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Nat Rev Genet. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

Nat Rev Genet. 2024 October ; 25(10): 679–697. doi:10.1038/s41576-024-00712-2.

Nuclear mRNA decay: regulatory networks that control gene expression

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Abstract

Proper regulation of mRNA production in the nucleus is critical for the maintenance of cellular homoeostasis during adaptation to internal and environmental cues. Over the past 25 years, it has become clear that the nuclear machineries governing gene transcription, pre-mRNA processing, pre-mRNA and mRNA decay, and mRNA export to the cytoplasm are inextricably linked to control the quality and quantity of mRNAs available for translation. More recently, an ever-expanding diversity of new mechanisms by which nuclear RNA decay factors finely tune the expression of protein-encoding genes have been uncovered. Here, we review the current understanding of how mammalian cells shape their protein-encoding potential by regulating the decay of pre-mRNAs and mRNAs in the nucleus.

Introduction

Proper quantitative and qualitative regulation of gene expression is essential for cellular homoeostasis and adaptation to stress. The expression of mammalian protein-encoding genes is generally viewed as a multistep process that includes gene transcription and pre-mRNA processing in the nucleus, mRNA export from the nucleus to the cytoplasm, and mRNA translation and mRNA decay in the cytoplasm. Decades of research have demonstrated that most, if not all, of these steps are regulated and functionally coupled, thereby dramatically increasing the ability for cells to finely and robustly tune gene expression (reviewed in refs. 1–4). Nonetheless, this multistep view of gene expression is simplified and has long underappreciated other aspects of RNA metabolism, such as the decay of pre-mRNA and mRNA in the nucleus.

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Author contributions

X.R. researched literature for the article. X.R. and L.E.M. contributed to the discussion of the content, wrote the manuscript, and edited and/or reviewed the manuscript before submission.

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Genetics* thanks Anita Corbett, who co-reviewed with Milo Fasken; François Bachand; and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Interest in nuclear RNA decay grew in the early 2010s when it became clear that genomes are transcribed pervasively by RNA polymerase II (RNAPII), producing many presumably nonfunctional, if not deleterious, non-coding RNAs (reviewed in refs. 5–7). Two eukaryotic nuclear RNA decay activities reduce the levels of these non-coding transcripts: XRN2 and, to a larger extent, the RNA exosome[8–12] (references include one non-peer-reviewed preprint) (Fig. 1).

XRN2 is an evolutionarily conserved 5′-to-3′ exoribonuclease that degrades 5′-monophosphorylated RNAs (Fig. 1a). XRN2-sensitive pervasive RNAs primarily consist of long (>1,000-nucleotide (nt)) nascent RNAs, that is, RNAs that have yet to be released from an association with RNAPII on chromatin[8]. XRN2 typically degrades pervasive nascent RNAs cleaved co-transcriptionally by either the cleavage and polyadenylation (CPA) complex[13] at polyadenylation signals (PASs) or the rixosome endonuclease[14]. XRN2-mediated decay of nascent RNAs also results in transcription termination via the ‘torpedo’ mechanism, whereby XRN2 catches up with and displaces elongating RNAPII (ref. 13) (Fig. 1a).

The RNA exosome, which should not be mistaken with the extracellular exosome vesicle, consists of an evolutionarily conserved non-catalytic barrel-shaped core, one or two 3′-to-5′ ribonucleases (DIS3 and RRP6) and structural proteins that cooperate with specialized protein adaptors (reviewed in refs. 5–7) (Fig. 1b). The RNA exosome degrades pervasive RNAs post-transcriptionally, that is, after they are released from RNAPII (ref. 8). Depending on their features, pervasive RNAs targeted for nuclear decay by the RNA exosome are preferentially recognized by one of at least two specialized protein adaptors, each of which is connected to the RNA exosome barrel via the RNA helicase MTR4 (refs. 9–12) (Fig. 1b): the nuclear exosome-targeting (NEXT) adaptor complex (Fig. 1c,d) or the poly(A) exosome-targeting (PAXT) adaptor ‘connection’ (Fig. 1e).

Both NEXT[15] and PAXT[16] recognize RNAPII-transcribed RNAs with a 5′-m^{7}GTP cap via mutually exclusive interactions between the ARS2 (also known as SRRT) constituent of the CBCA complex, that is, the largely nuclear CBP80–CBP20 cap-binding complex (CBC) bound by ARS2, and either the NEXT accessory protein ZC3H18 (ref. 17) (Fig. 1c), the NEXT accessory protein ZC3H4 (ref. 18) (Fig. 1d) or the PAXT scaffold subunit ZFC3H1 (refs. 19,20) (Fig. 1e). Notably, at least ZC3H18 directly contacts CBP80 in addition to ARS2 (ref. 21).

NEXT typically targets short (<1,000-nt) RNAs with non-polyadenylated 3′-ends[22,23] either via ZC3H18, whereby RNAs are cleaved and released from chromatin by Integrator[18,24] (Fig. 1c), or via ZC3H4, whereby RNAs are released presumably uncleaved from chromatin by Restrictor[18] (Fig. 1d). Nevertheless, it remains unclear which features, if any, discriminate NEXT-sensitive pervasive RNAs that are released by Integrator and targeted by ZC3H18 from those that are released by Restrictor and targeted by ZC3H4.

In contrast to NEXT, PAXT prefers short or long poly(A)$^+$ RNAs released from chromatin by the CPA complex. On one hand, the association of PAXT with ARS2 via ZFC3H1 may only be required for the decay of short poly(A)$^+$ RNAs with few exons[20]. Although ZC3H18

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co-immunoprecipitates with ZFC3H1 in an RNase-resistant way[16], whether ZC3H18 may also promote the connection of PAXT with ARS2 is unclear. On the other hand, the specificity of PAXT for poly(A)$^+$ RNAs, and possibly its targeting long RNAs[20], is mediated by the association of ZFC3H1 with nuclear poly(A)-binding proteins PABPN1 and/or ZC3H14 (refs. 16,25,26) (Fig. 1e). Importantly, PABPN1 mediates the decay of pervasive RNAs by stimulating RNA hyperadenylation in a pathway known as 'PABPN1-mediated and poly(A) polymerase α/γ-mediated decay' (PPD)[27,28]. PAXT and PPD RNA targets overlap, and knockdown of ZC3H3, a PAXT constituent[29], results in nuclear accumulation of hyperadenylated RNAs[30]. Thus, PAXT-mediated decay and PPD most probably belong to the same nuclear decay pathway. Notably, the association between ZFC3H1 with the CBCA and poly(A) binding proteins is largely RNase-sensitive[16]; hence, PAXT is usually referred to as a 'connection' rather than a 'complex'.

In *Saccharomyces cerevisiae*, specific sequences found in pervasive RNAs are recognized co-transcriptionally by the Nrd1–Nab3–Sen1 complex, which releases nascent RNAs from chromatin and recruits the Trf4/5–Air1/2–Mtr4 polyadenylation (TRAMP) adaptor to the RNA exosome to trigger RNA decay[12]. TRAMP-like complexes have been identified in metazoans[22], and we refer to these as mTRAMP. However, although mTRAMP constituents are present in heavy sucrose gradient fractions that include ZFC3H1 and lack PABPN1 (ref. 25), whether they control pervasive RNA decay and which RNA features they would recognize remain unknown[22] (Fig. 1f).

Owing to recent extensive efforts to characterize RNA decay pathways in the nucleus, it is now clear that nuclear RNA decay machineries also target pre-mRNAs and mRNAs to minimize the export of low-quality transcripts or quantitatively downregulate the expression of the genes from which they derive. Here, while reviewing key aspects of normal pre-mRNA metabolism in the nucleus, we describe how, in mammals, abnormal pre-mRNA metabolism produces aberrantly or inefficiently processed mRNAs and how these transcripts are targeted for decay. We then discuss how cells utilize co-transcriptional and post-transcriptional alternative processing of newly synthesized mRNAs to regulate their sensitivity to nuclear decay and shape protein-encoding gene expression, for example, during development and disease. We also review how epigenetic modifications and *cis*-acting DNA and RNA elements recruit or outcompete RNA decay machineries for quantity control of gene expression. Notably, we discuss throughout this Review the roles of XRN2, Restrictor and Integrator in transcription termination, which is intrinsically linked to nuclear pre-mRNA decay pathways[13,18,31,32]. We do not describe the mechanisms by which these and other nuclear RNA decay factors regulate other aspects of gene expression. However, we extend our understanding of nuclear pre-mRNA and mRNA decay pathways by presenting mechanisms by which nuclear non-coding transcripts are degraded. Although this Review focuses on mechanisms described for mammals, we also assess studies using fruitflies (*Drosophila melanogaster*), worms (*Caenorhabditis elegans*), budding yeast (*S. cerevisiae*), fission yeast (*Schizosaccharomyces pombe*), and plants to fill gaps in our understanding of mammalian pathways.

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Metabolism of nuclear mRNAs

Normal steps of nuclear mRNA metabolism include gene transcription (that is, transcription initiation, promoter-proximal pausing, pause release, transcription elongation and transcription termination; Fig. 2A–D,F), pre-mRNA processing (that is, pre-mRNA capping, splicing and CPA; Fig. 2B,D–F) and nuclear mRNA export[33] or sometimes nuclear mRNA decay or sequestration (Fig. 3). Below, we review how pre-mRNA processing steps are normally coupled to each other and to gene transcription, and how impaired processing or coupling of events produces aberrant mRNAs (Fig. 2B,D–F). Next, we discuss how pre-mRNA processing controls nuclear export and how export competes with nuclear decay or sequestration (Fig. 3). Finally, we review how aberrant mRNAs are targeted for nuclear decay to maintain nuclear RNA homoeostasis and prevent the production of nonfunctional or toxic proteins in the cytoplasm (Fig. 4).

Normal, alternative and aberrant pre-mRNA processing

Pre-mRNA capping controls premature transcription termination.—Pre-mRNA capping and acquisition of the CBP20–CBP80 CBC occurs during RNAPII promoter-proximal pausing (Fig. 2Ba) and influences virtually all aspects of nuclear mRNA metabolism[33–37], including transcription elongation and premature transcription termination (PTT).

First, the CBC promotes release of RNAPII from promoter-proximal pausing via the recruitment of the positive elongation factor P-TEFb[34,38] (Fig. 2C). Moreover, as more recently shown, Integrator competes with P-TEFb for recruitment to the CBC, which triggers promoter-proximal PTT[34] (Fig. 2Bb). At most if not all protein-encoding genes, Integrator suppresses pause release by recruiting protein phosphatase 2A, so as to dephosphorylate the RNAPII C-terminal domain and RNAPII-bound transcription elongation factors. Next, Integrator cleaves the nascent pre-mRNA via its catalytic subunit INTS11, thereby triggering promoter-proximal PTTand release ofa 20–60-nt ‘promoter-associated pre-mRNA’ from the chromatin template[31] (Fig. 2Bb). Although Integrator-mediated cleavage of promoter-associated pre-mRNAs attenuates the expression of short and lowly expressed genes[39,40], it may also terminate non-productive RNAPII to allow for transcription reinitiation and successful transcription elongation, thereby stimulating gene expression[41,42].

Second, if conserved with *S. cerevisiae*, the CBC may promote transcription elongation via physical connections with the processivity factor CDK12 (refs. 33,43) (Fig. 2Da). Third, the CBCA, which is formed by addition of ARS2 to the CBC after pause release, prevents intronic PTT mediated by CPA at non-canonical PASs[33], which produces ‘premature CPA mRNAs’ (Fig. 2Db). Encoded or regulated intronic CPA produces either stable non-coding RNAs or alternative mRNAs that diversify the proteome[44]. However, faulty premature CPA mRNAs, such as those produced by CBC deficiency, are unstable and their polyadenylation may be inefficient, especially for short transcripts[44] (see more in the sections that follow). Fourth, CBC-bound ARS2 may trigger intronic PTT via Restrictor[32], a newly discovered transcription termination pathway that does not involve CPA and produces non-adenylated ‘restrictor-released pre-mRNAs’[18] (Fig. 2Dc). These functions of the CBCA probably rely

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on interactions with the U1 small nuclear ribonucleoprotein particle (snRNP), the binding of which to 5′-splice sites prevents both types of intronic PTT in a mechanism called telescripting[32,45,46].

Pre-mRNA splicing, CPA, detained-intron pre-mRNA and readthrough pre-mRNAs.—Using triosephosphate isomerase pre-mRNA as a model, our lab was among the first in the 1990s to establish mechanistic connections between splicing events and CPA[47,48] (Fig. 2E). Recent long-read sequencing studies revealed that the extent of co-transcriptional splicing and how co-transcriptional splicing events and CPA are coordinated can vary between species, cell types and genes[49–51].

Co-transcriptional splicing and its coupling to CPA are influenced by key pre-mRNA processing factors: the CBC, exon–junction complexes (EJCs) and PABPN1. First, capping is required for efficient co-transcriptional splicing (Fig. 2Ec) and ‘canonical’ CPA[37] (Fig. 2Ed), thereby limiting the production of intron-containing ‘readthrough pre-mRNAs’, that is, pre-mRNAs with a 3′-end that extends beyond a canonical PAS (Fig. 2Fb). Capping most probably promotes splicing via a complex interaction network between the CBC and snRNPs[33] and promotes CPA as a consequence of promoting splicing. Second, splicing dynamics between *cis*-residing introns might be influenced by EJCs (Fig. 2Ec). Indeed, introns that are not spliced co-transcriptionally usually have weaker splice sites than do efficiently spliced introns[52,53], and the splicing of weak introns can be promoted by the deposition of EJCs upstream of exon–exon junctions that result from the splicing of neighbouring introns[54]. Third, CPA is positively and negatively influenced by PABPN1: although PABPN1 promotes CPA at canonical PASs (Fig. 2Ed), it suppresses premature CPA at PASs[55] that may reside within introns[56] (Fig. 2Db).

Detained-intron pre-mRNAs are a class of polyadenylated intron-containing pre-mRNAs in which only one or a few specific introns are poised for post-transcriptional splicing[57] (Fig. 2Ea). Notably, detained-intron pre-mRNAs are distinct from retained-intron RNAs, which are fully processed mRNAs in which one or more introns are identified as a part of an exon because of alternative co-transcriptional splicing. Many detained-intron pre-mRNAs constitute a nuclear reservoir of immature transcripts that can be rapidly and sustainably spliced post-transcriptionally, possibly in nuclear speckles, and sometimes more than 24 h after their synthesis — to maintain cellular homoeostasis or allow cellular adaptation to acute stimuli (reviewed in ref. 58). Nonetheless, as any terminated intron-containing pre-mRNAs, detained-intron pre-mRNAs are at least partially sensitive to nuclear decay (see section ‘Nuclear decay of faulty mRNAs’). Indeed, some detained-intron pre-mRNAs are ‘dead-end’ products destined to be degraded in the nucleus. Notably, some dead-end detained-intron pre-mRNAs can be post-transcriptionally spliced in response to stimuli, but their splicing products include cryptic ‘poison’ exons that trigger cytoplasmic nonsense-mediated mRNA decay (NMD) after nuclear export[59,60]. Interestingly, at least some ‘pairs’ of dead-end and mRNA-producing detained-intron pre-mRNAs that derive from genes with antagonizing functions are spliced post-transcriptionally in response to the same stimulus[59,60]. As one example of a co-regulated ‘pair’, post-transcriptional splicing of *OGA* and *OGT* detained-intron pre-mRNAs in response to high levels of O-linked *N*-acetylglucosamine generates productive *OGA* mRNA and NMD-sensitive *OGT* mRNA,

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thereby increasing and decreasing the cellular level of the enzyme that, respectively, removes (OGA) from or transfers (OGT) O-linked *N*-acetylglucosamine to target proteins[60].

Although CPA typically occurs co-transcriptionally, it was recently found that hundreds of intron-containing pre-mRNAs are subject to post-transcriptional CPA after initial co-transcriptional cleavage downstream of a weak distal PAS[61–63] (Fig. 2Fc,d). At least one example of stress-induced post-transcriptional CPA has been proposed to occur in paraspeckles[63]. However, it is unclear whether, like detained-intron pre-mRNAs, 3′-extended pre-mRNAs cleaved at a distal PAS generally constitute a nuclear reservoir of immature mRNAs that are post-transcriptionally processed in a regulated manner. Downstream-of-gene (DoG)-containing RNAs are another class of stress-induced readthrough pre-mRNAs. DoG RNAs have a 3′-end that is extended by >5,000−200,000 nts and may include sequences from a downstream 'read-in' gene, have undergone 3′-end cleavage, may or may not be polyadenylated, and typically retain introns, at least from the read-in gene[64–66]. Whether DoG RNAs can be post-transcriptionally processed is unclear.

Nuclear mRNA export

Each pre-mRNA processing step provides export competence by facilitating the assembly of transcription and export (TREX) complexes, which contain ALYREF, onto newly made mRNAs (Fig. 3b). According to a current model, a pool of ALYREF molecules is recruited to the CBC as early as promoter-proximal pausing (Fig. 2Ba) and then redistributed to EJCs upstream of exon–exon junctions[67] and possibly to nuclear poly(A)-binding proteins PABPN1 and ZC3H14 at poly(A) tails[68,69] (Fig. 2E). ALYREF, whose distribution throughout the mRNA indicates efficient pre-mRNA processing, promotes mRNA packaging and then nuclear export via the recruitment of additional TREX subcomplexes[70] during mRNA trafficking through nuclear speckles[58].

Although TREX promotes the export of processed mRNAs, features of misprocessed mRNAs directly inhibit their export (Fig. 3c). For example, a 5′-splice site motif in processed mRNAs blocks their export[71]. Mechanistically, retention of a 5′-splice site in intronic CPA pre-mRNAs or intron-containing pre-mRNAs was proposed to promote their nuclear sequestration via connections between U1 snRNP bound to the 5′-splice site and the PAXT scaffold ZFC3H1 (refs. 72,73). The ability of ZFC3H1 to form condensates prevents trafficking of RNAs through nuclear speckles and, thus, nuclear export[74]. By contrast, detained-intron pre-mRNAs, or at least detained-intron pre-mRNAs that are not dead-end transcripts and instead are poised for post-transcriptional splicing, are preferentially enriched in nuclear speckles, wherein they may gain export competence after splicing[75]. Recognition of the 3′-splice site by the splicing factor U2AF, which follows recognition of the 5′-splice site by U1 snRNP to form the early splicesome (E complex)[33], and inhibition of the B^{act} spliceosome complex, which forms after release of U1 snRNP and additional rearrangements[33], also contribute to nuclear retention of intron-containing pre-mRNAs[76]. However, the underlying mechanisms have not been characterized. Notably, the splicing of detained-intron pre-mRNAs was proposed to be delayed by stalling of the B^{act} complex[77]. Finally, PABPN1 (refs. 27,78) and possibly ZC3H14 (ref. 69) also prevent the nuclear export of unspliced mRNAs. This is despite antagonistic functions of PABPN1 and ZC3H14 on the

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control of poly(A) tail length, which in turn controls nuclear retention: although PABPN1 promotes poly(A) tail lengthening[79,80], and hyperadenylation (>250 nts) blocks nuclear export[76], ZC3H14 promotes poly(A) tail shortening[69,79] and a short poly(A) tail (~50 nts) promotes nuclear export[76].

Nuclear mRNA decay

Most normally processed mRNAs evade nuclear decay through localization to nuclear condensates or by efficient export to the cytoplasm. Nonetheless, some nuclear mRNAs are degraded in the nucleus by one or more pathways (Fig. 3a,d,e). As a first example, nuclear mRNAs encoding 'immediate early response transcription factors' can be degraded by PAXT or, possibly and to a lesser extent, by NEXT, presumably allowing for the rapid removal of mRNAs following transcription bursts[11,81] (Fig. 3d). However, whether these mRNAs are fully spliced and polyadenylated was not clearly demonstrated. As a second example, non-canonical histone mRNAs, which lack poly(A) tails and most often lack introns, are degraded by mTRAMP, thereby controlling cell cycling[82] (Fig. 3d). Third, inhibition of the deadenylase activity of poly(A)-specific ribonuclease (PARN) by the CBC is released in response to ultraviolet radiation (UV) stress, thereby preventing polyadenylation in the nucleus and promoting decay of housekeeping, as well as growth-related and differentiation-related mRNAs[83]. Fourth, in *C. elegans* XRN2 cooperates with the nuclear RNA-binding like-SM complex 2–8, which in yeast promotes nuclear pre-mRNA decapping[84], and possibly the poly(A)-binding protein PABP2 (the orthologue of human PABPN1) to degrade spliced mRNAs from developmental genes[85] (Fig. 3e).

Competition between nuclear mRNA decay and export

In contrast to most properly processed mRNAs, aberrant mRNAs such as premature CPA mRNAs[25,72], intron-containing pre-mRNAs[57], readthrough DoG RNAs[64] and hyperadenylated mRNAs that harbour a >250-nt poly(A) tail[76] are usually retained in the nucleus, wherein they exhibit half-lives as short as 10 min (refs. 57,64,86,87). Importantly, mRNAs and detained-intron pre-mRNAs poised for splicing are possibly stabilized by nuclear retention in nuclear speckles. Indeed, the RNA exosome is excluded from nuclear speckles, in which the negative exosome regulator NRDE2 further inhibits MTR4 (refs. 58,88). Stabilization of detained-intron pre-mRNAs in nuclear speckles may provide a longer window of time for their post-transcriptional splicing, which was also proposed to occur within speckles[58]. Notably, during meiotic prophase in plants, Cajal bodies were identified as another nuclear RNA condensate in which detained-intron pre-mRNAs accumulate and are potentially stabilized until they are post-transcriptionally spliced and exported to the cytoplasm at the appropriate developmental stage[89]. A mechanism resembling detained-intron pre-mRNA stabilization and post-transcriptional splicing was also observed during spermatogenesis in animals[53].

In *S. cerevisiae*, newly synthesized, but not bulk, nuclear poly(A)$^+$ RNAs are rapidly degraded following acute depletion of the RNA export factor Mex67p[90]. Correspondingly, in human cells, poly(A)$^+$ RNAs, including mature mRNAs, are stabilized and more efficiently exported to the cytoplasm when the RNA exosome is inactivated and ZFC3H1 is depleted[81]. As noted in section 'Nuclear mRNA export'), this function of ZFC3H1 may rely on its

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blocking RNA trafficking through nuclear speckles[74]. In fact, sorting of poly(A)$^+$ nuclear mRNAs for export or degradation occurs prior to localization to nuclear speckles[91].

Together, these findings indicate that nuclear export and decay activities compete in a way that is influenced by the timely processing of pre-mRNA into mRNA and the localization or sequestration of either molecule to subnuclear structures. Key underlying competition mechanisms involve those pre-mRNA processing factors that influence nuclear decay in addition to influencing export, including the CBCA, U1 snRNP, PABPN1 and ZC3H14 (Fig. 3). For example, the export factor ALYREF competes with the RNA exosome helicase MTR4 of PAXT and NEXT to associate with ARS2 (ref. 92). ALYREF recruitment may involve the CBCA auxiliary factor NCBP3 (ref. 35), the binding of which to ARS2 is mutually exclusive with that of the PAXT scaffold ZFC3H1 and a NEXT accessory protein, either ZC3H4 or ZC3H18 (refs. 17–20,33) (Fig. 3a). As a second example, although U1 snRNP promotes nuclear RNA sequestration (see section ‘Nuclear mRNA export’; Fig. 3c), it may promote RNA decay through its physical association with PAXT (via ZFC3H1) or NEXT (via ZC3H18)[72,73]. As a third example, although PABPN1 can either positively or negatively influence nuclear mRNA export (see section ‘Nuclear mRNA export’; Fig. 3b,c), PABPN1 also promotes PPD via PAXT[16,19,25–29,80]. As a last example, although ZC3H14 promotes PAXT-mediated decay of premature CPA mRNAs[26] (see more in the sections that follow), it also protects fully spliced mRNA from decay[93].

Nuclear decay of faulty mRNAs

As discussed in the previous sections, errors in or impaired mechanistic connections between gene transcription and the various pre-mRNA processing steps, including their relative rates, produce faulty mRNAs, which include prematurely terminated (Fig. 2Bb,Db,Dc) or intron-containing pre-mRNAs (Fig. 2Ea,Fb,Fc). Clearance of these export-incompetent faulty mRNAs is required to maintain nuclear RNA homoeostasis and, as they may leak into the cytoplasm, to prevent the production of nonfunctional or even toxic proteins.

Promoter-associated pre-mRNAs.—Early work has indicated a role for nuclear decapping factors (DCP1A, DCP2 or EDC3) and XRN2 in the co-transcriptional decay of nascent promoter-associated pre-mRNAs and subsequent PTT immediately downstream of the transcription start site of most protein-encoding genes[94,95]. However, acute depletion of XRN2 did not prevent promoter-proximal PTT in human cells[86,96], and siRNA-mediated depletion of XRN2 showed no effect on the levels of promoter-associated pre-mRNAs in *D. melanogaster* S2 cells[87]. Instead, Integrator recently emerged as the universal inducer of promoter-proximal PTT at protein-coding genes[31,40] (Fig. 2Bb). Promoter-associated pre-mRNAs cleaved by Integrator are typically capped and become oligoadenylated and eventually degraded by the RNA exosome, presumably in cooperation with mTRAMP, which has distributive polyadenylation activity[87,97,98] (Fig. 4a). Because as few as 1% of transcription initiation events may extend beyond promoter-proximal pausing[44], failure to degrade promoter-associated pre-mRNAs would undoubtedly result in unbalanced nuclear RNA homoeostasis.

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Premature CPA mRNAs.—Acute depletion of DIS3, EXOSC10 or XRN2 using the auxin-inducible degron system demonstrated that transcripts having undergone premature intronic polyadenylation are degraded in the nucleus at their 3′-end by the RNA exosome rather than at their 5′-end by XRN2 (ref. 86). Nonetheless, results also suggest that, for some but not all transcripts, XRN2 triggers premature intronic polyadenylation[86].

Decay of premature CPA mRNAs by the RNA exosome involves the essential 3′-to-5′ exoribonuclease and endoribonuclease DIS3 (but not the accessory 3′-to-5′ exoribonuclease RRP6 (ref. 86)) and either PAXT[11,25,72] or, when CPA occurs within the first intron, possibly NEXT[11] (Fig. 4b). PAXT recruitment to premature CPA mRNAs requires phosphorylation of ZC3H14 by the cyclin-dependent kinase CDK13 (ref. 26). Targeting premature CPA mRNAs for nuclear decay may also involve physical and functional interactions between U1 snRNP bound to a retained 5′-splice site and the PAXT scaffold ZFC3H1 or the NEXT accessory protein ZC3H18 (refs. 45,46,72), both of which also bind ARS2 (see in the previous sections). Providing additional support for the notion that PAXT-mediated decay and PPD are part of the same decay pathway, premature CPA mRNAs can be degraded following hyperadenylation[27] (Fig. 4b, left part). Premature CPA mRNAs that evade nuclear decay, at least by PAXT, can be exported and translated in the cytoplasm, thereby producing truncated proteins that induce proteomic stress[25,26]. Of note, CPA pre-mRNA translation is not expected to trigger cytoplasmic non-stop mRNA decay as it utilizes intronic stop codons[26].

Restrictor-released pre-mRNAs.—Restrictor-released pre-mRNAs constitute a new class of faulty mRNAs whose termination within a promoter-proximal intron by Restrictor is directly coupled to their decay by NEXT[18,32] (Fig. 4c). In this mechanism, ARS2-bound ZC3H4 first recruits the RNAPII-binding protein WDR82 to chromatin to form Restrictor and terminate transcription in introns[32]. Then, ZC3H4 disengages from WDR82 and recruits NEXT and the RNA exosome to the released RNAs to promote their decay[18]. Because Restrictor-released RNAs lack a poly(A) tail[18], whether they could leak into the cytoplasm and be translated, as has been shown for premature CPA mRNAs, is unclear[26].

Detained-intron pre-mRNAs and other presumably terminated intron-containing pre-mRNAs.—Studies of how the long-term depletion of decay factors impacts the levels of intron-containing reporter pre-mRNAs and endogenous detained-intron pre-mRNAs indicate that the retention of a 5′-splice site triggers decay at the RNA 3′-end by DIS3-containing and RRP6-containing RNA exosomes but not at the RNA 5′-end by XRN2 (refs. 99,100) (Fig. 4d). Additionally, the decay of intron-containing pre-mRNAs by the RNA exosome appears to occur in the nucleoplasm (that is, in the soluble nuclear fraction) and, to a lesser extent, in the insoluble nuclear fraction (which includes chromatin)[99]. Below, we review evidence that the RNA exosome degrades intron-containing pre-mRNAs via PAXT/PPD, (m)TRAMP and/or nuclear pore-associated proteins, and we then briefly discuss how NMD safeguards against the accumulation of intron-containing pre-mRNAs that evade nuclear decay.

A role for PABPN1 in the nuclear decay of polyadenylated intron-containing pre-mRNAs by the RNA exosome was first demonstrated for *S. pombe* in studies of the PABPN1 and

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RRP6 orthologues Pab2 and Rrp6 (ref. 101). Other studies in humans and *S. cerevisiae* have suggested that, although ZC3H14 protects fully spliced mRNAs from decay, ZC3H14 can detect spliceosome components on intron-containing pre-mRNAs to induce their decay by the RNA exosome[93], presumably via PAXT. Consistently, numerous detained-intron pre-mRNAs are degraded by PPD in the nucleus of human HEK293 cells[27]. Thus, the mechanism by which intron-containing pre-mRNAs are targeted for decay may be similar to how premature CPA mRNAs are degraded, that is, via cooperation between U1 snRNP, PABPN1, ZC3H14 and PAXT/PPD (Fig. 4d, left part).

In a different model deriving from studies of *S. cerevisiae*, serine–arginine-rich proteins 'sense' spliceosome constituents on unspliced pre-mRNAs to promote their degradation via TRAMP[102]. In support of this hypothesis applying to mammals (Fig. 4d, right part), robust RNase A-resistant association of mTRAMP with splicing factors, including serine–arginine-rich proteins and other snRNP factors, has been reported for human cells[22]. TRAMP-mediated nuclear mRNA decay in yeast does not require its oligoadenylation activity[103], raising the hypothesis that targeted mRNAs are already polyadenylated or oligoadenylated.

Finally, numerous studies in humans[100,104,105] and in *S. cerevisiae*[106] have shown that the nuclear pore-associated protein TPR, along with other nucleoporins, promotes the nuclear retention and decay of intron-containing pre-mRNAs in a mechanism that, at least in budding yeast[106], involves recognition of the 5′-splice site. However, another work in human cells indicates that TPR stabilizes short intronless mRNAs in the nucleus but is not involved in the nuclear retention of intron-containing pre-mRNAs[107]. Thus, how the nuclear RNA exosome cooperates with nucleoporins remains unclear.

Intron-containing pre-mRNAs that leak into the cytoplasm are usually targeted for NMD[52]. Therefore, although NMD functions as a fail-safe mechanism for the degradation of cytoplasmic intron-containing pre-mRNAs, taking cues from the upregulation of NMD efficiency in colorectal cancer cells with microsatellite instability[108], global evasion of intron-containing pre-mRNA decay in the nucleus might saturate the NMD pathway and prevent the degradation of the 5–10% of mammalian-cell mRNAs that are natural NMD targets[109].

Readthrough pre-mRNAs.—Long-term knockdown experiments suggest that readthrough transcripts deriving from a β-globin gene reporter harbouring a mutated CPA site and DoG RNAs, which arise owing to CPA-site readthrough, are degraded on chromatin by XRN2 (refs. 8,99). XRN2 may cooperate with a component of the decapping complex to degrade readthrough pre-mRNAs from their 5′-end cap. For example, depletion of DCP2 stabilized some, but not all, XRN2-sensitive intron-containing pre-mRNAs that accumulate after treatment with the splicing inhibitor spliceostatin A[99] (Fig. 4e, upper part). As inefficient co-transcriptional splicing generally results in inefficient CPA (see in the previous sections), we posit that these DCP2-targeted or XRN2-targeted intron-containing pre-mRNAs are nascent readthrough transcripts. Notably, because XRN2 can promote transcription termination of chromatin-bound pre-mRNAs not only after CPA but also after endonucleolytic cleavage independently of the CPA machinery[13,14,110,111], it is possible that

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XRN2 degrades 3′-cleavage products of these readthrough transcripts in a way that also promotes transcription termination.

Results from long-term knockdown experiments also suggest that reporter β-globin readthrough transcripts harbouring a mutated CPA site[99], as well as intron-containing readthrough cellular pre-mRNAs[112], are degraded by the RNA exosome in the nucleoplasm, that is, after release from chromatin[99] (Fig. 4e, lower part). Release from chromatin possibly involves the uncharacterized cleavage mechanism(s) that hypothetically generate XRN2-sensitive nascent pre-mRNAs. Although the mammalian adaptor of the RNA exosome required for the decay of readthrough RNAs is unknown, in *S. cerevisiae*, aberrant 3′-extended readthrough transcripts are targeted by TRAMP and rapidly degraded in conjunction with a distinct CBC-dependent and Rrp6p-dependent decay pathway called ‘degradation of mRNA in the nucleus’[113]. Supporting the idea that readthrough pre-mRNAs are also targeted for decay by mTRAMP (Fig. 4e, lower part), as shown for yeast TRAMP, human TRAMP-like complexes promote RNA 3′-end adenylation[114], and β-globin readthrough transcripts targeted by the RNA exosome have short oligo(A) tails (up to nine ‘As’ when the RNA exosome is inactivated)[115].

Finally, the 5′-to-3′ exoribonuclease DXO too has been implicated in the decay of readthrough pre-mRNAs, most probably in a co-transcriptional manner[37] (Fig. 4f). DXO-mediated decay seems to function in a pre-mRNA capping quality-control pathway and to target primarily, if not exclusively, intron-containing pre-mRNAs in which introns are retained as a result of inefficient or incomplete capping[37]. Nonetheless, owing to its large spectrum of catalytic activities, in addition to targeting m^7GTP-capping intermediates, DXO is also able to degrade RNAs with a variety of 5′-ends, including a 5′-monophosphate, a 5′-hydroxyl, a fully processed m^7GTP cap or other non-canonical RNA 5′-caps[116].

Regulation of nuclear mRNA decay

In this section, we review targeted mechanisms that cells utilize to sensitize mRNAs to, or protect mRNAs from, nuclear decay to quantitatively control gene expression in a gene-specific or a condition-specific manner during, for example, development and disease.

Epigenetic marks and their readers

Reversible ‘epigenic’ modifications of DNA and histones provide an important layer of gene regulation by controlling the accessibility and readability of genes by the transcription machinery. Although generally all protein-encoding and non-coding genes are transcriptionally controlled epigenetically, exciting research published in the past couple of years has shown that some epigenetic marks and their readers reinforce gene regulatory networks via promoting nuclear pre-mRNA or mRNA decay.

H3K27me3, H2AK119ub1 and the Polycomb repressive complexes.—Polycomb repressive complex (PRC)1 and PRC2 cooperate to silence genes whose inappropriate activation would otherwise lead to developmental problems or cancer[14,117]. In one epigenetic mechanism of transcriptional repression, de novo recruitment of PRC2 catalyses trimethylation of histone H3 at lysine 27 to generate H3K27me3, which triggers chromatin

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compaction and recruitment of PRC1, as well as more PRC2; PRC1 then catalyses mono-ubiquitination of histone H2A at lysine 119 to generate H2AK119ub1, which further triggers chromatin compaction and recruitment of both PRC1 and PRC2 (refs. 14,117). In a second mechanism that reinforces epigenetic gene silencing, PRC1 and PRC2 bind and recruit the rixosome at promoters harbouring H2AK119ub1 and H3K27me3 marks, the rixosome cleaves leaky pre-mRNAs possibly as early as during promoter-proximal pausing, and XRN2 degrades the resulting 3′-cleavage product and terminates spurious transcription[14] (Fig. 5a). Notably, PAXT also represses PRC-target genes, but the mechanism is indirect: in the absence of ZFC3H1, the accumulated nuclear poly(A)$^+$ RNAs sequester PRC2 away from its target genes, leading to their epigenetic derepression[118].

As noted in the section ‘Nuclear mRNA decay’, XRN2 represses developmental genes whose promoter harbours H3K27me3 marks also in *C. elegans*[85]. However, the underlying mechanism does not involve PRCs or the co-transcriptional decay of nascent pre-mRNAs but, most probably, the post-transcriptional decay of spliced mRNAs[85] in cooperation with DCP2 (Fig. 5b).

H3K4me3 and Integrator.—H3K4me3 is enriched at transcription start sites, wherein it promotes the transcription of genes that maintain cell identity via the recruitment of essential transcription initiation factors and, possibly independently, by counteracting repressive marks such as H3K27me3 (ref. 119). Acute depletion of the core subunits of the H3K4me3 writer SET1/COMPASS induces rapid loss of H3K4me3 at transcription start sites and, subsequently, the loss of Integrator, or at least its endonucleolytic subunit INTS11, which binds H3K4me3 directly or indirectly[119] (Fig. 5c). Consistent with studies using acute depletion of INTS11 (refs. 39,120), acute depletion of SET1/COMPASS results in the stabilization of RNAPII downstream of promoters and slower transcription elongation rates[119] (Fig. 5c). As the density of the activating epigenetic mark H3K4me3 at the transcription start site of protein-encoding genes anti-correlates with the recruitment of Integrator in *D. melanogaster*[98], the functional interaction between H3K4me3 and Integrator may have undergone divergent evolution.

H3K9me3 and the HUSH complex.—The human silencing hub (HUSH) complex sustains the epigenetic silencing of numerous transcriptional units through its ability to spread the repressive mark H3K9me3, thereby maintaining heterochromatin compaction. HUSH targets include active endogenous transposable elements (in particular retrotransposons), HIV retroviral insertions (which are structurally similar to the family of retrotransposons that harbour long terminal repeats), and hundreds of protein-encoding genes, including genes encoding zinc-finger proteins and evolutionarily young genes[121–124]. Recent works have shown that HUSH also represses transcriptionally active transposable elements[125] and HIV insertions[126] by promoting the decay of their transcripts via the recruitment of NEXT[125], PAXT[125,126] or the CCR4–NOT deadenylation complex scaffold CNOT1 (ref. 126) (Fig. 5d). Although there is no evidence that CCR4–NOT mediates nuclear RNA decay in mammals, *S. cerevisiae* Ccr4–Not physically and functionally interacts with Mtr4p and the nuclear RNA exosome to promote the decay of poly(A)$^+$ non-coding RNAs[127], and Ccr4−Not promotes the decay of transposable element-derived

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and telomere-derived transcripts in *Drosophila*[128]. While not experimentally demonstrated, it is enticing to hypothesize that HUSH-mediated repression of protein-encoding genes likewise involves RNA decay by the RNA exosome.

Alternative pre-mRNA processing

Unlike processed mRNAs, faulty mRNAs are usually retained and degraded in the nucleus (see in the previous sections). Thus, targeted inhibition of efficient pre-mRNA processing events leads to the downregulation of their associated genes. By contrast, enhancing pre-mRNA processing that is usually inefficient results in gene upregulation.

Premature cleavage of promoter-associated pre-mRNAs.—The transcriptional co-activator PGC1α, via its CBP80-binding motif[129], outcompetes Integrator for association with the CBC during promoter-proximal pausing, thereby promoting early transcription elongation over promoter-proximal PTT[34] (Fig. 6a). In mouse myoblasts, this mechanism transcriptionally activates short interferon-response genes to ensure timely skeletal muscle regeneration after injury[34]. These genes recruit PGC1α via promoter-bound nuclear receptor ERRα[34]. However, ERRα and PGC-1α also bind the promoter of other genes, at least in differentiated mouse myotubes[130,131], suggesting that additional gene-specificity mechanisms are at play. Alternatively, given that Integrator also increases transcription elongation rates[39,40] possibly by promoting transcription reiniation after release of stalled RNAPII, the cooperation of PGC1α, ERRα and CBP80 to outcompete Integrator may 'force' the release from pausing of poorly processive RNAPII, which could transcriptionally activate short genes only. PGC1α-dependent protection from pre-mRNA cleavage by Integrator during promoter-proximal pausing seems to function in a pre-mRNA capping, or at least CBC loading, quality-control pathway[34]. These findings support the idea that Integrator terminates non-productive transcription rather than transcription from normally paused RNAPII (refs. 41,42).

Binding of the N^6-methyladenosine (m^6A) readers YTHDC1 and/or hnRNPG to m^6A marks on promoter-associated pre-mRNAs also outcompetes Integrator-mediated cleavage, preventing promoter-proximal PTT[120] (Fig. 6a). However, the net impact of this mechanism on gene expression is unclear.

Premature CPA.—Cellular adaptation to stress involves a wide array of epigenetic, transcriptional, co-transcriptional and post-transcriptional signalling-mediated changes to gene expression[132]. For example, heat shock induces global premature CPA by inhibiting U1 telescripting[133]. Although many heat shock-induced premature CPA events generate stable shorter mRNAs, at least some generate unstable transcripts that are presumably degraded by the nuclear RNA exosome, thereby decreasing gene expression[133] (Fig. 6b). In a second example, degradation of premature CPA mRNAs was proposed to be initiated by PARN-mediated deadenylation in response to UV stress[83] (Fig. 6b). In a third example, mutations in CDK13 found in many cancers prevent the phosphorylation of ZC3H14, which results in the stabilization of premature CPA transcripts and proteomic stress (Fig. 6b).

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Alternative intron retention.—Intron retention is a widespread and regulated mechanism that finely tunes gene expression in a cell type-specific manner (reviewed in ref. 58). Differential expression and post-translational modifications of splicing factors control the levels of decay-sensitive detained-intron pre-mRNAs to repress or activate gene expression (Fig. 6c). As one example of controlled expression of a splicing factor, differential expression of the RNA-binding protein PTBP1 in neurons and non-neuronal cells mediates cell-specific expression of neurotransmitter genes through coordinated splicing of detained-intron pre-mRNAs. Indeed, whereas PTBP1 binding to the final introns of detained-intron pre-mRNAs in non-neuronal cells prevents their splicing and promotes their decay by the RNA exosome, PTBP1 loss in neurons alleviates intron retention and allows for sustained gene expression[100]. In a similar mechanism, human PABPN1 competes with the splicing factor SRSF10 for binding to an A-rich region in the 3′-UTR of *PABPN1* detained-intron pre-mRNA[134]. In a chain of events, excess PABPN1 inhibits splicing of the last intron of *PABPN1* detained-intron pre-mRNA, triggering its decay by the nuclear RNA exosome to maintain PABPN1 at homeostatic levels. In a last example, the double-stranded RNA-binding protein TARBP2 inhibits the splicing of introns that it binds at GC-rich structural elements, which causes intron-containing pre-mRNA decay by the RNA exosome via NEXT and/or by XRN2 (refs. 104,135). Consequently, overexpression of TARBP2 promotes lung cancer growth and the metastasis of breast carcinomas by destabilizing pre-mRNAs produced from cancer suppressor genes, for example *APP*, which encodes the metastasis-suppressor amyloid precursor protein[104,135].

As one example of detained-intron pre-mRNA decay controlled by the post-translational modification of a splicing factor, inhibition of the type II protein arginine methyltransferase PRMT5 in mouse or human cells reduces the expression of hundreds of genes, including oncogenes, by inhibiting post-transcriptional splicing of detained introns harbouring a weak 5′-splice site, thereby impeding brain cancer growth[136], limiting lymphoma progression[137] and improving mixed-lineage leukaemia survival[138]. The underlying mechanism probably involves methylation of the snRNP protein SmB, which promotes post-transcriptional splicing and/or inhibits detained-intron pre-mRNA decay[139]. Conversely, methylation of the TREX adaptor CHTOP by one or more type I protein arginine methyltransferases, which remain to be defined, promotes intron retention[139].

Alternative 3′-end processing.—Intron-containing pre-mRNAs produced by apoptosis-related and inflammation-related genes accumulate in the liver of liver-specific *Cnot1*-KO mice[140], raising the possibility that the CCR4–NOT complex destabilizes a subset of nuclear intron-containing pre-mRNAs by limiting their poly(A) tail length (Fig. 6d). Indeed, based on one non-peer-reviewed preprint, CNOT1 depletion leads to longer poly(A) tails in the nucleus[141]. Although the positive genetic interaction between CCR4–NOT and RRP6 described in yeast[142] suggests that CNOT1-targeted intron-containing pre-mRNAs are degraded by the RNA exosome, how this mechanism achieves such gene specificity is unclear.

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Recruitment of, or escape from, RNA decay factors

Cells also utilize RNA-binding proteins to recognize specific nuclear mRNAs and determine their fate by mediating the recruitment or activity of decay factors, by controlling their localization in subnuclear structures and/or by influencing their export competence (Fig. 7).

Subnuclear compartmentalization to promote or inhibit nuclear RNA decay.— A well-characterized mechanism by which an RNA-binding protein promotes the decay of specific mRNAs in a context-dependent manner involves the *S. pombe* RNA-binding protein Mmi1 (Fig. 7b). During vegetative growth, Mmi1 blocks the production of cytoplasmic mRNAs from meiosis-specific genes that are constitutively transcribed. To do this, Mmi1 binds near the 3′-end of detained-intron pre-mRNAs to promote their hyperadenylation by the PABPN1 orthologue Pab2, and it recruits the PAXT orthologue MTREC to trigger their nuclear decay[143,144]. Mmi1-mediated RNA decay occurs in Mmi1 decay foci, which are enriched in the RNA exosome and form via Mmi1 self-interaction assisted by a non-coding RNA homologue of enhancer of rudimentary, Erh1 (ref. 145). Alternatively, Mmi1 localizes meiotic detained-intron pre-mRNAs to the nuclear periphery via its binding to the nuclear envelope protein Lem2, which recruits MTREC via the serine–arginine-rich protein Iss10 (ref. 146). These pathways are regulated because inducing meiosis inhibits Lem2 activity[146] and triggers Mmi1 sequestration in inhibitory ‘Mei2 dots’[145], allowing for the timely expression of the targeted meiotic transcripts. The interaction between Lem2 and MTREC in *S. pombe* is recapitulated in humans via the orthologues LEM2 and PAXT[146], suggesting that this pathway is broadly conserved. Notably, Mmi1 also binds near the detained introns of meiotic pre-mRNAs to inhibit their splicing, but it is not required to promote RNA decay[143].

As an example of a similar mechanism in mammals, when mouse RAW 264.7 macrophages are stimulated with bacterial lipopolysaccharide, the RNA-binding protein nucleolin dampens the inflammatory response by recruiting pro-inflammatory pre-mRNAs containing intronic cytosine–uracil repeats to the nucleolus and delivering them to RRP6 for degradation by the RNA exosome[147] (Fig. 7c). By contrast, the binding of YTHDC1 to m^{6}A marks throughout exons, in addition to promoting nuclear RNA export (see in the sections that follow), sequesters cell cycle-related mRNAs in ‘nuclear YTHDC1-m^{6}A condensates’ (nYACs) away from PAXT and the RNA exosome[148] (Fig. 7d). Illustrating their biological importance, nYACs suppress the death and differentiation of acute myeloid leukaemia cells, thereby promoting leukaemia maintenance[148]. In a similar protective mechanism that is essential for the development of oocytes in mice, PABPN1 stabilizes poly(A)$^+$ mRNAs by sequestration into ‘nuclear poly(A) domains’ (NAPDs)[149] (Fig. 7k).

Control of nuclear mRNA export.—Numerous RNA sequences, structures and modifications have been shown to control nuclear mRNA export and, consequently, nuclear mRNA decay rates (reviewed in ref. 73). For example, m^{6}A generally promotes the nuclear export of mRNAs via the recruitment of the m^{6}A reader YTHDC1, which cooperates with the export licensing factor NXF1 (ref. 150) (Fig. 7d). However, a non-peer-reviewed preprint proposes that, when m^{6}A modifications are near an unused 5′-splice site of premature CPA pre-mRNAs, m^{6}A-bound YTHDC1 or YTHDC2 cooperates with ZFC3H1 and U1 snRNP to

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promote nuclear RNA retention and decay[151] (Fig. 7d). Thus, m^{6}A may differentially affect the fate of nuclear mRNAs depending on its location in the transcript.

Other examples of RNA modifications that control nuclear export are N^5-methylcytosine and adenosine-to-inosine editing. N^5-Methylcytosine is read by ALYREF to promote nuclear export[152] (Fig. 7e). Conversely, adenosine-to-inosine editing of double-stranded RNA structures promotes nuclear retention[73] (Fig. 7f). The RNA-binding protein interleukin enhancer binding factor 2 (ILF2) inhibits adenosine-to-inosine editing[153] and associates with TREX to promote the nuclear export and to prevent the MTR4-dependent nuclear decay of mRNAs encoding pluripotency transcription factors[154]. As ILF2 is induced by nicotine, this mechanism contributes to smoking-induced chemoresistance in oesophageal cancer[154].

An example of a structured RNA sequence that promotes mRNA export, thereby precluding nuclear decay, is provided by the NXF1-recruiting ‘constitutive transport element’ found in type D retroviral RNAs and an *Nxf1* mRNA isoform with a retained intron[155] (Fig. 7g). Conversely, splicing-inactive 5′-splice site motifs promote nuclear mRNA retention and decay[71] in a mechanism that is probably similar to the nuclear retention and decay of premature CPA mRNAs and intron-containing pre-mRNAs, both of which contain one or multiple 5′-splice sites (see section ‘Nuclear decay of faulty mRNAs’) (Fig. 7h).

Other mechanisms.—Viruses that infect humans have evolved strategies to protect their mRNAs and long non-coding RNAs (lncRNAs) from decay by the RNA exosome in the nucleus of their host cells using intricate mechanisms distinct from those described in the previous sections. The EB2 Epstein–Barr virus (EBV), which causes infectious mononucleosis, stabilizes intronless EBV mRNAs by counteracting SRSF3-mediated decay by NEXT[156] (Fig. 7i). By contrast, the ORF57 protein of Kaposi sarcoma-associated herpesvirus (KSHV), which causes Kaposi sarcoma, is orthologous to EBV EB2 and stabilizes HSHV mRNAs and lncRNAs by counteracting ARS2-mediated NEXT-independent decay[157] (Fig. 7i). For some but not all ORF57 targets, RNA stabilization also involves the recruitment of ALYREF to outcompete MTR4 at the CBCA (see in the previous sections) without involving ALYREF-promoted nuclear RNA export[157].

KSHV and other viruses also utilize a 3′-end *cis*-acting sequence called the ‘element for nuclear expression’, which forms a triple helix with the poly(A) tail of viral lncRNAs, thereby preventing their decay by PPD[27,158] (Fig. 7j). Such elements also stabilize human lncRNAs *MALAT1* and *NEAT1_2*, as well as cellular lncRNAs and mRNAs in plants, fungi and fish[158].

As a final albeit poorly characterized example, the human RNA-binding protein KHSRP protects *PSMD9* mRNA from 5′-to-3′ decay by XRN2 by bridging N^6,2′-*O*-dimethyladenosine at the *PSMD9* 5′-UTR and m^{6}A at a neighbouring enhancer RNA, thereby increasing radiotherapy resistance in bone-metastatic prostate cancer[159] (Fig. 7a).

Conclusions and future perspectives

As detailed in this Review, numerous studies reveal that nuclear RNA decay not only controls the levels of pervasively generated transcripts and the processing of non-coding

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RNAs but also, like cytoplasmic RNA decay, contributes in many ways to qualitatively and quantitatively controlling the expression of protein-encoding genes.

The 5′-to-3′ exonuclease XRN2 cooperates with decapping enzymes or possibly endonucleases to degrade nascent pre-mRNAs that fail to be cleaved and polyadenylated, such as owing to splicing defects. Another 5′-to-3′ exonuclease, DXO, manifests the same function as does XRN2, but when inefficient CPA is because of inefficient pre-mRNA capping. Recognition of immature but transcriptionally terminated mRNAs targeted for 3′-to-5′ decay by the RNA exosome requires at least one of three specialized adaptors: NEXT, PAXT or mTRAMP, each of which recognizes different but overlapping RNA features, such as the CBCA, RNA length, a poly(A) tail or intron retention. Recent insights from acute depletion of the NEXT or PAXT scaffold suggest that subcomplex compositions of RNA exosome adaptors may be at play[160]. Notably, PAXT-mediated decay most probably uses PPD, a decay pathway that involves RNA hyperadenylation triggered by PABPN1. Finally, PARN and possibly CCR4–NOT deadenylases contribute to the 3′-to-5′ decay of faulty nuclear mRNAs, but their connections with the RNA exosome are unclear. Although some determinants of exosomal RNA decay have been identified, understanding how each exosomal decay pathway recognizes export-incompetent mRNAs requires additional research.

Evidence that cells extensively use the RNA exosome and, to a lesser extent, XRN2 to control gene expression quantitatively is accumulating. For example, cells sensitize specific mRNAs to decay by inhibiting their processing and/or export or by recruiting decay factors in stimulus-responsive mechanisms. Conversely, sequestration of unprocessed mRNAs to nuclear condensates, or competition with decay factors, confers protection from decay by the RNA exosome. In particular, detained-intron pre-mRNA localization to nuclear speckles, which results in their immunity to exosomal decay, allows cells to draw on large nuclear stocks of pre-mRNAs in order to rapidly respond to intracellular and environmental cues. In other mechanisms, sequence-specific RNA-binding and DNA-binding proteins and histone marks control the cellular levels of specific mRNAs by recruiting or outcompeting decay factors co-transcriptionally or post-transcriptionally, or by influencing the subcellular localization of targeted mRNAs. The full diversity and complexity of gene-specific and stimulus-dependent nuclear RNA decay networks must still be unravelled.

Importantly, as shown for XRN2 (refs. 86,94), DIS3 (refs. 86,161), PAXT[160], NEXT[160] and Integrator[39–42,98,119,162], different conclusions have been drawn on the exact mechanism of action and the gene specificity of decay factors depending on whether loss-of-function experiments used long-term or acute depletion methods. Additionally, different conclusions have been reached for how decay factors degrade RNA targets when mRNA turnover was quantified using transcription inhibition, which often results in cell stress and uncouples transcription from decay, or metabolic labelling, which has less tendency to perturb cell homoeostasis[27,163]. With advanced methods, such as acute protein depletion and RNA metabolic labelling, and examinations of a larger repertoire of cell types, our understanding of nuclear RNA decay pathways in higher eukaryotes will undoubtedly be further characterized, and possibly redefined, in the coming years.

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Acknowledgements

We thank E. Wagner and P. Boutz for the critical comments on the manuscript. X.R. was supported by an American Heart Association Career Development Award (854100) and National Institutes of Health (NIH) R21 AG075368. This work was additionally supported by NIH R37 GM74593 and NIH R01 GM059614 (now NIH R35 GM149268) to L.E.M.

Glossary

Cajal bodies
Membraneless nuclear condensates, associated with the nucleolus, that have been implicated in the maturation of small nuclear ribonucleoproteins (snRNPs) and small nucleolar RNPs (snoRNPs), and telomerase assembly and trafficking.

Cap-binding complex
(CBC). Heterodimer of cap-binding proteins CBP20 and CBP80 acquired co-transcriptionally during promoter-proximal pausing of RNAPII. The CBC positively influences gene transcription, pre-mRNA processing, nuclear mRNA export, pioneer round(s) of translation and nonsense-mediated decay.

Cleavage and polyadenylation (CPA) complex
Modular multi-protein complex that recognizes polyadenylation signals and adjacent sequences in nascent RNAs to mediate their co-transcriptional cleavage and polyadenylation.

Enhancer RNA
Short unstable non-coding nuclear RNA that cooperates with distant promoters to activate gene transcription by, for example, promoting chromatin looping and formation of RNA condensates.

Exon–junction complexes
(EJCs). Heterogenous group of proteins deposited upstream of exon–exon junctions during pre-mRNA splicing that regulates multiple aspects of mRNA metabolism, including nuclear export, translation and nonsense-mediated decay.

Integrator
Metazoan-specific modular multi-protein complex that terminates pervasive RNA polymerase II (RNAPII)-mediated transcription, RNAPII-mediated transcription at diverse functional non-coding transcriptional units, and RNAPII-mediated transcription during promoter-proximal pausing at protein-encoding genes.

Nonsense-mediated mRNA decay
(NMD). Translation-dependent mRNA quality-control and quantity-control pathway that mediates the cytoplasmic decay of mRNAs terminating translation upstream of an exon–junction complex and/or a long or structured 3′-UTR.

Non-stop mRNA decay
Decay pathway triggered when ribosomes translate to the 3′-end of an mRNA because the mRNA lacks a translation termination codon.

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Nuclear speckles
Membraneless nuclear organelles rich in transcription, pre-mRNA processing and nuclear export factors, small nuclear RNAs, and poly(A)$^+$ RNAs. These structures are thought to function as storage centres and active sites of pre-mRNA processing.

Paraspeckles
Membraneless nuclear organelles containing the long non-coding RNA *NEAT1* and that influence nuclear RNA processes, such as by sequestering RNAs harbouring long double-stranded structures.

Poly(A)-specific ribonuclease
(PARN). 5′-End cap-binding and poly(A)-binding protein best characterized for its ability to stabilize non-coding RNAs, including microRNAs and the telomerase RNA, via the removal of oligo(A) tails.

Promoter-proximal pausing
RNAPII pausing ~20–60 nucleotides downstream of a transcription start site. It constitutes a quality control checkpoint that regulates the transcription of most, if not all, protein-encoding genes in higher eukaryotes.

Restrictor
ARS2-associated protein complex, composed of ZC3H4 and WDR82, that promotes transcription termination at non-coding genes or prematurely at protein-coding genes in a mechanism that probably does not require cleavage of the nascent RNA.

Rixosome
Multienzyme complex that is formed by the RIX1 complex, which includes a SUMO protease activity, and a RNase polynucleotide kinase complex.

RNAPII C-terminal domain
Tandem heptad repeats that extend the C-terminus of POLR2A, the largest RNAPII subunit, and whose complex phosphorylation status influences transcriptional and co-transcriptional processes.

Serine–arginine-rich proteins
RNA-binding proteins with serine-rich and arginine-rich domains that couple gene transcription, pre-mRNA splicing and mRNA export to the cytoplasm.

Transcription and export (TREX) complexes
A tetrameric assembly of four THO–UAP56 complexes recruited to spliced mRNA by ALYREF that licenses messenger ribonucleoproteins for nuclear export by NXF1–NXT1.

Transposable elements
Repetitive DNA sequences, largely restricted to inactivated retrotransposon insertions, that constitute up to 50% of mammalian genomes. Although intragenic transposable elements can harbour regulatory functions, intergenic transposable elements are largely parasitic and silenced.

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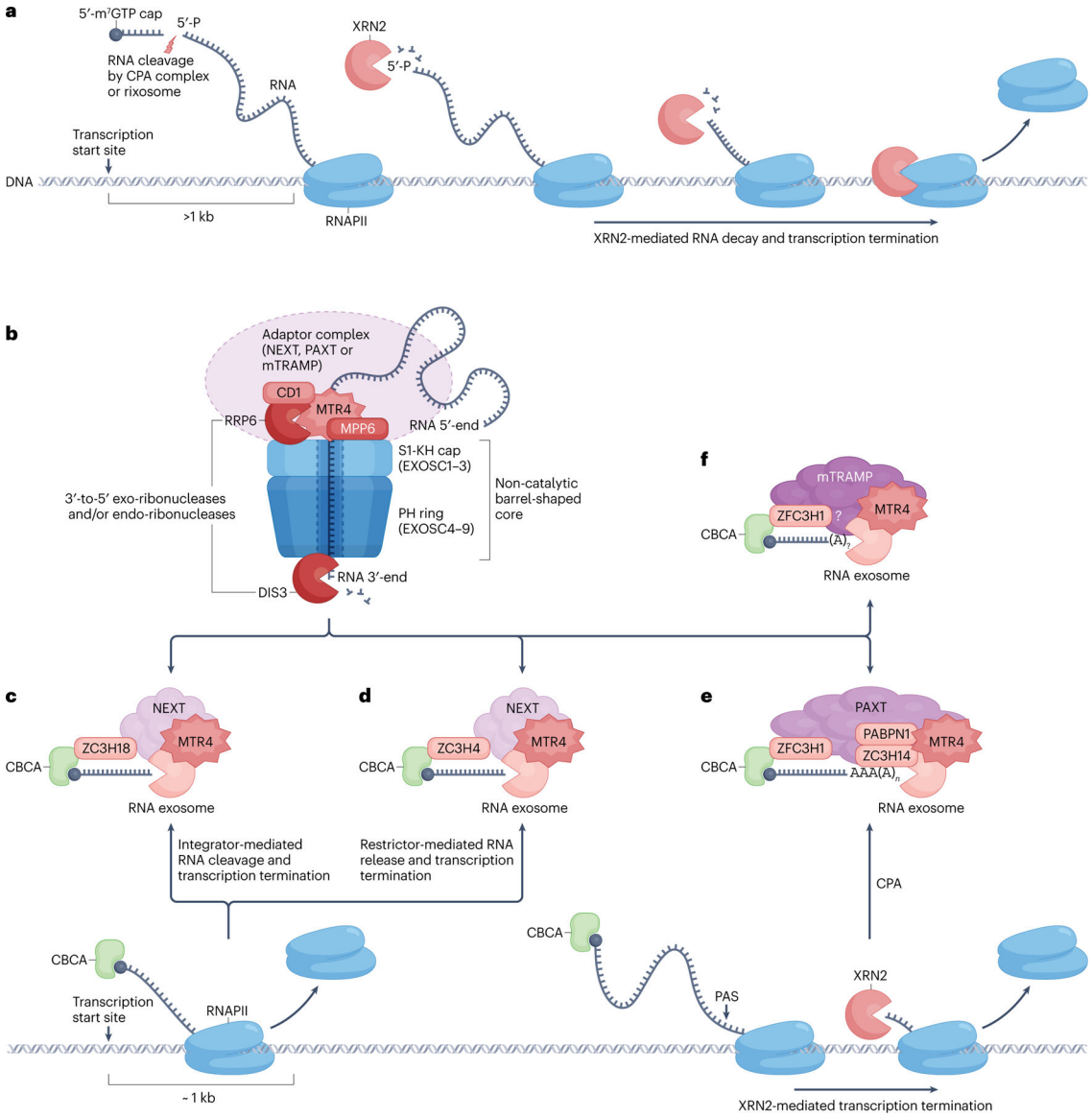

Fig. 1 |. Nuclear decay of pervasive RNAs by XRN2 or by the RNA exosome.

a, XRN2 degrades long (>1,000-nucleotide (nt)) nascent RNAs co-transcriptionally from a 5′-monophosphate (P) that can result from the endonucleolytic activity of the cleavage and polyadenylation (CPA) complex or the rixosome. In the ‘torpedo’ model of transcription termination, XRN2 chases after and eventually displaces elongating RNA polymerase II (RNAPII) from chromatin. **b**, The human nuclear RNA exosome consists of a non-catalytic barrel-shaped core (comprising ‘S1-KH cap’ and a ‘PH ring’), one or two ribonucleases (DIS3 and/or RRP6), and specialized structural proteins (CD1 and MPP6) that cooperate to recruit one of several protein adaptor complexes that include an RNA helicase (usually MTR4). In the nucleoplasm, typically the 3′-to-5′ RNA helicase MTR4 escorts the RNA targeted for decay to the S1-KH cap, which is the entry site of the RNA exosome barrel, MTR4 unwinds the RNA from its 3′-end, unwound RNA is threaded through the barrel channel, and the RNA is degraded by DIS3, which is a 3′-to-5′ exoribonuclease and

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endoribonuclease at the exit base of the PH ring. In the nucleolus, from which DIS3 is largely excluded, or in the nucleoplasm after long-term[161], but not rapid[86], depletion of DIS3, nuclease activity is provided by the 3′-to-5′ exoribonuclease RRP6, which localizes to the S1-KH cap. RRP6 also cooperates with the structural protein CD1 to recruit MTR4 to the RNA exosome and may facilitate RNA decay by widening and elongating the exosome channel and stimulating DIS3 activity. For RNA exosomes that target poly(A)$^+$ RNAs, S1-KH cap-bound MPP6 is required for DIS3-mediated decay, stimulates RRP6-mediated decay in the absence of DIS3, and can replace RRP6 to recruit MTR4 (ref. 161). **c**,**d**, Short (<1,000-nt) non-polyadenylated RNAs are typically targeted by the nuclear exosome-targeting (NEXT) adaptor complex via either ZC3H18 (part **c**), when RNAs are cleaved and released from chromatin by Integrator, or ZC3H4 (part **d**), when RNAs are released from chromatin by Restrictor. Both ZC3H18 and ZC3H4 bind the nuclear cap-binding complex–ARS2 (CBCA) in a mutually exclusive manner. **e**, Short and long polyadenylated RNAs produced by CPA are typically targeted by the poly(A) exosome-targeting (PAXT) connection via ZFC3H1 binding to the CBCA and PABPN1, and possibly ZC3H14, binding to the poly(A) tail. **f**, Metazoan Trf4/5–Air1/2–Mtr4 polyadenylation (TRAMP)-like complexes (mTRAMP) have been characterized and shown to degrade non-canonical histone mRNAs, which are typically not polyadenylated, as well as promoter-associated pre-mRNAs, which are oligoadenylated. However, if and how mTRAMP mediates the decay of pervasive RNAs in mammals, as TRAMP does in *Saccharomyces cerevisiae*, remains unknown. ‘?’ indicates that whether mTRAMP is recruited to the CBCA via ZFC3H1 was not experimentally tested. PAS, polyadenylation signal.

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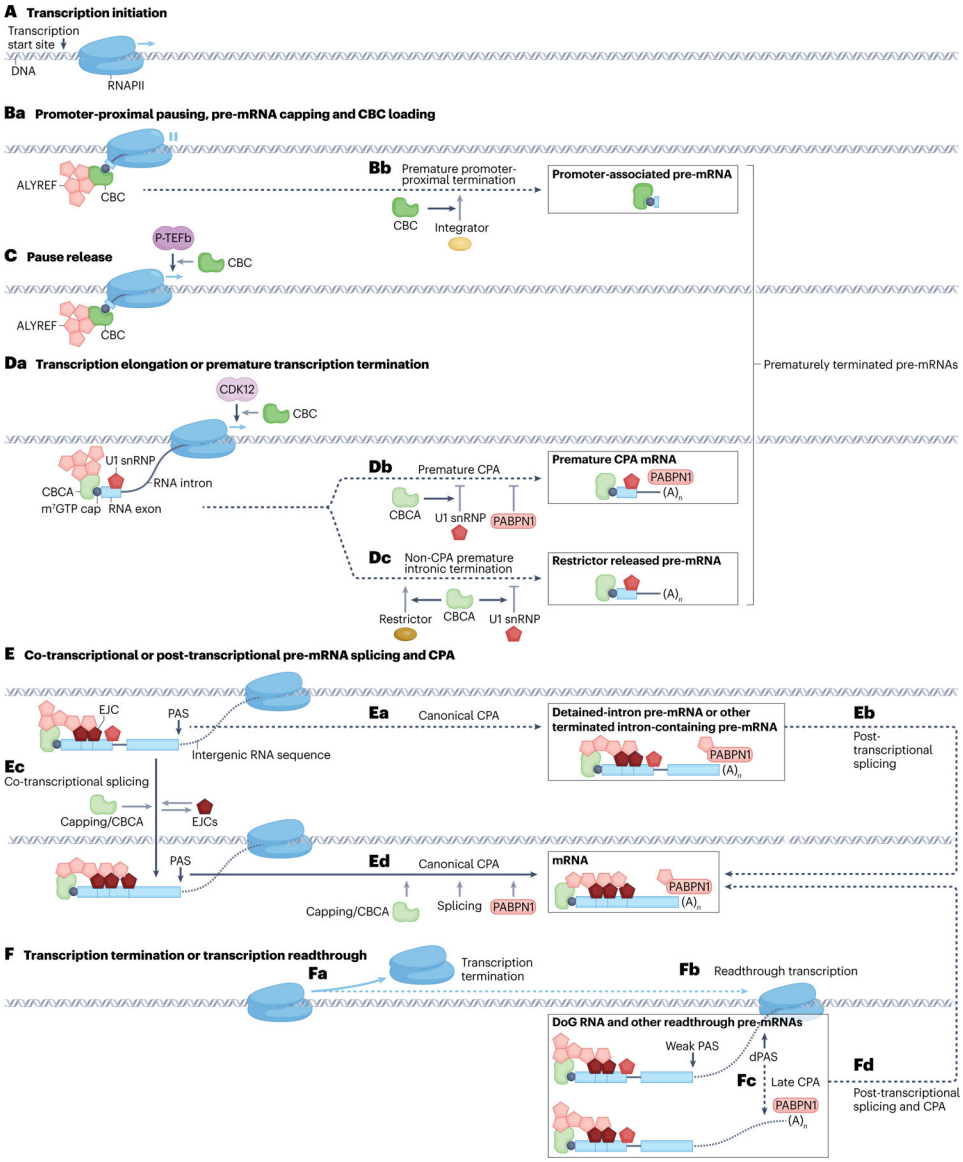

Fig. 2 |. Nuclear metabolism of pre-mRNAs, including the generation of abnormal mRNAs. **A–F**, The steps of gene transcription include RNA polymerase II (RNAPII) initiation from the transcription start site (part **A**), promoter-proximal pausing (which is concurrent with pre-mRNA capping and loading of the 5′-cap-binding complex (CBC) and possibly ALYREF) (part **Ba**), pause release by p-TEFb (after which the CBC recruits ARS2, to form the CBC–ARS2 complex (CBCA) (part **C**), transcription elongation (or premature transcription termination) (part **Da**), and transcription termination (or transcription readthrough) (part **F**). Co-transcriptional pre-mRNA processing includes capping (part **Ba**), co-transcriptional or post-transcriptional splicing (during which the U1 small nuclear ribonucleoprotein particle (snRNP) and other splicing factors are removed after promoting intron excision and exon ligation, and exon–junction complexes (EJCs) are deposited) (part **Ec**), and cleavage and polyadenylation (CPA), which may occur at a premature polyadenylation signal (PAS; part **Db**), canonical PAS (part **Ed**) or distal PAS (dPAS;

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part **Fc**), during which PABPN1 binds the 3′-poly(A) tail. **Bb**, Integrator-mediated transcript cleavage during promoter-proximal pausing triggers the premature termination of transcription and release of promoter-associated pre-mRNAs. **Db**, Premature intronic CPA releases premature CPA mRNAs. **Dc**, Restrictor-mediated premature termination of transcription within introns releases $p(A)^-$ mRNAs. **Ea**,**b**, Terminated intron-containing pre-mRNAs are produced when all introns are not spliced out before CPA occurs. Detained-intron pre-mRNAs are a class of terminated pre-mRNAs in which one or a few specific introns are retained and spliced post-transcriptionally in response to stimuli. **Fb**–**d**, Readthrough pre-mRNAs, which often include introns, are produced when a weak PAS is not recognized by the CPA machinery. At least some of these pre-mRNA species can be cleaved and polyadenylated at a distal PAS and undergo splicing post-transcriptionally.

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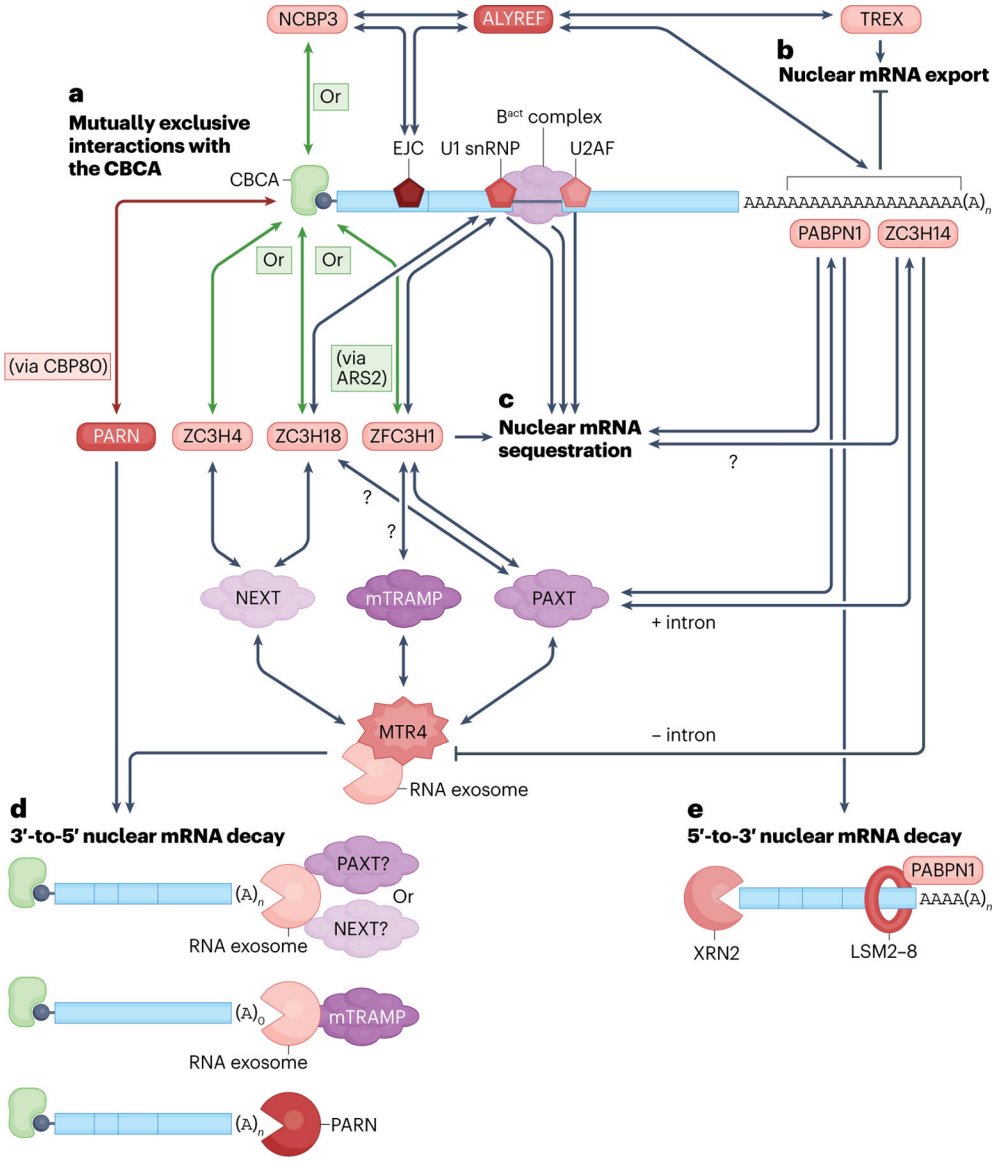

Fig. 3 |. Fate of nuclear mRNAs.

Ways that nuclear decay and nuclear export factors influence the fate of mature and immature nuclear mRNAs, for example, through mutually exclusive interactions with the cap-binding complex–ARS2 (CBCA) (part **a**): nuclear mRNA export (part **b**), nuclear mRNA retention (sequestration) (part **c**) or nuclear mRNA decay (parts **d**,**e**). The light blue boxes represent exons whereas the intervening lines represent introns. '?' depicts functional connections that have not been experimentally validated. EJC, exon–junction complex; mTRAMP, metazoan Trf4/5–Air1/2–Mtr4 polyadenylation; NEXT, nuclear exosome-targeting; PAXT, poly(A) exosome-targeting; snRNP, small nuclear ribonucleoprotein particle; TREX, transcription and export.

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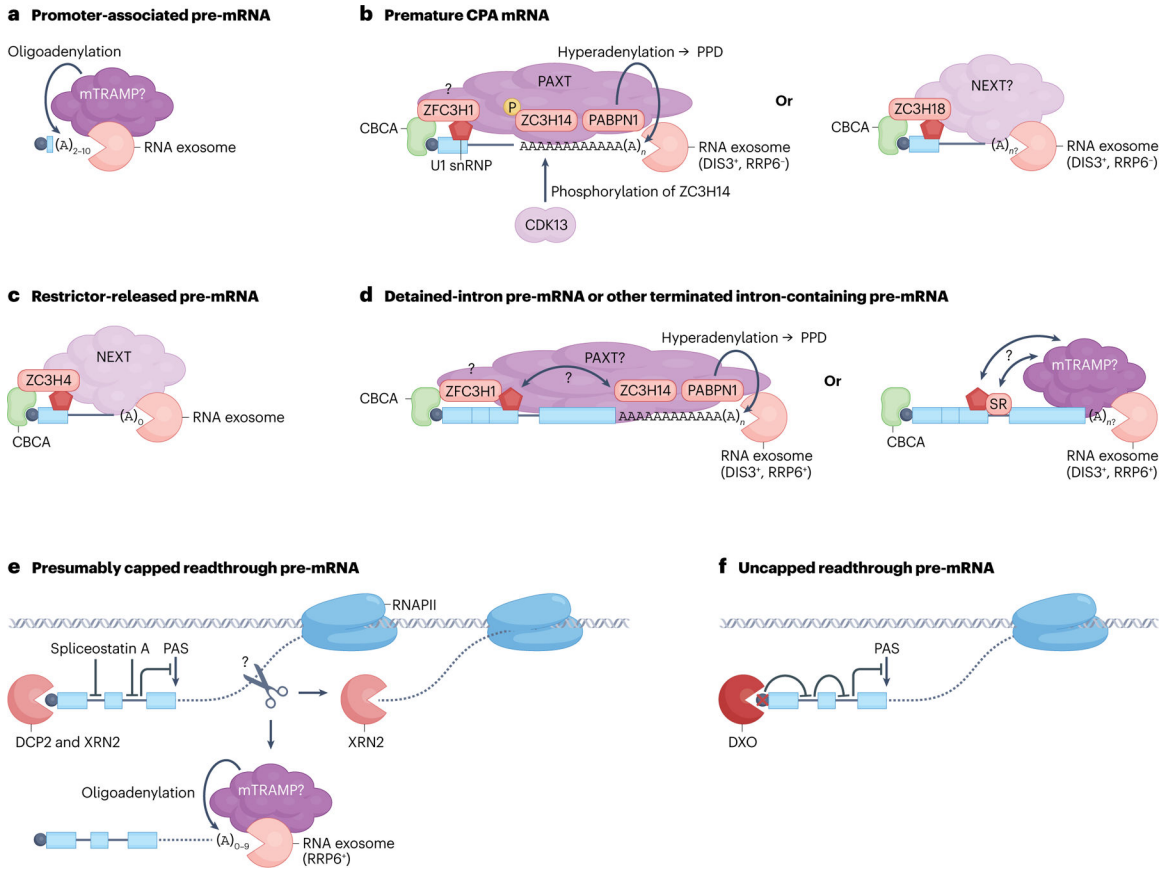

Fig. 4 |. Models for the nuclear decay of abnormal pre-mRNAs that have undergone transcription termination and release from chromatin or that remain chromatin-associated.

a, Promoter-associated pre-mRNAs cleaved by Integrator are oligoadenylated and presumably recruited to the RNA exosome via metazoan Trf4/5–Air1/2–Mtr4 polyadenylation (mTRAMP). **b**, Premature cleavage and polyadenylation (CPA) mRNAs are degraded by the RNA exosome that uses the exonuclease DIS3 (+) but does not use the exonuclease RRP6 (–), and is recruited via poly(A) exosome-targeting (PAXT), or possibly and less frequently, by nuclear exosome-targeting (NEXT). PAXT recruitment to premature CPA mRNAs is facilitated by CDK13-mediated phosphorylation of ZC3H14 and, possibly, by the binding of the scaffold subunit ZFC3H1 to the cap-binding complex–ARS2 (CBCA) complex and to U1 small nuclear ribonucleoprotein particle (snRNP). The nuclear poly(A)-binding protein PABPN1, which is a PAXT constituent, promotes mRNA hyperadenylation and ‘PABPN1-mediated and poly(A) polymerase α/ɣ-mediated decay’ (PPD). NEXT is recruited by ZC3H18 binding to the CBCA and U1 snRNP. **c**, Restrictor-released pre-mRNAs are degraded by the RNA exosome that is recruited by NEXT binding to the CBCA-associated and the U1 snRNP-associated protein ZC3H4. **d**, Detained-intron and other terminated intron-containing pre-mRNAs are degraded by the RNA exosome that contains the exonucleases DIS3 and RRP6 and is recruited via PAXT, or possibly mTRAMP. PAXT recruitment to detained-intron or intron-containing pre-mRNAs could be facilitated by ZC3H14, which might sense the presence of splicing factors, and by binding of ZFC3H1 to the CBCA and U1 snRNP. The PAXT constituent PABPN1 promotes

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mRNA hyperadenylation and PPD. Recruitment of mTRAMP could be mediated by splicing factors, including serine–arginine-rich (SR) proteins. **e**, Readthrough intron-containing pre-mRNAs that are presumably capped are degraded co-transcriptionally by XRN2 at a 5′-monophosphate either as a consequence of decapping by DCP2 or endonucleolytic cleavage (indicated by the scissor) that leaves a monophosphate at the 5′-end of a nascent 3′-cleavage product. In the case of the latter, the resulting 5′-cleavage product may be post-transcriptionally degraded by mTRAMP after mTRAMP-mediated oligoadenylation. **f**, Uncapped readthrough intron-containing pre-mRNAs are degraded co-transcriptionally by DXO. PAS, polyadenylation signal; RNAPII, RNA polymerase II. '?' depicts molecular determinants of the decay of faulty nuclear mRNAs or associated functional connections that have not been experimentally validated.

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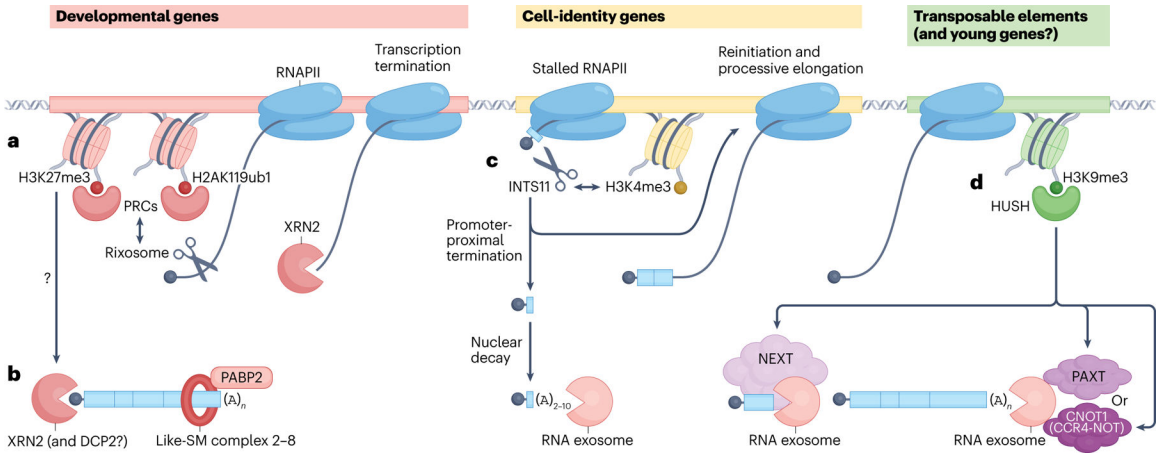

Fig. 5 |. Models for how epigenetic marks and their readers influence nuclear RNA decay.

a, H3K27me3 and H2AK119ub1 repressive marks recruit Polycomb repressive complexes (PRCs) to promote rixosome-mediated cleavage and, after decapping, XRN2-mediated decay of nascent pre-mRNAs from developmental genes. '?' depicts a functional connection that has not been experimentally validated. **b**, Alternatively, in *Caenorhabditis elegans*, H3K27me3 promotes XRN2-mediated decay of transcriptionally terminated mRNAs independently of PRCs. **c**, H3K4me3 recruits INTS11, that is, the endonucleolytic subunit of Integrator, to cleave promoter-associated pre-mRNAs from genes that maintain cell identity, releasing them from stalled RNAPII and allowing for transcription reinitiation and processive elongation. **d**, H3K9me3 repressive marks recruit the human silencing hub (HUSH) complex to promote nuclear exosome-targeting (NEXT-mediated), poly(A) exosome-targeting (PAXT)-mediated, or possibly CNOT1-mediated exosomal decay of RNAs from transposable elements and, possibly, mRNAs from evolutionarily young genes.RNAPII, RNA polymerase II.

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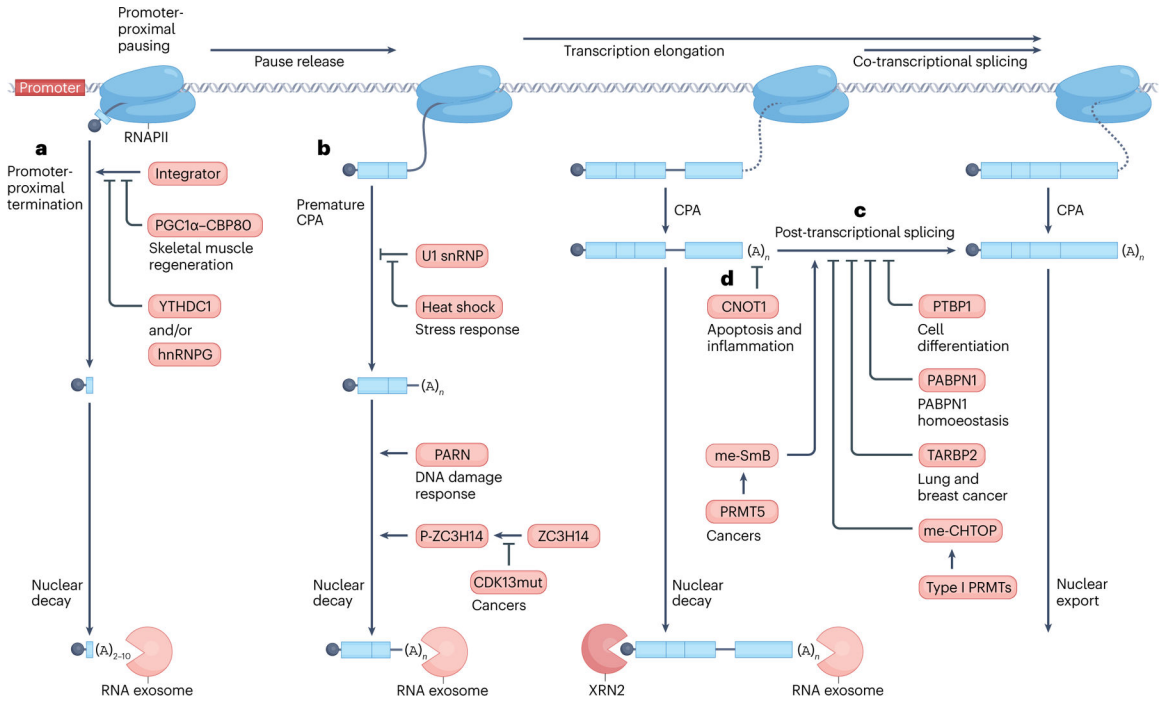

Fig. 6 |. Models for how alternative pre-mRNA processing influences nuclear RNA decay. **a**, Transcriptional co-activator PGC1α binding to CBP80 of the cap-bound nuclear cap-binding complex (CBC), such as the YTHDC1 or hnRNPG m^6A readers, prevents the premature cleavage of promoter-associated pre-mRNAs by Integrator. **b**, Heat shock promotes premature cleavage and polyadenylation (CPA), producing both stable and unstable premature CPA mRNAs. The deadenylase poly(A)-specific ribonuclease (PARN) promotes the decay of premature CPA mRNAs in response to UV-mediated DNA damage. Oncogenic CDK13 mutations (CDK13mut) prevent ZC3H14 phosphorylation and decay of premature CPA mRNAs. **c**, Different mRNA-binding proteins (PTBP1, PABPN1, TARBP2) and methylation of the snRNA-binding protein SmB or the TREX adaptor CHTOP by protein arginine methyltransferases (PRMTs) promote or repress post-transcriptional splicing of detained-introns, thereby influencing the fate of the targeted RNA. mRNA decay by XRN2 must occur after decapping. **d**, The CCR4–NOT scaffold CNOT1 may promote deadenylation and, thereby, decay by the RNA exosome of intron-containing pre-mRNAs in the nucleus. snRNP, small nuclear ribonucleoprotein particle.

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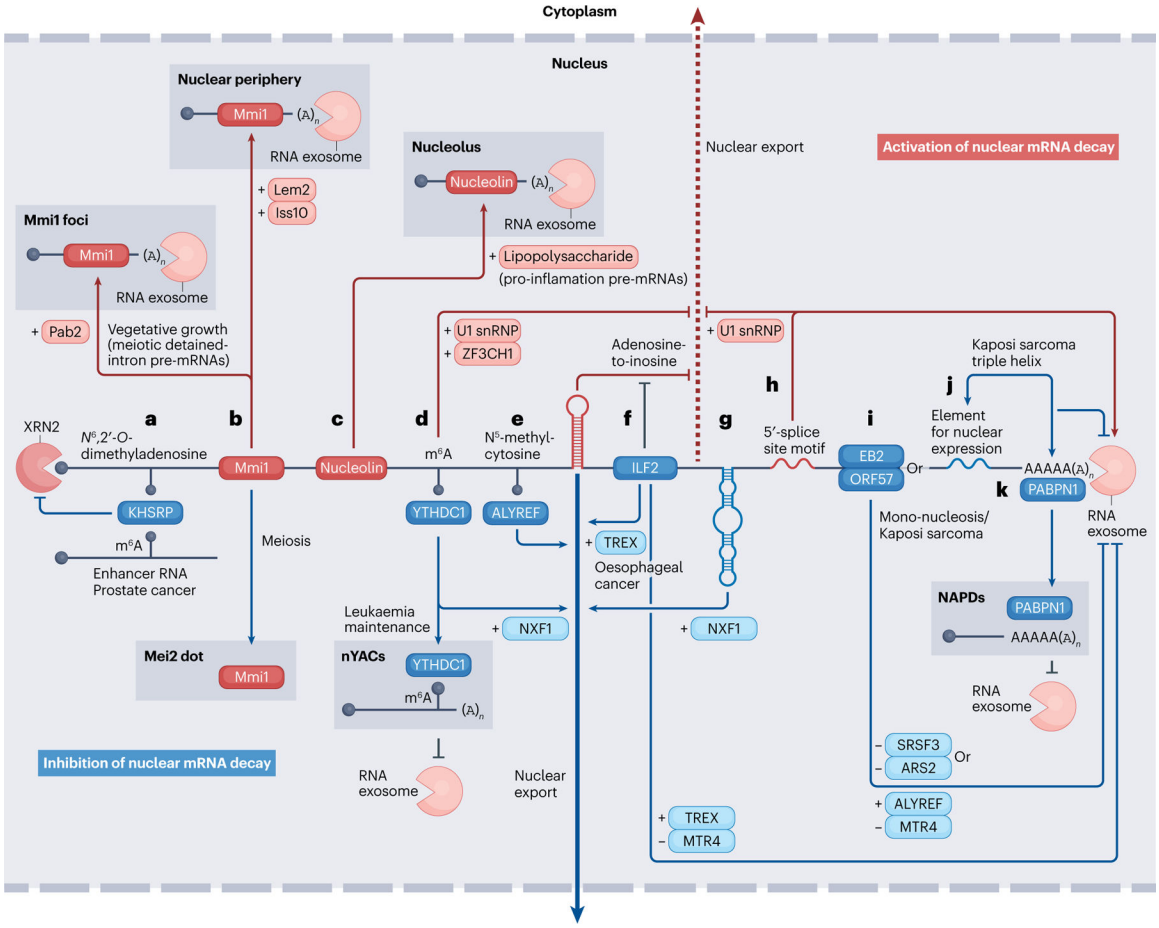

Fig. 7 |. Models for how RNA-binding proteins and cis-acting RNA elements influence the recruitment of, or the escape from, decay factors.

a, KHSRP connects N^6,2′-*O*-dimethyladenosine marks on *PSMD9* mRNA to m^{6}A marks on a neighbouring enhancer RNA, thereby repressing XRN2-mediated mRNA decay, which otherwise occurs after decapping. **b**, During vegetative growth in *Schizosaccharomyces pombe*, the RNA exosome degrades meiotic detained-intron pre-mRNAs that have been recruited to ‘Mmi1 foci’ or to the nuclear periphery by Mmi1. By contrast, Mmi1 is functionally repressed during meiosis by sequestration in ‘Mei2 dots’. **c**, To dampen the response to lipopolysaccharide, nucleolin recruits pro-inflammatory pre-mRNAs to the nucleolus to promote their exosomal decay. **d**, YTHDC1 prevents nuclear decay by sequestering mRNAs with N^6-methyladenosine (m^{6}A) marks in nuclear YTHDC1-m6A condensates (nYACs) and by promoting mRNA nuclear export (red broken arrow). When bound to m^{6}A near a 5′-splice site, YTHDC1 cooperates with U1 small nuclear ribonucleoprotein particle (snRNP) and ZFC3H1 to inhibit RNA export. **e**, ALYREF reads N^5-methylcytosine marks to promote nuclear mRNA export and, thereby, prevent nuclear mRNA decay. **f**, Interleukin enhancer binding factor 2 (ILF2) promotes nuclear mRNA export directly by recruiting transcription and export (TREX) and, possibly indirectly, by repressing adenosine-to-inosine editing. ILF2 also represses nuclear mRNA decay by facilitating the competition of TREX over MTR4. **g**, The ‘constitutive transport element’ of, for example, a *Nxf1* mRNA isoform recruits NXF1 to promote nuclear mRNA export. **h**,

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Possibly independently of m^6A, exonic 5′-splice site motifs cooperate with U1 snRNP to repress nuclear mRNA export and promote nuclear mRNA decay. **i**, Viral paralogs EB2 and ORF57 repress viral mRNA decay by interfering with SRSF3 or ARS2, respectively, and, sometimes in the case of ORF57, by facilitating the competition of ALYREF over MTR4. **j**, The element for nuclear expression forms an intramolecular triple helix with the poly(A) tail to inhibit exosomal mRNA decay. **k**, During oogenesis, PABPN1 prevents nuclear mRNA decay by sequestering $poly(A)^+$ mRNAs in nuclear poly(A) domains (NAPDs) away from the RNA exosome. Red and blue words or schematics refer to decay-promoting and decay-protecting elements, respectively. +, in cooperation with a given factor; −, by inhibiting a given factor.