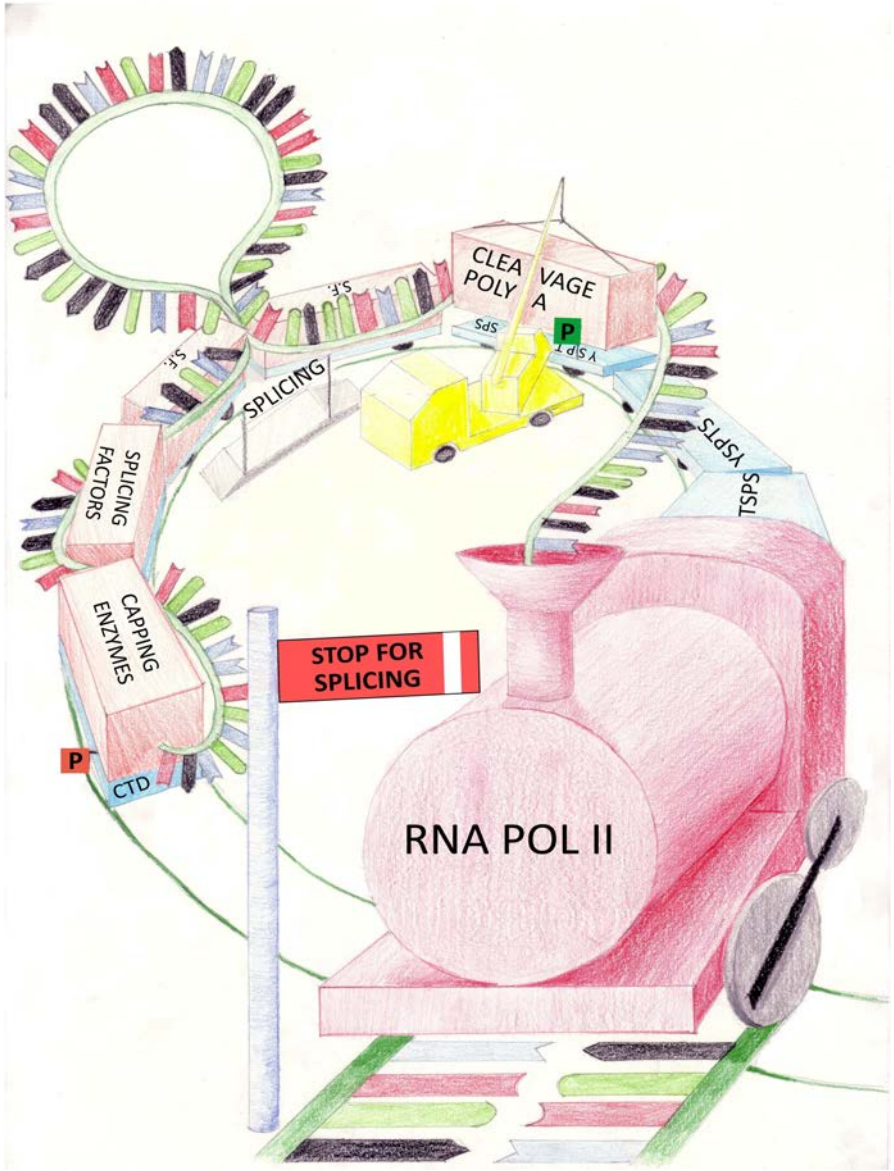
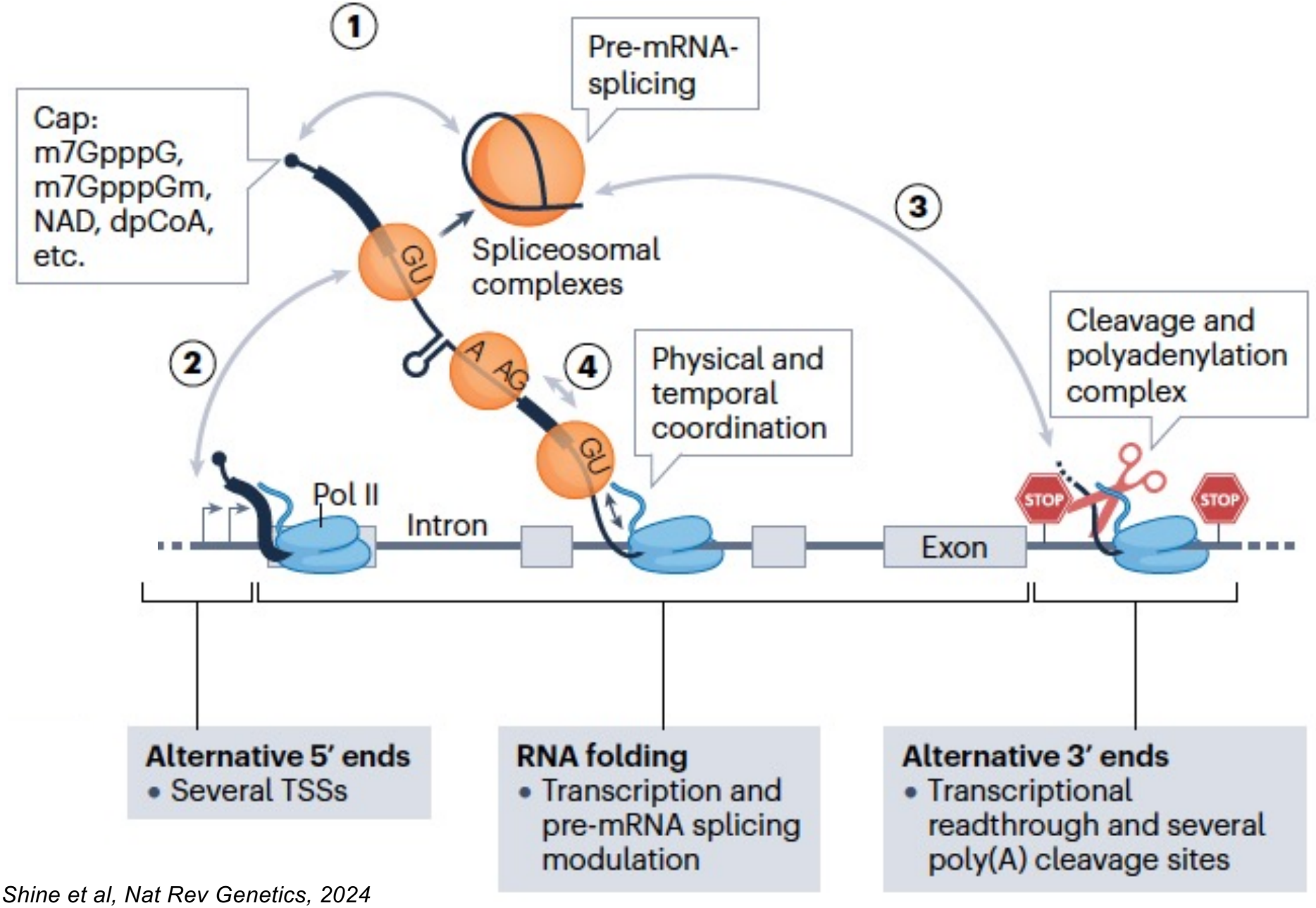
RNA PROCESSING

Co- or post- transcriptional?



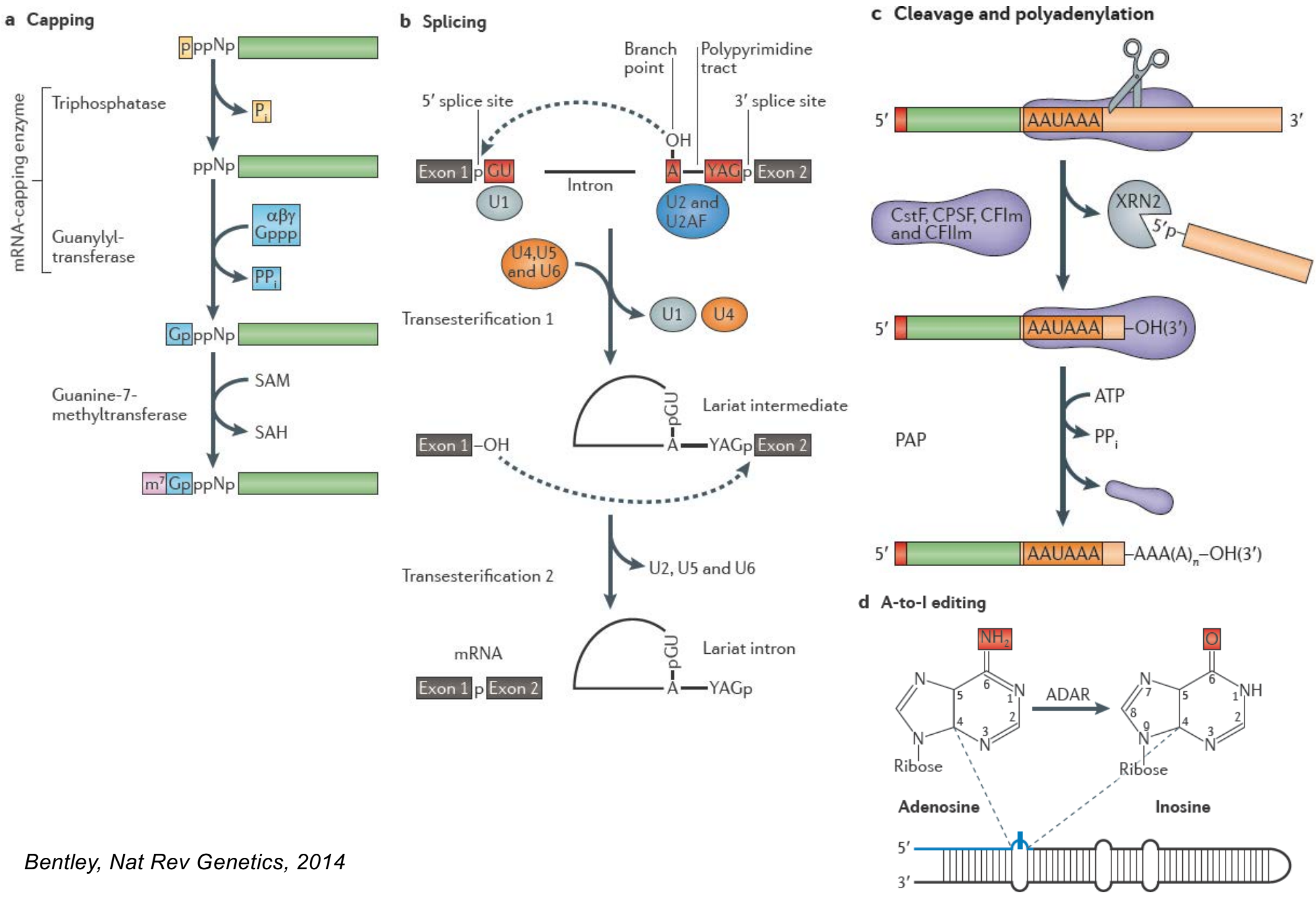
Chrissie Barrass, 2011, cover of Mol. Cell

Co-transcriptional mRNA processing steps



Shine et al, Nat Rev Genetics, 2024

Co-transcriptional mRNA processing steps

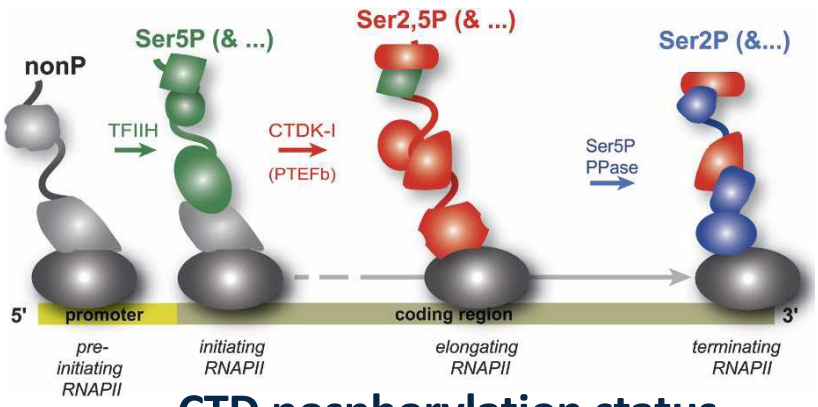


Bentley, Nat Rev Genetics, 2014

Co-transcriptional mRNA processing

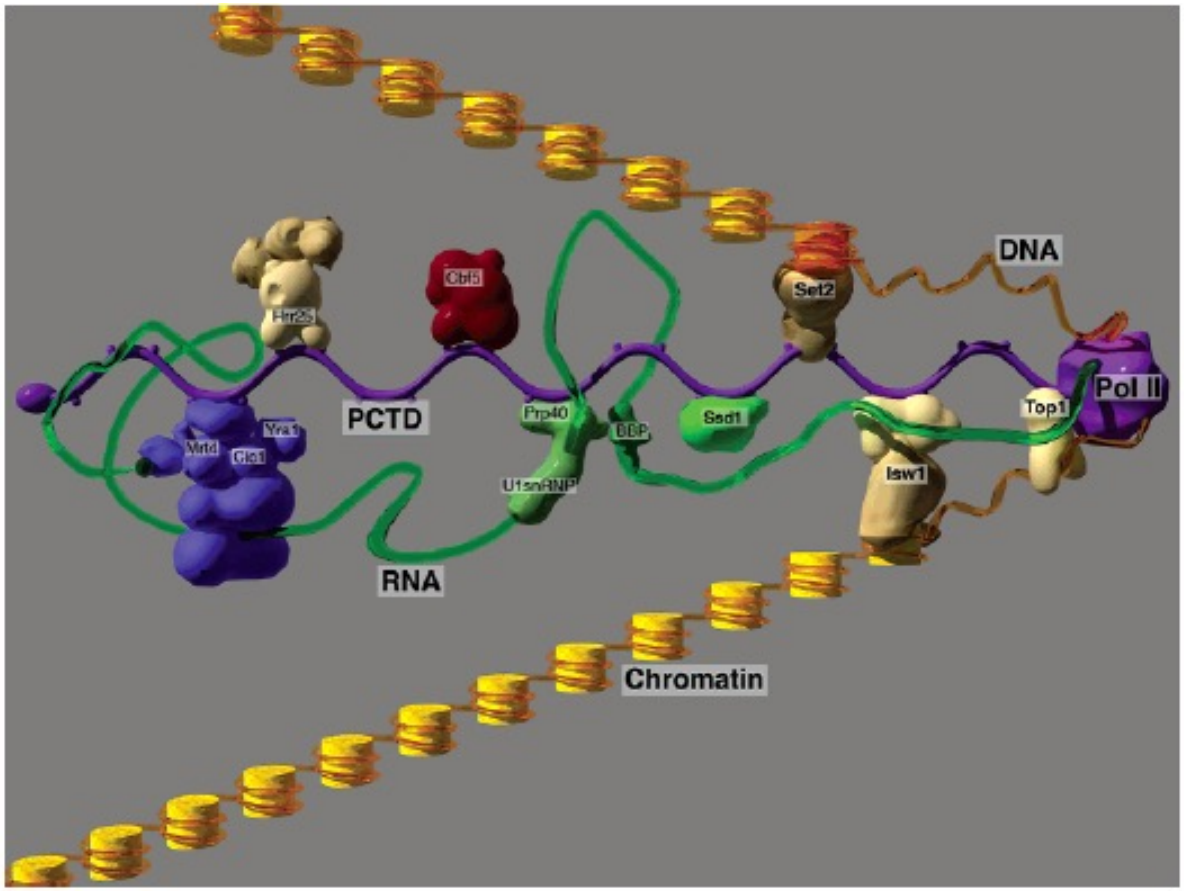
Phospho-CTD Associated Proteins

- **Transcription**
  - Mediator
  - Elongation factors
  - Termination factors
  - CTD Kinases/Phosphatases
- **chromatin structure**
  - Histone methyltransferases
- **RNA processing**
  - Capping enzymes
  - Splicing factors
  - Cleavage/polyadenylation factors
- **RNA export**
- **RNA degradation**
- **snRNA/snoRNA biogenesis**
  - Integrator
- **DNA metabolism**
- **protein synthesis and**
- **degradation**

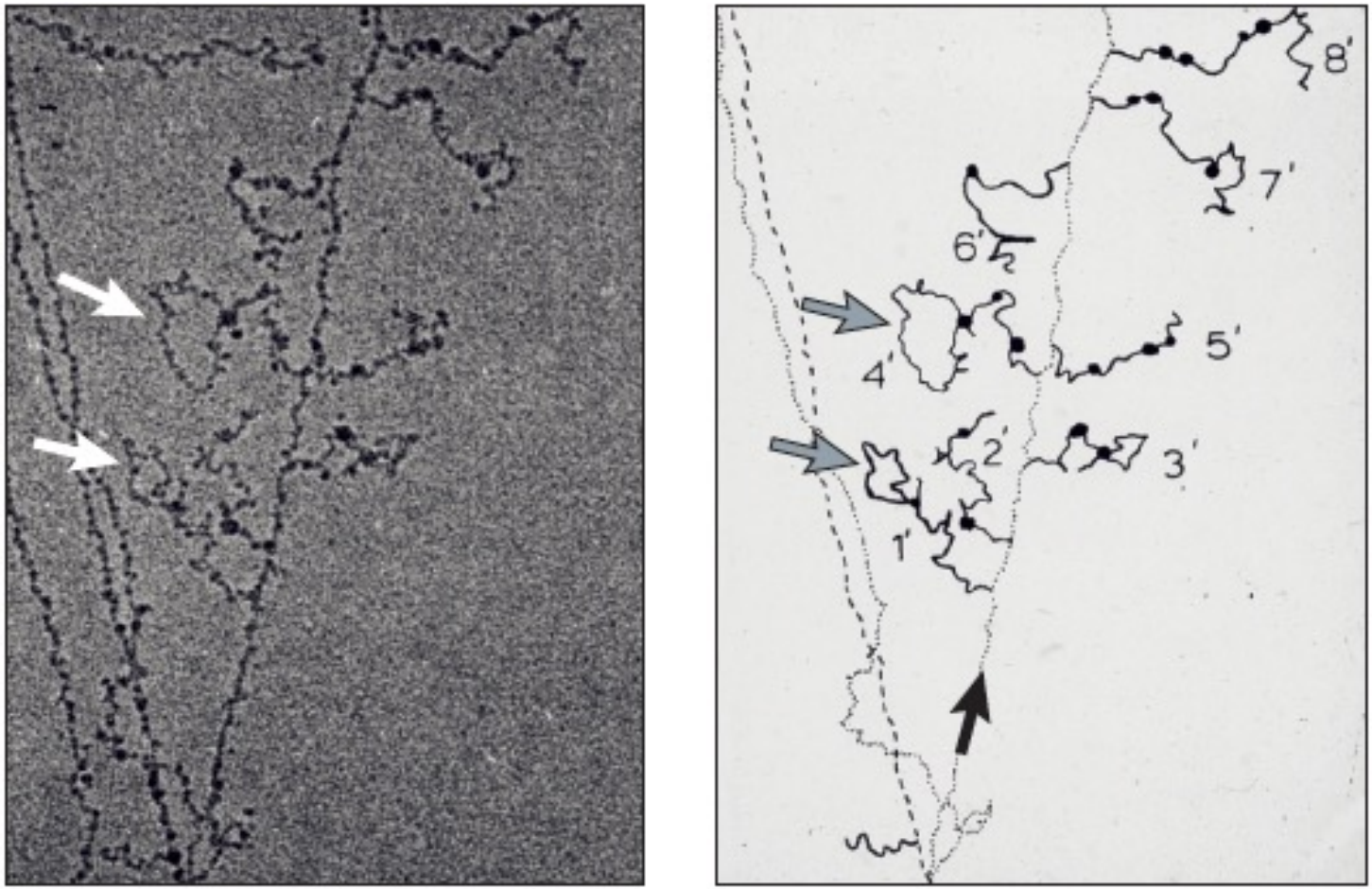


CTD posphorylation status

Phatnani and Greenleaf, 2006



Co-transcriptional mRNA processing

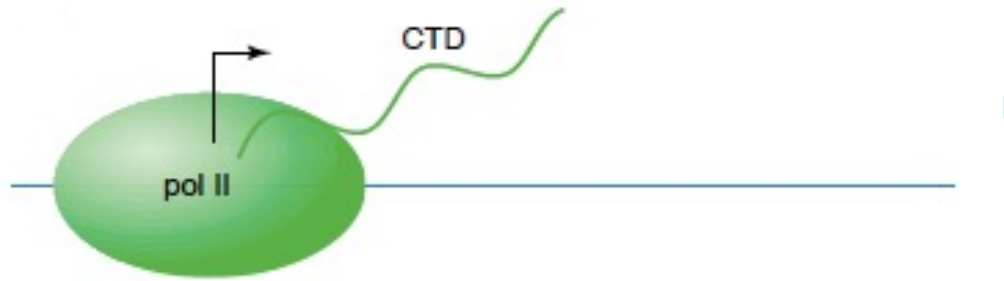


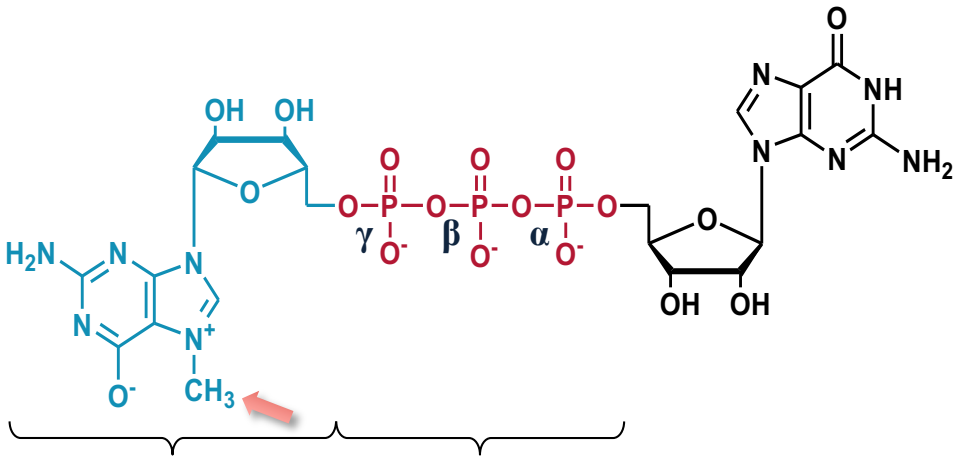
Bentley, Nat. Rev. Genetics, 2014

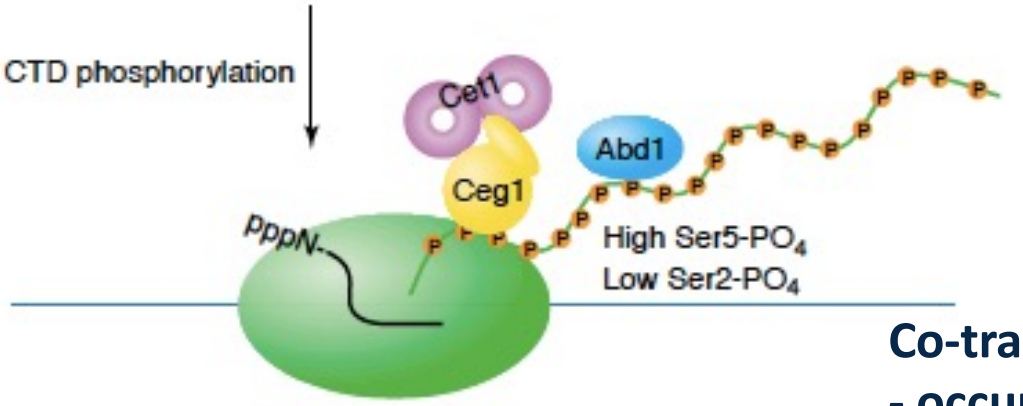
"Miller spread" electron micrograph *(D. melanogaster)*

DNA template + engaged Pol II + nascent RNA transcripts + bound proteins (blobs) + co-transcriptionally spliced out introns (arrows)

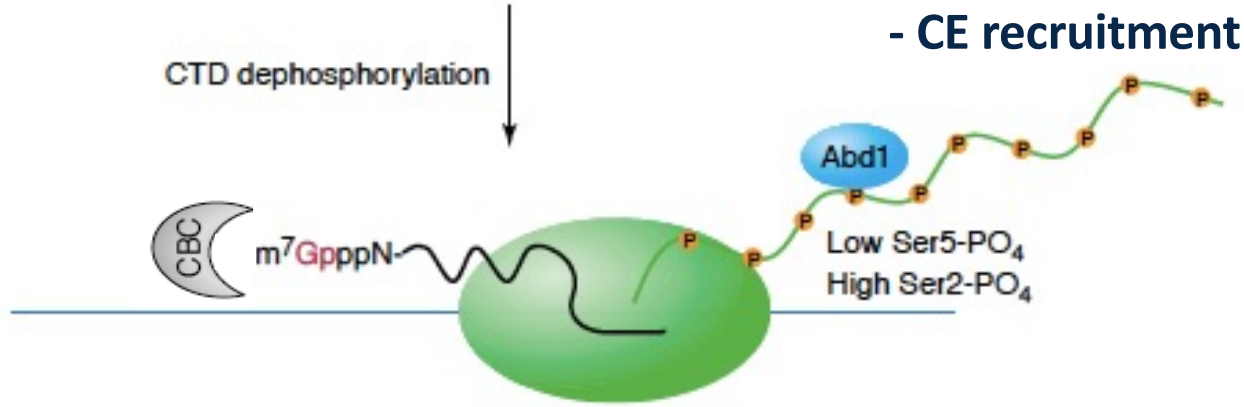
Capping







Co-transcriptional capping by Capping Enzymes
- occurs after the synthesis of 10-15 nt of RNA
- CE recruitment to CTD requires high Ser5-P



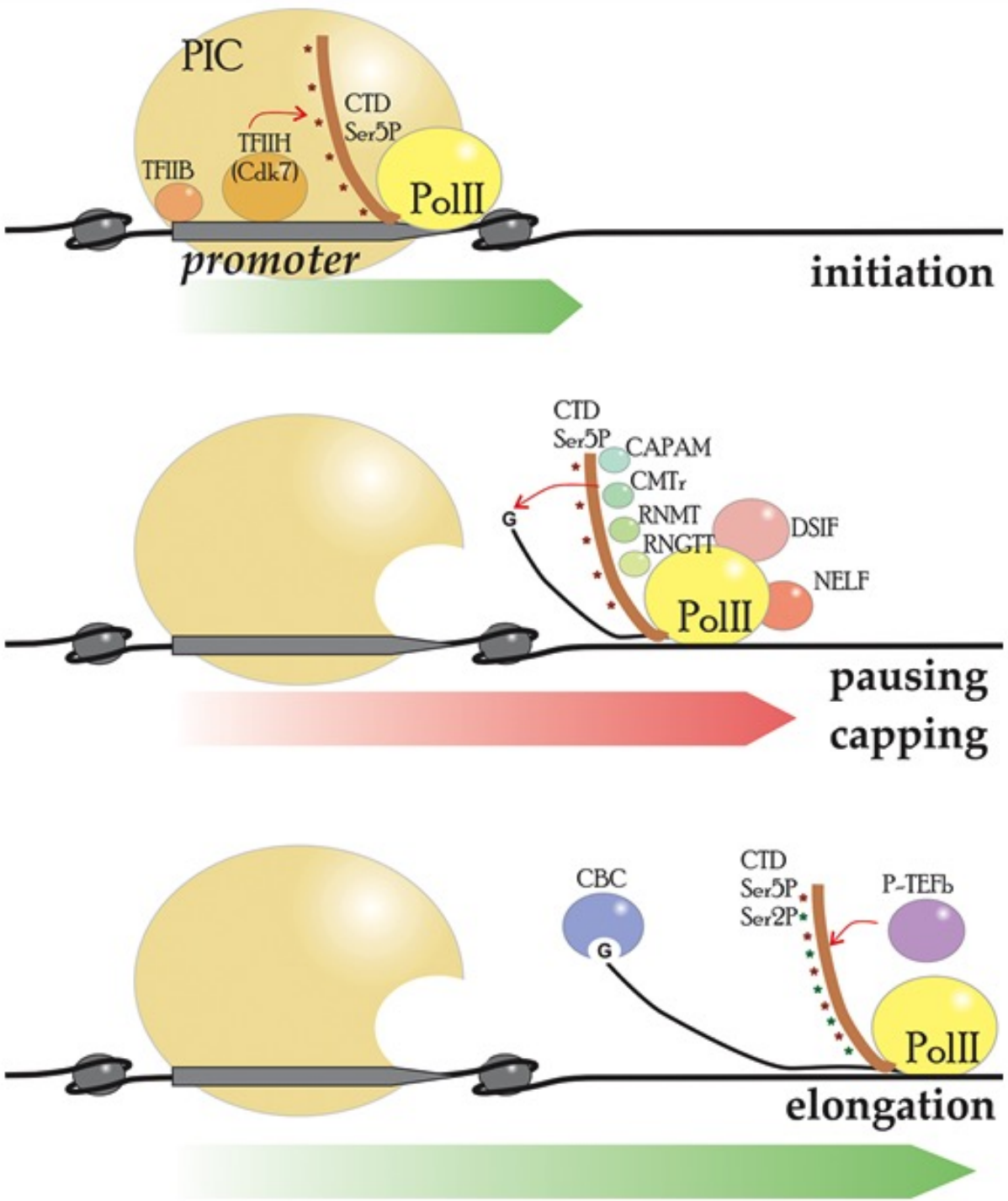
CE

Cet1 - RTase 5'RNA phopshatase
(inhibits re-initiation)
Ceg1 - GTase guanylyltransferase
Abd1 - N7MTase methyltransferase
(promotes early elongation)

CBC- cap binding complex

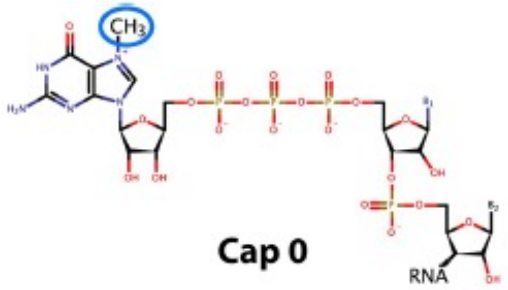
Guo and Lima, Curr Op Str Biol, 2005

Capping and transcription

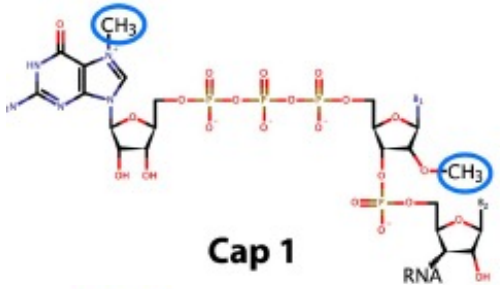


Kachaev et al, Biosci Rep., 2020

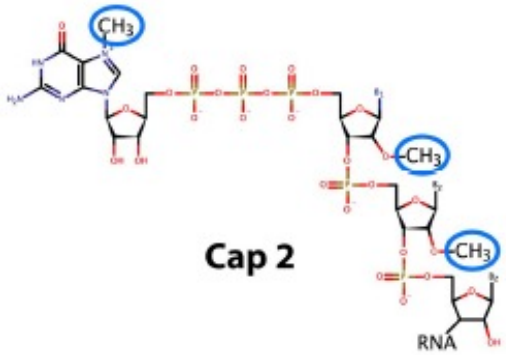
Cap and capping enzymes



mRNA
yeast
plants



mRNA
mammals



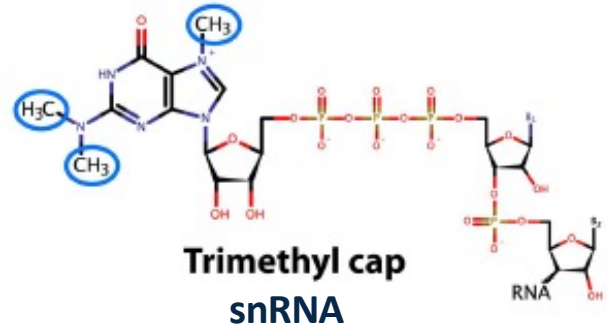
Capping Enzymes (CE)

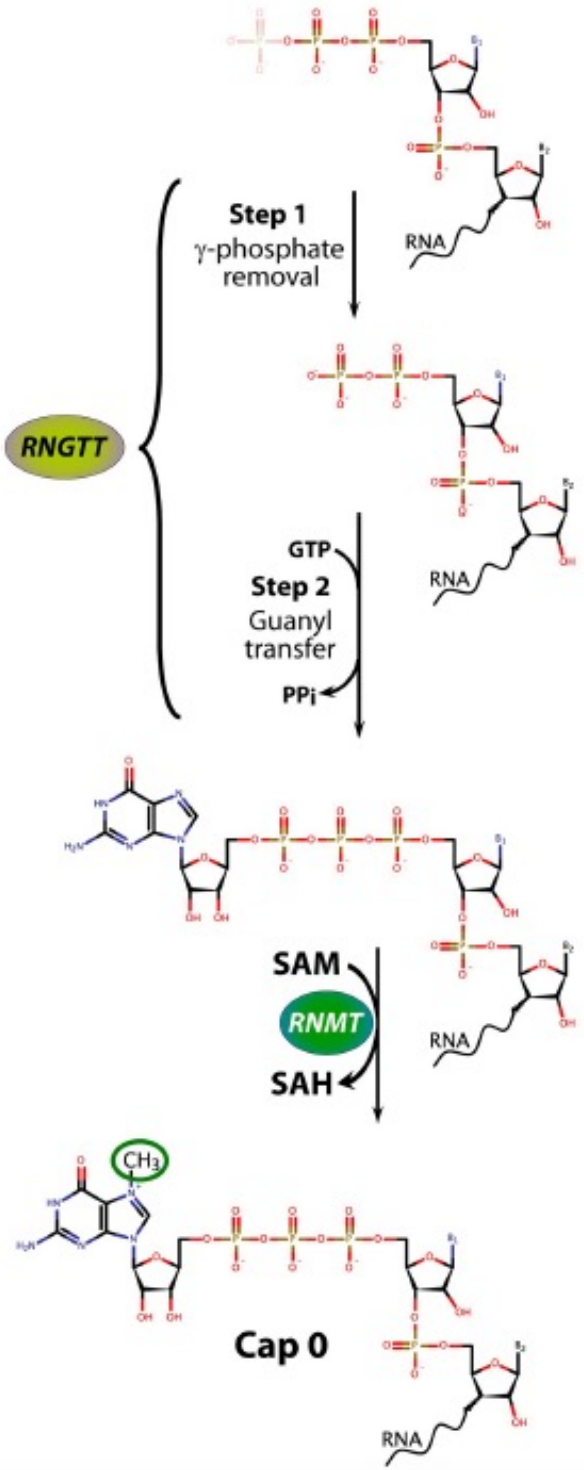
Yeast

Cet1 - RTase 5'RNA phopshatase
Ceg1 - GTase guanylyltransferase
Abd1 - N7MTase methyltransferase

Mammals

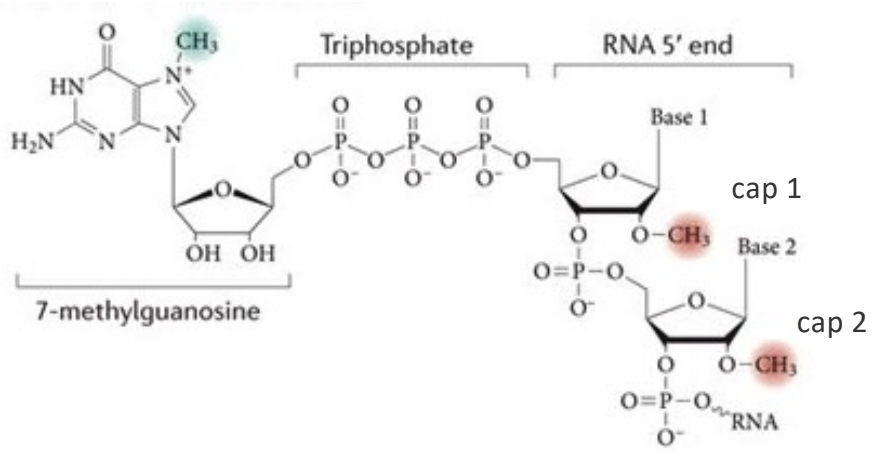
RNGTT - RTase and GTase
RNMT - N7MTase
CMTR1, CMTR2 - 2'OMTase





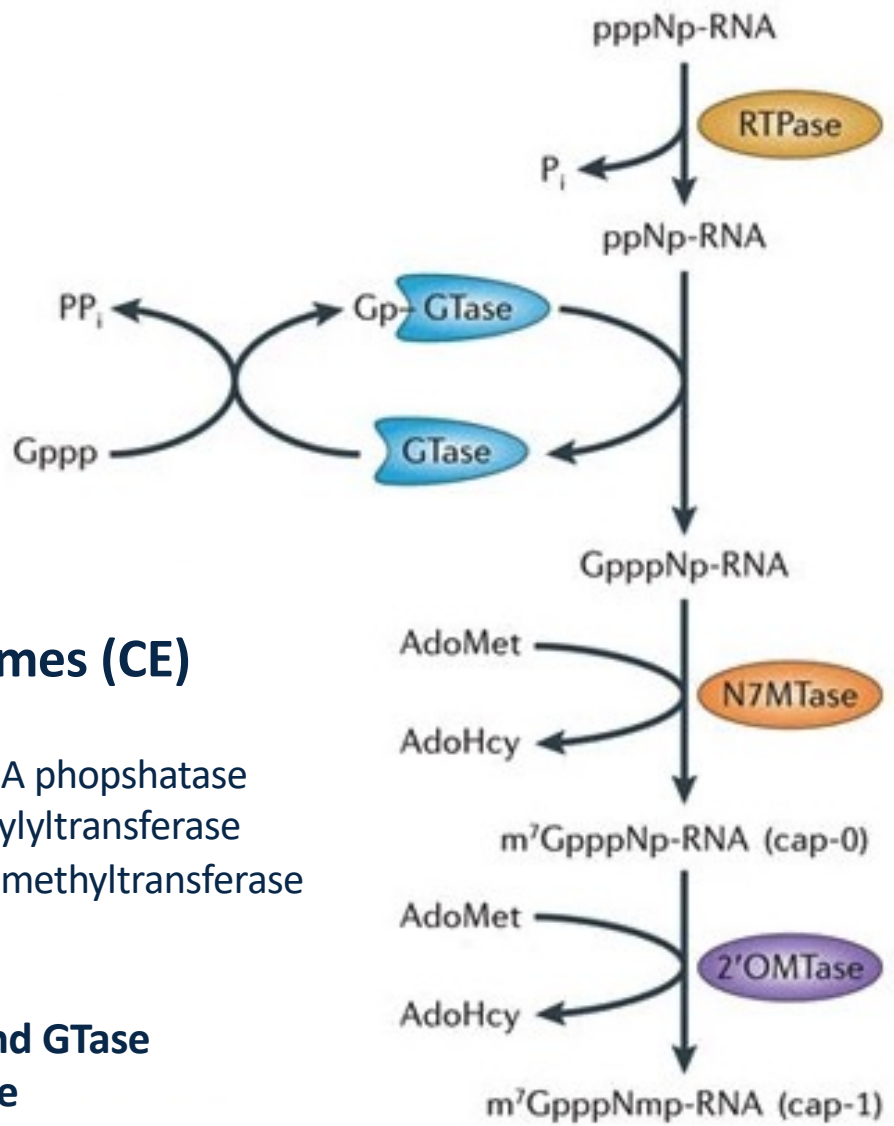
Borden et al, Cell Cycle, 2021

Cap and capping enzymes



Cap 0 (no 2'-O me)
yeast plants

Cap 1, Cap 2
mammals



Capping Enzymes (CE)

Yeast

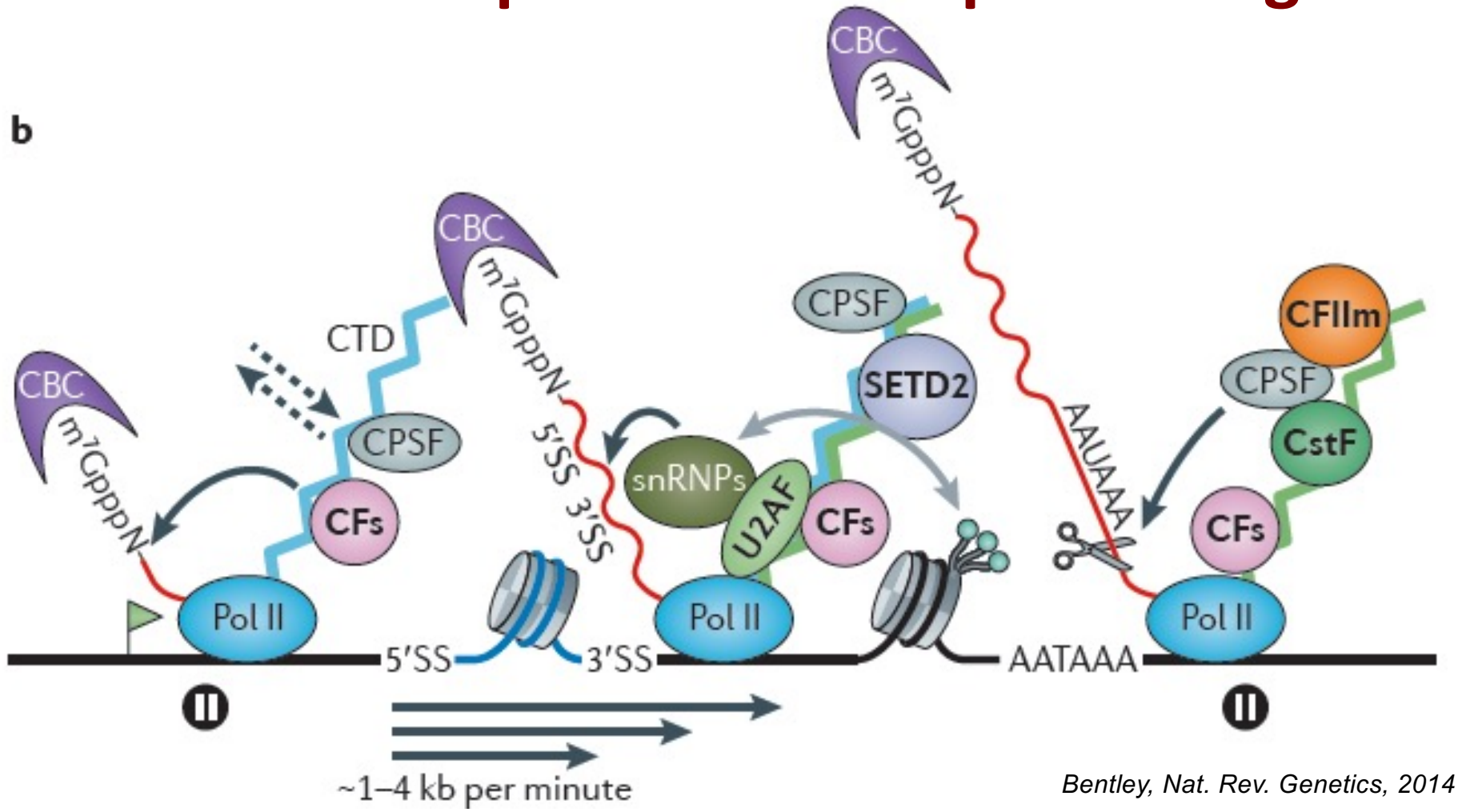
Cet1 - RTase 5'RNA phopshatase
Ceg1 - GTase guanylyltransferase
Abd1 - N7MTase methyltransferase

Mammals

RNGTT - RTase and GTase
RNMT - N7MTase
CMTR1, CMTR2 - 2'OMTase

Decroly et al, Nat Rev Micro, 2012

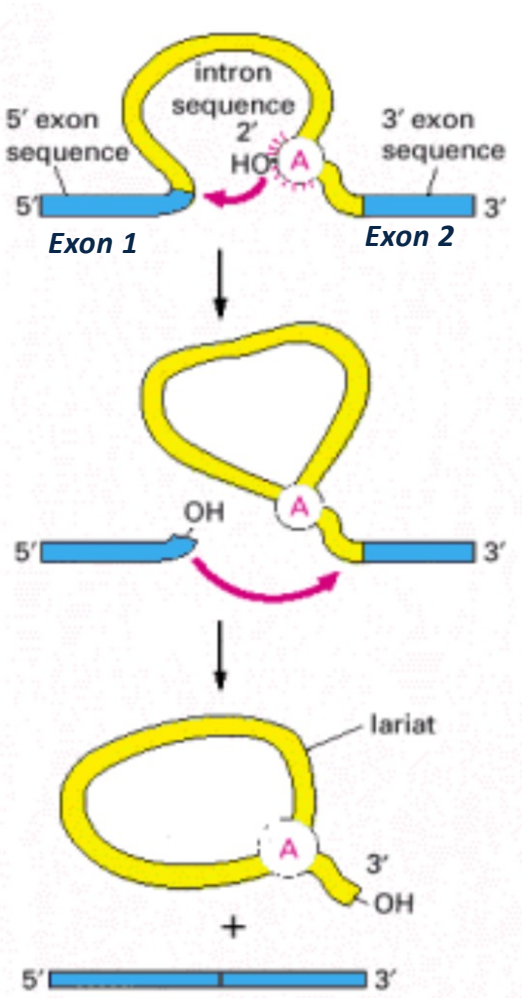
Co-transcriptional mRNA processing



Bentley, Nat. Rev. Genetics, 2014

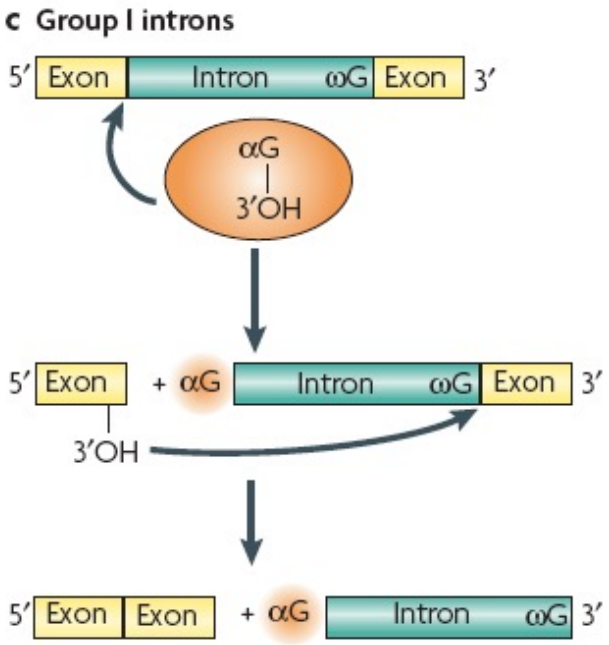
- **Chromatin** modified histones affect transcription and recruitment of processing factors
- **Pol II-CTD** recruits processing factors
  Capping enzymes, spliceosome, termination and 3' cleavage and polyadenylation machinery
- **Nascent RNA** 5' capped and with the 3' poly(A) site AAUAAA signal
- **Proteins** bound to CTD and/or RNA

SPLICING

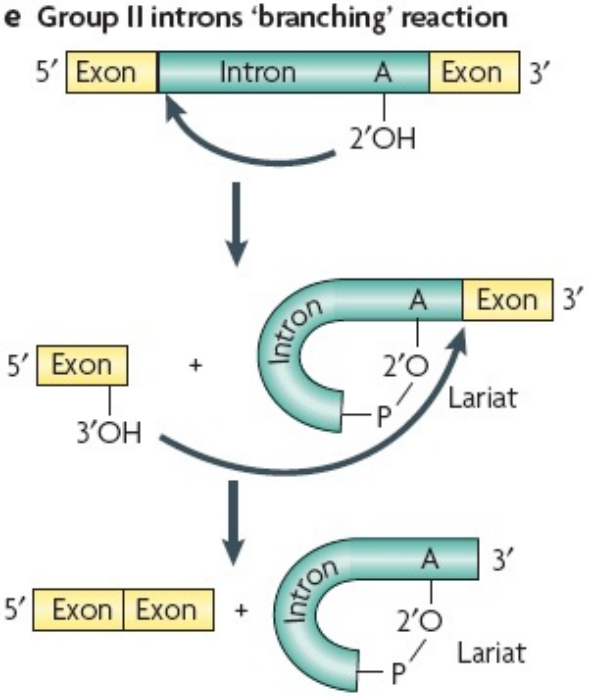


Eukaryotes

RIBOZYMES



organelles (fungi, plants), bacteria, mitochondria (animals)

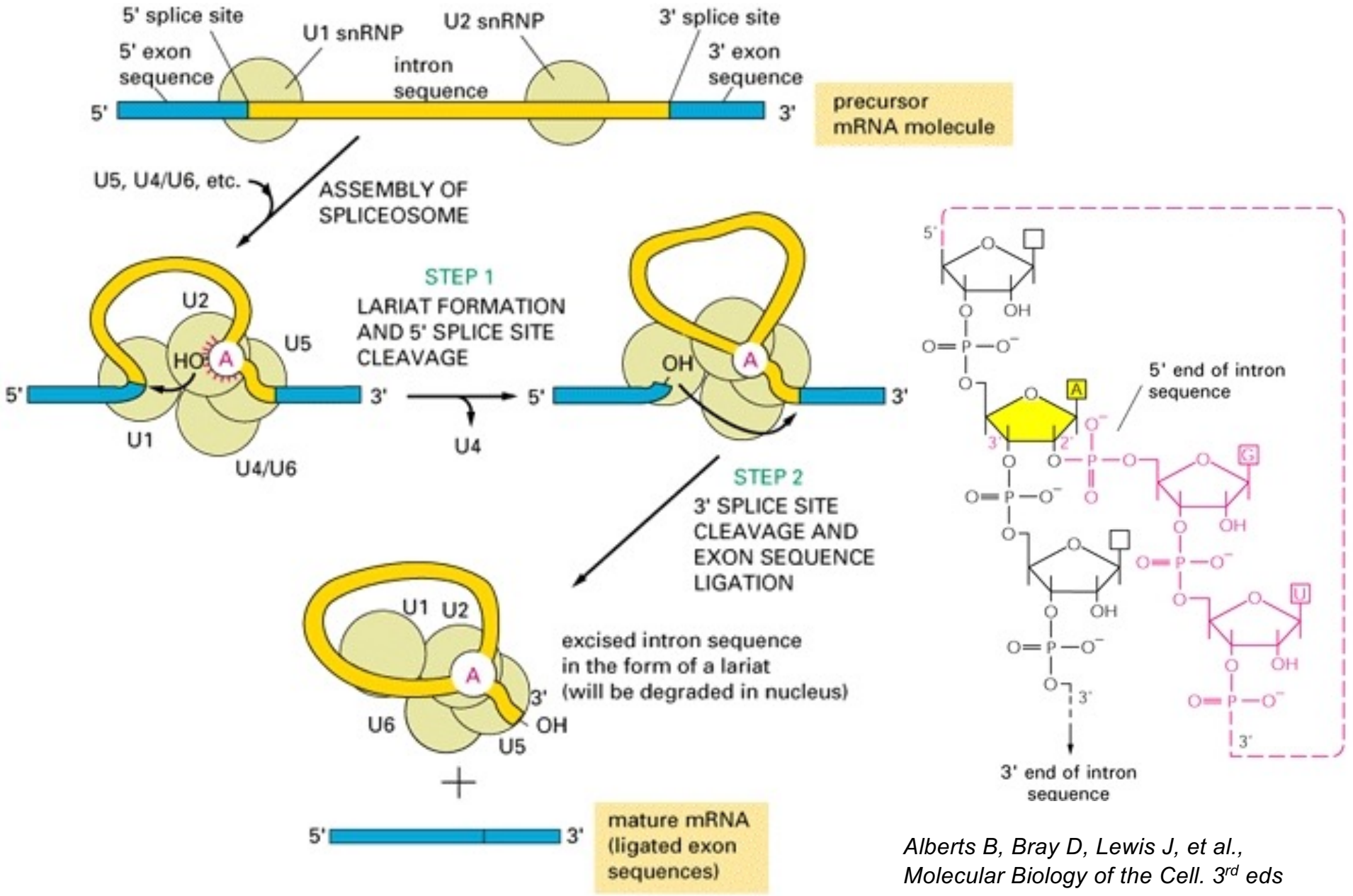


mRNA splicing-like organelles (fungi, plants), bacteria, archea

Serganov and Patel, Nat. Rev. Genet., 2007

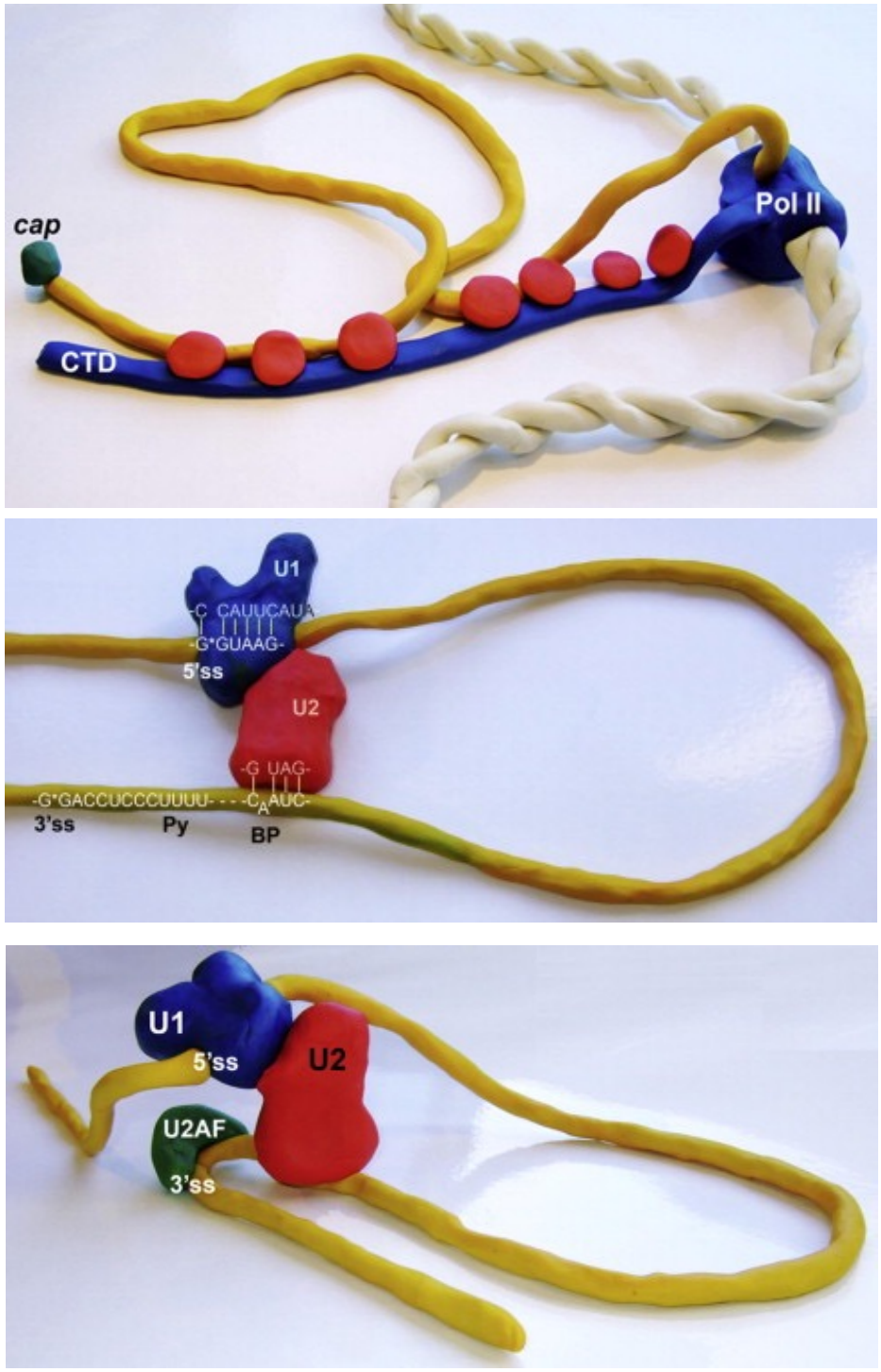
Two step mechanism: nucleophilic attack of the ribose 2'-OH group (branch point Adenosine, H_2O, Me^{2+}) on the phosphate

Pre-mRNA splicing



Alberts B, Bray D, Lewis J, et al., Molecular Biology of the Cell. 3rd eds

Pre-mRNA splicing



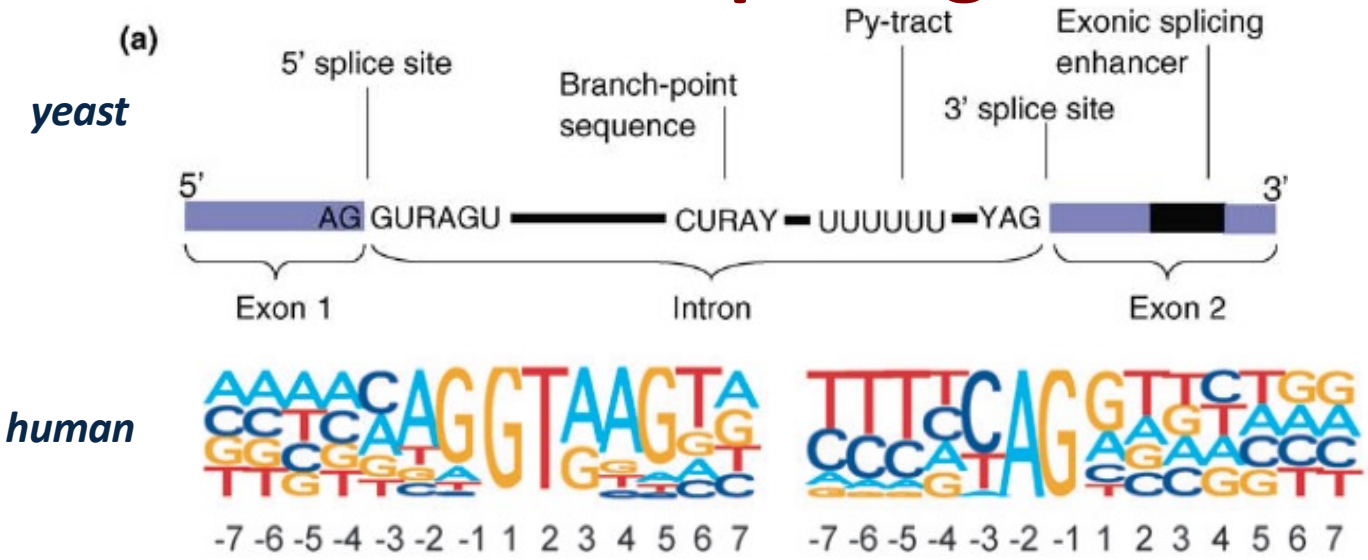
de Almeida and Carmo-Fonseca, FEBS Lett, 2008

Do you know that:

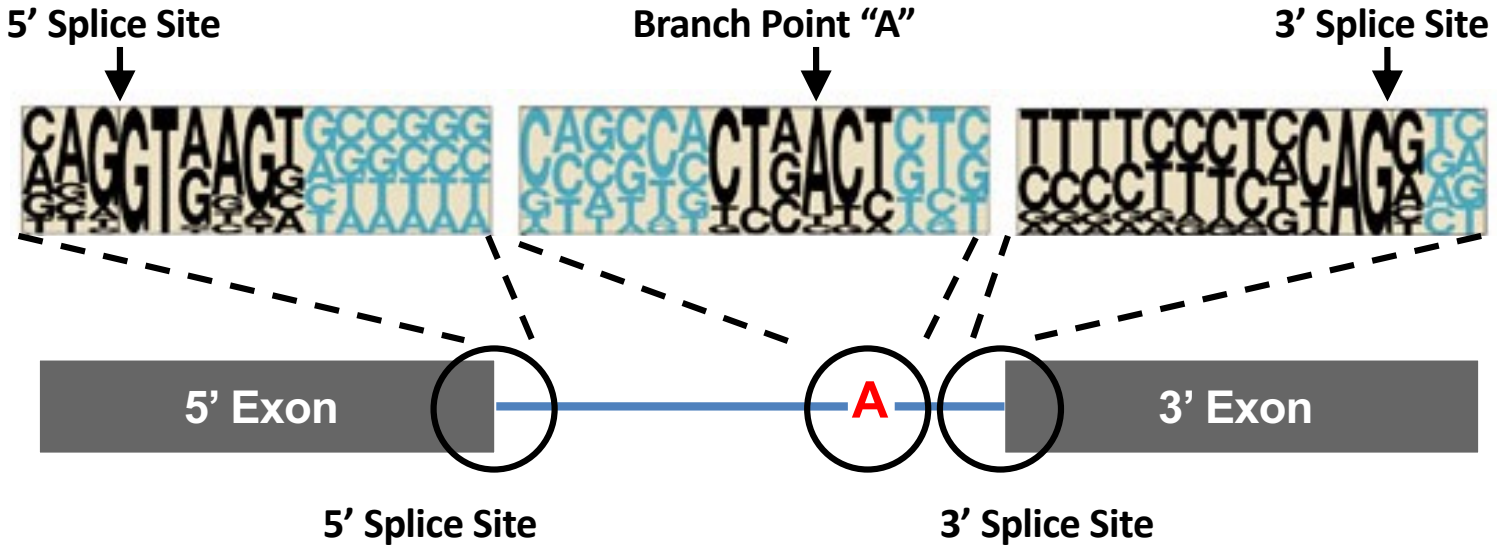
1. Average human pre-mRNA contain 27,000 nucleotides and 9 exons
2. Average exon contain 145 nucleotides
3. There are exons with only 3 nucleotides (one amino acid)
4. Average intron contain 3500 nucleotides
5. Average mRNA contains 1340 nucleotides, so only 5% of pre-mRNA ends up in mRNA
6. Dystrophin contains 3684 amino acids and is encoded by the largest human gene of 2.5 million nucleotides and 79 exons

Zbigniew Dominski, lectures 2008

Pre-mRNA splicing: *cis* elements



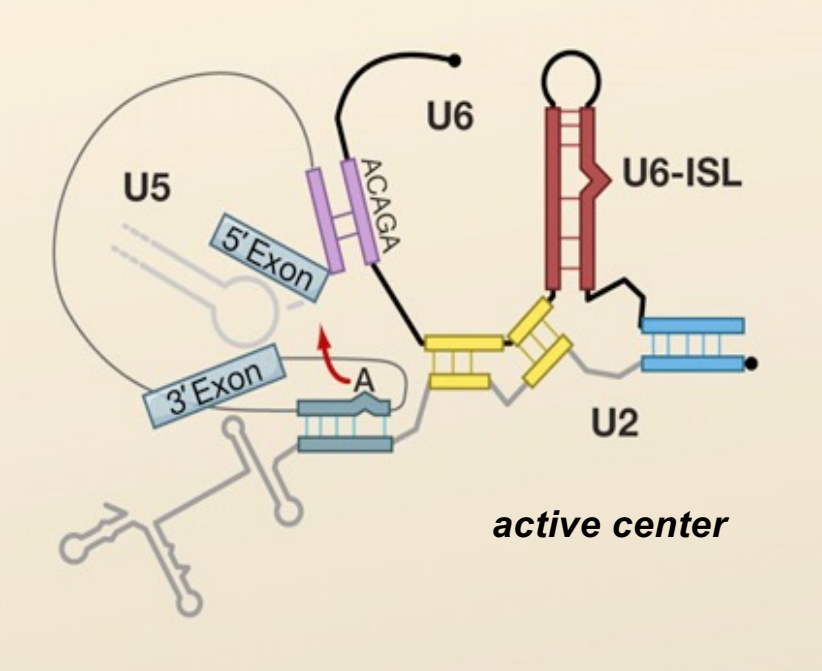
The consensus splicing sequences are not so conserved after all



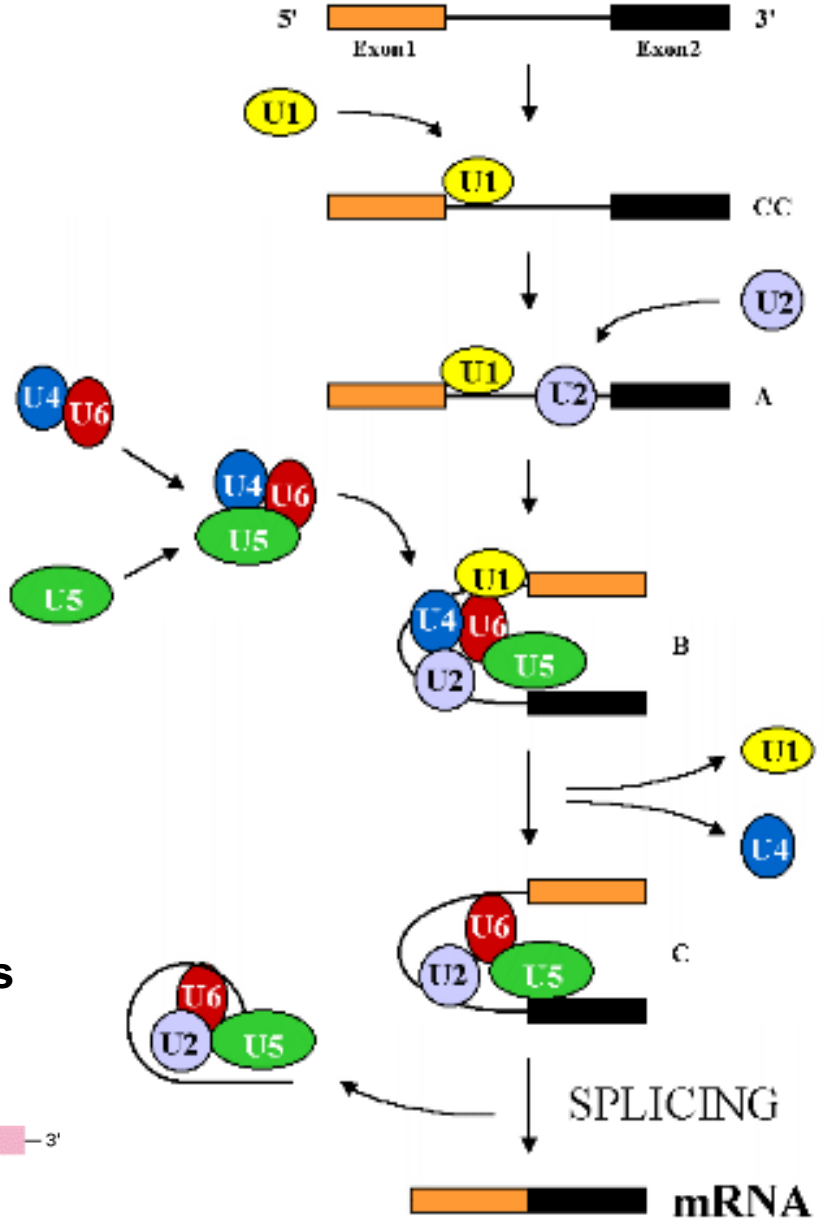
Zbigniew Dominski, lectures 2008

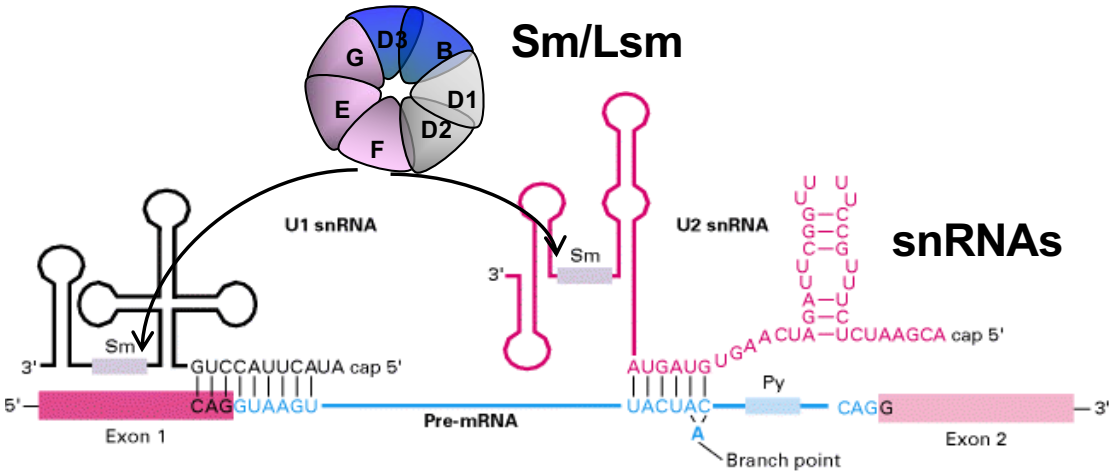
Warf and Berglund, 2010, TiBS; Reddy, Ann Rev Plant Biol, 2007

Pre-mRNA splicing: *trans* elements



5 snRNAs
U1, U2,
U4, U5,
U6

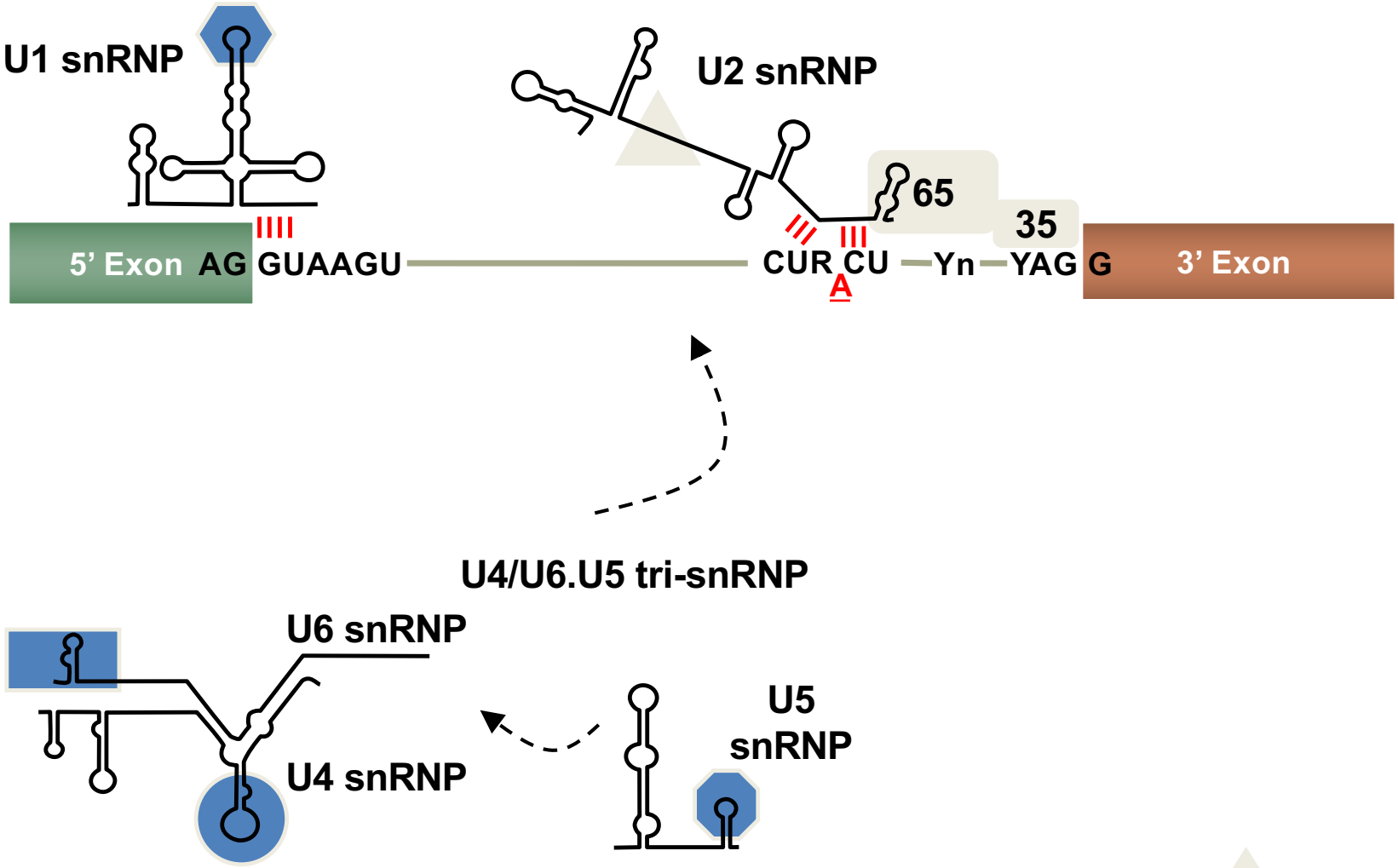




pre-mRNA::snRNA base-pairing

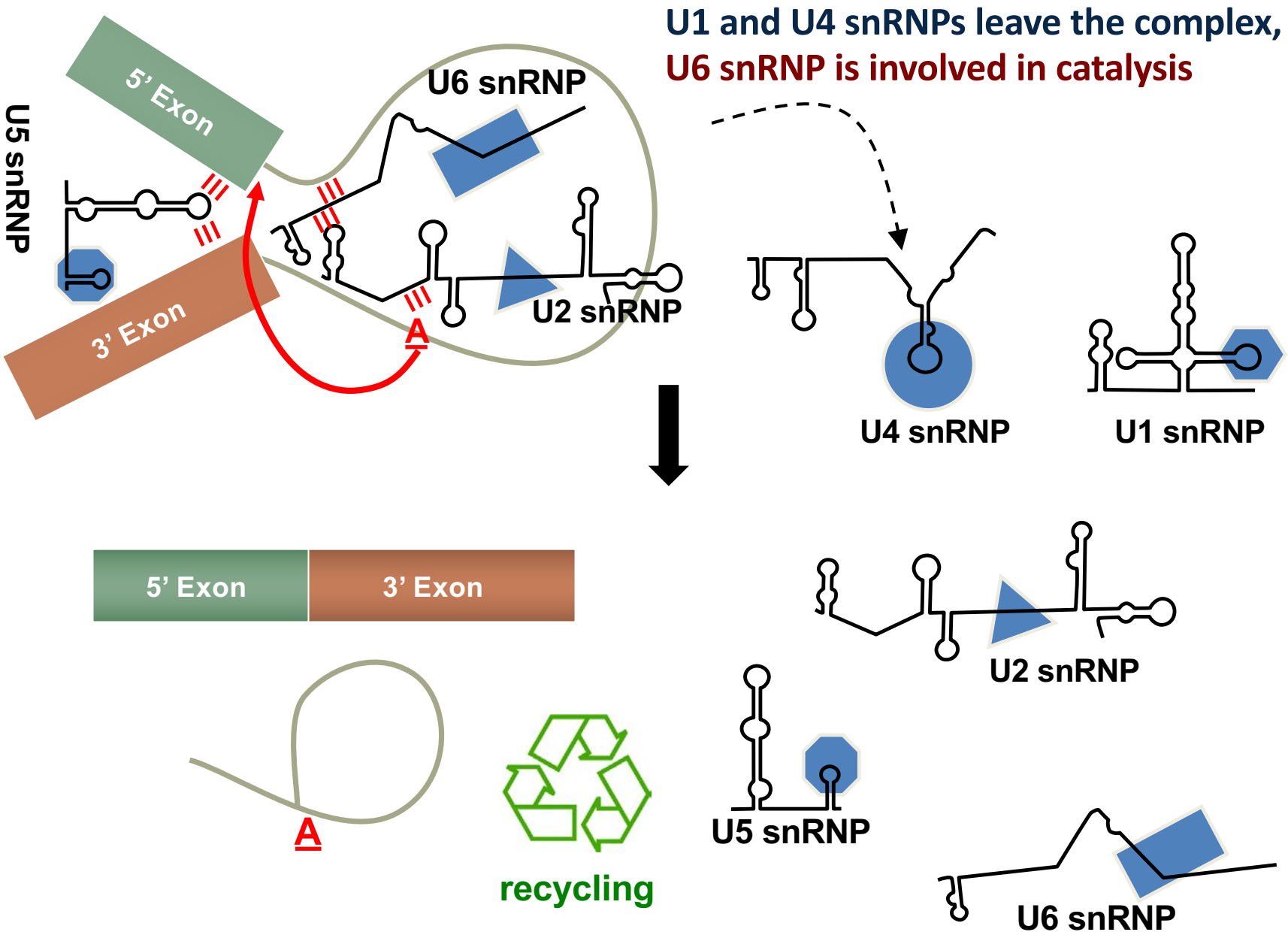
Warf and Berglund, 2010, TiBS; Reddy, Ann Rev Plant Biol, 2007

Structural rearrangements



Zbigniew Dominski, lectures 2008

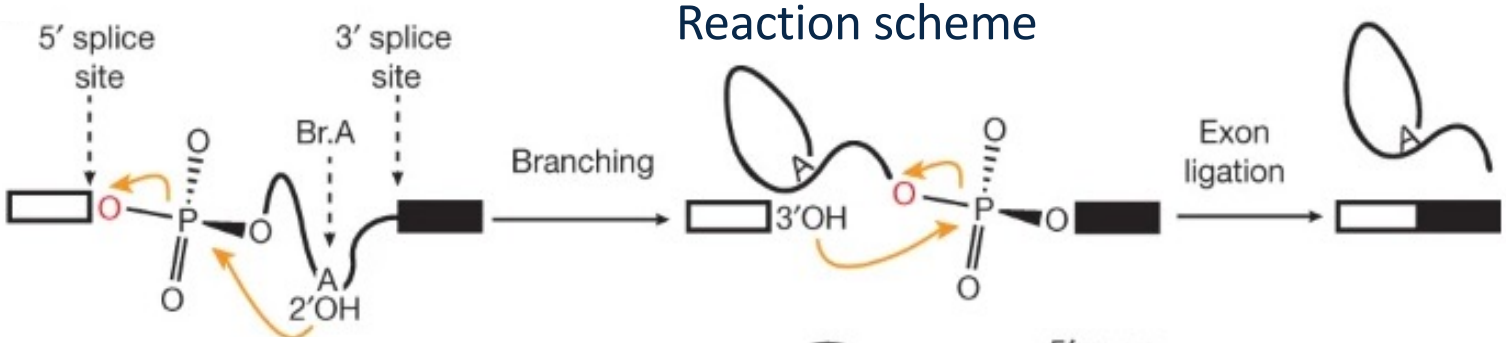
Structural rearrangements



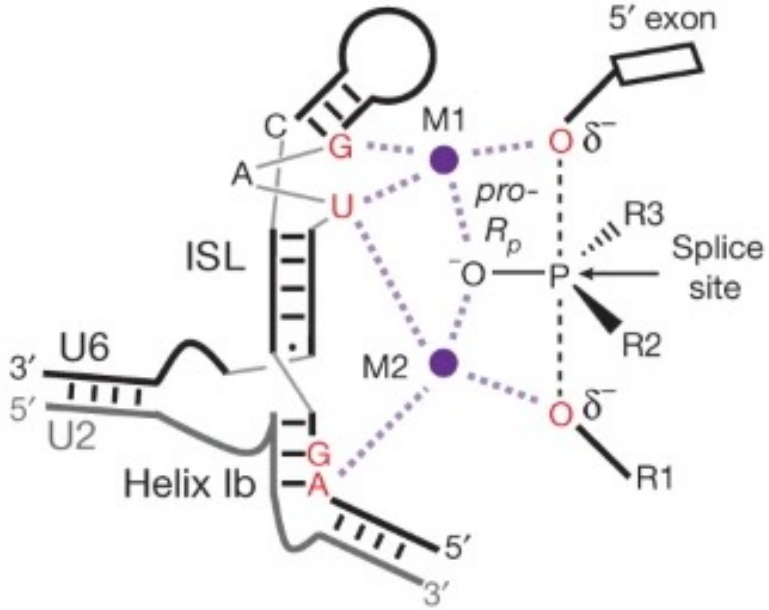
Zbigniew Dominski, lectures 2008

U6 snRNA is the catalytic spliceosome component

Reaction scheme



Catalytic RNA core: two-metal model for branching and exon ligation



nature

doi:10.1038/nature12734

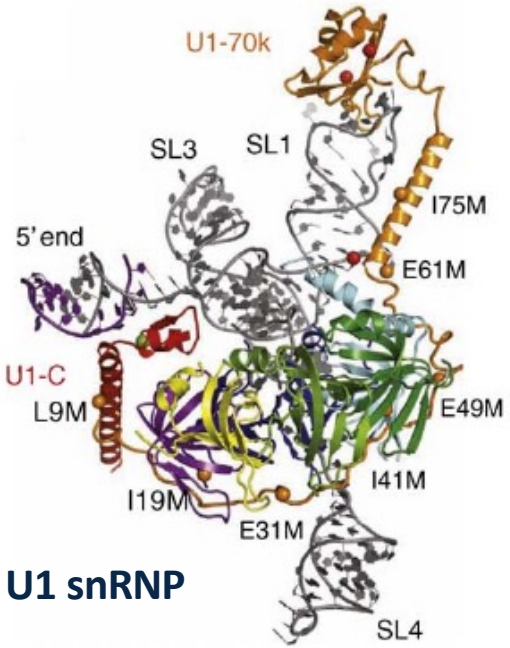
RNA catalyses nuclear pre-mRNA splicing

Sebastian M. Fica[1,2]*, Nicole Tuttle[3]*, Thaddeus Novak[4], Nan-Sheng Li[4], Jun Lu[3], Prakash Koodathingal[2], Qing Dai[3], Jonathan P. Staley[2] & Joseph A. Piccirilli[3,4]

For branching
R1 – 2'OH of branch A
R2 – the intron
R3 – *pro-*S_p oxygen
For exon ligation
R1 – 2' oxygen leaving group
R2 – *pro-*S_p oxygen
R3 – the 3' exon
Reactive oxygens - red

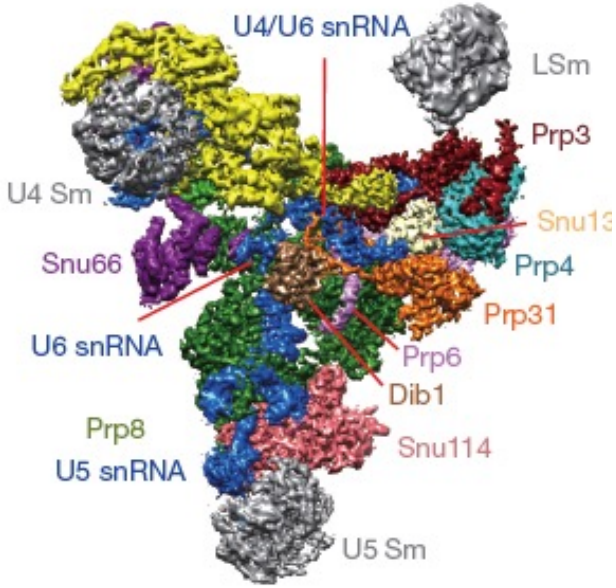
Spliceosome

Cryo-EM



U1 snRNP

Krummel et al, Nature, 2009

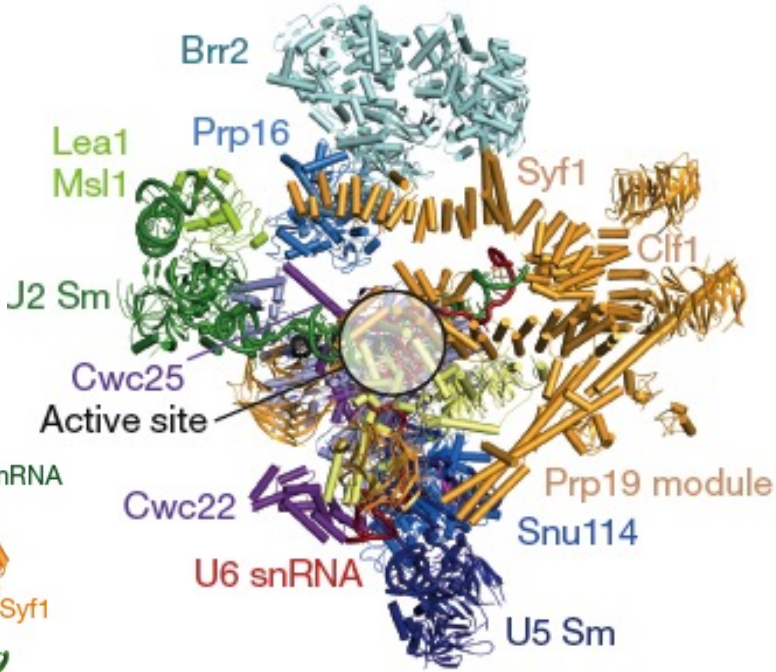


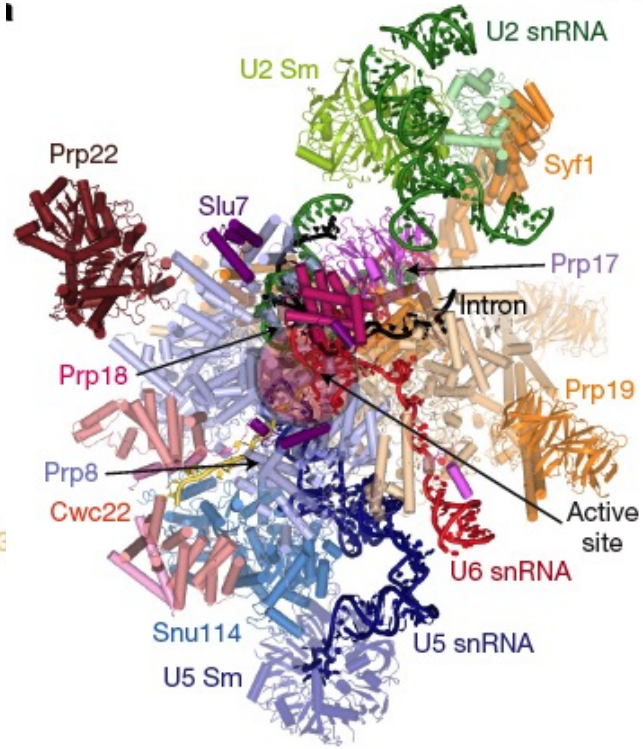
U4/U6.U5 tri-snRNP

Nguyen1, Galej et al, Nature, 2016*

C complex yeast

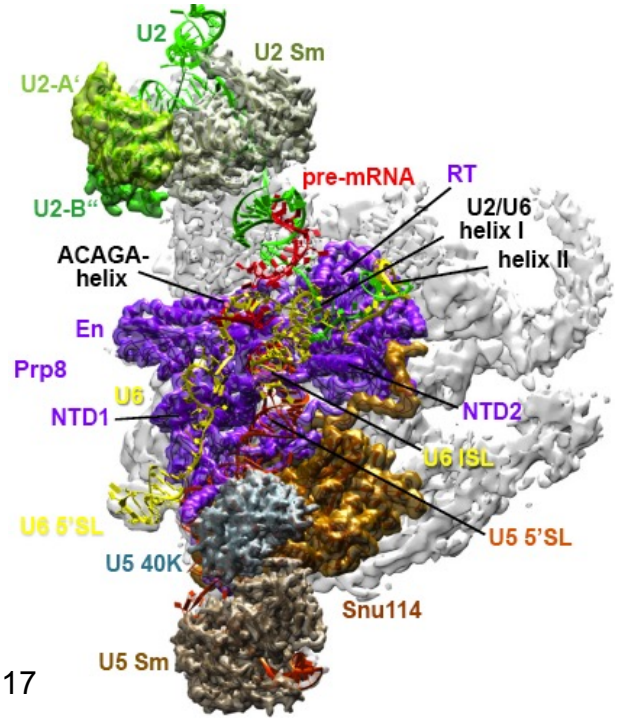
Galej et al, Nature, 2016





C* complex yeast

Fica et al, Nature, 2017

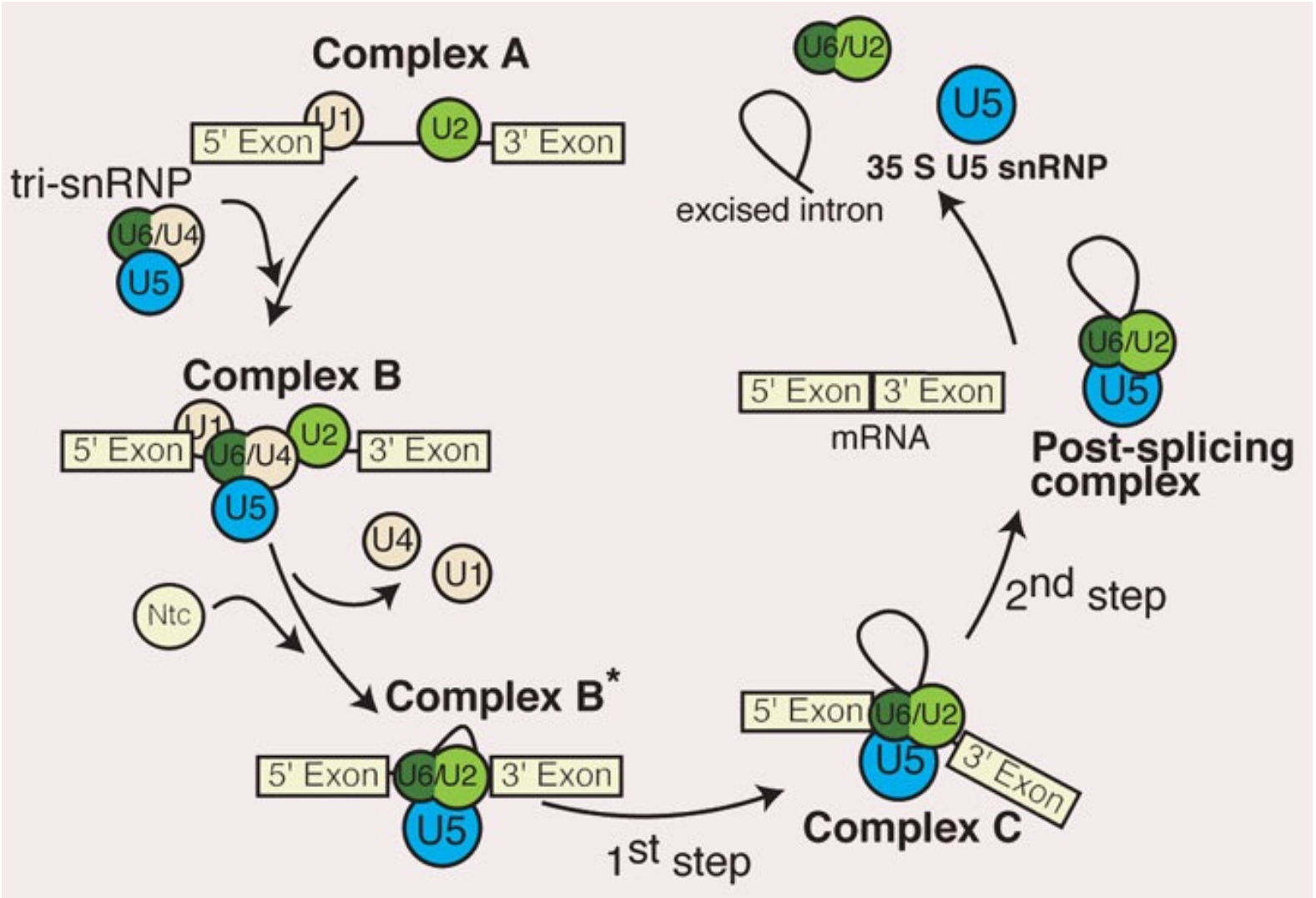


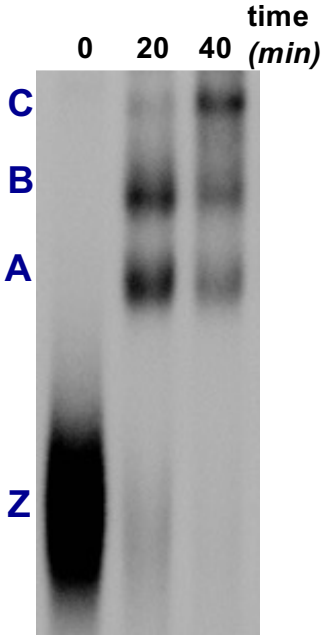
C* complex human

second step

Bertramet al, Nature, 2017

Spliceosomal complexes





native gel, labeled pre-mRNA

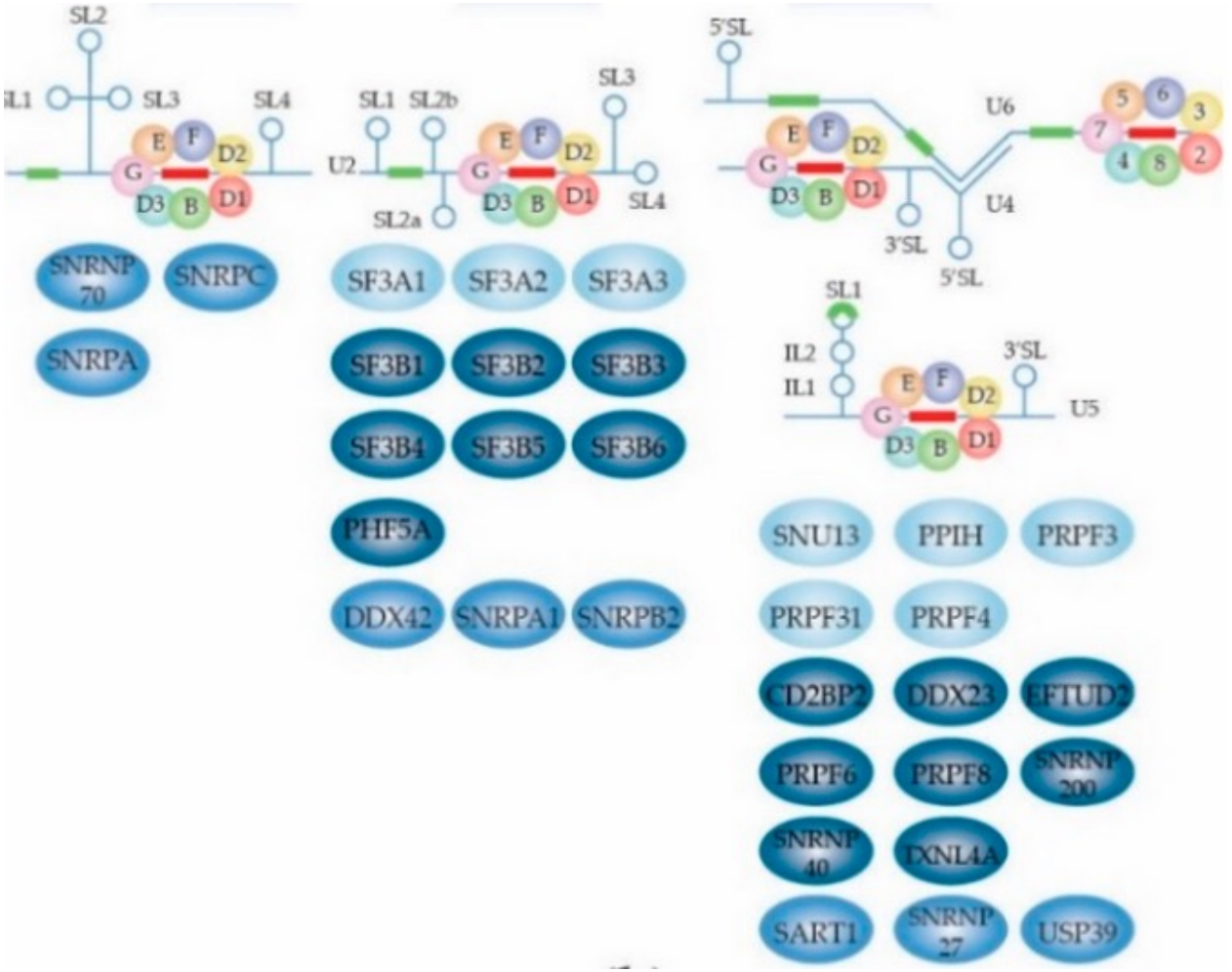
Crucial components of the spliceosome:

Prp8 U5 specific, contacts 5' ss, BP, 3'ss

Prp19 and NTC (the nineteen complex) important for catalytic activation

SF3a/SF3b stabilize the U2-BP interaction

snRNPs



Jurica, Curr.Op.Str.Biol., 2008

Jurica and Moore, Mol.Cell, 2003

Splicing factors

Human Name	Ensemble Accession	*S. cerevisiae* Name
snRNA		
U1 snRNA		
U2 snRNA		
U4 snRNA		
U5 snRNA		
U6 snRNA		
Core snRNP proteins		
SmB/B′	125835	Smb1
SmD1	167088	Smd1
SmD2	125743	Smd2
SmD3	100028	Smd3
SmE1	176773	Sme1
SmF1	139343	Smf1
SmG1	143977	Smx2
LSM2	111987	Lsm2
LSM3	170860	Lsm3
LSM4	130520	Lsm4
LSM5	106355	Lsm5
LSM6	164167	Lsm6
LSM7	130332	Lsm7
LSM8	128534	Lsm8
U1 snRNP specific proteins		
U1-70kD	104852	Snp1
U1-A	077312	Mud1
U1-C	124562	Yhc1
		Prp39
FBP11	123596	Prp40
		Snu56
		Nam8
		Snu71
		Snu65
U2 snRNP specific proteins		
U2-A′	131876	Lea1
U2-B″	125870	Msl1
SF3a60	nlm	Prp9
SF3a66	104897	Prp11
SF3a120	099995	Prp21
SF3b49	143368	Hsh49
SF3b145	087365	Cus1
SF3b130	nlm	Rse1
SF3b155	115524	Hsh155
p14	115128	Snu17

5 snRNAs

41 snRNP proteins

U5 snRNP specific proteins		
PRP8	174231	Prp8
U5-200kD	144028	Brr2
U5-116kD	108883	Snu114
U5-102kD	101161	Prp6
U5-100kD	174243	Prp28
U5-40kD	060688	
U5-15kD	141759	Dib1
U4/U6 snRNP specific proteins		
HPRP3	117360	Prp3
HPRP4	136875	Prp4
RY-1	124380	
USA-Cyp	171960	Cpr1
15.5 tri-snRNP	100138	Snu13
Miscellaneous splicing factors		
U2AF65	063244	Mud2
SF1	168066	Msl5
CBP20	114503	Cbc2
CBP80	136937	Sto1

> 70 splicing factors

Human Name	Ensemble Accession	*S. cerevisiae* Name
U2AF35	160201	
ASF/SF2	136450	
UAP56	173539	Sub2
PRP5	145833	Prp5
Tat-SF1	102241	Cus2
PTB	011304	
PRP19	110107	Prp19
PRP31	105618	Prp31
		Snt309
DDX16	137333	Prp2
PRP16	140829	Prp16
PRP17	168438	Prp17
SLU7	164609	Slu7
PRP18	165630	Prp18
PRP22	067596	Prp22
EWS	nlm	
		Prp38
PRP43	109606	Prp43
PRP24	075856	Prp24
DDX3	124487	Ded1
		Npl3

Proteins containing a DEAD/H box helicase motif		
HDB/DICE1	102786	
Abstrakt	146074	
eIF4a3	141543	
DDX35	101452	
DDX9	135829	
KIAA0052	039123	
p72	100201	
Proteins with homology to cis-trans prolyl isomerases		
CypE	084072	
KIAA0073	113593	
Cyp60	100023	
PPIL3b	115934	
PPIL1	137168	Cwc27 (Cwf27)
SDCCAG10	153015	
Additional proteins novel to splicing		
KIAA1604	163510	Cwc22 (Cwf22)
TIP39	100109	YLR424W
		Cwc21 (Cwf21)
G10	106245	Cwc14 (Cwf14)
FLJ10374	105248	Yju2 (Cwf16)
MGC13125	137656	Cwc26 (Cwf26)
ZNF183	125352	Cwc24 (Cwf24)
FLJ10634	104129	Cwc23 (Cwf23)
SF3b14b	100410	Rds3p
SPF31	126698	
CHERP	085872	
F23858	105705	
CA150	113649	
SF3b10	169976	
SR140	163714	
RBM5	003756	
E1B-AP5	105323	
FLJ10805	122692	
MFAP1	140259	
KIAA0560	021776	(Cwf11)
RED protein	113141	
Pinin	100941	
NOSIP	142546	
FLJ10206	076650	
PUF60	179950	
DGSI	100056	
Cactin	105298	
FRG1	109536	
PMSCL2	171824	
RBP 7	076053	
MGC23918	160799	(Cwf18)
SNP70	084463	
OTT	162775	
IMP3	136231	
PRP4 kinase	112739	
AcinusL	100813	
RNPC2	131051	
FLJ90157	033030	
NuMA	137497	

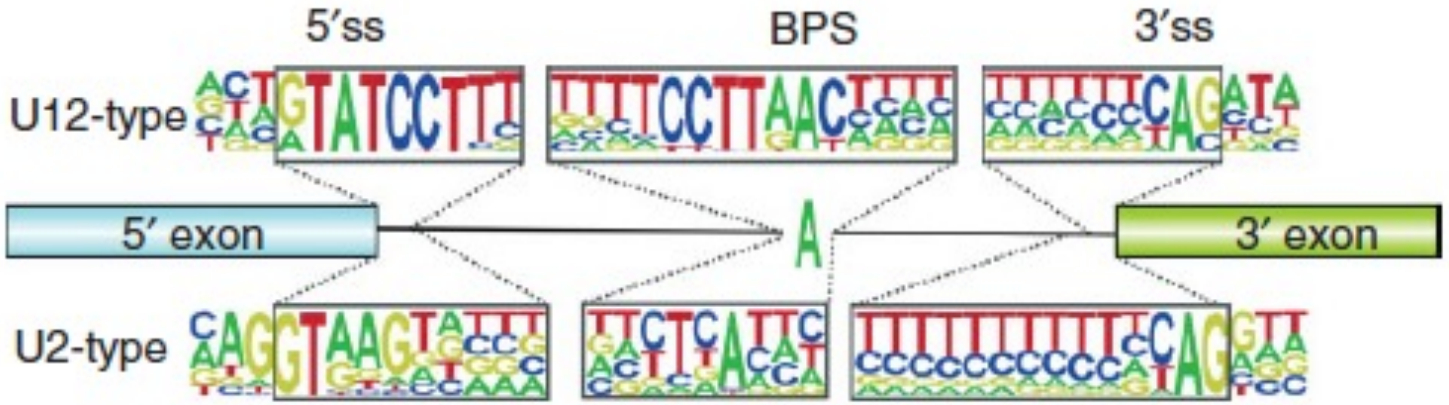
> 30 other proteins

Human Name	Ensemble Accession	*S. cerevisiae* Name
Proteins associated with the Prp19 complex		
CDC5	096401	Cef1 (Cdc5)
ISY1	172780	Isy1 (Cwf12)
SYF1	076924	Syf1(Cwf3)
CRN	101343	Clf1 (Cwf4)
GCIP-IP	117614	Syf2 (C3E7.13C)
PRL1	171566	Prp46 (Cwf1)
BCAS2	116752	(Cwf7)
		Ntc20
		Cwc2 (Cwf2)
Proteins with demonstrated roles in splicing		
SKIP	100603	Prp45 (Cwf13)
ECM2	086589	Ecm2 (Cwf5)
SART1	175467	Snu66
p68	108654	Dbp2
SPF45	134453	
SPF30	119953	
PSF	116560	
FLJ31121	146007	Snu23
SAD1	168883	Sad1
LUC7	007392	Luc7 (Luc7)
		Spp381
SR proteins		
SRm300	167978	
SRm160	133226	
SC35	161547	
SRp40	100650	
SRp55	124193	
SRp75	116350	
SRp30c	111786	
9G8	115875	
SRp54	116754	
SFRS10	136527	
SRp20	112081	
Proteins with roles in pre-mRNA metabolism pro		
REF	141592	Yra1
RNPS1	167971	
Y14	131795	
MAGOH	162385	
hTHO2	125676	Rlr1
hHPR1	079134	
HsKin17	151657	Rts2p
ASR2B	087087	
KIAA0983	100296	
C21orf66	159086	
PAB2	100836	
CF I-68kD	111605	
CF I-25kD	167005	
CPSF 160K	071894	

Minor spliceosome (U12-type)

Zbigniew Dominski, lectures 2008

~1% of pre-mRNAs have U12 type ATAC introns

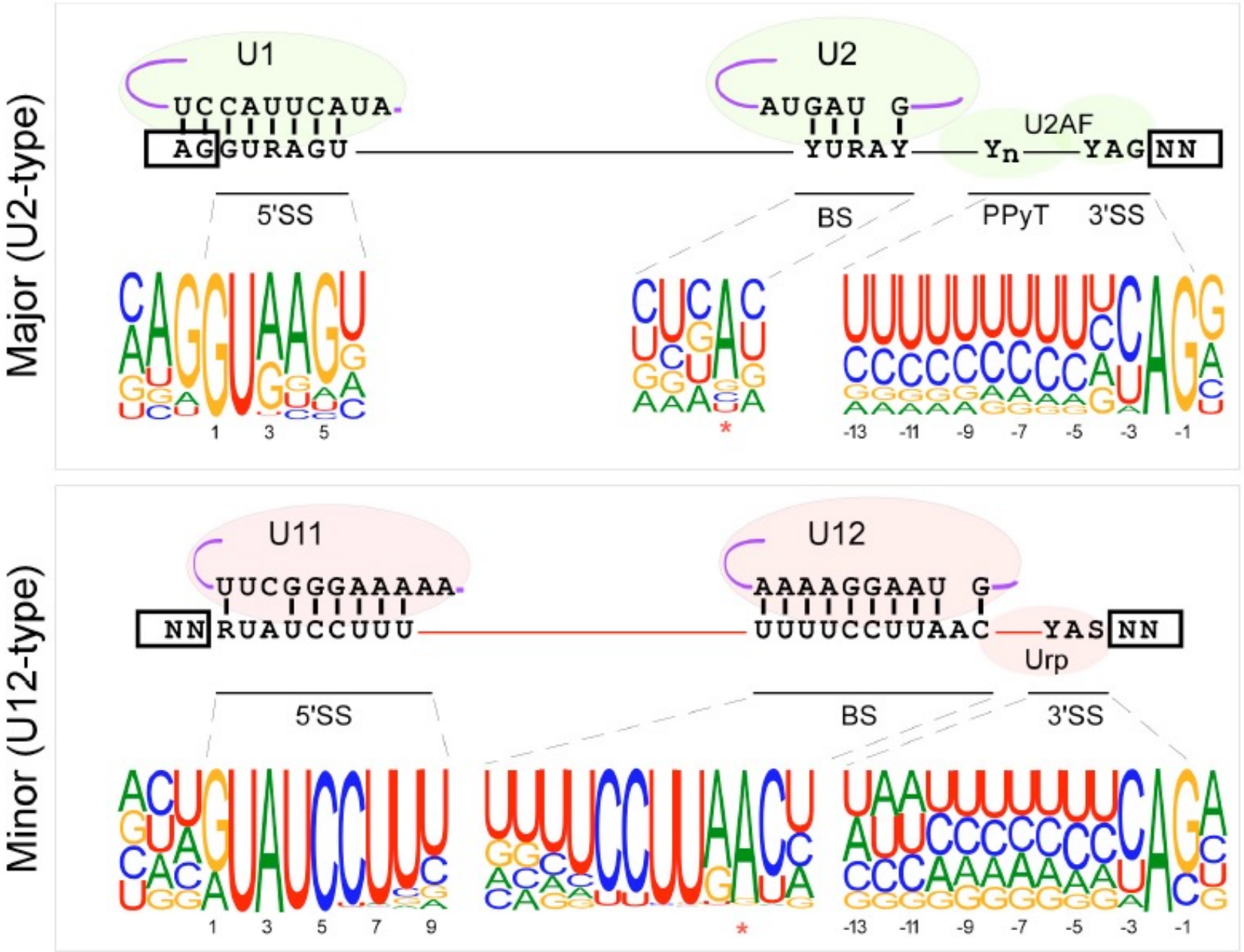


Turunen et al, WIREs RNA, 2023

Minor type of splicing depends on U4atac, U6atac, U11 and U12 snRNPs

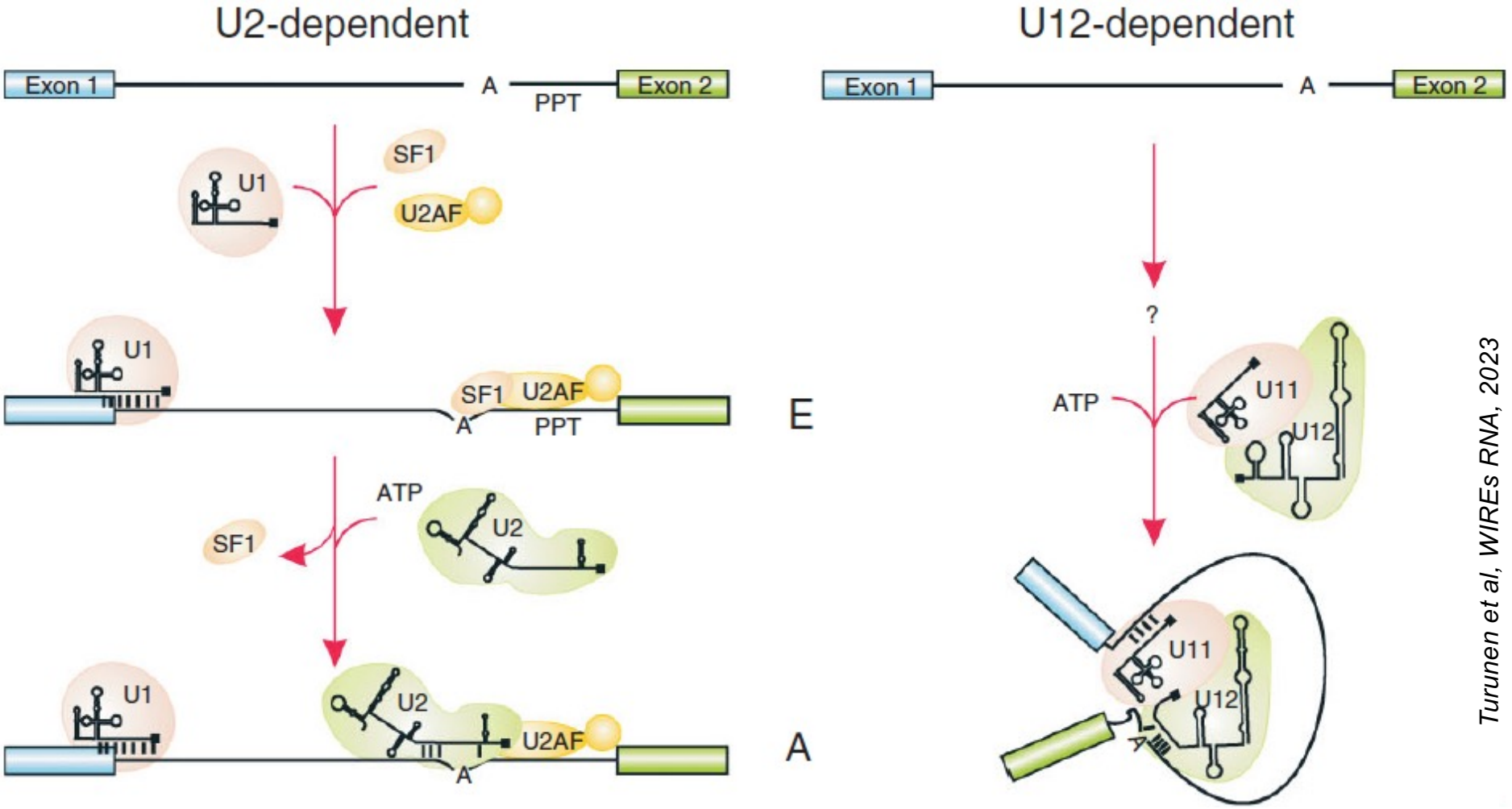
Minor splicing is essential for viability and development

Minor spliceosome (U12-type)



Ding et al, WIREs RNA, 2013

Minor spliceosome (U12-type)



Turunen et al, WIREs RNA, 2023

Konig et al, 2007, Cell

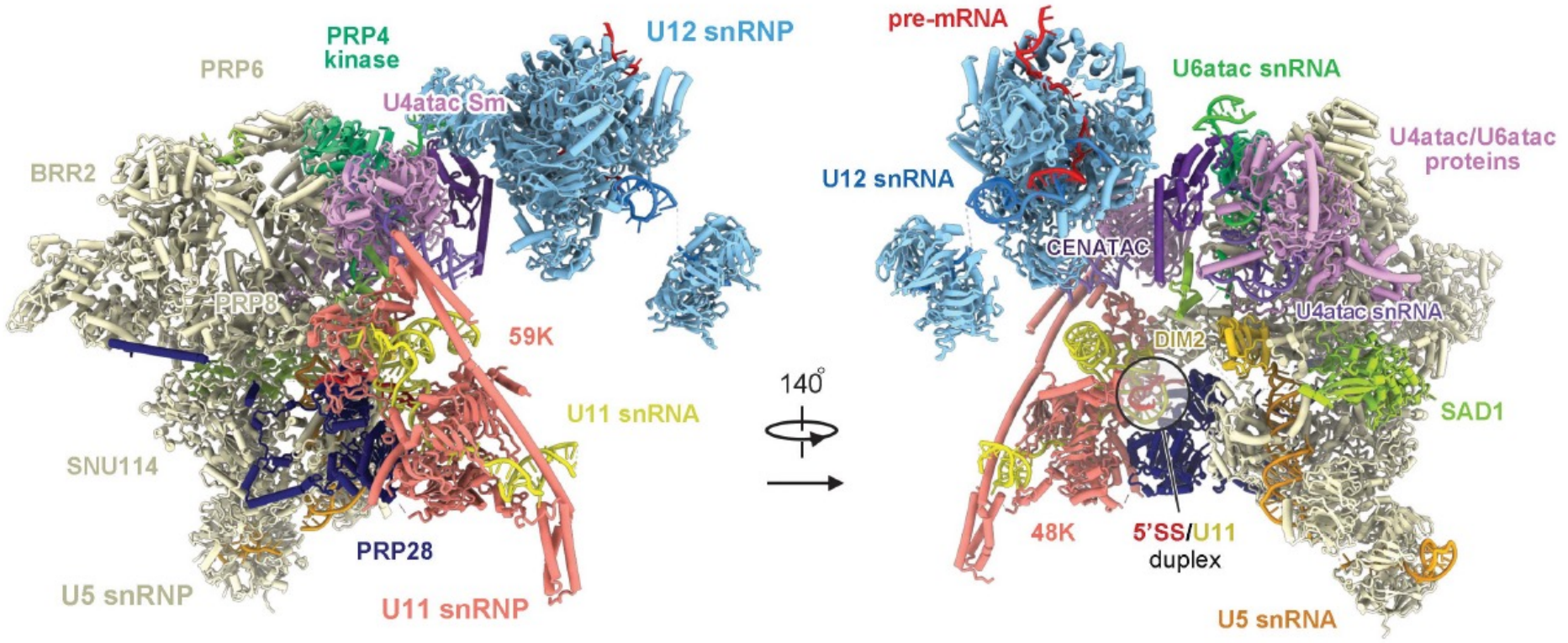
Splicing of U12 introns is slower.

~~**This often leads to an aberrant mRNA with unspliced U12 intron which leaves the nucleus and is spliced by the cytoplasmic minor spliceosome (not true).**~~

Splicing by U12-type spliceosome occurs in the nucleus and is often co-transcriptional.

Lack of minor splicing results in degradation of aberrant transcripts by NMD.

Minor spliceosome (U12-type)



U5 snRNP	
U5 snRNA	U5-40K
U5 Sm ring	PRP6
PRP8	DIM2
BRR2	PRP28
SNU114	DIM1
pre-mRNA	

U4atac/U6atac di-snRNP	
U4atac snRNA	
U4atac Sm ring	
PRP3	PRP4
PRP31	SNU13
CENATAC	
U6atac snRNA	
RBM42	27K

tri-snRNP specific
SAD1
SNU66

pre-B specific
PRP4 kinase

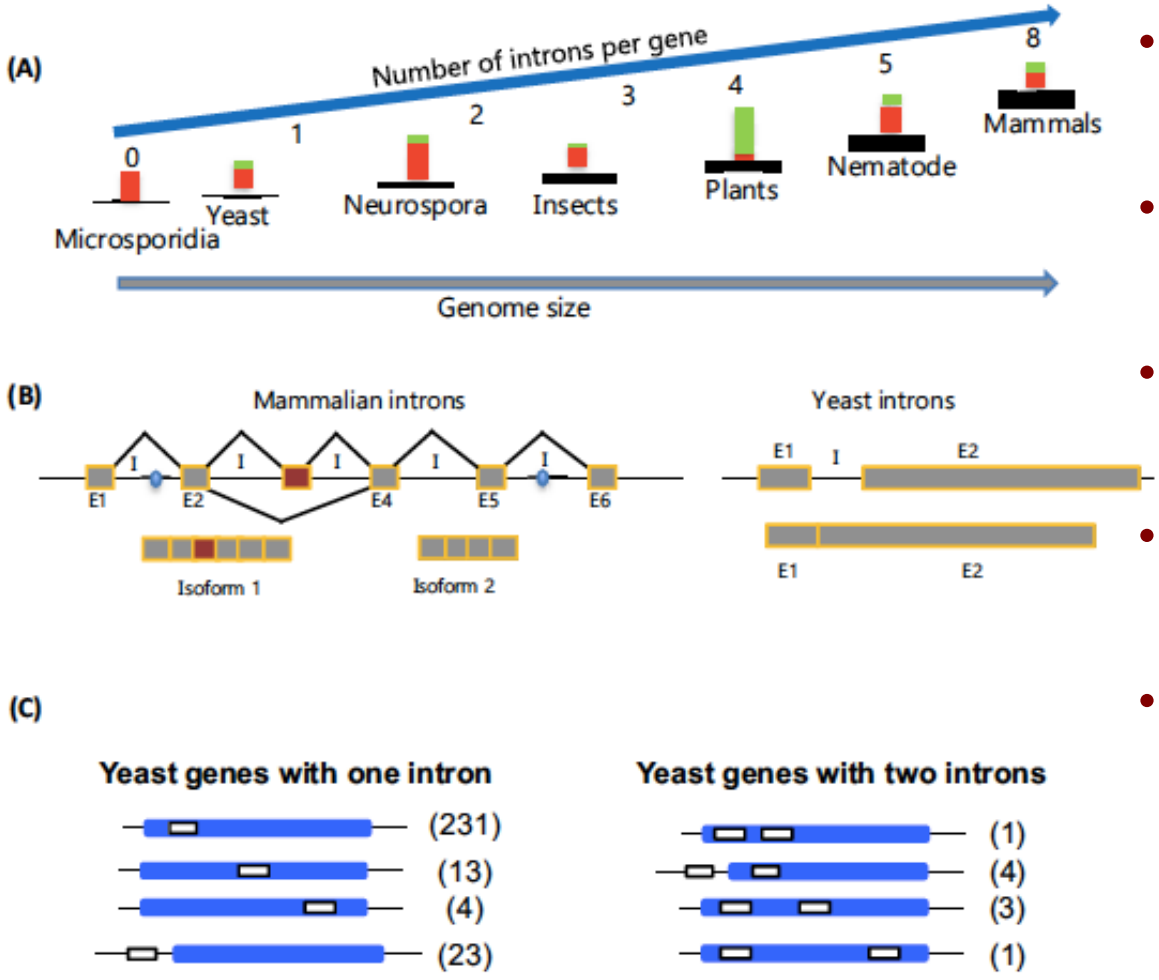
U11 snRNP	
U11 snRNA	U11-20K
U11 Sm ring	U11-25K
	U11-35K
	U11-48K
	U11-59K
U1-A	
U1-C	
U1-70K	

U12 snRNP		
Core	SF3B	
U12 snRNA	SF3b155	SCNM1
U12 Sm ring	SF3b145	
	SF3b130	SF3A
	SF3b49	SF3a120
	SF3b14a	SF3a66
U2-A'	SF3b14b	SF3a60
U2-B''	SF3b10	

Minor spliceosome contains specific proteins: U11 snRNP, DIM2, CENTAC, PRP4 kinase, SAD1, SNU66

Bai et al, 2012; 2024, Science

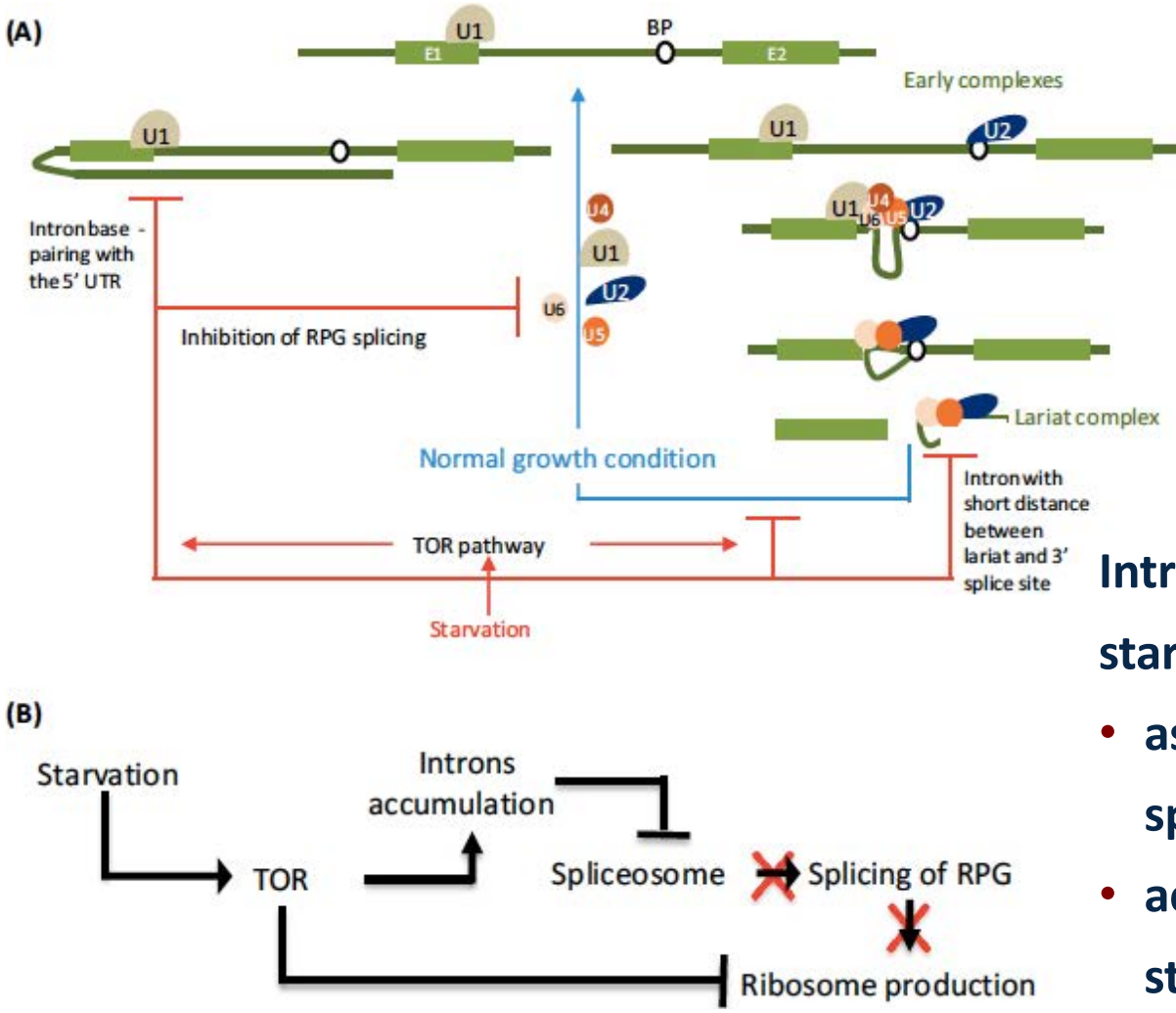
The meaning of introns



- **ncRNAs (snoRNA, miRNA) are often encoded in introns**
- **Maintaining introns is a burden for the cell**
- **Introns are enriched in yeast ribosomal protein (RP) genes**
- **Introns affect gene expression** (e.g. via NMD or as ncRNAs)
- **Introns accumulate in response to growth conditions when splicing is repressed** (starvation)

Parenteau and Abou Elela, TiG, 2019

The meaning of introns

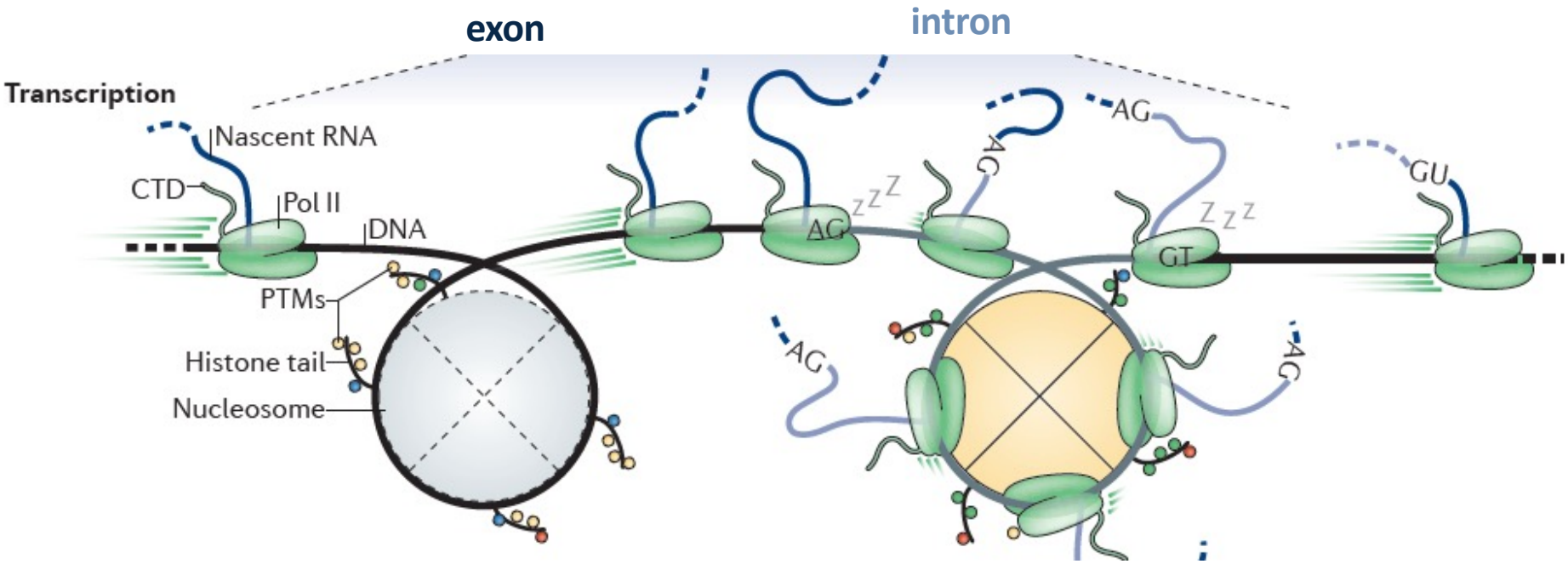


Parenteau and Abou Elela, TiG, 2019

Introns regulate splicing under starvation

- **as part of pre-mRNA or as free spliced linear introns**
- **accumulation of introns under starvation leads to repression of splicing of ribosomal protein genes (RPG) by sequestering spliceosome components**

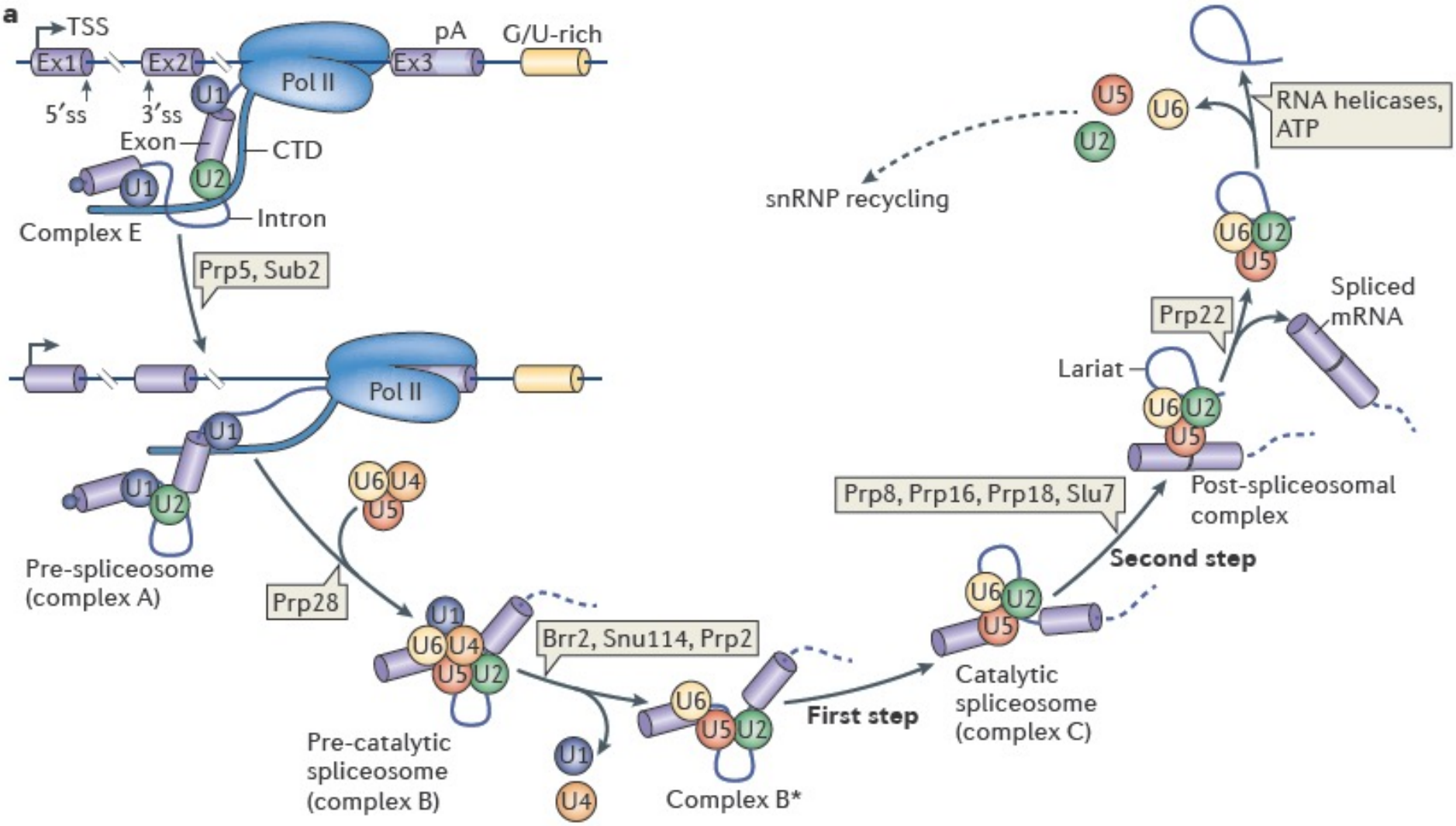
Transcription and splicing



PolII transcription varies along the gene.
Elongation rate is faster along introns and slower along exons.

Herzel et al, Nat Rev Mol Cell Biol, 2017

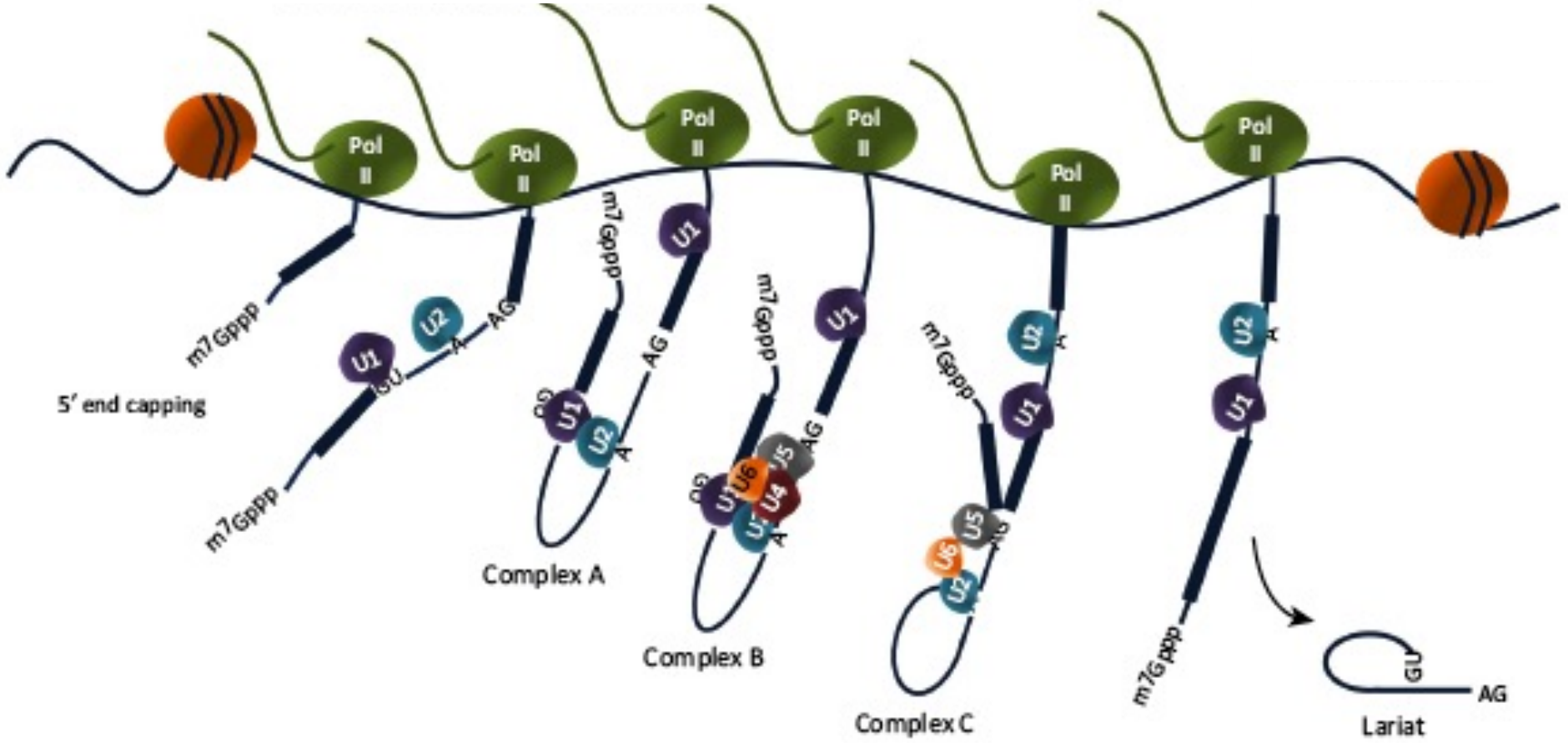
Transcription and splicing



Step-wise assembly of the spliceosome and catalytic steps of splicing

Matera and Wang, Nat Rev Mol Cell Biol, 2014

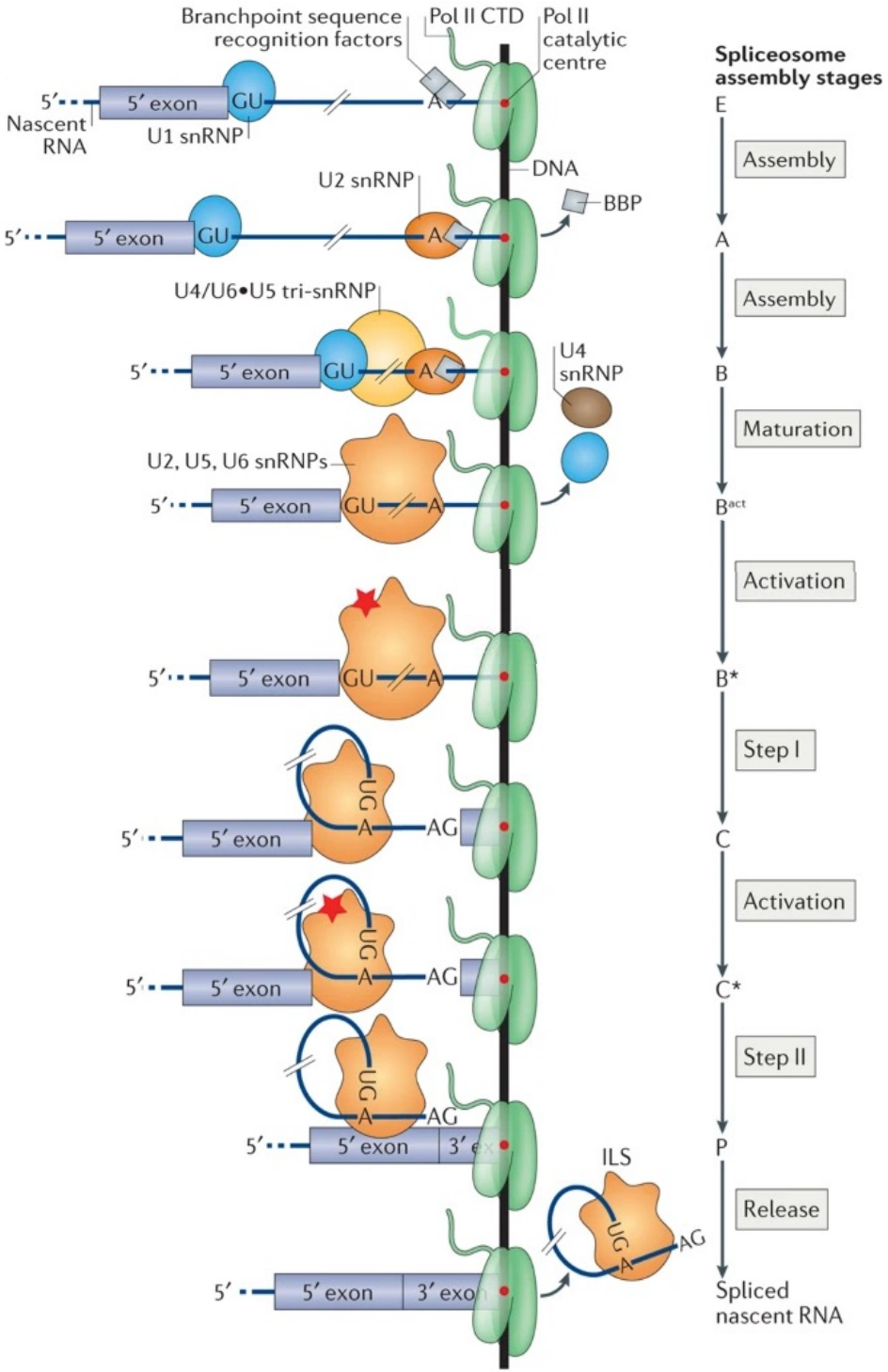
Pre-mRNA splicing is mainly co-transcriptional



Stepwise spliceosome assembly is co-transcriptional
Co-transcriptional splicing occurs at ~ 80%

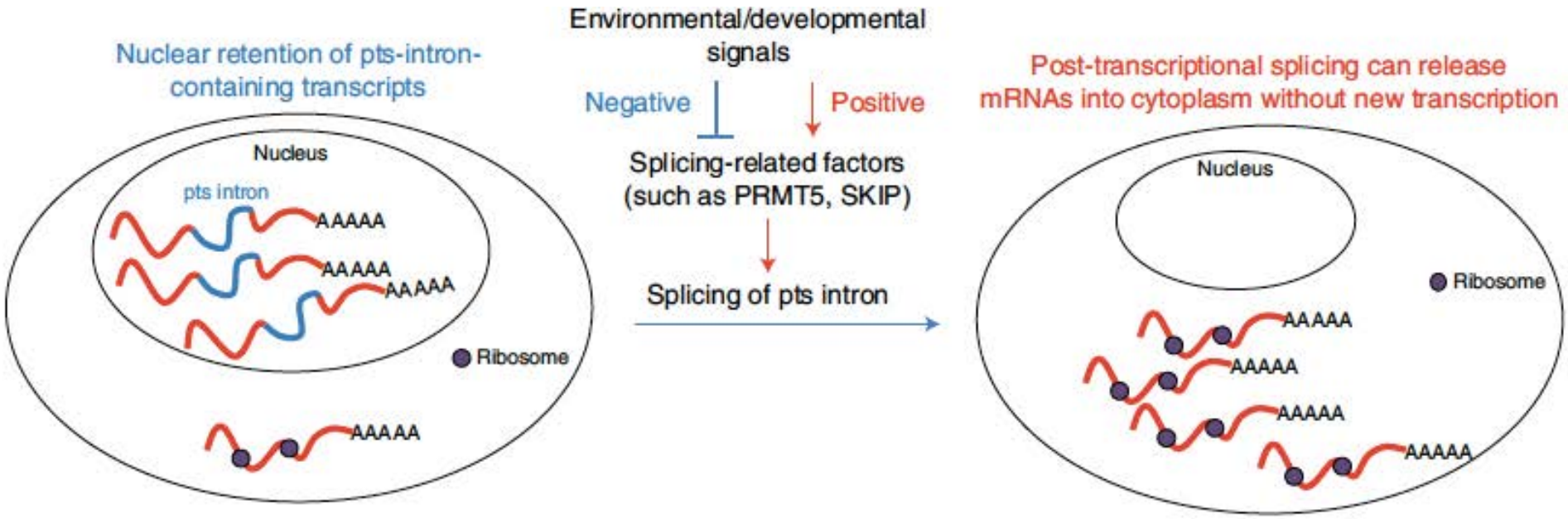
Wong et al., TiG, 2014

Co-transcriptional splicing



Herzel et al., Nat Rev Mol Cell Biol, 2017

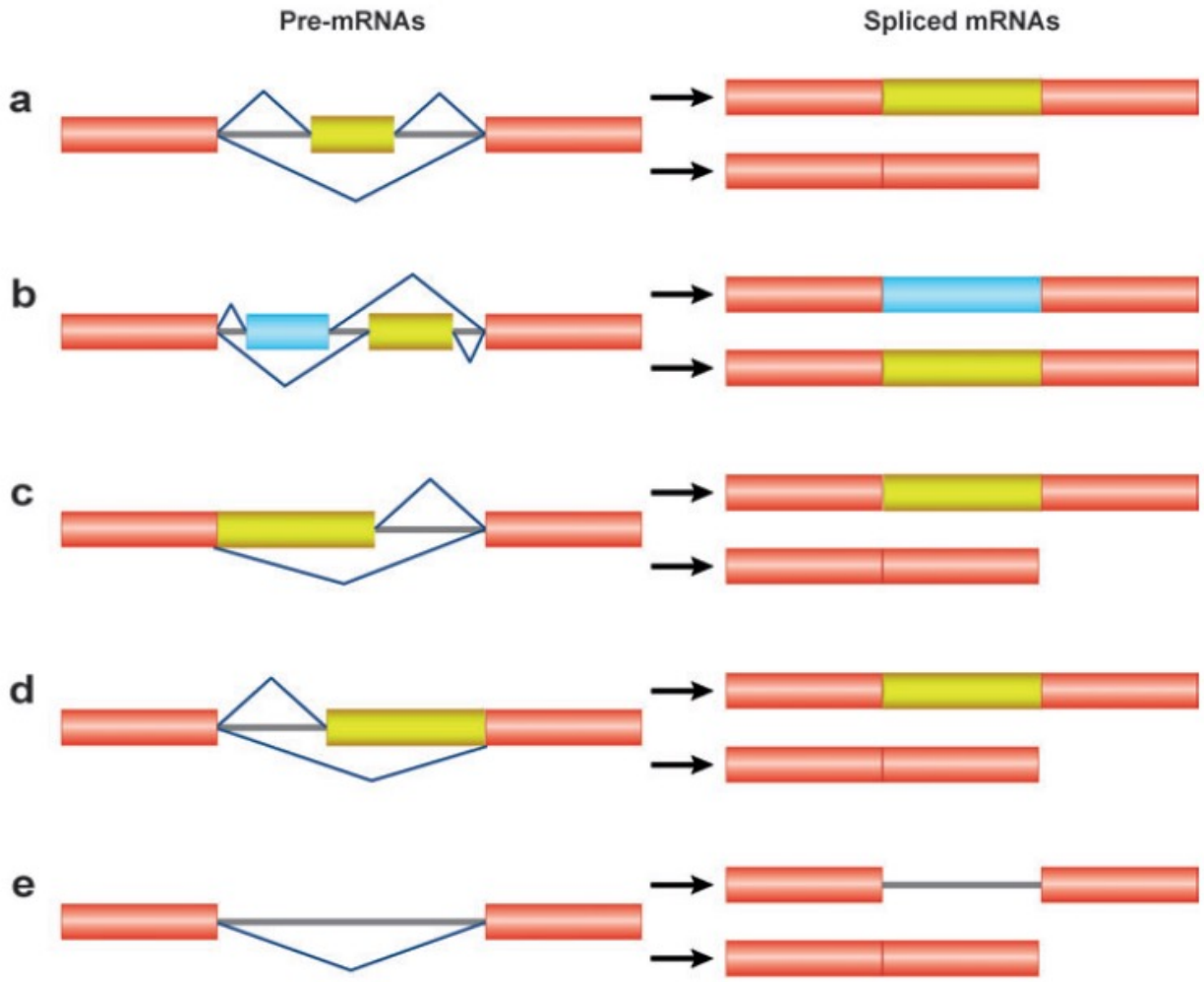
Co- vs post-transcriptional splicing



- Incompletely spliced and polyadenylated transcripts are detected on chromatin
- These are not released and exported to the cytoplasm and undergo post-transcriptional splicing
- Splicing of these introns is regulated in response to various environmental signals
- It represents additional layer of stress-related gene expression reprogramming
- Alternative introns are less efficiently spliced than constitutive introns
- Alternative introns are more often removed post-transciptionally

Jiaet al., Nat Plants, 2020

Alernative splicing (AS)



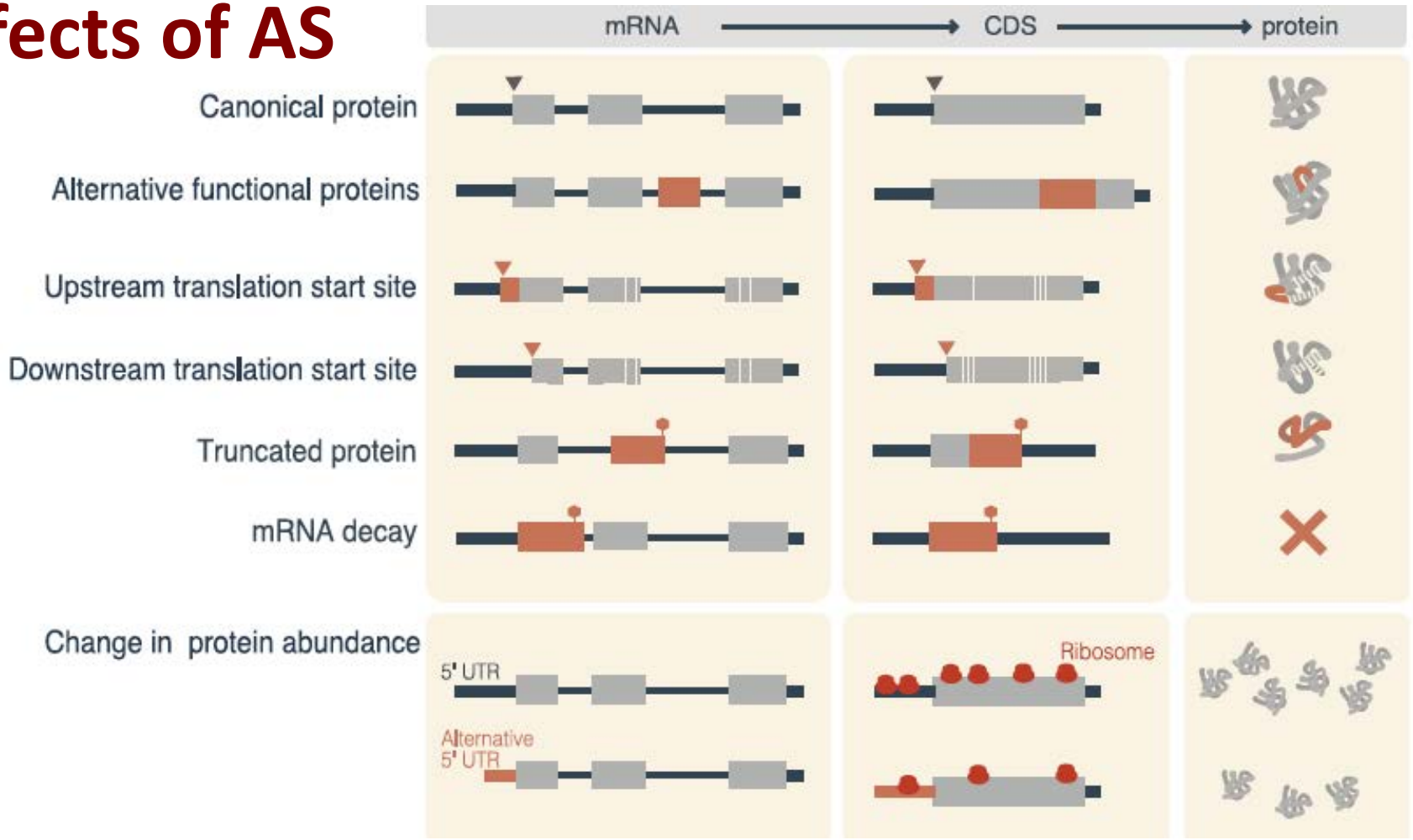
AS events

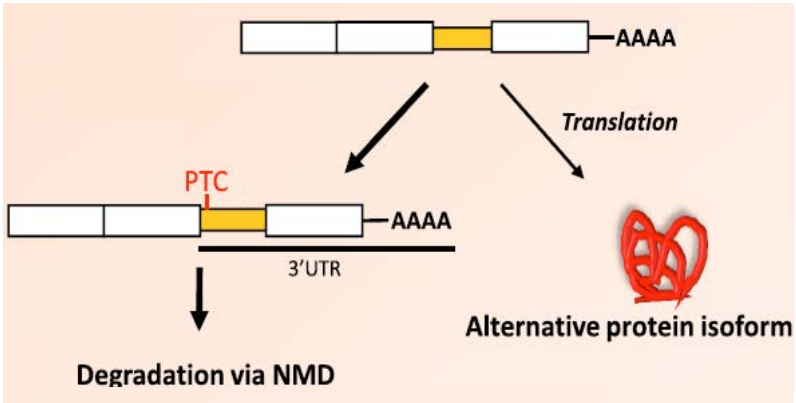
Exon skipping/ exon inclusion

Intron retention

Reddy, Ann Rev Plant Biol, 2007

Effects of AS





Reixachs-Solé and Eyras, WIREs RNA, 2021; Monteuuis et al, NAR, 2019

Some facts on AS

- **AS, widespread in higher eukaryotes, increases protein complexity**

(expression dependent on tissue type, cell cycle phase or stage of development; different level of biochemical activity; the presence of important regulatory domains)

- **75% of human and 50% of plant genes are estimated to produce AS events**
- **Average human pre-mRNA generates 3 different mRNAs**
- **AS is most common in neurons**
- **AS is linked with transcription**

- promoter structure contributes to AS

- transcription activators affect AS

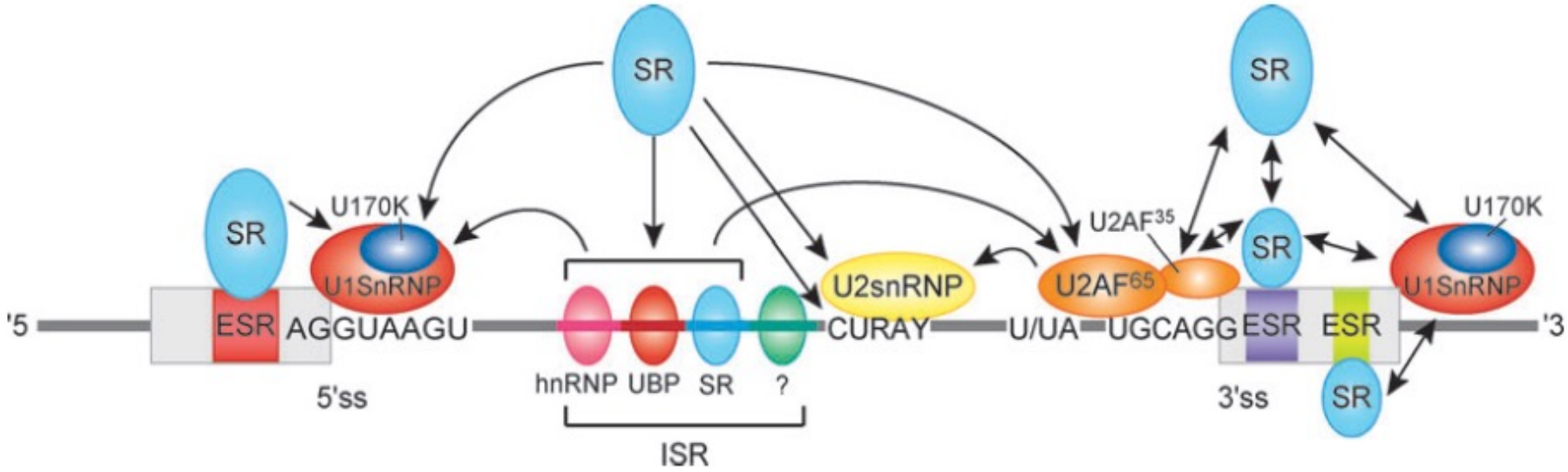
- elongation rate: slow transcription favors inclusion of alternative exons,

fast transcription promote exclusion of these exons

- **AS can affect mRNA stability and turnover:**

many alternatively spliced transcripts (> 30%) contain premature termination codons (PTC) that generate Nonsense Mediated Decay (NMD) substrates

Alernative splicing regulators



Exons and introns often contain sequences that facilitate or inhibit splice site usage.

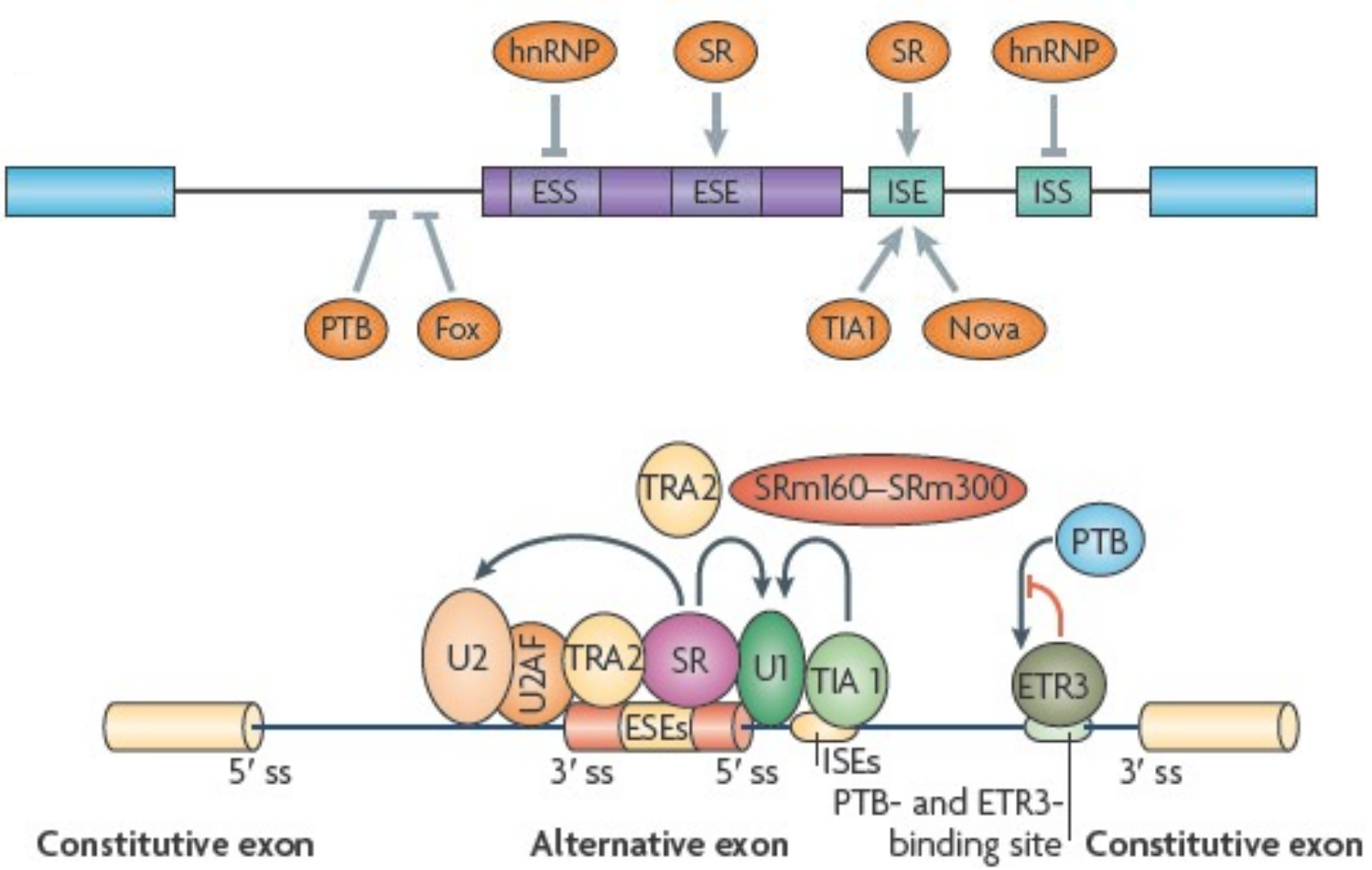
These elements bind splicing activators or repressors.

ESR – exonic splicing regulatory elements
ISR – intronic splicing regulatory elements
ESS/ISS – exonic/intronic splicing silencers
ESE/ISE – exonic/intronic splicing enhancers
SR – Ser/Arg rich proteins
PTB – polypyrimidine tract-binding proteins
hnRNP – heterogenous nuclear RNP

AS occurs at the level of recognition of splice sites and other regulatory elements by RNA-binding proteins

Reddy, Annu Rev Plant Biol, 2007

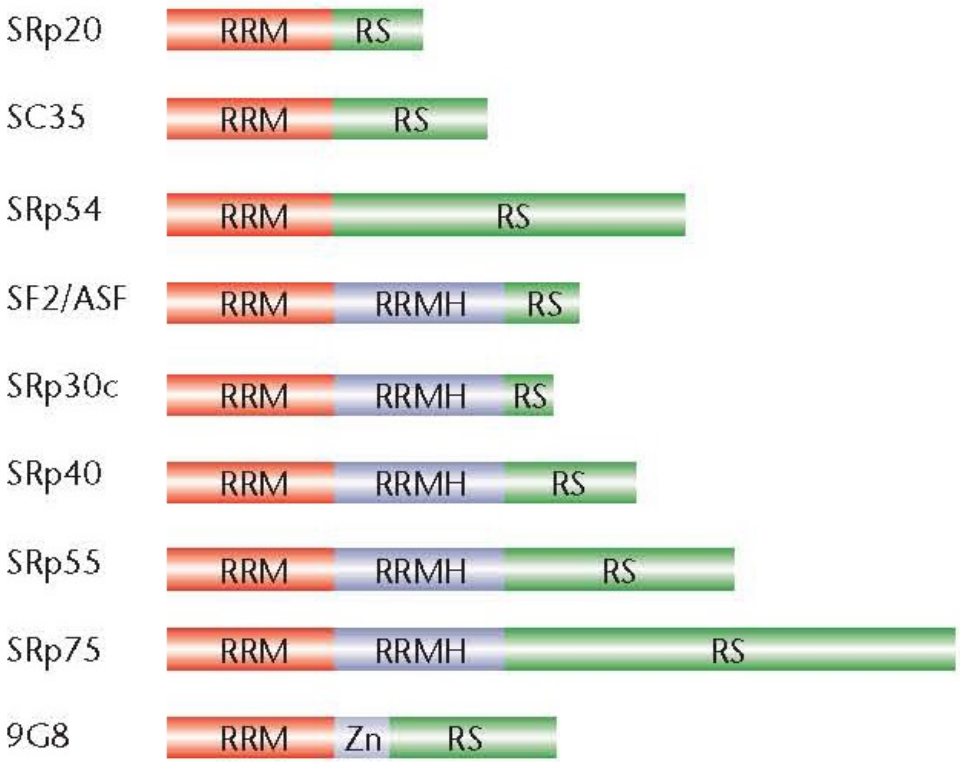
Alernative splicing



Keren et al, Nat.Rev.Genet., 2010

SR proteins bind to ESEs to stimulate the binding of U2AF to the upstream 3′ splice site (ss) or the binding of the U1 snRNP to the downstream 5′ ss.
SR proteins function with other splicing co-activators (TRA2) and the SR-related nuclear matrix proteins SRm160–SRm300.

Human SR proteins



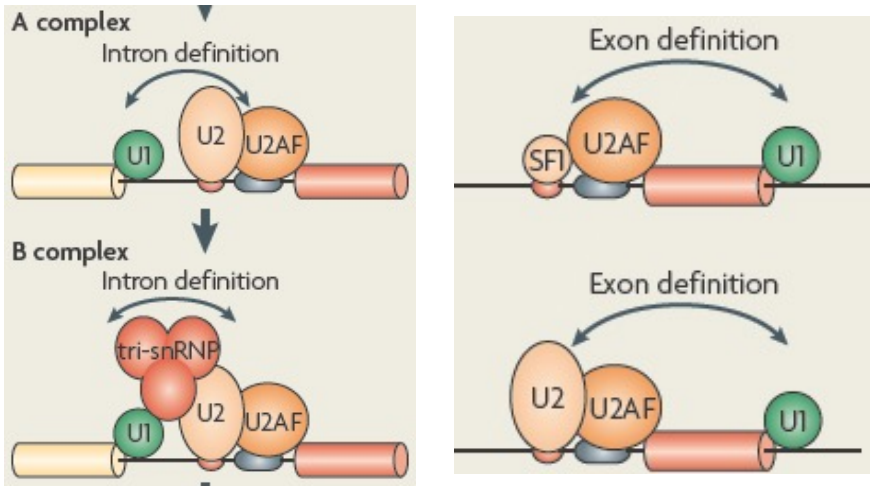
Protein	High-affinity binding site	Functional ESE
SRp20	WCWWC CUCKUCY	GCUCCUCUUCC CCUCGUCC
SC35	AGSAGAGUA GUUCGAGUA UGUUCSAGWU GWUWCCUGCUA GGGUAUGCUG GAGCAGUAGKS AGGAGAU	GRYYMCYR* UGCYGYY
9G8	$(GAC)_n$ ACGAGAGAY WGGACRA	
SF2/ASF	RGAAGAAC AGGACRRAGC	CRSMSGW*
SRp40	UGGGAGCRGUYRGCUCGY	YRCRKM*
SRp55		YYWCWSG*
TRA2β	$(GAA)_n$	

Zbigniew Dominski, lectures 2008

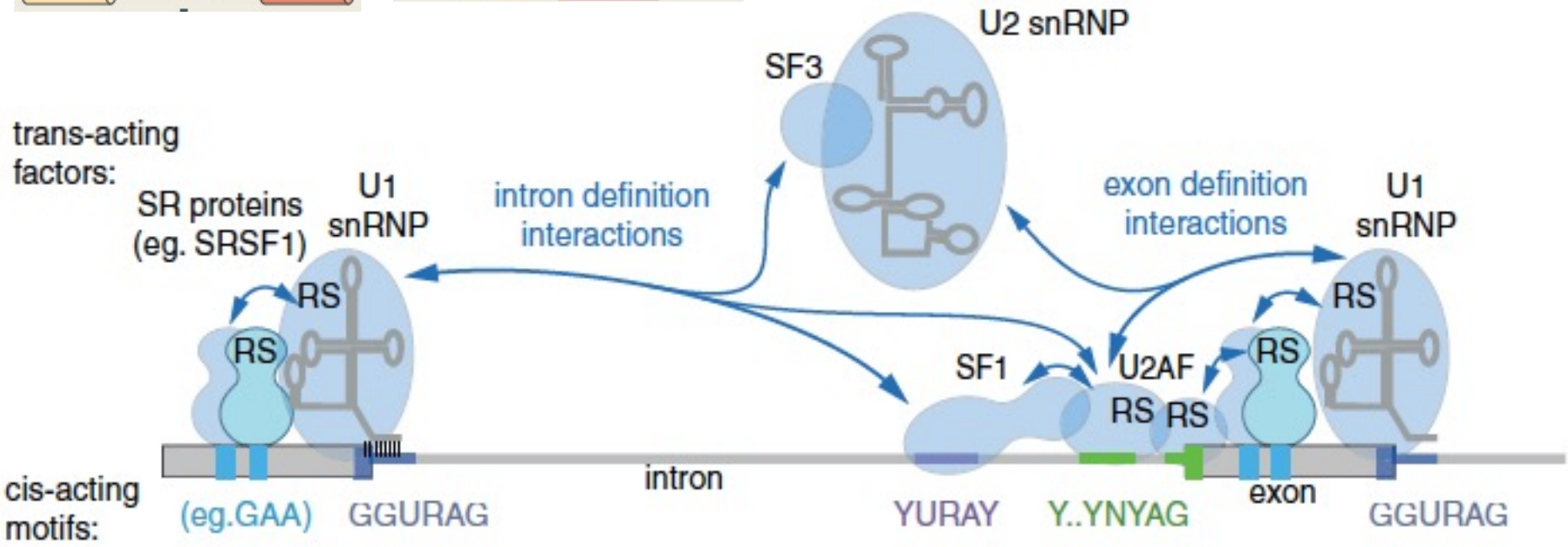
RRM: RNA recognition motif
RRMH: RRM homolog
second RNA-binding domain with a poor match to the RRM consensus

Alernative splicing

exon and intron interactions



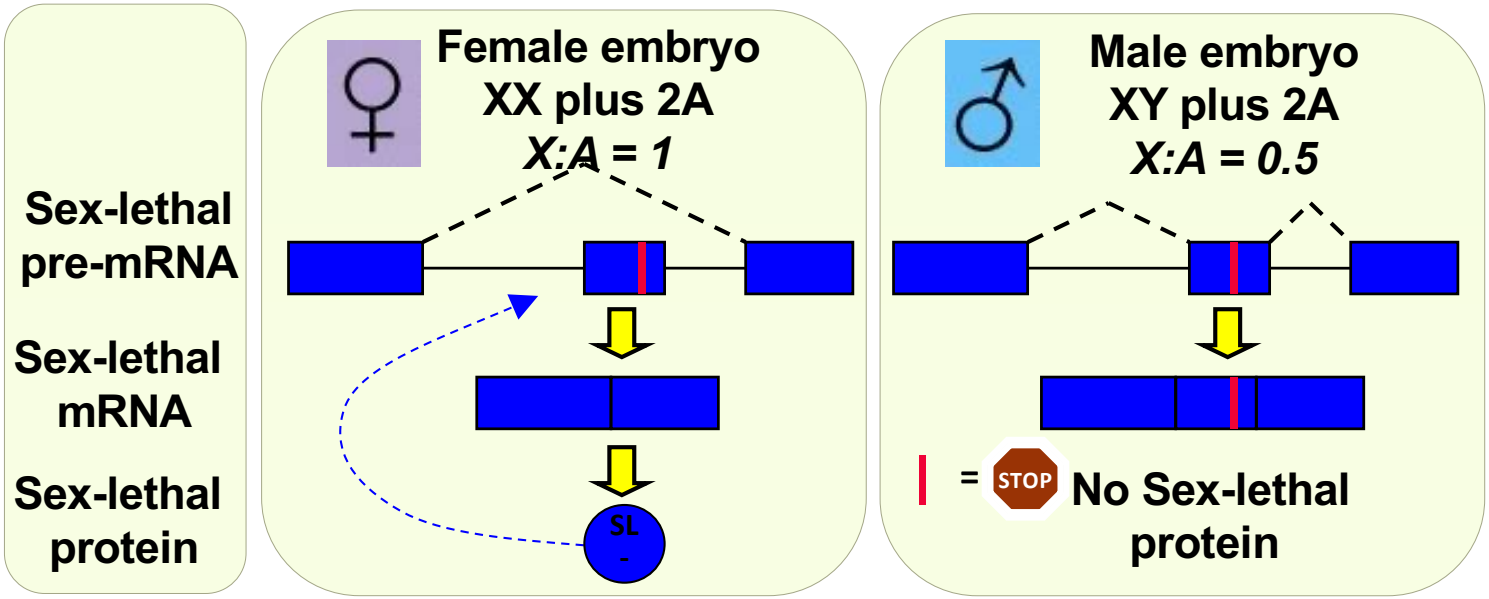
Communication between
3' and 5' splice sites



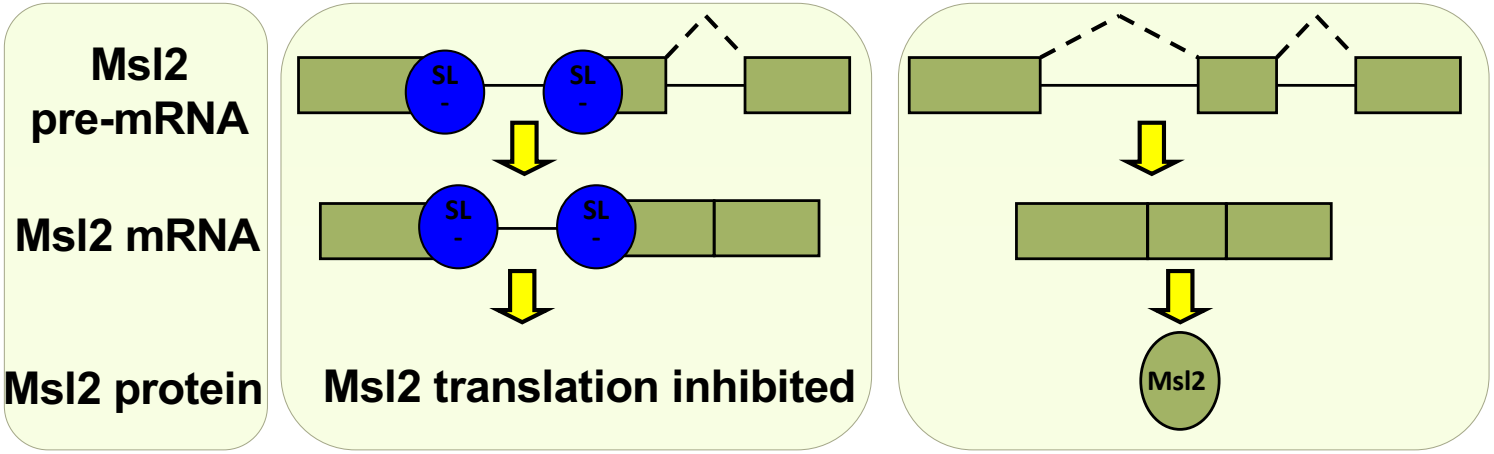
Chen and Manley, Nat Rev Mol Cell Biol, 2009; Ule and Blencowe, Mol Cell., 2019

AS: *Drosophila* sex determination

Zbigniew Dominski, lectures 2008



AS generates "Sex Lethal" protein in female embryos, a splicing inhibitor

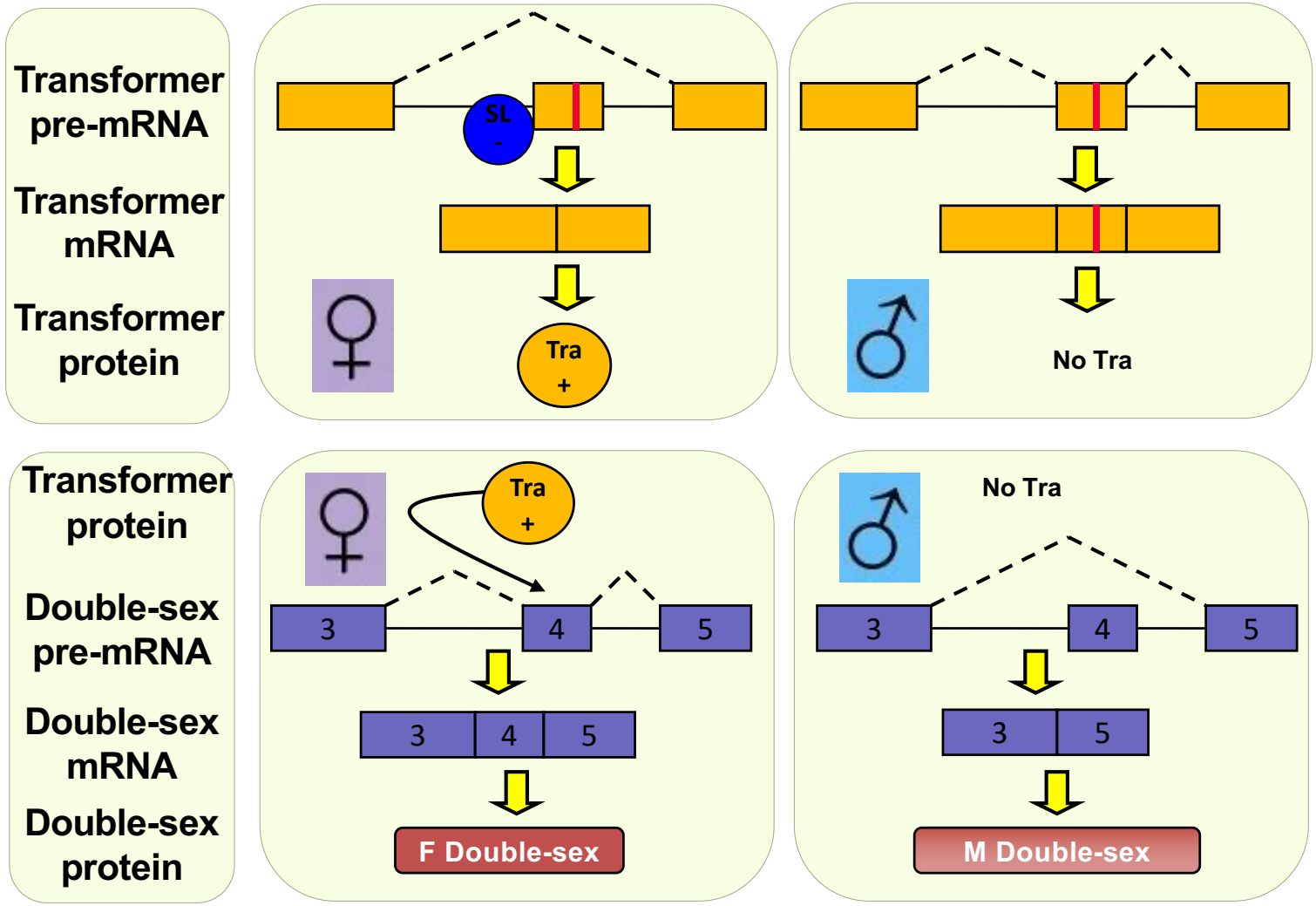


Sex Lethal controls AS of "Male-specific lethal 2" (Msl2) produced in males

AS: *Drosophila* sex determination

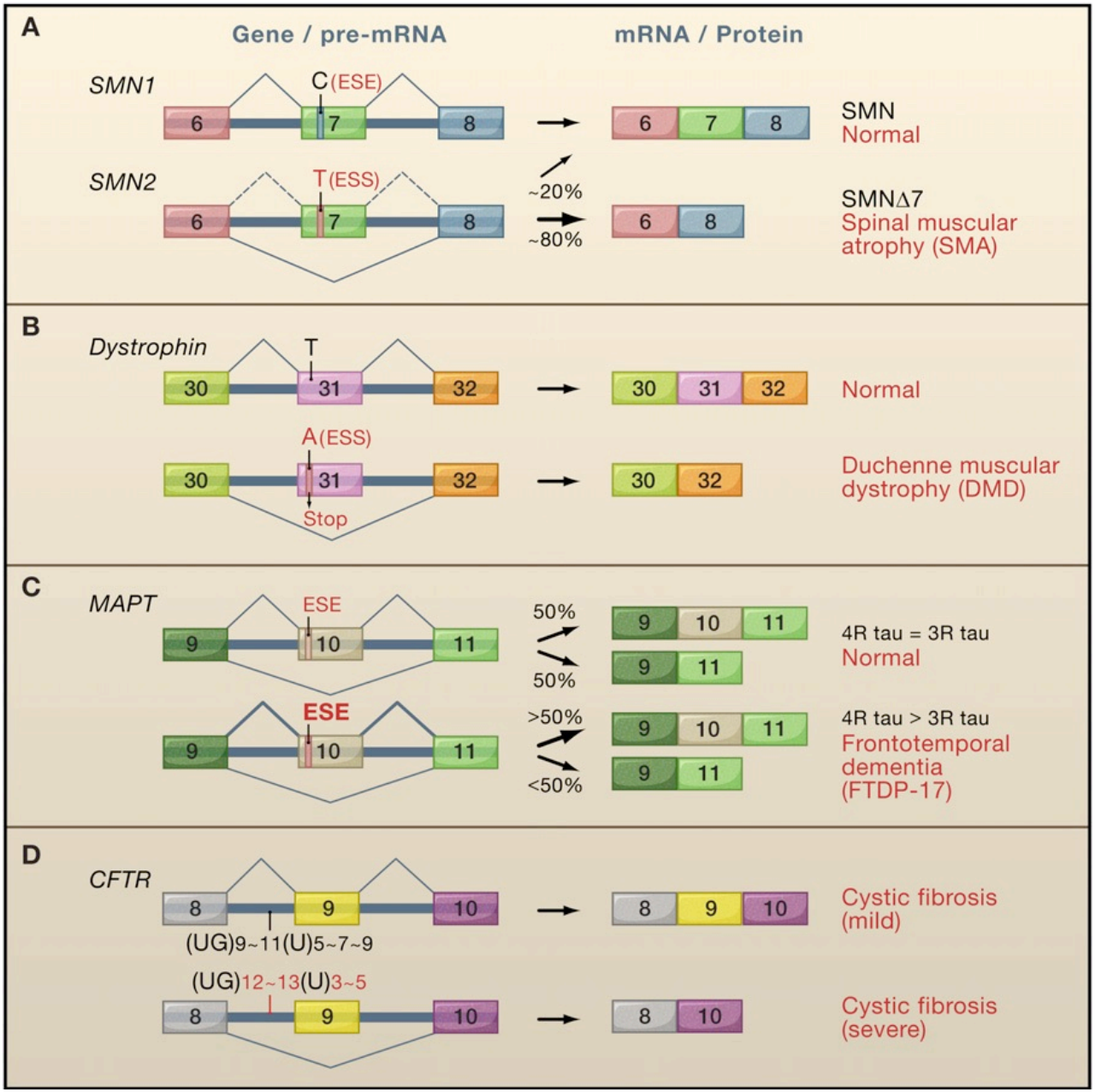
Zbigniew Dominski, lectures 2008

Sex Lethal modifies AS of Transformer pre-mRNA

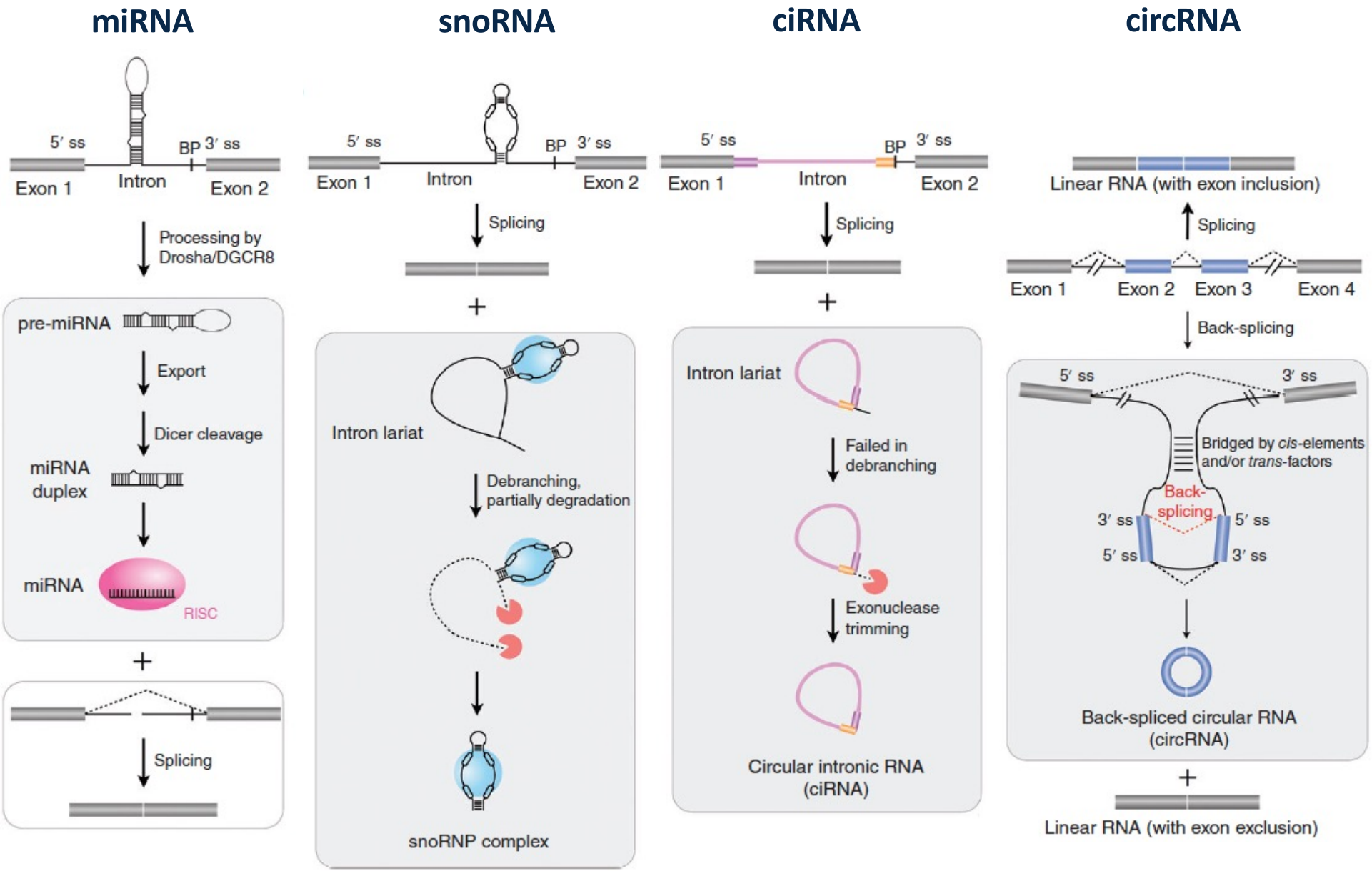


Tra – splicing activator - affects production of F/M Double-sex proteins: transcriptional factors controlling expression of female/male genes

AS and disease



Introns and ncRNAs

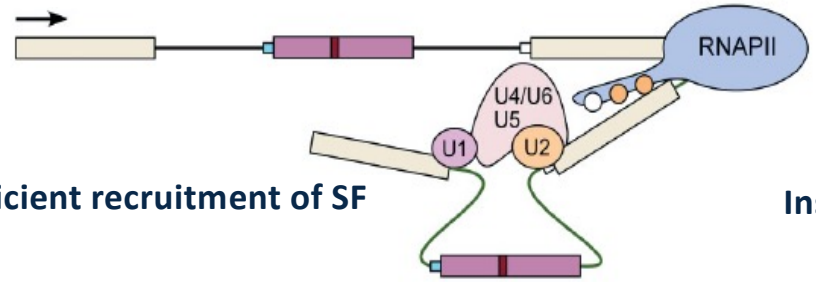


Yang, WIREs RNA, 2015

AS and transcription

CTD Ser-P and splicing

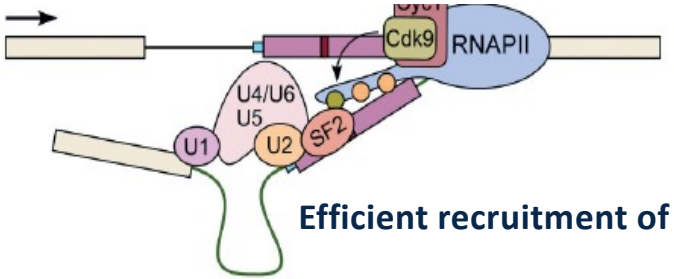
Inefficient Ser2 phosphorylation



Inefficient recruitment of SF

mRNA Skipping of a weak exon

Efficient Ser2 phosphorylation

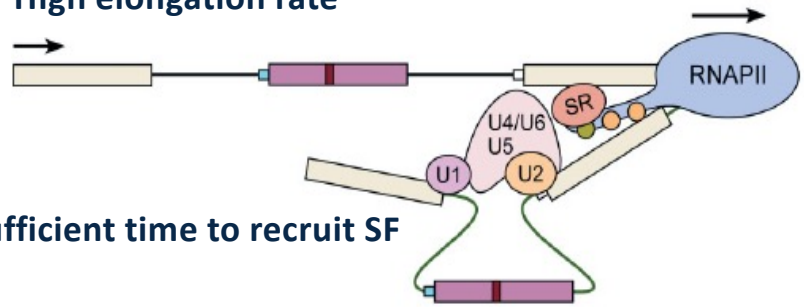


Efficient recruitment of SF

mRNA Inclusion of weak exon

Elongation rate and splicing

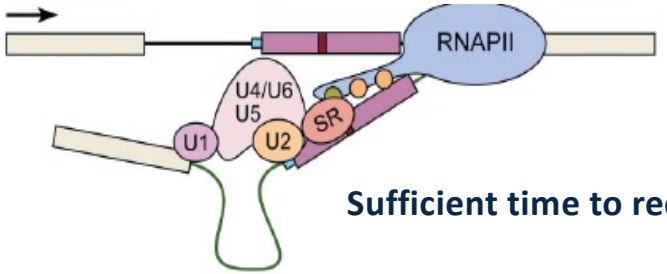
High elongation rate



Insufficient time to recruit SF

mRNA Skipping of a weak exon

Low elongation rate

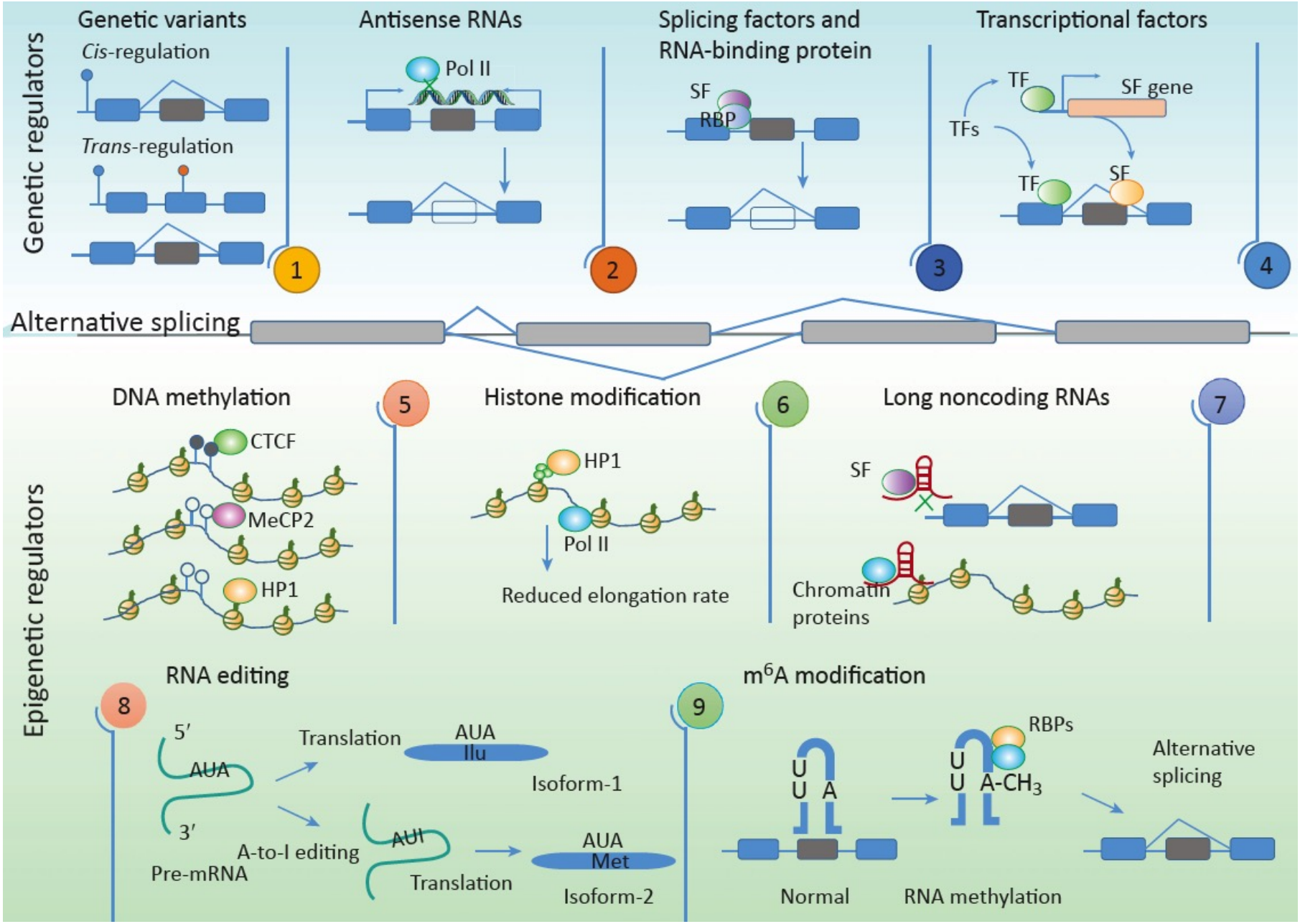


Sufficient time to recruit SF

Inclusion of a weak exon

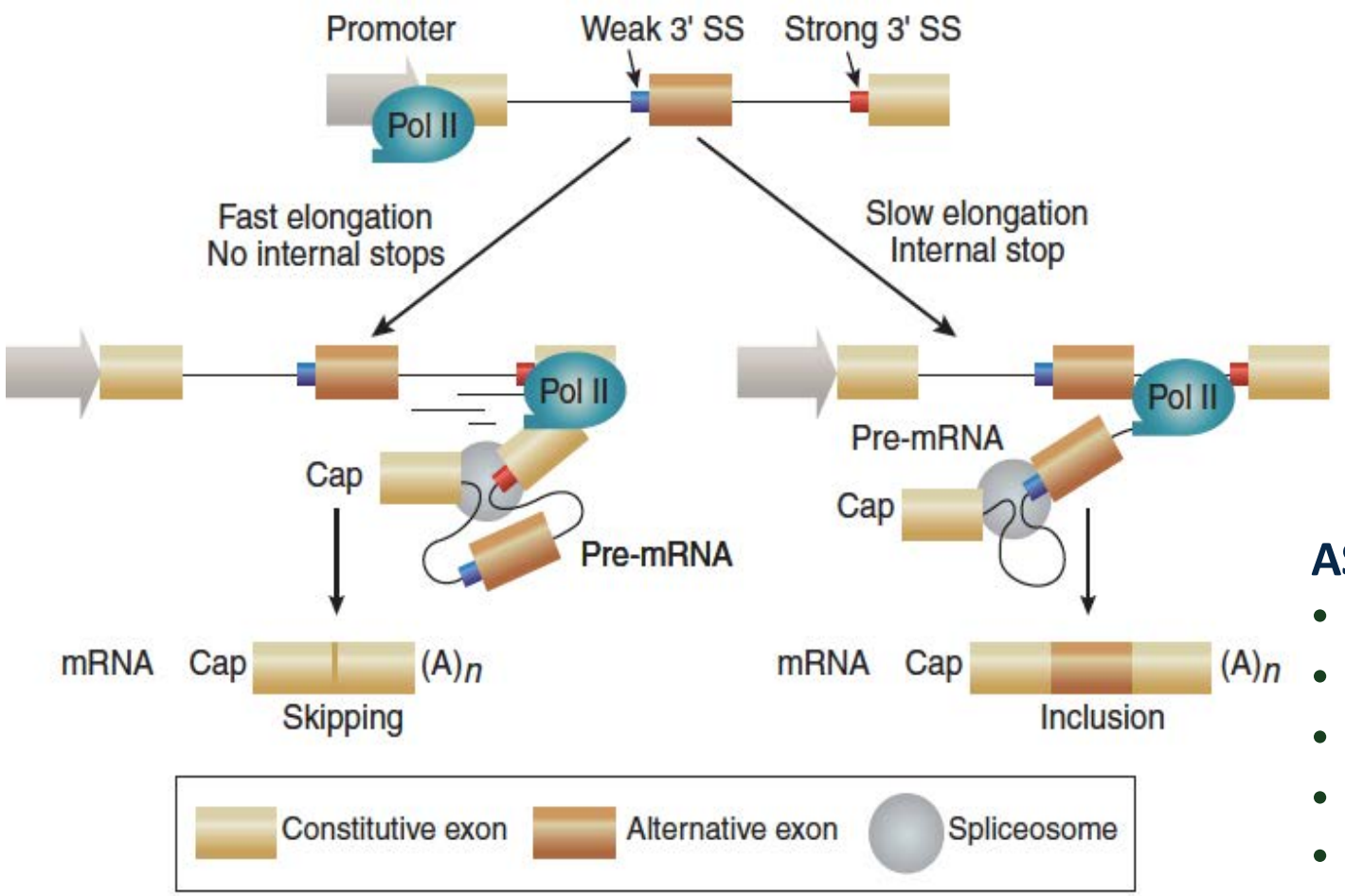
Lenasi and Barboric, WIREsRNA, 2013

Regulation of AS



Li et al, TiBS, 2018

Regulation of AS



AS is determined by

- **chromatin,**
- **Pol II elongation rate**
- **Pol II CTD-P status**
- **nascent RNA structure**
- **RNA binding proteins**
- **ncRNAs**
- **splicing factors**
  (e.g. SR proteins)

Kornblihtt, Nat Struct Mol Biol, 2009;
Herzel et al, Nat Rev Mol Cell Biol, 2017

AS in yeast *S. cerevisiae*

- **290 intron-containing genes (5%), most are single introns**
- **Introns are enriched in highly expressed genes**
- **Yeast has probably lost introns in many genes**
- **45 intron-containing genes are inefficiently spliced during vegetative growth**
- **Regulated splicing of 13 of the 20 intron-containing meiotic genes + *RPL30, YRA1, MTR2***
- **Regulated splicing/AS in most cases – intron retention**
- **Two genuine AS events for SRC1 and PTC7 that generate 2 proteins**

SRC1 splice variants (different 5' ss) give products of full and reduced activity

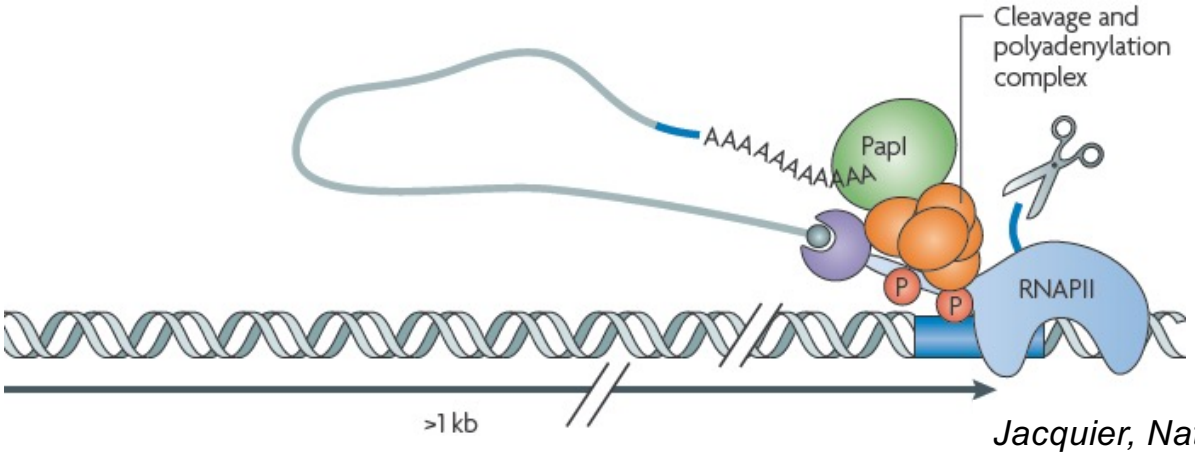
PTC7 AS results in different localization of proteins: product of unspliced mRNA localizes to the nuclear envelope, product of spliced mRNA to mitochondria

CLEAVAGE and POLYADENYLATION

mRNA 3’ end formation

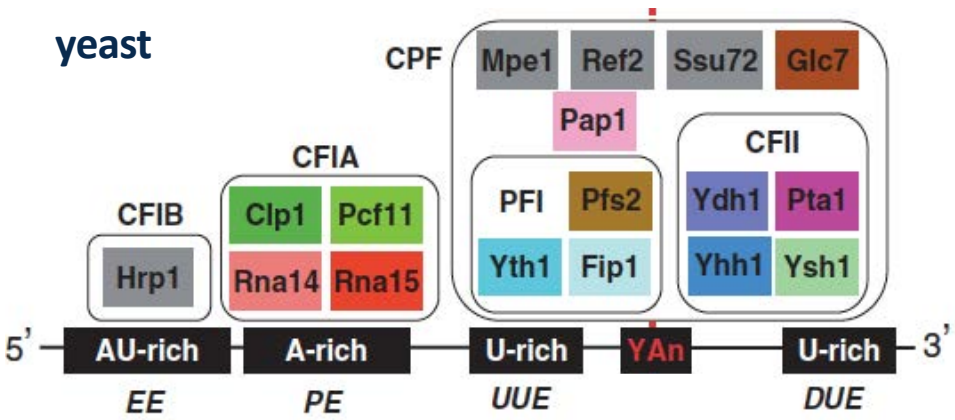
mRNA

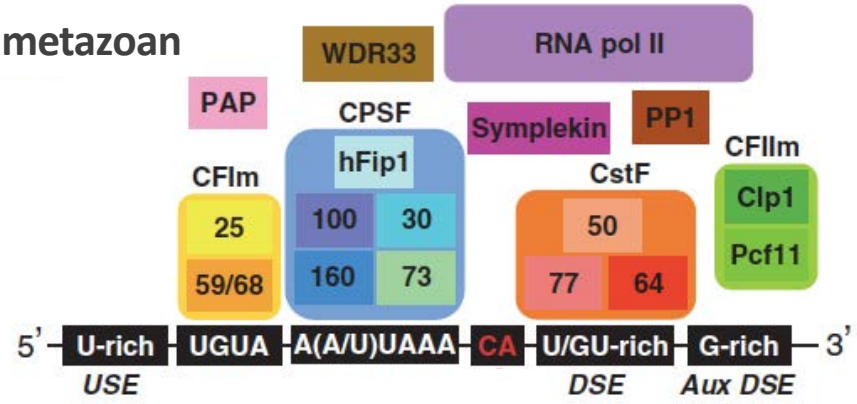
ncRNA



Jacquier, Nat. Rev. Genet, 2009

Cleavage and polyadenylation complex (CPA) recruited to CTD via Ser2-P

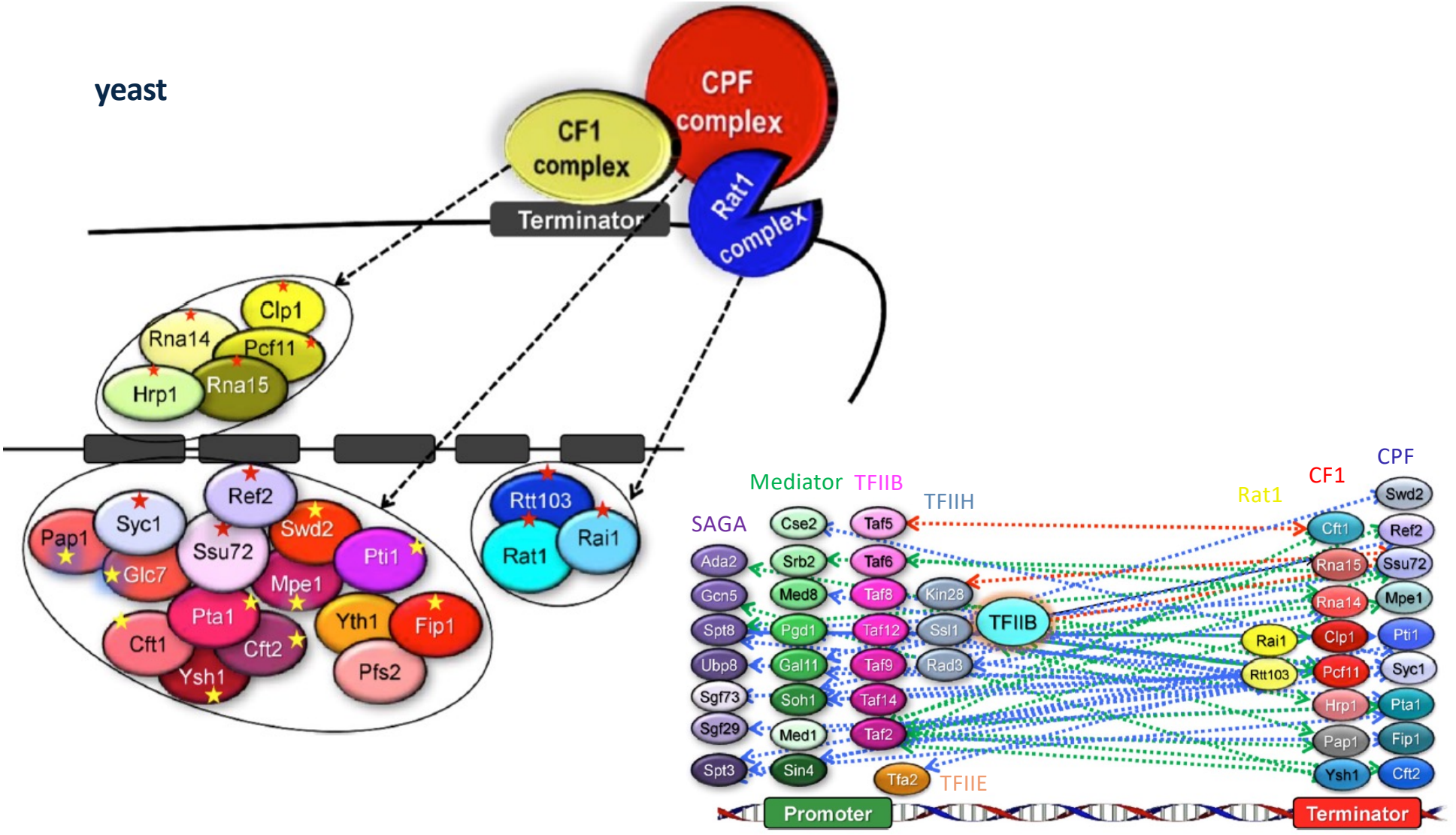




Cleavage by CPSF-73 (human), Brr5/Ysh1 (yeast)

Millevoi and Vagner, NAR, 2008

3’ end processing and termination



★ Termination factors that interact with initiation machinery

blue- generic interactions

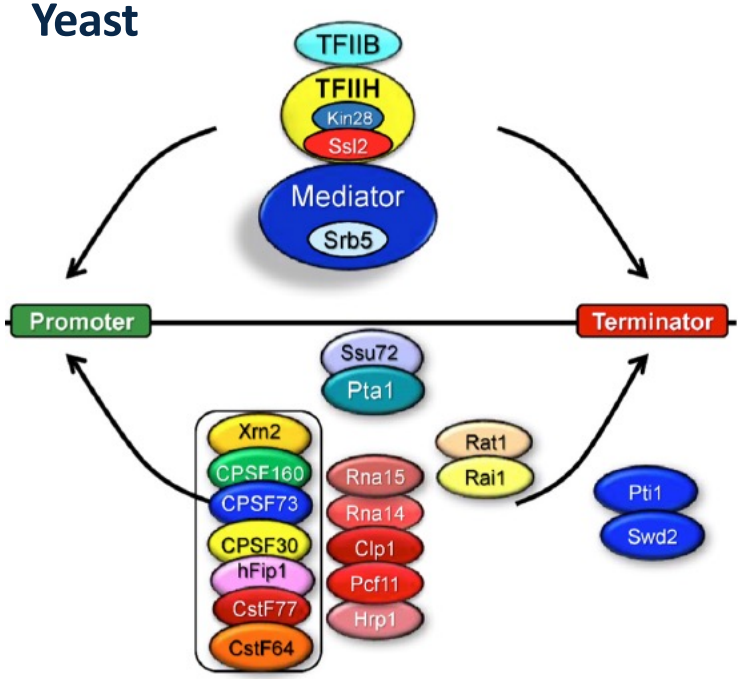
green- physical interactions

red - both

Al-Husini et al. BBA, 2020

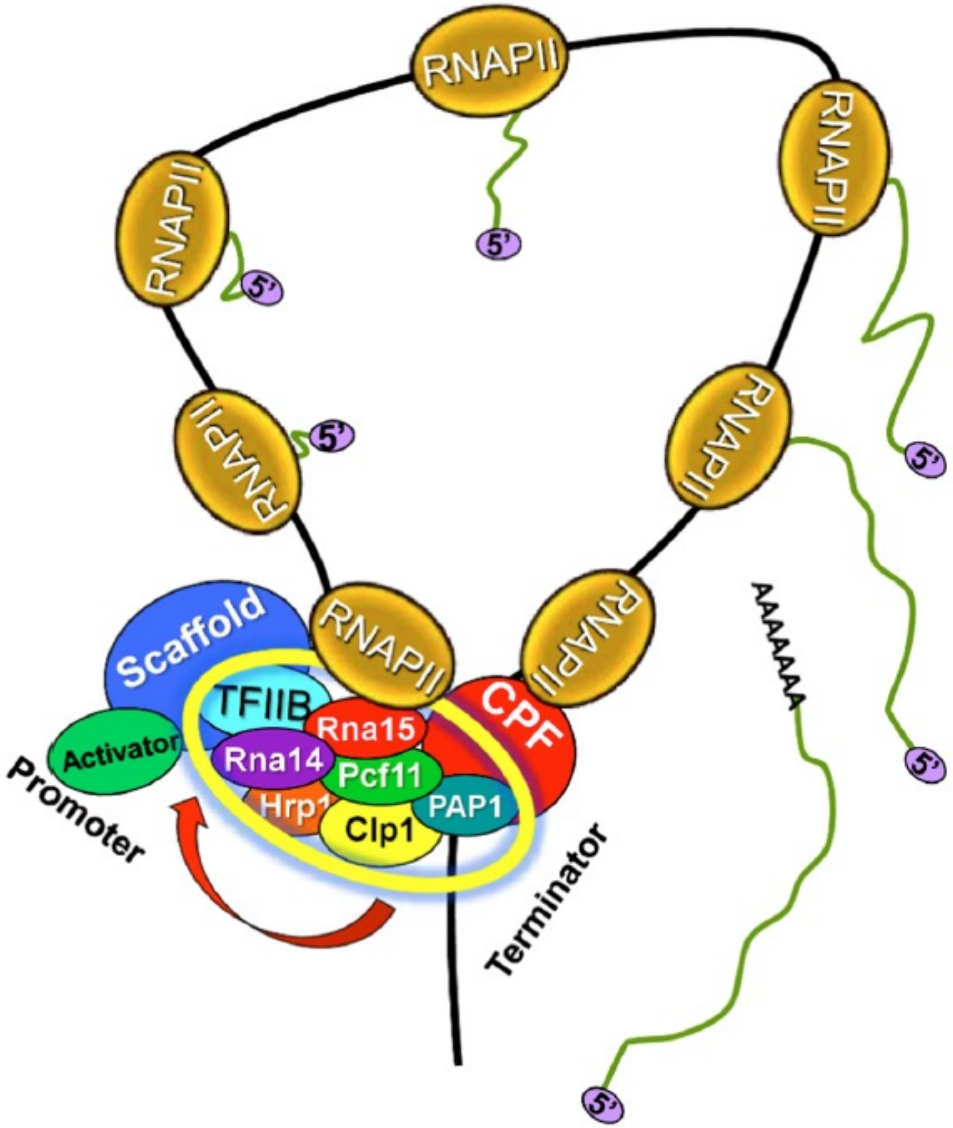
Initiation and termination are linked

Yeast



mammalian termination factors

Initiation factors are present on the terminator and termination factors on the promoter



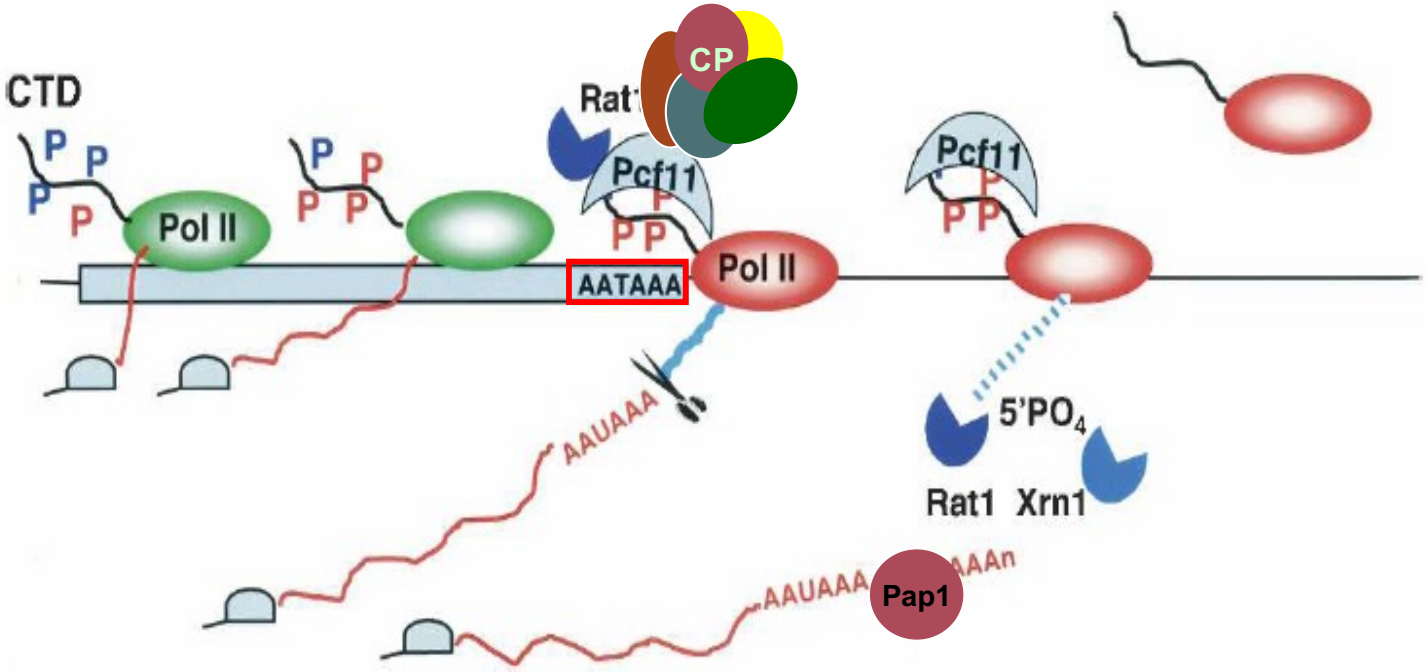
Gene looping is facilitated by the interaction of factors at the promoter and terminator

Al-Husini et al. BBA, 2020

Transcription termination

Hybrid allosteric- torpedo model

Yeast

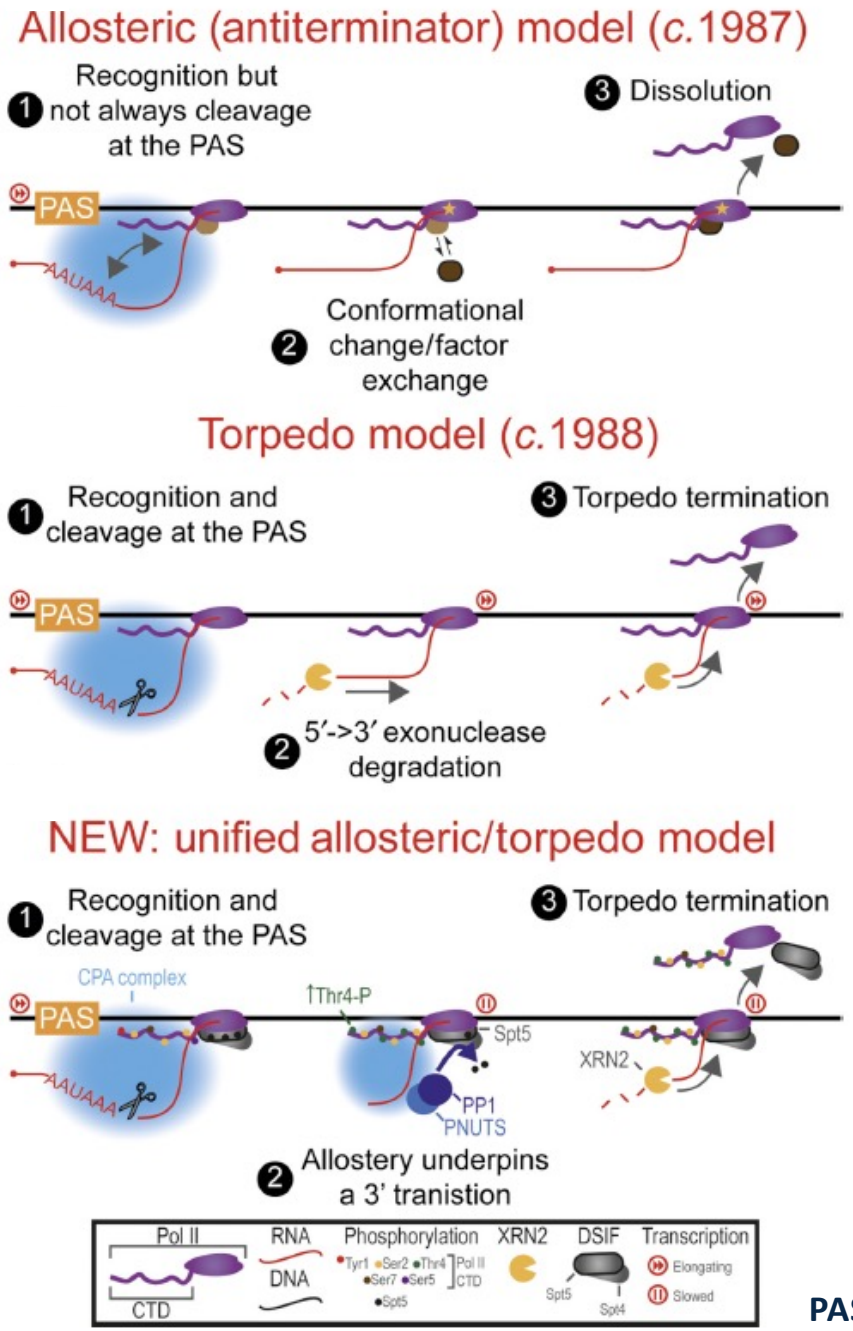


Luo and Bentley, Gene Dev, 2006

3'- end processing factors are recruited to Ser2-P CTD at 3' end of genes via CID (CTD-interacting domain). Pcf11 recruits CPA, Rtt103 recruits 5'-3' exonuclease Rat1/XRN2 and Rat1 activator Rai1 (in yeast).

Pcf11 and Rat1 coordinately contribute to the recruitment of 3'-end processing factors.

Evolution of the PAS-dependent Pol II termination model



PAS- poly(A) site

<u>Allosteric/anti-terminator model</u>

Conformational change or factors recruited to or dissociated from Pol II render it termination competent

<u>Torpedo model</u>

PAS cleavage generates a Pol-II-associated RNA that is degraded 5′ → 3′ by Rat1/Xrn2, leading to termination

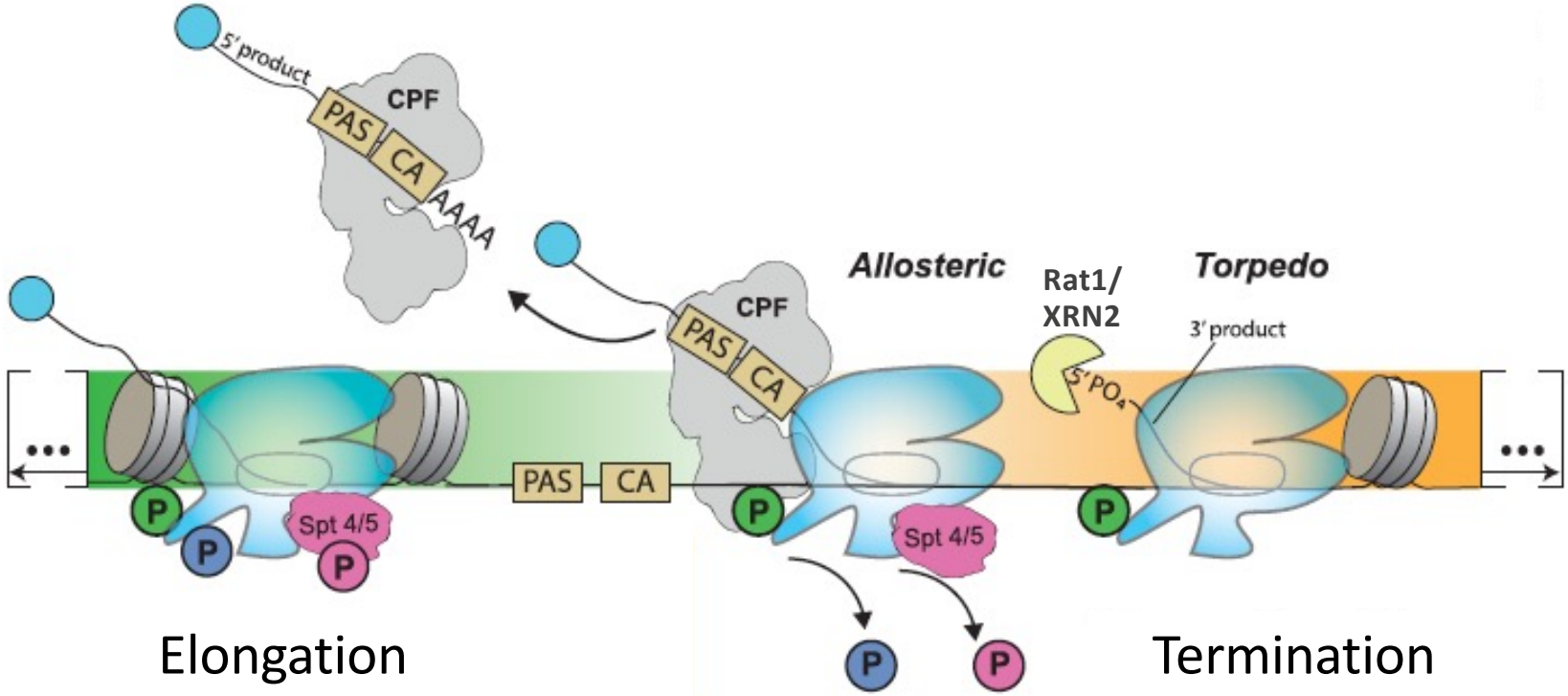
<u>Allosteric/torpedo model</u>

PAS cleavage promotes Pol II slowing, caused by dephosphorylation of SPT5 and promotes an allosteric switch. Paused Pol II is terminated by Rat1/XRN2, which degrades the nascent transcript after PAS cleavage

Eaton and West, TiG, 2020

Transcription termination

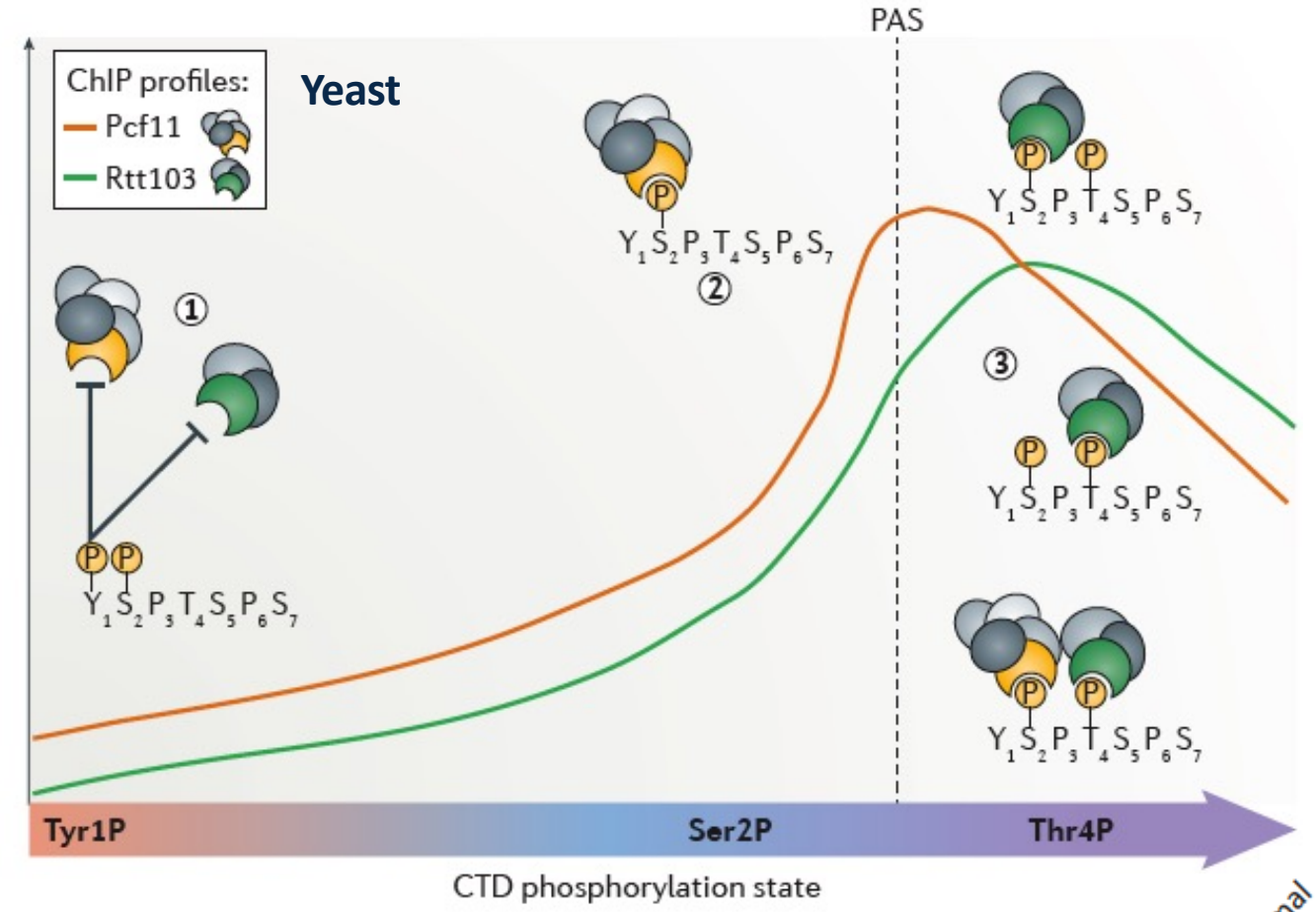
Hybrid allosteric- torpedo model

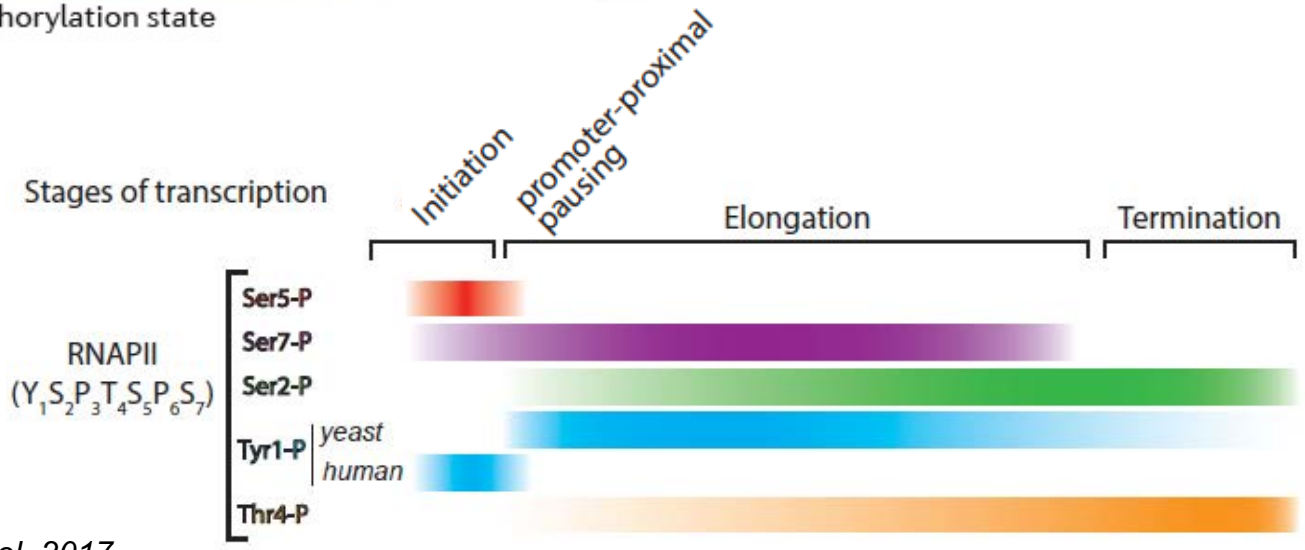


- **Transcription of the PAS recruits CPF/CPSF to RNA and transcribing Pol II.**
- **CPF/CPSF binding to the PAS promotes Pol II-CTD dephosphorylation by Glc7-Ref2/PP1-PNUTS, which allows recruitment of termination factors *(allosteric)*.**
- **CPSF-73 cleaves and releases the nascent pre-mRNA from Pol II.**
- **The 5’ end of mRNA is polyadenylated by poly(A) polymerase Pap1/PAP.**
- **The 5’ phosphate on the cleavage 3’ product is the substrate for the torpedo exonuclease Rat1/XRN2, which is required for termination *(torpedo)*.**

Eaton and West, TiG, 2020

Termination
CTD code



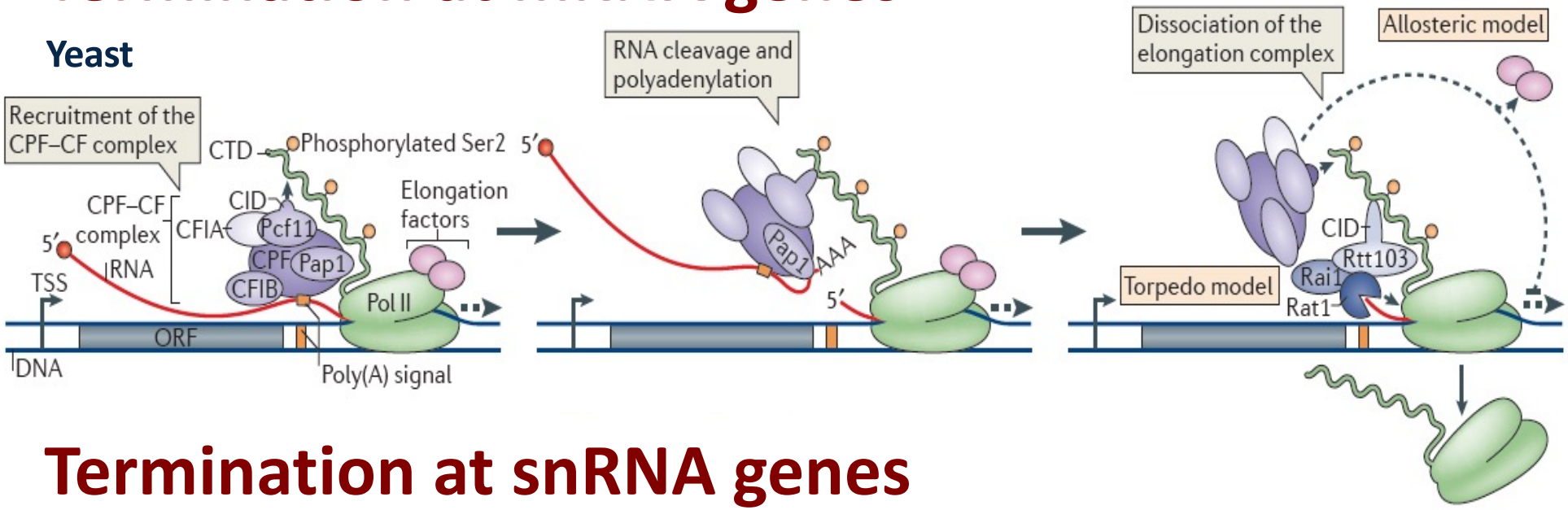


Rodrıguez-Molina et al, Mol Cell, 2022

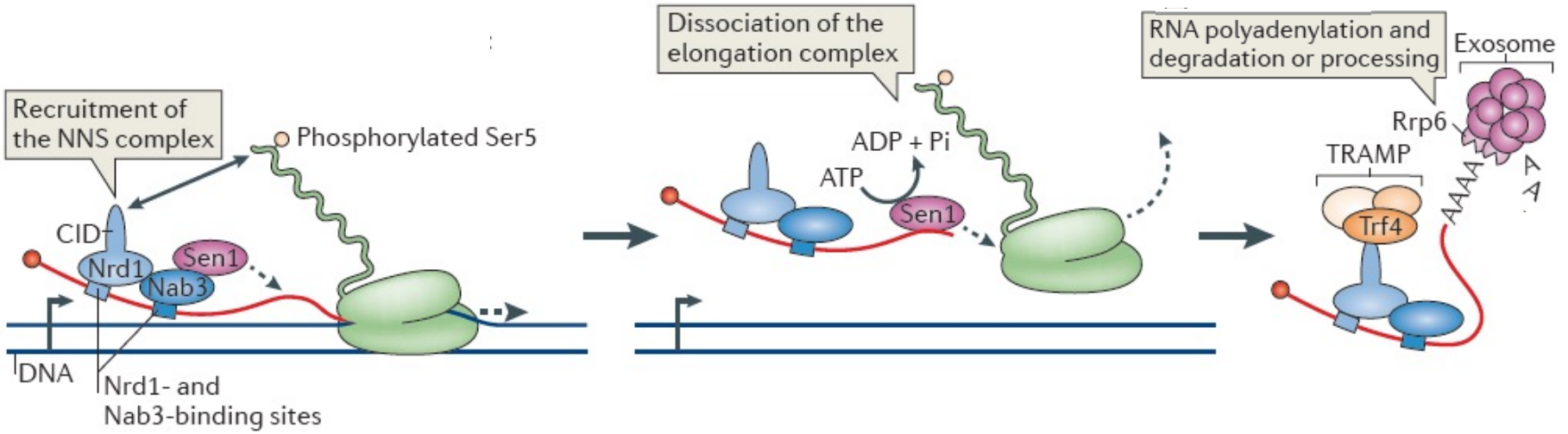
Harlan and Churchman, NatRevMolCellBiol, 2017

Termination at mRNA genes

Yeast



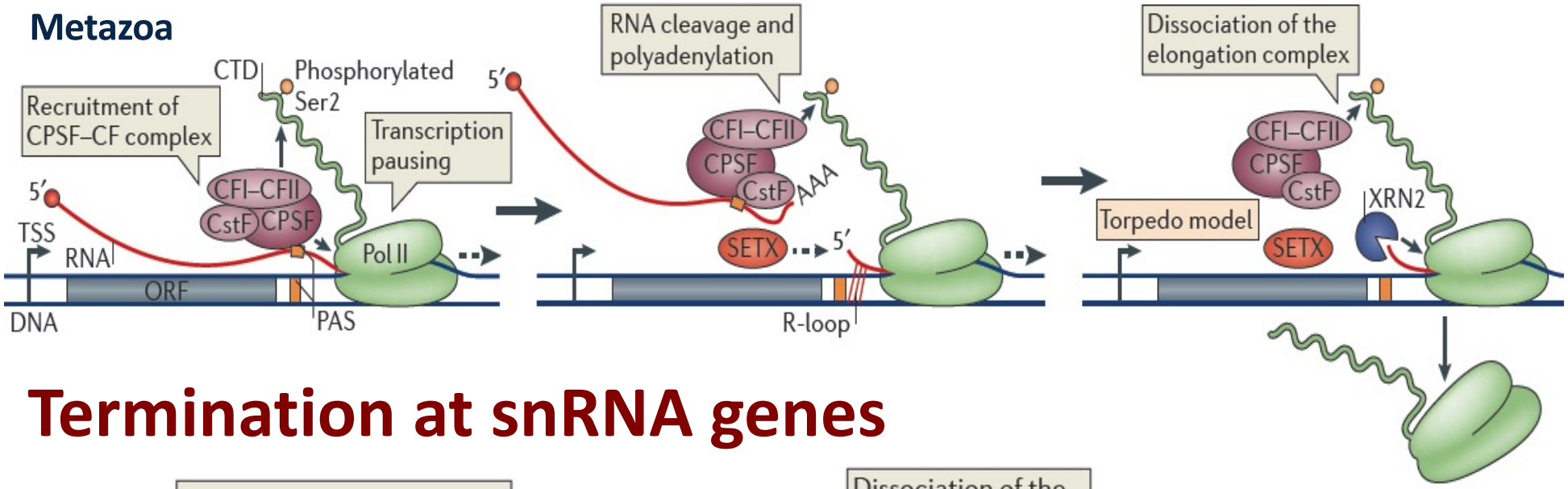
Termination at snRNA genes



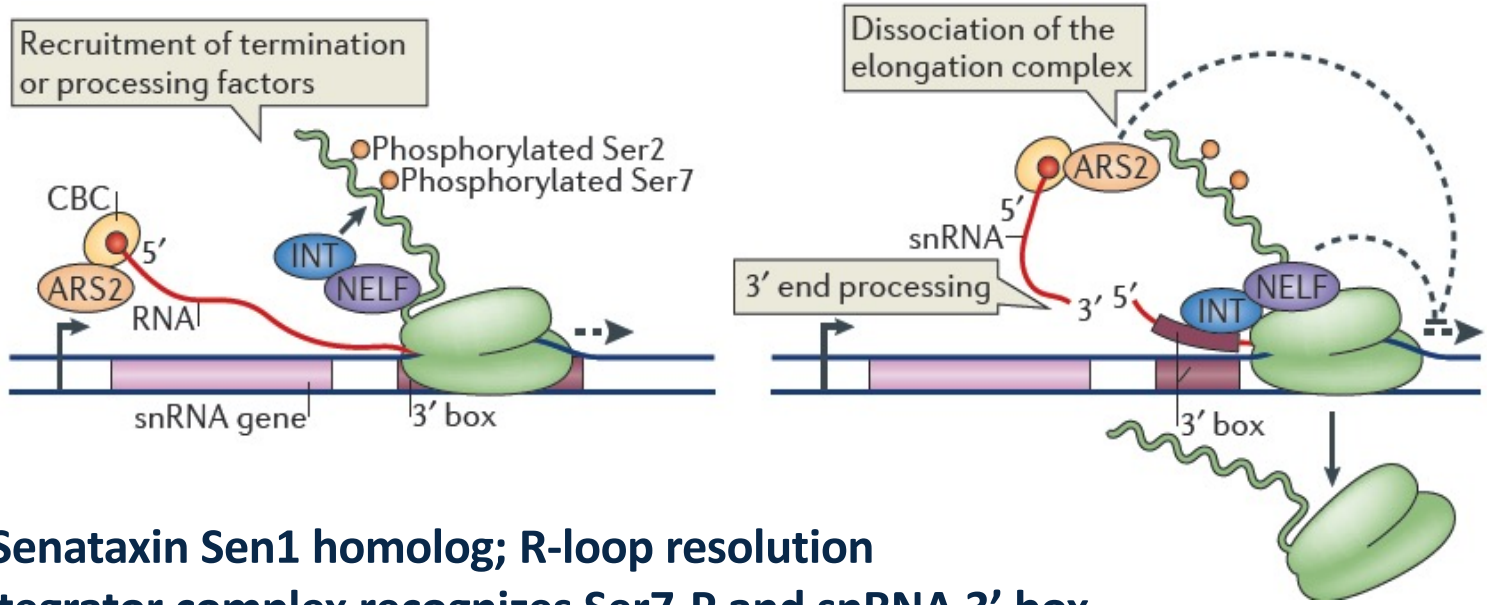
CPF-CF cleavage and polyadenylation complex
Pcf11 termination factor
Nrd1/Nab3 termination complex
Rat1/Rai1 5’-3’ exonuclease torpedo
Pap1 poly(A) polymerase
Mtr4/Trf4/Air1 TRAMP RNA surveillance complex

Poruua, Libri, Nat Rev Mol Cell Biol, 2015

Termination at mRNA genes



Termination at snRNA genes

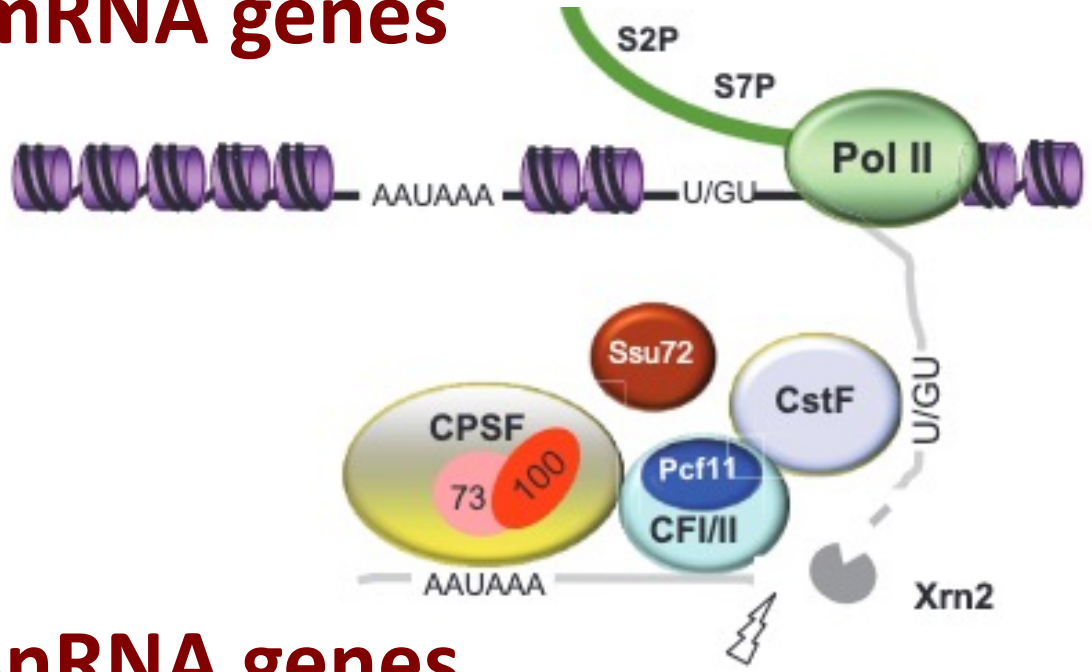


SETX – Senataxin Sen1 homolog; R-loop resolution
INT – Integrator complex recognizes Ser7-P and snRNA 3′ box
NELF – negative elongation factor **CBCA – CBC interacting with Ars2**

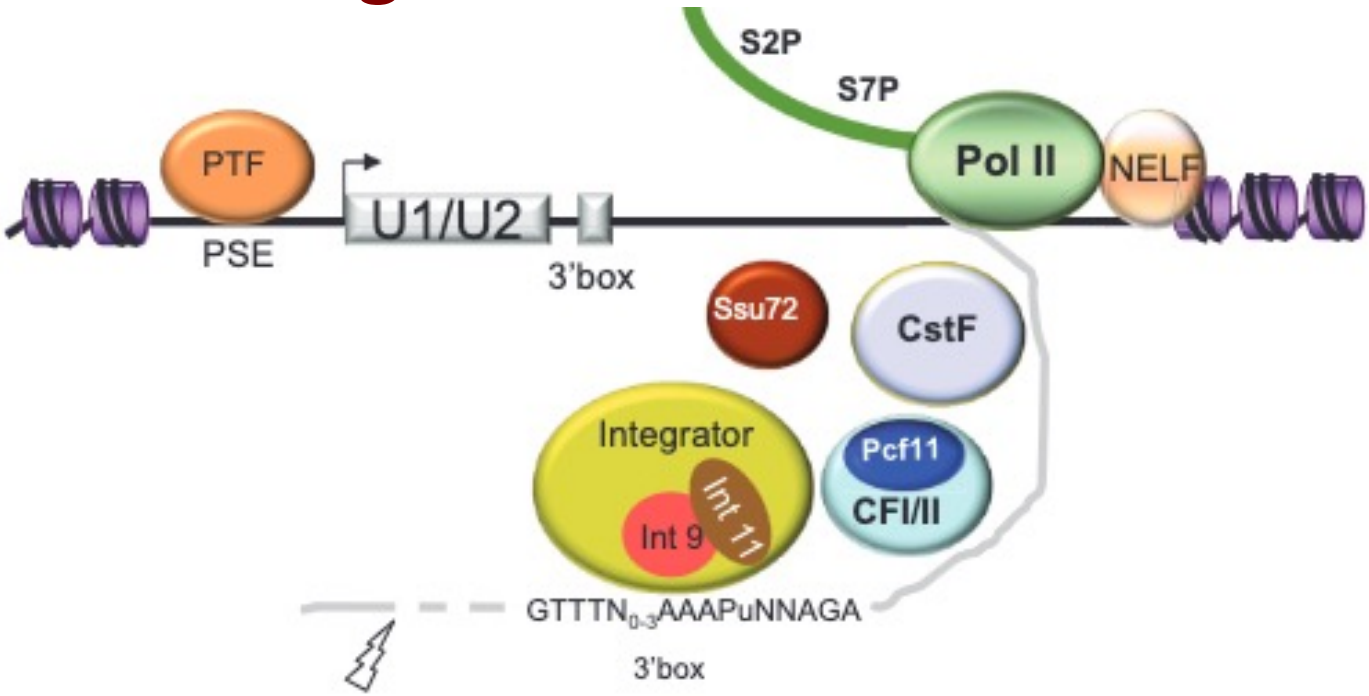
Poruua, Libri, Nat Rev Mol Cell Biol, 2015

Termination at mRNA genes

Metazoa



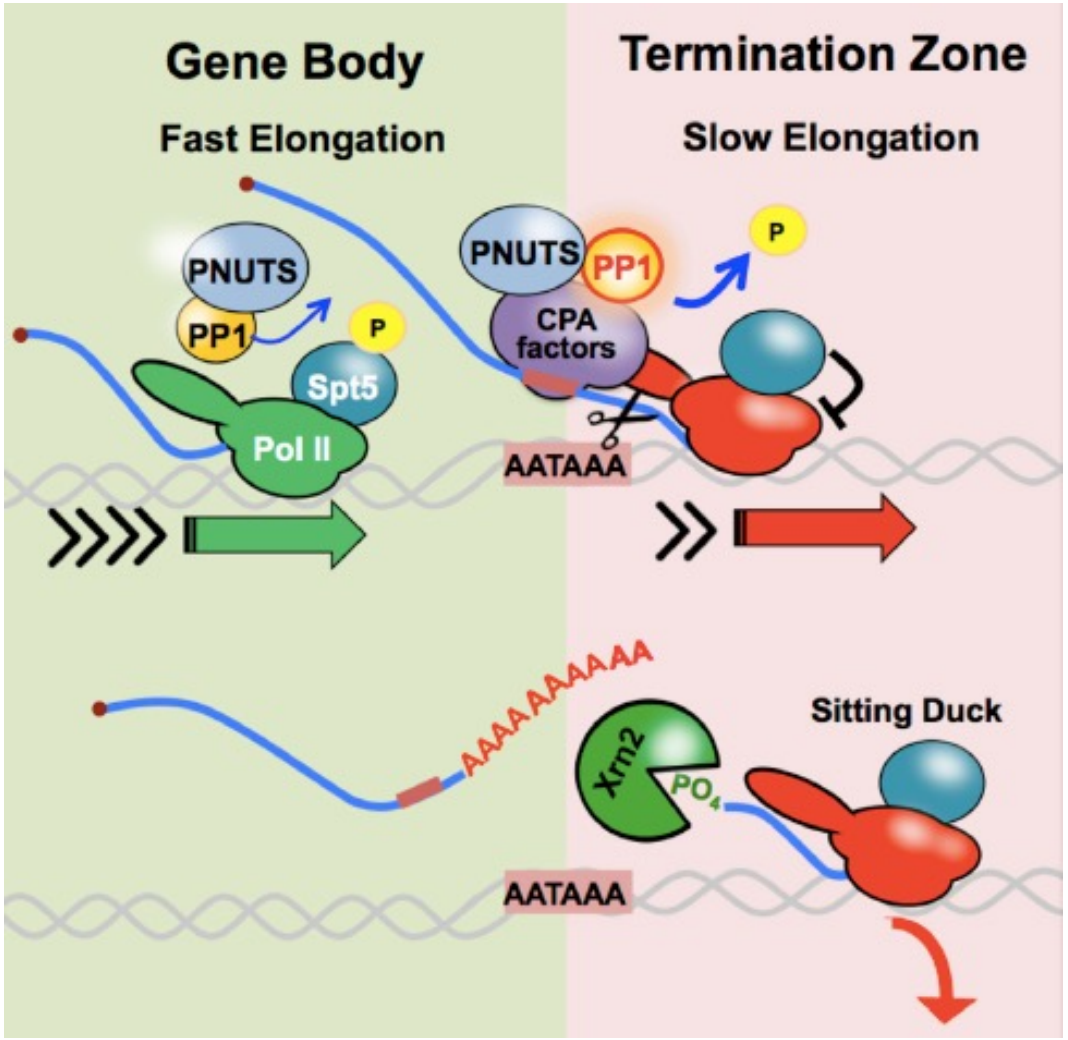
Termination at snRNA genes



O'Reilly et al, NAR, 2013

Termination at mRNA genes

Metazoa



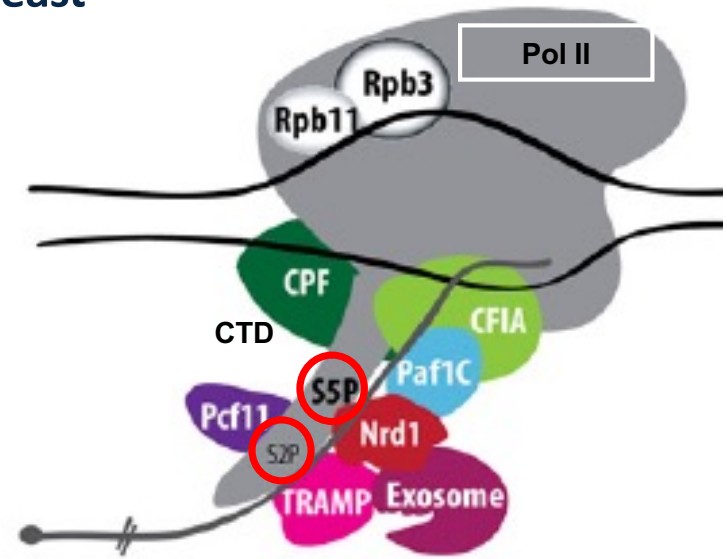
"Sitting Duck Torpedo" mechanism

- Pol II speed is limited by the PNUTS-PP1 phosphatase complex
- PNUTS-PP1 dephosphorylates the elongation factor Spt5
- Pol II decelerates in termination zones downstream of poly(A) sites
- Allosteric switch converts Pol II to a "sitting duck" and is tracked down and dislodged by Xrn2 "torpedo"

Cortazar et al, Mol Cell, 2019

Nrd1/Nab3/Sen1-dependent termination

Yeast



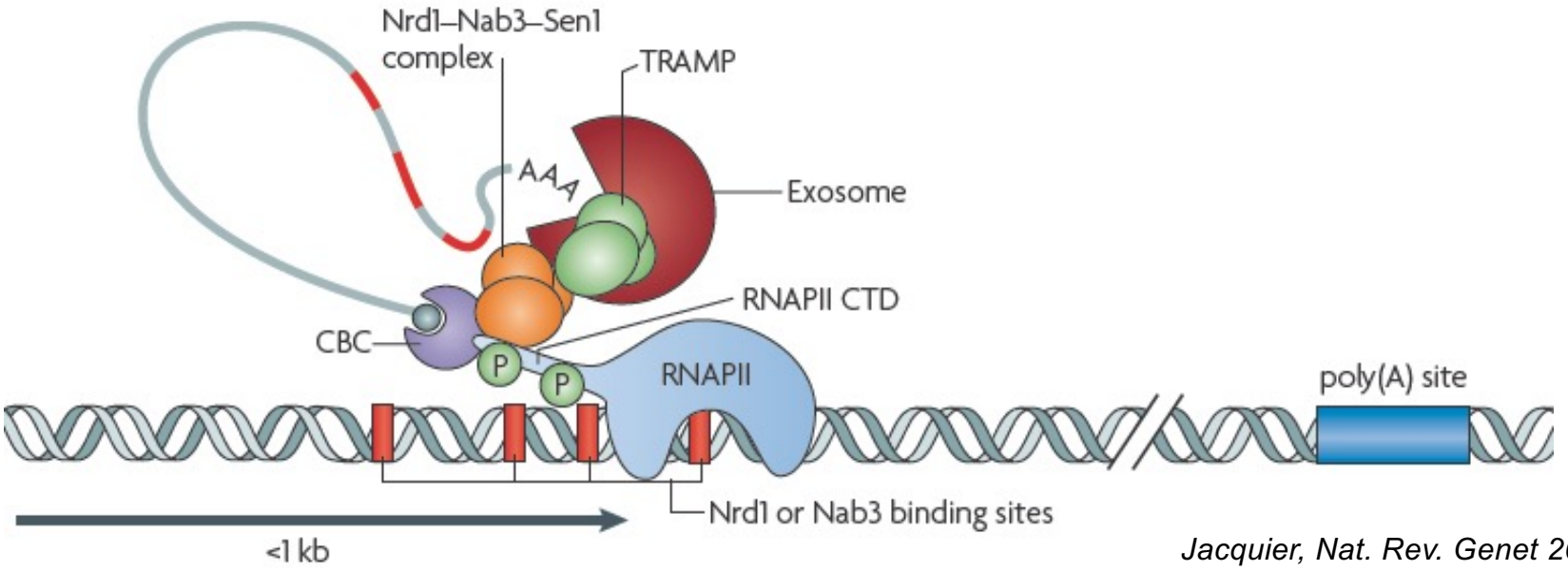
ncRNA

mRNA

Nrd1/Nab3/Sen1 complex (NNS)

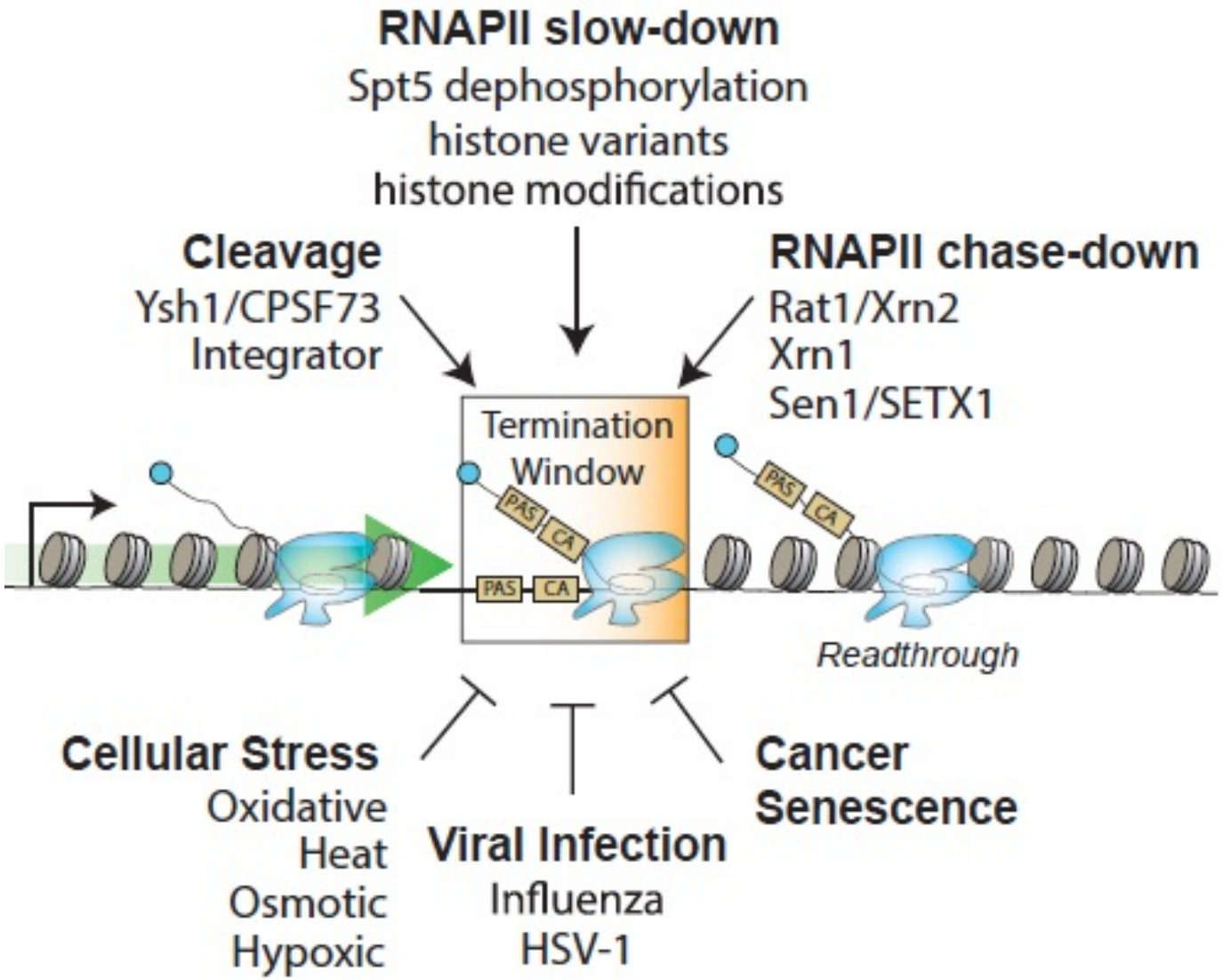
- **sn/snoRNAs**
- **CUTs**
- **short mRNAs** (< 600 nt)

Recruited to CTD via Ser5-P



Jacquier, Nat. Rev. Genet 2009

Transcription termination regulation



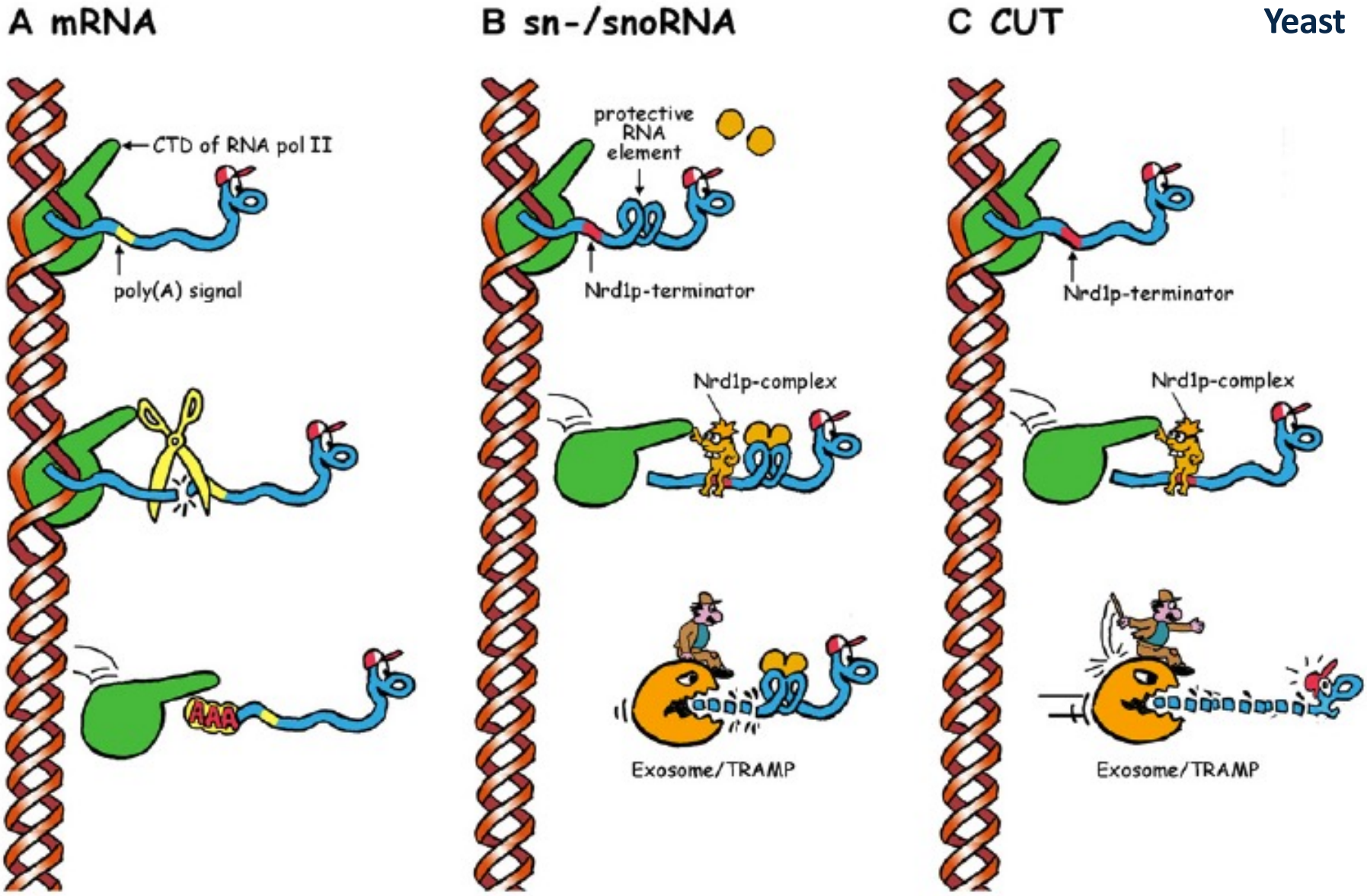
Pol II termination of different transcripts

Termination, processing and degradation pathways

Transcript	Termination pathway	Stability	Degradation factors
Yeast			
mRNA	CPF–CF and possibly Sen1	Stable	None
snRNA and snoRNA	NNS	Stable (3′ end processed)	TRAMP, Rrp6, exosome, Rex1 (3′ end processing)
CUT	NNS	Unstable	TRAMP, Rrp6, exosome
SUT	CPF–CF and possibly NNS	Partially unstable	Rrp6, exosome, Xrn1 (NMD)
XUT	CPF–CF	Unstable	Xrn1 (NMD)
RUT	Reb1 roadblock	Unstable	TRAMP, Rrp6, exosome
Metazoan			
mRNA	CPSF–CF and SETX	Stable	None
snRNA	Integrator complex, CBC–ARS2, PCF11 and NELF	Stable (3′ end processed)	Exosome (3′ end processing)
mRNAs encoding replication-dependent histones	CBC–ARS2	Stable	None
PROMPT	CPSF–CF and CBC–ARS2	Unstable	NEXT and exosome

Poruua, Libri, Nat Rev Mol Cell Biol, 2015

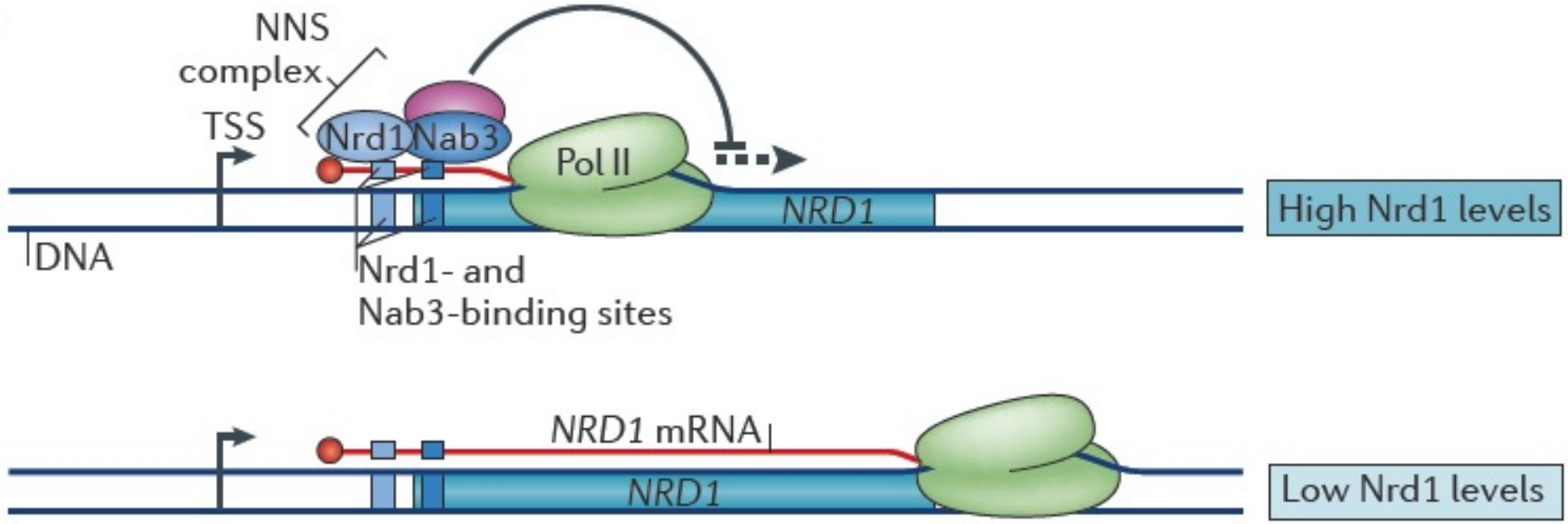
Pol II terminaton of different transcripts



Lykke-Andersen and Jensen, Biochemie, 2007

Attenuation of gene expression by NNS

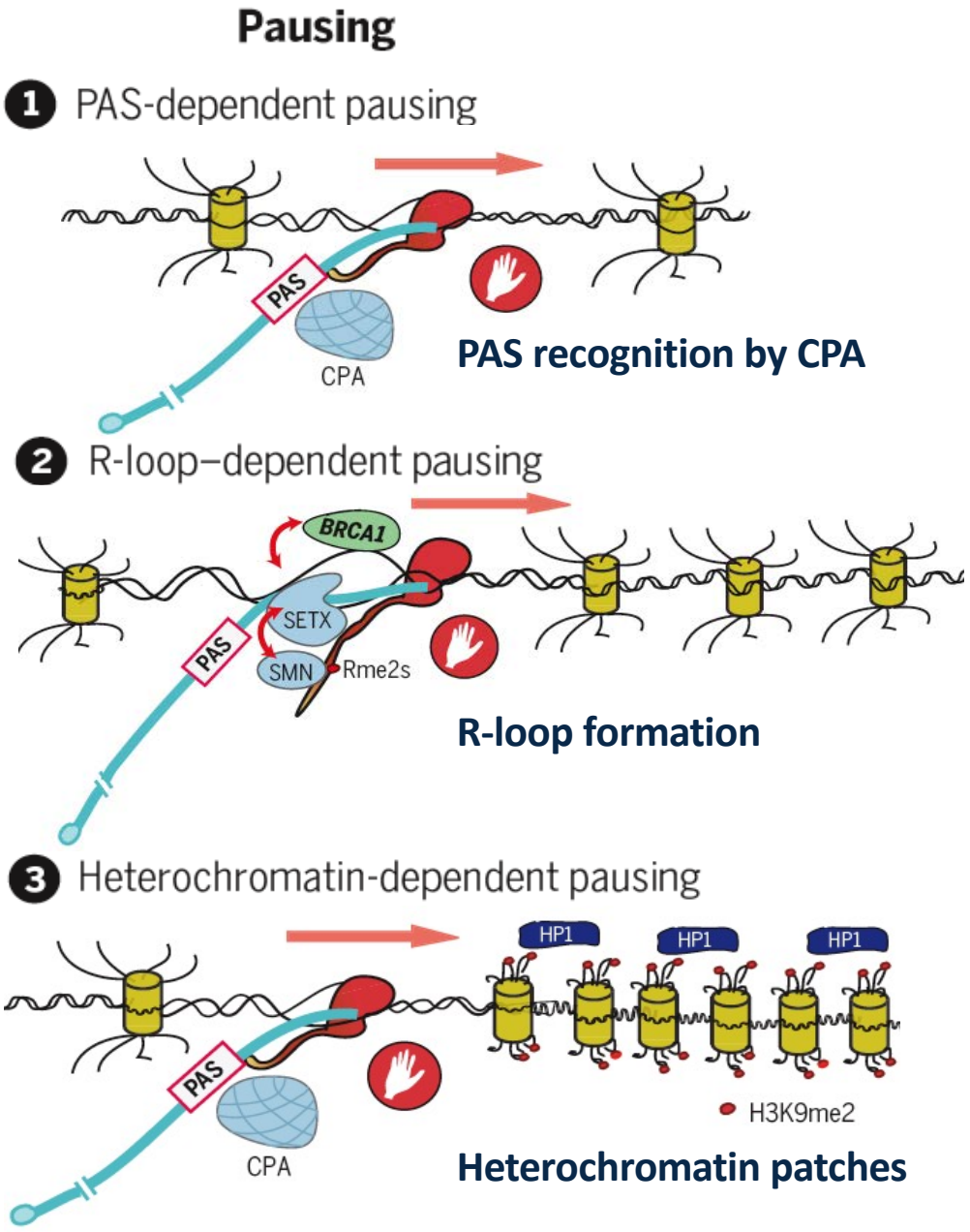
Yeast

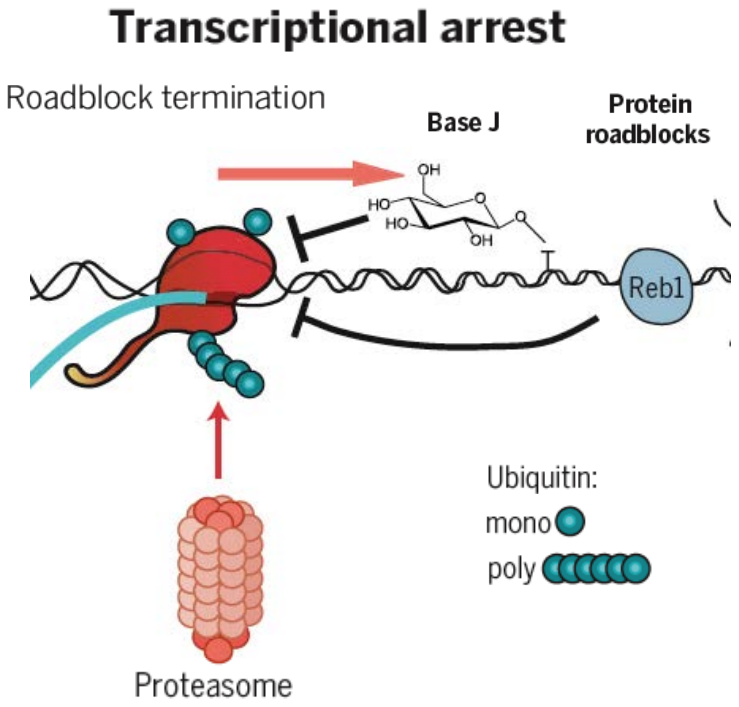


Binding of the NNS complex to Nrd1- and Nab3-binding sites in 5'UTR and 5' end of the *NRD1* gene promotes the NNS-mediated premature termination and exosomal degradation of the *NRD1* mRNA

Poruua, Libri, Nat Rev Mol Cell Biol, 2015

Transcription termination and Pol II pausing

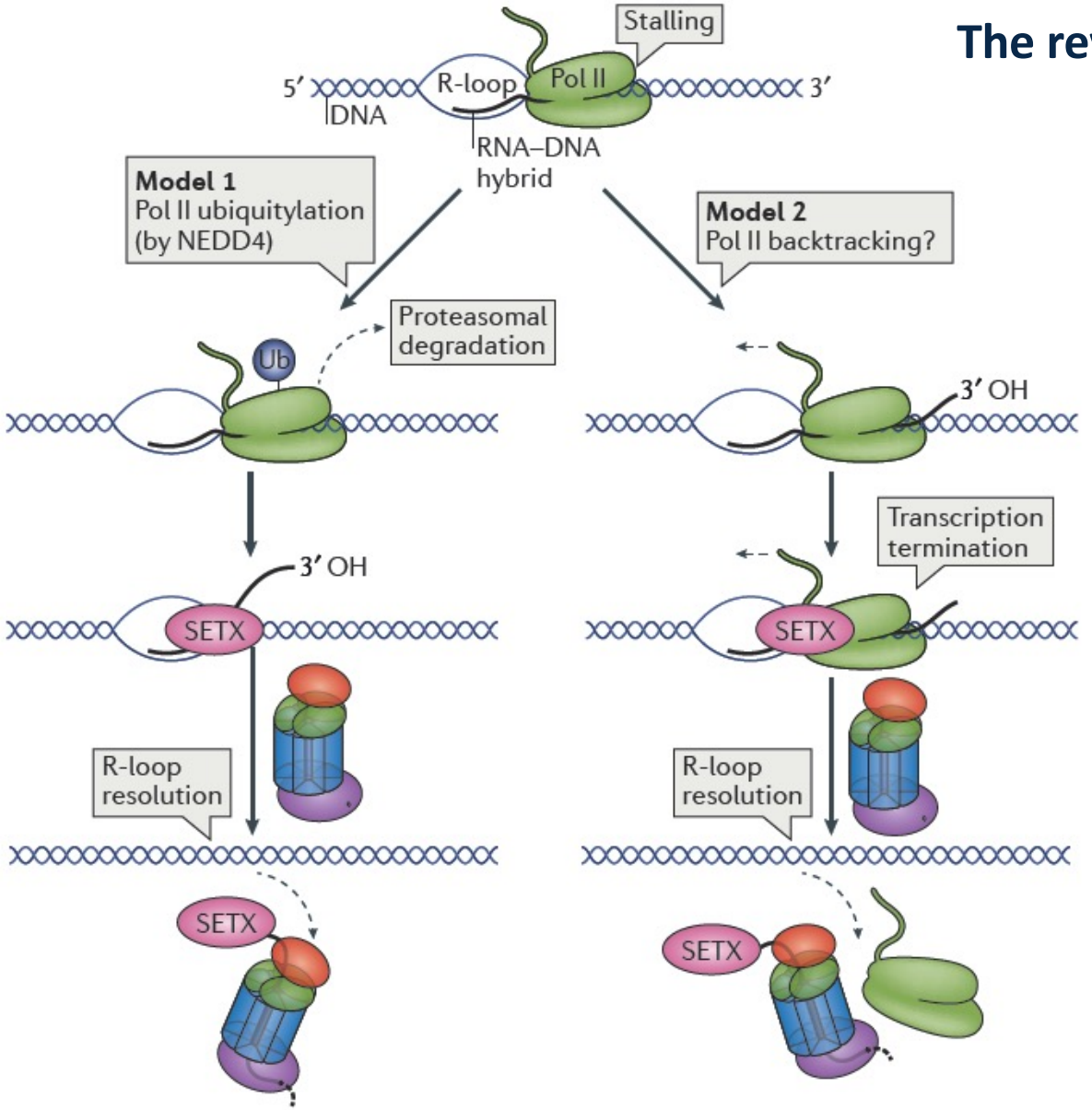




Pol II arrested by DNA damage or protein binding is ubiquitinated and degraded by the proteasome

Proudfoot, Science, 2016

Transcription termination and the exosome



Kilchert et al, Nat Rev Mol Cell Biol, 2016

The reverse torpedo model

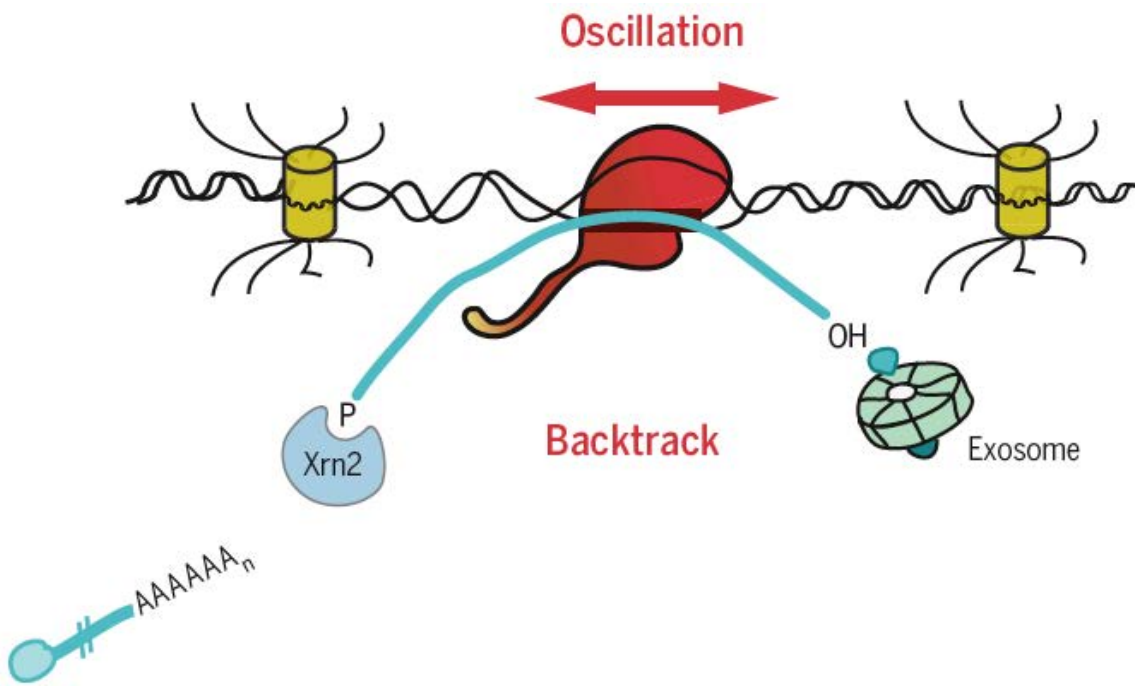
Pol II stalling and backtracking (e.g. by R-loops) **expose the 3'OH end of nascent transcript**

This attracts the exosome that degrades nascent transcript, causing Pol II displacement, R-loop resolution by SETX and transcription termination

The exosome-dependent reverse torpedo functions as a fail-safe mechanism of termination

Polymerase backtracking

Pol II forward and reverse torpedo

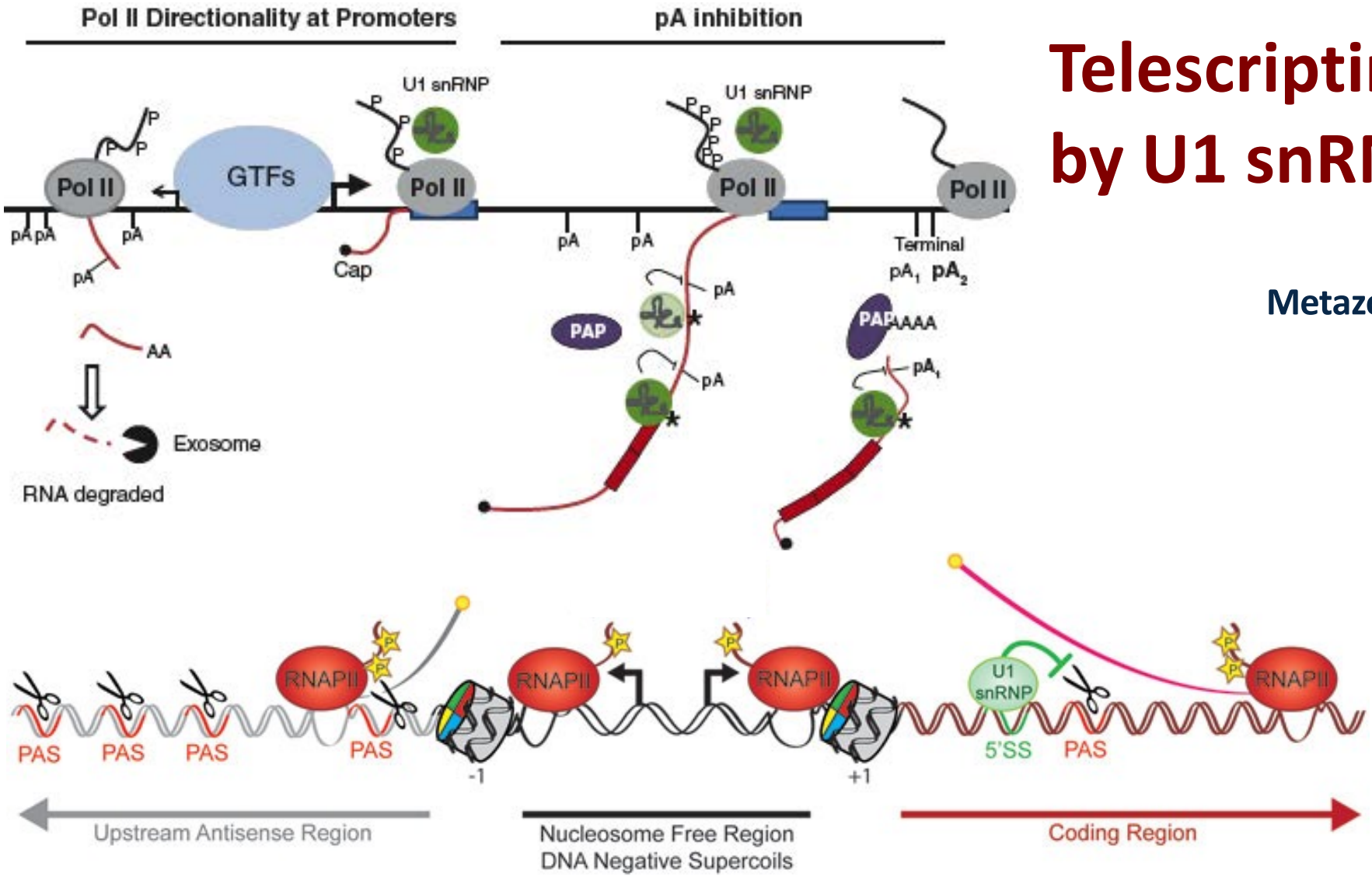


5’ P-end of the transcript (cleaved at PAS by CPA) is degraded by Rat1/XRN2.
3’ OH-end of the transcript after backtracking is degraded by the exosome.
Pol II displacement induces termination.

Proudfoot, Science, 2016

Telescripting by U1 snRNA

Metazoa



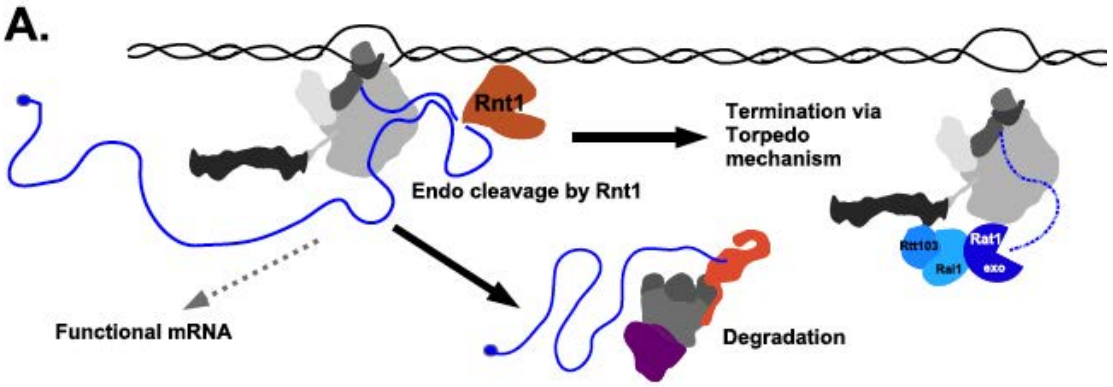
U1 acts as Pol II antiterminator to prevent premature termination.
U1 suppresses cryptic PAS in introns, provides transcription directionality, supports elongation and full-length protein expression.
Telescripting also leads to premature termination in the antisense direction and suppresses non-coding transcription

Guiro and O'Reilly WIREs RNA, 2015

Fail-safe transcription termination

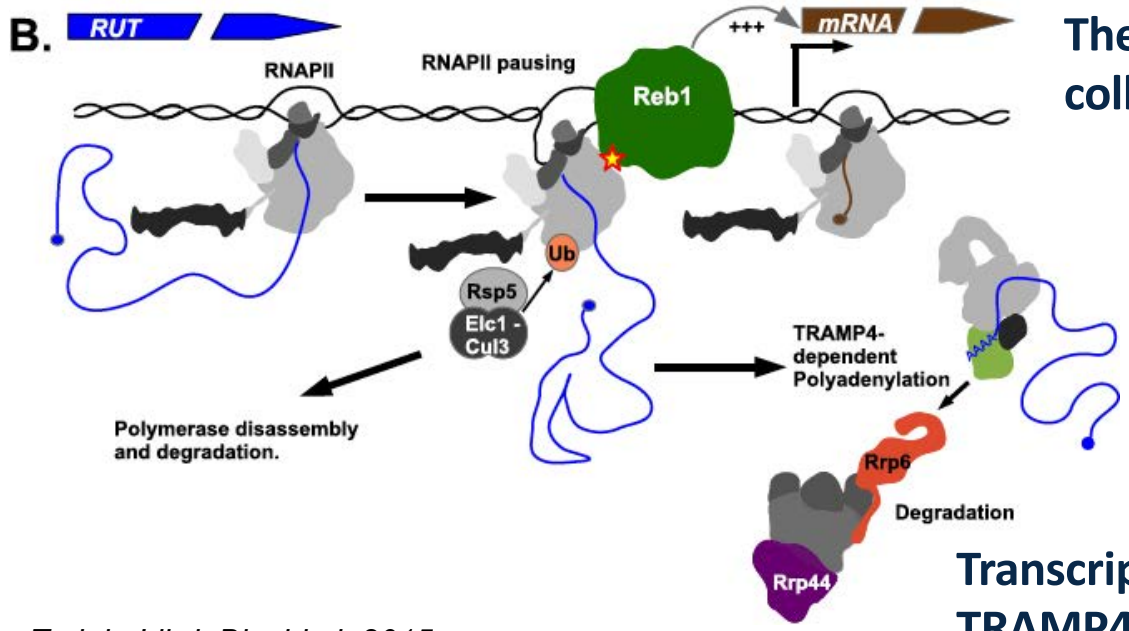
Rnt1-dependent termination

Yeast



Occurs after cleavage of the nascent transcript by Rnt1 via the torpedo mechanism

Road-block termination by the transcription factor Reb1



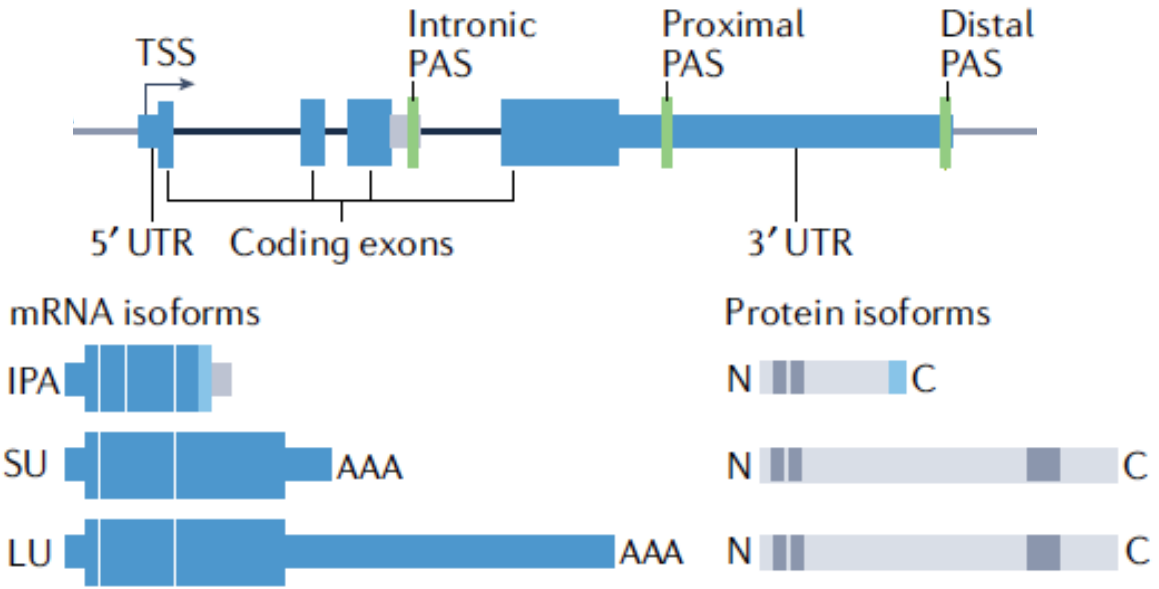
The elongating Pol II is arrested after collision with Reb1

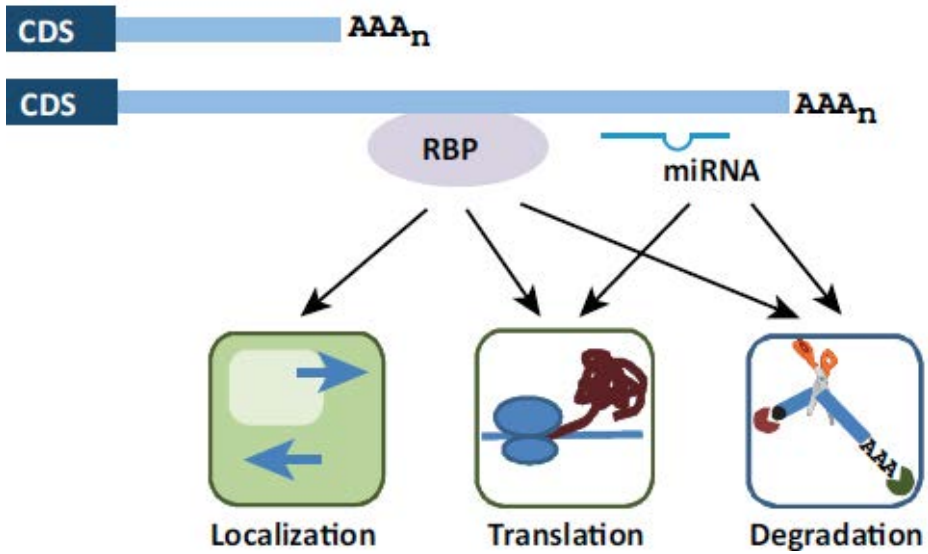
Ubiquitylation of Rpb1 by ubiquitin ligase Rsp5 and Elc1-Cul3 complex results in Pol II disassembly

Transcript is released, polyadenylated by TRAMP4 and degraded by the exosome

Tudek, Libri, Biochimi, 2015

Alternative cleavage and polyadenylation (APA)





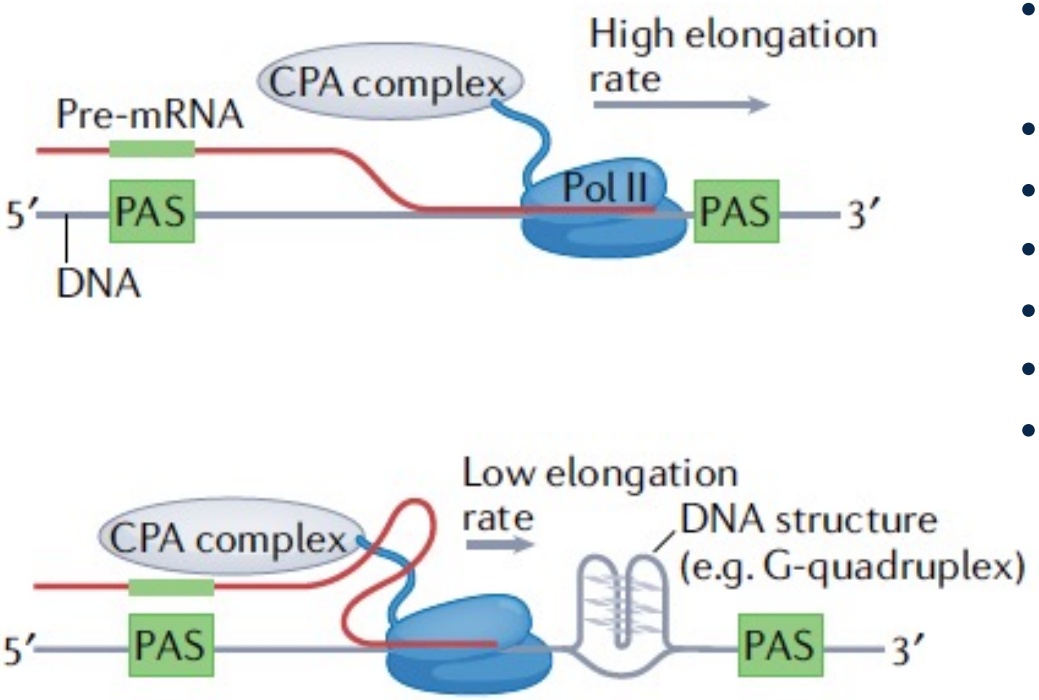
APA impacts the cellular transcriptome and proteome via RBPs and miRNAs

- Alternative 3′UTRs regulate mRNA stability and translation
- APA generates protein isoforms (PAS in exons)
- APA contributes to lncRNA diversity

Mitschka and Mayr Nat Rev Mol Cell Biol, 2022; Tian and Manley, TiBS, 2013

APA and transcription

Kinetic model



APA is modulated by different factors

- Chromatin structure and histone modification
- Pol II elongation dynamics
- Pol II CTD modification
- Pol II pausing and backtracking
- DNA sequence and topology (G4s)
- RBPs, CPA, splicing factors
- Transcription termination

APA dynamics under different biological conditions

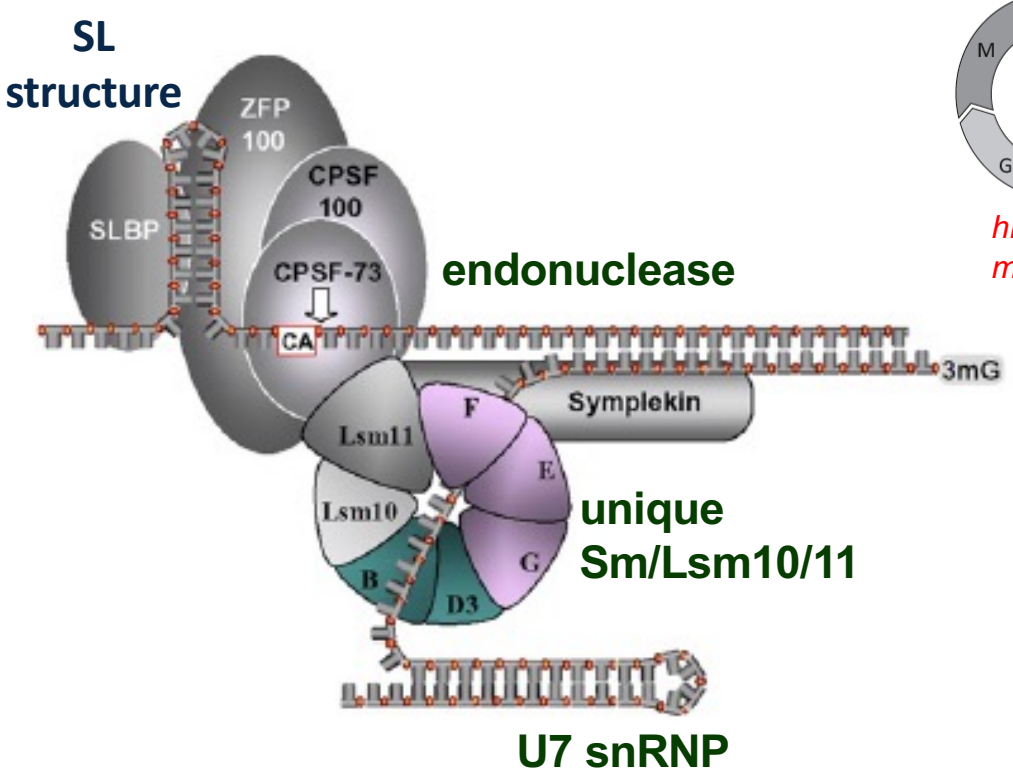
- Contributes to tissue specificity
- Controls response to extracellular signals
- Responds to growth and developmental conditions

Mitschka and Mayr Nat Rev Mol Cell Biol, 2022

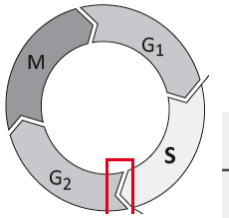
Histone mRNA 3’ end formation

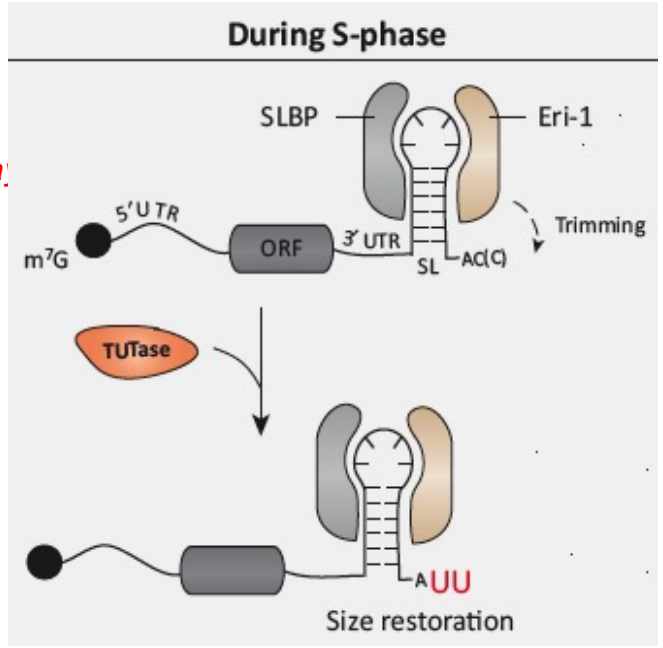
nonpolyadenylated

Metazoa



Dominski and Marzluff, Gene, 2007





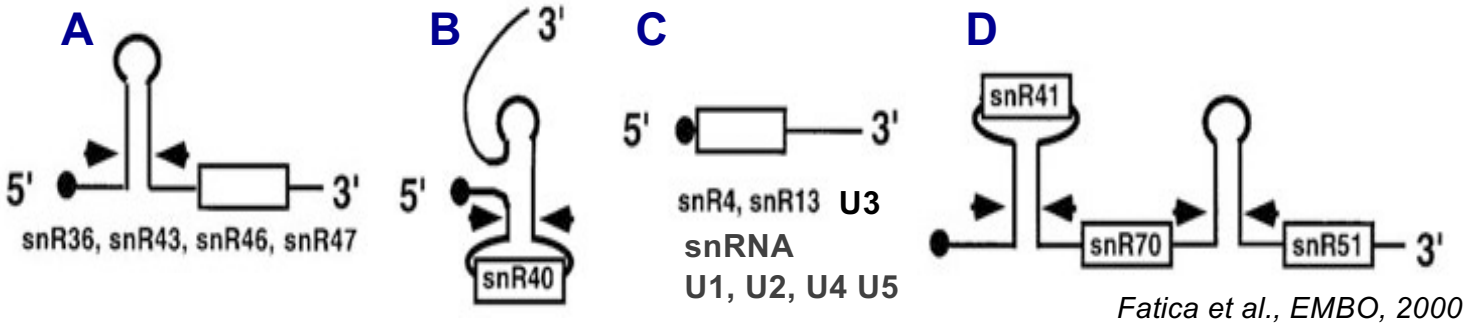
Scheer et al, TiG., 2016

- Histone pre-mRNA contains conserved stem-loop (SL) structure, recognized by the SLBP (SL-binding protein)
- SLBP, ZFP100 and HDE (histone downstream element) stabilize binding of U7
- U7 snRNP, specificaly Lsm11, recruits cleavage factors and the cleavage by endonuclease CPSF-73 generates mature 3’ end of histone mRNA

sn/snoRNA processing

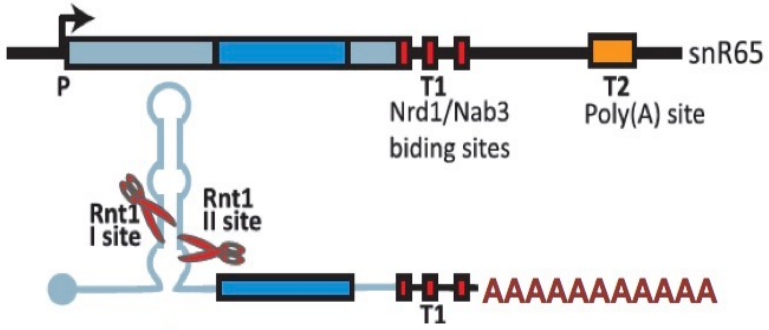
small nuclear and nucleolar RNAs

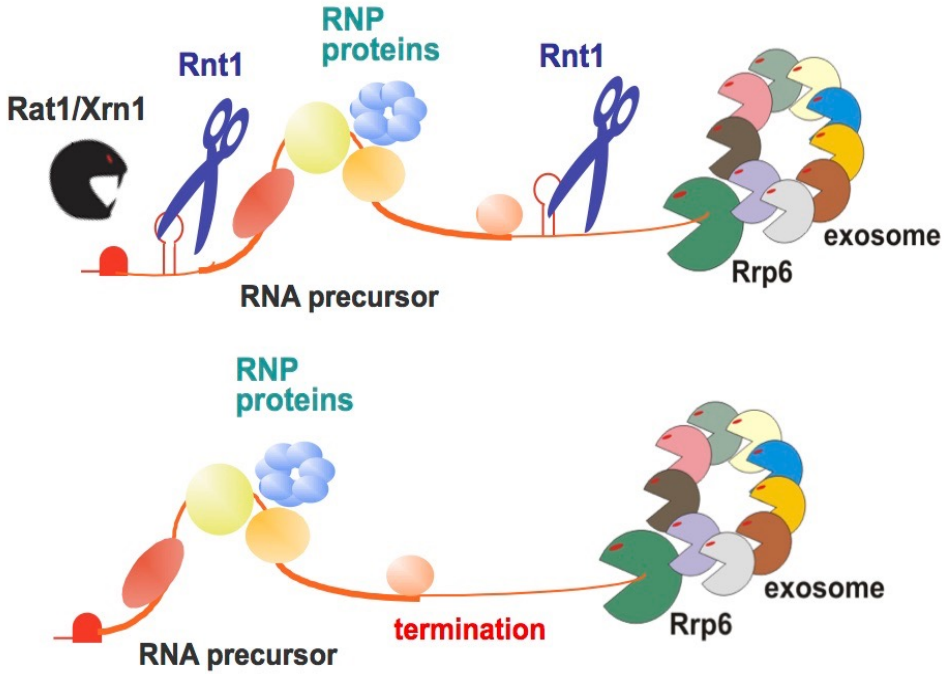
Yeast



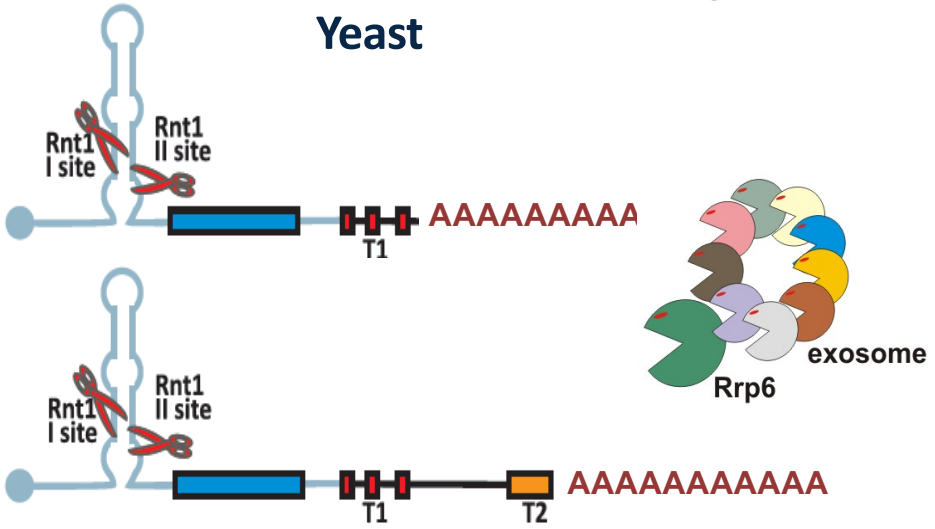
Fatica et al., EMBO, 2000

A, B, D types – 5' cap is removed by by Rnt1 and 5'- 3' exo processing by Rat1/Xrn1
C type – 5' cap is modified by modification (trimethylation) to TMG cap by Tgs1





sn/snoRNA processing

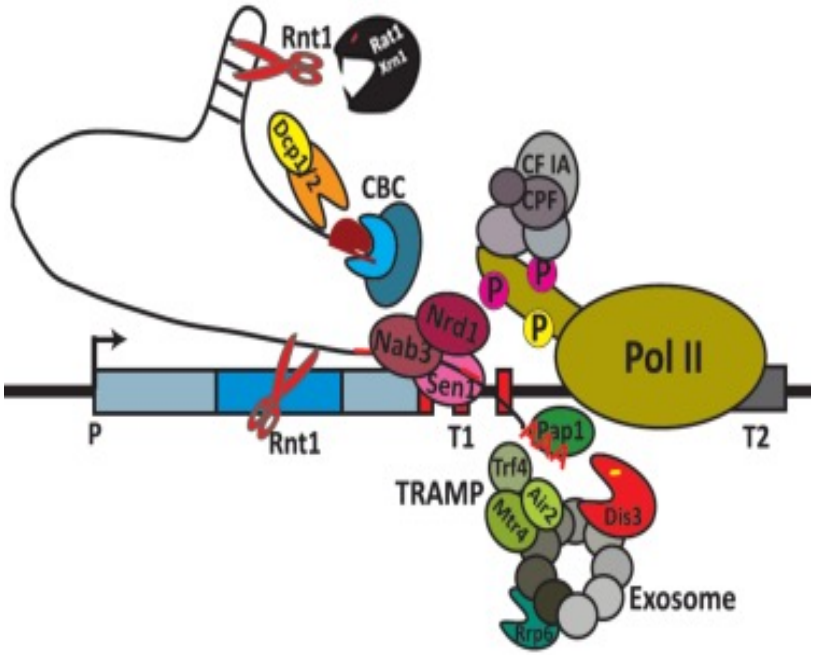


5'-end processing:

- **endonucleolytic cleavage by Rnt1**
- **exonucleolytic trimming by Rat1/Xrn1**

3'-end processing:

- **polyadenylation by TRAMP following termination**
- **exonucleolytic trimming by the exosome**



<u>Exosome: 3'- 5' exo/endo-nuclease</u>

- **complex of 10 core components (RNA BP)**
- **catalytically active hydrolytic Dis3/Rrp44** (RNase II)
- **nuclear cofactors: nuclease Rrp6** (RNase D)
  RNA helicase Mtr4, RNA BP Rrp47
- **cytoplasmic cofactors- Ski2-3-8 complex**
  RNA helicase Ski2, GTPase Ski7
- **Processing and/or degradation of almost all RNAs**

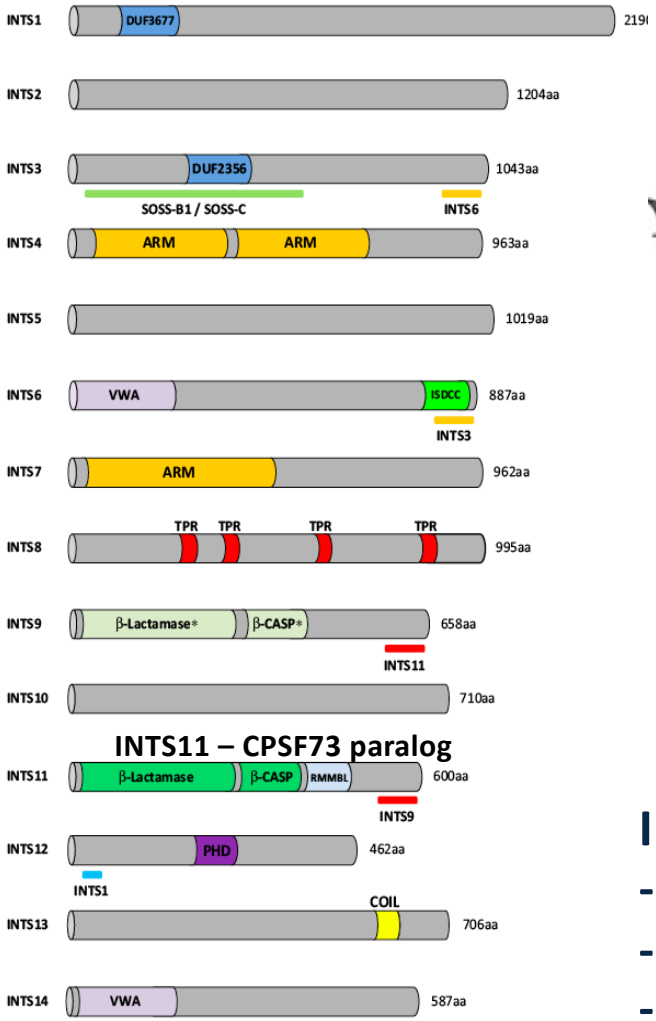
<u>TRAMP: nuclear surveillance</u>

<u>Trf</u>4/5	+	**<u>A</u>ir1/2**	+	**<u>M</u>tr4**
poly(A) polymerase		**RNA binding proteins**		**RNA DEVH helicase**

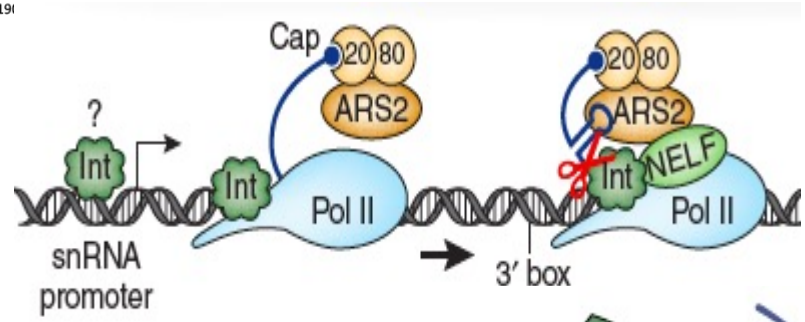
Metazoa

snRNA biogenesis

Integrator (INT) complex - snRNA 3’ end processing



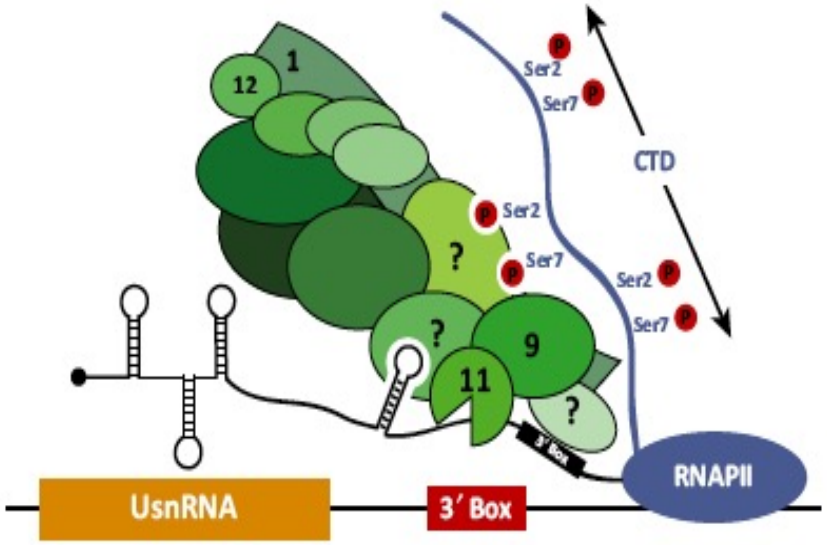
Baillat and Wagner., TiBS., 2015



INT - Integrator

CBCA = CBC+ARS2

NELF negative elongation factor



INT

- recruited contransctiptionaly to snRNA promoter
- interacts with Pol II CTD (Ser7-P/Ser2-P dyad)
- cleaves pre-snRNA at the 3’box

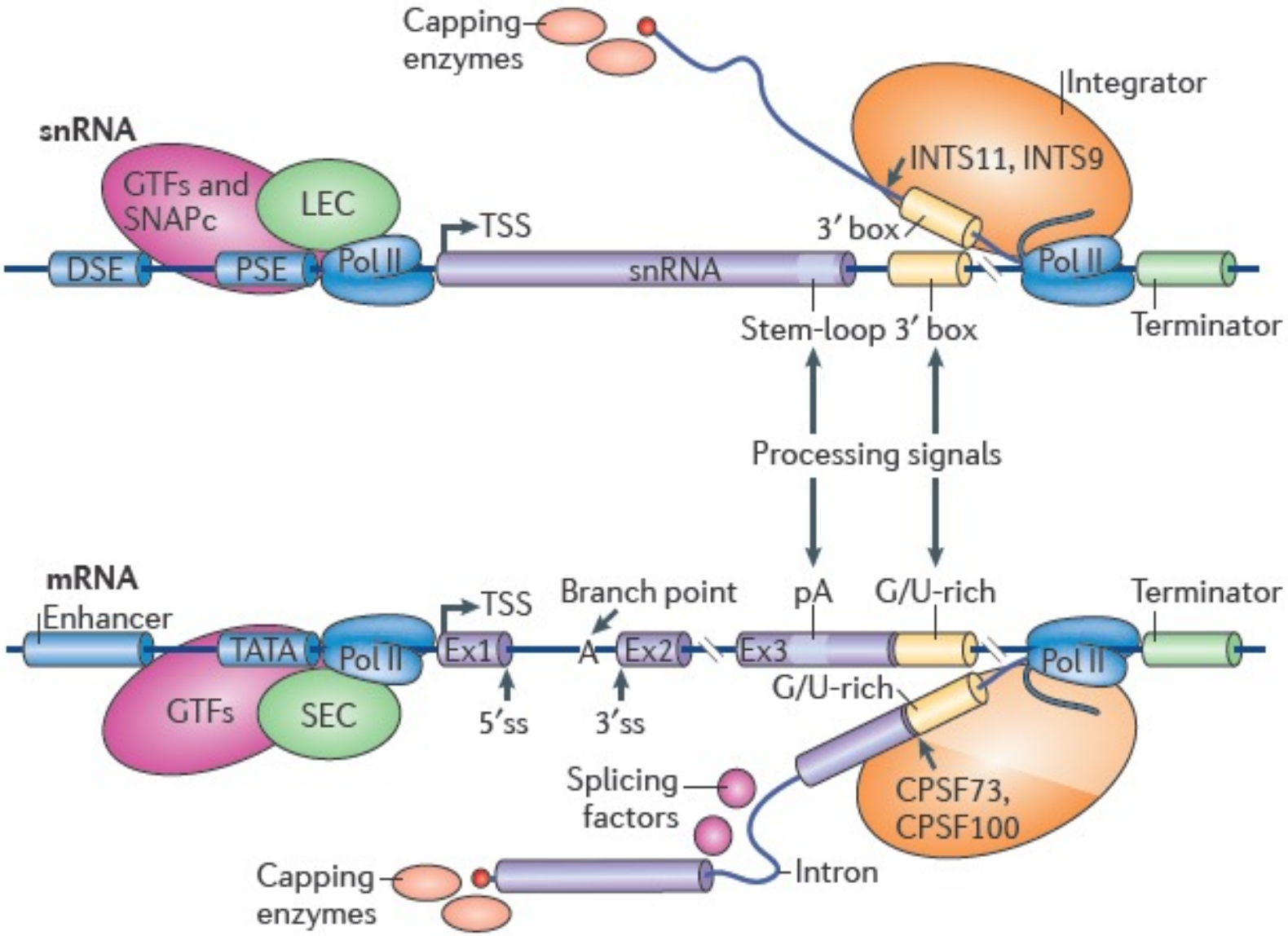
Termination and processing elements

snRNA-type promoter: DSE recruits transcription factors PSE bound by SNAPc (snRNA activating complex)

3’-box (GTTTN– AAARNNAGA)**, located 9–19 nt downstream of the snRNA 3’-end**

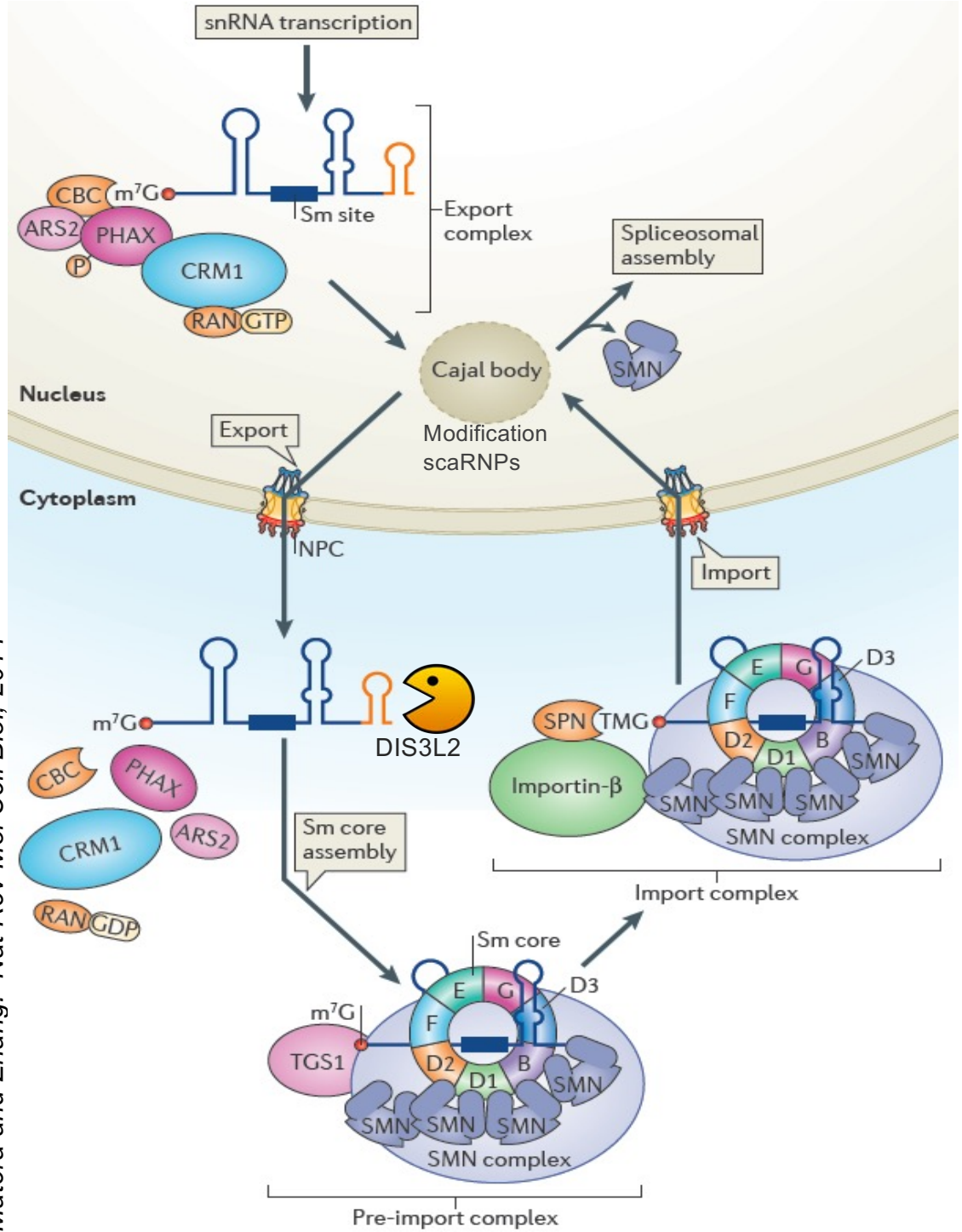
mRNA and snRNA processing

Metazoa



Matera and Zhang, Nat Rev Mol Cell Biol, 2014

snRNA biogenesis



Matera and Zhang. *Nat Rev Mol Cell Biol, 2014*

Nucleus

CBCA-bound to snRNA m^{7}G cap is recognized by export adaptor PHAX

PHAX recruits exportin CRM1-Ran-GTP, which exports snRNA to the cytoplasm

Cytoplasm

Export factors dissociate

snRNA 3’ ends are processed by 3’-5’ exonuclease DIS3L2

SMN complex associates with snRNA and promotes assembly with Sm core proteins

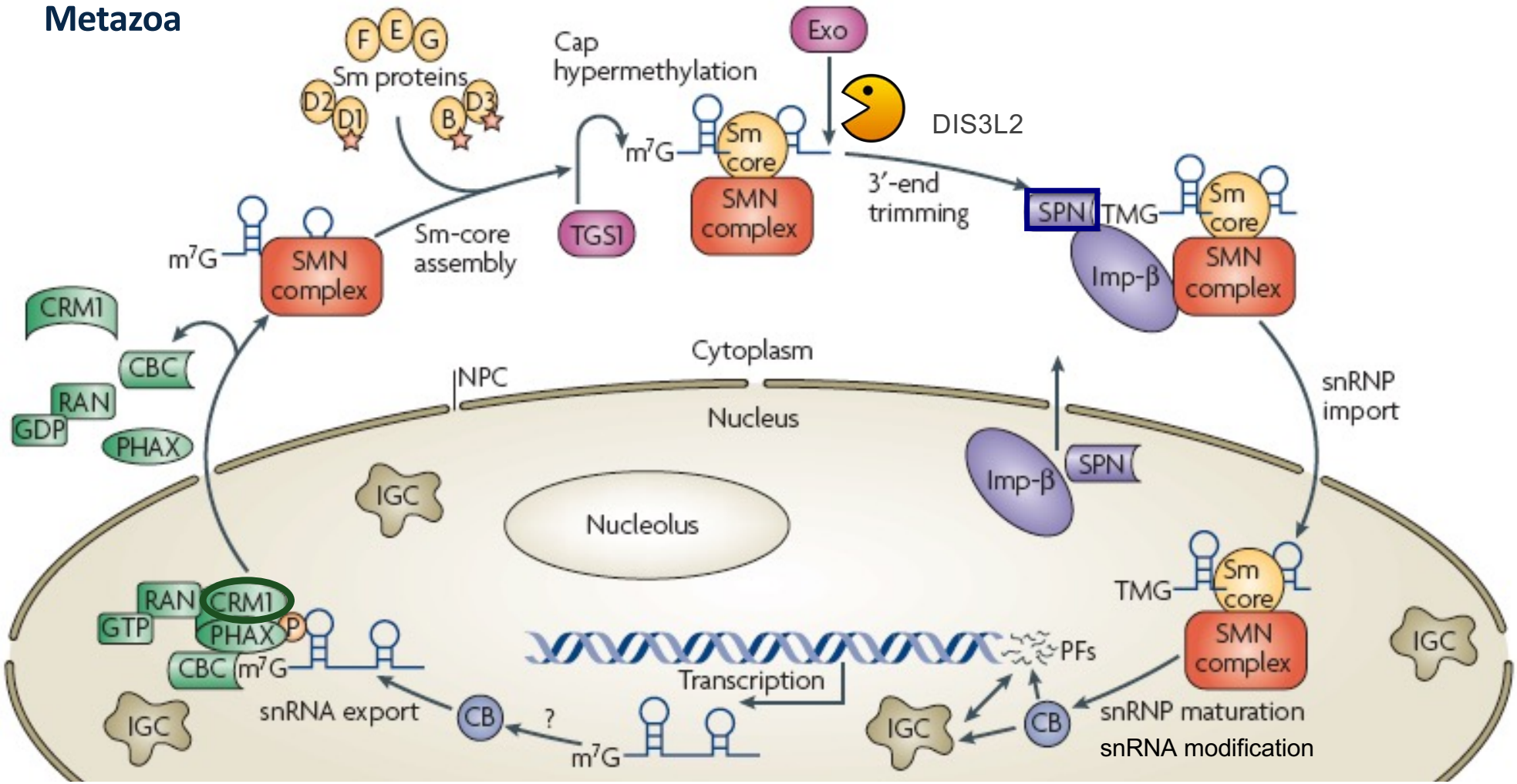
TGS1 hypermethylates m^{7}G cap to TMG cap that binds import adaptor snurportin SNP

Mature snRNP is exported to the nucleus by importin-β

snRNA is modified (2’-O-methylated and pseudouridylated in Cajal Bodies by boxC/D and box H/ACA scaRNPs

snRNA biogenesis

Metazoa



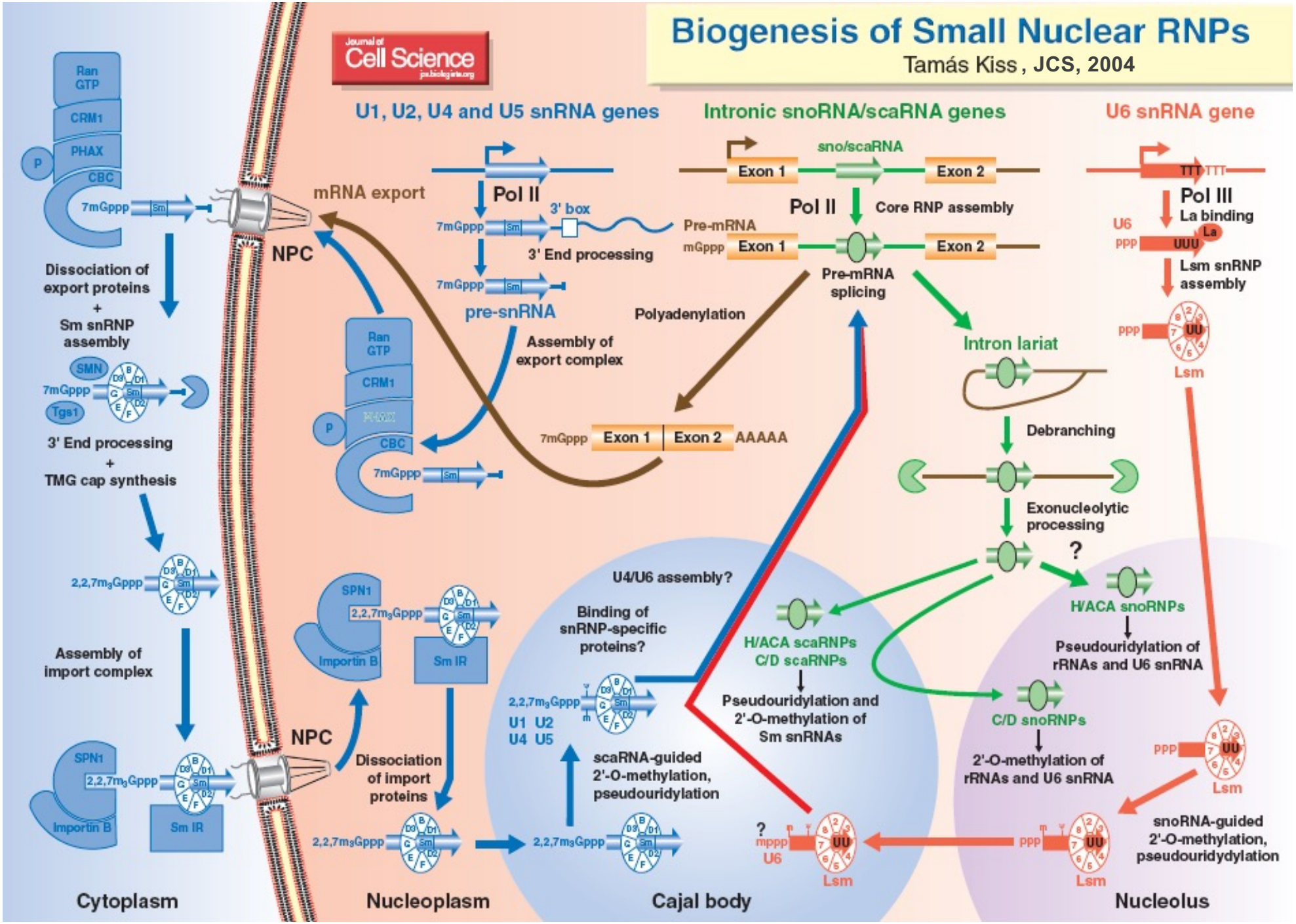
CRM1 - export receptor

PHAX(-P) - export adaptor, binds to CBC

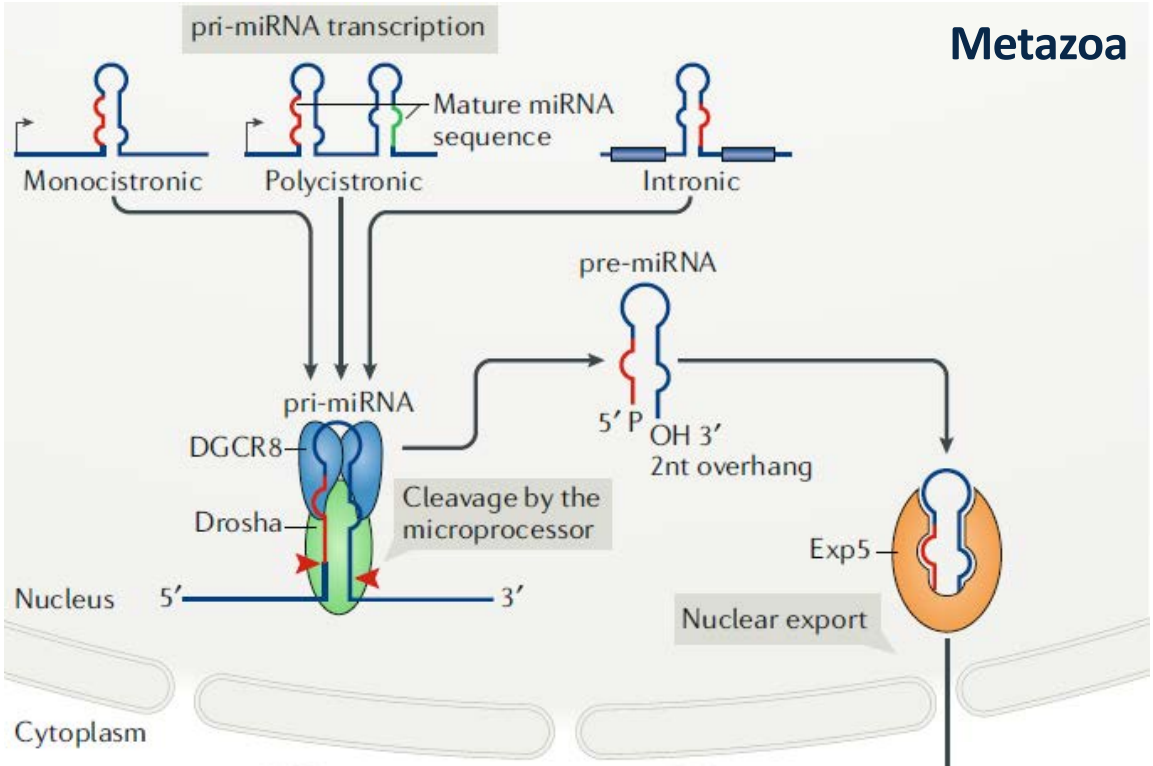
SMN - *Survival of Motor Neuron*, **binds snRNA and core Sm proteins to assemble mature snRNP**

TGS1 - *Trimethylguanosine Synthase*, **hypermethylates m^7G cap to 2,2,7-trimethylguanosine cap**

SPN - import adaptor snurportin; Imp-β - import receptor importin-β


Biogenesis of Small Nuclear RNPs
Tamás Kiss, JCS, 2004
Journal of Cell Science
U1, U2, U4 and U5 snRNA genes
Intronic snoRNA/scaRNA genes
U6 snRNA gene
Ran GTP
CRM1
PHAX
P
CBC
7mGppp
Sm
Dissociation of export proteins + Sm snRNP assembly
SMN
Tgs1
3' End processing + TMG cap synthesis
2,2,7m3Gppp
Assembly of import complex
SPN1
Importin B
Sm IR
NPC
mRNA export
Pol II
3' box
3' End processing
pre-snRNA
Assembly of export complex
Polyadenylation
sno/scaRNA
Exon 1
Exon 2
Pre-mRNA
mGppp
Core RNP assembly
Pre-mRNA splicing
AAAAA
Intron lariat
Debranching
Exonucleolytic processing
?
Pol III
La binding
U6
TTTTTT
UUU
La
Lsm snRNP assembly
Lsm
U4/U6 assembly?
Binding of snRNP-specific proteins?
U1 U2 U4 U5
scaRNA-guided 2'-O-methylation, pseudouridylation
Dissociation of import proteins
H/ACA scaRNPs
C/D scaRNPs
Pseudouridylation and 2'-O-methylation of Sm snRNAs
H/ACA snoRNPs
Pseudouridylation of rRNAs and U6 snRNA
C/D snoRNPs
2'-O-methylation of rRNAs and U6 snRNA
snoRNA-guided 2'-O-methylation, pseudouridydylation
mppp
UU
Cytoplasm
Nucleoplasm
Cajal body
Nucleolus

miRNA biogenesis



Microprocessor

Drosha (RNase III)

GGCR8 (dsRBP)

p68/DDX5 (helicase)

p72/DDX17 (helicase)

Dicer (RNase III)

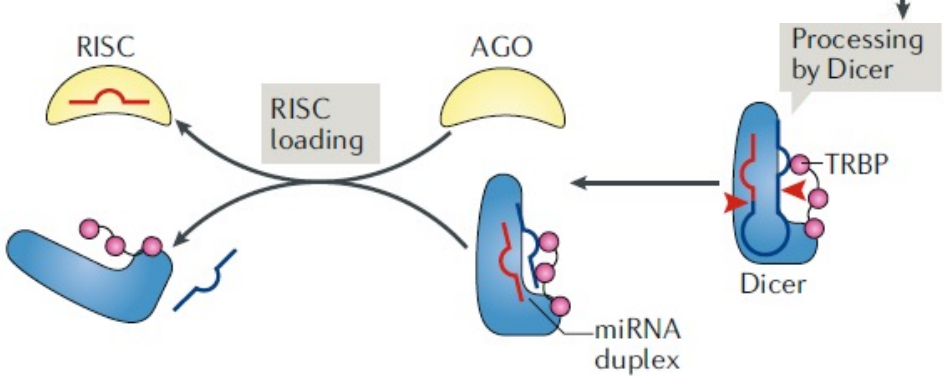
TRBP (dsRBP)

Exp5 (exportin)

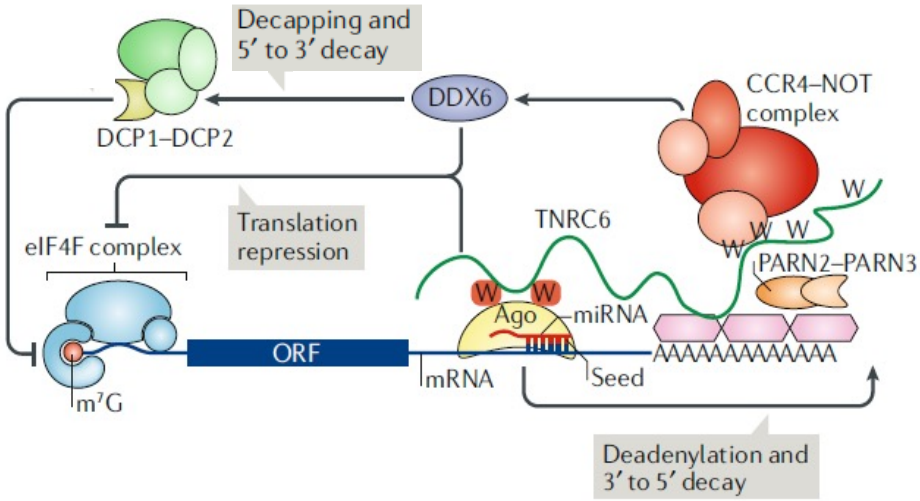
RISC RNA inducing silencing complex

miRNA

AGOs (Argonaute)

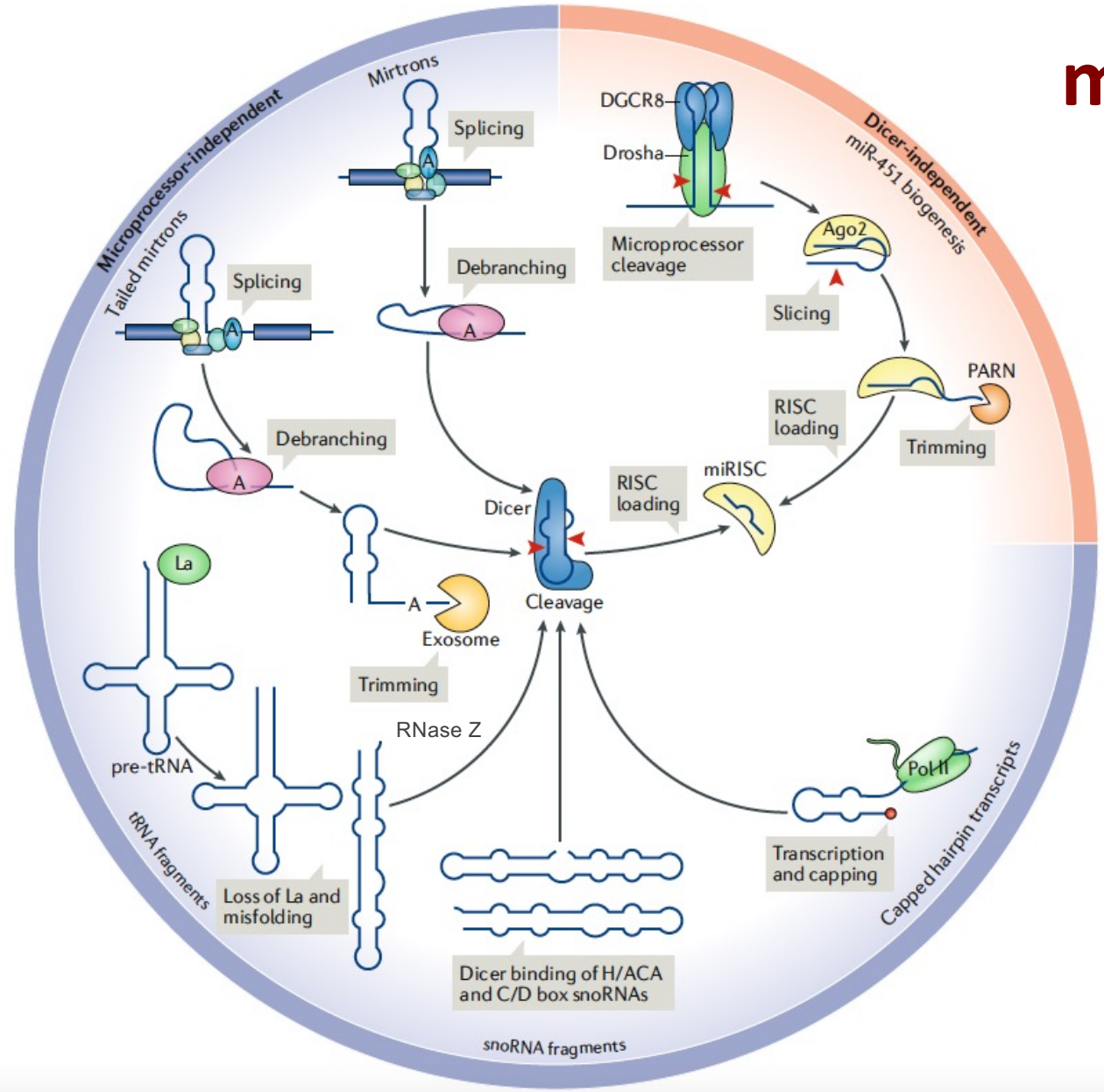


miRNA function



Treiber et al., Nat Rev Mol Cell Biol, 2018

Noncanonical miRNA biogenesis



Treiber et al., Nat Rev Mol Cell Biol, 2018

Microprocessor-independent mirtrons and tailed mirtrons are generated by splicing and lariat debranching

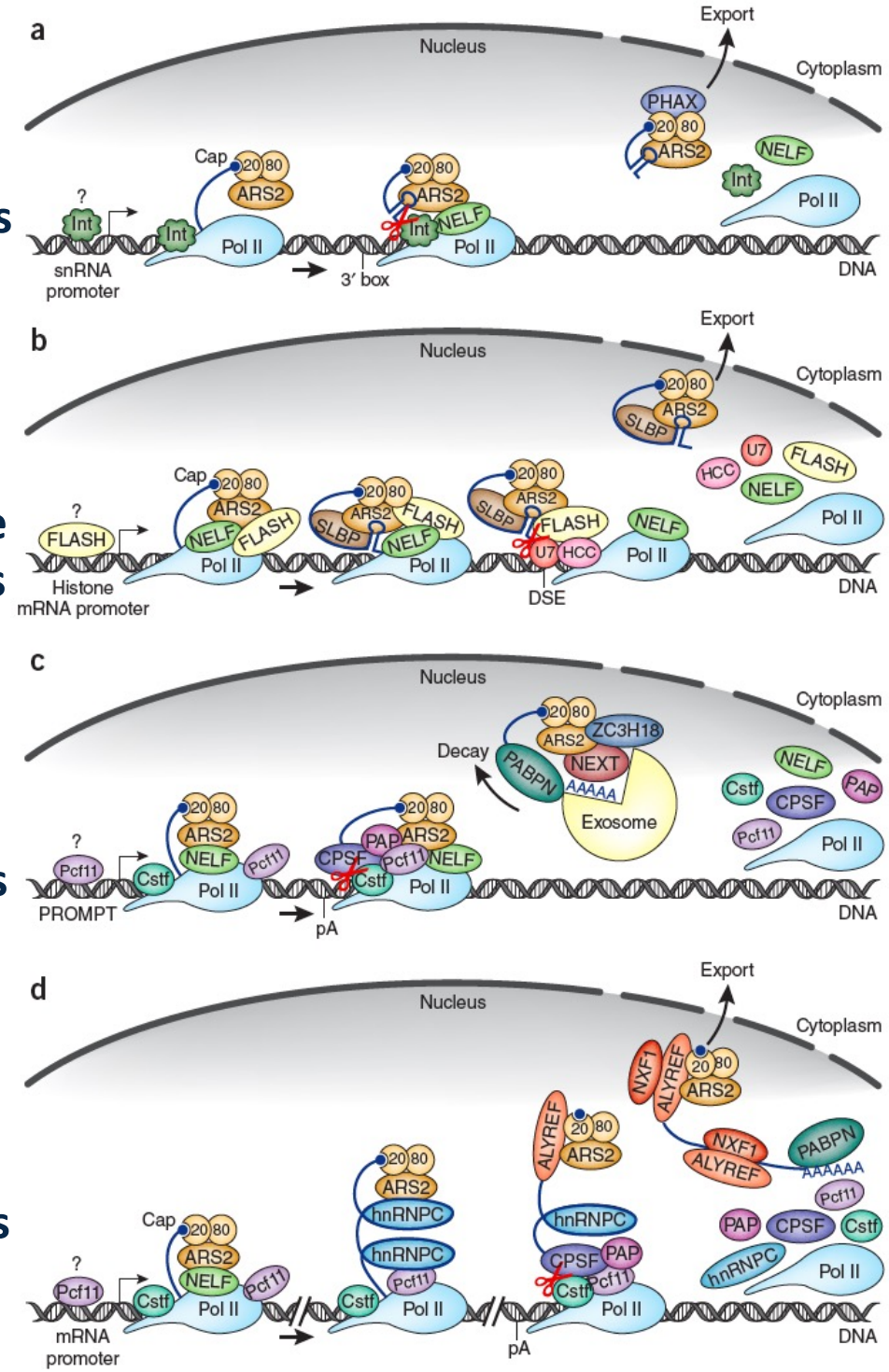
tRNAs which adopt hairpin-like structures mimic pre-miRNAs are processed to miRNAs

Some snoRNAs can be processed to miRNAs

Some Pol II transcripts are converted into m^7G capped miRNAs which are exported to the cytoplasm by Xpo1

Dicer-idependent processing involves pre-miRNA slicing by Ago2 and 3' trimming by PARN

Pol II transcript processing

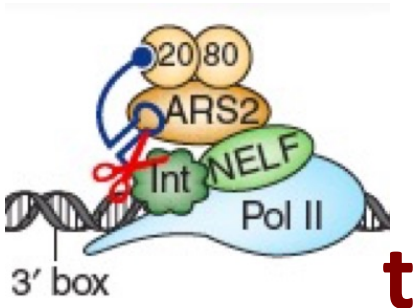


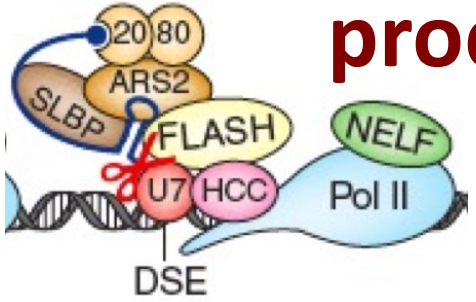
snRNAs

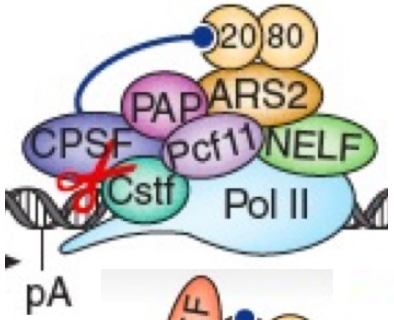
histone mRNAs

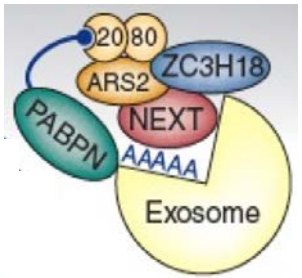
ncRNAs

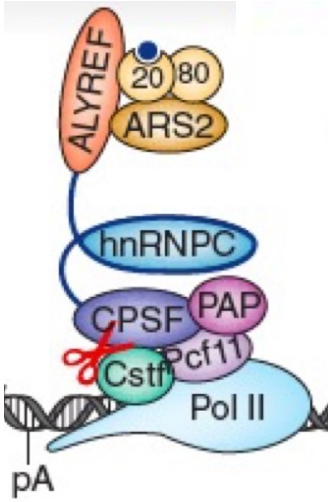
mRNAs





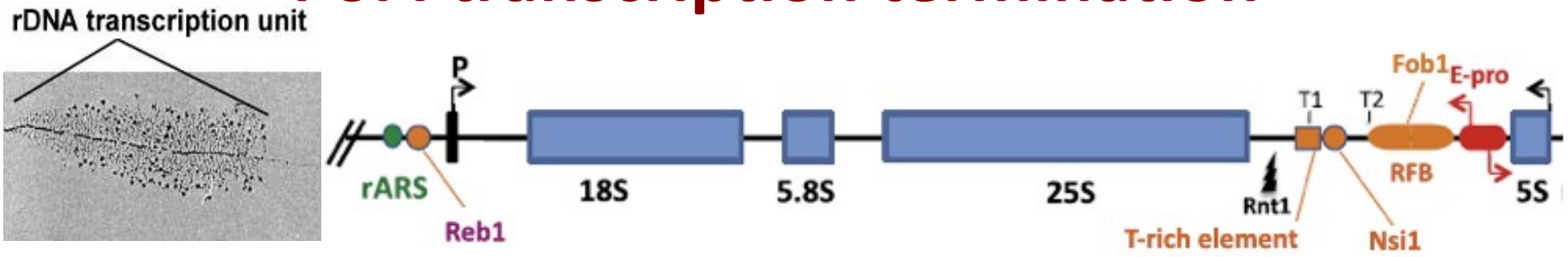




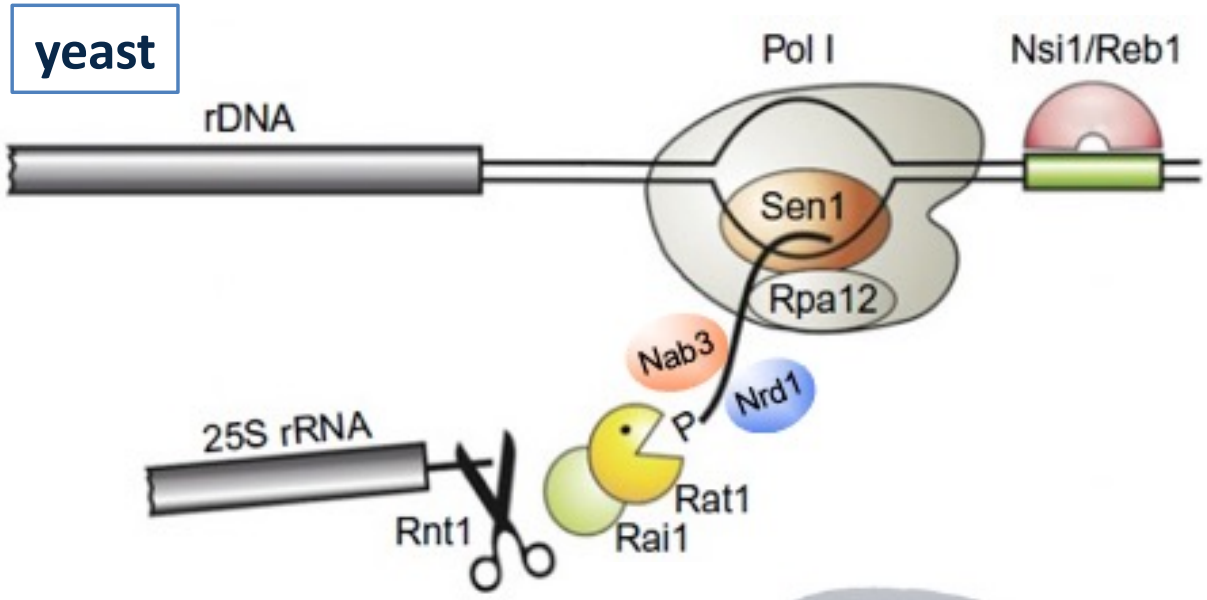


Müller-McNicoll and Neugebauer Nat. Struct. Mol. Biol, 2014

Pol I transcription termination

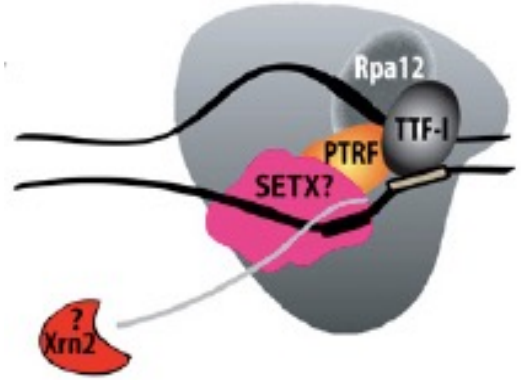


yeast



- **Pol I termination factors:**
- **DNA-binding protein Nsi1/Reb1**
- **Pol I subunit Rpa12**
- **endonuclease Rnt1**
- **RFB binding protein Fob1**
- **5'-3' exonuclease Rat1/Rai1**
- *(torpedo mechanism)*
- **RNA helicase Sen1**
- **Nrd1/Nab3 complex (??)**

mammalian

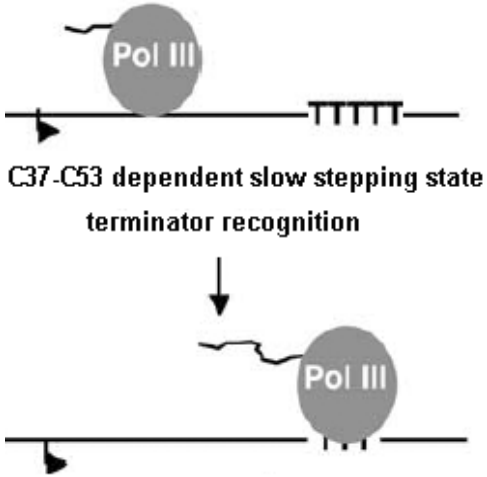


transcript release element
T-stretch + TTF-I pause site

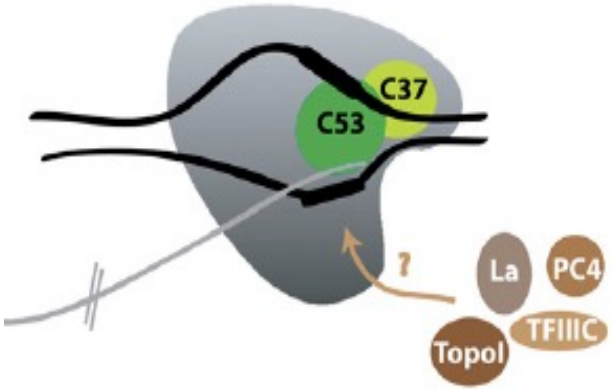
PTRF – release factor
SETX – helicase, Sen1 homolog
TTF-I – transcription termination factor I

Richard and Manley, GeneDev., 2009; Krzysztoń et al, Enzymes, 2012; Nemeth et al, BBA 2013

Pol III transcription termination



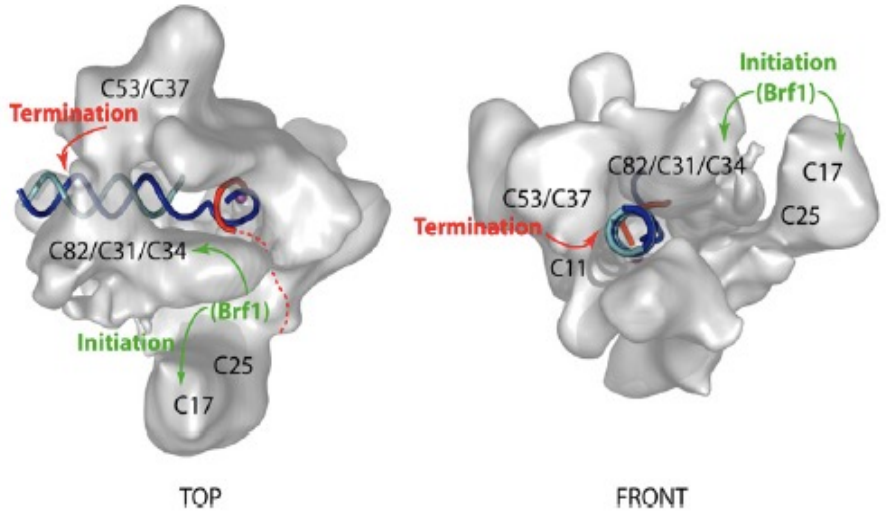
Landrieux et al., EMBO J., 2006



Richard and Manley, Gene Dev., 2009

- **Pol III pausing at oligo(dT) tract**
- **weak A:U hybrid at terminator**
- **backtracking** (blocks elongation)
- **Pol III subunits**
- **termination is coupled with processing**

Pol III EM structure



Fernadez-Tornero et al., Mol. Cell, 2007

- **<u>C1, C2</u> core subunits**

- Pol III pausing

- **<u>C37-C53</u> subcomplex**

- situated across the cleft near RNA exit

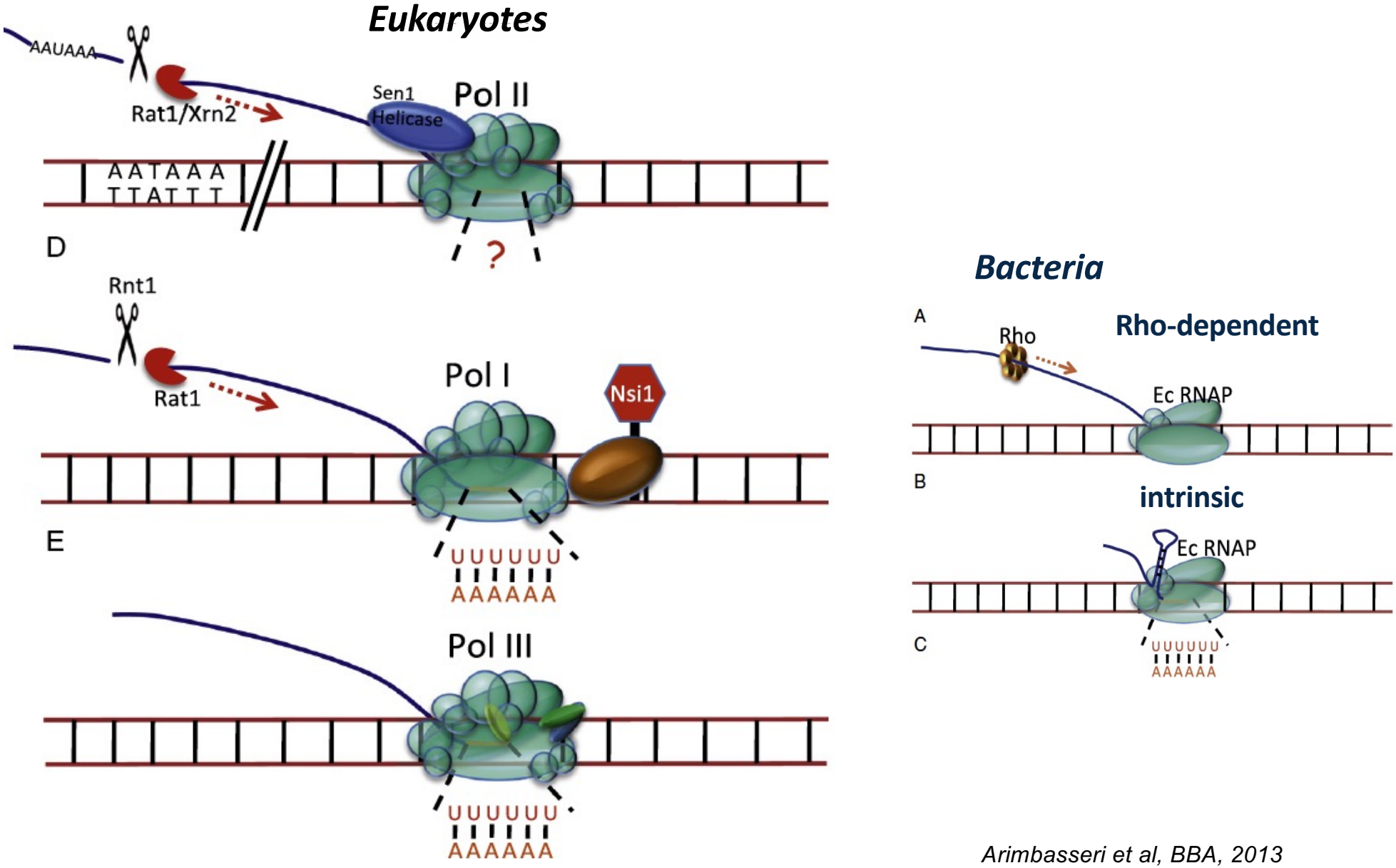
- reduces elongation rate

- **<u>C11</u> (*TFIIS*)**

- intrinsic 3' RNA cleavage activity

- faciliates recycling

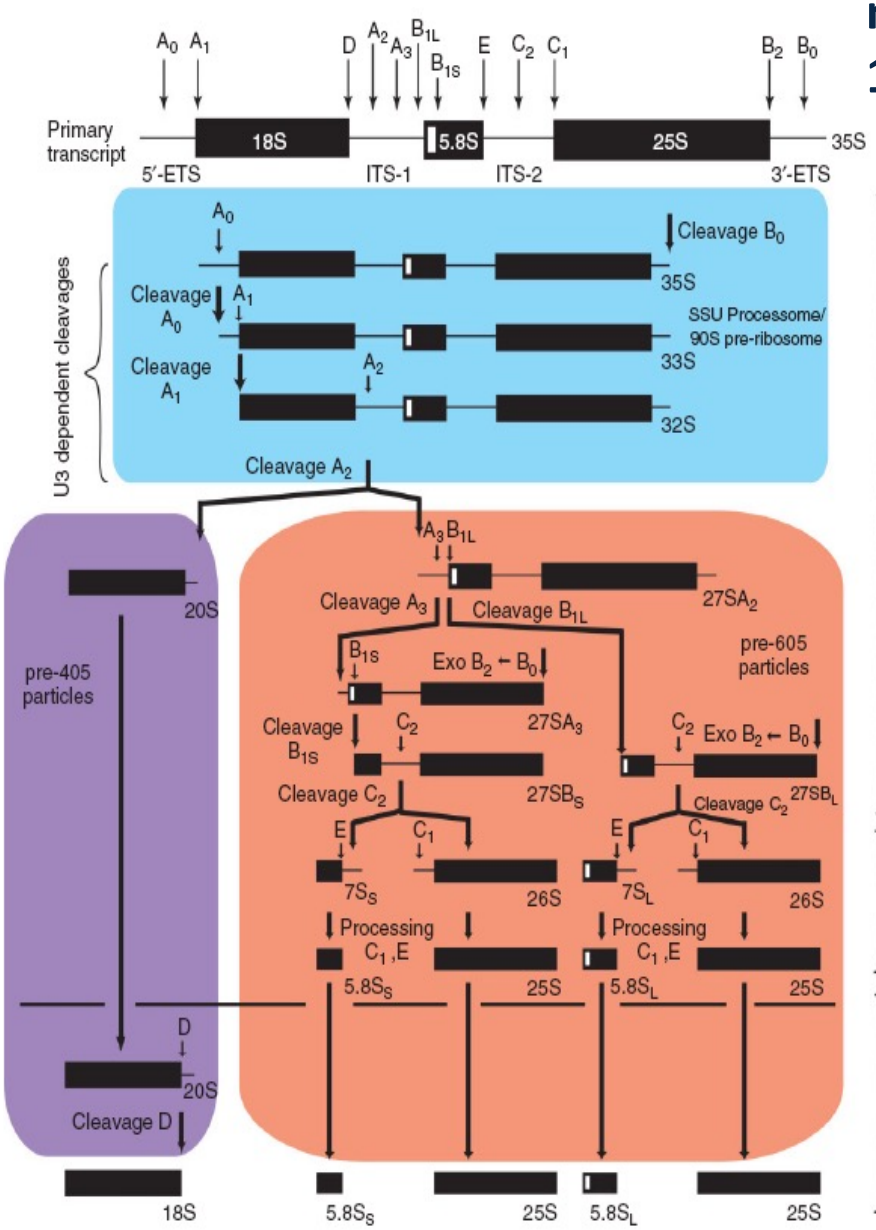
Pol I, Pol II, Pol III termination



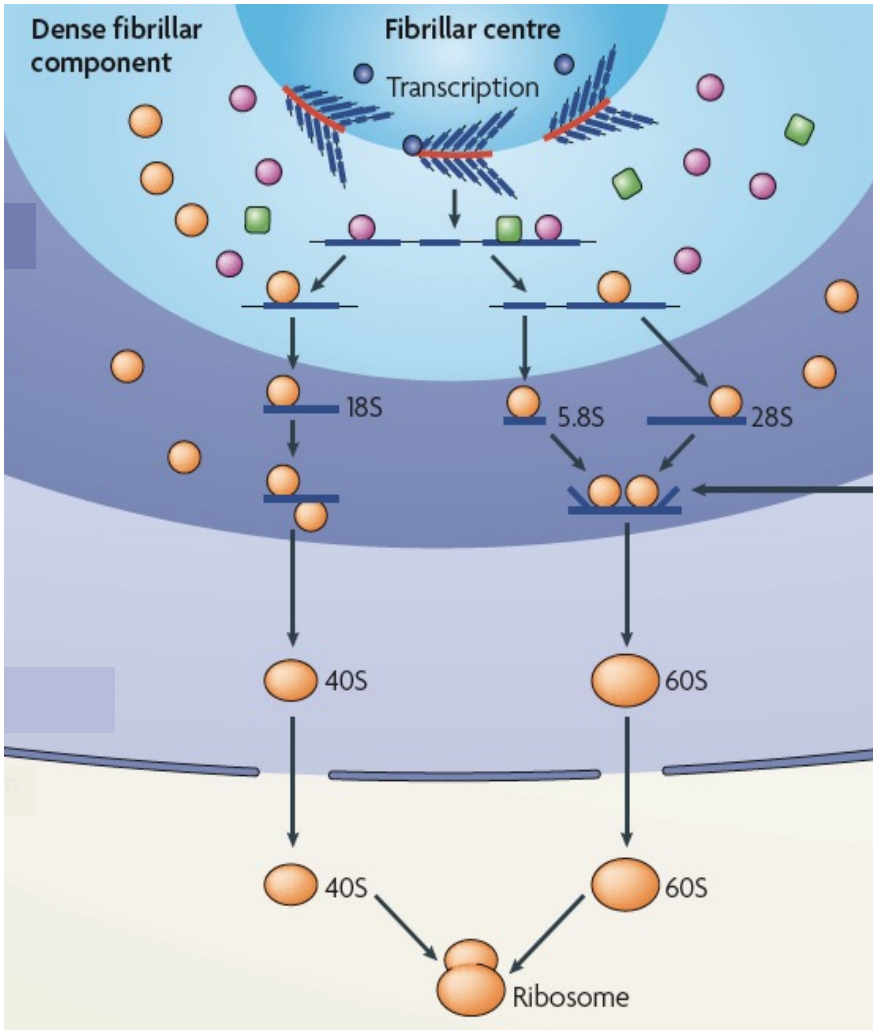
Arimbasseri et al, BBA, 2013

Pre-rRNA processing and modification

Making the ribosome takes approximately 200 non-ribosomal proteins
100 snoRNAs and 80 ribosomal proteins

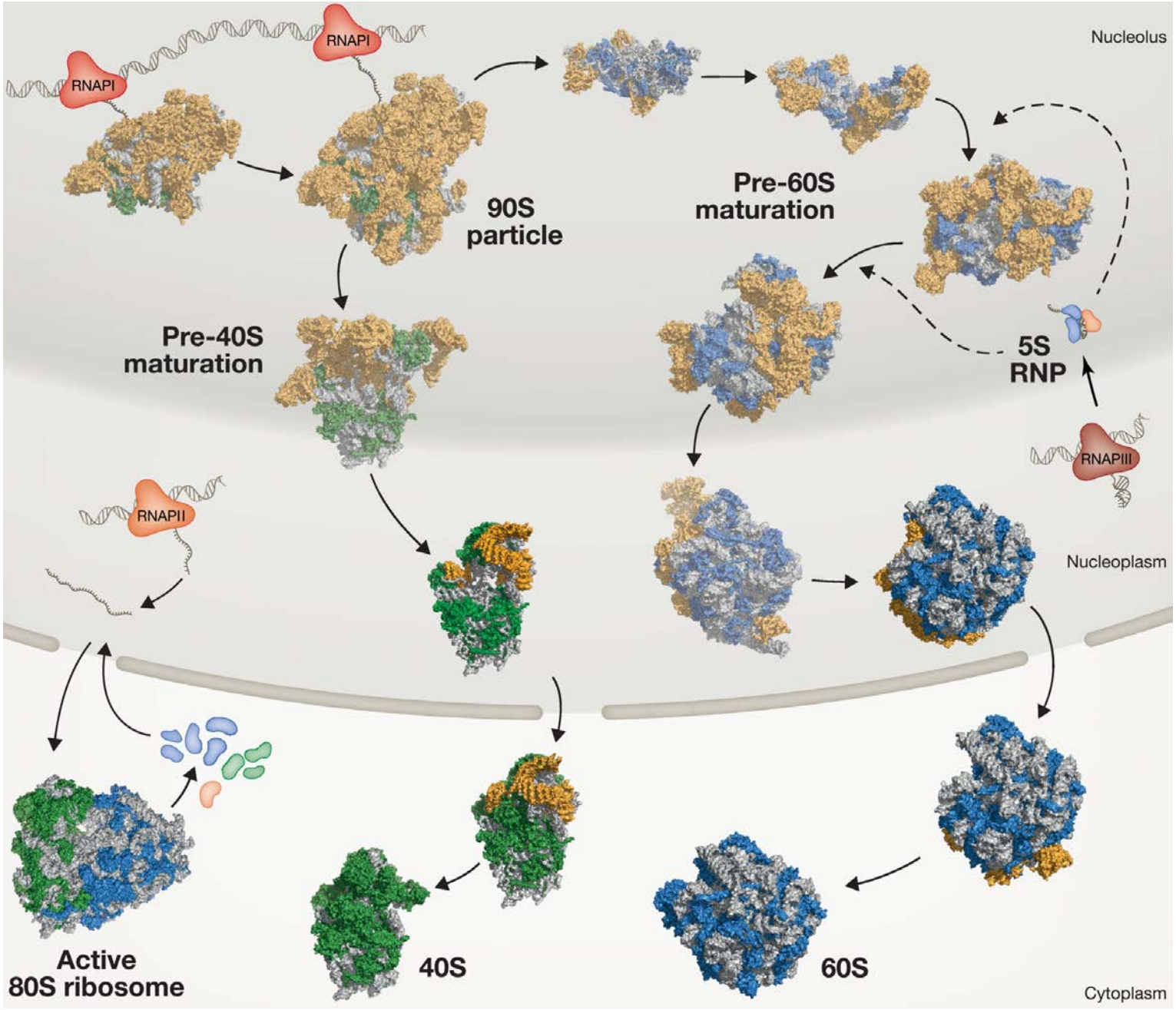


Phipps et al, WIRERNA., 2010



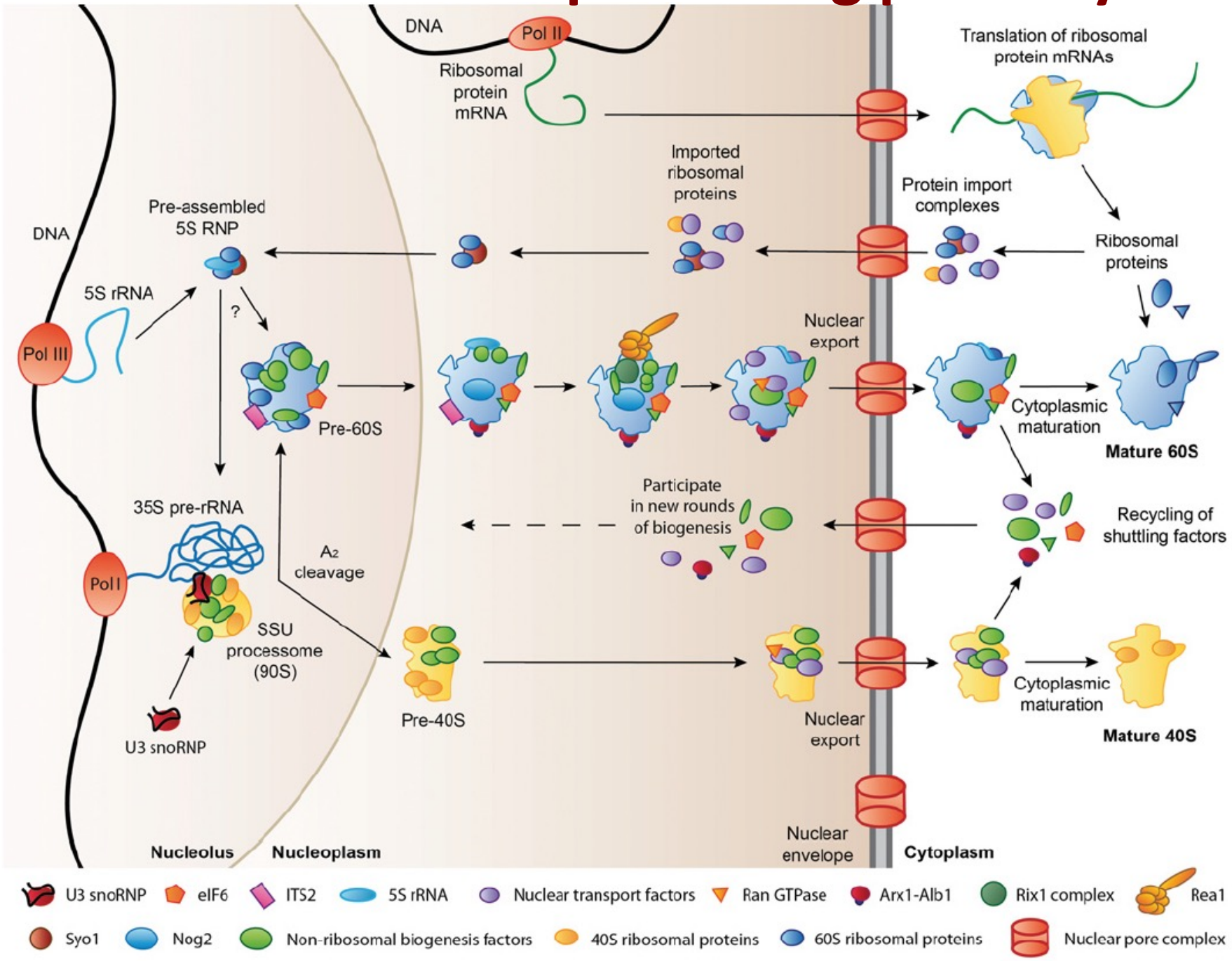
Boisvert et al, NatRevMolCellBiol., 2007

Pre-rRNA processing pathway



Dorner et al, EMBO, 2023

Pre-rRNA processing pathway



Greber, RNA, 2016

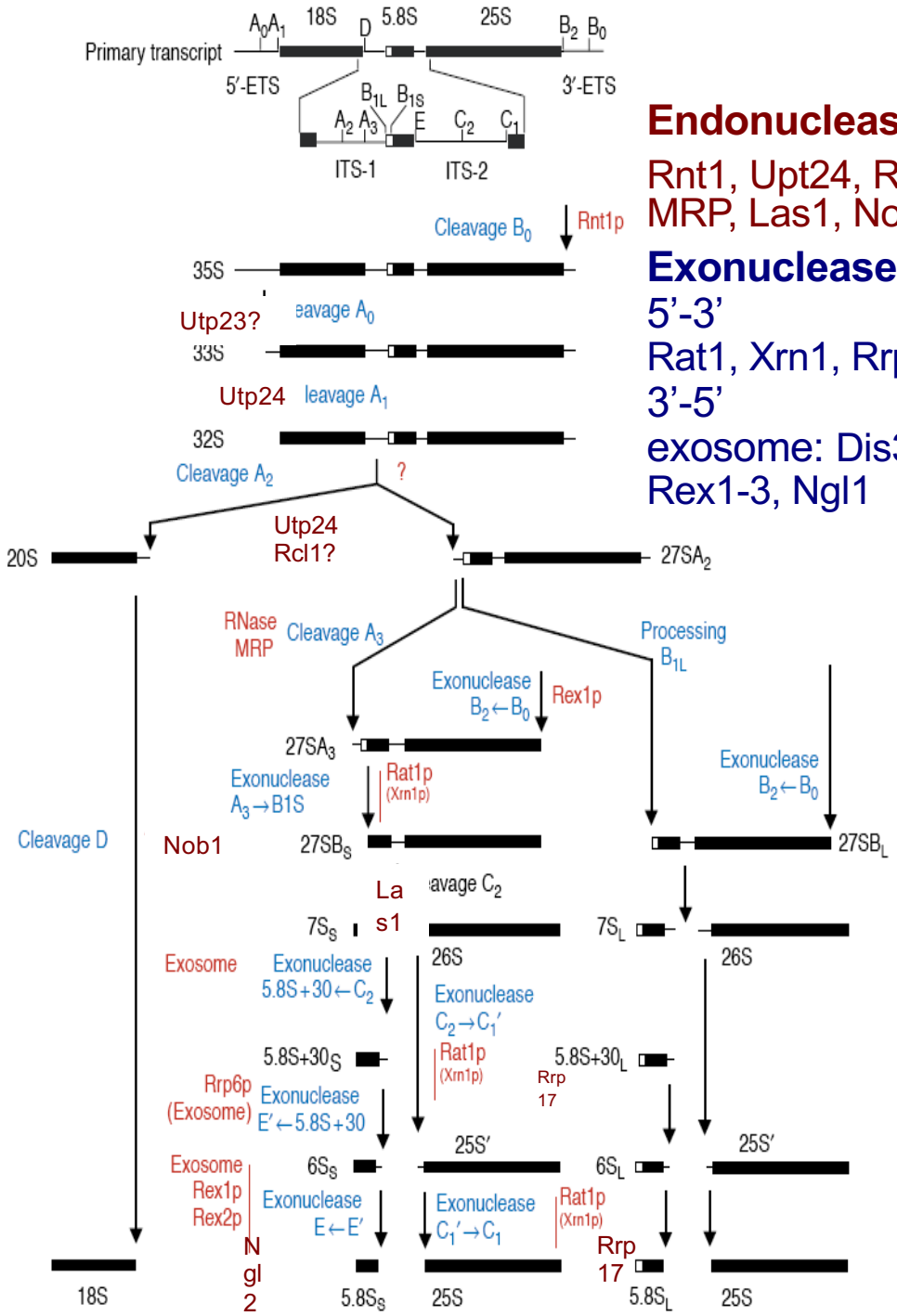
Pre-rRNA processing

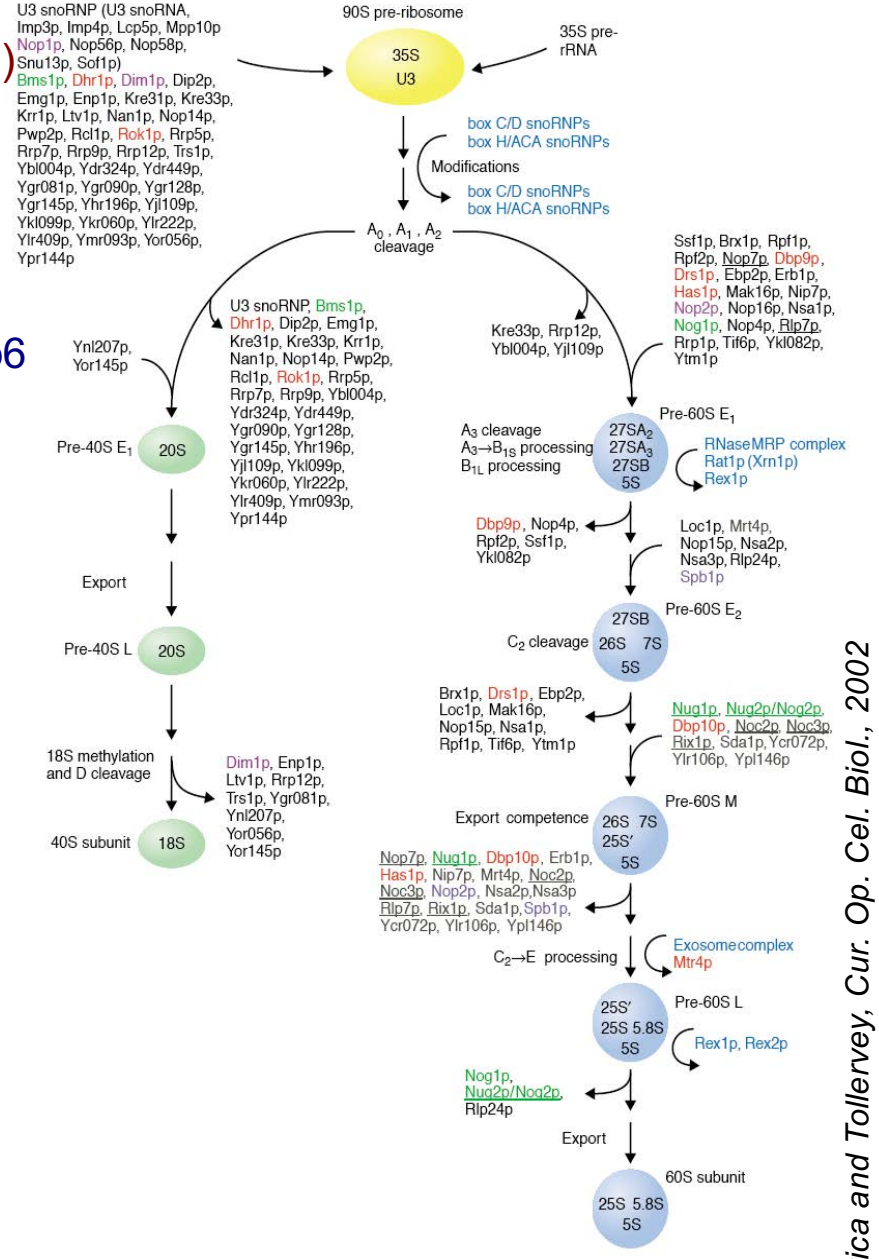
Endonucleases:

Rnt1, Upt24, Rcl1 (?)
MRP, Las1, Nob1

Exonucleases:

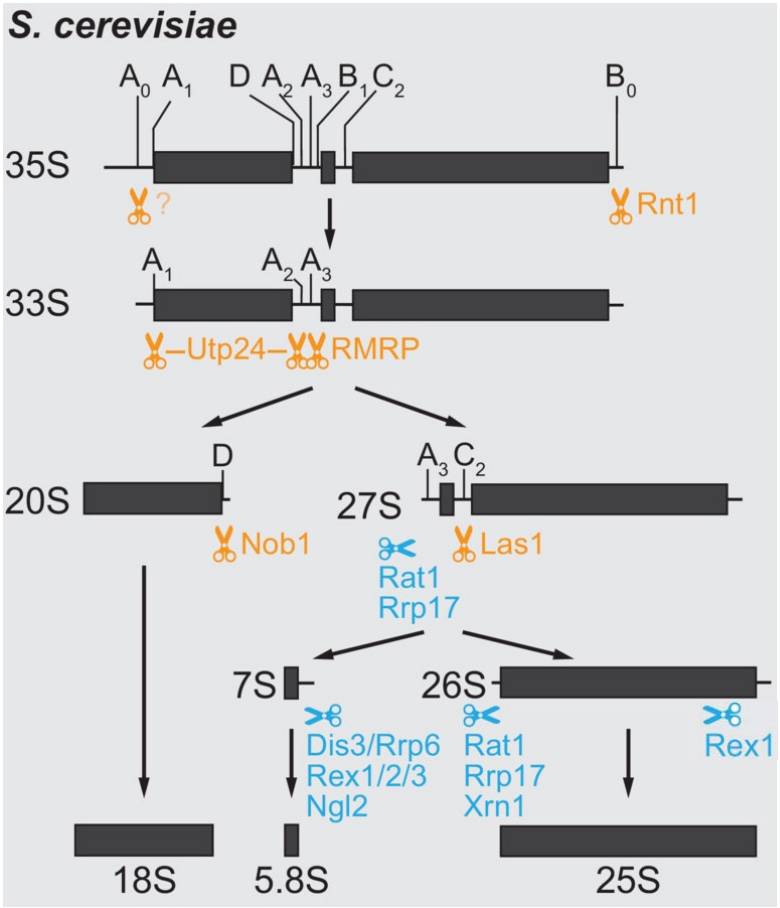
5'-3'
Rat1, Xrn1, Rrp17
3'-5'
exosome: Dis3, Rrp6
Rex1-3, Ngl1

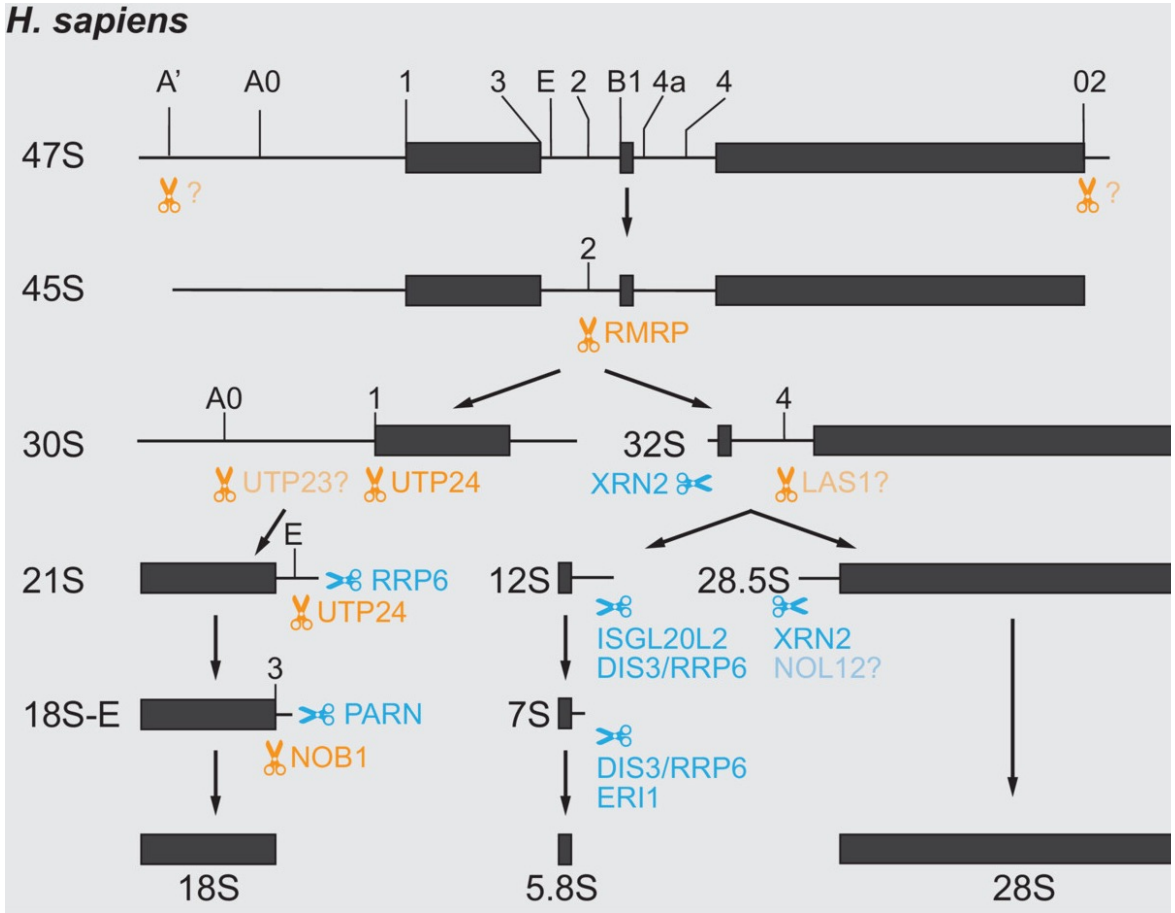




Fatica and Tollervey, Cur. Op. Cel. Biol., 2002

Pre-rRNA processing

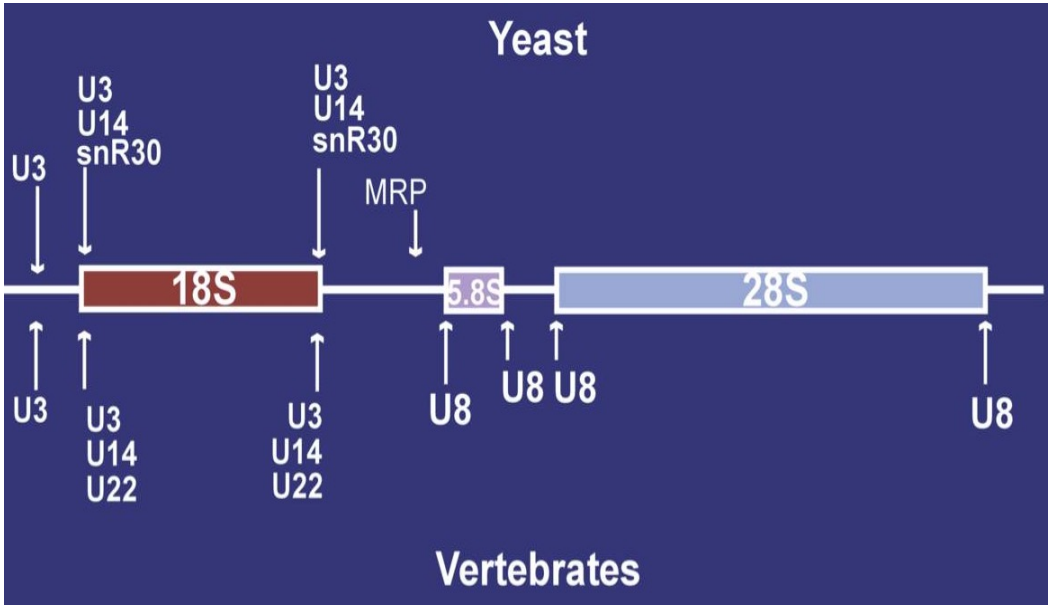


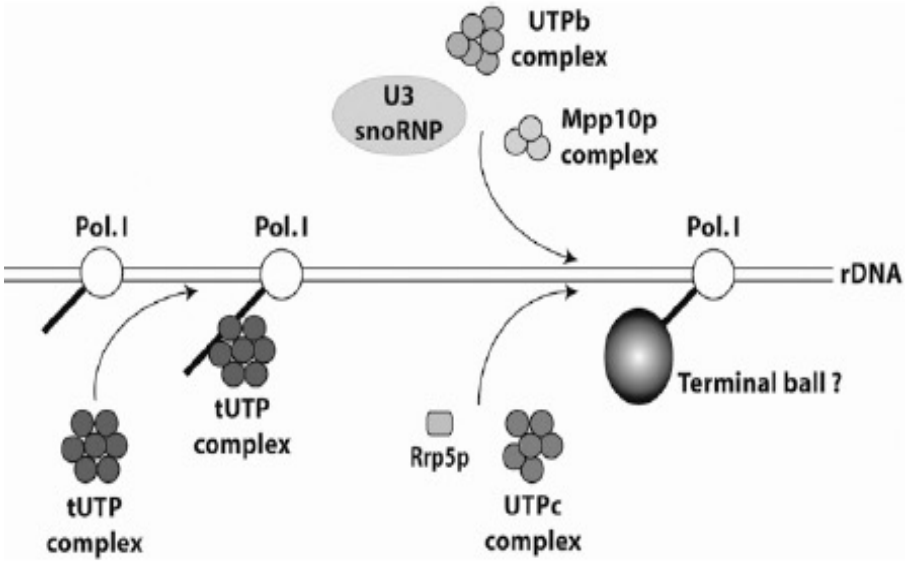


Dorner et al, EMBO, 2023

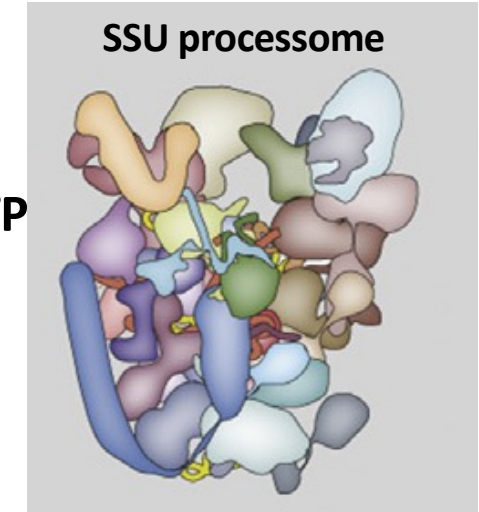
Pre-rRNA processing

Pre-rRNA processing requires snoRNAs (small nucleolar RNAs)

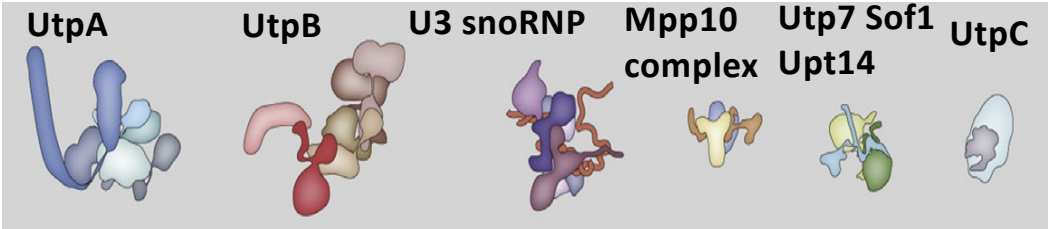




<u>Processome</u>
U3 snoRNP + UTP complex (UtpA+B+C) **+ other proteins**

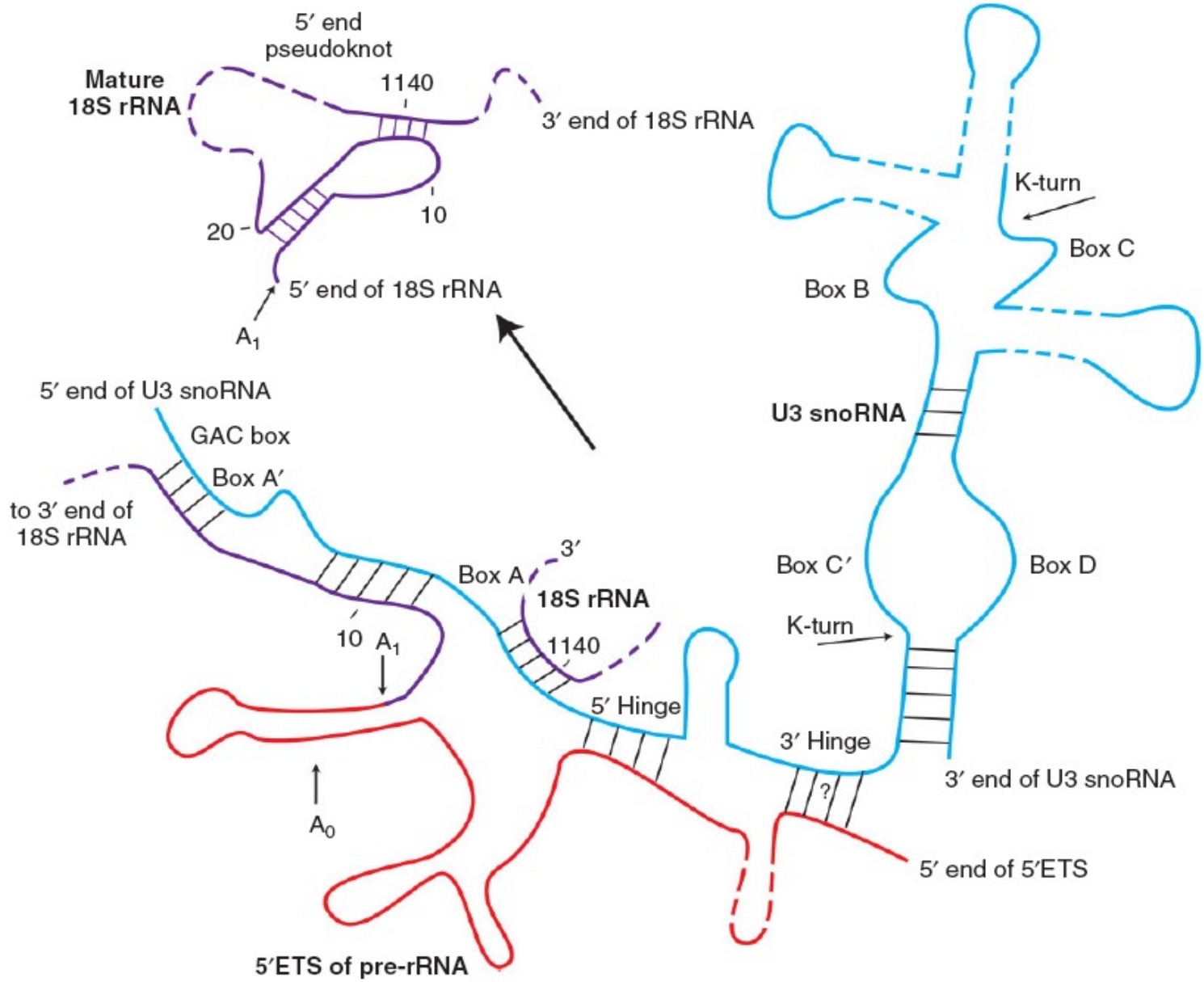


=



Henry et al, CMLS, 2008; Barandun et al, Curr Op Struct Biol 2018

Interaction of U3 snoRNA with pre-rRNA

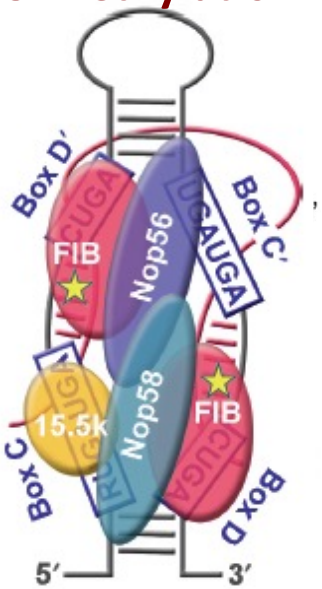


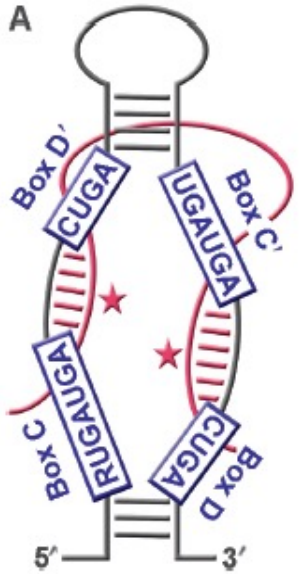
Phipps et al, WIRERNA., 2010

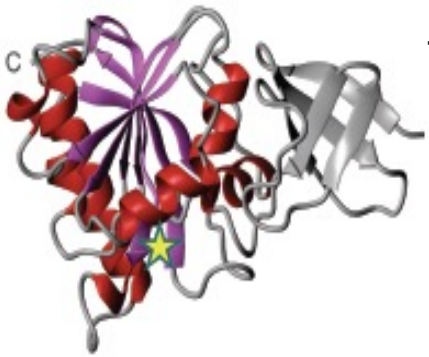
Pre-rRNA processing and modification

Early cleavages (A0-A2) in the pre-rRNA and modification of riboses (2'-OMe) and bases (pseudo-U) are carried by snoRNP complexes

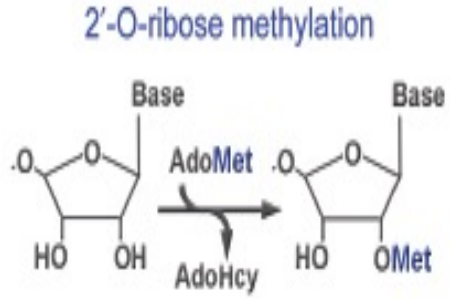
boxC/D: 2'-O-methylation



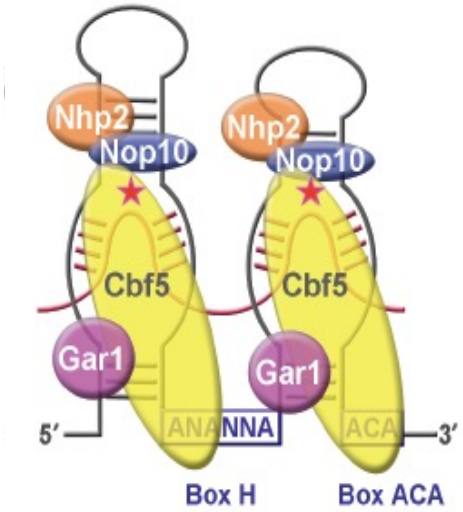


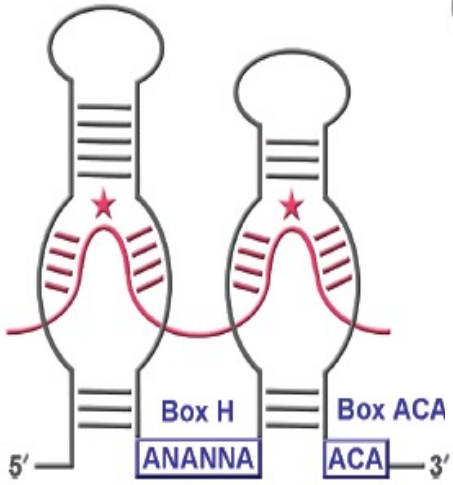


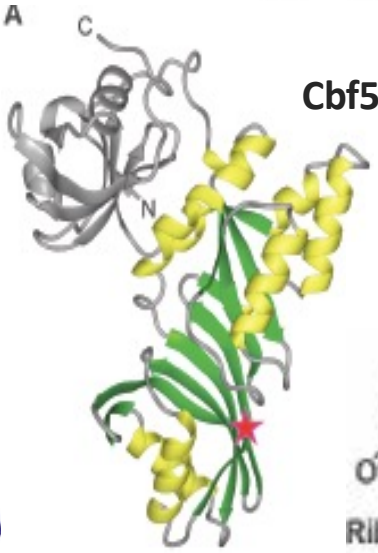
Nop1/ fibrillarin



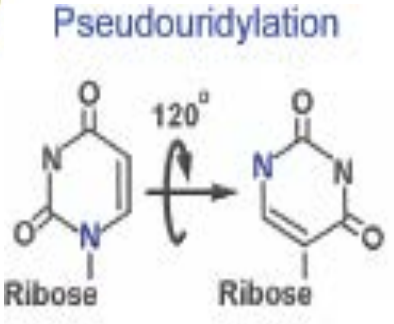
boxH/ACA: pseudouridylation







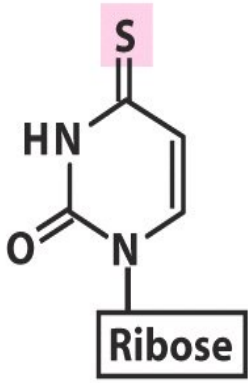
Cbf5



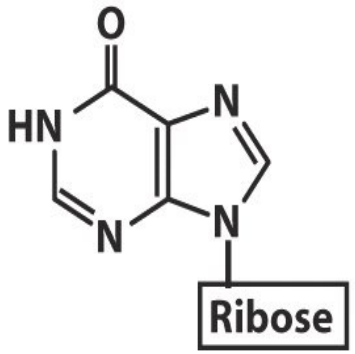
Reichow et al., NAR, 20027

RNA modification

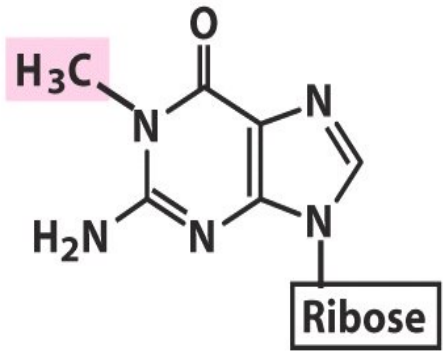
tRNAs, rRNAs, snRNAs and snoRNAs



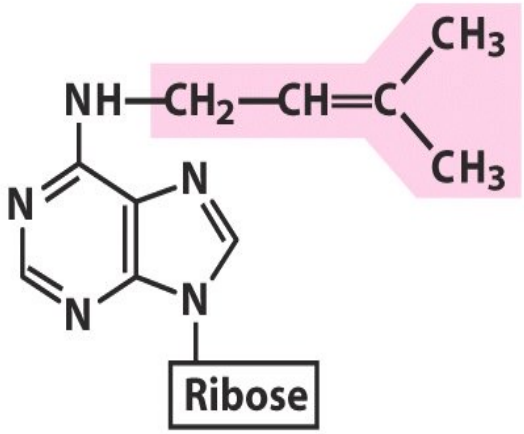
4-Thiouridine (S^4U)



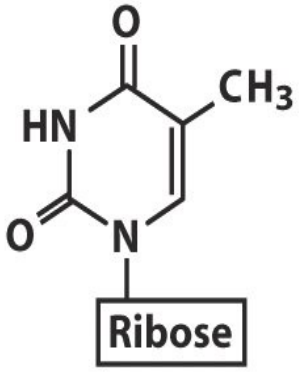
Inosine (I)



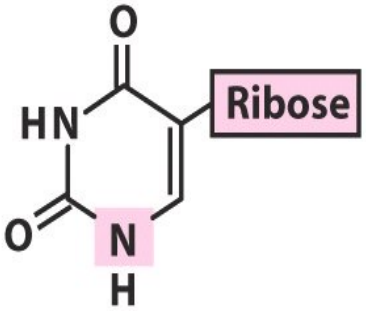
1-Methylguanosine (m^1G)



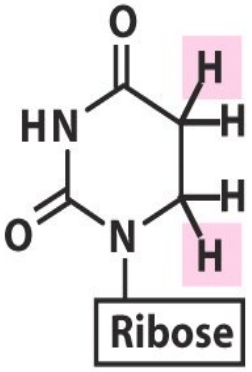
N^6-Isopentenyladenosine (i^6A)



Ribothymidine (T)



Pseudouridine (ψ)

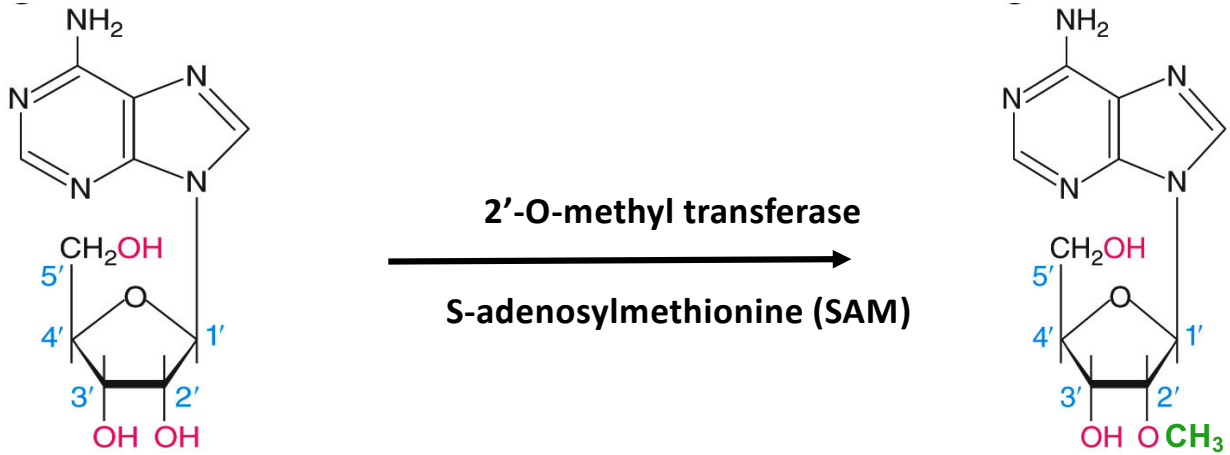


Dihydrouridine (D)

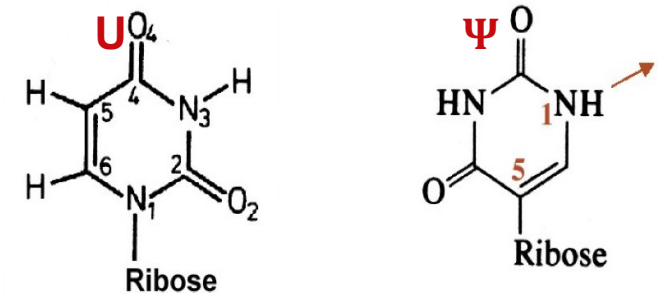
RNA modification

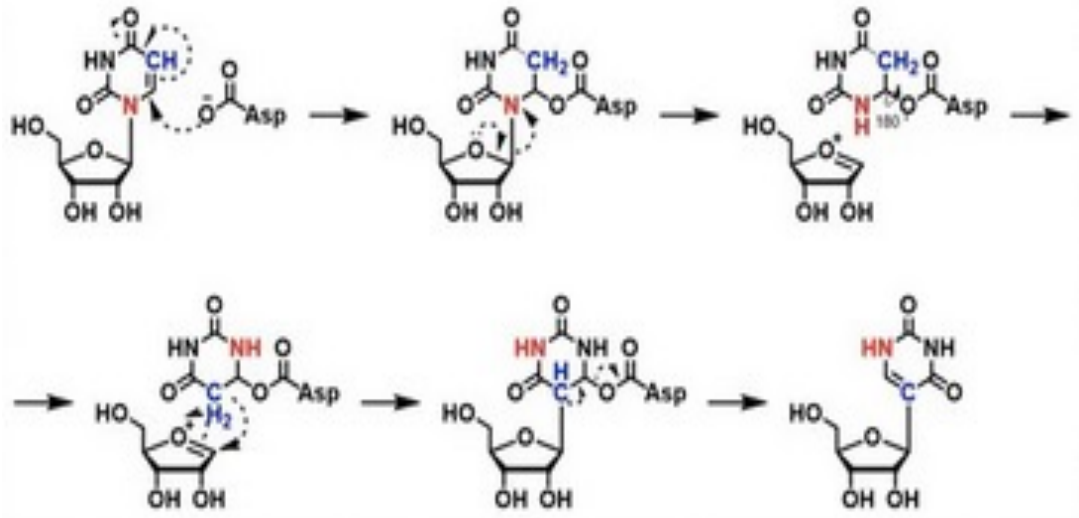
tRNAs, rRNAs, snRNAs and snoRNAs

1. 2'-*O*-methylation (modification of the ribose sugar)



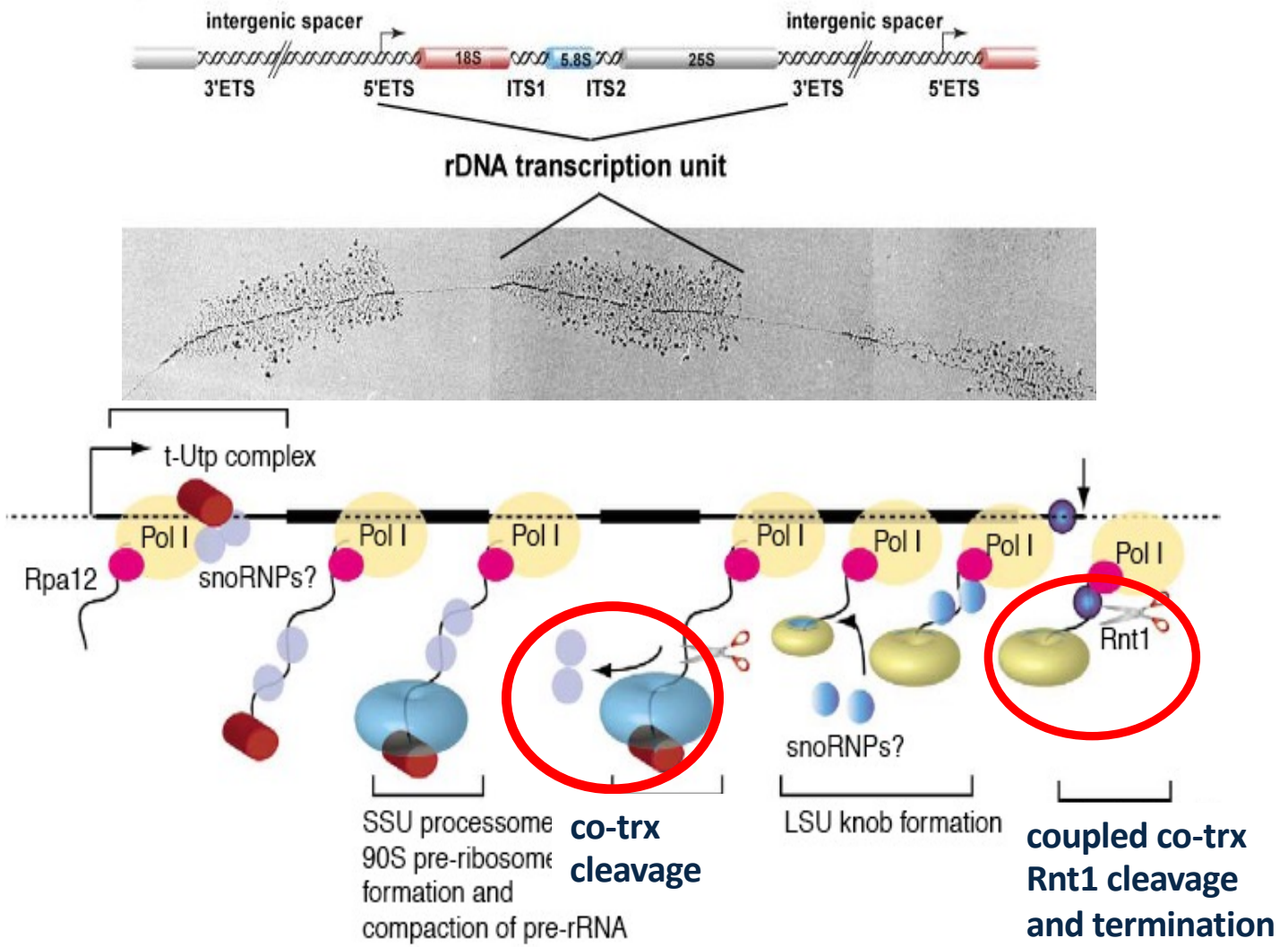
2. Conversion of uridine to pseudouridine by pseudouridine synthase





Common in rRNA, tRNA (up to > 1 %)

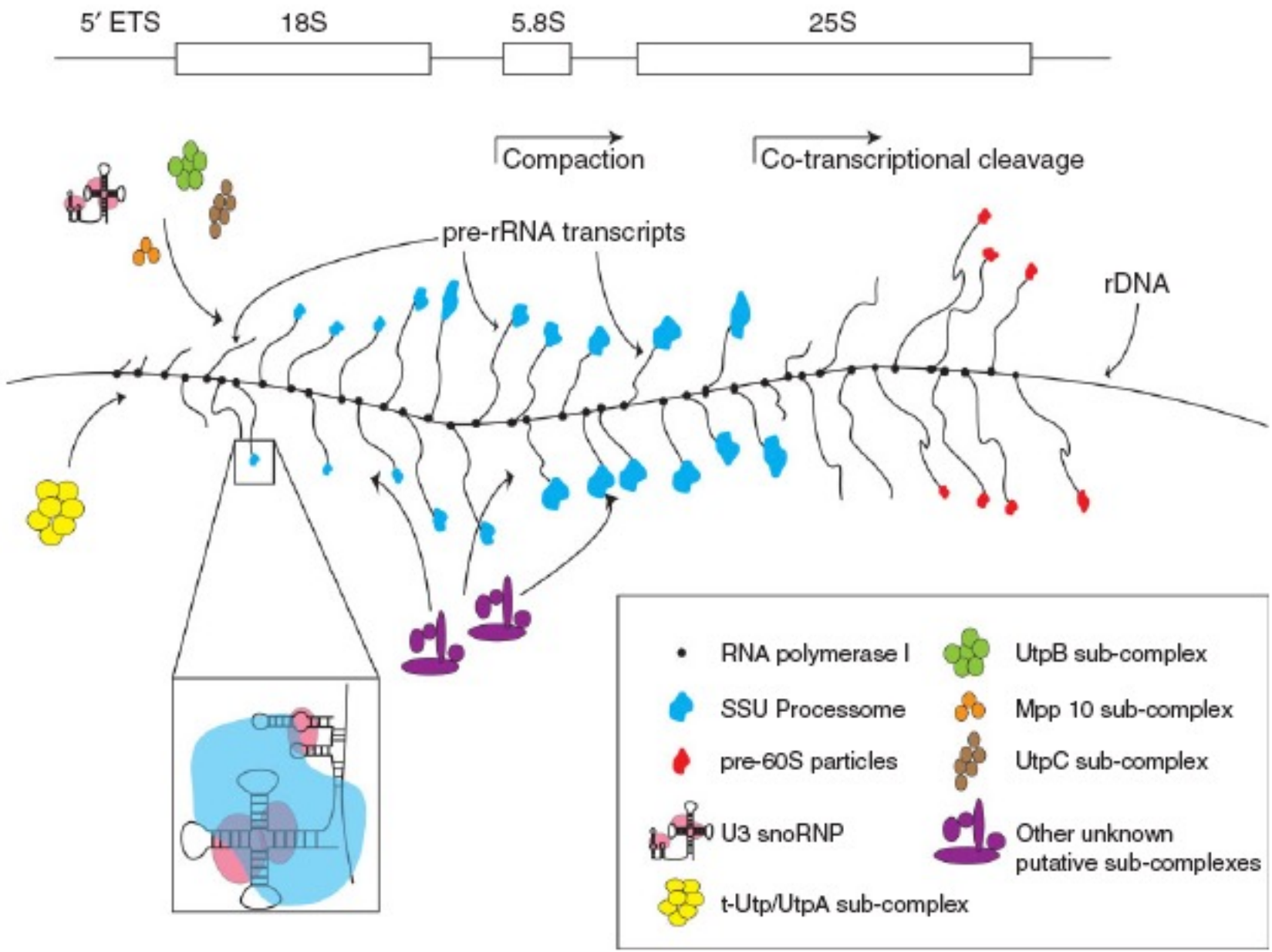
Pre-rRNA cotranscriptional processing



Granemman and Baserga, Curr.Op.CellBiol., 2005; Kos and Tollervey, Mol.Cell'10

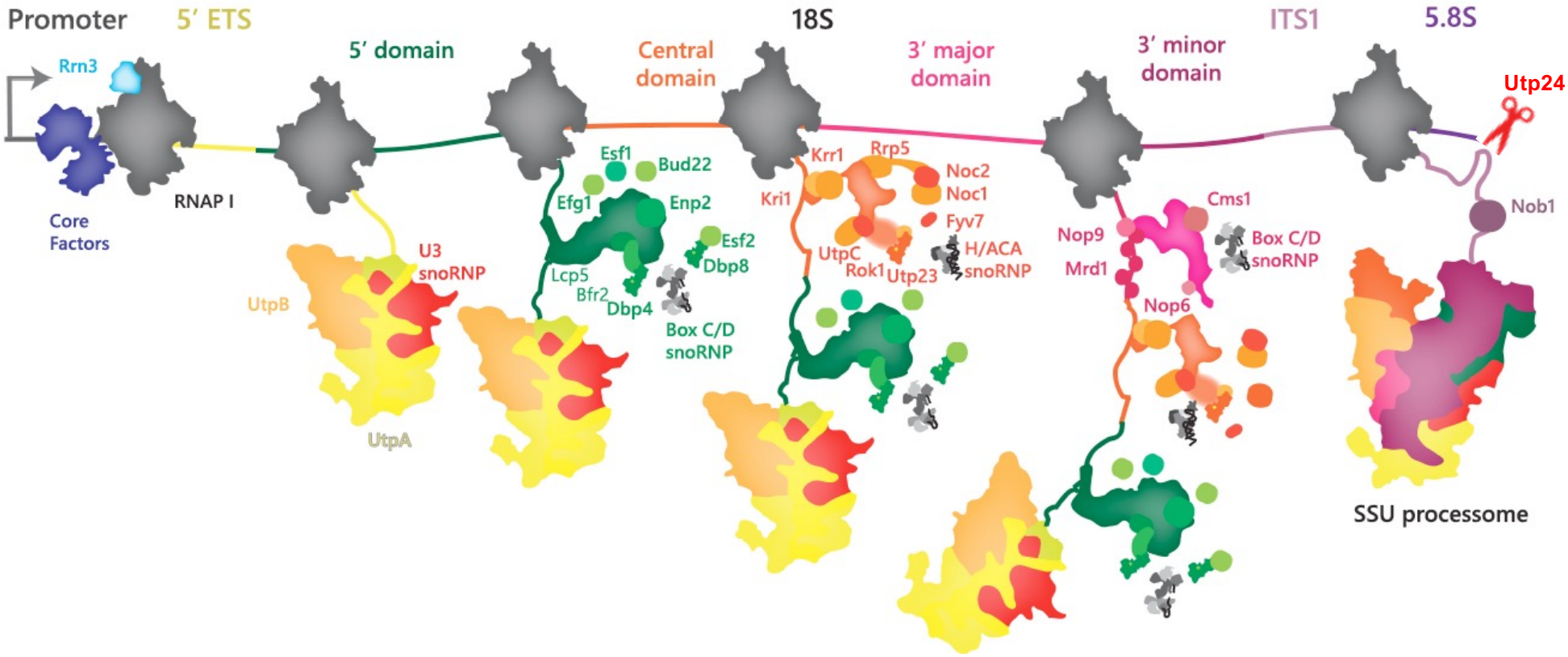
Cleavage dividing small and large subunits is largely co-transcriptional (70%)
Also rRNA modification (ribose methylation) is co-transcriptional and occurs on the nascent transcript, predominantly for the small subunit and partially for the large subunit.

Pre-rRNA cotranscriptional processing



Phipps et al, WIRERNA., 2010

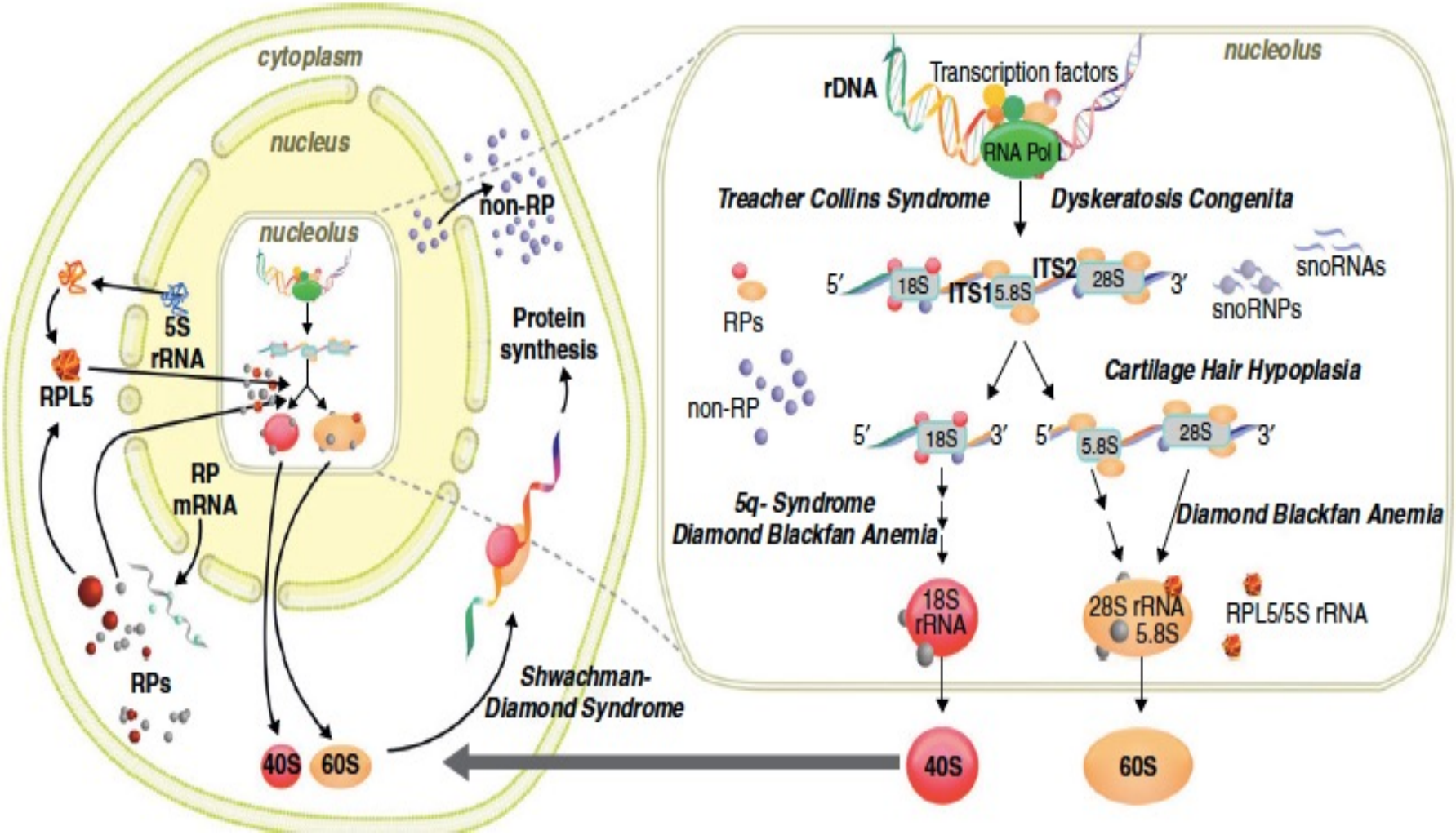
Pre-rRNA cotranscriptional processing



Pchaker-Margot, RNA., 2018

Ribosome and disease: ribosomopathies

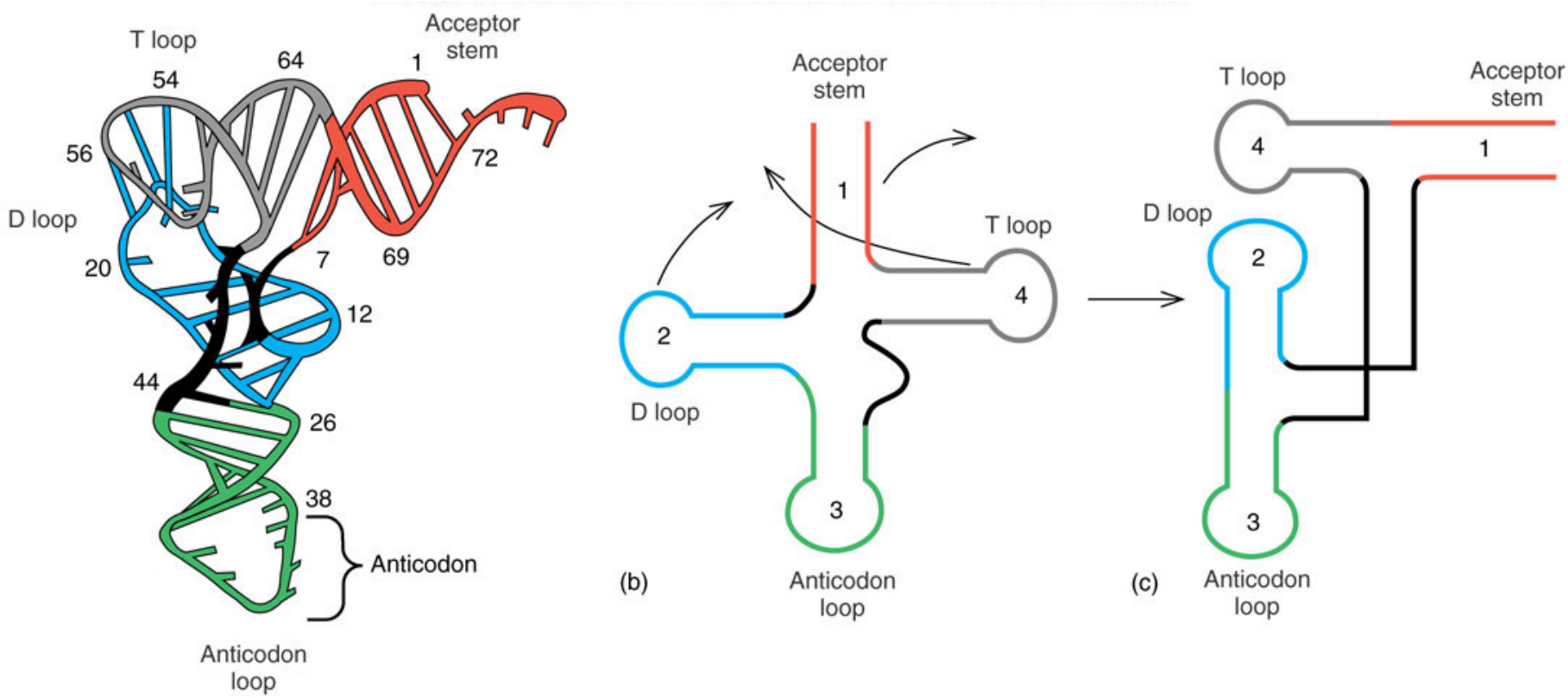
Diseases resulting from defects in rRNA processing and in expression of ribosomal proteins



Ribosomopathies: characterization and molecular defects

Disease	Genetic defect	Gene function	Congenital or acquired?	Clinical characteristics	Cancer risk	References
Diamond Blackfan Anemia (DBA)	RPS19, RPS24, RPL35a, RPS17, RPL5, RPL11, RPS7, RPS10, RPS26	Ribosomal proteins required for ribosome biogenesis	Congenital	Macrocytic anemia; growth retardation; craniofacial malformations; thumb, limb and heart defects	Myelodysplastic syndrome (MDS), AML and solid tumors	[12–22]
5*q*- syndrome	RPS14, one of ~40 genes in CDR	Ribosomal protein required for ribosome biogenesis	Acquired	Macrocytic anemia; micromegakaryocytosis and thrombocytosis	Acute myeloblastic leukemia (AML)	[23,24]
Shwachman–Diamond syndrome (SDS)	SBDS	Maturation of 60S ribosomal subunit and 60S–40S subunit joining	Congenital	Bone marrow dysfunction; pancreatic insufficiency; skeletal abnormalities; short stature	AML and MDS	[25–28]
X-linked dyskeratosis congenital (DKC)	DKC1	Nucleolar protein associated with snoRNPs. Modifies rRNA. Component of telomerase complex	Congenital	Skin and nail abnormalities; bone marrow failure	High risk of cancer	[29–31]
Cartilage hair hypoplasia (CHH)	RMRP	RNA component of RNase MRP complex. Cleaves precursor rRNA. Role in mitochondrial DNA replication	Congenital	Short-limbed dwarfism, hypoplastic hair, defective erythropoiesis and immunity	7-Fold higher incidence of cancer	[32–36]
Treacher Collins syndrome (TCS)	TCOF1 POLR1D POLR1C	Nucleolar protein with role in pre-ribosomal processing and ribosome biogenesis. RNA polymerase I and III components	Congenital	Craniofacial abnormalities	None known	[37–39,40•]

Pre-tRNA processing: 3D structure



- **All tRNAs share a common cloverleaf secondary structure and L-shaped tertiary structure.**
- **L shape maximizes stability by lining up base pairs in the D and anticodon stems, and base pairs in the T and acceptor stems**

Pre-tRNA processing

tRNA precursors:

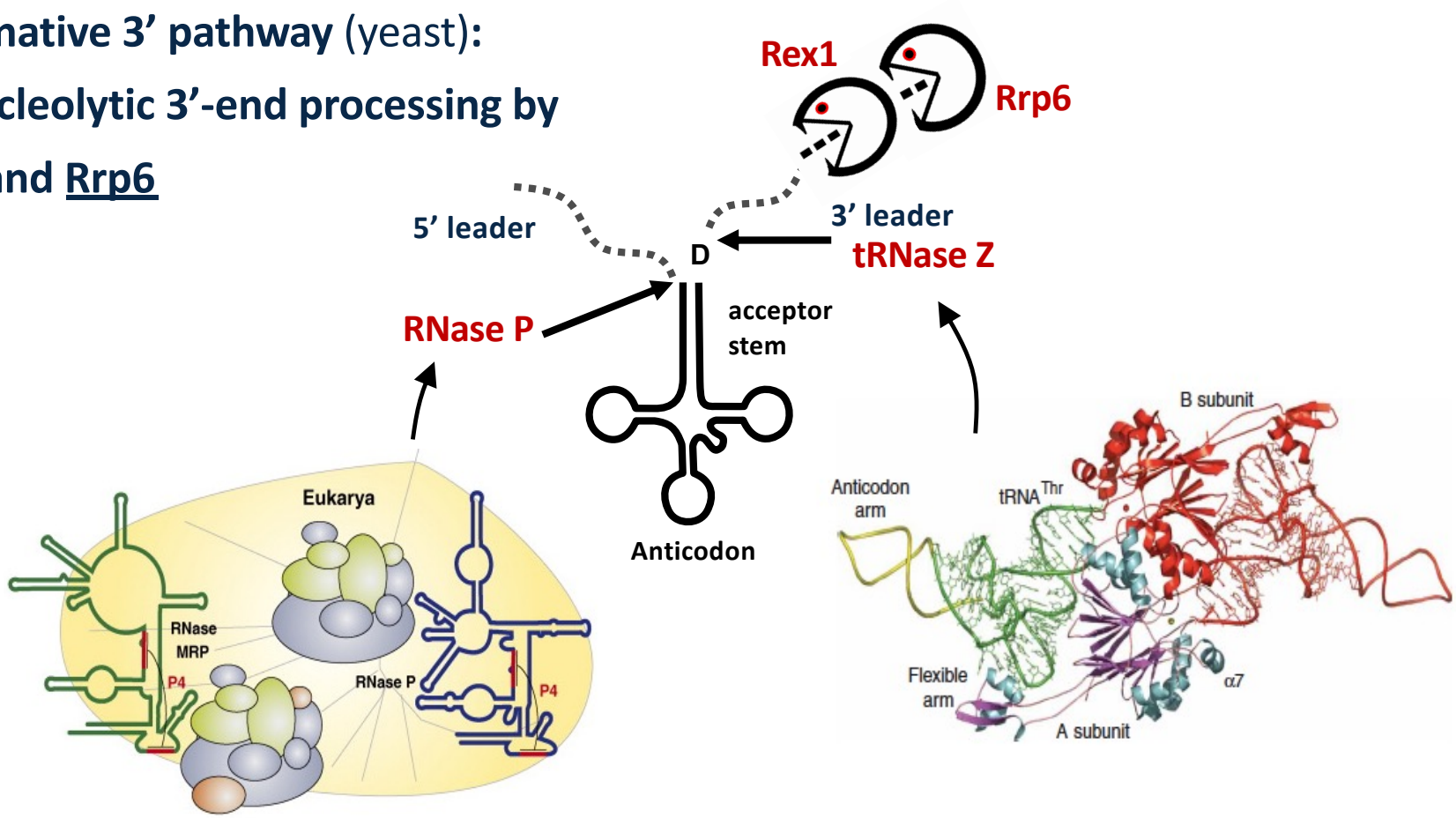
- 5’ end by RNAse P

- 3’ end by tRNase Z

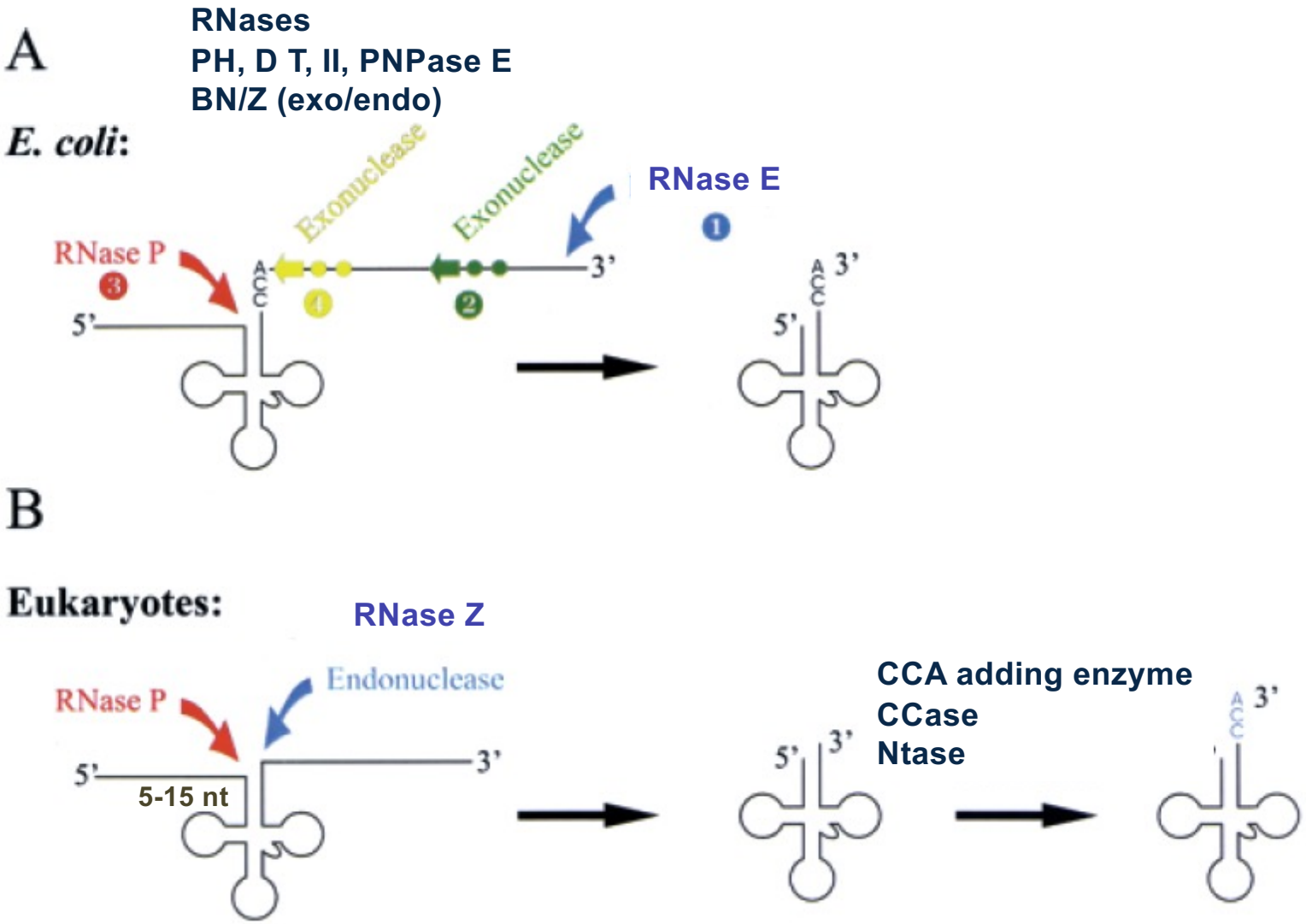
- alternative 3’ pathway (yeast)**:**

exonucleolytic 3’-end processing by

Rex1 and Rrp6



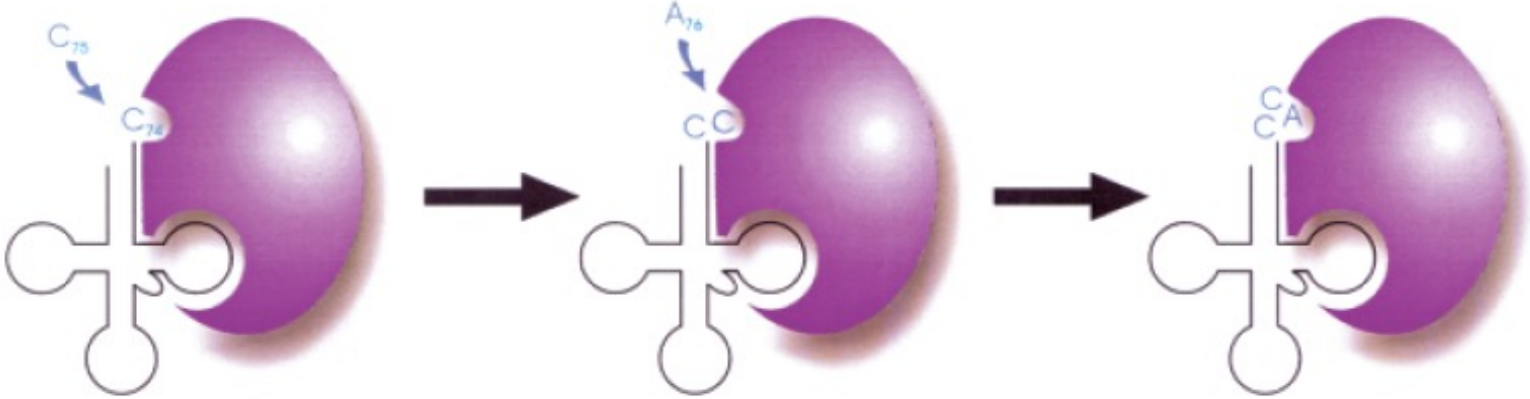
Pre-tRNA 5’ and 3’ processing



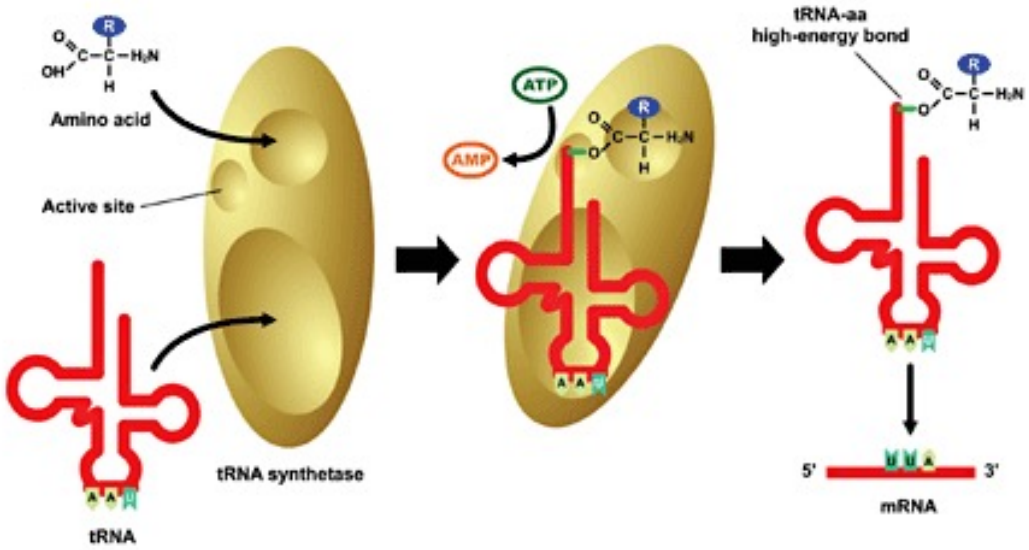
Schurer et al. Biol. Chem. 2001

tRNA Maturation

CCA template-independent addition by tRNA nucleotidyl-transferase *(collaborative)*
Addition of C74 and C75 uses CTP binding site, addition of ATP uses ATP binding site created by newly added Cs and CTP binding site



Aminoacylation by tRNA aminoacyl synthetases
two classes: class I and class II (aminoacylate 2'-OH and 3'-OH of A, respectively)

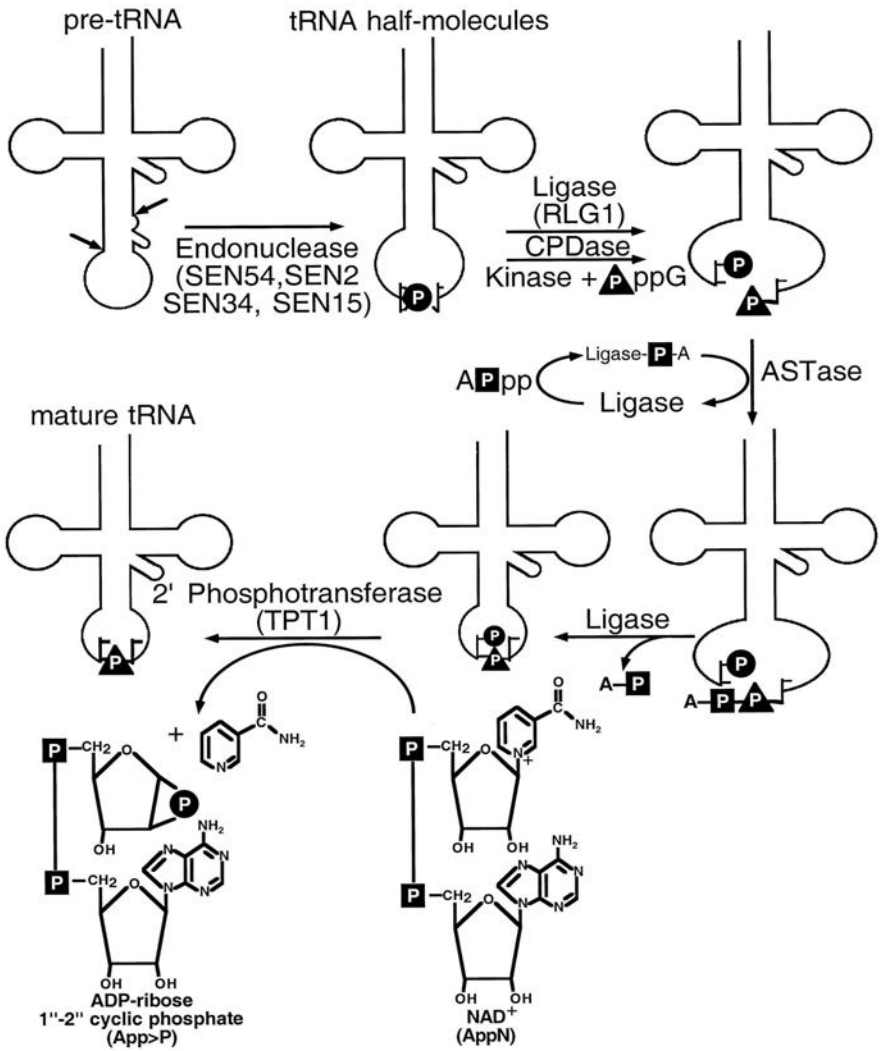


can occur in the nucleus and in the cytoplasm

Schurer et al, Biol.Chem., 2001

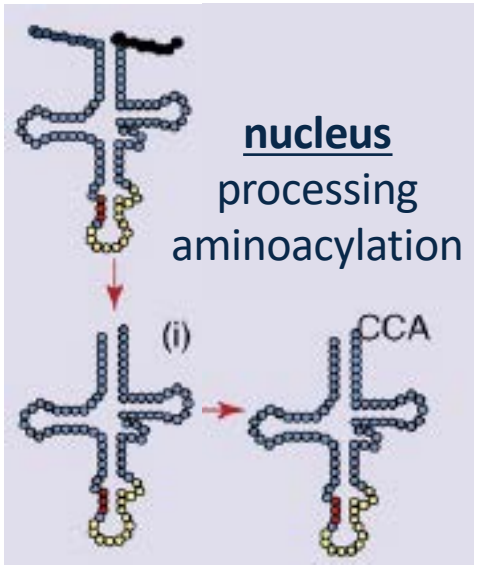
tRNA splicing

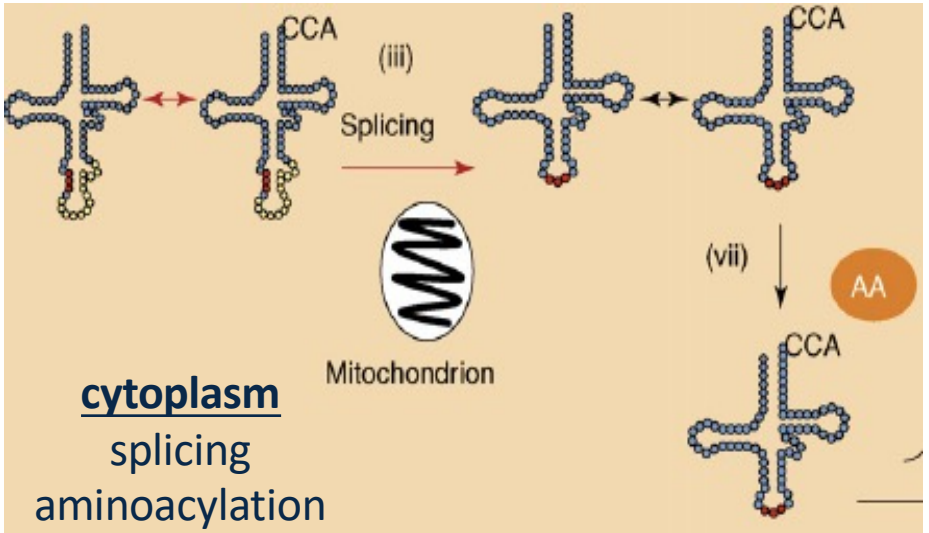
yeast



Abelson et al. J. Biol. Chem. 1998

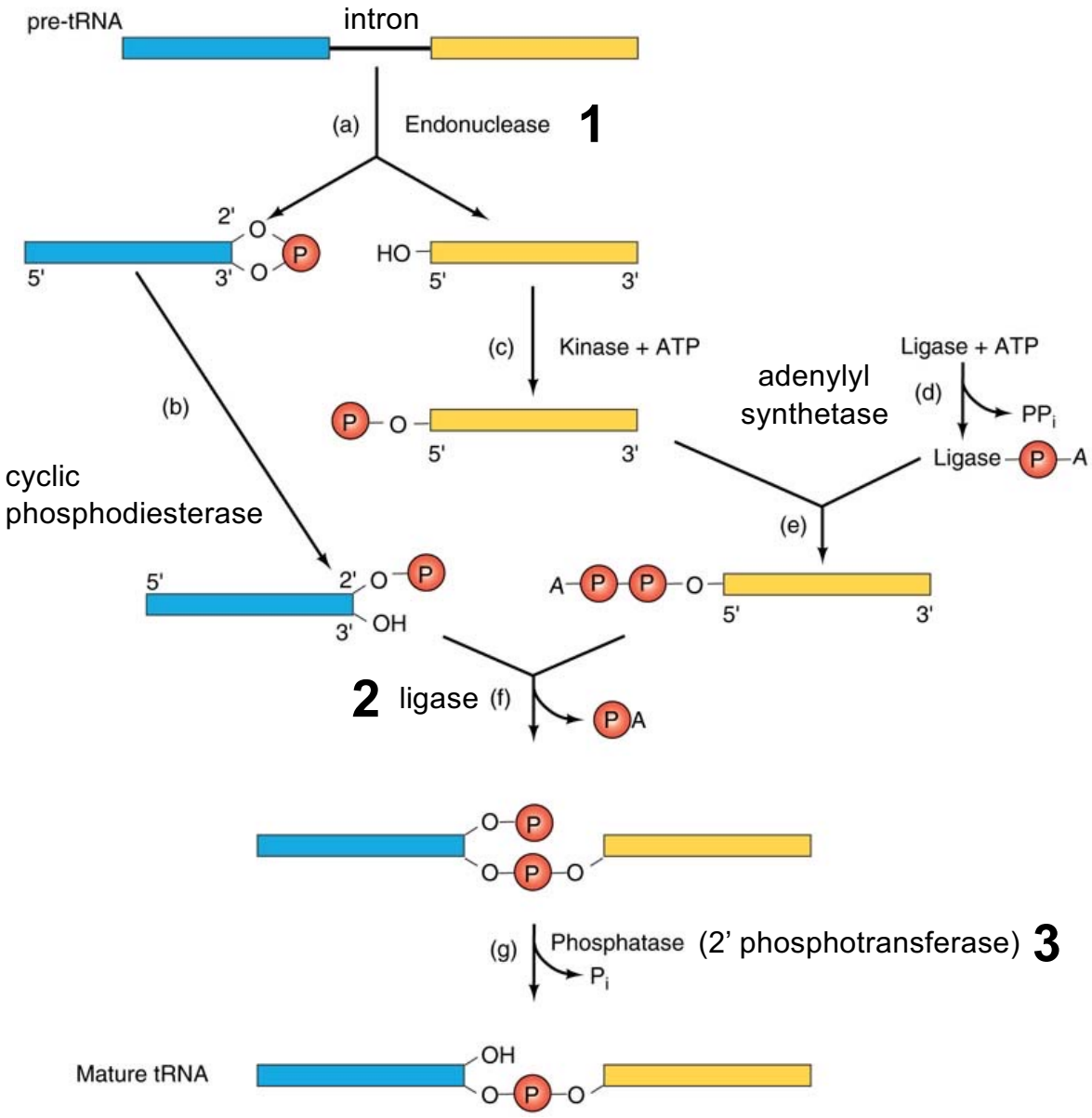
- **tRNA splicing occurs in the cytoplasm**
- **tRNA travels between nucleus and cytoplasm during processing steps**

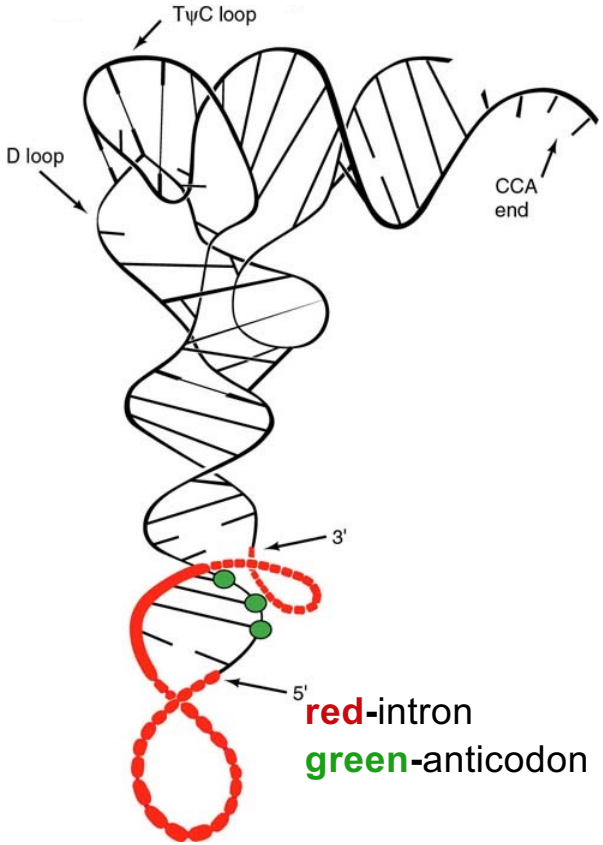




Hopper and Shaheen, TiBS,2008

Mechanism of tRNA splicing

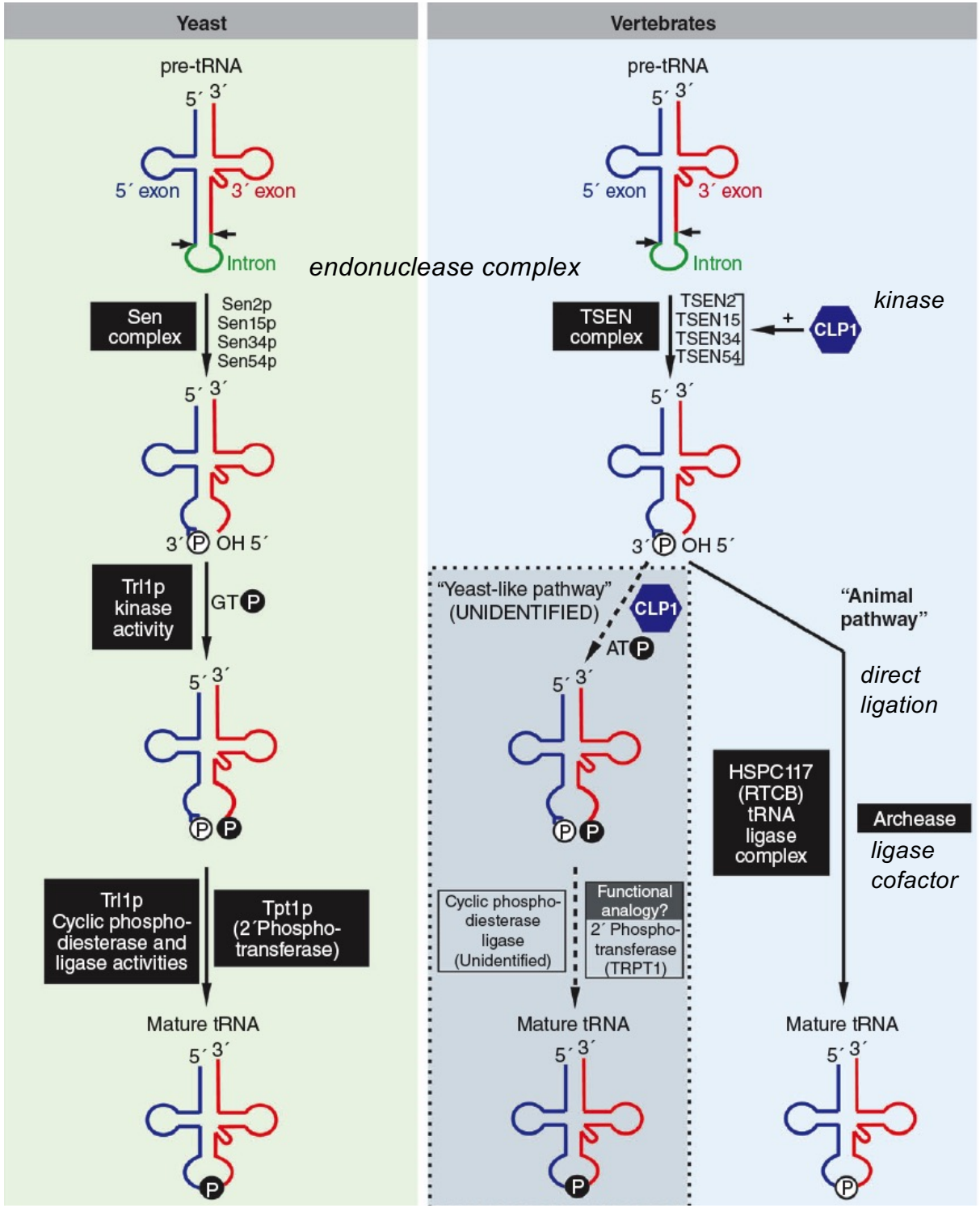




YEAST:
272 tRNA genes
59 contain introns

Abelson et al. J. Biol. Chem. 1998

tRNA splicing



Introns in tRNAs in yeast:

- are dispensable
(can be deleted)

- may control some tRNA modification
(pseudoU in anticodon in $tRNA^{Ile}$)

- ensure proper growth at some conditions
(deletion of some introns results in slow growth in respiratory conditions)

- may affect codon-anticodon pairing

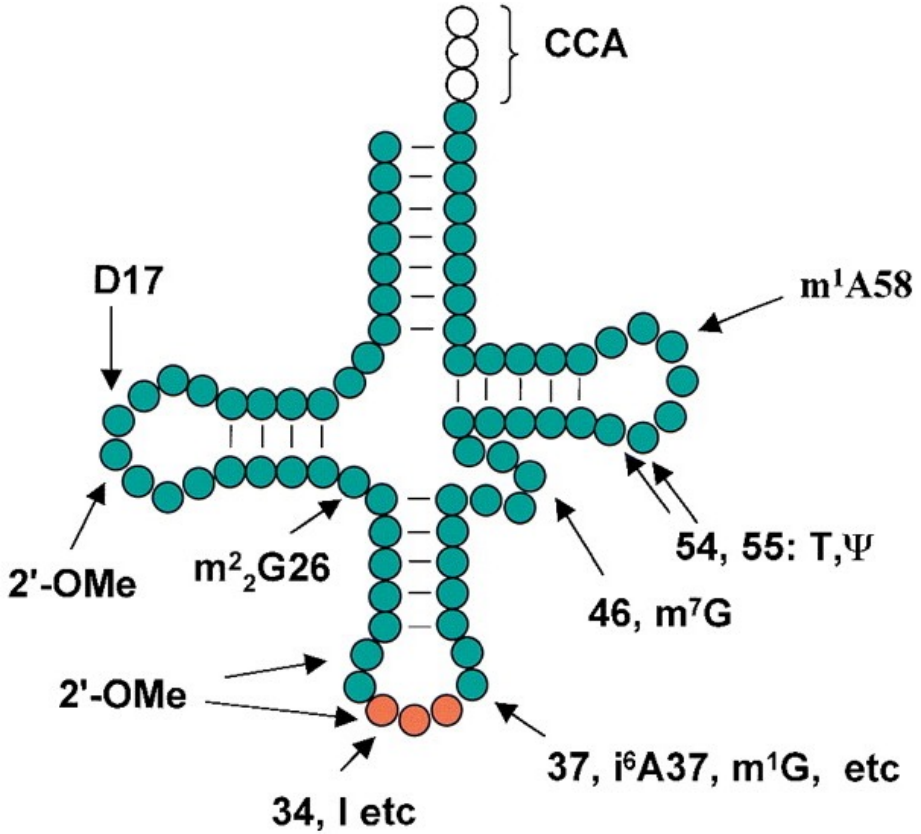
Weitzer et al, WIREs RNA,2015

Yeast gene	Isolation method	Modification
PUS1	genetics	Ψ27, 28, 34 (35), 36 (26, 65, 67 likely); U2 snRNP 44
PUS3 (DEG1)	bioinformatics	Ψ38, 39 cyt., mito.
PUS4	bioinformatics	Ψ55 cyt., mito.
PUS6	bioinformatics	Ψ31 cyt., mito.
PUS8	ND	Ψ32
TRM1	genetics; bioassay	m_2^2G26
TRM2	genetics, bioinformatics	m^5U54 cyt., mito.
TRM3	bioinformatics	Gm18
TRM4	bioinformatics	m^5C 34, 40, 48, 49
TRM5	bioinformatics	m^1G37, m^1I,yW
TRM7	bioinformatics	2′-*O*-Me 32 and 34
TRM8/TRM82	biochemical genomics	m^7G46
MOD5	genetics	i^6A37
GCD10, GCD14	genetics	m^1A58
TAD2, TAD3	bioinformatics	A34 to I34
DUS1,2	biochemical genomics; bioinformatics	D17 $tRNA^{Phe}$ (Dus1p, in vitro)
TAD1	bioinformatics	A37 to I37 $tRNA^{Ala}$
RIT1	genetics	2′-*O*-ribosyl phosphate at 64 of $tRNA_i^{Met}$

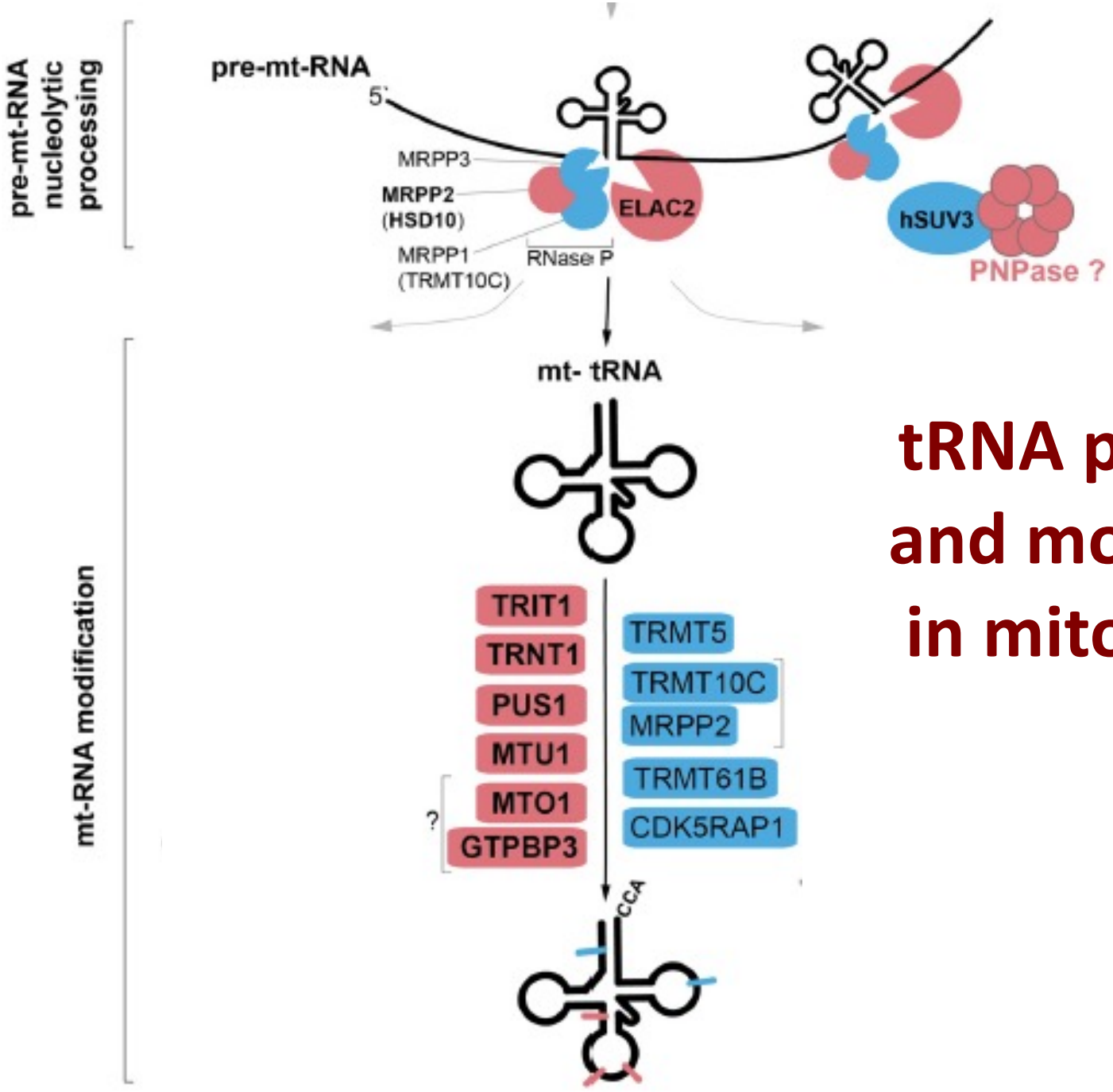
tRNA modification

Functions of modifications:

- **contribute to folding**
- **reinforce 3D structure**
- **provide stability**
- **facilitate alternative structures**
- **affect codon recognition** (wobble bp)
- **contribute to translation** (frameshifting)



Hopper and Phizicky, GeneGev. 2003



tRNA processing and modification in mitochondria

Powell et al, Front Genet . 2015

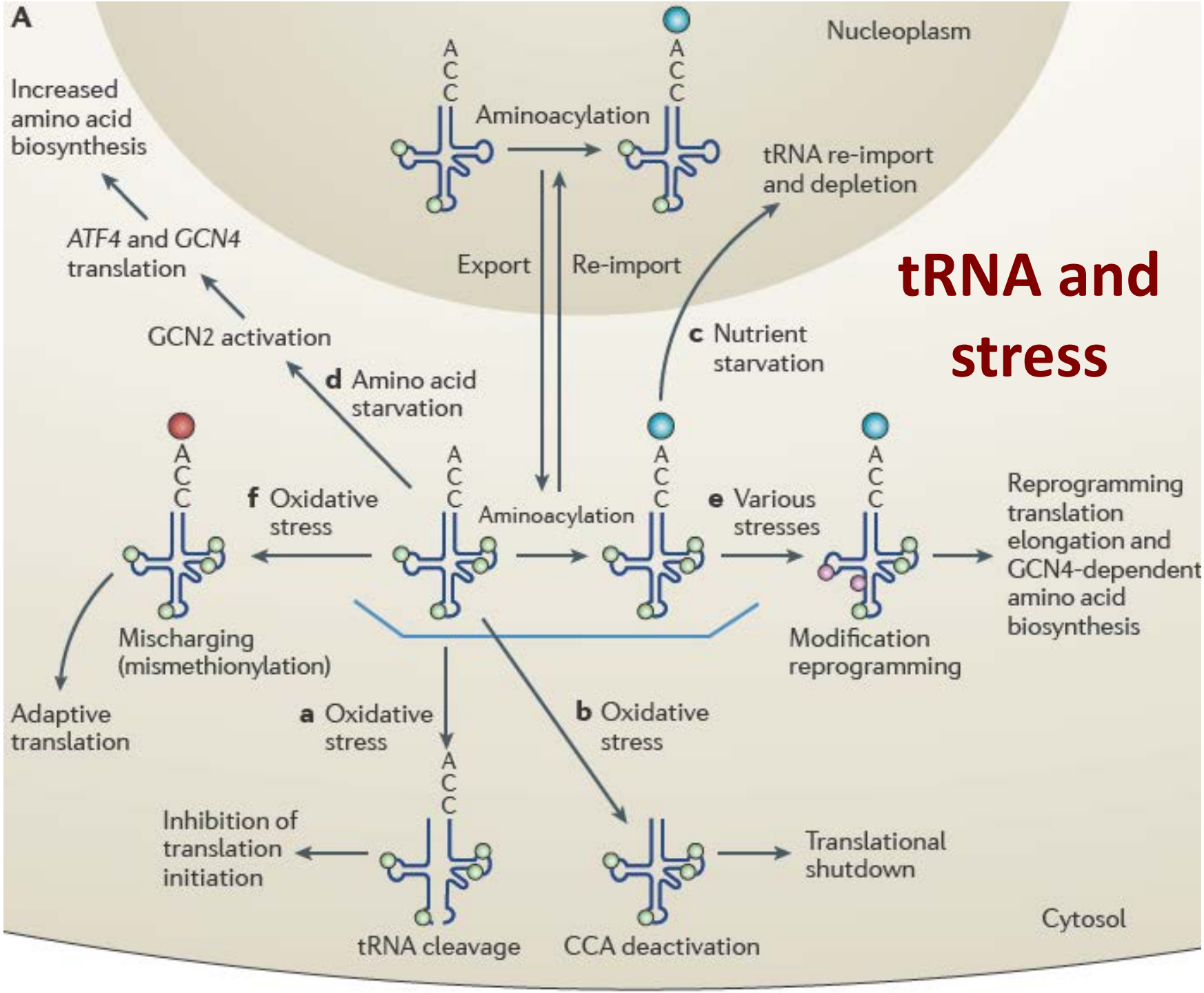
tRNA and disease

Disease type	Disease	Affected gene	Pathological effect
Mutations in tRNA genes			
Mitochondrial	Combined oxidative phosphorylation defect (COXPD)	*MT-TW*	Reduced $tRNA^{Trp}$(UCA) levels
		MT-TR	Reduced $tRNA^{Arg}$(UCG) levels
	Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS)	*MT-TL*	Impaired 3′ processing and reduced $tRNA^{Leu}$(UAA) levels
		MT-TL1	Lack of τm^5U34 modification and impaired translation of Leu codon UUG
		MT-TL1	Reduced activity of $tRNA^{Leu}$(UAA)
		MT-TH	Mutation in the D-stem leads to tRNA misfolding
	Myoclonic epilepsy with ragged-red fibres (MERRF)	*MT-TL1*	Lack of τm^5s^2U34 modification and impaired translation of Lys codons AAA and AAG
	Cardiomyopathy	*MT-TI*	Mutation in the D-stem leads to reduced tRNA levels
	Chronic ophthalmoplegia	*MT-TI*	T-stem mutations leads to misfolding and improper 3′ end processing
	Ragged-red fibres (RRFs)	*MT-TP*	Impaired mitochondrial function
	Cataract, spastic paraparesis and ataxia	*MT-TE*	Mutation in the T-stem disrupts conserved base pairing
	Neonatal death	*MT-TV*	Reduced tRNA levels
	Ataxia	*MT-TV*	Predicted to alter tRNA structure and function
		MT-TS2	Predicted to alter tRNA structure and function
	Myopathy	*MT-TD*	Unknown
		MT-TM	Impaired tRNA folding and reduced charging level
	Leigh syndrome	*MT-TW*	Unknown
	Hypertension	*MT-T1*	tRNA misprocessing and reduced $tRNA^{Ile}$(GAU) levels
		MT-TM	Reduced $tRNA^{Met}$(CAU) levels
Mutations in tRNA processing, charging and modification enzymes			
Metabolic	Type 2 diabetes mellitus	*CDKAL1*	Mistranslation of Lys codons AAA and AAG
		LARS2	Reduced charged $tRNA^{Leu}$ levels
Cancer	Breast cancer	*TRMT12*	Altered tRNA modification
Mitochondrial	Myopathy, lactic acidosis and sideroblastic anaemia (MLASA)	*YARS2*	Reduced aminoacylation
	Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL)	*DARS2*	Reduced aminoacylation
	Recessive ataxia	*MARS2*	Reduced aminoacylation and reduced protein synthesis
	Myopathy and infantile Charcot–Marie–Tooth syndrome	*AARS2*	Reduced aminoacylation
Neurological	Intellectual disability	*ADAT3*	Impaired A-to-I editing at tRNA position 34
	Dubowitz syndrome	*NSUN2*	Impaired modification of $tRNA^{Asp}$(GTC)
	Charcot–Marie–Tooth syndrome	*GARS*	Impaired aminoacylation
		AARS	Reduced aminoacylation and mischarging
		KARS	Impaired aminoacylation
	Dominant intermediate Charcot–Marie–Tooth syndrome	*YARS*	Gain of function of mutant YARS
	Pontocerebellar hypoplasia	*CLP1*	tRNA misprocessing and reduced tRNA levels
Others	Perrault syndrome	*HARS*	Reduced aminoacylation

Disease type	Disease	Affected gene	Pathological effect
Alterations in the tRNA pool accompanying diverse disease states			
Metabolic	Type 2 diabetes mellitus	Not directly related to specific tRNA-associated mutation	Increased aminoacylation of tRNAs
Cancer	Breast cancer	Not directly related to specific tRNA-associated mutation	Upregulation of tRNAs carrying polar and charged amino acids
	Multiple myeloma	Not directly related to specific tRNA-associated mutation	Increased tRNA levels
	Various carcinomas	Not directly related to specific tRNA-associated mutation	Increased translation of oncogenic genes
Neurological	Huntington disease	Not directly related to specific tRNA-associated mutation	Reduced charged $tRNA^{Gln}$(CUG) levels and reduced generation of *trans*-frame encoded species
Infection	Influenza A	NA	Alterations in translationally active tRNA pool
	Vaccinia	NA	Alterations in translationally active tRNA pool
	West Nile virus	NA	Increased aaRS expression
	Japanese encephalitis virus	NA	Increased aaRS expression
	HIV	NA	Alterations in the tRNA pool

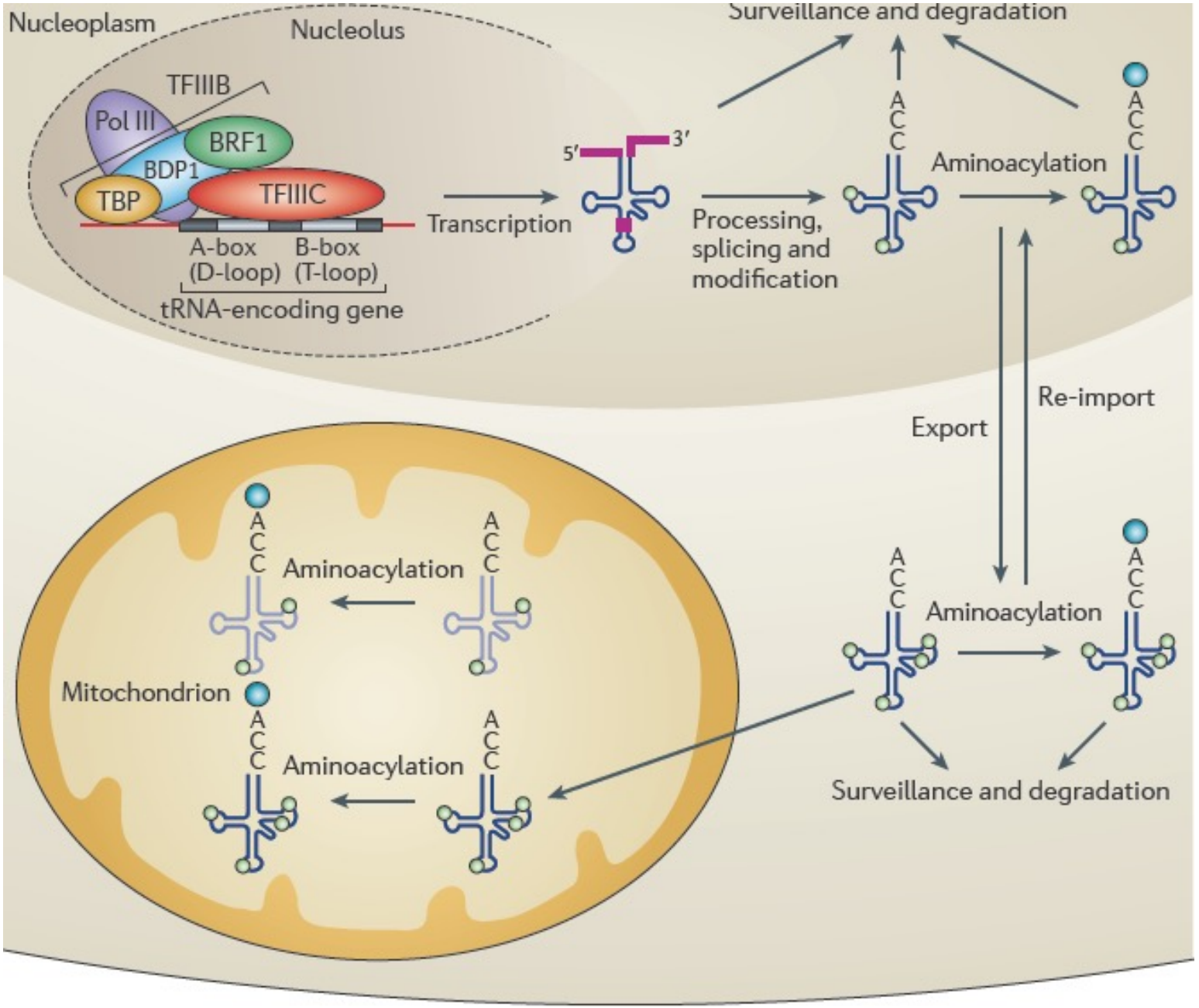
Kirchner and Ignatova, NatRevGenet, 2015

tRNA and stress



Kirchner and Ignatova, NatRevGenet, 2015

tRNA biogenesis: overview



Kirchner and Ignatova, NatRevGenet, 2015

TAKE-HOME MESSAGE

- RNA capping, splicing, 3' end formation, export occur, entirely or partly, cotranscriptionally
- Splicing is carried out by the spliceosome complex, with a catalytic center made of snRNAs (+ several protein components); U6 is a catalytic molecule
- Alternative splicing (AS), a highly regulated process (SR proteins), increases protein complexity but often generates NDM substrates
- Transcript 3' end formation is linked to transcription termination, both depend on Cleavage and Polyadenylation (CPA) complex or in yeast also Nrd1/Nab3 complex
- Alternative CP (APA) also contributes to the large pool protein variants
- RNA modification is largely post-transcriptional, but co-transcriptional cases (rRNA) also occur