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Review

RNA decay machines: The exosome ☆

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ABSTRACT

The multisubunit RNA exosome complex is a major ribonuclease of eukaryotic cells that participates in the processing, quality control and degradation of virtually all classes of RNA in *Eukaryota*. All this is achieved by about a dozen proteins with only three ribonuclease activities between them. At first glance, the versatility of the pathways involving the exosome and the sheer multitude of its substrates are astounding. However, after fifteen years of research we have some understanding of how exosome activity is controlled and applied inside the cell. The catalytic properties of the eukaryotic exosome are fairly well described and attention is now drawn to how the interplay between these activities impacts cell physiology. Also, it has become evident that exosome function relies on many auxiliary factors, which are intensely studied themselves. In this way, the focus of exosome research is slowly leaving the test tube and moving back into the cell.
The exosome also has an interesting evolutionary history, which is evident within the eukaryotic lineage but only fully appreciated when considering similar protein complexes found in *Bacteria* and *Archaea*. Thus, while we keep this review focused on the most comprehensively described yeast and human exosomes, we shall point out similarities or dissimilarities to prokaryotic complexes and proteins where appropriate.
The article is divided into three parts. In Part One we describe how the exosome is built and how it manifests in cells of different organisms. In Part Two we detail the enzymatic properties of the exosome, especially recent data obtained for holocomplexes. Finally, Part Three presents an overview of the RNA metabolism pathways that involve the exosome. This article is part of a Special Issue entitled: RNA Decay mechanisms.
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1. Part One: exosome structure

The eukaryotic exosome is composed of ten to eleven subunits, which can be divided into two major groups with regard to their structural and functional contributions. The first group encompasses nine proteins that form the 'exosome core'. They are all rather small polypeptides with molecular masses of 20–50 kDa. Three of these are built entirely of RNA binding domains and motifs, whereas the remaining six are single-domain proteins homologous to RNase PH, a homomultimeric enzyme found in *Escherichia coli*. The exosome core has a toroidal structure, strikingly similar to that of some prokaryotic complexes involved in RNA metabolism. It is most likely the evolutionarily oldest part of the exosome.

The second group of exosome components comprises two subunits, Dis3 and Rrp6, which associate with the core in different combinations, giving rise to isoforms of the complex. Dis3 and Rrp6 are both large multi-domain polypeptides with molecular masses around 100 kDa. They are quite different from each other and carry all catalytic activity of a typical eukaryotic exosome. The repertoire of catalytic subunits shows considerable diversity in different groups of *Eukaryota*, much more than the core subunits. Dis3 and Rrp6 should be viewed as protein families with substantially different members rather than the same proteins from different organisms. That being said, the Dis3 family is more conserved than the Rrp6 family.

1.1. The exosome core

The structure of the exosome core is well established, owing to the crystal structure obtained for the *Homo sapiens* complex [1]. Its nine subunits are arranged in a two-layered ring; the bottom layer is formed by six subunits homologous to bacterial RNase PH: Rrp41, Rrp42, Rrp43, Rrp45, Rrp46 and Mtr3, and referred to as the 'hexamer', while the top layer consists of the three RNA binding subunits, Rrp4, Rrp40 and Csl4, and is called the 'cap' (Fig. 1). Rrp4 and Rrp40 each contain one S1 and one KH RNA binding domain, and Csl4 consists of a KH domain and a zinc ribbon RNA binding motif. The cap subunits do not form contacts with one another but instead each is firmly attached to two subunits of the hexamer [1]. The central channel that traverses the ring along its pseudosymmetry axis is only wide enough to accommodate single-stranded RNA (ssRNA).

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The architecture of the exosome core is ancient and perhaps even predates the exosome itself as two complexes of prokaryotic origin have the exact same domain composition and spatial arrangement. One is the exosome found in most *Archaea*, sometimes called the 'exosome-like complex' [2,3] and the other is polynucleotide phosphorylase (PNPase) found in *Eubacteria* as well as in plant and vertebrate mitochondria [4]. In the archaeal exosome the hexamer consists of two RNase PH-like proteins, Rrp41 and Rrp42, each contributing three molecules [5–8]. The archaeal cap is composed of one or two proteins, depending on the species: Rrp4 is encoded in one operon with Rrp41 and Rrp42, and therefore it is present in all *Archaea* that have the exosome; Csl4 is encoded by a separate gene and therefore is absent from some species [3]. PNPase is encoded by a single gene and is a homotrimer with each polypeptide containing, one S1-, one KH- and two RNase PH domains. Thus, in these three complexes from different taxa the same structure is achieved with different genetic organization.

The hexamer of the archaeal exosome is a trimer of dimers, in which molecules of Rrp41 and Rrp42 proteins alternate around the central channel [8]. In PNPase the six RNase PH domains also form a trimer of dimers where one RNase PH domain has greater homology to Rrp41 and the other — to Rrp42 [4]. In the eukaryotic hexamer each of the RNase PH domains is encoded by a separate gene, but even here two alternating subgroups can be distinguished: one Rrp41-like (Rrp41, Rrp46, Mtr3) and another Rrp42-like (Rrp42, Rrp43, Rrp45) [1].

Components of the cap are somewhat less conserved than those of the hexamer. In PNPase the cap has three-fold symmetry as all S1- and KH domains are identical. In the eukaryotic exosome the domains come from separate proteins that differ slightly and one subunit is missing the S1 domain altogether [1]. The archaeal cap displays the greatest complexity. Since the hexamer is a trimer of dimers, it has three identical binding sites for cap subunits, and in principle these can be occupied by whichever protein is available. Indeed, the exosome of *Pyrococcus* sp. has been successfully reconstituted in vitro with different cap compositions: only Rrp4, only Csl4 or a mixture of the two [5]. In agreement, *Sulfolobus solfataricus* cells contain Csl4-enriched and Csl4-deprived subpopulations of the exosome, and Rrp4 and Csl4 co-purify with each other [9]. Notably though, the archaeal hexamer is stable on its own in vitro, unlike the eukaryotic one, in which neighboring dimers must be held together by cap subunits [1]. Such stability of the archaeal hexamer allows for some dynamics in cap composition and provides a potential regulatory mechanism as different cap proteins affect the catalytic properties of the hexamer differently [5,10].

It is reasonable to assume that the eukaryotic exosome core has evolved from the archaeal complex. However, the structure is probably too ancient to determine whether the archaeal or the eubacterial genetic arrangement is the original one. Regardless, the structural similarity extends to biochemical properties of the complexes and juxtaposition of the three provides an exciting glimpse into the history of RNA metabolic tools.

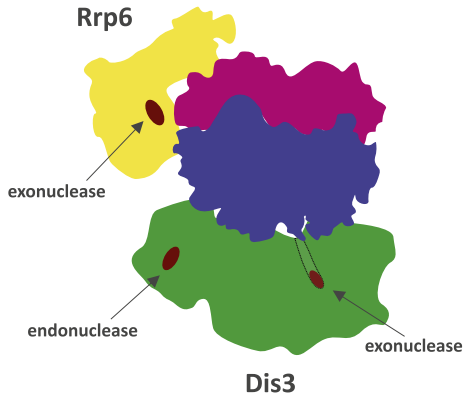

Fig. 1. Schematic structure of the eukaryotic RNA exosome. Side view with the hexamer in blue, the cap in magenta and the associated ribonucleolytic subunits in green (Dis3) and yellow (Rrp6). Endo- and exonuclease active sites are denoted in deep red. The position of Dis3 on the exosome core is extrapolated from the crystal structure of the *S. cerevisiae* Dis3p–Rrp41p–Rrp45p trimer [20] and the position of Rrp6 is inferred from the cryo-EM reconstruction of the *L. terantolae* exosome [29].

1.2. Dis3 ribonucleases

Dis3 proteins are homologous to RNase II and RNase R of *E. coli*, which makes them members of the RNR superfamily [11]. The RNR homology region of Dis3 proteins has the typical domain layout: two cold-shock domains (CSDs), an RNB domain and an S1 domain. CSD and S1 are ancient folds that non-specifically bind RNA, while RNB is a catalytic domain endowed with processive exoribonuclease activity. The RNB active site is built of four conserved aspartate residues grouped in one conserved sequence motif [11] and situated at the bottom of a narrow channel where it can only be accessed by ssRNA [12,13].

A distinguishing feature of the Dis3 family is a CR3 motif and a PIN domain located N-terminally to the RNR region [14–17]. PIN domains are found in many different proteins and can bear exo- or endoribonuclease activity [18,19]. The PIN active site consists of four acidic amino acid residues but, unlike in RNB, they are dispersed throughout the otherwise weakly conserved sequence of the domain. In Dis3 the PIN domain serves two purposes: one, it catalyzes endonucleolytic RNA decay; two, it attaches the protein to the bottom of the exosome core by a direct interaction with Rrp41 [15–17,20] (Fig. 1). The CR3 motif is named for its three conserved cysteine residues, but it also contains a conserved histidine residue. Although details are not clear, the motif also plays a role in the core–Dis3 interaction and is required for some of the cellular functions of the exosome [21,22].

1.3. Rrp6 ribonucleases

Rrp6 proteins belong to the large DEDD superfamily of nucleases, which includes enzymes of various functions, e.g. oligoribonuclease and proofreading domains of DNA polymerases [11]. All members share the catalytic DEDD domain, in which the active site consists of four conserved acidic amino acid residues (three aspartate and one glutamate, hence the name of the domain and the superfamily) located in three distinct and highly conserved motifs. Since this active site is much more exposed than that of the RNB domain, DEDD enzymes are distributive. Rrp6 has two more readily apparent features: the HDRC and PMC2NT domains, the exact functions of which are unknown [23,24]. The HDRC domain is crucial for full activity of the protein in vitro [23], while the PMC2NT domain appears to mediate the interaction of Rrp6 with its partner protein Rrp47 [25,26].

Rrp6 has high affinity for the exosome core, much higher than that of Dis3 [27]. In fact, due to the strong association of the *Saccharomyces cerevisiae* protein with nucleic acids, large scale purification of the whole exosome from a wild-type strain is difficult, a problem not encountered when using *rrp6Δ* cells (Dziembowski, unpublished). Human RRP6 also readily co-purifies with core subunits [28]. Although structural details of the interaction are unknown, cryo-electron microscopy (cryo-EM) data obtained with the *Leischmania terantolae* exosome suggests that Rrp6 binds near the cap [29] (Fig. 1).

1.4. Isoforms of the exosome

The exosome core displays little structural variability across *Eukaryota*, with two notable differences. First, human RRP45 is distinguished from its yeast and plant counterparts in that it has a long C-terminal extension. Second, in *Arabidopsis thaliana* genes encoding AtRRP40 and AtRRP45 are duplicated and therefore presumably give rise to two isoforms of each protein; *AtRRP45a* and *AtRRP45b* mutations are synthetically lethal

so the proteins are likely redundant [30]. The significance of these deviations is unknown.

All core subunits have the same subcellular localization in *S. cerevisiae* and *H. sapiens* [31–35] (Chlebowski and Lubas, unpublished). They are found in the cytoplasm and the nucleus, more concentrated in the latter, and enriched in nucleoli. Since core subunits tend to co-purify with 1:1 stoichiometries, even when non-core subunits or extraexosomal factors are used as baits, it is generally believed that the core complex remains assembled at all times, the only possible exception being the Csl4 subunit, which seems to be less stably associated with the exosome core [36,37].

In contrast to core subunits, some of the associated subunits are compartment-specific and give rise to different isoforms of the exosome. In *S. cerevisiae* Dis3p has the same localization pattern as the core, whereas Rrp6p is restricted to the nucleus [38]. This implies that the *S. cerevisiae* exosome exists in two isoforms: a ten-subunit cytoplasmic one (core + Dis3p) and an eleven-subunit nuclear one (core + Rrp6p + Dis3p).

The *H. sapiens* genome contains three homologues of the yeast *DIS3* gene. However, since one of them (*DIS3L2*) does not encode a full PIN domain, the Dis3 protein family only has two human members: DIS3 and DIS3-like (DIS3L) [34,35]. DIS3 has greater homology to yeast Dis3p than DIS3L does, but the domain composition is the same in both paralogues. This is also true for all known genomes of *Vertebrata* and *Echinodermata*, but not for those of other animals, plants, fungi or protists, which only encode one Dis3 protein [34]. Moreover, the subcellular localization of the two human paralogues is radically different: while DIS3L is strictly cytoplasmic, DIS3 is largely limited to the nucleus with only a small fraction in the cytoplasm. Notably, while the core subunits adhere to the usual subcellular distribution of exosome proteins, DIS3 is absent from nucleoli [34].

As in *S. cerevisiae*, human RPR6 is nuclear with the typical nucleolar enrichment. In addition, small quantities are also present in the cytoplasm [34]. A similar localization pattern is observed in *Drosophila melanogaster* [39] and *Trypanosoma brucei* [40], suggesting minor cytoplasmic functions of Rrp6 proteins in the corresponding taxa. However, co-immunoprecipitation (co-IP) studies suggest that interaction between RRP6 and the exosome core in the cytoplasm is rather rare (Lubas, unpublished). Taken together, three major exosome isoforms appear to exist in human cells: core + RRP6 in the nucleolus, core + RRP6 + DIS3 in the nucleoplasm and core + DIS3L in the cytoplasm. Other possible, less abundant isoforms are: nucleoplasmic core + DIS3 and cytoplasmic core + DIS3, core + RRP6 + DIS3 and core + RRP6 + DIS3L and core + RRP6.

Strikingly, plant genomes harbor only one gene of the *DIS3* family (although they do encode a PIN-less RNR protein as well) but three genes of the *RRP6* family [41]. AtRRP6L1 and AtRRP6L2 are both nuclear proteins, while AtRRP6L3 is strictly cytoplasmic. Interestingly, the gene duplication that gave rise to AtRRP6L1 and -2 seems to have occurred independently in three investigated plant genomes, yet these genes are still more similar to human and yeast *RPR6* than *AtRRP6L3* is; this third paralogue seems to be specific to plants. Moreover, only AtRRP6L2 has a PMC2NT domain, which is interesting, considering its importance for the interaction with exosome cofactors [25]. This suggests certain functional specialization of the nuclear paralogues. Whether AtDIS3 or any of the AtRRP6 proteins interact with the exosome core remains to be determined. The few co-IP attempts that used AtRRP4 or AtRRP41 as baits only yielded core proteins (all except AtRRP40B and AtRRP45A) [37]. However, complementation of the *S. cerevisiae rrp6Δ* mutation by AtRRP6L1 as well as exosome substrate accumulation upon *AtRRP6L2* gene disruption in *A. thaliana* both imply exosome-related functions of these two proteins [41].

2. Part two: catalytic properties and mechanisms of the exosome

Despite their structural similarity, the catalytic properties of the eukaryotic exosome core and the prokaryotic exosome core-like complexes are very different. While the latter are endowed with phosphorolytic ribonuclease activity residing in the RNase PH-like domains, the former is completely inactive. Instead, the exosome of *Eukaryota* has three hydrolytic ribonuclease activities, which are supplied by the associated subunits of the Dis3 and Rrp6 families. However, even though it has no catalytic activity, the exosome core forces a majority of substrates to be threaded through its central channel, which modulates substrate specificity and catalytic properties of Dis3 and Rrp6. Moreover, the catalytic subunits seem to influence each other as well.

2.1. Core components

In the exosome of *Archaea*, all catalytic activity comes from Rrp41 [5,8,42]. The catalytic amino acid residues are mutated in Rrp42, leaving three functional active sites in the nine-subunit complex. How these cooperate is unknown. The same is seen in PNPase, where only the Rrp41-like domain is catalytically active, further underscoring the common evolutionary history. Consistently, the two complexes have basically identical catalytic properties: they are processive, magnesium-dependent, 3′–5′ phosphorolytic ribonucleases. They use inorganic phosphate to attack the backbone of RNA and release nucleoside 5′-diphosphates. This reaction is easily reversible and the enzymes can extend 3′ ends of RNA in vitro if enough ADP is present [5,43]. The active sites are situated on the surface inside the central channel and substrates must enter the channel from the top side of the ring, through the cap, to be processed [2].

The archaeal hexamer retains ribonuclease activity when isolated, but it is enhanced and modulated by the cap: nine-subunit complexes of *S. solfataricus* work faster than the hexamer alone and produce different degradation patterns, depending on whether Rrp4 or Csl4 is used for reconstitution [5]. In fact, the product pattern produced by the Rrp41/Rrp42/Csl4 complex resembles that of a distributive enzyme. Moreover, Rrp4- and Csl4-containing exosomes of *Archaeoglobus fulgidus* exhibit different reaction kinetics [10]. Thus, the function of the cap seems to be recruitment of substrates and perhaps regulation of activity as well.

The exosome cores of *S. cerevisiae* and *H. sapiens* are devoid of catalytic activity due to point mutations in the active sites of all Rrp41-like subunits [1,27]. In plants Rrp41 has retained the active site and one report has demonstrated phosphorolytic activity of the *A. thaliana* protein [44]. The physiological role of this activity is unknown.

2.2. Dis3

Dis3 is an endoribonuclease and a 3′–5′ exoribonuclease. The endonuclease activity resides in the PIN domain [15–17], the active site of which is exposed to the solvent and consists of four acidic amino acid residues that coordinate two divalent metal cations (preferably manganese). The enzyme cannot cleave dsRNA but both circular and linear ssRNA are eligible substrates. A preference for substrates with phosphorylated 5′ termini has been reported [17]. Two of the four amino acids of the PIN active site are mutated in all Dis3l homologues [34]. As a result, Dis3 proteins are active endonucleases and Dis3l proteins are not. Plant Dis3 has not been characterized biochemically but all the crucial residues are conserved so endonuclease activity is expected. Destruction of PIN catalytic sites of DIS3L suggests that endonucleolytic decay is of minor importance in the cytoplasm of *Vertebrata*.

The exonuclease activity of Dis3 depends on four conserved aspartate residues that coordinate two magnesium cations to hydrolytically cleave the RNA backbone. Mutation of any one of these residues completely abolishes the activity [1,27]. The active site is located in the RNB domain, buried at the bottom of a narrow channel and can only be reached by ssRNA at least 7 nt long [12]. Dis3 exonucleases are highly processive. They attack 3′ ends of ssRNA and once bound they degrade substrate completely, releasing nucleoside 5′-monophosphates and leaving end products a few nucleotides long (this length varies slightly from one homologue to another) [34]. Dis3 proteins are highly active on single-

stranded substrates but can also unwind intra- or intermolecular secondary structures, as long as there is an unstructured region of at least 4–5 nt at the 3′ end [12,27].

All of these properties are shared by *E. coli* RNase R but not RNase II, which stops and dissociates immediately when it encounters secondary structures [45]. The difference between these two enzymes has been attributed to the catalytic RNB domain: in RNase R it is able to remain substrate-bound, without cleaving, long enough to take advantage of thermal breathing and unwind dsRNA by pulling on the molecule with the active site [46]. A slightly different mechanism has been proposed based on single-molecule unwinding assays of *S. cerevisiae* Dis3p and *E. coli* RNase R [47]. The force of the active site pulling on the substrate's 3′ end accumulates as elastic tension in the RNA and/or the protein, while the RNA duplex remains intact. About every 4 nt the tension reaches a threshold value and is released in a 'burst' to unwind about 4 nt of the duplex. The length of the unwinding step changes with temperature, suggesting that thermal breathing is involved [47]. These mechanisms are not mutually exclusive, they both state that substrate unwinding is driven by events happening away from the actual secondary structure and in principle could be transferred even further along the substrate.

2.3. Rrp6

Rrp6 proteins are distributive 3′–5′ exoribonucleases that cleave RNA hydrolytically and release nucleoside 5′-monophosphates [38]. Activity comes from the conserved DEDD domain, whose active site is solvent exposed [23,24] and consists of four conserved acidic amino acid residues coordinating two magnesium cations. The combination of a distributive mode of action with selectivity for ssRNA should preclude digestion of structured substrates. Surprisingly, Rrp6 proteins are able to break down structured substrates provided there is a single stranded region upstream of the structure [23]. This effect is slightly stronger for the human protein than for the yeast one. The mechanistic background for this activity is unknown.

2.4. Exosome holocomplexes

Even though yeast and human exosome core complexes are devoid of catalytic activity [1,27], they are essential for proper exosome function in vivo [31,48]. A recent study [49] concerning the activities of Dis3 and Rrp6 of *S. cerevisiae* in context of the core and one another demonstrated that all three ribonuclease activities are modulated by the exosome core because substrates are threaded through the central channel, and that Dis3 and Rrp6 interact functionally in the holocomplex. All these data are corroborated by results obtained at the Dziembowski laboratory for the exosome of the yeast *Chaetomium thermophilum* (Drążkowska et al., in press (http://dx.doi.org/10.1093/nar/gkt060)).

The exosome core has weaker RNA binding properties than the catalytic subunits and protein–RNA contacts in ten- or eleven-subunit complexes are largely due to the presence of Dis3 and Rrp6 [20,49]. Within Dis3, however, the PIN domain makes only little RNA contact compared to the RNR region [49], and when isolated separately, it binds RNA even weaker than the exosome core (Drążkowska et al., in press (http://dx.doi.org/10.1093/nar/gkt060)). RNA degradation is affected by the core in a similar manner: the activity of Rrp6 and both activities of Dis3 are diminished in the presence of the core, whereas the core + PIN complex is, in contrast, more active than the PIN domain alone. In general, RNA interaction with the exosome core becomes the rate-limiting step in RNA degradation by the holoexosome, likely because it precedes RNA interaction with the catalytic subunits. Furthermore, mutations that introduce steric hindrance or reverse surface charge within the central channel strongly inhibit all exosome activities in vitro as well as that of the core + PIN complex (Drążkowska et al., in press (http://dx.doi.org/10.1093/nar/gkt060)) [49]. Taken together this means that at least a large fraction of exosome substrates must pass through the central channel before they can reach the catalytic centers of the associated nucleases (Fig. 2). Such substrate threading closely resembles the *modus operandi* of PNPase and the archaeal exosome. While this may be surprising, considering the core complex of yeast and human has no catalytic activity, threading is supported by other biochemical and structural data, especially for the exonuclease activity of Dis3 [20,50]. Cryo-EM reconstruction of a substrate-bound core + Dis3 complex clearly shows electron density attributable to RNA at the entrance to the central channel [50]. The more detailed crystal structure of yeast Dis3p in complex with the Rrp41p–Rrp45p dimer suggests that the central channel of the core is roughly aligned with the channel of the Dis3p RNB domain [20] (Fig. 2A). The distance between the top and the bottom of the core channel is approximately the length of 30 nt of ssRNA and consistently the core + Dis3 complex requires not 4–5 nt but about 35 nt of ssRNA at the 3′ end of a substrate to initiate exonucleolytic decay [20]. Core-bound Dis3p can still unwind secondary structures in substrates. However, since dsRNA cannot pass through the core channel [20], unwinding most likely takes place at the entrance to the central channel, even though it is driven by the RNB domain.

The endonuclease activity of Dis3 (or of the PIN domain alone), which is purely distributive when the protein is not in complex, acquires some 3′–5′ directionality in the presence of the core, though it does not actually become processive [49]. This acquired directionality means that the PIN domain has easier access to the 3′ ends than to the 5′ ends of substrates, which makes sense if the RNA must pass through the channel to get to the place from which it can be transferred to the PIN domain (Fig. 2B). This could explain why the endo- and exonuclease activities are somewhat redundant in vivo. *S. cerevisiae* point mutants that lack only one of these activities are viable [15,16], although exonuclease-deficient variants exhibit strong growth phenotypes [27]. Complete depletion of Dis3p is lethal [48]. Both Dis3p activities can sustain exosome-mediated RNA decay in certain pathways [21], so perhaps the acquired directionality of the endonuclease can mimic exonuclease action to some extent. However, structural data shows that the PIN active site is exposed to the solvent and lies much farther from the channel exit than the RNB domain [20]. Also, there is no apparent route for the substrate from the channel to the PIN domain [50] and the endonuclease can cleave circular substrates [15], which have no way of passing through the core [1]. This all argues against a dependence of the endonuclease on substrate threading. Still, the biochemical data is compelling: blocking the channel diminishes both exo- and endonuclease activities of Dis3 [49]. Thus, reaching the PIN active site through the channel may be possible by structural rearrangement of Dis3 domains. However, blocking the channel inhibits exonuclease activity about twice as strongly as it inhibits the endonuclease activity, so apparently some substrates can reach the PIN domain directly and be cleaved internally, not in this quasi-exonuclease fashion [49] (Fig. 2B).

Previous research showed little dependency of Rrp6 activity on the exosome core [1]. Rrp6 and Dis3 can bind simultaneously to a single core complex, which makes it highly unlikely that they both occupy the central channel exit at the core bottom. Also, as mentioned earlier, Rrp6 seems to interact with the cap, placing it near the entrance of the channel [29]. However, there are openings in the exosome core, between the cap proteins and the RNase PH hexamer, that connect the central channel to the outside of the complex. These side channels are just wide enough to accommodate ssRNA and it was suggested that Rrp6 substrates, having entered the central channel through the cap as usual, turn into one of the side channels and to the active site of Rrp6p [49] (Fig. 2C). Threading of Rrp6 substrates through the central channel is further supported by experiments conducted on the eleven-subunit holocomplex (core + Rrp6 + Dis3). Here Rrp6p activity is unaffected when accompanied by wild-type Dis3p, but severely inhibited by an exonuclease-defective mutant version of Dis3p [49]. RNA cross-linking studies show that the core + Dis3p complex makes more RNA–protein contacts than core + Rrp6p [49]. Moreover, addition of mutant Dis3p to the core + Rrp6p complex results in diminished Rrp6p–RNA

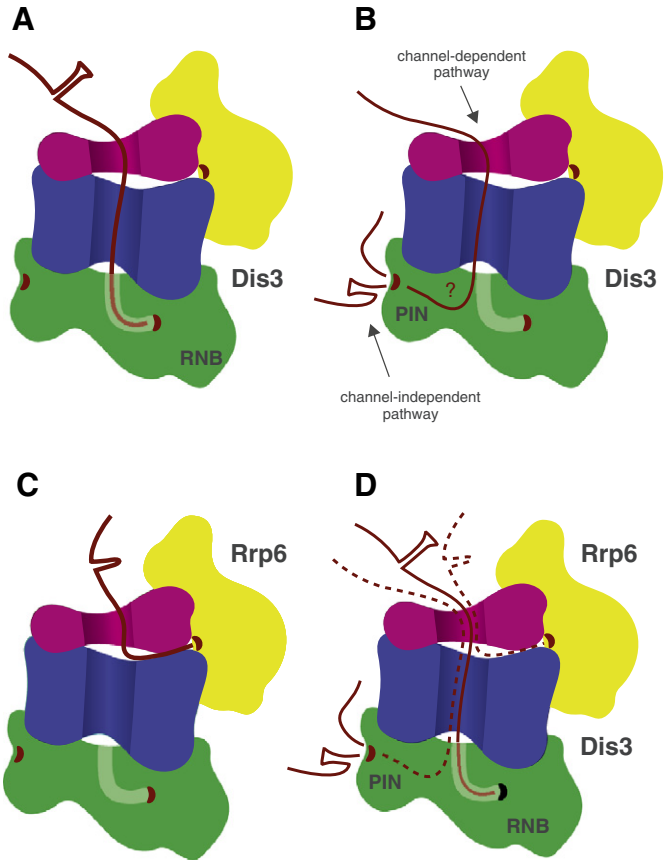

Fig. 2. Substrate threading through the exosome core. Cross-section view of the exosome complex. Double-stranded RNA cannot enter the central channel and secondary structures are unwound at the cap. A — the path to the Dis3 exonuclease active site leads through the entire central channel of the exosome core and into the channel of the RNB domain; B — RNA can access the Dis3 endonuclease active site either through the central channel (channel-dependent) or directly from the solvent (channel-independent); C — to get to the Rrp6 active site, the RNA passes through the top portion of the central channel and turns into one of the side channels between the cap and the hexamer; D — RNA trapped in an inactive RNB domain (solid line) blocks the channel, preventing RNA access to the PIN and DEDD active sites (dashed lines) and thereby inhibits Dis3 endonuclease and Rrp6 exonuclease activities, respectively; the PIN active site can still be accessed by the channel independent pathway.

cross-links. These observations are consistent with Dis3p, a processive enzyme, being able to associate with RNA more strongly than the distributive Rrp6p and indicate that an inactive Dis3p mutant sequesters RNA 3′ ends in the RNB domain and thus protects them from degradation by Rrp6p. Furthermore, blocking the central channel by the fruitless interaction of RNA with Dis3p makes it impossible for other substrates to reach the Rrp6p active site. The endonuclease activity of the Dis3p PIN domain is also affected this way [49] (Fig. 2D).

Another important feature of the eleven-subunit *S. cerevisiae* holoexosome is that Rrp6p enhances the activities of Dis3p [49]. The extent of this effect varies for different substrates and is as large as two orders of magnitude for poly(A) RNA. As a result, Dis3p is more active in the eleven-subunit complex than as a free protein. Curiously, the effect depends on Rrp6p presence rather than its activity as wild-type and catalytically inactive Rrp6p influence both endo- and exonuclease activities of Dis3p similarly. The mechanistic rationale for this enhancement is currently unknown.

Overall, the enzymatic properties of exosome holocomplexes are primarily dictated by the relevant catalytic subunits. However, all three activities are strongly influenced by the threading of substrates through the core complex. The most striking effect of the core is its ability to bestow quasi-processive properties onto the Dis3p endonuclease as well as a considerable interdependence between the catalytic subunits. RNAs threaded through the exosome core have two choices: follow the channel to Dis3 at the bottom or take an early turn towards Rrp6 (Fig. 2A and C). How this choice is made remains to be elucidated. The substrates' destination appears to be purely stochastic in vitro [49], but perhaps in vivo it is determined by additional factors.

Another important question is how the three activities cooperate in vivo. What little data there is comes from *S. cerevisiae*, where Dis3p and Rrp6p work sequentially in the maturation of 5.8S rRNA, one of the first exosome functions described. After excision from the polycistronic pre-rRNA precursor transcript 5.8S molecules are trimmed by Dis3p, leading to an intermediate with a 30 nt extension relative to the mature molecule, which is in line with RNB domain substrates being threaded through the central channel. The 5.8S + 30 molecule is then trimmed further by Rrp6p, which in turn leaves only a few nucleotides that are removed by the extraexosomal distributive Rex nucleases [38,51]. As for the RNA decay functions of the exosome, some of them can be sustained by either the endo- or exonuclease activity of Dis3p but others require the exonuclease specifically [21]. Also, the endonuclease activity apparently cleaves loops in structured substrates to introduce new entry points for the exonuclease [15,52].

3. Part three: the exosome in vivo

Considering its complicated structure and array of catalytic activities, the exosome is far more than just another nuclease. It targets RNAs of virtually all classes at various stages of their lives, from maturation through constant quality control to final turnover. Its functions are, among others, maturation of 3′ ends of rRNA and snoRNAs, degradation of unstable RNAs generated by pervasive and read-through transcription, removal of excised introns and other RNA maturation by-products, quality control of RNA in the nucleus as well as in the cytoplasm, and finally complete RNA decay, both constitutive and regulated. Thus, the range of processes involving the exosome is vast and its number of substrates is enormous. To cope with this overwhelming diversity the exosome takes advantage of a network of auxiliary factors.

3.1. General exosome behavior in the cellular environment

Different tasks of the exosome require different modes of action. RNA processing involves precise trimming of substrates, while degradation demands their prompt and complete removal. RNA surveillance calls for voracious enzymes to dispose of the slightest transgressors yet must not endanger proper molecules. Assigning one enzyme, no matter how complex, to all these tasks may seem counterproductive. How the exosome handles such diversity has slowly been clarified over the past years and although many details are lacking, it appears that the exosome rarely engages its substrates directly and that its activity in vivo is suppressed by depriving it of eligible substrates. Different classes of exosome substrates are recognized by specialized adapters and brought to the exosome. The exosome itself is rarely involved in the decision process that commits a molecule for degradation and in most cases it can be viewed as a common effector of the many pathways of RNA processing/degradation/surveillance.

Keeping exosome-mediated decay in check is achieved by the core complex and may be one of its key functions. The core markedly reduces degradation rates of the catalytic subunits by impairing their RNA binding abilities [49] and thereby puts high demands on potential substrates, at least for Dis3, which is forced to ignore molecules without long unstructured 3′ ends [20]. 'Healthy' mature RNAs exist as ribonucleoprotein (RNP) complexes and consequently display compact quaternary structures, in which RNA 3′ ends are covered by proteins or embedded in secondary structures and thereby escape the exosome's attention. On the other hand, faulty RNAs have difficulties assuming their native conformations or forming RNPs, resulting in weaker RNA–RNA and/or RNA–protein interactions, thereby exposing unstructured 3′ ends [53]. An example here is hypomodified *S. cerevisiae* tRNAs, which can be recognized and

degraded by Dis3p [54]. To facilitate efficient decay, molecules are often cleaved by endonucleases to produce new 3′ ends for the exosome to attack [55]. However, since the exosome's ability to recognize substrates is relatively weak in vivo, degradation of most, if not all substrates involves auxiliary factors, often termed activators or cofactors. These are single proteins or protein complexes that interact with the exosome to recruit it to substrates and/or make them eligible for degradation. Some of these hold RNA helicase and poly(A) polymerase activities. See Fig. 3 for a summary of the functions and cofactors of the exosome.

3.2. The exosome in the cell nucleus

3.2.1. Main cofactors of the nuclear exosome

A key auxiliary factor of the nuclear exosome is the DEVH-box RNA helicase Mtr4 [56]. It localizes strictly to the nucleus with nucleolar enrichment and directly associates with the exosome. In human cells – and perhaps also in yeast – the association is tight enough to co-purify MTR4 in stoichiometric amounts with exosome [28,57]. In *S. cerevisiae*, Mtr4p is also part of the trimeric Trf4/5p-Air1/2p-Mtr4p polyadenylation (TRAMP) complex, which includes a zinc knuckle RNA binding protein (Air1p or Air2p) and a non-canonical poly(A) polymerase (Trf4p or Trf5p) [58–60]. TRAMP facilitates and/or enhances exosome activity due to the helicase activity of Mtr4p as well as the addition of unstructured oligo(A) stretches to RNA 3′ ends by Trf4/5p. These two activities are intricately coordinated: the adenylation by Trf4p is frequently paused for Mtr4p to attempt RNA binding, which can only occur with 3′ extensions of 5–6 nt or longer [61]. As soon as the extensions are sufficiently long, Mtr4p attaches to the substrate and further polyadenylation is hampered. This ensures optimal unwinding activity, as Mtr4p works better on short 3′ extensions and quite poorly on longer ones. Also, Mtr4p unwinding activity is stimulated in the presence of Trf4p and Air2p [62].

The human version of Mtr4 forms at least two distinct complexes [28]. As in yeast, it is found in the nucleoplasm and in nucleoli but it has different interaction partners in these compartments. In the nucleoplasm MTR4 associates with two putative RNA binding proteins, RBM7 and ZCCHC8, to form the Nuclear Exosome Targeting (NEXT) complex. In the nucleolus MTR4 binds TRF4-2 (homologue of Trf4p) and the putative RNA binding protein ZCCHC7 (closest human homologue of Air1p and Air2p) in a complex referred to as 'human TRAMP' proper, although whether it is actually the human version of yeast TRAMP has not been confirmed by extensive functional assays. Regardless, MTR4 ensures that both complexes interact with the exosome and their differential localization within the human nucleus suggests that TRAMP functions have been subdivided between them much like some nuclear and cytoplasmic functions of the exosome have been divided between the DIS3 and DIS3L proteins.

S. cerevisiae TRAMP acts on most nuclear exosome substrates: aberrant mRNA, pre-rRNA, sn/snoRNA, defective tRNA and the large and diverse group of products of pervasive transcription (e.g. cryptic unstable transcripts (CUTs), stable unannotated transcripts (SUTs), Xrn1p unstable transcripts (XUTs)) [52,63–67]. Some of these are marked for complete degradation while others undergo exosome-mediated maturation. These pathways are not always distinct and the final outcome of TRAMP–exosome action is often a result of competition between substrate protection and degradation. This is well exemplified by the exosome-mediated maturation of snRNAs and snoRNAs. Most yeast snoRNAs are encoded by independent genes (rather than situated within introns of protein coding genes or polycistronic precursors as is usual for their mammalian counterparts) and their transcription terminates at RNA binding sites recognized by the Nrd1p–Nab3p–Sen1p complex [68,69]. The Nrd1p protein also binds to RNA polymerase II (RNAPII), which allows Nrd1p–Nab3p–Sen1p to recruit the exosome and TRAMP to the RNA 3′ end. The RNA is then alternately oligoadenylated and trimmed until protective proteins are encountered and the final snoRNP is formed. If protective proteins are not incorporated, the trimming may turn into complete degradation as seen for faulty transcripts [69].

Nrd1p–Nab3p–Sen1p is also involved in terminating RNAPII-mediated transcription of CUTs, which are relatively short RNAs (up to 400 nt) originating mostly from intergenic regions [58,70–72]. As with snoRNA genes, Nrd1p–Nab3p–Sen1p recruits TRAMP and the exosome, however, CUTs are devoid of protective features and thus are degraded so rapidly that they usually evade detection in wild-type cells [52,73]. A similar class of transcripts, promoter upstream transcripts (PROMPTs), has been identified in human cells [74]. PROMPT levels are not affected by depletion of 'human TRAMP' subunits, hTRF4-2 and ZCCHC7, but their decay depends on the NEXT complex [28]. How NEXT is recruited to RNAPII transcripts is unclear since human homologues of the yeast Nrd1p and Nab3p factors have not been identified.

3.2.2. Other nuclear cofactors

The TRAMP and NEXT complexes are not the only exosome cofactors in the cell nucleus. Two other proteins, Mpp6p and Rrp47p (MPP6 and C1D in humans), also co-purify with yeast and human exosomes [75,76]. They are both nuclear proteins enriched in nucleoli that exhibit RNA binding properties; Rrp47p/C1D binds structured substrates and Mpp6p/MPP6 shows strong preference for pyrimidine-rich sequences. Both proteins interact directly with Rrp6 [77]. The mechanisms dictating Rrp47p- or Mpp6p-dependent exosome recruitment are unknown, but in *S. cerevisiae* the two proteins seem at least partly redundant; their absence has similar effects on CUT stability and rRNA biogenesis and a double deletion of the two genes is lethal [78,79].

3.2.3. Rrp6 in quality control

Two recent studies have demonstrated that Rrp6 is associated with transcriptionally active genes in *S. cerevisiae* and the salivary glands of *Chironomus tentans* [80,81]. Moreover, in *C. tentans* the protein accompanies the mRNA as it is prepared for export and only detaches near the nuclear envelope. Also, in *S. cerevisiae* Rrp6p has been shown to be responsible for the accumulation of robustly synthesized mRNAs at their transcription sites in strains where 3′ end formation, mRNP formation or mRNA export is impaired [82]. These mRNAs are sequestered, often in a hyperadenylated state, to give more time for their proper maturation as their premature release can be harmful to the cell [83]. In light of these data Rrp6 appears as a central player in nuclear quality control of mRNA.

3.3. The exosome in the cytoplasm

3.3.1. Cytoplasmic RNA surveillance

The cytoplasmic exosome is usually assisted by the superkiller (SKI) complex [84], which in *S. cerevisiae* consists of the DEVH family RNA helicase Ski2p, the tetratricopeptide protein Ski3p and two molecules of the WD repeat protein Ski8p. Ski3p and Ski8p are RNA binding proteins [85–87]. The SKI complex is necessary for exosomal degradation of mRNAs subject to RNA interference (RNAi) [88] and for decay of several kinds of faulty mRNAs: ones with premature termination codons (PTCs; the nonsense mediated decay (NMD) pathway), ones lacking termination codons altogether (the non-stop decay (NSD) pathway) and ones where ribosomes stall (the no-go decay (NGD) pathway) [87]. In yeast the interaction between the exosome and SKI is mediated by the Ski7p protein [89]. How the human exosome and SKI interact remains unknown, since a human version of Ski7p does not appear to exist. However, Ski7p has a paralogue – Hbs1p – that is involved in the NSD and NGD pathways, and Hbs1p has a human counterpart – HBS1L – that co-purifies with the exosome and perhaps has taken over the functions of Ski7p [34,90].

NMD, NSD and NGD systems all detect defects that interfere with translation efficiency [91]. In NMD, mRNA bearing a PTC is recognized

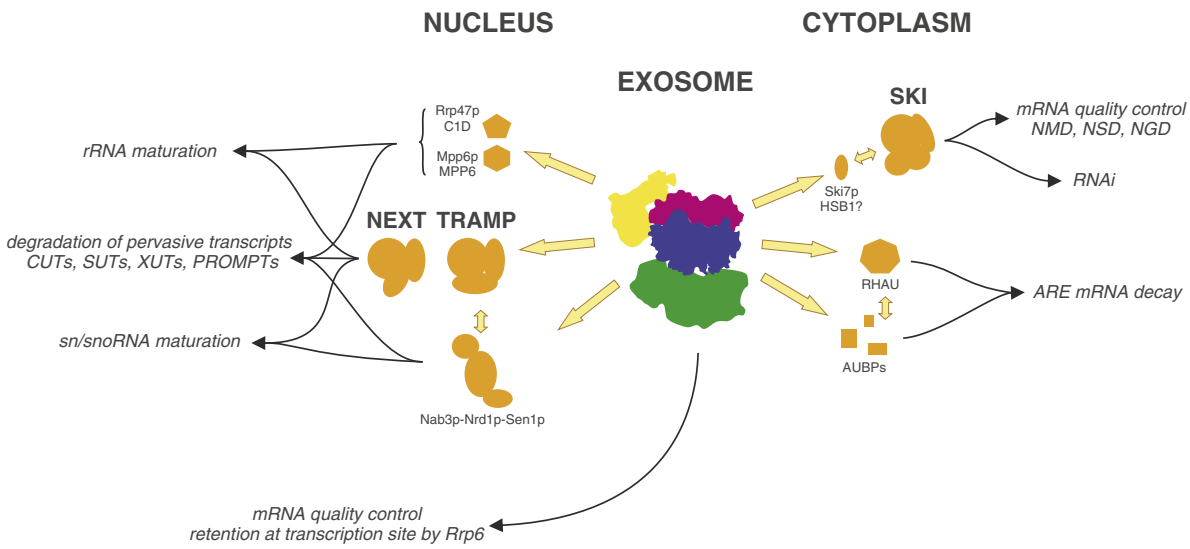

Fig. 3. Map of the exosome's cellular functions and cofactors. Large and small arrows denote interaction and functional involvement, respectively. See text for details.

by Upf proteins, deadenylated or cleaved and then SKI and the exosome are recruited to destroy it [92]. In NSD, ribosomes that stall at 3′ ends of mRNA are identified by a domain of Ski7p that resembles ribosome release factors, followed by SKI and the exosome [93]. In NGD, ribosomes stop translation because of rare codons or stable secondary structures. Here, recognition occurs by the proteins Dom34p and Hbs1p (the Ski7p paralogue), after which the obstructing secondary structure is cleaved by an unknown endonuclease, enabling mRNA degradation by SKI–exosome [94,95].

3.3.2. mRNA turnover

Regular cytoplasmic RNA decay involves 5′–3′ degradation by the Xrn1 protein and 3′–5′ degradation by the exosome [96]. These two pathways are largely redundant and can act on the same classes of RNAs. 3′–5′ decay is usually preceded by gradual shortening of the poly(A) tail, whereupon the exosome can attack the unprotected 3′ end and break the molecule down to a few nucleotides. A slightly different mode of mRNA decay involves the exosome engaging the still polyadenylated 3′ end.

Some mammalian mRNAs bear regulatory AU-rich elements (AREs) in their 3′ untranslated regions. These recruit ARE-binding proteins (AUBPs), which usually lower the stability of the mRNA in question. AREs account for the rapid destabilization of many mRNAs, e.g. in the regulated expression of cytokines and other inflammatory proteins [97,98]. The exosome was shown to interact with some AUBPs [99] or even some AREs directly [100]. Degradation of ARE containing RNAs can also involve the DExH family RNA helicase RHAU [101], which is recruited by some AUBPs and interacts with the exosome as well as poly(A)-specific ribonuclease (PARN). The helicase activity of RHAU has been suggested to clear protective proteins from transcripts, thus enabling their quick degradation. Interestingly, RHAU has two isoforms, one of which is strictly cytoplasmic and the other mostly nuclear [101]. Finally, in RNAi molecules are endonucleolytically cleaved by the RISC complex, creating points of entry for exonucleases. The products of this cleavage are removed by Xrn1 and the exosome, again assisted by the SKI complex [88].

4. Conclusion

With its multi-subunit structure and three different ribonuclease activities, tightly regulated and applied to vastly different purposes (house-keeping and regulatory), the exosome is a truly amazing machine. While its importance was never questioned, it is only in recent years that its functional diversity has been fully appreciated, and still new surprising roles of the exosome are being uncovered [102,103]. The various auxiliary systems that govern exosome substrate recruitment form an intricate functional network with a research field of its own. Yet, questions remain and outlooks are many. Now that the biochemical relationships between the three ribonuclease activities have been established, it will be interesting to see how substrates are sorted between them. By elucidating this, exosome research would come full circle: from biological function through biochemical characterization back to intracellular activity. Some structural features of the exosome also remain elusive: the relationship between the core and Rrp6 and its mechanistic implications; structures of exosome–cofactor supercomplexes; details of substrate transition from cofactor to exosome; etc. Finally, *DIS3* mutations recently discovered in multiple myeloma patients provide new medical relevance to exosome research. More exciting times are sure to come.

Note added in proof

When this article was in press, a study by Makino et al. (http://dx.doi.org/10.1038/nature11870) revealed new information about the structure of the holoexosome of S. cerevisiae, specifically the binding site of Rrp6 and details of substrate threading through the core complex. We strongly suggest that the article be read along with this review.

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