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Review

Cellular responses to RNA damage

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SUMMARY

RNA plays a central role in protein biosynthesis and performs diverse regulatory and catalytic functions, making it essential for all processes of life. Like DNA, RNA is constantly subjected to damage from endogenous and environmental sources. However, while the DNA damage response has been extensively studied, it was long assumed that RNA lesions are relatively inconsequential due to the transient nature of most RNA molecules. Here, we review recent studies that challenge this view by revealing complex RNA damage responses that determine survival when cells are exposed to nucleic acid-damaging agents and promote the resolution of RNA lesions.

INTRODUCTION

DNA and RNA encode and transmit genetic information along the central dogma of molecular biology,[1] enabling not only protein synthesis but also catalytic and regulatory functions of non-coding RNAs. How cells respond when the integrity of DNA is challenged by genotoxic agents such as UV irradiation has been studied for decades. Hence, we have a detailed understanding of the cellular DNA damage response (DDR) that coordinates DNA repair, cell cycle progression, and cellular survival.[2] However, most sources of DNA damage act pleiotropically and also affect RNA.[3] Nonetheless, the consequences of RNA damage have been largely overlooked, due to the assumption that damaged RNA poses a negligible challenge for cellular integrity because RNA can simply be degraded and resynthesized. This view has changed with several recent studies showing that mRNA damage induces translation-dependent signaling cascades that dominate the immediate cellular response following exposure to "DNA-damaging" agents.[4,5] The consequences of persistent RNA damage-induced signaling are severe, ranging from inflammation and cell death to whole-genome doubling (WGD) events.[5–10] Moreover, the discovery of a pathway dedicated to the resolution of mRNA crosslinking damage[11,12] and the identification of a mammalian RNA repair ligase[13] demonstrate that cells possess mechanisms for the detection and resolution of specific RNA lesions.

Here, we review the emerging evidence for a coordinated cellular response to RNA damage in human cells and examine its crosstalk with DDR pathways. We propose two key roles for the RNA damage response: first, in the face of severe acute nucleic acid damage, the RNA damage response is crucial to ensure the momentary functioning of the damaged cell by resolving damaged RNA molecules; second, the detection of RNA lesions acts as a sentinel for concurrent DNA damage.

SOURCES OF RNA DAMAGE

DNA and RNA are both susceptible to chemical modifications, either of their nucleobases or sugar-phosphate backbone. Importantly, RNA is generally more vulnerable to damage due to its primarily single-stranded nature, whereas DNA is protected through base pairing and chromatinization (Figure 1).[14,15] Additionally, cytosolic RNA is exposed to the relatively more oxidizing conditions of the cytoplasm, in contrast to the more reductive nucleoplasm.[16] In the cytosol, RNA faces further risk of damage from reactive oxygen species (ROSs) leaking from mitochondria, as well as other reactive agents that enter cells from the environment (Figure 1).

Oxidation and alkylation of the nucleobase, the phosphodiester backbone, and the 2′-hydroxyl group of the ribose moiety damages RNA (Figure 1).[17] The most common oxidative RNA lesion is 8-oxo-guanine (8-oxoG), but other nucleobase modifications and abasic sites arise frequently as well.[17,18] Alkylating agents such as the chemotherapeutic drug temozolomide, which is used to treat several brain cancers, cause different types of RNA lesions, for example, N1-methyladenosine (m^1A) and O6-methylguanosine (m^6G).[3,19] Additionally, UV irradiation causes diverse photolesions, including uracil photoproducts and covalent RNA-RNA and RNA-protein crosslinks.[20,21] Such crosslinks are also induced by metabolic bifunctional crosslinkers, such as formaldehyde or acetaldehyde (Figure 1).[11,12] Formaldehyde is produced in substantial quantities during one-carbon metabolism and as a consequence of various cellular demethylation reactions.[22] Acetaldehyde is generated in the liver upon consumption of alcohol and is the primary cause of ethanol toxicity.[23] Various additional relevant sources of RNA

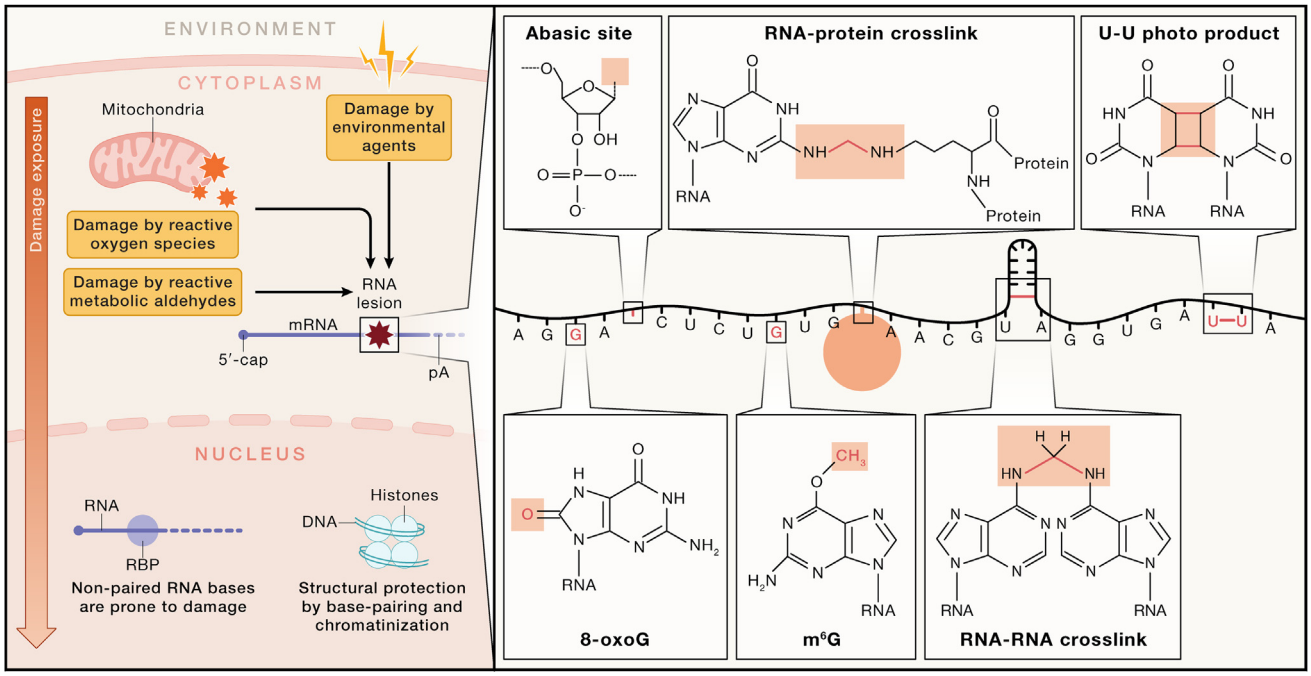

Figure 1. Chemical diversity of RNA lesions

RNA is highly susceptible to environmental and endogenous damage and lacks the 3-fold protection provided to DNA by nuclear sequestration, base pairing, and chromatinization. While RNA is also bound by RNA-binding proteins (RBPs), the protection provided is likely less pronounced due to the dynamic nature of the involved interactions.

damage, including chemotherapeutic drugs, tobacco smoke, and environmental pollution, produce chemically diverse lesions.[3,24] RNA integrity is further challenged by damaged ribonucleotides. For example, spontaneous deamination of adenosine triphosphate leads to the formation of inosine triphosphate (ITP). To remove ITP, ITP pyrophosphatase (ITPase) hydrolyzes it into inosine monophosphate. This sanitization of the nucleotide pool is compromised in patients with infantile multisystem disorder, which is caused by germline mutations in the gene encoding ITPase.[25] If ITPase is defective, ITP accumulates, leading to its incorporation into RNA. During translation, inosine in mRNAs is likely to be decoded as guanine, resulting in missense and nonsense alterations. The resulting impediment of faithful translation presumably underlies the severe pathology observed in affected individuals.[25] In addition to deamination, the ribonucleotide pool can also be damaged by oxidation.[26] This is counteracted by the nucleotide pool sanitizing enzyme MTH1,[27] which hydrolyzes 8-oxoGTP to prevent RNA incorporation. If sanitization fails or RNA is damaged by other sources, the consequences for the cell can be severe.

CONSEQUENCES OF RNA DAMAGE

RNA lesions affect almost every step in the life cycle of an RNA molecule, from transcription and splicing to translation and post-transcriptional gene regulation (Figure 2). Transcription fidelity is compromised by oxidative damage to the nucleotide pool,[26] which leads to the incorporation of 8-oxoGTP into nascent RNA by RNA polymerases.[28] Following transcription, selection of splice sites and removal of introns during splicing depends on correct base pairing of snRNAs within small nuclear ribonucleoproteins (snRNPs).[29] RNA lesions that affect base pairing thus compromise splicing fidelity, resulting in splicing defects and intron retention following UV irradiation.[30] Analogously, it is likely that functions of other short non-coding RNAs that rely on precise base pairing, including microRNAs (miRNAs), small interfering RNAs (siRNAs), and Piwi-interacting RNAs (piRNAs), are perturbed by RNA damage as well.[31,32] RNA lesions also affect the processing of ribosomal RNA (rRNA) and the assembly, maturation, and function of ribosomes. Oxidative damage of rRNA impacts protein synthesis in bacteria[33,34] and disrupts rRNA processing in eukaryotes,[35] while certain platinum-based chemotherapeutic drugs prevent efficient ribosome biogenesis in human cells.[36] rRNA integrity can additionally be affected by chemotherapeutic nucleoside analogs that cause damage upon incorporation into rRNA, ultimately leading to the degradation of faulty ribosomes.[37] Translation itself is affected by RNA damage, as it depends on the catalytic and structural functions of rRNA and on the establishment of correct base pairing between amino-acyl tRNAs and mRNA.[38] The presence of m^6G at the first or second position in codons decreases the accuracy of tRNA selection, ultimately leading to miscoding.[39,40] In addition, translation of oxidized or alkylated mRNAs stalls ribosomes in bacteria[19,41] and eukaryotes.[41,42] Similarly, bulky RNA lesions such as mRNA-protein crosslinks (mRPCs) within the coding region of mRNA cause translation stress by stalling translating ribosomes.[7,11,12] These severe consequences necessitate a fast and effective cellular response to RNA damage.

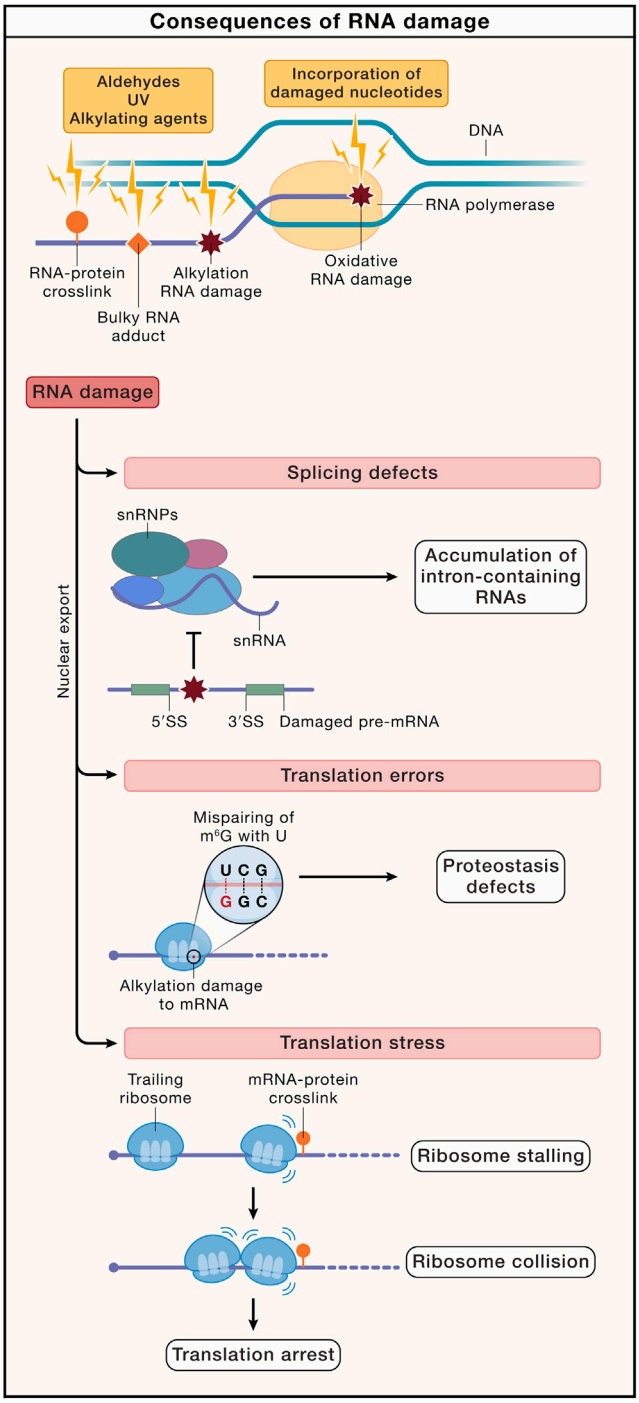

Figure 2. Consequences of RNA damage

RNA damage affects all stages during the life cycle of an RNA molecule. Misincorporation of damaged nucleotides by RNA polymerases or nascent RNA damage interferes with productive splicing (top). Mispairing of damaged mRNA bases with tRNA can lead to amino acid misincorporation and subsequent proteostasis defects (middle). mRNA lesions block progression of translating ribosomes (bottom).

RESPONSES TO RNA DAMAGE

The diverse nature of RNA damage makes it challenging for cells to sense specific lesions. An overarching theme of nucleic acid damage sensing is the detection of stalled molecular machineries as a proxy. DNA damage is detected and resolved by replication- and transcription-coupled pathways, which are initiated when DNA and RNA polymerases, respectively, stall at DNA lesions.[43,44] Stalling not only promotes repair but can also lead to a local and global shutdown of DNA and RNA synthesis.[45–48] A corresponding principle has emerged for the detection of RNA lesions that stall elongating ribosomes. Ribosome stalling and the subsequent collisions with trailing ribosomes elicit a rapid signaling response, shutting down protein synthesis and alerting cells to the presence of RNA damage.[4,5,7,11,12] Hence, RNA damage can be detected due to its detrimental consequences on mRNA translation.

The ribotoxic stress response (RSR) and the integrated stress response (ISR) have emerged as two major RNA damage response pathways that are activated by ribosome collisions,[4] regulating cell cycle progression, activation of proinflammatory pathways, and induction of cell death.[5,6,9,49] The RSR was initially identified as a translation-coupled stress response pathway triggered by certain ribotoxins, such as ricin or sarcin, and protein synthesis inhibitors such as anisomycin.[50] Subsequent work identified UV irradiation as a potent inducer of the RSR, implicating RNA damage as a source of activation.[51] The RSR is a MAP kinase signaling cascade initiated by the ribosome-associated MAP3K ZAKα.[52] ZAKα is activated when ribosomes stall and collide at mRNA lesions induced by UV,[4] nitric oxide,[53] ROS,[8] or metabolic aldehydes[11,12] (Figure 3A). ZAKα activation ultimately leads to phosphorylation of the stress-activated protein kinases (SAPKs) JNK and p38. SAPKs stall cell cycle progression by inhibition of cyclin-dependent kinases (CDKs).[54] In response to UV irradiation, p38 can initiate a G2 arrest via phosphorylation of MAPKAP kinase-2 (MK-2) and subsequent phosphorylation and inhibition of CDC25B. Inhibition of CDC25B was shown to block activation of cyclin A/CDK2, all of which occurs independently of the canonical DDR.[55–57] Additionally, SAPKs can mediate cell cycle arrest through stabilization of the CDK inhibitor p21.[58] Consequentially, failure to resolve stalled ribosomes induces a persistent G2 arrest.[7] Of note, continuous ZAKα-mediated p38 activation and the resulting inhibition of cyclin A/CDK1 and CDK4/6 can lead to premature APC/C^{Cdh1} reactivation, driving cells into G2 exit and subsequent mitotic bypass. This results in a new round of DNA replication without prior cell division, causing endoreplication.[6] In parallel to p38/MK2-mediated cell cycle checkpoint regulation, ZAKα can induce cell death through initiation of p38-dependent pyroptosis or JNK-dependent apoptosis.[5,9,59]

The ISR acts in concert with the RSR and is activated by GCN2 in response to ribosome collisions (Figure 3B).[4,60,61] In addition to GCN2, three other kinases can initiate the ISR in response to different types of stressors,[62] HRI activates the ISR upon heme deprivation and mitochondrial damage, while PERK when cells experience endoplasmic reticulum stress. PKR activates the ISR upon sensing of double-stranded RNA (dsRNA), which can arise as a consequence of UV-induced damage to nascent RNA.[30] All four kinases induce the ISR through phosphorylation of the

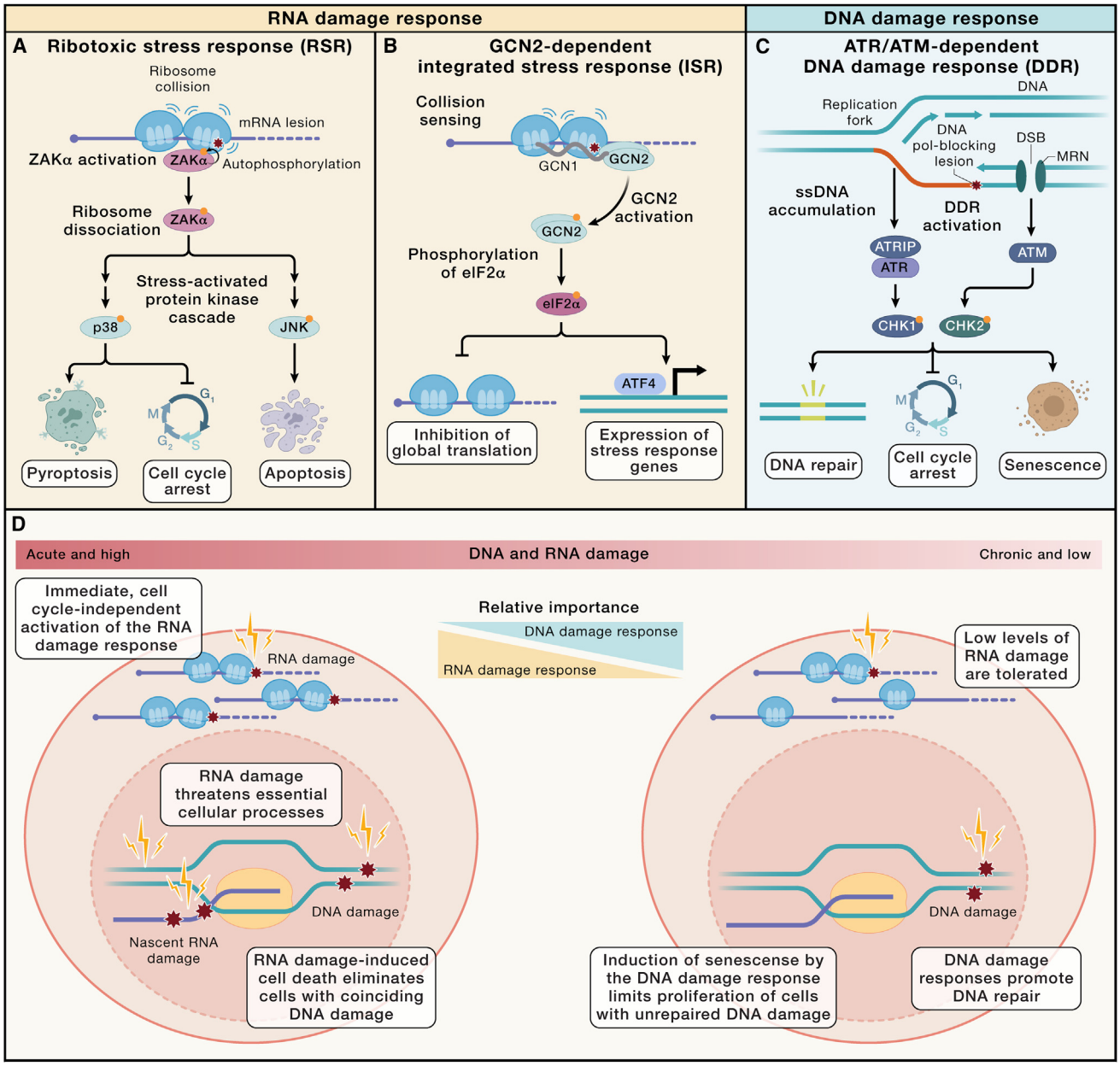

Figure 3. Signaling responses to RNA and DNA damage

(A) mRNA lesions induce ribosome collisions, which in turn activate the MAP3K ZAKα, triggering the ribotoxic stress response. Downstream of ZAKα activation, MAP kinases p38 and JNK trigger various cell fate decisions ranging from cell cycle arrest to cell death.

(B) RNA damage-induced ribosome collisions activate the kinase GCN2, downstream of the collision sensor protein GCN1. GCN2 phosphorylates eIF2α, thereby triggering the integrated stress response, which entails a global translation shutdown and a concurrent expression of specific stress response genes.

(C) Lesions within DNA activate DNA damage response kinases. ATR is activated by ssDNA, which is sensed by the ATR interaction partner ATRIP. ATM can be activated by the MRN complex that senses DNA double-strand breaks. ATR and ATM activate CHK1 and CHK2, respectively, promoting DNA repair, arresting the cell cycle, and potentially inducing senescence.

(D) The relative importance of the RNA or DNA damage response is determined by the intensity of damage. During acute and high doses of nucleic acid damage, the rapid sensing of RNA lesions by translating ribosomes activates the RNA damage response. During chronic and low doses of nucleic acid damage, the DNA damage response prevails, promoting DNA repair or inducing senescence to prevent the proliferation of cells with unrepaired DNA lesions.

α-subunit of translation initiation factor eIF2 (eIF2α). As a result, global translation shuts down, while translation of ISR transcription factors, like ATF4, is induced via de-repression of an inhibitory uORF, steering gene expression toward stress response genes.[63–65] GCN2 phosphorylates eIF2α not only by sensing uncharged tRNAs during starvation[66,67] but also upon induction of mRNA damage.[4,12,68] Like ZAKα, GCN2 is activated by ribosome collisions, which occur after mRNA damage.[4,60] Activation

requires the sensor protein GCN1 that binds to collided ribosomes, promoting recruitment and activation of GCN2,[69] which is further enhanced by its binding to the ribosomal P-stalk.[70,71] GCN2 provides cells with resistance to UV and limits ribosome collisions and, thus, RSR activation under stress conditions.[5,72] While the ISR is generally considered to be protective, it can lead to expression of pro-apoptotic factors and ultimately cell death.[49,62]

INTEGRATION OF DNA AND RNA DAMAGE RESPONSES

Research on the DDR was conducted over decades under the assumption that it is primarily the response to DNA lesions that determines survival when cells are exposed to genotoxic agents. Here, we integrate the resulting paradigms with emerging insights on the RNA damage response.

DNA lesions activate a sophisticated DDR network that orchestrates cellular DNA repair activities, cell cycle checkpoints, and survival decisions in response to DNA damage (Figure 3C). The DDR is initiated by members of the phosphoinositide-3-kinase-related protein kinase (PIKK) family.[73] Ataxia-telangiectasia and Rad3-related kinase (ATR) is recruited by its interaction partner ATRIP to single-stranded DNA (ssDNA) that accumulates when replication forks encounter lesions in template DNA.[74] By contrast, ataxia-telangiectasia mutated (ATM) is recruited to DNA double-strand breaks (DSBs) via the MRE11-RAD50-NBS1 (MRN) complex, which senses DNA ends.[75] Upon activation of ATM or ATR, downstream phosphorylation cascades are initiated by the transducer kinases CHK2 or CHK1, respectively.[76,77] The subsequent phosphorylation of CDC25 phosphatases prevents dephosphorylation and activation of CDKs.[78] Additionally, ATM and CHK2 both phosphorylate and stabilize p53, leading to p21 expression, which also inhibits CDKs.[79] As a consequence, cells arrest at the G1/S or G2/M transitions of the cell cycle and thereby provide time for DNA repair to occur.[80] If cells fail to repair DNA lesions during this transient cell cycle arrest or if they are exposed to extensive amounts of damage, persistent CHK2 activation will lead to continuous expression of p21, which ultimately induces cellular senescence.[81,82] In parallel, p53 stabilization leads to transcription of pro-apoptotic target genes, resulting in permeabilization of the mitochondrial outer membrane, thereby promoting apoptosis.[83] Accordingly, it is often discussed in the literature that persistent activation of the DDR leads to cell death, but experimental data supporting this notion are sparse.

The canonical understanding of the DDR was challenged by the observation that loss of ZAKα, and thus the RSR, leads to resistance to high doses of UV irradiation.[5] This observation suggests that upon acute exposure to UV, apoptosis is primarily driven by the induction of RNA damage and not DNA damage. By contrast, loss of ATM or ATR activity leads to severe sensitivity to various genotoxic agents,[84,85] highlighting fundamental differences in how both signaling networks determine the fate of cells experiencing complex nucleic acid damage. The DDR is important to prevent cell cycle progression before DNA repair has been completed. In the absence of DDR activation, cells initially continue to proliferate even in the presence of unrepaired DNA lesions, potentially leading to genetic aberrations and loss of chromosomes. While in most cells this will result in a reduction of fitness, the associated genetic changes may cause malignant transformation of individual cells. In addition to promoting DNA repair in the first instance, the DDR further prevents tumorigenesis by inducing cellular senescence if DNA repair is not successful.[86,87] In mice with reduced DNA repair capacity or increased endogenous DNA damage, DDR-induced senescence can lead to tissue dysfunction, which can be suppressed by deletion of p53.[23,88] However, and in line with the tumor-suppressive role of the DDR, p53 loss does not suppress the underlying genome instability and can therefore lead to an increased cancer burden.[23] In general, it thus appears that the role of the DDR is particularly prominent when cells experience low dose or chronic DNA damage (Figure 3D). The response to RNA damage, on the other hand, seems to dominate in situations of acute and severe damage.[5,55] Such damage can be efficiently and rapidly sensed by translating ribosomes independently of cell cycle status. The resulting activation of ISR and RSR elicits an immediate stress response that ensures the recovery and continuation of essential cellular functions. Moreover, RNA damage likely serves as the proverbial canary in the coalmine that alerts cells to the presence of coinciding DNA damage. The induction of apoptosis by continuous activation of the ZAKα-dependent RSR in response to persistent RNA damage is thus an efficient strategy to limit the proliferation of cells that also bear substantial amounts of potentially tumorigenic DNA lesions, independently of p53 or cell cycle status. In agreement with this idea, the off-target inhibition of ZAKα-induced cell death by certain cancer drugs has been linked to secondary UV-induced tumorigenesis and cutaneous squamous cell carcinoma.[89]

In addition to the parallel operation of DNA and RNA damage responses, the sensing of DNA lesions in the nucleus can indirectly trigger ISR and RSR activation. This crosstalk is mediated by the tRNA endoribonuclease SLFN11[90] that responds to DNA damage (Figure 4). While the precise signal for SLFN11 activation remains elusive, it is likely activated by ssDNA that accumulates in the presence of DNA damage.[91] Once activated, SLFN11 specifically cleaves nascent and mature leucine tRNAs recognizing UUA codons.[90,92] The resulting depletion of tRNA-Leu-UUA leads to ribosome stalling on the corresponding codons and ensuing ribosome collisions. The subsequent GCN2-dependent activation of the ISR leads to a global translation shutdown, while the concurrent ZAKα-dependent RSR activation triggers p53-independent apoptosis.[90] Its ability to trigger apoptosis in response to DNA damage makes SLFN11 a key determinant of cellular sensitivity to DNA-damaging agents such as camptothecin or cisplatin.[93] Consequentially, SLFN11 expression strongly correlates with the response of cancer patients to treatment with camptothecin- or cisplatin-derivatives and is often lost in tumors that do not respond to chemotherapy.[94]

Collectively, these insights paint a picture of a highly interconnected nucleic acid damage response that integrates information from networks that respond to DNA and RNA damage to inform cellular survival decisions.

SEQUESTRATION OF RNA DAMAGE

A key function of the DDR is to facilitate efficient DNA repair, for example through direct reversal or lesion excision.[95,96] While DNA must be repaired to preserve genetic information, cells

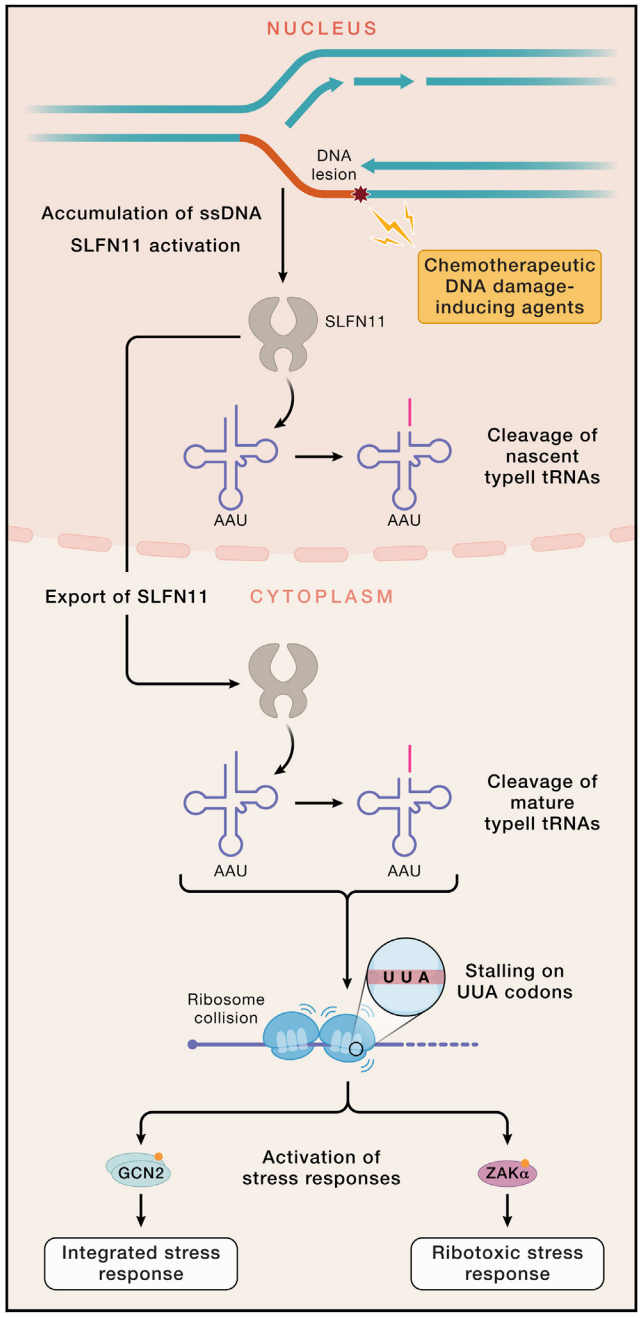

Figure 4. DNA damage-induced activation of translation-coupled stress responses

The tRNA endoribonuclease SLFN11 links DNA damage responses with activation of RNA damage response pathways. ssDNA that accumulates because of DNA damage activates SLFN11, which then specifically cleaves tRNAs-UUA-Leu in the nucleus and the cytoplasm. The lack of corresponding tRNAs leads to the stalling of ribosomes at UUA codons, inducing ribosome collisions and subsequent activation of the ribotoxic stress response and integrated stress response.

have a wider range of options to deal with damaged RNAs to prevent the disruption of crucial cellular processes such as splicing or translation (Figure 5).

The toxicity of damaged mRNA molecules can be limited through their sequestration. mRNAs damaged by alkylation or oxidation are sequestered in membrane-less organelles such as stress granules or P-bodies.[97,98] These granules contain non-translated mRNAs and RNA-binding proteins and form in cells that experience various types of stresses. Unique cytoplasmic RNA damage-induced stress granules appear upon UV irradiation (Figure 5A).[30] These granules contain primarily mis-spliced nascent mRNAs and dsRNA species and are marked by the presence of the RNA helicase DHX9. Interestingly, DHX9-positive granules form predominantly in daughter cells of UV-irradiated parental cells following mitosis. In the daughter cells, sequestration by DHX9 is important to prevent an innate immune response against cytosolic dsRNA.[30] Even in the absence of exogenously induced RNA damage, DHX9 deficiency leads to the accumulation of cytosolic dsRNA and innate immune responses,[99] presumably caused by the accumulation of endogenous RNA damage.

In addition to preventing the aberrant activation of immune sensors, sequestrating damaged RNA in stress granules may help to prevent the translation of problematic mRNAs. The sequestration of oxidized mRNAs is likely promoted by RNA-binding proteins such as YB-1 that binds 8-oxoG-containing RNA[100] and has been shown to initiate the formation of stress granules in response to arsenite-induced oxidative stress.[101] Interestingly, RNA enriched in these stress granules is highly modified with m^6A and m^7G.[102–105] Reader proteins for both modifications are essential for the formation of stress granules[102–104,106] and to target correspondingly modified mRNAs to these compartments.[103,106] Given that UV irradiation induces m^6A[107] and alkylating agents induce m^7G,[98] it seems plausible that these modifications play a general role in regulating the sequestration of damaged RNAs. Indeed, sodium arsenite-induced m^7G modifications target RNAs to stress granules.[105] Once formed, stress granules act as a triage center for mRNA. Sequestered mRNAs can in principle be transferred back into the cytosol and reused for translation but can also be degraded via the autophagy pathway.[30,108]

RESOLUTION OF mRNA DAMAGE

Degradation is a straightforward strategy to dispose of damaged mRNAs. Cells have multiple mRNA surveillance and quality control mechanisms at their disposal that can degrade aberrant transcripts. Historically, defined model substrates containing secondary structures, truncations, premature stop codons, or lacking stop codons have been used to study mRNA degradation.[109] Notably, however, the recognition principles of all three major mRNA quality control pathways—nonsense-mediated decay (NMD), non-stop decay (NSD), and no-go decay (NGD)—are all perfectly suited to also recognize damaged RNAs.[109,110,111] NMD responds to premature stop codons, which can arise from aberrant splicing events, which in turn can be a consequence of UV crosslinking damage on nuclear pre-mRNAs.[30] NSD is initiated when ribosomes reach the far 3′-end of an mRNA, which can happen due to the lack of a stop codon but also upon induction of RNA breaks.[112] Finally, NGD is activated when roadblocks within mRNA, such as strong

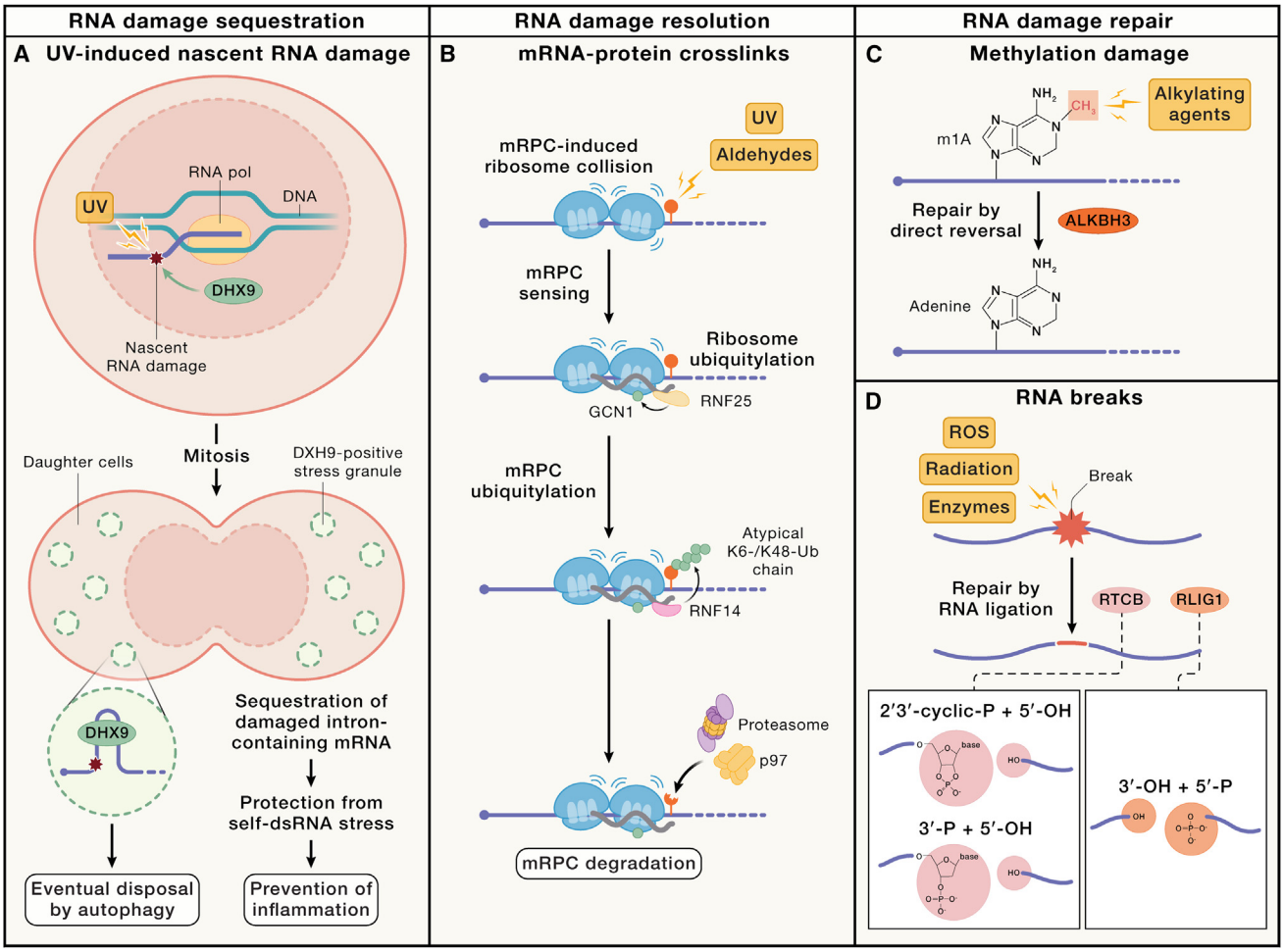

Figure 5. Cellular processes mitigating the toxicity of RNA damage

(A) The toxicity of UV-induced nascent RNA damage is limited by sequestration into DHX9-positive stress granules. The sequestration of damaged RNAs prevents the accumulation of cytoplasmic dsRNA after mitosis and averts activation of inflammatory responses in daughter cells.

(B) UV- and aldehyde-induced mRNA-protein crosslinks (mRPCs) are resolved in a translation-coupled manner. Stalling and subsequent collisions of ribosomes at mRPCs are sensed by the collision sensor protein GCN1. E3 ligase RNF25 is recruited to ubiquitylate ribosomal proteins, followed by ubiquitylation of the mRPC by E3 ligase RNF14. The modification with atypical K6-/K48-linked ubiquitin chains then triggers proteasomal degradation of the adduct, supported by the ubiquitin-dependent segregase p97.

(C) RNA methylation damage caused by alkylating agents can be directly reverted by dioxygenase ALKBH3.

(D) RNA breaks can be repaired by dedicated RNA repair ligases. RTCB ligase repairs RNAs bearing 5′-hydroxyl and 2′,3′-cyclic phosphate or 3′-phosphate termini. RLIG1 promotes ligation of clean 5′-phosphate and 3′-hydroxyl RNA ends.

hairpin structures, prevent ribosomal progression.[113] As such, NGD is likely activated by the multitude of RNA lesions that stall ribosomes. Indeed, NGD has a critical role in clearing mRNAs damaged by ROS or alkylation.[42] All three pathways are cytoplasmic and rely on translating ribosomes to sense faulty mRNAs and ultimately lead to exosome- or XRN1-mediated RNA decay.[110] In the nucleus, un-spliced and incorrectly adenylated transcripts can be targeted by exosome- and XRN2-mediated decay.[114]

In conjunction with translation-coupled decay of faulty mRNAs, the partially synthesized nascent peptide chains must be degraded, which is accomplished through ribosome-associated quality control (RQC). This pathway is initiated by the binding of the ubiquitin E3 ligase ZNF598 (Hel2 in yeast) to an interface formed between two collided ribosomes, leading to the subsequent ubiquitylation of ribosomal proteins eS10, uS10, and uS3.[115–118] In a second step, the affected ribosomes are split,[119–121] and the released nascent polypeptide is degraded by the proteasome, depending on ubiquitylation by the ubiquitin E3 ligase Listerin (Ltn1).[122–124]

In addition, a pathway has recently been identified that resolves covalent mRPCs that arise upon treatment of cells with UV irradiation or metabolic aldehydes (Figure 5B).[11,12] This quality control process is initiated when elongating ribosomes encounter covalent protein adducts within the coding regions of mRNA. The crosslinked protein stalls the elongating ribosome, eventually causing collisions with trailing ribosomes. While these collisions activate the ISR and RSR in a GCN2- and

ZAKα-dependent manner, respectively, they also promote the proteolytic degradation of the crosslinked protein. To this end, the collision sensor GCN1 recruits two ubiquitin E3 ligases. One of these, RNF25, ubiquitylates specific lysine residues on the ribosome, including lysine residues on eS31.[125,126] This modification appears to be important to support the function of the second recruited E3, RNF14.[125,126] RNF14 modifies the crosslinked protein with atypical K6- and K48-linked ubiquitin chains.[11,12] Of note, RNF14 does not only target mRPCs but also entrapped translation factors.[125,126] Ubiquitylation targets the crosslinked protein for proteasomal degradation, which is in addition supported by the ubiquitin-dependent segregase p97.[11] However, the fate of the damaged mRNA following degradation of the protein adduct remains currently unclear.

RESOLUTION OF rRNA AND tRNA DAMAGE

In addition to serving as sensors for mRNA damage, ribosomes can be subjected to damage themselves. rRNA damage disrupts ribosome function and ensuing impairment of protein homeostasis, necessitating distinct quality control pathways, collectively termed non-functional rRNA decay (NRD). NRD was initially discovered in yeast and resolves aberrant rRNA, ensuring that only properly assembled and fully functional ribosomes participate in translation. Two conceptually different pathways are known for NRD: ribosomal 40S subunits with damage to their decoding site in the 18S rRNA can still fully assemble and translate but lead to ribosome stalling and initiate NRD in a translation-coupled manner.[127–129] Ribosomal protein uS3 is ubiquitylated, triggering the subsequent dissociation of the faulty small subunit and elimination of the problematic 18S rRNA.[130,131] NRD in mammalian cells appears to rely on the GCN2-dependent ISR to promote 18S rRNA degradation.[132] Additionally, translation-coupled ubiquitylation of uS3 and uS5 by RNF10 is important to promote 40S subunit degradation.[133,134] By contrast, the resolution of defective 60S subunits containing non-functional 25S rRNA is translation-independent, as indicated by experiments in yeast.[128] Aberrant 25S rRNA is cleared by exosome-mediated degradation in the cytoplasm,[128] while defective 60S ribosomal particles are targeted by proteasomal degradation to prevent assembly of faulty 80S ribosomes.[135,136]

While tRNA damage has been investigated only scantly, research on the consequences of defective tRNA modifications in yeast provides insights into the principles of cellular tRNA quality control. tRNAs undergo diverse modifications that are critical for their stability and function. Disrupting these modifications leads to the elimination of tRNAs through the rapid-tRNA decay (RTD) pathway involving the cytosolic exonuclease Xrn1 and nuclear exonuclease Rat1.[137,138] In the nucleus, abnormal pre-tRNAs are degraded upon polyadenylation by the TRAMP complex followed by exosome-mediated decay.[139]

REPAIR OF RNA DAMAGE

Degrading damaged RNA molecules is an effective strategy to prevent the toxic consequences of RNA lesions, but it results in the irreversible loss of the affected RNA. By contrast, repairing damaged RNA molecules would eliminate the need for their energetically expensive resynthesis, reducing the metabolic burden required for transcriptome maintenance and ribosome biogenesis.

Indeed, viral and human nucleic acid dioxygenases of the AlkB family have been proposed to repair alkylated RNA molecules (Figure 5C).[140,141] ALKBH3 directly reverts methylated bases in single-stranded RNA,[141,142] thereby preventing translation defects upon cellular exposure to methylating agents.[143] While the precise regulation of ALKBH3-dependent RNA repair is not well understood, it has been linked to the stalling of ribosomes at the lesion and their subsequent splitting by the helicase ASCC3 during RQC.[98] Interestingly, ALKBH3-dependent DNA repair also depends on ASCC3 and is tightly connected to RNA damage. ALKBH3 repairs DNA in nuclear speckles, requiring the activity of the ubiquitin E3 ligase RNF113A.[144] RNF113A is activated upon binding to methylated RNA, which is required for the formation of nuclear ASCC3-ALKBH3 foci and thus repair of methylated DNA.[145] Hence, efficient DNA repair can be linked to the presence of coinciding proximal RNA damage.

In addition to direct reversal, damaged RNA bases can be excised by lesion-specific glycosylases. Single-strand-selective monofunctional uracil-DNA glycosylase 1 (SMUG1) targets not only 5-hydroxymethyluracil in DNA but can also act on RNA, such as the RNA component of telomerase, hTERC.[146] In addition to its role in RNA processing for telomere maintenance, SMUG1 is localized to the nucleoli—the location of rRNA synthesis—and its depletion leads to increased amounts of 5-hydroxymethyluracil in 28S and 18S rRNA and reduced amounts of mature rRNA, underscoring an essential role in rRNA quality control.[147] Base excision by SMUG1 yields abasic sites in RNA. In DNA, abasic sites are further processed by the endonuclease APE1, enabling repair by the DNA single-strand break repair machinery. APE1 has been reported to also process abasic sites in RNA,[148] but the relevance of this reaction has not been investigated in eukaryotes. Interestingly, however, a recent study in bacteria indicated that the ribosomal protein Rps3 acts as an endonuclease that incises abasic sites within mRNAs.[149] While this activity appears to protect cells from oxidative and UV-induced stress,[149] the fate of the resulting RNA break remains uncertain.

Breaks in RNA can indeed be repaired (Figure 5D). The Hen1-Pnkp heterotetramer repairs ribotoxin-induced tRNA breaks in bacteria by religating cleaved tRNA termini.[150] This process entails the transfer of a methyl group to the 2′-hydroxyl group of the cleaved tRNA, which protects against re-cleavage by the ribotoxin.[151] The RNA ligase Rtcb similarly ligates rRNA and tRNA breaks bearing 5′-hydroxyl and 2′,3′-cyclic phosphate or 3′-phosphate termini.[152–155] The same enzymatic activity is employed by the mammalian homolog RTCB during the unconventional splicing of XBP1-mRNA upon induction of ER stress[156,157] and to repair RNA breaks induced by a CRISPR-based RNA editing system.[158] However, given that this type of repair requires specific RNA termini, it is unlikely that it constitutes a general RNA break repair system. Intriguingly, however, RLIG1 was recently identified as the first human RNA ligase that promotes ligation of clean 5′-phosphate and 3′-hydroxyl RNA ends, protecting cells against the toxic effect of oxidative RNA damage.[13]

This discovery indicates the presence of an RNA break repair pathway in mammals. Understanding how RNA breaks would be positioned by such a system to ensure faithful repair remains a critical question. It seems plausible that ligation-dependent repair of RNA breaks would be particularly suited for repairing highly structured RNAs, such as tRNAs, in which the affected termini are likely to be positioned correctly due to the inherent folding of the molecule. Consistent with this idea, RLIG1 loss is associated with aberrant tRNA levels in mouse brains.[159]

How damaged RNAs are sensed independently of translation, the choice between degradation and repair of damaged RNAs, and how both processes are regulated in physiological and pathological situations are important future questions.

RNA DAMAGE RESPONSES IN HUMAN DISEASE

The existence of complex RNA damage resolution and repair pathways highlights the importance of minimizing the toxic effects of RNA lesions. Hence, it is not surprising that RNA damage and the associated cellular response are closely connected to a range of human diseases.

The ZAKα-dependent activation of the RSR at stalled and collided ribosomes is a key nexus that integrates various pathological conditions caused by compromised RNA integrity. UVB irradiation drives the expression of proinflammatory genes in skin keratinocytes,[160] which express the innate immune sensor NLRP1. ZAKα and p38 phosphorylate NLRP1 in response to UVB-induced RNA damage.[9,161] The phosphorylation of NLRP1 promotes inflammasome formation, triggering proinflammatory signaling and pyroptosis upon acute sunburn[9] (Figure 6A). As a consequence, patients with *NLRP1* gain-of-function mutations are susceptible to inflammatory skin disorders and skin cancers.[162] Of note, ZAKα-dependent activation of the RSR drives rapid dermal inflammation, skin thickening, and JNK-dependent apoptosis in response to UVB also in mice,[10], where NLRP1 is not subjected to p38-mediated activation.[9] Proinflammatory signaling and the development of autoimmune diseases also arise when components of the NMD pathway are defective,[163] but the identity of the causative damaged or otherwise aberrant RNA species has not been established. Nonetheless, RNA damage and the downstream signaling responses are emerging as highly relevant sources of endogenous inflammation.

In addition to driving proinflammatory signaling, persistent activation of the ZAKα-dependent RSR can lead to excessive cell death, affecting tissue integrity and organismal fitness. Consequently, loss of ZAKα activity in animal models slows the development of metabolic aging hallmarks caused by oxidative RNA damage.[8] Moreover, in a mouse model of high-fat, high-sugar diet-induced obesity, ROS-induced RSR activation promotes various metabolic dysfunctions, including blood glucose intolerance and liver steatosis.[8] In further agreement, loss of ZAKα leads to reduced adiposity and lower fat content in the liver,[164] implicating the RSR as a potential therapeutic target to treat obesity-related disorders (Figure 6B). Oxidative RNA damage may be particularly relevant in neurons, which are especially susceptible to oxidative stress due to their high metabolic needs.[165] Indeed, oxidized RNA molecules accumulate in patients with aging-related neurodegenerative diseases.[166–168] Furthermore, ribosome pausing and collisions—which can be caused by RNA damage—increase during aging, leading to the activation of RQC.[169] If cellular quality control becomes overwhelmed, proteostasis is disrupted, threatening all organismal functions.[169] Consequentially, loss of RQC factors, such as LTN1 or NEMF, leads to neurodegeneration and neuromuscular disease in mice.[170,171] However, the underlying sources of increased ribosome pausing and collisions during aging, and whether they include accumulation of RNA damage, have not been determined.

Induction of RNA damage is likely also responsible for at least some of the therapeutic effects of certain chemotherapies. Cancer cells rely on efficient translation to produce sufficient proteins to sustain their rapid growth rates. Therefore, targeting protein synthesis, particularly ribosome biogenesis, has been proposed as a potential treatment strategy.[172] In support, some chemotherapeutics traditionally considered to be genotoxic agents appear to kill cancer cells rather by their ability to disrupt ribosome biogenesis.[36] Oxaliplatin, a platinum-based agent that induces DNA and RNA crosslinking damage, disrupts nucleolar integrity, thereby indirectly impairing rRNA synthesis and ribosome biogenesis.[36,173] Similarly, the chemotherapeutic drug 5-fluorouracil (5-FU) causes cytotoxicity not only by inhibiting dNTP synthesis by targeting thymidylate synthase (TYMS),[174] but also by impairing ribosome biogenesis and protein translation upon incorporation into RNA.[37,175]

An intriguing additional possibility is that chemotherapy-induced RSR activation during cancer treatment may promote relapse and treatment resistance by promoting WGD. Approximately a third of all cancers undergo WGD, also known as endoreplication. WGD occurs due to mitotic bypass and genome duplication without cell division, resulting in a polyploid state.[176] Cancers with WGD tend to be more metastatic, drug-resistant, and have worse overall prognosis compared with non-WGD cancers[177] (Figure 6C). Persistent RSR signaling has the ability to induce WGD events by promoting premature G2 exit and mitotic bypass.[6]

In summary, RNA damage and the associated signaling responses are central to various pathological contexts, which also highlights the potential of targeting RNA damage response networks for improved and novel treatments.

CONCLUDING REMARKS

An efficient response to the various flavors of RNA damage is vital to maintain the diverse cellular functions of RNA molecules, some of which can be exceptionally long-lived.[178] Despite the fundamental importance of RNA, the mechanistic understanding of RNA damage sensing, resolution, and repair lags far behind our knowledge of DNA repair. How RNA damage response pathways, like the RSR and ISR, control cell fate following the induction of RNA lesions in physiological and pathological situations is an exciting future topic. A particularly interesting question is how the RNA damage response crosstalks with established DDR networks to regulate cell death, cell cycle progression, and inflammation in response

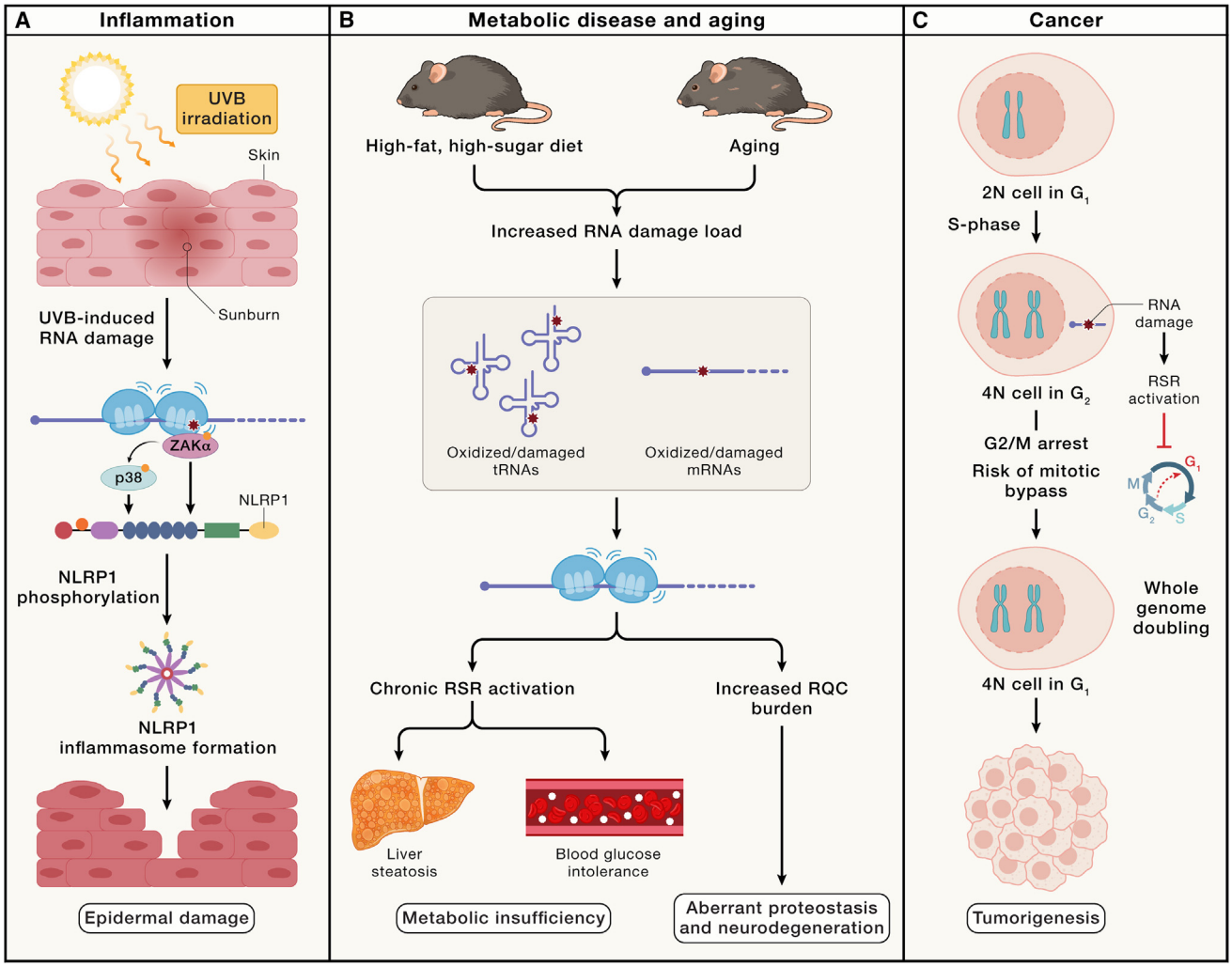

Figure 6. RNA damage and disease

(A) UVB-induced RNA damage activates the ribotoxic stress response in keratinocytes, resulting in phosphorylation of the skin-specific innate immune sensor protein NLRP1 by ZAKα and p38. NLRP1 phosphorylation promotes inflammasome formation, driving proinflammatory signaling and pyroptosis upon acute sunburn.

(B) Higher RNA damage load in metabolic diseases and aging induces ribosome stalling and subsequent collisions. The corresponding persistent activation of the RSR and the continuous overload of RQC likely contribute to metabolic insufficiency or aberrant proteostasis in metabolic diseases, neurodegeneration, and aging.

(C) RNA damage-induced ribosome collision leads to persistent activation of the RSR, which can result in cell cycle arrest, mitotic bypass, and whole-genome doubling events. Cancer cells with WGD are more metastatic, drug-resistant, and have an overall worse prognosis.

to genotoxic agents that induce both RNA and DNA damage. The pleiotropic nature of these agents makes it challenging to determine the specific cellular responses caused by RNA damage. Therefore, it will be critical to develop and deploy experimental systems that enable the specific induction of RNA damage, such as the mimicry of RNA crosslinking damage by metabolic labeling with 4-SU followed by photoactivation.[11,12] An important issue to be addressed using such methodologies is to what degree lesions within mRNA, rRNA, tRNA, and other non-coding RNAs contribute to the cellular RNA damage response. Extending our knowledge of translation-dependent RNA damage responses and revealing yet unknown translation-independent mechanisms will improve our understanding of various human diseases and provide opportunities for novel therapeutic strategies.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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