

The exosome, plugged

The correct processing, quality control and turnover of cellular RNA molecules is crucial to many aspects of cell physiology. The exosome—a large molecular assembly with exoribonuclease activity—has emerged as a crucial component in many, if not most, stages of RNA metabolism, including degradation and maturation of ribosomal RNA, RNA quality control and turnover of cytosolic messenger RNA (reviewed by Houseley *et al.*, 2006). Exosomes are found in eukaryotes and archaea. The eukaryotic core exosome consists of six polypeptides with sequence similarity to the phosphate-dependent 3'→5' exoribonuclease RNase PH and three protein K homology (KH) and/or S1-domain-containing RNA-binding proteins (Fig 1A; Mitchell *et al.*, 1997). Additional subunits include the RNase R homologue Rrp44/Dis3 (yeast exosome) and the RNase D homologue Rrp6 (nuclear isoform). The archaeal (a) exosome exhibits a simplified subunit composition and is assembled from three copies each of two RNase PH-like proteins (aRrp41 and aRrp42), aRrp4 or aCsl4 (Evguenieva-Hackenberg *et al.*, 2003).

X-ray crystallographic studies have revealed that the archaeal exosome is a globular structure with a central processing chamber (Fig 1B; Buttner *et al.*, 2005). Three phosphorolytic active sites are formed in the PH ring at the interfaces of alternating aRrp41 and aRrp42 subunits (Lorentzen & Conti, 2005; Lorentzen *et al.*, 2005). The PH ring is capped by aRrp4 and/or aCsl4 subunits, which form a potential RNA recognition platform. Circumstantial evidence has indicated a possible RNA path by which RNA reaches the active sites through a narrow 'S1 pore', located in the trimeric ring of the S1 domains of aRrp4 or aCsl4.

In this issue of *EMBO reports*, Conti and colleagues now provide direct structural evidence for this model (Lorentzen *et al.*, 2007). The authors determined the structure of the *Sulfolobus solfataricus* core exosome in complex with a cleverly designed RNA substrate that can only be partly trimmed owing to its secondary structure. This revealed two different RNA-binding sites: RNA was observed in the middle of the neck and in the three phosphorolytic active sites (Fig 1B). With a width of only 8–10 Å, the neck is too narrow to allow more than one single-stranded RNA molecule or structured RNA to enter, suggesting these forms are sterically excluded from the S1 pore. These results now provide answers to two important questions: how is the exosome regulated and why is the exosome so processive? The threading of RNA through the neck suggests that part of the regulation of the archaeal exosomes is based on the provision of suitable, single-stranded RNA substrates. Such RNA could be provided by specific cofactors. In addition, tight encircling of RNA could be a main determinant for the high processing ability of the exosome.

Are these archaeal features also relevant for eukaryotic exosomes? Lima and colleagues reported a structural and functional analysis of the nine-subunit core of the human exosome (Liu *et al.*, 2006). This complex is a processive phosphorolytic exonuclease *in vitro* that, although structurally highly similar to archaeal exosomes, has only a single phosphorolytic site, located in the Rrp41–Rrp45 dimer. In light of the work of Conti and co-workers reported in this issue of *EMBO reports*, it will be interesting to also test the functional role of the neck to see whether RNA enters the processing chamber of the human exosome through the S1 pore.

Remarkably, the nine-subunit core of the yeast exosome has no phosphorolytic activity (Dziembowski *et al.*, 2007; Liu *et al.*, 2006). Instead, hydrolytic processive and distributive exonuclease activities could be observed for the ectopic components Rrp44 and Rrp6. Consistent with this conclusion, a point mutation in the putative active site of yeast Rrp41 did not lead to detectable phenotypes *in vivo*. All RNase PH-like subunits are essential in yeast; therefore, these data suggest that the architecture of the yeast core exosome is still required, perhaps to act as a platform for RNA recognition and the assembly of co-activators and additional exonucleolytic activities (Dziembowski *et al.*, 2007).

Several co-activators of the exosome have been identified in *Saccharomyces cerevisiae*, and their structural and functional analysis together with the core exosome should soon produce exciting results. In the cytoplasm, the superkiller (SKI) complex is involved in 3'→5' decay of mRNA and interacts with the exosome through Ski7, a GTP-binding protein (Araki *et al.*, 2001; van Hoof *et al.*, 2000). Nuclear RNAs are adenylated by the Trf4–Air2–Mtr4 polyadenylation (TRAMP) complex (LaCava *et al.*, 2005; Vanacova *et al.*, 2005; Wyers *et al.*, 2005). The SKI and TRAMP complexes contain homologous, putative RNA helicases that might unwind secondary structures before their degradation. Additional functions of these co-activator complexes include the recruitment of the exosome to its RNA targets. For example, Ski7 is required to degrade mRNAs that do not contain a stop codon and is sequence-related to the eukaryotic translation release factor 3 (van Hoof *et al.*, 2002). Therefore, Ski7 might recruit exosomes to stalled ribosomes.

In future, it will be important to understand if and where these co-activators interact with the core exosome. One possibility is the array formed by various S1, KH and other potential macromolecular recognition domains. Of course, these domains are also expected to interact with RNA but *S. cerevisiae* Rrp40 and Rrp4 have low intrinsic affinities for RNA outside the exosome (Oddone *et al.*, 2007), although aRrp4 contributes to RNA binding by the archaeal exosome (Buttner *et al.*, 2005). In addition, recent results indicate that the activity of eukaryotic exosomes is dependent on the RNA sequence, a feature that is not understood from the present structural information (Anderson *et al.*, 2006). *In vitro*, human and yeast exosome complexes preferentially degrade RNA substrates that contain AU-rich elements, which are often located in 3' untranslated regions of eukaryotic mRNA transcripts (Liu *et al.*, 2006). In the human exosome, this preference is an intrinsic property of isolated RRP41/45

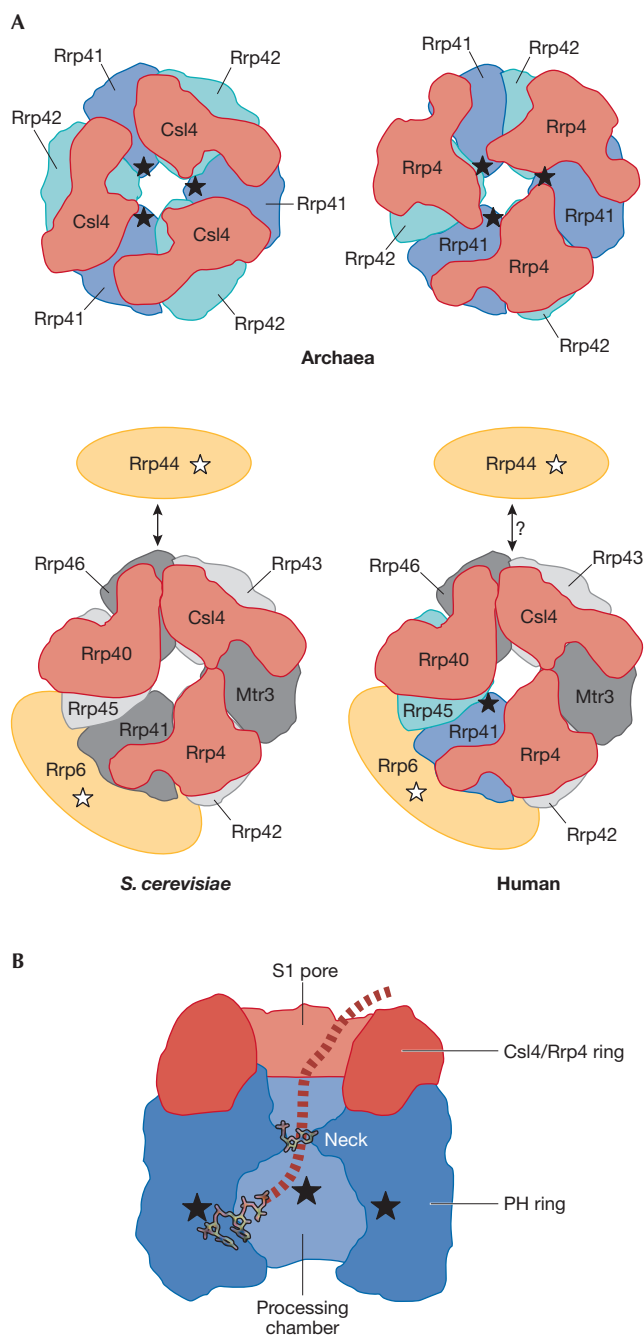


Fig 1 | Comparison of exosomes in archaea and eukaryotes. (A) Schematic comparison of subunit composition of archaeal and yeast exosomes (viewed from the caps of Rrp4/Rrp40/Csl4). Identified active sites are indicated by black (phosphorolytic) or white (hydrolytic) asterisks. Inactive phosphorolytic subunit pairs are shaded grey. The position of Rrp6 is inferred from native mass spectrometry (Hernandez *et al.*, 2006). Archaeal (a) exosomes might have additional subunits and mixed aRrp4/aCsl4 trimers *in vivo* (Walter *et al.*, 2006). (B) Schematic model for archaeal exosome (in a cut-open view) with the observed path of RNA into the processing chamber. RNA probably enters through a narrow neck that restricts entry to unstructured RNA. Therefore, RNA decay by the archaeal exosome probably involves steric exclusion of structured RNA.

and thus does not depend on other RNA-recognition domains. Therefore, there is still much to be learned to fully understand all the 'nuts and bolts' of these multisubunit exosomes.

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