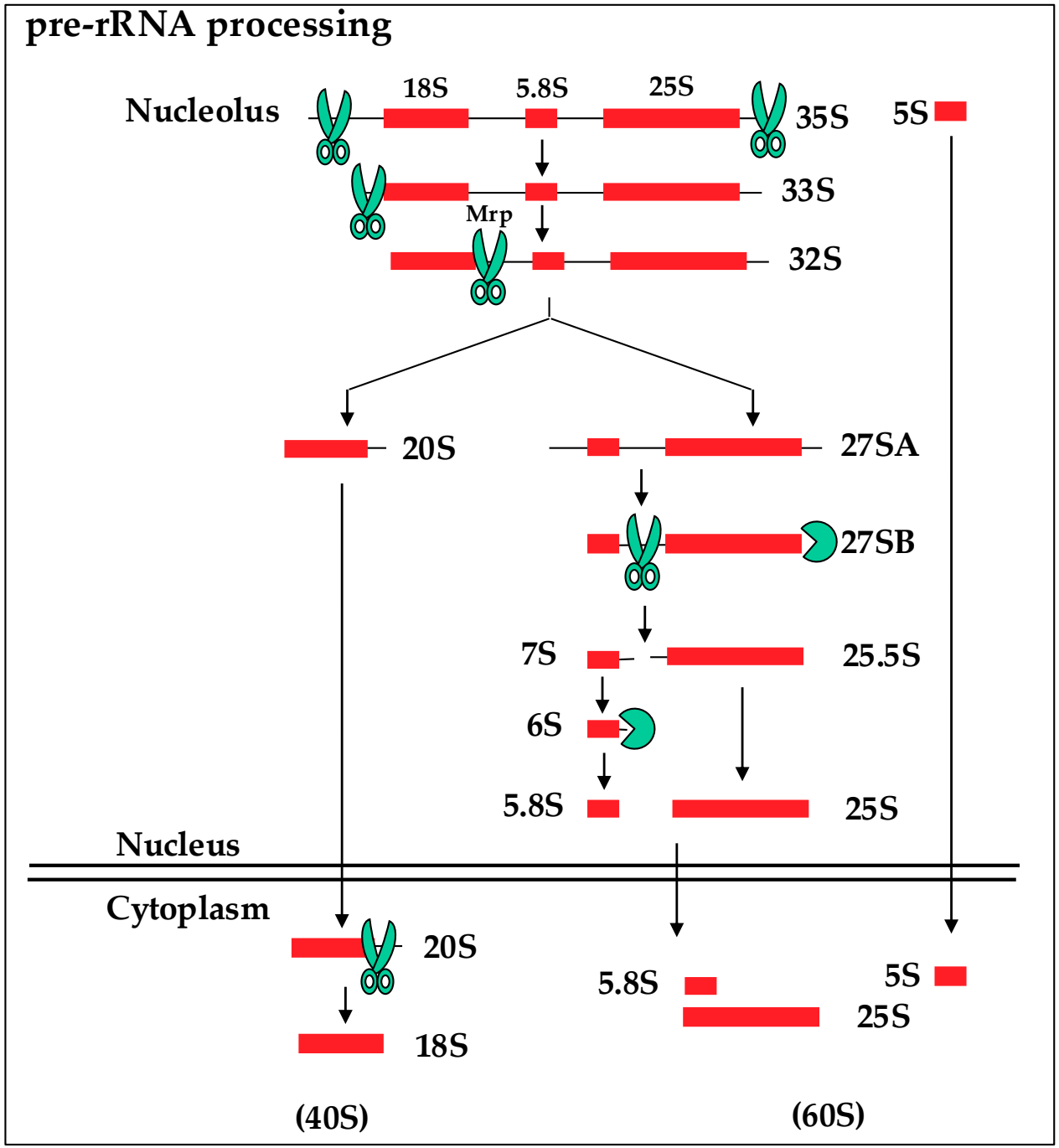
RNA Processing

Eukaryotic cells contain many different RNA species:

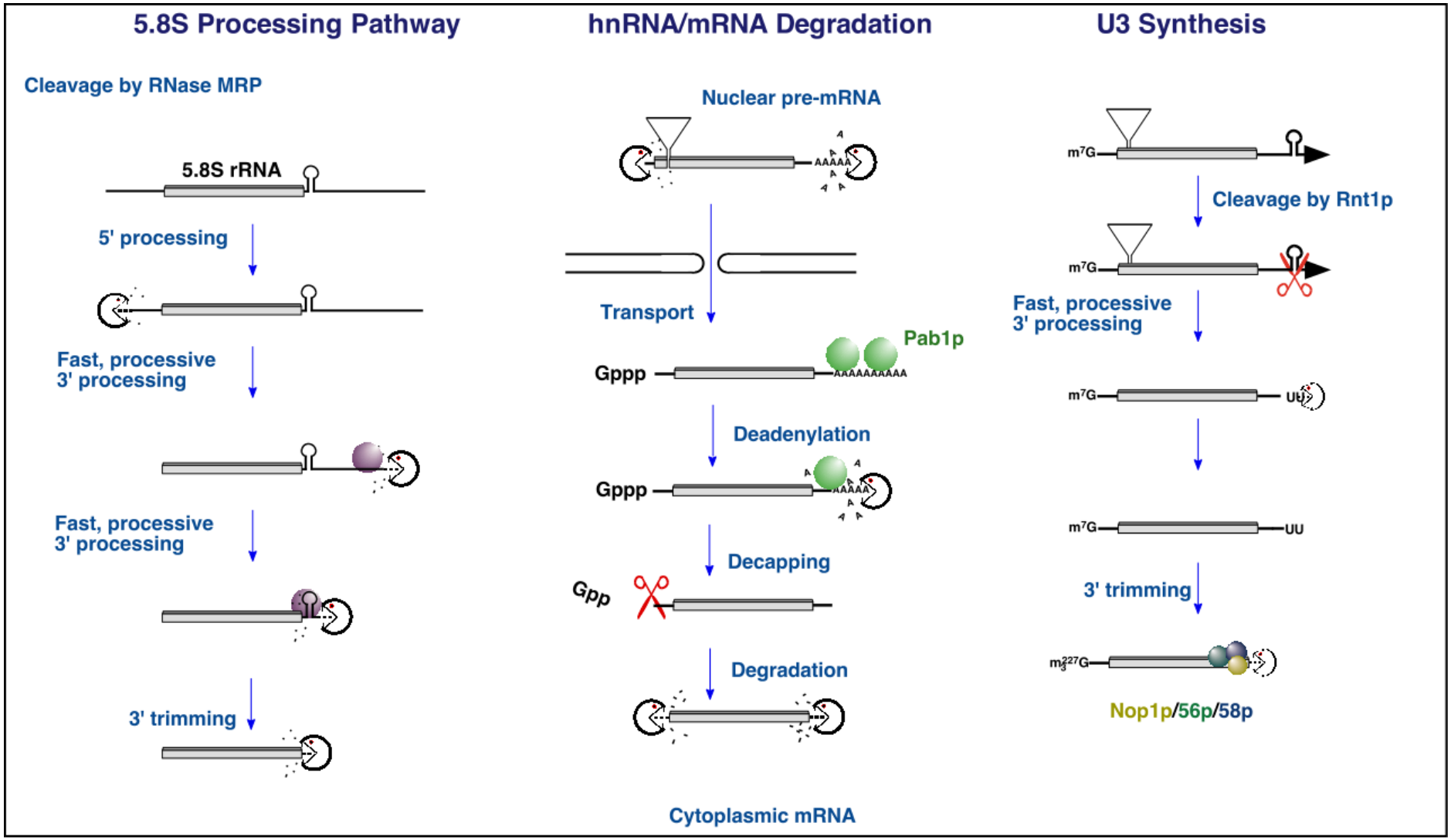
- **mRNA**
- **rRNA**
- **tRNA**
- **Small nuclear RNAs (snRNAs)**
- **Small nucleolar RNAs (snoRNAs)**
- **Signal recognition particle (SRP)**
- **RNase P and MRP**
- **Telomerase RNA**
- **microRNA and piRNA**
- **lncRNA**

- Almost all stable RNAs are generated by post-transcriptional processing

RNA Processing/degradation



RNA Processing/degradation



RNA Processing

•It has long been assumed that specific RNA processing machinery would be adapted for the processing of specific substrates - that there would be, for example, "pre-rRNA processing enzymes" or " mRNA degradation machinery" etc.

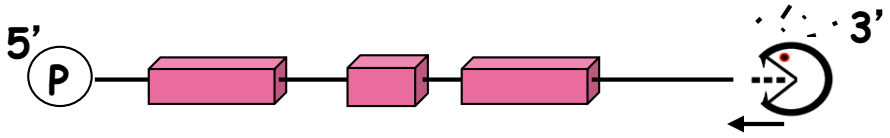
•In fact, it does not appear that eukaryotic RNA processing is organized in this way.

•The processing and turnover of RNA species involves a set of common enzymes and cofactors.

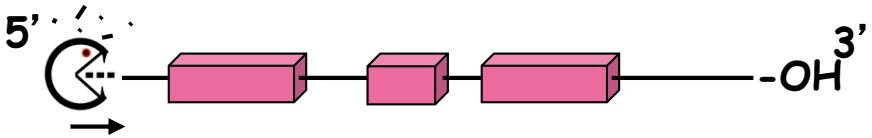
•These are recruited in different combinations to many different substrates.

RNA maturation and/or degradation Enzymes

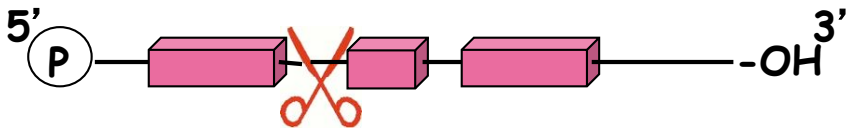
- 3’ ⟶ 5’ exoribonucleases:



- 5’ ⟶ 3’ exoribonucleases:



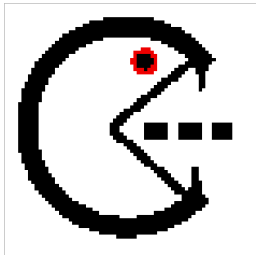
- Endonucleases:



Yeast 5’ ->3’ exonucleases

Rat1p (Xrn2) Predominately nuclear

pre-rRNA processing
pre-rRNA spacer degradation
snoRNA processing
pre-mRNA degradation
Transcription termination

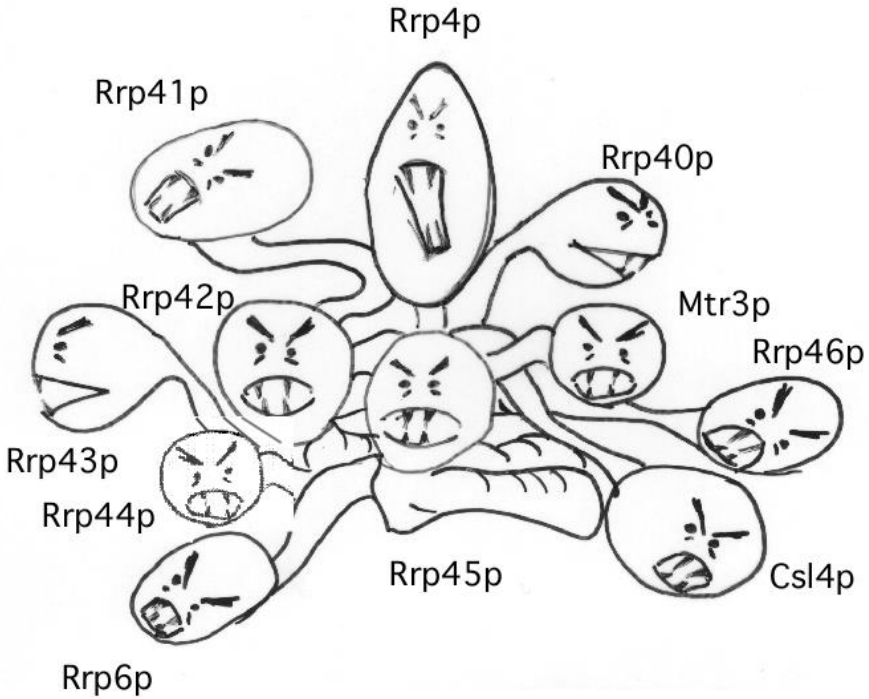


Xrn1p Predominately cytoplasmic

mRNA degradation

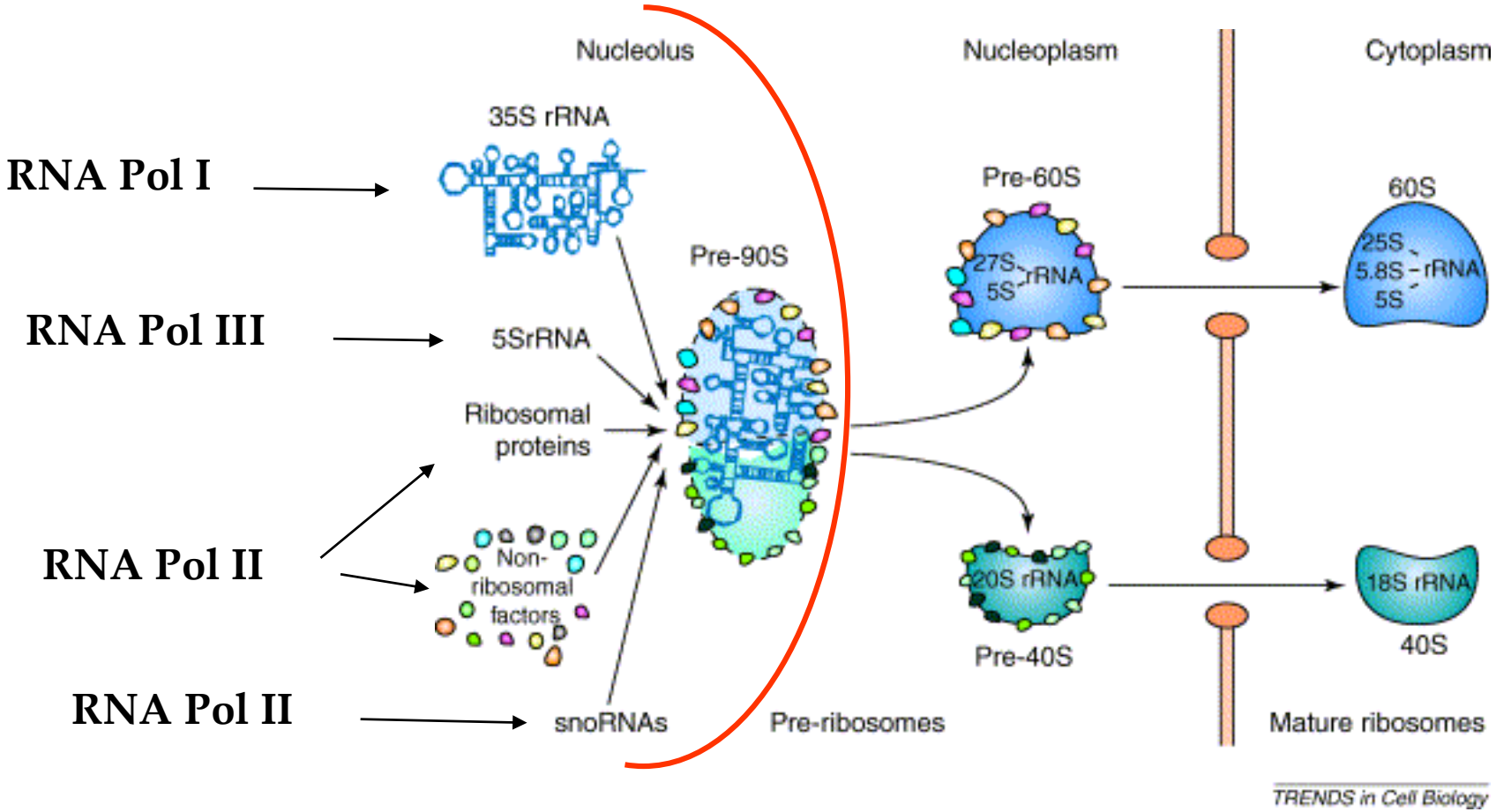
Yeast 3' ->5' exonucleases

The exosome complex



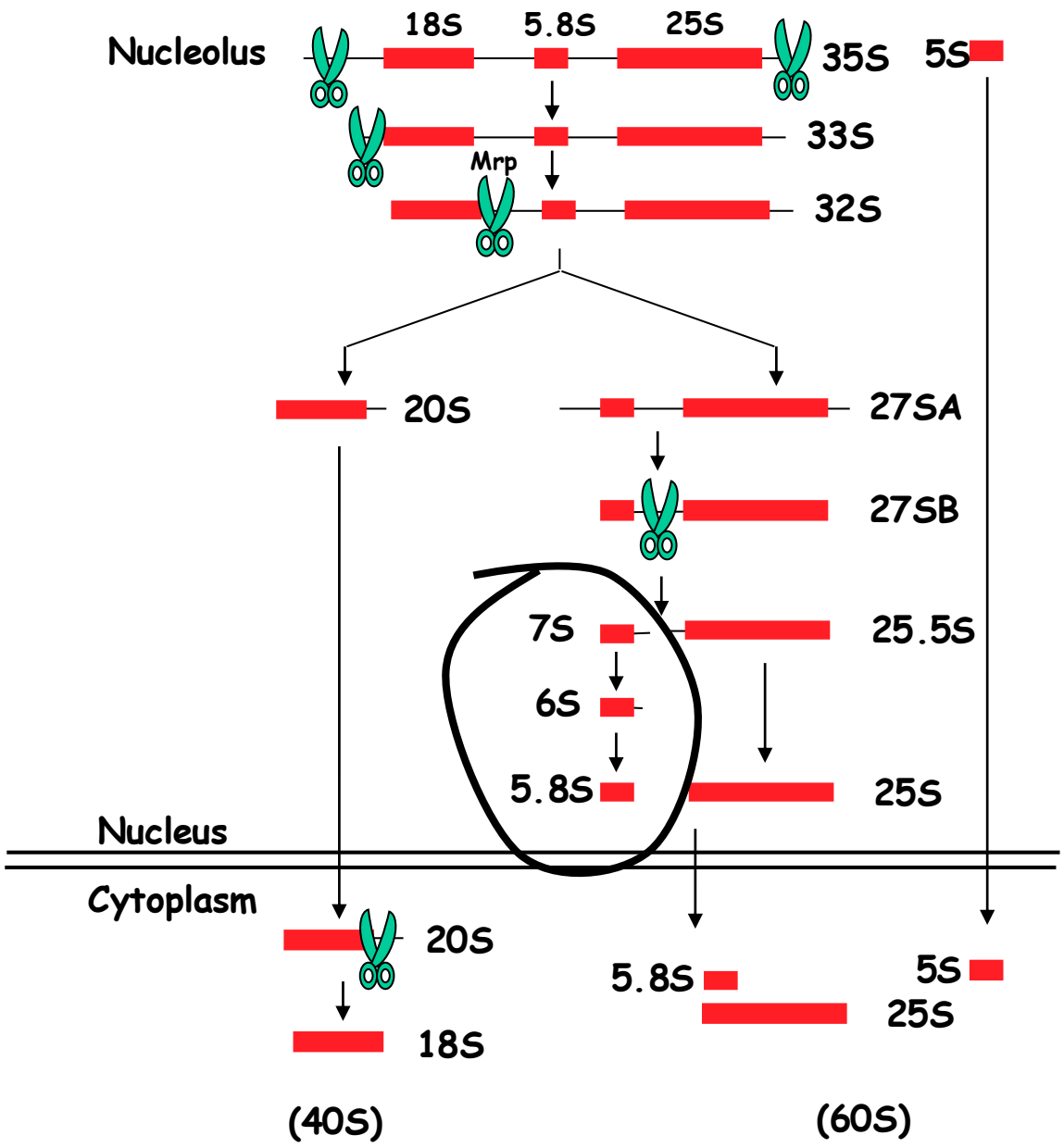
- pre-rRNA processing
- pre-rRNA spacer degradation
- snRNA processing
- snoRNA processing
- pre-mRNA degradation
- mRNA deadenylation
- mRNA degradation
- pervasive transcription

Ribosome synthesis



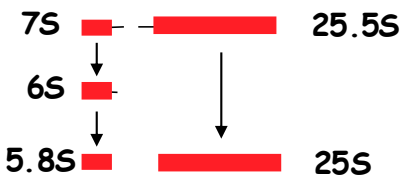
- Eukaryotic cells produce from 4000 to 10000 ribosome/minut
- rRNA transcription is about 60-70% of total RNA transcription
- rRNA molecules are about 80-90% of total RNA

Pre-rRNA processing

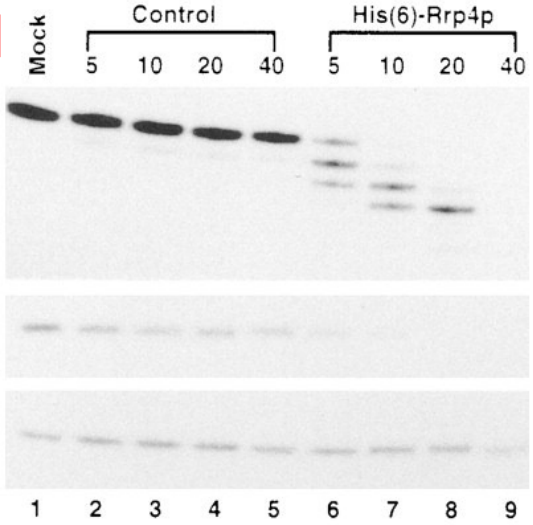


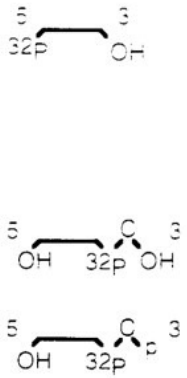
1997-The first Exosome component

- The first eukaryotic 3'->5' exonuclease has been demonstrated in yeast (1)
- In S. cerevisiae, the rrp4-1 mutation causes defective 3′ maturation of 5.8S rRNA (2) and confers a temperature-sensitive growth phenotype (3)



1





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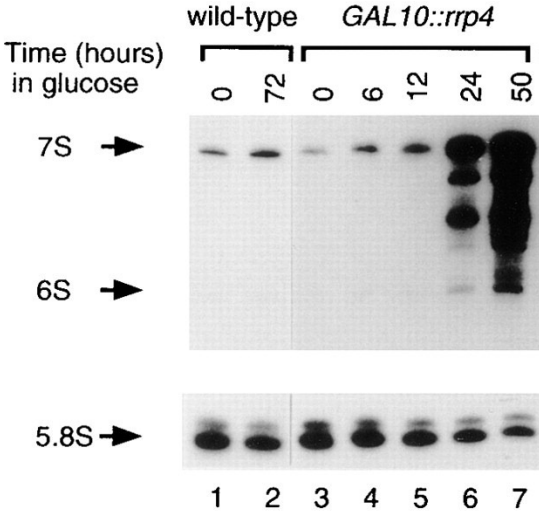
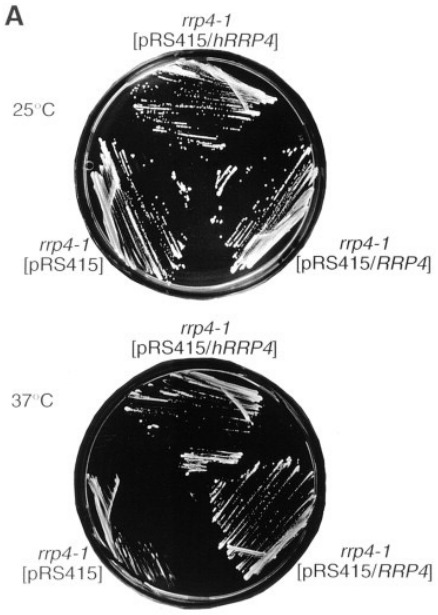
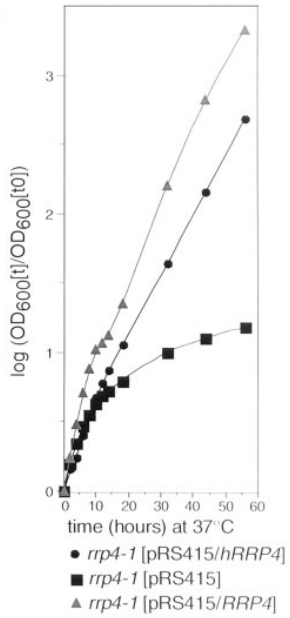




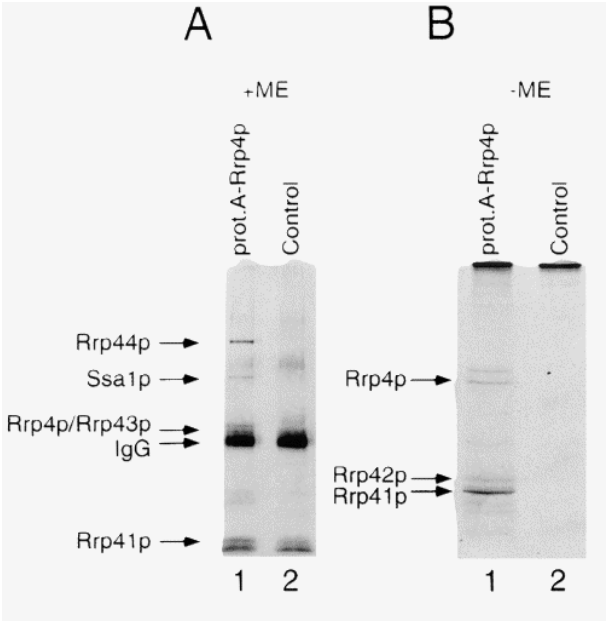


Mitchell et al., 1997. Cell

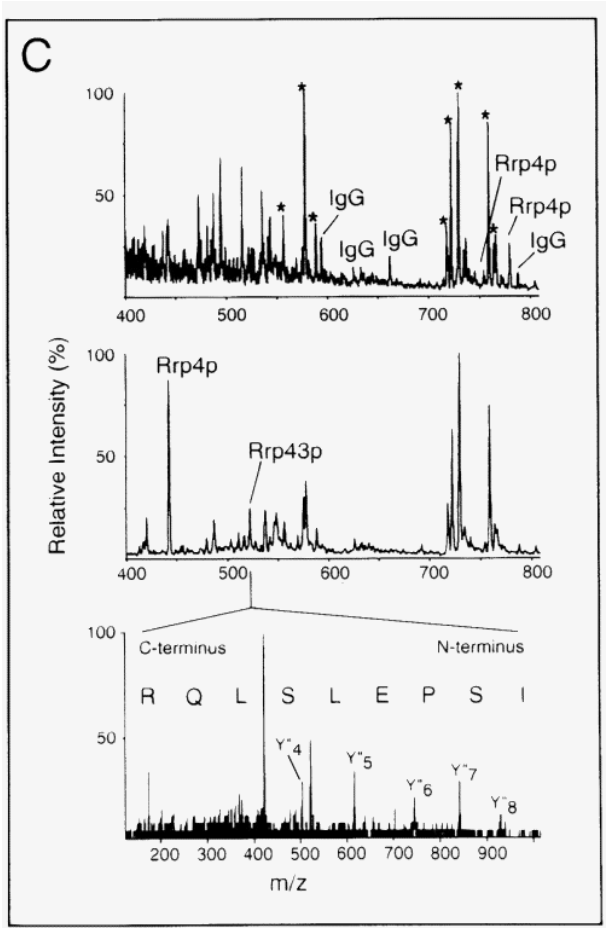
Four proteins copurify with yeast Rrp4p

- **Rrp41p, Rrp42, Rrp43 and Rrp44p**

ProtA-tagging and immunoprecipitation

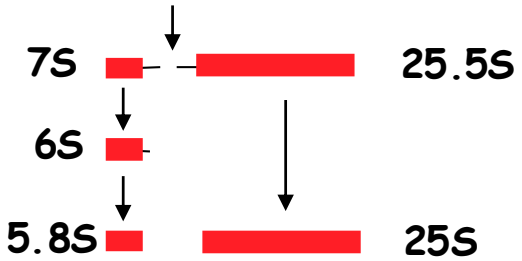


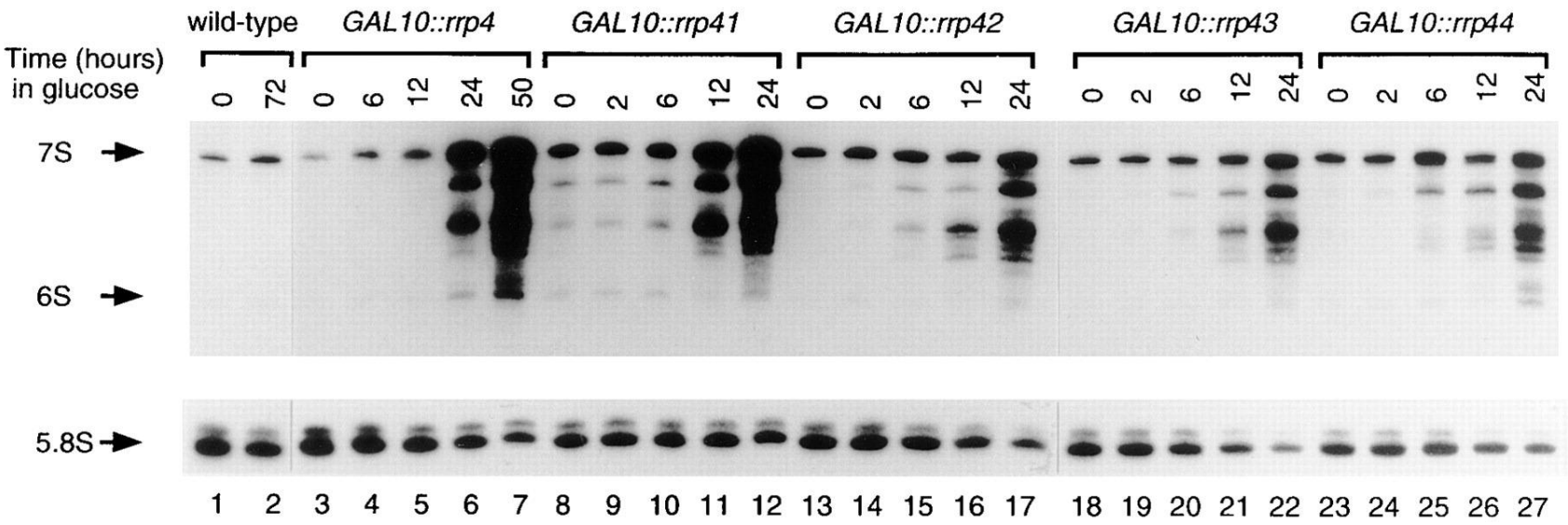
Mass spectrometry



Mitchell et al., 1997. Cell

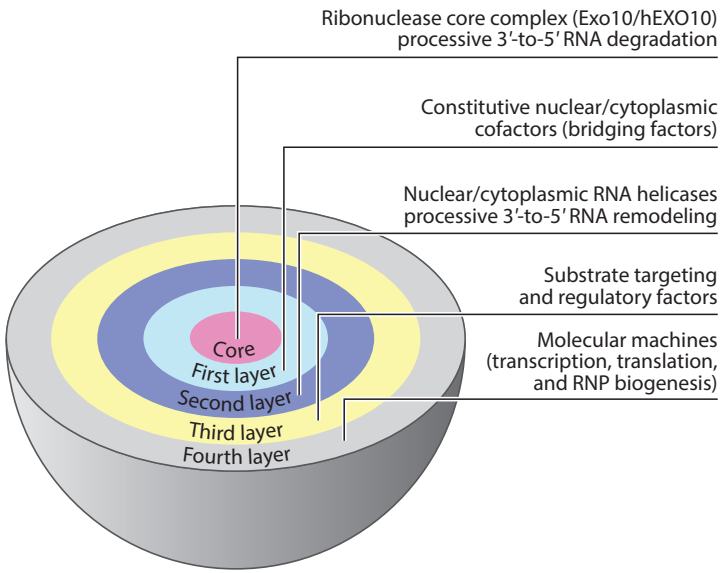
Depletion of Each Component of the Exosome Inhibits 5.8S rRNA Maturation





Versatile compositions of nuclear and cytoplasmic exosome holo-complexes.

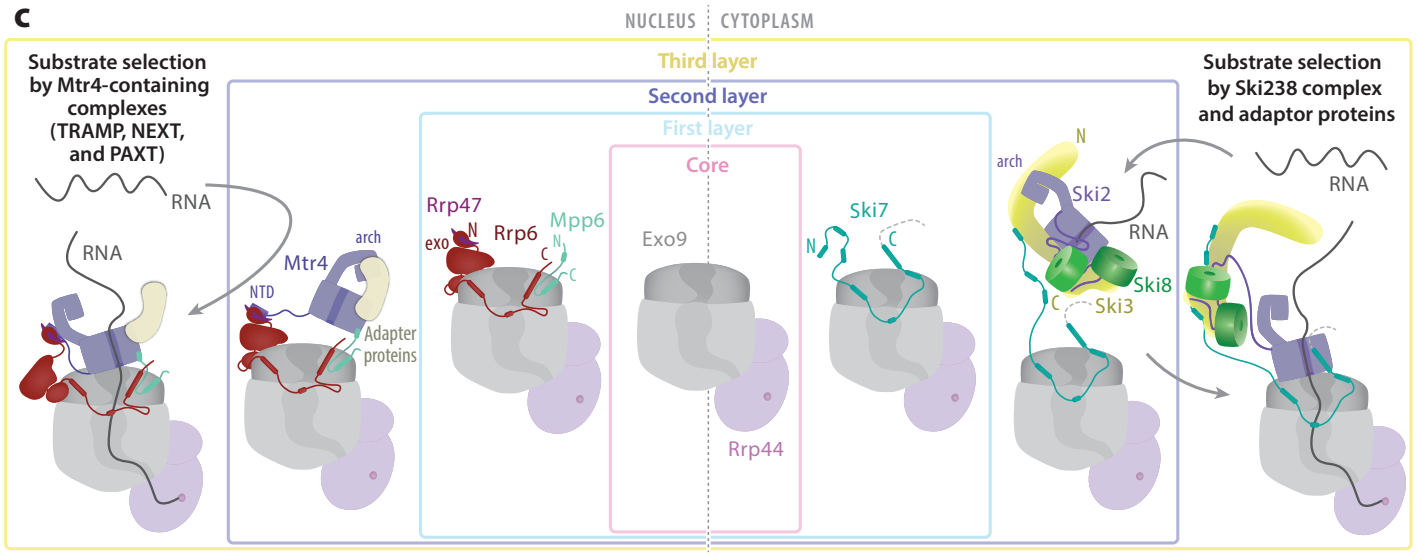
a



b

		S. cerevisiae	*H. sapiens*
Core	**Exo9 cap** S1/KH domain proteins	Csl4 and Ski4 Rrp4 Rrp40	EXOSC1 and hCSL4 EXOSC2 and hRRP4 EXOSC3 and hRRP40
	Exo9 ring/barrel S1/KH domain proteins	Rrp41 and Ski6 Rrp46 Mtr3 Rrp42 Rrp43 Rrp45	EXOSC4 and hRRP41 EXOSC5 and hRRP46 EXOSC6 and hMTR3 EXOSC7 and hRRP42 EXOSC8 and hRRP43 EXOSC9 and hRRP45
	Nuclease	Rrp44	hDIS3 and hDIS3L
First layer	**Nuclear**	Rrp6 Rrp47 and Lrp1 Mpp6	EXOSC10 and hRRP6 C1D hMPP6
	Cytoplasmic	Ski7	HBS1L3 and hSKI7
Second layer	**Nuclear**	Mtr4 Ski2	hMtr4, SKIV2L2, and MTREX hSKI2 and SKI2VL
	Cytoplasmic		
Third layer	**Nuclear**	PAP2 and Trf4/5 Air1/2 Nop53 Utp18	PAPD5 and TENT4B ZCCHC7 NOP53 UTP18 NVL NRDE2 RBM7 ZCCHC8 ZFC3H1 RBM26/27 ZC3H3 PABPN1
	Cytoplasmic	Ski3 Ski8 Ska1	hSKI3 and TTC37 hSKI8 and WDR61q AVEN FOCAD

c



Components of the exosome

Exo-10

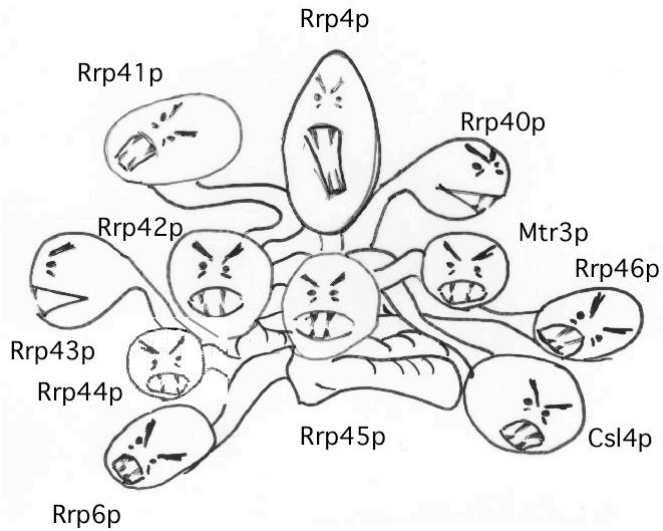
Exo-9

Protein	Gene	Phenotype	Domains	Mammalian Homologues	Comments
Rrp4p*	YHR069c	Essential	S1 and KH **RNA BD**	hRrp4p 43% (52%)	hRrp4p complements *rrp4-1*
Rrp40p	YOL142w	Essential	S1 and KH **RNA BD**	hRrp40p 35% (48%)	Homologous to Rrp4p
Rrp41p/Ski6p*	YGR195w	Essential	**RNase** PH	hRrp41p 35% (55%)	hRrp41p complements *GAL::rrp41*
Rrp42p	YDL111c	Essential	**RNase** PH	hRrp42p 25% (51%)	
Rrp43p	YCR035c	Essential	**RNase** PH		
Rrp45p	YDR280w	Essential	**RNase** PH	PM-Scl 75 38% (64%)	Human KIAA0116 and *OIP2* also homologous
Rrp46p	YGR095c	Essential	**RNase** PH	hRrp46p 35% (48%)	
Mtr3p*	YGR158c	Essential	**RNase** PH		
Csl4p	YNL232w	Essential	S1 and KH **RNA BD**	hCsl4p 48% (56%)	hCsl4p complements *csl4-1*
Rrp44p/Dis3p*	YOL021c	Essential	**RNase R** (RNase II family),	hDis3p 45%	hDis3p complements *dis3-81* and contains a PIN domain(endonuclease)
Rrp6p*	YOR001w	ts-lethal	**RNase** D	PM-Scl 100 32% (52%)	Component only of nuclear complex

* exos *in vitro*

1997-The Exosome complex

Deletion of any of the ten core subunits is lethal in yeast, and depletion of each of the individual subunits gives very similar phenotypes. These results, combined with the analysis of the nuclease activities of partially purified exosomes and individual recombinant subunits (Dis3/Rrp44, Rrp4 and Rrp41), suggest that the exosome core is a **multinuclease complex.**

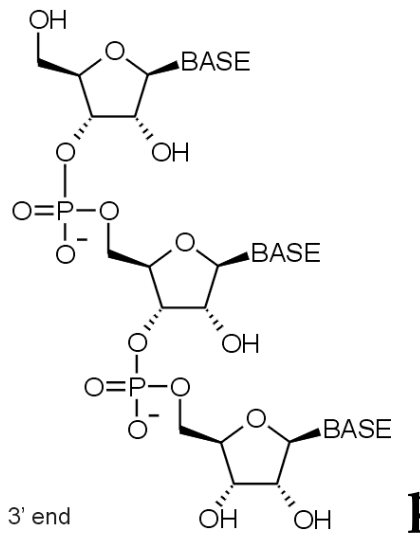


Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M. & Tollervey, D. The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′->5′ exoribonucleases. *Cell* **91**, 457–466 (1997).

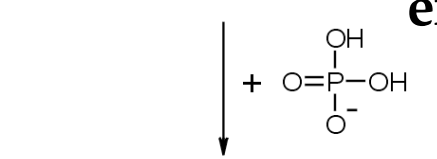
Components of the exosome

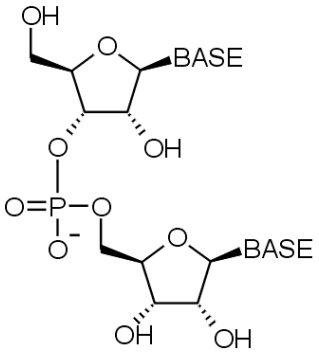
The **RNase PH-like exosome proteins**, belong to the RNase PH family of RNases and are **phosphorolytic exonuribonuclease**, which utilize inorganic phospate to remove nucleotide from the 3'-end of RNA

Dis3/Rrp44 and **Rrp6** belongs to the RNase R and RNase D families, respectively, and are **hydrolytic exonuribonuclease**, which utilize water to cleave the nucleotide bond



phosphorolytic exonuribonuclease





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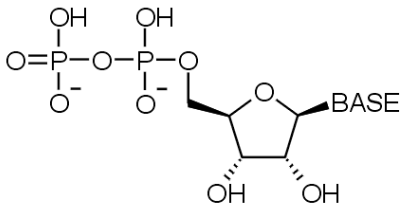
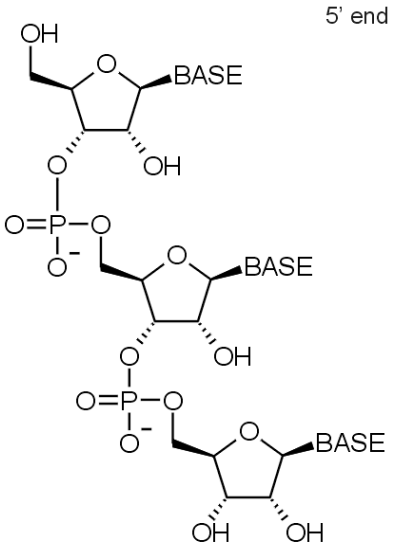
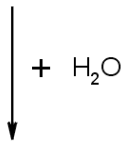
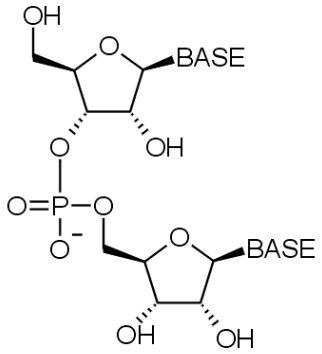


NDP





hydrolytic exonuribonuclease



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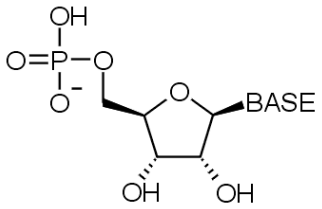
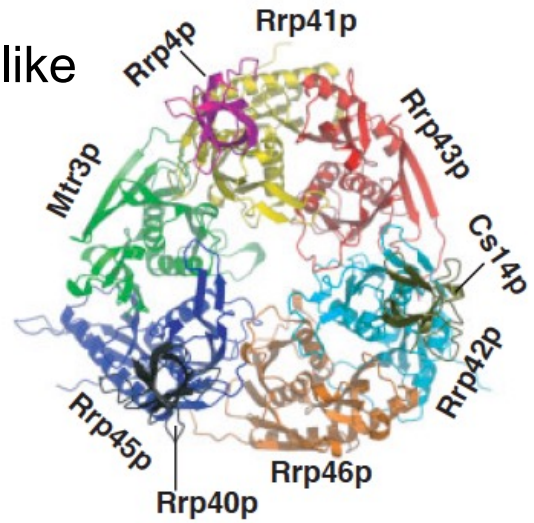
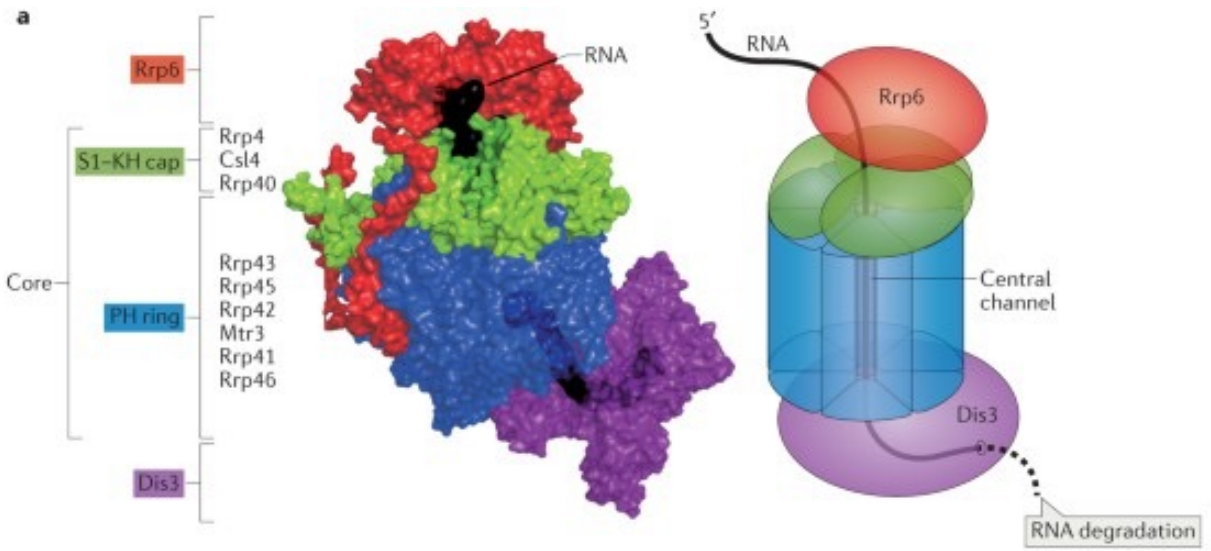


NMP

The yeast Exosome: 3’-5’ decay

Barrel like



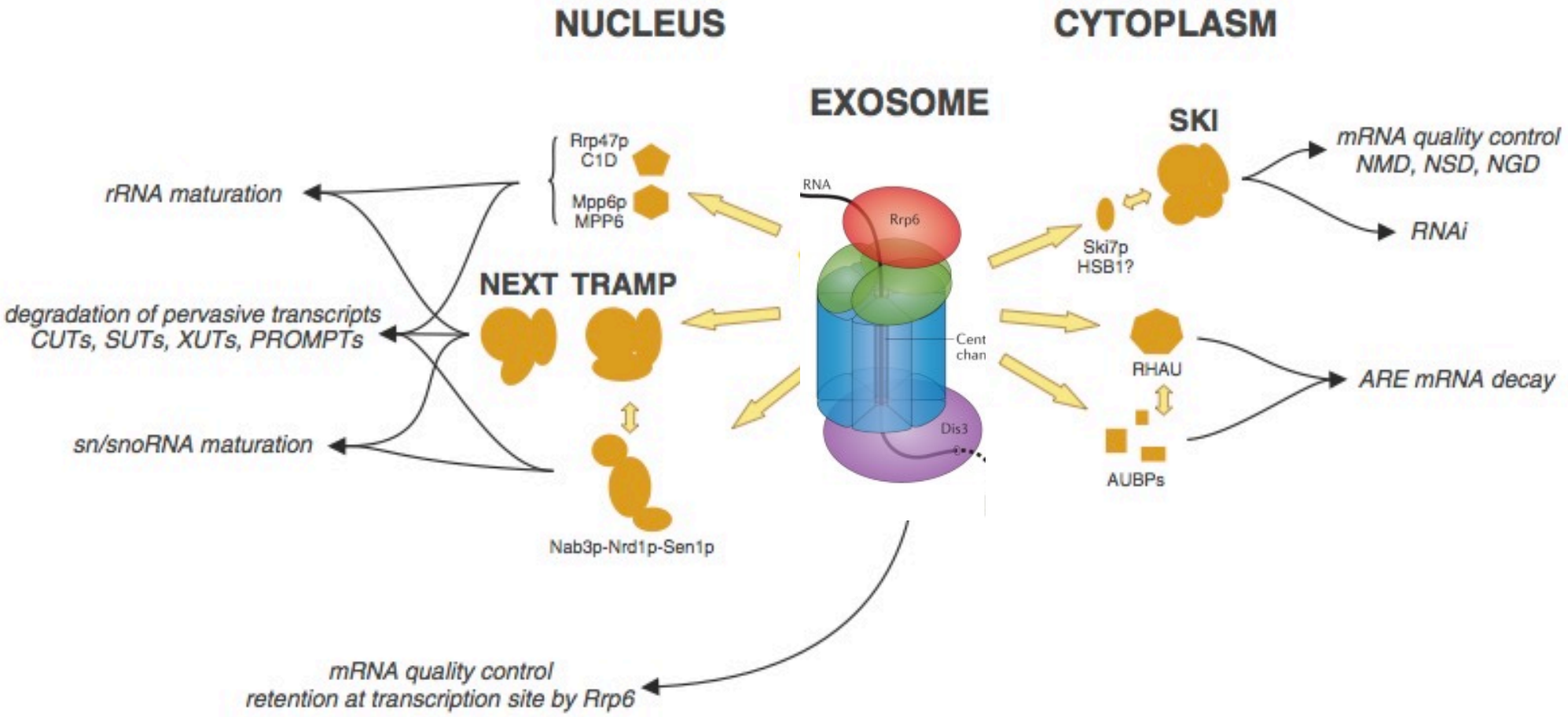


The eukaryotic exosome is a **major ribonuclease for RNA decay and processing.**
The repertoire of catalytic subunits varies among Eukaryota.
Some catalytic subunits are compartment-specific and give rise to exosome isoforms.

In *S. cerevisiae*, the exosome is the only essential 3’-5’ exoribonuclease, which is highly conserved in eukaryotic kingdom, with exosome core forming **a barrel-like assembly** (6 subunits RNase PH-like– Rrp41, Rrp42, Rrp43, Rrp45, Rrp46, Mtr3) and cap (3 subunits – Rrp4, Rrp40, Csl4 (S1/KH domains)).

In yeast, humans, and most other eukaryotes, all exosome RNase PH- like subunits have lost the ability to perform the phosphorolytic reaction due to subtle changes in their corresponding active sites

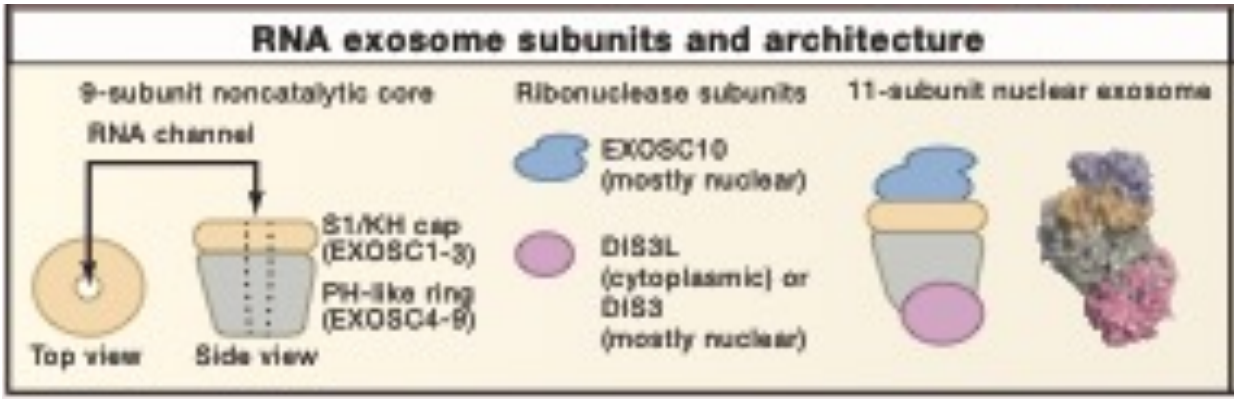
Exosome's cellular functions and cofactors



The core complex in *S. cerevisiae*, which is the best studied model in exosome research, lacks catalytic activity which is instead provided by a stable interaction with **Dis3p** (Rrp44), **a nuclease possessing exonucleolytic activity**. However, DIs3/Rrp44 posseses also a PIN domain features a characteristic endoribonuclease active site, albeit exhibiting rather modest activity

Dis3p is also present in the **nucleus**, where the exosome core associates with an additional catalytic subunit, Rrp6p.

The human Exosome: 3′-5′ decay



Only one homologue of Rrp6p exists in humans (**EXOSC10**/RRP6) **is localised in** the **nucleolus**, but there are three proteins of the DIS3 (Rrp44) family: **DIS3, DIS3L and DIS3L2.**

DIS3 is primarily localized in the **nucleus**, and is the closest ortholog to yeast Rrp44,

DIS3L is cytoplasmic, is 20 times less abundant than hDIS3 reflecting the smaller pool of exosome substrates in the cytoplasmic transcriptome. hDIS3L has lost the endonuclease site in the PIN domain but contains an additional N-terminal extension that interacts with hEXO9 and human hRRP45. This in turn forms the binding platform for recruiting the hSKI7 cytoplasmic cofactor.

hDIS3L2 is also restricted to the cytoplasm, lacks the PIN domain and accordingly does not associate with the exosome, instead functioning in other **exosome-independent** cytoplasmic RNA degradation pathway

Mutations in the RNA exosome component gene *EXOSC3* cause pontocerebellar hypoplasia and spinal motor neuron degeneration

Jijun Wan[1,24], Michael Yourshaw[2,24], Hafsa Mamsa[1], Sabine Rudnik-Schöneborn[3], Manoj P Menezes[4], Ji Eun Hong[1], Derek W Leong[1,23], Jan Senderek[3,5], Michael S Salman[6,7], David Chitayat[8,9], Pavel Seeman[10], Arpad von Moers[11], Luitgard Graul-Neumann[12], Andrew J Kornberg[13], Manuel Castro-Gago[14], María-Jesús Sobrido[15,16], Masafumi Sanefuji[17], Perry B Shieh[1], Noriko Salamon[18], Ronald C Kim[19,20], Harry V Vinters[1,21], Zugen Chen[2], Klaus Zerres[3], Monique M Ryan[13], Stanley F Nelson[2,21,22] & Joanna C Jen[1]

nature genetics

EXOSC8 mutations alter mRNA metabolism and cause hypomyelination with spinal muscular atrophy and cerebellar hypoplasia

Veronika Boczonadi[1,*], Juliane S. Müller[1,*], Angela Pyle[1,*], Jennifer Munkley[1,*], Talya Dor[2], Jade Quartararo[3], Ileana Ferrero[3], Veronika Karcagi[4], Michele Giunta[1], Tuomo Polvikoski[5], Daniel Birchall[6], Agota Princzinger[7], Yuval Cinnamon[2,8], Susanne Lützkendorf[9], Henriett Piko[4], Mojgan Reza[1], Laura Florez[10], Mauro Santibanez-Koref[1], Helen Griffin[1], Markus Schuelke[9], Orly Elpeleg[2], Luba Kalaydjieva[10], Hanns Lochmüller[1], David J. Elliott[1], Patrick F. Chinnery[1], Shimon Edvardson[2] & Rita Horvath[1]

nature COMMUNICATIONS

Germline mutations in *DIS3L2* cause the Perlman syndrome of overgrowth and Wilms tumor susceptibility

Dewi Astuti[1,9], Mark R Morris[1,2,9], Wendy N Cooper[1], Raymond H J Staals[3], Naomi C Wake[1], Graham A Fews[4], Harmeet Gill[1], Dean Gentle[1], Salwati Shuib[1], Christopher J Ricketts[1], Trevor Cole[4], Anthonie J van Essen[5], Richard A van Lingen[6], Giovanni Neri[7], John M Opitz[8], Patrick Rump[5], Irene Stolte-Dijkstra[5], Ferenc Müller[1], Ger J M Pruijn[3], Farida Latif[1] & Eamonn R Maher[1,4]

nature genetics

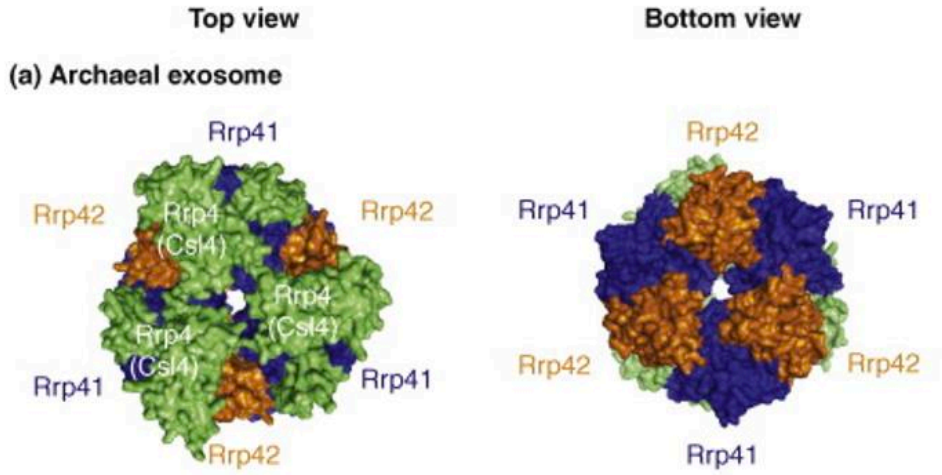
The RNA exosome complex degrades expanded hexanucleotide repeat RNA in *C9orf72* FTLD/ALS

Yuya Kawabe, Kohji Mori*, Tomoko Yamashita, Shiho Gotoh & Manabu Ikeda

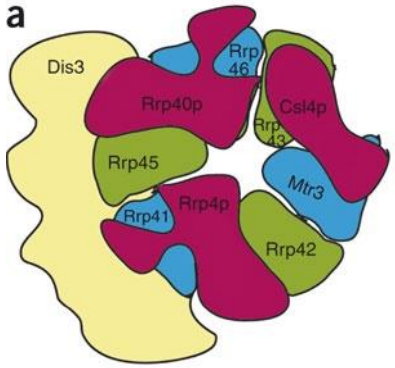
THE EMBO JOURNAL

The yeast exosome model

The **archaeal exosome** has a ring structure, with six **RNase PH** domains forming a ring, on top of which **three RNA-binding domains** are positioned. Based on the archaeal structure, a similar architecture is predicted for the homologous eukaryotic exosomes in agreement with existing two-hybrid data, direct protein-protein interaction assays and phylogenetic sequence conservation.

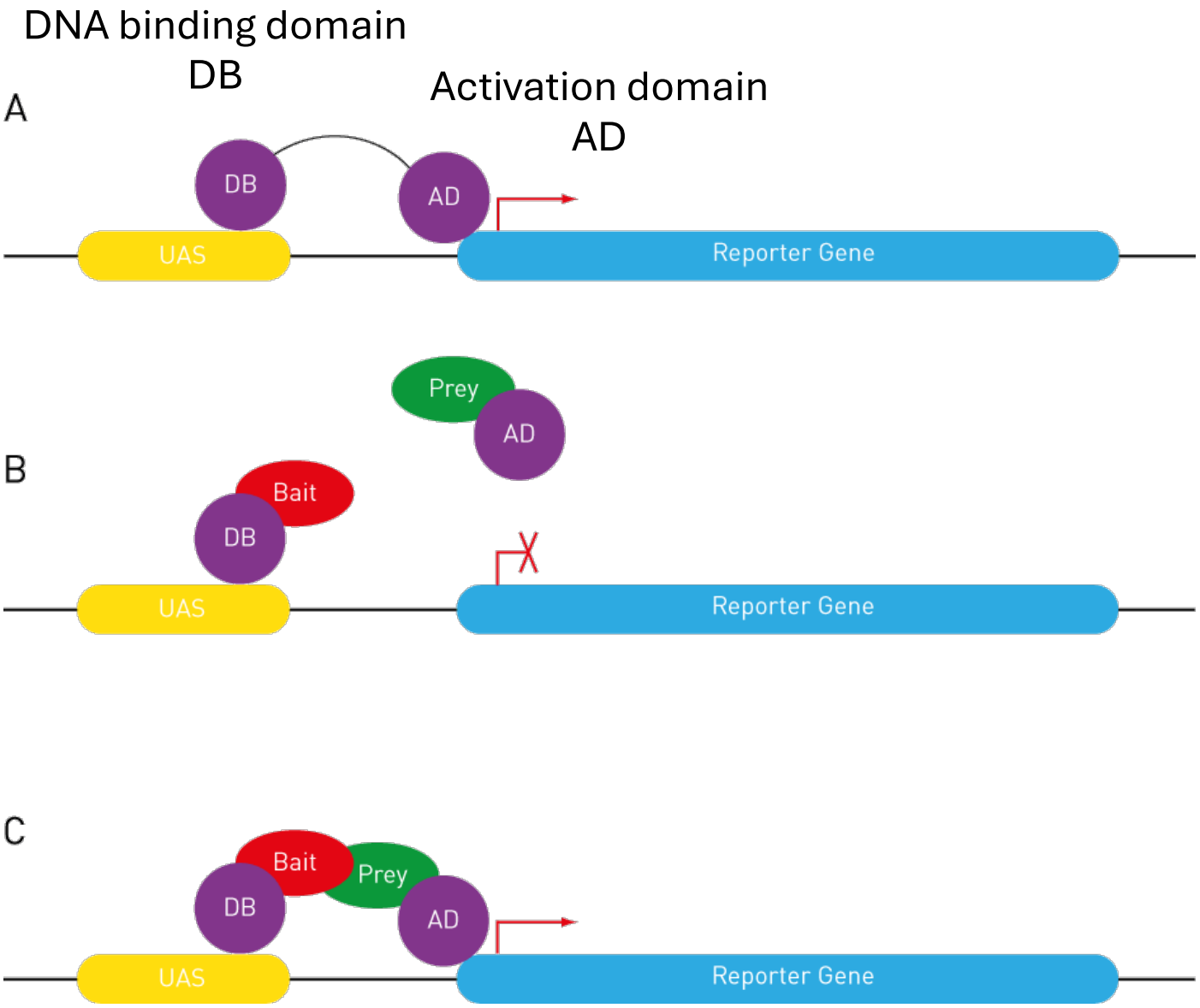


S.solfataricus

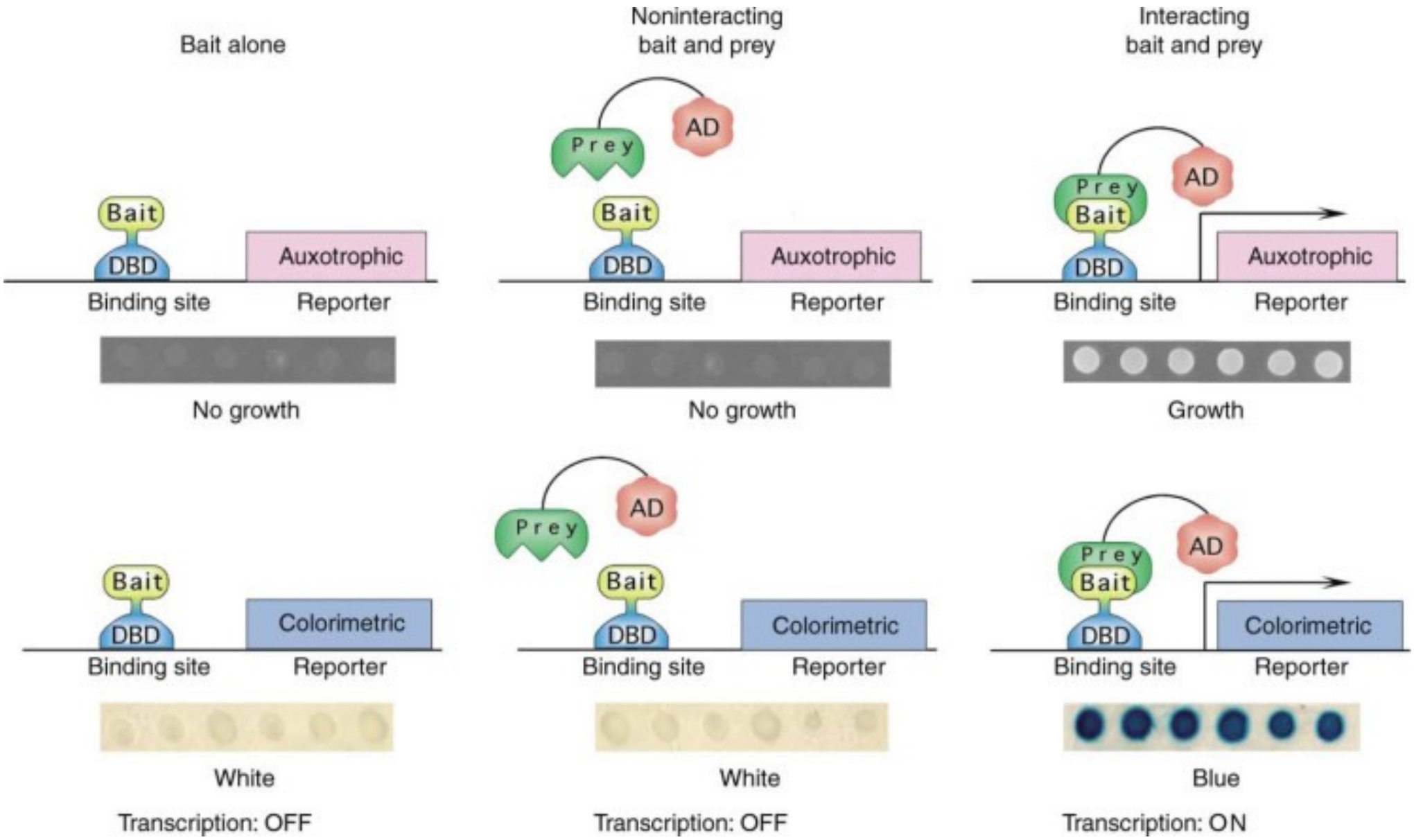


S.cerevisiae

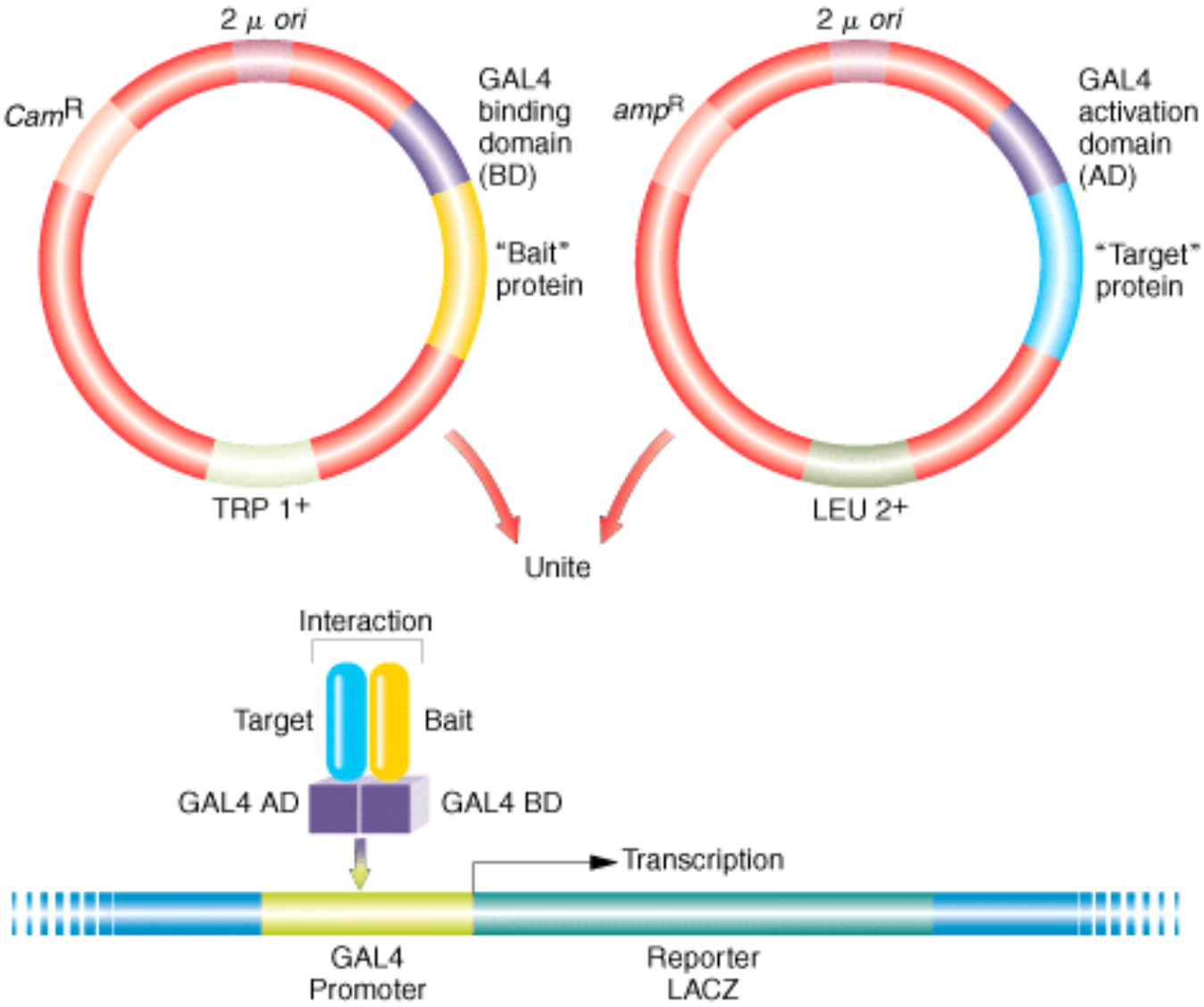
Two-Hybrid System



Two-Hybrid System

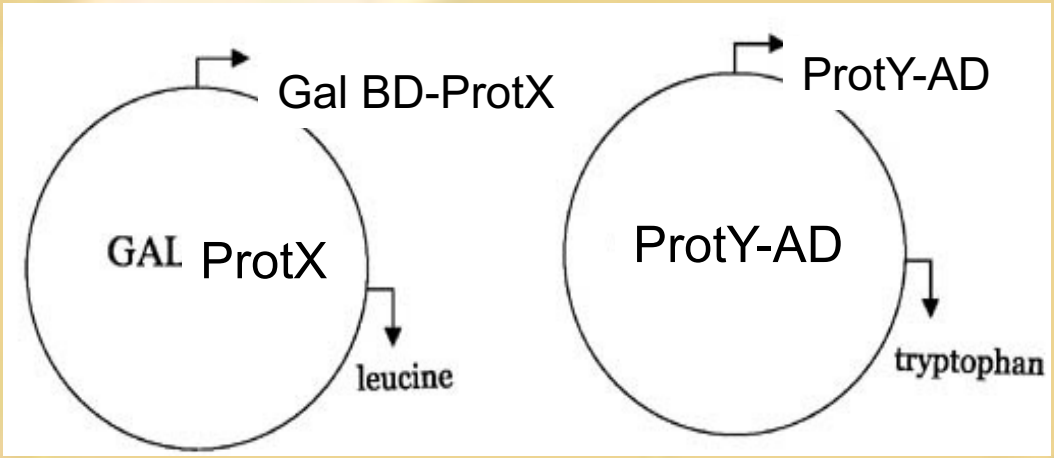


2 Hybrid System

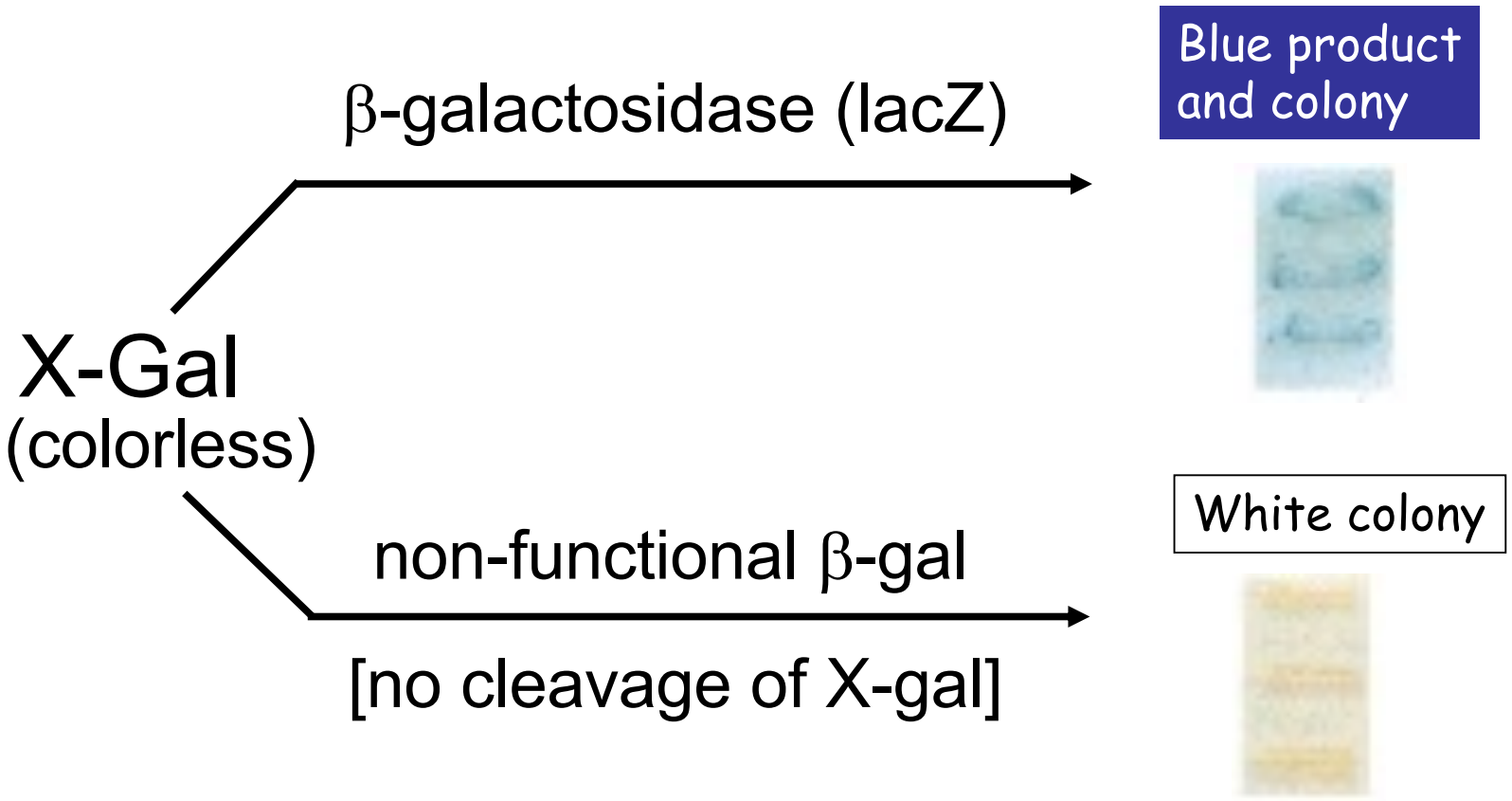


2 Hybrid System

leu^{-}, trp^{-}



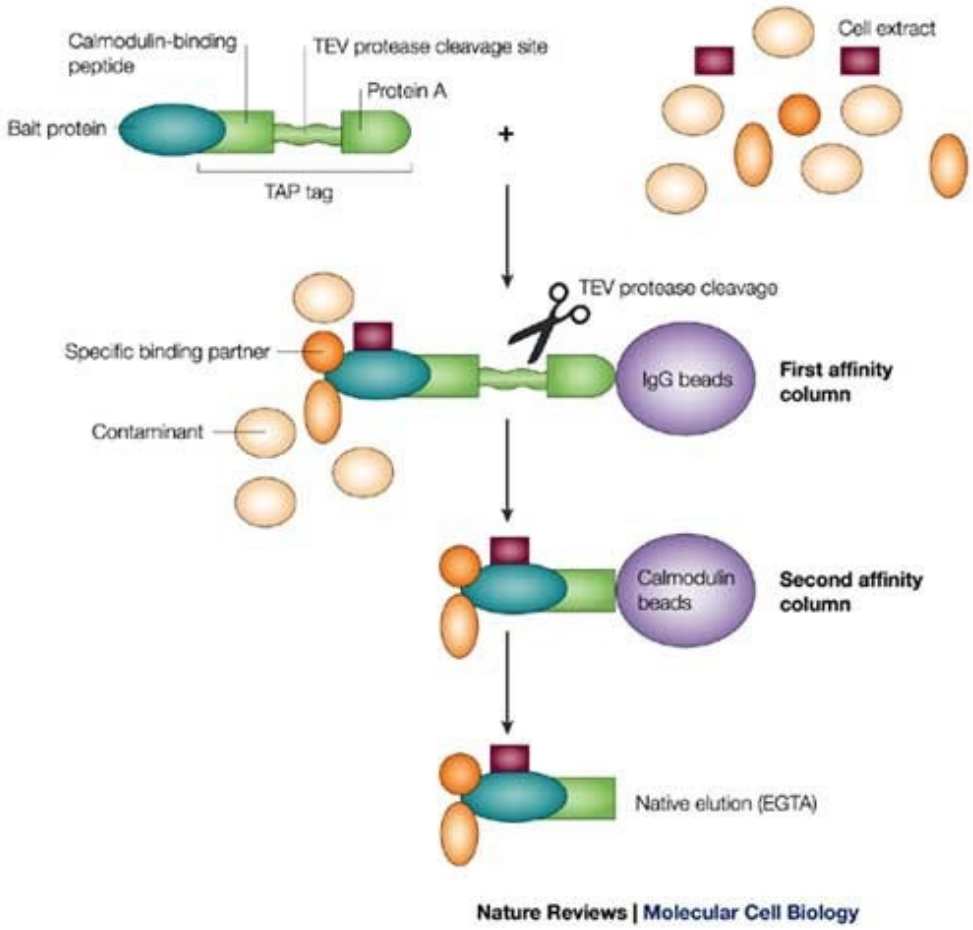
Blue-White Screening



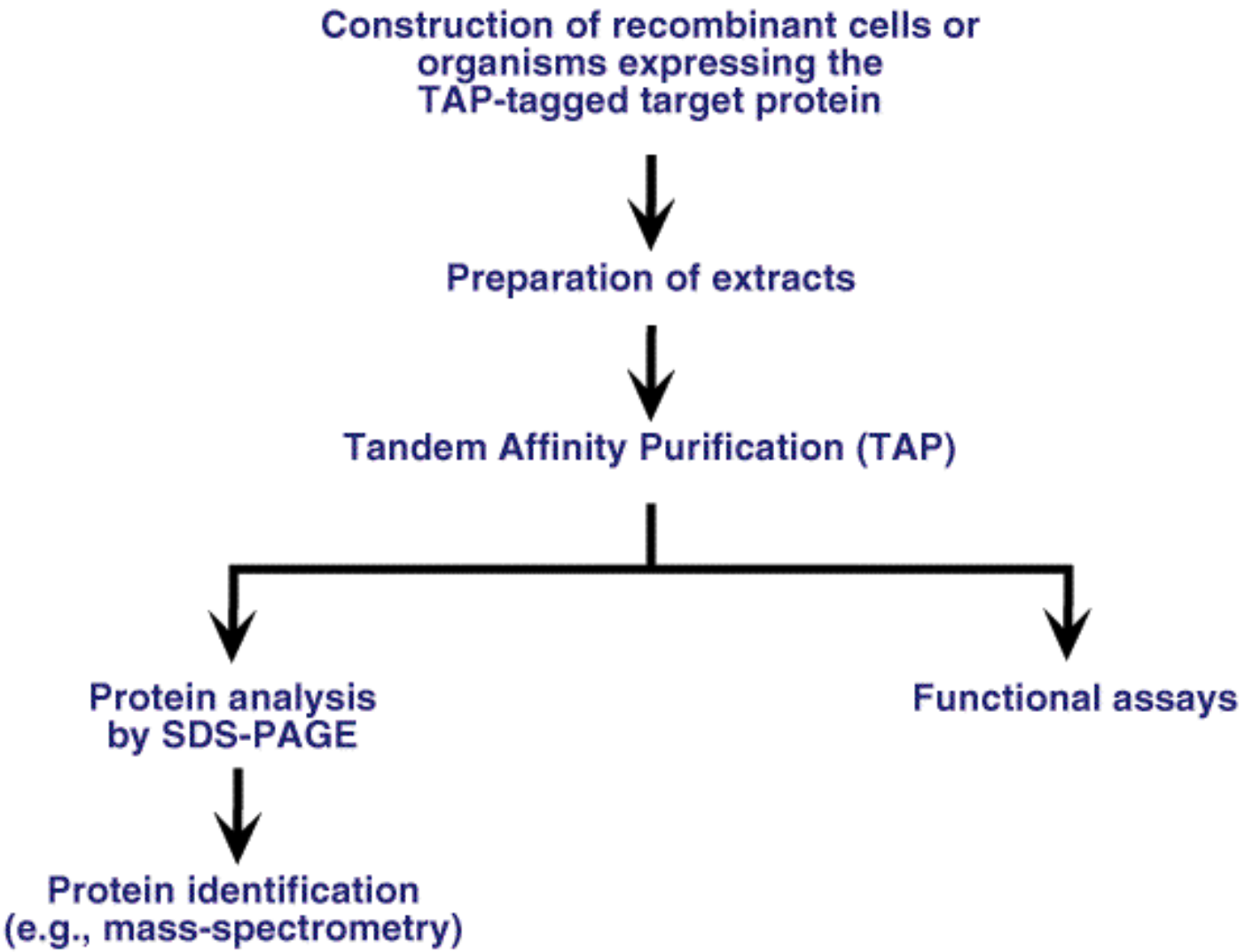
X-Gal = 5-bromo-4-chloro3-indolyl-β-D-galactopyranoside

1999-2000 TAP-tagging is invented

It is a two-step affinity purification system for isolating native multiprotein complexes

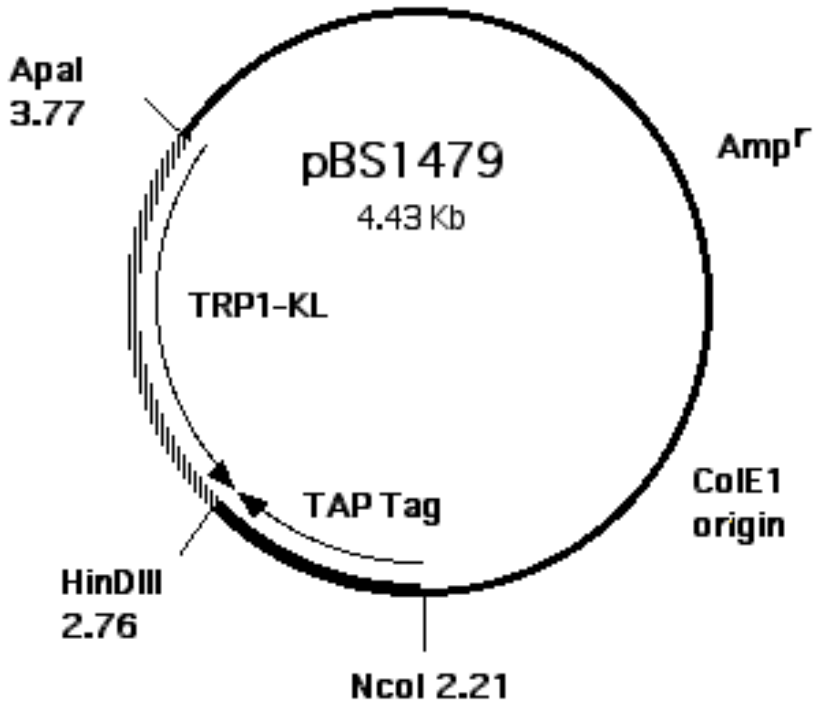


TAP tagging

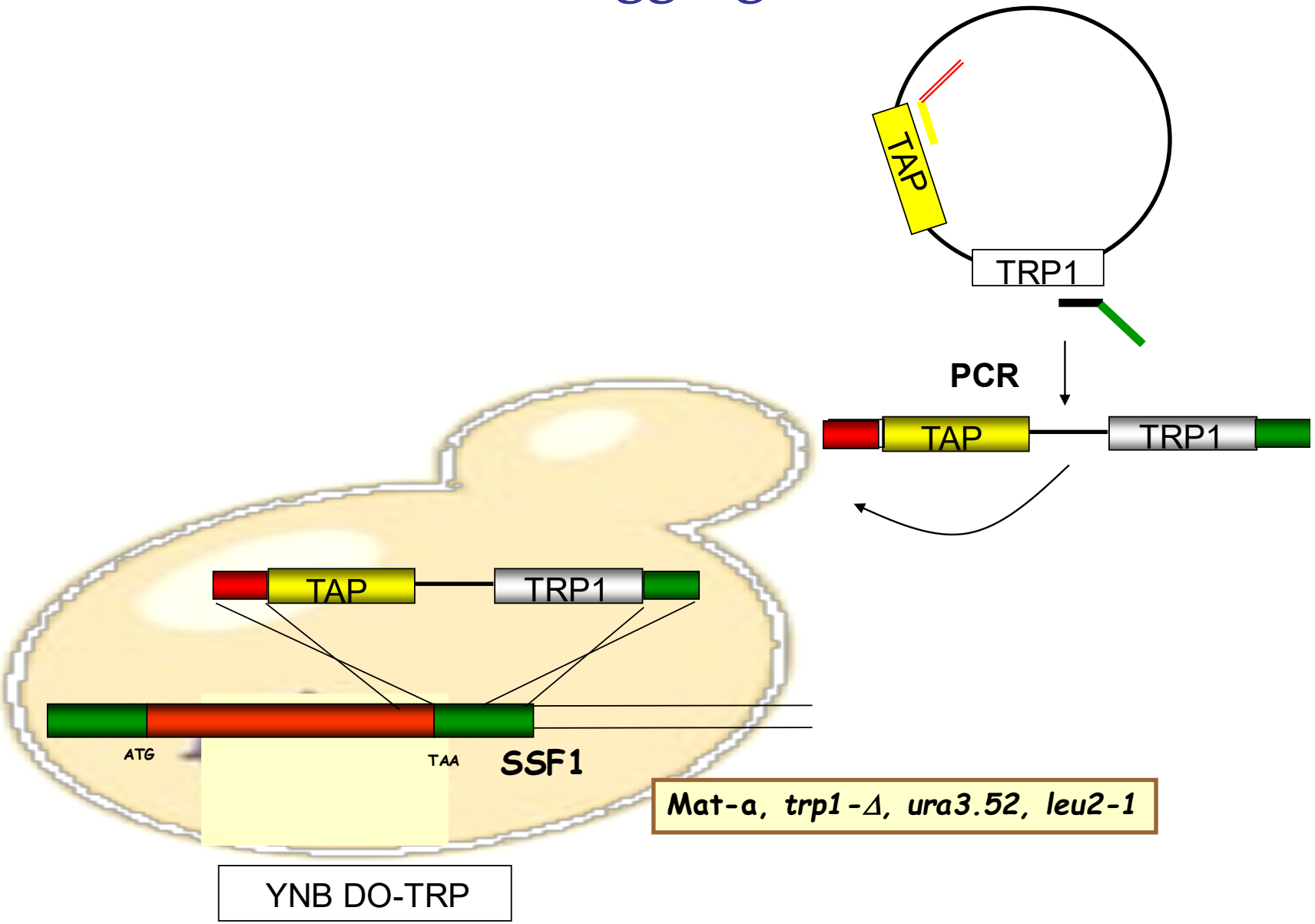

Construction of recombinant cells or organisms expressing the TAP-tagged target protein
Preparation of extracts
Tandem Affinity Purification (TAP)
Protein analysis by SDS-PAGE
Protein identification (e.g., mass-spectrometry)
Functional assays

TAP tagging

TAP plasmid:

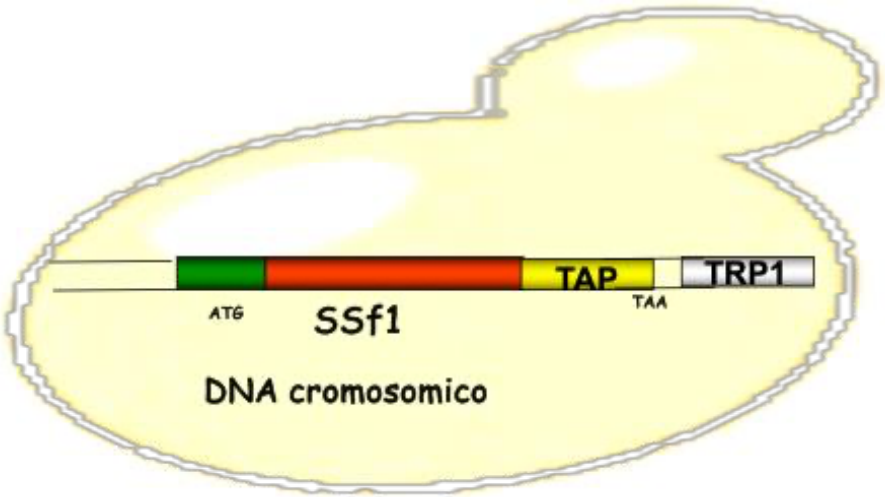


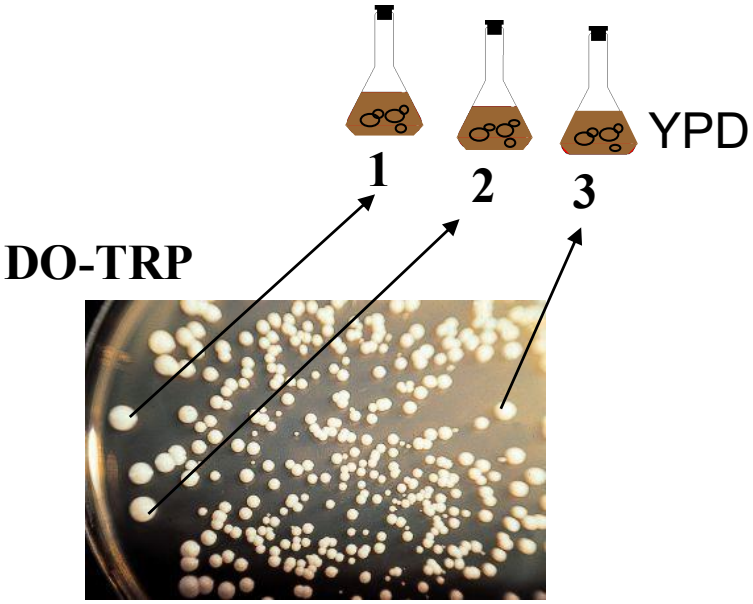
TAP tagging

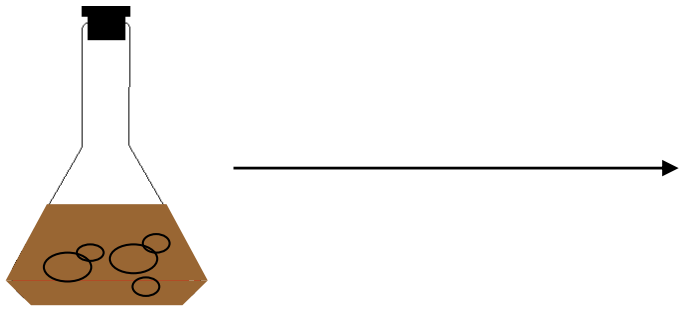

TAP
TRP1
PCR
TAP
TRP1
TAP
TRP1
ATG
TAA
SSF1
Mat-a, trp1-Δ, ura3.52, leu2-1
YNB DO-TRP

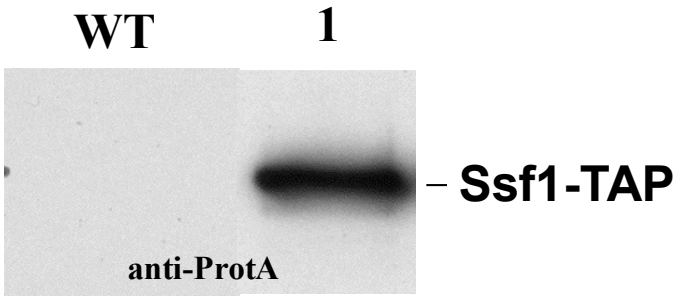
TAP tagging

Ssf1-TAP : Mat-a, *trp1-D, ura3.52, leu2-1, Ssf1-TAP::TRP1*





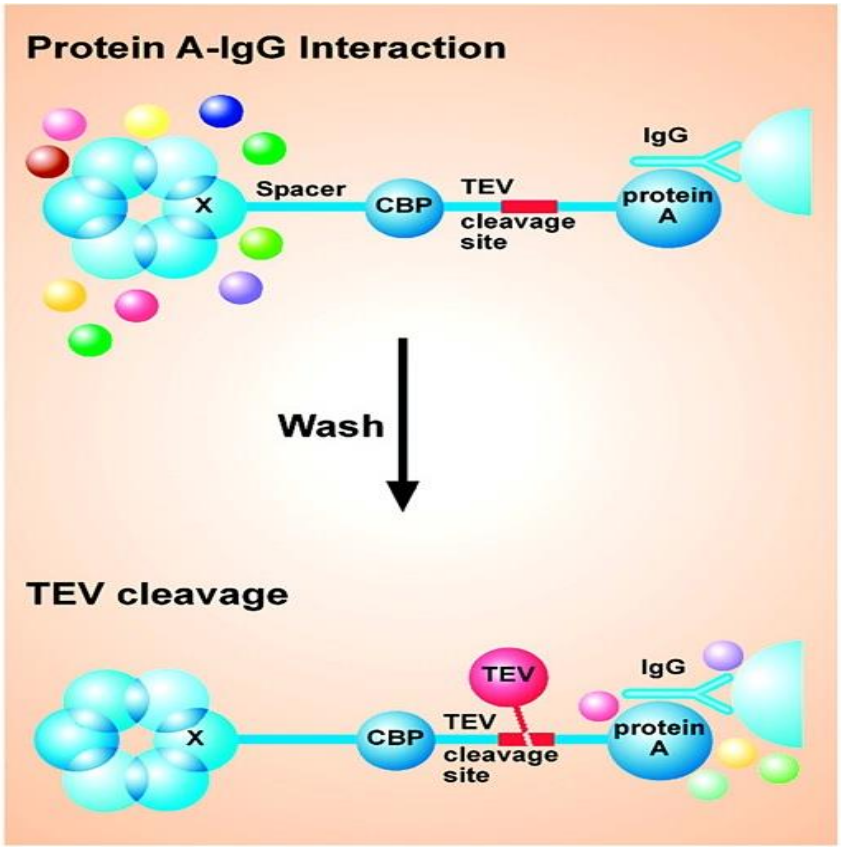




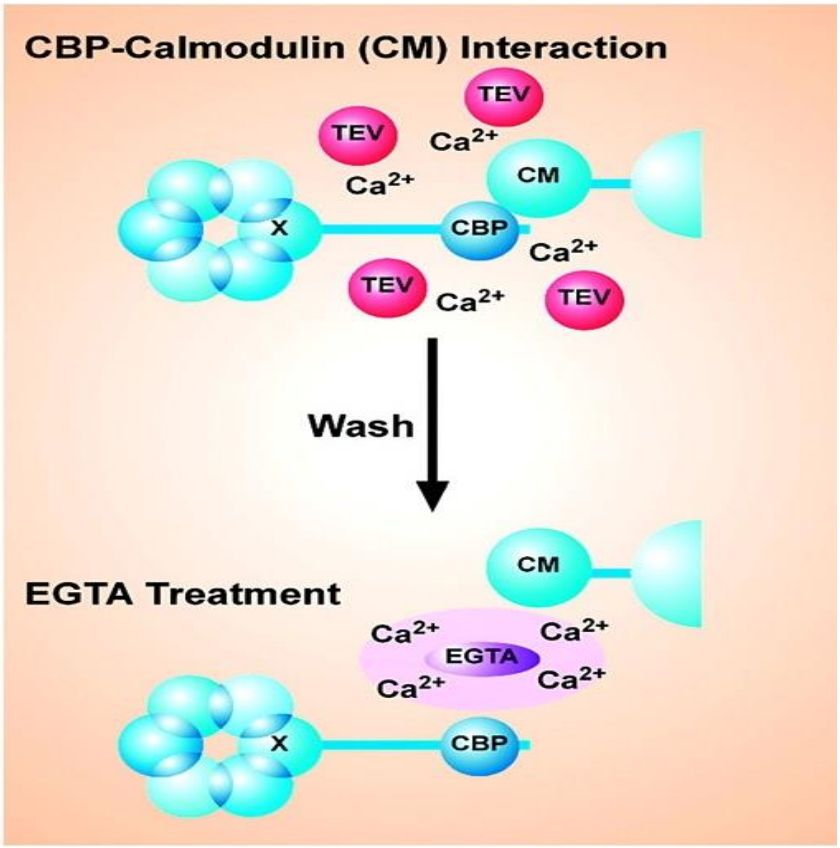
Western blot

Purificazione TAP

Prima Purificazione



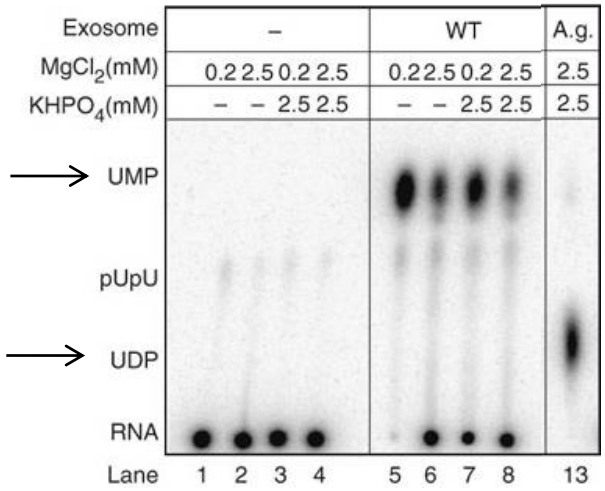
Seconda Purificazione



2007-Exosome complex revised

The yeast exosome core acts only hydrolytically

They purified the yeast exosome complex from a strain expressing a TAP-tagged *DIS3* allele and an *RRP6* deletion. RNA degradation experiments resulted in a hydrolytic activity, revealed by the production of UMP, but produced no detectable UDP, which would have indicated a phosphorolytic activity (see A.g.).



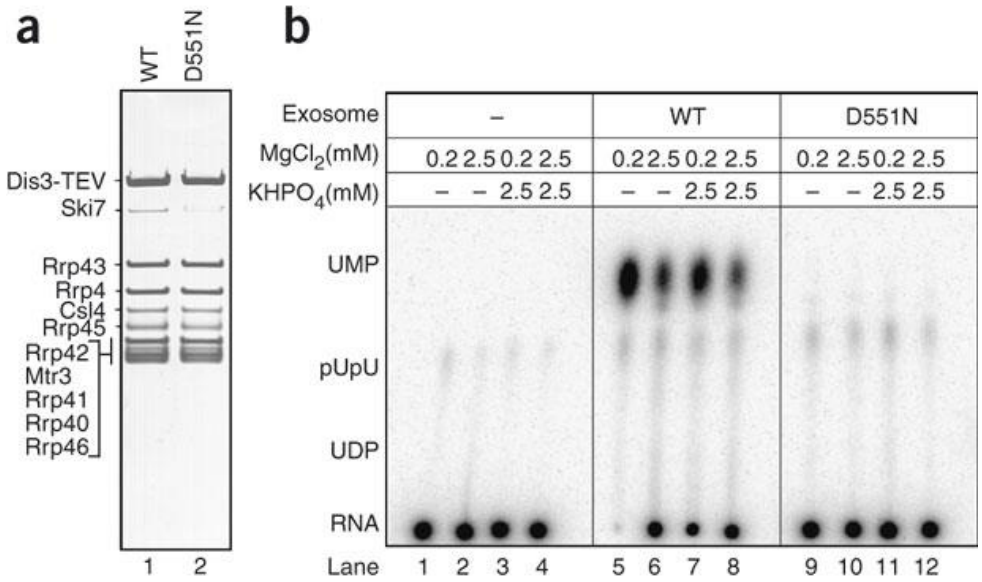
RNA degradation experiments using a RNA substrate internally labeled with [-^{32}P]UTP and thin-layer chromatography

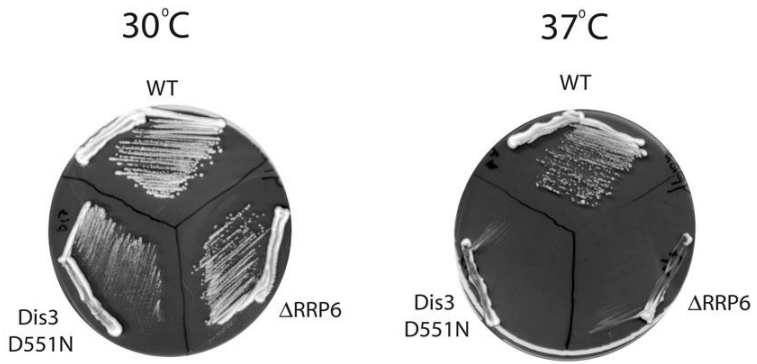
A.g.=the recombinant archaeal Rrp41-Rrp42 dimer (phosporolytic)

2007-Exosome complex revised

Dis3/Rrp44* mutation blocks core exosome activity *in vitro:

To test whether Dis3 is responsible for the exosome core activity, they introduced a mutation in the putative exo-catalytic site (D551N) into the TAP-tagged *DIS3* allele. *Dis3* mutation abolished all detectable exoribonucleolytic activity and gives growth phenotype defects.



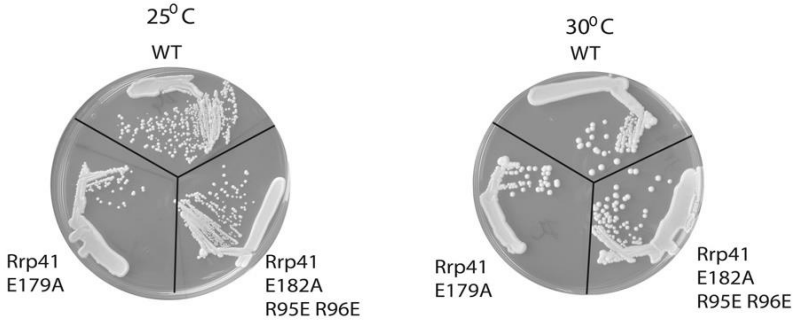


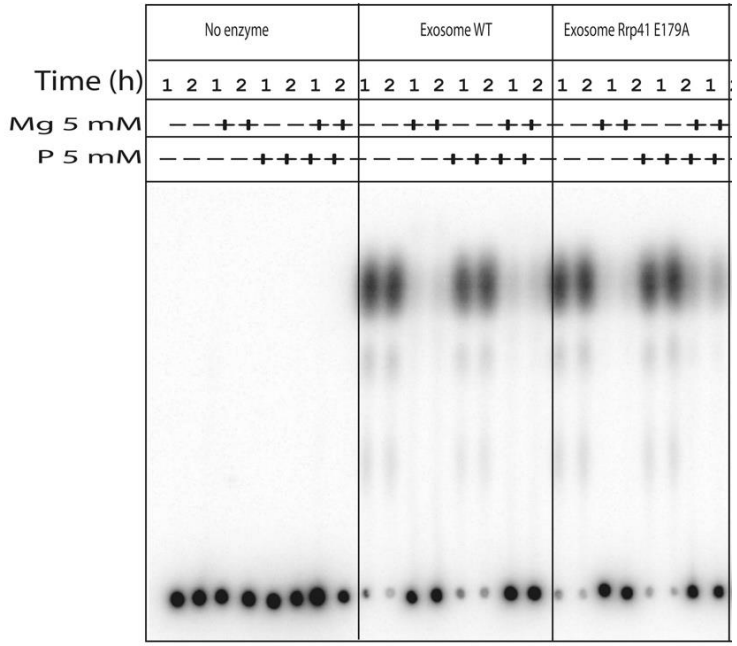
Dziembowski et al., 2007. Nature Struct. Mol. Biol

2007-Exosome complex revised

***rrp41* point mutants behave like wild-type yeast:**

The other subunit of the hexameric ring that contains most of the residues essential for a phosphorolytic activity (PH domain) is **Rrp41** (active in *S. solfataricus*). Several mutations were introduced in potentially active sites of Rrp41, and their effects on growth and exosome activity were analyzed. The mutation of the potentially catalytic Glu179 to alanine (a mutation shown to completely abolish archaeal exosome) did not result in a growth phenotype different from wild-type yeast. Finally, a purified exosome core containing the E179A-mutated Rrp41 subunit was as active as the wild-type exosome core in *in vitro* RNA degradation assays



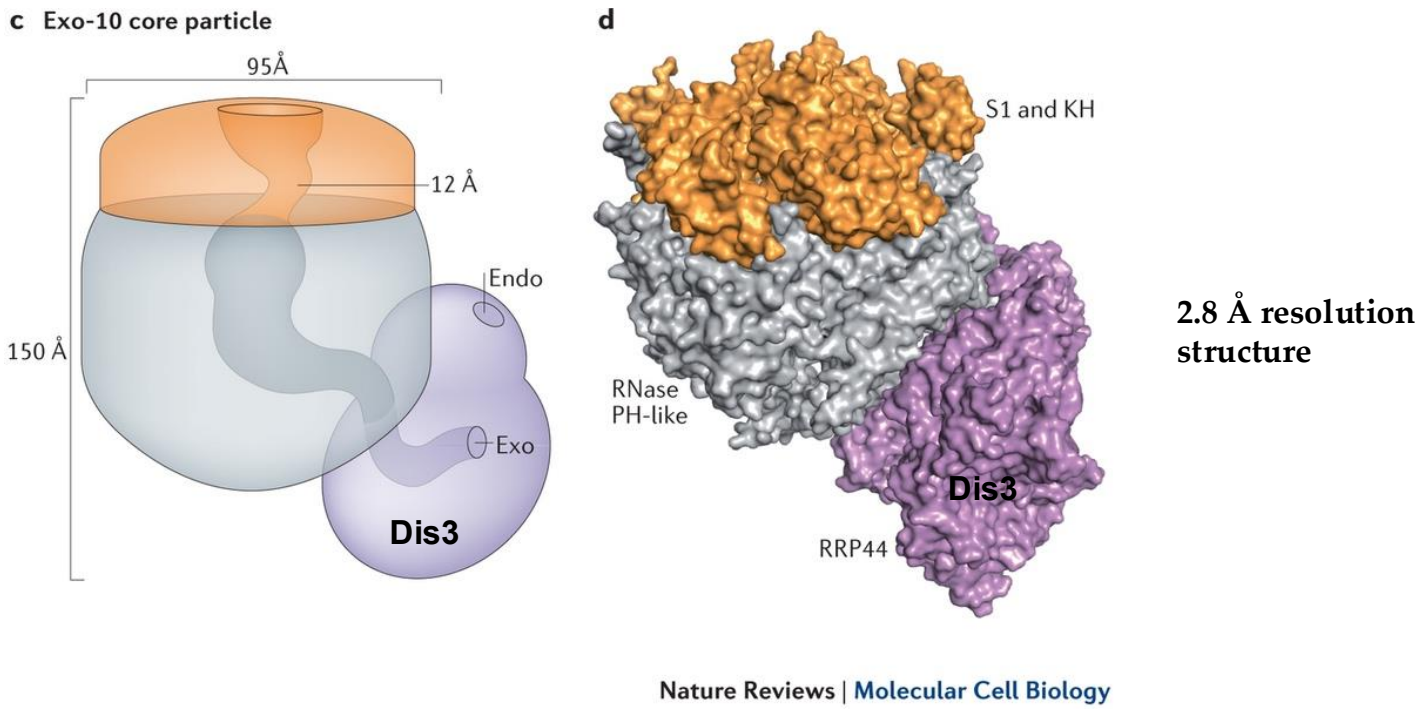


Dziembowski et al., 2007. Nature Struct. Mol. Biol

2007-Exosome complex revised

The observation that the main exosome core nuclease activity originates from Dis3 is surprising, given that the nine remaining subunits, which are predicted to adopt an archaeal exosome/PNPase–like ring structure, are essential. One could imagine that an ancient phosphorolytic enzyme of archaeal origin was responsible for 3'->5' RNA degradation and processing in primordial eukaryotic cells. During the course of evolution, additional hydrolytic nucleases having eubacterial origins, now encoded by ***DIS3/RRP44*** and ***RRP6***, could have become associated with the original complex. The requirement for the phosphorolytic activity of this new assembly would have decreased slowly as the new hydrolytic nuclease(s) assumed the catalytic function, possibly because of a lower requirement for fast energy recycling in more slowly growing eukaryotic cells. However, **interactions with factors regulating and targeting the exosome**, which occurred through the original ring structure, would have remained essential, thus preventing the complete disappearance of the corresponding subunits, resulting in selective pressure to maintain a given structure rather than catalytic features.

2013-Yeast Exosome complex structure



2.8 Å resolution structure

In the **Exo-10** complex, single-stranded RNA substrates enter the barrel-like structure of **Exo-9** via a pore at the centre of the S1 and KH domains. The RNA is then threaded with sequence-unspecific interactions via an internal channel to the exoribonuclease site of the catalytic subunit**, DIS3/RRP44**. Although the cylindrical structure of the eukaryotic exosome is catalytically inert, it is used to channel and restrain an unfolded substrate for processive rounds of cleavage.

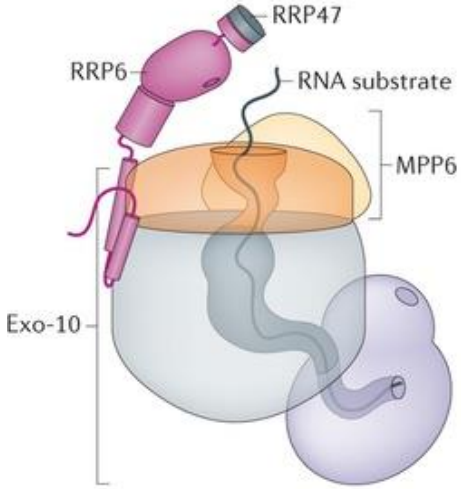
Cofactors for the exosome complexes

FIRST LAYER (Rrp6 and Ski complex**)**

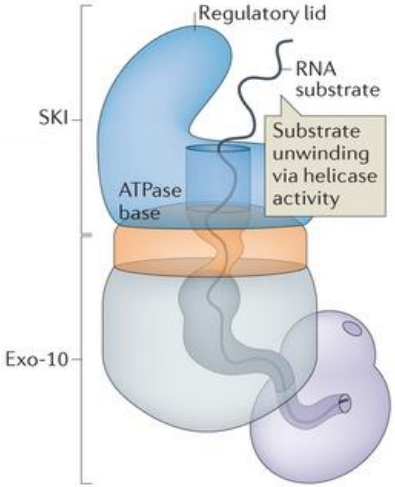
SECOND LAYER (Transient RNA Helicases: Mtr4 and Ski2**)**

The 3′-5′ RNA helicases Ski2 (in the cytoplasm) and Mtr4 (in the nucleus) are central components in the exosome cofactors networks for their roles in unwinding RNAs and/or remodeling ribonucleoprotein (RNP) complexes, making the transcripts accessible to the Exo10 exoribonuclease site.

Cofactors for the exosome complexes



Rrp6-mediated RNA cleavage uses a two-metal ion mechanism, but its RNase activity is distributive instead of processive



Nuclear exosome:

Mpp6- a cofactor that functions in the maturation of 5.8S rRNA, the degradation of CUTs and pre-mRNA surveillance

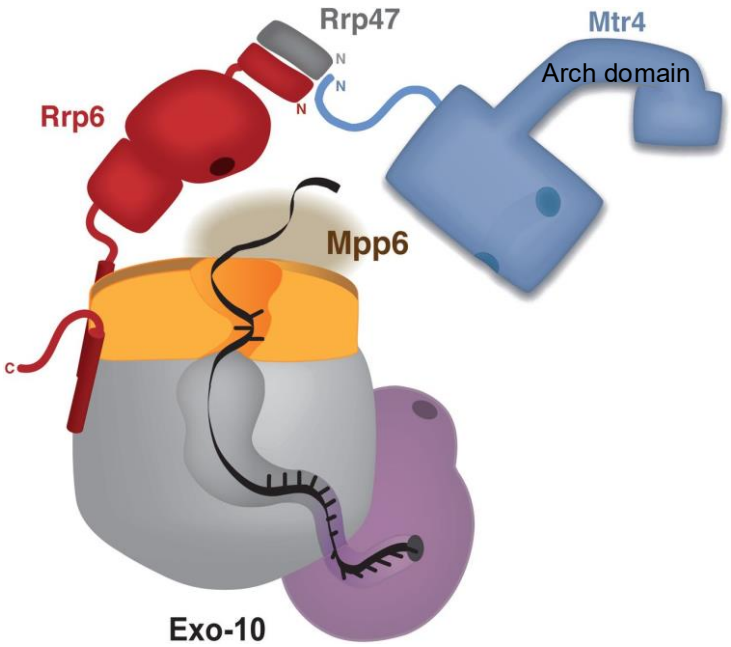
Dob1p/Mtr4p - DEAD box putative ATP-dependent RNA translocase (helicase)

Cytoplasmic exosome:

Ski2p - DEAD box putative ATP-dependent RNA translocase (helicase)

Ski7p - putative GTPase homologous to translation factors (e.g. EF-Tu and EF-G)

Cofactors for the exosome complexes



Nuclear exosome

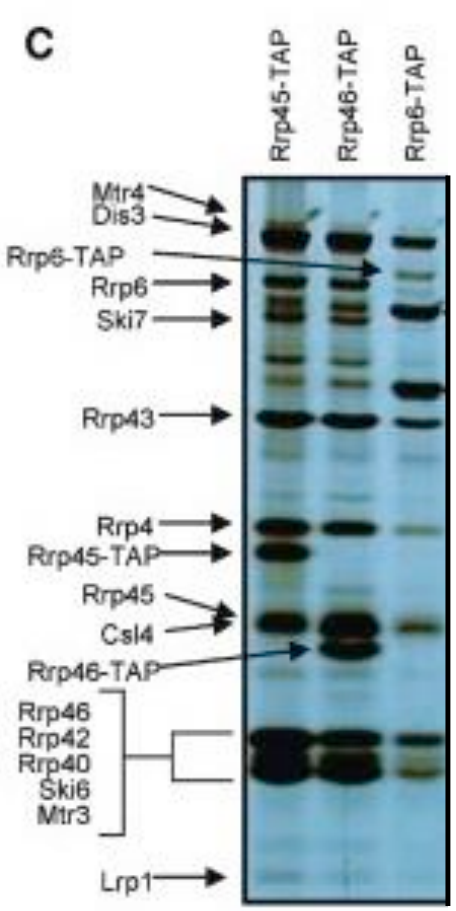
Dob1p/Mtr4p - DEAD box putative ATP-dependent RNA translocase (helicase)

Function:

- pre-rRNA processing
- pre-rRNA spacer degradation
- snRNA processing
- snoRNA processing
- pre-mRNA degradation
- degradation of pervasive transcription

Dob1p/Mtr4 phisically interacts with the exosome

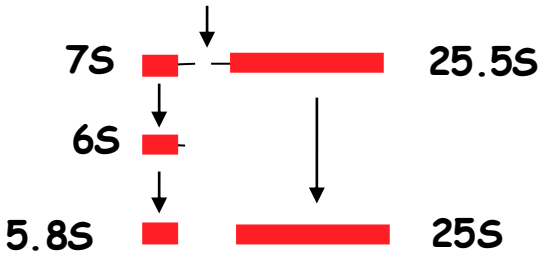
TAP-purification

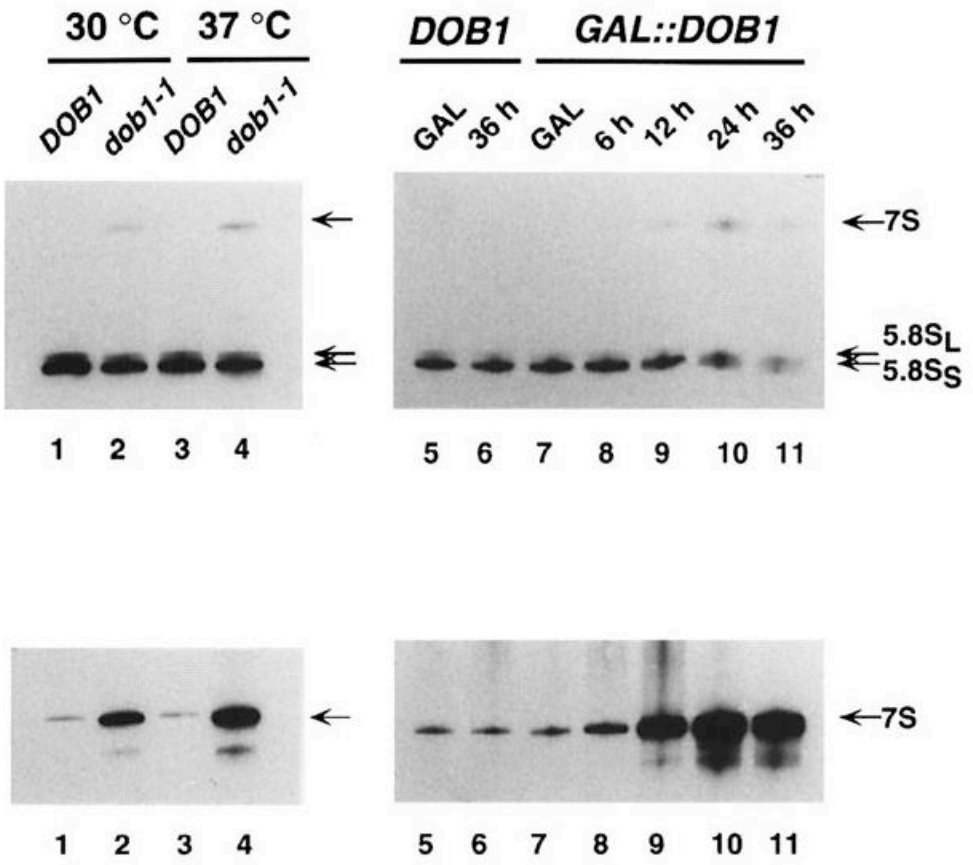


Dob1/Mtr4 is NOT sufficient to stimutate exosome activity *in vitro*

Cofactors for the exosome complexes

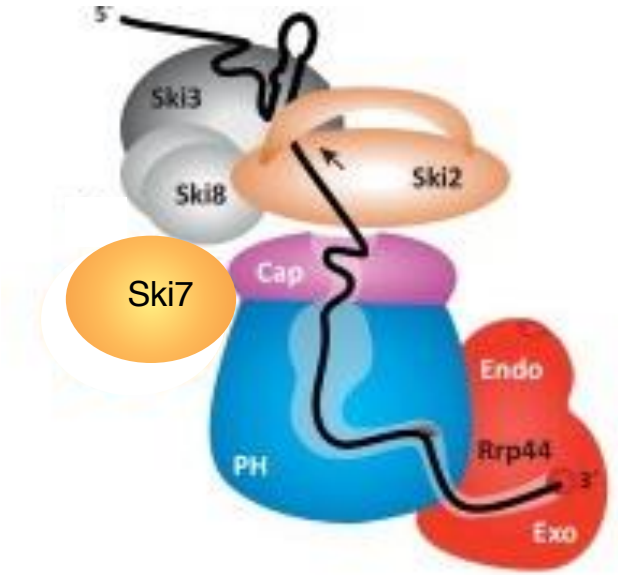
DOB1/MTR4: putative ATP-dependent RNA helicase





This defect resembles the phenotype described for rrp4-1

Cofactors for the exosome complexes



Cytoplasmic exosome

Ski2p - DEAD box putative ATP-dependent RNA translocase (helicase)

Ski7p - putative GTPase homologous to translation factors (e.g. EF-Tu and EF-G)

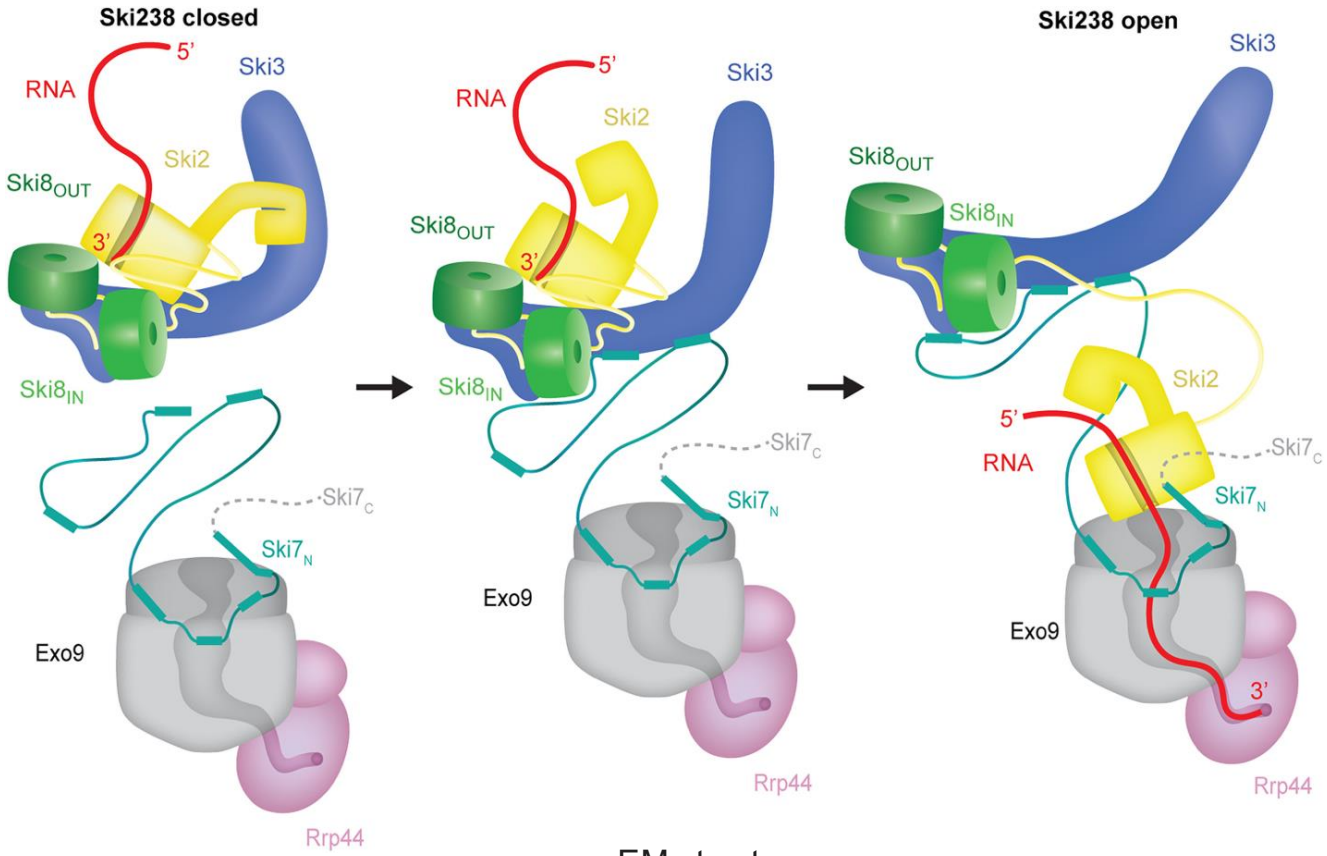
The Ski7 protein is recognized as the bridge between the exosome and the Ski2-Ski3-Ski8 complex in cytoplasmic mRNA decay pathways

Function:

•mRNA degradation

Although cytoplasmic Ski7 and nuclear Rrp6 share little to no sequence similarity, with only a short motif in common, they are recognized by the same surfaces of Exo9, notably in a mutually exclusive manner, rationalizing how cells prevent the cytoplasmic exosome complex (Exo11c) from being imported into the nucleus

Working model of the Ski2/3/8-Ski7-Exo10-mediated 3′-5' RNA decay in eukaryotes

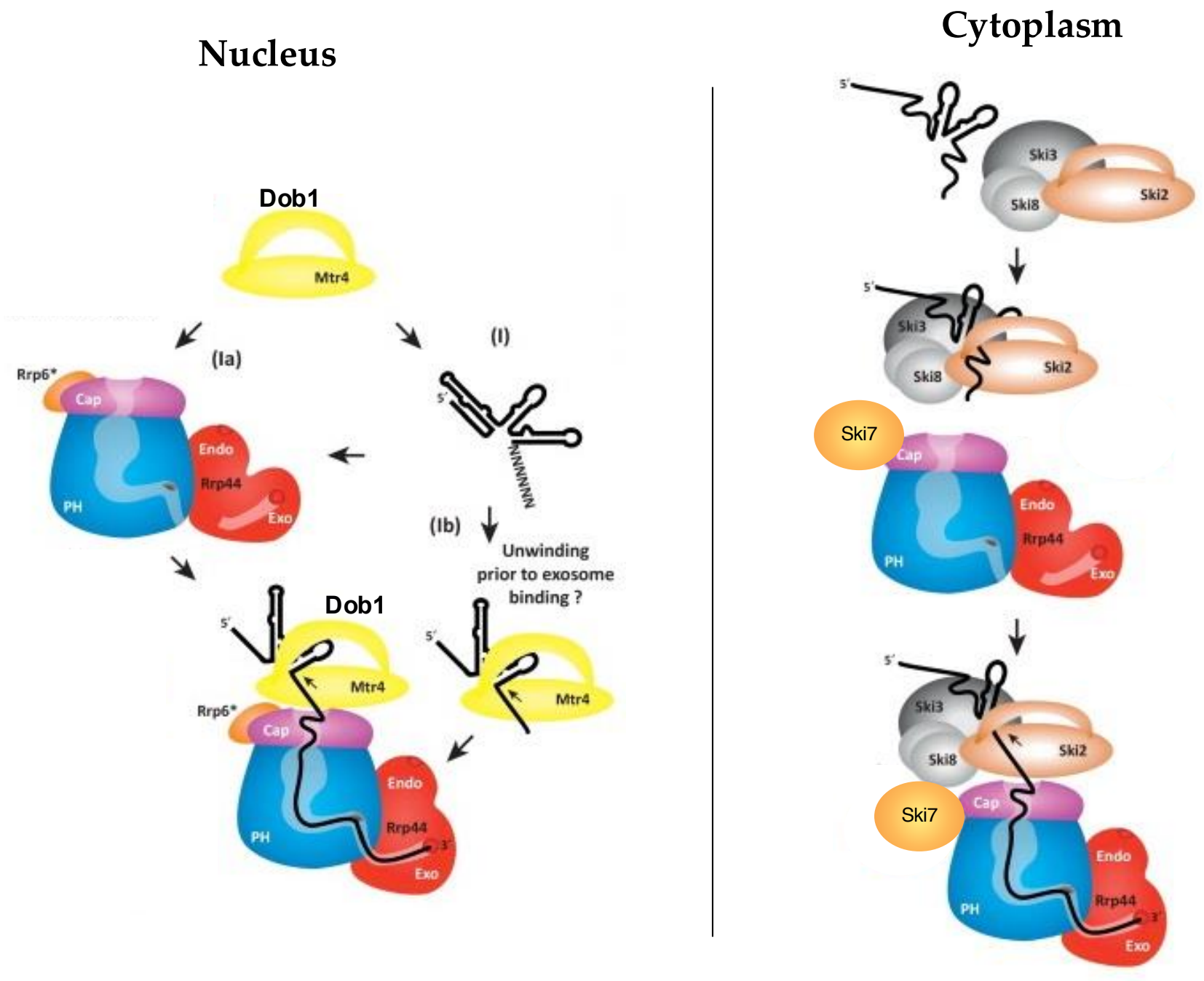


cryo-EM structures

Keidel et al. 2023. Mol Cell

The closed-state Ski2/3/8 can bind the 3′ end of RNA but must undergo conformational changes to the open state to interact with Ski7-Exo10 in order to form a continuous channel that may be traversed by RNA.

Cofactors for the exosome complexes



RNA Processing

Eukaryotic cells contain many different RNA species:

- **mRNA**
- **rRNA**
- **tRNA**
- **Small nuclear RNAs (snRNAs)**
- **Small nucleolar RNAs (snoRNAs)**
- **Signal recognition particle (SRP)**
- **RNase P and MRP**
- **Telomerase RNA**
- **microRNA and piRNA**
- **lncRNA**

- Almost all stable RNAs are generated by post-transcriptional processing

THIRD LAYER (Substrate Adaptors and Regulators of Helicases)

How can so few RNase activities adapt to so many classes of RNAs?

The Mtr4 and Ski2 helicases interact with adaptor proteins to regulate RNA delivery to the exosome exoribonuclease core. These adaptors serve as a hub that tunes helicase activity and substrate targeting for exosome-mediated RNA degradation.

Mtr4 associates with a variety ofadaptors, reflecting the diverse types of exosome substrates in the nucleus [e.g., rRNA, transfer RNAs (tRNAs), precursor mRNAs (pre-mRNAs), and noncoding RNAs].

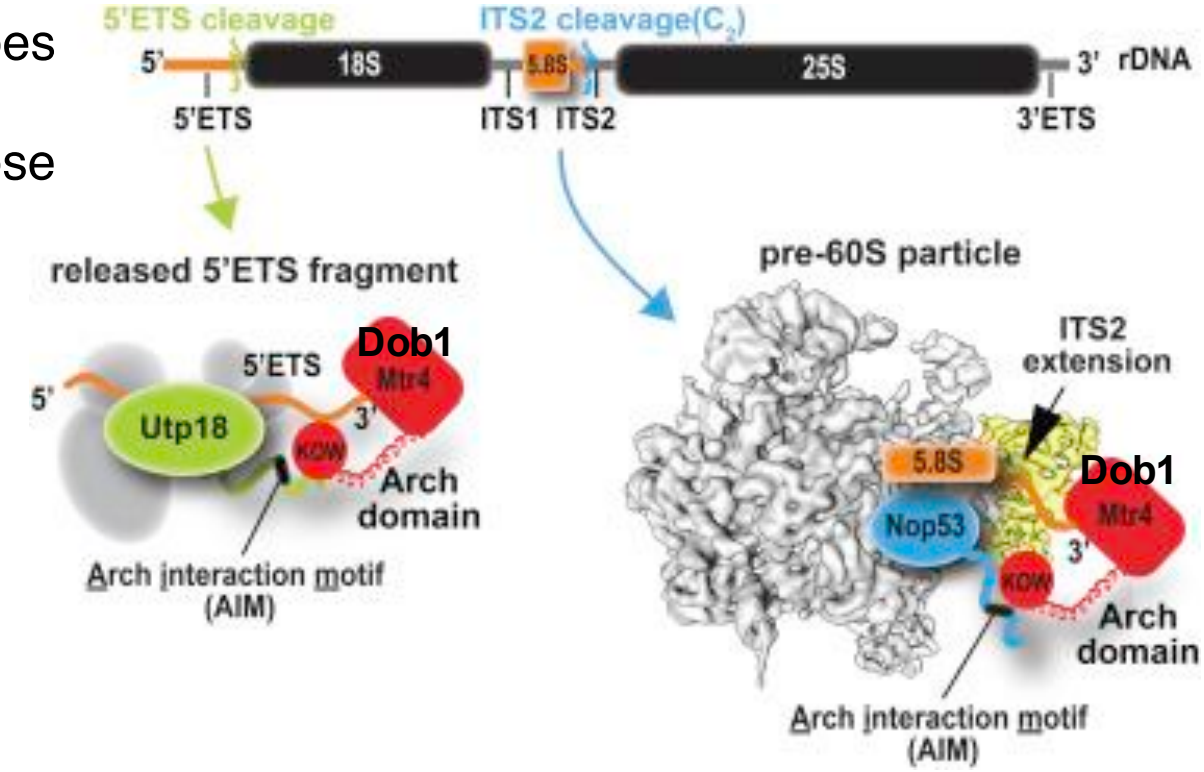
In contrast, Ski2is embedded in a single complex in the cytoplasm, Ski2-Ski3-SKI8, the presence of a predominant exosome substrate in this subcellular compartment (i.e., mRNA).

Structural studies on the helicase module of yeast Mtr4 and Ski2 first revealed a conserved architectural organization with a DExH catalytic core and a regulatory arch domain which can adopt different conformations

The Exosome Is Recruited to RNA Substrates through Specific Adaptor Proteins

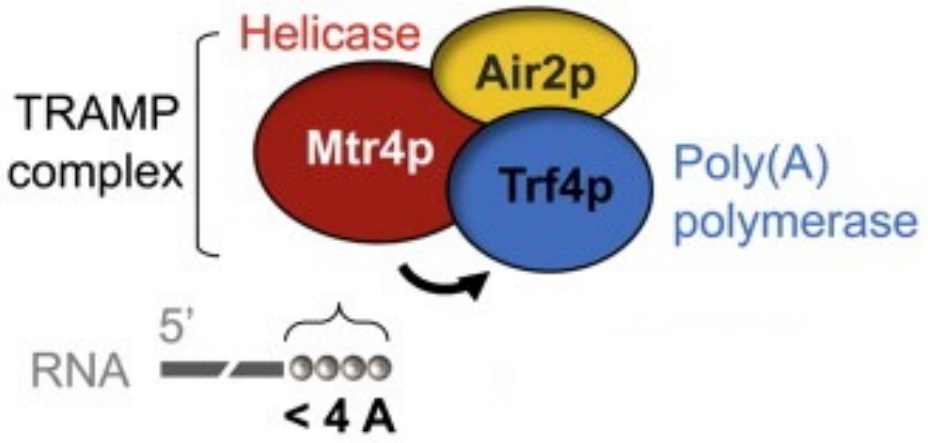
Nop53, the yeast homolog of the tumor suppressor **PICT1**, targets Mtr4/Dob1 to pre-ribosomal particles for exosome-mediated processing, while a second adaptor **Utp18** recruits Mtr4/Dob1 to cleaved rRNA fragments destined for degradation by the exosome. Both Nop53 and Utp18 contain the same consensus motif, through which they dock to the "arch" domain of Mtr4 and target it to specific substrates

The simplest types of Mtr4-binding adaptors are those containing short arch-interacting motifs (AIMs)



Thoms et al., 2015 Cell

The Trf4p/Air2p/Mtr4p polyadenylation complex (TRAMP)



Substrates: tRNAs, snRNAs, snoRNA, CUTs

In human cells, the orthologous TRAMPcomplex (hMTR4-PAPD5-ZCCHC7) is exclusively localizedin the nucleolus and involved in rRNA processing under normal cellular conditions (Fasken

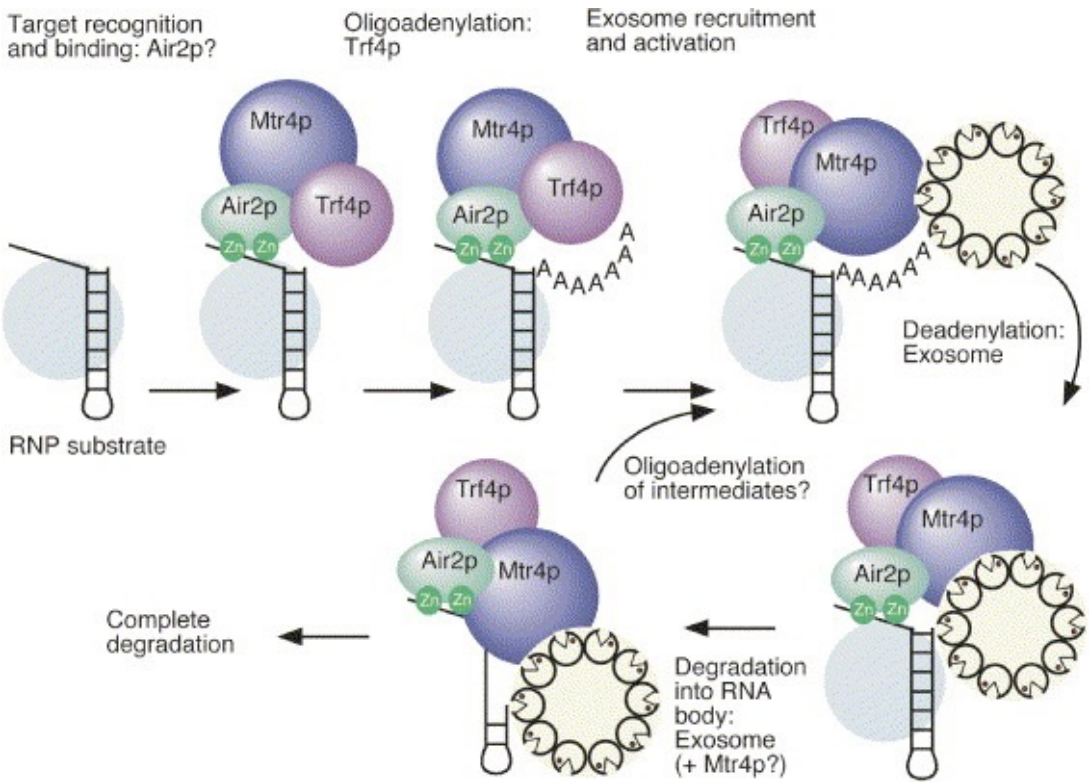
Roles of the TRAMP Complex in RNA Degradation

- 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.

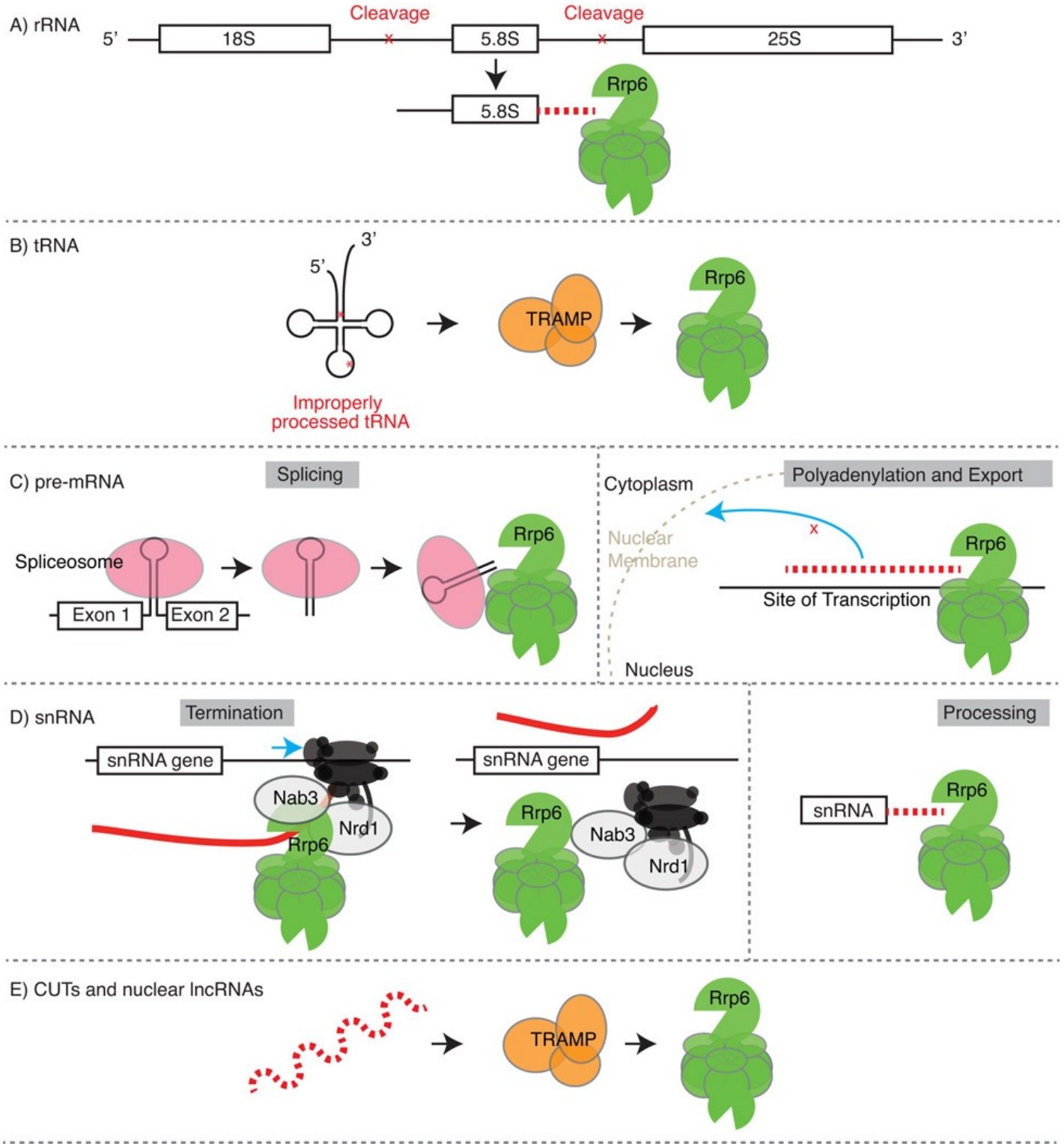
-The zinc finger domains of Air2p is involved in substrate binding. The RNA is then polyadenylated by Trf4p.

- 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.

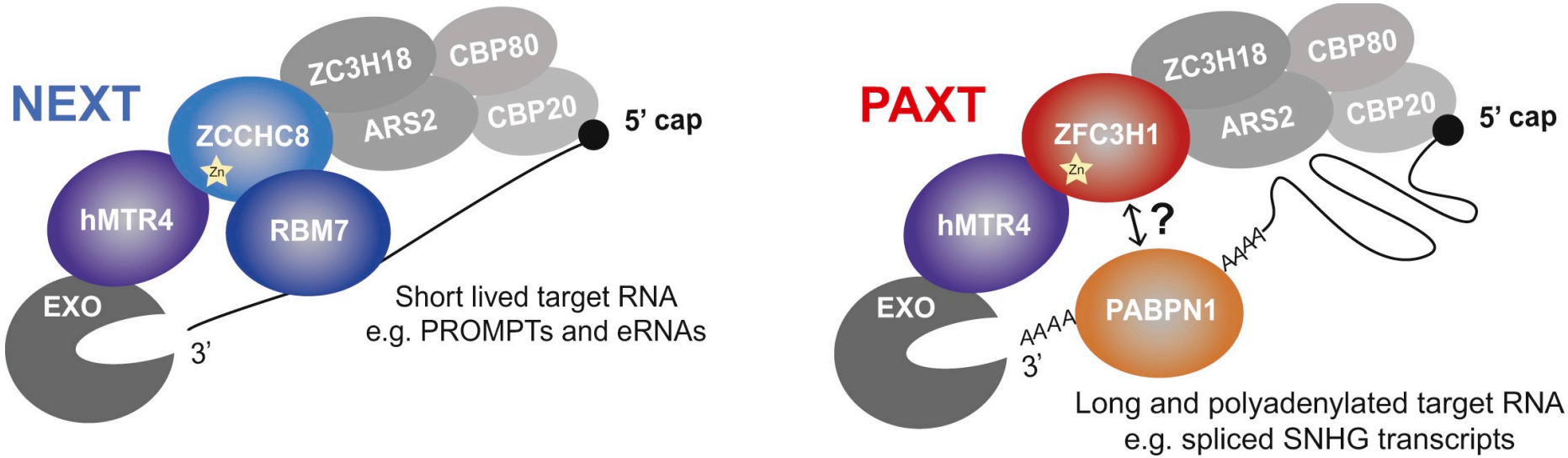
- Helicase activity of Mtr4p is important for dissociation or remodeling of stable RNP structures to allow passage of the exosome.



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

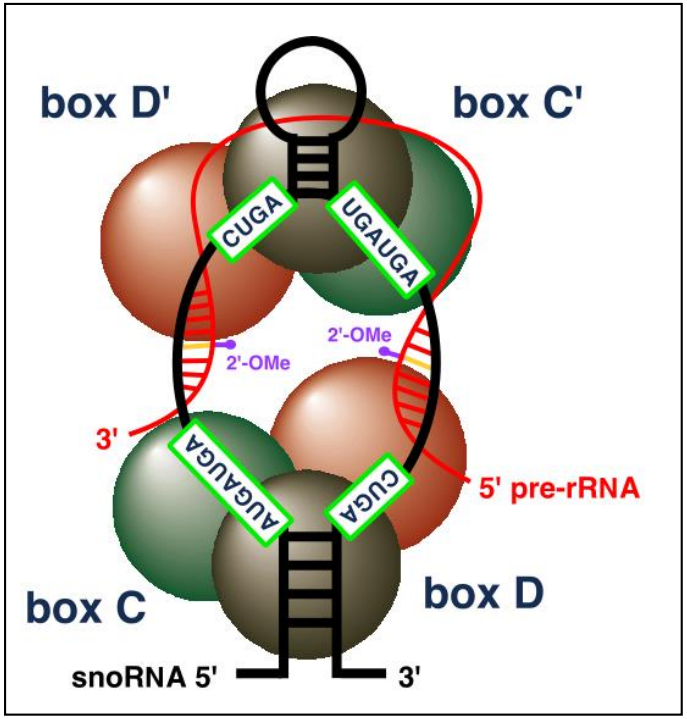

A) rRNA
5'
18S
Cleavage
5.8S
Cleavage
25S
3'
5.8S
Rrp6
B) tRNA
3'
5'
Improperly processed tRNA
TRAMP
Rrp6
C) pre-mRNA
Splicing
Spliceosome
Exon 1
Exon 2
Rrp6
Cytoplasm
Polyadenylation and Export
Nuclear Membrane
Rrp6
Site of Transcription
Nucleus
D) snRNA
Termination
snRNA gene
Nab3
Nrd1
Rrp6
snRNA gene
Rrp6
Nab3
Nrd1
Processing
snRNA
Rrp6
E) CUTs and nuclear lncRNAs
TRAMP
Rrp6

In human cells, additional hMTR4 adaptor complexes have been identified and exhibit an even greater level of complexity, with large multidomain scaffold proteins that serve as platforms for diverse interactions.
Among them, the **NEXT (nuclear exosome targeting**) complex is a key nuclear exosome adaptor that mediates the degradation of nonpolyadenylated (pA-) RNAs from spurious transcription, of snoRNA precursors, of telomerase RNA during maturation



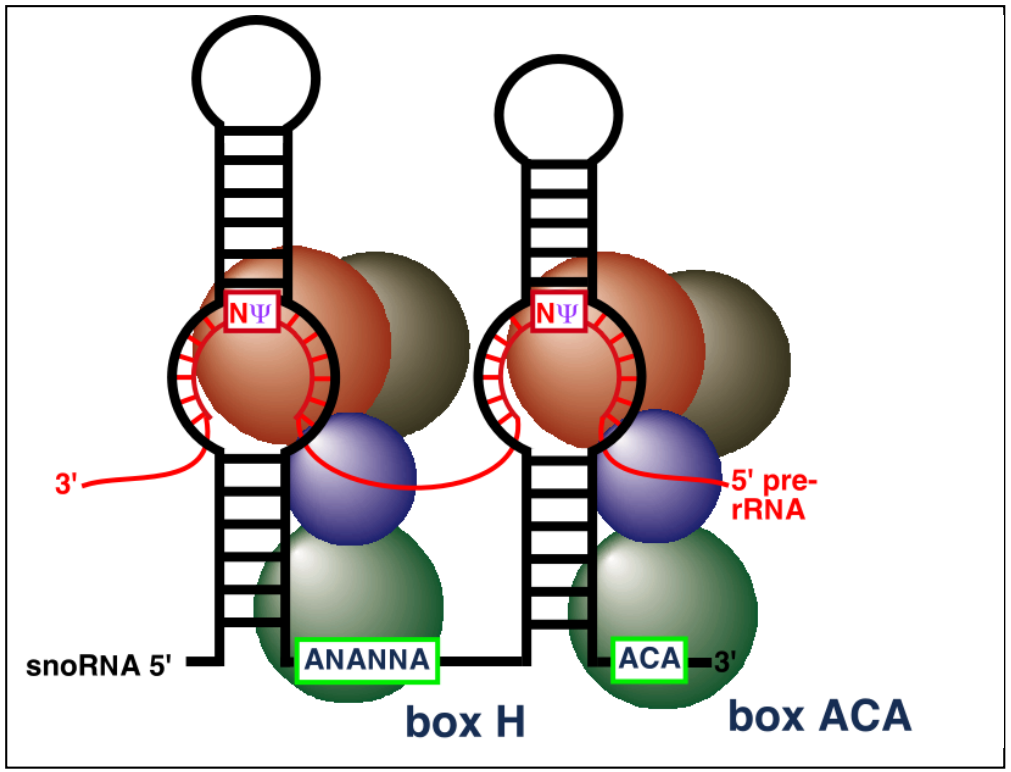
The NEXT complex functions in synergy with **PAXT (pA-tail-exosome-targeting),** which preferentially directs longer and polyadenylated (pAC) Pol II transcripts to the exosome for degradation, thereby preventing RNAs arising from cryptic transcription events from reaching the cytoplasm.

Box C + D snoRNAs
Direct 2'-O-Methylation



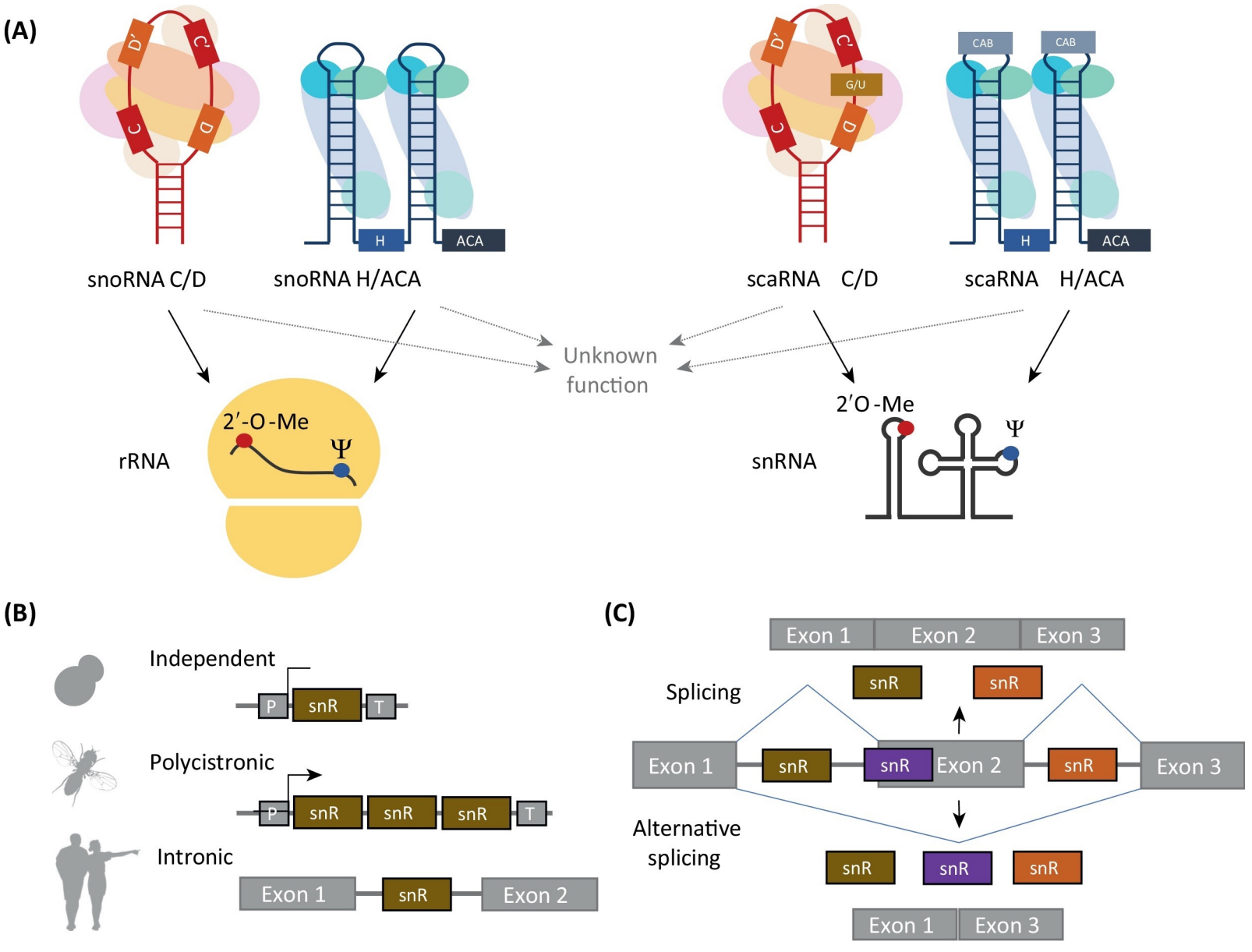
Nop1p/Fibrillarin
Nop56p
Nop58p
Snu13p

Box H + ACA snoRNAs
Direct Ψ Formation



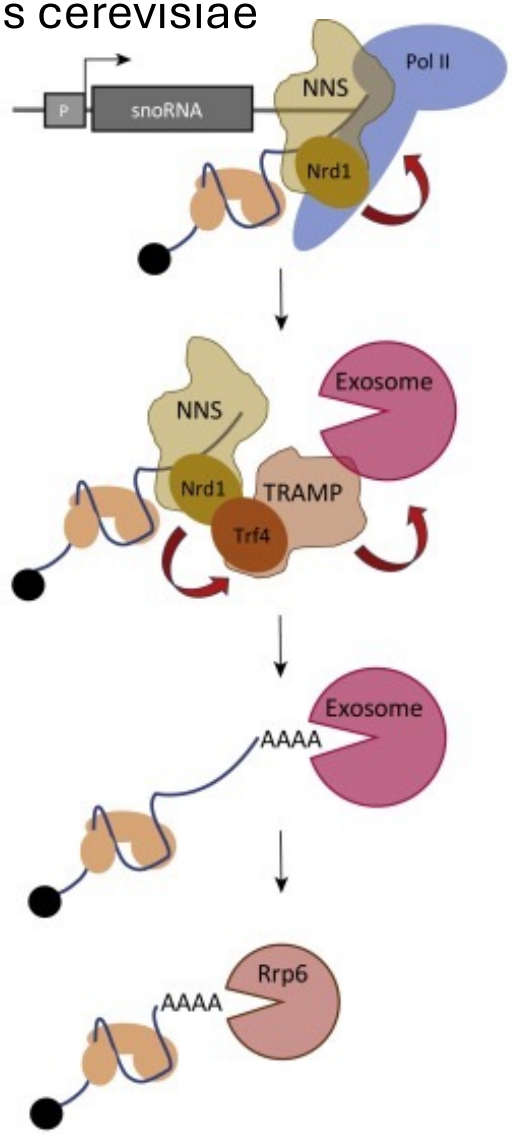
Cbf5p/Dyskerin
Nhp2p
Gar1p
Nop10p

Classes and Genomic Organisation of Small Nucleolar RNAs (snoRNAs)



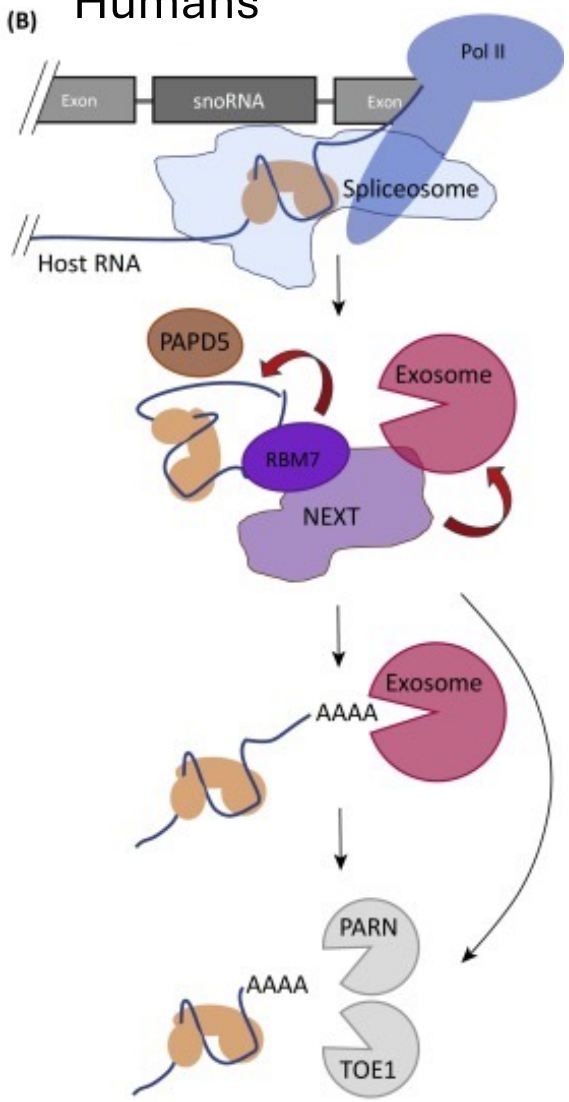
Trends in Genetics

Saccaromyces cerevisiae



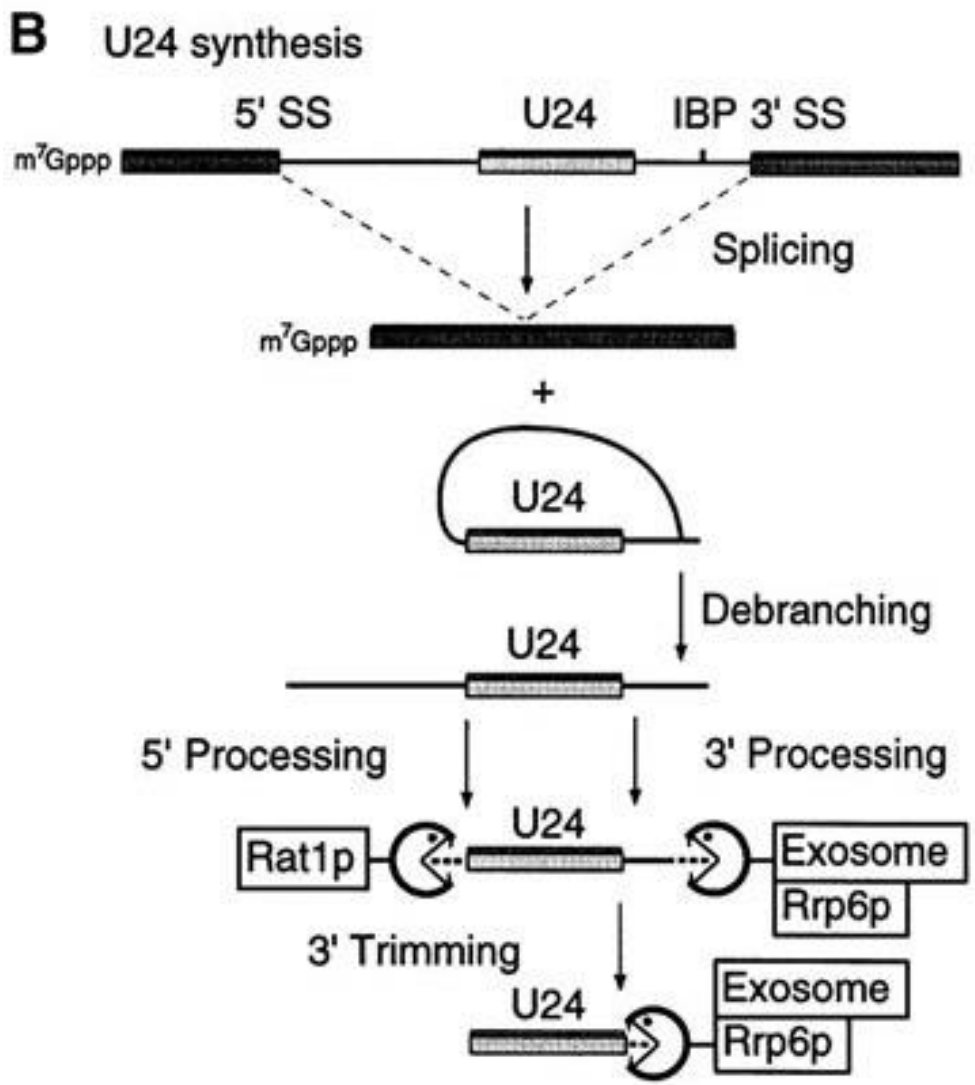
The Nrd1-Nab3-Sen1 (NNS) complex terminates transcritpion and the pre-snoRNA is bound by NNS. Nrd1, through interaction Trf4, recruits the TRAMP complex, which in turn recruits the nuclear exosome. The exosome removes 3′ extensions in rounds of subsequent oligoadenylation and exonucleolytic trimming. The last nucleotides are digested by the exosome cofactor exonuclease Rrp6.

(B) Humans



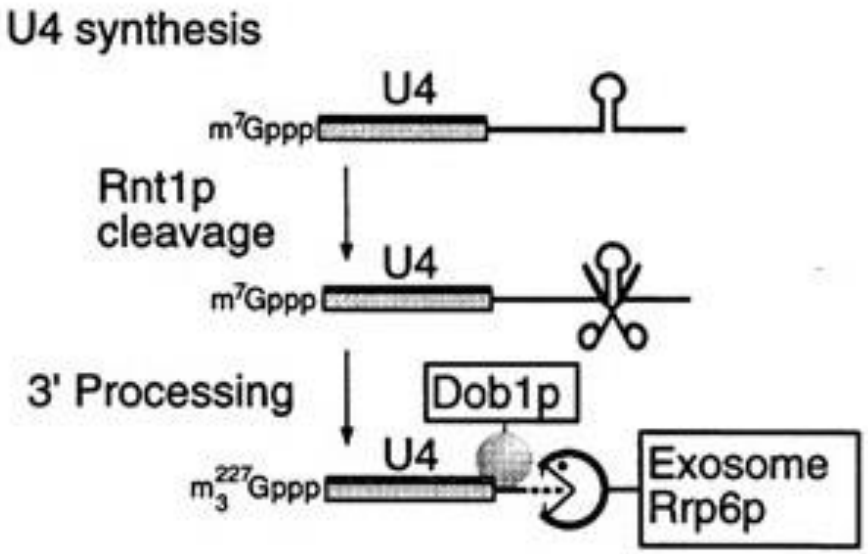
The exosome is recruited to intron-encoded snoRNA via the NEXT component RBM7. Trimming of the 3′ end is most likely performed by a combined action of the poly(A) polymerase PAPD5, exosome, and deadenylases PARN and TOE1

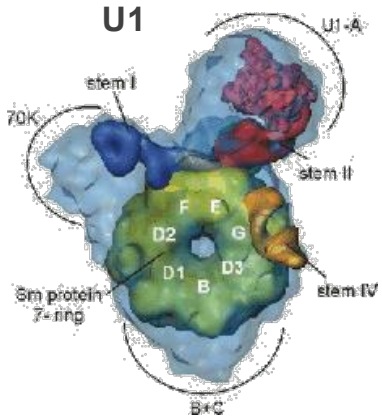
Function of the exosome in snoRNA synthesis

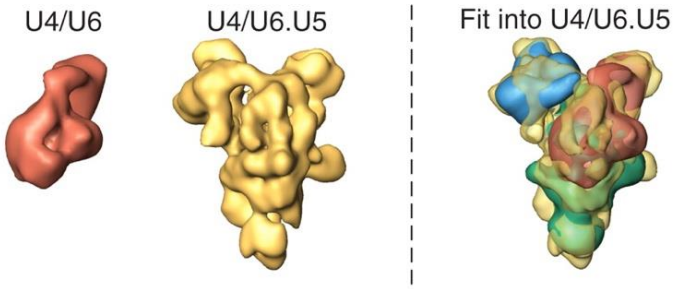


Exosome and Rrp6 recruitment mode to these precursors has not been described.

Function of the exosome in snRNA synthesis

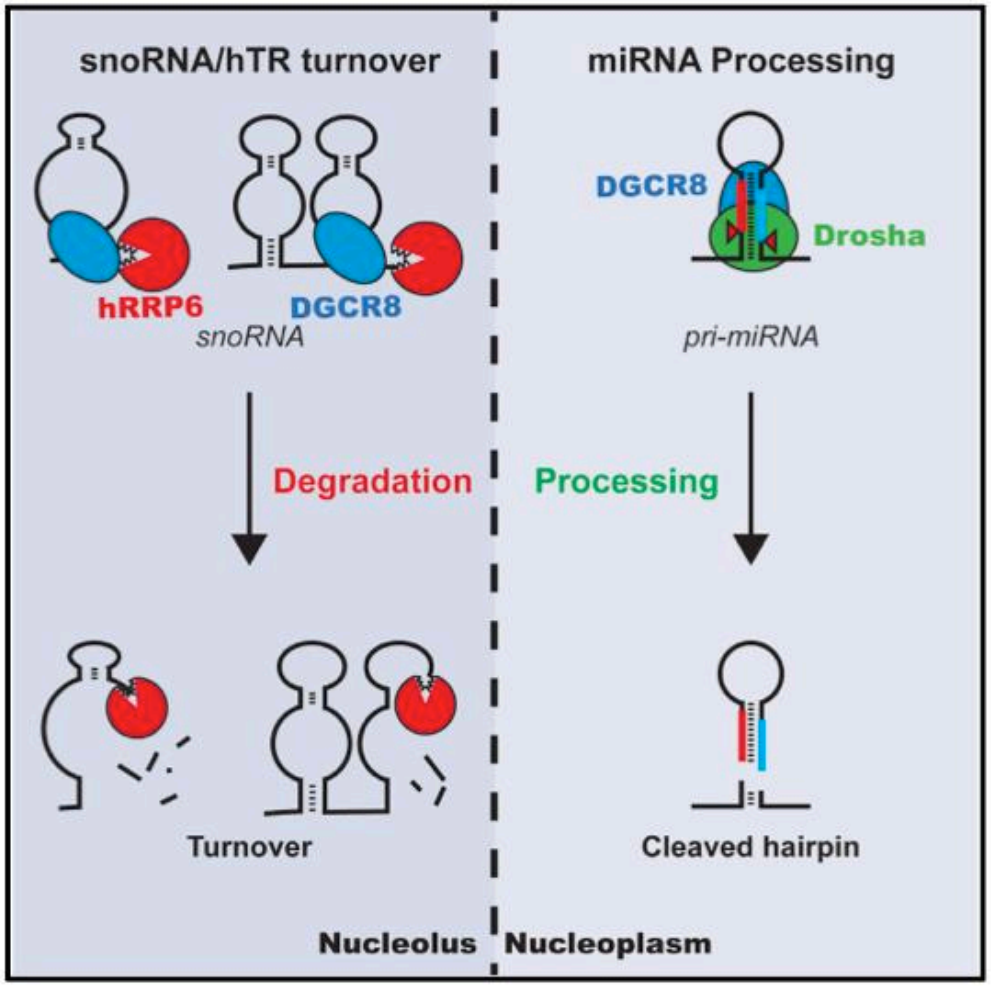






The Exosome Is Recruited to RNA Substrates through Specific Adaptor Proteins

- DGCR8 forms an alternative complex with the hRRP6-containing form of the exosome.
- DGCR8 acts as an adaptor to recruit the exosome to target structured RNAs.
- The DGCR8/hRRP6 complex also controls the stability of human telomerase RNA.



Macias et al., 2015. Mol Cell