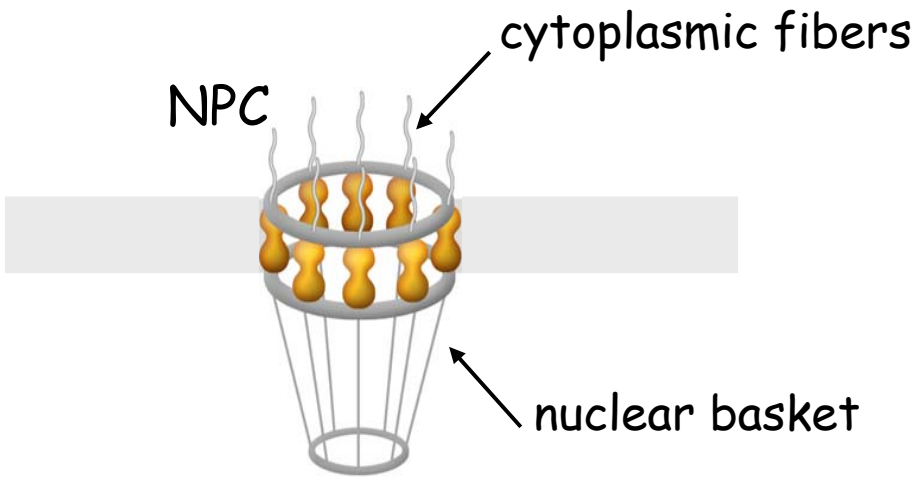
RECAP FROM MONDAY AND TUESDAY LECTURES

Nucleo-cytoplasmic transport factors:

interact with the target macromolecule (signal recognition)
interact with the NPC (nucleoporin Phe - Gly repeats)

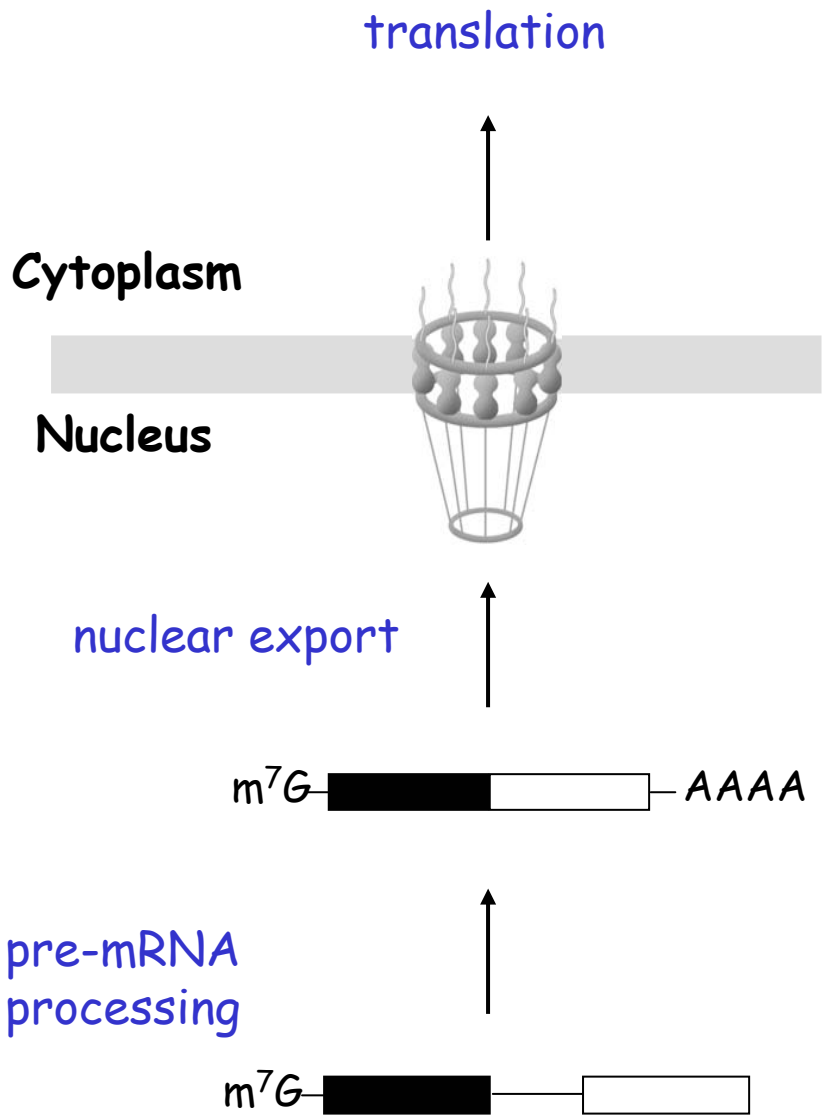


Nucleocytoplasmic transport factors:

Karyopherins (importins/exportins)
import proteins / export tRNA, snRNA, rRNA
regulated by the GTPase Ran

NTF2 import Ran

Search for mRNA export factors



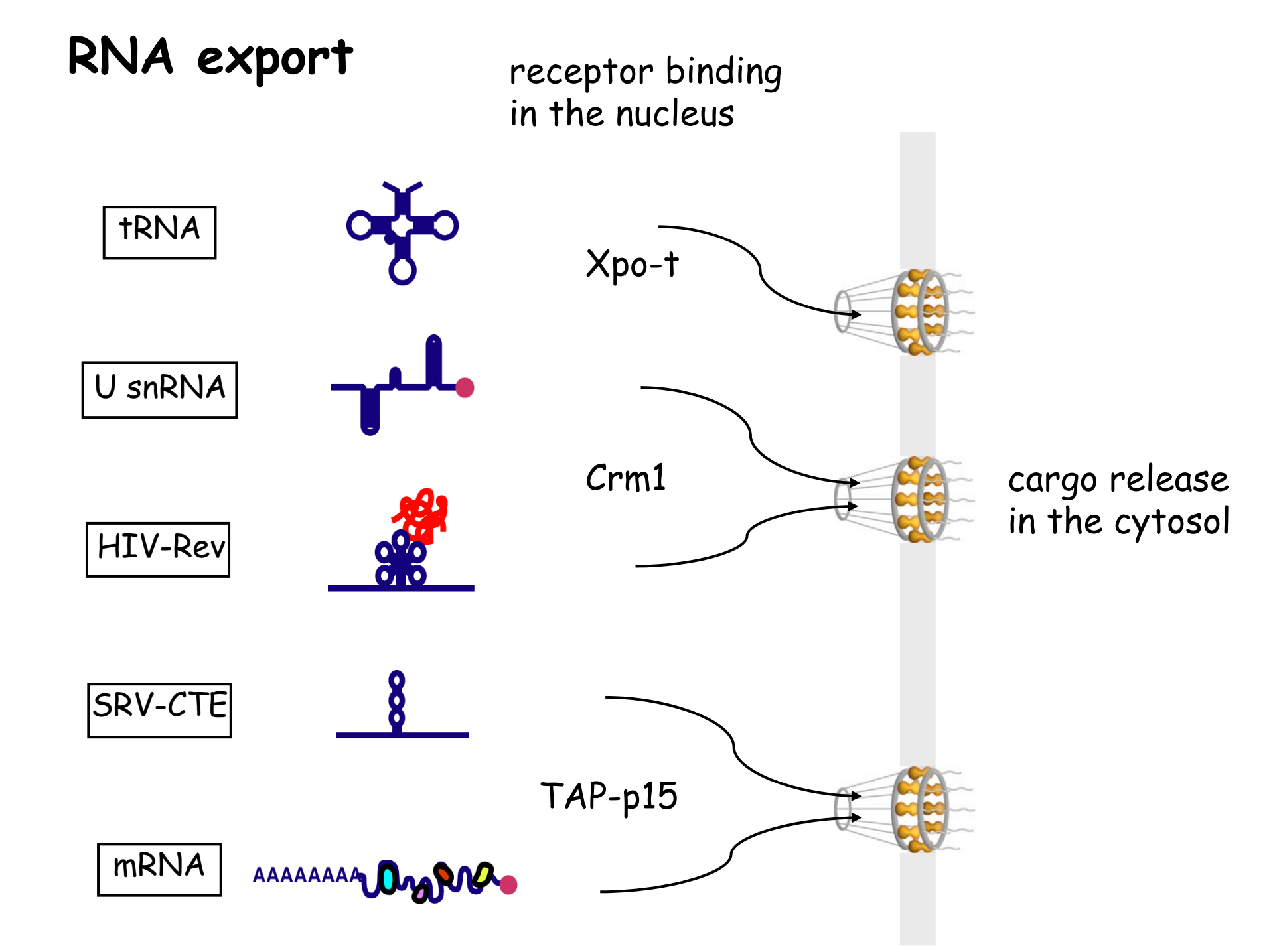
yeast genetics:

poly(A+) accumulation
interaction with nucleoporin
- Mex67p — Segref et al.,'97
- Dbp5p — Snay-Hodge et al.,'98
- Gle2p/Rae1 — Murphy et al.,'96; Brown et al.,'95

retroviruses:

- Crm1 (exportin) - HIV-1 — Fornerod et al.,'97
- TAP (Mex67p homologue) - SRV (CTE RNA) — Grüter et al.,'98

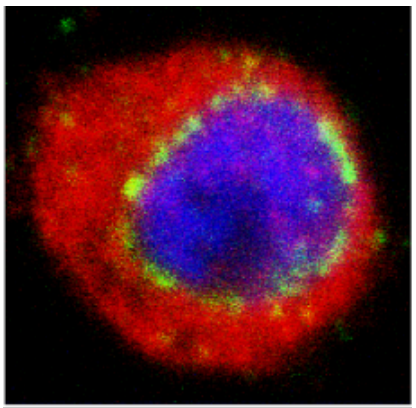
RNA export


receptor binding
in the nucleus
tRNA
U snRNA
HIV-Rev
SRV-CTE
mRNA
AAAAAAAA
Xpo-t
Crm1
TAP-p15
cargo release
in the cytosol

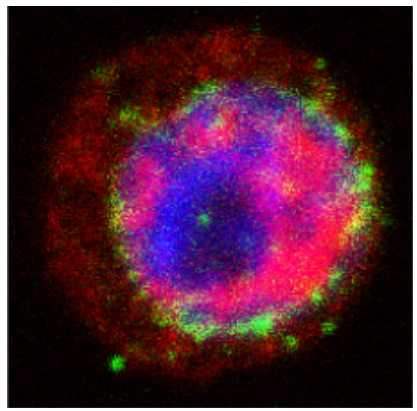
Both TAP and p15 are essential for bulk mRNA export

Knockdown of *D.melanogaster* TAP or p15 results in nuclear accumulation of bulk polyadenylated RNAs

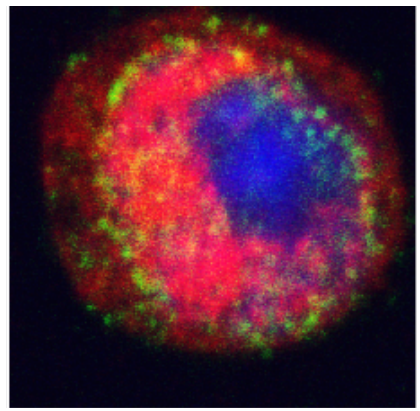
control



TAP ds RNAi



p15 ds RNAi



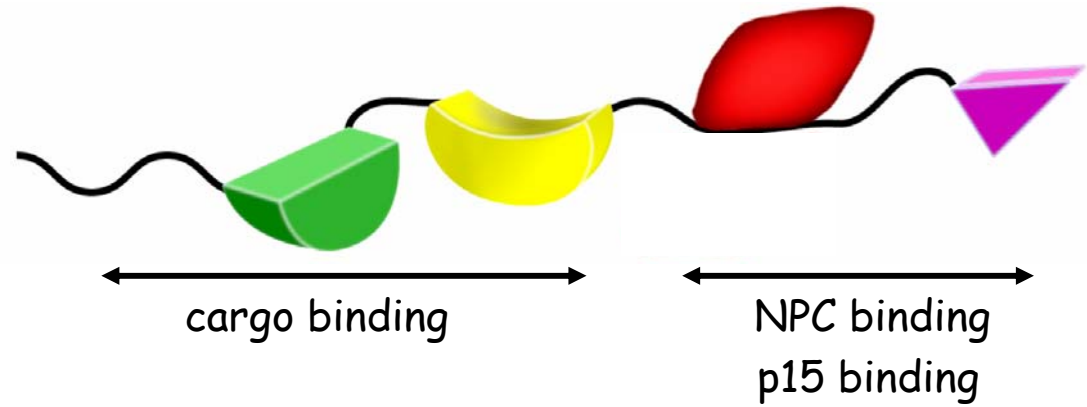
oligo(dT) *in situ* hybridization

poly-$(A)^+$

DNA

nucleoporins

The TAP subunit of the mRNA export receptor is conserved (human NXF1 and yeast Mex67)



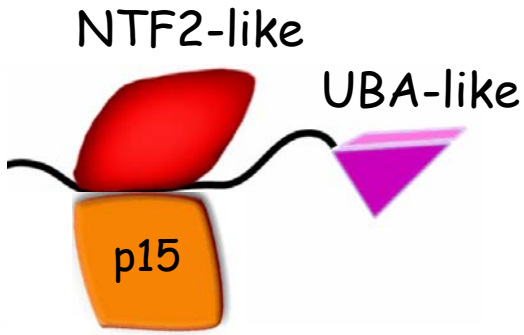
The p15 subunit of the mRNA export receptor is not conserved (human p15 / yeast Mtr2)

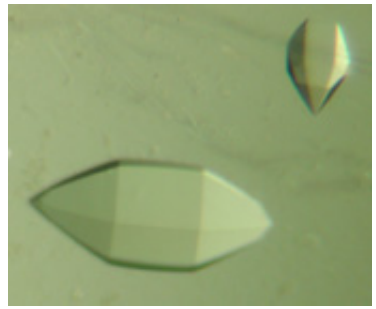
How does it recognize RNA cargoes?

How does it recognize nuclear pore complexes?

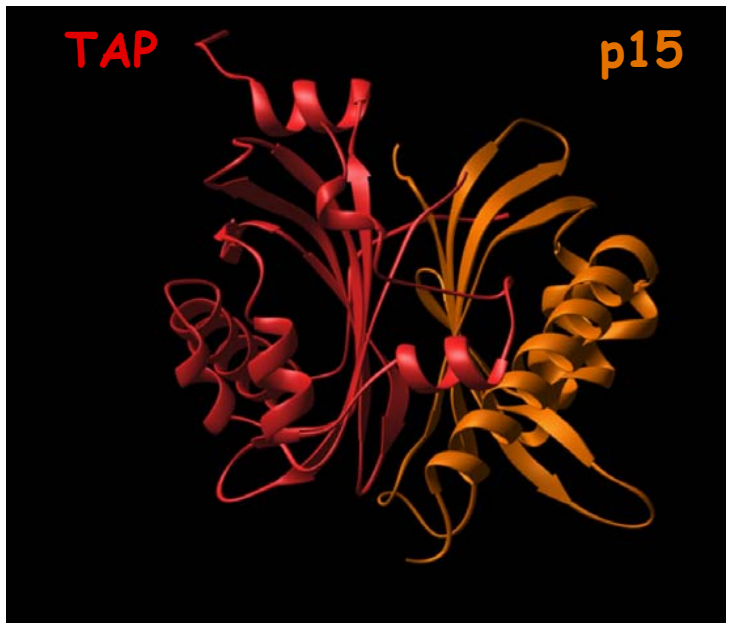
What is the function of p15? Mtr2?

TAP C-terminal portion: interacts with nucleoporins and p15

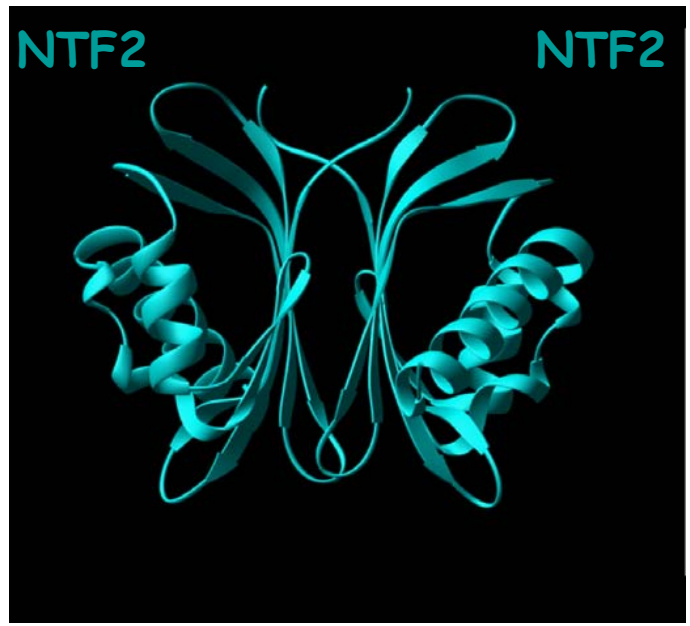




1.9Å resolution
Rfree 22.5 %

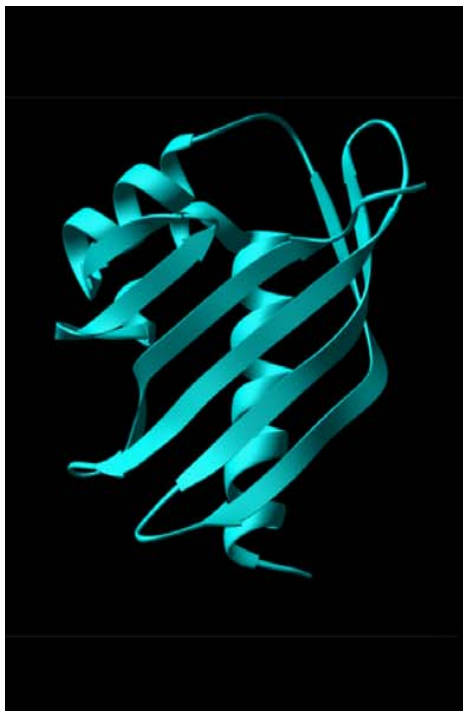


TAP-p15 heterodimer
(Fribourg et al., 2001)



NTF2-NTF2 homodimer
(Stewart et al., 1998)

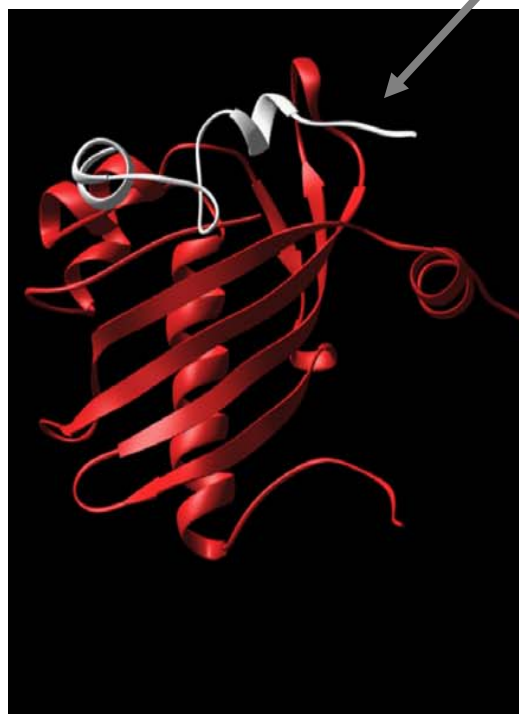
Structural similarity of NTF2-like domains



NTF2

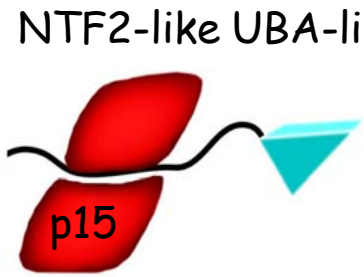
p15

22% seq.id. with NTF2
rmsd 1.9Å (113 Cα)



TAP

13% seq.id. with NTF2
rmsd 2.5 Å (95 Cα)



UBA-like domain function: NPC binding
ΔUBA-like domain: 10% export activity

ΔNTF2-like domain: no export activity
NTF2-like domain: function ????

Extrapolate functional similarity from structural similarity?

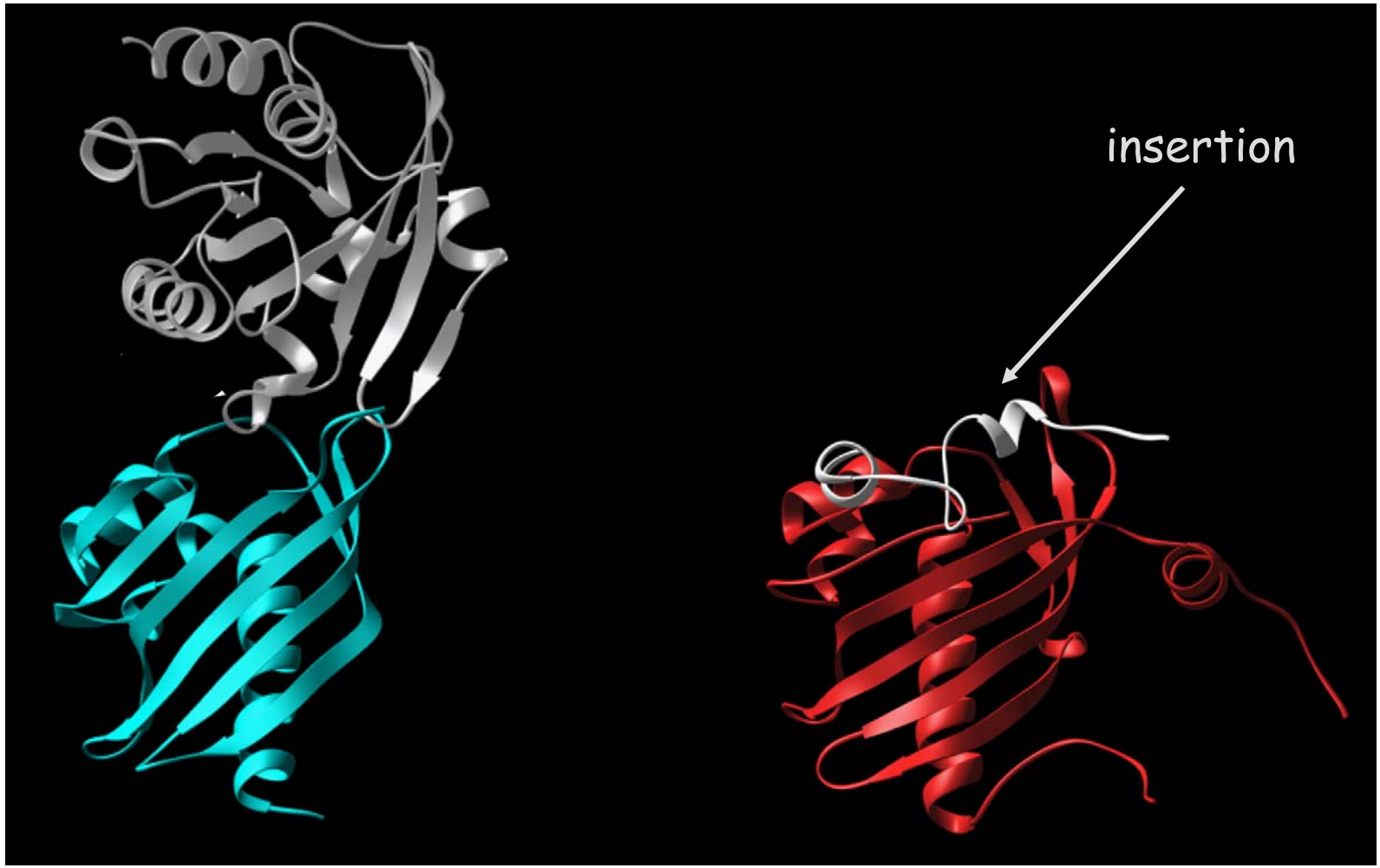
NTF2 is the Ran import factor
binds RanGDP
binds nucleoporins

RanGDP

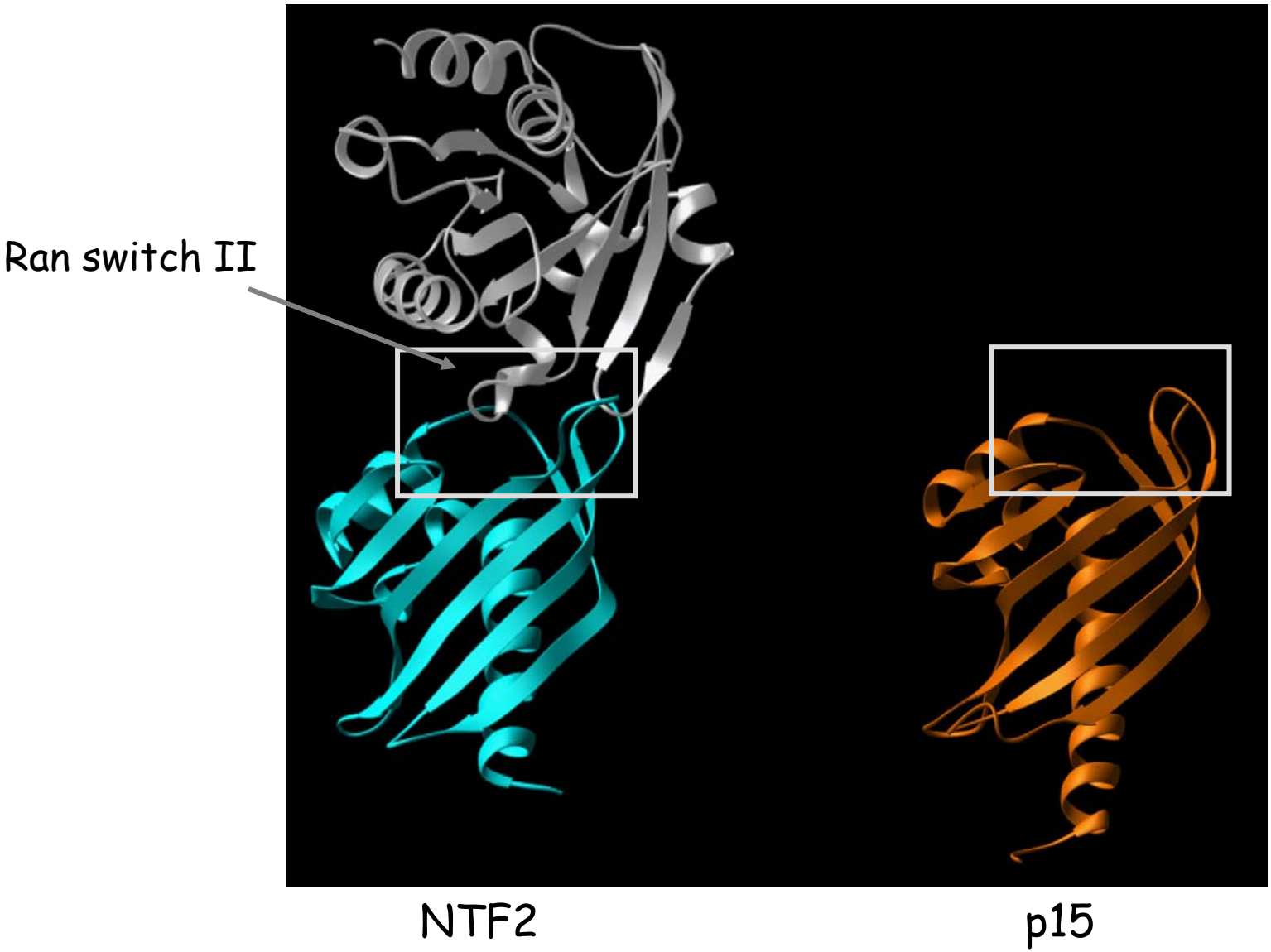
NTF2

Ran regulator to most transport pathways
?mRNA export pathway?

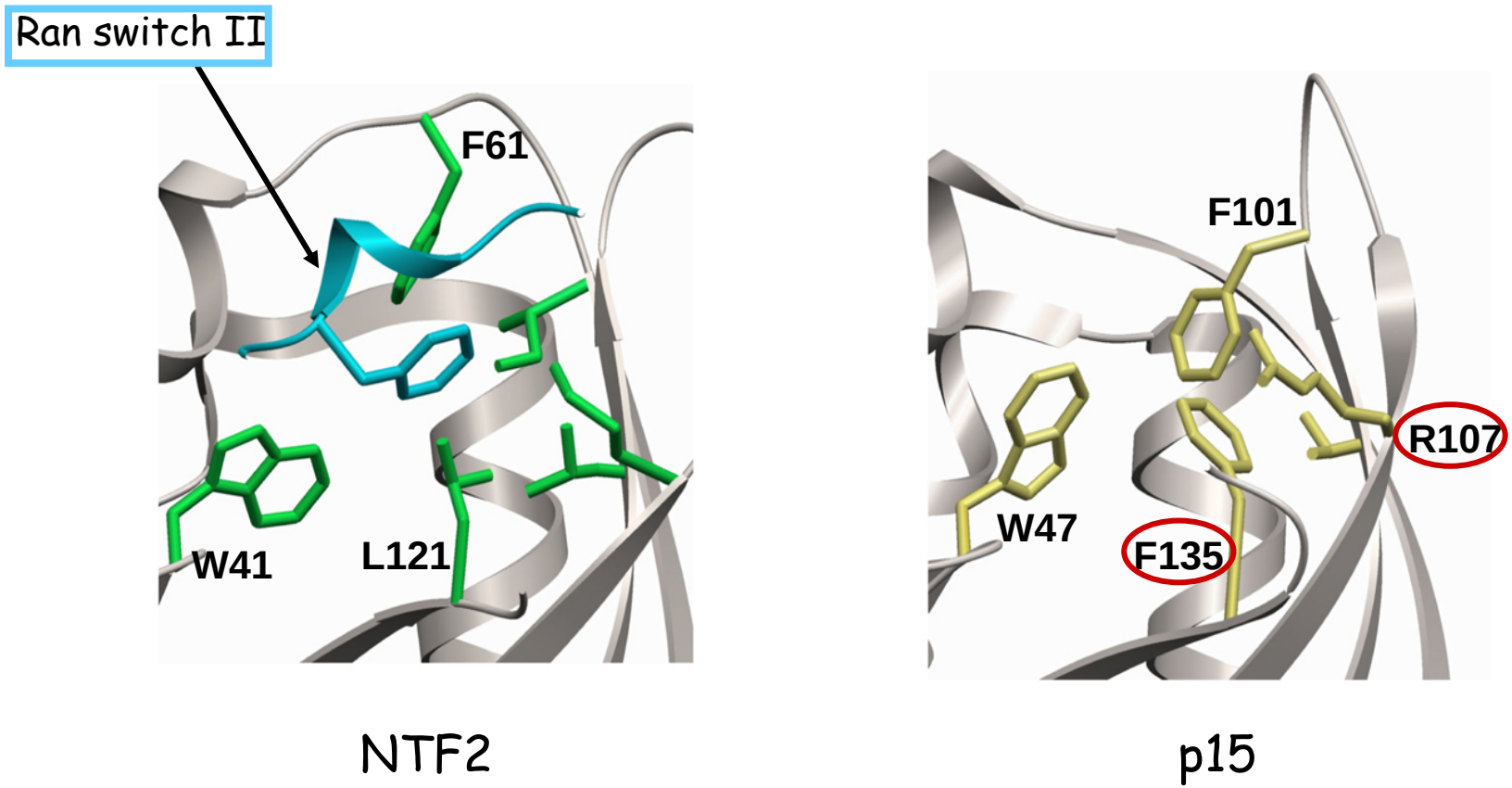
TAP structure is incompatible with Ran binding



p15 structure is incompatible with Ran binding



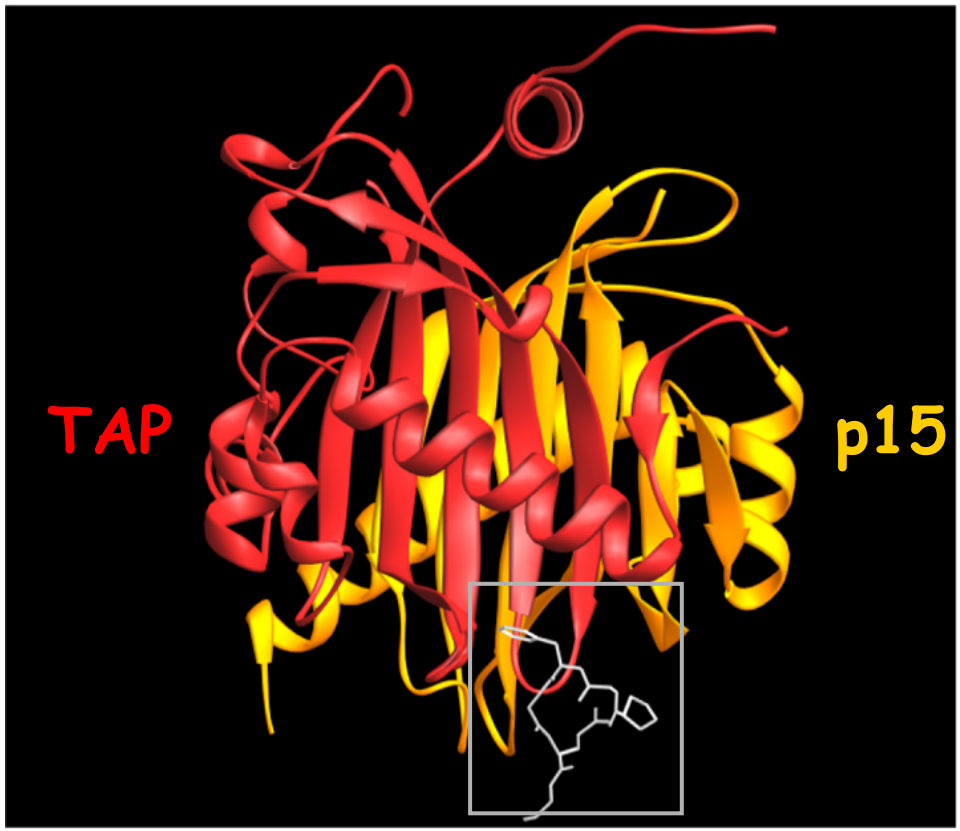
The Ran-binding pocket of NTF2 is occluded in p15

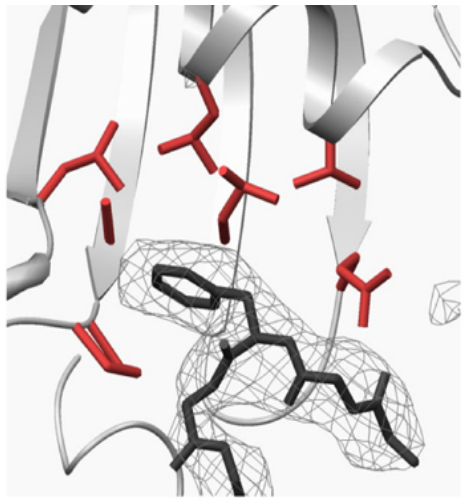


NTF2

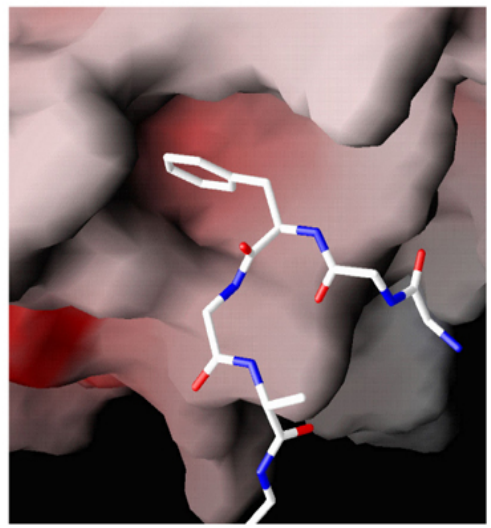
p15

TAP NTF2-like domain binds Phe-Gly repeat nucleoporins

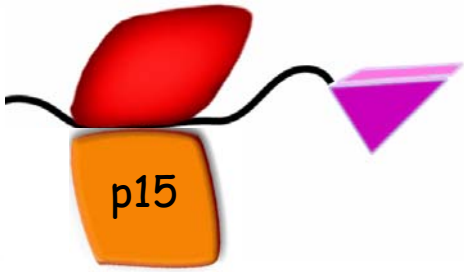




2.8 Å
*R*free 26.9%

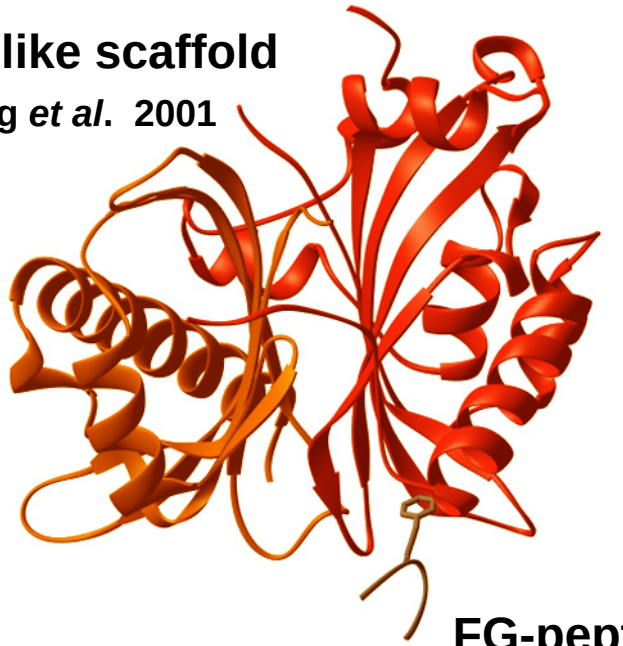


TAP:p15 directly interact with Phe-Gly containing nucleoporins via two structurally unrelated domains



NTF2-like scaffold

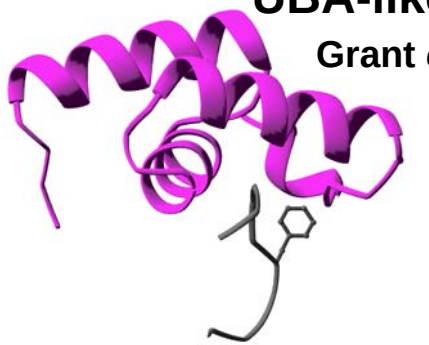
Fribourg *et al*. 2001



FG-peptide

UBA-like domain

Grant *et al*. 2003



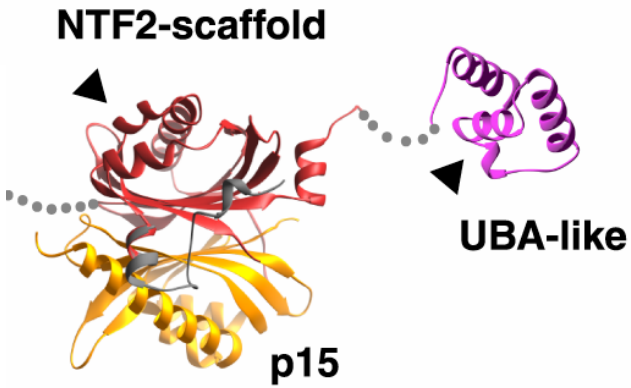
FG-peptide

Recognition of nucleoporins:

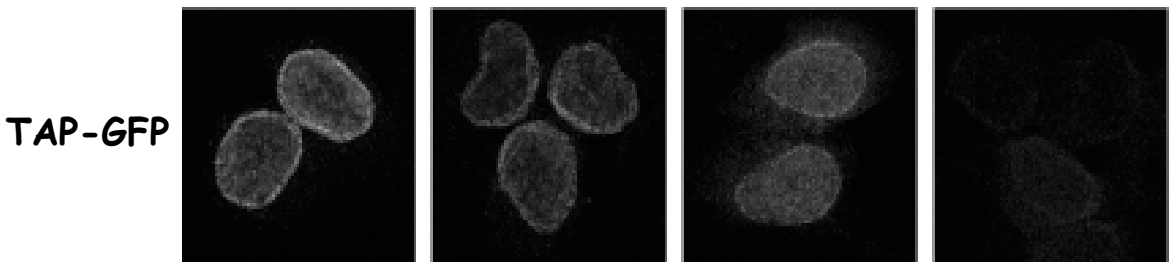
Phe residue: hydrophobic interactions

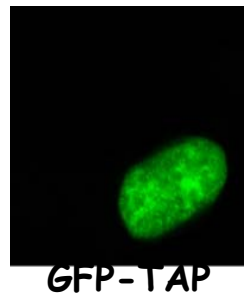
Gly residue: conformational flexibility

TAP has two nucleoporin binding sites

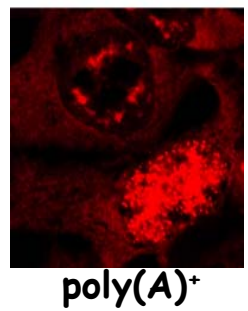


Mutation of nucleoporin-binding sites: nuclear envelope localization

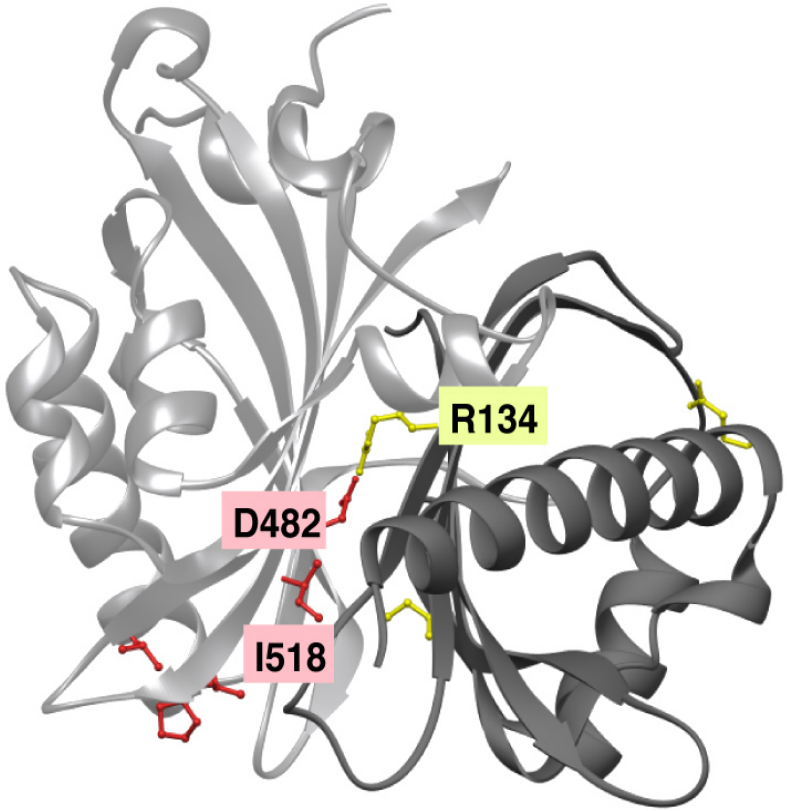




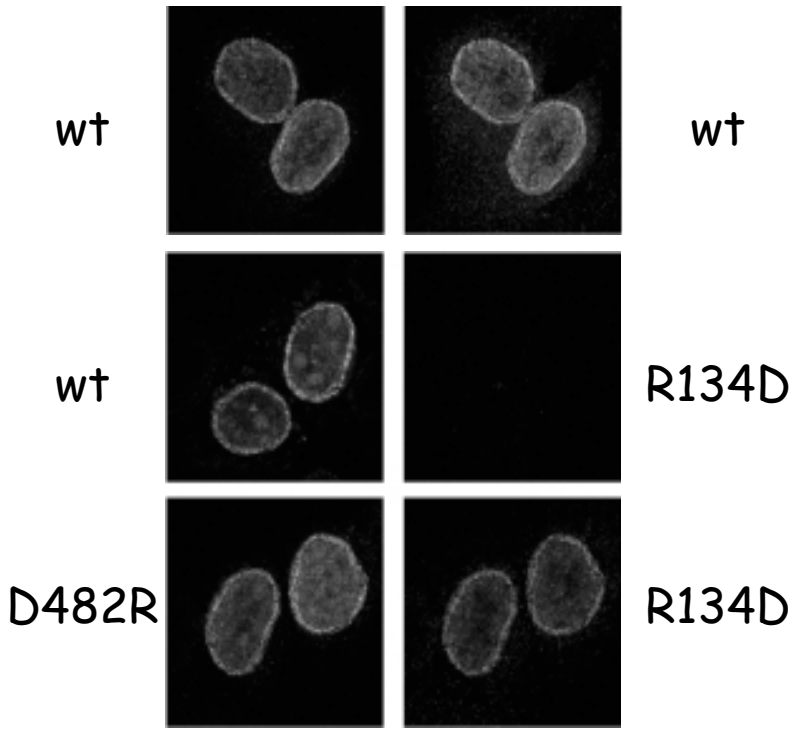
Double mutant overexpression:
inhibition of bulk poly(A)⁺RNA



p15 is recruited to the NPC by TAP



Nuclear envelope localization

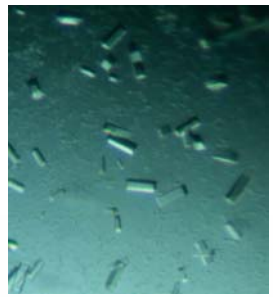


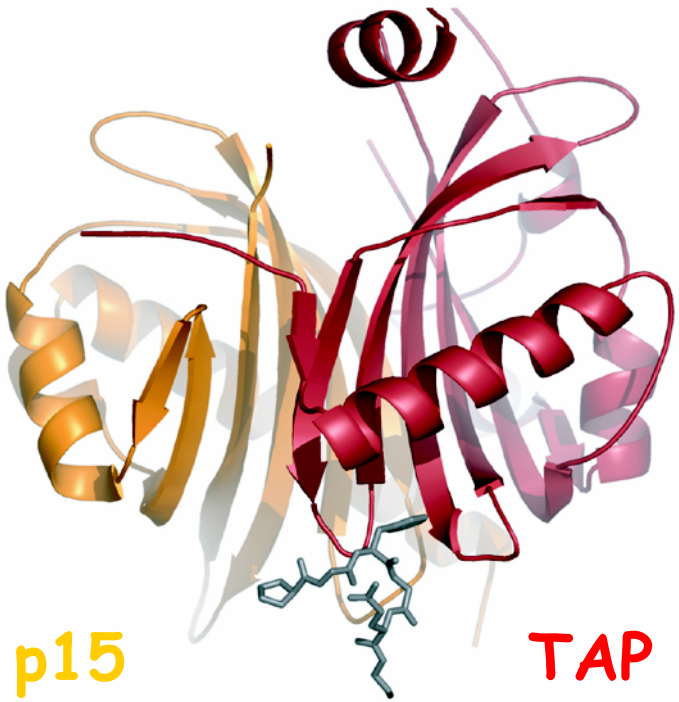
salt bridge swap

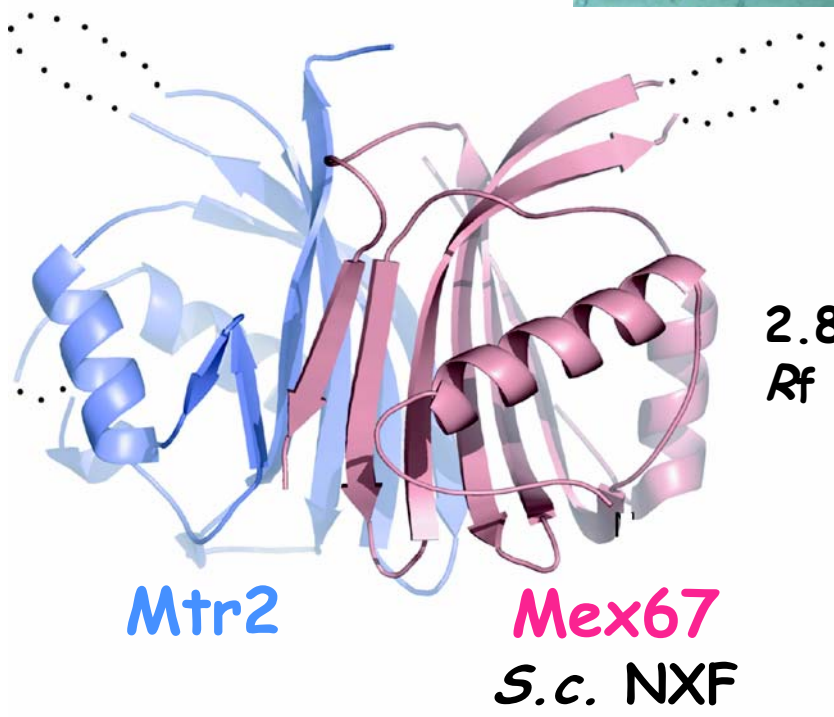
the function of p15 is structural

TAP is conserved from lower to higher eukaryotes
Human TAP and yeast Mex67 (NXF)

The smaller subunit of the mRNA export factor is not conserved
Human p15 and yeast Mtr2: functional homologues
no sequence homology





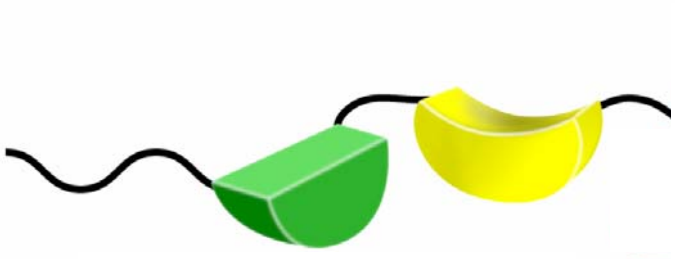


2.8 Å
Rf 26.2%

Fribourg and Conti, 2003

Human p15 and yeast Mtr2: structural homology

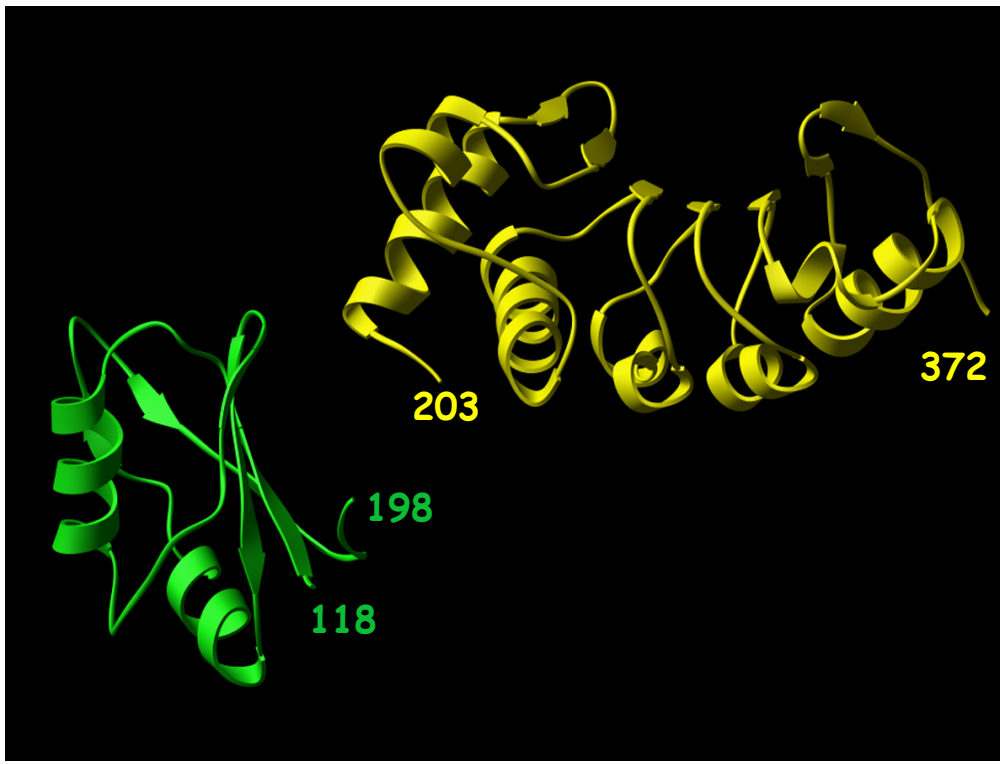
TAP N-terminal portion: cargo binding



Cellular mRNA (indirect binding via adaptors)
Viral CTE RNA (direct binding to human TAP)

RBD domain
RNA-binding

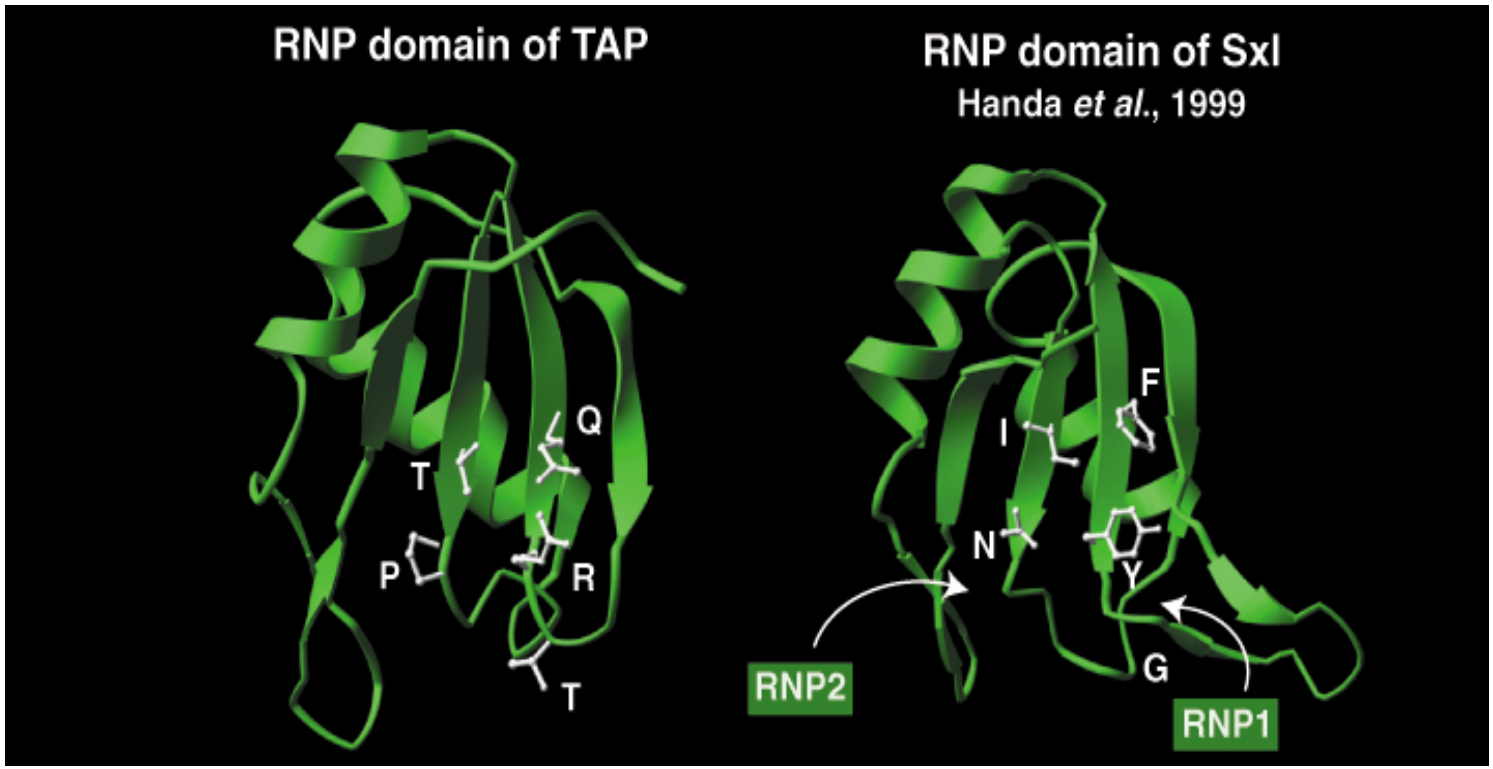
LRR domain
conserved human/yeast



2.9 Å resolution
Rfree 27.8 %

Liker et al. 2000

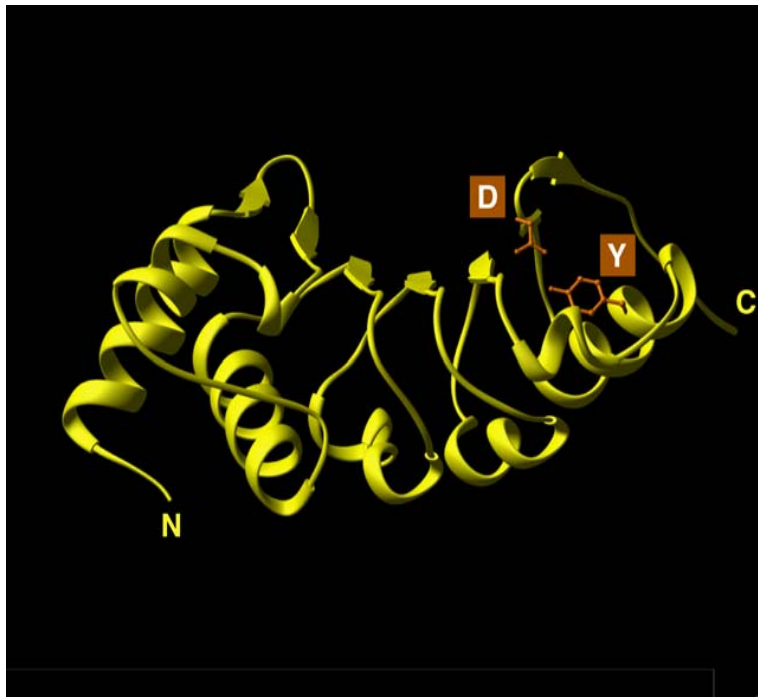
RNA binding domain of TAP:
canonical structure, non canonical sequence



	RNP2	RNP1
Sxl	... LIVNYL	... YGYAFVDF
U2B"	... IYINNM	... RGQAFVIF
TAP	... ITIPYG	... NTRAQFFV

deviation from consensus

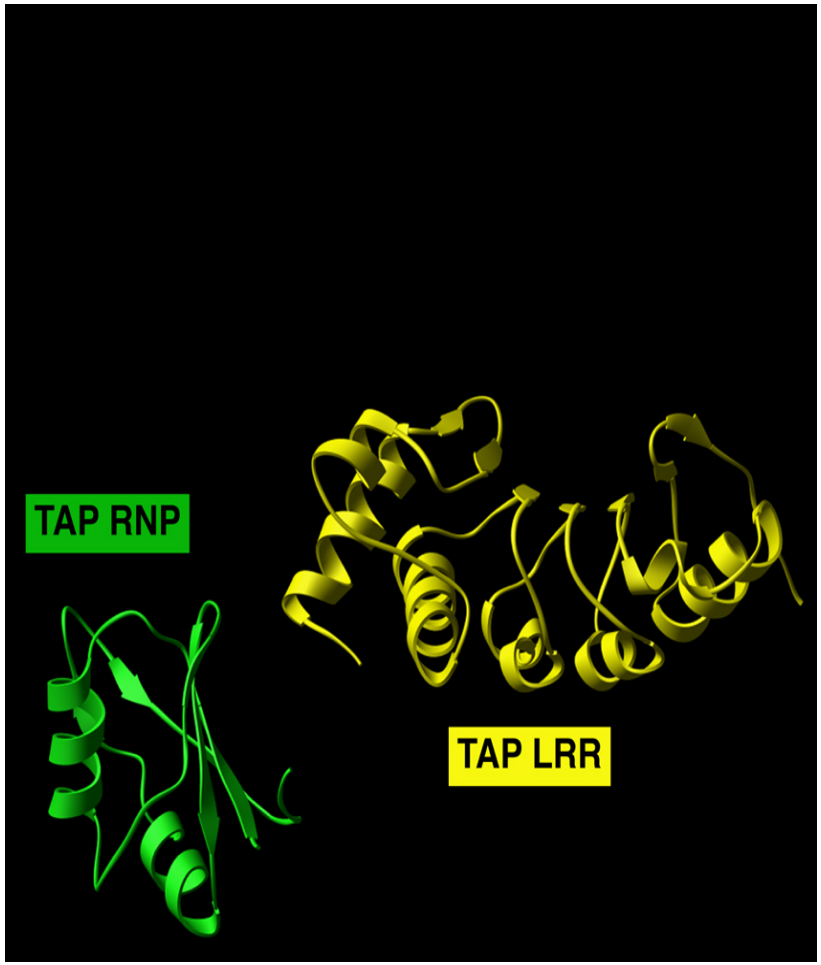
LRR binding domain of TAP:
structural homology to U2A'



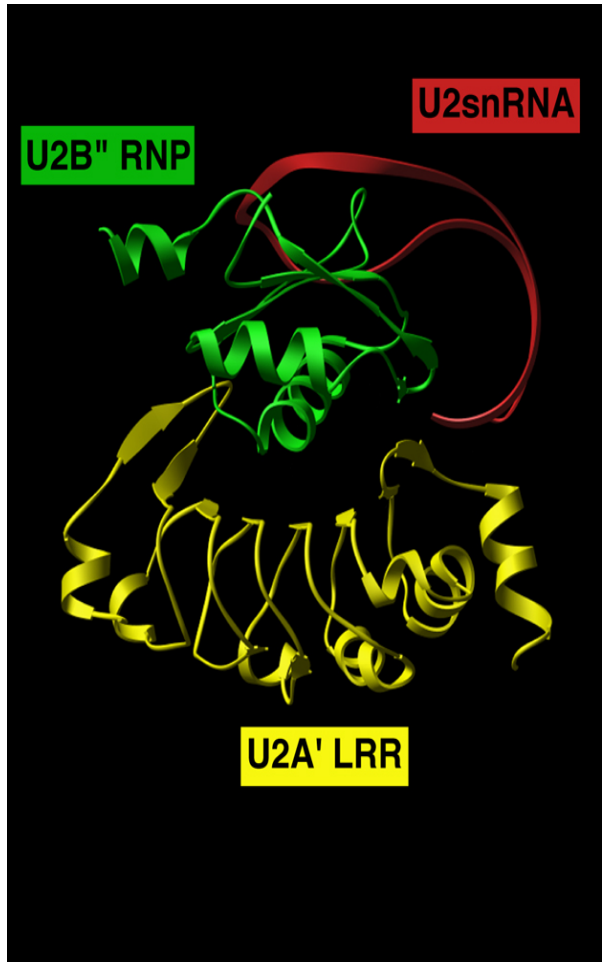
4 leucine-rich-repeats
in TAP

5 leucine-rich-repeats
in U2A'
(Price et al., 1998)

Structural homology



TAP RNP-LRR domains

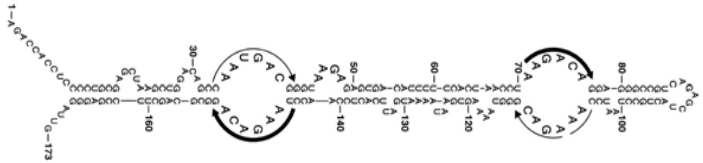


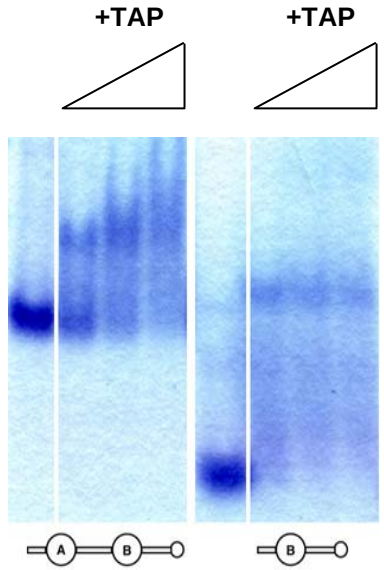
RNP-LRR domains
binding to U2 snRNA
(Price et al., 1998)

TAP RNP and LRR domains bind a viral RNA directly

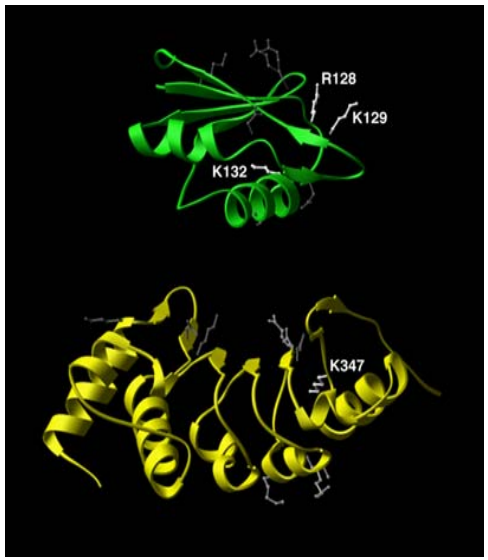
Viral CTE RNA

CTE-RNA:

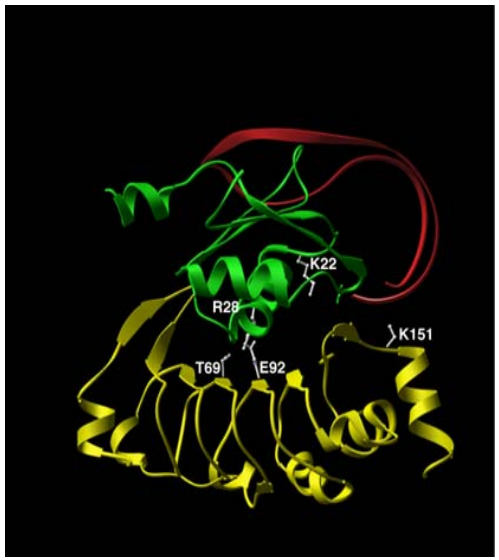




TAP: RNP and LRR domains required (*in cis*)
mapped the surface by mutagenesis



TAP

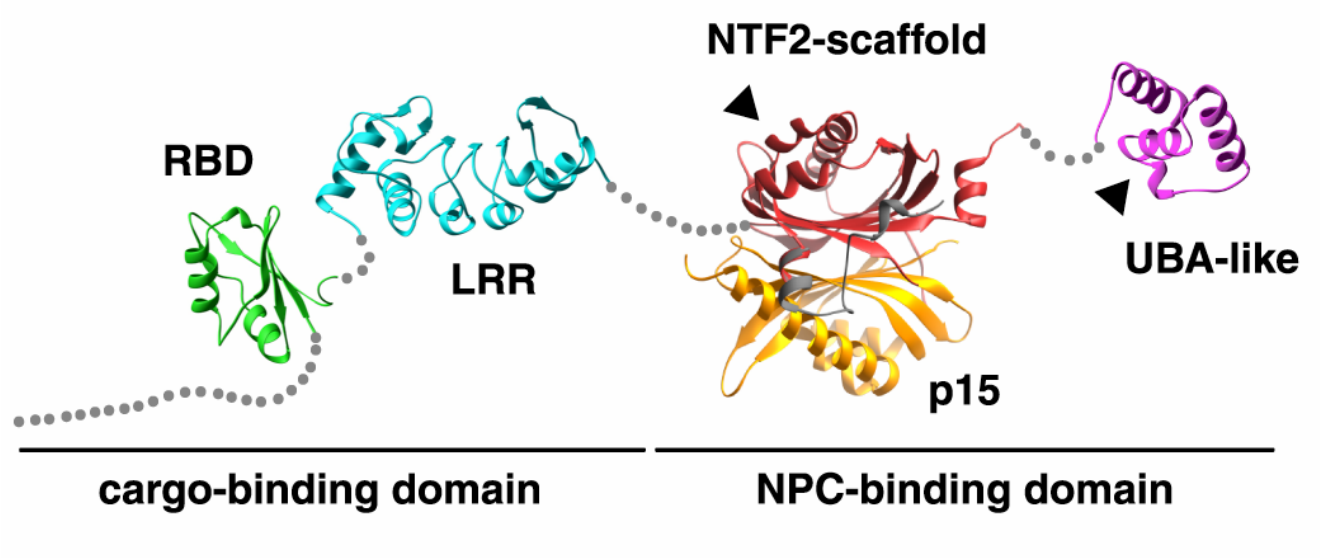


Spliceosomal complex
(Price et al., 98)

Cellular mRNA

TAP: RNP not strictly required (not conserved)
conserved LRR domain as an adaptor?

mRNA export receptor TAP:p15 heterodimer



TAP: conserved and essential LRR, NTF2-like and UBA-like domains

recognizes Phe-Gly nucleoporin repeats at two sites

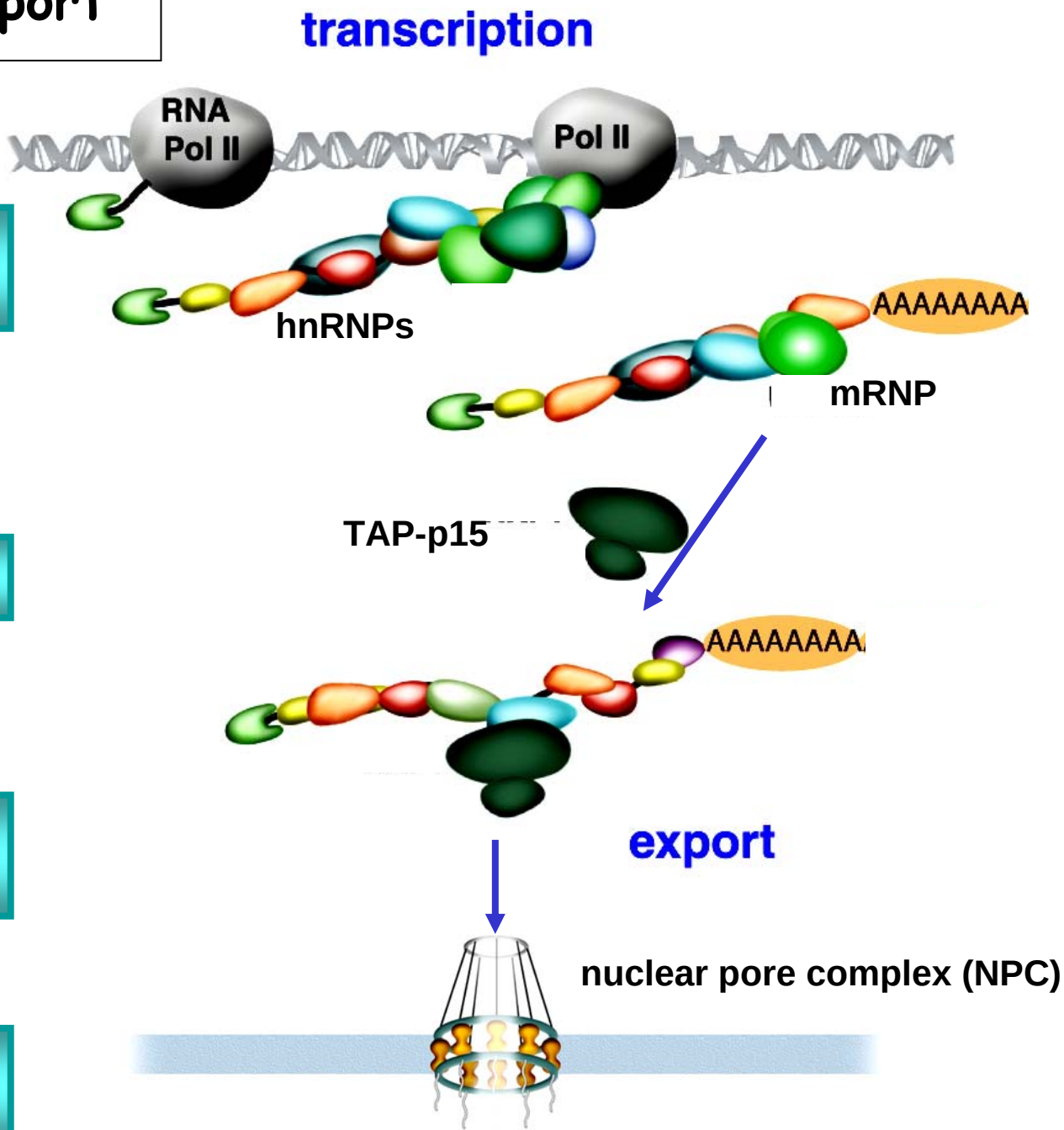
The function of p15 is structural in maintaining a proper fold (Mtr2?)

It is not regulated by Ran

mRNA release?

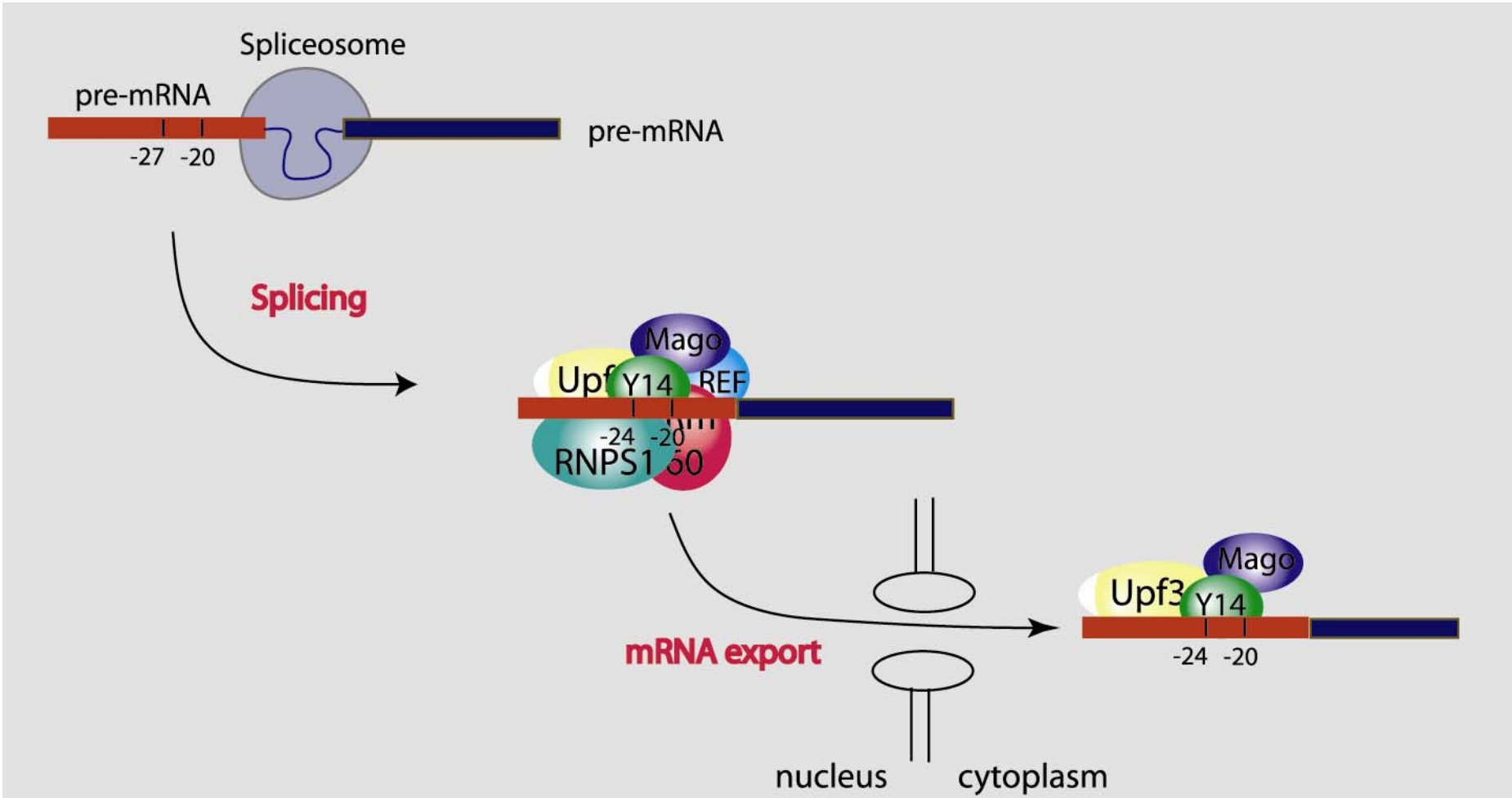
mRNA binding? Adaptor proteins?

mRNA nuclear export



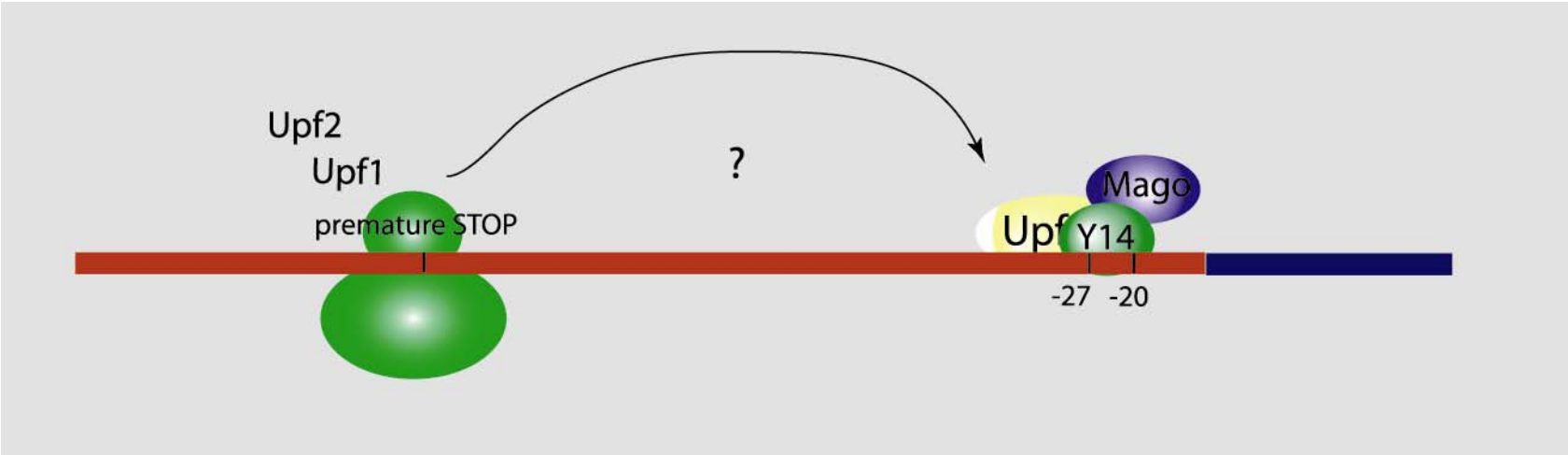
Adaptors: the exon-exon junction complex?

EJC: protein complex deposited upstream of splice junctions



EJC proteins in mRNA metabolism: splicing, export, NMD, localization

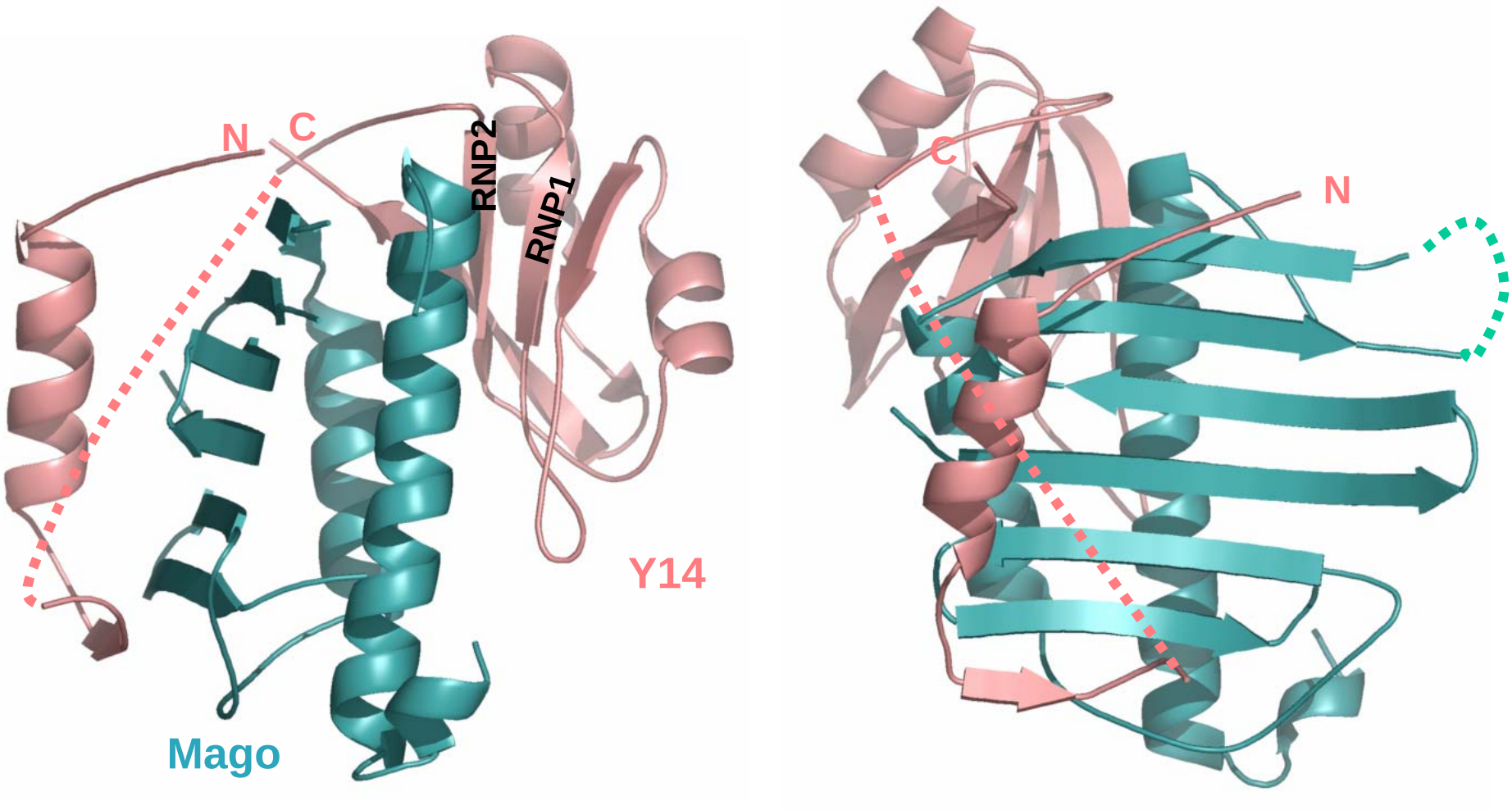
Mago and Y14: at the core of the EJC
form a complex in vitro and in vivo
from sequence: Y14 contains an RBD
involved in *oskar* mRNA localization
involved in nonsense mediated mRNA decay



Normal case: displacement of EJC proteins: no NMD, stable mRNA

Aberrant case (PTC): recruitment of NMD factors: mRNA degradation

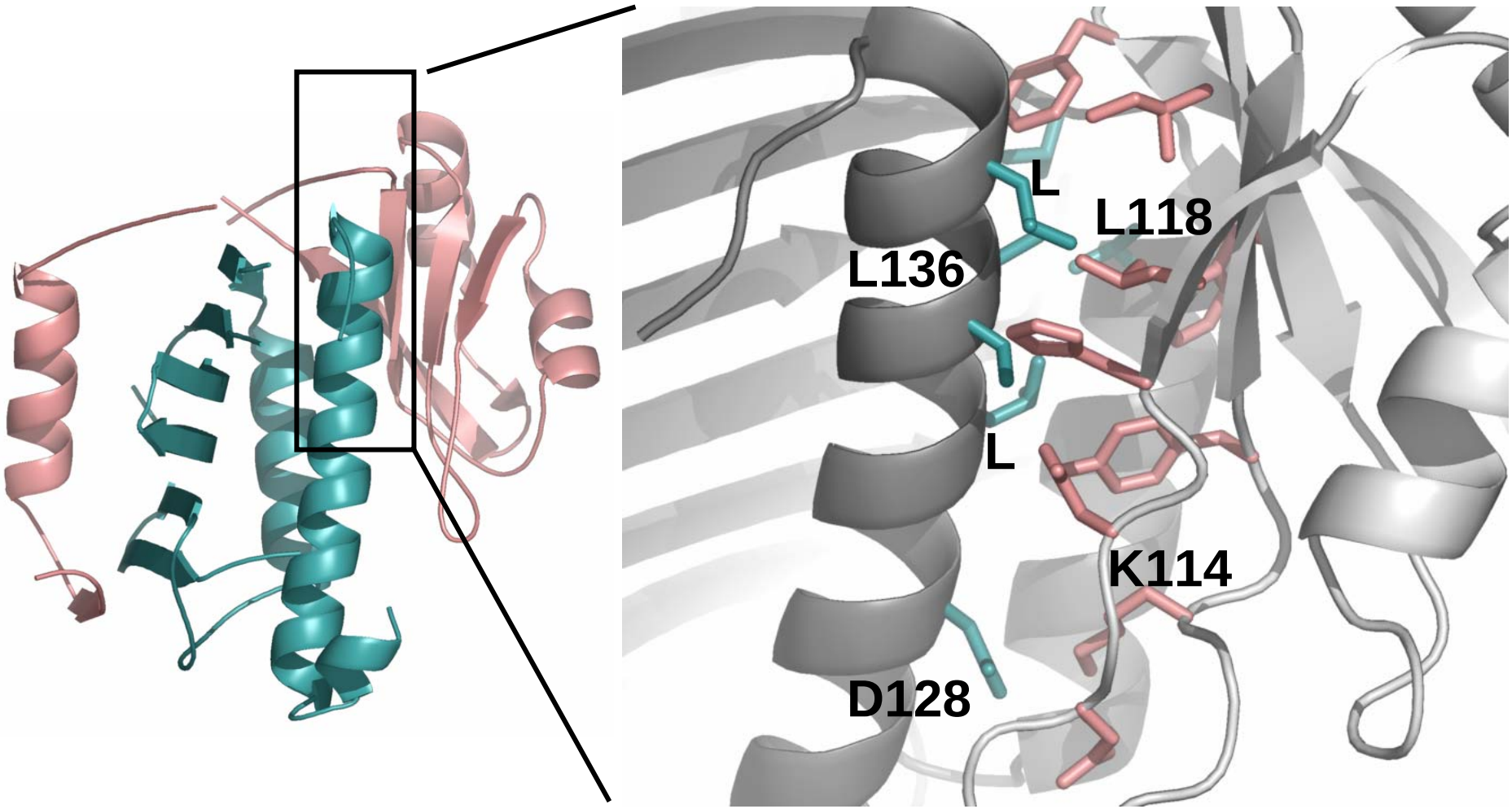
Mago-Y14 complex



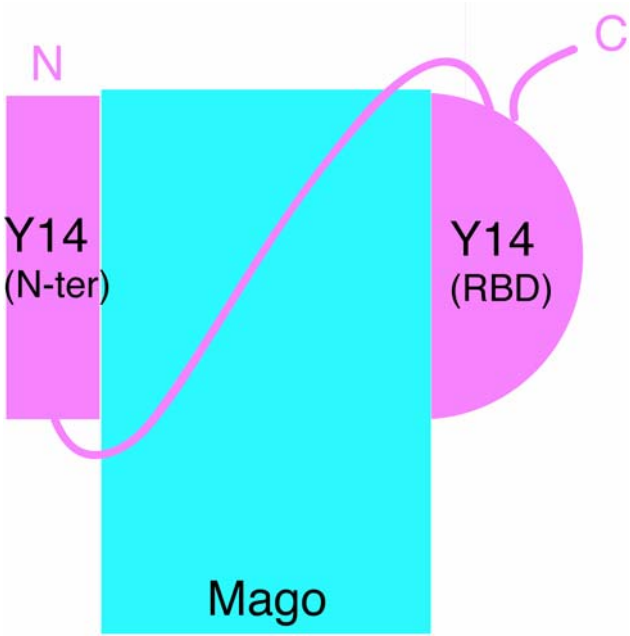
Mago is sandwiched between two domains of Y14

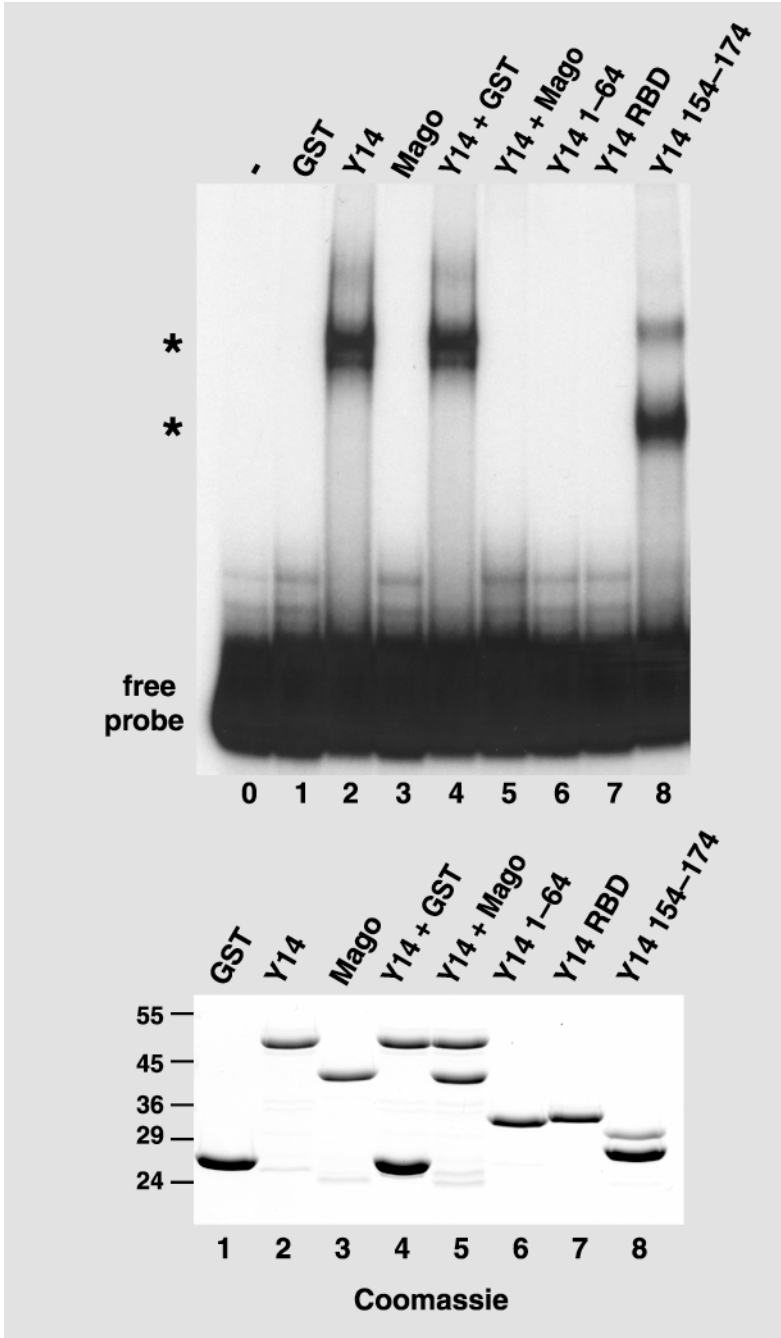
The RBD of Y14 binds the protein Mago

tight interaction interface

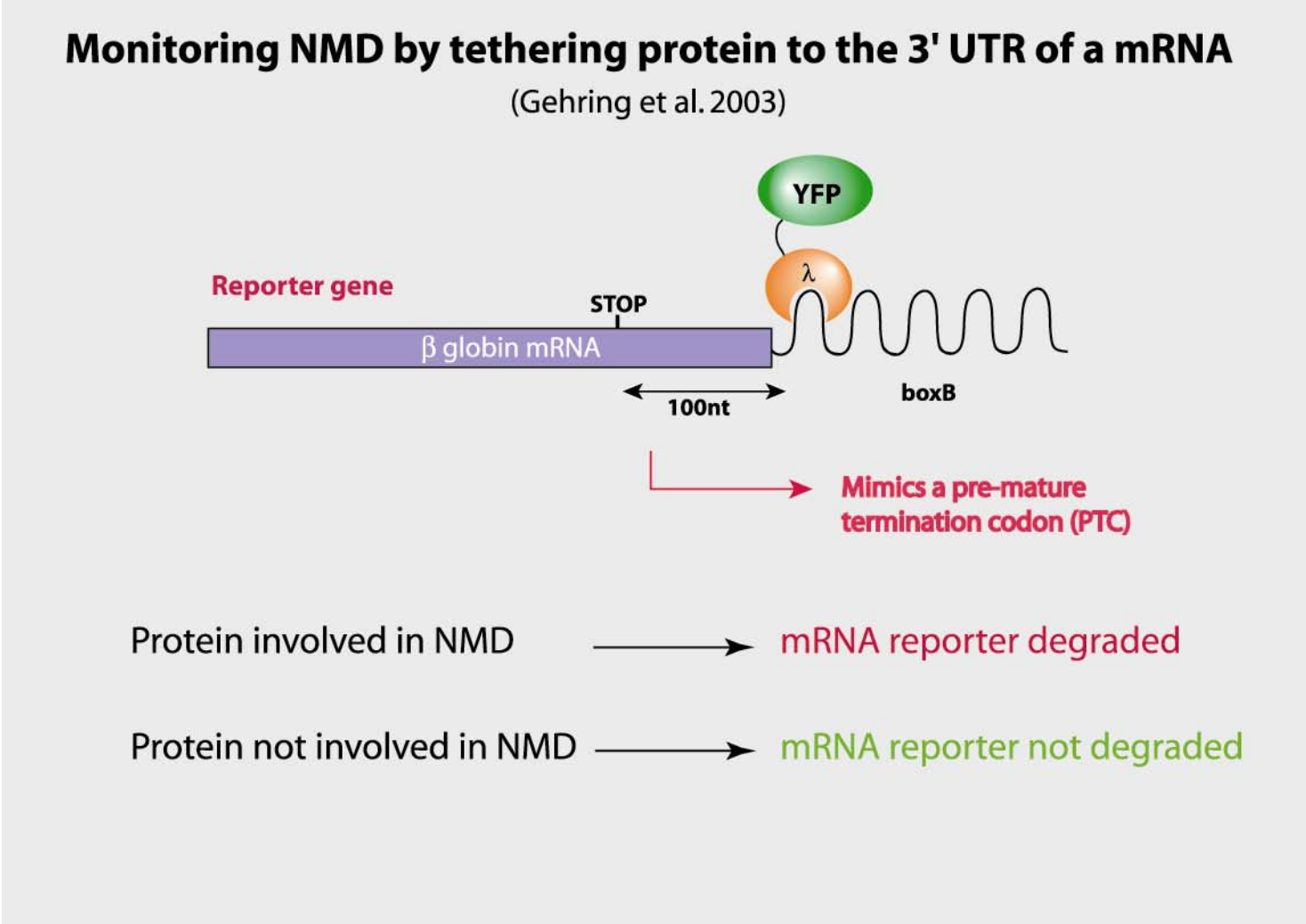


How does it contact RNA?

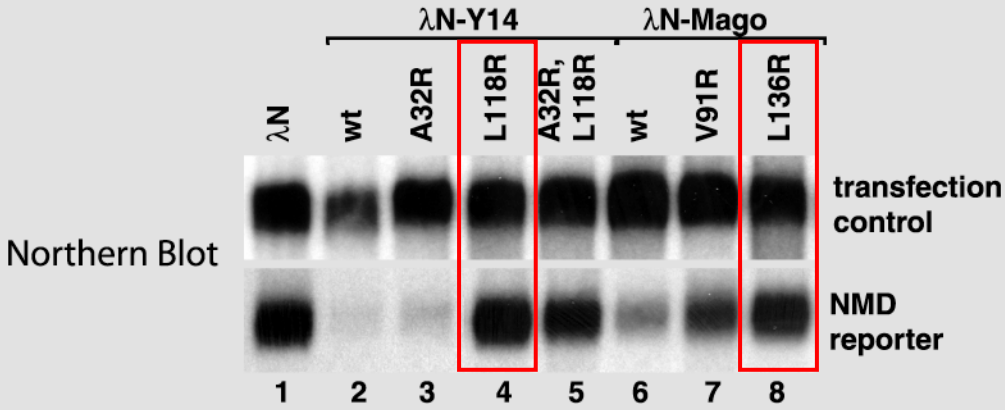


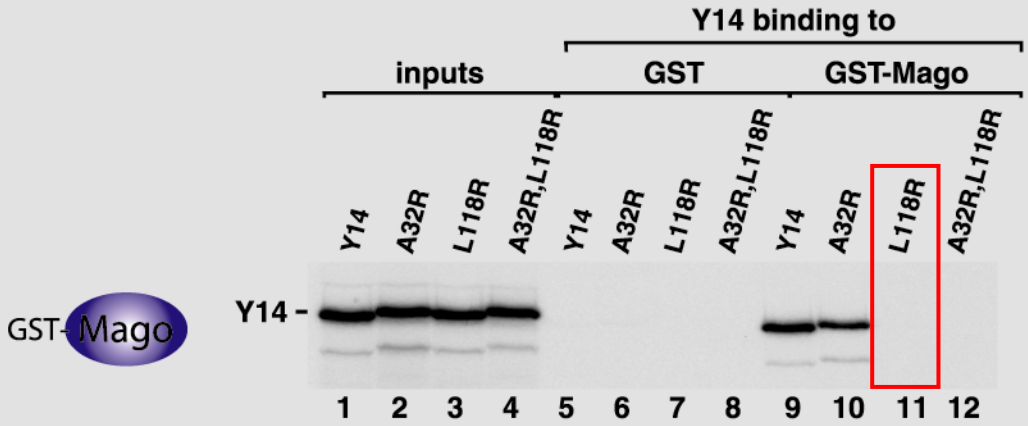


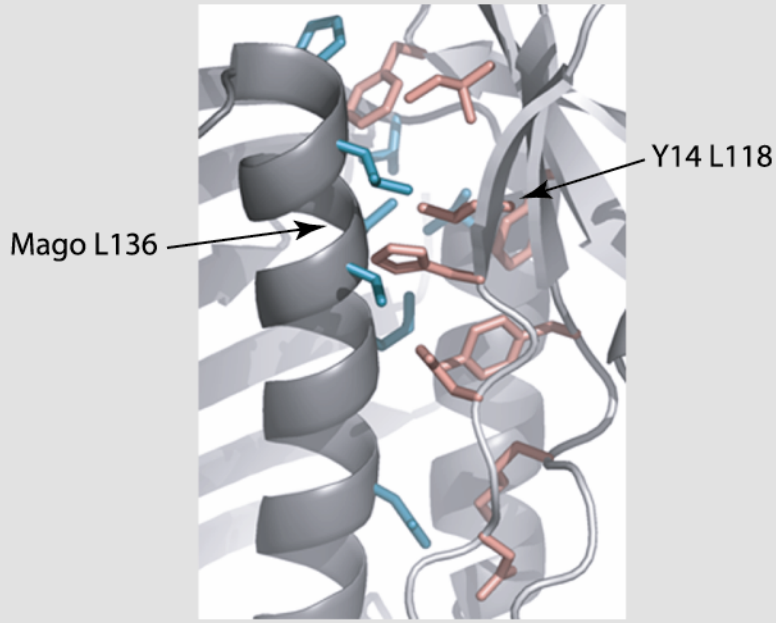
How does it work in nonsense-mediated mRNA decay?

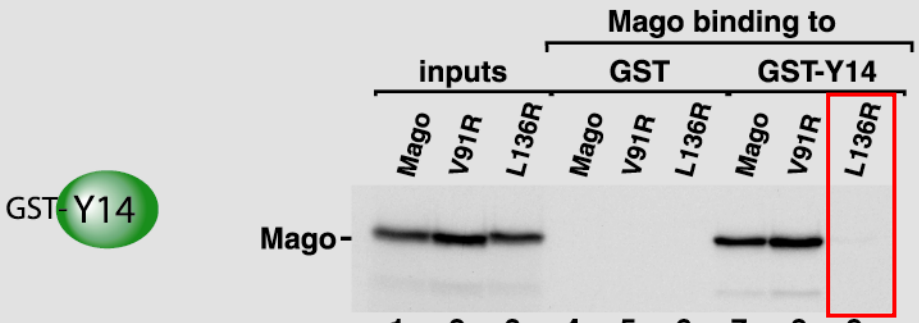


NMD is affected by disruption of Y14 - Mago heterodimer

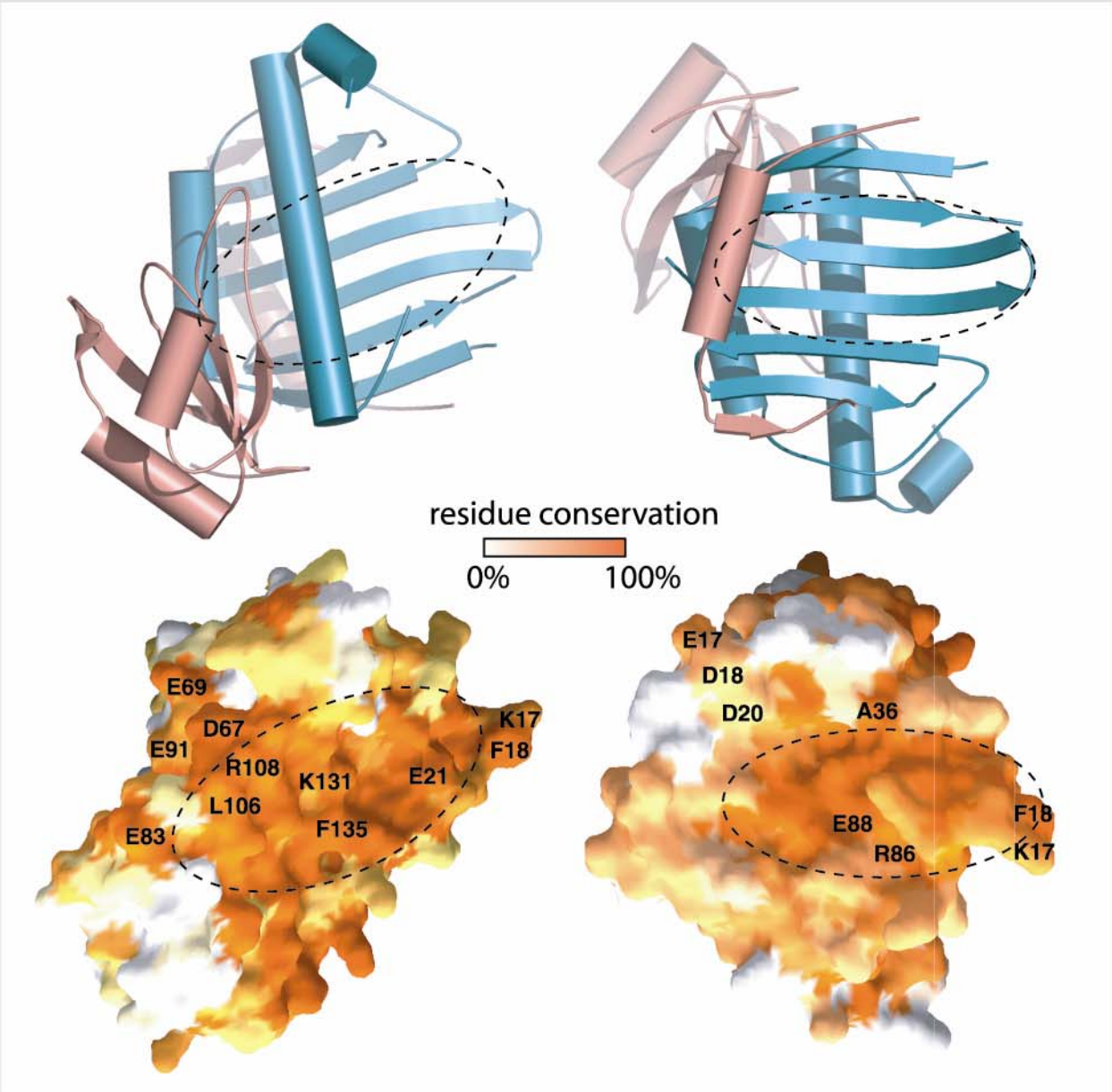




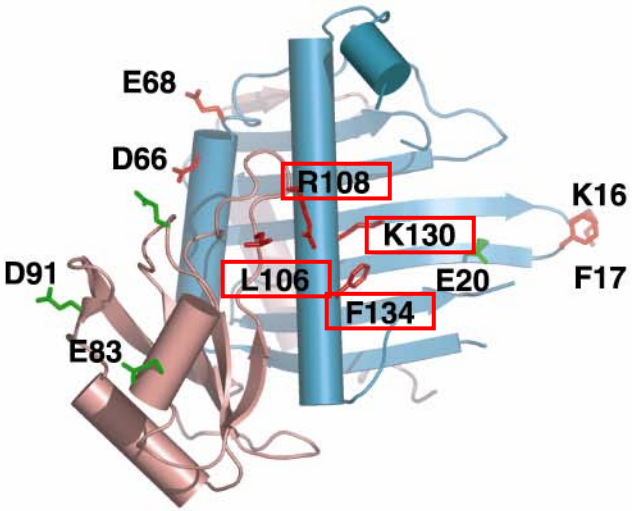


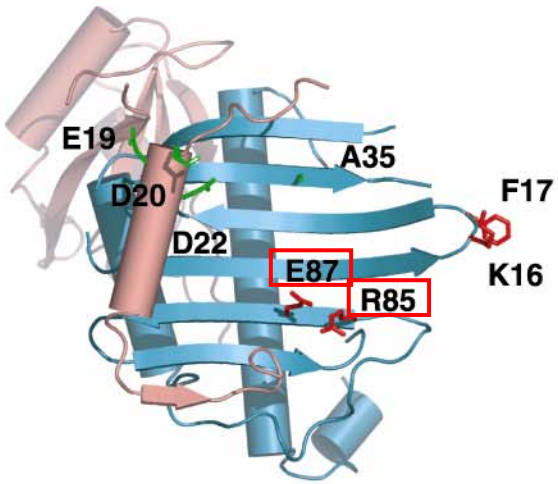


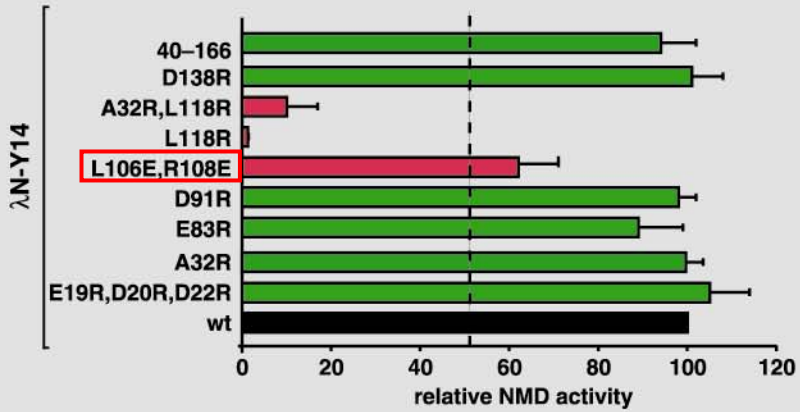
A highly conserved surface

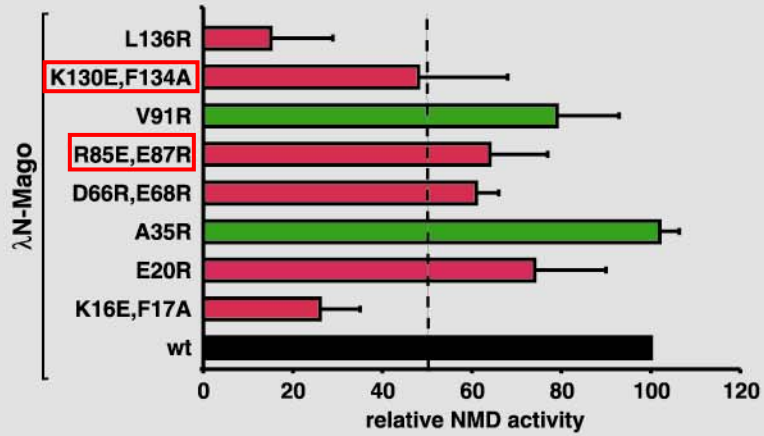


A highly conserved surface involved in NMD





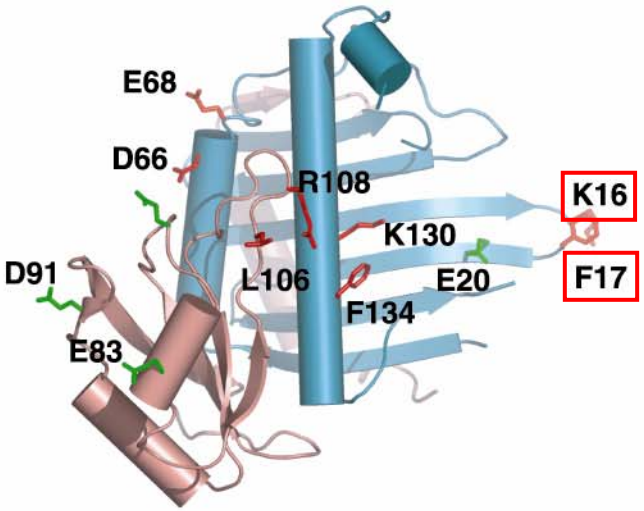


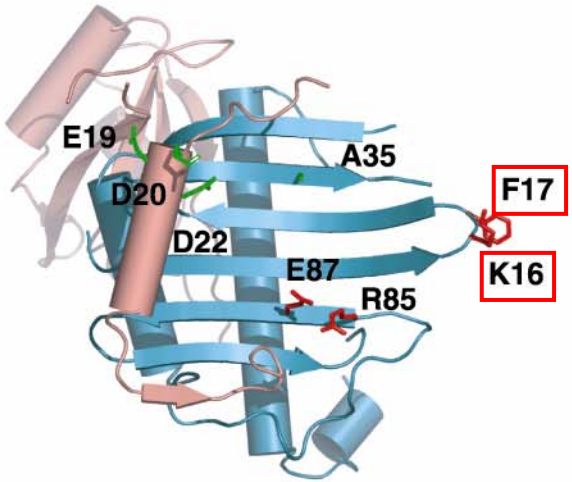


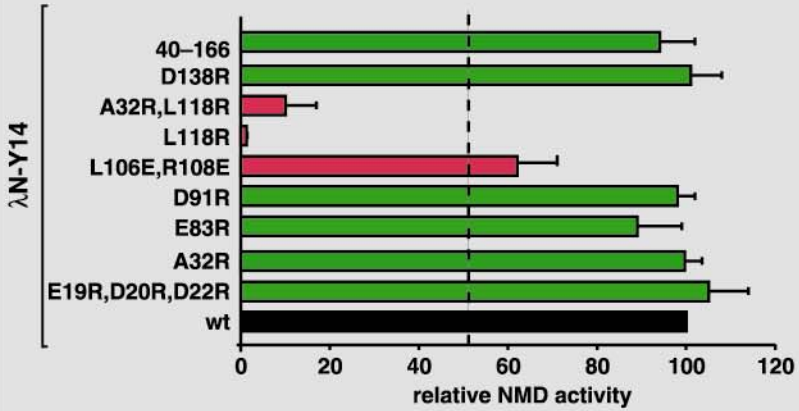
No effect on NMD

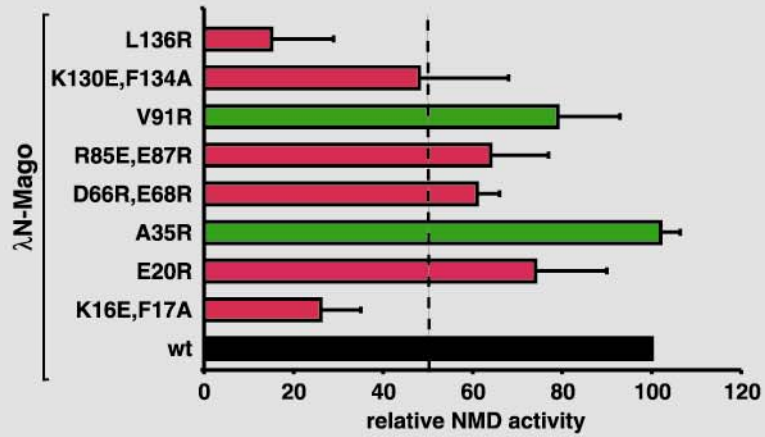
Alteration of NMD

A highly conserved surface involved in NMD



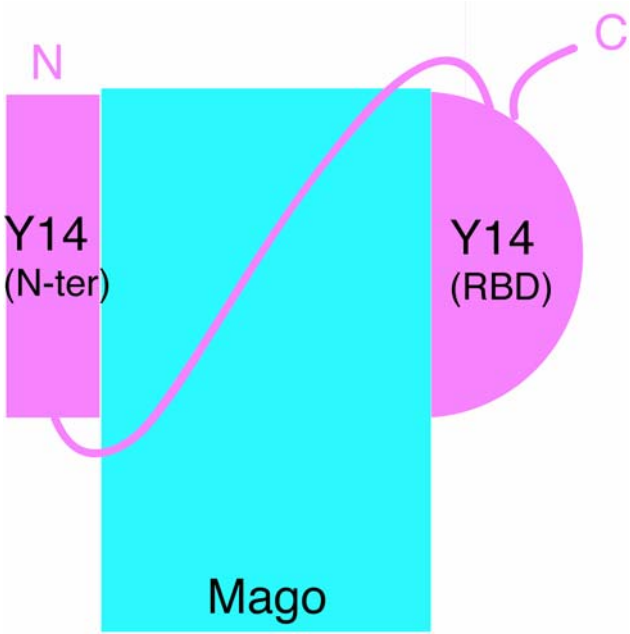






No effect on NMD

Alteration of NMD

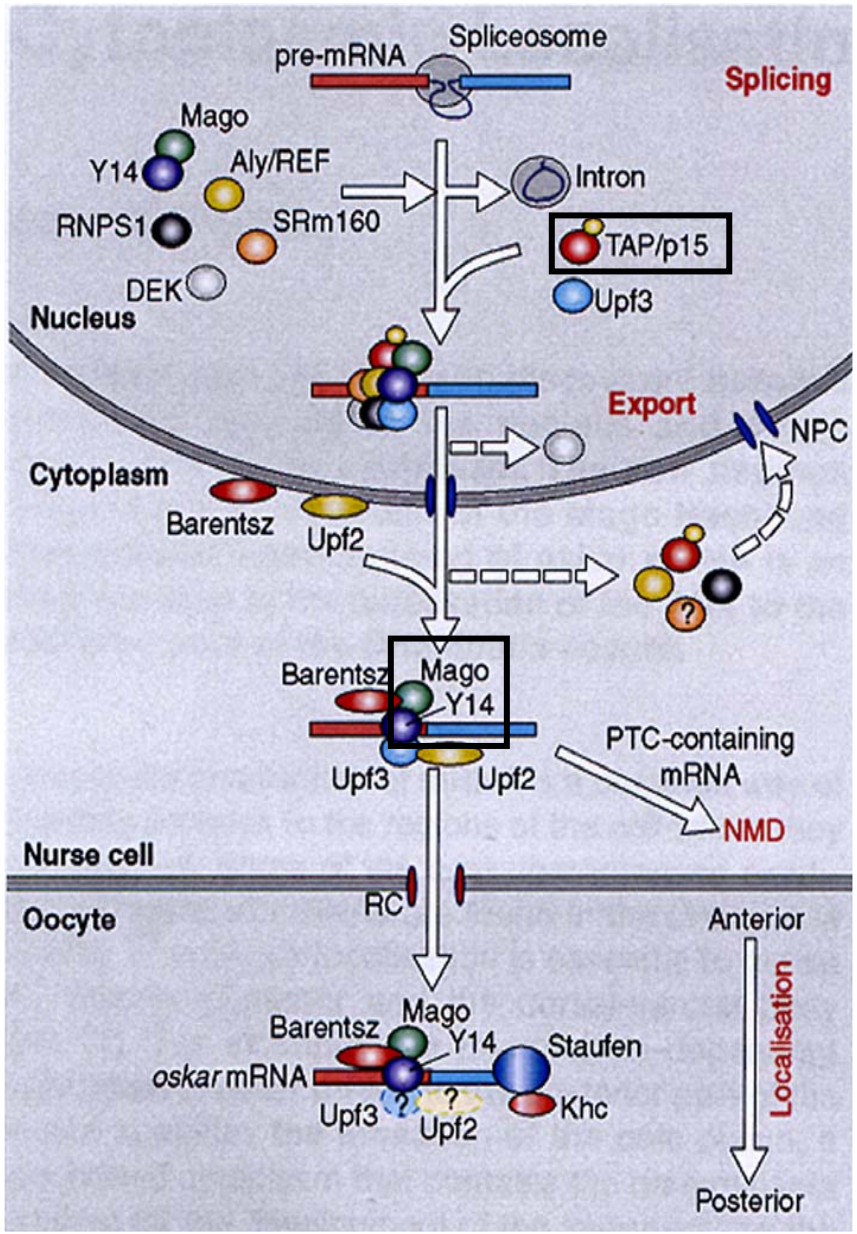


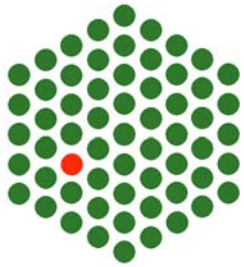
Both Y14 and Mago are required for nonsense mediated mRNA decay

Provide a binding platform for recruitment of the NMD machinery

Same surface is involved in both NMD and oskar mRNA localization

Current view of mRNA export





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