Introduction

The variety and amount of RNA molecules that are produced in a cell is remarkable. There are mRNAs from thousands of genes as well as many RNA molecules that are not translated into proteins and function directly as RNAs. These non-coding RNAs include tRNAs (transfer RNAs involved in translation), rRNAs (ribosomal RNAs), snRNAs (spliceosomal small nuclear RNAs), snoRNAs (small nucleolar RNAs involved in processing of rRNAs) and a diversity of other RNAs that influence processes ranging from chromosome replication to mRNA translation [1]. In addition, RNA molecules can be combined with proteins and form an RNP (ribonucleoprotein) complex. Similarly to proteins, many RNAs are only functional when folded into a complex structure. In addition to the case of mRNAs, non-functional mutated RNA can also arise by synthesis from mutant genes, transcriptional errors, premature termination, post-transcriptional edition events and mistakes in RNA processing. These errors may result in RNA misfolding and/or failure to assemble with proteins to form functional RNPs. The recognition of defective or non-functional RNAs leads to rapid decay, and the regulation of cellular mRNA levels can be achieved via post-transcriptional degradation. Two main pathways that are responsible for the RNA surveillance have been identified in eukaryotes: either the RNA is degraded from the 5′-end by the exonuclease Rat1 (Xrn2 in humans) and Xrn1 [2], or from the 3′-end by the so called exosome [3].

The RNA exosome

The exosome is a multiprotein complex with 3′→5′ exonuclease activity. It degrades many RNAs that are targeted by surveillance activities in the nucleus as well as in the cytoplasm. In addition, it is responsible for the precise trimming of 3′-ends of several nuclear RNA precursors, including maturation of ribosomal RNA [4–11]. Exosome complexes are found in archaea and eukaryotes and are, to some extent, homologous with the bacterial PNPase (polynucleotide phosphorylase). The PNPases, homologues of which are also found in mitochondria and chloroplasts, are therefore often referred as exosome-like complexes. All exosomes and the PNPases contain domains that are structurally highly related. The eukaryotic core exosome consists of six polypeptides with sequence similarity to the phosphate-dependent 3′→5′ exoribonuclease RNase PH (pleckstrin homology) and three protein-K-homology (KH) and/or S1-domain-containing RNA-binding proteins [3]. Additional subunits include the RNase R homologue Rrp (ribosomal RNA processing protein) 44/Dis3 (identified as the constant binding partner of the yeast exosome) and the RNase D homologue Rrp6 (only present in the nuclear isof orm). The archaeal exosome exhibits a simplified subunit composition and is assembled from three copies each of two RNase PH-like proteins (Rrp41 and Rrp42), Rrp4 and/or Csl4 [12].

The role of the exosome in all organisms and especially the differences in enzymatic activity from a phosphate-dependent RNase in bacteria and archaea to a hydrolytic RNase in eukaryotes are of widespread interest. The determination of the crystal structures of the bacterial PNPase [13], the archaeal exosome [14,15] and the human exosome [16] have helped to gain insights into modes of function of nuclease activity of exosome complexes (Figure 1). All complexes have a globular double-doughnut-like
structure with a central hole. The core ring (shown in green) of the complex is formed by the six RNase PH-type subunits. In archaea, three Rrp41 subunits alternate with three Rrp42 subunits, which are, in eukaryotes, replaced by six different proteins (three Rrp41 homologues, Rrp41, Rrp46 and Mtr3, and three Rrp42 homologues, Rrp42, Rrp43 and Rrp45) in a very similar orientation. This core ring binds three copies of Rrp4 and/or Csl4 subunits in archaea and two Rrp4 homologues (Rrp4 and Rrp40) together with one Csl4 subunit in eukaryotes. The equivalent domains in the bacterial PNPase are only partly visible in the crystal structure and probably quite flexible. Csl4, Rrp4 and Rrp40 consist of three structural domains, an N-terminal domain that anchors them on to the RNase PH domain core, a central S1 domain and a C-terminal KH domain (Rrp4 or Rrp40) or zinc-ribbon domain (Csl4). The three S1 domains are situated in the centre of the cap trimer and frame a pore. The KH domains of Rrp4 and Rrp40 and the ZnR (zinc-ribbon) domains of Csl4 are situated on the periphery of the cap ring. As homologues of KH, S1 and ZnR domains are typically involved in nucleic acid binding, this KH/S1/ZnR array might form a large RNA-recruitment surface. Although high-resolution structural evidence for the interaction of RNA with the KH or S1 domains is still lacking, removal of these domains together with a mutation that restricts access into the phosphorolytic active sites drastically removes RNA binding [14,17].

### Architectural model for regulated RNA degradation and putative role for helicase cofactors

On the basis of these structures, a working model for RNA degradation by the archaeal exosome is as follows: substrate RNA may bind to the cap proteins (on top of the complex) followed by a threading through the neck of the processing chamber into the active sites. Cleaved nucleotides might leave through the PH pore at the bottom. Recent new structural studies provide evidence to prove this model: RNA–exosome complexes with bound RNA molecules have been crystallized [18,19]. Lorentzen et al. [18] used the *Sulfolobus* Rrp4 exosome and an RNA molecule with secondary structured parts at the 5′-end, which is designed to be only partially trimmed rather than totally degraded. Navarro et al. [19] used the *Pyrococcus* six-subunit exosome and a decameric...
polyadenylated RNA. Both groups could see the first four RNA bases bound tightly to the active site in a horizontal orientation. As expected, the interactions between protein and RNA appear to be sequence unspecific. In addition, the authors could see electron density for one RNA base at the narrowest constriction of the central channel, in the neck of the processing chamber. At this position, a conserved arginine residue points towards the pore and binds the RNA base via a charged interaction. Preliminary results obtained by us using small-angle X-ray scattering indicate that the cap proteins indeed bind to structured RNA (S. Hartung and K.-P. Hopfner, unpublished work).

With a width of only 8–10 Å (1 Å = 0.1 nm), this neck is too narrow to allow more than one single-stranded RNA molecule or structured RNA to enter, suggesting that these forms are sterically excluded from the S1 pore. 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. Several of these potential cofactors have been identified in eukaryotes, the TRAMP complexes (Trf4–Air2–Mtr4p polyadenylation complex) as well as the Ski complex, and they contain with Mtr4 (TRAMP complexes) and Ski2 (Ski complex) subunits with nucleic acid helicase sequence motifs [20–24]. A putative cofactor with helicase activity is still lacking in archaea, however. A homologue of eukaryotic Ski2, Hεl308a, turned out to be a DNA repair helicase [25,26]. Owing to the substantial homology with yeast Ski2 and Mtr4, the crystal structure of Hεl308a bound to an unwound duplex might serve as a good first model for understanding the unwinding of duplex nucleic acids by this Ski2-related helicase [27]. In addition, the archaean exosome can be co-purified with a polypeptide that shares homology with the bacterial-type primase DnaG. The biochemical activity and mechanistic role in the context of the exosome complex of this archaean DnaG homologue needs to be explored. However, it is interesting to note that TRAMP4 and TRAMP5 complexes, which are cofactors of the nuclear exosome in yeast, possess a subunit with polyadenylation activity [28–30], presumably to generate a signal for subsequent degradation of the RNA by the exosome. Whether DnaG, with its homology with primases, has RNA polymerization activity, and whether this activity is required for the physiological function of the exosome, is a point that should be addressed in future studies.

Processivity and evolution of biochemical features

The crystal structures of the archaean exosome in complex with short RNA substrates gave a snapshot of RNA binding to the active sites [19,31]. To resolve better the RNA-degradation activity of the archaean exosome, more refined assays for degradation processivity by the complex are required, coupled to a quantitative analysis. With the determination of association and degradation rate constants, it appears that the RNA binds to the complex relatively slowly, but the initial binding is followed by a much faster degradation of the substrate (S. Hartung and K.-P. Hopfner, unpublished work). This mechanism may ensure high processivity. We can observe this processivity only for RNA molecules that are longer than approx. 10–11 bases, probably because these molecules are long enough to reach from the active site through the neck of the processing chamber and are attached via the above-mentioned arginine residue. These results indicate that binding of RNA in the neck region is an important determinant for high processivity of the archaean exosome. Such an architecture-implicit processivity could also be important in the context of eukaryotic exosomes. Recent results have shown that human and yeast exosomes lost phosphorolytic activity in the RNase PH-like domains, while the hydrolytic activity is provided by RNase II-like cofactors [32]. The mechanistic function of the phosphorolytic ‘inactive’ exosome core remains to be shown, but the integrity of the core is clearly important for the function of the exosome in yeast. It is possible that the core is only a scaffold for RNA recognition or assembly of the other hydrolytic activities. However, an alternative possibility is that the exosome core encapsulated RNA similarly to the archaean exosome and thus provides means for processivity and regulated degradation of non-structured RNA. If this is the case, it remains to be shown why exosomes have switched RNA degradation activity from one active site (phosphorolytic in the core complex) to another (hydrolytic in cofactors/ectopic subunits) during evolution. One has to keep in mind that the phosphorolytic active site can also efficiently polymerize RNA by adding nucleotide diphosphates to the 3′-end of RNA and releasing phosphate [33,34], and polymerization against degradation depends in vitro on the concentration of free phosphate and nucleotide diphosphates. This intrinsic dual activity is likely to be very difficult to regulate, and the archaean exosome can be seen as a mediator between a free NDP and phosphate pool and (unstable) heteropolymeric tails. Certainly, such an activity would compromise the regulation of RNA stability and decay via stable poly(A) tails in eukaryotes. Thus, whereas archaean exosomes can degrade or polymerize RNA, the evolution of eukaryotes, with their perhaps greater need for controlled RNA metabolism and important regulatory role of stable poly(A) tails, may have required separation of these two functions. As a consequence, separated (more or less irreversible) hydrolytic nuclease and polyadenylation activities may have evolved (Figure 2). Thus one possibility for the evolutionary process is that the active sites within the processing chamber were disabled and the exosome complex was expanded to a ten-subunit core, now containing an ectopic hydrolytic nuclease.

Although, through the emergence of new polyadenylating enzymes and new hydrolytic RNases, the phosphorolytic activity of the exosome core might not be needed any longer, the cap proteins might still play a role in substrate binding and recognition. Furthermore, the architecture of the processing chamber could obviously help to regulate degradation of structured RNA molecules. Recent results for
Figure 2 | Displaced localization of active sites from archaea to eukaryotes

Black stars indicate the active sites that changed from phosphate-dependent RNase and polymerase activity within the processing chamber (green) to hydrolytic RNase activity in the additional subunit Rrp44 (blue) and polyanhydrolation activity in the associated TRAMP complex. Different varieties of cap proteins (red, orange and pink) emerged during evolution from an internal RNA-binding surface in the bacterial PNase and two different cap proteins in archaea to a set of three different caps in eukaryotes. The path of the RNA in the eukaryotic exosome is still speculative. NDP, nucleotide diphosphate.

References

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