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REVIEW ARTICLE

Nuclear RNA surveillance: no sign of substrates tailing off

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Abstract

The production of cellular RNAs is tightly regulated to ensure gene expression is limited to appropriate times and locations. Elimination of RNA can be rapid and programmed to quickly terminate gene expression, or can be used to purge old, damaged or inappropriately formed RNAs. It is elimination of RNAs through the action of a polyadenylation complex (TRAMP), first described in the yeast *Saccharomyces cerevisiae*, which is the focus of this review. The discovery of TRAMP and presence of orthologs in most eukaryotes, along with an increasing number of potential TRAMP substrates in the form of new small non-coding RNAs, many of which emanate from areas of genomes once thought transcriptionally silent; promise to make this area of research of great interest for the foreseeable future.

Keywords: *RNA degradation; RNA processing; polyadenylation; polyA tail; exosome; transcriptome*

Introduction

Transcription of genomic DNA into RNA produces a nucleic acid that must go through a series of processes before it can become mature and function properly. For example, messenger RNA undergoes capping, splicing, 3′ end cleavage and maturation. Three of the four ribosomal RNAs (rRNA) are transcribed as a polycistronic RNA that is then processed via endonucleolytic cleavage and exonucleolytic trimming to create large and small rRNAs. Transfer RNA (tRNA) is produced as a precursor with 5′ and 3′ end extensions and sometimes intervening sequences that are enzymatically removed during maturation. Small nuclear RNA (snRNA) and small nucleolar RNA (snoRNA) are transcribed as precursors independently or as part of a larger and unrelated transcription unit and nucleolytically processed to remove sequences unimportant for RNA structure or function. The discovery of many small noncoding RNAs (ncRNAs), including miRNAs, siRNAs and numerous others that do not fall in the first two classes, has fueled a great many studies into how these ncRNAs are expressed, processed and function in cellular metabolism. It is not unexpected that mistakes will occur during synthesis and processing of all RNAs, but how these mistakes are recognized and determine an RNA's fate of repair or destruction is just beginning to be uncovered.

Tramp

Trf4p/Air2p/Mtr4p polyadenylation complex (TRAMP) was first identified in the single cell eukaryote *Saccharomyces cerevisiae* as an oligomeric protein complex using Mtr4p as bait in a two-hybrid screen (LaCava et al., 2005), where proteins that physically interact with Mtr4p *in vivo* can be identified, and as a complex that copurified with affinity tagged Trf4p from yeast whole cell extract (Vanacova et al., 2005). Other proteins that were found to interact with Trf4p are Hul4p, a HECT domain protein that is a putative E3 ubiquitin ligase and Prp16p, an ATP-dependent RNA helicase required for pre-mRNA splicing (Schwer and Guthrie, 1991). Several studies have demonstrated a functional relationship between Trf4/5p, Mtr4p and Air2/1p, but no biological relevance has been described for Trf4p interaction with Hul4p or Prp16p.

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Table 1. The two best BLAST matches with Trf4p from *S. cerevisiae* from *Homo sapiens* (h), *Mus musculus* (m) and *Bos taurus* (b). The Trf4.1, Trf4.2 designation is arbitrarily assigned.

Protein	Database ID refseq	Gene ID/chrom	Protein length amino acids	% identity yTrf4/yTrf5
yTrf4p	CAA99134	15	589	100/47
yTrf5p	CAA96217	14	625	47/100
hTrf4.1	NP 008930.1	11044/5	542	18/17
hTrf4.2	NP 001035374/5.1	64282/16	442/489*	20/20
mTrf4.1	XP 134422.8	214627/8	680	21/22
mTrf4.2	NP 941002	210106/16	542	18/18
bTrf4.1	XP 001256516	789894/18	467	21/21
bTrf4.2	XP 601308.3	523016/20	853	18/19

* denotes the presence of an additional exon in the mRNA coding for 47 additional amino acids.

TRAMP exists in two forms, TRAMP4 (Trf4p, Air2/1p, Mtr4p) (LaCava et al., 2005; Vanacova et al., 2005; Wyers et al., 2005) and TRAMP5 (Trf5p, Air1p, Mtr4p) (Houseley and Tollervey, 2006) and each one contributes to RNA surveillance in the nucleus/nucleolus of yeast cells. The TRAMP complexes can be considered cofactors of the nuclear exosome, a multi-subunit complex (Mitchell et al., 1997) that functions to process or degrade RNAs.

Trf4/5p

DNA topoisomerase I related function (Trf4p) was initially discovered and characterized in a synthetic lethal screen with a topoisomerase I conditional null mutant (Sadoff et al., 1995). Subsequent studies demonstrated that *S. cerevisiae* also contains a structurally and functionally related protein to Trf4p, Trf5p (Castano et al., 1996). The Trf proteins of *S. cerevisiae* are members of a large family of nucleotidyltransferases (Aravind and Koonin, 1999), and because of several DNA synthesis-related phenotypes of *trf4* mutants and the enzymatic activity of purified Trf4p, Trf4p was renamed DNA polymerase Kappa, polκ (Wang et al., 2000). Recent studies have been unable to reproduce the initial reports of Trf4p polymerizing DNA strands (Haracska et al., 2005; Vanacova et al., 2005), so its role as a DNA polymerase is in question. In a series of elegant genetic experiments, it was shown that *trf4*^{ts} *trf5Δ* and *rrp6Δ* triple mutants accumulate DNA replication-dependent histone mRNAs to unusually high levels, leading to histone subunit imbalance and genome instability during the cell cycle (Reis and Campbell, 2007). The involvement of Trf4/5p in maintaining histone stasis begins to explain why phenotypes attributed to *trf4* mutants initially supported its role as a DNA polymerase.

Trf4 and Trf5p are 65% identical in *S. cerevisiae* and it is likely they evolved after genome duplication and gene specialization (Kellis et al., 2004). The presence of highly related Trf proteins from yeast to humans is well documented (Aravind and Koonin, 1999), but only Cid14p from *Scchizosaccharomyces pombe* has been shown to be functionally homologous to the Trf proteins from *S. cerevisiae* (Saitoh et al., 2002; Win et al., 2006). *S. pombe*, a yeast distantly related to *S. cerevisiae*, contains several nucleotidyltransferases that resemble canonical polyA polymerases, all of which have been attributed to some aspect of RNA metabolism in the nucleus or cytoplasm, reviewed in Stevenson and Norbury (2006). One of these polyA polymerases, Cid13p, is required to maintain normal levels of dNTPs by stabilizing ribonucleotide reductase mRNA, and it was also shown to possess polyadenylation activity *in vitro*, along with its *S. cerevisiae* structural homolog, Trf4p (Saitoh et al., 2002). Cid14p possesses good sequence homology to *S. cerevisiae* Trf4/5p, complements growth defects of a strain bearing a *trf4*^{ts} *top1Δ* double mutation and is found in a complex including *S. pombe* Mtr4p and Air2p (Win et al., 2006). The role of Trfs in all other organisms is unknown, but the conservation of two Trfs (Table 1) in most mammals suggests strongly that there will be functional similarities between the Trfs of *S. cerevisiae* and those found in higher eukaryotes.

Air1/2p

Air1/2p, like Trf4/5p, are expressed from distinct genes as remnants of the whole genome duplication that occurred in *S. cerevisiae* (Kellis et al., 2004). *AIR1* was originally identified in a two-hybrid screen for proteins that physically interact with the RGG domain of Npl3p, an mRNA binding protein involved in messenger RNA 3′ end formation and export (Shen et al., 1998; Inoue et al., 2000). Air1/2p share 45% amino acid identity and have five repeated zinc-binding ring finger domains which make up approximately 30% of the protein. Available protein sequence data suggest that *S. pombe* has one Air protein, and the best Air protein candidate from Mus musculus indicates a different organization of two out of five putative zinc binding domains (Figure 1). Mouse Airp has the same zinc-binding domain size (14 amino acids), but only three of the five zinc domains maintain the $Cx_2Cx_4Hx_4C$ architecture (Figure 1), calling to question whether this is a true ortholog of *S. cerevisiae* Air1/2p. Ring finger/knuckle proteins have a wide range of functions, one of which is nucleic acid binding, but it is unknown whether the ring fingers motifs in Air1/2p

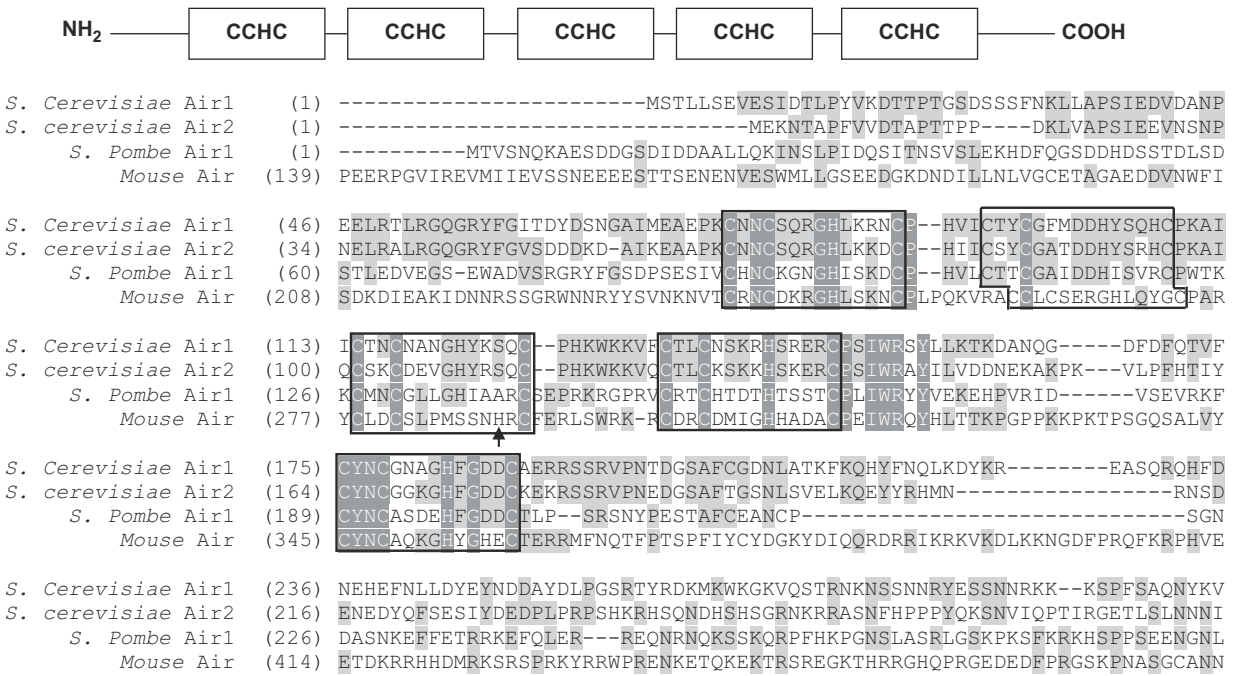

Figure 1. An alignment of Air proteins from *Schizosacharomyces pombe* and *Mus musculus* that were obtained by BLAST using *Saccharomyces cerevisiae* Air2p as a query. The potential zinc-binding domains (boxes) are indicated for each protein and the amino acid identities are shaded. *Mus musculus* Air has two isoforms; shown is isoform 1.

bind nucleic acids. Air1p and Air2p form stable complexes with Trf4p/Trf5p and Mtr4p (LaCava et al., 2005; Vanacova et al., 2005; Wyers et al., 2005), but the role of Air1/2p in TRAMP has not been determined, although it has been shown that recombinant Trf4p is polyadenylation incompetent unless pre-incubated with Air1/2p (Vanacova et al., 2005).

Npl3p is methylated by Hmt1p (McBride et al., 2005), and Air1p was shown to physically interact with Hmt1p and inhibit methylation of Npl3p *in vitro* (Inoue et al., 2000). The methylation of Npl3p by Hmt1p facilitates its nucleocytoplasmic export, presumably as an mRNP (McBride et al., 2005). If TRAMP localizes along with the exosome to transcriptionally active protein coding genes, as has been suggested (Hilleren et al., 2001; Saguez et al., 2008; Vodala et al., 2008), Hmt1p methylation of Npl3p could be inhibited by Air1/2p, and thereby block or reduce the maturation and export of aberrant mRNAs that then become substrates of TRAMP-mediated nuclear surveillance (Figure 2).

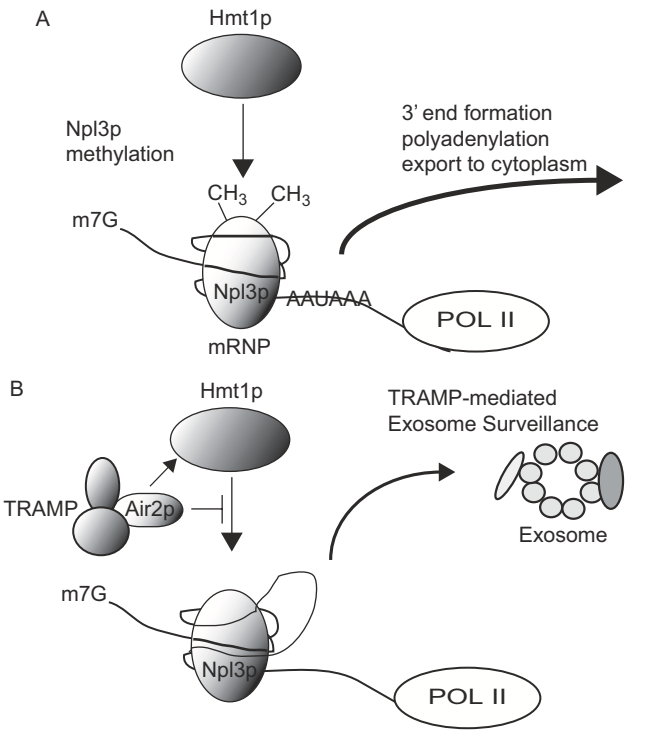

Figure 2. A model depicting TRAMP-mediated surveillance of mRNA. (A) Pre-mRNA transcription, 3′ end formation and polyadenylation are shown, and after successful processing the mRNP is exported to the cytoplasm. (B) In cases of improper processing, incomplete or aberrant packaging, TRAMP-mediated surveillance inhibits export by blocking Npl3p methylation by Hmt1p. The inhibition of Npl3p methylation could lead to TRAMP mediated exosome degradation.

Mtr4p

Mtr4p/Dob1p was identified by two contemporaneous studies. First, a genetic screen in yeast designed to identify proteins required for mRNA nucleocytoplasmic transport found genes *mtr1-mtr17* that accumulate nuclear polyA+ RNA at a non-permissive temperature (Kadowaki et al., 1994). Independently, *MTR4/DOB1* was identified as a

mutation that requires overexpression of translation initiation factor 4B, yeast *TIF3*, for survival (de la Cruz et al., 1998). Mtr4p is a member of the DExH box RNA helicase superfamily 2 (SF2), which contains the majority of known RNA helicases and is further organized into subfamilies based on the conserved motif DExD/H. For an extensive review of helicases see Tanner and Linder (2001) and the references therein. Mtr4p is in the same subfamily as the highly homologous (38% identity) Ski2p RNA helicase, both of which contain DEVH in the signature motif II, one of six motifs organized into two domains that have been used to identify putative RNA helicases. The homology of Ski2p with Mtr4p is not just structural; Ski2p functions to facilitate exosome-dependent mRNA turnover (Anderson and Parker, 1998), but in the cytoplasm, rather than the nucleus, where Mtr4p functions in RNA processing and degradation by the nuclear exosome. Mtr4p possesses RNA-dependent ATPase activity (Bernstein et al., 2008; Wang et al., 2008) and can also hydrolyze dATP but not other NTPs (Bernstein et al., 2008; Wang *et al.*, 2008). In the presence of ATP/dATP Mtr4p can displace RNA or DNA from an RNA–RNA or RNA–DNA duplex where the RNA strand has at least three unpaired nucleotides, but it cannot displace DNA or RNA from a duplex where the DNA strand contains unpaired nucleotides (Anderson, unpublished data). Mtr4p helicase activity is restricted to a 3′–5′ direction *in vitro* (Wang et al., 2008), which is consistent with its role in facilitating RNA processing/degradation by the nuclear exosome. It is not clear whether Mtr4p exists outside of the TRAMP complex in cells, meaning studies of Mtr4p alone do not necessarily reflect how it functions in the context of TRAMP.

TRAMP nuclear surveillance and processing substrates

TRAMP-mediated exosome processing and degradation of RNA is well established, but there still remains a question of how substrates are identified and marked for processing or degradation by the nuclear exosome. There is also some confusion about the role of TRAMP-mediated polyadenylation; whether it signals turnover, processing or both. It seems likely that polyadenylation by TRAMP may serve more than one function, leading to degradation or processing, depending on the RNA substrate. This section is devoted to describing the known substrates of TRAMP-mediated nuclear RNA surveillance, and a model of how TRAMP and the exosome may distinguish between RNAs to be degraded or processed is proposed.

The exosome

The core exosome, identical in the nucleus and cytoplasm, is composed of nine subunits that form a ring structure (Liu et al., 2006) and one subunit, Rrp44p/Dis3p, that is associated on an outside surface away from the pore formed by the other nine subunits (Wang et al., 2007). The ring pore size is too small to allow entry of highly structured RNAs, so it has been postulated that TRAMP provides a single strand adenosine polymer that can easily fit into the exosome pore. During exonucleolytic degradation of the polyA tail, Mtr4p would remove highly ordered RNA structures following the primer polyA tail that would otherwise preclude its entrance into the pore. This working model of exosome structure/function came under question when Rrp44p was shown to be: (1) the only active exonuclease in the yeast core exosome (Dziembowski et al., 2007); (2) sufficient to recognize and degrade TRAMP adenylated hypomodified $tRNA_i^{Met}$ *in vitro* (Schneider et al., 2007); and (3) not near enough to the pore to support such a model (Wang et al., 2007). In addition to the core subunits, the nuclear exosome also contains the 3′–5′ exonuclease Rrp6p and the accessory factors Rrp47p and Mpp6p (Mitchell et al., 2003; Milligan et al., 2008). Rrp47p and Mpp6p each play roles in processing and degradation of some, but not all, RNAs known to be exosome substrates, implying accessory factors may provide a means to limit processing or degradation to appropriate RNAs.

Exosome accessory factors

Purified exosome complexes do not possess robust exonuclease activity *in vitro* and this led to a search for factors that could stimulate its degradation activity. TRAMP4 stimulated *in vitro* degradation of small RNA substrates, including tRNA, by the exosome (LaCava et al., 2005; Vanacova et al., 2005); more recently, recombinant Nrd1p, a member of the Nrd1p-Nab3p alternative 3′ end processing pathway of Pol II transcripts (Steinmetz and Brow, 1998; Conrad et al., 2000; Steinmetz et al., 2001), was shown to recruit the exosome and stimulate degradation of transcripts bearing Nrd1p binding sites (Vasiljeva and Buratowski, 2006). Depleting any one of the exosome core subunits, Rrp6p or Mtr4p/Dob1p, results in accumulation of 3′ extended and polyadenylated snRNA and snoRNA (Allmang et al., 1999; van Hoof et al., 2000b), but at the time it was not understood if these represented processing intermediates or polyadenylated RNAs stalled in a degradation pathway. It was not a surprise that these RNAs had polyA tails since they are both products of RNA polymerase II and should be polyadenylated by the canonical polyA polymerase Pap1; this was further supported by the finding that most polyadenylated forms of snoRNAs in an *rrp6Δ* strain lost their A tails at 37°C in strains also carrying a *pap1-1* temperature-sensitive allele (van Hoof et al., 2000b). More recently, it was shown that polyadenylation of the 3′ extended U4 snRNA is dependent

on Trf4p, challenging the original observation. It was concluded from that study that Trf4p-dependent polyadenylation plays an important role in the processing of U4 snRNA (Egecioglu et al., 2006).

TRAMP4 and TRAMP5

TRAMP exists in two forms – TRAMP4 is composed of Trf4p, Air2p, and Mtr4p, whereas TRAMP5 is composed of Trf5p, Air1p, and Mtr4p (Houseley and Tollervey, 2006). TRAMP4, for the most part, seems to be involved in the surveillance of RNAs that are generated in the nucleoplasm, which is consistent with the localization of Trf4p to the nucleus (Huh et al., 2003), but TRAMP5 appears to be devoted more to surveillance of nucleolar produced/localized RNAs, again consistent with localization of Trf5p predominantly to the nucleolus (Huh et al., 2003). The localization of Trf4p and Trf5p may not be limited to their respective compartments, as there are examples where deletion of the corresponding gene causes accumulation of RNAs that are not processed in the nucleolus (Trf5p) or the nucleus (Trf4p) (Egecioglu et al., 2006; Houseley and Tollervey, 2006). Interestingly, a prediction of protein levels in cells is consistent with Trf4p (7550 copies/cell) playing a larger role in RNA surveillance than Trf5p (2240 copies/cell) (Ghaemmaghami et al., 2003), and this has experimental support (Egecioglu et al., 2006; Kadaba et al., 2006). Oddly, overexpression of Trf5p can replace Trf4p to restore degradation of a hypomodified $tRNA_i^{Met}$, even though deletion of *TRF5* does not effectively block degradation of hypomodified $tRNA_i^{Met}$ to the same extent as deleting *TRF4* (Kadaba et al., 2006). Are there strictly limited roles for Trf4p and Trf5p in RNA surveillance? One can imagine a scenario where Trf4p is the main polyA polymerase in TRAMP-mediated surveillance and Trf5p, due to its reduced abundance and its nucleolar localization, specializes in surveillance of rRNAs. The polyA polymerase activities of Trf4p and Trf5p are presumably identical and this might explain why overexpression of Trf5p eliminates the requirement of Trf4p in tRNA surveillance, if Trf5p can readily form TRAMP complexes with the other members of TRAMP4 – Air2p and Mtr4p.

Transfer RNA

Nuclear RNA surveillance through the activities of TRAMP and the exosome was first described as a mechanism for eliminating hypomodified initiator $tRNA^{Met}$ ($tRNA_i^{Met}$) (Kadaba et al., 2004; 2006). In yeast lacking a functional tRNA 1-methyladenosine (m^1A) methyltransferase (*TRM6*) (Anderson et al., 2000), all tRNAs are missing the m^1A modification (hypomethylated) (Anderson et al., 1998). The steady-state level of hypomethylated $tRNA_i^{Met}$ is greatly diminished, probably due to a destabilization of a tertiary structure in $tRNA_i^{Met}$ that is believed to be unique to this tRNA (Basavappa and Sigler, 1991). In a surprise finding, the mechanism used to degrade the hypomodified $tRNA_i^{Met}$ initiates with 3′ end polyadenylation (Kadaba et al., 2004; 2006), followed by exosome degradation (Figure 3), which is reminiscent of mRNA turnover in bacteria (Kushner, 2002). This may be a uniquely nuclear pathway for tRNA turnover, but hypomodified $tRNA_i^{Met}$ is also modestly stabilized in strains lacking Ski2p (Anderson, unpublished data) or when the rapid turnover pathway more recently described (Chernyakov et al., 2008) is defective (Anderson, unpublished data), indicating that not all tRNA turnover occurs in the nucleus.

tRNAs become substrates of TRAMP-mediated surveillance when hypomodified and now it appears perturbation of pre-tRNA 3′ end processing as well as 3′ end trimming of pre-tRNAs retaining introns can also lead to TRAMP-mediated surveillance. While tRNA 3′ end processing in not well understood in *S. cerevisiae*, it is believed that it can occur through either an endo or exonucleolytic step. Rex1p, a member of the DEDD riboexonluclease family which is required for 3′ end processing of $tRNA^{Arg}$ from the dimeric pre-$tRNA^{Arg\text{-}Asp}$ (Piper and Straby, 1989; van Hoof et al., 2000a), has recently been found to process pre-$tRNA_i^{Met}$ in which the 3′ extension prior to the terminating $U_{(5\text{-}7)}$ tract is 6–7 nucleotides long (Anderson, unpublished data). Deletion of Rex1p leads to accumulation of polyadenylated $tRNA_i^{Met}$ in an otherwise wild-type strain, and this effect is exacerbated if $tRNA_i^{Met}$ lacks the m^1A58 modification (Anderson, unpublished). In addition, intron containing pre-tRNAs with processed ends accumulate in strains lacking a functional TRAMP4 or the nuclear exosome subunit Rrp6p, and this accumulation is dependent on Rex1p and blocked by overexpression of the pre-tRNA 3′ end processing protein, Lhp1p (Copela et al., 2008).

These two observations seem at odds at first glance. In one case, loss of Rex1p results in an increase in TRAMP-mediated RNA surveillance of hypomodified pre-$tRNA_i^{Met}$ (Anderson, unpublished) and in the other case, the presence of Rex1p is required for targets of TRAMP-mediated RNA surveillance to accumulate (Copela et al., 2008). Because so little is known about the order of pre-tRNA processing events, these potentially opposing data may reflect differences in the temporal processing of pre-tRNAs with introns and those without. Alternatively, since both pre-tRNAs under consideration exhibit slowed processing, the difference may just be individual examples of how a delay of pre-tRNA processing can lead to tRNA surveillance. Finally, it is also reasonable to consider that as a normal course of production, some pre-tRNAs are subjected to TRAMP-mediated degradation because of poor formation of tertiary structure, or a failure to interact productively

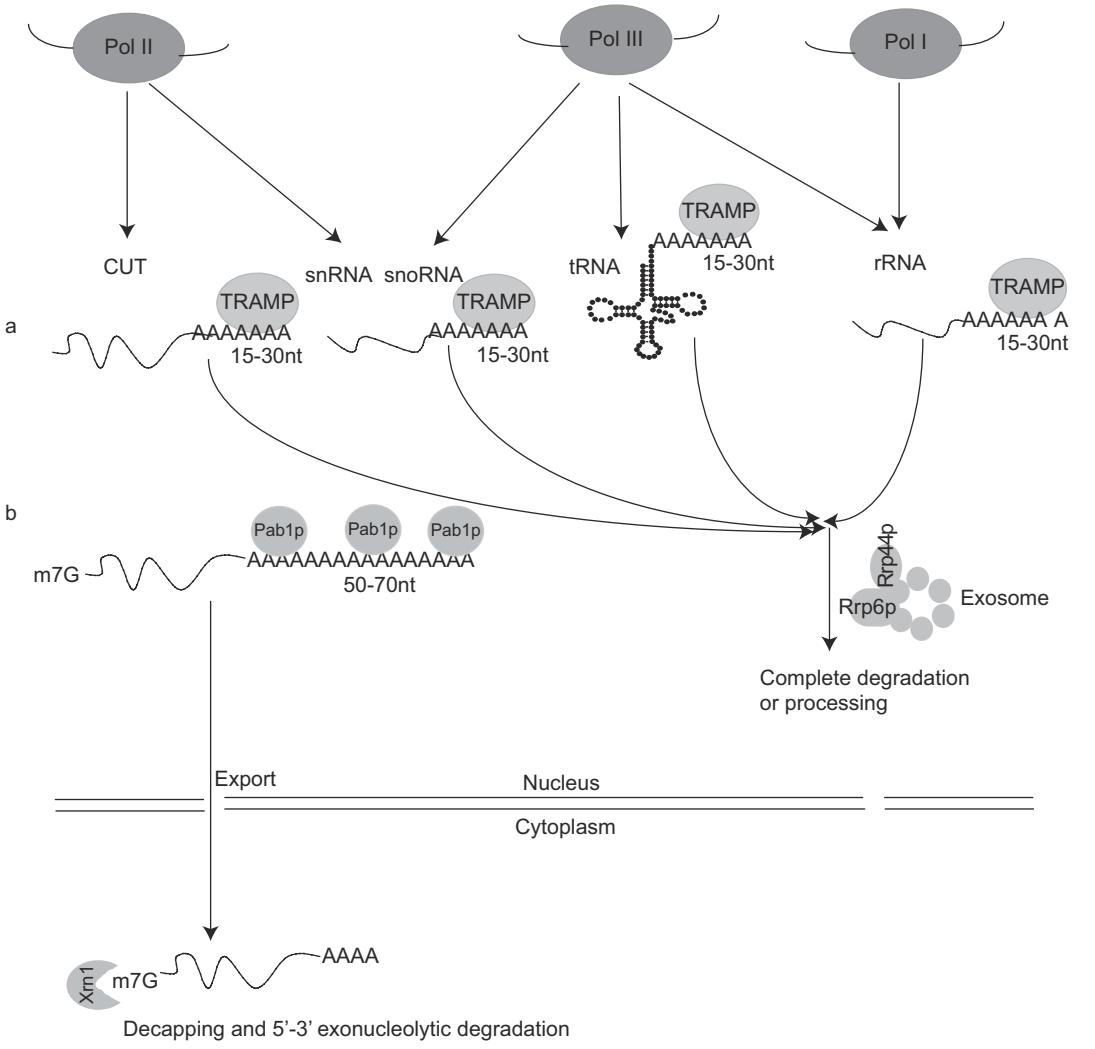

Figure 3. TRAMP mediated surveillance of small noncoding RNAs (ncRNA). RNAs produced by all three polymerases can be subjected to TRAMP-mediated surveillance or processing. Shown are ncRNAs and processing intermediates with short (15–30 nucleotides) or long (50–70 nucleotides) polyA tails. The RNAs with short polyA tails become substrates of the nuclear exosome through TRAMP-mediated surveillance, whereas full-length CUTs with long polyA tails are bound by polyA tail binding protein (Pab1p) and exported to the cytoplasm where they undergo degradation by the 5′–3′ exonuclease Xrn1p.

with proteins needed for complete processing or modification. The direct link of pre-tRNA turnover to 3′ end formation of pre-tRNAs may not be universal to all RNAs under TRAMP-mediated surveillance, but it does provide a point to consider when exploring RNA surveillance in general.

Cryptic unstable transcripts

Interest in the nuclear surveillance pathway and its potential impact on cellular metabolism gained momentum when small RNAs of unknown function were first identified in strains lacking a functional exosome (Wyers et al., 2005), and it has continued to increase as more has been learned about the interplay between small RNAs and various cellular macromolecules and machines.

A new classes of non-coding RNA under TRAMP-mediated nuclear exosome surveillance were identified in yeast cells (Wyers et al., 2005). The so-called cryptic unstable transcripts (CUTs) originate from inter- and intragenic regions that generally do not contain typical ncRNAs (Wyers et al., 2005; Davis and Ares, 2006), and their discovery demonstrated that this relatively primitive eukaryotic organism exhibits an unexpected transcriptional complexity. CUTs were initially thought only to be degraded through the combined activity of TRAMP4 and the nuclear exosome (Wyers et al., 2005; Davis and Ares, 2006), but now there is evidence that some CUTs are degraded in the cytoplasm by decapping and 5′–3′ degradation by Xrn1p (Figure 3) (Thompson and Parker, 2007). The biological significance of most CUTs remains a mystery, but a few CUTs or noncoding antisense transcripts have been attributed to controlling

gene expression (Martens et al., 2004; 2005; Hongay et al., 2006; Camblong et al., 2007). One such example is *SRG1*, which is transcribed in a tandem orientation upstream of the *SER3* protein coding gene, and when transcribed blocks binding of *SER3* transcriptional activators. *SRG1* RNA is degraded in the nucleus and cytoplasm (Thompson and Parker, 2007), but the significance of its degradation in regulating *SER3* expression is not clear.

In the fission yeast *S. pombe*, the Trf4p ortholog Cid14p has been shown to be required for heterochromatic gene silencing (Buhler et al., 2007). *cid14Δ* strains that have a ura4+ reporter construct inserted near centromeres or other transcriptionally inactive loci show increased expression of ura4+. This observation led to the conclusion that polyadenylation of ura4+ from silent loci by Cid14p contributes to degradation, either by the silencing RNA or exosome degradation pathways, depending on the loci. This recent work expands the reach of TRAMP to include control of nuclear RNA transcript levels in order to maintain chromatin. As detailed mechanisms of these observations become available, appreciation for transcription of non-protein coding genes will be heightened and will likely continue to provide surprises.

PolyA has a tail to tell

The cytoplasmic degradation of CUTs has been recently described (Thompson and Parker, 2007; Berretta et al., 2008), although the degradation of these transcripts is not without influence from TRAMP and the nuclear exosome. This highlights a key question, the answer of which may help reveal the importance of the different fates of CUTs. The common feature of these RNAs is the presence of a polyA tail at their 3′ ends. It is interesting that most full-length CUTs seem to be degraded predominantly in the cytoplasm (Thompson and Parker, 2007). Full-length CUTs possess polyA tails that are synthesized by the canonical polyA polymerase, and the predicted length of these tails is between 50 and 90 nucleotides (Zhao et al., 1999). The polyA tail lengths generated by TRAMP *in vivo* were shown to be between 15 and 30 nucleotides (Figure 3) (Kadaba et al., 2006), but this is probably not accurate since the cDNAs were created using oligo $d(T)_{16}$ for first strand synthesis. *In vitro*, TRAMP is capable of polyadenylating substrate RNAs with up to 200 adenosines (Vanacova et al., 2005), but this likely does not reflect the length of TRAMP-generated polyA tails *in vivo*. If the two pathways differ in the length of polyA tails generated, this could be a means of distinguishing between RNAs for export from those to be degraded. The distinction would most likely require protein partners, most of which could be in TRAMP or the exosome. It will be interesting to see if polyA tails tell the final story of an RNA's fate.

Epilogue

The largest and most important unanswered question is how does TRAMP recognize/distinguish RNAs that are destined to be degraded by surveillance versus those that are not? With the increasing number and diversity of substrate RNAs for TRAMP-mediated surveillance, it is hard to imagine how RNA sequence plays a pivotal role in the process. If TRAMP-mediated surveillance is an active process, there will certainly be factor(s) that target specific RNAs for degradation. The discovery of one such factor would help us better understand this RNA surveillance mechanism. If TRAMP-mediated surveillance is passive, then RNAs that simply fail to proceed through processing could be targets of RNA surveillance. These two possibilities are not mutually exclusive, and ultimately it may turn out that both proteins and a failure to process are important in determining which RNAs are subjects of TRAMP-mediated RNA surveillance.

Acknowledgments

***Declaration of interest*:** The authors report no conflicts of interest.

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