The multifunctional RNase XRN2

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Abstract

Different classes of RNA function in various cellular processes, and their biogenesis and turnover involve diverse RNases for processing and degradation. XRN2 is a 5′→3′ exoribonuclease that is evolutionarily conserved in eukaryotes. It is predominantly localized in the nucleus and recognizes single-stranded RNA with a 5′-terminal monophosphate to degrade it processively to mononucleotides. In the present paper, we review functions of XRN2 and its cofactors in maturation, surveillance and activity control of several classes of RNA such as pre-mRNA (precursor mRNA), rRNA and snoRNA (small nucleolar RNA).

XRN2 is an evolutionarily conserved 5′→3′ exoribonuclease related to XRN1

XRN2 was originally identified in the budding yeast Saccharomyces cerevisiae as a gene mutated in cells with a poly(A)⁺ RNA (polyadenylated RNA) nuclear export defect, and accordingly named RAT1 for ribonucleic acid trafficking protein 1 [1]. Orthologous proteins were subsequently identified in other eukaryotes [2–4], and mouse Xrn2 was shown to complement the lethality of an XRN2 null mutation in Schizosaccharomyces pombe [3], indicating widespread conservation throughout evolution (Figure 1). XRN2/RAT1 is additionally known as TAP1, HKE1 and DHM1 [3,5,6], but we will use XRN2 throughout the present review for clarity.

XRN2 has regions homologous with the 5′→3′ exoribonuclease XRN1 [1,7] (Figure 1), and both possess comparable exonuclease activity in vitro: they recognize single-stranded RNA with a 5′-terminal monophosphate and degrade it processively to mononucleotides [6,8,9]. XRN2 is predominantly localized in the nucleus [10], whereas XRN1 is localized in the cytoplasm [11]. Johnson [10] showed that XRN2 with a mutation in a putative NLS (nuclear localization signal), which was mislocalized to the cytoplasm, could complement defects in cytoplasmic RNA turnover and sporulation of S. cerevisiae XRN1 mutants. Conversely, XRN1 fused to the SV40 (simian virus 40) large T antigen NLS, which accumulated in the nucleus, could restore viability of XRN2 mutant cells, suggesting that catalytic activities of XRN2 and XRN1 are comparable in vivo and that their cellular roles are largely determined by subcellular localization. Nonetheless, even a nuclear XRN1 cannot substitute for all activities of XRN2 [12], suggesting some intrinsic differences. Moreover, although XRN2 and XRN1 show redundancy in processing of some RNA substrates, XRN2, but not XRN1, is essential in S. cerevisiae, indicating important roles of XRN2 in nuclear RNA processing and turnover (see below).

Degradation for RNA maturation

rRNA maturation

A pre-rRNA (precursor rRNA) transcript is produced by Pol I (RNA polymerase I) as a single RNA molecule, which comprises 18S, 5.8S and 25S/28S (in yeast/mammals respectively) rRNAs and two ETS (external transcribed spacer) (5'ETS and 3'ETS) and two ITS (internal transcribed spacer) (ITS1 and ITS2) sequences (Figure 2A). XRN2 has been reported to play critical roles in maturation of 5.8S and 25S/28S rRNAs as well as the degradation of some spacer fragments that are excised during rRNA maturation in yeast [1,5,13,14], ciliates [15], plants [16] and mammals [17]. Involvement of XRN2 in rRNA processing was first shown in an S. cerevisiae XRN2 temperature-sensitive mutant [1]. Between two isoforms of yeast 5.8S rRNA, 5.8S(S) and 5.8S(L), the level of the major isoform 5.8S(S) decreased upon shift to restrictive temperature. Later, the 5.8S(S) rRNA was shown to be generated by endonucleolytic cleavage at the upstream site by MRP and subsequent trimming to its 5′-end by XRN2, whereas the 5.8S(L) isoform was shown to be dominant when the endonucleolytic cleavage was inhibited [18,19] (Figure 2A).

Involvement of XRN2 in 25S rRNA maturation was also first shown in yeast. After endonucleolytic cleavage at the upstream site, XRN2 rapidly trims the extension to generate the 5′-end of 25S rRNA [14] (Figure 2A). In both 5.8S and 25S rRNA maturation, 5′-end trimming can be performed by the 5′→3′ exonuclease Rrp17 in parallel with XRN2 [20] and also by Xrn1 when XRN2 is inactive [14,19], suggesting redundancy among these RNases. Exonucleolytic trimming...
by XRN2 to generate the 5′-ends of 5.8S and 28S rRNAs is likely to be conserved in mammals. XRN2 knockdown by RNAi (RNA interference) resulted in accumulation of 5′-extended 5.8S and 28S rRNAs in mouse LAP3 cells [17].

5.8S and 25S/28S rRNAs are major components of the 60S ribosomal subunit, and some protein components of the pre-60S particles are required for efficient maturation of these rRNAs ([21] and references therein). Among them, depletion of NOP4 or NOP15 in yeast reduced association of XRN2 with upstream sites of 5.8S and 25S rRNAs, indicating that these proteins promote XRN2 entry possibly through structural remodelling of pre-rRNA [21]. How XRN2 completes trimming precisely at the 5′-ends of these rRNAs is unknown, but a similar structural remodelling mechanism might be responsible.

**snoRNA (small nucleolar RNA) maturation**

During ribosome synthesis in the nucleolus, snoRNAs associate with pre-rRNA and participate in its processing and modification [22]. XRN2 has been reported to be required for biogenesis of several snoRNAs in yeast (Figure 2B). Following cleavage by the endoribonuclease Rnt1, XRN2 trims 5′ extensions of snR190 and U14 snoRNAs and seven methylation guide snoRNAs, snR72–snR78 [13,23,24]. XRN2 also trims the 5′ extension of U24 snoRNA excised from an intron of the BEL1 gene transcript [13]. Simultaneous deletion of XRN1 enhanced accumulation of 5′-extended intermediates of these snoRNAs in XRN2-depleted cells, suggesting some contribution of XRN1 to snoRNA maturation.

**mRNA transcription termination**

Transcription by Pol II (RNA polymerase II) consists of three stages: initiation, elongation and termination. XRN2 has been reported to be required for transcription termination in yeast and mammals [25,26]. After Pol II transcribes a poly(A) signal, the transcript is cleaved by cleavage factors, and the 5′-fragment is polyadenylated. Pol II remains associated with DNA and continues transcription further downstream. XRN2 is thought to degrade the 3′ transcript until it collides with Pol II to induce its dissociation from DNA (Figure 2C). However, degradation of a 3′ transcript by XRN2 was shown to be insufficient for termination both in vivo and in vitro [12,27]. Moreover, both XRN2 and Xrn1 can degrade a 3′ transcript, but only XRN2 is required and competent for termination in yeast [12]. Luo et al. [12] showed that XRN2 and a cleavage factor Pcf11 are mutually required for association with a poly(A) site and suggested a model in which co-operative association of XRN2 and cleavage factors with Pol II causes poly(A) site cleavage, and degradation of the downstream transcript [12]. Note that, at least in mammalian cells, involvement of XRN2 in Pol II transcription termination downstream of poly(A) sites appears to be limited to specific genes rather than reflecting a general mechanism [28,29]. In yeast, transcription termination by XRN2 appears to not be essential, because ectopic expression of nuclear Xrn1 can restore growth of an XRN2 mutant but not transcription termination at an ADH4 (alcohol dehydrogenase 4) reporter gene [12].

XRN2 has also been reported to be involved in termination of pre-rRNA transcription by Pol I [30,31], intergenic primary miRNA (microRNA) transcription by Pol II [32] and premature termination of Pol II transcription [29,33,34].

**Degradation for RNA quality control**

**Degradation of aberrant pre-rRNA**

Given the essential function of ribosomes, the quality of rRNAs must be surveyed and defective species removed. At least some fractions of aberrant pre-rRNAs are polyadenylated and targeted for degradation in yeast [35,36]. Although the 3′→5′ exoribonuclease complex exosome plays a major role in this degradation, XRN2 is also involved [14]. Depletion of XRN2 enhanced the accumulation of polyadenylated pre-rRNAs and decreased...
Figure 2 | Schematic representation of XRN2 functions in rRNA biogenesis, snoRNA biogenesis and Pol II transcription termination

Processing steps by other factors are omitted for clarity. (A) 5.8S and 25S/28S rRNA biogenesis. Pre-rRNA is transcribed as a single RNA comprising 18S, 5.8S and 25S/28S rRNA sequences, two ETSs and two ITSs. XRN2 trims 5′ extensions of 5.8S and 25S/28S rRNAs following endonucleolytic cleavage at the A3 and C2 sites respectively. (B) snoRNA maturation. Top panel: snR190 and U14 snoRNAs are encoded in a single RNA transcript. Following cleavage by the endoribonuclease Rnt1, XRN2 trims 5′ extensions of snR190 and U14 snoRNAs. Bottom panel: U24 snoRNA is encoded in an intron of the BEL1 gene transcript. Following splicing, XRN2 trims its 5′ extension. (C) The torpedo model for Pol II transcription termination. Pol II continues transcription beyond the poly(A) signal. Cleavage downstream of poly(A) signal makes an entry site for XRN2 to degrade the downstream transcript. XRN2 collides with Pol II and induces dissociation of Pol II and the upstream transcript from the DNA template.

their 5′ heterogeneity in strains deleted of the exosome subunit Rrp6, whereas a deletion of XRN2 alone had no effect. In mammalian cells, on the other hand, XRN2 knockdown by RNAi led to accumulation of aberrant pre-rRNA species, indicating more prominent roles of XRN2 [17].

Degradation of pre-mRNA (precursor mRNA)

As errors in pre-mRNA processing could lead to production of harmful proteins, these aberrant species should be eliminated. Unspliced pre-mRNAs are degraded rapidly from both 5′- and 3′-ends by XRN2 and the nuclear exosome respectively in yeast, with the exosome playing a predominant role, although environmental conditions may tilt this balance [37].

In human cells, XRN2 appears to be more important for degradation of aberrant pre-mRNA products than the exosome. Davidson et al. [38] showed that plasmid-derived β-globin gene transcripts deficient in poly(A) site processing or transcription termination accumulated in chromatin fractions of XRN2-depleted HeLa cells. They also observed accumulation of several endogenous pre-mRNAs in chromatin fractions of XRN2-depleted cells treated with spliceostatin A, a splicing inhibitor. These results suggest that aberrant pre-mRNAs are degraded co-transcriptionally by XRN2 in human cells.

Degradation of hypomodified tRNA

tRNAs undergo numerous post-transcriptional modifications including methylation. As modifications are often important for proper tRNA function, including accurate translation [39], they must be a target of surveillance. In fact, mature tRNAArg(CCG) lacking two methylations due to deletion of two tRNA methyltransferases was shown to be rapidly degraded and deacylated in yeast [40]. Chernyakov et al. [41] found that XRN2 and Xrn1 participate in this degradation and deacylation. How XRN2 and Xrn1 are involved in deacylation, and whether this mature tRNA surveillance occurs in the nucleus or the cytoplasm are unknown.

Degradation for RNA level and/or activity control

Degradation of nuclear pre-mRNA

The levels of mRNAs are critical in determining protein amounts and thus tightly regulated by turnover both at pre-mRNA and mRNA stages. Bousquet-Antonelli et al. [37] showed that pre-mRNAs are degraded actively in the nucleus by XRN2 and the exosome, and that pre-mRNA degradation and splicing compete for mRNA production in S. cerevisiae. mRNA was also shown to be degraded in the nucleus. Das et al. [42] used S. cerevisiae strains with a nuclear export defect and showed that nuclear-retained mRNAs are rapidly degraded. This degradation is suppressed by depletion of XRN2 or the exosome, although its significance under normal conditions is not clear. Similar to aberrant pre-mRNA degradation, the exosome plays major roles in these processes.

Regulation of telomere length

Telomeres are added to chromosome ends to maintain genome stability [43]. Telomerase maintains telomere length by adding telomere units to the 3′-end of chromosomes, which are otherwise shortened during every round of DNA replication. Transcription of telomeres yields TERRA (telomeric repeat-containing RNA), which associates with telomeres. Luke et al. [44] found that XRN2 degrades TERRA in S. cerevisiae. XRN2 depletion caused TERRA...
accumulation and inhibited telomerase-dependent telomere elongation. The latter was bypassed by overexpression of RNase H, which degrades the RNA of DNA–RNA hybrids, suggesting that TERRA inhibits telomerase activity and that XRN2 regulates telomere elongation through degradation of TERRA.

Control of miRNA levels and activities

miRNAs guide miRISCs (miRNA-induced silencing complexes) to their target mRNAs to suppress translation or to induce degradation. miRNAs are generally stable and persist for many hours or days [45]. However, some miRNAs have been reported to decay rapidly in specific situations [45]. Chatterjee and Großhans [46] found that XRN2 degrades let-7 and some other miRNAs in Caenorhabditis elegans. XRN2 depletion by RNAi increased mature let-7 miRNA levels without affecting its precursor levels, and reduced accumulation of let-7 target mRNAs, suggesting that XRN2 does not merely scavenger non-functional miRNAs, but controls miRNA levels and activities through degradation. In vitro, wild-type C. elegans lysate released miRNAs from miRISC more efficiently than XRN2-depleted lysate, indicating that XRN2 promotes miRNA release from miRISC by an unknown mechanism.

XRN1 was also shown to degrade miRNAs [47]. Presence of target miRNAs promoted miRISC residency of miRNAs and protected them from degradation by XRN2 and XRN1 [47]. In which cellular compartment these RNases degrade miRNA, as well as their target specificity, are unclear. Whether XRN2 can degrade miRNAs in other organisms is yet to be determined, although XRN1 has been implicated in degradation of miR-382 in HEK (human embryonic kidney)-293 cells [48].

Degradation of other RNA species

Production of TSSa (transcription start site-associated) RNA

Mammalian cells have a fraction of small RNA derived from regions flanking promoters of protein-encoding genes, therefore called TSSa RNAs [49]. TSSa RNAs are 16–30 nt long and thought to be nascent transcripts protected by Pol II stalled near TSSs (transcription start sites) [49,50]. Interestingly, 5′-ends of most TSSa RNAs do not correspond to the first nucleotides of Pol II transcripts, indicating degradation by ribonucleases. Valen et al. [50] showed that 3′-ends of TSSa RNAs peaked between 30 and 40 nt downstream of the TSSs and that depletion of both XRN2 and XRN1 lowered the peak and increased a proportion of corresponding RNAs that are longer than 30 nt and have 5′-ends mapped to TSS. These observations suggest that XRN2 and/or XRN1 degrades nascent transcripts from 5′-ends until reaching the position protected by stalled Pol II. Whether this degradation induces dissociation of Pol II from pausing sites is unknown.

Elimination of excess shRNA/siRNA

Excess introduction of shRNA/siRNA into cells may cause toxicity. Wei et al. [51] found that expression of shRNA/siRNA that targets no mRNA induced expression of NPGPx (non-selenocysteine-containing phospholipid hydroperoxide glutathione peroxidase), an endoplasmic reticulum-resident protein, in human HEK-293T cells and mouse embryonic fibroblasts and that this NPGPx expression is important for releasing cellular stress. They found that XRN2 covalently bound NPGPx and that XRN2 degraded non-targeting siRNAs in an NPGPx-dependent manner. How NPGPx regulates XRN2 in this process, and whether this is relevant to any physiological process, is unclear.

Cofactors and regulators of XRN2

Rai1 (Rat1-interacting protein)

In purification of XRN2 from whole yeast cell extracts for activity of poly(A)+ RNA degradation, a 45-kDa protein was co-purified [9]. Named Rai1, this protein was found to enhance processivity of XRN2 to degrade RNA and to stabilize XRN2 activity in vitro without possessing nuclease activity itself [52]. A yeast rai1 deletion mutant is viable, but shows a defect in 5.8S rRNA biogenesis, which is complemented by an additional copy of XRN2 [53]. Unexpectedly, a structural study revealed an additional function of Rai1. Xiang et al. [52] found a pocket for a divalent cation in the Rai1 structure and biochemically showed that Rai1 has pyrophosphohydrolase activity for 5′-triphosphorylated RNA. Given that XRN2 cannot degrade 5′-triphosphorylated RNA [9], this finding raises a possibility that Rai1 preprocesses 5′-triphosphates of RNA into 5′-monophosphates for XRN2 and that this mechanism might function as a quality control, e.g. to eliminate uncapped mRNAs. Thus Rai1 possesses dual functions: stimulation of XRN2 exononuclelease activity and pyrophosphohydrolase activity for 5′-triphosphorylated RNA.

Rai1 is homologous with C. elegans DOM-3 and human DOM3Z [53]. However, although DOM3Z also has a pocket for a divalent cation and might have pyrophosphohydrolase activity, it and other metazoan homologues are unlikely to associate with XRN2 owing to a lack of residues that are in the XRN2-binding interface of Rai1 [52].

Tw12

The Tetrahymena Ago/Piwi protein Twi12 has recently been identified as a cofactor of XRN2. Couvillion et al. [15] identified XRN2 and Tan1 (Twi-associates novel 1) in Twi12 immunoprecipitates and named the ternary complex TXT (Twi12–XRN2–Tan1). Purified TXT complexes had exoribonuclease activity for a 5′-monophosphorylated, but not 5′-triphosphorylated, RNA substrate. The TXT complex is predominantly nuclear, localizing to both the micronucleus and the macronucleus, and Twi12 needs to bind small 3′ fragments of tRNA for nuclear localization. On Twi12...
depletion, XRN2 was mislocalized to the cytoplasm and also lost exoribonuclease activity, suggesting that the exoribonuclease XRN2 functions exclusively as a component of the TXT complex, although the contribution of Tan1 is yet to be found. Depletion of Twi12, and also of XRN2, decreased mature 5.8S rRNA levels and caused accumulation of pre-rRNA processing intermediates, suggesting that the TXT complex is required for maturation of 5.8S rRNA as has been reported for XRN2 in other organisms.

**Conclusion and perspective**

The evolutionarily conserved exonuclease XRN2 regulates various cellular processes through degradation of RNA, including maturation, quality control and activity regulation of different classes of RNA. How XRN2 is targeted to, and recognizes, these diverse RNA substrates is not clear. As XRN2 degrades 5′-monophosphorylated RNA with little apparent sequence-specificity in vitro [9], there are probably other factors that guide XRN2 to its substrates. XRN2 depletion causes growth arrest in *S. cerevisiae* [1] and developmental arrest, sterility and moulting defect in *C. elegans* ([46,54], and T. Miki, S. Riegeger and H. Grohans, unpublished work). Single nucleotide polymorphisms that affect XRN2 expression were shown to determine susceptibility to spontaneous lung tumour in mouse and human, suggesting its involvement in cancer development [55]. However, it remains to be established which cellular functions of XRN2 are responsible for these physiological defects. None of the RNA degradation processes mentioned above has been able to account individually for even the essential function of XRN2 in yeast. The multifunctional nature of XRN2 makes it difficult to relate one cellular function to the function of XRN2 in yeast. The identification of one physiological role, and it is possible that combinations of some functions are responsible. The identification of additional XRN2-interacting partners may help to address these issues.

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**References**


10. Johnson, A.W. (1997) Rat1p and Xrn1p are functionally interchangeable exoribonucleases that are restricted to and required in the nucleus and cytoplasm, respectively. Mol. Cell. Biol. 17, 6122–6130


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