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OVERVIEW

Nuclear sorting of RNA

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Abstract

The majority of the mammalian genome is transcribed by RNA polymerase II, yielding a vast amount of noncoding RNA (ncRNA) in addition to the standard production of mRNA. The typical nuclear biogenesis of mRNA relies on the tightly controlled coupling of co- and post-transcriptional processing events, which ultimately results in the export of transcripts into the cytoplasm. These processes are subject to surveillance by nuclear RNA decay pathways to prevent the export of aberrant, or otherwise "non-optimal," transcripts. However, unlike mRNA, many long ncRNAs are nuclear retained and those that maintain enduring functions must employ precautions to evade decay. Proper sorting and localization of RNA is therefore an essential activity in eukaryotic cells and the formation of ribonucleoprotein complexes during early stages of RNA synthesis is central to deciding such transcript fate. This review details our current understanding of the pathways and factors that direct RNAs towards a particular destiny and how transcripts combat the adverse conditions of the nucleus.

This article is categorized under:
RNA Export and Localization > Nuclear Export/Import
RNA Turnover and Surveillance > Turnover/Surveillance Mechanisms
RNA Interactions with Proteins and Other Molecules > Protein–RNA Interactions: Functional Implications

KEYWORDS
RNA decay, RNA exosome complex, RNA retention, RNA sorting

1 | INTRODUCTION

Mammalian genomes produce a variety of RNA subtypes (Djebali et al., 2012; Wu, Yang, & Chen, 2017). Although these contain the same basic ribonucleotide building blocks, cellular machineries are able to distinguish and separate transcripts for their final destinies. For example, mRNA, snoRNA, and unstable long noncoding RNA (lncRNA) are accurately sorted for export to the cytoplasm, localization to subnuclear compartments or for decay, respectively (Chin & Lécuyer, 2017; Palazzo & Lee, 2018). Unsurprisingly, accurate processing and localization of RNA are therefore crucial biogenic steps, the malfunction of which can result in dysregulation of the transcriptome and ultimately contribute to disease (Hurt & Silver, 2008). For newly synthesized RNA, the nucleus acts as a sorting bin, comprising a harsh environment of endo- and exoribonucleolytic activities (Schmid & Jensen, 2018). Hence, transcripts must be processed sufficiently fast and associate with a proper complement of RNA binding proteins (RBPs) to escape nuclear decay. This in turn ensures that only those ribonucleoprotein (RNP) complexes, which pass the checks and balances of nuclear quality control, are sufficiently robust for any downstream activities.

Here, we focus on RNA polymerase II (Pol II)-derived transcripts, which include coding RNAs (mRNA) as well as ncRNA, such as small nuclear/nucleolar RNA (sn/snoRNA), microRNA (miRNA), and the broad encompassing category of

lncRNA. Coding transcripts are generally exported to the cytoplasm and bound by ribosomes to serve in protein translation. In contrast, many ncRNAs function through molecular interactions with proteins in RNP complexes in both the nucleus and the cytoplasm. The primary mechanism to guide RNAs to their ultimate fate is through associations of *trans*-acting RBPs, which can be recruited via sequence motifs, structural elements, or RNA modifications, such as the 5′,7-methylguanosine (m^7G) cap or the 3′-polyadenylation (pA) tail (Palazzo & Lee, 2018). The majority of these interactions manifest during transcriptional and early RNA processing events and provide a selection for transcripts into their respective downstream pathways. It has been suggested that relevant molecular interactions work in a combinatorial fashion, where an assortment of productive and destructive factors compete to define the ultimate outcome for any particular RNA (Giacometti et al., 2017; Schulze, Stein, Rettel, Nanao, & Cusack, 2018). For a functional RNA, it needs to localize to its site of cellular action, which may comprise a cellular compartment such as the cytoplasm for exported mRNAs, or a niche localization, such as *XIST* RNA retention on the inactive X-chromosome (Galupa & Heard, 2015). Moreover, some RNAs are shuttled between multiple subcellular compartments as part of their maturation pathways. For example, most snRNAs are exported to the cytoplasm to undergo final processing and assembly with seven-member proteins before re-entry to the nucleoplasm and final accumulation in Cajal bodies (CABs; Kiss, 2004). In contrast, nonfunctional, or aberrant, RNAs are efficiently removed shortly after their synthesis to prevent their processing and/or progress into specific locales where they might cause undesired off-target effects.

In this review, we highlight early RNA processing events in mammalian cells, with an emphasis on human cells unless otherwise specified, and discuss how they drive the dynamic competition that define RNA fate. Moreover, we describe RNA/RNP features that increase the tendency of transcripts to be retained in the nucleus and we discuss how they may evade nuclear degradation to maintain copy numbers compatible with function.

2 | MAIN BODY

2.1 | RNA processing: Orchestration of export versus decay

RNA processing events create mature products while being themselves subject to the watchful eye of surveying quality control. The addition of a 5′ cap, removal of introns and 3′ end formation mostly occur cotranscriptionally on nascent RNA. However, the mere act of processing is not enough for nuclear export, as many ncRNAs undergo similar processing steps but are ultimately retained in the nucleus or degraded. Thus, while early maturation processes contribute to determining RNA fate, it is ultimately the combinatorial action of multiple factors that guide transcripts towards their endgame. Given this dynamic nature of RNP assembly, it is likely that transcript fate is not completely fixed from sequence but rather is dictated by the sum and timing of dynamic molecular interactions, forming in the particular cell type in question.

2.2 | Capping: Creating a platform for early factor binding

The addition of a m^7G cap to the 5′ end of a nascent RNA is a pioneering determinant of transcript fate. This modification is exclusive to Pol II transcripts as the enzymes necessary for cap formation are recruited via the serine-5 phosphorylated C-terminal domain, uniquely found in this enzyme (Cho, Takagi, Moore, & Buratowski, 1997; McCracken et al., 1997). The 5′ cap serves dual functions for RNA fate. First, the m^7G modification readily protects nascent transcripts from degradation by nuclear 5′–3′ exoribonuclease enzymes (Parker & Song, 2004). Second, the cap structure provides a *cis*-element, that recruits the highly conserved cap binding complex (CBC) heterodimer, consisting of NCBP1 (CBP80) and NCBP2 (CBP20) (Gonatopoulos-Pournatzis & Cowling, 2014). NCBP2 directly binds the m^7G cap, whereas NCBP1 acts as an adaptor protein, mediating the contact to downstream effector proteins/complexes (Giacometti et al., 2017; Izaurralde et al., 1994; Mazza, Ohno, Segref, Mattaj, & Cusack, 2001; Schulze & Cusack, 2017). The CBC is proposed to bind all capped nascent transcripts, regardless of their ultimate localization or utility, and it acts as a central player in RNA sorting by forming diverse, and competitive, interactions with proteins involved in productive as well as destructive activities. CBC–RNA interactions include those to precursor and processed forms of mRNAs, replication dependent histone (RDH) mRNAs, pre-snRNAs, a few independently transcribed snoRNAs and a multitude of lncRNAs, comprising stable but also spurious unstable transcripts, such as promoter upstream transcripts (PROMPTs, also known as upstream antisense RNAs [uaRNAs]) and enhancer RNAs (eRNAs) (Schulze & Cusack, 2017). Given the diverse destinies of these commonly CBC-bound transcript classes, they must require other factors to further define their biotype.

Early CBC-interacting proteins include the negative elongation factor E (NELF-E) component of the multisubunit NELF complex and the highly conserved metazoan protein ARS2 (SRRT) (Andersen et al., 2013; Narita et al., 2007; Schulze &

Cusack, 2017). Along with the DRB sensitivity-inducing factor, the NELF complex is involved in promoter-proximal pausing of Pol II, which allows for 5′ capping of the nascent RNA before switching to the elongation phase of transcription (L. Core & Adelman, 2019; Mandal et al., 2004; Scruggs et al., 2015). ARS2 forms a trimeric complex with the CBC (termed the CBC-ARS2 [CBCA] complex; Andersen et al., 2013; Hallais et al., 2013), which can act as a mediator of both RNA transport and decay pathways (Andersen et al., 2013; Hallais et al., 2013; Schulze et al., 2018). NELF-E and ARS2 occupy the same site within a binding pocket, formed by NCBP1 and NCBP2, and are thought to be mutually exclusive CBC interactors, possibly representing different time points of the early transcription process (Schulze & Cusack, 2017). As NELF is involved in promoter-proximal Pol II pausing and ARS2 is a platform for RNA sorting, it is presumed that these proteins bind the CBC sequentially, with NELF bound first and later exchanged by ARS2, which then remains bound from the elongation phase onwards (Figure 1a).

CBCA-bound RNAs can be subsequently packaged into a variety of RNPs. Factors that promote RNA sorting into functional RNPs include the nuclear export factor ALYREF and the phosphorylated adaptor for RNA export (PHAX), which are involved in linking CBCA-bound mRNA and snRNA/snoRNA to their respective export and localization pathways (Figure 1b; Fan et al., 2017; Gromadzka, Steckelberg, Singh, Hofmann, & Gehring, 2016; Hallais et al., 2013; Ohno, Segref, Bachi, Wilm, & Mattaj, 2000). ALYREF associates with CBCA-bound pre-mRNA through NCBP1 and recruits the transcription/export machinery (TREX) to promote nuclear export via the nuclear export factor 1 (NXF1/TAP) (Figure 1c,i) (Cheng et al., 2006; Masuda et al., 2005; Shi et al., 2017). ALYREF also binds to the exon junction complex (EJC) and recent data suggests that the initial recruitment of ALYREF by the CBC leads to its transfer throughout the RNA at sites adjacent to bound EJCs (Viphakone et al., 2019). This highlights that proper cap formation, together with efficient splicing, cements ALYREF binding and mark these transcripts towards nuclear export. However, this is not yet a full commitment as exemplified by nuclear retained lncRNAs, such as *XIST*. These transcripts can be capped, spliced and bound by export factors, including ALYREF and CHTOP, but still show comparatively reduced binding of NXF1 compared to exported mRNAs (Viphakone et al., 2019). Other signals, bound by proteins that mediate *XIST* retention, such as HNRNPU, must therefore overrule the drive towards export (Sakaguchi et al., 2016). Additionally, how ALYREF distinguishes stable RNAs destined for export from RNAs destined for decay is yet to be fully determined. One clue is that unstable RNAs, such as PROMPTs, eRNAs and some lncRNAs, are often not spliced, preventing the stable deposition of ALYREF (Schlackow et al., 2017; Viphakone et al., 2019) and thus prolonging nuclear residence time contributing to decay (Fan et al., 2017).

It has been suggested that transcript length plays a role in the sorting of smaller functional RNAs, such as snRNAs and capped snoRNAs, from mRNA. PHAX binds to the CBCA complex (forming the CBC-ARS2-PHAX [CBCAP] complex; Andersen et al., 2013; Hallais et al., 2013) to promote the nuclear export of snRNAs and the intranuclear localization of snoRNAs to CABs and nucleoli (Figure 1c,ii) (Boulon et al., 2004; Mourao, Varrot, Mackereth, Cusack, & Sattler, 2010; Ohno et al., 2000; Segref, Mattaj, & Ohno, 2001). For transcripts longer than 300 nt, PHAX was proposed to be displaced by a tetrameric complex of the hnRNPC protein, which then directs pre-mRNAs towards nuclear export via ALYREF (McCloskey, Taniguchi, Shinmyozu, & Ohno, 2012; Ohno, Segref, Kuersten, & Mattaj, 2002). However, this is somewhat at odds with recent iCLIP data, showing PHAX association with a large repertoire of longer RNAs, including lncRNAs and mRNAs (Giacometti et al., 2017). This therefore led to the suggestion that CBC-associated complexes are highly dynamic and undergo constant remodeling until other cues are sensed to form a signature that ultimately defines the transcript (Figure 1b).

RDH mRNAs are highly transcribed during S-phase of the cell cycle to provide histone proteins essential for packaging of newly replicated DNA into chromatin. These transcripts are capped but not polyadenylated. Instead, they are cleaved after a conserved sequence in the 3′ UTR, that forms a stem loop structure (discussed in Section 2.4; Marzluff & Koreski, 2017). Correct biogenesis of RDH mRNAs depends on their interaction with the FLICE associated huge protein (FLASH), which also interacts with the CBCA (Figure 1c,iii) (Hofmann et al., 2006; Kiriyama, Kobayashi, Saito, Ishikawa, & Yonehara, 2009; O'Sullivan et al., 2015; Schulze et al., 2018). RDH mRNA 3′ end processing differs from canonical mRNA processing and this, in partnership with FLASH at the 5′ end, likely commits these transcripts for maturation. Again, the recruitment of FLASH to ARS2 requires the competition against ALYREF and other CBCA interactors (Schulze et al., 2018), which may be aided by the localized transcription of RDH mRNA loci in the physical vicinity of CABs where high concentrations of RDH mRNA processing factors U7 snRNP and FLASH reside (Barcaroli et al., 2006; Frey & Matera, 1995; Handwerger & Gall, 2006; Ogg & Lamond, 2002; Schulze et al., 2018).

While CBCA interactions promote productive RNA pathways, ARS2 is also capable of directing RNAs towards nuclear retention and decay (Andersen et al., 2013; Schulze et al., 2018). Transcripts meeting this fate include PROMPTs, eRNAs, 3′ extended sn/snoRNAs and longer polyadenylated transcripts, including spliced snoRNA host gene lncRNAs (Andersen et al., 2013, Andersson et al., 2014; Lubas et al., 2015; Meola et al., 2016; Ntini et al., 2013; Ogami et al., 2017). In doing so, ARS2

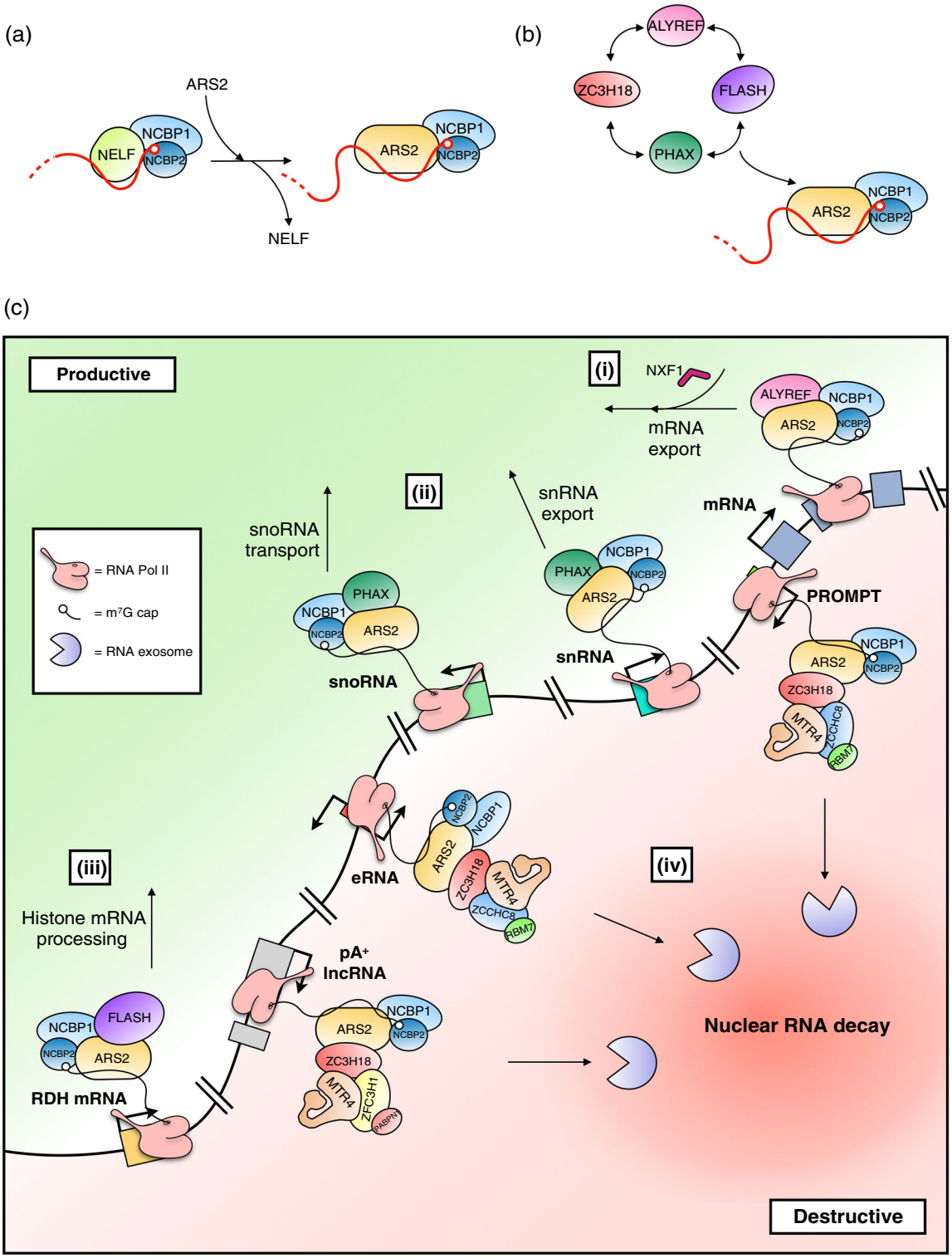

FIGURE 1 Cap binding complex (CBC) associating factors provide a first step in defining Pol II transcript fate. The formation of CBC-containing RNPs is dynamic due to mutually exclusive interactions of various adaptors. (a) Nascent CBC-bound RNAs are targeted by the negative elongation factor (NELF) complex, involved in early transcriptional pausing. As transcription enters the elongation stage, NELF is replaced by ARS2 to form the CBCA complex. (b) The CBCA complex provides a platform for subsequent competitive interactions of ALYREF, PHAX, FLASH, and ZC3H18, hereby initializing the creation of RNP identity. (c) ARS2 in turn interacts with RNP adaptors that connect to downstream productive or destructive pathways: (i) the TREX component ALYREF promotes nuclear export of mRNAs via the NXF1 export receptor; (ii) smaller RNAs, such as snRNAs and independently transcribed snoRNAs, are bound by CBCA and PHAX, forming the CBCAP complex, which connects these transcripts to their respective export and localization pathways; (iii) downstream processing of RDH mRNAs is facilitated through association of FLASH to ARS2, and (iv) pervasively transcribed transcripts, such as eRNAs, PROMPTs and many lncRNAs, are targeted for nuclear decay via the interaction of the CBCA complex with ZC3H18, which in turn recruits nuclear exosome targeting complexes NEXT and/or PAXT (see Figure 4 for further detail)

connects with nuclear decay machineries (described in Section 2.5) for example via the zinc finger protein ZC3H18, which aids in targeting transcripts for rapid degradation via the ribonucleolytic RNA exosome complex (Figure 1c,iv) (Andersen et al., 2013; Meola et al., 2016; Silla, Karadoulama, Mąkosa, Lubas, & Jensen, 2018; Winczura et al., 2018). Keeping with the theme of competitive binding, the interaction of ZC3H18 with the CBCA is mutually exclusive with PHAX binding (Giacometti et al., 2017). Thus, the CBCA is in perpetual flux in binding to interchangeable productive as well as destructive adaptors (Figure 1b,c). This early RNP remodeling appears then to be responsive to other surrounding signals such as mRNA splicing, which enhances the propensity of ALYREF binding, or such as local surroundings, which brings RDH mRNA transcription near CABs with a high local concentration of FLASH.

2.3 | Splicing: Enhancing export

Splicing often occurs during the elongation phase of Pol II transcription and it has long been accepted that the presence of an intron enhances RNA export (Carrillo Oesterreich et al., 2016; Luo & Reed, 1999). A reason for this is presumably that the spliceosome directly interacts with a number of export factors, including the RNA helicase UAP56, which aids in the loading of TREX during co-transcriptional processing (Fleckner, Zhang, Valcárcel, & Green, 1997; Sträßer et al., 2002). However, cDNA derived RNAs lacking introns can also be export competent, demonstrating that splicing is not fundamental for export, but rather facilitating in combination with other mechanisms (Palazzo & Akef, 2012). Consistently, naturally intronless RNAs are still able to recruit TREX and NXF1/TAP (Rodrigues et al., 2001; Taniguchi & Ohno, 2008), likely due to their coupling with other processing machineries, such as the interaction between the CBC and ALYREF as well as the 3′ end processing factors of RDH mRNAs and NXF1 (Marzluff, Wagner, & Duronio, 2008; Nojima, Hirose, Kimura, & Hagiwara, 2007). Still, intronless cDNA-derived transcripts are less efficiently exported compared to their spliced endogenous counterparts. Classic examples are the *β-globin* and *Fushi tarazu* (FTZ) reporter transcripts, whose cDNA-derived versions are retained in the nucleus and degraded (Buchman & Berg, 1988; Luo & Reed, 1999). This could again be interpreted as an issue of timing: In the absence of splicing, delayed export kinetics allow time for nuclear decay to intervene. In addition, it has been reported that the 3′ end of *β-globin* RNA contains a nuclear retention element, that can be overcome by splicing of the transcript (Akef, Lee, & Palazzo, 2015). Such *cis*-elements are not well understood, but their presence appears to actively promote nuclear RNA confinement (discussed in Section 2.8).

Taken together, the balance between export, retention and decay can be tipped in favor of export as a result of splicing because it reduces nuclear residence time by promoting the loading of TREX components and their resulting formation of export-ready RNPs. This in turn discourages decay as export simply precedes the assembly of nuclear degradation complexes on such RNAs. For more stable nuclear RNAs that are spliced, these transcripts require active mechanisms to overcome the drive to export (described in Section 2.7).

2.4 | RNA 3′ end formation: Termination and protection

The final step in RNA processing is the 3′ end formation of the nascent transcript and the termination of Pol II transcription. These events are often coupled and several pathways are implicated (Porrua, Boudvillain, & Libri, 2016; Porrua & Libri, 2015; Proudfoot, 2016). As with RNA 5′ end processing and its stimulation of formation of distinct CBC-containing complexes, 3′ end processing of RNA influences RNP formation and therefore transcript fate. The establishment of particular 3′ end processing complexes is directed by *cis*-elements within the 3′ end of the nascent RNA (Mandel, Bai, & Tong, 2008). Canonical 3′ end processing of mRNA utilizes the 3′ end cleavage and polyadenylation (CPA) machinery, which is typically recruiting factors to promote nuclear export (Chan, Choi, & Shi, 2011; Xiang, Tong, & Manley, 2014). Several ncRNAs employ CPA but alternative transcription termination/3′ end processing pathways are also at play, exemplified by for example, snRNAs (Matera, Terns, & Terns, 2007). Generally, RNA 3′ ends require some form of stability elements to ensure their protection towards exonucleolytic attack. The classical example here is the pA tail and its attached polyA binding proteins (PABs). However, transcripts lacking pA tails have also adapted ways to ensure nuclear stability.

The major 3′ end formation pathway in metazoans comprises RNA cleavage by the CPSF73 endonuclease followed by polyadenylation of the produced 3′ end by polyA polymerase (PAP). These processes are carried out by multiple protein complexes, including the CPA specificity factor (CPSF), cleavage stimulatory factor (CstF), Cleavage factors I and II (CFI and CFII) and PAP (Mandel et al., 2008). Assembly of the necessary factors on the nascent pre-RNA is directed by the presence of a consensus polyadenylation signal (PAS), AAUAAA (or some of its variants), found upstream of the cleavage site (Proudfoot, 2011). This is aided by less conserved enhancing elements, the G/U rich downstream element (DSE) and the U-

rich upstream auxiliary element (UAE) (Figure 2,i; Zhao, Hyman, & Moore, 1999). The 3′ end processing machinery can be physically and functionally linked to export factors such as ALYREF (S. A. Johnson, Cubberley, & Bentley, 2009; Shi et al., 2017), THOC5 (TREX) (Katahira et al., 2013; Tran, Koch, & Tamura, 2014), and NXF1/TAP (Ruepp et al., 2009). Combined with previously formed CBC subcomplexes on the nascent RNP, this further mediates nuclear export.

As mentioned, RDH mRNAs do not contain genomic encoded PAS sequences and consequently these transcripts are not polyadenylated. Instead, they harbor a conserved stem-loop sequence, that is bound by the stem-loop binding protein (SLBP) and a purine-rich histone downstream element (HDE), which base pairs with the U7 snRNA (Figure 2,ii; Marzluff & Koreski, 2017). As with canonical mRNAs, RDH mRNAs are also cleaved by CPSF73, which, in this case, is recruited by SLBP and the U7 snRNP. SLBP remains bound to RDH mRNAs during transcription, export and translation, and is proposed to confer protection to their 3′ ends (Wilusz, 2016). Moreover, depletion of SLBP results in the nuclear retention of histone transcripts, demonstrating its relevance for export (Marzluff et al., 2008; Sullivan, Mullen, Marzluff, & Wagner, 2009). However, nuclear retained RDH transcripts remain stable even in the absence of SLBP and therefore must employ additional means to avoid decay (Sullivan et al., 2009). Nuclear export of RDH mRNA occurs via the NXF1/TAP pathways, which in this case depends on the interaction with the U7 snRNP (Eckner, Ellmeier, & Birnstiel, 1991; Erkmann, Sànchez, Treichel, Marzluff, & Kutay, 2005; Williams, Ingledue, Kay, Marzluff, & Marzluff, 1994). More recently it was also shown that ALYREF binds to RDH mRNA adjacent to its stem loop and interacts with SLBP to facilitate export (Fan et al., 2019). Interestingly, CBC bound factors, ARS2 and NELF, have also been implicated in RDH mRNA 3′ end formation, suggesting a possible functional crosstalk between 5′ ends and 3′ ends for these short RNAs (Gruber et al., 2012; Hallais et al., 2013; Iasillo et al., 2017; Narita et al., 2007). Despite this, and the fact that RDH mRNAs utilize a distinct 3′ end processing machinery with only a subset of CPSF factors, they ultimately appear to couple with the same export machinery employed by polyadenylated RNAs.

3′ end formation of snRNAs and independently transcribed snoRNAs is carried out by the multisubunit integrator (INT) complex (Baillat et al., 2005). Similar to the PAS and stem loop motifs of mRNAs and RDH mRNAs, snRNA 3′ end processing is believed to depend on a conserved sequence element, termed the 3′ box, being recognized by the INTS9 and INTS11 homologs of CPSF73 and CPSF100, respectively (Figure 2,iii; Baillat et al., 2005; Ezzeddine et al., 2011). As for RDH mRNAs, ARS2 and NELF have been implicated in snRNA 3′ end formation (Andersen et al., 2013; Hallais et al., 2013; Iasillo et al., 2017), although the mechanism still remains to be elucidated. snRNAs are exported from the nucleus as 3′ extended precursors, which are subjected to further trimming in the cytoplasm and assembled into snRNPs before returning to the nucleus for final maturation in CABs (Shaw, Eggleton, & Young, 2008). Whether 3′ end processing plays any role in snRNA export is not clearly understood, but it is tempting to speculate that proper 3′ end formation by INT may act as a promotional signal to commit the competitive binding of the snRNA-specific export factor PHAX to the CBCA-bound complex at the 5′ end of the transcript (Giacometti et al., 2017).

Curiously, 3′ end formation of a number of stable nuclear lncRNAs, including *NEAT1* and *MALAT1*, involves an alternative 3′ end processing pathway orchestrated by the RNaseP enzyme normally involved in the 3′ end cleavage of Pol III transcribed tRNA precursors (Kirsebom, 2007; Figure 2,iv). In consequence, these transcripts are not polyadenylated but instead exploit genome-encoded U- and A-rich sequences, which engage in 3′ end triple helical structures (Wilusz, 2016; Y. Zhang, Yang, & Chen, 2014). Seemingly, this confers protection against 3′–5′ exonucleases and promotes stability in the nucleus. Similar triple helical structures are found in the *PAN* lncRNA from Kaposi's sarcoma-associated herpesvirus (KSHV) and in other viral RNAs (Mitton-Fry, DeGregorio, Wang, Steitz, & Steitz, 2010; Tycowski, Shu, Borah, Shi, & Steitz, 2012). The absence of canonical 3′ end processing machineries and the lack of splicing likely ensures that these lncRNAs are not loaded with export factors. Furthermore, *NEAT1* and *MALAT1* appear to be actively retained in the nucleus by binding to specific nuclear proteins (discussed in Section 2.7), which may also compete against export and decay factors.

While stable RNAs predominantly succeed in connecting their processing to cellular localization pathways, the processing of unstable RNAs appears to instead trigger their decay (Andersen et al., 2013). The finer details behind termination of spurious unstable RNAs are somewhat unclear as both CPA and INT complexes, along with ARS2, have been reported to be involved (Almada, Wu, Kriz, Burge, & Sharp, 2013; Iasillo et al., 2017; Lai, Gardini, Zhang, & Shiekhattar, 2015; Ntini et al., 2013). In addition, these RNAs are proposed to have multiple, sporadic termination points with a high number of early PASs and relatively low intronic content in comparison to more stable RNAs (Almada et al., 2013; L. J. Core et al., 2014; Duttke et al., 2015; Ntini et al., 2013). Taken together this hints that such RNA species may undergo inefficient or aberrant 3′ end processing, producing unprotected 3′ ends, that are obvious targets for 3′–5′ exonucleases, such as the nuclear exosome complex (Andersson et al., 2014; Y. Chen et al., 2016; Flynn, Almada, Zamudio, & Sharp, 2011; Preker et al., 2008).

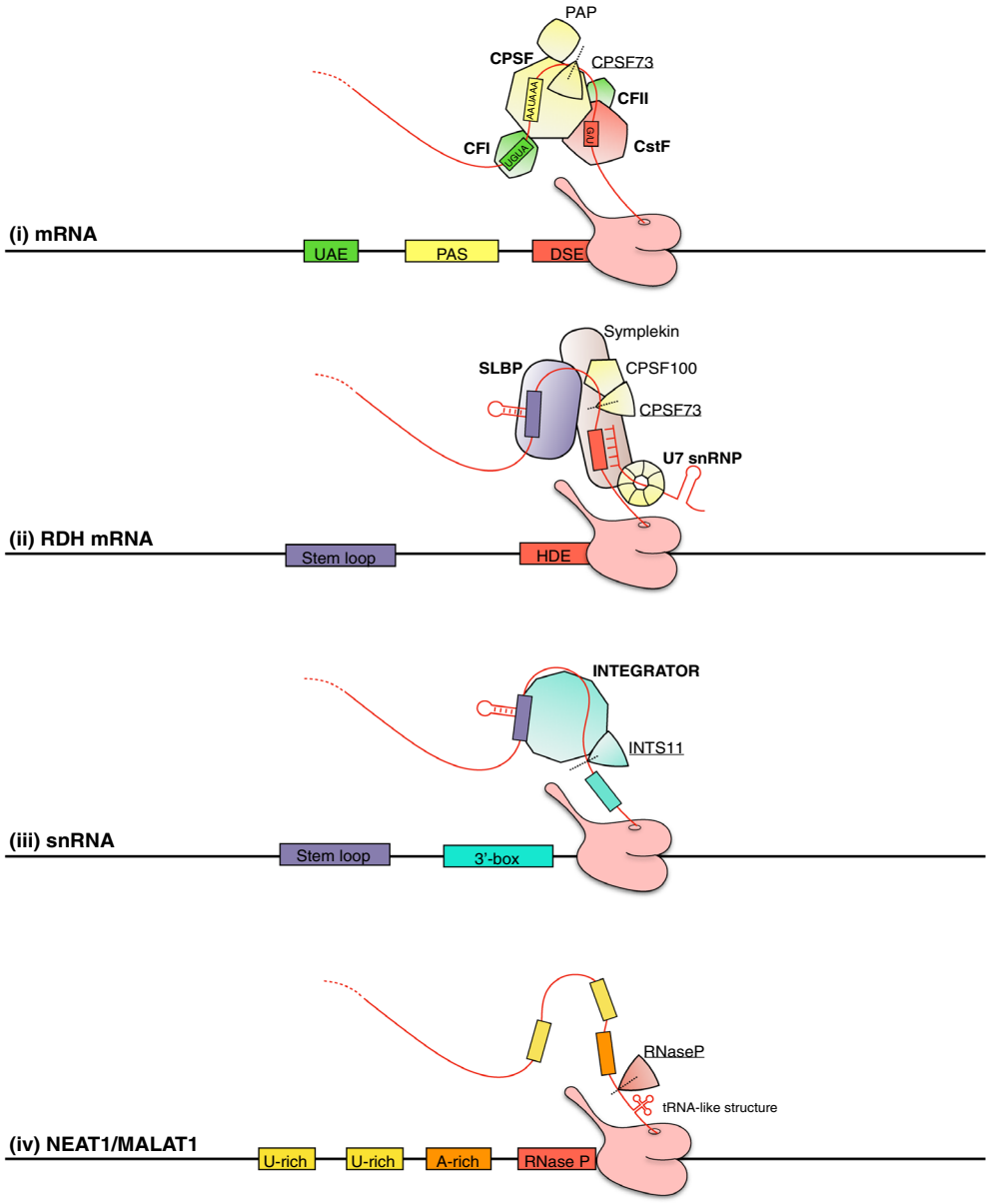

FIGURE 2 Cleavage and termination complexes. The recruitment and action of distinct cleavage and 3′-end processing complexes is influenced by *cis*-elements within the 3′-end of the nascent RNA. (i) Canonical mRNAs utilize the cleavage and polyadenylation machinery, predominantly guided by the presence of a polyadenylation signal (PAS), aided by an upstream auxiliary element (UAE) and downstream element (DSE). Together, these recruit the cleavage and polyadenylation factor (CPSF), cleavage factors I and II (CFI, CFII) and cleavage stimulatory factor (CstF). RNA cleavage is carried out by CPSF73 and subsequently transcripts are polyadenylated by PAP. (ii) RDH mRNAs contain conserved stem loop sequences and histone downstream elements (HDEs), which are bound by the stem loop binding protein (SLBP) and the U7-snRNP, respectively. Cleavage is also carried out by CPSF73 and the resulting 3′ end remains bound to SLBP throughout downstream processing. (iii) snRNAs and a small number of independently transcribed snoRNAs are processed by the integrator (INT) complex. This is recognized by conserved stem loop and 3′ box motifs in the nascent transcript. Cleavage is carried out by the CPSF73 homolog INTS11. (iv) The 3′ end of *MALAT1* and *NEAT1* transcripts are processed by tRNA biogenesis factors due to presence of an RNaseP sequence element. These RNAs form an unusual triple helical structure owing to complementary U- and A-rich sequences upstream of the cleavage site

2.5 | The nuclear decay machinery: Maintaining control

All of the RNA processing pathways discussed are constantly challenged by RNA decay activities, largely governed by exonucleases, which degrade transcripts from their 5′- or 3′-ends and therefore require free accessibility. Terminal modifications and/or the binding of RBPs provide protection from surveying exonucleases and thereby promote stability in the nucleus (Figure 3).

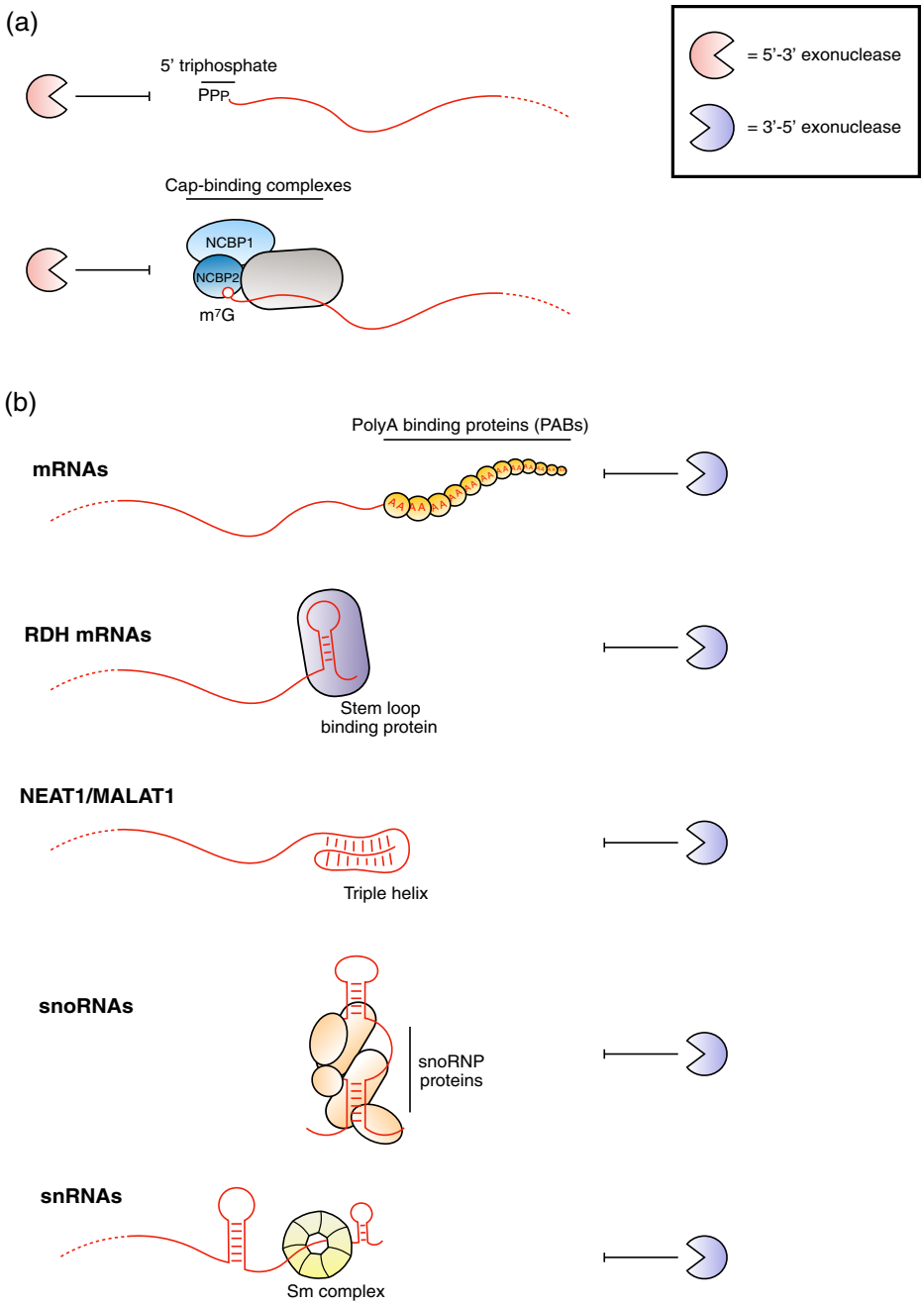

FIGURE 3 Defense against exonucleases. To survive the harsh environment of surveying nuclear exonucleases, RNAs require protective elements at their 5′ and 3′-ends. (a) 5′-end protection. Initiating nascent RNAs have a 5′ triphosphate, which is incompatible with 5′–3′ exonucleolytic decay. This 5′-end is matured with a m7G cap, which is subsequently bound by the cap binding complex (CBC), providing additional protection. (b) 3′-end protection. Most mRNAs are protected at their 3′-ends by the polyA tail and its bound polyA binding proteins (PABPs). Nonpolyadenylated transcripts, such as replication dependent histone (RDH) RNAs, harbor distinct stem loop structures bound by the stem loop binding protein (SLBP) to provide transcript stability. Some nuclear retained lncRNAs, such as *NEAT1* and *MALAT1*, form unique triple helical 3′-ends, owing to genomic encoded A- and U-rich stretches, which are inaccessible for 3′–5′ exonucleases. Small nucleolar RNAs (snoRNAs) are tightly bound into snoRNP structures, shielding their 3′ ends. Small nuclear RNAs (snRNAs) are bound by a group of Sm proteins in the cytoplasm. These constitute a heptameric ring complex that forms on the RNA before reimport to the nucleus

Nuclear decay, carried out by the 5′–3′ exonuclease XRN2, is important for quality control of uncapped RNA, including the removal of spliced out introns and the downstream fragments following pre-RNA 3′ end cleavage. The 5′ ends of nascent Pol II transcripts are tri-phosphorylated and provides initial protection from XRN2, which requires a monophosphate 5′ end for activity. Subsequent m7G capping and binding of the CBC reinforces shielding against XRN2 (Miki & Großhans, 2013) (Figure 3a). Transcripts with aberrant 5′ ends, due to their improper capping or methylation, are subjected to quality control by the decapping and exonuclease protein, DXO (Jiao, Chang, Kilic, Tong, & Kiledjian, 2013). This decapping mechanism leaves a 5′ monophosphate, which provides a suitable substrate for XRN2. Resultingly, cells depleted of DXO accumulate transcripts with aberrant caps. Interestingly, these RNAs are also inefficiently spliced and polyadenylated, suggesting that biology at the RNA 5′ end provides a crucial early mark to prevent aberrant transcripts from moving through normal processing (Jiao et al., 2013).

A central component of nuclear 3′–5′ exonucleolytic pathways is the essential RNA exosome complex, involved in the regulation of the majority of cellular transcripts (Kilchert, Wittmann, & Vasiljeva, 2016; Mitchell, Petfalski, Shevchenko, Mann, & Tollervey, 1997; Schmid & Jensen, 2018). The nuclear exosome participates in both RNA processing and complete

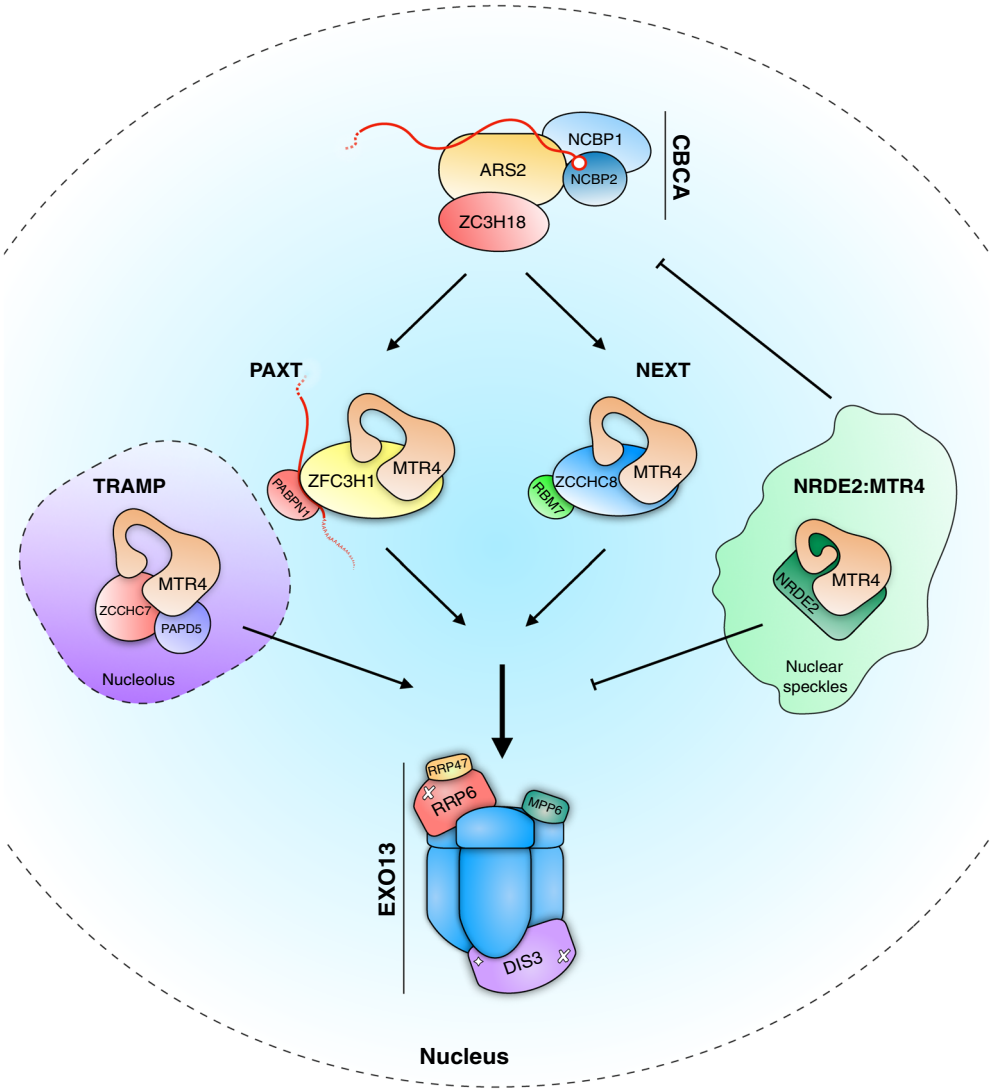

FIGURE 4 Nuclear exosome targeting complexes. The ribonucleolytic nuclear exosome complex is regulated by interactions with distinct targeting and inhibitory complexes/factors. The core exosome (EXO9) forms a catalytically inert barrel structure and associates with the exonuclease RRP6 and the exo/endonuclease DIS3 (EXO11) along with the cofactors RRP47 and MPP6 (EXO13). Transcripts are targeted to the exosome via adaptor complexes, forming mutually exclusive interactions with the RNA helicase MTR4. In the nucleoplasm, these include the nuclear exosome targeting (NEXT) complex (MTR4:ZCCHC8:RBM7) and the polyA exosome targeting (PAXT) connection (MTR4:ZFC3H1:PABPN1). Both NEXT and PAXT can be physically connected to capped RNAs via the CBCA adaptor protein ZC3H18. The TRAMP adaptor complex (MTR4:ZCCHC7:PAPD5) is restricted to the nucleolus and is predominantly engaged with the exosome in rRNA processing. To dampen RNA decay in nuclear speckles, sites of productive mRNA processing and export factors, MTR4 is bound by the NRDE2 protein. This locks MTR4 in a closed conformation and blocks its interactions with the CBCA and EXO13

degradation, owing to the endo- and exonuclease activities from its associated ribonucleases DIS3 and RRP6 (Figure 4, bottom). These enzymes are active in the processing of precursor transcripts, the removal of transcriptional by-products and the decay of otherwise nuclear retained RNAs (Schmid & Jensen, 2018). To aid in the recognition of its plethora of transcript targets, the exosome associates with adaptor complexes that recognize specific transcripts and recruit them for decay. While the core exosome complex is well conserved across the eukaryotic domain, the number and composition of adaptor complexes can vary between lower and higher eukaryotes (Schmid & Jensen, 2018). A central factor here is the RNA helicase MTR4 (also known as SKIV2L2 or MTREX), which engages in all known adaptor complexes, including the mammalian-specific nuclear exosome targeting (NEXT) complex (constituting MTR4-ZCCHC8-RBM7), the polyA exosome targeting (PAXT) connection (constituting MTR4-ZFC3H1 and the RNA-dependent interaction with PABPN1) and the nucleolus-specific hTRAMP complex (constituting MTR4-PAPD5-ZCCHC7; Lubas et al., 2011, 2015; Meola et al., 2016; Silla et al., 2018;

Figure 4). NEXT primarily functions to target unprocessed transcripts, such as PROMPTs, eRNAs and 3′ end extended snRNA and snoRNAs (Lubas et al., 2015, 2011; Preker et al., 2008). PAXT, on the other hand, targets longer polyadenylated transcripts, seemingly through the RNA polyA tail-dependent association with PABPN1 (Beaulieu, Kleinman, Landry-Voyer, Majewski, & Bachand, 2012; Bresson & Conrad, 2013; Bresson, Hunter, Hunter, & Conrad, 2015; Meola et al., 2016; Ogami et al., 2017). Finally, both NEXT and PAXT can be connected to CBC-containing RNPs via an interaction with the zinc finger protein, ZC3H18, which bridges an interaction to the CBCA complex (Figure 4) (Andersen et al., 2013; Giacometti et al., 2017; Winczura et al., 2018). Similar nuclear targeting complexes formed around MTR4 homologs are found in budding and fission yeast with the TRAMP and MTREC complexes respectively functioning in the removal of unstable transcripts via the exosome (Kilchert et al., 2016).

2.6 | Duel of the fates: Fighting to avoid decay

From early conception, RNAs are in a constant battle between processing and decay. During the RNP maturation process, the combination of bound factors will direct the transcript towards a specific path. However, time can tip the scales again, since prolonged nuclear exposure may favor assembly, and activation, of decay factors (Schmid & Jensen, 2018). A key example of such battle of processes is the mutual competition between MTR4 and ALYREF for association with CBCA-bound RNAs, the outcome of which plays a causative role in fate commitment (Fan et al., 2017; Giacometti et al., 2017; Schulze et al., 2018). This balance can be tipped to favor either pathway depending on the local environment, such as other export or decay factors already bound as a result of processing. Furthermore, shuttling to subnuclear compartments can play a role in the downstream processing of RNAs. Many factors involved in mRNA export, including ALYREF, are enriched in nuclear speckles, which thereby serve as hubs for productive RNA processing. It has been proposed that mRNAs are shuttled through nuclear speckles for assembly into export-ready mRNPs (Akef, Zhang, Masuda, & Palazzo, 2013; Dias, Dufu, Lei, & Reed, 2010; Mor et al., 2016). A recent report even suggested that to further outrun nuclear decay, exosome activity is inhibited in nuclear speckles through the negative regulation of MTR4 by the nuclear RNAi defective-2 (NRDE2) protein (J. Wang, Chen, et al., 2019). That is, NRDE2 is not only enriched in nuclear speckles, but it also physically interacts with MTR4 to lock it in a 1:1 stoichiometry, which blocks key residues of MTR4 involved in its recruitment to capped RNAs via the CBCA as well as to the exosome (Figure 4). This combinatorial inhibition is suggested to promote productive export of mRNAs by providing a decay-free zone in nuclear speckles (J. Wang, Chen, et al., 2019).

RNA decay systems can also be dampened as an evasion mechanism by viruses to initiate infection and conquer their host cells. These mechanisms have mainly been observed in the cytoplasm (Dickson & Wilusz, 2011; Hogg, 2016), there are also instances of viral RNAs that can evade nuclear decay pathways. The KSHV expresses the ORF57 protein (also known as Mta), which is important in all steps of viral biogenesis (Malik & Schirmer, 2006). Interestingly, however, it appears to selectively increase the nuclear stability of KSHV RNAs in infected mammalian cells. KSHV transcripts are produced as short, capped, and polyadenylated species, through the hijacking of host RNA biogenesis mechanisms, but are generally intronless (Zheng, 2003). As such, they would be obvious targets of the nuclear decay machinery, but reports have shown that ORF57 binds KSHV RNAs and prevents their decay by impeding the recruitment of MTR4-containing complexes (Conrad, Shu, Uyhazi, & Steitz, 2007; Conrad & Steitz, 2005; Ruiz, Hunter, & Id, 2019). This may occur by an active recruitment of ALYREF by ORF57 (Stubbs, Hunter, Hoover, & Conrad, 2012), which then outcompetes MTR4 for interactions with CBCA-bound viral RNA, owing to their mutual exclusivity with ARS2. In turn, it might accelerate KSHV RNA export and escape from the nucleus. A similar mechanism is employed by retroviruses such as Mason-Pfizer monkey virus (M-PMV) and human immunodeficiency virus (HIV), which utilize a constitutive transport element and the viral export-promoting protein HIV-Rev, respectively, to accelerate nuclear export (Ernst, Bray, Rekosh, & Hammarskjöld, 1997; Felber, Hadzopoulou-Cladaras, Cladaras, Copeland, & Pavlakis, 1989). Still, a number of KSHV transcripts appear to be stable, independent of their nuclear export, and therefore must engage with alternate factors to evade decay (Ruiz et al., 2019).

In relation to these themes, recent reports have demonstrated that depletion of decay factors can shift the balance of transcripts towards export. Specifically, inactivation of the nuclear exosome triggers the accumulation of pA^+ RNAs in nuclear foci distinct from other subnuclear bodies, such as nucleoli, speckles, or paraspeckles (Fan et al., 2018; Silla et al., 2018). These nuclear pA^+ RNA foci are enriched with components of the PAXT connection, including MTR4, ZFC3H1 and PABPN1, involved in the decay of polyadenylated transcripts (Silla et al., 2018). Since formation of foci critically depends on the presence of the ZFC3H1 protein, with co-depletion of the core exosome factor RRP40 and ZFC3H1 resulting in ALYREF-dependent export of some RNAs normally targeted for decay (Ogami et al., 2017; Silla et al., 2018), this suggests that ZFC3H1 acts as a pA^+ RNA nuclear retention factor in a competitive manner with export factors such as ALYREF.

Whether such nucleoplasmic sorting occurs prior to passing through nuclear speckles for final licensing for export remains to be determined (Fan et al., 2018; Silla et al., 2018).

Nuclear retention factors, like ZFC3H1, may act by keeping pA^+ RNA nuclear and thereby allowing enough time for decay complexes to assemble (Meola & Jensen, 2017; Schmid & Jensen, 2018). A such timed system would in turn allocate RNA export pathways only a margined window to act before decay would take over. A particularly interesting player here is PABPN1, which serves dual roles in productive RNA pathways along with its described role in RNA decay (Beaulieu et al., 2012; Bresson & Conrad, 2013; Bresson et al., 2015; Z. Chen, Li, & Krug, 1999; Kühn, Buschmann, & Wahle, 2017; Kühn et al., 2009; Lemay et al., 2010). PABPN1 is engaged in pA tail biogenesis where it stimulates PAP during the polyadenylation process and protect the fully adenylated 3′ end (Eckmann, Rammelt, & Wahle, 2011). However, the transient interaction of PABPN1 with ZFC3H1 may, over time, tilt pA^+ RNAs towards decay, pending their increased nuclear residence time (Meola & Jensen, 2017; Schmid & Jensen, 2018).

2.7 | Stable nuclear transcripts

Functional nuclear RNAs have adapted to survive the harsh surroundings in which they operate. The majority of such Pol II-derived transcripts are noncoding such as snRNAs, snoRNAs, and lncRNAs. However, mammalian nuclei also retain a repertoire of pre- and processed mRNAs, which are thought to function as "stockpiles" to be subsequently released to the cytoplasm upon stimuli such as stress or differentiation (Mauger, Lemoine, & Scheiffele, 2016; Pimentel et al., 2016; Shalgi, Hurt, Lindquist, & Burge, 2014). Independent of their specific biotype, nuclear transcripts require stabilizing mechanisms to prevent decay. These include binding to RBPs, localization to subnuclear RNA structures or association with chromatin.

Conventional RBPs, such as the CBC and PABPs, provide early protection against exonucleolytic decay (Figure 3). For longer term stability in the nucleus, transcripts form tight RNP assemblies. For example small RNAs, such as snRNA and snoRNAs, contain conserved sequence elements that are bound by dedicated factors to form strong and functional RNP assemblies (Dupuis-Sandoval, Poirier, & Scott, 2015; Kiss, 2004; Watkins & Bohnsack, 2012). These elements can also contribute to the intracellular shuttling of these transcripts. As previously mentioned, the initial export of snRNAs, as part of their maturation, is dependent on the CBCA-interactor, PHAX. Similarly, three independently transcribed snoRNAs, *U3*, *U8*, and *U13* are directed to CABs for maturation mediated by the CBCA and PHAX. The majority of snoRNPs are further shuttled to their sites of function in nucleoli in a process depending on the box C/D and H/ACA motifs along with a terminal stem structure (Lange, Ezrokhi, Amaldi, & Gerbi, 1999; Narayanan, 1999). A subset of snoRNAs, the scaRNAs, are retained in the CABs, mediated by a common CAB box motif in H/ACA scaRNAs or a long UG repeat in box C/D scaRNAs (Marnef, Richard, Pinzón, & Kiss, 2014; Richard et al., 2003).

While snRNAs and snoRNAs have clear nuclear functions, roles of many lncRNAs remain putative. Stable nuclear lncRNAs evade decay through a number of mechanisms. An example is provided by the metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*, also known as *NEAT2*) and the long isoform of the nuclear enriched transcript 1 (*NEAT1*, also known as *MENβ*). Both of these transcripts are highly transcribed by RNA Pol II. Moreover, they neither undergo splicing nor conventional 3′ end processing and therefore lack canonical polyA tails (Wilusz, Freier, & Spector, 2008). Instead, a tRNA-like motif in their 3′ termini is recognized and cleaved by the endonuclease RNAse P (Sunwoo et al., 2008). The resulting mature 5′ end fragments contain genomic-encoded A-rich sequences and two upstream U-rich motifs, that form protective triple helical structures (Figure 3b) (J. A. Brown et al., 2014; J. A. Brown, Valenstein, Yario, Tycowski, & Steitz, 2012; Wilusz et al., 2012). So far, it is unknown if this 3′ end formation occurs co-transcriptionally or post transcriptionally. *MALAT1* has two isoforms in mouse and humans with drastically different abundances. The longer form has two well conserved PAS sites at the 3′ end, but is present at very low levels whereas the shorter form is generated by RNaseP and is the predominant transcript species (Wilusz et al., 2008). It is not clear if the long isoform is the precursor transcript that is rapidly processed into the shorter form or if it represents transcripts arising from low levels of readthrough transcription. *MALAT1* and *NEAT1* presumably also garner protection by accumulating in speckles and paraspeckles, respectively (Clemson et al., 2009; Hutchinson et al., 2007; Sasaki, Ideue, Sano, Mituyama, & Hirose, 2009; Sunwoo et al., 2008), which, as described above, may be shielded from nuclear decay pathways (J. Wang, Chen, et al., 2019). Along with nucleoli and CABs, nuclear speckles and paraspeckles are subnuclear compartments that occupy the interchromatin space and concentrate specific proteins and RNAs into dedicated sites of specific activity (Sleeman & Trinkle-Mulcahy, 2014). Nuclear speckles are present near sites of transcription and are enriched for pre-mRNA splicing factors (Spector & Lamond, 2011), a subset of which *MALAT1* has been suggested to be required to recruit (Bernard et al., 2010; Tripathi et al., 2010). Retention of *MALAT1* in speckles depends on two distinct sequence elements, which are also involved in binding to the nuclear speckle enriched protein RNPS1 (Figure 5;

Miyagawa et al., 2012). Consistently, these regions were identified in a recent high-throughput screen for RNA sequence elements that promote nuclear retention (Shukla et al., 2018).

NEAT1 RNA is a central integral component of paraspeckles, which are often close to but still distinct from nuclear speckles and enriched for specific RBPs from the Drosophila behavior/human splicing (DBHS) family (Clemson et al., 2009; Knott, Bond, & Fox, 2016; Sasaki et al., 2009; Sunwoo et al., 2008). It has been suggested that paraspeckle formation occurs in close proximity to the *NEAT1* gene itself and that its active transcription is required for paraspeckle maintenance (Clemson et al., 2009; Mao, Sunwoo, Zhang, & Spector, 2011). *NEAT1* is directly bound by DBHS proteins, including SFPQ and NONO, during early transcription (Figure 5; Fox & Lamond, 2010). This binding likely competes with export factors, thereby promoting retention. Multiple SFPQ and NONO molecules bind to *NEAT1* and multimerization of these assemblies induces liquid phase separation and the aggregation of paraspeckles (Figure 5; Knott et al., 2016; Nakagawa, Yamazaki, & Hirose, 2018). In addition to accumulating *NEAT1*, paraspeckles retain hyper edited mRNAs, although this has been subject to debate. Such hyper edited transcripts contain inverted repeat sequences, such as Alu elements, that form double-stranded RNA regions and are subjected to adenosine to inosine (A-to-I) editing by adenosine deaminase actin on RNA (ADAR) enzymes (L.-L. Chen & Carmichael, 2009; L.-L. Chen, DeCerbo, & Carmichael, 2008; Nishikura, 2016; Prasanth et al., 2005). Some

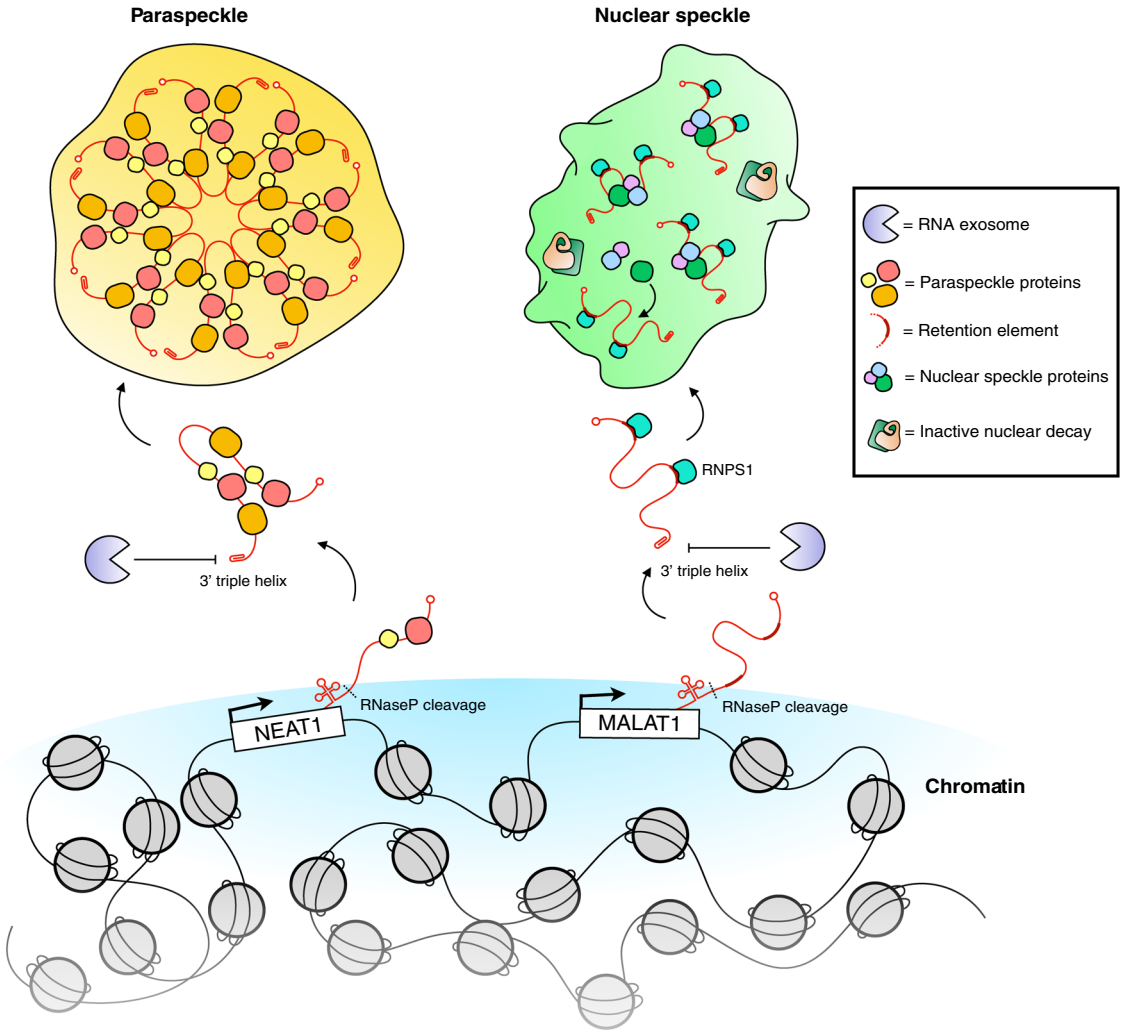

FIGURE 5 Escaping decay in subnuclear compartments. For continued survival in the nucleus, some RNAs are sequestered into RNP aggregates such as nuclear speckles and paraspeckles as exemplified by *MALAT1* and *NEAT1*, respectively. (Left) *NEAT1* harbors a triple helical structure at its 3′ end and engages co-transcriptionally with paraspeckle factors, such as DBHS protein family members. *NEAT1* itself plays an integral role in the structure and maintenance of paraspeckles, which are subsequently involved in the retention of other RNAs. (Right) *MALAT1* also possesses a protective triple helical 3′-end. Two identified sequence elements are crucial for the retention of *MALAT1* in the nucleus and bind to the nuclear speckle protein RNPS1. *MALAT1* is not intrinsic to the structure of nuclear speckles but is proposed to function in the recruitment of mRNA processing factors to these subnuclear compartments

extensively modified mRNAs have been reported to be recognized by DBHS proteins, such as PSPC1, NONO, and MATR3, which bind A-to-I converted hairpin structures and can aggregate these transcripts in paraspeckles (Mao et al., 2011; Prasanth et al., 2005). This process is suggested to be a mechanism that regulates gene expression through retention and these transcripts can be released to the cytoplasm upon certain cellular stimuli. However, other reports have shown that RNAs containing modified Alu elements can be efficiently exported and translated, suggesting that retention is not fully dependent on such elements (Capshew, Dusenbury, & Hundley, 2012; Hundley, Krauchuk, & Bass, 2008). Despite this, one such example is the mouse specific *Ctn* mRNA, which is retained in paraspeckles due to a hyper modified Alu element in its 3′ UTR. Under stress conditions, this retention element is cleaved off and the mRNA is exported to the cytoplasm where it appears to be translationally competent (Anantharaman et al., 2016; Prasanth et al., 2005). Although the cleavage mechanisms and the polyadenylation status of the cleaved transcript are yet to be determined, it has been speculated that it is subjected to posttranscriptional cleavage/polyadenylation, where the *Ctn* RNA is revisited by CPSF under stress conditions to generate an active transcript.

XIST is one of the most intensively studied functional nuclear lncRNAs due to its role in X-chromosome inactivation. Curiously, *XIST* is capped, spliced and polyadenylated, yet retained in the nucleus (Brockdorff et al., 1992; C. J. Brown et al., 1992; Clemson, McNeil, Willard, & Lawrence, 1996). Consistently, early experiments reported that *XIST* shows only weak binding to nuclear export factors, is undetectable in the cytoplasm and does not shuttle in heterokaryon assays (Cohen & Panning, 2007). This suggests that nuclear localization is due to active retention efficiently competing with export. The *XIST* RNA is transcribed during early development from the X-inactivation center (XIC), but solely from the future silenced X-chromosome (Xi). The transcript subsequently spreads over the X-chromosome in *cis* and triggers a cascade of Xi silencing events, including the deposition of repressive chromatin marks, DNA methylation, the removal of Pol II, the removal of active histone marks and a complete repackaging of the chromosome (Figure 6a) (Augui, Nora, & Heard, 2011; Lee & Bartolomei, 2013; Matarazzo, Cerase, & D'Esposito, 2008). Numerous studies have shown that this function of *XIST* is not specific to the X-chromosome but that translocated expression to other chromosomes results in similar silencing effects (Allderdice, Miller, Miller, Klinger, & Opitz, 1978; Hall, Clemson, Byron, Wydner, & Lawrence, 2002; Leisti, Kaback, & Rimoin, 1975). Thus, *XIST* function likely depends on its local proximity of transcription.

The nuclear retention of *XIST* is due to interactions with nuclear proteins and chromatin following transcription. These interactions and aggregations may shield the RNA from decay machineries, promoting its long-term nuclear stability. An ongoing endeavor is to identify *cis*- and *trans*-acting features, serving to retain *XIST* in the nucleus (Chu et al., 2015; McHugh et al., 2015; Minajigi et al., 2015). Through such efforts, the heterogeneous RNP, hnRNPU (also known as SAF-A), was shown to mediate interactions between *XIST* and chromatin through RNA and DNA binding domains, respectively (Figure 6; Fackelmayer, Dahn, Renz, Ramsperger, & Richter, 1994; Hasegawa et al., 2010; Romig, Fackelmayer, Renz, Ramsperger, & Richter, 1992; Sakaguchi et al., 2016). Furthermore, the CDKN1A interaction protein CIZ1 was reported to anchor *XIST* on the Xi via a highly repetitive RNA element in *XIST* termed Repeat E (Sunwoo, Colognori, Froberg, Jeon, & Lee, 2017). Similar repetitive sequences, such as the conserved Repeat A element, have also been proposed to retain *XIST* on chromatin, but in a manner independent of HNRNPU and CIZ1 (Chigi, Sasaki, & Sado, 2017; Hendrich, Brown, & Willard, 1993; Nesterova et al., 2001; Y. Wang et al., 2019; Wutz, Rasmussen, & Jaenisch, 2002). These, and possibly additional, connections appear to work together to anchor *XIST* at chromatin during and following its transcription. As *XIST* undergoes "mRNA-like" processing, these retention factors compete efficiently with any export factors that may be recruited during capping, splicing and 3′ end formation.

2.8 | Retention elements: Nuclear anchors

cis-elements involved in mRNA localization to the cytoplasm have been vastly described, whereas the search for *cis*-elements that co-ordinate the localization of lncRNAs is still developing. Key examples include the aforementioned regions of *MALAT1* and *XIST*, which promote localization to nuclear speckles and chromatin, respectively (Miyagawa et al., 2012; Y. Wang et al., 2019). A similar example comes with the nuclear retained lncRNA *FIRRE*, that associates with chromatin through a 156-nt repeating RNA domain (RRD) bound by hnRNPU (Figure 6b; Hacisuleyman et al., 2014). Finally, retention elements have also been identified in the lncRNAs *BORG* and *MEG3*, and subsequent transplantation of these motifs to otherwise cytoplasmic RNAs was sufficient to relocate these to the nucleus (Azam et al., 2019; B. Zhang et al., 2014).

While, the above mentioned examples have all been revealed on a case-by-case basis, more global approaches have also been taken to identify common motifs involved in nuclear retention of transcripts (Carlevaro-Fita et al., 2019; Gudenas & Wang, 2018; Lubelsky & Ulitsky, 2018; Shukla et al., 2018; Yin et al., 2018; Zuckerman & Ulitsky, 2019). Recent high-

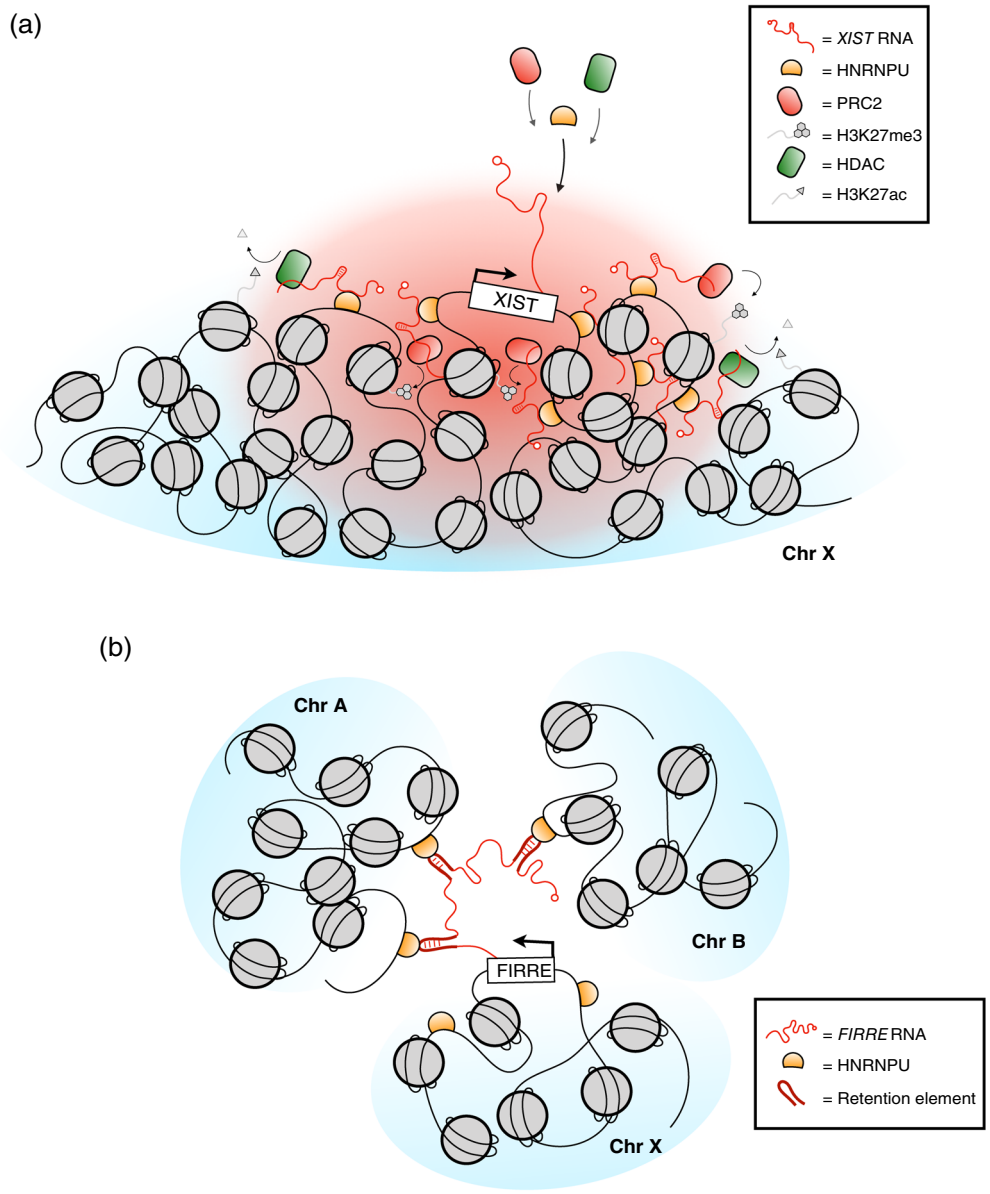

FIGURE 6 Nuclear retention on chromatin. Several lncRNAs are actively retained in the nucleus by sequestration on chromatin. This is exemplified by the well-characterized lncRNAs *XIST* and *FIRRE*. (a) While *XIST* is processed analogous to mRNAs, it is actively anchored in the nucleus through interactions with heterogeneous nuclear ribonucleoproteins (hnRNP), such as hnRNPU, which mediates RNA:chromatin interactions. *XIST* further recruits epigenetic factors such as PRC2 and histone deacetylases (HDACs) to aid in silencing the X-chromosome. (b) The *FIRRE* lncRNA has been shown to mediate interchromosomal interactions between the host X chromosome and other genomic locations. An identified repeating domain, acting as a retention element, is involved in binding to hnRNPU and mediating the interaction with chromatin

throughput approaches employed a systematic design using tiled overlapping sequences to screen for *cis*-acting motifs (Lubelsky & Ulitsky, 2018; Shukla et al., 2018). A similar method from the Shen lab used random fragmentation of candidate genes to generate their screen for elements controlling RNA-chromatin retention (Yin et al., 2018). The Massively Parallel RNA assay (MPRNA) by Shukla et al. (2018) took advantage of the sequences of 37 lncRNAs, with known localizations in both nuclear and cytoplasmic compartments, to generate approximately 12,000 overlapping barcoded oligonucleotides. These sequences were then fused to reporter constructs, known for their native cytoplasmic localizations, and transfected into HeLa cells before sequencing either nuclear or total cellular RNA (Shukla et al., 2018). Approximately 110 unique RNA tags were identified, that showed a significant relative enrichment in the nuclear fraction. Subsequent motif analysis of the putative retention signals showed the enrichment of cytosine rich sequences (Shukla et al., 2018). A parallel study from Lubelsky and Ulitsky (2018) utilized a similar methodology with a tiling-based screen of lncRNAs, 3′ UTRs of nuclear enriched mRNAs and homologs of *MALAT1*. This approach identified 19 regions within 14 transcripts, that exhibited increased nuclear localization and also included a cytosine rich motif derived from Alu repeat elements (RCCTCCC, R=A/G). This motif element, referred to as short interspersed nuclear element (SINE)-derived nuclear RNA localization (SIRLOIN), was further shown to bind the abundant nuclear protein hnRNPK, consistent with its known preference for C-rich motifs (Choi et al., 2009; Lubelsky & Ulitsky, 2018). The screen from Yin and colleagues (Yin et al., 2018) more specifically focussed on identifying *cis* elements involved in RNA association with chromatin. This method, termed RNA element for subcellular localization by

sequencing (RELseq), took short sequences of known chromatin-associated RNAs and fused them to reporters before fractionating and sequencing. This screen, and subsequent mutational analysis, identified a 7-nt motif U1 binding motif suggesting that the U1 snRNP is involved in chromatin retention of these RNAs. This notion was supported by a related study, showing that nuclear retention of the *MEG3* lncRNA depends on U1 snRNP components (Azam et al., 2019). Depletion of hnRNPK or U1-snRNP factors resultingly disturbed the nuclear localization of RNAs containing SIRLOIN motifs or U1 recognition motifs respectively (Azam et al., 2019; Lubelsky & Ulitsky, 2018; Yin et al., 2018). However, fusion of the C-rich element identified in the MPRNA study was not sufficient to retain a known cytoplasmic RNA in the nucleus (Shukla et al., 2018). This is likely due to the artificial nature of the utilized construct and because such retention elements will be in functional competition with export factors, often requiring additional elements, or activities, to retain a host RNA in the nucleus. We assume that these mechanisms act in concert with other processes described throughout this review to affect RNA fate.

SIRLOIN elements are likely to be abundant in the genome due to their derivation from repetitive SINE elements. SINEs, along with other transposable elements (TEs), make up a considerable fraction of mammalian genomes and their repetitive nature has been proposed to be a major contributor to the evolution of genomic features. Curiously, in comparison to mRNAs, exonic TEs appear to be overrepresented in lncRNAs with estimates of ~40 and ~83%, respectively (Kelley & Rinn, 2012). It has therefore been proposed that elements shared between lncRNAs are derived from neo-functionalized TEs (Carlevaro-Fita et al., 2019; R. Johnson & Guigo, 2014). These so-called repeat insertion domain of lncRNAs (RIDLs) are predicted to have roles in forming molecular interactions contributing to the localization of transcripts, as observed for SIRLOIN elements (R. Johnson & Guigo, 2014; Lubelsky & Ulitsky, 2018). A high-throughput study aiming to correlate the presence of RIDLs with RNA nuclear localization identified three TEs (L2b, MITb, and MIRc), that positively correlate with the nuclear retention of their host transcripts (Carlevaro-Fita et al., 2019). As with the other identified elements, the presence of a RIDL is a likely contributor to lncRNA nuclear localization but it probably functions in parallel with additional retention mechanisms, and it will require further high throughput approaches to determine how these act together in assumed combinatorial fashions.

3 | CONCLUSIONS

In recent years, work has begun to uncover mechanisms underlying the nuclear sorting of RNAs and what components aid in discriminating subsequent localization within the cell or targeting for decay. From their early transcriptional conceptus, RNAs are subjected to dynamic and rapid exchanges of mutually exclusive factors, that decidedly "lock" in a terminal pathway when combined with other elements that drive RNAs towards the same fate. All Pol II transcripts can be targeted for nuclear decay, but efficient processing, rapid export and/or protective elements may allow the escape from such processes. Indeed, decay can be thought of as the default state of RNAs and it is an uphill battle to escape this destiny. While this may seem wasteful, in the long run, perhaps even measured in evolutionary time, it ensures the survival of only high quality RNA, thus maintaining a stable and operational transcriptome. Still, despite our recent comprehension of nuclear decay pathways and their interconnections with RNA productive factors, the timing, mechanism and recruitment underlying this interplay remain to be fully understood.

Similarly, all Pol II transcripts likely have the potential to be exported from the nucleus. But when this process is outcompeted by nuclear retention elements or nonoptimal processing, providing insufficient shielding, this will lead to RNA decay. Our understanding of export pathways has been widely broadened by several years of investigation, yet, insights into nuclear retention mechanisms are not fully realized. Recent identification of *cis*-retention elements is a warranted step in this direction, although the complexes, and means by which they act upon these elements, still need be identified and characterized. This will be greatly aided by high throughput screening and gene editing approaches. The majority of our present knowledge stems from classical model organism-based research in "steady state" systems. While this provides fundamental backbone to these fields, it will be compelling to understand how these processes are regulated in more dynamic and biologically relevant states such as cellular differentiation or transformation into cancer cells.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

William Garland: Conceptualization; writing-original draft; writing-review and editing. **Torben Heick Jensen:** Conceptualization; supervision; writing-review and editing.

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