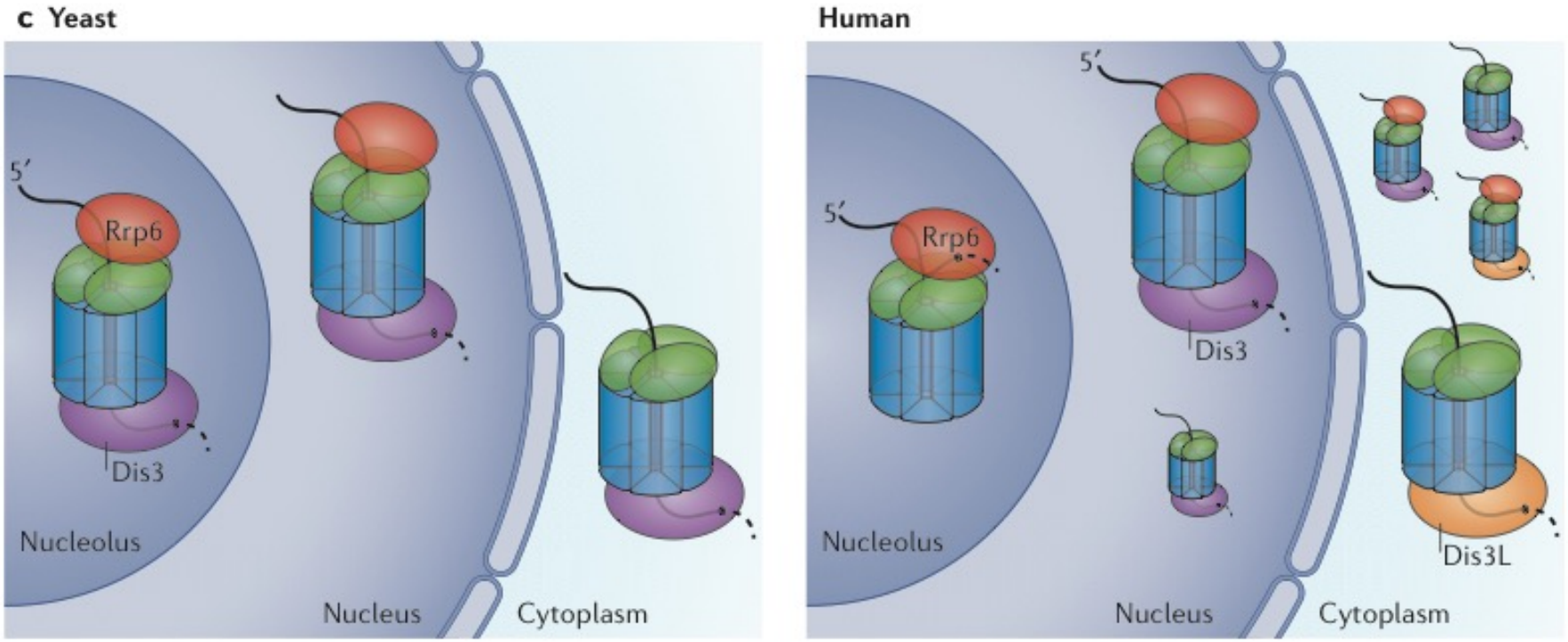
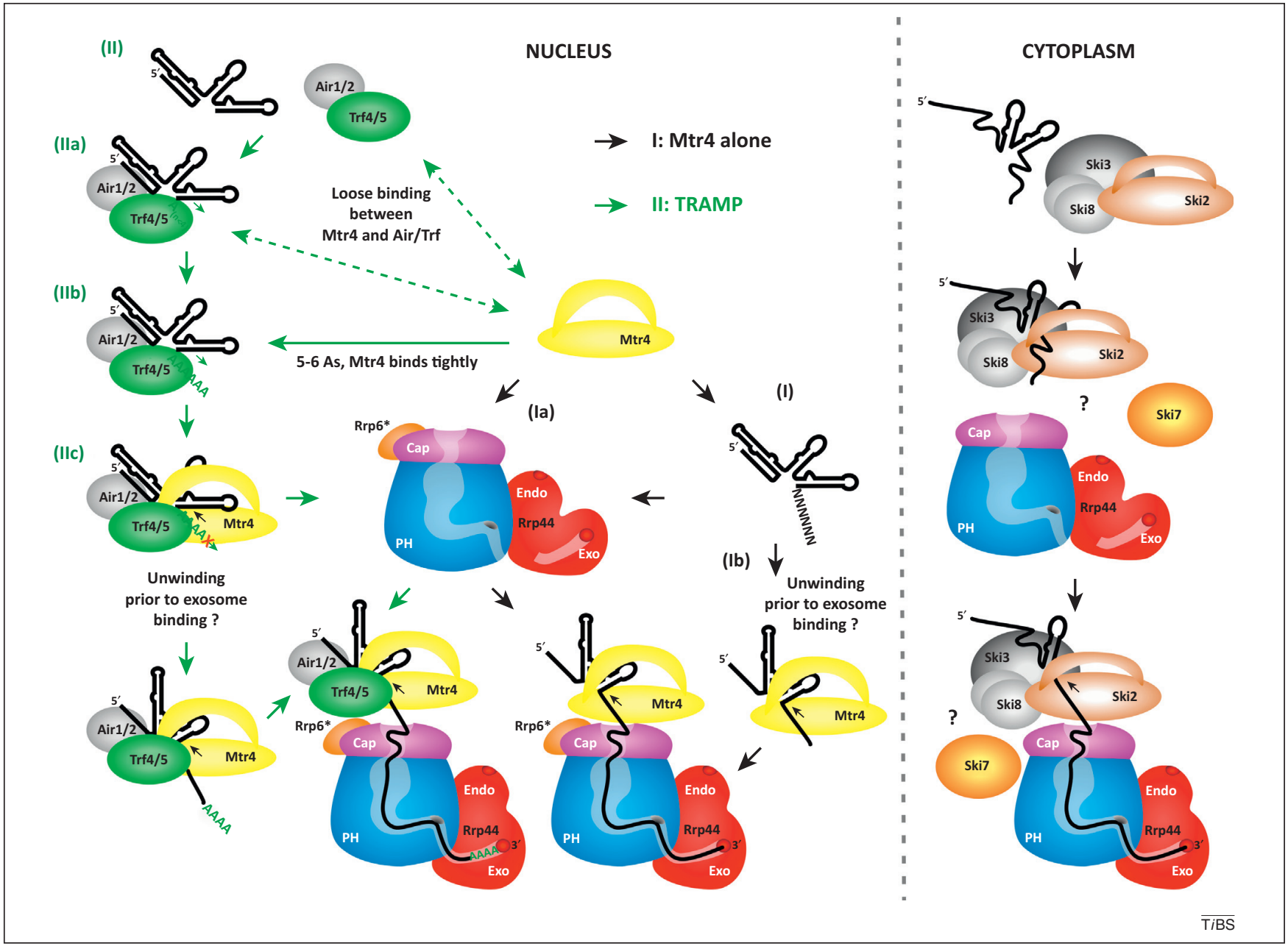
The Trf4p/Air2p/Mtr4p polyadenylation complex (**TRAMP**)

The composition of exosome complexes differs between yeast and human.

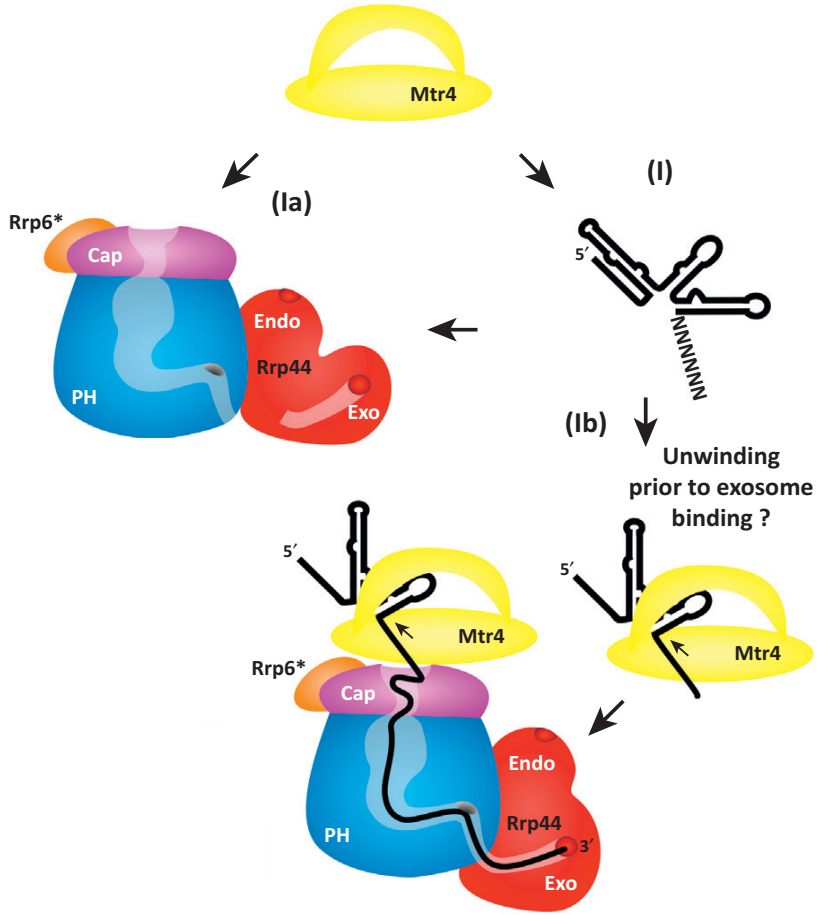


In *S. cerevisiae* (left), exosome complexes with the Rrp6 subunit are exclusively localized in the nucleus, whereas exosomes with Dis3 can be found throughout the cell. In human cells (right), however, Dis3 is excluded from the nucleolus. The Dis3 homologue Dis3L also associates with the exosome core and is restricted to the cytoplasm. In addition, small amounts of exosomes with different exonuclease subunits are present in the cytoplasm (indicated by relative sizes).

Cofactors for the exosome complexes



Looking for Dob1/Mtr4 interacting proteins



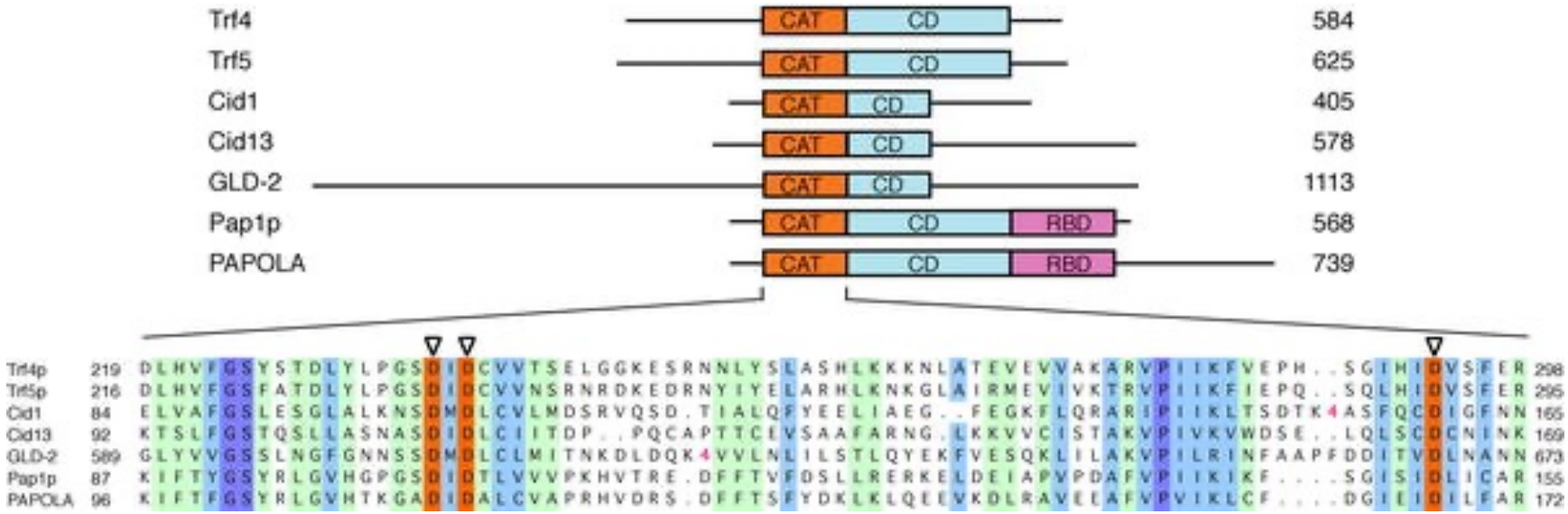
- Two hybrid system
- TAP purification

The TAP purified exosome does not work efficiently in vitro, even in the presence of Dob1.

Trf4p and Trf5p

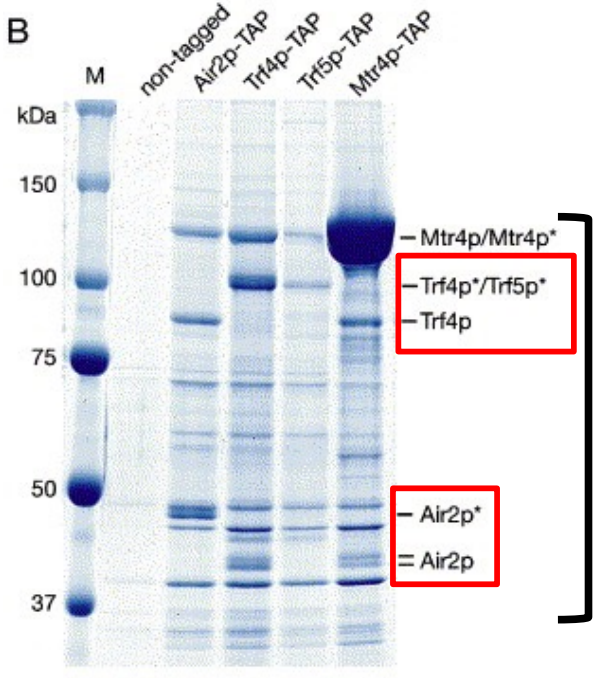
•Trf4p and Trf5p are related to the β-nucleotidyltransferase superfamily and share several characteristic features with canonical poly(A) polymerases

•Trf4p and Trf5p lack a recognizable RNA-binding domain.



The TRAMP Complex

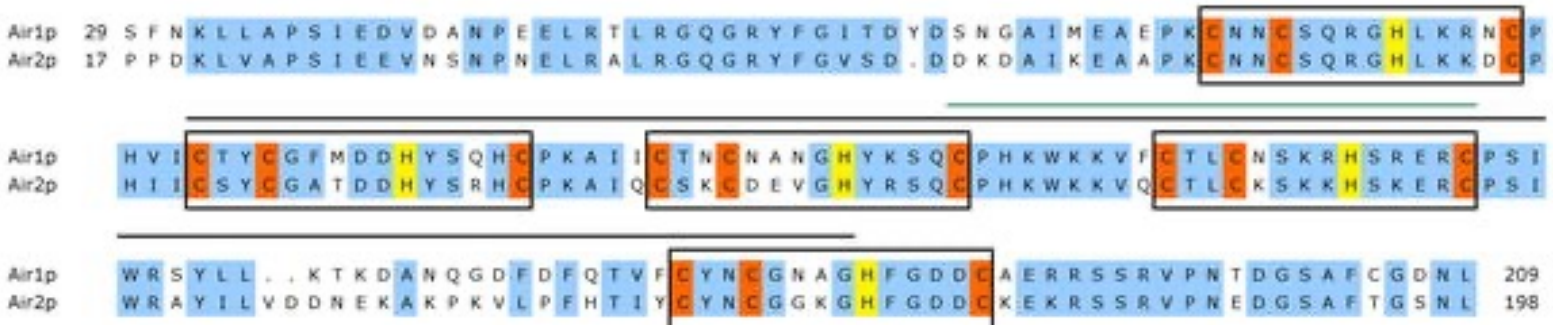
Mtr4-TAP
Trf4-TAP
Trf5-TAP
Air2-TAP



Mtr4=Dob1

TRAMP

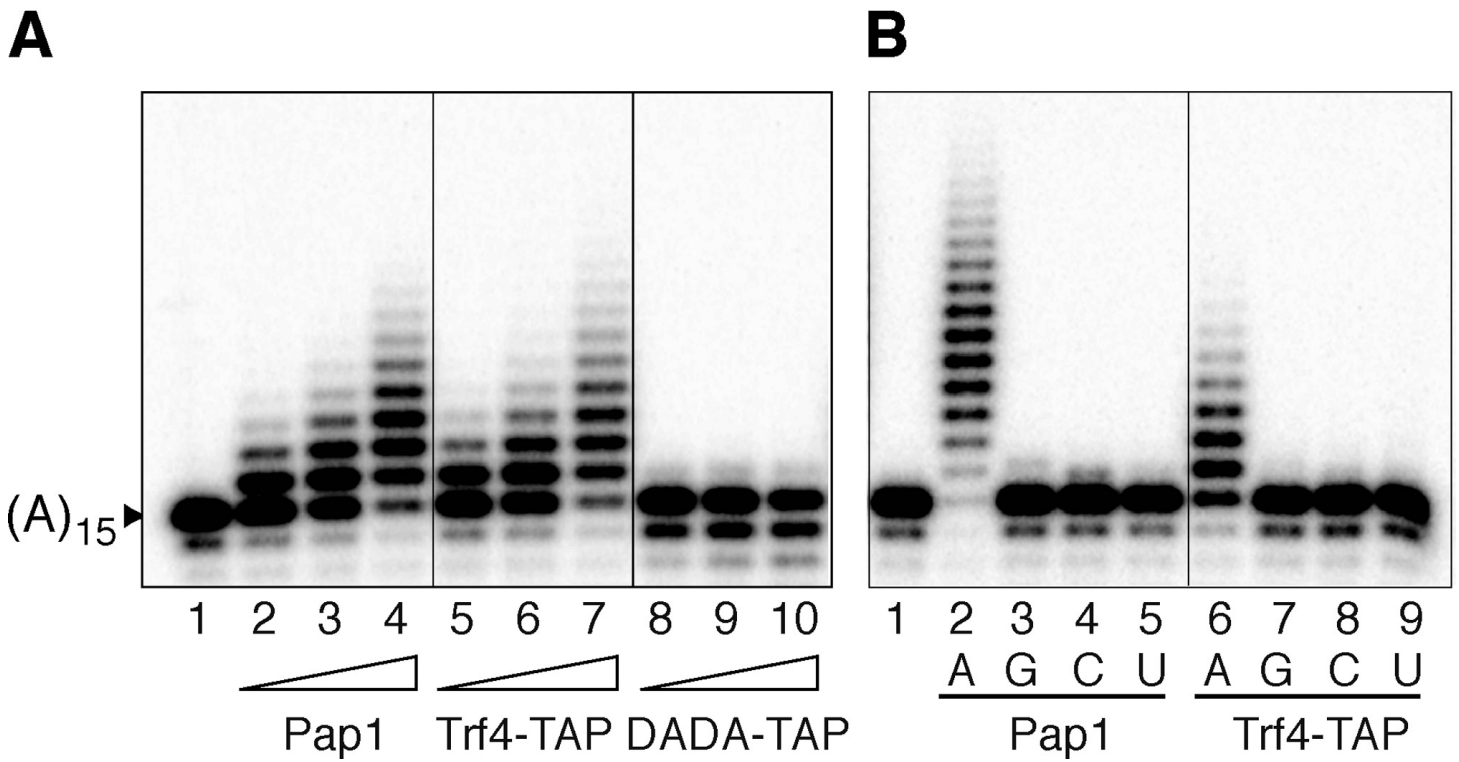
Air1 and Air 2 are two putative RNA binding proteins (non essential)



LaCava et al., 2005. Cell

The TRAMP Complex polyadenylates RNA

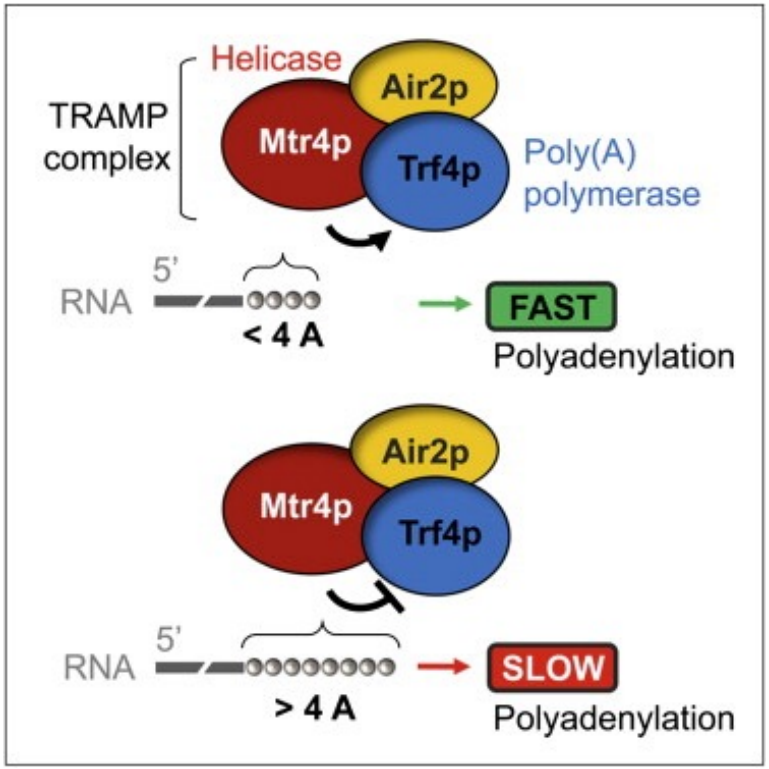
Polyadenylation activity associated with the TRAMP complex (purified with TAP-TRF4). *In vitro* transcribed 5′-end labeled RNA was incubated with the affinity-purified TRAMP and analyzed by electrophoresis.



DADA is a mutant of Trf4 mutant with the aspartic acid residues 236 and 238 changed to alanines

The RNA Helicase Mtr4/Dob1 Modulates Polyadenylation in the TRAMP Complex

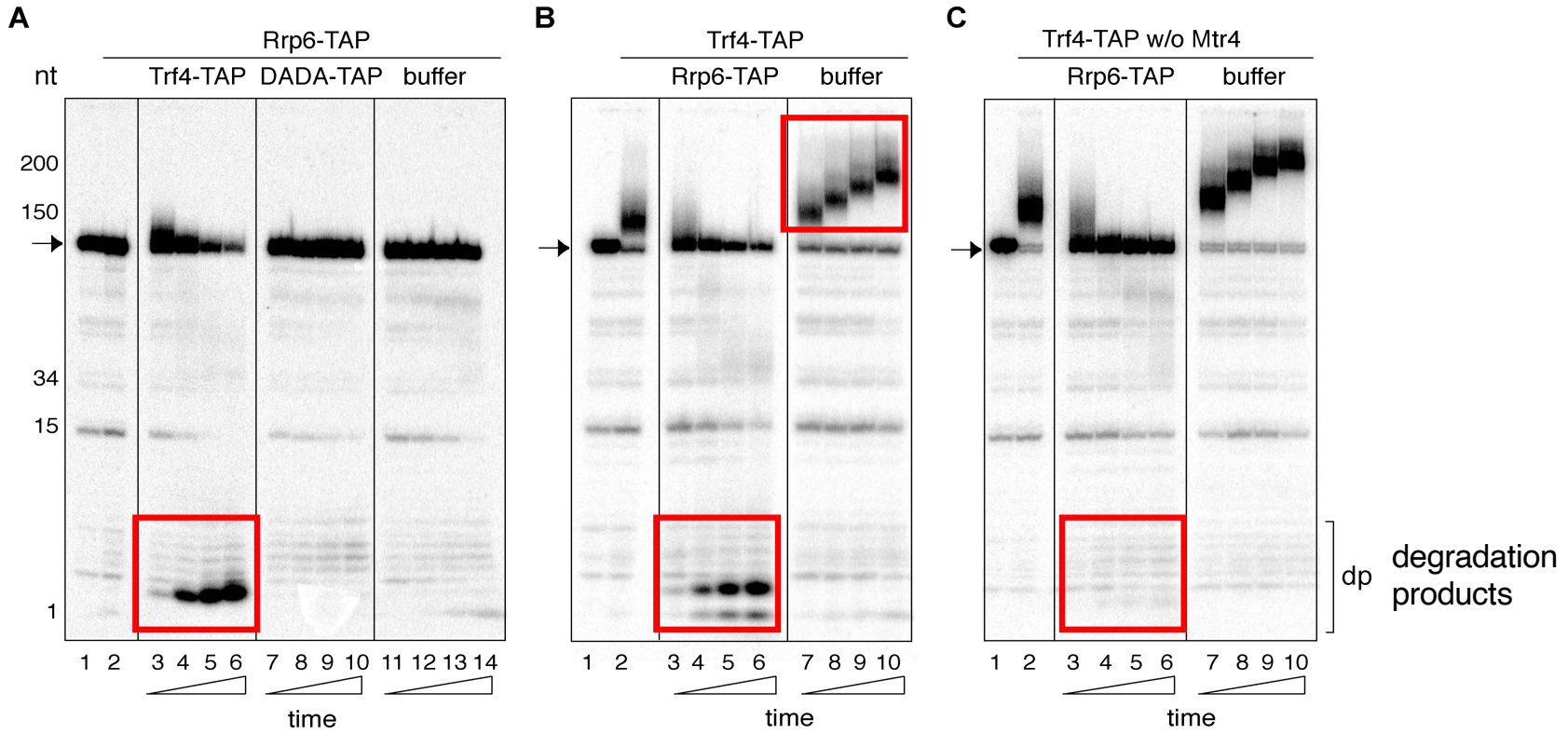
- The TRAMP polyadenylation complex restricts adenylation after 3 to 4 adenosines
- Restriction of the poly(A) tail length is controlled by the RNA helicase **Mtr4/Dob1**
- **Mtrp/Dob1** detects the number of 3′-terminal adenosines
- **Mtr4/Dob1** limits polyadenylation by controlling the activity of Trf4p poly(A) polymerase
- **PABP** needs at least 25 A for association with polyA



Jia et al., 2011. Cell.

The TRAMP Complex Stimulates RNA Degradation by the Exosome *in vitro*

The 5′-end-labeled unmodified $tRNA_i^{Met}$ was pre-adenylated for 30 min with Rrp6-TAP or Trf4-TAP or Trf4-TAP/w/o Mtr4



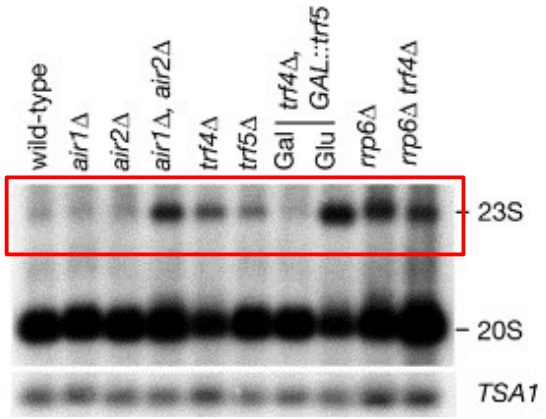
(**A**) In a coupled exosome/polyadenylation assay, 5′-end-labeled RNA was incubated with affinity-purified Rrp6-TAP followed by addition of 50 ng of wild- type (Trf4-TAP), mutant complex (DADA-TAP), or buffer A (buffer).

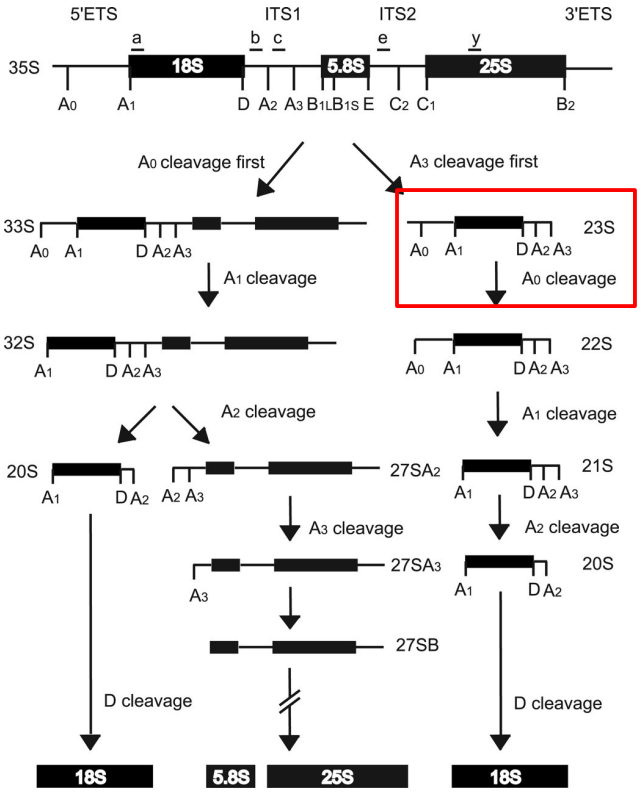
(**B**) The RNA was pre-adenylated with affinity-purified Trf4-TAP complex. Then exosome complex (Rrp6-TAP) or buffer A (buffer) was added, and the reactions were continued as in (A).

(**C**) Depletion of Mtr4p results in incomplete degradation.

The TRAMP Complex Is Required for Normal RNA Processing and Degradation *In Vivo*

Northern hybridization of 23 Spre-rRNA. Strains with GAL-regulated alleles were grown in galactose and transferred to glucose for the times indicated. The TSA1 mRNA was used as a loading control.





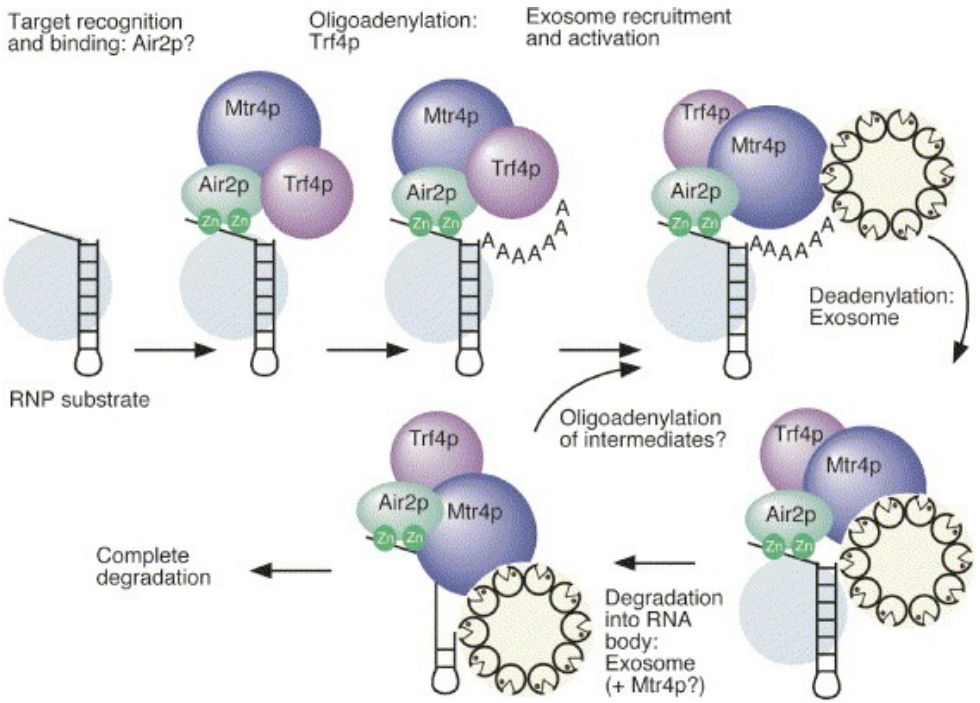
Roles of the TRAMP Complex in RNA Degradation

- The TRAMP complex interacts with RNAs or RNP complexes, making them targets for degradation. For most substrates other than tRNAs, this will primarily be via protein:protein interactions.

-The zinc finger domains of Air2p is involved in substrate binding. The RNA is then polyadenylated by Trf4p.

- Exosome recruitment and activation requires the intact TRAMP complex. The activated exosome then rapidly deadenylates the RNA and can penetrate into regions of stable structure.

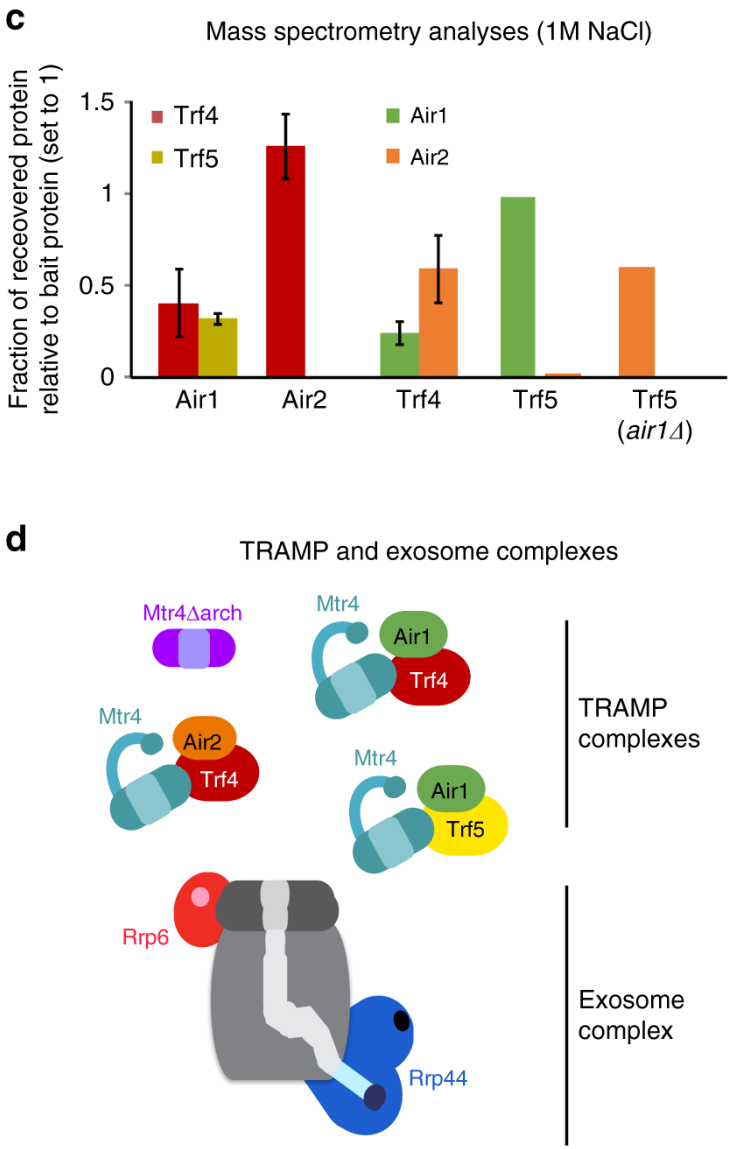
- Helicase activity of Mtr4p is important for dissociation or remodeling of stable RNP structures to allow passage of the exosome.



LaCava et al., Cell 121: 713-724 (2005)

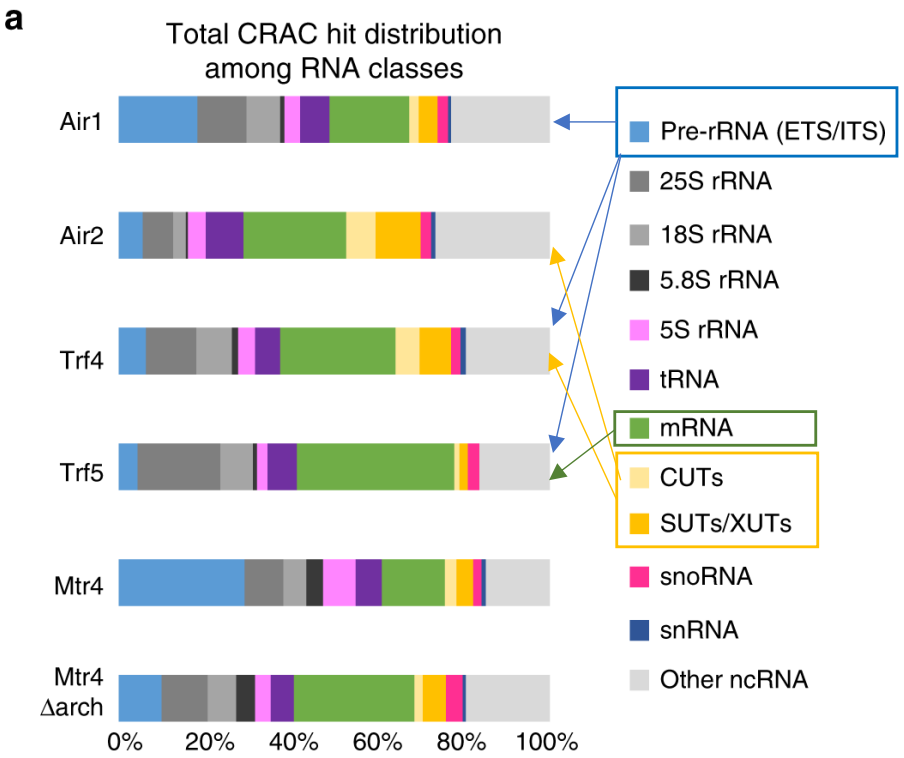
Three distinct TRAMP complexes are detected in vivo

Air1, Air2, Trf4, and Trf5 were each tagged with His6-TEV-protein A.



The TRAMP complexes exhibit distinct substrate preferences

UV-cross-linking followed by protein purification and sequence analysis of cDNAs (CRAC)

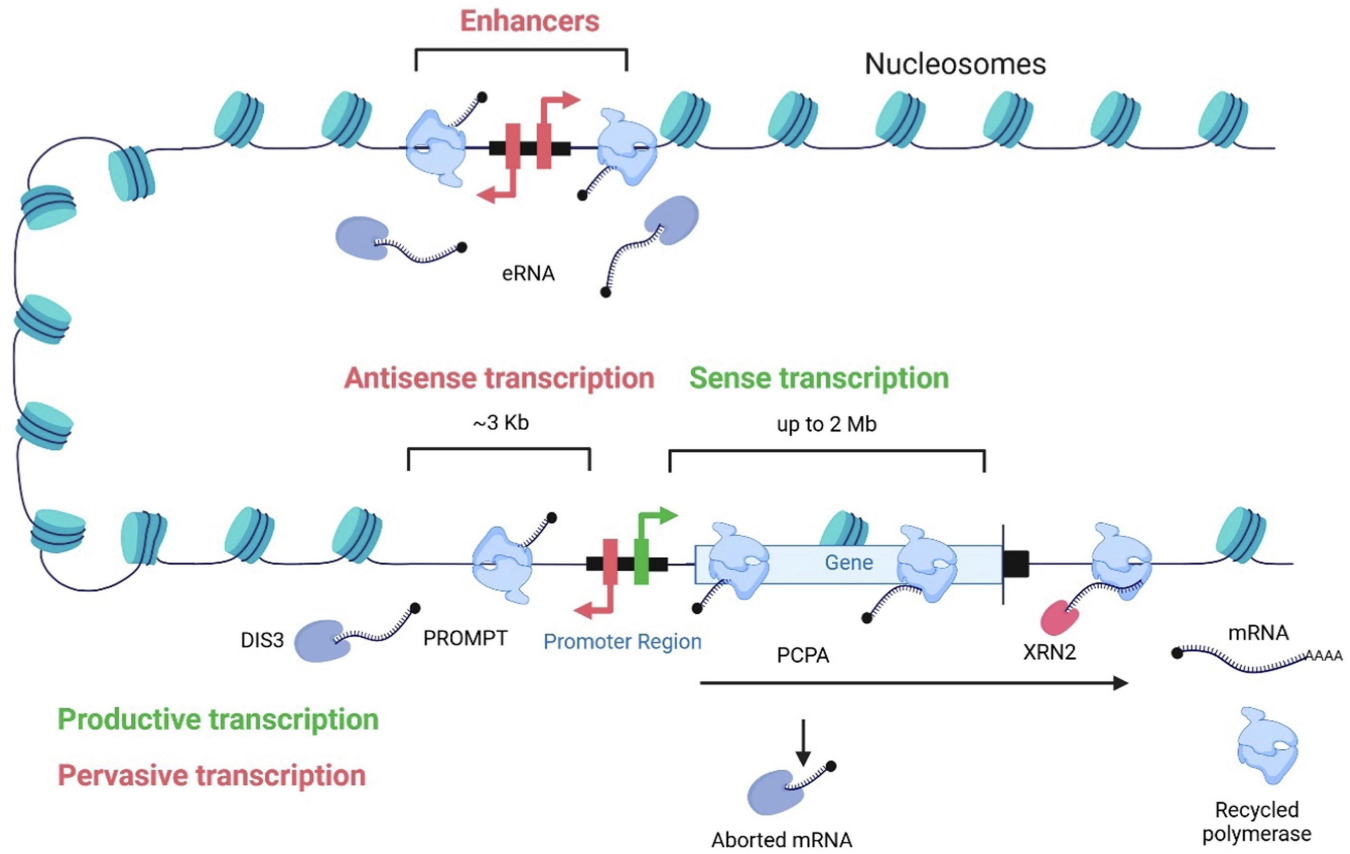


The Air1/2 and Trf4/5 pairs show some functional redundancy, since the single mutants are viable, whereas double mutants are inviable or severely growth impaired, depending on strain background. However they show sequence divergences that are much greater than most duplicated gene pairs in yeast, suggesting that the functions of the different forms of the yeast TRAMP complex may also have diverged and developed distinct specificities in vivo.

Delan-Forino, et al . *Nat Commun* (2020)

Pervasive transcription (PT)

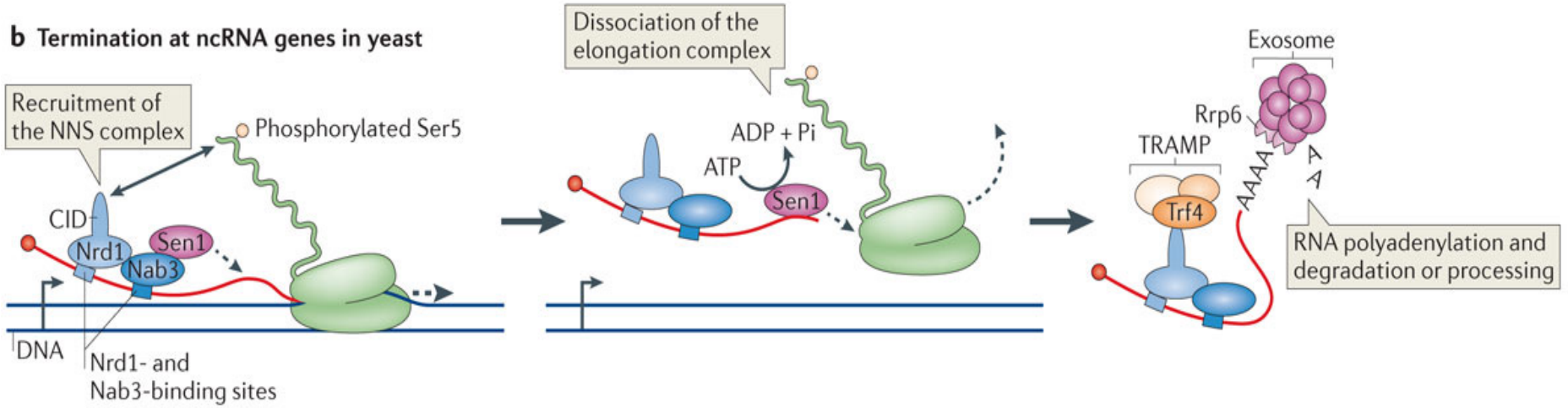
PT can be defined as any transcription that occurs at a genomic region that does not generate classical "functional" RNAs. Regions of the genome that are susceptible to PT contain a low number of nucleosomes – so-called nucleosome depleted regions (NDR).



Entsell and West, 2024 JMB

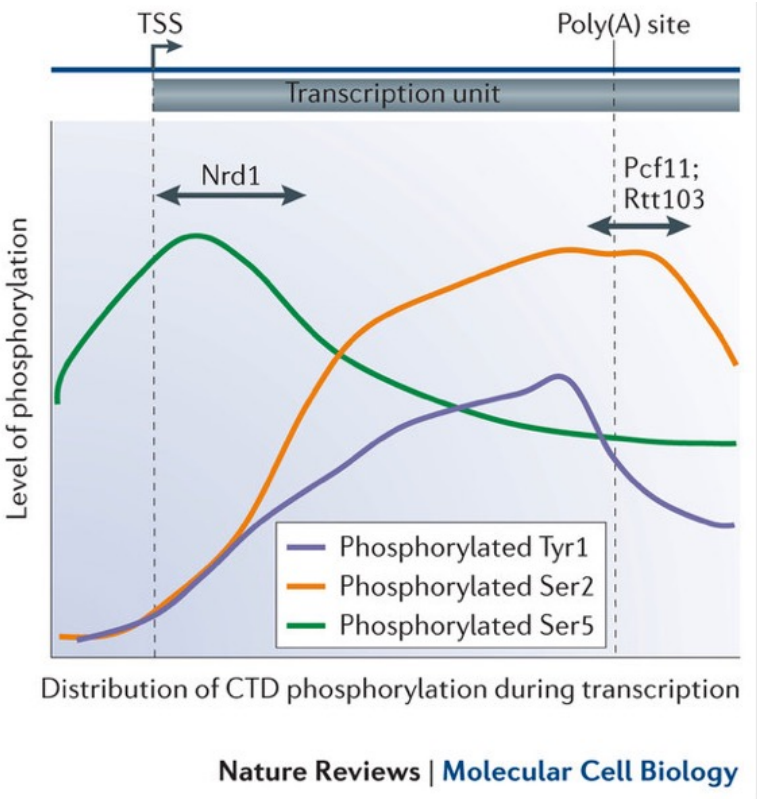
Termination of ncRNAs: the NNS-dependent pathway

In *S. cerevisiae*, the **Nrd1–Nab3–Sen1 (NNS) complex** is responsible for transcription termination at genes encoding **snRNAs** and **snoRNAs** and at **cryptic unstable transcripts (CUTs)**. The essential NNS complex contains two RNA-binding proteins, Nrd1 and Nab3, and the conserved superfamily I RNA and DNA helicase Sen1. Cleavage of the primary transcript has never been demonstrated for this termination pathway, and release of the polymerase occurs by a mechanism that strictly requires the action of the helicase Sen1 (similar to bacterial Rho-dependent termination).
An important and distinctive trait of the transcripts produced by NNS-dependent termination is that they are targeted by the nuclear exosome rapidly after their release.



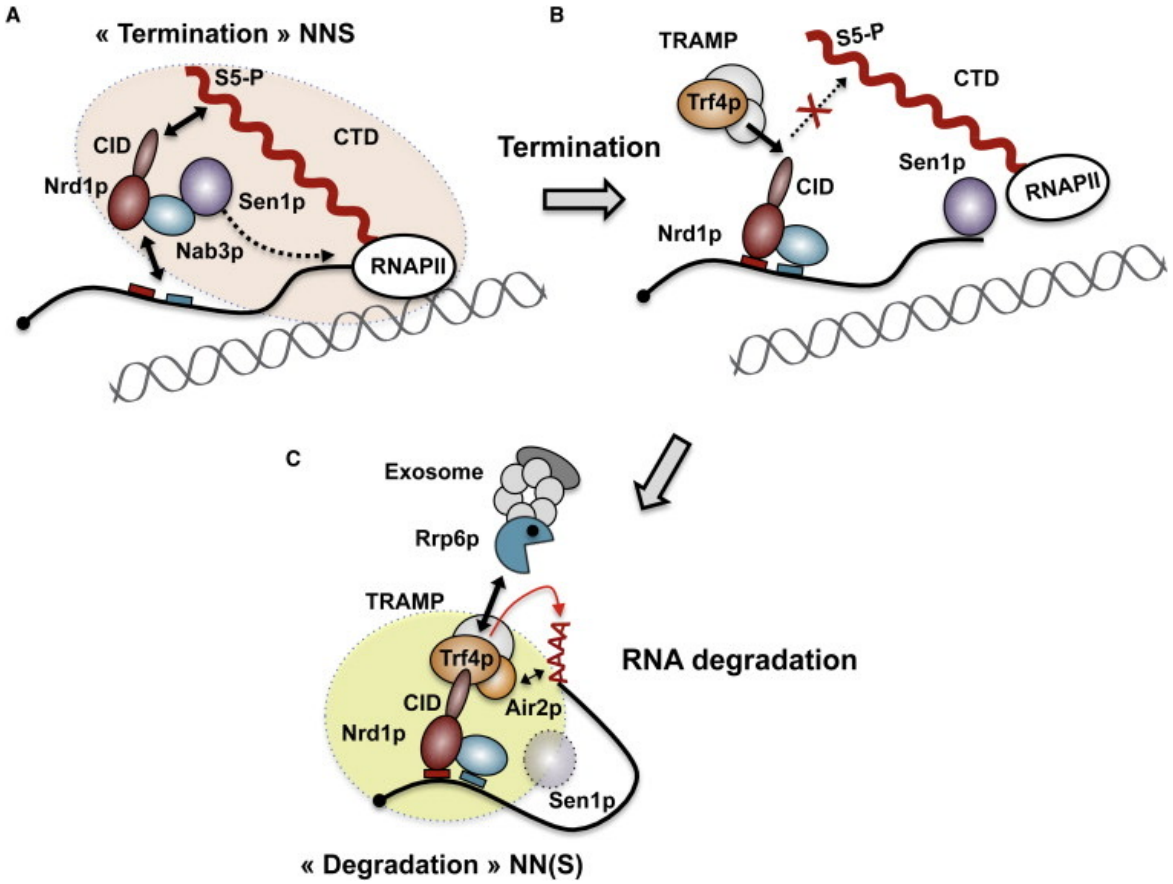
The CTD of RNA Pol II and termination

The most relevant modifications for transcription termination are phosphorylation at Tyr1, Ser2, Ser5 and Ser7, which alter the binding specificity of termination factors. For instance, in yeast the CPF complex component Pcf11 interact preferentially with the Ser2P form of the CTD only when Tyr1 is dephosphorylated. Conversely, Nrd1 recognizes the Ser5P form of the CTD, which predominates early in transcription, but only before the phosphorylation of Tyr1, which helps to restrict the recruitment of the NNS complex to the early stages of transcription.

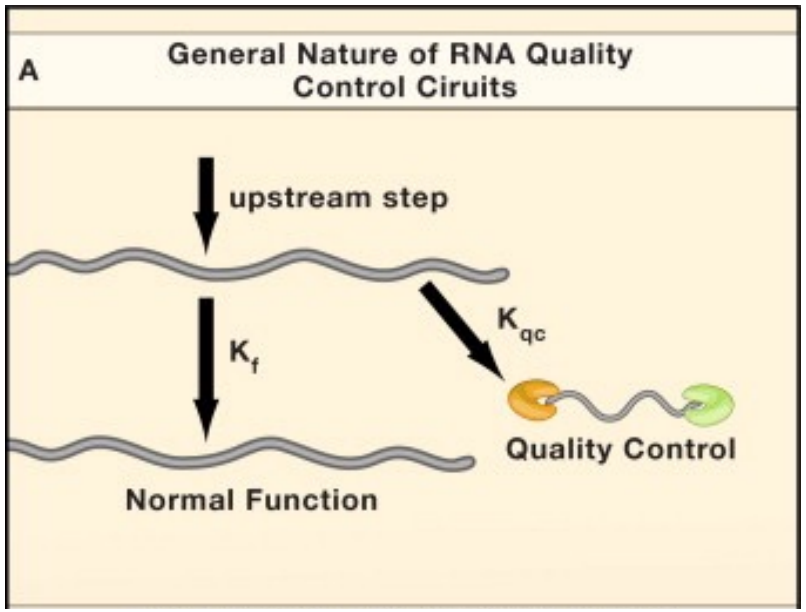


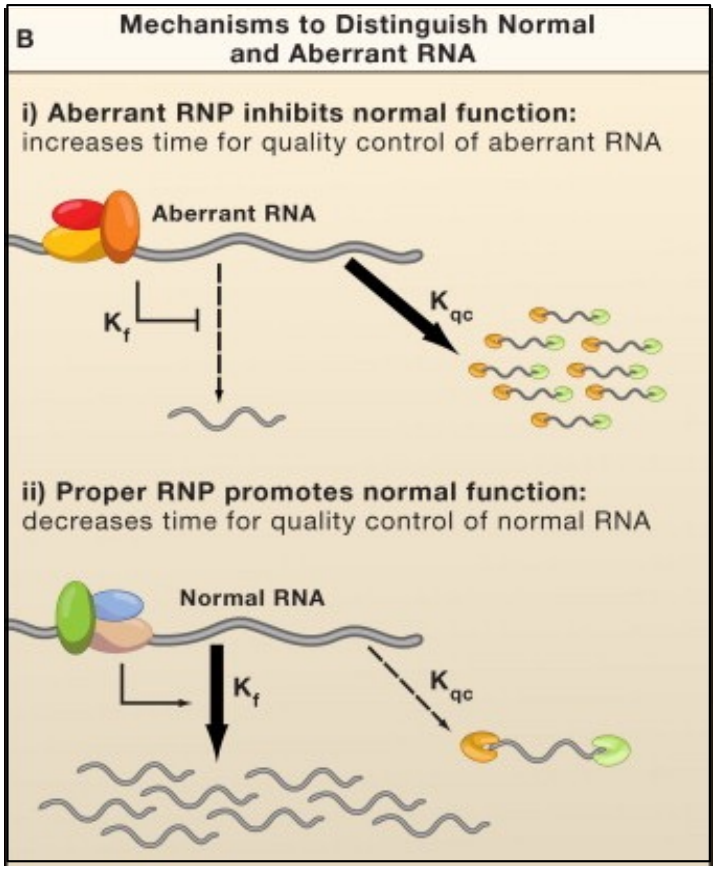
Model for the Coordination of Transcription Termination with RNA Degradation at NNS

After interacting with the Ser5P CTD for the termination step, Nrd1 recruits TRAMP through the direct recognition of a CTD mimic — known as the **Nrd1-interacting motif (NIM)** — in the TRAMP component Trf4. The sequential (and mutually exclusive) interaction of Nrd1 with the CTD and Trf4 contributes to the temporal coordination of termination with degradation.

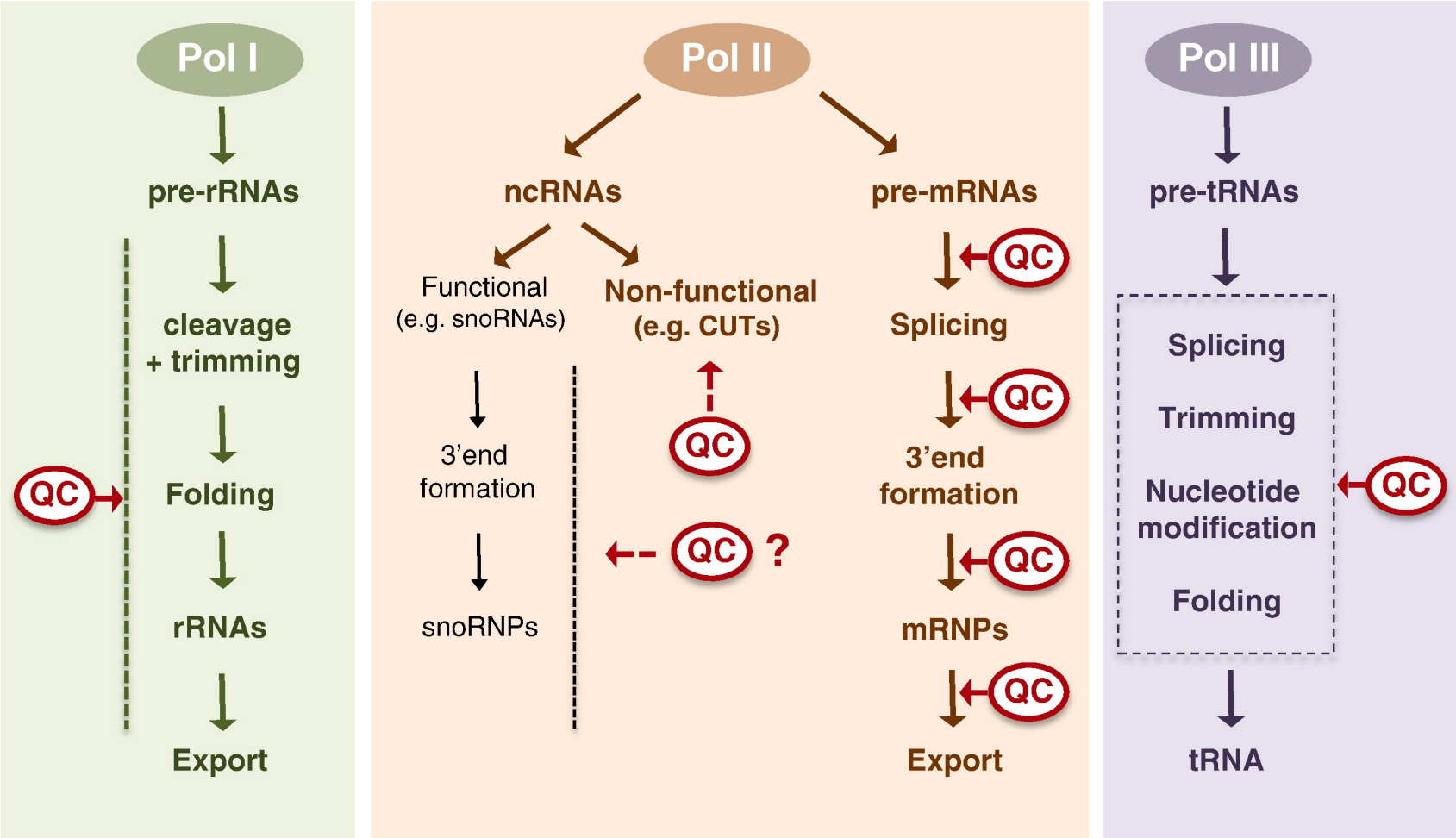


RNA quality control





A summary of quality control (QC) pathway for transcripts produced by the three polymerases.



Nuclear mRNA quality control

• Multiple processing and localization steps are required for proper mRNA maturation following the synthesis of the primary transcript.

• At a minimum, these include **capping**, **splicing**, **3´-end cleavage**, **polyadenylation**, and **nuclear export**.

• If each of these 5 processes are 90% efficient, **only ~50% of mRNAs will be properly matured.**

• What happens to the improperly processed transcripts?

– Do they get sent to the cytoplasm for protein synthesis anyway?

– Do they get degraded before they can make potentially defective proteins?

Nuclear mRNA surveillance occurs at checkpoints during maturation

•mRNA synthesis involves a series of processing steps to synthesize a mature transcript that are proofread in parallel **by nuclear surveillance mechanisms at each step.**

•The splicing and processing components as well as export factors are **co-transcriptionally recruited to the nascent transcript, and the correct mRNA forms the core of an export-competent mRNP** that is eventually exported and expressed in the cytoplasm.

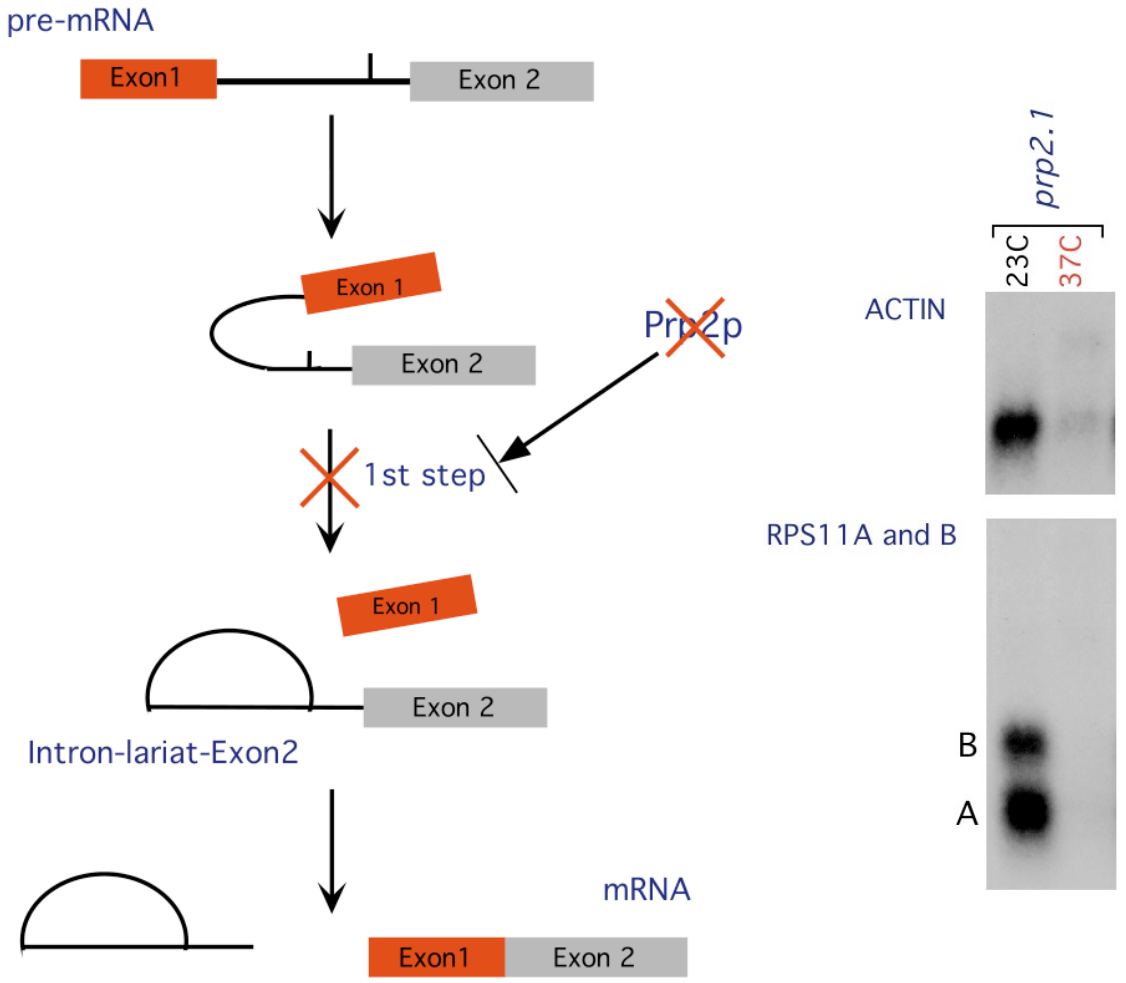
•Alternatively, aberrant synthesis or processing leads to **retention of the defective transcript.** These retained transcripts subsequently undergo exosome-mediated degradation.

Degradation of nuclear pre-mRNA

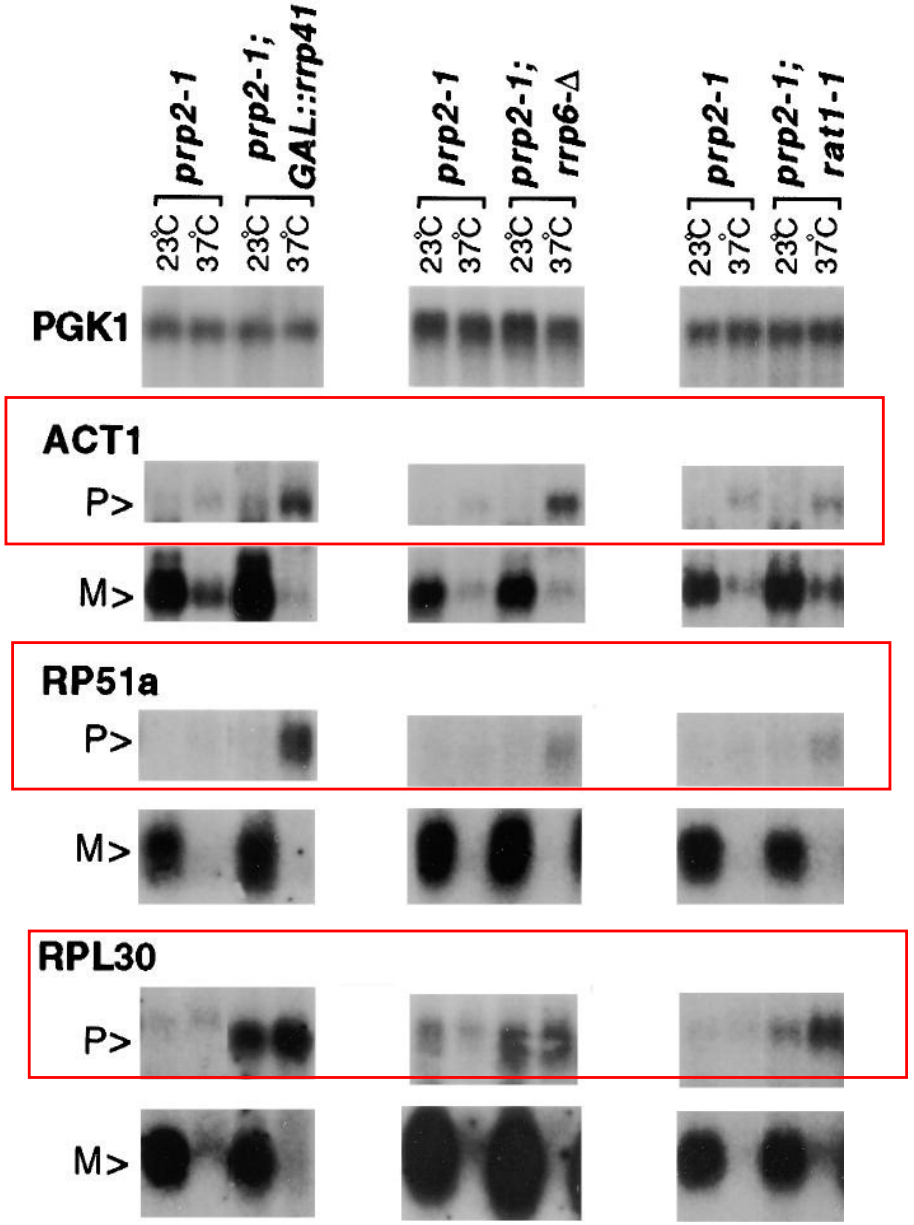
- Turnover data indicates that degradation of nuclear transcripts is very active in eukaryotic cells.
- Only around **2%** of the pre-mRNA synthesized is converted to cytoplasmic mRNA.

mRNA decay mutes the splicing defects of spliceosome component mutations

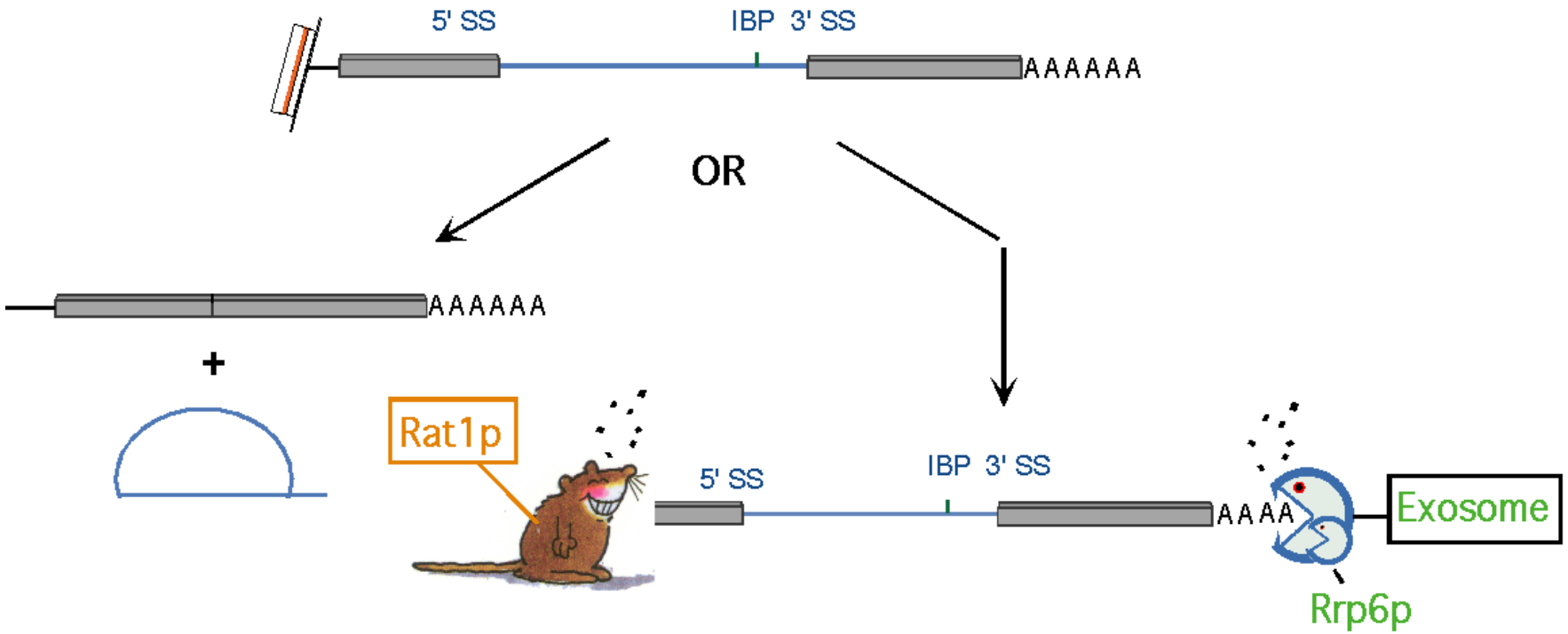
Mutations in yeast Prp2p (a DEXH-box ATPase essential for spliceosome activation) inhibit splicing, but the lack of unspliced pre-mRNA accumulation



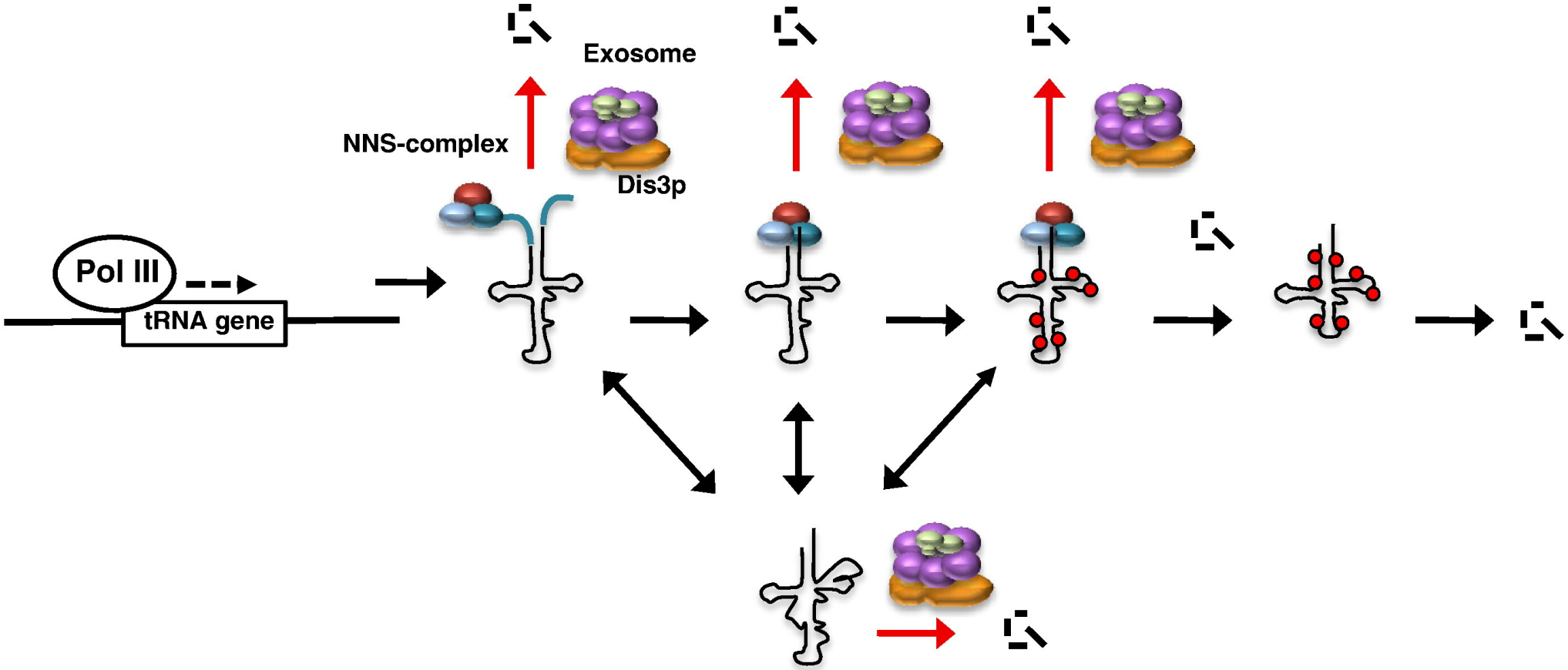
Inactivation of Rrp41p, Rrp6p, or Rat1p Stabilizes Pre- mRNAs in a Splicing-Deficient Strain



Pre-mRNA degradation and splicing compete in the nucleus

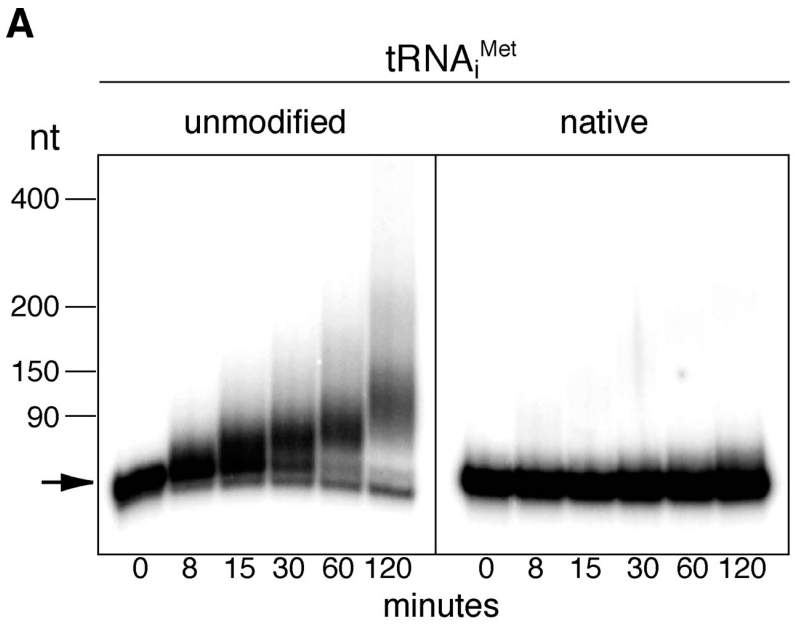


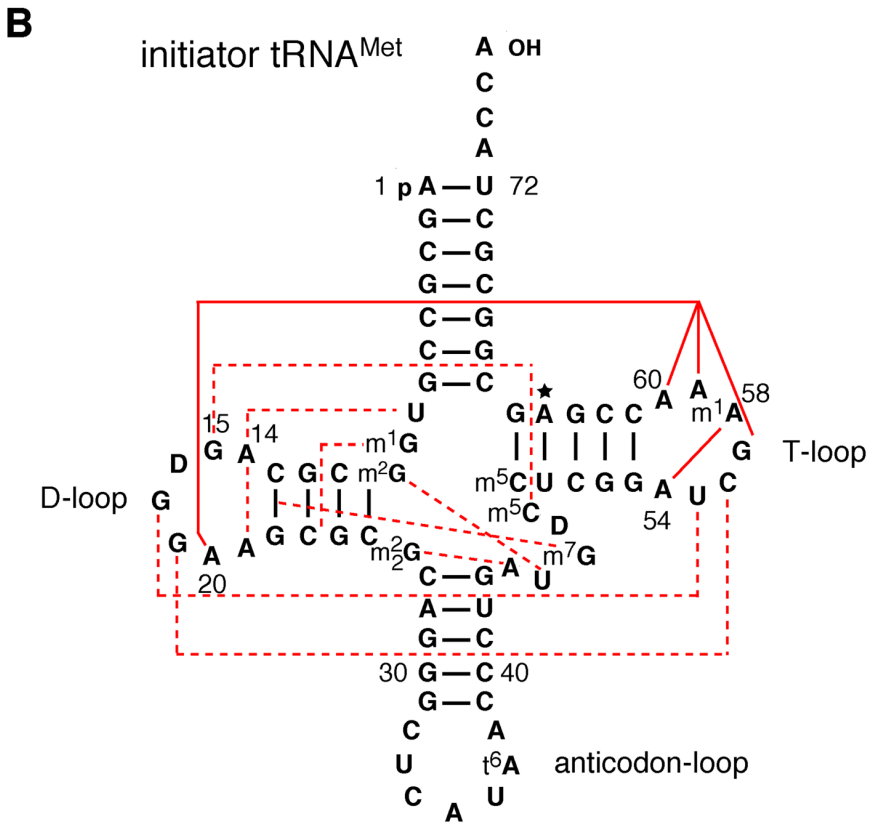
TRAMP targets not properly structured and Unmodified tRNAs



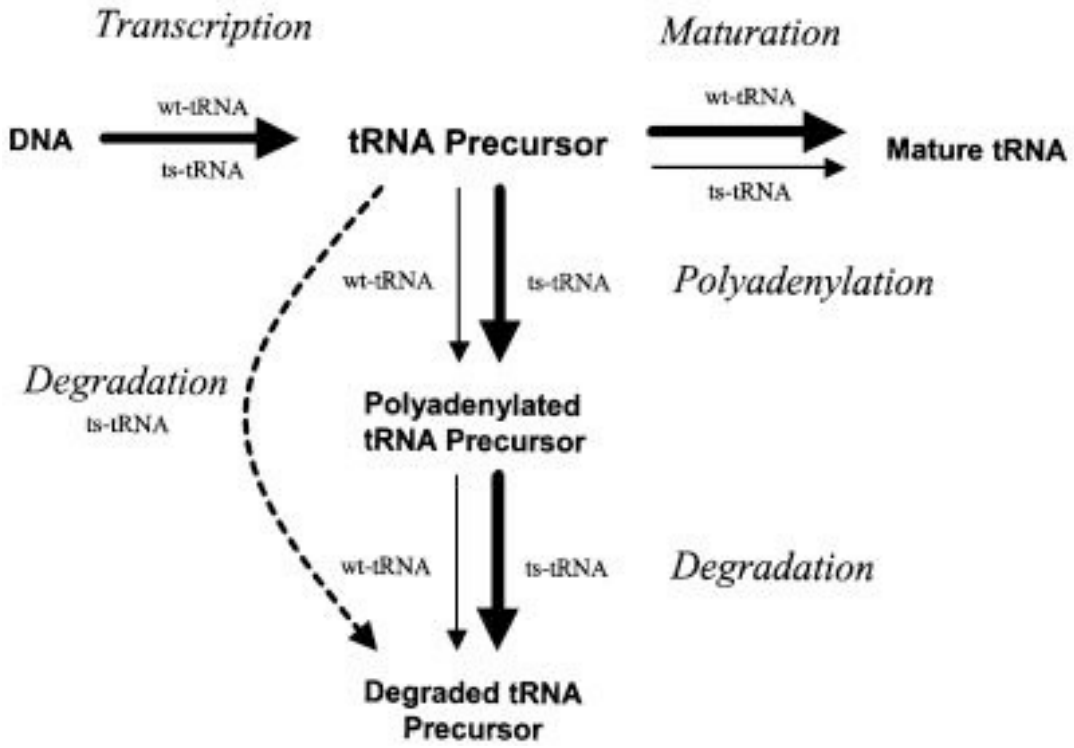
TRAMP Complex Preferentially Polyadenylates Unmodified tRNA

Polyadenylation assay with Trf4p-TAP and unmodified and native tRNAiMet as substrates. The 5′-end-labeled tRNAs were incubated with 50ng of Trf4 complex for times indicated and resolved by gel electrophoresis.





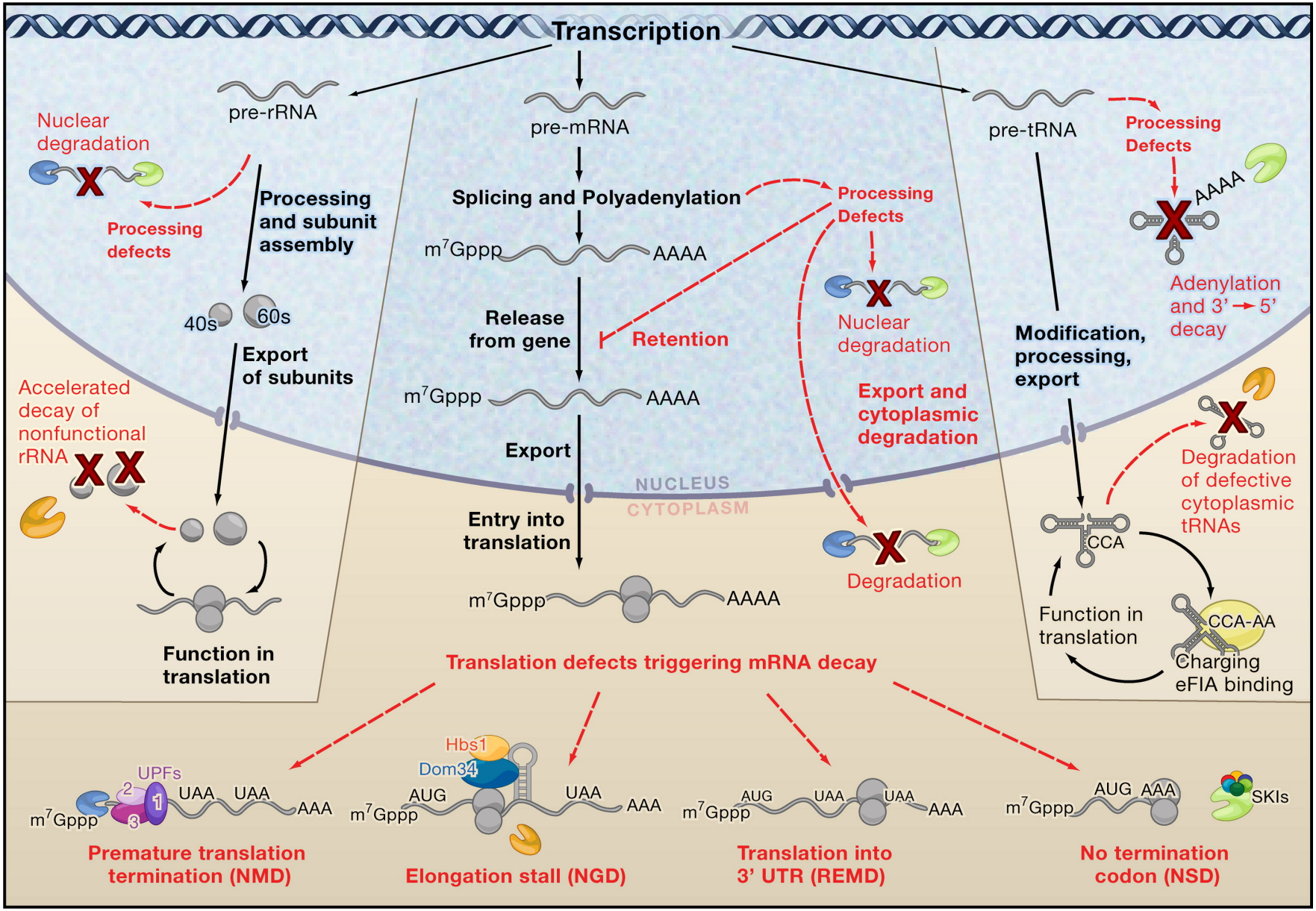
Polyadenylation is also involved in stable RNA degradation in bacteria



Model for quality control of stable RNA synthesis in bacteria. Genes encoding normal (wt-tRNA) and defective (ts-tRNA) RNAs are transcribed with equal efficiency to generate tRNA precursors. However, whereas wt-tRNA precursor is rapidly converted to its mature form by the processing RNases, maturation of the defective precursor is greatly slowed. As a consequence, the defective tRNA precursor is first subject to polyadenylation by poly(A) polymerase and is then degraded by PNPase.

Li et al., EMBO J 21: 1132-1138 (2002)

RNA quality control



RNA quality control in human

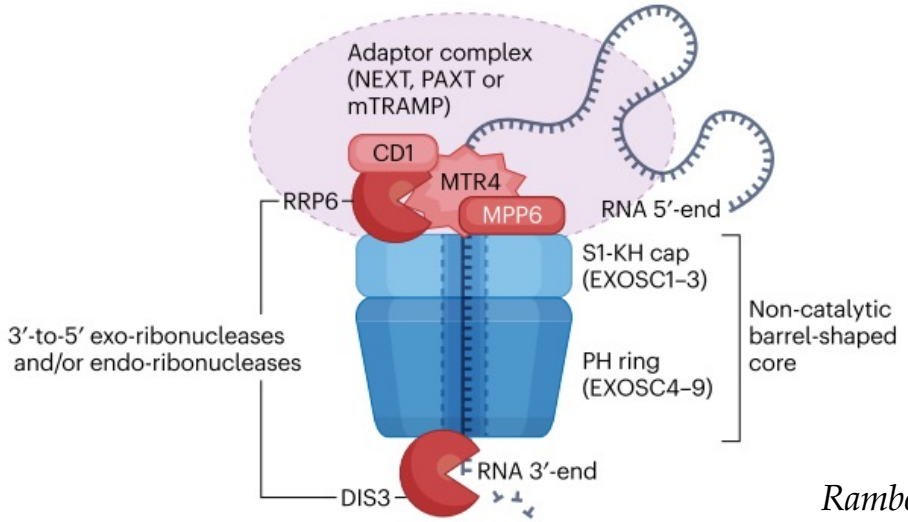
Human cells express at least three complexes that each contain MTR4 and a zinc-finger protein. These include

1. **TRAMP,** which is predominately nucleolar in humans,
2. **NEXT** (Nuclear EXosome Targeting) which is nucleoplasmic
3. **PAXT** (Poly(A) eXosome Targeting) complexes, which is nucleoplasmic

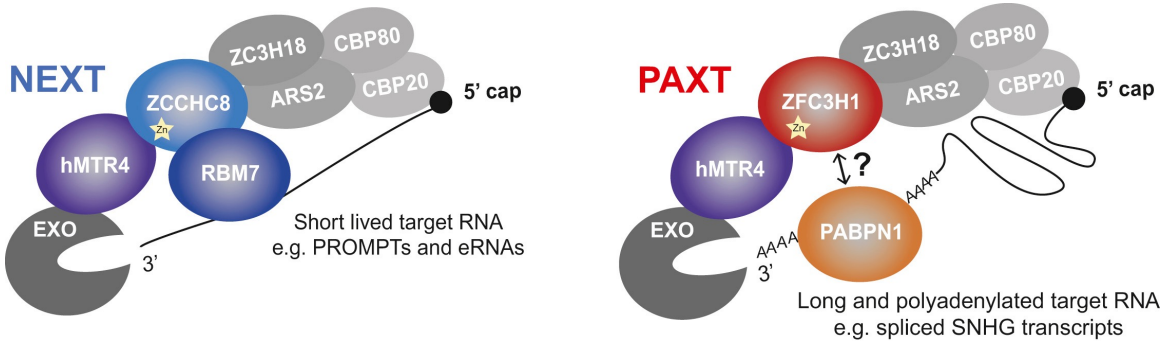
Budding yeast appears to lack NEXT and PAXT homologs, but the TRAMP complexes are present in both the nucleolus and nucleoplasm.

Human exosome adaptors

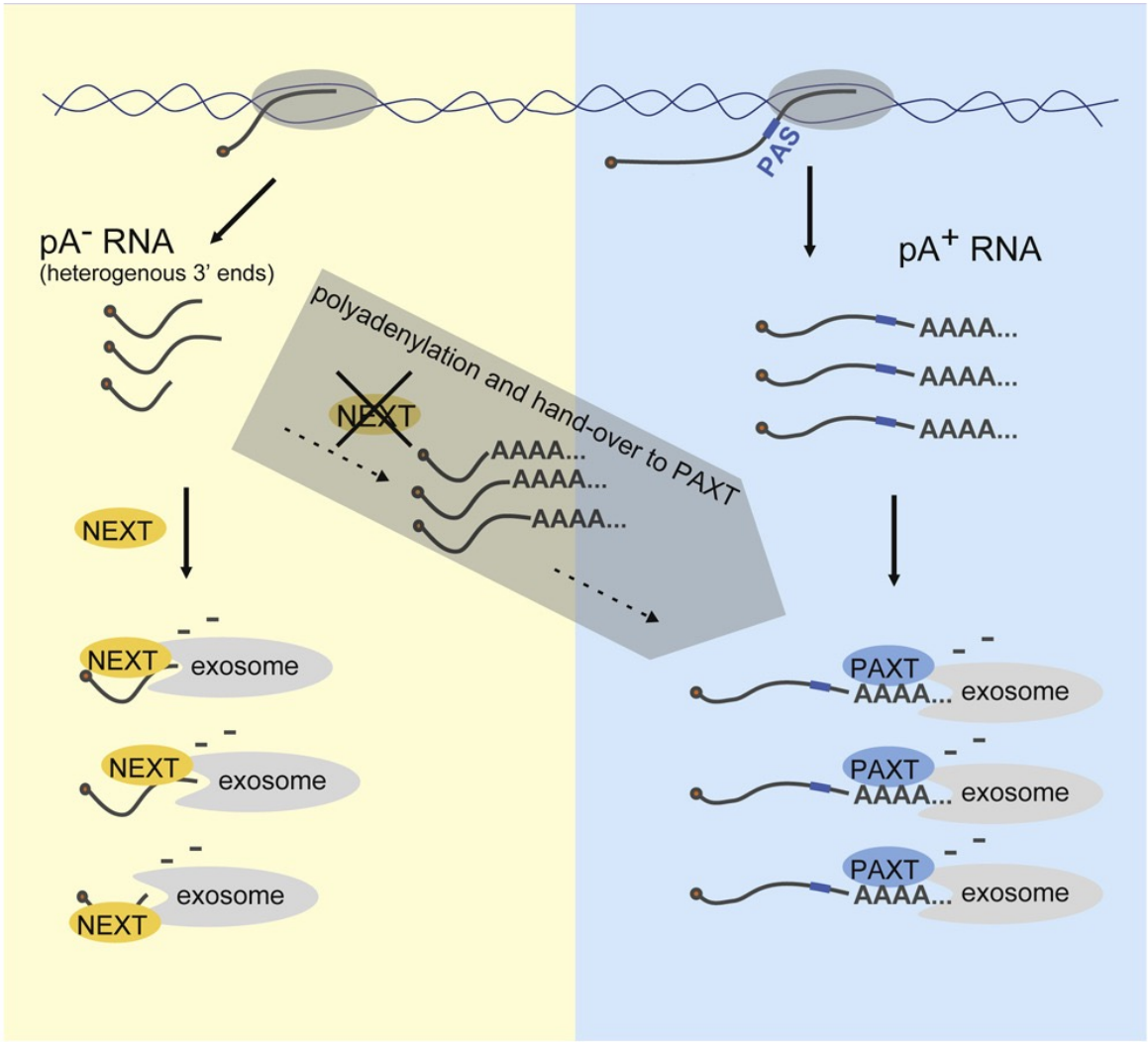
The RNA exosome degrades pervasive RNAs post-transcriptionally , that is, after they are released from RNAPII . Depending on their features, pervasive RNAs targeted for nuclear decay by the RNA exosome are preferentially recognized by one of at least two specialized protein adaptors, each of which is connected to the RNA exosome barrel via the RNA helicase **MTR4/DOB1**: the **nuclear exosome-targeting (NEXT)** adaptor complex, **the poly(A) exosome-targeting (PAXT)** adaptor complex or the **metazoan TRAMP (mTRAMP).**



Rambout & Maquat, 2024. Nature Rev. Gen.



Nuclear RNA exosome trageting

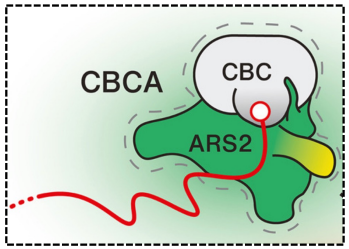


NEXT substrates arise from heterogenous and predominantly pA⁻ 3′ ends. In contrast, PAXT targets harbor well-defined pA⁺ 3′ ends defined by canonical pA site use.

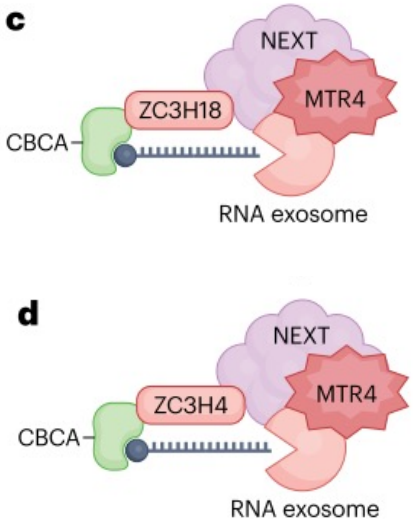
Human exosome adaptors

Both NEXT and PAXT recognize RNAP II-transcribed RNAs with a 5′-m7GTP cap via mutually exclusive interactions between the ARS2 constituent of the CBCA complex, that is, the largely nuclear CBP80–CBP20 cap-binding complex (CBC) bound by ARS2, and either the NEXT accessory protein **ZC3H18**, the NEXT accessory **protein ZC3H4** or the PAXT scaffold subunit **ZFC3H1**.

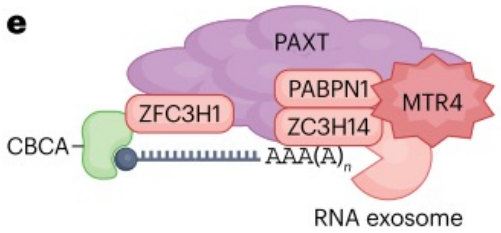
CBCA complex



NEXT complex

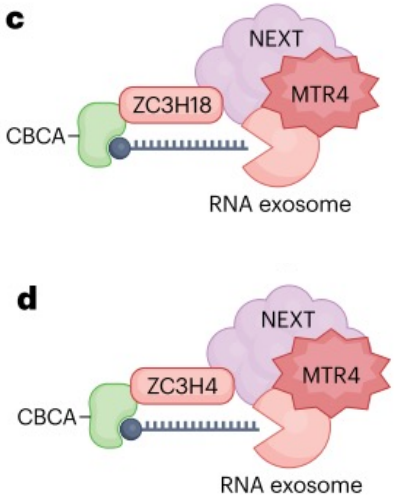


PAXT complex

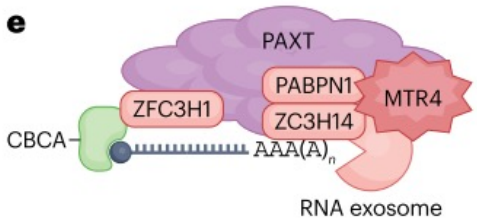


Human exosome adaptors

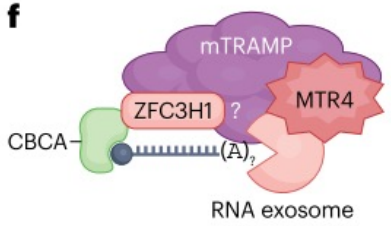
NEXT complex



PAXT complex

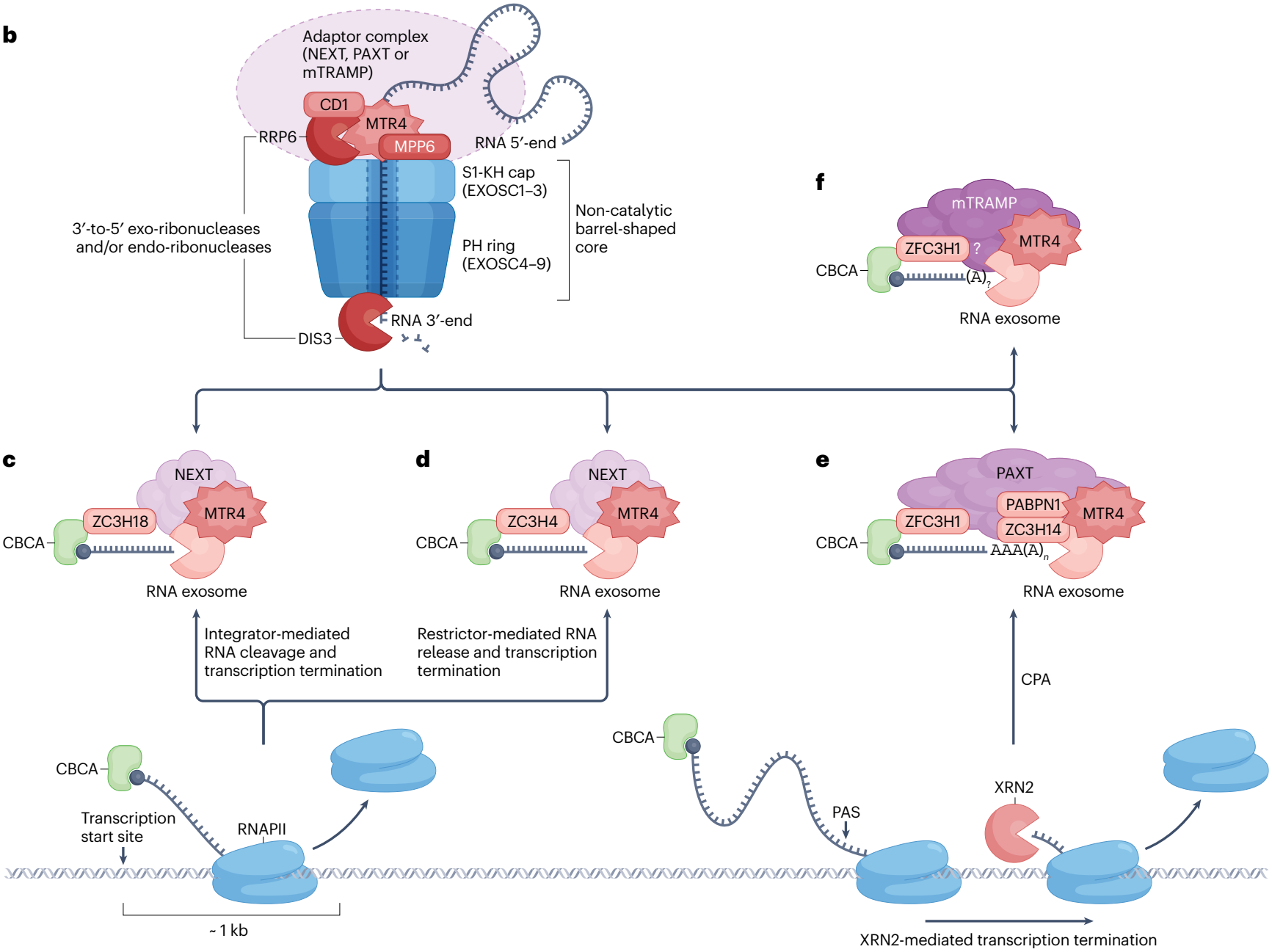


mTRAMP complex

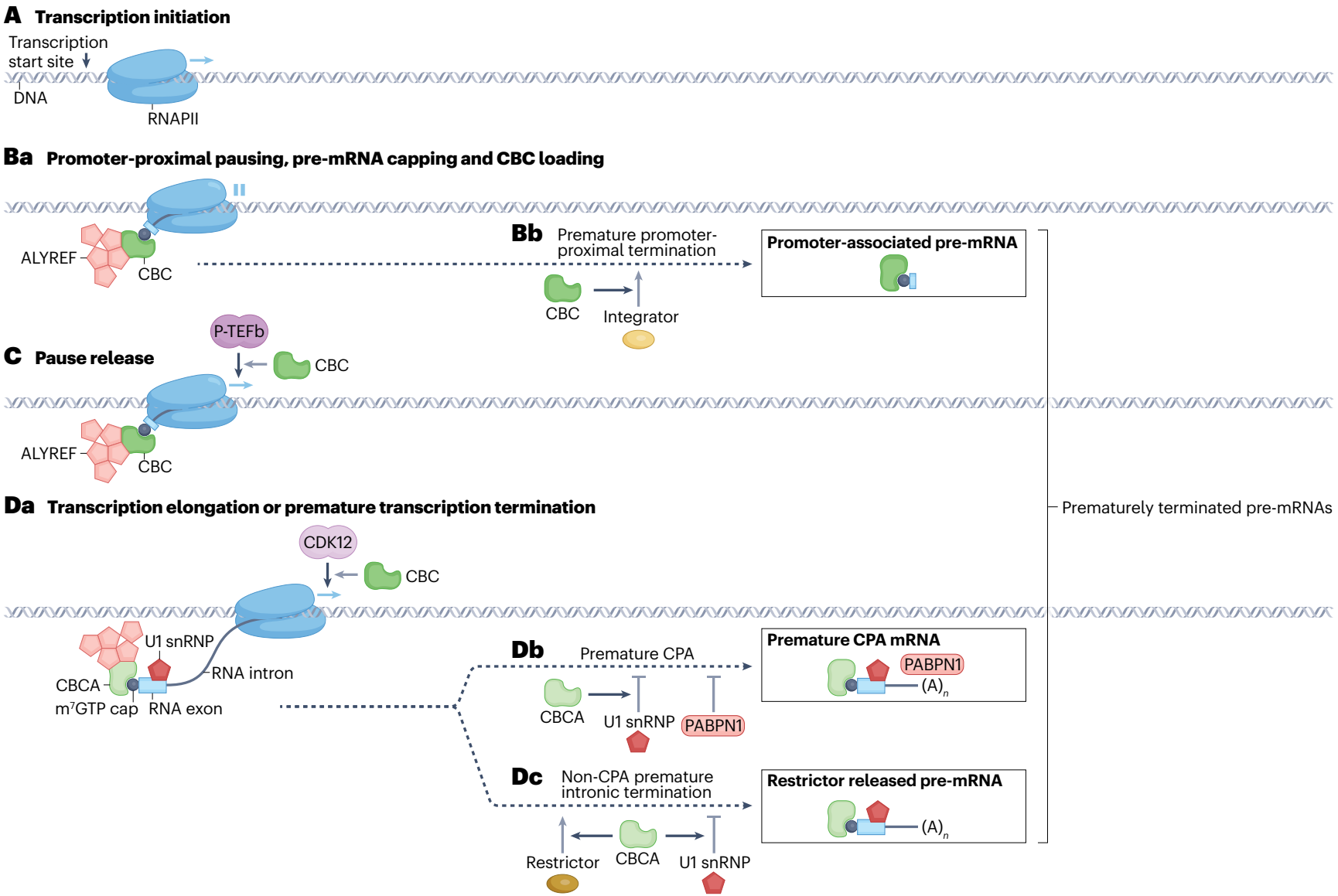


Short (<1,000-nt) non-polyadenylated RNAs are typically targeted by the NEXT adaptor complex via either ZC3H18 (c), when RNAs are cleaved and released from chromatin by Integrator, or ZC3H4 (d), when RNAs are released from chromatin by Restrictor. Short and long polyadenylated RNAs produced by CPA are typically targeted by PAXT connection via ZFC3H1 binding to the cap binding complex (CBCA) and PABPN1, and possibly ZC3H14, binding to the poly(A) tail. Metazoan Trf4/5–Air1/2–Mtr4 polyadenylation (TRAMP)-like complexes (mTRAMP) have been characterized and shown to degrade non-canonical histone mRNAs, which are typically not polyadenylated, as well as promoter-associated pre-mRNAs, which are oligoadenylated.

Human exosome adaptors

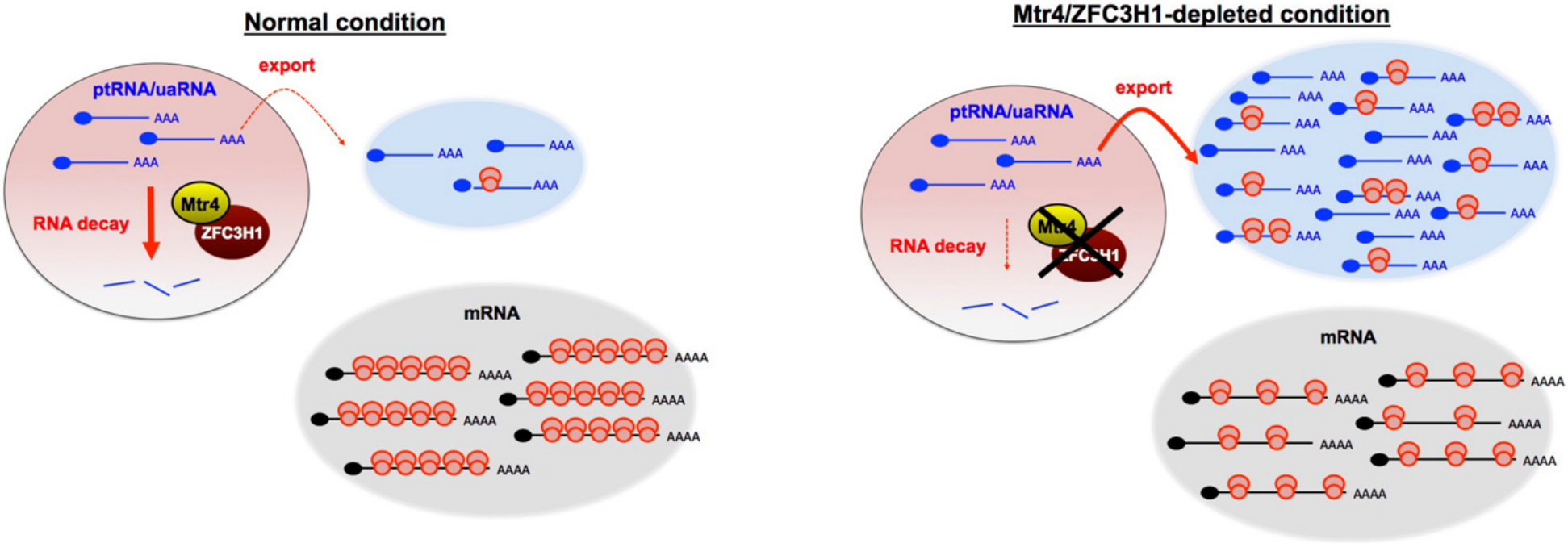


Nuclear metabolism of pre-mRNAs



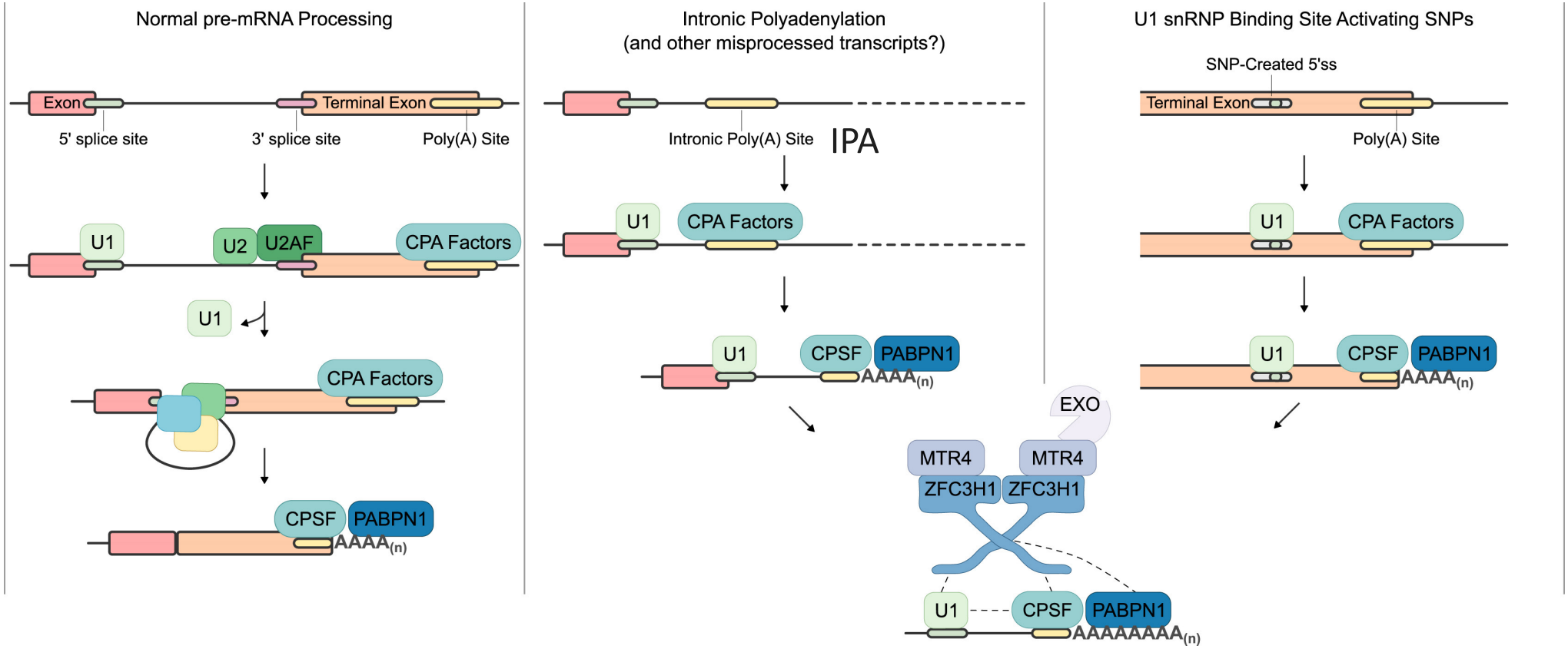
PAXT facilitates turnover of aberrant nuclear RNAs to prevent their cytoplasmic transport and global translational repression

Loss of the PAXT complex results in stabilization of prematurely terminated RNAs (ptRNAs) and upstream antisense RNAs (uaRNAs), which are normally rapidly degraded in the nucleus, and these RNAs are then transported to the cytoplasm. The exported RNAs become ribosome-associated and overwhelm the translational machinery, which leads to disruption of the quantitative balance between available ribosomes and translatable RNAs.



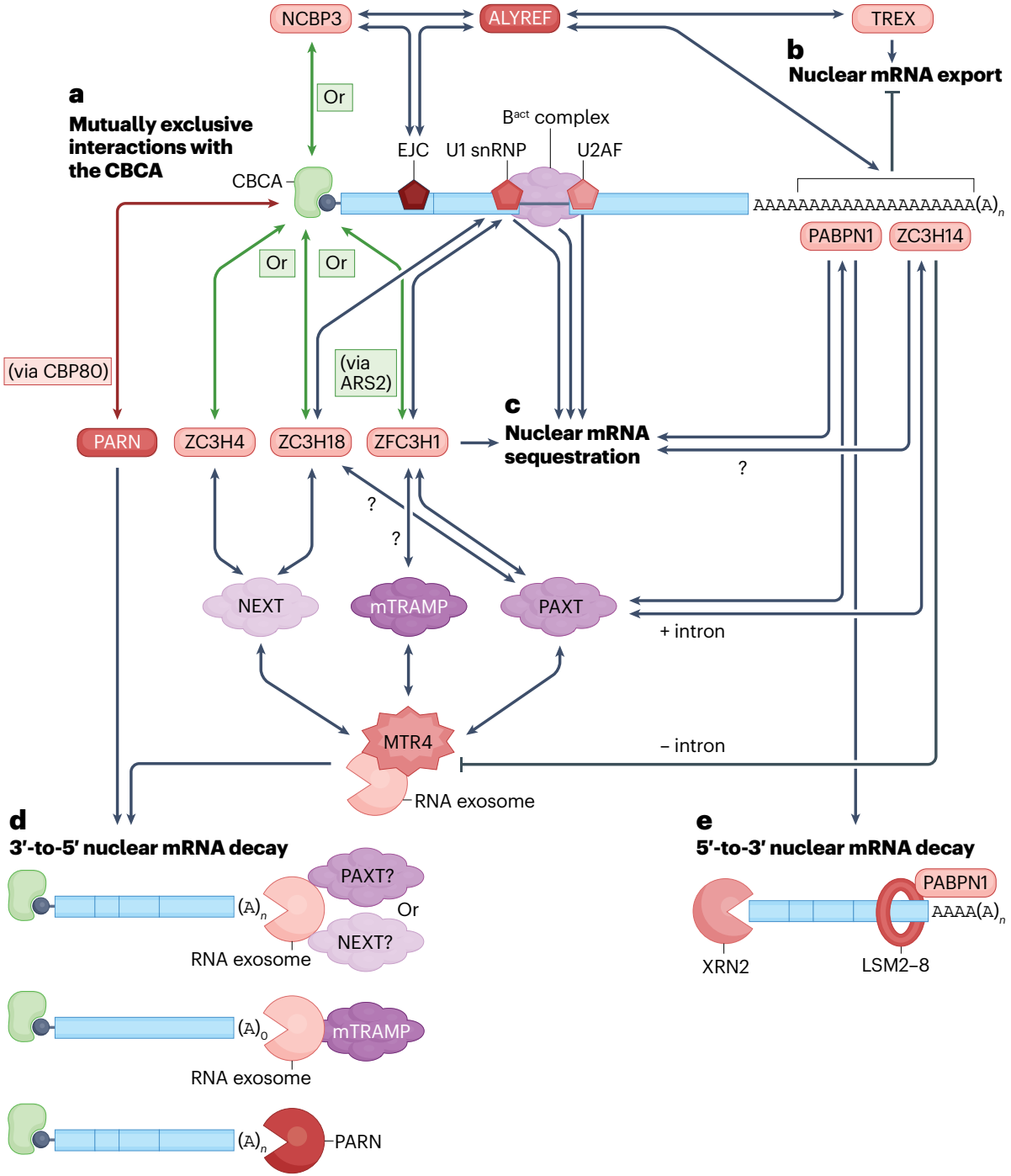
A nuclear RNA degradation code (NRDC) is recognized by PAXT for eukaryotic transcriptome surveillance

PAXT-mediated RNA degradation is induced by the combination of a 5′ splice site (ss) and a poly(A) sites but not by either sequence alone. These sequences are bound by U1 snRNP and cleavage/polyadenylation factors, which, in turn, cooperatively recruit PAXT. This mechanism may contribute to human diseases because SNPs in the 3′ UTR of the human genes that create 5’ ss induce PAXT-mediated RNA degradation.

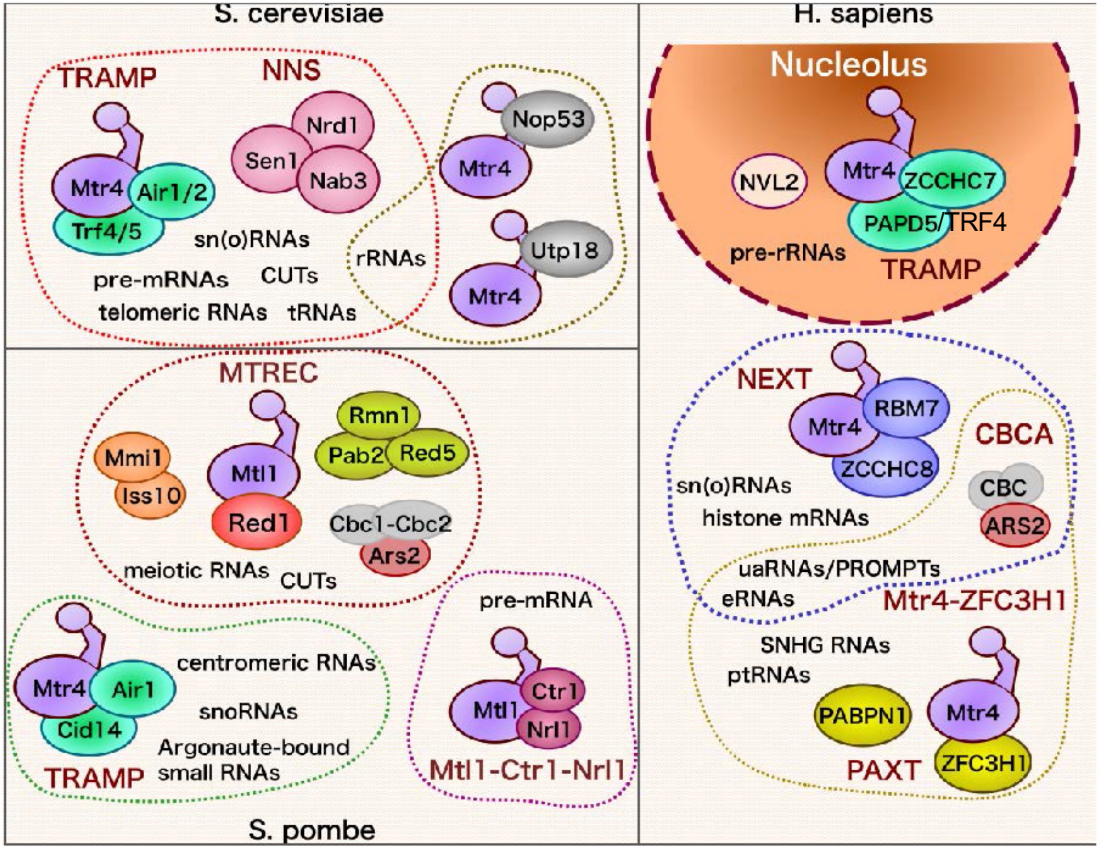


There is an inverse correlation between the 5′ ss strength and RNA stability and that stable IPA transcripts tend to have weaker 5′ ss

Fate of nuclear mRNAs.



Dob1/Mtr4-containing exosome adaptor complexes in yeasts and humans



The RNA helicase Dob1/Mtr4 participates in multiple distinct exosome adaptor complexes to complete degradation and/or processing of specific RNA substrates.

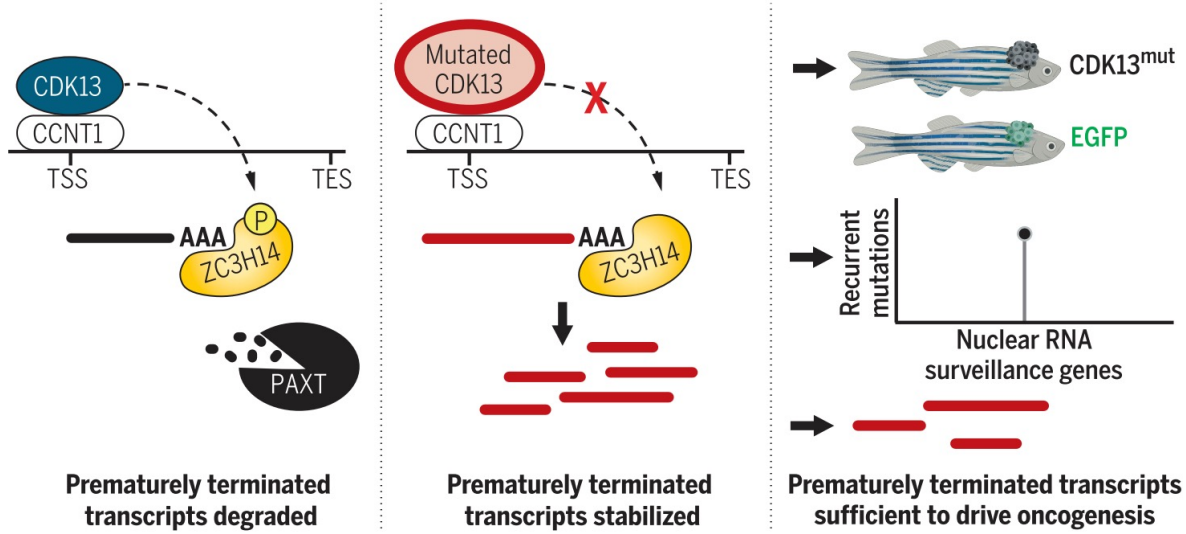
Science
AAAS

Oncogenic *CDK13* mutations impede nuclear RNA surveillance

2023

Megan L. Insco*, Brian J. Abraham, Sara J. Dubbury, Ines H. Kaltheuner, Sofia Dust, Constance Wu, Kevin Y. Chen, David Liu, Stanislav Bellaousov, Anna M. Cox, Benjamin J. E. Martin, Tongwu Zhang, Calvin G. Ludwig, Tania Fabo, Rodsy Modhurima, Dakarai E. Esgdaille, Telmo Henriques, Kevin M. Brown, Stephen J. Chanock, Matthias Geyer, Karen Adelman, Phillip A. Sharp, Richard A. Young, Paul L. Boutz, Leonard I. Zon*

RNA surveillance pathways detect and degrade defective transcripts to ensure RNA fidelity. We found that disrupted nuclear RNA surveillance is oncogenic. Cyclin-dependent kinase 13 (CDK13) is mutated in melanoma, and patient-mutated CDK13 accelerates zebrafish melanoma. CDK13 mutation causes aberrant RNA stabilization. CDK13 is required for ZC3H14 phosphorylation, which is necessary and sufficient to promote nuclear RNA degradation. Mutant CDK13 fails to activate nuclear RNA surveillance, causing aberrant protein-coding transcripts to be stabilized and translated. Forced aberrant RNA expression accelerates melanoma in zebrafish. We found recurrent mutations in genes encoding nuclear RNA surveillance components in many malignancies, establishing nuclear RNA surveillance as a tumor-suppressive pathway. Activating nuclear RNA surveillance is crucial to avoid accumulation of aberrant RNAs and their ensuing consequences in development and disease.



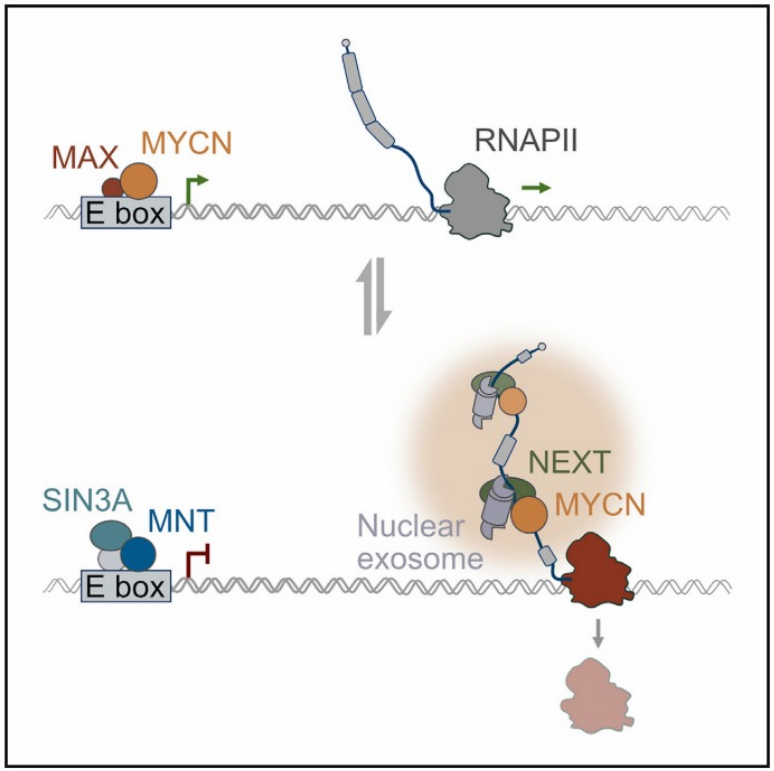
2024

Molecular Cell

Article

The MYCN oncoprotein is an RNA-binding accessory factor of the nuclear exosome targeting complex

Graphical abstract



Authors

Dimitrios Papadopoulos, Stefanie Anh Ha, Daniel Fleischhauer, ..., Gabriele Büchel, Seychelle M. Vos, Martin Eilers

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In brief

Papadopoulos et al. discover that MYCN, a chromatin-associated oncoprotein, engages RNA *in vitro* and in cells. DNA- and RNA-bound MYCN are mutually exclusive states with distinct interactomes and functions. The latter state facilitates RNA processing and prevents replication stress, thereby exposing a targetable vulnerability of aggressive MYCN-driven tumors.

MYCN engages the nuclear RNA exosome to prevent conflicts between the DNA replication fork and RNAPII in MYCN-driven neuroblastoma cells.

The exosome assumes an additional function specifically in MYCN-driven tumor cells by maintaining productive transcription for a large group of genes required for progression through the S and G2 phases of the cell cycle and preventing double-strand break accumulation and stalling of replication forks in MYCN-driven tumor cells.

Human Molecular Genetics, 2016, Vol. 25, No. 14 2985–2996

ORIGINAL ARTICLE

Altered RNA metabolism due to a homozygous *RBM7* mutation in a patient with spinal motor neuropathy

Michele Giunta[1,†], Shimon Edvardson[2,†], Yaobo Xu[1], Markus Schuelke[3], Aurora Gomez-Duran[1], Veronika Boczonadi[1], Orly Elpeleg[2], Juliane S. Müller[1,†] and Rita Horvath[1,†,*]

RBM7 is a component of the NEXT complex. Moreover, mutations in the exosome subunits EXOSC8 and EXOSC3 cause pontocerebellar hypoplasia, spinal muscular atrophy (SMA) and central nervous system demyelination.

These studies indicate that impaired RNA metabolism may underlie the clinical phenotype by fine tuning gene expression which is essential for correct neuronal differentiation.

Genes Dev. 2016 Jul 15;30(14):1658-70.

A conserved virus-induced cytoplasmic TRAMP-like complex recruits the exosome to target viral RNA for degradation

Jerome M. Molleston,[1] Leah R. Sabin,[1] Ryan H. Moy,[1] Sanjay V. Menghani,[1] Keiko Rausch,[1] Beth Gordesky-Gold,[1] Kaycie C. Hopkins,[1] Rui Zhou,[2] Torben Heick Jensen,[3] Jeremy E. Wilusz,[4] and Sara Cherry[1]

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While hMTR4 and hZCCHC7 are normally nuclear, infection by cytoplasmic RNA viruses induces their export, forming a cytoplasmic complex that specifically recognizes and induces degradation of viral mRNAs.