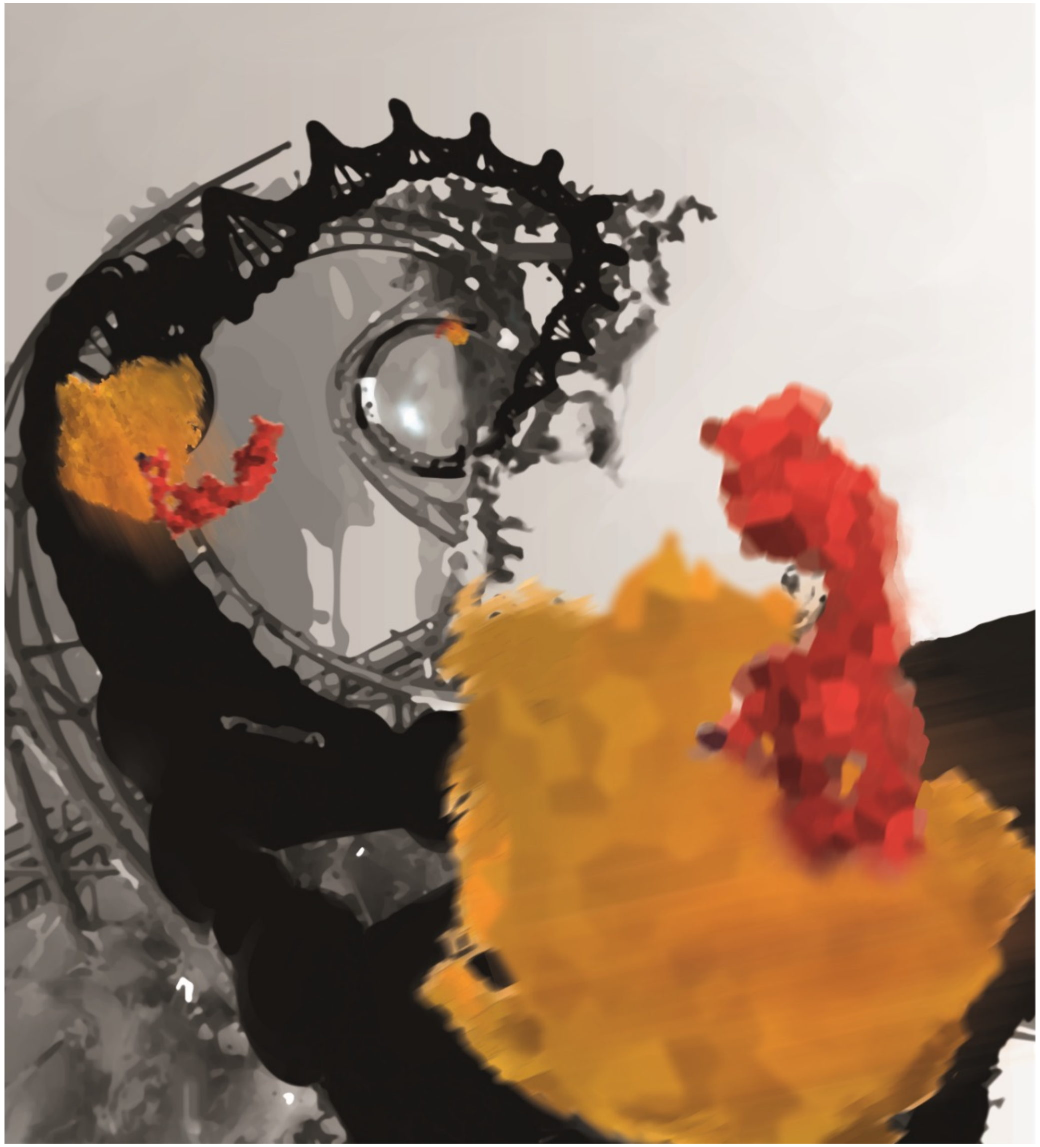


INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
POLISH ACADEMY OF SCIENCES

Biosynthesis of rRNA and tRNA

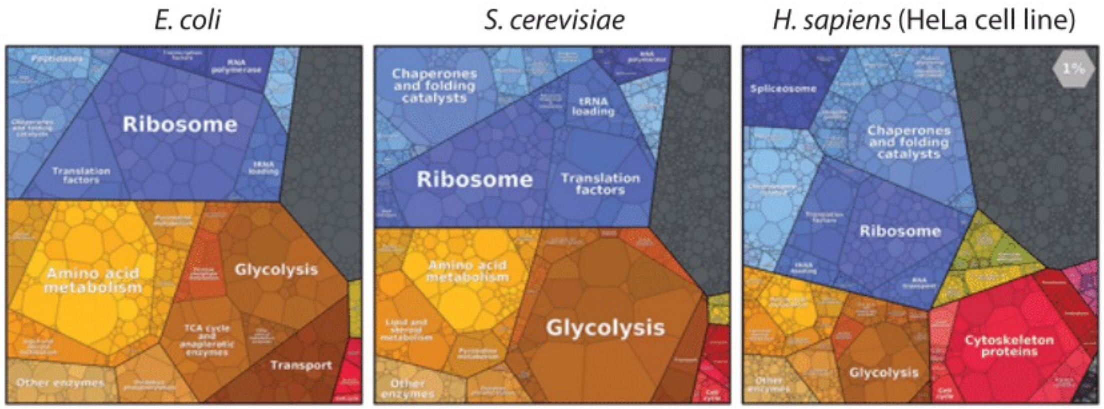
Tomasz W. Turowski

UW

22.04.2024

RNA/protein composition:

Proteins:



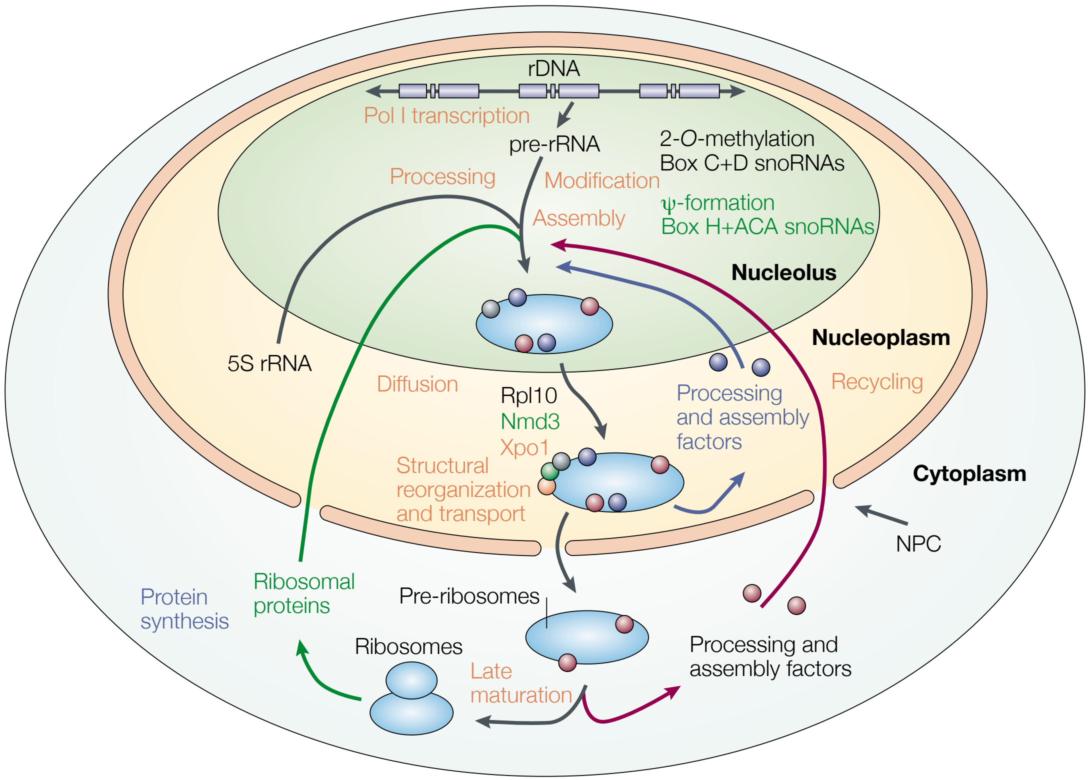
RNA:

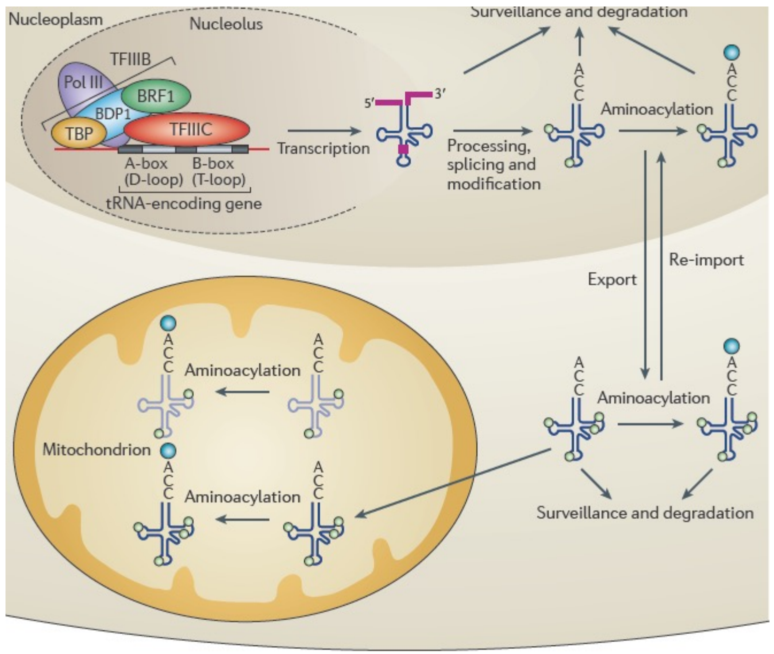
- rRNA 80%
- tRNA 15%
- mRNA 2-5%

RNA synthesis: The road from the nucleus to the cytoplasm

rRNA **tRNA**

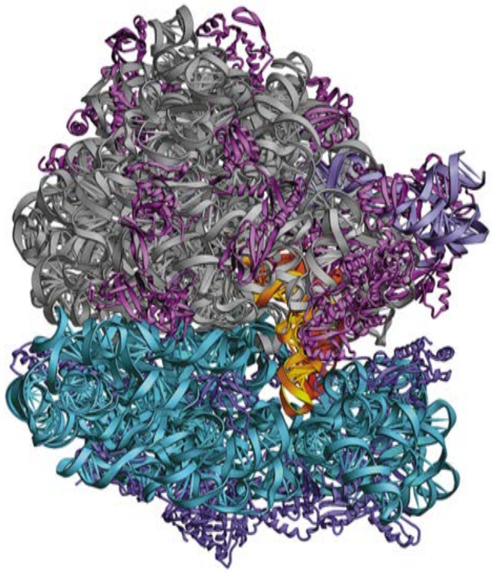
The pathway is best characterized in the yeast *Saccharomyces cerevisiae*





Kirchner and Ignatova, NatRevGenet, 2015

Ribosome biosynthesis



Ribosome synthesis rates

	Ribosomes	Division time	Ribosome/min
HeLa	3,300,000	24h = 1,440 min	2,300
S.cerevisiae	200,000	100 min	2,000

- In yeast ~70% of Pol II transcription is devoted to making ribosomal protein mRNAs and 80% of all RNA in the cell is ribosomal RNA

The Ribosome

The ribosome is composed of ribosomal RNA (rRNA) and ribosomal proteins.

~ 200 non-ribosomal proteins
~ 100 snoRNAs
~ 80 ribosomal proteins

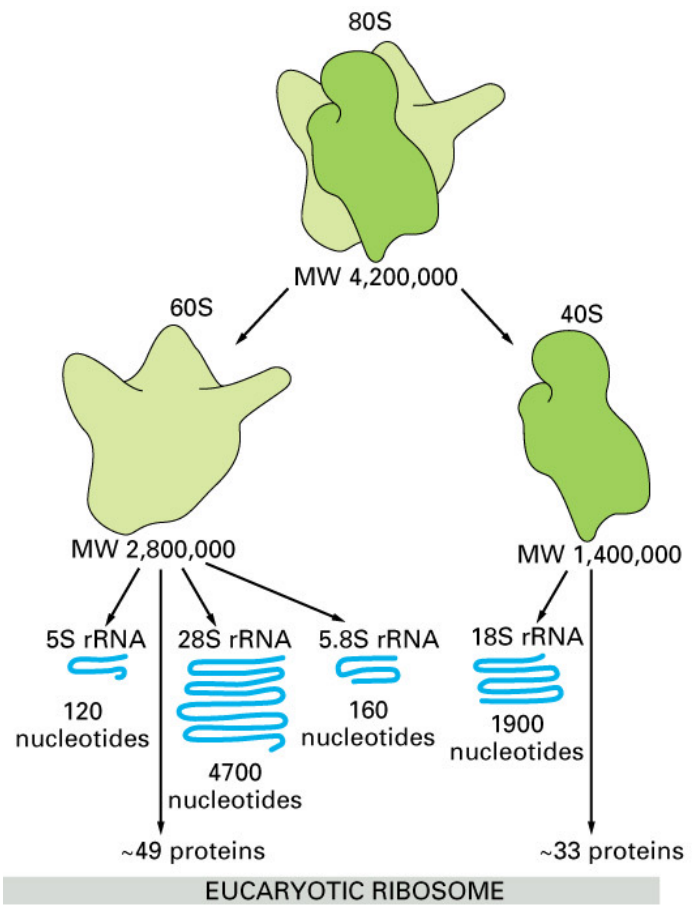
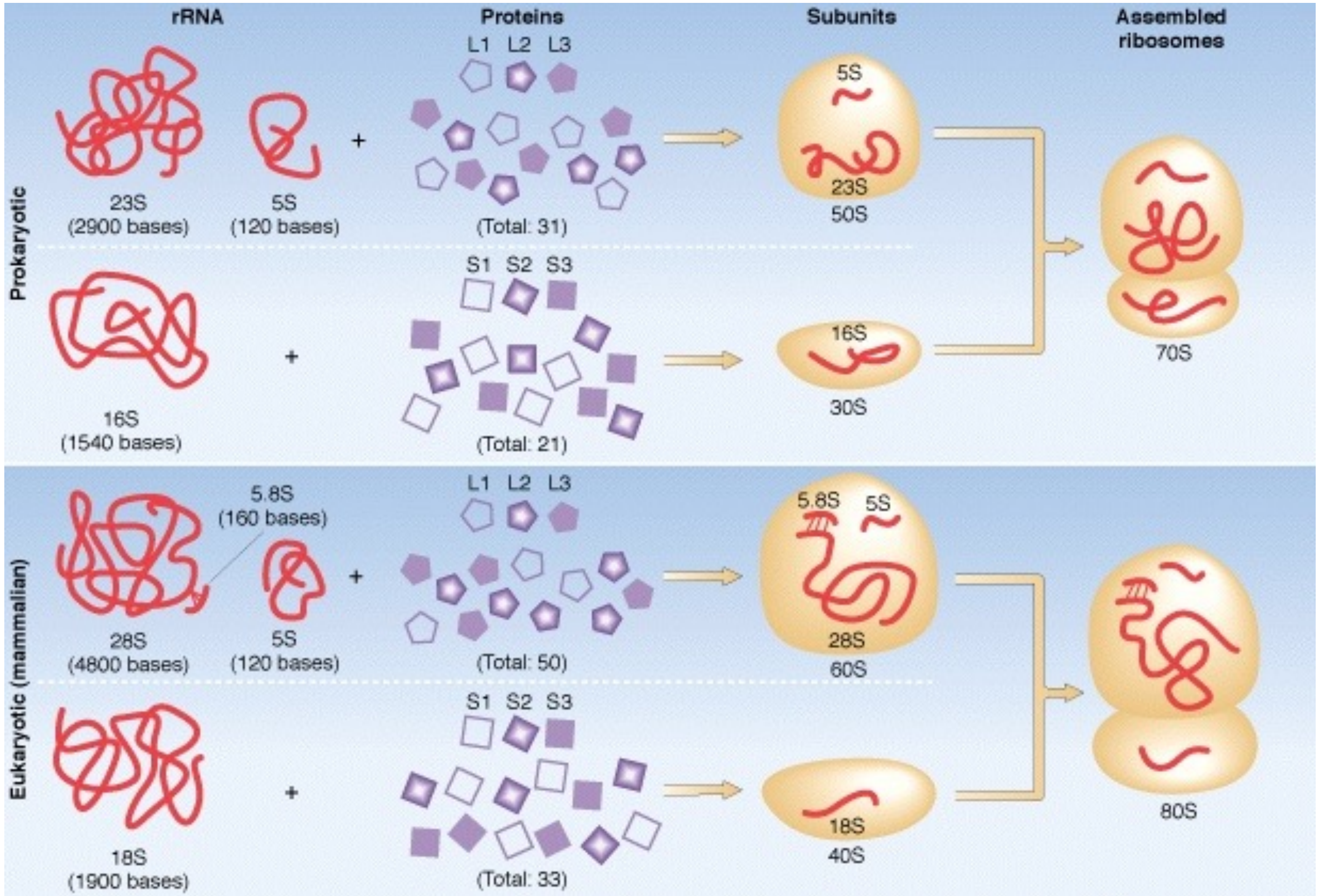


Figure 6–63 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

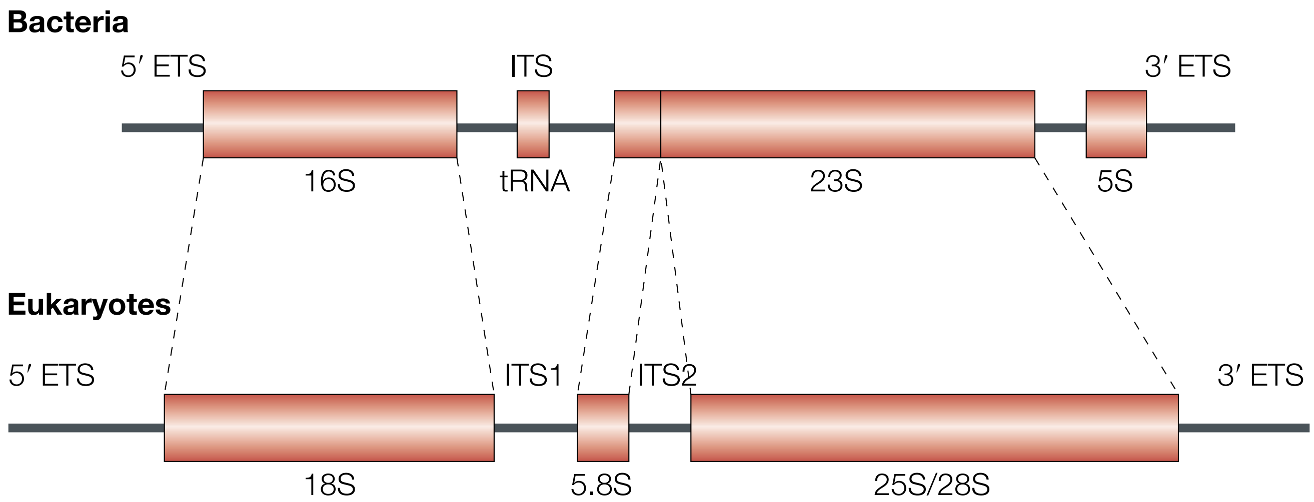
Prokaryotic vs Eukaryotic Ribosome

Prokaryotic vs Eukaryotic Ribosome

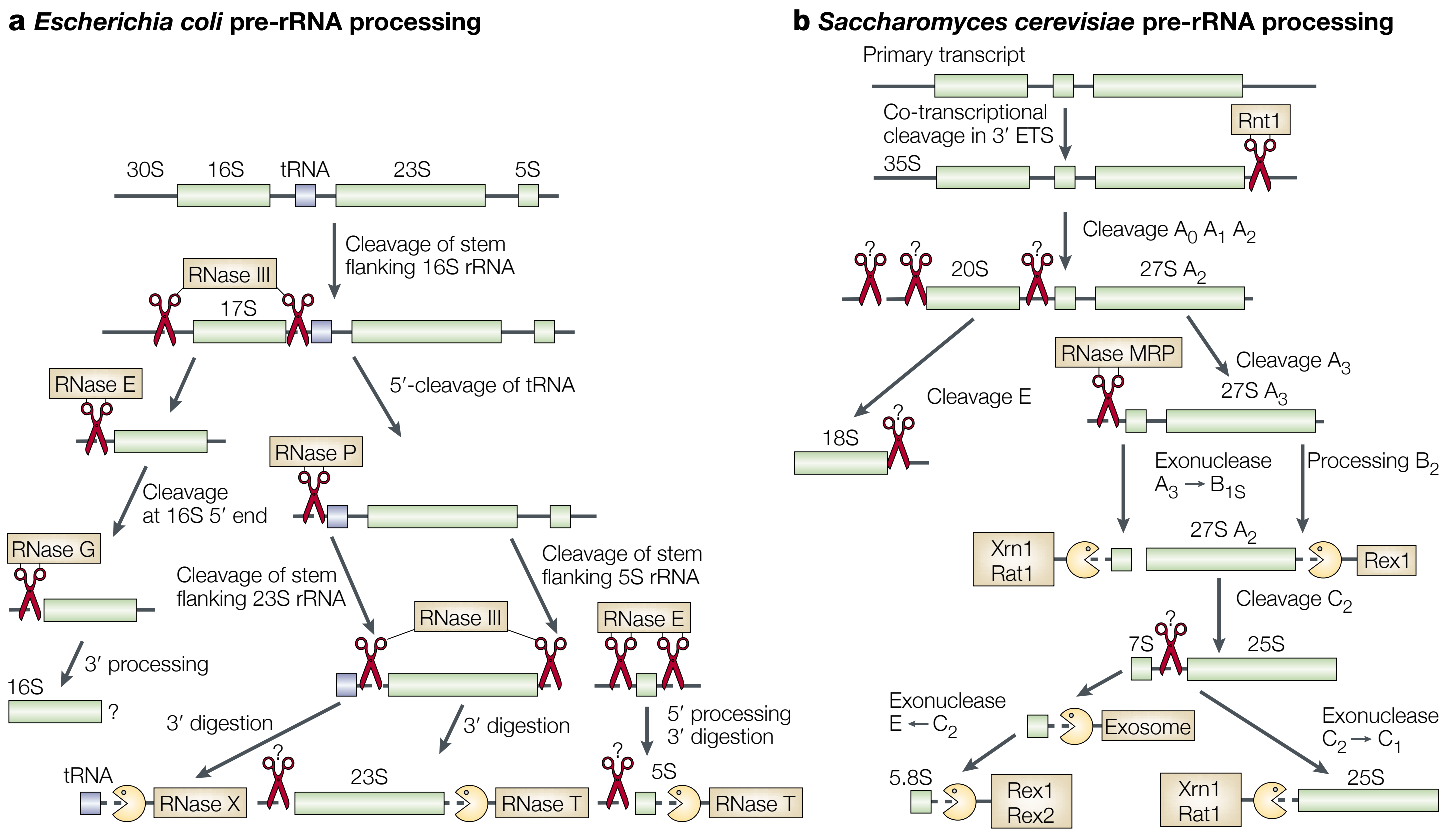


Ribosomal RNA Processing

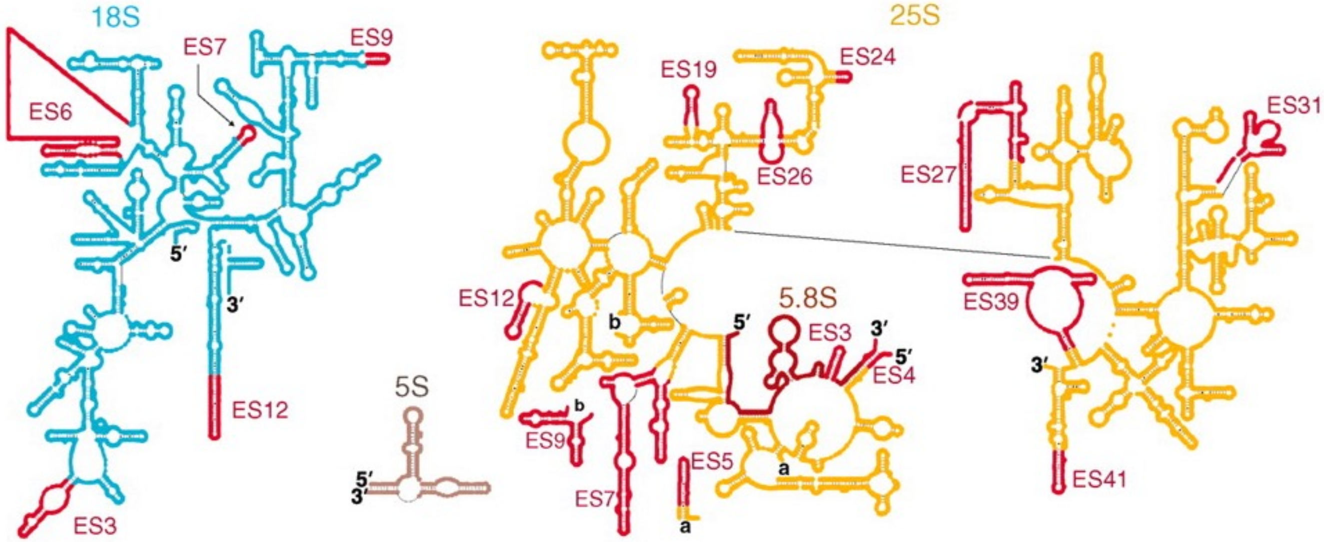
- rRNA genes of both eukaryotes and bacteria are transcribed as larger precursors and must be processed to yield rRNAs of mature size
- Several different rRNA molecules are embedded in a long precursor and each must be cut out



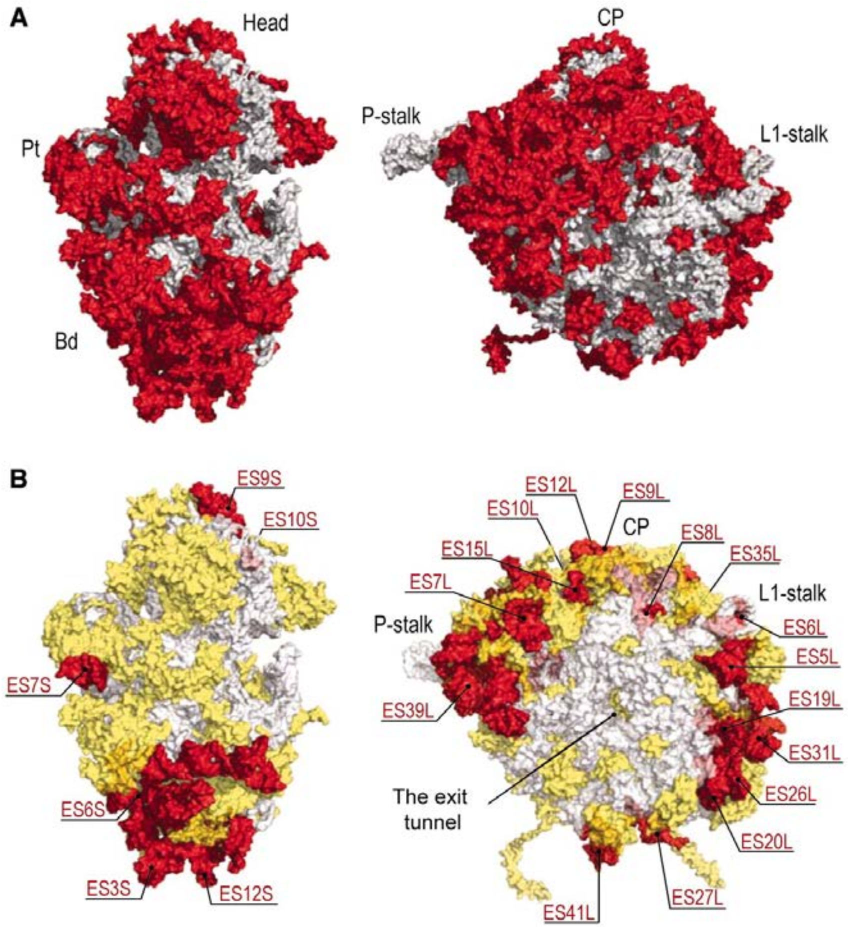
Ribosomal RNA Processing



Eukaryotic rRNAs contain expansion segments (ES)

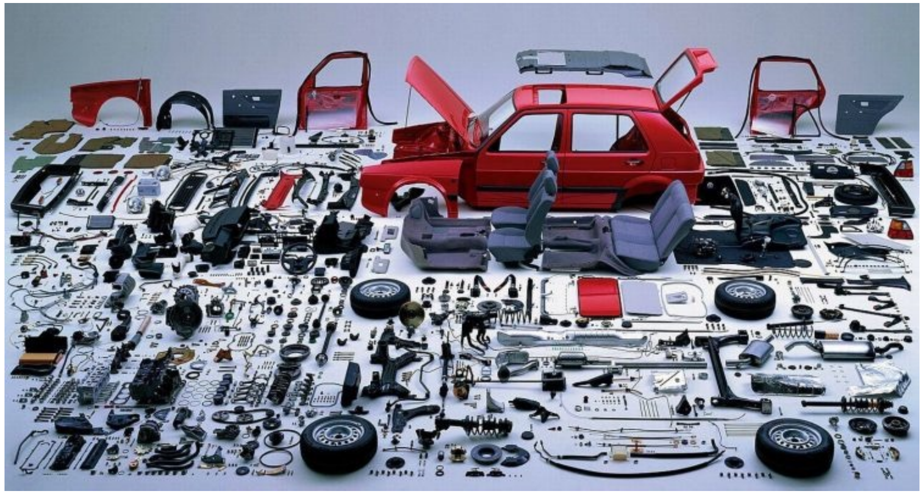


Ribosomal RNA expansion segments and variable regions



Ben-Shem et al Science 2011

Ribosome biosynthesis:

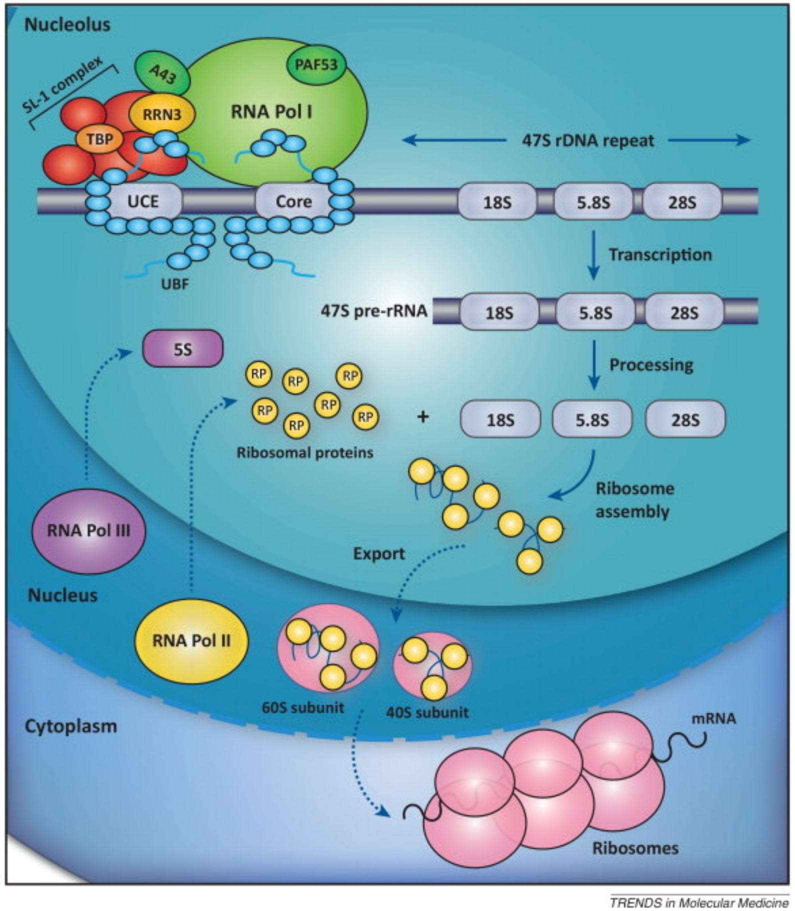


?

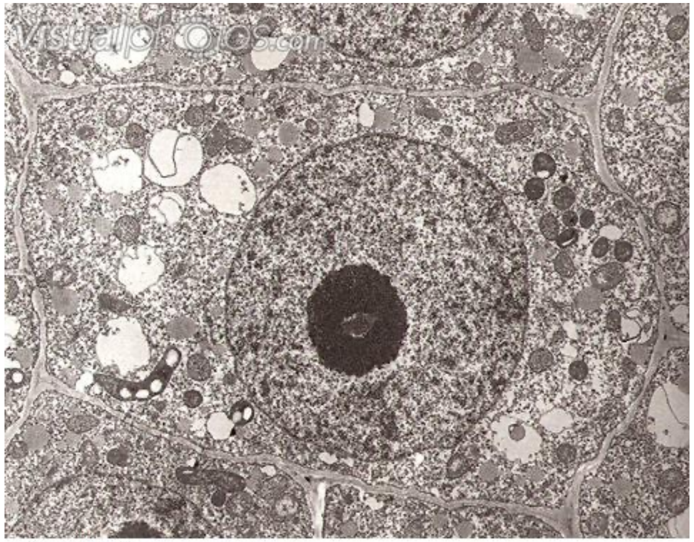
Ordering
Positioning
Quality Control (QC)

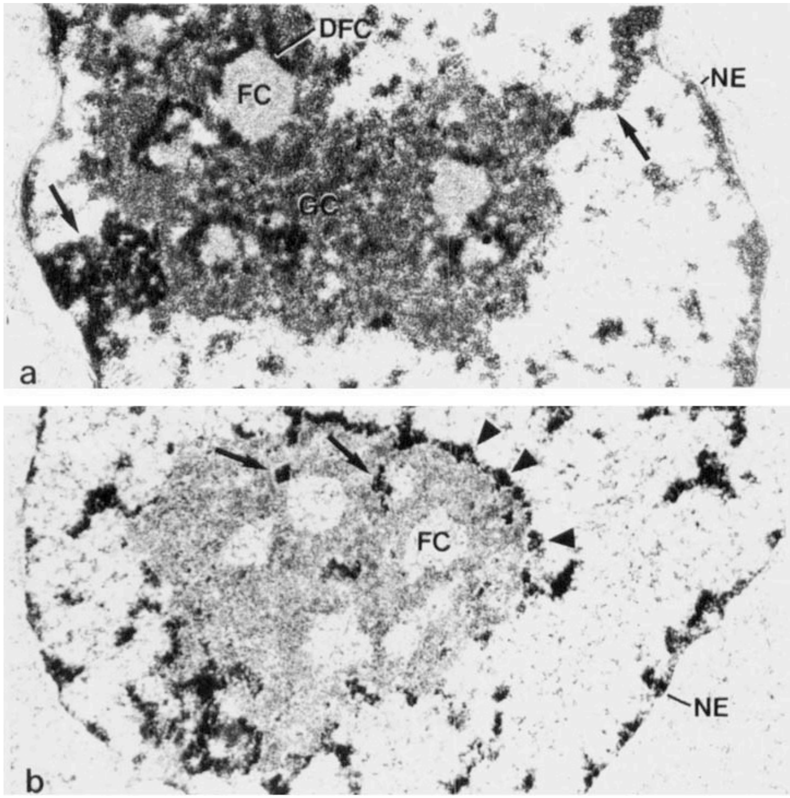
Ribosome assembly: the Nucleolus

RNAPI transcription



The nucleolus

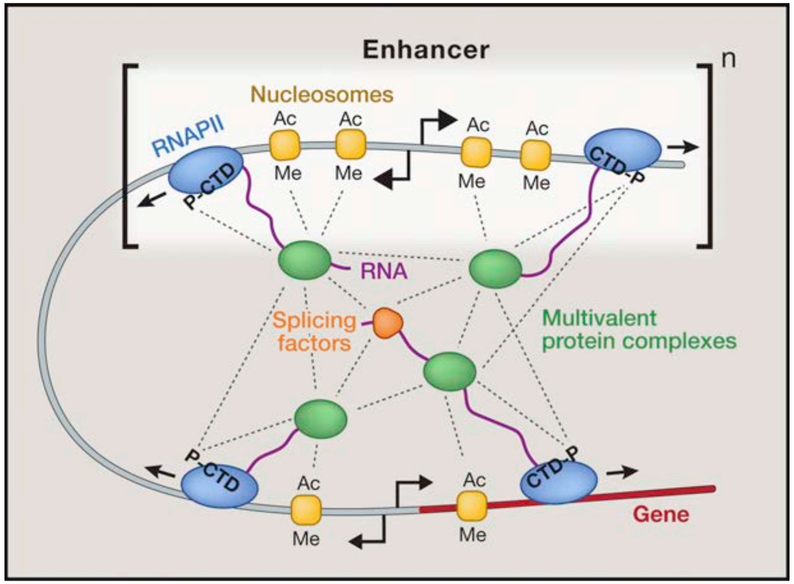


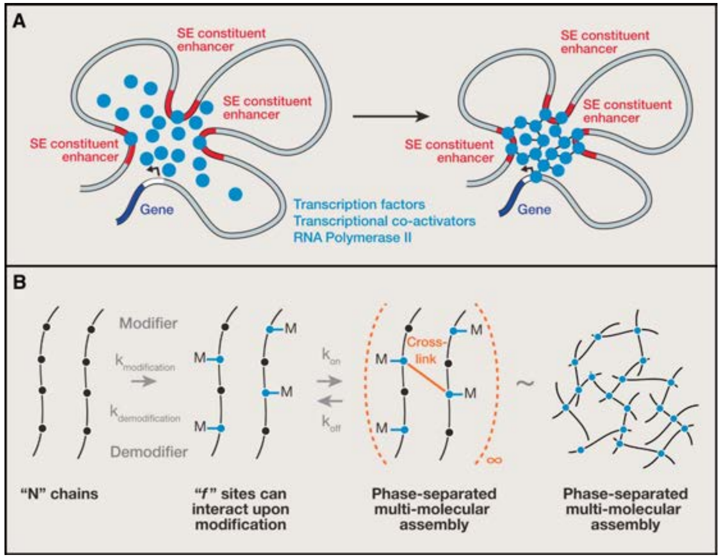


electron micrograph

Ribosome assembly: the Nucleolus

The nucleolus is liquid droplet

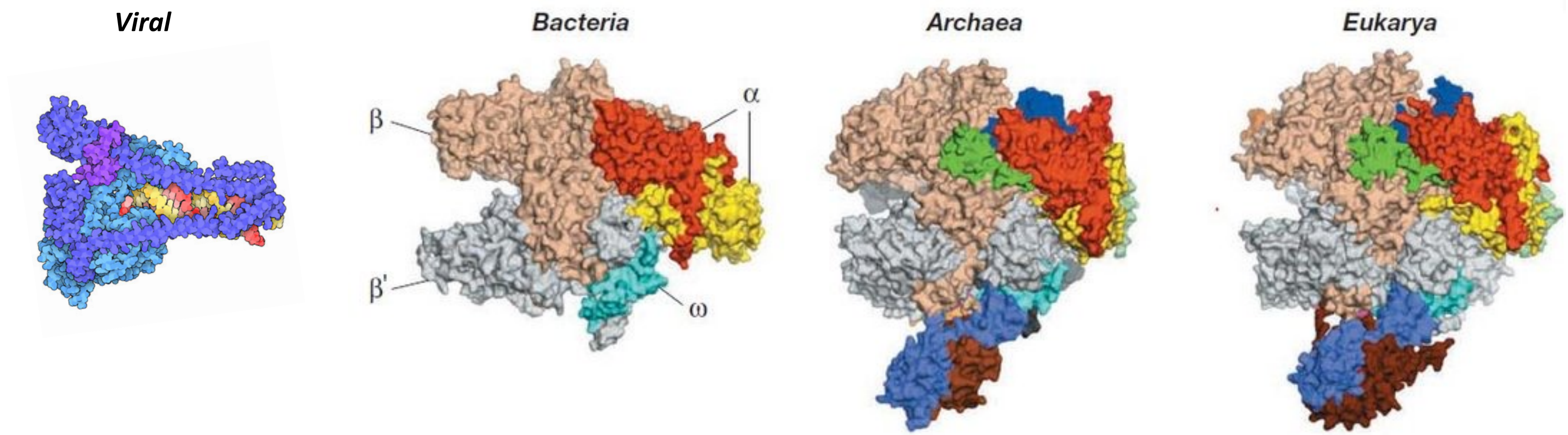




Hnisz et al Cell 2013

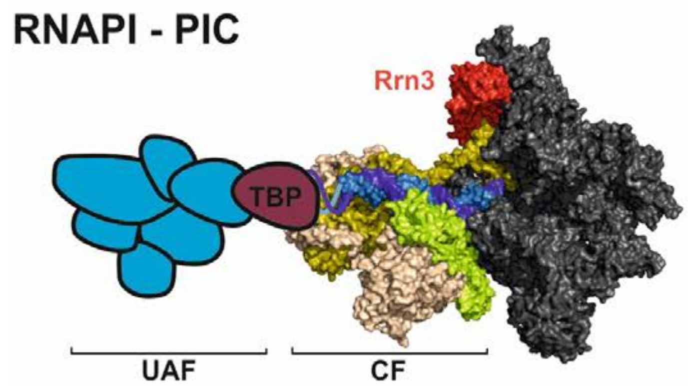
Basic mechanisms of transcription

- Catalized by RNA polymerases
- Composed of initiation, elongation and termination
- Determines fate of mature transcripts



Pre-rRNA transcription

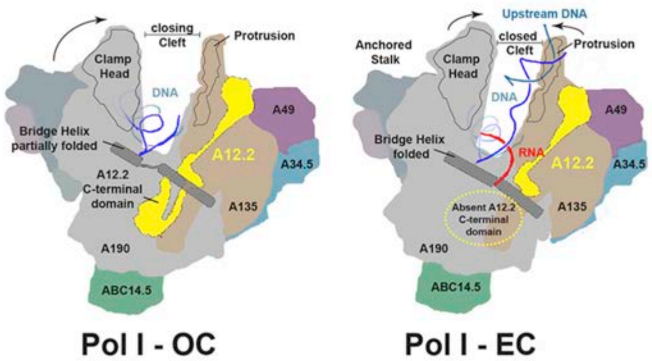
RNA polymerase I transcribes rDNA transcription



RNAPI-specific transcription factors (UAF, CF, Rrn3)

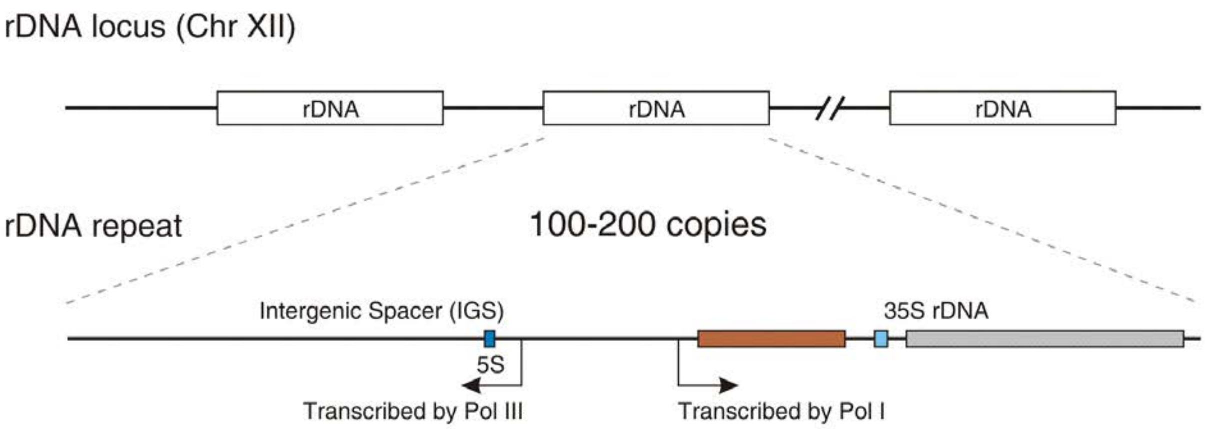
Variable cleft architecture

Rpa12-mediated cleavage



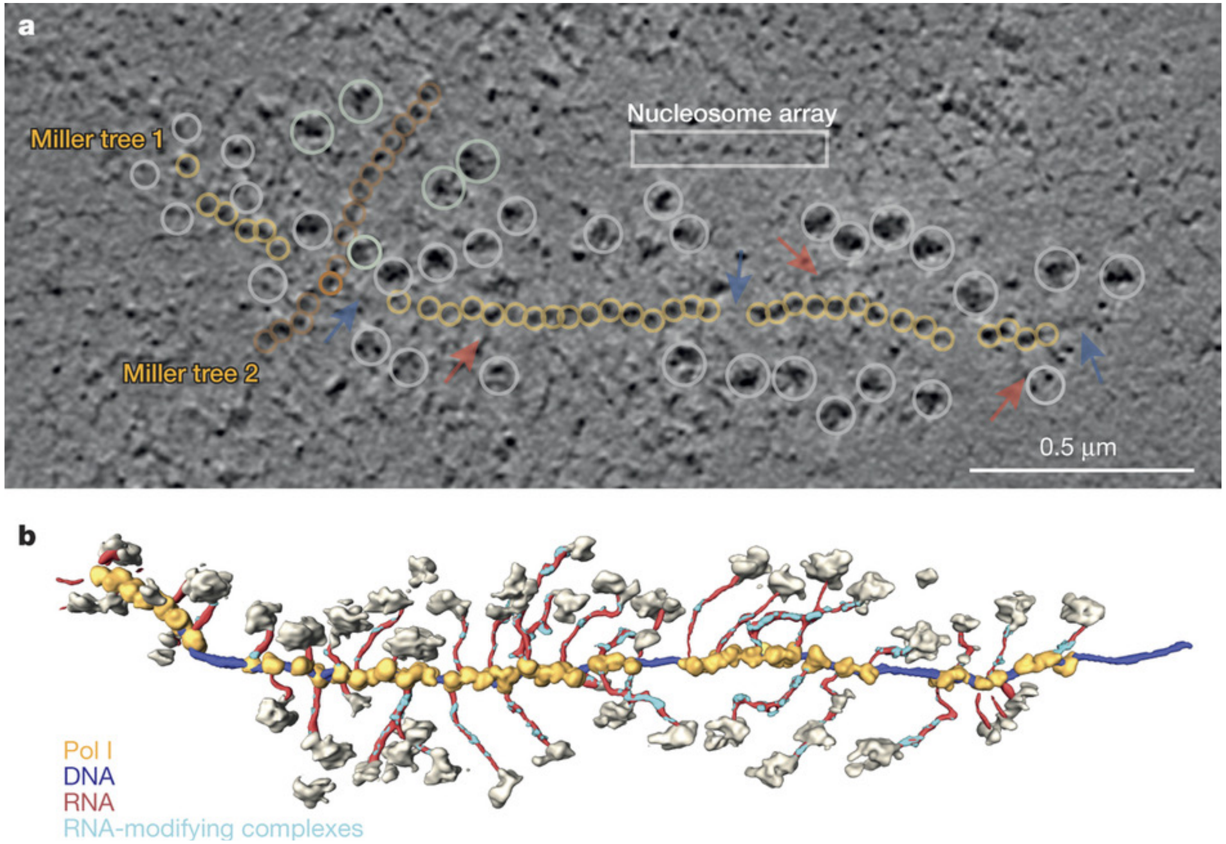
Pre-rRNA transcription

rDNA transcription unit



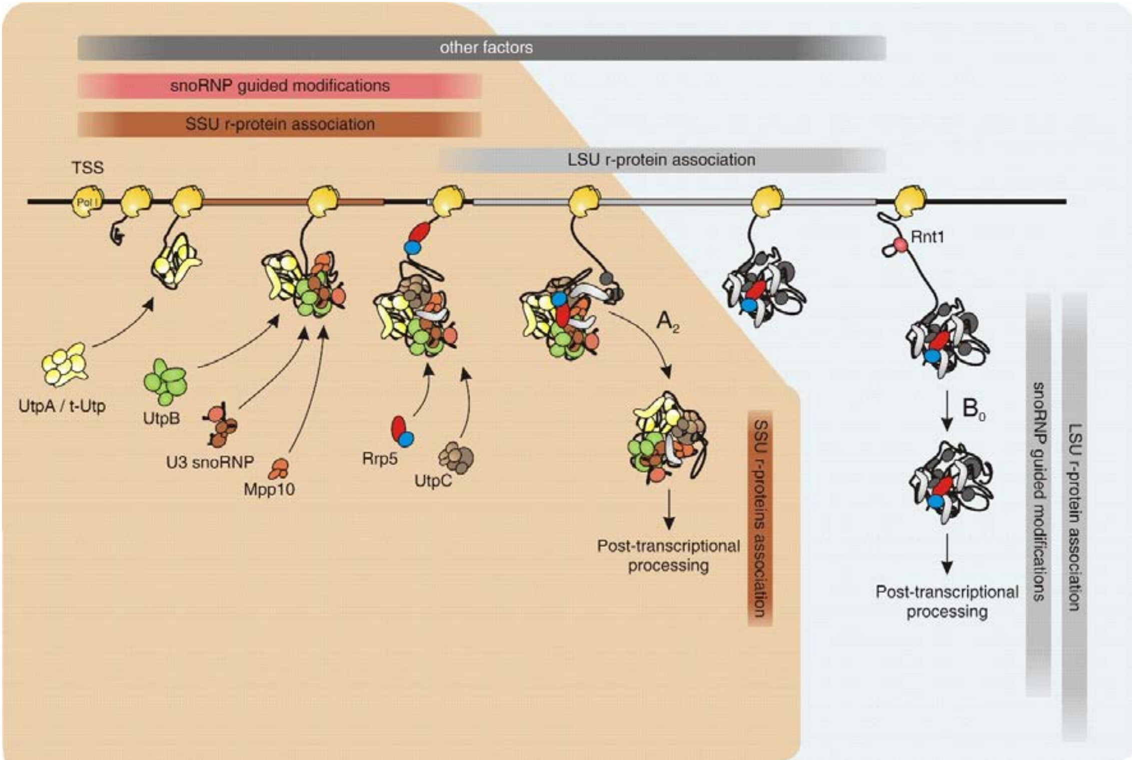
Ribosome assembly: the Nucleolus

Ribosome assembly and rRNA processing occurs co-transcriptionally



Ribosome assembly: the Nucleolus

Ribosome assembly and rRNA processing occurs co-transcriptionally



SSU processome **LSU processome**

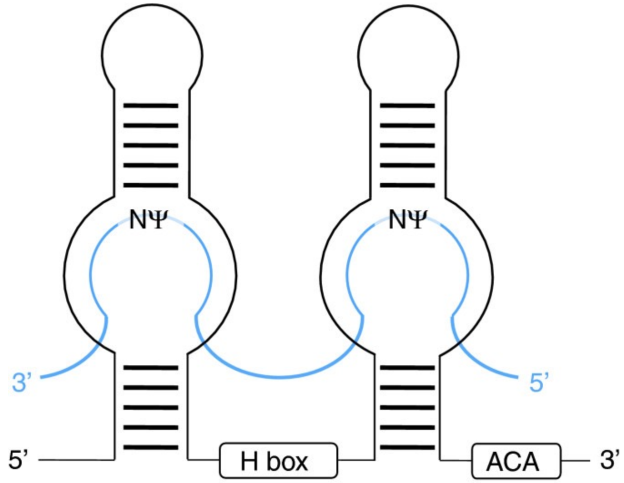
Co-transcriptional:

70% of A2 cleavage
Rnt1 cleavage

Base modifications
R-protein association

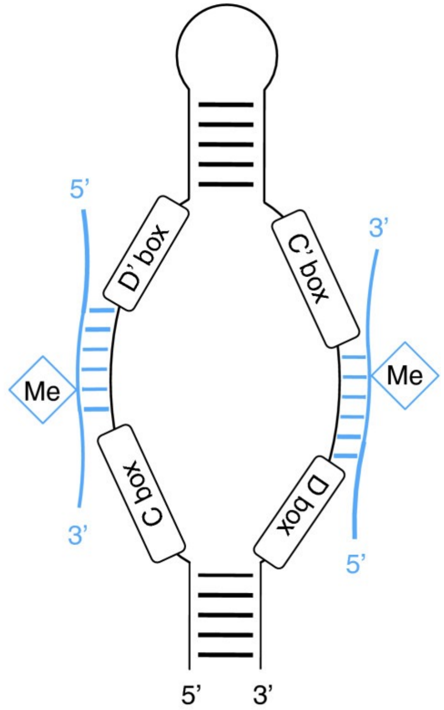
Ribosome assembly: the Nucleolus

Modification of rRNA by snoRNPs



Eukaryotes:
Cbf5
Gar1
Nop10
Nhp2

Archaea:
Cbf5
Gar1
Nop10
L7Ae



Eukaryotes:
Fibrillarin (Nop1)
Nop56
Nop58
15.5kDa/Snu13

Archaea:
Fibrillarin
Nop5
L7Ae

rRNA processing

Endonucleases:

Rnt1, Upt24, MRP, Las1, Nob1

Exonucleases:

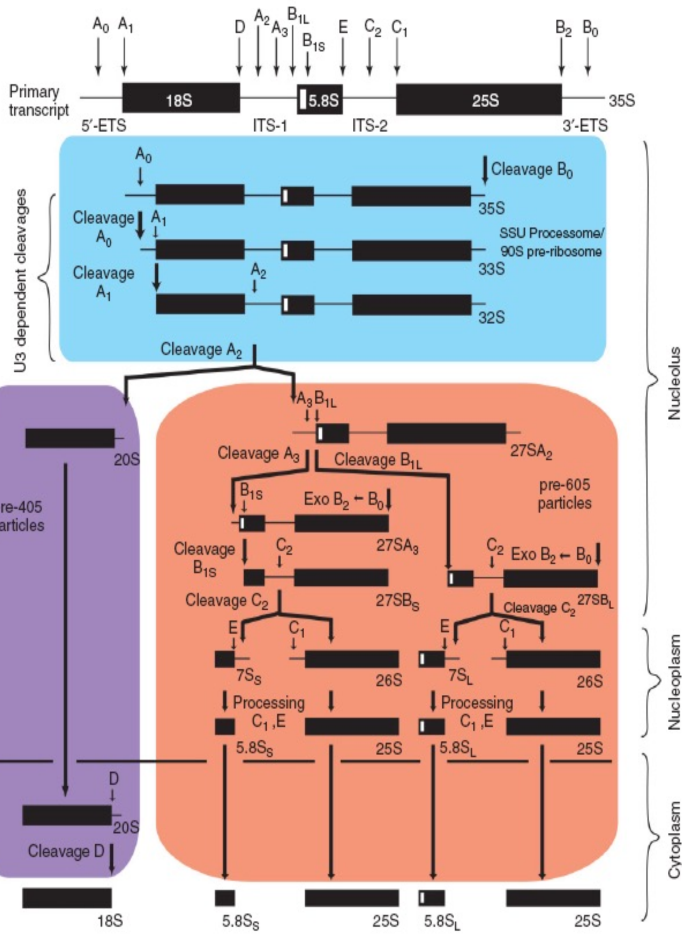
5'-3'

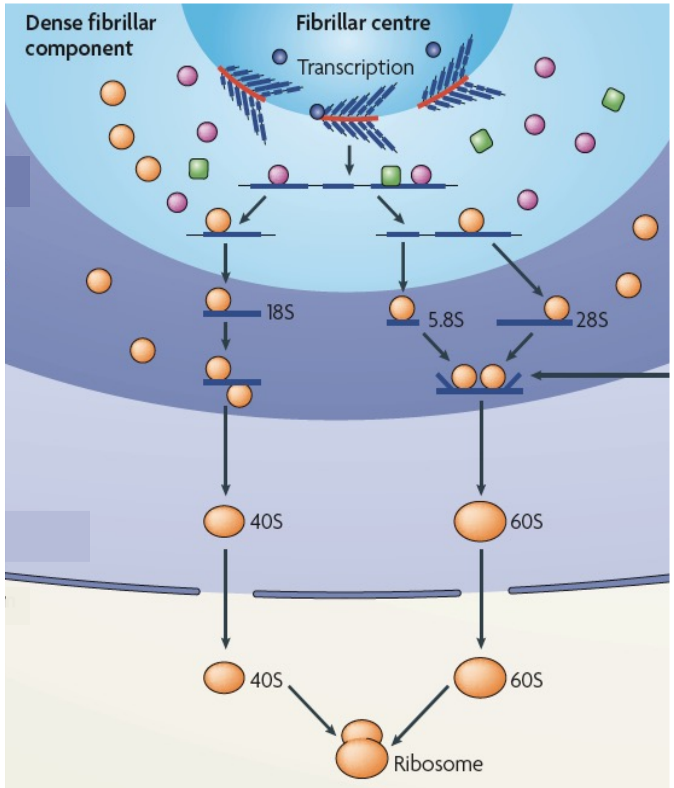
Rat1, Xrn1, Rrp17

3'-5'

exosome: Dis3, Rrp6

Rex1-3, Ngl1

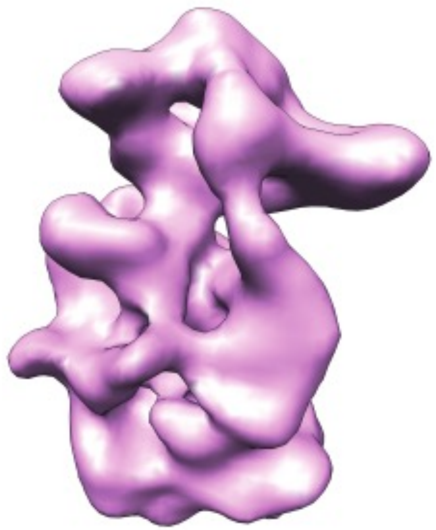




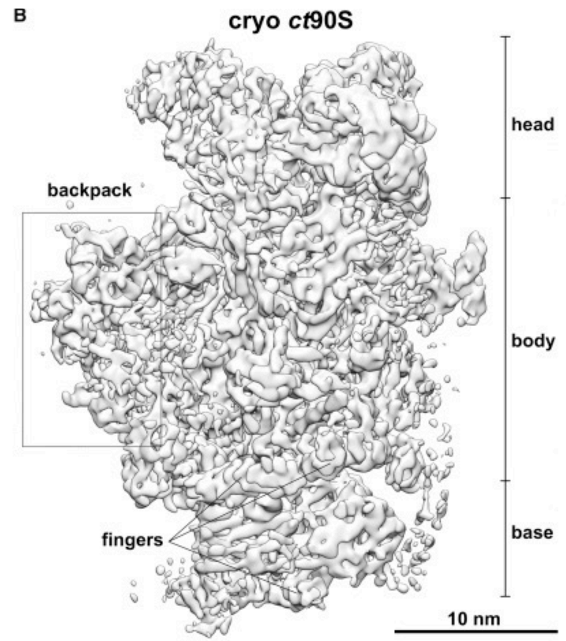
Phipps et al, WIRERNA., 2010

Boisvert et al, NatRevMolCellBiol., 2007

Ribosome assembly factors



High resolution cryo-EM revealed

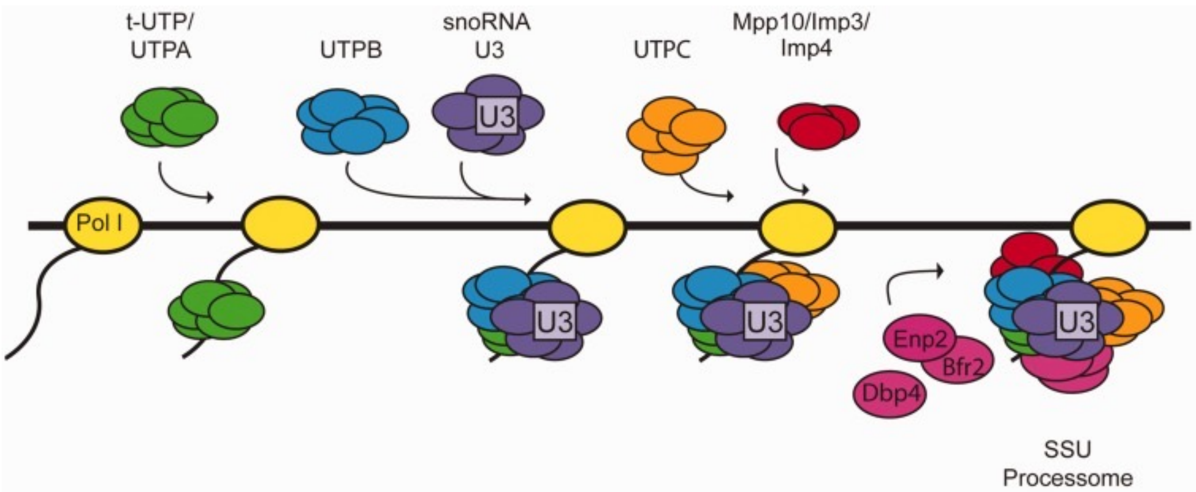


Ribosome assembly factors

Small subunit (SSU) processome

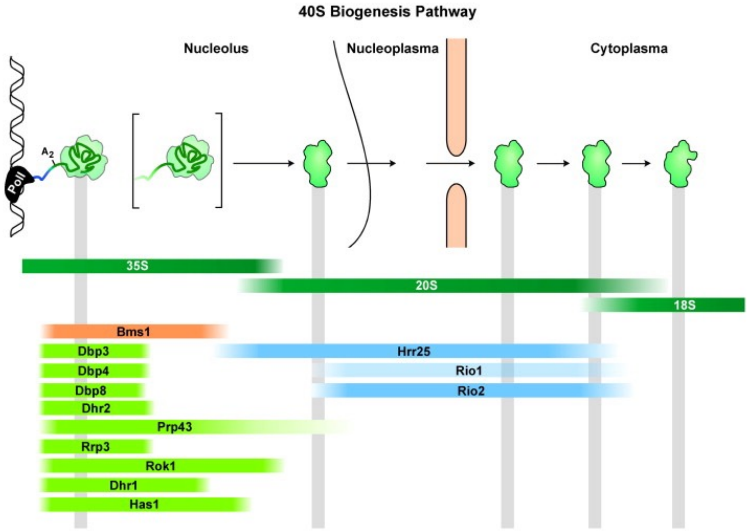
TABLE 1 | Composition of the SSU Processome

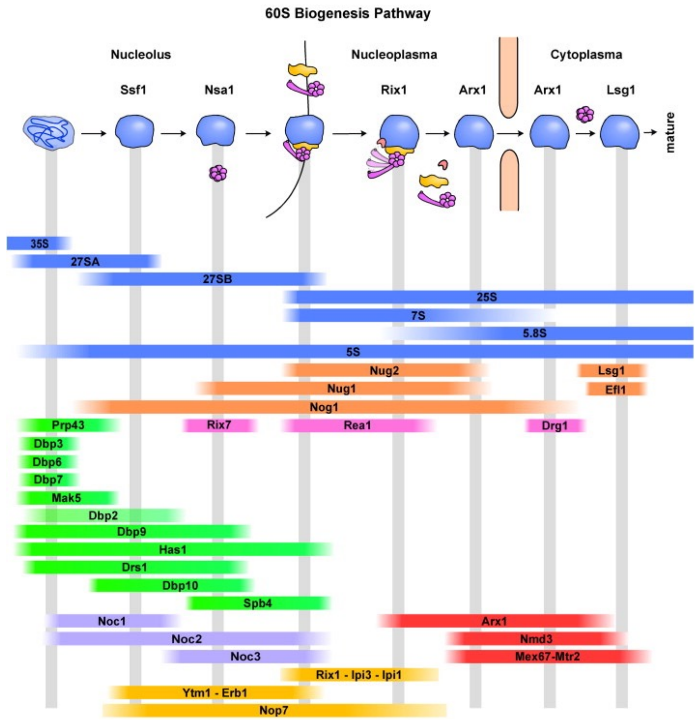
Complex	Subcomplex	Protein Names
U3 snoRNP	Box C/D	Nop1, Nop56, Nop58, Snu13
	Mpp10	Imp3, Imp4, Mpp10
		Rrp9
UtpA/t-Utp		t-Utp4, t-Utp5, t-Utp8, t-Utp9, t-Utp10, t-Utp15, t-Utp17
UtpB		Utp1, Utp6, Utp12, Utp13, Utp18, Utp21
UtpC		Rrp7, Utp22
		Rrp36
	CK II	Cka1, Cka2, Ckb1, Ckb2
Other		Utp2, Utp3, Utp7, Utp11, Utp14, Utp16, Noc4, Utp20, Utp23, Utp24, Utp25, Utp30, Bms1, Dbp8, Dhr1, Dhr2, Emg1, Krr1, Rcl1, Rok1, Rrp3, Rrp5, Sof1, Dbp4, Enp1, Esf1, Esf2, Fal1, Fyv7, Gno1, Has1, Kre33, Lcp5, Ltv1, Mrd1, Nop9, Nsr1, Pfa1, Prp43, Sgd1, Slx9, Ygr251



Ribosome assembly factors

Many “assembly” factors are required to build a ribosome
Most of these have an unknown function.

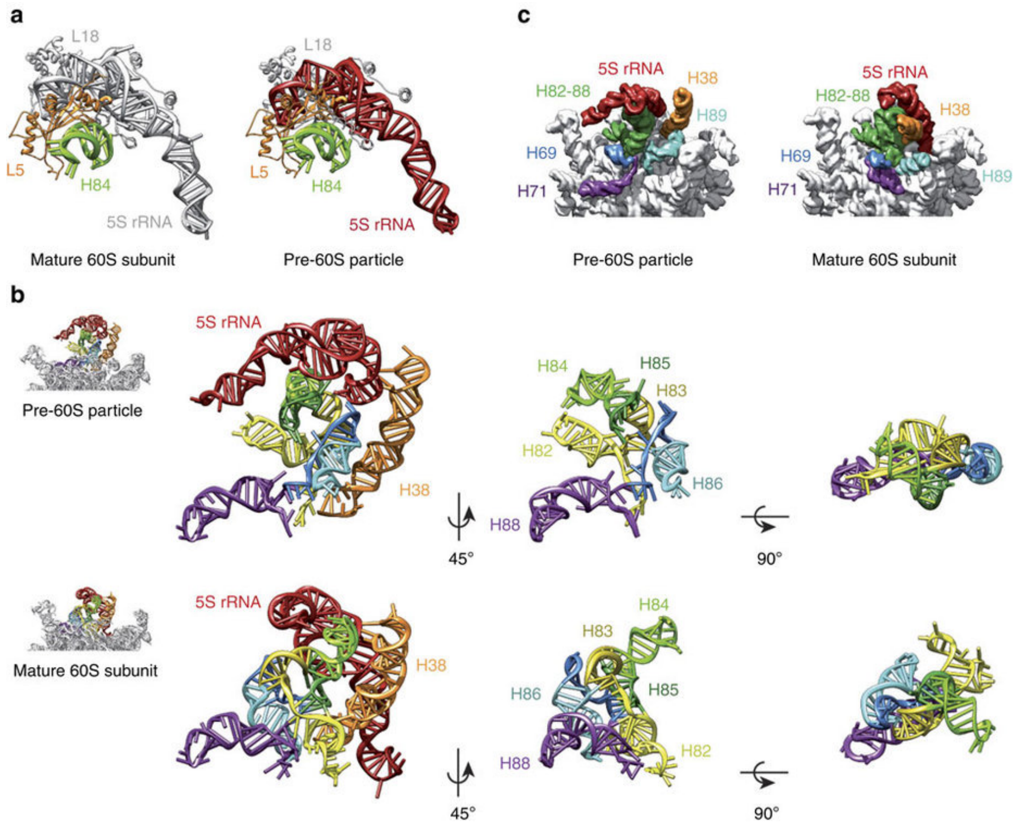




ATPases, GTPases, helicases

Ribosome assembly: structural maturation

5S RNP rotation in 60S ribosome biogenesis



In pre-60S particle the 5S rRNA and its associated ribosomal proteins L18 and L5 (5S ribonucleoprotein (RNP)) are rotated by almost 180° when compared with the mature subunit

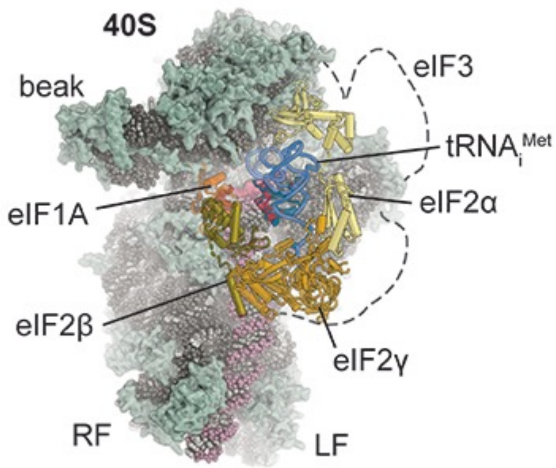
Ribosome assembly factors

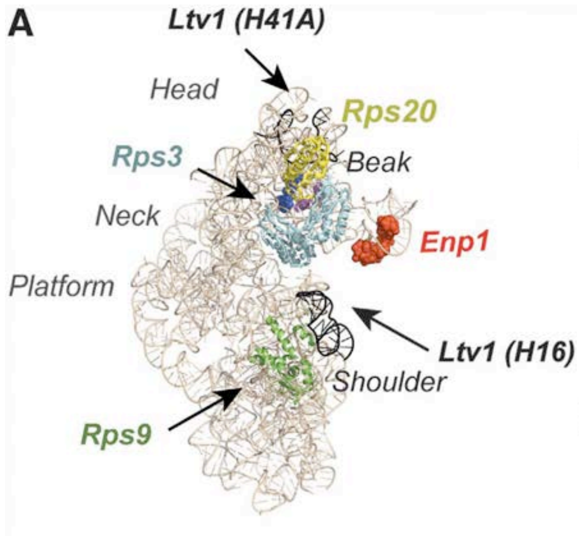
Many overlap with sites where translation initiation factors bind

Some overlap with sites where other assembly factors bind
(to provide order of assembly)

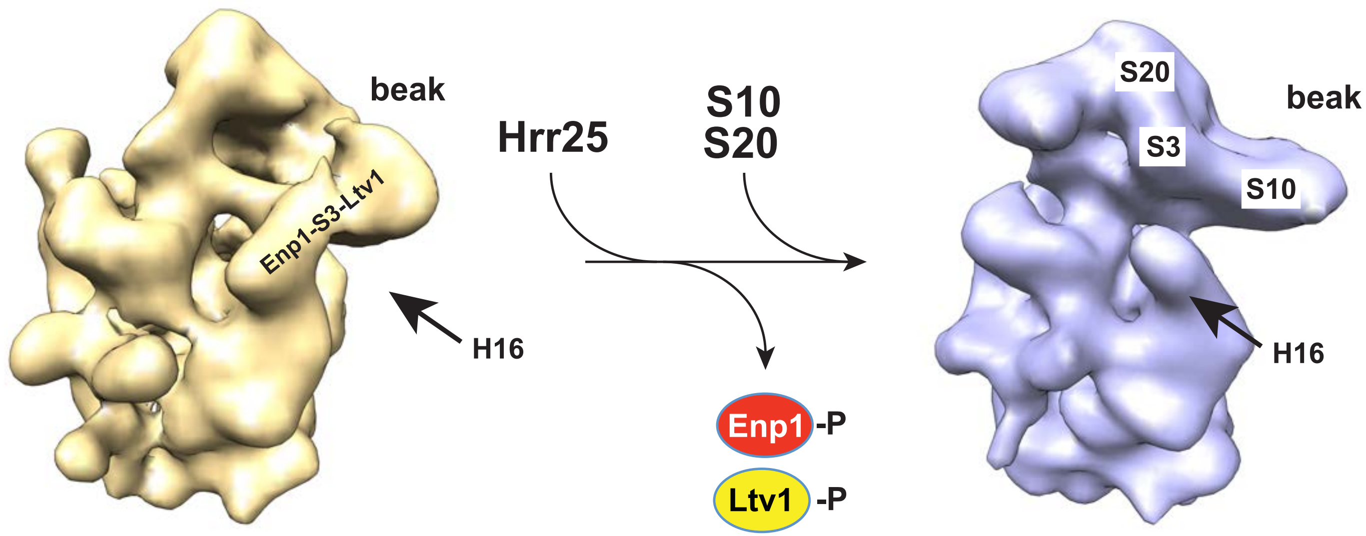
Ribosome assembly: the Cytoplasm

Translation initiation factors binding sites





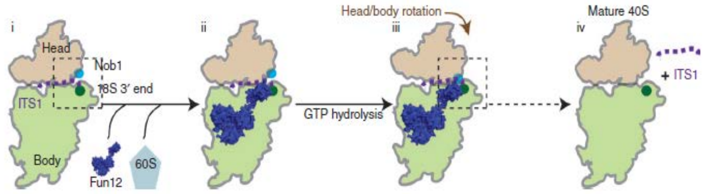
Ribosome assembly: the Cytoplasm



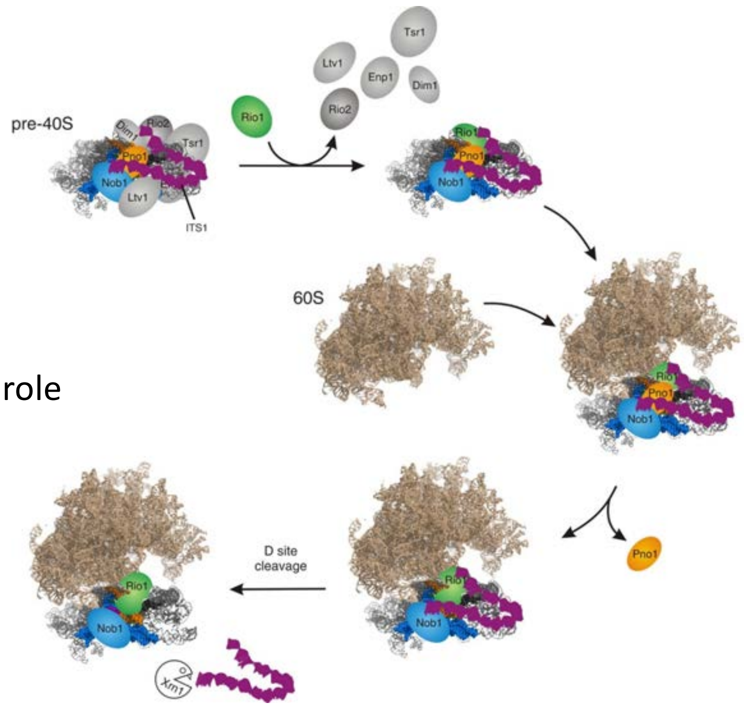
Ribosome assembly: the Cytoplasm

Two pathways of pre-rRNA proccesing: GTP and ATP-dependent

GTP dependent pathway – GTPase eIF5b



ATP dependent pathway – ATPase Rio1

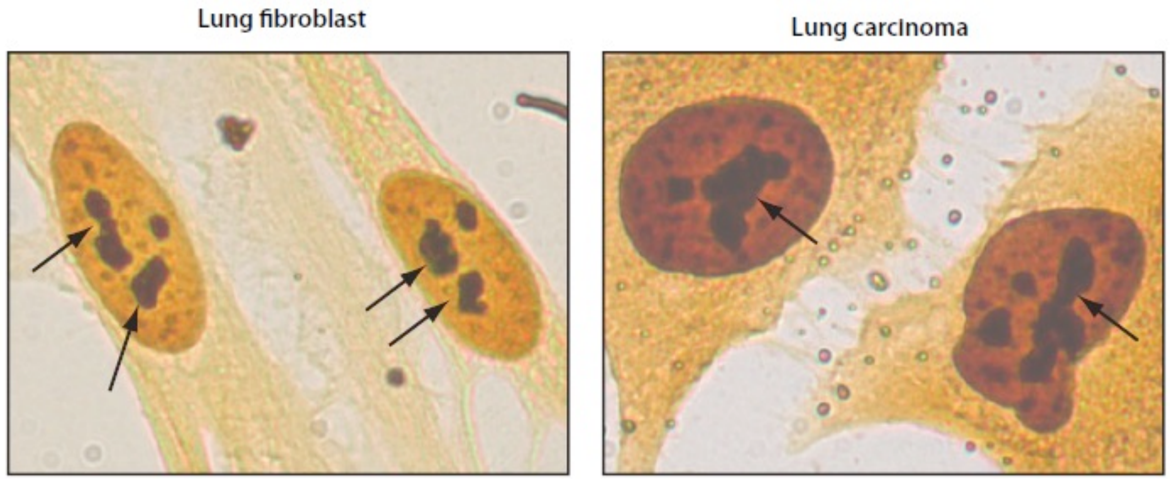


Late pre-40S maturation has a proofreading role

Ribosome synthesis and diseases

Ribosome synthesis and cancer

The size of nucleoli correlates with rRNA synthetic activity, and nucleolar hypertrophy is a diagnostic marker for cancer.



Annu. Rev. Pharmacol. Toxicol. 2010. 50:131–56

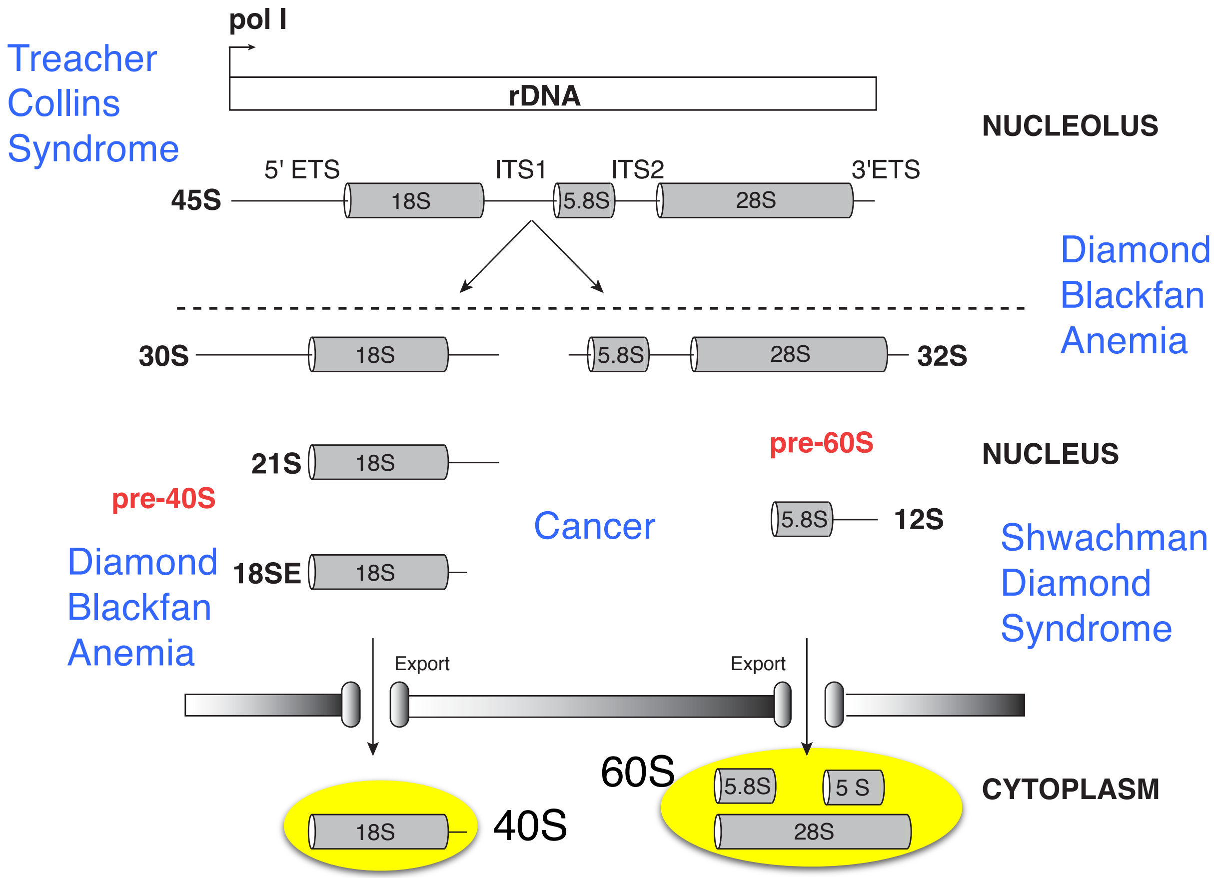
Pre-rRNA transcription as a target for anticancer drugs

Table 1 Approved anticancer drugs that may partially exhibit their therapeutic potential through inhibition of rRNA biogenesis

Drug	Target	Mode of action	Ref.
Actinomycin D	GC-rich duplex DNA	Intercalates in GC-rich regions of rDNA and inhibits at low concentrations elongation of Pol I transcription	(126)
Cisplatin	Adjacent guanosines in DNA	Forms crosslinks in DNA that possess high affinity for HMG-containing proteins. Hijacks UBF from its site of action, thus inhibiting Pol I transcription	(120)
Irinotican/Topotican	Topoisomerase I	Traps Topoisomerase I to rDNA leading to DNA strand breaks and inhibition of Pol I transcription	(127)
Mitomycin C	Guanosines in 5′-CpG-3′motifs	Inhibits Pol I transcription by alkylating guanosines and inducing interstrand crosslinks in rDNA	(128)
5-Fluorouracil	Thymidylate synthase	Incorporation of 5-FU in 47S pre-rRNA inhibits processing of pre-rRNA	(129)
Temsirolimus	mTORC1	Inhibits rRNA synthesis by interfering with mTORC1 activity	(130)

Ribosome synthesis and diseases

Ribosome assembly defects leads to “Ribosomopathies”



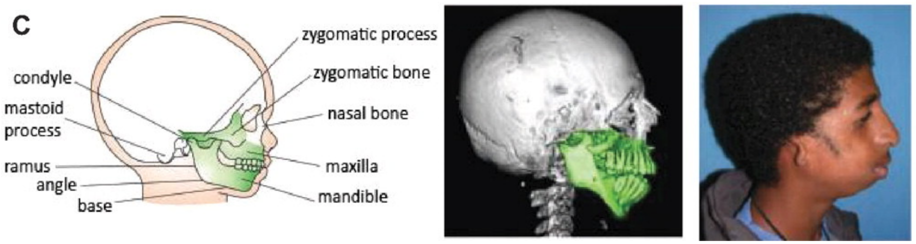
Ribosome synthesis and diseases

Selected physical abnormalities seen in ribosomopathies

Diamond Blackfan Anemia

- Congenital disorders
- Haploinsufficiency

Dyskeratosis Congenita

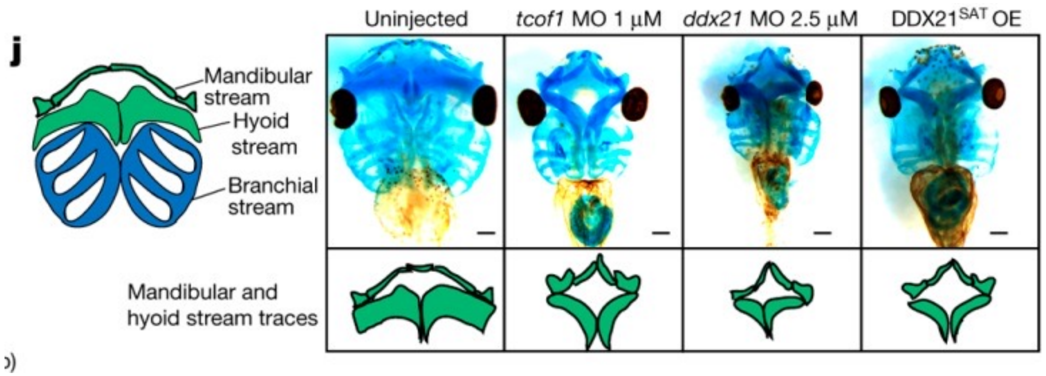


Treacher Collins

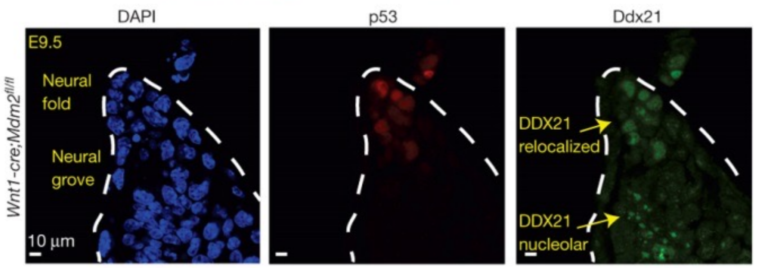
Narla A , Ebert B L Blood 2010;115:3196-3205
Hannan et al. Biochimica et Biophysica Acta 1829 (2013) 342–360

Ribosome synthesis and diseases

The functions of DDX21 are linked to rRNA synthesis levels and altered by TCS-associated perturbations



Xenopus laevis cranial cartilages



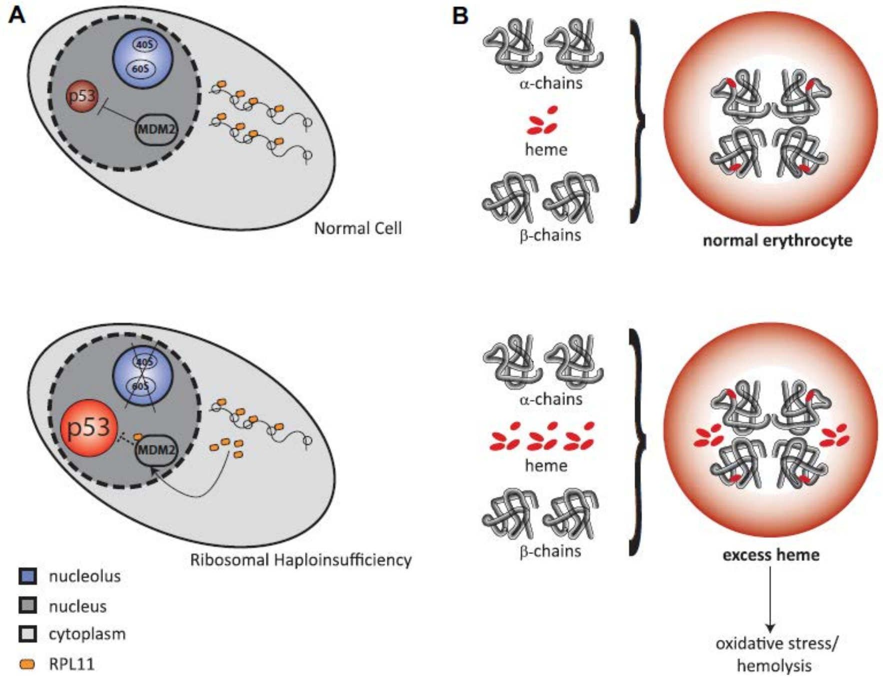
(E)9.5 mouse embryos

Neural crest cells are sensitive to p53 stabilization

Calo et al. Nature 2018

Ribosome synthesis and diseases

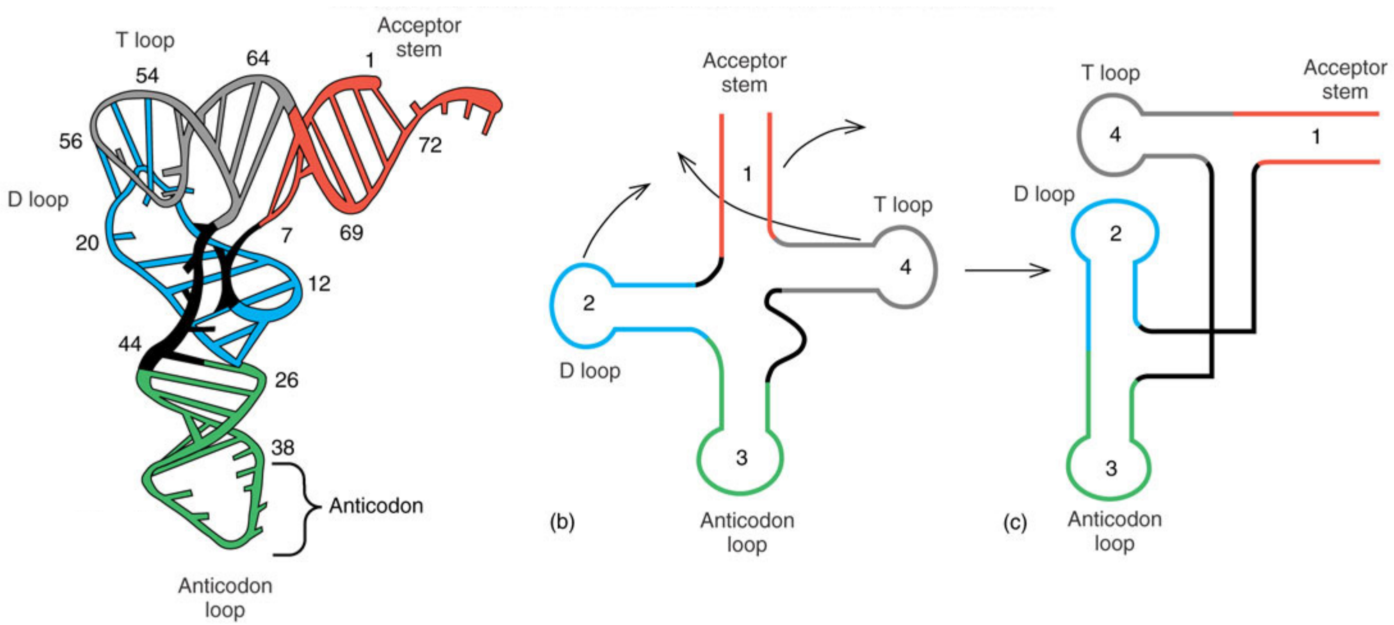
Potential mechanisms for the cellular consequences of ribosomal haploinsufficiency



Narla A , Ebert B L Blood 2010;115:3196-3205

Synthesis of tRNA

tRNA molecule

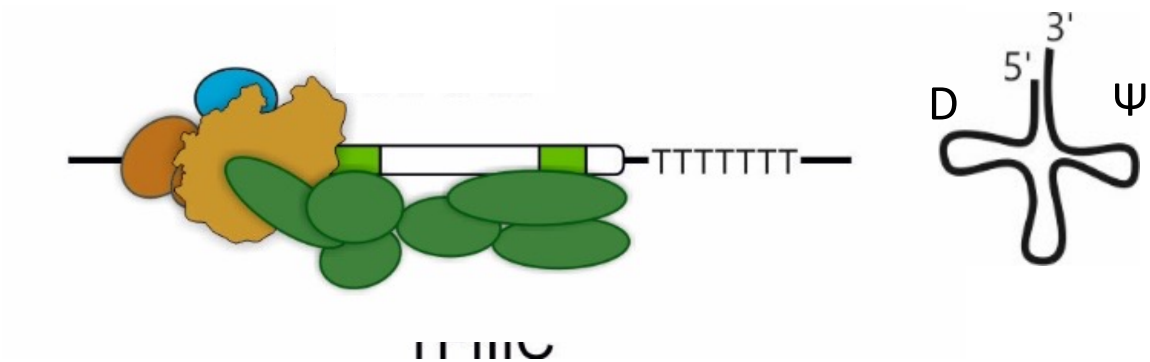


•**All tRNAs share a common cloverleaf secondary structure and L-shaped tertiary structure.**

• **L shape maximizes stability by lining up base pairs in the D and anticodon stems, and base pairs in the T and acceptor stems**

tRNA biogenesis: transcription

RNAPIII transcription

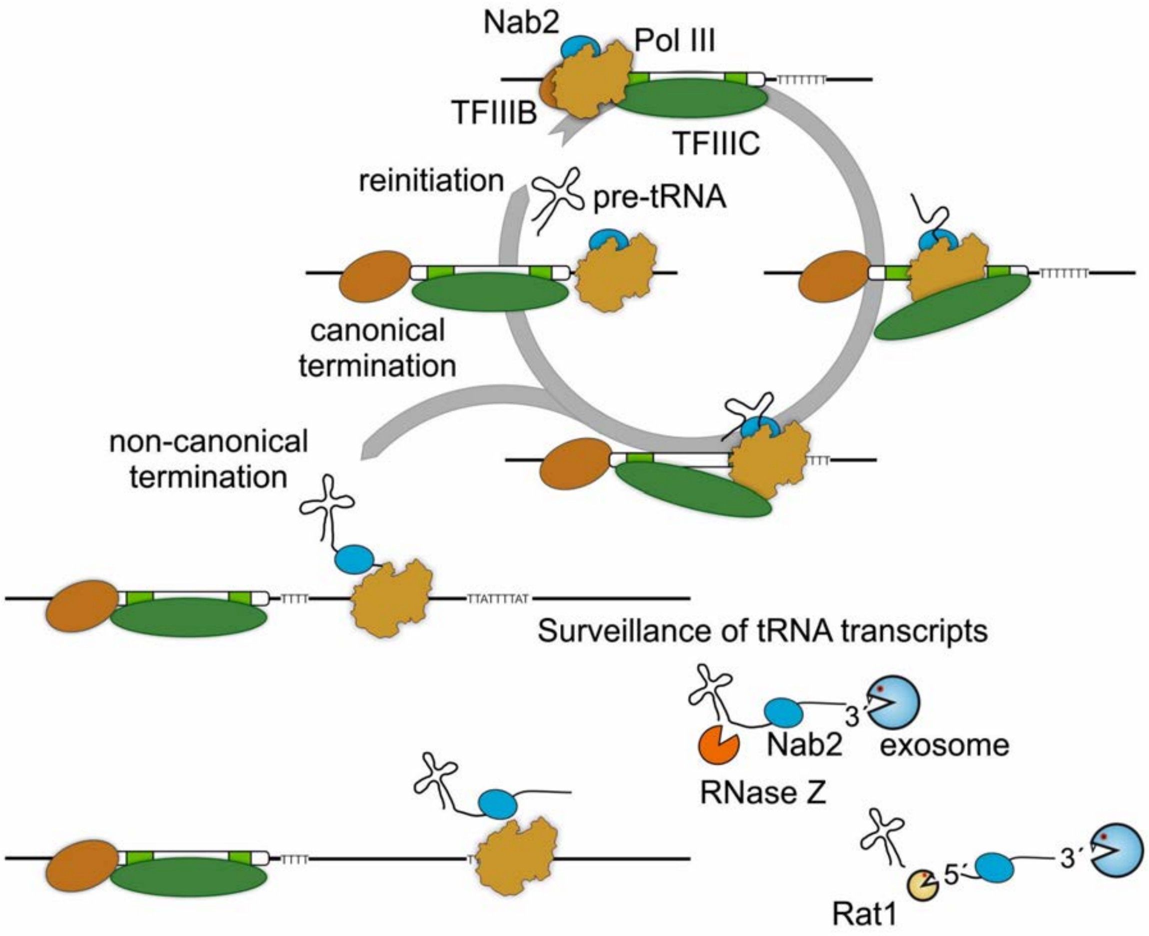


TFIIIC recognizes internal promoter elements

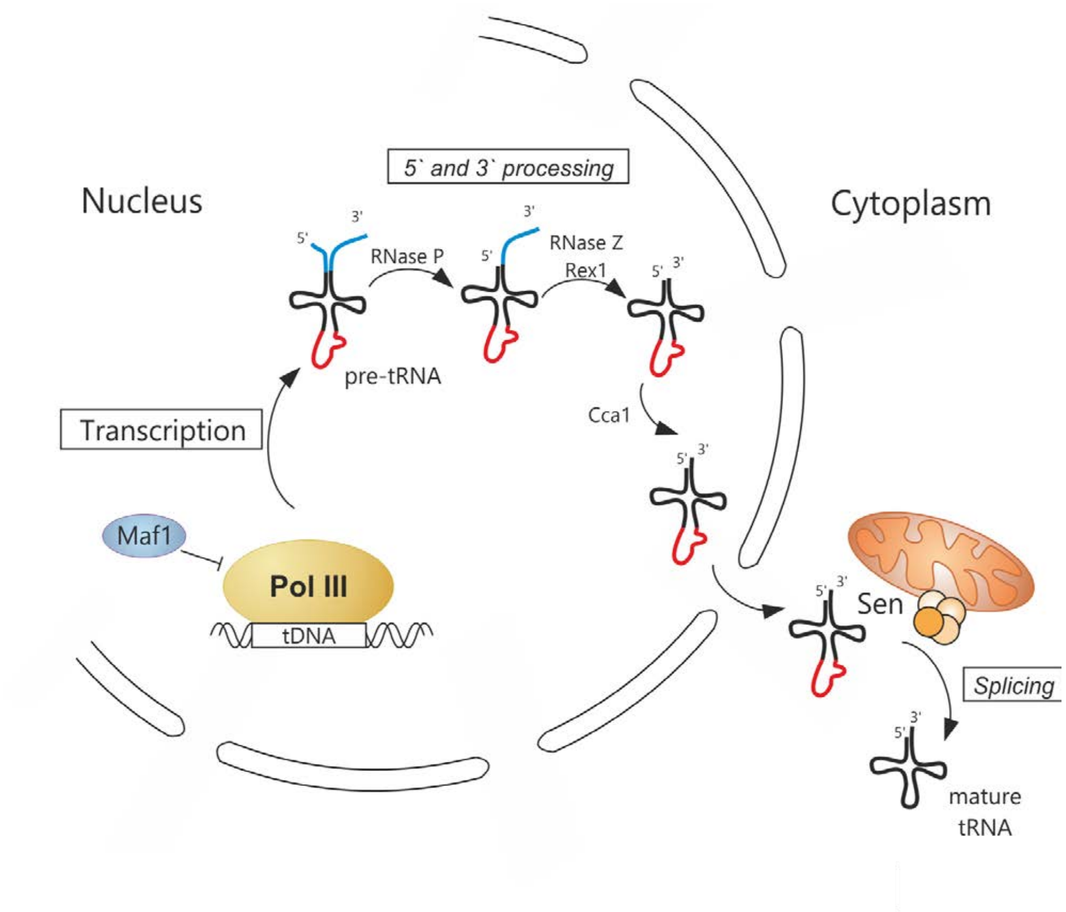
TFIIIC recruits TFIIIB

TFIIIB recruits Pol III -> all together constitute initiation complex of Pol III

tRNA biogenesis: RNAPIII transcription cycle



tRNA biogenesis: processing

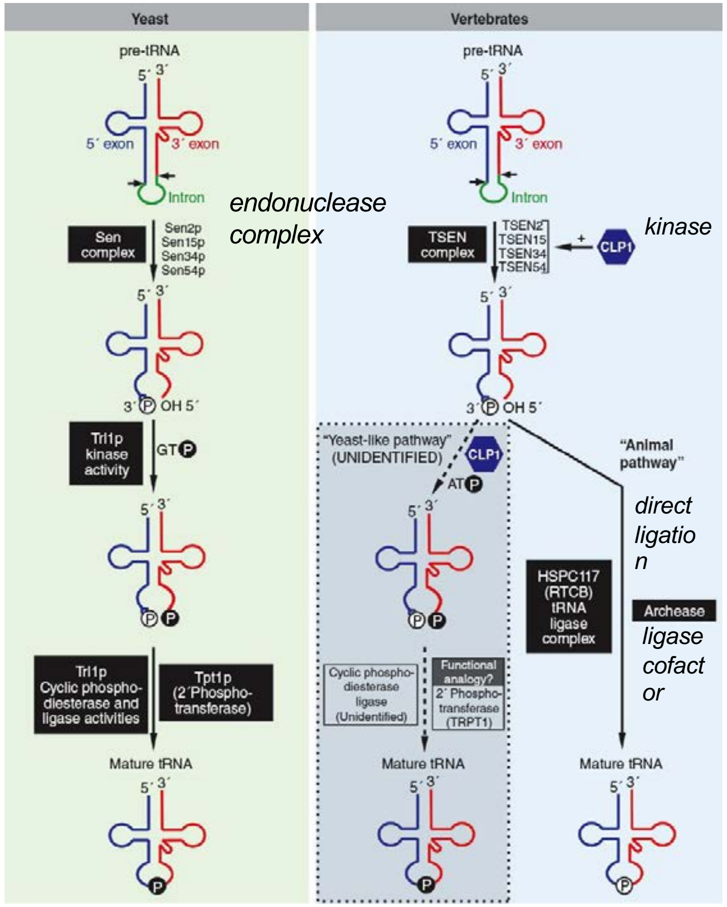


Nucleus:
5’ end endo cleavage – RNase P
3’ end endo cleavage – RNase Z
3’ exo trimming Rex1-3
CCA addition by Cca1

Cytoplasm:
Splicing SEN complex

- **tRNA travels between nucleus and cytoplasm during processing steps**
- **mature tRNA can be imported to the cytoplasm by retrograde pathway**

tRNA biogenesis: splicing



Weitzer et al, WIREs RNA,2015

Introns in tRNAs in yeast:

- are dispensable
(can be deleted)

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

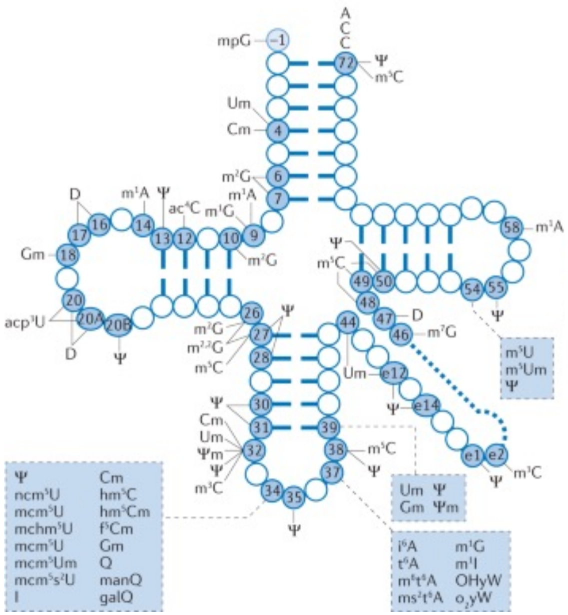
- ensure proper growth at some conditions
(deletion of some introns results in slow growth in respiratory conditions)

- may affect codon-anticodon pairing

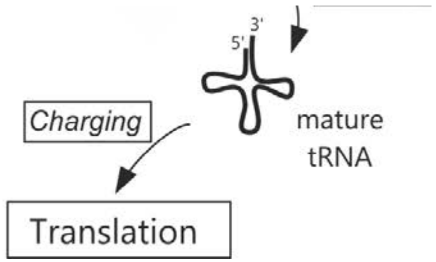
tRNA biogenesis: processing and charging

Can occur in the nucleus and in the cytoplasm

Base modifications:

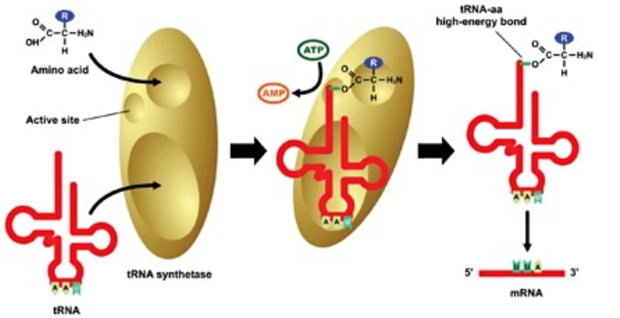


tRNA aminoacylation



Aminoacylation by tRNA aminoacyl synthetases

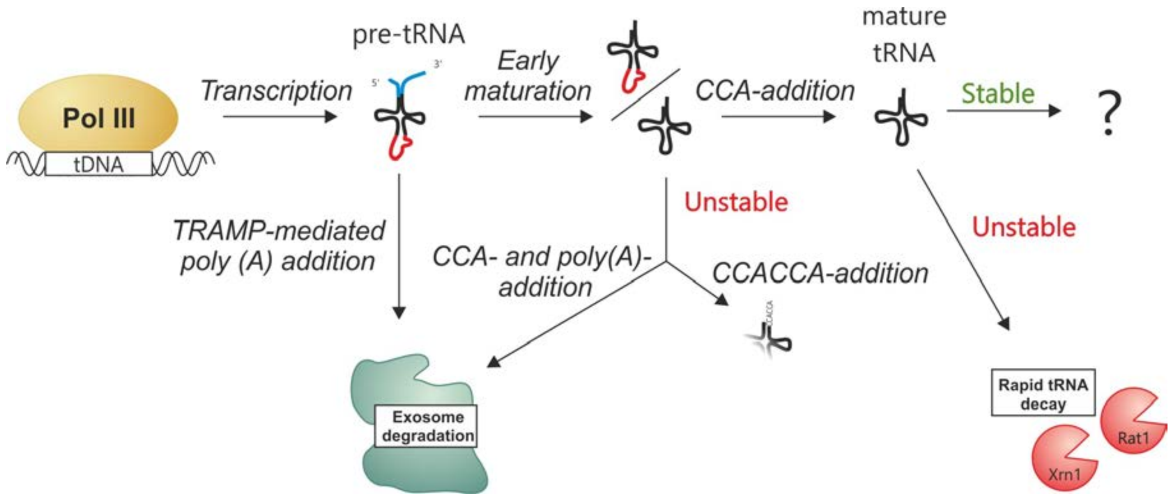
two classes: class I and class II (aminoacylate 2'-OH and 3'-OH of A, respectively)



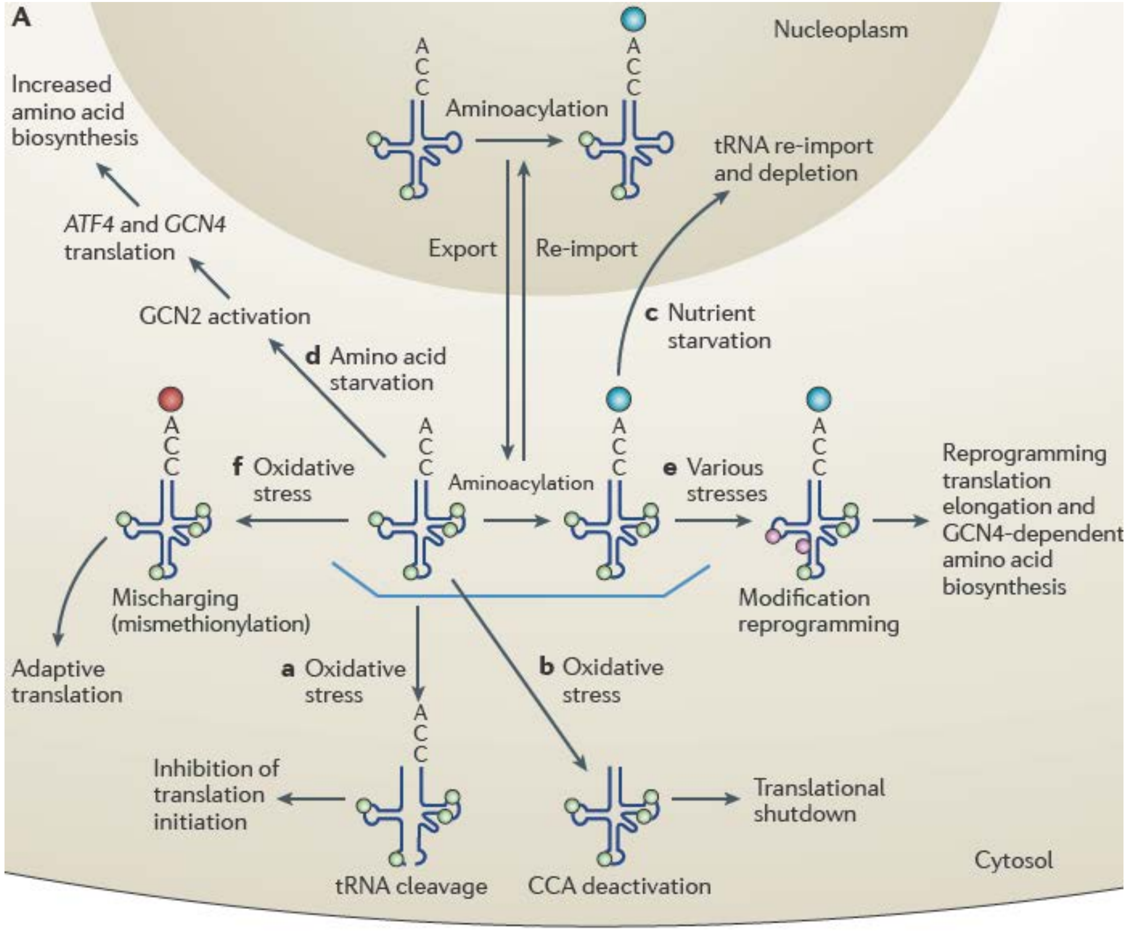
Suzuki 2021 *Nat Rev Mol Cel Biol*

Schurer et al 2001 Biol Chem

tRNA biogenesis: surveillance



tRNAs and stress



Kirchner and Ignatova, NatRevGenet, 2015

Thank you