

Poring over exosome structure

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Almost all RNA molecules are processed by RNases to form mature RNAs. In addition, many RNAs are degraded, either because they are no longer needed or because they are aberrant. All of these functions—RNA processing, normal RNA degradation and RNA quality control—are carried out by the eukaryotic RNA exosome complex. In this issue of *EMBO reports*, the Lorentzen group provide structural insight into the eukaryotic exosome and the mechanism by which it degrades RNA from 3' to 5' (Malet *et al*, 2010).

The crystal structures of overlapping parts of the eukaryotic exosome (Liu *et al*, 2006; Bonneau *et al*, 2009) and the related bacterial PNPase (Symmons *et al*, 2000) and archaeal exosome (Lorentzen *et al*, 2007) have been solved, and show that these RNA-degrading machines from the three domains of life have a similar structure (Fig 1). They are all composed of a ring of six RNase PH domains, one side of which has a cap that contains putative RNA-binding domains. Although this overall structure is conserved, the way that it is formed is not. Bacterial PNPase is a homotrimer of which each monomer contains two RNase PH domains, an S1 domain and a KH domain. The archaeal PH ring consists of three copies of two proteins and the cap is made of three copies of either one of two proteins. Finally, the eukaryotic exosome core is composed of nine proteins: six with one RNase PH domain each and three cap proteins.

In PNPase and the archaeal exosome, substrates enter the PH ring from the cap-side. The putative RNA-binding domains of the cap are therefore probably important for controlling entry to the PH ring. In both archaea and bacteria, the active sites are on the inner side of the PH ring and thus the ribonucleic catalysis occurs inside the central channel. However, in humans and yeast each of the RNase PH domains have point mutations that make the exosome ring catalytically inactive (Dziembowski *et al*,

2007). Instead, catalysis is carried out by a tenth subunit—Rrp44/Dis3—which binds to the PH ring on the opposite side to the cap proteins (Bonneau *et al*, 2009; Wang *et al*, 2007). This organization made it unclear whether RNA also enters the central channel of the exosome in eukaryotes (Fig 1), or whether substrate RNAs directly access the catalytic subunit.

Malet and colleagues now provide structural information that resolves this by reconstituting the ten-subunit yeast exosome and analysing its structure with electron microscopy, in the presence and absence of RNA. This analysis suggests that the RNase PH

ring of the exosome is stable, but that the cap and catalytic subunits are more flexible than previously appreciated. It is the first structural evidence that in eukaryotes RNA is threaded through the central channel before being degraded by Rrp44.

Flexibility within the exosome

The study by Malet and collaborators suggest that there are three levels of flexibility in the eukaryotic exosome. First, in the composition of the exosome; not all nine subunits of the structural core are needed to assemble it. Biochemical studies have shown that the cap proteins were required to form a stable

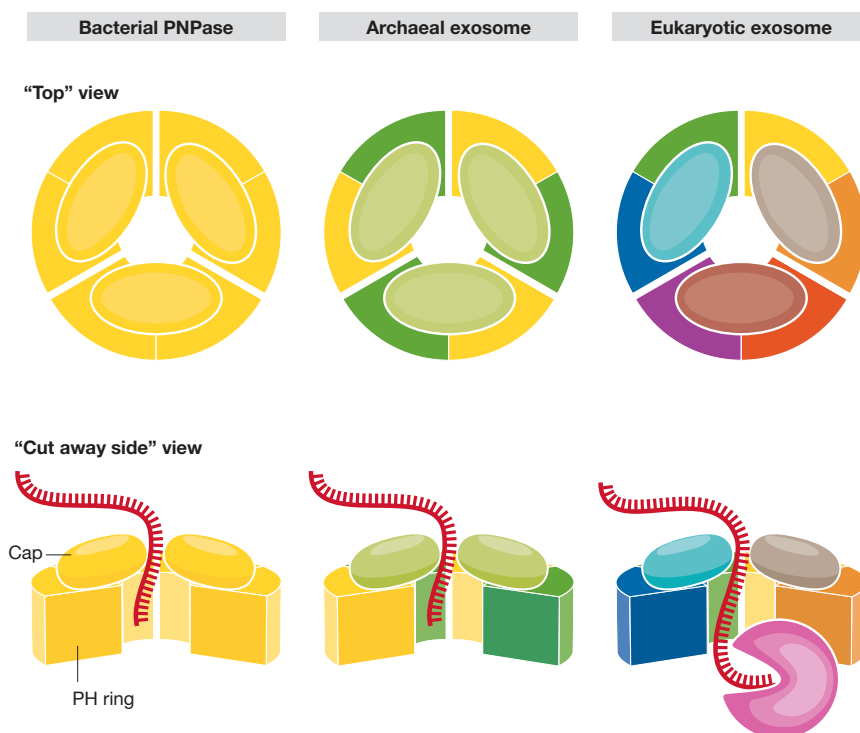


Fig 1 | Exosome structures. The bacterial PNPase (left), the archaeal exosome (middle) and eukaryotic core exosome (right) have a common overall structure. The top panels are schematic views from above, showing the cap proteins. The bottom panels show a view from the side, with one-third of the exosome cut away to reveal the RNA in the central channel.

exosome (Liu *et al*, 2006), but genetic evidence suggested that not all of the three subunits were equally important (Schaeffer *et al*, 2008), which is now confirmed and extended by the results of biochemical experiments (Malet *et al*, 2010). The authors were able to assemble stable exosome complexes with any two of the cap proteins and also with one of the cap proteins—Rrp40—alone. Thus, two minimal core exosomes can be formed *in vitro*, one contains the RNase PH ring and the cap protein Rrp40, whereas the other contains the RNase PH ring and the cap proteins Rrp4 and Csl4. Both of these minimal exosomes are able to interact with the catalytic subunit and RNA. It has been proposed previously that more than one conformation of the exosome exists in fly cells (Andrulis *et al*, 2002) and that some mutations in yeast Csl4 disrupt only specific exosome functions (Schaeffer *et al*, 2009; van Hoof *et al*, 2000), which is consistent with the existence of multiple forms of the exosome. Whether the seven-mer and eight-mer minimal exosomes described by the Lorentzen group are formed and function *in vivo* remains to be determined.

The second level of exosome flexibility occurs in the cap proteins. The amino-terminal domains of Rrp40 and Csl4 show no clear density, suggesting that their position is flexible. Cap protein flexibility has already been found in the archaeal exosome (Lorentzen *et al*, 2007) and the S1/KH domains of PNPase (Symmons *et al*, 2000), and these results therefore show that it is common to all three domains of life. Further flexibility is apparent when comparing the structures obtained by Malet and colleagues in the presence or absence of RNA. In the presence of RNA, extra density can be observed in the cap, which can be explained if the RNA binding loops of the cap proteins become ordered. The authors suggest that three conserved, positively charged residues are important for this interaction between Rrp4 and the substrate RNA, and showed that substituting them with negatively charged residues is lethal (Malet *et al*, 2010). The authors propose that this mutation disrupts an interaction with RNA that is required *in vivo*, but they have not demonstrated that RNA binding is affected nor have they excluded other possible explanations, such as that the mutated Rrp4 is unable to form an exosome *in vivo*.

More flexibility is revealed on the other side of the PH ring, in the catalytic subunit of the exosome, Rrp44. Rrp44 contains an N-terminal endonuclease domain—which

seems to be relatively static—and a more flexible carboxy-terminal part that resembles bacterial RNase II, as both contain three RNA-binding domains—CSD1, CSD2 and S1—and a catalytic domain. Three previous crystal structures of RNase II and Rrp44 (Frazao *et al*, 2006; Lorentzen *et al*, 2008; Bonneau *et al*, 2009) had different orientations of CSD1 and CSD2 relative to the rest of the protein. Malet *et al* (2010) found that CSD1 is slightly shifted compared with previous structures and that CSD2 does not display well-ordered density in the absence of RNA, but becomes more ordered in its presence (Malet *et al*, 2010). Thus, the RNA-binding domains of Rrp44, and perhaps other RNase II family members, are more flexible than initially thought.

Conserved RNA channelling

In addition to revealing a surprising level of flexibility, the Lorentzen group provide the first structure, to our knowledge, that suggests RNA channelling is conserved in all three domains of life. The resolution of the electron microscopic structures is not sufficient to provide a detailed view of single-strand RNA, but the authors show extra density near the cap proteins, which is probably the double-strand 5' end of the RNA (Malet *et al*, 2010). The presence of several RNA-binding loops in the cap proteins could also contribute to this density. On incubation with RNA, additional density is also seen in the RNase PH ring, suggesting that the RNA goes through the channel, resulting in conformational changes to exposed loops of the PH-ring subunits. The addition of RNA also results in density changes that seem to be due to movement of CSD2 in Rrp44. Thus, although the electron microscopic studies cannot visualize single-strand RNA directly, they do show changes in density in the cap, PH ring and catalytic subunit, strongly suggesting that RNA can go through the exosome channel *in vitro*. The same Rrp4 mutations suggest that this route could be important for exosome function *in vivo*.

Many questions about the structure and function of the exosome remain. If the cap proteins are not required for exosome formation, is there more than one exosome core complex in the cell and, if so, what are their respective functions? How would such arrangement be regulated by the cell? The study by Malet and collaborators suggests that RNA channelling occurs. Is this true for all exosomal substrates, or are some substrates channelled through the core, while others

access the catalytic domain directly? The best evidence for RNA channelling *in vivo* comes from the analysis of 5.8S rRNA processing, which occurs through an intermediate with a 30-nucleotide 3' extension that matches the distance between the top of the cap proteins and the Rrp44 active site. However, similar intermediates have not been detected for other exosome reactions. One interpretation of these results is that the 5.8S precursor is channelled through the PH ring, but other exosome substrates are not. The relevance of channelling to exosome function *in vivo* is also unclear in the light of the orientation of other catalytic domains in the exosome. As noted, the catalytic subunit also contains an endoribonuclease domain, the active site of which is pointed away from the central channel (Bonneau *et al*, 2009). The nuclear form of the exosome can also associate with an additional exonuclease subunit. Although the position of this subunit is unknown, one study suggests that it is not near the channel exit (Cristodero *et al*, 2008). Thus, there is only evidence for channelling to one of the three catalytic domains, yet these domains have overlapping functions. The structural studies of PNPase, and the archaeal, human and yeast exosomes have greatly contributed to our understanding of exosome function. We eagerly await what the next picture of the exosome will reveal, as well as future studies that correlate these structural insights with specific RNA processing and degradation reactions *in vivo*.

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