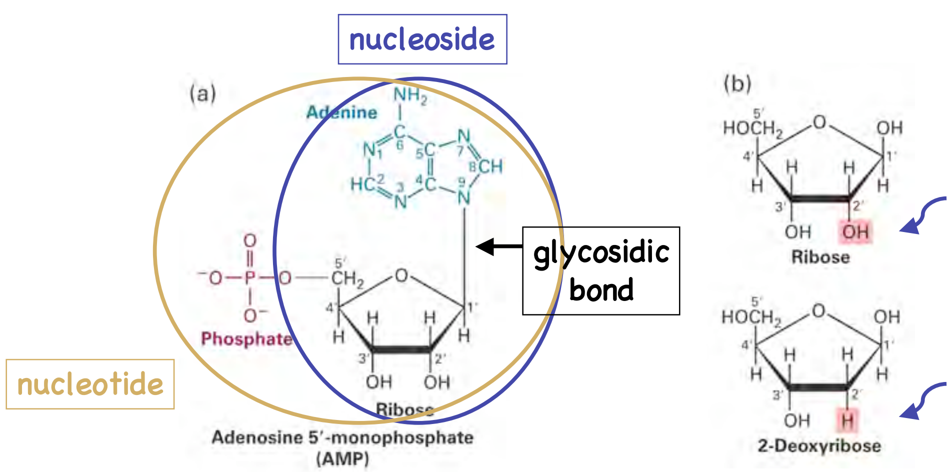
Patterns and principles of RNA structure

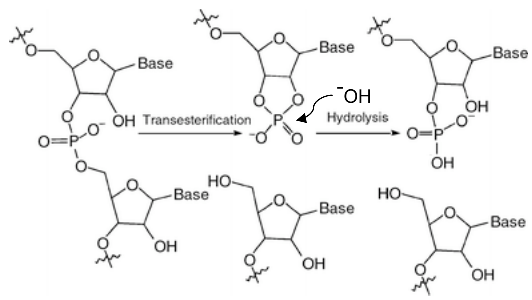
RNA structure can be specific, stable and complex.
(As a result, RNA mediates specific recognition and catalytic reactions.)

Principles/ideas--RNAs contain characteristic 2° and 3° motifs
- Secondary structure--stems, bulges & loops
  - Coaxial stacking
  - Metal ion binding
- Tertiary motifs (Pseudoknots, A-A platform,
  - tetraloop/tetraloop receptor,
  - A-minor motif, ribose zipper)

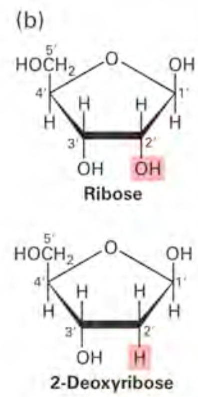
RNA vs. DNA



RNA vs. DNA: who cares?



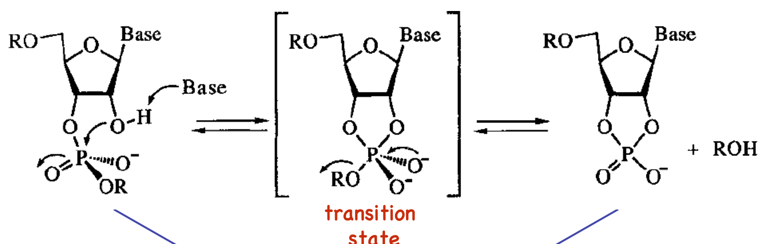
Base-catalyzed RNA cleavage!



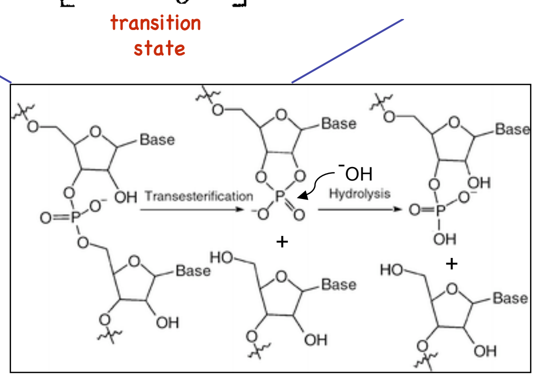
Unstable backbone

Stable backbone

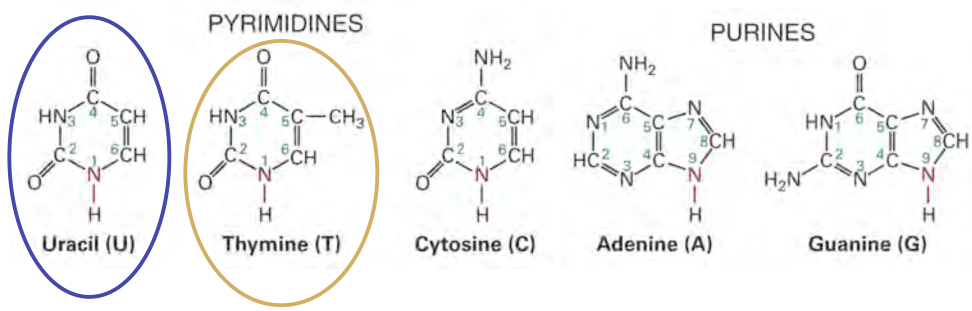
RNA transesterification mechanism



Base-catalyzed RNA cleavage!



Different bases in RNA and DNA



RNA only

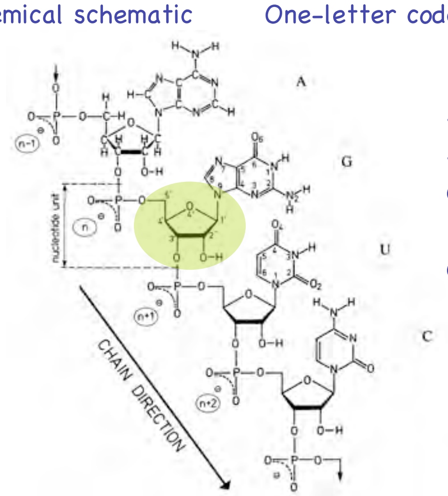
DNA only

DNA and RNA

RNA chain is made single stranded!

Chemical schematic   One-letter code

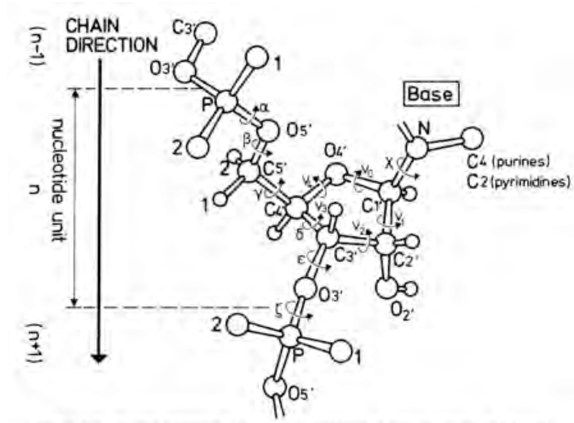
dsRNA can block protein synthesis and signal viral infections



ssDNA can signal DNA damage and promote cell death

Chain is directional. **Convention: 5' → 3'.**

Six backbone dihedral angles (α–ζ) per nucleotide in RNA and DNA

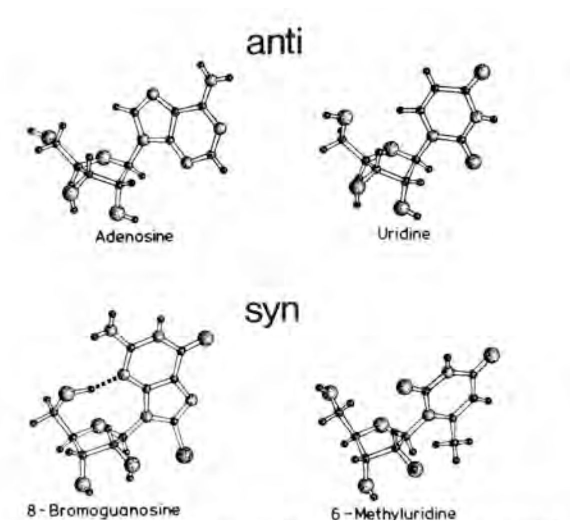


Is ssDNA floppy or rigid? Is RNA more or less flexible than ssDNA?

Two orientations of the bases: Anti and syn

DNA and RNA

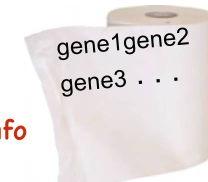
Absent from undamaged dsDNA



-OH, what a difference an O makes!

Different functions of

DNA and	RNA
Stores genetic info	Stores genetic info
ssDNA signals cell death	ssRNA OK
	E.g. mRNA = gene copy
dsDNA OK	dsRNA ("A" form) signals infection, mediates editing, RNA interference, . . .
Double helical (B form)	Forms complex structures
Supercoiled	Enzymes (e.g. ribosome), Binding sites & scaffolds, Signals, Templates (e.g. telomeres)



Examples of RNA structural motifs

Secondary structures

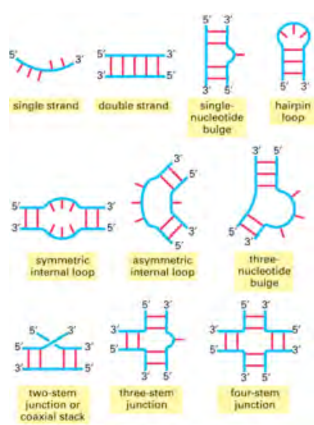


Figure 6-94. Molecular Biology of the Cell, 4th Edition.

- Stem, bulge, loop
- 4-helix junction
- Tetraloop
- Pseudoknot
- Sheared AA pairs
- Purine stacks
- Metal binding sites
- A-A platform
- Tetraloop receptor
- A-minor motif
- Ribose zipper
- . . .

Tertiary structures

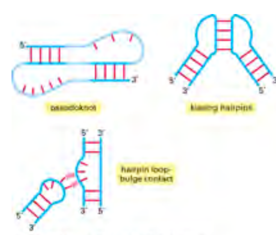
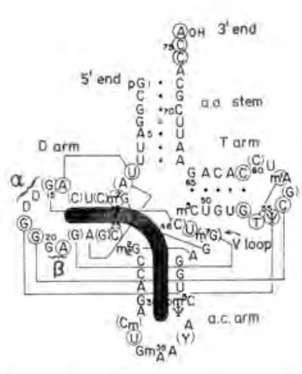
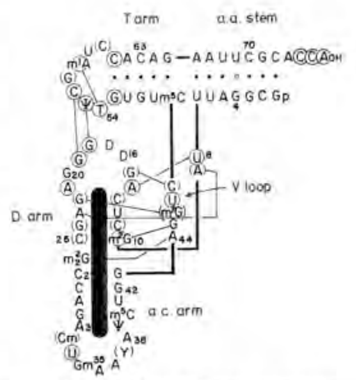


Figure 6-95. Molecular Biology of the Cell, 4th Edition.

Cloverleaf representation of yeast Phe tRNA





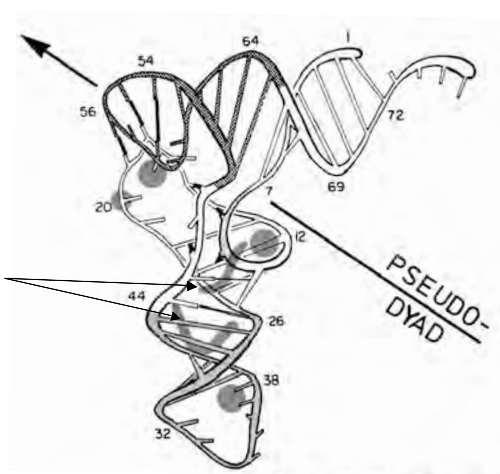
"Cloverleaf" conserved
in all tRNAs

Coaxial stacking of adjacent
stems forms an L-shaped fold

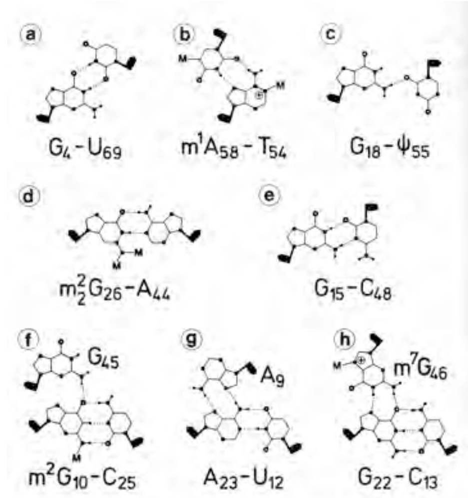
Schematic drawing of yeast Phe tRNA fold

Mg^{2+} (balls)

Spermine

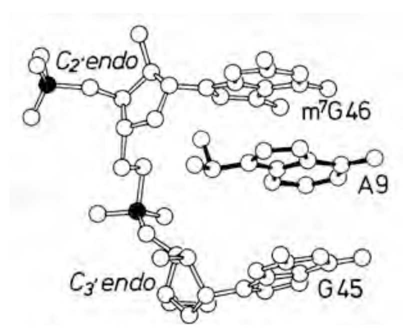


Non-WC base pairs and base triples in yeast



LOTS OF BASE COMBOS!!
Enable alternate backbone orientations:

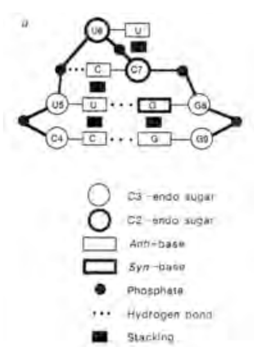
A9 intercalates between adjacent G45 and m^7G46 in yeast tRNA Phe



Examples of RNA structural motifs

Tetraloop
Pseudoknot
4-helix junction
Sheared AA pairs
Purine stacks
Metal binding sites
A-A platform
Tetraloop receptor
A-minor motif
. . .

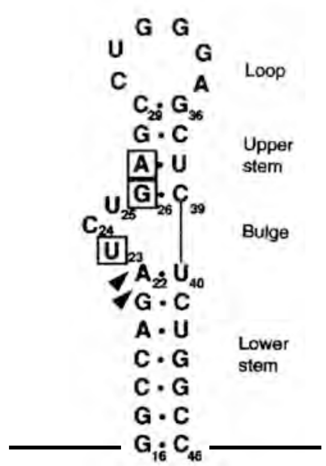
UNCG tetraloop

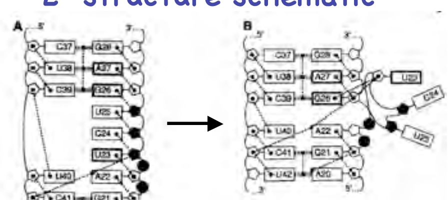


Stabilizes attached stem

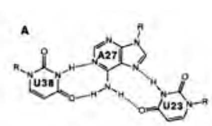
HIV TAR RNA mediates Tat binding

2° structure schematic

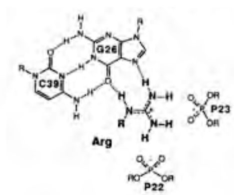




Coaxial stacking



Base triple

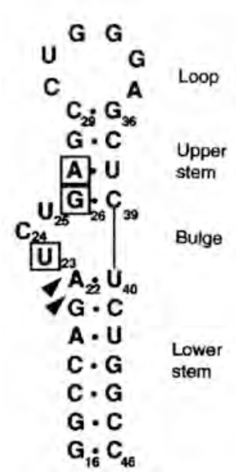


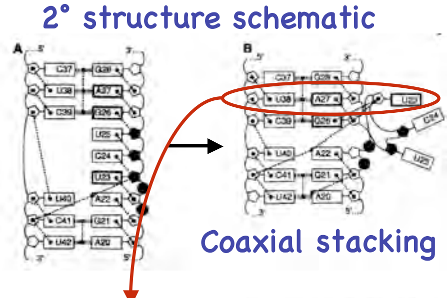
Arg binds GC bp

Nomenclature for secondary structure: stem, loop & bulge

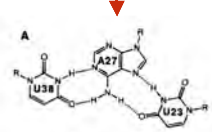
HIV TAR RNA mediates Tat binding

2° structure schematic

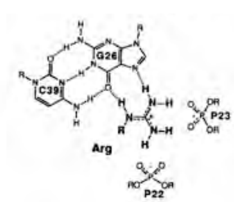




Coaxial stacking

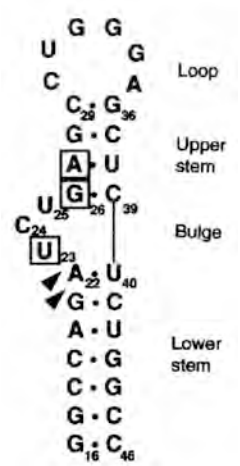


Base triple



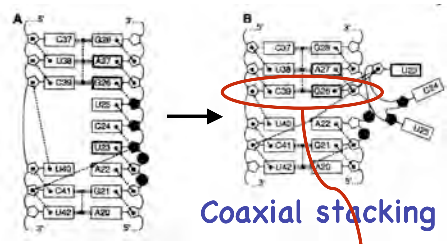
Nomenclature for secondary structure: stem, loop & bulge

HIV TAR RNA mediates Tat binding

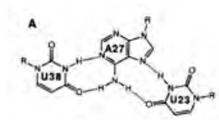


Nomenclature for secondary structure: stem, loop & bulge

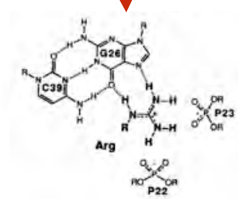
2° structure schematic



Coaxial stacking

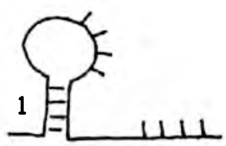


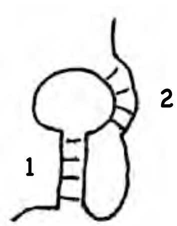
Base triple



Arg binds G26/C39 bp

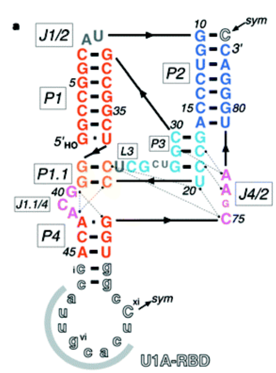
Pseudoknots



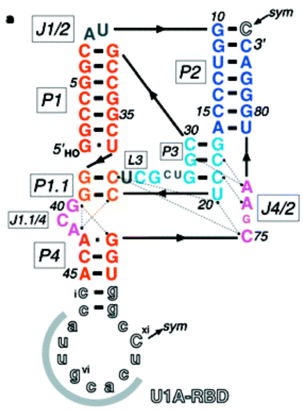


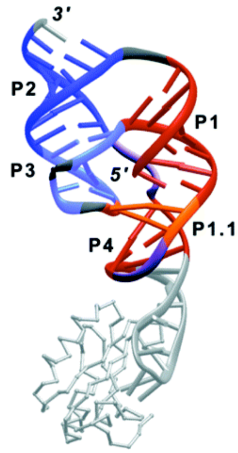
Bases in loop of stem 1 form stem 2 (with bases outside stem 1)

HDV ribozyme forms a double pseudoknot

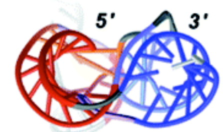


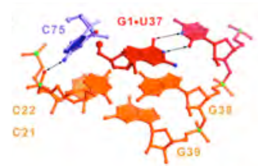
Hepatitis Delta Virus (HDV) ribozyme double pseudoknot





"Top" view

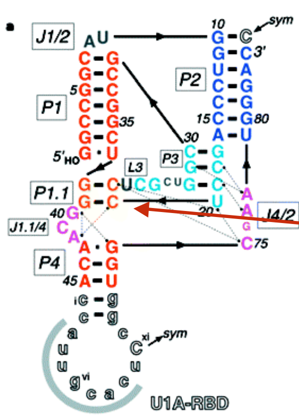


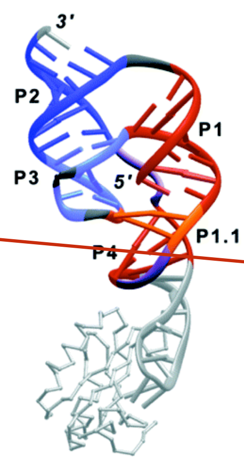


2° structure schematic

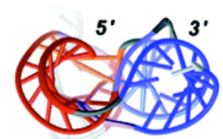
U1A protein cocrystals

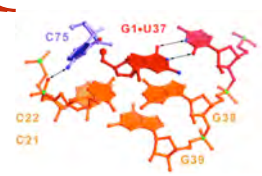
Hepatitis Delta Virus (HDV) ribozyme double pseudoknot





"Top" view



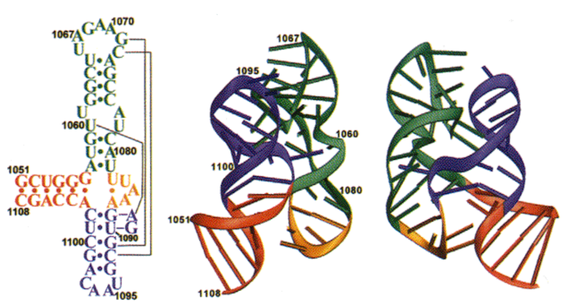


2° structure schematic

U1A protein cocrystals

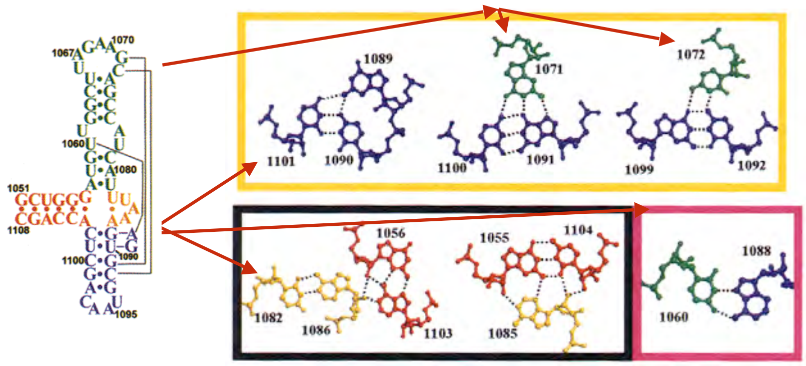
Four-helix junction: L11 protein binding site in 23S RNA

Four-helix junction: L11 protein binding site in 23S RNA



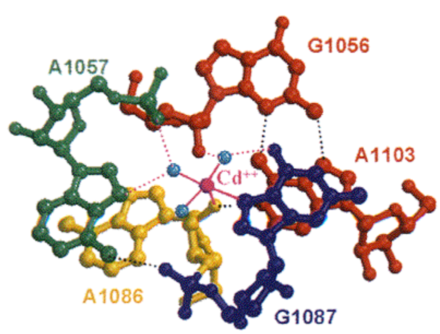
Four helices emerge from a central wheel.
The four double-helical stems form two coaxial stacks.
The two stacks have irregular but complementary shapes.
The helices knit together to form a compact globular domain.

Base triples in the L11 4-helix junction



Bulge and loop mediate long-range tertiary interactions.
The riboses of A1084-A1086 (all A's) form a "ribose zipper.
A1086 adopts a syn conformation to facilitate tight sugar packing.

Metal ions stabilize the L11 RNA 4-helix junction

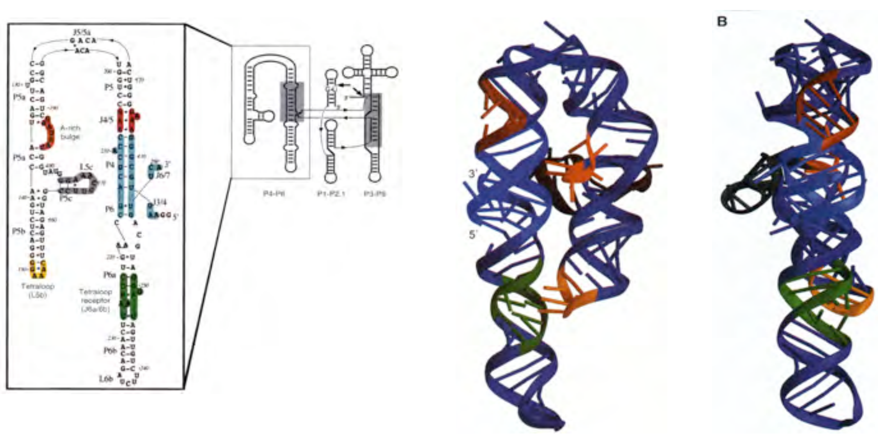


Mg^{2+} ions (gold balls)
Cd^{2+} ions (magenta)
Hg^{2+} (rose)

RNA interactions of the central Cd^{2+} ion

P4-P6 Domain of the Group I ribozyme

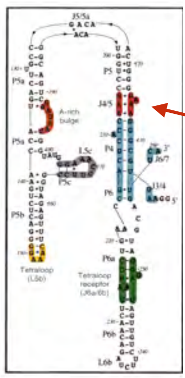
P4-P6 Domain of the Group I ribozyme



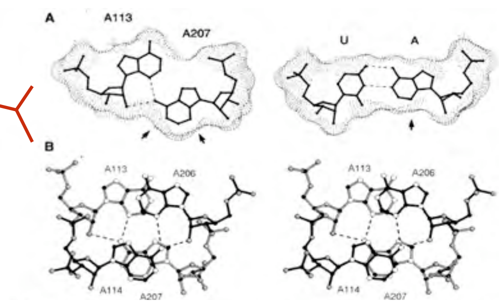
Two helical stacks are arranged parallel to each other.
The structure is one helical radius thick.
Two regions of 3° interactions between the two helical stacks.

1. Tetraloop/Tetraloop-receptor.
2. A-rich, single-stranded loop and the minor groove of the opposing helix.

Tertiary interactions in the P4-P6 domain



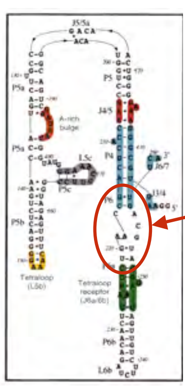
Sheared AA   Standard AU



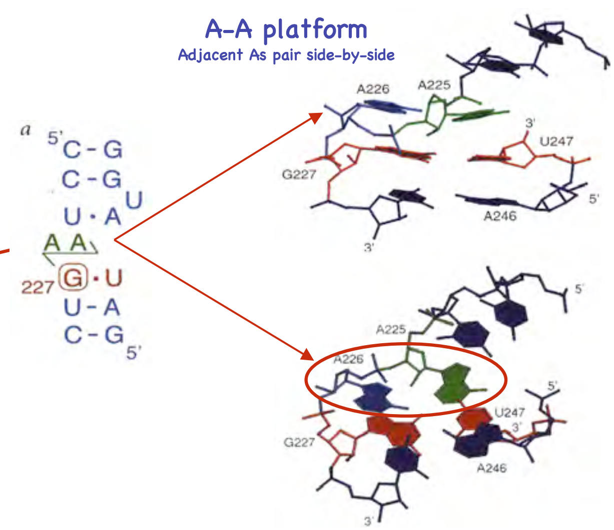
Sheared AA bps fill minor groove

Cross-strand purine stack.

Tertiary interactions in the P4-P6 domain



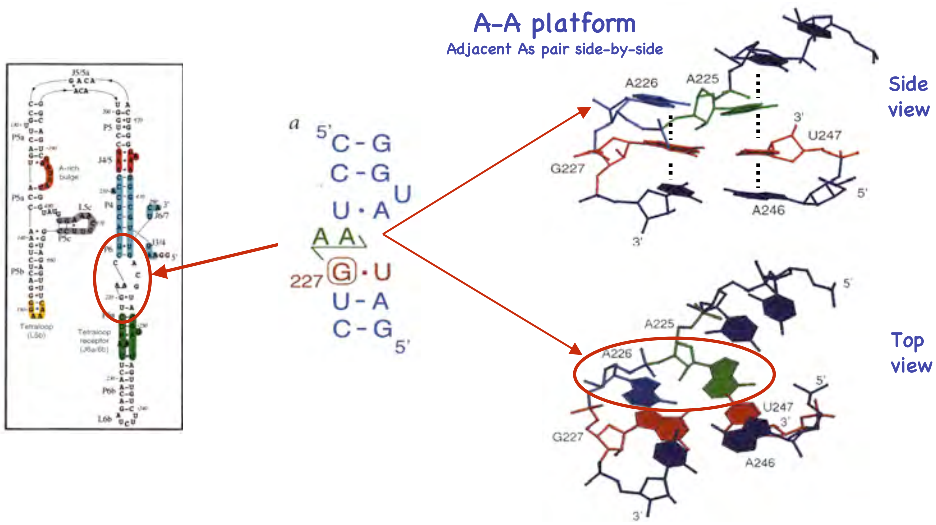
A-A platform
Adjacent As pair side-by-side



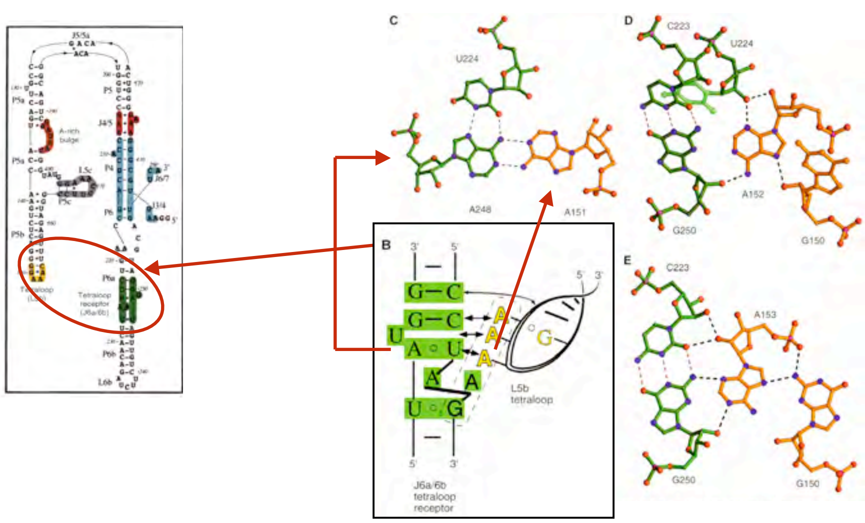
Side view

Top view

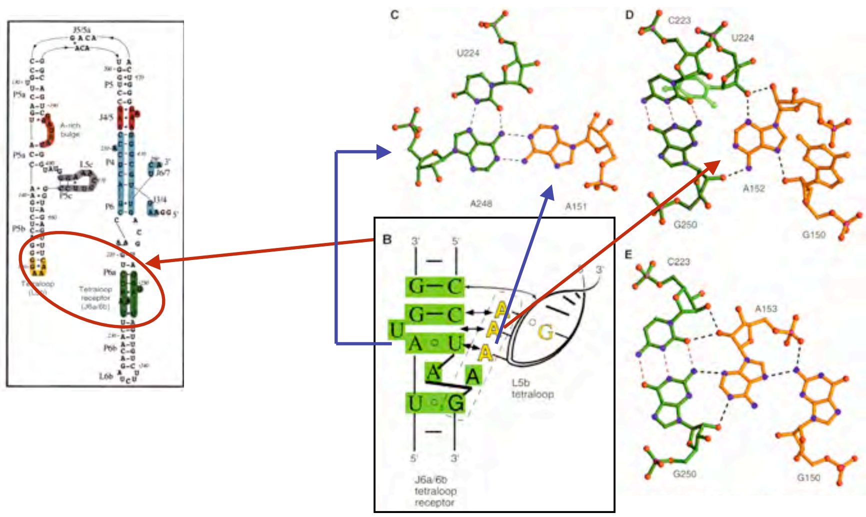
Tertiary interactions in the P4-P6 domain



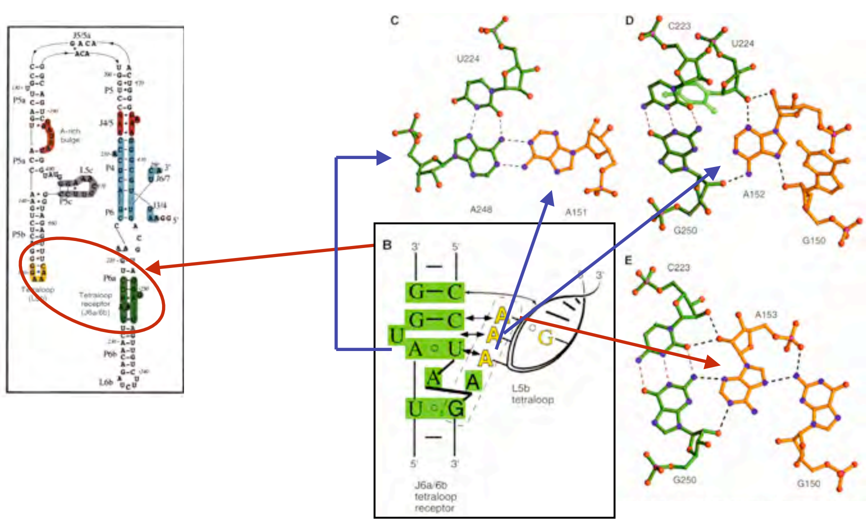
Tertiary interactions in the P4-P6 domain



Tertiary interactions in the P4-P6 domain



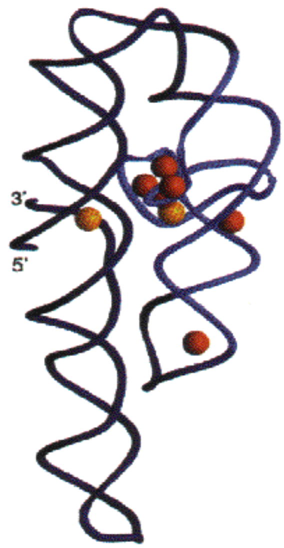
Tertiary interactions in the P4-P6 domain

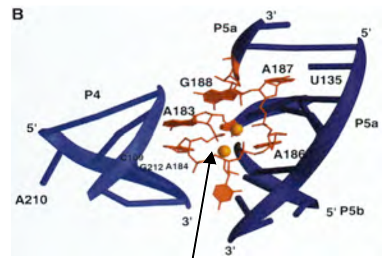


Metal ion core in the P4-P6 domain

Divalent metal ions (Mg^{2+}) are required for proper folding.

These ions bind to specific sites and mediate the close approach of the phosphate backbones

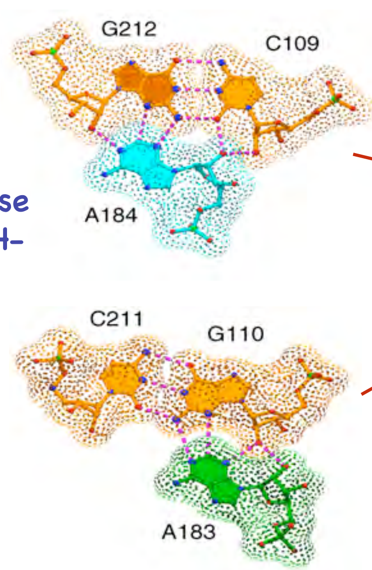


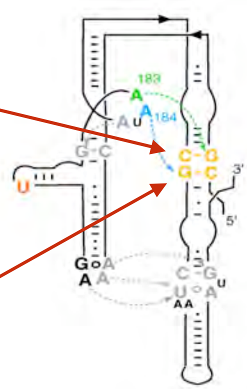


At one position in the molecule the phosphate backbone turns inward and coordinates two metal ions.

Adenosine-minor-groove base triples: the A-minor motif

A fills minor groove & ribose 2′ OH forms H-bonds



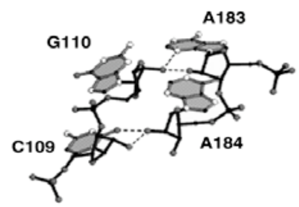


Adjacent base-triples bring together RNA strands

Hydrogen bonds between adjacent backbone atoms create a "ribose zipper"

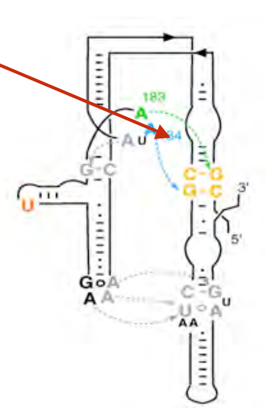
Ribose Zipper

2'-OH Mediated Interactions
A183 - G11 and A184 - C109



Mutant	ΔΔG (kcal/mole)
A183 deoxy	0.6
A184 deoxy	1.4

Deoxynucleotides destabilize P4-P6



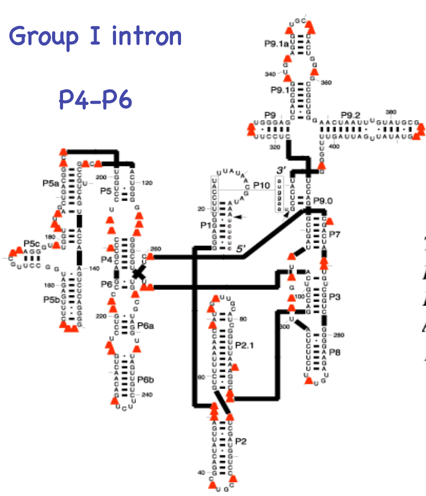
The A-minor motif is widespread

Conserved As are abundant in unpaired regions of structured RNAs.

Single Stranded Adenosines

Group I intron

P4-P6



% of As in "single-stranded regions

Tetrahymena intron	62/129	48%
E. coli RNase P	55/126	44%
B. Subtilis RNase P	69/159	43%
Archea 16S RNA	233/530	44%
E. coli 16S RNA	252/608	41%

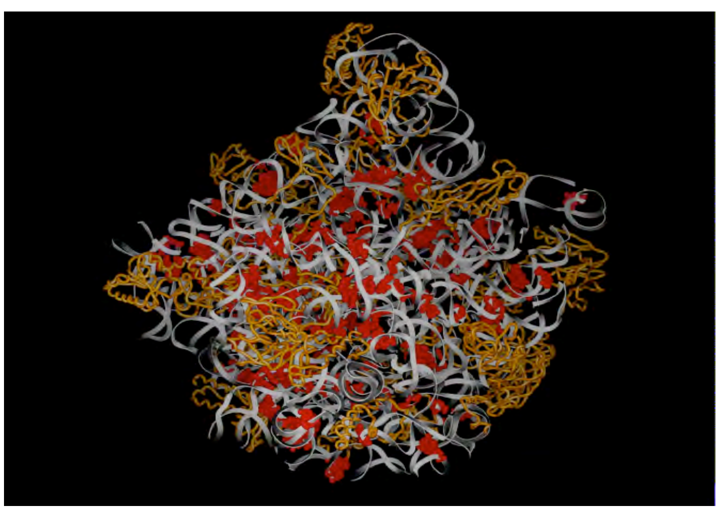
What happens in very large RNAs?

E. Coli 16S rRNA secondary structure

<u>% of As in "single-stranded regions</u>

E. Coli 16S rRNA	40%
Tetrahymena group I intron	48%
B. Subtilis RNase P	43%
Archeal 16S rRNA	44%
HCV IRES element	27%

A-minor motifs are the predominant tertiary interaction in the 50S ribosomal subunit



Summary

1. RNA structure can be specific, globular, stable and complex. (As a result, RNA mediates specific recognition and catalytic reactions.)
2. Secondary structures include stems, bulges, and loops.
3. Tertiary motifs include base triples, pseudoknots, A-A platforms, the tetraloop/tetraloop receptor, A-minor motifs, ribose zippers
4. Principles: stems and loops conserved,
   many non-WC base contacts,
   coaxial stacking,
   metal ion binding,
   H-bonding of ribose 2′ OH, and
   repeated “motifs”.