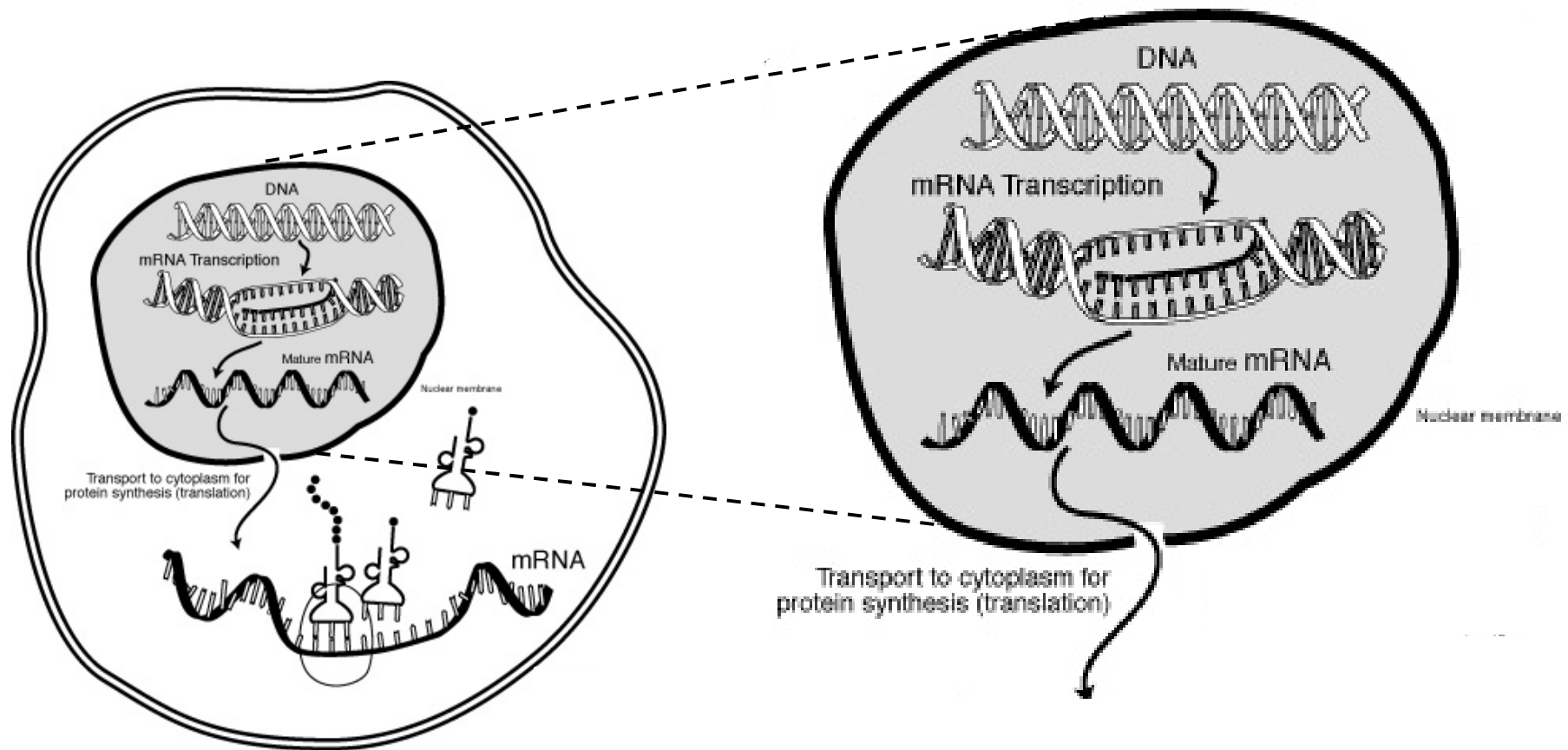
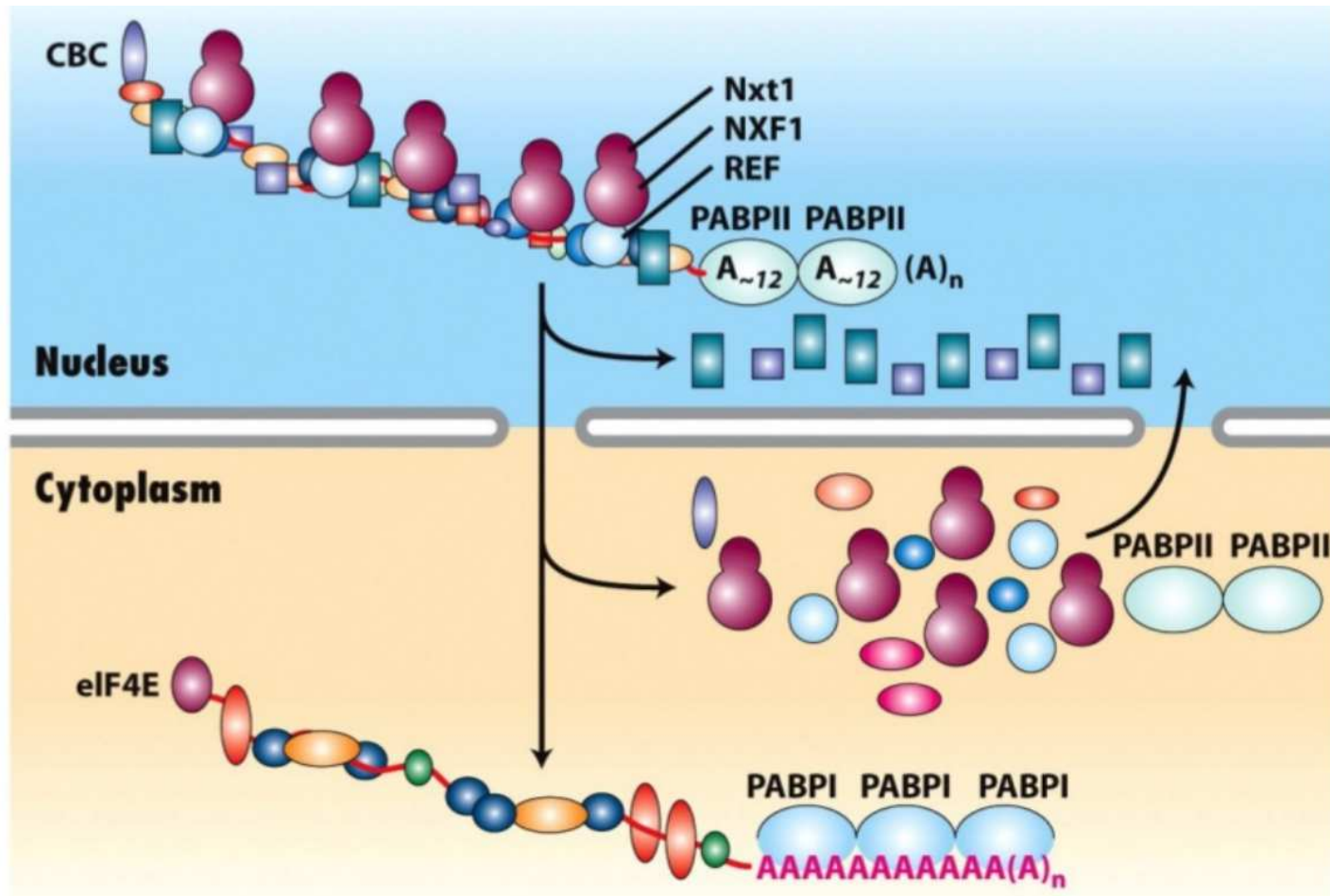


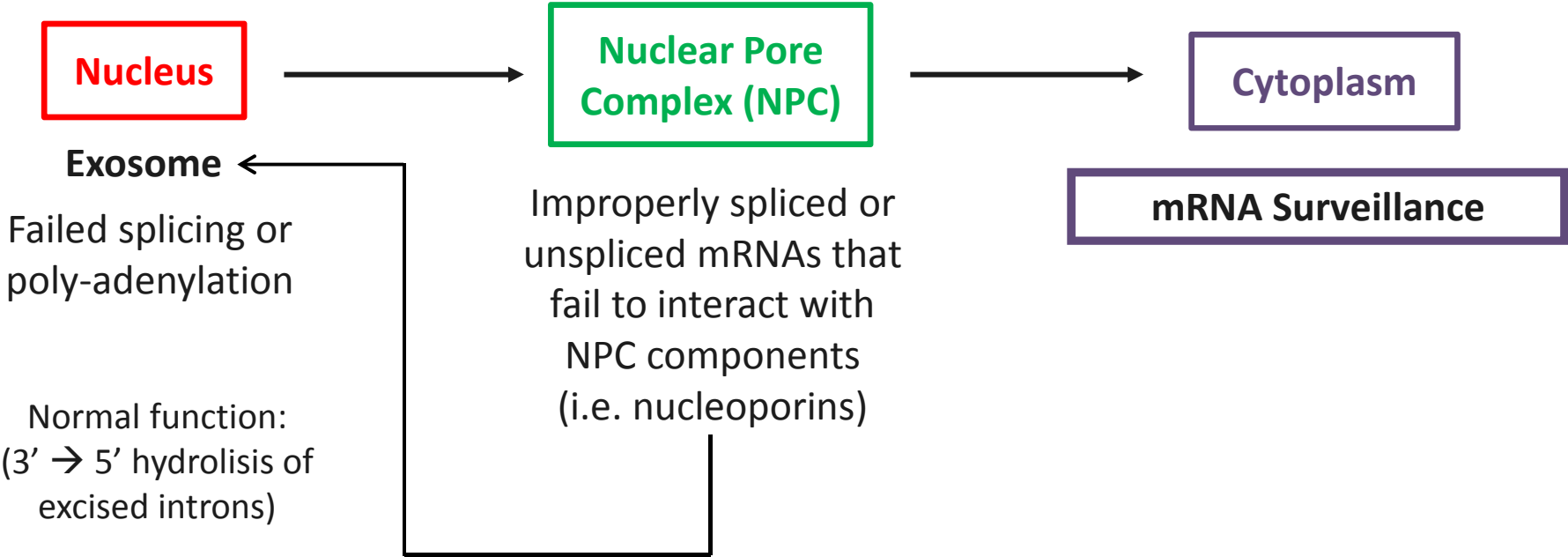
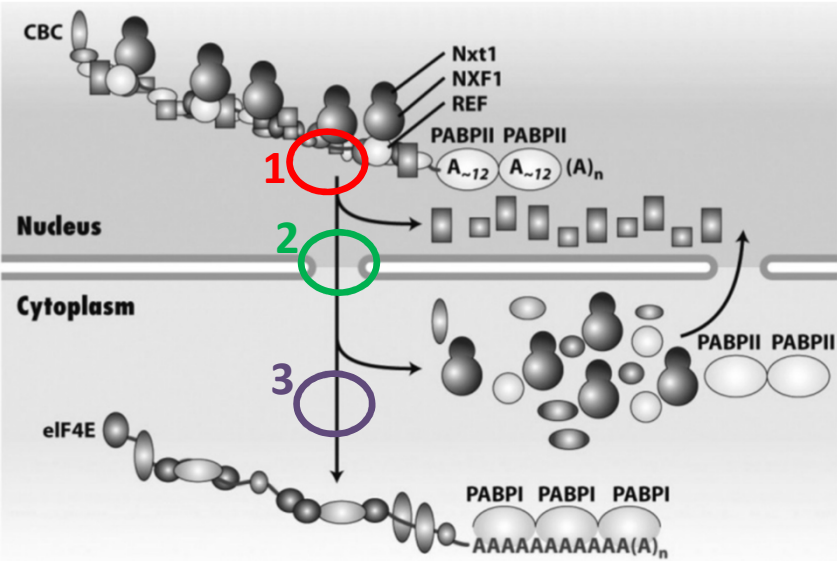
mRNA biogenesis and quality control



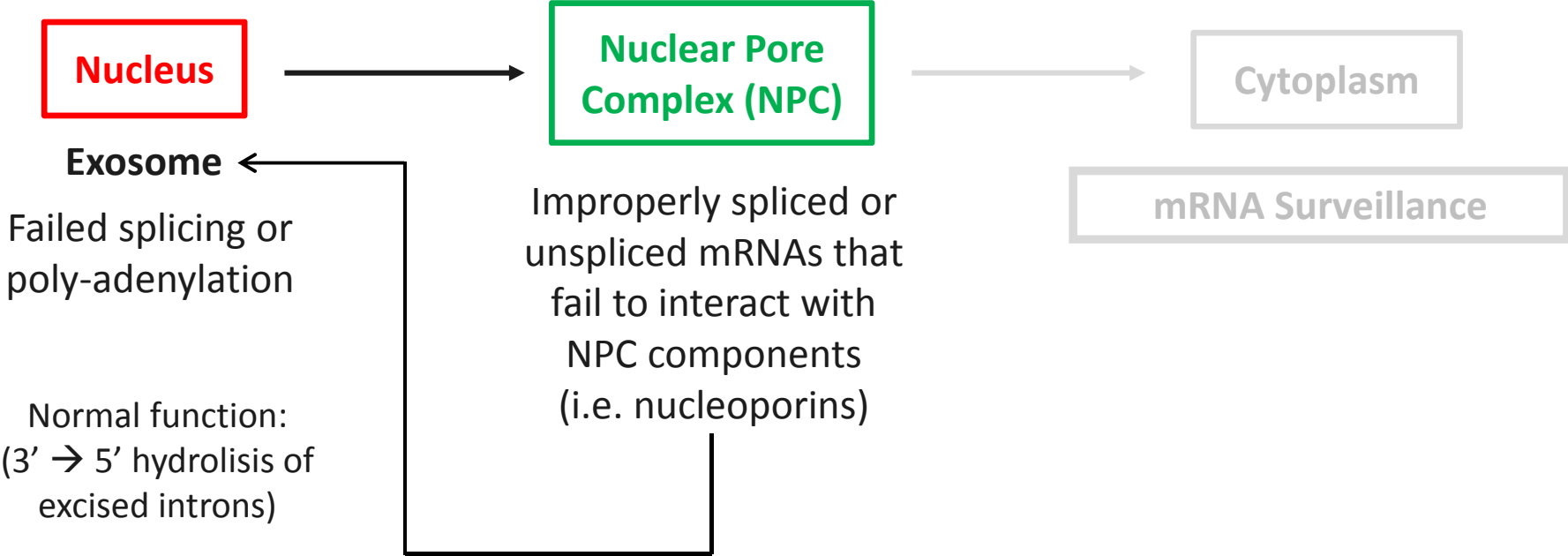
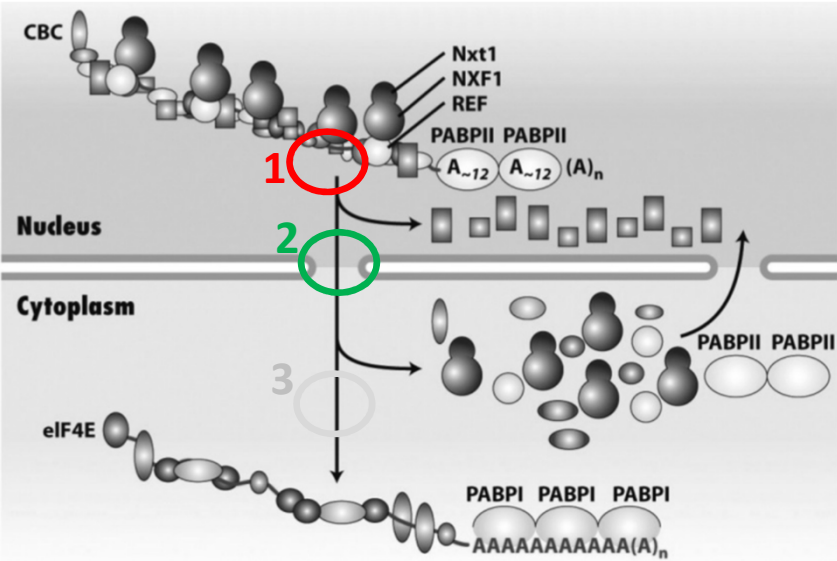
Transport of mRNA across nuclear envelope in eukaryotes



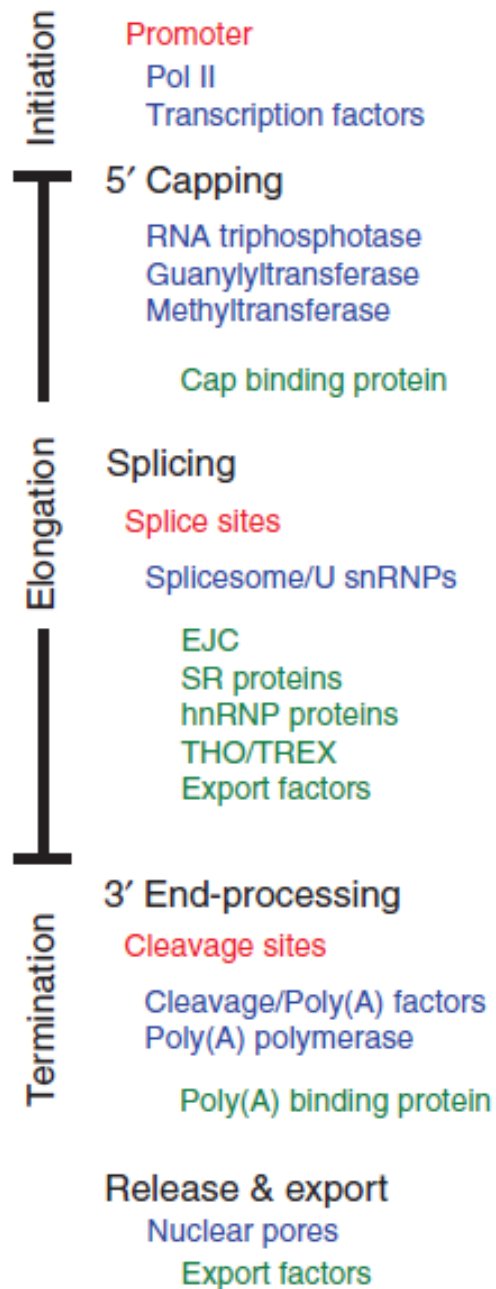
Quality control mechanisms ensure fidelity to mRNA biogenesis



Quality control mechanisms ensure fidelity to mRNA biogenesis



mRNA processing is tightly coupled to transcription

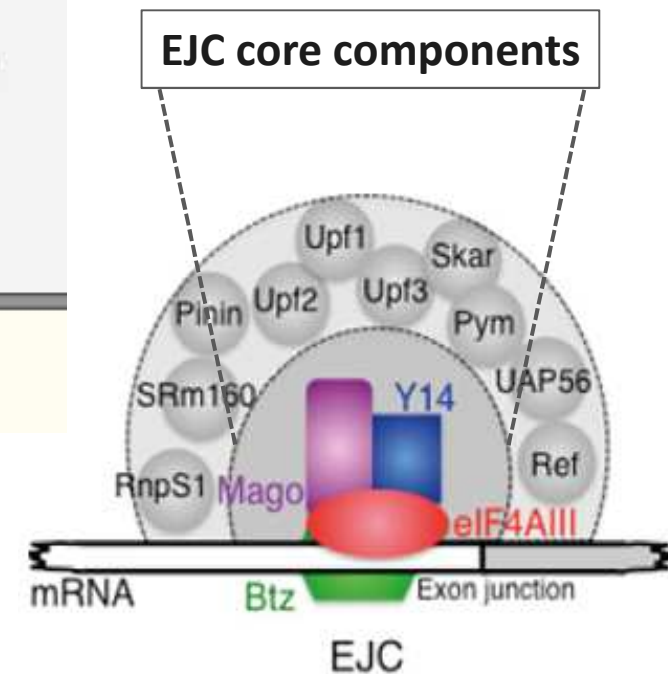
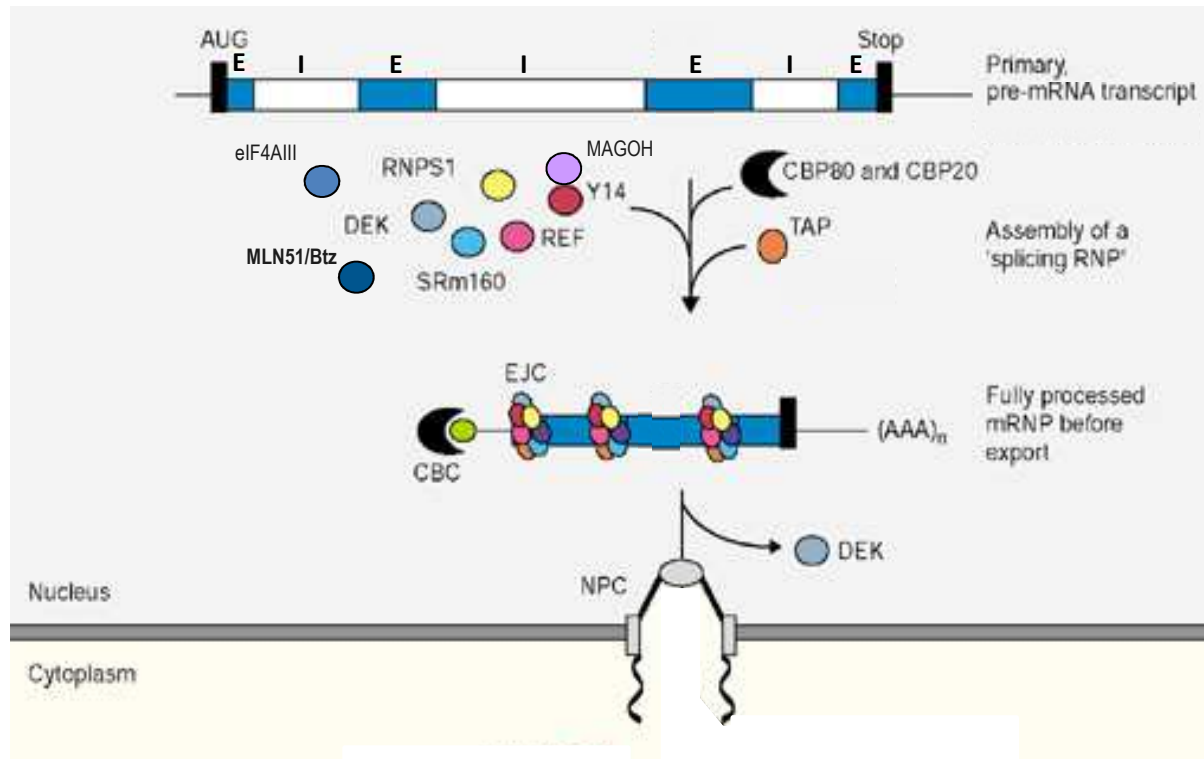


Legend:

- Processing steps
- Functional processing sequences
- Components of processing machinery
- Factors loaded onto transcripts upon processing

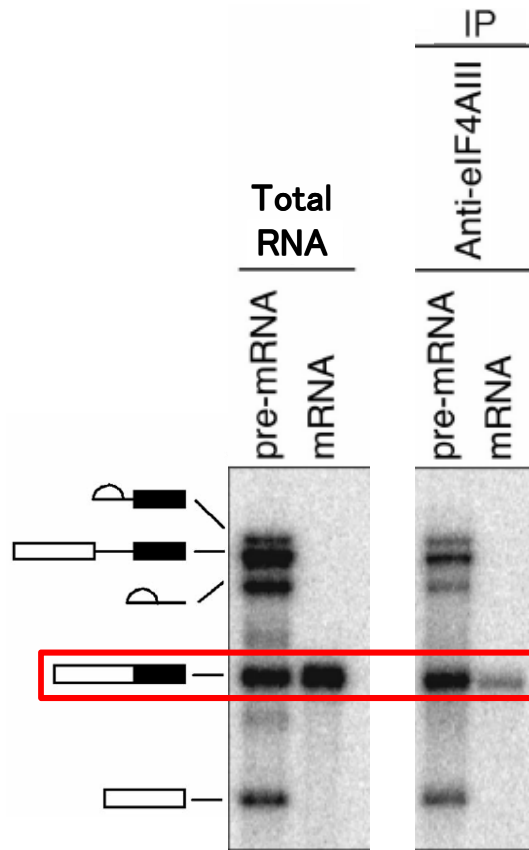
The Exon-Junction Complex (EJC) as a splicing hallmark

Multi-protein complexes deposited during splicing at 20-24 nucleotides upstream of each exon-exon junction



eIF4AIII is a key component in mRNA processing

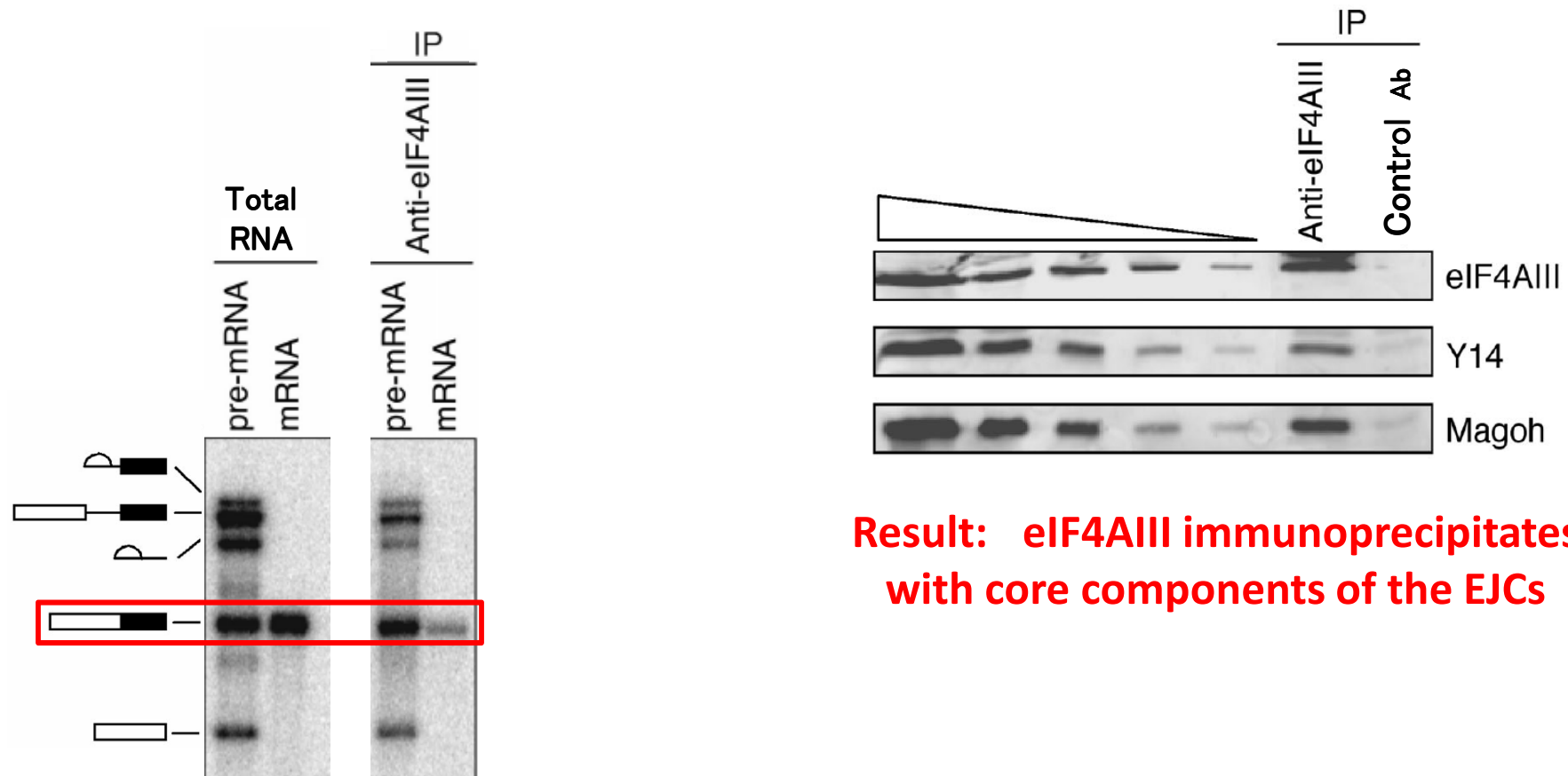
1st Experiment: *in-vitro* splicing with ³²P-labeled pre-mRNAs and mRNAs incubated with cell nuclear extract, followed by immunoprecipitation (IP) with anti-eIF4AIII antibodies



Result: eIF4AIII, in addition to being part of the spliceosome, remains associated with spliced mRNA

eIF4AIII is a key component of Exon-Junction Complexes

2nd Experiment: Immunoprecipitation (IP) of RNase A-treated cell extracts with anti-eIF4AIII or control Ab followed by western blotting analysis with anti-eIF4AIII, anti-Y14 and anti-Magoh Abs

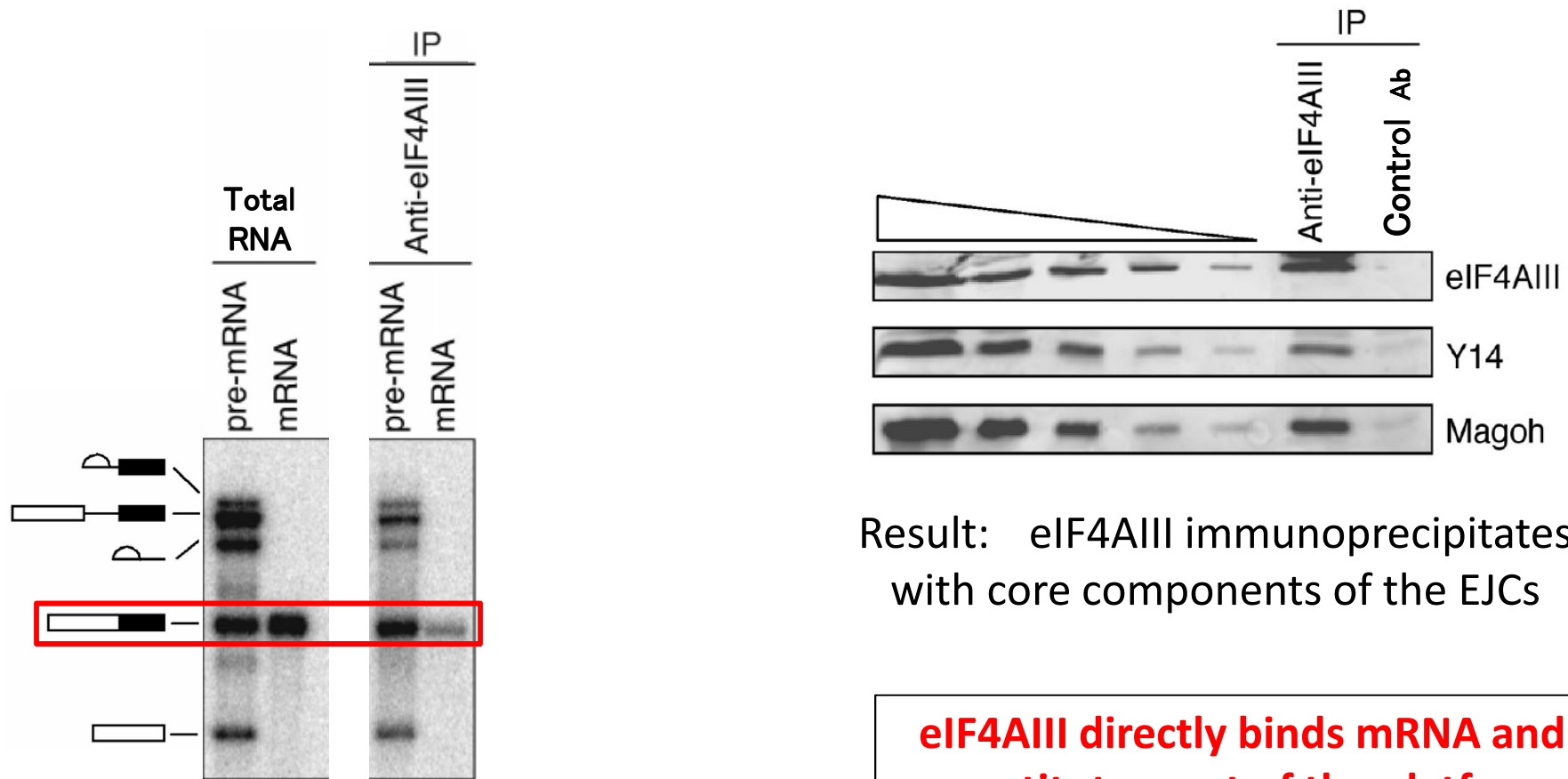


Result: eIF4AIII immunoprecipitates with core components of the EJs

Result: eIF4AIII, in addition to being part of the spliceosome, remains associated with spliced mRNA

eIF4AIII is a key component of Exon-Junction Complexes

2nd Experiment: Immunoprecipitation (IP) of RNase A-treated cell extracts with anti-eIF4AIII or control Ab followed by western blotting analysis with anti-eIF4AIII, anti-Y14 and anti-Magoh Abs



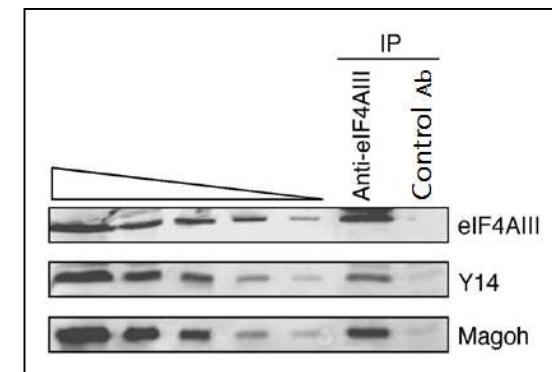
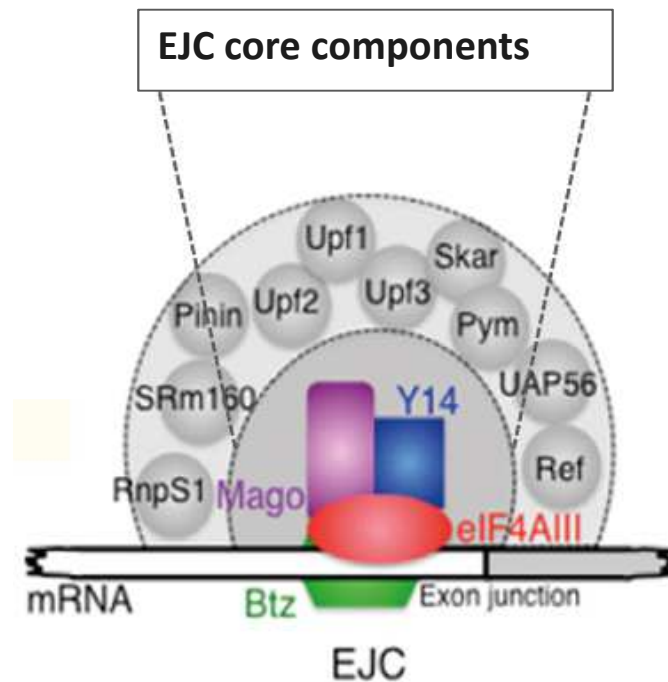
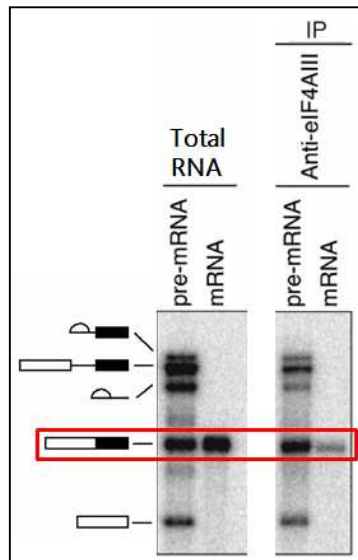
Result: eIF4AIII, in addition to being part of the spliceosome, remains associated with spliced mRNA

Result: eIF4AIII immunoprecipitates with core components of the EJC

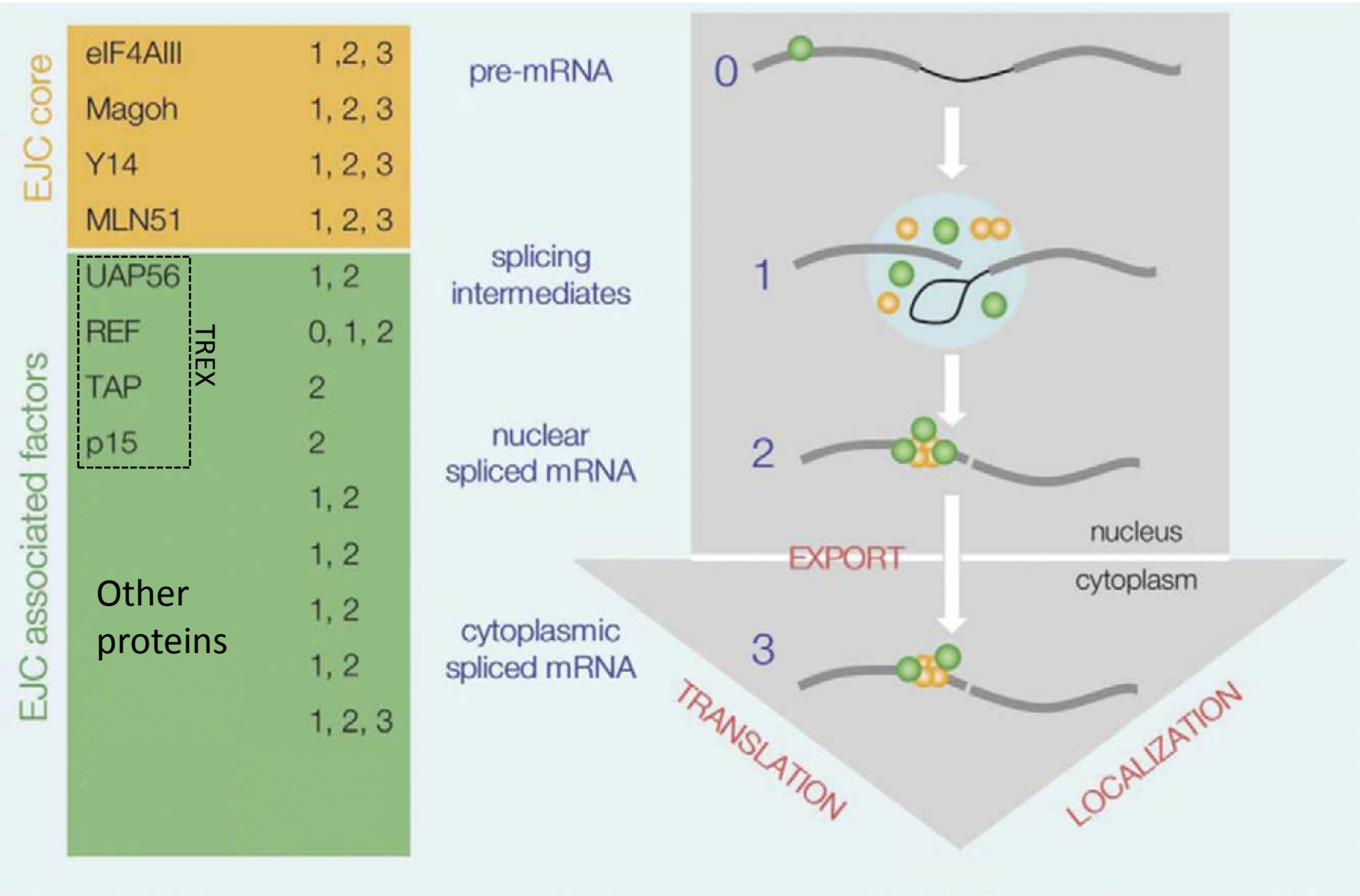
eIF4AIII directly binds mRNA and constitutes part of the platform anchoring other EJC proteins to spliced mRNAs.

These data indicate that **eIF4AIII**

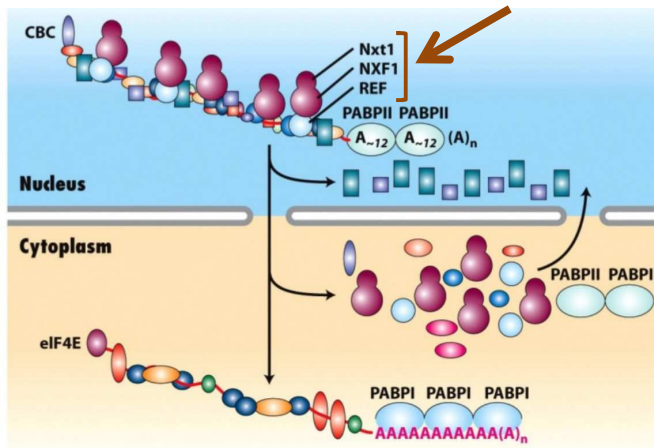
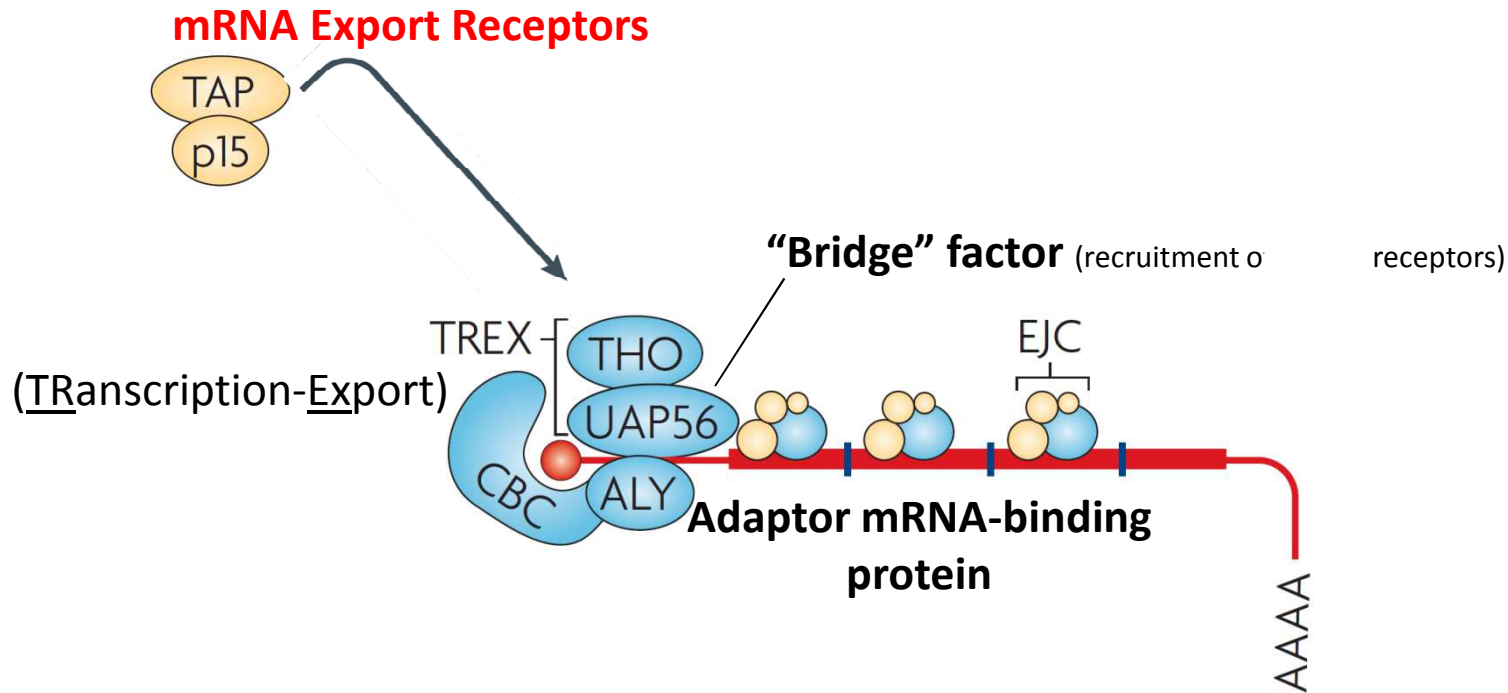
- i) directly binds pre-mRNA**
- ii) is located on spliced mRNAs**
- iii) interacts with core components of the EJC**



The EJC components interact with pre-mRNA at different stages

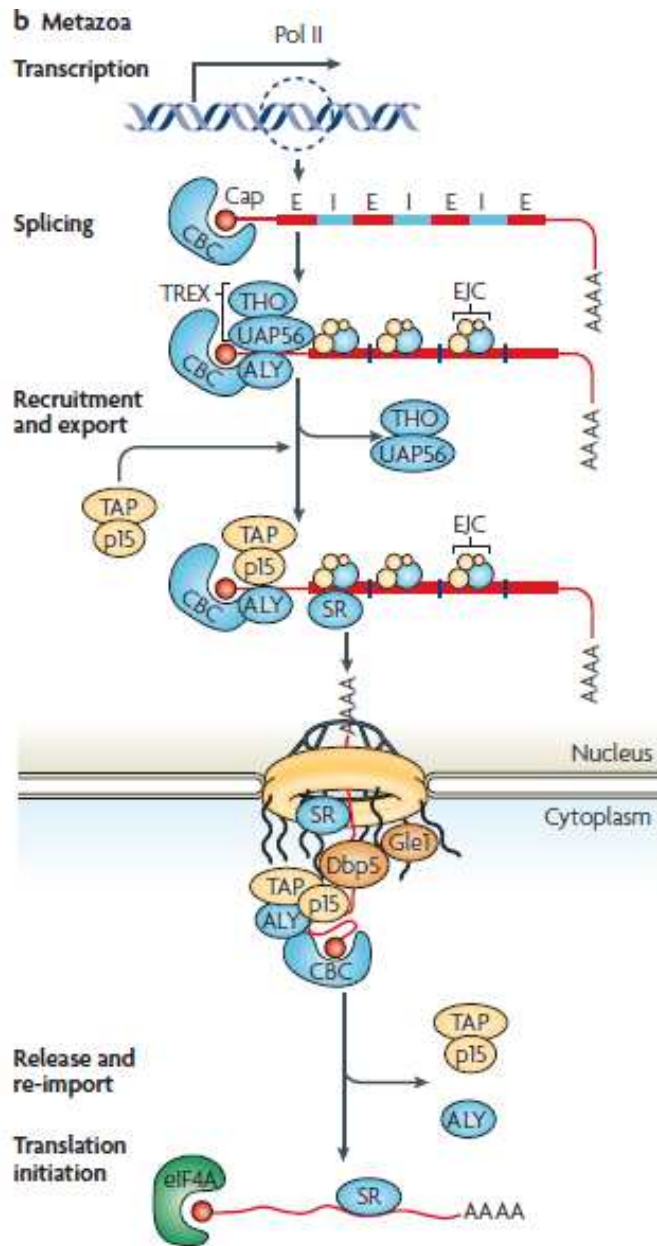


General mechanism of mRNA export pathway



Humans		Yeast
ALY/REF	Adaptors “Bridge” Factors	Yra1
UAP56		Sub2
TAP/Nxf1	Export Receptors	Mex67
P15/Nxt1		Mtr2

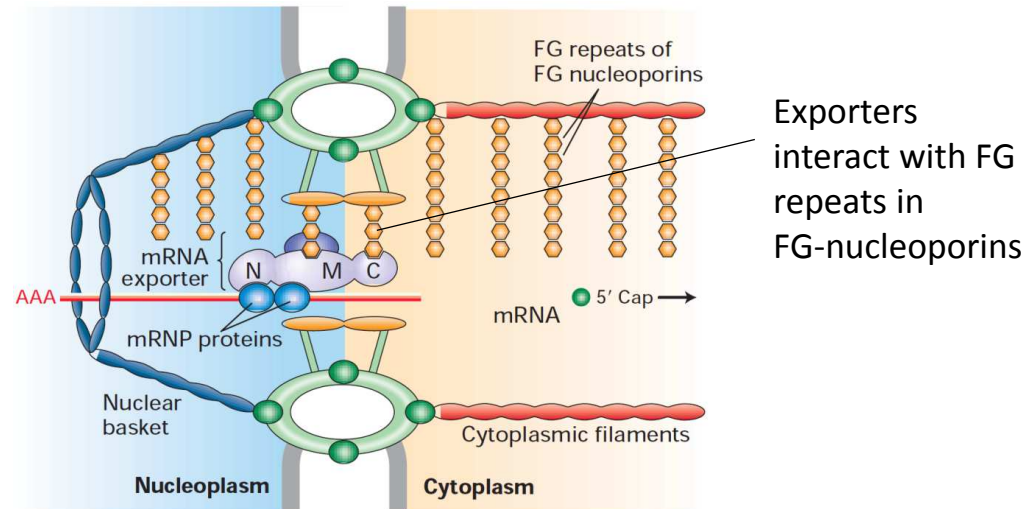
Splicing-coupled mRNA export in mammals



EJCs recruit the mRNA export machinery and link splicing with export

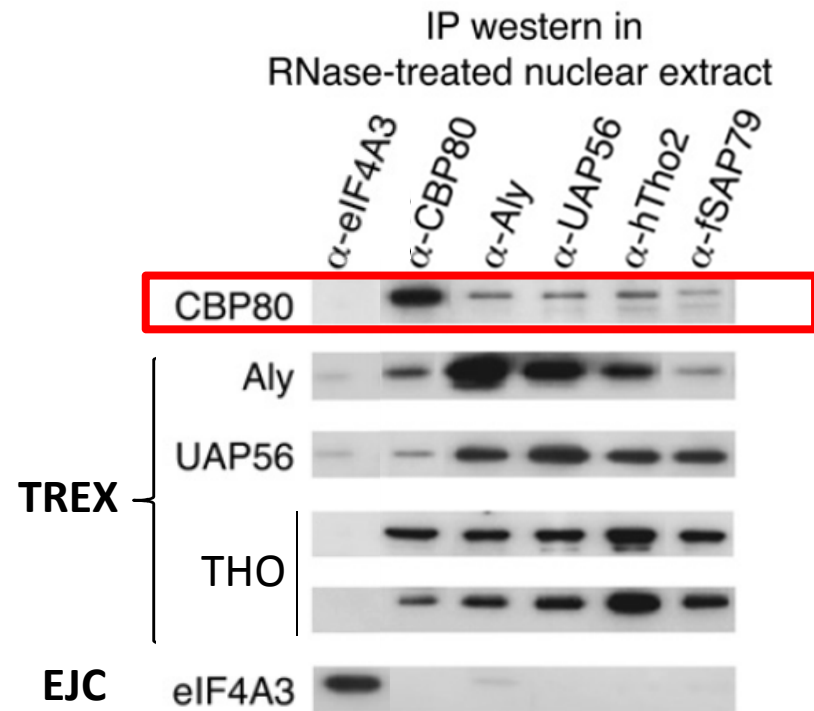
CBP80 is required for the splicing- and cap-dependent recruitment of TREX at the 5' prime of mRNA

mRNA transcripts are exported in the 5' → 3' direction



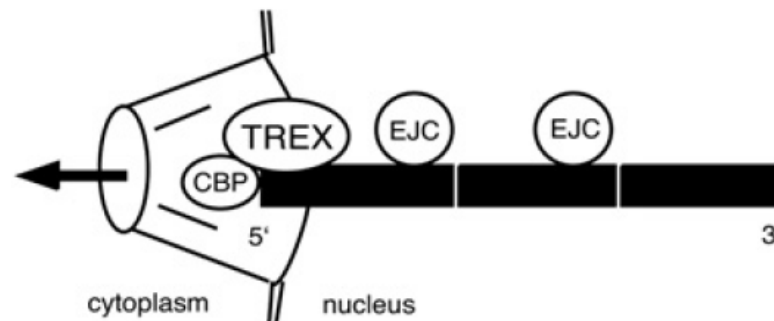
TREX interacts with CBP80 via protein-protein interactions

Experiment: Immunoprecipitation from RNase-treated nuclear extracts and western blotting



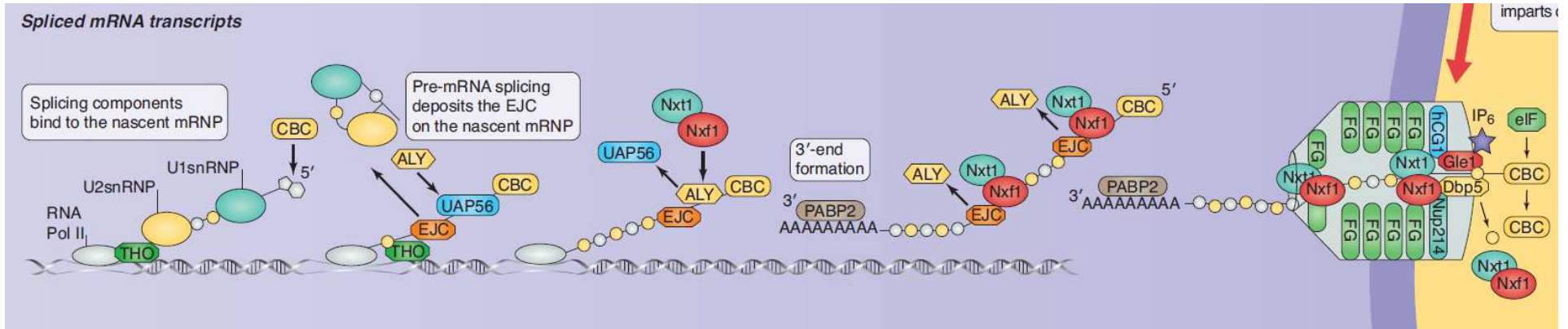
CBP80 coimmunoprecipitates with the **TREX components** but not with EJs.

Model for directional (5' to 3') translocation of mRNA



Nuclear Quality Control – The exosome

Normally processed transcripts



Improperly processed transcripts (*i.e.* failed splicing or poly-adenylation)

