



## Review

## Cytoplasmic RNA decay pathways - Enzymes and mechanisms

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## ABSTRACT

RNA decay plays a crucial role in post-transcriptional regulation of gene expression. Work conducted over the last decades has defined the major mRNA decay pathways, as well as enzymes and their cofactors responsible for these processes. In contrast, our knowledge of the mechanisms of degradation of non-protein coding RNA species is more fragmentary. This review is focused on the cytoplasmic pathways of mRNA and ncRNA degradation in eukaryotes. The major 3' to 5' and 5' to 3' mRNA decay pathways are described with emphasis on the mechanisms of their activation by the deprotection of RNA ends. More recently discovered 3'-end modifications such as uridylation, and their relevance to cytoplasmic mRNA decay in various model organisms, are also discussed. Finally, we provide up-to-date findings concerning various pathways of non-coding RNA decay in the cytoplasm.

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## 1. Introduction

Regulation of gene expression in eukaryotic cells occurs at multiple levels. Among them, control of mRNA decay rates in the cytoplasm plays a vital role in supporting transcriptional regulation. The significance of mRNA turnover is underscored by observations from genome-wide studies showing that mRNA steady-state levels do not directly correlate with the rate of transcription [1]. On the other hand, the coordinated mRNA stability of groups of transcripts encoding proteins involved in specific metabolic pathways was demonstrated, which introduced the concept of mRNA decay regulons [2].

mRNA half-lives differ significantly between various transcripts in all eukaryotic organisms investigated so far [3]. Interestingly, decay rates for some mRNAs seem to be conserved between different species to some extent [4]. Notably, mRNAs encoding housekeeping proteins tend to have considerably longer half-lives than those encoding regulatory proteins [5].

The importance of post-transcriptional processes in the regulation of gene expression is further accentuated by the fact that multiple parallel, partially redundant, mRNA decay pathways operate in the cytoplasm. These mechanisms include the action of endoribonucleases, initiating degradation through internal cleavage within the transcript sequence, or exoribonucleases, which digest mRNA from either end. Moreover,

the action of nucleases requires prior activation, which is also achieved through several means. To prevent indiscriminate decay, activating mechanisms are dependent on a number of *cis*-acting mRNA sequence elements, as well as a large repertoire of *trans*-acting protein/ncRNA regulatory factors, which specifically recruit degradative enzymes to their respective targets.

Several lines of evidence indicate that mRNA degradation is intimately coupled to translation. First, initiation of mRNA decay usually requires destabilization of the closed loop mRNA structure, mandatory for efficient protein synthesis. Thus, reducing translation initiation results in enhanced mRNA degradation.

Second, multiple mRNA quality control pathways depend on ongoing translation. As examples, ribosomes may stall in the presence of a premature termination codon in the ORF (open reading frame), or secondary structures, blocking their progression along the message, which trigger nonsense-mediated decay (NMD) or no-go decay (NGD) cytoplasmic mRNA surveillance pathways, respectively, whereas mRNAs lacking stop codons are degraded *via* the non-stop decay (NSD) mechanism. Herein, we will focus on the general mRNA decay pathways in the cytoplasm that are critical to establishing the final levels of functional proteins. Quality control pathways removing aberrant transcripts, which could produce potentially harmful proteins, will be referred to only occasionally. Therefore, the reader is strongly encouraged to consult recently published relevant review dealing with this topic [6].

The third connection between mRNA decay rates and translation is reflected by the observation that the codon content of the transcript has some impact on how quickly it is degraded. It has been found recently that mRNAs comprising optimal codons (those that are decoded

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more efficiently and are thus translated faster) have long half-lives. On the other hand, transcripts composed of sub-optimal codons, undergoing slower translation, are significantly less stable [7]. This has been demonstrated so far in *S. cerevisiae*, but it is likely that such mechanism of fine-tuning gene expression may well be common for all eukaryotes. Ribosome pausing during translation that may lead to accelerated mRNA degradation has been also shown recently in both invertebrate and vertebrate cells on reporters containing repetitive tracts of AAA codons. Transcripts coding for proteins with such polybasic runs were translated considerably less efficiently and displayed shorter half-lives than control mRNAs lacking these sequences [8]. Polylysine-coding tracts may lead to three possible scenarios: ribosomal frameshifting consolidated with NMD, which results in the reduced output of wild-type protein; frameshifting with synthesis of both out-of-frame and wild-type protein; and non-resolved stalling, consolidated by endonucleolytic cleavage of mRNA and reduction in wild-type protein levels, as in the NGD pathway [8].

During its lifecycle, an mRNA is coated with different proteins through various stages of gene expression, forming mRNPs (messenger ribonucleoproteins) which are subjected to dynamic remodeling, affecting mRNA turnover in both default and quality control decay pathways. Another connection between mRNA degradation and translation is reflected by the existence of cytoplasmic mRNP foci, such as processing bodies (P-bodies) which are sites where non-translatable transcripts accumulate together with mRNA decay factors. These messages can either undergo degradation (P-bodies) or be translocated to other cytoplasmic substructures, called stress granules, and re-enter translation. We will not discuss the influence of P-bodies and stress granules formation on mRNA metabolism in depth, since this area of research has been also summarized in a review paper [9]. Nonetheless, it should be noted that the question of whether an mRNA is to be degraded, stored or translated – which is largely dependent on the mRNA flow between these two types of mRNP granules – is of considerable importance to its fate. However, the extent to which P-bodies contribute to mRNA decay may differ between species and has been often debated due to the observation that transcripts can be degraded co-translationally while still associated with polysomes [10].

It should be also noted that, in addition to these links with translation, experimental data suggest that mRNA decay may also be coupled to other stages of gene expression such as transcription [11]. Interrelations between these various processes ensure that mRNA levels are tightly controlled in a coordinated fashion from the initiation of transcription to the late stages of gene expression.

Protein-coding transcripts constitute only a tiny fraction of the transcriptome. Eukaryotic genomes are pervasively transcribed, which gives rise to a number of distinct non-coding RNA (ncRNA) molecules, grouped into functional classes. Some of them, like PROMPTs (promoter upstream transcripts) in human cells are highly unstable and could only be detected after interfering with the mRNA degradation machinery. As we have demonstrated recently, their degradation occurs in the nucleus and is largely dependent on the major catalytic subunit of the RNA exosome complex – DIS3 protein [12]. On the other hand, other regulatory ncRNAs exemplified, but not limited to endo-siRNA (endogenous small-interfering RNAs) and miRNA (microRNAs) molecules, perform their functions in the cytoplasm, which is also the site of their degradation. Interestingly, while the mechanisms of decay initiation may differ between mRNAs and ncRNAs, the exoribonucleases executing the ultimate phase of the process are shared by both superfamilies of transcripts.

This review focuses on the cytoplasmic pathways of mRNA and ncRNA decay, with an emphasis on the recently identified or more deeply characterized mechanisms. The article is divided into two parts. Part One is dedicated to the key players governing RNA degradation in the cytoplasmic compartment and details the ways of mRNA decay initiation. Part Two presents mechanisms activating ncRNA degradation in the cytoplasm.

## 2. Part one: cytoplasmic pathways of mRNA decay

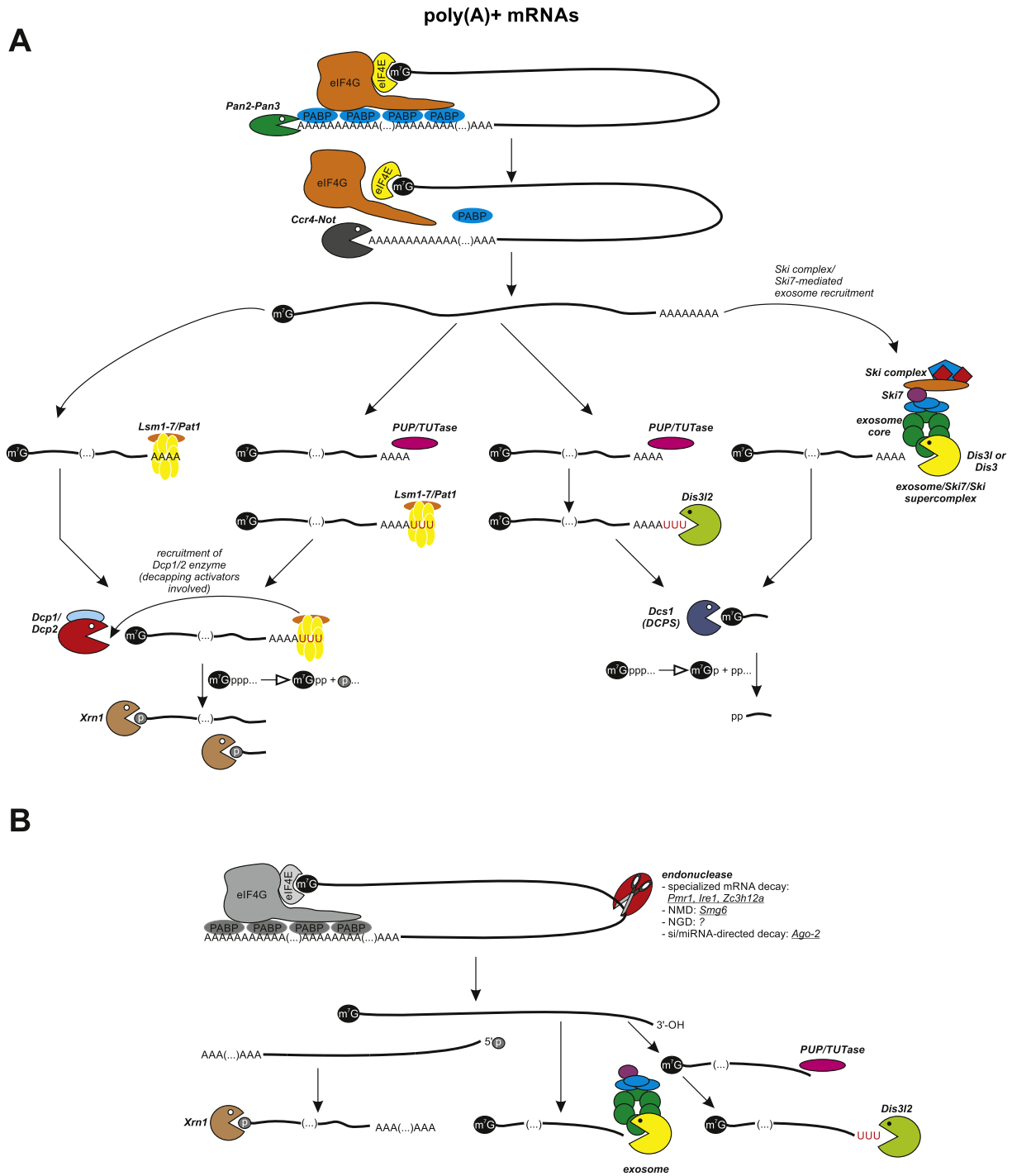
Productive translation initiation is dependent on the formation of the closed-loop mRNA structure, due to interactions between poly(A)-binding proteins (PABPs) associated with the 3'-terminal poly(A) tail and the cap-binding complex, associated with the 5'-terminal cap (Fig. 1A). In particular cases, endonucleolytic cleavage of mRNA can overcome the protective influence of these interactions and trigger decay (Fig. 1B). Involvement of endoribonucleases, such as Pmr1, Ire1, Zc3h12a (MCPIP1), Smg6 or the siRNA RISC (RNA-induced silencing complex) in the decay of specific transcripts has been well-documented, but is beyond the scope of this review and has been thoroughly discussed elsewhere [13]. Generally, mRNA decay is initiated by deprotection of the transcript, which requires modification of mRNA ends (Fig. 1A). As described below, this can be achieved in multiple ways. For the majority of mRNAs, poly(A) tail shortening – deadenylation – is an event signaling that mRNA should be destined to degradation (Fig. 1A). Shortened 3'-terminal adenosine extensions serve as a landing pad for the Lsm1–7/Pat1 complex, which stimulates cap hydrolysis, followed by Xrn1-mediated decay in the 5'–3' direction (Fig. 1A); alternatively, the multisubunit RNA exosome complex may further degrade the oligo(A) tail and continue the 3'–5' decay into the transcript body (Fig. 1A). While deadenylation and decapping were long considered as consecutive events, providing accessibility of the exoribonucleases to the mRNA termini, it is now known that cap removal can be uncoupled from poly(A) tail shortening. Therefore, rates of deadenylation, decapping or exoribonucleolytic digestion can each be limiting to the overall transcript degradation speed.

In recent years, increasing evidence has accumulated that the requirement of deadenylation for mRNA degradation can be bypassed through the untemplated addition of uridine-rich (or sometimes mixed uridine/cytidine) stretches to the 3'-terminus of a transcript. Similar to deadenylation, 3'-uridylation either stimulates decapping via the Lsm1–7/Pat1 assembly or activates decay pathways dependent on the 3'–5' exoribonuclease, Dis3l2, related to the major catalytic subunits of the exosome complex, but working independently (Fig. 1A). These and other effects of modifications of the mRNA termini status will be described in more detail in the following sections. A separate section is dedicated to non-polyadenylated replication-dependent histone mRNAs, which are protected at their 3'-ends by the stem-loop structure, but uridylation, Lsm1–7/Pat1 complex, Xrn1 and Dis3l2 also seem to participate in their decay. Below we describe the major players and processes governing cytoplasmic mRNA degradation.

### 2.1. Deadenylation

Deadenylation has been long considered as a rate-limiting step in the mRNA degradation process, crucial for activation of cytoplasmic mRNA decay in both 5'–3' and 3'–5' directions. This has been most extensively demonstrated in *S. cerevisiae*, the eukaryotic model organism most commonly utilized in studies related to mRNA metabolism. In this species, uridylyltransferases are absent and shortening of a poly(A) tail beyond a certain limit, associated with removal of bound proteins, is the predominant event activating degradation of mRNA.

The regulation of mRNA poly(A) tail length is a dynamic process, involving activities of canonical poly(A) polymerases (PAPs) and poly(A)-specific 3' exonucleases (deadenylases), extending or shortening 3'-terminal adenosine extensions, respectively, allowing for precise control of mRNA stability. The importance of deadenylation in the determination of mRNA decay rates is reflected by the existence of multiple distinct enzymes able to trim poly(A) tails, acting at different phases of reaction, and a variety of regulatory factors influencing their catalytic activities and selectively recruiting them to their cellular targets (reviewed in [14]).



**Fig. 1.** An overview of cytoplasmic poly(A) + mRNA decay pathways. A – degradation mechanisms initiated by disruption of the closed circle mRNA structure and deprotection of mRNA termini. The roles of deadenylation, 3'-terminal uridylation, Ski complex/Ski7 protein and decapping in the stimulation of 3'-5' exonucleases (Dis3/Dis31 associated with the exosome core or Dis312) and 5'-3' exonuclease (Xrn1) are described in Sections 1.1.1–1.1.6. B – mRNA degradation in specialized and quality control pathways can be also initiated by endonucleolytic cleavage within the transcript body. This leads to the exposure of unprotected 3' and 5' termini on the proximal and distal products, respectively, which become accessible to the exosome and Dis312 or Xrn1, respectively.

It is now established, that cytoplasmic deadenylase activity comes predominantly from Ccr4-Not and Pan2-Pan3 complexes (reviewed in [15]) (Fig. 1A).

In all eukaryotes studied, Ccr4-Not complex is a major deadenylase, believed to play an important role during different stages of the RNA lifecycle in various organisms [16]. Not1 protein serves as a structural scaffold, to which additional subunits are docked with varied composition depending on organism, but canonical ones can be distinguished, namely: Ccr4, Caf1/Pop2, Caf40, Not2 and Not3/5 [15]. Not1 is the only essential

protein in yeast, however multiple synthetic lethal phenotypes can be observed when different Ccr4-Not subunits are deleted, pointing to the overall importance of the entire complex [17]. Two subunits of the Ccr4-Not complex exert catalytic activity, namely Ccr4 and Caf1/Pop2, both acting as 3'-5' poly(A)-specific exoribonucleases (major and minor, respectively), with the prevailing hypothesis that these deadenylases act exclusively as parts the Ccr4-Not complex and not on their own [15].

Ccr4 owes its activity to an EEP (endonuclease-phosphatase)-type domain [14]. Mammalian genomes encode several

homologues of Ccr4, including CNOT6 (Ccr4a), CNOT6L (Ccr4b) [18] as well as more distantly related proteins: Nocturnin (NOC/Ccr4c), ANGEL2 (Ccr4d) and ANGEL1 (Ccr4e) [19]. Ccr4p is a subunit of the *S. cerevisiae* Ccr4-Not complex [15] and its structure is consistent with the architecture of human homologue [20], which explains the preference of this enzyme for a poly(A) substrate. Moreover, Ccr4p deadenylase activity is inhibited *in vitro* by PABPs [21].

Caf1/Pop2, the second catalytic subunit of the Ccr4-Not complex, has an RNase DEDD (Asp-Glu-Asp-Asp) domain [14] and is widely conserved across the eukaryotic kingdom as *A. thaliana* possesses many related proteins [22], and three Caf1 homologues have been identified in human: CNOT7 (hCaf1/hCaf1a), CNOT8 (hPOP2/hCaf1b) and the more distant CAF1Z [18,19]. Human CAF1Z, in addition to its deadenylation activity, catalyzes 3'-5' exoribonucleolytic decay, shuttles between cytoplasm and nucleus and localizes to nuclear foci (Cajal bodies) [19].

As mentioned earlier, a second deadenylation activity in eukaryotes is provided by the Pan2-Pan3 complex (Fig. 1A), which does not degrade poly(A) tails completely *in vitro* or *in vivo* [15], so it is thought to be responsible for initial poly(A) tail trimming. In both yeast and humans it is composed of two proteins: Pan2p/Pan3p and PAN2/PAN3 respectively, which are conserved in other eukaryotes [15], with Pan2 acting as a catalytic subunit, similarly to Caf1 belonging to the DEDD family of exoribonucleases [14]. Pan2 shows a preference for poly(A) substrate and is stimulated by PABPs both in yeast and mammals [15]. Additionally, yeast and human Pan2 interacts with Pan3, and human PAN3 mediates PAN2 recruitment to mRNA through PABPs [15]. Recently, structures of Pan2-Pan3 complexes from *Neurospora crassa* [23] and *S. cerevisiae* [24] were published, indicating its asymmetrical architecture with 1:2 Pan2:Pan3 stoichiometry. *In vitro* experiments conducted in *Drosophila* cells showed that in the absence of PAN3, tethering of PAN2 results in accumulation of deadenylated intermediates, which disappear upon PAN3 tethering [23], suggesting that PAN3 is important not only for initial PAN2 recruitment, but also for eliciting further steps, resulting in complete mRNA degradation.

The existence of additional deadenylases, namely PARN, Nocturnin and Angel1/2 further expands the repertoire of poly(A) tail length regulators in eukaryotes (reviewed in [25]). In sharp contrast to deadenylases described above, it is currently believed that these enzymes do not act within larger protein assemblies.

PARN belongs to the DEDD family of nucleases [14] and is conserved in many eukaryotic species (including *S. pombe*, *Arabidopsis*, *C. elegans*, *Xenopus* and mammals) but is absent in *S. cerevisiae* and *Drosophila* [25]. It specifically degrades the poly(A) tail and binds to the 5'-terminal methylguanosine cap structure, which stimulates the deadenylase activity and processivity of the enzyme [26]. In addition to cytoplasmic localization, PARN is also present in the nucleus, as shown in humans and *Xenopus* [25]. PARN acts as a homodimer, demonstrated by its crystal structure, and was found to be essential for embryogenesis in *A. thaliana*, but dispensable in *S. pombe* [25]. In *C. elegans*, loss of one homologue (PARN1) reduces fertility and in *X. leavis* PARN participates in oogenesis, while in mammals it acts during the NMD process and regulates a distinct subset of mRNAs [25].

Nocturnin (Ccr4c), Angel1 (Ccr4e) and Angel2 (Ccr4d) are distant Ccr4p homologues, belonging to the EEP superfamily with a conserved catalytic domain, but without a region responsible for interaction with Ccr4-Not complex [15]. Deadenylation activity of Nocturnin has been shown in few organisms [25], and it was suggested to play a role in the regulation of circadian rhythms, in inflammatory responses and nutrient metabolism in mice and several other species, but its direct RNA targets have not been identified, and it is uncertain whether deadenylation activity actually contributes to its biological functions [25]. Even less information is available for Angel1 and Angel2 proteins, besides confirmed interaction involving Angel1 and eIF4E in HeLa cells [27] and complex formation

between human homologues of Angel2 and Caf1z, shuttling between the nucleus and cytoplasm [19].

The relative contribution of the deadenylases, especially Ccr4-Not and Pan2-Pan3 complexes to overall deadenylation activity is not entirely clear, but it is established that it varies depending on the organism. Mammalian CAF1 has activity towards poly(A) substrate *in vitro* [28], and is also important for deadenylation *in vivo* [29], but the actual enzymatic activity of its yeast counterpart *in vivo* is a matter of debate [28]. It is now assumed that the majority of *S. cerevisiae* deadenylating activity comes from Ccr4p, for which there is evidence of its ability to digest poly(A) tails largely on its own [21,30]. This is in contrast to experiments in *Drosophila*, where Caf1 homologue is a major deadenylation subunit of the Ccr4-Not complex [31]. Situation is also different in *Aspergillus nidulans*, in which Ccr4p and Caf1p apparently play different roles in mRNA decay with the former responsible for basal deadenylation, whereas the latter mediating the regulated deadenylation of specific transcripts [32]. In *C. elegans*, deletion of both Ccr4 and Caf1 homologues induced global deadenylation defects [33], and it was shown that in human cells, both CNOT6 (Ccr4a) and CNOT7 (hCaf1) are required for efficient constitutive deadenylation, with CNOT7 being more important in some conditions [29]. The presence of additional Ccr4 homologues in mammals makes their regulation more intricate, as CNOT7 and CNOT8, similarly to CNOT6 and CNOT6L, were found to be mutually exclusive components of different Ccr4-Not complexes in humans [18]. In HTGM5 cells, CNOT7 and CNOT8 were shown to have redundant roles [34], and CNOT6/CNOT6L as well as CNOT7/CNOT8 were subsequently demonstrated to regulate distinct subsets of genes in MCF7 cells [35]. A separate study reported that CNOT6 and CNOT6L have distinct influences on NIH 3T3 cell proliferation [36], so their activities may be subject to cell-type specific regulation.

Disruption of the gene encoding Pan2p in yeast showed that it is not essential for survival, and resulted only in increased levels of mRNAs with longer poly(A) tails [37]; similarly, the Pan2 homologue in *Drosophila* only weakly contributes to *hsp70* mRNA deadenylation [38]. However, double deletion of both Ccr4p and Pan2p results in a complete block of deadenylation and severe growth defect in yeast [30]. Likewise, double knockdown of Caf1 and Pan2 homologues in *Drosophila* inhibits deadenylation more severely than knockdown of Caf1 homologue alone [38]. Pan2 is generally thought to act prior to activities of the Ccr4-Not complex during mRNA deadenylation, which is a bi-phasic process particularly in mammalian cells, as shown by experiments conducted on NIH3T3 fibroblast cell line [39] (Fig. 1A). In the first phase, PAN2 shortens the poly(A) tail in a distributive manner [39] (Fig. 1A). Further deadenylation is carried out by CCR4 (Fig. 1A), which initially acts processively, but switches to a distributive mode when the tail gets trimmed to approximately 45 nt [39]. Activation of CCR4 deadenylase is most likely associated with PABPs displacement from the poly(A) tail, which both inhibits PAN2 and stimulates CCR4 [21]. Notwithstanding, some experiments suggest that in certain cases Ccr4-Not and Pan2-Pan3 may have partially overlapping functions. Since the physiological consequences of knockout or knockdown of Ccr4-Not complex subunits are far stronger than in the case of Pan2-Pan3 dimer, the former seems to be the only deadenylase essential for control of mRNA homeostasis.

Since deadenylation not only stimulates mRNA decay, but also disrupts the closed circle mRNA structure required for efficient translation (Fig. 1A), removal of the poly(A) tail leads to translational repression [40]. This adds another layer of complexity to deadenylation-mediated regulation of gene expression. Importantly, deadenylation in mammalian cells is required for the formation of P-bodies, which are cytoplasmic granules containing transcripts targeted for degradation and storing translationally silenced mRNAs [41]. Owing to its reversibility, deadenylation significantly impacts the decision between different possible mRNA fates: degradation, storage or translation. Furthermore, the importance of deadenylation in the control of mRNA turnover is

underscored by the fact that it serves as a signal initiating decay of protein-coding transcripts in a variety of more specialized degradation pathways, such as NMD [6], miRNA-mediated decay and ARE-mediated decay [15].

Interestingly, both the constituents of deadenylase complexes (Ccr4-Not and Pan2-Pan3) and factors implicated in the pathways mentioned above were found in P-bodies [15]. However, P-body formation was not a prerequisite for deadenylation in mammalian cells [41]. Moreover, mRNAs with shortened poly(A) tails were retained on polysomes in yeast [10], indicating that deadenylation indeed precedes formation of these cytoplasmic granules. Polysomal deadenylation was also observed in *Drosophila*, suggesting that while deadenylase enzymes may be enriched in P-bodies, the process itself occurs co-translationally [42]. In concordance, Ccr4-Not subunits were demonstrated to associate with polysomes in yeast [43].

It is uncertain how exactly deadenylation is initiated in the case of regular mRNA turnover. It is known that the human PAN3 protein partner of PAN2 deadenylase, catalyzing the first phase of the process, interacts with PABPs [44], but this may not be sufficient to trigger poly(A) tail shortening. It was demonstrated in HeLa cells that deadenylation is coupled to translation termination and requires eRF3, but also depends on the recruitment of deadenylases by PABPs and proteins of the BTG/TOB family [45]. The latter were shown in independent studies to function as activators of general mRNA deadenylation [29]. In the mammalian NMD pathway, CCR4-NOT complex is recruited by a dimer of SMG5 and SMG7 factors [6], which triggers deadenylation and exonucleolytic degradation, redundant with SMG6-mediated endonucleolytic decay. The choice between these alternative PTC-containing transcript degradative pathways depends on the UPF1 helicase phosphorylation pattern in mammalian cells [46]. In the case of ARE-mediated decay, one of the mRNA destabilizing ARE-binding proteins (TTP) associating with 3'-UTRs of unstable mRNAs, such as those coding for TNF $\alpha$  or several proto-oncogenes (c-Fos, c-Myc), was demonstrated to interact with the CNOT1 subunit of the major human deadenylase complex [15]. Degradation of transcripts containing related regulatory sequences, such as GU-rich elements (GREs), is similarly initiated by attracting proteins which interact with such motifs, such as human CELF1 (CUGBP1) or its homologues, which promote deadenylation [47]. In turn, deadenylase recruitment in miRNA-mediated mRNA decay is dependent on GW182 protein in several organisms [15]. Other examples of RNA-binding proteins that recognize particular sequences within either the coding mRNA region or in the 3'-UTR, and subsequently trigger deadenylation are members of the Puf family, Smaug and Roquin proteins [15].

In summary, deadenylation plays an important role in the initiation of mRNA decay in numerous instances, including default turnover of stable transcripts, quality control mechanisms and specialized degradation pathways. In all these cases, it is subject to multifaceted regulation, resulting from an interplay between poly(A) tail and bound PABPs, deadenylases, *cis* elements residing within protein-coding transcripts and *trans* regulatory factors comprising both non-coding RNAs and proteins.

## 2.2. The role of exosome and Ski complexes in the 3'-5' decay of normal mRNAs and cytoplasmic mRNA surveillance

Deadenylation leads to the release of PABPs from the mRNA 3'-end (Fig. 1A), which allows for a direct attack by 3'-5' exoribonucleases. A major eukaryotic exoribonuclease degrading transcripts from 3'-end is the RNA exosome complex (Fig. 1A). In yeast, exosome-mediated 3'-5' decay pathway, although functional, does not play a major role in the control of mRNA stability, and decapping-dependent 5'-3' pathway prevails (see below). In contrast, the exosome is a crucial effector in the NSD, as well as contributes to NGD and NMD quality control paths. In other model systems, the exosome seems to contribute more significantly to cytoplasmic mRNA homeostasis.

In *S. cerevisiae*, the exosome is the only essential 3'-5' exoribonuclease (reviewed in [48]), which is highly conserved in eukaryotic kingdom, with exosome core forming a barrel-like assembly (6 subunits – Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, Mtr3) and cap (3 subunits – Rrp4, Rrp40, Csl4). In terms of structure and domain composition, it resembles exosome-like complexes from *Archaea* and phosphorylase (PNPase) from *Eubacteria* and mitochondria [48]. However, the cores of the two latter RNA-degrading enzymes have catalytic properties, in sharp contrast to eukaryotic exosome (with possible exception in plants [49]). The core complex in *S. cerevisiae*, which is the best studied model in exosome research, lacks catalytic activity which is instead provided in the cytoplasm by a stable interaction with Dis3p, a nuclease possessing both endo- and exonucleolytic activity [48]. Dis3p is also present in the nucleus, where the exosome core associates with an additional catalytic subunit, Rrp6p [48]. There are two Dis3p homologues in *A. thaliana*, but only one (RRP44A) can be considered as its functional equivalent and is present predominantly in the nucleus, whereas the second one (RRP44B/SOV) does not interact with the exosome core and localizes exclusively in the cytoplasmic compartment [50]. One of the three RRP6-like *A. thaliana* proteins, RRP6L3, is localized in the cytoplasm [51], however its association with the exosome core and contribution to cytoplasmic mRNA turnover have not been studied. Only one homologue of Rrp6p exists in humans (EXOSC10/RRP6), but there are three proteins of the DIS3 family: DIS3, DIS3L and DIS3L2 [52]. Of these, DIS3L and DIS3L2 are cytoplasmic, DIS3 is primarily localized to the nucleus, and EXOSC10 to the nucleolus. DIS3L2, similar to the plant RRP44B/SOV, does not possess a domain responsible for interaction with exosome core [53] and is responsible for functioning of an exosome-independent, conserved cytoplasmic RNA degradation pathway (see below).

Following 3'-5' decay mediated by the exosome, and presumably also mediated by Dis312 (see below), the remaining mRNA fragment with its 5'-cap (m7GpppG) is degraded by scavenger decapping enzymes: Dcs1p in *S. cerevisiae*, DCPS in mammals and possibly also in other organisms, as it is evolutionarily conserved (reviewed in [54]) (Fig. 1A). DCPS is capable of efficiently hydrolyzing capped RNA substrate when its length does not exceed 10 nt [54]. Human DCPS localizes to both cytoplasm and nucleus, in contrast to its mostly cytoplasmic homologues from lower eukaryotes [55], suggesting that mammals may have adopted DCPS to act in both compartments, as it was shown that the enzyme functions in pre-mRNA splicing, due to its ability to control cap structure concentrations [55]. This hypothesis is further supported by the fact that, while yeast with *DCS1* deletion are viable, mice homozygous for mutation in the *DCPS* gene are embryonically lethal [56]. Only in yeast is Dcs1p activity regulated by Dcs2p [54]. Additionally, besides degrading an mRNA fragment after 3'-5' decay, a role for DCPS in removal of the final 5'-3' degradation product (m7GDP) was proposed [57]. The same authors also reported a novel enzyme with scavenger decapping activity, called Aph1p in yeast and its homologue FHIT in humans [57].

Activity of the exosome *in vivo* depends on the presence of specific activators, such as the Ski complex – a hetero-tetramer composed of ATP-dependent helicase Ski2p, Ski3p and two copies of the Ski8p protein, functioning in the yeast cytoplasm [58] (Fig. 1A). In this model organism, deletion of *Ski* genes induces synthetic lethality when 5'-3' decay pathway is also blocked [58]. Homologues of Ski complexes were identified in other eukaryotes, sometimes with additional or different functions than in *S. cerevisiae* [59]. Additionally, *S. cerevisiae* possesses a protein called Ski7p, acting as a bridge between Ski complex and the exosome [60] (Fig. 1A), which function together in bulk mRNA decay and in NSD [6,60]. During degradation of normal mRNAs only the N-terminal domain of Ski7p, responsible for interaction with the exosome, is needed [60], in contrast, both N- and C-terminal domains of this protein are required for NSD [6]. In the course of NSD in yeast, the C-terminal domain of Ski7p recognizes a stalled ribosome with an empty aminoacyl-(RNA-binding) site (A site), which triggers

exosome recruitment leading to subsequent deadenylation-independent mRNA degradation, owing to endo- and 3'-5' exonuclease catalytic activities of Dis3p [6,61]. The C-terminal domain of Ski7p is homologous to GTPase domains present in eRF3 and eEF1 [62] (eukaryotic translation termination and elongation factors), however it is not known whether GTP-hydrolyzing activity itself is needed for NSD, with recent data suggesting that Ski7p is a GTP-binding protein rather than a GTP-hydrolyzing enzyme [62]. Alternatively, when Ski7p is absent, mRNA that is a substrate for NSD, can be degraded by decapping-dependent 5'-3' decay [63]. NSD could be also activated by the presence of premature polyadenylation and cleavage at incorrect/cryptic sites inside an ORF or endonucleolytic cleavage within the ORF [6].

Interestingly, the yeast Ski7p homologue, Hbs1p, shares a similar C-terminal domain and functions in NGD [64], as *S. cerevisiae* Hbs1p and Ski7p are probably a result of gene duplication. Strikingly, other yeast species (with the exception of *S. pombe*) use alternative splicing mechanisms instead, which enables generation of two mRNAs, coding for Ski7- and Hbs1-like proteins from a single gene locus [59] and a similar mechanism is probable in plants [59]. It was shown recently [65,66] that in human cells the situation is quite analogous in that single gene produces several alternatively spliced isoforms with one isoform responsible for connecting exosome and Ski complexes, thus acting as a functional homologue of the yeast Ski7p during bulk mRNA decay (HBS1LV3), and the second isoform possibly functioning in translational quality control (HBS1LV1) ("Hbs1" is the name used in the reference [67]).

In contrast to NSD, ribosome stalling during NGD does not occur at the end of mRNA, and can be attributed to many distinct factors such as the presence of secondary structures, pseudoknots, rare codons, sequences encoding stretches of basic amino acid residues or RNA-oxidation [6,64]. Similarly to NSD, the NGD pathway does not require deadenylation [64], but is in turn largely dependent on endonucleolytic cleavage and Dom34p/Hbs1p proteins [64]. The Dom34p/Hbs1p complex stimulates endonucleolytic cleavage by an unknown endonuclease (however this is not the only mechanism, as cleavage could also occur independently of Dom34p/Hbs1p [68]), and subsequently cleaved fragments with free 3' and 5' termini are further degraded by the exosome-Ski complex-Ski7p and Xrn1p, respectively [64]. Dom34p/Hbs1p complex, showing an overall shape similar to that of eRF1-eRF3-GTP [68], dissociates ribosomes both during NGD/NSD and stimulates degradation of NSD-mRNAs and 5'-fragments resulting from cleavage during NGD *in vivo* [69].

Research concerning NSD and NGD has been done mostly in yeast, particularly *S. cerevisiae*, and information about these mechanisms in higher eukaryotes is scarce – NSD was shown to occur in mammals [67], NGD in *Drosophila* [70] and possibly plants [71]. Additionally, although Dom34p and Hbs1p are non-essential for yeast survival, they are widely conserved across the eukaryotic kingdom, possibly functioning in mRNA surveillance mechanisms [72]. This is exemplified by the *Drosophila* homologue of Dom34p, PELOTA that can complement Dom34p deletion in yeast [70]. Dual participation of Dom34p/Hbs1p in both NSD and NGD indicates that these quality control pathways are more interconnected than initially thought, as well as it was suggested that the NGD substrate, once internally cleaved, may represent an NSD target [73]. From one perspective, these described mechanisms prevent production of truncated and non-functional proteins from faulty mRNAs, and from another, they allow for recycling/degradation of ribosomes stalled on aberrant transcripts. An additional degradation mechanism associated with stalling induced by non-functional rRNA is described below (see Section 1.2.2).

### 2.3. mRNA uridylation

Over the past few years it has become evident that not only deadenylation, but also extension of the 3'-ends of protein-coding

transcripts with stretches of uridine residues, *i.e.* uridylation, may serve as an initial signal triggering mRNA decay in the cytoplasm.

The human genome encodes 7 non-canonical RNA nucleotidyltransferases, with some of them preferentially adding uridine instead of adenine, functioning more as terminal uridylyltransferases (TUTases) or poly(U) polymerases (PUPs), rather than true PAPs [74]. Uridylating enzymes have been found in all eukaryotes, with the exception of *S. cerevisiae* [75]. TUTases are mainly cytoplasmic, except TUTase-1/RET1, which is mitochondrial [76] but cytoplasmic localization of this enzyme was also reported [77]; similarly U6 TUTase was thought to be restricted to the nucleus, but was recently demonstrated to localize also to cytoplasm in human cells [78]. Silencing of TUTases in HeLa cells does not induce lethality, except for U6 TUTase [77].

Before the discovery of cytoplasmic uridylation, this process had been known to regulate metabolism of only selected RNA species in other cellular compartments. For instance, human U6 TUTase is responsible for uridylation of U6 snRNA in the nucleus, which is necessary for its stability, preventing adenylation-mediated decay, and required for production of the splicing-competent, functional, mature U6 snRNP [79]. Furthermore, several TUTases were shown to play an important role in kinetoplast mitochondrial RNA metabolism in *Trypanosoma*. First, guide RNAs (gRNAs) must complete their maturation cycle by RET1 TUTase-mediated uridylation in order to be able to participate in mRNA editing. On the other hand, TUTases (RET2 and MEAT1) are involved in mRNA editing based on U insertion/deletion, necessary to establish the correct sequence of the open reading frame. Moreover, mRNAs in trypanosomatoid mitochondria have A/U-rich 3'-terminal tails (synthesized by both PAP and PUP), which are essential for their translational competence [80].

3'-terminal uridylation has also been implicated in the regulation of non-polyadenylated replication-dependent histone-coding transcripts in mammalian cells (see Section 1.1.7). Furthermore, addition of short uridine stretches to the 3'-end of proximal fragment generated by miRNA-mediated mRNA cleavage in *Arabidopsis* and mouse cells was also reported [81]. Interestingly, extension of the 3'-ends with oligouridine tails was correlated with trimming of the mRNA cleavage product 5'-ends, suggesting that such modification stimulates degradation in the 5'-3' direction, thus preventing translation of truncated, cleaved messages [81].

That uridylation may play a prominent role in the control of poly(A)<sup>+</sup> mRNA stability in the cytoplasm was first demonstrated from studies in *S. pombe*. First, Cid1 non-canonical nucleotidyltransferase implicated in the S-M checkpoint control, previously misidentified as poly(A) polymerase, was demonstrated to preferentially add polyuridine extensions to the 3'-termini of RNA substrates *in vitro*, both as a recombinant protein and when purified in its native form [74]. Furthermore, Cid1 PUP activity significantly outcompeted PAP activity in the case of protein purified from fission yeast. This suggested that some regulatory factors present in the cell enhance its uridylyltransferase activity, while inhibiting poly(A) polymerase activity, and that Cid1 acts as a PUP *in vivo*. In addition, similar biochemical properties for the human Cid1 ortholog, TUTase-7 (ZCCHC6), were observed, and independent studies reported that proteins with poly(U) polymerase activity are also present in *A. thaliana* and *C. elegans* [74,82]. Most importantly, Cid1 was demonstrated to be responsible for 3'-terminal uridylation of *S. pombe* actin mRNA specifically upon S-phase arrest, which provided evidence that mRNA uridylation in fission yeast indeed occurs *in vivo* [74]. Together, these results were the first indications that uridylation might be an evolutionary conserved modification of polyadenylated mRNAs in eukaryotic cells.

Several hypotheses were initially put forward to explain the function of uridylation in the regulation of mRNA turnover [74]. Unexpectedly, while most of these focused on the possible influence of the U-rich extension in controlling the access of 3'-5' exoribonucleases, follow-up studies showed that poly(U) tails primarily stimulate decapping and subsequent degradation in the 5'-3' direction (Fig. 1A). Numerous

mRNA decay intermediates identified in *S. pombe* were found to be devoid of 5'-methylguanosine caps and contained poly(A) tails, indicative of the existence of a deadenylation-independent decapping mechanism, acting on at least a subset of protein-coding transcripts [83]. Importantly, the length distribution of poly(A) tails was similar for capped and uncapped *act1* transcripts, while for other mRNAs a varying level of dependence of decapping on deadenylation was observed, which indicated that deadenylation is not a prerequisite for degradation [83]. Furthermore, Cid1-dependent uridylation of polyadenylated mRNAs apparently stimulated decapping (see below), suggesting that uridylation and deadenylation play partially redundant roles in triggering decapping-mediated degradation, most likely converging on the recruitment of Lsm1–7/Pat1 complex [83] (Fig. 1A). Indeed, decapped, adenylated transcripts with 3'-terminal U-tails were found to accumulate upon *lsm1* deletion [83]. The stimulation of cap removal via 3'-terminal uridylation and Lsm1–7/Pat1-mediated recruitment of other decapping factors is evolutionarily conserved, since it was demonstrated that short oligo(U) tracts promote decapping in mammalian cell extracts in comparison with non-uridylated transcripts, and that they are preferentially bound by a particular subcomplex. This subcomplex comprises components of the LSM complex, as well as DCP2 decapping enzyme and its regulatory partners, DCP1 and EDC4 decapping enhancer [84] (see Section 1.1.4 for details on decapping machinery). Moreover, LSM1 depletion from the extract significantly reduced oligo(U)-dependent stimulation of decapping [84]. Yeast Lsm1p–7p–Pat1p protein assembly was demonstrated not only to bind to oligo(A) tails with higher affinity than to poly(A) tails, but the presence of a U-rich fragment in the vicinity of the RNA molecule 3'-end was shown to enhance binding of this complex *in vitro* [85]. Further supporting an overlap between functions of uridylation and deadenylation in mRNA decay, it was shown that in *S. pombe* each of the two processes is enhanced when the other is impaired, thus demonstrating that they can compensate for one another [83]. Interestingly, U-rich extensions found on decapped mRNAs were only 1–2 nucleotides long, *i.e.* much shorter than tails synthesized by Cid1 or orthologous PUPs *in vitro* [74, 82, 83], indicating that the uridytransferase activity must be precisely controlled *in vivo* and that short stretches of uridine residues are sufficient to elicit mRNA degradation. An independent study showed that the efficiency of decapping in mammalian cell extract correlates with the length of the uridine tail up to 5 added nucleotides. A plateau is observed after exceeding this length, whereas even monouridyl extensions effectively stimulated decapping [84]. Several lines of evidence pointed to the conclusion that oligouridylation occurs prior to decapping, including significant accumulation of capped, uridylated decay by-products upon impairment of decapping [83]. The involvement of Cid1 PUP activity in the control of mRNA stability *in vivo* was demonstrated by analyzing transcripts half-lives, which increased substantially in a *cid1* deletion strain [83]. It was postulated that uridylation-dependent decay may be of particular importance in *S. pombe*, since poly(A) tails present on its mRNAs are considerably shorter than in other eukaryotes [83].

While the 3'-oligo(U) tract stimulates decapping, it also inhibits decay in the 3'-5' direction *in vitro* [84]. It was therefore proposed that oligouridylation not only enhances the decapping-mediated pathway, but also establishes 5'-3' directionality of mRNA degradation. This may be through protection of the U-rich stretch from 3'-5' trimming, most likely secured by Lsm1–7/Pat1 complex binding, as in the case of deadenylated 3'-termini of mRNAs [85]. In agreement with the plausible protective role of oligo(U) tails, it was reported that addition of U-rich extensions to oligoadenylated mRNAs in *A. thaliana* prevents their further shortening [86]. Since this phenomenon was also observed for polysome-associated mRNAs, it was proposed to ensure 5'-3' polarity of co-translational degradation. Strikingly, impairment of the functions of the URT1 enzyme, responsible for this modification, resulted in increased levels of the 3'-truncated messages, but it was not accompanied by changes in mRNA stability [86]. On the other

hand, the hypothesis that uridylation interferes with mRNA decay in the 3'-5' direction has been recently challenged by the discovery of U-preferring Dis3L2 exoribonuclease, as well as by global transcriptomic analyses in human cells, demonstrating that uridylated mRNAs accumulate upon depletion of both DIS3L2 and exosome components (see part 1.6.).

#### 2.4. Deadenylation- and uridylation-dependent mRNA decapping

Deadenylated or uridylated mRNA, instead of being directly degraded by the 3'-5' pathway can undergo decapping (reviewed in [87]) (Fig. 1A). Despite initial confusing reports concerning the identity of the enzyme responsible for decapping, it is now well-established that the main decapping activity is provided by Dcp2 protein [87], which catalyzes hydrolysis of the cap structure, rendering transcript vulnerable to nucleolytic attack by Xrn1 (see below) (Fig. 1A).

While both yeast and mammalian Dcp2 are active *in vitro* on their own [87], *in vivo* they require additional decapping activators, known as enhancers of decapping (EDCs) for stimulation of their activity. Most of the EDCs are conserved over evolution, albeit some are present only in specific organisms, and one of the best studied is Dcp1p in yeast (Fig. 1A), which enhances intrinsic enzymatic activity of Dcp2p [87]. Similarly, in human cells, decapping activity was strongly impaired when Dcp1 homologue binding to DCP2 was prevented [88]. In *S. cerevisiae* Dcp2p interacts with Dcp1p, constituting a decapping holoenzyme with a catalytic and regulatory subunit, respectively, however deletion of either *DCP1* or *DCP2* is not lethal, but mRNA degradation in such strains is impaired [89]. Recombinant Dcp1p/Dcp2p complexes prefer longer RNA molecules as substrates [87], and the addition of cap analogs does not inhibit decapping [90], suggesting that not only the cap structure, but also the RNA body is important for initiation of decapping, which is further confirmed by structural analysis of the yeast Dcp1p/Dcp2p complex [91]. Structural data also show that *S. cerevisiae* Dcp1p may stimulate Dcp2p activity, not through mediation of RNA binding, but by changing Dcp2p conformation from an inactive open state to an active closed one [91]. Interestingly, residues involved in the interaction between Dcp1p and Dcp2p are not conserved in higher eukaryotes [91] corroborating observations that interaction between Dcp1 and Dcp2 homologues in human cells is either very weak [88] or occurs only in cell extracts [92], suggesting that additional proteins may be required for its stabilization, as shown in mammalian cells [88].

The crystal structure of yeast Dcp1p demonstrated the presence of a surface responsible for binding of other decapping regulatory proteins, although it does not always mediate interaction of EDCs with Dcp2, as shown by Lai et al. [93]. There exist a plethora of additional EDCs, exemplified by *S. cerevisiae*, where Edc1p, Edc2p, Pat1p, Scd6p, Edc3p, Dhh1p and Lsm1p–7p complex are additionally present [94]. Yeast EDCs bind to Dcp2p [94] but differ in mechanisms of decapping enhancement. Scd6p, Dhh1p and Pat1p repress translation directly, which enhances decapping because translation and decapping are in competition. Edc1p–3p and Pat1p are able to stimulate Dcp2p catalytic activity directly, additionally Pat1p serves as a scaffold for recruitment of other proteins, including Lsm1p–7p heptamer [94]. In agreement with its multiple roles in the regulation of decapping, Pat1p deletion induces the most severe decapping defect among known yeast EDCs [94]. Lsm1p–7p complex forms a ring, binds to shortened poly(A) tail after deadenylation and enhances interaction of Dcp2p with mRNA [94]. As mentioned earlier, Lsm1p–7p together with Pat1p also bind to uridine stretches near the 3' end of transcript [85], explaining why Lsm1–7/Pat1 complex stimulates decapping following both mRNA deadenylation and uridylation.

Multiple interactions among yeast EDCs and between EDCs and Dcp2, were reported [94, 87], as well as the fact that Xrn1 ribonuclease interacts with EDCs [94], which provides direct connection between decapping and 5'-3' degradation. Decapping can be not only enhanced by EDCs, but also inhibited by cap binding proteins [95], which is

consistent with translation being in competition with decapping. However, this is not an entirely universal statement, since, firstly, mRNA can be decapped in the course of translation [10], and secondly, translationally-repressed mRNA may not undergo decapping [96].

Generally, Dcp1/Dcp2 are deposited on mRNAs as parts of a ribonucleoprotein complex, and the composition of this complex varies in different organisms. In addition to conserved and folded domains present in EDCs, unstructured regions are also important for the assembly of decapping machinery [97]. While Edc1p and Edc2p are specific to *S. cerevisiae*, Edc3, Pat1, Lsm1–7, Scd6 and Dhh1 have homologues in many other eukaryotic organisms [87]. On the other hand, *S. cerevisiae* lacks another decapping enhancer, called Edc4, homologues of which are present in metazoans [87] and also in *S. pombe* [98]. Recently, human 4E-T protein was suggested to be a novel component of the decapping machinery, facilitating decay through bringing DCP2 into proximity of the 5'-cap structure [99]. Crystal structures of many decapping enhancers or their fragments have been solved [87].

Apart from the well-studied, canonical Dcp2 decapping enzyme, a novel protein displaying this activity was recently identified in mammalian cells (NUDT16) [100] and subsequently found to be differentially utilized during specific mRNA degradation processes in comparison to DCP2 [101]. Very recently, NUDT3 was also shown to act as a decapping enzyme *in vivo*, specifically on transcripts coding for proteins involved in cell migration, thus establishing a third mammalian enzyme able to catalyze cap hydrolysis [102]. Since mammals and other eukaryotic species possess additional proteins homologous to NUDT3 that display decapping activity *in vitro*, it is possible that the future research will reveal additional decapping enzymes functioning *in vivo*.

### 2.5. Degradation of decapped mRNA by Xrn1

This major canonical degradation pathway starts from the 5'-end of the transcript and begins following decapping, after which RNA is exposed to exonucleolytic degradation exerted by Xrn1 nuclease (reviewed in [103] (Fig. 1A).

In yeast and in most animals, there are two enzymes belonging to the XRN family: Xrn1, localized mainly in the cytoplasm, and Xrn2 (in yeast better known as Rat1p), functioning in the cell nucleus [103]. In *S. cerevisiae* only the *RAT1* gene is essential, whereas *XRN1* deletion causes slowed growth, sporulation defects, impaired DNA recombination and other phenotypes [103]. That Xrn1p function is not entirely indispensable for life is likely a result of redundancy between different mRNA decay pathways operating in the cytoplasm.

All enzymes of the XRN family contain two conserved regions, CR1 and CR2, at their N-terminus, separated by a segment of more diverse sequence. CR1 comprises 7 strictly evolutionary preserved acidic amino acids, the role of which is to coordinate two  $Mg^{2+}$  cations essential for catalysis [104]. Substitutions of these amino acids lead to the loss of ribonucleolytic activity. Protein sequences situated downstream of the CR2 region are less conserved, nonetheless the parts immediately adjacent to the CR2 module are essential for enzymatic activity, while deletion of more C-terminal fragments does not impair catalysis [103].

Proteins of the XRN family are  $Mg^{2+}$ -dependent, processive 5'-3' exoribonucleases. This activity can be also supported to some extent by supplying  $Mn^{2+}$  ions as a cofactor. XRN enzymes generally prefer single-stranded, unstructured RNA substrates bearing monophosphate at the 5'-end [103], explaining why Xrn1p activity on mRNAs is inhibited by the 5'-cap structure and stimulated after its removal during decapping. Similarly, the 5'-hydroxyl group or the 5'-terminal triphosphate renders RNA less susceptible to Xrn1p-mediated decay *in vitro* [105], and the presence of a stable secondary structure inhibits the exoribonucleolytic activity of both Xrn1p and Rat1p [103].

Apart from yeast Xrn1p, homologues in *Drosophila melanogaster* (Pacman/XRN1), *A. thaliana* (XRN4) and human cells (XRN1) were also studied [103], and demonstrated to interact with decapping machinery, as mentioned earlier. In *S. cerevisiae*, degradation mediated

by Xrn1p is a prevailing pathway responsible for mRNA decay, confirmed by the fact that deletion of Xrn1p results in the genome-wide increased stability of mRNAs [106,107]. However, it also plays a role in mRNA synthesis, which highlights an interplay between transcription and decay, the molecular basis of which is far less understood. Two studies [106,107] reported that Xrn1p contributes to buffering of mRNA levels (*i.e.* mRNA levels are rendered stable because changes in synthesis or degradation rate are balanced by corresponding modulation in the opposite process), however, these reports, while agreeing in Xrn1p contribution to buffering, indicate different mechanistic explanations. One report suggests that it indirectly represses transcription through regulation of Nrg1 transcriptional repressor levels [106], while the second implies the opposite, showing that it has a direct and stimulating effect on transcription initiation and elongation [107]. Consistent with its role in both synthesis and degradation of transcripts, Xrn1p shuttles between nucleus and cytoplasm as shown by Haimovich et al. [107]. Subsequent studies from the same group [108] pointed out that, although Xrn1p influences the transcription rate genome-wide, it has a strong bias towards the most actively transcribed genes, *i.e.* encoding components of the translational machinery, suggesting an important function in the regulation of genes essential for optimal growth. In addition, another publication discovered Snf1p as a kinase, regulating activity of many proteins involved in mRNA metabolism, including Xrn1p [109], but is not established if and how this regulation impacts transcriptome buffering. The model proposed by Sun et al. [106] is somewhat inconsistent in that deletion or impairment of Xrn1p enzymatic function resulted in the increase of *nrg1* mRNA levels, which should lead to repression of transcription rather than stimulation. The differences between these studies [106, 107,109] are thoroughly discussed by Braun and Young [11]. Further research is needed to clarify these issues and to determine whether a similar mechanism of coupled RNA synthesis-degradation occurs in higher eukaryotes.

### 2.6. Uridylation-dependent 3'-5' mRNA decay by Dis3L2 exonuclease

As mentioned above, decapping/5'-3' decay is not the only possible mechanism of degradation of the 3'-uridylylated mRNAs. Since the uridine tails added by *S. pombe* Cid1 *in vivo* were significantly shorter than the extensions synthesized *in vitro*, the existence of a poly(U)-specific 3'-5' exoribonuclease was anticipated. The mutually opposite actions of PUP activity and the nuclease were envisaged to control the length of 3'-terminal U-rich stretches in cells, in a manner similar to balancing poly(A) tail lengths by adenylating enzymes and deadenylases. Intriguingly, recent studies carried out in both *S. pombe* and mammalian cells revealed that Dis3L2 nuclease, a paralogue of the exosome complex Dis3/Dis3L catalytic subunits, rather than controlling uridine-tail length, preferentially degrades 3'-uridylylated mRNAs in the 3'-5' direction [53, 110] (Fig. 1A). This was inferred both from the *in vivo* accumulation of uridylylated mRNA decay intermediates and uridylylated poly(A)<sup>+</sup> mRNAs upon impairment of Dis3L2 function, and from *in vitro* biochemical assays, in which the presence of 3'-terminal U-stretches boosted Dis3L2 activity on RNA substrates [110–112]. Thus, Dis3L2 was proposed to be the central player of the novel, exosome-independent mRNA decay pathway in the cytoplasm. Apart from identification of this degradation path as a parallel to the exosome-mediated cytoplasmic decay of protein-coding messengers, these studies also determined that uridylation can affect mRNA fate in two different, possibly redundant, ways. In addition to activation of decapping and 5'-3' degradation, uridylation apparently also stimulates mRNA decay in the other direction by enhancing Dis3L2 exoribonuclease activity (Fig. 1A).

Dis3L2 is absent from *S. cerevisiae*, but was shown to participate in mRNA degradation in *S. pombe* [110], plants [113] and human cells [53], working on its own, instead of being part of any molecular complex in mentioned organisms, pointing to its independence of the exosome [53]. Its plant homologue, RRP44B/SOV, was identified as a suppressor



of a severe phenotype observed when decapping machinery was impaired [113]. In *S. pombe*, it was demonstrated that deleting Dis3L2 together with Xrn1 or Lsm1, but not the simultaneous impairment of Ski cytoplasmic exosome activator, resulted in negative genetic interactions, indicating that the decapping-dependent Xrn1-mediated 5'-3' decay and Dis3L2-dependent 3'-5' mRNA degradation pathways probably cooperate on the same pools of transcripts [110]. In concordance, synergistic influence of *dis3L2Δ* in the background of either *xrn1Δ* or *lsm1Δ* on mRNA accumulation and increased half-lives were observed, which was not the case for the *dis3L2Δski2Δ* double mutant [110]. Additional evidence for possible cooperation between exoribonucleolytic activities of XRN1 and DIS3L2 in the degradation of the same substrates came from studies in human cells, which showed that both nucleases interact with one another and with polysomes [53]. Furthermore, *S. pombe* Dis3L2 was reported to localize to the cytoplasmic foci adjacent to P-bodies, containing Xrn1 as one of the major protein constituents, while DIS3L2 downregulation in human cells affected P-body formation, albeit in a different way than in the case of XRN1 depletion [53,110].

Importantly, 3'-RACE (rapid amplification of cDNA ends) analysis of *S. pombe adh1* mRNA showed that while a minority of poly(A)<sup>+</sup> molecules were found to be mono- or diuridylated, accumulation of 3'-terminally truncated transcripts, equipped with longer oligo(U) extensions (comprising up to 7 nucleotides), was observed in the *dis3L2Δlsm1Δ* strain [110]. It was hypothesized that in the wild-type cells, Dis3L2 may assist in the elimination of non-polyadenylated, trimmed messengers, which escaped protection from the 3'-5' degradation by the Lsm1–7/Pat1 complex, and are marked for degradation by addition of U-rich extensions to their 3'-ends [110]. In mammalian cells, DIS3L2 was implicated in ARE-mediated decay, as well as in bulk mRNA degradation, and – very recently – in degradation of variety of ncRNAs [114–116]. It is worth noting that high-throughput transcriptomic analyses in human cells revealed numerous mRNA targets shared by DIS3L2 and XRN1, corroborating previous observations favoring the hypothesis that these two enzymes cooperate with each other [53].

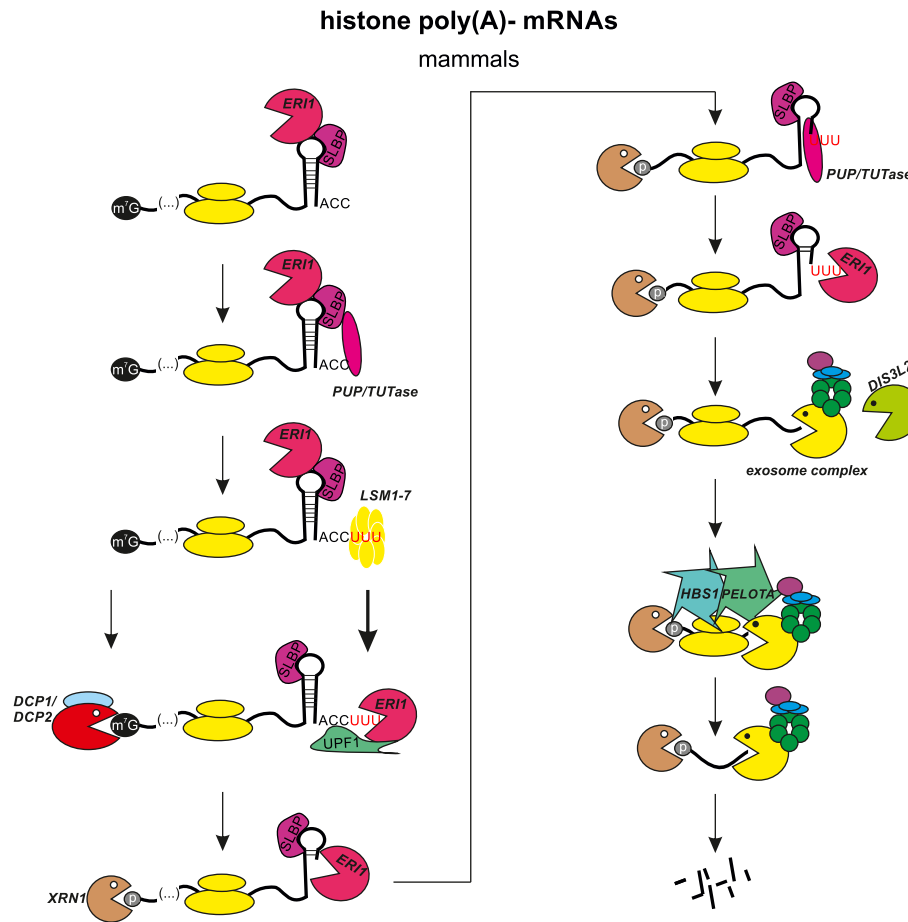
Similar to experiments in *S. pombe*, the DIS3L2 mammalian orthologue was found to specifically bind oligouridylated RNA species, indicating that the uridylation-mediated DIS3L2-dependent mRNA degradation mechanism may be conserved in higher eukaryotes [111,117]. Indeed, widespread uridylation of poly(A)<sup>+</sup> mRNAs has been recently detected in mammalian cells using a dedicated, newly developed, high-throughput TAIL-seq technique [118]. Short (1–4 nucleotides) U-rich extensions were found mostly on deadenylated messengers and a negative correlation was observed between uridylation frequency and mRNA stability [118,119]. The recently solved structure of a mouse DIS3L2 co-crystal including uridine homooligomer explained the molecular basis of the enzyme specificity towards oligo(U) substrates [112]. Furthermore, TUTase-4 (ZCCHC11) and TUTase-7 (ZCCHC6) proteins, non-canonical nucleotidyltransferases homologous to fission yeast Cid1, were identified as enzymes responsible for the uridylation of mRNA 3'-ends in human cells [119]. In concordance with previous findings, siRNA-mediated TUTase-4/7 depletion resulted in decreased mRNA turnover rates. U-rich extensions were added more efficiently to shortened poly(A) tails by TUTase-4/7 both *in vivo* and *in vitro*. It was therefore concluded that in human cells, in contrast to *S. pombe* (possessing intrinsically shorter poly(A) tails), mRNA deadenylation precedes uridylation [119]. Importantly, a similar temporal relationship between poly(A) tail shortening and uridylation was reported in plants [86,120]. In addition, human TUTase-4/7 were demonstrated to have an ability to measure the length of poly(A) tails which, together with the observed inhibition of their uridylation activities by poly(A) binding proteins, explained why the longer poly(A) tails on human mRNAs are rarely oligouridylated [119]. Since uridylation of transcripts bearing short oligo(A) tails was enhanced upon downregulation of XRN1, exosome and DIS3L2 – three major exoribonucleases participating in cytoplasmic mRNA decay – it suggested that uridylated mRNAs can be degraded by multiple, redundant pathways.

The most direct link between mRNA uridylation and stimulation of DIS3L2 activity *in vivo* in higher eukaryotes has been demonstrated through the recent identification of an apoptotic mRNA decay pathway in human cells [121]. mRNA degradation, affecting transcripts coding for proteins involved in a variety of processes, was detected as an early event during apoptosis, induced by different signals and in various cell types, more than 10 years ago. Nevertheless, the identities of enzymes responsible for this phenomenon remained largely unknown. A recent paper by Thomas et al. confirmed previous observations that the global mRNA decay is indeed a hallmark of apoptosis, occurring prior to phosphatidylserine externalization and DNA fragmentation, and dependent on the permeabilization of the outer mitochondrial membrane [121]. Inspection of the mRNA decay intermediates' termini revealed the presence of untemplated U-rich stretches at the 3'-ends, the number of which decreased following siRNA-mediated depletion of TUTase-4 or TUTase-7. This indicated that these two TUTases contribute to uridylation of mRNA degradation intermediates [121]. Interestingly, TUTase downregulation correlated with reduced annexin V staining and caspase 3 cleavage, demonstrating that mRNA uridylation contributes to apoptosis [121]. Since some of the mRNA decay products were not decapped, it was concluded that their degradation must proceed in the 3'-5' direction. Indeed, DIS3L2 silencing resulted in the increased length of 3'-terminal uridine extensions, inhibition of mRNA degradation and suppression of cell death. Conversely, DIS3L2 overexpression enhanced apoptosis. These results showed that DIS3L2 is an important player in the apoptotic mRNA decay pathway [121]. It should be emphasized that the treatment of cells not subjected to apoptotic stimuli with siRNA against DIS3L2 also enhanced mRNA uridylation, in agreement with the proposed role of this 3'-5' exoribonuclease in the general regulation of mRNA turnover [121]. This has been recently confirmed in our laboratory by transcriptomic analyses of human cell lines expressing DIS3L2 with a catalytic mutation [114].

## 2.7. Degradation of non-polyadenylated histone mRNAs

The only known examples of metazoan mRNAs that are non-polyadenylated are histone-encoding mRNAs, belonging to highly conserved group of transcripts, with unique mechanism ensuring their proper degradation. Mammalian histone mRNAs turnover is highly cell-cycle dependent, as the abundance of these transcripts increases approximately 40-fold during replication in the S-phase, and then decreases again. This post-transcriptional regulation in humans is possible owing to the specific architecture of histone mRNAs, including the presence of a stem-loop (SL) structure within the 3'-UTR, which binds SLBP protein and ERI1 ribonuclease [122] (Fig. 2). Initiation of degradation is mediated by SLBP interaction with TUTase, which adds an oligo(U) tail, attracting the LSM1–7 complex [77] (Fig. 2). Different TUTases have been implicated in this process: initially TUTase-1 (mitochondrial poly(A) polymerase) and TUTase-3 (cytoplasmic poly(A) polymerase PAPD4/GLD2) [77], but more recently TUTase-4 (ZCCHC11) as well [123]. Because the latter is localized only in the cytoplasmic compartment and endowed exclusively with poly(U) polymerase activity, TUTase-4 would be the most likely enzyme involved in histone mRNA decay. However, the latest report unexpectedly pointed towards the role of TUTase-7 rather than TUTase-4 in the uridylation of histone mRNA 3'-ends [124].

Upon LSM1–7 binding, stimulation of decapping and Xrn1-dependent 5'-3' decay and/or degradation in the 3'-5' direction by ERI1 and exosome occurs (Fig. 2). In the latter pathway, which was suggested to prevail in this case, repetitive rounds of ERI1-mediated decay and TUTase-dependent uridylation allow for SL removal, which is followed by degradation by the exosome [77,125] (Fig. 2). UPF1 protein is also required for histone mRNA degradation, possibly due to its helicase activity, which may allow for opening of the SL structure [125] (Fig. 2). UPF1 stimulates mRNA degradation in the NMD



**Fig. 2.** Schematic presentation of poly(A)- histone-coding mRNA decay pathway in mammalian cells. See Section 1.1.7 for details on ERI1 3'-5' exonuclease action and the role of repetitive uridylation in the removal of stem-loop (SL) structure, recruitment of decapping factors mediated by LSM1–7 complex and the involvement of exosome, DIS3L2 and HBS1/PELOTA heterodimer in the degradation process.

surveillance pathway [126] and similarly to NMD, UPF1 is phosphorylated by SMG1 kinase during histone mRNA degradation [127]. It was also proposed that ribosomes stalled during histone mRNA decay may be removed by the HBS1-PELOTA complex [128] (Fig. 2). Recently, it was shown that replication-dependent histone mRNAs are DIS3L2 substrates [53,114], thus DIS3L2 may be a component of an additional pathway for histone mRNA decay (Fig. 2), however mechanistic details are unknown, other than the stimulating role of oligo(U) tails in DIS3L2-mediated degradation.

### 2.8. Other non-canonical 3'-end modifications and their relevance to cytoplasmic mRNA decay in various model organisms

While the impact of deadenylation and oligouridylation on the regulation of general, decapping-mediated 5'-3' or exosome- and Dis3L2-dependent mRNA decay pathways has been the focus of many research groups worldwide, a number of studies additionally indicate that: 1) uridylation does not always accelerate mRNA degradation; 2) other mRNA 3'-terminal modifications such as oligoadenylation, CUCU-addition, cytidylation and guanylation, exist. The following subsections are dedicated to several specific instances linking different 3'-untemplated nucleotide additions with variable outcomes with regard to mRNA stability control.

#### 2.8.1. Non-destabilizing effects of uridylation in *Arabidopsis* and starfish oocytes

Recent study performed in *Arabidopsis* revealed that uridylation can exert yet another effect on mRNA stability besides stimulation of

degradation associated with decapping or activation of Dis3L2 3'-5' exoribonuclease. Similar to the case in mammalian cells, TAIL-seq experiments revealed that mRNA uridylation in *A. thaliana* is a widespread process [120]. URT1-mediated uridine addition was found not only to protect deadenylated mRNAs from further trimming, but also to control the extent of deadenylation [86,120]. Extension of oligo(A) tails containing 13–15 nucleotides with uridines up to a length of 15–18 residues, served to repair deadenylated termini, which was sufficient to stimulate re-binding of PABPs [120]. Furthermore, PABPs were demonstrated to limit the size of U-rich extensions on deadenylated ends, likely through indirect inhibition of URT1-mediated tail elongation [120]. These observations suggest that deadenylation and the concerted action of URT1 and PABPs are antagonistic processes, which together define the length of deadenylated tails and the future fate of transcripts bearing oligo(A) extensions of their 3'-termini [120]. Based on the TAIL-seq data, mRNAs with oligoadenylated tails comprising less than 13 nt are further deadenylated and subjected to 5'-3' or 3'-5' decay, rather than subject to repair associated with uridylation and PABPs binding, which might be simply inefficient when deadenylation occurs beyond a certain limit [120].

These findings imply that the functions of cytoplasmic uridylation in poly(A)<sup>+</sup> mRNA turnover may not be restricted solely to destabilizing protein-coding transcripts, although it is not known whether a uridylation/PABPs-dependent mechanism of counteracting deadenylation is conserved in other eukaryotic species. On the other hand, URT1 or another enzyme (e.g. HESO1 uridylyltransferase, involved primarily in plant miRNA U-tailing) can still mark *A. thaliana* mRNAs with short oligo(A) tails by uridylation, targeting them for decay in

one of the pathways described in other organisms. Although this requires further experimental verification, URT1-independent uridylation of messages, playing a distinct function from the repair of deadenylated mRNA ends, has also been reported in *Arabidopsis* [120].

A somewhat different impact of oligouridylation on the regulation of mRNA poly(A) tail length has been reported very recently in starfish oocytes [129]. Namely, short oligo(A) tails of the majority of translationally silent *cyclin B* mRNA were found to be modified by addition of several (most frequently two) uridine residues. Treatment of oocytes with 1-methyladenine hormone, inducing meiotic reinitiation, led to extension of poly(A) tails [129]. Notably, inspection of the 3'-terminal sequences revealed that two types of polyadenylated *cyclin B* transcripts co-existed following hormonal stimulation. One of them contained poly(A) tails downstream of the U-stretch, indicating that removal of the U-tail is not indispensable for efficient re-adenylation. In turn, the second group of mRNAs was characterized by the absence of antecedent oligo(U) extension and 3'-trimming of the transcript body [129]. These results and other presented experimental evidence suggest that, rather than inducing mRNA decay, uridylation may be required to maintain some transcripts in a translationally inactive state. Although the exact mechanisms underlying addition of poly(A) tails to oligouridylated mRNAs upon hormonal stimulation remain to be explored in detail, these observations indicate that the repertoire of uridylation functions in the regulation of mRNA turnover may be broader than initially anticipated.

### 2.8.2. Cytoplasmic mRNA oligoadenylation

It has been widely accepted that the addition of 3'-terminal adenosines in the form of poly(A) tails stabilizes protein-coding transcripts in the cytoplasm. The known cases of oligoadenylation-mediated destabilization of coding and non-coding RNA molecules, attributed to the activity of non-canonical poly(A) polymerases, occur in the nuclear compartment where such processes are an important part of RNA surveillance [130]. However, there are several clues that oligoadenylation may similarly promote RNA decay in the cytoplasm.

The first indication for the role of oligoadenylation in the activation of mRNA degradation in the cytoplasm came from studies in *Chlamydomonas reinhardtii*, which documented that the proximal, and in some instances, 3'-terminally truncated products of RISC-mediated endonucleolytic cleavage, contain untemplated oligo(A) tails at their 3'-ends, synthesized by the non-canonical nucleotidyltransferase, MUT68 [131]. It was proposed that the observed oligoadenylation most likely stimulates exosome-mediated 3'-5' degradation of such mRNA decay intermediates [131]. In regard to the functionally analogous pathway described in plants, this implies that, depending on the organism, mRNA decay intermediates arising after cleavage carried out by RISC may undergo either oligouridylation or oligoadenylation. This triggers mRNA degradation, albeit in the opposite directions: 5'-3' or 3'-5', respectively [81,131].

Interestingly, MUT68 was also shown to uridylate a fraction of both siRNAs and miRNAs, thus stimulating their degradation by the RRP6 exosome catalytic subunit [132] and *in vitro* assays showed that MUT68 nucleotidyltransferase displays preference towards ATP and UTP [132]. Apparently, the NTP specificity of this enzyme is somehow dictated by the nature of the RNA substrate, but the mechanism underlying differences of MUT68 activity towards mRNAs and small regulatory RNAs is unknown. The dual role of MUT68 in the regulation of RNAi pathway in algae clearly deserves further investigation.

Transient addition of A-rich tails was also reported in the cytoplasm of human cells, for rRNA degradation intermediates and truncated  $\beta$ -actin mRNA. Since the decay products accumulated upon downregulation of exosome catalytic subunits (DIS3 and DIS3L), it was concluded that they are degraded in the 3'-5' direction [133].

That the exosome is mainly responsible for the degradation of 3'-oligoadenylated mRNA decay intermediates in the cytoplasm has been also recently proposed based on studies in *Drosophila* cells, regarding

degradation paths of *Hsp70* mRNA [134]. Similar to other short-living mRNAs, this transcript is degraded predominantly by the deadenylation-dependent decapping and XRN1 activity in wild-type cells. However, a minor decay pathway related to exosome action, was also demonstrated to exist [38], and to be utilized in *Drosophila* cells particularly when the major, 5'-3' degradation pathway is blocked [38,134]. Addition of oligo(A) tails 2–3 nt in length to mRNA fragments terminating upstream of the major poly(A) site was shown to be dependent on the non-canonical TRF4-1 PAP, localized mainly in the cytoplasm [134]. The possible involvement of the exosome, but not the 5'-3' pathway, in the degradation of such transcripts was proposed based on the fact that depletion of the former increased their number, while DCP2 downregulation led to the opposite effect, indicating that decapping is disfavored [134]. Furthermore, these mRNA decay intermediates co-fractionated with polysomes, consistent with the observed cellular localization of TRF4-1, thus supporting the conclusion that oligoadenylation facilitates exosome-mediated mRNA degradation in the cytoplasm [134].

### 2.8.3. Extension of mRNA 3'-ends with other residues (CUCU modification, cytidylation, guanylation)

Apart from deadenylation, uridylation and oligoadenylation, 3' termini of mRNAs can undergo other posttranscriptional modifications based on non-templated addition of nucleotides such as cytidine and guanine.

Protein-coding transcripts in the filamentous fungus *A. nidulans* were demonstrated to be modified by extension of poly(A) tails with short C/U stretches [135]. Similar to uridylation in *S. pombe*, an enzyme responsible for CUCU addition in *A. nidulans*, CutA, is a member of the non-canonical nucleotidyltransferase family. Furthermore, the role of CUCU modification appears to be functionally equivalent to uridylation, since the presence of C/U-rich extensions most likely triggers deadenylation-independent decapping of polyadenylated mRNAs when the function of the Ccr4-Not complex is disrupted [135]. In the wild-type strain, this modification occurs when poly(A) tails are shortened to approximately 15 nt, suggesting that deadenylation and CUCU addition act in concert to efficiently stimulate decapping. Importantly, CutA dysfunction leads to impaired decapping and increased mRNA half-lives [135]. It is thus conceivable that C/U-rich extension serves as a platform for factors such as Lsm1–7, which, similarly to the case of the uridylation-dependent decay mechanism, recruits decapping machinery. The involvement of CutA-mediated C/U-tagging in the degradation pathway associated with decapping was further supported by the observation that *cutA* deletion leads to the impaired P-body formation [32]. The importance of mRNA 3'-end C/U modification is underscored by the fact that a second nucleotidyltransferase, CutB, ensures residual pyrimidine tagging in *A. nidulans* when the *cutA* gene is deleted [135,136]. This is similar to the situation in *S. pombe*, where, although Cid1 is predominantly responsible for mRNA uridylation, some level of uridylation is maintained in *cid1*  $\Delta$  strain, pointing to the possible involvement of a second, yet-unidentified poly(U) polymerase in this process [83].

Whether CUCU modification, like uridylation, plays a role in global mRNA turnover, remains to be determined. The efficiency of pyrimidine tagging in *A. nidulans* was shown to be largely dependent on NMD and protein components of this quality control pathway, such as Upf1 [136]. Furthermore, experimental evidence was provided for the role of C/U-tagging in the clearance of ribosomes terminating at premature stop codons, which, together with decapping and 5'-3' degradation, precludes re-initiation of translation on PTC-containing messages [135, 136]. However, it cannot be excluded that wild-type transcripts may also be targeted for C/U-mediated decay. Following CutA/B activation by Upf1 recruited to the terminating ribosome through interaction with eRF3 at the normal stop codon, an NMD-like mechanism of translation termination may proceed on deadenylated transcripts [135,136]. Importantly, C/U 3'-tagging does not seem to be restricted to *A. nidulans*, as it was also observed in *A. thaliana* [135].

Due to the presence of long poly(A) tails at the 3'-end of mRNAs and the use of oligo(dT)-based priming methods, 3'-terminal extensions comprising stretches of nucleotides other than adenosine escaped detection and, until recently, their significance has been underestimated in high-throughput RNA-seq analyses. TAIL-seq approaches revealed that 3'-end mRNA modifications other than uridylation are common. A significant fraction (over 60%) of poly(A)<sup>+</sup> protein-coding transcripts in mouse NIH 3T3 and human HeLa cells were found to be guanylated [118]. The role of 3'-terminal guanylation remains to be explained, however, its presence on poly(A) tails longer than 40 nucleotides and the observed positive correlation between frequency of G-tailing and mRNA half-lives, suggested that the occurrence of G-rich stretches may inhibit deadenylation and thus increase transcript stability [118]. Similar analyses performed in *A. thaliana* confirmed that mRNA 3'-guanylation occurs in plants as well [120]. Furthermore, a fraction of reads presenting 3'-terminally cytidylated transcripts, was also identified in both human cells and plants [118,120], demonstrating that cytidylation is probably also conserved among eukaryotes.

### 3. Part two: non-coding RNA decay in the cytoplasm

In addition to mRNAs, the human genome encodes many non-protein coding RNA classes (ncRNAs), some with well-defined functions (like tRNA and rRNA), but others arising as pervasive transcription products, which are often quickly degraded in the nucleus, but may also be exported to the cytoplasm [137]. In the case of RNA polymerase II products, they may share degradation mechanisms with mRNA turnover because of a similar transcript architecture but RNA polymerase III also produces a variety of ncRNAs [138]. Moreover, RNAs present in cells are not only direct products of polymerase activity, but also the result of not yet fully understood processing events [139]; among them are multiple examples of non-coding transcripts devoid of poly(A) tails, and these RNAs do not follow canonical mRNA degradation pathways starting with deadenylation. Nevertheless, apart from deadenylases, the majority of enzymes involved in decay are shared between mRNA and ncRNA. In some cases, specific mechanisms ensuring that these transcripts are degraded properly have been identified, but for many other RNAs, their decay pathways remain unknown. Below, different mechanisms of degradation of various classes of ncRNAs are discussed.

#### 3.1. Transfer RNA (tRNA)

tRNAs, involved in translation processes and representing approximately 10% of the total RNA, carry 80% of identified nucleotide modifications types and are highly structured, which generally protects them from ribonucleolytic degradation. Indeed, these are very stable molecules, as their half-lives extend to days [140] and little is known about their turnover in normal cellular conditions. Interestingly, in mammalian tumor tissue, subsets of tRNA have much faster turnover than in normal cells [141], but the reason remains unidentified. In yeast, tRNA levels seem to be controlled by Dis3p/exosome, but the relative contribution of nuclear decay of precursors and degradation of mature forms in the cytoplasm is also not known [142], with most of our knowledge of tRNAs decay resulting from studies of defective tRNAs.

*S. cerevisiae* tRNAs lacking certain modifications may be susceptible to rapid tRNA degradation (RTD) conducted by nuclear Rat1p and cytoplasmic Xrn1p [143] (Fig. 3A). Structurally unstable tRNAs and tRNA-like small RNAs can be degraded by an alternative pathway: tRNA nucleotidyltransferase can add CCACCA sequence instead of the canonical CCA triplet, which results in rapid degradation due to cooperation between Dis3p and Xrn1p [144] (Fig. 3A). Localization of these mechanisms is not well defined, because (as mentioned earlier) yeast Xrn1p is mostly cytoplasmic, but observed also in the nucleus [108]. Additionally, uridylation may also be a mechanism for decay, as was shown for mammalian mascRNA, which is an ncRNA with tRNA-like

structure [145]. tRNA molecules in many organisms are also a source of a variety of shorter transcripts, resulting from cleavage, known as tRNA-derived RNA fragments (tRFs) [139]. These are sometimes highly abundant, and while their biogenesis, function and implications in diseases has been studied, little is known about their degradation. However, work done in Vanacova's laboratory suggests that uridylylated tRFs might be DIS3L2 substrates in human cells (Ustianenko et al., 2016).

#### 3.2. Ribosomal RNA (rRNA)

rRNA biogenesis is a very complex process, consuming a considerable proportion of cellular energy, with mature rRNAs constituting approximately 80% of total cellular RNA. rRNA molecules are very stable and degraded only in certain conditions, as shown in *S. cerevisiae*, where rRNAs with mutations at bases important for translation show decreased stability, while degradation of wild-type rRNA is undetectable [146]. This is a component of the eukaryotic quality control system termed "nonfunctional rRNA decay" (NRD) that ensures produced ribosomes are functional and can be linked to removal of non-functional rRNA associated with disease in human [147]. Despite similar functions, elimination of 18S rRNA with a faulty decoding site (18S NRD) and 25S rRNA harbouring a mutated peptidyl transferase center (25S NRD) use two distinct processes in *S. cerevisiae* (Fig. 3B). 18S NRD utilizes Dom34p/Hbs1p, the same protein complex used in NGD/NSD, thus coupling these quality control mechanisms [148] (Fig. 3B). The exosome core contributes to both 18S and 25S NRD, while Ski7p and Xrn1 only contribute to 18S NRD [148] (Fig. 3B). It is not established how the exosome is recruited to the 25S NRD substrate, taking into account that Ski7p is normally indispensable for it. In addition, 25S NRD is independent of translation elongation, unlike NSD/NGD/18S NRD, and occurs before the 60S subunit is engaged in translation [148].

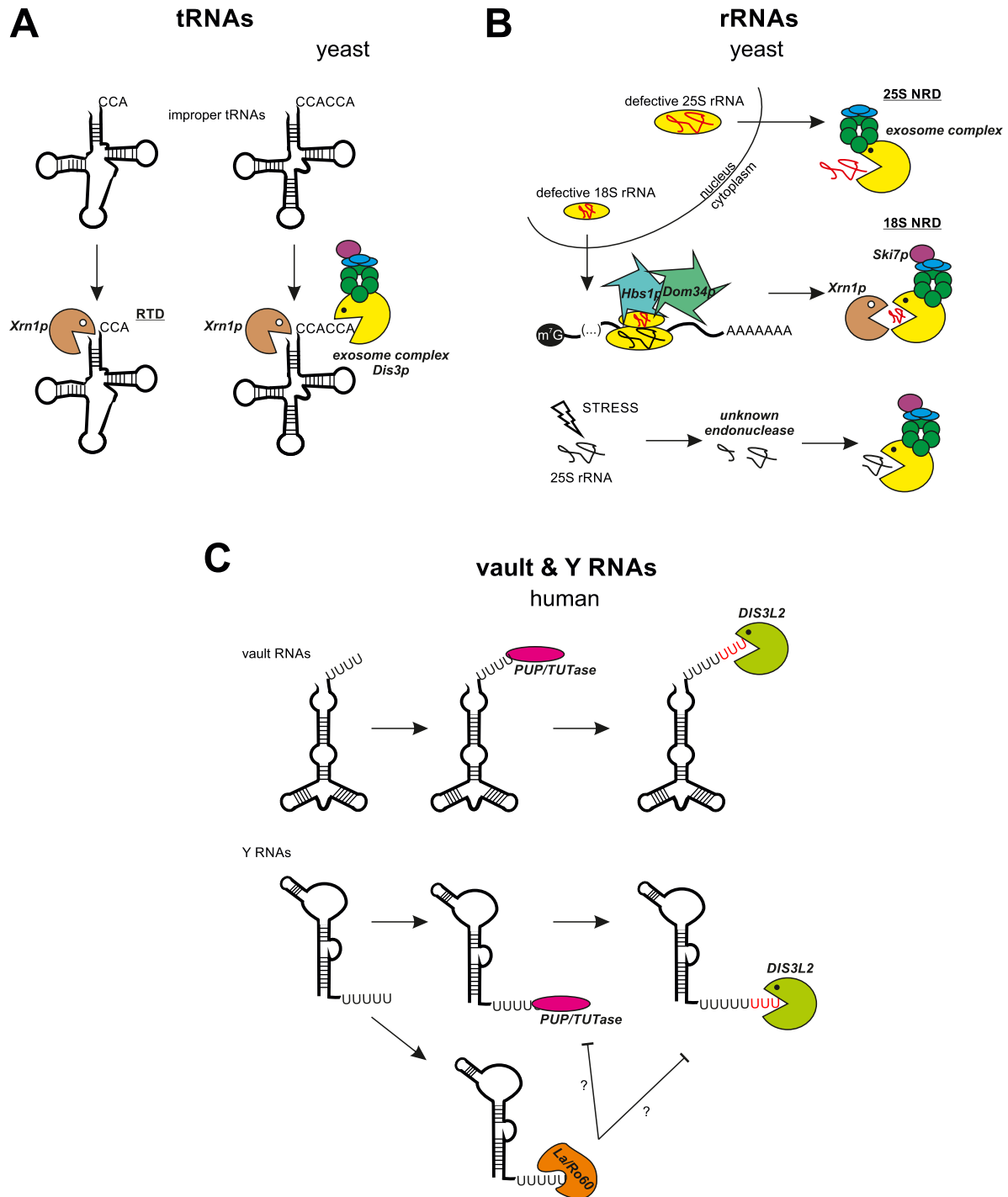
Mature yeast ribosomes may be also a target of selective autophagy, coined "ribophagy", wherein ribosomes are engulfed in vesicles and transported for degradation to vacuoles upon starvation [149], the process that was also shown to occur in plants during rapid cell growth [150] and in mammalian cells [151]. Additionally, rRNA was shown to be degraded through endonucleolytic cleavage with the help of the exosome, during apoptotic stress in yeast [152] (Fig. 3B). Despite mentioned reports, relationships between NRD, ribophagy and stress-induced rRNA degradation require further elucidation.

#### 3.3. Vault RNA and Y RNA

Vault and Y RNAs are products of polymerase RNA III, whose functions in cells are not yet fully established, nevertheless these transcripts have been associated with several biological processes.

Vault RNAs are short ( $95 \pm 20$  nt) ncRNAs, encoded in humans by four genes (*vtRNA1-1*, *vtRNA1-2*, *vtRNA1-3* and *vtRNA2-1*), as well as one additional vault pseudogene, with fraction (~20% of the total population) associated with proteins forming so-called vault particles, while the rest distributed mainly in the cytoplasm, however with possible nuclear localization in some cases [153]. Although the sequences of vault RNAs are not highly conserved between species (except RNA polymerase III promoter elements) and they may differ substantially in length, their overall secondary structures are similar [153].

Y RNAs are similarly short RNA molecules ( $100 \pm 20$  nt). There are four Y RNAs (Y1, Y3, Y4 and Y5) in humans (expression from Y pseudogenes was also reported), with approximately half of the Y RNA population forming RNPs complexes with Ro60, La and several other proteins, that function in RNA stability and quality control (reviewed in [154]). While Y RNAs were initially discovered in the cytoplasm (Y comes from "cytoplasmic"), and this localization was further confirmed by numerous studies, it is now known that Y

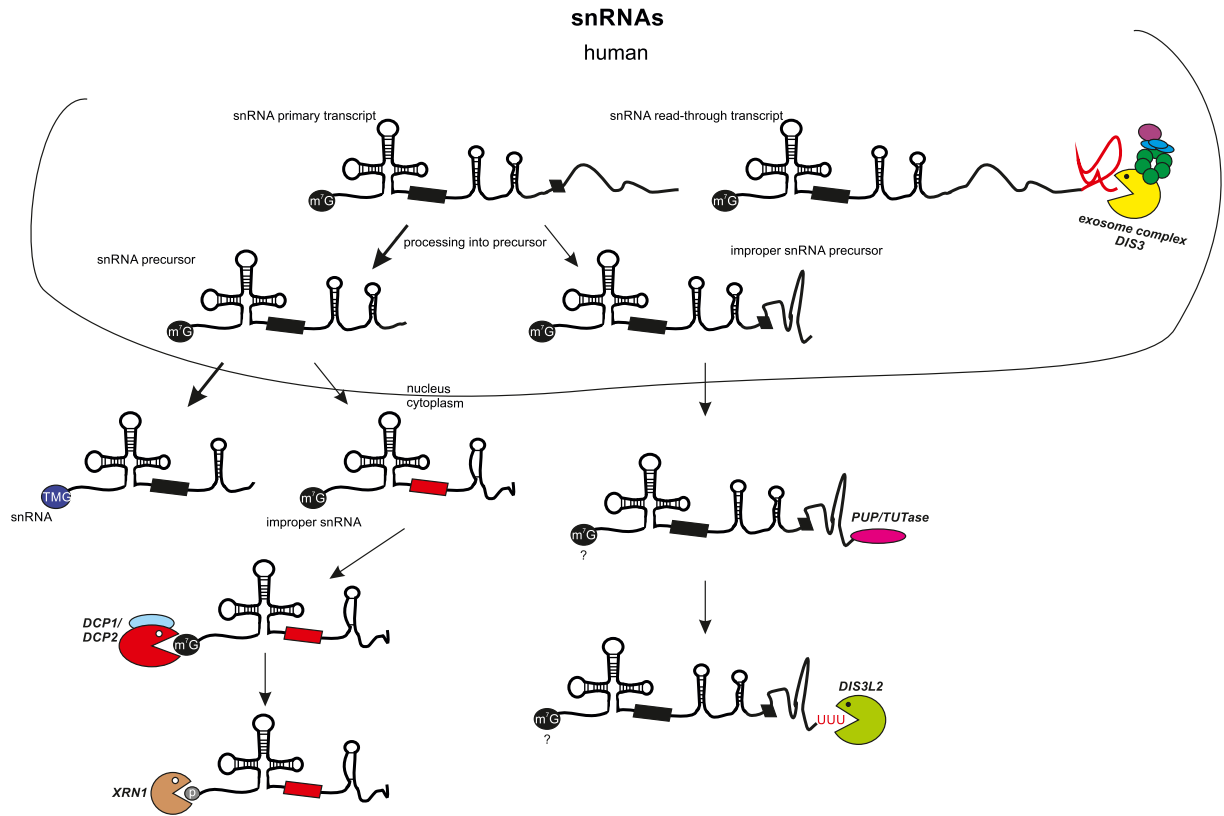


**Fig. 3.** Cytoplasmic pathways of tRNA (A), rRNA (B), vault RNA and Y RNA (C) degradation. See Sections 1.2.1, 1.2.2, and 1.2.3 for details, respectively. A – aberrant yeast tRNAs can undergo RTD (rapid tRNA decay), or be removed followed addition of CCACCA sequence by tRNA terminal nucleotidyltransferase. B – defective rRNAs (18S and 25S) are degraded in yeast via respective, distinct, NRD (non-functional rRNA decay) mechanisms; in addition, stress-induced endonucleolysis can also initiate rRNA decay. C – vault and Y RNAs are short, structured, human transcripts, degraded most likely by uridylation-stimulated DIS3L2 exonucleolytic activity; La and Ro60 proteins possibly exert inhibitory effect on the decay process.

RNAs are present in both the nuclear and cytoplasmic compartments [154]. Y RNAs are conserved among vertebrates and similar ncRNAs are also present in other metazoans such as insects and nematodes [154].

Apart from being products of polymerase III and existing as parts of RNPs, vault and Y RNAs share more common features such as strong secondary structures [153,154], and further processing to smaller fragments, namely svRNAs and YsRNAs [139].

Little is known about the degradation mechanism of either vault or Y RNAs. Ro60 and its homologues were shown to influence Y RNA accumulation [155], which suggests that they may protect Y RNAs from degradation; La protein was reported to exert a similar effect [156] (Fig. 3C). Very recently, results of both *in vitro* and *in vivo* experiments from our group implicated that a mechanism based on uridylation is involved in their decay in human cells [114] (Fig. 3C). TUTase-4 and TUTase-7 are engaged in uridylation of these transcripts,



**Fig. 4.** Quality control of human snRNA biogenesis. See Section 1.2.4 for a more detailed description. snRNA read-through transcripts can be degraded by the exosome/DIS3 already in the nucleus. snRNAs defective in snRNP formation are degraded in the cytoplasm by decapping/XRN1 5'-3' pathway. 3'-extended snRNA precursors may escape from the nucleus to the cytoplasm, where they are removed by DIS3L2, following uridylation. m7G – monomethylated cap; TMG – hypermethylated cap.

while DIS3L2 was identified as an enzyme involved in their degradation (Fig. 3C). Nonetheless, it is currently unknown if vault- and Y RNA-derived fragments are also DIS3L2 substrates.

### 3.4. Small nuclear RNA (snRNA)

As their name suggests, small nuclear RNAs are predominantly localized to the nucleus, where they participate mainly in splicing reactions (however there are also snRNAs with different functions, like U7 snRNA, which functions in histone pre-mRNA processing). Nevertheless, following transcription from snRNA loci in the nucleus, in most organisms (with exception of *S. cerevisiae* and *Trypanosomes*) the transcripts are exported to the cytoplasm, where they associate with the survival motor neuron complex (SMN), that orchestrates additional modifications of snRNA transcripts such as cap hypermethylation and trimming (reviewed in [157]), leading to formation of snRNPs. Quality control pathways for snRNAs defective in snRNP formation exist, and such snRNAs were shown to be degraded in the nucleus and cytoplasm (Dcp2p-Xrn1p) in *S. cerevisiae*, and in the cytoplasm (DCP2/DCPS-XRN1) in mammals [158] (Fig. 4). Furthermore, additional surveillance pathways are present, at least in human cells, as results from our group indicate that improperly processed snRNA precursors undergo DIS3L2-mediated degradation in an uridylation-dependent

manner in the cytoplasm [114] (Fig. 4). Additionally, incorrectly terminated transcripts from snRNA loci (“read-through snRNAs”), which are normally degraded by DIS3 (Fig. 4), may also escape to the cytoplasm [114].

### 3.5. Pre-microRNA (pre-miRNA) and microRNA (miRNA)

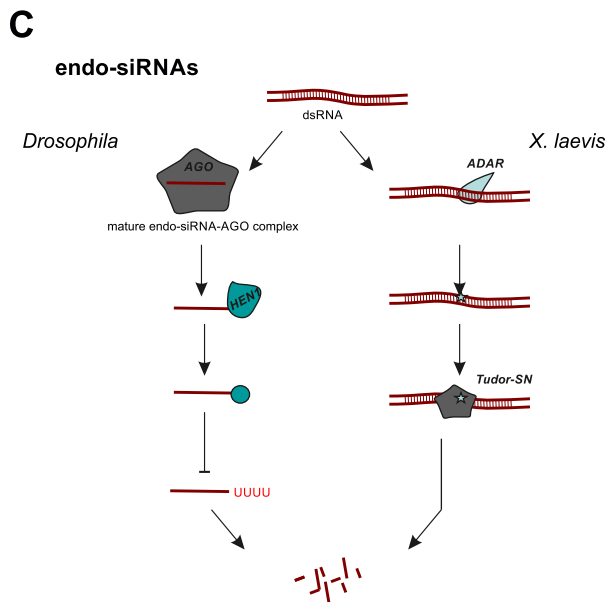
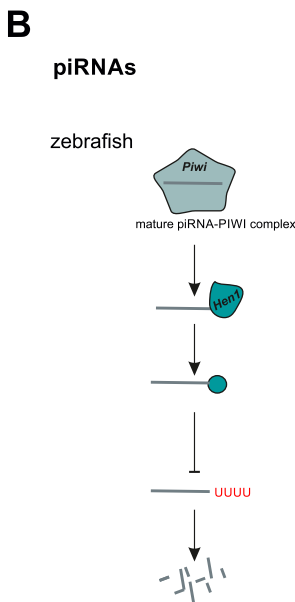
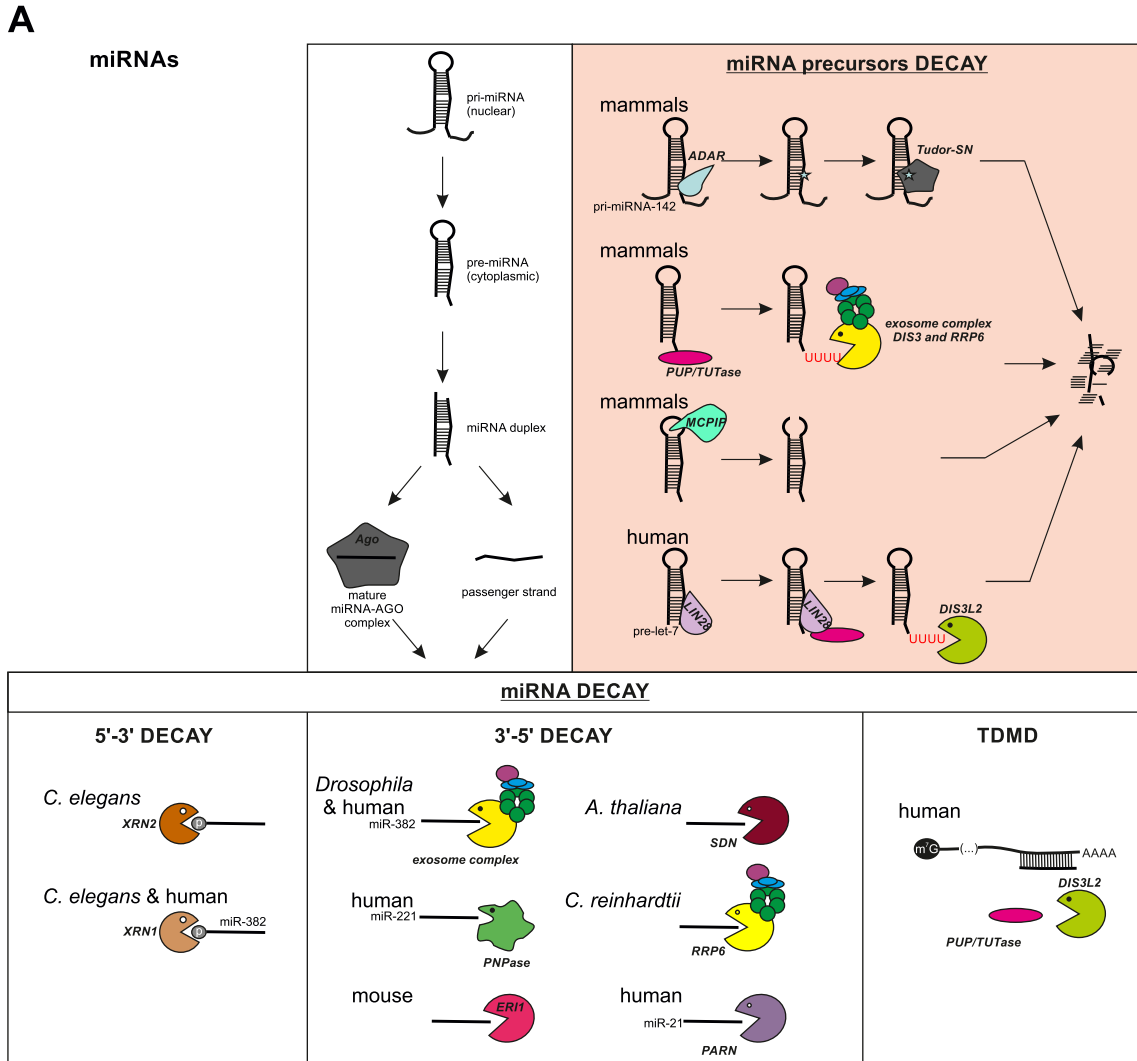
MicroRNAs, exerting potent post-transcriptional control of gene expression, undergo a complicated biogenesis, resulting in production of intermediate transcripts, such as primary miRNA (pri-miRNA), precursor miRNA (pre-miRNA) and, eventually, miRNA-duplexes (Fig. 5A). In light of their important functions, levels of these molecules are precisely controlled, including post-transcriptional level. Among the above-mentioned intermediates of miRNA biogenesis pathways, pre-miRNAs and miRNA-duplexes localize to the cytoplasm, while pri-miRNA are normally present in nucleus, however in mammals they may escape to cytoplasm and be targeted by adenosine deaminases that act on RNA (ADARs), which results in their modification and degradation by Tudor staphylococcal nuclease (Tudor-SN), a subunit of the RISC complex [159] (Fig. 5A).

Pre-miRNAs are products of Drosha-mediated cleavage, resulting in stem-loop structures of approximately 70 nt in length. In a recent paper,

**Fig. 5.** Decay pathways for pre-miRNAs and miRNAs (A), piRNAs (B) and endo-siRNAs (C). See Sections 1.2.5, 1.2.6, and 1.2.7 for details, respectively. A – miRNA biogenesis is a multi-step pathway, beginning with the synthesis of pri-miRNAs, which are processed to pre-miRNAs in the nucleus. Pre-miRNAs are then exported to the cytoplasm and further processed to miRNA duplexes. Each of the miRNA biogenesis intermediates can undergo degradation in the cytoplasm, since pri-miRNAs are known to escape to the latter compartment. Such pri-miRNAs can be degraded by Tudor-SN, following modification by ADAR. In turn, pre-miRNAs are known to be degraded by exosome/DIS3/RRP6 in conjunction with uridylation, or undergo MDC1P endonucleolytic cleavage. Pre-miRNAs from let-7 family are oligouridylated by TUTases with the help of LIN28, and subsequently degraded by DIS3L2. Mature miRNAs can be degraded from both 5'-end (by enzymes from XRN family) and 3'-end (by various exonucleolytic activities, including exosome, RRP6, PARN, ERI1, PNPase, SDN), depending on the organism and miRNA species. In human cells, target-mediated miRNA degradation (TDMD) path, dependent on uridylation and DIS3L2 activity, was discovered. B – piRNAs are protected from degradation through association with Piwi proteins, as well as owing to Hen1-mediated 2'-O-methylation of the 3'-terminus; piRNAs lacking this modification undergo uridylation and degradation by an unknown exonuclease. C – endo-siRNAs degradation is controlled similarly to piRNAs; in addition, dsRNA being the source of endo-siRNAs can be modified by ADAR and thus targeted to degradation by Tudor-SN.

mammalian pre-miRNAs were found to undergo degradation by the exosome subunits RRP6 and DIS3, in cooperation with cytoplasmic TUTases (TUTase-4/7) (Fig. 5A), despite that the main localization of these nucleases is nucleolar and nuclear, respectively [160]. Further-

more, current knowledge strongly suggest that uridylated pre-miRNA should be degraded by DIS3L2 in the exosome-independent manner, while DIS3 and RRP6 may degrade nuclear pool of precursors. Further research is needed to resolve these issues.



Degradation of pre-miRNAs can have a significant physiological influence, as shown by two examples. The first example concerns mammalian MCPIP1 endoribonuclease, which cleaves the loop of pre-miRNA, rendering it inaccessible for further processing [161] (Fig. 5A). The second example regards let-7 pre-miRNA, biogenesis of which is precisely controlled, as miRNAs from the let-7 family exert tumor suppression functions and are necessary for stem cell renewal. It was shown in mammalian cells [111,117] that LIN28 recruits TUTase-4 (ZCCHC11) and TUTase-7 (ZCCHC6), which results in let-7 pre-miRNA oligouridylation (Fig. 5A), attracting DIS3L2 and stimulating decay of the precursor transcript, thus inhibiting mature let-7 biogenesis (Fig. 5A). Similarly, stability of let-7 in *C. elegans* is regulated in an uridylation-dependent manner [162].

miRNA-duplexes consist of two strands (a guide and a passenger; usually the former exerts activity, while the latter is degraded), that are loaded into Ago proteins forming RISC complex, and must be separated in order to function in silencing. Unwinding of the strands can occur through either Ago2-mediated cleavage-dependent or cleavage-independent mechanisms [163], resulting in displacement of the passenger strand that is then vulnerable to degradation in the absence of RISC protection. On the other hand, guide strands, protected by RISC, are generally believed to be very stable molecules, with average half-lives of miRNAs in mammals as long as 119 h (~10-fold longer than typical mRNAs). However, there are examples of miRNAs with short half-lives, which may be a result of specific miRNA decay, depending on developmental and cellular context, and miRNA stability may also be controlled by nucleotide additions/modifications, specific intrinsic destabilizing sequence elements and interaction with additional proteins (reviewed in [164]). For example, mouse miR-122 is stabilized through adenylation by the cytoplasmic poly(A) polymerase GLD-2, a TUTase-2 homologue [165], while uridylation of miRNA 3'-ends in algae stimulates their degradation *in vivo* [132]. Normally, plant miRNAs are substrates of the HEN1 enzyme, introducing 2'-O-methylation at their 3' end, which blocks uridylation and therefore stabilizes the miRNAs [166]. Influence of tailing on overall miRNAs stability may be indirect, in that modified miRNA may exhibit altered RISC incorporation properties [167], which may expose them to ribonucleases more or less frequently.

Mature miRNA strands were shown to be degraded in the 5'-3' direction by XRN2 in *C. elegans* [168], or by XRN1 in human cells [169] and in *C. elegans* [170] (Fig. 5A). They are also degraded in the 3'-5' direction through the activities of the exosome in humans [169] and *Drosophila* [171], polynucleotide phosphorylase (PNPase) in human cells [172], ER11 in mouse [173], small RNA degrading nucleases (SDNs) in *Arabidopsis* [174], RRP6 in *C. reinhardtii* [132], and PARN in human cells [175] (Fig. 5A). Importantly, most ribonucleases target only a small subset of miRNAs, pointing to specificity of these decay mechanisms and furthermore, there are conflicting reports regarding the role of target recognition by miRNAs on their stability. miRNAs could be either protected from degradation ("target-mediated miRNA protection" TMMP [170]) or degraded ("target-mediated miRNA degradation" TDMD [176]), depending on the stoichiometry and degree of complementarity between miRNAs and mRNAs. Very recently, DIS3L2 and probably TUT1, were shown to play an important role in the TDMD mechanism in mammals [78] (Fig. 5A). In this particular case, complete complementarity of miRNA with the target induced uridylation and subsequent DIS3L2-mediated degradation of miRNA.

In conclusion, there are multiple ways of mature miRNAs decay, as well as degradation mechanisms for their different precursor forms, acting together with transcriptional regulation to control the levels of these small molecules. Nevertheless, there is still an area for future research to uncover the relationships between these pathways and to resolve the issue of conservation of multiple enzymes operating in different studied organisms.

### 3.6. PIWI-interacting RNA (piRNA)

piRNAs are small non-coding RNAs, expressed predominantly in animal gonads and functioning primarily in maintaining genome integrity through repression of repetitive elements by transcriptional and posttranscriptional silencing mechanisms. They are slightly longer than miRNAs (24–31 nt), and their name is derived from the fact of their association with Piwi proteins (Fig. 5B), which belong to the Argonaute family that includes Ago proteins associating with miRNAs and endo-siRNAs in RISCs. piRNA biogenesis differs between species, in mammals beginning with transcription of a piRNA cluster by RNA polymerase II, although some piRNAs originate from 3'-UTRs of protein-coding transcripts [177]. After initial processing in the nucleus, piRNA precursors are exported to the cytoplasm and cleaved to form piRNA intermediates, which are subsequently loaded into PIWI proteins, where they undergo further trimming and modifications forming mature piRNAs, which additionally may be amplified during a process known as the ping-pong cycle [177]. Compared to miRNA, much less is known about degradation of piRNAs and their precursors. The only well-studied degradation mechanism involves the Hen1 enzyme, which methylates the 3'-end of mature piRNAs in a manner similar to plant miRNAs [178] (Fig. 5B). In zebrafish, this modification was shown to protect piRNAs from uridylation that would result in their degradation by a yet-unknown 3'-5' exoribonuclease [179] (Fig. 5B). Methylation of piRNAs is widely conserved in the animal kingdom, as it was revealed that loss of Hen1 results in reduced piRNA levels not only in zebrafish [179], but also in other species [178]. Furthermore, it was shown that, like miRNAs protected by RISC, piRNA transcripts are stabilized by binding to PIWI in mouse [180] and *C. elegans* [181].

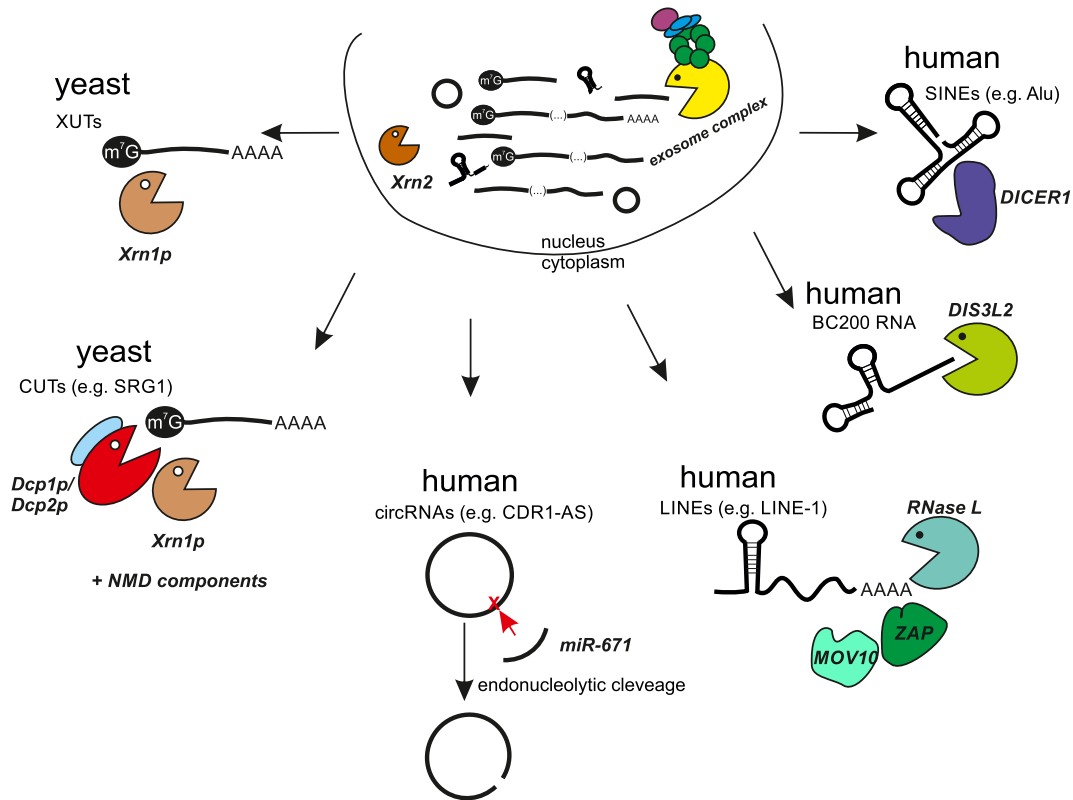
### 3.7. Endogenous small-interfering RNAs (endo-siRNAs)

Endogenous small-interfering RNAs are a class of small ncRNAs, which is similar to miRNAs in terms of size and binding to Ago proteins, but in contrast to miRNAs, endo-siRNAs are generated from intrinsic, long, double-stranded transcripts (dsRNAs) (Fig. 5C). Endo-siRNAs function in the regulation of gene expression and protection from endogenous and exogenous transcripts, and have been identified in *Arabidopsis*, *Drosophila*, *C. elegans* and mouse. In mammals, their biogenesis starts with long dsRNAs, which are further processed to shorter double-stranded RNAs, exported to the cytoplasm and loaded onto Ago proteins, thus forming RISCs. These small RNAs are the least well studied, in comparison to described above miRNA and piRNA, but nevertheless, it was shown that AGO binding stabilizes endo-siRNA in *C. elegans* [182], and that Hen1 homologue participates in maintaining endo-siRNA stability in rice [183] (Fig. 5C). A similar situation was observed in *Drosophila*, where endo-siRNAs in flies lacking Hen1 homologue possess 3'-untemplated nucleotide additions, consisting mainly of uridines [176]. Interestingly, dsRNA can be targeted by ADARs and subjected to extensive covalent modification (hyper-editing), which may result in their cleavage by Tudor-SN (Fig. 5C), as shown in *X. laevis* [184].

Eukaryotes produce plentiful amounts of small RNAs up to 30–40 nt in length, as shown by the results of small RNAs deep profiling conducted in *Drosophila*, presented recently by Wen et al. [185]. In addition to known small RNAs mentioned above (miRNAs, piRNAs, endo-siRNAs), some new classes have been discovered, such as uncharacterized ~28-nt RNAs derived from atypical hairpins produced in the *Ascaris* nematode [186]. In some cases, the traditional distinctions between the small RNAs groups become blurred, as exemplified by *Tetrahymena* scnRNAs interacting with Piwi homologues, which formally defines them as piRNA, but undergoing different biogenesis pathway [187]. Nevertheless, further research is needed not only to characterize the small ncRNA transcriptome, assign biological functions to particular transcripts, but also to reveal their degradation mechanisms.



## products of pervasive transcription



**Fig. 6.** Degradation of pervasive transcription products in eukaryotic cells. See Section 1.2.8 for details. A large fraction of eukaryotic genomes is transcribed, giving rise to a variety of unstable ncRNA species, which are degraded by different mechanisms. While many of them undergo exosome- and/or Xrn2-mediated degradation already in the nucleus, some of them escape to the cytoplasm, where their decay takes place. For instance, degradation of XUTs and CUTs in yeast is dependent on Xrn1p, and decay of the latter may require also decapping enzyme and some NMD factors. CircRNAs (circular RNAs) in human cells do not have free ends, so their degradation is initiated by endonucleolytic cleavage, possibly assisted by miRNA. ncRNAs originating from repetitive DNA elements, such as LINES or SINEs are degraded by distinct, not yet fully explored mechanisms, involving the action of RNase L, MOV10 helicase and ZAP protein (LINES) or DICER1 (Alu family of SINEs). BC200 Alu-related element is in turn degraded with the help of DIS3L2.

### 3.8. XUTs and other cytoplasmic non-coding RNA species resulting from pervasive transcription

Apart from non-coding RNAs with well-defined identities (like Y RNAs) and/or functions (like rRNAs), which are described above, there exist a multitude of other species, for which we have little knowledge about their biological roles. As genomes are pervasively transcribed, there is a need for degradation of arising ncRNAs. Initially, such products were observed in *S. cerevisiae* upon depletion of components of the RNA degradation machinery, because usually these RNAs are undetectable in wild-type cells, as shown by the example of Xrn1-sensitive unstable transcripts (XUTs), which were found to accumulate upon depletion of Xrn1p [188] (Fig. 6). More examples of pervasive transcription products were discovered both in yeast and in higher eukaryotes and many of them are synthesized by RNA polymerase II and, as such, they may be capped and polyadenylated (including antisense and intergenic transcripts, transcripts produced from pseudogenes or spurious initiation sites). In addition, length of these RNAs varies, with traditional nomenclature distinguishing “small” (for transcripts shorter than 200 nt) and “long” non-coding RNAs (lncRNAs) (for molecules longer than 200 nt). Among these, XUTs are thought to be exclusively cytoplasmic, but stability of other ncRNAs expected to be nuclear is also Xrn1-dependent, which suggests that they may escape from nuclear degradation machinery to the cytoplasm and undergo degradation in the latter compartment [188]. Another example of ncRNAs previously considered as exclusively nuclear are transcripts from the *SRG1* locus in yeast, belonging to CUT family (cryptic unstable transcripts) that were found

to be degraded by decapping and 5'-3' degradation by Xrn1p, some with additional help from NMD machinery [189] (Fig. 6). Thus, *SRG1* transcripts constitute an example of cytoplasmically degraded intergenic ncRNA, but there are more RNAs of this kind, as shown by further analysis, indicating that their decay mechanism requires Dcp1p, Xrn1p or NMD components [189] (Fig. 6). As the NMD process necessitates translation, and some ncRNAs were found to be associated with polyribosomes, they may enter translation, providing new mechanisms for protein evolution [189]. Different groups confirmed the finding that cytoplasmic pathways play a role in the degradation of ncRNAs in several organisms [190,191].

An interesting category of transcripts resulting from pervasive expression comprises RNA molecules originating from repetitive DNA elements, which is especially interesting given that up to two-thirds of the human genome consists of repeated sequences of various types, but mostly dominated by transposable elements. A recent study reported on the transcriptional landscape of repetitive elements in human cells [192], showing that transcripts derived from Short Interspersed Nuclear Elements (SINEs) and Long Interspersed Nuclear Elements (LINES) are present in the cytoplasm. Human representatives of SINE retrotransposons are members of the Alu family, some of which were shown to be expressed and degraded by DIS3 [12]. Additionally, Kaneko et al. reported that DICER1 (a protein engaged in small RNA biogenesis) participates in the degradation of RNA from specific Alu repeats in human retinal pigmented epithelium [193] (Fig. 6) and another group demonstrated that autophagy contributes to SINE and LINE retrotransposon RNA degradation [194]. One Alu-related element

which is not only expressed, but also has an established biological function, is a primate-specific BC200 (Brain Cytoplasmic RNA, 200 nt) and its rodent counterpart, BC1 (Brain Cytoplasmic 1 RNA, 152 nt). These transcripts are most abundant in neurons, however our group found that BC200 accumulates in HEK293 cells upon DIS3L2 mutation, suggesting that BC200 is DIS3L2 target [114] (Fig. 6). Remarkably, Alu sequences may serve as an origin of smaller transcripts, namely small cytoplasmic Alu (scAlu), and results from our laboratory indicate that they may be in turn subjected to DIS3-mediated degradation [114]. Regarding LINE transposons, which were shown to be capable of active retrotransposition, human LINE-1 RNA was found within stress granules together with MOV10 helicase [195] and zinc-finger antiviral protein (ZAP) [196] (Fig. 6), as well as it was reported that their exogenous expression resulted in LINE-1 transcript downregulation by yet unknown mechanisms. Additionally, expression of RNase L also reduced LINE-1 RNA levels [197] (Fig. 6).

Circular RNAs (circRNAs) are a specific class of RNAs belonging to a larger group of lncRNAs, with characteristic feature consisting in a linkage between 5' and 3' ends through a covalent bond. These RNAs are abundant, present in different species (*A. thaliana*, *C. elegans*, *D. melanogaster*, and *H. sapiens*), sometimes conserved, and often expressed in a cell-specific manner. Because of an absence of free 5' and 3' ends, they do not undergo exonucleolytic degradation, which may explain their increased stability [198]. Nevertheless, exogenous siRNA delivery resulted in their decay [198] and one study stated that miRNA action (miR-671), resulting in endonucleolytic cleavage is involved in the degradation of a circular antisense RNA (CDR1-AS) in HEK293 cells [199] (Fig. 6). A different report suggested that circRNAs may be eliminated from cells via extracellular vesicles [200], but otherwise little is known about circRNA degradation.

#### 4. Outlook

RNA degradation systems are ubiquitous and present in all living organisms, as their activity guarantees removal of transcripts that are no longer needed and may even be harmful for cell homeostasis. Despite many significant differences in cytoplasmic degradation pathways between experimental systems, enlightened in this review, there are some common principles, beginning with three major types of degrading enzymes – namely 3'-5' exoribonucleases, 5'-3' exoribonucleases and endoribonucleases, which have been found in all eukaryotes. Additionally, the idea of protection of RNA ends also seems to be conserved between species, as is also the obligation for decay activation, which is conducted by many auxiliary factors, including deadenylases and decapping machinery (as only dysfunctional/unwanted RNAs have to be degraded). Similarly, RNA modifying enzymes such as poly(A) or poly(U) polymerases are found in majority of organisms, and their activities exert potent effect on regulation of RNA turnover. Another conserved and prevailing feature of the RNA decay systems is their efficiency, proved by existence of many ncRNA species detectable only when functions of the degradation apparatus are disturbed. The importance of the RNA decay process itself is further illustrated by multiplicity of enzymes able to catalyze the same reaction, and different levels of functional redundancy between them. It would be of high interest to identify cell- or tissue-specificity of degradation machinery, and look for distinct substrates for any given enzyme engaged in RNA decay. This is exemplified by metazoan and higher plant genomes, which encode various differentially expressed catalytic subunits of deadenylases, as well as multiple non-canonical poly(A) and poly(U) polymerases.

Our knowledge of RNA decay pathways in the cytoplasm of eukaryotic cells has increased significantly in recent years mostly due to substantial technological advances, which were particularly crucial for analysis of RNA metabolism in higher eukaryotes. The

role of uridylation itself has begun to emerge only recently, and with novel approaches such as TAIL-seq it is now possible to investigate other 3'-terminal RNA modifications, for which we have now limited information (e.g. guanylation), in different model systems.

While in the case of mRNAs we have pieced together several mechanisms involving individual enzymes or complexes, what is missing is detailed knowledge of the contributions of different pathways at the level of the whole mRNA transcriptome. This is especially true for higher eukaryotes, for which a large fraction of available data is based on siRNA depletions. Analysis of CRISPR-generated knockouts combined with deep sequencing approaches will help to cover this gap. Additionally, it would be crucial to understand how these complicated networks change depending on environmental conditions, developmental stage or other factors, as well as at both cellular and organismal level.

In contrast to mRNAs, much less is known about the decay of non-coding RNA species, with investigated pathways summarized in this review. Some of ncRNAs, such as tRNA and rRNA molecules are extremely stable, which makes analyses of their decay quite difficult and only quality control or stress-induced degradation pathways have been described in some detail. Less stable RNA species, such as pervasive transcription products, were discovered only recently and we are just starting to understand their biogenesis and possible functionality. Further research is clearly needed to comprehensively understand the regulation of their stability as – for instance – the decay mechanisms of circular RNA are enigmatic. In the case of miRNA and their precursors, different decay pathways have been suggested for each model system studied. It would be of great interest to resolve the issue whether the major, conserved degradation pathway exists that is common for many miRNAs in distinct organisms, or the decay mechanisms for different miRNA species or subfamilies have diversified during evolution. Not much information about RNA turnover has been provided to date also for other small ncRNAs, including well-known ones (i.e. piRNAs), not to mention other, more recently described, ncRNA classes. Finally, there are ncRNAs transcribed by RNA polymerase III, such as Y and vault RNAs, which have been lately found to be degraded in humans by DISL3L2 in an uridylation-dependent manner, but it remains to be established how this degradation is conducted in other species, as well as to identify decay pathways for other RNA polymerase III products. In addition, some transcripts (e.g. tRNAs or vault RNAs) can also be processed into smaller RNAs fragments and no information about their degradation is currently available.

Additionally, the topic of interconnection between different stages of gene expression poses a significant challenge for future studies. Even though association between RNA degradation and translation is underlined by multiple examples mentioned in this review, relationships with transcription are much more enigmatic and controversial.

Although structures of many factors involved in RNA degradation have been already solved, for others, including ones that have been newly identified, as well as whole multiprotein assemblies, architecture is unknown, while only resolving this issue would give us detailed insights into mechanisms of their action and shed light on the evolution of degradation machinery.

In this review we referred to diseases connected with RNA metabolism only occasionally, nevertheless this is topic of significant importance, as the contribution of impaired RNA degradation to development of human disorders is widely known. Moreover, studies in this research area may result in medical progress with the benefits for the patients. In aggregate, although the major RNA decay factors and their biochemical activities have been established, comprehensive knowledge about mRNA and ncRNA homeostasis in the cytoplasm of eukaryotic cells will be a focus of study in the future, paving the way for novel therapeutic approaches.

## Glossary

ARE	<i>adenylate-uridylate-rich (AU-rich) element</i> ; regions with frequent adenine and uridine found in 3' UTR of mRNAs, determining their stability	rRNA	<i>ribosomal RNA</i> ; type of ncRNA, component of ribosome
BC1	<i>brain cytoplasmic 1 RNA</i> ; type of ncRNA, expressed mainly in mice neuronal cells	SINE	<i>short interspersed nuclear element</i> ; type of interspersed repeats present in the genome, belonging to nonautonomous transposons (not coding protein needed for their transposition)
BC200	<i>brain cytoplasmic RNA, 200 nt</i> ; type of ncRNA, expressed mainly in human neuronal cells	snRNA	<i>small nuclear RNA</i> ; type of ncRNA involved primarily in splicing
circRNA	<i>circular RNA</i> ; type of ncRNA with linked 5' and 3' ends	snRNP	<i>small nuclear ribonucleoprotein</i> ; RNP with snRNA as a RNA component
CUT	<i>cryptic unstable transcript</i> ; type of ncRNA resulting from pervasive transcription in yeast, similar to XUT, but typically degraded by different mechanism	tRF	<i>tRNA-derived RNA fragment</i> ; type of ncRNA arising from tRNA
EDC	<i>enhancer of decapping</i> ; protein that enhances process of decapping	tRNA	<i>transfer RNA</i> ; type of ncRNA involved in translation through delivering amino acids for protein synthesis
endo-siRNA	<i>endogenous small-interfering RNA</i> ; type of small ncRNA typically 20–23 nt in length, generated from double-stranded transcripts, functioning both in regulation of gene expression and repression of transposable elements	TUTase	<i>terminal uridylyl transferase</i> ; type of enzyme transferring uridines onto 3' end of target transcript
eRF	<i>eukaryotic release factor</i> ; type of a protein participating in the termination of translation	UTR	<i>untranslated region</i> ; region of mRNA that is typically not translated
miRNA	<i>microRNA</i> ; type of small ncRNA typically 21–24 nt in length, generally generated from primary miRNA transcripts and functioning in regulation of gene expression		
mRNA	<i>messenger RNA</i> ; protein coding RNA		
mRNP	<i>messenger ribonucleoprotein</i> ; RNP with mRNA as an RNA component		
ncRNA	<i>non-coding RNA</i> ; non-protein-coding RNA		
NGD	<i>no-go decay</i> ; mRNA surveillance mechanism, typically resulting from the ribosome stalling		
NMD	<i>nonsense-mediated decay</i> ; mRNA surveillance mechanism, typically resulting from the presence of premature termination codons		
NRD	<i>nonfunctional rRNA decay</i> ; rRNA surveillance mechanism		
NSD	<i>non-stop decay</i> ; mRNA surveillance mechanism, typically resulting from the lack of in-frame stop codon		
LINE	<i>long interspersed nuclear element</i> ; type of interspersed repeats present in the genome, belonging to autonomous transposons (encoding protein needed for their transposition)		
lncRNA	<i>long non-coding RNA</i> ; type of ncRNA typically longer than 200 bp		
ORF	<i>open reading frame</i> ; the sequence of nucleotides predicted to code for a protein or a peptide		
PAP	poly(A) <i>polymerase</i> ; type of enzyme synthesizing adenine tracts		
P-body	<i>processing body</i> ; foci present in the cytoplasm of the eukaryotic cell containing enzymes involved in mRNA degradation		
piRNA	<i>PIWI-interacting RNA</i> ; type of small ncRNA typically 24–31 nt in length, interacting with PIWI proteins and generally functioning in maintaining genome stability through repression of transposable elements		
PROMPT	<i>promoter upstream transcript</i> ; type of ncRNA resulting from pervasive transcription in human cells (bidirectional transcription from promoter region)		
PUP	poly(U) <i>polymerase</i> ; type of enzyme synthesizing uridine tracts		
XUT	<i>Xrn1-sensitive unstable transcript</i> ; type of ncRNA resulting from pervasive transcription in yeast, typically degraded by Xrn1 enzyme		
RACE	<i>rapid amplification of cDNA ends</i> ; PCR-based molecular biology technique for revealing cDNA ends		
RISC	<i>RNA-induced silencing complex</i> ; multiprotein RNP complex with endo-siRNA or miRNA as an RNA component, used to recognize target RNA during silencing process		
RNP	<i>ribonucleoprotein</i> ; complex of proteins and RNA		

## Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

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