The multitasking polyA tail: Nuclear RNA maturation, degradation and export

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Summary

A polyA (pA) tail is an essential modification added to the 3'ends of a wide range of RNAs at different stages of their metabolism. Here, we describe the main sources of polyadenylation and outline their underlying biochemical interactions within the nuclei of budding yeast *Saccharomyces cerevisiae*, human cells and, when relevant, the fission yeast *Schizosaccharomyces pombe*. Polyadenylation mediated by the *S. cerevisiae* Trf4/5 enzymes, and their human homologues PAPD5/7, typically leads to the 3'end trimming or complete decay of non-coding (nc) RNAs. In contrast, the primary function of canonical pA polymerases (PAPs) is to produce stable and nuclear export-competent mRNAs. However, this dichotomy is becoming increasingly blurred, at least in *S. pombe* and human cells, where polyadenylation mediated by canonical PAPs may also result in transcript decay.

Introduction

The RNA 3'end pA tail is a post-transcriptional modification discovered in the seventies to occur on both eukaryotic and prokaryotic transcripts [1-5]. Polyadenylation of eukaryotic mRNAs, mediated by canonical PAPs, was found to result in the production of long polyadenosine chains and initially shown to contribute to transcript stability, processing, nuclear export and translation [6,7]. Prokaryotic RNA pA tails, on the other hand, were found to be shorter and only present in a small fraction of the RNA population, making their initial studies more cumbersome [6,8,9]. Moreover, prokaryotic pA tails were shown to mainly be involved in the degradation and quality control of RNA and, in addition to their production by bacterial PAP, to also be produced by polynucleotide phosphorylase (PNPase) in a sequential process of degradation and readenylation. This marked divide between eukaryotic and prokaryotic pA tail biology was later narrowed considerably when it was discovered that the *S. cerevisiae* Trf4/5-Air1/2-Mtr4 Polyadenylation (TRAMP) complex can destabilize a range of transcripts by the addition of short, and 'bacterial-like', oligoA tails, targeting RNAs to the 3'-5' exonucleolytic nuclear exosome complex [10-12]. Additionally, it is now clear that pA binding proteins (PABs) can participate in the decay of transcripts with longer pA tails both in yeast and in mammals [13-19]. In this review, we focus on the nuclear roles of the pA tail in yeast and human cells and how it directs RNAs to different fates, depending on its kinetics of synthesis, its length and its context of associated factors.

The pA tail stimulates mRNP formation and nuclear export

Finding the cleavage site

Cleavage and polyadenylation of the mRNA 3'end is an intrinsic part of the transcription termination process for eukaryotic protein-coding loci and is operated by a conserved complex called Cleavage and Polyadenylation Factor (CPF) in *S. cerevisiae* and Cleavage and Polyadenylation Specificity Factor (CPSF) in human. The multi-subunit nature of CPF/CPSF confers RNA binding-, RNA endonuclease-, protein phosphatase- and PAP-activities to the complex (see Figure 1A for details). In *S. cerevisiae*, CPF is assisted by the Cleavage Factor I (CFI) complex, while this activity in human cells is divided between the Cleavage Stimulation Factor (CstF) and the Cleavage Factors I and II (CFIm/CFIIm). These CFI-like complexes dually interact with RNA polymerase II (RNAPII) and the nascent RNA, which contributes to the proper selection of the RNA cleavage site [20-22]. *S. cerevisiae* encodes one PAP enzyme called Pap1, while this activity in human cells is divided between the α/γPAPs (PAPOLA and PAPOLG) and the recently identified Star-PAP (TUT1). Additionally, human cells encode for the testis specific PAPOLB protein. All these enzymes bind to the Cft1/CPSF160 subunit of CPF/CPSF (Figure 1A). Pap1 and its orthologs PAPOLA/PAPOLG also contact Fip1/FIP1, which is particularly important for the regulation of PAP processivity [23,24]. In contrast, Star-PAP interacts with the CPSF73 orthologue of the yeast Ysh1 endonuclease [25,26].
The cleavage/polyadenylation process initiates by the recruitment of CPF/CPSF and the associated complexes to the pA site within the nascent RNA and to the C-terminal domain (CTD) of RNAPII. The nature of an optimal pA site sequence varies between species but generally is composed of several short motifs, which for efficient recruitment of the 3'end processing complex need to be sequential and strictly separated spatially. In human cells, the cleavage site is located between an AAUAAA hexamer consensus sequence and a GU/U-rich Downstream Sequence Element (DSE).

The strength of the pA site also depends on other upstream U-rich and downstream G-rich elements. In *S. cerevisiae*, this consensus motif is organized differently, with the RNA cleavage site located between two U-rich sequences and assisted by upstream A- and AU-rich elements [20]. Recruitment of CPF/CPSF to the pA site is further facilitated by the interaction of Pcf11/PCF11 with RNAPII (Figure 1A). Taken together, these events lead to Ysh1/CPSF73-mediated endocleavage of the RNA. pA sites are often degenerate with several motifs occurring sequentially, so that a locus can produce RNA isoforms with different 3'ends, often displaying distinct half-lives and localizations. In human cells such different isoforms can be preferentially polyadenylated by either PAPOLA/G or Star-PAP [25-28].

Synthesizing the tail

After endocleavage, a pA tail is added to the 3'end of the upstream cleavage fragment. Nascent pA tails have a tightly constrained species-specific length, which is required for efficient mRNA nuclear export. Any alterations to the required number of adenosines added may lead to nuclear retention of the transcript and its ensuing decay. Hence, mechanisms exist that regulate pA tail length, but although this process has been studied for over two decades, any *in vivo* mechanism is still speculative. This is in part due to deadenylation processes, which occur in the nucleus as well as in the cytoplasm, making it challenging to experimentally distinguish the impact of nuclear *vs.* cytoplasmic factors.

In vitro and *in vivo* studies show that the efficiency of PAP-mediated polyadenylation depends on the strength and composition of the pA site and on the activity of regulatory factors. Pap1/PAPOLA activity *in vitro* is greatly enhanced in the context of the CPF/CPSF complex [24,29,30], most likely due to tethering of the PAP to the substrate. Indeed, the AAUAAA sequence element has been shown *in vitro* to be required for specific recruitment of the CPSF complex. Moreover, the upstream and downstream elements can additionally enhance CPSF binding to the substrate [24,31,32]. Fip1/FIP1 may also regulate PAP enzymatic activity, although the underlying mechanism seems to differ between organisms. In the case of *S. cerevisiae*, Fip1 can conditionally inhibit Pap1 activity *in vitro* on an A12 oligonucleotide, presumably serving to reduce any unspecific polyadenylation [33,34]. In contrast, human PAPOLA polyadenylation is strongly distributive and FIP1 appears required to stimulate PAP activity and does so most optimally on targets containing a strong U-rich upstream element [24]. Human PAP activity *in vitro* and *in vivo* is also stimulated by the nuclear PAB, PABPN1 [30,35]. The presence of both CPSF and PABPN1 synergistically stimulates polyadenylation by decreasing the off rate of the PAP enzyme from the target RNA. In doing so, this complex is capable of synthesizing a ~250 nt long pA tail without dissociating from the transcript substrate. It is therefore likely that PAPOLA, in the early stages of polyadenylation, only interacts with CPSF, leaving the polyadenylation reaction less processive until the first PABPN1 molecule has bound the nascent tail. This may create a time-window for regulation of the process, possibly defining the fate of the RNA as discussed below. After synthesis of the polymer beyond these ~250 nt the pA addition reaction is strongly inhibited and changes from a highly processive to a distributive mode. The reason for this change in processivity is elusive. PABPN1 has been shown to form spherical structures when bound to long adenosine polymers, and it has been suggested that formation of such structures during the polyadenylation reaction might interfere with PAP activity. Alternatively, long pA tails might impair the CPSF-PAP interaction, leading to a decrease in complex processivity [36].

PABPN1 has no obvious ortholog in *S. cerevisiae* (Figure 1B), so it is possible that the CPF complex is sufficient for controlling Pap1 processivity. However, additional mechanisms of PAP-stimulation and tail-length restriction have also been proposed. Some of these efforts have employed whole cell extracts derived from selected mutant strains to study the chemistry of polyadenylation and have led to somewhat contradicting models, probably due to the mixing of nuclear and cytoplasmic factors. *S. cerevisiae* harbors two major PABs, Pab1 and Nab2 [37,38]. Initial models postulated that Pap1/CPF would interact to processively polyadenylate the RNA 3'end, while an interaction of Pab1 with the growing oligoadenosine chain and with CFI would restrict the tail length to the 70-80 nt characteristic for *S. cerevisiae* cells [39]. It was later shown that Pab1 could inhibit A-tail extension *in vitro* on an A12 oligonucleotide but only in high concentrations [34], possibly reflecting competition of Pab1 with Pap1 in binding to the substrate. However, this seeming control of pA tail length was also suggested to result from Pab1-mediated recruitment of the Pan2/Pan3 deadenylase complex [40]; only Pab1 and Pan2/Pan3 are predominantly cytoplasmic and thus probably mediate tail length regulation in this compartment. Instead, the predominantly nuclear Nab2p was suggested as a possible tail-length restriction factor [41-43], and in a more recent study Nab2 was shown to be required both *in vivo* and *in vitro* to protect newly made RNA from decay by the nuclear ribonucleolytic RNA exosome [17]. Pap1 and PAPOLA are both less processive when outside the context of the CPF complex and even though the yeast enzyme is more robust on its own [24,29] both PAPs rely on the CPF/CPSF for polyadenylation initiation. This feature might leave a window of opportunity for shifting the fate of the newly made RNA towards decay rather than full polyadenylation at the early steps of 3'end processing [17]. Importantly, the models for Pab1 and Nab2 function in pA tail biogenesis are not mutually exclusive as both proteins shuttle between the nucleus and cytoplasm [44,45], potentially allowing Pab1-mediated pA tail restriction of at least some transcripts. Moreover, *S. cerevisae* pA tails can also be extended by Trf4-mediated polyadenylation [46]. This is, however, only apparent in nuclear exosome-impaired cells and it is therefore not clear whether nuclear 3'-5' decay partakes in pA tail restriction in a wild-type context. In human cells PABPN1 is functionally connected to the exosome (see below) and a nuclear exosome-dependent pA tail restriction process is therefore possible but has not been reported.

pA tail-guided RNA export

It is generally assumed that proper synthesis of the pA tail stimulates the assembly of an export competent mRNP. Studies in *S. cerevisiae* indicate that a minimum of 48 DNA-encoded adenosines enhances RNA export without any apparent contribution from the CPF complex [47]. Consistently, inhibition of PABPN1 by the influenza virus NS1A protein, or otherwise PABPN1 depletion, results in the nuclear accumulation of pre-mRNAs with short pA tails [48,49]. However, the pA tail itself is not the only player involved in RNA export. Several proteins are recruited to the nascent transcript by RNA binding and/or via interaction with RNAPII. These factors induce RNA remodeling steps to form mature export-competent mRNPs. Interestingly, some export factors are also required for proper pA tail synthesis. For example in *S. cerevisiae* the integrity of the conserved THO complex (harboring the Tho2, Hpr1, Mft1, Thp2 and Tex1 proteins) [50] is important for maintaining normal levels of the polyadenylation factor Fip1 and deletion of THO components leads to inefficient polyadenylation and mRNA decay [51]. A generally accepted model explaining a contribution of the pA tail to mRNA export stipulates that recruitment of the main export adaptor, the Mex67/Mtr2 heterodimer in *S. cerevisiae* (NXF1/NXT1 or TAP-p15 in human) is enhanced by binding to Nab2 [52] (Figure 1A). Other adaptors for Mex67/Mtr2 are the Yra1 protein (ortholog of human Aly/REF) and other subunits of the Transcription-Export (TREX) complex (composed also of the THO complex and the Sub2 helicase) [53,54], and Npl3, an abundant SR-like *S. cerevisiae*-specific RNA binding protein [55]. Moreover, several mRNP components, including Mex67 and Nab2, interact with constituents of the Nuclear Pore Complex (NPC), contributing to export efficiency. Export directionality is achieved by the Dbp5/DDX19B helicase, which mediates the release of Nab2 and possibly other export factors from the RNA. This step is enhanced by other mechanisms such as Npl3 phosporylation [56-62]. Although the network of Nab2 interactions places the protein and the pA tail centrally in the RNA export process, Nab2 depletion on its own only slightly impairs export as for example compared to mutation of Mex67 [41,63]. In addition to the aforementioned activity of other Mex67/Mtr2 recruiters, this could also be due to redundant Nab2 and Pab1 export functions, as adding a nuclear localization signal (NLS) to Pab1 partially rescues viability of *nab2Δ* cells [41]. As Nab2 is not orthologous to human PABPN1 (Figure 1B), it is not yet clear if this mechanism is fully conserved. PABPN1 was proposed to contribute to Aly/REF recruitment to the 3'end of mRNAs [64]. PABPN1 also shuttles between the nucleus and the cytoplasm [48,65] and it has therefore been proposed that it accompanies the mRNA during export and is thereafter exchanged with cytoplasmic PABPC1 [66,67]. However, how this might occur is not fully understood and more investigation is required to elucidate the exact function of the pA tail and PABPN1 in mRNP export.

pA tails assist the 3'end trimming and complete degradation of nuclear RNA

Polyadenylation can stimulate or mediate RNA decay in the nucleus by different means depending on its source. Transcripts that are terminated and polyadenylated by the CPF/CPSF complex might be directed towards degradation conditioned by their nuclear retention and such decay is often mediated by PABs. The mechanisms provoking nuclear retention have not been fully described, though some examples from both *S. cerevisiae* and human cells involve the sensing of pre-mRNA splicing defects. Other polyadenylated transcripts that fall prey to nuclear decay include, lncRNAs as well as shorter transcripts like some 'PROMoter uPstream Transcripts (PROMPTs)' and products of Premature Cleavage and Polyadenylation (PCPA). In contrast to CPF/CPSF-mediated events, non-canonical polyadenylation instigated by the TRAMP complexes can target RNAs produced by all three polymerases and is independent of PABs. Instead, this pathway is driven by the tight co-operation of the TRAMP complexes with the nuclear RNA exosome. RNAs that are targeted by TRAMP are mainly non-coding and range from stable RNAs such as rRNAs, sn-/snoRNAs and tRNAs to 'Cryptic Unstable Transcripts (CUTs)'.

PABs target nuclear-retained transcripts

A clear example of the degradative function of pA tails mediated by PABs has been studied in *S. pombe,* which displays functional similarities to the human system and have yielded valuable insight. Pab2, an ortholog of human PABPN1, is seemingly not involved in the control of pA tail synthesis in *S. pombe* [35]. Instead, Pab2 and Pla1, the ortholog of human PAPOLA, are both part of an RNA exosome co-factor complex called 'Mtl1-Red1 core' (MTREC) or 'Nuclear RNA silencing' (NURS) (Figure 2A) [68-71]. MTREC/NURS is composed of several modules organized around Mtl1, a homologue of the Mtr4/MTR4 helicase, and the Red1 protein, and these factors mediate contact to the *S. pombe* exosome via the Rrp6 exonuclease The distinct MTREC/NURS modules are suggested to target different exosome substrates, including meiotic mRNAs during mitosis [72-74], CUTs and unspliced pre-mRNAs [71].

A related interaction network has emerged from studies in human cells (Figure 2B, Table 1), where complexes functionally homologous to MTREC modules target distinct classes of RNAs. Interestingly, PABPN1 has been implicated, highlighting the likely relevance of pA tails. The protein was first described to target selected ncRNAs and pre-mRNAs for degradation by the exosome in a process depending on PAPOLA/PAPOLG and dubbed the 'PABPN1 and PAPα/γ-mediated decay (PPD)' pathway [14-16]. In subsequent work, PABPN1 was linked to the exosome via the Zn-finger protein ZFC3H1 and MTR4, forming a stable dimer, which associates with PABPN1 in a partially RNA-dependent manner in the so-coined 'Poly(A) RNA eXosome Targeting (PAXT)' connection [19,75]. As the PAXT and PPD pathways share substrates, they may act redundantly or even be overlapping. In addition to its functional interaction with the nuclear exosome, PABPN1 has been reported to partake in another process were polyadenylation is linked to a degradative activity, namely the mechanism that promotes maturation of the human telomerase RNA (hTR),

where the pA-specific ribonuclease PARN, together with PABPN1, processes hTR into its mature form [76-78]. Of note, the PABPN1 dependent pathways are not solely responsible for degrading cryptic transcripts in human cells where a well described parallel pathway is mediated by the 'Nuclear Exosome Targeting (NEXT)' complex [79,80], which likely does not target polyadenylated RNA (Figure 2B and see below). Nevertheless, in human, as in fission yeast, the MTR4/Mtl1 helicase provides a 'hub' that brings different decay complexes together, some of which contain PABs. Finally, *S. cerevisiae* Nab2 has also been implicated in pre-mRNA decay via its interactions with the splicing machinery. Nab2 was suggested to exert quality control and prevent the ultimate export of pre-mRNA in a process requiring an interaction with the Mlp1-Mlp2 proteins of the NPC nuclear basket. The Nab2 human ortholog ZC3H14 was also shown to interact with the splicing machinery, though its participation in nuclear RNA decay has not yet been addressed (Figure 1A, Table 1) [18,46,60,81].

Oligoadenylation by TRAMP triggers exosome-mediated exonucleolysis

The conserved TRAMP complex

The *S. cerevisiae* TRAMP complex is composed of a pA polymerase, Trf4 (alias Pap2) or Trf5 (DNA topoisomerase I related function), a zinc knuckle (ZnK) and RNA binding protein Air1 or Air2 (arginine methyltransferase interacting RING finger) and the Mtr4 helicase, belonging to the DExD/H family (Figure 3). It can exist as two isoforms: TRAMP4 (Trf4/Air2/Mtr4) and TRAMP5 (Trf5/Air1/Mtr4), though some reports suggest that TRAMP4 might alternatively contain Air1. The Trf4/5 proteins are unable to bind and adenylate RNA without Air1 or Air2 [11], which contact a domain in Trf4/5 adjacent to their catalytic sites via the Air1/2 terminal ZnK4-5 [82]. Outside of their known domains, the Trf and Air proteins are both largely unstructured but contain short epitopes at their N-termini, that bind Mtr4 in a cooperative manner. This binding positions the exit of the helicase domain towards the PAP domain of Trf [83-85].
In human cells, orthologues of TRAMP components (Table 1) have been reported to interact with each other and with RRP6, which suggests that the overall structure of the TRAMP complex and its association with the nuclear exosome is conserved. The pA polymerase activity is provided by the 'PAP-associated domain containing proteins 5 and 7' (PAPD5 and PAPD7 aka TRF4-2 and hTRF4-1). PAPD5 and PAPD7 interact independently with ZCCHC7, orthologue of Air1/2 and with MTR4 and RRP6 [79,86]. PAPD5 contains a basic RNA binding motif at its C-terminus and is capable of adenylating RNA substrates *in vitro* without any co-factors [87]. However, it is likely that, for efficient activity and/or specificity, it requires ZCCHC7 *in vivo*. The TRAMP complex is also conserved in *S. pombe* and is composed of the pA-polymerase Cid14, the RNA binding protein Air1 and the helicase Mtr4, a paralog of Mtl1 [88]. *S. pombe* also contains another non-canonical PAP, Cid12, which is not part of a TRAMP-like complex (see below).

TRAMP is an important co-factor of the RNA exosome

Transcripts adenylated by TRAMP are rapidly degraded by the RNA exosome (Figure 3), a conserved complex that constitutes the main source of nuclease activity in eukaryotic nuclei and is also active in the cytoplasm. The exosome core consists of a ring of six catalytically inert proteins with polynucleotide phosphorylase (PNPase) homology and three RNA binding proteins attached to one side, coined the cap. The core and the cap form a central channel ending at the 3'-5' hydrolytic exonuclease catalytic site of Rrp44 (alias Dis3) [89-91]. Rrp44 also contains an endonuclease site [92], that can access RNA independent of the core [93,94], but which has limited *in vivo* significance [95]. Rrp6, a nuclear specific 3'-5' exonuclease, and its co-factor Rrp47, are attached opposite to Rrp44 via interactions with the Csl4, Rrp43 and Mtr3 core subunits, which form the cap [91]. In all studied species, the exosome requires co-factors for substrate handling and here the TRAMP complex is the best understood. In *S. cerevisiae*, Mtr4 physically connects Trf4/5-Air1/2 with the exosome via binding to Rrp6 and Rrp47 [96]. Curiously, in human cells ZCCHC7 and PAPD5 interact with Rrp6 independently of MTR4 [86].

Oligoadenylation enhances the unwinding activity of Mtr4

The *S. cerevisiae* and human TRAMP-associated PAPs adenylate RNA 3'ends in a non-processive manner [10,87]. In the case of the *S. cerevisiae* complex, *in vitro* studies have shown that the TRAMP adenylation and helicase activities act in a cooperative manner to unwind structured RNAs. The Trf/Air dimer cannot adenylate blunt-ended dsRNA but requires an overhang of at least 1-3 nt [82,85,97]. On the other hand, Mtr4 requires a minimal 5 nt overhang, preferably of adenosines, to conduct its helicase activity. In turn, an A-stretch of 5 nt reduces the affinity of TRAMP towards ATP, limiting further adenylation [97]. Indeed, the average size of TRAMP-produced A tails *in vivo* appears to be 4-5 nt [98], however, it is not clear whether this is only due to the chemistry of TRAMP-mediated adenylation or whether exosome mediated tail shortening also plays a role [46]. Regardless, these data indicate that the TRAMP complex provides adenylation to stimulate the unwinding activity of Mtr4. The channel formed by the core and cap of the exosome can accommodate a 25-30 nt long RNA [91,99]. Thus, the primary function of TRAMP appears to be to produce a ssRNA long enough to be threaded through the exosome core.

Non-canonical polyadenylation targets diverse RNAs

The *S. cerevisiae* Trf and Air proteins are found in both the nucleoplasm and nucleolus and impact the maturation and expression of transcripts produced by all three RNA polymerases. Individual deletion of *TRF* or *AIR* genes does not significantly affect yeast growth at permissive conditions, however, loss of both *TRF4* and *TRF5* is synthetically lethal

and concomitant deletion of *AIR1* and *AIR2* results in severe growth impairment [100,101]. Thus, TRAMP4 and TRAMP5 appear to act redundantly in the processing of some essential RNAs, the identities of which remain unknown. Trf4 and Trf5 were both proposed to play a role in the decay of incorrectly processed rRNA and tRNA precursors [10,98,102-104], and to be required for the regulation of histone mRNA levels [105]. In addition, the Trf proteins have specialized sets of substrates with Trf4 targeting some mRNAs but mostly sn/snoRNAs, CUTs and Ty1 retrotransposons, while Trf5 is primarily involved in regulating mRNA abundance [10,12,106]. Interestingly, Trf4-mediated adenylation is dispensable for the maturation or decay of many substrates [106], which is probably because the Trf/Air dimer can stimulate Mtr4 unwinding activity in an adenylation-independent manner; at least *in vitro* [107]. Both of the Air proteins bind RNA rather unspecifically and may therefore rely on other factors for their substrate recruitment. Indeed, the specificity of TRAMP4 for ncRNAs is mediated by its association with the Nrd1-Nab3-Sen1 (NNS) complex, which recognizes short sequence motifs enriched in CUTs and snRNAs [98]. Simultaneously, Nrd1 binds to the RNAPII CTD and hereby mediates Sen1 helicase recruitment, which induces transcription termination. In a subsequent step, Nrd1 binds Trf4, which stimulates RNA decay, possibly by enhancing TRAMP4 adenylation activity or by stabilizing the TRAMP complex on the transcript, which allows efficient exosome recruitment (Figure 3) [108-110].

The human Air homolog, ZCCHC7 is strictly localized to nucleoli [79]. In contrast, while also localized to nucleoli, human PAPD5 and MTR4 are robustly present in the cell nucleoplasm. This differential localization of human TRAMP subunits is reflected in the repertoire of their RNA targets. PAPD5 and ZCCHC7 interact with proteins involved in rRNA processing and have been shown to mediate turnover of pre-rRNA 5'ETS fragment [79,86]. PAPD5 also binds to splicing factors [79,111], but the function of this connection still remains incompletely explored.

The *S. pombe* TRAMP subunit Cid14 is exclusively localized to nucleoli where it participates in the removal of aberrant tRNAs and rRNAs [112]; (Figure 2A). Another fission yeast polyA polymerase takes part in heterochromatin formation. In *S. pombe* production of small interfereing RNAs (siRNAs) is mediated by the RNA-induced transcriptional silencing (RITS) complex, which interacts with the RNA-directed RNA polymerase complex (RDRC, Table 1). RDRC is composed of the non-canonical polyA polymerase, Cid12, the helicase Hrr1 and the RNA-directed RNA polymerase, Rdp1. The Cid12 protein itself is required for heterochromatin formation, though it is unclear what is the role of its polyA-polymerase activity [113,114]. Curiously, impairment of nucleolar TRAMP function and its resulting accumulation of abundant aberrant tRNAs and rRNAs has been shown to direct the siRNA machinery towards nucleolar RNAs in an unspecific manner, thus perturbing heterochromatin formation at the usual loci [88,115].

In conclusion, all human and yeast TRAMP complexes participate in the decay of nucleolar targets [86,98,102-104,112,116]. However, it appears that *S.cerevisiae* Trf4/5 also have broad nucleoplasmic activities, which in *S. pombe* and human cells are are mediated by other complexes organized around MTR4: MTREC, NEXT and PAXT, respectively. Additionally, in the case of S. pombe, non-canonical polyadenylation mediates heterochromatin formation.

How can pA tail status help distinguish unstable from stable RNA?

In this review, we have described two opposing outcomes deriving from the addition of a pA tail to the 3'end of an RNA. Some adenylation events are coupled to exonuclease-mediated 3’end maturation or degradation, while others facilitate the stabilization of the RNA and its export to the cytoplasm. One critical question is therefore how RNAs that are destined for nuclear export, such as mRNAs, are distinguished from those that are normally degraded, such as PROMPTs, PCPA RNAs [117] and CUTs. *S. cerevisiae* has evolved an elegant and unique system in which cryptic transcripts are specifically terminated and marked for TRAMP/exosome degradation by the dedicated NNS complex. This, however, appears different in human and *S. pombe* cells where coding, and at least some cryptic, transcripts are 3’end processed by the CPF/CPSF pathway. Thus, the difference between export-competent and nuclear-degraded RNAs must lie in the differential recruitment of proteins to these RNAs. However, though the question is still not fully explored, the answer does not seem to be fully provided by the differential recruitment of export *vs*. degradation factors. Firstly, the PAXT subunit PABPN1 is supposedly recruited to all these RNAs as an intrinsic component of the CPSF complex, and secondly, the RBM7 subunit of the NEXT complex appears to bind all nascent RNAs alike [118]. Thus other features must contribute to selecting RNAs for decay. One to consider here is transcript length. *S. cerevisiae* CUTs, human PROMPTs and PCPA transcripts are all short in comparison to their mRNA counterparts. For CUTs this feature has a key bearing in promoting their transcription termination as Nrd1 binds the RNAPII CTD phosphorylated at serine 5 residues, a hallmark of early transcription activity. A conceptually analogous mechanism might be operating in human cells through the CBC, which is physically close to the 3’ends of the short PROMPT/PCPA RNAs and might elicit degradation by its efficient recruitment of the RNA exosome via the CBCN complex (Figure 2B) [80].

Such probable propensity of short transcripts to be degraded does, however, not explain how nuclear decay of longer transcripts, which are terminated by the CPSF pathway and bound by PABPN1, can occur. A ‘nuclear-timer’ model has therefore been proposed, suggesting that PABPN1 binding to newly synthesized RNAs initially yields protection, while decay will only be promoted if the RNA has a prolonged residence time in the nucleus, providing sufficient time for RNA exosome recruitment [119,120]. Several mechanisms, such as export kinetics or splicing, could contribute to selective nuclear retention. With the exception of specific highly expressed genes, which upon activation might translocate towards the NPC [121], most mRNAs diffuse stochastically within the interchromatin matrix until reaching the nuclear exit [122]. Thus, some transcripts may reside in the nucleus for a sufficient amount of time to be targeted for decay. Studies in the human system have shown that various mRNAs exhibit different nuclear export rates and

depending on the cell type and environmental condition some mRNAs might reside longer in the nucleus than in the cytoplasm, which was proposed to play a role in reducing the transcriptional noise resulting from gene-activation bursts [123]. Exact mechanistic aspects of selective transcript retention remain elusive but are probably also relevant for the degradation of improperly processed transcripts that are not exported with sufficient kinetics. An obvious feature contributing to nuclear retention time is splicing. In *S. cerevisiae*, the NPC is actively engaged in retaining unspliced mRNAs in the nucleus via the Mlp1 and Mlp2 proteins located on the nuclear face of the pore complex [81]. While this has not been reported to occur in the human system, it has been shown that intron excision stimulates RNA export [124]. Moreover, some poorly processed transcripts with nuclear localization are stabilized in cells overexpressing a dominant-negative PABPN1 mutant [125], which indicates that slow splicing could lead to nuclear retention and degradation mediated by PABPN1.
The length of the pA tail itself might also be a key factor that promotes nuclear retention as a critical length of the adenosine chain is required for promoting export [47]. In *S. cerevisiae*, CUTs, which are destined for nuclear decay, are on average short-tailed, which is a consequence of the low Trf4 processivity and the physical interaction of the TRAMP complex with the exosome. Human PAP is a distributive enzyme in the early phase of its polyadenylation activity and prior to PABPN1 recruitment. This feature might result in formation of a fraction of RNAs harbouring only short pA tails, which would be more susceptible to decay. It is therefore tempting to speculate that polyadenylation of some unstable human ncRNAs might be negatively regulated to further their decay. This regulation could depend on sequence elements. The FIP1 protein is most efficient at stimulating PAPOLA activity when the RNA template is U-rich. In a similar manner, other CPSF associated proteins, like CFIm, CFIm68 and CFIm59, have the capacity to bind RNA in a sequence specific manner and their recruitment regulates Fip1 function in alternative polyadenylation [32]. Polyadenylation efficiency can also be influenced by other activities such as splicing, which has been shown to stimulate 3'end processing [32,126-128]. The CPSF complex is recruited to the nascent RNA by binding to RNA and to the RNAPII CTD phosphorylated as serine 2 [129]. It is therefore also possible that a polymerase that has not yet entered into a state of productive elongation, marked by robust serine 2 phosphorylation status, does not support formation of a fully competent polyadenylation machinery. Further studies will show whether any of these aspects of the polyadenylation process explain the de-stabilization of transcripts destined for nuclear decay.
Polyadenylation is a 3'end modification that can direct RNA fate towards stability and export or processing and decay, depending on the context in which it is produced. TRAMP-mediated adenylation is always coupled to exonucleolysis, whereas CPF/CPSF A-tailing may induce export or decay, depending on still ill explained mechanisms. While some of the decisive potential for CPF/CPSF-targets appears to relate to PABs, an equally important, but less explored, role might be exercised by negative regulation of PAP activity, potentially impacting export efficiency and allowing more time for nuclear decay.

List of abbreviations:

PAP – polyA polymerase; PABP – polyA-binding protein; CUT – Cryptic Unstable Transcript; PROMPT - PROMoter uPstream Transcript; PCPA RNA – Premature Cleavage and Polyadenylation RNA; TRAMP - Trf4/5-Air1/2-Mtr4 Polyadenylation complex; hTRAMP – human TRAMP; NNS – Nrd1-Nab3-Sen1 complex; ncRNA - non-coding RNA; CPF – Cleavage and Polyadenylation complex; CFI – Cleavage Factor I; CPSF – Cleavage and Polyadenylation Specificity Factor, RNAPII – RNA polymerase II; PAXT – PolyA RNA exosome Targeting connection; PPD – PABPN1 and PAPα/γ-mediated decay; NEXT – Nuclear EXosome Targeting complex; MTREC - MTl1-Red1 Core; NURS - NUclear RNA Silencing; THO – suppressor of the transcriptional defect of Hpr1 by overexpression; TREX – Transcription and Export complex.

Additional Information

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Figure and table captions

Fig.1 Model of CPF/CPSF-mediated mRNA 3'end formation and nuclear export
A. Schematic overview of 3'end cleavage, polyadenylation and export factors from *S. cerevisiae* (left panel) and human (right panel) cells. Factors with no human or *S. cerevsiae* orthologs with a similar function in polyadenylation and export, respectively, are marked with dashed outlines. In some cases the human ortholog has not yet been studied in the context of cleavage and polyadenylation. The CPF and CPSF complexes are composed of three basic modules (inspired from model in [22]): (i) the endonuclease module organized around the Ysh1/CPSF73 enzyme supported by Cft2/CPSF100 and Mpe1; (ii) the RNAPII phosphatase module, the catalytic activity of which is mediated by the Glc7/PP1 and Ssu72 subunits of the 'associated with Pta1' (human symplekin protein – SYM*) complex (APT: Pta1, Swd2, Ref2, Pti1, Syc1); and (iii) the pA polymerase module, containing the Pap1/PAPOLA/PAPOLG/Star-PAP enzymes, which are recruited to this module mainly by an interaction with Fip1/FIP1, but also with Cft1/CPSF160, Pfs2/WDR33 and Yth1/CPSF30. The yeast CPF complex is further assisted by Cleavage Factor I (CFI: Clp1, Hrp1, Pcf11, Rna14, Rna15), while the human CPSF complex is supported by the CFI-like sub-complexes: cleavage stimulation factor (CstF: CstF50/77/64), cleavage factor II (CFIIm: CLP1, PCF11) and the human-specific cleavage factor I (CFIm: CFIm25/59/68). The CPF and CPSF complexes are recruited dually by binding to specific sequences in the nascent RNA and to the RNAPII CTD. In *S. cerevisiae* cells polyadenylation is shown to be stimulated by the THO complex (indicated by the arrow) and in human cells by PABPN1 (arrow). The formation of an export competent mRNP starts co-transcriptionally with association of the cap-binding complex (CBC, CBP20/80). In yeast Npl3, TREX and Nab2 loading enhances recruitment of the export adaptor Mex67/Mtr2, while in human only the TREX subunit Aly/REF stimulates nuclear export. Successful synthesis of a long pA tail and mRNP assembly leads to release of the newly made RNA from the site of transcription and its translocation to the nuclear pore. Mex67/NXF1 interaction with core FG-nuclear pore factors and Nab2 interactions with the Gfd1 NPC subunit stimulates export. Nab2 binding to Mlp1/Mlp2 promotes retention of unspliced transcripts. An mRNP associated helicase Dbp5/DDX19B is activated by interaction with the Gle1/GLE1 NPC subunit located at the cytoplasmic face and contributes to the displacement of Nab2 and possibly other nuclear mRNP subunits from the transcript. Other mechanisms also contribute to recycling of export factors, such as phosphorylation in case of Npl3. This allows for the release of the transcript into the cytoplasm. Human PABPN1 shuttles between the nucleus and the cytoplasm, but the mechanism by which it is exchanged by cytoplasmic PABPC1 is still unclear.
B. Schematic overview of domain and motif organizations of the main CPF-associated non-orthologous PABs in *S. cerevisiae* (left panel - Nab2) and human (right panel – PABPN1) cells highlights the diverse functions of these proteins in pA tail length control and RNA nuclear export. PABPN1 binds RNA using an RNA Recognition Motif (RRM), while Nab2 recognizes RNA via its three distal zinc fingers (ZnFs) [43]. PABPN1 interacts directly with and stimulates PAPOLA activity, while Nab2 binds the NPC subunits Mlp1/2 and Gfd1 through its N-terminal domain. Moreover, PABPN1 interacts with the spliceosome subunit SKIP [130]. The region responsible for nuclear import of Nab2 is located within the RGG domain [61].

Fig. 2. Nuclear RNA decay pathways in *S. pombe* and human cells
A. Schematic overview of the different modules of the *S. pombe* MTREC/NURS complex. Mtl1 and Red1 are central proteins that are suggested to facilitate the recruitment of the various modules to the RNA exosome. The Mmi1-Iss10 module, assisted by Pab2, plays a key role in the removal of meiotic mRNAs during vegetative growth. The modules binding the RNA 5'cap (Cbc1-Cbc2-Ars2) and 3'end pA-tail (Pab2-Rmn1-Red5) mediate the removal of CUTs. Finally, the Mtl1–Ctr1–Nrl1 complex interacts with the spliceosome and is involved in the recognition and degradation of unspliced or mis-spliced RNA. Also depicted is the *S. pombe* TRAMP complex, composed of Cid14, Air1 and the Mtl1 paralog Mtr4. This complex targets nucleolar tRNAs and rRNAs for decay.
B. Schematic overview of nucleoplasmic human complexes involved in RNA targeting. The cap binding complex (CBC), composed of CBC20 and CBC80, is bridged via the ARS2 and ZC3H18 proteins to the NEXT complex composed of the RNA binding protein RBM7, the Zn-finger protein ZCCHC8 and MTR4, forming the CBC-NEXT (CBCN) complex [79,80]. The NEXT complex, which does not appears to have a *S. pombe* orthologues module, targets short cryptic RNAs, like PROMPTs and enhancer RNAs (eRNAs), for exosomal decay. PABPN1, on the other hand, targets longer ncRNAs and pre-mRNAs, which are polyadenylated by PAPOLA/PAPOLG, via the PPD and/or PAXT pathways. PAXT can also interact with the CBC via ARS2 and ZC3H18. The human TRAMP-like complexes,

composed of the RNA-binding protein ZCCHC7, MTR4 and one of the pA-polymerases PAPD5 or PAPD7, mediate the decay and processing of nucleolar rRNAs.

Figure 3. Schematic overview of *S. cerevisiae* TRAMP4/5 complexes and their physical relationships with the NNS and exosome complexes.
TRAMP4 and TRAMP5 can both be recruited to their targets via Air1/2-mediated RNA binding. In addition, TRAMP4 recruitment to some ncRNAs is enhanced by the direct binding of Trf4 to the Nrd1 subunit of the NNS complex. TRAMP4/5 complexes contact the exosome via Mtr4. TRAMP4 interaction with the exosome can be further enhanced via binding of Nab3 to Rrp6 [131].

Figure 1

A

S. cerevisiae

RNAPII
TREX
THO
Tex1
Thp2
Tho2
Hpr1
Mft1
Cbp20/80
Dbp5
Npl3
Yra1-Sub2
CFI
Clp1
Hrp1
Pcf11
Rna14
Rna15
Yth1
Pfs2
Fip1
Pap1
Cft1
PolyA polymerase
phosphatase
Nab2
Mex67 Mtr2
Cft2
Ysh1
Glc7
Ref2
Swd2
Mpe1
Pta1
Pti1
Ssu72
endonuclease
Mlp1/2
FG-Nuclear Porines
Gle1
Dbp5
Gfd1
ATP
ADP+ Pi
ATP
ADP
P
Pab1
AAAAAAAAAAAAAAAA

HUMAN

RNAPII
TREX
THO
THOC5
THOC6
THOC7
THOC3
THOC1
DDX19B
Cbp20/80
Aly/REF-UAP56
hCFIm
25-25
59
68
CstF
CstF50
CLP1
CstF77
PCF11
CstF64
CPSF30
WDR33
FIP1
CPSF160
PolyA polymerase
phosphatase
PAPOLA/G
Star-PAP
PABPN1
NXF1 NXT1
CPSF73
PP1
CPSF100
SYM*
SSU72
endonuclease
AAAAAAAAAAA
TPR
GLE1
DDX19B
ATP
ADP+ Pi
PABPC1
AAAAAAAAAAAAAAAA

B

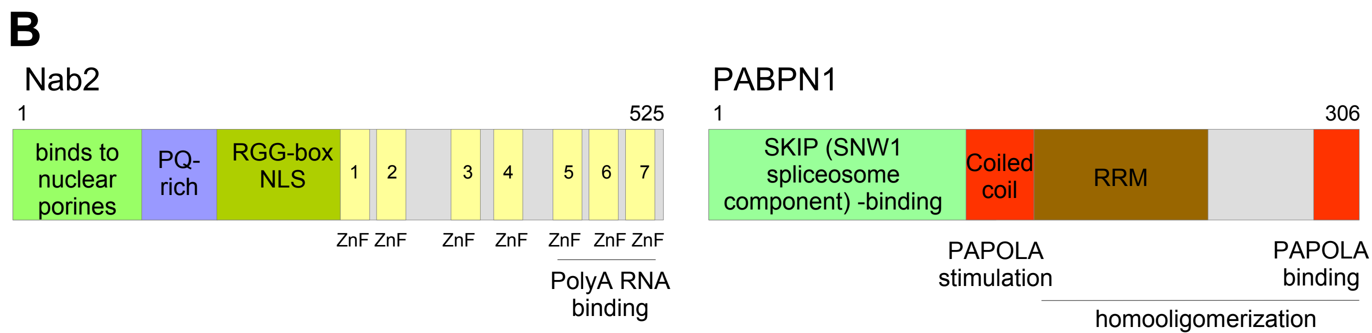
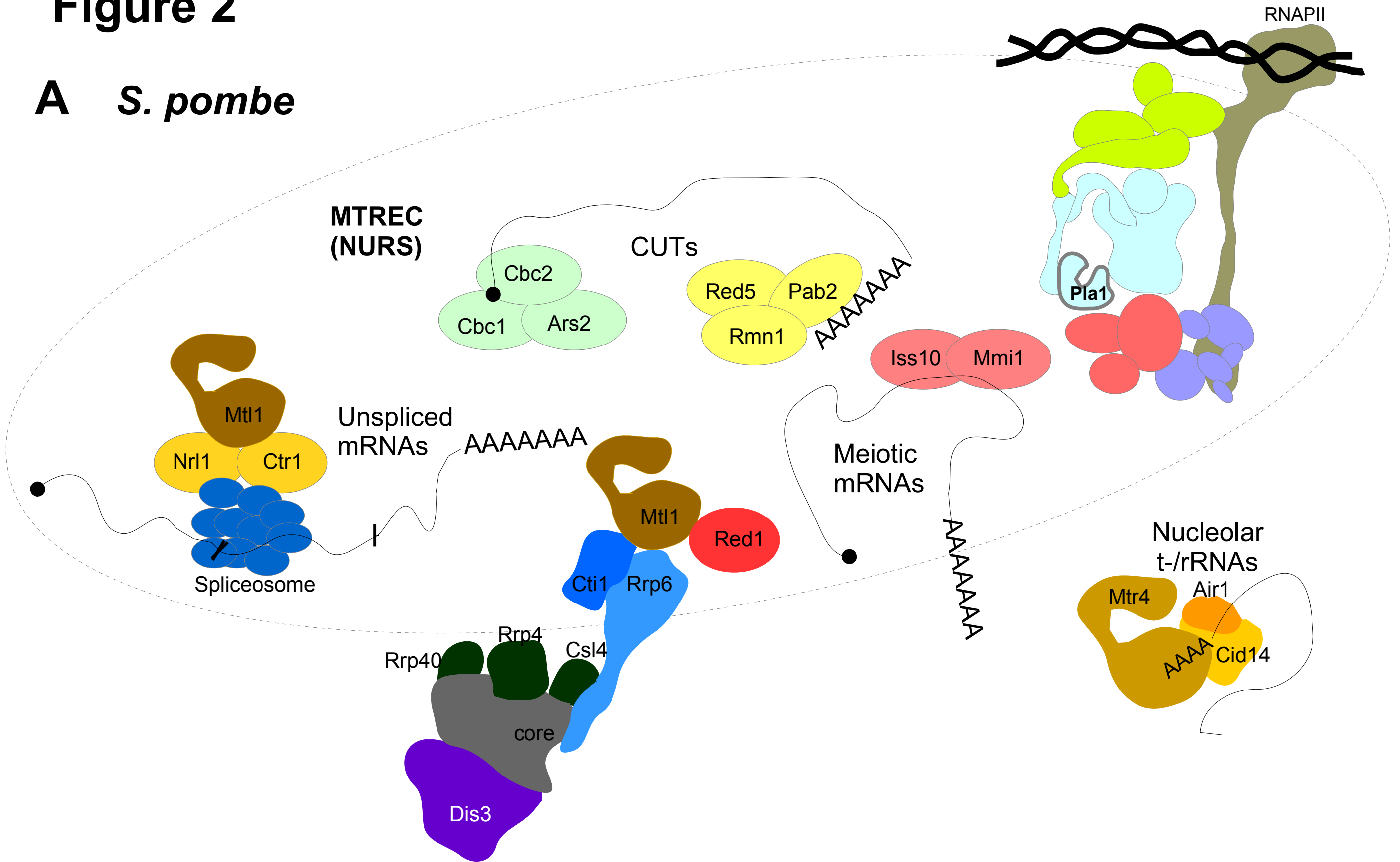


Figure 2



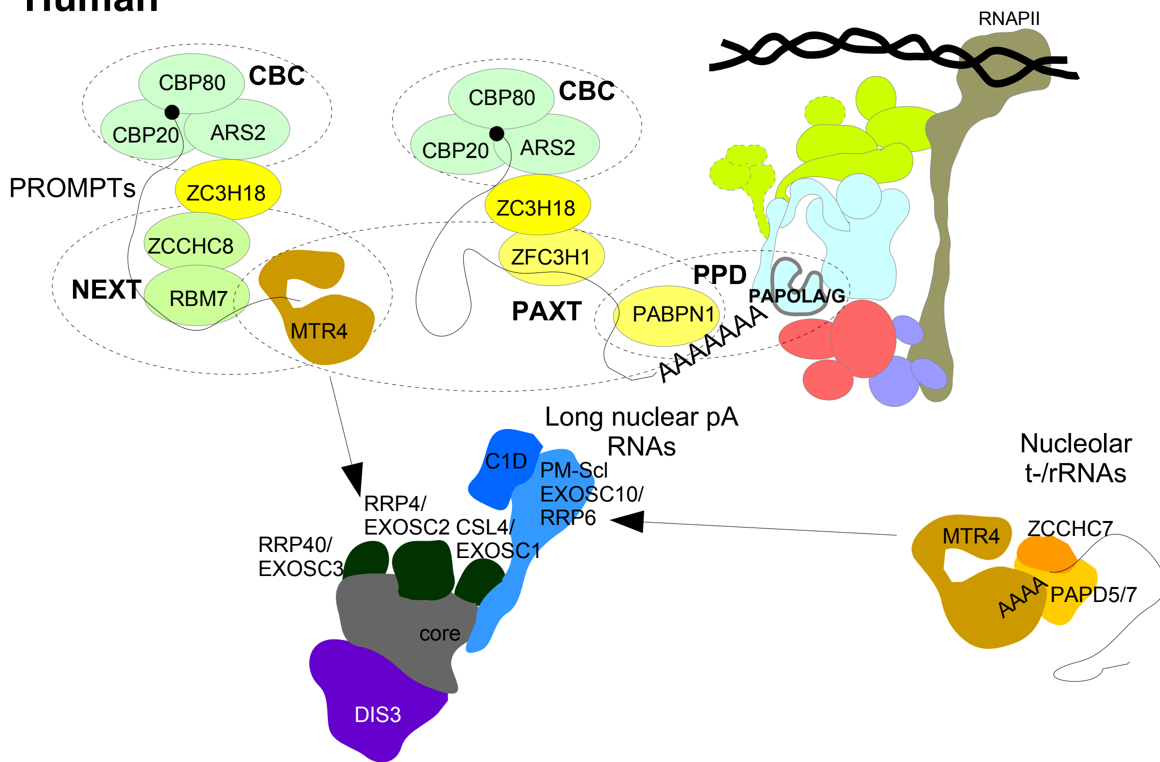


Figure 3

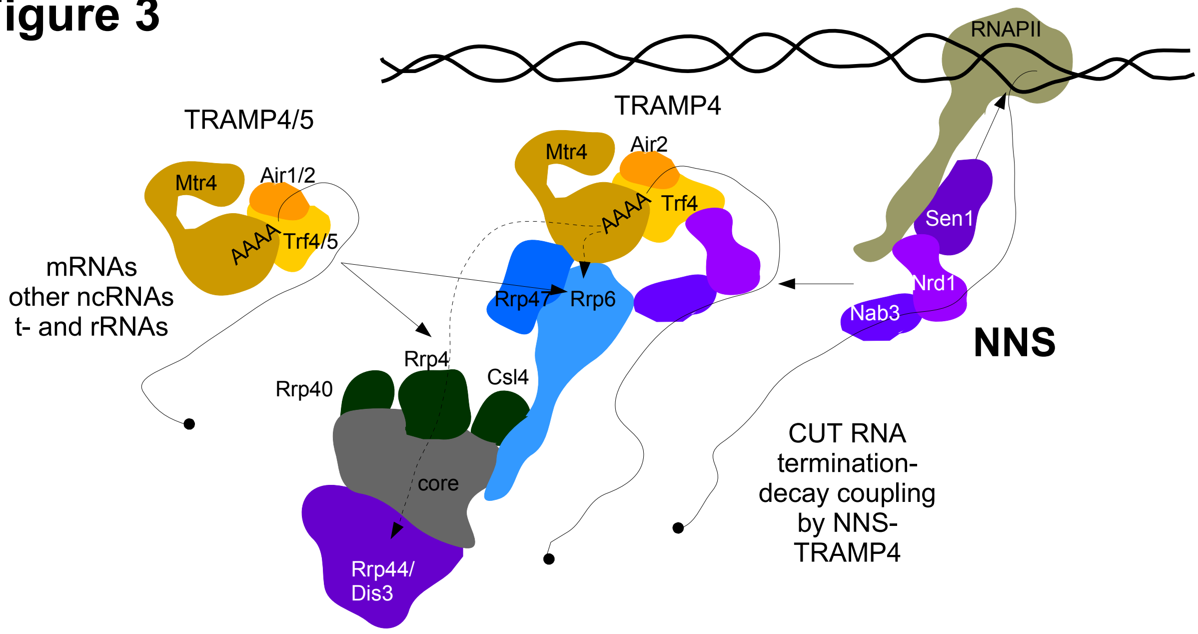


Table 1 Ortholog proteins involved in nuclear RNA decay in *S. cerevisiae*, *S. pombe* and human cells.

S. cerevisiae		***S. pombe***		***H. sapiens***			**Main activity:**
Protein	**Complex**	**Protein**	**Complex**	**Protein**	**Complex**		
Trf4 Trf5	TRAMP4 TRAMP5	Cid14	TRAMP	PAPD5 (TRF4-2) PAPD7 (TRF4-1)	TRAMP		Non-canonical polyA-polymerase
Air1/Air2		Air1		ZCCHC7			RNA binding (zinc knuckle)
Mtr4		Mtr4		MTR4 (SKIV2L2)			Helicase
		Mtl1	MTREC/ NURS				
-		Ars2		ARS2		CBCA	CBC binding
Sto1 (Cbp80)		Cbc1		CBP80	CBC		Cap binding complex large subunit
Cbp20 (Cbc2)		Cbc2		CBP20			Cap binding complex small subunit
-		-		ZCCHC8	NEXT (with MTR4)		Zinc finger; linker between MTR4 and RBM7
-		-		RBM7			RNA binding (RNA Recognition Motif)
-		-		ZC3H18	-		RNA binding (zinc finger); linker between CBCA and NEXT or PAXT
-		Red1		ZFC3H1	PAXT (with MTR4)		RNA binding (zinc finger)
Sgn1		Pab2		PABPN1		PPD	pA RNA binding (RNA Recognition Motif)
Pap1		Pla1		PAPOLA/G			Canonical polyA polymerase
-		Rmn1		Rbm26/27			pA RNA binding (RNA Recognition Motif)
-		Red5		ZC3H3			RNA binding (zinc finger)
-		Iss10		-			Associates with meiotic transcripts
Pho94		Mmi1		YTHDF1/2/3			YTH domain
-		Ctr1		CCDC174			Telomerase regulatory factor Ctr1
-		Nrl1		NRDE2			Spliceosome-associated protein Nrl1
Hrp1		Msi2		MSI1/2 DAZAP1			mRNA cleavage factor complex subunit (predicted in human)
Nrd1	NNS	Seb1		SCAF4 SCAF8			RNA binding (RNA Recognition Motif), Rpb1 binding: CTD-S5P in *S. cerevisiae* and CTD-S2P in *S.pombe* and human
Nab3		Nab3		RALY/RALYL HNRNPC/HNRNPCL1/2/3/4			RNA binding (RNA Recognition Motif)
Sen1		Sen1		SETX			Helicase
-		Cid12	RDRC	-			Non-canonical polyA-polymerase
-		Hrr1		ZNFX1			Hrr1: helicase ZNFX1: RNA binding (zinc finger domain)
-		Rdp1		-			RNA-directed RNA polymerase
Nab2		Nab2		ZC3H14			RNA binding (zinc finger domain) interaction with nuclear porines