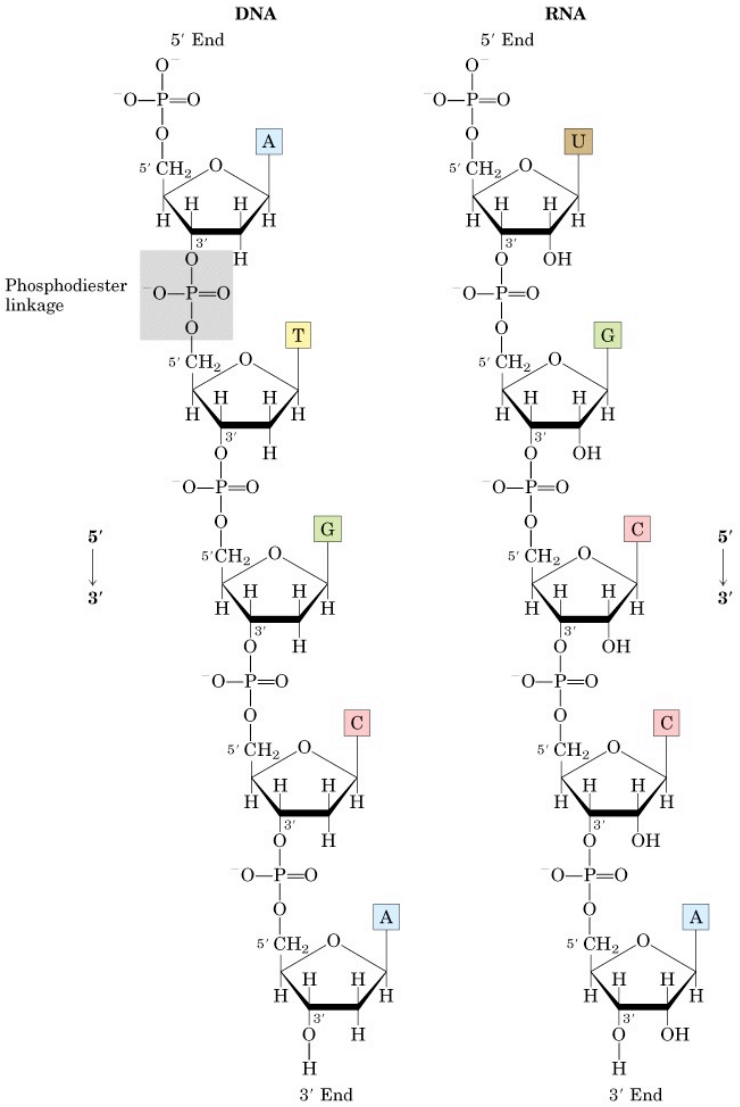
RNA overview

RNA is reactive due to its 2’ OH group

RNA is the first read-out of the genetic information
RNA is more than a messenger, RNA ‘interprets’ the genetic information
RNA is processed, which changes the readout of the genetic information
RNA can have enzymatic activity
RNA is structural more diverse than DNA
Proteins have evolved that stabilize the structure of RNA

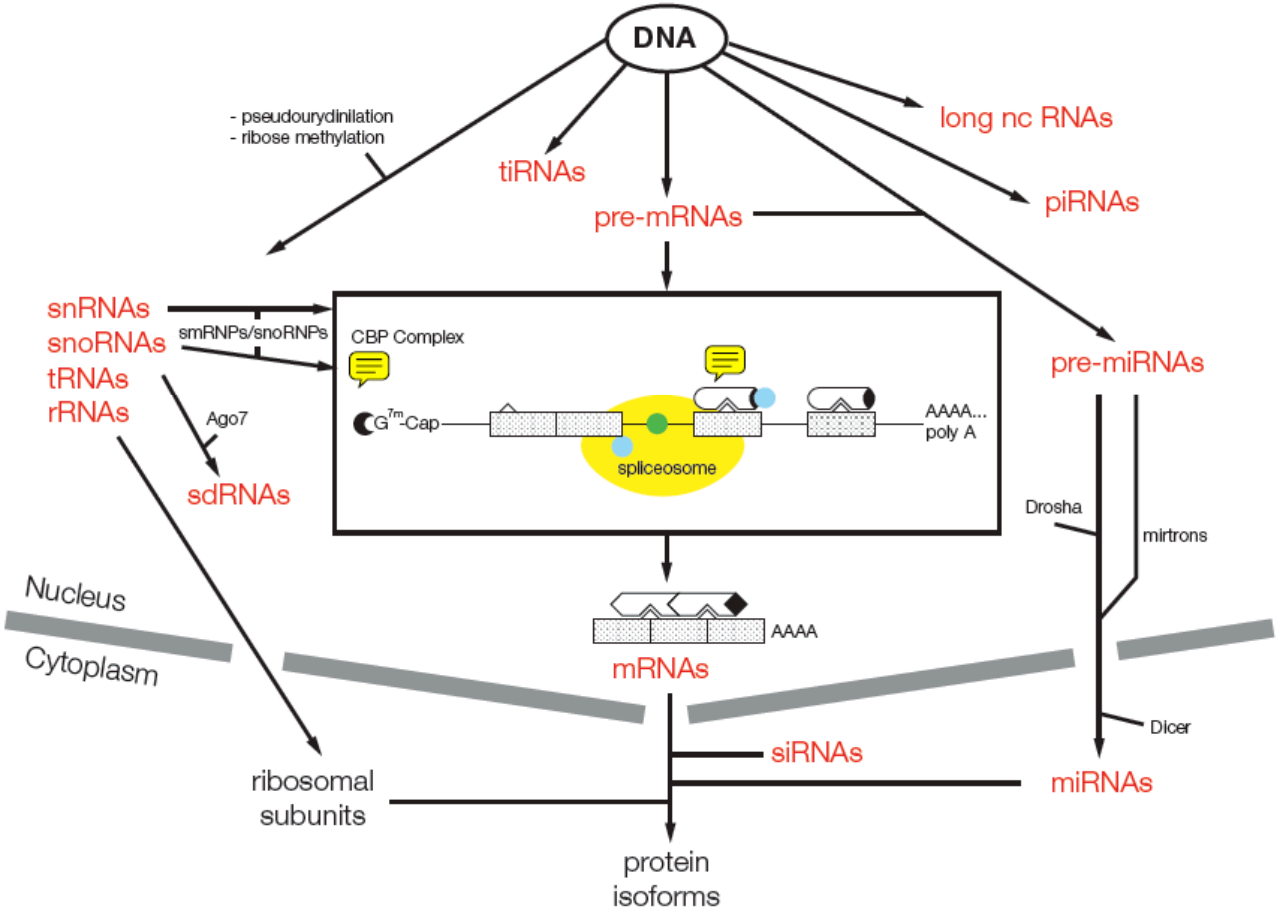
Literature: The RNA World, Cold Spring Harbor Press 2005

Structure of RNA and DNA



What are the differences between RNA and DNA structures?

Types of RNAs



Where do RNAs come from?
What are the differences between DNA and RNA polymerases?
What do all RNAs have in common?

Tiling array data /ENCODE project: 93% of DNA in the ENCODE region is transcribed Birney et al., 2007, Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature *447*, 799-816.	VS	Most of this RNA is in regions coding for proteins Bakel et al. PLoS Biol. 2010 May 18;8(5):e1000371.

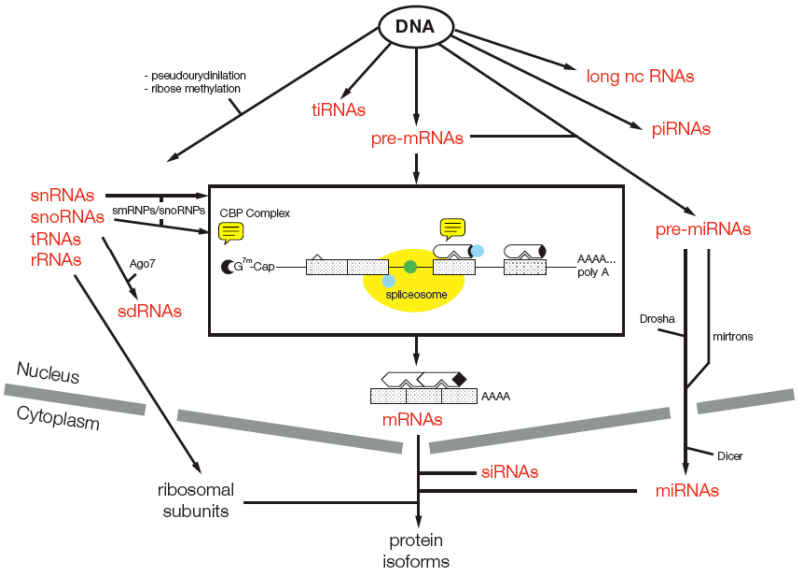
Types of RNAs

Most RNAs are noncoding:

Ribosomal RNA, 60S, 20S		Protein synthesis, Ribozyme
tRNA (transfer)		Protein synthesis,
tiRNA (tiny),		Fragments from tRNAs, block translation
tiRNAs (transcription induced)		Associated with promoter, RNA polII backtracking, nucleosome marking
snoRNA (small nucleolar) C/D Box		Guide for RNA methylation
snoRNA (small nucleolar) H/ACA Box		Guide for RNA pseudouridinilation
sdRNAs, psnoRNAs snoRNA derived RNAs, processed snoRNAs		Unclear, some work in alternative splicing
snRNA (small nuclear)		RNA processing
snRNAs, SM class (Stephanie Smith)		Pre-mRNA splicing
snRNAs LSM (Like sm)		Pre-mRNA splicing (U6 associated), RNAse P processing
miRNAs		Regulate translation (*and more*) 20-23 nt
siRNA		Small interfering RNAs 22 nt
piRNA (piwi interacting, Piwi (P-element induced wimpy testis in *Drosophila*)		Male Germline, 26-31 nt long
Long nc RNAs (non coding)		Chromatin structure (Xist)

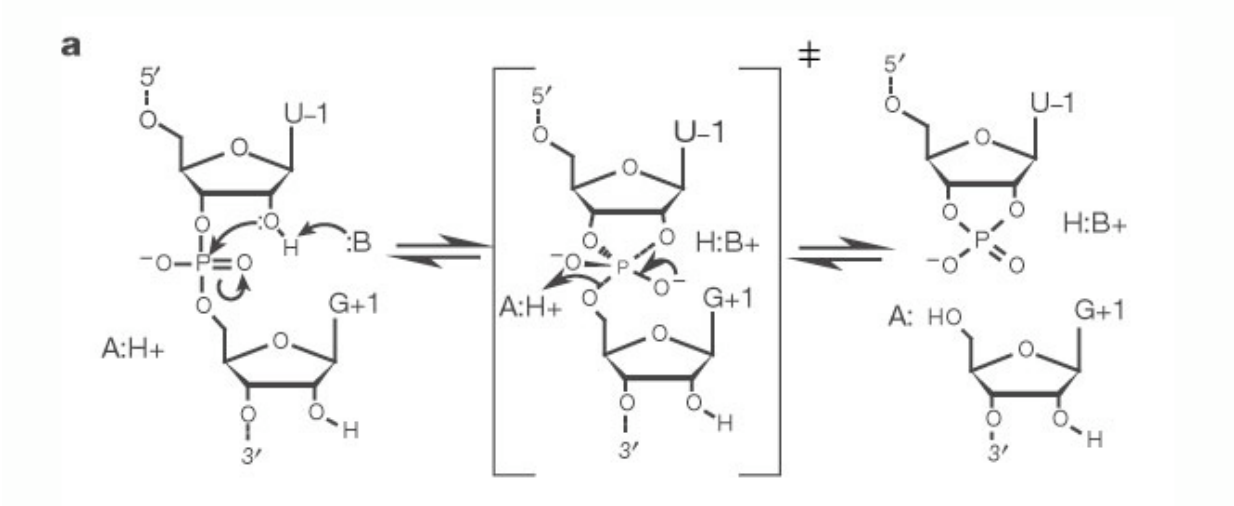
mRNAs are coding

mRNA (messenger)		Encodes protein

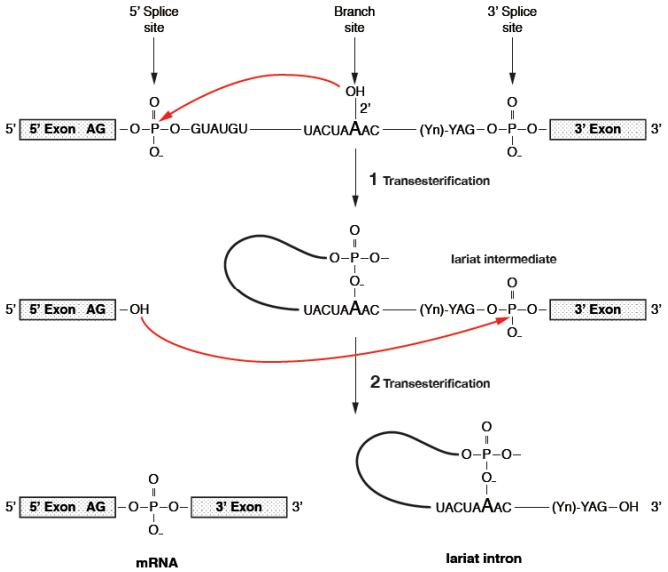


This list is growing due to better analysis techniques

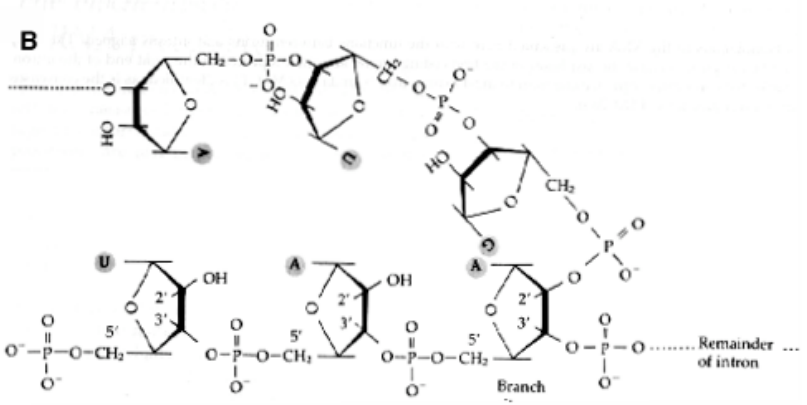
The free 2’ OH group makes RNA more reactive



2’ 3’ cyclic phosphate
After cleavage



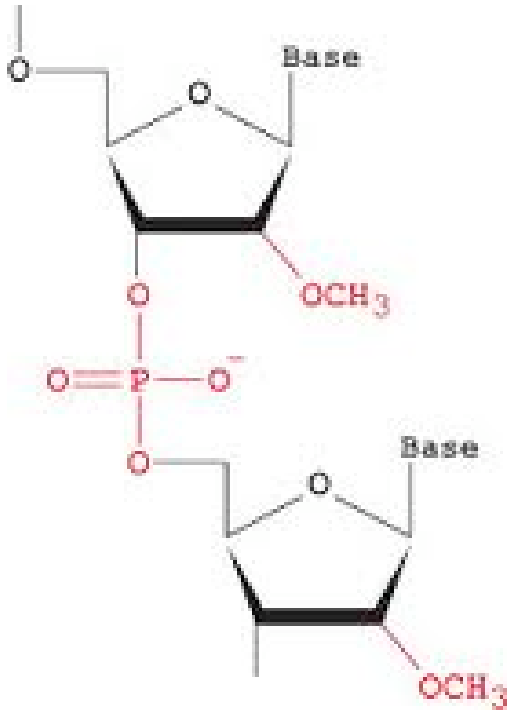
2’ 5’ lariat structure, ligation



How can you make RNA more stable?

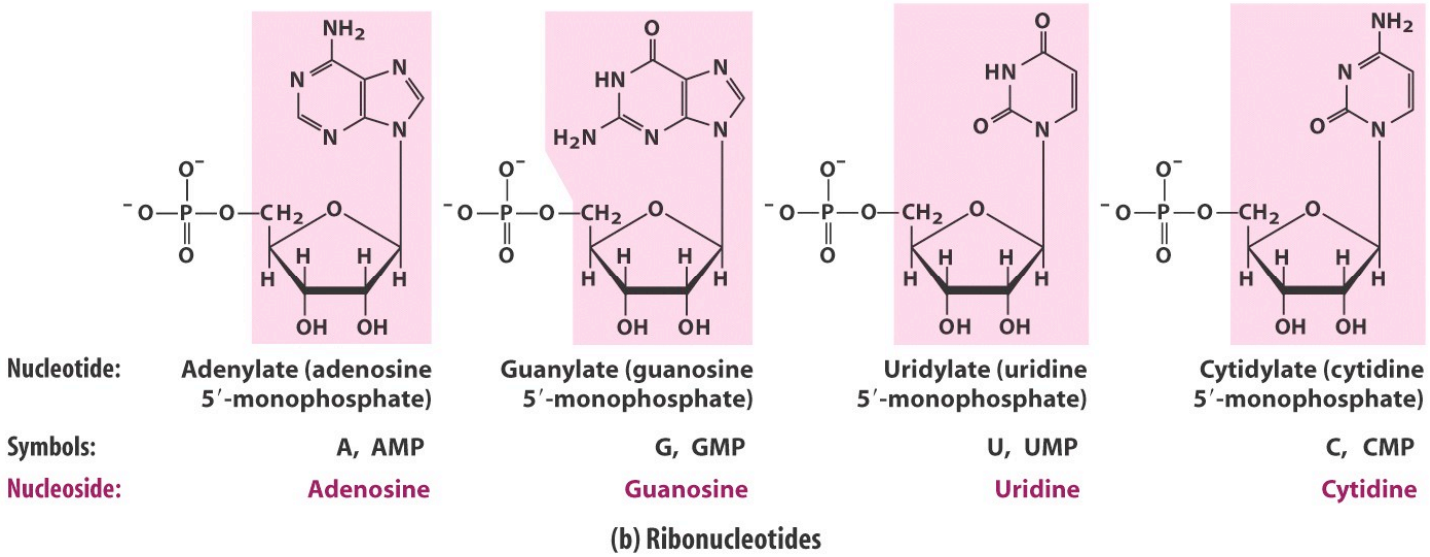
[The chemical repertoire of natural ribozymes](#) Jennifer A. Doudna & Thomas R. Cech*Nature* **418**, 222-228(11 July 2002)

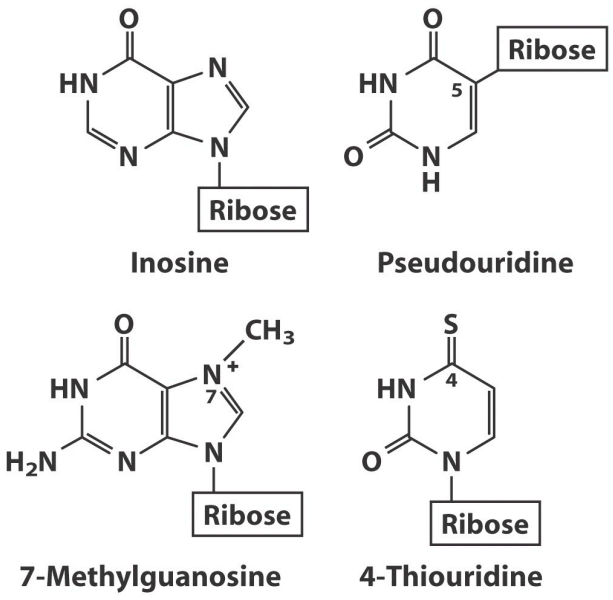
RNA is chemically more diverse than DNA

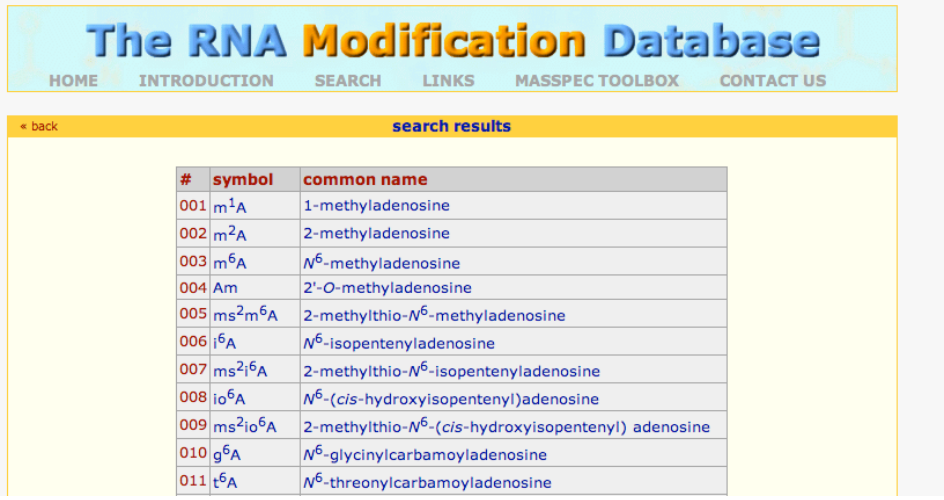


What is the effect of a methyl group?
Why is RNA not converted into DNA?
What kind of oligos do you use to change RNA Processing?

How many bases are in RNA?





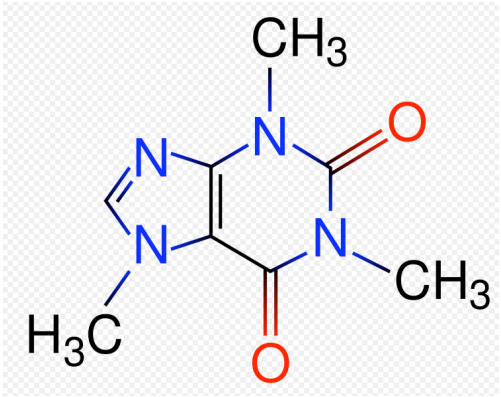


#	symbol	common name
001	m^1A	1-methyladenosine
002	m^2A	2-methyladenosine
003	m^6A	N^6-methyladenosine
004	Am	2'-*O*-methyladenosine
005	ms^2m^6A	2-methylthio-N^6-methyladenosine
006	i^6A	N^6-isopentenyladenosine
007	ms^2i^6A	2-methylthio-N^6-isopentenyladenosine
008	io^6A	N^6-(*cis*-hydroxyisopentenyl)adenosine
009	ms^2io^6A	2-methylthio-N^6-(*cis*-hydroxyisopentenyl) adenosine
010	g^6A	N^6-glycinylcarbamoyladenosine
011	t^6A	N^6-threonylcarbamoyladenosine

Currently 107 entries

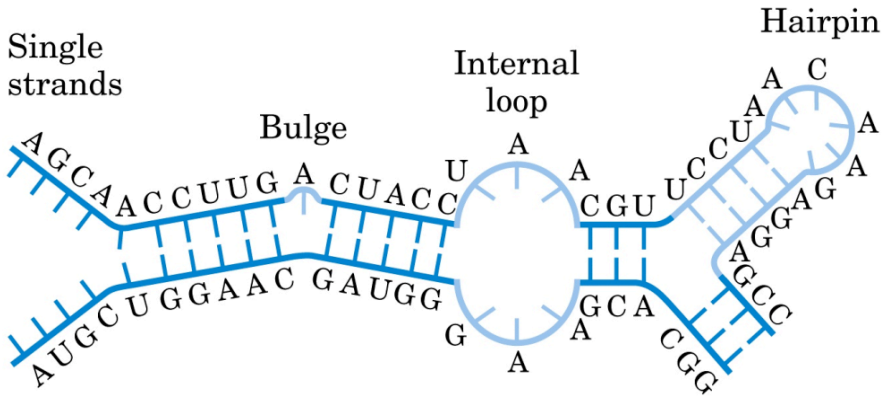
http://rna-mdb.cas.albany.edu/

RNA forms strutures



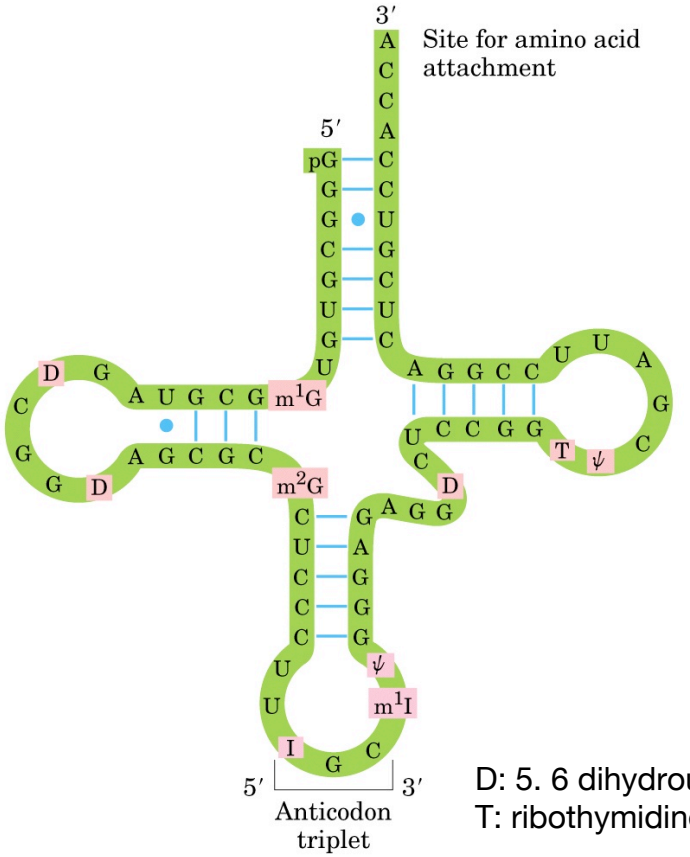
What is this?
Chemical properties?

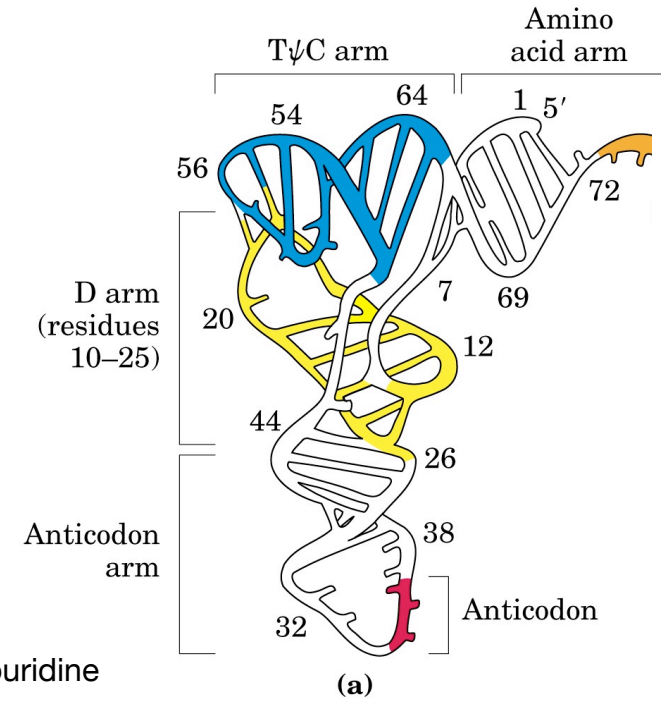
RNA forms secondary and teriary structures

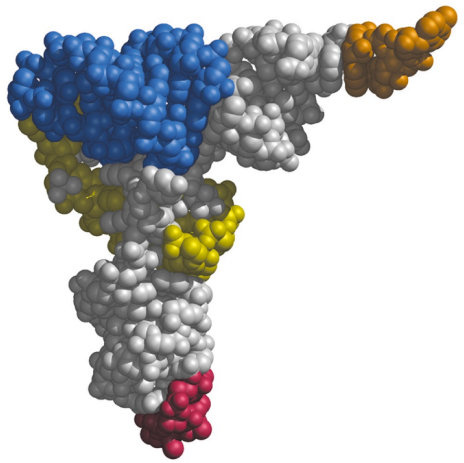


Why does RNA forms these structures?
What kind of teriary structure does DNA form?

Secondary and teriary structure of tRNA







D: 5. 6 dihydrouridine
T: ribothymidine

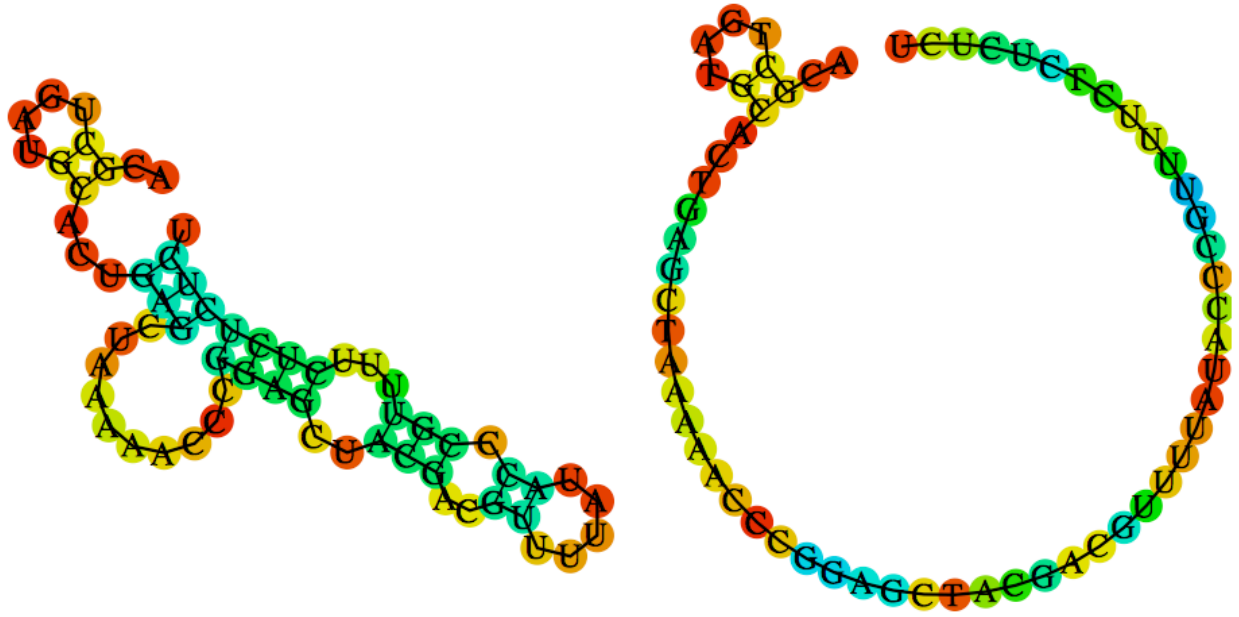
RNAs have additional base pairing: G-U and many others,
RNAs for stable teriary structures that are important for function

How can you change/melt the structure?

Prediction of secondary RNA structures

http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
RNA fold

ACGCUGAUGCACUGAGCUAAAAACCCGGAGCUACGACGUUUUAUACCCGUUUUCUCUCUCU



There is typical more than one RNA structure
Why is there some much tRNA and rRNA processing?

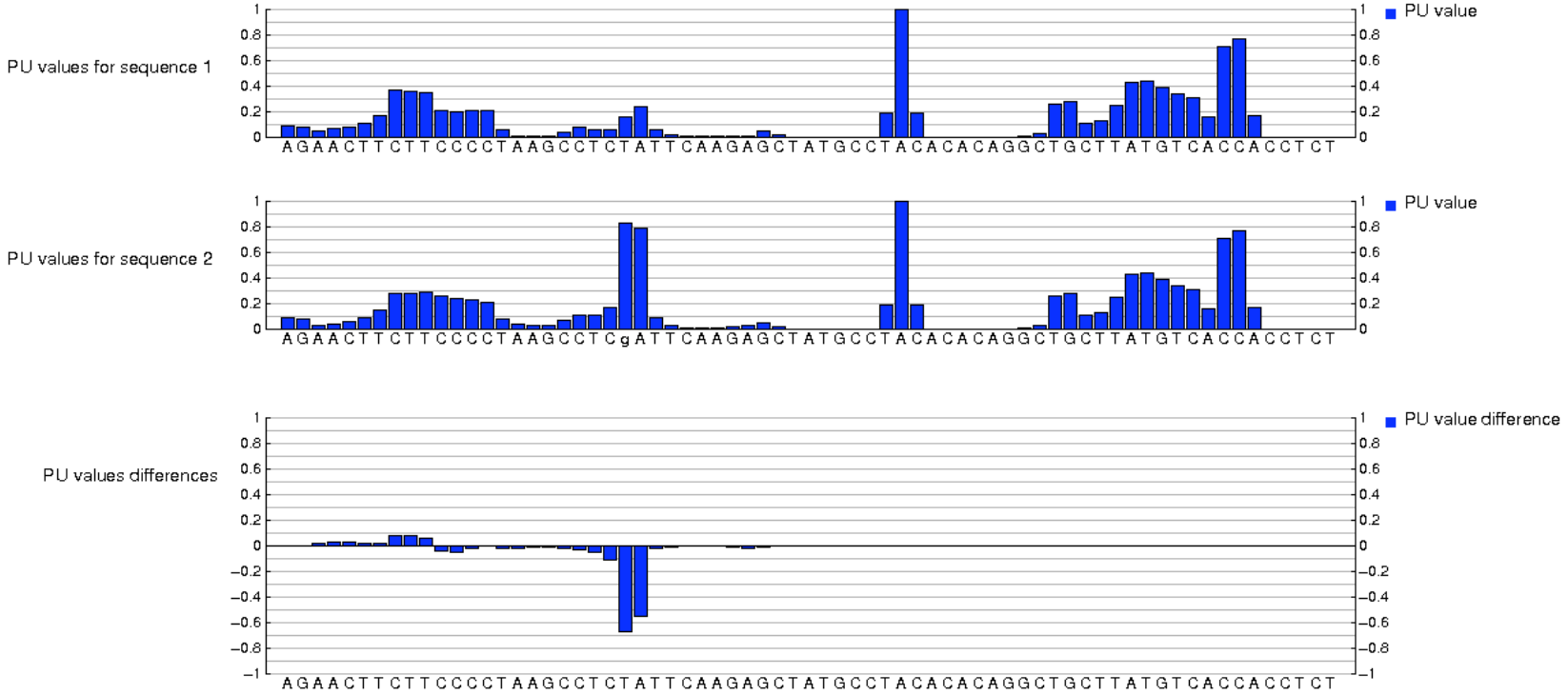
Statistical prediction of (m)RNA structure

http://biwww2.informatik.uni-freiburg.de/Software/NIPU/

PU: probability of being unpaired

Example: DMD c.884G>T

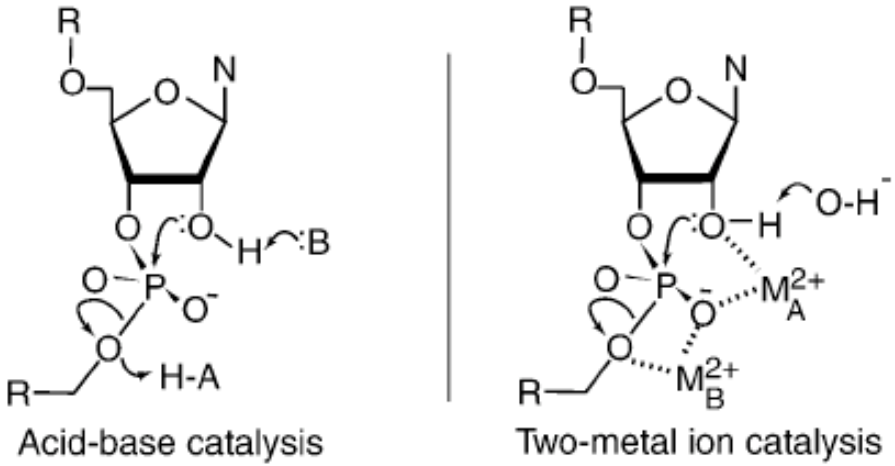
TccccctcctctctatccactcccccaaacccttctctgcagATCACGGTCAGTCTAGCACAGGGATATGAGAGAACTTCTTCCCCTAAGCCT
CgATTCAAGAGCTATGCCTACACACAGGCTGCTTATGTCACCACCTCTGACCCTACACGGAGCCCATTTCCTTCACAGgtctgt
caacatttactctctgttgtacaaaccagagaact



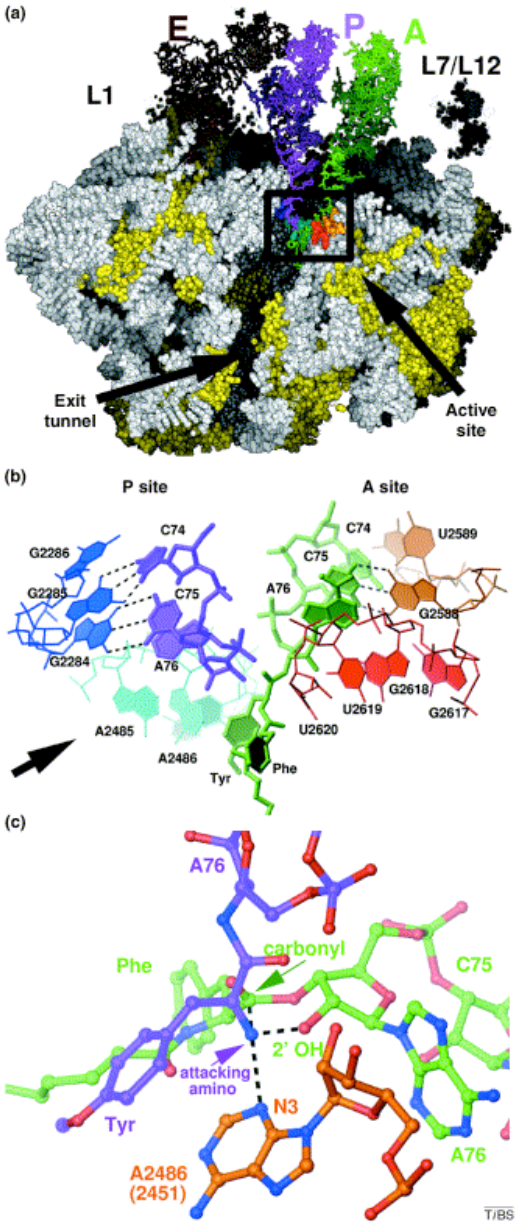
What happens if a single-stranded RNA binding protein binds to the G>T region?

RNA can be catalytic active: Ribozymes

Principle: form ‘stable’ RNA structure,
Use 2’ OH as a reactive group



Annu. Rev. Biochem. 2000. 69:597–615



RNA, the first macromolecular catalyst: the ribosome is a ribozymeThomas A. Steitz and Peter B. Moore[1], TIBS

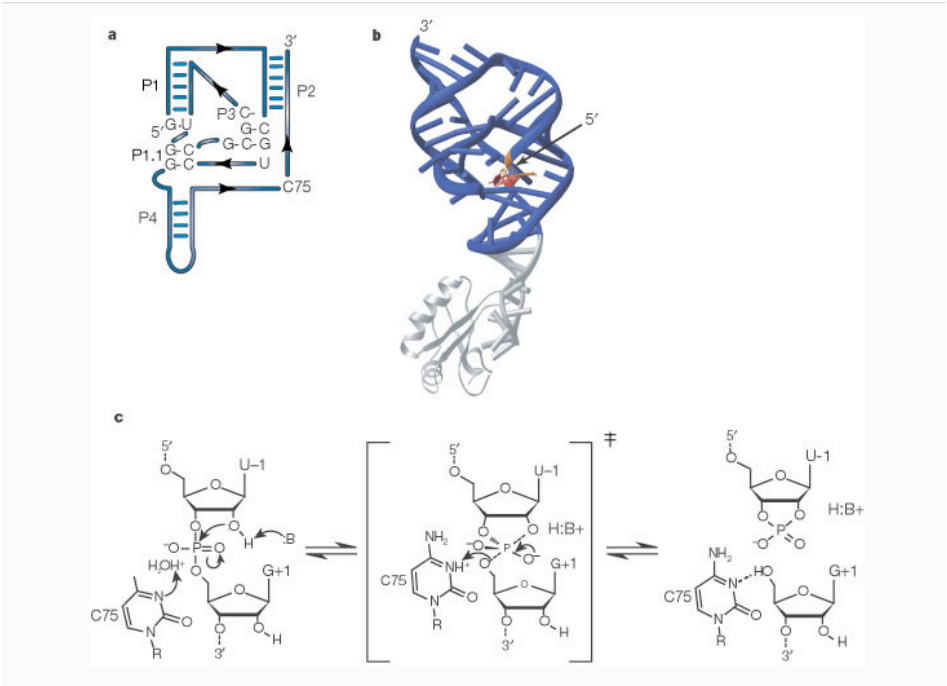
More example of ribozymes

Table 1 Naturally occurring ribozymes and ribonucleoprotein enzymes

Ribozyme	Sequenced examples	Size (nt)	Activity (reaction product)
Hammerhead	11	40	Self-cleavage via transesterification (2′,3′ cyclic phosphate)
Hepatitis delta virus	2	90	
Hairpin	1	70	
Varkud satellite	1	160	
Group I intron	>1,500	210	Self-splicing via transesterification (3′-OH)
Group II intron	>700	500	
RNase P*	>500	300	Pre-tRNA processing via hydrolysis (3′-OH)
Spliceosome* (U2+U6 snRNAs)	70,50	180,100	RNA splicing via transesterification (3′-OH)
Ribosome* (23S rRNA)	>900	2,600	Peptidyl transfer (amide)

Number of sequenced examples is a snapshot as of 2002 and is influenced by DNA-sequencing strategies and database upkeep; it may provide a rough indication of relative abundance. RNAs in any group vary in size; the size provided here indicates the lower end of the length distribution for the natural examples. See www.rna.icmb.utexas.edu and www.jwbrown.mbio.ncsu.edu/RnaseP/.

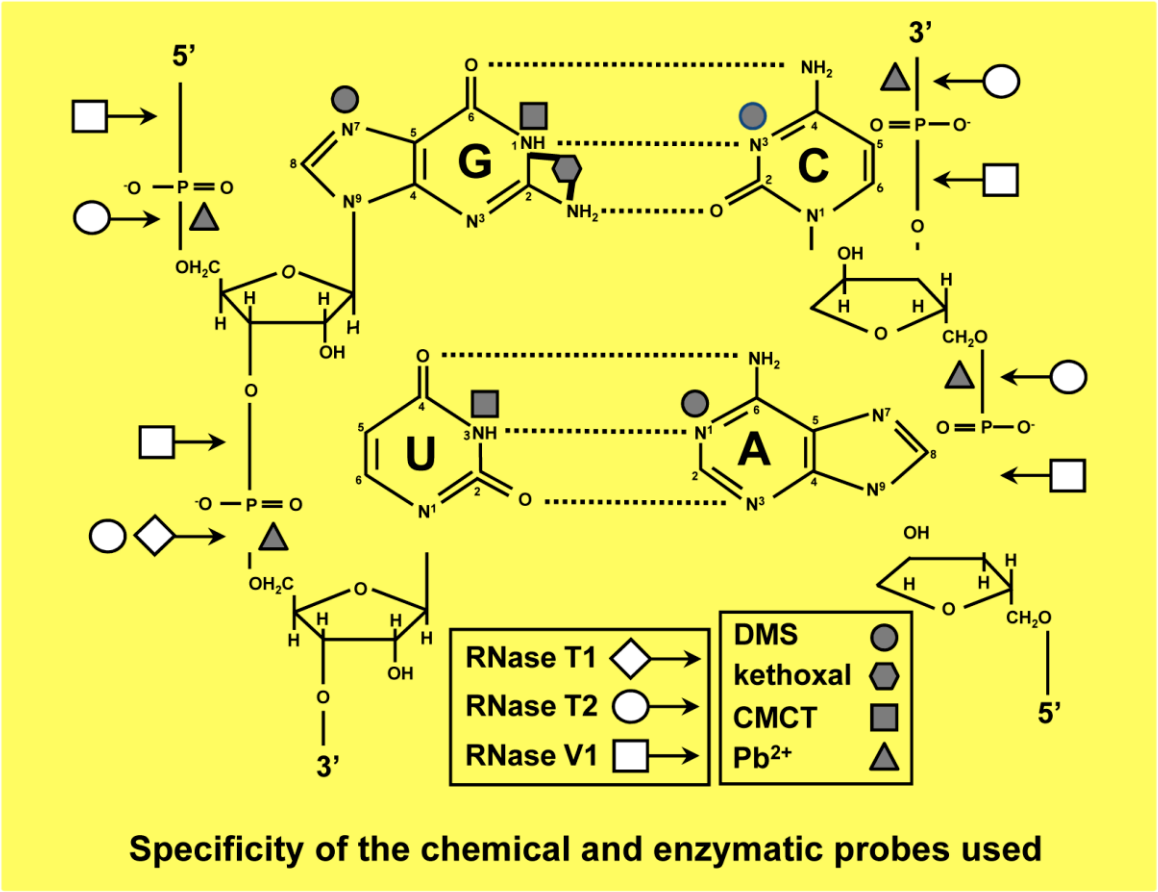
*Ribonucleoprotein enzymes. RNase P: bacterial and archaebacterial RNAs have the relevant activity in the absence of protein. Spliceosome: U2 and U6 small nuclear RNAs (snRNAs) alone show an activity related to the natural activity. Ribosome: no activity has yet been observed with protein-free, large-subunit rRNA.



Bases are involved in catalysis

[The chemical repertoire of natural ribozymes](#) Jennifer A. Doudna & Thomas R. Cech*Nature* **418**, 222-228(11 July 2002)

RNAs undergo enzymatic modification: RNAses



RNAse A: single stranded C, U

RNAse H: RNA in DNA/RNA doublex

RNAse I: single stranded C, U, G, A

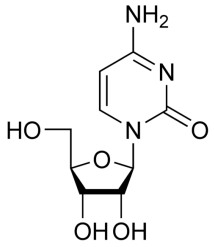
RNAse III: dsRNA

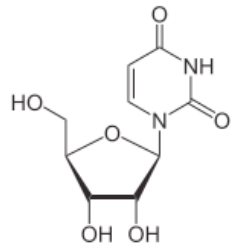
RNAse P: tRNA precursor

RNAse L: interferon induced

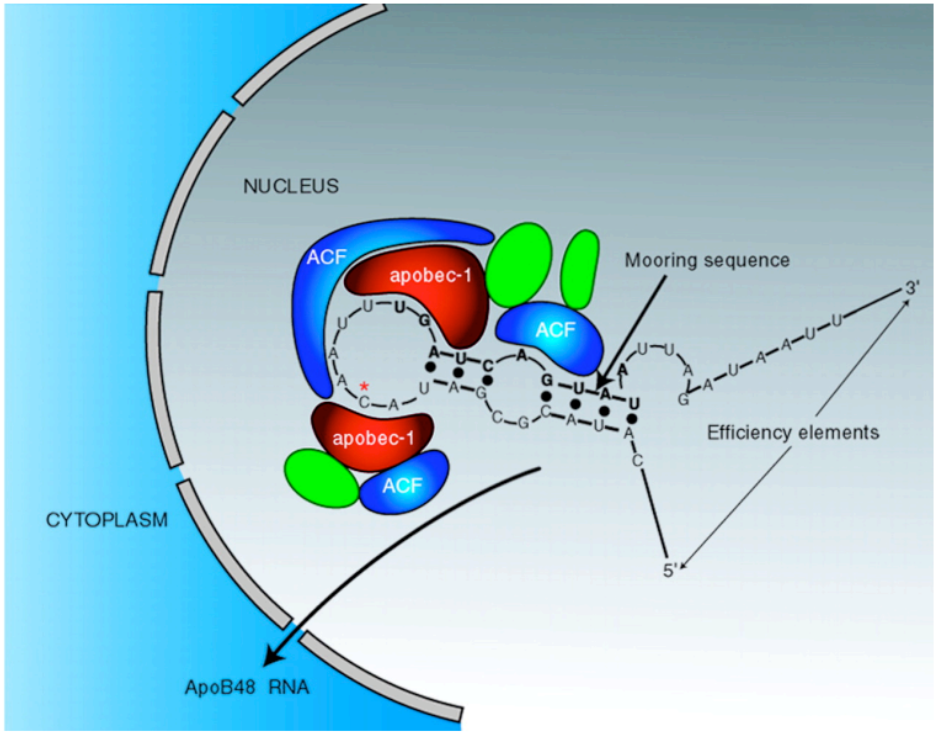
How do cells protect their RNAs?
How can you use these RNAses?

RNAs undergo enzymatic modification: Deamination/1

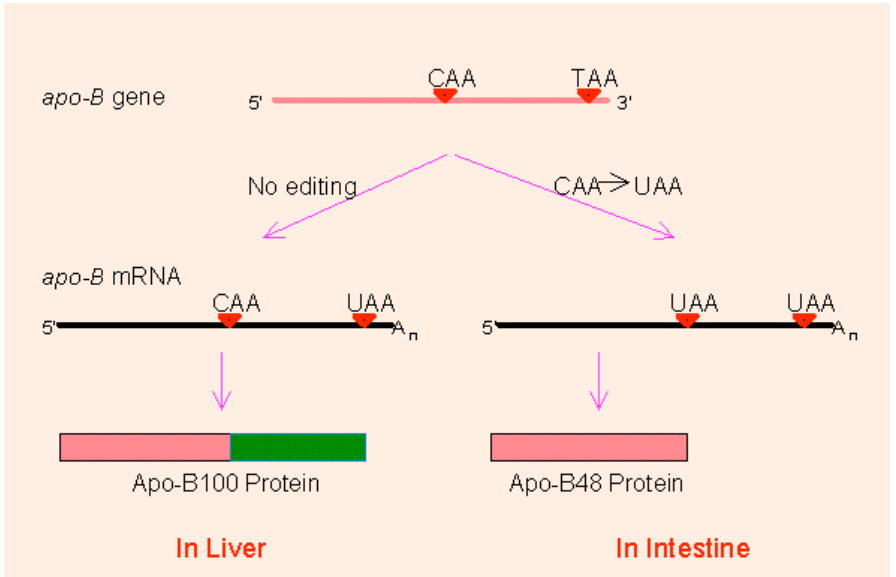




C-> U editing, apobec-1 + ACF
apobec-1 complementation factor



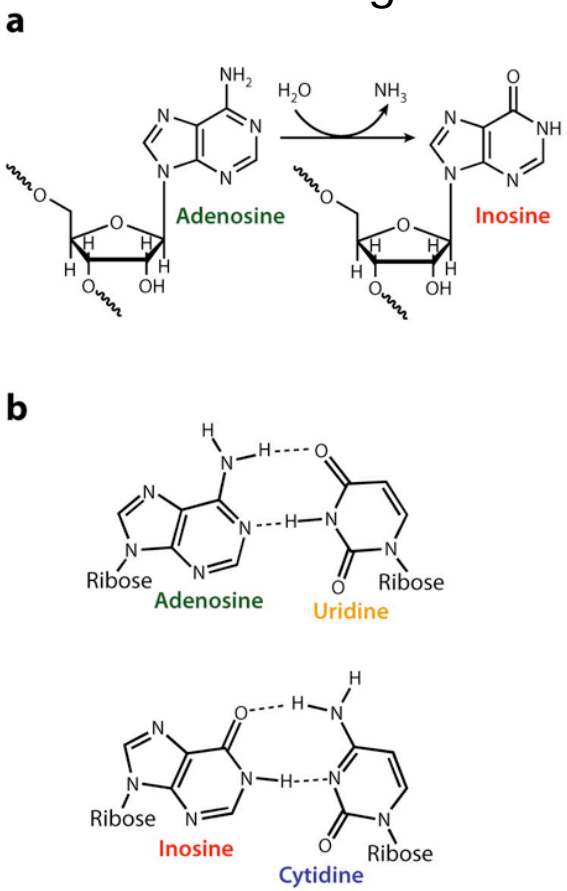
Blanc V , Davidson N O J. Biol. Chem. 2003;278:1395-1398



Tissue-specific editing of RNA, example of how genetic information can be interpreted

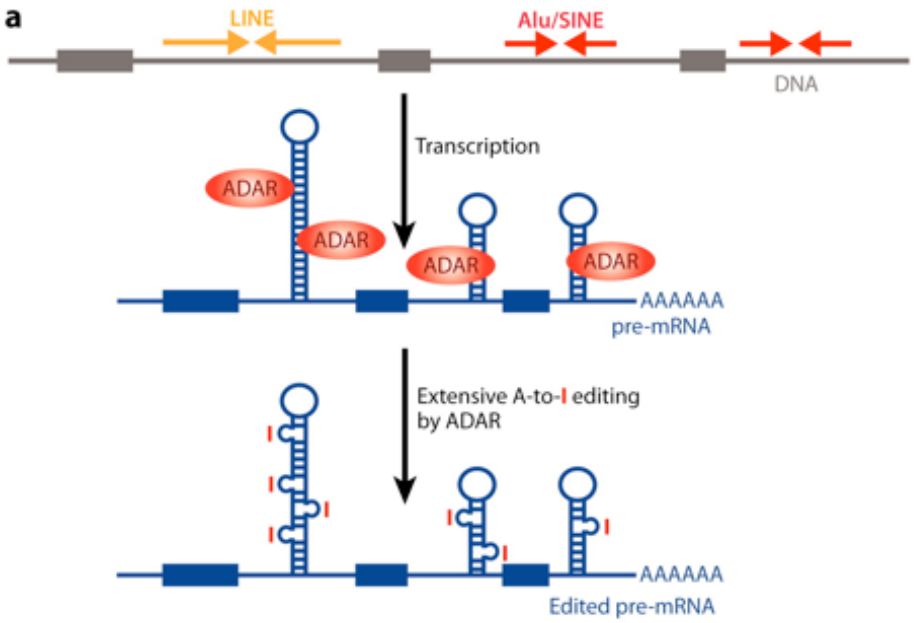
RNAse undergo enzymatic modification: Deamination /2

A->I Editing



Nishikura K. 2010.
Annu. Rev. Biochem. 79:321–49

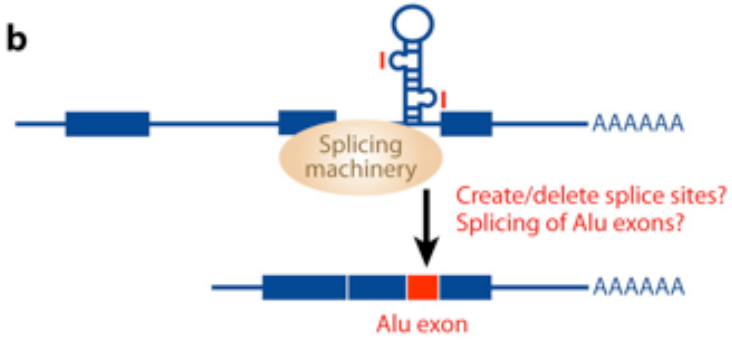
ADAR adenosine desaminase acting on RNA,
Needs double stranded RNA



How is an ‘edited A’ interpreted by the ribosome?

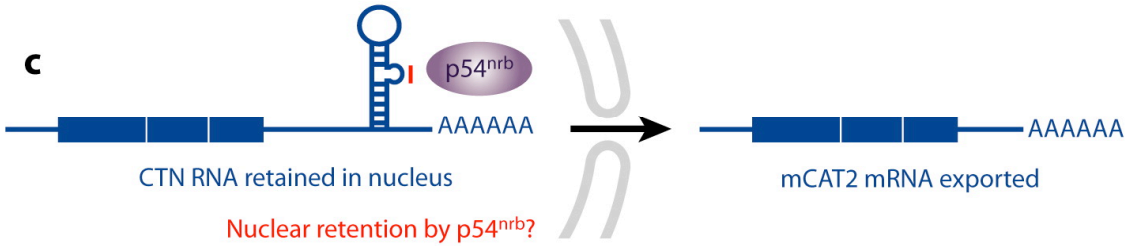
Why is there so much double stranded RNA in humans?

Function of ADARs

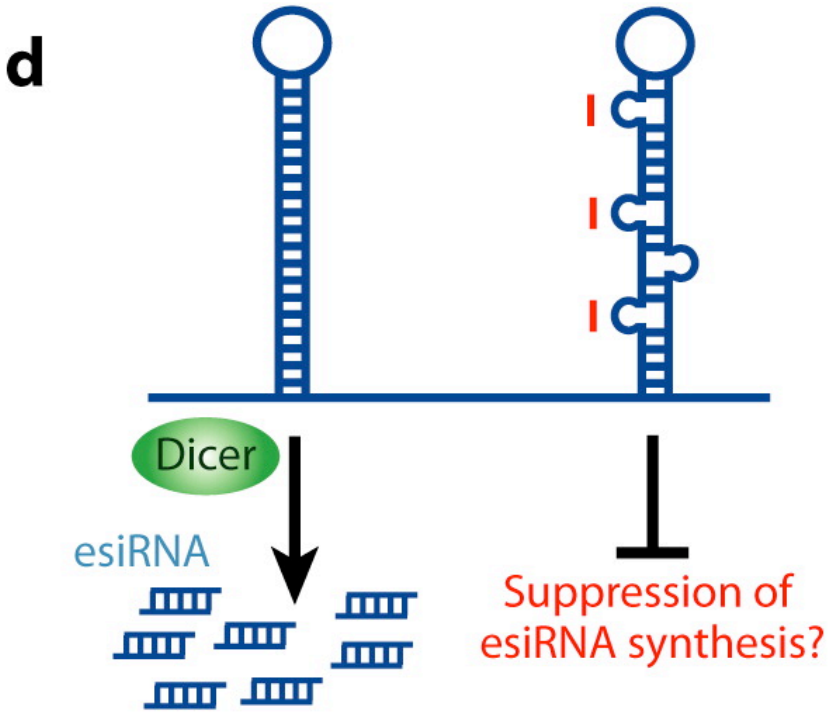


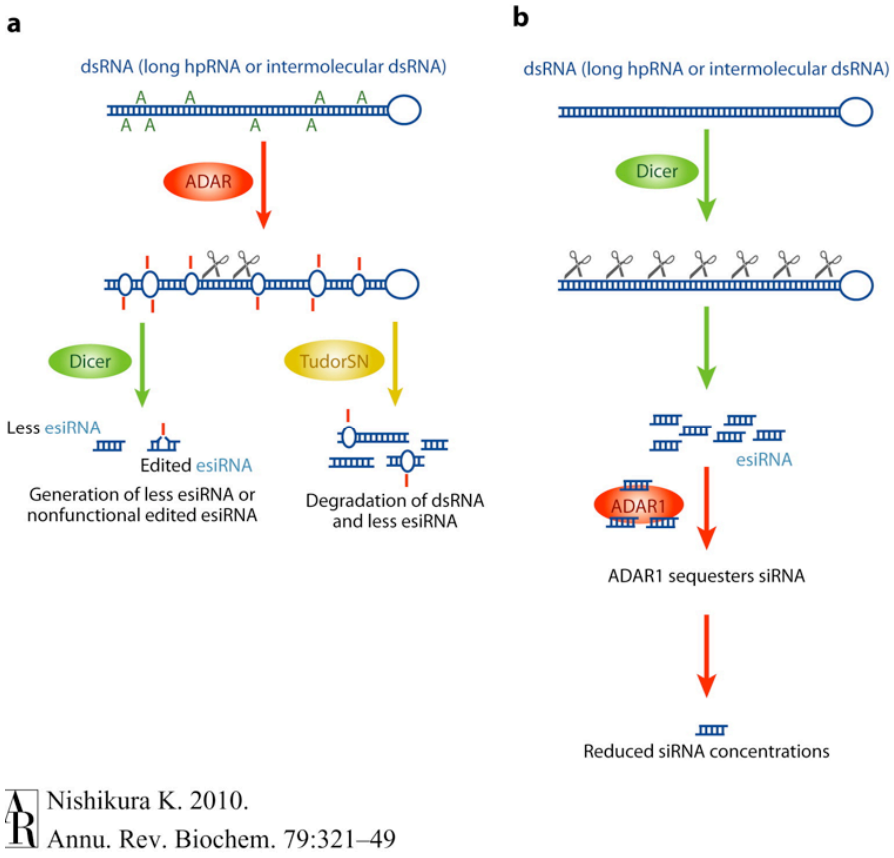
Splice sites are similar to:
yyyyycagG
CAGgtaagta

How could
yyyyycaaG
CAGataagta be made into splice sites?



ADARs reduce endogenous siRNAs





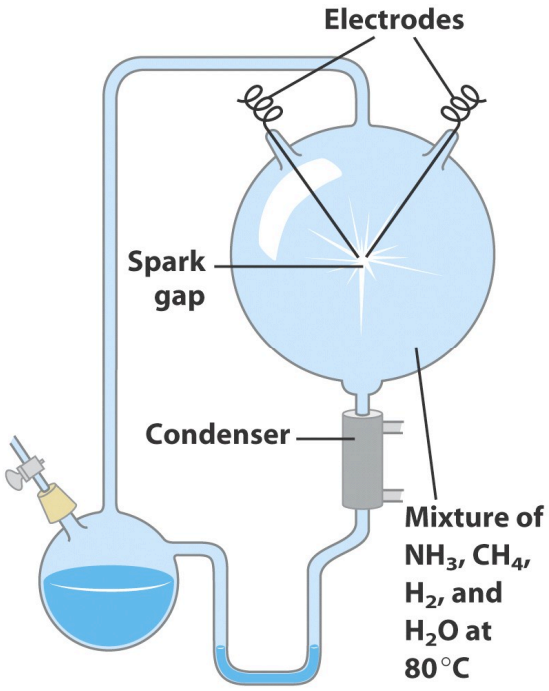
Nishikura K. 2010.
Annu. Rev. Biochem. 79:321–49

esiRNA: Endogenous siRNA

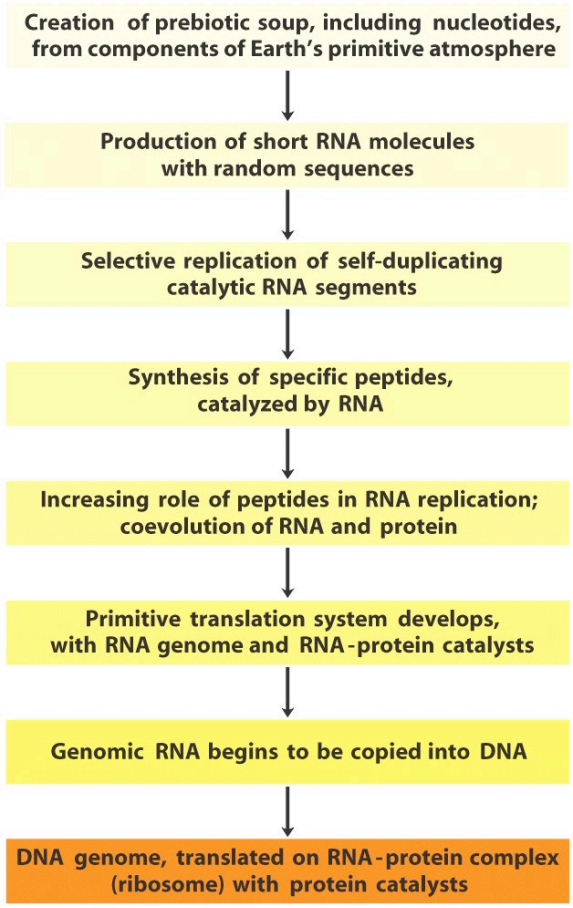
dsRNA with I-U pairs resistant to dicer
->less siRNAs
ADAR1 sequesters siRNA

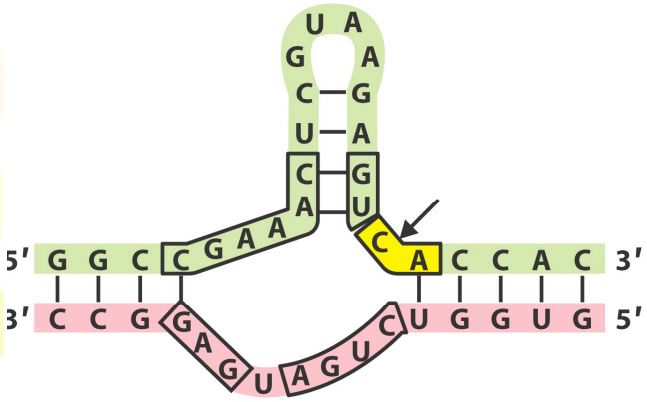
In which cell lines would you preferably do your siRNA experiments?

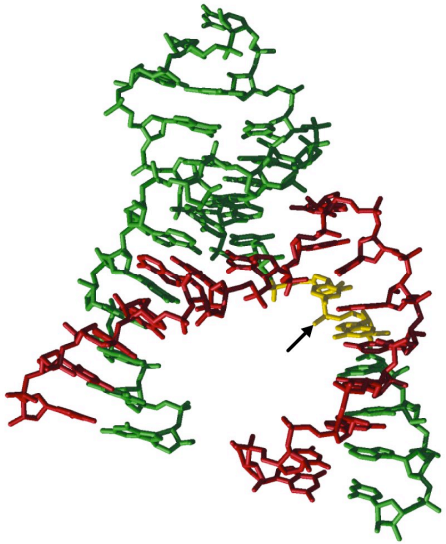
There (used) to be an RNA world



Miller/Urey





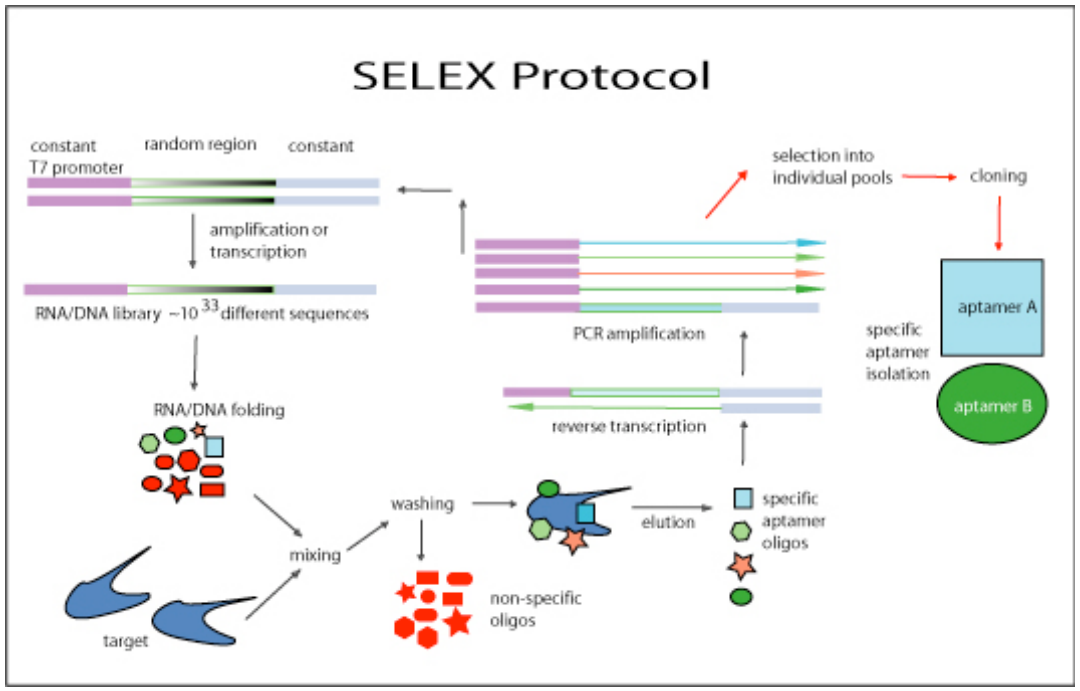


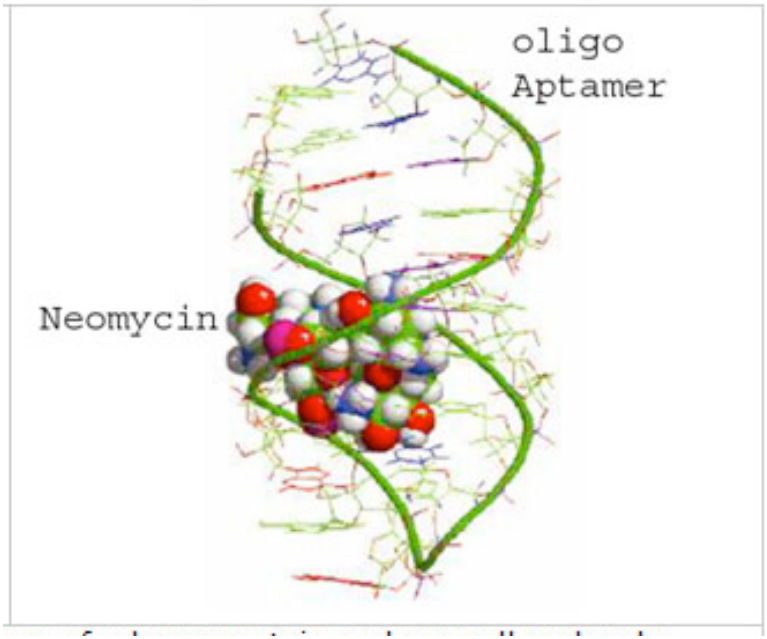
Self-splicing, Hammerhead ribozyme

Creation of macromolecules that undergo selection process, biocatalysts

How are dNTPs made from the cell?

SELXEX: and echo of the RNA world

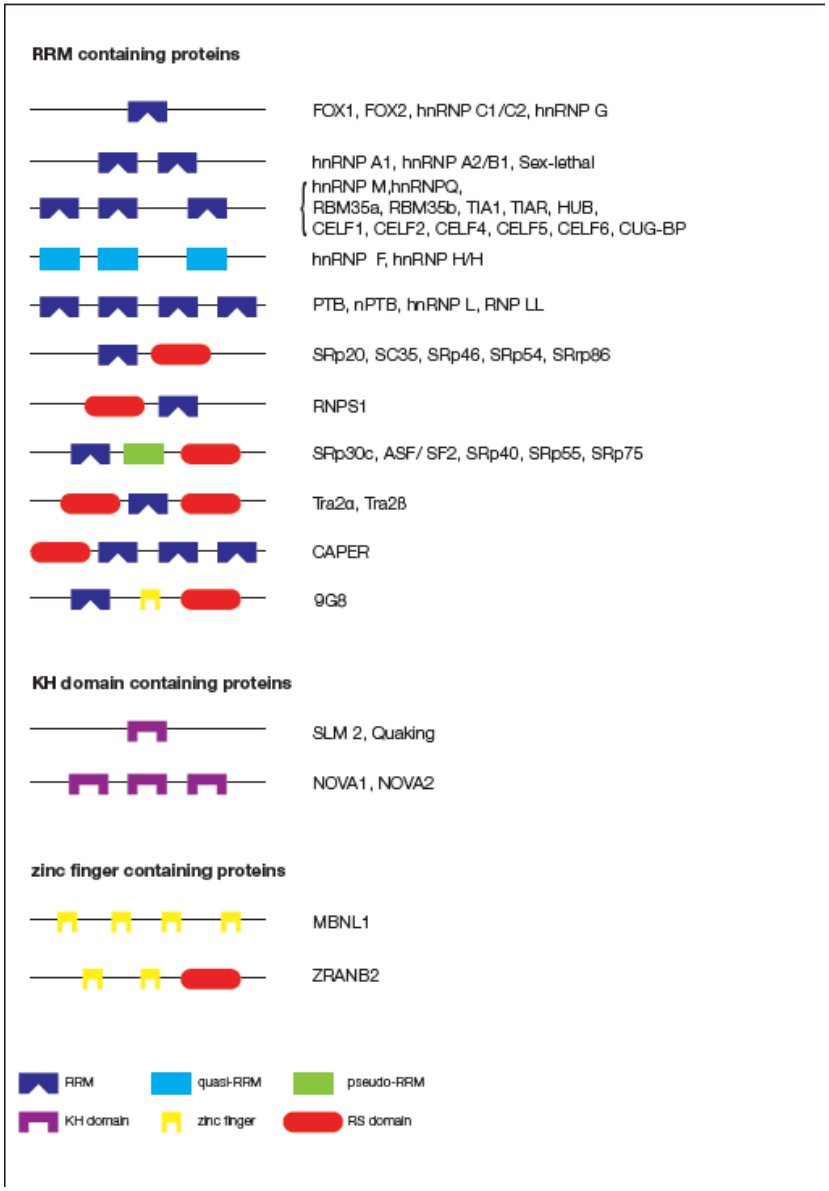




SELEX: Systematic Evolution of Ligands by Exponential Enrichment

Make an RNA molecule binding to any target
Clinic: anti VEGF (Macugen)
Scientific: aptamers, ‘RNA polymerases”

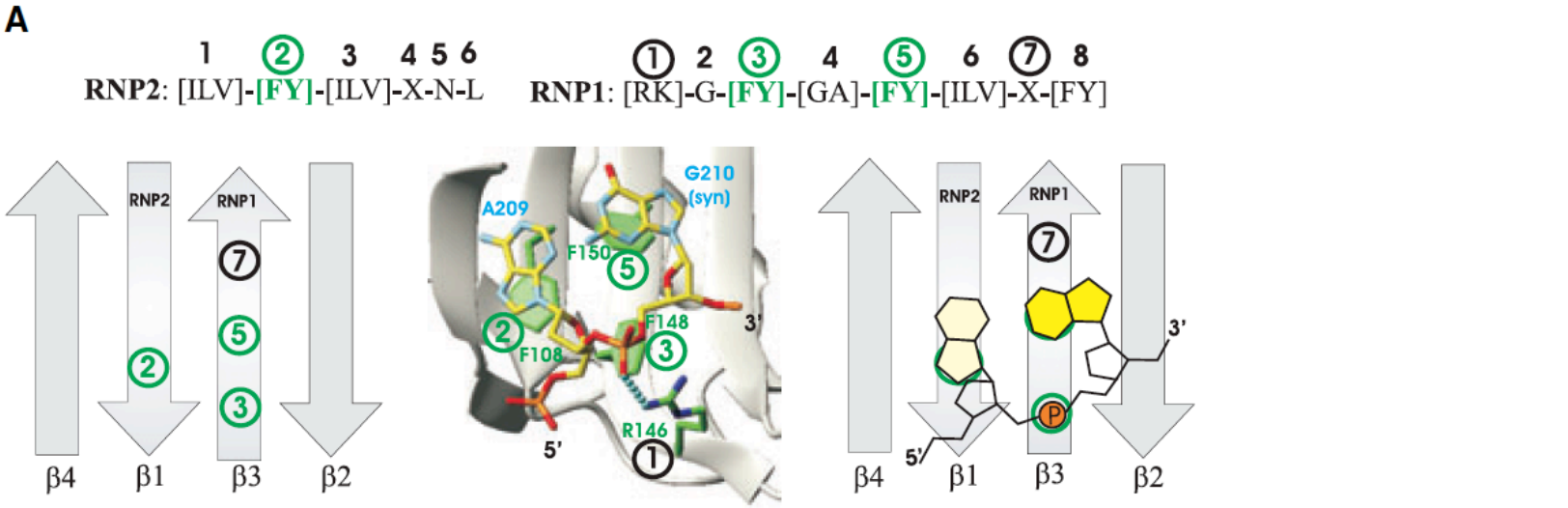
RNA and the protein world

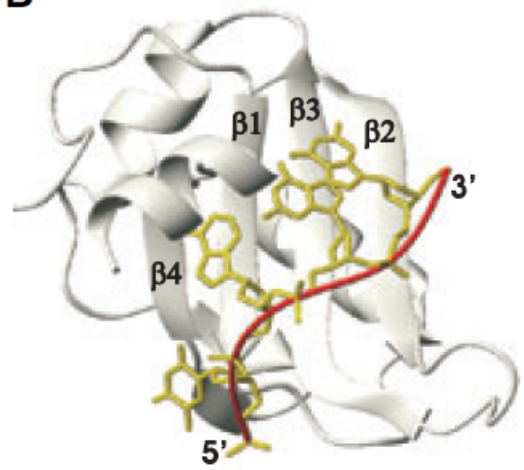


RRM “RNA recognition motif’ is the most frequent protein domain in humans
497 RRM in about 25,000 proteins (2%)

Beta alpha beta beta alpha beta fold
Binds only single stranded RNA

Structure of the RRM

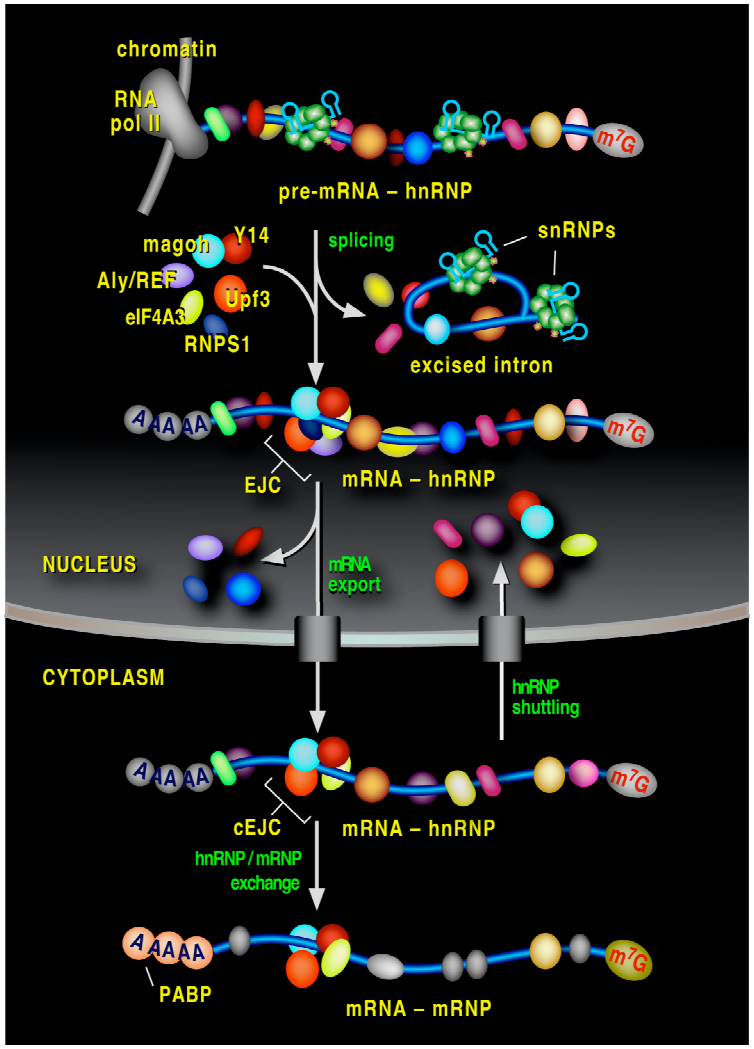




Limited sequence specificity
Kd low nM to 20 μM

The RNA recognition motif, a plastic RNA-binding platform to regulate post-transcriptional gene expression.Christophe MarisÜ, Cyril DominguezÜ, FrÈdÈric H.-T. Allain, FEBS J.

RNA is covered with proteins in the cell



Summary and outlook

RNA is more complex and reactive than DNA
There are more than 100 RNA bases due to enzymatic modifications
RNA forms secondary and teriary structures that can act as enzymes
The ribosome is an RNA enzyme
The ‘message’ of RNA molecules can be altered by enzymes
RNA is covered with proteins (or in secondary structure)
THe RRM (RNA recognition motif) is the most frequent human protein domain

How to identify RNAs: modern transcriptome analysis
Example of RNAs: siRNA, miRNAs
Example of a ribozyme: the spliceosome
Where to find information: gene expression databases

Homework: fold RNA of your choice using the Vienna package and the NIPU server