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Review

RNA recognition by 3′-to-5′ exonucleases: The substrate perspective

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Abstract

The 3′-to-5′ exonucleolytic decay and processing of a variety of RNAs is an essential feature of RNA metabolism in all cells. The 3′–5′ exonucleases, and in particular the exosome, are involved in a large number of pathways from 3′ processing of rRNA, snRNA and snoRNA, to decay of mRNAs and mRNA surveillance. The potent enzymes performing these reactions are regulated to prevent processing of inappropriate substrates whilst mature RNA molecules exhibit several attributes that enable them to evade 3′–5′ attack. How does an enzyme perform such selective activities on different substrates? The goal of this review is to provide an overview and perspective of available data on the underlying principles for the recognition of RNA substrates by 3′-to-5′ exonucleases.

Keywords: Exonuclease; 3′-to-5′ exonuclease; Exosome; RNA decay; RNA stability; RNA processing

1. Introduction

Exoribonuclease activities play major roles in both prokaryotic and eukaryotic cells. First, exonucleases are vital for many pathways of RNA decay. RNA levels are determined not only by their rate of synthesis, but also by the rate of degradation. Thus, RNA turnover rates are an integral component of the control of gene expression. Importantly, 3′-to-5′ exonucleases (3′ exos) play both general and regulated roles in RNA decay. Second, aberrant RNA molecules are inevitably generated during transcription and processing. Several 3′ exos are essential to the removal of these aberrant transcripts from the cell. Finally, transcription does not terminate precisely at the 3′ end of a transcript, thus 3′ processing is essential to the maturation of both mRNAs and non-coding RNAs. 3′ exos have been implicated in many such processing events.

Not surprisingly, given the destructive activity of these enzymes, they experience various regulatory restrictions. Most will recognize only single-stranded 3′ RNA ends, others have sequence specificity, and often stimulatory co-factors are closely associated with the core enzymatic activity. In this review, we will discuss the various characterized 3′-to-5′ exonuclease activities, their substrate specificities and regulation. Given that the exosome, a large complex of exoribonucleases present in archaea and eukaryotes, is perhaps the most prolific 3′-to-5′ exonuclease activity known, we will devote a significant proportion of our discussion to this enzyme complex.

2. Roles of 3′ exos in eukaryotes

In all three kingdoms of life, the processing and decay of RNA by 3′ exos is an essential pathway. The functions of 3′ exos are surprisingly well conserved, although the factors involved are significantly more complex in eukaryotes.

2.1. RNA processing by 3′ exos

In eukaryotic cells, the vast majority of 3′ exo activity is contributed by the exosome, a complex of nine core and several auxiliary components. Since transcription termination does not occur precisely at the 3′ end of mature transcripts [53,61,63], this activity contributes to the 3′ end trimming/processing of several classes of RNAs in the nucleus. Deletion or mutation of exosome components leads to accumulation of transcripts with extended 3′ ends including 5.8S rRNA, snRNAs (e.g. U4 and U5) and

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snoRNAs (e.g. U14, U18, U24 that are excised from larger precursors) [1,2,68]. Other 3′ exos may also contribute to the 3′ end trimming of 5S, U4 and MRP RNAs [81]. Interestingly, the exosome also contributes to an alternative pathway of 3′ end formation for mRNAs [31,77]. Finally, the exosome contributes directly or indirectly to a variety of RNA processing events. In the pathway of rRNA maturation, for example, exosome mutations directly lead to elevated levels of the 5′ external transcribed spacer region and indirectly to the reduction of cleavage efficiency at a variety of steps in the processing of the large ribosomal RNAs [1,2].

Likewise, in prokaryotes, 3′ exos have been shown to play a role in processing of small stable RNAs, and rRNA. In *Pseudomonas syringae*, RNase R is involved in 3′ trimming of the 16S and 5S rRNAs [62] whilst in *E. coli*, 3′ exos, particularly RNase T and RNase PH, are essential for 3′ maturation of tRNA and other small RNAs [41,42].

Finally, a 3′ exo, 3′hExo, has recently been found associated with histone mRNAs. 3′hExo was initially thought to be involved in degradation of these transcripts [18], but it now seems more likely that it is required for cytoplasmic 3′ trimming [56].

2.2. 3′ exos and mRNA degradation

In eukaryotic cells, the degradation of most mRNAs starts with deadenylation, which is performed by a variety of poly(A)-specific 3′ exos [25]. The mRNA body undergoes degradation either by the exosome (3′-to-5′ pathway) or by cleavage of the 5′ cap followed by 5′-to-3′ degradation by the Xrn1p exoribonuclease (5′-to-3′ pathway) [25]. In addition to its contribution to general mRNA stability, the exosome also contributes to regulated mRNA decay mediated by AU-rich elements [12,55] as well as to quality control of gene expression by degrading defective transcripts, such as mRNAs that have premature translation termination codons [75], or those which lack termination codons altogether [79]. The exosome is also involved in the degradation of the extensive array of cryptic nuclear RNA polymerase II transcripts that have recently been described [50]. Finally, other 3′-to-5′ exonucleases also contribute to specialized mRNA decay mechanisms. For example, the interferon-induced ISG20 3′ exo has antiviral activity [21].

In prokaryotic cells, 3′ exo activity is just as important to mRNA decay. A complex termed the degradosome is integral to the process [10]. The degradosome comprises an endonuclease (RNase E), a helicase (RhlB), enolase, and a 3′ exo (polynucleotide phosphorylase, PNPase). Decay usually initiates with endonucleolytic cleavage, followed by polyadenylation of mRNA fragments by poly(A) polymerase. These fragments are then substrates for 3′-to-5′ decay by PNPase and other 3′ exos [10,13].

2.3. The exosome and RNA interference

There is growing evidence that 3′-to-5′ exonucleases may also play an important and complex role in RNA interference pathways. First, the exosome has been implicated as a means to degrade the RNA fragments generated by RNAi-mediated cleavage [58]. Second, it has been suggested that 3′hExo [18] and its putative *Caenorhabditis elegans* homologue, ERI-1 [35], might play a role in down-regulation of RNA interference by degrading small interfering RNAs (siRNAs) since ERI-1 was identified by genome-wide scanning for mutants of *C. elegans* with enhanced RNA interference. Third, ERI-1 may play a positive role in the RNAi response by interacting with Dicer where it assists in the accumulation of several endogenous siRNAs and regulates the response to exogenous double-stranded RNAs [19]. In this model, ERI-1 binds to short stem-loops of endogenous RNAs and removes unpaired 3′ nucleotides, generating the structure required for synthesis of double-stranded RNA species which can be cleaved by Dicer to initiate the RNA interference cascade [19].

3. Classes of 3′–5′ exonucleases

3′ exos cleave phosphodiester bonds through either a hydrolytic or a phosphorylytic mechanism resulting in production of nucleotide monophosphates or nucleotide diphosphates, respectively. There are four major classes of characterized 3′ exos: the RNR, DEDD, and PDX superfamilies all have members in eukaryotic, archaeal and bacterial kingdoms, whilst the RRP4 family is exclusive to eukaryotes and archaea [92].

3.1. RNR superfamily

The RNAseR/RNAse II 3′ exos are non-specific, highly processive hydrolytic enzymes that bear three putative OB-fold type RNA-binding domains (one S1 domain and two cold-shock domains). Both enzymes have been implicated in decay of bacterial mRNAs. The bacterial RNase R is somewhat unique among 3′ exos in that it can degrade structured RNAs on its own, provided that there is a 3′ single-stranded extension of more than seven nucleotides [83]. RNase II, in contrast, can only degrade single-stranded RNAs [15].

In eukaryotes, the RNR superfamily is represented by Rrp44/Dis3, the catalytic component of the yeast exosome [20,44], and by Dss1, the active subunit of the yeast mitochondrial degradosome [48].

3.2. DEDD superfamily

These hydrolytic enzymes are named for the four invariant amino acids required for activity and are represented by RNase T, RNase D and oligoribonuclease (*orn*) in prokaryotes. The bacterial RNases T and D contribute to 3′ maturation of several small stable RNAs [41,42] whilst *orn* is required for recycling of short oligonucleotide fragments 2–7 nt long generated by other 3′ exo activities [27]. RNase T differs from other 3′ exos in that it can recognize very short single-stranded regions of only 1–2 nucleotides [93]. This makes it the major processing enzyme for several small stable RNAs in *E. coli*.

In eukaryotes there are several different DEDD superfamily 3′ exos with various functions. The Rex proteins and Rrp6/PM-Scl100, like bacterial RNase T, are involved in 3′ maturation events, such as those of 5S rRNA, U4 snRNA and RNase P RNAs [8,80,81]. Rrp6/PM-Scl100 is in fact an essential