RNA Processing

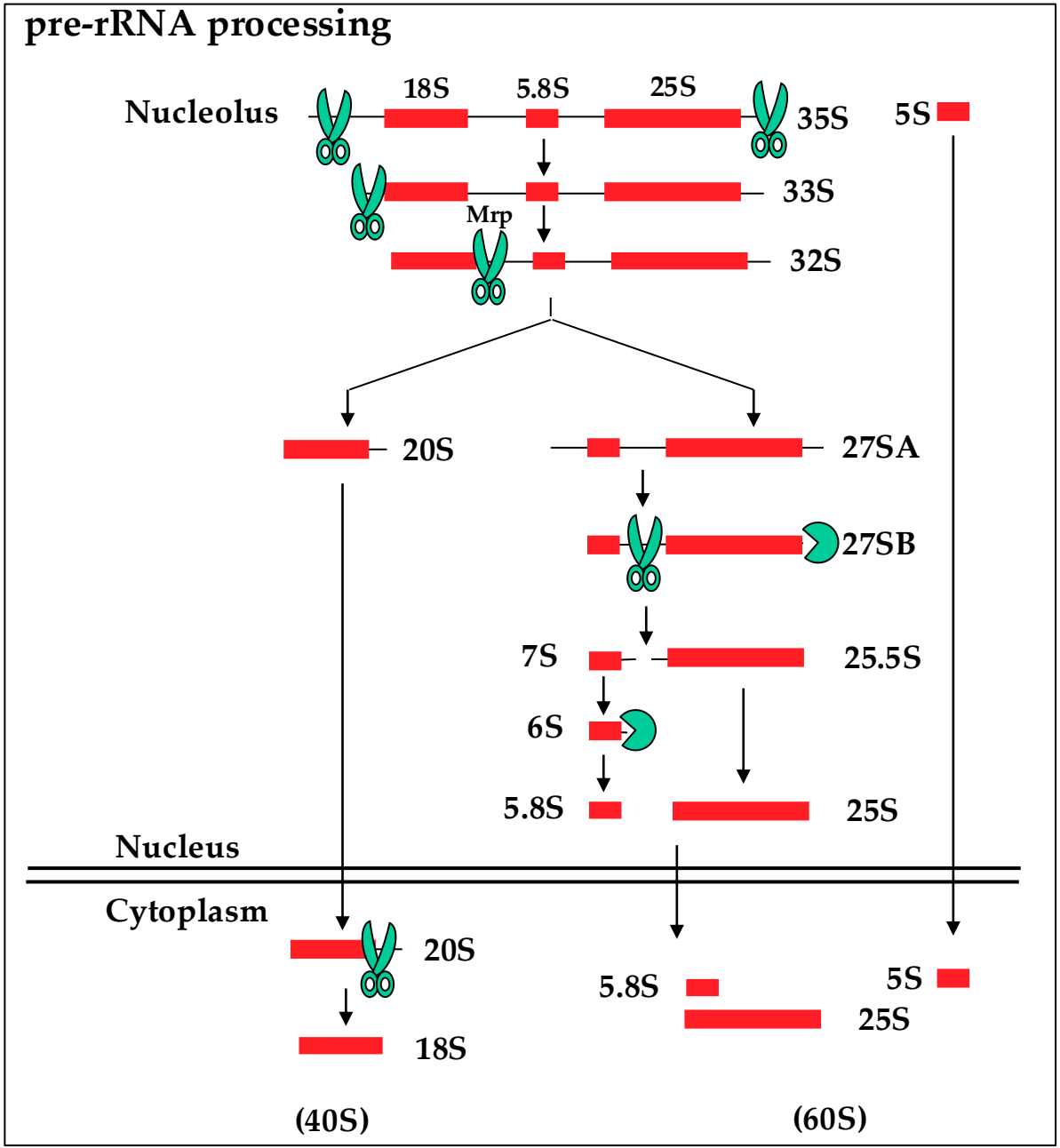
RNA Processing

Eukaryotic cells contain many different RNA species:

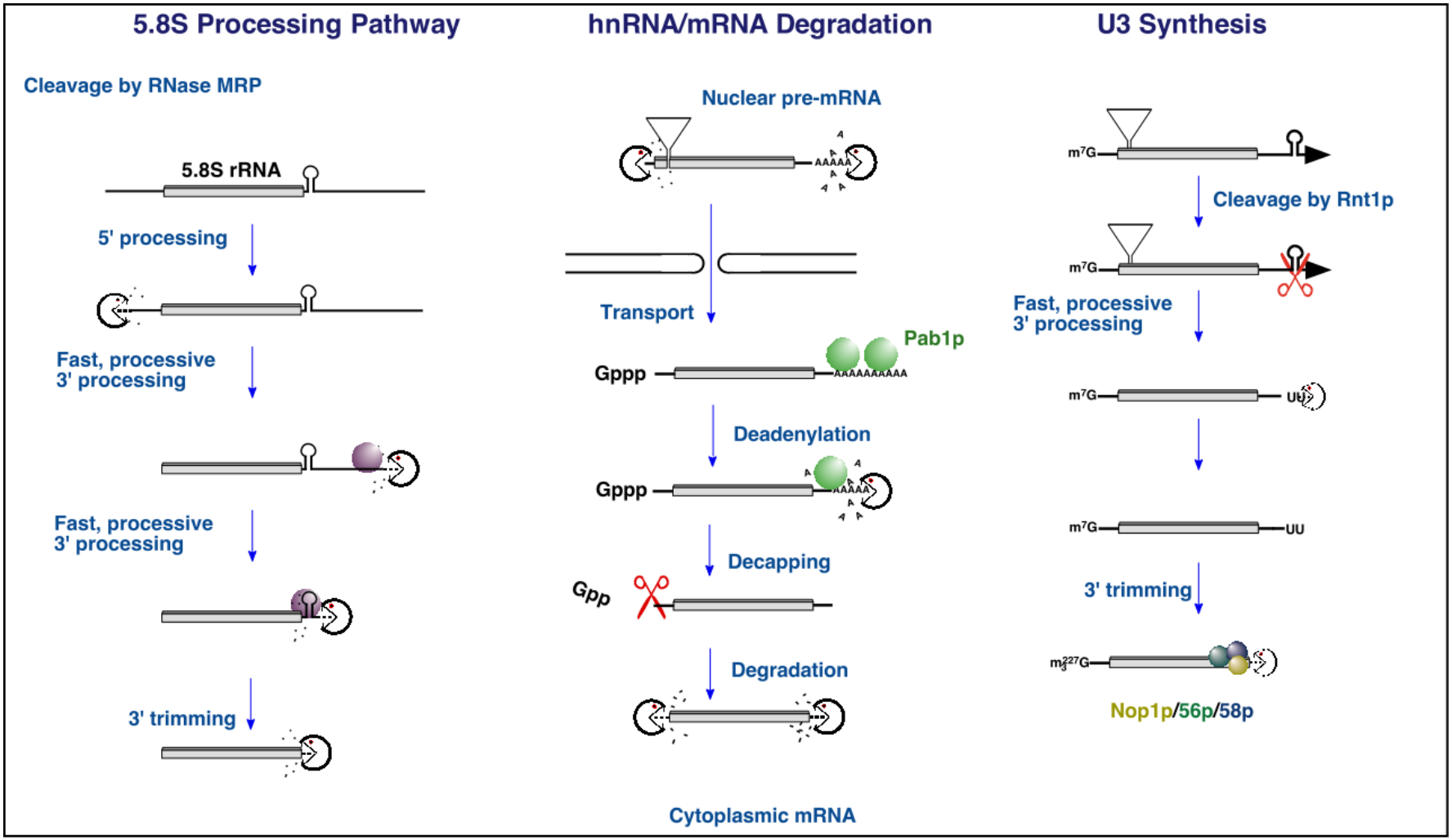
- **mRNA**
- **rRNA**
- **tRNA**
- **Small nuclear RNAs (snRNAs)**
- **Small nucleolar RNAs (snoRNAs)**
- **Signal recognition particle (SRP)**
- **RNase P and MRP**
- **Telomerase RNA**
- **microRNA and piRNA**
- **lncRNA**

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

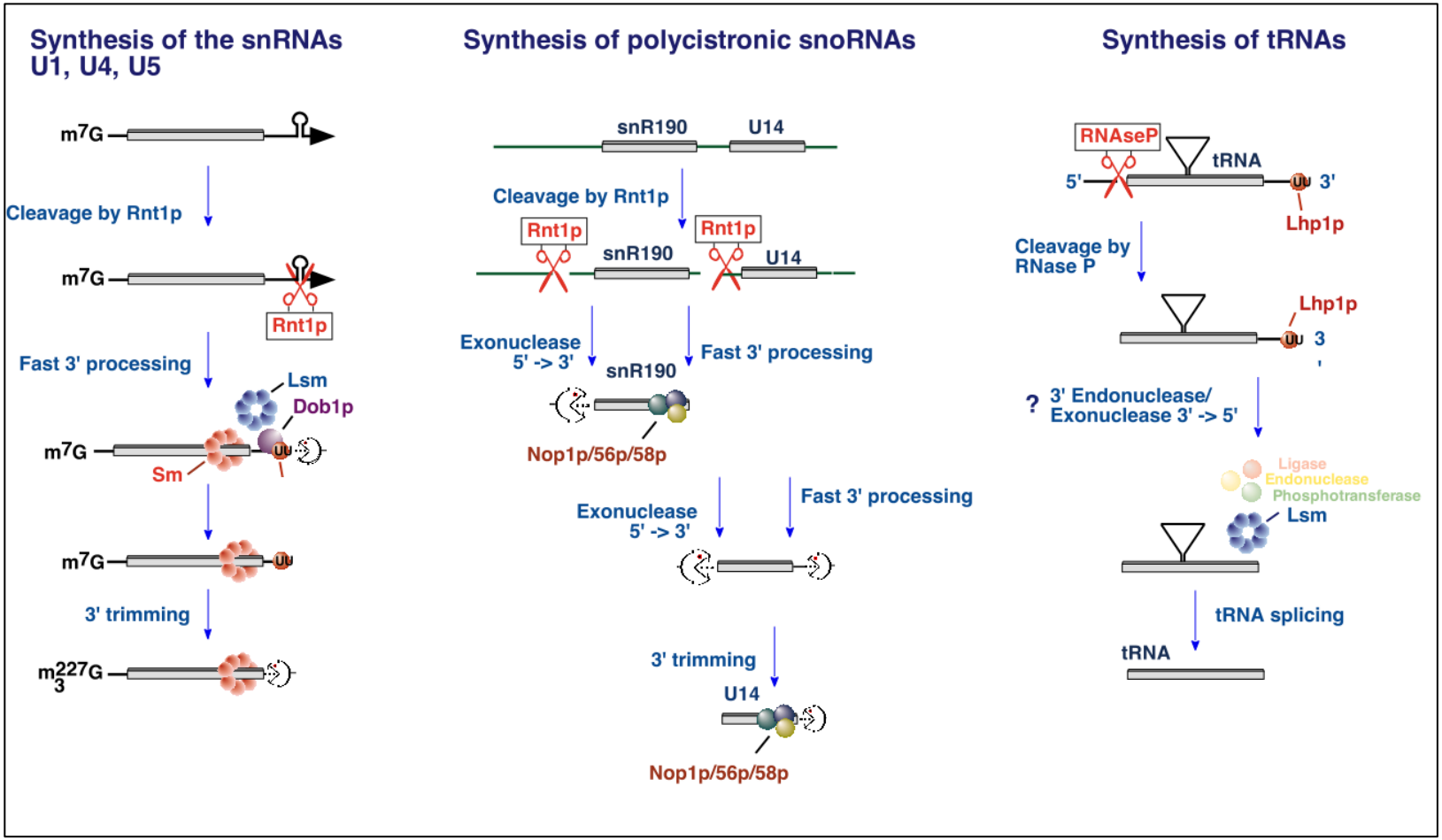
RNA Processing/degradation



RNA Processing/degradation



RNA Processing/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.

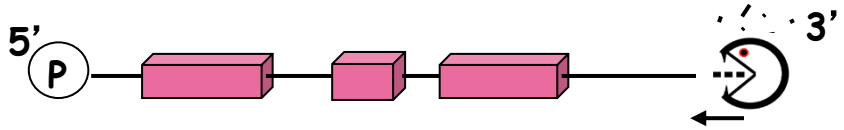
•In fact, it does not appear that eukaryotic RNA processing is organized in this way.

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

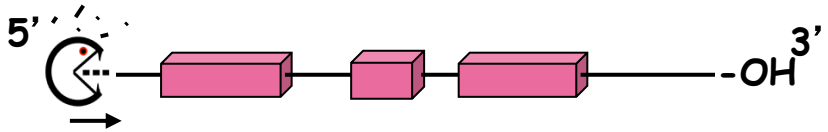
•These are recruited in different combinations to many different substrates.

RNA maturation and/or degradation Enzymes

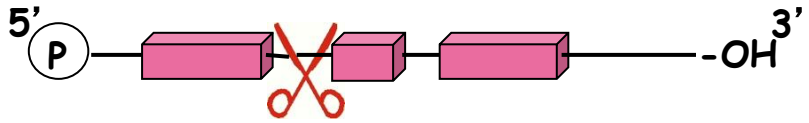
- 3’ ⟶ 5’ exoribonucleases:



- 5’ ⟶ 3’ exoribonucleases:



- Endonucleases:

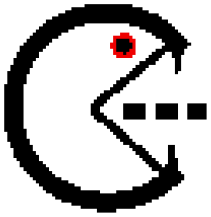


Yeast 5’ ->3’ exonucleases



Rat1p (Xrn2) Predominately nuclear

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

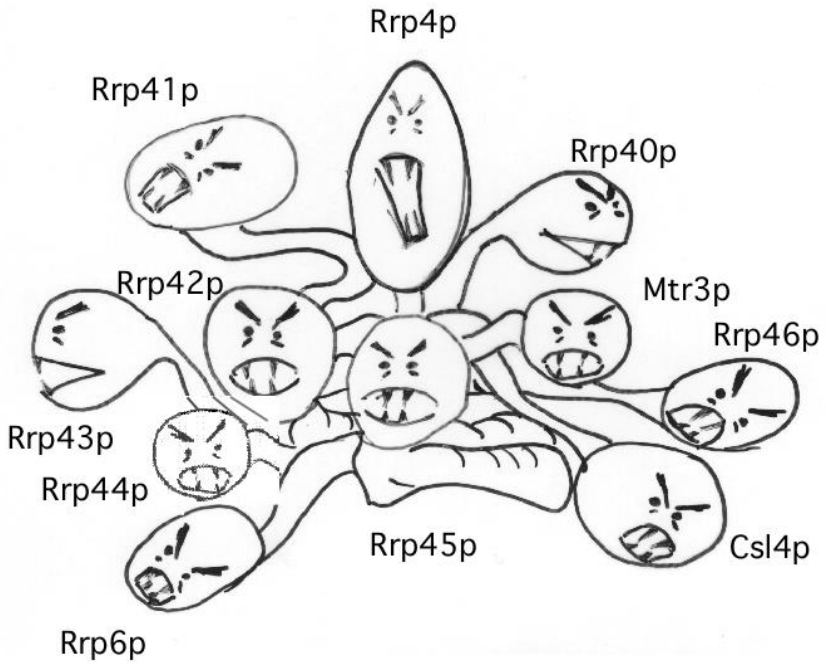


Xrn1p Predominately cytoplasmic

mRNA degradation

Yeast 3’ ->5’ exonucleases

The exosome complex



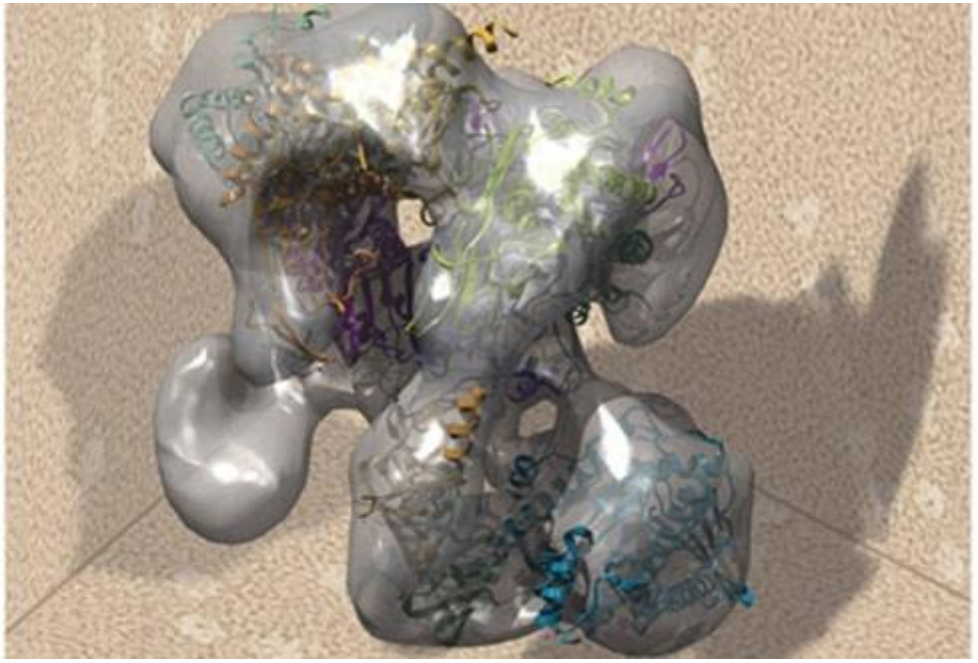
- pre-rRNA processing
- pre-rRNA spacer degradation
- snRNA processing
- snoRNA processing
- pre-mRNA degradation
- mRNA deadenylation
- mRNA degradation
- pervasive transcription

Endonucleases

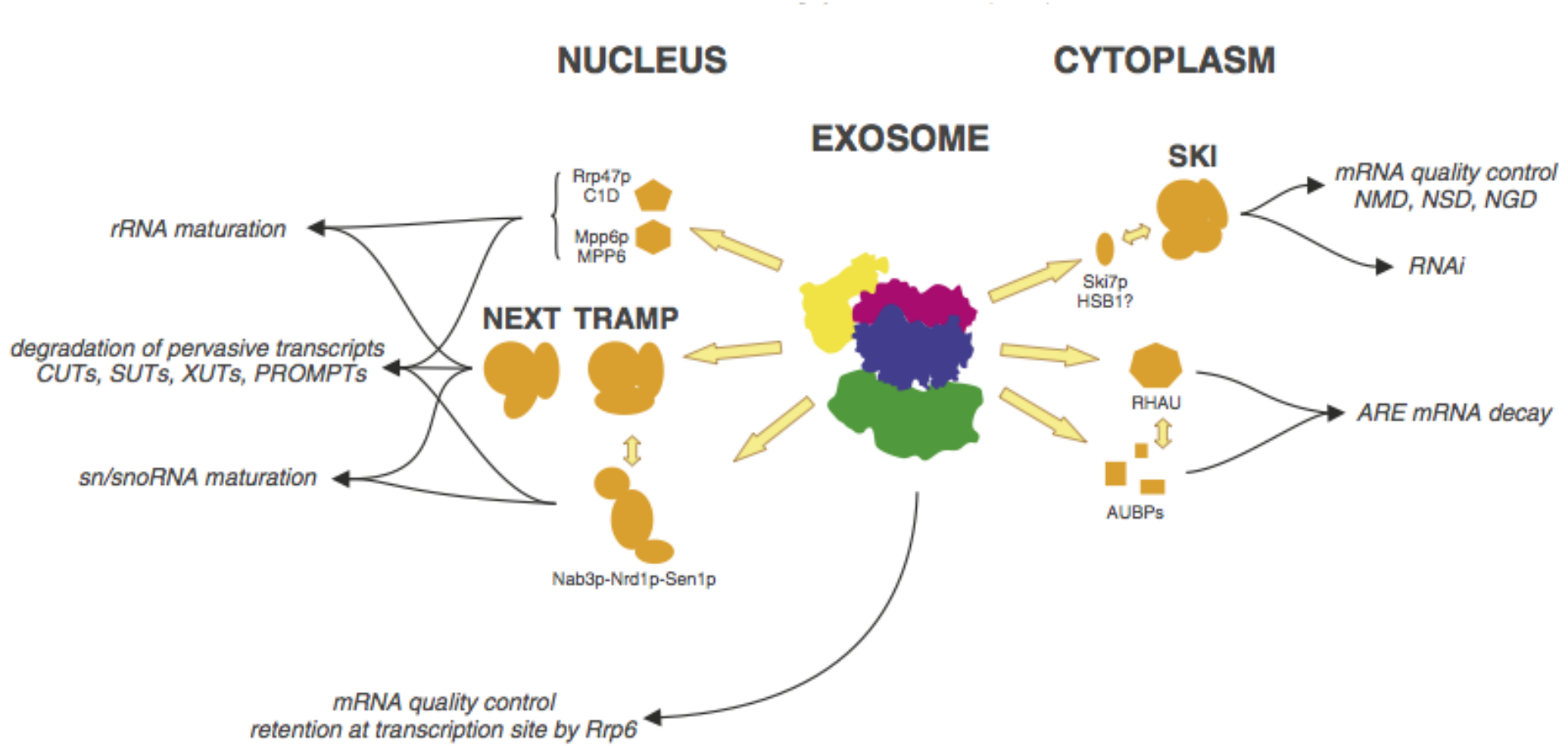
Yeast	Vertebrates	*Drosophila*	Plants	
Dis3/Rrp44	DIS3/RRP44	Dis3	Dis3	PIN CSD1 CSD2 RNB S1 — RNA process./degrad
Swt1	SWT1	Swt1	Swt1	PIN — nuclear mRNA quality control
Ysh1	CPSF-73	CPSF-73	CPSF-73	β-lac β-casp — 3'-end mRNA process.
Trz1	tRNase Z/ELAC	tRNase Z	tRNase Z	β-lac β-lac — RNA process.
-	XendoU	XendoU	XendoU	EndoU — snoRNA process.
-	SMG6	Smg6	Smg6	PIN — mRNA quality control
Nob1	NOB1	Nob1	Nob1	PIN — pre-rRNA processing
Utp24	UTP24	Utp24	Utp24	PIN — pre-rRNA processing
Rnt1/RNase III	RNase III (Drosha)	RNase III (Drosha)	RNase III	EndoND dsRBD — RNA processing
-	Dicer	Dicer	Dicer	DExD DExD 283 PAZ EndoND EndoND dsRBD
-	AGO	Ago	Ago	N PAZ MID PIWI — miRNA process./function
-	C3PO/TRAX	C3PO/Trax	C3PO/Trax	TRAX — siRNA processing
-	-	-	RNase E	S1 S1 5'sen H DNase I Small — RNA degradation (Chlor.)
-	-	-	Csp41	SDR — RNA degradation (Chlor.)

Domain description: The PIN is a domain of potential endoribonuclease activity which shares common fold with FLAP nuclease, RNase H, and Fen1 endonuclease. RNB is a catalytic domain of the RNAse II/R family of processive exoribonucleases. The S1 is an ancient nucleic acid binding domain; in the case of Dis3 proteins S1 domains are involved in substrate recruitment. The β-lac is a metallo-β-lactamase-fold endonuclease catalytic domain. The β-CASP is a noncatalytic domain associated with metallo-β-lactamase-fold endonucleases. The EndoU is a unique fold endonuclease catalytic domain. The dsRBD is a double-stranded RNA binding domain. The EndoND is a catalytic domain of the RNAse III superfamily of endonucleases. The DExD are domains specific for RNA helicases and RNA-dependent ATPases. The PAZ domain is responsible for binding to the 3' end of the dsRNA. The PIWI are RNase H-like domains responsible for Argonaute slicing activity. The MID domain forms a binding pocket for 5'phosphate. The Trax is an α-helical domain active as an endonuclease which forms a heterodimer with translin. The 5' sen is a domain specific for RNase E family members, responsible for the recognition of the 5' end of RNA. The DNase I is a catalytic domain of RNase E. Small is a domain with structural function, specific for RNase E proteins. The SDR domain of Csp41 chloroplast endoribonuclease belongs to the Rossmann-fold short-chain dehydrogenase/reductase superfamily.

The Exosome complex



Exosome's cellular functions and cofactors



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

nature genetics

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]

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

nature COMMUNICATIONS

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]

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

nature genetics

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]

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

THE EMBO JOURNAL

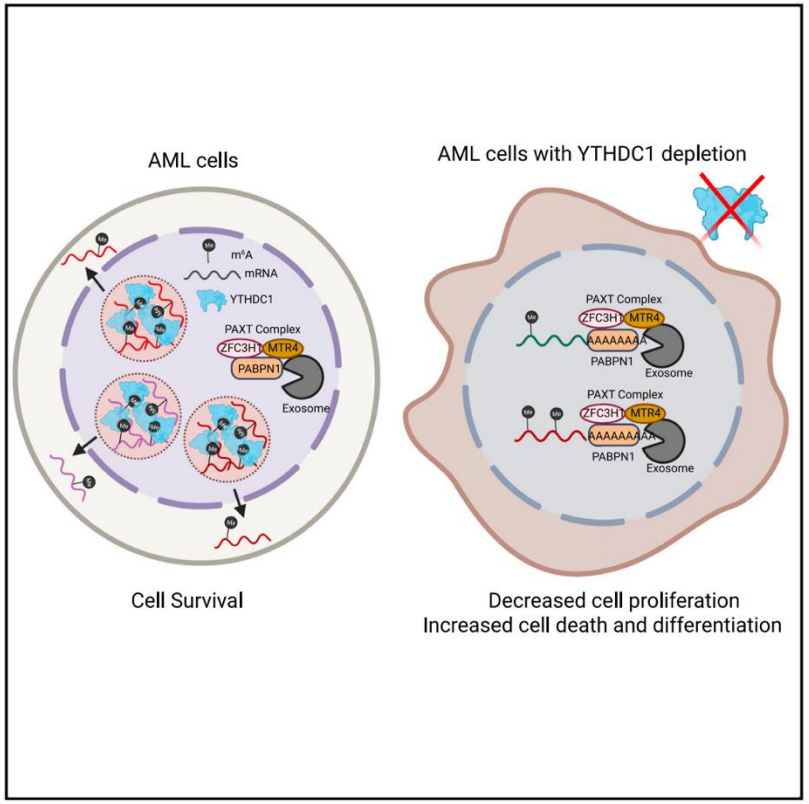
Yuya Kawabe, Kohji Mori*, Tomoko Yamashita, Shiho Gotoh & Manabu Ikeda

Article

Cancer Cell

N^6-Methyladenosine on mRNA facilitates a phase-separated nuclear body that suppresses myeloid leukemic differentiation

Graphical abstract



Authors

Yuanming Cheng, Wei Xie, Brian F. Pickering, ..., Dinshaw J. Patel, Samie R. Jaffrey, Michael G. Kharas

Correspondence

kharasm@mskcc.org

In brief

Using AML cell lines and patient samples Cheng et al. identify a requirement for YTHDC1 in myeloid leukemogenesis. YTHDC1 undergoes liquid-liquid phase separation by binding to m6A to form dynamic nuclear condensates. These nuclear bodies are increased in myeloid leukemia cells and protect mRNAs from the PAXT-exosome complex.

nature immunology

The SKIV2L RNA exosome limits activation of the RIG-I-like receptors

Sterling C Eckard[1], Gillian I Rice[2], Alexandre Fabre[3,4], Catherine Badens[3,5], Elizabeth E Gray[1], Jane L Hartley[6], Yanick J Crow[2] & Daniel B Stetson[1]

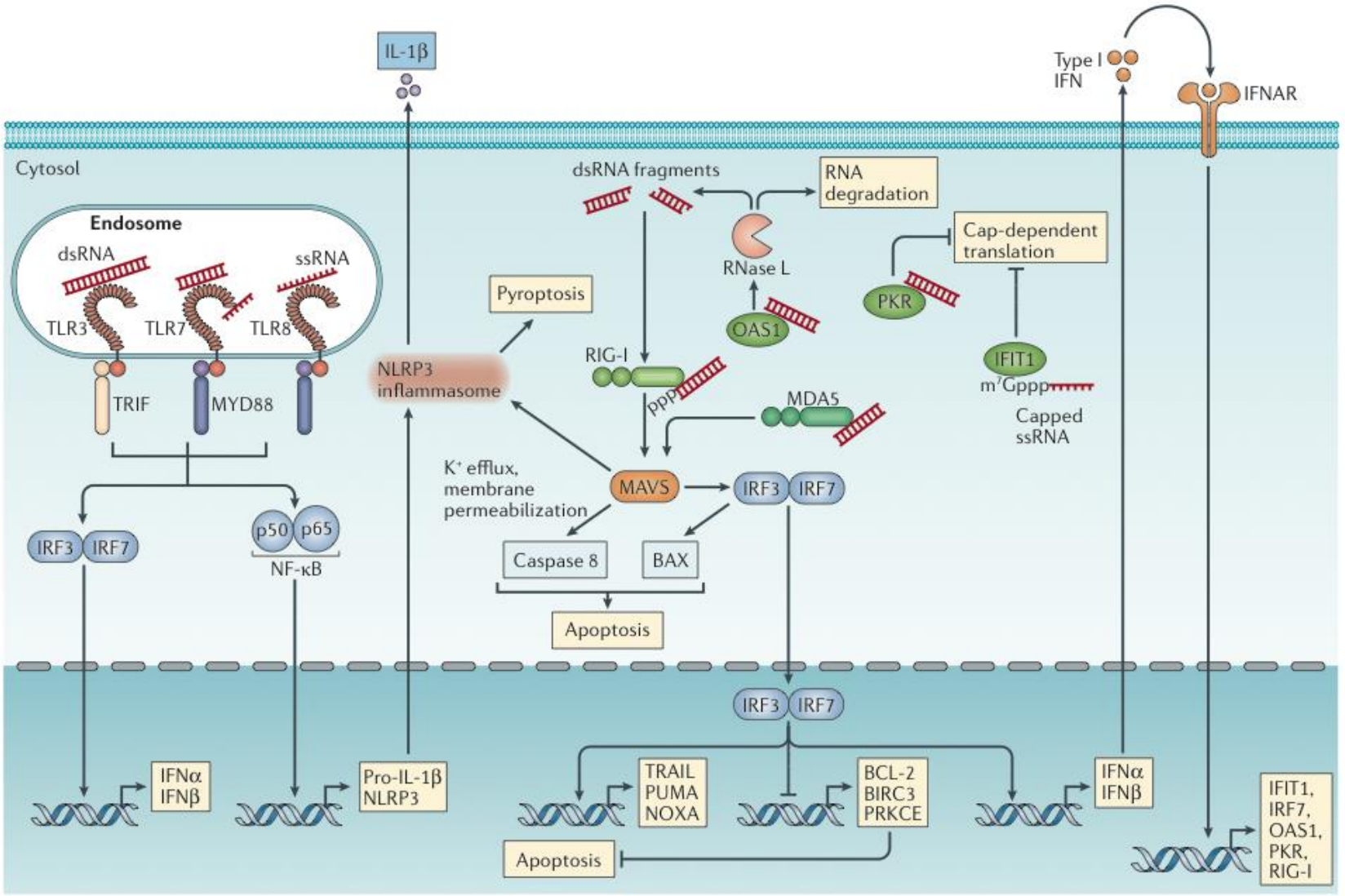
SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

B CELLS

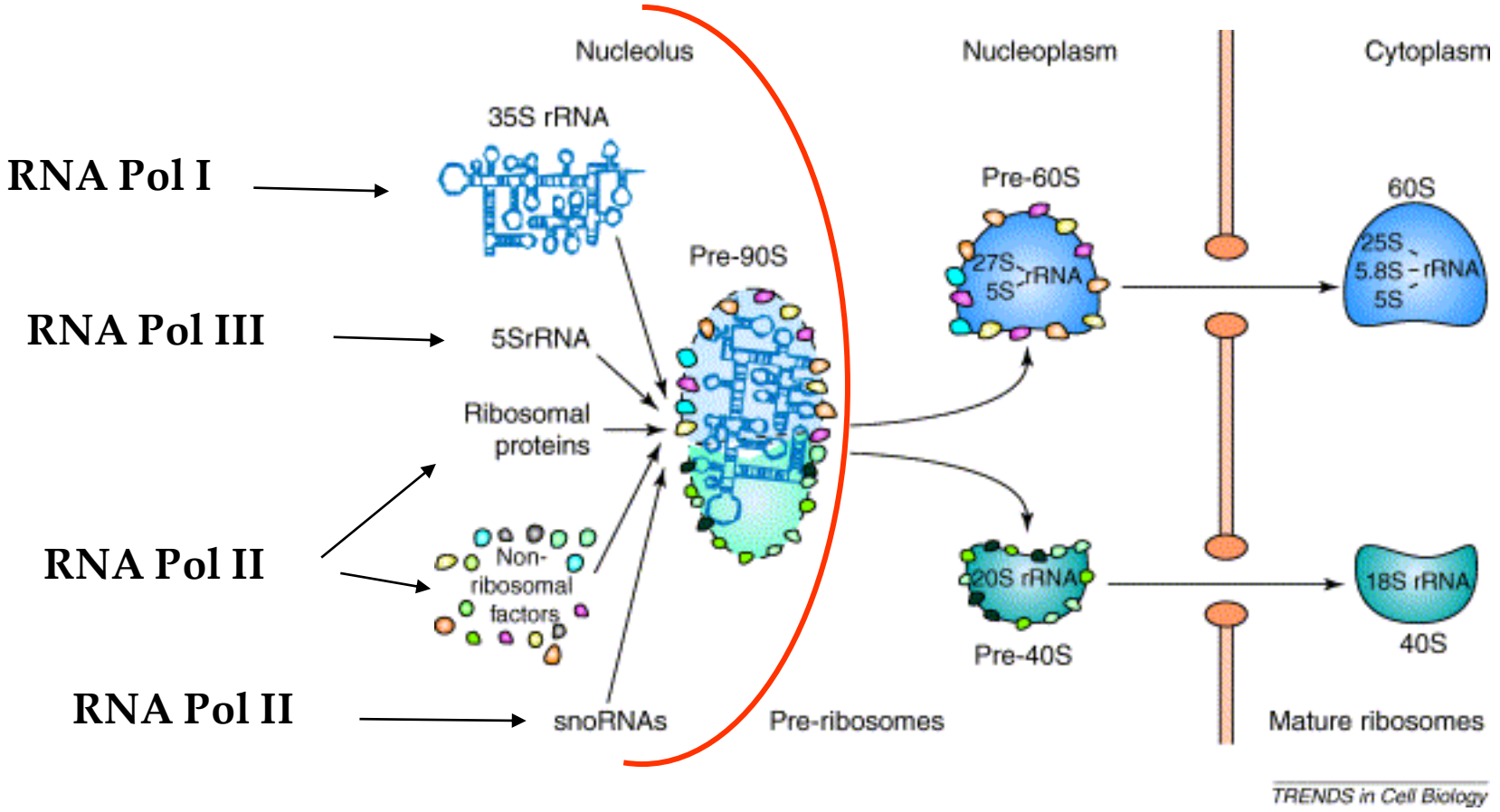
RNA exosome drives early B cell development via noncoding RNA processing mechanisms

Brice Laffleur[1]*†‡, Carolina R. Batista[1]‡, Wanwei Zhang[1]‡, Junghyun Lim[1], Biao Yang[1], Delphine Rossille[2]†, Lijing Wu[1], Jerson Estrella[1], Gerson Rothschild[1], Evangelos Pefanis[3], Uttiya Basu[1]*§

Nucleases rapidly degrade most self nucleic acids before they can be sensed by nucleic acid receptors

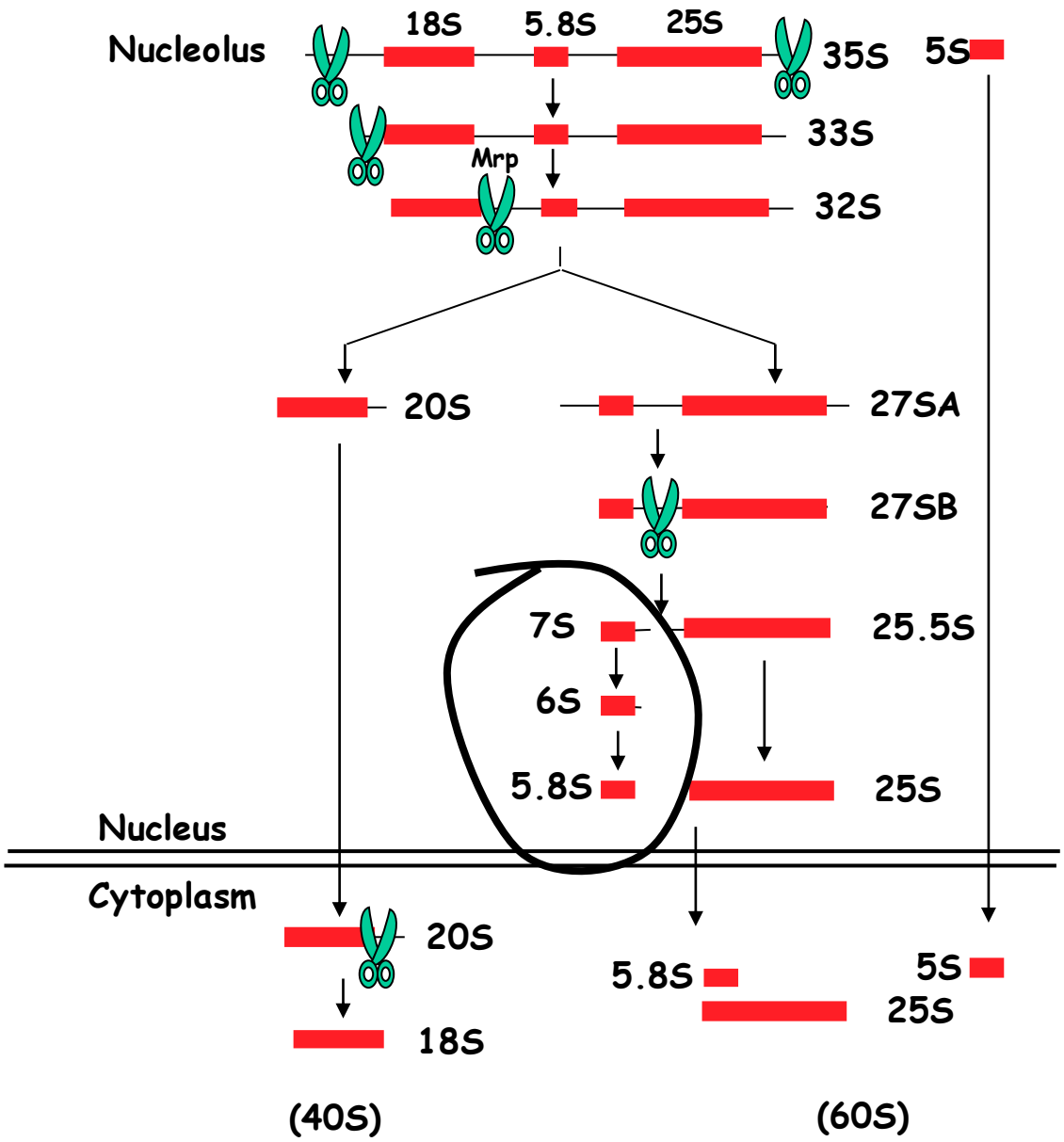


Ribosome synthesis



- 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

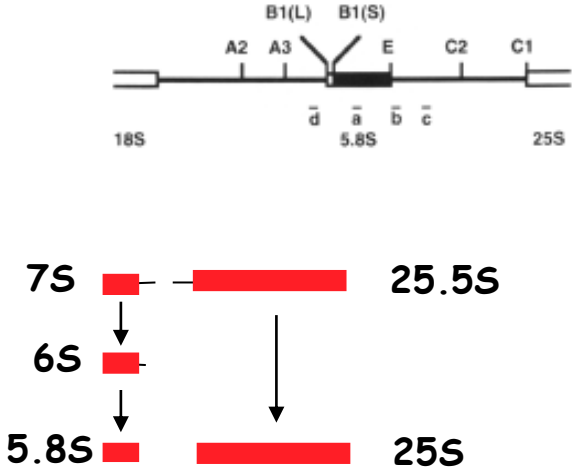
Pre-rRNA processing

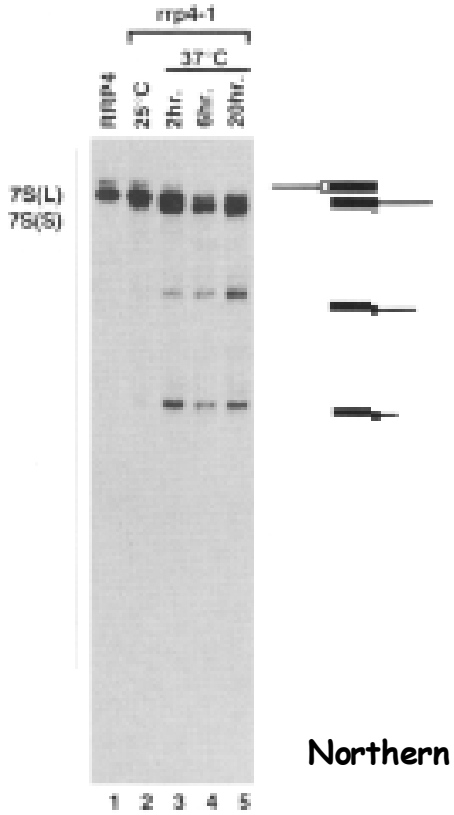


1997-The first Exosome component

•The first eukaryotic 3′->5′ exonuclease has been demonstrated in yeast

•In *S. cerevisiae*, the **rrp4-1 mutation** causes defective 3′ maturation of 5.8S rRNA and confers a temperature-sensitive growth phenotype



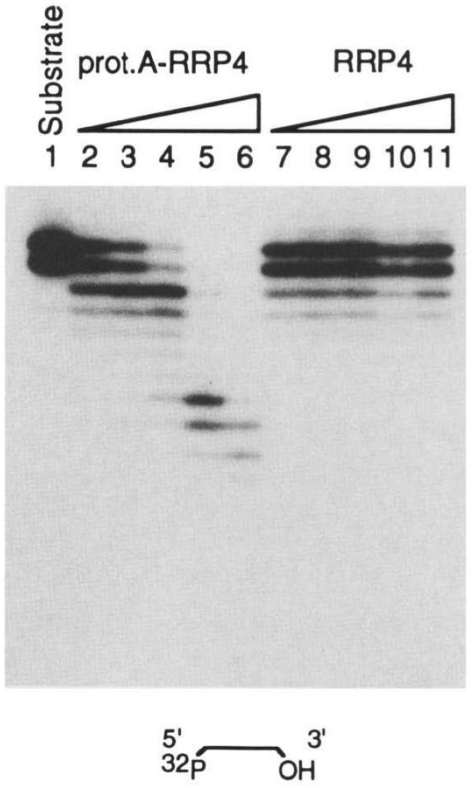


Northern

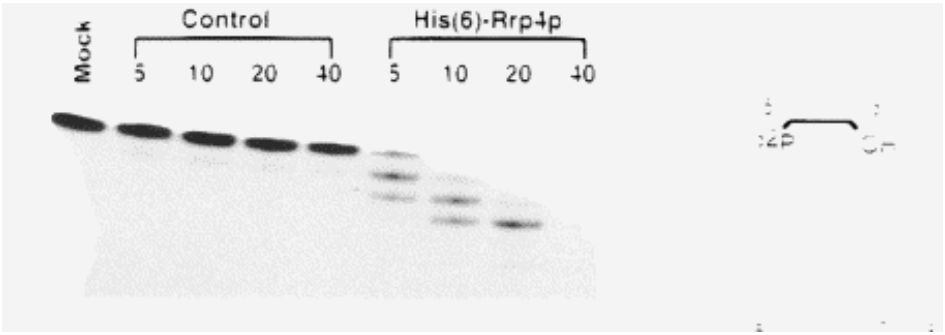
Mitchell et al., 1997. Cell

Rrp4 shows 3′->5′ esonuclease activity *in vitro*

Assays of IP from protA strains



Assays of recombinant Rrp4



Mitchell et al., 1997. Cell

Analysis of protein complexes by gradient centrifugation

Protein extract

↓

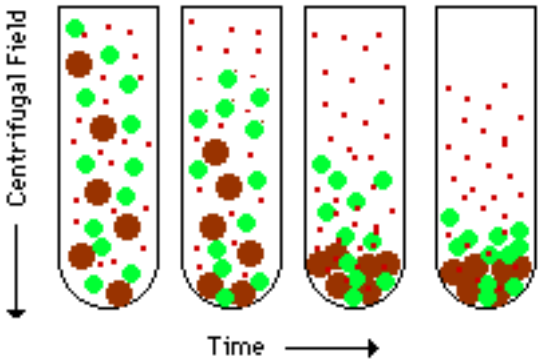
Gradient

↓

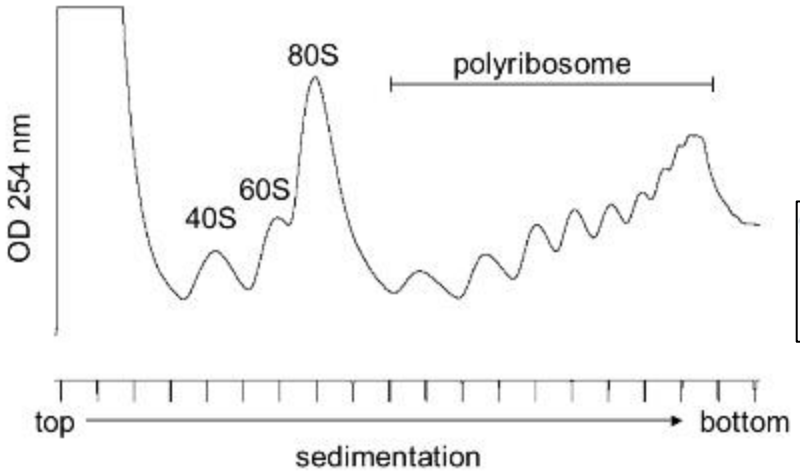
Ultracentrifugation (100.000 g)

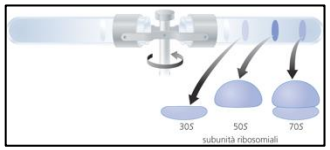
↓

Fractions collection

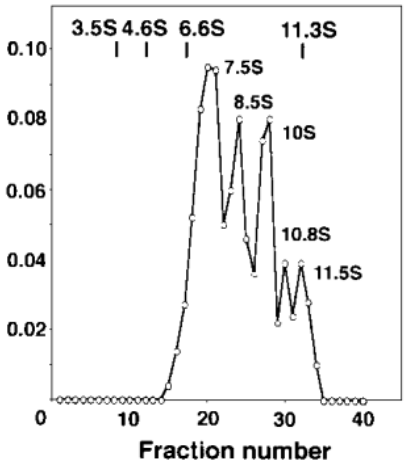


sucrose (10%-50%)





glycerol (10%-50%)



Svedberg Unit

(From Theodor Svedberg, Nobel price in 1926, inventor of the analytical ultracentrifuge)

The *sedimentation coefficient* s of a particle is used to characterize its behaviour in sedimentation processes, notably centrifugation. It is defined as the ratio of a particle's sedimentation velocity to the acceleration that is applied to it. The sedimentation speed v_t (in ms^{-1}) is also known as the terminal velocity. It is constant because the force applied to a particle by gravity or by a centrifuge (measuring typically in multiples of tens of thousands of gravities in an ultracentrifuge) is cancelled by the viscous resistance of the medium through which the particle is moving. The applied acceleration a (in ms^{-2}) is the centrifugal acceleration rw^2; w is the angular velocity of the rotor and r is the distance of a particle to the rotor axis (radius).

The viscous resistance is given by the Stokes' law: $6\pi\eta r_0 v$ where η is the viscosity of the medium, r_0 is the radius of the particle and v is the velocity of the particle.

$$s = \frac{v_t}{a} \qquad v_t = \frac{mr\omega^2}{6\pi\eta r_0} \longrightarrow s = \frac{v_t}{r\omega^2} = \frac{m}{6\pi\eta r_0}$$

For most of the biological particles, sedimentation coefficients are very small values and, by convention, their unit value is:

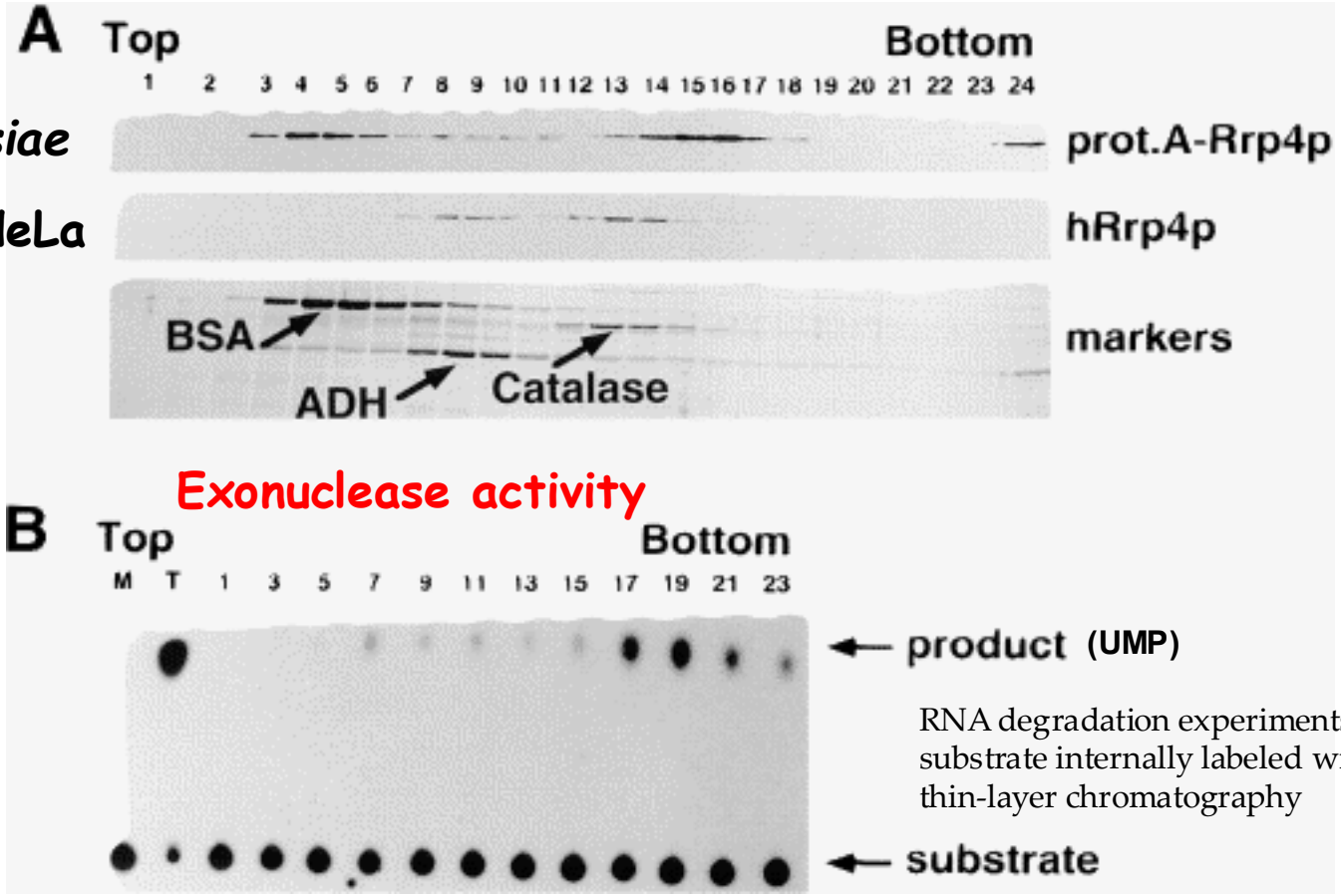
10^{-13} s = *Svedberg unit (S)*

For example, a molecule of rRNA having a sedimentation coefficient equal to $5x10^{-13}$ s has a value of 5S

Bigger particles sediment faster and have higher sedimentation coefficients. Sedimentation coefficients are, however, not additive.

The Rrp4p-Associated Exonuclease Activity Is Found in a Complex

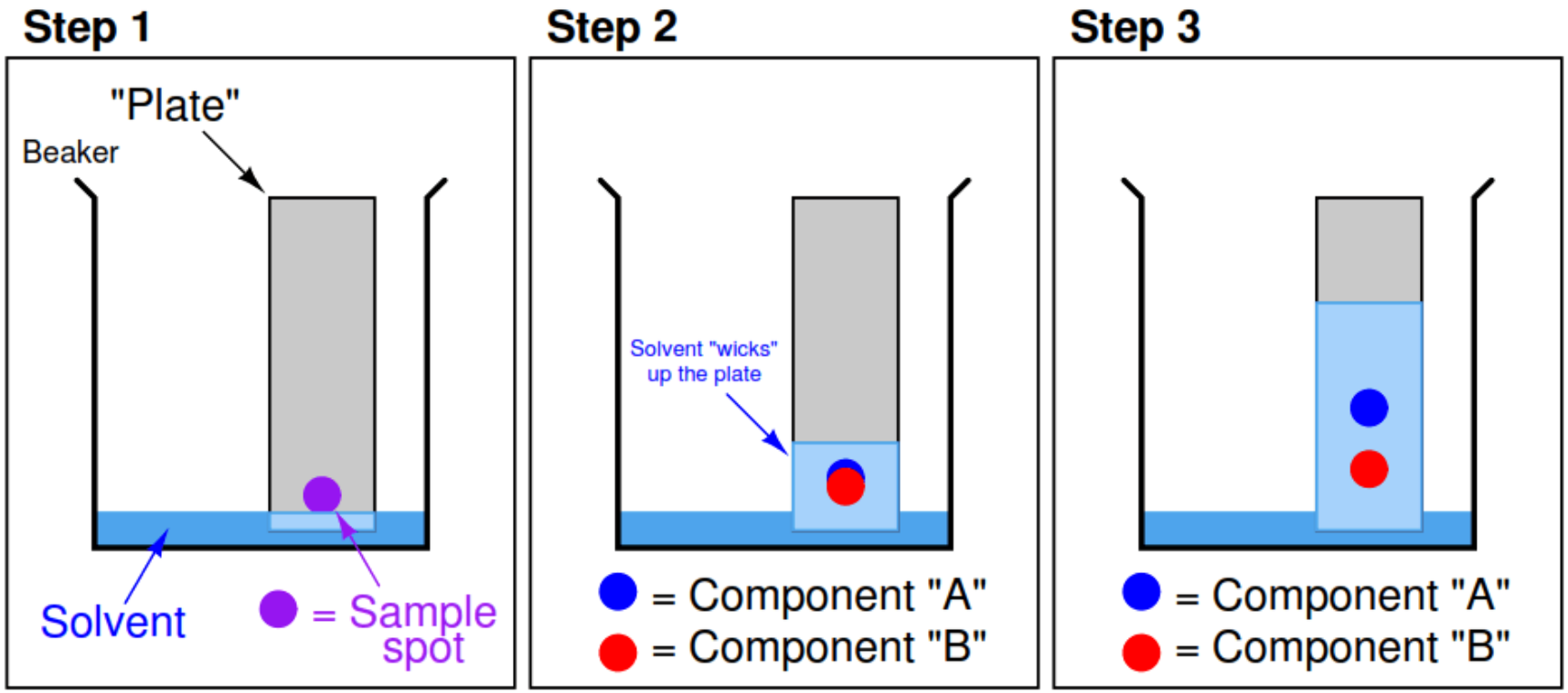
10%-30% glycerol gradient



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

Mitchell et al., 1997. Cell

Thin-layer chromatography



RNA

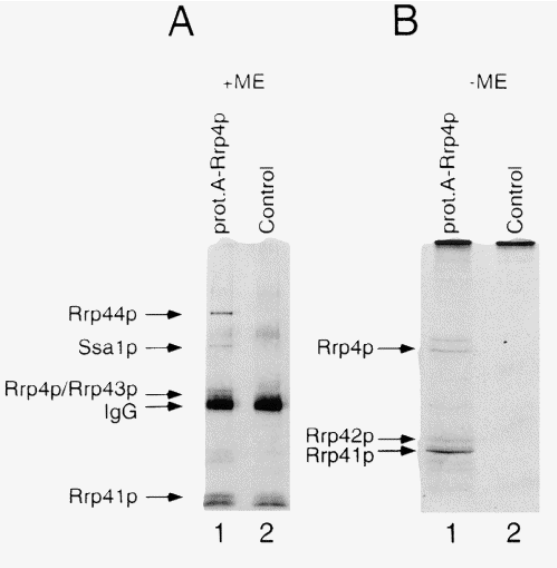
mono-nucleotide

di-nucleotide

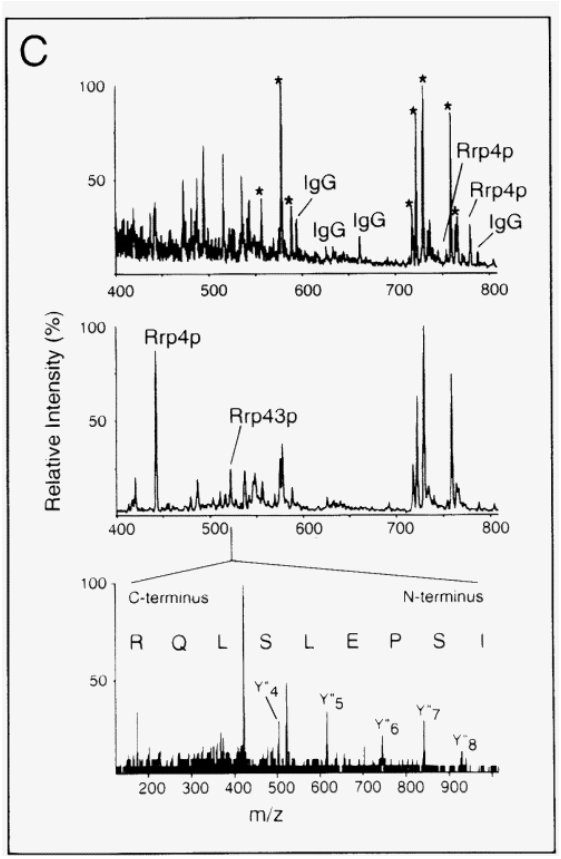
Four proteins copurify with yeast Rrp4p

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

ProtA-tagging and immunoprecipitation

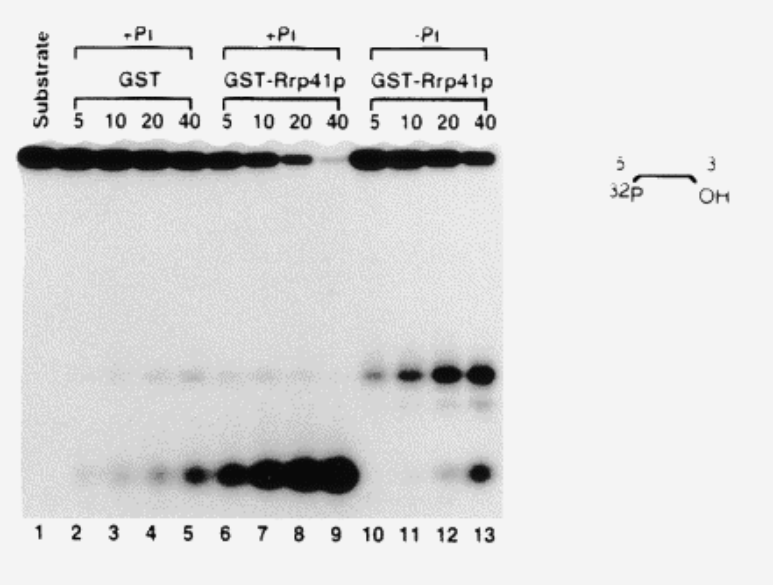


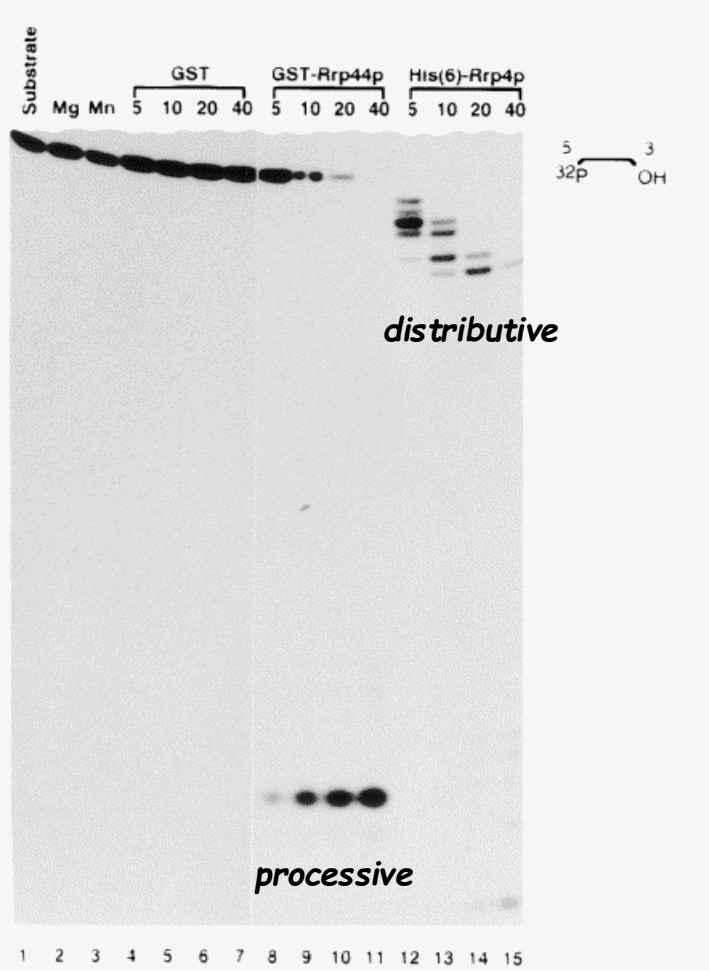
Mass spectrometry



Mitchell et al., 1997. Cell

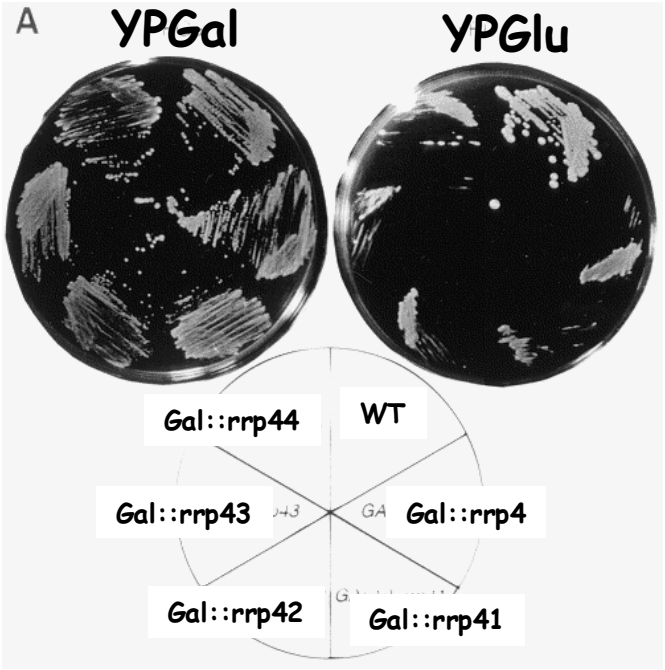
Recombinant Rrp41p and Rrp44p Exhibit 3′→5′ Exoribonuclease Activity *In Vitro*

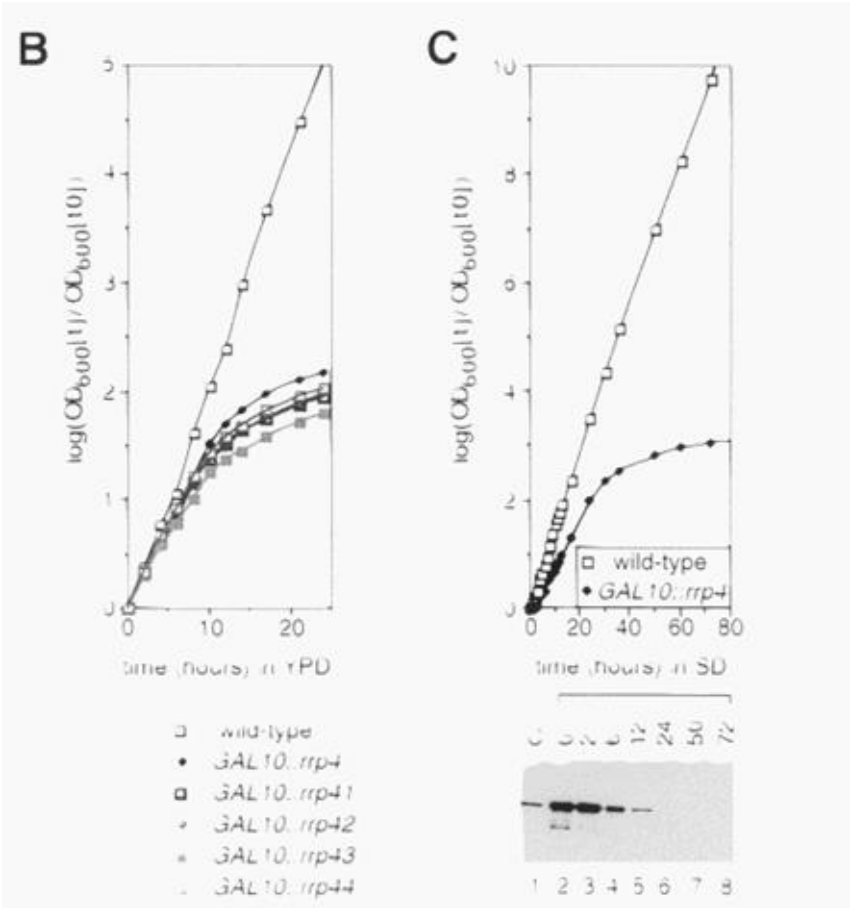




Mitchell et al., 1997. Cell

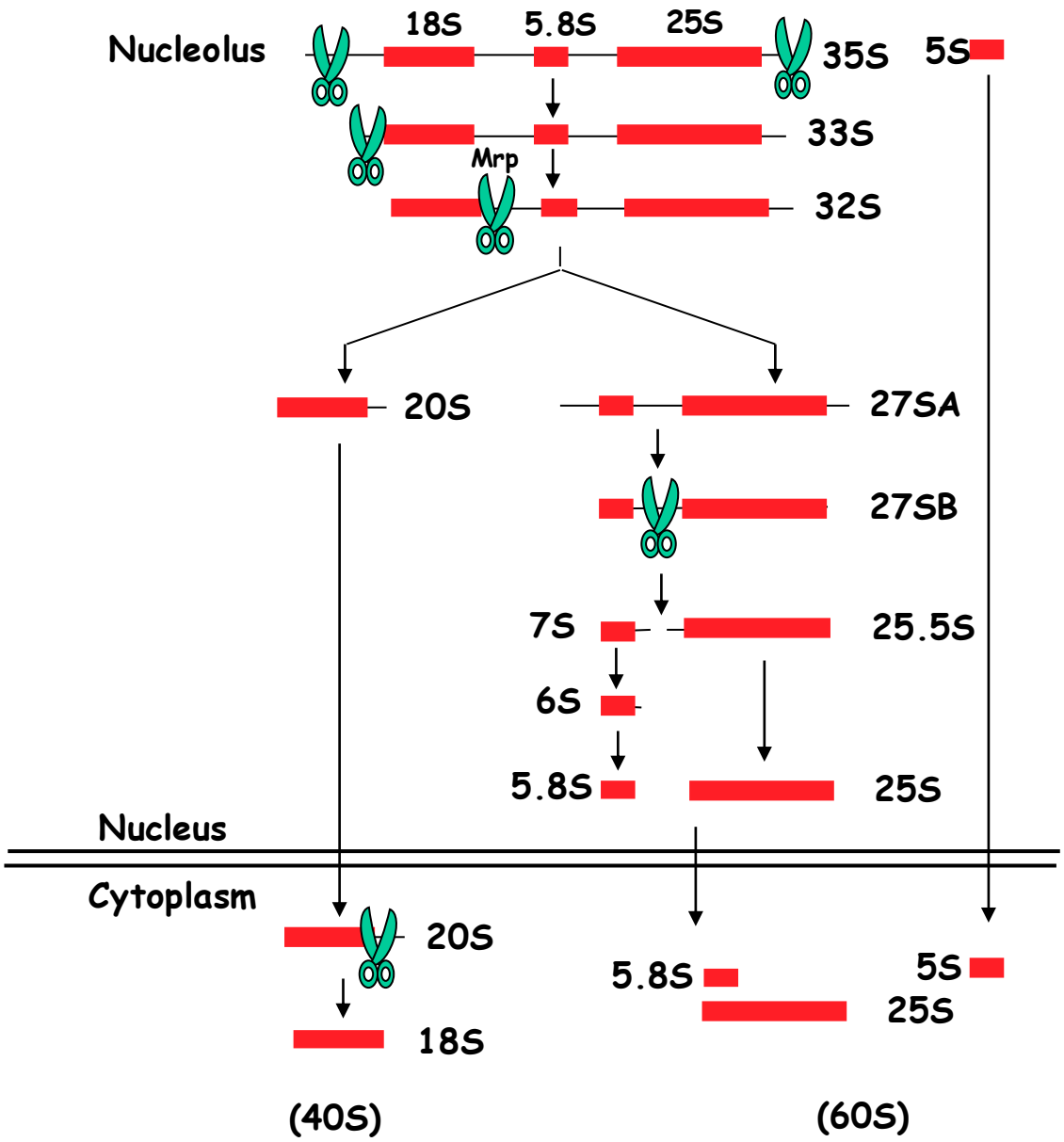
All components are required for cell growth



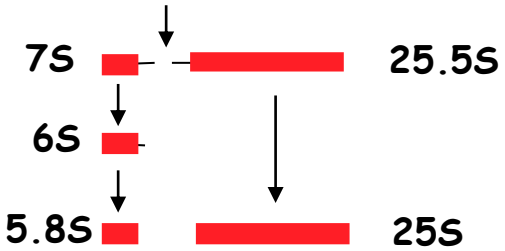


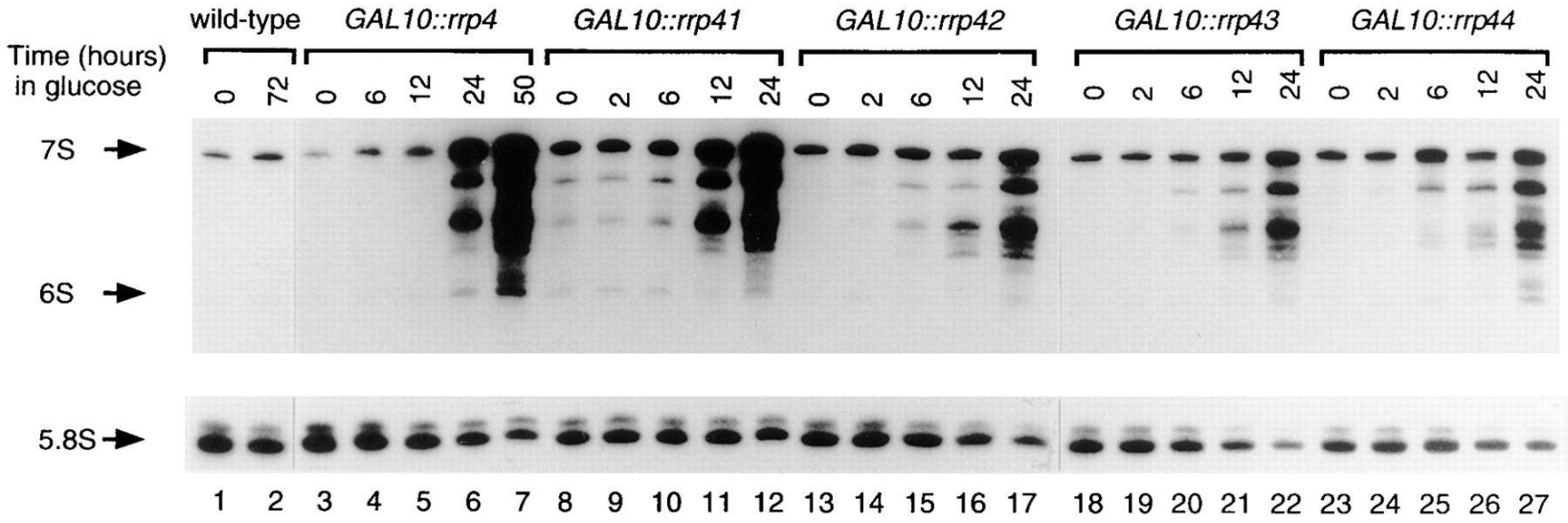
Mitchell et al., 1997. Cell

Pre-rRNA processing



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

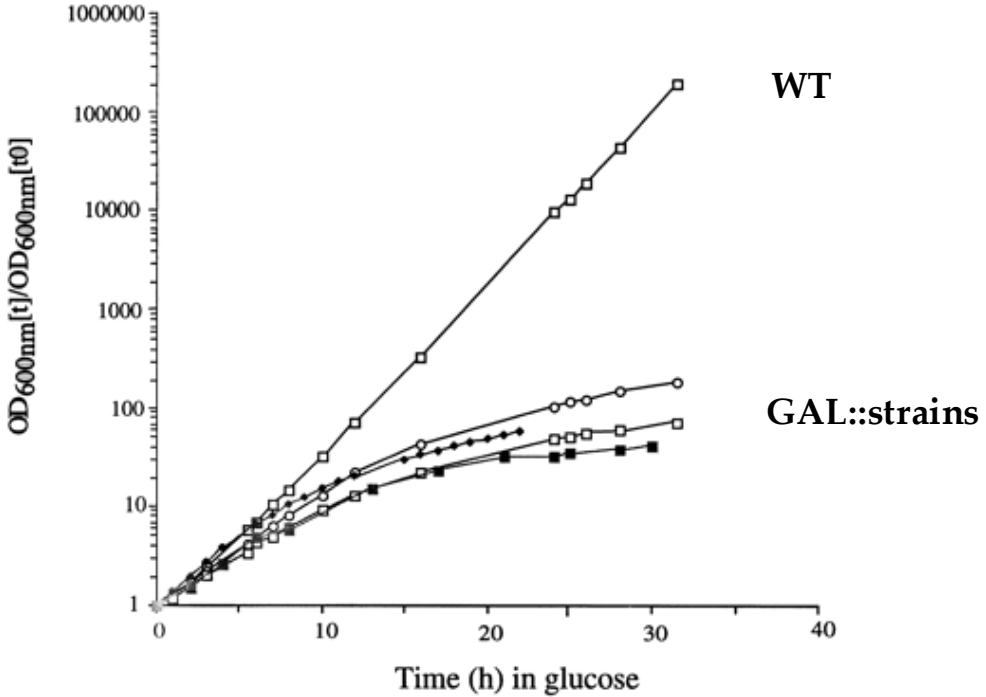




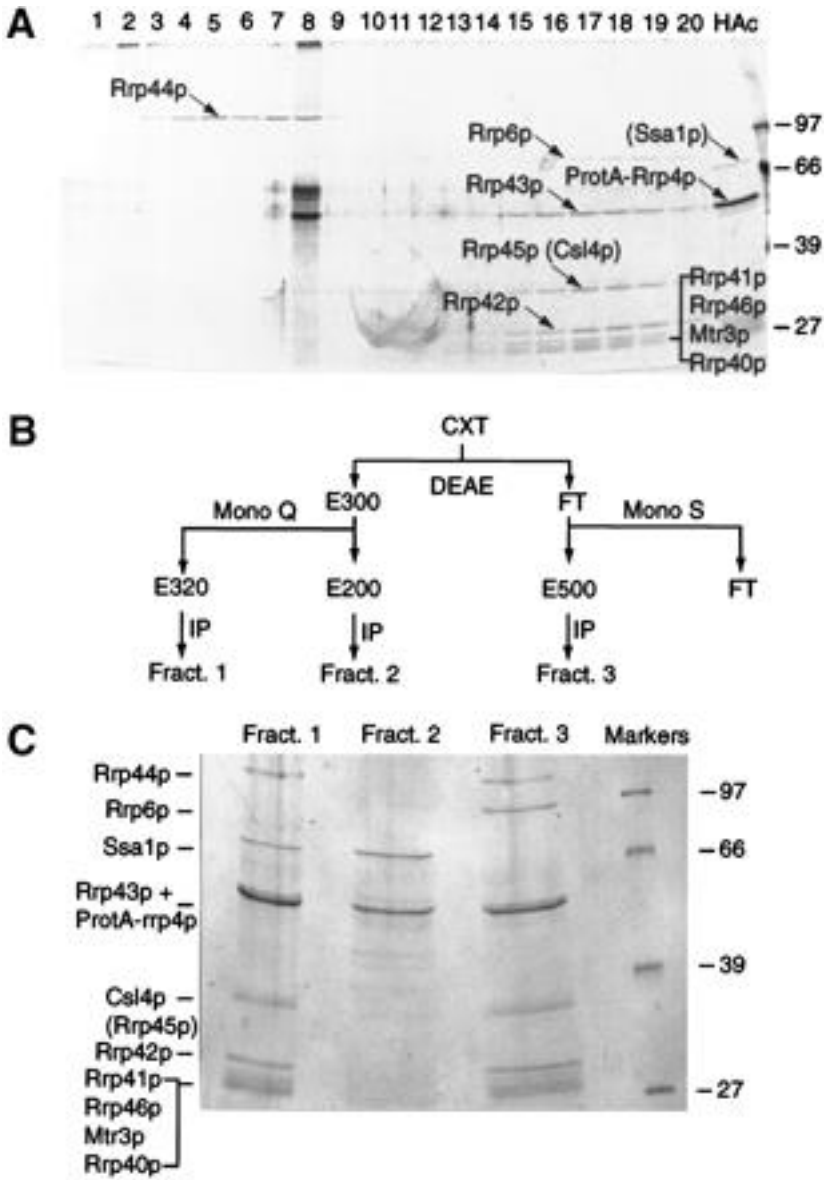
Identification of new components of the exosome complex

•**Rrp41p, Rrp42p, and Rrp43p** were homologous to *E. coli* ***RNase PH***

•the yeast genome contains three other putative open reading frames (ORFs) with homology to RNase PH**: YDR280w (*RRP45*), *YGR095c (RRP46), and *YGR158c (MTR3).*** <u>They are all essential genes.</u>

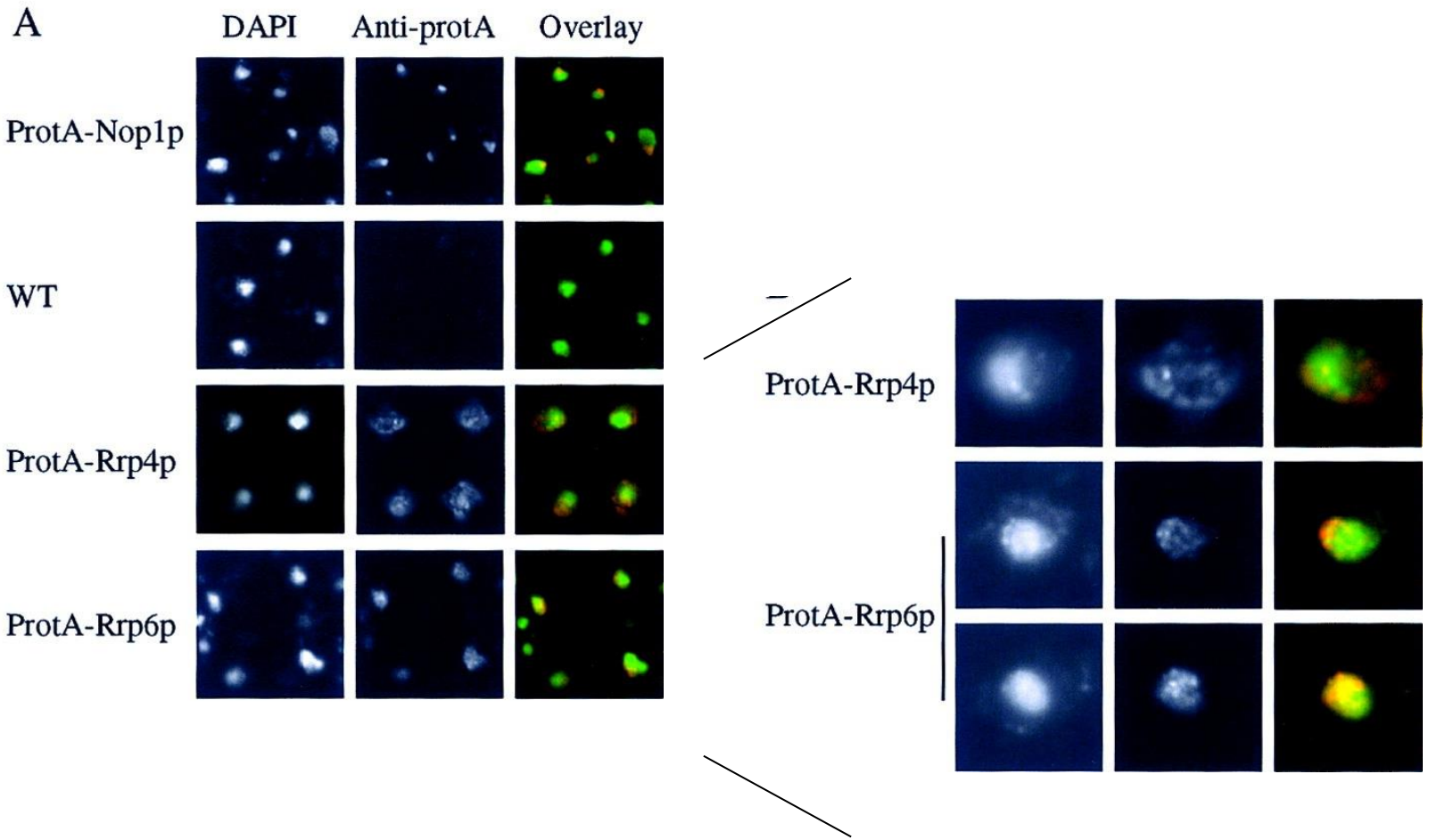


Biochemical Fractionation of the ProtA-Rrp4p IP



Cellular localization

•**Rrp6 is the only component restricted to the nucleus and non essential (TS)**



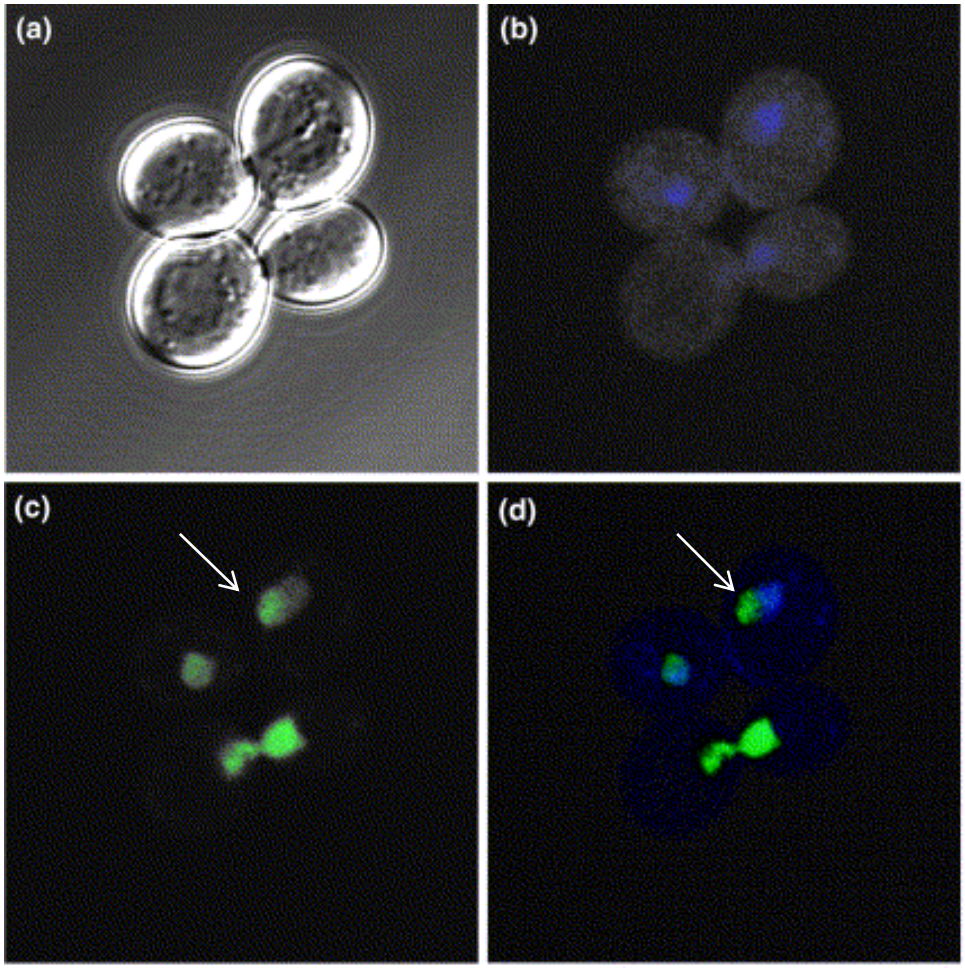
Nuclear and Nucleolar distribution of Rrp6p using Rrp6p-GFP fusion protein in yeast cells

a) Visible field (DIC)

b) DNA strain for nucleus (Hoescht)

c) GFP-Rrp6p

d) Overlay of GFP-Rrp6p and DNA



Components of the exosome

Exo-10

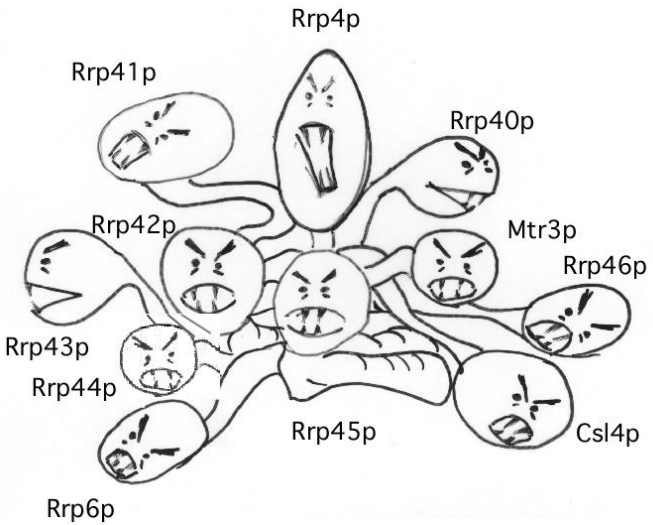
Exo-9

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.**

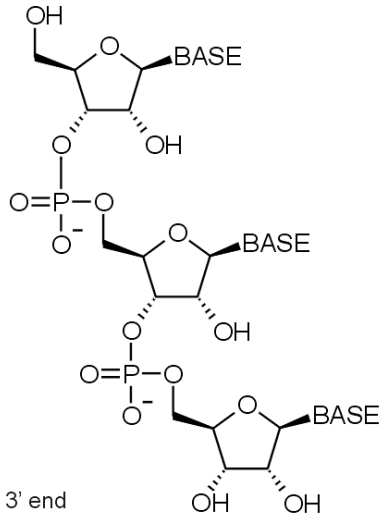


Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M. & Tollervey, D. The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′->5′ exoribonucleases. *Cell* **91**, 457–466 (1997).

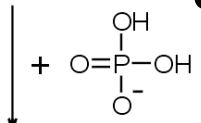
Components of the exosome

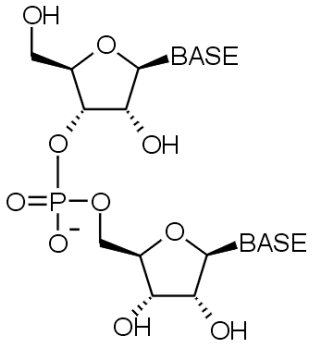
The **RNase PH-like exosome proteins**, belong to the RNase PH family of RNases and are **phosphorolytic exonuribonuclease**, which utilize inorganic phospate to remove nucleotide from the 3'-end of RNA

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

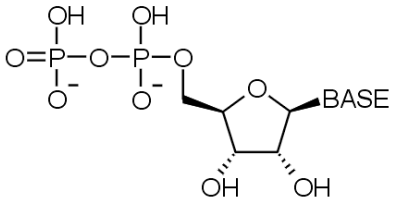


phosphorolytic exonuribonuclease

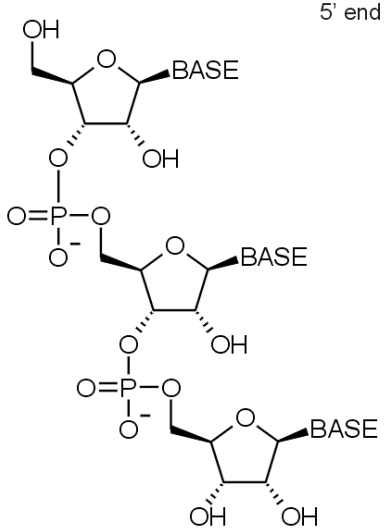




+

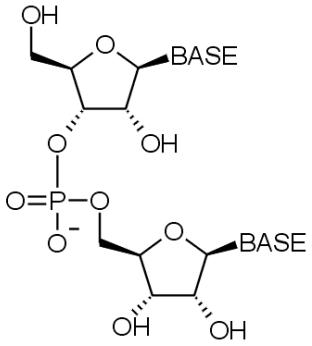


NDP



+ H_2O

hydrolytic exonuribonuclease



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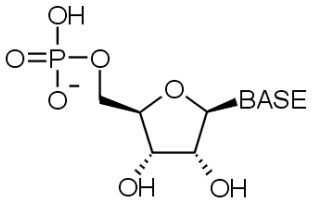
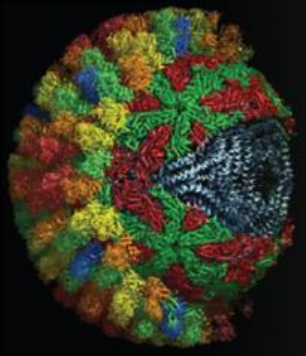


NMP

Structural biology tecniques



X-Ray Crystallography

sample must be crystallized in a solid frozen structure

any size macromolecule

atomic resolution but crystallization may take years and damage protein structure

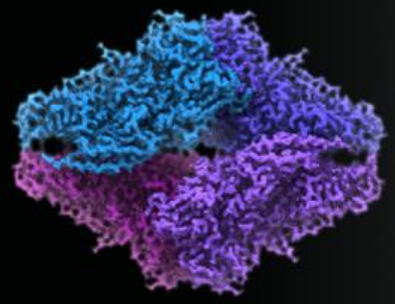


Nuclear Magnetic Resonance

sample must be dissolved and radioactively labelled

small molecules

closer to real protein structure but larger proteins can not be resolved



Cryo-Electron Microscopy

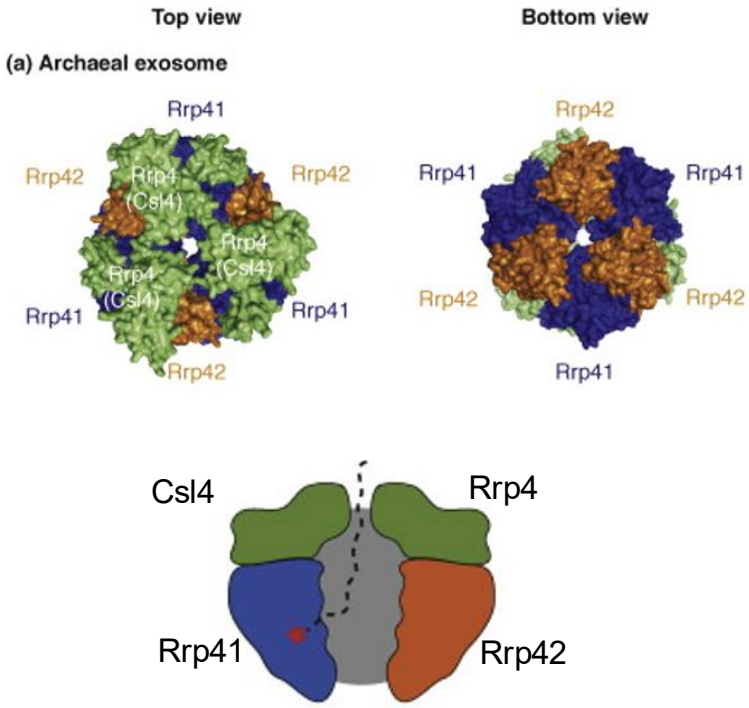
sample is frozen in its native state

any size macromolecule

near-atomic resolution, fast sample preparation

2005-The archaeal Exosome structure

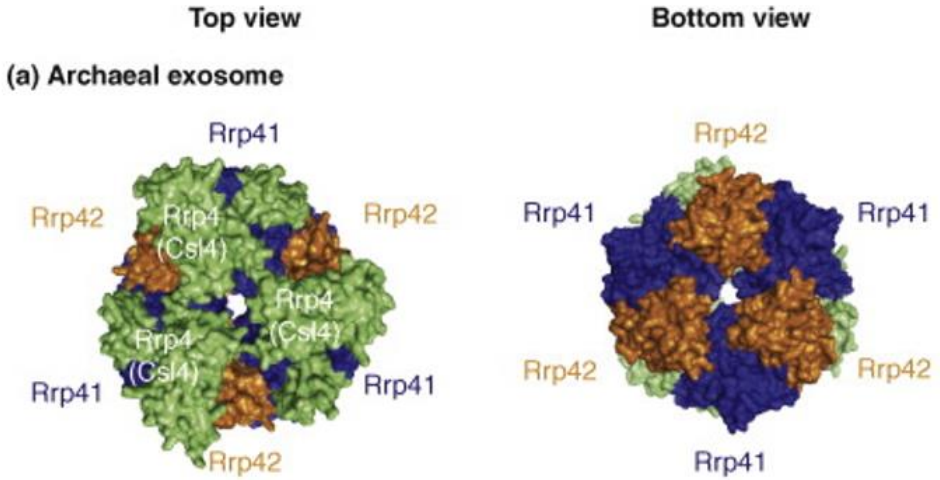
The *Sulfolobos solfataricus* complex has a simpler subunit composition with two RNase PH subunits (Rrp41 and Rrp42) and two S1 homologs (Rrp4 and Csl4), but has a similar molecular mass (250 kDa). The 2.8-Å-resolution structure of the RNase PH core of the *S. solfataricus* exosome shows that pairs of Rrp41 and Rrp42 trimerize to form a ring structure.



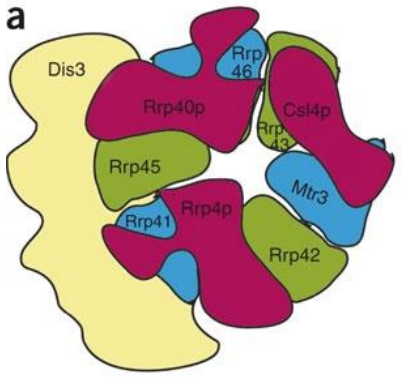
Lorentzen et al., 2005. Nature Struct. Mol. Biol

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.



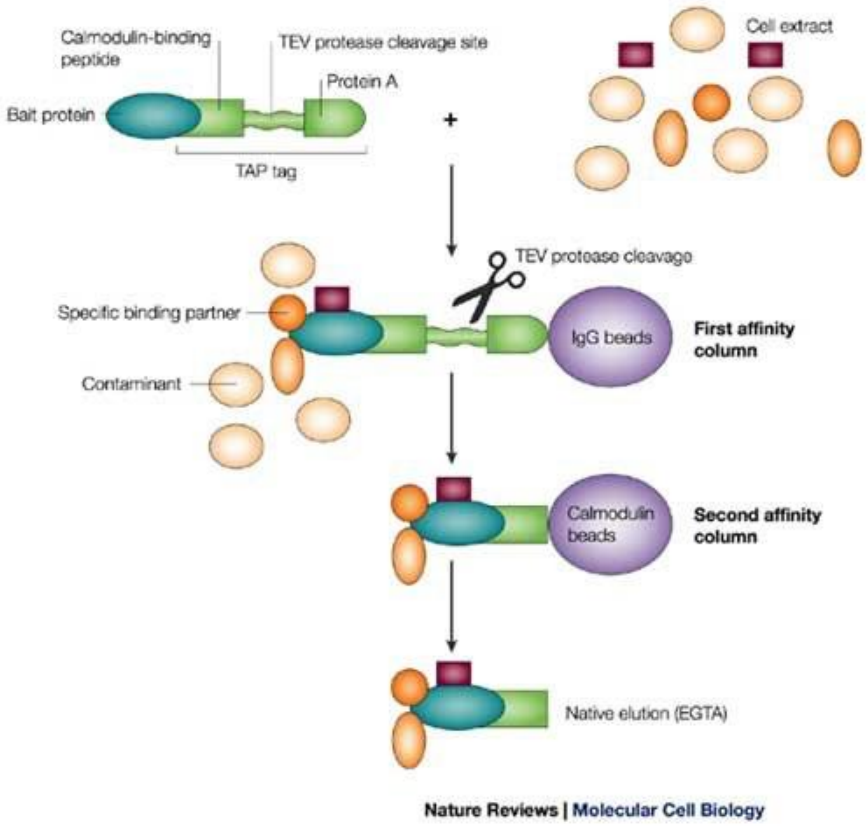
S.solfataricus



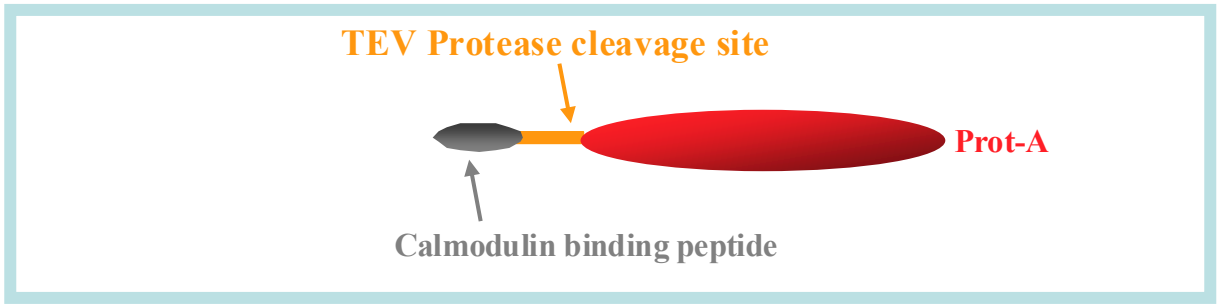
S.cerevisiae

1999-2000 TAP-tagging is invented

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



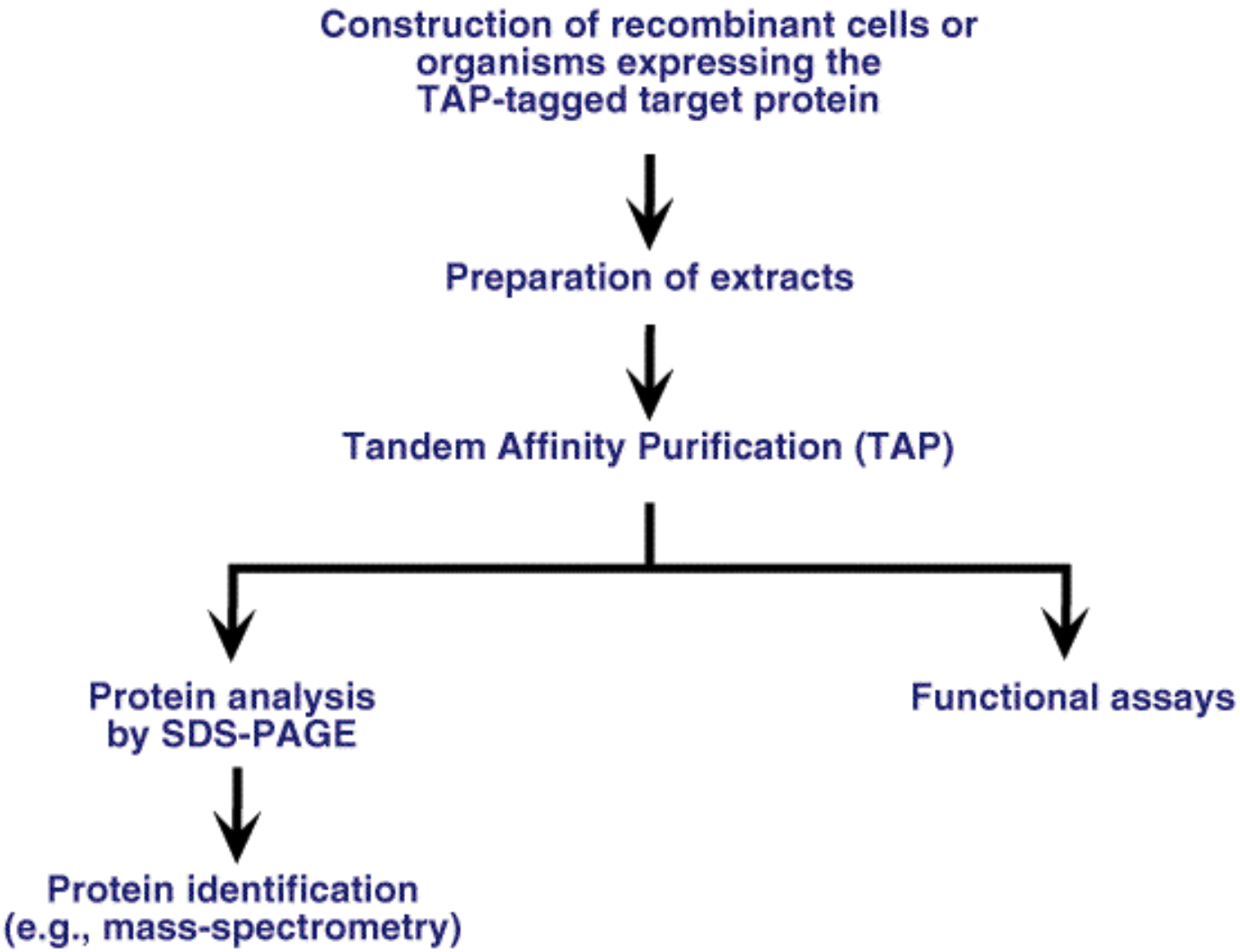
TAP tag



30KDa

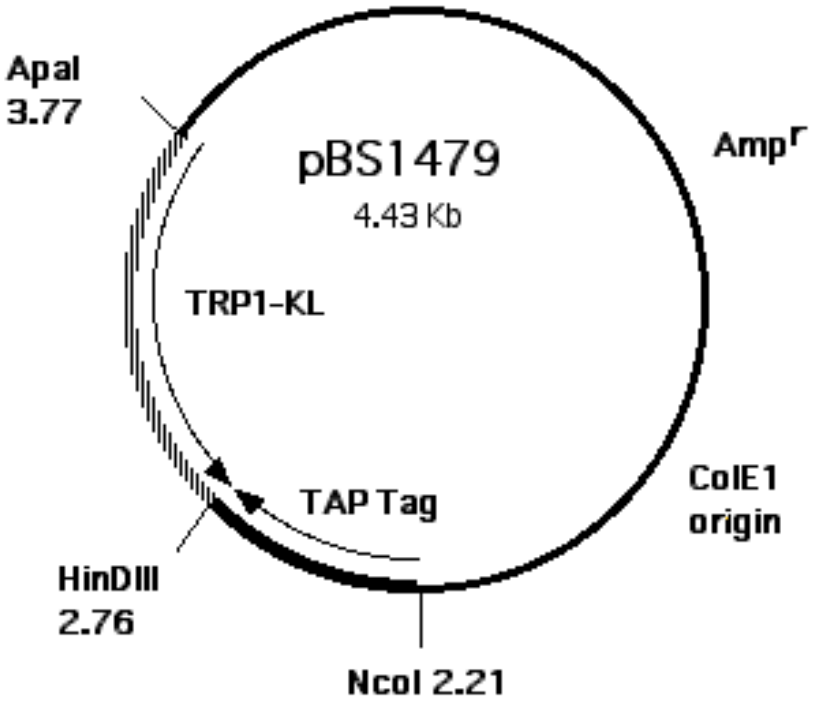
```
            <spacer><-CBP tag
gGA GGA TCC Atg gaa aag aga aga tgg aaa aag aat ttc ata gcc gtc tca gca gcc aac
        S   M   E   K   R   R   W   K   K   N   F   I   A   V   S   A   A   N

                                        CBP tag-><--            Spacer          -->
cgc ttt aag aaa atc tca tcc tcc ggg gca ctt gat tat gat att cca act act gct agc
R   F   K   K   I   S   S   S   G   A   L   D   Y   D   I   P   T   T   A   S

<----------TEV site--------><Spacer><- Protein A
gag aat ttg tat ttt cag ggt GAG ctc AAA ACC GCG GCT CTT GCG CAA CAC GAT GAA GCC
E   N   L   Y   F   Q   G   E   L   K   T   A   A   L   A   Q   H   D   E   A

GTG GAC AAC AAA TTC AAC AAA GAA CAA CAA AAC GCG TTC TAT GAG ATC TTA CAT TTA CCT
V   D   N   K   F   N   K   E   Q   Q   N   A   F   Y   E   I   L   H   L   P

AAC TTA AAC GAA GAA CAA CGA AAC GCC TTC ATC CAA AGT TTA AAA GAT GAC CCA AGC CAA
N   L   N   E   E   Q   R   N   A   F   I   Q   S   L   K   D   D   P   S   Q

AGC GCT AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GAT GCT CAG GCG CCG AAA GTA GAC
S   A   N   L   L   A   E   A   K   K   L   N   D   A   Q   A   P   K   V   D

AAC AAA TTC AAC AAA GAA CAA CAA AAC GCG TTC TAT GAG ATC TTA CAT TTA CCT AAC TTA
N   K   F   N   K   E   Q   Q   N   A   F   Y   E   I   L   H   L   P   N   L

AAC GAA GAA CAA CGA AAC GCC TTC ATC CAA AGT TTA AAA GAT GAC CCA AGC CAA AGC GCT
N   E   E   Q   R   N   A   F   I   Q   S   L   K   D   D   P   S   Q   S   A

AAC CTT TTA GCA GAA GCT AAA AAG CTA AAT GGT GCT CAG GCG CCG AAA GTA GAC GCG AAT
N   L   L   A   E   A   K   K   L   N   G   A   Q   A   P   K   V   D   A   N

            protein A-> STOP
TCC GCG GGG AAG TCA ACC TGA AGC TTG ATA TCG AAT TCC TGC AGC CCG GGG GAT CCA CTA
S   A   G   K   S   T   *
```

TAP tagging

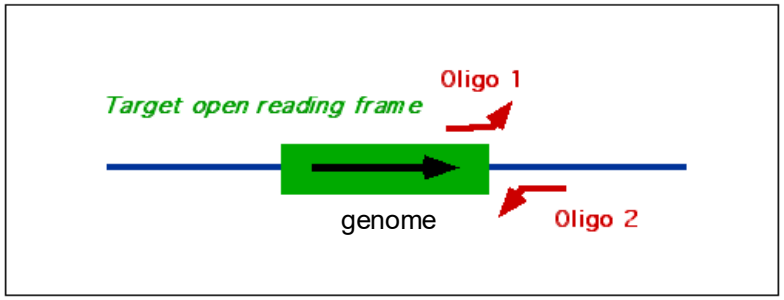

Construction of recombinant cells or organisms expressing the TAP-tagged target protein
Preparation of extracts
Tandem Affinity Purification (TAP)
Protein analysis by SDS-PAGE
Functional assays
Protein identification (e.g., mass-spectrometry)

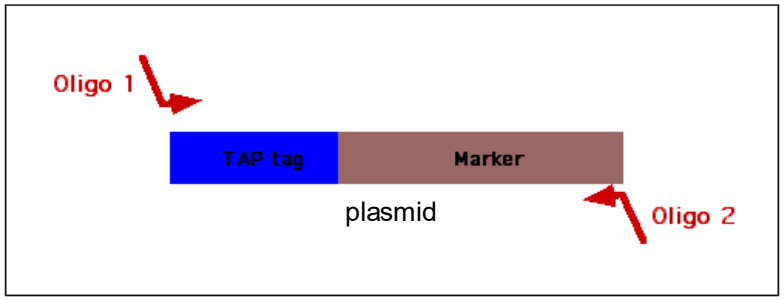
TAP tagging

TAP plasmid:



TAP tagging





Example

Start Codon *Stop Codon*

---ATG---AAA AAT---XXX---GGT GAG TAG----------

----------ACG---XXX---GGA---

0-50 nt

Oligo 1 (5',3')

AAA AAT---XXX---GGT GAG TCCATGGAAAAGAGAAG

Yeast genomic 43-48 nt — Constant priming site

! Be careful not to include the Stop codon and to maintain the reading frame !

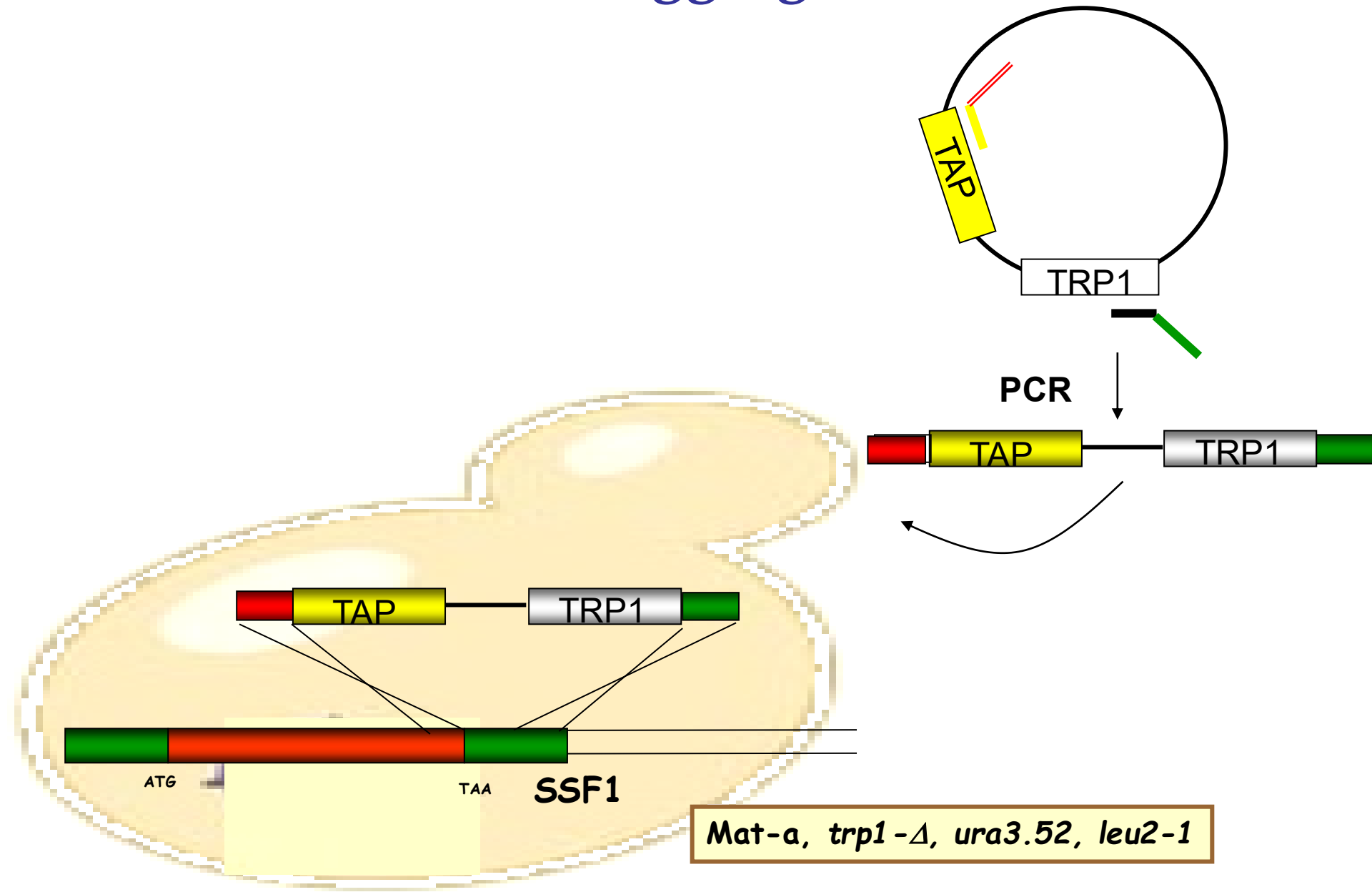
Oligo 2 (5',3')

AGG---XXX---GCA TACGACTCACTATAGGG

Yeast genomic 43-48 nt — Constant priming site

! Pay attention not to alter reading frames downstream of your gene on BOTH strands !

TAP tagging

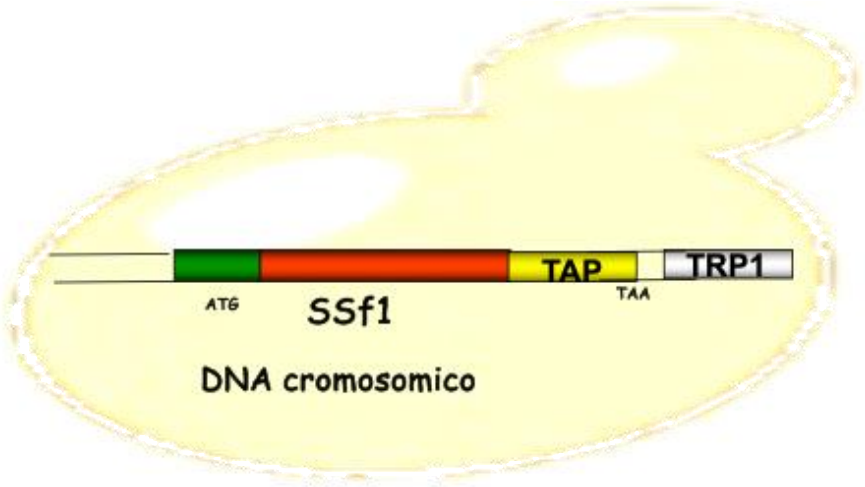


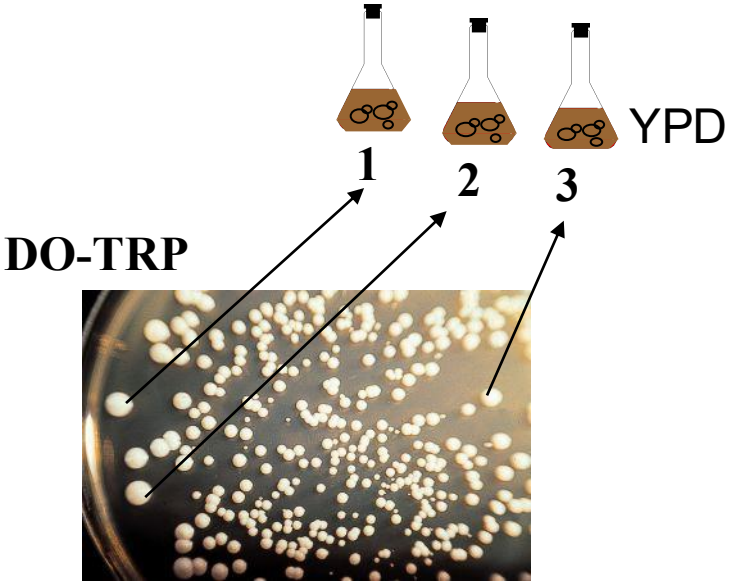
Mat-a, trp1-Δ, ura3.52, leu2-1

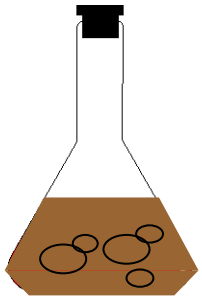
YNB DO-TRP

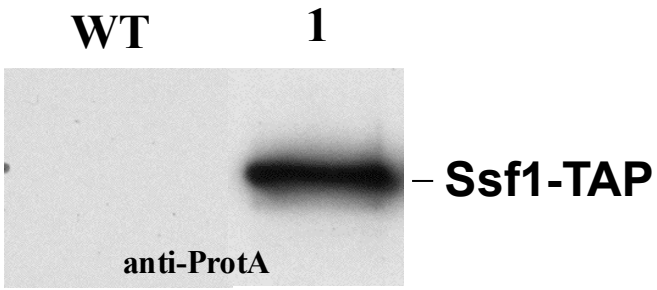
TAP tagging

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





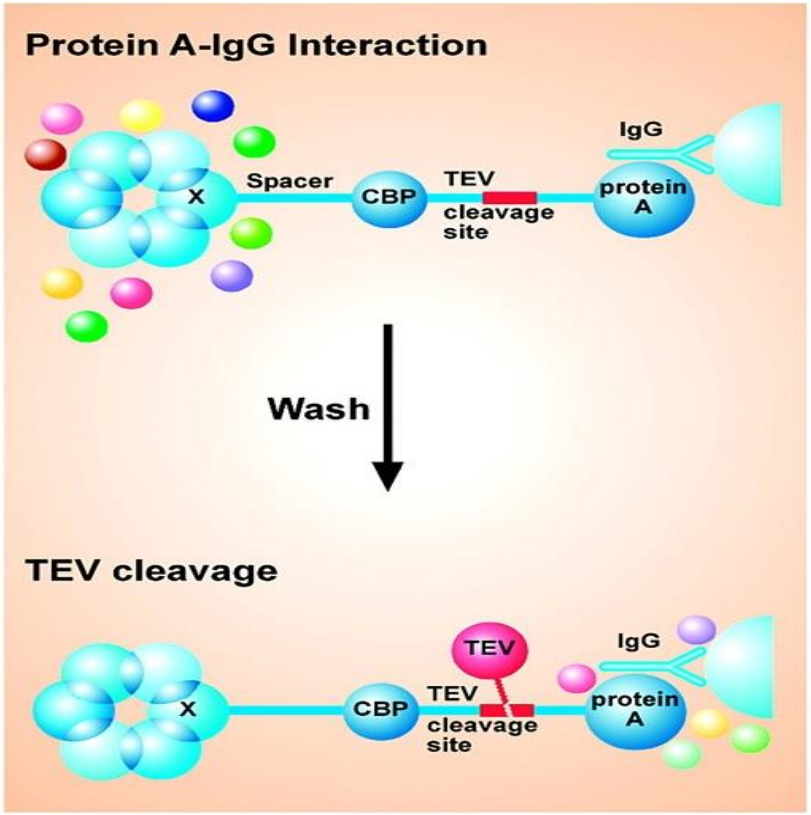




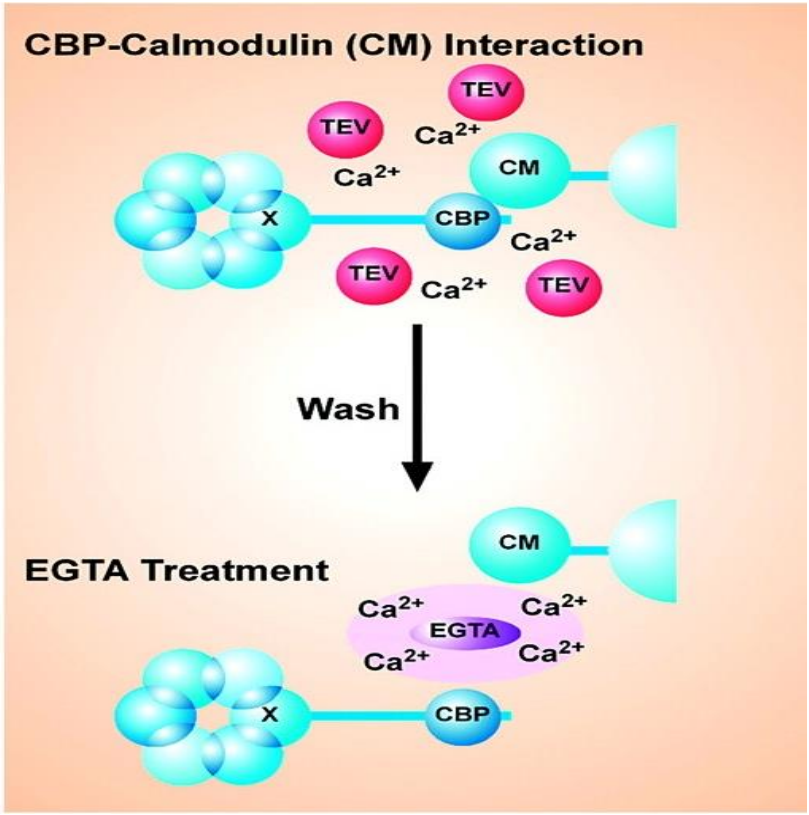
Western blot

Purificazione TAP

Prima Purificazione



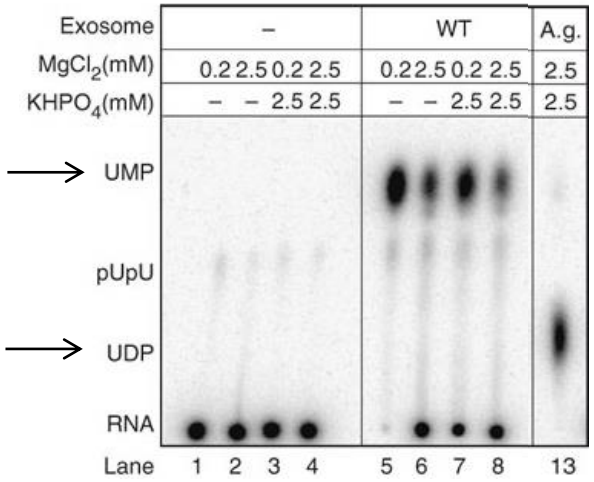
Seconda Purificazione



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.).



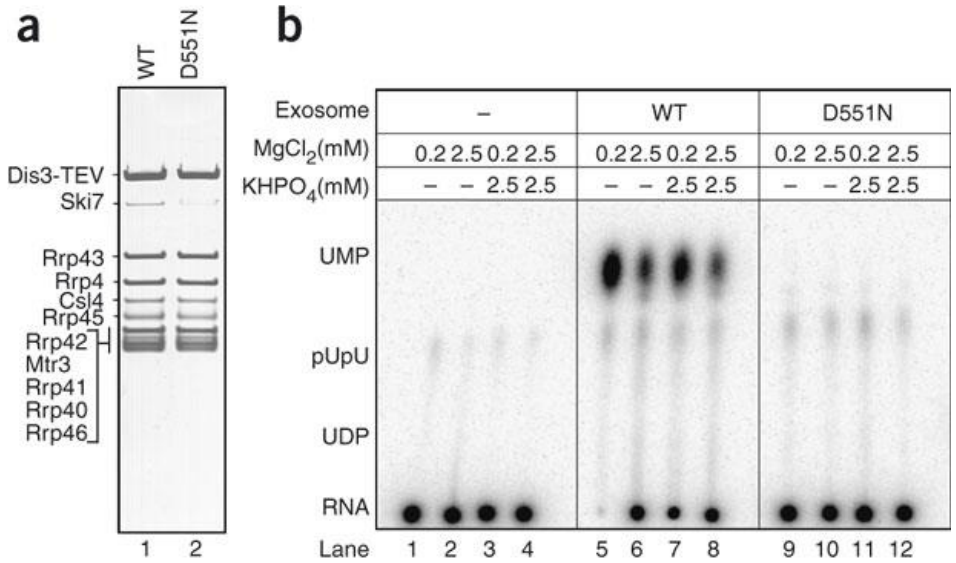
RNA degradation experiments using a RNA substrate internally labeled with [-^{32}P]UTP and thin-layer chromatography

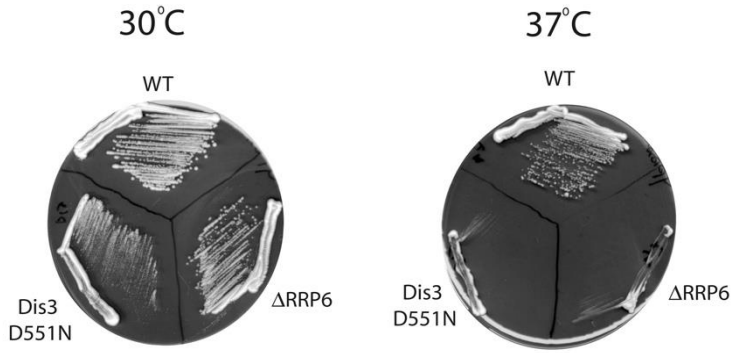
A.g.=the recombinant archaeal Rrp41-Rrp42 dimer (phosporolytic)

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.



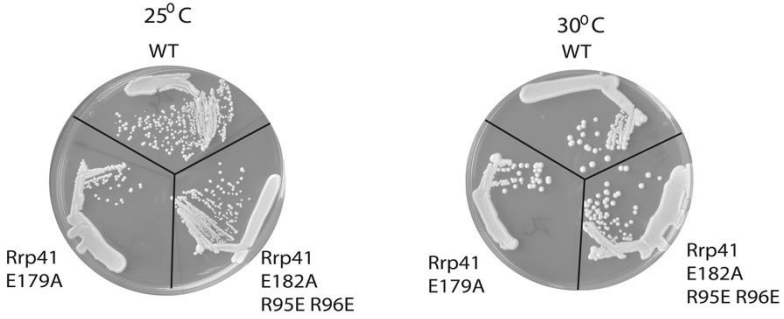


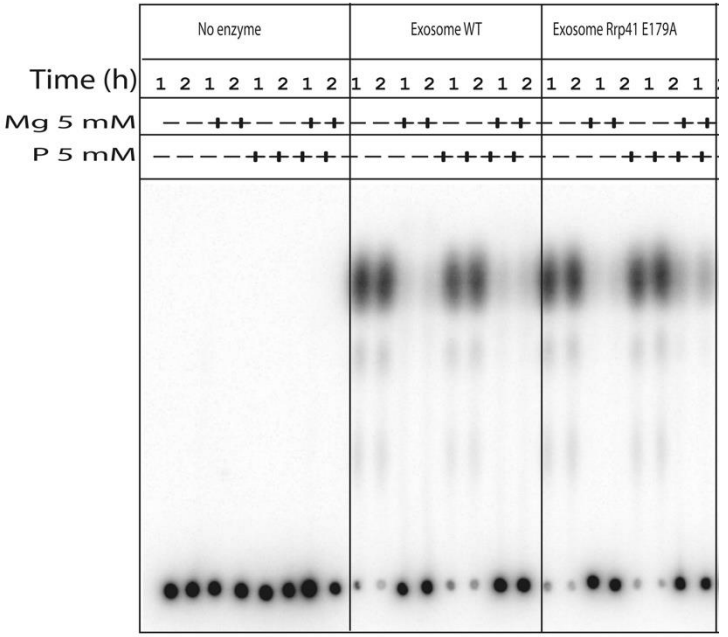
Dziembowski et al., 2007. Nature Struct. Mol. Biol

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



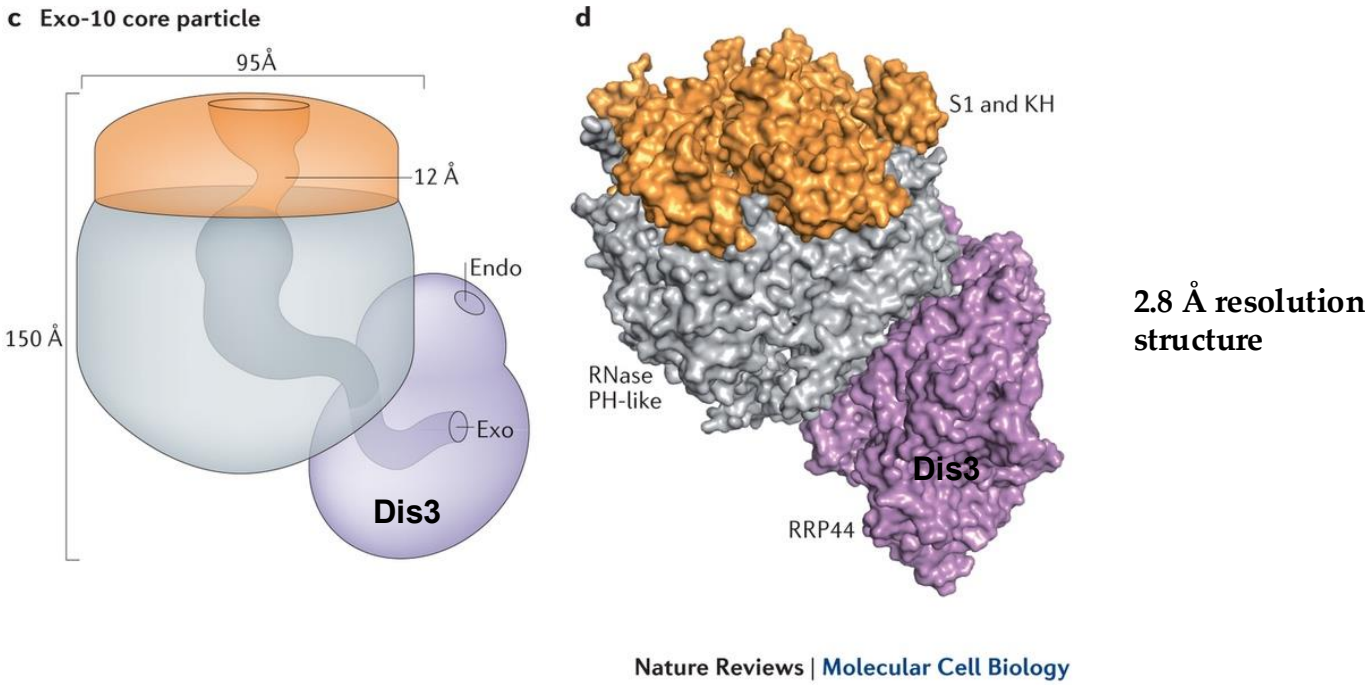


Dziembowski et al., 2007. Nature Struct. Mol. Biol

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

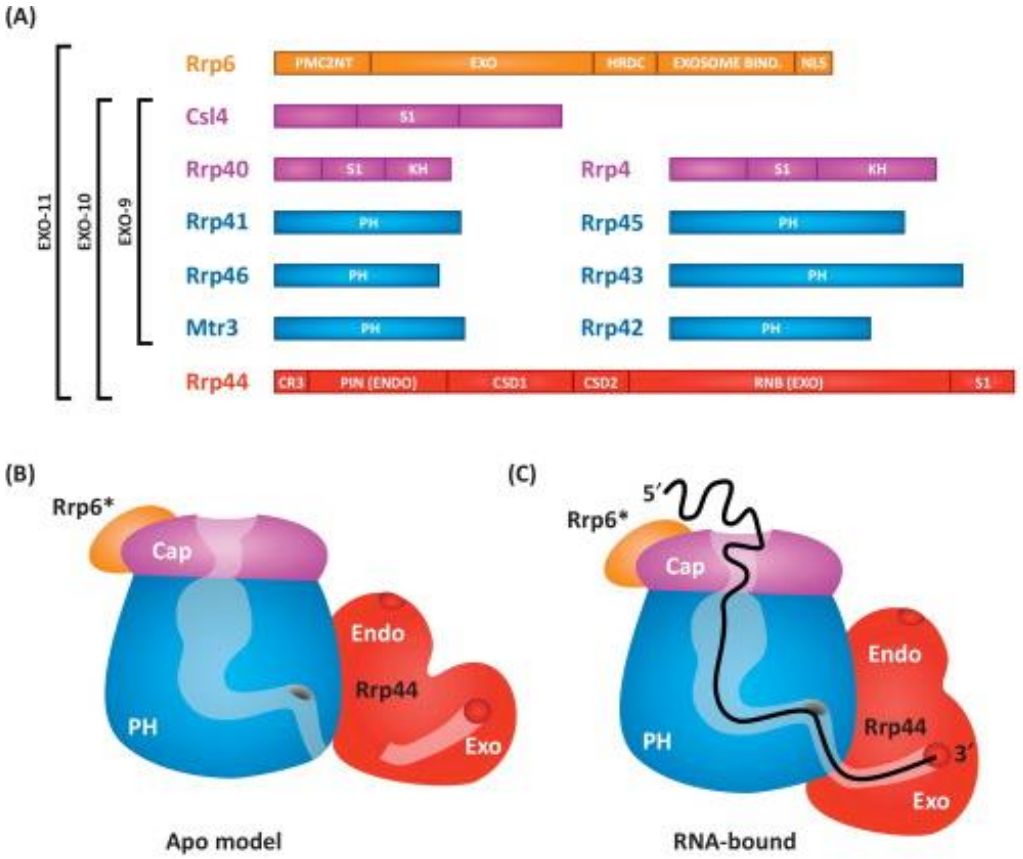


2.8 Å resolution structure

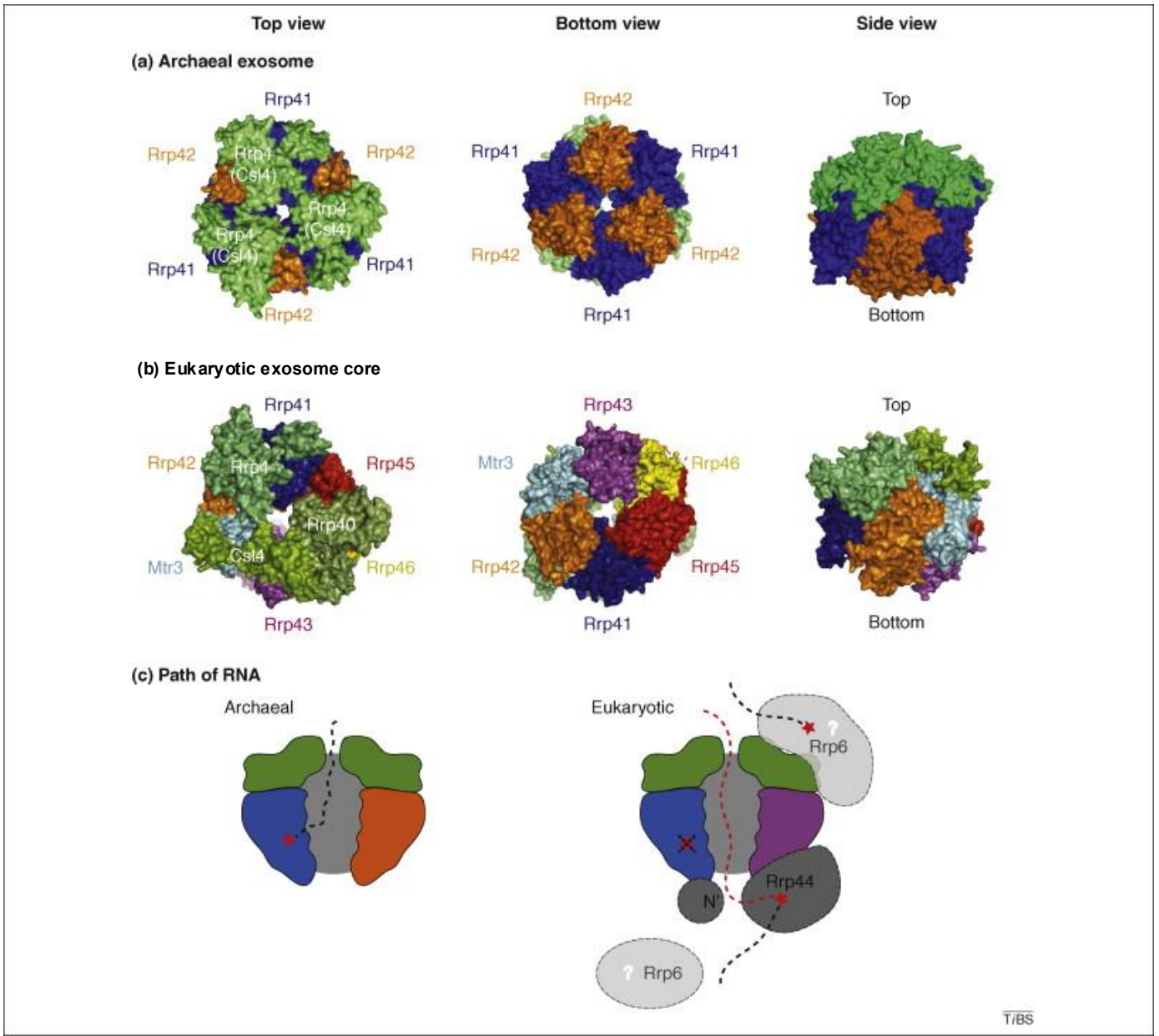
In the **Exo-10** complex, single-stranded RNA substrates enter the barrel-like structure of **Exo-9** via a pore at the centre of the S1 and KH domains. The RNA is then threaded with sequence-unspecific interactions via an internal channel to the exoribonuclease site of the catalytic subunit, **DIS3/RRP44**. 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.

The Exosome complex

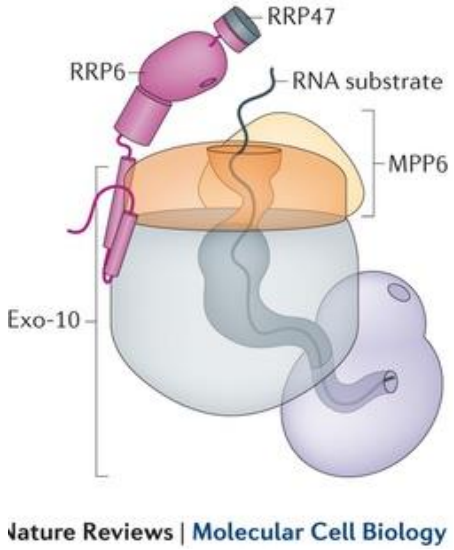
Features	Eukaryotic exosome
Functions	RNA quality control, RNA turnover and RNA processing
Localization	Cytoplasm and nucleus
Estimated abundance in yeast	600 Exo-10 particles per cell* (REF. 90), 6,000 Exo-9 particles per cell‡ (REF. 90)
Core complex	Exo-10
Year discovered	1997 (REF. 8)
Year the complete atomic structure was solved	2013 (REF. 22)
Number of protein subunits	10
Molecular mass	420 kDa
Oligomeric state	Monomer
Architecture	Two stacked rings composed of six RNase PH-like proteins, three S1 and KH-domain containing proteins and one RRP44 molecule (with PIN and RNase II-like regions)
Number of active sites	One
Activity	Exoribonuclease and endoribonuclease
Active subunits	RRP44
Products	Single nucleotide monophosphates and a residual four-nucleotide fragment
Processivity	Yes



Structure of RNA exosome complexes



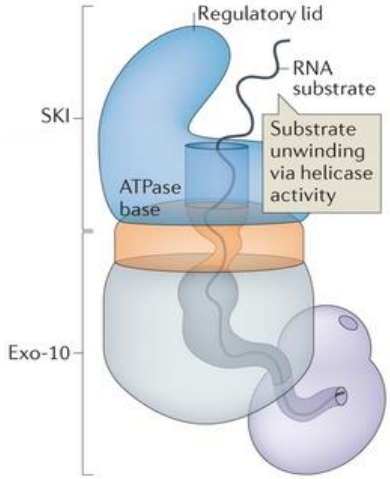
Cofactors for the exosome complexes



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)

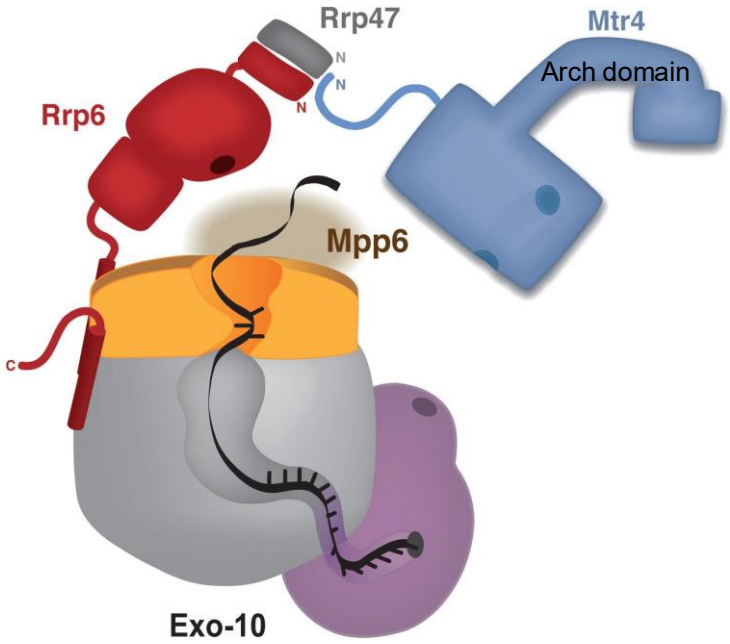


Cytoplasmic exosome:

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

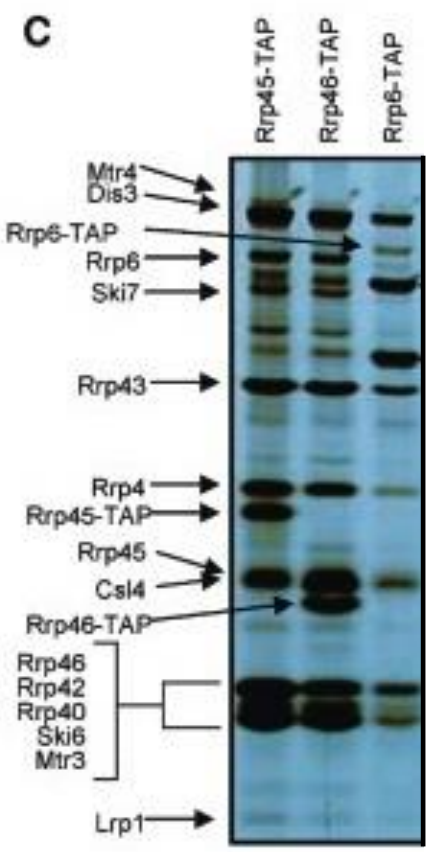
Dob1p/Mtr4p - DEAD box putative ATP-dependent RNA translocase (helicase)

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

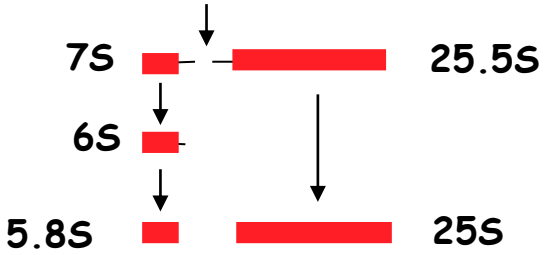
TAP-purification

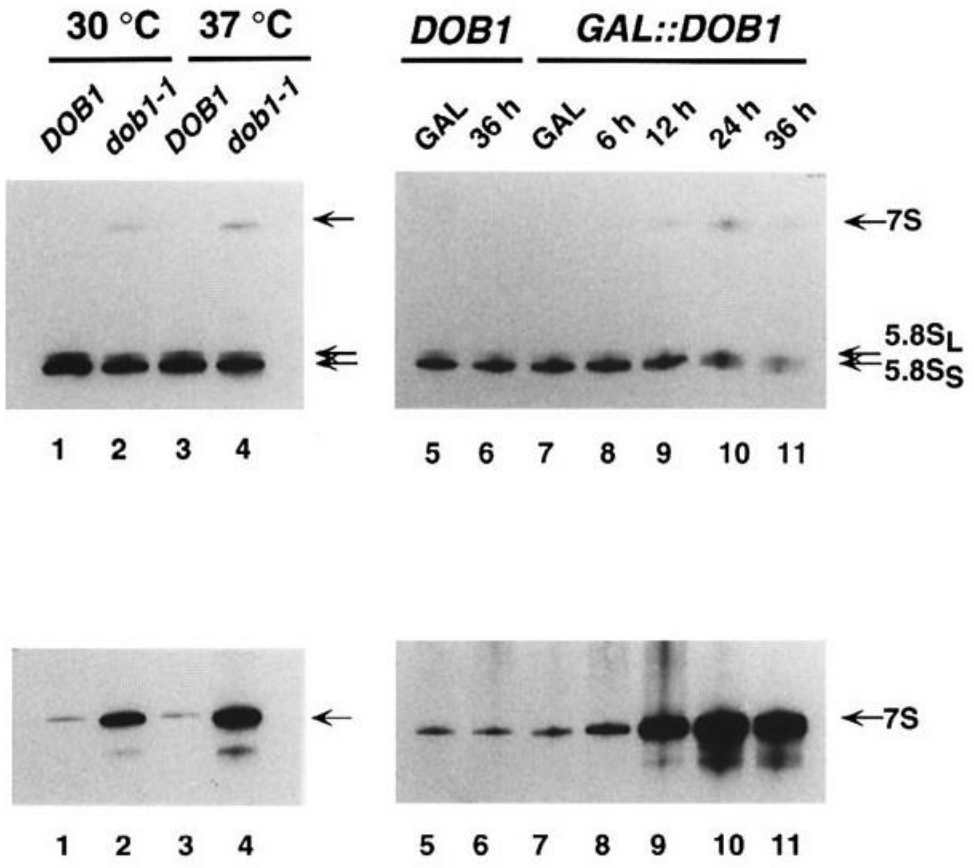


Dob1/Mtr4 is NOT sufficient to stimutate exosome activity *in vitro*

Cofactors for the exosome complexes

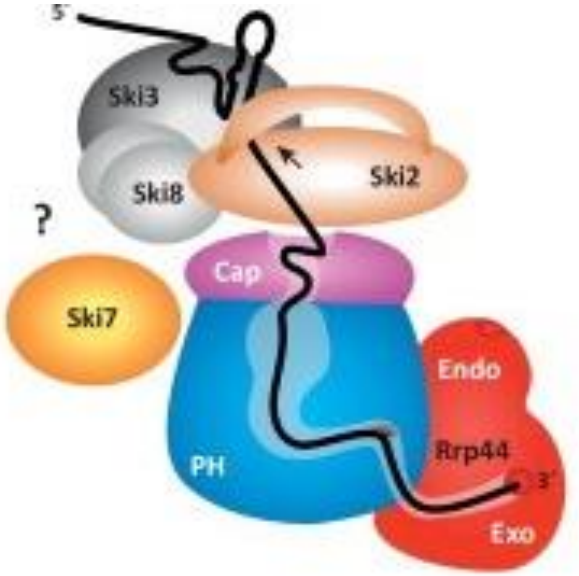
DOB1/MTR4: putative ATP-dependent RNA helicase





This defect resembles the phenotype described for rrp4-1

Cofactors for the exosome complexes



Cytoplasmic exosome

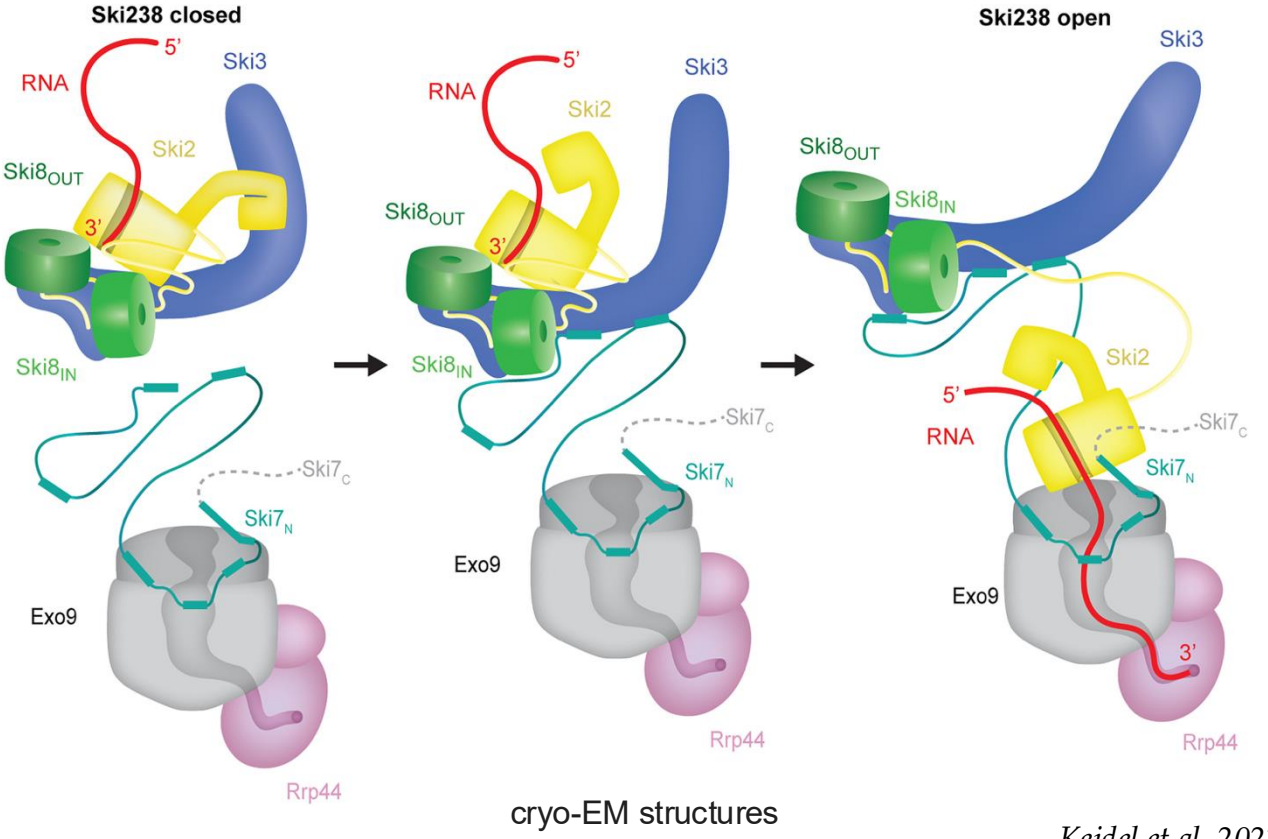
Ski2p - DEAD box putative ATP-dependent RNA translocase (helicase)

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

Function:

- mRNA degradation

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

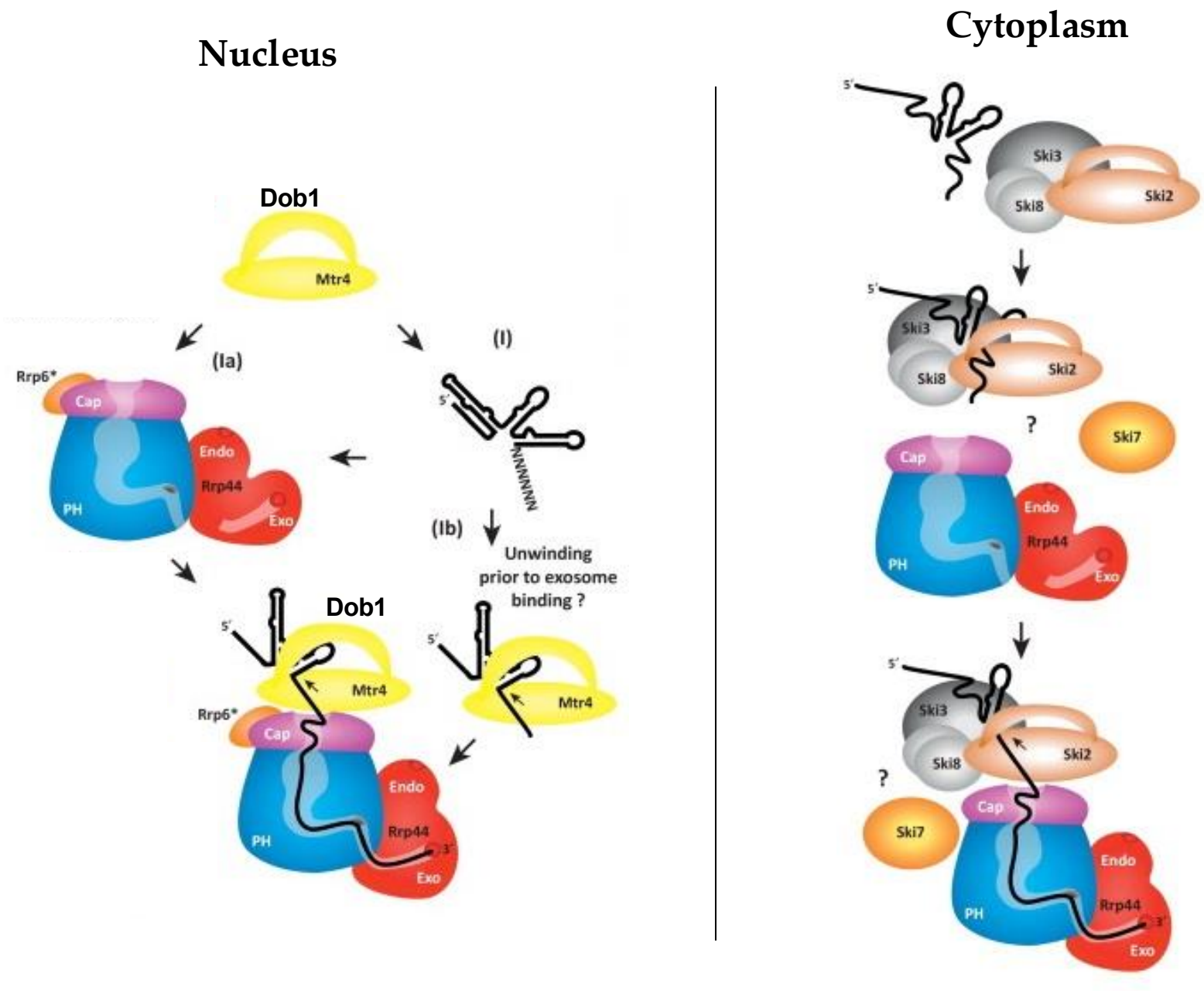


cryo-EM structures

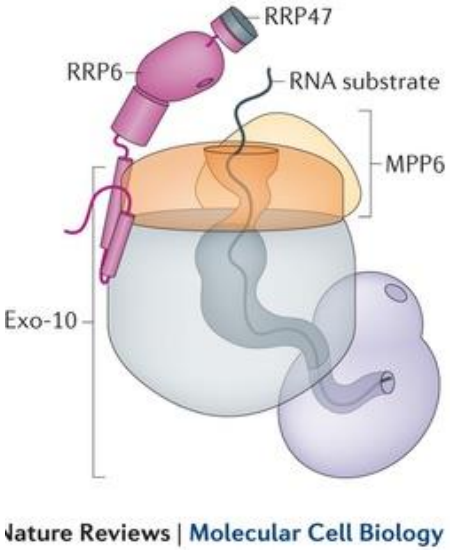
Keidel et al. 2023. Mol Cell

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

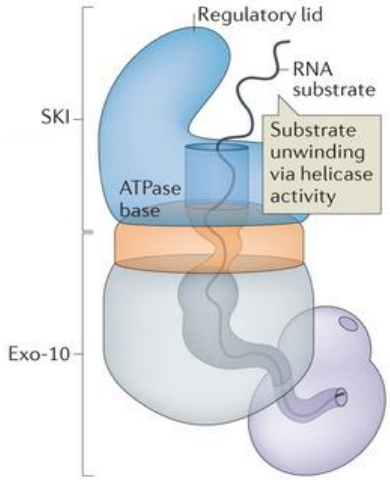


Functions of the exosome complexes



Nuclear exosome

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



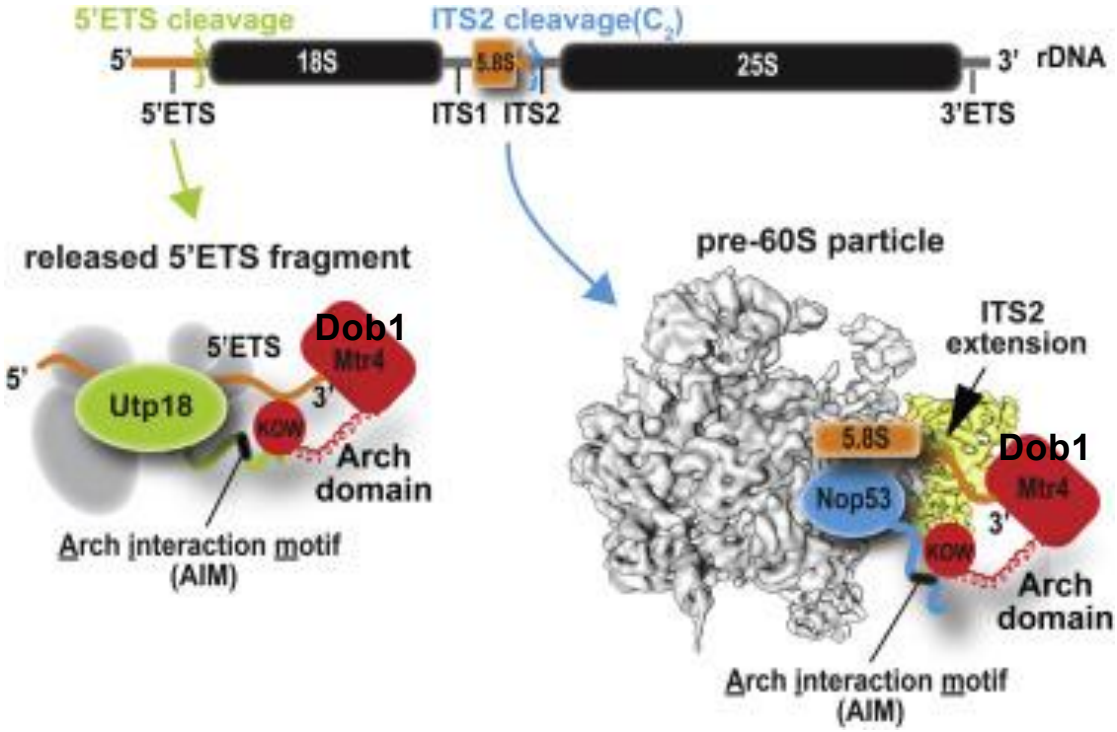
Cytoplasmic exosome

- mRNA degradation

How do so few RNase activities process so many classes of RNA?

The Exosome Is Recruited to RNA Substrates through Specific Adaptor Proteins

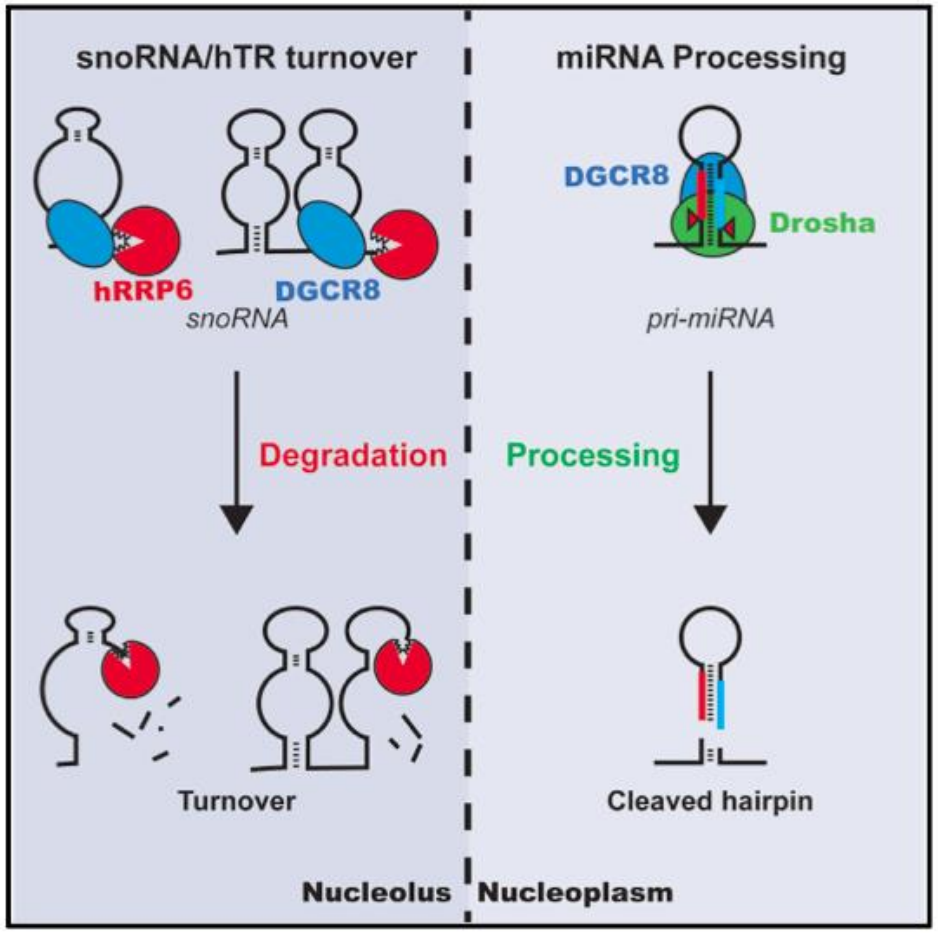
Nop53 and Utp18 proteins target Mtr4/Dob1 to pre-ribosomal particles for exosome-mediated processing. Both Nop53 and Utp18 contain the same consensus motif, through which they dock to the "arch" domain of Mtr4 and target it to specific substrates



Thoms et al., 2015 Cell

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

Assembly into RNPs Prevents Exosomal Degradation of ncRNAs

Steric Blockade Strategy:

Nascent pre-ncRNAs escape destruction only by binding core protein subunits to form **Ribonucleoprotein Particles (RNPs)**.

Structural Barrier:

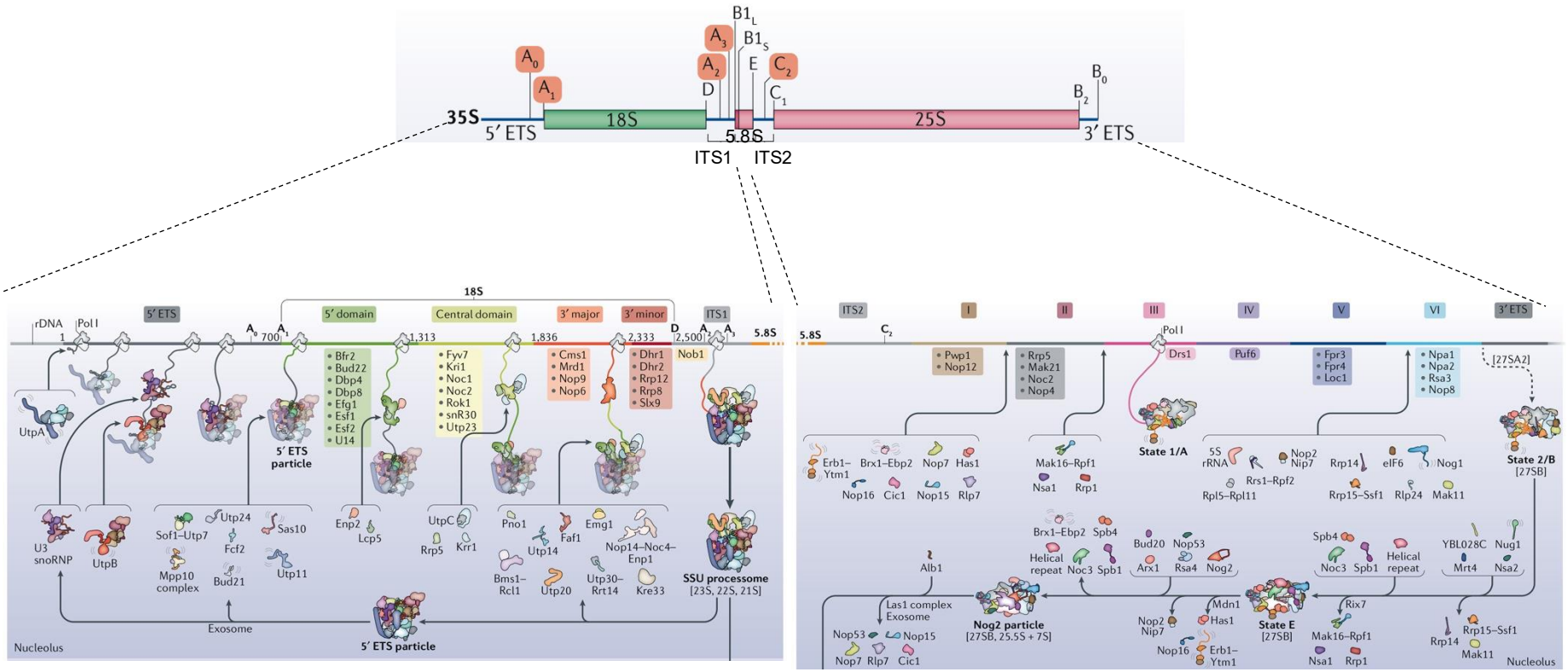
Assembled protein cores physically block the entry channel of the exosome or stop its catalytic progress at defined nucleotides.

Unassembled / Defective ncRNAs:

Naked or misfolded transcripts are targeted for **rapid degradation**.

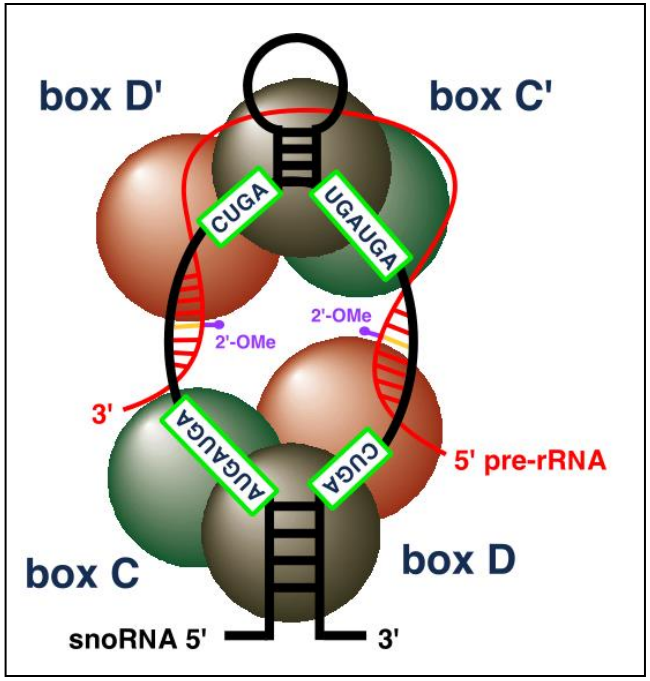
Assembly of the ribosomal subunits

The early stages of nucleolar ribosome biogenesis occur co-transcriptionally. Over 200 trans-acting non-ribosomal factors—including individual proteins, multi-protein complexes, and small nucleolar ribonucleoproteins (snoRNPs)—are required for the assembly and maturation of the small and large ribosomal subunits.



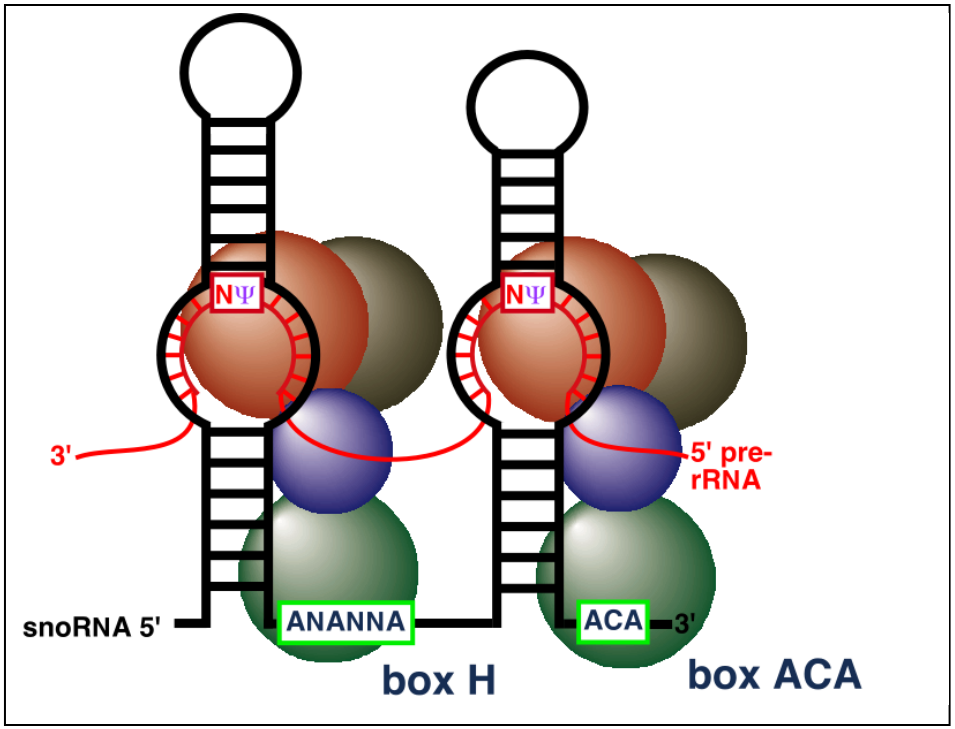
Klinghe & Woolford, 2022. Nat Rev Mol Cell Biol.

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



Nop1p/Fibrillarin
Nop56p
Nop58p
Snu13p

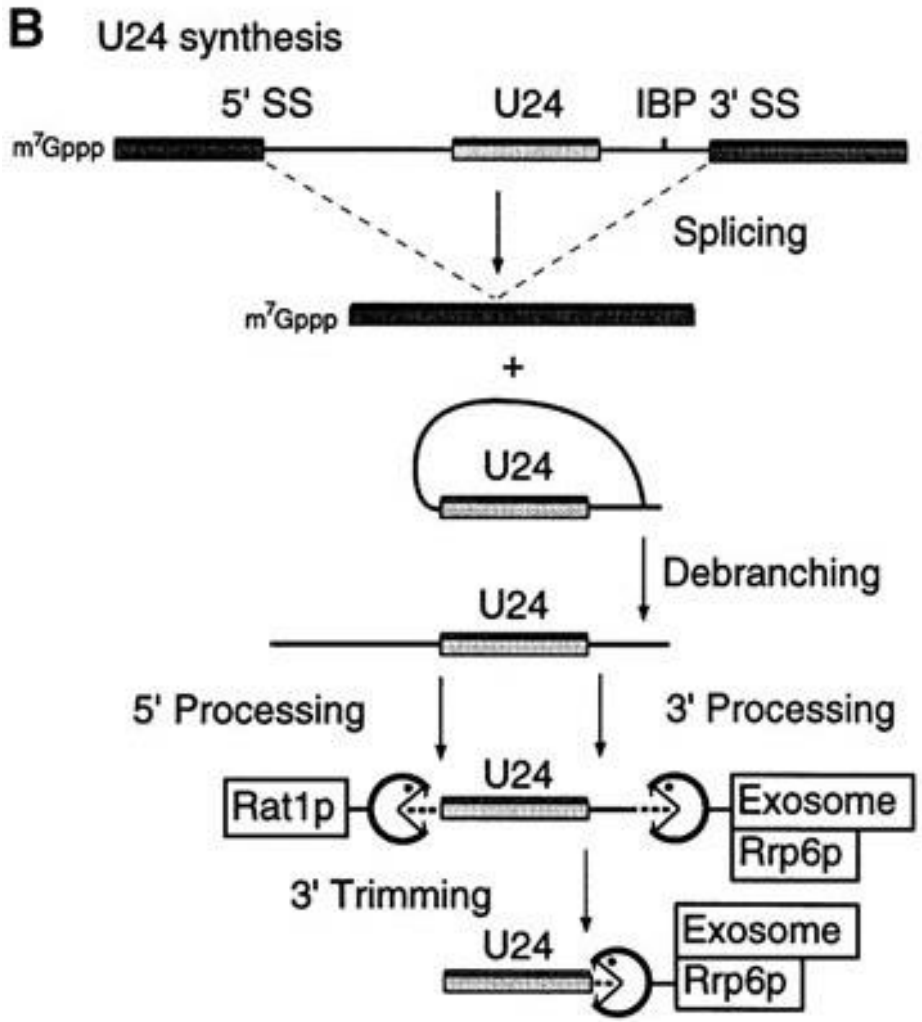
Box H + ACA snoRNAs
Direct Ψ Formation



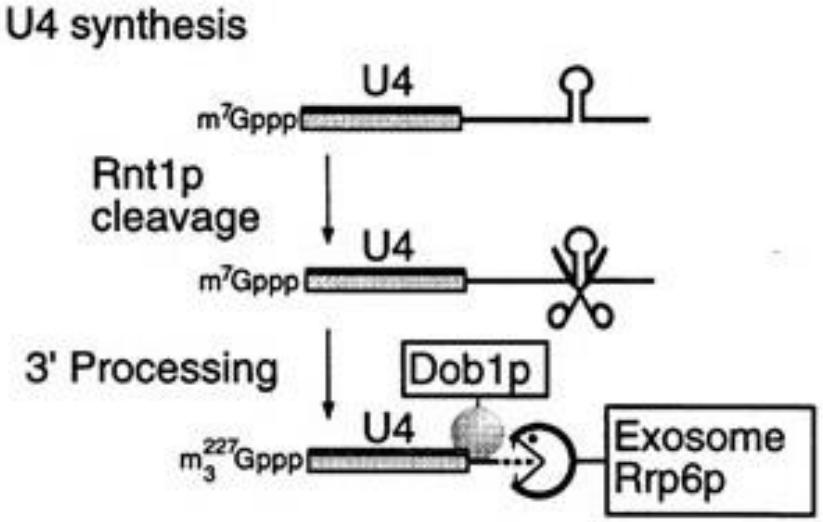
Cbf5p/Dyskerin
Nhp2p
Gar1p
Nop10p

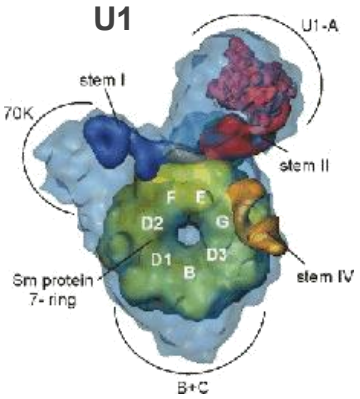
WELLCOME TRUST CENTRE *for* CELL BIOLOGY

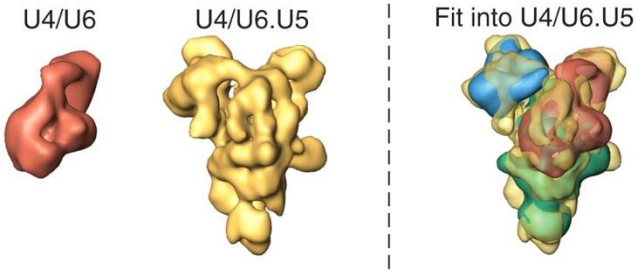
Function of the exosome in snoRNA synthesis



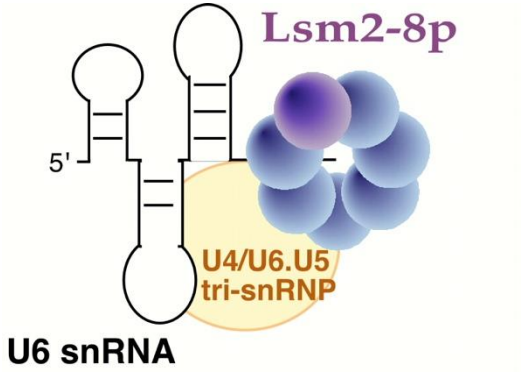
Function of the exosome in snRNA synthesis







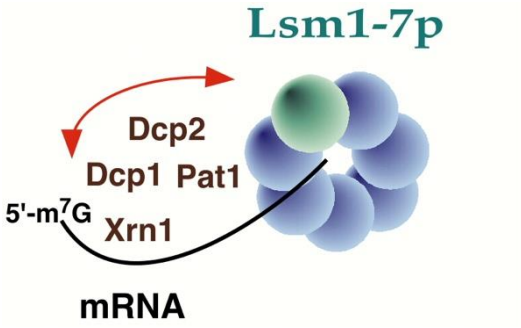
Protection of ncRNAs from Exosomal Degradation by LSM Complexes



Associates with U6 snRNA

Required for U6 RNA accumulation and U6 snRNP biogenesis

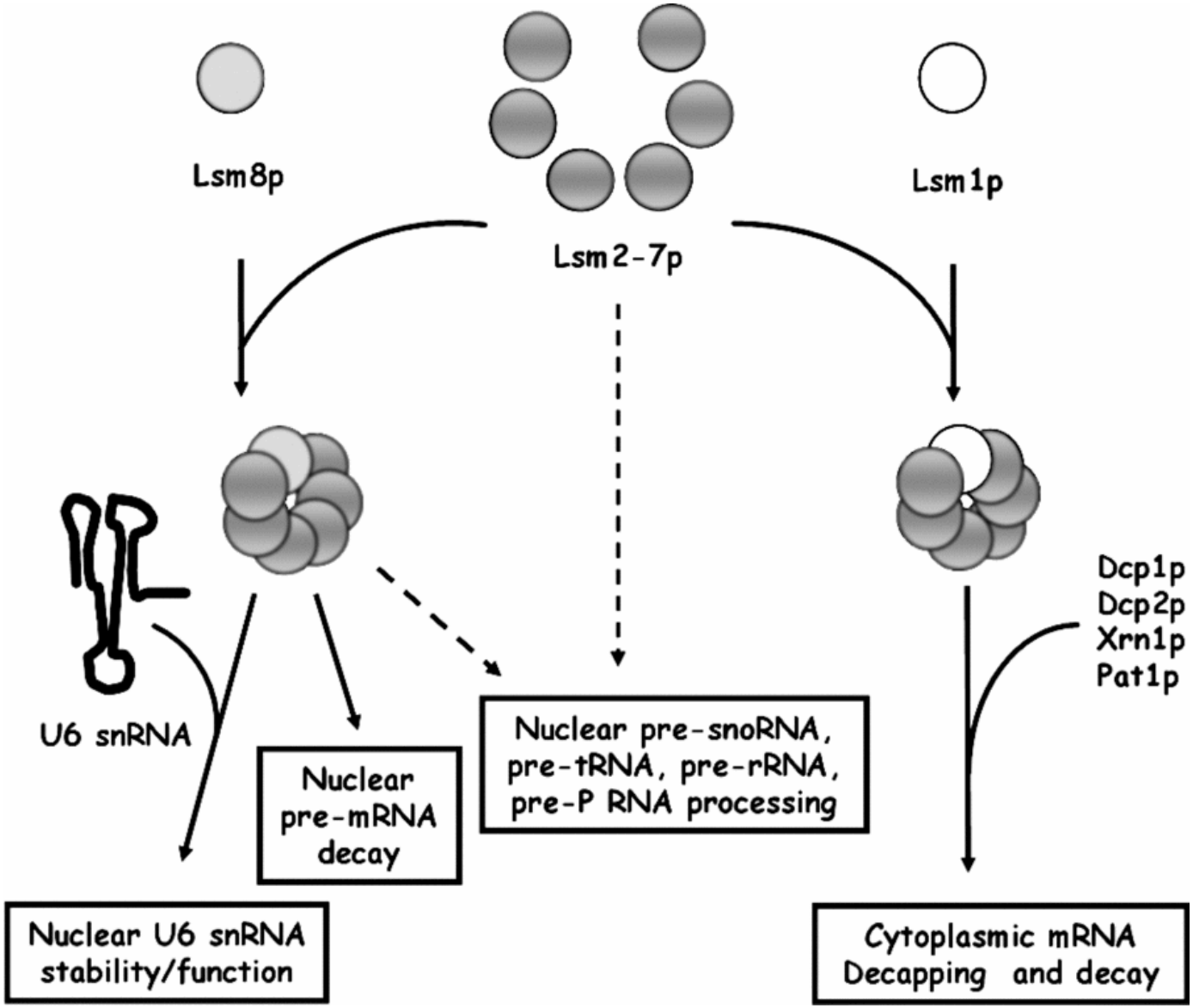
Interacts with the U4/U6.U5 tri-snRNP



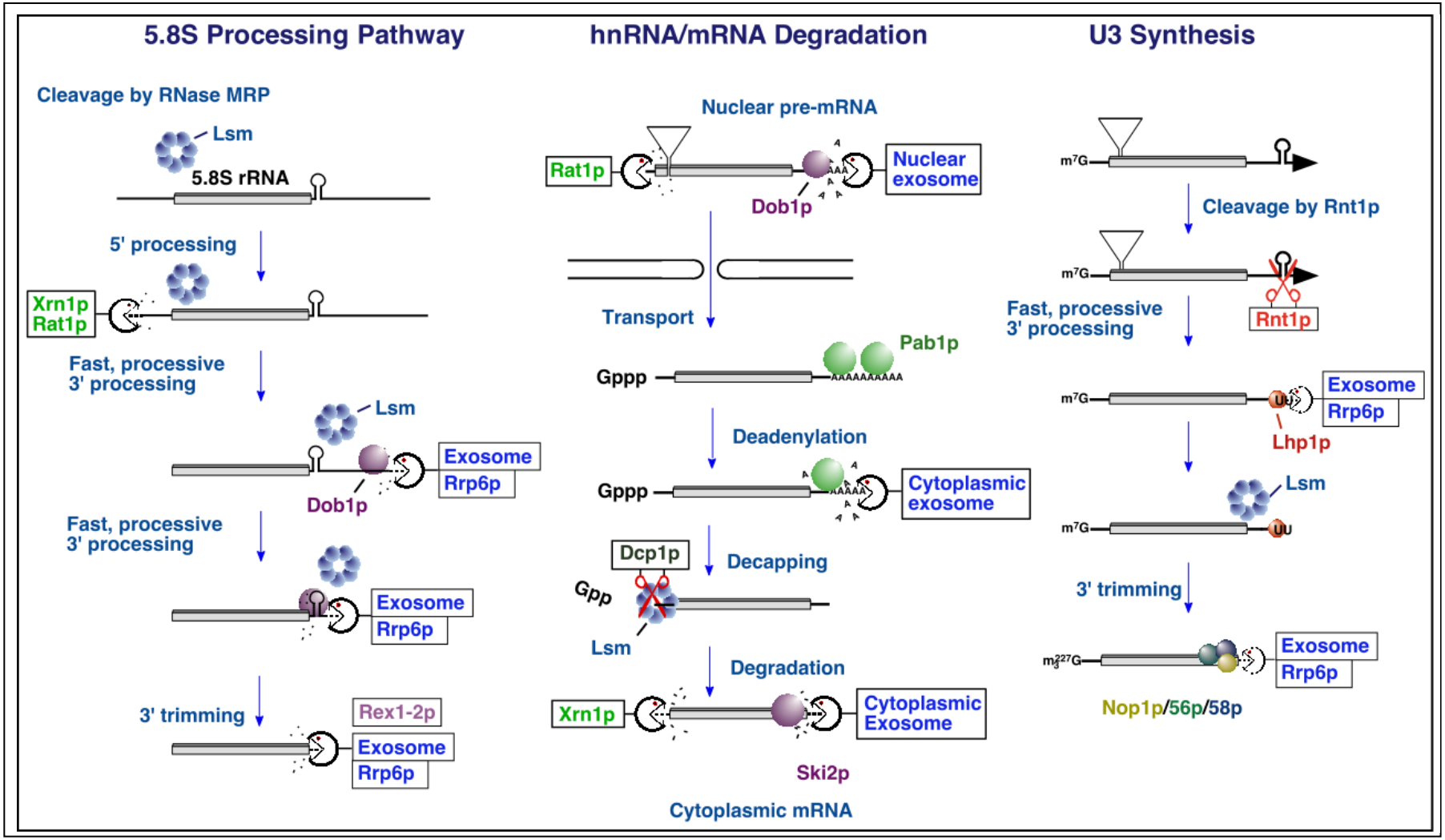
Functions in mRNA decapping and decay

Interacts with components of the mRNA decapping and degradation machinery

There are at least two separate Lsm protein complexes



Common factors in RNA processing pathways



Common factors in RNA processing pathways

