RNA Export

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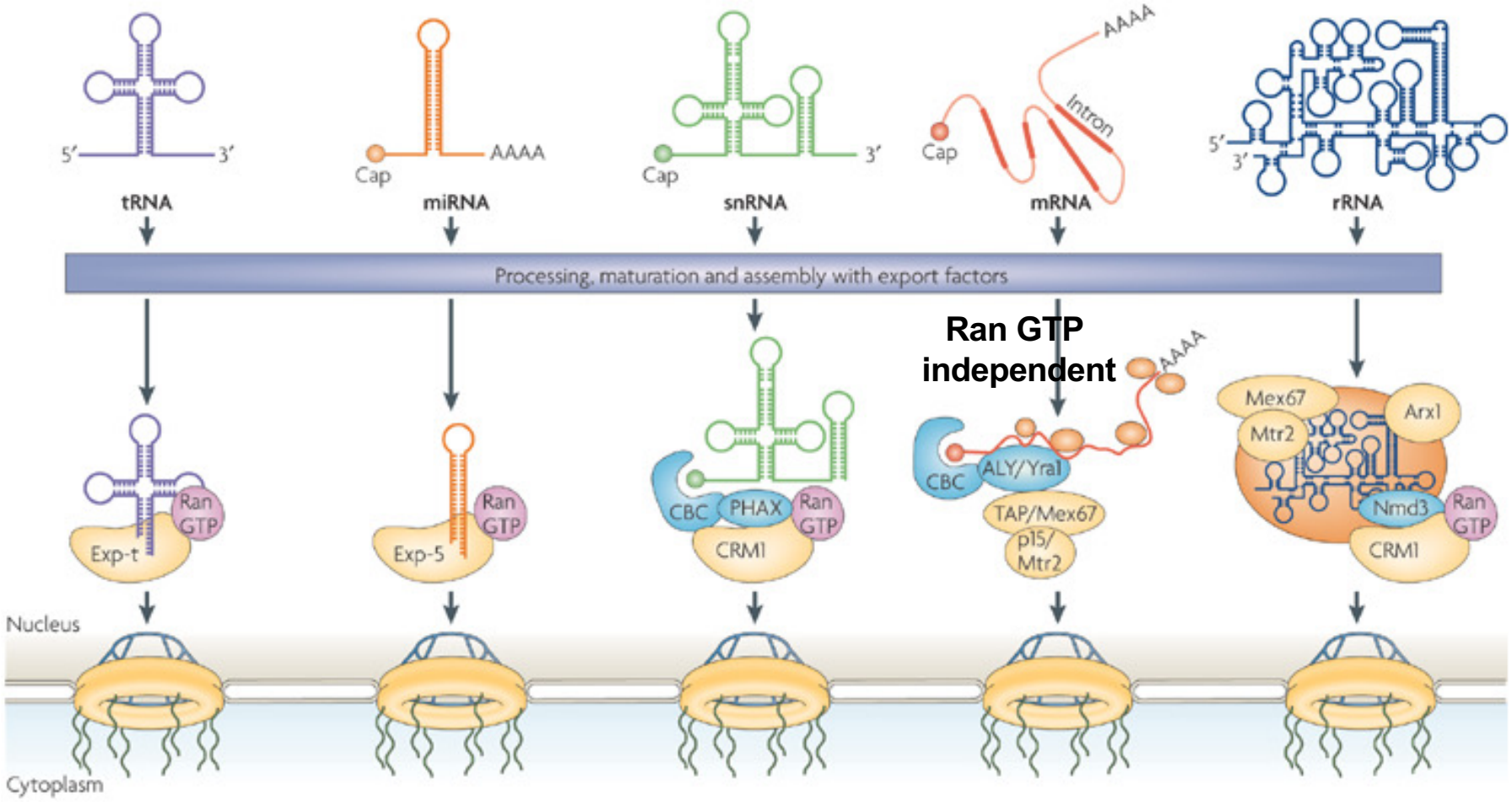
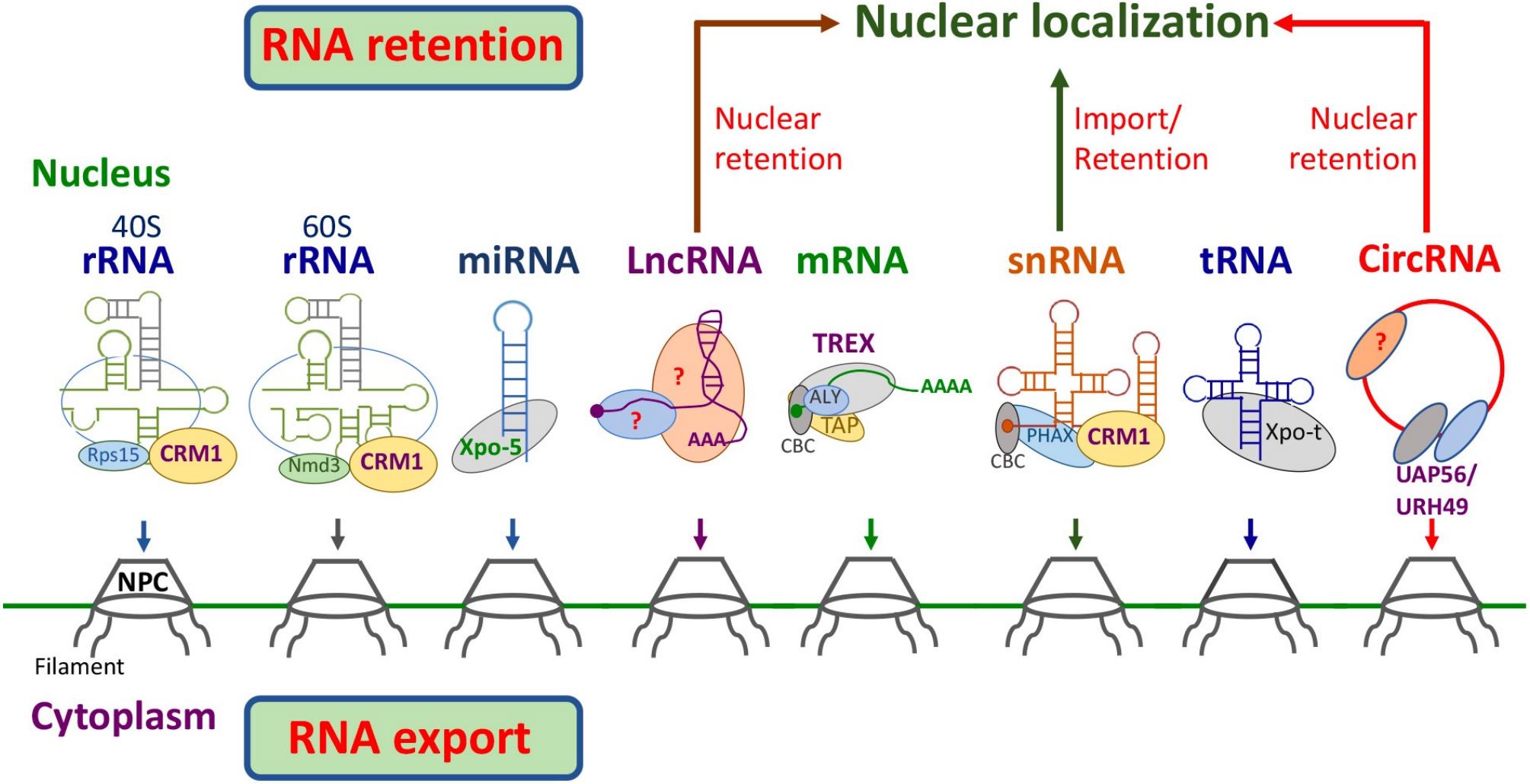
•**Small RNAs** (**tRNAs**, **microRNAs**) follow simple export routes by binding directly to **export receptors**

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



Messenger RNAs, tRNAs, rRNAs, snRNAs, most of the lncRNAs, and circular RNAs are exported to the cytoplasm, but some lncRNAs and circular RNAs are retained in the nucleus and snRNAs are re-imported to the nucleus after processing in the cytoplasm

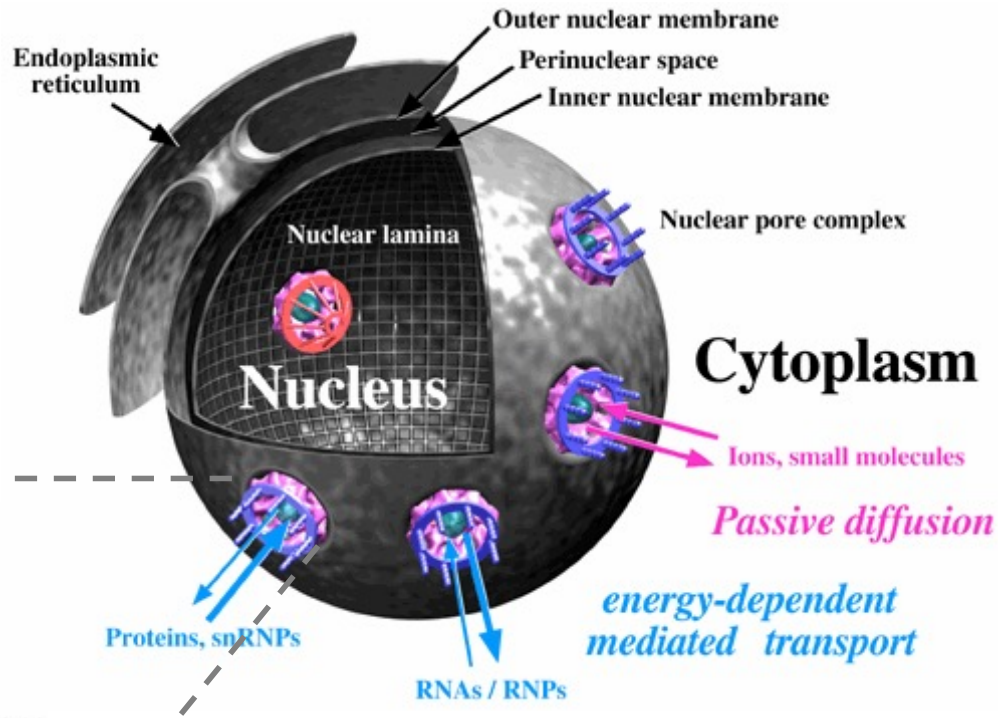


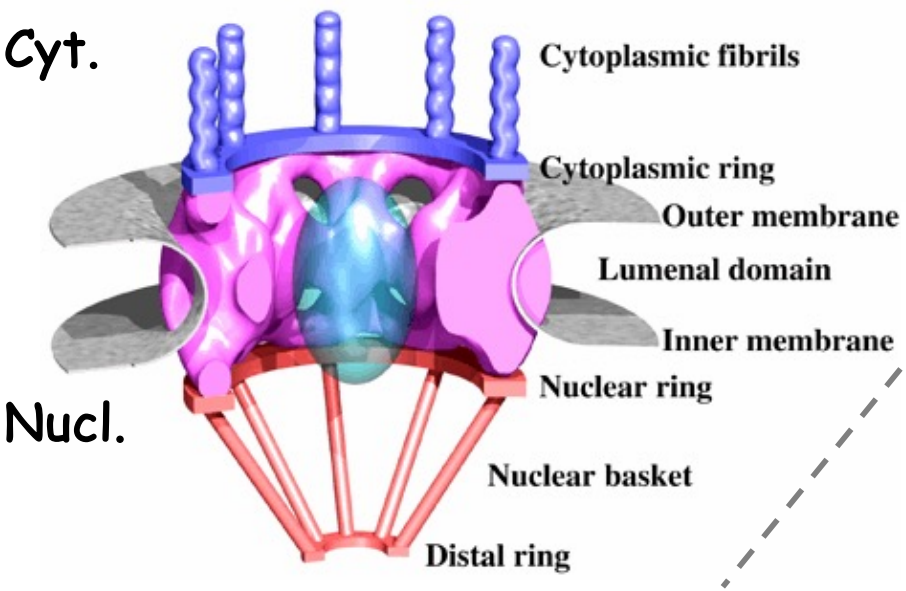
Nuclear Pore Complex (NPC)

•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

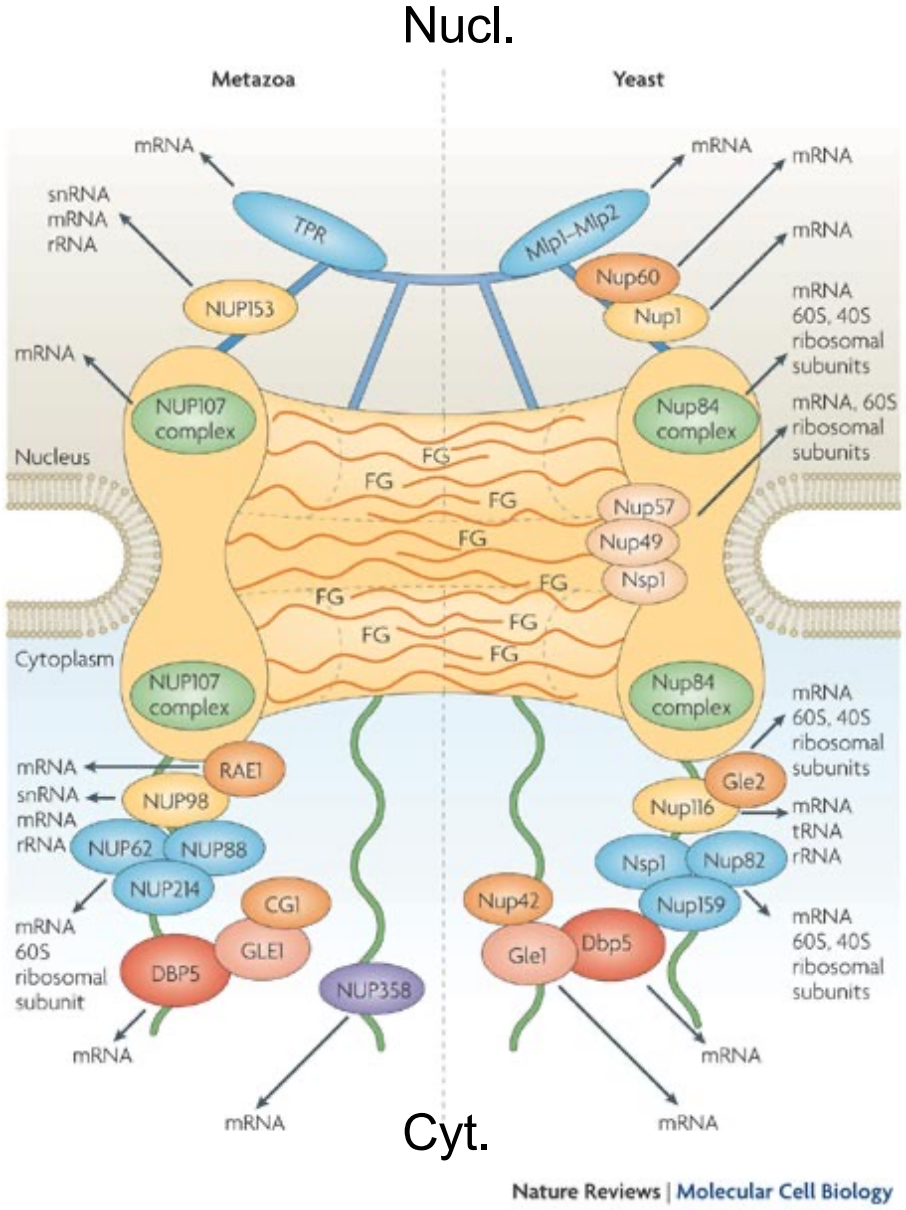
•Ions and small molecules use passive diffusion (<20-40KDa/5 nm diameter)

•Proteins and RNPs use <u>energy-dependent mediated transport</u>





Nucleoporins



•**Nucleoporins** are grouped in three major classes:

1. **FG nucleoporins**: contain Phe-Gly-rich repeat. They are present in the transport channel and mediate the passage of soluble transport receptors, small molecule diffusion, but blocks large molecules.

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

3. **Nups**. These are integral membrane proteins that anchor the NPC to the membrane

Transport Receptors

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

• **karyopherins** recognize a short peptide signal on a cargo protein, either a **nuclear localization signal** (NLS) or a **nuclear export signal** (NES)

•Typically, karyopherins that import cargo are called **importins** and karyopherins that export cargo are called **exportins**

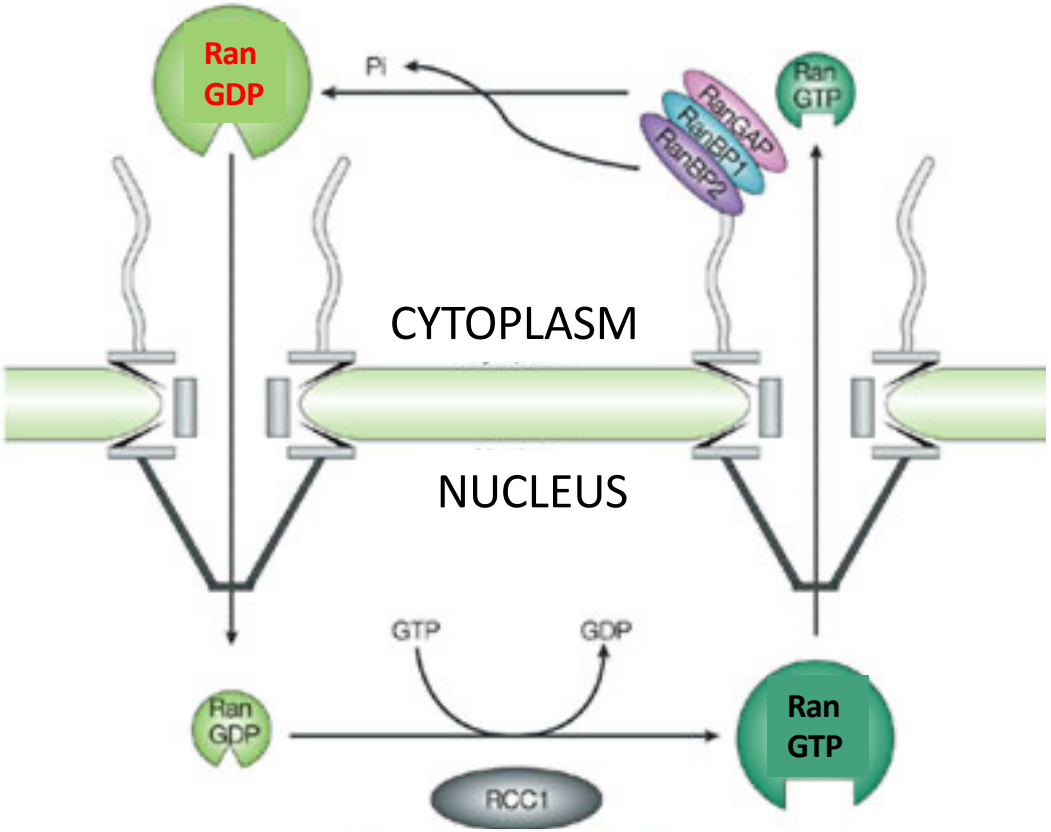
•karyopherins can recognize nucleotide motifs in RNA cargoes, which also enables them to export RNAs.

•A feature of karyopherins is their regulation by the **small GTPase Ran**

Small GTPase RAN

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

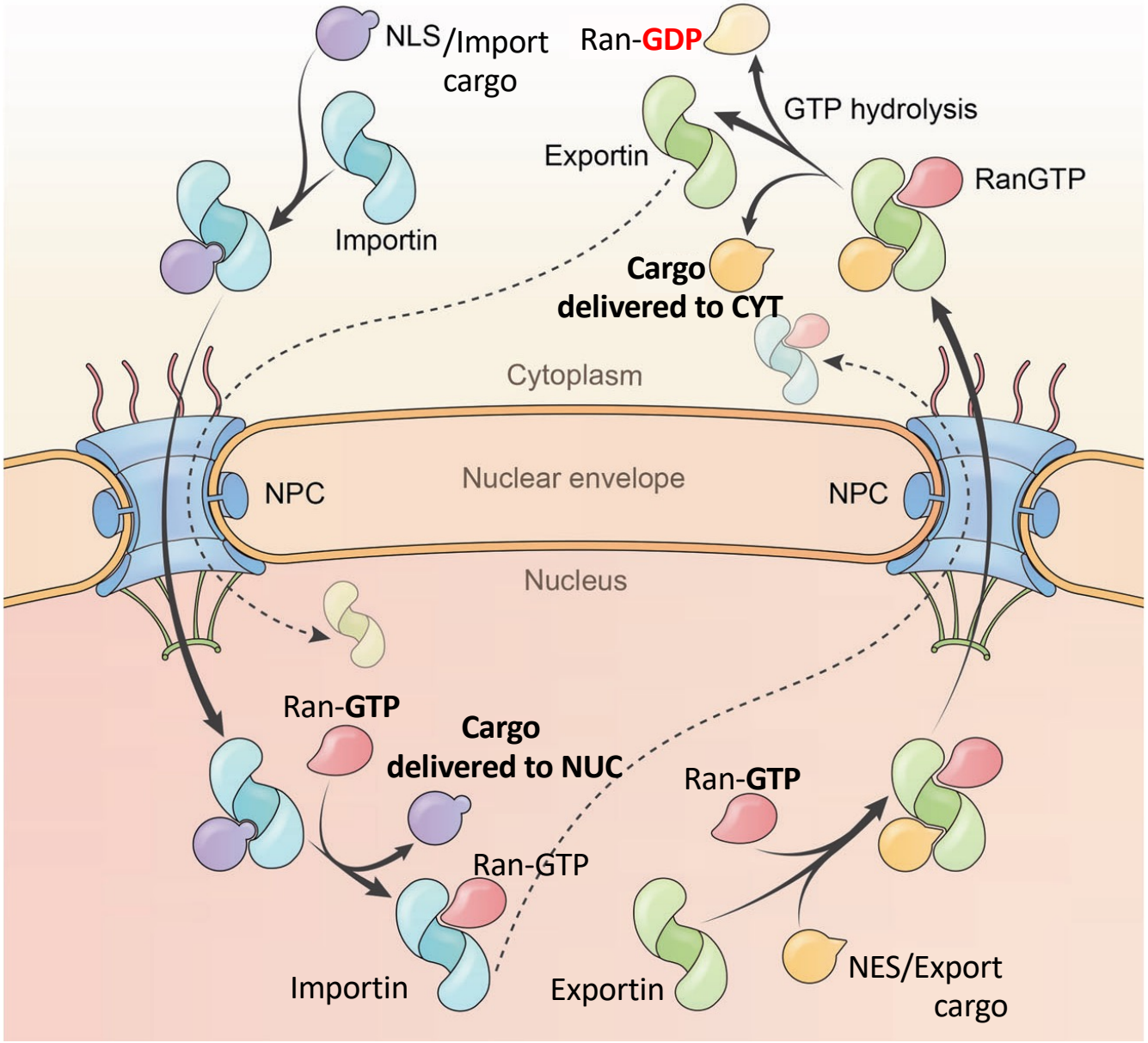


https://www.nature.com/articles/nrm2255

Nuclear import and export cycles through the NPC

•**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

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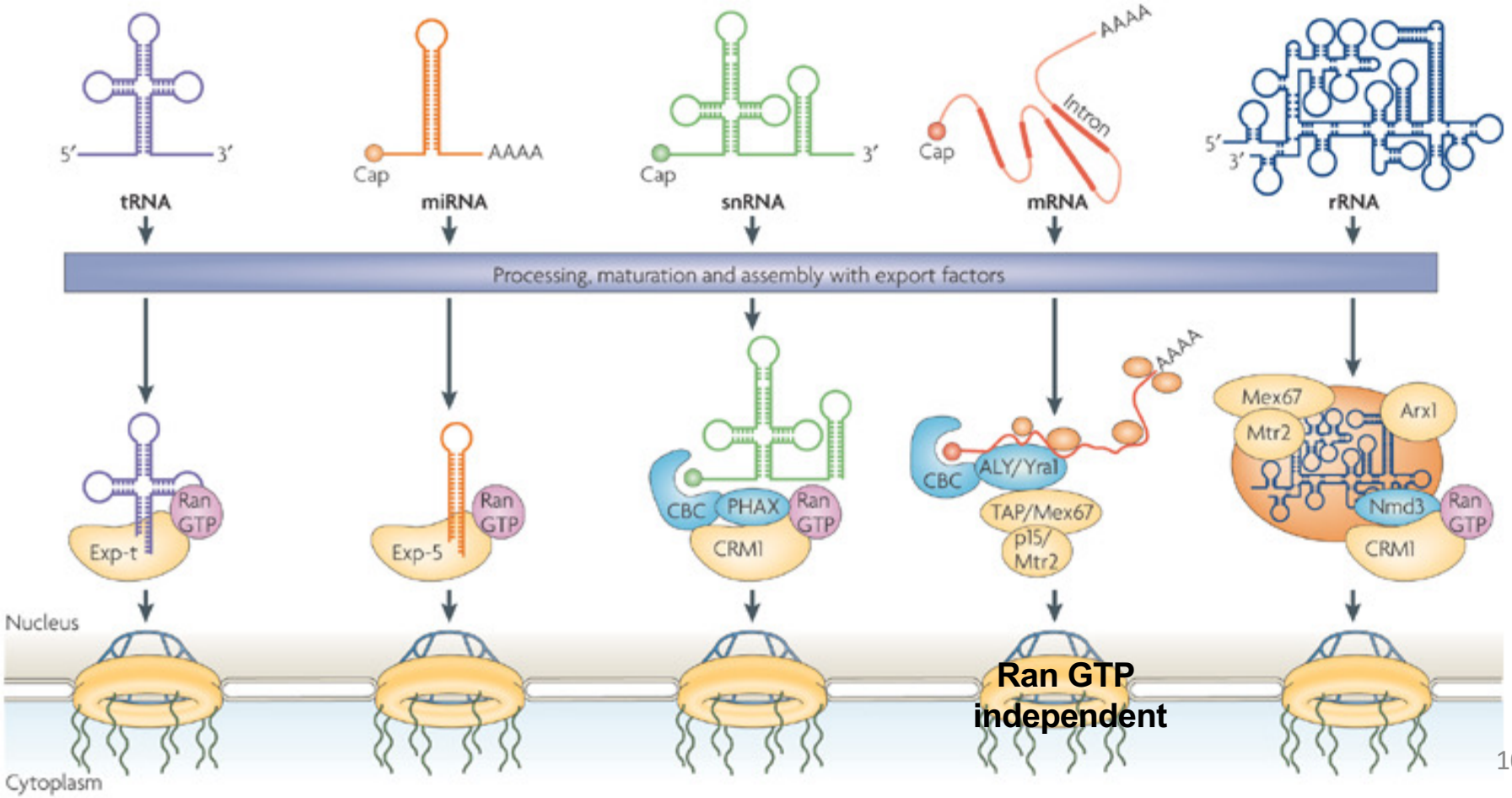
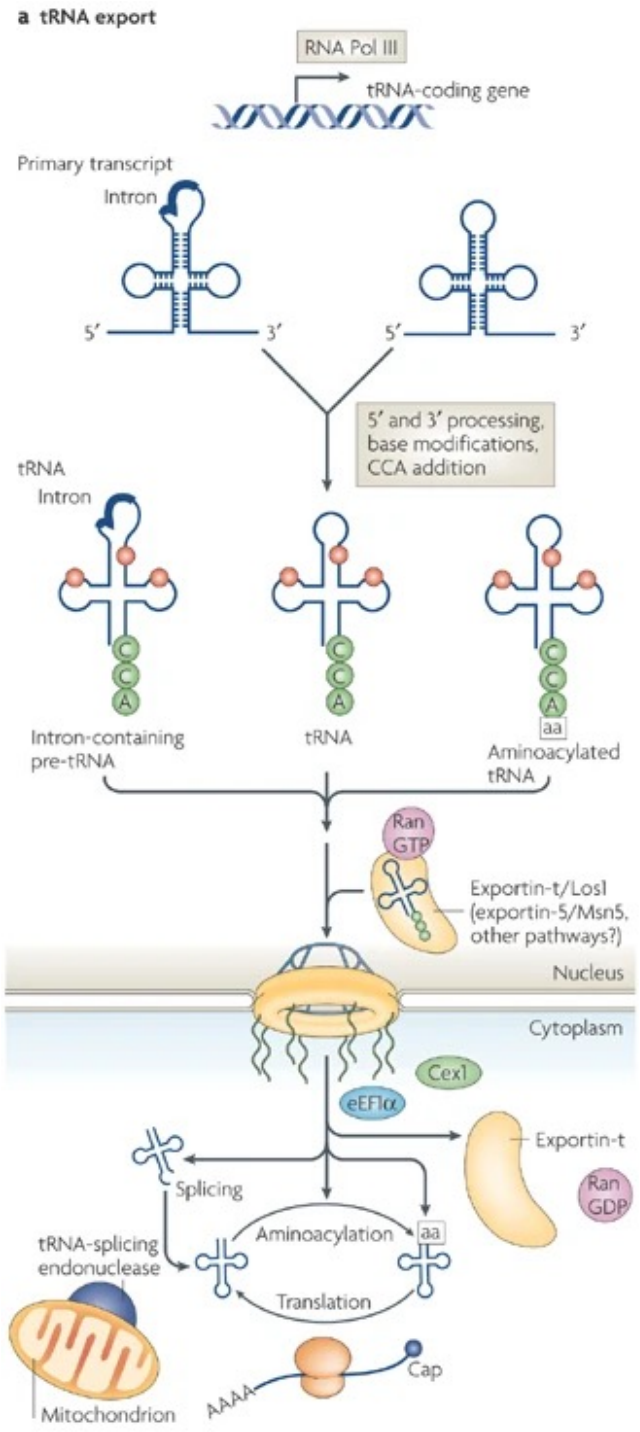
•Large RNAs (rRNAs,mRNAs) assemble into complicated ribonucleoprotein (RNP) particles and recruit their exporters via class-specific adaptor proteins.



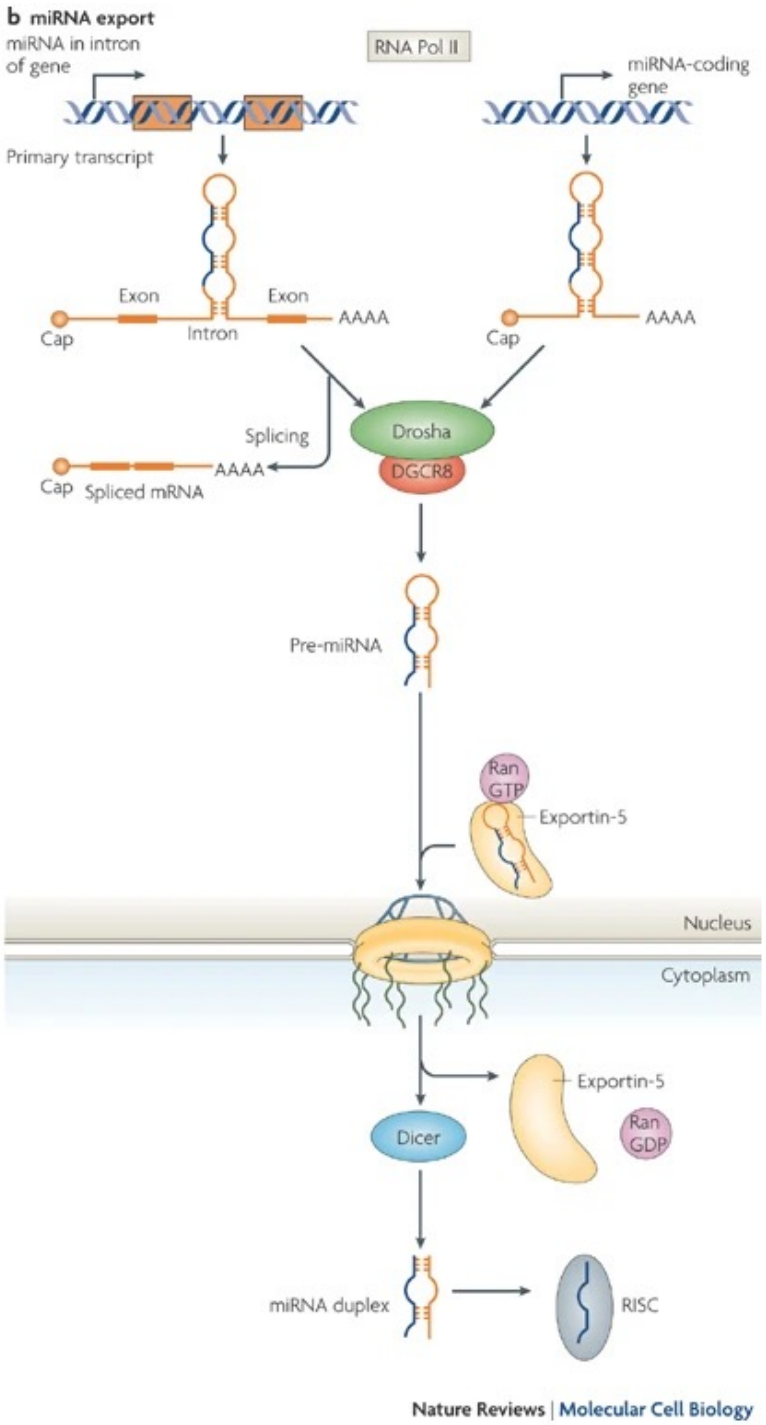
tRNA EXPORT



tRNAs 80 nt long RNAs can undergo about 200 chemical 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. Indeed, in yeast the SEN complex responsible for the spicing is located on the cytoplasmic surface of mitochondria.

miRNA EXPORT

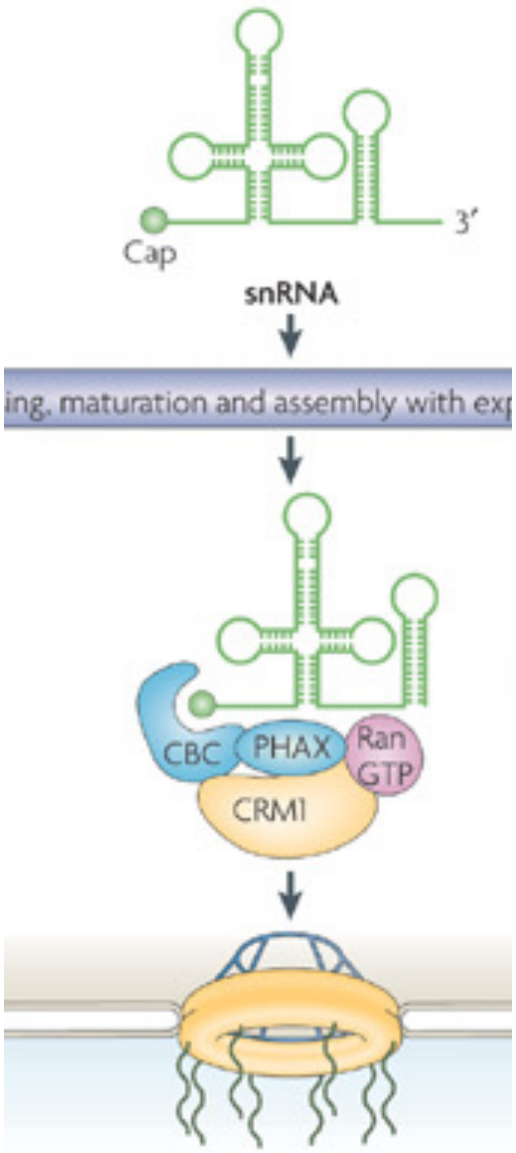


The ~65-nucleotide **pre-miRNA** is exported to the cytoplasm in a RanGTP-dependent manner by Exportin-5, a member of the karyopherin family.

During the first maturation step Drosha enzyme 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 in the Cytoplasm.

After release in the cytoplasm upon GTP hydrolysis on Ran, the pre-miRNA hairpin is released and further cleaved by Dicer.

snRNA EXPORT

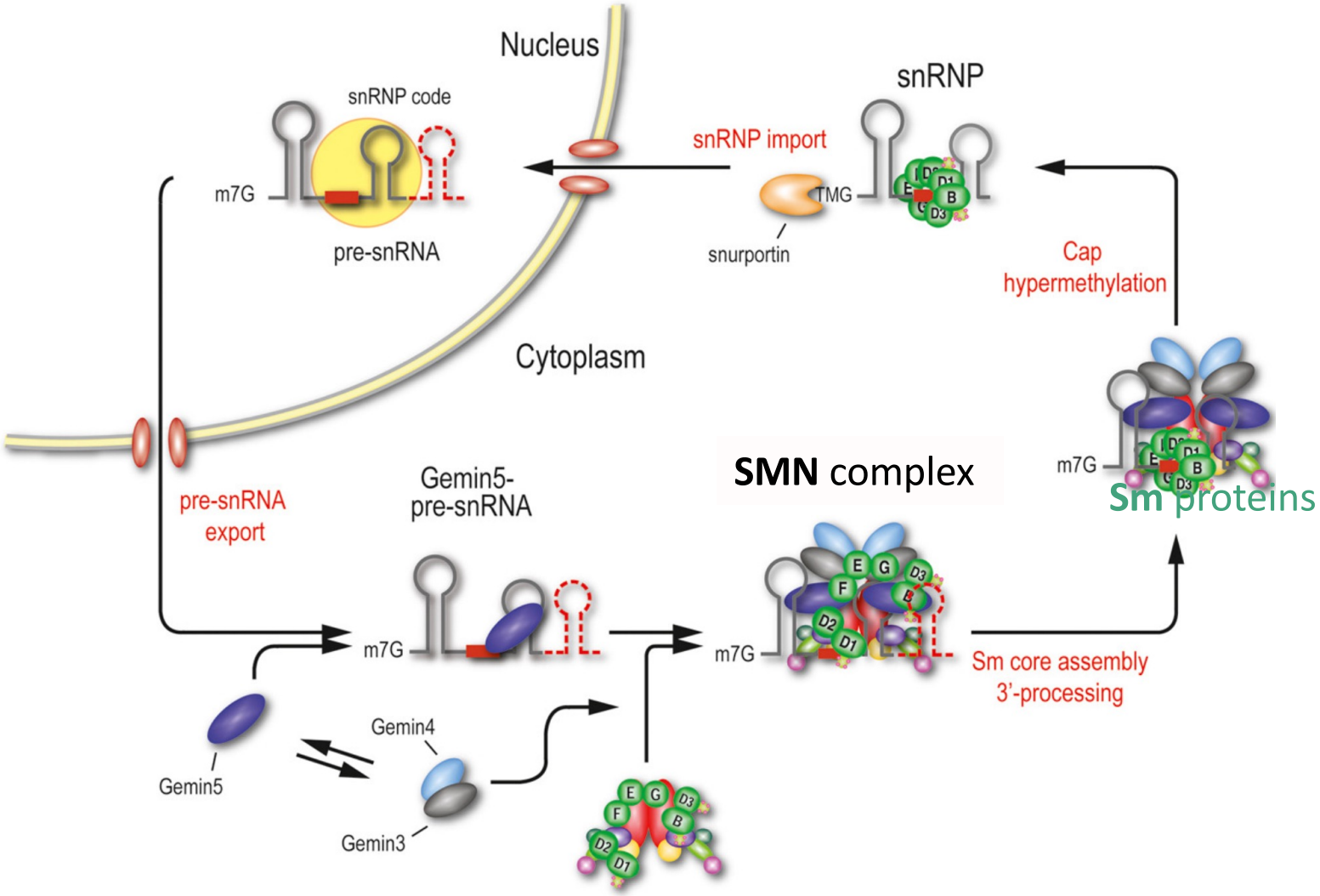


snRNAs have a nuclear and a cytoplasmic phase. Their maturation is completed in the cytoplasm (except for U6 transcribed by Pol III).

Their specific exportin is CRM1 (exportin-1) which does not directly interact with the snRNA cargo, but requires the cap-binding complex (CBC) and a NES-containing adaptor protein called PHAX (phosphorylated form) to be targeted to the 5’ cap of the snRNA5

After export to the cytoplasm, GTP hydrolysis of Ran and dephosphorylation of PHAX are necessary to efficiently dissociate the export complex and release the snRNA

Once in the cytoplasm, the **survival of motor neurons (SMN)** complex facilitates exonucleolytic removal of the 3′ trailer sequences and the assembly of the exported snRNA with a heteroheptameric ring of **Sm proteins**, which bind to a conserved Sm-binding site that is present on each snRNA. Binding of Sm proteins induces trimethylation of the cap. The trimethylated cap and the associated Sm proteins provide a composite nuclear targeting signal for subsequent nuclear import of the mature snRNPs. After re-import into the nucleus, snRNPs together with numerous other splicing factors assemble into the functional spliceosome53.



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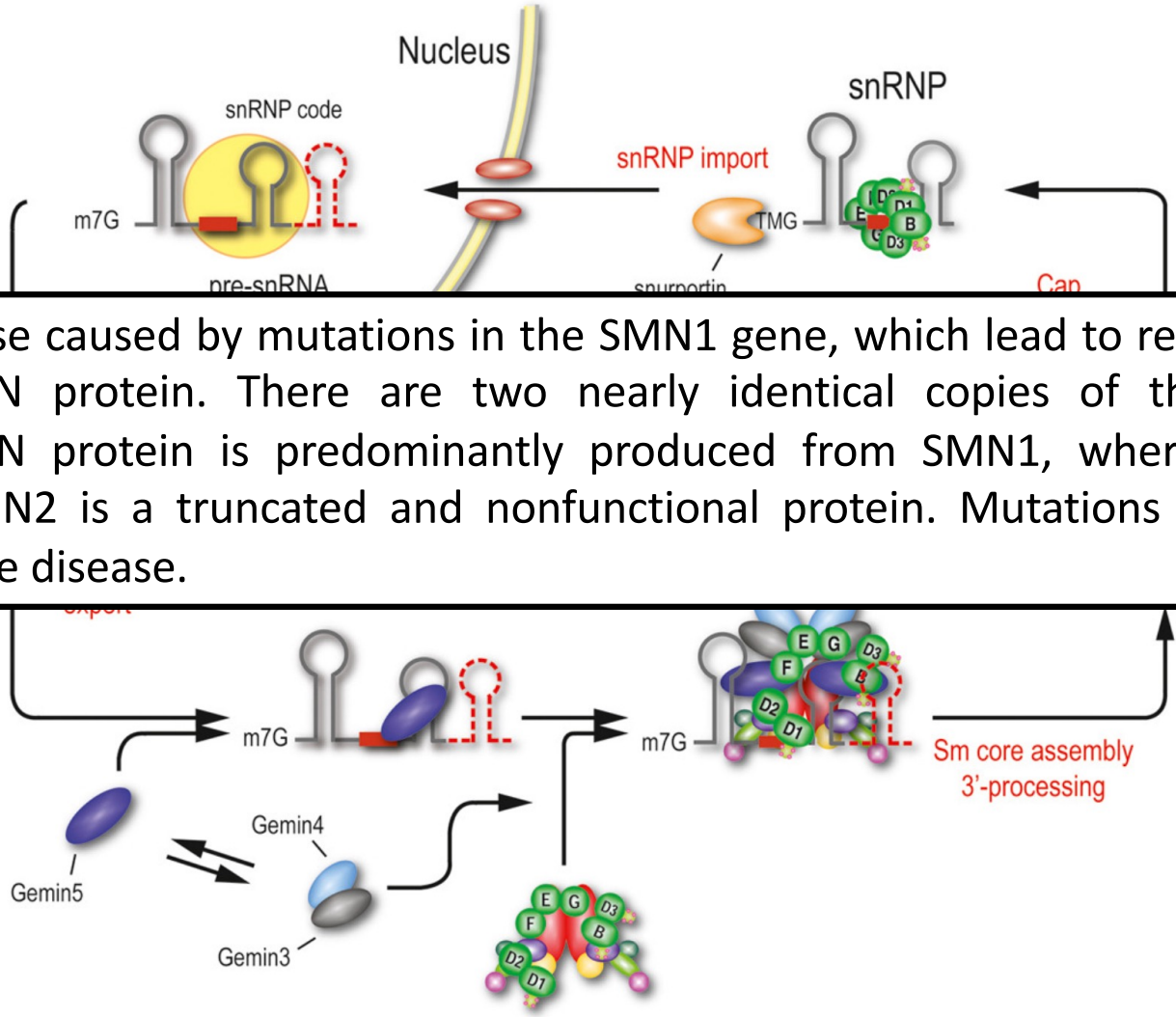
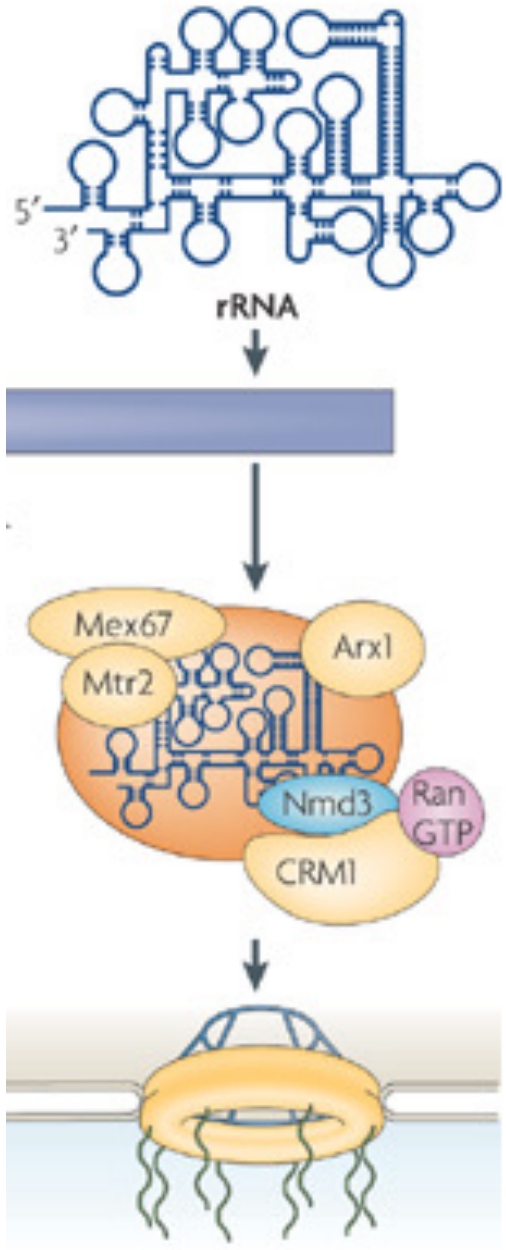


SMA is a disease caused by mutations in the SMN1 gene, which lead to reduced levels of functional SMN protein. There are two nearly identical copies of the SMN gene. Functional SMN protein is predominantly produced from SMN1, whereas the major product of SMN2 is a truncated and nonfunctional protein. Mutations that inactivate SMN1 cause the disease.

rRNA EXPORT



rRNA associate to the ribosomal subunits inside the nucleus.

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

- CRM1/Exportin 5 (RAN-GTP-dep.)
- MEX67-MTR2 (yeast), 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. Crm1 recognises the nuclear export signal (NES) of ribosome-bound NMD3 in a RanGTP-dependent manner

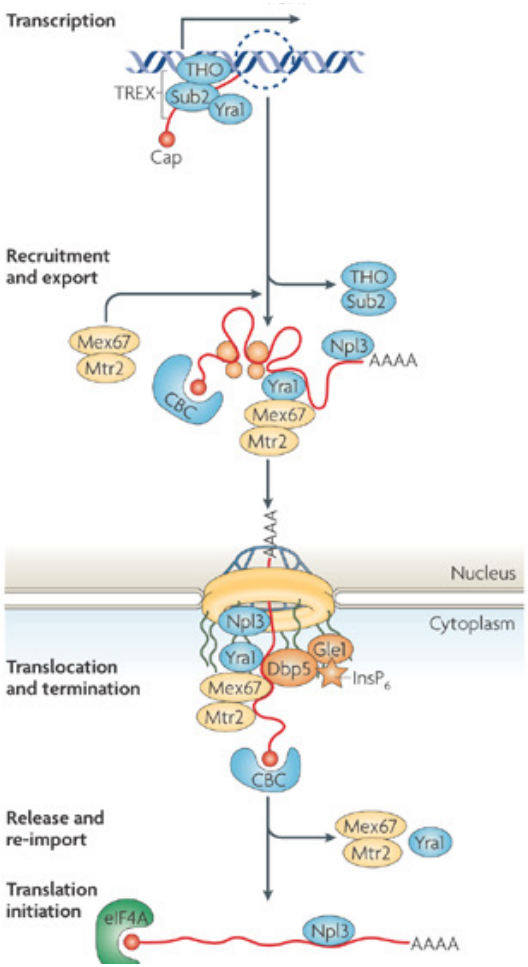
In yeast Mex67/Mtr2 is involved in the export of pre-60S particles together with additional export factors. Crm1 is necessary for nuclear export of the pre-40S ribosome tigether with Rio2 (with NES),the export adapter slx9 and RanGTPc

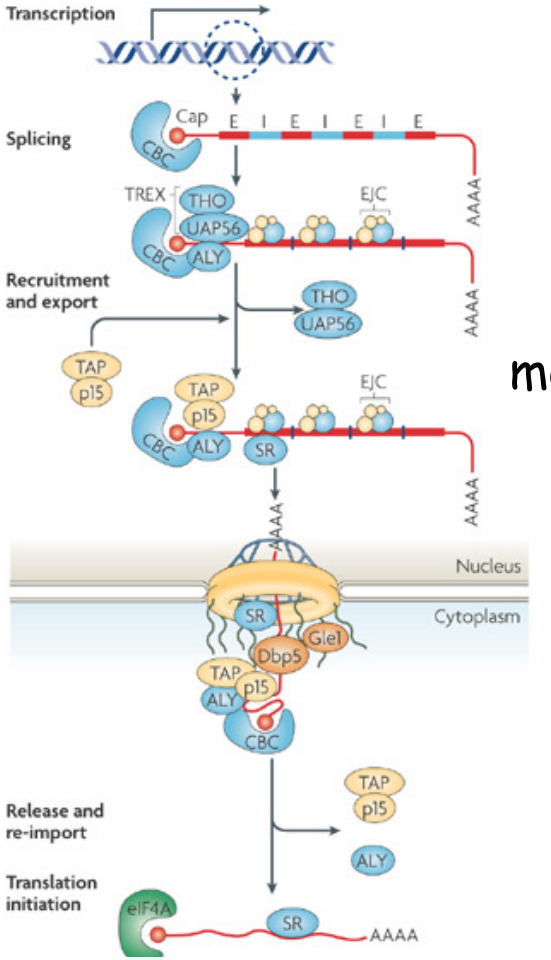
mRNA Export

•mRNAs are channelled into the specific export pathway coordinately with their processing and assembly into messenger (m)RNPs.

•Among the factors bound to the pre-mRNAs are also export adaptors that serve to establish a physical bridge between the mRNA molecule and its export receptor.

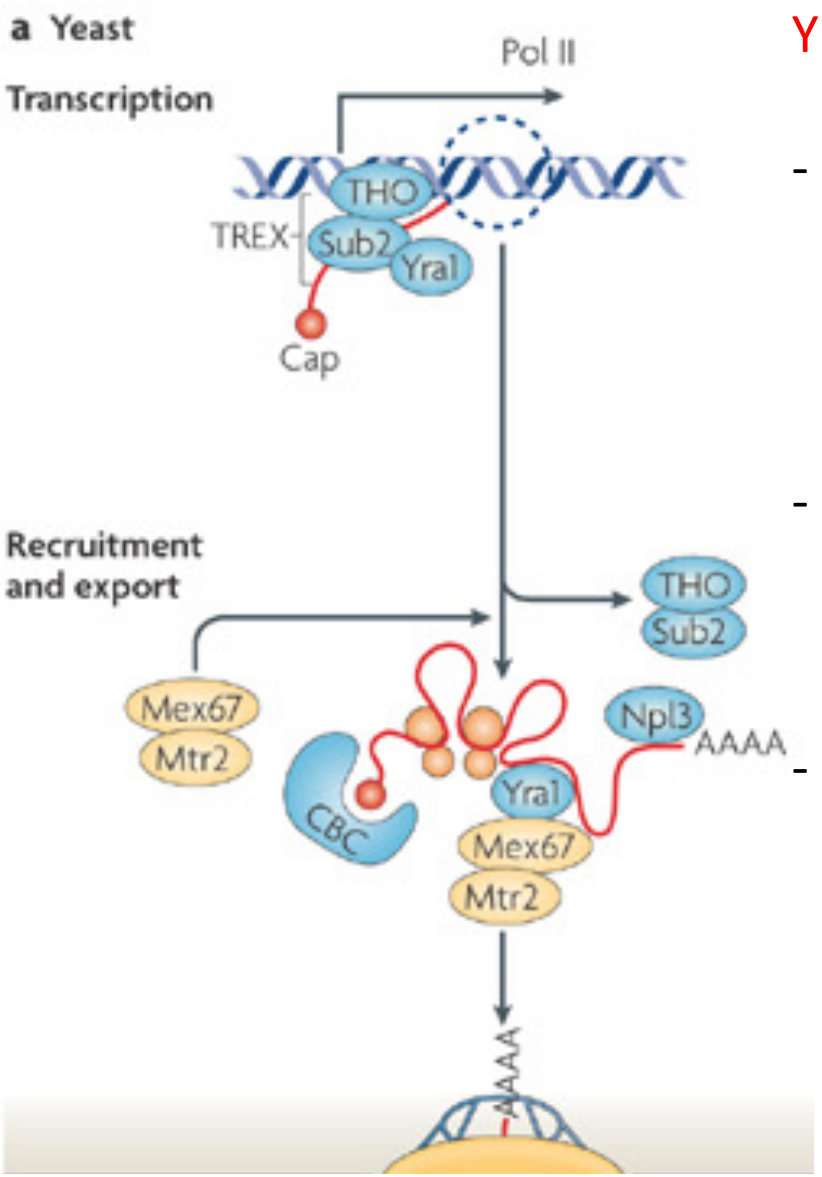
yeast





metazoa

mRNA EXPORT: differences between YEAST and METAZOA



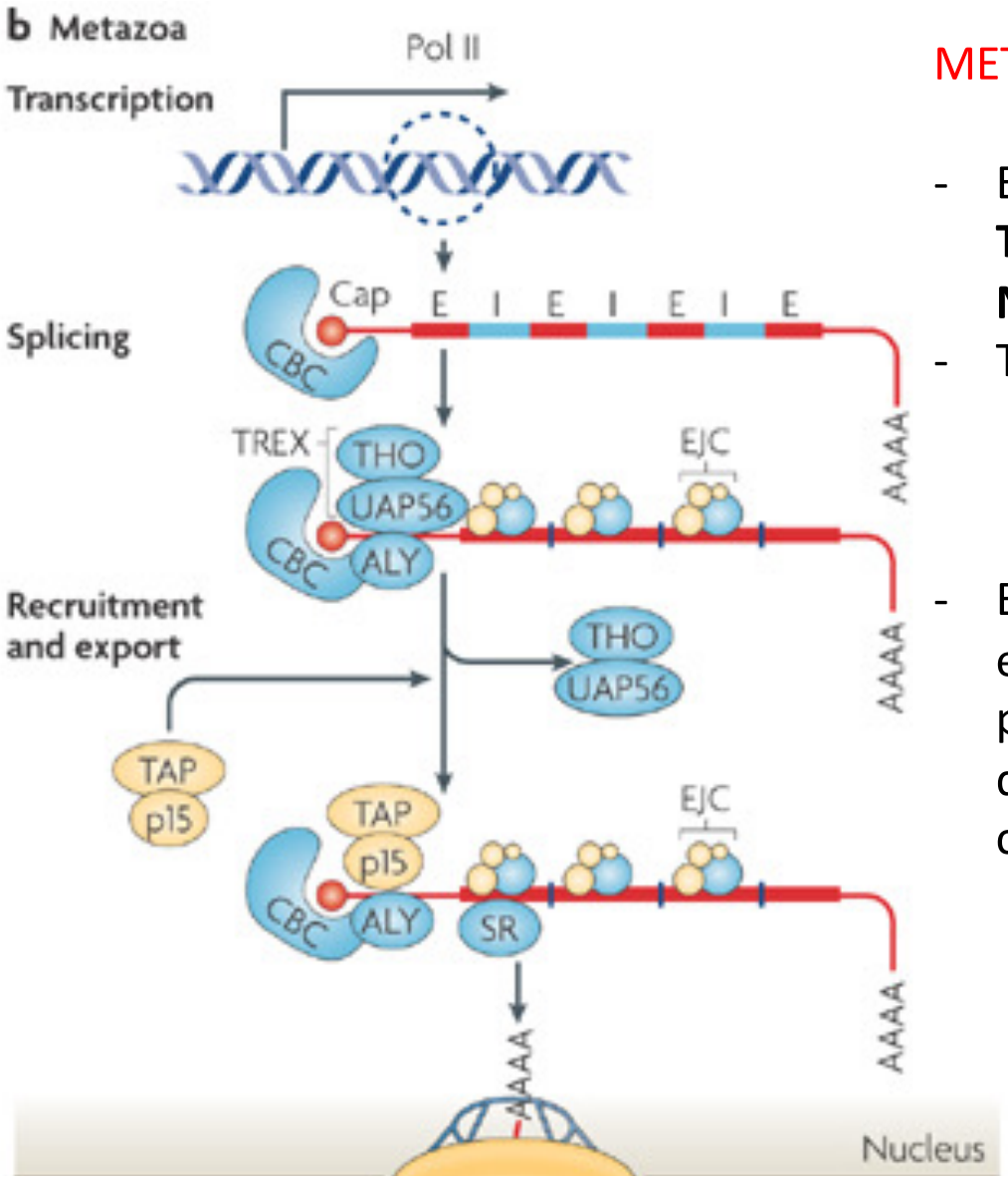
YEAST:

- 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.

mRNA Export



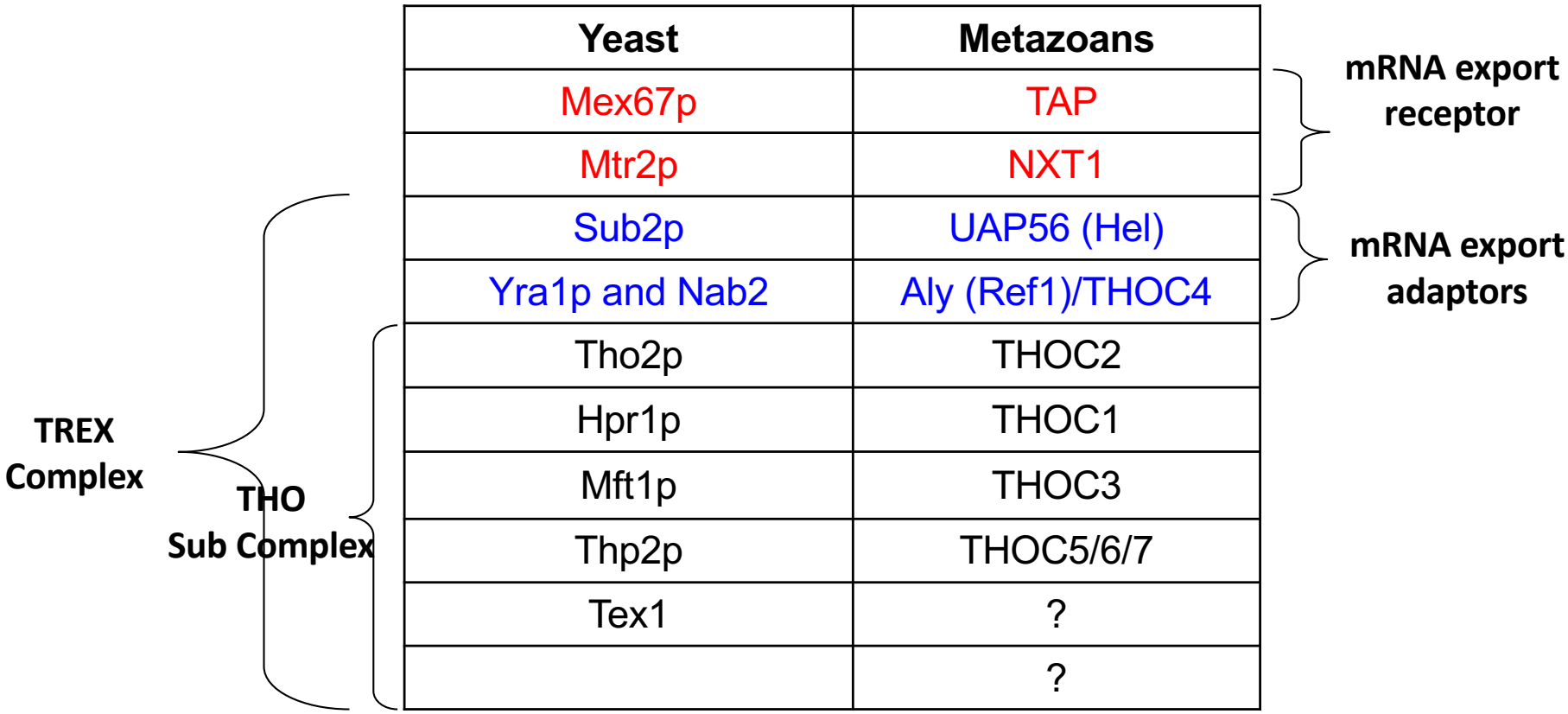
METAZOA:

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

- Besides its physiological role in cellular mRNA export, human TAP transports a set of viral pre-mRNAs to the cytoplasm by binding directly to specific viral RNA elements called constitutive transport elements (CTEs)

Factors that influence mRNA export

The coupling of nuclear mRNA biogenesis to the recruitment of the export machinery is largely coordinated by the transport/export complex (TREX)



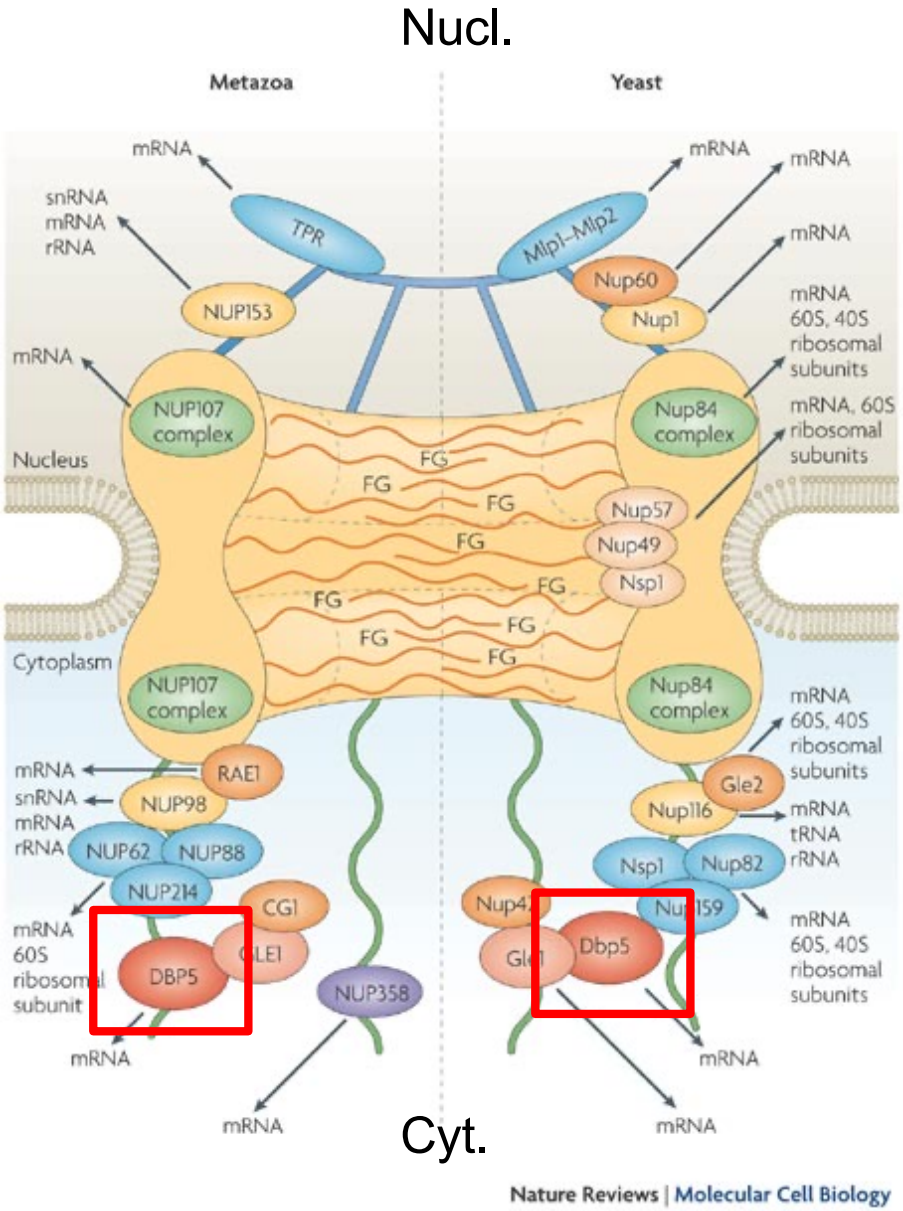
mRNA EXPORT

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

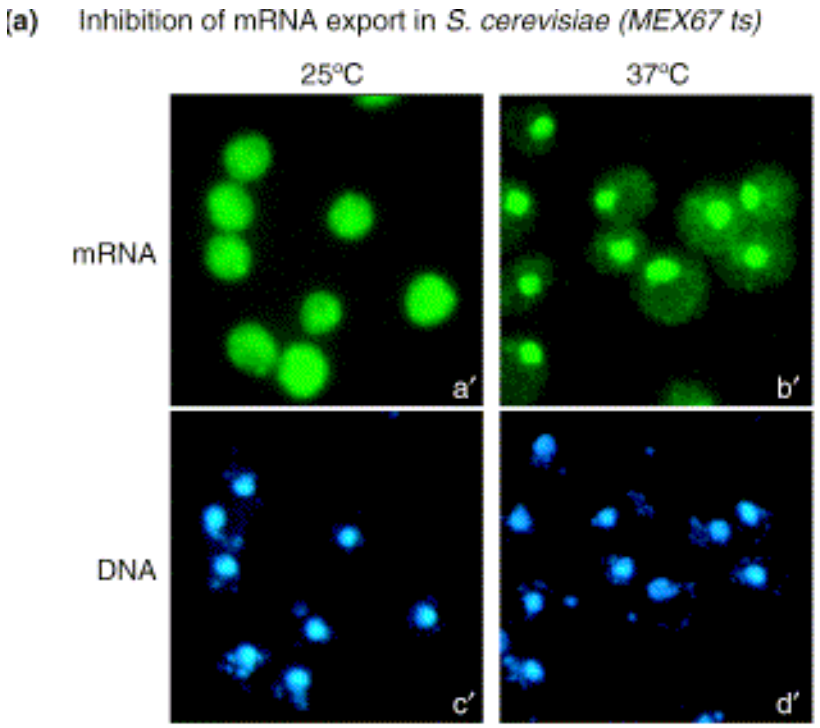
Both in yeast and in metazoa, mRNA export is **RNA-GTP independent**.

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

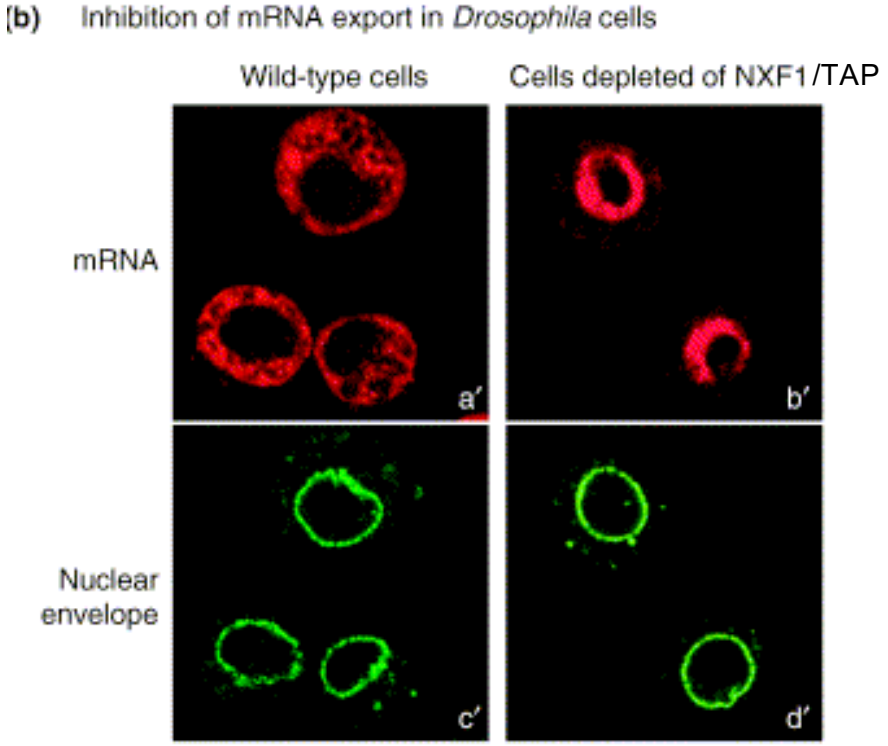
DBP5 is located in the **cytoplasmic** part of the nucleopore and removes the exporters from the mRNA.



Mex67p and TAP (NXF1) are essential for export of bulk mRNA



Shifting the mex67^{ts} strain to the restrictive temperature (37° C) causes nuclear mRNA accumulation.

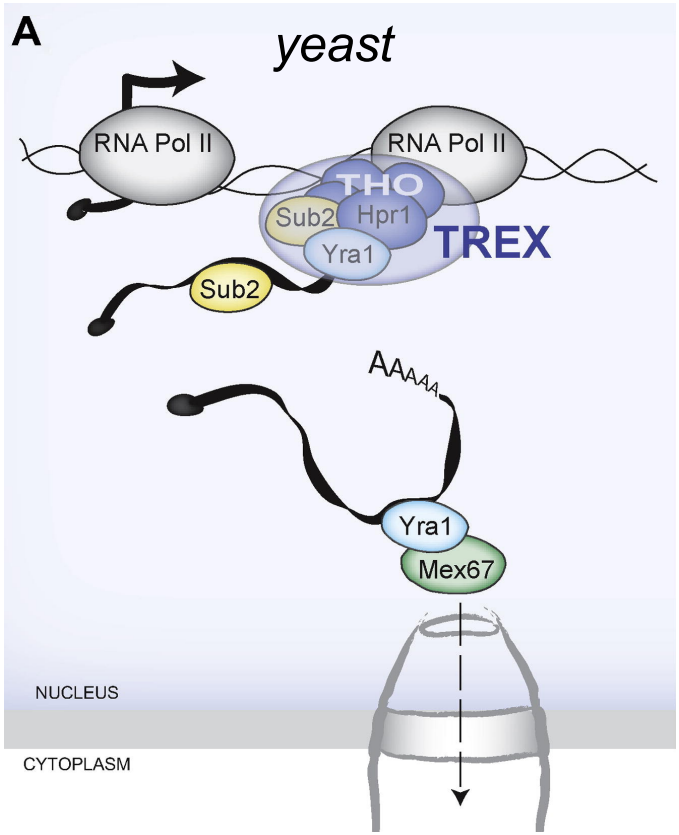


Depletion of Drosophila cells of NXF1(TAP) by double stranded RNA inhibition causes nuclear mRNA accumulation.

In both experiments, poly(A) mRNA was visualized by in situ hybridization with a fluorescently-labeled oligo-dT probe

The Yra1 and Aly/REF adaptors

•Because of its low affinity and low specificity for binding mRNAs, Mex67/TAP requires adaptor proteins to interface with mature transcripts ready for export. So far, the best-characterized adaptor for Mex67/TAP is the essential **Yra1** protein in yeast or **Aly/REF** in higher eukaryotes.

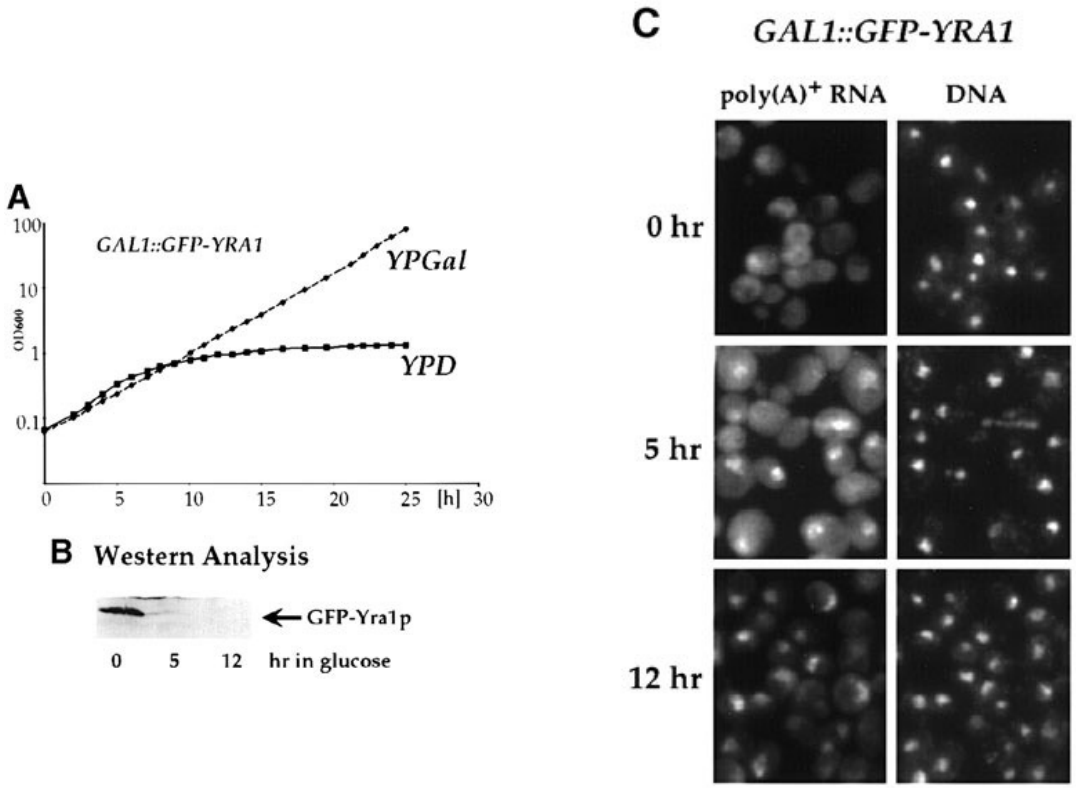


•**Thus, Yra1 and ALY/REF form a bridge between an upstream-acting RNA-binding protein and a downstream-acting mRNA export receptor.**

The Yra1 and the adaptor Sub2

- Yra1 was identified as an interactor of Mex67p

- Yra1p-depleted cells accumulated poly(A)$^+$ RNA inside the nucleus.

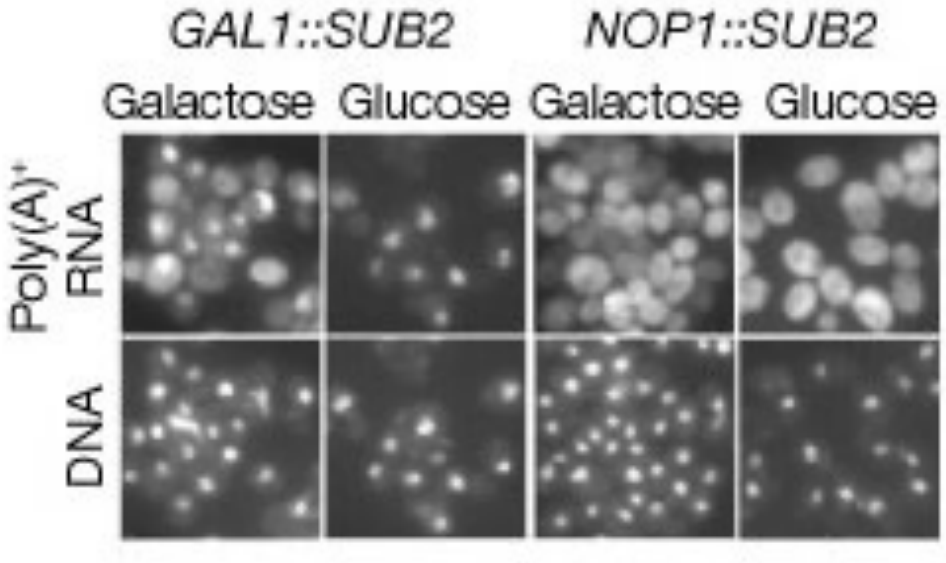


poly(A) mRNA was visualized by in situ hybridization with a fluorescently-labeled oligo-dT probe

Sub2p

•Sub2 (UAP56 in human) was identified as an interactor of Yra1 and it is a member of the DEAD box family of RNA helicases

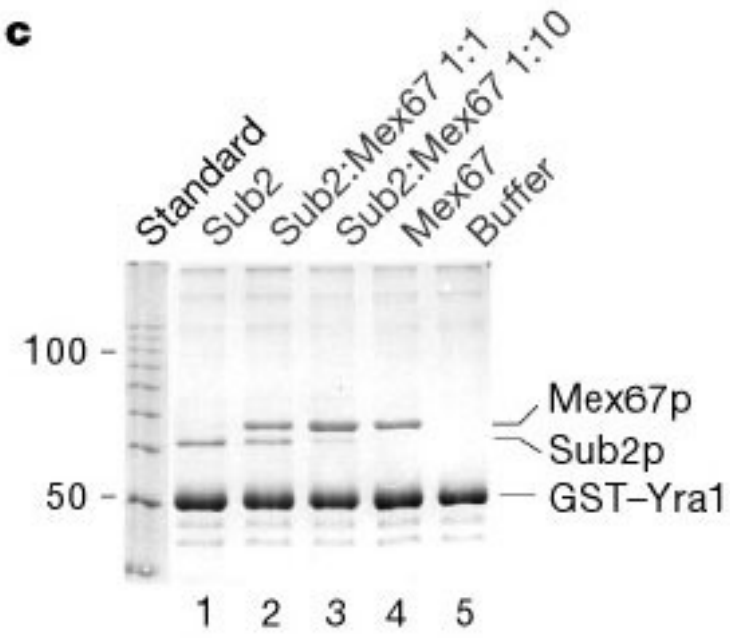
•Sub2p-depleted cells accumulated poly(A)$^+$ RNA inside the nucleus.



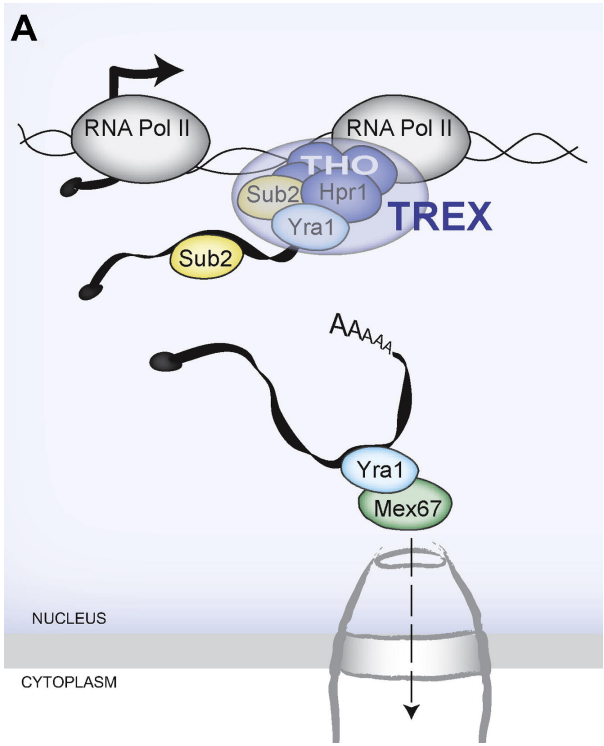
poly(A) mRNA was visualized by in situ hybridization with a fluorescently-labeled oligo-dT probe

Mex67p competes with Sub2p for binding to Yra1p.

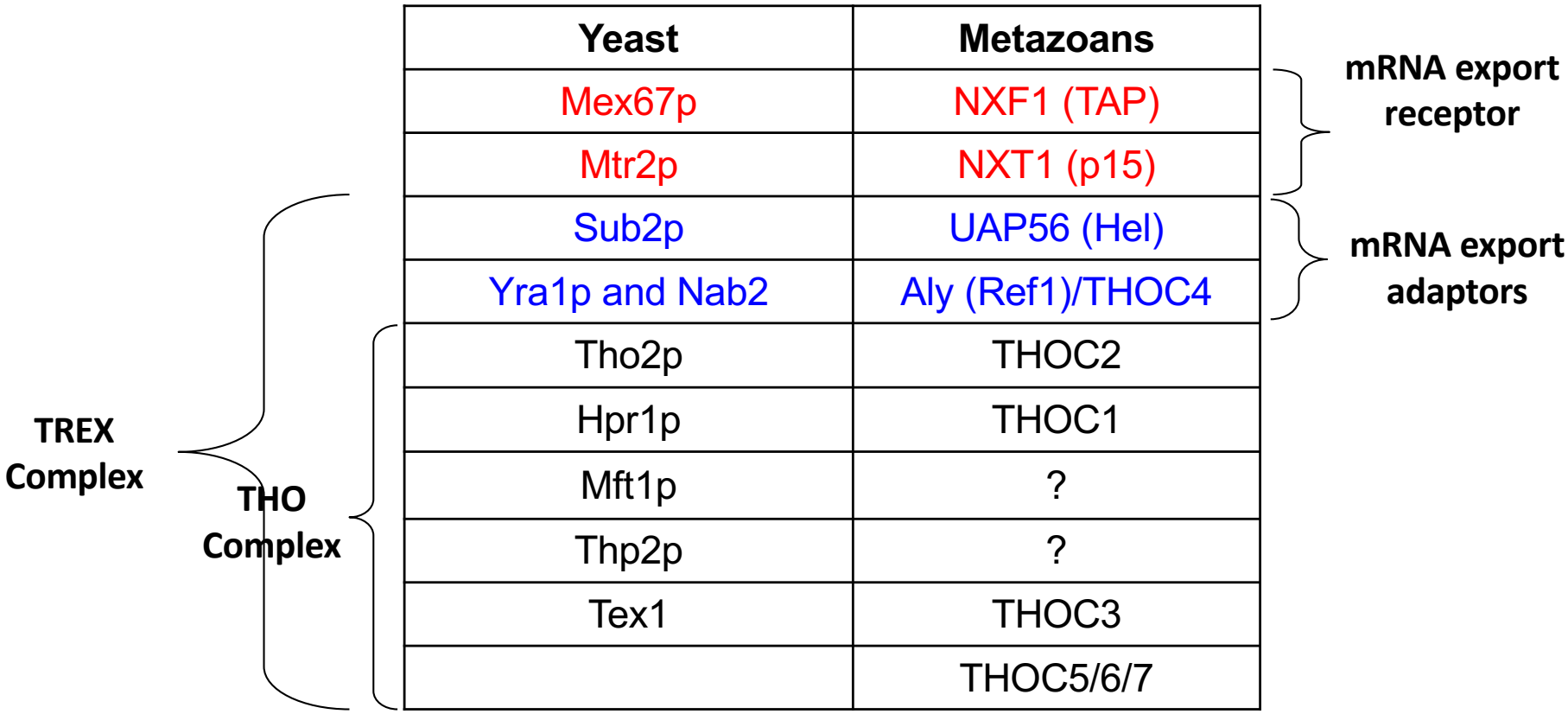
•Mex67p competed with Sub2p for binding to Yra1p when Mex67p and Sub2p were present in equimolar amounts



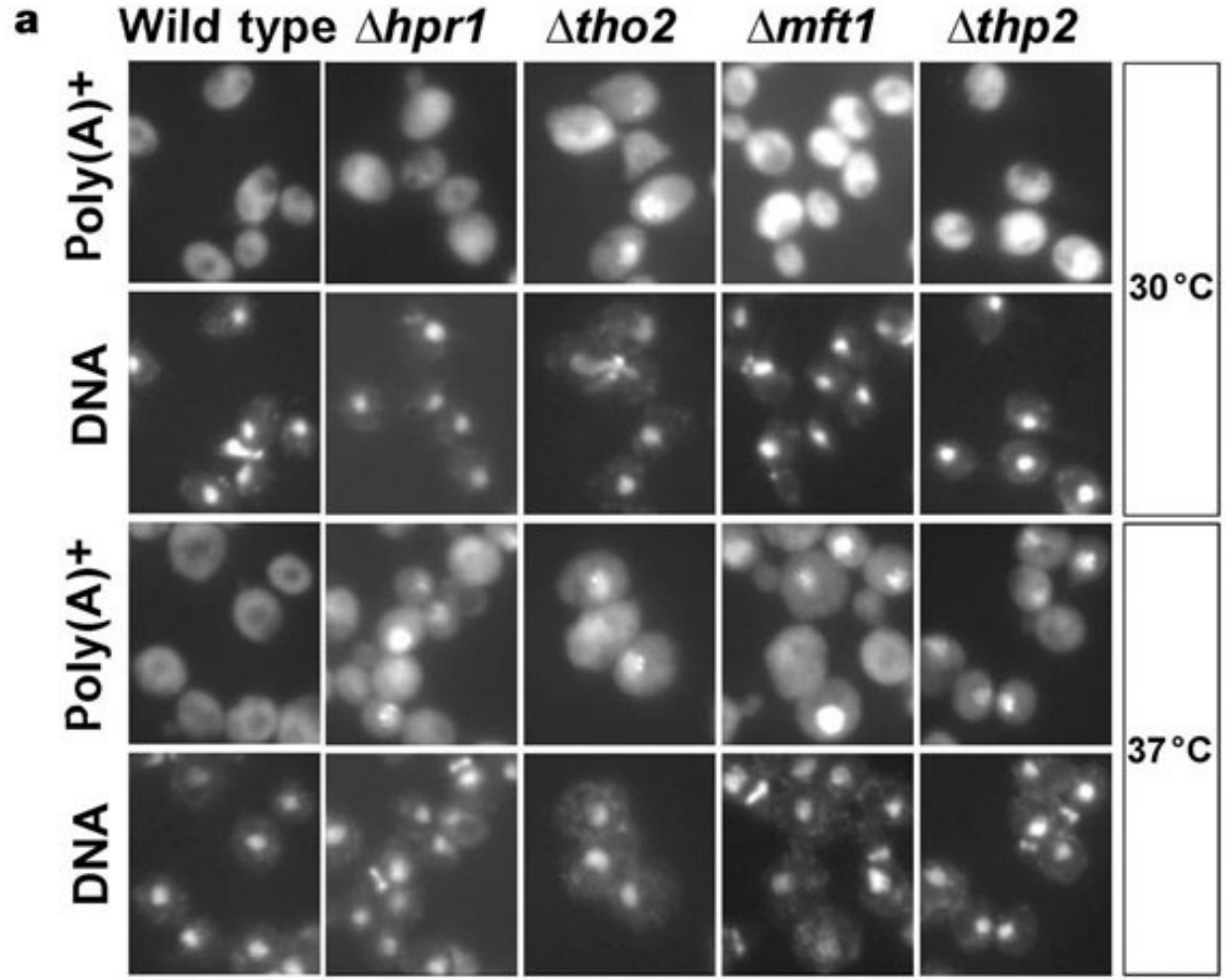
Competition assay



Factors that influence mRNA export



Deletion of Tho2, Hpr1, Mft1 and Thp2 results in mRNA export defect



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

The TREX complex contains THO and 2 mRNA export factors

- In yeast, the multi-subunit TREX complex plays a role in coupling transcription to mRNA export. This complex contains the mRNA export factors **Sub2p** and **Yra1p** as well as the **THO complex**, which functions in transcription elongation.

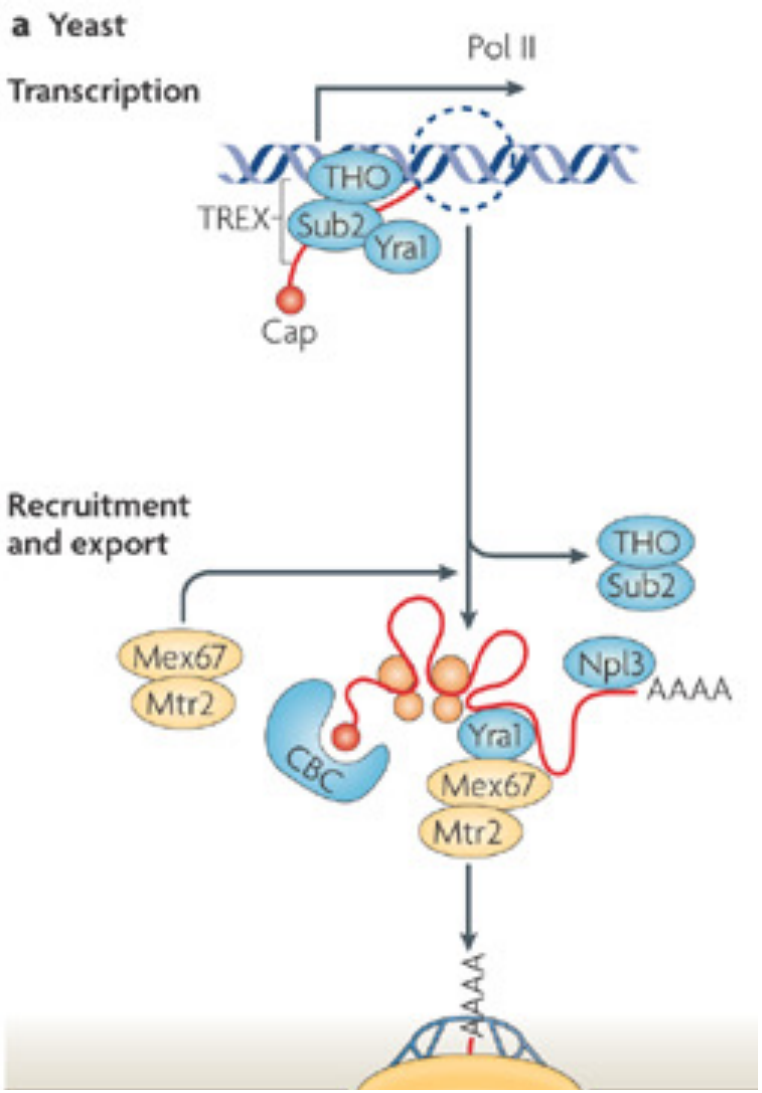
mRNA EXPORT: YEAST
In the nucleus…

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

YRA1 (Adaptor) is the RNA binding protein which bridges mRNA and the exporter MEX67

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

SUB2 is a RAN-helicase recurited by the THO elongation complex during transcription.



mRNA EXPORT: YEAST
In the nucleus…

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

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

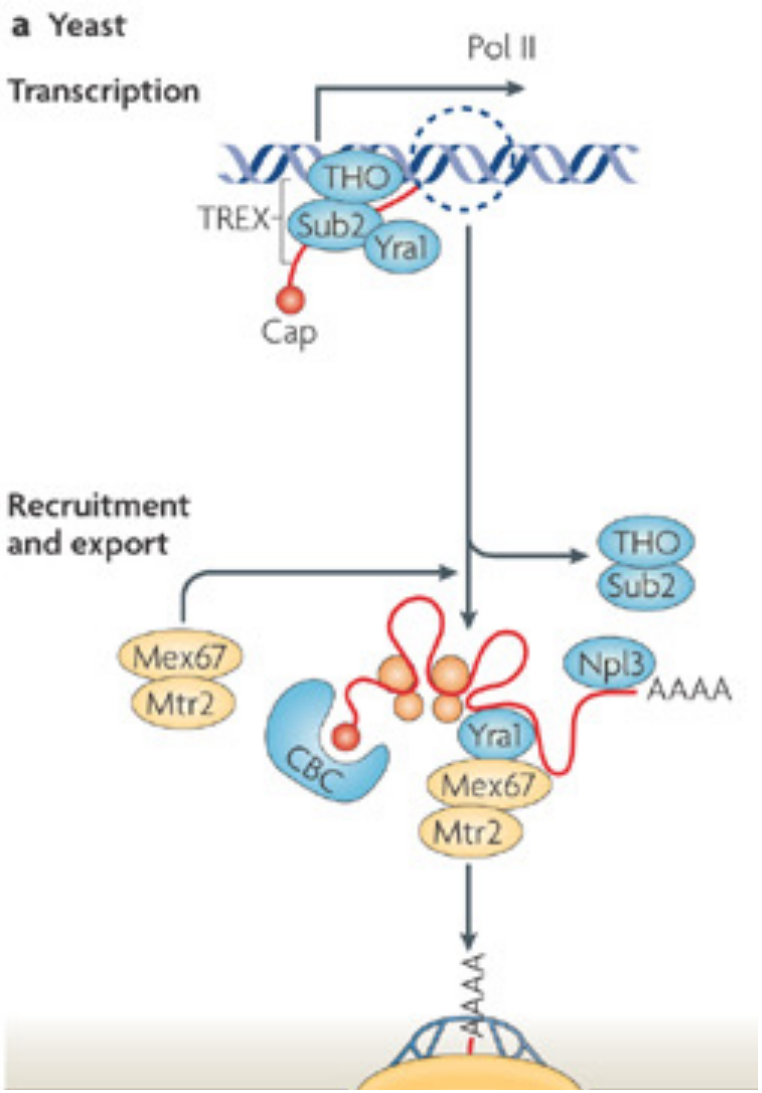
SUB2 interacts with YRA1 **(provides competence for the export to the mRNA)**

SUB2 is a RAN-helicase recurited by the THO elongation complex during transcription.

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

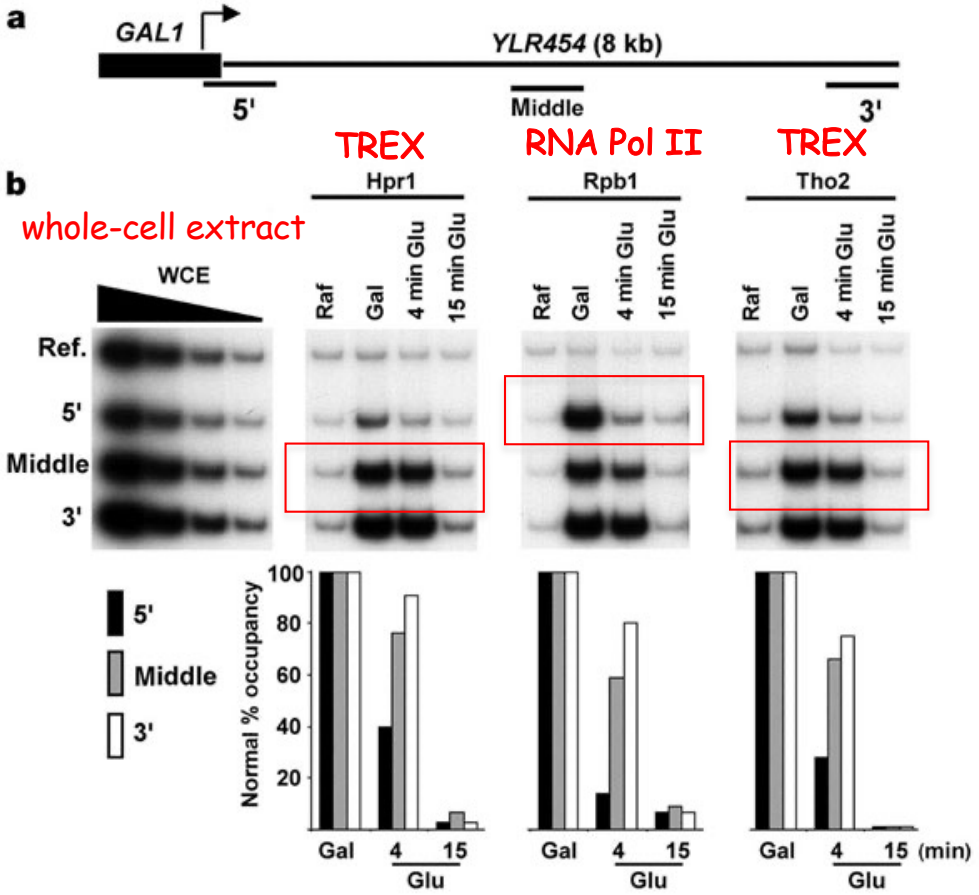
2. At the end of transcription, **SUB2 detaches** from the mRNA and YRA1 -> **YRA1 + MEX67** -> **mRNA ready to be exported.**

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



TREX complex operates in coupling transcription elongation to mRNA export

•The TREX complex is specifically recruited to the transcribing gene and travels with the polymerase during transcriptional elongation.



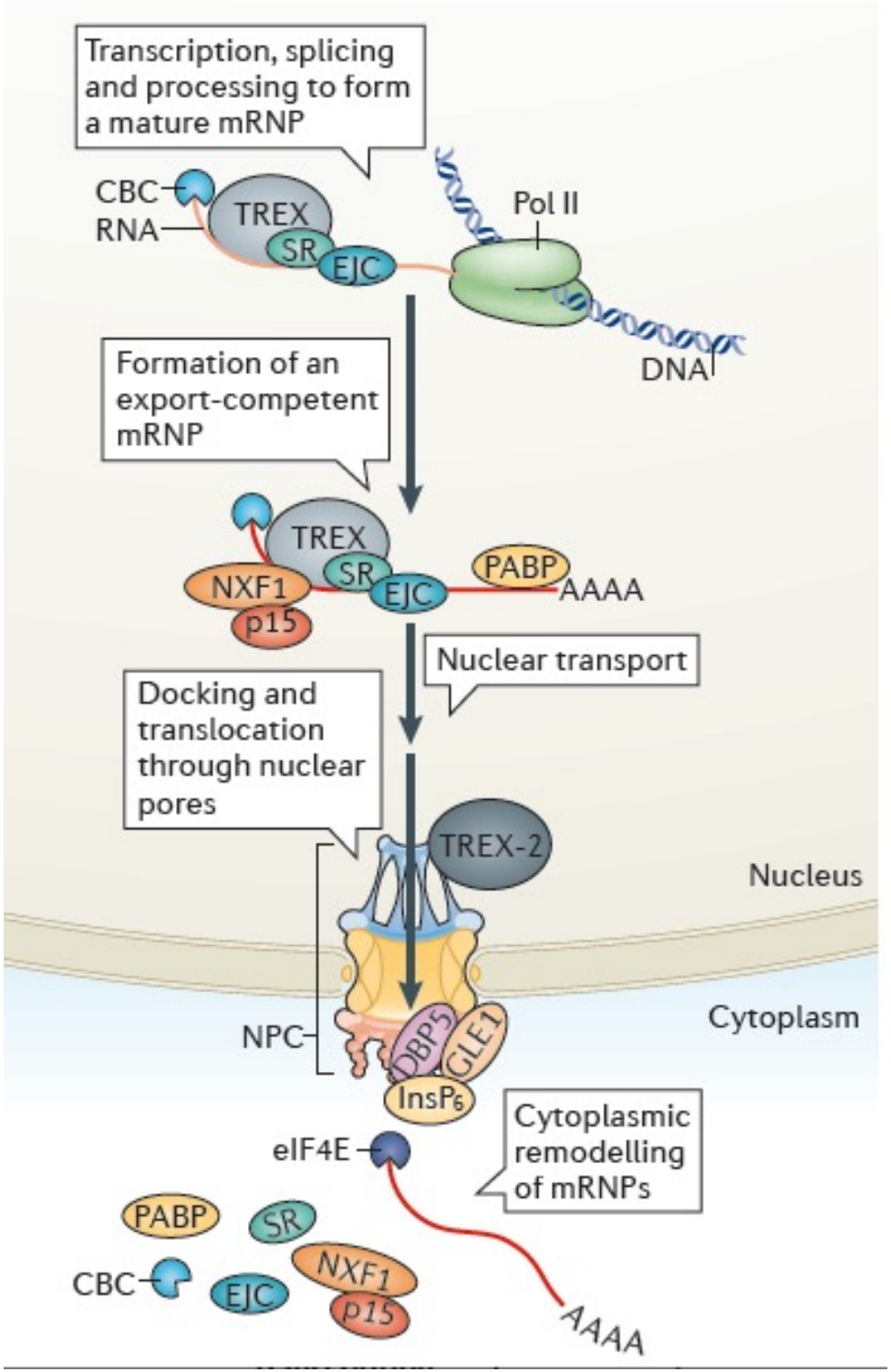
chromatin immunoprecipitation assay in strains containing a long yeast gene (ORF YLR454) under the control of the regulatable GAL1 promoter

The TREX complex contains THO and 2 mRNA export factors

- Human TREX contains Aly/REF, UAP56, and the human counterpart of the yeast THO complex. The human THO complex only associates with spliced mRNA and not with unspliced pre-mRNA.

- Recent data indicate that recruitment of the human TREX complex to spliced mRNA occurs by a splicing-coupled mechanism rather than by the direct transcription-coupled mechanism that occurs in yeast.

- In human ALY/REF is rectuited to the mRNP via UAP56 during splcing in an ATP dependent manner



In Metazoa TREX complex is recruited to the transcribing gene by different mechanisms:

1. CAP-dependent manner: CAP binding subunit CBP80 interacts with the ALY/REF component of TREX. Suggenting why mRNAs are exported in a 5'➔3' direction.

2. Splicing-dependent manner: UAP56 interacts with the splicing factors U2AF2 and together with Aly/REF and TAP-p15 interatct with the exon-junction complex which is deposited as a consequence of splicing 20–24 nucleotides upstream of every exon–exon junction in the spliced mRNA.

3. transcription dependent manner: SPT6 elongation factor recruits IWS1 to act ad bridging protein for Aly/REF

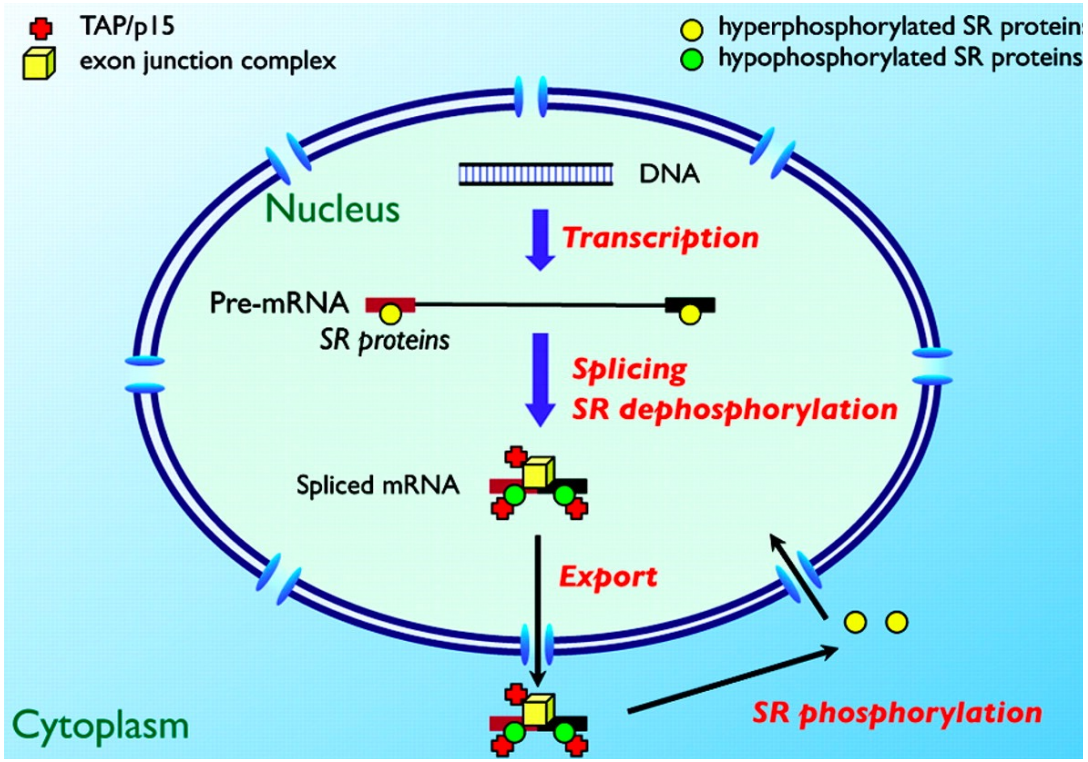
Export competency is linked to splicing in metazoa: the Serine/arginine-rich (SR) proteins

•**TAP interacts preferentially with shuttling SR proteins that are hypophosphorylated.**

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

They become partially dephosphorylated during the splicing reaction and more avidly bind TAP (NXF1). **The phosphorylation state of bound SR proteins contributes to the ability of the export machinery to discriminate between spliced and unspliced mRNPs**

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

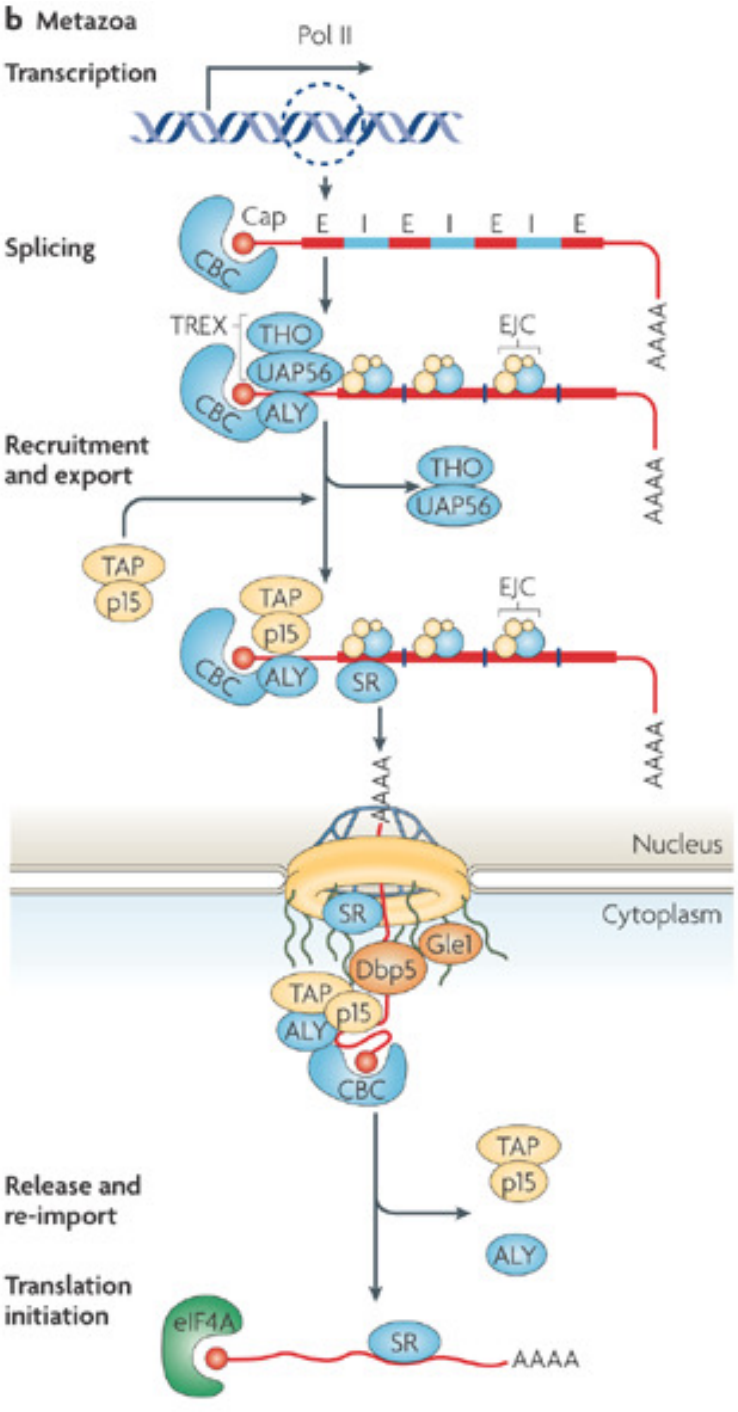
1. Metazoan TREX contains Aly/REF (YRA1), UAP56 (SUB2), and the metazoan counterpart of the yeast THO complex.

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

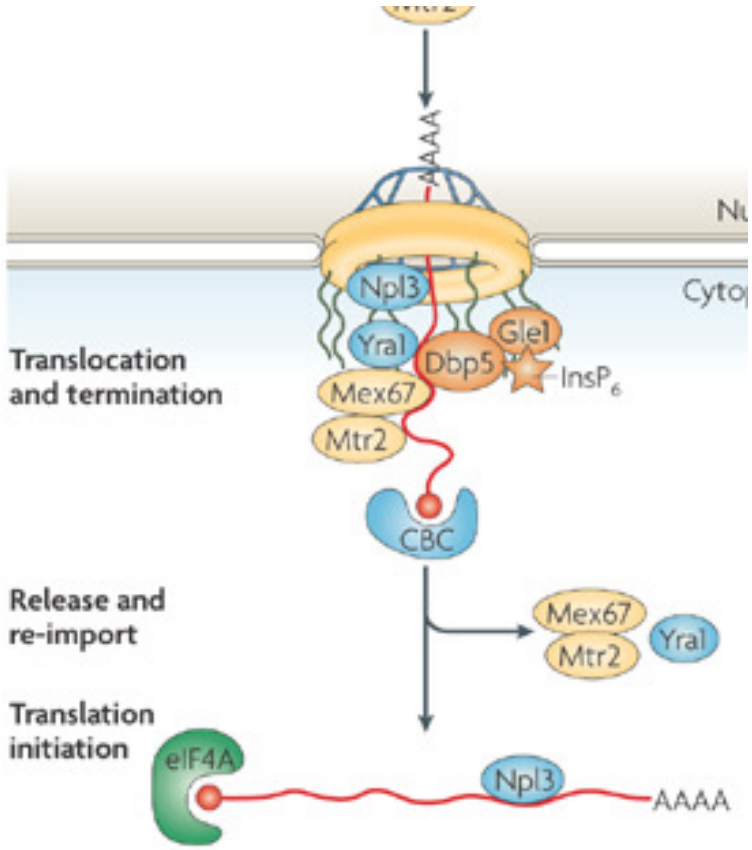


Directionality and termination of mRNA export

•ATP-dependent RNA helicases such as **Dbp5** which are involved in mRNA export, could trigger an irreversible ATP-driven mRNP rearrangement at the cytoplasmic side of the NPC

•Dbp5 exhibits a very low ATP-dependent RNA-helicase activity, which can be stimulated by **Gle1**, an essential mRNA export factor that is also asymmetrically located at the cytoplasmic nuclear pore filaments

•Maximal stimulation of the ATPase activity of Dbp5 requires the signalling molecule **inositol heaxakisphospate** (**$InsP_6$**) which regulates the interaction between Gle1 and Dbp5

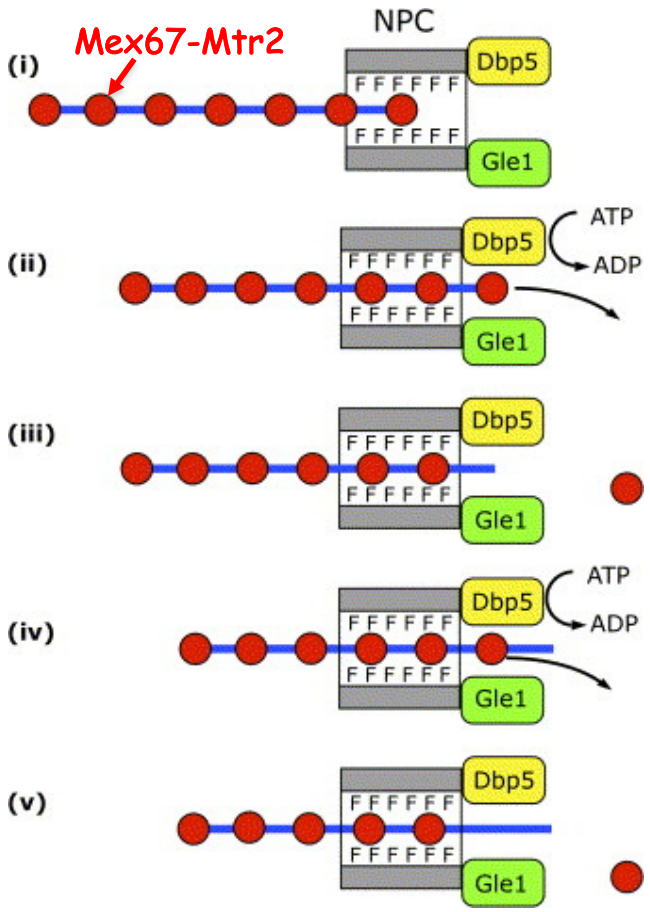


Directionality and termination of mRNA export

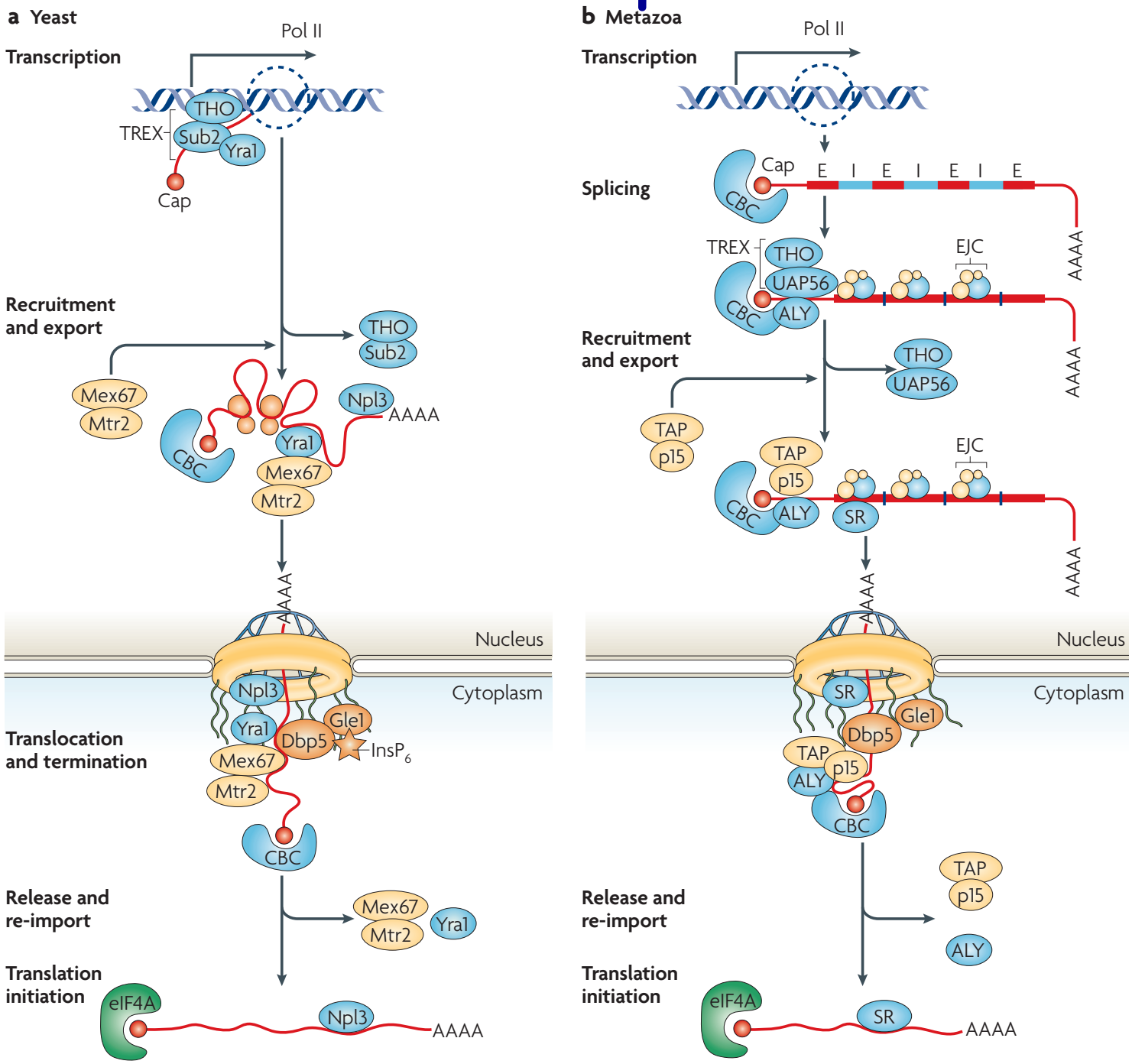
•Interactions between **Mex67:Mtr2** and **FG-nucleoporins (F)** that line the NPC transport channel facilitate movement of the mRNP

•When one of the Mex67:Mtr2 complexes reaches the cytoplasmic face of the NPC, it is removed from the mRNP by the **DEAD-box helicase Dbp5**, the ATPase activity of which is stimulated by **Gle1** and **$InsP_6$**

•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

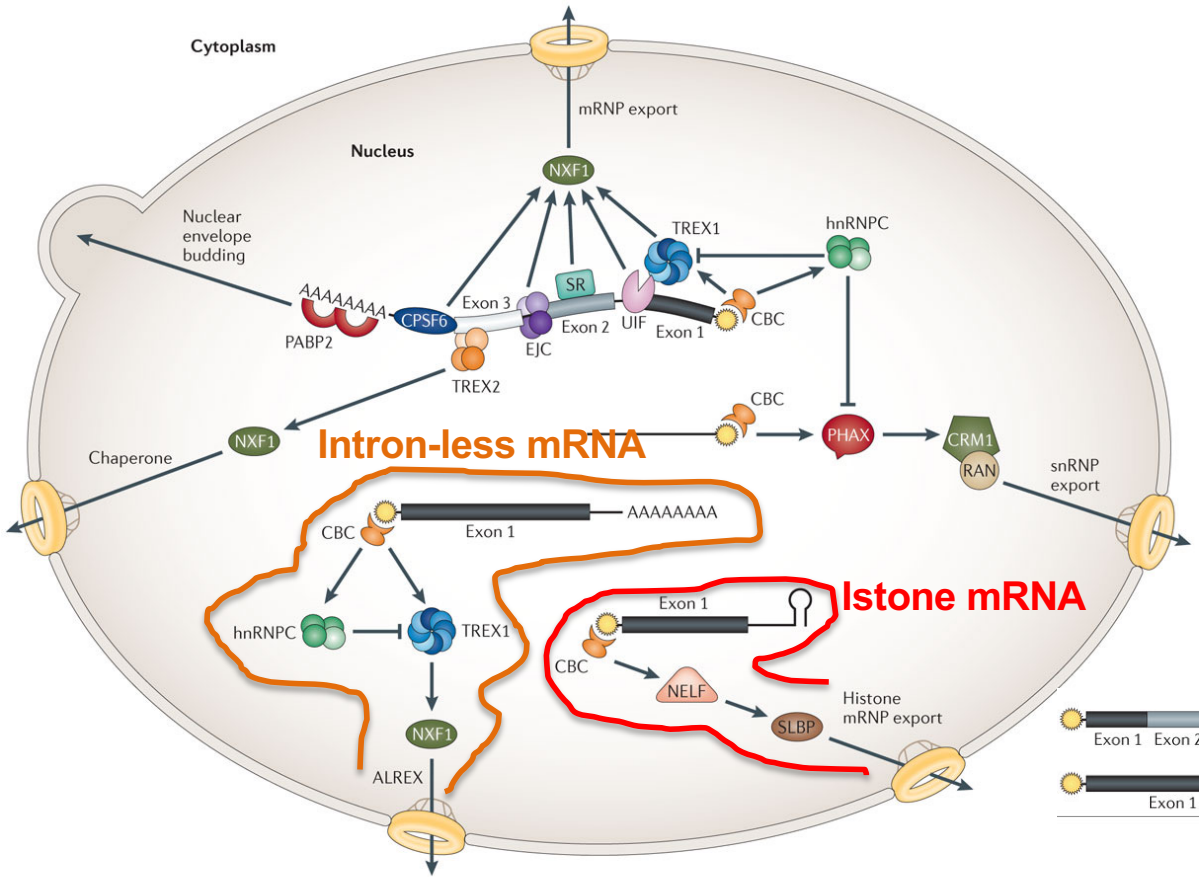

a Yeast
Transcription
Pol II
THO
TREX
Sub2
Yra1
Cap
Recruitment and export
THO
Sub2
Mex67
Mtr2
Npl3
AAAA
CBC
Yra1
Mex67
Mtr2
AAAA
Nucleus
Cytoplasm
Translocation and termination
Npl3
Yra1
Gle1
Dbp5
InsP6
Mex67
Mtr2
CBC
Release and re-import
Mex67
Mtr2
Yra1
Translation initiation
eIF4A
Npl3
AAAA
b Metazoa
Transcription
Pol II
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
ALY
CBC
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

Alternative mRNA export (AREX)

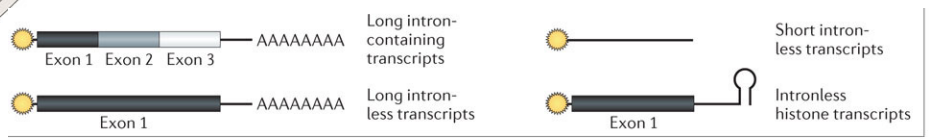
TREX also participates in the nuclear export of several **intronless transcripts** independently of splicing.

Efficient export requires the presence of GC-rich export-promoting sequences at the 5′ end of these transcripts. Recruitment of the TREX complex and **UAP56** to the 5′ end of intronless mRNAs (e.g. heat-shock protein 70) occurs through interaction with the cap-binding complex (CBC).

This alternative mRNA export pathway, which is termed **alternative RNA export (ALREX)**, requires NXF1 (TAP) and the CBC.



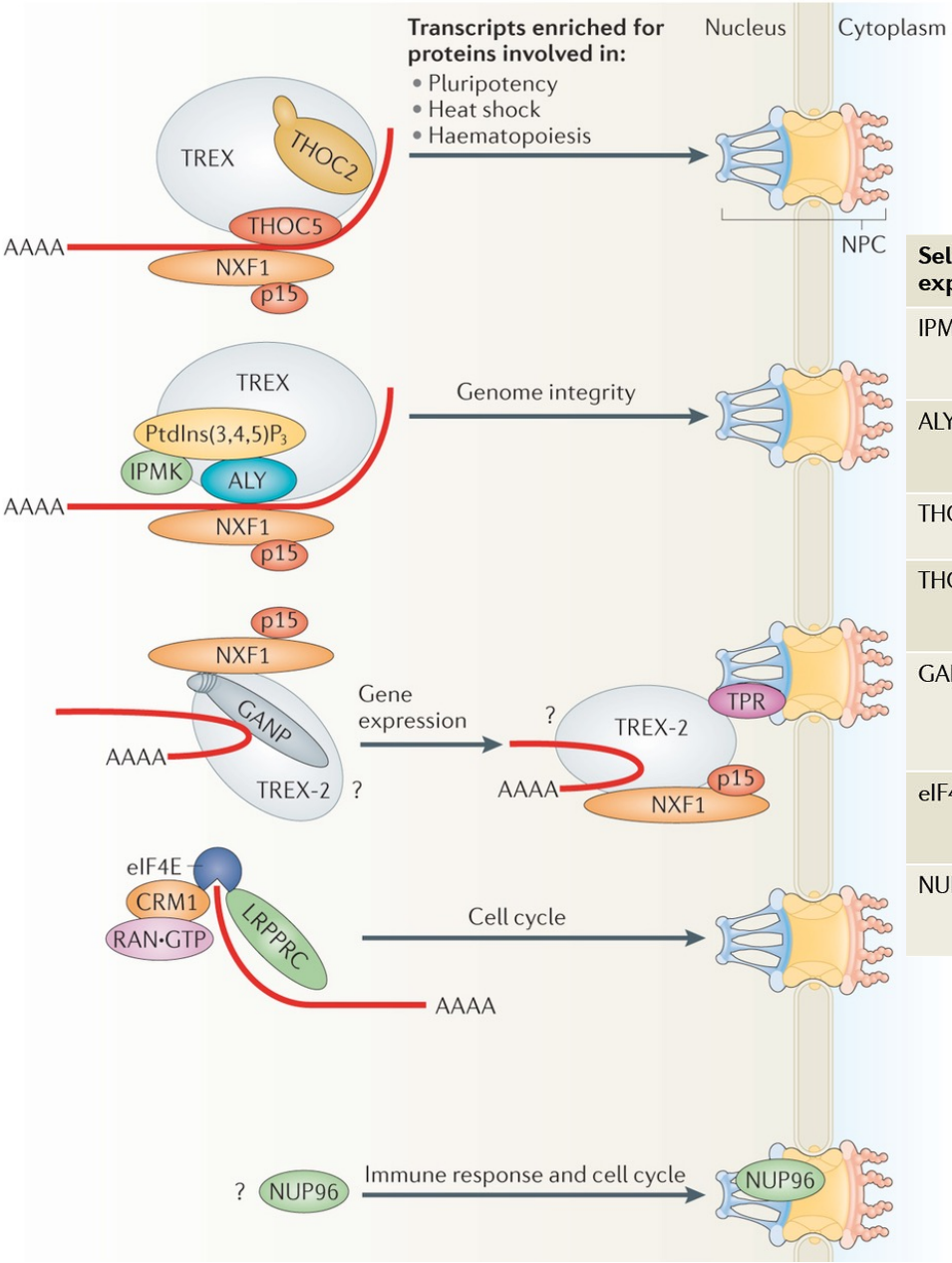
By contrast, **histone mRNAs**, which also lack introns, are exported to the cytoplasm by the **stem-loop-binding protein (SLBP)** which is recruited to the 3′ end of histone transcripts through interaction with the **CBC** and **negative elongation factor (NELF)**



Nature Reviews | Genetics

Another complex, which recently was shown to connect transcription with export of the matured mRNA is TREX-2, composed of Sac3, Thp1, Sem1, Sus1 and Cdc31. TREX-2 together with the SAGA complex are involved in docking transcribing genes to the NPC in a process called“gene gating” that allows preferential export of these mRNAs.

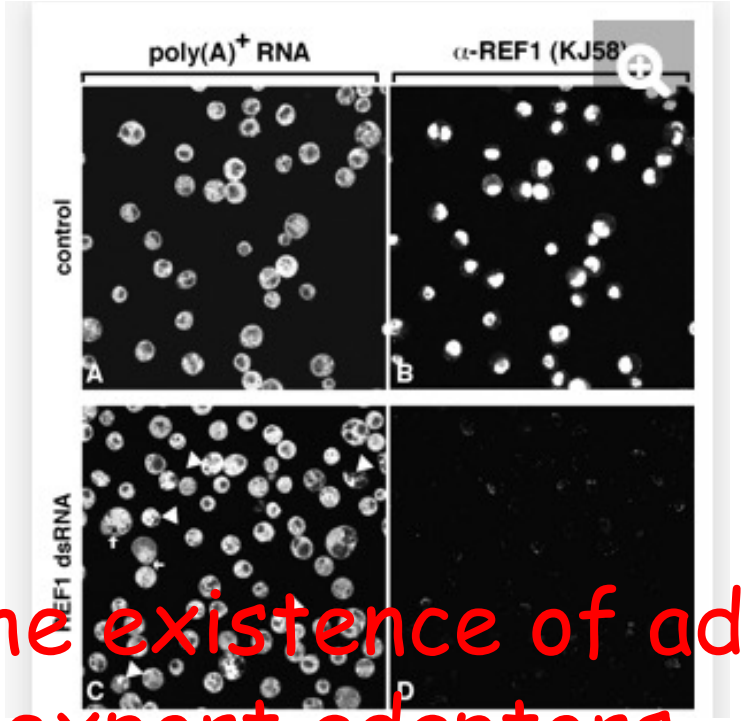
Control of mammalian gene expression by selective mRNA export



Selective mRNA export factor	Component of TREX or TREX-2	Function	Biological pathway (transcript examples)
IPMK	Neither	Kinase involved in synthesis of inositol phosphates and PtdIns(3,4,5)P_3	Genome duplication and repair; cell cycle (*RAD51*, *CHEK1* and *FANCD2*)
ALY	TREX	RNA-binding adaptor of TREX	Genome duplication and repair; cell cycle; heat shock (*RAD51*, *CHEK1*, *FANCD2* and HSP70)
THOC2	TREX	Core subunit of THO and TREX	Maintenance of pluripotency (*NANOG*, *SOX2* and *KLF4*)
THOC5	TREX	RNA-binding metazoa-specific subunit of TREX	Maintenance of pluripotency; haematopoiesis; heat shock (*NANOG*, *SOX2*, *KLF4* and HSP70)
GANP	TREX-2	Scaffold protein for TREX-2; exports a subset of mRNA, the transport of which depends on NXF1	Gene expression; RNA processing (*SP1*, *RPS23* and *ARPP19*)
eIF4E	Neither	Binds to m^7G cap of mRNA; export is CRM1-dependent and NXF1-independent	Cell proliferation and cell survival (cyclin D1 and *MYC*)
NUP96	Neither	Component of nuclear pore complex	Immune response; cell cycle (MHC I, MHC II, β2 microglobulin and *CDK6*)

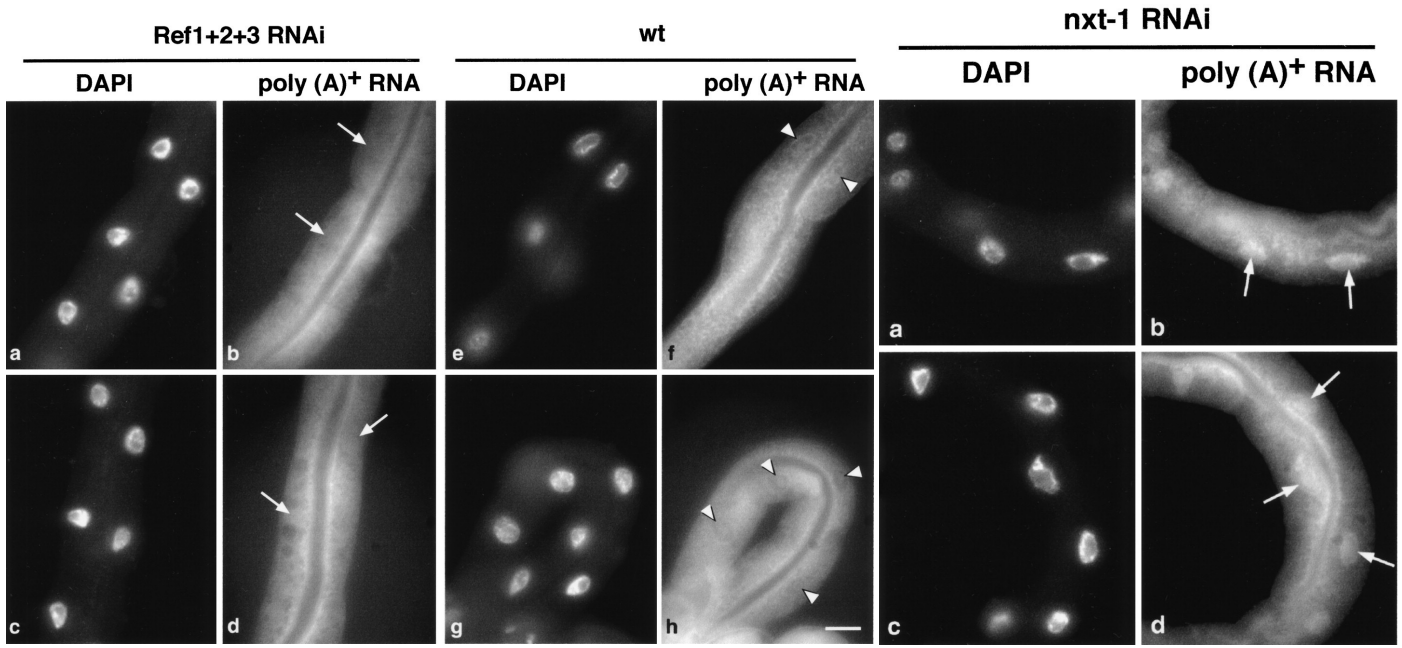
In metazoans,Aly/REF, in contrast to Yra1, which is essential for mRNA export in yeast, is required but not essential for bulk cellular mRNA export

REF1/Aly is dispensable for mRNA export in Drosophila cells.



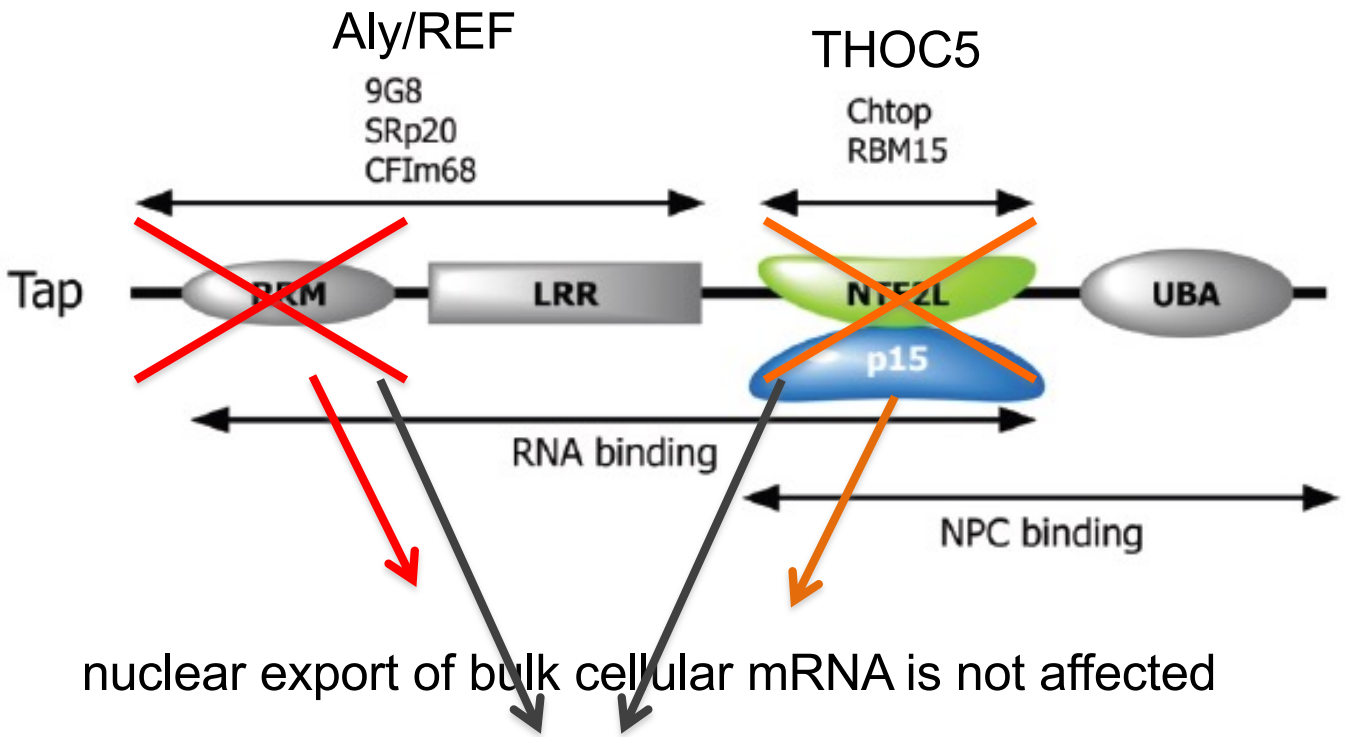
This suggests the existence of additional mRNA export adaptors

simultaneous suppression of all three C. elegans Ref genes did not result in accumulation of poly(A)+ mRNA in the nucleus..



Contribution of TREX to selective mRNA export

THOC5, a metazoan-specific RNA-binding component of the TREX complex, interacts as Aly/REF factor, with TAP.

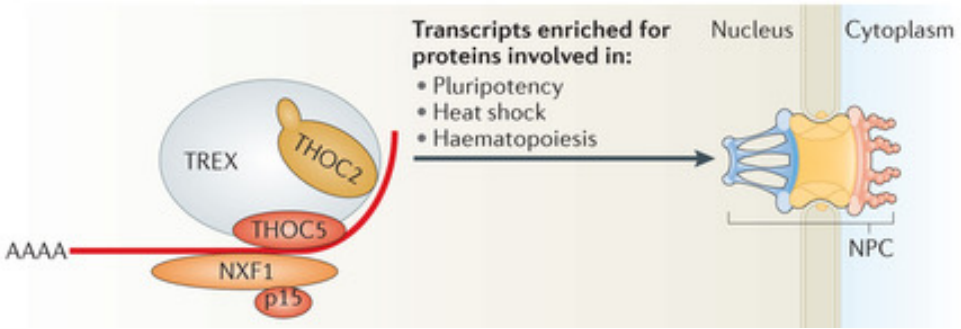


nuclear export of bulk cellular mRNA is not affected

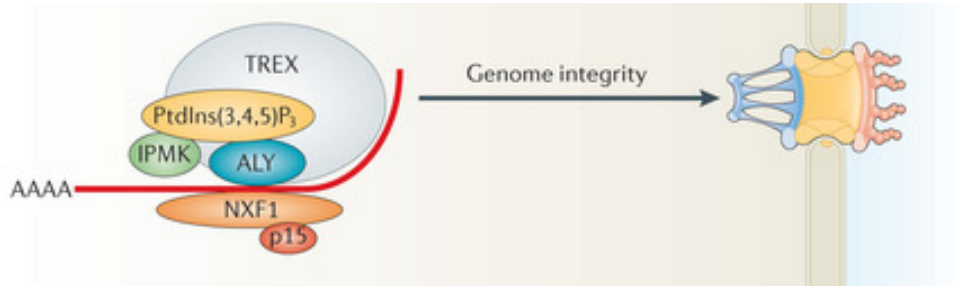
severely block the mRNA binding and export activities of Tap

simultaneous knock down of Aly/REF and THOC5 block the nuclear export of mRNA

Taken together, these data suggest that, at least in mammalian cells, the TREX component Thoc5 could be the factor that cooperates with Aly/REF.
Recent studies indicated that the nuclear export of only a subset of genes is impeded in mammalian cells under THOC5 depleted condition



THOC5 preferentially regulates the nuclear export of mRNAs encoding factors that are crucial for haematopoietic development.
Together with THOC2 can regulate the balance between stem cell specification and differentiation by regulating the nuclear export of NANOG and SOX2 mRNAs. Indeed, expression of THOC2 and THOC5 correlates with the pluripotent state of embryonic stem (ES) cells,
Together with ALY also promote the export of mRNA that encodes heat shock 70 kDa proteins (HSP70s), which are crucial factors in the response to heat stress



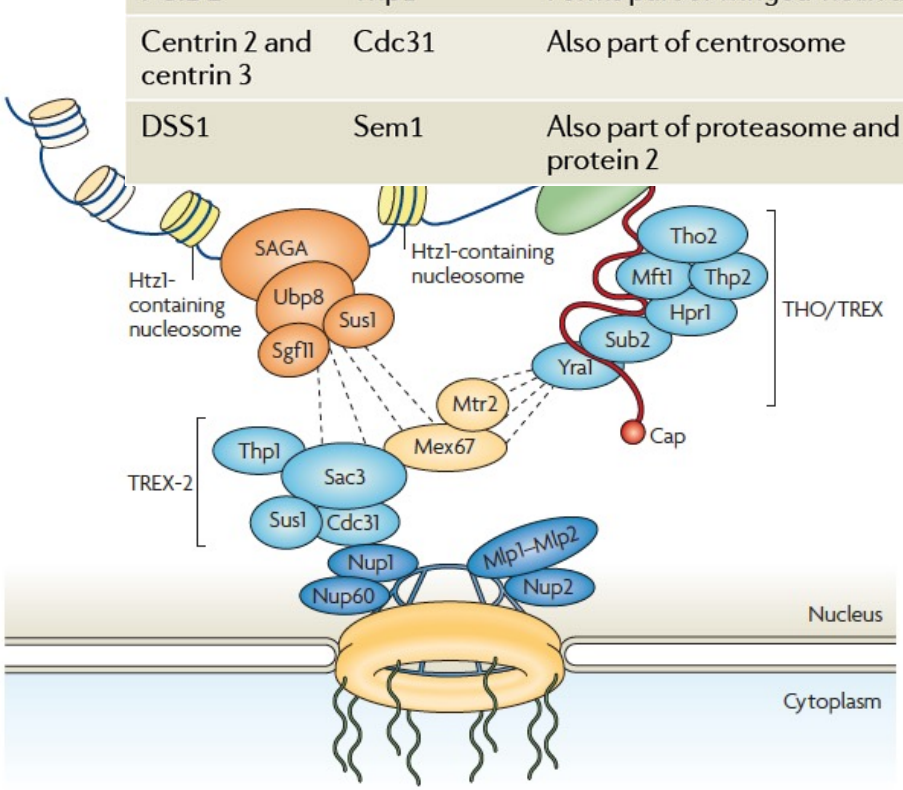
IPMK and ALY preserve genome integrity by controlling the nuclear export of transcripts encoding proteins that are essential for accurate genome duplication and repair

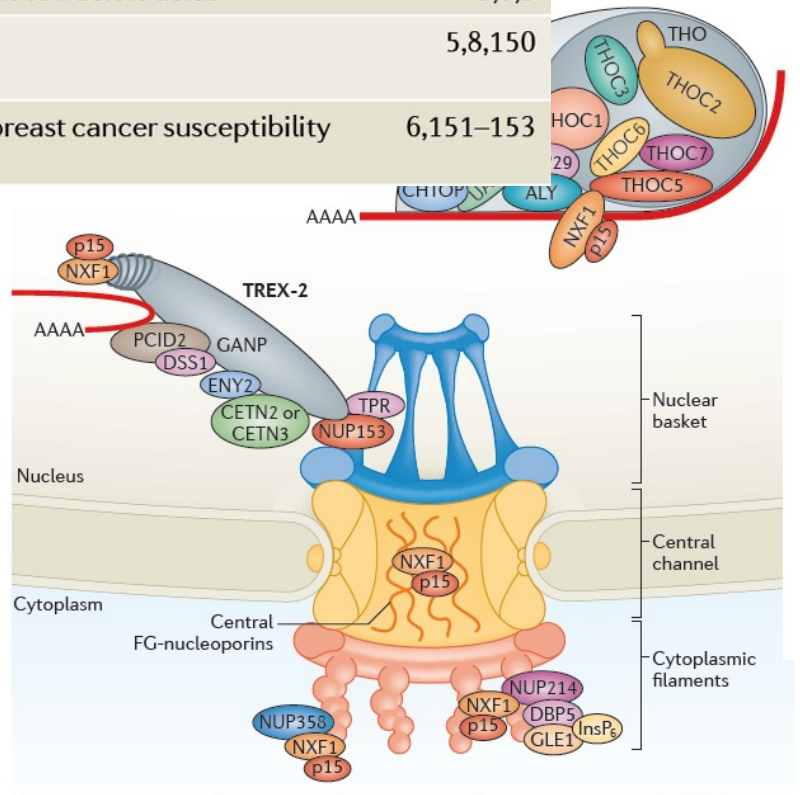
Contribution of TREX-2 to mRNA export selectivity

GANP promotes the nuclear export of classes of mRNA that are involved in gene expression, such as those involved in mRNA processing and splicing, and mRNP and ribosome biogenesis.

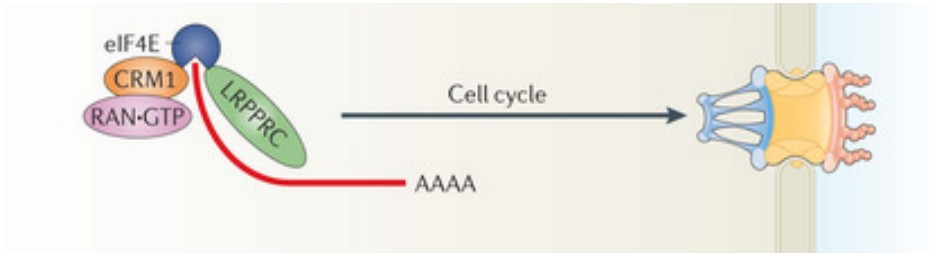
One possibility is that GANP (and hence TREX-2) mediates a priority fast-track export route for transcripts that control cell behaviour, presumably facilitating rapid adaptation to changing cellular environments.

TREX-2			
GANP	Sac3	Scaffold for TREX-2 complex; has domain with homology to nucleoporins	5,7,9,12,14
ENY2	Sus1	Also subunit of SAGA transcription activation complex	5,7,102
PCID2	Thp1	Forms part of winged-helix domain that binds to nucleic acids	5,6,9
Centrin 2 and centrin 3	Cdc31	Also part of centrosome	5,8,150
DSS1	Sem1	Also part of proteasome and interacts with breast cancer susceptibility protein 2	6,151–153





Crm1 dependent mRNA Export



•The general RanGTP-dependent protein export receptor **CRM1** can be involved in the nuclear export of a subset of transcripts, such as mRNAs of several protooncogenes and cytokines, that contain AU-rich elements.

•CRM1 itself does not bind to RNA, instead recruiting NES-containing adaptor proteins that bind directly to RNA or to other RBPs. For example, AU-rich elements are recognized by RBP Hu-antigen R (HuR; also known as ELAVL1) and its protein ligands, which interact with CRM1

•CRM1 acts in the nuclear export of a number of unspliced and partially spliced viral mRNAs. These viral mRNAs can bind adaptor proteins that contain NESs (for example, HIV Rev, adenovirus E1b 55 kDa), thereby targeting the transport receptor CRM1.

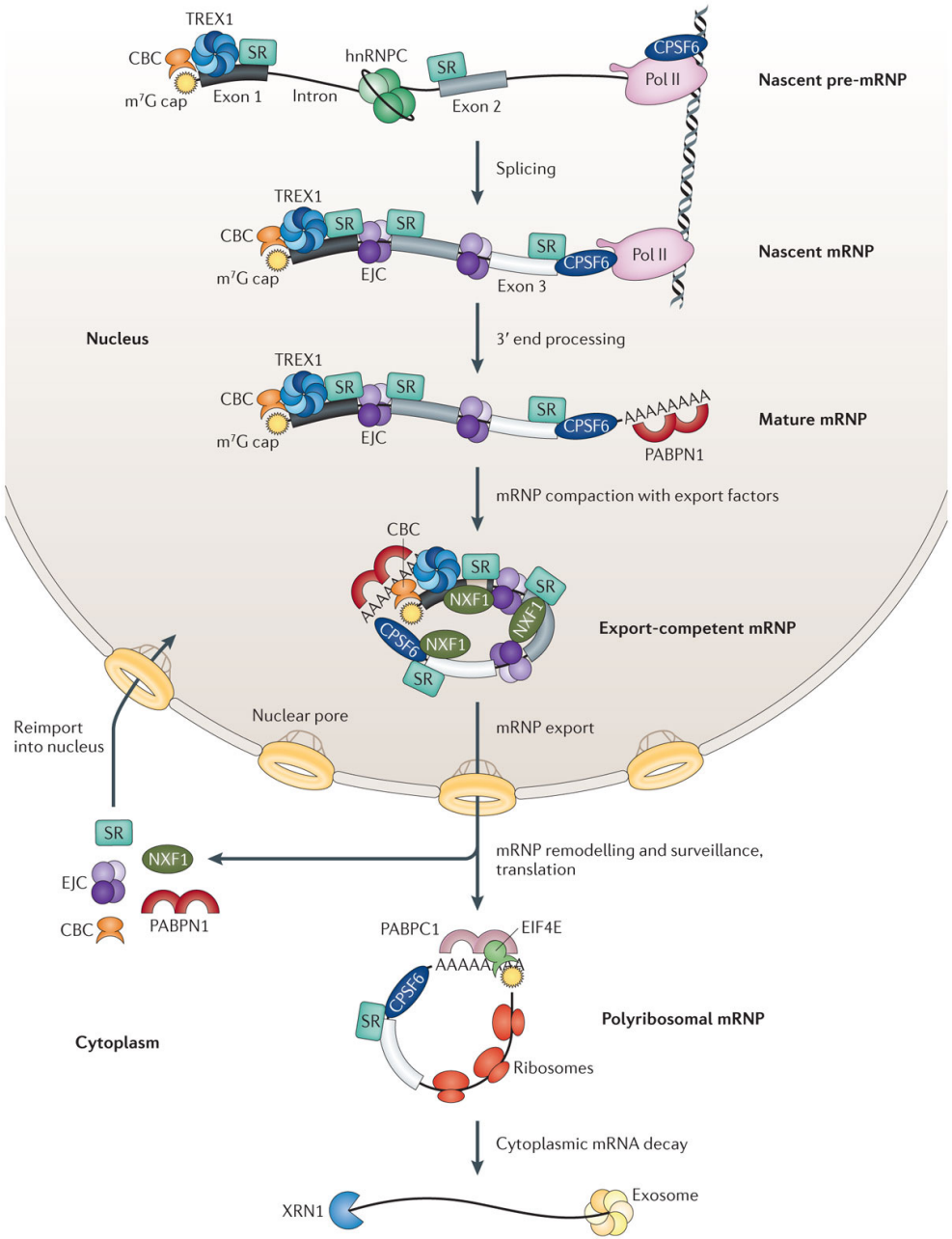
Collectively, these recent findings indicate that specific mRNA export factors can modulate diverse processes such as DNA repair, haematopoiesis and maintenance of pluripotency, thus demonstrating the potential impact that selective mRNA export can have on biological function.

The finding that TREX components ALY and THOC5 selectively export transcripts encoding proteins that mediate such essential emergency responses as DNA repair by homologous recombination and the heat shock response emphasizes the importance of selective mRNA export for cell survival.

Whereas THOC2 and THOC5 expression in ES cells are important for the maintenance of pluripotency16, ALY and UAP56 are not required.

This raises the possibility that cell type-specific TREX complexes may exist and regulate selectivity

Messenger RNA (mRNA) is never alone

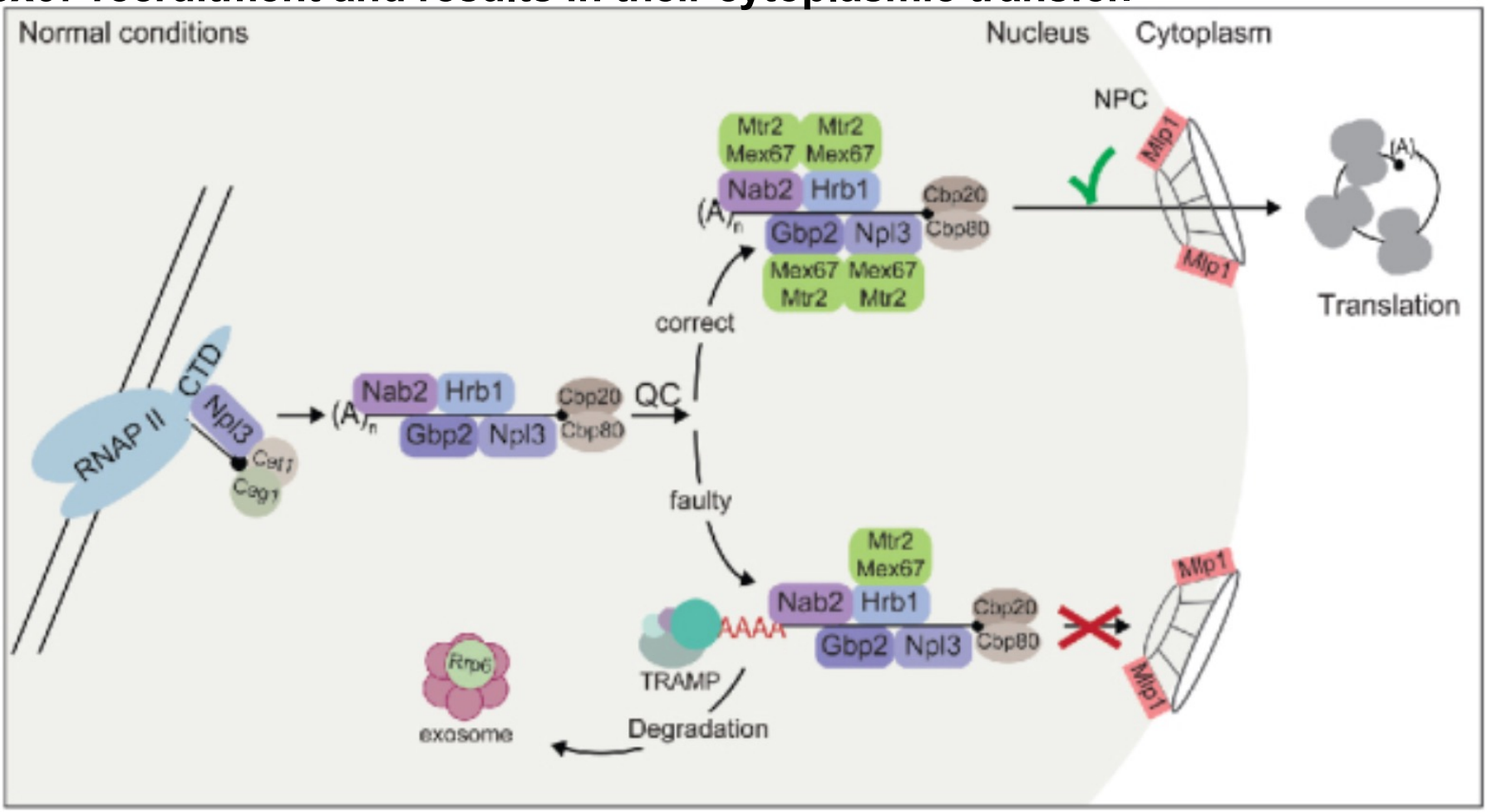


mRNA is coated and compacted by **RNA-binding proteins (RBPs)**, forming large messenger ribonucleoprotein particles (mRNPs). RBPs assemble on nascent and mature mRNAs. They serve as structural elements for mRNP packaging and modify the output of gene expression at all steps of the mRNA life cycle: transcription, splicing, 3′ end processing, capping, **nuclear export**, localization, translation and mRNA stability.

mRNA EXPORT: YEAST mRNA quality control and export checkpoint

Maturing mRNAs bind the guard proteins to prevent Mex67 association until the transcripts are fully processed. Completed maturation is signaled by Mex67 recruitment and results in their cytoplasmic transfer.

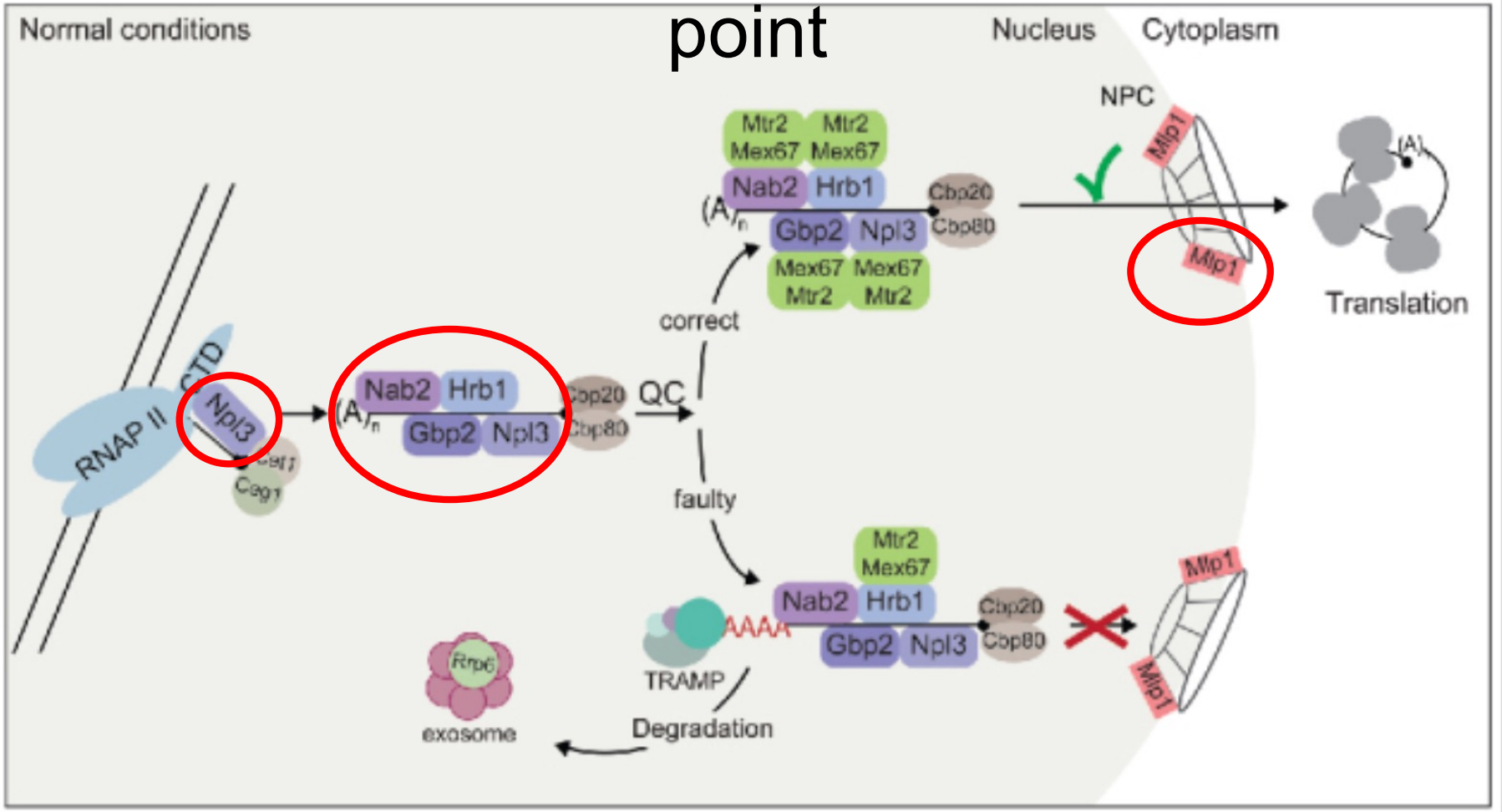
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/



mRNA quality control in the nucleus: **Guard proteins** control mRNA maturation and nuclear export. Every maturation step results in association of guard proteins that recruit the export receptor Mex67-Mtr2 to the correct mRNA and support degradation by the TRAMP/exosome pathway for faulty transcripts. Finally, Mlp1 controls proper Mex67 decoration of the guard proteins to allow nuclear export.

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

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

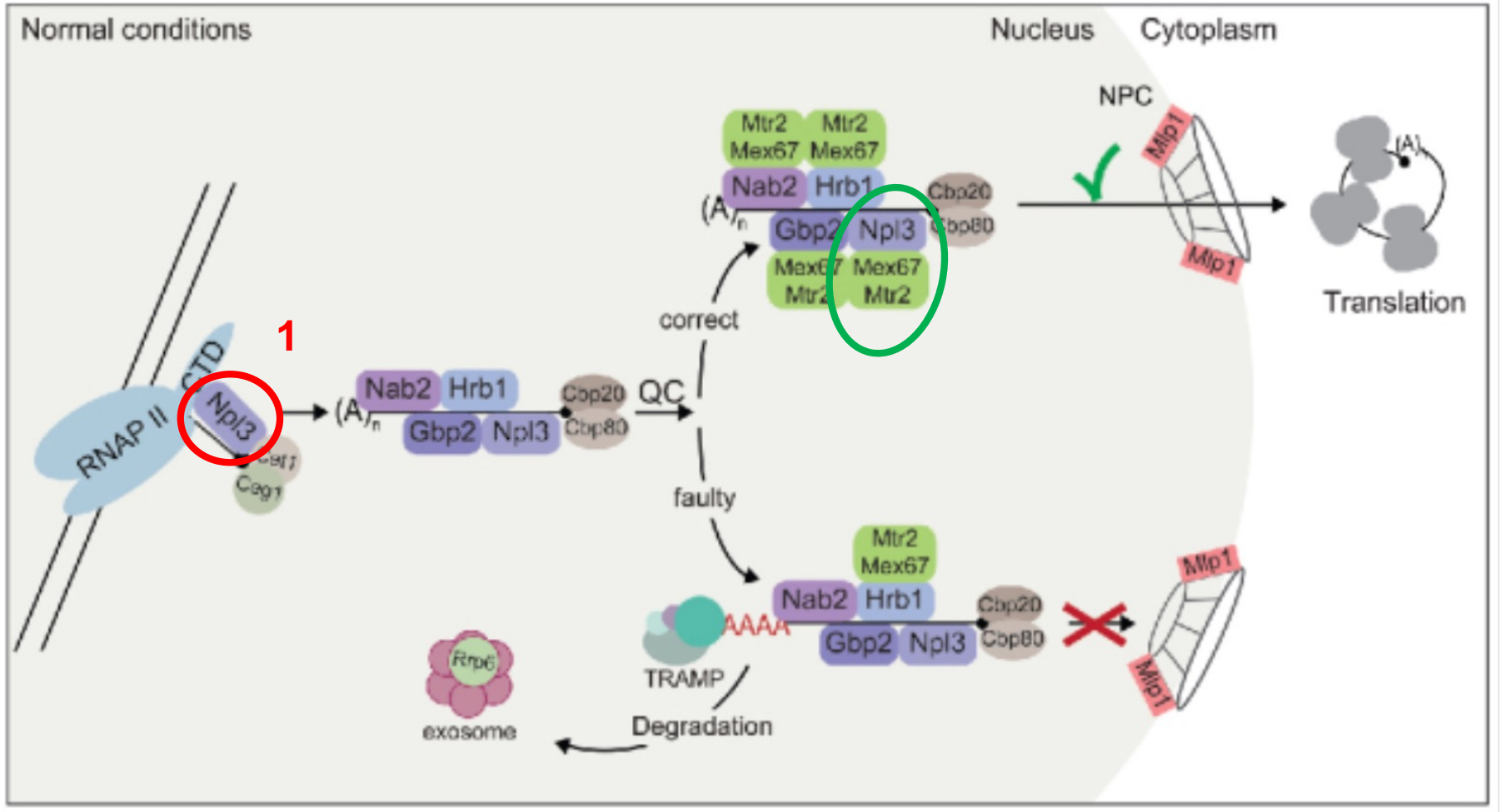


Serine/arginine (SR)-rich proteins Npl3, Gbp2 and Hrb1 and the poly(A)-binding protein Nab2 -> **guard proteins, they shuttle with mRNAs.**

Mlp1: last step in export chek-point

mRNA EXPORT: YEAST

The **Npl3** binds the nascent RNA and to the early spliceosome, being the first checkpoint

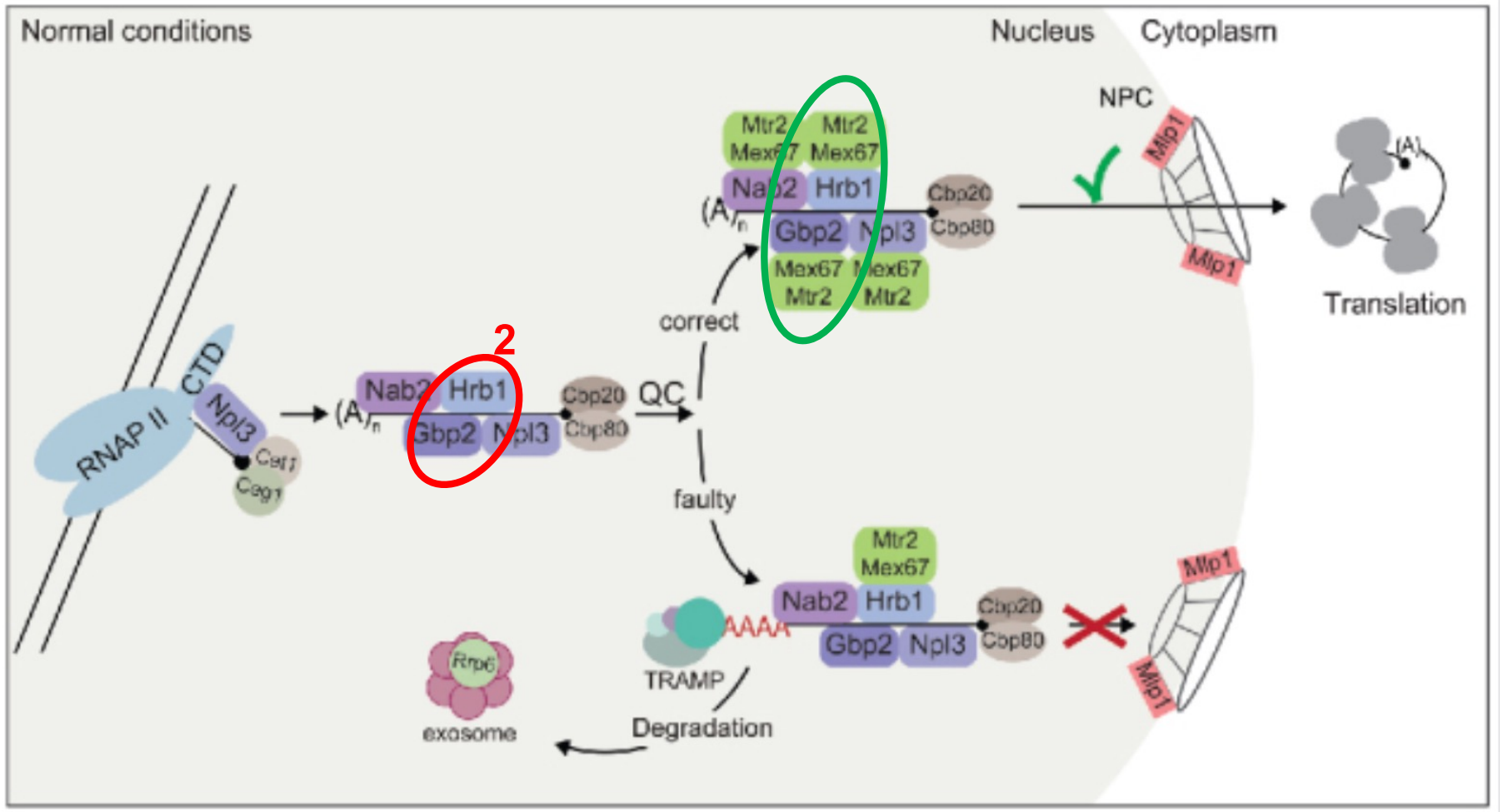


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/

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 quality control and export check-point

Hrb1 and **Gbp2** interacts with the later spliceosome and directly recruit **Mex67** after this second checkpoint,

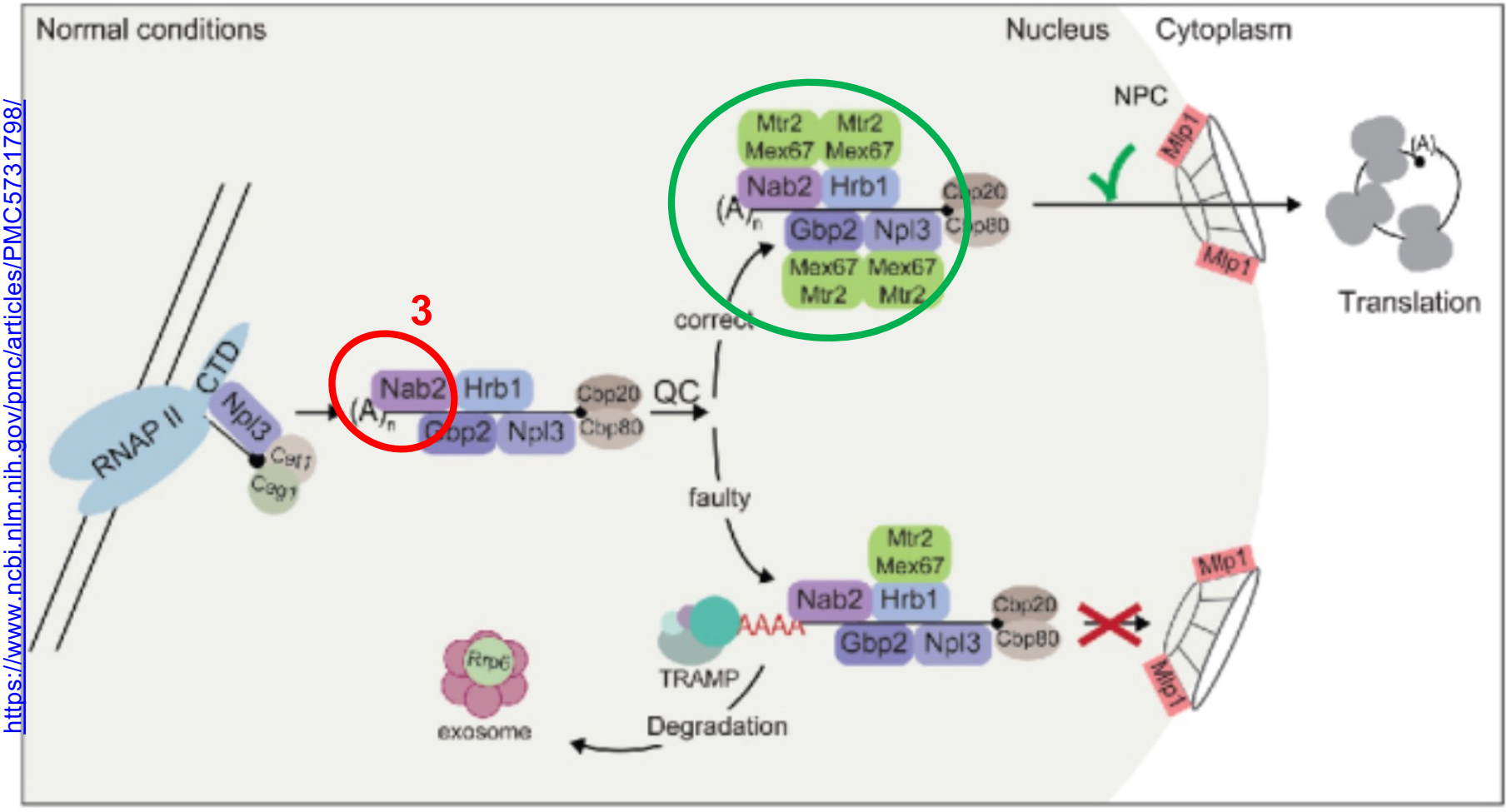


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/

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 quality control and export check-point

The last processing step that results in decoration of the mRNA with the guard proteins is the formation of the 3’ end and synthesis of the poly(A) tail.

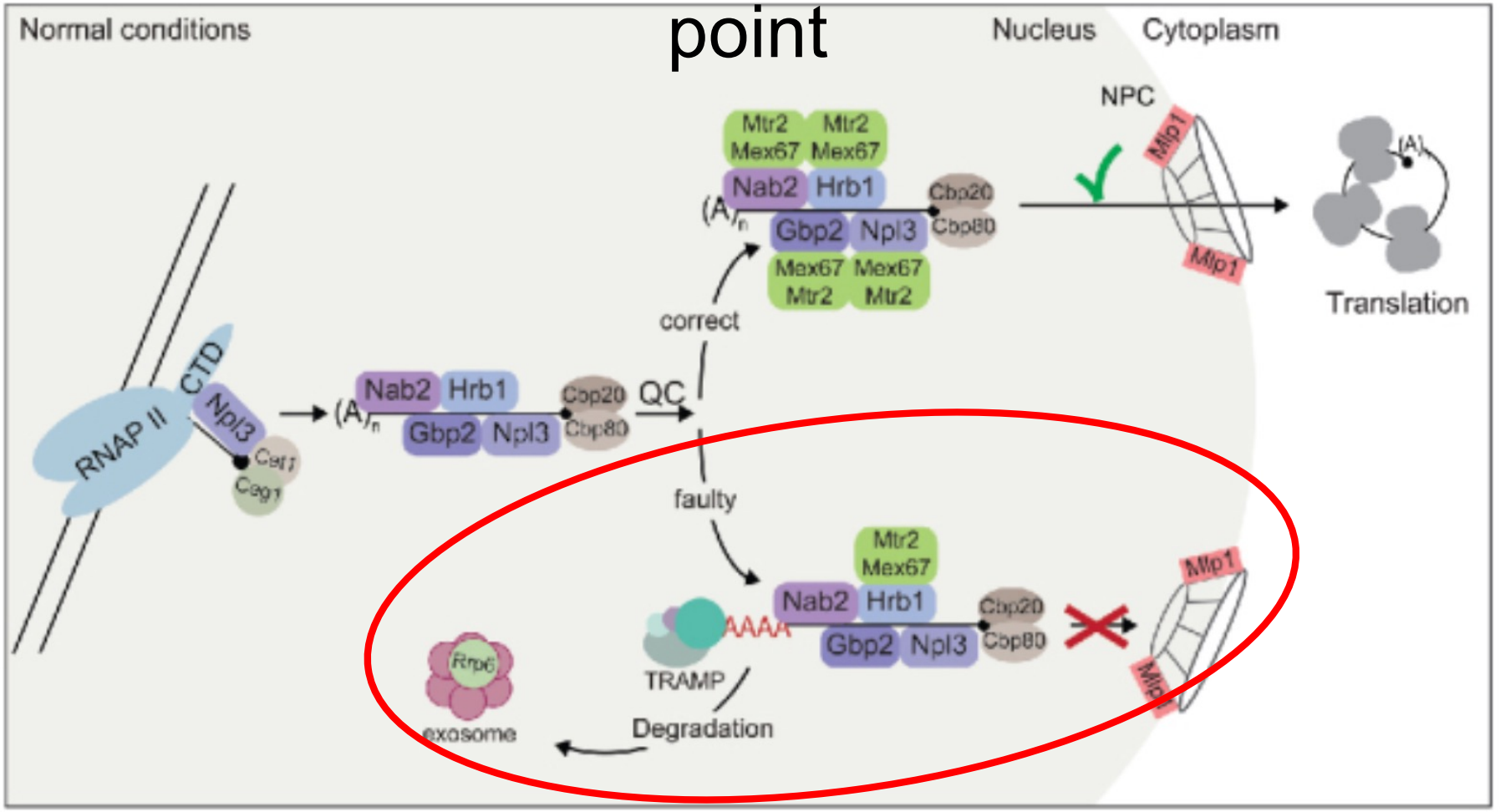


https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/

the poly(A)-binding protein **Nab2**, together with its mainly cytoplasmic homolog Pab1, controls length and quality of the 3’ tail.

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

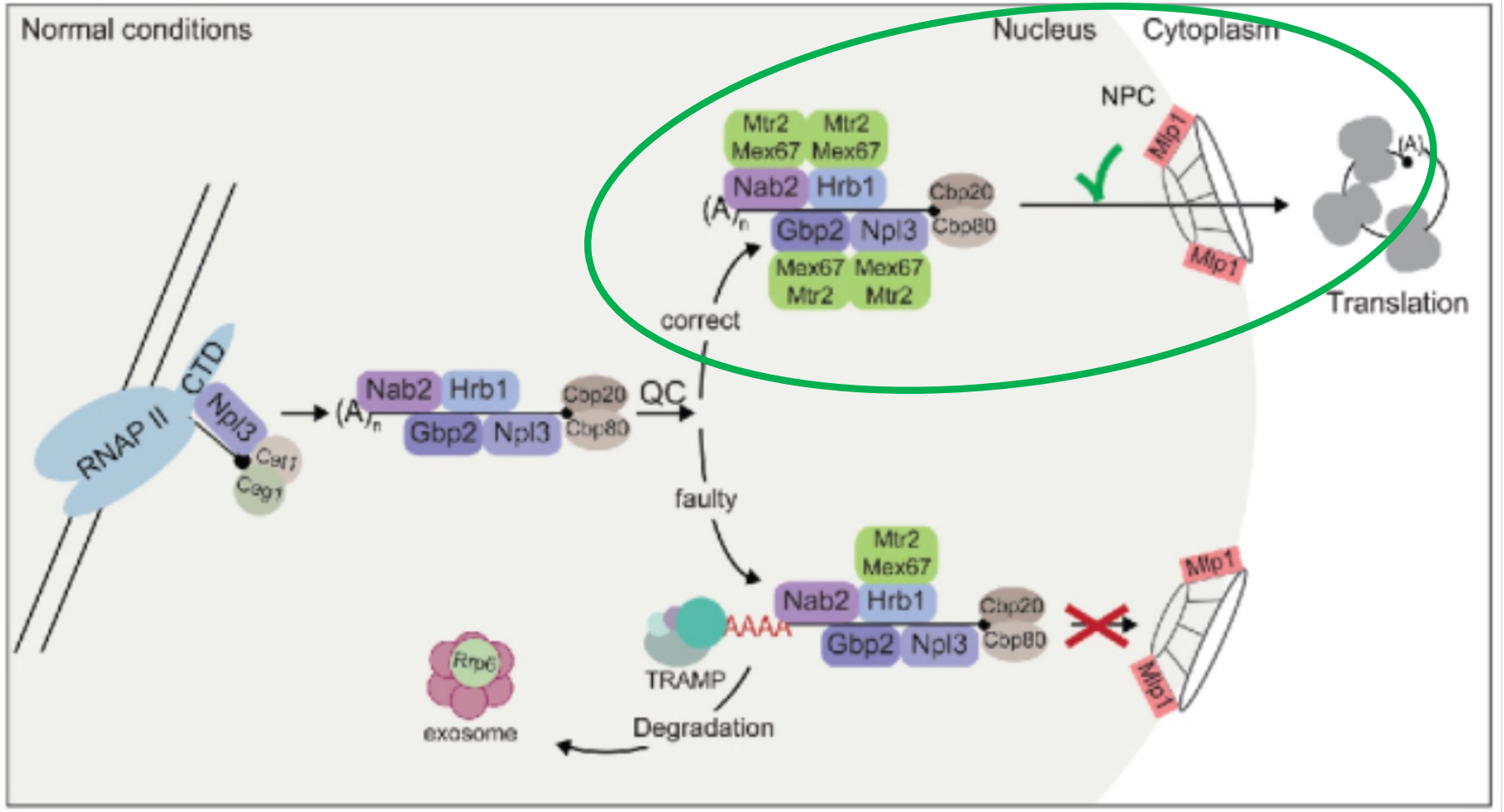
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731798/



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 containing Rrp6**

mRNA quality control and export check-point

At the nuclear basket, Mlp1 and the highly homologous nucleoporin Mlp2 are the last nuclear factors involved in retaining erroneous transcripts.



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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**.

This elaborated system ensures efficient translation of correct mRNAs and prevents the translation machinery to deal with an overwhelming number of suboptimal or even defective transcripts.

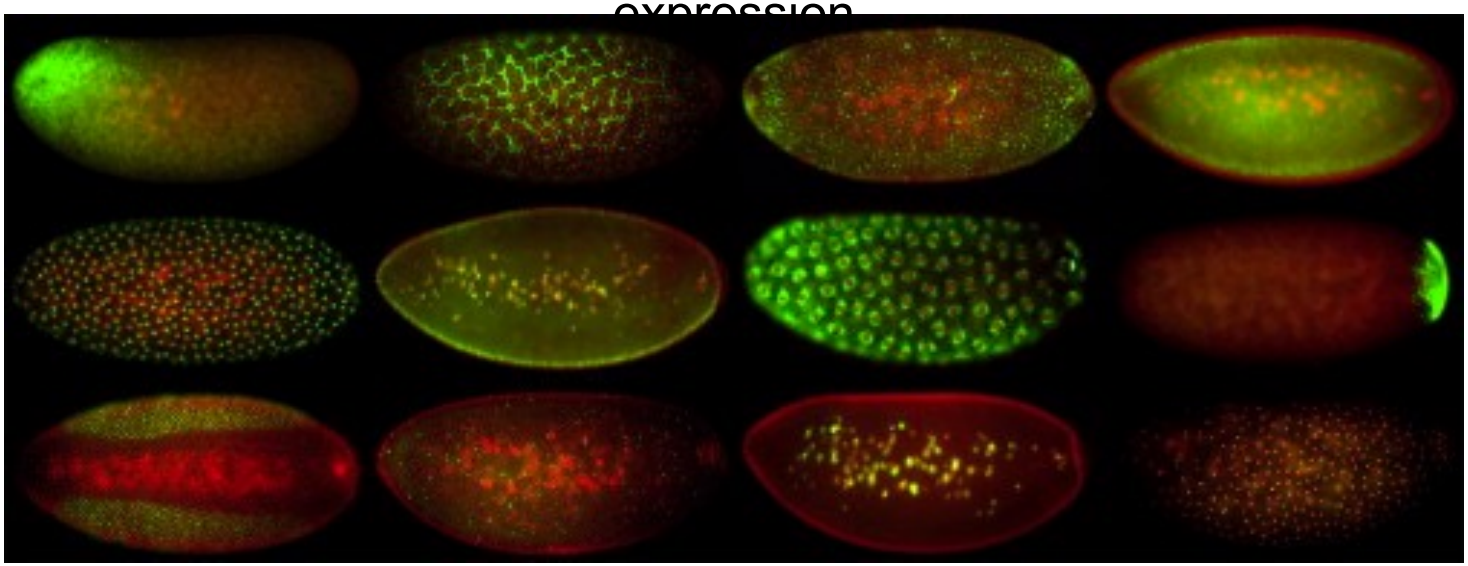
There is growing evidence that not only in yeast but also in mammals mRNA adaptor proteins act as guards and are tightly linked to correctness and thus quality of mRNA expression (https://doi.org/10.1101/gad.276477.115).

Cytoplasmic non- or wrongly processed mRNAs that are translated into missfunctional proteins threaten homeostasis and have in general a detrimental effect on cellular growth.
In the same way as a lack of quality control is harmful to the cell, an overexpression of the guard proteins has the same adverse effect on cellular fitness in yeast, as it possibly even leads to a retention of correct mRNAs.

Defects or overexpression of human homologues of thecguard proteins (ZC3H14, as a human Nab2 ortholog), or thecSR-proteins SRSF1, SRSF3 and SRSF7, which also accompanycthe mRNAs to the cytoplasm and interact with TAP-p15, are known to cause several diseases, including cancer and neurodegenerative diseases, cardiovascular diseases or conditions like neuronal dysfunction

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

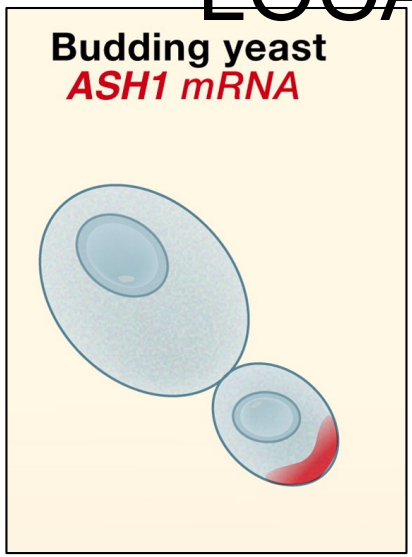
ADVANTAGES OF REGULATING GENE EXPRESSION BY mRNA LOCALIZATION

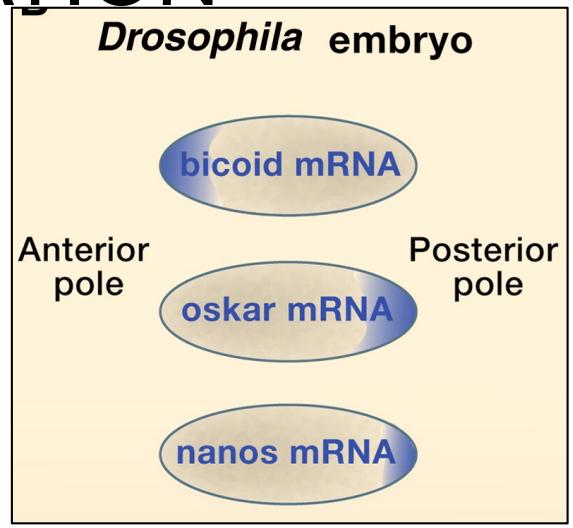
1. **High temporal resolution**: fast response to stimuli, by regulating translation of on-site mRNAs

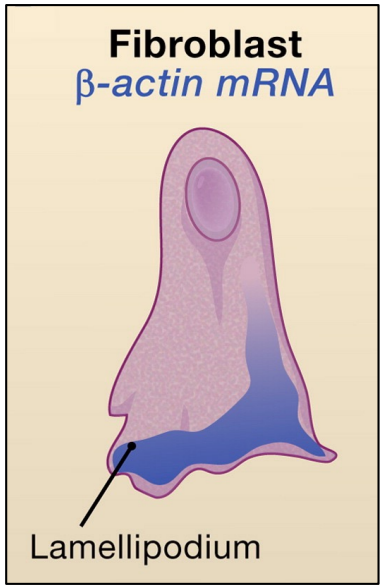
2. Localized mRNA translation leads to **protein accumulation** in a specific cytoplasmic district -> more efficient than having translating mRNAs elsewhere and then transporting proteins to a distinct site

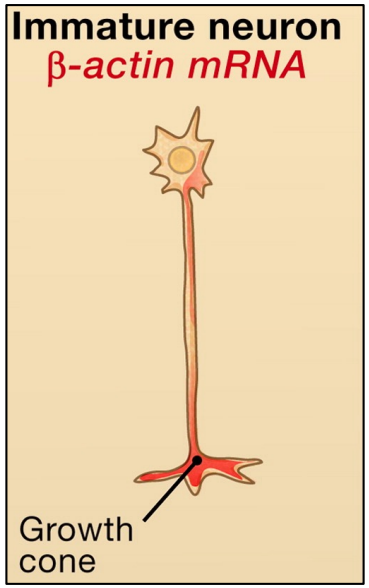
3. Local translation of proteins **protects** the cell from proteins that might be toxic in other cell compartments

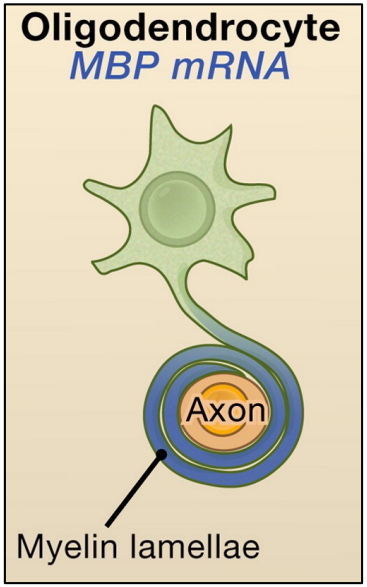
mRNA SUBCELLULAR LOCALIZATION





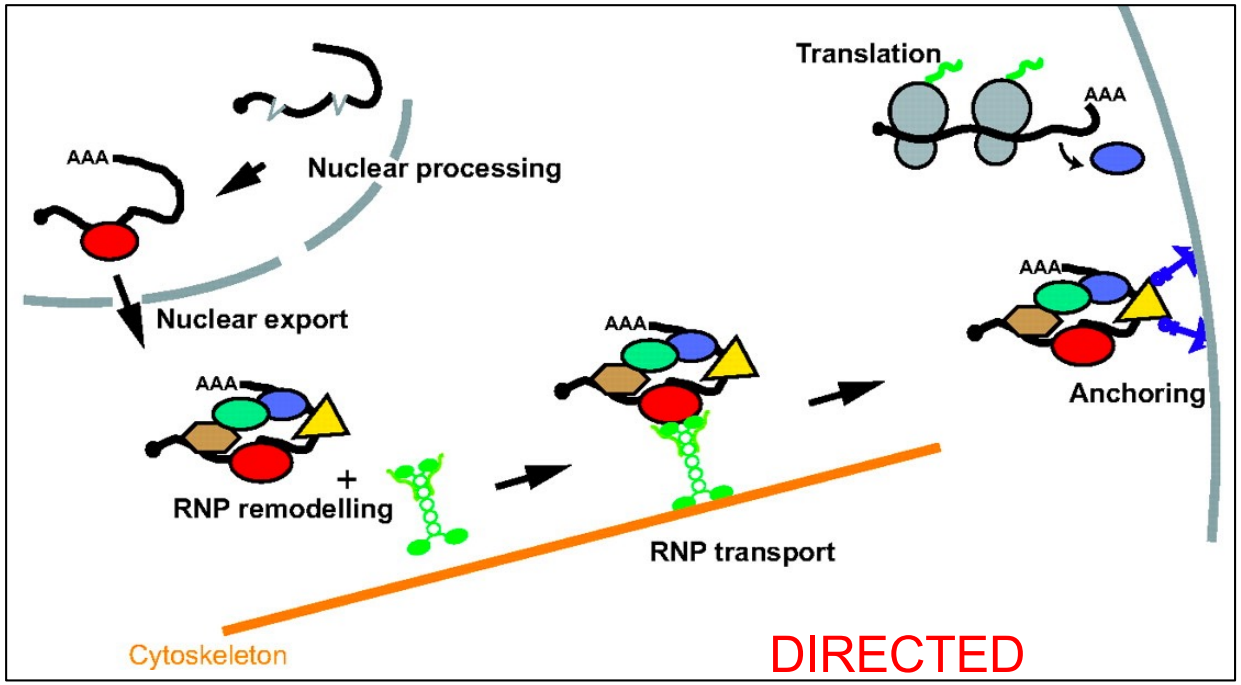






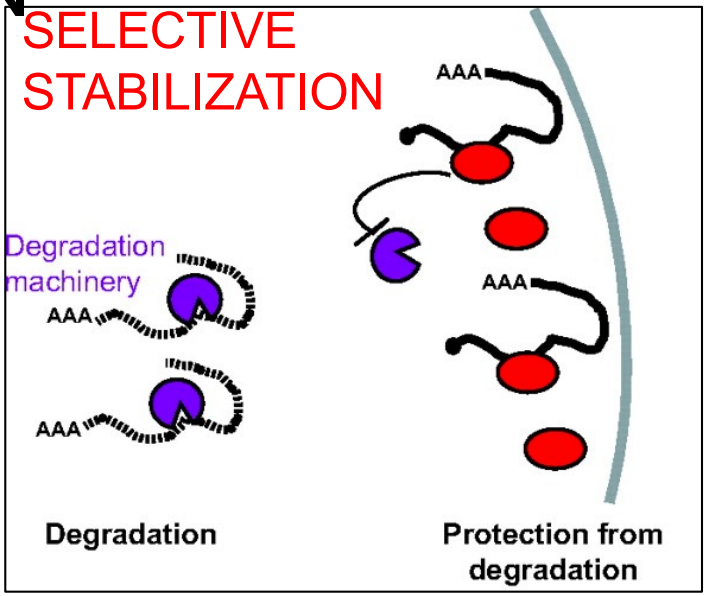
https://www.cell.com/fulltext/S0092-8674(09)00126-

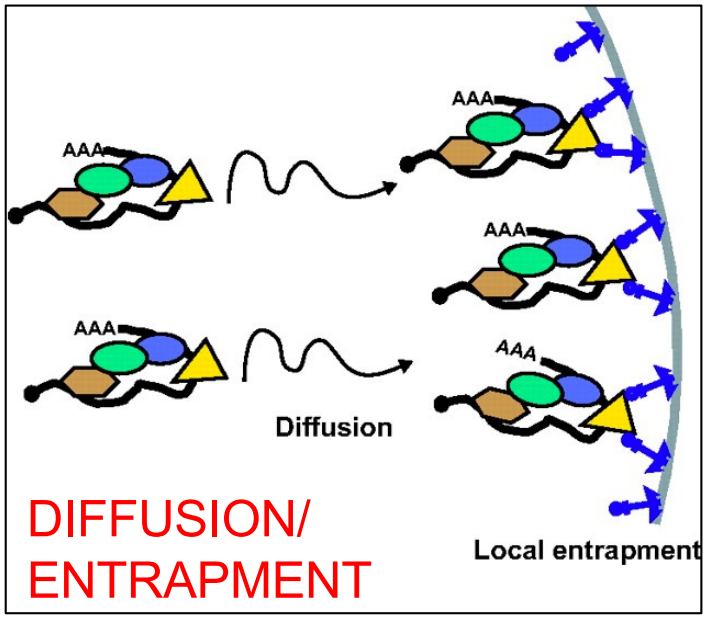
MECHANISMS OF mRNA LOCALIZATION



DIRECTED TRANSPORT

SELECTIVE STABILIZATION





DIFFUSION/ ENTRAPMENT

LOCALIZATION ELEMENTS OR «ZIPCODES»

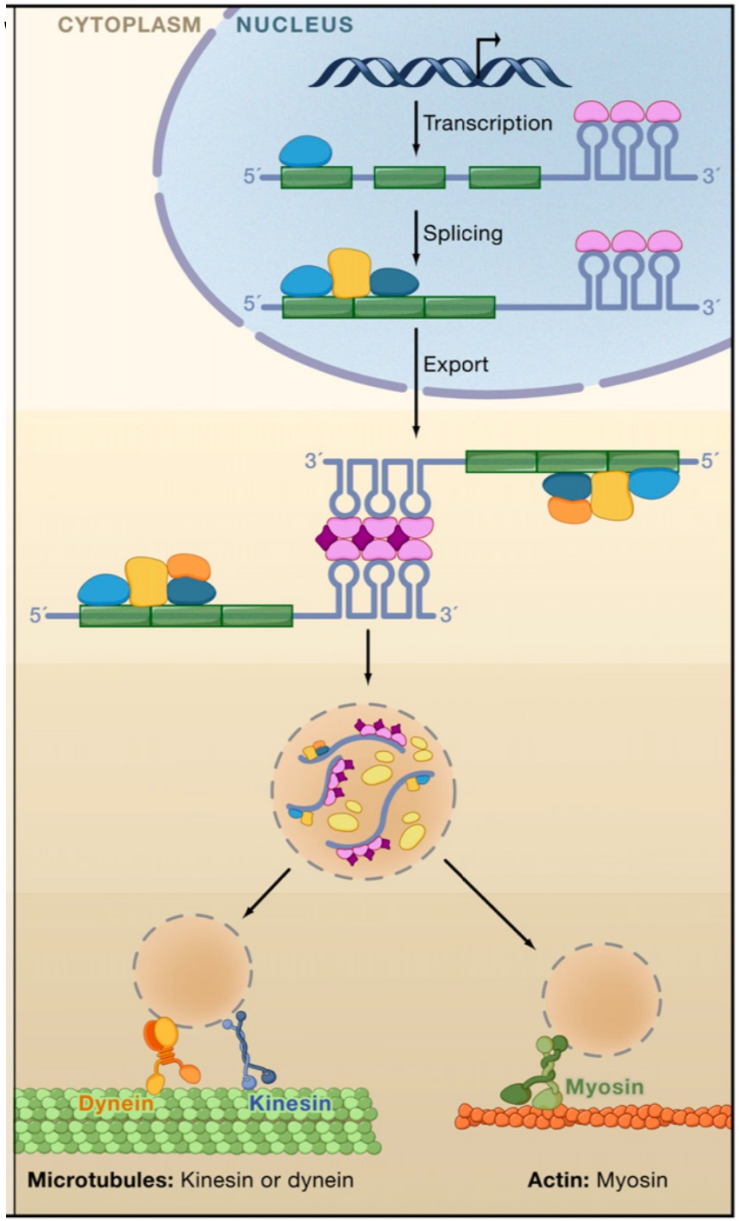
1. **<u>Cis-acting</u> elements** in the mRNA (sequence forming secondary structures, usually stemp-loops)

2. Usually found in the 3'UTR sequence

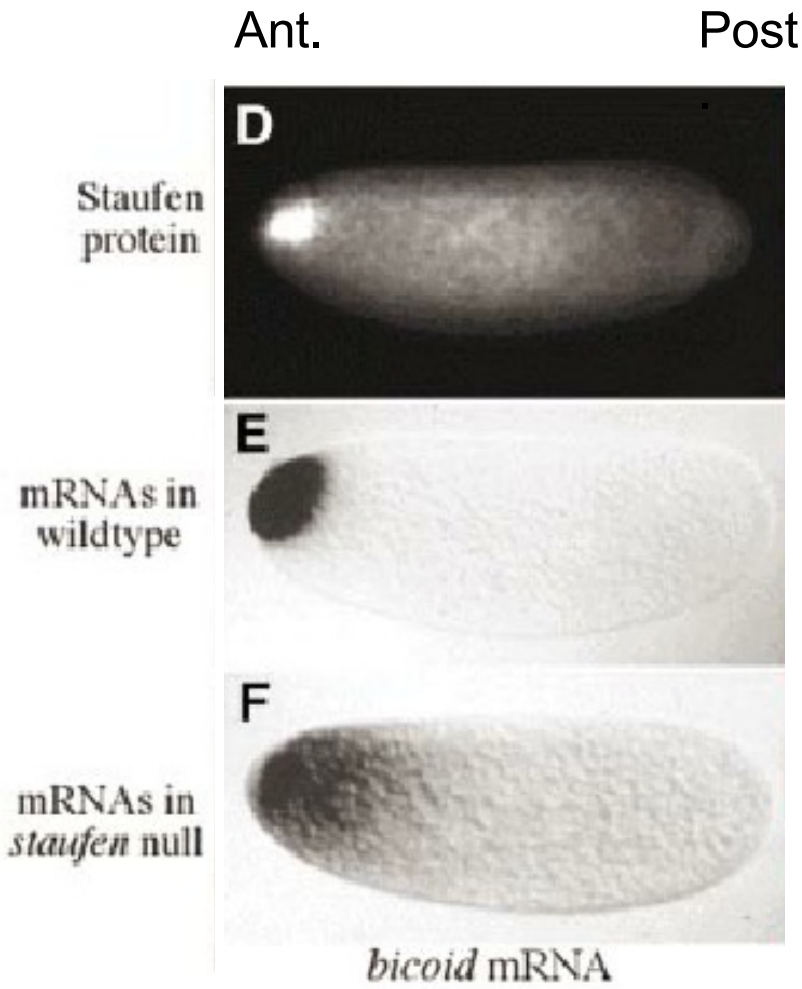
3. Variable length: from 6 nt up to hundreds of nt

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

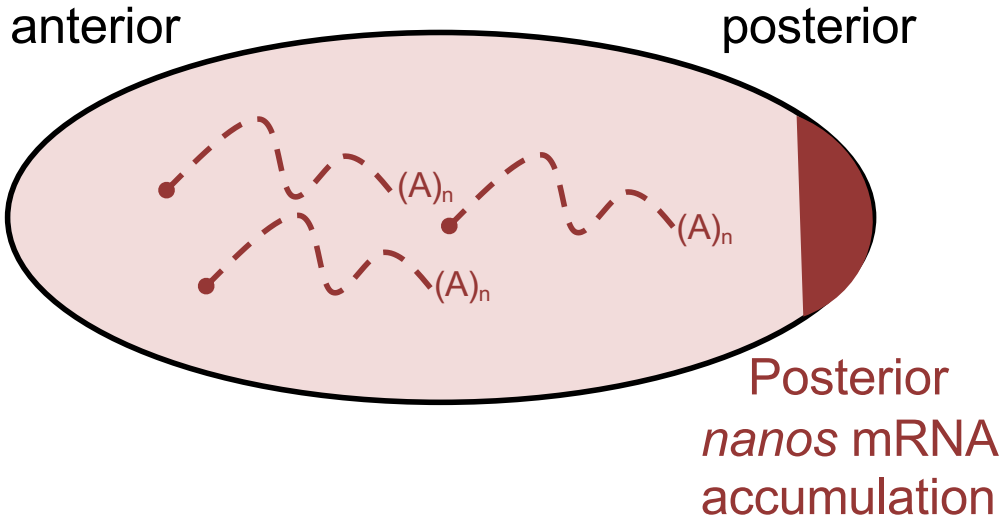


1. Cis-acting elements: 625nt long region in the *bicoid* mRNA containing several localization elements (BLE). BLE1: stem-loop essential for anterior localization. *bicoid* mRNA dimerizes through stem-loop structure

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



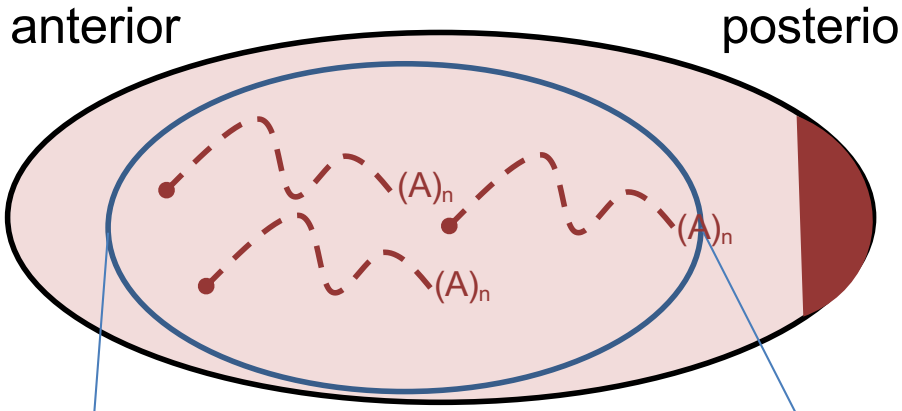
1. Nanos is required for posterior specification of Drosophila embryo

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

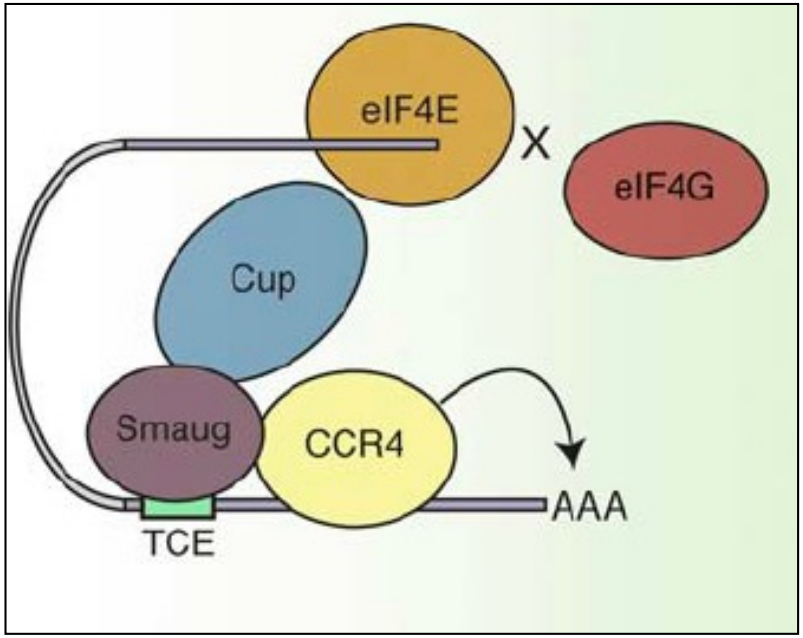
3. Only 4% of *nanos* mRNA is localized at the posterior pole, but it is stable, while the *Nanos* mRNA elsewhere in the embryo is degraded thanks to Smaug protein

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

SELECTED STABILIZATION OF *nanos* mRNA

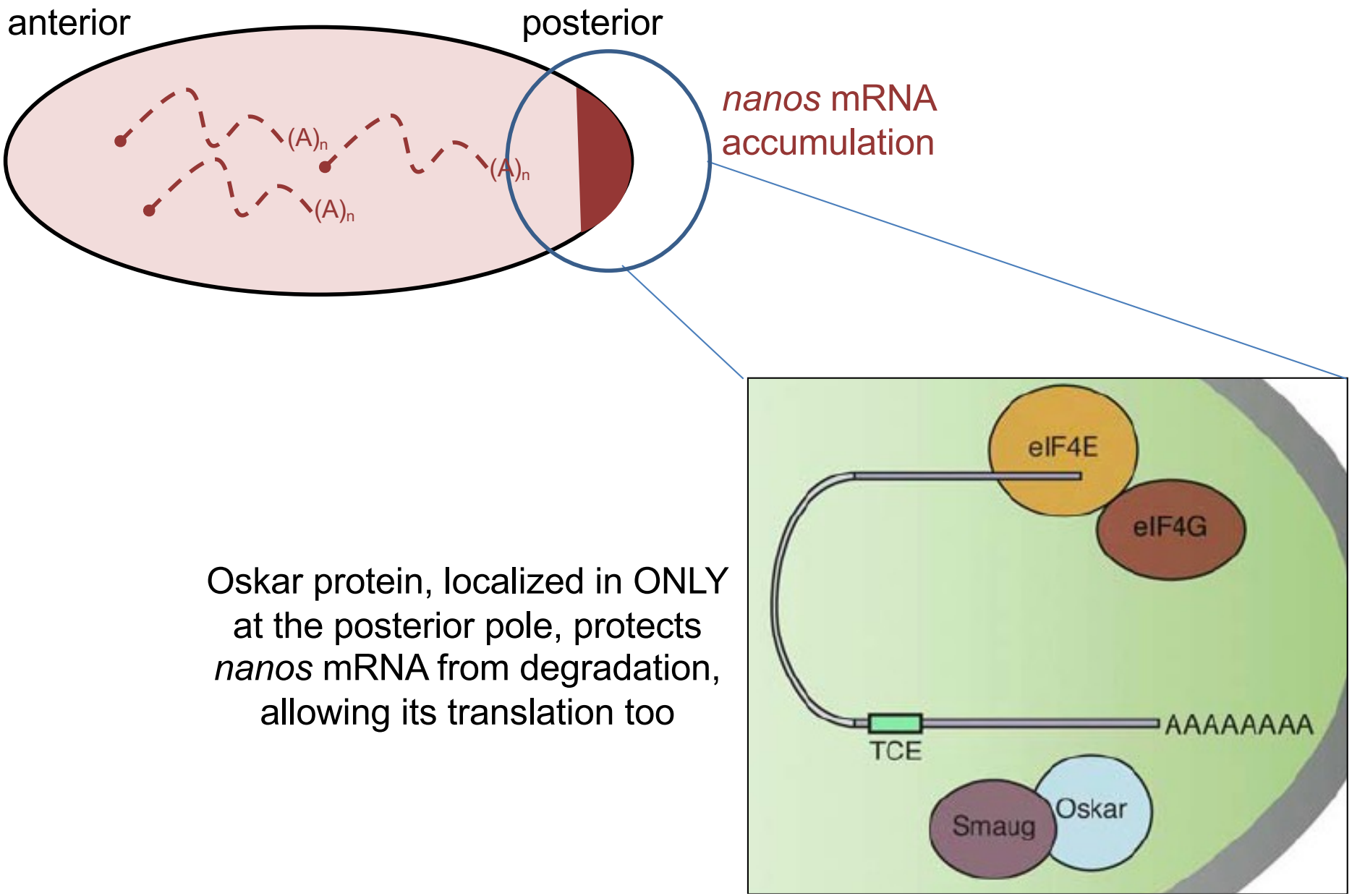


nanos mRNA accumulation



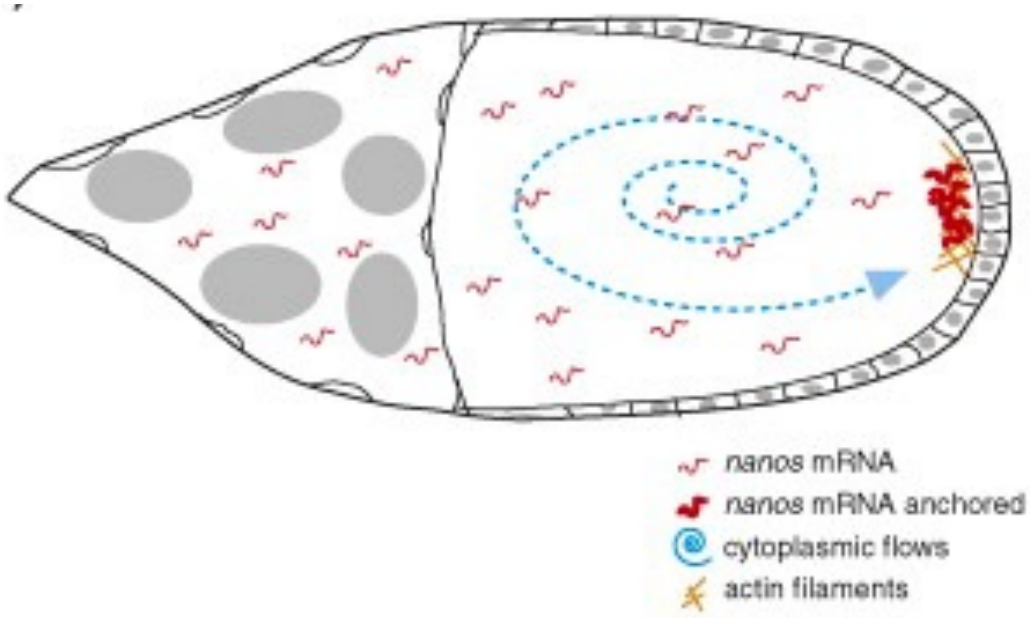
Translational repression and degradation through deadenylation

SELECTED STABILIZATION OF *nanos* mRNA



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

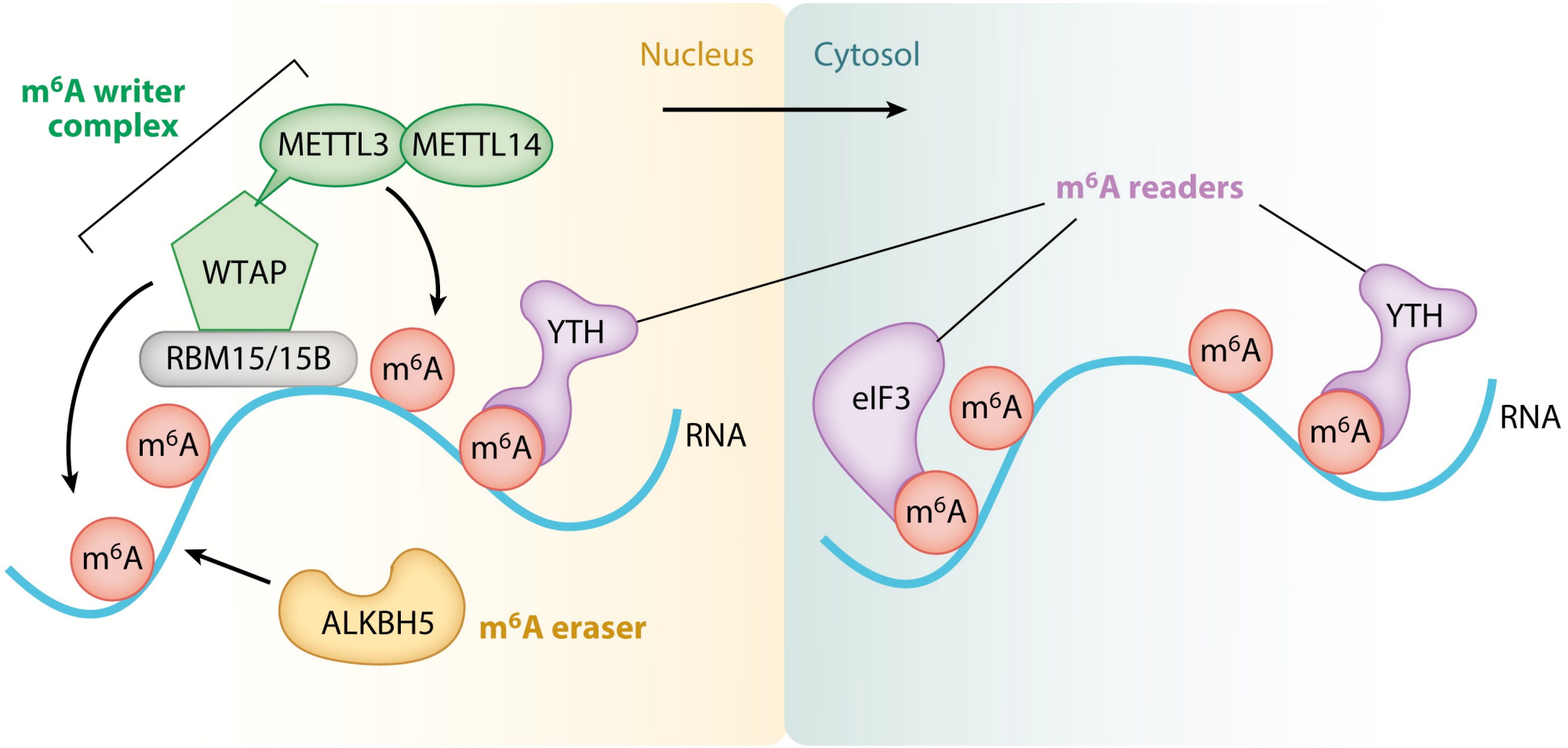
DIFFUSION/ENTRAPMENT OF *NANOS* mRNA



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

m6A RNA modification machinery



Meyer KD, Jaffrey SR. 2017.
Annu. Rev. Cell Dev. Biol. 33:319–42

The m6A writer complex interacts with the TREX complex aiding in the export of specific mRNAs