Origins and activities of the eukaryotic exosome

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Summary

The exosome is a multi-subunit 3′-5′ exonucleolytic complex that is conserved in structure and function in all eukaryotes studied to date. The complex is present in both the nucleus and cytoplasm, where it continuously works to ensure adequate quantities and quality of RNAs by facilitating normal RNA processing and turnover, as well as by participating in more complex RNA quality-control mechanisms. Recent progress in the field has convincingly shown that the nucleolytic activity of the exosome is maintained by only two exonuclease co-factors, one of which is also an endonuclease. The additional association of the exosome with RNA-helicase and poly(A) polymerase activities results in a flexible molecular machine that is capable of dealing with the multitude of cellular RNA substrates that are found in eukaryotic cells. Interestingly, the same basic set of enzymatic activities is found in prokaryotic cells, which might therefore illustrate the evolutionary origin of the eukaryotic system. In this Commentary, we compare the structural and functional characteristics of the eukaryotic and prokaryotic RNA-degradation systems, with an emphasis on some of the functional networks in which the RNA exosome participates in eukaryotes.

Key words: Exosome function, Exosome structure, RNA exosome

Introduction

When it was first discovered, the RNA exosome was described as an elusive complex consisting of several 3′-5′ exonucleases (Allmang et al., 1999b; Mitchell et al., 1997). The idea that many exonucleases could work together in a single functional unit made it very difficult to understand how the regulation of this complex could be achieved. Today, looking back at ten years of combined functional and structural studies, it is clear that the exosome is a highly organised and regulated macromolecular machine that has only a few enzymatically active components. The new insights provide pieces of a puzzle that promises to teach us not only about RNA processing and degradation in general, but also about how eukaryotic cells regulate the activity and fidelity of gene expression at a very fundamental level. In this Commentary, we compare multi-subunit RNA-degradation complexes in bacteria, archaea and eukaryotes to trace the evolutionary origins of the enzymatic activities of the exosome, the repertoire of which has been expanded by the recent discovery in *Saccharomyces cerevisiae* that the exosome has an associated endonucleolytic activity (Lebreton et al., 2008; Schaeffer et al., 2009; Schneider et al., 2009). We describe how the basic nucleolytic functions found in prokaryotes have evolved to participate in the more complex and compartmentalised pathways that are found in eukaryotic cells. During the course of evolution, some of the exosome components in eukaryotes have become inactive structural subunits. This has resulted in a sophisticated machine that is capable of dealing with a wide range of RNA transactions, more of which are continuously being identified.

Structural organisation of the exosome core

Structure determination of the archaeal and eukaryotic exosomes allowed a detailed analysis of the architecture of the complexes, and showed for the first time that the core forms a ring-shaped structure (Buttner et al., 2005; Liu et al., 2006; Lorentzen et al., 2005). In eukaryotes, the ring consists of three distinct heterodimers built from six different proteins, Rrp41-Rrp45, Rrp46-Rrp43 and Mtr3-Rrp42 (Hernandez et al., 2006; Lehner and Sanderson, 2004; Liu et al., 2006) (Fig. 1). However, the ring of six proteins is not stable on its own in vitro; it requires three additional proteins – Rrp4, Csl4 and Rrp40 – as a cap to form a stable ‘core exosome’ (Liu et al., 2006). In addition to stabilising the complex, the cap proteins contain RNA-binding domains of either the KH (K-homology domain) (Grishin, 2001) or S1 (ribosomal protein S1) type (Suryanarayana and Subramanian, 1984). The archaeal exosome is a simplified version of the eukaryotic complex that only consists of two different proteins, Rrp41 and Rrp42, which are arranged as a homotrimer of heterodimers and capped by a combination of Rrp4 or Csl4 proteins (Buttner et al., 2005; Evguenieva-Hackenberg et al., 2003; Lorentzen et al., 2005) (Fig. 1). The eukaryotic and archaeal ring subunits are related both structurally and by sequence to bacterial RNase PH, an enzyme that uses inorganic phosphate to mediate cleavage of RNA (Deutscher et al., 1988). RNase PH itself forms a ring of six subunits similar to the exosome, and is essential for the 3′ end maturation of transfer RNA (tRNA) molecules in bacteria (Deutscher et al., 1988; Ishii et al., 2003). But unlike the exosome, the RNase PH ring is a homo-hexamer, with neighbouring subunits in an inverted orientation with respect to each other (Fig. 1). Interestingly, a third RNA-processing and -degradation complex known as polynucleotide phosphorylase (PNPase) forms a similar ring structure (Leszczyniecka et al., 2002; Littauer and Kornberg, 1957; Shi et al., 2008; Symmons et al., 2000). PNPase is present both in bacteria and in higher eukaryotes (Leszczyniecka et al., 2002), where it is located in the mitochondria (Piwowarski et al., 2003), suggesting that the eukaryotic enzyme might have been derived from bacteria through endosymbiosis. PNPase is a homotrimer of subunits that each contain two RNase PH-type domains, organised in an inverted arrangement similar to the individual subunits of RNase PH. In addition, each PNPase monomer contains two RNA-binding domains in the C-terminus: one S1 type and one KH type, which are similar to those found in the eukaryotic exosome cap proteins.

The existence of two similar ring-shaped RNA degradation complexes in bacteria (RNase PH and PNPase) suggests that one was derived from the other via gene duplication or lateral gene transfer. However, phylogenetic analysis shows that RNase PH is more closely related to the second catalytic domain of PNPase than the two domains of PNPase are to each other, suggesting that the proteins co-evolved from a common primordial single-domain enzyme (Leszczyniecka et al., 2004). That enzyme also evolved into two archaeal (*Rrp41* and *Rrp42*) and six eukaryotic genes (*Rrp41*, *Rrp42*, *Rrp45*, *Rrp46*, *Rrp43* and *Mtr3*). In fact, the eukaryotic genes can be organised into two groups based on their similarity to either archaeal *Rrp41* (eukaryotic *Rrp41*, *Rrp46* and *Mtr3*) or *Rrp42* (eukaryotic *Rrp42*, *Rrp43* and *Rrp45*) (Lorentzen et al., 2005). This indicates that the eukaryotic exosome evolved from a single RNase PH-type ancestor by several subsequent gene duplication events, the first of which produced *Rrp41* and *Rrp42*, which later diversified into the six individual proteins. The finding that the cap proteins are present in both eukaryotes and archaea also indicates common ancestry. However, a strict requirement of the cap for stability of the exosome core seems to be a development that is specific to eukaryotes (Buttner et al., 2005; Liu et al., 2006; Lorentzen et al., 2005).

The exosome core is catalytically inactive in yeast and humans

The homo-hexameric RNase PH has three phosphorolytic active sites on each side of the ring, whereas in PNPase, this is reduced to a single active site per subunit (three active sites per complex) (Ishii et al., 2003; Symmons et al., 2000) (Fig. 1). This is reminiscent of the archaeal exosome, where the Rrp41-Rrp42 heterodimers each contribute a single active site to the ring (Lorentzen et al., 2005). Originally, the exosomes in *S. cerevisiae* and humans were believed to consist of a similar arrangement of active RNase PH domains but, surprisingly, it turns out that the phosphorolytic activity of the exosome ring has been lost in most eukaryotes (Dziembowski et al., 2007; Liu et al., 2006). This finding has a major impact on our understanding of the function and regulation of the eukaryotic exosome, because it presumably reduces the core to a protein scaffold that organises the enzymatic activities and facilitates substrate recruitment. However, this picture is not universally true, because plants appear to have retained the phosphorolytic activity of Rrp41, although it is not currently known whether the subunit is directly involved in RNA degradation (Chekanova et al., 2000).

RNA degradation in bacteria

In bacteria, most RNA turnover appears to proceed via a combination of endonucleolytic cleavage and 3′-5′ exonucleolysis. However, the recent discovery of the first bacterial 5′-3′ exonuclease, RNase J, means that even simpler organisms use the full range of RNA degradation enzymes in their metabolism (Mathy et al., 2007). mRNA degradation in bacteria is initiated by recognition of the 5′ triphosphate structure, which is cleaved to 5′ monophosphate and pyrophosphate by the enzyme RppH (Deana et al., 2008) (Fig. 2). The free 5′

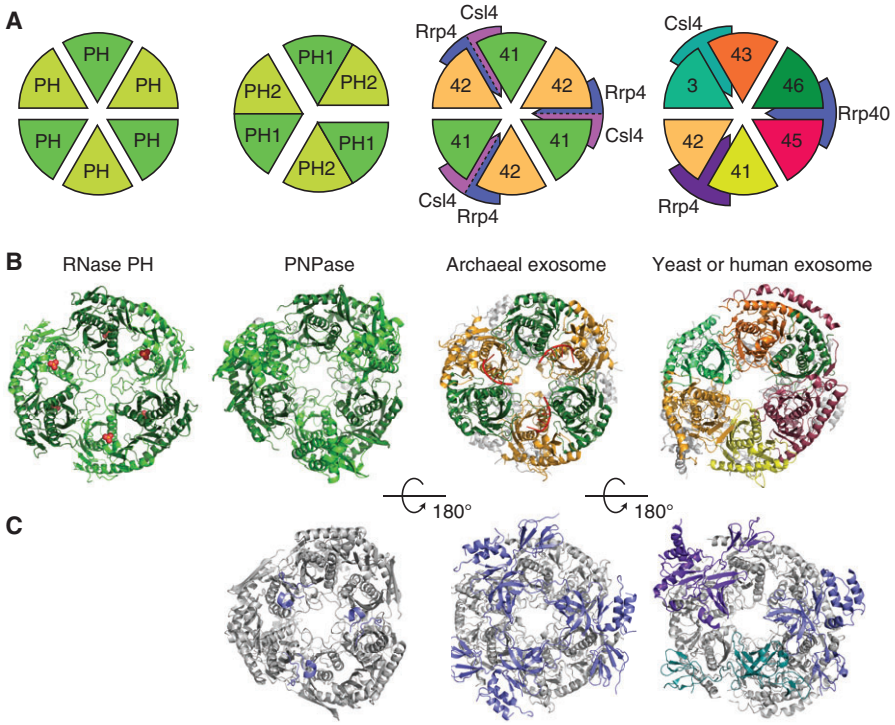

Fig. 1. Common architecture of RNA degradation complexes. (A) Schematic diagrams illustrate the subunit composition and orientation in bacterial RNase PH (dark green and light green show the inversely oriented arrangement of neighbouring subunits), bacterial PNPase (combined wedges illustrate PH domains from the same polypeptide chain), the archaeal exosome complex and the *S. cerevisiae* and human exosome complex. The diagrams show the complexes from 'underneath', with the RNA-binding cap of the archaeal exosome and *S. cerevisiae* and human exosome shown as extra wedges behind (blue and purple). In the case of the archaeal exosome, the two-tone wedges indicate that the three RNA-binding proteins in each complex can be either Rrp4, Csl4 or a combination of these. (B) Actual structures of complexes shown in the same orientation, with domains in similar colours to those in (A). The structures shown are PDB 1UDN [RNase PH (Ishii et al., 2003)], 1E3H [PNPase (Symmons et al., 2000)], 2JEA [archaeal exosome (Lorentzen et al., 2007)] and 2NN6 [human exosome (Liu et al., 2006)]. The RNA-binding caps are displayed in the background coloured in grey. Active sites are illustrated by the red colouration of the inorganic phosphate (for RNase PH) or RNA substrate (for the archaeal exosome). (C) Anterior views of PNPase, the archaeal exosome and the *S. cerevisiae* and human exosomes, with RNA-binding cap proteins shown in blue, purple and cyan. For PNPase, the blue ribbon shows the small part of the S1 domain that is resolved in the current structure (Symmons et al., 2000).

monophosphate then recruits the endonuclease RNase E, which cleaves the downstream mRNA body into two halves (Callaghan et al., 2005). The 5′ fragment undergoes additional endo-cleavages that are catalysed by RNase E, and the fragments are subsequently degraded in the 3′-5′ direction by the combined action of PNPase and another 3′-5′ exonuclease, RNase II. In cases where the 3′ end of an mRNA molecule is protected by a stem-loop structure, the fragment is initially oligoadenylated by bacterial poly(A) polymerase, PAP1, before being degraded by the combined action of PNPase and RNase R, a 3′-5′ exonuclease that is capable of degrading secondary structure (Cheng and Deutscher, 2002) (Fig. 2). Both RNase II and RNase R belong to the RNase R (RNR) superfamily of single-subunit, processive, hydrolytic 3′-5′ exonucleases, which have a common structure and exhibit little sequence specificity (Cheng and Deutscher, 2002; Cheng and Deutscher, 2003; Frazao et al., 2006; Zuo et al., 2006). Of these, RNase II appears to be the main 3′-5′ exonuclease in *Escherichia coli* and might be responsible for as much as 90% of all RNA turnover (Deutscher and Reuven, 1991). Therefore, even with a wide range of both phosphorolytic and hydrolytic enzymes available, degradation by hydrolysis seems to be predominant in bacteria.

Rrp44 functions both as a 3′-5′ exonuclease and as an endonuclease

If the core components lack enzymatic activity, then which associated enzymes are responsible for the activity of the eukaryotic RNA exosome? During eukaryotic evolution, an ancestral RNR-type nuclease evolved into the enzyme Rrp44 (also known as Dis3), which is stably associated with the exosome core in both the nucleus and the cytoplasm in *S. cerevisiae* (Lorentzen et al., 2008; Mitchell et al., 1997). Furthermore, it appears that Rrp44 is the only active subunit of the *S. cerevisiae* cytoplasmic exosome, because a single active-site mutation in the enzyme renders the entire complex inactive (Dziembowski et al., 2007). However, recent data have revealed that Rrp44 also is an active endonuclease (Lebreton et al., 2008; Schaeffer et al., 2009; Schneider et al., 2009). RNase II and Rrp44 share a common architecture consisting of two cold-shock domains (CSDs) followed by the exonuclease domain and a C-terminal RNA-binding domain of the S1 type. But in addition, Rrp44 in both *S. cerevisiae* and higher eukaryotes has acquired a pilT N-terminal (PIN) domain, which is a coiled-coil helical motif that was originally thought to also harbour exonuclease activity (Arcus et al., 2004; Wall and Kaiser, 1999). Despite this prediction, PIN domains – including

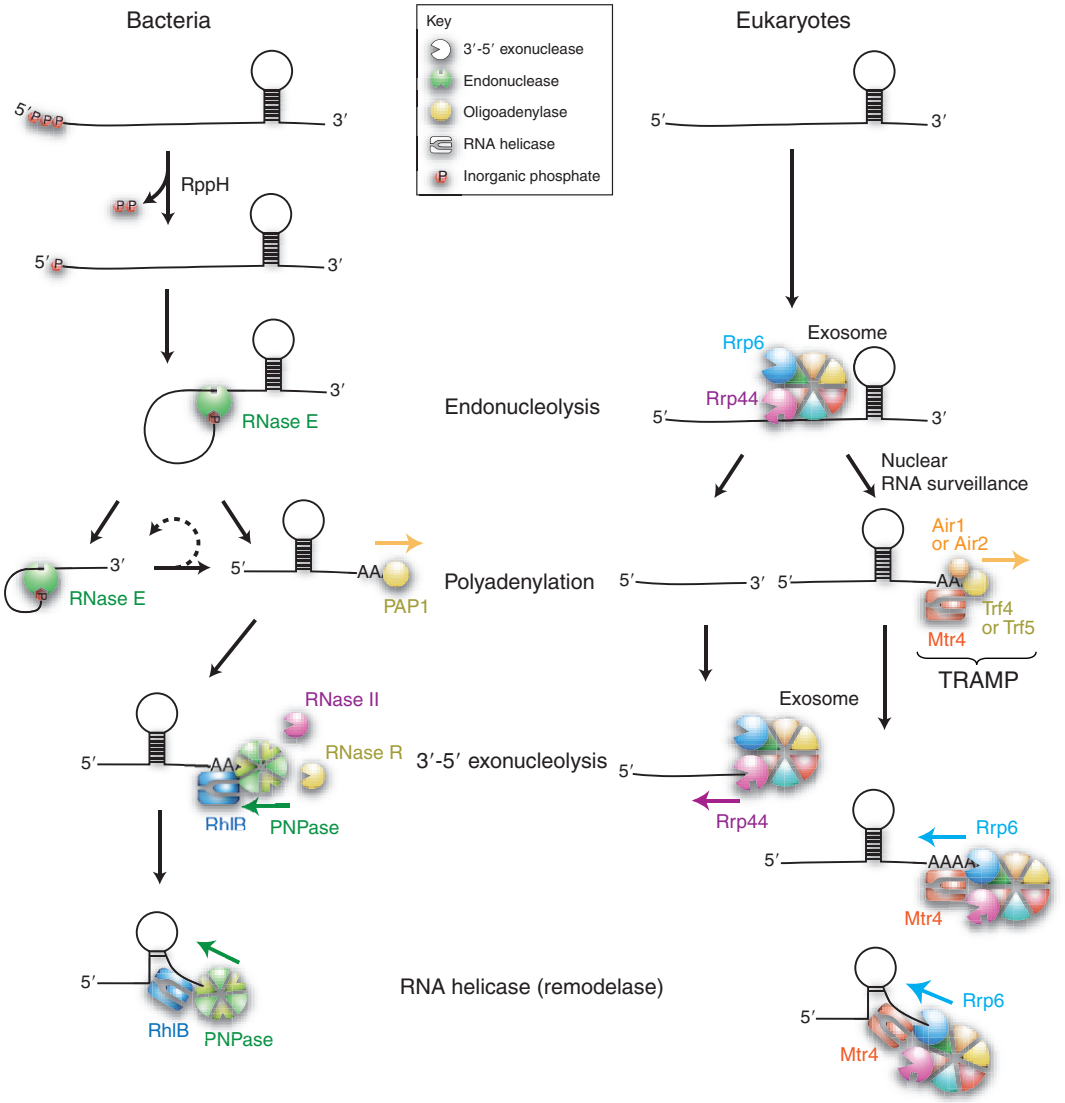

Fig. 2. RNA degradation pathways in bacteria and eukaryotes. RNA degradation pathways in some bacteria (such as *E. coli*) and in eukaryotes require four enzymatic activities: endonucleolysis, oligoadenylation, exonucleolysis and RNA helicase (remodelase) activity. In some bacteria (left), RNA turnover is initiated by the recognition of the 5′ monophosphate and subsequent endonucleolytic cleavage by RNase E. The 5′ fragment undergoes additional endo-cleavages, whereas the 3′ fragment is oligoadenylated by PAP1 when secondary structure elements are present. The oligo(A) sequence then serves as a tag for recruitment of the bacterial degradosome containing both PNPase and the helicase RhlB (which degrades the RNA completely) or for degradation by RNase II or RNase R. In eukaryotes (right), RNA degradation can also be initiated by endo-cleavage by the exosome component Rrp44 and by 3′ oligoadenylation by the nuclear TRAMP complex. The association of the RNA helicase Mtr4 with the exosome allows for the degradation of secondary structure elements. Note that the degradation of fragments upstream of an endocleavage (which might also contain secondary structure elements) can also involve Mtr4 (not shown).

that of Rrp44 – were shown to function as endonucleases (Eberle et al., 2008; Fatica et al., 2004; Glavan et al., 2006; Huntzinger et al., 2008; Lebreton et al., 2008; Schaeffer et al., 2009; Schneider et al., 2009). As an interesting analogy to the bacterial system, endonucleolysis by Rrp44 also appears to be stimulated by a 5′ phosphate on the target RNA (Schaeffer et al., 2009). With the limited availability of active subunits, the newly discovered activity of Rrp44 might well turn out to be crucial for eukaryotic exosome function by enhancing the versatility of the complex, and suggests that many new targets of its activity have yet to be discovered.

An RNase-D-like 3′-5′ exonuclease is associated with the nuclear exosome

Most bacteria contain an additional type of 3′-5′ exonuclease, RNase D, which is involved in the processing of several species of stable RNAs, including tRNAs and 5S ribosomal RNA (Li et al., 1998; Zuo et al., 2005). RNase D enzymes consist of a DEDD-type exonuclease domain in addition to one or more helicase and RNase D C-terminal (HRDC) domains (Liu et al., 1999). The exact function of the HRDC domains is not known, but they might regulate substrate binding either by guiding RNAs into the active site or by preventing the degradation of certain RNA structural elements (Liu et al., 1999; Midtgaard et al., 2006; Zuo et al., 2005). Eukaryotes contain several enzymes derived from RNase D, one of which is the *S. cerevisiae* protein Rrp6, which is restricted to the nucleus and associates with the exosome (Briggs et al., 1998; Mitchell et al., 1997). The human Rrp6 homologue, known as the PM/Scl100kDa auto-antigen, however, appears to be present both in the nucleus and in the cytoplasm (Chen et al., 2001; Lejeune et al., 2003; van Dijk et al., 2007). The eukaryotic RNase D enzymes have diversified compared with their bacterial homologues and thus Rrp6 is almost twice as large as bacterial RNase D. In *S. cerevisiae*, a long N-terminal extension covers the catalytic core and creates an interaction surface for the RNA-binding protein Rrp47 (also known as C1D in humans and Lrp1 in *S. cerevisiae*) (Midtgaard et al., 2006; Mitchell et al., 2003; Stead et al., 2007). The interaction of the exosome with Rrp47 and another co-factor, MPP6, appears to be crucial for the recognition of special RNA elements, such as double-stranded RNA or single-stranded, pyrimidine-rich sequences (Milligan et al., 2008; Schilders et al., 2005; Stead et al., 2007) (Fig. 3). Additionally, Rrp6 takes part in mRNA quality control by degrading aberrant transcripts near the site of transcription (Burkard and Butler, 2000; Hilleren et al., 2001; Libri et al., 2002; Torchet et al., 2002). This process also requires a poly(A) polymerase and is reminiscent of the bacterial PAP1-RNase R-PNPase system.

The eukaryotic exosome is stimulated by helicase and poly(A) polymerase activities

As mentioned above, most RNA-degradation systems require additional enzymatic activities for proper function. In bacteria, PNPase and RNase E associate directly with the DEAD-box RNA helicase RhlB and possibly with PAP1 in a complex known as the bacterial degradosome (Py et al., 1994; Py et al., 1996; Raynal and Carpousis, 1999; Xu and Cohen, 1995) (Fig. 2). This association is thought to facilitate the degradation of difficult substrates, such as highly structured or protein-coated RNAs. This probably involves unwinding or remodelling by the helicase, and the addition of a single-stranded, non-structured tail to the 3′ end of the RNA by the poly(A) polymerase, which thereby provides a suitable anchor for 3′-5′ exonucleolytic attack (Carpousis, 2007). Interestingly, a similar system has been discovered in eukaryotic nuclei, where the exosome associates with the so-called TRAMP complex that consists of a DEAD-box helicase (Mtr4) and a poly(A) polymerase (Trf4 or Trf5), in addition to one of two RNA-binding proteins, Air1 or Air2 (Houseley and Tollervey, 2006; LaCava et al., 2005; Vanacova et al., 2005; Wyers et al., 2005) (Fig. 2). The helicase Mtr4 has also been found to associate directly with the exosome and probably provides a physical link between the exosome and TRAMP (Chen et al., 2001; Milligan et al., 2008; Peng et al., 2003; Schilders et al., 2007). The combined action of TRAMP and the exosome has convincingly been demonstrated in *S. cerevisiae* by the targeted degradation of hypo-modified tRNA (Kadaba et al., 2004; Kadaba et al., 2006; Schneider et al., 2007; Vanacova et al., 2005). Putative homologues of the constituent proteins exist in human cells (Martin and Keller, 2007), in which TRAMP-like oligoadenylation activity has also been demonstrated (West et al., 2006). Surprisingly, TRAMP can also direct the degradation of certain aberrant RNAs by the exosome in an oligo(A)-independent manner (Houseley et al., 2007; LaCava et al., 2005; Rougemaille et al., 2007; Wyers et al., 2005).

It is still a mystery how TRAMP identifies aberrant substrates in an enormous pool of functional RNA molecules, because the nature of RNA abnormalities varies widely. Although TRAMP was originally thought to target only substrates that are destined for degradation, it has recently been suggested that it is also involved in 3′-end processing of stable RNAs (Allmang et al., 1999a; Dez et al., 2006; Egecioglu et al., 2006; Houseley and Tollervey, 2006; Kadaba et al., 2004; Kadaba et al., 2006; Kuai et al., 2004; Mitchell et al., 2003; van Hoof et al., 2000). Thus, as has been suggested for RNA surveillance in bacteria, it is likely that TRAMP and the exosome associate with and ‘survey’ the entire RNA population, but are only allowed access to the 3′ ends of transcripts that lack secondary structures or RNA-binding proteins (Li et al., 2002).

Contrary to the oligo(A) tails produced by TRAMP, the longer poly(A) tails found on stable and functional mRNAs are produced by the canonical, cellular poly(A) polymerase Pap1. In this case, the tail takes the role of stabilising the RNA, mainly because of tightly bound poly(A)-binding proteins (PABPs) that protect the 3′ end from deadenylation and subsequent exosomal degradation (Wang et al., 1999; Wormington et al., 1996). However, when the normal formation of a messenger ribonucleoprotein (mRNP) complex (which contains the pre-mRNA) is impaired, polyadenylation by Pap1 does allow nuclear degradation by the exosome (Houseley et al., 2007; Saguez et al., 2008; van Hoof et al., 2000). In this way, inefficiently processed mRNAs are intercepted by the exosome, retained or degraded to prevent aberrant mRNPs from reaching the translational machinery (Rougemaille et al., 2007; Saguez et al., 2008). The molecular basis for the interaction between the nuclear exosome and the RNA-processing machinery, however, is not fully understood.

In the cytoplasm, other co-factors with similar activities associate with the exosome, such as the Ski complex containing the Ski2 RNA helicase (Araki et al., 2001). However, no poly(A) polymerase has been reported to influence the activity of the cytoplasmic exosome. By contrast, it was recently shown that oligouridylation might be the cytoplasmic equivalent of nuclear oligoadenylation. An example is the controlled turnover of histone mRNAs [which naturally lack poly(A) tails] at the end of S-phase in human cells, which has been shown to involve the exosome and oligouridylation of the 3′ end of the mRNAs – a process that is carried out by terminal RNA uridylyltransferases (TUTases) (Mullen and Marzluff, 2008). The oligo(U) tails are responsible for the recruitment of the Lsm

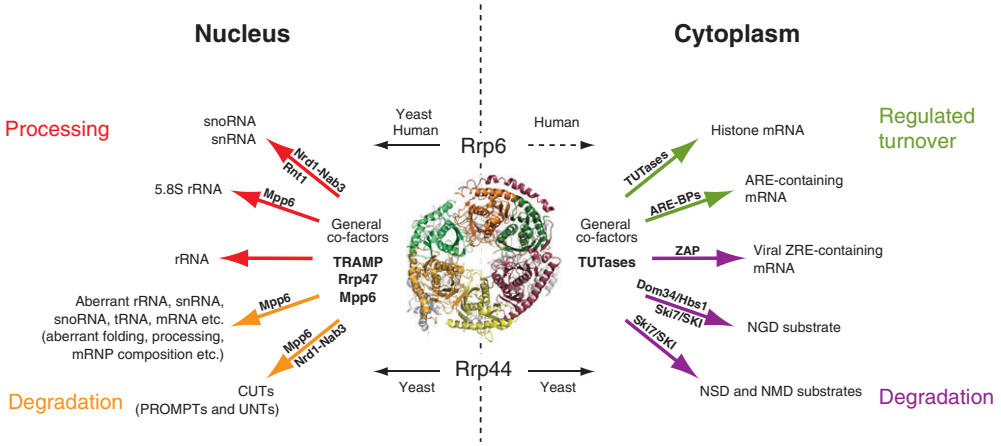

Fig. 3. Diverse functions of the eukaryotic exosome. The nuclear and cytoplasmic processes maintained by the eukaryotic exosome are listed on the left and right, respectively. The active nucleases Rrp6 and Rrp44 are indicated above and below the core exosome. Arrows point to the subcellular compartments in which the nucleases have been shown to localise. Also indicated are the organism(s) in which this localisation has been determined. The dashed arrow pointing to the cytoplasm for human Rrp6 indicates that this localisation pattern is still disputed. The compartmentalised processes are further subdivided into pathways of nuclear RNA processing (red arrows) and degradation (orange arrows) on one side, and regulated cytoplasmic RNA turnover (green arrows) and degradation (purple arrows) on the other. Co-factors that are directly linked to the action of the exosome on specific RNA substrates are indicated above and below the arrows (although other pathway-specific factors might be involved in each case). Putative general nuclear and cytoplasmic co-factors are indicated immediately to the left and right of the exosome, respectively. TRAMP and Rrp47 are necessary for the degradation of all listed nuclear species of RNA, whereas it is unclear whether Mpp6 is required for all nuclear exosome functions (Milligan et al., 2008). TUTases have been shown to be necessary for only histone mRNA degradation, and their putative role as universal cytoplasmic co-factors is speculative (Mullen and Marzluff, 2008). Some substrates and co-factors shown are not discussed in the main text: Rnt1 is an endonuclease that cleaves at the base of stem-loop structures that are present in some snRNAs, snoRNAs and pre-mRNAs, and can provide a free 3′ end for the exosome. It co-purifies with the Nrd1-Nab3 transcription termination complex, and their activities appear to be coordinated (reviewed by Lykke-Andersen and Jensen, 2007; Schmid and Jensen, 2008). A large group of mRNAs with AU-rich instability elements (AREs) in their 3′ UTRs are subject to tight expression control via regulated cytoplasmic turnover. Following stimulation, these ARE-containing mRNAs can be rapidly eliminated by the active recruitment of degradation factors, including the exosome, via ARE-specific adaptor proteins (Chen et al., 2001; Lykke-Andersen and Wagner, 2005; Mukherjee et al., 2002). Similarly, the antiviral protein ZAP can recruit the exosome, and thereby mediate the degradation of certain viral RNAs that contain ZAP-binding elements (ZREs) (Guo et al., 2007). The eRF3/EF1A homologue HBS1 acts together with the endonuclease and eRF1 homologue, Dom34, to mediate the endo-cleavage and subsequent degradation of no-go decay mRNA substrates (Doma and Parker, 2006).

complex, which promotes mRNA decapping and its subsequent degradation; however, in analogy to nuclear oligo(A) tails, they might also act as anchors for the cytoplasmic exosome (Mullen and Marzluff, 2008). This idea is supported by the observation that the cytoplasmic exosome-associated nuclease, Rrp44, prefers AU-rich sequences over oligo(A) in vitro (Liu et al., 2006). Oligouridylation might therefore be a way of distinguishing cytoplasmic RNAs that are destined for rapid turnover within a pool of stable, polyadenylated mRNAs (Fig. 3).

The nuclear exosome is closely associated with the transcription machinery

The exosome is involved in RNA processing and the quality control of transcripts produced by all three RNA polymerases (I, II and III). This means that most, if not all, RNA molecules encounter the exosome at some stage before reaching their mature form (Fig. 3). In a similar way to bacterial RNase D and RNase PH, the eukaryotic exosome takes part in the early biogenesis of stable RNAs, such as ribosomal RNA and eukaryotic-specific small nuclear (snRNAs) and small nucleolar RNAs (snoRNAs) (reviewed by Schmid and Jensen, 2008). But, in addition, the nuclear exosome has recently been found to be responsible for the degradation of a large group of intrinsically unstable transcripts that originate from intergenic regions of eukaryotic genomes (Chekanova et al., 2007; Davis and Ares, 2006; Neil et al., 2009; Preker et al., 2008; Wyers et al., 2005; Xu et al., 2009). In *S. cerevisiae*, the exosome and TRAMP are also recruited during transcription of short RNA polymerase II transcripts such as snRNAs, snoRNAs as well as so-called cryptic unstable transcripts (CUTs) via the transcription termination factor complex Nrd1-Nab3 (Vasiljeva and Buratowski, 2006). This complex, which minimally consists of the RNA-binding proteins Nrd1 and Nab3, and the helicase Sen1, binds to RNA polymerase II and mediates transcription termination after recognition of specific RNA sequence elements (Carroll et al., 2007; Carroll et al., 2004; Conrad et al., 2000; Steinmetz and Brow, 1998; Steinmetz et al., 2001). During this process, TRAMP and the exosome are allowed immediate access to the exposed 3′ end of the RNA. For stable snRNAs and snoRNAs, degradation stops at protein-bound secondary structures and therefore becomes a processing and maturation step for these RNAs (Allmang et al., 1999a; van Hoof et al., 2000). Other substrates, such as CUTs, do not possess these protective elements and are removed completely (Arigo et al., 2006; Thiebaut et al., 2006). Putative homologues of the Nrd1-Nab3 complex proteins exist in human cells, but functional data are currently not available. However, in higher eukaryotes, the involvement of the complex in the termination and processing of snoRNA and snRNA might have been rendered obsolete, because most vertebrate snoRNAs are encoded within introns (reviewed by Kiss et al., 2006). Moreover, the reported transcriptional termination mechanism of human snRNA genes involves the so-called integrator complex, which is unrelated to Nrd1-Nab3 (Baillat et al., 2005).

The cytoplasmic exosome is recruited to ribosomes that are stalled on substrate mRNAs

The Ski2 helicase, which is related to Mtr4, is an essential co-factor for cytoplasmic exosome activity in *S. cerevisiae* (Anderson

and Parker, 1998; Araki et al., 2001; Mitchell and Tollervey, 2003). The Ski2 helicase is part of the larger SKI complex, which also contains Ski3 and Ski8 (Brown et al., 2000; Zhu et al., 2005). The SKI complex is involved in the turnover of aberrant mRNAs with defects that are detected during translation, such as premature stop codons, missing stop codons and translation elongation stalling sites, which are degraded by the nonsense-mediated decay (NMD), non-stop decay (NSD) and no-go decay (NGD) pathways, respectively (reviewed by Doma and Parker, 2007). Although the 5′-3′ exonuclease Xrn1 has an important role in cytoplasmic mRNA degradation, the exosome also contributes significantly to this process (Fig. 3). Ski7 interacts both with the SKI complex and the exosome (Araki et al., 2001), and is a general co-factor in these processes (Doma and Parker, 2006; Frischmeyer et al., 2002; Gatfield and Izaurralde, 2004; Mitchell and Tollervey, 2003; Takahashi et al., 2003; van Hoof et al., 2002). Owing to its homology with the GTP-binding translation release factor eRF3 and elongation factor EF1A, Ski7 is thought to bind the ribosomal A site of stalled ribosomes and recruit the exosome for degradation of the aberrant mRNA (Benard et al., 1999; Clement and Lykke-Andersen, 2006). In the NMD and NGD pathways, the initial recognition of stalled ribosomes occurs via the canonical release factors and the eRF3-like protein HBS1, respectively. However, the subsequent degradation of the mRNA substrate involves Ski7 and the exosome (Doma and Parker, 2006; Mitchell and Tollervey, 2003; Takahashi et al., 2003; van Hoof et al., 2002).

Concluding remarks

Recent scientific progress on the structure and function of the eukaryotic exosome has revolutionised our understanding of the complex, its constituent subunits and associated co-factors. Although different in composition to its prokaryotic counterparts, it now appears that most RNA 3′-5′ degradation complexes require similar enzymatic activities for their function – more specifically, their exonucleolytic, endonucleolytic, polymerase and helicase activities. The requirement for association with these diverse activities may explain in part why the eukaryotic exosome has a complicated structure, when its actual nuclease activities are mediated by only one or two co-factors. The exosome must be versatile but at the same time retain a high level of specificity. Versatility is achieved by multiple enzymatic activities, and by juggling exonucleolysis versus endonucleolysis. As the exosome has at the same time evolved a level of specificity sufficient to avoid the destruction of functional RNAs, the activities of its subunits and co-factors must be tightly regulated. Presumably the non-catalytic core of the exosome has a role here.

Studies of eukaryotic exosome function have also led to the ground-breaking discovery of previously unidentified widespread RNA polymerase II transcriptional activity in the form of CUTs, promoter upstream transcripts (PROMPTs) and upstream non-coding transcripts (UNTs) (Chekanova et al., 2007; Preker et al., 2008; Wyers et al., 2005). Most of these unstable transcripts can only be detected when the exosome is inhibited, and their cellular functions appear to be quite diverse. For example, the transcription of some CUTs is necessary for chromatin to remain in a repressed state in *S. cerevisiae* (Houseley et al., 2007; Vasiljeva et al., 2008), whereas the transcription of others is involved in regulating gene expression through transcriptional interference or attenuation (Davis and Ares, 2006; Kopcewicz et al., 2007; Kuehner and Brow, 2008; Martens et al., 2004; Martens et al., 2005; Steinmetz et al., 2006; Thiebaut et al., 2008; Thiebaut et al., 2006). In humans, PROMPTs have been shown to influence local DNA modification patterns in a few selected cases (Preker et al., 2008; Wang et al., 2008). The elucidation of these unconventional non-coding transcripts opens up a whole new area of research and underscores the broad range of RNAs that are targeted by the exosome. Although today we understand the basic structure and function of eukaryotic exosomes, unexpected findings such as these imply that the future of exosome research holds yet more surprises.

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