Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex

Binyamin Zuckerman, Maya Ron, Martin Mikl, Eran Segal, Igor Ulitsky

Virginia Anna Gazziero, Giusy Lovallo, Monica Rossin

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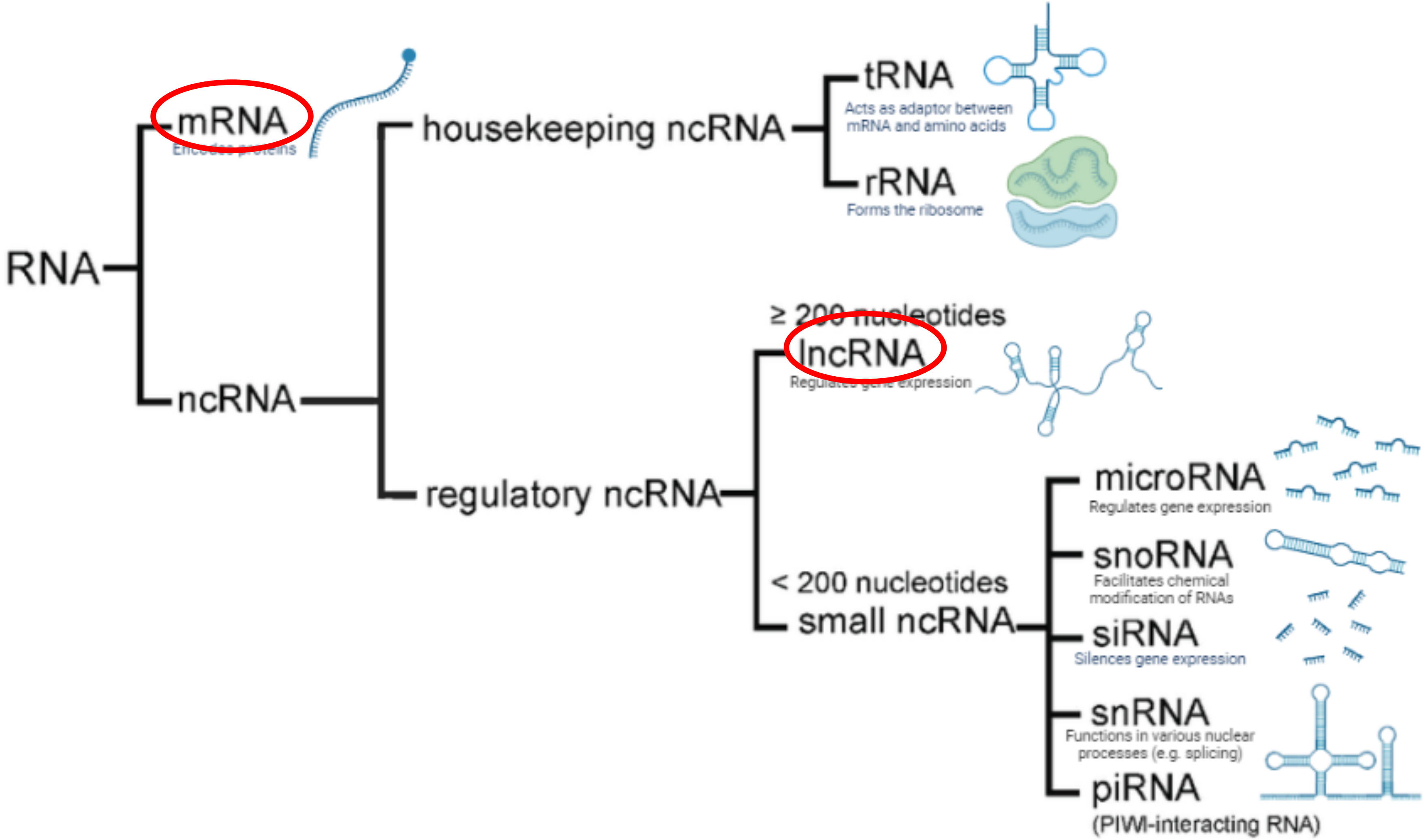
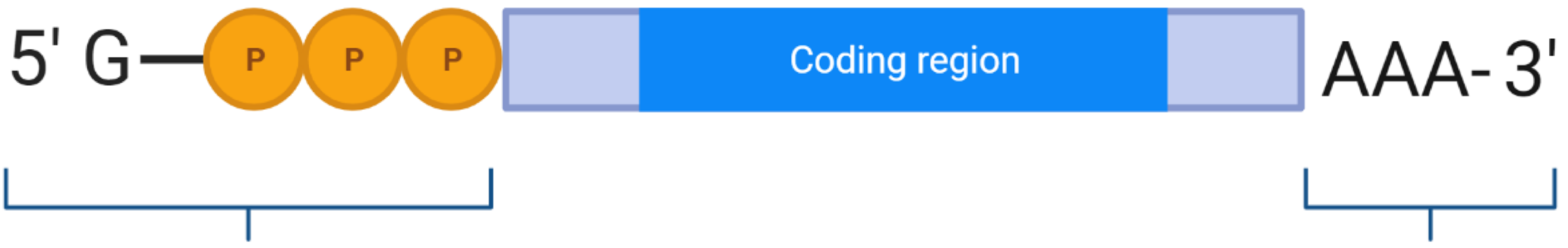
DI TRIESTE

PART ONE

mRNA and lncRNA characteristics


RNA
mRNA
Encodes proteins
ncRNA
housekeeping ncRNA
tRNA
Acts as adaptor between mRNA and amino acids
rRNA
Forms the ribosome
regulatory ncRNA
≥ 200 nucleotides
lncRNA
Regulates gene expression
< 200 nucleotides
small ncRNA
microRNA
Regulates gene expression
snoRNA
Facilitates chemical modification of RNAs
siRNA
Silences gene expression
snRNA
Functions in various nuclear processes (e.g. splicing)
piRNA
(PIWI-interacting RNA)

Messanger RNA



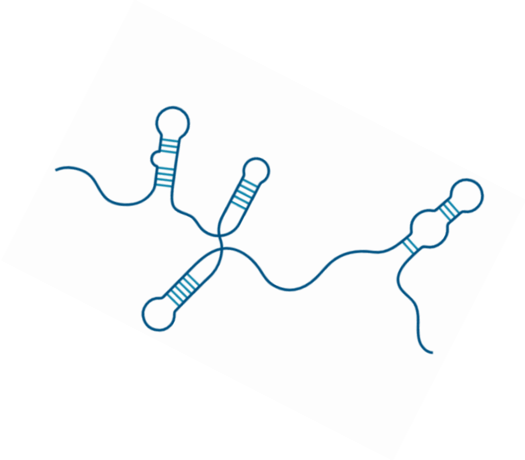
Cap

- Recogniction of mRNA in protein biosynthesis
- Prevent from 5'-exonucleases activities

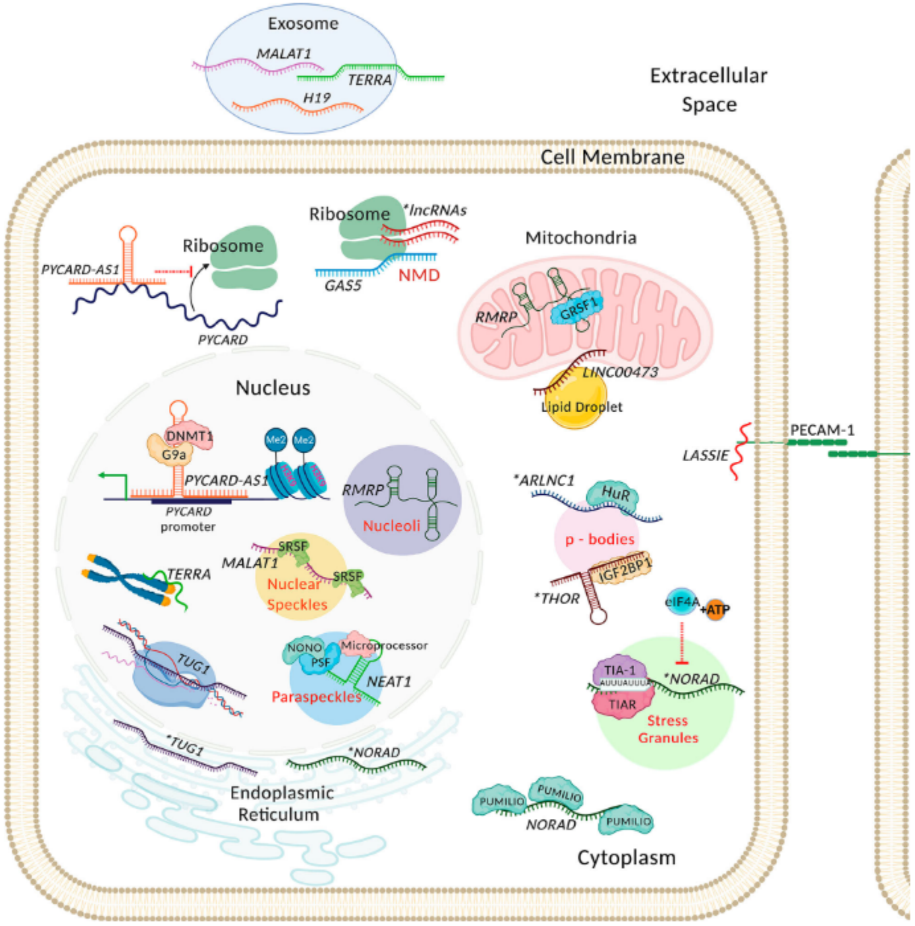
Poly A-Tail

- Prevent from 3'-exonucleases activities
- Helps in exporting mRNA from nucleus to cytosol

Long non-coding RNA

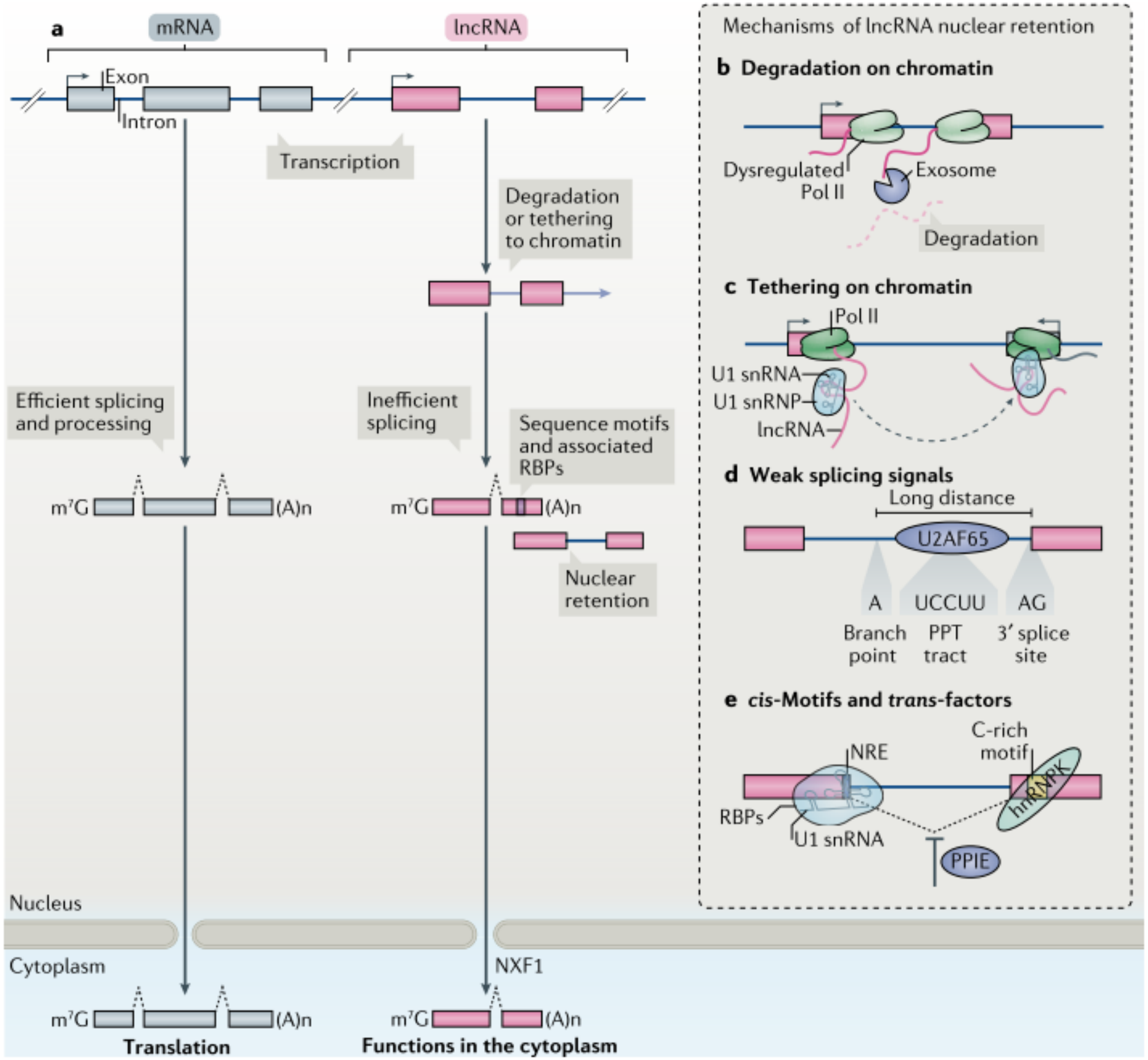


- Longer than 200 nucleotides
- Are not translated into functional proteins
- Depending on location, sequence, morphology, structure and function features, lncRNAs can be cathegorized into different groups



Bridges et al., 2021

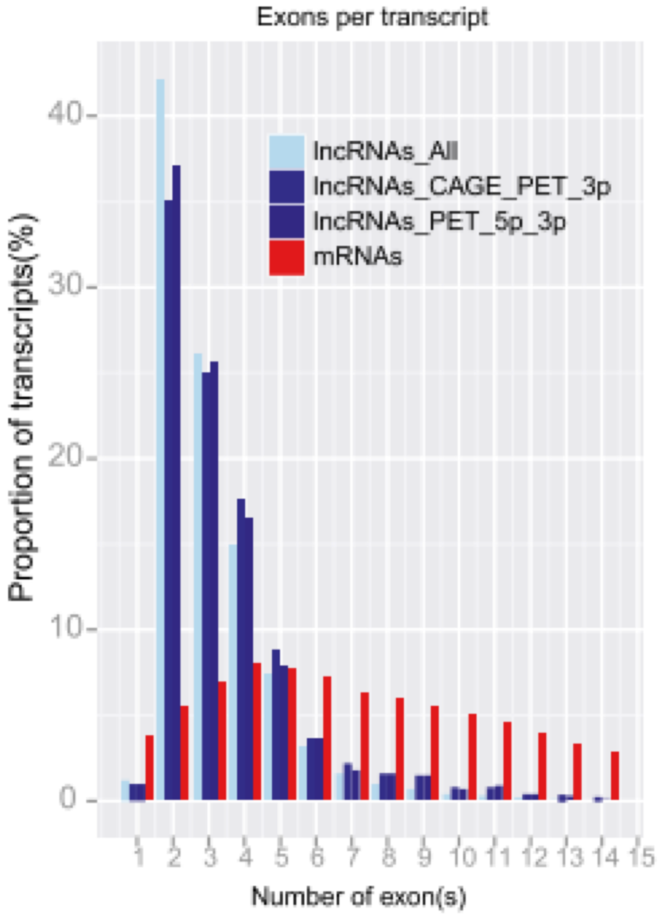
mRNA vs lncRNA



Statello 2021

lncRNAs

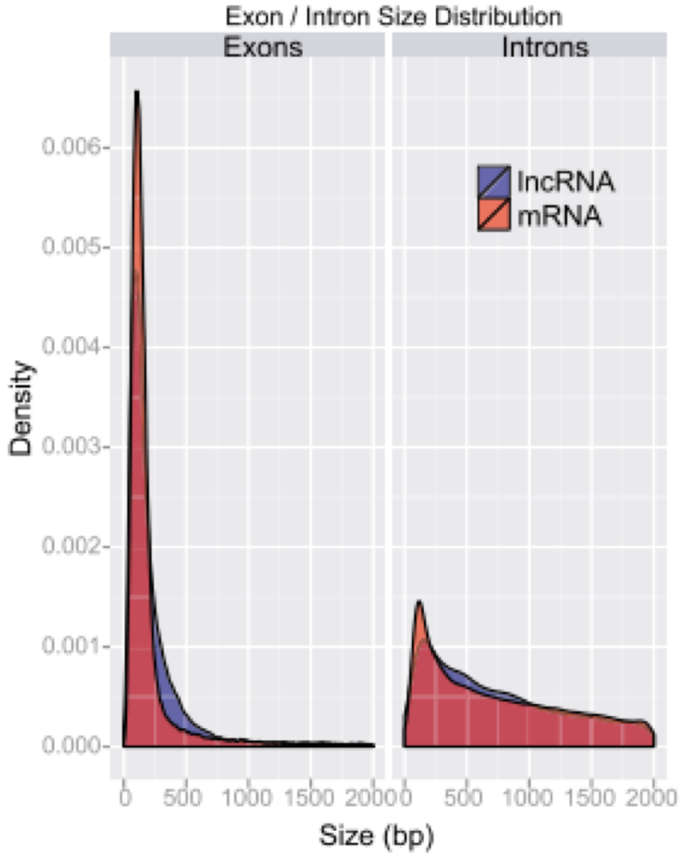
lncRNAs have less exons (tipically two)



(Derrien et al., 2012)

lncRNAs

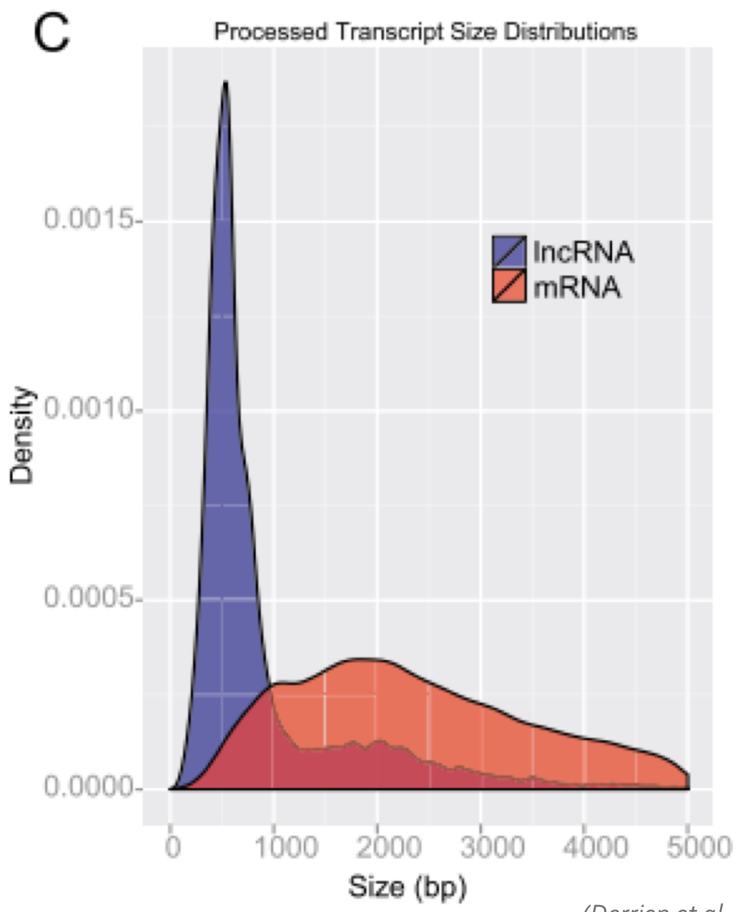
lncRNAs have longer exons



(Derrien et al., 2012)

lncRNAs

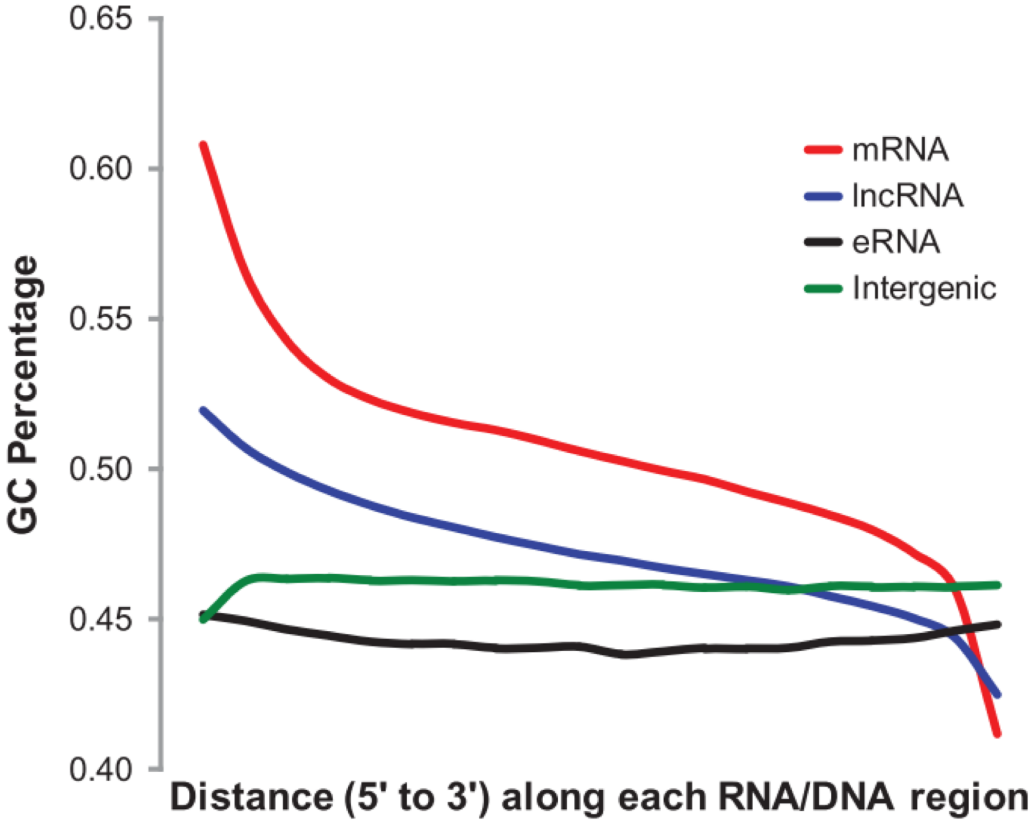
lncRNAs are usually shorter than mRNAs



(Derrien et al., 2012)

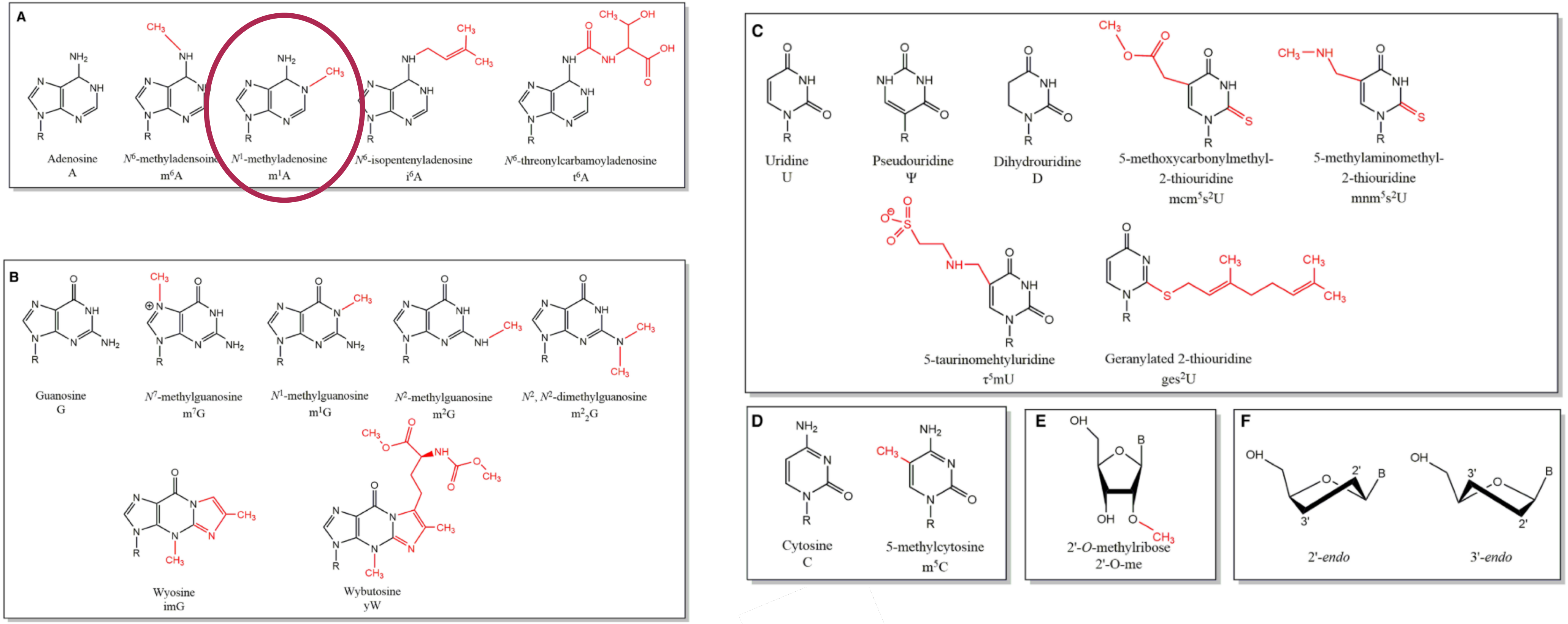
lncRNAs

lncRNAs tend to have less GC content than mRNA



(Palazzo & Kang, 2021)

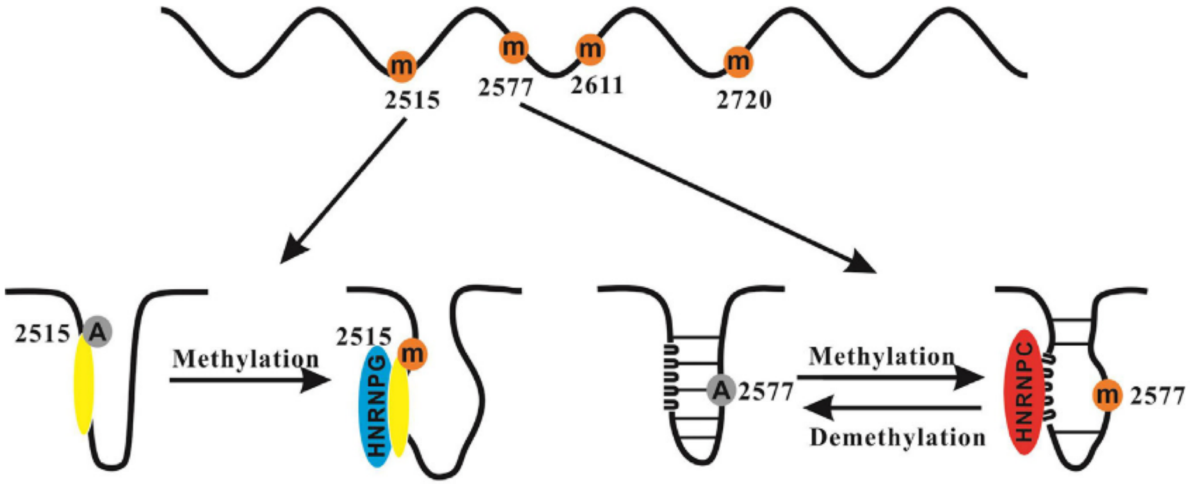
RNA editing

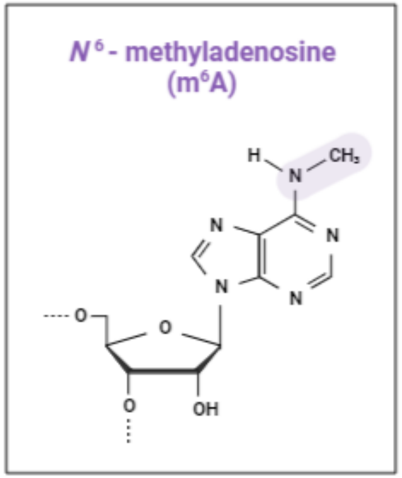


→ lncRNAs and mRNAs share some modifications

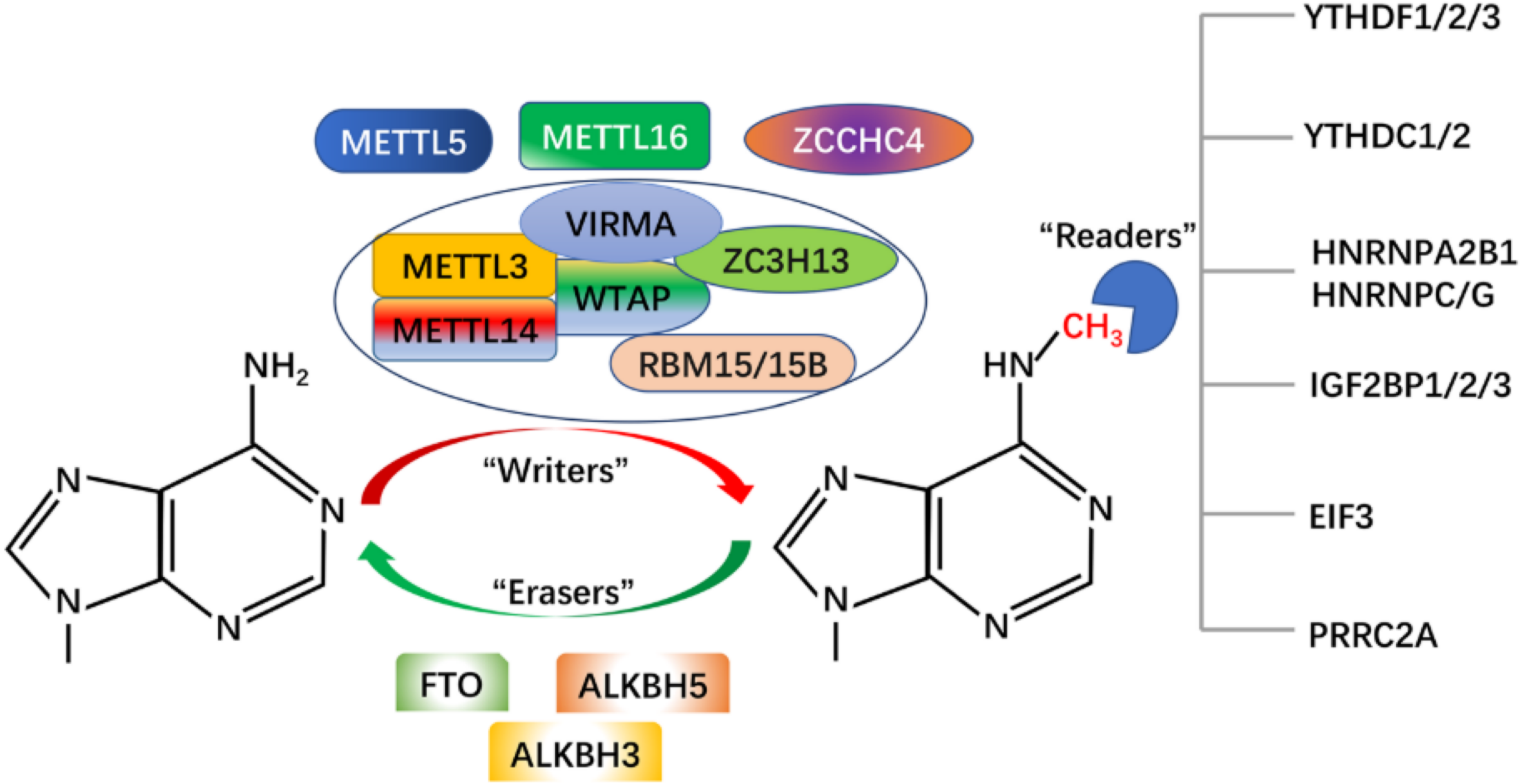
N^6 – methyladenosine (m^6A)

- N^6 -methyladenosine in mRNA N^6 -methyladenosine (m^6A) is the more prevalent internal modification in eukaryotic mRNA, occurring on an average of three sites per given mRNA molecule.
- m6A can be also found in lncRNAs



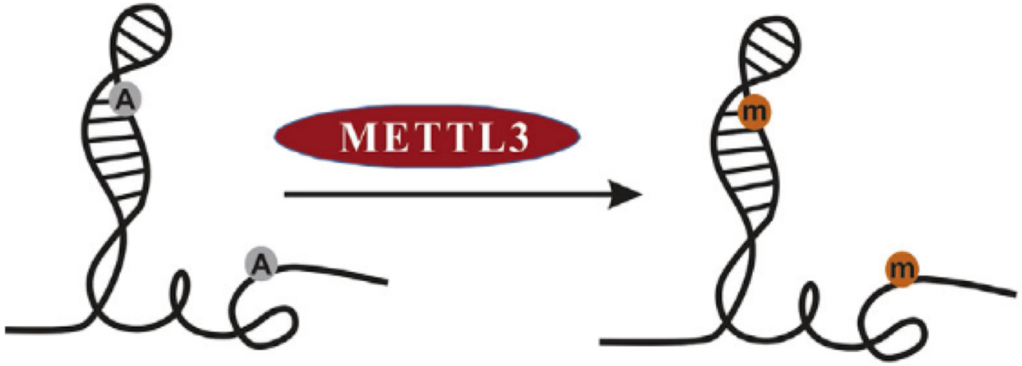


m6A



METTL5
METTL16
ZCCHC4
VIRMA
METTL3
ZC3H13
WTAP
METTL14
RBM15/15B
NH2
N
N
N
N
"Writers"
"Erasers"
FTO
ALKBH5
ALKBH3
"Readers"
HN
CH3
YTHDF1/2/3
YTHDC1/2
HNRNPA2B1
HNRNPC/G
IGF2BP1/2/3
EIF3
PRRC2A

m6A effects in lncRNAs

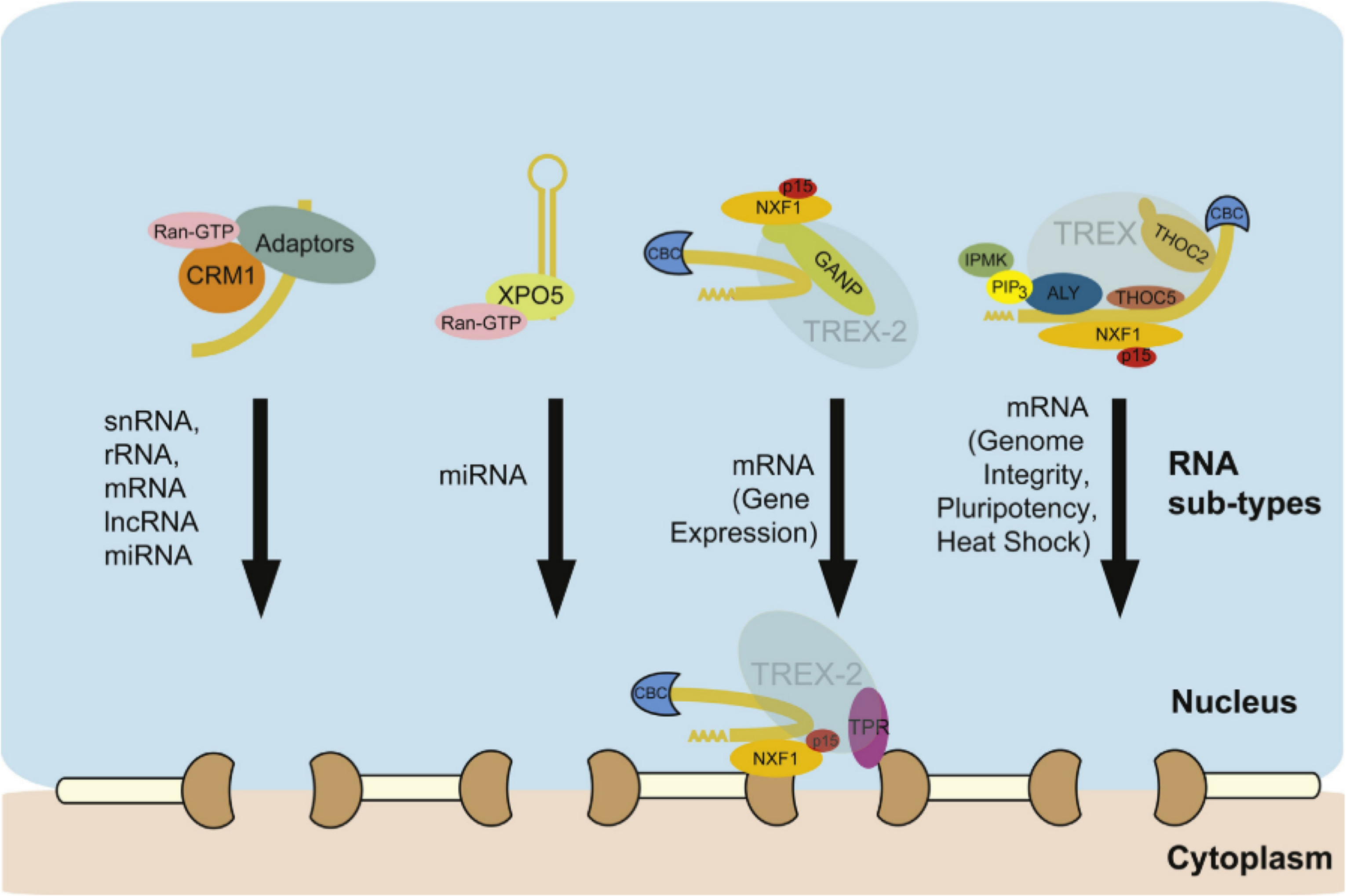


- Changing in the structure of lncRNAs and their interactions with proteins
- Could mediate gene transcription repression
- Alters subcellular distribution of lncRNAs
- Regulates lncRNAs stability

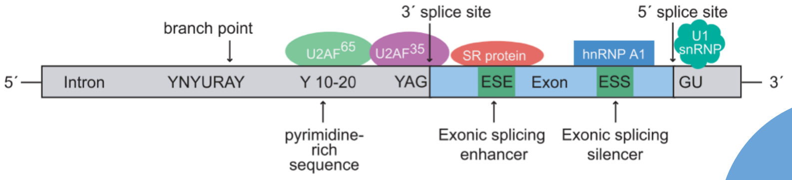
PART TWO

Nuclear export of RNA

Principal export mechanisms



Elements involved in RNA export or retention



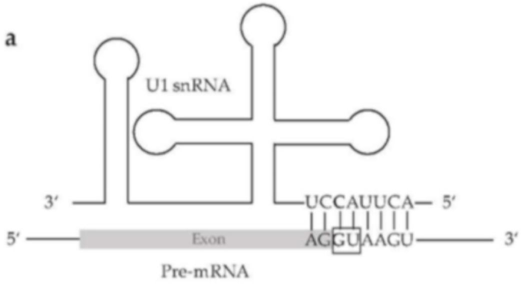
Elements involved in RNA export

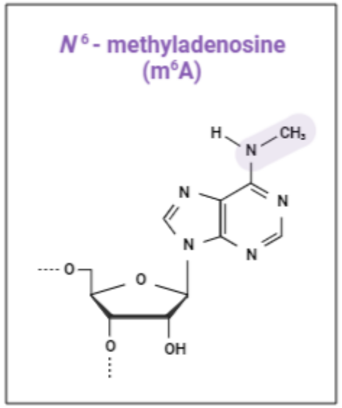
Exonic Splicing Enhancers (ESEs)

U1 binding site

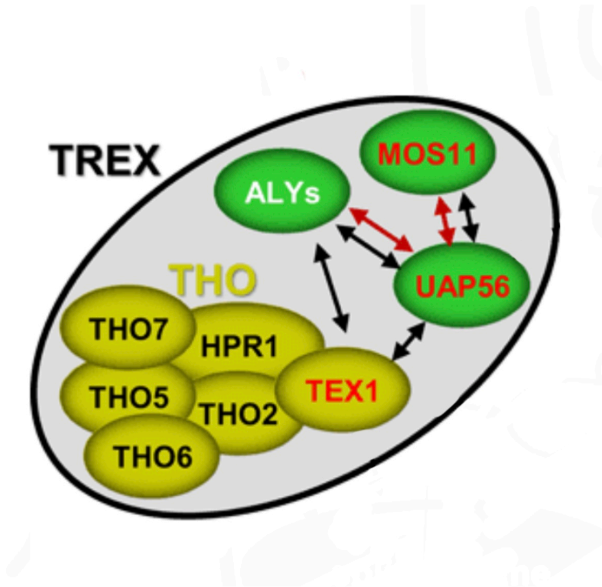
m6A presence

Cytoplasmatic Accumulation Region (CAR)

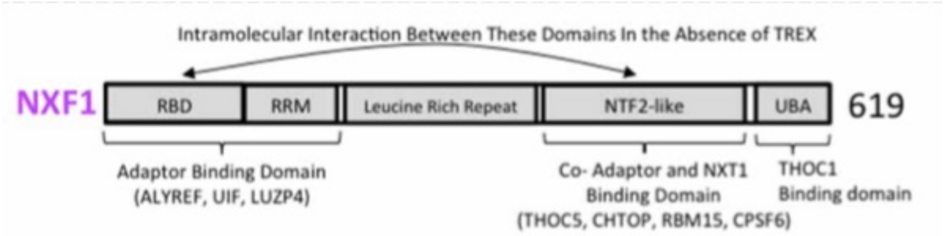


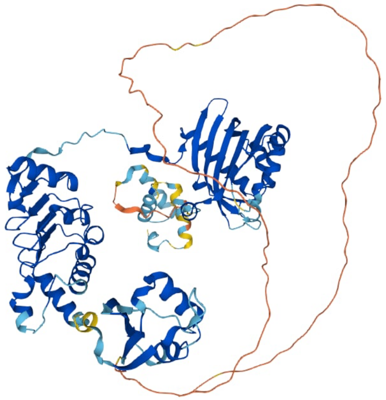


TREX complex



NXF1

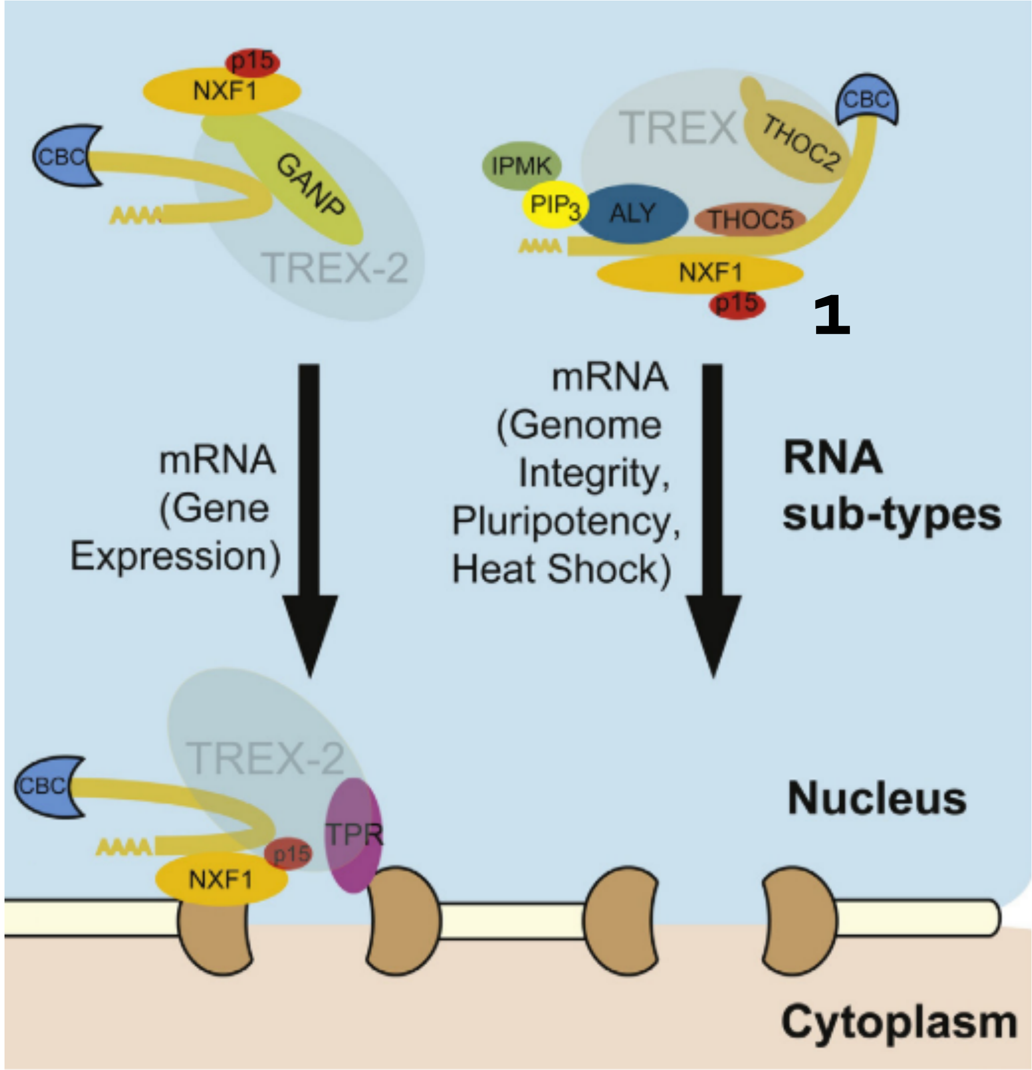




TREX plays a central role in mRNA export and this activity is governed by its ability to act as a binding platform for NXF1.

mRNAs nuclear export pathway

1. **TREX**
2. TREX-2
3. a subset of mRNAs involved in proliferation, survival and metastasis use CRM1



Williams et al.. 2018

mRNAs nuclear export pathway

1. TREX
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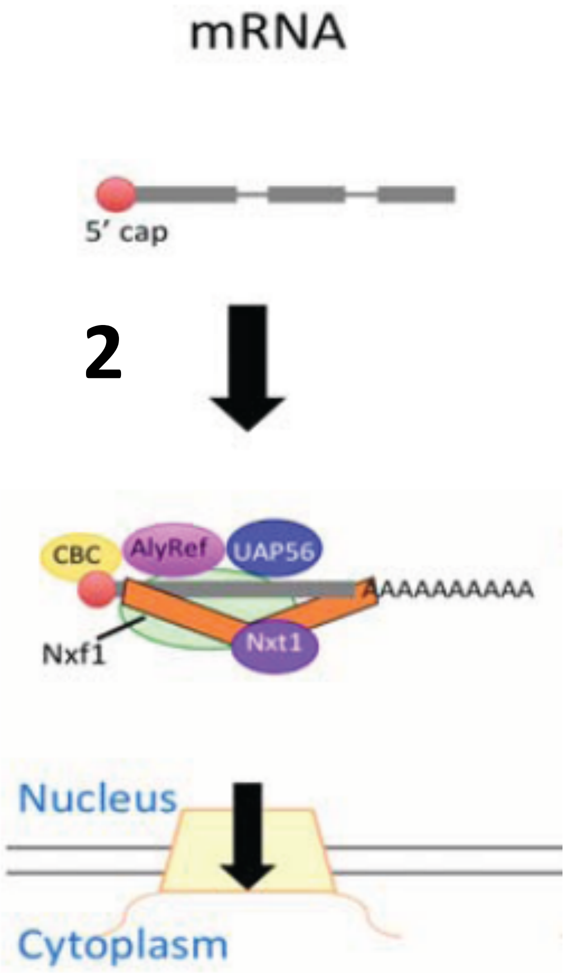
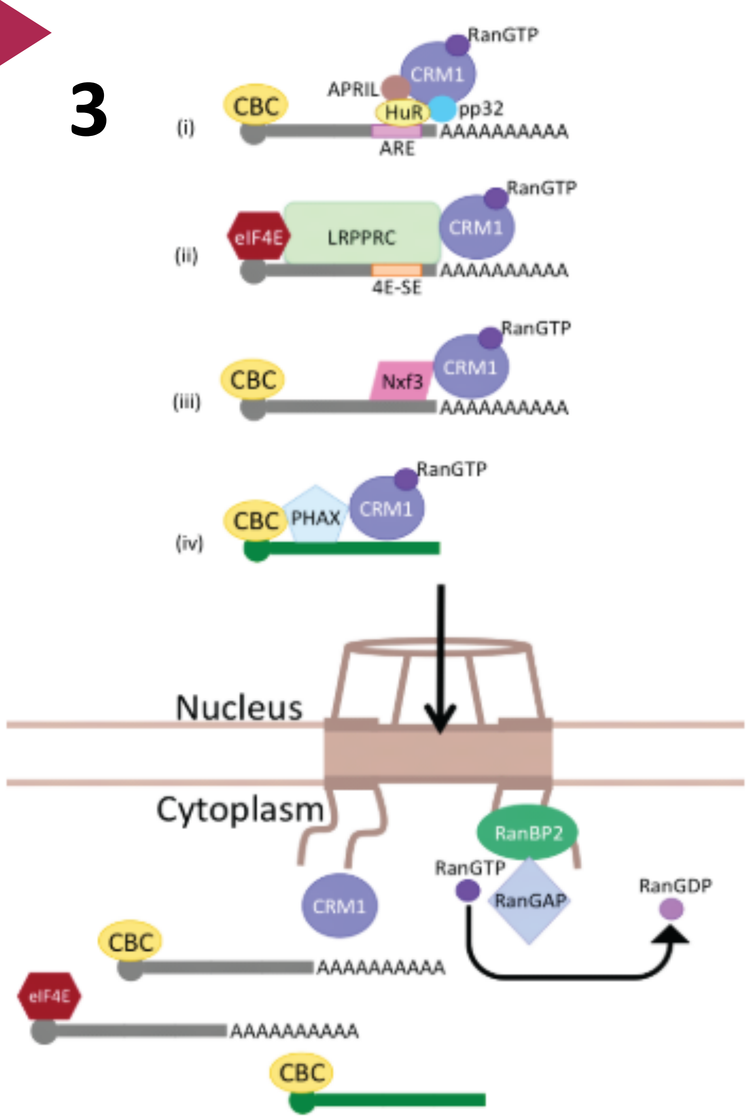


Williams et al., 2018

mRNAs nuclear export pathway

1. TREX
2. TREX-2
3. **A subset of mRNAs involved in proliferation, survival and metastasis use CRM1**

3

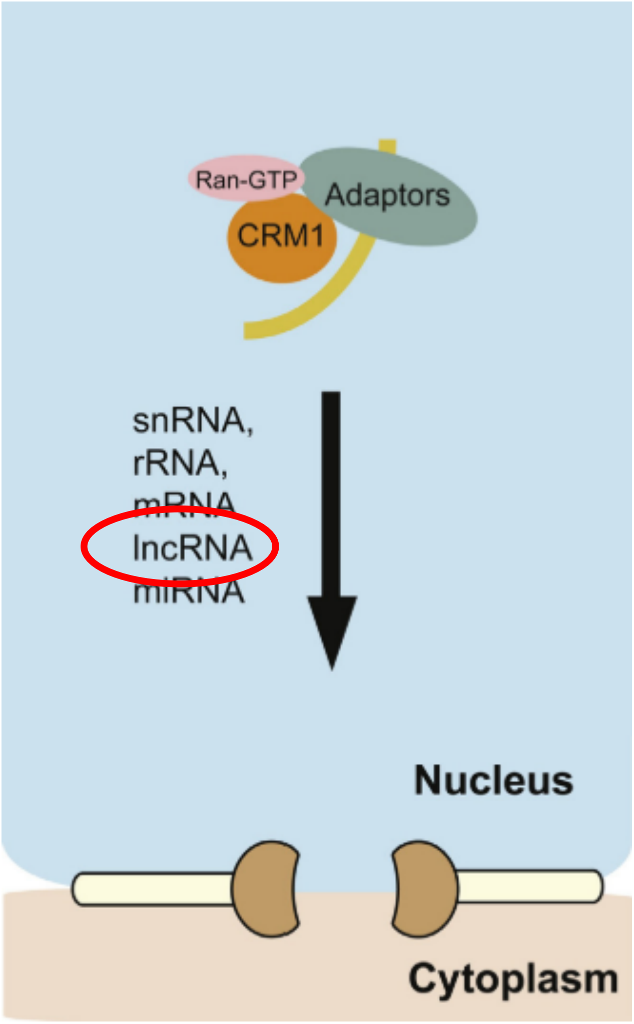


CITAZIONE

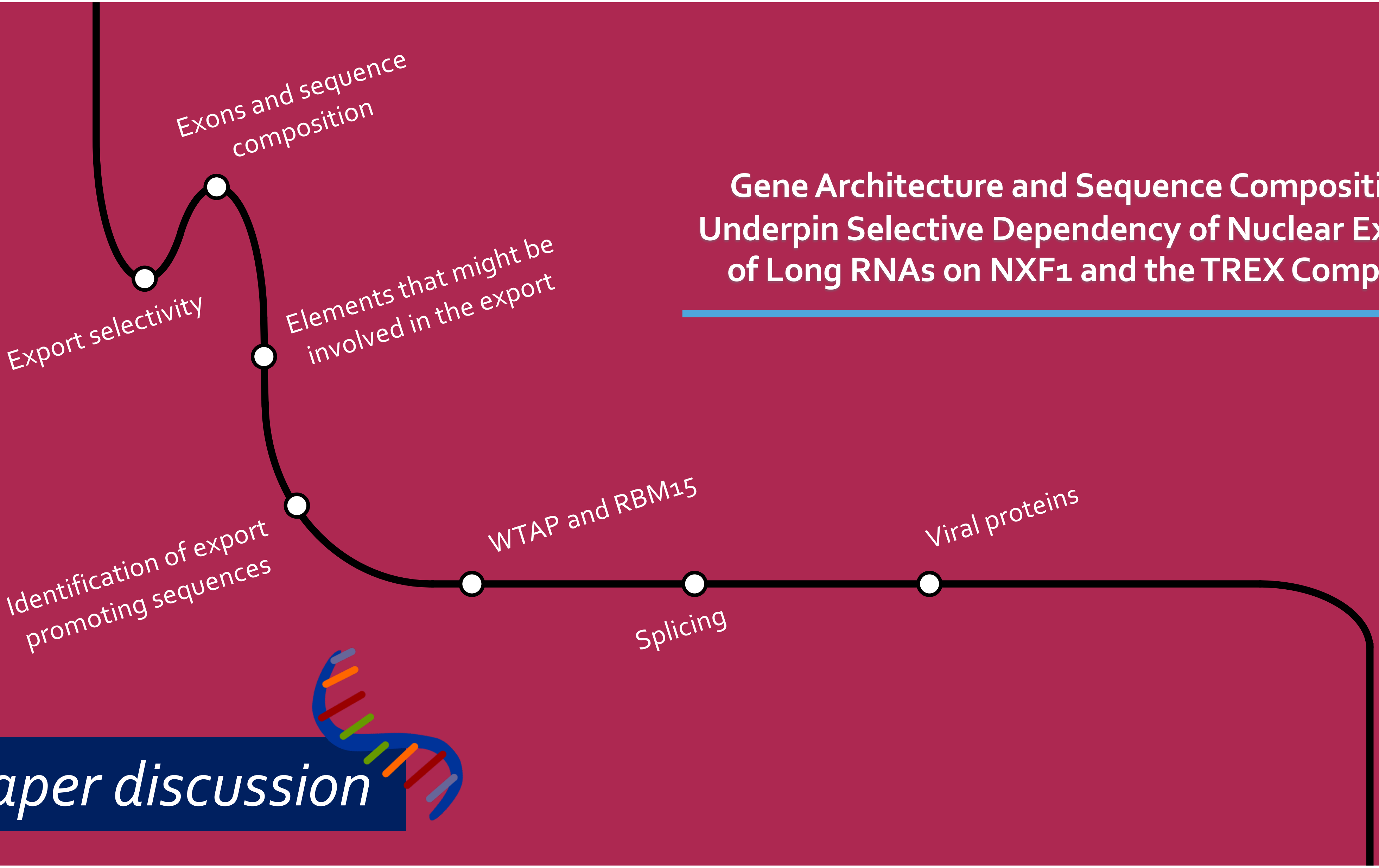
lncRNAs nuclear export pathway

It is known that some lncRNA use CRM1 for their export

Could they use NXF1 too?



Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex



Export selectivity

Exons and sequence composition

Elements that might be involved in the export

Identification of export promoting sequences

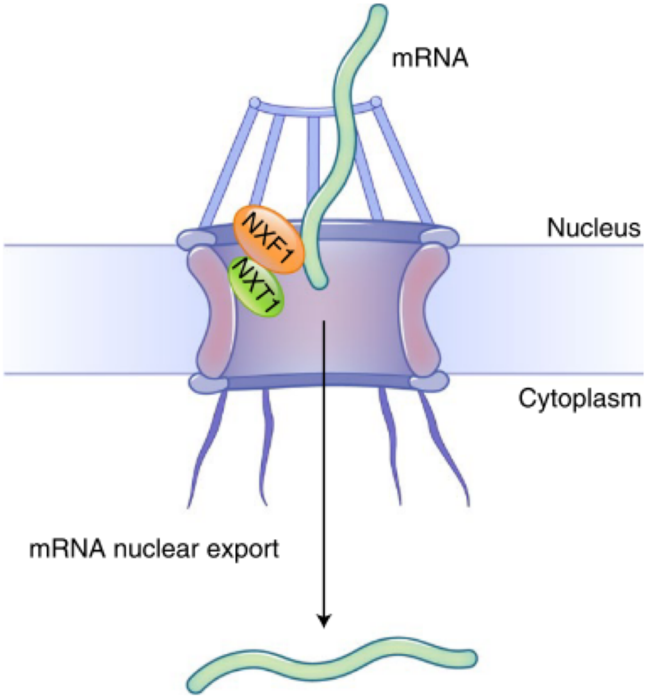
WTAP and RBM15

Splicing

Viral proteins

Paper discussion

Efficiency of the nuclear export



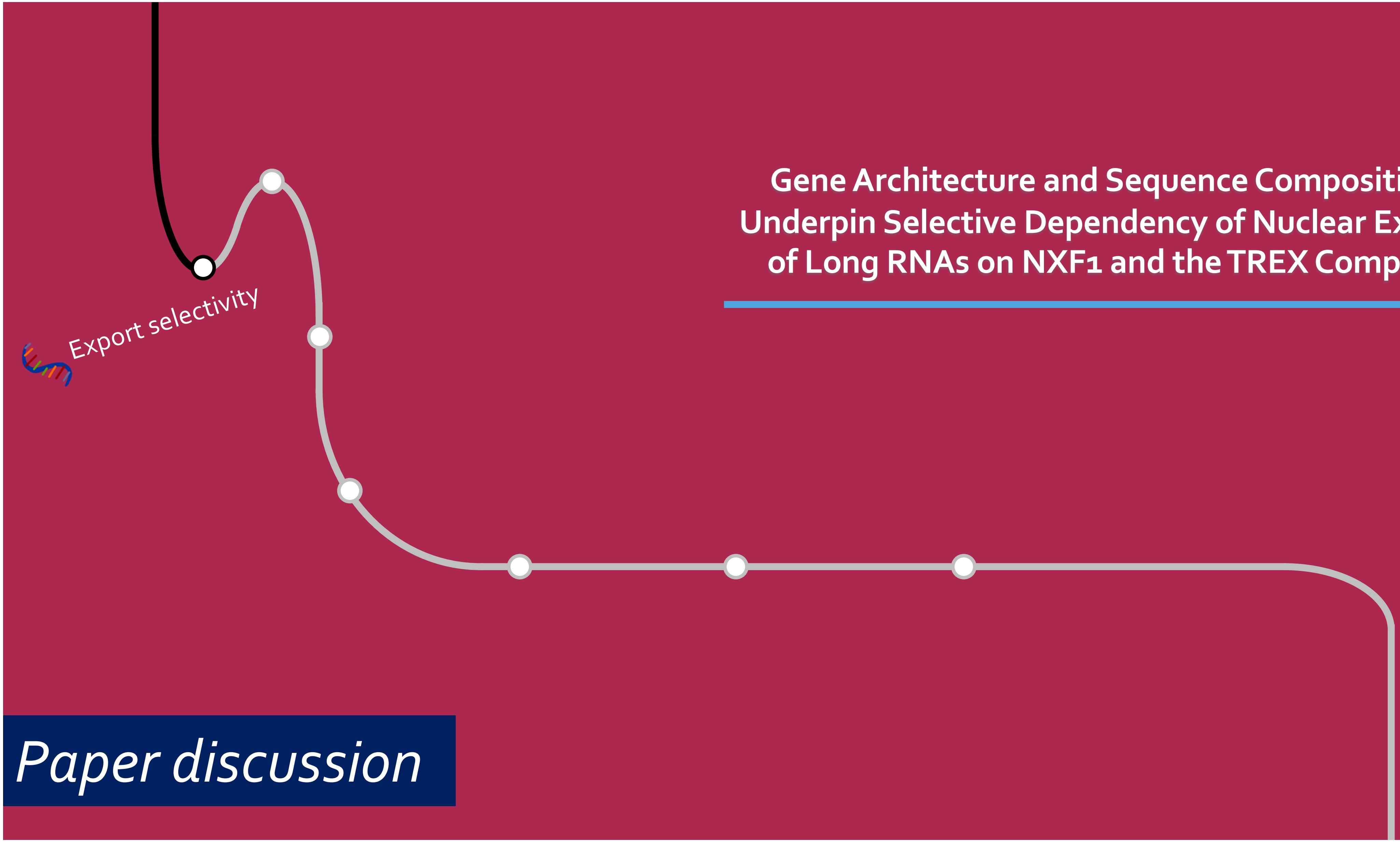
Splicing density and efficiency

Lenght of the RNA

Sequence composition

Chromatin environment

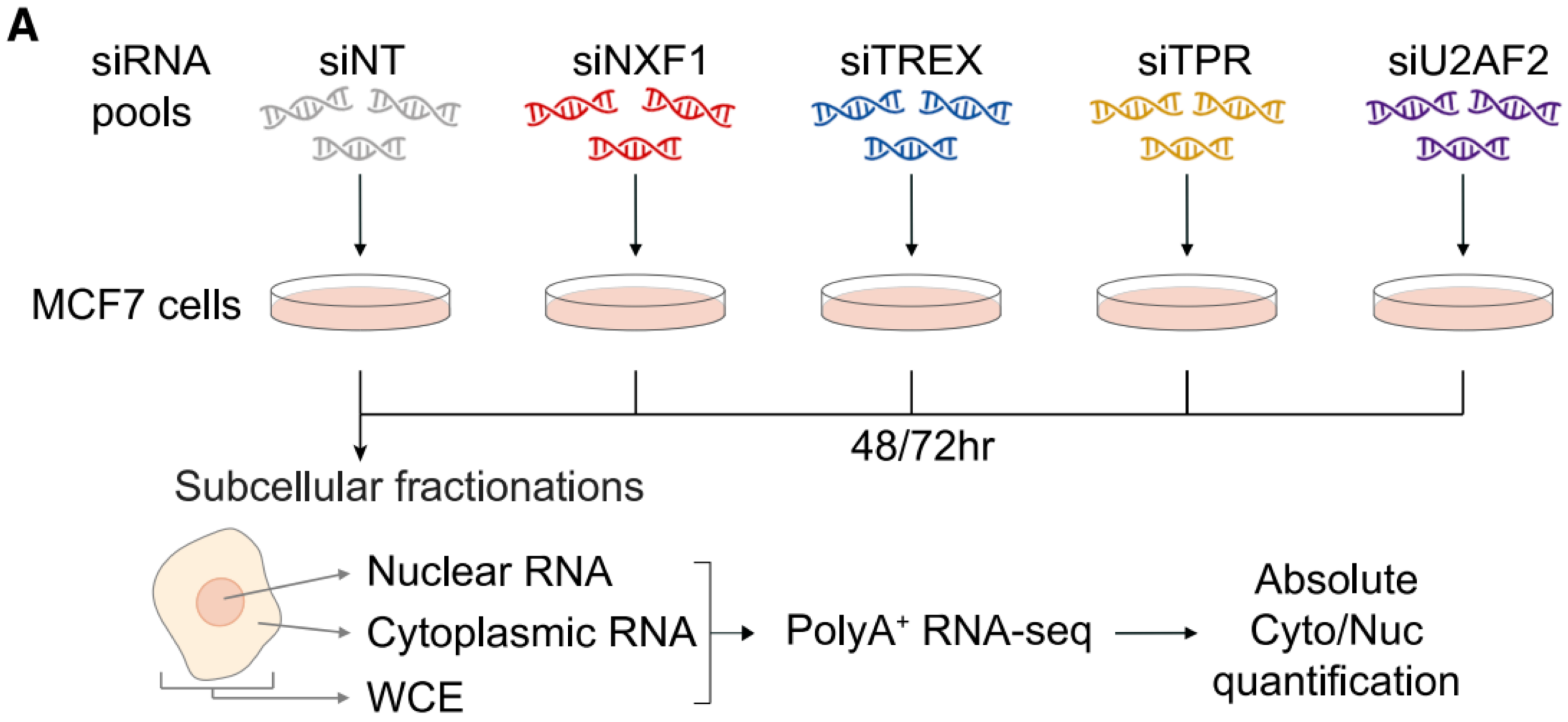
Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex



Paper discussion

Selectivity of canonical RNA export factors

Workflow



A

siRNA pools

siNT

siNXF1

siTREX

siTPR

siU2AF2

MCF7 cells

48/72hr

Subcellular fractionations

Nuclear RNA

Cytoplasmic RNA

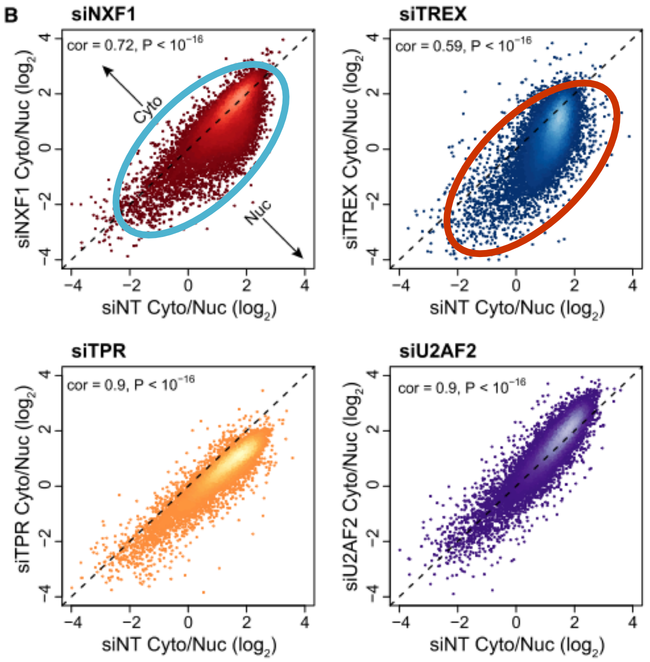
WCE

PolyA$^+$ RNA-seq

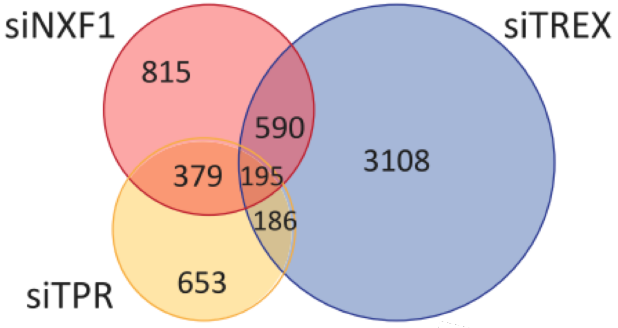
Absolute Cyto/Nuc quantification

Results

1) Subcellular localization

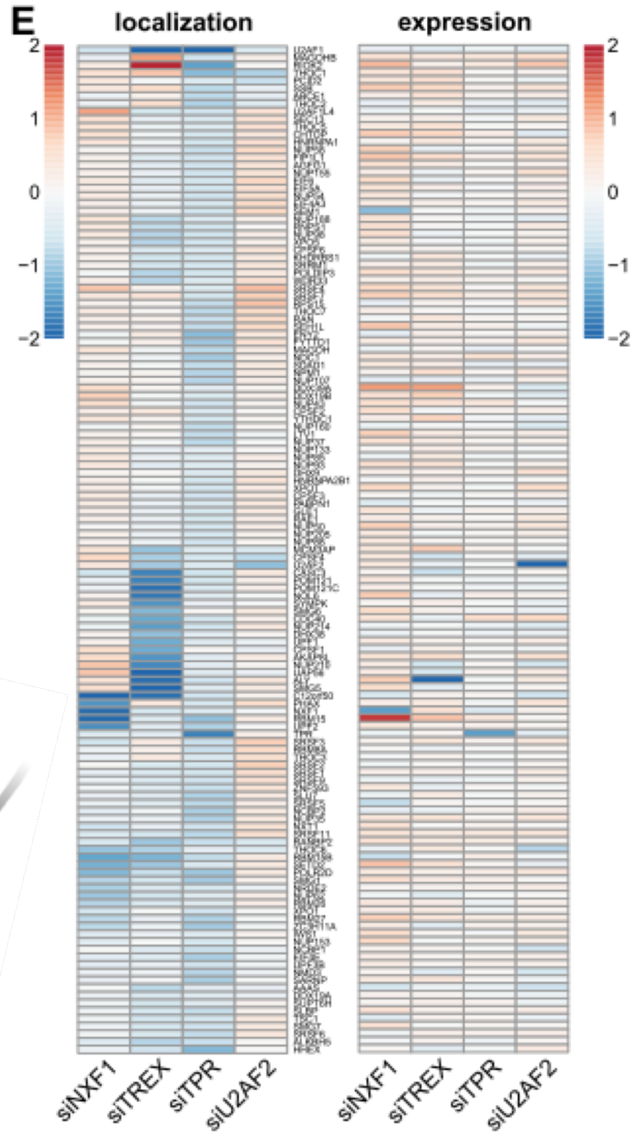


2) Groups affected



Gene Ontology analysis of «RNA export from nucleus»

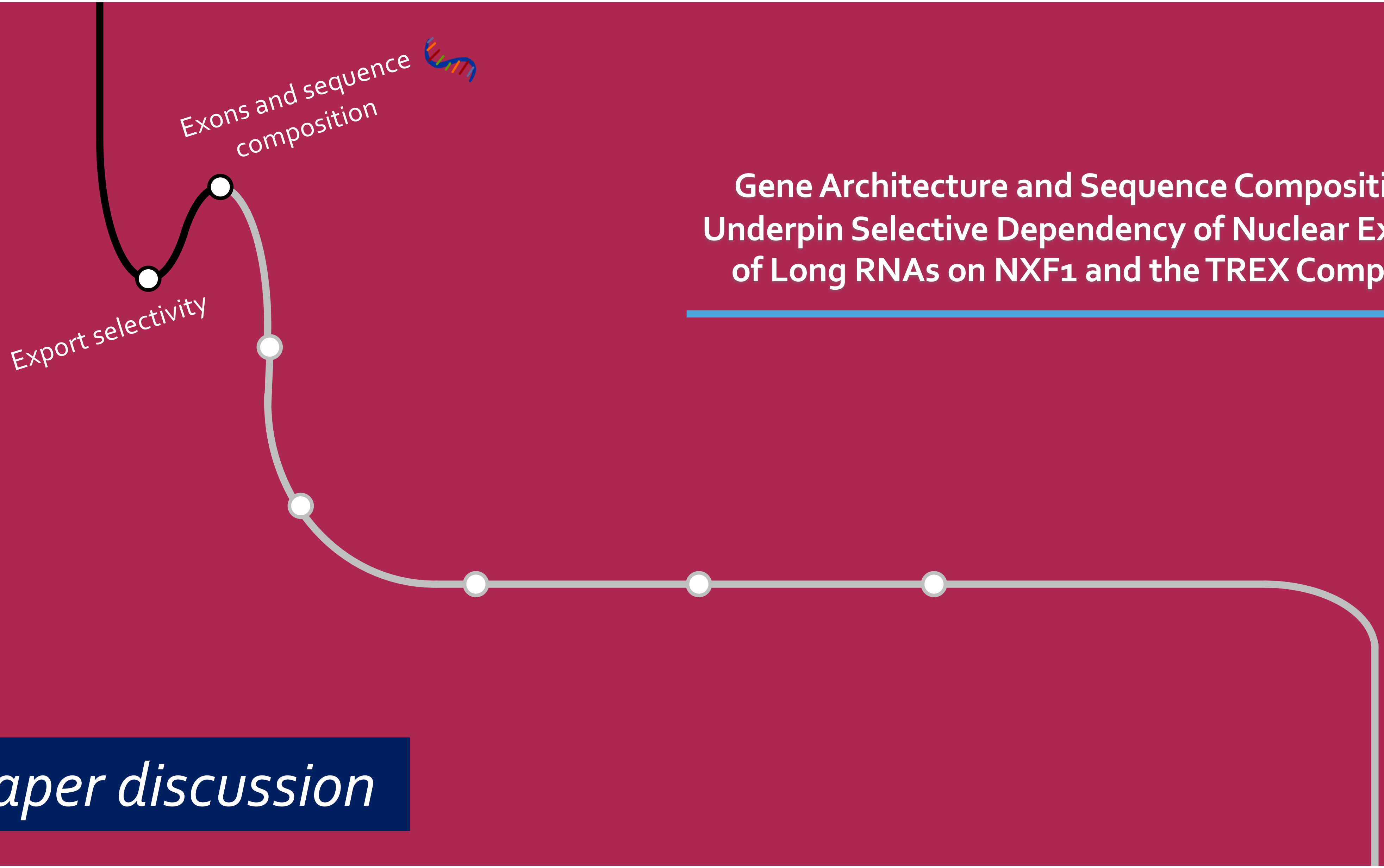
3) Feedback effects



RBM15 is induced by NXF1 depletion

Possible compensation mechanism?

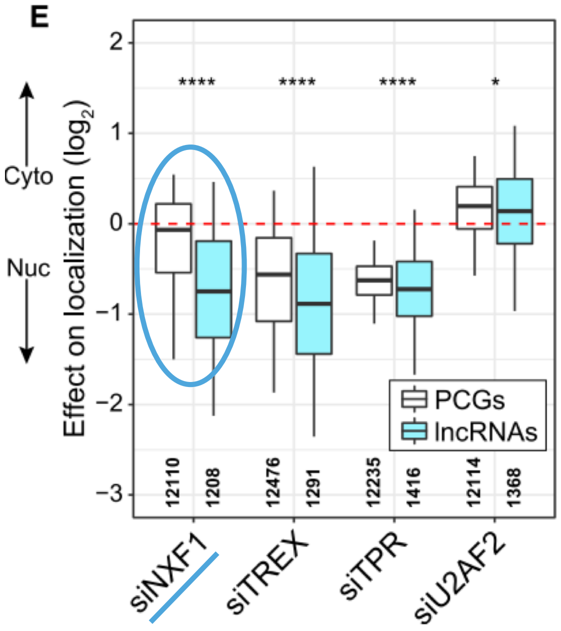
Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex


Exons and sequence composition

Export selectivity

Paper discussion

Protein Coding Genes VS lncRNAs

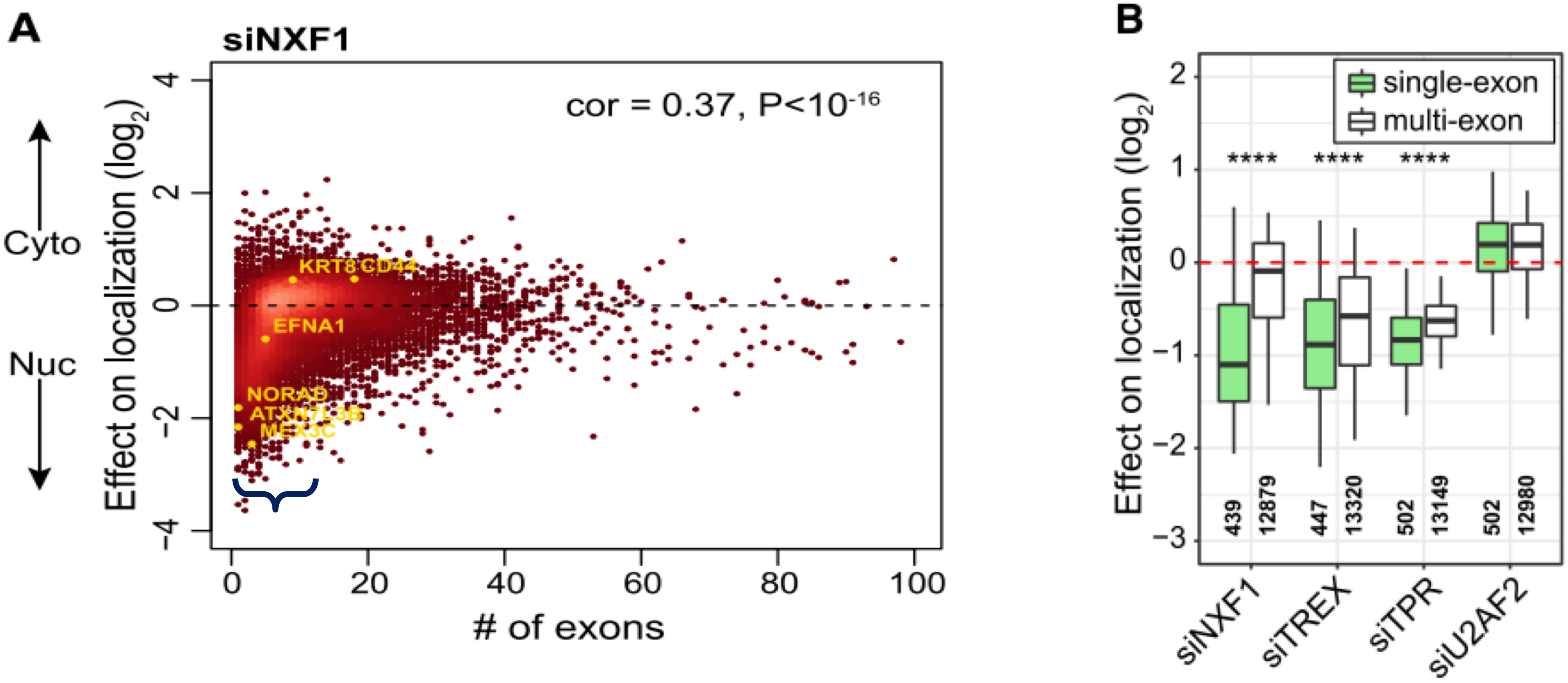


lncRNA are more sensitive to NXF1 depletion than PCGs

There might be specific features that explain this differency

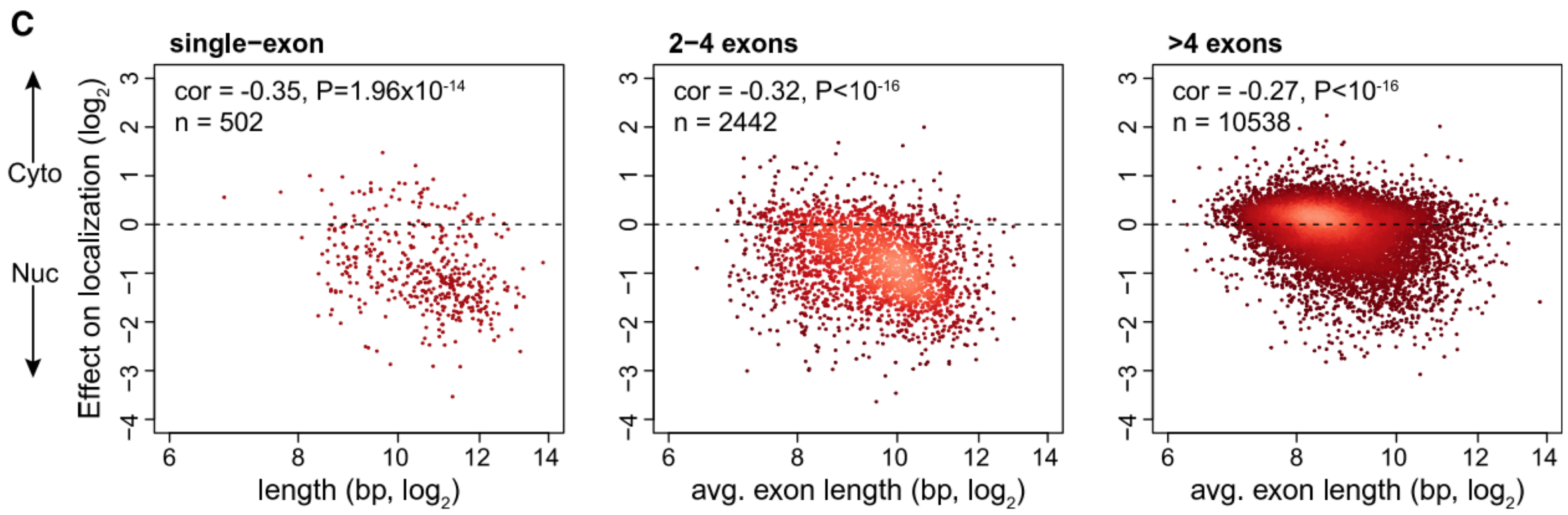
- Number of exons
- Exons lenght
- GC content
- Elements that might promote the export

Number of exons



Nuclear enrichment of transcripts with few exons

Exons lenght

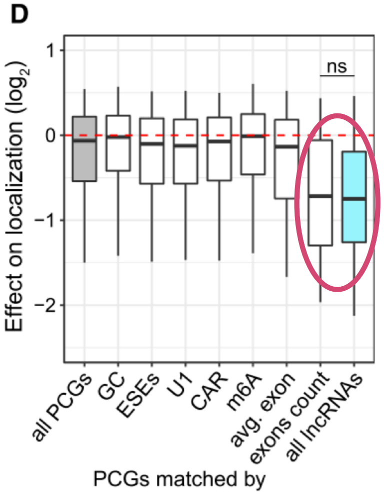


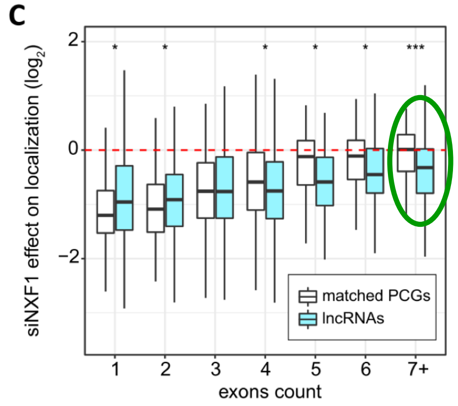
Transcripts with longer exons are more affected by NXF1 depletion

Gene architecture and sequence

PGCs mathching to lncRNAs were sampled

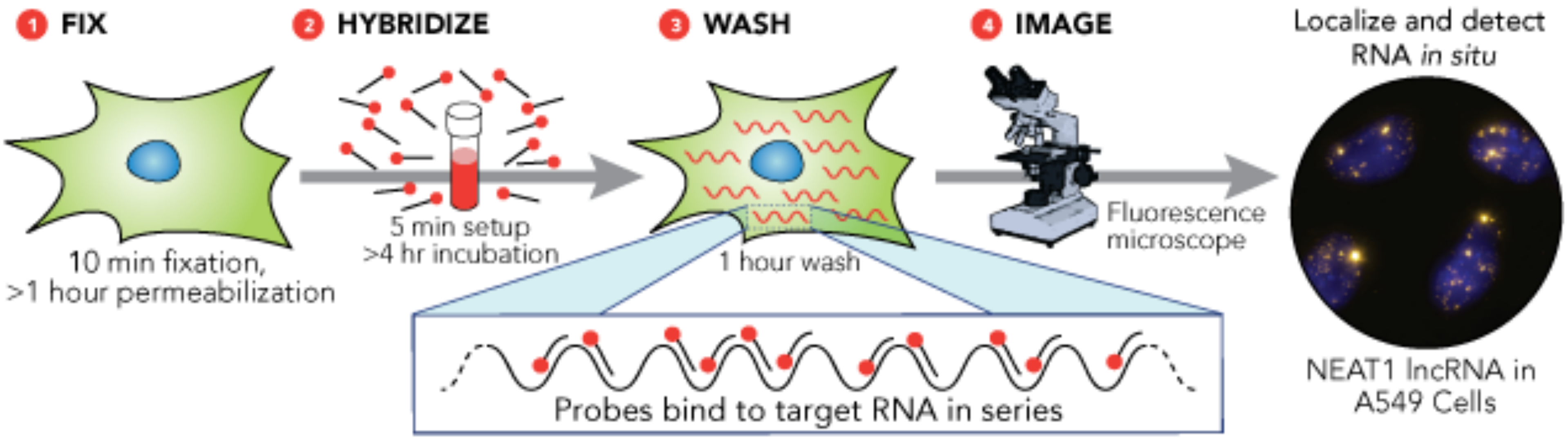
- CG content
- Exon count
- Average exonic lenght
- Enrichment with sequence features associated by previous studies with nuclear RNA export





The different sensitivity to NXF1 depletion is associated with differences in the number of exons

Single molecule FISH analysis

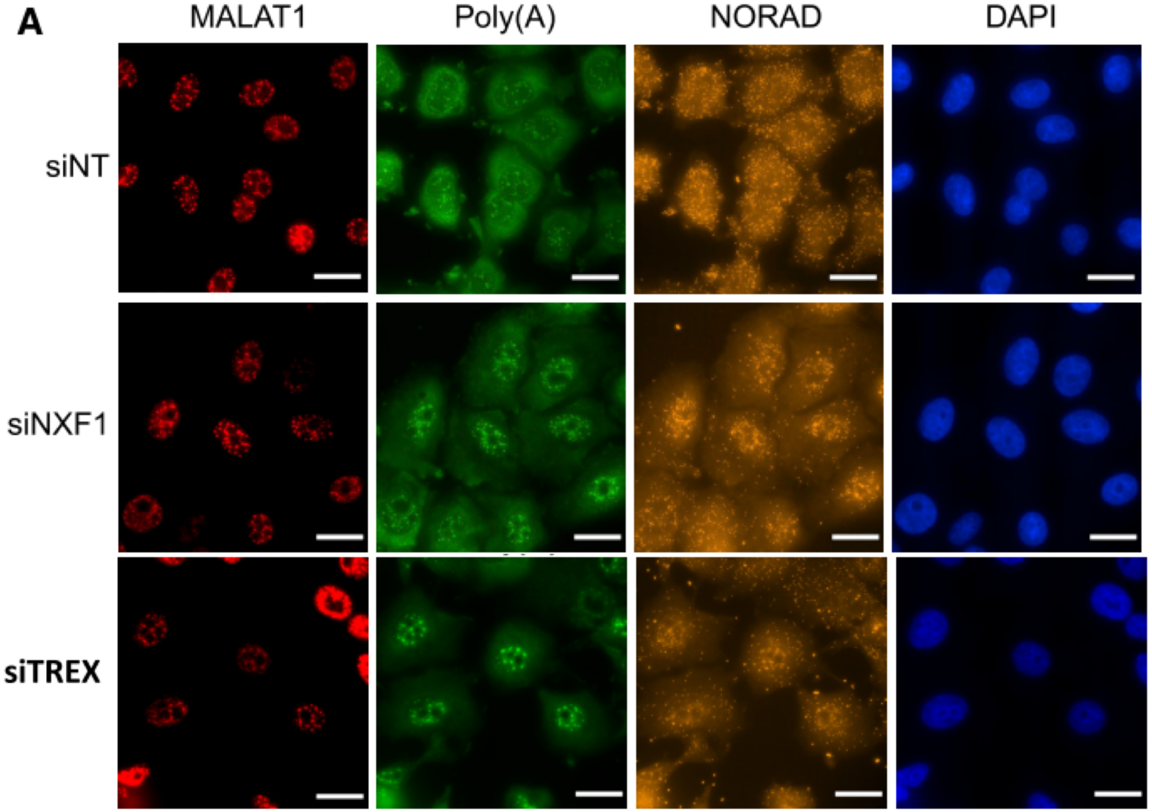


Single molecule FISH analysis

siNXF1 and siTREX → smFISH analysis

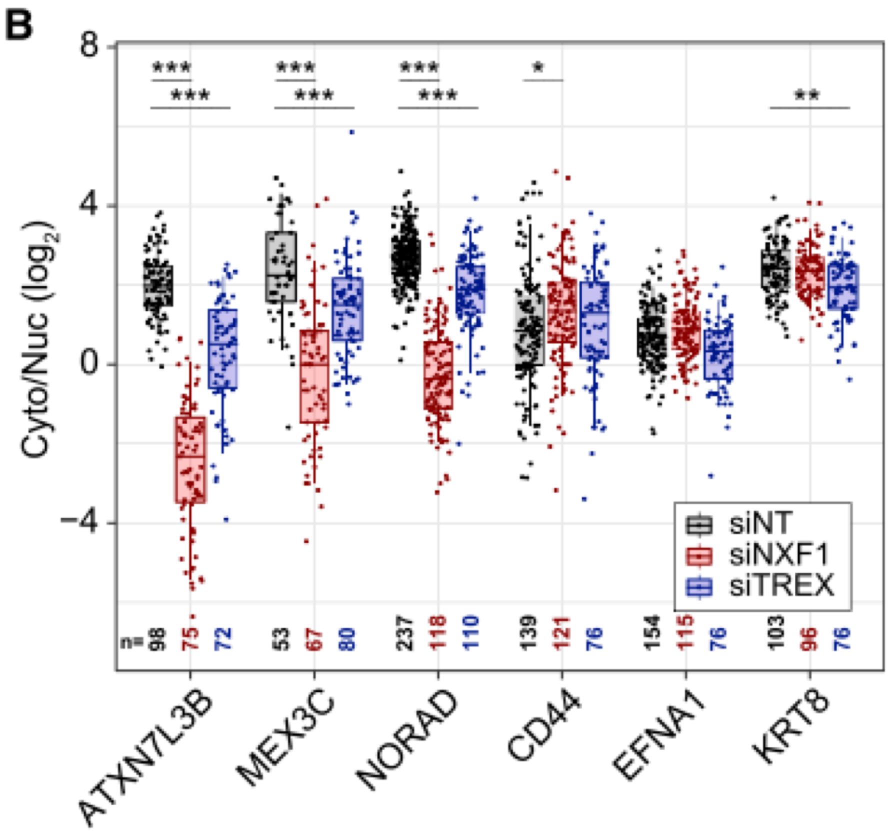
NXF1 sensitive gene

- NORAD, an a mostly cytopl
- ATXN7L3B, ar
- MEX3C, a P intron



multi-exon and tive genes

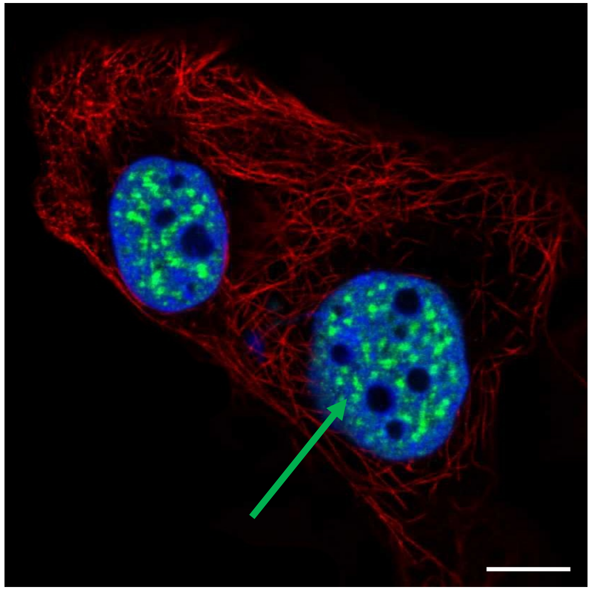
Single molecule FISH analysis



Transcripts with few exons are retained in the nucleus after NXF1 or TREX depletion

Subnuclear distribution of RNA

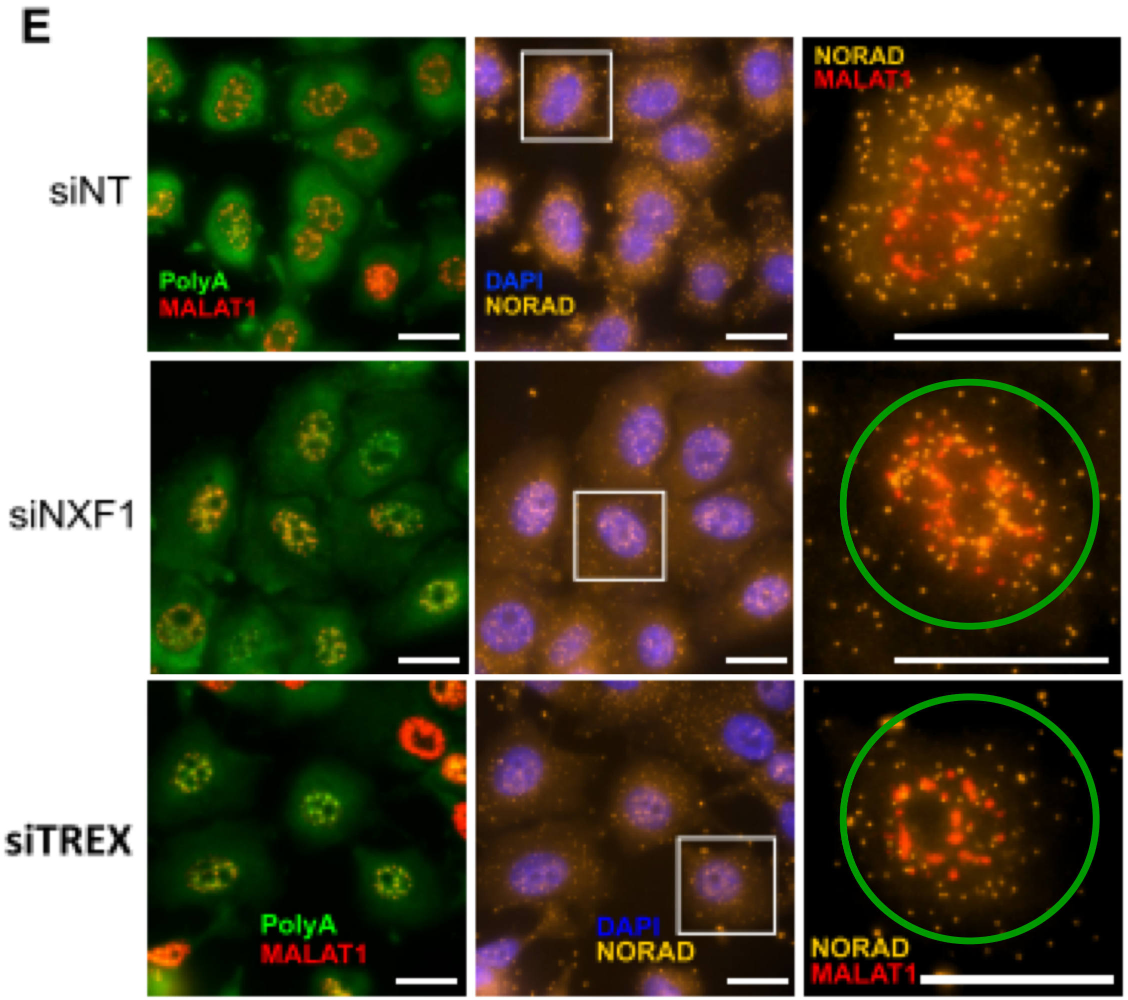
Nuclear speckles or interchromatin granule clusters (green)



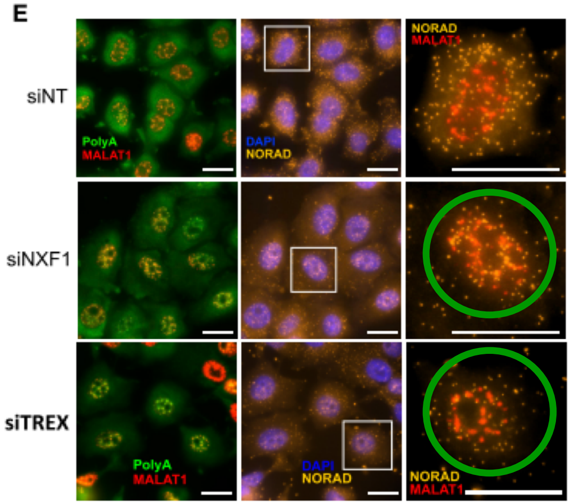
(From *The Human Protein Atlas*)

- Enriched in pre-mRNA splicing factors
- Located in the interchromatin regions of the nucleoplasm of mammalian cells
- Involved in the export of intronless transcripts

Subnuclear distribution of RNA



Subnuclear distribution of RNA



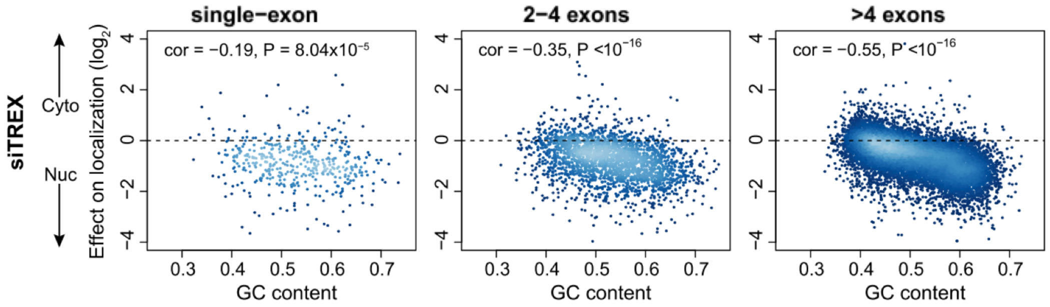
NORAD accumulates in nuclear speckles after NXF1 depletion, but not in TREX-depleted cells

↓

Export factors depletion differencially affects the subnuclear RNA distribution

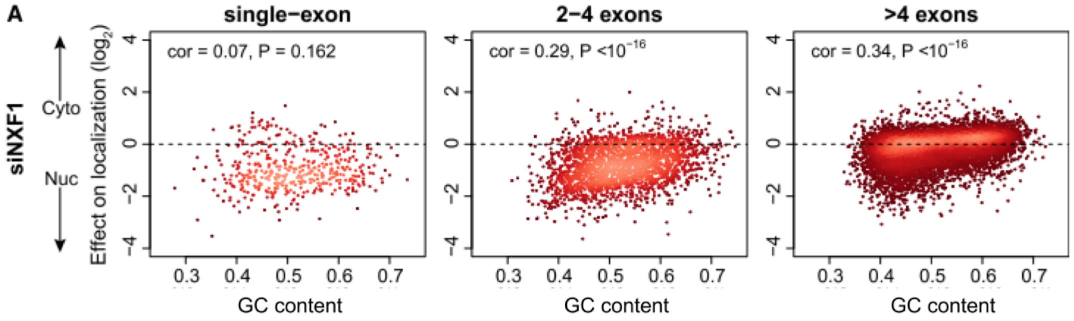
Differencial nuclear enrichment with siTREX and siNXF1

siTREX



Correlation between nuclear enrichment after TREX depletion and G/C content

siNXF1



Correlation between nuclear enrichment after NXF1 depletion and **A/U content only for multi-exon genes**

Single-exon transcripts, transcripts with long exons and A/U-rich multi exons transcripts depend on NXF1 for their export

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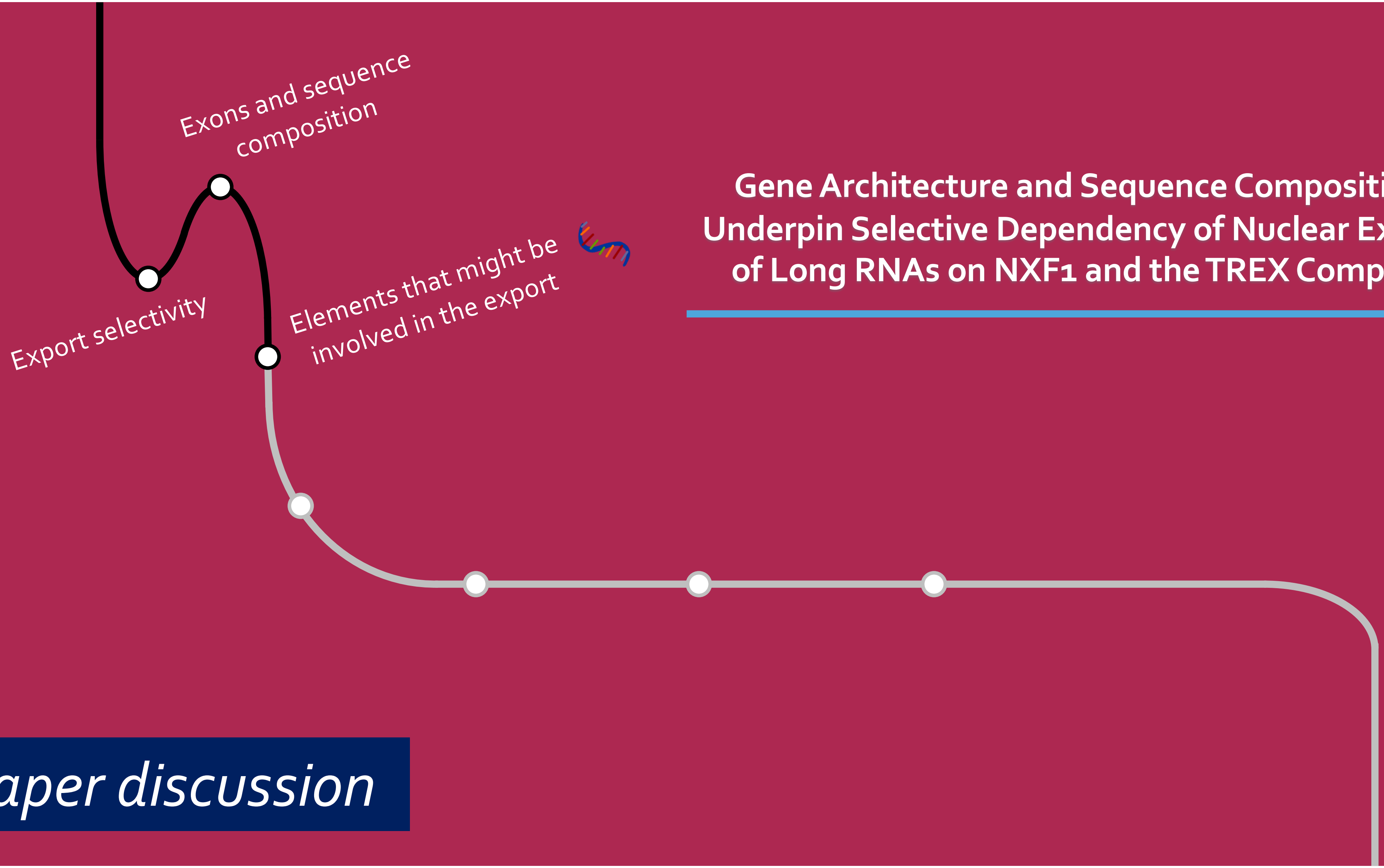


Exons and sequence composition

Export selectivity

Elements that might be involved in the export

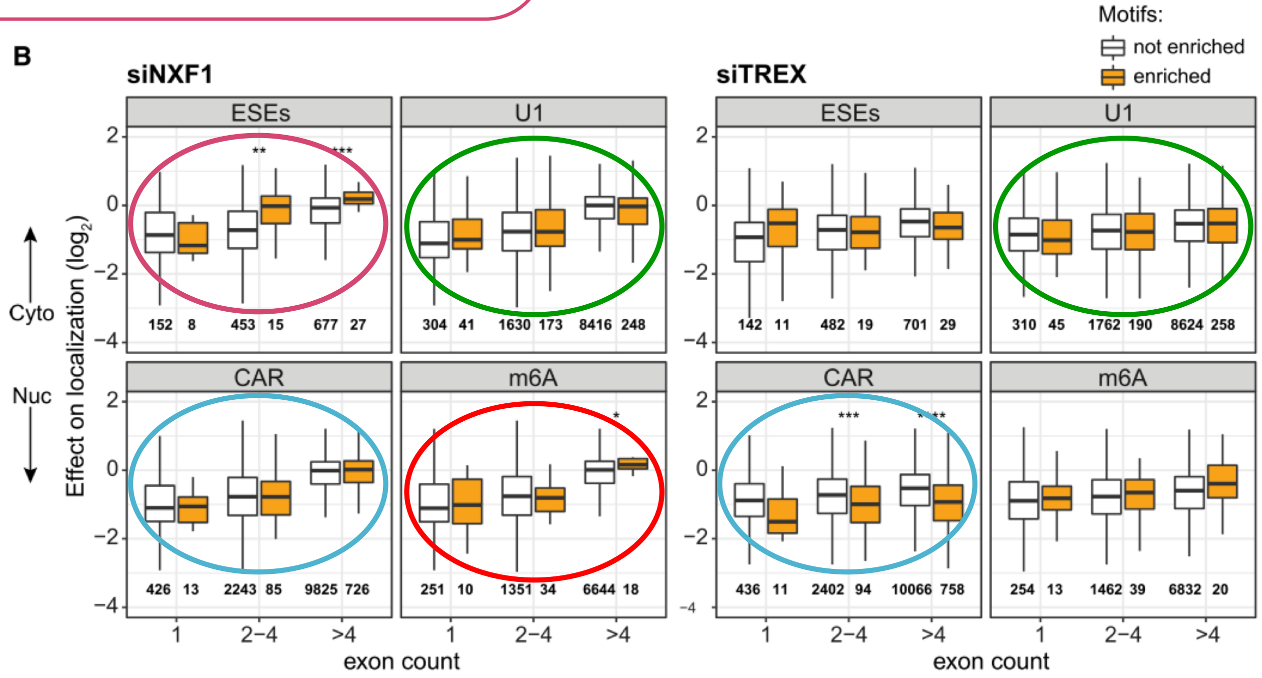
Paper discussion

Potential export associated elements

siNXF1 and siTREX ⟶ **Analysis of the enrichment of lcnRNAs whit specific elements**

Recruitment of splicing related factors is partially involved in the protection against NXF1 depletion

U1 binding site had minor effect



CAR is relevant for nuclear export, but not specifically for the export of single-exon transcripts

m6A is highly present in genes retained in the nucleus after NXF1 depletion

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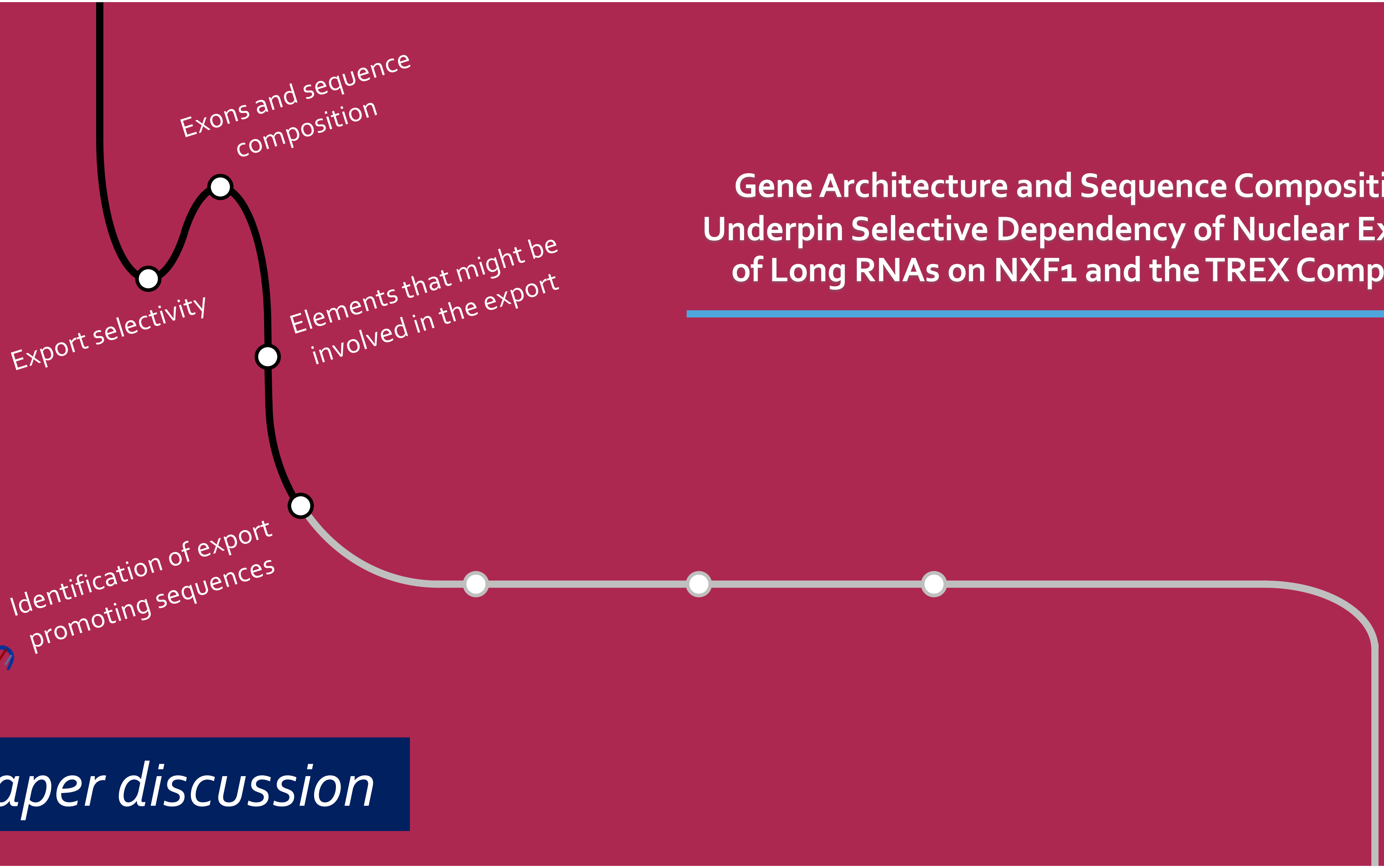
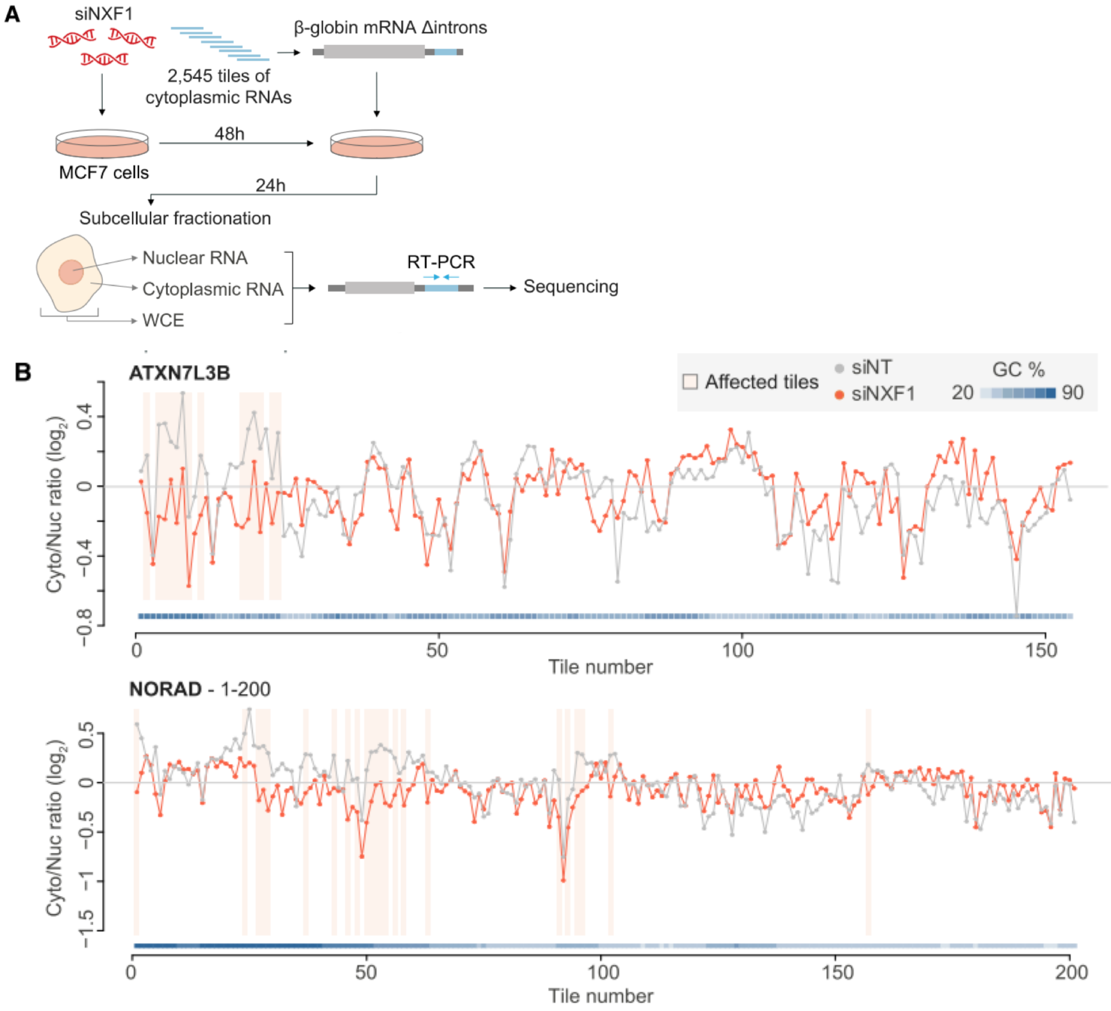


Paper discussion

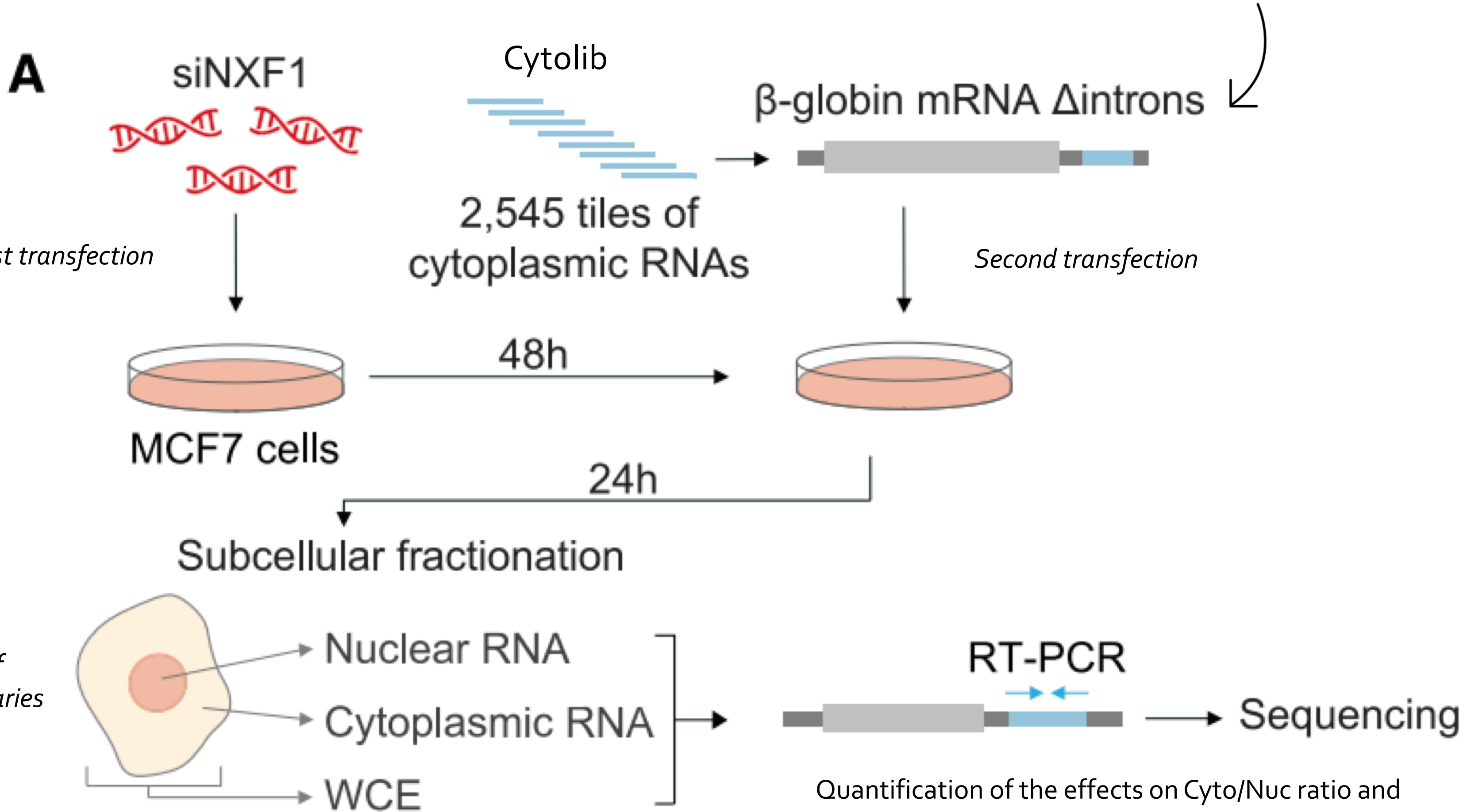
Export of genes with few exons

Nuclear export of intronless transcripts is NXF1-dependent

Is it promoted by specific sequences?



Workflow



Relatively inefficiently exported

First transfection

Second transfection

Preparing of sequencing libraries

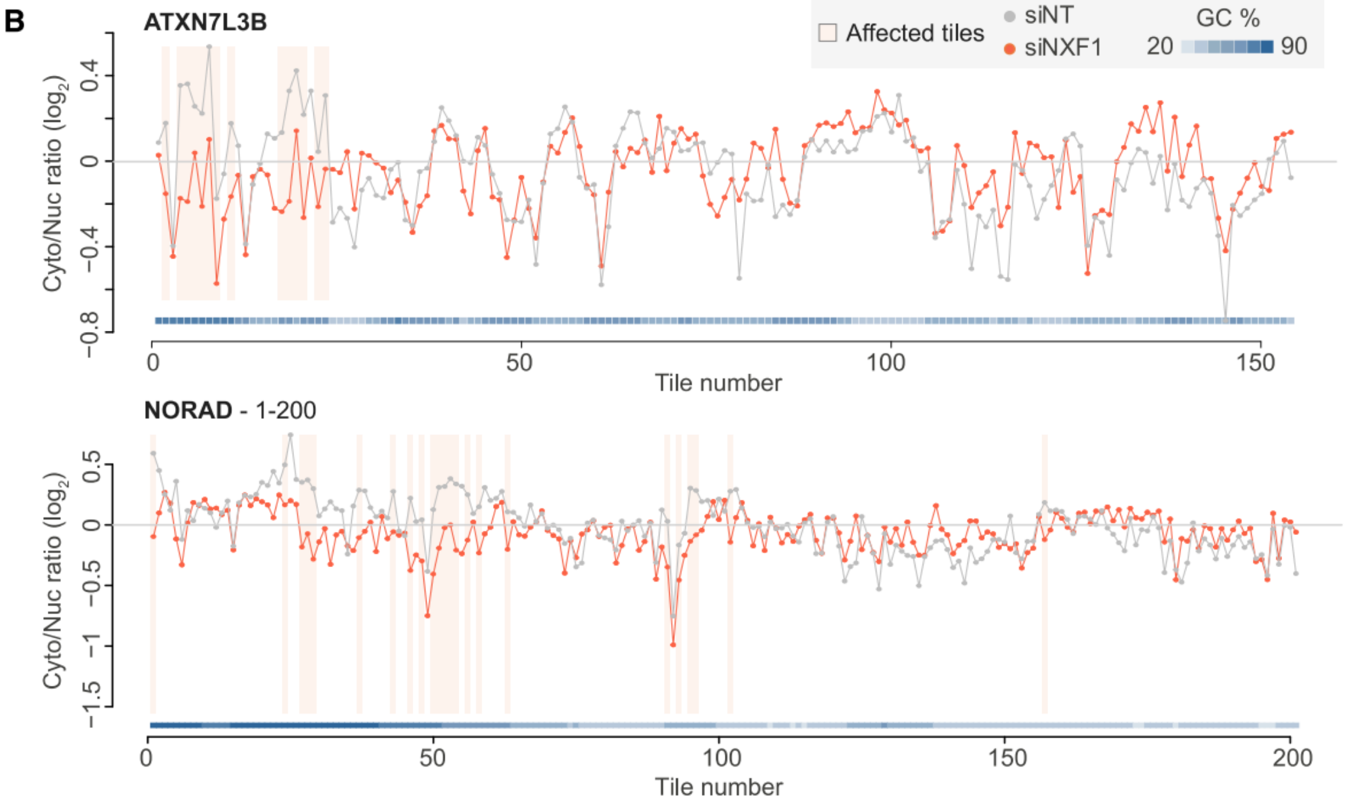
Quantification of the effects on Cyto/Nuc ratio and expression levels

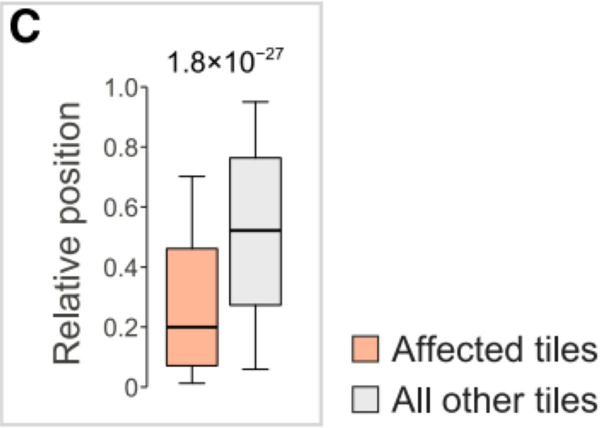
Combined the results from five biological replicates

257 sequences from all the genes tested exhibited significant sensivity to NXF1 depletion

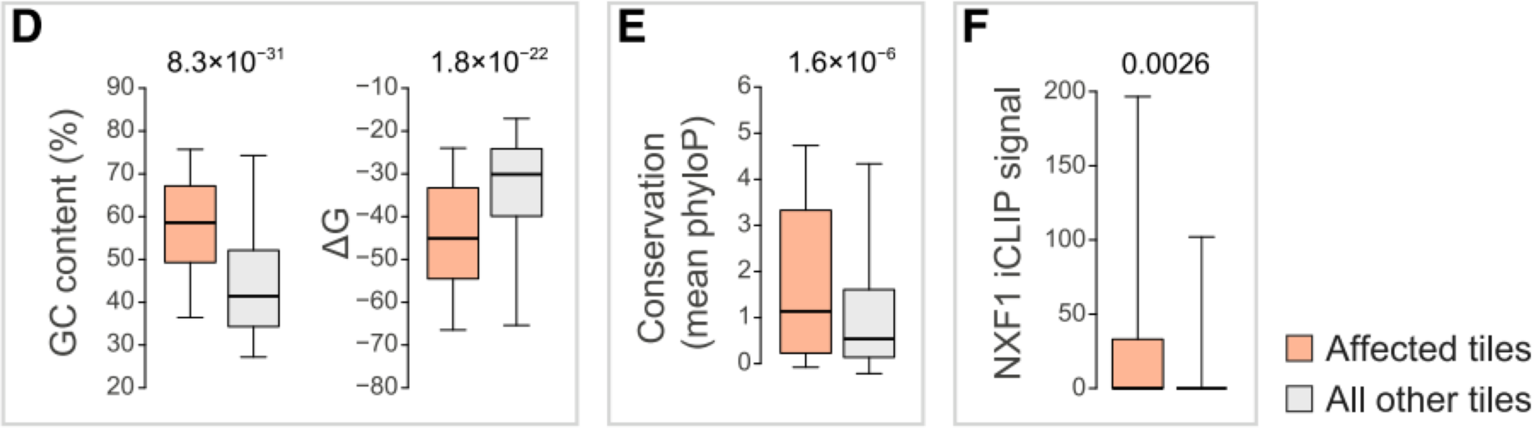
181 tiles associated with nuclear enrichment

76 tiles associated with cytoplasmic enrichment





Characteristics of tiles driving NXF1-dependet export



More G/C rich and predicted to form more stable secondary structures

Evolutionally conserved

↓

Important function

Able to recruit NXF1

lncRNA with one or few exons that are efficiently exported contain focal regions able to drive the NXF1-dependent export

Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex

Export selectivity

Exons and sequence composition

Elements that might be involved in the export

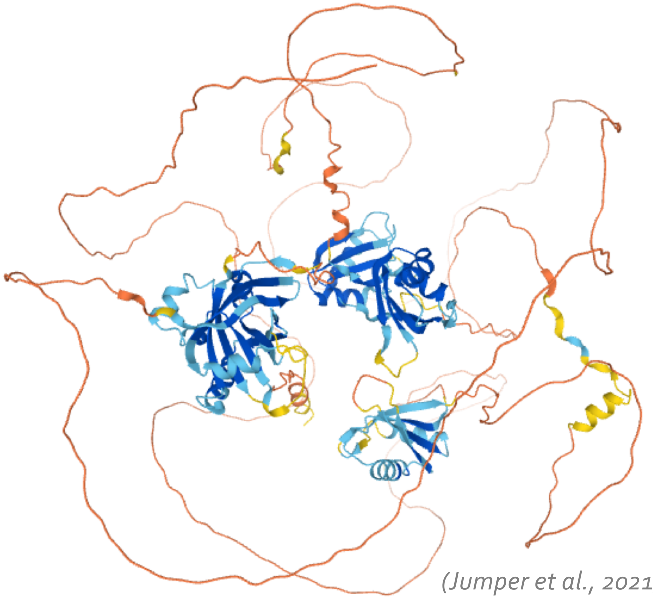
Identification of export promoting sequences

WTAP and RBM15

Paper discussion

Identification of proteins binding the tiles

Intersection between the 181 tiles associated with nuclear enrichment and enhanced CLIP data from ENCODE

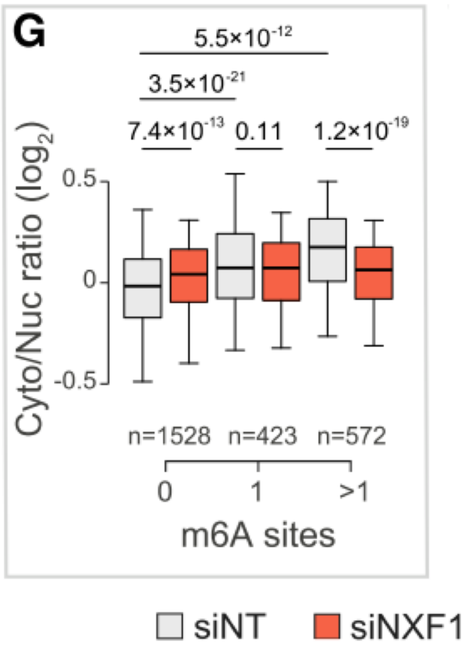


(Jumper et al., 2021)

RBM15
Implicated in NXF1-dependent export

RBM15 and m6A involvement

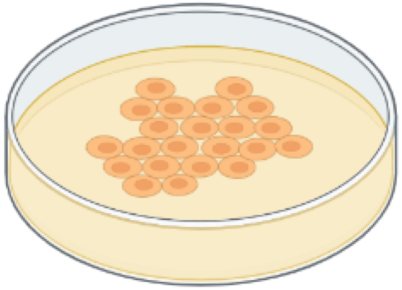
Tiles that overlap experimentally determined m6A sites significantly increased export in an NXF1-dependent manner

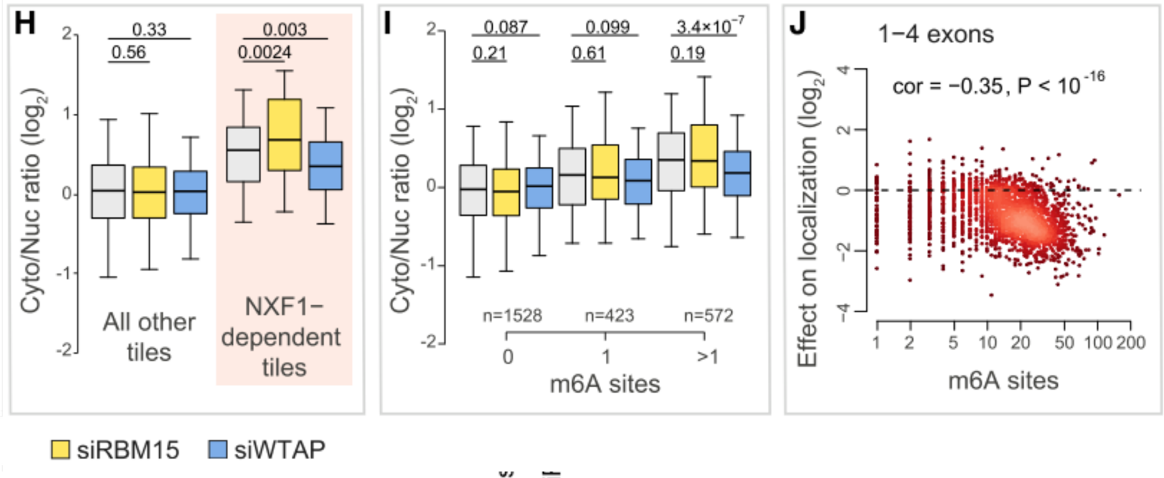


RBM15 and m6A might be involved in nuclear export

RBM15 and m6A involvement

Knock down of RBM15, RBM15B and WTAP

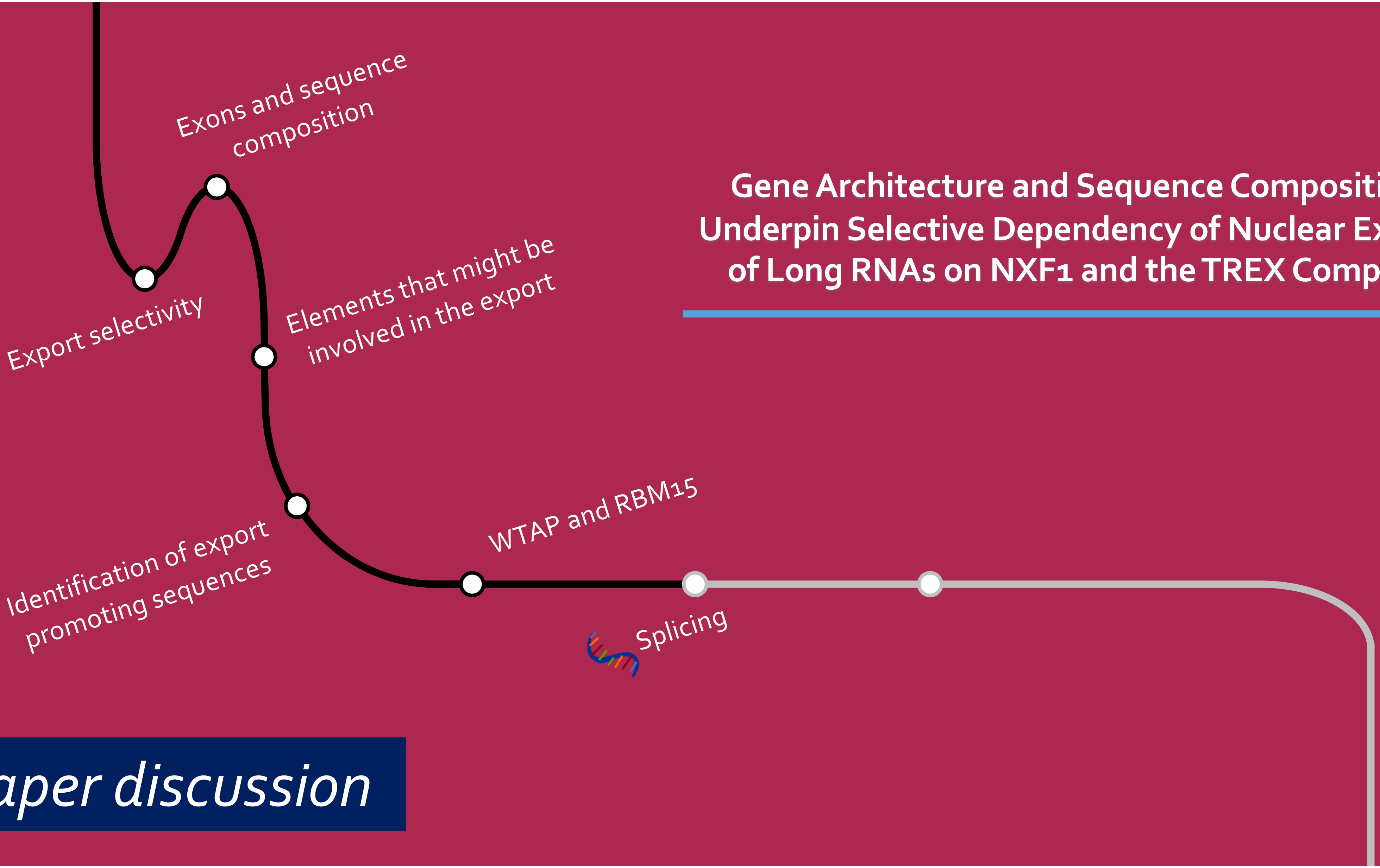




Analysis of the localization of Cytolib tiles after KO

m6A is crucial for export of lncRNA, in particular for those with few exons

Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex



Export selectivity

Exons and sequence composition

Elements that might be involved in the export

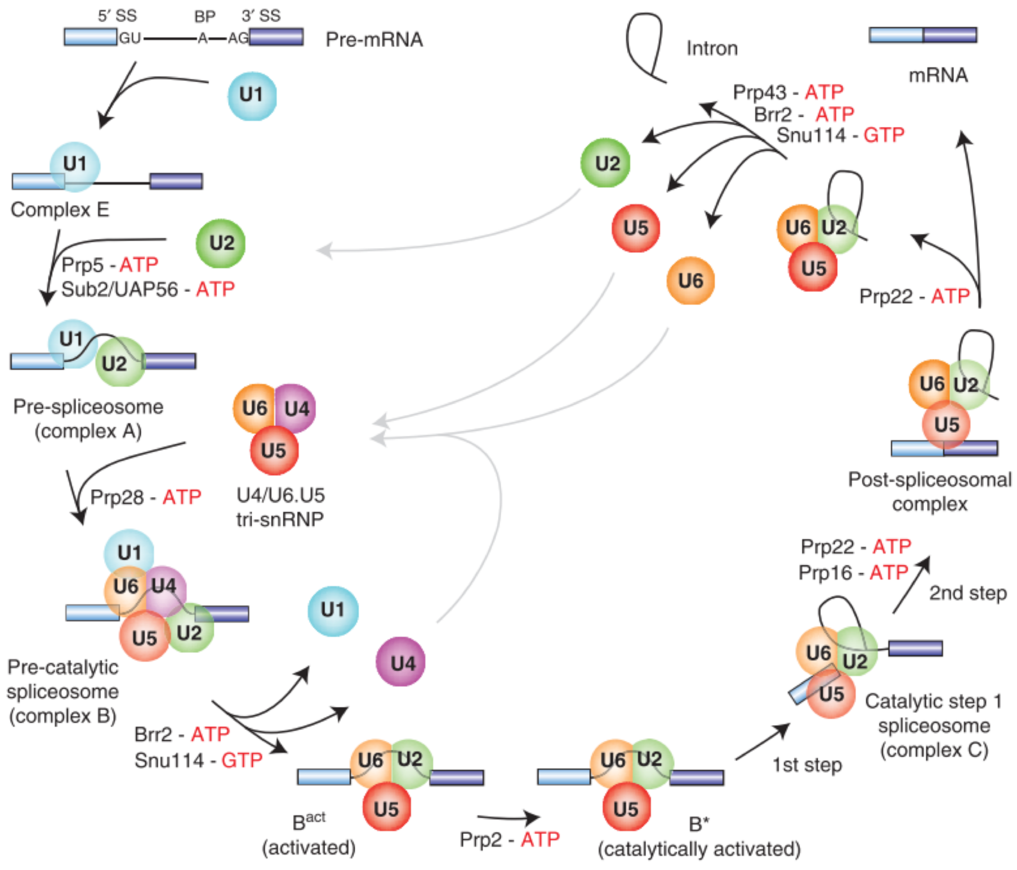
Identification of export promoting sequences

WTAP and RBM15

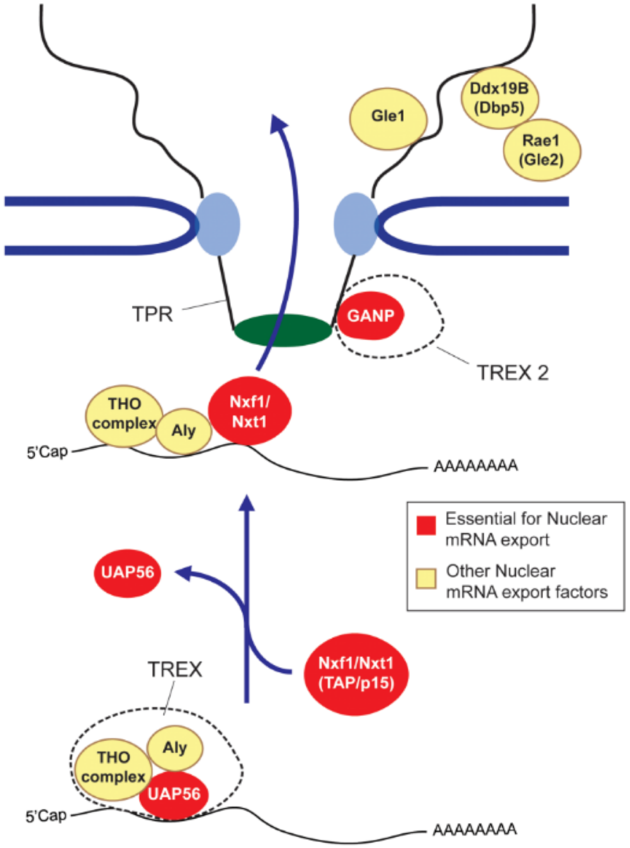
Splicing

Paper discussion

Splicing efficiency and NXF1 export



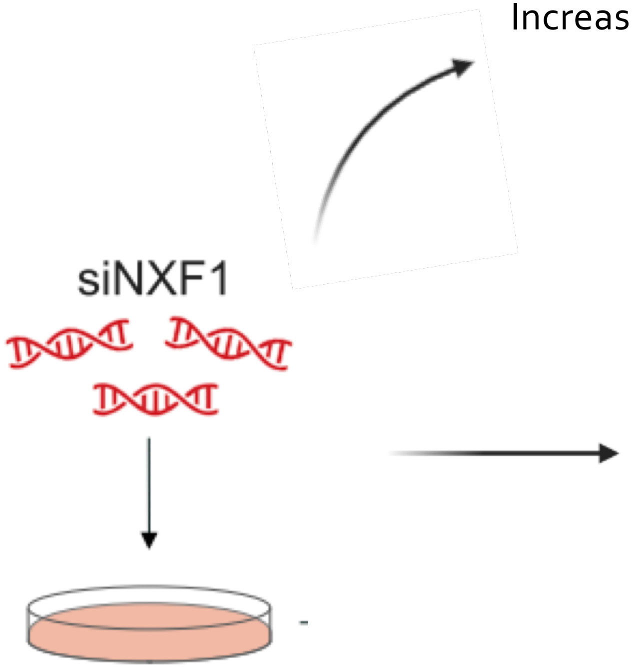
(Will & Lührmann, 2011)

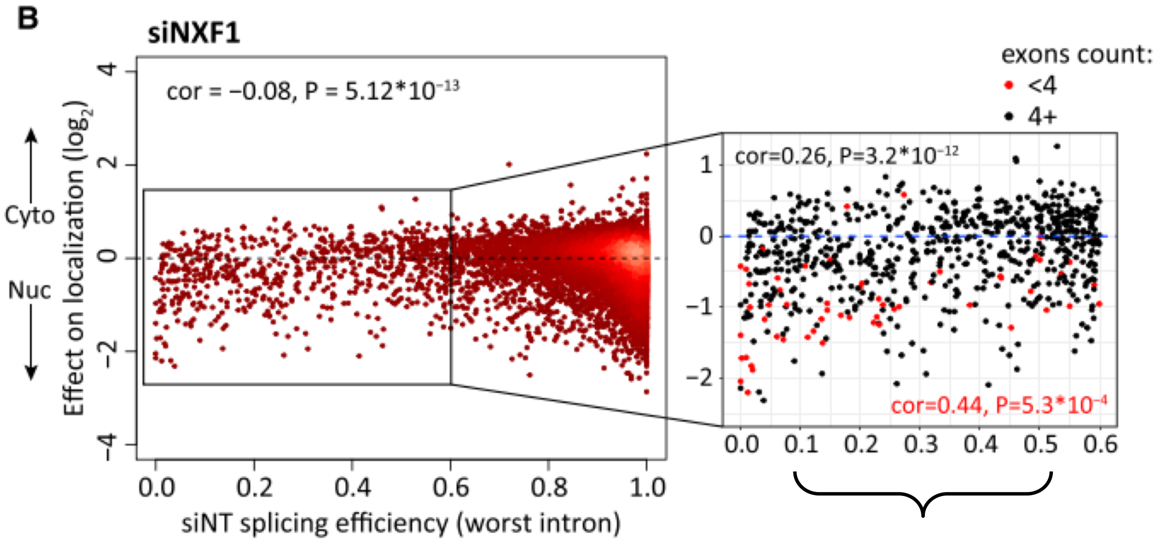


(Palazzo & Lee, 2018)

Testing the relationship

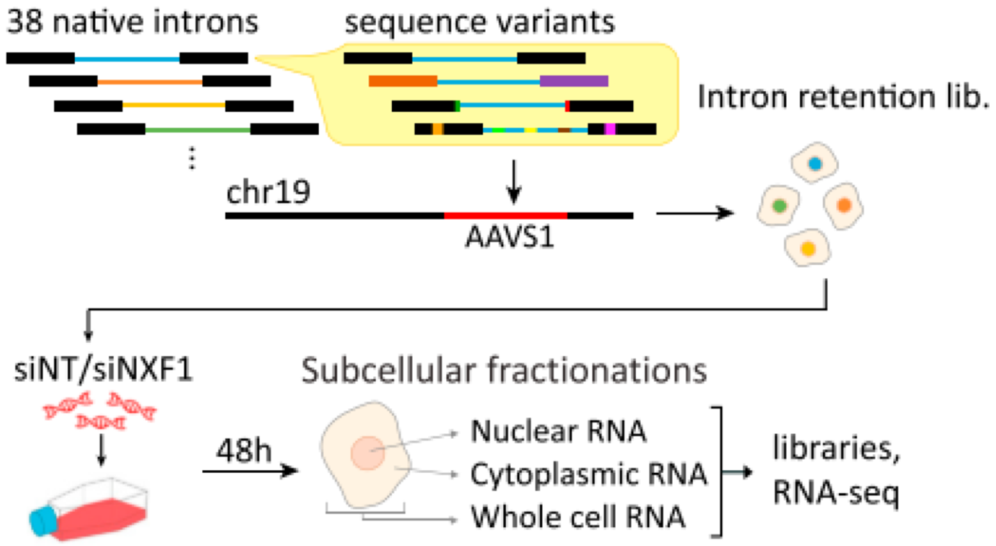
Increase in splicing efficiency in the nuclear fraction

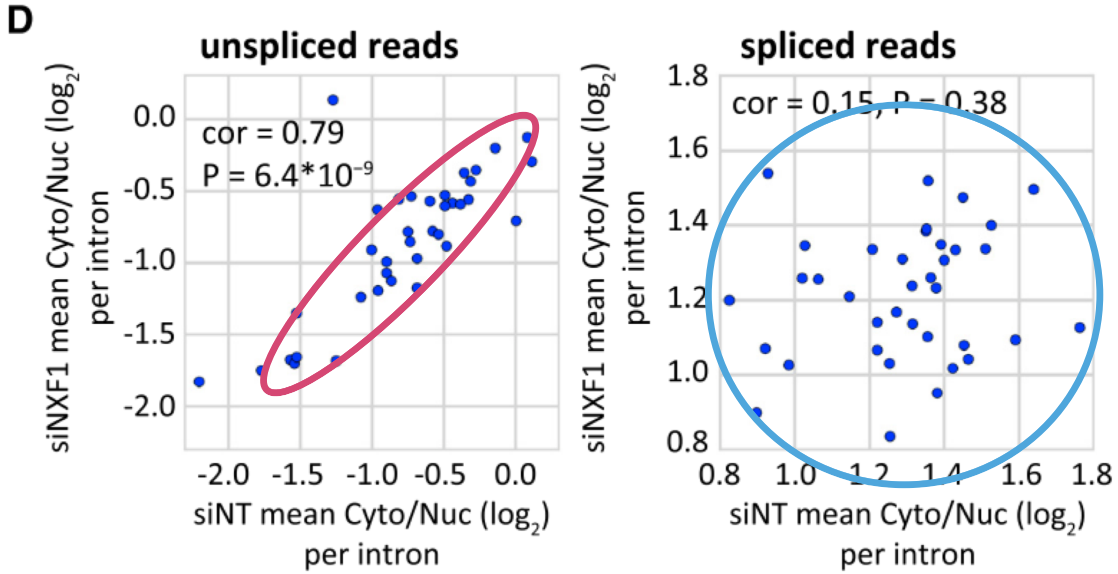


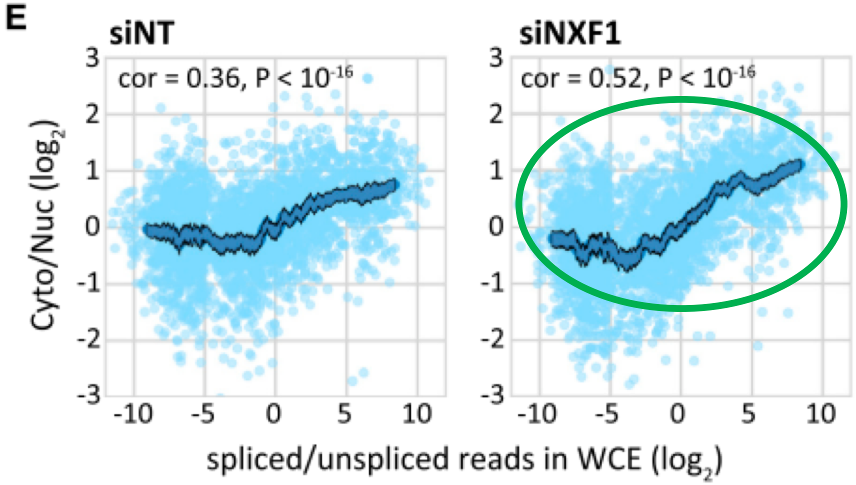


Transcripts with retained introns

Association between inefficient splicing and nuclear retention upon depletion of NXF1







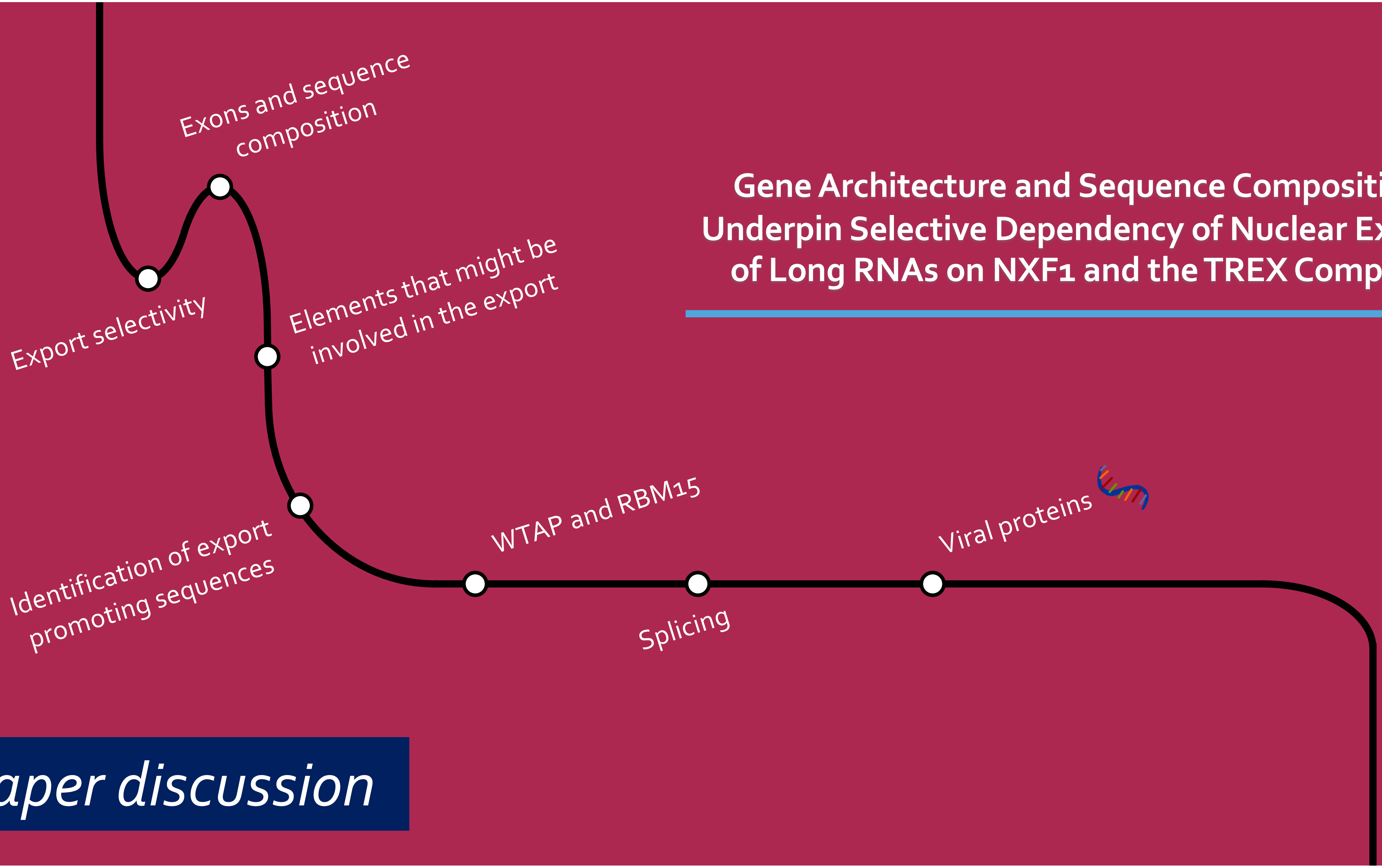
Increased correlation betweend splicing efficiency and Cyto/Nuc ratio

Splicing enhances non NXF1 nuclear export

Low splicing efficiency of some transcripts might activate NXF1-dependent nuclear export

(Maybe to overcome nuclear retention sequences in their introns)

Gene Architecture and Sequence Composition Underpin Selective Dependency of Nuclear Export of Long RNAs on NXF1 and the TREX Complex



Export selectivity

Exons and sequence composition

Elements that might be involved in the export

Identification of export promoting sequences

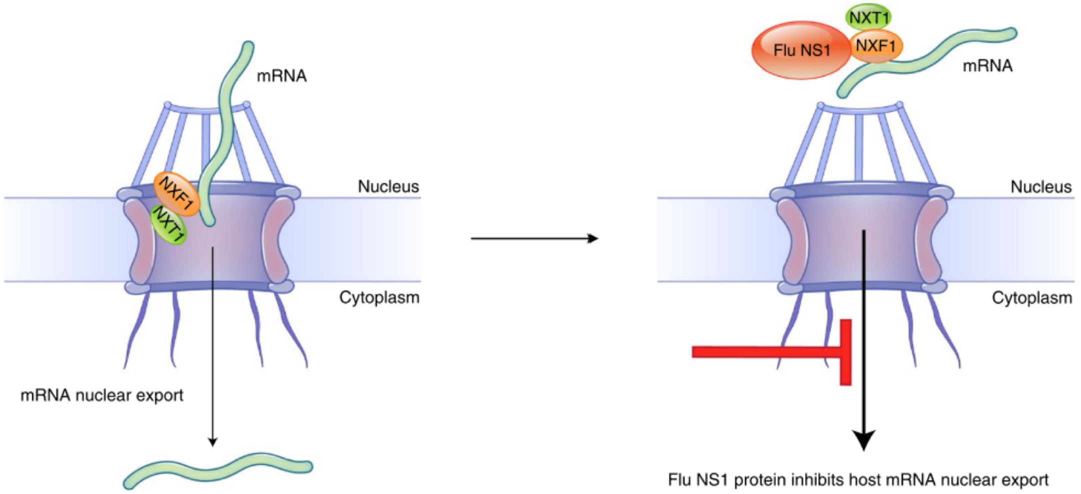
WTAP and RBM15

Splicing

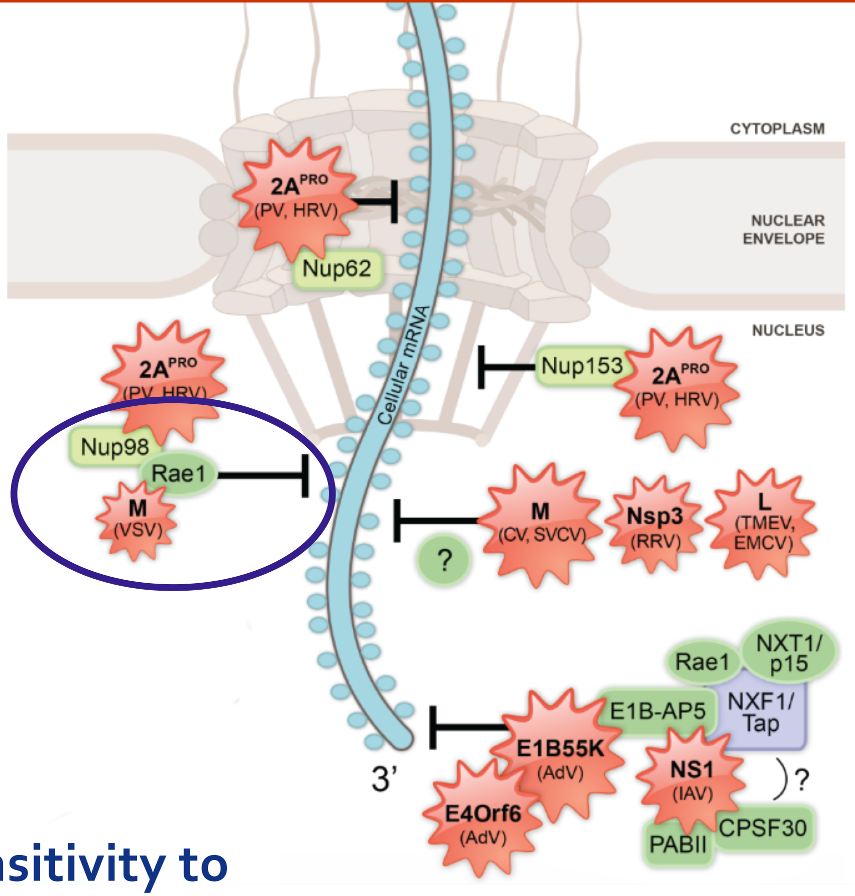
Viral proteins

Paper discussion

Viral infections and NXF1 export



(Zhang et al., 2019)

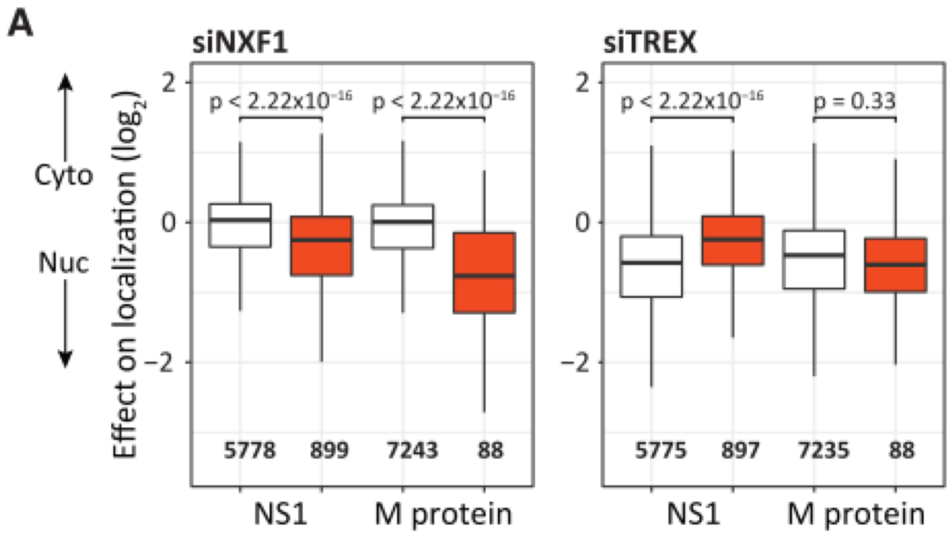


(Kuss et al., 2013)

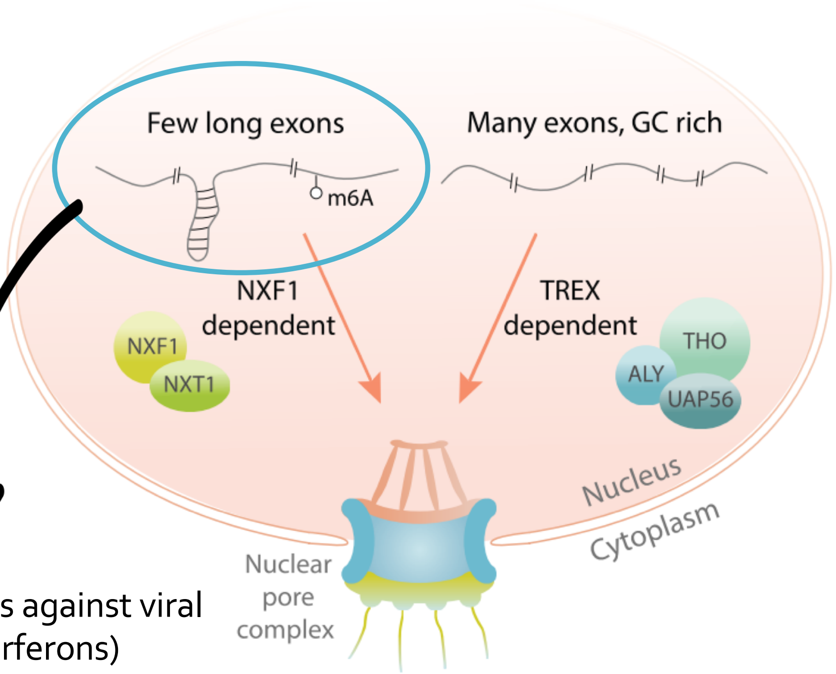
Export block occurs with selectivity both for viral and host mRNa in infected cells

→ **Correlation with different sensitivity to NXF1 depletion?**

Results



NXF1 is used for export of RNA with few exons



Overlap between genes retained in the nucleus in presence of M protein and NS1 and those retained after NXF1 depletion

Immediate-early genes against viral infection (*ie*: interferons)

The selective sensitivity to NXF1 has consequences during viral infections

Conclusions

NXF1 dependent lncRNAs export

- Low splicing efficiency might activate it
- Splicing enhances non-NFX1 export
- m6A is important for export of lncRNAs with few exons
- Exons number regulate sensitivy to NXF1 depletion
- NXF1 is necessary for export of
  - Single exon transcripts
  - Transcripts with long exons
  - A/U-rich multi-exons transcripts
- lncRNA exported contain focal regions to drive NXF1 dependent export

Thanks!