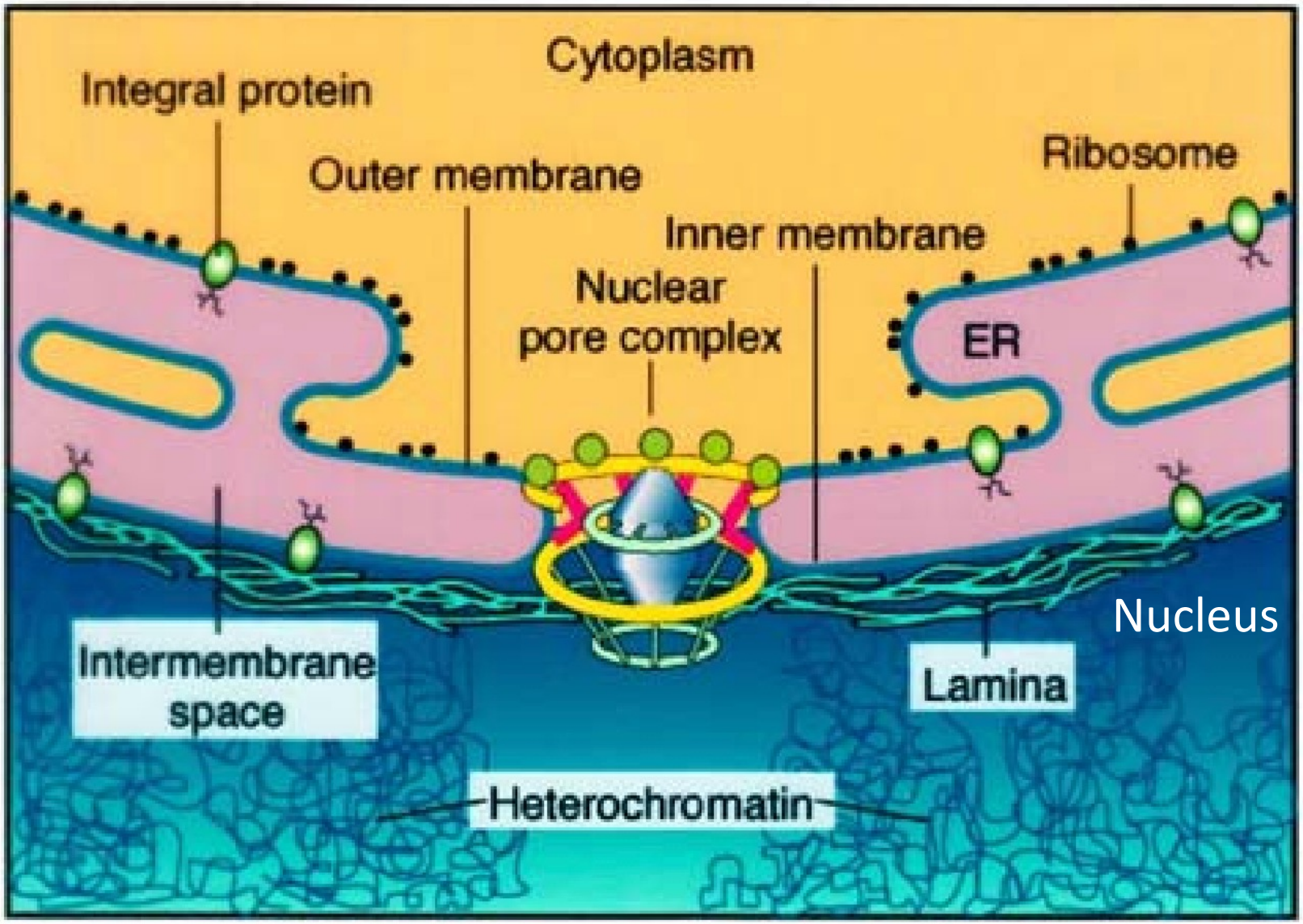
RNA TRANSPORT AND LOCALIZATION

SUMMARY

- RNA **EXPORT/IMPORT** (TRANSPORT FROM THE NUCLEUS TO THE CYTOPLASM AND *VICEVERSA*)

- RNA **LOCALIZATION** IN SUBCELLULAR DISTRICTS

RNA EXPORT

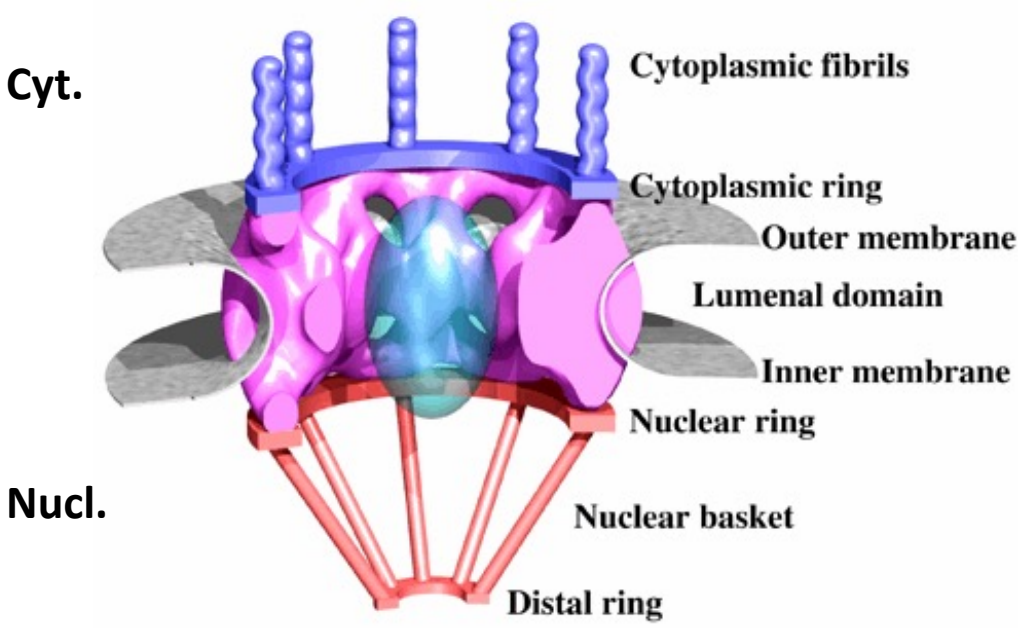

Cytoplasm
Integral protein
Outer membrane
Ribosome
Inner membrane
Nuclear
pore complex
ER
Nucleus
Intermembrane
space
Lamina
Heterochromatin

THE NUCLEAR PORE COMPLEX

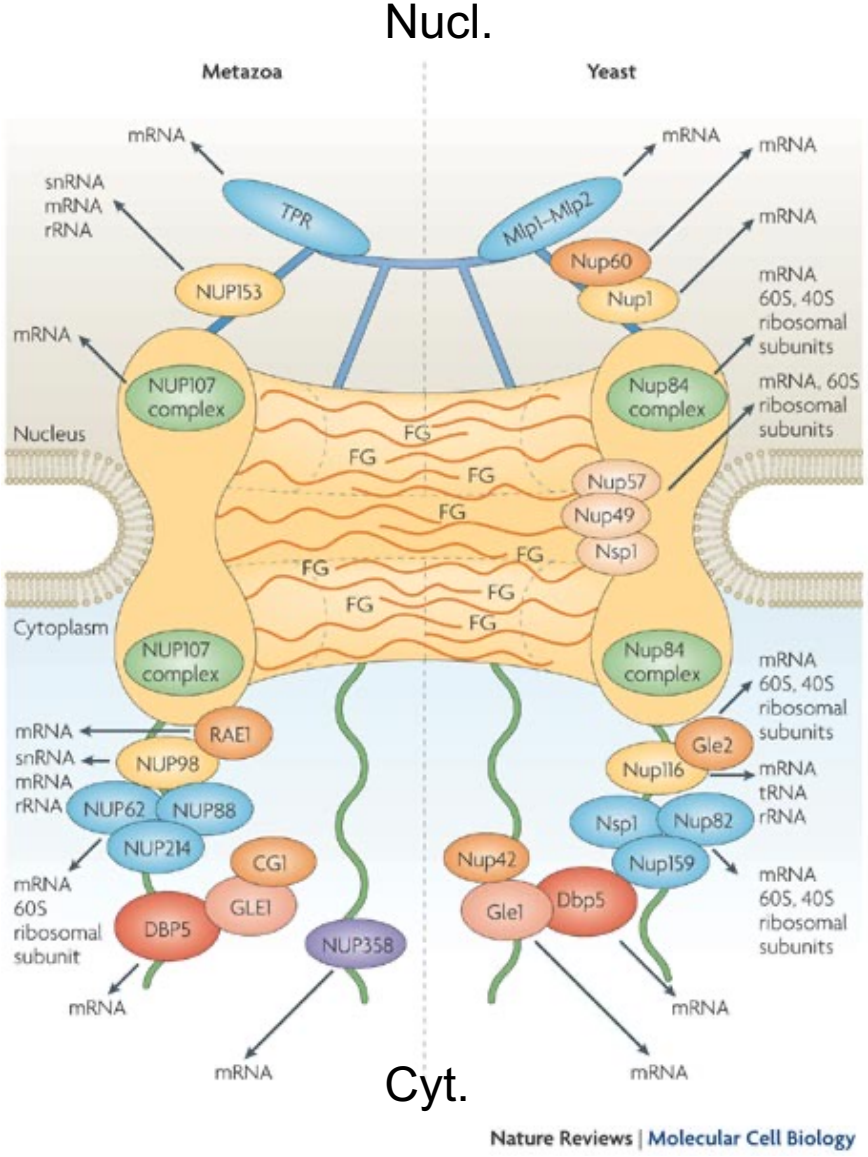
1. It is a complex (**60 MDa** in yeast and **125 MDa** in metazoa) formed by 30 different **nucleoporins** that exist in 8 or 16 copies per NPC

2. Ions and small molecules use passive diffusion (<20-40KDa/5 nm diameter)

3. Proteins and RNPs use **energy-dependent** mediated transport

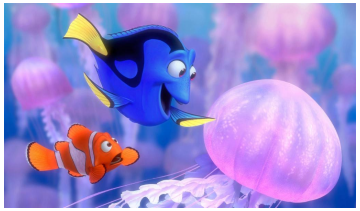


THE NUCLEOPORINS



3 CLASSES:

1. **FG nucleoporins**: contain Phe-Gly-rich repeat. They are present in the <u>transport channel</u> and form a gelatinous structure which allows small molecule diffusion, but blocks large molecules.



2. **Nucleoporins devoid of FG-repeat**. These are <u>structural costituent</u> of the NPC that interact with transport receptors.

3. **Nups**. These are <u>integral membrane proteins</u> that anchor the NPC to the membrane

TRANSPORT RECEPTORS: export and import

1. Transport through NPCs requires a family of conserved **transport receptors** (also known as **karyopherins**)

2. Karyopherins that import cargo in the nucleus are called **importins** and karyopherins that export cargo are called **exportins.**

3. Karyopherins recognize a short peptide signal on a cargo **protein**, either a **nuclear localization signal** (NLS) or a **nuclear export signal** (NES)

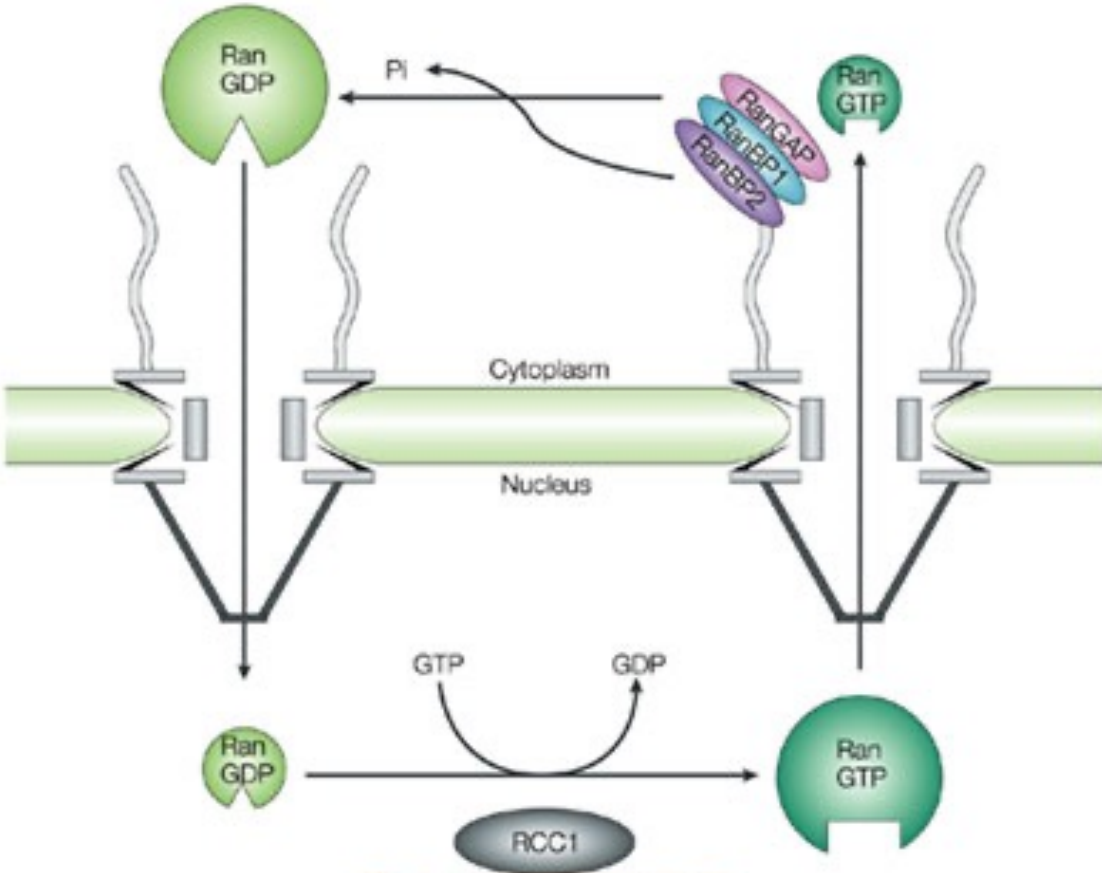
4. Karyopherins can recognize nucleotide motifs in **RNA** cargoes (except mRNAs), which also enables them to export RNAs.

5. Karyopherins are regulated by the **small GTPase Ran (energy producer)**

THE SMALL GTPase RAN DETERMINES TRANSPORT DIRECTION

Ran exists in a GTP-bound state in the nucleus and a GDP-bound state in the cytoplasm.

The RanGTP–RanGDP gradient across the nuclear membrane is generated by the action of two regulators, **RanGEF/RCC1** (Ran-GDP-exchange factor) in the nucleus and **RanGAP** (Ran-GTPase-activating protein) in the cytoplasm, and creates a driving force for directional nucleocytoplasmic transport processes

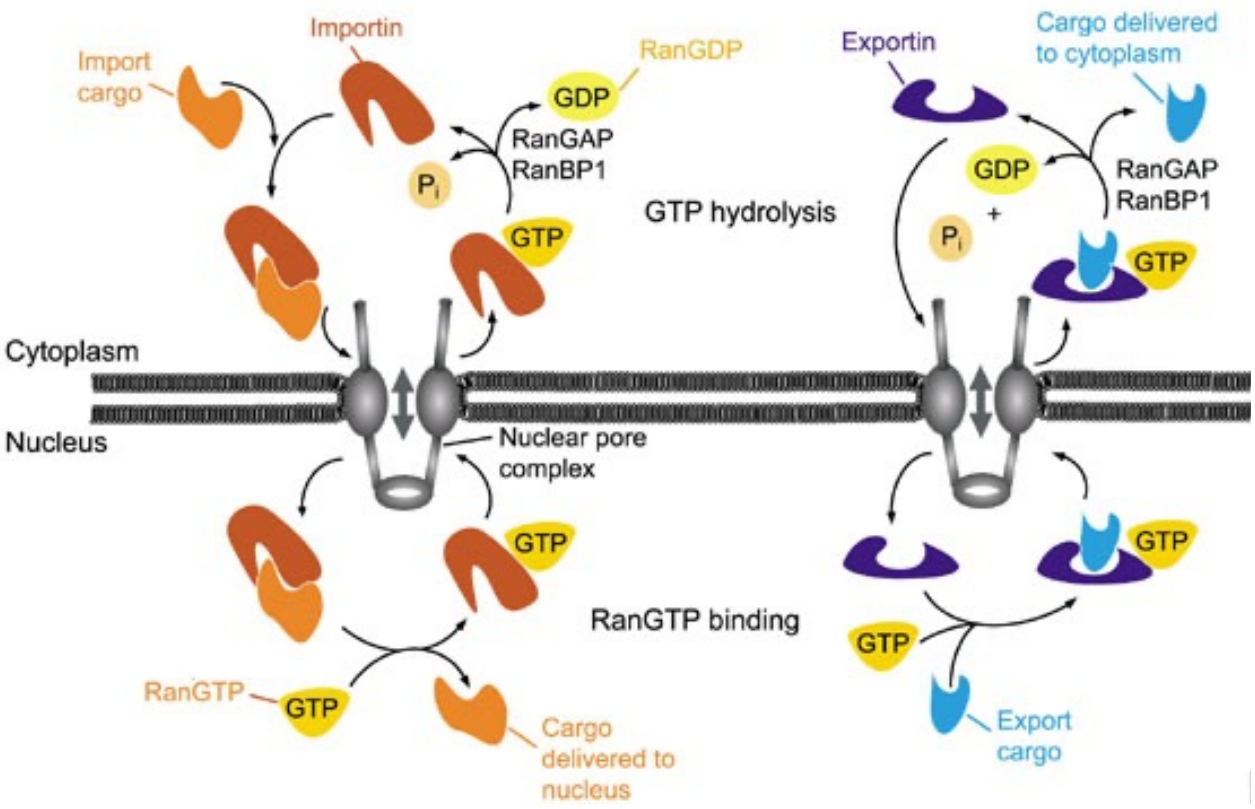


https://www.nature.com/articles/nrm2255

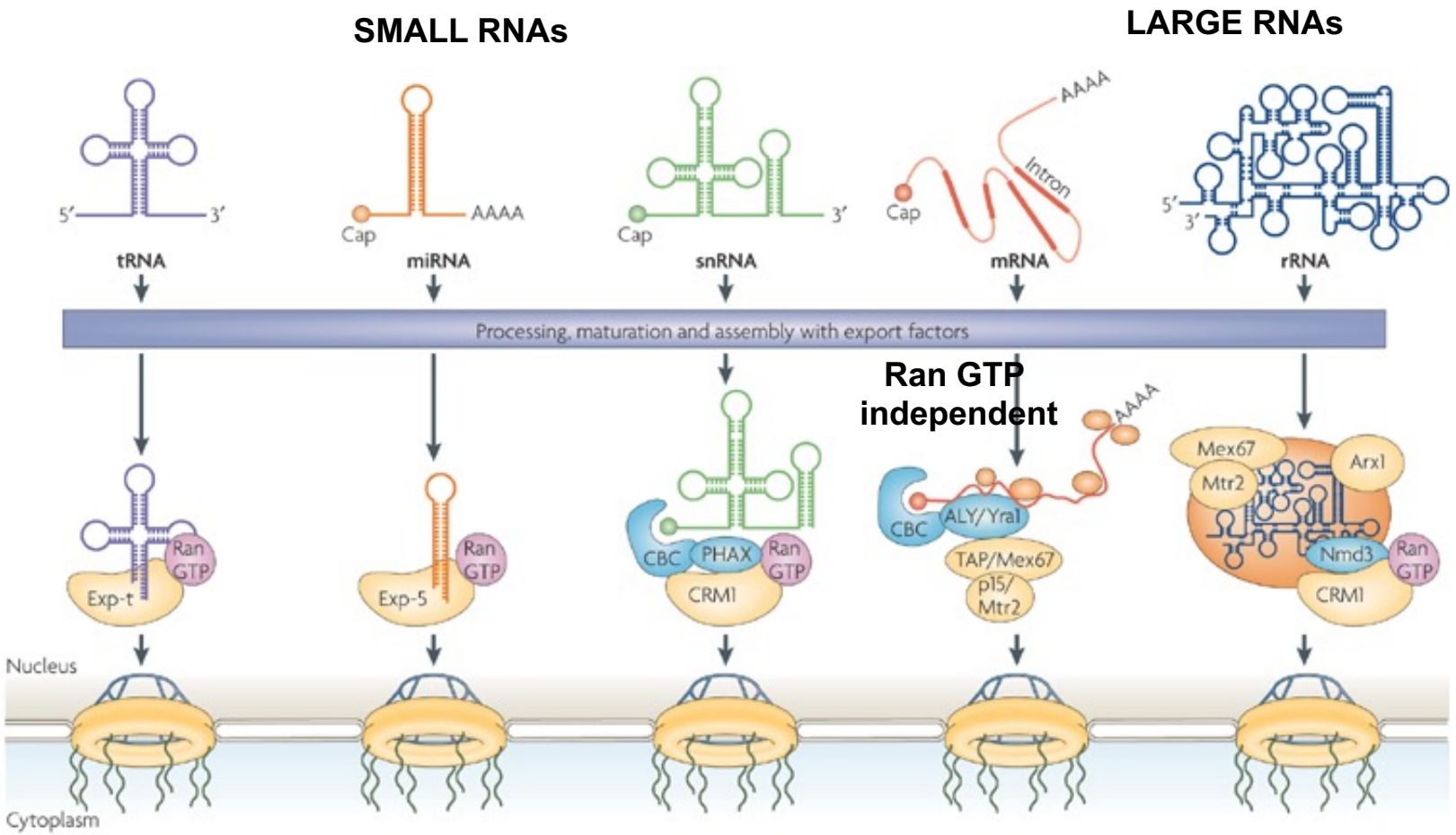
NUCLEAR IMPORT AND EXPORT

•**Importins** bind cargo in the cytoplasm and release it after transport into the nucleus upon binding of RanGTP

•**Exportins** bind nuclear cargo only together with RanGTP, and this ternary complex is translocated to the cytoplasm, where it dissociates upon hydrolysis of RanGTP by RanGAP.



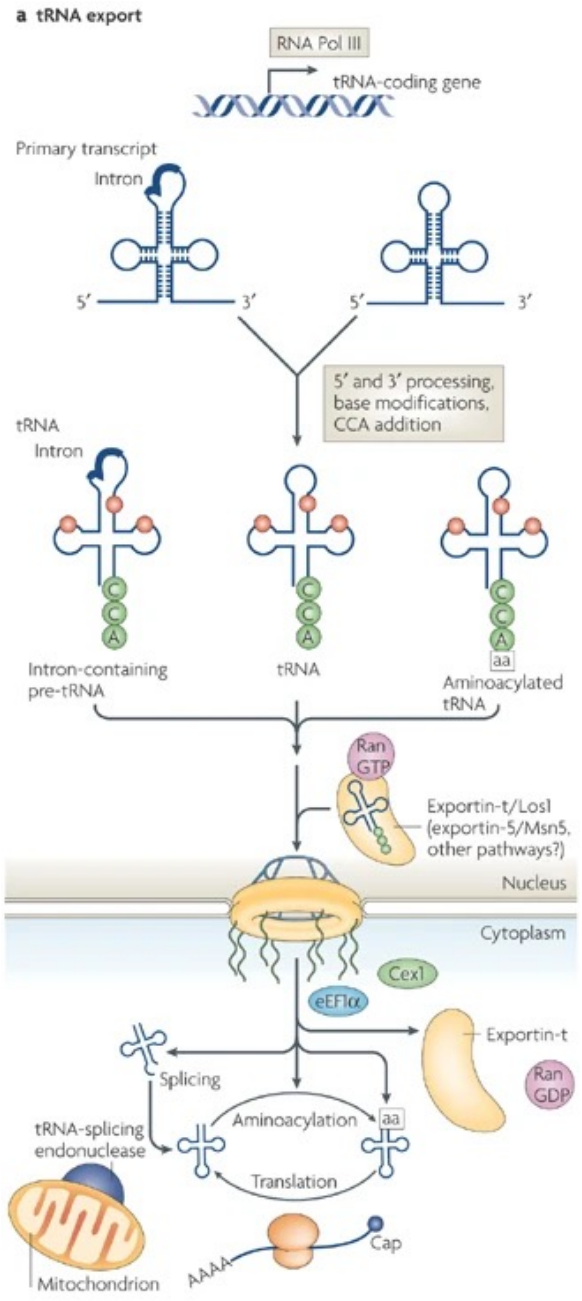
RNA EXPORT OVERVIEW



•**Small RNAs** (**tRNAs**, **microRNAs**) follow simple export routes by <u>binding directly to export receptors</u>

•**Large RNAs** (**rRNAs**, **mRNAs**) assemble into complicated ribonucleoprotein (RNP) particles and <u>recruit their exporters via class-specific adaptor proteins</u>.

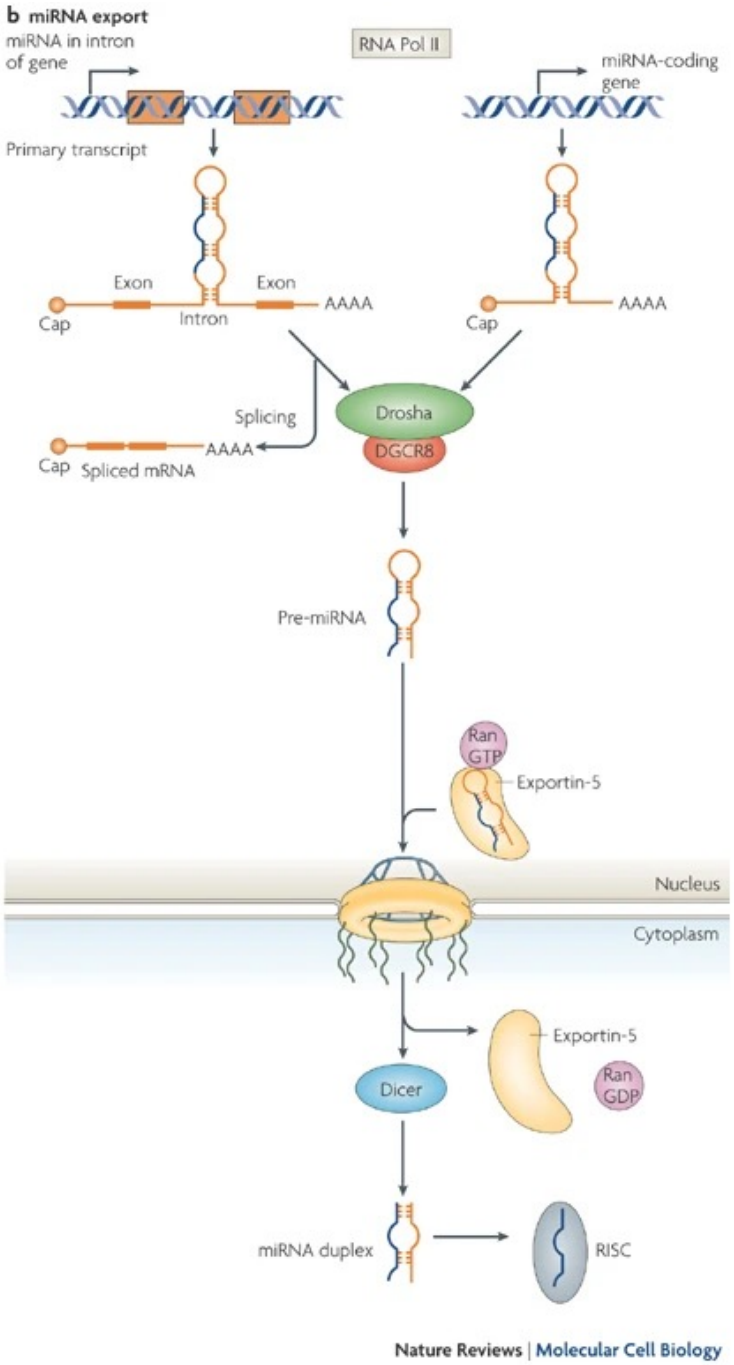
tRNA EXPORT



tRNA: 80 nt. tRNAs can undergo about 200 modifications. Exportins only recognize correct and nearly mature tRNAs. tRNA specific exportin is Exp-t (Los1 in Yeast). However, exportin-t does not discriminate between intron-containing and spliced tRNAs.

Exp-5 has great affinity for pre-miRNA (stem and loop structure), but it can recognize also some tRNAs.

Additional tRNA export routes exist, linked to the aminoacylation machinery: no clear export receptor candidate has as yet been identified



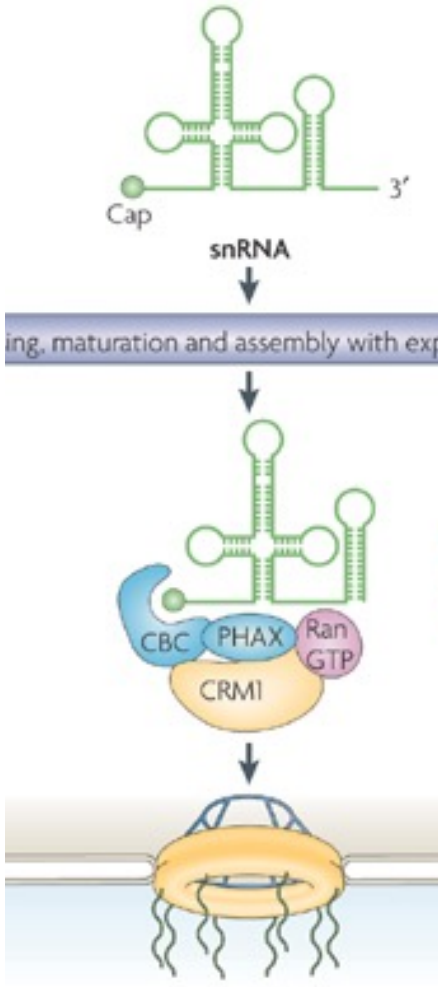
miRNA EXPORT

The ~65-nucleotide pre-miRNA is exported to the cytoplasm in a RanGTP-dependent manner by exportin-5, a member of the karyopherin family.

After release in the cytoplasm upon GTP hydrolysis on Ran, the pre-miRNA hairpin is further cleaved by Dicer, another type III RNase that produces a ~22-nucleotide miRNA duplex.

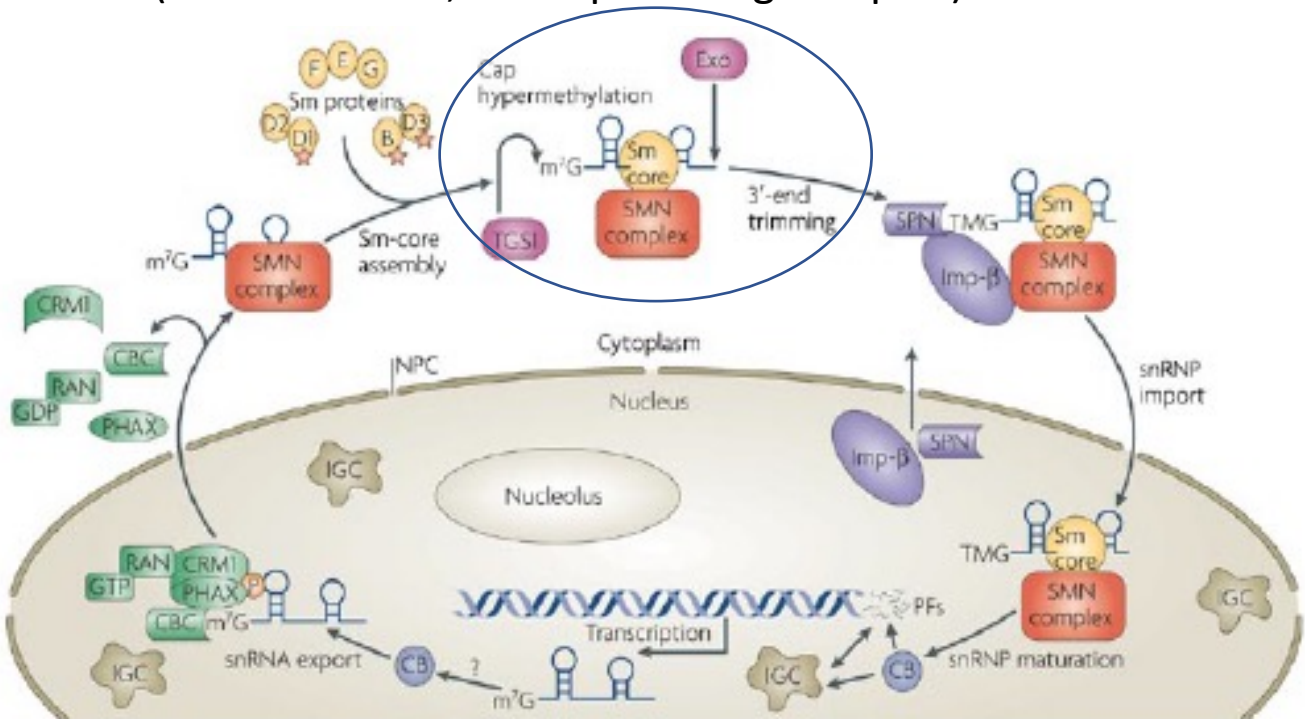
Drosha generates a double-stranded RNA minihelix with a ~2-nucleotide 3′ overhang, the unique structure of which is recognized both by exportin-5 and the downstream-acting processing enzyme Dicer.

snRNA EXPORT

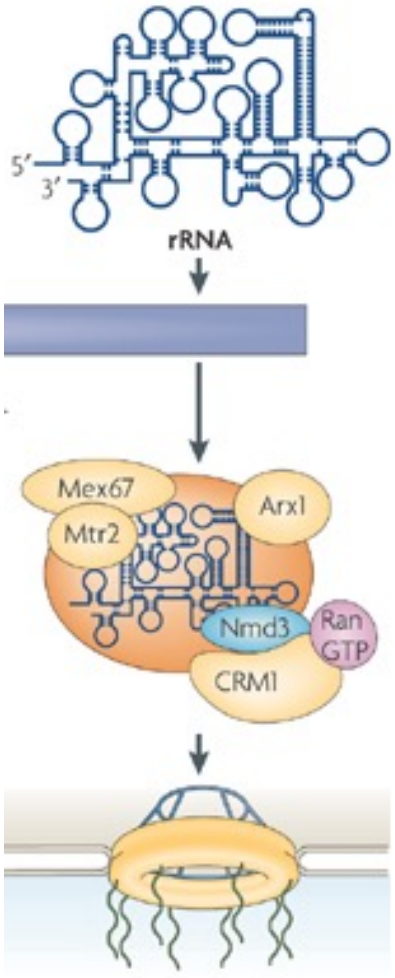


snRNAs have a nuclear and a cytoplasmic phase. Their maturation is completed in the cytoplasm (except U6).

Their specific exportin is CRM1 (exportin-1). It recognize adaptor proteins (PHAX and CBC, the cap-binding complex).



rRNA EXPORT



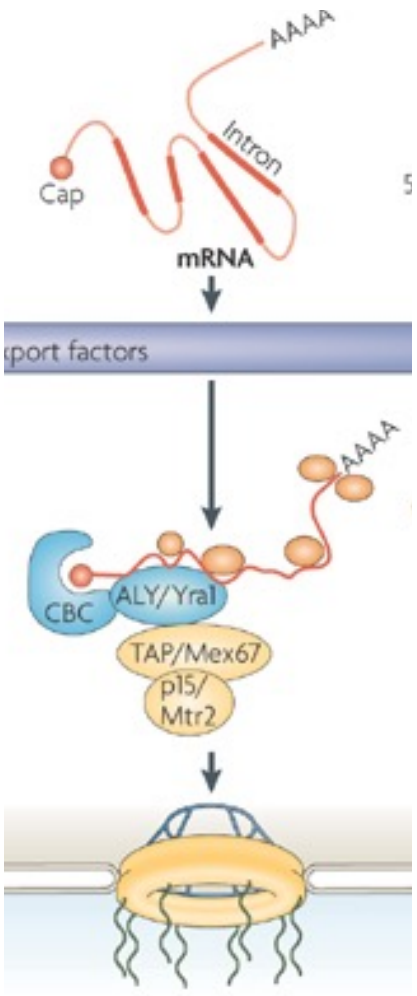
rRNA associate to the ribosomal subunits inside the nucleus.

Ribosomal subunit export has to be very efficient: 2 export systems:

- CRM1 (RAN-GTP-dep.)
- MEX67-MTR2, RAN-GTP-indep. (used by mRNAs).

In mammals, the pre-60S subunit is exported by Crm1 or Exportin 5 (Xpo5), whereas the pre-40S subunit is exported by only Crm1. Nmd3 functions in the export of pre-60S with Crm1. It remains unclear whether Nxf1, a homolog of Mex67, functions as the nuclear export receptor for rRNA.

mRNA EXPORT: differences between YEAST and METAZOA



YEAST:

- The export protein is **Mex67** together with **Mtr2,** which recognize the nucleoporins. The Mex67-Mtr2 complex associate to the mRNP complex.
- Mex67 does not recognize the mRNA directly, but recognizes adaptor proteins, such as **Yra1**.
- The export is connected to **TRANSCRIPTION**: the adaptor protein is associated to the mRNA since its transcription.

METAZOA:

- Export proteins and adaptors are conserved: **TAP–p15 complex** (also known as **NXF1–NXT1**).
- The export is connected to **SPLICING**.

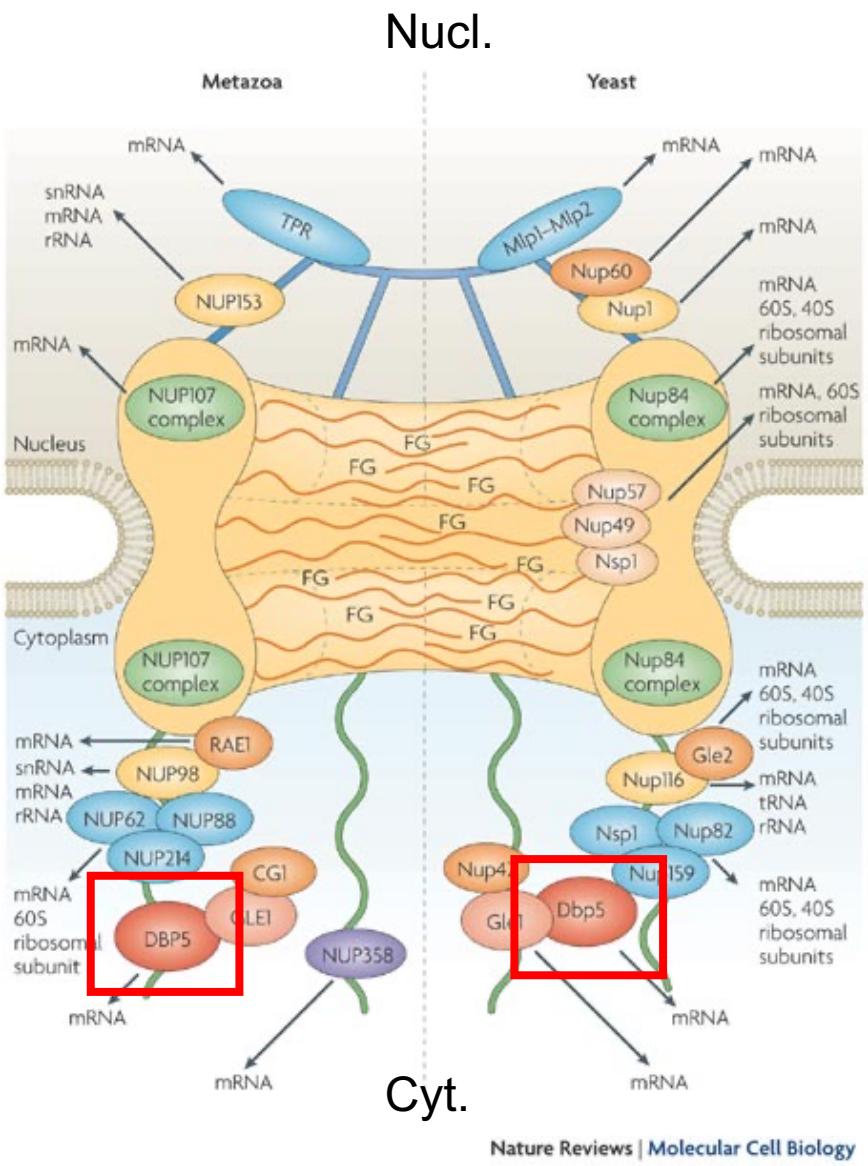
mRNA EXPORT

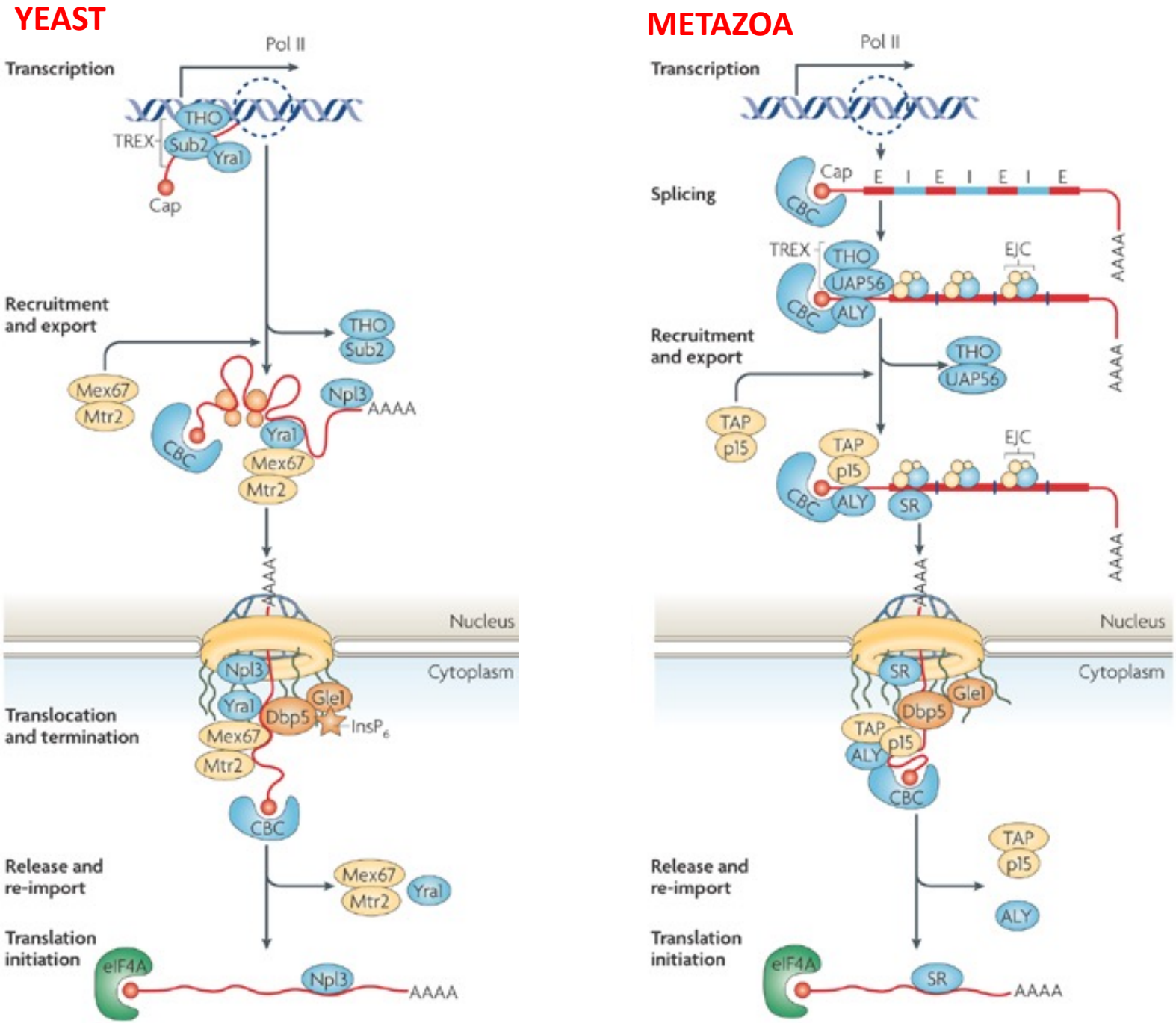
The conserved mRNA **exporter** is structurally **unrelated** to the karyopherins, but it can physically interact with the Phe-Gly-rich repeats of FG nucleoporins.

Both in yeast and in metazoa, mRNA export is **RNA-GTP independent**.

The directionality of the transport is given by **DBP5**, both in yeast and in metazoa.

DBP5 is located in the **cytoplasmic** part of the nucleopore and removes the exporters from the mRNA.

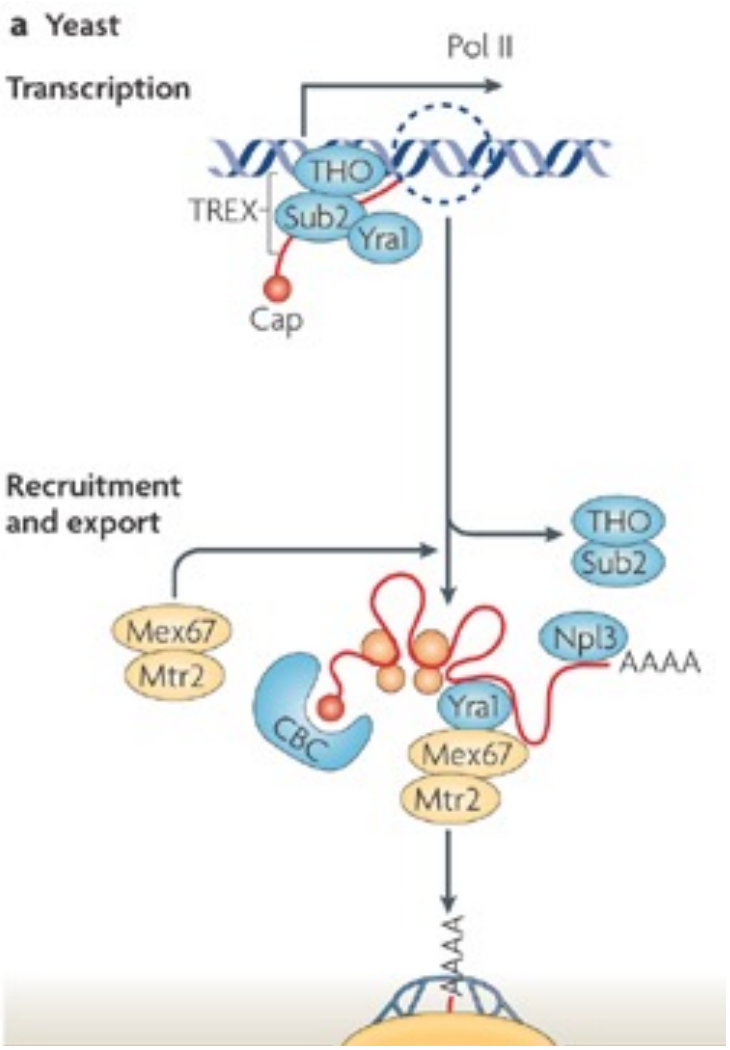



YEAST
Pol II
Transcription
THO
TREX
Sub2
Yra1
Cap
Recruitment and export
THO
Sub2
Mex67
Mtr2
Npl3
AAAA
CBC
Yra1
Mex67
Mtr2
AAAA
Nucleus
Cytoplasm
Translocation and termination
Npl3
Yra1
Gle1
Dbp5
InsP6
Mex67
Mtr2
CBC
Release and re-import
Mex67
Mtr2
Yra1
Translation initiation
eIF4A
Npl3
AAAA
METAZOA
Pol II
Transcription
Splicing
Cap
E I E I E I E
CBC
TREX
THO
UAP56
ALY
EJC
AAAA
Recruitment and export
THO
UAP56
TAP
p15
TAP
p15
CBC
ALY
SR
EJC
AAAA
AAAA
Nucleus
Cytoplasm
SR
Gle1
Dbp5
TAP
p15
ALY
CBC
Release and re-import
TAP
p15
ALY
Translation initiation
eIF4A
SR
AAAA

mRNA EXPORT: YEAST
In the nucleus…

The exporter MEX67-MTR2 has **not** RNA binding domains

MEX67 interactor with RNA binding domains is **YRA1 (Adaptor,** ALY or REF in metazoa**): a** bridge between the mRNA and the exporter



mRNA EXPORT: YEAST
In the nucleus…

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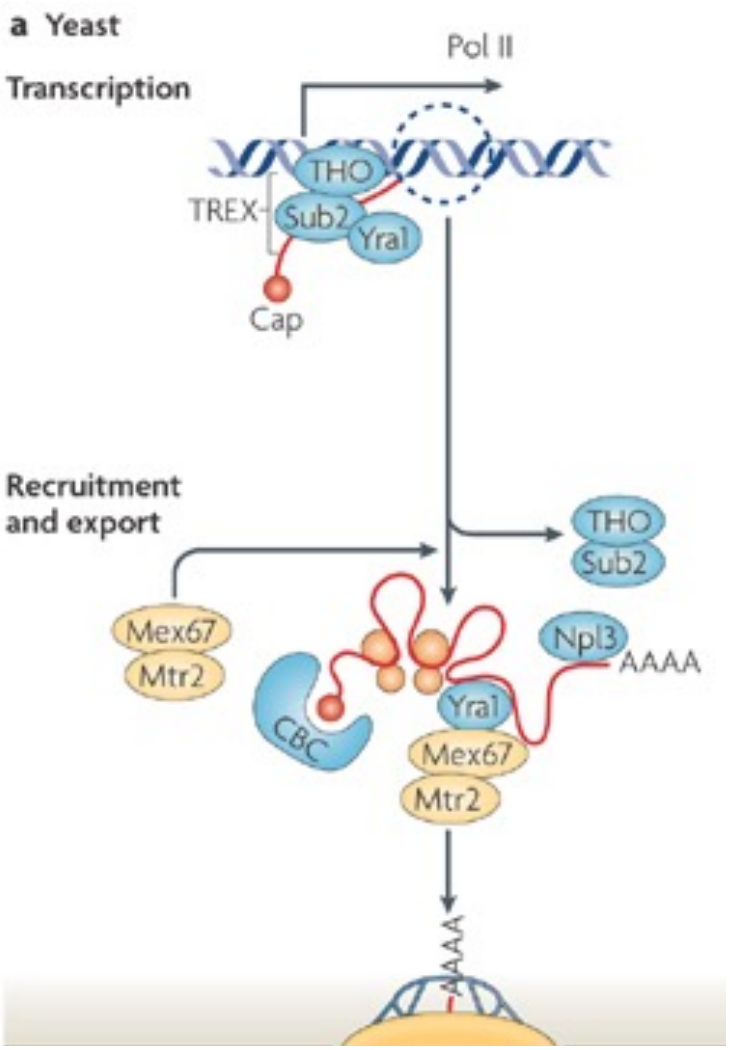
MEX67 interactor with RNA binding domains is **YRA1 (Adaptor): a** bridge between the mRNA and the exporter

SUB2 (UAP56 in metazoa) interacts with YRA1 **(provides competence for the export to the mRNA)**

SUB2 is a RNA-helicase interacting with THO complex (elongation complex).



mRNA EXPORT: YEAST
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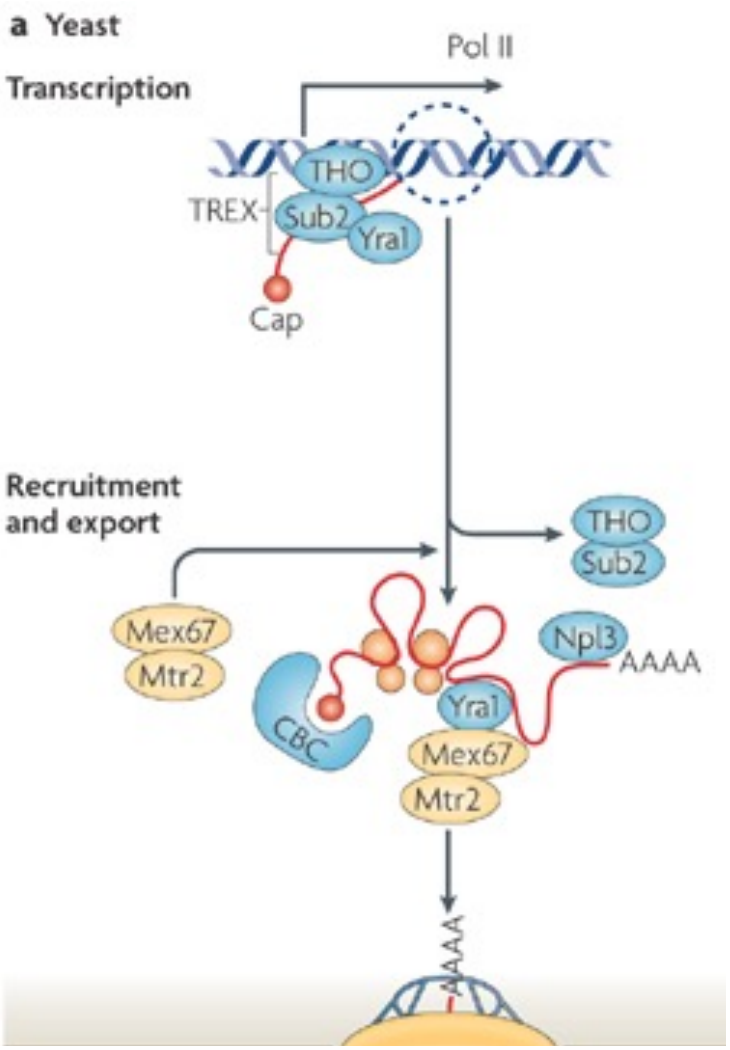
SUB2 interacts with YRA1 **(provides competence for the export to the mRNA)**

SUB2 is a RAN-helicase interacting with THO complex (elongation complex).

1. When YRA1 interacts with SUB2, it cannot bind MEX67 -> **mRNA+SUB2+YRA1= not ready for the export**



mRNA EXPORT: YEAST
In the nucleus…

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SUB2 interacts with YRA1 **(provides competence for the export to the mRNA)**

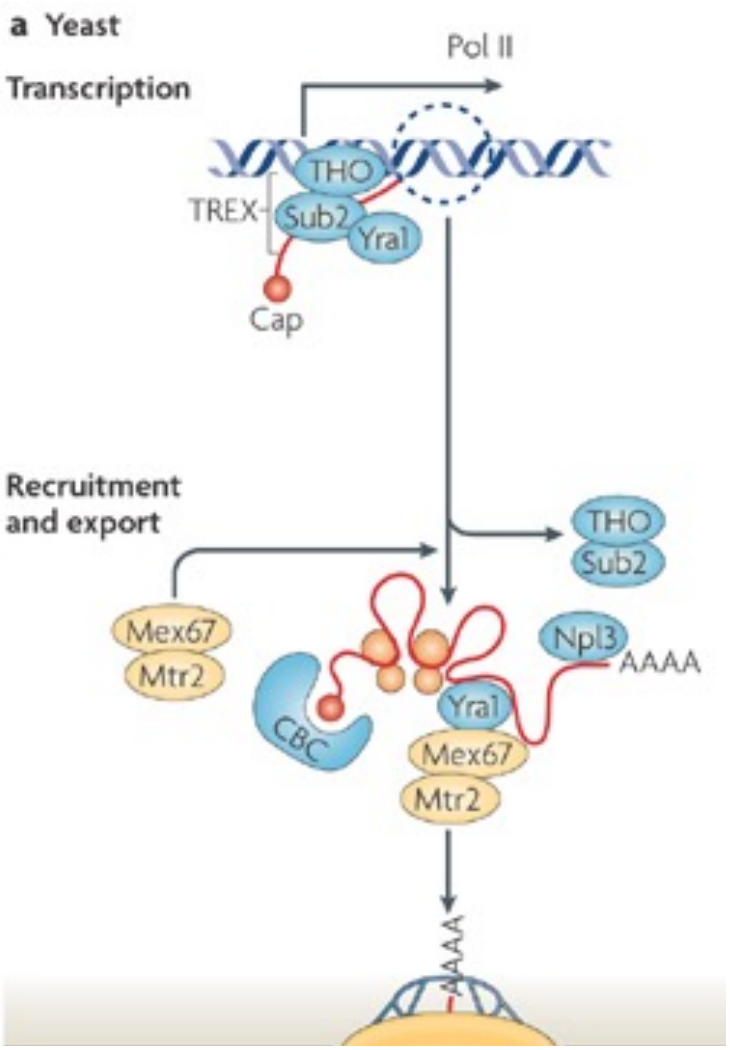
SUB2 is a RAN-helicase interacting with THO complex (elongation complex).

1. When YRA1 interacts with SUB2, it cannot bind MEX67 -> **mRNA+SUB2+YRA1= not ready for the export**

2. At the end of transcription, **SUB2 detaches** from the mRNA and YRA1 -> **YRA1 + MEX67** -> **mRNA ready to be exported.**

THO elongation complex is therefore important also for export: **THO+SUB2+YRA1= TREX** complex

TREX complex associates to the mRNA **during transcription** elongation, in <u>yeast</u>



mRNA EXPORT: YEAST
In the cytoplasm…

YEAST in the Cytoplasm:

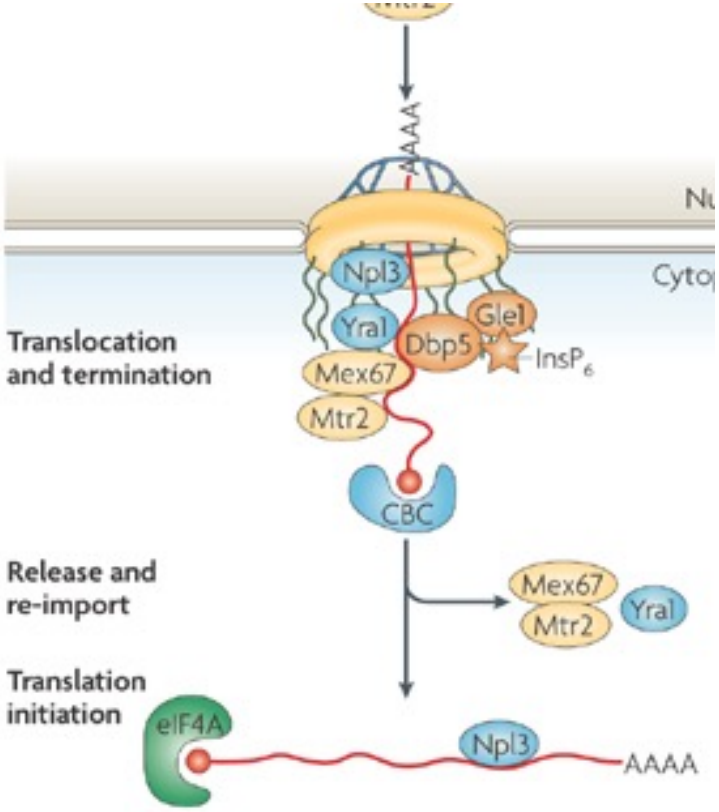
Interactions between Mex67-Mtr2 and FG- nucleoporins facilitate movement of the mRNP 1.

DBP5 is the helices which provides directionality to the export.

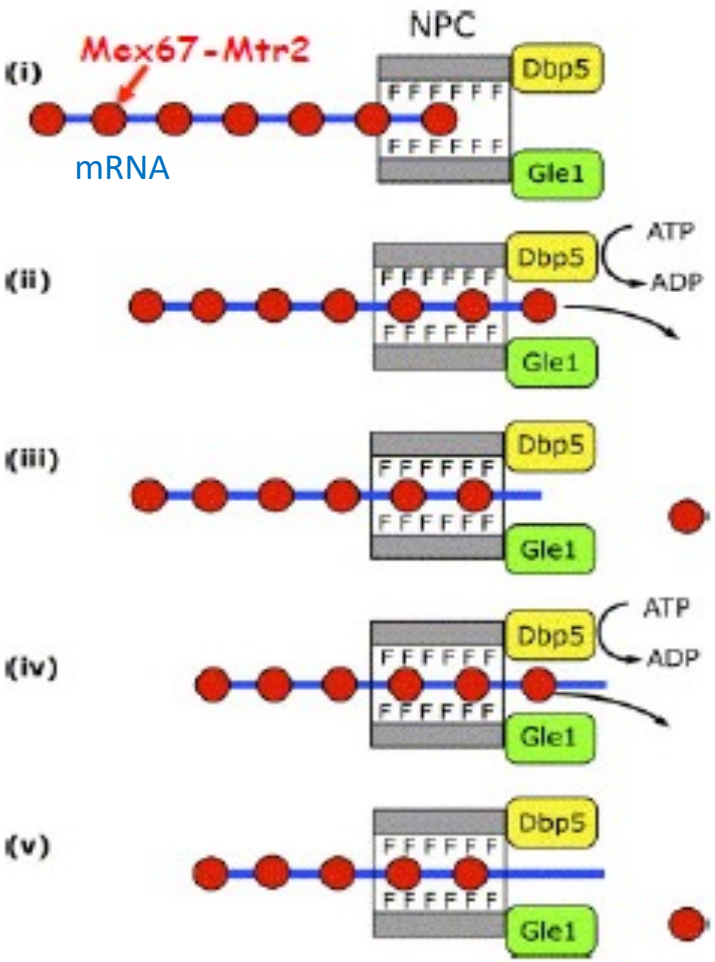
To be activated, DBP5 requires **GLE1** (associated to the nucleopore) and **InsP6** (inositole exakiphosphate).

DBP5 hydrolyzes ATP to produce energy for the export.

When one of the Mex67-Mtr2 complexes reaches the cytoplasmic face of the NPC, it is removed from the mRNP by Dbp5 2**. Removal of Mex67-Mtr2 prevents this segment of the mRNP from moving back into the transport channel and so functions as a molecular ratchet.**



mRNA EXPORT: YEAST
At the cytoplasmic side of the nucleopore…

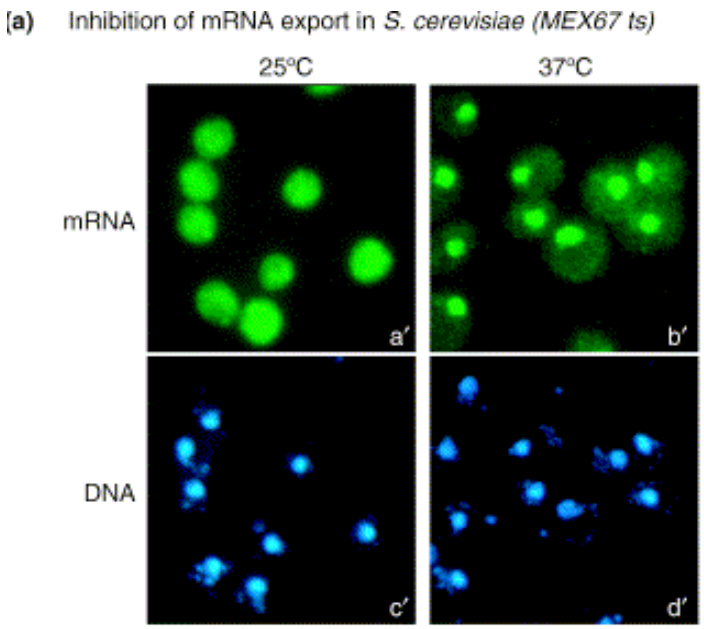


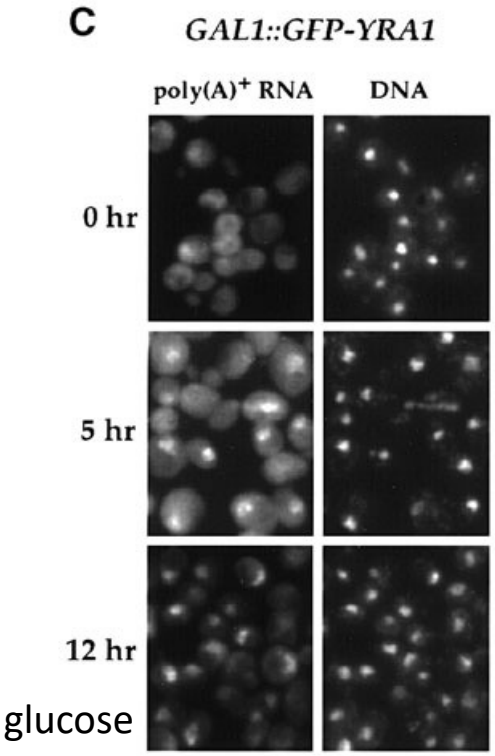
Interactions between **Mex67-Mtr2** and **FG-nucleoporins** facilitate movement of the mRNP

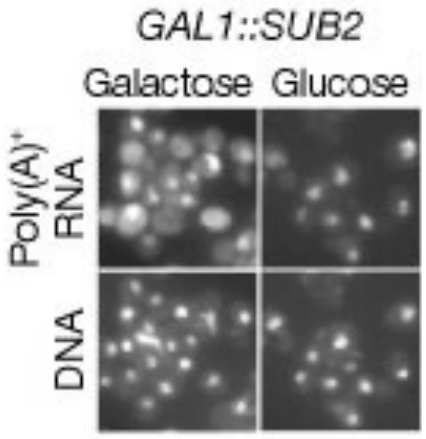
1. When one of the Mex67-Mtr2 complexes reaches the cytoplasmic face of the NPC, it is **removed from the mRNP by Dbp5**

2. **Removal of Mex67-Mtr2 prevents this segment of the mRNP from moving back** into the transport channel and so functions as a molecular ratchet.

mRNA EXPORT: conditional mutants in yeast

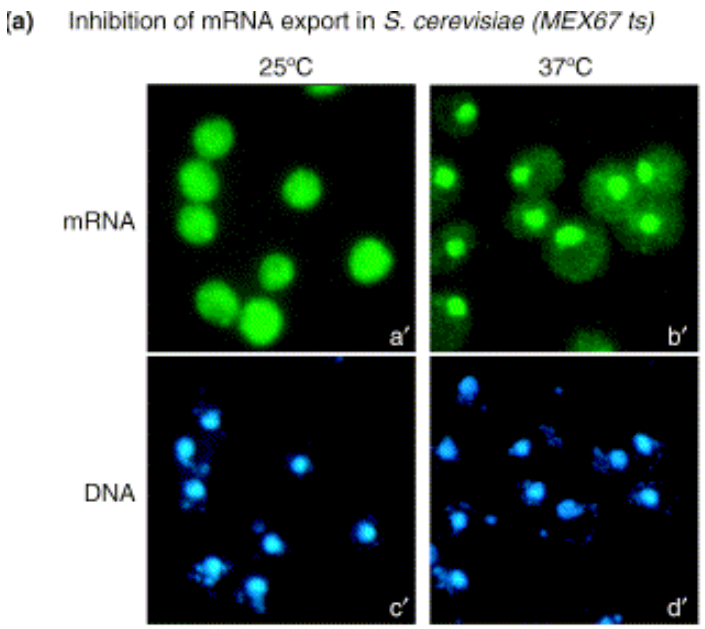


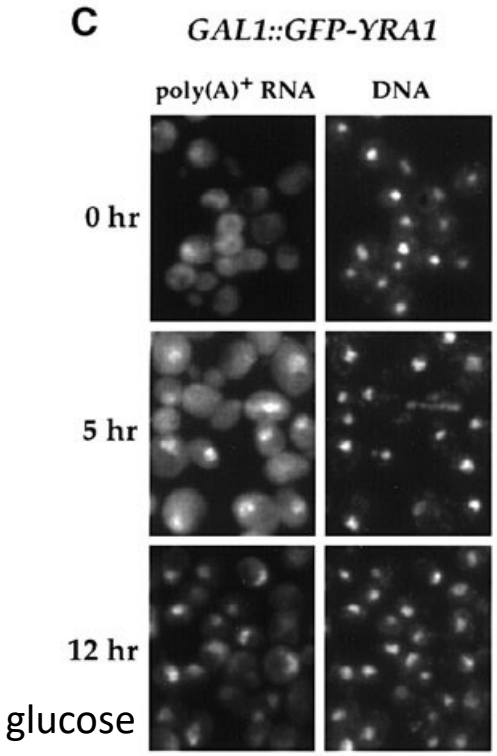


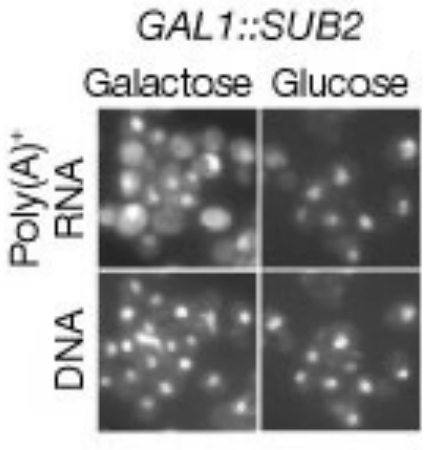


GAL1 promoter: induced by galactose and repressed by glucose

mRNA EXPORT: conditional mutants in yeast



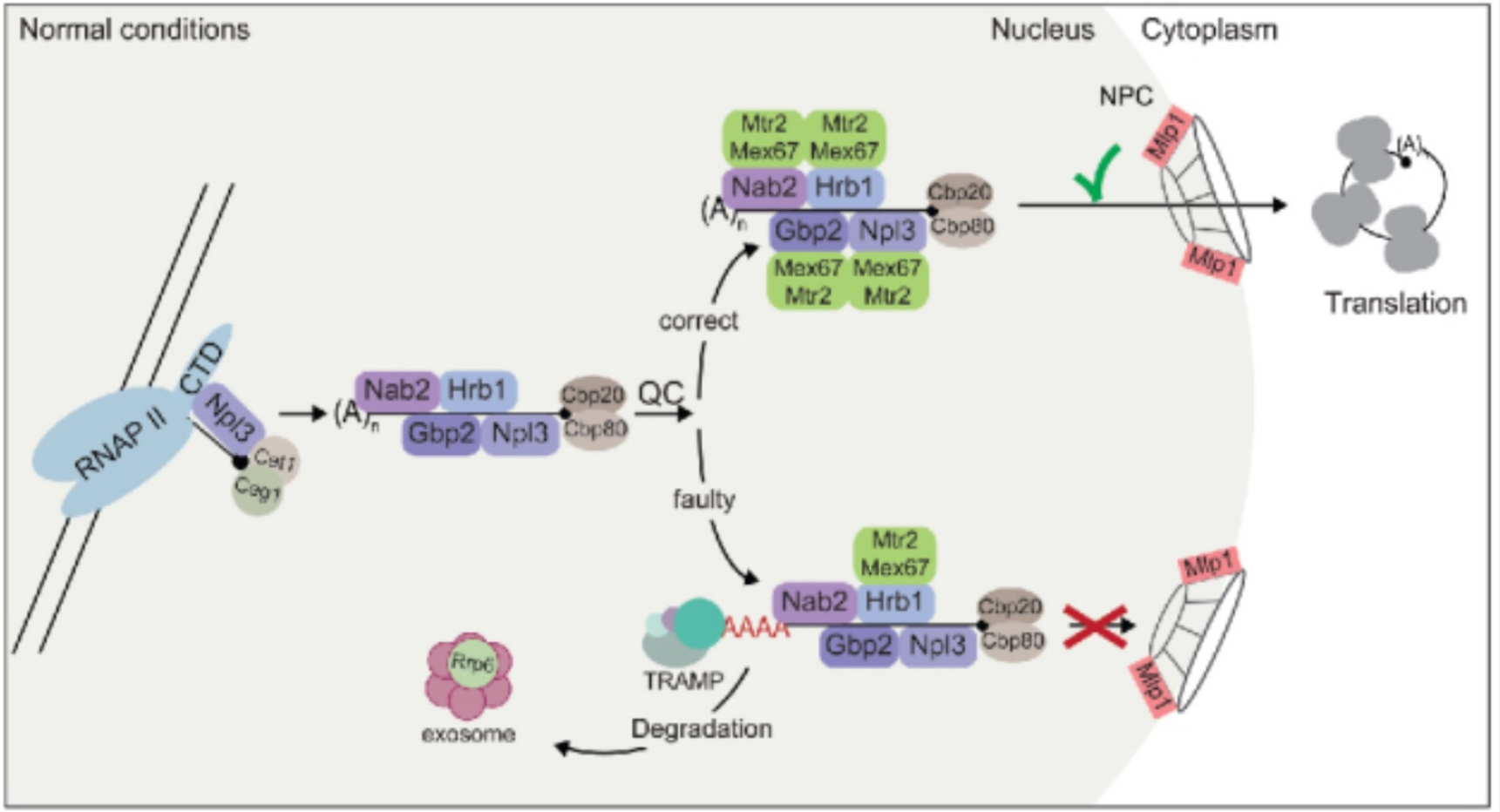




Poly(A) mRNA was visualized by in situ hybridization with a fluorescently-labeled oligo-dT probe

mRNA EXPORT: YEAST
mRNA quality control and export check-point

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/



mRNA quality control in the nucleus: each step in mRNA maturation is **controlled** and involves the recruitment of **adaptor** proteins that **interact with the correct mRNA and the exporter MEX67-MTR2**, accompanying the mRNA from the nucleus to the cytoplasm (**shuttling** proteins)

mRNA EXPORT: YEAST
mRNA quality control and export check-point

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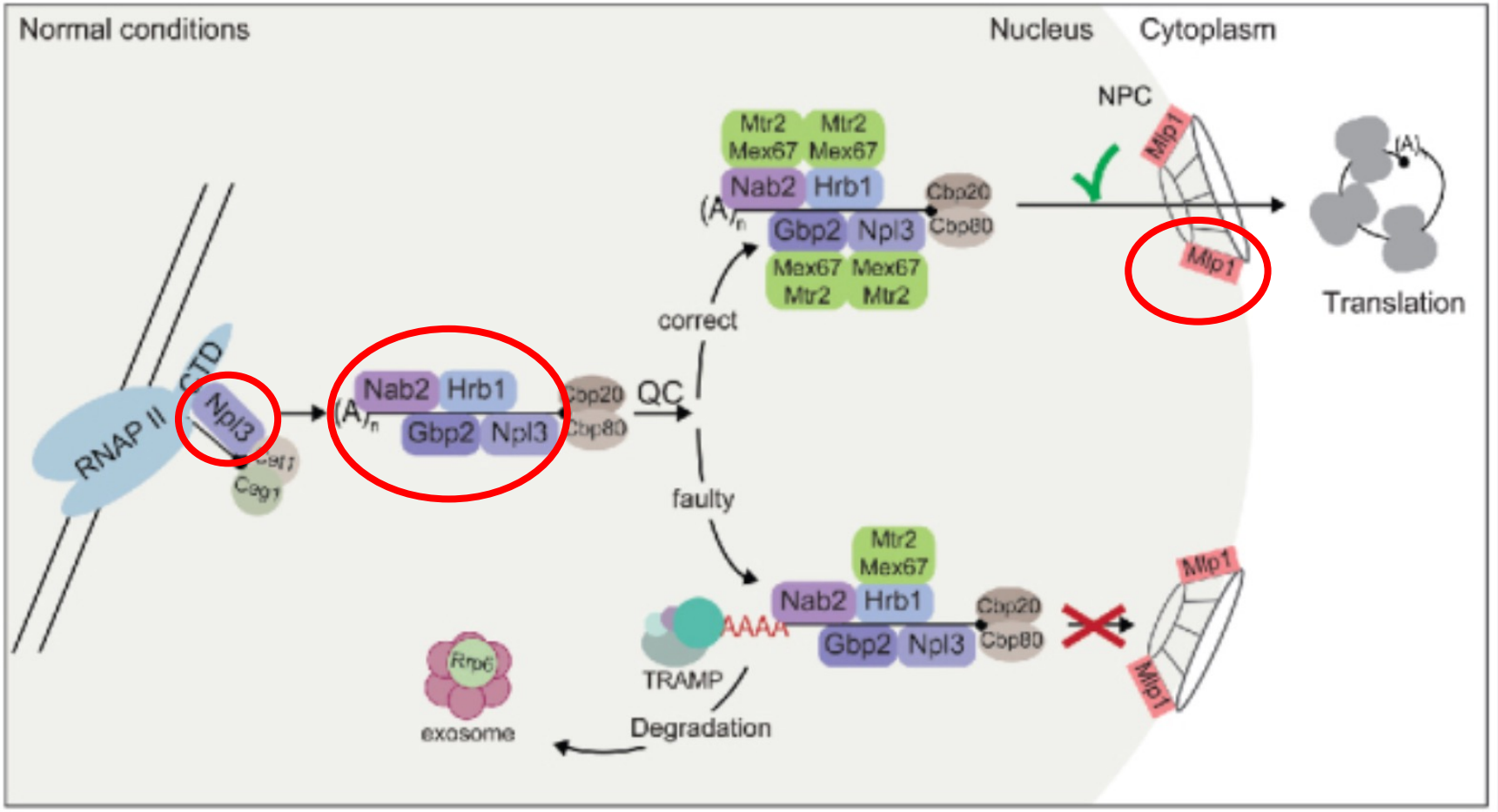

Serine/arginine (SR)-rich proteins Npl3, Gbp2 and Hrb1 and the poly(A)-binding protein Nab2 -> **guard proteins, they shuttle with mRNAs**.

Mlp1: last step in export chek-point

mRNA EXPORT: YEAST
mRNA quality control and export check-point

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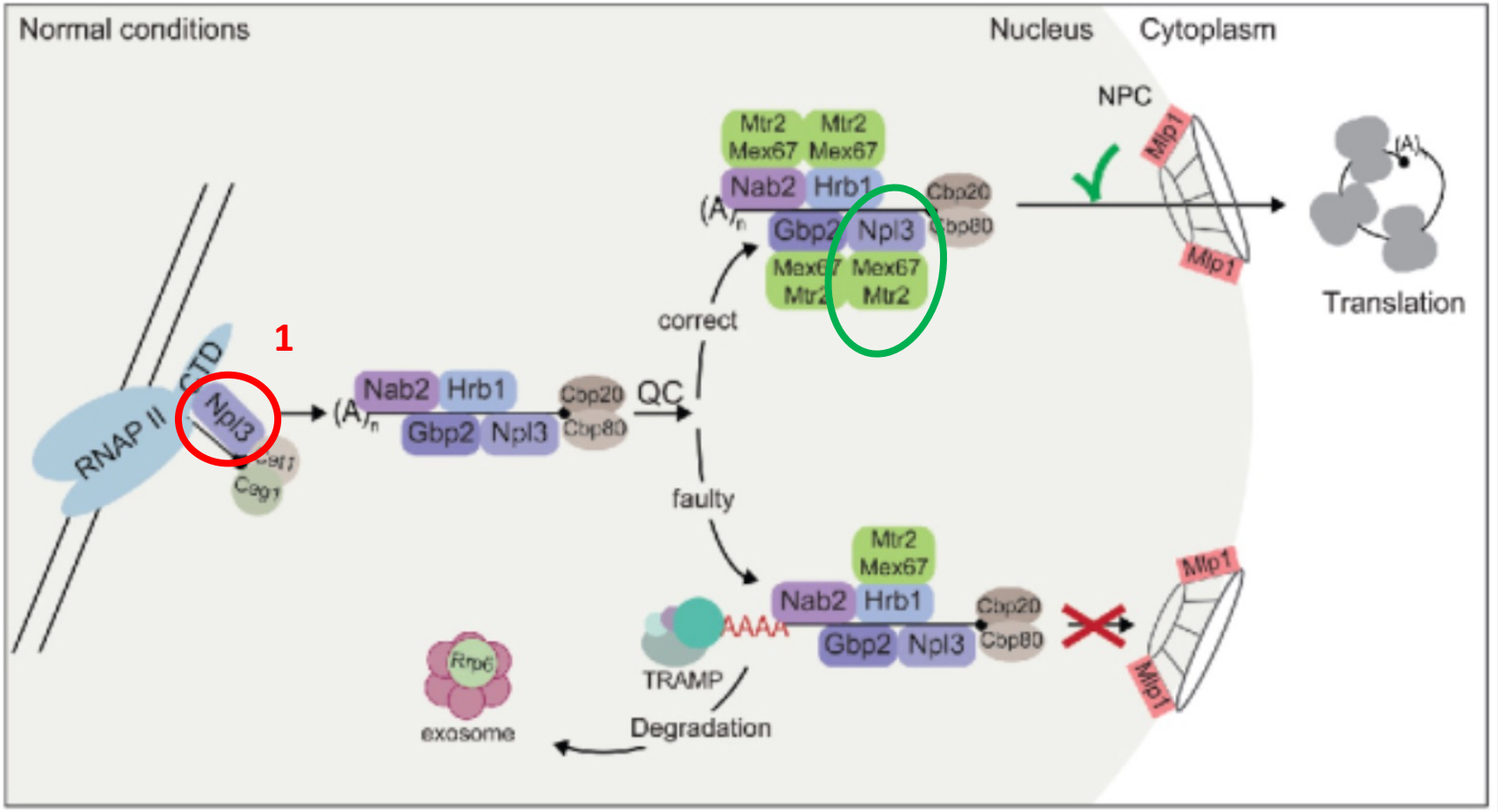
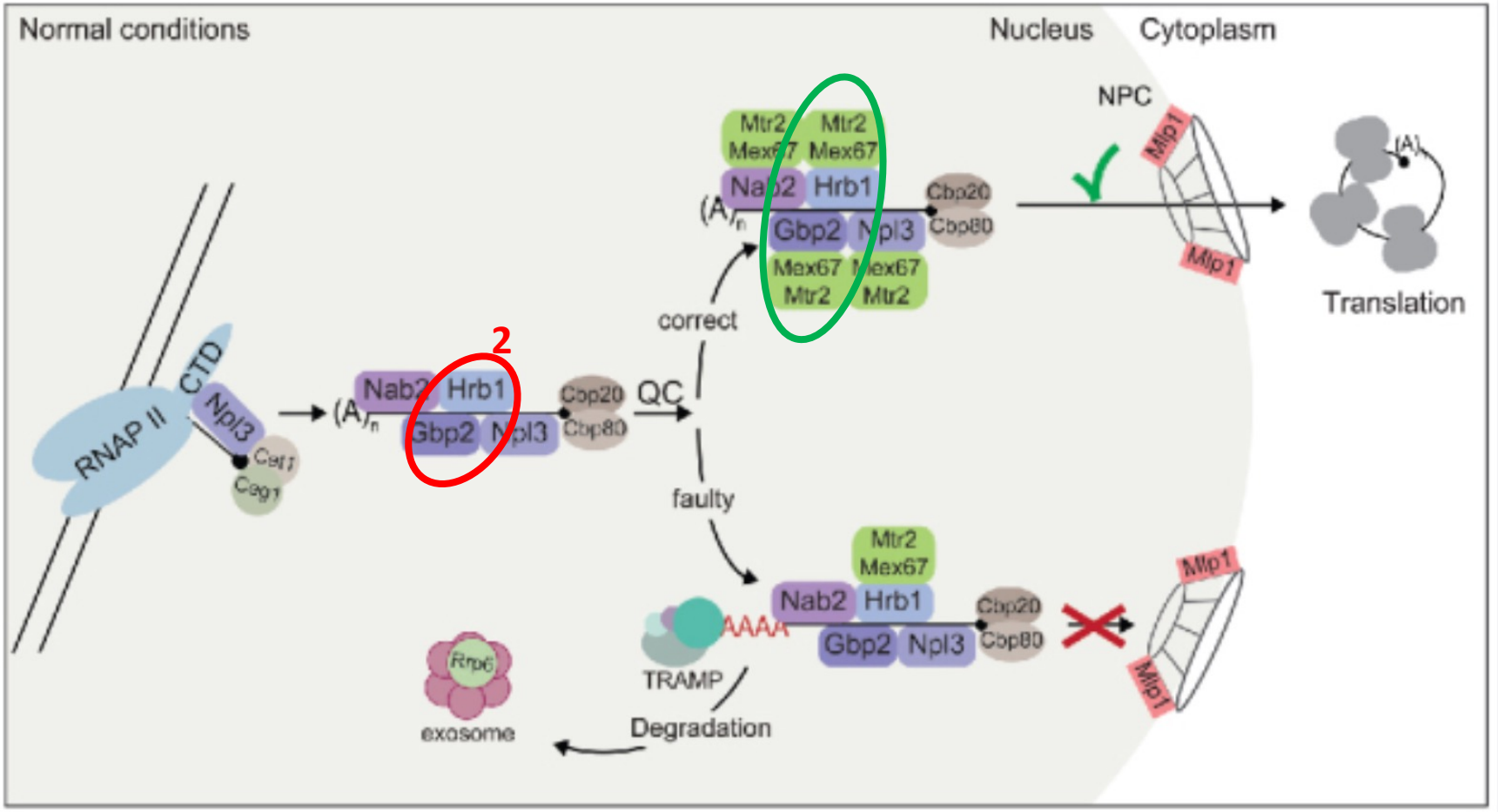


Npl3: contacts a **newly emerging mRNA** as it interacts with the **RNAPol-II** as well as with the **CBC**. Npl3 supports efficient splicing by interacting with the **early spliceosome.**

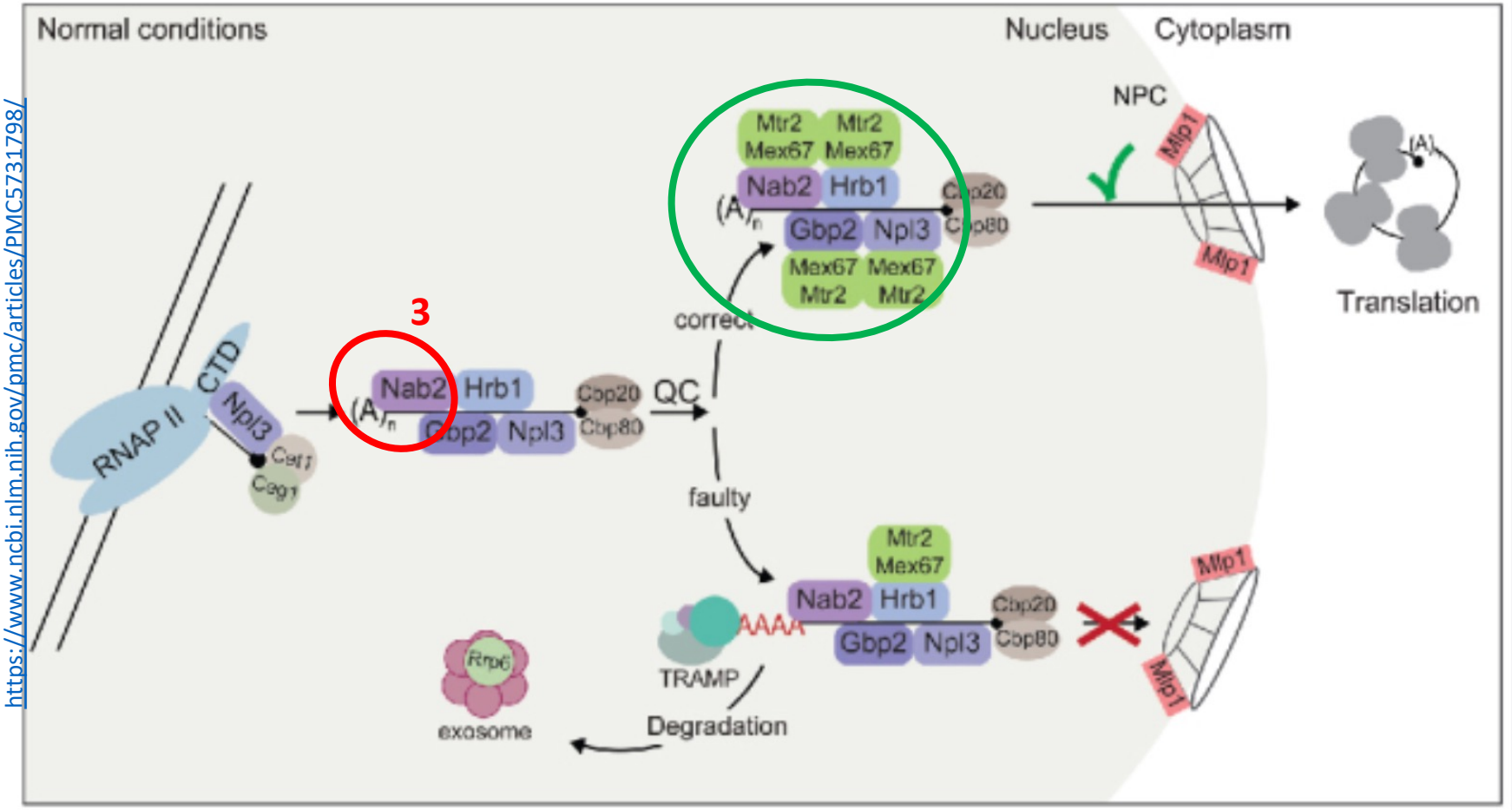
mRNA EXPORT: YEAST
mRNA quality control and export check-point

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/



Correct splicing is controlled by **Gbp2 and Hrb1** that interact with the **late spliceosome**. These proteins are loaded co-transcriptionally by the TREX complex, and recruit the exporter Mex67, in case the mRNA is processed **properly**

mRNA EXPORT: YEAST
mRNA quality control and export check-point

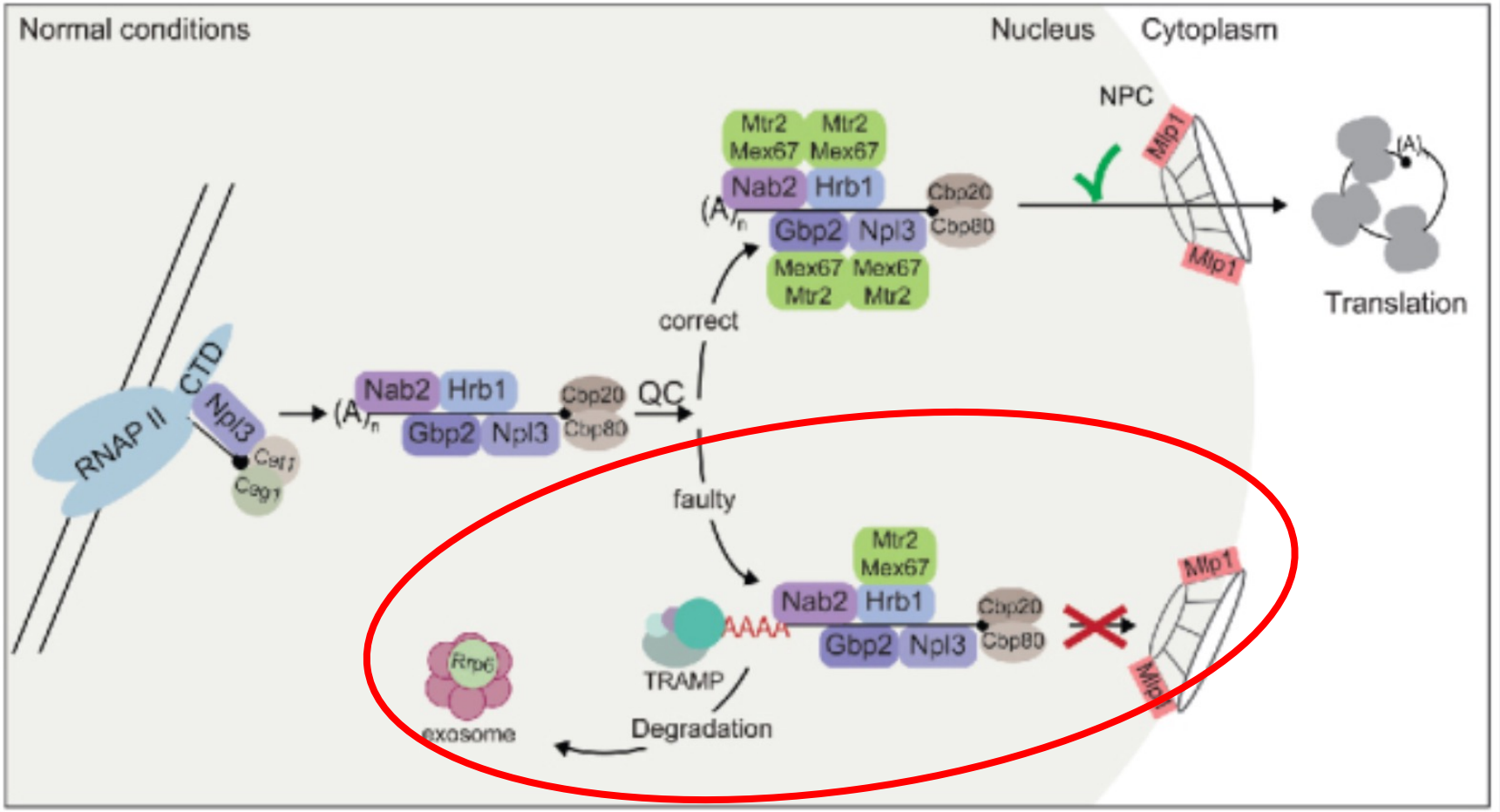


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/

The last processing step is the formation of the **3' end and of the poly(A) tail**.
The poly(A)-binding protein **Nab2** controls length and quality of the 3' tail.

mRNA EXPORT: YEAST
mRNA quality control and export check-point

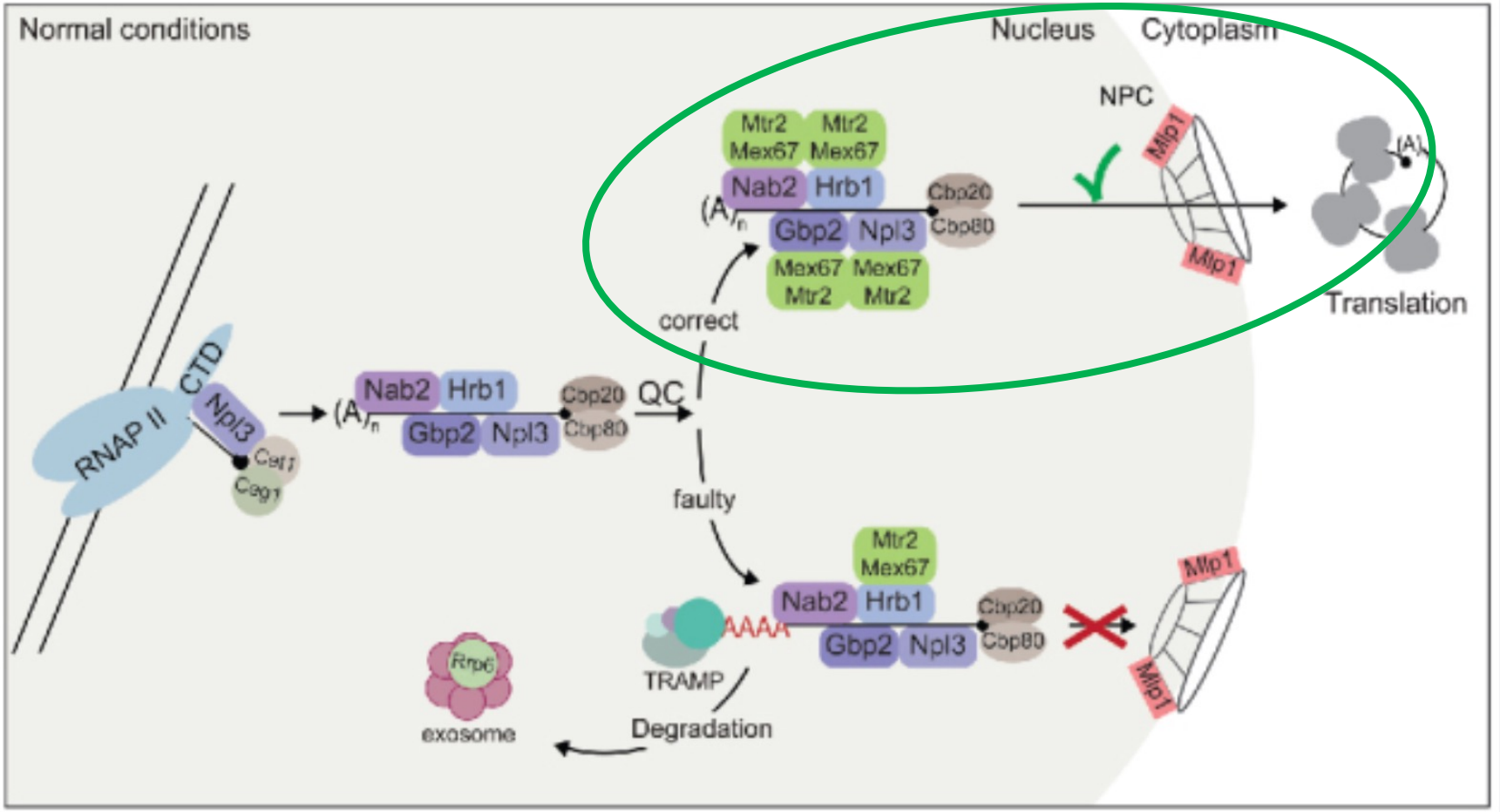
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/



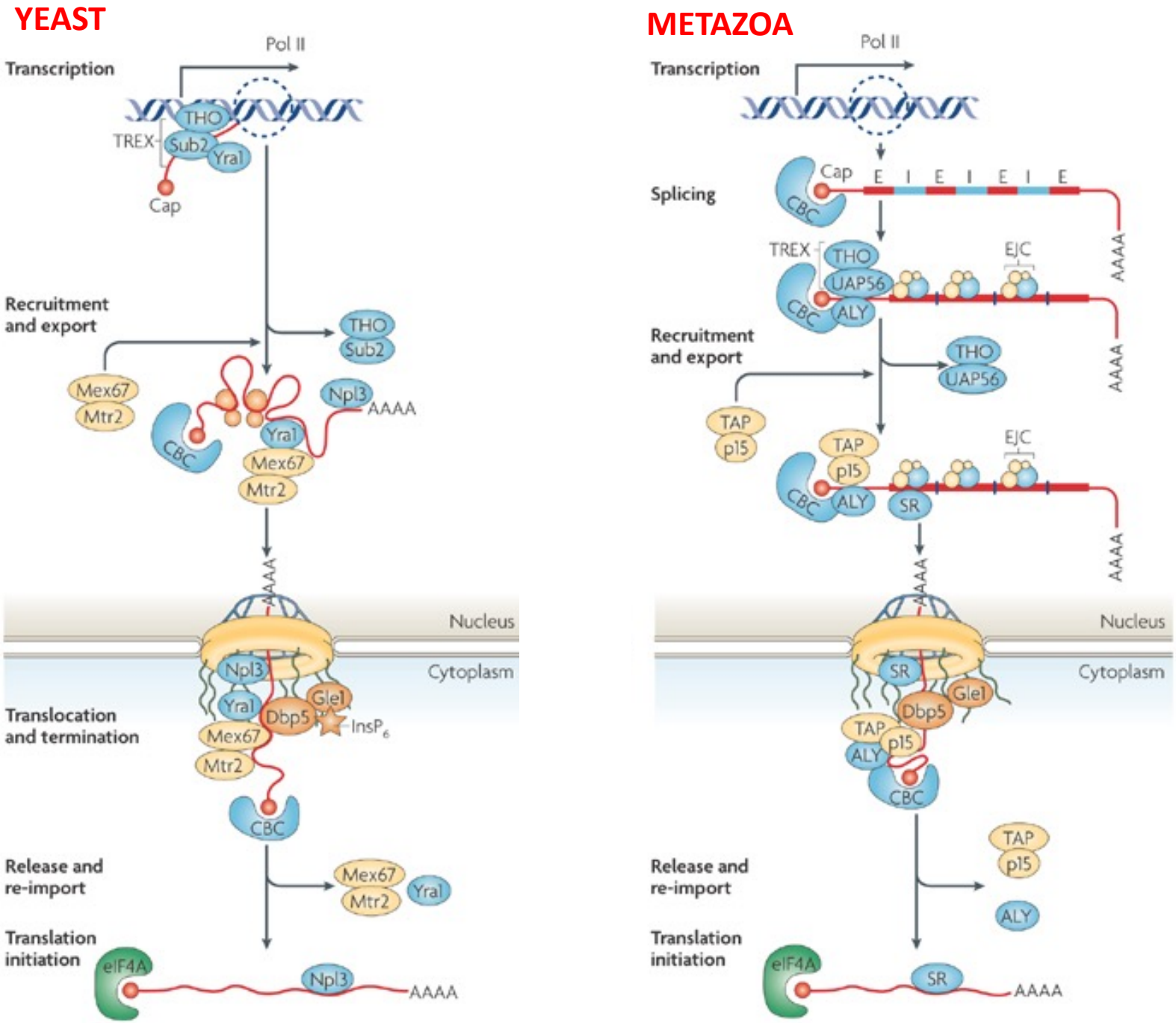
The nuclear removal of **faulty RNAs** relies on the **TRAMP** (Trf4/5, Air1/2, Mtr4) **complex** that marks these RNAs with a **short oligo(A) tail** for subsequent **degradation by the nuclear exosome**

mRNA EXPORT: YEAST
mRNA quality control and export check-point

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/



At the NPC, **Mlp1 and Mlp2** are the last nuclear factors involved in retaining erroneous transcripts. Mlp1 was shown to interact with the guard proteins, to **control the Mex67-guard-protein interactions**, before letting them pass and **retains the mRNA, if no or insufficient Mex67 is bound to the guard proteins**.


YEAST
Pol II
Transcription
THO
TREX
Sub2
Yra1
Cap
Recruitment and export
THO
Sub2
Mex67
Mtr2
Npl3
AAAA
CBC
Yra1
Mex67
Mtr2
AAAA
Nucleus
Cytoplasm
Translocation and termination
Npl3
Yra1
Gle1
Dbp5
InsP6
Mex67
Mtr2
CBC
Release and re-import
Mex67
Mtr2
Yra1
Translation initiation
eIF4A
Npl3
AAAA
METAZOA
Pol II
Transcription
Splicing
Cap
E I E I E I E
CBC
TREX
THO
UAP56
ALY
EJC
AAAA
Recruitment and export
THO
UAP56
TAP
p15
TAP
p15
CBC
ALY
SR
EJC
AAAA
AAAA
Nucleus
Cytoplasm
SR
Gle1
Dbp5
TAP
p15
ALY
CBC
Release and re-import
TAP
p15
ALY
Translation initiation
eIF4A
SR
AAAA

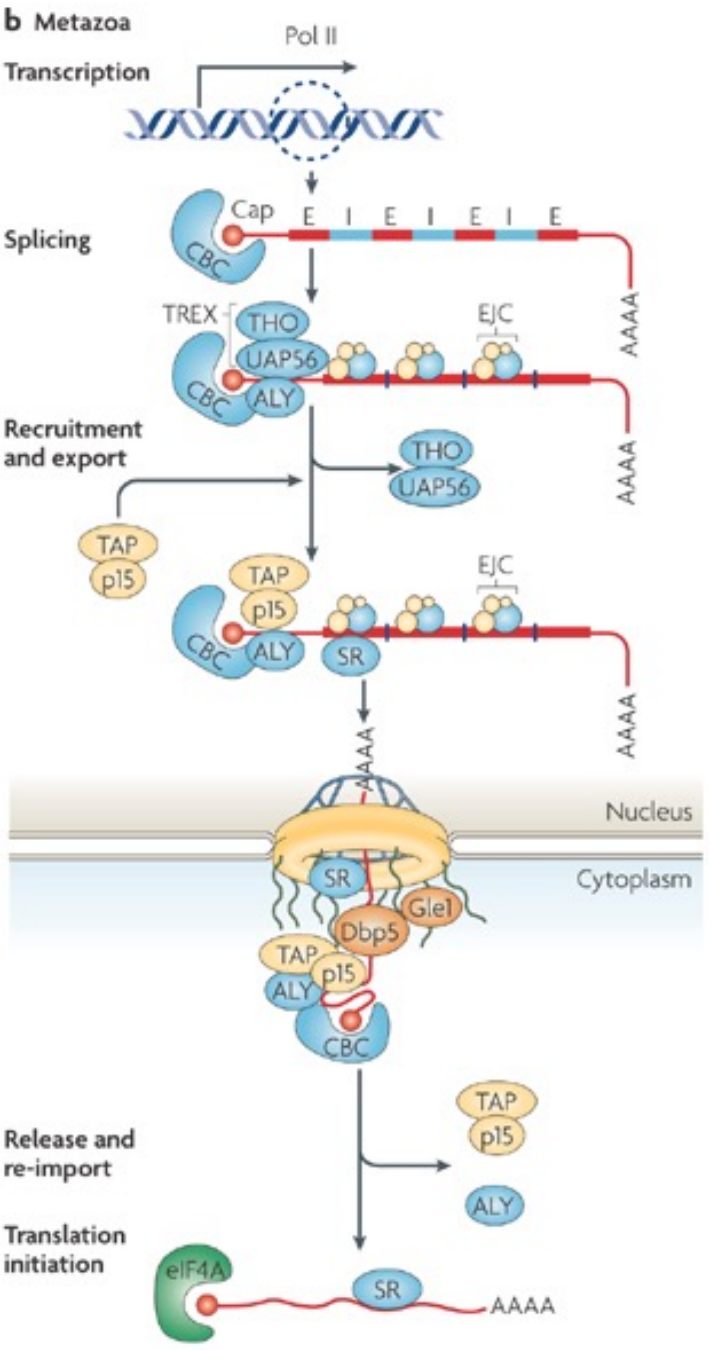
1. Metazoan TREX contains Aly/REF (YRA1), UAP56 (SUB2), and the metazoan counterpart of the yeast THO complex.

2. Human TREX complex binds only to **spliced mRNAs** by a **splicing-coupled mechanism,** rather than by the direct transcription-coupled mechanism that occurs in yeast.

3. TREX is recruited by the **cap-binding complex** and by the **exon-junction-complex**

4. In human ALY/REF (YRA1) is recruited to the mRNP **via UAP56 (SUB2) during splicing, in an ATP dependent manner**

5. Aly/REF, in contrast to Yra1, which is essential for mRNA export in yeast, is required but **not essential** for bulk cellular mRNA export. This suggests the existence of additional mRNA export adaptors in metazoa



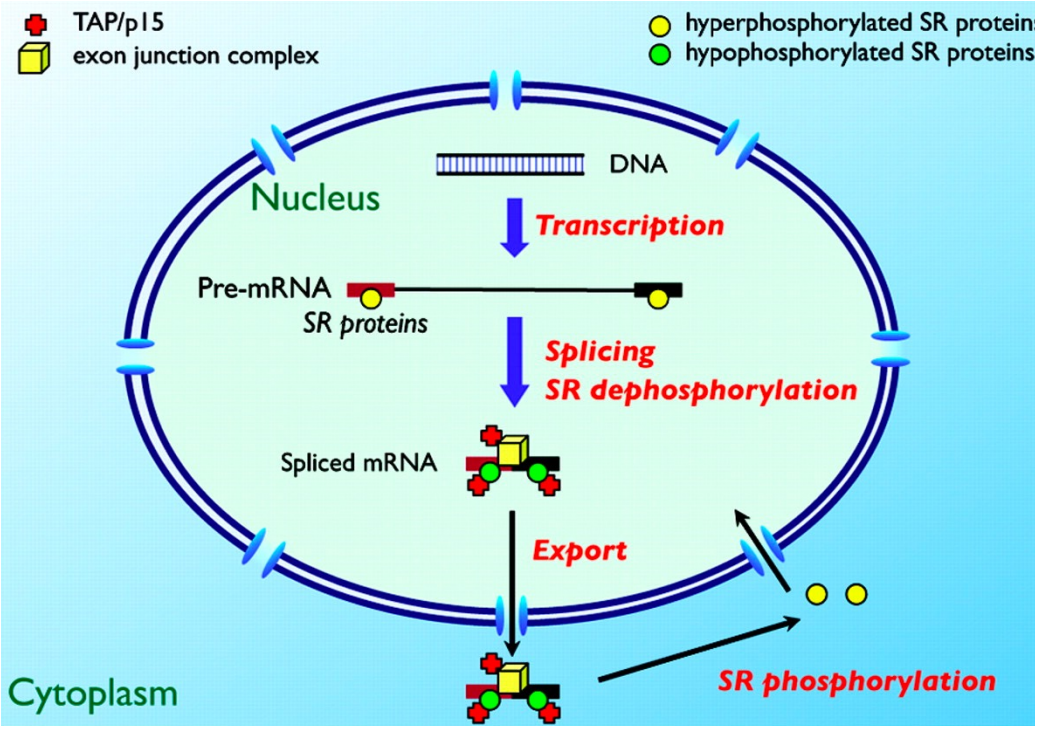
mRNA EXPORT: METAZOA
Serine/arginine-rich (SR) proteins

Hyperphosphorylated SR proteins are recruited to **pre-mRNA** molecules at exonic enhancers.

During splicing, SR proteins are **hypophosphorylated** but remain associated with the spliced mRNP.

Together with the exon junction complex, which contains REF1, they **recruit multiple copies of TAP (homologue of Mex67)** , thereby increasing the efficiency of export of spliced mRNP.

In the **cytoplasm**, **rephosphorylation** of the SR protein adapters results in their **dissociation** from mRNP complexes and in their recycling to the **nucleus**.



https://www.pnas.org/content/101/26/9666

mRNA EXPORT: exceptions and selective transport

1. TREX1 also participates in the nuclear export of several **intronless transcripts** independently of splicing (e.g. heat-shock protein 70 mRNA) .

Efficient export requires the presence of **GC-rich export-promoting sequences** at the **5' end** of these transcripts.

Recruitment of the **TREX1** complex to the 5' end of intronless mRNAs occurs through interaction with the cap-binding complex (CBC).

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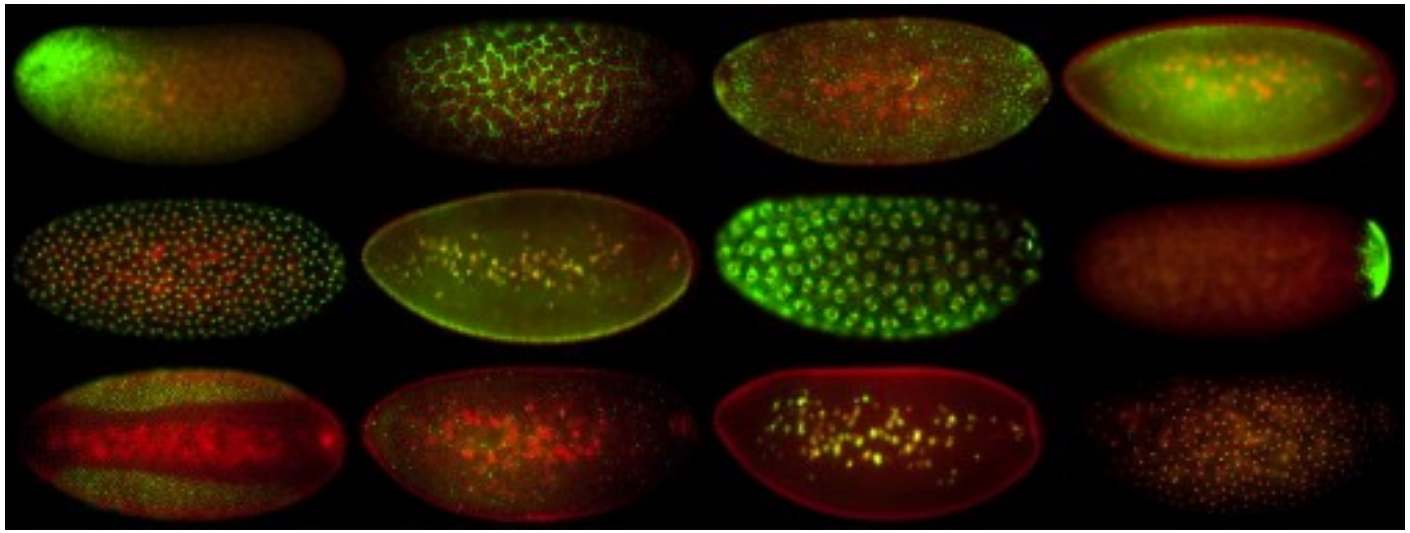
3. The RanGTP-dependent exporter **CRM1** transport specific mRNAs of **viral mRNAs** and also several **protooncogenes and cytokines**, that contain AU-rich elements. AU-rich elements are recognized by **HuR and its protein ligands**, which interact with CRM1

mRNA SUBCELLULAR LOCALIZATION

mRNA localization and regulated translation allow spatio-temporal regulation of gene expression



«High-resolution fluorescent in situ analysis of 25% of mRNAs encoded by the *Drosophila* genome revealed that 71% of these display striking patterns of subcellular localization in early embryos. Some of these patterns are illustrated in this montage of photomicrographs, in which nuclei are in red and **mRNAs in green**.»

https://www.cell.com/fulltext/S0092-8674(09)00126-3

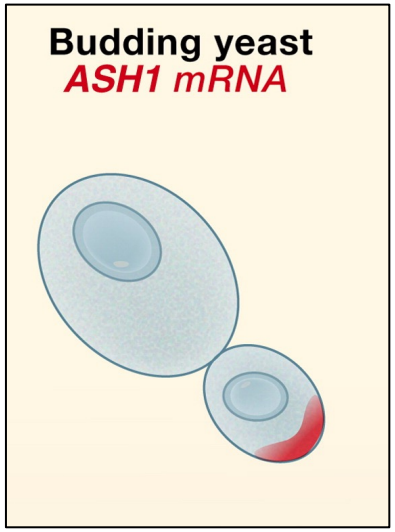
ADVANTAGES OF REGULATING GENE EXPRESSION BY mRNA LOCALIZATION

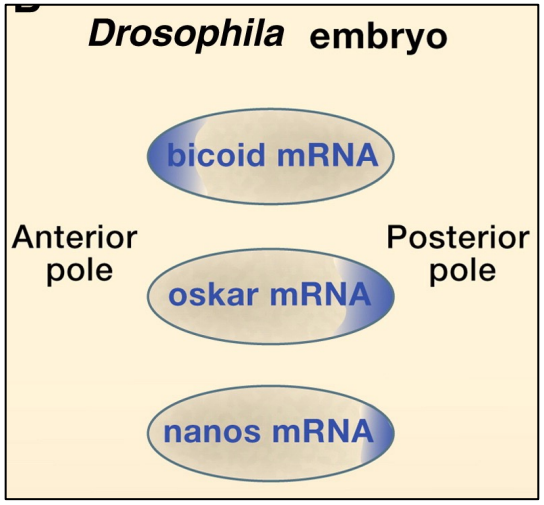
1. **High temporal resolution**: fast response to stimuli, by regulating translation of on-site mRNAs

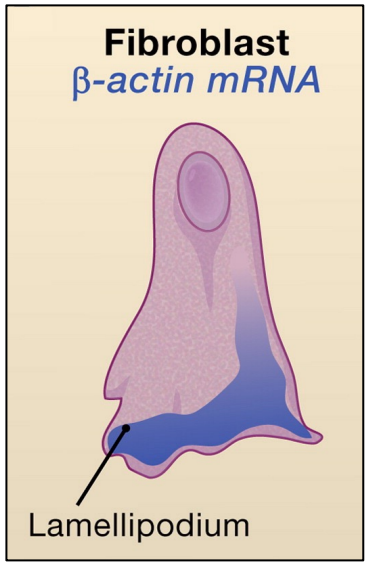
2. Localized mRNA translation leads to **protein accumulation** in a specific cytoplasmic district -> more efficient than having translating mRNAs elsewhere and then transporting proteins to a distinct site

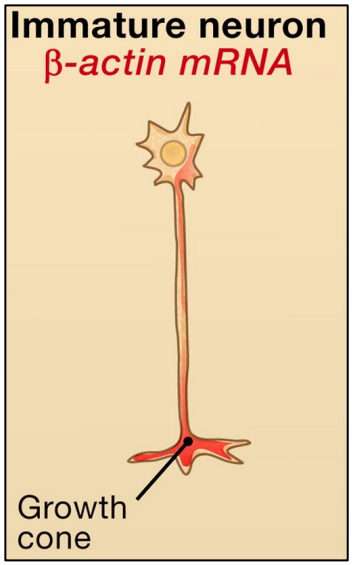
3. Local translation of proteins **protects** the cell from proteins that might be toxic in other cell compartments

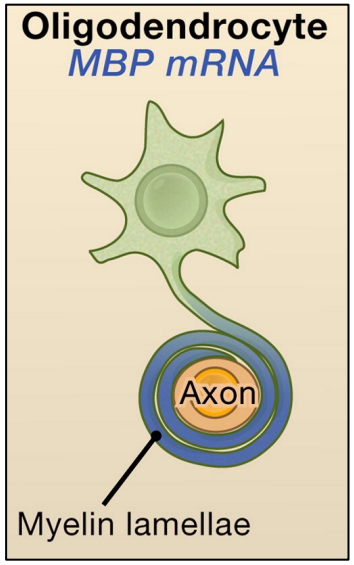
mRNA SUBCELLULAR LOCALIZATION











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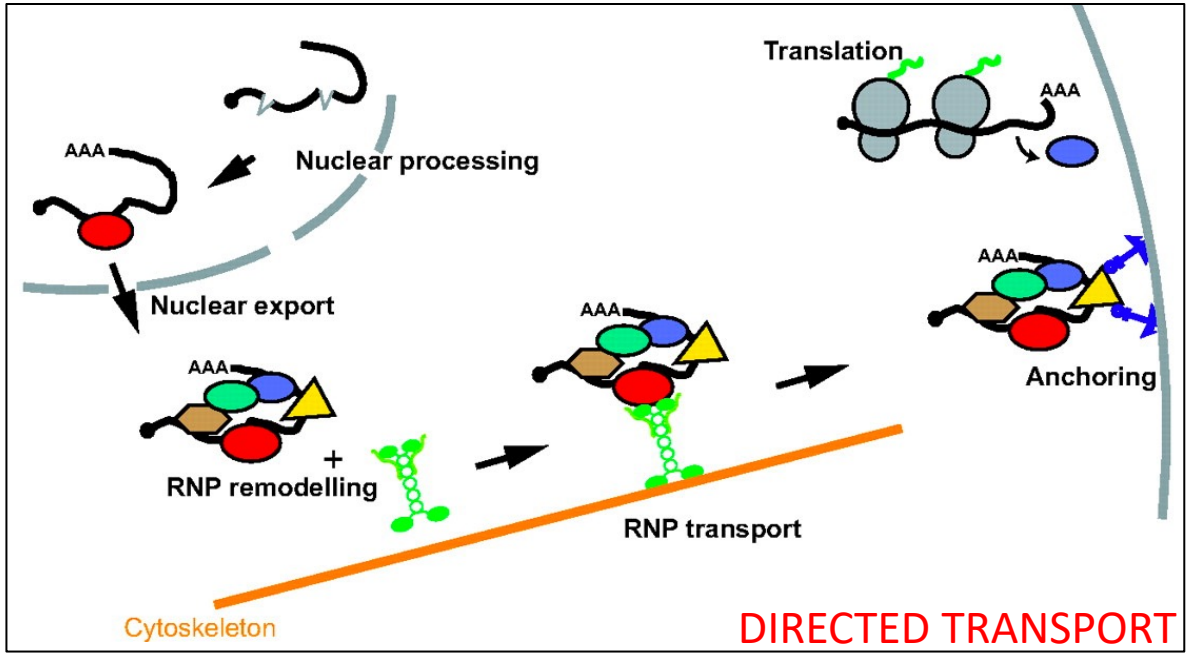
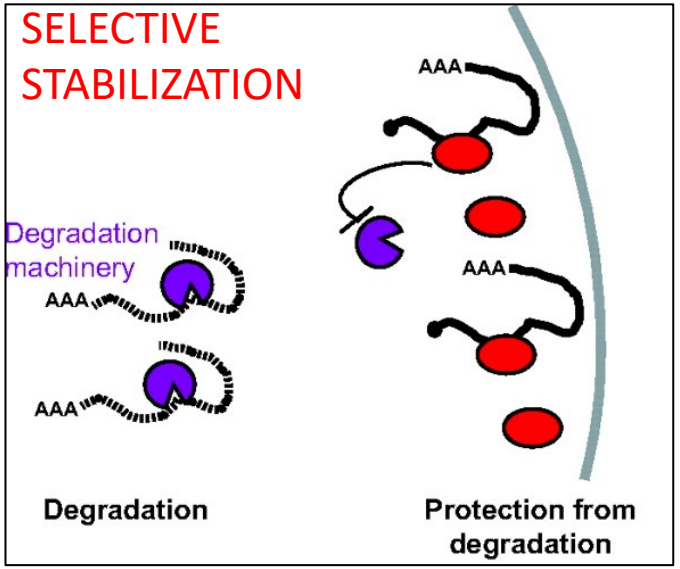
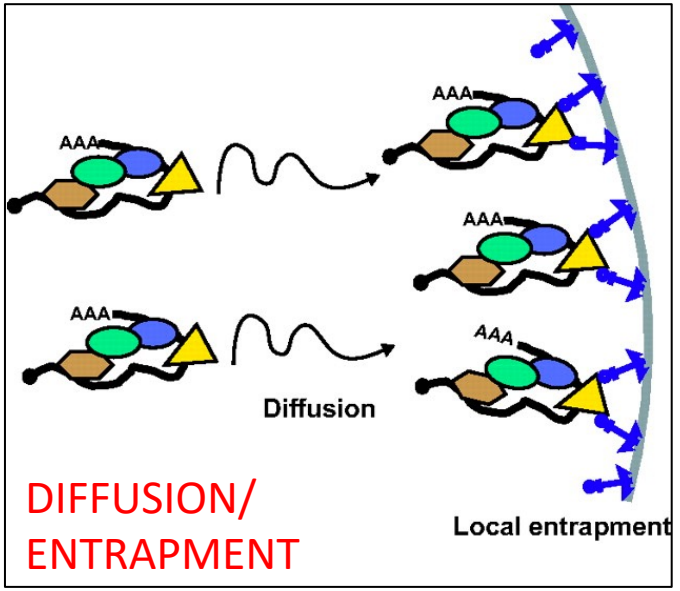
MECHANISMS OF mRNA LOCALIZATION







LOCALIZATION ELEMENTS OR «ZIPCODES»

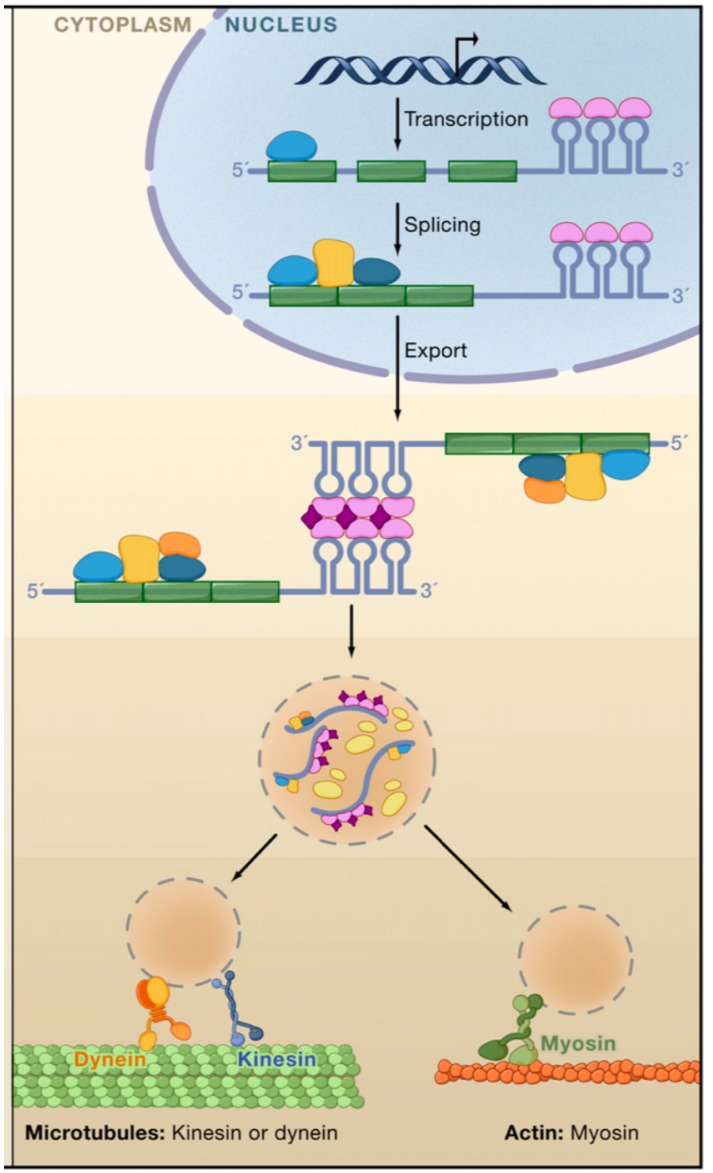
1. **<u>Cis-acting</u> elements** in the mRNA (sequence forming secondary structures, usually stemp-loops)

2. Usually found in the 3'UTR sequence

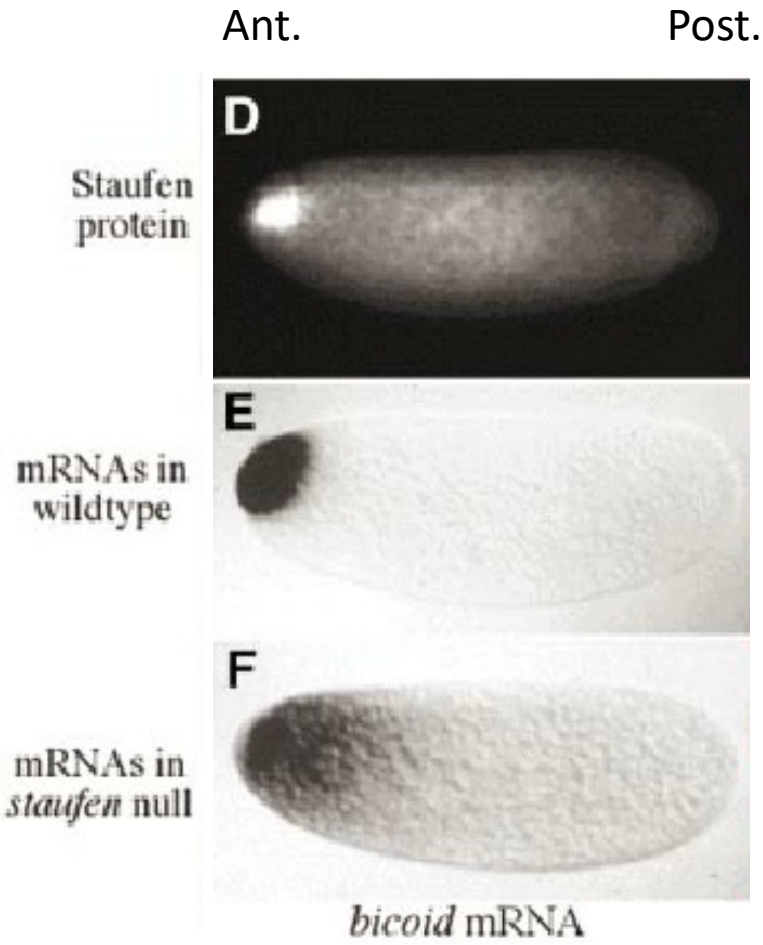
3. Variable length: from 6 nt up to hundreds of nt

4. Recognized by specific RNA-binding proteins that both regulate mRNA localization and translation (**<u>trans-acting</u> elements**)

5. The mRNAs + RNA-binding proteins (RNPs) in many cases form a part of a larger complex called «**RNA transport granul**e» which is transported to its final destination in the cell



DIRECTED TRANSPORT OF *bicoid* mRNA

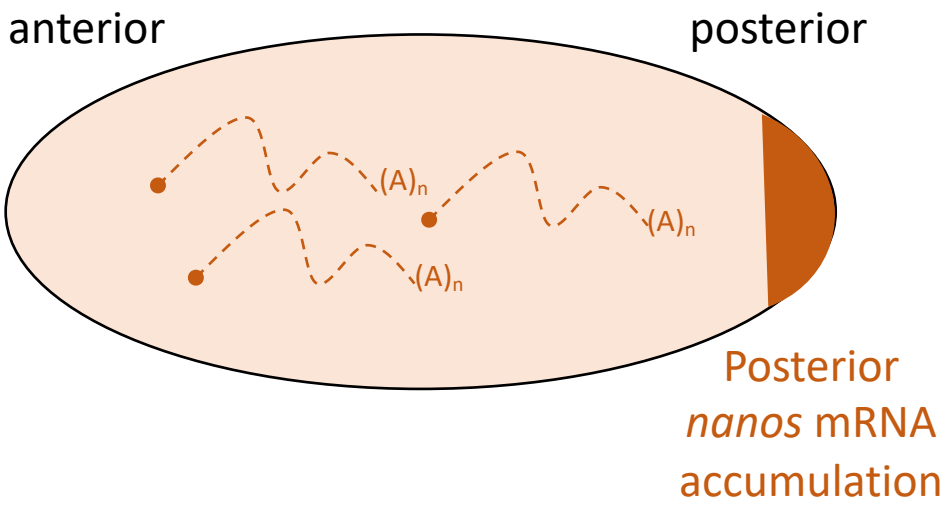


1. Cis-acting elements: 625nt long region in the *bicoid* mRNA containing several localization elements (BLE). BLE1: stem-loop essential for anterior localization. *bicoid* mRNA dimerizes through stem-loop structure

2. Trans-acting elements: *bicoid* mRNA dimerization is essential for Staufen binding, which is necessary for anterior localization

3. Directed transport along **microtubule**

SELECTED STABILIZATION OF *nanos* mRNA



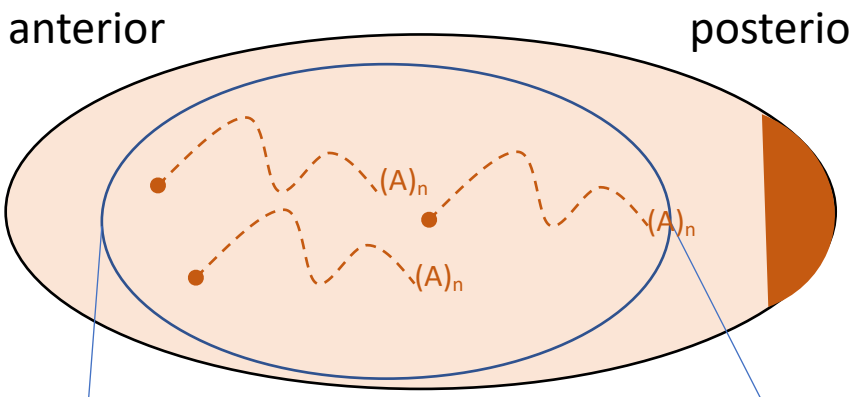
1. Nanos is required for posterior specification of Drosophila embryo

2. Its posterior localization and localized translation are guaranteed mainly by the “selected stabilization” mechanism

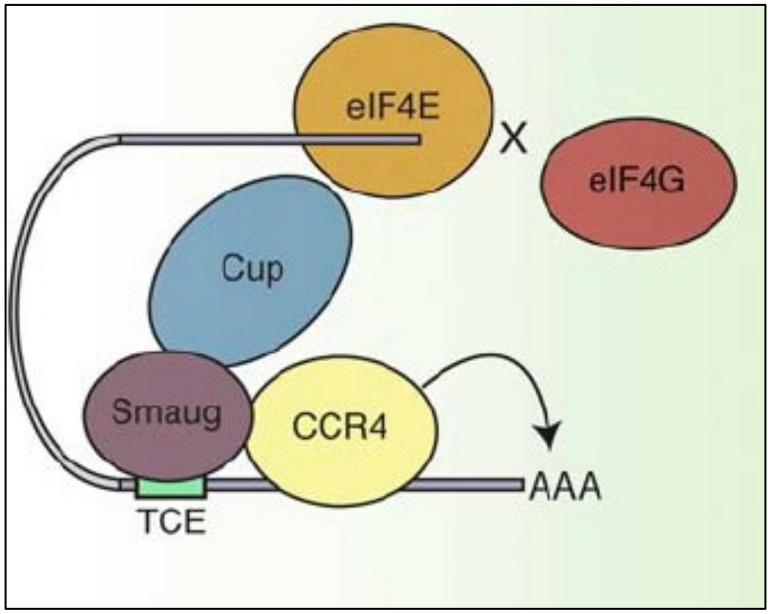
3. Only 4% of *nanos* mRNA is localized at the posterior pole, but it is stable, while the *Nanos* mRNA elsewhere in the embryo is degraded thanks to Smaug protein

4. Smaug binds sequence elements in *nanos* 3’UTR

SELECTED STABILIZATION OF *NANOS* mRNA

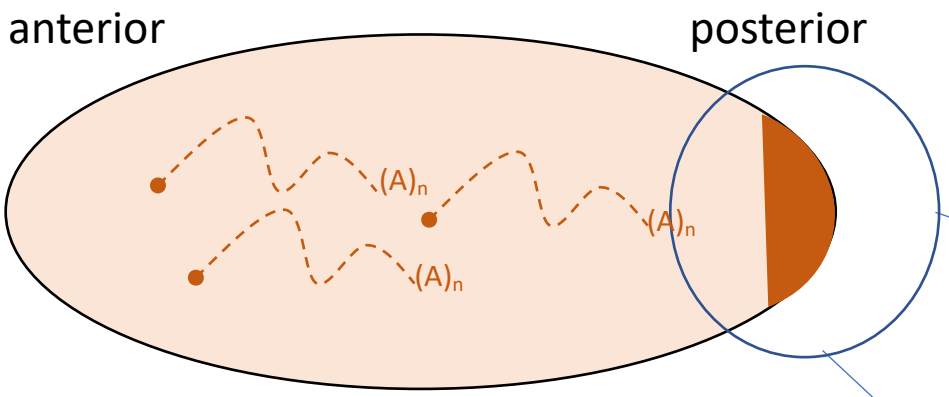


nanos mRNA accumulation



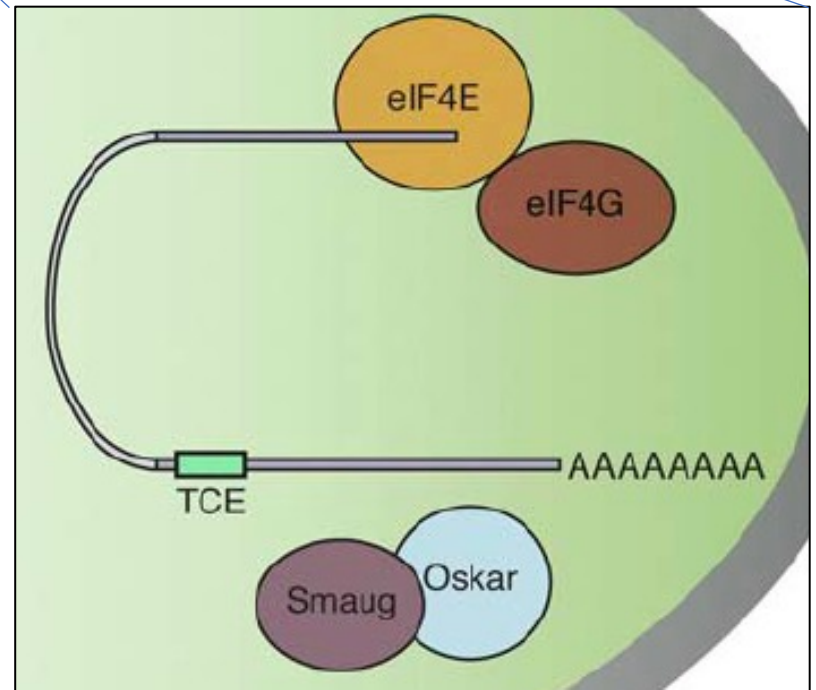
Translational repression and degradation through deadenylation

SELECTED STABILIZATION OF *NANOS* mRNA

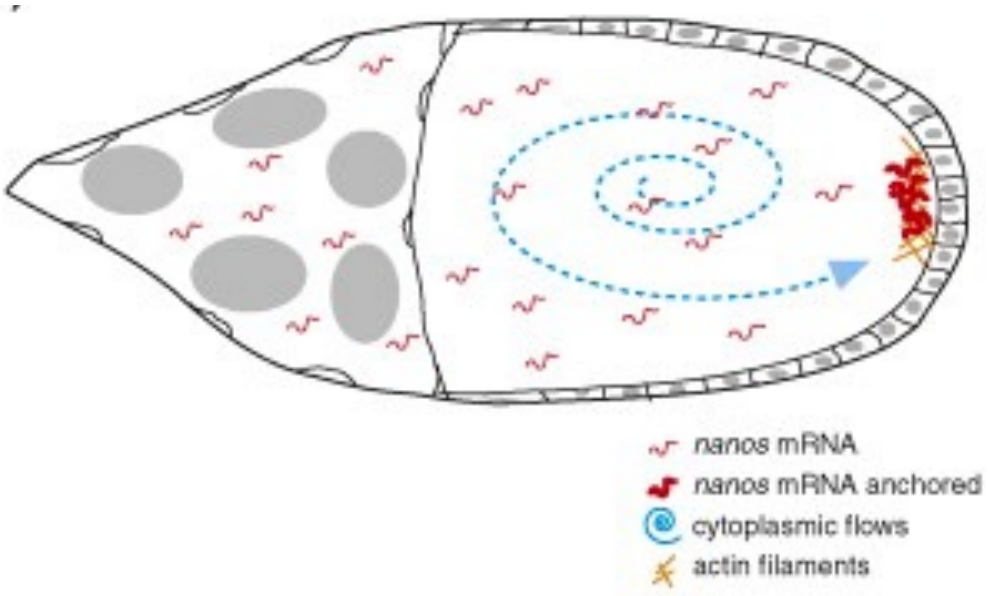


nanos mRNA accumulation

Oskar protein, localized in ONLY at the posterior pole, protects *nanos* mRNA from degradation, allowing its translation too



DIFFUSION/ENTRAPMENT OF *NANOS* mRNA



1. Another mechanism also contributes to *nanos* localization in the late stages of oogenesis

2. Strong cytoplasmic flows move *nanos* mRNA throughout the oocyte so that it can encounter a specialized Actin-based anchor at the posterior pole

Further datails and Suggested Readings:
https://www.nature.com/articles/nrm2255
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4377836/
https://www.cell.com/fulltext/S0092-8674(09)00126-3
https://www.pnas.org/content/101/26/9666
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/