Cell, Vol. 99, 347–350, November 12, 1999, Copyright ©1999 by Cell Press

The Exosome: A Proteasome for RNA?

Minireview

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The eukaryotic cell contains a wide variety of RNA species that are either processed from 3′-extended precursors or degraded in a 3′-to-5′ direction. How 3′-to-5′ processing is controlled for different transcripts and distinguished from complete 3′-to-5′ degradation of an RNA molecule is unknown. Surprisingly, a single complex of multiple 3′-to-5′ exonucleases identified by the Tollervey lab, termed the exosome, catalyzes many of these reactions. For example, the exosome trims 5.8S rRNA from a 3′-extended precursor and functions in the 3′-to-5′ degradation of mRNA. In this review, we discuss the organization and functions of the exosome.

The presence of multiple exonucleases in the exosome complex is analogous to a number of proteases both in prokaryotes and eukaryotes, such as the proteasome, that assemble in large complexes. In addition, both the exosome, and the proteasome, require ATPases for their functions (see below, and reviewed in Gottesman et al., 1997; Baumeister et al., 1998; DeMartino and Slaughter, 1999). These similarities suggest that there may be a fundamental advantage to the compartmentalization of degradative enzymes by their assembly into larger complexes.

What Is the Exosome?

Based on copurification, the yeast exosome is a protein complex that consists of a core of at least ten proteins (Rrp4p, Rrp40p to Rrp46p, Mtr3p, and Csl4p; Table 1; Allmang et al., 1999a). The stoichiometry of the different subunits is unknown, but the sedimentation of the exosome in glycerol gradients (300–400 kDa; Mitchell et al., 1997) is consistent with a single copy of each subunit. Strikingly, all ten subunits have been proposed to be active 3′-to-5′ exoribonucleases (Allmang et al., 1999a). Six of the exosome subunits (Rrp41p, Rrp42p, Rrp43p, Rrp45p, Rrp46p, and Mtr3p) appear to be 3′-to-5′ phosphorolytic enzymes, since they are related to the 3′-to-5′ exoribonucleases RNase PH and PNPase from *Escherichia coli* (Mian, 1997). These *E. coli* enzymes function in the decay of mRNA and the processing of other RNAs. Unlike hydrolases they utilize phosphate as an attacking group during RNA digestion and produce nucleotide 5′ diphosphates (NDPs). In support of these proposed activities, recombinant Rrp41p is a phosphate-stimulated exonuclease that produces NDPs (Mitchell et al., 1997). In contrast, Rrp44p is related to the 3′ hydrolases RNase II and RNase R from *E. coli* (Mian, 1997) and recombinant Rrp44p has 3′-to-5′ exonuclease activity that releases nucleotide 5′ monophosphates (NMPs; Mitchell et al., 1997). Recombinant Rrp4p, purified from *E. coli* also has 3′ exoribonuclease activity that releases NMPs, although it is not obviously related to known exonucleases (Mitchell et al., 1997). Finally, Rrp40p and Csl4p may be active 3′ exoribonucleases, since these proteins show sequence similarity to Rrp4p (Allmang et al., 1999a). However, it is unknown what the catalytic domain of Rrp4p is, and the homology with Rrp40p and Csl4p may be restricted to an S1 RNA–binding domain.

Each of the core exosome subunits is an essential protein (Mitchell et al., 1997; Allmang et al., 1999a). This is presumably because defects in one exosome subunit cause a failure to assemble the exosome properly, and the exosome per se is required for viability. This conclusion is supported by the observations that inactivation of any core component generally gives similar defects in exosome-dependent events (Mitchell et al., 1997; Jacobs Anderson and Parker, 1998; Allmang et al., 1999a, 1999b; van Hoof et al., 2000). In addition, there does not appear to be a substantial free pool of exosome subunits (Mitchell et al., 1997; Allmang et al., 1999a). This hypothesis would be similar to what has been observed for the proteasome. Here catalytic subunits are essential for structural reasons, but individual active sites are not essential (reviewed in Baumeister et al., 1998). However, two observations raise the formal possibility that exosome subunits may have distinct essential functions independent of the entire complex. First, in contrast to subunits of the proteasome, which require assembly for activity, isolated exosome subunits show exonucleolytic activity (see above). Second, homologous human cDNAs can complement at least some of the phenotypes of yeast strains carrying mutations in the *RRP4*, *RRP44*, or *CSL4* genes. This suggests that either the exosome subunits are sufficiently conserved to allow assembly between different species, or that exosome subunits may be able to function individually. To resolve these issues, the analysis of mutant alleles of exosome subunits that separate exonucleolytic activity from assembly will be needed.

Similar to the proteasome, the exosome is present in both the nucleus and the cytoplasm. This conclusion is based on immunolocalization of core exosome subunits and biochemical fractionation (Kinoshita et al., 1991; Mitchell et al., 1997; Allmang et al., 1999a; Zanchin and Goldfarb, 1999). However, the nuclear exosome has an additional subunit, Rrp6p, which is yet another active 3′-to-5′ exoribonuclease (Allmang et al., 1999a; Burkard and Butler, 2000). Rrp6p is the only exosome subunit that is not essential for viability, although rrp6Δ strains have strong defects in all the known nuclear exosome functions (see below).

Several lines of evidence indicate that the exosome is conserved in eukaryotes. For example, the majority of the exosome subunits identified in yeast have strong homologs in other eukaryotes. Moreover, two observations indicate that these homologs do form an exosome complex in other eukaryotes. First, the human homologs of Rrp6p (PM-Scl100), Rrp4p, and Rrp45p (PM-Scl75) are found in the PM-Scl particle, which appears to be the human exosome (Allmang et al., 1999a). Second, the *Schizosaccharomyces pombe* homolog of Rrp44p, termed Dis3p, is also found in a complex similar in size to the exosome (Noguchi et al., 1996).

Table 1. Exosome Structure

Subunit	Similarity	In Vitro Activity	Notable Homologs
Core subunits			
Rrp4p	S1 RNA BP	3′ exo hydrolase	human Rrp4 complements yeast mutant and is part of PM-Scl
Rrp40p	S1 RNA BP		
Rrp41p/Ski6p	RNase PH	3′ exo phosphorolase	
Rrp42p	RNase PH		
Rrp43p	RNase PH		
Rrp44p/Dis3p	RNase II	3′ exo hydrolase	human and *S. pombe* DIS3 complement yeast mutant
Rrp45p	RNase PH		human PM-Scl75
Rrp46p	RNase PH		
Mtr3p	RNase PH		
Csl4p	S1 RNA BP		human CSL4 complements yeast mutant
Nuclear subunits			
Rrp6p	RNase D	3′ exo hydrolase	human PM-Scl100
Associated factors			
Mtr4p	RNA helicase		
Ski2p	RNA helicase		
Ski3p	TPR domains		
Ski8p	WD domains		

Despite this conservation, there may be differences in exosome structure between species. For example, the EST database contains sequences for six human RNase PH-like genes, similar to the six found in yeast, but without clear orthologous pairs of human and yeast genes (Allmang et al., 1999a). More strikingly, the *Caenorhabditis elegans* genome codes for only three proteins related to RNase PH (B0564.1, F37C12.13, and C14A4.5). Similarly, the yeast exosome contains the related Rrp4 and Rrp40 proteins, but the *C. elegans* genome has only one corresponding gene (F56C6.4). One possibility is that the three *C. elegans* RNase PH homologs and the Rrp40 homolog are present in two copies per exosome, creating a similar overall exosome structure. Such structural variation would be analogous to the difference between archaebacterial and eukaryotic proteasomes. Archaebacterial proteasome core particles consist of 14 copies of two different subunits, while eukaryotic proteasome core particles contain two copies of 14 different subunits (reviewed in Baumeister et al., 1998; DeMartino and Slaughter, 1999).

Many of the exosome components have distant homologs in eubacteria and archaebacteria. However, at least in *E. coli*, they do not appear to assemble into an exosome-like complex, although this has not been extensively studied. *E. coli* does contain a complex, named the degradosome, that contains several proteins including polynucleotide phosphorlyase (a 3′-to-5′ exoribonuclease), RNase E (an endonuclease), and RhlB (an RNA helicase) (see Carpousis et al., 1999 for review).

What Does the Exosome Do?

The exosome, like the proteasome, performs both processing of some substrates to shorter forms and the complete degradation of other substrates. The exosome is required for nuclear 3′ trimming reactions that produce the mature 3′ end of several stable RNAs. For example, yeast strains with conditional defects in exosome subunits accumulate 3′-extended 5.8S RNA and show a loss of mature 5.8S RNA over time (Briggs et al., 1998; Mitchell et al., 1997). Similarly, several snoRNAs and snRNAs accumulate as 3′-extended, and in some cases, polyadenylated forms in strains with exosome mutations (Allmang et al., 1999b; van Hoof et al., 2000). In this case, the exosome may be required both for deadenylation of the snoRNA and for further 3′ trimming. Since all of these processing reactions require 3′-to-5′ exonucleolytic function, and this is the biochemical activity of the exosome, it is likely that the exosome is the actual nucleolytic complex carrying out the process.

The exosome is also required for the degradation of some RNAs. For example, the 3′-to-5′ degradation of poly(A)$^-$ mRNAs in the cytoplasm is inhibited in strains lacking exosome function (Jacobs Anderson and Parker, 1998). This cytoplasmic function may be important as an antiviral defense, since mutations in exosome subunits, or other proteins required for 3′-to-5′ mRNA degradation, lead to increased abundance of some viral genomes in yeast (Matsumoto et al., 1990; Jacobs Anderson and Parker, 1998). The exosome is also required for the degradation of the 5′ external transcribed spacer region of the primary transcript for rRNA (de la Cruz et al., 1998; Allmang et al., 1999a).

The exosome is likely to have additional functions. Two observations suggest that the exosome may also be involved in a nuclear mRNA degradation process. First, mutations in some exosome components have been observed to lead to the accumulation of polyadenylated transcripts in the nucleus as assessed by in situ hybridization with an oligo(dT) probe (Kadowaki et al., 1994). This suggests that these mutants are defective either in the transport, processing, or degradation, of some nuclear polyadenylated transcripts, although whether these polyadenylated transcripts correspond to mRNAs, polyadenylated snoRNAs, or both, is not yet clear (van Hoof et al., 2000). Second, mutations in a component of the nuclear exosome (i.e., Rrp6p) were identified as suppressors of a conditional defect in poly(A) polymerase (Briggs et al., 1998). This suppression could be explained if the nuclear exosome functions to degrade poly(A)$^-$ mRNA, which is produced in the poly(A) polymerase mutant (Burkard and Butler, 2000). The exosome has also been suggested to function in the degradation of pre-rRNA transcripts that are unable to be properly processed (Zanchin and Goldfarb, 1999). Other likely

functions include the deadenylation of the telomerase RNA and the degradation of excised and debranched introns.

Why An Exosome?

The multiple exonucleases in the exosome complex and its diversity of functions raises several issues. First, why are so many exonucleases in a single complex? Second, what is the relationship of the different exonucleases within the exosome to its different functions? Understanding these issues will ultimately require knowledge of the structure of the exosome, which is not yet available. However, some hypotheses can be drawn from the available data, and from consideration of exosome function.

Some advantages for the assembly of multiple degradative enzymes into a single complex have come from the analysis of the proteasome. In this case, the advantages of assembly are tightly linked to the structure of the proteasome wherein the active sites are arranged within a central cavity that is not freely accessible to macromolecules. This sequestration of active sites reduces the rate at which incorrect substrates are cleaved. Conversely, by having a committed step of entry into the degradative compartment it may decrease the chance of terminating processing or degradation prematurely. In addition, the sequestration of active sites allows for the separation of substrate recognition from the actual degradation, thereby allowing more flexible regulation of both. If the active sites of the exosome subunits are similarly arranged in an internal cavity, then these advantages are likely to apply to the exosome as well.

Part of the explanation for the presence of multiple exonucleases in the exosome may be that not all of the subunits are active. For comparison, the eukaryotic proteasome consists of 14 related subunits, but only three are thought to be active proteases while the others perform structural roles (reviewed in Baumeister et al., 1998; DeMartino and Slaughter, 1999). In contrast, all four exosome subunits examined to date have shown active nuclease activity, suggesting that at least the majority of the exosome subunits will be active exonucleases.

One simple hypothesis is that the assembly of multiple exonucleases into the exosome allows both for their coordinate regulation, and for the delivery of a diversity of biochemically distinct exonucleases to a given substrate simply by interaction of the given RNA substrate with the exosome. This is analogous to the proteasome where the individual active sites in the proteasome are thought to act on the same substrate proteins, although each protease has a preference for peptide bonds flanked by different amino acids (reviewed in Baumeister et al., 1998; DeMartino and Slaughter, 1999). In this view, the individual exonucleases in the exosome might perform distinct steps on a single substrate, have preferential activity on particular substrates, or be differentially active under different growth conditions.

Evidence in support of different exonucleases performing distinct steps in the processing of a single RNA has come from two phenotypes of rrp6Δ strains. First, while other exosome mutants lead to the accumulation of long 3′ extensions on snoRNAs, rrp6Δ strains are completely blocked at the removal of the last few nucleotides of the 3′-extended precursor (van Hoof et al., 2000; Allmang et al., 1999b). This suggests that while other exonucleases in the exosome can trim the snoRNA to near the 3′ end, Rrp6p is specifically required for the removal of the last few nucleotides. Second, rrp6Δ strains accumulate 5.8S RNA with a 30-nucleotide 3′ extension (Briggs et al., 1998), while core exosome mutants accumulate longer forms of 5.8S precursors (Mitchell et al., 1997; Allmang et al., 1999b). Moreover, strains defective in both core exosome function and Rrp6p accumulate the longer species (Allmang et al., 1999b). This suggests that the core exosome can effectively trim the 5.8S RNA to the +30 form, but that Rrp6p preferentially functions to process the 5.8S species past this point. These observations argue that the core exosome and the associated Rrp6p function to perform distinct processing steps on the same RNA substrate. It is possible that there will be distinct steps, or reactions, carried out by individual members of the core exosome as well. Examination of this possibility will require the construction and analysis of specific mutations that inactivate a single exosomal nuclease without affecting the assembly and function of the remainder of the exosome. However, it should be noted that in cases where the product of one enzyme is the substrate for the next enzyme, it would be highly advantageous to have the multiple enzymes in a single complex.

RNA–Exosome Interactions

What determines whether the exosome degrades a substrate completely, as it does with mRNA, or processes the substrate to a shorter form, as it does with 7S pre-rRNA? One possibility is that processing substrates contain distinct secondary structures or bound proteins that stop the exosome. This hypothesis is supported by the observation that very strong secondary structures can stall exosome digestion during mRNA degradation in vivo (Jacobs Anderson and Parker, 1998). Alternatively, if the different exosome subunits have distinct roles then the processing versus decay fate may not be inherent to the substrate, but could be determined by the exonuclease that acts on it. For example, mRNAs that need to be degraded might be targeted to a processive exonuclease, while processing substrates might be targeted to a distributive exonuclease. Consistent with this possibility, isolated Rrp4 is distributive in vitro, while isolated Rrp41 and Rrp44 appear to be more processive in vitro (Mitchell et al., 1997), although how these properties relate to their activities in the exosome complex is not clear.

A related issue is how the variety of RNA substrates is recognized by the exosome. One key to understanding the interaction of the exosome with its substrates is that each function of the exosome identified to date is dependent on either Mtr4p or Ski2p. Ski2p and Mtr4p are closely related members of the superfamily II of RNA helicases. Members of this family interact with RNA and utilize ATP hydrolysis to promote conformational changes, either in RNA structure or possibly in RNA–protein interactions (for review, see de la Cruz et al., 1999). Defects in nuclear Mtr4p inhibit the processing of 5.8S rRNA, snRNA, and snoRNA, as well as degradation of the 5′ external transcribed spacer region of the pre-rRNA (de la Cruz et al., 1998; Allmang et al., 1999b;

van Hoof et al., 2000). Conversely, 3′-to-5′ mRNA degradation by the exosome is dependent on the Ski2p protein (Jacobs Anderson and Parker, 1998), which is found in the cytoplasm (Qu et al., 1998).

There are two related ways in which Ski2p and Mtr4p could function. First, these helicases could unwind secondary structure in the RNA or disrupt RNA–protein interactions. This activity would create unprotected RNA that then could be degraded by the exosome. A related possibility is that the Ski2p and Mtr4p proteins do not simply unwind inhibitory secondary structures, but may also couple the unfolding of the substrates to delivery to the exosome. This hypothesis is suggested by the fact that either Ski2p or Mtr4p is required for each of the known exosome reactions, even though many substrates do not have any obvious secondary structure. This function would be analogous to a proposed function of AAA ATPases that associate with the proteasome. According to this proposal, these ATPases may act both to unfold proteins and to transport the polypeptide backbone into the active site of the proteasome. Similarly, Mtr4p and Ski2p could deliver RNA substrates into the active sites of the exosome.

This latter hypothesis predicts that Mtr4p and Ski2p not only interact with RNA, but also interact with the exosome. This association with the exosome might be indirect. For example, Ski2p might interact with the exosome through Ski3p and Ski8p. These two proteins are required for exosome-mediated mRNA degradation (Jacobs Anderson and Parker, 1998). No physical interaction has been reported yet between Mtr4p or Ski2p and the exosome, but Ski2p and Mtr4p association with the exosome might be transient, or dependent on RNA and/or ATP. It will be important in future work to determine if these proteins interact with the exosome under certain conditions.

Perspective

The discovery of the exosome has identified an important RNA processing/degradation machine in eukaryotic cells. Moreover, given the diversity of roles for the exosome that have already been identified in the past two years, it is almost certain that the exosome will have additional functions that have not yet been determined. Important issues for future work will be to understand the structure and organization of the exosome, how substrates interact with the exosome, and what the role of the Ski2 and Mtr4 ATPases are in exosome-mediated RNA processing or degradation. A combination of genetic analysis of various exosome alleles, structural studies on the exosome, and biochemical analysis of exosome activities should help solve these questions and should further our understanding of self-compartmentalizing degradative enzymes.

Selected Reading

Allmang, C., Petfalski, E., Podtelejnikov, A., Mann, M., Tollervey, D., and Mitchell, P. (1999a). Genes Dev. *13*, 2148–2158.

Allmang, C., Kufel, J., Chanfreau, G., Mitchell, P., Petfalski, E., and Tollervey, D. (1999b). EMBO J. *18*, 5399–5410.

Baumeister, W., Walz, J., Zuhl, F., and Seemuller, E. (1998). Cell *92*, 367–380.

Briggs, M.W., Burkard, K.T., and Butler, J.S. (1998). J. Biol. Chem. *273*, 13255–13263.

Burkard, K.T.D., and Butler, J.S. (2000). Mol Cell Biol., in press.

Carpousis, A.J., Vanzo, N.F., and Raynal, L.C. (1999). Trends Genet. *15*, 24–28.

de la Cruz, J., Kressler, D., Tollervey, D., and Linder, P. (1998). EMBO J. *17*, 1128–1140.

de la Cruz, J., Kressler, D., and Linder, P. (1999). Trends Biochem. Sci. *24*, 192–198.

DeMartino, G.N., and Slaughter, C.A. (1999). J. Biol. Chem. *274*, 22123–22126.

Gottesman, S., Maurizi, M.R., and Wickner, S. (1997). Cell *91*, 435–438.

Jacobs Anderson, J.R., and Parker, R.P. (1998). EMBO J. *17*, 1497–1506.

Kadowaki, T., Chen, S., Hitomi, M., Jacobs, E., Kumagai, C., Liang, S., Schneiter, R., Singleton, D., Wisniewska, J., and Tartakoff, A.M. (1994). J. Cell Biol. *126*, 649–659.

Kinoshita, N., Goebl, M., and Yanagida, M. (1991). Mol. Cell. Biol. *11*, 5839–5847.

Matsumoto, Y., Fischel, R., and Wickner, R.B. (1990). Proc. Natl. Acad. Sci. USA *87*, 7628–7632.

Mian, I.S. (1997). Nucleic Acids Res. *25*, 3187–3195.

Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M., and Tollervey, D. (1997). Cell *91*, 457–466.

Noguchi, E., Hayashi, N., Azuma, Y., Seki, T., Nakamura, M., Nakashima, N., Yanagida, M., He, X., Mueller, U., Sazer, S., and Nishimoto, T. (1996). EMBO J. *15*, 5595–5605.

Qu, X., Yang, Z., Zhang, S., Shen, L., Dangel, A.W., Hughes, J.H., Redman, K.L., Wu, L.C., and Yu, C.Y. (1998). J. Biochem. (Tokyo) *123*, 883–890.

van Hoof, A., Lennertz, P., and Parker, R. (2000). Mol Cell Biol., in press.

Zanchin, N.I., and Goldfarb, D.S. (1999). Nucleic Acids Res. *27*, 1283–1288.