8-23-2005

RNA Turnover: Unexpected Consequences of Being Tailed

James T. Anderson
Marquette University, james.anderson@marquette.edu

Accepted version. *Current Biology,* Vol. 15, No. 16 (August 23, 2005): R635-R638. DOI.

Curent Biology, Vol. 15, No. 16 (August, 2006) : R635-R638. DOI.

RNA Turnover: Unexpected Consequences of Being Tailed

James T. Anderson
Department of Biological Sciences, Marquette University, Milwaukee, WI

In eukaryotic cells, the 3′ poly(A) tails found on mRNA influence their stability and translation. The discovery of a second nuclear poly(A) polymerase complex has fueled a series of reports defining a new and unexpected role for 3′ end poly(A) tails in the nuclear surveillance and turnover of noncoding RNAs and intergenic mRNAs of unknown function.

The life cycle of RNA begins with transcription and ends upon degradation; in between are several processing steps that are required for maturation and function. One aspect of RNA maturation is 3′ end processing, which for most RNA occurs through endonucleolytic cleavage, and/or exonucleolytic digestion, and subsequent addition of polyadenylate (poly(A)) tails to mRNA and CCA to tRNA, or no addition in the case of rRNA, snRNA and snoRNAs. The synthesis of mRNA 3′ poly(A) tails is carried out by a conventional nuclear localized poly(A) polymerase, and the functions of poly(A) tails are to stabilize, facilitate localization and enhance translation of mRNAs.

Recent studies have confirmed the identity of a second nuclear poly(A) polymerase in yeast that is evolutionarily conserved throughout eukaryotes. Genetic and biochemical characterization of this poly(A) polymerase, Trf4p, and its associated factors[1,2] establishes a new function for poly(A) tails in targeting the degradation of aberrant non-coding RNAs — tRNA, rRNA and snRNA — and intergenic mRNAs of unknown function.[3]

The first poly(A) polymerase enzymatic activity, Pap1, was identified in *Escherichia coli*, but the identification of poly(A)$^+$ RNAs in bacteria lagged behind this discovery. As it turns out, a small percentage of bacterial mRNA is polyadenylated by bacterial Pap1, and in some cases polyadenylation is associated with increased mRNA turnover. Noncoding RNAs can also be polyadenylated in *E. coli*, and the presence of the poly(A) tail on noncoding RNAs has been shown to control the expression of a regulatory RNA and target a mutant tRNA for degradation (reviewed in[4,5]).

Interestingly, Piper *et al*.[6] identified 3**′** end poly(A) tails on yeast 5S rRNA in a mutant strain lacking a putative endonuclease required for proper 5S rRNA 3**′**end processing. More recently, polyadenylation of snRNA, snoRNA and 5S rRNA by the conventional poly(A) polymerase was observed in mutant strains lacking one or more subunit of the exosome,[7,8,9] a multisubunit protein complex involved in many aspects of RNA metabolism.[10]

While these observations were suggestive, their significance remained enigmatic until recently, when a number of laboratories began investigating Trf4p, a novel yeast poly(A) polymerase, and the effects of Trf4p-derived poly(A) tails on mRNA and noncoding RNA expression. Trf4p belongs to the polymerase **β**-type nucleotidyltransferase superfamily;[11] other members are DNA polymerase Xs, tRNA CCA adding enzymes, terminal deoxynucleotidyltransferases, Gld2 and Trf5p, a functionally redundant protein in yeast which exhibits 48% amino acid identity with Trf4p (my unpublished observation).

While conducting a genetic screen in *Schizosaccharoymces pombe* designed to uncover genes involved in replicational response, Saitoh *et al*.[12] identified Cid13p, a cytoplasmic poly(A) polymerase related to *Saccharomyces cerevisiae*Trf4/5p. During this work they showed that affinity purified tagged-Trf4p possesses an intrinsic poly(A) polymerase activity *in vitro*.

The role of Trf4p as a poly(A) polymerase *in vivo* was first implied from a genetic suppressor analysis used to identify genes required for the instability of a tRNA$_i^{Met}$ lacking one modified nucleotide, 1-methyladenosine 58 in the T**Ψ**C loop. Along with *TRF4*, *RRP44* and *MTR4* were also found to be required for degradation of the hypomethylated tRNA$_i^{Met}$.[13] Rrp44p is a subunit of the exosome which functions in the nucleus and cytoplasm[10] and Mtr4p is a putative ATP-dependent RNA helicase that influences the processing and degradation of many RNAs by the nuclear exosome.[14]

In a sophisticated series of experiments, Kadaba and Krueger[13] demonstrated that the hypomethylated pre-tRNA$_i^{Met}$ is polyadenylated, and that the length and abundance of the poly(A) tail is strongly influenced by the presence of excess Trf4p. These results established a firm relationship between the polyadenylation of the hypomethylated tRNA$_i^{Met}$ by Trf4p and degradation of the polyadenylated tRNA by the exosome.

In accordance with the genetic relationship between *TRF4* and *MTR4*,[13] Trf4p, Mtr4p and a putative RNA binding protein, Air2p,[15] form a stable complex *in vivo.*[16] Air2p shows 41% amino acid identity with Air1p, curiously reminiscent of the structural similarity between Trf4p and Trf5p.

The Trf4p/Air2p/Mtr4p polyadenylation complex (TRAMP) has been the focus of recent studies which have demonstrated that TRAMP purified from yeast whole cell extracts has poly(A) polymerase activity[1,2] that can polyadenylate small RNAs, including tRNA *in vitro* (Figure 1), which in turn stimulates degradation of the adenylated RNA by the exosome.

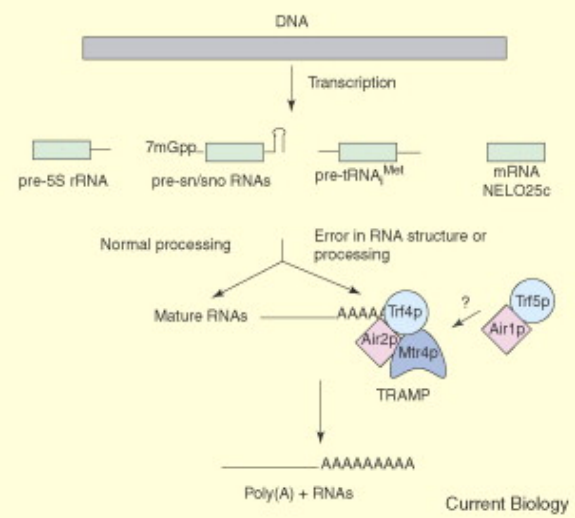

Figure 1. TRAMP polyadenylates RNAs to trigger their degradation.

After transcription, pre-5S rRNA, sn/snoRNAs, pre-tRNA and mRNAs that do not pass a quality control point, because of a perturbation of structure or error in processing, are recognized and polyadenylated by TRAMP, a complex of Trf4p (poly(A) polymerase), Air2p (RNA binding) and Mtr4p (putative ATP dependent RNA helicase). It is not known what role the highly related proteins Trf5p and Air1p play in this pathway so they have been shown on the side highlighted by a question mark.

LaCava *et al.*[1] and Vanacova *et al.*[2] independently identified the components of TRAMP by affinity purification of Trf4p and mass spectrometry analysis of the copurifying proteins.[1,2] They both found that Trf4p, Mtr4p and Air2p form stable and stoichiometric complexes. Purified TRAMP exhibits significant poly(A) polymerase activity when presented with a small RNA substrate and ATP, and this activity is ablated if mutations are introduced that replace two key glycine amino acids in the nucleotidyltransferase catalytic core of Trf4p with alanine.[1,2]

The presence of Trf5p and Air1p, in addition to Trf4p and Air2p, when Mtr4p-TAP is used for purification of TRAMP has made it hard to discern the role each of these proteins play in TRAMP function. It has brought to light some important unanswered questions about the role of Trf4p/Trf5p and Air1p/Air2p in the formation of TRAMP. Purification of Trf5p-TAP resulted in the copurification of Mtr4p, but no Air1p or Air2p were detectable and no polyadenylation activity was detected,[1,2] leaving the question what is the role of Trf5p in TRAMP function?

When Air1p-TAP was affinity purified, Mtr4p and, to a lesser extent, Trf4p copurified with Air1p and this complex had a low-level of polyadenylation activity.[2] Recombinant Trf4p failed to polyadenylate an RNA substrate unless it was incubated together with recombinant Air1p or Air2p and the RNA,[2] indicating that Air1p or Air2p provide the RNA-binding activity of TRAMP complexes.

As Trf5p and Air1p are marginally required for adenylation and degradation of a tRNA substrate *in vitro* and for the adenylation and degradation of hypomethylated $tRNA_i^{Met}$ *in vivo* (my and S. Kadaba's unpublished data), do Trf5p, Air1p and Mtr4p form a distinct complex from Trf4p, Air2p and Mtr4p that functions similarly to polyadenylate an unidentified substrate, or do they have a completely different function? Consistent with the idea that their function may be to modify different substrates, Trf5p-GFP was predominantly localized to the nucleolus, while Trf4p-GFP was distributed throughout the nucleus.[17]

LaCava *et al.*[1] and Vanacova *et al.*[2] showed that providing the exosome with a prepolyadenylated tRNA or purified TRAMP lacking Mtr4p was insufficient to convey degradation of the RNA substrate beyond deadenylation. This is striking in the face of the observation that TRAMP does not require ATP to stimulate exosome degradation of a nonadenylated pre-tRNA substrate.[1] What is the function of a poly(A) tail on the degradation of RNA substrates by the exosome? On the one hand it appears that the tRNA substrate requires adenylation for degradation by the exosome to proceed efficiently. This is in agreement with the *in vivo* data demonstrating that hypomethylated $tRNA_i^{Met}$ is not polyadenylated or efficiently degraded in strains lacking a functional Trf4p (S. Kadaba's and my unpublished data).

It is not clear from the reports whether stimulation of tRNA degradation by TRAMP in the absence of added ATP results in the same processive degradation observed when the exosome, TRAMP and ATP are incubated together with the substrate, or if substrate degradation stalls and intermediate sized degradation products accumulate.

It is possible that initial rounds of degradation occur slowly in the absence of poly(A) and cannot reach completion unless initiated by adenylation and multiple subsequent rounds of adenylation as has been proposed (Figure 2).[1,2,3] If this hypothesis is true, then the 3' poly(A) might be functioning as an unstructured fuse to prime the more difficult task of degrading the highly structured parts of RNA targets, but in its absence RNA degradation is slow and unprocessive. This is consistent with the observed accumulation of degradation intermediates seen when RNA is incubated with the exosome alone.[1,2]

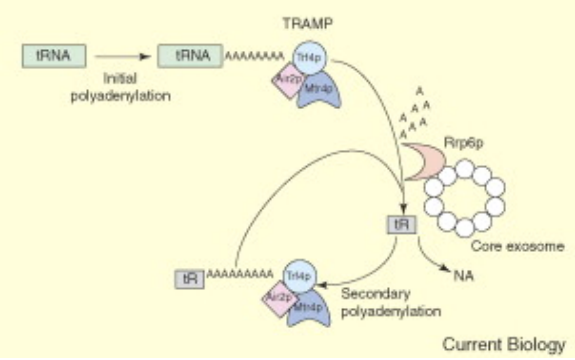

Figure 2. Proposed model for degradation of poly(A)$^+$ tRNA by exosome.

A defective tRNA is polyadenylated by TRAMP, which stimulates degradation by the exosome. This initial round of degradation may result in stalling by the exosome at highly structured regions of the tRNA, leaving an intact but shortened tRNA (tR). This shortened tRNA undergoes a secondary round of adenylation by TRAMP, which stimulates the exosome to re-engage and continue degradation of this substrate RNA. This cycle continues until the tRNA is completely degraded.

The work of Wyers *et al*.[3] expands our understanding of TRAMP and the exosome to include turnover of intergenic mRNAs. Intergenic mRNAs were discovered in studies characterizing the transcriptional topography of whole genomes, which ultimately led to the birth of the 'transcriptome'. The intergenic mRNAs identified in this work[3] are products of RNA polymerase II, as they contain a 5′ cap identical to coding mRNAs and a mutation that blocks RNA polymerase II function reduced their abundance. One intergenic mRNA, *NEL025c*, was found to accumulate in yeast as heterogeneous length poly(A)$^+$species in a strain lacking Rrp6p, a subunit of the exosome that is restricted to the nucleus.

Wyers *et al*.[3] went on to show that the heterogeneity of these mRNAs was due to a difference in their 3′ termini and that polyadenylation of these shortened mRNAs is dependent on TRAMP. Are these mRNAs functional or do they represent transcriptional noise? Whether they are transcriptional noise or functional mRNAs, yeast have developed and retained a mechanism for regulating their abundance by oligoadenylation, which hastens their degradation.

The role of polyadenylation to facilitate degradation of RNA now stretches from aberrantly structured or processed noncoding RNA to intergenic mRNAs. Is it possible this mechanism of RNA turnover is more general and used to degrade aged RNAs? New evidence suggests that tRNAs undergo retrograde movement from the cytoplasm back to the nucleus ([18] and H. Shaheen and A. Hopper, personal communication). Could this return to the nucleus by tRNA represent a method of colocalizing tRNA with a highly efficient RNA degrading machine to enable tRNA turnover?

One of the key molecular questions to be answered is that of how TRAMP distinguishes between a substrate that is to be adenylated and degraded, such as $tRNA_i^{Met}$ lacking m^1A58, and one that should not, such as $tRNA_i^{Met}$ possessing m^1A58. If recognition of a tRNA designated for degradation is conferred by the absence of m^1A58, then all tRNAs that normally contain m^1A58 having lost this modified nucleotide should be degraded, but this is not seen (my unpublished data).

Vanacova *et al.*[1] showed that $tRNA_i^{Met}$ isolated from a wild-type yeast strain cannot be polyadenylated by TRAMP while the fully unmodified form of the same tRNA made by *in vitro* transcription can. Given this result, it is more likely that a loss of tRNA tertiary structure triggers adenylation of the hypomethylated $tRNA_i^{Met}$*in vivo* and not the lack of m^1A58.[13,19] This is consistent with the finding that TRAMP efficiently adenylated a $tRNA^{ala}$ containing two point mutations predicted to disrupt its structure while the wild-type $tRNA^{ala}$ was inefficiently adenylated *in vitro.*[2] This result also implies that all the components needed to recognize aberrantly structured RNAs are present within purified TRAMP.

The same structural perturbations may be required for the adenylation of snRNA, snoRNA and 5s rRNAs, but since most of these RNAs have been characterized as full-length or their 5′ and 3′ ends remain uncharacterized this seems unlikely for this set of RNAs. These new findings promise to provide insights into how nuclear RNA surveillance plays an important role in regulating eukaryotic gene expression.

References

1 J. LaCava, J. Houseley, C. Saveanu, E. Petfalski, E. Thompson, A. Jacquier, D. Tollervey. **RNA degradation by the exosome is promoted by a nuclear polyadenylation complex.** Cell, 121 (2005), pp. 713-724

2 S. Vanacova, J. Wolf, G. Martin, D. Blank, S. Dettwiler, A. Friedlein, H. Langen, G.Keith, W. Keller. **A new yeast poly(A) polymerase complex involved in RNA quality control.** PLoS Biol., 3 (2005), p. e189

3F. Wyers, M. Rougemaille, G. Badis, J.C. Rousselle, M.E. Dufour, J. Boulay, B.Regnault, F. Devaux, A. Na mane, B. Seraphin, *et al.* **Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase.** Cell, 121 (2005), pp. 725-737

4 S.R. Kushner. **mRNA decay in *Escherichia coli* comes of age.** J. Bacteriol., 184 (2002), pp. 4658-4665

5 Z. Li, S. Reimers, S. Pandit, M.P. Deutscher. **RNA quality control: degradation of defective transfer RNA.** EMBO J., 21 (2002), pp. 1132-1138

6 P.W. Piper, J.A. Bellatin, A. Lockheart. **Altered maturation of sequences at the 3¢ terminus of 5S gene transcripts in a Saccharomyces cerevisiae mutant that lacks a RNA processing endonuclease.** EMBO J., 2 (1983), pp. 353-359

7 L. Kuai, F. Fang, J.S. Butler, F. Sherman. **Polyadenylation of rRNA in Saccharomyces cerevisiae.** Proc. Natl. Acad. Sci. USA, 101 (2004), pp. 8581-8586

8 C. Allmang, J. Kufel. **Functions of the exosome in rRNA, snoRNA and snRNA synthesis.** EMBO J., 18 (1999), pp. 5399-5410

9 A. van Hoof, P. Lennertz, R. Parker. **Yeast exosome mutants accumulate 3'-extended polyadenylated forms of U4 small nuclear RNA and small nucleolar RNAs.** Mol. Cell. Biol., 20 (2000), pp. 441-452

10 P. Mitchell, E. Petfalski, A. Shevchenko, M. Mann, D. Tollervey. **The exosome: a conserved eukaryotic RNA processing complex containing multiple 3'→5' exoribonucleases.** Cell, 91 (1997), pp. 457-466

11 L. Aravind, E.V. Koonin. **DNA polymerase beta-like nucleotidyltransferase superfamily: identification of three new families, classification and evolutionary history.** Nucleic Acids Res., 27 (1999), pp. 1609-1618

12 S. Saitoh, A. Chabes. **Cid13 is a cytoplasmic poly(A) polymerase that regulates ribonucleotide reductase mRNA.** Cell, 109 (2002), pp. 563-573

13 S. Kadaba, A. Krueger. **Nuclear surveillance and degradation of hypomodified initiator $tRNA^{Met}$ in S. cerevisiae.** Genes Dev., 18 (2004), pp. 1227-1240

14 J. de la Cruz, D. Kressler, D. Tollervey, P. Linder. **Dob1p (Mtr4p) is a putative ATP-dependent RNA helicase required for the 3' end formation of 5.8S rRNA in Saccharomyces cerevisiae.** EMBO J., 17 (1998), pp. 1128-1140

15 K. Inoue, T. Mizuno, K. Wada, M. Hagiwara. **Novel RING finger proteins, Air1p and Air2p, interact with Hmt1p and inhibit the arginine methylation of Npl3p.** J. Biol. Chem., 275 (2000), pp. 32793-32799

16Y. Ho, A. Gruhler, A. Heilbut, G.D. Bader, L. Moore, S.L. Adams, A. Millar, P. Taylor, K. Bennett, K. Bouti lier, *et al.* **Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry.** Nature, 415 (2002), pp. 180-183

17 W.K. Huh, J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, E.K .O'Shea. **Global analysis of protein localization in budding yeast.** Nature, 425 (2003), pp. 686-691

18 A. Takano, T. Endo, T. Yoshihisa. **tRNA actively shuttles between the nucleus and cytosol in yeast.** Science, 309 (2005), pp. 140-142

19J. Anderson, L. Phan, R. Cuesta, B.A. Carlson, M. Pak, K. Asano, G.R. Bjork, M.Tamame, A.G. Hinnebusc h. **The essential Gcd10p-Gcd14p nuclear complex is required for 1-methyladenosine modification and maturation of initiator methionyl-tRNA.** Genes Dev., 12 (1998), pp. 3650-3662