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Regulation of long non-coding RNAs and genome dynamics by the RNA surveillance machinery

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Abstract

Much of the mammalian genome is transcribed, generating long non-coding RNAs (lncRNAs) that can undergo post-transcriptional surveillance whereby only a subset of the non-coding transcripts is allowed to attain sufficient stability to persist in the cellular milieu and control various cellular functions. Paralleling protein turnover by the proteasome complex, lncRNAs are also likely to exist in a dynamic equilibrium that is maintained through constant monitoring by the RNA surveillance machinery. In this Review, we describe the RNA surveillance factors and discuss the vital role of lncRNA surveillance in orchestrating various biological processes, including the protection of genome integrity, maintenance of pluripotency of embryonic stem cells, antibody–gene diversification, coordination of immune cell activation and regulation of heterochromatin formation. We also discuss examples of human diseases and developmental defects associated with the failure of RNA surveillance mechanisms, further highlighting the importance of lncRNA surveillance in maintaining cell and organism functions and health.

Non-coding RNAs (ncRNAs) are a type of RNA that are not translated into protein and include small ncRNAs (such as microRNAs) in the range of 15–30 nucleotides[1–3] and long ncRNAs (lncRNAs), which are generally considered as longer than 200 nucleotides[4]. LncRNAs include a variety of transcripts, all of which can modulate gene expression in specific ways based on cell type, developmental stage and function[5–7] (Box 1). As only a fraction of lncRNAs may be biologically relevant to a given process, cells use RNA surveillance pathways to identify and degrade most lncRNAs.

RNA surveillance is necessary for genome stability and gene expression[8–10]. Whereas mechanisms of mRNA surveillance have been extensively reviewed[11,12], less is known about the surveillance of the diverse types of lncRNA. Although the various components of the RNA surveillance pathways are still being characterized, it is known that endoribonucleases, 5′-exoribonucleases and 3′-exoribonucleases can degrade nascent lncRNAs, often with the

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help of RNA helicases, which can unwind the non-coding transcripts. The many RNA surveillance factors are vital to the well being of the cell and the organism[13–15].

This Review discusses the various mechanisms of nuclear lncRNA surveillance most tightly associated with transcription, initially giving an introduction to the major groups of lncRNA surveillance machinery. This is followed by a discussion of major molecular functions of lncRNA surveillance in the nucleus, such as regulation of antisense transcripts, genome integrity, chromatin state and nuclear architecture. Studies regarding the biological relevance of RNA surveillance machinery are then discussed in the context of immune–pathogen interplay and embryonic stem cell differentiation. Finally, the global importance of RNA surveillance is made clear in the discussion of human diseases and disorders associated with loss of or mutations in RNA surveillance components. The findings associated with RNA surveillance function at multiple levels of biology, from molecular mechanisms to human disease, highlight the importance of RNA surveillance machinery and of understanding the mechanisms of lncRNA turnover.

The RNA surveillance machinery

We first discuss the most prominent nuclear factors that have demonstrated ties to lncRNA processing and genome integrity. Their function is often related to handling harmful nucleic acid structures such as the R-loops (three-stranded structures consisting of an RNA–DNA hybrid and the remaining, displaced strand of DNA) that can form during transcription (Fig. 1), thereby underlying the importance of RNA surveillance to neutralize such threats to genome integrity.

Endonucleases.

Ribonuclease H1 (RNase H1) and RNase H2 are important regulators of genome stability (Table 1). Both enzymes process R-loops, as their depletion has been linked to R-loop accumulation in budding yeast, whereas their overexpression has been linked to R-loop depletion[16,17]. The RNase H enzymes are endonucleases that degrade the RNA strand of the RNA–DNA hybrid (Fig. 1) and, especially RNase H2, are required for the removal of harmful ribonucleotides from DNA strands[18]. The role of RNase H2 and RNase H1 in protecting genome integrity has been established by studies showing that they suppress hyper-recombination coupled with DNA breakage[19–21]. The use of catalytically inactive RNase H proteins as markers for R-loops[22] has further validated their ubiquitous role in R-loop binding and resolution. Importantly, loss of this arm of RNA surveillance has been linked to disease, as discussed below.

3′ Exoribonucleases.

The eukaryotic RNA exosome is a 3′→5′ exoribonuclease complex, which was initially discovered in budding yeast and shown to degrade ribosomal RNA and other small RNAs[23,24]. The 11-subunit exosome complex is highly conserved, with regard to structure and function, between budding yeast and mammals[25,26]. The RNA exosome is present in the cytoplasm and in the nucleus as a nine-subunit core complex, with two subunits — the mammalian proteins exosome component 10 (EXOSC10) and DIS3 (also known as RRP44)

— bearing RNase activity[27,28] (Fig. 1). The exosome is targeted to specific nascent and recently transcribed ncRNAs by nuclear targeting complexes comprising the DExH-box helicase MTR4 and associated accessory proteins. There are two mammalian RNA exosome-associated nuclear targeting complexes, one located in the nucleoplasm and the other in the nucleolus. In the nucleoplasm, MTR4, zinc finger CCHC domain-containing protein 8 (ZCCHC8) and RNA-binding protein 7 (RBM7) make up the nuclear exosome targeting (NEXT) complex, which is responsible for the RNA exosome-mediated degradation of various types of ncRNAs[14,29,30] (Fig. 1). This role renders RNA surveillance a prominent factor in regulating gene expression and in the maintenance of genome integrity[31–34].

5′ Exoribonucleases.

The major 5′→3′ exoribonucleases of RNA surveillance in mammals are the homologues 5′–3′ exoribonuclease 1 (XRN1) in the cytoplasm and XRN2 in the nucleus[35] (Fig. 1). Both proteins were initially discovered and characterized in budding yeast[36,37] and shown to function in ribosomal RNA processing[38] and tRNA decay[39] as well as in mammalian cells in mRNA turnover[40–42]. XRN2, in particular, has been associated with other nuclear RNA surveillance mechanisms in mediating transcription termination through the ‘torpedo model’[43], wherein RNA polymerase II (Pol II) continues transcribing following cleavage of the mRNA beyond (3′ of) the poly(A) site[43,44]. XRN2 then degrades the superfluous transcript 5′→3′ until it catches up with Pol II and mediates its dissociation from the DNA template[43–45]. This has been demonstrated in the *ACTB* gene, where transcription through the poly(A) site and proximal genetic elements promote Pol II pausing and XRN2-mediated transcription termination[46].

XRN2 also functions in transcription termination with other components of the RNA surveillance machinery. R-loops form at G-rich transcription pause sites, where the displaced DNA strand is more likely to form secondary structures such as the four-stranded G-quadruplexes, which are unwound by the RNA helicase senataxin (SETX) to facilitate XRN2-mediated transcription termination[47] (Fig. 1). Other involved factors include the microprocessor complex (better known for its role in microRNA maturation) and EXOSC10, indicating the existence of a transcription termination complex that includes various components of the RNA surveillance machinery[48]. XRN2 and transcription termination are vital for the preservation of genome integrity; XRN2 and other factors, such as SETX, are recruited to R-loops for RNA degradation and prevention of DNA double-stranded breaks (DSBs)[49]. It remains unclear whether XRN2 also has a role in repairing DSBs. These observations highlight the functional importance of 5′ exonuclease activity in maintaining an effective DNA damage response and in the prevention of genome instability.

RNA helicases.

R-loops can present a threat to genome integrity by leaving a displaced single-stranded DNA (ssDNA) vulnerable to damage, and therefore degradation of the nascent transcripts is a crucial regulator of R-loop-mediated genome instability. The processing of R-loops by endoribonucleases and exoribonucleases requires the unwinding of the associated RNA and DNA strands by various helicases[50] (Table 1). The human RNA helicase SETX has a role in

XRN2-mediated transcription termination and transcription-associated replication fork integrity[47,48,51]. SETX and related DEAxQ-like domain-containing helicase Aquarius are vital for R-loop processing and genome stability[52–54]. The DExH helicase MTR4 is a nuclear cofactor of the RNA exosome in RNA surveillance; targeted RNA surveillance and optimal RNA exosome activity require association with MTR4 helicase and the NEXT complex[55]. Biochemical and genetic analyses of R-loop-associated somatic mutations, together with structural data generated by cryo-electron microscopy, show that MTR4 and the RNA exosome (along with SETX) form a complex that unwinds RNA–DNA hybrids and double-stranded RNA (dsRNA) to promote RNA exosome-mediated ncRNA degradation (discussed below). Several other DEAH-box (DHX) and DEAD-box (DDX) helicases have roles in the maintenance of genome stability[56] (Table 1). For example, the RNA helicase DHX9 is involved in R-loop processing and prevention of DNA damage[57,58] (Fig. 1), and DDX1 has been implicated in resolving RNA G-quadruplexes in immunoglobulin class switch recombination (CSR)[59] (Fig. 2).

The activities of ncRNA surveillance

In this section, we discuss the molecular functions of lncRNA (and ncRNA) processing by RNA surveillance and the consequences of their absence.

Sense and antisense transcription.

A key regulator of protein-coding transcripts are antisense transcripts, many of which are associated with gene promoters or initiated from gene bodies (often from intragenic enhancers)[32]. Promoter-associated or gene body-associated antisense RNAs can modulate the expression of neighbouring or overlapping genes by multiple mechanisms such as histone modifications[60], DNA methylation[61], and co-transcriptional effects, such as RNA polymerase collisions and formation of secondary DNA structures[32,62].

Antisense and sense transcripts can regulate each other in various manners to control gene expression. The mutual promotion of sense and antisense transcription is exemplified in antisense transcription start site RNAs (TSSa-RNAs), the transcription of which is divergent and the expression directly correlated with that of their sense mRNAs through the use of shared promoters[63]. Conversely, the expression of sense and antisense transcripts can be inversely correlated when the transcription of one transcript results in the suppression of the other. For example, transcription of the lncRNA *Airn* (antisense of IGF2R non-protein coding RNA) overlaps with the *Igf2r* gene, suppressing *Igf2r* transcription[64]. A recent study has suggested another possible relationship between sense and antisense transcripts, in which mRNA transcription can promote transcription of antisense RNA through an R-loop-dependent mechanism[65].

The aforementioned correlations between sense and antisense transcripts indicate that RNA surveillance mechanisms could be important in regulating gene expression mediated by antisense transcription, particularly in relation to R-loops. Indeed, in the absence of RNA exosome activity, TSSa-RNAs accumulate at bidirectionally transcribed promoters (and enhancer RNAs (eRNAs; Box 1) accumulate at bidirectionally transcribed enhancers)[32]. The TSSa-RNA-accumulating bidirectional promoters show a marked increase in R-loop

stability, suggesting that the RNA exosome is required for efficient degradation of antisense RNA in R-loops at divergent promoters.

In the absence of RNA surveillance, an antisense RNA-dependent R-loop structure was observed in the promoter and in the gene body of Myc[32], which was also found to be extended in size in the absence of Integrator complex activity (measured by loss of the recruiter protein SPT6)[66]. The Integrator is an endoribonucleolytic complex that was shown to regulate transcription by cutting nascent mRNAs to mediate premature transcription termination and Pol II dissociation[67]. Loss of Integrator function results in the expression of extended transcription start site-associated lncRNAs owing to defects in transcription termination and an increase in R-loop size and accumulation at divergent promoters, similar to the previous findings in cells lacking RNA exosome function[32,66]. A similar correlation between the function of the RNA exosome and the Integrator complex has been identified also for eRNA[33,68]. The role of the RNA exosome and the Integrator complex in regulating the expression of antisense ncRNAs at gene promoters and other regulatory elements illuminates the importance of RNA surveillance for gene regulation.

Maintaining genome integrity.

Over the course of the cell cycle, genome integrity is constantly engendered by DNA damage, mutations, and chromatin breakage and rearrangements[69]. Such genomic insults can be stimulated by normal cellular processes, such as DNA replication and transcription, and are efficiently processed and cleared to prevent deleterious outcomes such as unregulated DNA break-associated recombination[70]. Genomic instability can lead to cancer development if repair and regulatory pathways are not intact[71,72]. DNA replication is a considerable source of genome instability, as the replisome encounters many ribonucleotides and protein barriers that can stall the replication fork and cause DNA vulnerability. Defects in pathways that monitor and aid replication result in replication-associated genome instability[73–75]. The mechanisms of replication regulation and maintenance have been described extensively in the literature[76–79].

NcRNAs, especially nascent ncRNAs, are a considerable source of genome instability[33,66]. Likewise, R-loops are a common by-product of transcription and are widely implicated in transcription-associated genome instability[80]. The current model of R-loop formation during transcription is the ‘thread back’ model, whereby the nascent RNA strand exits the RNA polymerase and reanneals to the template DNA strand, leaving the displaced DNA strand vulnerable to damage[80,81] (Fig. 2a). DNA features, such as high GC content, negative super-coiling and DNA nicks, stabilize R-loops and prolong the exposure of the ssDNA to damaging agents[82,83]. R-loops are also stabilized by G-rich sequences that can form G-quadruplexes, which inhibit reannealing of the DNA[84,85]. When not properly processed, transcription-associated R-loops can lead to genome instability in the form of DSBs and deleterious recombination[86,87] (Fig. 2a).

Among the helicases relevant for maintaining genome stability are members of the DDX family[59,88], the R-loop and G-quadruplex helicase DHX9 (reF.[57]), and SETX[53,89]. Loss of SETX in mouse spermatocytes resulted in the accumulation of R-loops and in apoptosis[52]. In neurons, SETX interacts with the RNA exosome to decrease R-loop burden; individuals

with *SETX* mutations suffer from ocular degeneration, leading to ataxia with oculomotor apraxia 2 (AOA2; also known as spinocerebellar ataxia with axonal neuropathy 2) (Table 1). The molecular mechanism of AOA2 is not completely understood, but it is clear that the underlying *SETX* mutations uncouple the interaction between SETX and the RNA exosome[90].

Programmed DNA mutagenesis.

B cells diversify their antibody gene repertoire through three remarkable DNA alteration processes. Immature B cells in the bone marrow undergo V(D)J recombination, and peripheral B cells undergo CSR and somatic hypermutation (SHM) following antigen exposure. Collectively, these phases of developmentally programmed DNA mutagenesis occur in the immunoglobulin (Ig) gene loci and involve processes that, in the absence of RNA surveillance, can cause genome instability at other regions of the mammalian genome. CSR and SHM require the activity of the ssDNA deaminase enzyme activation-induced cytidine deaminase (AID), which identifies ssDNA on the template and non-template strands of Ig switch regions to induce nicks that accumulate and form DSBs. Two such programmed DSBs are then synapsed to undergo DNA recombination, leading to isotype selection and CSR.

The R-loop-processing role of the RNA exosome in orchestrating CSR is arguably its most thoroughly characterized function in the immune system[32,91]. CSR requires transcription of ncRNA at switch sequences in the Ig locus of B cells, and AID recruitment and activity at this site facilitates DSB formation and recombination[92] (Fig. 2b). Transcription-associated R-loops persist at transcribed switch sequences owing to the high guanine content of the non-transcribed DNA strand, which stabilizes DNA structures such as stem-loops and G-quadruplexes. The RNA exosome, which processes the switch region transcripts, recruits AID to the ncRNA template strand to deaminate and ultimately nick it[91,92] (Fig. 2b). In addition to the activity of the RNA exosome complex, RNase H1 is capable of degrading the switch region transcript to facilitate R-loop resolution and CSR[17]. It has also been postulated that the AID protein is associated with a G-quadruplex in the nascent ncRNA[93] that needs to be unwound by the G-quadruplex helicase DDX1 prior to AID recruitment to the switch sequences[59] (Fig. 2b). The AID-associated G-quadruplex RNA could be an RNA exosome substrate upon its unwinding by DDX1, though the mechanism of AID–RNA interaction is currently being evaluated[32,93,94]. Following AID recruitment and catalysis of ssDNA nicks on both strands of switch sequences, the dU bases are then recognized and processed by either the base excision repair or mismatch repair pathways[95]. The ensuing DSB can then recombine with a downstream switch sequence that has undergone the same process (Fig. 2b).

Importantly, the switch region ncRNA must be degraded to efficiently process the R-loop and create a DSB[13,32,96]. It is also possible that unwinding and degradation of the switch region transcript leads to misalignment of the two switch region DNA strands owing to the formation of stable G-quadruplexes and other DNA structures. This may be followed by aberrant base pairing of long stretches of dG–dC sequences to create long stretches of ssDNA ‘bubbles’ that ultimately recruit AID (Fig. 2c). The functions of RNA surveillance

factors at switch sequences are prime examples of the importance of RNA surveillance in maintaining the balance between programmed genetic instability and genome integrity. Although the mechanism of DNA mutagenesis by AID during SHM is still under investigation, it is already clear that the RNA surveillance machinery is important for programmed DNA mutagenesis and breaks during antibody gene diversification.

DNA double-stranded break repair.

RNA exosome-deficient embryonic stem cells (ESCs) accumulate DNA breaks, γH2AX foci (marker of DSB repair response) and aberrant chromosomal rearrangements[33]. Specifically, when the RNA exosome subunit EXOSC3 was depleted in ESCs, γH2AX foci appeared at sites of higher accumulation of exosome substrates such as enhancers. Analysis of metaphase chromosomes revealed an accumulation of translocation-dependent chromosomal alterations following RNA exosome depletion. These observations indicate that loss of the RNA exosome leads to deficiency in DNA damage repair.

A recent study[97] has attributed a role for the RNA exosome subunit EXOSC10 in identifying R-loop-associated lncRNAs formed at DNA breaks as a result of pre-existing or DNA break-induced transcription, as well as in processing these lncRNAs to generate ssDNA available for the recruitment of replication protein A (RPA). RPA recruitment to ssDNA overhangs (that are free of exosome-sensitive lncRNAs) primes the strand-invasion step and DSB repair by homologous recombination (Fig. 3a). The targeting of the ssDNA-binding protein RPA to DSB sites is impaired, and DNA end resection is overstimulated in EXOSC10-depleted cells[97]. By contrast, defects in DNA end resection are restored by transcription inhibitors, and RNase H1 overexpression restores the RPA recruitment defect caused by EXOSC10 depletion. These observations compel us to suggest that clearance of newly synthesized lncRNAs at DSB sites is required for RPA recruitment (Fig. 3a). Moreover, it is likely that DNA end resection and assembly of the homologous recombination machinery depends on lncRNA transcription at DSB sites.

Another prominent example of the role of RNA surveillance proteins in DNA damage repair is the role of SETX in resolving RNA–DNA hybrids at DSB sites and limiting aberrant DNA rearrangement. RNA–DNA hybrids were shown to accumulate on DSB-flanking chromatin and have a narrow overlapping SETX binding distribution[53]. Moreover, SETX promotes recruitment of the recombinase RAD51, thereby minimizing illegitimate re-joining of distant DNA ends and preventing chromosome translocations[53]. Taken together, these studies highlight different mechanisms by which RNA surveillance functions to regulate DSB repair and limit the number of deleterious chromosome translocations.

Chromatin regulation.

In the yeast *Schizosaccharomyces pombe*, lncRNAs generated from centromeric DNA by ‘pervasive transcription’ self-hybridize to form dsRNAs, which then associate with components of the heterochromatinization machinery to initiate transcription silencing[98–101]. RNA surveillance factors have a role in regulating centromeric silencing through RNA interference (RNAi)-dependent and RNAi-independent pathways[102]. Whereas transcripts synthesized in euchromatin regions are properly processed by the canonical

poly(A) polymerases to be exported to the cytoplasm for translation, transcripts from heterochromatin regions are processed by a non-canonical poly(A) polymerase (Cid14) and are eventually identified and degraded by the RNA exosome complex or undergo degradation through RNAi[102] (Fig. 3b). Deletion of the RNA exosome factor Rrp6 (the *S. pombe* homologue of EXOSC10) leads to accumulation of the antisense and sense centromeric transcripts by RNA-dependent RNA polymerase and formation of dsRNAs, which are processed to promote centromere silencing[101].

An equivalent role of RNA surveillance proteins in regulating heterochromatin-associated transcripts is yet to be elucidated in mammalian cells. Nevertheless, RNA exosome function in heterochromatin has been evaluated in mammalian cells. Loss of EXOSC3 in ESCs has been shown to reduce the levels of the gene-silencing heterochromatin modification histone H3 Lys9 dimethylation (H3K9me2) at a selected set of silent enhancers, and loss of EXOSC10 can suppress the formation of facultative heterochromatin at certain genes owing to a decrease in H3K27 methylation levels[33]. The recently characterized MiCEE complex is composed of the microRNA let-7d, the ribosomal RNA processing factor C1D, EXOSC10 and EZH2 (the catalytic subunit of PRC2)[103]. In the process of degrading lncRNAs that are divergently transcribed from promoters of active genes, the RNA exosome and MiCEE recruit PRC2 to catalyse H3K27 trimethylation and gene silencing at the periphery of the nucleolus (Fig. 3c). Another recent study has also uncovered a link between the RNA exosome and PRC2-mediated chromatin modification through the RNA exosome cofactor poly(A)-tail RNA exosome targeting (PAXT) complex (Table 1). PAXT, which comprises MTR4, the zinc-finger protein ZFC3H1 and poly(A) binding protein nuclear 1 (PABPN1), binds polyadenylated nuclear transcripts and targets them for degradation by the RNA exosome[30]. ESCs lacking ZFC3H1 and PAXT function exhibit defective PRC2 function and aberrant gene expression[104]. The function of PRC2 was hindered by RNA accumulation, suggesting that the main role of PAXT in facilitating RNA exosome-mediated RNA degradation is also a crucial regulator of PRC2 function and chromatin status. These preliminary studies suggest that the RNA exosome and ncRNA surveillance have a central role in chromatin modification and gene expression.

Nuclear architecture.

The 3D organization of chromatin is linked with transcription regulation. The genome is organized into topologically associating domains (TADs), defined as genomic regions where regulatory elements and genes primarily interact with each other rather than with sequences in other TADs[105,106]. Structurally, chromatin is organized into loops that drive (and are driven by) promoter–enhancer interactions and gene expression. TAD borders are enriched in binding sites of the transcriptional repressor CTCF, the structural maintenance of chromosomes complex cohesin, the mediator of Pol II transcription (Mediator) complex and their associated proteins[107,108]. Transcription of RNA, and mainly of ncRNA, can coordinate chromatin topology. For example, a class of transcription-activating ncRNAs is thought to be necessary for chromosome looping, potentially facilitating interactions between CTCF–cohesin complexes and gene promoters[109,110]. In B cells, the RNA exosome is important for the interaction through chromatin looping of 3′ regulatory region super-enhancer (3′RR; which is the enhancer of the Ig locus) with other regions of the B cell

genome, including a distal enhancer RNA-expressing element, lncRNA-CSR^{IgA} (reF.[33]). In the absence of proper degradation of lncRNA-CSR^{IgA}, its physiologically relevant topological association with 3′RR is perturbed, leading to decreased CSR (ref.[33]; G. Rothschild and U. Basu, unpublished results). Future studies should interrogate whether the RNA exosome and other RNA surveillance components have a role in the overall regulation of genome topology.

The organization of the nucleus is also shaped by liquid–liquid phase separation, which can drive the formation of membraneless nuclear bodies with various (often unknown) functions in gene regulation. LncRNAs, and RNA in general, are important for phase separation, suggesting the need to evaluate how RNA surveillance-sensitive lncRNAs can mediate phase separation to alter gene expression[111]. Though a definitive role for RNA surveillance in phase separation is yet to be determined, the finding that the PAXT complex (which is often associated with the RNA exosome) is required for the formation of specific nuclear foci[112] suggests that the RNA exosome may be involved in the formation and function of membraneless nuclear bodies.

Biological roles of lncRNA surveillance

In this section, we discuss how ncRNA surveillance is important for orchestrating various physiological processes.

Controlling pathogens and the immune system.

LncRNAs regulate the expression of immune genes, the differentiation of immune cells and the ability to mount an inflammatory response to infection[113,114]. LncRNAs and other ncRNAs are also important in controlling bacterial and viral pathogenesis[115]. In *Salmonella enterica* serovar Typhimurium (*Salmonella*), the intergenic, antisense RNA *AmgR* is vital for growth in a low Mg^{2+} environment and for host control of *Salmonella* virulence[116]. LncRNAs also function in immune evasion and pathogen-mediated damage to the host. Kaposi's sarcoma-associated herpesvirus (KSHV), a tumorigenic virus in epithelial cells, expresses a number of lncRNAs that facilitate its life cycle, the transformation of host cells and cancer development[117–119]. These and many other examples highlight the capacity of lncRNAs to dramatically affect the host–pathogen relationship.

Given the importance of lncRNA expression for the host immune system and pathogen infectivity, lncRNA surveillance is prominent in the host–pathogen interplay. RNA stability is linked with the functional dynamics of lncRNAs[120,121]. In the case of *Salmonella* infections, the accumulation of lncRNA in the nucleus was a direct consequence of *Salmonella*-associated loss of the host RNA helicase MTR4 and the RNA exosome subunit EXOSC10 (reFs[122,123]) (Fig. 4a). Upon *Salmonella* infection and cellular replication, normally unstable lncRNAs, likely involved in the immune response, were stabilized and expressed in the nucleus, ultimately resulting in *Salmonella* clearance[122]. The 3′→5′ RNA degradation machinery was primarily involved in this change in lncRNA stability, as loss of MTR4 mediated lncRNA accumulation owing to failure to recruit the RNA exosome[122] (Fig. 4a). Although the full details of this mechanism are yet be elucidated, it is direct evidence for the involvement of RNA surveillance in host–pathogen interactions.

Contrary to the mechanism seen in *Salmonella* infection, counteracting host lncRNA-associated inflammatory responses is a type of immune evasion employed by pathogens. In the case of KSHV infection, the viral protein ORF57 was found to prevent degradation of viral RNA in the nucleus, but the mechanism of RNA degradation by the host and the mechanism of ORF57-mediated protection against degradation remained unknown[124]. However, recent investigations have revealed that ORF57 protects viral transcripts from degradation by blocking their interactions with MTR4, potentially implicating the RNA exosome in antiviral responses[125] (Fig. 4b). The RNA exosome and its cofactors have also been implicated in antiviral activity in *Drosophila melanogaster*[126] and in HIV transcription in human cells[127]. Several other RNA surveillance factors are involved in counteracting viral infections[128].

Maintenance of embryonic stem cells and differentiation.

The interplay between ESC pluripotency and differentiation is complex and relies heavily on transcription regulation and plasticity. ESCs are characterized by widespread, genome-wide transcription allowing them to quickly shift to any specific gene-expression programme upon differentiation to any cell type[129]. Several types of ncRNAs have a role in maintaining ESC pluripotency. Long non-coding eRNAs can regulate ESC differentiation and must be regulated themselves for pluripotency maintenance[130]. Endogenous retroviral elements, such as long interspersed nuclear element 1 (LINE1) and long terminal repeat (LTR) transcripts, can influence pluripotency, with evidence to support an association between LTRs and eRNAs[131,132]. Divergent transcription of lncRNAs and mRNAs can also mediate stem cell differentiation[133,134], and lncRNAs can both be a product of pluripotency-maintenance transcription factors and profoundly affect their expression[130,135].

The crucial role of lncRNAs in regulating ESC transcription and fate points to the importance of lncRNA surveillance in regulating ESC pluripotency and differentiation. The RNA exosome has been shown in multiple studies to control ESC gene expression and maintenance of the pluripotency state. Gene expression is globally deregulated in EXOSC3-deficient and in EXOSC10-deficient mouse ESCs, and the levels of silencing chromatin modifications decrease in regions of eRNA expression[33]. In RNA exosome-deficient mouse ESCs, genes with higher transcriptional activity in the differentiated state showed greater exosome sensitivity as measured by transcript accumulation in the deficient ESCs, suggesting a role for the RNA exosome in downregulating differentiation-associated genes in the ESC state[136]. Furthermore, upon depletion of the RNA exosome in human ESCs, the levels of mRNAs and of lncRNAs (such as LINE transcripts) that are normally expressed in later stages of embryonic development were increased, further supporting the role of the RNA exosome in regulating ESC differentiation[137]. In the future, the functional relationship between the expression of LINE transcripts and ESC pluripotency should be evaluated. Overall, these studies establish a role for RNA surveillance in regulating ESC pluripotency and differentiation.

RNA surveillance-related diseases

As RNA surveillance is crucial for essential cellular processes, it stands to reason that there would be a correlation between mutations in genes encoding RNA surveillance factors and human diseases. Indeed, defects in RNA surveillance have been identified in a number of human disorders and diseases, especially in neurodegenerative disorders, immunological disorders and cancer (Table 1).

Neurodegenerative disorders.

Several RNA surveillance mechanisms have been linked to neurodegenerative and neuromuscular disorders. Mutations in various subunits of the RNA exosome have been found to associate with neurodegenerative disorders in model organisms and in humans[138]. Missense mutations in the *EXOSC8* gene, encoding a core component of the RNA exosome, cause cerebellar and corpus callosum hypoplasia, hypomyelination and spinal motor neuron disease[139]. Missense mutations in the exosome cap subunit *EXOSC2* gene were associated with retinitis pigmentosa, progressive hearing loss, premature ageing and mild intellectual disability[140]. The first direct tie between the RNA exosome and human disease was the discovery that mutations in the integral cap subunit *EXOSC3* gene correlate with pontocerebellar hypoplasia and associated spinal motor neuron degeneration[141–144]. Mutations in the *RBM7* gene, a subunit of the exosome-associated NEXT complex, also cause pontocerebellar hypoplasia-like symptoms and spinal muscular atrophy[145]. The DNA/RNA helicase SETX has also been implicated in neurodegenerative disorders; mutations in the *SETX* gene are linked to AOA2 and with amyotrophic lateral sclerosis, both marked by muscle weakness and degeneration[146,147]. This phenotype is suggested to be caused by the crucial role of SETX in transcription termination and R-loop processing[47,89,148].

Immunological disorders.

Aicardi–Goutières syndrome is both a neurological disorder and an autoimmune disease, which shares a certain resemblance with systemic lupus erythematosus (SLE)[149]; both diseases are marked by type I interferon (IFN)-mediated inflammation and loss of self-tolerance, resulting in an antibody response against nucleic acids associated with dying cells[149]. RNase H2 defects have been implicated in Aicardi–Goutières syndrome by studies using an RNase H2 knockout mouse model and patient samples[150,151]. RNase H2 mutants give rise to increased nucleic acid damage and accumulation, which activate the type I IFN response[152–154]. In the context of RNase H2 deficiencies, type I IFN-mediated inflammation is induced in response to damaged DNA that activates innate immunity through the nucleic-acid-sensing cGAS–STING pathway, which primarily functions as a viral DNA sensor[154]. Similarly, RNase H2 is important for removing aberrant ribonucleotides from DNA, and loss of RNase H2 results in the accumulation of damaged DNA, which stimulates inflammation and leads to systemic auto-immunity[151]. The RNA exosome-associated RNA helicase MTR4 is implicated in suppressing a similar inflammation signalling pathway, intracellular RNA-mediated RIG-I[155]. These studies highlight the role of RNA surveillance in modulating the activation of cell-intrinsic mechanisms of inflammatory responses.

Cancer.

RNA surveillance has an important role in balancing genomic integrity and dynamics, which, if lost, can quickly result in cancer development. The RNA exosome catalytic subunit DIS3 has specifically been implicated in cancer development. Loss-of-function mutations in the *DIS3* gene are found in individuals with multiple myeloma, which is a mature B cell (plasma cell) malignancy[156]. Interestingly, *DIS3* mutations coincide with IgH translocations in many individuals with multiple myeloma, suggesting that the role of the RNA exosome in regulating B cell translocations may also be implicated in cancer development[32,157]. RNA surveillance defects in R-loop and DSB processing are potential risks for cancer development as they have been shown to result in R-loop accumulation and to threaten genome stability[158]. The Ig locus is a prime example of the potential negative outcomes of deregulated R-loop and DSB processing. AID-mediated R-loop and DSB formation in appropriate switch regions is necessary for on-target CSR[159], and development of cancers such as lymphomas is marked by off-target translocations between IgH and proto-oncogenes, such as *MYC* or *BCL2*, which support constitutive expression of the oncogene[160]. RNA surveillance mechanisms regulate AID targeting, R-loop processing and subsequent on-target recombination, thereby demonstrating their importance in cancer prevention. Ongoing research may identify additional mutations in RNA surveillance factors implicated in the development of a variety of malignancies.

Conclusions and future perspective

The role of lncRNA surveillance in preventing cellular and developmental defects and diseases highlights their biological importance. Currently, many studies of various physiological processes, such as immune system development and function, stress-associated apoptosis and developmental neurobiology, do not sufficiently consider the effects of lncRNA turnover and overlook the considerable cellular effects of ncRNAs. It is now becoming clear that many aberrations in mammalian gene regulation, cell function and development occur owing to overexpression of lncRNAs in the absence of RNA surveillance. Higher levels of lncRNAs interfere with gene regulation, and RNA surveillance has a role in titrating lncRNA levels down to non-existent or physiologically tolerable levels.

In addition to the RNA surveillance mechanisms discussed here, other RNA surveillance mechanisms exist, which are still less understood. For example, multiple types of RNA modifications could modulate RNA degradation, localization and other surveillance mechanisms. Most of these modifications have largely been studied in mRNA, the most widely studied being N^6-methyladenosine, but much is yet to be characterized in terms of N^6-adenosine methylation in lncRNAs[161].

Similar to RNA modification is RNA editing, which is a post-transcriptional modification that introduces changes in the RNA sequence. One of the most prevalent types of RNA editing is mediated by adenosine deaminase acting on RNA (ADAR), which are enzymes that convert adenosine (A) into inosine (I) in dsRNA, mostly of non-coding origin[162]. The role of ADAR enzymes in RNA surveillance was first noted when a lack of ADAR1-mediated RNA editing in mammals was shown to cause symptoms that mimic viral infection[163,164]. Further investigations demonstrated that defective ADAR1 function leads to

spontaneous activation of PKR and MDA5, which are pattern recognition receptors (PRRs) for viral dsRNA[165]. These studies suggest that ADAR1 editing of endogenous, mostly ncRNA prevents the activation of PRRs.

How A-to-I editing shields RNAs from PRR detection is not fully understood. In mammals, the vast majority of ADAR1 editing takes place in the abundant Alu repetitive elements residing in non-coding regions of mRNAs (untranslated regions, introns)[162,165]. One model is that A-to-I editing destabilizes the structure of the endogenous dsRNA formed by inverted Alu repeats, resulting in their evasion from detection by PRRs[163]. Alternatively, A-to-I editing might lead to the degradation of RNAs or may recruit other RNA binding proteins that shield RNAs from PRR detection. Taken together, the investigations into ADAR1 suggest that, alongside RNA degradation, mammals adapted the RNA editing machinery to ensure that cellular ncRNAs do not trigger an abnormal immune response.

There are many yet uncharacterized processes in which RNA surveillance mechanisms might be employed. The role of RNA and RNA surveillance in phase separation-driven mechanisms, for example, warrants immediate characterization. There is also potential for a shift or expansion to a broader conceptual understanding of the roles of ncRNAs and RNA surveillance in currently well-studied processes. Transcriptome diversity between single cells will be very informative about RNA surveillance in heterogeneous populations of cells undergoing development. Owing to the relatively short lifespan of ncRNAs and the established expression and turnover mechanisms associated with these, it is possible that, with further understanding of the underlying dynamics, ncRNAs may be recognized as central ‘on–off’ switches of gene expression. The challenge remains to uncover and characterize more ncRNA functions and further strengthen the burgeoning research of ncRNA surveillance.

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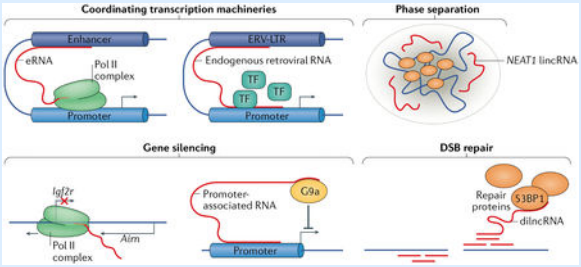
Box 1 |

Functions of different long non-coding RNA species

A major function of long non-coding rNas (lncrNas) is the coordination of gene-expression machinery. enhancer rNas (erNas) are lncrNas transcribed from enhancers; they are key to facilitating interactions between the enhancers and their target promoters through 'chromatin looping'[166] (see the figure). Transposable elements derived from endogenous retroviruses (ERVs) are remnants of ancient viral infections and are abundant in mammalian genomes[167]. although ERV transcripts are regulated by surveillance mechanisms, they can also have profound effects on gene expression, for example, through the assembly of transcription factors (TFs). ERV long terminal repeat (ERV-LTR) transcripts contain binding sites for several TFs, and studies in placental development have attributed a role for ERV-LTRs in enhancer-like recruitment and assembly of the RNA polymerase II (Pol II) complex at developmental genes[168,169]. Finally, some membraneless nuclear bodies, which can form through liquid–liquid phase separation, coordinate the transcription machinery around genes of interest[170]. For example, the long intergenic non-coding RNA (lincRNA) *NEAT1* is required for the formation and structural integrity of paraspeckles by acting as a scaffold for paraspeckle proteins[171,172].

Another vital function of lncRNAs is gene silencing. among the best characterized silencing non-coding RNAs (ncRNAs) is the lincRNA X inactive specific transcript (*Xist*), which is essential for X chromosome inactivation. *Xist* spreads in *cis* over the X chromosome, from which it is transcribed to initiate inactivation[173]. Promoter-associated transcripts are ncRNAs that have been identified for their role in gene silencing by recruitment of histone-modifying enzymes to gene promoters to activate or repress gene expression[174]. For example, in mice, the promoter-associated ncRNA *Airn* (antisense of IGF2R non-protein coding RNA) can recruit the histone methyltransferase G9a to the gene *Igf2r* and silence it[175]. *Airn* can also silence *Igf2r* by transcriptional interference of *Airn* with the *Igf2r* promoter[64].

Aside from gene expression, lncRNAs also have roles in DNA damage repair. For example, transcription at sites of DNA double-stranded breaks (DSBs) results in production of DSB-induced lncRNA (dilncRNA), which can recruit DNA repair proteins such as p53-binding protein 1 (53BP1)[176].



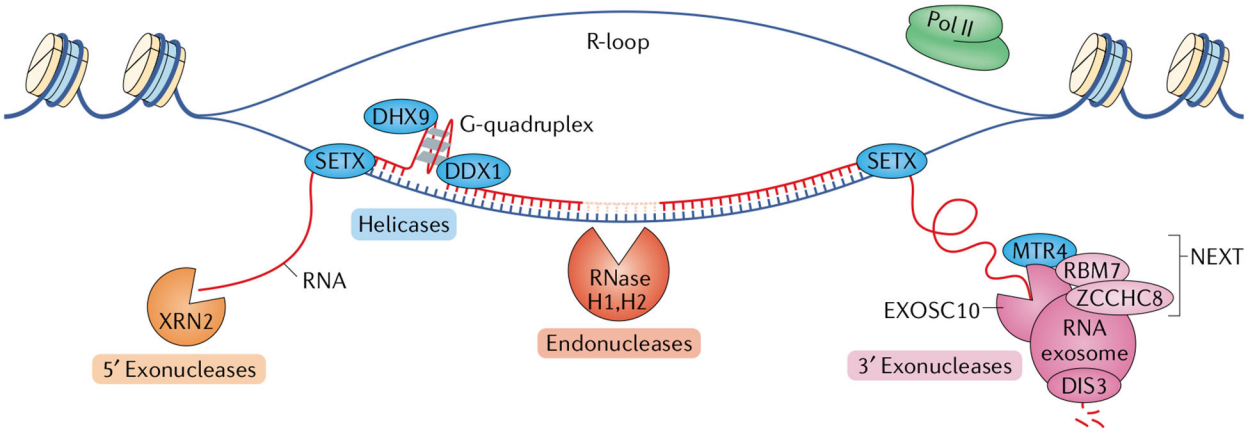


Fig. 1 |. Transcription-associated RNA surveillance.

Several components of RNA surveillance are associated with the transcription of both coding and non-coding RNAs; these include factors for RNA degradation and for RNA–DNA and RNA–RNA unwinding that facilitate said degradation (not shown). The three major degradation machineries are 5′ exonucleases, endonucleases and 3′ exonucleases. Depicted here are the components that are most important for transcriptional surveillance. The proteins 5′–3′ exoribonuclease 1 (XRN1) and XRN2 are the primary 5′ exoribonucleases. XRN2 is located in the nucleus, making it most relevant for transcription-associated 5′ exonucleolytic RNA degradation. XRN2 and the helicase senataxin (SETX) function together in mediating transcription termination (not shown). The ribonuclease H (RNase H) enzymes are the primary endoribonucleases in mammalian cells. RNase H1 is located both in the mitochondria and in the nucleus, whereas RNase H2, which is the main source of transcription-associated endonuclease activity, is located only in the nucleus. The 3′ exoribonuclease most tightly associated with transcription of both coding and non-coding RNA is the RNA exosome complex. Its main catalytic subunit is DIS3, though exosome component 10 (EXOSC10) also has catalytic activity. The RNA exosome often associates with the nuclear exosome-targeting (NEXT) complex comprising RNA-binding protein 7 (RBM7), the scaffolding protein zinc finger CCHC domain-containing protein 8 (ZCCHC8) and the RNA helicase MTR4, which is required for efficient RNA exosome activity. In some cases, such as in the process of class switch recombination in B lymphocytes, SETX joins MTR4 in unwinding RNA–DNA and RNA–RNA substrates to promote RNA exosome activity. Several other RNA helicases function in transcription-associated RNA surveillance, including the DEAD-box (DDX) and DEAH-box (DHX) helicases, which help prevent DNA damage. For example, DDX1 and DHX9 can each unwind G-quadruplex structures to facilitate R-loop resolution. Pol II, RNA polymerase II.

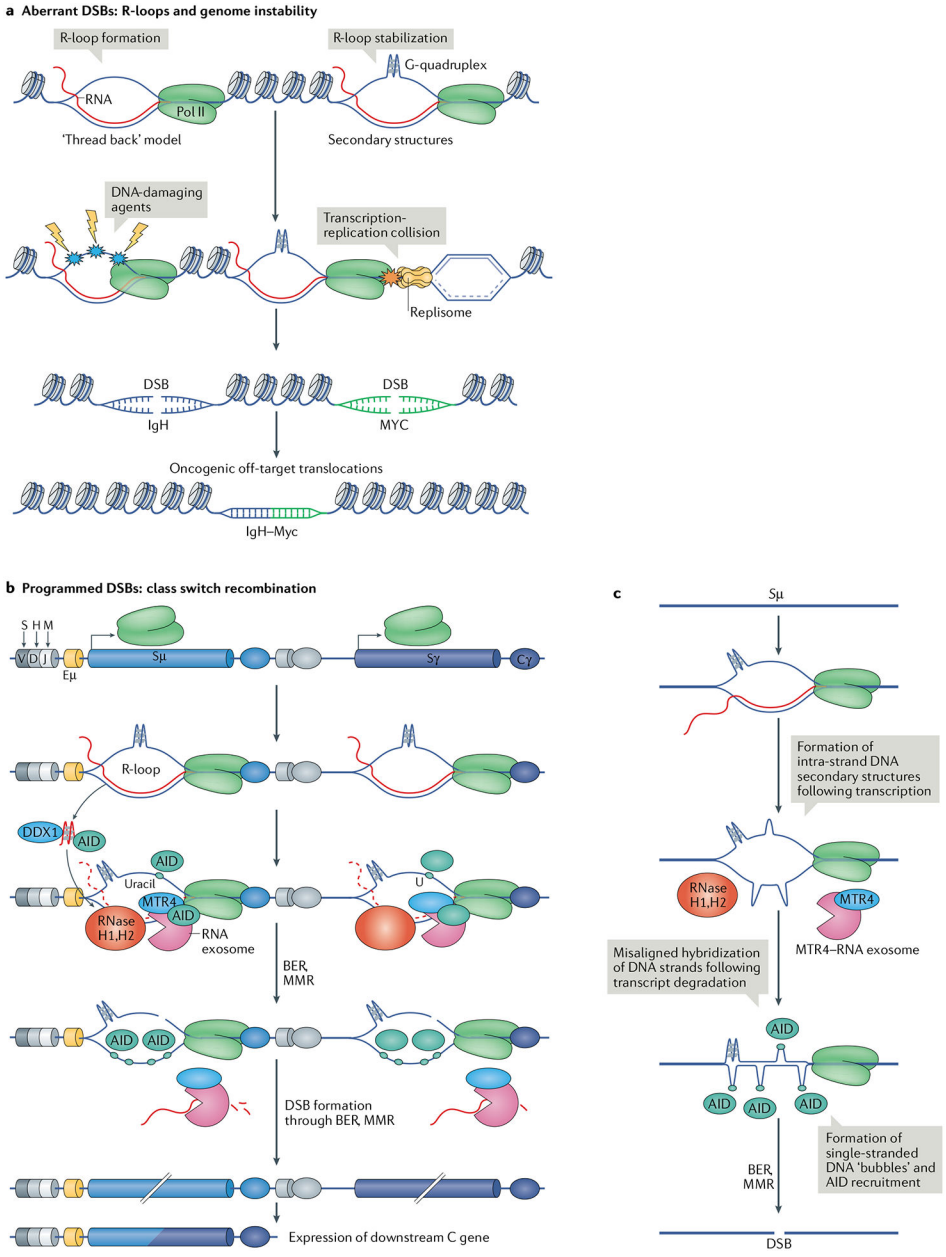


Fig. 2 |. RNA surveillance between deleterious and programmed DNA mutagenesis.

a | The current model of R-loop formation is 'threading back' of the nascent RNA into the DNA. R-loops are stabilized by the formation of secondary DNA structures, such as G-quadruplexes, on the exposed single-stranded DNA (ssDNA). R-loop formation and stabilization is a threat to DNA integrity because the ssDNA is vulnerable to DNA-damaging agents. R-loops and the associated RNA polymerase II (Pol II) can also pose a threat during DNA replication in the form of collisions between Pol II and the replisome. Consequently, R-loop persistence increases the threat of formation of aberrant DNA double-stranded breaks (DSBs), which can cause potentially oncogenic DNA translocations such as the common oncogenic translocation between the immunoglobulin heavy chain locus (IgH) and Myc. **b** | During class switch recombination of the immunoglobulin locus, transcription of

non-coding RNA in switch sequences upstream of constant (C) genes leads to the formation of R-loops. The RNA exosome complex and other enzymes are recruited to these R-loops along with activation-induced cytidine deaminase (AID). R-loop formation allows AID to deaminate cytidines on the non-template strand. AID access to the template strand is enabled by the RNA exosome and ribonuclease (RNase) H1 and RNase H2, which degrade the nascent RNA strand of the RNA–DNA hybrid. Owing to the ability of the non-template DNA strand to form stable secondary DNA structures, the two DNA strands are slow to reanneal; this increases the availability of ssDNA substrates for AID and mutagenesis. G-quadruplex-forming, nascent non-coding RNAs at switch sequences are bound by AID, and this binding and RNA degradation by the RNA exosome are enhanced by the unwinding of the RNA by the helicase DEAD-box 1 (DDX1). Processing of AID-associated mutations in the switch sequence by the base excision repair (BER) or mismatch repair (MMR) pathway leads to DSB formation, ultimately resulting in recombination and expression of the downstream constant gene. **c** | Another possible mechanism of AID recruitment and DSB formation involves transcription in the Sμ segment, which is rich in dG–dC sequences, by Pol II and R-loop formation. The non-transcribed DNA strand, exposed as ssDNA, forms a G-quadruplex that ‘crunches’ the DNA strand and promotes misalignment of the two DNA strands. Upon degradation of the non-coding RNA transcript by the RNA exosome and RNase H enzymes, aberrant dG–dC pairing results in the formation of ‘bubbles’ of ssDNA, which act as AID substrates, thereby resulting in the formation of a DSB.

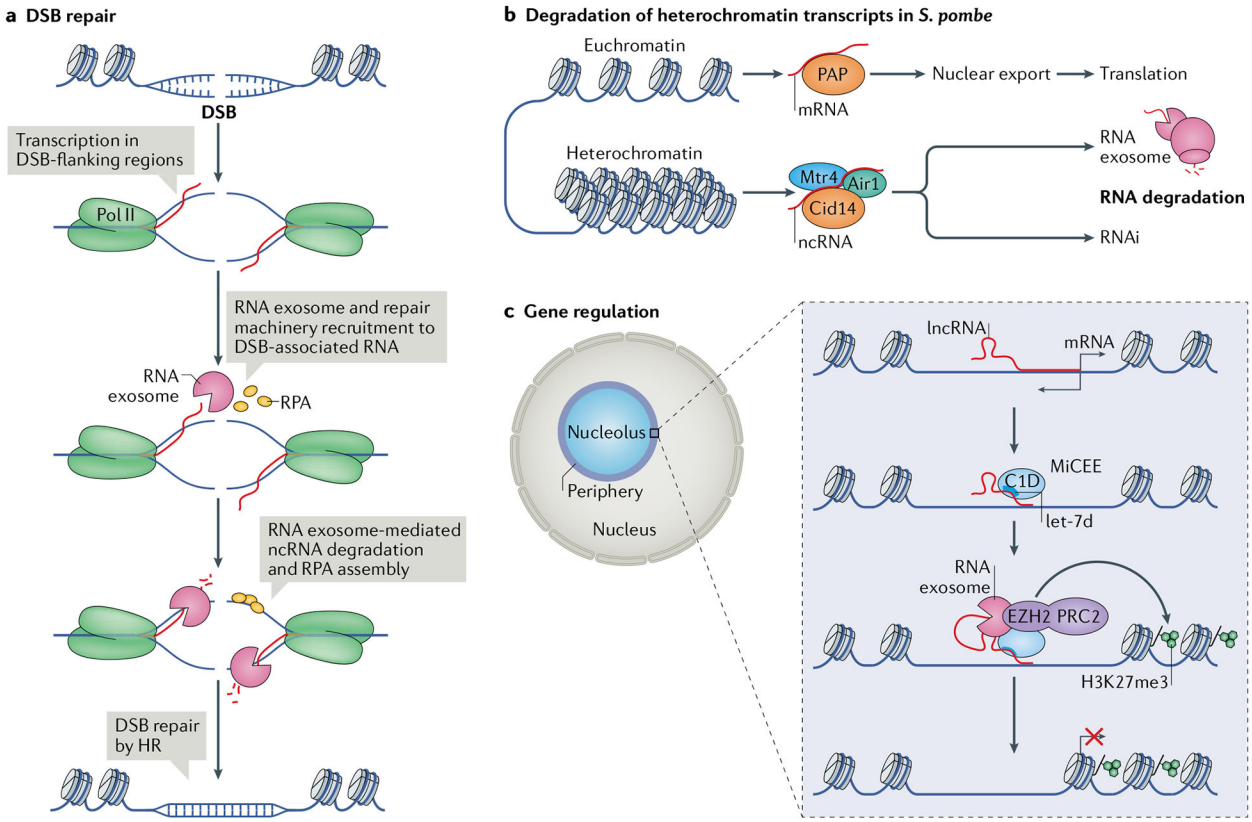


Fig. 3 |. Functions of RNA surveillance in DNA damage repair and chromatin biology.

a | RNA surveillance factors such as the RNA exosome can function in DNA double-stranded break (DSB) repair[97]. Upon DSB formation, the DSB-flanking regions are transcribed and the RNA exosome is recruited to the DSB-associated nascent transcripts along with DNA repair factors such as replication protein A (RPA), which binds to single-stranded DNA (ssDNA). Degradation of the non-coding RNA (ncRNA) by the RNA exosome is coupled with ssDNA binding by RPA, which recruits the homologous recombination (HR) repair machinery and leads to repair of the DSB. **b** | In the yeast *Schizosaccharomyces pombe*, the RNA exosome has a role in degrading heterochromatin-derived transcripts in parallel to RNA interference (RNAi)-mediated degradation[102]. Euchromatin-derived mRNAs undergo processing by poly(A) polymerase (PAP), are exported from the nucleus and undergo translation in the cytoplasm. By contrast, heterochromatin transcripts are processed by the non-canonical poly(A) polymerase Cid14 in association with Mtr4 and Air1. The polyadenylated heterochromatin-derived transcripts are then degraded either by the RNA exosome, the RNAi pathway or both. **c** | Bidirectional transcription from gene promoters produces, in addition to mRNAs, long non-coding RNAs (lncRNAs) that can be bound by the microRNA let-7d–C1D–EXOSC10–EZH2 (MiCEE) complex. EZH2 is the catalytic component of Polycomb repressive complex (PRC2), which catalyses histone H3 Lys27 trimethylation (H3K27me3) and transcriptional silencing[103]. Pol II, RNA polymerase II.

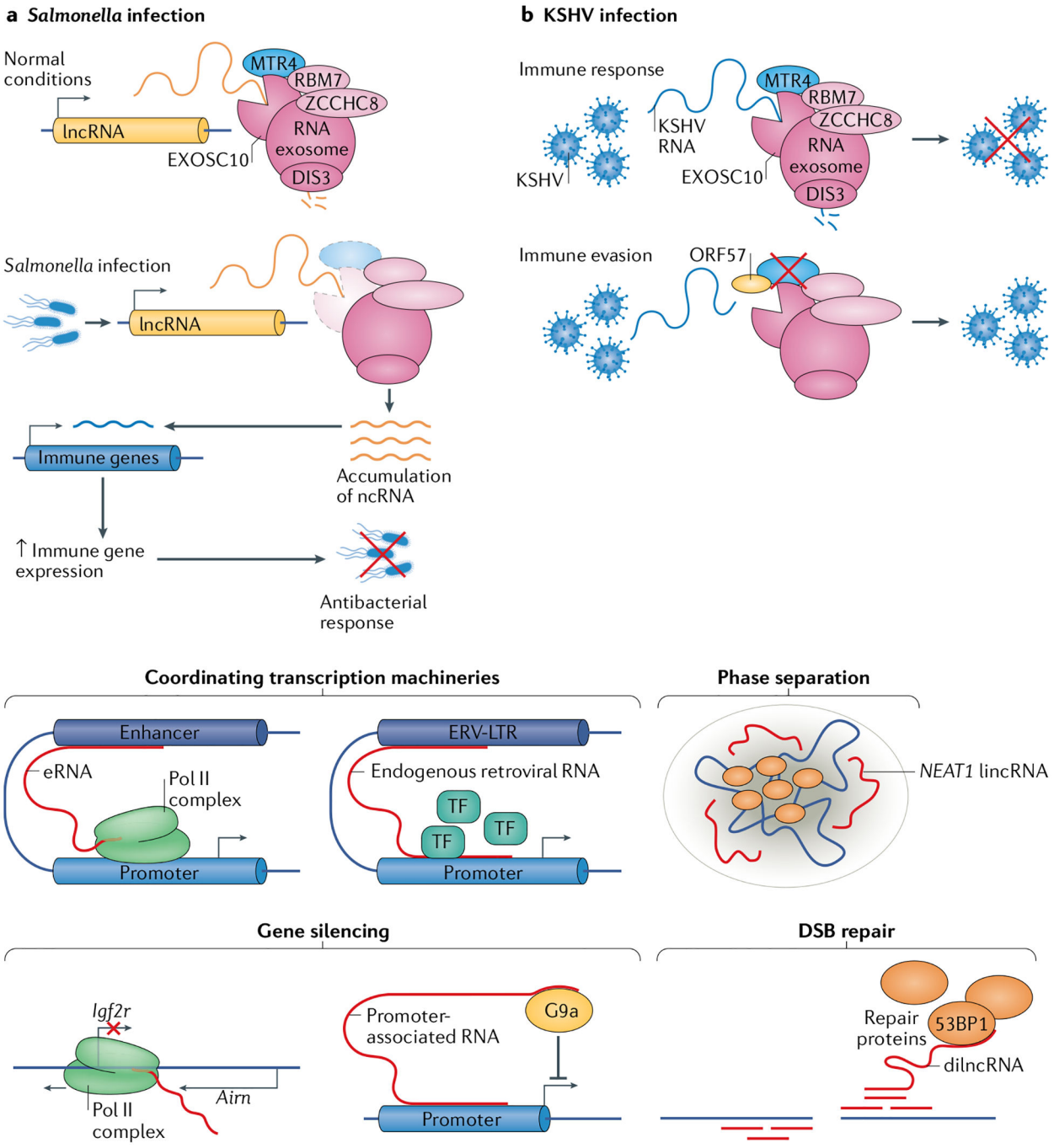


Fig. 4 |. The biological relevance of RNA surveillance.

a | The role of RNA surveillance in bacterial infection is highlighted in the regulation of RNA exosome function in response to *Salmonella enterica* serovar Typhimurium (*Salmonella*) infection. The RNA exosome normally degrades many long non-coding RNA (lncRNA) transcripts. Following *Salmonella* infection, exosome component 10 (EXOSC10) and the RNA exosome-associated helicase MTR4 are degraded, resulting in lncRNA accumulation. These transcripts then activate transcription of immune genes and increase the immune response, thereby clearing the *Salmonella* infection. The specific mechanisms by

which this process occurs are as yet unknown. **b** | A role for RNA surveillance in viral infection was uncovered in studies of host–pathogen interactions in Kaposi's sarcoma-associated herpesvirus (KSHV) infection. KSHV infection is kept at bay by the degradation of viral transcripts by the RNA exosome. However, KSHV has a counter measure: the viral protein ORF57, which prevents access of RNA (including viral transcripts) to the RNA exosome, specifically by blocking access to MTR4. This allows the expression of viral proteins and viral persistence. ncRNA, non-coding RNA; RBM7, RNA-binding protein 7; ZCCHC8, zinc finger CCHC domain-containing protein 8.

Table 1 |

Machineries of non-coding RNA surveillance

Activity	Factor	Main function	Main subunits: related diseases
Endonuclease	RNase H1	Mitochondrial RNA processing, DNA replication	–
	RNase H2	Nuclear R-loop processing, ribonucleotide excision repair	Aicardi-Goutieres syndrome[150,151]
	Integrator complex	Transcription termination	–
3′–5′ Exonuclease	RNA exosome complex	Nuclear and cytoplasmic RNA degradation, R-loop processing	DIS3: multiple myeloma[156]; EXOSC2: retinitis pigmentosa[140]; EXOSC3: pontocerebellar hypoplasia type 1, spinal motor neuron disease[141–144]; EXOSC8: cerebellar and corpus callosum hypoplasia, spinal motor neuron disease[139]; EXOSC10
	PARN	Po1y(A)-specific ribonuclease	Dyskeratosis congenita[177,178]; familial pulmonary fibrosis[179]
5′–3′ Exonuclease	XRN1	Cytoplasmic RNA degradation	–
	XRN2	Nuclear RNA degradation, transcription termination	–
Helicase	MTR4	Processing double-stranded RNA and RNA–DNA hybrids	–
	Senataxin (SETX)	R-loop processing, transcription termination	Ataxia with oculomotor apraxia type 2 (REF[146]); amyotrophic lateral sclerosis[147]
	Aquarius	R-loop processing	–
	DDX1	R-loop processing, G-quadruplex processing	–
	DHX9	R-loop processing, G-quadruplex processing	–
	DDX5	R-loop resolution with XRN2	–
	DDX19	RNA export, R-loop processing	–
	DDX21	R-loop processing	–
	DDX23	R-loop processing	Adenoid cystic carcinoma[88]
Cofactors	NEXT complex	Nuclear cofactor of the RNA exosome, primarily targeting nascent transcripts	MTR4, RBM7: pontocerebellar hypoplasia type 1-like and spinal muscular atrophy[145]; ZCCHC8: familial pulmonary fibrosis[180]
	PAXT complex	Nuclear cofactor of the RNA exosome, targeting poly(A)-tailed transcripts	MTR4, PABPN1, ZFC3H1

DDX, DEAD-box; DHX, DEAH-box; EXOSC, exosome component; NEXT, nuclear exosome targeting; PABPN1, poly(A) binding protein nuclear 1; PARN, poly(A)-specific ribonuclease; PAXT, poly(A)-tail exosome targeting; RBM7, RNA-binding protein 7; RNase, ribonuclease; XRN, 5′–3′ exoribonuclease; ZCCHC8, zinc finger CCHC domain-containing protein 8; ZFC3H1, zinc finger C3H1 domain-containing protein.