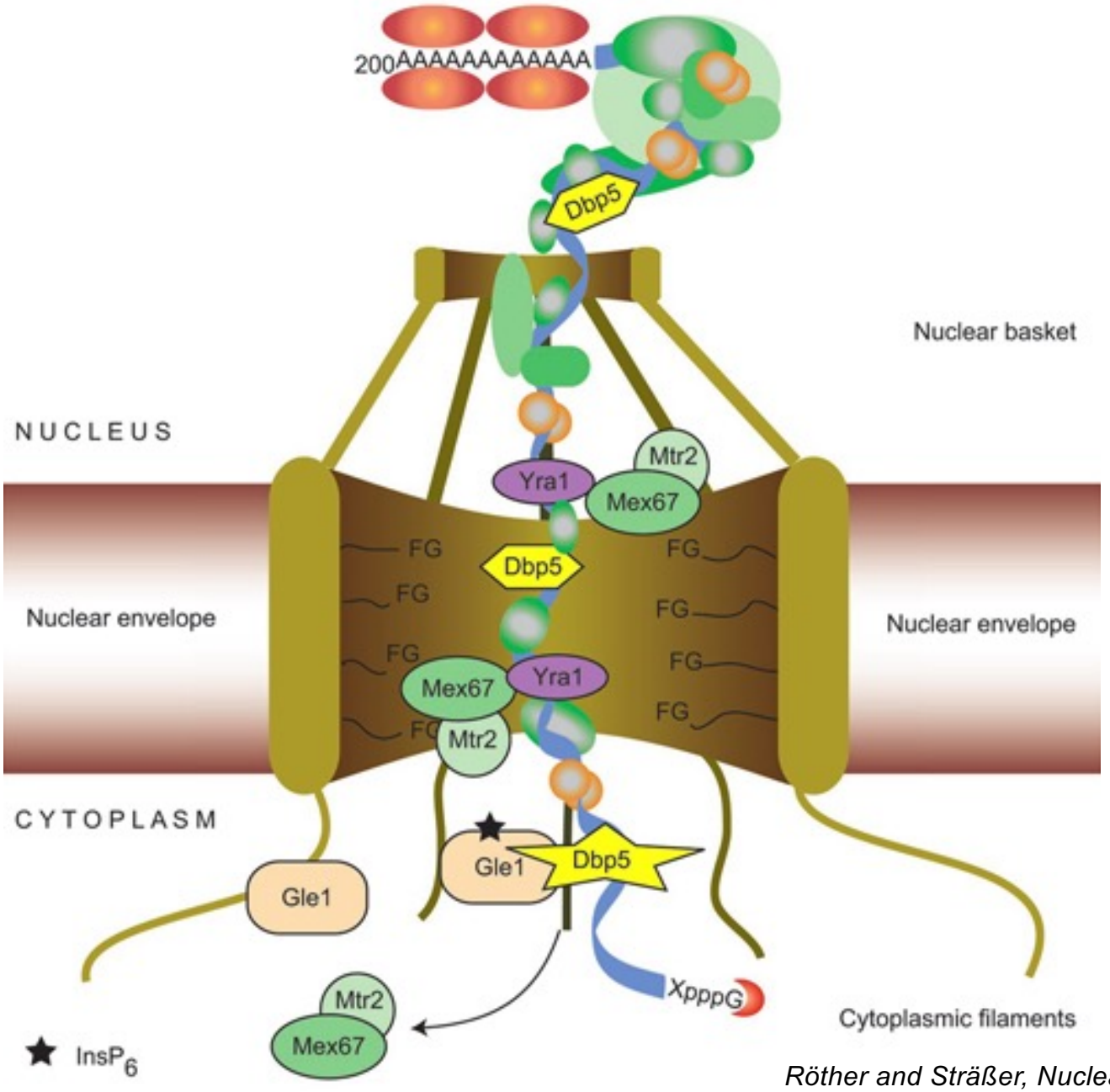
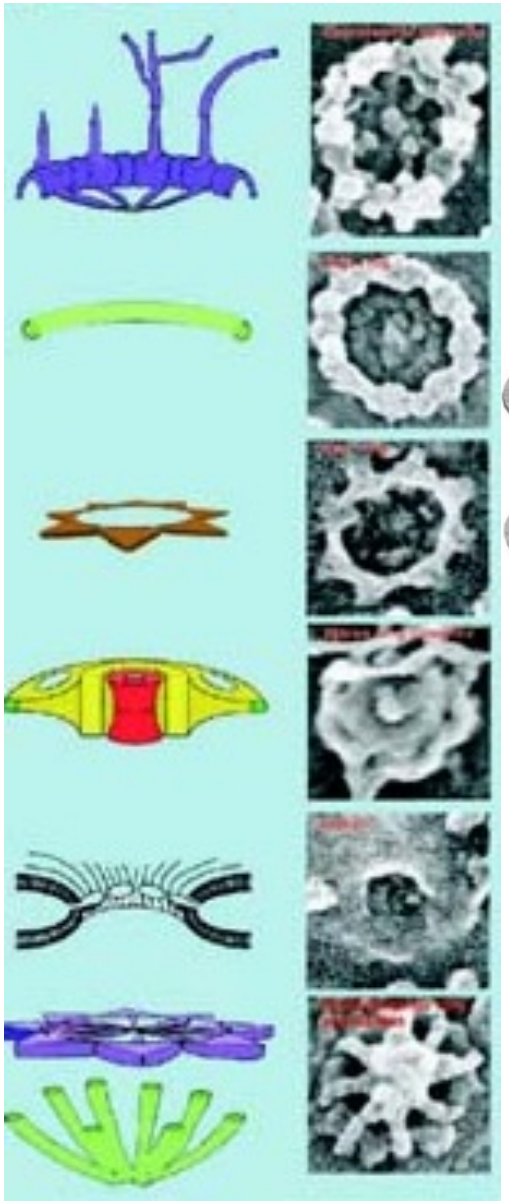
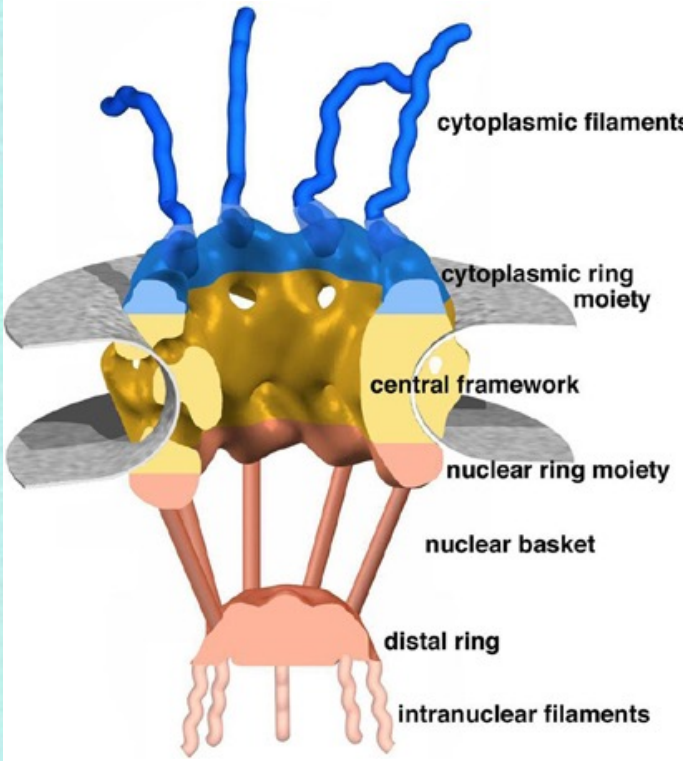
RNA EXPORT

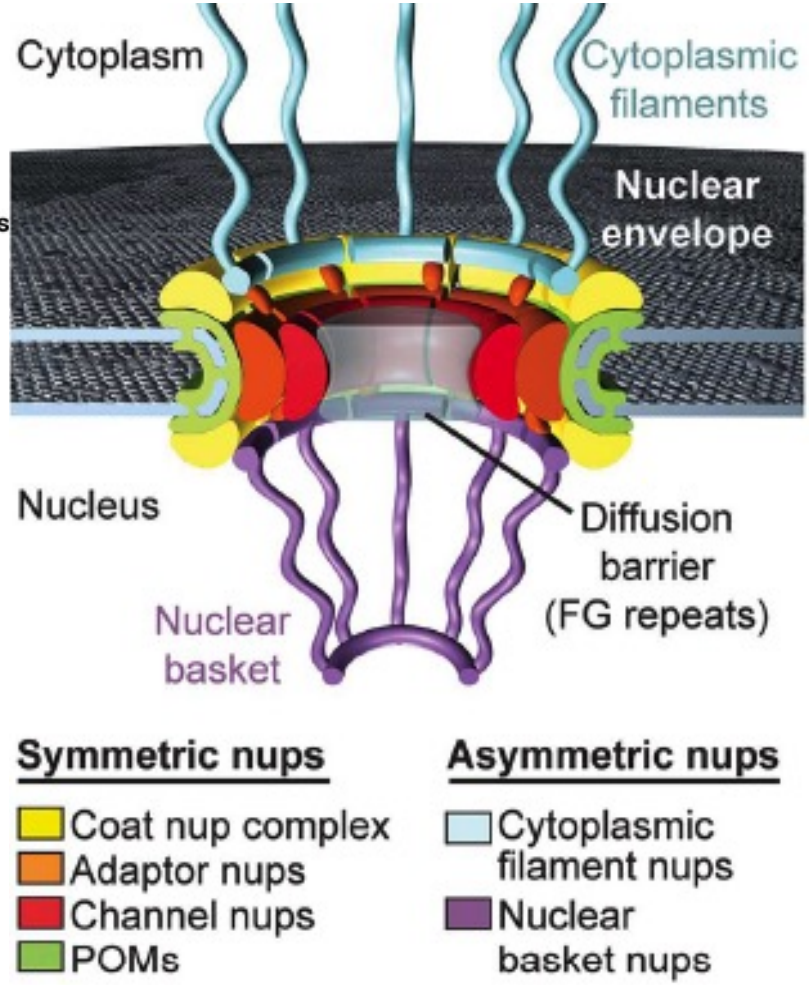


Röther and Sträßer, Nuclear Transport,2009

NUCLEAR PORE (NP)



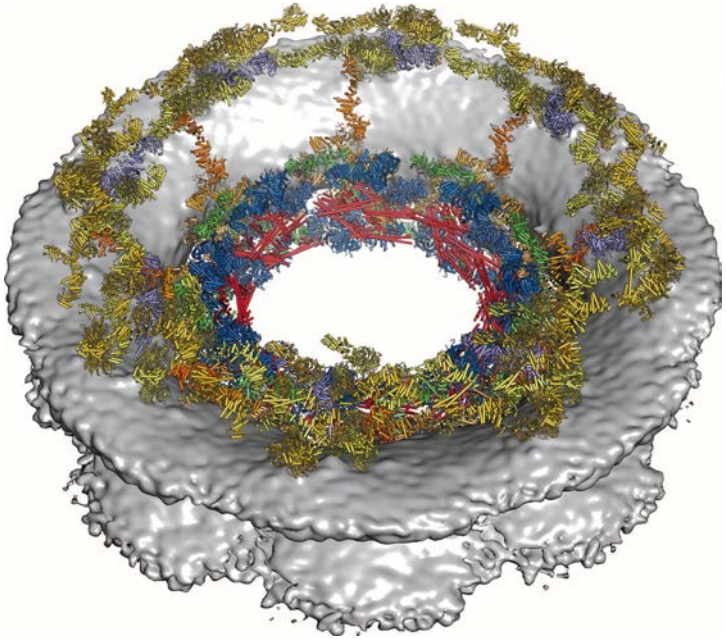


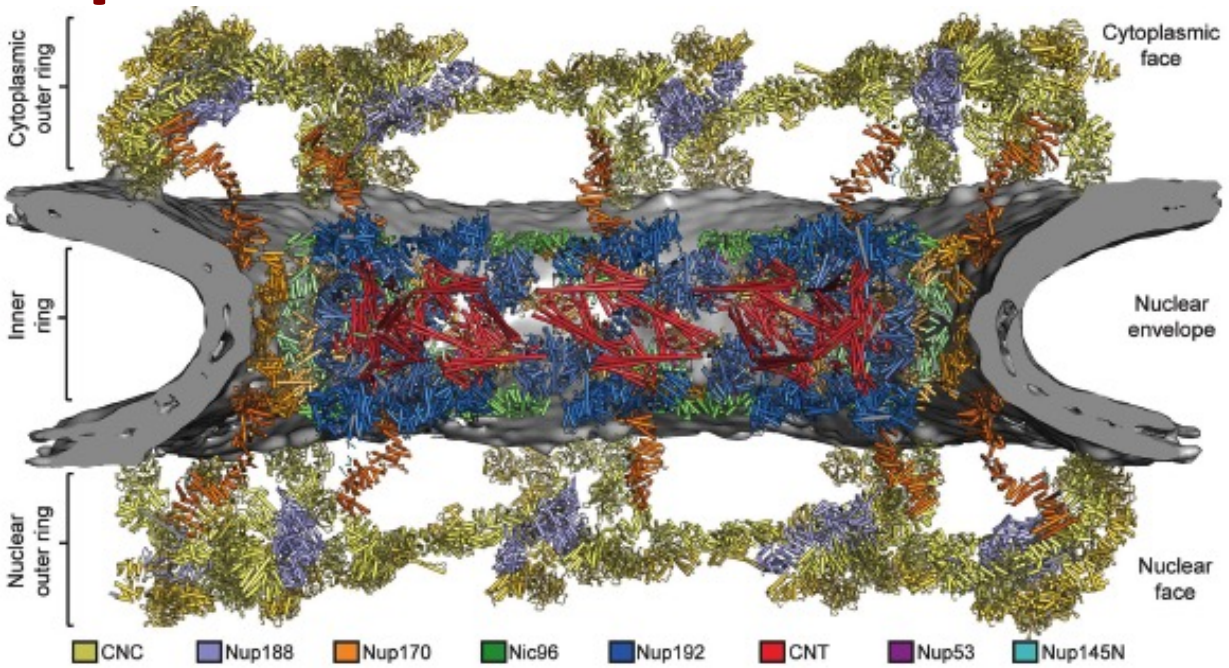


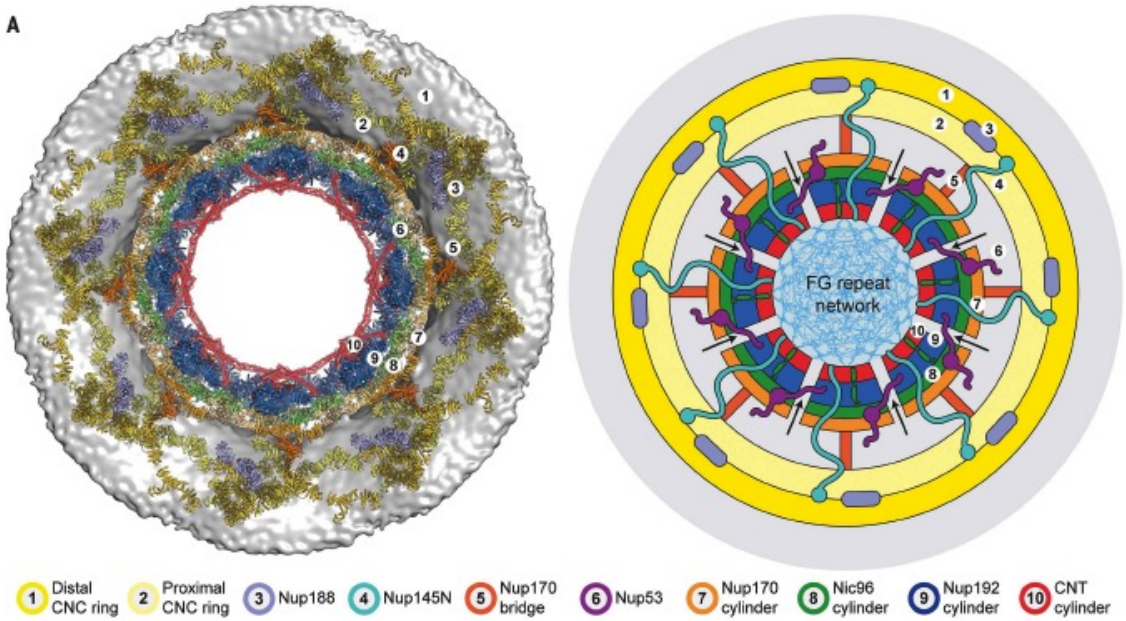
NUPs – nucleoporins
POM – pore membrane proteins
FG – nucleoporins with Phe-Gly-rich repeats

Lin et al, Science 2016

Nuclear pore architecture

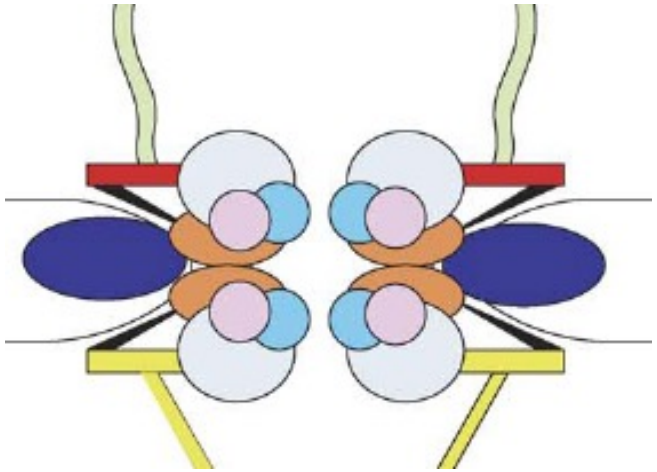




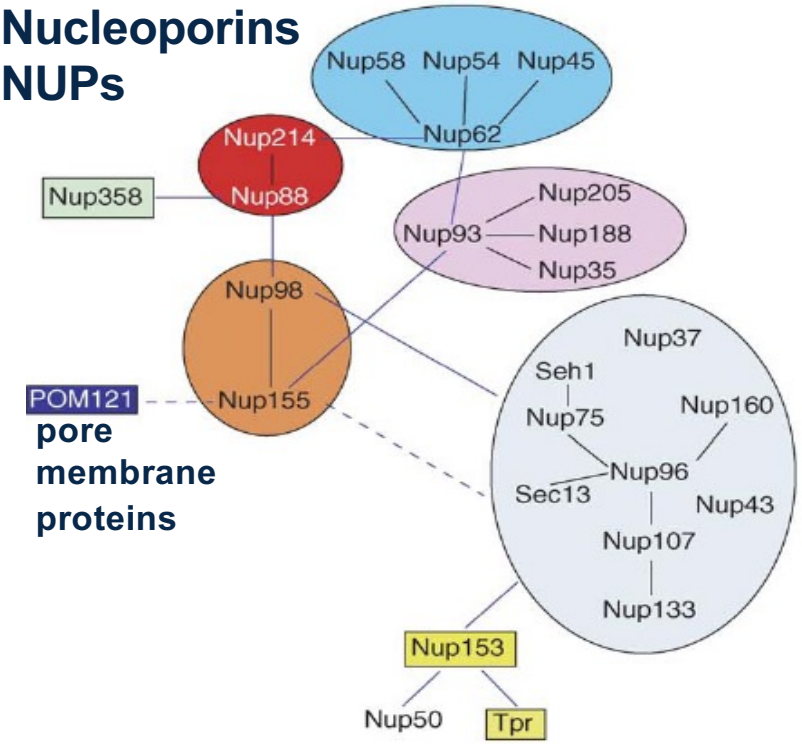


Lin et al, Science 2016;
Kosinski et al, Science 2016

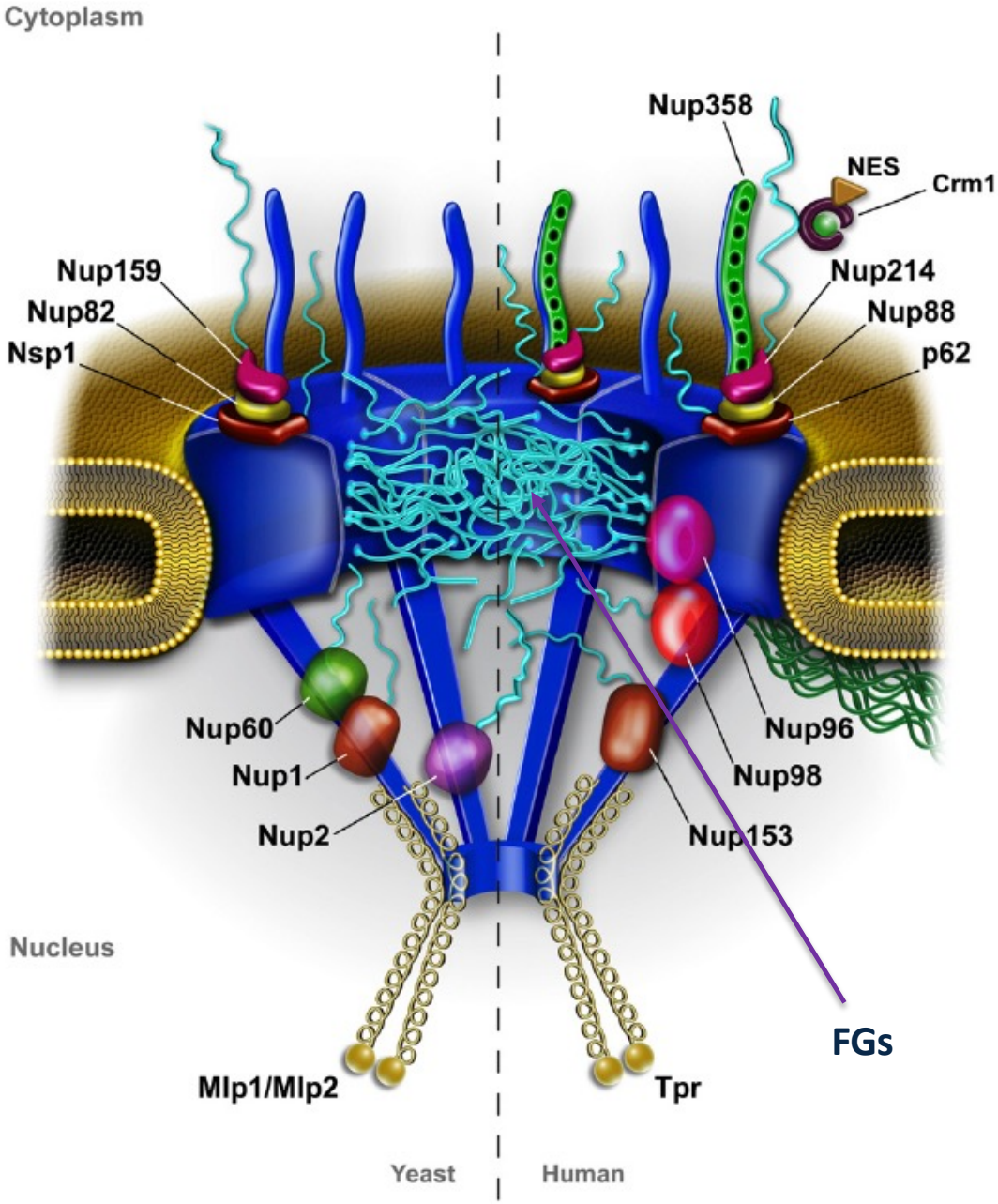
Nuclear pore complex (NPC)



nuclear pore proteins
Nucleoporins
NUPs



pore membrane proteins



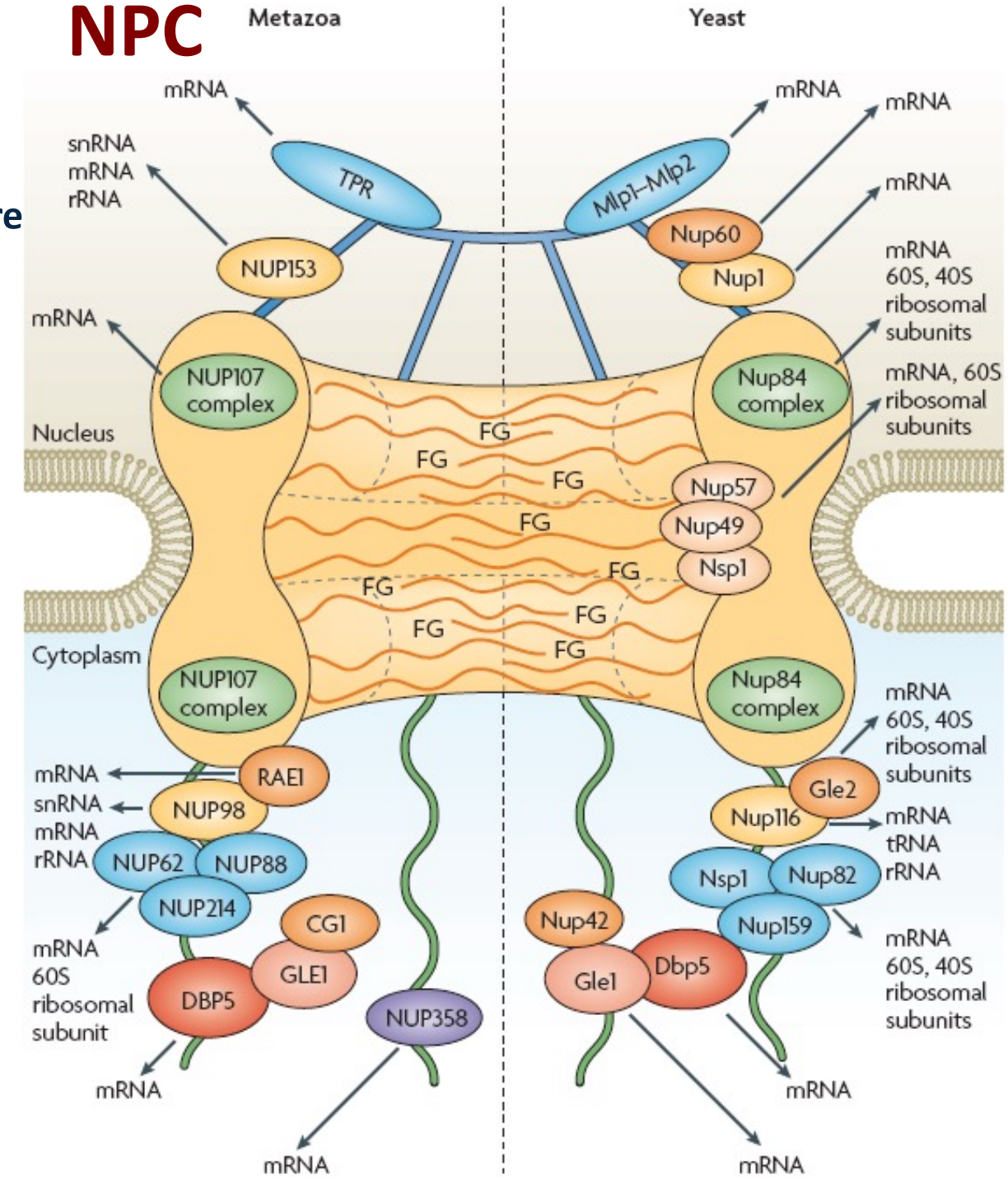
Köhler and Hurt, Cell,2010; Lim and Fahrenkrog, Cur.Op.Cel.Biol, 2006

- **large complex embedded in the nuclear envelope**
- **~125 nm diameter,**
- **125/60 MDa in metazoa/yeast**
- **8-fold symmetrical core structure**
- **~30 nucleoporins**

(8, 16 or even 32 copies per NPC)

FG nucleoporins contain Phe-Gly-rich repeats

NPC

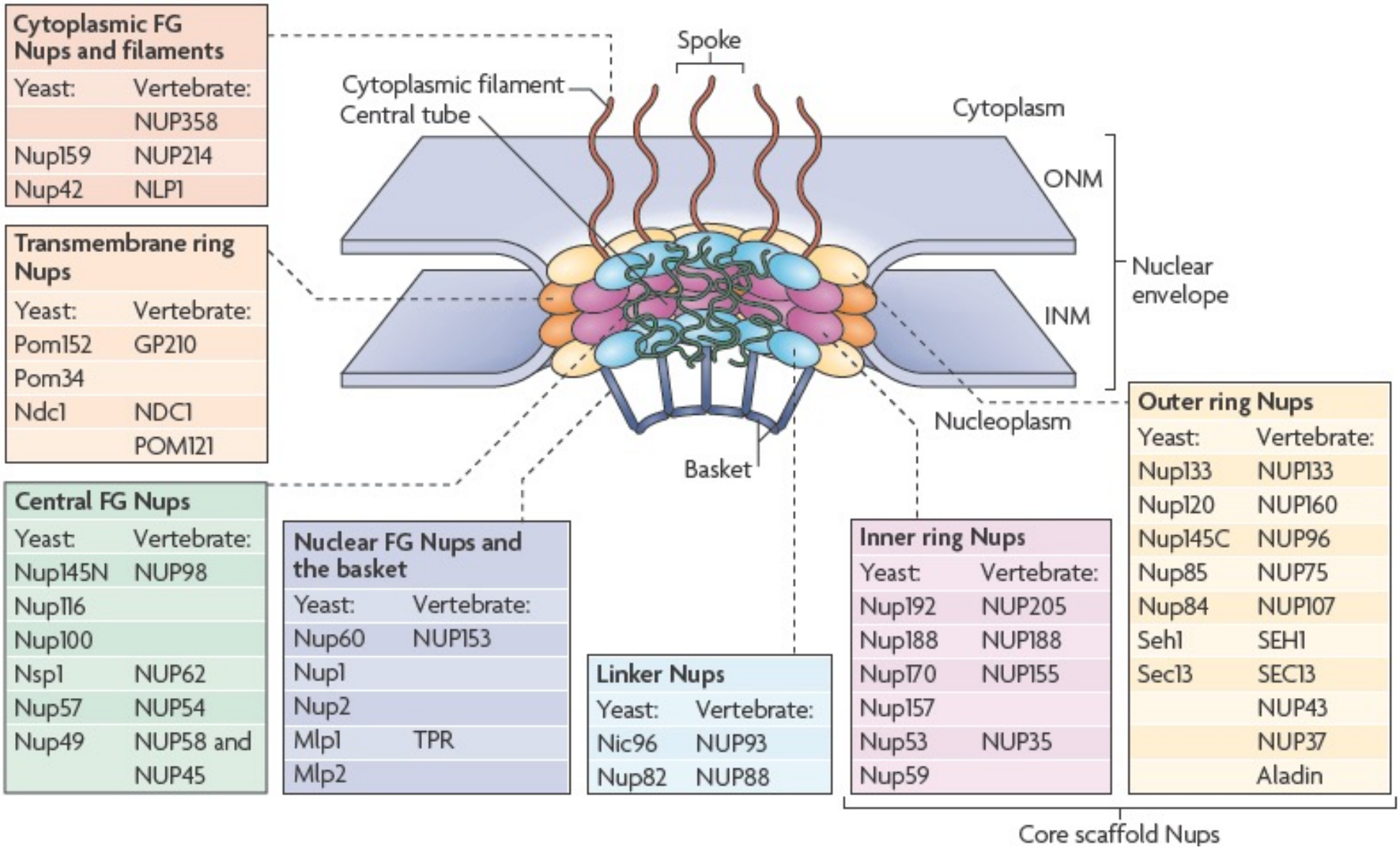


Nucleoporins

	POMs	non-FG nups	FG nups
Cytoplasmic fibrils		Nup82	Nup159 Nup42
Central framework & transport conduit	Pom34 Pom152 Ndc1	Nup157 Nup170 Nup188 Nup192 Nup84 Nup85 Nup120 Nup133 cNup145 Sec13 Seh1 Cdc31 Nic96 Gle1 Gle2	Nup49 Nup57 Nsp1 Nup100 nNup145 Nup116 Nup53 Nup59
Nuclear basket			Nup60 Nup1 Nup2

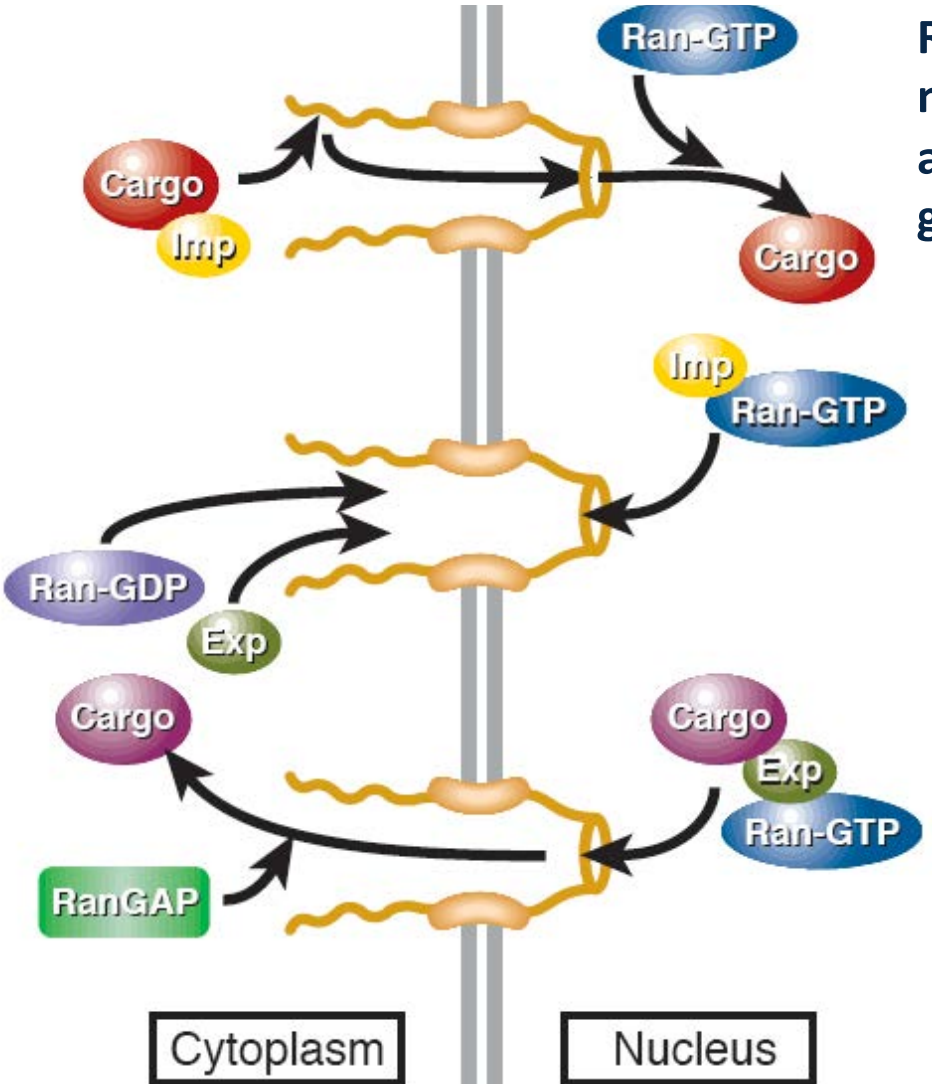
Köhler and Hurt, Nat.Rev.Mol.Cel.Biol, 2007

NPC

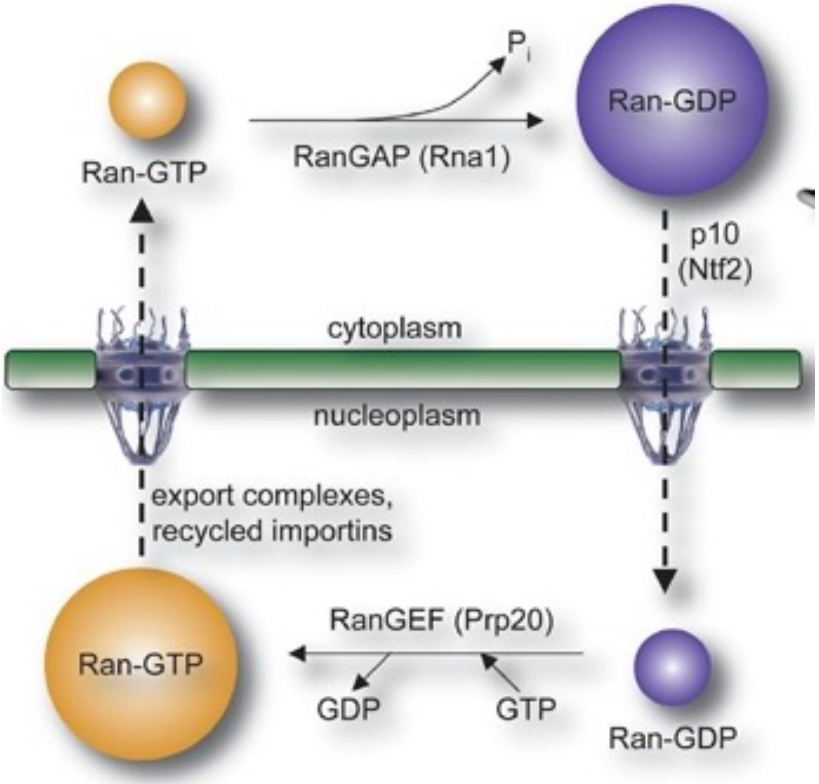


Strambio-De-Castillia et al., Nat.Rev.Mol.Cel.Biol,2007

Nucleoplasmic transport



Ran-GTP binds cargo/Exp or Imp in the nucleus. GTP hydrolysis releases the target and Ran-GDP in the cytoplasm. Ran-GDP goes back to the nucleus.

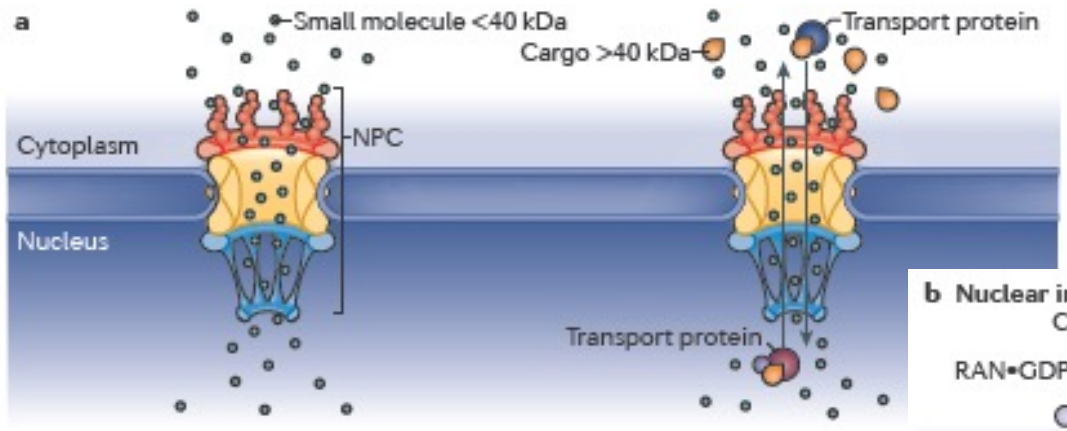


Cullen, J.Cell Sci.,2003; Patel, 200

The directionality of transport is governed by Ran-GTP gradient

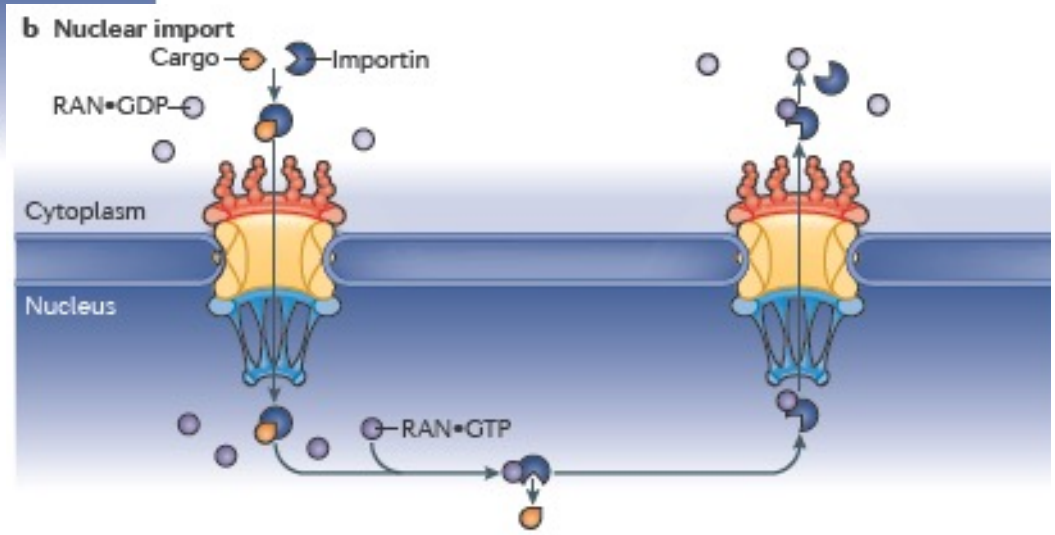
Asymmetric distribution of **RanGEF** *(Ran Guanine nucleotide Exchange Factor)* in the **nucleus** and **RanGAP** *(Ran GTPase activating protein)* in the **cytoplasm** ensures that **Ran-GTP** form is mainly in the **nucleoplasm** and **Ran-GDP** form in the **cytoplasm**.

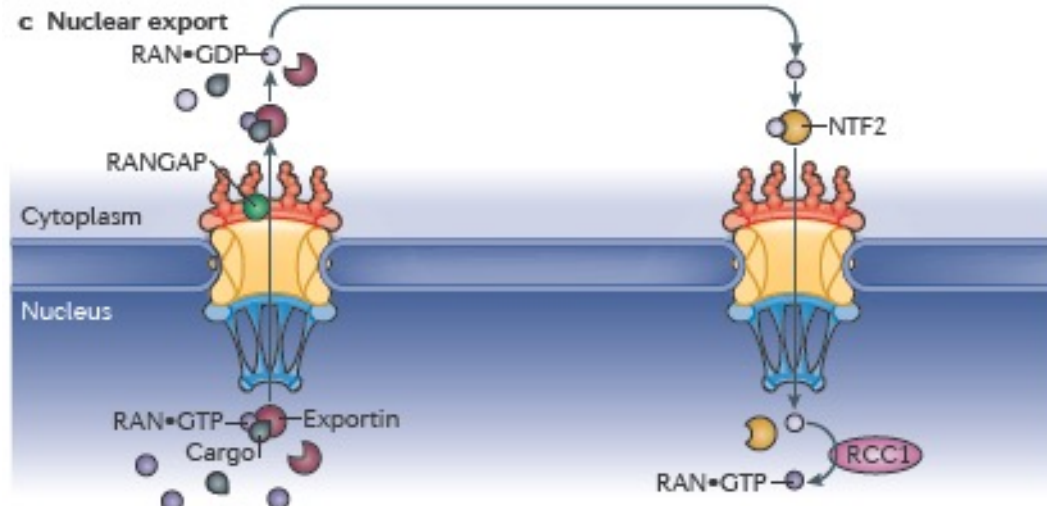
Nucleoplasmic transport



< 40 kDa
passive
diffusion

> 40 kDa
active transport
via transport
receptors



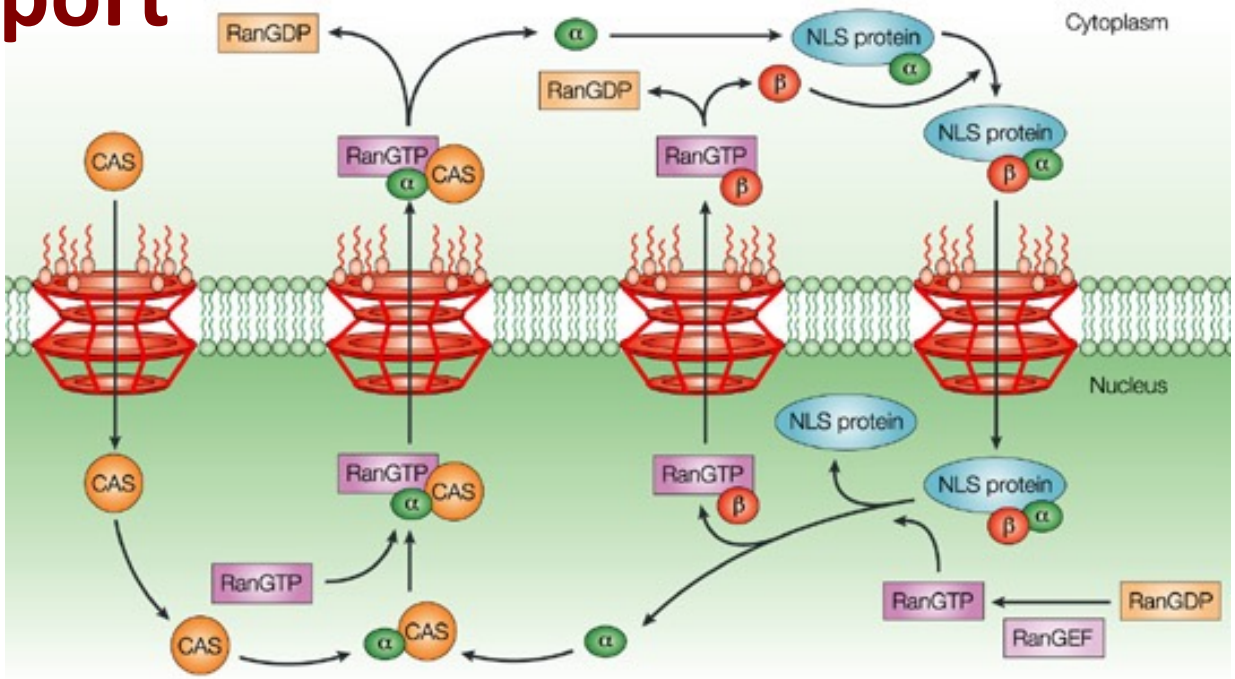


Protein transport

NLS

Nuclear Localization Signal

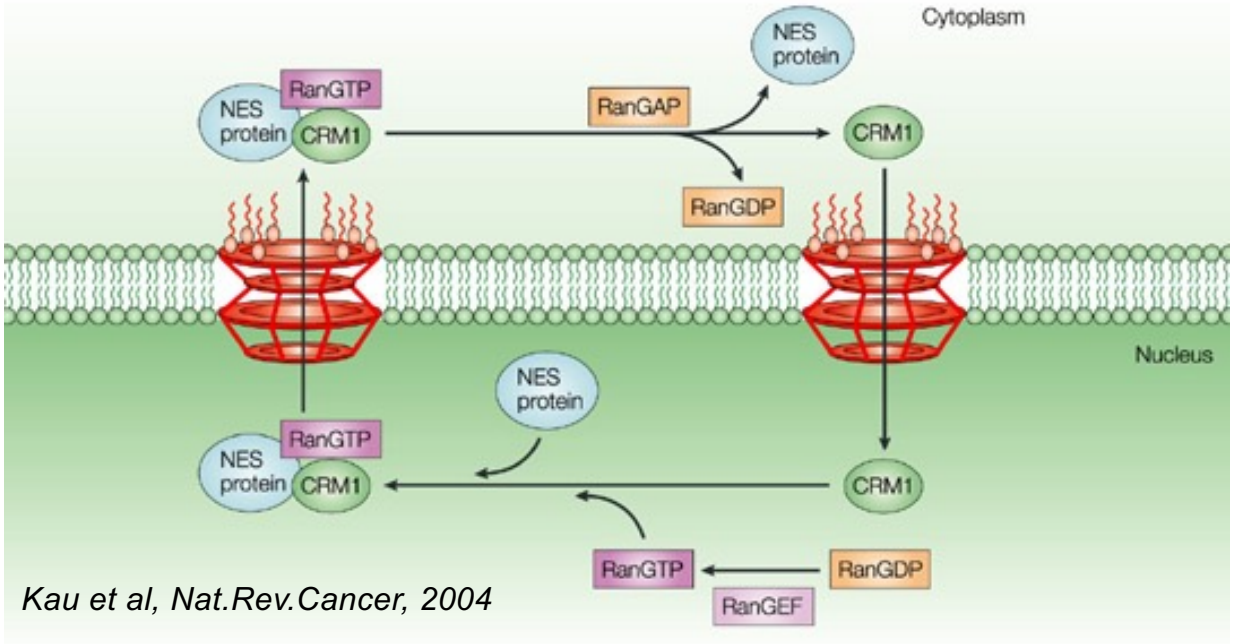
(binds Importins)



NES

Nuclear Export Signal

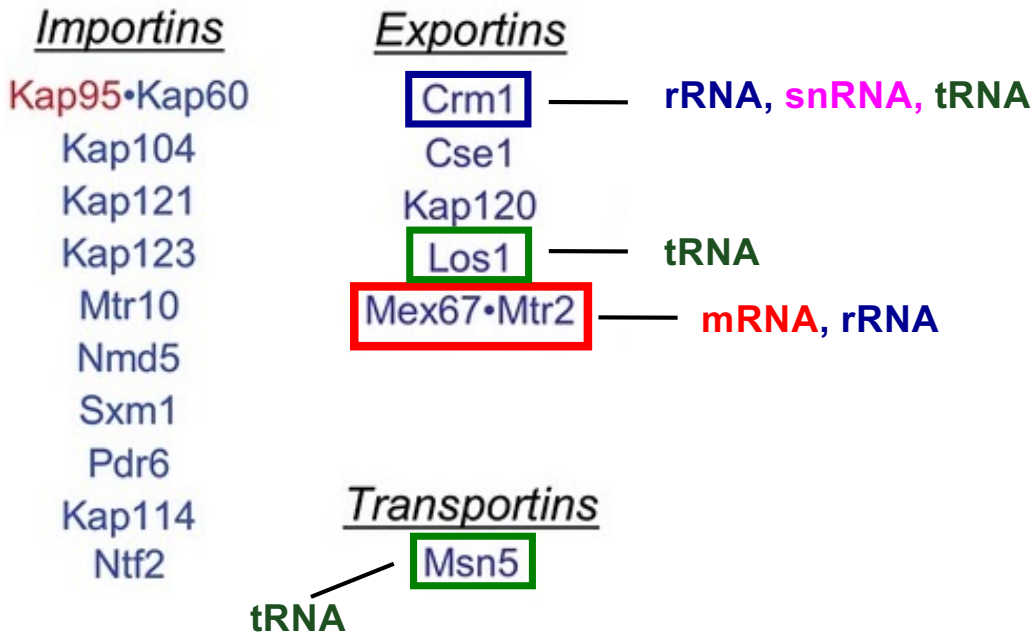
(binds Exportins and Ran-GTP)



Kau et al, Nat.Rev.Cancer, 2004

Nucleoplasmic transport

Transport factors in yeast



Transport receptor	mammals
Kapβ1	
Kapβ1–Kapα complex (Impβ–Impα)	
Kapβ1–snurportin complex	
Kapβ1–XRIPα complex	
Karyopherin-β–Imp7 heterodimer	
Karyopherin-β–RanBP8 heterodimer	
Kapβ2 (transportin)	
Karyopherin-5	
Transportin SR	
CRM1 (exportin)	
CAS	
Exportin-t	

In general, transport is mediated by members of the karyopherin family of nuclear transport factors, importins and exportins, and depends on RanGTP-RanGDP gradient.
Export of tRNA, rRNA, miRNA and snRNA uses karyopherin exportins.
mRNA export uses Mex67-Mtr2 (TAP-p15) export receptors unrelated to karyopherins and is independent of the Ran cycle.

mRNA nuclear export machinery

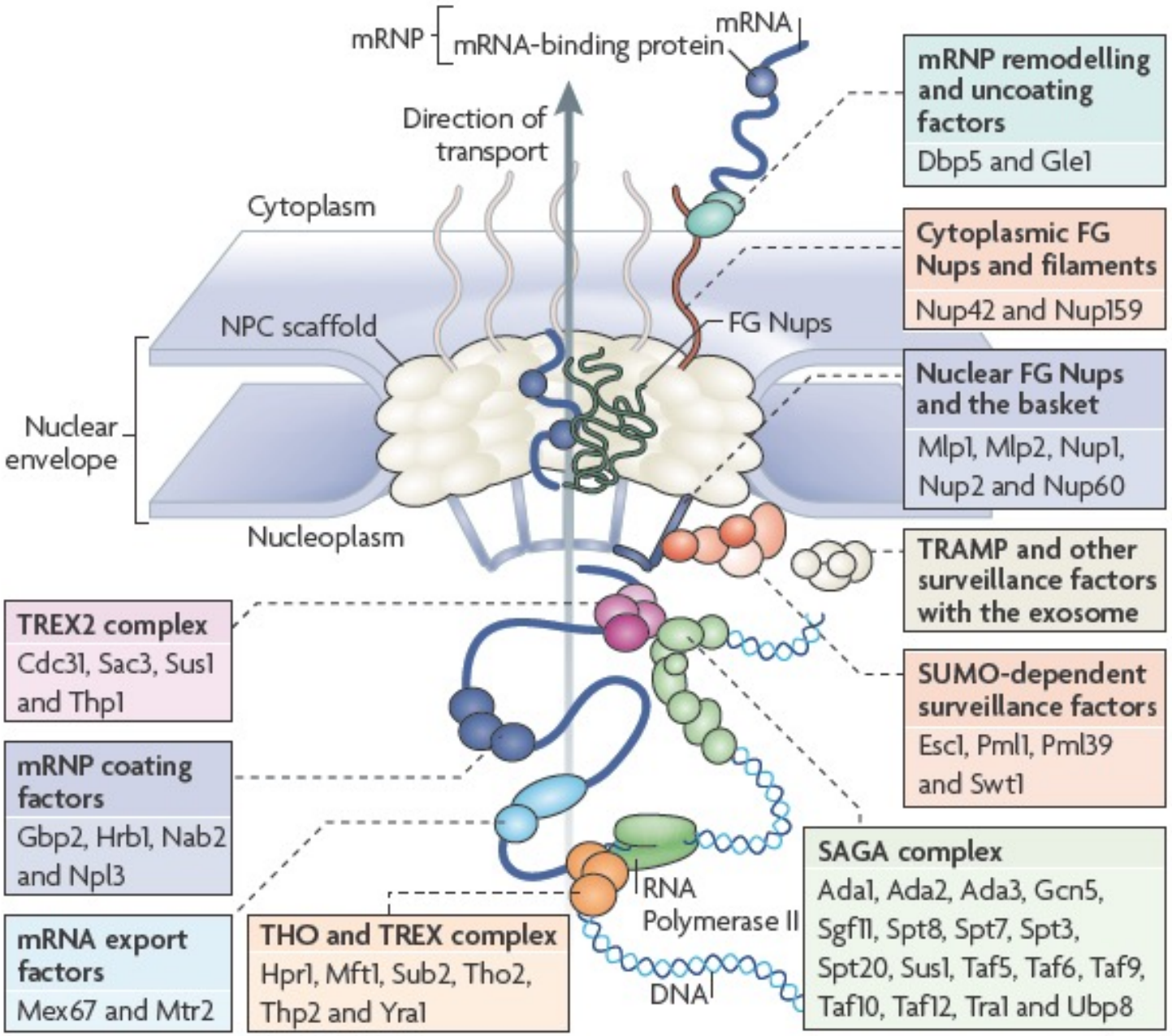
Component		Function
Yeast	Metazoan	
Mex67–Mtr2	NXF1–NXT1	Facilitates bulk mRNA transport through NPCs
Yra1	ALY (REF)	Adaptor linking Mex67–Mtr2 to mRNA
Sub2	UAP56	DEAD-box helicase involved in assembly of export-competent mRNPs
Nab2	–	Binds polyA-mRNA and Mlp1; modulates length of 3′ polyA tail
Mlp1	TPR	Nuclear basket protein to which Nab2 binds
TREX	TREX	Complex involved in coordinating transcription and
TREX-2	TREX-2	Complex that targets actively expressing genes to NPCs
Dbp5 (Rat8)	DDX19	DEAD-box helicase involved in disassembly of mRNP export complex at NPC cytoplasmic face
Gle1	GLE	Enhances Dbp5 activity
Gfd1	–	Enhances Dbp5 activity
Nup159 (Rat7)	NUP214	Located on NPC cytoplasmic face; binds Dbp5

Mex67-Mtr2 – major mRNA export factor; Mtr2 – required for Mex67 association with NPC
Yra1 – export adaptor between Mex67 and mRNA
Nab2 – poly(A) binding protein; Npl3 - RS, shuttling RNA-binding protein
Sub2 – helicase, assembles mRNP, recruits cotranscriptionally Yra1 to mRNAs
Dbp5 – remodels mRNPs as they emerge from NPC
Sac3 – associates with Sub2 and Mex67-Mtr2, in complex with Tho1 (trx elongation)
Gle2 – NPC-associated mRNA export factor binds to NPCs via Nup116
Mtr10 – importin for Npl3

THO/TREX and **TREX-2 complexes – coordinate transcription, processing and export**
EJC (metazoan)

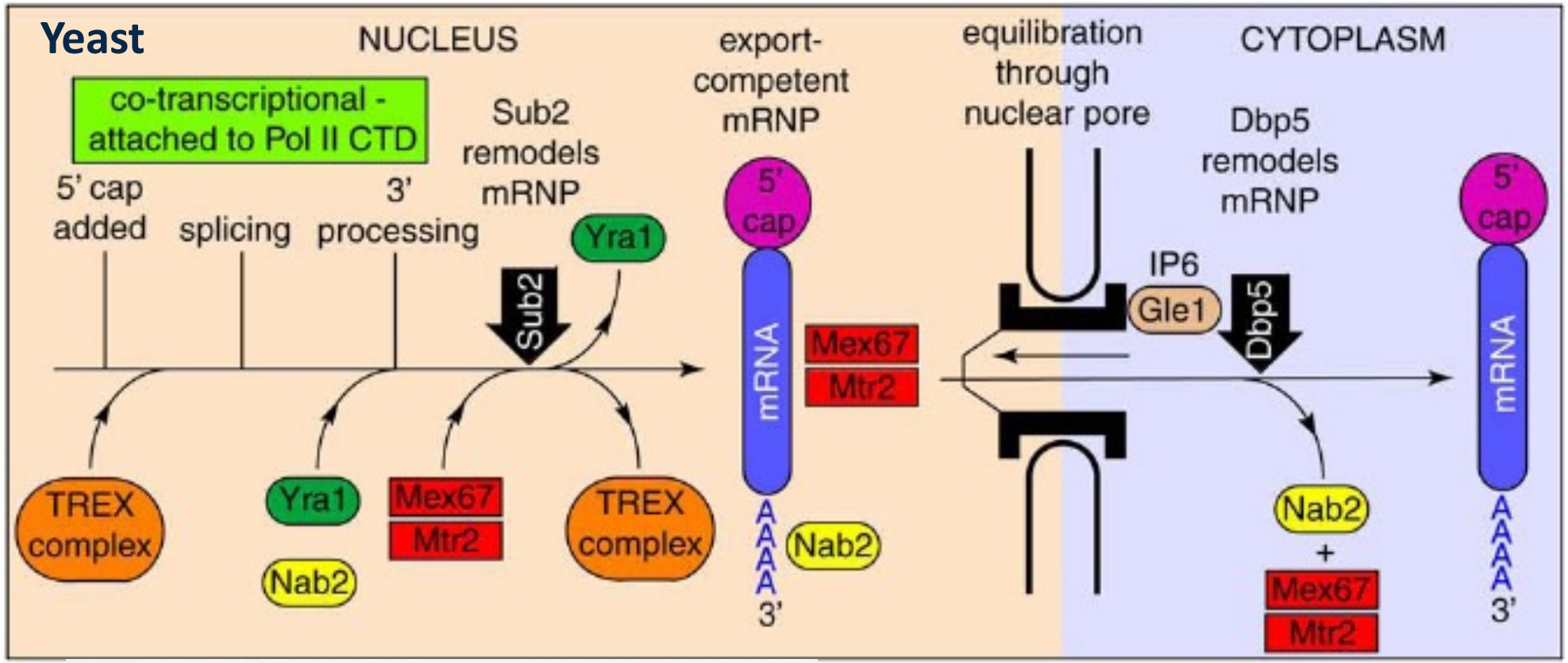
Murray, TiBS,2010

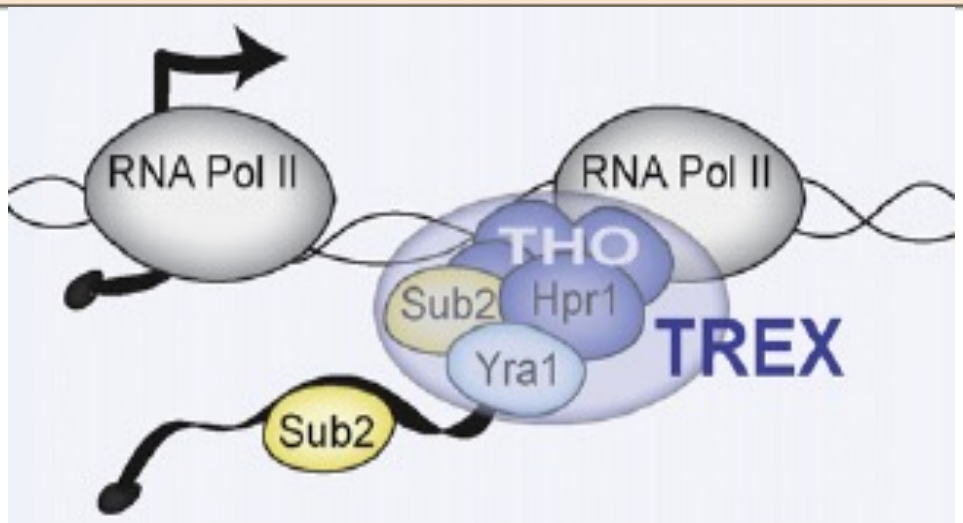
mRNA export – all factors



Strambio-De-Castillia et al., Nat.Rev.Mol.Cel.Biol,2007

RNA export – co- or post- transcriptional





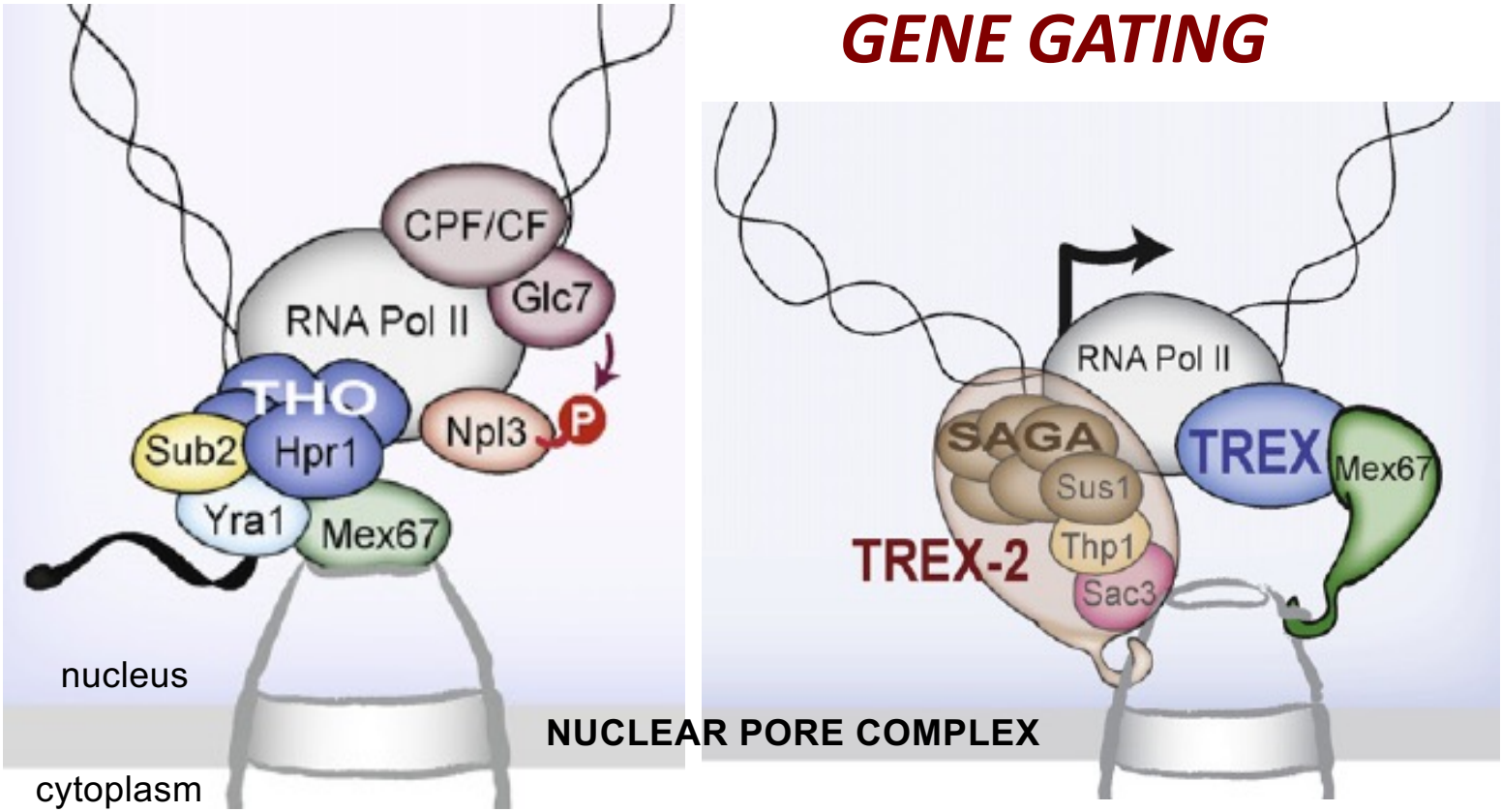
mRNA export machinery is recruited co-transcriptionally to nascent transcripts via Pol II

Murray, TiBS,2010;
Iglesias and Stutz, FEBS Lett, 2008

Co-transcriptional mRNA export

GENE GATING

Yeast



Iglesias and Stutz, FEBS Lett, 2008

SAGA histone acetyltransferase complex (including **Spt, Ada, Gcn5**); transcription activation

THO mRNP biogenesis and export: **Hpr1, Mft1, Tho2** and **Thp2** (human **THOC1-7**)

TREX transcription-export complex: **THO/Sub2/Yra1,** interacts with NPC via Mex67-Mtr2

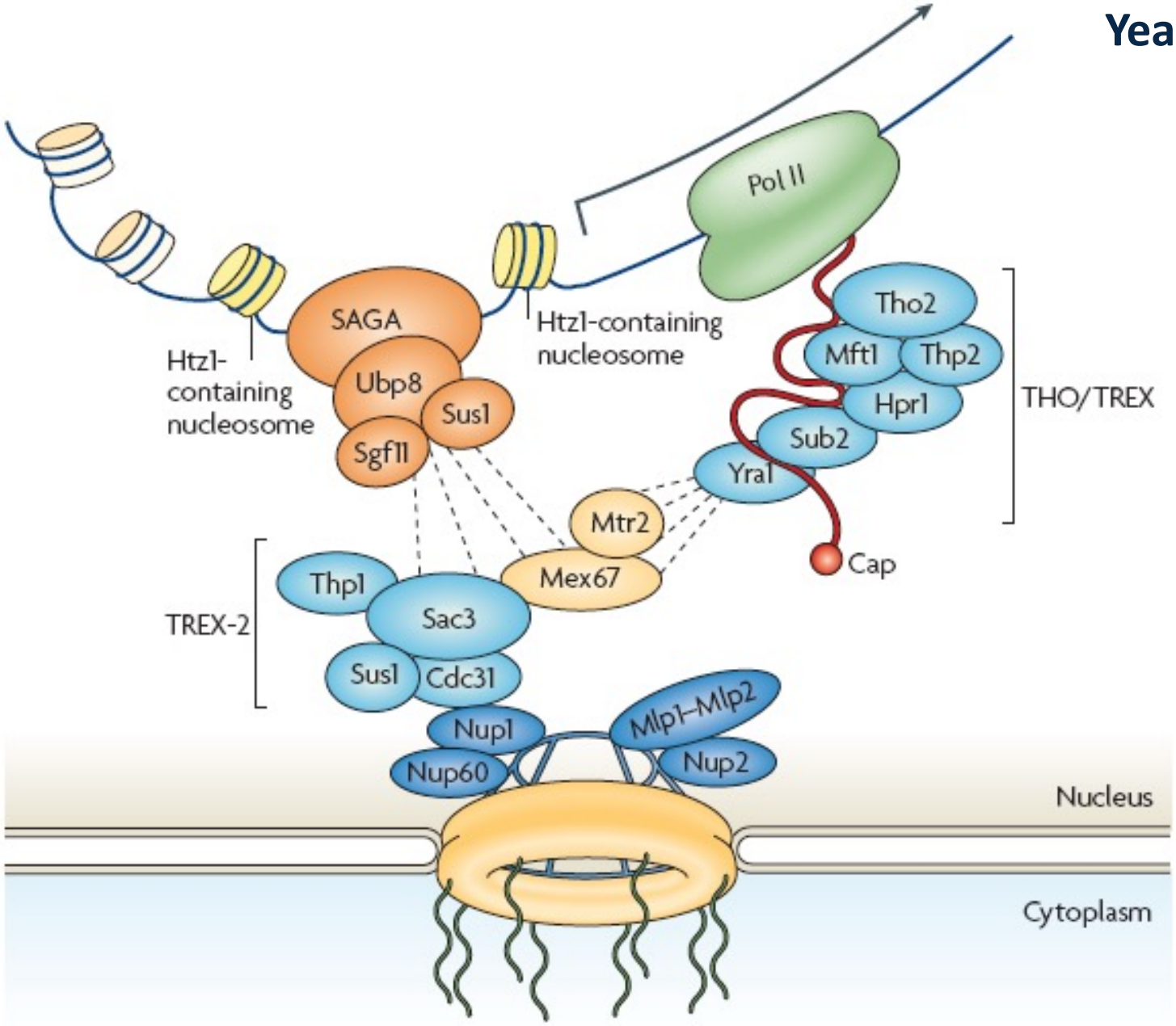
TREX-2 transcription-export complex: **Cdc31/Thp1/Sac3** and **Sus1** from **SAGA**

TREX-2 and TREX complexes link transcription (Pol II via THO, initiation complex SAGA via Sus1) to export receptors (Mex67, Yra1) and NPC

Co-transcriptional mRNA export

GENE GATING

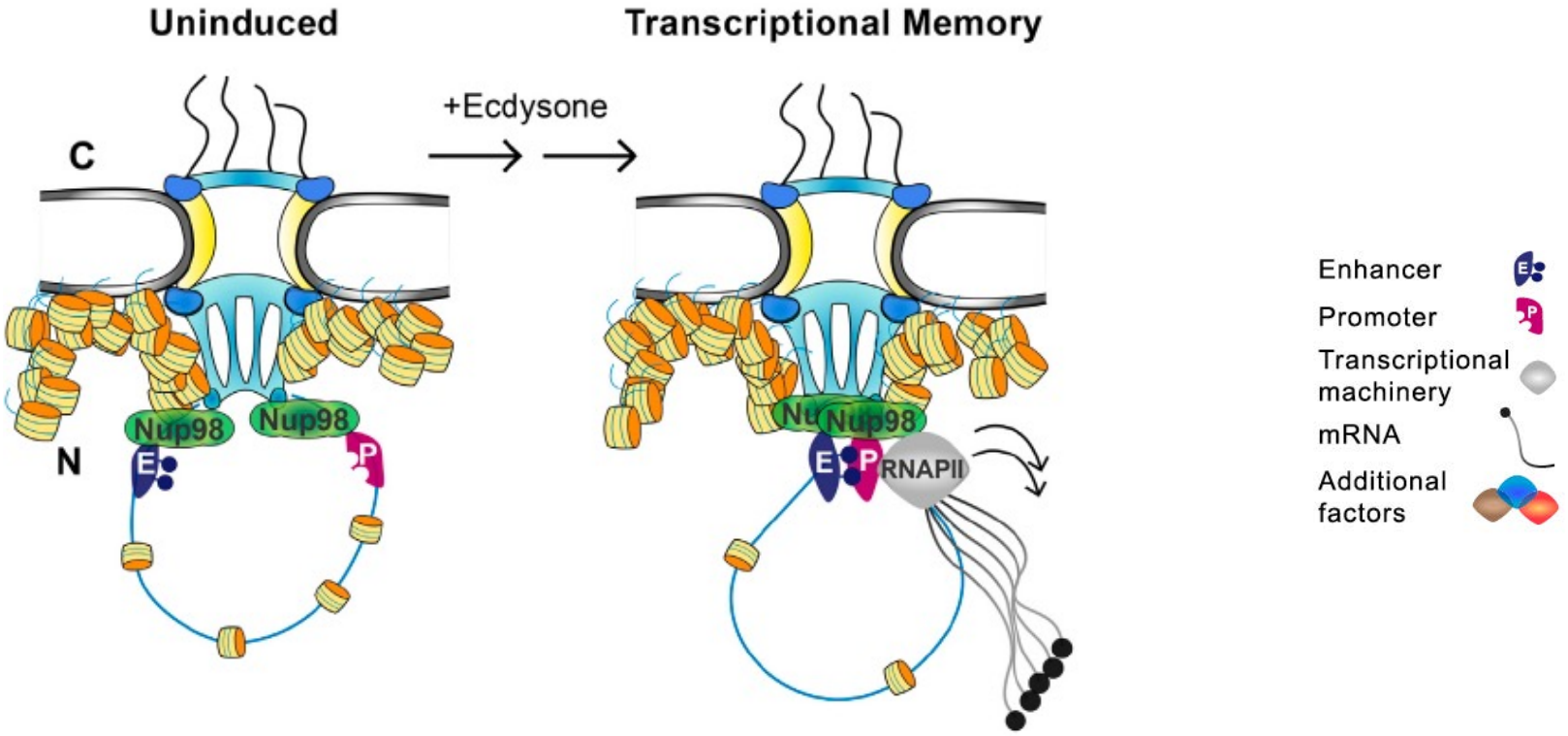
Yeast



Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

GENE GATING

Metazoa

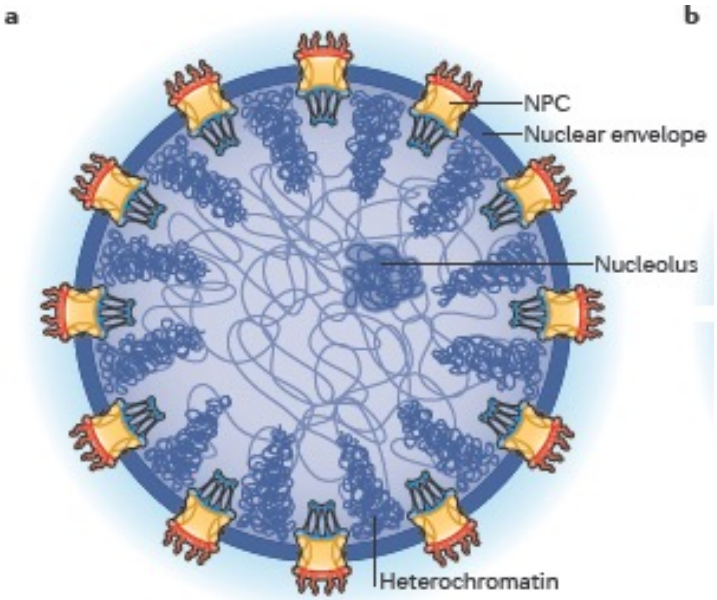


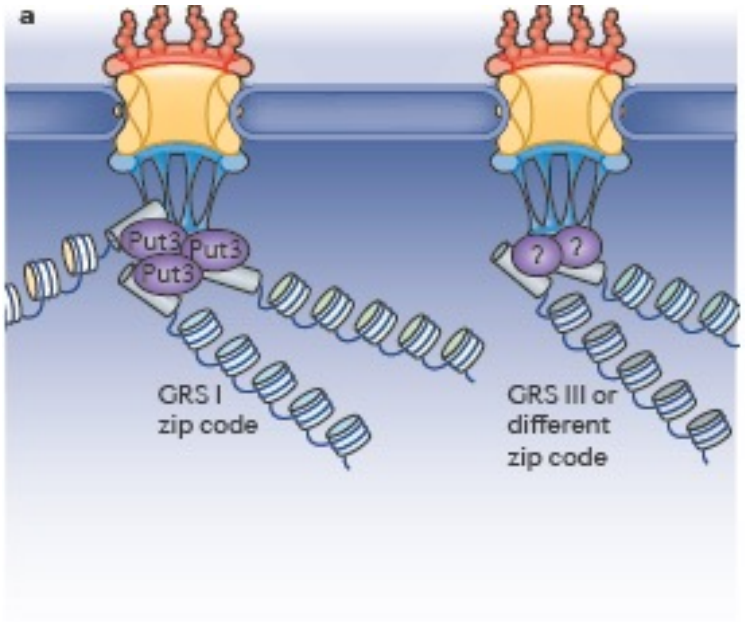
- Nuclear pore proteins (NUPs) bind promoters and enhancers in *Drosophila*
- Nup98 mediates enhancer-promoter looping of inducible genes
- Inducible genes stably associate with nuclear pores in silent and active states

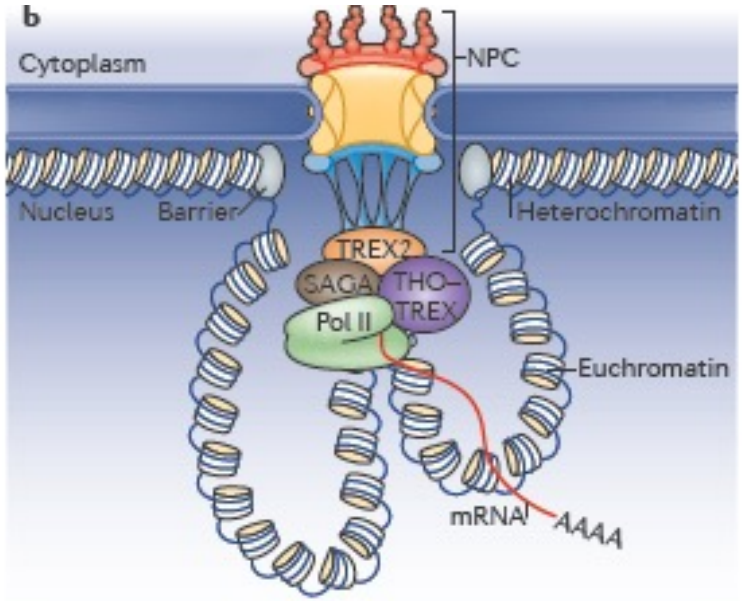
Pascual-Garcia et al, Mol Cell 2017

Active euchromatin is targeted to the NPC

Heterochromatin is excluded from the NPC







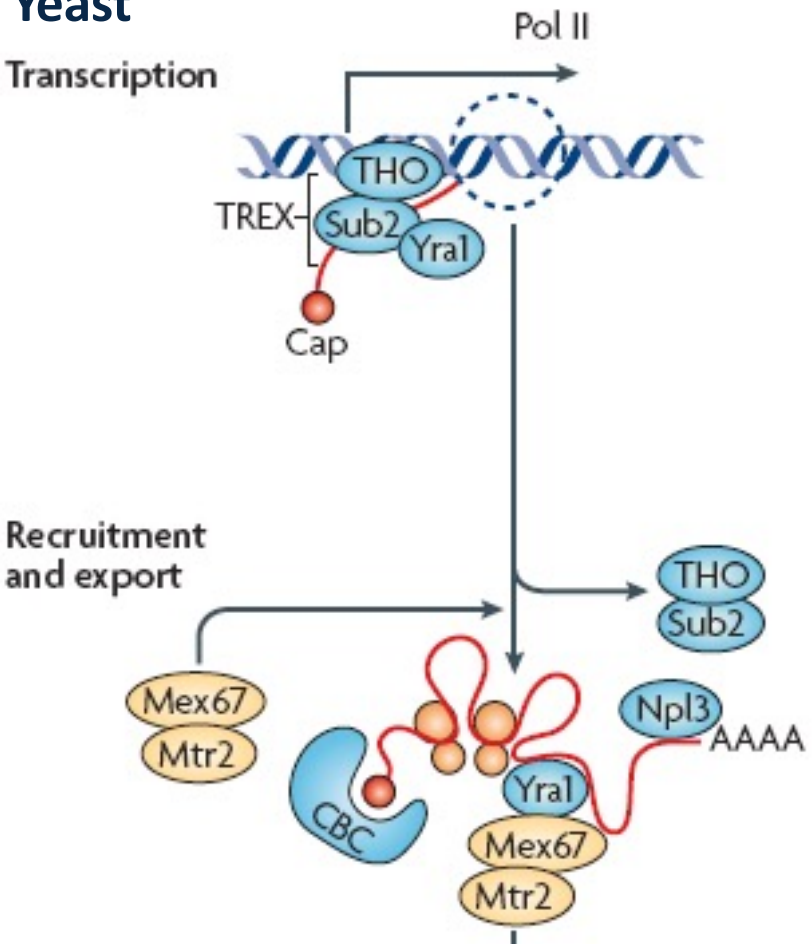
cis-acting DNA zip codes (GRS, gene recruitment sequences), are located in gene promoters and interact with NPC

SAGA, TREX-2 and THO/TREX interact with NPC

mRNA export (nuclear side)

transcription-coupled

Yeast

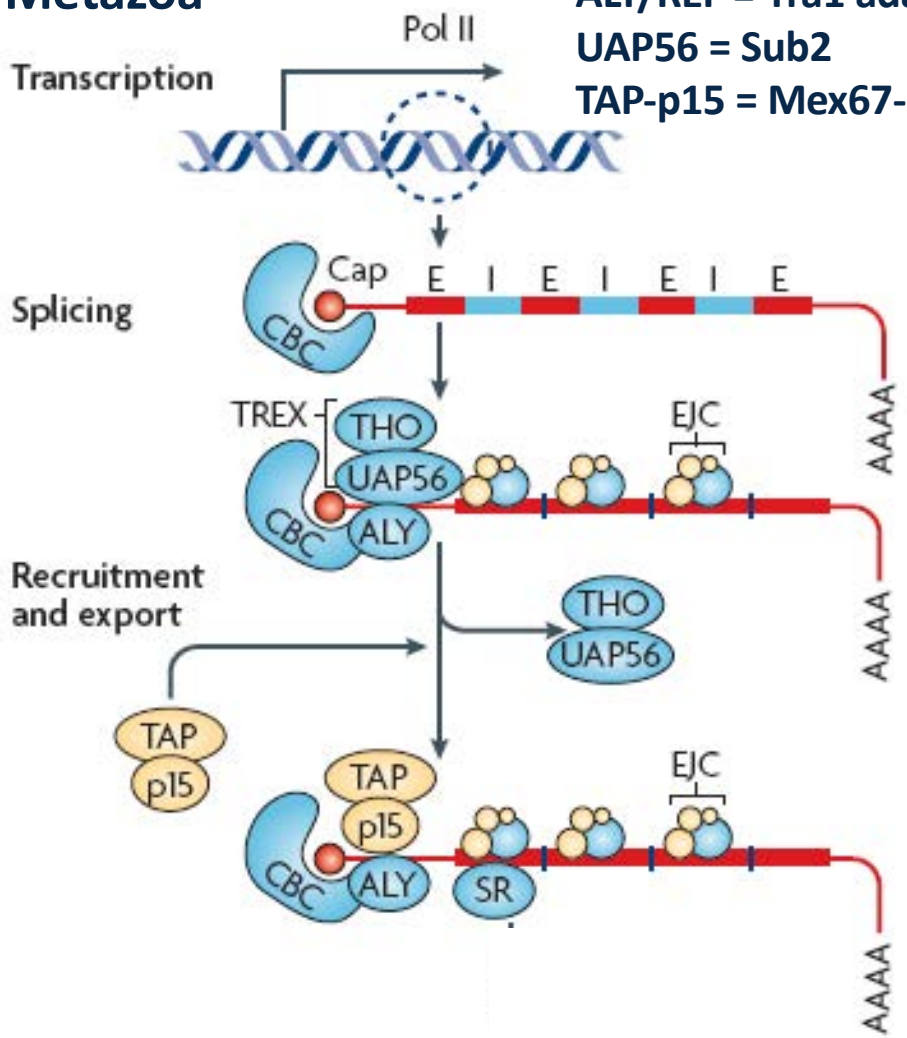


The nascent transcript is co-transcriptionally assembled onto pre-mRNP by the THO/TREX complex (with Yra1 and Sub2).
The Mex67-Mtr2 mRNA export receptor is recruited to the mRNP via adaptor Yra1.

splicing-coupled

Metazoa

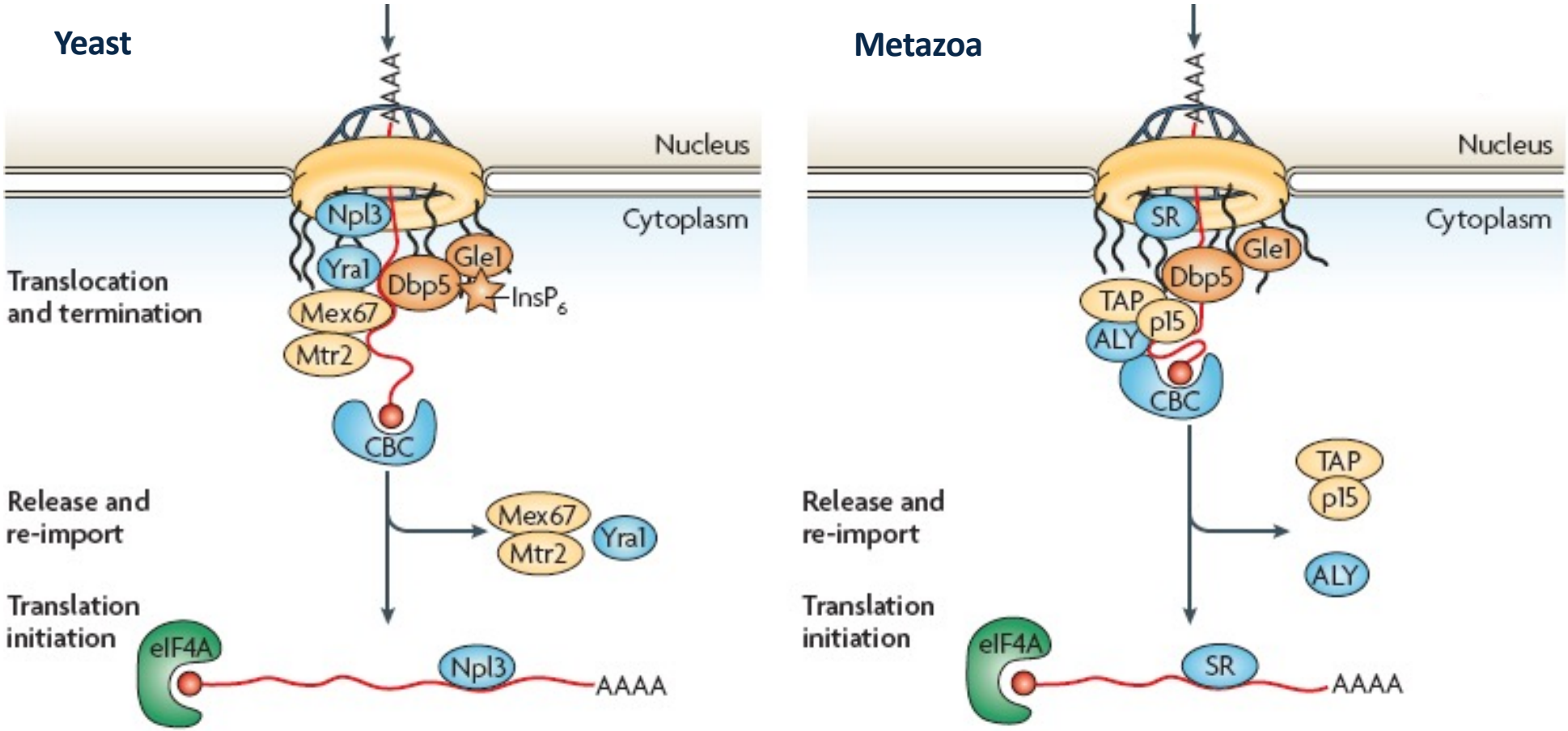
ALY/REF = Yra1 adaptor
UAP56 = Sub2
TAP-p15 = Mex67-Mtr2



Human TREX is recruited to mRNP in a splicing- and cap-dependent modes.
ALY/REF, UAP56 and TAP-p15 associate with EJC.

Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

mRNA export (cytoplasmic side)



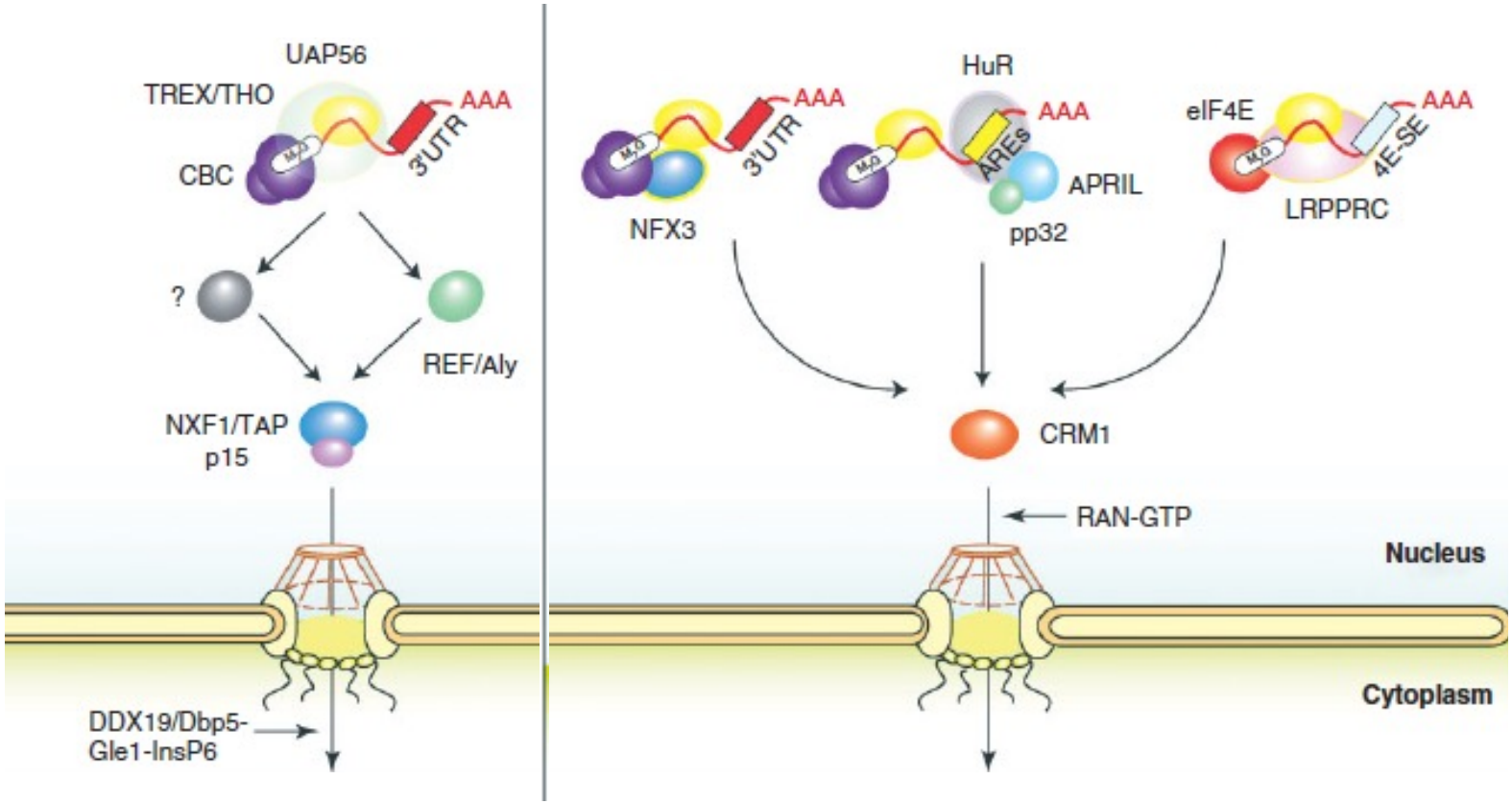
Unidirectional movement of RNPs from the nucleus to the cytoplasm requires RNP remodeling and release by RNA helicases and GTPases.

mRNP is remodeled and released from NPC by the ATP-dependent RNA helicase Dbp5. Dbp5 activity is stimulated by Gle1 activator and the signaling molecule inositol hexakisphosphate ($InsP_6$).

Mex6-Mtr2 dissociates and mRNA is recruited to the translation initiation machinery via cap-eIF4A.

Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

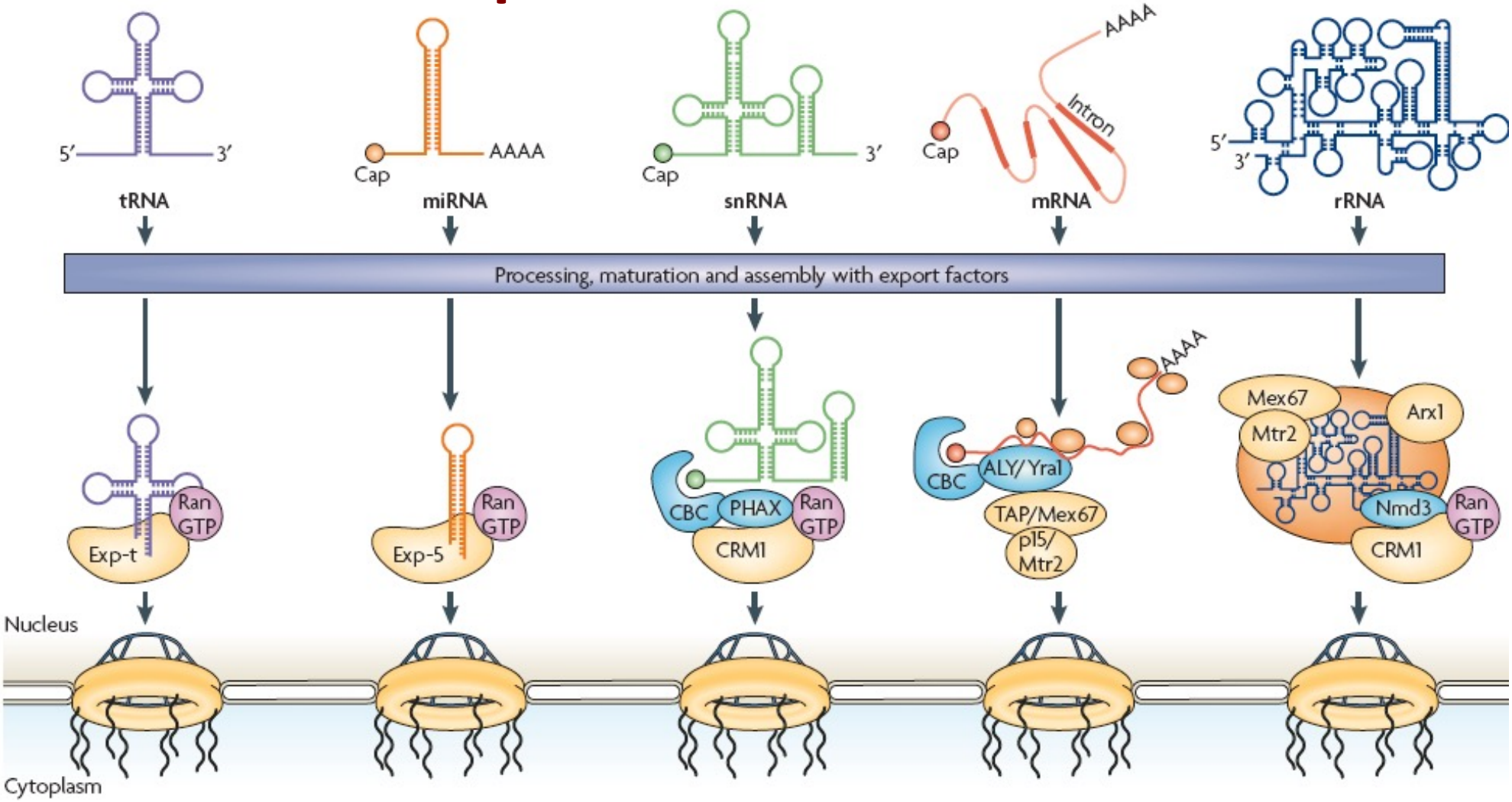
mRNA nuclear export - summary



Unique features of mRNA export:

- **Mex67-Mtr2 (TAP-p15) transport receptors are structurally unrelated to karyopherins and independent of the RanGTP-RanGDP gradient.**
- **mRNA export receptors cooperate with other factors: adaptors (Yra1/ALY/REF, SR proteins) and release factors**
- **some mRNAs can be exported via the Crm1 RanGTP-dependent pathway** (protooncogenes, cytokines with AU-rich elements, viral mRNAs).

Export of other RNAs

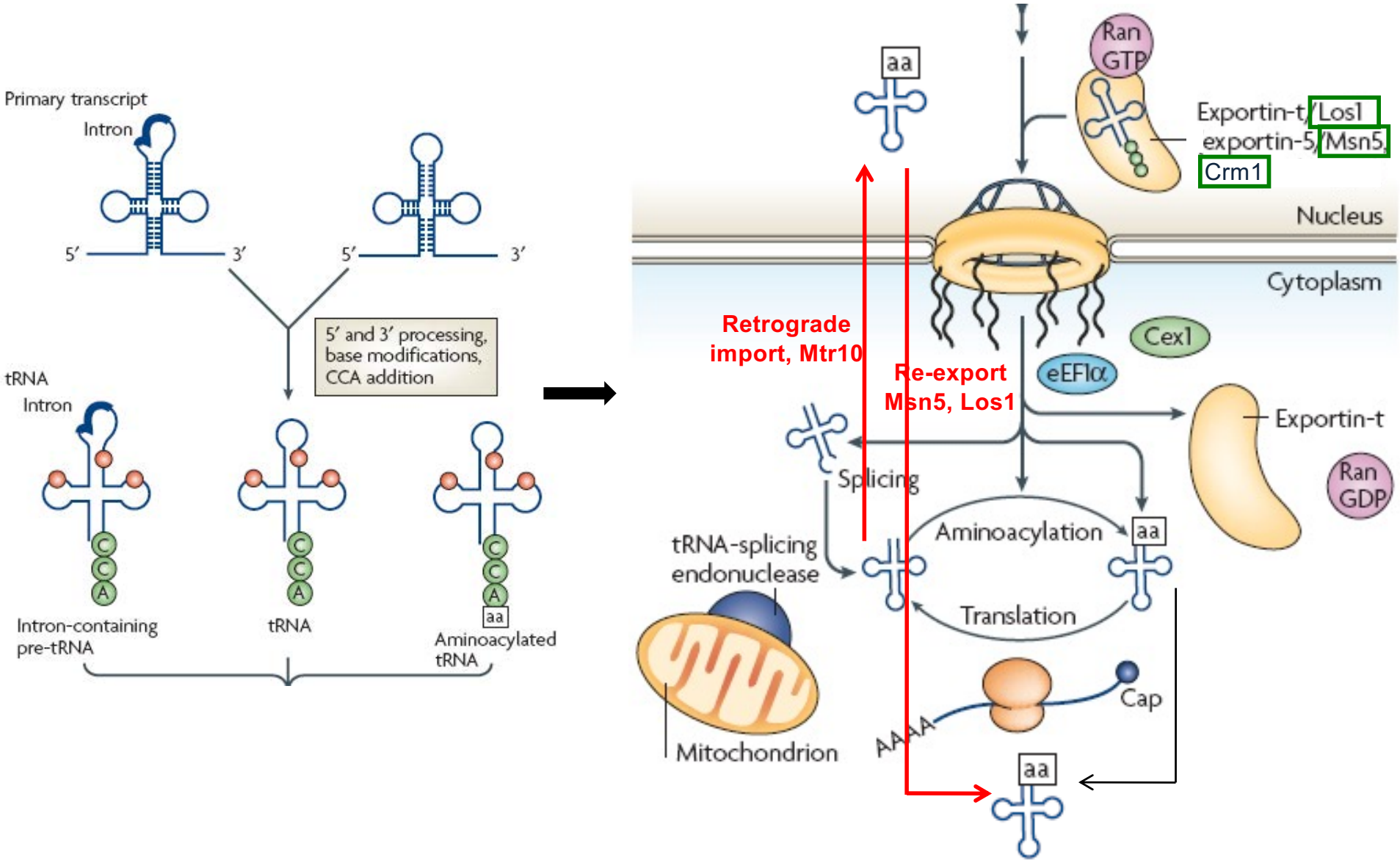


- **Similar general scheme, involves exportins** (karyopherin family) **and Ran cycle.**
- **mRNA export mechanistically different: uses a transport receptor unrelated to karyopherins and does not directly depend on the RanGTP-RanGDP gradient. mRNA export receptors cooperate with other factors: adaptors, release factors**

Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

tRNA export

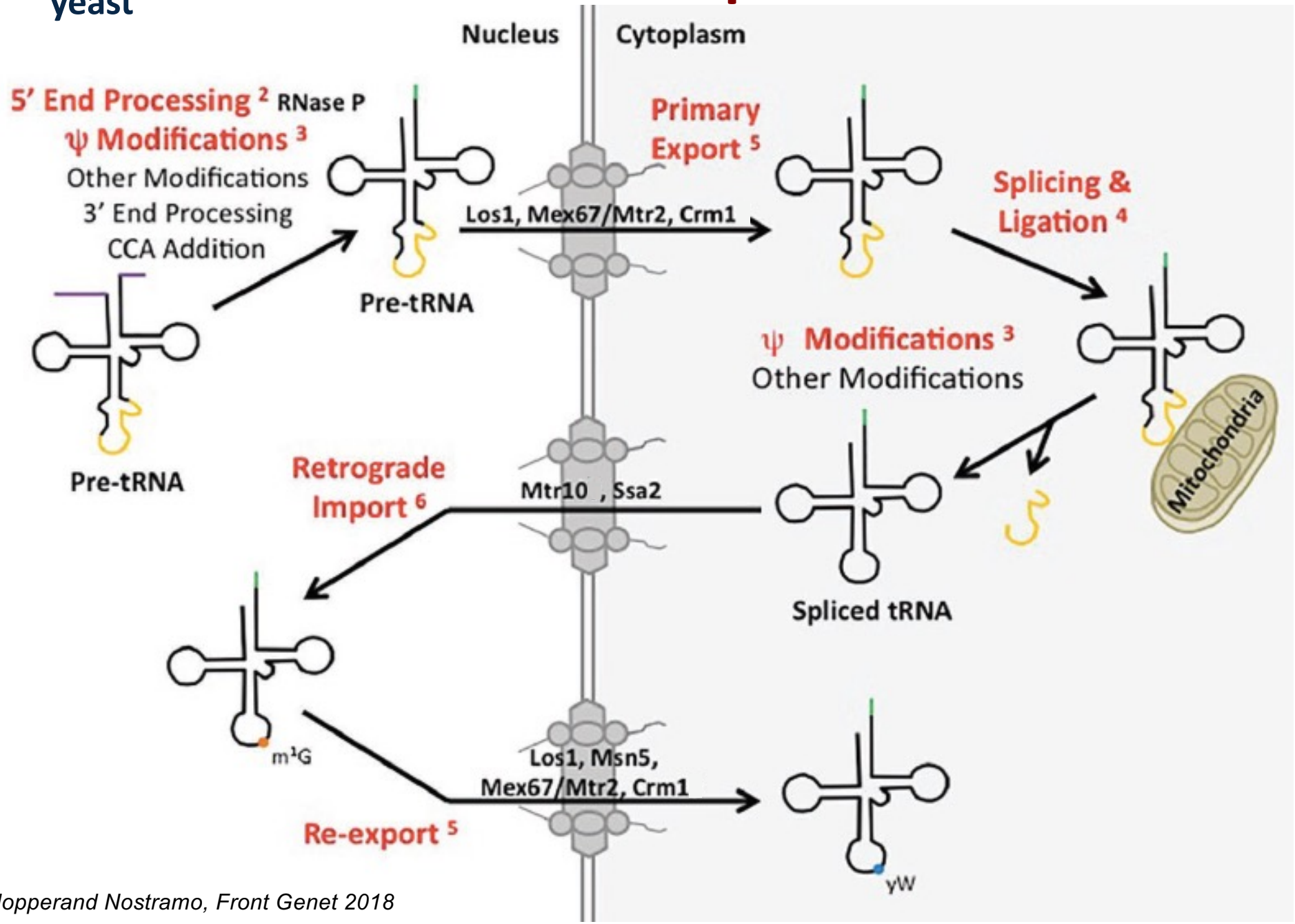
yeast



Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

tRNA export

yeast

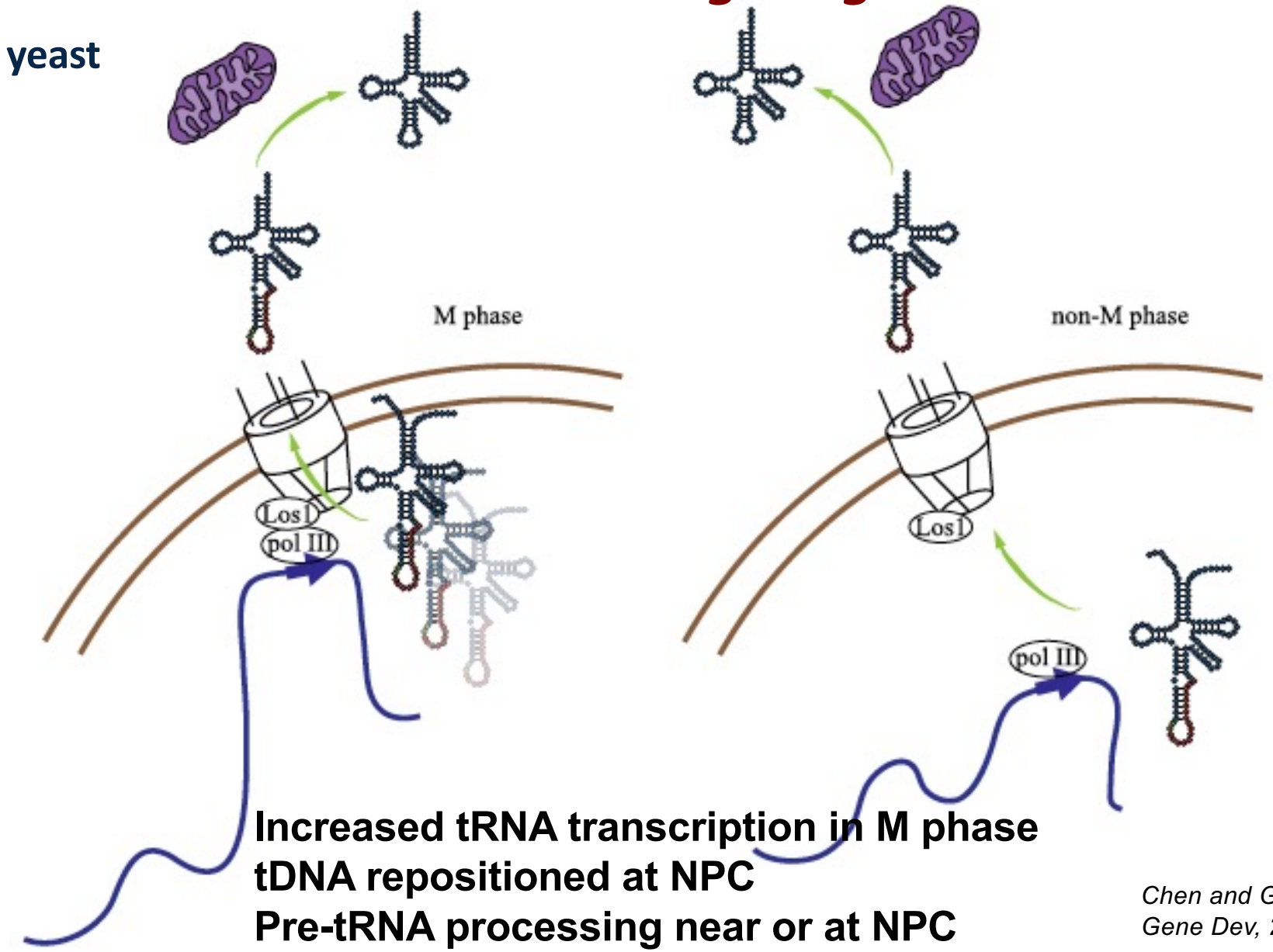


Hopperand Nostramo, Front Genet 2018

Co-ordinated tRNA transcription and export

tRNA gating

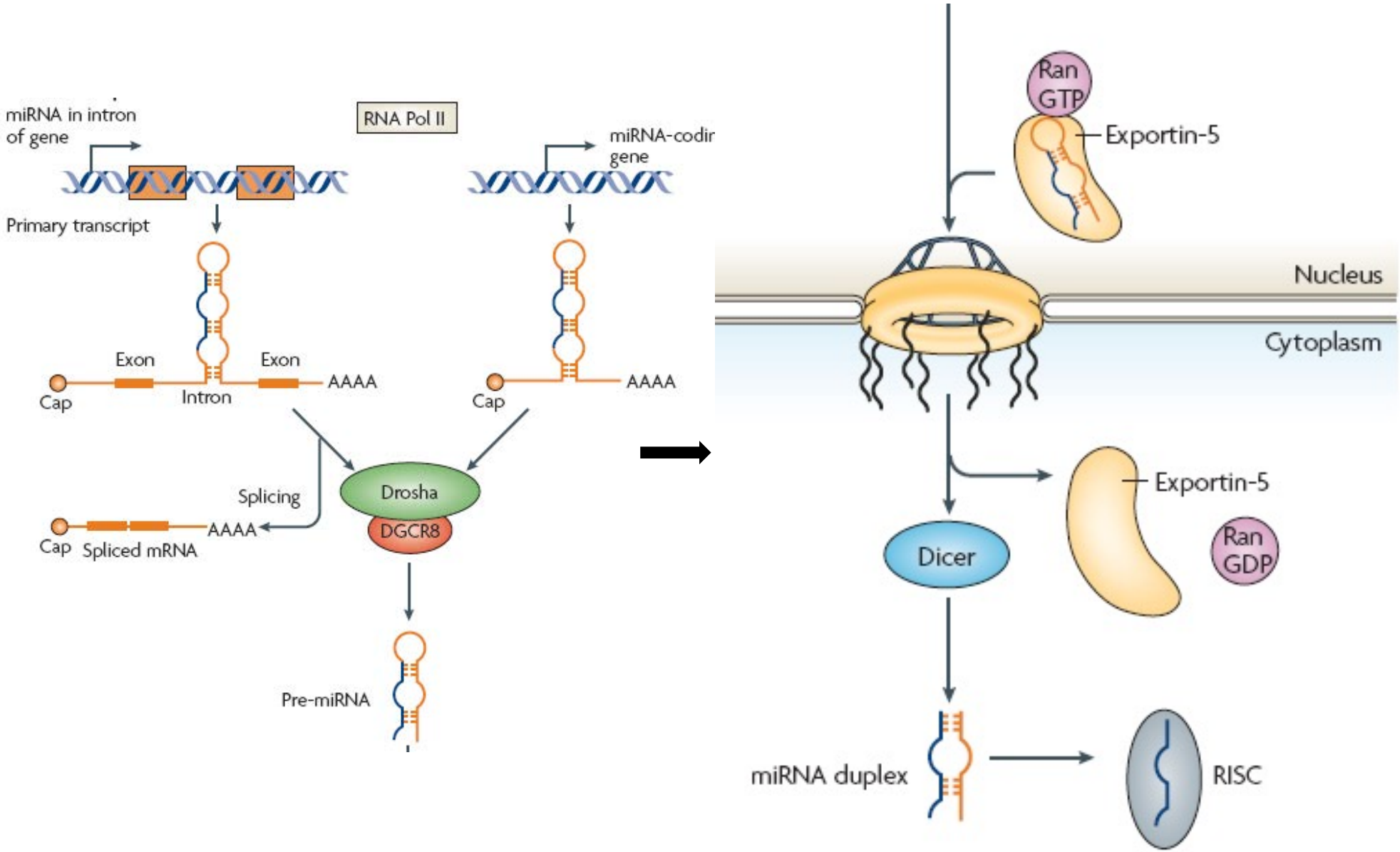
yeast



Increased tRNA transcription in M phase
tDNA repositioned at NPC
Pre-tRNA processing near or at NPC

Chen and Gartenberg
Gene Dev, 2014

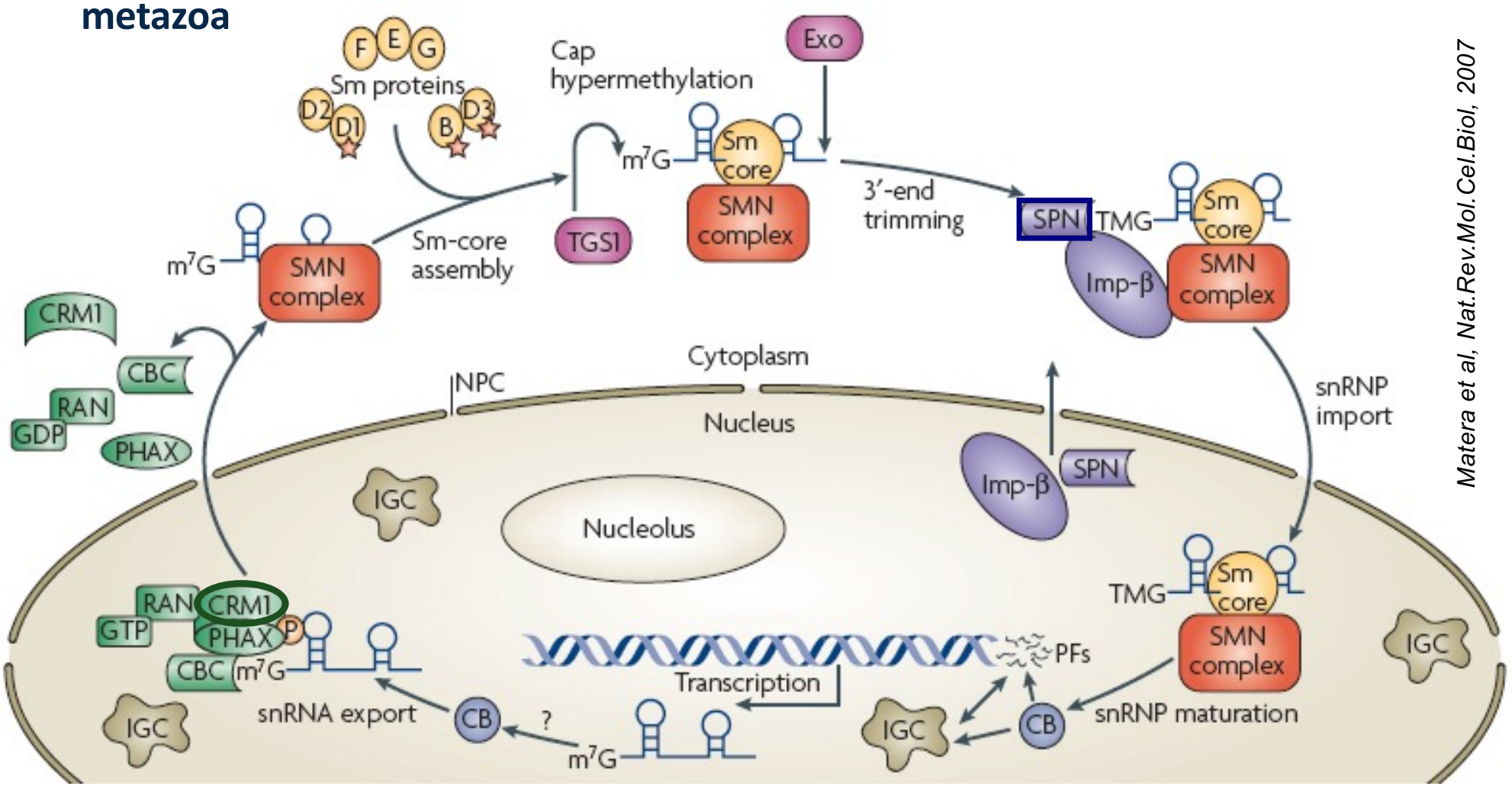
miRNA export



Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

snRNA export

metazoa

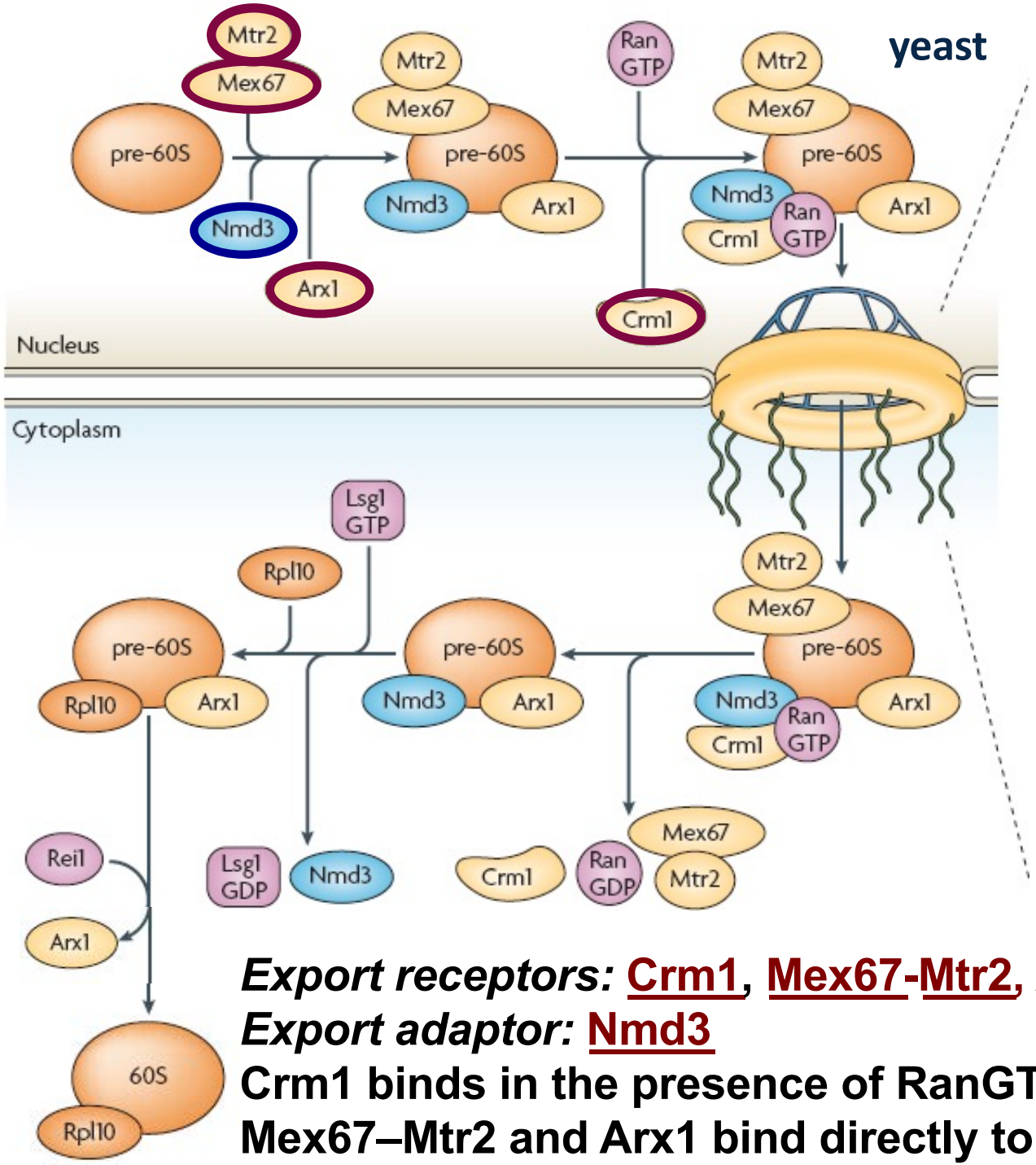


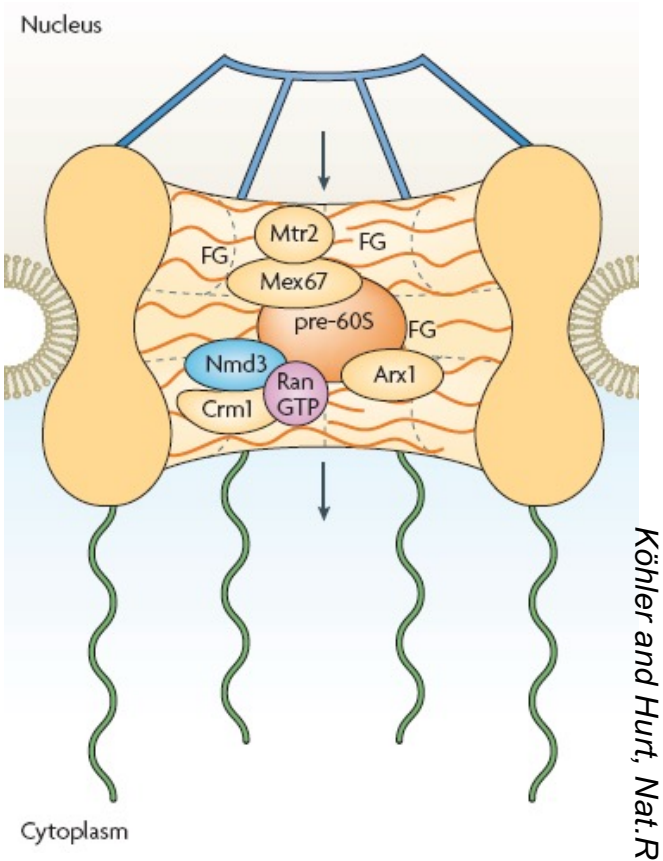
Matera et al, Nat.Rev.Mol.Cel.Biol, 2007

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 m7G cap to 2,2,7-trimethylguanosine cap
SPN - import adaptor snurportin; Imp-β - import receptor importin-β

rRNAexport

yeast





Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

Export receptors: **Crm1, Mex67-Mtr2, Arx1**

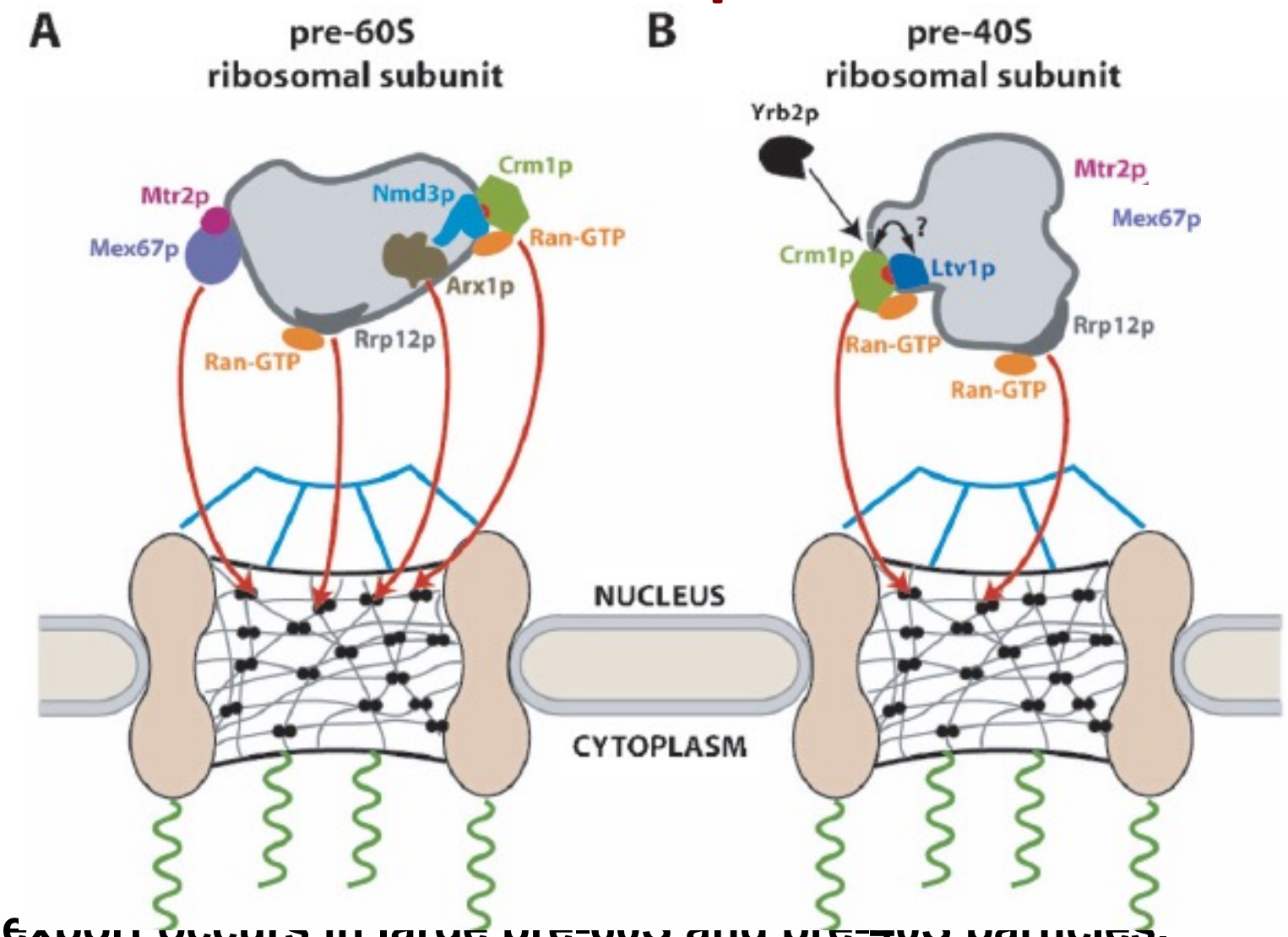
Export adaptor: **Nmd3**

Crm1 binds in the presence of RanGTP to Nmd3 (contains NES)

Mex67–Mtr2 and Arx1 bind directly to pre-60S

rRNAexport

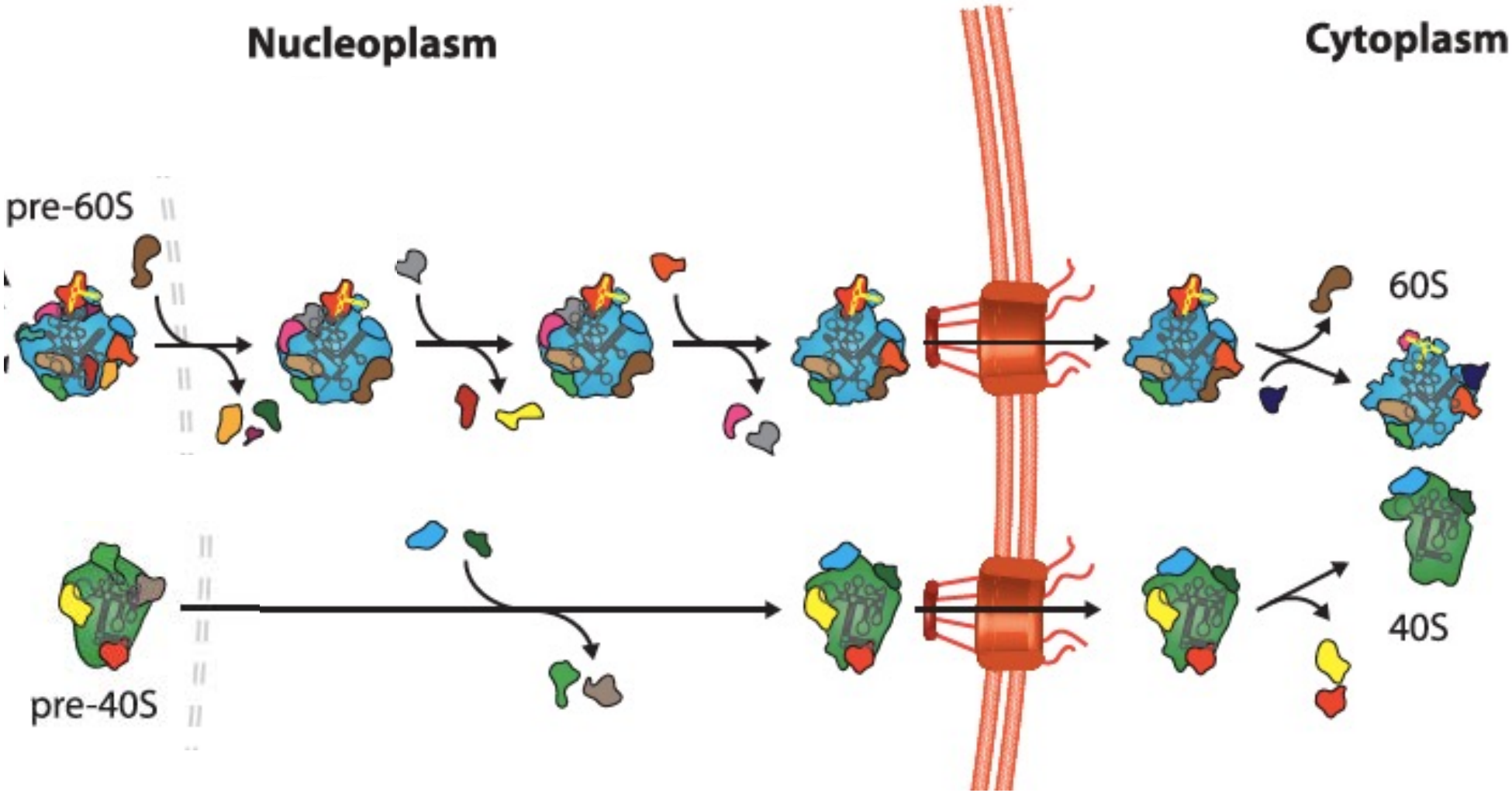
yeast



rRNA export occurs in large pre-60S and pre-40S particles.
It is accompanied by massive RNP rearrangements (changes in protein composition from non-ribosomal to ribosomal components) **and last processing steps in the cytoplasm**

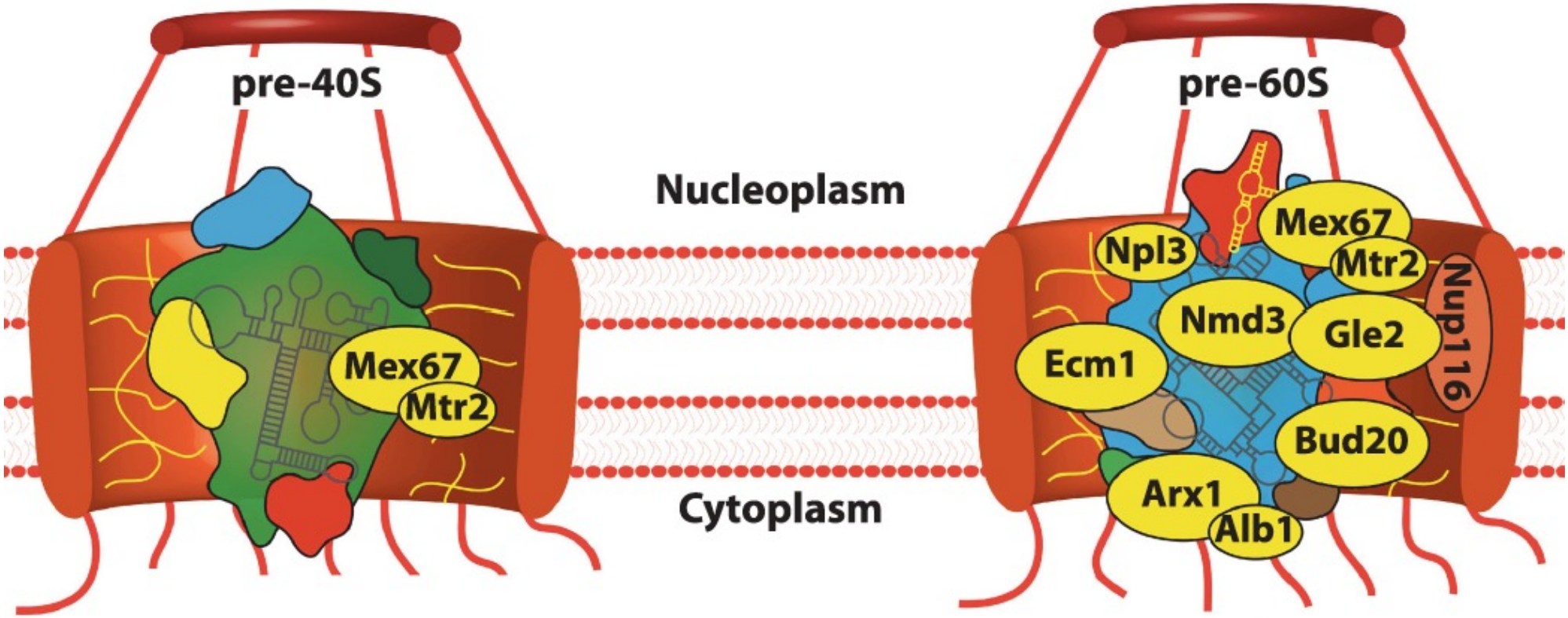
Henras et al., Cell.Mol.Life Sci.,2008

rRNAexport



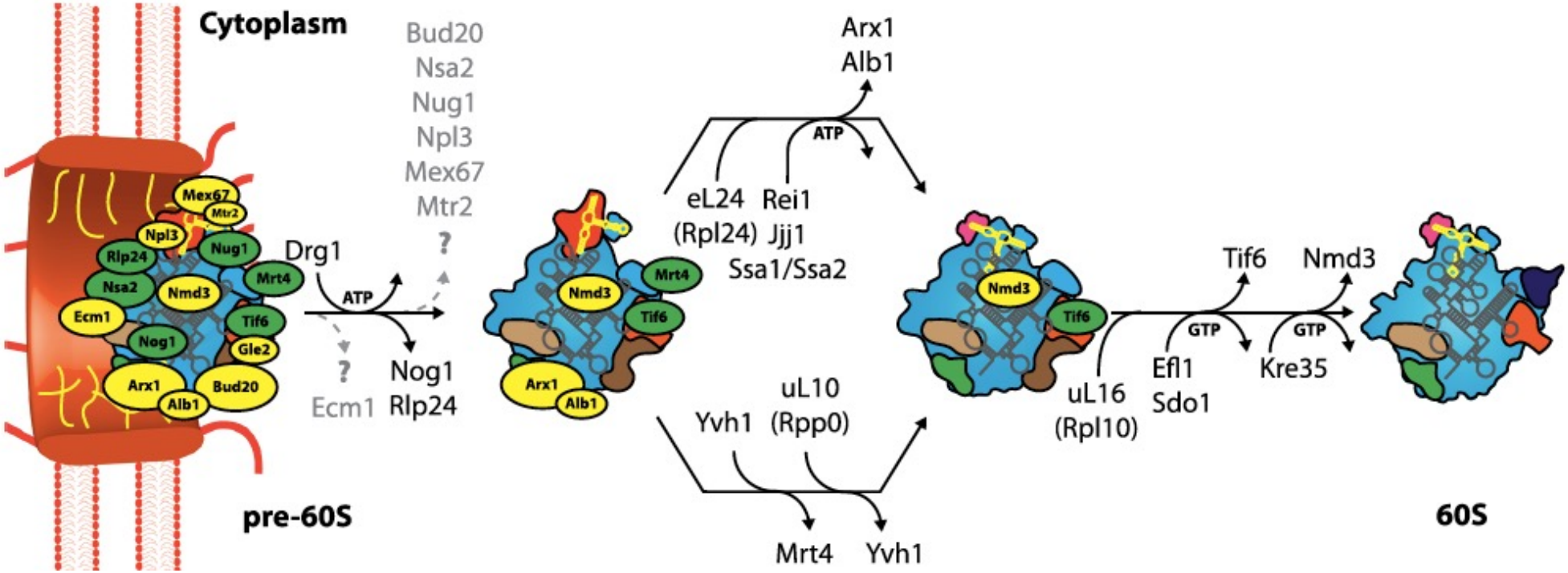
Gerhardy et al., Chromosoma, 2014

rRNAexport



Gerhardy et al., Chromosoma, 2014

Cytoplasmic maturation/export termination of pre-60S



Gerhardy et al., Chromosoma, 2014

TAKE-HOME MESSAGE

- **RNA export initiates by co-transcriptional recruitment of several export factors**
- **RNA export occurs in RNP particles and requires various nuclear transport factors: importins and exportins**
- **Each type of RNA employs a specific export pathway but their components** (adaptors, receptors) **often overlap**
- **Most export pathways require energy: Ran-GTP to Ran-GDP hydrolysis, except for the mRNA export via Mex67-Mtr2**
- **Also release of mature RNP into the cytoplasm uses energy of ATP-dependent helicases or GTPases**