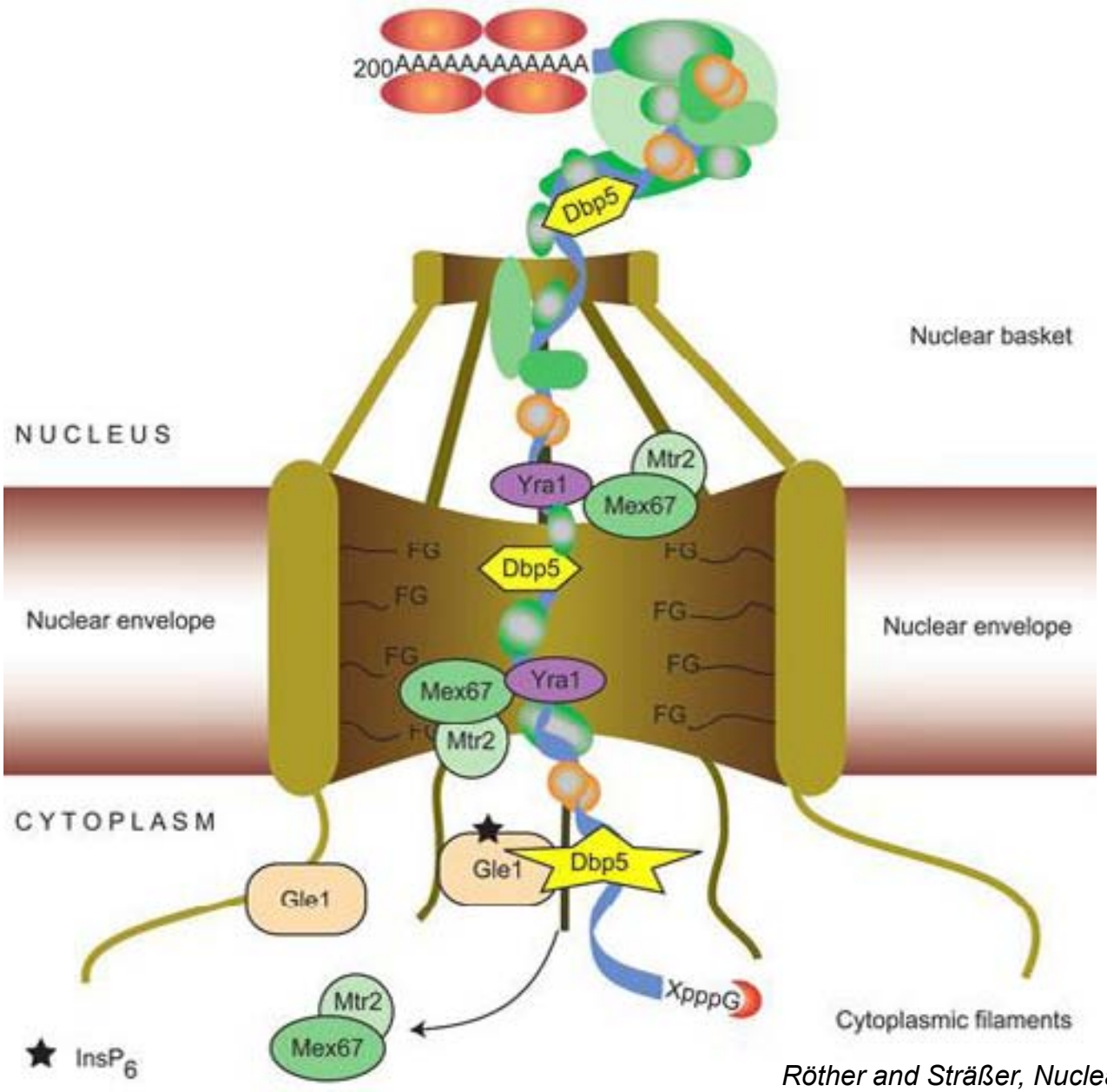
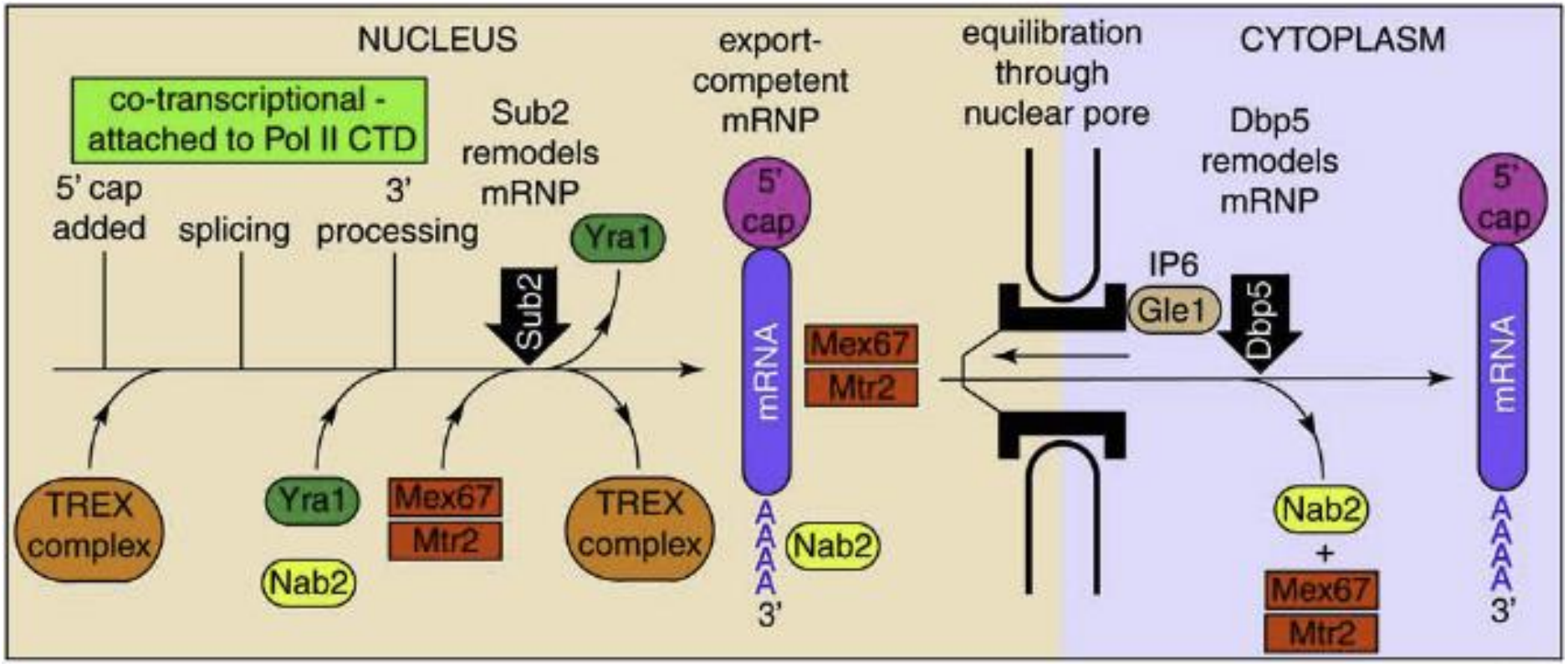
RNA EXPORT



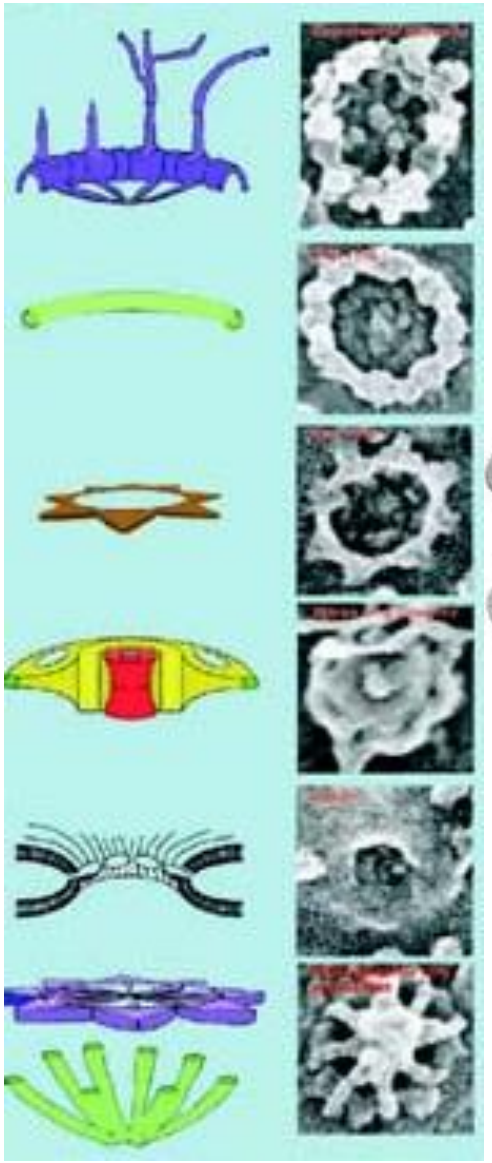
Röther and Sträßer, Nuclear Transport,2009

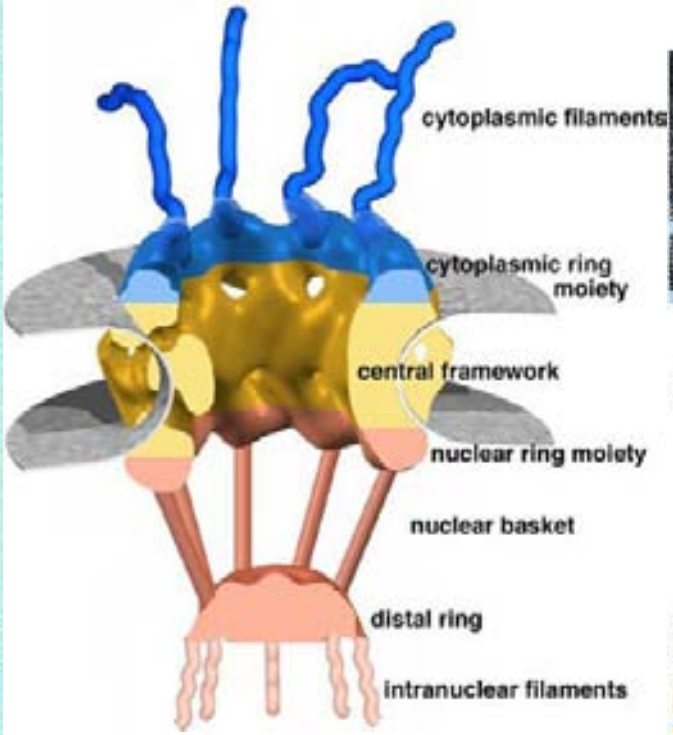
RNA EXPORT – co- or post- transcriptional

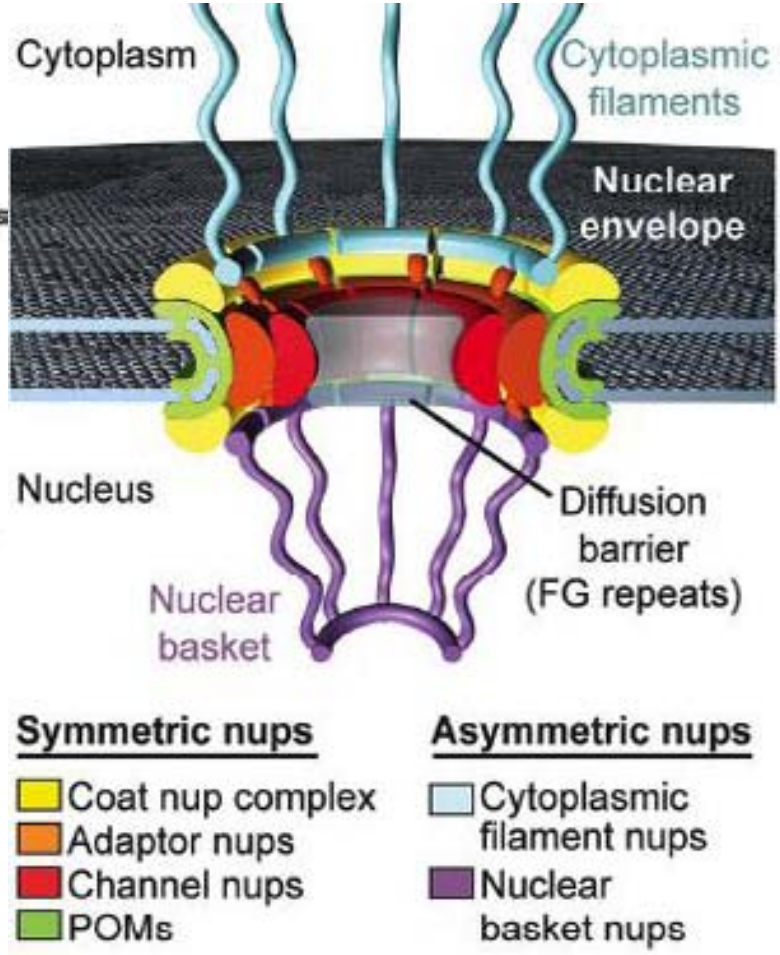


Murray, TiBS,2010

NUCLEAR PORE (NP)

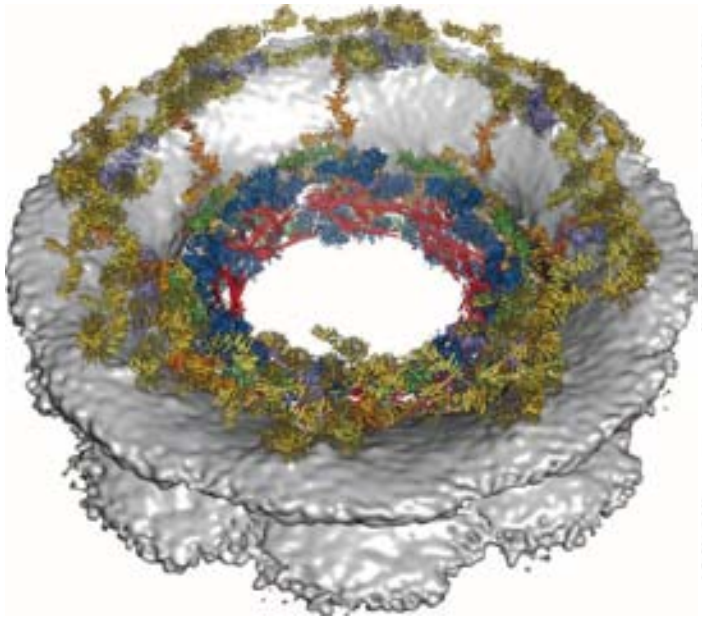


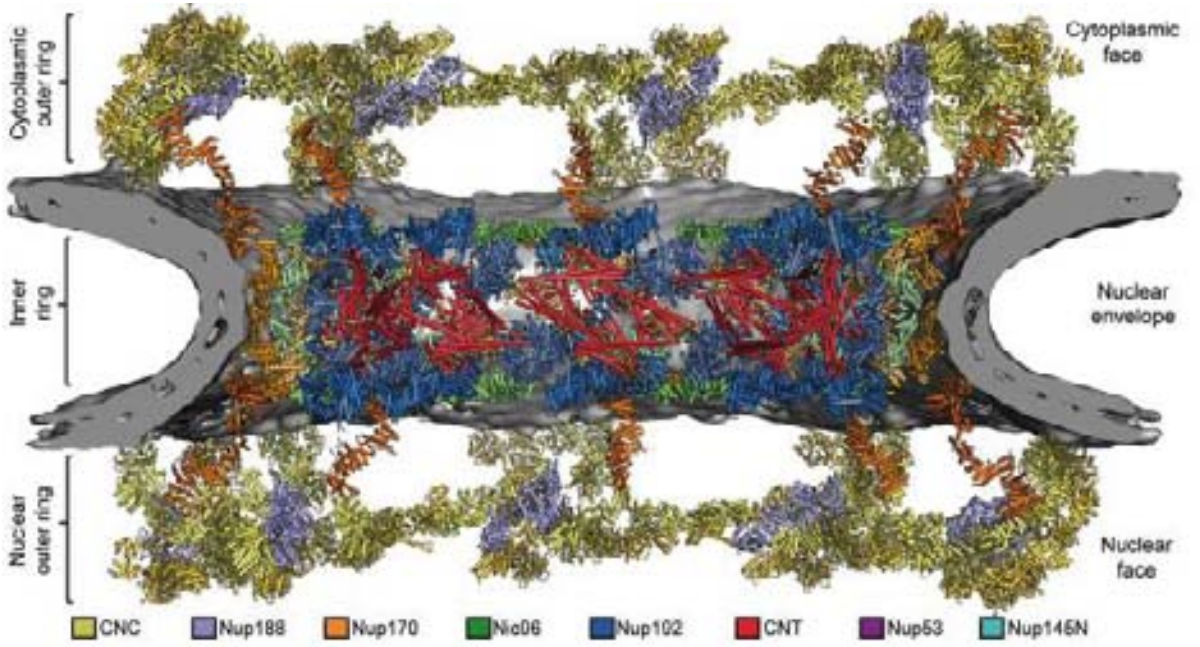


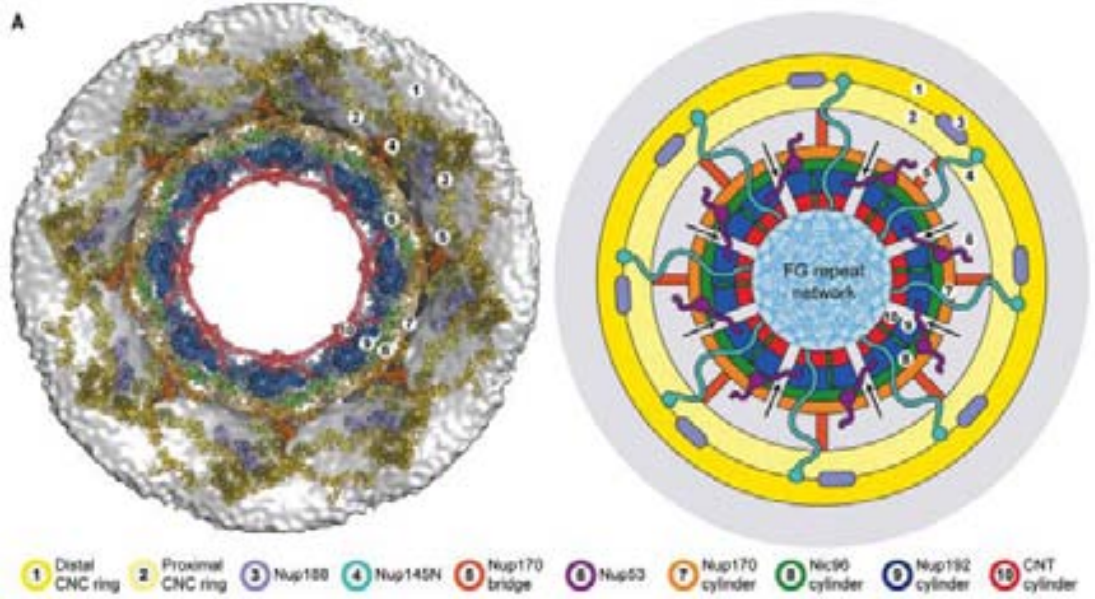


Lin et al, Science 2016

NUCLEAR PORE ARCHITECTURE

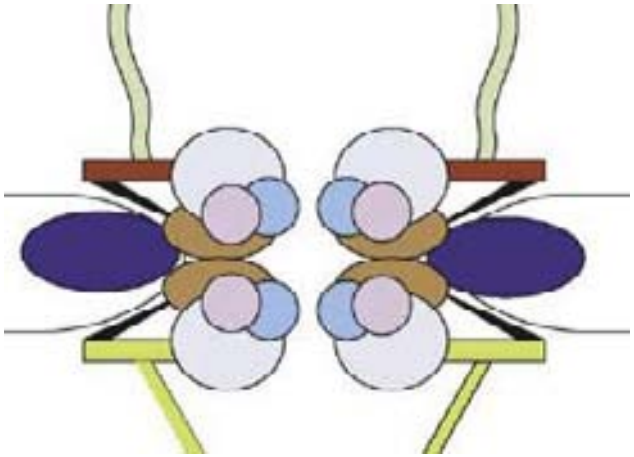




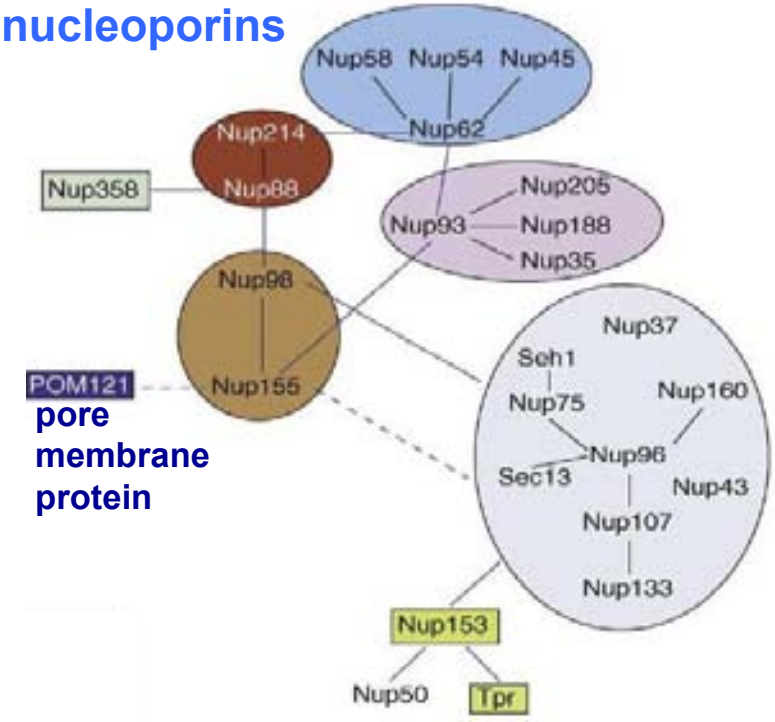


Lin et al, Science 2016;
Kosinski et al, Science 2016

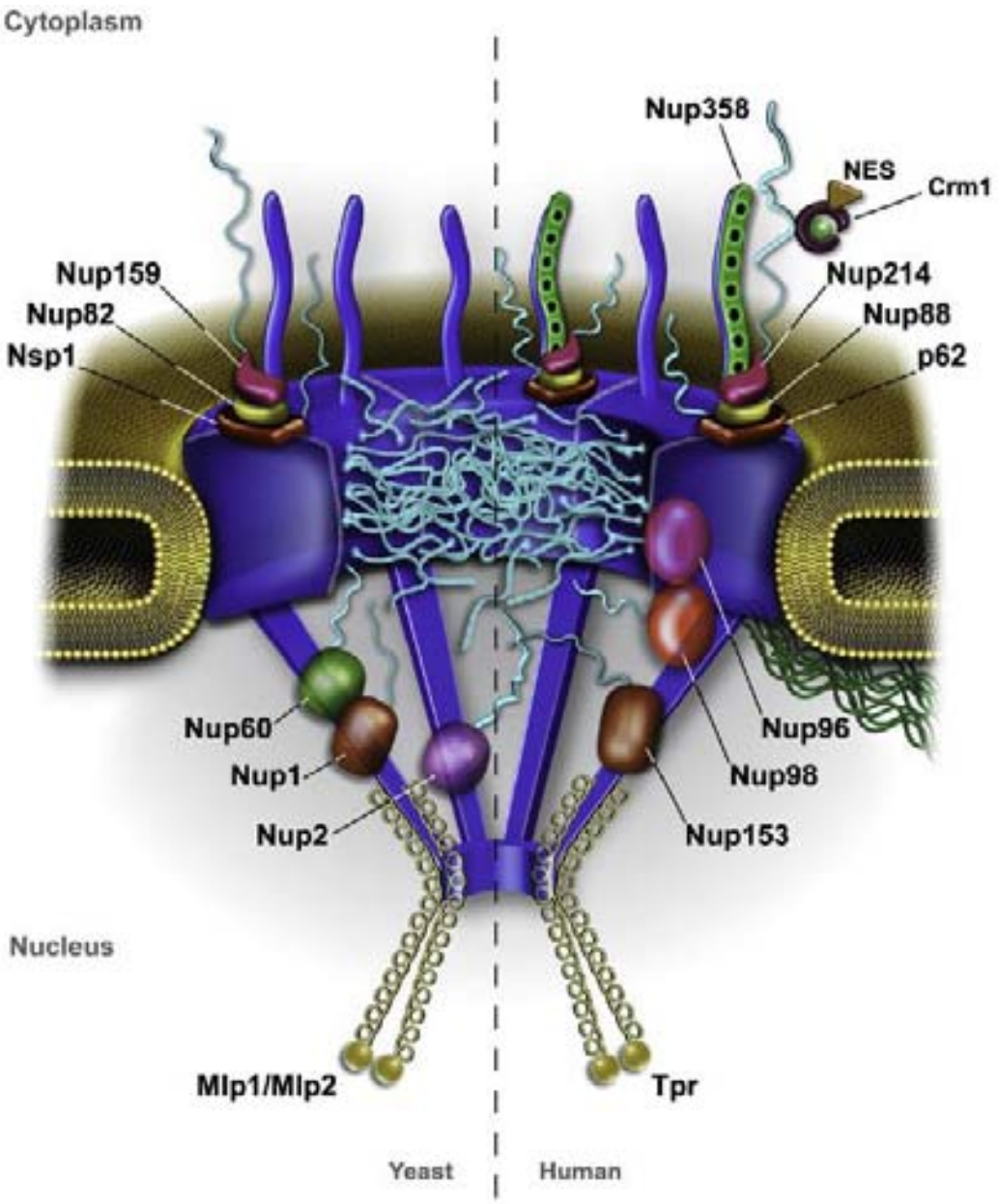
NUCLEAR PORE COMPLEX (NPC)



nuclear pore proteins
nucleoporins



pore membrane protein



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**

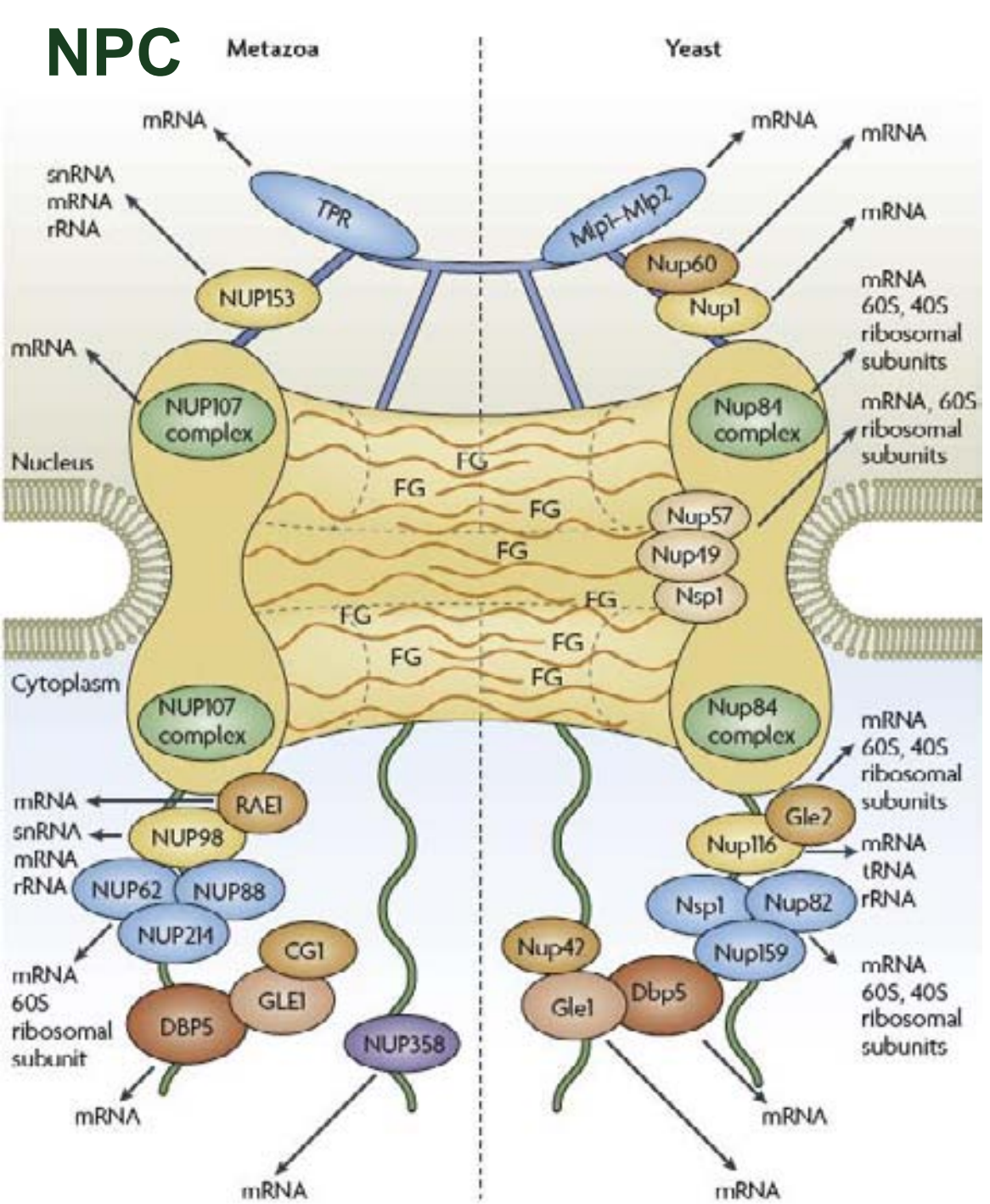
(8, 16 or even 32 copies per NPC)

FG nucleoporins contain Phe-Gly-rich repeats

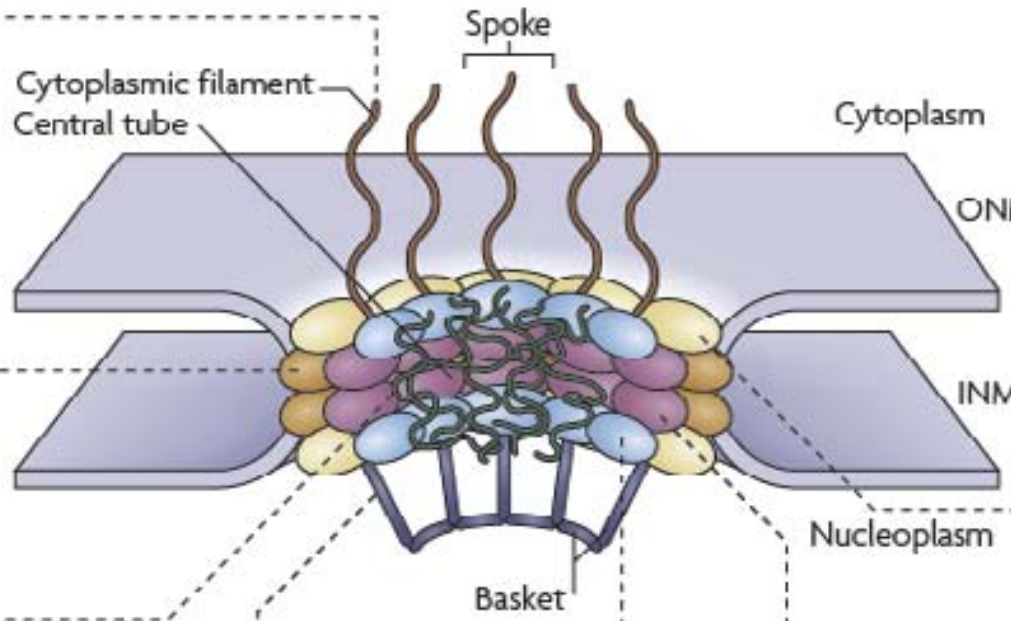
Nucleoporins

	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



Cytoplasmic FG Nups and filaments	
Yeast:	Vertebrate:
	NUP358
Nup159	NUP214
Nup42	NLP1

Transmembrane ring Nups	
Yeast:	Vertebrate:
Pom152	GP210
Pom34	
Ndc1	NDC1
	POM121

Central FG Nups	
Yeast:	Vertebrate:
Nup145N	NUP98
Nup116	
Nup100	
Nsp1	NUP62
Nup57	NUP54
Nup49	NUP58 and NUP45

Nuclear FG Nups and the basket	
Yeast:	Vertebrate:
Nup60	NUP153
Nup1	
Nup2	
Mlp1	TPR
Mlp2	

Linker Nups	
Yeast:	Vertebrate:
Nic96	NUP93
Nup82	NUP88

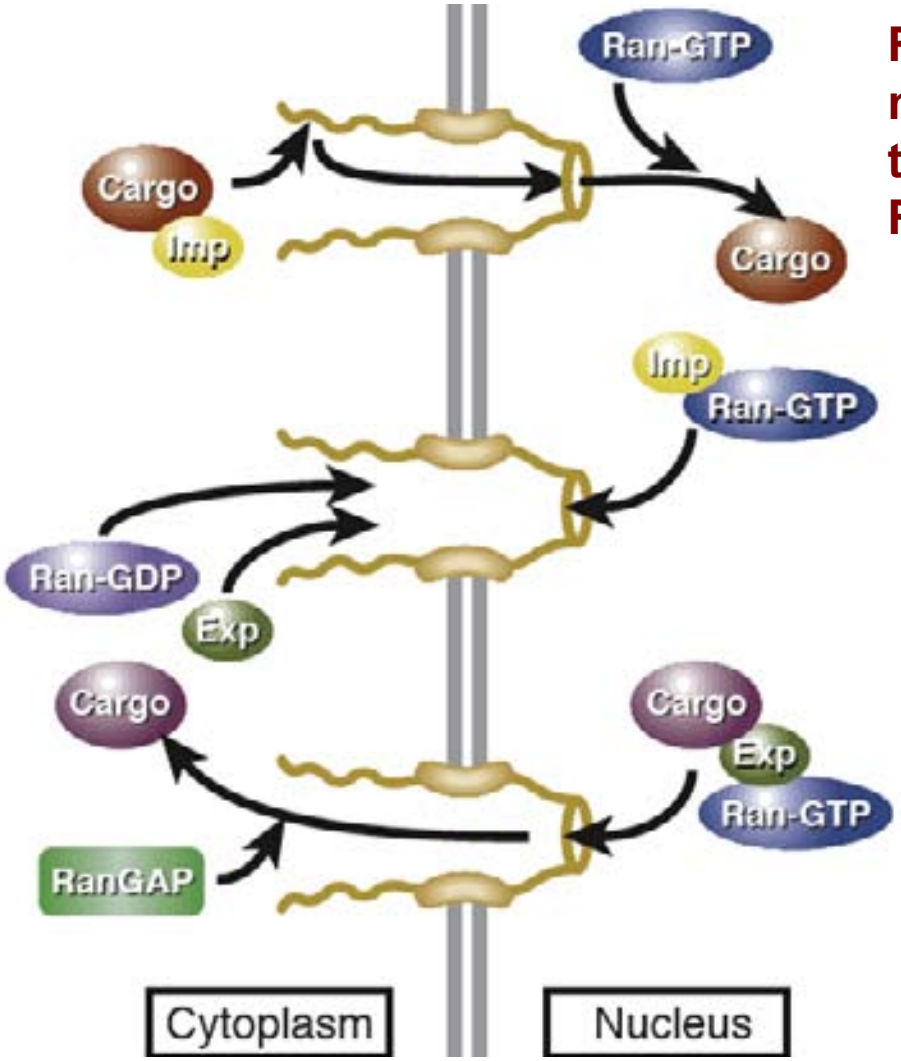
Inner ring Nups	
Yeast:	Vertebrate:
Nup192	NUP205
Nup188	NUP188
Nup170	NUP155
Nup157	
Nup53	NUP35
Nup59	

Outer ring Nups	
Yeast:	Vertebrate:
Nup133	NUP133
Nup120	NUP160
Nup145C	NUP96
Nup85	NUP75
Nup84	NUP107
Seh1	SEH1
Sec13	SEC13
	NUP43
	NUP37
	Aladin

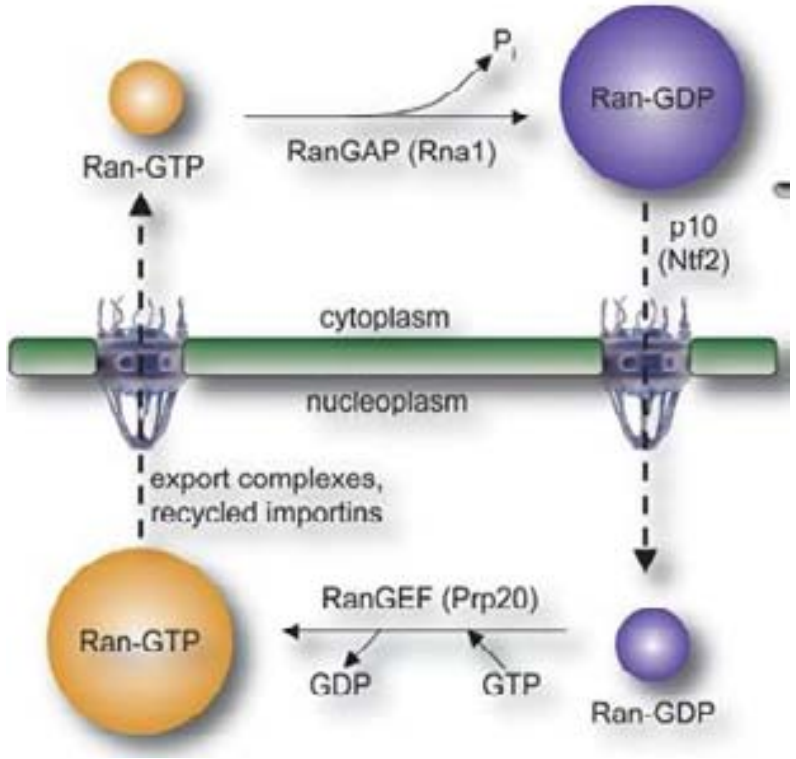
Core scaffold Nups

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**.

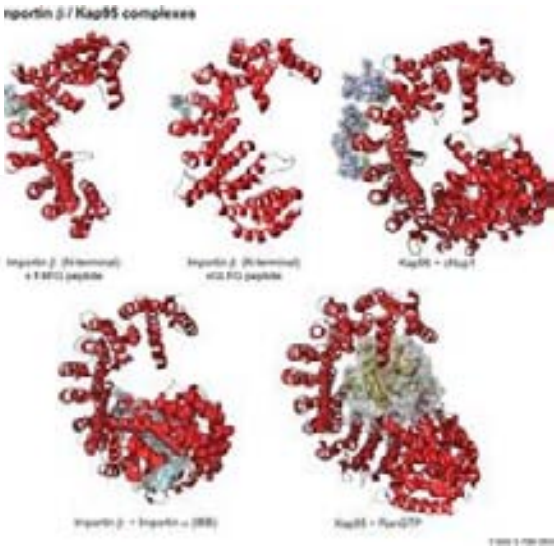
NUCLEOPLASMIC TRANSPORT

Karyopherins yeast

Importins	*Exportins*	
Kap95•Kap60	Crm1	rRNA, snRNA
Kap104	Cse1	
Kap121	Kap120	
Kap123	Los1	tRNA
Mtr10	Mex67•Mtr2	mRNA, rRNA
Nmd5		
Sxm1		
Pdr6		
Kap114		
Ntf2		

Transportins

Msn5 — tRNA



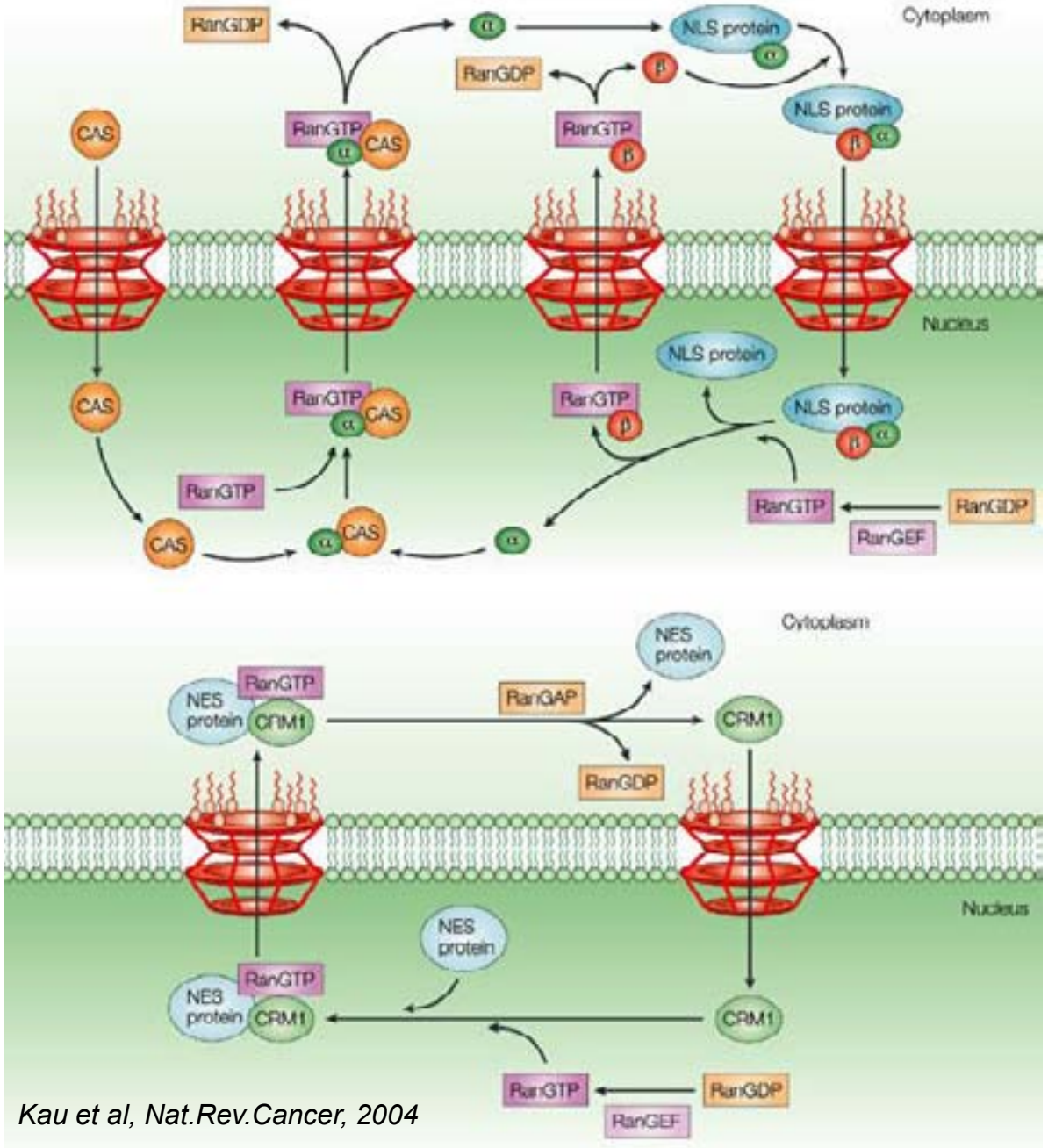
Transport receptor	mammals
Kapβ1	
Kapβ1–Kapα complex (Impβ–Impα)	
Kapβ1–snurportin complex	
Kapβ1–XRIPα complex	
Karyopherin-β–Imp7 heterodimer	
Karyopherin-β–RanBP8 heterodimer	
Kapβ2 (transportin)	
Karyopherin-5	
Transportin SR	
CRM1 (exportin)	
CAS	
Exportin-t	

Transport is mediated by members of the karyopherin family of nuclear transport factors: importins and exportins

PROTEIN TRANSPORT

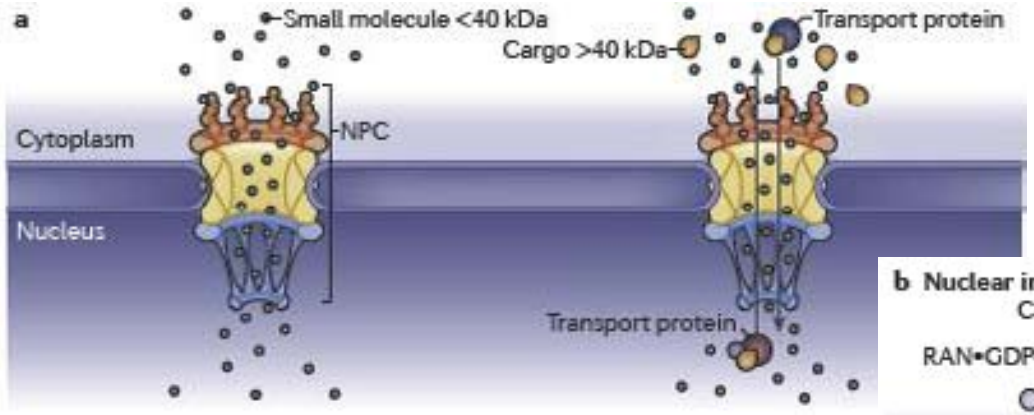
NLS
Nuclear Localization Signal
(binds Importins)

NES
Nuclear Export Signal
(binds Exportins and Ran-GTP)



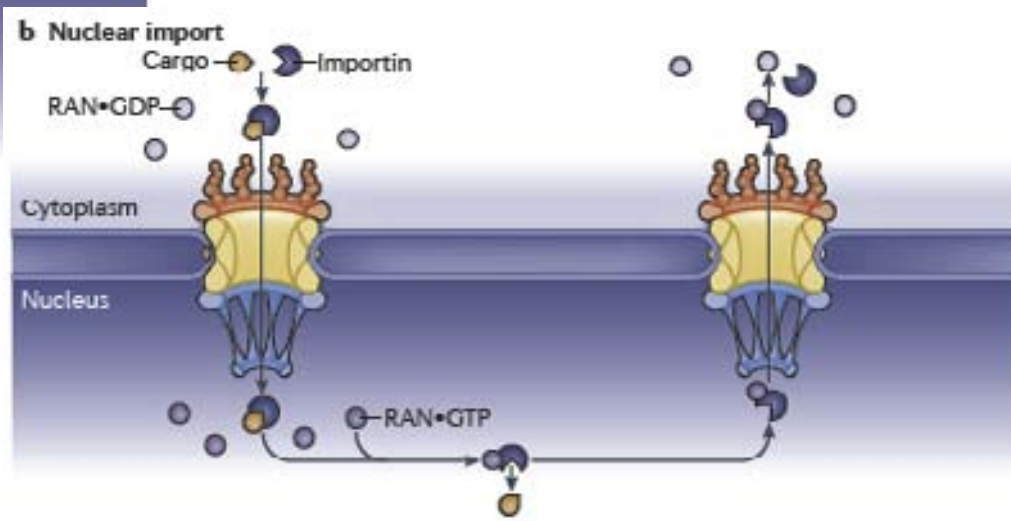
Kau et al, Nat.Rev.Cancer, 2004

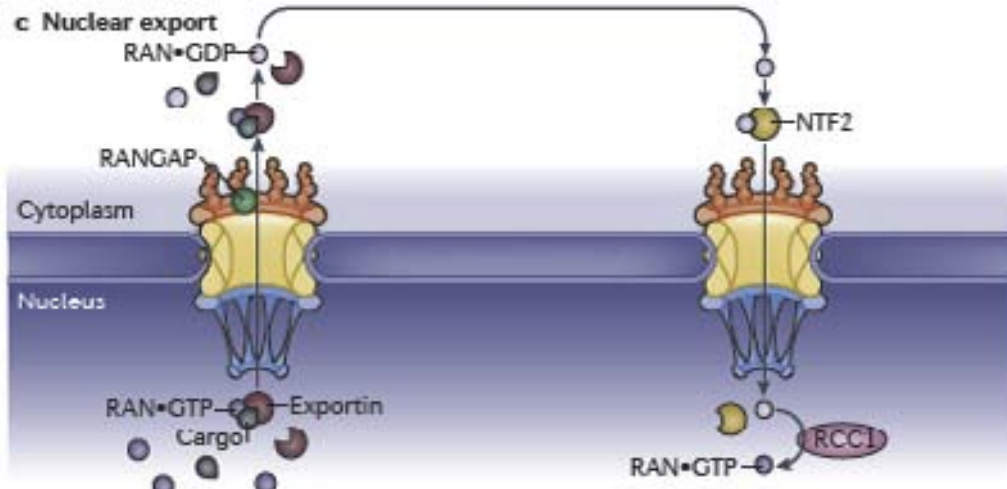
NUCLEOPLASMIC TRANSPORT



< 40 kDa
passive diffusion

> 40 kDa
active transport via transport receptors (NLS)





mRNA NUCLEAR EXPORT MACHINERY

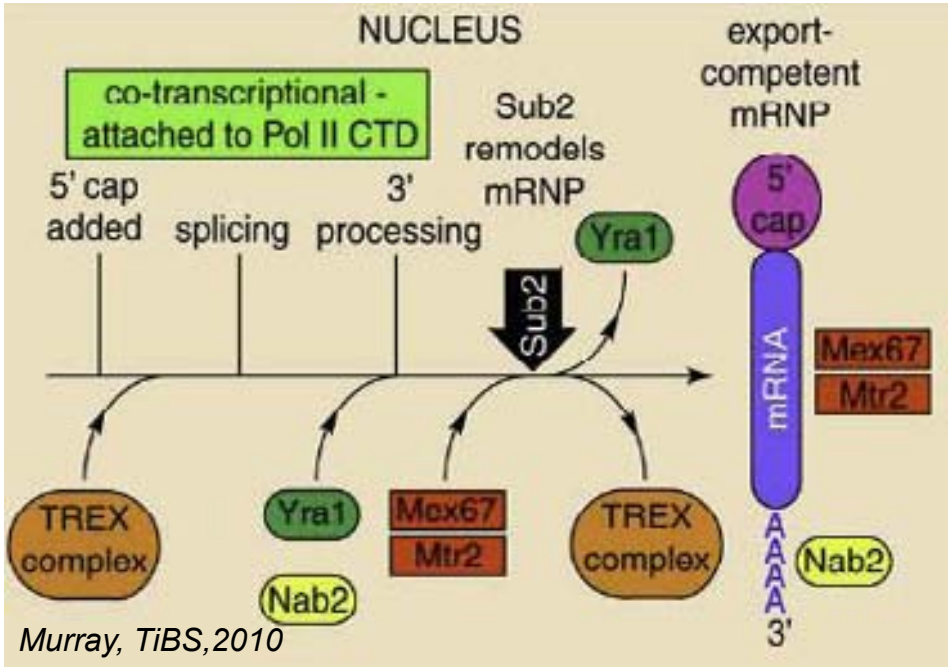
Component		Function
Yeast	Metazoan	
Mex67–Mtr2	NXF1–NXT1	Facilitates bulk mRNA transport through NPCs
Yra1	ALY (REF)	Adaptor linking Mex67–Mtr2 to mRNA
Sub2	UAP56	DEAD-box helicase involved in assembly of export-competent mRNPs
Nab2	–	Binds polyA-mRNA and Mlp1; modulates length of 3′ polyA tail
Mlp1	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

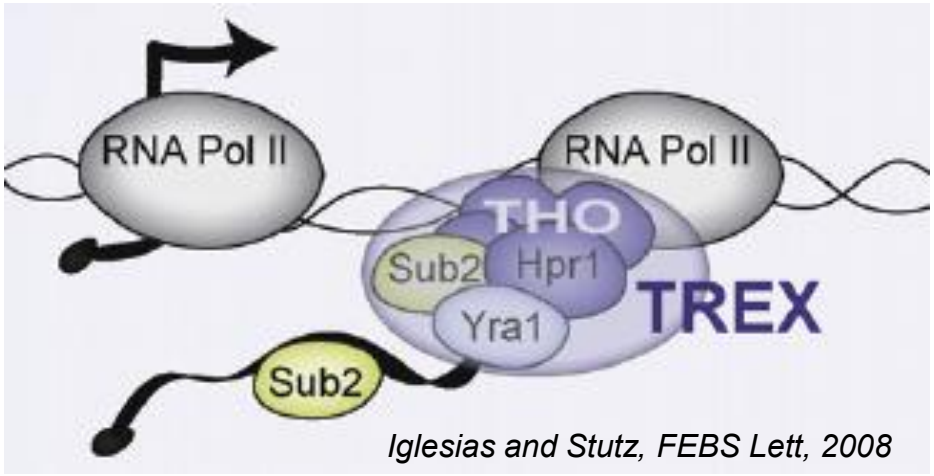
THO/TREX and TREX-2 complexes – coordinate trx, processing and export
EJC (metazoan)

Murray, TiBS,2010

Co-transcriptional mRNA EXPORT
(yeast)

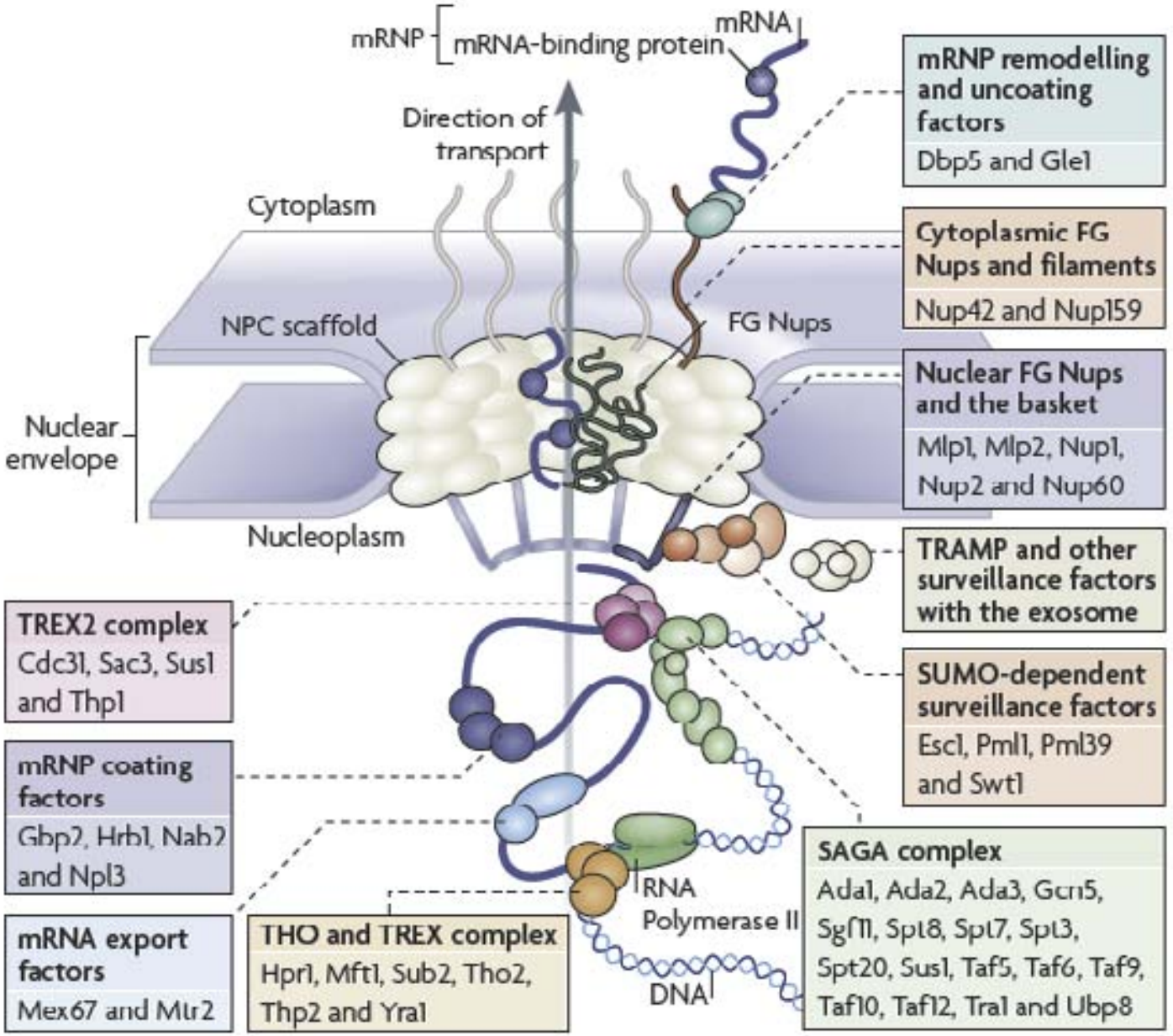


Murray, TiBS,2010



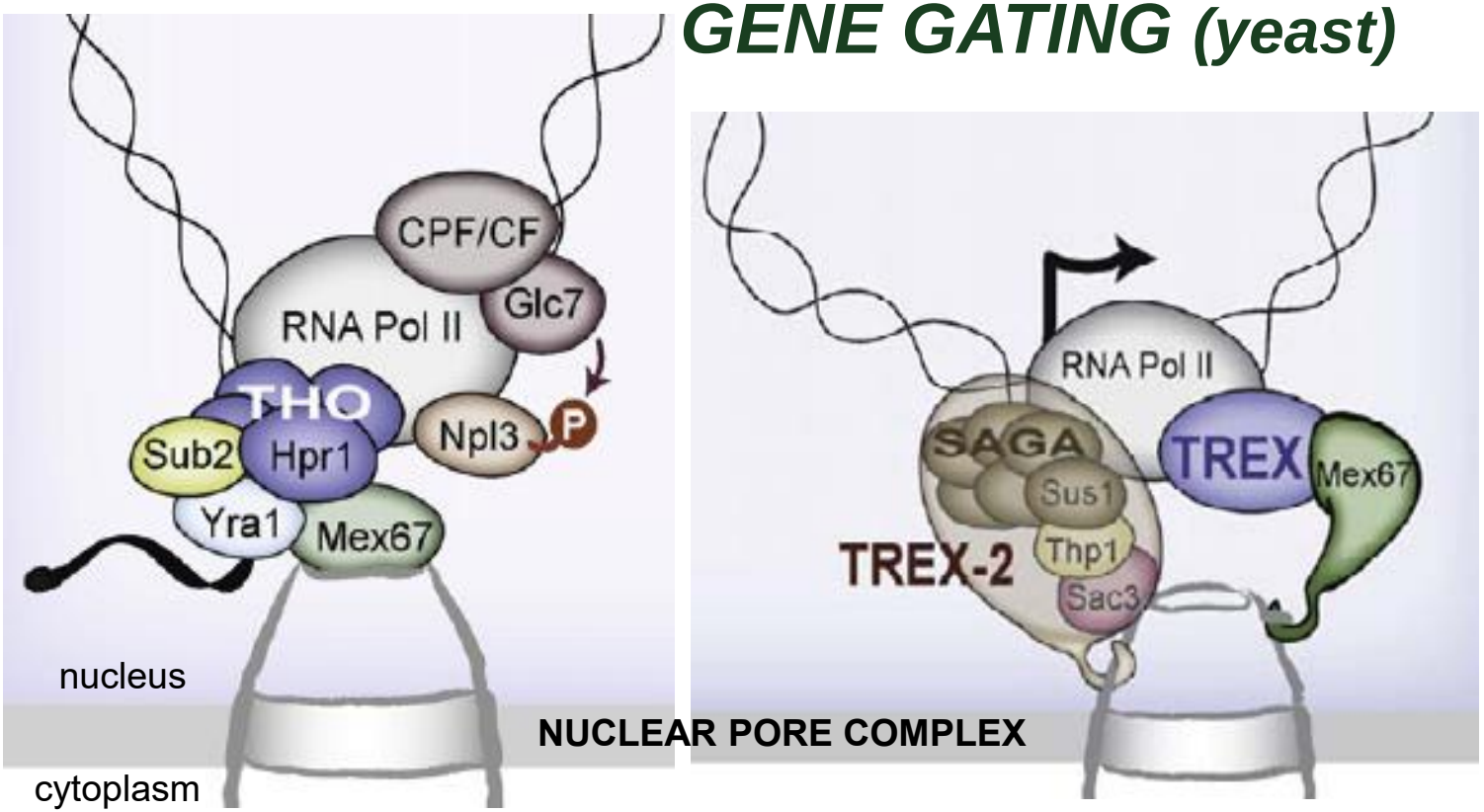
Iglesias and Stutz, FEBS Lett, 2008

mRNA EXPORT – ALL FACTORS



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

Co-transcriptional mRNA EXPORT:
GENE GATING (yeast)



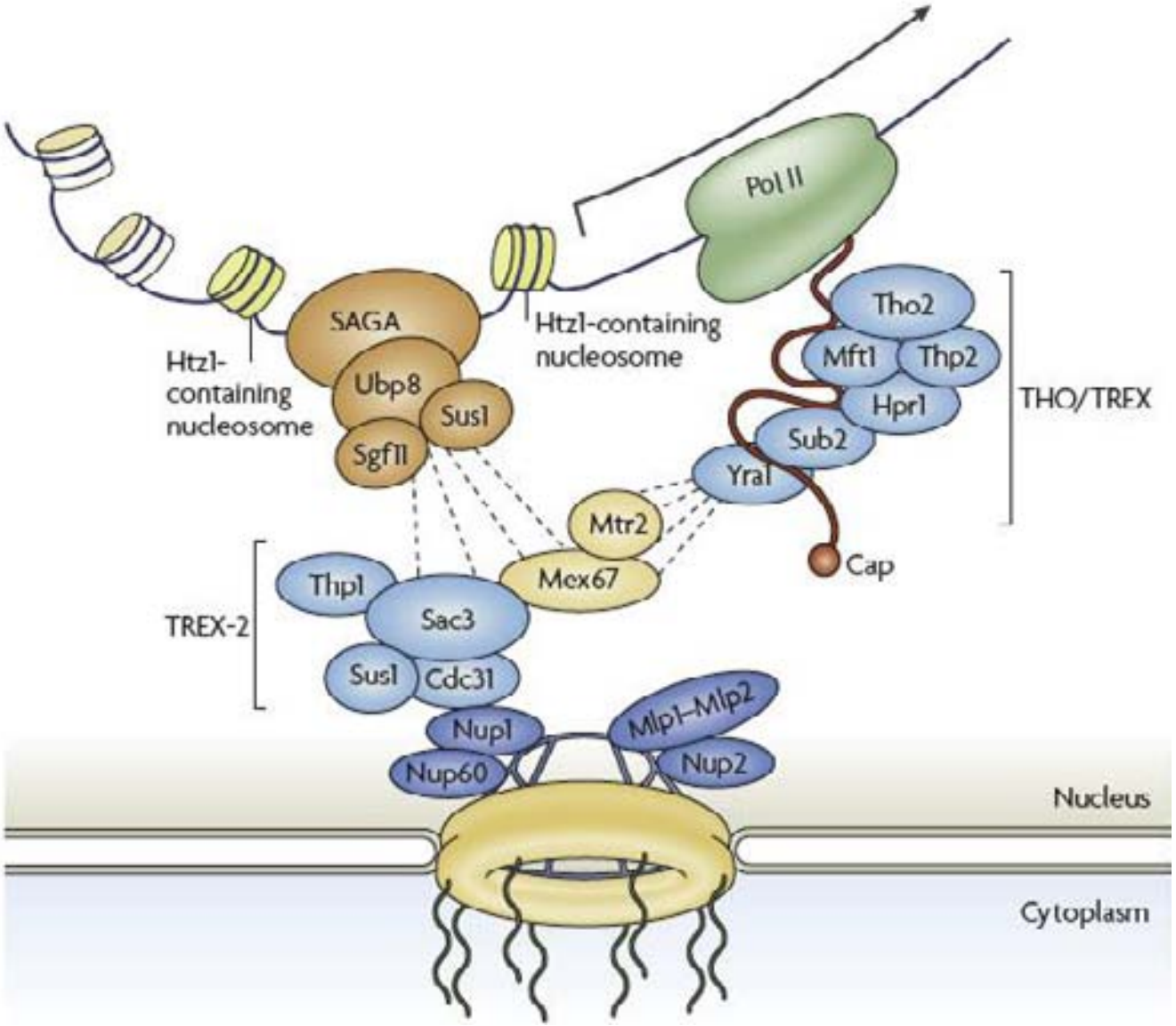
Iglesias and Stutz, FEBS Lett, 2008

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

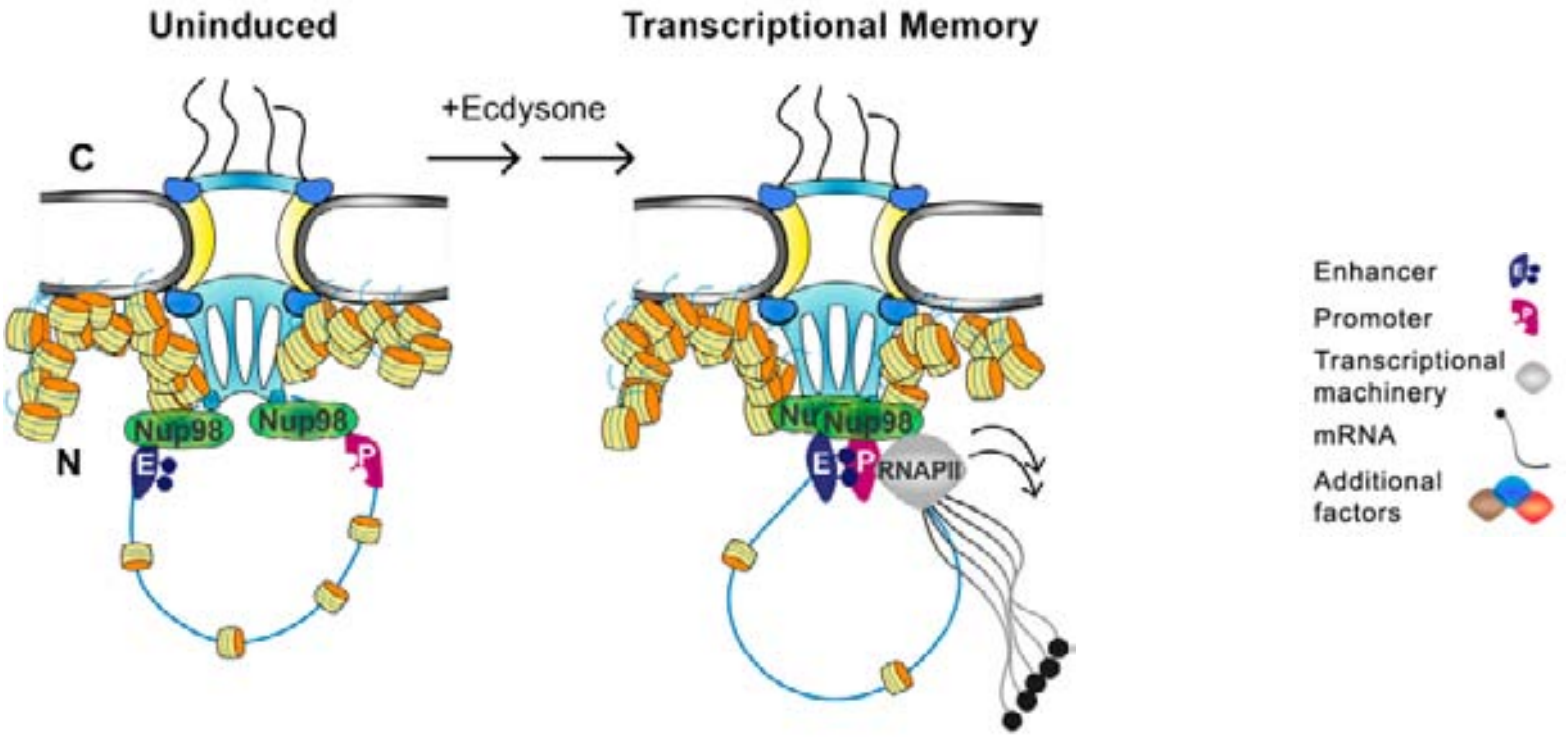
TREX-2 and TREX complexes link transcription (Pol II via THO, initiation complex SAGA via Sus1) to export receptors (Mex67, Yra1) and Nuclear Pore Complex

Co-transcriptional mRNA EXPORT:
GENE GATING (yeast)



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

GENE GATING (metazoa)

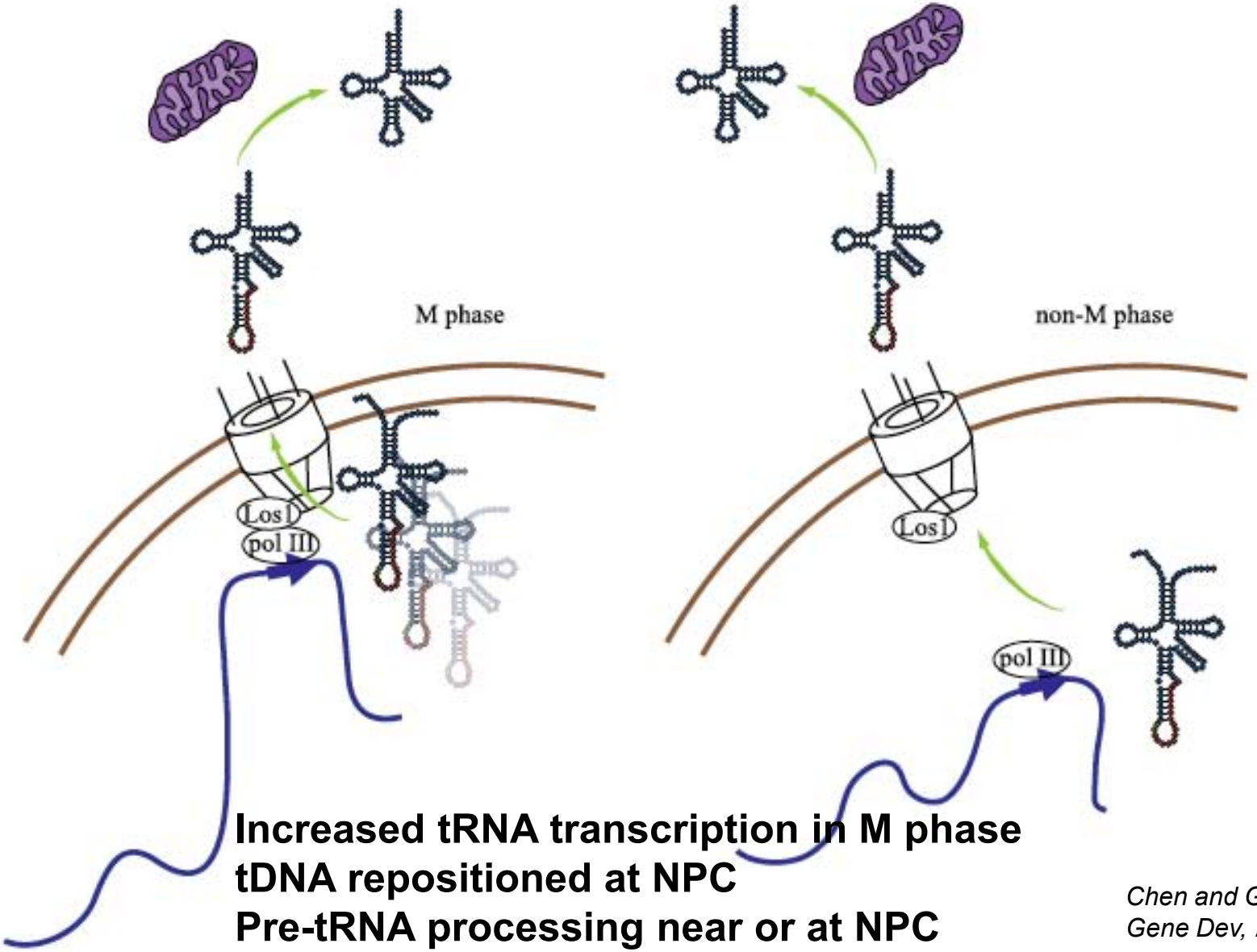


- **Nuclear pore proteins (Nups) bind promoters and enhancers in *Drosophila***
- **Nup98 mediates enhancer-promoter looping of inducible genes**
- **Inducible genes stably associate with nuclear pores in silent and active states**

Pascual-Garcia et al, Mol Cell 2017

Co-ordinated tRNA TRANSCRIPTION and EXPORT:

tRNA GATING (yeast)



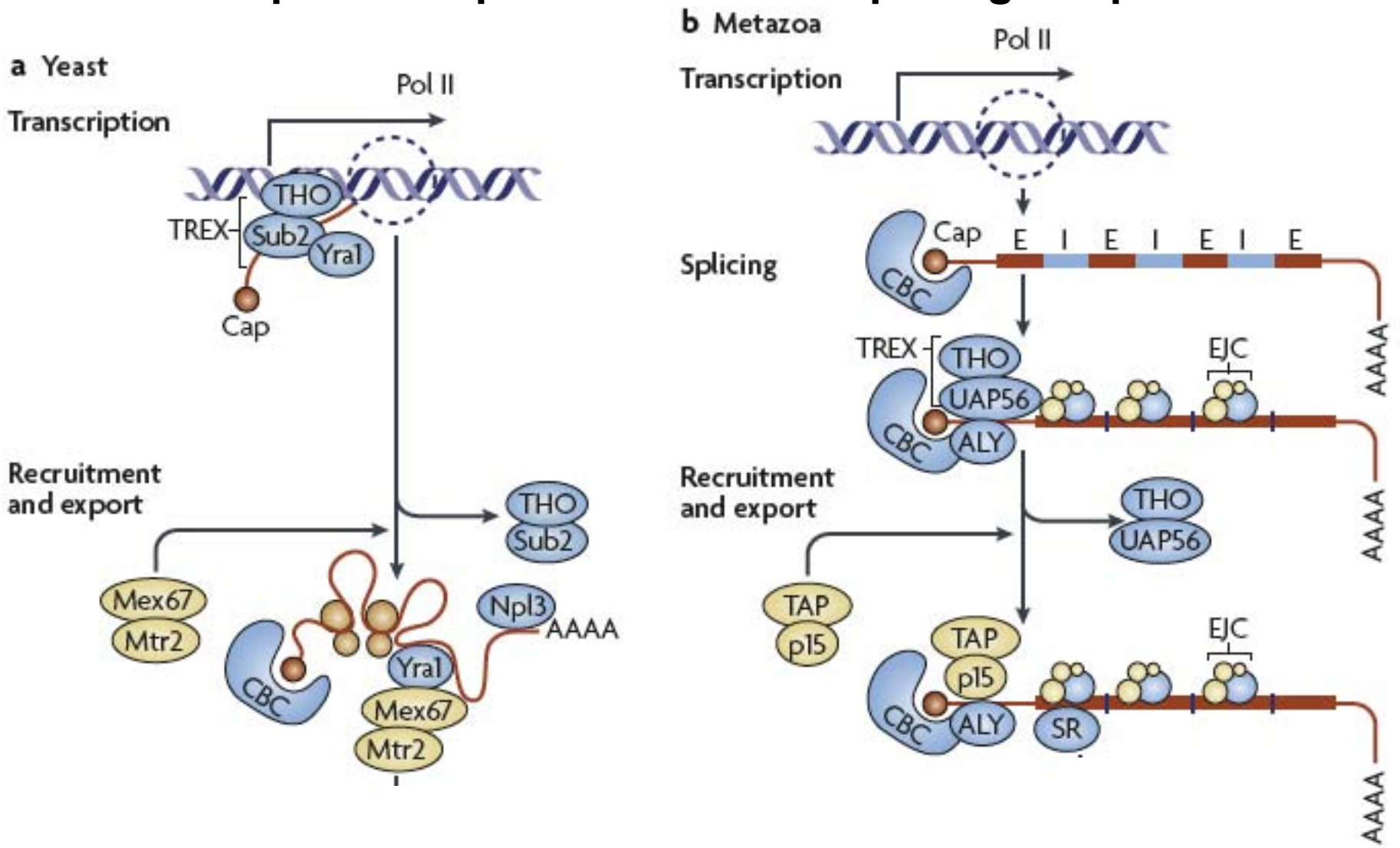
Increased tRNA transcription in M phase
tDNA repositioned at NPC
Pre-tRNA processing near or at NPC

Chen and Gartenberg
Gene Dev, 2014

mRNA EXPORT (nuclear side)

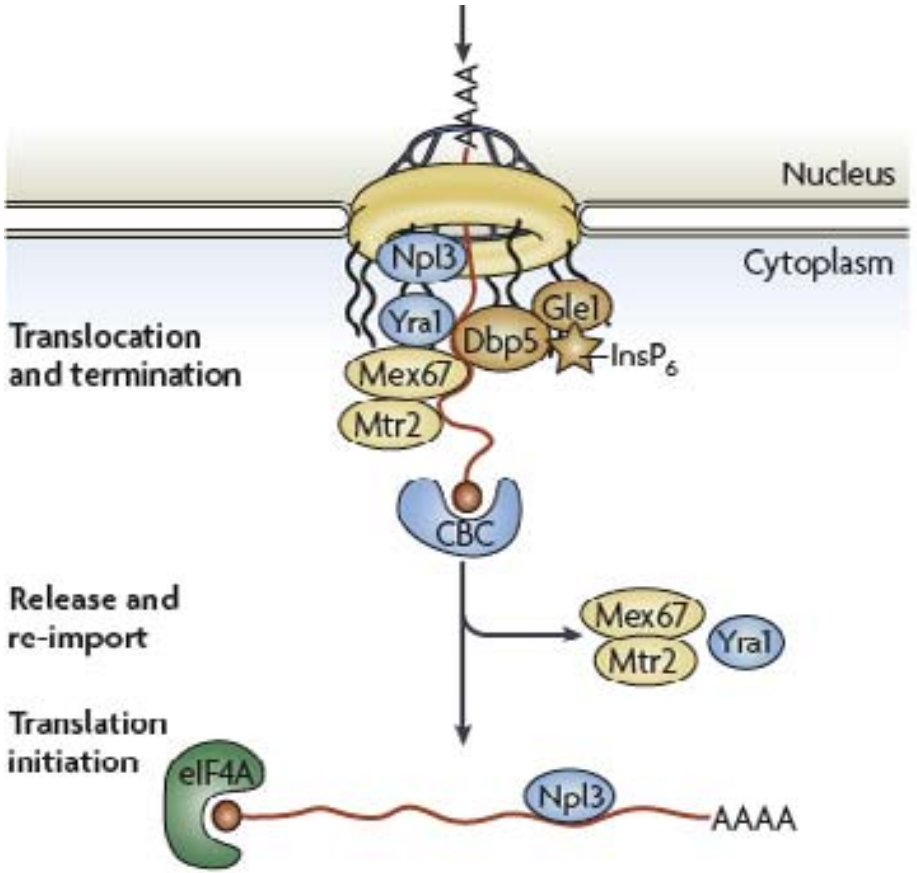
transcription-coupled

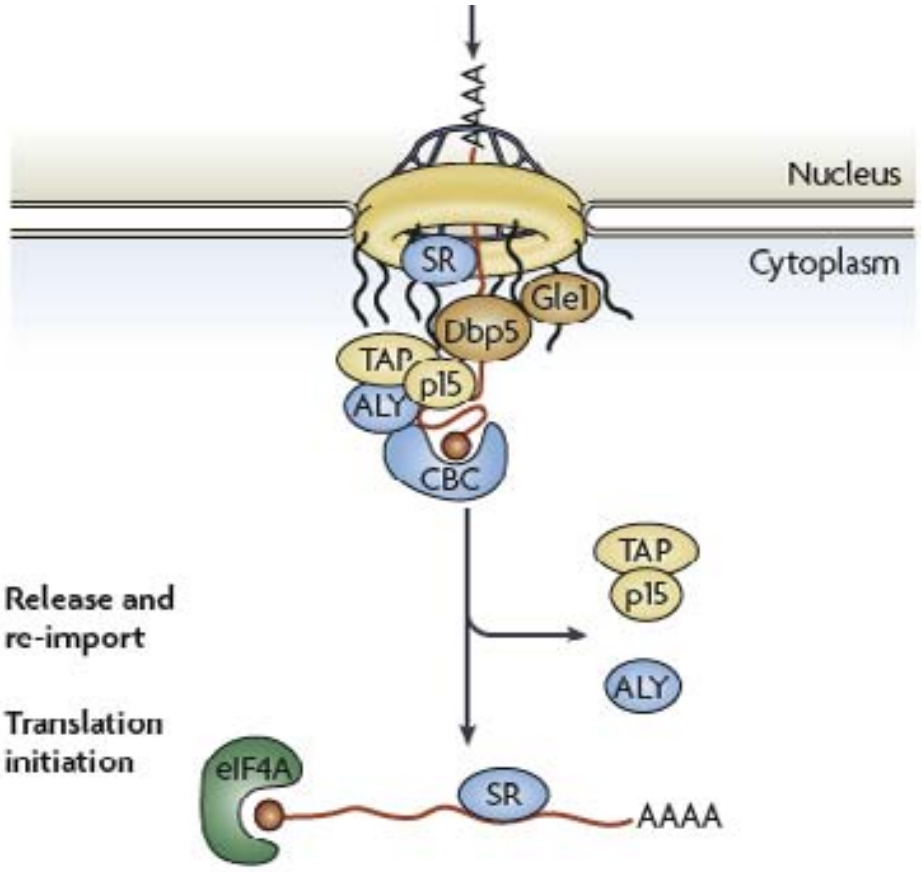
splicing-coupled



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

mRNA EXPORT (cytoplasmic side)



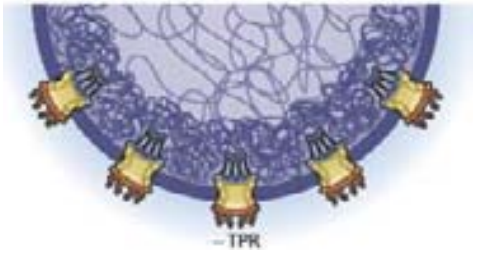


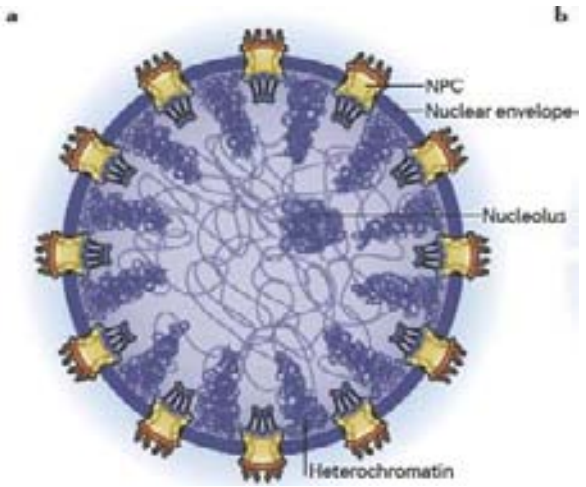
Unique features of mRNA export:

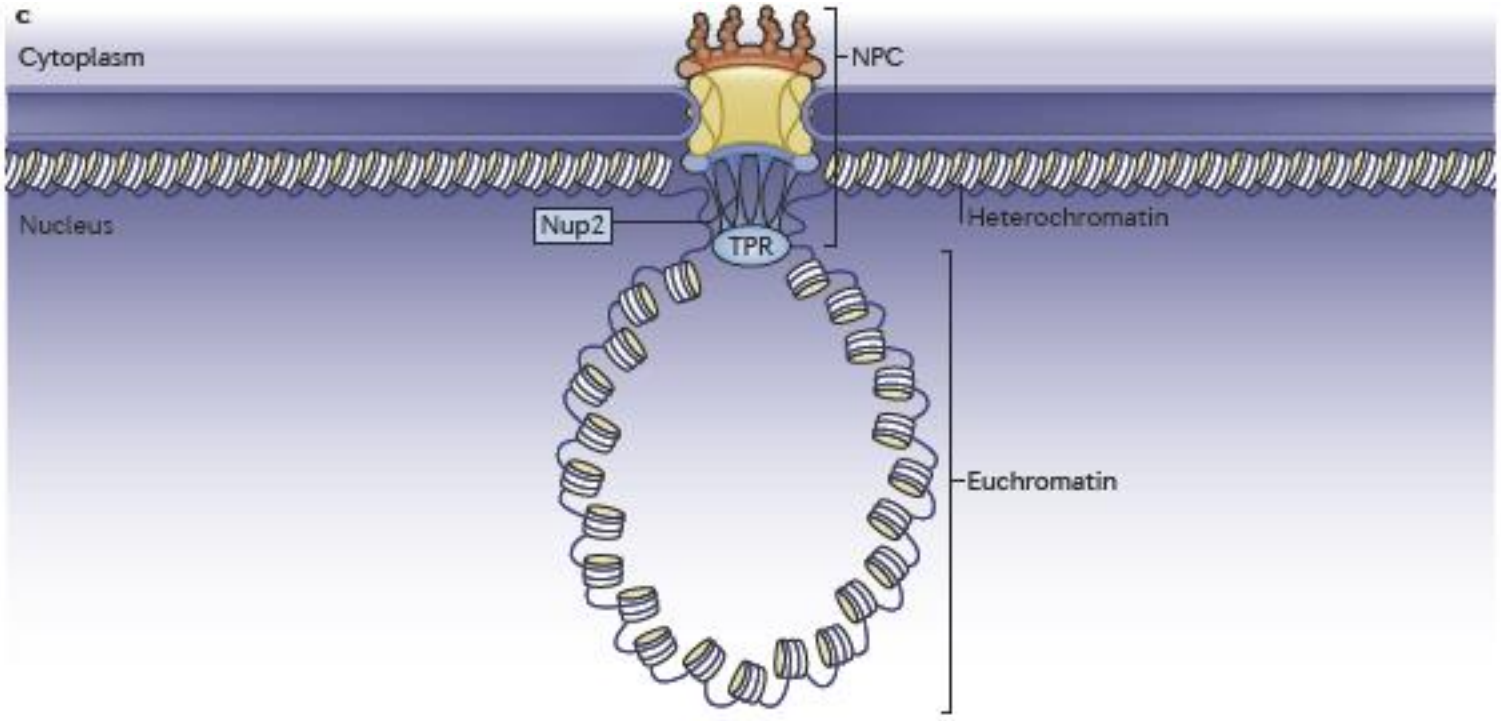
- **Mex67-Mtr2 (TAP-p15) transport receptor structurally unrelated to karyopherins, independent of the RanGTP-RanGDP gradient.**
- **mRNA export receptors cooperate with other factors: adaptors (Yra1/ALY/REF, SR proteins), release factors**
- **some mRNAs can be exported via the Crm1 RanGTP-dependent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

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

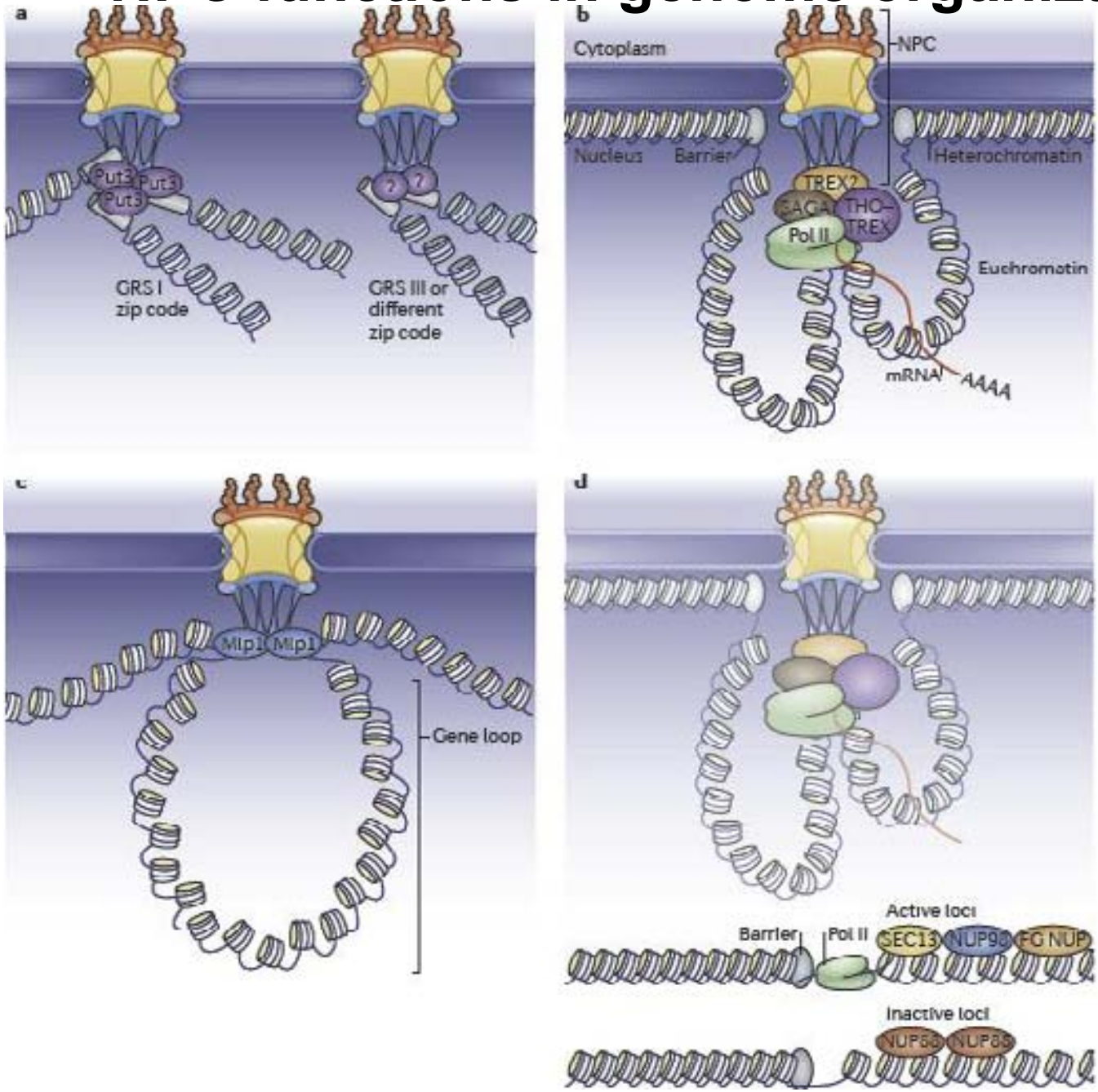
Peripheral heterochromatin is excluded from the NPC



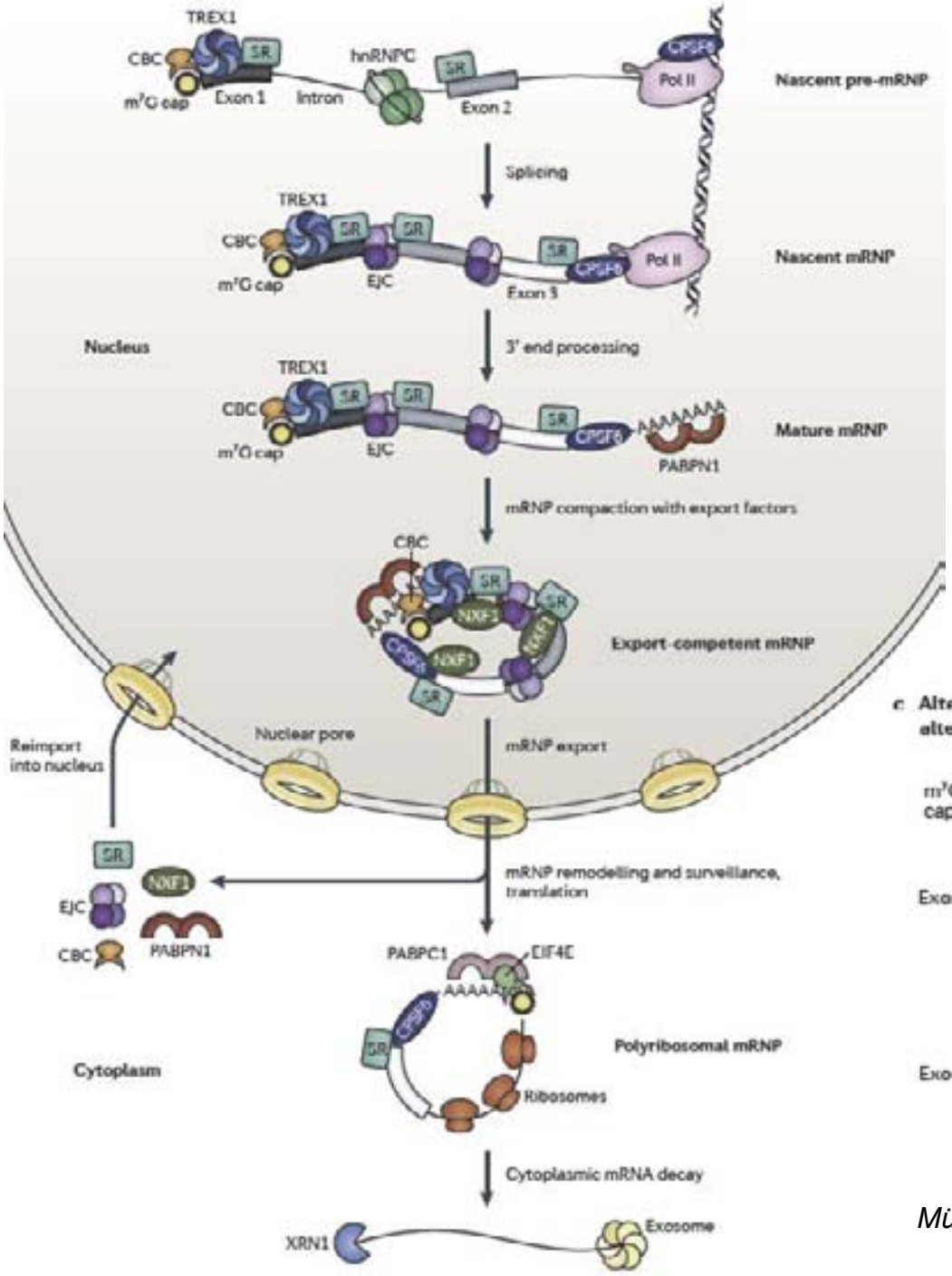


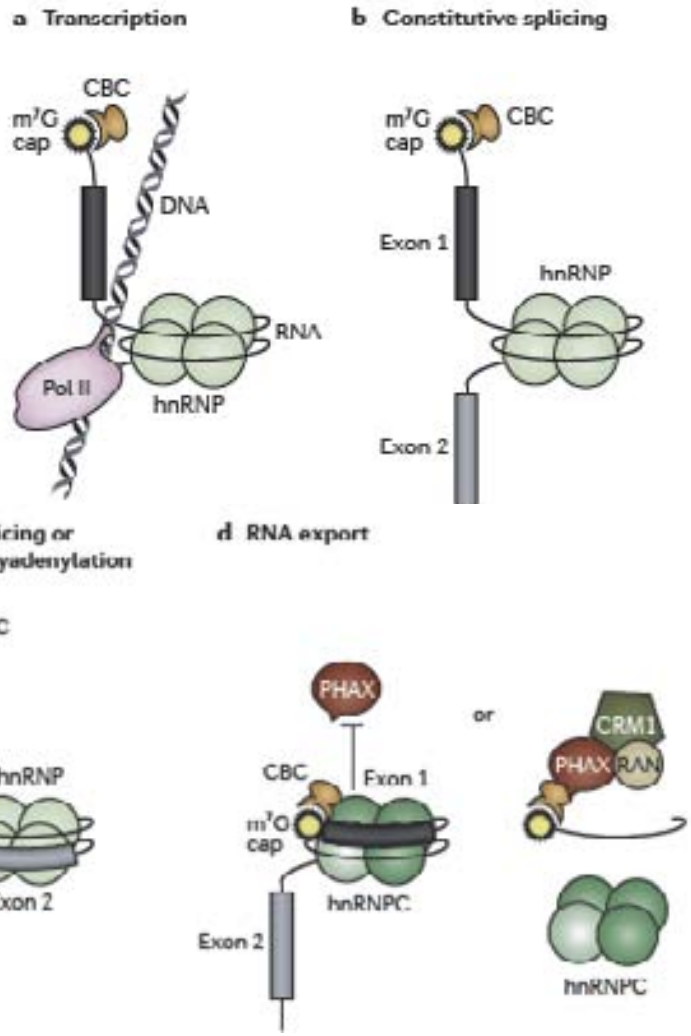


NPC functions in genome organization and gene expression



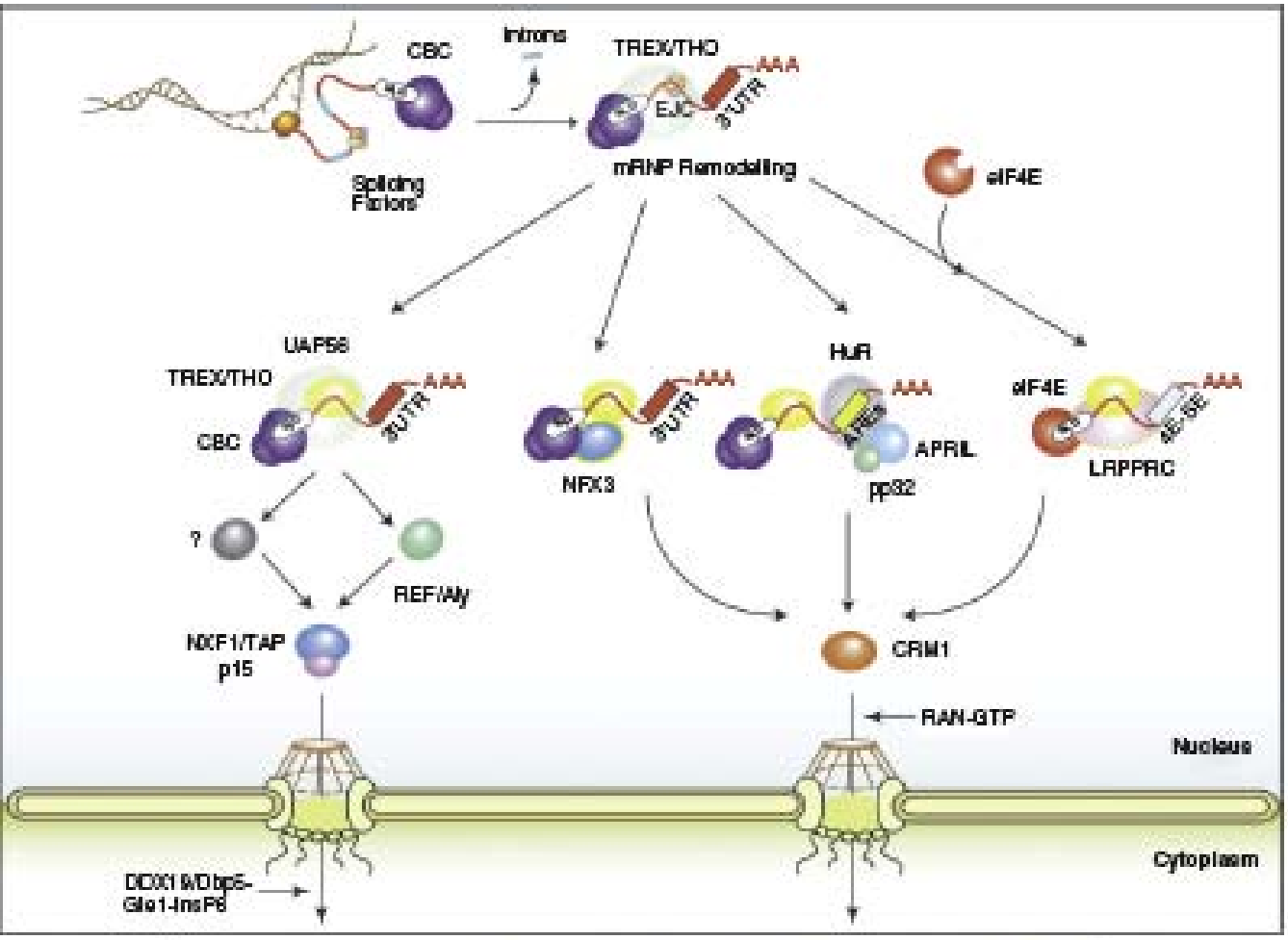
mRNP from TRX to DECAY



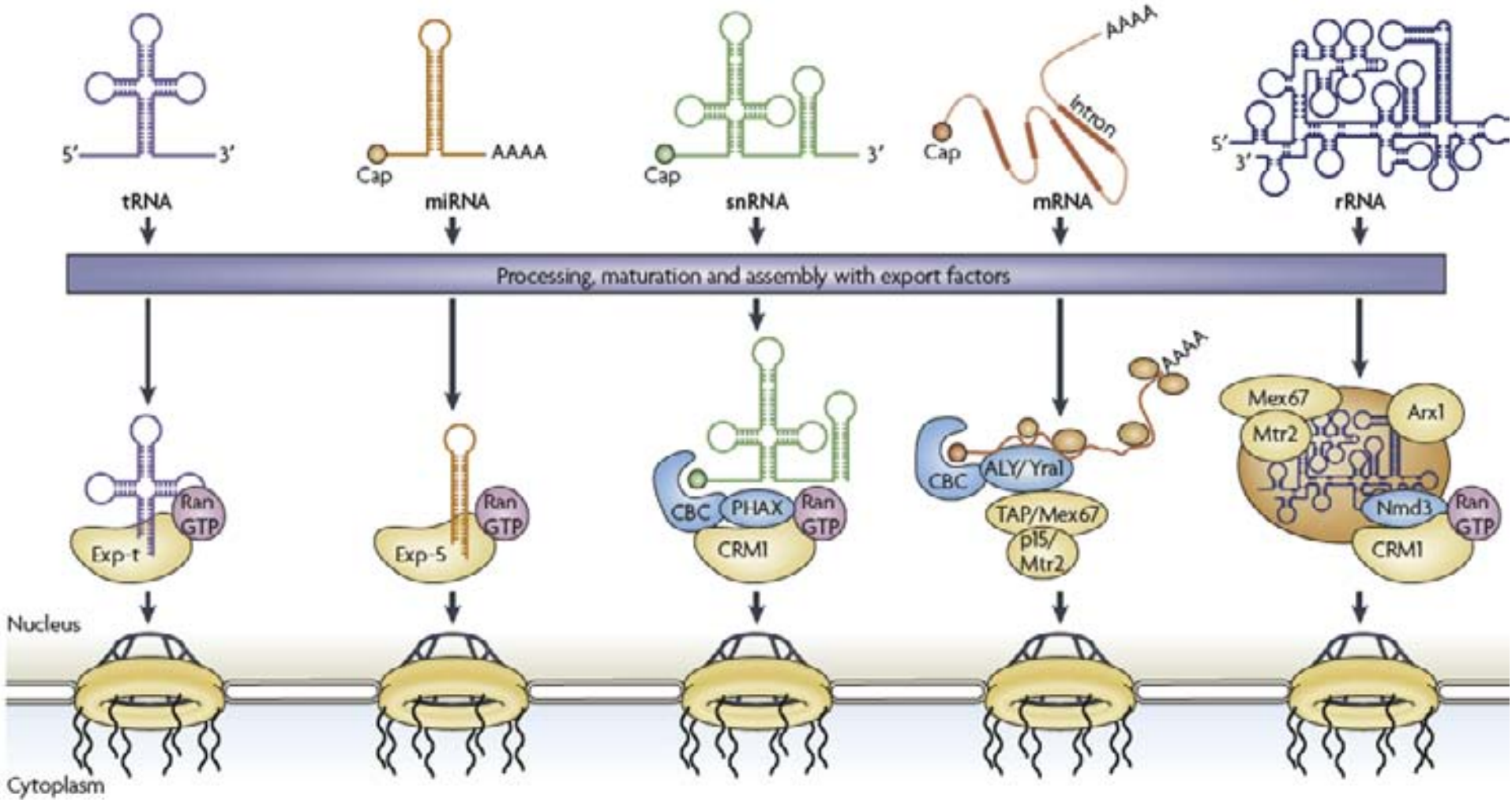


Müller-McNicoll and Neugebauer, Nat.Rev.Genet.,2013

mRNP from TRX to DECAY



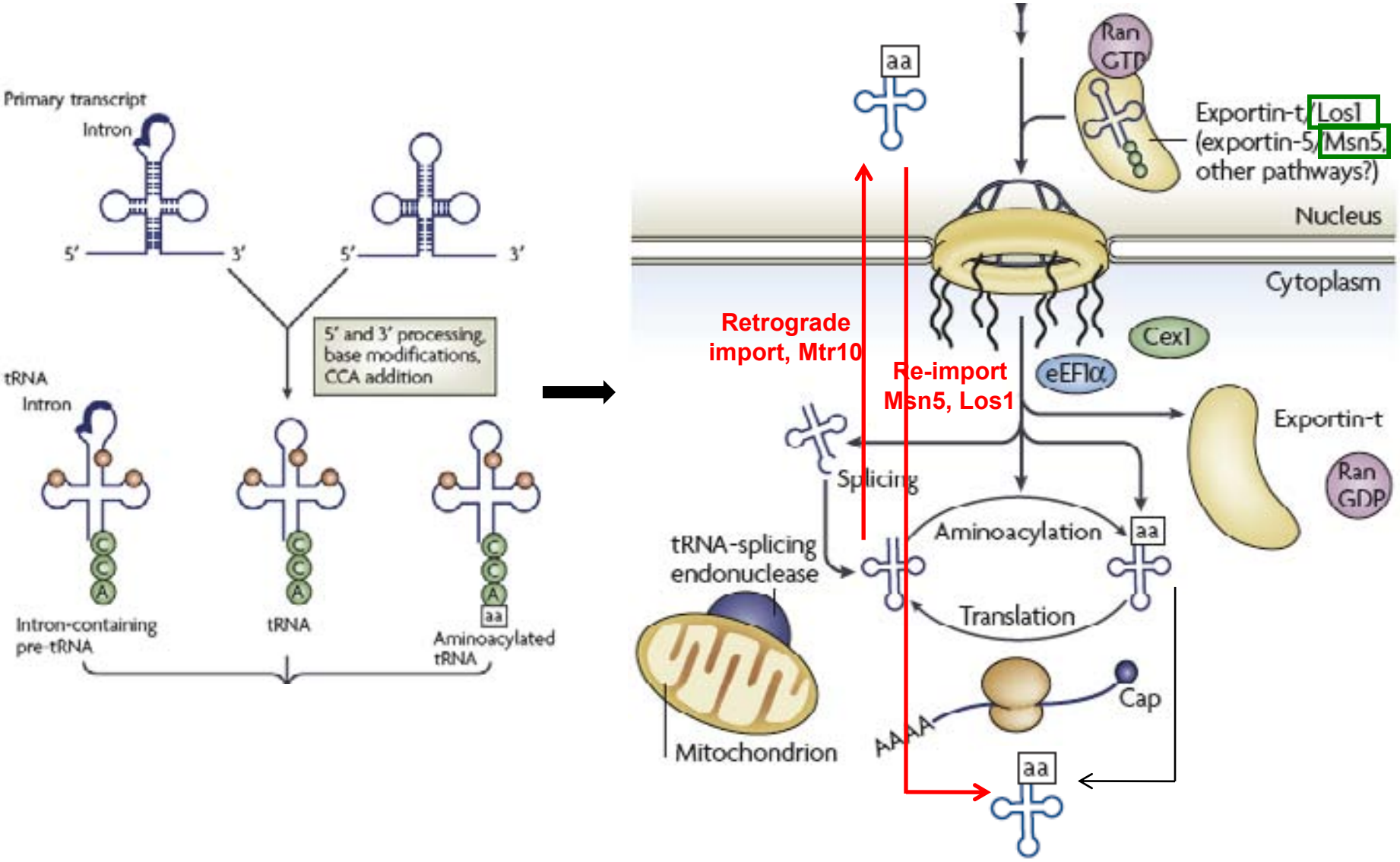
EXPORT of other RNAs



- **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**

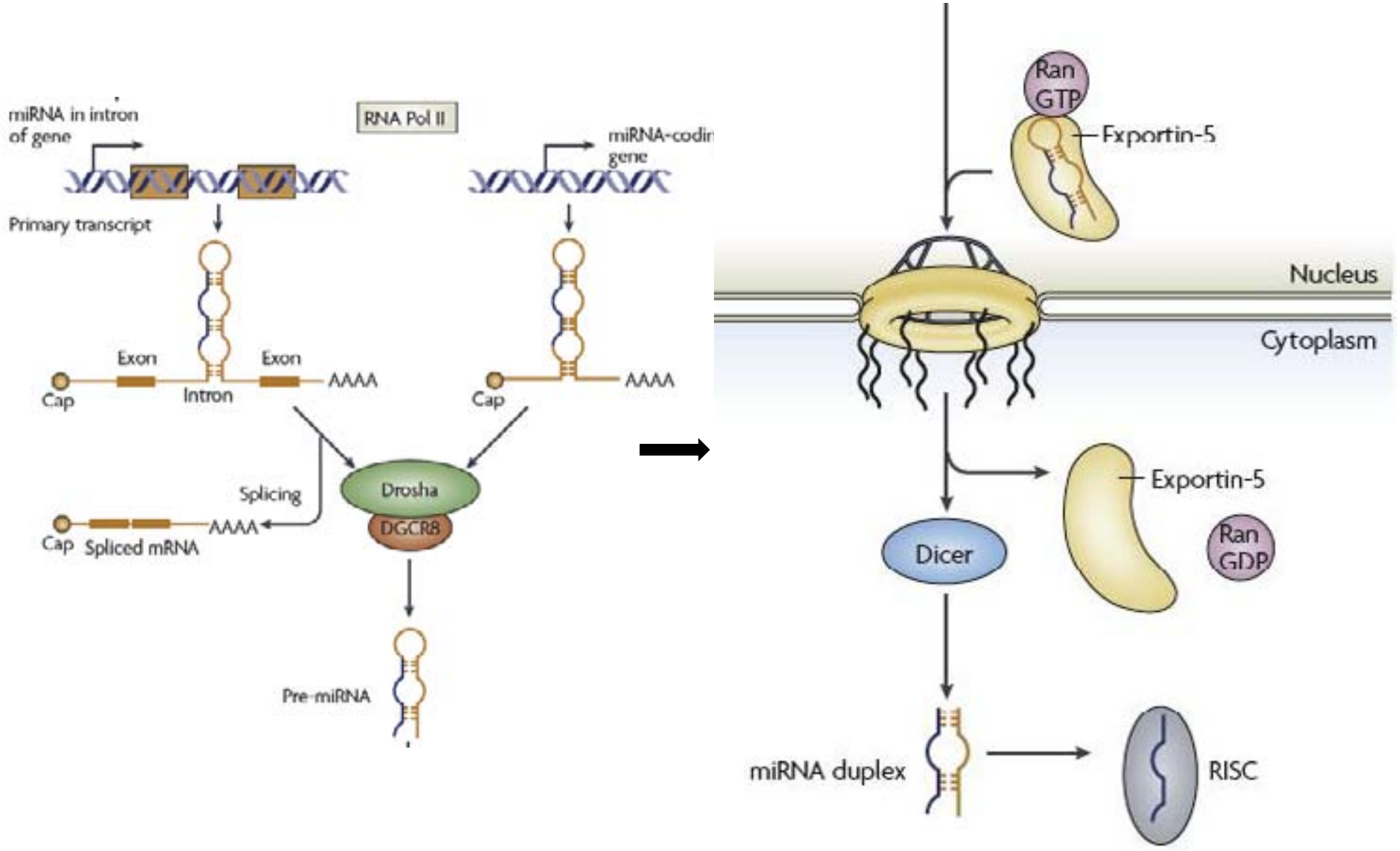
Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

tRNA EXPORT

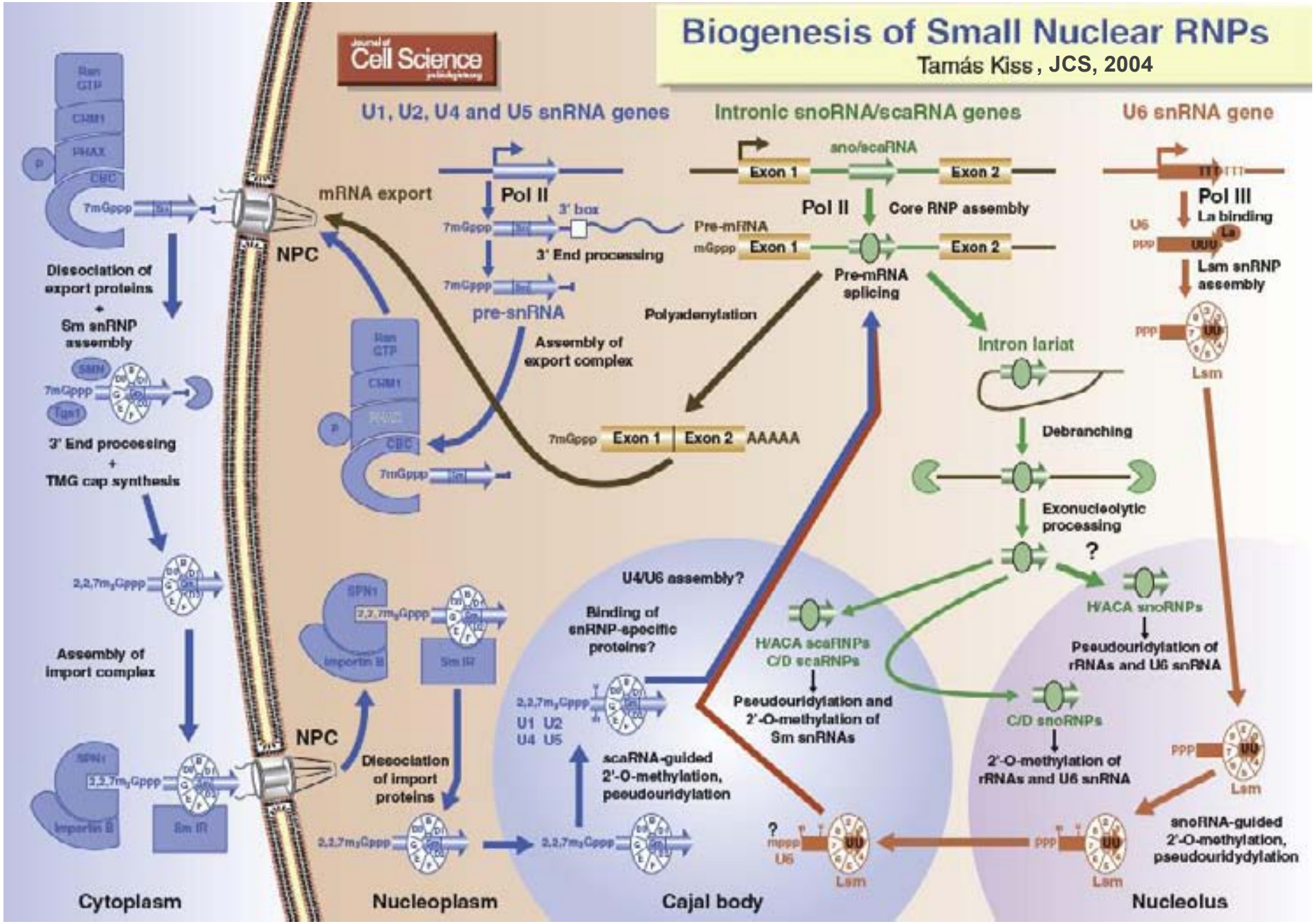


Köhler and Hurt, Nat.Rev.Mol.Cel.Biol,2007

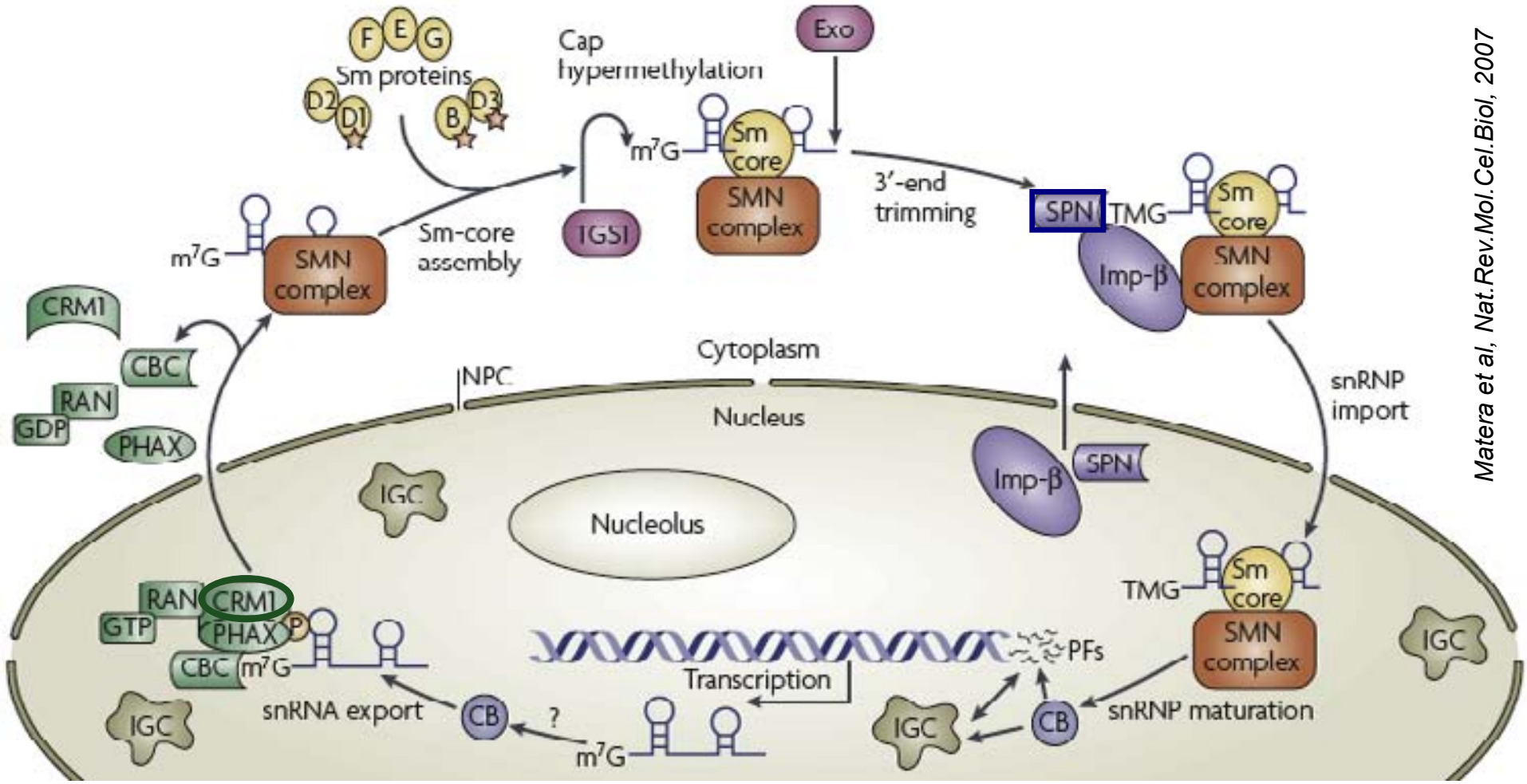
miRNA EXPORT



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


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
Pol II
3' box
3' End processing
pre-snRNA
mRNA export
NPC
Assembly of export complex
Dissociation of export proteins
Sm snRNP assembly
3' End processing
TMG cap synthesis
Assembly of import complex
Dissociation of import proteins
Pre-mRNA
Pre-mRNA splicing
Core RNP assembly
Polyadenylation
Intron lariat
Debranching
Exonucleolytic processing
Pol III
La binding
Lsm snRNP assembly
Lsm
U4/U6 assembly?
Binding of snRNP-specific proteins?
U1 U2 U4 U5
scaRNA-guided 2'-O-methylation, pseudouridylation
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, pseudouridylation
Cytoplasm
Nucleoplasm
Cajal body
Nucleolus

snRNA EXPORT *(metazoa)*



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

CRM1 - export receptor

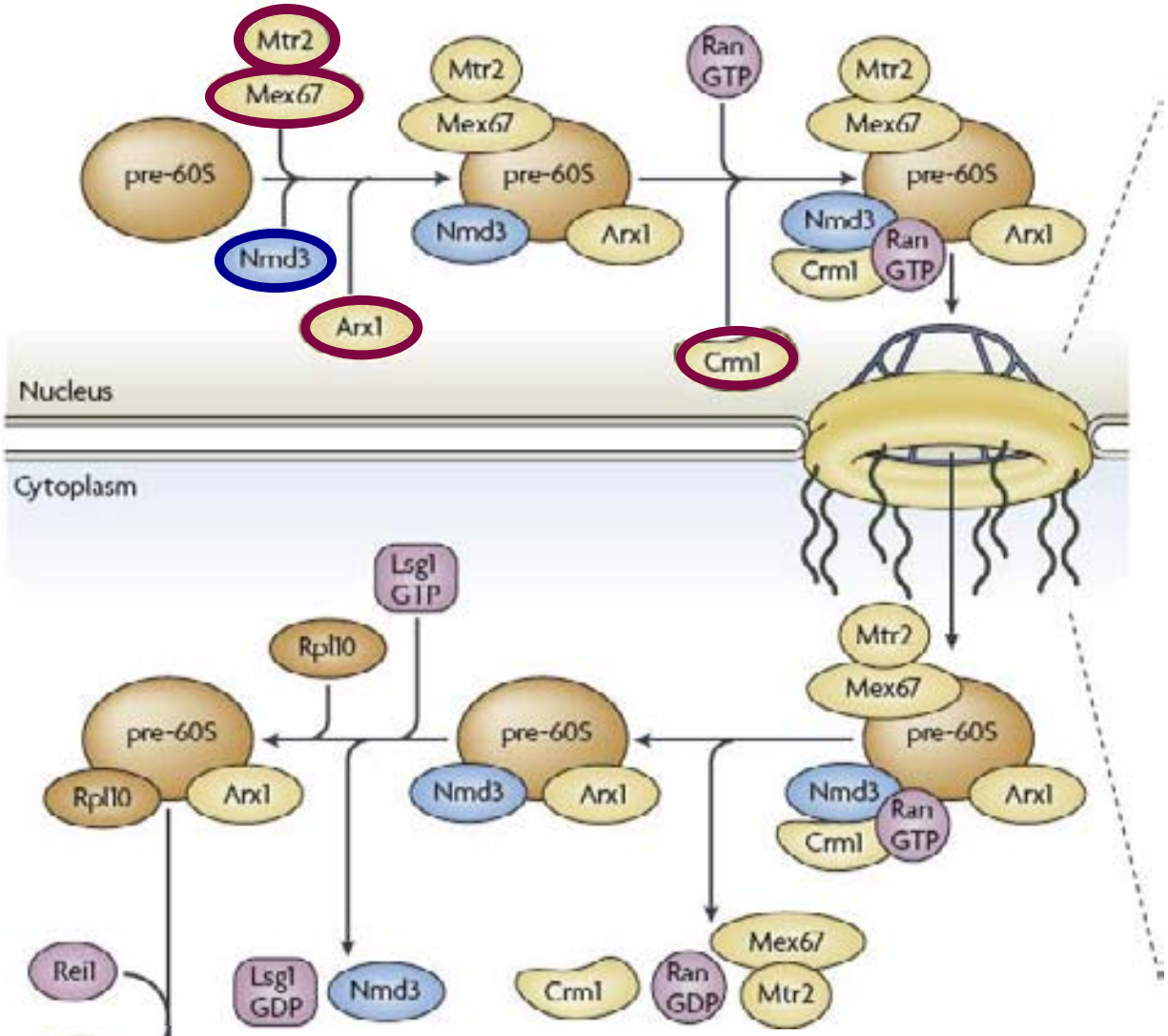
PHAX(-P) - export adaptor, binds to CBC

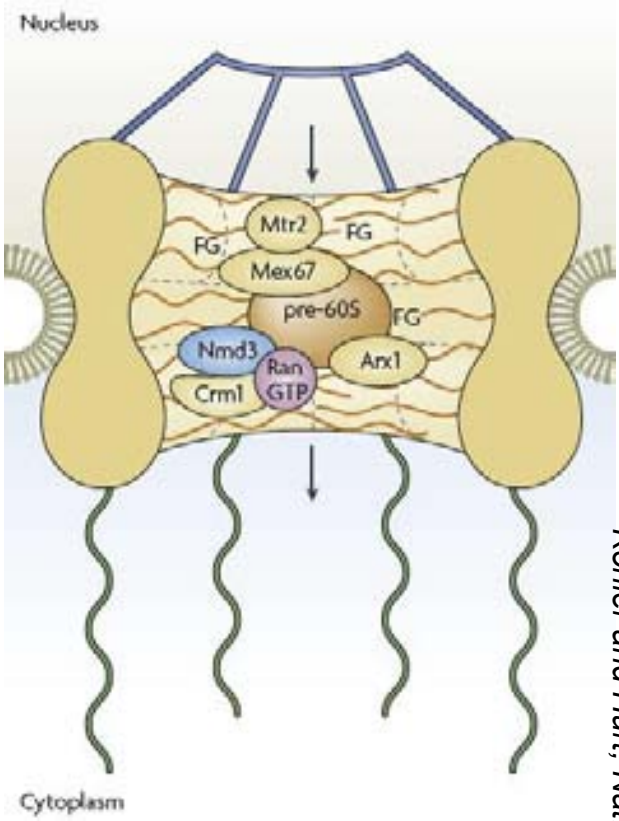
SMN - *survival of motor neuron*, binds snRNA and core Sm proteins to assemble mature snRNP

TGS1 - *trimethylguanosine synthase*, hypermethylates m7G cap to 2,2,7-trimethylguanosine cap

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

rRNA EXPORT





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

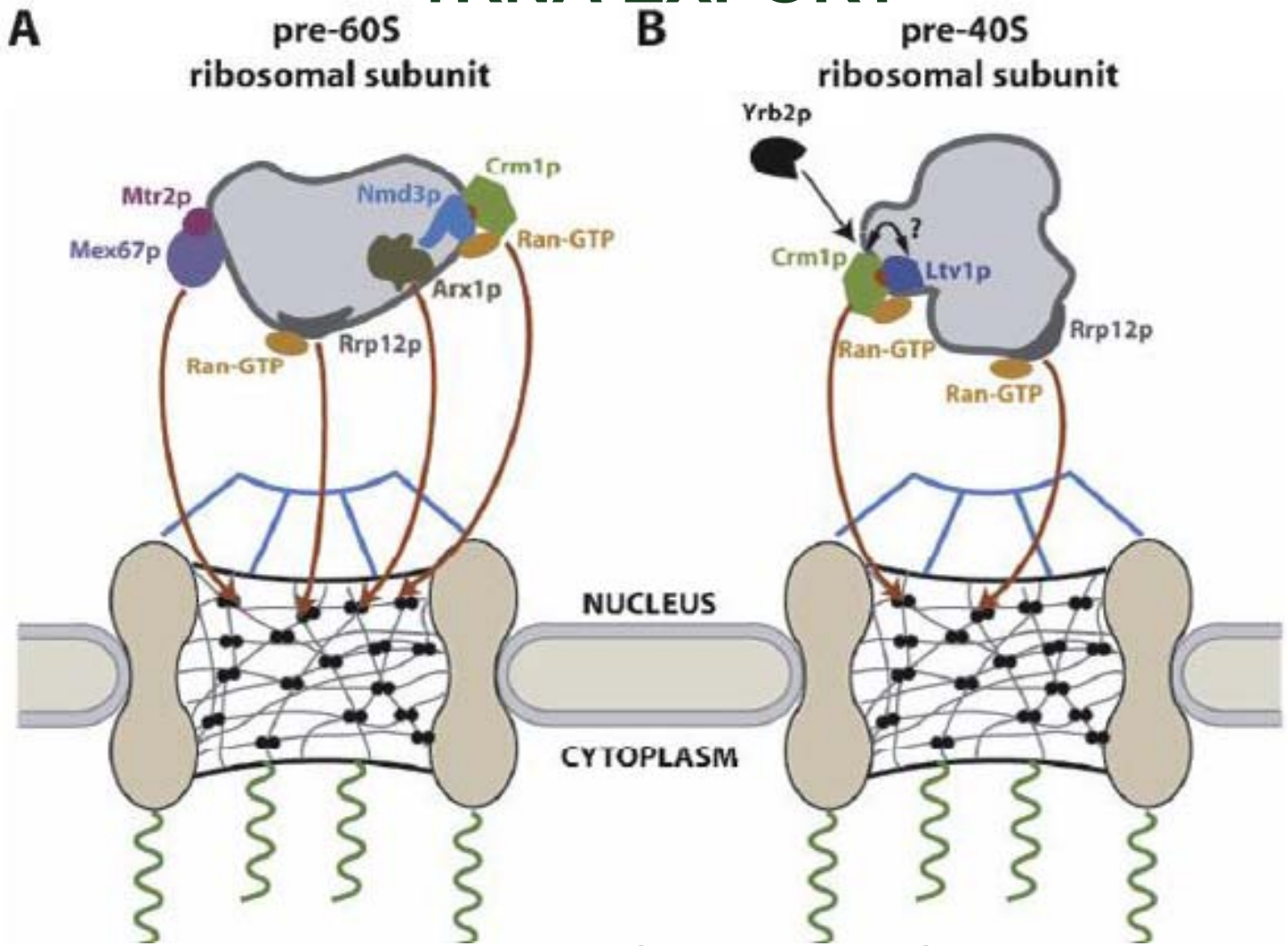
Export adaptor: **Nmd3**

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

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

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

rRNA EXPORT



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**

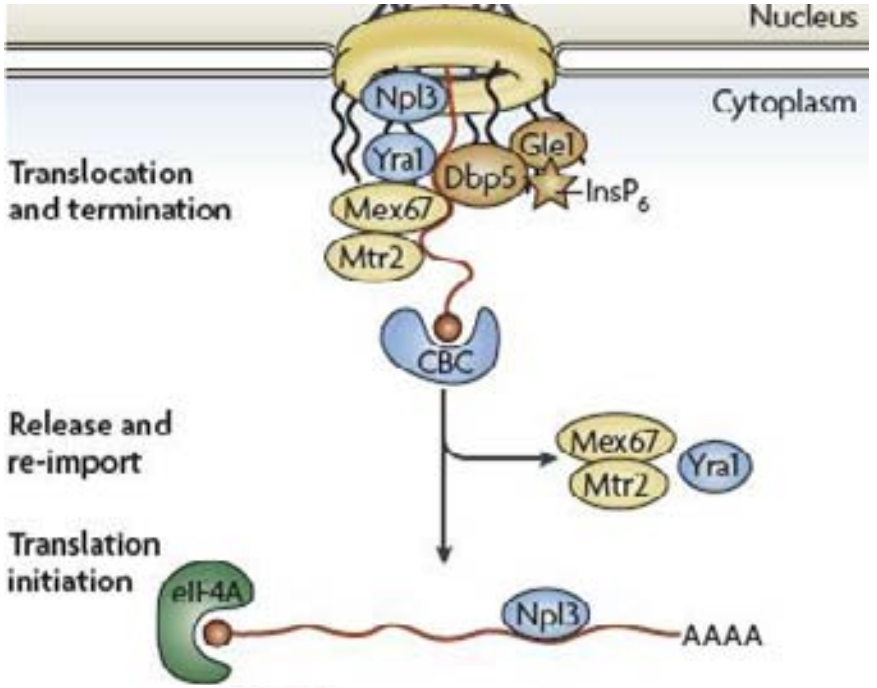
Henras et al., Cell.Mol.Life Sci.,2008

TERMINATION OF EXPORT

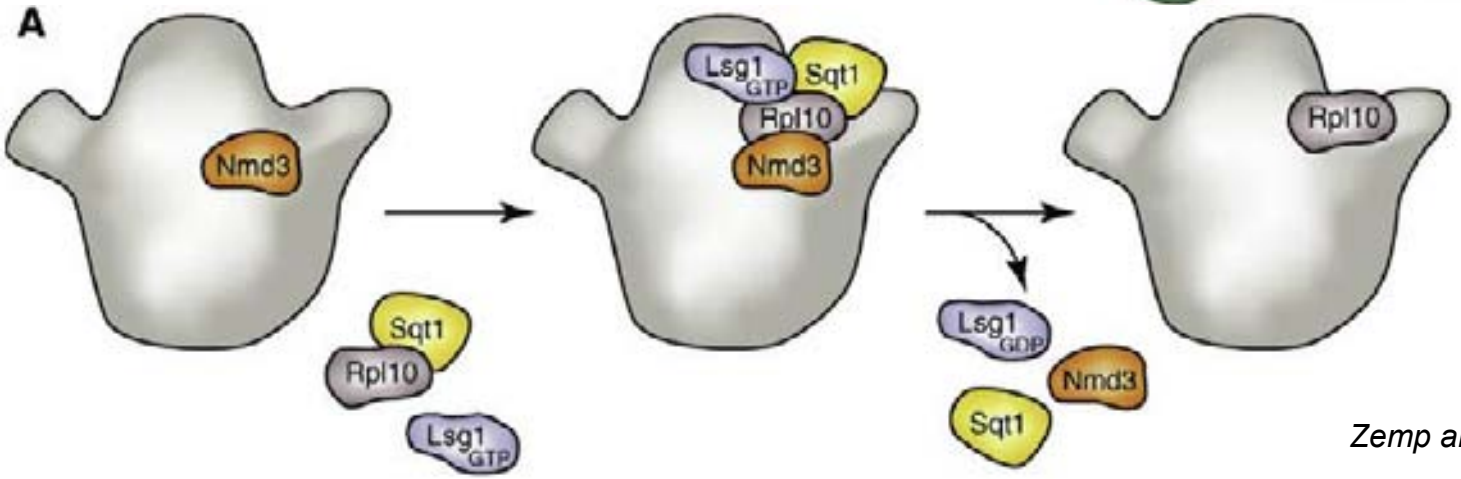
Unidirectional movement of RNPs from the nucleus into the cytoplasm requires RNP remodeling and release by RNA helicases and GTPases

mRNP release by Dbp5 helicase, Gle1 activator (export factor**) and the signaling molecule inositol hexakisphosphate** ($InsP_6$, stimulates ATP activity of Dbp5)

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



Ribosome release by Lsg1 GTPase



Zemp and Kutay, FEBS Lett,2007

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** (karyopherins)**: importins and exportins**

- **Each type of RNA employs a specific export pathway but their components** (adaptors, receptors) **often overlap**

- **Export requires energy: Ran-GTP to Ran-GDP hydrolysis**

- **Also release of mature RNP into the cytoplasm uses energy of ATP-dependent helicases or GTPases**