TABLE 11-1 Half-Lives of Messenger RNAs

Cell	Cell Generation Time	mRNA Half-Lives* Average	mRNA Half-Lives* Range Known for Individual Cases
Escherichia coli	20–60 min	3–5 min	2–10 min
Saccharomyces cerevisiae (yeast)	3 h	22 min	4–40 min
Cultured human or rodent cells	16–24 h	10 h	30 min or less (histone and *c-myc* mRNAs) 0.3–24 h (specific mRNAs of cultured cells)

*For information on specific mRNA half-lives for *E. coli*, see A. Hirashima, G. Childs, and M. Inouye, 1973, *J. Mol. Biol.* **119**: 373; for yeast, see L.-L. Chia and C. McLaughlin, 1979, *Mol. Gen. Genet.* **170**:137; and for mammalian cells, see M. M. Harpold, M. Wilson, and J. E. Darnell, 1981, *Mol. Cell Biol.* **1**:188.

Protein-RNA complex structures involved in pre-mRNA processing

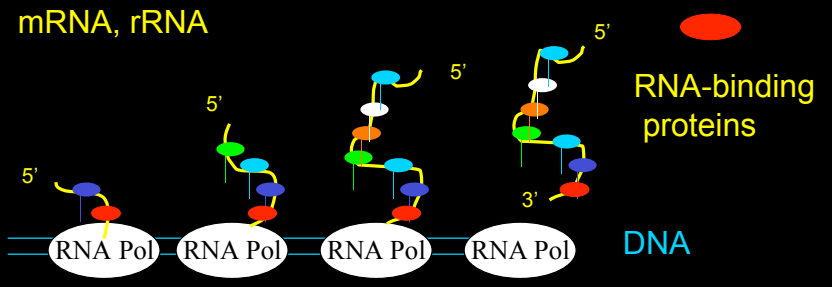
Gene expression and regulation

DNA → RNA → protein

Transcription factors Methylation

?

Phosphorylation Modification



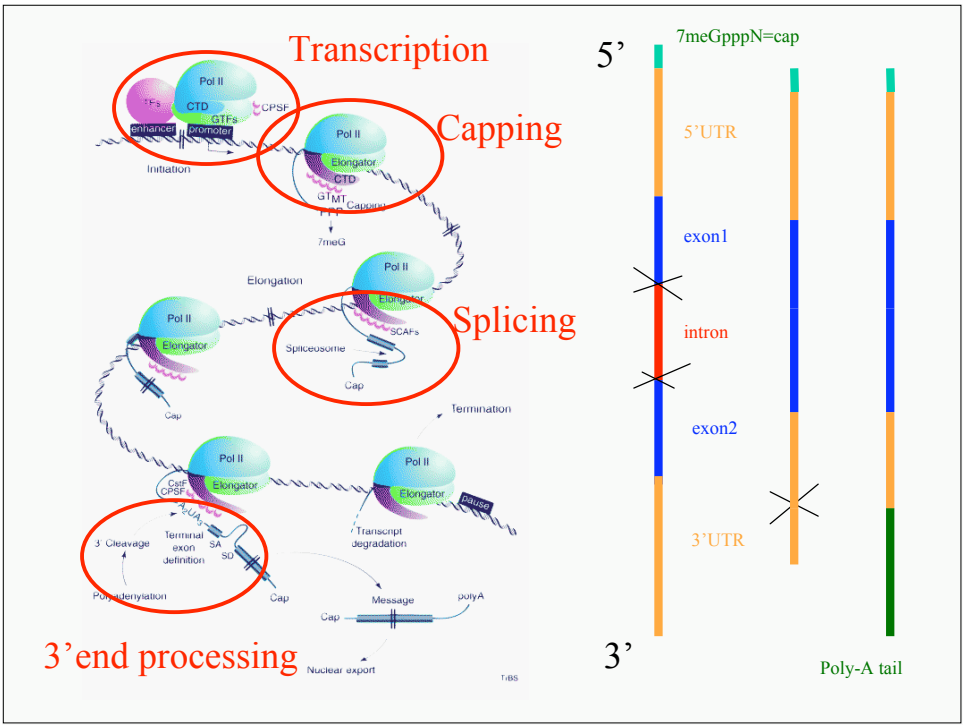
- RNA packaging, stability
- 5' capping
- splicing
- 3' end processing

(cleaveage and polyadenylation)

Alternative-splicing

RNA editing

- export
- translation



Biophysical, chemical approach

RNA binding proteins of two types:

- enzymes

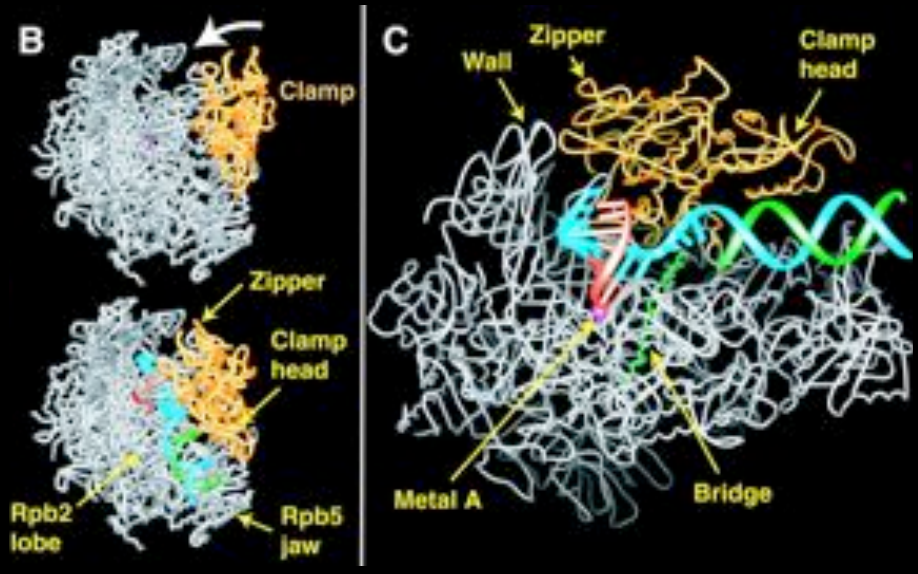
polymerase, nuclease, modifying enzymes

- binding proteins

protection, folding (chaperone), gene regulation

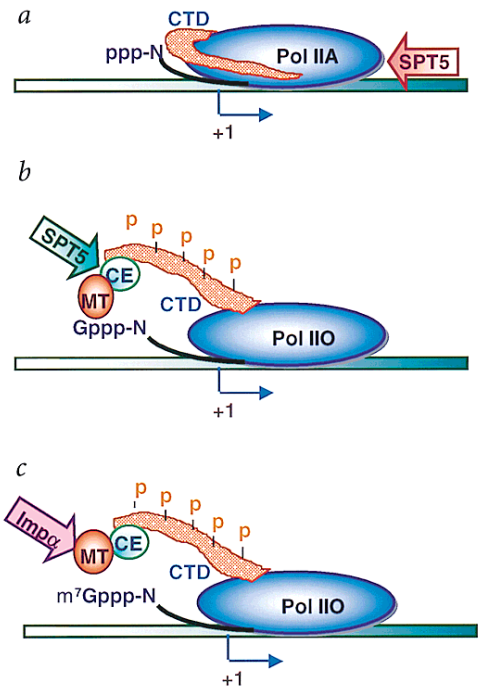
Protein-RNA structure of the constitutive mRNA processing machinery

Yeast Pol II-mRNA-DNA complex



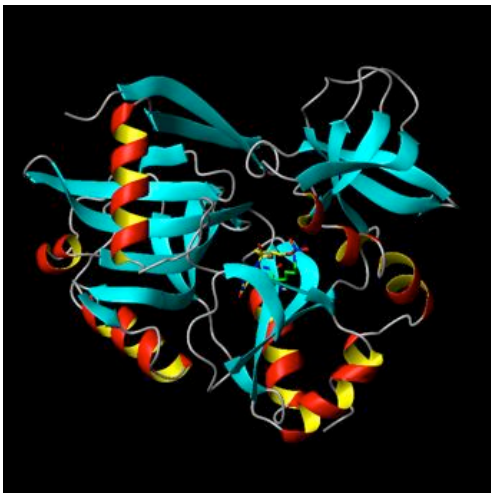
Gnatt et al and Kornberg, Science 2001

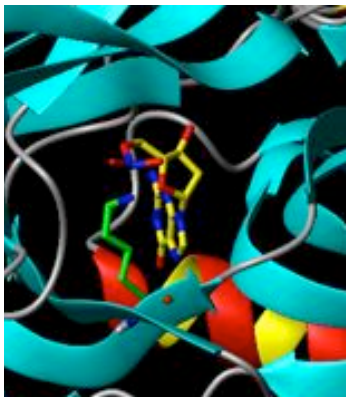
5’ capping



Mechanism of capping (viral capping enzyme)

PP-RNA + GMP → G-PPP-RNA

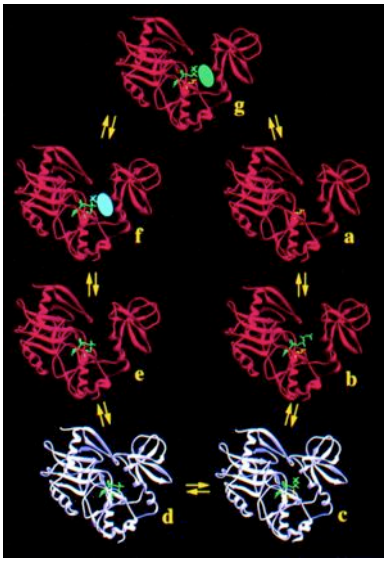


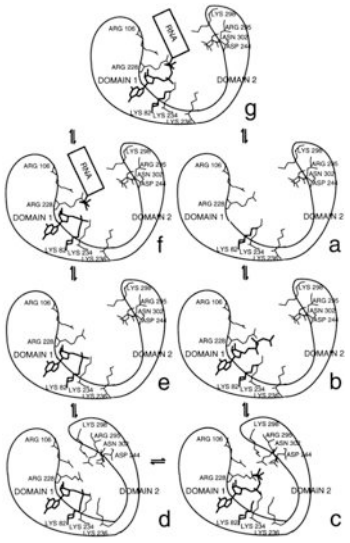


Hakansson et al et Wigley, Cell 1997

Mechanism of capping (viral capping enzyme)

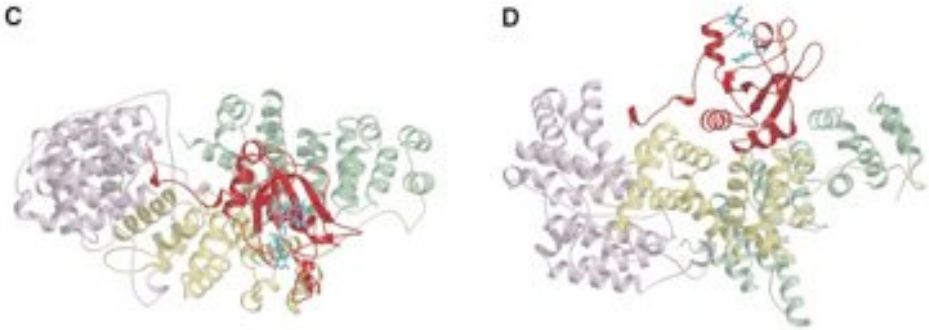
PP-RNA + GMP → G-PPP-RNA





Hakansson et al et Wigley, Cell 1997

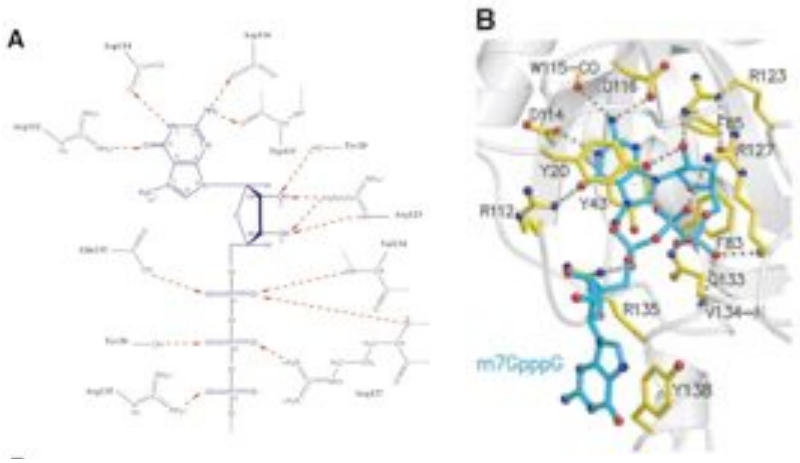
5'cap binding protein, CBP20-CBP80



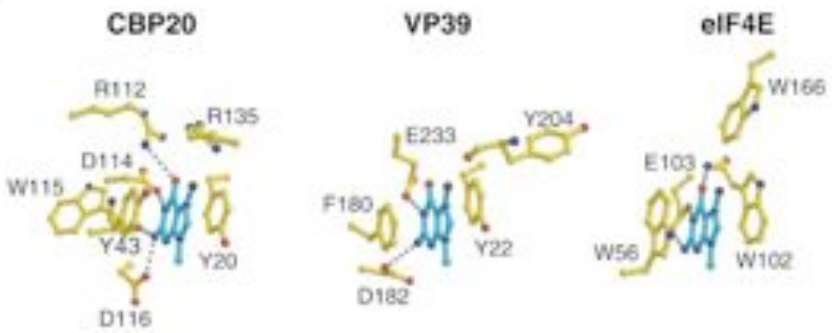
CBP20-CBP80

Mazza et al, EMBO J (2002)

CBP20-m7GpppG contacts

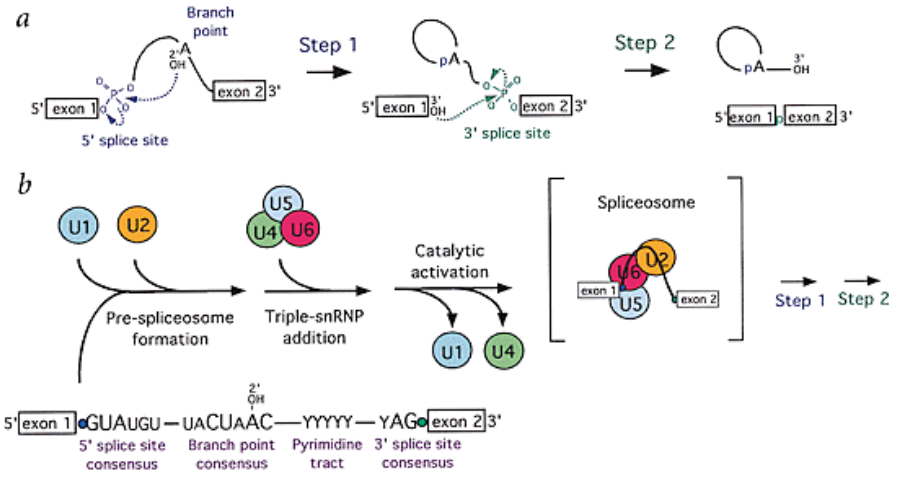


Comparison with other 5'cap binding proteins



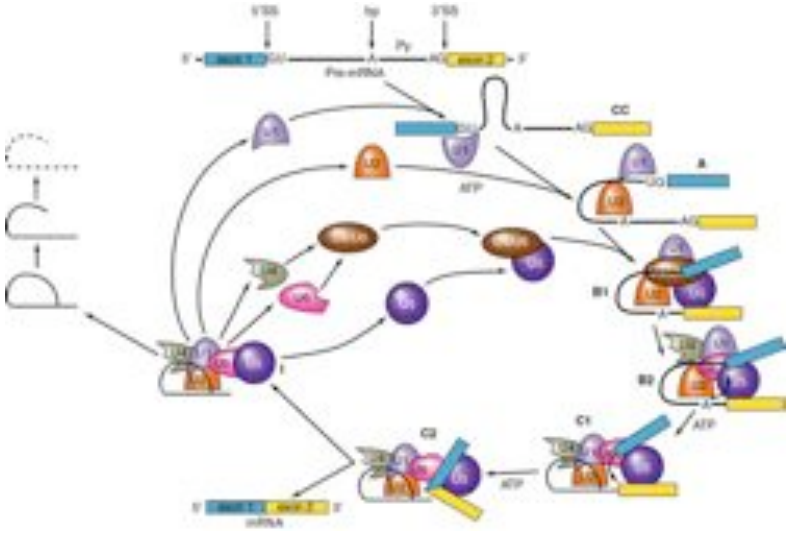
vaccinia virus

Constitutive splicing

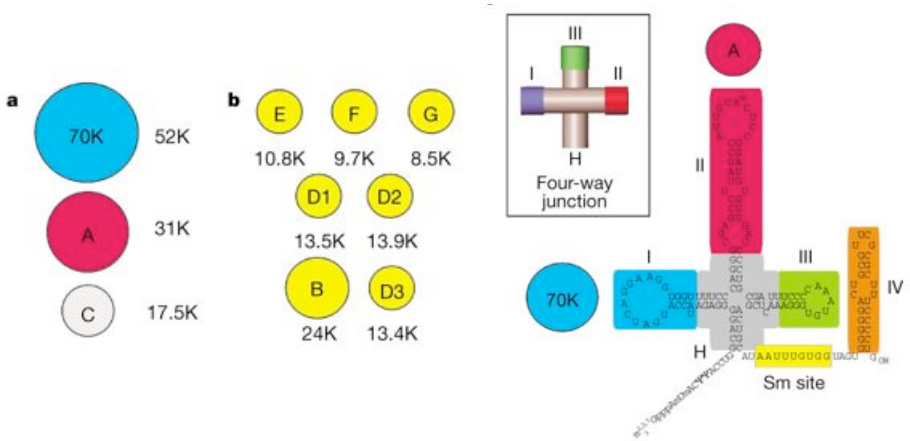


Collins & Guthrie, Nat.Struct.Biol 2000

Pre-mRNA splicing

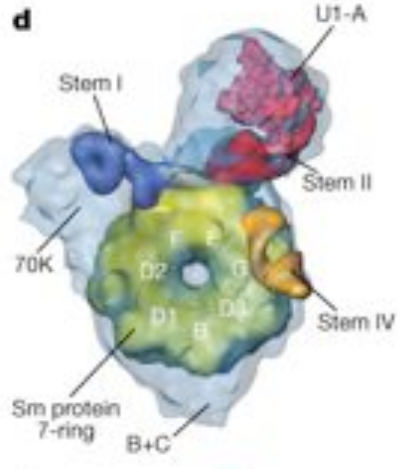


U1snRNP, single particle cryoEM



Stark et al, Nature (2001)

U1snRNP, single particle cryoEM



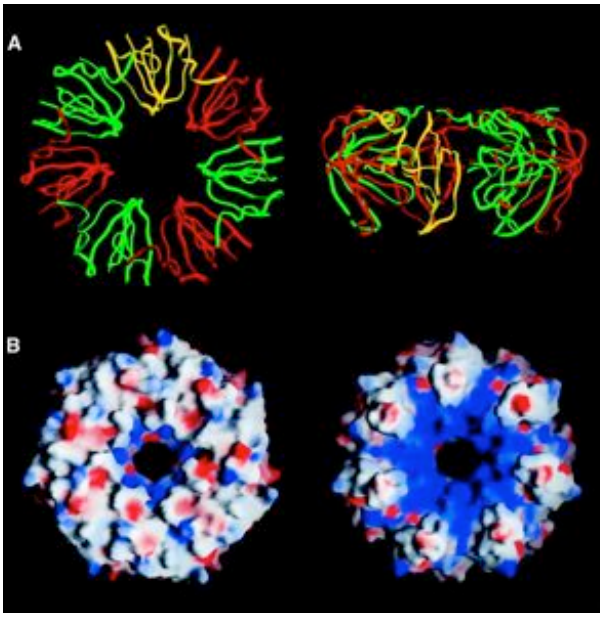
U1 snRNA

Stark et al, Nature (2001)

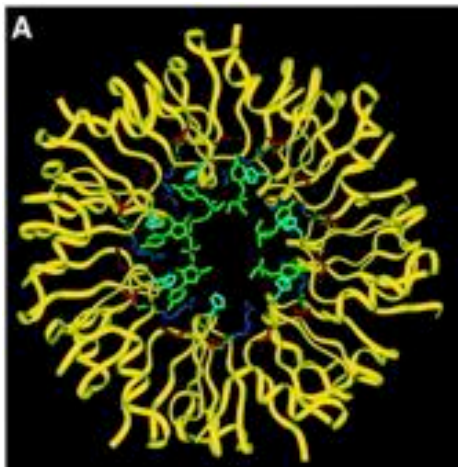
U1A-U1snRNA SLII

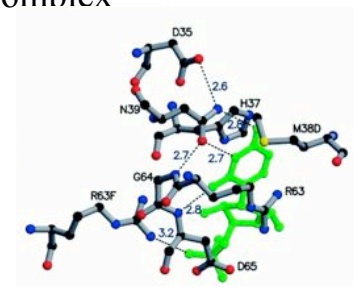
Oubridge et al, Nature (1994)

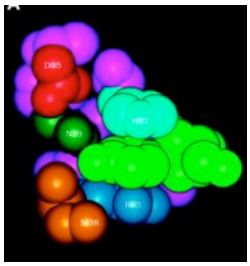
SM proteins-RNA complex, Archaea, Toro et al, EMBO J (2001)



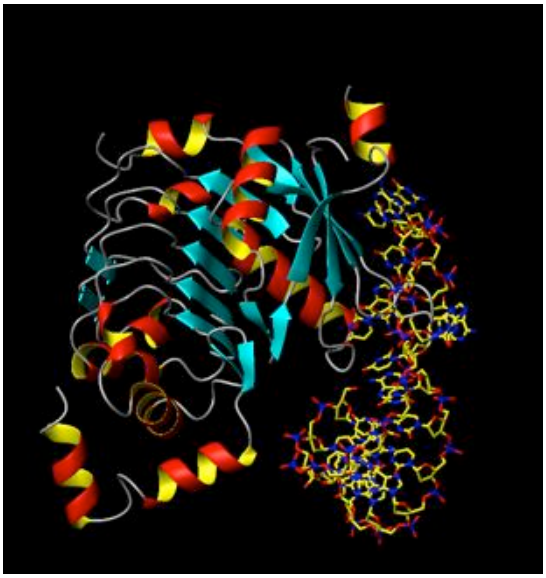
SM protein-RNA complex

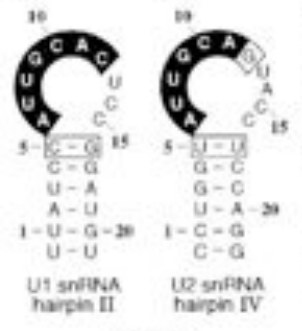






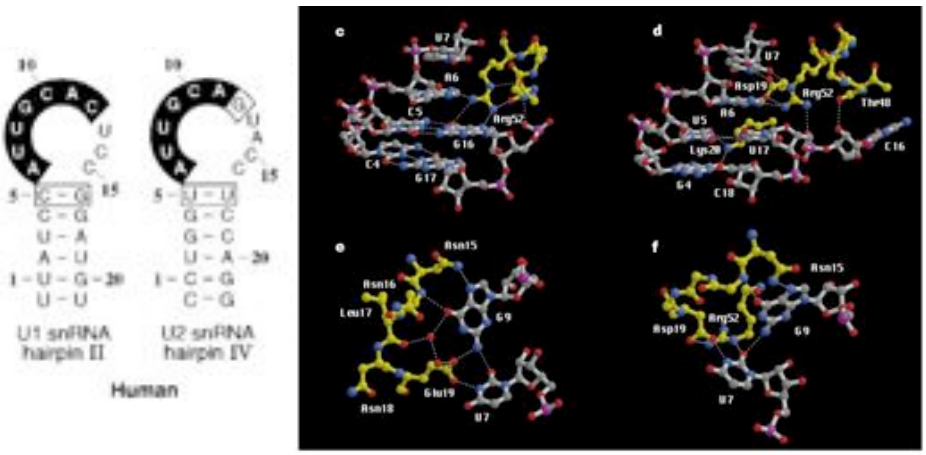
U2A-U2B''-U2snRNA stem-loop IV





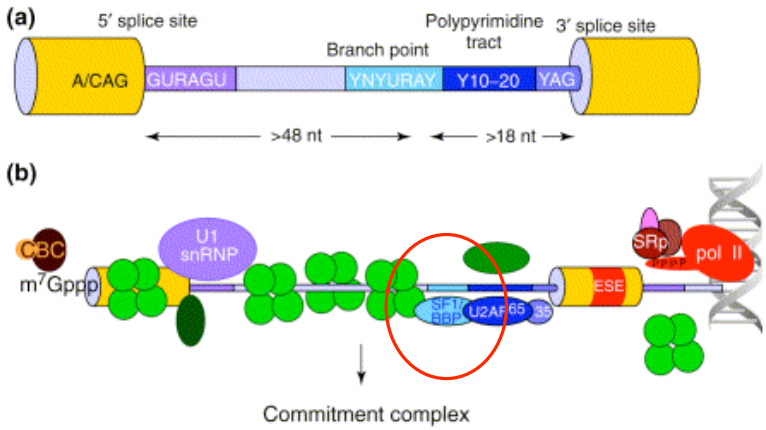
Price et al, Nature (1998)

U2A-U2B''-U2snRNA stem-loop IV

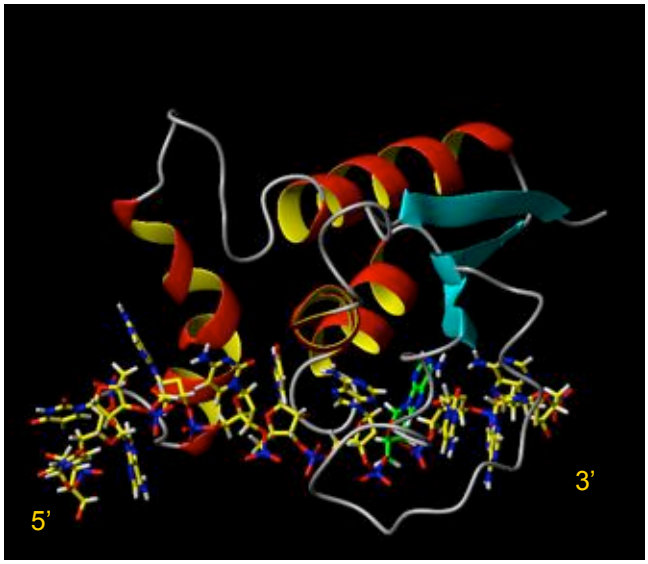


Price et al, Nature (1998)

The commitment complex

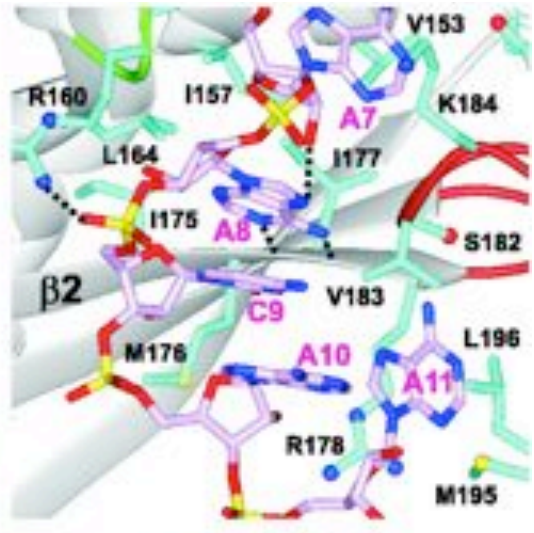


SF1-branch point complex



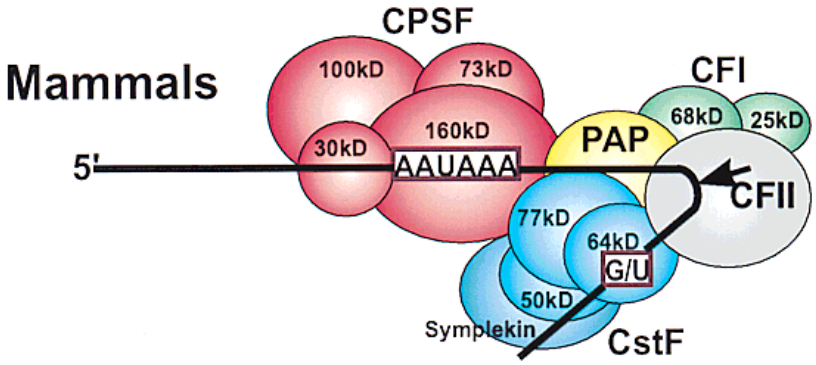
Liu et al, Science (2001)

SF1-branch point (A8) complex

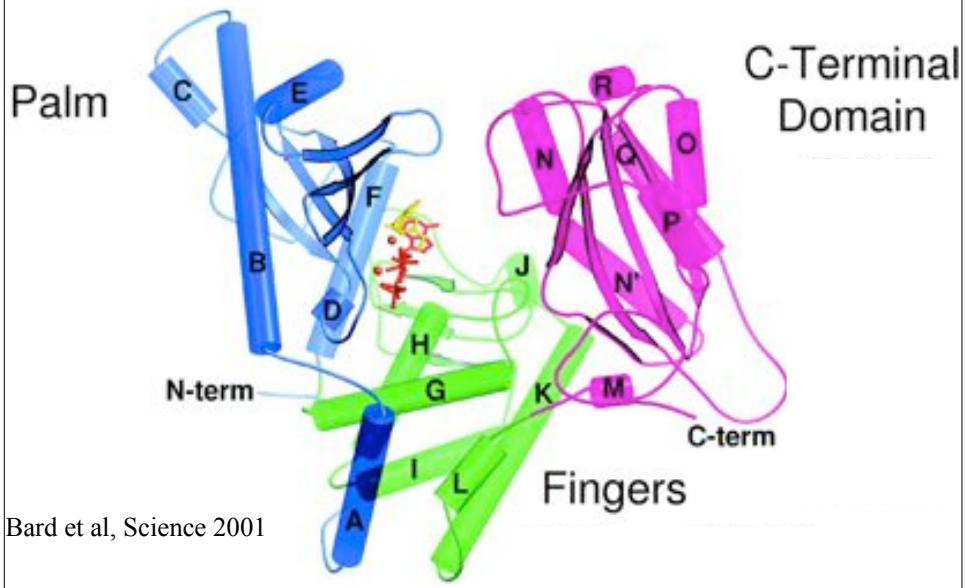


3’ end proceessing

Cleavage and polyadenylation



Yeast PolyA Polymerase-dATP-dATP complex



Bard et al, Science 2001

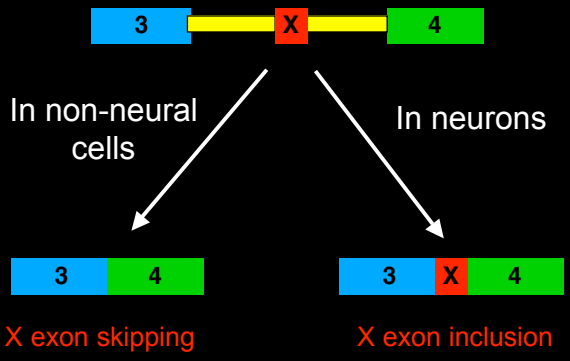
Poly A binding protein

(Deo et *al*, Cell, v98 1999)

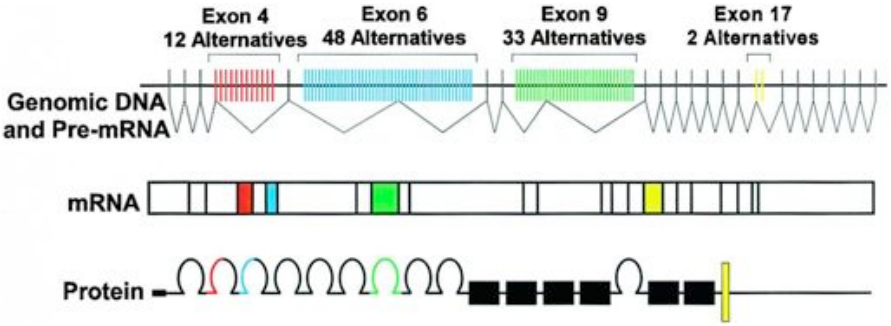
Protein-RNA structures of the regulated mRNA processing machinery

Post-transcriptional gene regulation
Unique to each gene

Alternative splicing

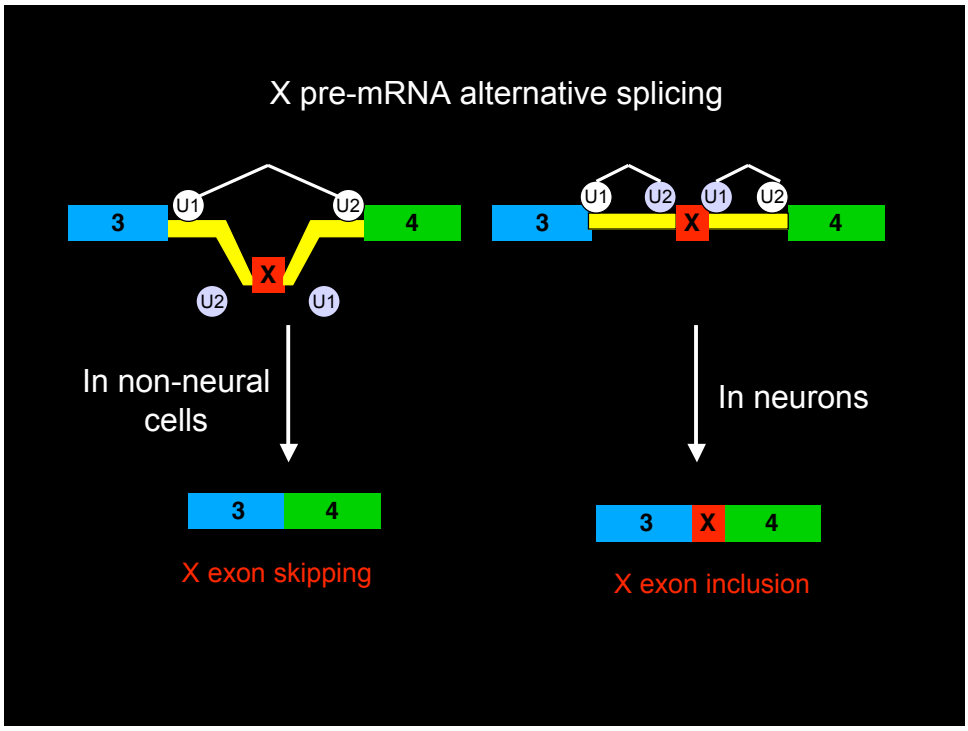


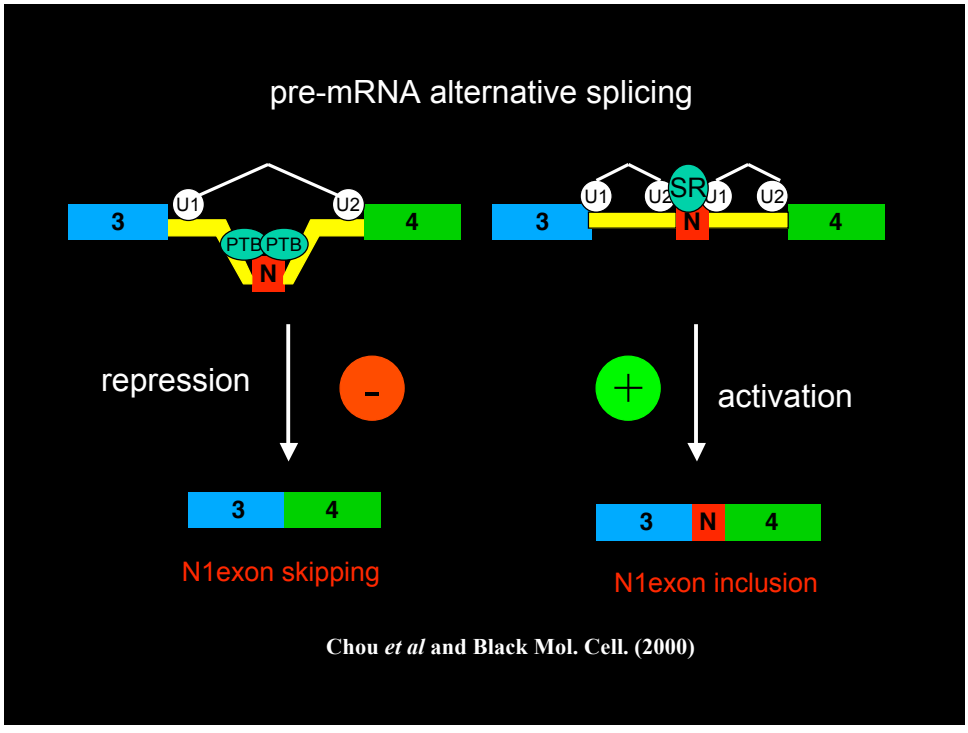
Alternative-splicing of the DSCAM gene



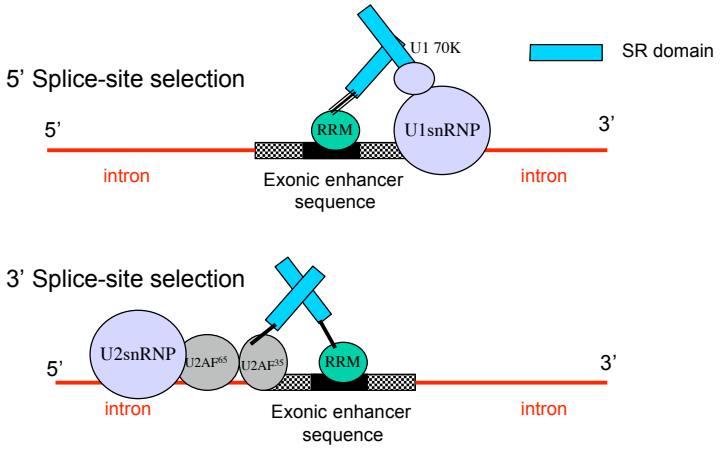
Can produce up to 38016 different protein isoforms

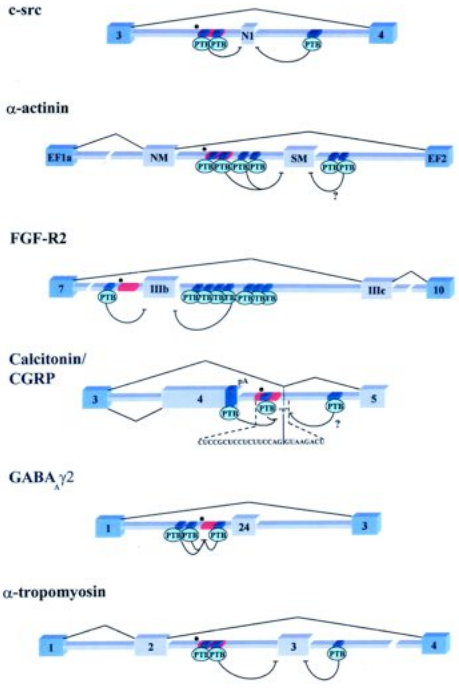
From Black D.L. *Cell* (2000), v103 p367


X pre-mRNA alternative splicing
U1
U2
3
4
X
In non-neural cells
In neurons
X exon skipping
X exon inclusion


pre-mRNA alternative splicing
U1
U2
3
4
PTB
N
SR
repression
activation
N1exon skipping
N1exon inclusion
Chou et al and Black Mol. Cell. (2000)

Exon definition by SR proteins





PTB is a general Splicing repressor

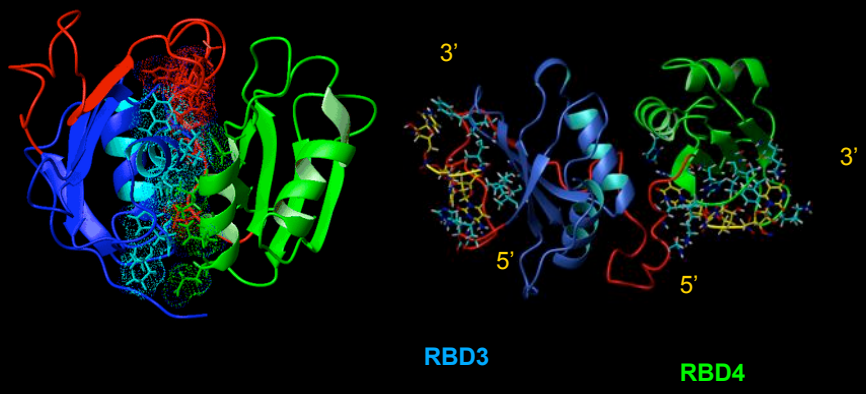
-

Pyr tract

Pyr tract near Branch point

Wagner et al (2001)
Mol Cell Biol **21**, 3281 - 3288

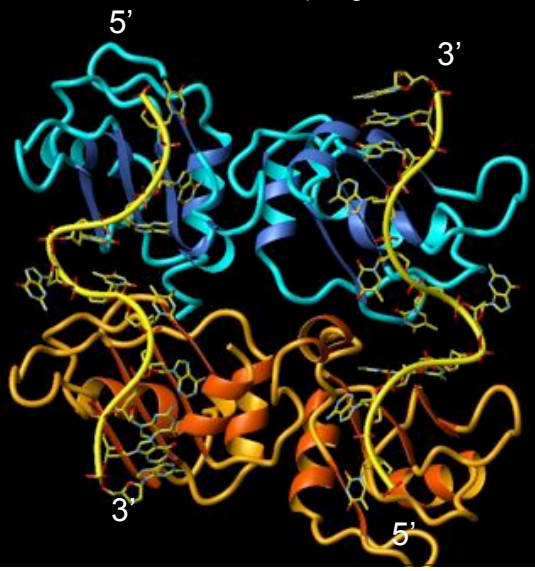
Model for splicing repression by PTB



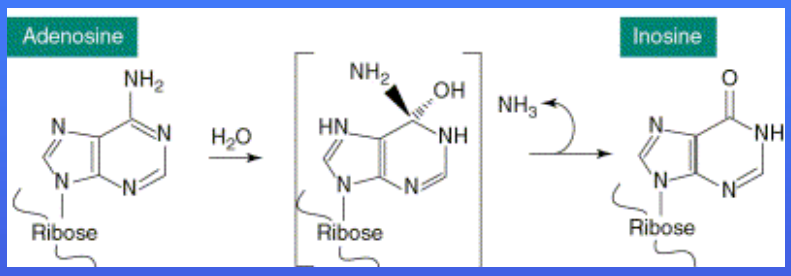
Splicing repression

hnRNPA1- DNA

(Ding et *al*, Gene & Dev, 1999)



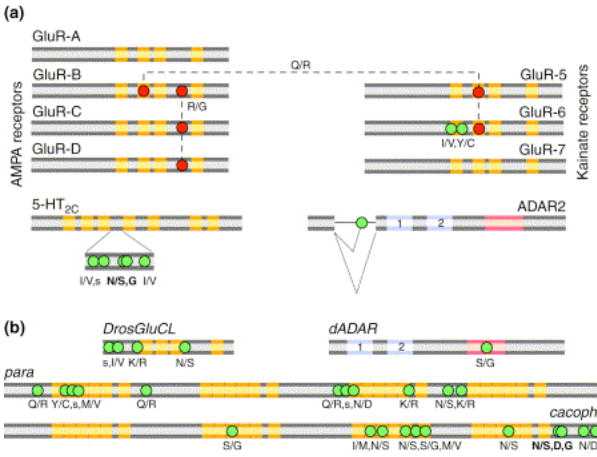
RNA editing by adenine deamination



A → I

read as G

Pre-mRNA editing by Adenosine deamination

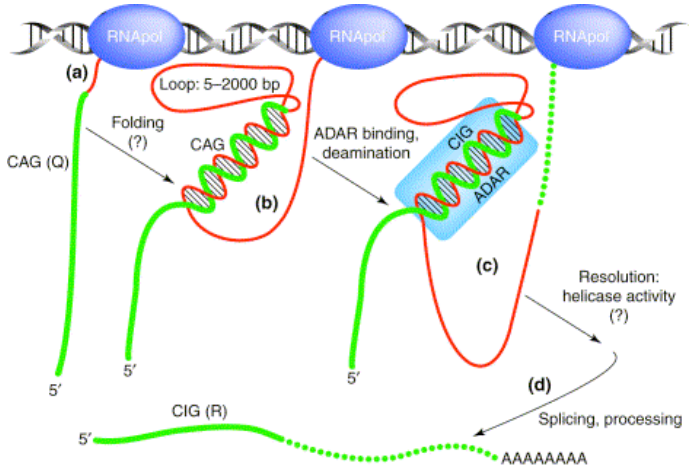


Human genes

Drosophila genes

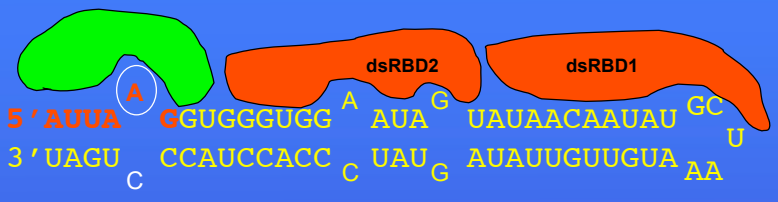
From Reenan *Trends in Genetics* (2001), v17 p53

Editing mechanism by ADAR



From Reenan *Trends in Genetics* (2001), v17 p53

GluR-B pre-mRNA



Arg (**AGG**) to Gly (**GGG**)

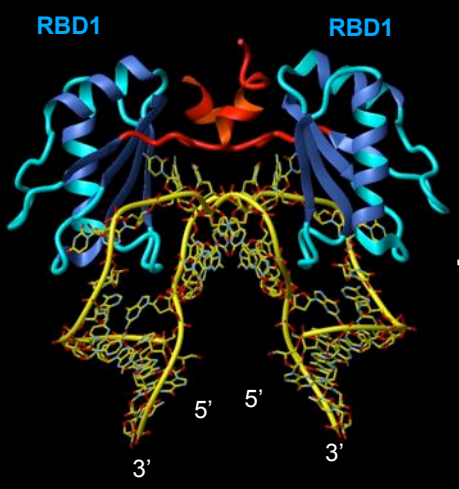
ADAR2

RNA binding domain **Deaminase domain**

Drosophila *Paralytic* gene

13 alternative exons
+
11 RNA editing sites
=
> 1 000 000 potential isoforms

Gene regulation at the 3'end



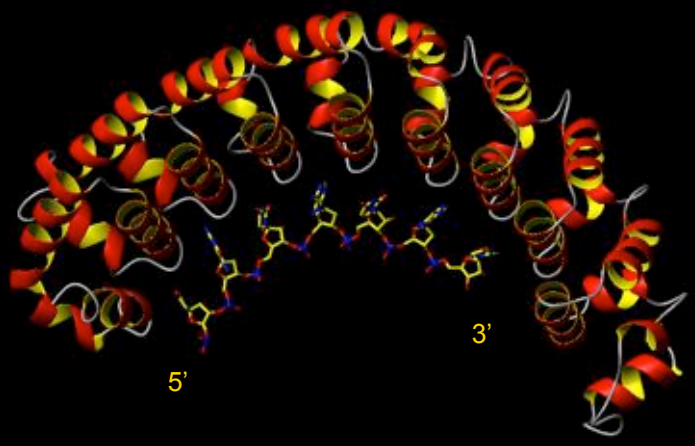
U1A-3’UTR

(Varani et *al*, Nature Struct Biol, v7 2000)

Direct interaction with the PAP and inhibition

Gene regulation by a feed back mechanism

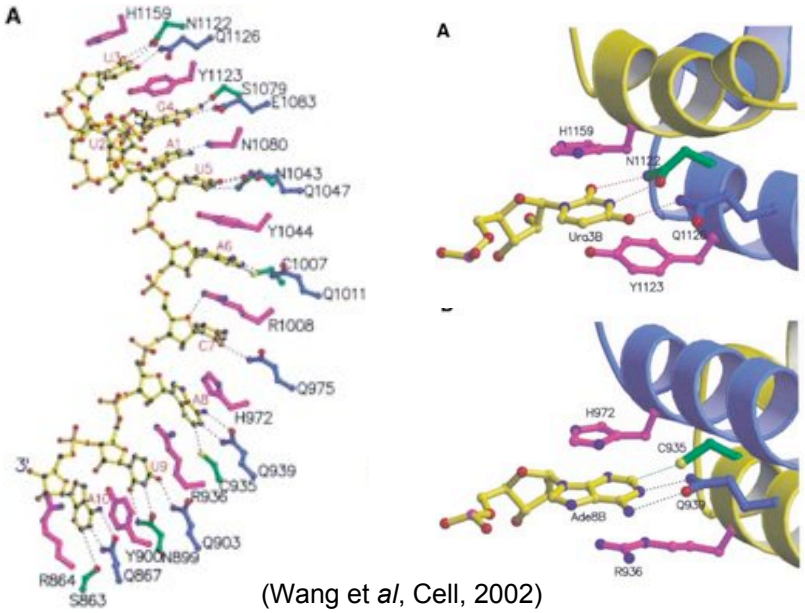
Pumilio domain



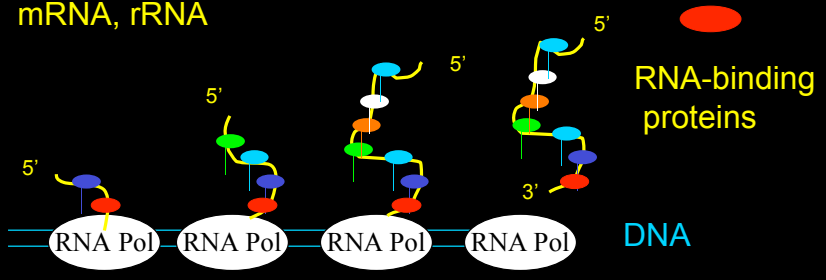
hPUM-UGUAUAU

(Wang et *al*, Cell, 2002)

Pumilio domain



(Wang et *al*, Cell, 2002)



protection, folding (chaperone), gene regulation

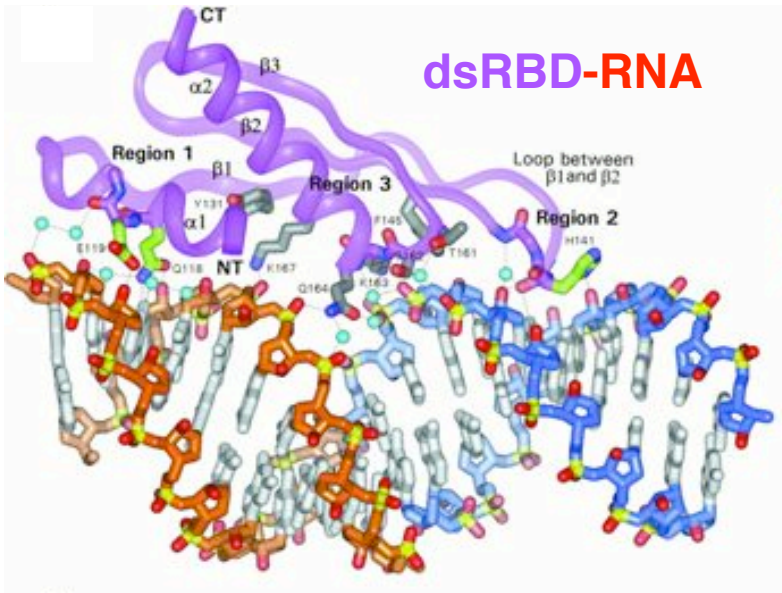
RNA binding specificity

RNA binding proteins:

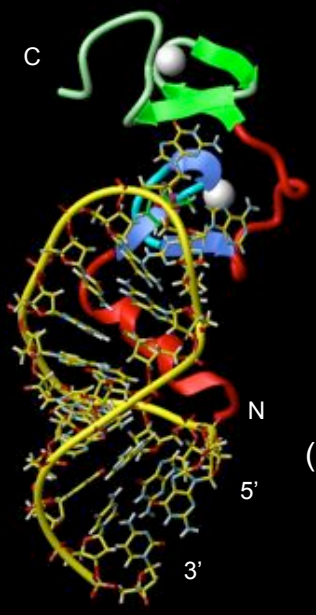
multidomain protein

RGG
(Gar)
SR
Dimerisation

RBD/RRM/RNP
KH, Sam
dsRBD
Arg-rich
Zinc knuckle
Zinc finger



Ryter and Schultz, Embo J, 1998



HIV-1 RNA packaging

HIV-1Nucleocapsid-SL3

(De Guzman et *al*, Science, 1998)

Arg-rich-RNA

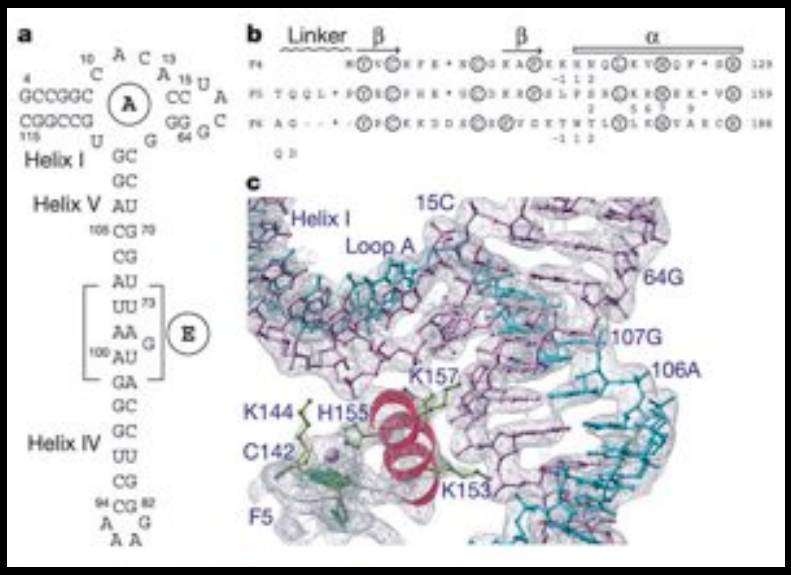
HIV rev-RRE

Battiste et al, and Williamson Science 1996

BIV tat-TAR

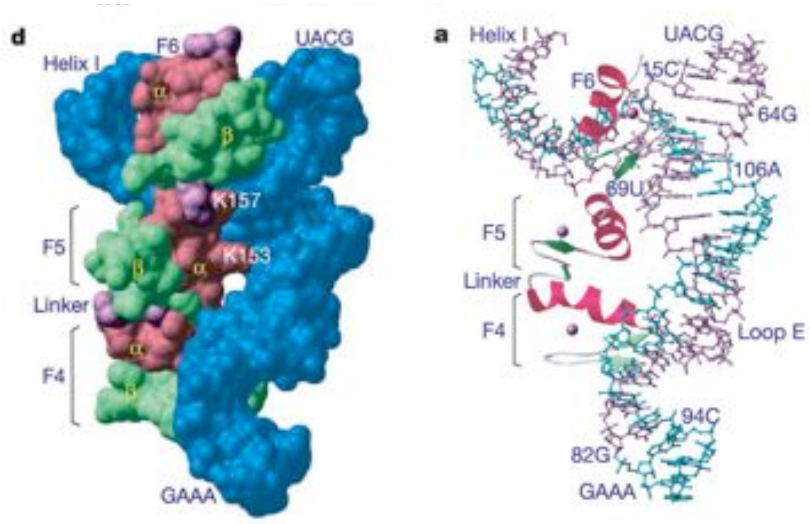
Puglisi et al, Science 1996

Zinc finger-RNA



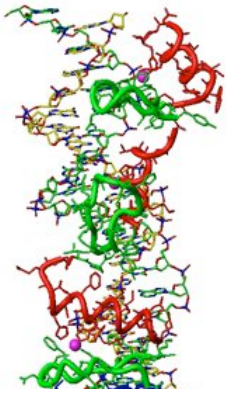
(Lu et *al*, Nature , 2003)

Zinc finger-RNA



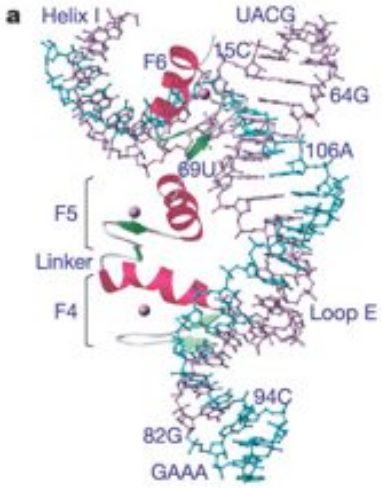
(Lu et *al*, Nature , 2003)

Zinc finger-DNA



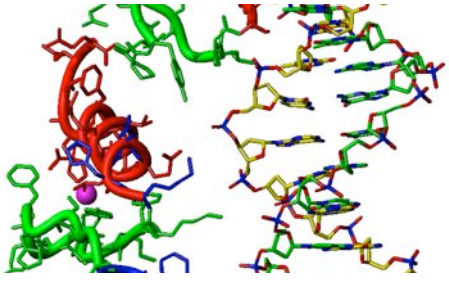
(Nolte et *al*, PNAS , 1998)

Zinc finger-RNA



(Lu et *al*, Nature , 2003)

Zinc finger4-DNA

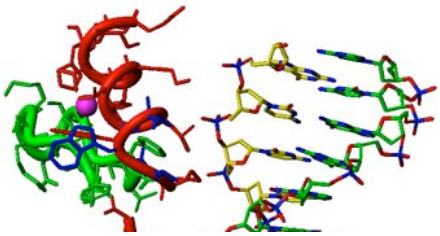


(Nolte et *al*, PNAS , 1998)

Zinc finger4-RNA

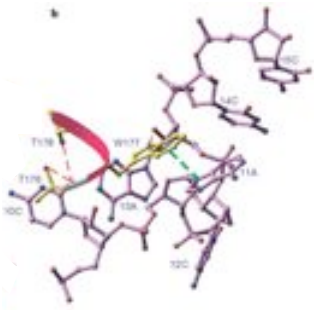
(Lu et *al*, Nature , 2003)

Zinc finger6-DNA



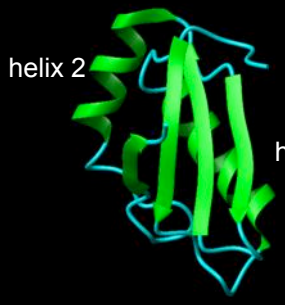
(Nolte et *al*, PNAS , 1998)

Zinc finger6-RNA

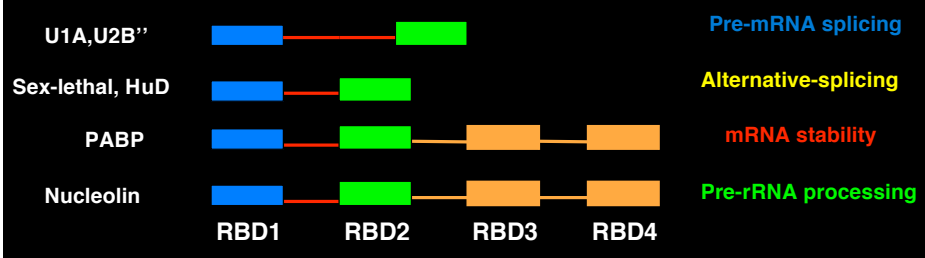


(Lu et *al*, Nature , 2003)

RNA Binding Domain or RNP domain



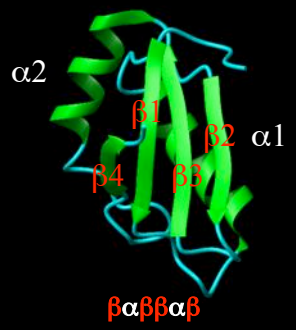
The most common RNA binding modules found in 224 proteins (324 modules) (0.5-1% of the genes)



RNA Binding Domain or RNP domain

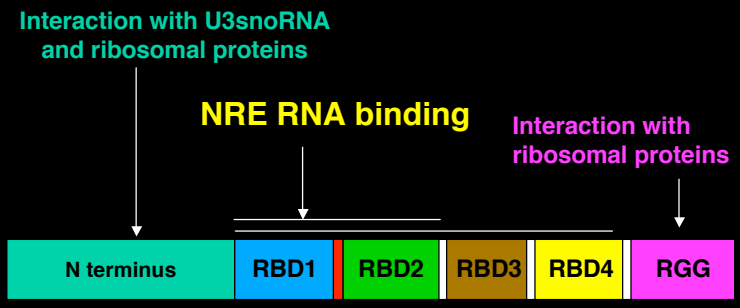
75-85 residue long

β1	U(F/Y)U*NL
α1	***L***F
β2	G*U**Z*U
β3	(R/K)G(F/Y)(A/G)(F/Y)V*F
α2	Z**AU**
β4	G*U*U**

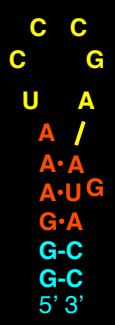


U hydrophobic
Z non charged
* any residue

Nucleolin, the most abundant nucleolar protein

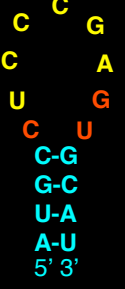


Nucleolin RNA Targets



sNRE
5-20nM

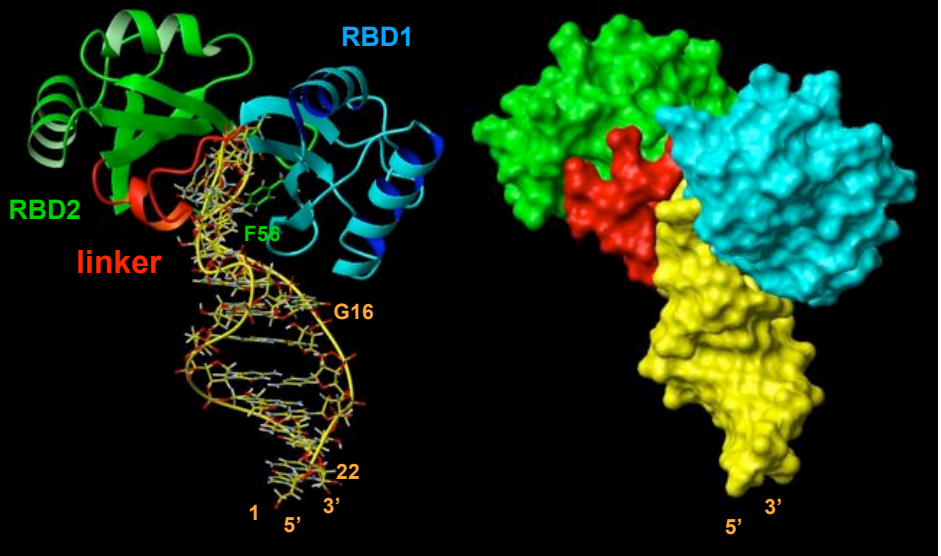
B1
10-50nM
(5'ETS)
Mouse

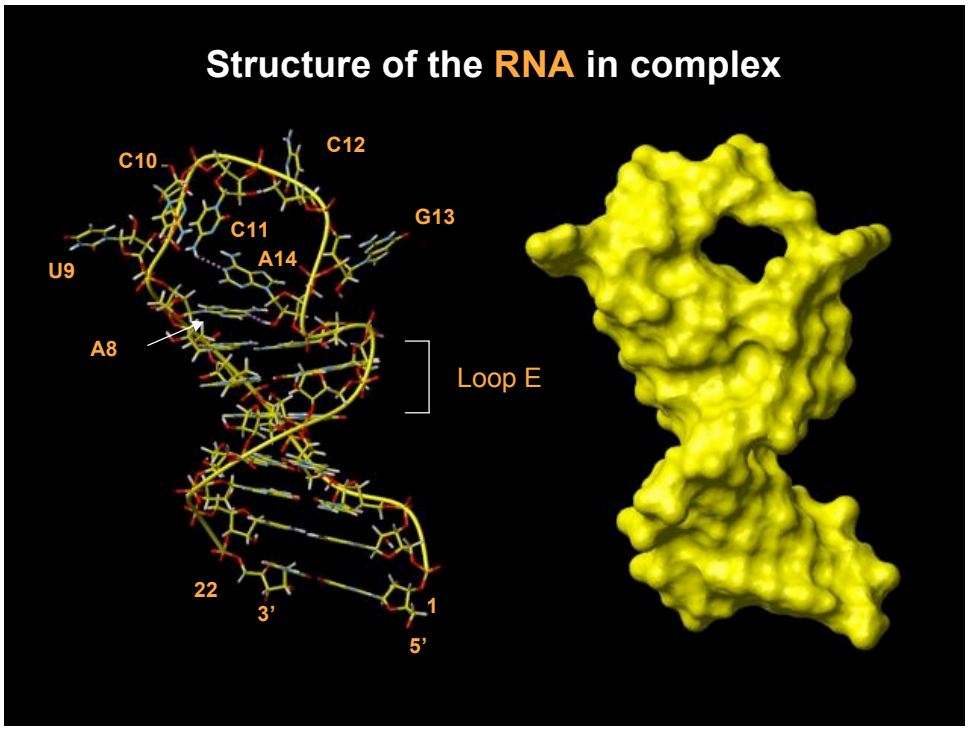


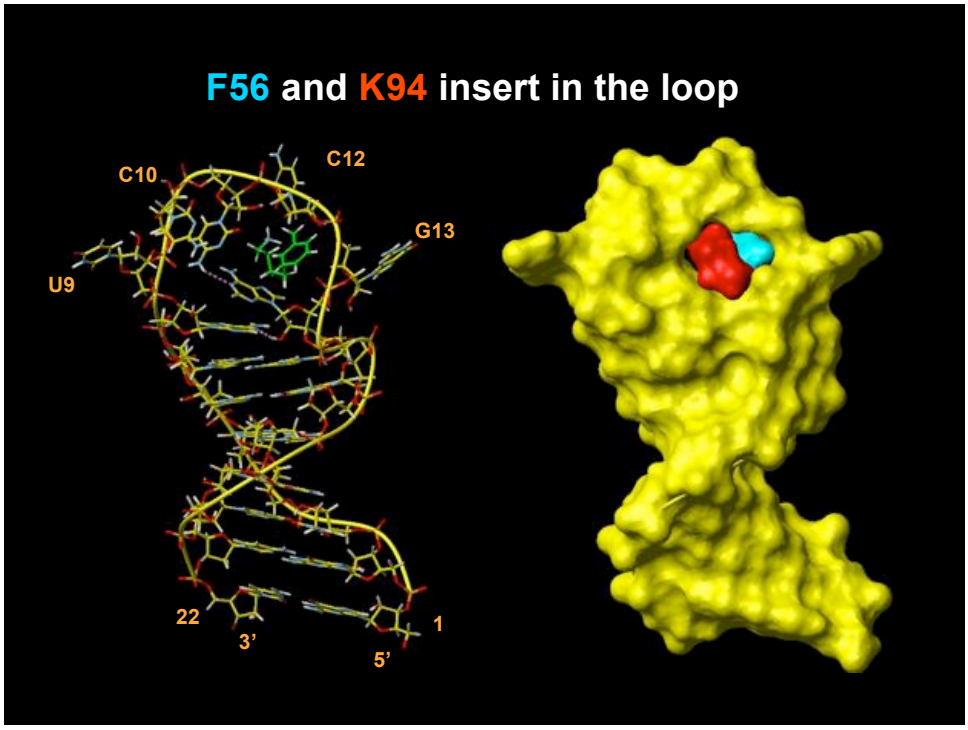
B2
50-100nM
(5'ETS)
Mouse

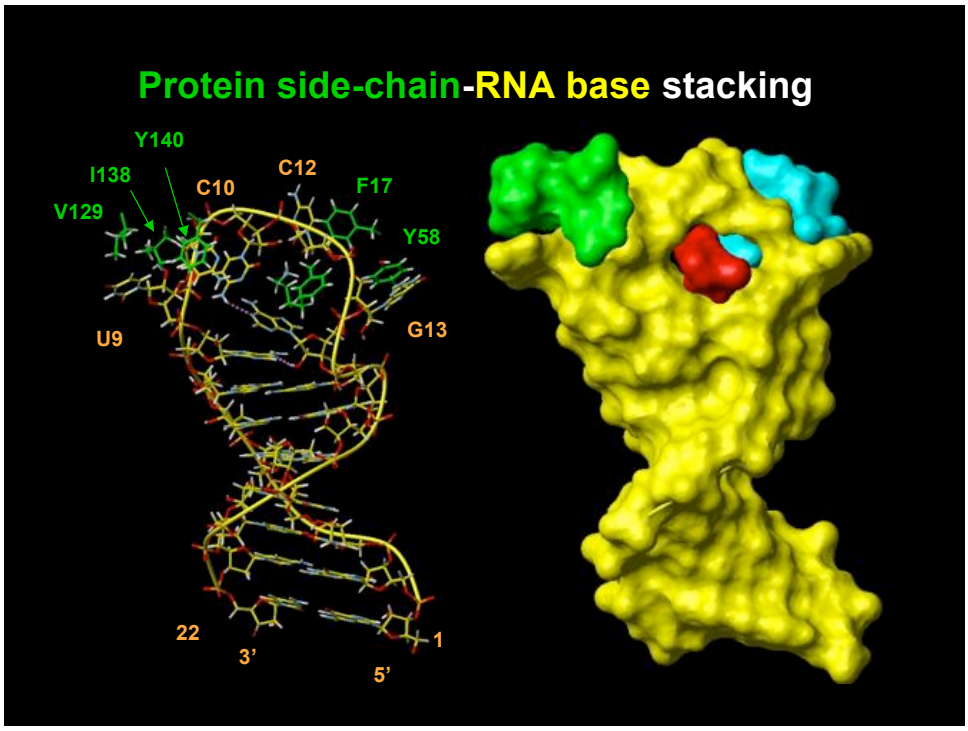
Consensus NRE

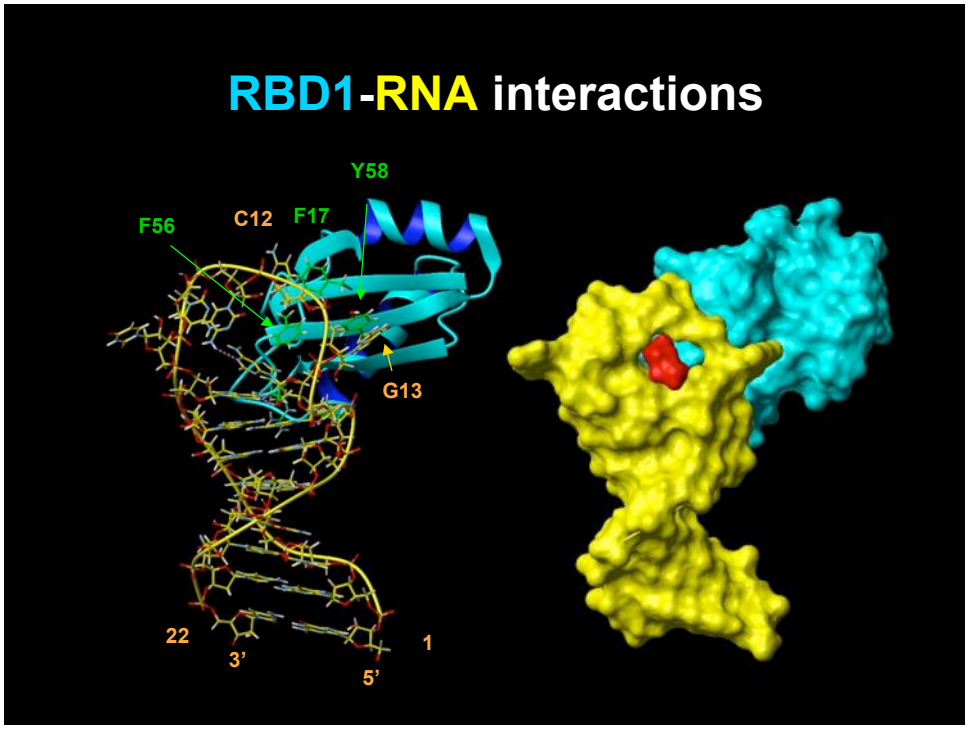
RBD2-RNA-RBD1 "sandwich"



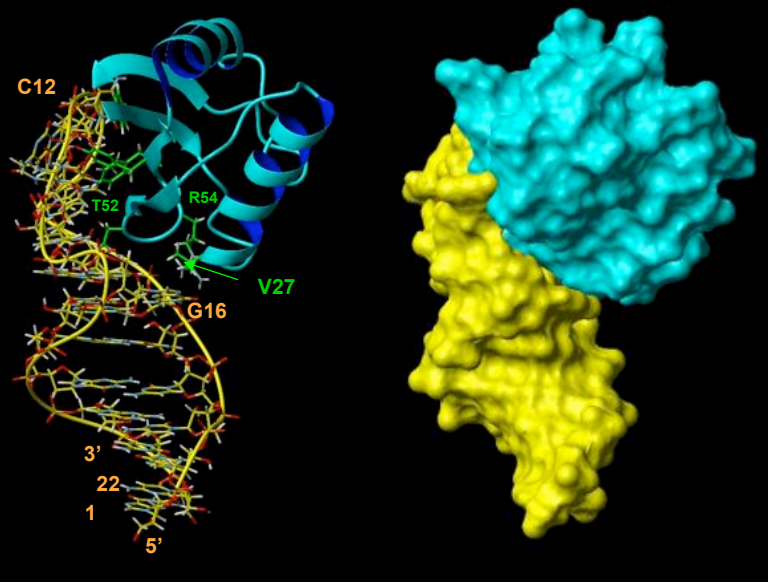

Structure of the RNA in complex
C10
C12
G13
C11
A14
U9
A8
Loop E
22
3'
1
5'


F56 and K94 insert in the loop
C10
C12
G13
U9
22
3'
1
5'

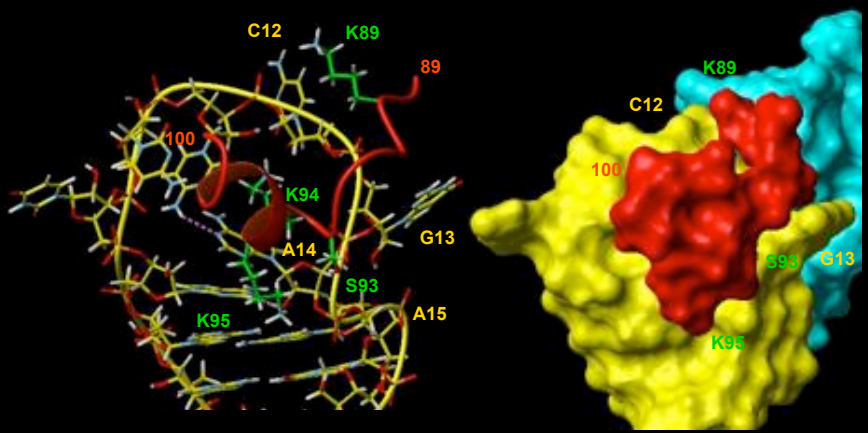

Protein side-chain-RNA base stacking
Y140
I138
V129
C10
C12
F17
Y58
U9
G13
22
3'
5'
1


RBD1-RNA interactions
Y58
F56
C12
F17
G13
22
3'
5'
1

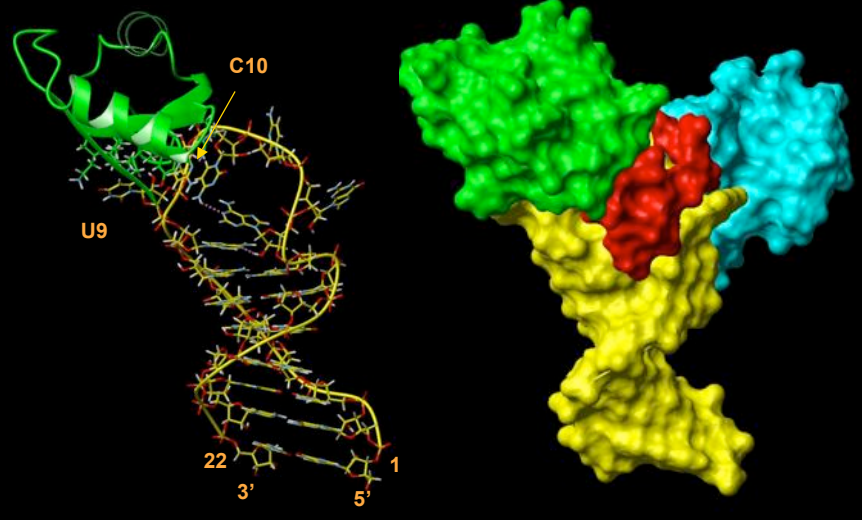
RBD1-RNA stem interactions



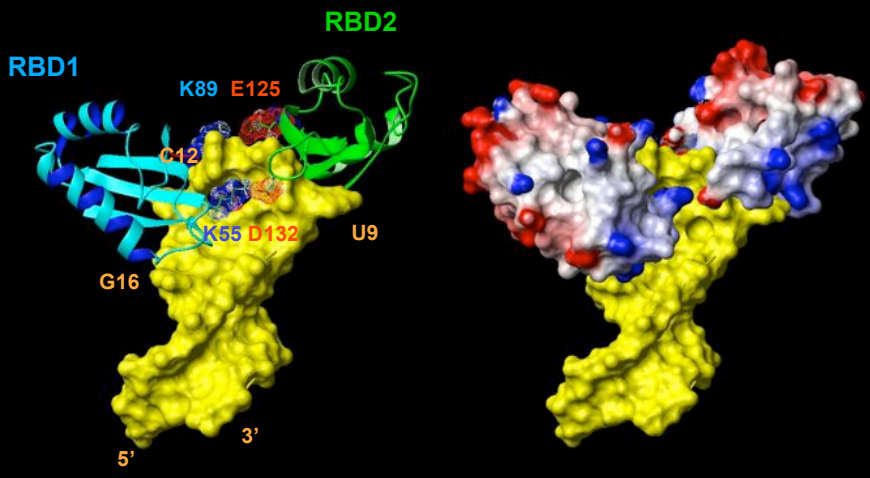
linker-RNA interactions

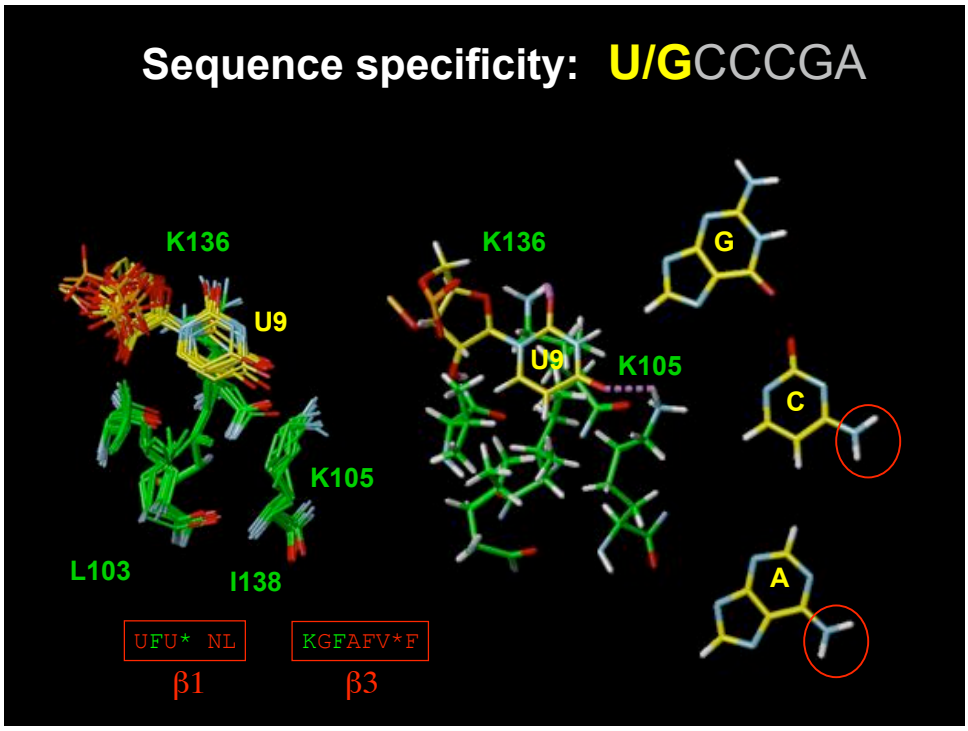


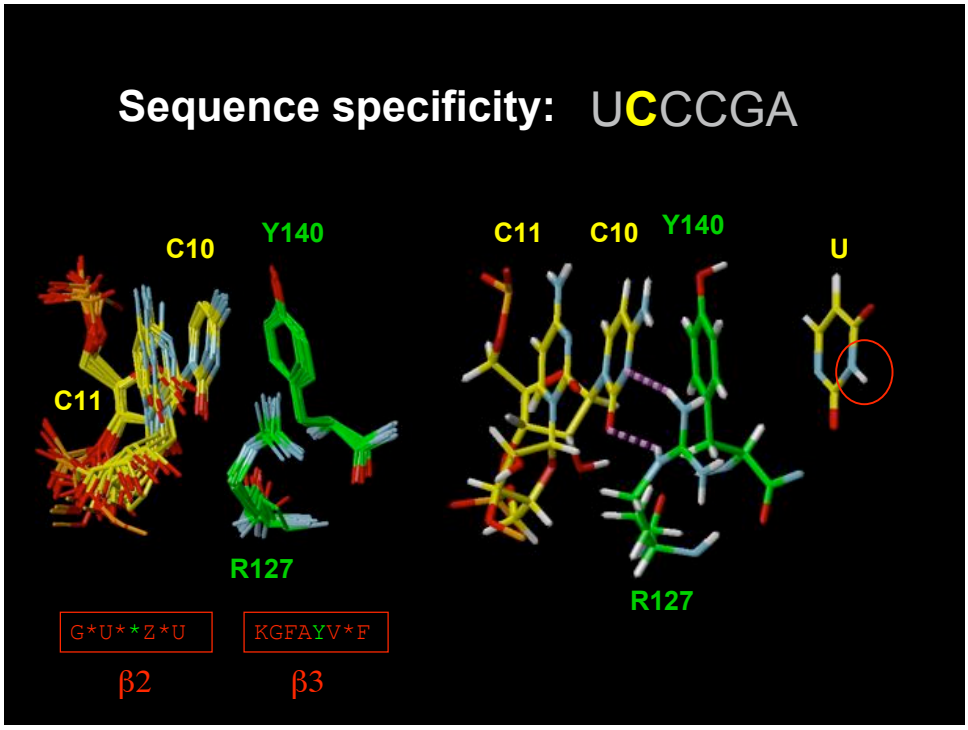
RBD2-RNA interactions



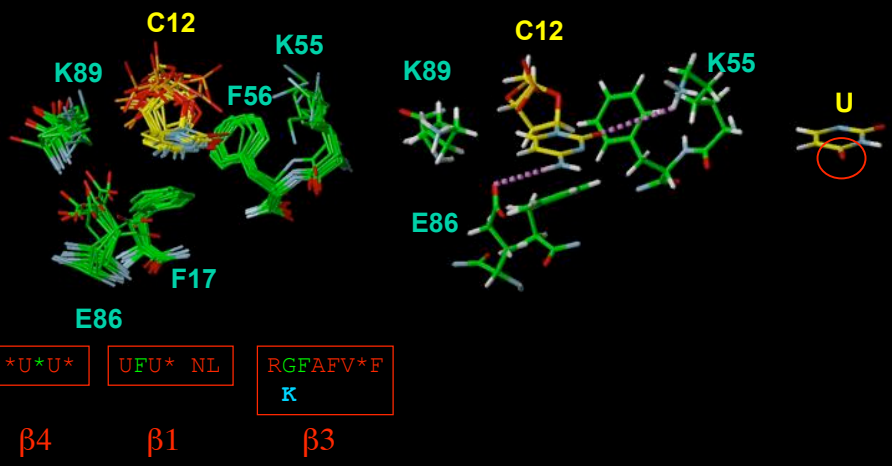
RBD1-RBD2 interactions



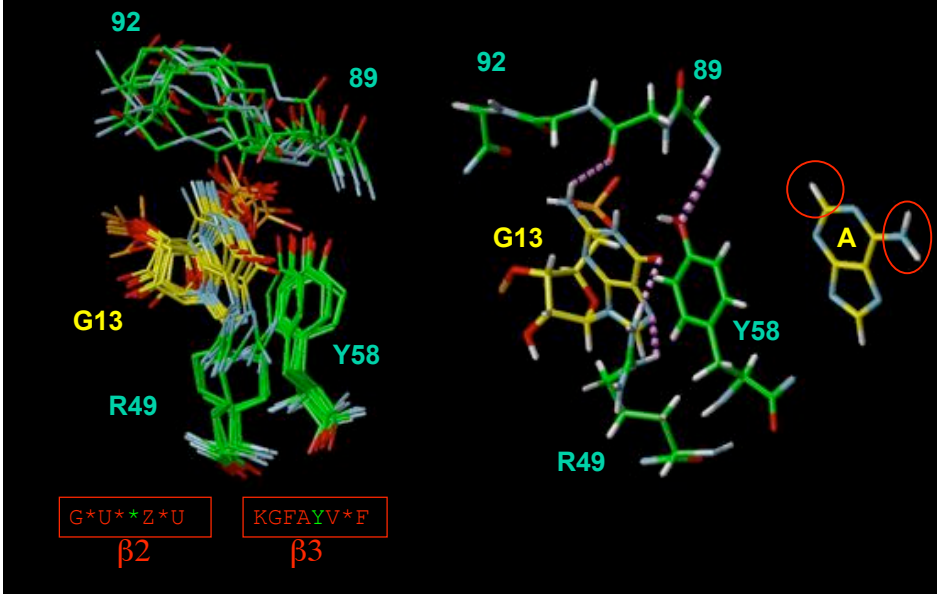

Sequence specificity: U/GCCCGA
K136
U9
K105
L103
I138
UFU* NL
β1
KGFAFV*F
β3
K136
G
U9
K105
C
A

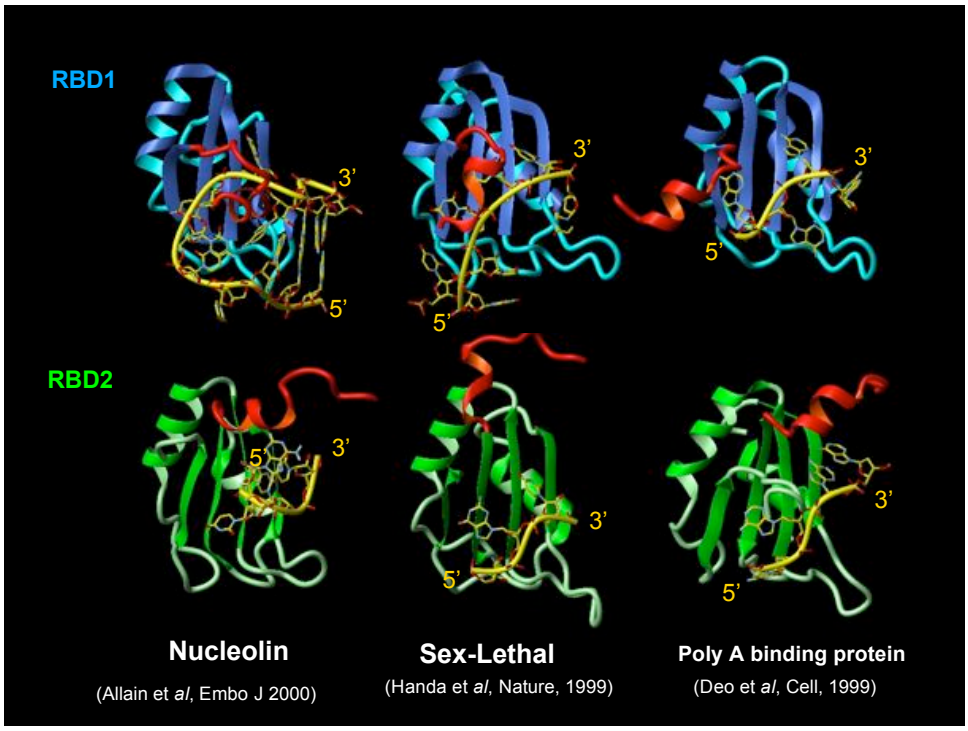

Sequence specificity: UCCCGA
C10
Y140
C11
R127
G*U**Z*U
β2
KGFAYV*F
β3
C11
C10
Y140
U
R127

Sequence specificity: UCC**C**GA



Sequence specificity: UCCC**G**A




RBD1
RBD2
3'
5'
3'
5'
3'
5'
5'
3'
3'
5'
3'
5'
Nucleolin
(Allain et *al*, Embo J 2000)
Sex-Lethal
(Handa et *al*, Nature, 1999)
Poly A binding protein
(Deo et *al*, Cell, 1999)