

RNA oxidation: Role of polynucleotide phosphorylase in the quality control of oxidized RNA

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Abstract

Reactive oxygen species is one of the most common cellular RNA damaging agents in living organisms. A growing number of studies show a strong correlation between oxidatively damaged RNA and human diseases, predominantly age-related neurodegenerative disorders. Oxidized RNAs impair the fundamental cellular processes including gene regulatory activities and protein synthesis. Molecular characterization of oxidized RNA such as understanding the sources of RNA oxidation, their mechanism of action, and cellular consequences may help to unravel their involvement in the pathogenesis of human diseases. Several proteins and factors with potential function to control RNA oxidation have been identified. Here, we will discuss the role of oxidized RNA binding protein polynucleotide phosphorylase (PNPase) in the quality control of oxidized RNA. PNPase is an evolutionarily conserved 3'-5' exoribonuclease having multifaceted RNA regulatory roles. Apart from binding to oxidized RNA, PNPase reduces the level of RNA oxidation and protects cells during oxidative stress. In this review, we discuss RNA oxidation and its quality control process with a specific focus on PNPase in regulating oxidized RNA.

Keywords: Reactive oxygen species (ROS), oxidative stress, RNA oxidation, PNPase, quality control pathways, aging, age-related neurodegenerative diseases

1. Introduction

Most life forms on earth rely on some forms of oxygen for their existence. Although oxygen is an essential component, it is also metabolized to produce reactive oxygen species (ROS)¹. ROS are highly unstable and reacts with cellular components including the biomolecules in its vicinity. In eukaryotes, ROS are primarily generated as a byproduct of oxidative phosphorylation inside mitochondria. In addition, they are also produced by cellular organelles such as peroxisomes, lysosomes, endoplasmic reticulum (ER)^{2,3}, and enzymatic reactions in the cell membrane. Exogenous sources of ROS are ionizing and ultraviolet radiations, chemotherapeutic agents, air pollutants, transition metals, and certain foods³. ROS can be either free radicals such as superoxides ($\cdot\text{O}_2$) and hydroxyl radicals ($\text{HO}\cdot$) or nonradical molecules such as hydrogen peroxide (H_2O_2), and singlet oxygen ($^1\text{O}_2$). ROS can be beneficial to the cellular system as they trigger the onset of various cell signaling cascades^{4,5} and

host defense when present at optimum level^{6,7}. When the levels of ROS exceed the physiologically tolerable range, they can disrupt the fundamental cellular processes such as transcription⁸, RNA processing⁹, translation¹⁰, and RNA export¹¹. One of the many reasons of such alteration could be that ROS induces the oxidative modification and damage of cellular biomolecules such as proteins, lipids, DNA and RNA. Such alteration may eventually lead to cell death and trigger the pathogenesis of various human diseases^{12,13}.

ROS are neutralized by the antioxidant defense mechanism which is also known as the first line of cellular defense against ROS. The defense mechanism involves both enzymatic or non-enzymatic factors¹⁴. Some of the common enzymatic antioxidants are catalase, superoxides dismutase (SOD), and glutathione peroxidases whereas ascorbic acid and lipoic acid, carotenoids, and tocopherols are non-enzymatic

antioxidants. Despite the cellular antioxidant defense system, ROS can escape these processes under sub-optimal cellular conditions such as nutrient starvation, endoplasmic reticulum stress, and oxidative stress. Also, ROS can accumulate when the functions of such antioxidants are impaired. Such impairments can lead to disease states. For example, the mutation in superoxide dismutase 1 (SOD1) enhances the level of ROS which is associated with the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) (reviewed in¹⁵).

Although the oxidative modifications and oxidative damage of most of the biomolecules such as DNA, proteins, and lipids by ROS have been extensively characterized, RNA research has just begun to be recognized. This could be due to the notion that RNAs have a very short half-life which undergo rapid turnover before causing deleterious effects on the cellular system. But some RNAs have a half-life of more than an hour and some even days, hence they have ample opportunities to cause deleterious activities before their turnover. In the subsequent sections, we will discuss how RNA could be targeted by ROS. We then discuss the consequences of such ROS-derived RNA oxidation and highlight its relevance in the pathogenesis of human diseases. Next, we describe the mechanisms by which cells control the level of oxidized RNA, with a specific focus on the role of polynucleotide phosphorylase (PNPase). Finally, we will conclude with open questions that remain to be addressed by future studies towards the therapeutic intervention of oxidized RNA associated diseases.

2. Reactive oxygen species (ROS) and RNA damage

RNA can be the target of various nucleic acid damaging agents such as ionizing radiation, ultraviolet radiation, xenobiotics, therapeutic drugs, and reactive nitrogen species (RNS), and ROS¹⁶. Damage due to ROS is perhaps the most prominent intracellular source of damage to the RNA^{17,18,19}. Compared to other biomolecules such as DNA and proteins, RNA is more

vulnerable to oxidative damage²⁰. Such elevated levels of RNA oxidation could be due to that RNA are single-stranded and less protected by binding proteins¹⁹. Also, RNAs are exported in the cytoplasm where the level of ROS is higher than that of the nucleus. In addition, unlike DNA repair mechanisms, no such mechanism has been characterized in repairing oxidatively damaged RNA. ROS can oxidatively modify the bases, phosphate backbone, or sugar moiety of an RNA, however, the modification of bases has been widely characterized. More than 20 different purine and pyrimidine oxidative base modifications have been reported in DNA and this list is growing²¹. Since RNA shares the majority of the nucleobases with DNA, it is highly likely that such modifications could also be present in RNA²². Among many oxidative base modifications, hydroxyl (OH) induced guanosine base modification, 8-oxoguanosine (8-oxoG) is one of the most commonly characterized and also highly mutagenic base modifications¹⁸. The level of 8-oxoG is also used as a common biomarker of RNA oxidation in most studies. However, the actual level of RNA oxidation should be higher than the level of 8-oxoG as ROS has tendency to oxidatively modify all RNA bases. Moreover, oxidative stress can also cleave the nucleobases that results in the formation of AP RNAs²³. Thus, AP site can be another marker of RNA oxidation which can be measured using aldehyde reactive probe (ARP) assay²⁴.

Oxidative stress can target all different types of RNA species including transfer RNA (tRNA), ribosomal RNA (rRNA), and messenger RNA (mRNA)(for details see a recent excellent review²⁵). The extent of RNA oxidation can vary significantly under oxidative stress and pathological condition²⁶. In humans, for instance, the level of mRNA and rRNA oxidation were found to be much higher in mild cognitive impairment (MCI), Alzheimer's Disease (AD), amyotrophic lateral sclerosis (ALS)^{27,28,29,30}. In *E. coli* cells, rRNAs are oxidized to a relatively lesser extent than non-ribosomal RNA under normal growth condition³¹. Interestingly, when cells were exposed to an exogenous oxidant such as

hydrogen peroxide, both the ribosomal and non-ribosomal RNA had an equal increase in the level of RNA oxidation. Moreover, rRNA and non-rRNA in their native and denatured state had an equal increase in the level of RNA oxidation, suggesting that all RNA types can be oxidized regardless of their higher-order structure or their association with proteins. Oxidative stress also can oxidatively damage non-coding RNAs such as microRNA³². Oxidation of microRNA has been shown to misrecognize its target and leads to apoptosis³³ and cardiac hypertrophy³⁴. It should be noted that the extent of oxidative modification of microRNAs is not uniform as miR-184³³ and miR-1b³⁴ were preferentially oxidized over other types of miRNAs when they were examined under similar conditions.

3. Consequences of RNA oxidation

The robustness and spryness of cellular processes and their proper functioning depend on the quality of their individual components. When the integrity and quality of RNA is threatened by ROS, they alter cellular metabolism and homeostasis. Besides modification of RNA components, oxidative stress also induces RNA strand scission. Exposure of eukaryotic and prokaryotic cells such as *E. coli*, cultured human cells, and yeast cells to oxidants has been shown to induce cleavage of rRNA^{31,35,36,37}, as well as tRNAs^{38,39,40}. Increased accumulation of 3'UTR RNA fragments were observed in ageing brains which were associated with ROS⁴¹. Oxidized RNA has also been shown to induce errors in the fundamental cellular processes such as RNA export¹¹, translation^{27,42,43,29,44,45,46}, transcription^{47,48,49,50}, and microRNA mediated gene regulation^{32,33}. At the cellular level, a strong correlation between RNA oxidation and cell death has been established^{35,31}. Treatment of *E. coli* or cultured human cells with oxidants such as hydrogen peroxide increases the level of RNA oxidation and decreases cell viability^{35,51}. Although the mechanism that links RNA oxidation with cell death has not been well-established, recent studies show that oxidized RNA activates the apoptotic associated pathways. For instance,

Ishii and colleagues demonstrated that poly (C)-binding protein 1 (PCBP1) recognizes highly oxidized RNA and activates the apoptosis-related factors such as caspase-3 and cleavage of Poly [ADP-ribose] polymerase 1 (PARP1)⁵². Moreover, Tanaka et al showed that cytochrome c (cyt c) cross-links with oxidized RNA and facilitate its dissociation from the mitochondria to the cytoplasm to induce a mitochondrial mediated apoptosis⁵³. In addition, microRNA mediated oxidation such as miR-184 also triggers the apoptotic pathways by interacting with the 3'UTR of Bcl-xL and Bcl-w³³. In another study in yeast, mutating of decapping enzyme *Kluyveromyces lactis* Lsm4p (KILsm4P), increased the level of oxidized RNA and induces apoptosis when left unrepaired, they induce apoptosis⁵⁴. These findings suggest that oxidized RNA trigger cell death which may be due to their inability to perform many cellular processes including the activation of apoptosis-related pathways.

4. RNA Oxidation and human diseases

Oxidized RNAs are strongly associated with a wide range of human diseases, predominantly age-related neurodegenerative disorders^{55,26,56,57,19,58}. The level of RNA oxidation in pathological conditions such as Alzheimer's disease (AD)^{28,59}, Parkinson's disease (PD)⁶⁰, Dementia with Lewy bodies⁶¹, Amyotrophic Lateral Sclerosis (ALS)²⁹, and multiple sclerosis⁶² was found to be significantly higher than that of age-matched healthy controls. These neurodegenerative disorders are commonly associated with neuronal death, which could result from the production of aberrant and truncated proteins due to oxidized mRNAs. In most of these cases, neurons exhibited increased RNA oxidation before their death^{29,63} and such oxidation of RNA is observed in the early stages of these neurodegenerative disorders^{64,29,63}. The brain is one of the metabolically active organs that consumes almost 20% of the total oxygen in the body⁶⁵. Higher oxygen metabolism could produce an elevated level of ROS. Moreover, the increased level of ROS could also be associated with a higher number of mitochondria,

polyunsaturated fatty acids, modest antioxidant defense system, and a higher level of redox-active transition metals^{65,66}. As briefly discussed in the earlier section, the deleterious effect of ROS is controlled by the cellular antioxidant defense system. However, any alternation in the fine balance between ROS level and antioxidant defense mechanism could oxidatively damage the biomolecules such as RNA that ultimately led to these disorders.

Besides neurodegenerative disorders, increased RNA oxidation level has also been observed in other pathological conditions such as diabetes⁶⁷, schizophrenia⁶⁸, bipolar 1 disorder (BD1)^{69,70}, atherosclerosis⁷¹, hereditary hemochromatosis⁷², colorectal cancer⁷³, prion disease⁷⁴, Down's syndrome⁷⁵, and aneurysmal subarachnoid haemorrhage⁷⁶ indicating that RNA oxidation could be an important event associated with a multitude of human diseases. These findings suggest that RNA oxidation could potentially be used as a diagnostic biomarker for these wide ranges of human diseases^{77,78,79}. Currently, several assays and approaches have been used in the detection and quantification of oxidized RNA. These approaches rely on the use of liquid chromatography coupled with mass spectrometry (LC-MS/MS), comet assay, immunoassay, and ELISA (for details, see the recent review⁸⁰). Multiple human samples such as urine, blood, tissues have been used for the detection and quantification of oxidized RNA.

One of the possible therapeutic approaches of oxidized RNA associated human diseases can be to control the levels of the formation of oxidized RNA in the cellular system. The level of oxidized guanine residue in DNA (8-oxo-dG) was evaluated in response to certain drugs (see review⁷⁷). Plant extract based supplements⁸¹ and antioxidants⁸² reduced the levels of DNA/RNA and protein oxidation suggesting that they could help to prevent oxidative processes. Hereafter, we will discuss the cellular mechanism of dealing with oxidatively damaged RNA.

5. The quality control mechanism of oxidized RNA

RNA oxidation poses a significant threat to cells and, therefore, cells need to recognize and eliminate them from the cellular system. A number of cellular mechanisms could be involved in the quality control of oxidized RNA^{18,46}. Oxidized RNA may be initially recognized and sequestered from the normal RNA pool by a number of oxidized RNA binding proteins and factors¹⁸. In 2001, Hayakawa et.al discovered a high binding affinity of polynucleotide phosphorylase (PNPase) to oxidized RNA while searching for *E. coli* proteins that bind to oxidized RNA⁸³. The detailed molecular mechanism of bacterial PNPase and its human homolog (PNPT1) in dealing with oxidized RNA will be discussed in later sections. In 2002, Hayakawa et al further demonstrated yet another protein, Y-box-binding protein (YB-1), having a high binding affinity to RNA containing 8-oxoG⁸⁴. Once the oxidized RNAs are recognized and bound by oxidized RNA binding proteins, they may either be repaired by RNA repair enzymes or degraded by ribonucleases [Figure 1]. The final product after the degradation of oxidized RNA may be either re-incorporated during RNA synthesis or eliminated out of the body in the form of urine or other bodily fluids. However, the incorporation of 8-oxoG into new RNA is a rare event as cells have developed several mechanisms to block this process. First, RNA polymerases in both *E. coli*⁸⁵ and human⁸⁶ are less efficient in utilizing 8-oxoGTP compared to normal GTP. Second, oxidized nucleoside tri- (8-oxo-GTP) and di - (8-oxo-GDP) phosphates are hydrolyzed to 8-oxo-GMP by several hydrolyzing enzymes such as MutT, MTH1, NUDT5, MTH2, and MTH3 (NUDT18) (reviewed in¹⁸). Finally, unlike conversion of GMP to GDP by guanylate kinase (GK), it does not convert 8-oxo-GMP to 8-oxo-GDP⁸⁶. Aside from decay factors and RNA binding proteins in controlling oxidatively damaged RNA, RNA granules such as stress granules (SG) and P-bodies may have an important role in further processing and eventual decay⁸⁷. The direct involvement of

these RNA granules in the regulation or turnover of oxidatively damaged RNA remains to be characterized.

The cellular mechanisms for handling oxidatively damaged RNA may involve recognition, binding, sequestration, repair, degradation of oxidized RNA, and blocking the incorporation of oxidized ribonucleotides to newly synthesized RNA¹⁸. Recently, two promising discoveries have been made in the quality control of oxidized RNA^{46,88,89,90,91}. The first describes a mechanism mediated by the no-go-decay (NGD) pathway⁸⁸. During translation, when a ribosome encounters a modified nucleotide such as 8-oxoG, it stalls on that specific position of an mRNA containing such modified nucleobase. This recruits multiple factors that eventually regulate the fate of the nascent truncated polypeptide, the ribosome, and the damaged mRNA. Yan et al showed that modification to even a single nucleotide in the mRNA such as 8-oxoG is enough to activate the NGD pathway⁸⁸. Once the NGD pathway is activated, the exoribonuclease XRN1 degrades the mRNA from its 5'-end. Interestingly, they showed that upon deletion of XRN1, there was an increased accumulation of 8-oxoG suggesting an important role of XRN1 in the exonucleolytic digestion of oxidatively damaged RNA. This finding supports the previous reports where disruptions in the NGD pathway led to an increased accumulation of 8-oxoG⁴². Recently, it has been discovered that endoribonucleases, CUE2⁹², and NONU-1⁹³ are involved in the cleavage of mRNAs near the stalled ribosomes in *S. cerevisiae* and *C. elegans*, respectively. These endoribonucleases may also be involved in the cleavage of oxidized mRNAs undergoing ribosome stalling.

The second mechanism involves the RNA binding proteins (RBPs) such as AUF1 and/or PCBP1 [See ref.^{18,94} for the list of all proteins involved in binding and regulating oxidized RNA]. These proteins recognize and bind to oxidized RNA. These RBPs may catalyze oxidized RNA turnover on their own or may recruit other factors. For

instance, AUF1 has been shown to detect and bind to moderately oxidized RNA (RNA containing only a few 8-oxoGs)⁸⁹ and target them to downstream factors involved in RNA decay pathways⁸⁹. On the other hand, PCBP1 recognizes heavily oxidized RNA and induces the activation of factors involved in apoptosis^{52,90}. These findings have enhanced our understanding of molecular factors involved in the quality control of oxidized RNA. However additional studies are required to deepen our knowledge of these mechanisms. For instance, the current evidence suggests that no-go decay and ribosome quality control pathways maybe activated when mRNAs are oxidized even at the single nucleotide base modification⁸⁸, while AUF1 or PCBP1 may come into play when RNA is moderately and heavily oxidized, respectively⁶⁴. More empirical evidence is required to substantiate this observation. Since PNPase is an exoribonuclease, theoretically it may be associated with both the NGD and AUF/PCBP1 pathways in the quality control of oxidized RNA. The byproduct of those pathways may be eventually cleared out from the cellular system with the exoribonucleases as shown in Figure 1. The involvement of exoribonuclease at the 5' end of RNA pathway has been discussed⁸⁸ and PNPase might be another important exoribonuclease at the 3'-end for the cumulative removal of oxidatively damaged RNA. In the subsequent section, we will discuss the multifaceted RNA regulatory role of polynucleotide phosphorylase (PNPase) with a focus on the quality control process of oxidized RNA.

6. PNPase in regulating oxidized RNA and cell protection during oxidative stress

PNPase is a 3'-5' exoribonuclease that belongs to the PDX family of enzymes^{95, 96}. It was first discovered in *Azobacter vinelandii* and its function was reported to synthesize polynucleotides (RNA) from 5'-nucleosides diphosphates (dNDPs)⁹⁷. Later, the primary function of PNPase was identified to catalyze the phosphorolysis of ribonucleotides from its 3' to 5' end^{98,99}. PNPase is widely known for its 3'-5'

exoribonuclease activity and its phosphorolysis activity was found to be more favorable in the presence of elevated levels of inorganic phosphates (Pi)¹⁰⁰. However, at the low concentration of inorganic phosphate, PNPase adds a heteropolymeric tail to single-stranded RNA (ssRNA) in a template-independent manner⁹⁸. Therefore, PNPase is considered a unique exoribonuclease having a dual function in both the degradation and synthesis of RNA. In *E. coli*, PNPase forms a degradosome complex that is primarily composed of RNA helicase RhlB, endoribonuclease RNase E, and glycolytic enzyme enolase^{101,102,103}. Such degradosome complex plays an essential role in the degradation and quality control process of structured RNAs. PNPase also works in

association with HF-I protein (Hfq) and poly (A) polymerase I (PAP I) as well as independent of its RNA helicase partner RhlB¹⁰⁴. PNPase is an evolutionarily conserved protein in bacteria, plants, flies, mammals, and humans. It consists of five conserved domains, two RNase PH domains (RPH1 and RPH2), two RNA binding domains (KH homology and S1), and an alpha subunit (α) in between RPH1 and RPH2 domains [Figure 2A]. In plants and human, PNPase has additional mitochondrial and chloroplast translocating signals at its C-terminal end. Crystal structure of trimeric hPNPase shows that RPH forms the hexameric ring-like structure whereas KH forms the pore¹⁰⁵. The KH traps the long RNA 3' tail that is directed to the RNase PH channel for further degradation.

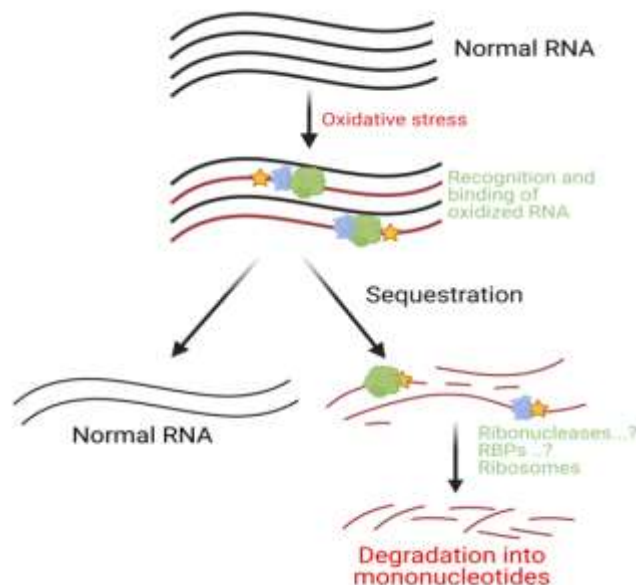


Figure 1. Oxidative stress induces oxidative modifications in RNA. Oxidized RNA may be sequestered from the normal RNA pool by oxidized RNA binding and recognition factors. These sequestered RNAs may eventually undergo turnover due to ribonucleases and ribosomes mediated decay.

Hayakawa and colleagues first demonstrated the involvement of PNPase in the quality control of oxidized RNA in 2001⁸³. Using two-dimensional gel analysis in conjunction with the gel-shift assay, they showed that PNPase binds to oligonucleotides containing 8-oxoG with high affinity and protects against ribonuclease RNase A. It is also the first study to show that PNPase responds to cellular oxidative processes as PNPase mutant *E. coli* strains were hyper

resistant to paraquat, a drug that induces oxidative stress. In 2006, the same group demonstrated a similar role of the human homolog of polynucleotide phosphorylase (hPNPase) in binding to 8-oxoG containing RNA¹⁰⁶. These findings provided an important insight into the cellular mechanisms of handling oxidatively damaged RNA. Since PNPase is an exoribonuclease, its high binding affinity to

oxidized RNA may eventually degrade oxidized RNAs.

PNPase in cultured human cells reduces the level of 8-oxoG and improves cell viability during oxidative stress³⁵. Such reduction in 8-oxoG is likely due to degradation of oxidized RNA mediated by hPNPase. Later, the protective role of PNPase against oxidative stress was confirmed in *E. coli* cell⁵¹. Unlike hyper resistance of PNPase lacking *E. coli* cells to paraquat⁸³, Wu and colleagues demonstrated hypersensitivity towards oxidative stress (hydrogen peroxide)⁵¹. Interestingly, such protective function of PNPase is directly associated with the level of RNA oxidation (8-oxoG). A similar role of PNPase has also been characterized in other bacterial strains such as *Deinococcus radiodurans*¹⁰⁷ and *Yersinia pestis* although the protective function of the later strain was dependent on its degradosome¹⁰⁸.

As mentioned earlier, hPNPase has an additional mitochondrial translocation signal (MTS) that translocate PNPase to mitochondria¹⁰⁹. Since a majority of the total RNA is present in the cytoplasm, PNPase localization may impact its regulatory role in controlling cytoplasmic oxidized RNA. Also, mitochondria are the predominant intracellular sources of ROS, and they may have a differential effect on RNA inside mitochondria and cytoplasm. For this, the levels of RNA oxidation inside mitochondria and cytoplasm were examined under normal and oxidative stress conditions. The RNA oxidation level was observed to be higher in mitochondrial RNA than that of cytoplasmic RNA as measured by the number of 8-oxoG¹¹⁰. The knockdown and overexpression of hPNPase showed a significant change in the level of 8-oxoG inside mitochondria and cytoplasm only when cells were exposed to oxidants. This finding further substantiates that PNPase protects cell viability by regulating the level of oxidized RNA during stress conditions. Since hPNPase forms a degradosome complex with human RNA helicase

(hSUV3) and the complex is involved in regulating mRNAs inside mitochondria, hSUV3 could also facilitate hPNPase in controlling RNA oxidation^{111,112}. However, the knockdown of hSUV3 didn't change the levels of 8-oxoG under both normal and oxidative stress conditions¹¹⁰. This suggests that hSUV3 does not have any role in facilitating hPNPase to control oxidized RNA.

PNPase also has evolutionarily conserved domains such as KH and S1 RNA binding domain, RPH1 and RPH2 catalytic domains and alpha subunit [Figure 2A]. These different domains constitute the formation of pores such as S1 pore, KH pore, and RPH pore [Figure 2B]. S1 pore may facilitate the binding of oxidized RNA that may eventually help to direct the oxidized RNA to RPH1 pore for the eventual catalysis and degradation. To provide a further mechanistic understanding of PNPase on oxidatively damaged RNA, the role of an individual domain of PNPase in regulating the 8-oxoG level was examined. Interestingly, the levels of 8-oxoG were significantly increased when cells were transfected with plasmids containing S1, RPH1, and RPH2 deleted hPNPase mutants. The result suggests that the S1 binding domain and RPH1/RPH2 catalytic domains are required for binding, and catalytic activities, respectively¹¹³. Furthermore, a recent computational modeling study that used multi-nanosecond molecular dynamics simulation¹¹⁴ demonstrated the role of PNPase in recognizing 8-oxoG at the atomic level¹¹⁵. The study showed that PNPase interacts more favorably with 8-oxoG than non-oxidized Guanosine as per the per-nucleotide energy calculation, stacking contact with specific amino acids of the PNPase subunit, and stronger hydrogen bonding with 2'-OH group. The SFF (S76-F77-F78) groove forms the binding pocket for 8-oxoG that discriminates the 8-oxoG from normal guanosine. Their computationally designed mutational analysis at the SFF groove further reinforces the notion that PNPase has a high binding specificity to 8-oxoG.

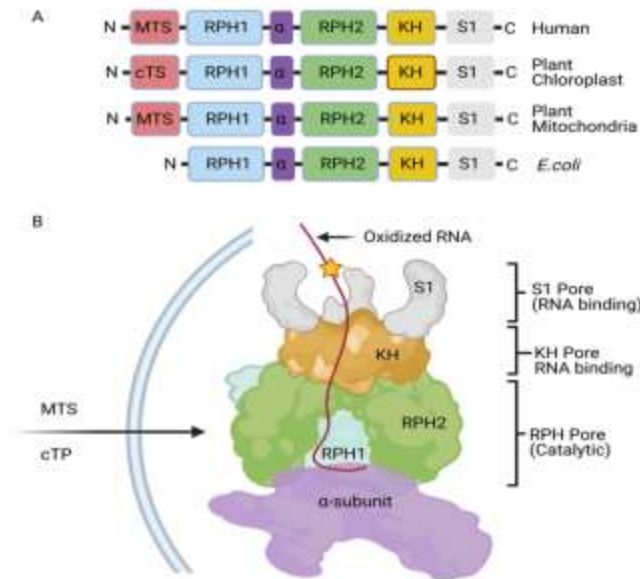


Figure 2. Proposed model of PNPase in controlling RNA oxidation. **A.** Evolutionarily conserved domains of PNPase in Humans, plants, and *E. coli*. **B.** Domain organization of PNPase as described in^{116,117,105}. In humans, MTS will translocate PNPase to mitochondria whereas in plants, MTS and cTP will translocate it to the mitochondria and chloroplast, respectively. The oxidized RNA may be first recognized by S1 and KH RNA binding domains and channel it to RPH catalytic pore for its degradation.

Increased body of evidence supports the notion that PNPase plays a crucial role in controlling oxidized RNA in both prokaryotes and eukaryotes. Biochemical assay^{83,51} and computational simulation at the atomic level¹¹⁵ show that it preferentially recognizes and binds to 8-oxoG containing RNA with high affinity. Overexpression and knockdown of PNPase in both *E. coli* and cultured human cells controls the level of 8-oxoG providing firm evidence that it directly regulates oxidized RNA in the cells. Furthermore, cells lacking PNPase were hypersensitive to oxidative stress suggesting that it is an essential ribonuclease in protecting cells under stress conditions. Although it forms a degradosome complex with RNA helicases such as RhlB in *E. coli* and hSUV3 in eukaryotic cells, these helicases do not seem to facilitate PNPase in controlling oxidized RNA. One of the intriguing features is that PNPase binds to synthetic RNA but does not degrade them⁸³ whereas it reduces the level of 8-oxoG *in vivo*⁵¹. Hence, the open question remains whether PNPase recruits other factors to degrade oxidized RNA. PNPase has two RNA binding domains (KH and S1) and two

catalytic domains (RPH1 and RPH2). What triggers PNPase's RNA binding domains to demarcate the binding of oxidized RNA from other aberrant RNA remains to be characterized. PNPase has a dual role as a 3'-5' exoribonuclease and 3'-terminal oligonucleotide polymerases. The reduction of 8-oxoG levels by PNPase inside cells suggests that the exoribonuclease activity is dominant over polymerase activity. However, PNPase may recruit other factors to degrade oxidized RNA after its binding. Hence, the role of other proteins cannot be undermined unless direct evidence of PNPase exoribonuclease activity on oxidized RNA is established.

7. Conclusion and future perspectives

An increasing number of studies show that RNA oxidation poses a significant threat to living organisms. RNA oxidation could be a major player of human aging⁵⁸ and various diseases. This could be due to the defects in molecular and cellular processes associated with oxidized RNAs such as protein translation, RNA strand scission, abasic site formation, and cell death. Yet, the relationship between RNA oxidation-associated disease and their clinical applications is not fully

established. A better understanding of the role of oxidized RNA in human diseases could be discerned by studying the distribution pattern of oxidized RNA (8-oxoG) as has been done for DNA. In DNA, the profiling of oxidized deoxyguanosine (8-oxodG) modification was first studied by Nakabeppu and colleagues in 2006¹¹⁸. Subsequent studies show that the sites of 8-oxodG modifications are not consistent¹¹⁹ instead they are enriched in the gene body and promoter regions when compared to the intergenic regions (5'UTR and 3'UTR)^{120,121,122,123}. Similar information for RNA could enhance our understanding of the role of RNA oxidation in various diseases. In RNA, this type of global analysis of the distribution of oxidation has been reported by few studies in yeast¹²⁴ and lung cells¹²⁵. Further studies using approaches such as high throughput long-read RNA sequencing strategies (third-generation sequencing) could significantly enhance our understanding of RNA oxidation and human diseases

As for the proteins and factors involved in the quality control mechanisms of oxidized RNA, a handful has been well characterized while others are still being discovered. Current evidence points to a crucial role of PNPase. PNPase. Studies in both *E. coli* and cultured human cells have shown that PNPase is involved in controlling oxidized RNA and thus resulting in cell protection during oxidative stress. Functional defects of PNPase, for instance, due to mutation may interfere with its role in controlling oxidized. This may result in the increased accumulation of oxidized RNA which may lead to, or further, aggravate the pathogenesis of oxidized RNA-associated human diseases.

The growing evidence of the association of RNA oxidation with multiple human diseases warrants further studies into this area. Future studies can probe into how oxidized RNA can be employed as a routine clinical biomarker of various diseases. Also, since PNPase plays an important role in the quality control of oxidized RNAs, further studies should be directed at fully

deciphering the pathways associated with PNPase. This can also inform the development of new therapeutic agents that enhance the function of PNPase.

Acknowledgments: We thank the members of Emmanouil Maragkakis lab for helpful discussion on this manuscript.

Abbreviations/nomenclature

¹ O ₂	Singlet oxygen
8-oxoG	8-hydroxyguanosine
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ARP	Aldehyde reactive probe assay
ATP	Adenosine triphosphate
AUF1	AU-rich element RNA-binding protein 1
BD1	Bipolar disorder 1
CNS	Central nervous system
DAZAP1	DAZ Associated Protein 1
DNA	Deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
ER	Endoplasmic reticulum
ES7	Expansion segment 7
H ₂ O ₂	Hydrogen peroxide
HO [*]	Hydroxyl groups
HPLC	High-performance liquid chromatography
hSUV3	Human RNA helicase
Kilsm4p	Kluyveromyces lactis Lsm4p
Lsm4p	U6 snRNA-associated Sm-like protein
mRNA	Messenger RNA
MTS	Mitochondrial translocation signal
O ₂ ⁻	Superoxide
ONOO ⁻	Peroxynitrite
PARP1	Poly [ADP-ribose] polymerase 1
PCBP1	poly C binding protein one
PD	Parkinson's disease
PNPase	polynucleotide phosphorylase
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rRNA	Ribosomal RNA

ssRNA	Single stranded RNA
tRNA	Transfer RNA
UTR	Untranslated region
UV	Ultraviolet

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