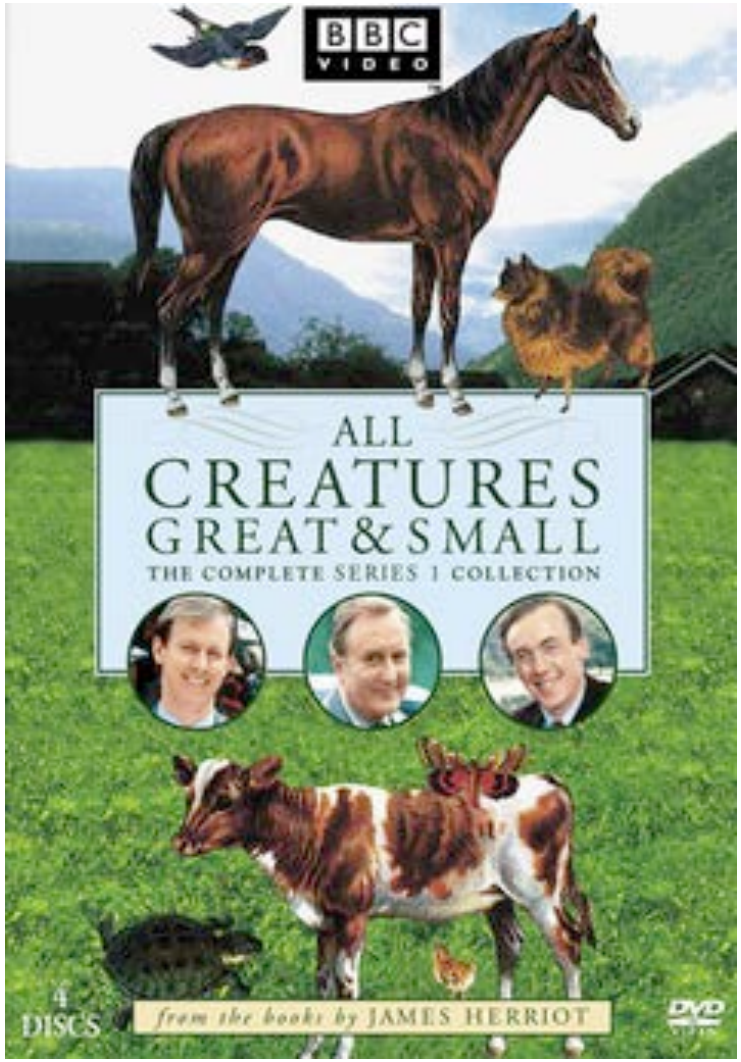
All RNAs great and small



Nascent transcripts

Co-transcriptional and post-transcriptional processess

Gene loops and Rloops

Splicing

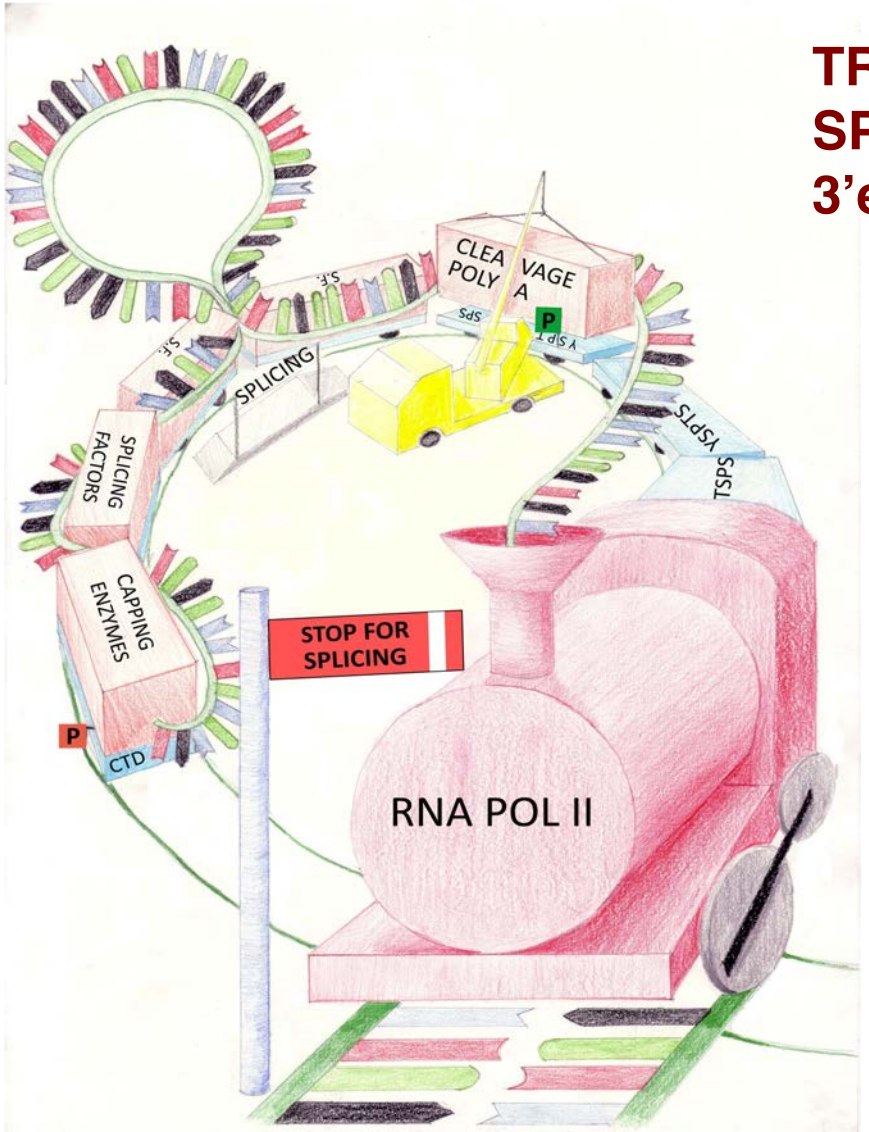
3' end formation

Translation cycle

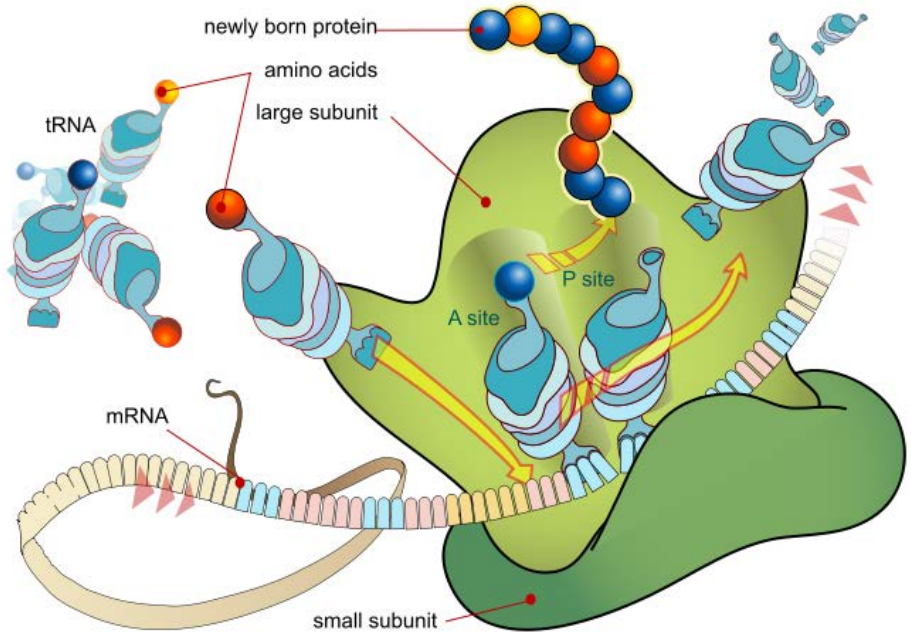
RNA enzymes and complexes

Institute of Genetics and Biotechnology
University of Warsaw

RNA MACHINERIES

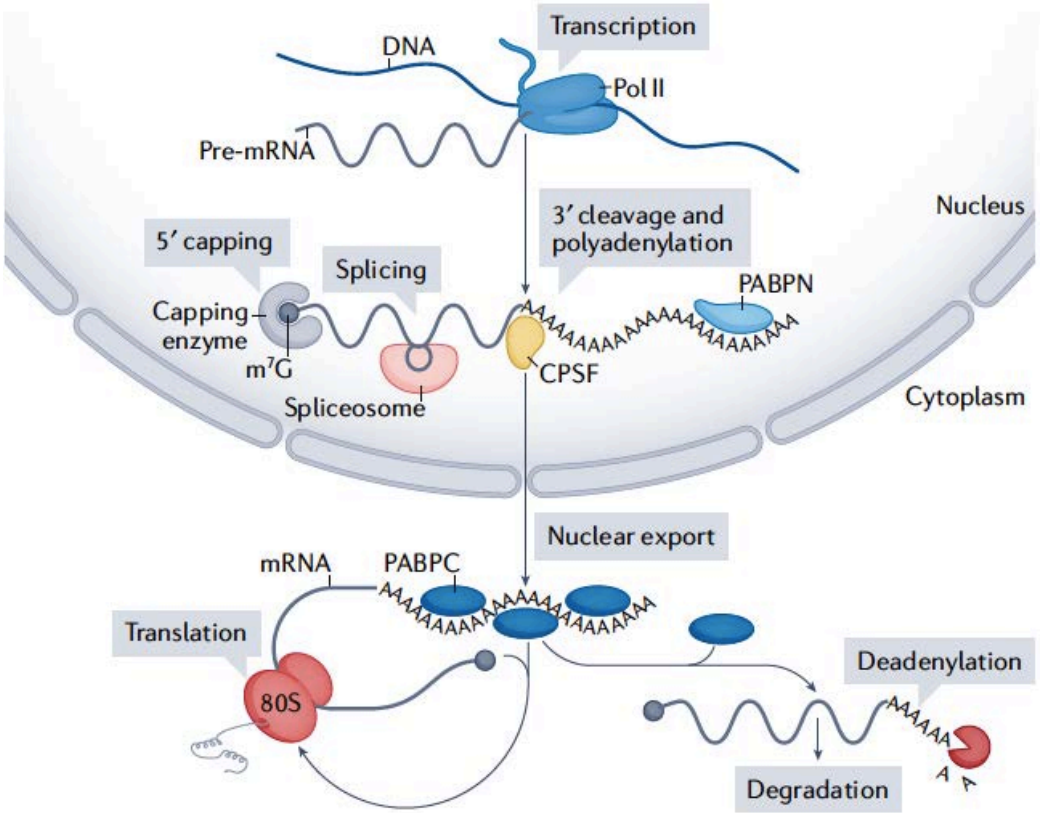


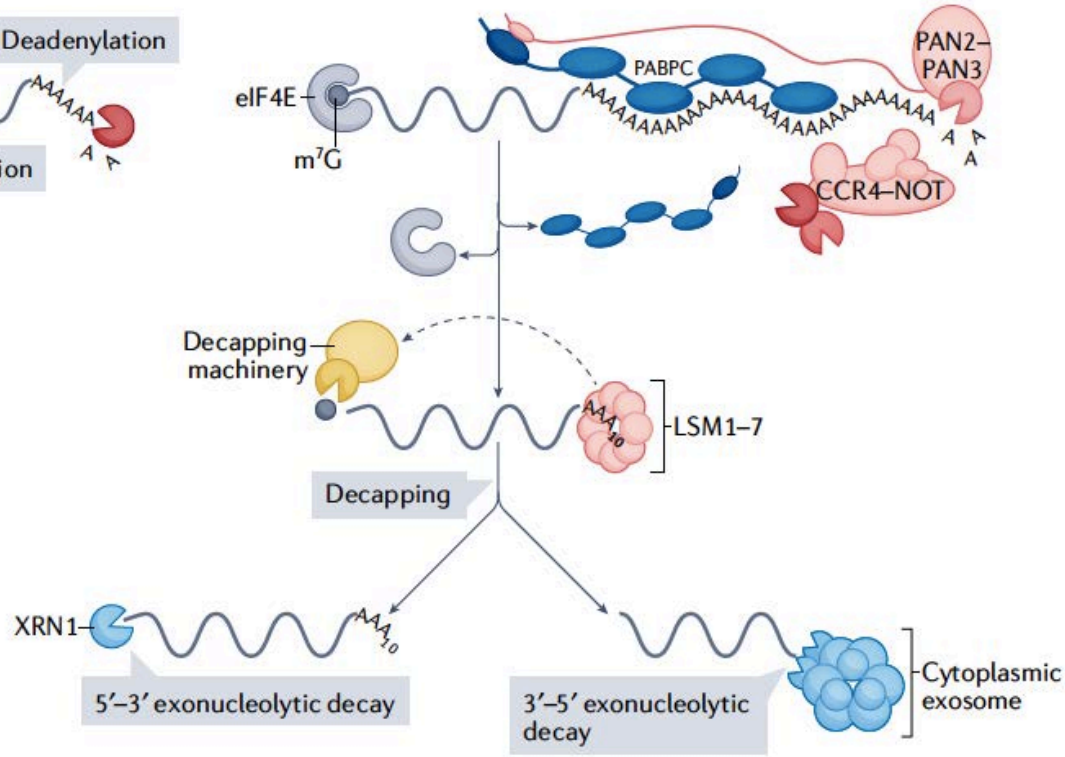
TRANSCRIPTION – RNAP
SPLICING – SPLICEOSOME
3'end FORMATION – CPA



TRANSLATION – RIBOSOME
DEGRADATION

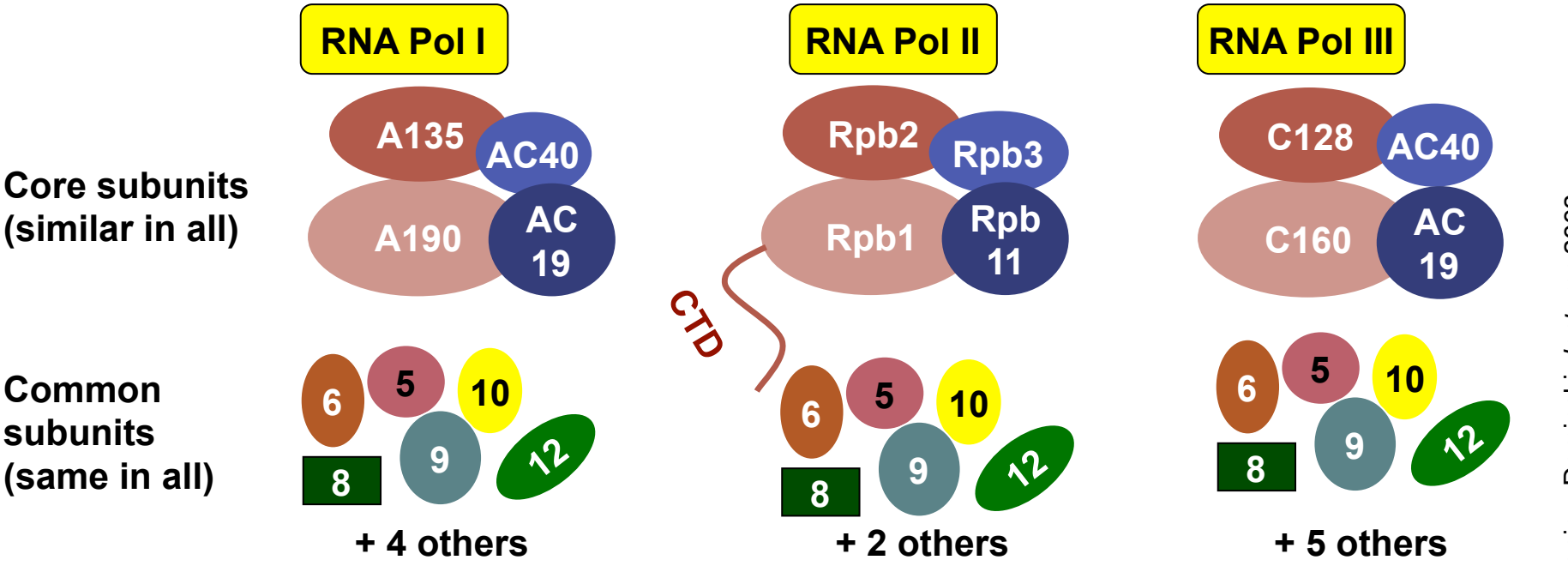
mRNA LIFECYCLE





Passmore and Coller Nat Rev Cell Mol Biol, 2021

RNA POLYMERASES



Zbigniew Dominski, *lectures* 2008

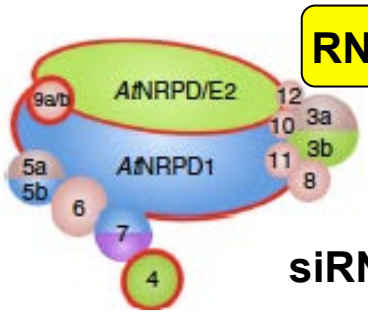
RNAs:

ribosomal RNA
35S precursor contains
18S, 5.8S and 25S rRNAs

mRNA , most snRNAs (U1, U2, U3, U4, U5, U11, U12, U4atac), snoRNAs, microRNAs, telomerase RNA, ncRNAs

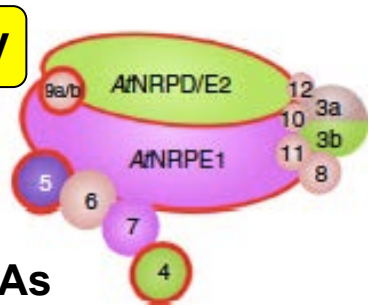
tRNA, 5S rRNA, U6 snRNA, U6atac snRNA, 7SK RNA, 7SL RNA, RNase P RNA, RNase MRP RNA

Additional plant Polymerases



Involved in transcriptional gene silencing

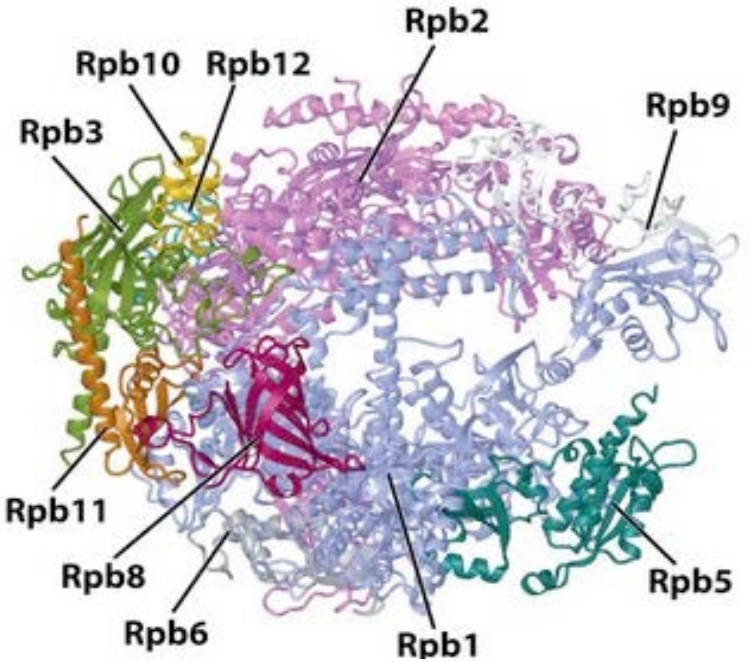
siRNAs



lncRNAs

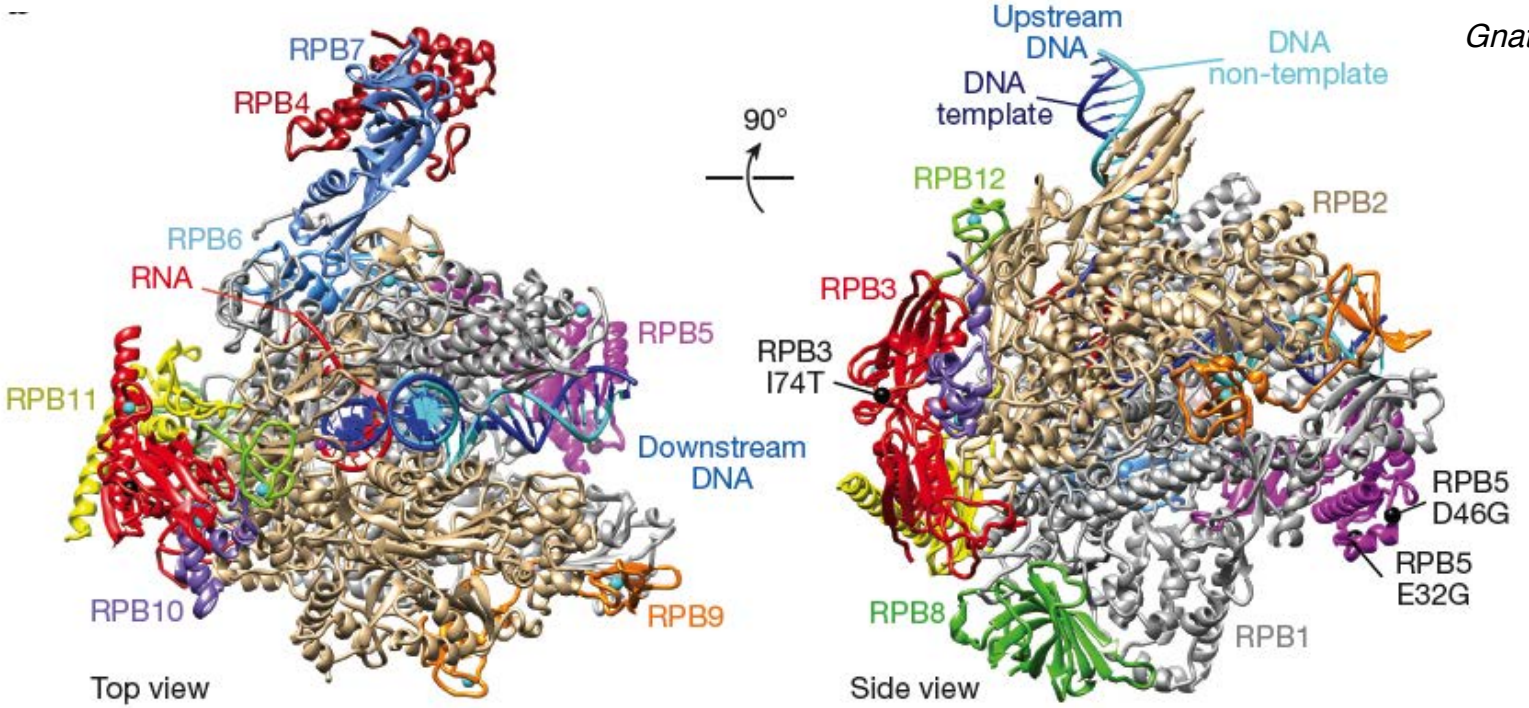
Yeast Pol II

- 12 subunits
- core by specific **Rpb1–3**, **9** and **11**
- **Rpb5–6, 8, 10** and **12** – shared by Pol I-III
- specific subcomplex **Rpb4/7** not essential
- associated factors RAP74, RAP30 (TFIIF)



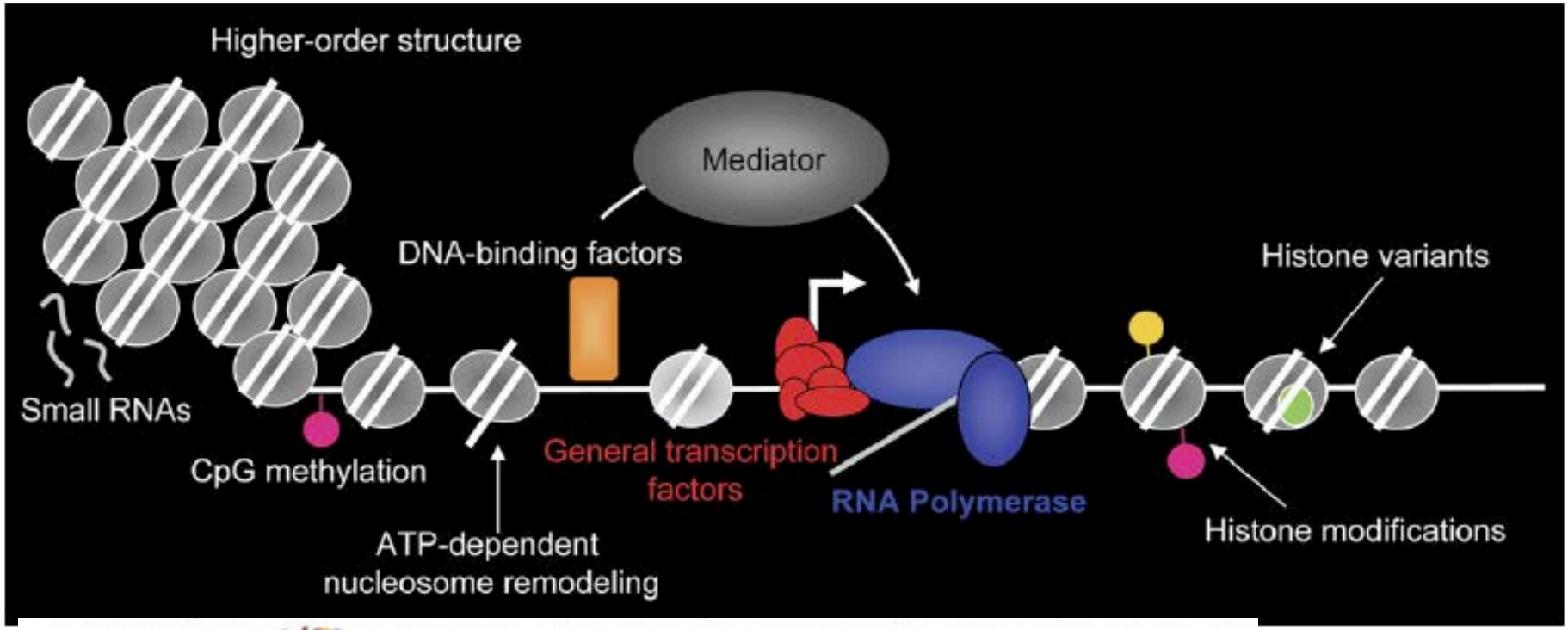
Gnatt et al, Science, 2001

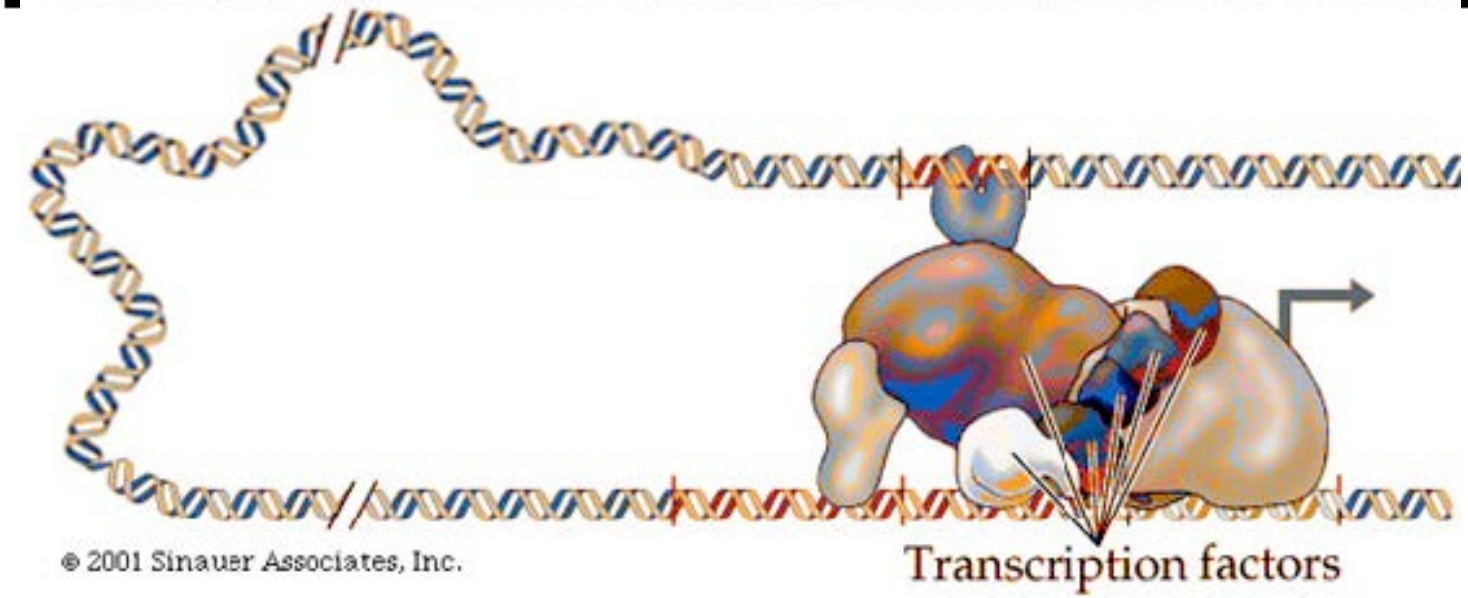
Mammalian Pol II



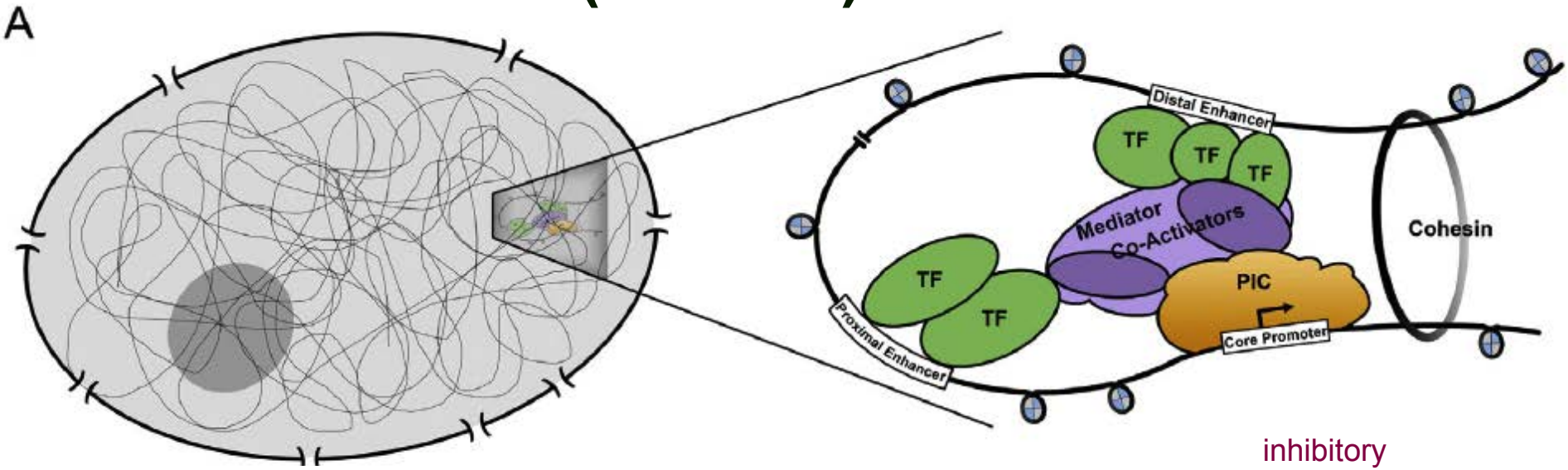
Berneckyet al, 2016, Nature

Pol II (RNAPII) in the cell

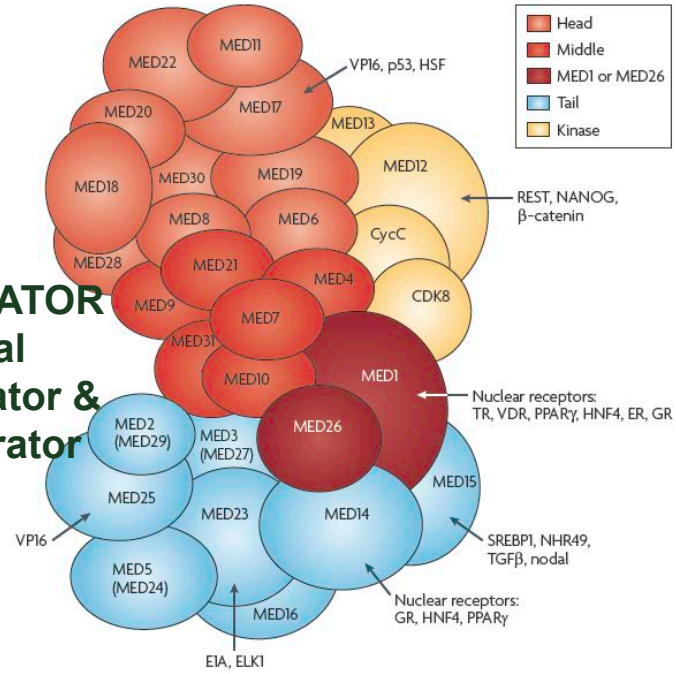




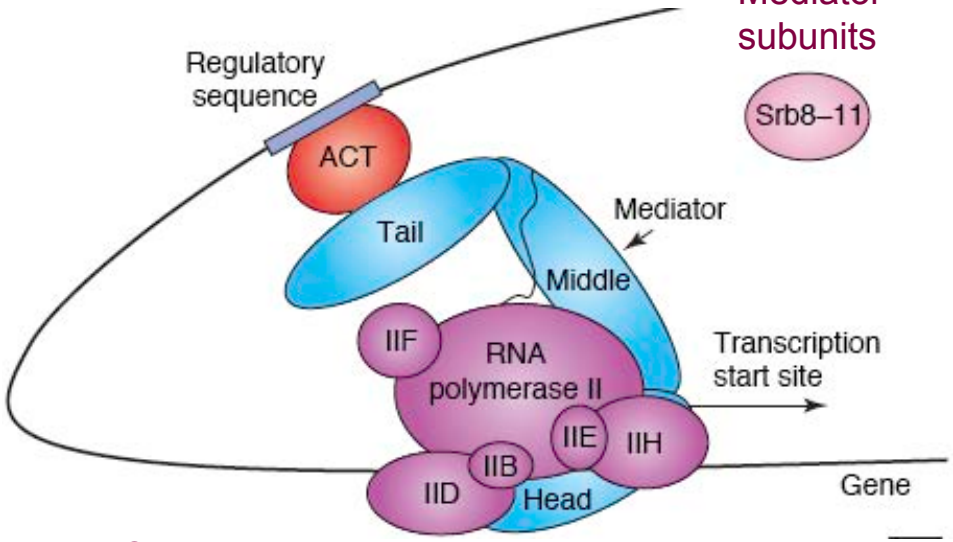
Pol II (RNAPII) in the cell



MEDIATOR central activator & integrator



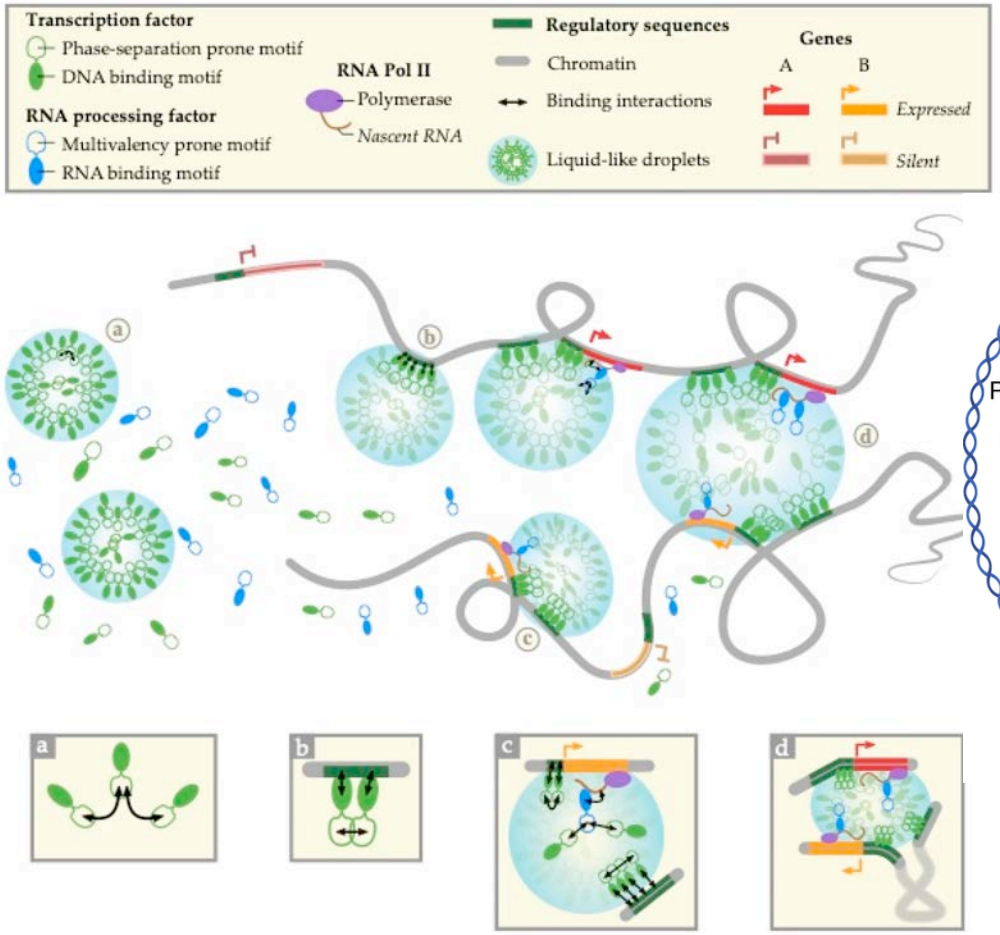
inhibitory Mediator subunits

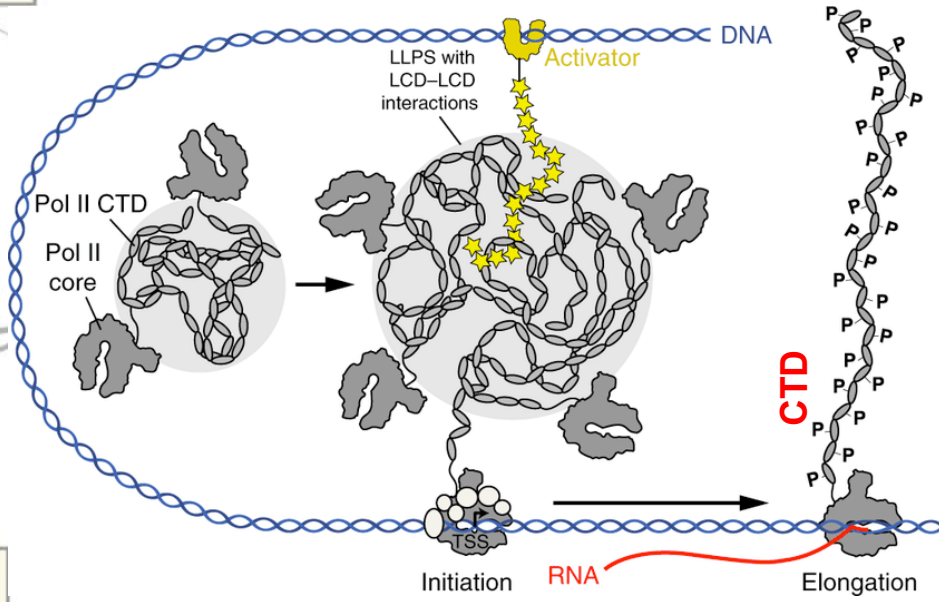


ACT - trx activator
II B D E H F – trx factors

Bjorklund and Gustafsson, 2005, TiBS; Danino et al. BBA, 2015

Pol II (RNAPII) in the cell





LLPS, droplets

Liquid-liquid phase separation

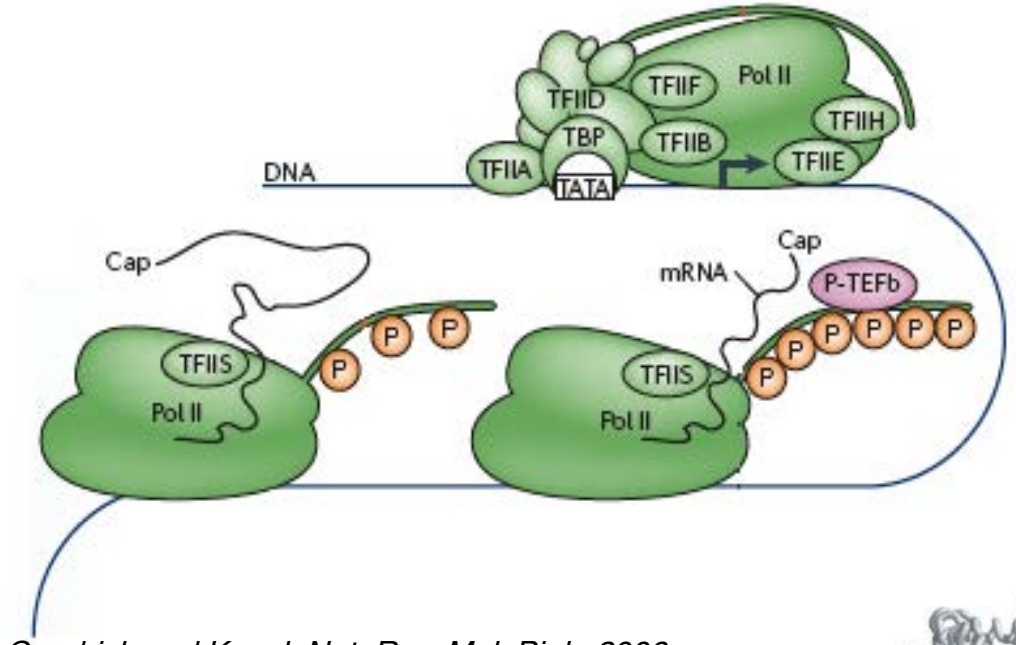
Transcriptional condensates are formed by phase-separation self-assembly driven by IDR (Intrinsically Disordered Region)-containing proteins (e.g. CTD in Pol II)

CTD-driven phase separation

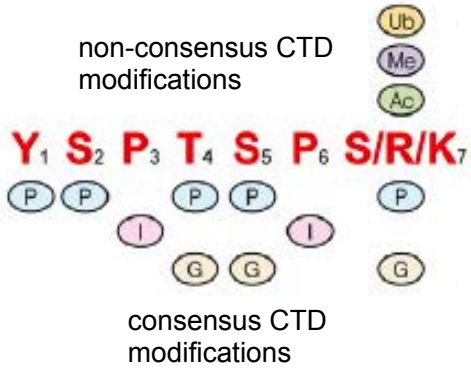
Activators recruit/nucleate Pol II hubs near promoters. Initiation-coupled CTD phosphorylation removes individual Pol II enzymes for transcription elongation.

Lesne et al.,2019 Genes
Boehning et al, 2018, Nat Struct Mol Biol

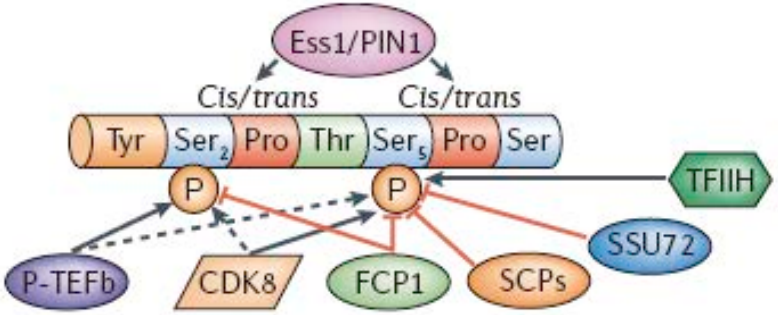
Pol II C-terminal domain (CTD)



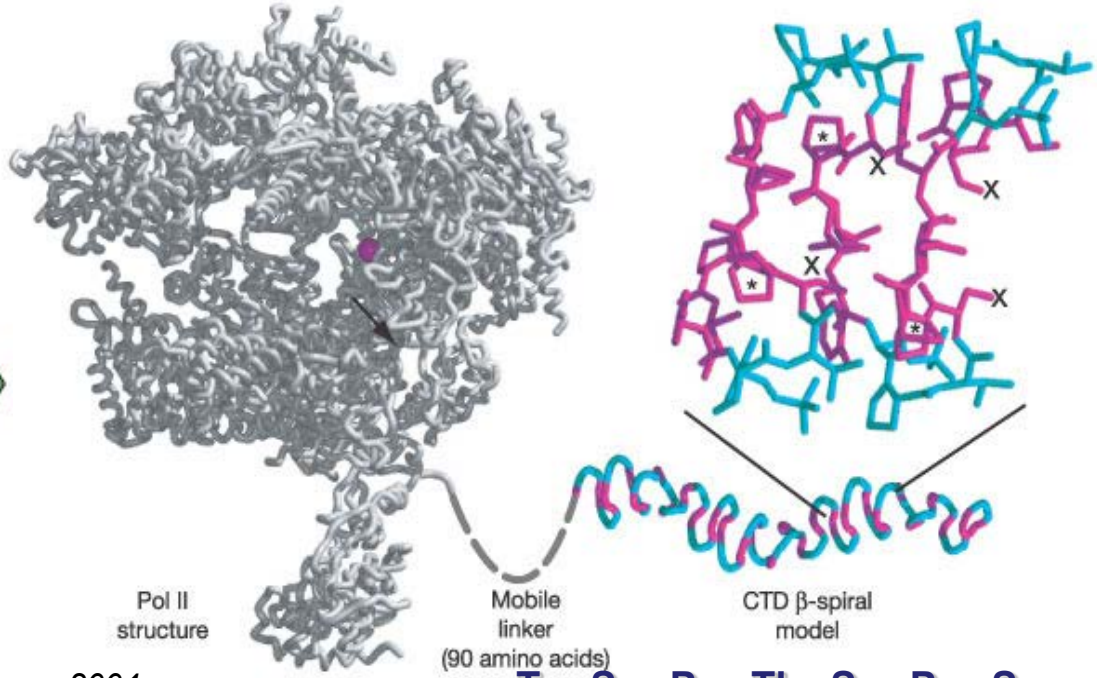
Goodrich and Kugel, Nat. Rev. Mol. Biol., 2006



26 (yeast) – 52 (human) repeats



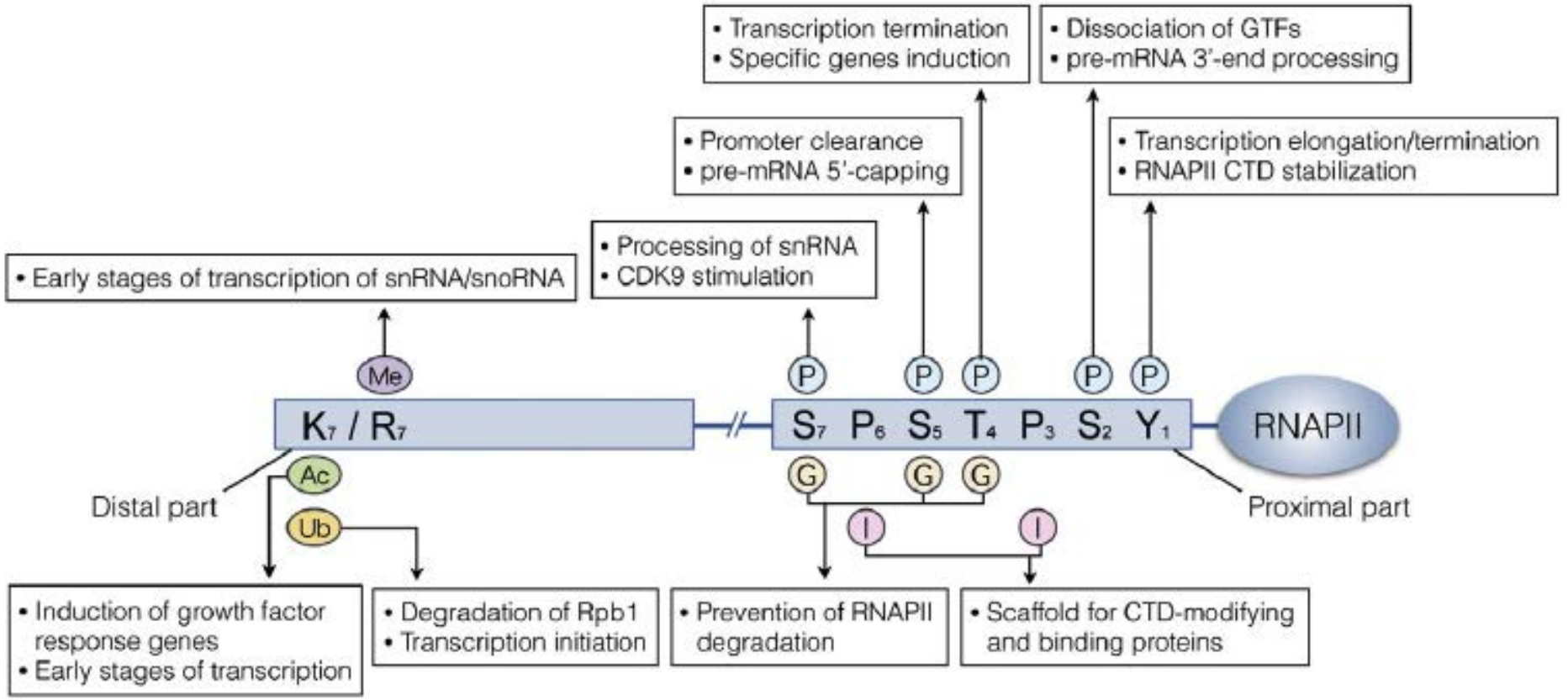
Saunders et al, 2006, Nat.Rev.Mol.Cel.Biol



Meinhart and Cramer, 2004

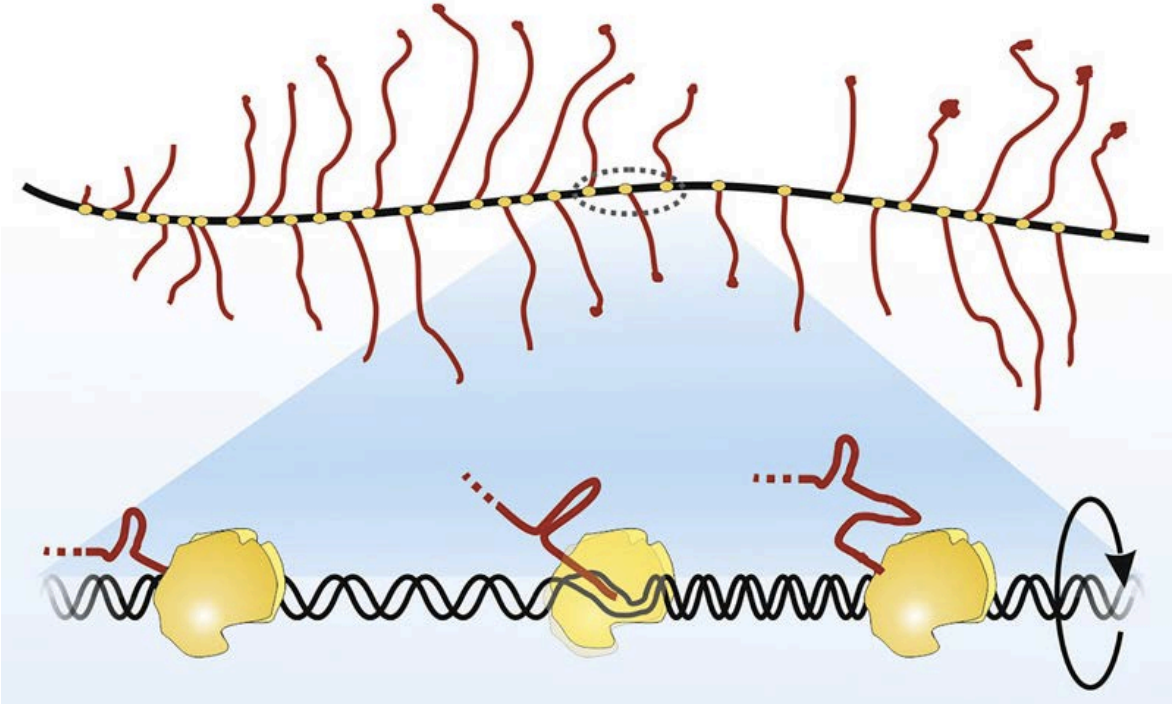
$Tyr_1Ser_2Pro_3Thr_4Ser_5Pro_6Ser_7$

CTD CODE



Srivastava and Ahn, Biotechnol Adv 2015

NASCENT TRANSCRIPTS

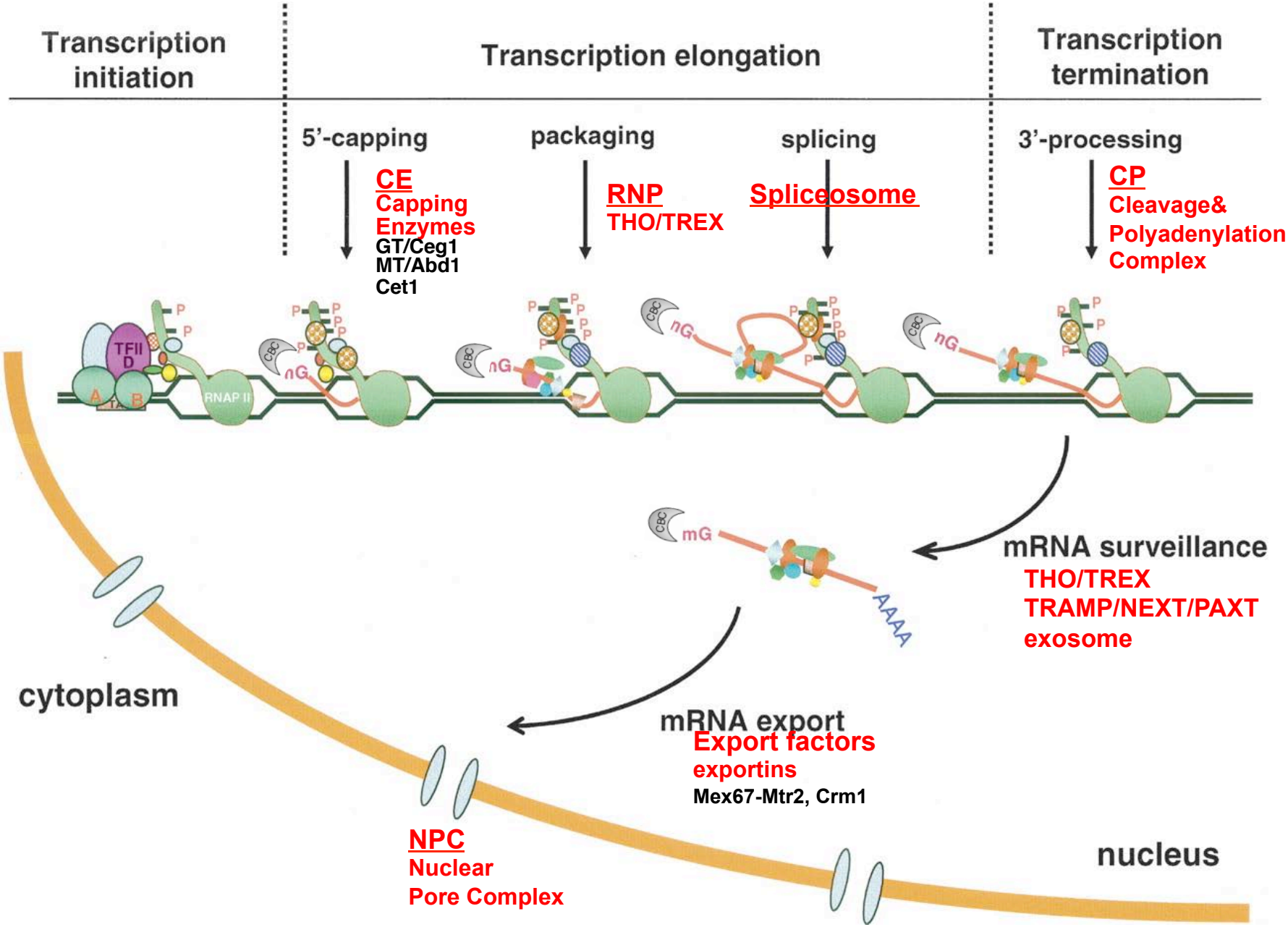


Nascent transcript = during formation, newly formed, still bound by polymerase

- nascent RNAs couple RNA processing with transcription elongation and chromatin modification
- nascent RNAs modulate binding of proteins to regulatory elements (chromatin)
- regulatory effects of nascent transcripts can be enhanced by gene looping
- high concentrations of nascent RNAs can initiate formation of nuclear bodies
- sometimes the function is conferred by nascent transcription (activity) and not the transcript itself

Turowski et al., Mol Cell, 2020

CO-TRANSCRIPTIONAL PROCESSES

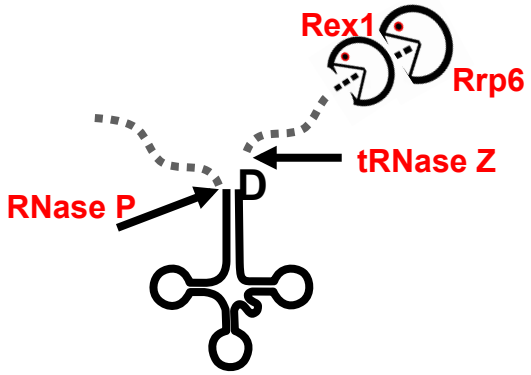


Li and Manley, Genes Dev, 2006

POST-TRANSCRIPTIONAL PROCESSES

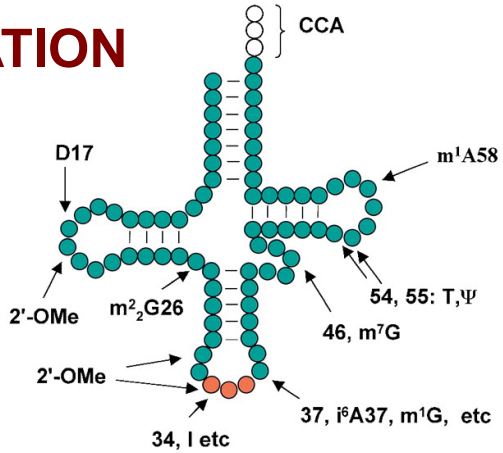
tRNA PROCESSING

- 5’ end by RNAse P
- 3’ end by tRNase Z or
- by exonuclease Rex1 and Rrp6



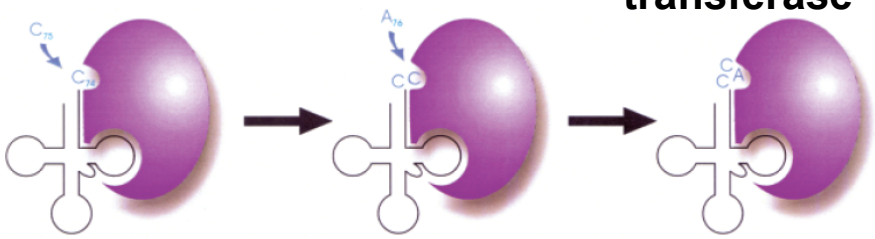
tRNA MODIFICATION

by RNA modifying enzymes



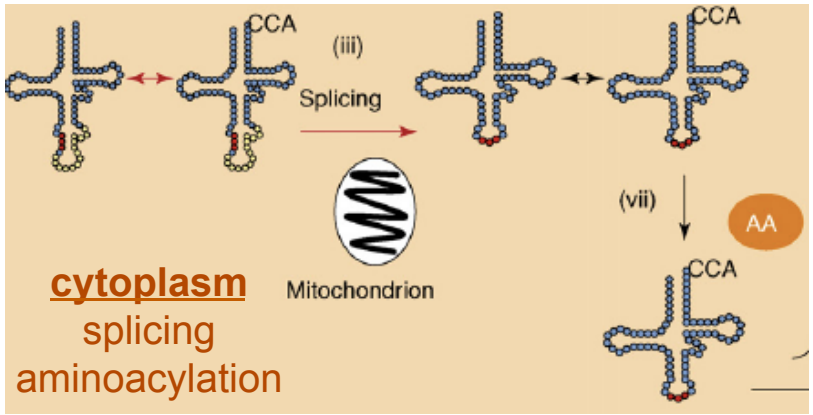
tRNA CCA ADDITION

by tRNA nucleotidyl-transferase



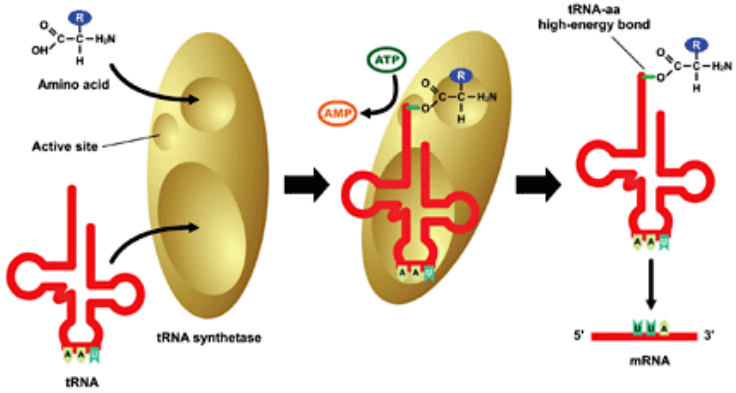
tRNA SPLICING

In the cytoplasm on the mitochondrial membrane *(YEAST!!)*



Hopper and Shaheen, TiBS,2008

tRNA AMINOACYLATION



by tRNA aminoacyl synthetases

GENE LOOPING

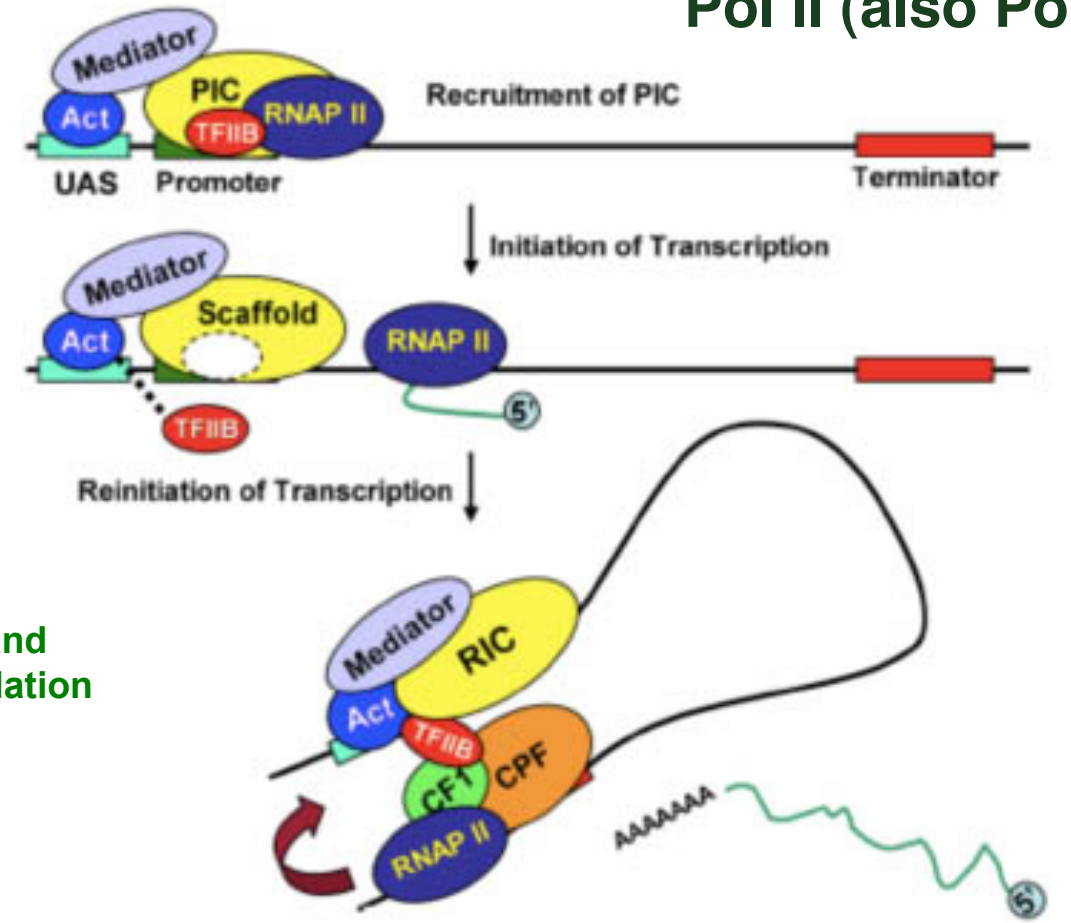
Pol II (also Pol I)

Ansari and Hampsay, GDev, 2005; El Kaderi et al., JBC, 2009

PIC Preinitiation Complex

Scaffold transcription factors (TFIID, A, E, H)

CF1, CPF Cleavage and Polyadenylation Complex

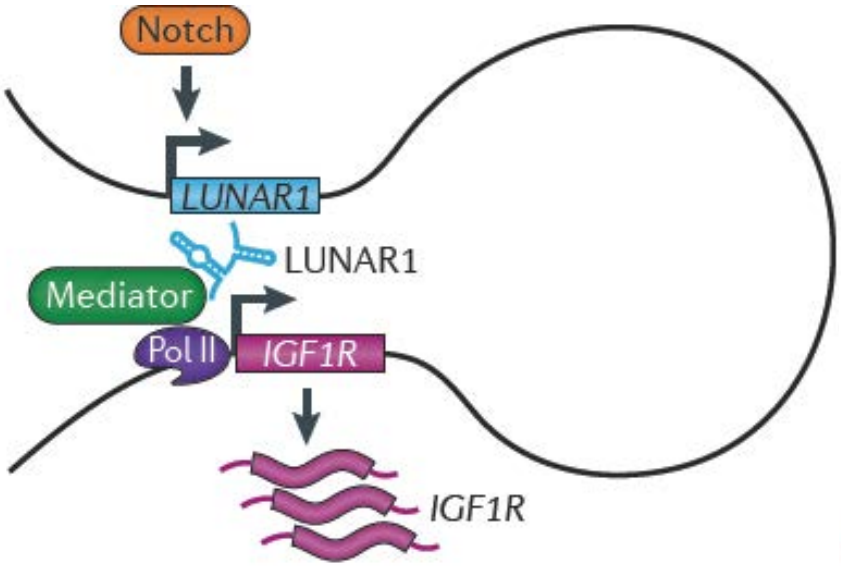


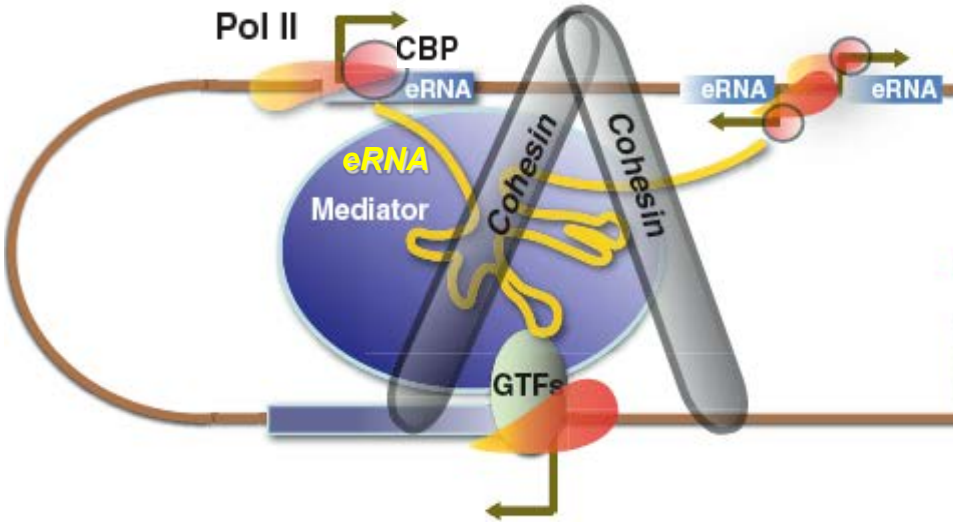
Loop formation requires interaction between factors at the promoter (THIIB) and terminator (Rna15 from CF1) /in mammals: transcription factors, nuclear receptors, insulators, chromatin remodellers, Polycomb, architectural proteins/

Loop function: facilitation of transcription reinitiation of PolII, but also repression of gene expression (PcG, DNA methylation)

GENE LOOPING

via Mediator and enhancer RNAs (eRNAs)



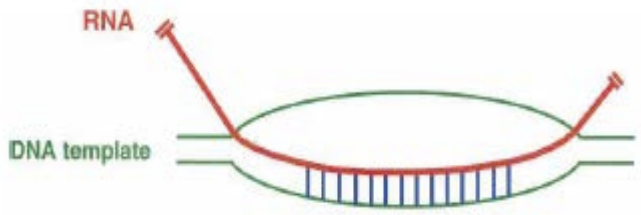


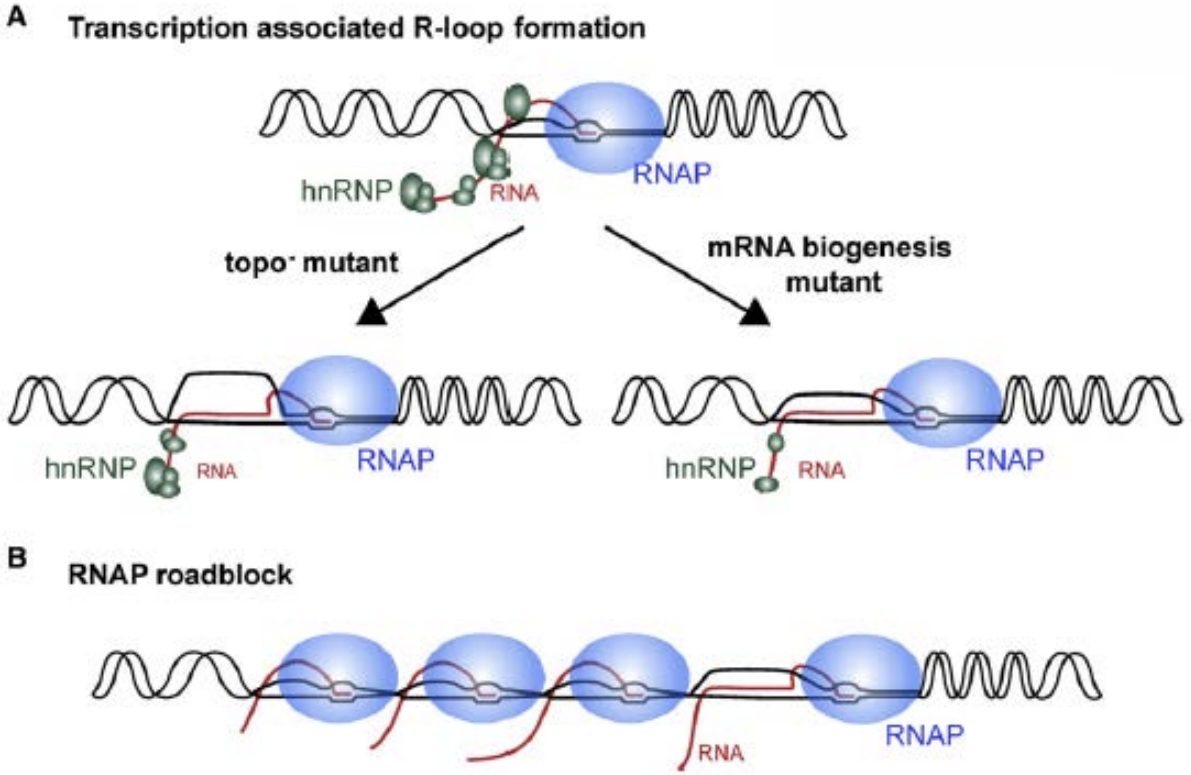
Some eRNAs (e.g. *LUNAR1* near the IGF1R locus) **mediate chromosome looping between enhancers and nearby genes via Mediator or MLL protein complexes**

Quinn and Chang, Nat Rev Genet 2015;
Lai and Shiekhattar, Curr Op Gene Dev 2014

R-LOOPs

DNA::RNA hybrids formed during transcription before RNP packaging





R-loops

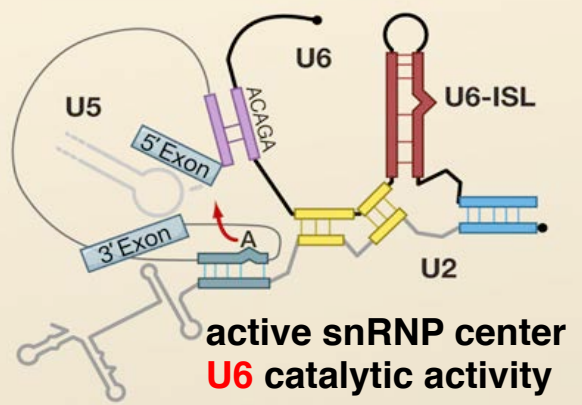
- accumulate in RNP biogenesis mutants (*tho*, *sen1*, mRNA export)

- negative effects: polymerase stalling, termination defects, replication fork stalling, DNA damage, genetic instability

- prevented by topoisomerases, helicase Sen1, THO complex, resolution (cleavage) by RNase H

Aguilera and García-Muse, Mol Cell, 2012

SPLICEOSOME

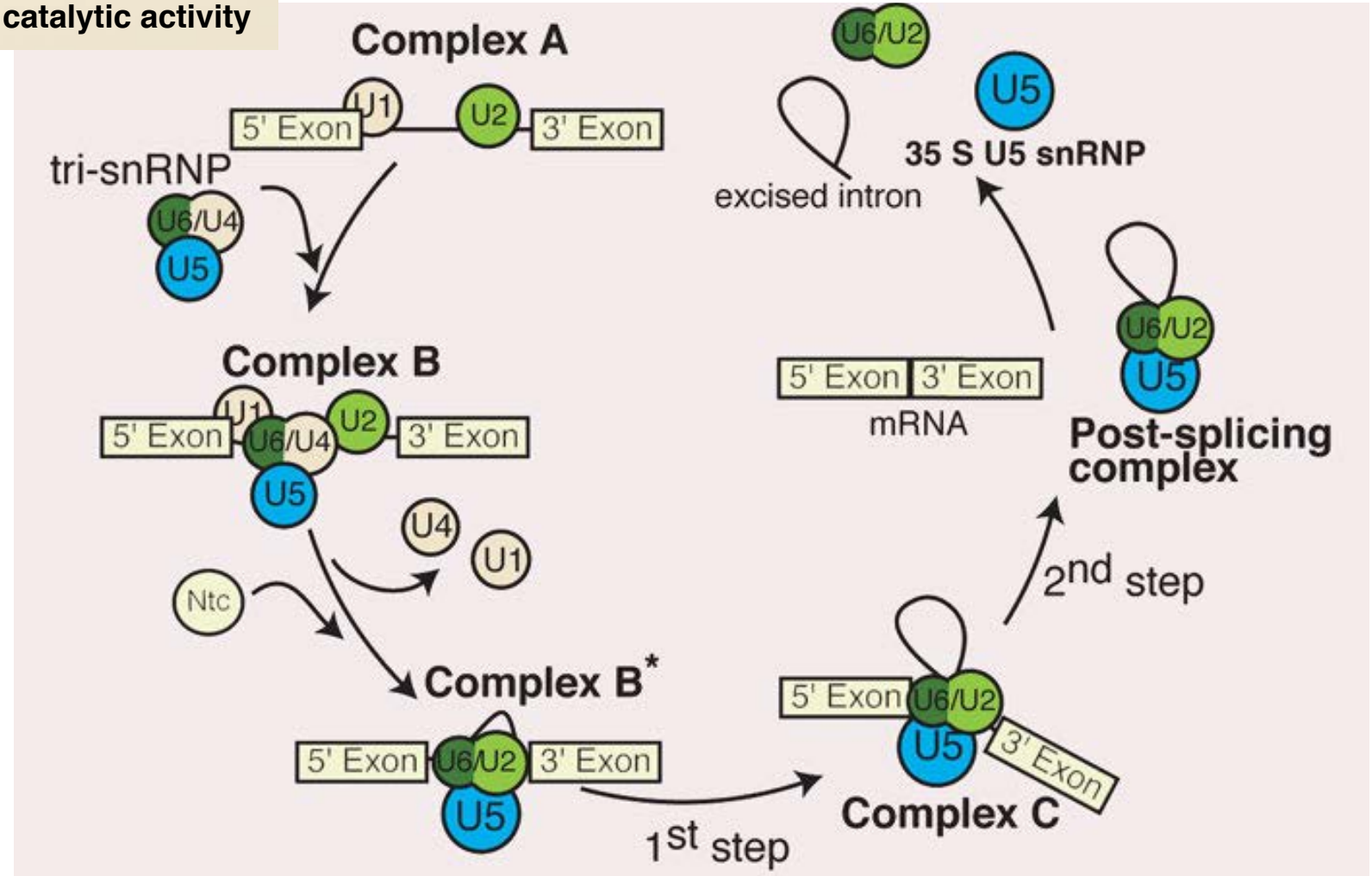


5 snRNAs: U1, U2, U4, U5, U6

Core Sm or LSM (U6) proteins **1.7 – 3 MDa**

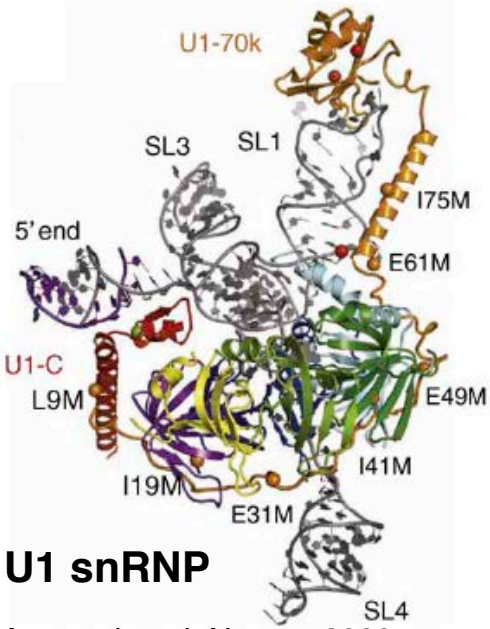
Specific snRNP proteins

Splicing factors



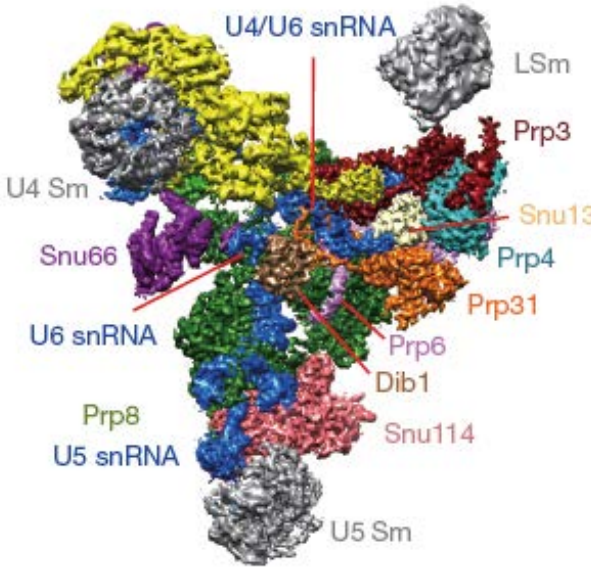
Warf and Berglund, 2010, TiBS; Reddy, Ann.Rev.PlantBiol., 2007

SPLICEOSOME
Cryo- EM



U1 snRNP

Krummel et al, Nature, 2009

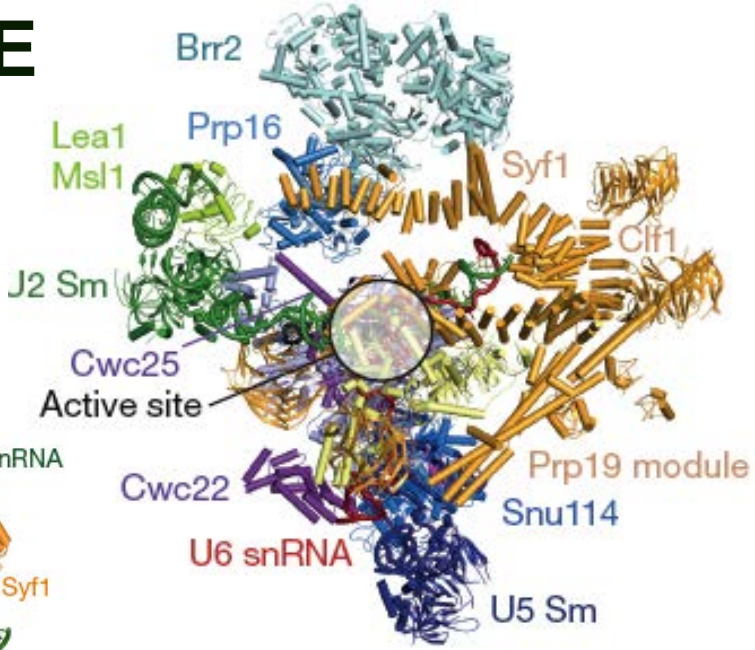


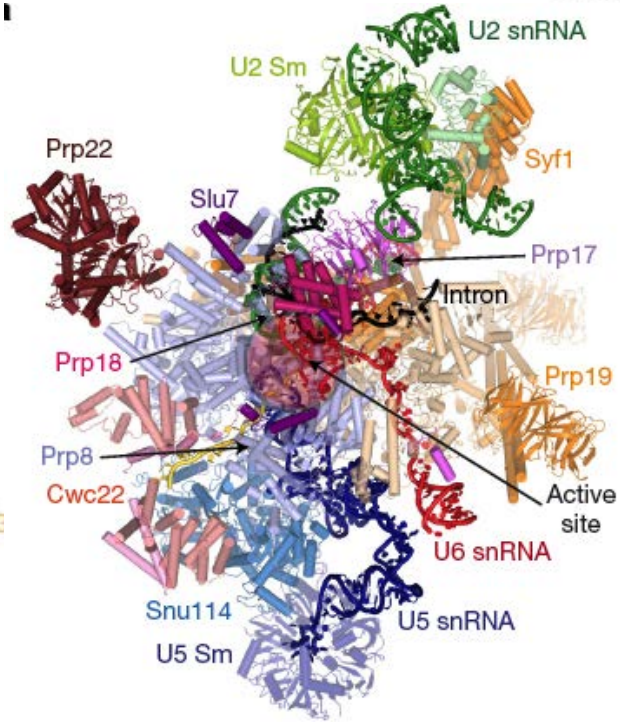
U4/U6.U5 tri-snRNP

Nguyen1, Galej et al, Nature, 2016*

C complex yeast

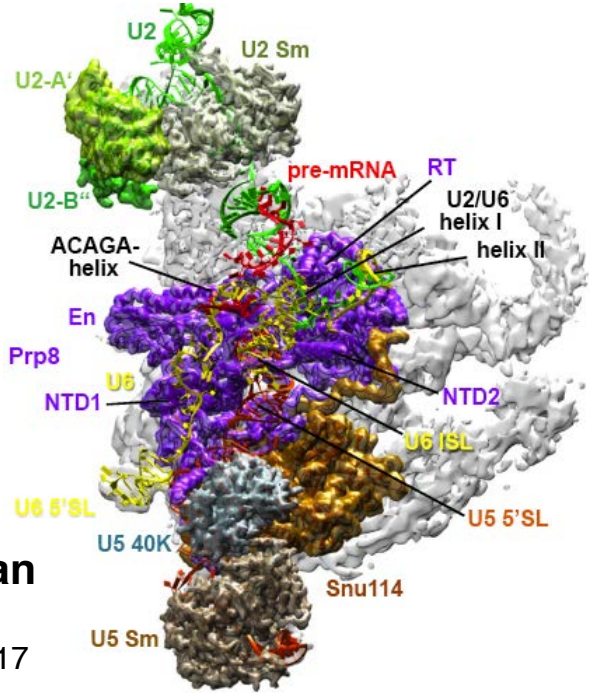
Galej et al, Nature, 2016





C* complex yeast

Fica et al, Nature, 2017

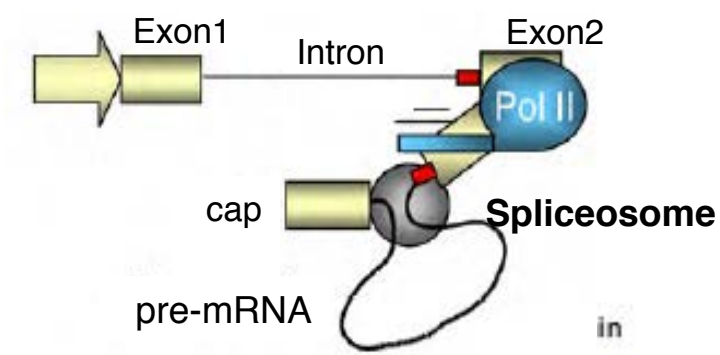


C* complex human

second step

Bertramet al, Nature, 2017

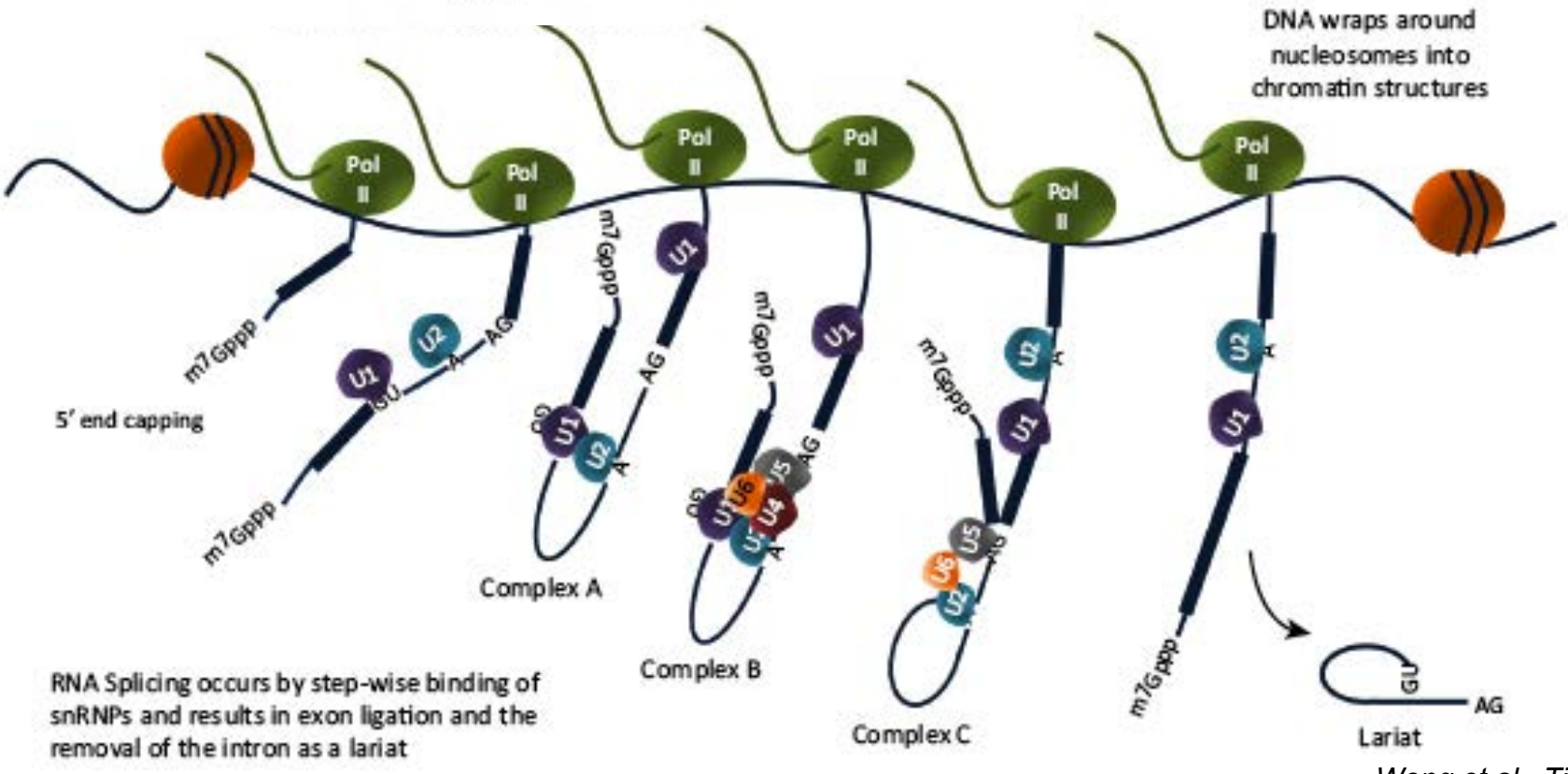
SPLICING: co-transcriptional process



- spliceosome assembly (Ser5-P)
- majority of splicing (up to 70–80%)

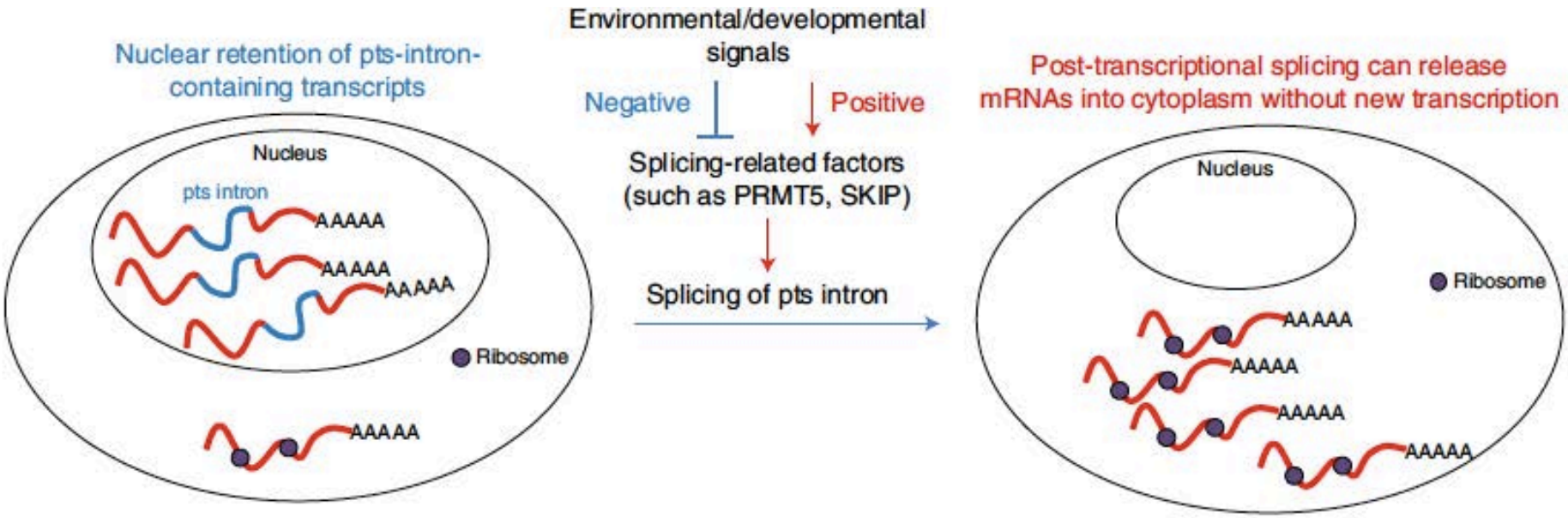
Munoz et al., TiBS, 2009

in



Wong et al., TiG, 2014

Co-trx vs post-trx splicing

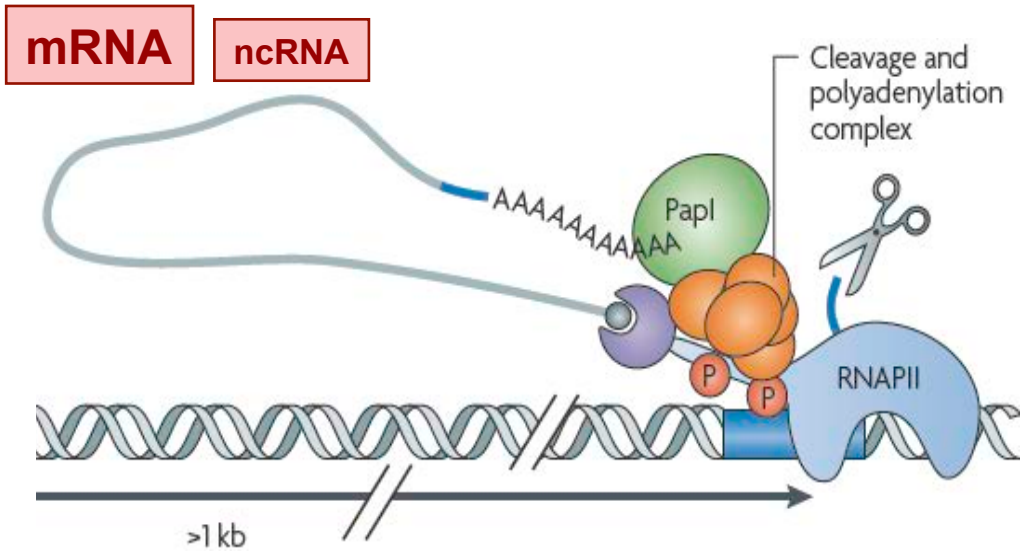


Nanopore-based profiling of chromatin-bound RNA

- **Incompletely spliced and polyadenylated transcripts are detected on chromatin**
- **They 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**

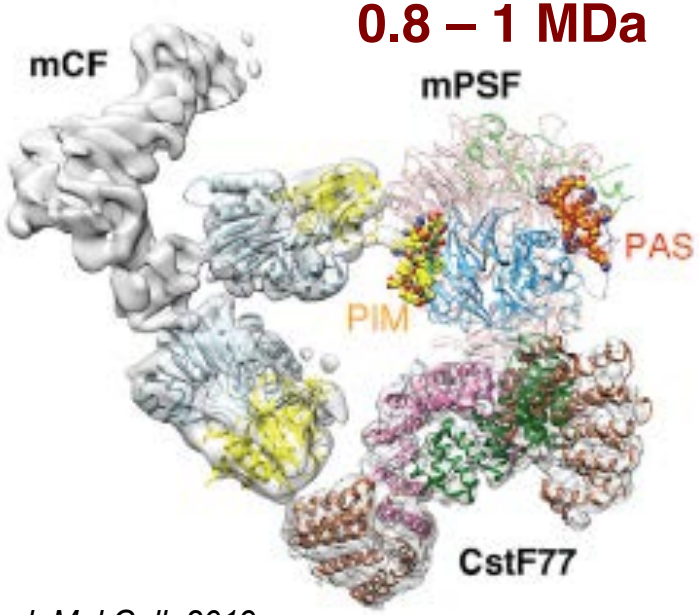
Jiaet al., Nat Plants, 2020

CPA Cleavage and Polyadenylation

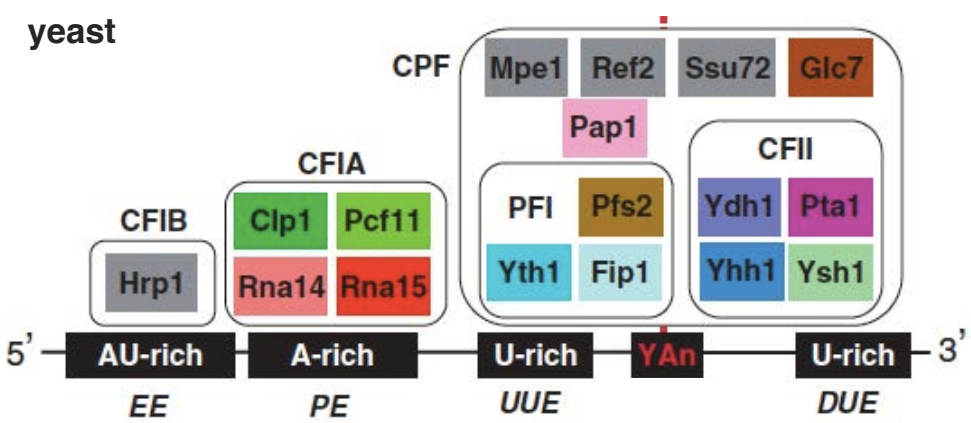


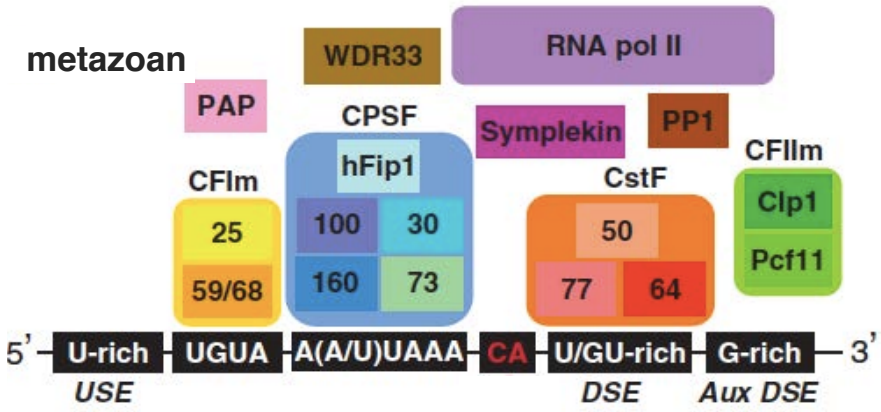
Jacquier, Nat. Rev. Genet, 2009

0.8 – 1 MDa



Zhang et al, Mol Cell, 2019

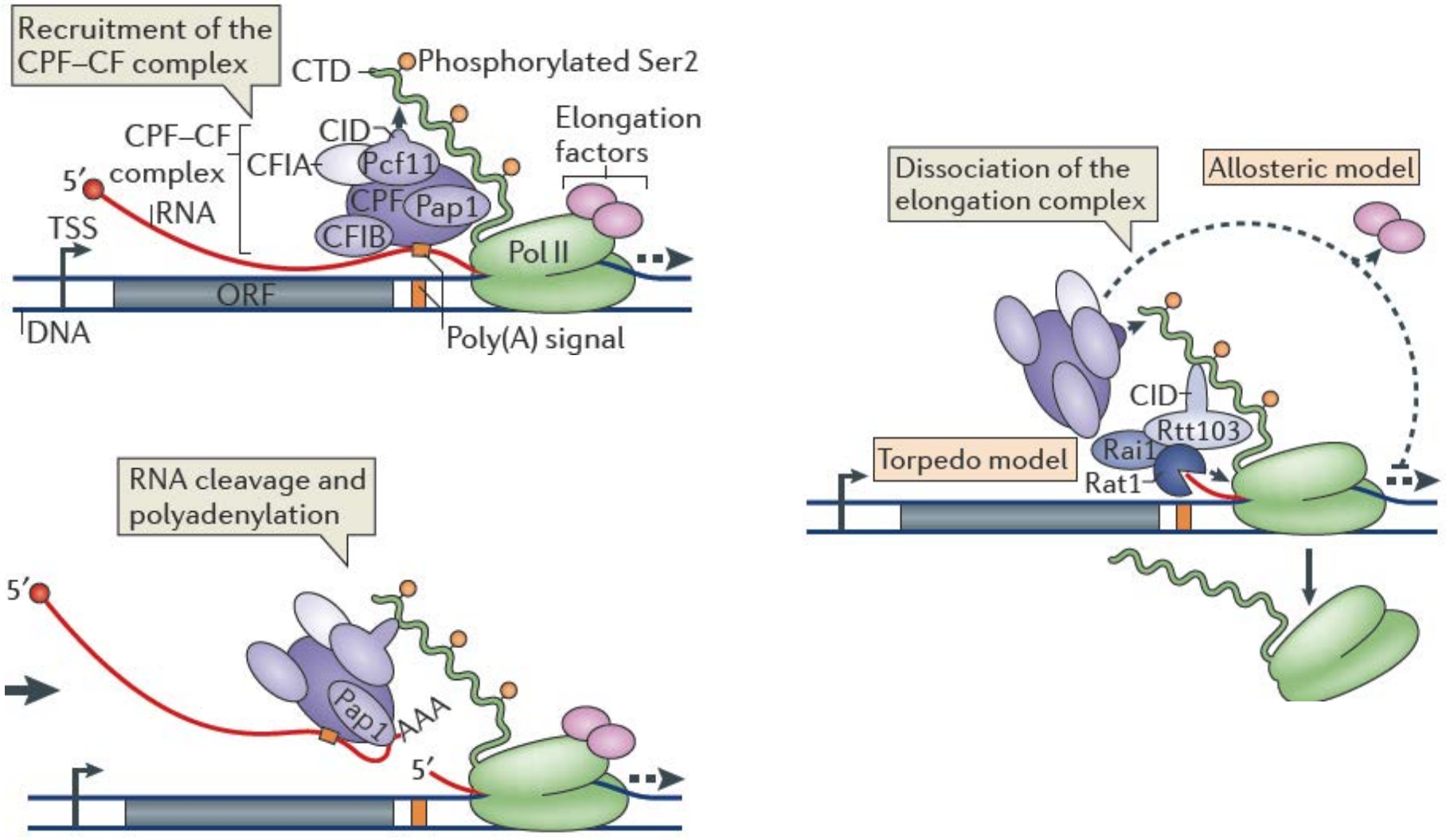




Cleavage by CPSF-73 (human), Brr5/Ysh1 (yeast)

Millevoi and Vagner, NAR, 2008

CPA: mRNA 3' end formation transcription termination at mRNA genes



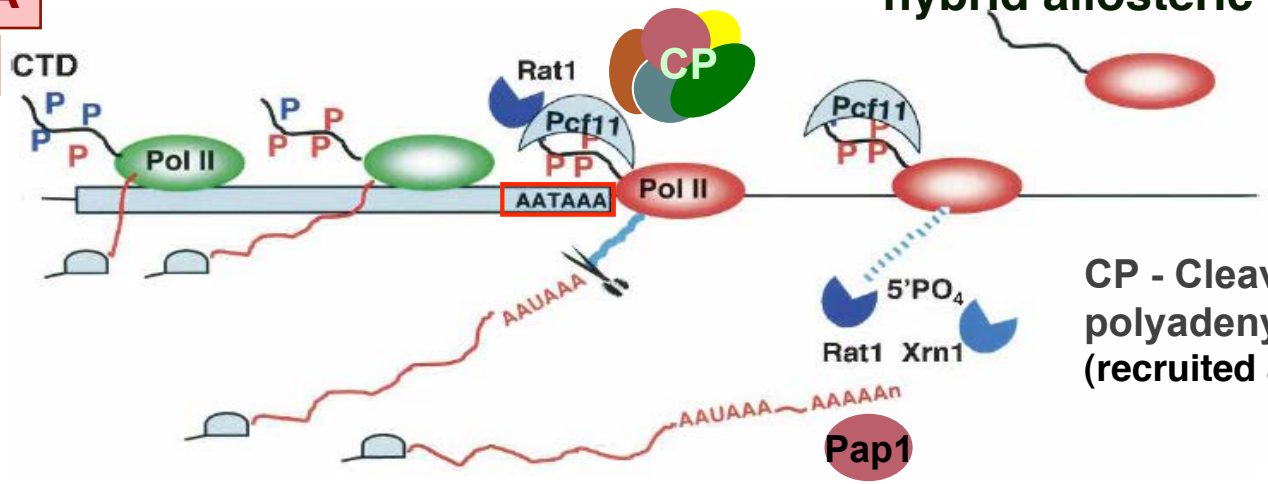
Poruua, Libri, Nat Rev Mol Cell Biol, 2015

POL II TRANSCRIPTION TERMINATION

mRNA

ncRNA

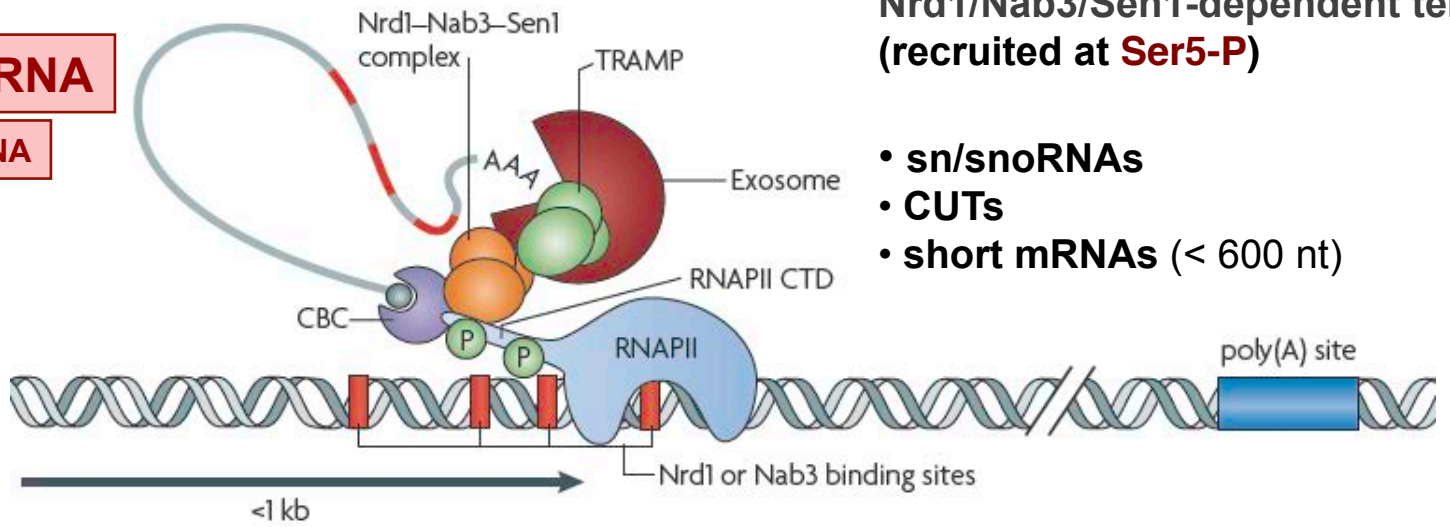
hybrid allosteric- torpedo model



CP - Cleavage and polyadenylation complex (recruited at Ser2-P CTD)

ncRNA

mRNA



Nrd1/Nab3/Sen1-dependent termination (recruited at Ser5-P)

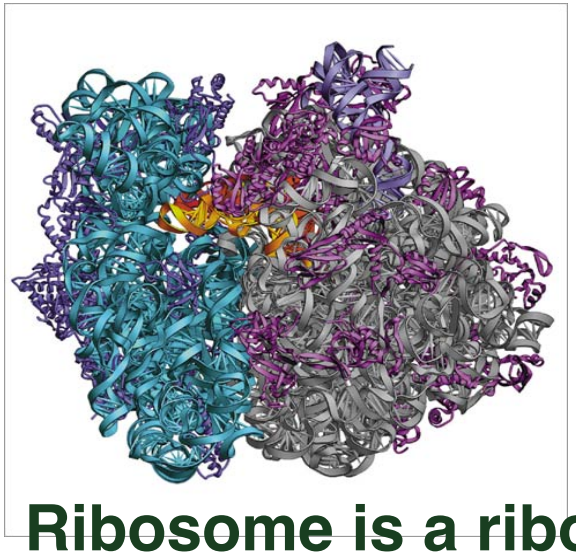
- **sn/snoRNAs**
- **CUTs**
- **short mRNAs** (< 600 nt)

Jacquier, Nat. Rev. Genet 2009

Luo and Bentley, Gene Dev, 2006

[Lecture on transcription termination by Michał Koper]

RIBOSOME



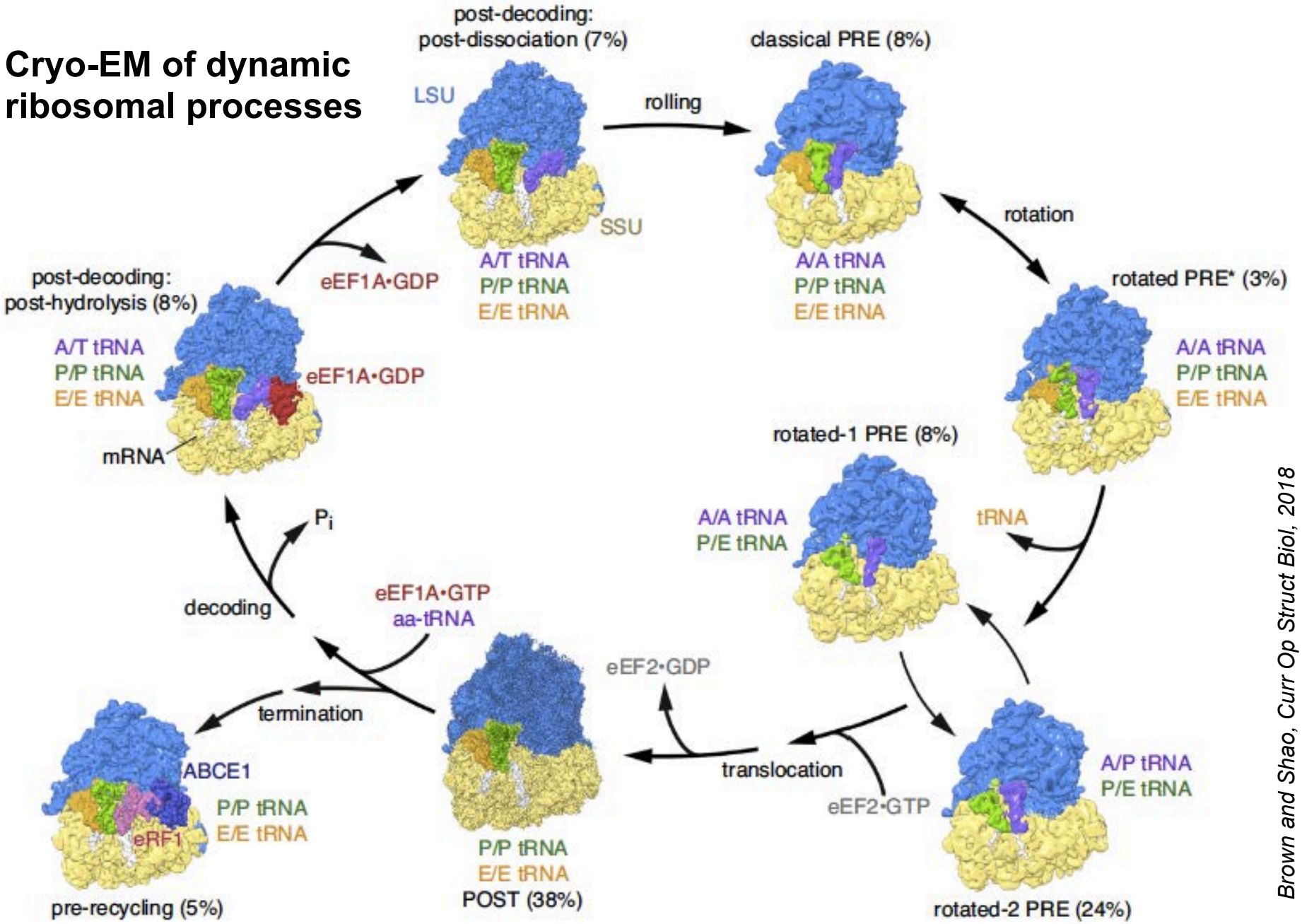
3.3 MDa (yeast) – 4.3 MDa (humans)

Ribosome is a ribozyme

- No ribosomal protein with a peptidyl transferase (PT) activity
- Drugs (chloramphenicol) that inhibit PT bind to the 25S rRNA (PT loop)
- Mutations that provide resistance to these drugs map to the PT loop
- Nearly all (99%) of proteins can be stripped from the large subunit and it still retains the PT activity
- Only RNA chains are close enough to the PT center (structure)
- Ribosomal proteins are important for ribosome stability and integrity, but NOT for catalysis

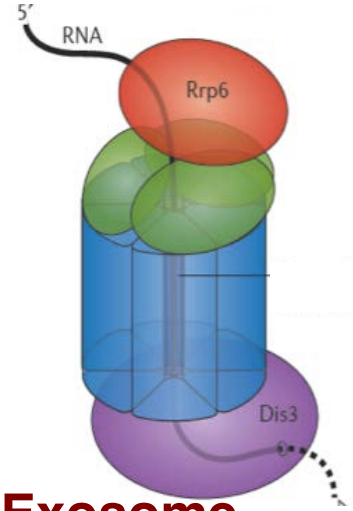
TRANSLATION CYCLE

Cryo-EM of dynamic ribosomal processes

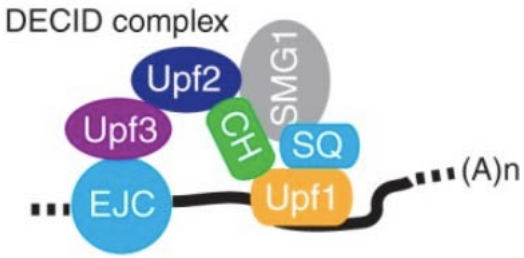


Brown and Shao, Curr Op Struct Biol, 2018

RNA ENZYMES AND COMPLEXES

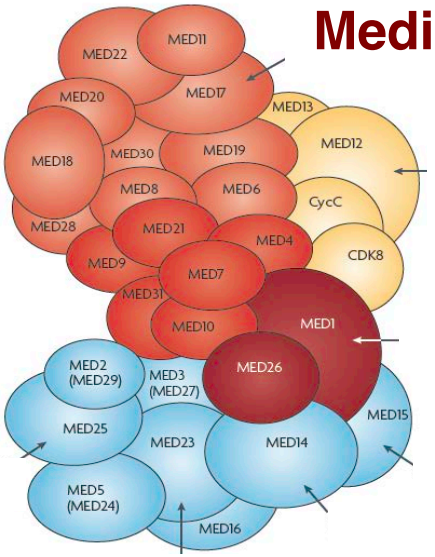


Exosome

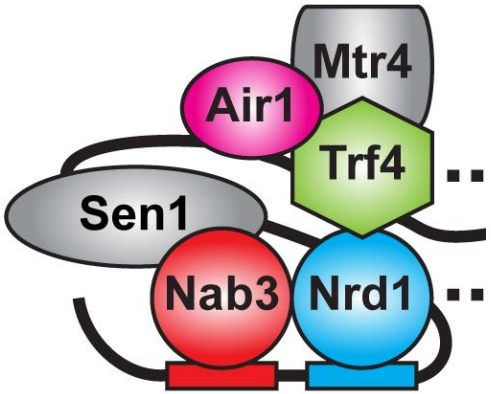


NMD

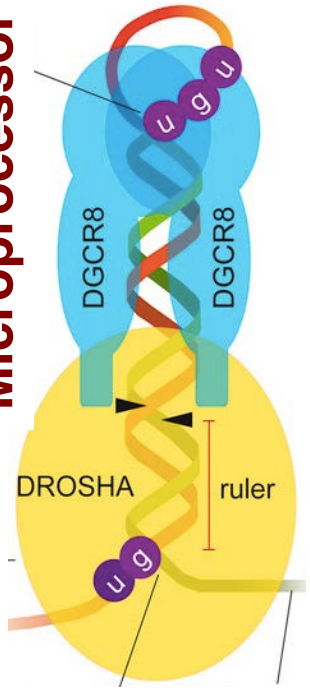
Mediator



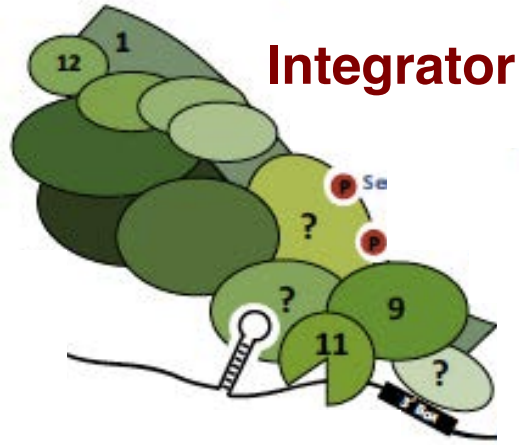
TRAMP/NNS



Microprocessor



Integrator



RNA PROCESSING and DECAY machinery: RNases

Protein	Function	Characteristics
Exonucleases 5'→3'		
Xrn1	cytoplasmic, mRNA degradation	processsive
Rat1	nuclear, pre-rRNA, sn/snoRNA, pre-mRNA processing and degradation	
Rrp17/hNol12	nuclear, pre-rRNA processing	
Exosome 3'→5'	multisubunit exo/endo complex	subunits organized as in bacterial PNPase
Rrp44/Dis3	catalytic subunit	Exo/PIN domains, processsive
Rrp4, Rrp40	pre-rRNA, sn/snoRNA processing, mRNA degradation	
Rrp41–43, 45–46	participates in NMD, ARE-dependent, non-stop decay	
Mtr3, Ski4		
Mtr4	nuclear helicase cofactor	DEAD box
Rrp6 (Rrp47)	nuclear exonuclease (Rrp6 BP, cofactor)	RNAse D homolog, processsive
Ski2,3,7,8	cytoplasmic exosome cofactors. SKI complex	helicase, GTPase
Other 3'→5'		
Rex1–4	3'-5' exonucleases, rRNA, snoRNA, tRNA processing	RNase D homolog
DXO	3'-5' exonuclease in addition to decapping	
mtEXO 3'→5'	mitochondrial degradosome	RNA degradation in yeast
Suv3/ Dss1	helicase/ 3'-5' exonuclease	DExH box/ RNase II homolog
Deadenylation		
Ccr4/NOT/Pop2	major deadenylase complex (Ccr, Caf, Pop, Not proteins)	Ccr4- Mg^{2+} dependent endonuclease
Pan2p/Pan3	additional deadenylases (poliA tail length)	RNase D homolog, poly(A) specific nuclease
PARN	mammalian deadenylase	RNase D homolog, poly(A) specific nuclease
Endonucleases		
RNase III		
-Rnt1	pre-rRNA, sn/snoRNA processing, mRNA degradation	dsRNA specific
-Dicer, Drosha	siRNA/miRNA biogenesis, functions in RNAi	PAZ, RNA BD, RNase III domains
Ago2 Slicer	mRNA cleavage in RNAi	
SMG6	mRNA cleavage in NMD	PIN domain
RNase P	5' tRNA end processing	RNP complex
RNase MRP	pre-rRNA processing	RNP complex, similar to RNase P
RNase L	rRNA degradation in apoptosis	oligo 2–5A dependent $(ppp(A2'p)_nA)$
ELAC2/Trz1	3' tRNA endonuclease	PDE motif and Zn^{2+}-binding motif
Utp24 Nob1 Las1	pre-rRNA processing at sites A0, D and C2	

Eukaryotic auxiliary factors

Protein	Function / Characteristics
5'→3' decay: decapping	
Dcp1/Dcp2	Dcp2- pyrophosphatase catalytic activity, Nudix domain, Dcp1- protein binding
Hedls/Ge-1/Edc4	decapping cofactor, WD40 domain
Edc1,2,3	decapping enhancers, stimulate cap binding/catalysis, Edc1–2 (yeast), Edc3 (all eykaryotes)
Dhh1	DexD/H ATPase, decapping activator by translation repression
Lsm1–7	decapping activator, heptameric complex, binds mRNA 3' end-U rich tracts
Pat1	decapping activator by translation repressio
DXO	pyrophoshohydrolase, 5' decapping endonuclease, deNADding, 5'OH hydrolase
TRAMP complex: nuclear RNA surveillance, polyadenylation-dependent degradation	
Trf4/Trf5	nuclear alternative poly(A) polymerases
Mtr4	DEAD box helicase
Air1/Air2	RNA binding proteins, also nuclear exosome cofactor
Nrd1-Nab3-Sen1 complex: PolII termination of small RNAs, TRAMP-depdendent degradation	
Nrd1	Pol II C-terminal domain (CTD) binding, RNA binding
Nab3	RNA binding
Sen1	RNA helicase

Next lecture

RNA enzymes and complexes

RNA granules and subcellular structures

RNA decay