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REVIEW

N4-acetylcytidine and other RNA modifications in epitranscriptome: insight into DNA repair and cancer development

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ABSTRACT

N4-acetylcytidine (ac4C) is a post-transcriptional RNA modification that plays a crucial role in the epitranscriptome, influencing gene expression and cellular function. This modification occurs at the cytosine base, where an acetyl group is installed to the nitrogen at the 4th position (N4). This co-transcription modification affects RNA stability, RNA structure, and translation efficiency. Recent studies have uncovered a potential link between RNA modifications and DNA repair mechanisms, suggesting that ac4C-modified or methylated RNAs may interact with factors involved in DNA repair pathways; thus, influencing the cellular response to DNA damage. Dysregulation of modified RNAs, including ac4C RNA, has been implicated in cancer development, where aberrant levels of these RNAs may contribute to oncogenic transformation by altering genome stability and the expression of key genes regulating cell proliferation, cell cycle progression, and apoptosis. Understanding the dynamics of modified RNAs offers promising insights into the role of epitranscriptome in DNA repair processes and cancer treatment.

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N-acetylcytidine; RNA modifications; NAT10; DNA damage repair; epigenetics; epitranscriptomics

1. Introduction

Ribonucleic acid (RNA), is a vital molecule involved in various cellular processes, including coding, regulation, and expression of genes. One of the fascinating aspects of RNA biology is the presence of numerous chemical modifications that can influence its structure, stability, and function. Among these modifications, N4-acetylcytidine (ac4C) has garnered attention due to its role in enhancing the stability and functionality of RNA molecules. In human cells, the installation of ac4C in distinct types of RNA is regulated via the function of N-acetyltransferase 10 (NAT10) which is considered as eukaryotic lysine-specific acetyltransferase also regulating the function of microtubules, histones, and cell division. NAT10 is considered an important target in cancer therapy due to its overexpression in many tumor cells. Concerning the important role of NAT10 in the development of serious human diseases, here, we summarize the known information about the structure, nuclear distribution, and function of both the NAT10 acetyltransferase and N4-acetylcytidine modification in RNA. Also, in parallel with the function of ac4C RNA in gene expression regulation and DNA damage response, we discuss the role of methylated RNAs in these fundamental nuclear processes.

2. Structure of N4-acetylcytidine

N4-acetylcytidine (ac4C) is a modified nucleoside in which an acetyl group (–COCH3) is attached to the nitrogen atom at the fourth position of the cytidine base [1,2]. This modification alters the chemical properties of cytidine, affecting how RNA molecules interact with proteins and other nucleic acids. The acetyl group introduces a degree of bulkiness and polarity, which can impact RNA structure and function. The biosynthesis of ac4C is a multi-step enzymatic process [3]. It begins with the recognition of specific cytidine residues within RNA by enzymes, known as acetyltransferases. In eukaryotes, the enzyme responsible for this modification is N-acetyltransferase 10 (NAT10) [4,5]. NAT10 transfers an acetyl group from acetyl coenzyme A (acetyl-CoA) to the nitrogen atom at the fourth position of cytidine, forming ac4C [6]. This enzymatic modification is highly specific and ensures that ac4C is incorporated in precise regions within RNA molecules. It is known that the antisense sequence of short nucleolar RNA (snoRNA) is essential for NAT10 binding to the target sequence to be ac4C modified in 18S rRNA [5,7]. The ac4C modification of transfer RNA (tRNA), is mediated by THUMPD1 which binds to the tRNA and facilitates NAT10 functioning at the D-arm structure of tRNA (Ser) and tRNA (Leu) [5,8]. Besides tRNA and ribosomal RNA (rRNA), N4-acetylcytidine is found in various types of RNA, including messenger RNA (mRNA), and has also been identified in long non-coding RNA (lncRNA) [9,10]. In mRNA, ac4C is typically found in coding sequences and untranslated regions (5′ UTRs), where it can influence translation efficiency and mRNA stability [11].

3. Functional properties of N4-acetylcytidine in distinct types of RNA

One of the primary functions of ac4C is to enhance RNA stability. The acetyl group can protect RNA from degradation

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Article highlights

- **Key Role of N4-acetylcytidine (ac4C) in RNA Biology**
  - This modification occurs at the cytosine base, where an acetyl group is added to the nitrogen at the 4th position (N4).
  - N4-acetylcytidine (ac4C) is a pivotal post-transcriptional RNA modification that contributes significantly to the epitranscriptome.
- **Functional Impact of ac4C on RNA Stability and Translation**
  - ac4C enhances RNA stability and influences RNA structure, optimizing its functional integrity.
  - It improves translation efficiency, thereby playing a critical role in protein synthesis and cellular function.
- **Emerging Connections Between ac4C and DNA Repair**
  - Recent evidence suggests that ac4C-modified RNA interacts with key factors in DNA repair pathways.
  - These interactions may modulate cellular responses to DNA damage, linking RNA modification to genomic stability.
- **Implications of Dysregulated ac4C in Cancer**
  - Aberrant levels of ac4C in RNA have been associated with cancer development and progression.
  - Dysregulation contributes to oncogenesis by altering genome stability and affecting the expression of genes involved in cell proliferation, cycle control, and apoptosis.
- **Promising Avenues for Future Research**
  - Investigating the role of ac4C in epitranscriptomics offers potential insights into its dual roles in DNA repair mechanisms and cancer biology.
  - Understanding ac4C dynamics could pave the way for novel therapeutic strategies targeting this RNA modification in cancer and related conditions.

by nucleases, thereby increasing the half-life of the RNA molecules. In rRNA, ac4C contributes to the proper assembly and function of ribosomes [4,11]. In tRNA, ac4C is commonly located in the anticodon loop, playing a crucial role in accurate codon recognition and translation fidelity, and in lncRNA, for example, the NAT10-mediated ac4C modification promotes stabilization and increased expression. This phenomenon was observed in lncRNA CTC-490G23.2, studied in primary esophageal squamous cell carcinoma (ESCC) [9]. Structural and functional stability is also important for mRNA, as it allows for sustained translation and protein production [12]. By altering the local structure of the mRNA, ac4C can affect ribosome binding and movement along the mRNA, ultimately impacting the rate and fidelity of protein synthesis. To this fact, it was found that ac4C in such RNAs can promote both translation initiation and peptide chain elongation [11]. These observations contribute to the fact that translation is a precisely regulated cellular process, representing a final step of the transcription-translation signaling cascade leading to protein production. This central dogma of molecular biology ensures cellular homeostasis and proper response to changes in environmental conditions [13].

As mentioned above, ac4C is present in various types of RNA, with significant roles in all of them [3–5,9,14,15]. In detail, in mRNA, ac4C is found in both coding sequences and untranslated regions (UTRs) [11,14]. Its strategic installation can influence the translation process by modulating ribosome interaction and movement along the mRNA. In t-RNA, ac4C is predominantly located in the anticodon loop. This positioning is vital for the accurate pairing of tRNA with mRNA codons, ensuring fidelity of protein synthesis. Via ac4C modification, translation of the genetic information from mRNA to proteins is regulated in the following ways: ac4C in the anticodon loop stabilizes codon-anticodon interactions. This stabilization ensures that the correct amino acids are incorporated into the synthesizing polypeptide chain. Also, the presence of ac4C reduces the likelihood of translational errors, such as frame-shifting or misincorporation of amino acids, which prevents the formation of aberrant proteins [11,16]. Also, very important is the presence of ac4C in ribosomal RNA (rRNA). This post-transcription (co-transcription) modification contributes to the proper assembly and function of ribosomes, the molecular machines responsible for the final protein synthesis in the endoplasmic reticulum. In this case, ac4C can modulate the dynamics of ribosome movement along the mRNA. It may influence pause sites where the ribosome temporarily halts, affecting the elongation phase of translation. These pauses can be crucial for the co-translational folding of proteins or the recruitment of specific chaperones. The ac4C modification can also impact translation termination by affecting the interaction of release factors with the mRNA. Efficient termination is essential for proper protein synthesis and to prevent the production of incomplete or aberrant proteins. During stress conditions, such as heat shock or nutrient deprivation, the modification pattern of RNA can change, leading to the selective translation of stress-response proteins [17–19].

4. DNA damage response and repair

DNA damage can occur due to various internal and external factors, such as the above-mentioned stress conditions, environmental agents (UV radiation, chemical pollution), replication errors, or oxidative stress. If not repaired, DNA damage can lead to mutations causing cancer, or cell death. To counteract this, cells have evolved a sophisticated DNA Damage Response (DDR) system that detects and repairs damaged DNA, maintaining genomic integrity. According to the type of DNA lesions, several repair mechanisms develop, including base excision repair (BER) recognizing Single-Strand Breaks (SSBs) presenting in one strand of the DNA helix. These breaks are generally less harmful and are repaired by this pathway (Figure 1(a)). In the damaged genome, there is the existence of other DNA lesions, including base modifications, representing chemical changes to the DNA bases, such as methylation or oxidation. These chemical changes can also be repaired by the BER mechanism. On the other hand, crosslinks between DNA strands can block replication and transcription. These are repaired by Nucleotide Excision Repair (NER) (Figure 1(b)) or Homologous Recombination (HR). Another repair mechanism, called Mismatch Repair (MMR) corrects base-pairing mismatches that occur during DNA replication (Figure 1(c)). In the genome, the most deleterious are Double-Strand Breaks (DSBs) in both strands of the DNA helix. These DNA lesions are repaired by Non-Homologous End Joining (NHEJ) or HR repair pathways (Figure 1(d)). In these cases, proteins like ATM (Ataxia-Telangiectasia Mutated) and ATR (ATM and Rad3-Related) act as sensors for DNA damage. ATM is activated primarily by DSBs, while ATR responds to a variety of DNA

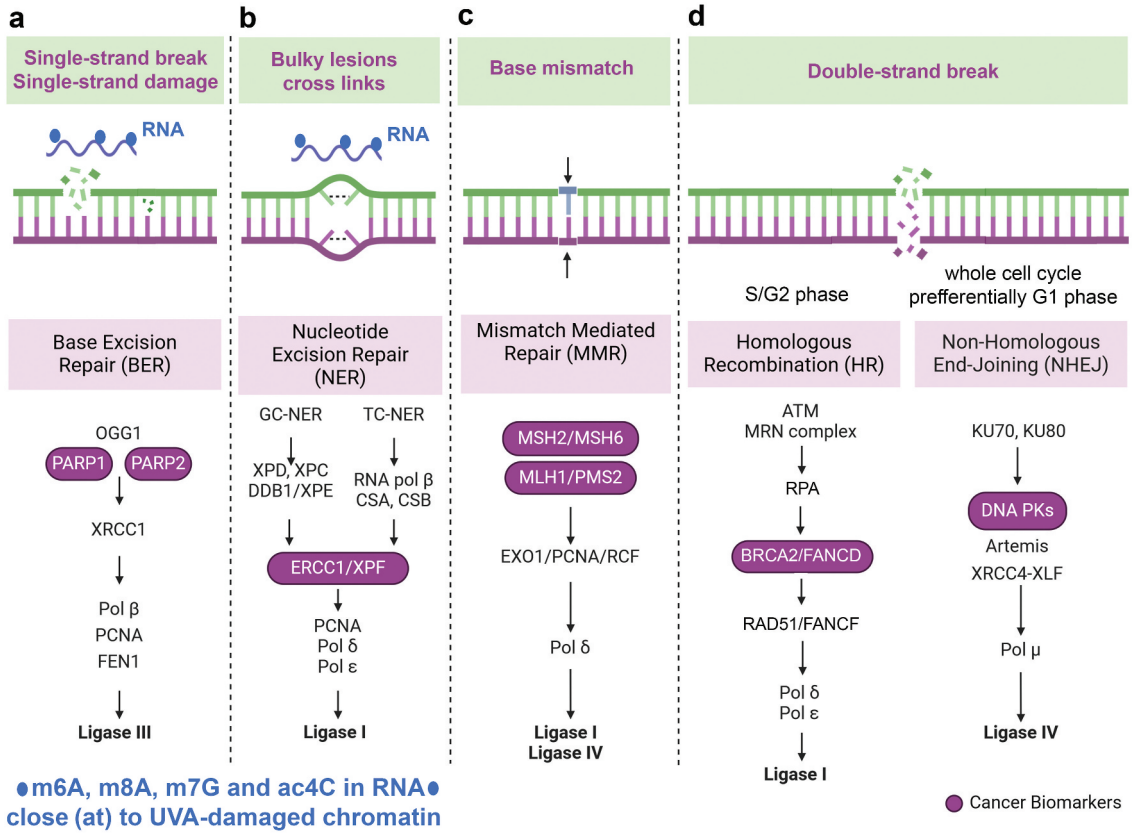

Figure 1. DNA damaged response pathways. panels show (a) Single-strand break/single-strand DNA damage – the Base Excision Repair (BER) mechanism, (b) Bulky lesions cross-links – the Nucleotide Excision Repair (NER) pathway, (c) Base mismatch – the mismatch Mediated Repair (MMR), (d) Double strand breaks repair - homologous recombination (HR) or non-homologous end joining (NHEJ) repair pathways. BioRender template was adopted. Pictorials were added in panels A and B showing RNA modifications contributing to DDR. Created with BioRender.com.

lesions, especially those caused by replication stress [20,21]. Once DNA damage is detected, a cascade of phosphorylation occurs, which is mediated by ATM, ATR, and DNA-PK (DNA-Dependent Protein Kinase). These kinases activate downstream effectors like the checkpoint kinases CHK1 and CHK2, which halt the cell cycle, allowing time for repair [22]. It is known that the cell cycle is arrested at specific checkpoints (G1/S, intra-S, or G2/M) to prevent the propagation of damaged DNA. In this case, the p53 protein, a critical tumor suppressor, plays a central role by enforcing cell cycle arrest, especially at the G1/S checkpoint [23]. Cell cycle phases also decide which DDR pathway will be activated. It applies especially to the NHEJ pathway which is an error-prone process, not requiring a homologous DNA template, but HR represents a high-fidelity repair mechanism for DSBs that uses a sister chromatid as a template. It is known that HR is restricted to the S and G2 phases of the cell cycle when a sister chromatid is available, while NHEJ preferentially appears in the G1 phase [24]. Defects in DNA repair mechanisms are associated with various diseases, most notably cancer [25]. For example, mutations in crucial DNA repair factors, BRCA1 and BRCA2, increase the risk of breast and ovarian cancers. Understanding DDR and repair pathways has led to the development of targeted therapies, such as PARP inhibitors. The use of these chemotherapeutic drugs is a promising strategy because many DNA repair pathways are PARP-dependent [26]. Thus, continued research in this field holds promises for advancing cancer therapies, with less DNA damaging effects.

5. Overview of nucleotide excision repair

NER pathway is a highly conserved DNA repair mechanism that detects and removes a wide range of DNA lesions, including those caused by UV radiation, such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6-4PPs) [27]. NER operates through a cut-and-patch process that excises a short single-stranded DNA segment containing the lesion and then fills the gap with newly synthesized DNA. NER is broadly divided into two sub-pathways: Global Genome NER (GG-NER) which operates throughout the genome, scanning and repairing lesions on both transcribed and non-transcribed DNA. The second mechanism is the Transcription-Coupled NER (TC-NER) that specifically targets lesions on the transcribed strand of active genes, ensuring that transcription can proceed unhindered. In the case of GG-NER, the XPC (xeroderma pigmentosum, complementation group C) complex, often in conjunction with RAD23B and CETN2, is primarily responsible for detecting DNA distortions across the genome. However, XPC does not directly recognize the lesion itself; instead, it senses the helical distortion or destabilized DNA caused by the lesion [28]. Phosphorylation of XPC at serine 94, mediated by casein kinase II (CK2), regulates its recruitment and ubiquitination, underscoring a post-translational mechanism essential for repairing UV-induced DNA damage [29]. TC-NER is initiated when RNA polymerase II stalls at a lesion during transcription. Stalling triggers the recruitment of the CSA (Cockayne syndrome A) and CSB (Cockayne syndrome B)

proteins, which displace RNA polymerase and attract other NER factors to the site of damage [30] (Figure 1(b)). The accessibility of DNA in chromatin can affect the efficiency of NER. Chromatin remodelers and histone modifications play a role in making DNA lesions accessible to NER machinery. NER activity is modulated throughout the cell cycle, with certain phases (e.g., G1 and G2) showing higher repair activity due to the increased time available for repair before DNA replication or mitosis. NER is essential for maintaining genomic stability and preventing mutations. Defects in NER can lead to several genetic disorders, including Xeroderma Pigmentosum (XP) which is a rare autosomal recessive disorder caused by mutations in NER genes (e.g., XPC, XPA, XPB, XPD). Patients with XP exhibit extreme sensitivity to UV light and a high predisposition to skin cancers due to defective GG-NER. Another autosomal recessive disorder represents Cockayne Syndrome (CS) which is characterized by growth retardation, neurological defects, and photosensitivity and arises from mutations in TC-NER genes (e.g., CSA, CSB). Unlike XP disease, CS is not associated with an increased cancer risk. Also, trichothiodystrophy (TTD) is a disease characterized by brittle hair, intellectual impairment, and photosensitivity, often resulting from mutations in the XPB, XPD, or TTDA genes, which are components of the TFIIH complex [31].

The understanding of NER has significant implications for cancer therapy [32,33]. From the view of chemotherapy resistance, cancer cells with proficient NER can repair the DNA damage caused by chemotherapeutic agents like cisplatin, leading to drug resistance. Targeting NER pathways, or using selective inhibitors that block specific NER components, is a strategy under investigation to sensitize tumors to therapy. Tumor cells with defective NER (e.g., due to mutations in XP genes) may be particularly sensitive to agents that induce bulky DNA lesions. Exploiting these vulnerabilities can lead to more effective and personalized cancer treatments. Based on the mentioned facts, the investigation of how NER efficiency changes with age and contributes to age-related diseases is essential [34]. Understanding how environmental factors impact NER activity and contribute to carcinogenesis, as well as investigating the regulation of NER during development and across various cell types, could uncover tissue-specific vulnerabilities in radiotherapy and chemotherapy. This knowledge may pave the way for more targeted therapeutic strategies.

6. A short overview of the base excision repair (BER): the essential mechanism for DNA damage repair

DNA is constantly under threat from various endogenous and exogenous agents that cause damage to its structure. One of the most frequent types of damage results from spontaneous base modifications, oxidative stress, and environmental factors, leading to small, non-helix-distorting lesions. It is known that the BER pathway is essential for ensuring genome stability and preventing mutations that could lead to cancer and other diseases [35] (Figure 1(a)). BER is a highly conserved cellular process responsible for the repair of small lesions in DNA, such as oxidized bases (e.g., 8-oxoguanine), deaminated bases (e.g., uracil resulting from cytosine deamination), alkylated bases (e.g., 3-methyladenine), and single-strand breaks. These lesions can arise from oxidative stress, and radiation and can be caused by metabolic byproducts. If unrepaired, they can cause mutations, transcriptional blocks, or even double-strand breaks. BER acts to exercise the damaged base and restore the correct nucleotide sequence [36]. BER can be divided into two sub-pathways: short-patch repair and long-patch repair [35]. Both involve a sequence of coordinated steps to remove damaged bases and restore DNA integrity. The first step in BER is the recognition of the damaged base by DNA glycosylases. These enzymes are specialized to detect specific types of damaged bases and cleave the N-glycosidic bond, releasing the damaged base and leaving behind an abasic site (AP site; apurinic/apyrimidinic site). There are various glycosylases, each recognizing specific lesions [37]. Examples include OGG1 (8-oxoguanine DNA glycosylase). Also, the BER mechanism removes oxidized guanine bases, including UNG (Uracil-DNA glycosylase). In this case, excises uracil residues that result from cytosine deamination or detects and excises adenines misincorporated opposite 8-oxoguanine. Once the damaged base is removed, the glycosylase leaves an AP site, marking the next step in the repair process [35].

After base excision, an apurinic/apyrimidinic (AP) endonuclease, such as APE1, cleaves the phosphodiester bond at the AP site [38]. This incision creates a single-strand break, with a deoxyribose phosphate (dRP) residue that must be removed before DNA synthesis can proceed. The next step involves the removal of the dRP residue, which is crucial for proper repair. This is typically carried out by DNA polymerase β (Pol β), a key enzyme in BER [39]. Pol β has both dRP lyase activity, which removes the sugar-phosphate backbone at the AP site, and polymerase activity, which fills in the gap with the correct nucleotide. The final step in BER depends on whether the repair involves short-patch or long-patch pathways. For instance, short-patch BER involves the incorporation of a single nucleotide by Pol β, which fills the gap left after dRP removal. And long-patch BER, in some cases, Pol β or other polymerases (e.g., Pol δ or Pol ε) synthesize a short stretch of 2–10 nucleotides. This requires Flap Endonuclease 1 (FEN1) to remove the displaced DNA flap [40]. The repair of the damaged genome is completed by DNA ligase I or DNA ligase III, which seals the nick in the DNA backbone, restoring the integrity of the DNA strand [41].

Besides the above-mentioned factors, the BER pathway is regulated by post-translational modifications. Enzymes involved in BER, such as Pol β and APE1, undergo phosphorylation, acetylation, and ubiquitination to modulate their activity. Also, BER proteins interact with each other and with proteins involved in other DNA repair pathways, such as nucleotide excision repair (NER) and mismatch repair (MMR). Thus, the response to different types of DNA damage is highly coordinated. Also, chromatin remodeling factors contribute to the access of repair proteins to DNA in the context of global chromatin. Chromatin remodelers and histone modifications help open chromatin regions around damaged sites, facilitating repair enzyme recruitment

[42,43]. Worth mentioning is also the fact that the BER pathway is PARP dependent and many proteins, including XRCC1 or PCNA, contribute to the efficiency of this molecular mechanism (Figure 1(a)).

It is known that the defect in the BER mechanism can lead to a variety of diseases, especially cancer, due to the accumulation of DNA lesions that cause mutations. For example, mutations in OGG1 lead to an accumulation of 8-oxoguanine, which can result in G-to-T transversions [44], a common mutation in cancer. Also, APE1 deficiency leads to neurodegenerative disorders, as AP sites are highly toxic and can lead to neuronal cell death if not properly repaired [45]. Moreover, oxidative stress and chronic inflammation, which generate high levels of reactive oxygen species (ROS), are common in cancer, aging, and neurodegenerative diseases. The BER pathway plays a critical role in countering this damage. Based on this fact, the role of BER in repairing DNA lesions makes it a target for therapeutic intervention, particularly in cancer treatment. Some strategies are the following: It is possible to inhibit key enzymes in the BER pathway (e.g., Pol β or APE1), which can increase the sensitivity of cancer cells to DNA-damaging agents like radiation or chemotherapy, and it leads to increased cell death. Also, tumors with defective DNA repair pathways (e.g., defective homologous recombination) may rely more heavily on BER for survival. Targeting BER in such tumors could lead to synthetic lethality, selectively killing cancer cells while sparing normal cells [46].

7. Post-translational modifications of histones play a pivotal role in regulating DNA damage repair

DNA is not freely floating in the nucleus but is packaged into higher-order structure chromatin, where histones serve as the core proteins around which DNA winds. Histone post-translational modifications (PTMs) dynamically alter chromatin structure, influencing accessibility to the underlying DNA. One of the most significant cellular processes influenced by histone PTMs is the DNA damage response (DDR). It is known that histone PTMs occur primarily on the N-terminal tails of the core histones (H2A, H2B, H3, and H4), but they can also occur on their globular domains, and linker histone H1. The main types of PTMs include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, and citrullination [47]. Each of these modifications can influence chromatin structure either by directly altering histone-DNA interactions or by recruiting effector proteins that bind specific modified residues. When DNA is damaged, the chromatin structure needs to be remodeled to allow repair machinery access to the site of damage. Chromatin relaxation is often the first step in making DNA more accessible. PTMs of histones play a central role in orchestrating this chromatin remodeling. Additionally, histone modifications act as signaling platforms, recruiting DNA repair proteins to the site of damage. Several histone modifications are directly involved in the sensing and repair of damaged DNA. These modifications can either signal the presence of a damaged genome or recruit specific repair proteins to the site of the lesion. One of the most well-known histone modifications in response to DNA damage is the phosphorylation of H2AX, a variant of histone H2A. Upon DSBs, H2AX is phosphorylated on serine 139 by kinases such as ATM, ATR, and DNA-PK. This phosphorylated form, known as γH2AX, spreads over megabase regions of chromatin surrounding the break in DNA. The γH2AX mark serves several functions. It acts as a platform for the recruitment of DDR proteins, such as MDC1 (mediator of DNA damage checkpoint protein 1) and 53BP1 (tumor suppressor p53-binding protein 1), facilitating genome repair. Also, it is known that γH2AX enhances chromatin relaxation, allowing greater accessibility for complexes of DNA repair proteins. γH2AX is also involved in the amplification of the DDR signal, ensuring efficient detection and repair of DNA damage [48–51].

Histone acetylation, particularly on H3 and H4, is generally associated with transcriptional activation and chromatin relaxation. In the context of DNA damage, acetylation plays a key role in chromatin de-condensation to allow the repair machinery access to the damaged sites. For example, acetylation of lysine 56 on histone H3 (H3K56ac) is particularly important during replication-associated DNA repair [52]. It is enriched during the S phase of the cell cycle and facilitates chromatin assembly after DNA damage repair. Acetylation of lysine 16 on histone H4 (H4K16ac) is also critical for chromatin relaxation in response to DNA injury, particularly recognized by HR repair [53]. Acetyltransferase (HAT), such as Tip60, has a very important role in this process. It was described that Tip60 is recruited to the site of the damaged genome, where this protein acetylates histones to promote chromatin relaxation [54].

Among others, histone methylation can either promote or suppress the DNA damage response, depending on the residues modified. For instance, methylation of lysine 36 on histone H3 (H3K36me) is associated with transcriptional elongation and repair via TC-NER. This modification is also recognized by the DDR protein, MSH6, which is involved in mismatch repair. On the other hand, di-, and tri-methylation of lysine 20 on histone H4 (H4K20me2/me3) is a key signal for double-strand break (DSB) repair [55–57]. To this fact, 53BP1, an important mediator of NHEJ, recognizes di-methylated or tri-methylated H4K20, promoting the repair of DSBs through the NHEJ pathway. In these cases, methyltransferases and demethylases modulate histone marks dynamically, either amplifying or resolving the DDR as needed.

Also, the ubiquitination of histones, particularly H2A and H2B, plays a crucial role in DNA repair. Unlike its role in protein degradation, histone ubiquitination is non-degradative and instead functions as a signal for chromatin remodeling and recruitment of repair proteins. Ubiquitination of H2A at lysine 119 (H2AK119ub) is mediated by the RNF8-RNF168 ubiquitin ligase complex. This modification is crucial for the recruitment of repair proteins such as BRCA1, which is involved in homologous recombination repair [58]. Moreover, Ubiquitination of H2B at lysine 120 (H2B K120ub) is also involved in chromatin remodeling during DDR and facilitates subsequent methylation of H3K79, which is important for DNA repair processes [59,60]. One of the most fascinating aspects of histone PTMs is their ability to interact with one another, a phenomenon known as the "histone code." For example, phosphorylation of H2AX can promote ubiquitination of H2A, which in turn facilitates the recruitment of

downstream repair proteins. Similarly, acetylation of H4K16 can enhance the effect of H4K20 methylation in promoting DSB repair [61].

This crosstalk ensures a highly coordinated and dynamic response to DNA damage, integrating multiple signaling pathways and repair mechanisms to maintain genomic stability. Based on the above-mentioned facts, we conclude that histone post-translational modifications are critical regulators of the DNA damage response, influencing both chromatin structure and the recruitment of DNA repair machinery. Through modifications such as phosphorylation, acetylation, methylation, and ubiquitination, cells can efficiently detect, signal, and repair DNA damage, ensuring genomic integrity. Understanding the complex network of histone PTMs in DDR not only provides insight into fundamental cellular processes but also highlights potential therapeutic targets for diseases such as cancer, where DNA repair mechanisms are often dysregulated.

8. The role of RNA modifications including N4-acetylcytidine in DNA damage response

Effective DNA damage response (DDR) mechanisms are essential for maintaining genomic integrity and preventing diseases such as cancer. While much of the focus has traditionally been on the proteins and pathways directly involved in recognizing and repairing DNA damage. However, also recent research has highlighted the role of RNA modifications in this process. One such modification, N4-acetylcytidine (ac4C), has emerged as a key player in the DNA damage response [62–64]. In the case of DNA damage repair, we have recently published that a pronounced acetylation of N4-cytidine, especially in small RNAs appears after UV irradiation. Recently we suggested that ac4C-modified RNAs are integrated into so-called R-loops, in which RNA acetylation causes de-condensation of chromatin in the vicinity of DNA lesions. Such arranged, modified and damaged chromatin is better recognized by DNA repair factors [63]. To this fact, we found that recruitment of ac4C-modified RNA to UVA-microirradiated chromatin was dependent on poly (ADP-ribose) polymerase 1 (PARP-1) function, but independent of the function of the NAT10 acetyltransferase [63]. To this fact, other authors [64] showed that NAT10 plays a role in the repair of UVB-induced DNA lesions. This study claims that NAT10 regulates the expression of the DDB2 gene by affecting its mRNA stability. From this view, it is known that UV-damaged DNA binding protein (UV-DDB), a heterodimeric protein, consisting of DDB1 and DDB2, regulates the canonical repair pathway, NER. UV-DDB complex recognizes and binds to sites of UV-induced DNA lesions. Also, it has been shown that this heterodimeric protein exhibits stronger binding affinity to a short DNA duplex with abasic sites, compared to a DNA substrate containing cyclobutane pyrimidine dimers (CPDs). This observation finally suggests a potential role for UV-DDB in BER mechanism [64,65].

Altogether, it is likely that ac4C-modified RNAs directly affect the pool of repair proteins by stabilizing the mRNAs from which they originate. This is particularly relevant for DNA damage-related genes, including those involved in homologous recombination and nucleotide excision repair. Also, NAT10 acetyltransferase could interact with chromatin and facilitate the recruitment of repair factors to damaged sites in the genome. Alternatively, NAT10 could contribute to DNA repair processes via its interaction with DNA repair proteins. Understanding this regulatory axis has implications for further studies on cancer biology and therapeutic strategies targeting NAT10 or related RNA modifications.

From the view of repair processes in the genome, the regulatory effects are also ascribed to additional post-transcription (co-transcription) modifications of RNA [66–68]. More than a hundred different RNA modifications have been revealed in distinct types of RNA, including N6-methyladenosine (m6A), N1-methyladenosine (m1A), 5-methylcytosine (m5C), internal 7-methylguanosine (m7G), 2′-O-methylation (2′-OMe), pseudouridine (ψ), uridylation or ADP-ribosylation. Also, adenosine deamination to inosine has been described [69]. It is known that the appearance of these modifications is catalyzed by specific writers and erasers, while RNA biochemical features are recognized by regulatory proteins called readers [70,71]. An example of intensively studied RNA modification is the methylation of adenosine, N6-methyladenosine, (m6A) whose appearance is mediated via the function of methyltransferase complex formed by METTL3 and METTL14 proteins, alternatively by METTL16 or other writers [72–75]. A specific eraser of this RNA modification is the FTO demethylases (Fat Mass and Obesity-associated protein) or the ALKBH5 protein [76,77]. From the view of epigenetic readers, m6A in RNA is recognized by the following proteins: YTH domain family of proteins (YTHDF1–3 and YTHDC1–2), insulin-like growth factor 2 mRNA-binding protein (IGF2BP), heterogeneous nuclear ribonucleoproteins A2/B1 (HNRNPA2B1), or proline-rich and coiled-coil containing protein 2A (PRRC2A) [78–81]. However, many other RNA modifications exist and are regulated in similar epigenetic/epitranscriptomic ways. For instance, we recently described that RNAs modified on N6-methyladenosine/N8-methyladenosine (m6A/m8A), 7-methylguanosine (m7G), N4-acetylcytidine (ac4C) but not N1-methyladenosine (m1A), or 2,2,7-methylguanosine (m3G/TMG) recognize UVA-microirradiated genome (Table 1) and [63,82–84].

In the case of DNA damage repair, also worth mentioning is the existence of so-called R-loops that are DNA/RNA hybrids likely playing a role in both mRNA processing and also in DNA damage repair (DDR), especially mediated by homologous recombination recognizing double-strand breaks (Figure 2(a)) [67,86–93]. The function of R-loops in DDR is not clear and needs additional studies. For example, it was suggested that epigenetically modified RNA (for example methylated or acetylated) is integrated into damaged double-stranded DNA to form a three-stranded structure in which RNA represents a template for the repair of damaged DNA [67,88,93] (Figure 2(a,b)). Indeed, in Kovarikova et al (2023) [63] we observed an increased pool of ac4C-modified RNAs in both the nucleoplasm of UVC-irradiated cells and UVA-microirradited chromatin. This observation implies the existence of the functional role of ac4C RNA in DRR. However, this DDR-related mechanism seems to be NAT10 independent ([63] and see Figure 2(b) showing fluorescence images representing original unpublished data). To this fact, Liu et al. [94], showed that mutation of PARylation residues

Table 1. RNA modifications and their role in the repair of UVA-damaged chromatin. Selected co-transcription modifications of distinct types of RNA and their role in DNA damage response are shown. Changes in the pool of modified RNAs after irradiation can be observed inside both the cell nucleus and the cytoplasm.

RNA modification	Level in microirradiated nuclear region ROI/whole UVC-irradiated cell populations	Described function	Preferential cellular localization	References
ac4C (N4-acetylcytidine)	Increased level in ROI 30 s – 45 min. postirradiation/increased level in the nucleus after UVC)	Regulation of RNA stability and translation	nucleus/ nucleolus	[63]
m1A (N1-methyladenosine)	Decreased level in the cytoplasm	The N1-methylation confers a positive charge on the nucleobases	cytoplasm	[82]
m6A (N6-methyladenosine)	Increased level in ROI – 30 s-15 min postirradiation/increased in the nucleus and reduced level in the cytoplasm after UVC)	Regulation of RNA stability and functions	cytoplasm	[82]
m8A (N8-methyladenosine)	Increased level in ROI – 30 s-15 min. postirradiation	Regulation of RNA stability and functions	nucleus	[83]
m3G/TMG (2,2,7-methylguanosine)	Decreased level in ROI	Regulation of stability, nuclear export, and assembly	nucleus	[82]
m7G (7-methylguanosine)	Increased level in ROI, 2 min – 1 h after microirradiation	Regulation of stability, translation, and protection	cytoplasm	[84]

on NAT10, pharmacological inhibition of PARP1 activity, or depletion of PARP1 causes translocation of the NAT10 acetyltransferase from nucleoli to the nucleoplasm, which appears after genome injury. In this case, we observed that the localization of NAT10 in nucleoli is not affected by UV radiation [63].

9. The m6A, m8A, m7G modifications in RNA and their role in UVA-damaged chromatin

Ultraviolet A (UVA) radiation, with wavelengths ranging from 315 nm to 400 nm, can penetrate the skin and induce various types of cellular damage, including DNA lesions and chromatin structure alterations [95,96]. As mentioned above, post-transcriptional RNA modifications have been implicated in diverse regulatory mechanisms in the context of DNA damage and chromatin remodeling (Table 1). These modifications may influence how cells respond to UVA-induced damage [63,83,84,97,98]. It is known that m6A is the most prevalent internal modification on mRNA and plays crucial roles in RNA metabolism, including stability, splicing, and translation [99]. Upon UVA radiation, chromatin undergoes significant stress, and RNA modifications can help regulate the transcriptional response [100,101]. m6A modifications on specific RNA molecules help modulate the DNA damage response by regulating the stability and localization of transcripts involved in the repair process. Following UVA-induced damage, m6A-modified transcripts may be selectively stabilized or degraded to facilitate efficient repair of lesions, especially through mechanisms like nucleotide excision repair (NER) [98]. To this fact, Zhang et al. [102] showed that the METTL3-m6A RNA-YTHDC1 mediated pathway influences the accumulation of DNA-RNA hybrids at DSBs sites, which recruit RAD51 and BRCA1, proteins of homologous recombination (HR) repair. To this fact, we additionally observed that m8A RNA-protein interactions could be involved in recruiting or modulating DDR-related proteins to UVA-damaged chromatin [83]. This may influence chromatin compaction and facilitate repair mechanisms. We suggest that RNA modifications at the m8A position may impact RNA stability in damaged cells. Also, we presuppose that modified RNAs contribute to the BER mechanism accompanied by active DNA demethylation. In this process, γH2AX stabilizes m6A/m8A-positive RNA-DNA hybrid loops via its interaction with m8A RNAs. In this case, R-loops might represent basic three-stranded structures recognized by PARP-dependent non-canonical m6A/m8A-mediated DNA repair pathway [83].

We also showed that m7G, as a well-known modification found at the 5′ cap of eukaryotic mRNA, plays a role not only in mRNA stability and translation initiation but also in DNA damage repair. We observed that irradiation by UV light increases the m7G RNA pool in the cytoplasm and the microirradiated genome (Table 1) [84]. Also, Chen et al. [103] demonstrated that m7G-modified mRNAs of DDR genes could serve as important biomarkers for predicting the prognosis of patients with colon cancer, which results from improperly repaired DNA. And, in a model of radiation-induced pulmonary fibrosis (RIPF), a complication after radiotherapy of the thorax, the authors investigated rosmarinic acid, which reduced the transcription and translation efficiency of sphingosine kinase 1 in lung fibroblasts by decreasing the N7-methylguanosine modification of tRNA [104].

10. The role of ac4C RNA in diseases and its diagnostic or therapeutic potential

The involvement of ac4C in DDR has significant implications for diseases characterized by genomic instability. Aberrant ac4C levels or dysregulation of its modifying enzyme, NAT10, could lead to non-physiological DDR, which contributes to tumorigenesis. Targeting ac4C pathways may offer therapeutic strategies enhancing the sensitivity of cancer cells to DNA-damaging agents; thus, improving the efficacy of chemotherapy and radiotherapy. Also, ac4C levels in RNA might serve as biomarkers for cancer diagnosis or prognosis, helping to identify patients with defective DDR mechanisms. From this view, it was published by Luo et al. [62] that cisplatin, a widely used cytostatic drug, and γ-radiation significantly up-regulate NAT10 expression [62,105,106]. Overall, the dysregulation of the NAT10 gene expression or protein activity has been linked to various cancers. Overexpression of NAT10 can lead to aberrant cell proliferation inducing a pronounced tumor growth, while its inhibition can sensitize cancer cells to DNA-damaging agents. Also, inhibitors of NAT10 are being explored as potential anti-cancer drugs. This fact highlights NAT10 as a potential

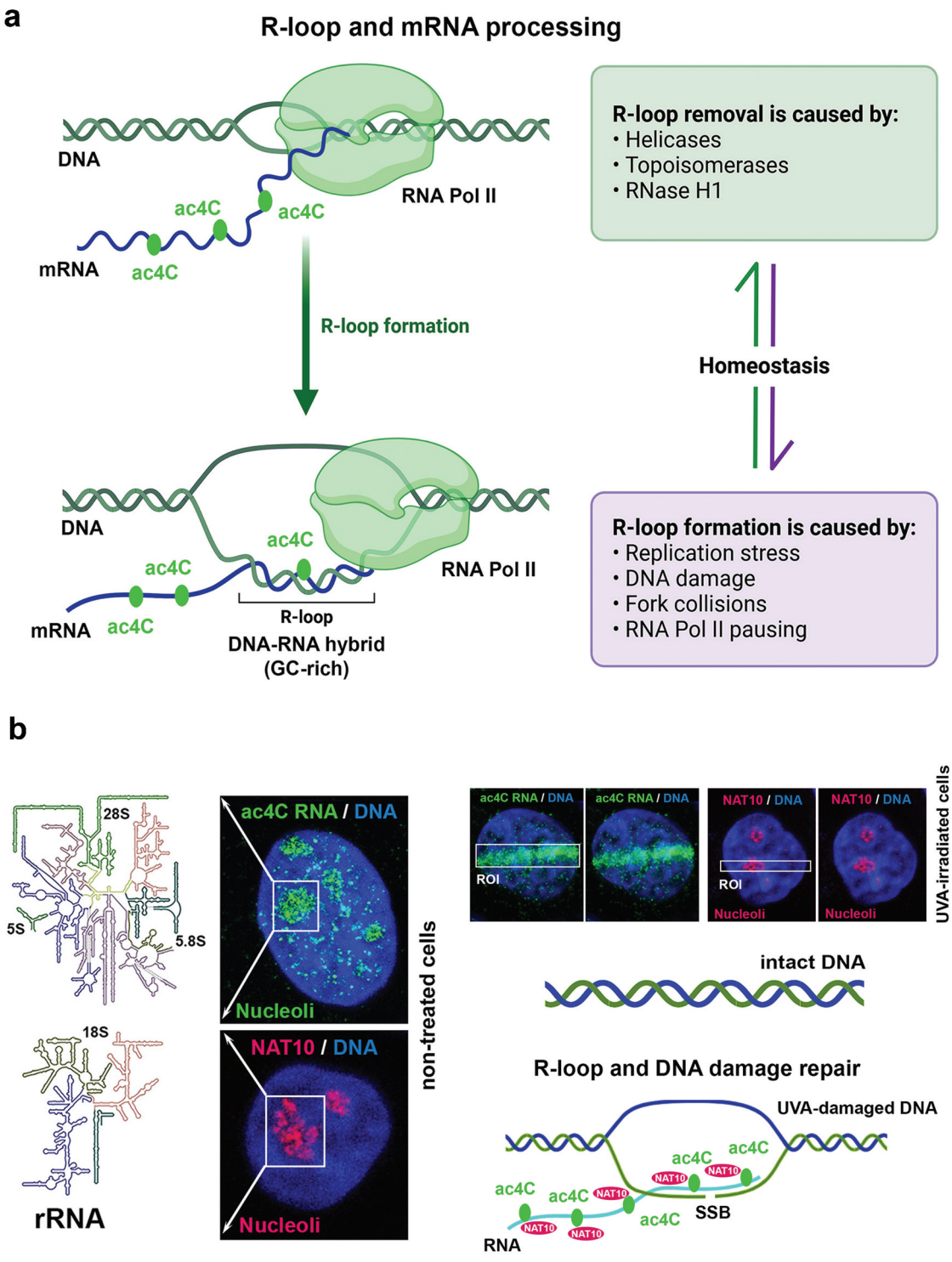

Figure 2. The suggested role of R-loops in the genome. (a) The R-loop is a structure composed of a duplex RNA-DNA and a single-chain DNA. R-loops can form during transcription upon the invasion of the newly formed RNA into the DNA duplex to remove the non-template string. R-loops likely play regulatory roles in genomes, but they can cause genomic instability, especially when replication forks or translation complexes collide with these R-loops. The template shown originates from BioRender and was adapted from the figure created by Dr. Christoph Grunseich from NIH/NINDS and Dr. Vivian Cheung from the University of Michigan. Panel (b) Shows rRNA and it is adapted from Petrov, et al. [85], and the database https://rnacentral.org/rna/URS0000ABD8B3/9606?tab=2d, the right panel in b shows the nuclear localization of ac4C in RNA and NAT10 acetyltransferase in nucleoli of HeLa human cervical carcinoma cells. Panel (b) Also shows a very high level of ac4C RNA (green fluorescence) that appears in UVA-damaged chromatin, but NAT10 acetyltransferase (red fluorescence) is not recruited to micro-irradiated DNA lesions [63]. The lower right panel (b shows a simple schematic illustration of intact DNA and a suggestion of R-loops when single-strand DNA break (SSB) appears. An integrated RNA is ac4C-positive. Here, we used the R-loop template (created with BioRender.com), into which ac4C modifications in RNA were added. Fluorescence images in panel B represent original unpublished data.

therapeutic target. For example, in prostate cancer cells, NAT10 contributes to the function of DNA replication initiation [107]. Also, NAT10 has been implicated in the regulation of cellular aging and senescence. By disrupting the ac4C RNA modification pathway, such inhibitors can reduce cancer cell proliferation and enhance the efficacy of existing treatments. From this view, high-throughput screening and structure-based drug design are being utilized to develop specific inhibitors or modulators of NAT10 activity, aiming to precisely specify its function in various diseases, including different types of tumors.

11. Anti-cancer-directed therapeutic strategies aimed at RNA modifications

Studies on RNA modifications offer a promising avenue for innovative therapies in DNA damage-related diseases, including cancer. From this view, the design and synthesis of small molecules targeting modified RNAs, including m6A or ac4C seem to be very promising. Such epigenetic-based chemical compounds could affect writers (e.g., METTL3), erasers (e.g., FTO), or readers (e.g., YTH domain proteins); and thus, could modulate DDR processes. For example, an inhibitor of the METTL3 methyltransferase enhances the sensitivity of cancer cells to DNA-damaging agents [108]. Also, inhibitors of RNA methyltransferases, NSUN2, could modulate cell survival in response to DNA damage [109]. A very important thing is the fact that therapeutics aiming at RNA modification pathways could synergize with traditional treatments, including chemotherapy or radiotherapy by enhancing their efficacy or reducing drug resistance. Together, recent research implies that future research should be directed to the achievement of selective modulation of RNA modifications without off-target effects. Also, safe delivery systems for RNA-modifying agents and other epigenetically based drugs should be developed and successfully applied in biomedicine.

12. Conclusion

N4-acetylcytidine (ac4C) and other RNA modifications play a multifaceted role in cancer progression and the DNA damage response, influencing the stability and translation of DDR-related mRNAs, the function of non-coding RNAs, the recruitment of repair proteins, and the regulation of apoptosis. These functions underscore the importance of RNA modifications in maintaining genomic stability and cellular homeostasis. Here, we also discuss the role of acetylated RNA in DDR not only from the view of our knowledge of RNA biology but also from the view of promising experimental approaches strengthening error-free DNA repair, which is essential not only in physiological conditions but also in pathophysiological states, including malignancies and their therapy.

13. Future perspective

The exploration of RNA acetylation and RNA methylation and their implications in cancer treatment and DNA damage repair is a rapidly evolving field, poised for transformative breakthroughs in the coming years. Future research is likely to focus on the development of small-molecule modulators or inhibitors specifically targeting RNA acetyltransferases (methylase) and deacetylases (demethylases). These tools could offer precision medicine approaches, allowing tailored interventions based on a patient's RNA acetylation landscape. Advanced omics technologies, particularly single-cell transcriptomics, and epitranscriptomics, will likely refine our ability to map RNA acetylation at unprecedented resolution. This will enable the identification of key acetylation marks associated with therapy resistance and aggressive tumor phenotypes, opening pathways for biomarker discovery and novel therapeutic targets.

In the realm of DNA damage repair, the crosstalk between RNA modifications and repair mechanisms represents a fertile ground for investigation. Future studies may uncover how RNA acetylation modulates the recruitment and activity of DNA repair machinery, particularly under genotoxic stress. Such insights could lead to combination therapies that exploit vulnerabilities in cancer cells' repair pathways, enhancing the efficacy of current treatments like radiotherapy and chemotherapy. Moreover, the potential for RNA acetylation to serve as a diagnostic or prognostic biomarker warrants extensive clinical exploration. Liquid biopsy techniques, for instance, could leverage RNA acetylation patterns to monitor disease progression or predict therapeutic responses with high accuracy. From this view, an interdisciplinary collaboration among molecular biologists, chemists, and clinicians will be essential to overcome current limitations in detecting and modulating RNA acetylation and other RNA modifications. While challenges remain, including the complexity of RNA modifications and their diverse roles in cellular processes, the next decade promises to harness the therapeutic potential of RNA acetylation, ultimately improving outcomes for patients with cancer and other DNA damage-related conditions.

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Author contributions

Eva Bártová wrote the manuscript and designed graphical illustrations; Lenka Stixová revised the graphical illustrations, and reference list and discussed the chapter on the role of ac4C RNA in damage response. Alena Svobodová Kovaříková is responsible for immunofluorescence data in Figure 2(b). All authors critically reviewed and revised the manuscript and approved the final version for submission.

Disclosure statement

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