under oxidative stress; however, this function seems to be dependent on the assembly of PNPase in the degradosome of *Y. pestis*[39].

The protective roles of bacterial PNPase against oxidative stress are probably due to the enzyme's involvement in the elimination of oxidized RNA, including specifically binding 8-oxo-G oligoribonucleotides and oxidized RNA with high affinity, and reducing 8-oxo-G levels under oxidative stress. However, it should be noted that PNPases are multifunctional, and its antioxidant property can be related to other activities such as DNA repair[59,60].

PNPase is widely distributed across organisms of all kingdoms[61]. Human PNPase (hPNPase) is predominantly localized in mitochondria in the intermembrane space[62,63,64]. Interestingly, hPNPase and the RNA helicase hSUV3 form a complex in human mitochondria, similar to the yeast mitochondrial RNA degradosome[65]. hPNPase has been shown to maintain the homeostasis of poly(A) tail of some mt-mRNAs, probably through deadenylation of these RNAs[66]. In cell cultures, hPNPase knockdown stabilizes mitochondrial mRNA[67], c-myc mRNA and miRNAs[61,63], suggesting a role for hPNPase in the degradation of these RNAs. When hPNPase or hSUV3 is knocked down in HeLa, mt-mRNAs encoding COX1, CYTB, ATP8/6 and ND5 were specifically stabilized[67]. The intermembrane localization of hPNPase complicates the issue on whether or not hPNPase directly degrades these RNA through its 3′-5′ exoribonuclease activity, so other possibilities cannot be ruled out. Like *E. coli* PNPase, hPNPase binds 8-oxo-G RNA with high affinity[68], prompting the study on the potential role of hPNPase in controlling oxidized RNA in human cells.

When hPNPase was knocked down, HeLa cells responded to the H_2O_2 treatment with a higher 8-oxo-G content in RNA and lower growth than the control; when hPNPase was overexpressed, it had the opposite effects[69]. More research is necessary to elucidate whether hPNPase directly participates in degradation of 8-oxo-G RNA or works by maintaining mitochondrial stability under oxidative stress. This finding adds to the growing list of biological functions of hPNPase[61]. This initial study suggests that PNPase may have a general role in controlling RNA oxidation among various organisms.

1.3. The mammalian Apurinic/apyrimidinic endonuclease 1 (APE1) protects human cells from oxidative stress

APE1 is a multifunctional enzyme that participates in DNA repair and redox regulation of transcriptional factors. APE1 binds DNA and RNA and cleaves abasic double-stranded DNA and single-stranded RNA. Recently, APE1 has been shown to copurify with a number of proteins involved in ribosome biogenesis and RNA processing, including nucleophosmin (NPM1)[70]. APE1 forms stable interaction with both NPM1 and RNA depending on the 33 N-terminal residues of APE1. Such interaction causes APE1 to accumulate in the nucleolus. *In vitro*, NPM1 stimulates APE1's endonuclease activity on abasic DNA but inhibits its activity on abasic RNA, suggesting that NPM1 is able to differentially modulate APE1 function on DNA repair and RNA damage control.

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Several lines of evidence suggest that APE1 plays an important role in RNA quality control under oxidative stress, probably through degradation of oxidized RNA. First, when APE1 is knocked down in HeLa cells, H_2O_2 treatment causes greater increase of 8-oxo-G levels in both total RNA and 18S rRNA than in the control cells[70]. Second, H_2O_2 treatment disrupts the interaction between APE1 and NPM1, suggesting that oxidative stress may activate APE1's cleavage of damaged RNA by removing NPM1[70]. This was further explained by the observation that under oxidative stress, the N-terminal lysine residues responsible for APE1/NPM1 interaction undergo acetylation, causing dissociation of the proteins[71]. Third, H_2O_2 treatment of APE1-knockdown cells resulted in a significant reduction in protein synthesis and cell growth, and an increase in apoptosis, consistent with deleterious effects of increased RNA oxidation[70]. Fig. 2 describes a possible APE1-mediated RNA quality control mechanism under oxidative stress.

2. Repair of oxidized RNA

In contrast to the well-known DNA repair mechanisms, repair of oxidized RNA has not been reported. However, one can speculate that specific repair mechanisms for oxidized RNA may exist because repair of other forms of RNA damage has been described. For instance, human and bacterial oxidative demethylases, are able to repair alkylation damage in both DNA and RNA, proving that RNA base damage is repairable[72]. In addition, during tRNA restriction-repair, RNA ligases seal cleavages in the anticodon loop[73]. It has long been known that truncated tRNA lacking part or all of the 3′ CCA sequence can be repaired by tRNA nucleotidyl transferase[74]. This CCA repair mechanism can potentially be used to repair tRNA molecules lacking the 3′ CCA sequence as a result of oxidation. Therefore, repair could be a potentially important mechanism for converting oxidized RNA into normal, functional RNA (Fig. 1).

3. Recognition and sequestration

As shown in Fig. 1, oxidized RNA may become nonfunctional and are subject to repair and degradation. It is possible that the repair and degradation activities work on oxidized RNA whenever the substrate becomes accessible, such as when the RNA molecules dissociate from the functional assembly. Alternatively, specific factors may exist to bind and mark the oxidized molecules, and recruit repair/degradation activities. The latter model is attractive because it makes sense for cells to invest in such an important solution to efficiently remove oxidized RNA molecules from the functional sites and to eliminate them specifically. Although compelling evidence is lacking, this idea of recognition and sequestration is supported by the findings: (1) proteins showing high affinity to oxidized RNA protect cells against oxidative stress (see below); and (2) oxidized RNA is specifically reduced independent of normal RNA turnover[25]. Fig. 3 describes a proposed cellular pathway responsible for recognizing and sequestering oxidized RNA molecules prior to their elimination. Some factors potentially involved in recognition of oxidized RNA are presented in Table 2.

Several proteins have been found to bind oxidized RNA specifically. Initially, PNPase was identified in *E. coli* cell extract to have a higher binding affinity to poly(8-oxo-G•A) than to

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a normal oligonucleotide[55]. It was later shown that PNPase-deficient *E. coli* cells show reduced viability and increased 8-oxo-G levels in RNA under oxidative stress[56]. A similar study exhibited that the human homologue of PNPase, hPNPase, also binds the 8-oxo-G oligoribonucleotide with specificity[68]. Over-expression of hPNPase promotes the viability of HeLa cell and reduces the level of 8-oxo-G in response to H_2O_2 treatment, whereas knockdown of hPNPase shows the opposite effects[69]. Further studies are necessary to elucidate how hPNPase functions in cell protection and 8-oxo-G reduction.

Similar search has also identified that the mammalian Y box-binding protein 1 (YB-1 protein) specifically binds poly(8-oxo-G•A) but not the control poly(U•A), depending on the protein's central and C-terminal RNA binding domains[75]. YB-1 is a multifunctional protein that binds both RNA and DNA, playing a significant role in transcriptional and translational regulation, DNA repair, drug resistance, and stress response to extracellular signal[76]. The full-length YB-1 rendered *E. coli* cells more resistant to the oxidant paraquat, while a truncated YB-1 lacking the RNA binding domains failed to do so[75]. This suggests a role for YB-1 in the metabolism of oxidized RNA and cell survival under oxidative stress through its specific binding to 8-oxo-G in RNA.

More recently, several other human proteins were shown to bind oxidized RNA with specificity. HeLa cell extract was incubated with normal and oxidized poly(A)+ RNA. The proteins bound to RNA were pulled down using biotinylated oligo(dT) and streptavidin particles, and were then identified using Mass-Spec. Among the identified proteins, HNRNPD exhibited the highest affinity to oxidized RNA, whereas SF3B4, HNRNPC and DAZAP1 shown affinity similar to that of hPNPase[77]. Importantly, knockdown of HNRNPD or HNRNPC increased sensitivity of HeLa to H_2O_2[77].

Ro autoantigen, a ring shaped RNA binding protein, has also been implicated in RNA handling during environmental stress conditions. In *Xenopus* oocytes, Ro binds to misfolded 5S rRNA with 3′ extensions[78,79], suggesting a role for Ro autoantigen in novel RNA quality control pathways. The Ro-ortholog Rsr proteins are found in bacteria. Rsr protects *D. radiodurans* under UV irradiation[80]. More recently, Rsr was shown to form a complex with PNPase and a non-coding RNA in *D. radiodurans*, and helps degradation of RNA by binding to the RNA and deliver it to PNPase[81]. These results suggest that Ro proteins are able to recognize aberrant RNA and send them to degradation machinery. Such activities may also participate in the recognition and sequestration of oxidized RNA.

4. Blocking incorporation of oxidized nucleotides into RNA

Oxidized nucleotides can be generated by degradation of oxidized RNA[1]. Nucleotides may also be oxidized in cells[82]. Under oxidative stress, oxidized nucleosides and nucleotides are likely increased, and oxidized RNA could potentially be generated from incorporation of oxidized nucleotides in RNA synthesis. When 8-oxo-deoxyguanosine triphosphate (8-oxo-dGTP) and 8-oxo-guanosine triphosphate (8-oxo-GTP) are used during DNA and RNA synthesis, it can be inserted at positions where the opposite nucleotide in the template is "A" or sometimes "U" or "G"[83,84]. This would not only create oxidized DNA or RNA from synthesis, but also introduce mutations to the nucleic acids. Currently, there is strong

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evidence for the existence of molecular mechanisms that block incorporation of oxidized nucleotide into RNA at various levels. So far, the data is focused on a limited number of oxidized nucleotides, mainly 8-oxo-G; however, such mechanisms should also exist for other oxidized nucleotides. The reported activities that control RNA quality at this level are summarized below and are outlined in Fig. 1.

4.1. RNA polymerase discriminates G and 8-oxo-G

It looks like the ability to block incorporation of oxidized RNA is built into the RNA polymerase itself. The *E. coli* RNA polymerase exhibits a ten-fold decrease in efficiency in utilizing 8-oxo-GTP as opposed to GTP during RNA synthesis[84]. Human RNA polymerase II incorporates 8-oxo-GTP at only 2% of the rate of GTP incorporation[85]. It remains to be investigated whether this is a common property for RNA polymerases and whether other oxidized nucleotides are also discriminated by RNA polymerases.

4.2. Breakdown of oxidized nucleoside tri- and di-phosphate

The *E. coli* MutT protein specifically hydrolyzes both 8-oxo-dGTP and 8-oxo-GTP, which can otherwise be incorporated into DNA and RNA[84,86,87,88]. Recently, MutT1 and ADPRase in *Mycobacterium tuberculosis* have also been shown to degrade 8-oxo-dGTP, 8-oxo-GTP and 8-oxo-GDP[89]. Such *in vivo* cleaning of the nucleotide pool decreases oxidation and mutation in both DNA and RNA. More recently, MTH1, a human homologue of MutT, has been shown to convert 8-oxo-GTP and 8-oxo-GDP to 8-oxo-GMP[85,90]. MTH1 probably also degrades oxidized adenosine tri- and di-phosphate[91]. Another enzyme, NUDT5, can also convert 8-oxo-GDP into 8-oxo-GMP[90], thus reducing the conversion of 8-oxo-GDP to 8-oxo-GTP by nucleotide-diphosphate kinase. In addition, MTH2 and MTH3 (NUDT18) confer activities similar to that of MTH1 in degrading 8-oxo-dGDP and 8-oxo-GDP[92]. These activities minimize the level of 8-oxo-GTP within the cell.

4.3. Guanylate kinase blocks conversion of 8-oxo-GMP to 8-oxo-GDP

8-oxo-GDP can still be converted to 8-oxo-GTP by the activities of nucleoside-diphosphate kinase (NDK) or adenosine-diphosphate kinase (ADK) with little difference from the conversion of GDP to GTP[88,90]. Therefore, it is crucial that the former is not regenerated from its degradation product 8-oxo-GMP. Conversion of normal GMP to GDP is carried out by guanylate kinase. This enzyme, however, does not carry out the conversion of 8-oxo-GMP to 8-oxo-GDP[85]. As discussed above, 8-oxo-GMP can be produced by direct oxidation of GMP, dephosphorylation of 8-oxo-GDP and 8-oxo-GTP, or degradation of oxidized RNA. Once 8-oxo-GMP is produced, it is trapped at this level without the ability to be phosphorylated. 8-oxo-GMP can finally be converted to 8-oxo-guanosine or 8-oxo-guanine, which can be removed from the cells. Indeed, both 8-oxo-guanosine and 8-oxo-guanine are detected in urine[20].

5. Remarks

Coping with oxidized RNA is inarguably an important issue for all living organisms. Recent advances have revealed diverse mechanisms and suggested a number of factors in this process. However, we have probably just begun to see the tip of the iceberg since much

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more may have been evolved. Many of the pathways and factors may play important roles in maintaining healthy life.

It is likely that different mechanisms are used to deal with different oxidized RNAs. For instance, oxidized mRNA and stable RNA may be broken down by different RNA degradation pathways. Some RNAs, such as rRNA in the ribosome, may experience more repair than other RNA in order to save the energy of rebuilding the complex.

The basic mechanisms described here may combine with other cellular processes and activities in protecting RNA from oxidation. Molecular assemblies and cellular processes may segregate and organize the removal of oxidized RNA with high efficiency. For instance, stress granule and P-body may effectively organize the elimination of oxidized RNA through autophagy[93]. In addition, other cellular mechanisms can protect RNA from oxidation. Antioxidant mechanisms form the first line of defense against ROS and may play a primary role in RNA protection[1,5]. Spatial separation of RNA in the nucleus, cytoplasm and organelles may ensure that some RNAs are more protected from oxidation than others by restricting the RNA's accessibility to oxidants.

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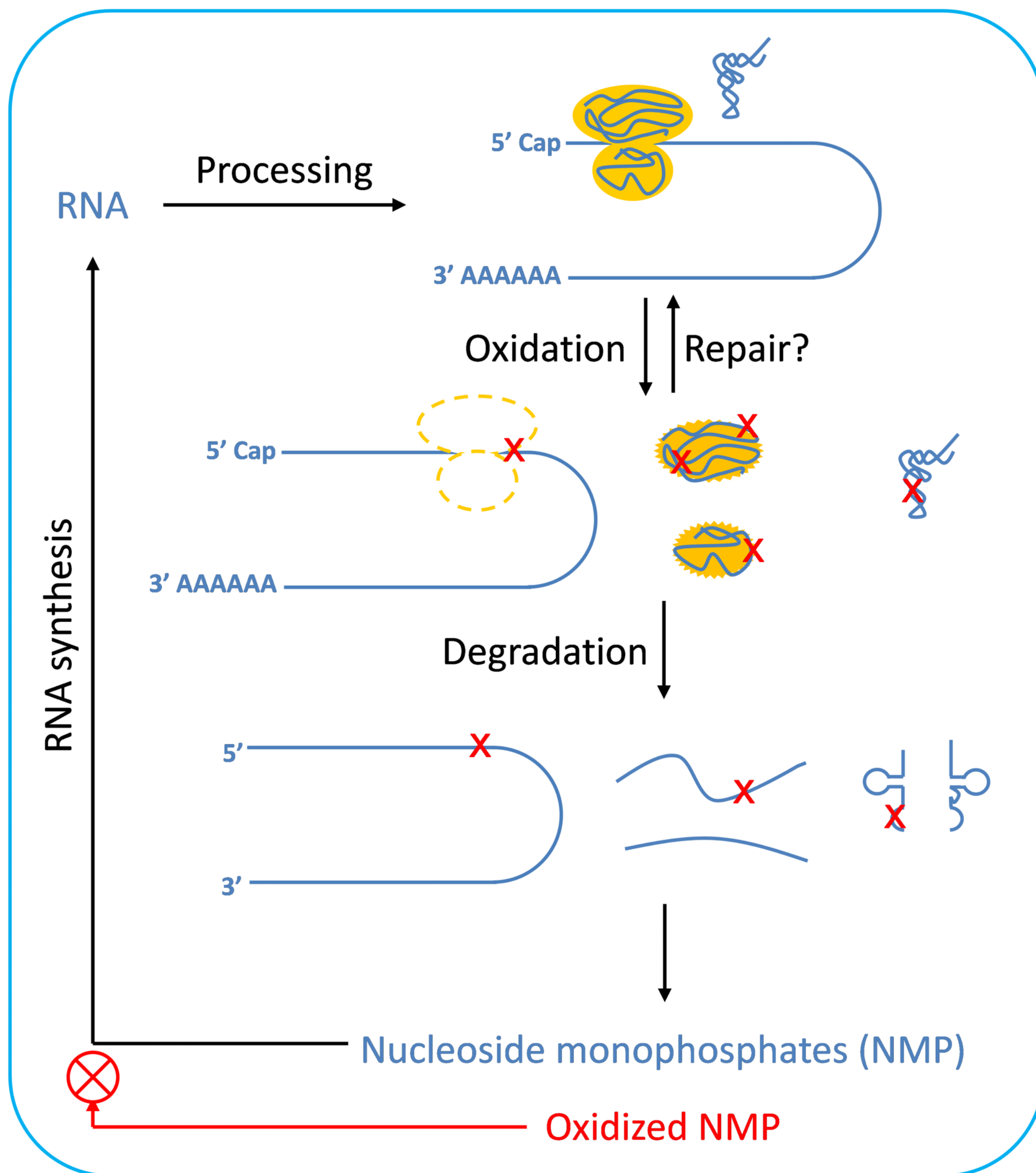

Figure 1. Molecular mechanisms for reducing oxidized RNA in cells

RNA may become non-functional once is oxidized. At the early stage after oxidation modification, the RNA may possibly be reverted to normal, functional RNA by repair activities. Oxidized RNA is also subjected to degradation, which is responsible to irreversibly eliminate probably the majority of oxidized RNA. The oxidized nucleotides resulted from degradation are blocked from transcription, reducing the formation of oxidized RNA by synthesis. RNA molecules are shown as blue lines. Oxidized residues in RNA are marked by a red “X”.

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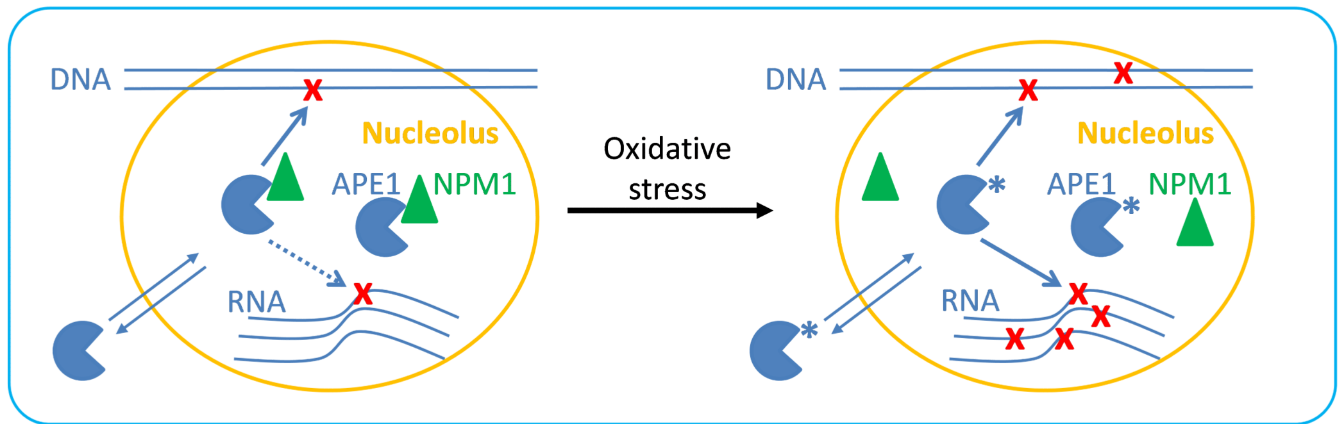

Figure 2. Predicted action of APE1 on oxidized RNA which is activated by oxidative stress
Under normal conditions, APE1 interacts with NPM1 and RNA and accumulates in the nucleolus. APE1 activity on damaged RNA is inhibited by binding NPM1[70]. Oxidative stress induces acetylation of lysine residues (marked by *) at the N-terminus of APE1[71], resulting in the dissociation of APE1 and NPM1 and activation of APE1 on oxidized RNA. RNA and DNA molecules are shown as blue lines. Oxidized residues are marked by a red "X".

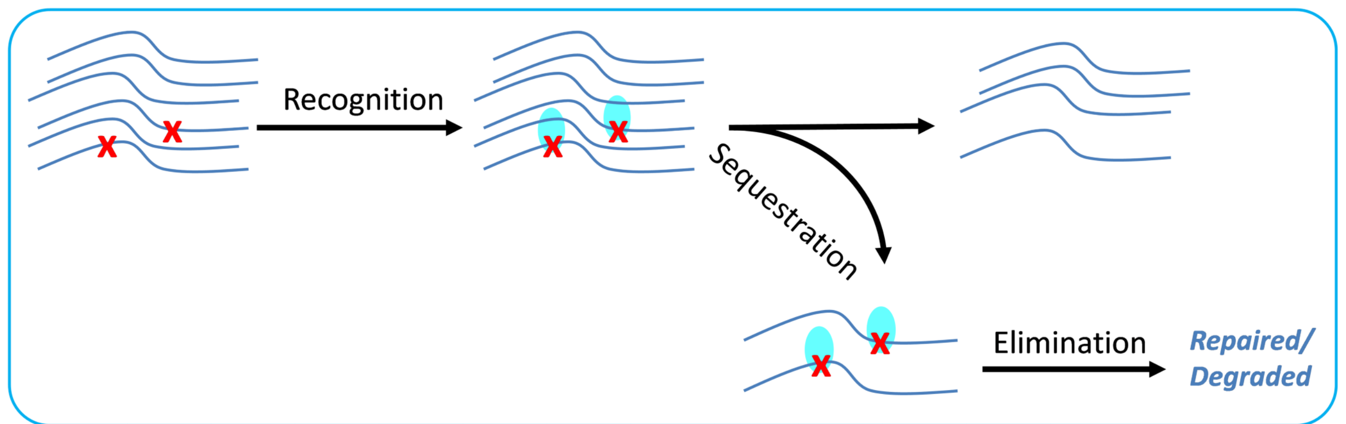

Figure 3. Recognition and sequestration target oxidized RNA for elimination

In the recognition step, oxidized RNA molecules can be marked by proteins that specifically bind them. After being recognized, oxidized RNA may be separated from normal RNA either physically or functionally in the sequestration step. Recognition and sequestration may help recruiting repair/degradation activities that will eventually eliminate oxidized RNA. RNA molecules are shown as blue lines. Oxidized residues in RNA are marked by a red "X". Oxidized RNA-binding proteins are shown as light blue ovals.

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Table 1

Steady state levels of RNA oxidative damage in E. coli in response to H2O2 treatment

Growth condition	8-oxoG/10^5 G*	8-oxo-G RNA†	Damaged RNA‡	Cell viability (CFU)*
Normal aeration	1.0 ± 0.1	0.25%	2.4%	(set to 100%)
Plus 1 mM H_2O_2	3.9 ± 0.1	0.98%	9.3%	83%
Plus 5 mM H_2O_2	10.9 ± 0.5	2.7%	24%	42%

*8-oxo-G levels were determined 15 min after addition of H_2O_2 to exponentially growing cultures. CFU was determined after treatment with H_2O_2 for 60 min.

†Based on the assumption that the length of RNA is 1 kb in average, the GC content is 50%, and 8-oxo-G is randomly distributed so that the percentage of 8-oxo-G containing RNA can be described by Poisson distribution.

‡Based on the assumption that total damage occurrences can be 10 times of 8-oxo-G content.[25] (Reference 25, p. 58, reproduced by permission of De Gruyter).

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Table 2
Factors potentially involved in reducing oxidized RNA

Categories	Potential Functions	F actors / pathways
Ribonucleases	Degradation of oxidized RNA	*E. coli* PNPase [55,56], RNase E[94]; human PNPase[69], mammalian APE1[70,71]. Poly(A) polymerase, RNA helicases[36].
Factors facilitating RNA degradation	Facilitating degradation of oxidized RNA	
RNA quality control pathways	Degradation of non-functional oxidized RNA	
RNA repair enzymes	Conversion of oxidized nucleotides to normal residues in RNA	
Oxidized RNA-binding Proteins	Recognition and sequestration	*E. coli* PNPase[55,56]; YB-1[75], hPNPase[68]; HNRNPD[77], SF3B4[77], HNRNPC[77] and DAZAP1[77].
Enzymes for nucleotide metabolism	Block incorporation of oxidized nucleotides into RNA	*E. coli* MutT[84,86,87,88], *M. tuberculosis* MutT1 and ADPRase[89]; human MTH1[85,90,91], MTH2 and MTH3 (NUDT18)[92], NUDT590, guanylate kinase[78,88].
RNA Polymerases	Discrimination of oxidized nucleotides during RNA synthesis	*E. coli* RNA polymerase[84], mammalian RNA polymerase II[85].