CHAPTER 7

FUNCTIONS OF THE CYTOPLASMIC EXOSOME

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Abstract: The exosome consists of a core of ten essential proteins that includes the ribonuclease Rrp44p and is present in both the cytoplasm and nucleus of eukaryotic cells. The cytoplasmic exosome has been extensively characterized in the budding yeast *Saccharomyces cerevisiae* and some characterization of its metazoan counterpart indicates that most functional aspects are conserved. These studies have implicated the cytoplasmic exosome in the turnover of normal cellular mRNAs, as well as several mRNA surveillance pathways. For this, the exosome needs a set of four proteins that do not partake in nuclear exosome functions. These cofactors presumably direct the exosome to specific cytoplasmic RNA substrates. Here, we review cofactors and functions of the cytoplasmic exosome and provide unanswered questions on the mechanisms of cytoplasmic exosome function.

INTRODUCTION

The eukaryotic exosome is present in both the nucleus and cytoplasm and carries out a variety of RNA processing and degradation reactions. The nuclear and cytoplasmic forms of the exosome contain the same 10 essential subunits as reviewed in other chapters in this book. Also described in other chapters in this book are the various RNA processing and degradation events catalyzed by the nuclear exosome, with the help of a number of nuclear cofactors. In this chapter we will focus on the cytoplasmic roles of the exosome and on four cofactors solely required for cytoplasmic functions of the exosome.

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THE CYTOPLASMIC EXOSOME REQUIRES FOUR COFACTORS

Three genetic screens have been performed that identified cofactors of the cytoplasmic exosome. Two screens for host mutants that affect the killer virus system (see below) identified exosome subunits as well as four proteins named superkiller, or Ski, proteins.[1,2] These four Ski proteins were later shown to be cytoplasmic exosome cofactors.[3,4] The cytoplasmic exosome is also required for the rapid degradation of nonstop mRNAs (see below). A genetic screen of the genome-wide collection of yeast knock-outs for mutants defective in nonstop mRNA decay identified the same four *SKI* genes.[5] In this nonstop mRNA decay screen, a number of other mutants also showed smaller effects on the expression of nonstop mRNAs, but these additional mutations do not appear to be in exosome cofactors. Since the killer virus screen identified many alleles of each of the four *SKI* genes and the genome-wide nonstop decay screen identified the same four genes, it appears that there are only four cofactors of the cytoplasmic exosome. Various experiments have shown that these four Ski proteins are needed for all known functions of the cytoplasmic exosome.[3,6-8] This differs from the nuclear exosome cofactors, which are more numerous and mostly appear to be required for only a subset of exosome functions (with the exception of Mtr4p). An additional difference is that the cytoplasmic exosome cofactors are not required for viability, while many nuclear exosome cofactors and the exosome subunits themselves are essential. This indicates that the activity of the cytoplasmic exosome is not essential, presumably because its role overlaps with that of Xrn1p (see below). Since deletion of the SKI genes does not appear to affect the distribution of the exosome between the nucleus and cytoplasm (A. van Hoof and Roy Parker; unpublished data), the Ski proteins are most likely directly involved in exosome function.

The Ski Complex

Three of the Ski proteins form a Ski complex, consisting of Ski2p, Ski3p and two copies of Ski8p.[9,10] Ski2p is the only Ski protein with a catalytic function, while Ski3p and Ski8p contain motifs thought to be needed for protein-protein interactions. Ski2p is a putative DExH-box RNA helicase and mutating the DEVH motif to AEVA disrupts Ski2p function, suggesting that Ski2p is catalytically active and that this activity is required for its function.[11] Ski2p contains 1287 amino acids and the C-terminal 960 amino acids resemble the nuclear exosome cofactor Mtr4p. The structure of Mtr4p was recently solved and revealed four domains that are shared with a family of eukaryotic and archaeal RNA and DNA helicases (the Ski2-like family of DExH helicases) and thus form a helicase core.[12] In addition, a fifth domain shared between Mtr4p and Ski2p has been termed the arch domain, because it forms an arch-like structure on one side of the helicase core.[12] This arch domain is present in all eukaryotic Ski2p and Mtr4p orthologs, but not in any other protein, distinguishing exosome-associated RNA helicases from other helicases. Although the Mtr4p arch domain is required for exosome-mediated RNA decay and processing, its molecular functions and the function of the Ski2p arch, are not yet clear.[12] Mtr4p and Ski2p also contain a large conserved surface area, which has been suggested to interact with the conserved surface of the cap proteins of the exosome.[12] The N-terminal 320 residues of Ski2p do not show any sequence similarity with Mtr4p and likely provide functions required for Ski2p, but not Mtr4p. Consistent with this, Wang et al.[11] used yeast two-hybrid and co-immunoprecipitation experiments to show that this region of Ski2p is necessary and sufficient for interaction with Ski3p and Ski8p. Therefore, the Ski complex

seems to have a general helicase core and two accessory domains. The first accessory domain is an arch domain that is shared only with exosome-associated RNA helicases, while the second accessory domain is a Ski complex-specific part and consists of Ski3p, two copies of Ski8p and the N-terminus of Ski2p.

Although the function of cytoplasmic exosome cofactors has not been extensively studied in other eukaryotes, such as humans, the available evidence suggests that the function of all four is conserved in Metazoans. The *Drosophila* Ski2p (a.k.a. twister), Ski3p and Ski8p are required for cytoplasmic 3′ to 5′ mRNA degradation and thus carry out essentially the same function as the yeast orthologs.[13-15]

Most eukaryotes, including humans, have two RNA helicases of the Mtr4p/Ski2p group with one more closely resembling Ski2p and the other more closely resembling Mtr4p (unpublished observations), however the very early diverging genera *Trypanosoma*, *Leishmania* and *Giardia* have only one recognizable ortholog that is most similar in sequence to Mtr4p (unpublished observations and ref. 16). It is not entirely clear whether, in these species, one protein carries out both the Mtr4p and Ski2p function, or whether they only need Mtr4p or Ski2p function. The *T. brucei* protein resembles Mtr4p in that when overexpressed and tagged it is mainly nuclear,[16] it forms a TRAMP complex[17] and knocking down its expression causes a defect in rRNA processing.[16] In addition, *T. Brucei* lacks readily identifiable orthologs of Ski3p and Ski8p. Thus, while most eukaryotes have a Ski complex, *T. brucei* may lack Ski complex function. Interestingly, the exosome in *T. brucei* is mostly cytoplasmic[18] and thus may function independently of a Ski complex.

Ski7p

In addition to the Ski complex, the cytoplasmic exosome also requires Ski7p, which is composed of two functional domains. The N-terminus of Ski7p interacts with the cytoplasmic exosome and the Ski complex[19] and is needed for exosome-mediated degradation of both normal and nonstop mRNAs.[8,19] Interestingly, this region appears unique to Ski7p orthologs and is not similar to any known protein. The C-terminal domain of Ski7p is homologous to the translation factors eEF1A and eRF3[4,19,20] and is important in nonstop mRNA decay (see below; ref. 8).

While the evolutionary history of the Ski complex is straightforward, the yeast *SKI7* gene has a peculiar history. About 100 million years ago, the ancestor of yeast duplicated its genome. Subsequently, most of the duplicated genes were lost, but both *SKI7* and its paralog *HBS1* were maintained.[21,22] As a consequence of this duplication, *S. cerevisiae* and its close relatives have both a *SKI7* gene and an *HBS1* gene, but most other eukaryotes have only one corresponding gene that presumably performs the functions of both *SKI7* and *HBS1*. Consistent with this, the single *S. kluyveri* gene can perform both *SKI7* and *HBS1* function.[23] In addition, knocking down the expression of the *Drosophila HBS1/SKI7* ortholog inhibits cytoplasmic exosome function, although to a lesser extent than knocking down a component of the Ski complex (leading to a 2-fold increase in the abundance of an mRNA decay intermediate, compared to a 10-fold increase after Ski complex knockdown; ref. 14).

In conclusion, most, but not all, eukaryotes have orthologs of the four *SKI* genes identified in yeast and these orthologs presumably carry out the same functions. The function of the yeast *SKI* genes has been characterized using genetics and based on these analyses, the Ski complex and Ski7p are thought to recruit the cytoplasmic exosome to specific RNA substrates. However, their biochemistry and how they act on the molecular level

is still largely unexplored. The structures of Mtr4p[12] and Ski8p[24] and future biochemical approaches should be very useful in increasing our understanding of the function of the cytoplasmic exosome cofactors.

THE EXOSOME FUNCTIONS IN ONE OF TWO GENERAL PATHWAYS FOR CYTOPLASMIC mRNA DEGRADATION

Two general pathways of mRNA degradation have been identified using *S. cerevisiae* as a model system and both pathways appear conserved in most other eukaryotes (Fig. 1). The initiating and rate-limiting step in both pathways is the shortening of the poly(A) tail in a process termed deadenylation. Following deadenylation, transcripts can be degraded from their 5′- and 3′-ends. The 5′ to 3′ mRNA decay pathway is initiated by removal of the 5′ cap by Dcp2p.[25-27] This exposes the 5′-end of an mRNA to degradation by the

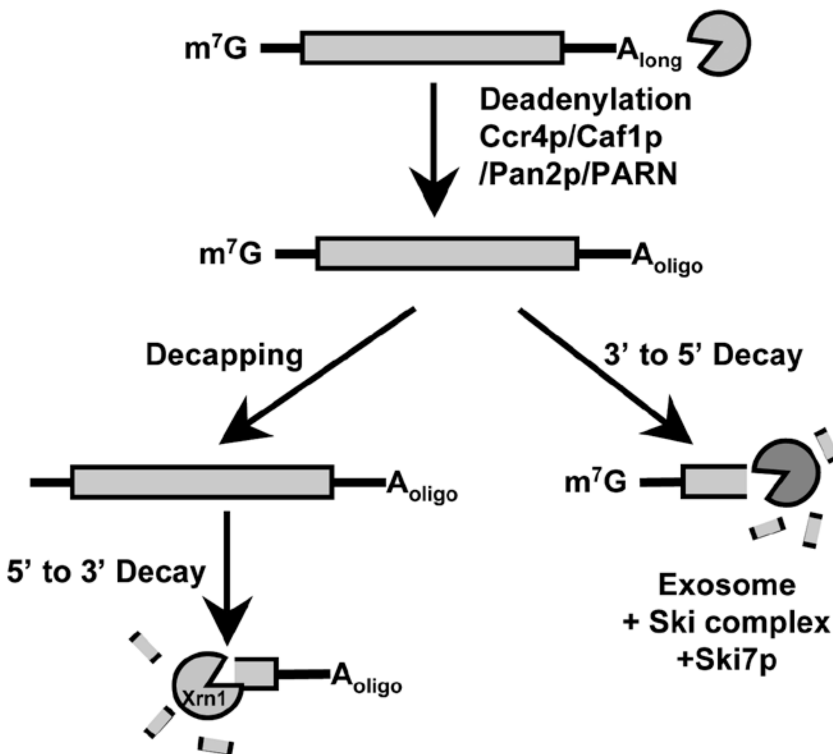

Figure 1. The degradation of eukaryotic mRNAs is generally initiated by removal of the poly(A) tail, which can be carried out by various deadenylases (Ccr4p, Caf1p, Pan2p and/or PARN). Deadenylation can be followed by removal of the cap structure, which makes the RNA susceptible to the 5′ to 3′ exoribonuclease Xrn1p. Alternatively, deadenylation can be followed by 3′ to 5′ degradation by the exoribonuclease activity of the cytoplasmic exosome. This activity of the exosome also requires Ski7p and the Ski complex.

5′ to 3′ exoribonuclease Xrn1p.[12,28-30] Deadenylation can also trigger the degradation of an mRNA by the cytoplasmic exosome.[3] Following exosome-mediated decay of the body of the transcript, the scavenger decapping enzyme, Dcs1p in yeast and DcpS in humans, removes the 5′ cap from the remaining oligonucleotide.[31,32]

These two general decay pathways are redundant and mRNAs can be degraded either by the decapping or the exosome pathway. Consistent with this, neither pathway is essential for yeast viability, but simultaneous inactivation of both mRNA degradation pathways is lethal.[3,33] Although mRNAs can be degraded by either pathway, normal yeast mRNAs are mostly degraded through the decapping pathway, with the exosome making a smaller contribution. This conclusion is based on the observation that several normal cellular mRNAs are stabilized in mutants of the decapping enzyme or of Xrn1p, but not in mutants defective in cytoplasmic exosome function. Careful determination of key decay characteristics suggests that exosome-mediated decay is about 2-5 fold slower than decapping-mediated decay in yeast.[34] Quantification of this difference required the use of null mutants that are unavailable in most other organisms, thus it is not clear whether this 2-5 fold rate difference is conserved, or whether there are conditions or other eukaryotic cells where the relative importance is reversed.

The exosome contains both endoribonuclease and 3′ to 5′ exoribonuclease domains[35-37] and the 3′ to 5′ exoribonuclease activity is the major contributor to the exosome's mRNA decay function. This conclusion is based on two observations. First, a mutation that inactivates the 3′ exoribonuclease domain leads to accumulation of mRNA decay intermediates (in a strain background also lacking decapping activity; ref. 38). Second, this same 3′ exoribonuclease mutation is synthetic lethal with deletion of the 5′ to 3′ exoribonuclease Xrn1p, while a mutation inactivating the endoribonuclease activity of the exosome is not.[37]

Although all mRNAs are subject to both mRNA decay pathways, individual mRNAs are degraded at different rates. For example, mammalian transcripts that contain AU-Rich Elements (ARE) in their 3′ untranslated region are more rapidly degraded.[39,40] These AREs are recognized by ARE-binding proteins, including KSRP and TTP, which also interact with the exosome to promote rapid mRNA degradation.[41-43] Thus, sequence-specific RNA binding proteins can accelerate the decay of specific mRNAs by recruiting the exosome to these mRNAs.

THE CYTOPLASMIC EXOSOME FUNCTIONS IN mRNA SURVEILLANCE

In addition to degrading "normal" cellular transcripts and mRNAs with specific sequences, the cytoplasmic exosome is also involved in the quality control of mRNAs in the cytoplasm. In these specialized mRNA surveillance pathways, exosome cofactors and adaptor proteins distinguish aberrant mRNAs from normal mRNAs and ultimately direct these aberrant transcripts to the exosome for rapid degradation. Most importantly, the cytoplasmic exosome is required for the rapid degradation of mRNAs that lack a stop codon. The cytoplasmic exosome also contributes to several other mRNA surveillance pathways, including the degradation of mRNAs with premature stop codons and mRNA fragments that are generated by various endoribonucleases. Not surprisingly, the rate-limiting step of deadenylation is bypassed in these mRNA surveillance pathways to rapidly rid the cell of these potentially harmful transcripts.

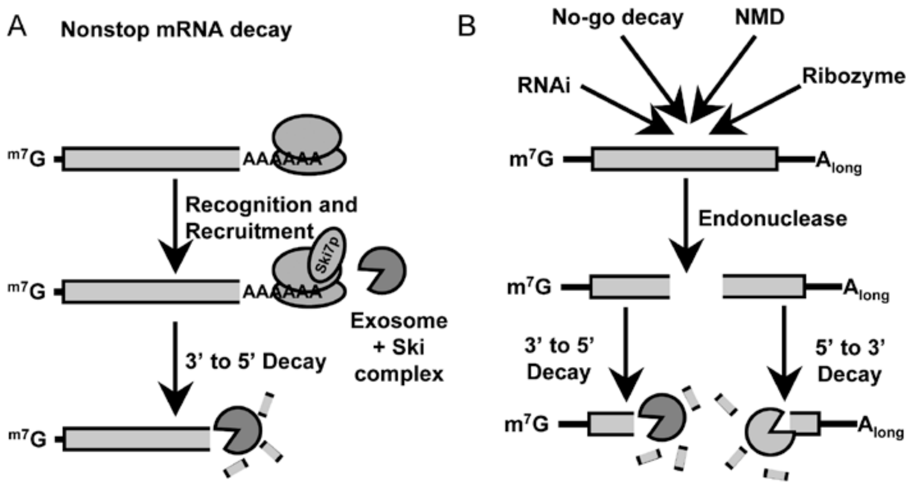

Figure 2. A) mRNAs that lack a stop codon are recognized by the C-terminal domain of Ski7p. This recruits the exosome and the Ski complex, which mediates their degradation. B) A variety of triggers can cause an mRNA to be cleaved by an endonuclease. The resulting 5′ cleavage products are degraded by the cytoplasmic exosome. This activity of the exosome also requires Ski7p and the Ski complex.

Nonstop mRNA Degradation

Mutations that inactivate the cytoplasmic exosome have little effect on the stability of normal mRNAs, but dramatically stabilize mRNAs that lack a stop codon.[8,44] These types of transcripts can arise from mistakes in gene expression, including genetic mutations, defects in transcription, or premature polyadenylation, due to inaccurate 3′ end formation or through the use of a cryptic polyadenylation site. In the current model of nonstop mRNA decay, the translating ribosome reads through the poly(A) tail and stalls at the 3′ end of the mRNA (Fig. 2A). Based on its homology to translation factors, the C-terminus of the cytoplasmic exosome cofactor Ski7p is thought to recognize the stalled ribosome with an empty A-site. Consistent with this, deletion of this C-terminal domain stabilizes nonstop mRNAs, but does not affect other exosome functions. Along with the Ski complex, Ski7p recruits the exosome to rapidly degrade the transcript from the 3′-end.[8] For a more detailed review of nonstop mRNA decay see Wilson et al.[45]

Nonsense-Mediated mRNA Degradation (NMD)

mRNAs that contain a premature nonsense codon are rapidly degraded by an mRNA surveillance pathway called nonsense-mediated mRNA degradation (NMD). How these mRNAs are distinguished from normal mRNAs is not yet clear, although the spatial relationship between the termination codon and various other features of the mRNA have been implicated.[46-49] The recognition and/or decay of these NMD targets requires a set of three Upf proteins that are conserved in most eukaryotes.[50-54] In yeast, NMD targets are predominantly degraded by decapping and 5′ to 3′ decay.[55] Nonsense transcripts are also degraded, to a lesser extent, by the cytoplasmic exosome.[7,56] The key observation

supporting this conclusion is that nonsense mRNAs are more stable in *xrn1*Δ *upf1*Δ or *dcp1-2 upf1*Δ double mutants than in *xrn1*Δ or *dcp1-2* single mutants.[7,56]

Degradation of Endoribonuclease Products

While eukaryotes contain two general mRNA decay pathways that are mediated by exoribonucleases, in specific cases mRNA decay can be initiated by endoribonuclease cleavage (Fig. 2B). One example of mRNA decay initiated by endonucleolytic cleavage is found in the NMD pathways in *Drosophila* and humans.[13,57,58] Here, nonsense transcripts are endonucleolytically cleaved by SMG6, which generates a 5′- and 3′-degradation fragment. The 5′-fragment is degraded by the cytoplasmic exosome, in a manner that also requires the Ski complex. The 3′-degradation fragment is degraded by the 5′ to 3′ exoribonuclease Xrn1p.[13,57,58]

A second example of mRNA decay that is initiated by endonucleolytic cleavage is a process termed no-go decay. No-go decay is triggered by translational pauses caused by secondary structures such as stem loops in the mRNA, but can also be activated to a lesser extent by pseudoknots and rare codons.[6] In the current model of no-go decay, Dom34p and Hbs1p promote, but are not absolutely required for, the endonucleolytic cleavage of the transcript in the vicinity of the stalled ribosome. After cleavage, 5′ and 3′ fragments of the translational stall site are released.[6] The 5′-fragment is degraded by the exosome and this reaction also requires the Ski complex and Ski7p, while the 3′-fragment is degraded by Xrn1p.

A third example of mRNA decay that is initiated by endoribonucleolytic cleavage is RNAi. In RNAi, double-stranded RNA (dsRNA) is processed by Dicer into 21-22 nucleotide small interfering RNAs (siRNAs).[59-62] These siRNAs are then incorporated into the RNA-induced silencing complex (RISC), which can endonucleolytically cleave target transcripts at a site complementary to the siRNA.[63,64] The 5′- and 3′-fragments generated by endonucleolytic cleavage in RNAi in *Drosophila* cells are degraded by the cytoplasmic exosome and Xrn1p, respectively. The 3′ decay of the 5′-fragment also requires the Ski complex.[14]

Finally, endoribonucleolytic cleavage can be initiated by hammerhead ribozymes. This can either occur because of natural ribozyme sequences in an mRNA,[65,66,67] or because of artificially introduced ribozymes. Meaux and van Hoof[68] used either a hammerhead ribozyme, or a mutated group I intron to artificially generate cleaved mRNAs in yeast. The 5′ cleavage product of these reactions was degraded by the cytoplasmic exosome, with the help of cytoplasmic exosome cofactors. These 5′ cleavage products were very unstable in wild-type cells (half-life 1 minute). In most of the other endoribonucleolytic cleavage reactions mentioned above, the cleavage products are not detectable unless the activity of the cytoplasmic exosome is reduced, suggesting that the degradation of these cleavage products by the exosome is also very rapid.

THE ANTIVIRAL FUNCTION OF THE CYTOPLASMIC EXOSOME

Many yeast strains contain the L-A virus and the M satellite RNA. The M satellite RNA encodes a protein toxin that is secreted from infected cells that kills uninfected cells. Thus, this toxin gives infected cells an advantage in their competition with uninfected cells for nutrients. Mutants in the *SKI* genes were discovered as "superkiller" mutants,

or mutants that had an increase in viral toxin production.[2] Specifically, it was shown that disruption of any of the *SKI* genes resulted in an increase in viral copy number and increased toxin production.[1,69,70] These observations suggest that the Ski proteins have an antiviral function and regulate the replication of RNA viruses.[71] It was first hypothesized that the Ski proteins inhibit translation of nonpoly(A) viral mRNA. This hypothesis was based on the observation that only RNA that lacked a poly(A) tail were affected by *ski* mutations.[71,72] In addition, Ski7p is homologous to translation factors, suggesting that at least Ski7p has a role in translation.[20] More recent studies, however, have shown that a major function of the Ski proteins is in the decay of cytoplasmic mRNAs (see above). From these results an alternative mechanism for the Ski genes appears more likely: Instead of regulating translation, the Ski proteins target mRNAs that lack a poly(A) tail, including viral RNA, for exosome-mediated degradation. An increase in viral RNA stability would allow an increase in toxin production, thus explaining the superkiller phenotype. The M and L-A viral mRNAs resemble the endoribonuclease cleavage products discussed above, since both lack a poly(A) tail. This raises the possibility that recognition of endoribonuclease cleavage products and viral RNAs uses the same molecular mechanism. If such a common mechanism exists, it may have initially evolved as an antiviral defense, or as an mRNA surveillance pathway to correct errors in gene expression. A deeper understanding of the recognition mechanism is required to provide insight in this area.

Eukaryotes have numerous pathways that recognize viral RNAs and trigger antiviral defenses. Typical eukaryotic mRNAs are single-stranded, have a 5′ cap and a 3′ poly(A) tail. Host cells initiate innate immune defense pathways in response to RNAs that lack these specific characteristics. Examples of this include the recognition of dsRNA by toll-like receptors, by MDA5, by PKR and by 2-5A synthetase in mammalian cells (reviewed in refs. 73-75). Other eukaryotes use the RNAi machinery to recognize dsRNA and defend against viruses (e.g., refs. 76, 77-79). The human RIG-I recognizes uncapped RNAs that end in a triphosphate, possibly with some sequence specificity.[80-82] The exosome-mediated decay of unadenylated mRNAs may similarly be considered an innate immune pathway. Whether viral mRNAs in other eukaryotes are similarly targeted is unknown.

CONCLUSION AND FUTURE PERSPECTIVES

The cytoplasmic exosome plays a dual role in eukaryotic gene expression. First, the exosome regulates gene expression by participating in general mRNA turnover to degrade transcripts that are no longer needed. Second, the exosome acts as a quality control mechanism to maintain the fidelity of gene expression by rapidly degrading aberrant transcripts. Specifically, the cytoplasmic exosome degrades transcripts that lack termination codons in the nonstop mRNA decay pathway and contributes to the degradation of transcripts that have premature termination codons or that have been cleaved. Importantly, the Ski complex and Ski7p in yeast, are needed for both general mRNA degradation and for mRNA surveillance in the cytoplasm.

Despite extensive studies of the cytoplasmic exosome in mRNA degradation, important questions remain unanswered. First, how is the exosome recruited to RNA substrates? In each of the mRNA surveillance pathways described here, one or more proteins recognize an aberrant RNA, which is then targeted for rapid degradation by the exosome. It is not yet known how these proteins and exosome cofactors recruit and/or potentially activate the nuclease activities of the exosome. Second, what is the relationship between the

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cytoplasmic and nuclear exosome? Does the exosome shuttle between the two compartments, or are there separate pools of nuclear and cytoplasmic exosomes? Where is the exosome assembled and how is it targeted to the nucleus, cytoplasm and nucleolus? Third, is the recently characterized endoribonuclease activity of the Rrp44p PIN domain involved in any of the functions of the cytoplasmic exosome? Fourth, are there any proteins that protect exosome substrates from degradation? All of the proteins and cytoplasmic exosome cofactors described here aid in mRNA destabilization. However the data reviewed here, do not exclude the possibility that there are proteins that stabilize cytoplasmic exosome substrates. Fifth, is the cytoplasmic exosome involved in other cytoplasmic RNA surveillance pathways? Future studies are needed to answer these questions.

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NOTE ADDED IN PROOFS

A second paper describing the Mtr4p structure was recently published, which confirms the domain structure of Mtr4p and Ski2p.[83]

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