R E V I E W

Current perspectives on the role of TRAMP in nuclear RNA surveillance and quality control

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Abstract: The TRAMP complex assists the nuclear exosome to degrade a broad range of ribonucleic acid (RNA) substrates by increasing both exoribonucleolytic activity and substrate specificity. However, how the interactions between the TRAMP subunits and the components of the nuclear exosome regulate their functions in RNA degradation and substrate specificity remain unclear. This review aims to provide a summary of the recent findings on the role of the TRAMP complex in nuclear RNA degradation. The new insights from recent structural biological studies are discussed.

Keywords: TRAMP, nuclear exosome, NEXT, RNA surveillance

Introduction

TRAMP complex is one of the most well-characterized nuclear exosome cofactors, which enhances the activity and substrate specificity of the exosome.[1] In *Saccharomyces cerevisiae*, TRAMP complex is a heterotrimeric complex consisting of a poly(A) polymerase (either Trf4p or Trf5p); a zinc-knuckle ribonucleic acid (RNA)-binding protein (either Air1p or Air2p); and an RNA helicase Mtr4p.[2,3] The main function of TRAMP is to assist the nuclear exosome to degrade a large variety of RNA substrates, such as hypomodified initiator tRNA$_i^{Met}$, abnormally processed ribonucleic RNAs (rRNAs),[4–6] cryptic unstable transcripts (CUTs),[7–10] long noncoding RNAs (lncRNAs),[11] micro-RNAs (miRNAs),[12,13] and normal by-products of RNA metabolism such as spliced-out introns,[14,15] and to be involved in the many other RNA processes such as the maturation steps in precursor RNA processing and transfer RNA (tRNA) editing.[16] Therefore, it is not surprising that the TRAMP complex is also directly involved in many RNA processing pathways such as splicing,[15,17,18] RNA export,[19,20] and heterochromatic gene silencing,[21,22] and is even indirectly involved in the maintenance of genomic stability.[9,23,24] This review summarizes recent findings on molecular mechanisms underlying how the TRAMP complex regulates nuclear RNA surveillance.

Trf4p and Trf5p – non-canonical nuclear poly(A) polymerases

Trf4p and Trf5p are two highly similar (45% identity and 72% similarity) poly(A) polymerases.[24] Previous studies showed that they exhibited negative genetic interaction with the DNA topoisomerase Top1p, and they were named as topoisomerase-related function (Trf) 4p and Trf5p.[23,24] As a result, Trf4p and Trf5p were suggested to act as DNA polymerases that play a role in genome stability.[25–27] Many different mechanisms for their contribution in genomic instability were proposed, for example, through the

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regulation of the levels of histone messenger RNA (mRNA)[28] and non-protein coding RNA transcripts (ncRNAs),[9,29] the R-loop-mediated transcription-associated recombination,[30] and the recruitment of replication protein A.[31] In subsequent research, the importance of Trf4p and Trf5p in RNA degradation intermediates were demonstrated, and the fact that their poly(A) polymerase activity is required for RNA degradation.[32,33]

As the TRAMP complex is composed of either Trf4p or Trf5p, two distinct TRAMP complexes (TRAMP4 and TRAMP5, named for the presence of Trf4p or Trf5p, respectively) co-exist in *S. cerevisiae*. The cellular level of Trf4p is approximately three times higher than that of Trf5p. That may be the reason why deletion of *TRF5* generates no obvious phenotype, whereas loss of *TRF4* leads to a slow-growth phenotype.[28] As deletion of both *TRF4* and *TRF5* are inviable,[24] it is highly possible that Trf4p and Trf5p have overlapping functions.[5,14] However, recent studies demonstrated that deletion of *TRF4* and *TRF5* affected barely overlapping sets of substrates.[14,32,34,35]

Despite considerable structural similarities to the catalytic and central regions of the canonical poly(A) polymerase, both Trf4p and Trf5p lack the RNA-binding domain and rely on their RNA-binding partners, AIR proteins (Air1p or Air2p), to bind to their substrates. It was supported by chromatin immunoprecipitation analyses that the absence of Air2p impeded the recruitment of Trf4p and Trf5p to transcribing genes.[36]

Air1p and Air2p – zinc knuckle RNA-binding proteins

Both Air1p and Air2p were originally identified by a yeast-two hybrid screening, as proteins physically interacting with Hmt1p. Air1p inhibits Npl3p methylation by suppressing Hmt1p's activity.[19] AIR proteins were hence named accordingly as arginine methyltransferase-interacting RING finger proteins.[19] A recent protein–protein interaction study demonstrated that Air2p could be methylated by Hmt1p and that the direct interaction between Npl3p and Air2p was significantly increased in the presence of active Hmt1p.[37]

The amino acid sequences of Air1p and Air2p are highly similar (45% identity and 71% similarity). They have five zinc knuckle (ZnK) motifs that are critical for the binding to RNA and the assembly of the TRAMP–RNA complex.[38,39] That exposed guanosines on RNA loops or in the single-stranded RNAs are recognized by the ZnK motifs of AIR proteins was shown by the quantitative solution-binding assay using fluorescence anisotropy experiments.[39] This previous

study also demonstrated that Air2p bonded to unstructured oligo(A) 15 RNA substrate with a dissociation constant in the lower micromolar range, but did not bind to oligo(dA) 15 DNA substrate.[39] The function of ZnK motifs on AIR proteins is not only important for RNA binding, but also for protein–protein interaction. Based on mutation analysis and nuclear magnetic resonance titration experiments, the fourth and fifth ZnK motifs of Air2p are associated with the central domain of Trf4p, and the interaction is essential for TRAMP4 polyadenylation activity.[39,40]

Since no change in the growth rate of *S. cerevisiae* cells lacking either Air1p or Air2p was evident, while double deletion led to very severe growth defects,[19] early publications[2,14] assumed that the functions of Air1p and Air2p were redundant. However, by RNA deep sequencing, distinct accumulation patterns of polyadenylated RNAs in the cells lacking either Air1p or Air2p were observed.[41] The findings clearly indicated that Air1p and Air2p targeted specific transcripts for polyadenylation and degradation by the nuclear RNA exosome, resulting in profound physiology effects on the cell.[41] In brief, ablation of Air1p causes the loss of the 2 μ plasmid and 2 μ CUTs, but ablation of Air2p decreases the levels of mRNAs encoding proteins for glucose transport and metabolism, while it elevates the levels of mRNAs involved in iron import.[41]

Mtr4p – RNA helicase

Mtr4p (also known as Dob1p) was identified as an RNA helicase critical for the regulation of TRAMP-mediated degradation by unwinding secondary structures of target RNAs and modulating the polyadenylation in the TRAMP complex.[42,43] The Mtr4p subunit of TRAMP was initially discovered as a protein that, when mutated, would result in an mRNA transport (mtr) defect with the accumulation of polyadenylated RNAs in the nucleus. It was then shown to be required for the nuclear exosome-dependent synthesis of 5.8S rRNA from its 7S precursor.[42] As Mtr4p is a member of the DExH-box RNA helicases family, Mtr4p may accelerate RNA degradation by the nuclear exosome through unwinding the RNA duplexes.[44,45] In addition, it has been suggested that Mtr4p is also responsible for the proper recruitment of TRAMP-targeted RNAs to the nuclear exosome via the direct physical interaction between Mtr4p and the components of the nuclear exosome.[29,46]

Molecular mechanism of the TRAMP complex

The current model states that both helicase and poly(A) polymerase activities of the TRAMP complex are

required,[45] but not essential,[46,47] for its function. Based on the findings from isothermal titration calorimetry, the dimerization of Trf4p and Air2p is likely required for efficient assembly with Mtr4p.[39] Trf4p and Air2p form the poly(A) polymerase subcomplexes first, and the N-terminal low-complexity regions of Trf4p and Air2p bind to the DExH adenosine triphosphate (ATP)ase core domain of the Mtr4p in a cooperative manner.[48] A similar conserved region in the N-terminus of Trf5p is required for Mtr4p interaction.[3]

The interactions between the subunits of the TRAMP complex are important for the coordination between helicase and polyadenylation activities of TRAMP.[43] On one hand, Trf4p with Air2p stimulates the RNA helicase activity of Mtr4p through increasing its ATP affinity.[49] On the other hand, Mtr4p detects the number of 3′ adenosines added and inhibits polyadenylation by controlling the activity of Trf4p.[43] Mtr4p is also shown to bind to RNAs with adenosines tighter than other sequences.[44,50] Mtr4p only fully binds when the 3′ extensions contain at least 5–6 nucleotides.[49] Therefore, during polyadenylation, the binding affinity of Mtr4p to target substrate increases and induces slower dissociation of TRAMP from terminal adenosines.[43]

The model was supported by previous findings that polyadenylation by Trf5p was also markedly increased in strains lacking the RNA helicase Mtr4p[51] or with mutated Mtr4p.[39,43] That may be the reason why RNAs adenylated by the TRAMP complex contain significantly shorter poly(A) tails than the canonical poly(A) polymerases.[52,53] These findings collectively suggested that the TRAMP complex polyadenylates the 3′ ends of aberrant noncoding RNAs, and subsequently provides a docking site for the exosome to initiate RNA decay.[40] However, in some cases, RNA degradation does not require the polyadenylation activity of TRAMP.[14,47,54]

Targeting RNAs for degradation: recognition of aberrant sequences

As mentioned in the "Molecular mechanism of the TRAMP complex" section, the TRAMP complex processes a broad range of RNAs produced by all three RNA polymerases (RNAP I, II and III). In the TRAMP complex, only the AIR proteins (Air1p and Air2p) have RNA-binding domains. Therefore, it is highly possible that the RNA-binding domains of Air1p and Air2p determine the substrate specificity of the TRAMP complex.[2,40,41,51,55,56] The subcellular localizations of the TRAMP complexes may also contribute to the different substrate specificities of TRAMP4 and TRAMP5 complexes.

The TRAMP5 complex localizes mainly in the nucleolus,[36,57] while the TRAMP4 complex is found throughout the entire nucleus in *S. cerevisiae*.[58,59] That may be the reason why TRAMP5 is recruited co-transcriptionally to nucleolar surveillance machinery and is responsible for the degradation of aberrant 23S RNA.[60,61]

In addition, the physical binding of interacting proteins to TRAMPs controls differential substrate specificity.[41] For example, TRAMP4, but not TRAMP5, is co-purified with Nrd1p and Nab3p,[62,63] which are part of the Nrd1p–Nab3p–Sen1p (NNS) complex that bind to the nuclear exosome to mediate transcription termination of non-polyadenylated RNAs. The Nrd1p–Trf4p interaction is required for this activity.[63] According to the current model, Nrd1p interacts with RNAPII and Trf4p in a mutually exclusive manner.[63] The C-terminal repeat domain (CTD) interacting domain (CID) of Nrd1p mediates the transition of the NNS complex from RNAPII to the Trf4p of the TRAMP complex.[63] Nrd1p interacts with Trf4p and stimulates the RNA polyadenylation by the TRAMP complex.[63] Hence, the dynamic interactions among RNAPII, Nrd1p, and Trf4p regulate the NNS targets by promoting proper 3′ end formation of small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs), and the degradation of unstable ncRNAs and CUTs in the nucleus.[54,63]

Interestingly, Air1p is shown to be co-purified with the poly(A) binding protein Hrb1p,[64] and Trf4p binds to the splicing factor Prp16p.[55] In addition, Hrb1p and Gbp2p genetically and physically interact with Mtr4p, and play an important role in the quality control of spliced mRNAs; whether TRAMP4 is specialized for quality control of spliced mRNAs remains to be explored.

Modulation of the RNA exosome by TRAMP

Although the nuclear exosome is an active ribonuclease in vivo, purified nuclear exosome only showed weak activity in vitro, particularly toward highly structured RNA substrates.[2] The TRAMP complex increases the accessibility of the RNA 3′ ends to nuclear exosome through polyadenylation. The 3′ oligo-adenosine tail then serves as docking for the exosome and unwinds the structured RNA. Although there is evidence demonstrating the requirement of ATP and polyadenylation of RNA substrates for the TRAMP complex to enhance the activity of the exosome,[2,4,55] both poly(A) polymerase and the helicase activities of the TRAMP complex seem dispensable for the enhancement of RNA degradation by the exosome in vitro.[47] Interestingly, one of

the components of nuclear exosome Rrp6p plays an unexpectedly critical role in the enhancement of RNA degradation by purified exosomes via the TRAMP complex in vitro.[47]

Recent crystallographic analysis revealed that the N-terminal domains of Rrp6p and Rrp47p form a highly intertwined structure, which creates a groove that binds the N-terminus of Mtr4p.[46] In agreement with previous findings,[65] overexpression of the core domains of Mtr4p is sufficient to suppress the growth defect of *rrp6Δ* and thus restore specific rrp6Δ RNA defects.

The TRAMP complex may enhance RNA degradation and specificity of the exosome via co-transcriptional recruitment.[36] Co-transcriptional recruitment of the exosome cofactors is required to enhance the degradation of RNA substrates by the exosome.[2,36,55,66] The transcription elongation complex (THO) (which is involved in RNAPII transcription elongation and messenger ribonucleoprotein [mRNP] export) was suggested to be responsible for the maintenance of TRAMP occupancy at sites of snoRNA transcription in fission yeast.[67] It is interesting to further explore whether the THO complex is also important for the recruitment of the TRAMP complex to the transcription sites for other RNA substrates.

Conservation of TRAMP-mediated processes

Components of the TRAMP complex are highly conserved from yeast to mammals. *Schizosaccharomyces pombe*'s TRAMP complex, consisting of Cid14p, Air1p, and Mtr4p, is functionally homologous to the TRAMP complex in *S. cerevisiae*.[68,69] Interestingly, *S. pombe CID1* family of non-canonical poly(A) polymerases has six members (Cid1p, Cid11p, Cid12p, Cid13p, Cid14p, and Cid16p).[70] As Cid14p is constitutively nucleolar and is required for polyadenylation and degradation of *S. pombe* rRNAs, Cid14p is considered as the functional orthologue of *S. cerevisiae* Trf4p and Trf5p.[71] Unlike *trf4–trf5* double mutants in budding yeast, *S. pombe* lacking *CID14* is viable, though it suffers from an increased frequency of chromosome mis-segregation.[68] In addition, *S. pombe* Cid14p is required for proper silent RNA (siRNA) generation through the RNA interference pathway.[21,72]

Similar to *S. cerevisiae*, the human TRAMP complex also consists of three subunits: a helicase hMtr4p, a non-canonical poly(A) polymerase hPAPD (PAP-associated domain-containing) 5 or hPAPD7, and a ZnK protein hZCCHC7.[73] Humans also contain two TRF homologues, hPAPD5 and hPAPD7, both of which exhibit 37% identity to the catalytic domain of *S. cerevisiae* Trf4p. Human Air1/2p orthologues of hZCCHC7 share 35% and 34% identity to yeast Air1p

and Air2p, respectively. In addition, hMtr4p (also named as SKIV2L2) exhibits 51% total identity to yeast Mtr4p. Recent studies showed that hMtr4p precipitates contain hZCCHC7p and hPAPD5p, which strongly suggests that humans have functionally related yeast TRAMP complex in the processing and surveillance of rRNA, snRNA, snRNA, mRNA, and miRNA.[38,73] However, in contrast to yeast Trf4p, the C-terminus of hPAPD5p contains a stretch of basic residues that is involved in binding the RNA substrate, which enables hPAPD5p to carry out its function without a separate RNA-binding subunit, such as AIR proteins in *S. cerevisiae*.[74]

hMtr4p also forms a heterotrimer with a putative RNA-binding protein hRbm7p and a zinc knuckle RNA-binding domain-containing protein hZCCHC8, known as nuclear exosome targeting (NEXT) complex. The NEXT complex contributes to the degradation of promoter upstream transcripts (PROMPTs), which are produced upstream of the promoters of actively transcribing protein-coding genes.[73] The recruitment of NEXT to PROMPT regions is through NEXT's physical interaction with a cap-binding complex (CBC). The CBC–NEXT subcomplex mediates the RNAPII-derived transcription termination and suppresses the expression of PROMPTs in humans.[75] Interestingly, the localization of the human TRAMP complex is only restricted to the nucleolus, and the NEXT complex is found in other parts of the nucleoplasm.[73] The presence of the TRAMP complex and the NEXT complex in different subnuclear localizations suggests that compartmentalization of nuclear exosome cofactors may be necessary to cope with the more diverse transcripts.

In addition, a recent study demonstrated that the RNA-binding component of the NEXT complex, Rbm7p, could be phosphorylated by p38MAPK/MK2.[76] Phosphorylated Rbm7p has lower RNA-binding capacity to nuclear non-coding RNA (such as PROMPTs) than its unphosphorylated form, and hence increases the stability of nuclear noncoding RNA for the stress-dependent modulations of the noncoding transcriptome.[76]

Conclusion and perspectives

The advances in protein binding and structural analysis have advanced our knowledge of the molecular mechanisms of the TRAMP complex in nuclear RNA surveillance (Figure 1). However, many fundamental questions remain to be addressed. For example, how does the TRAMP complex determine the fate of different types of RNA? How does the TRAMP complex coordinate with other RNA processing events via protein–protein interactions? How is the TRAMP complex co-transcriptionally recruited, especially for the RNAPI- and
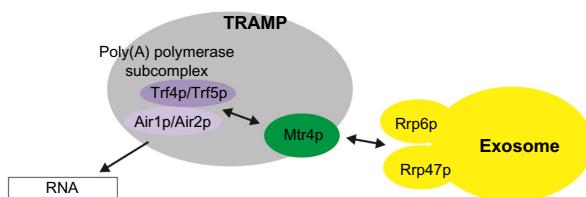
Figure 1 Model of interactions between RNA, TRAMP, and the exosome. RNA substrate bound by Air1p or Air2p is oligo-adenylated by poly(A) polymerase Trf4p or Trf5p until sufficient binding sites are generated for the helicase Mtr4p. Mtr4p is loosely associated with the poly(A) polymerase subcomplex in the TRAMP complex. Mtr4p can only fully bind when the 3′ extensions contain at least 5–6 nucleotides. Therefore, during polyadenylation, the binding affinity of Mtr4p to target substrate increases. This increased affinity might contribute to Mtr4p inhibiting further oligo-adenylation and might induce slight slowing of TRAMP dissociation from the substrate. TRAMP recruits the exosome for RNA degradation through the interaction between Mtr4p and Rrp6p/Rrp47p. Mtr4p also forms a complex with other proteins, such as with Hrb1p and Gbp2p, to mediate quality control of spliced transcripts in yeast, and with hRbm7p and hZCCHC8, as NEXT complexes to degrade PROMPTs.
Abbreviations: RNA, ribonucleic acid; NEXT, nuclear exosome targeting; PROMPT, promoter upstream transcript.

RNAPIII-derived transcripts? Can the activity and specificity of the TRAMP complex be regulated by post-translational modifications?

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Disclosure

The authors report no conflicts of interest in this work.

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