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Oxidative RNA Damage and Neurodegeneration

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Abstract: Although cellular RNA should be subject to the same oxidative insults as DNA and other cellular macromolecules, oxidative damage to RNA has not been a major focus in investigating the magnitude and the biological consequences of the free radical damage. However, because RNA is mostly single-stranded and its bases are not protected by hydrogen bonding and are less protected by specific proteins, RNA may be more susceptible to oxidative insults than DNA. Thereafter, oxidative damage to protein-coding RNA or non-coding RNA will potentially cause errors in proteins or dysregulation of gene expression. While less lethal than mutations in genome, such non-acutely lethal insults to cells might be associated with underlying mechanisms of several human diseases, especially chronic degeneration. Recently, oxidative RNA damage has been described in several neurodegenerative diseases including Alzheimer disease, Parkinson disease, dementia with Lewy bodies, and prion diseases. Of particular interest, oxidative RNA damage is a feature in vulnerable neurons at the very earliest-stages of these diseases, suggesting that RNA oxidation may actively contribute to the onset or to the development of disease. Mechanistically speaking, an increasing body of evidence suggests that the detrimental effects of oxidative RNA damage to protein synthesis are attenuated, at least in part, by the existence of mechanisms that avoid the incorporation of the damaged ribonucleotides into the translational machinery.

Further investigations toward understanding of the consequences and processing mechanisms related to oxidative RNA damage may provide significant insights into the pathogenesis and therapeutic strategies for neurodegenerative and other degenerative diseases.

Keywords: Alzheimer disease, 8-hydroxyguanosine, neurodegeneration, oxidative damage, Parkinson disease, RNA

INTRODUCTION

Neurodegenerative diseases are common and associate with aging; for example, the prevalence in the United States per 1,000 elderly is 65 for Alzheimer disease (AD) and 9.5 for Parkinson disease (PD), and the annual incidence per 100,000 of general population is 1.6 for amyotrophic lateral sclerosis (ALS) [1]. Many lines of evidence have indicated that oxidative damage is involved in the pathogenesis of neurodegenerative diseases including AD, PD, and ALS [2-9]. Indeed, oxidatively modified products of nucleic acids (e.g., 8-hydroxydeoxyguanosine, 8-hydroxyguanosine) and proteins (e.g., 3-nitrotyrosine, protein carbonyls), as well as products by lipid peroxidation (e.g., 4-hydroxynonenal, F_2-isoprostane, malondialdehyde) and glycoxidation (e.g., carboxymethyl-lysine, pentosidine), all known markers of oxidative damage, have been demonstrated in the affected lesions in the post-mortem central nervous system (CNS) or pre-mortem cerebrospinal fluid, plasma or serum, and urine from the patients with these diseases [2-9]. The increased levels of oxidative damage in such neurodegenerative diseases are often accompanied by the reduced levels of anti-oxidative defense mechanisms in the same subjects [3,5]. Remarkably, a number of known genetic and environmental factors of the neurodegenerative diseases, namely disease-specific gene mutations, risk-modifying

gene polymorphisms, and risk-modifying life-style factors are closely associated with oxidative damage [3,6,9], which implicates a pathogenic role of oxidative damage in the process of neurodegeneration. Despite the abundant evidence for an involvement of oxidative insults as an early-stage of the neurodegenerative process, interventions such as the administration of one or a few antioxidants have been, at best, modestly successful in clinical trials. The complexity of the metabolism of reactive oxygen species (ROS) suggests that such interventions may be too simplistic and requires more integrated approaches not only to enrich the exogenous antioxidants but also to up-regulate the multilayered endogenous anti-oxidative defense systems [8,9]. Indeed, it is clear that there is a considerable need for a better understanding of the association between ROS metabolism and neurodegeneration, one which may constitute a breakthrough in the treatment of neurodegenerative diseases.

Although RNA should be subject to the same oxidative insults as DNA and other cellular macromolecules, oxidative damage to RNA has not been a major focus in investigating the magnitude and the biological consequences of ROS. Because RNA is mostly single-stranded and its bases are not protected by hydrogen bonding and is probably less protected by specific proteins, RNA may be more susceptible to oxidative insults than DNA [10-12]. That RNA is a vulnerable target is also a reasonable

proposition given the relative cellular abundance of RNA and the subcellular distribution of RNA that locates in the vicinity of mitochondria, the primary source of ROS [10]. Given these factors, it is not surprising that, using high-performance liquid chromatography coupled with electrochemical detector (HPLC-ECD) or with electrospray tandem mass spectrometry (HPLC-MS/MS), greater oxidation to RNA than to DNA has been shown in an experiment with isolated DNA and RNA [13] as well as in cell lines and tissue, namely, human leukocytes [14], human skin fibroblasts [15], human lung epithelial cells [16] and in rat liver [13,17]. Urinary excretion of an oxidized form of ribonucleoside in healthy humans and rats [18] not only suggests substantial RNA oxidation in normal metabolism but also the existence of a repair mechanism for the damaged RNA.

It is now becoming evident that RNA molecules are not only intermediates for the transfer of genetic information from DNA to proteins but that RNA is also a key player in many mechanisms controlling expression of genetic information [19-22]. Given the view that RNAs are undergoing a "renaissance", we and others have developed a hypothesis that RNA damage is involved in the pathomechanisms of neurodegeneration [10-12,20-22]. Here we review recent studies demonstrating RNA oxidation in vulnerable neuronal populations in several neurological diseases and

discuss the biological significance of the damage to RNA and possible cellular mechanisms against such RNA damage.

POSSIBLE IMPACT OF THE "RNA RENAISSANCE" ON NEURODEGENERATIVE DISEASE RESEARCH

Recent progress in genetics has revealed an expanding universe of RNA beyond its classical function as intermediates in protein synthesis according to "Central Dogma" that describes the transcription from DNA to messenger RNA (mRNA) and translation from mRNA to proteins. Indeed, it is now evident that only a minority of genetic transcripts (2-3% in human) code for proteins. Non-coding RNA (ncRNA), rather than being "junk", can function directly in structural and catalytic activities and also appear to play a critical role in regulating the timing and rate of gene expression [19-22]. Of particular note, the complexity of an organism correlates poorly with the number of protein coding genes, however, complexity is highly correlated with the number of ncRNAs [23]. Furthermore, the increasing variety of ncRNAs being identified in the CNS suggests a strong connection between the biogenesis, dynamics of action, and combinational regulatory potential of ncRNAs and the complexity of the CNS [21,22]. Therefore, further advances in studies on the mechanisms and

consequences of RNA damage and its surveillance may have a significant impact on understanding of the pathophysiology of currently unresolved complex diseases including neurological and psychiatric diseases [21-24].

In two of the most frequent neurodegenerative diseases, AD and PD [1], gene mutations cause hereditary-forms of the diseases and the mutations are associated with protein aggregates (e.g., amyloid-β aggregation in senile plaques in AD and α-synuclein aggregation in Lewy bodies in PD) that form hallmark pathologies in the affected brains [8,25]. However, the majority of the patients with AD and PD have sporadic disease and the etiology of the diseases is largely unknown. However, as we have reviewed here, oxidative damage to neuronal RNA is not only a common feature of AD, PD and associated neurodegenerative diseases but also an early-stage event in the pathological cascade of the diseases, which suggests an involvement of RNA damage in the pathogenic mechanisms of these neurodegenerative diseases. While RNA damage would be less lethal for cells than mutations in the genome, such moderate, non-acutely lethal insults to cells might be associated with underlying mechanisms of several human diseases, especially chronic degeneration.

RNA OXIDATION IN VARIOUS NEUROLOGICAL DISEASES

The disruption of transcriptional or translational fidelity in neurons leads to the accumulation of aberrant or misfolded proteins and neuronal death [26,27], which suggests a role of RNA damage in the underlying mechanisms of neurological diseases. Oxidative damage to DNA has been well studied and several classes of products such as base oxidation and fragmentation products (e.g., single- and double-strand breaks), inter/intra-strand cross-links, DNA-protein cross-links, and sugar fragmentation products are identified [28,29]. However, few studies have focused on oxidative damage to RNA and only limited kinds of oxidatively modified bases in RNA have been reported previously [30-35]. Among multiple adducts of nucleoside oxidation, adducts of deoxyguanosine (1) and guanosine (2), i.e., 8-hydroxydeoxyguanosine (8-OHdG) (3) and 8-hydroxyguanosine (8-OHG) (4) are two of the best characterized and studied forms of DNA and RNA oxidation, respectively (Fig. (**1**)) [13-17].

The availability of highly specific antibodies with 8-OHdG and 8-OHG has enabled us to perform *in situ* approaches to examine nucleoside oxidation in post-mortem brain samples taken from patients with neurological diseases [36,37]. In 1999, increased levels of 8-OHdG/8-OHG were demonstrated in the vulnerable neuronal populations in post-mortem brains of patients with AD and PD [10,38]. In AD and PD, the neuronal 8-OHdG/8-OHG showed cytoplasmic predominance, which

suggested that either mitochondrial DNA and/or cytoplasmic RNA in neurons were major targets of oxidative damage. However, because the neuronal 8-OHdG/8-OHG immunoreactivity in AD brain were diminished greatly by RNase pretreatment but not by DNase pretreatment, the oxidized nucleoside was predominantly associated with RNA rather than DNA [10]. This notion was further supported by the immunoelectron microscopic observation that most of the oxidized nucleoside was localized to ribosomal structures [39].

Similar RNA oxidation in neuronal cytoplasm was observed in brain samples of patients with Down syndrome [40], dementia with Lewy bodies [41], Creutzfeldt-Jakob disease [42], and subacute sclerosing panencephalitis [43], as summarized in Table 1. The oxidative damage to RNA was demonstrated not only in sporadic-forms of the diseases but also in familial-forms of AD [44] and prion diseases, i.e., familial Creutzfeldt-Jakob disease and Gerstmann-Strausler-Scheinker disease [42,45]. Furthermore, nuclear DNA oxidation and cytoplasmic RNA oxidation were observed in brains of patients with a genetic defect of nucleotide excision repair mechanism, *xeroderma pigmentosum*, showing cutaneous hypersensitivity to sunlight and progressive neurological disturbances [46]. Furthermore, RNA oxidation was demonstrated in muscle cells of patients with rimmed vacuole myopathy [47] as well as

in the smooth muscle cells and endothelial cells of human atherosclerotic plaques [48]. Although these lesions are out of the nervous system, myopathies with rimmed vacuoles show a striking similarity in pathological changes with the brains of AD [47,49] and atherosclerosis is associated with an increased risk and pathology formation of AD [50], which suggests the pathogenic relation between oxidative RNA damage and AD-type neurodegeneration.

These immunocytochemical studies demonstrating neuronal RNA oxidation in were followed by biochemical detection of the oxidized nucleoside in AD brain with immunoblot approaches [51-55]. Shan et al. [51,52] used northwestern blotting with a monoclonal anti-8-OHG antibody, to isolate and identify oxidized RNA species and showed that a significant amount of brain poly $(A)^+$ mRNA species were oxidized in AD. The oxidation to mRNA was further confirmed by cDNA synthesis and Southern blotting of the immunoprecipitated mRNA species. Densitometric analysis of the Southern blot results revealed that 30-70% of the mRNAs from AD frontal cortices were oxidized, while only 2% of the mRNAs were oxidized in age-matched normal controls [52]. Interestingly, reverse transcription polymerase chain reaction (RT-PCR) and filter array analyses of the identified oxidized mRNAs revealed that some species were more susceptible to oxidative damage in AD, while no common motifs or

structures were found in the oxidatively susceptible mRNA species. Some of the identified known oxidized transcripts were related to AD, which included p21ras, mitogen-activated protein kinase (MAPK) kinase 1, carbonyl reductase, copper/zinc superoxide dismutase (SOD1), apolipoprotein D, calpains, but not amyloid-β protein precursor or tau [51]. Although these studies by Shan et al. [51,52] focused on mRNA species that account for only a few percent of total cellular RNA, Honda et al. [53] and Ding et al. [54,55] reported that ribosomal RNA (rRNA), extremely abundant in neurons, contained 8-OHG in AD brain. Remarkably, rRNA showed higher binding capacity to redox-active iron than transfer RNA (tRNA), and consequently the oxidation of rRNA by the Fenton reaction formed 13 times more 8-OHG than tRNA [53].

Of note, both immunocytochemical studies [9,10,38,41] and biochemical studies [51,54] revealed that the regional distribution of RNA oxidation in the brain was consistent with the selective neuronal vulnerability in each neurological disease. There were increased levels of 8-OHG in the hippocampus and cerebral neocortex in AD as well as in the substantia nigra in PD, while no alteration in 8-OHG levels was found in the cerebellum in both AD and PD compared with controls [9,10,38,51,54]. Immunocytochemical approaches further enabled confirmation that the oxidized RNAs were localized predominantly in neuronal cells compared with glial cells [10,38-41,44].

In addition to the brain, significantly increased levels of the oxidized RNA nucleoside, 8-OHG, have been identified in cerebrospinal fluid collected from patients with AD and PD [56-58] as well as in serum of PD patients [57], which indicates that 8-OHG is a possible biomarker of the diseases. As we describe in the following section, 8-OHG may have diagnostic utility as an early-stage marker of the diseases or a marker predicting conversion from the prodromal stage into early-stage of the diseases.

NEURONAL RNA OXIDATION IN EXPERIMENTAL CONDITIONS

Experimental studies with rodent have shown that neuronal RNA oxidation (8-OHG) and spatial memory deficit are observed in old animals [59] as well as animals with intermittent hypoxia [60]. In both aging and hypoxia models, antioxidants or mitochondrial metabolites can reduce both oxidative damage and the spatial memory deficit. In the C57BL/6J mice, even young animals (10-12 weeks old) show substantial levels of spontaneously oxidized RNA (8-OHG) in neurons of the hippocampus and the substantia nigra [61], which contrasts the observations in human control brains with no apparent level of RNA oxidation at younger ages [10]. It may be interesting to see if the levels of RNA oxidation are different among several mammalian species particularly

with reference to the maximum life spans of the species which negatively correlates with the levels of DNA oxidation [62].

Animal models of neurodegeneration exposed to neurotoxins demonstrate an involvement of RNA oxidation in the degenerative pathway. Animals treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) showing degeneration in nigrostriatal dopaminergic neurons are widely used as an experimental model of PD [63]. In MPTP-treated mice, there is a significant increase in neuronal 8-OHG in the substantia nigra [61]. Also, kainic acid-mediated excitotoxicity, a model for neurodegeneration [64] is also associated with increased levels of 8-OHG in neurons and glial cells of the hippocampus [65].

A strong genetic link between oxidative damage and neurodegeneration has been suggested by the finding that about 20% of patients with familial ALS are caused by a mutation in SOD1, a metalloenzyme that catalyzes the dismutation of the toxic superoxide ($O_2^{\bullet -}$) to hydrogen peroxide (H_2O_2) [66]. Although several lines of evidence indicates that the involvement of the SOD1 mutation in the neurodegeneration seems to be a toxic gain of function rather than a loss in the SOD1 activity [5], a transgenic mice model of ALS expressing Gly93Ala-SOD1 mutation that develops an ALS phenotype [67] shows increased RNA oxidation in the motor neurons of the spinal cord [52,68].

Cell culture experiments also suggest an association between increased RNA oxidation and neurodegeneration [69,70]. In an experimental model using cell culture mixed astrocyte and neuron cultures [69], DNA and RNA oxidation have been observed following proteasome inhibition that is associated with several neurodegenerative features such as protein aggregation, activated apoptotic pathways, and induction of mitochondrial disturbances. Interestingly, in this proteasome inhibition model, neurons underwent larger increases in nucleic acid oxidation compared to astrocyte cultures, and RNA appeared to undergo a greater degree of oxidation than DNA, which was exactly identical in AD brain [10]. Various neurodegenerative diseases including AD, PD, and ALS are associated with compromises in the ubiquitin-proteasome system that affect multiple aspects of RNA metabolism and promote RNA pathology [71]. Another recent study using primary rat cortical cultures has shown that exposures to H_2O_2 and other oxidative insults cause neuronal RNA oxidation and subsequent neuronal death [70]. This model has clearly demonstrated a chronological primacy of neuronal RNA oxidation in the process of neurodegeneration.

RNA OXIDATION: AN EARLY-STAGE EVENT IN THE PROCESS OF NEURODEGENERATION

Because RNA oxidation is involved in a wide variety of neurological diseases (Table 1), it may be considered a common event in the neurodegenerative pathway that occurs in late-stages of the diseases and is simply epiphenomenal. However, that is not the case in AD and PD, and probably in ALS (Table 2). In fact, there is considerable evidence supporting an early involvement of RNA oxidation in the pathological cascade of neurodegeneraton, especially in AD. For instance, RNA oxidation has been observed in post-mortem brains of cases with early-stage AD [9,39], a presymptmatic case with familial AD mutation [44], Down syndrome cases with early-stage AD pathology [40], and subjects with mild cognitive impairment (MCI) who possibly represent prodromal stage of AD [54,55]. Furthermore, the increased level of RNA oxidation in cerebrospinal fluid is more prominent in cases with shorter duration of AD and PD [56,58] as well as in AD cases with higher scores on a cognitive scale [56]. Recent studies of MCI subjects have also demonstrated increased oxidation/nitration to protein and lipid peroxidation in post-mortem brain [72,73], increased lipid peroxidation in cerebrospinal fluid, plasma and urine [74], increased DNA oxidation in peripheral leukocytes [75] as well as decreased plasma antioxidant vitamins and enzymes [76] and decreased plasma total antioxidant capacity [77]. As for ALS, neuronal RNA oxidation has not been reported in analysis of samples from patients with ALS. However,

significantly increased RNA oxidation has been observed in motor neurons, a presymptomatic stage, before motor neuron death, of spinal cord from the Gly93 Ala-SOD1 transgenic animal model of familial ALS [52,68]. Of note, an early-stage involvement of neuronal RNA oxidation is not only evident in age-associated neurodegenerative diseases, but also in cases with subacute sclerosing panencephalitis that is caused by persistent measles virus infection in the CNS and is pathologically accompanied with brain atrophy and neurofibrillary tangles [43]. From a clinical perspective, the notion of an early involvement of oxidative damage in the pathogenesis of these degenerative diseases should have a great importance to the establishment of diagnostic tools and therapeutic targets, as we have reviewed recently [9,78,79].

The chronological primacy of neuronal RNA oxidation in the process of neurodegeneration has been clearly demonstrated also in a model of primary rat cortical cultures [70]. In the time course after various oxidative insults to the cultures, RNA oxidation occurs primarily in a distinct group of neurons that die later. Together with an observation of decreased protein synthesis due to the oxidized RNA, this study suggests that neuronal RNA oxidation is neither a consequence of dying neurons nor a harmless epiphenomenon but is an early event that precedes neuronal death and contributes to it [70].

While protein carbonyls, lipid peroxidation products and glycoxidation products are relatively stable at the site of generation due to the formation of cross-links and consequent resistant to degradation, oxidized RNAs are likely turned over more rapidly compared to the other oxidatively modified macromolecules. Therefore, the detection of most oxidative markers using *in situ* approaches in tissues affected by disease (protein carbonyls, lipid peroxidation products or glycoxidation products) indicates the "history" of oxidative damage. On the other hand, RNA oxidation reflects the "steady-state balance" of oxidative damage at a "snapshot" point [10,80]. In accordance with this concept, protein carbonyls, lipid peroxidation products such as 4-hydroxynonenal and F_2-isoprostane, and a glycoxidation product carboxymethyl-lysine have been demonstrated in neurons with and without associated pathology in AD brains [81-84]. These data likely reflect the occurrence of damage throughout the early- and advanced-stages of neurodegeneration ("history" of the damage). These observations contrast remarkably with the pattern of the RNA oxidation, a "steady-state" marker, which is prominent in neurons without pathology and is present in lesser amounts in neurons containing pathology [39,40]. Among the markers for oxidative damage, 3-nitrotyrosine may be another steady-state marker of oxidative damage. 3-nitrotyrosine is formed by a modification of tyrosine residue of proteins by an attack of peroxynitrite

($ONOO^-$), a powerful oxidant produced from the reaction of $O_2^{\bullet-}$ with nitric oxide ($NO^\bullet$), and is not known to accumulate in cells. As such, it is not surprising that intracellular level of 3-nitrotyrosine parallels the level of 8-OHG in AD and Down syndrome brains [39,40].

SOURCES OF REACTIVE OXYGEN SPECIES (ROS) RESPONSIBLE FOR CELLULAR RNA OXIDATION

The brain is especially vulnerable to oxidative damage because of its high content of easily peroxidizable unsaturated fatty acids, high oxygen consumption rate (accounting for 20-25% of total body oxygen consumption but less than 2% of total body weight), and relative paucity of antioxidant enzymes compared with other organs (e.g., the content of catalase in brain is only 10-20% of liver and heart) [85,86]. Given this environment, neurons are continuously exposed to ROS such as $O_2^{\bullet-}$, H_2O_2, and hydroxyl radical (•OH) that are produced from the mitochondrial electron transport chain through normal cellular metabolism [85-87]. •OH can diffuse through tissue only in the order of several nanometers [88] and $O_2^{\bullet-}$ is hardly permeable through cell membranes [89]. In consideration of the widespread damage to cytoplasmic RNA in neurodegenerative diseases, RNA species are likely attacked by •OH, which is formed

from the reaction of highly diffusible H_2O_2 [90] with redox-active metals through the Fenton reaction [53]. In the AD brain, disrupted mitochondria likely play a central role in producing abundant ROS as well as supplying redox-active iron into the cytosol [8,91-93]. Indeed, ribosomes purified from AD hippocampus contain significantly higher levels of redox-active iron compared to controls, and the iron is bound to rRNA [53]. Therefore, mitochondrial abnormalities coupled with metal dysregulation of metal homeostasis are key features closely associated with ROS formation responsible for the RNA oxidation in AD [94]. Interestingly, mitochondrial abnormalities [95,96] and metal ion dysregulation [97,98] are also found in the substantia nigra of PD making this mechanism a common theme in neurodegenerative cascades.

RNA OXIDATION AND THE BIOLOGICAL CONSEQUENCE

More than 20 different types of oxidatively altered purine and pyrimidine bases have been detected in nucleic acids [28,29,35,99]. However, since guanine is the most reactive of the nucleic acid bases [32], it is not surprising that the oxidized base, 8-hydroxyguanine is the most abundant among the oxidized bases [11]. The 8-hydroxyguanine-containing nucleoside, 8-OHG, can be formed in RNA by direct oxidation of the base and also by the incorporation of the oxidized base from the

cytosolic pool into RNA through the normal action of RNA polymerase (Fig. (**2**)) [32,99]. Not only 8-OHG but also 8-hydroxyadenosine, 5-hydroxycytidine, and 5-hydroxyuridine have been identified in oxidized RNA [32], which may have altered pairing capacity and thus be at the origin of erroneous protein production. Indeed, the 8-hydroxyguanine can pair with both adenine and cytosine, and thus oxidized RNA compromises the accuracy of translation [99,100].

The biological consequence of oxidatively damaged mRNA species has been investigated *in vitro* by expressing them in cell lines. Oxidized mRNAs lead to loss of normal protein level and protein function, and potentially produce defective proteins leading to protein aggregation, a common feature of neurodegenerative diseases [51]. In a recent study, polyribosome analysis indicates that oxidized bases in mRNAs cause ribosome stalling on the transcripts or slow the translation process, which leads to a decrease of protein expression [70]. When oxidized and non-oxidized luciferase RNAs were subjected to translation in rabbit reticulocyte lysates and the fractions were analyzed by northern blot, the oxidized RNA samples showed a decreased amount of free monosomes and an increased amount of RNA-associated polyribosomes compared to the non-oxidized RNA samples [70]. In another recent study, the translation of oxidized mRNA in cell lines causes the accumulation of short polypeptides, resulting

from the premature termination of the translation process of the oxidixed mRNA and/or the proteolytic degradation of the modified protein containing the translation errors due to the oxidized mRNA [101]. Coincidently, oxidative damage to *Escherichia coli* 16S rRNA results in the formation of short cDNA by the RT-PCR [102]. The biological consequence of ribosomal oxidation have also been investigated *in vitro* using translation assays with oxidized ribosomes from rabbit reticulocytes and show a significant reduction of protein synthesis [53]. Notably, studies on brains of subjects with AD and MCI have demonstrated ribosomal dysfunction associated with oxidative RNA damage [54,55]. Isolated polyribosome complexes from AD and MCI brains show a decreased rate and capability for protein synthesis without alteration in the polyribosome content. Decreased rRNA and tRNA levels and increased 8-OHG in total RNA pool, especially in rRNA, are accompanied by the ribosomal dysfunction, while there is no alteration in the level of initiation factors [54].

These findings have indicated that RNA oxidation has detrimental effects on cellular function whether the damaged RNA species are coding for proteins (mRNA) or performing translation (rRNA and tRNA). It is noteworthy that studies on some anti-cancer agents have shown that RNA damage can lead to cell-cycle arrest and cell death, much as DNA damage does. RNA damage may cause cell death via either

pathway involving p53-dependent mechanism associated with inhibition of protein synthesis or p53–independent mechanism different from inhibition of protein synthesis [103].

COPING WITH RNA DAMAGE

Degradation of RNA plays a central role in RNA metabolism and damaged RNA can be removed through degradation by ribonucleases (RNase), but selective degradation activity for oxidized RNA has not been established for known RNases [12,104]. Oxidative stress induces cytoplasmic mRNA processing bodies (P-bodies), the site of active degradation of mRNA [105], which is linking with an induction of another cytoplasmic structure called “stress granules” [106]. In contrast to mRNAs with rapid turnover, stable RNAs, consisting primarily of rRNAs and tRNAs and encompassing 98% of total cellular RNA, may be protected against RNase action by tertiary structure, assembly into ribonucleoprotein complex, or even blocking the RNA’s 3’ terminus [104].

Until recently, it has been considered that damaged RNA may be only degraded rather than repaired. However, Aas et al. [107] has suggested that the cells have at least one specific mechanism to repair RNA damage, indicating that cells may

have a greater investment in the protection of RNA than previously suspected [11,103,108]. Indeed, alkylation damage in RNA is repaired by the same mechanism as a DNA-repair, catalyzed in the bacterium *Escerichia coli* by the enzyme AlkB, and in humans by the related protein [107]. Alk B and its homologue hABH3 cause hydroxylation of the methyl group on damaged DNA and RNA bases, and thus directly reverse alkylation damage. Alk B and hABH3, but not hABH2, repair RNA, since Alk B and hABH3 prefer single-stranded nucleic acids while hABH2 acts more efficiently on double-stranded DNA [107]. DNA damage can be repaired not only by the mechanism of direct reversal of the modified bases but also base excision repair mechanism. Specific DNA glycosylases excise the damaged base and DNA polymerases replace the nucleotide [103,109]. However, because the excision repair generally requires a complementary strand, the mechanism is not likely efficient at the RNA level [108].

Cells have mechanisms of dealing with nucleotide damage other than direct repair and excision repair, which seems to be useful for defense against oxidative damage to both DNA and RNA. Because oxidation of nucleotides can occur in the cellular nucleotide pool and the oxidized nucleotide can be incorporated into DNA and RNA, the mechanism avoiding such incorporation of the oxidized nucleotide is

involved in coping with nucleic acid damage (Fig. (**2**)) [11,12,103]. MutT protein in *Escerichia coli* and its mammalian homologues MutT homologue 1 (MTH1) and Nudix type 5 (NUDT5) proteins participate in this error-avoiding mechanism by hydrolyzing the oxidized nucleoside diphosphates and /or triphosphates to the monophosphates [99,100,109-112]. Indeed, the increase in the production of erroneous proteins by oxidative damage is 28-fold over the wild type cells in *Escerichia coli mutT* deficient cells, which is reduced to 1.2- or 1.4-fold by the expression of MTH1 or NUDT5, respectively [99]. Correspondingly, MTH1 deficiency augments the RNA oxidation induced by kainic acid treatment in MTH1-null mouse [65]. An increased expression of human MTH1 in the vulnerable neuronal populations has been demonstrated in the post-mortem brains of AD [113] and PD [114], which may indicate a compensatory up-regulation of the MTH1 against oxidative stress [112]. In addition to the degradative activity of MTH1 and NUDT5, several enzymes involved in nucleotide metabolism show a discriminator activity against the oxidized nucleotides (Fig. (**2**)). Guanylate kinase (GK), an enzyme that converts GMP to GDP, is inactive on 8-OH-GMP, while nucleotide diphosphate kinase (NDK), an enzyme that converts GDP to GTP, fails to show such discriminating function [110]. Similarly, ribonucleotide reductase (RNR), an enzyme that catalyzes reduction of four naturally occurring ribonucleoside diphosphates,

is inactive on conversion of 8-OH-GDP to 8-OH-dGDP, which avoid incorporation of the oxidized nucleotide into DNA synthesis [110]. The final gatekeeper discriminating the oxidized nucleotide from normal nucleotide is RNA polymerase that incorporates 8-OH-GTP into RNA at a much lower rate compared to the normal GTP incorporation [12,100].

Then, one important question is whether cells have machinery to deal with oxidatively damaged nucleotides that are contained in RNA, because RNA can be directly oxidized even if the incorporation of oxidized nucleotides into RNA is blocked. Recently, proteins that bind specifically to 8-OHG-containing RNA have been reported, namely, *Escerichia coli* polynucleotide phosphorylase (Pnp) protein and human PNP [115,116] as well as human Y box-binding protein 1 (YB-1) [117]. The binding of the specific protein likely makes the 8-OHG-containing RNA resistant to nuclease degradation [115]. However, it has been proposed that these proteins may recognize and discriminate the oxidized RNA molecule from normal ones, thus contributing to the fidelity of translation in cells by sequestrating the damaged RNA from the translational machinery [115-117]. The human PNP protein binds to 8-OHG-containing RNA preferentially and cellular amounts of human PNP protein decrease rapidly by exposure to agents inducing oxidative stress, while amounts of other proteins in the cells do not

change after the treatments [117]. Recently, human YB-1 was demonstrated to be a component of P-bodies where active degradation of mRNA occurs. YB-1 is translocated from P-bodies to stress granules during oxidative stress, which suggests a dynamic link between P-bodies and stress granules under oxidative stress [118].

It is possible that the RNA quality control mechanisms are defective or inefficient in cancer cells as well as cells of neurodegenerative diseases. Further elucidation of the mechanisms of repair or avoidance of RNA damage and their potential role in preventing human diseases might provide new approaches to a number issues of life science that have evaded resolution, while it has not been the major focus in investigation for a long period [11,12,108].

CONCLUSION

Involvement of RNA oxidation in the process of neurodegeneration has been demonstrated in vulnerable neuronal population in neurodegenerative diseases such as AD and PD as well as several cellular and animal models of neurodegeneration. Particular emphasis should be placed on the early-stage involvement of RNA oxidation in the process of neurodegeneration, which suggests a primary role of RNA oxidation in the pathomechanisms. Indeed, oxidized RNA is associated with a disturbance in protein

synthesis *in vitro* and *in vivo*. Although there are only a small number of studies suggesting the existence of coping mechanisms for RNA damage at present, the known mechanisms may be the tip of iceberg of cellular investment in counteracting the RNA damage. Understanding of the consequences and cellular handling mechanisms of the oxidative RNA damage may provide clues to both the basic pathophysiology and the therapeutic strategies of the neurodegenerative diseases.

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Figure Legends

Fig. (**1**). Markers for oxidative damage to nucleosides of DNA and RNA. (1) Deoxyguanosine (a DNA nucleoside), (2) Guanosine (a RNA nucleoside), (3) 8-Hydroxydeoxyguanosine (8-OHdG) (an oxidized DNA nucleoside), (4) 8-Hydroxyguanosine (8-OHG) (an oxidized RNA nucleoside).

Fig. (**2**). Quality control of RNA synthesis from 8-OHG-containing nucleotides and possible surveillance mechanism for oxidized RNA in mammalian cells. 8-OHG-containing RNA can be generated by direct oxidation to RNA as well as by incorporation of oxidized ribonucleotide, 8-hydroxy-guanosine-triphosphate (8-OH-GTP), which can be generated by direct oxidation of GTP as well as by phosphorylation of 8-hydroxy-guanosine-diphosphate (8-OH-GDP) by nucleotide diphosphate kinase (NDK). Remarkably, several enzymes involved in nucleotides metabolism show discriminating function against incorporation of oxidized nucleotide into nucleic acids. Guanylate kinase (GK), an enzyme converts GMP to GDP, is inactive on 8-hydroxy-guanosine-monophosphate (8-OH-GMP). Similarly, ribonucleotide reductase (RNR), an enzyme converts GDP to dGDP, is inactive on 8-OH-GDP, which avoid incorporation of the oxidized nucleotide into DNA synthesis. Additionally, RNA

polymerase incorporates 8-OH-GTP into RNA at much lower rate compared with the incorporation of GTP. Furthermore, MutT homologue 1 (MTH1) has the potential to hydrolyze 8-OH-GTP or 8-OH-GDP to 8-oxoGMP and Nudix type 5 (NUDT5) can hydrolyze 8-OH-GDP to 8-oxoGMP, which drastically reduces the possibilities that the oxidized nucleotides are incorporated into RNA. When 8-OHG is misincorporated into RNA, it may cause direct errors in translation or inadequate regulation of protein synthesis and cells may switch from translation to degradation by sequestrating the oxidized RNA with specific binding proteins such as polynucleotide phosphorylase (PNP) and Y-box binding protein 1 (YB-1). The existence of repair mechanism for oxidized RNA remains to be elucidated.

Table 1. Summary of Studies on RNA Oxidation in the Central Nervous System

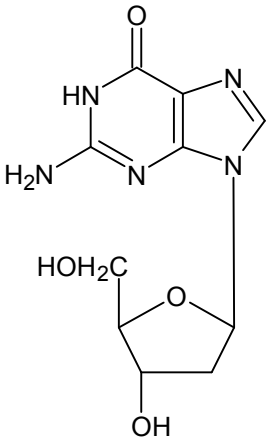
Year	*Human neurological diseases*	*Materials / Procedures*	*Authors*
1999	Alzheimer disease	Brain (hippocampus/cerebral cortex) / ICC	Nunomura et al.[10]
	Parkinson disease	Brain (substantia nigra) / ICC	Zhang et al.[38]
2000	Down syndrome	Brain (cerebral cortex) / ICC	Nunomura et al.[40]
2001	Alzheimer disease	Brain (hippocampus/cerebral cortex) / IEM	Nunomura et al.[39]
2002	Dementia with Lewy bodies	Brain (hippocampus/cerebral cortex) / ICC	Nunomura et al.[41]
	Familial / sporadic Creutzfeldt-Jakob disease	Brain (cerebral cortex) / ICC	Guentchev et al.[42]
	Subacute sclerosing panencephalitis	Brain (cerebral cortex) / ICC	Hayashi et al.[43]
	Alzheimer disease	Cerebrospinal fluid / HPLC	Abe et al.[56]
	Parkinson disease / multiple system atrophy	Cerebrospinal fluid / ELISA	Kikuchi et al.[57]
2003	Alzheimer disease	Brain (hippocampus/cerebral cortex) / IB and RT-PCR (mRNA)	Shan et al.[51]
	Parkinson disease	Cerebrospinal fluid / HPLC	Abe et al.[58]
2004	Familial Alzheimer disease	Brain (cerebral cortex) / ICC	Nunomura et al.[44]
2005	Alzheimer disease	Brain (hippocampus) / IB and RT-PCR (rRNA)	Honda et al.[53]
	Xeroderma pigmentosum (group A)	Brain (globus pallidus) / ICC	Hayashi et al.[46]
	Gerstmann-Straussler-Scheinker disease	Brain (hippocampus/cerebral cortex) / ICC	Petersen et al.[45]
	Alzheimer disease / mild cognitive impairment	Brain (cerebral cortex) / IB and RT-PCR (rRNA)	Ding et al.[54]
Year	*Experimental conditions*	*Materials / Procedures*	*Authors*
2002	Old rat (Fischer 344, over 24.5 months old)	Brain (hippocampus) / ICC	Liu et al.[59]
2003	Adult rat exposed to intermittent hypoxia	Brain (hippocampus) / ICC	Row et al.[60]
2004	Culture neuron under proteasome inhibition	Mixed astrocyte and neuron cultures / ICC and IB	Ding et al.[69]
	Transgenic mouse model of familial amyotrophic lateral sclerosis (SOD1-G93A)	Spinal cord (motor neuron) / ICC	Chang et al.[68]
2006	Normal mouse (C57BL/6J , 10-12 weeks old)	Brain (hippocampus, substantia nigra) / ICC	Yamaguchi et al.[61]
	Mouse treated with MPTP, a model of Parkinson disease	Brain (substantia nigra) / ICC	Yamaguchi et al.[61]
	Mouse treated with a excitotoxin, kainic acid	Brain (hippocampus) / ICC	Kajitani et al.[65]
	MTH1-null mouse treated with kainic acid	Brain (hippocampus) / ICC	Kajitani et al.[65]
2007	Culture neuron exposed to H_2O_2, glutamate, or amyloid-β	Primary rat cortical neuronal cultures / ICC, IB and RT-PCR (mRNA)	Shan et al.[70]

ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; IB, immunoblot; ICC, immunocytochemistry; IEM, immunoelectronmicroscopy; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTH1, MutT homologue 1; RT-PCR, reverse transcription polymerase chain reaction; SOD1, copper/zinc superoxide dismutase

Table 2. Summary of Evidence Suggesting Temporal Primacy of RNA Oxidation in the Process of Neurodegeneration

Materials/Subjects	Findings
Post-mortem brains of patients with Alzheimer disease	●RNA oxidation is more prominent in cases with lesser amounts of amyloid-β plaque deposition or shorter disease duration[39]. ●RNA oxidation is more prominent in hippocampal neurons free of neurofibrillary tangles compared to neurons with neurofibrillary tangles[39]. ●RNA oxidation is increased in a presymptomatic case with presenilin-1 gene mutation[44].
Post-mortem brains of subjects with mild cognitive impairment	●RNA oxidation is increased in brains of subjects with mild cognitive impairment, who at least in part, represent a prodromal stage of dementia[54].
Post-mortem brains of patients with Down syndrome	●RNA oxidation precedes amyloid-β plaque deposition in a series of Down syndrome brains, a model of Alzheimer-type neuropathology[40].
Post-mortem brains of patients with subacute sclerosing panencephalitis	●RNA oxidation is observed in cases with shorter disease duration, while lipid peroxidation is observed in cases with longer disease duration[43].
Cerebrospinal fluid of patients with Alzheimer disease	●RNA oxidation is more prominent in cases with shorter disease duration or higher scores in mini-mental state examination[56].
Cerebrospinal fluid of patients with Parkinson disease	●RNA oxidation is more prominent in cases with shorter disease duration[58].
Spinal cord of transgenic mice expressed the mutation (SOD1-G93A) in familial amyotrophic lateral sclerosis	●RNA oxidation is increased in motor neurons at presymptomatic stage (2-month-old) when motor neurons look like still healthy[68].
Primary rat cortical neuronal cultures exposed to oxidative insults	●RNA oxidation is an early event that far precedes neuronal death in the process of neurodegeneration[70].

SOD1, copper/zinc superoxide dismutase



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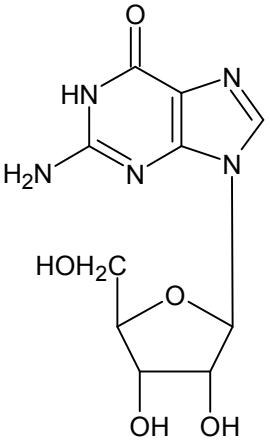

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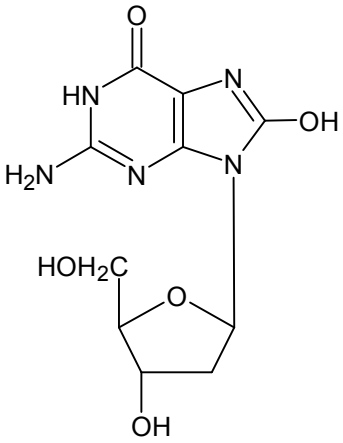

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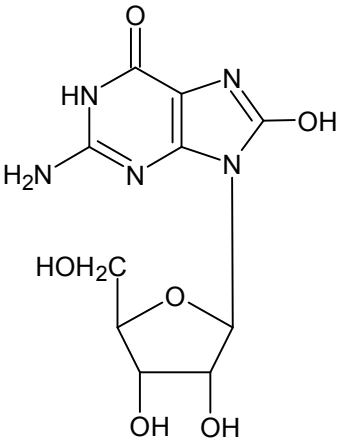

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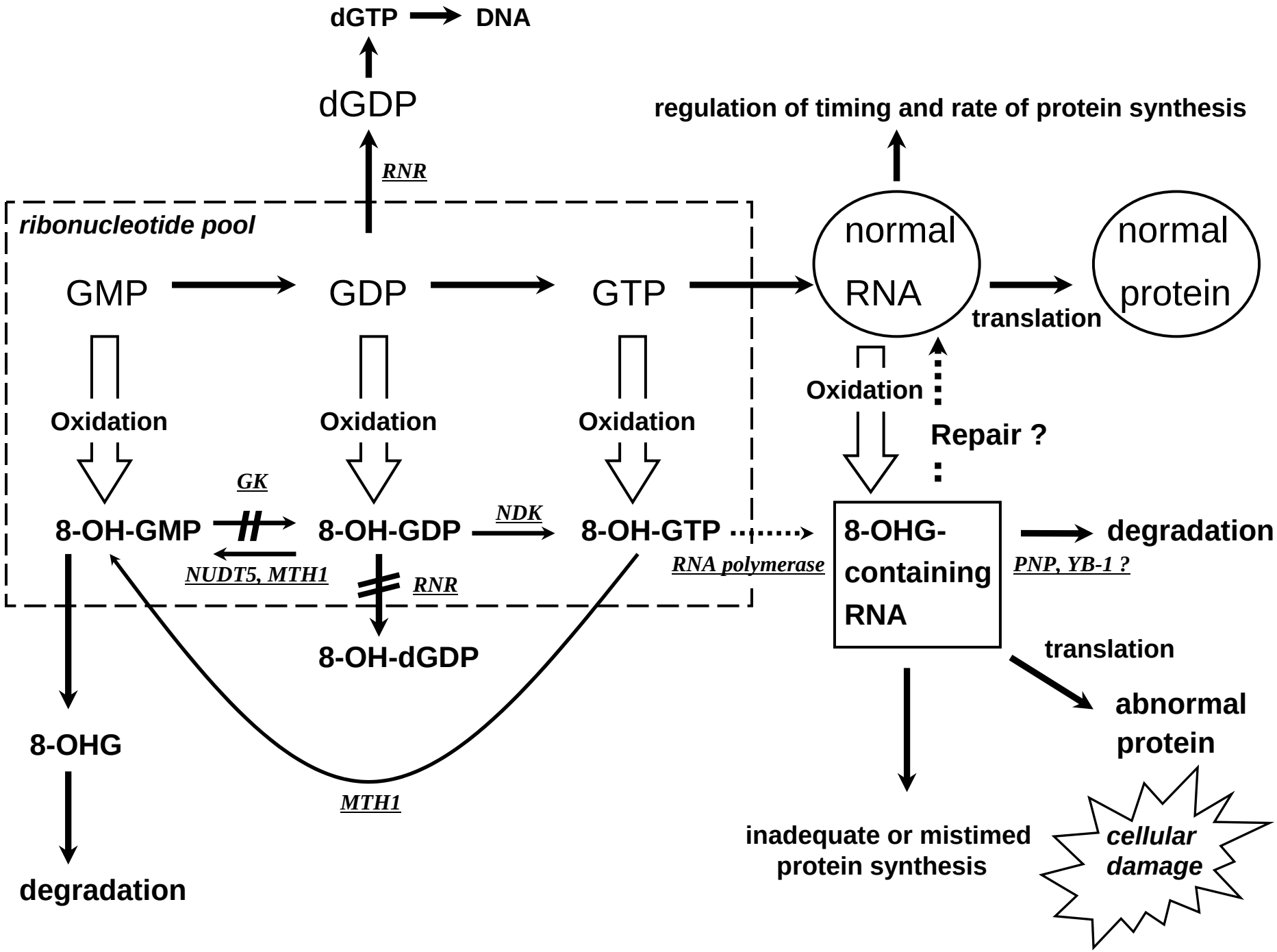
dGTP
DNA
dGDP
RNR
ribonucleotide pool
regulation of timing and rate of protein synthesis
normal RNA
normal protein
translation
GMP
GDP
GTP
Oxidation
Oxidation
Oxidation
Oxidation
Repair ?
GK
8-OH-GMP
8-OH-GDP
NDK
8-OH-GTP
RNA polymerase
8-OHG-containing RNA
degradation
PNP, YB-1 ?
NUDT5, MTH1
RNR
8-OH-dGDP
translation
abnormal protein
8-OHG
MTH1
inadequate or mistimed protein synthesis
cellular damage
degradation