Biochemistry 410 - RNA Section

Learning objectives:

- Coding and Non-Coding RNAs, How to detect RNAs?
- secondary and tertiary structures of RNA and how to study them
- RNA-based enzymes: ribozymes (e.g., the ribosome) – the 'RNA world'
- RNA molecules as sensors: riboswitches
- RNA processing
- The control of mRNA translation
- The regulation of the translation of specific mRNAs
- microRNAs and RNA interference/gene silencing

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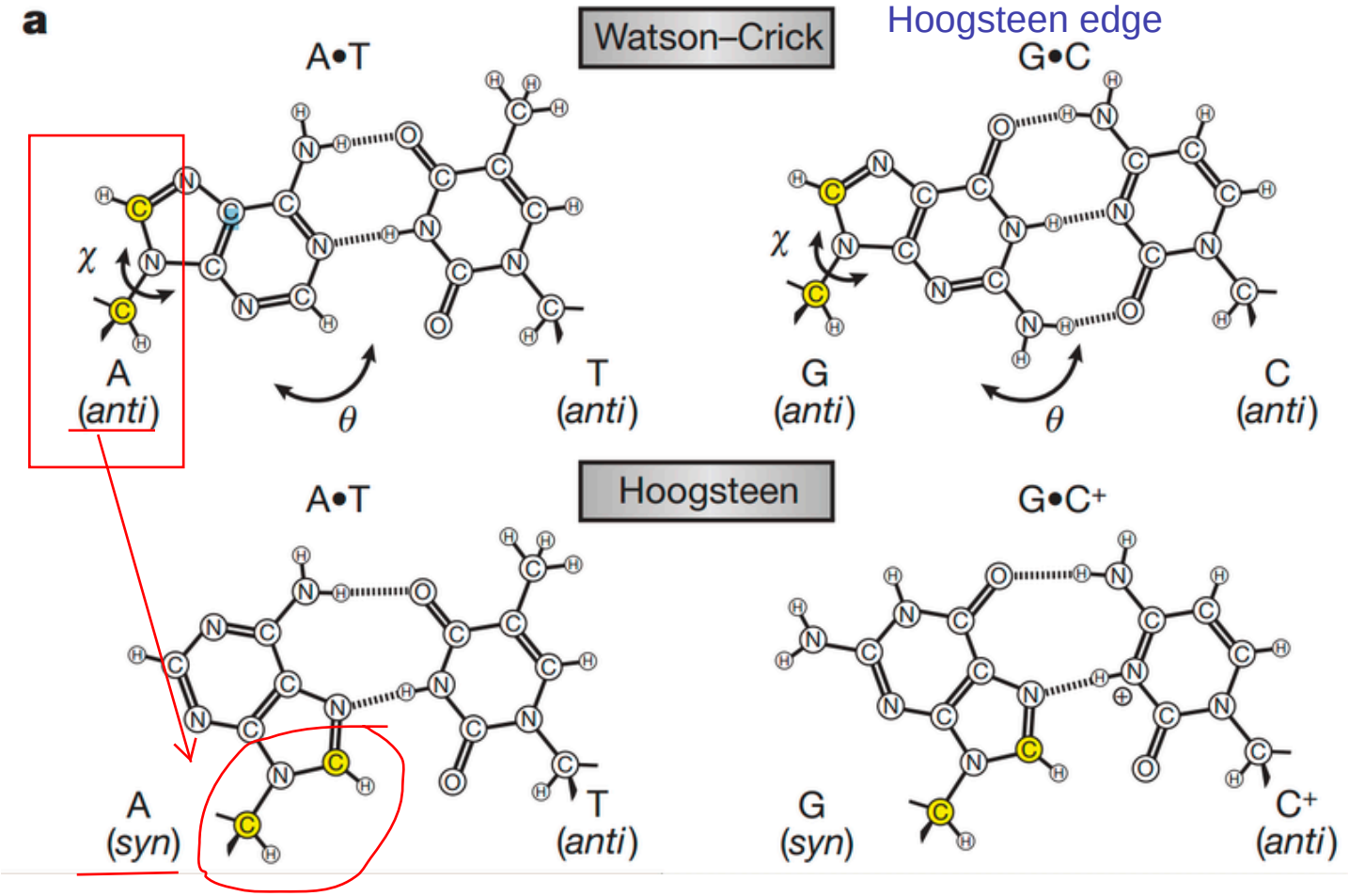
Non-canonical Basepairing

be able to recognize Watson-Crick vs Hoogsteen edge



backbone must be changed a little bit in order to accommodate this anti-syn change (don't need to know more)

498 | NATURE | VOL 470 | 24 FEBRUARY 2011

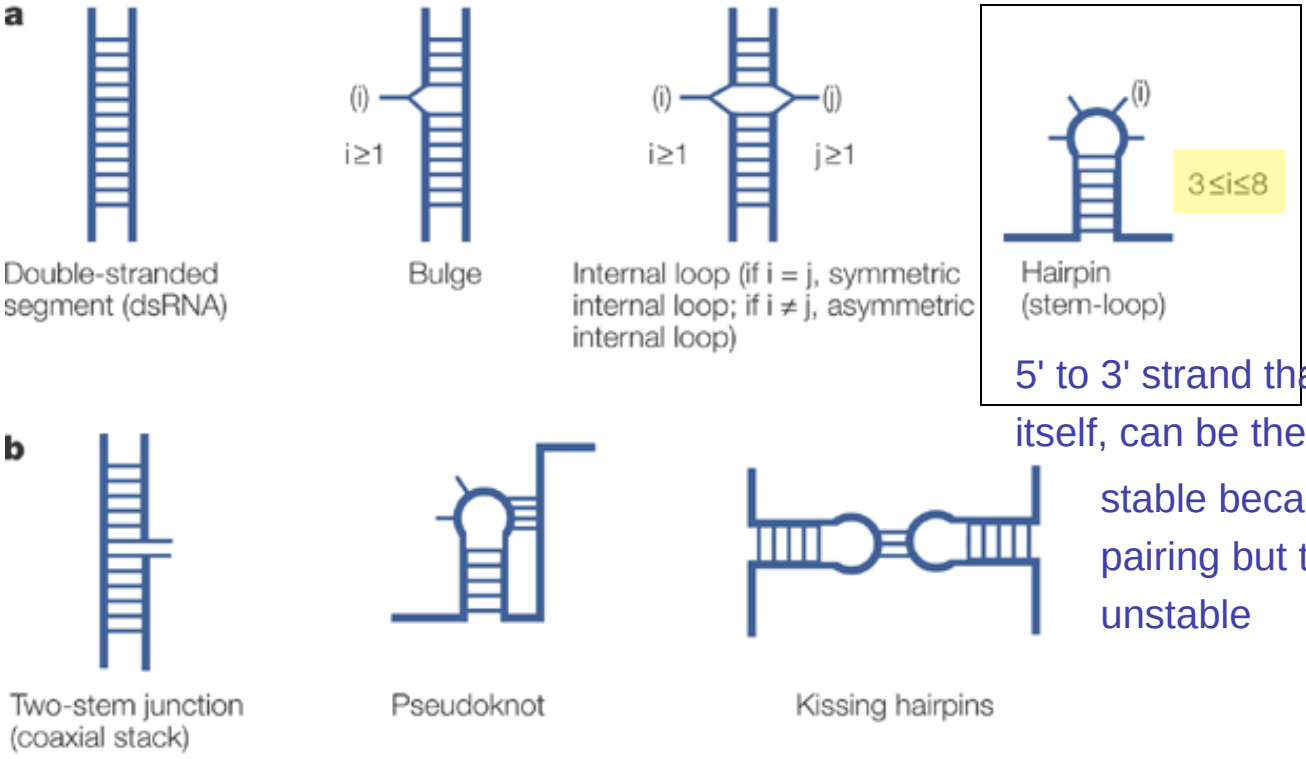
3 nucleotides can interact at the same junction

RNA can fold and is dynamic

secondary (local) and tertiary (long-distance interactions)....not exclusive

RNA fold that determines binding to RNA or protein and can be catalytic!

Common RNA Structural Motifs



triloop
tetraloop

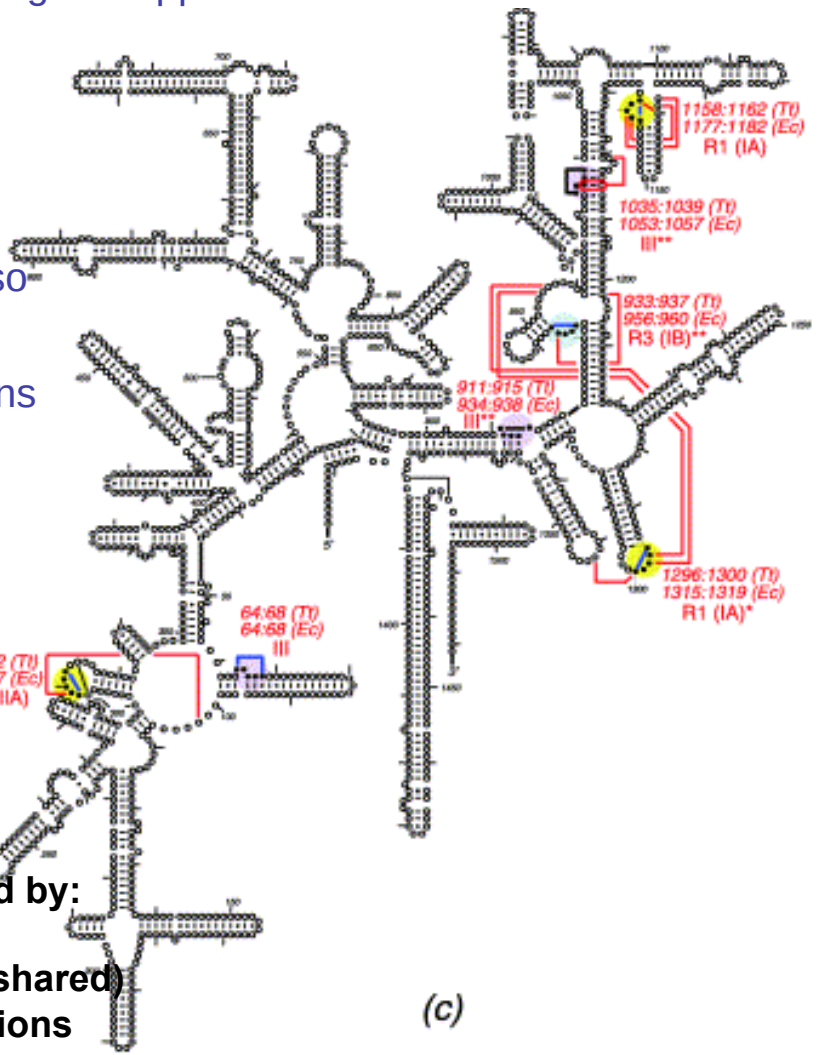
5' to 3' strand that folds back on itself, can be thermodynamically stable because of base-pairing but the loop is unstable

Nature Reviews | Molecular Cell Biology

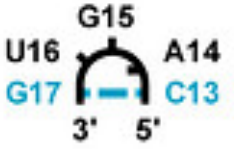
Triloop

can interact with other regions of the RNA using the flipped out nucleotides

16S rRNA



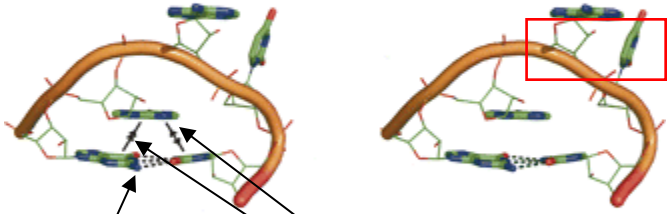
Triloop



1aqo

nucleotides can also flip out for tertiary structure interactions

Stereoviews of triloop
used to show the 3D structure



not very strong, but stabilizes it

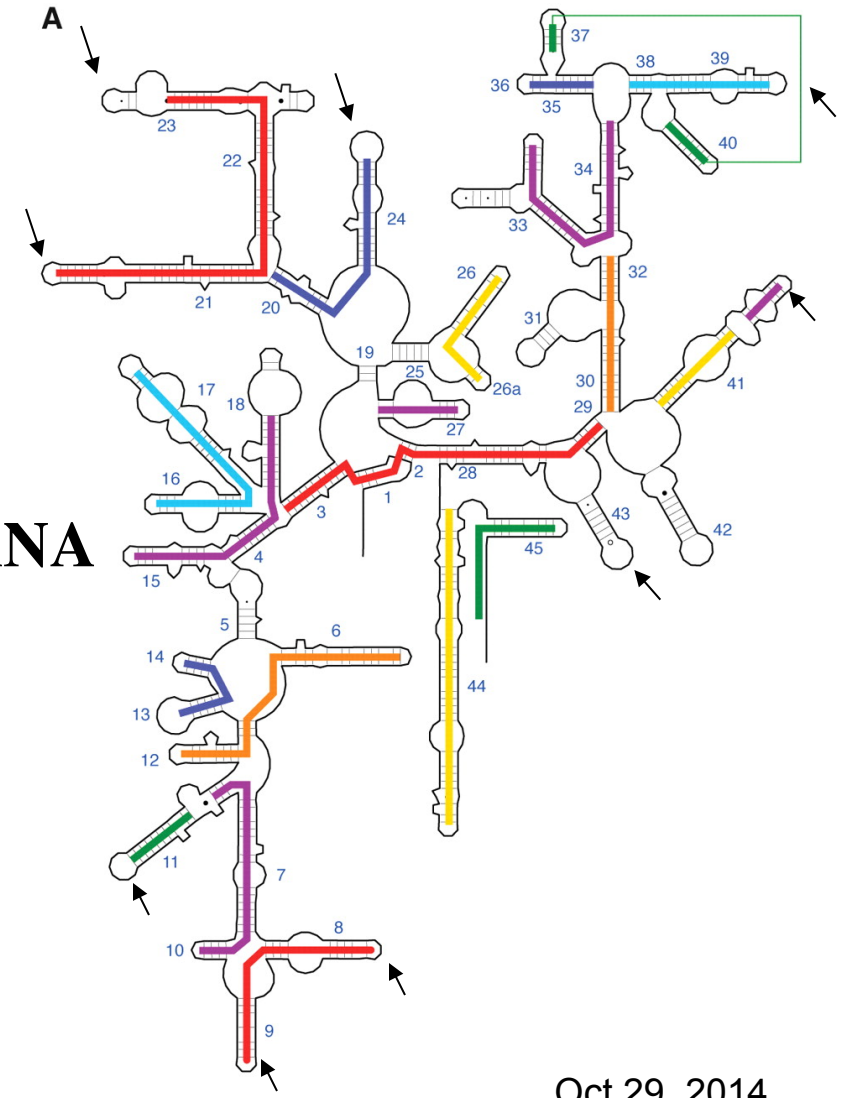
H bonding (Watson-Crick base pairing)

Base stacking stabilized by:
-hydrophobic,
-π orbitals (electrons are shared)
-van der Waals interactions

Oct 29, 2014

RNA. 2007 Sep;13(9):1537-45. Epub 2007 Jul 25

Tetraloop Hairpin very common motif for tertiary structure



16S rRNA

- one of the most common are tetraloops, GNRA and UNCG, in rRNA

G and A interact with each other

- very thermodynamically stable because the first and last bases of tetraloop hydrogen bond

For GNRA.....G and A

For UNCG.....U and G

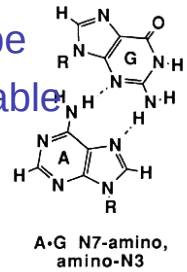
Tetraloops

5' -GNRA-3'

(N = any nt; R = a purine)

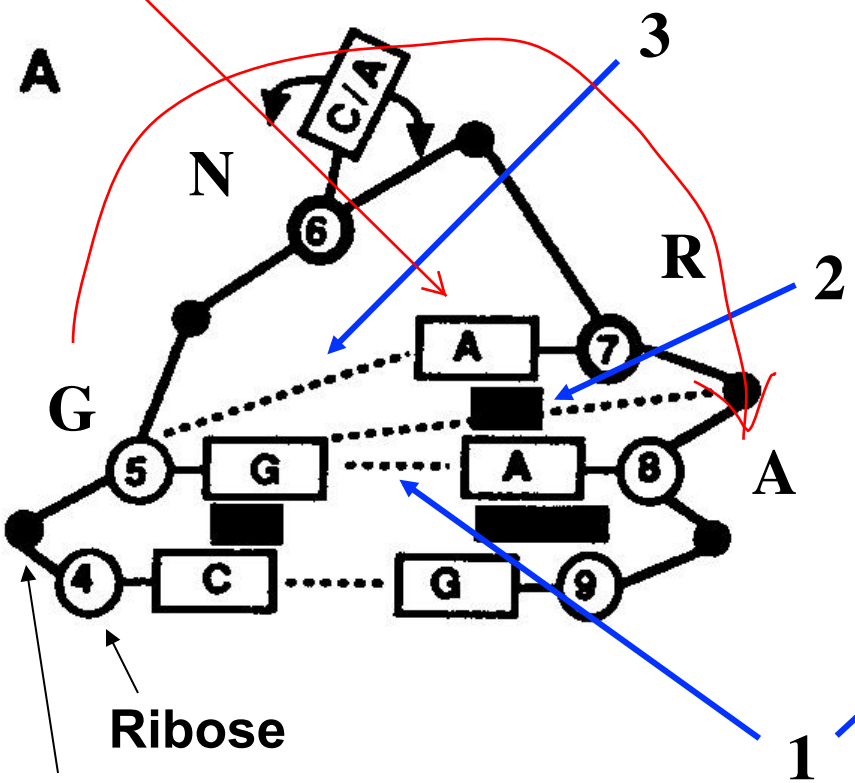
2) base stacking (x3 here) stabilized by pi orbitals and hydrophobic interactions, and van der wals

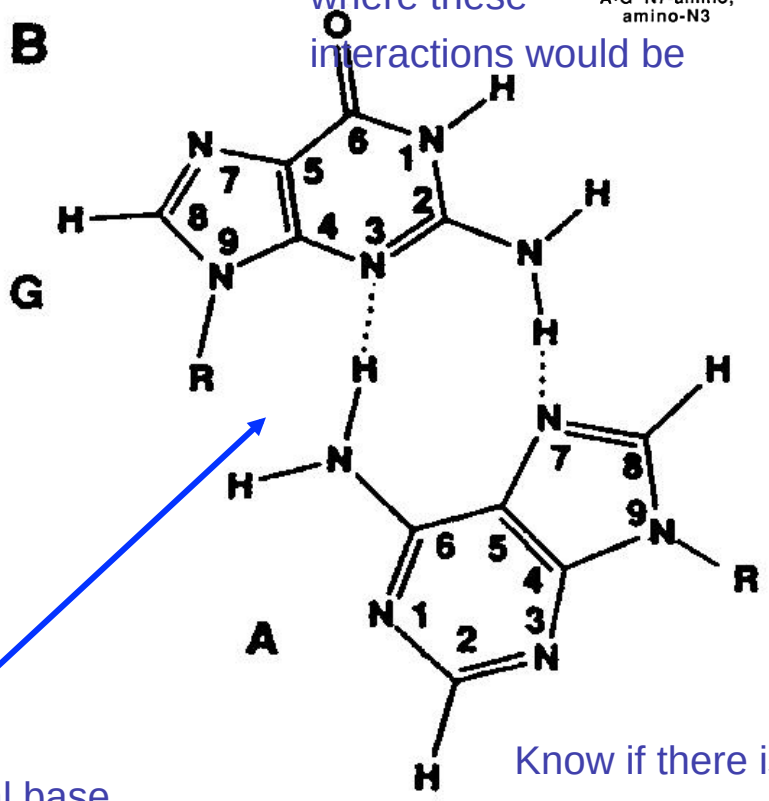
don't memorize these structures but now that GNRA is a common motif, and be able to say why it is stable



and point out where these interactions would be

3) adenine can H bond with the #5 ribose





non canonical base pairing, stabilized via hoogsteen edge

Know if there is base interaction or actually Hoogsteen edge

J. Mol. Biol. (1996) **264**, 968–980

Tetraloops

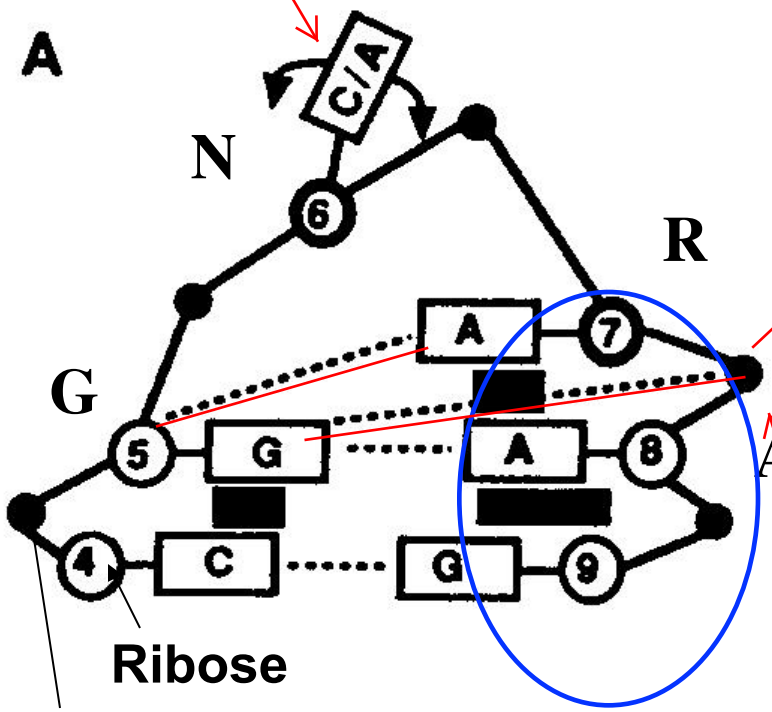
just 4 nucleotides can lead a LOT of stability to this tetraloop

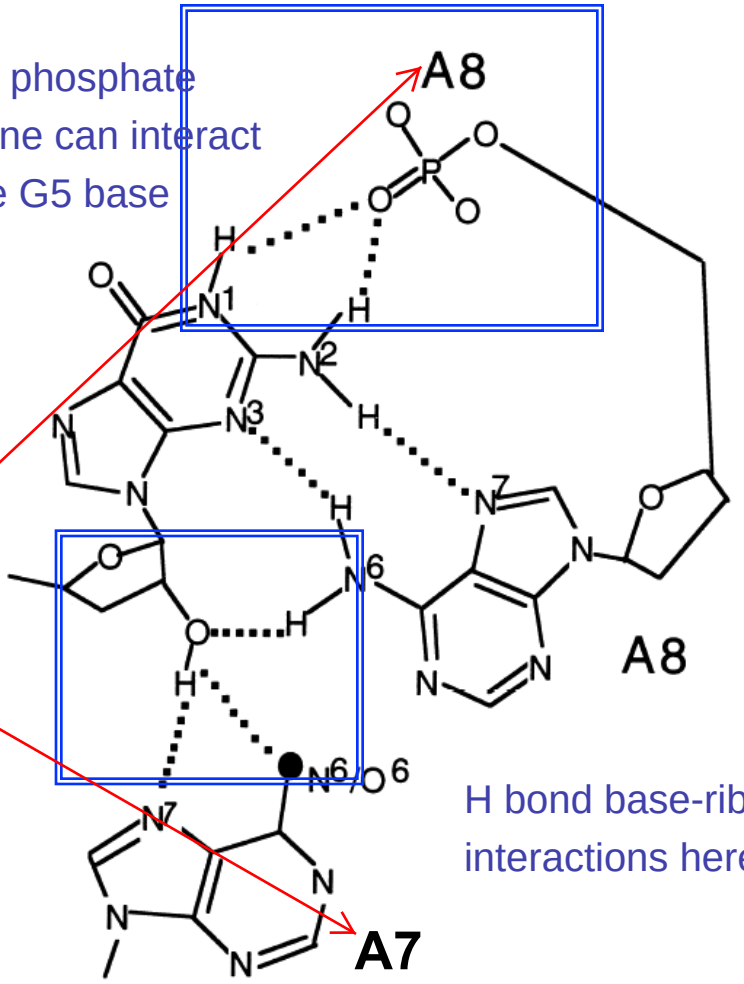
** really holds the RNA together

5' -GNRA-3'

this nucleotide can flip out too (can be any nucleotide, not just C or A)

H bond phosphate backbone can interact with the G5 base





H bond base-ribose interactions here

this phosphate connects A7 and A8

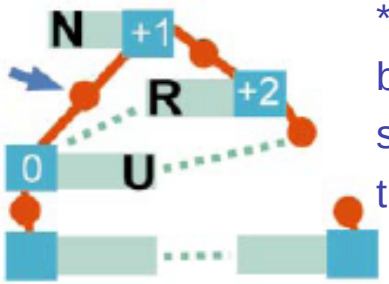
in anticodon loop of tRNA

U-Turn Motif (Uridine turns)

often found in loops

can sometime be without the U

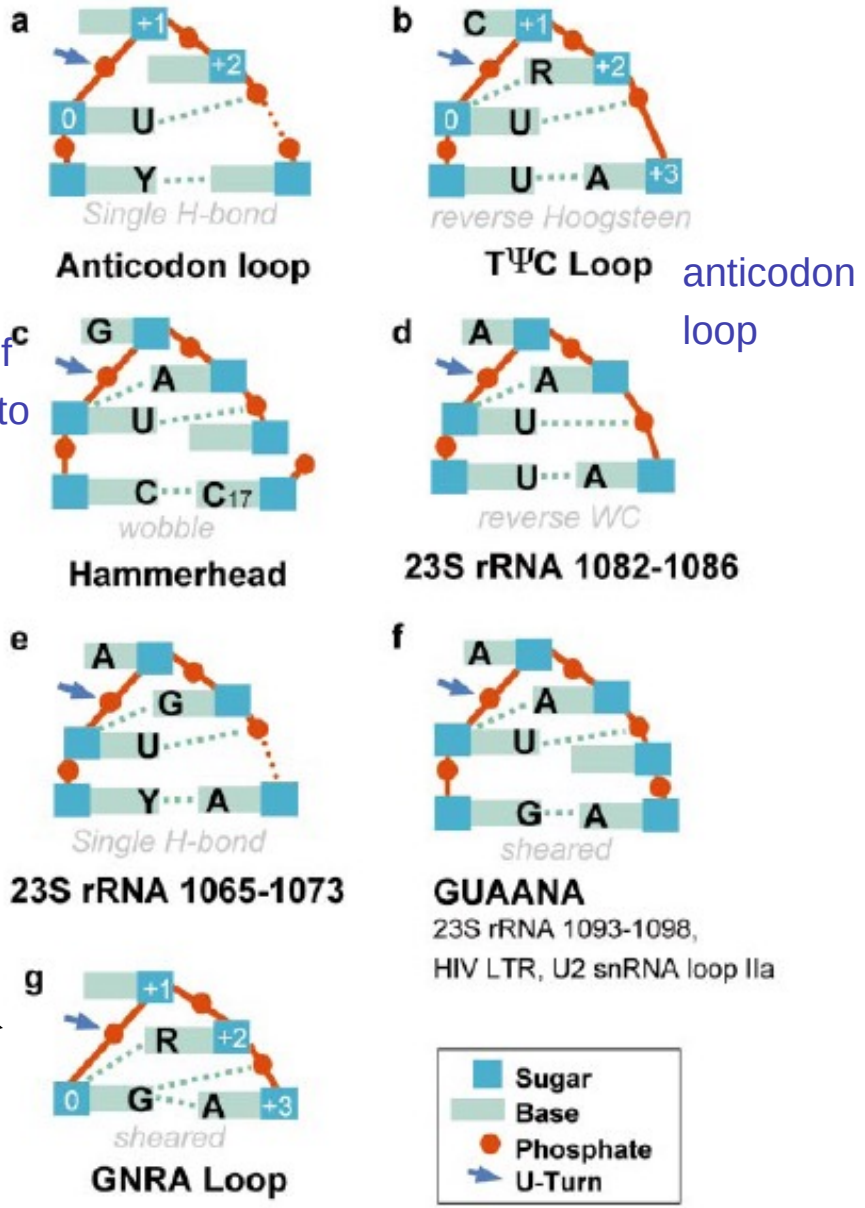
Sharp Phosphate Turn in Phosphate Backbone



** sharp phosphate backbone, instead of smooth region, due to the U

UNR consensus

very similar



anticodon loop

J Mol Biol. 2000 Jul 21;300(4):791-803

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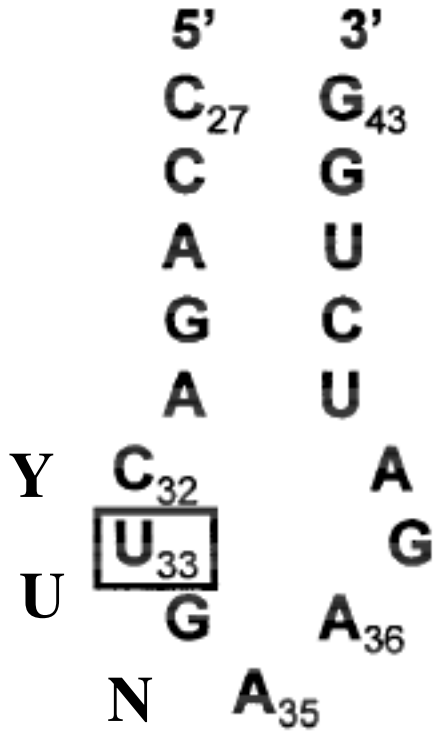
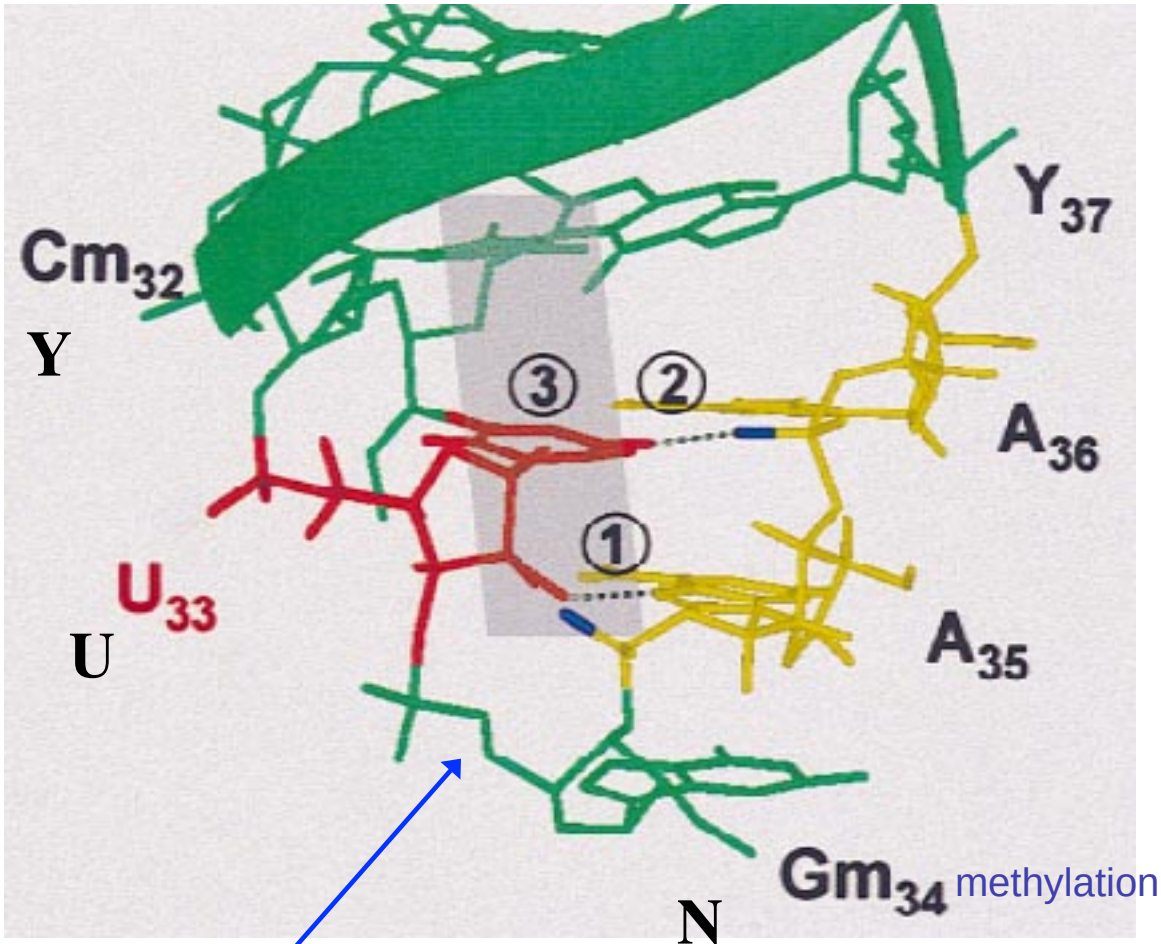
U-turns
(uridine turn)

phenylalanine anti-codon (UUU)





methylation

at least 3 nucleotides, therefore U turn

Especially sharp turn in phosphate backbone

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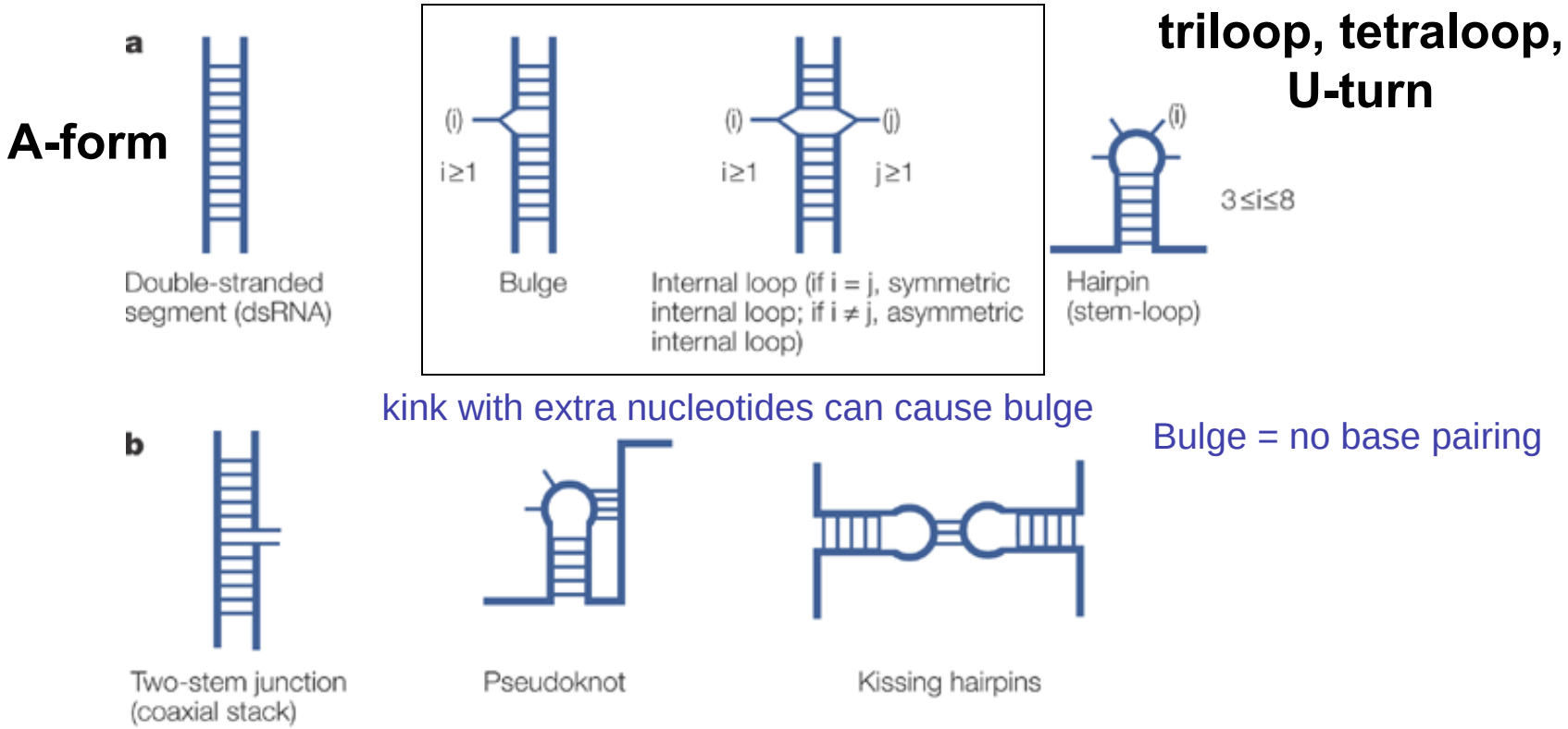
RNA can fold and is dynamic

- secondary (local) and tertiary (long-distance interactions)

RNA fold that determines binding to RNA or protein and can be catalytic!

Common RNA Structural Motifs



triloop, tetraloop, U-turn

kink with extra nucleotides can cause bulge

Bulge = no base pairing

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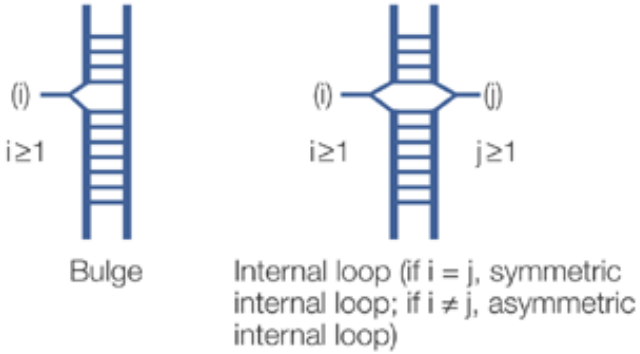
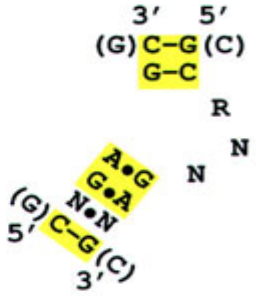


all somewhat the same

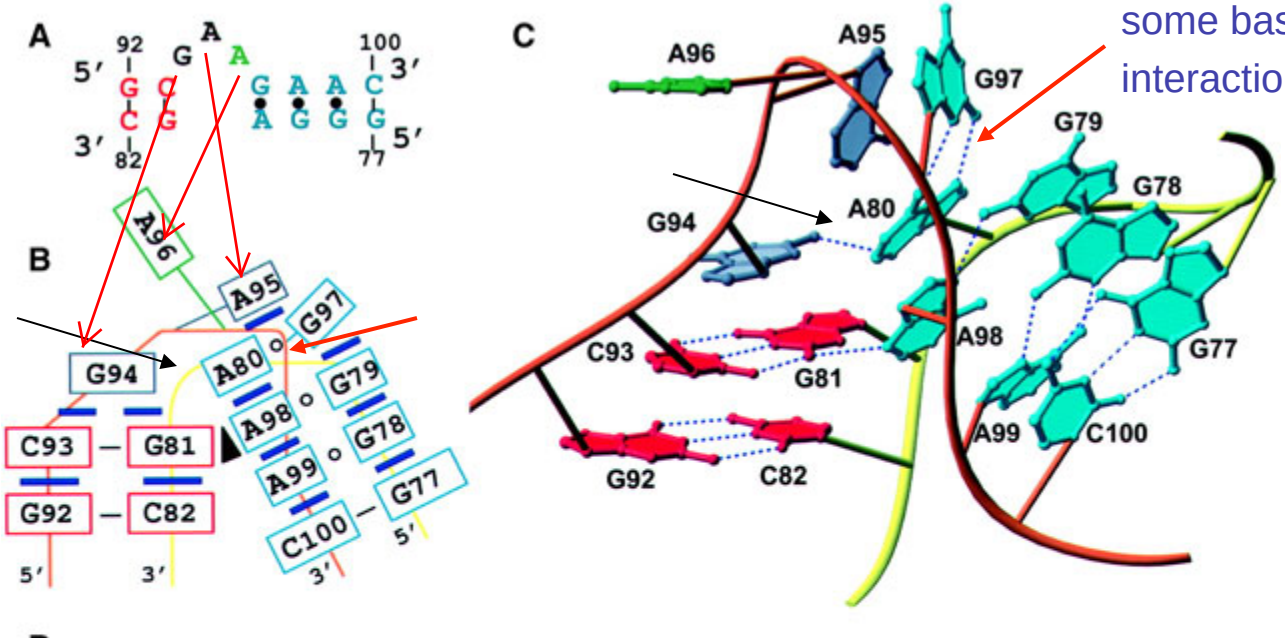
Types: K-turn, S-turn, hook-turn cross-strand purine stack, Adenosine platform

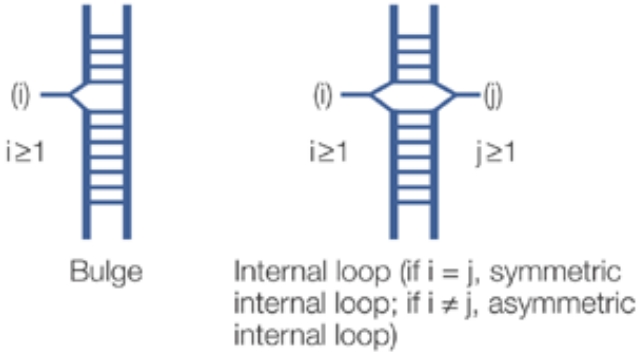


RNN

Consensus K–turn

some base stack interaction at A80

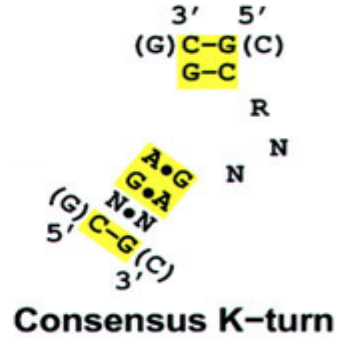




Bulge

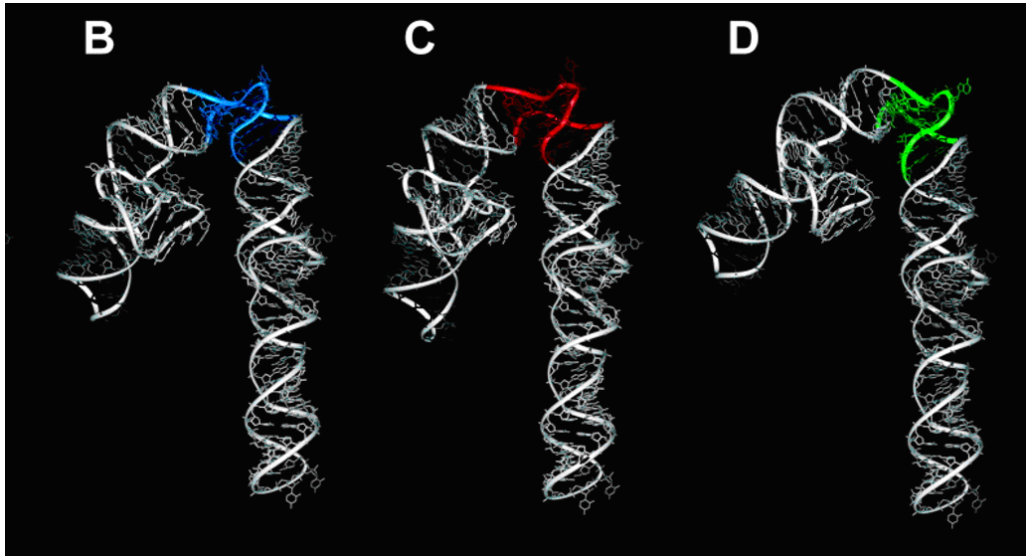
Internal loop (if i = j, symmetric internal loop; if i ≠ j, asymmetric internal loop)

Types: K-turn, S-turn, hook-turn cross-strand purine stack, Adenosine platform



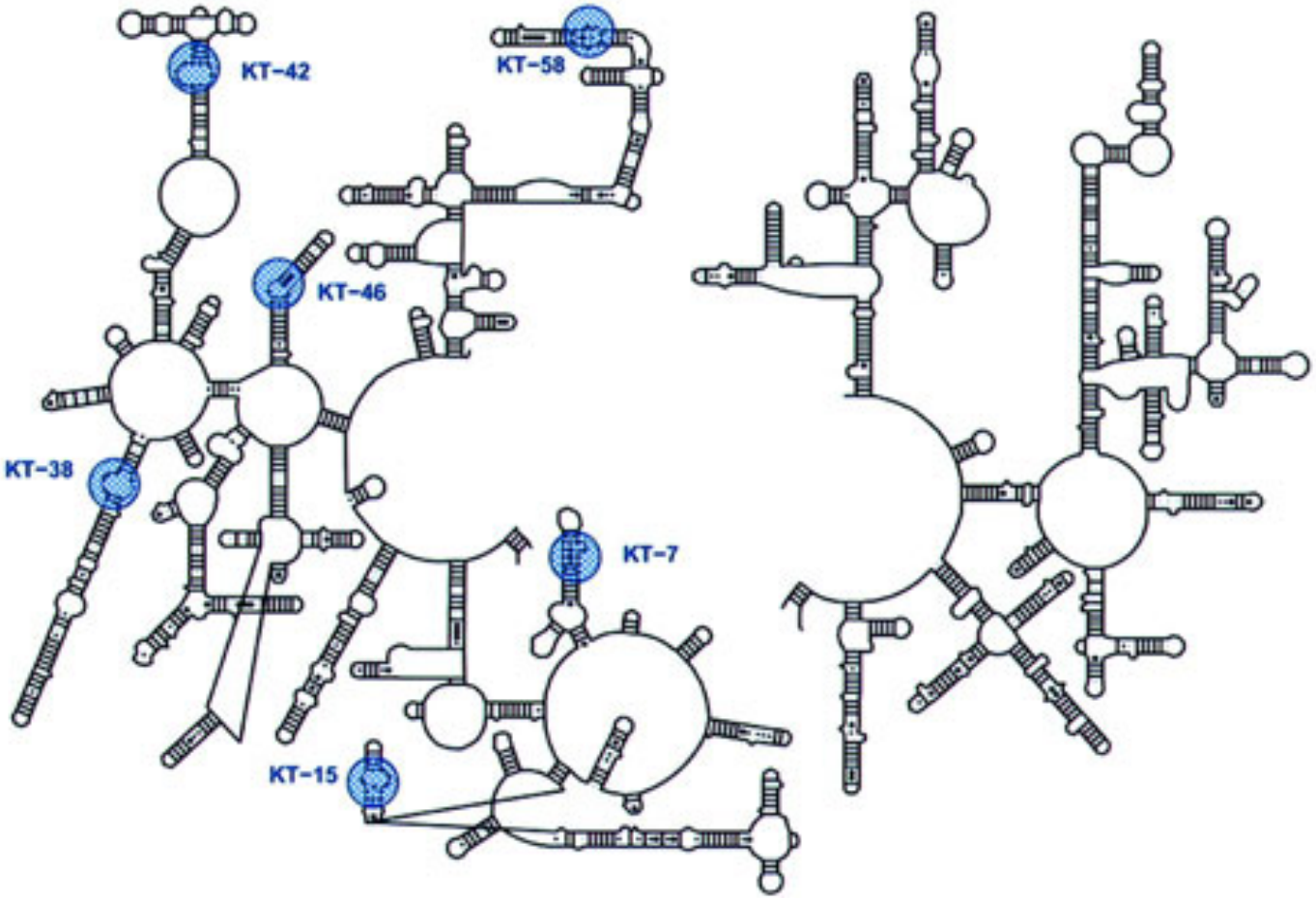
Consensus K–turn

all consensus K turns



Examples of K-turn

K-turns in 23S rRNA



K-turns, like most of bulges and loops, can be involved in tertiary structur interactions and protein interactions

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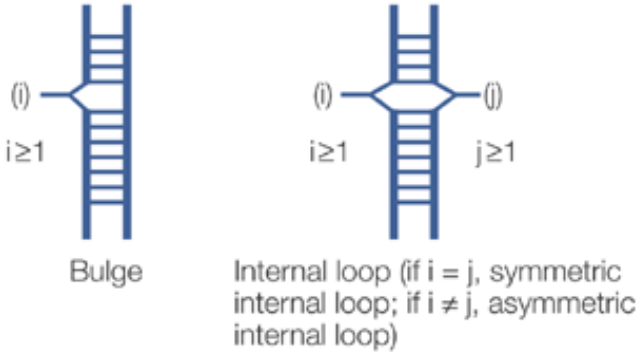
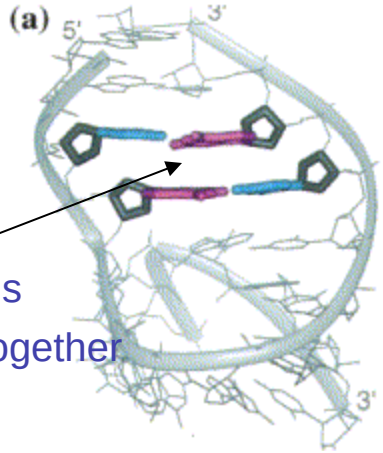
The EMBO Journal **(2001) 20, 4214–4221**

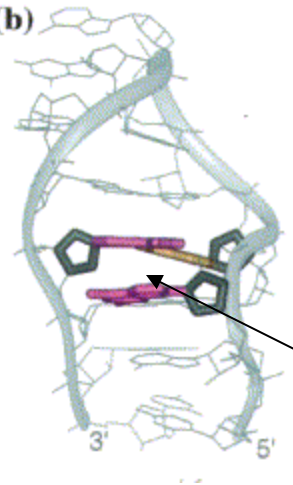
What stabilizes these hairpin loops, internal loops?



- hydrogen bonding within base pairing and ribose groups
- base stacking interlocking RNA strands

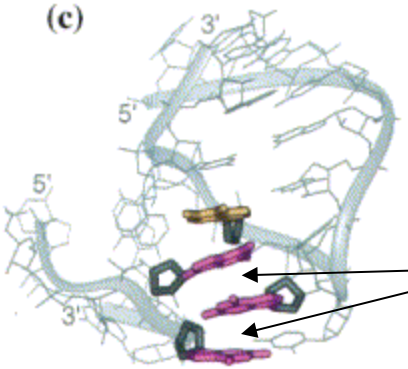


multiple interactions needed to hold it together



some overlap of bases without h bonding (in the same strand here)

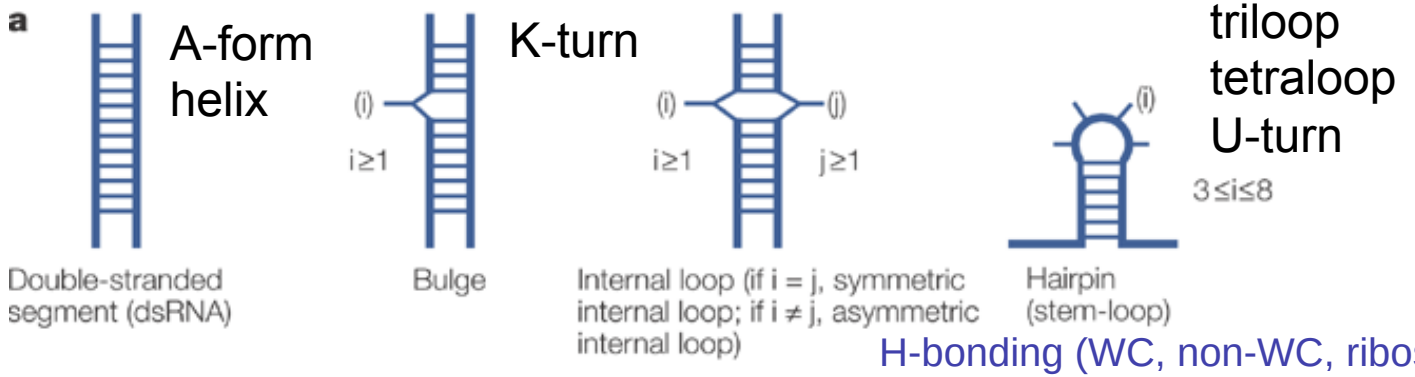
base stacking (intra and inter) (same or cross strand stacking



a) ColE1 dimer loop
b) sarcin/ricin loop in 28S rRNA
c) junction of T and D loop of tRNA

stacking of bases from different strands

RNA Secondary Structures



H-bonding (WC, non-WC, ribose, phosphate)

base stacking (inter and intra strand)

bulges and loops involved in teritiary structures

Tertiary structures

- long distance interactions between RNA strands, normally between secondary structure motifs

STRUCTURAL MOTIFS IN RNA

P. B. Moore Annu. Rev. Biochem. 1999. 68:287–300

Stitching Together RNA Tertiary Architectures

Thomas Hermann* and Dinshaw J. Patel*

J. Mol. Biol. (1999) **294**, 829–849

tetraloop/tetraloop receptor, A-minor motifs, and ribose zippers

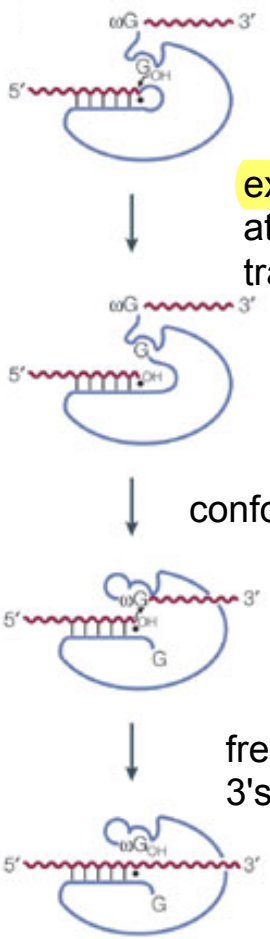
group 1 self-splicing intron

Tetrahymena Group I intron in pre-rRNA

In 1982, Tom Cech et al discovered that an intron within a pre-rRNA from *Tetrahymena thermophila* can catalyze its own cleavage (called self-splicing) to form the mature rRNA product.

The Nobel Prize in Chemistry 1989

Thomas R. Cech &
Sidney Altman



Folding requires Mg^{+2}

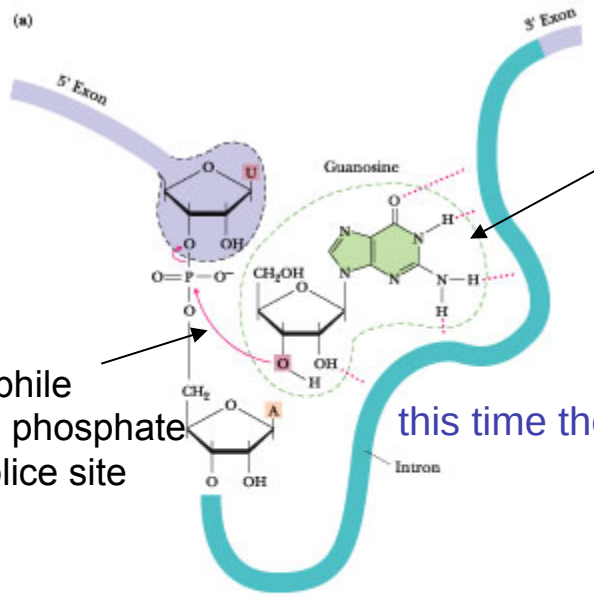
exogenous guanosine
attacks 5' splice site via
transesterification reaction

exons = red

blue = introns

conformational change

free 3'OH attacks
3'splice site



Exogenous
Guanosine

nucleophile
attacks phosphate
at 5' splice site

this time the 3' OH attacks back

doesn't use any proteins, and

the RNA splices itself out! (catalytic RNA)

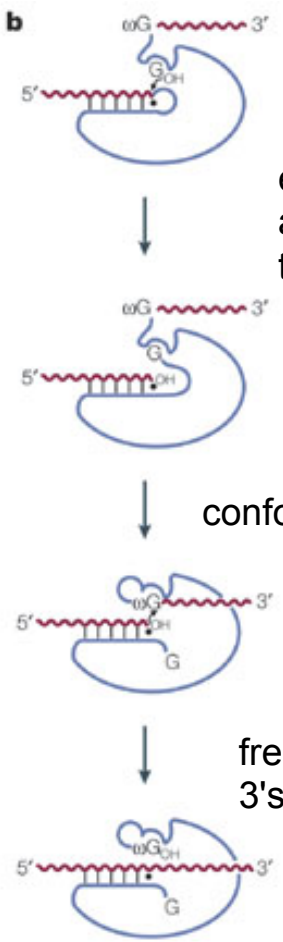
Lehninger, Biochemistry Ed. 5 pp 1035-1036

THE CATALYTIC DIVERSITY OF RNAS

Martha J. Fedor‡ and James R. Williamson*‡§*

Tetrahymena Group I intron in pre-rRNA

Tetrahymena - ciliated protozoa
Group I intron - found in rRNA, tRNA, and mRNA of bacterial, mitochondrial and chloroplast genes. Also in rRNA of lower eukaryotes.

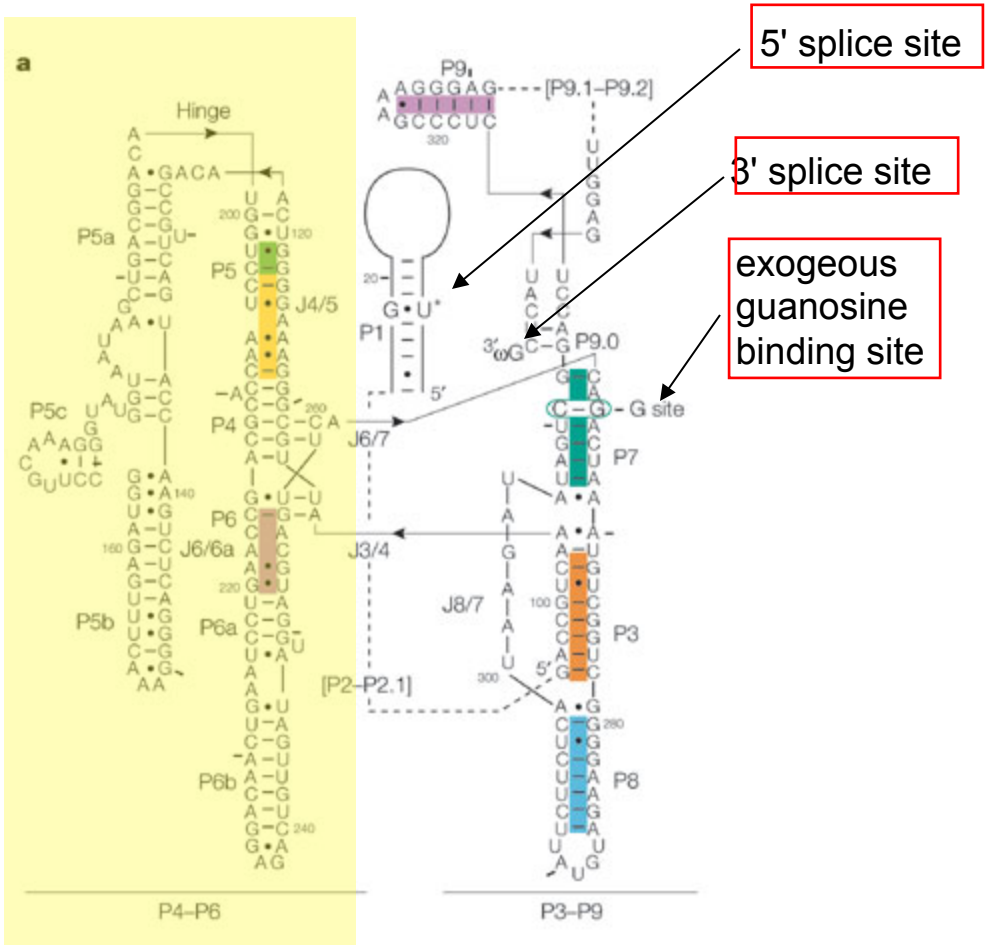


Folding requires Mg^{+2}

exogenous guanosine attacks 5' splice site via transesterification reaction

conformational change

free 3'OH attacks 3'splice site



held together by tertiary structure interactions

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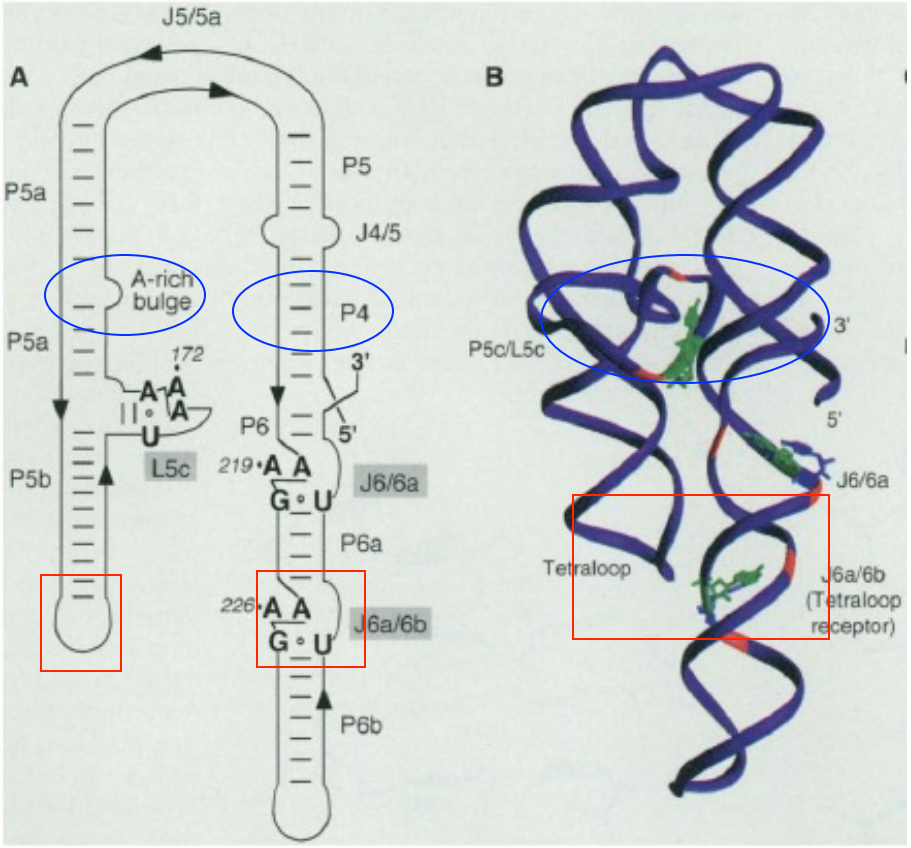
Group I intron P4-P6 domain

Helical stacks are arranged parallel to each other.



1) tetraloop/tetraloop receptor

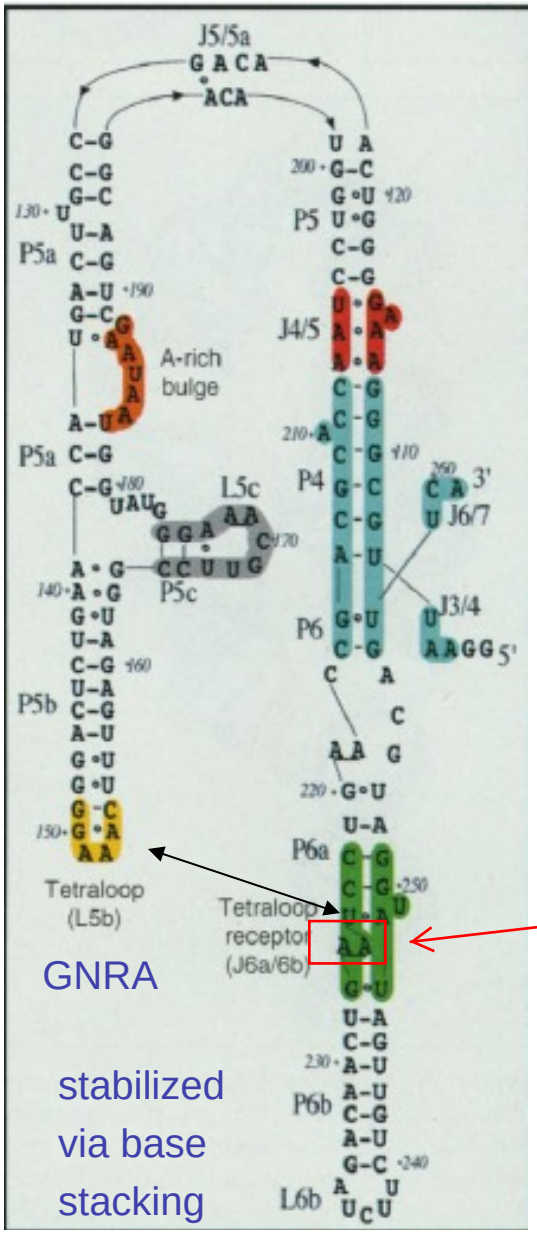
2) A-rich, single-stranded loop and the minor groove of the opposing helix

3) Ribose zipper

Crystal Structure of a Group I Ribozyme Domain: Principles of RNA Packing
Author(s): Jamie H. Cate, Anne R. Gooding, Elaine Podell, Kaihong Zhou, Barbara L. Golden, Craig E. Kundrot, Thomas R. Cech, Jennifer A. Doudna
Source: *Science*, New Series, Vol. 273, No. 5282 (Sep. 20, 1996), pp. 1678-1685
Published by: American Association for the Advancement of Science
Stable URL: http://www.jstor.org/stable/2891992

(GNRA)

1) tetraloop/tetraloop receptor

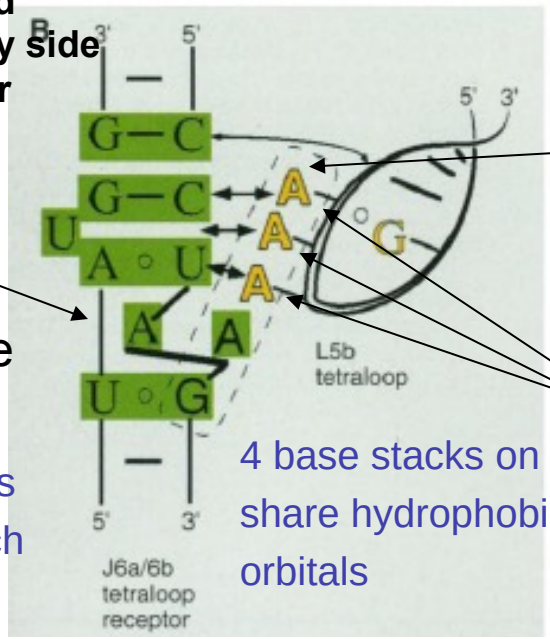


GNRA

stabilized via base stacking interactions, and some A's stick out

Adenosine 226 and 225 lie next side by side and open up minor groove

adenosine platform (2° structure motif)



4 A and G are stacked on top of each other to stabilize the interaction

adenosines flip out

4 base stacks on top of each other to share hydrophobic interactions pi orbitals

bulging adenines interact with each other and act as landing pad

side by side not across from each other

bulge

- Triple adenosines are flipped out to interact with CU

Interaction requires an Adenosine Platform within the tetraloop receptor

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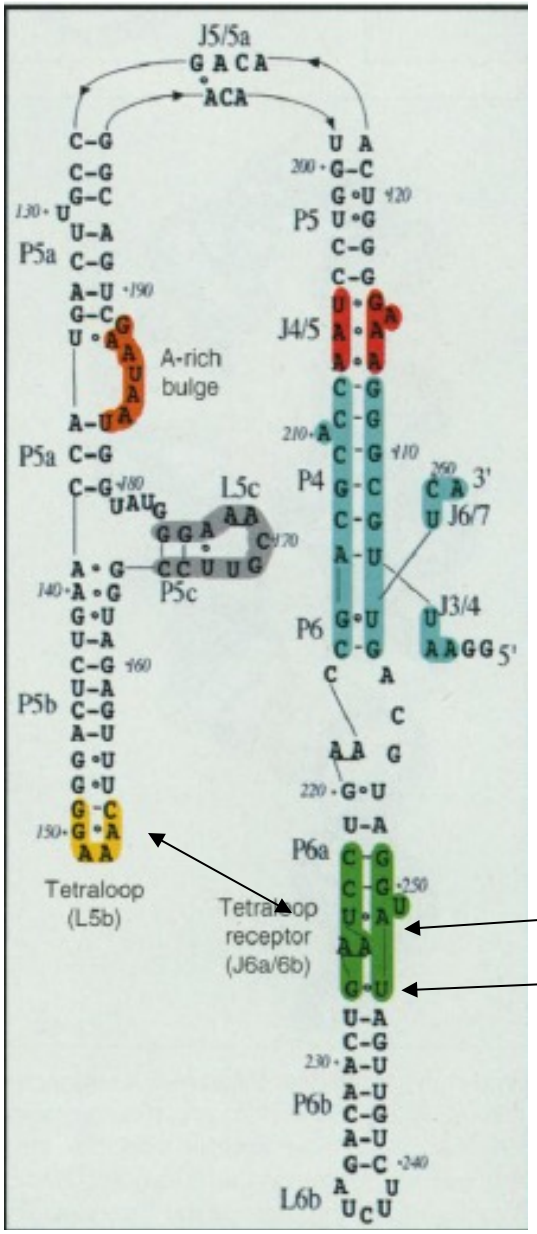
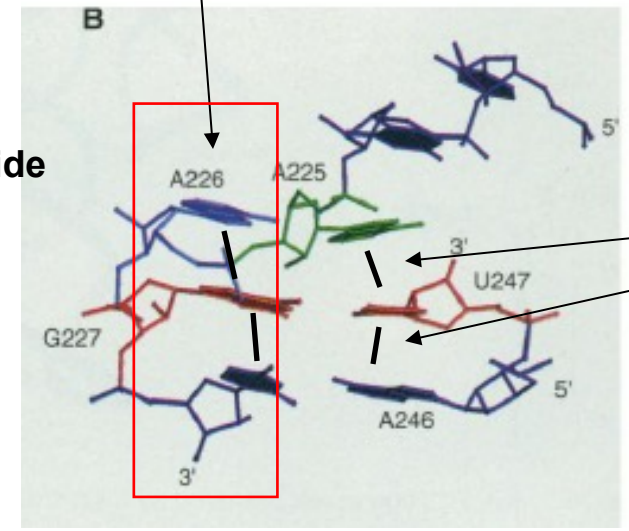
Adenosine Platform



Face of Adenine is open for interaction

Adenosine 226 and 225 lie next side by side and open up minor groove



base stacks

A G and U stacked on top of each other

Internal bulge of adenosines

G227, U247 and A246 also play a role

base stacking stabilizing it so it opens up

Interaction requires an Adenosine Platform within the tetraloop receptor

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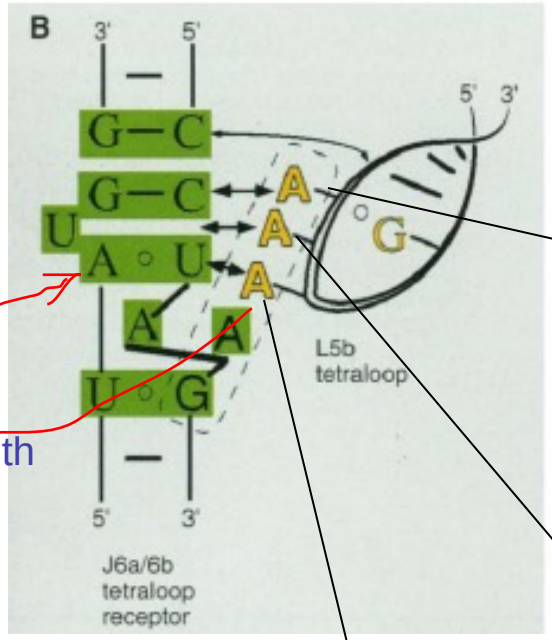
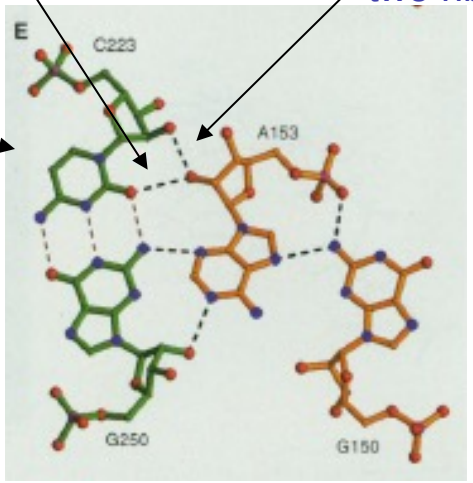
Extensive base pairing between the tetraloop and tetraloop receptor



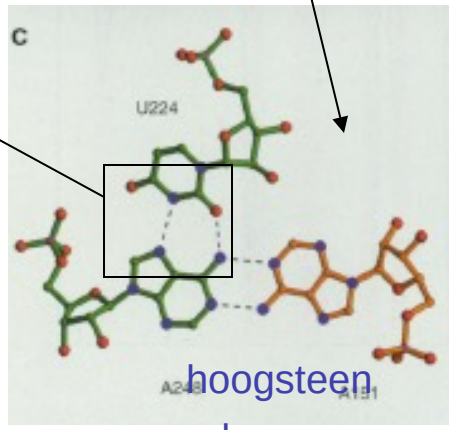
interacting with this A

H-bonding between ribose and base

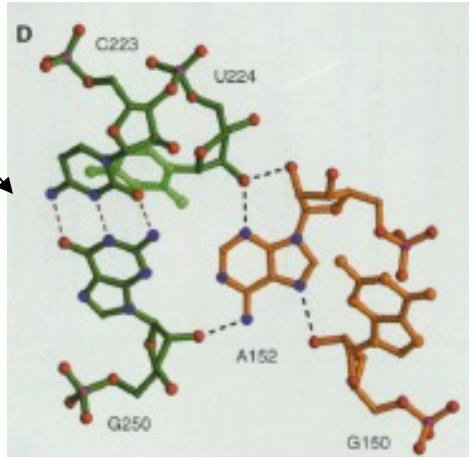
H bonding between two riboses



A-U reverse Hoogsteen



hoogsteen edge



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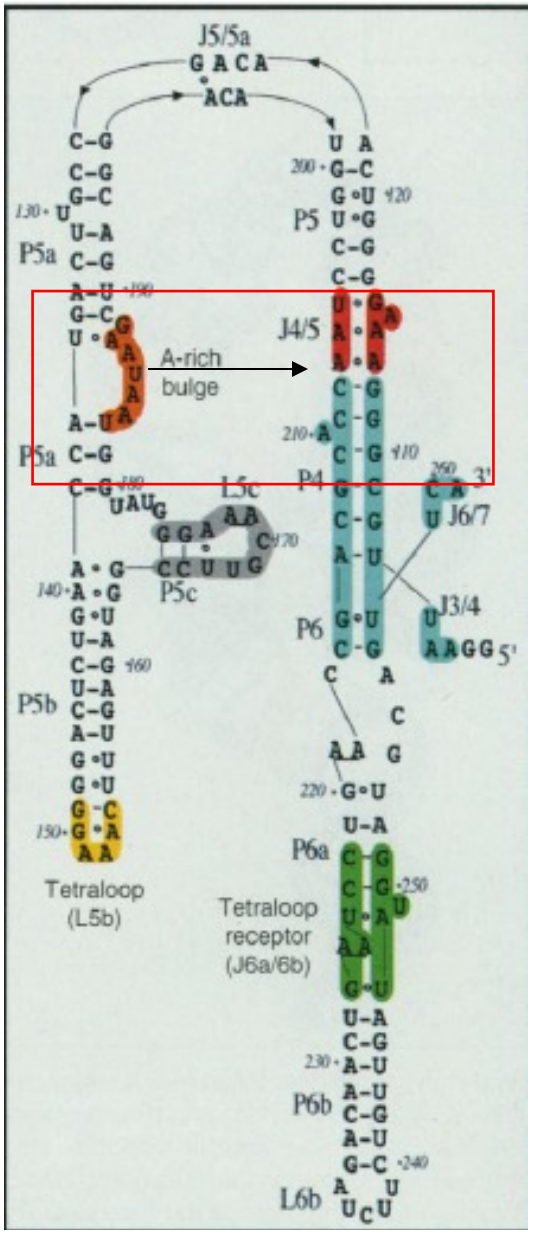
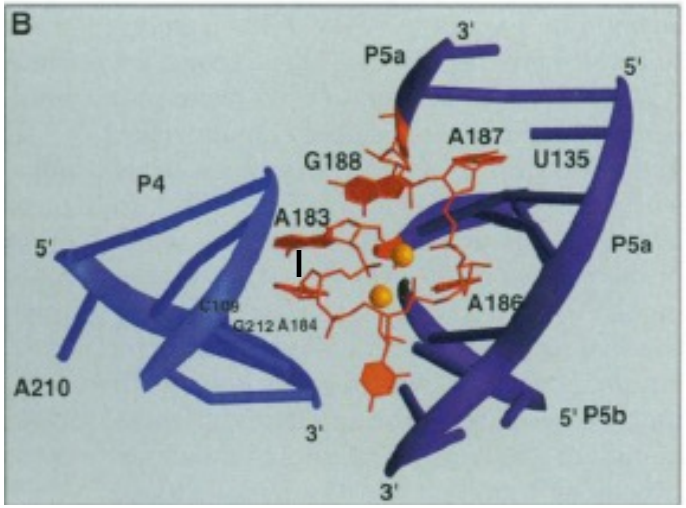


A-rich bulge interacts with minor groove of J4/5 and P4 helix



Bases flip out (AAUAA)

Adenosines are involved in stacking interactions and hydrogen bonding

How does A-rich interact with the minor groove?

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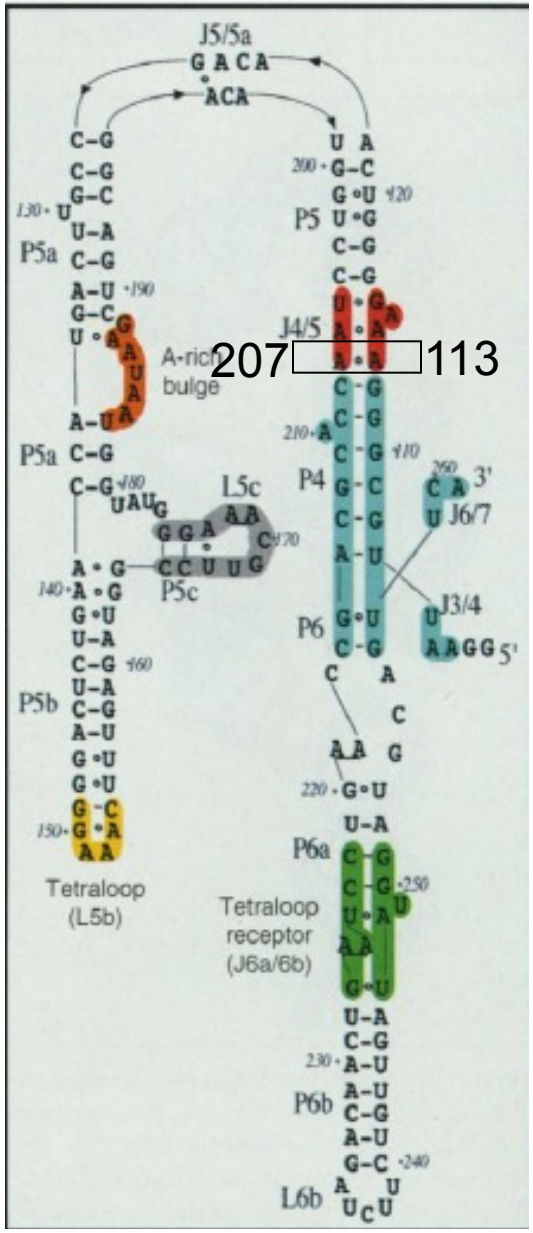
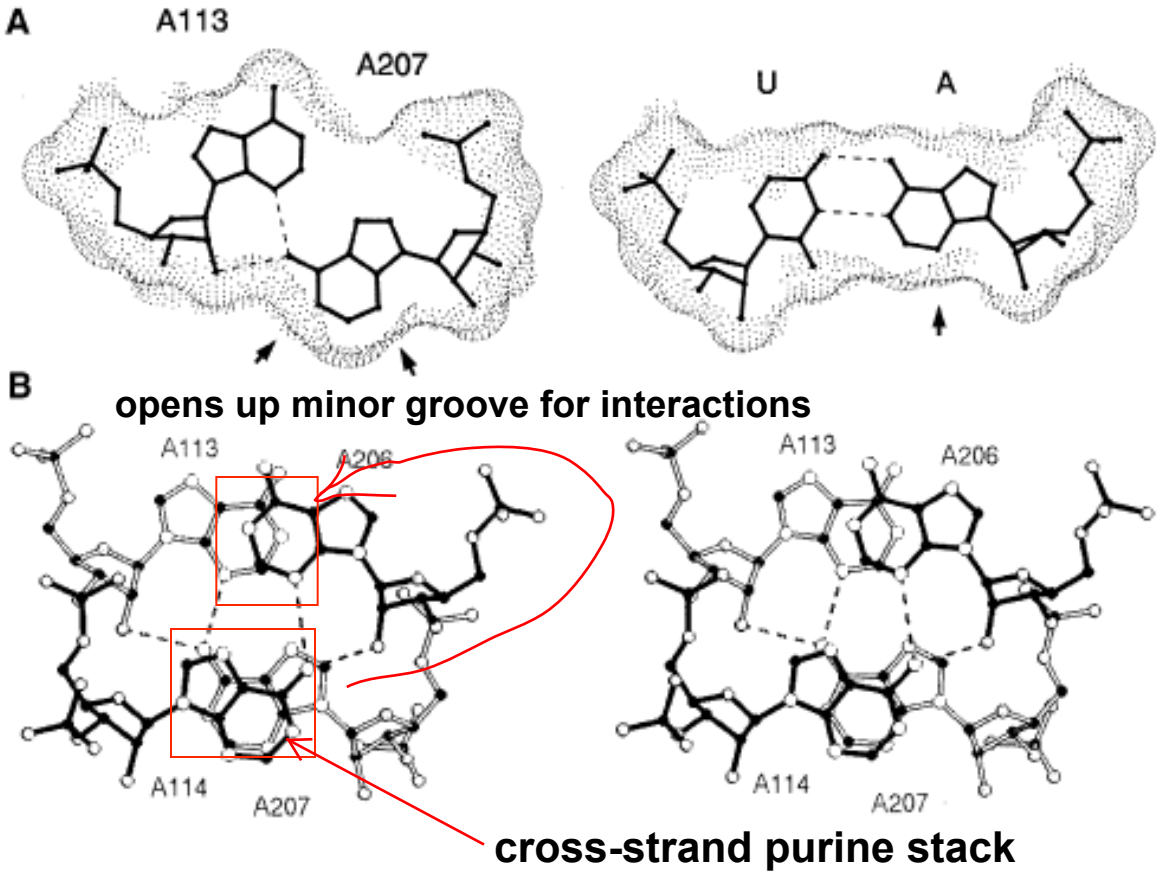
How does A-rich interact with the minor groove?



Sheared A-A bp **Standard A-U bp**



stack allow the top A's to interact

- Hydrogen bonding and stacking of bases stabilize this conformation....opens up minor groove

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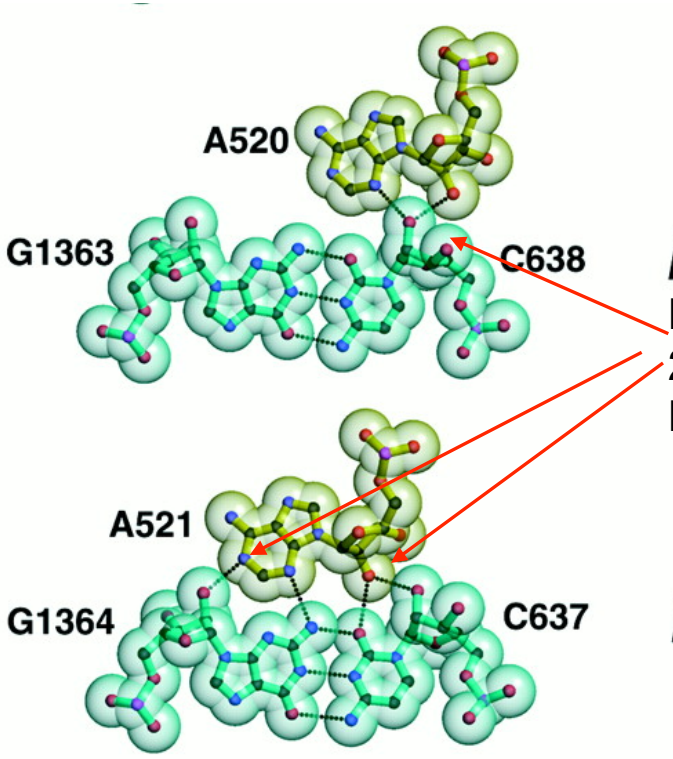
A-Minor Interactions

stick long distance interactions together

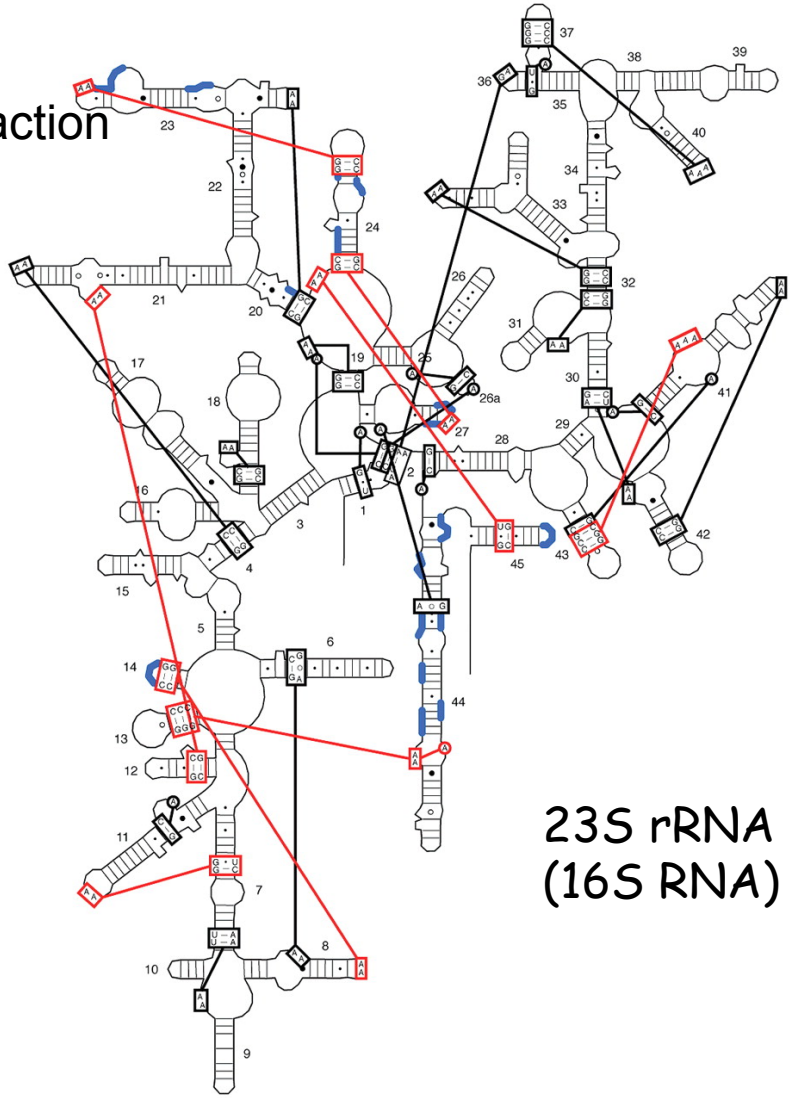
-Adenines inserted into the minor groove
-A-bulge interaction is a type of A-minor interaction
-Often in clusters of adenines and
can be in loop or bulges



//

Extensive
2'OH ribose
H bonding

/

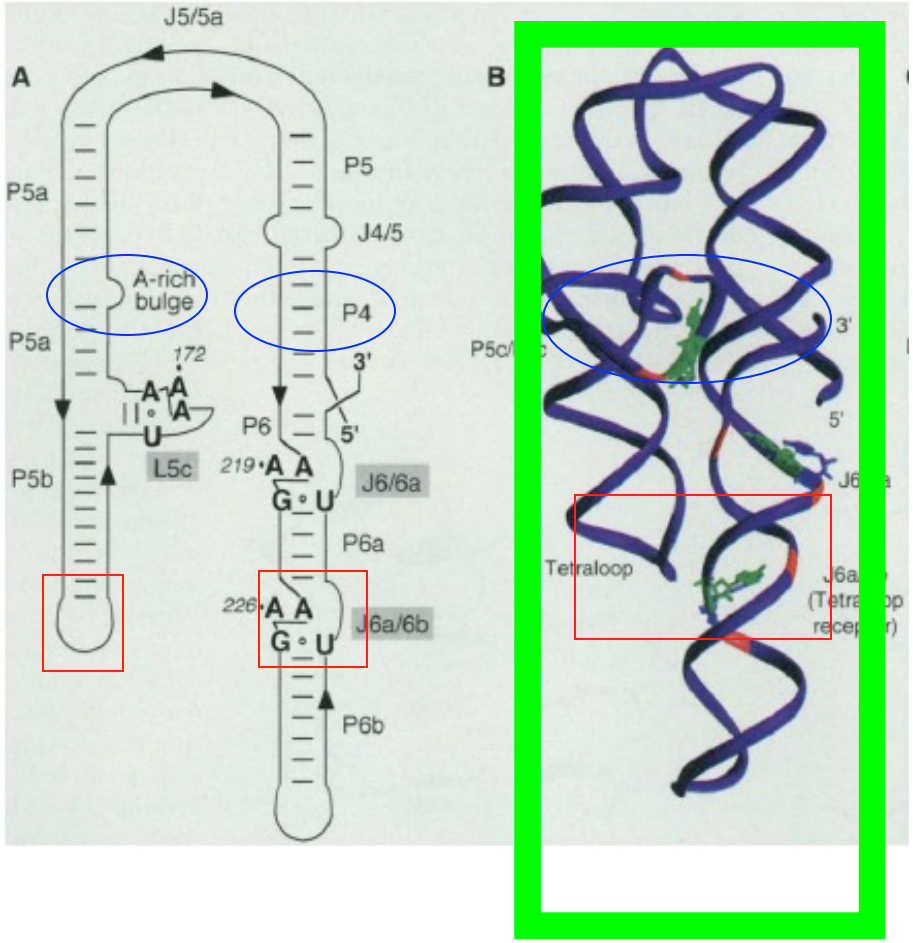


23S rRNA
(16S RNA)

A common motif in the 23S rRNA
Not WC basepairing

Group I intron P4-P6 domain

Helical stacks are arranged parallel to each other.

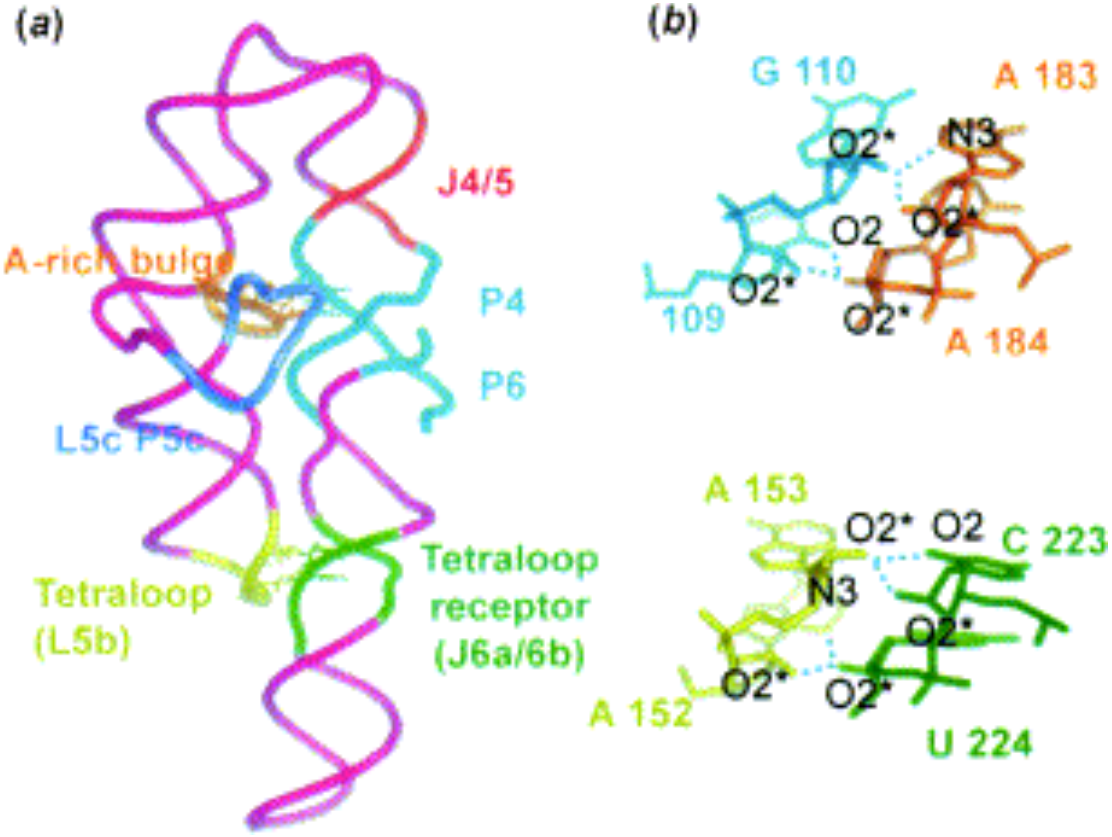


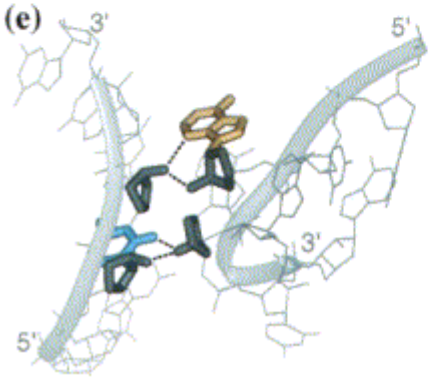
1) tetraloop/tetraloop receptor

2) A-rich, single-stranded loop and the minor groove of the opposing helix

3) Ribose zipper

Interactions result in tight packing of the helices
Ribose Zippers



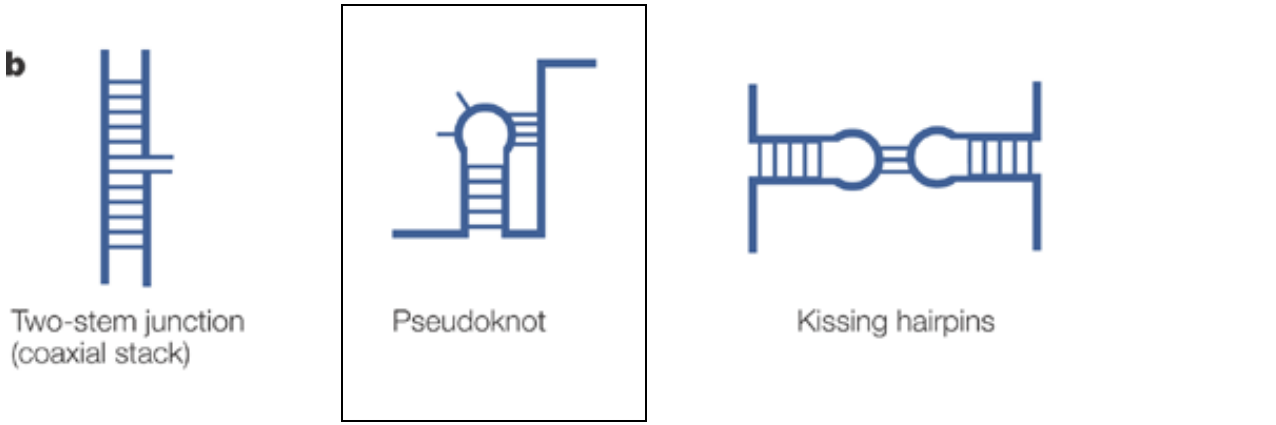


<u>Ribose zippers</u>: closely packed RNA strands, the 2'OH of ribose can hydrogen bond

Tertiary structures

- long distance interactions between RNA strands, normally between secondary structure motifs

tetraloops, A-minor interactions, ribose zippers



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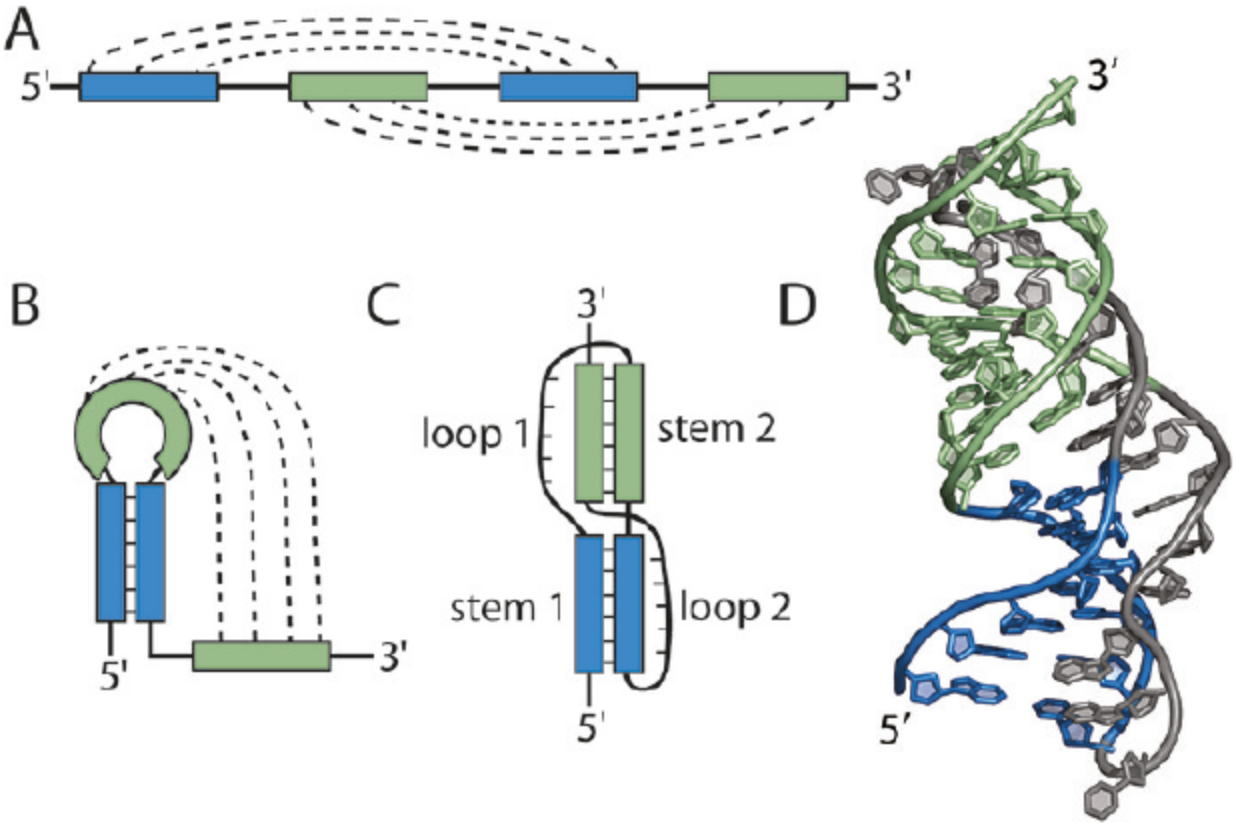
‘Pseudoknots’



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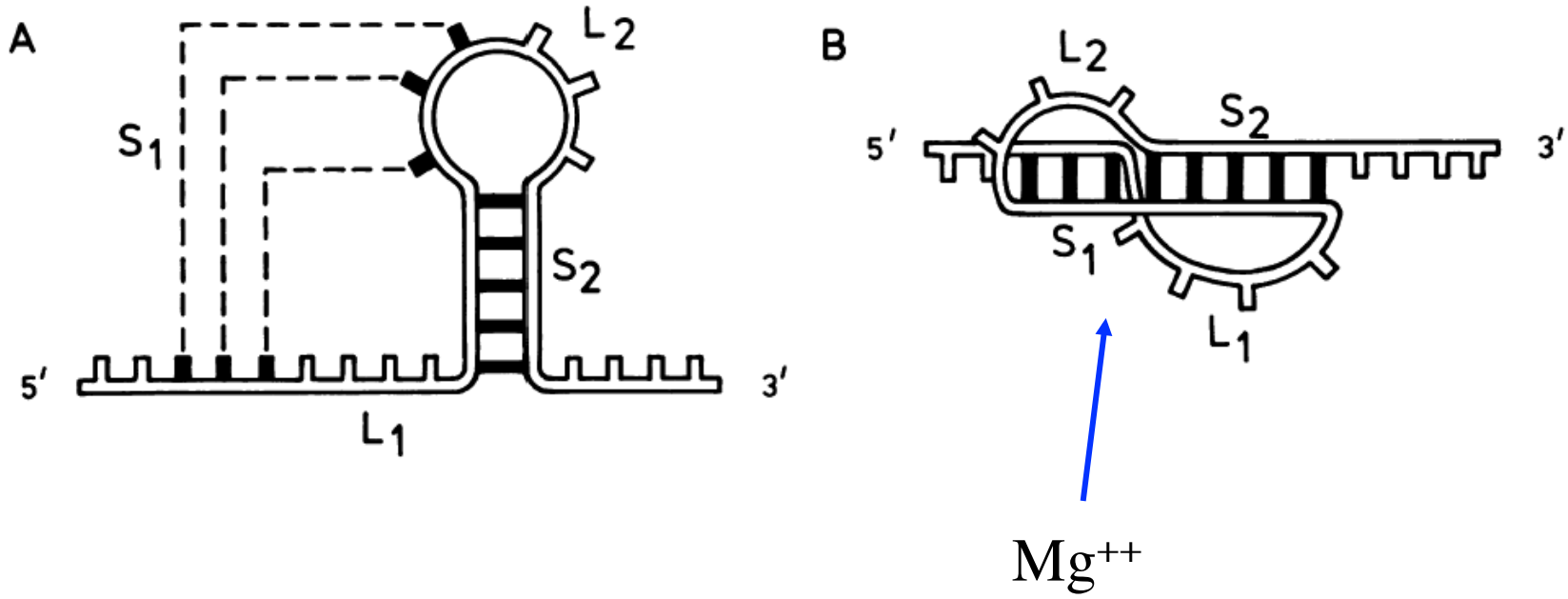
'Pseudoknots'



Found in rRNA, telomerase RNA

Also in viruses, important for translation (i.e. IRES), viral frameshifting

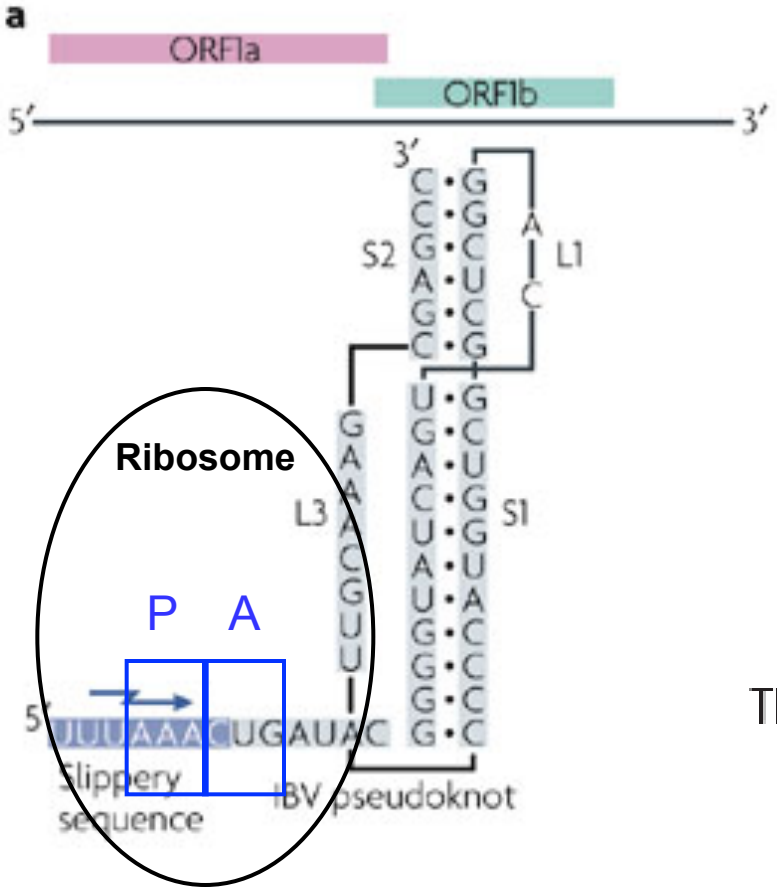
Viral RNA pseudoknots: versatile motifs in gene expression and replication

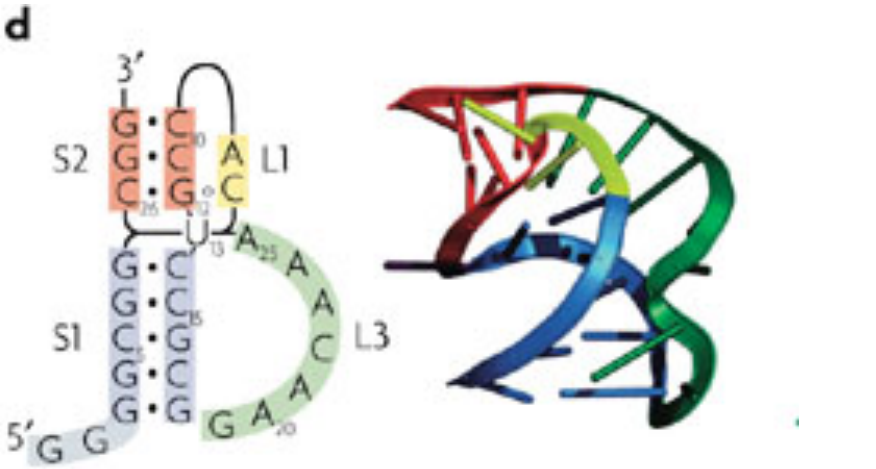
598 | AUGUST 2007 | VOLUME 5

Ian Brierley, Simon Pennell‡ and Robert J. C. Gilbert§*

Nature reviews Microbiology

Frameshift requires a ribosome pseudoknot and a slippery sequence





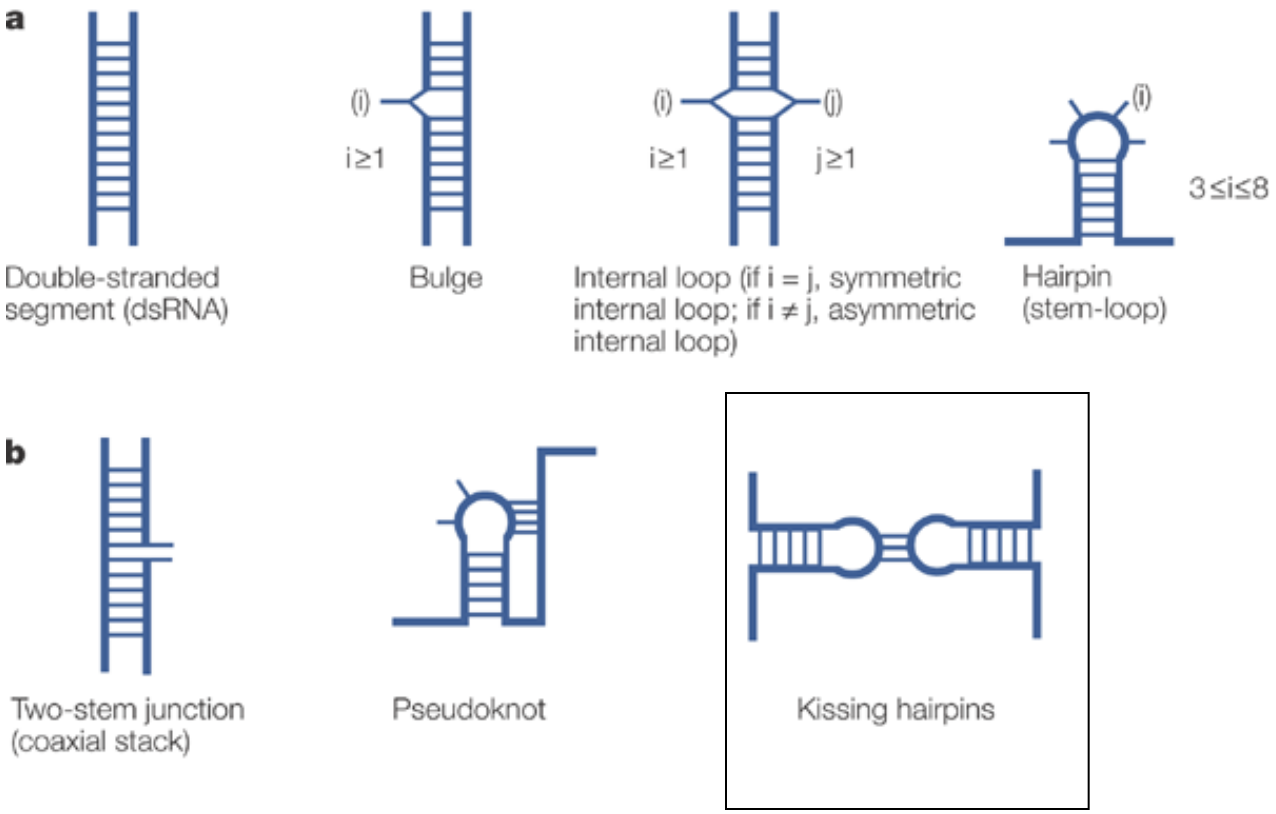
The beet western yellows virus (BWYV) pseudoknot

coronavirus Infectious Bronchitis Virus (IBV)
- efficient 14-27% frameshifting

Frameshift pseudoknots important for retroviruses (ex. HIV) and coronaviruses (ex. SARS)

Tertiary structures

- long distance interactions between RNA strands, normally between secondary structure motifs



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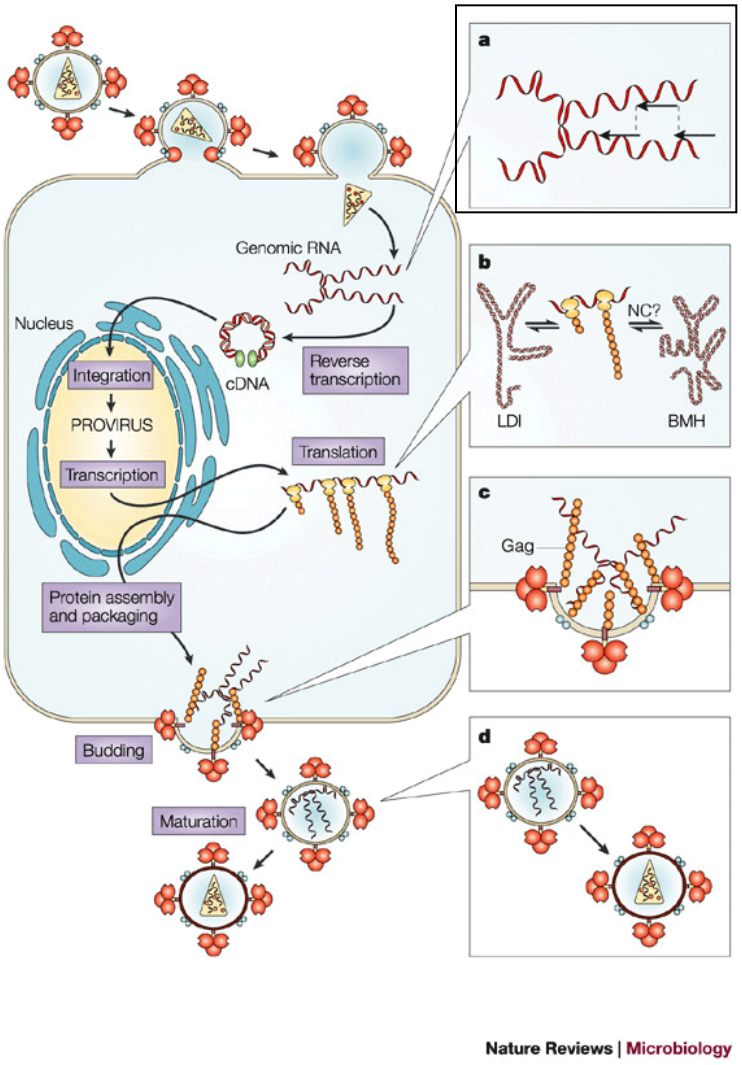
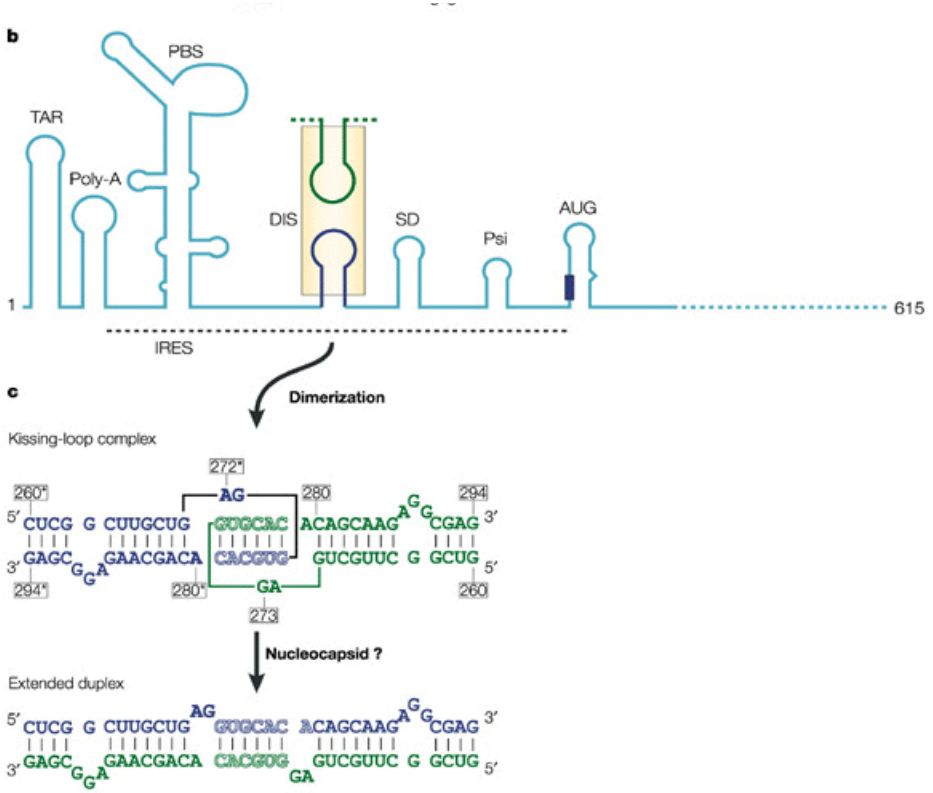
Kissing loop interactions involved in HIV life cycle



HIV dimerization required for replication, recombination, possibly translation



Kissing Loop Interactions important for HIV replication

Kissing hairpins

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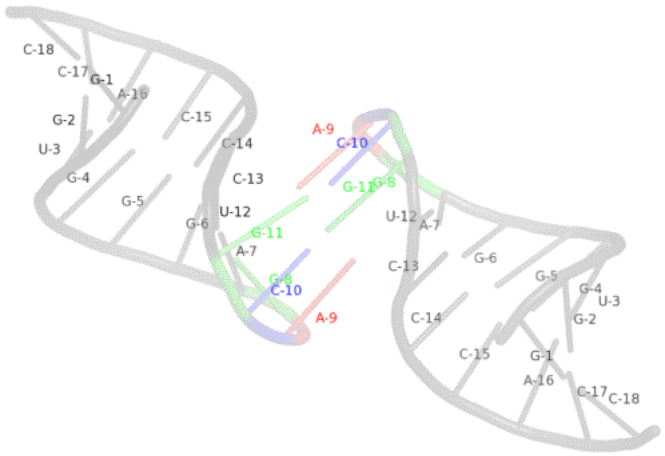
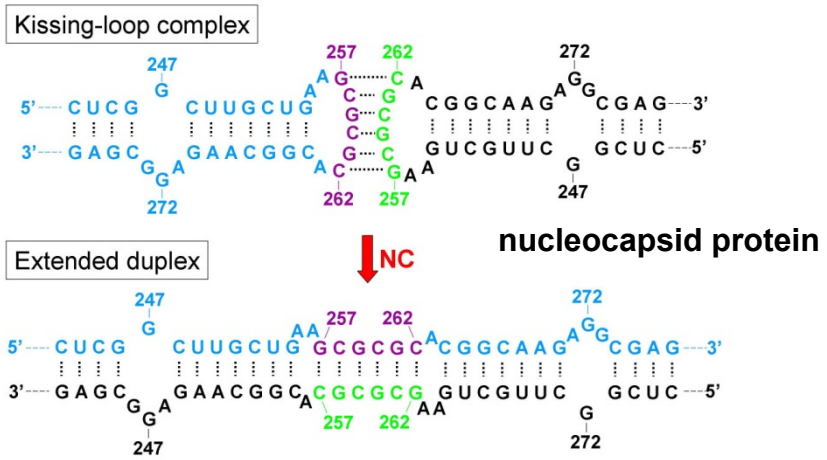
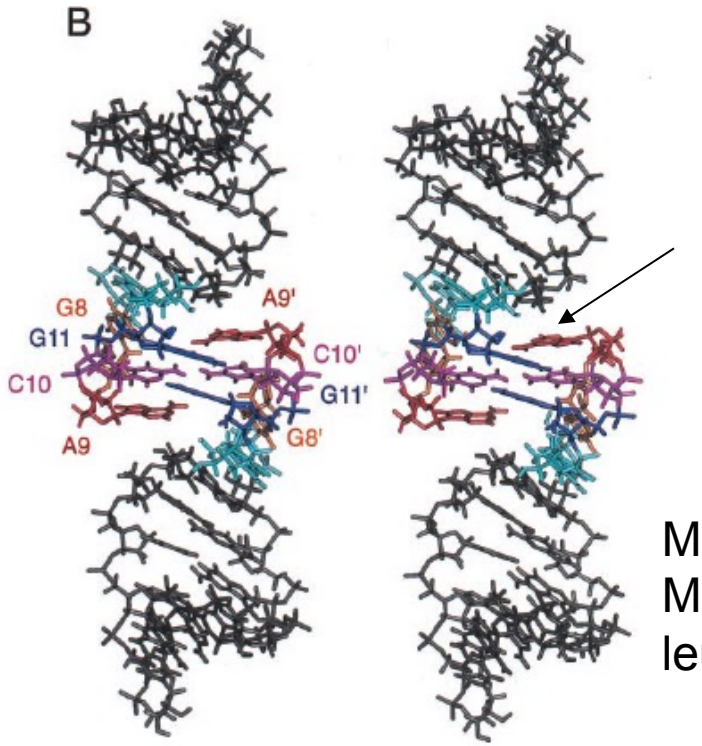


2 tetraloop interactions



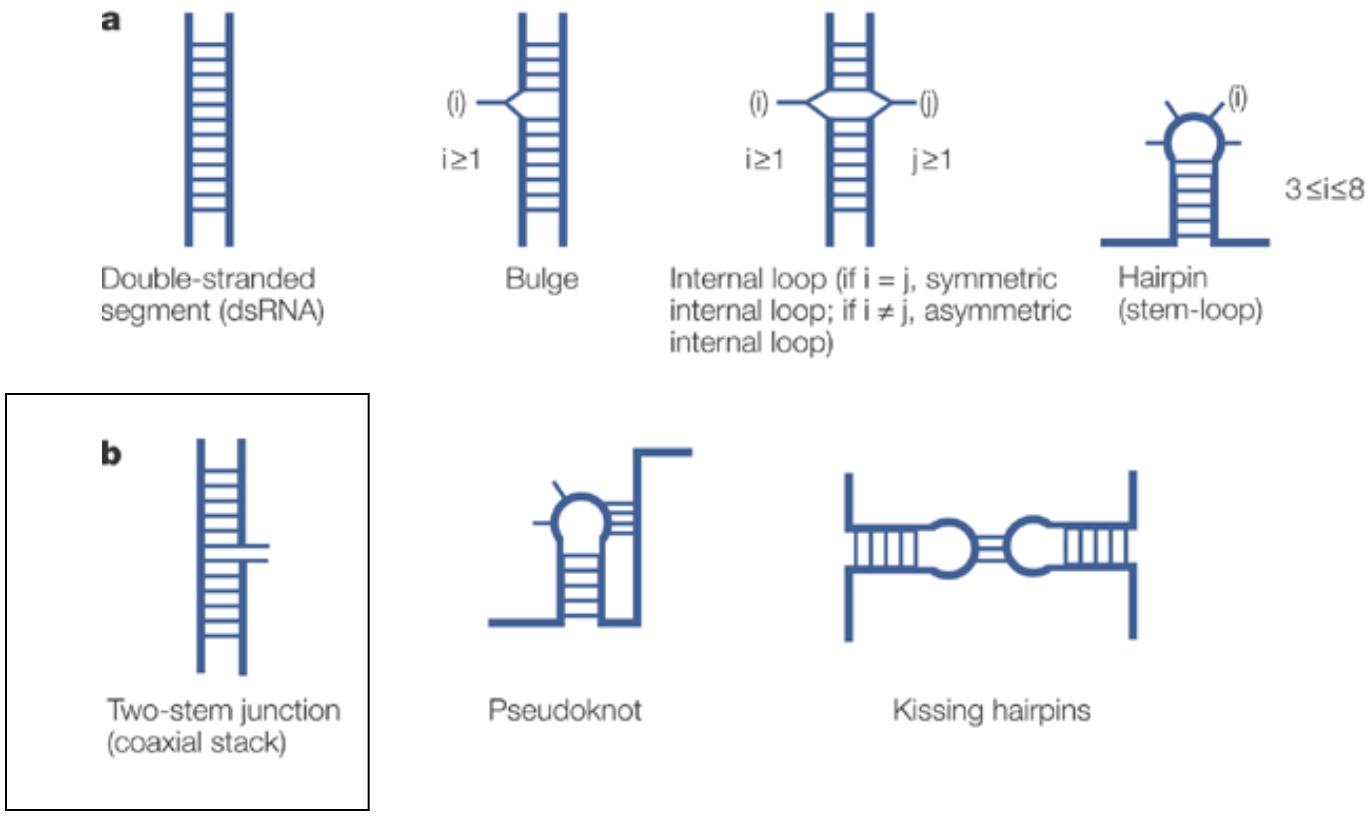


MMLV
Moloney murine
leukemia virus

C

Tertiary structures

- long distance interactions between RNA strands, normally between secondary structure motifs



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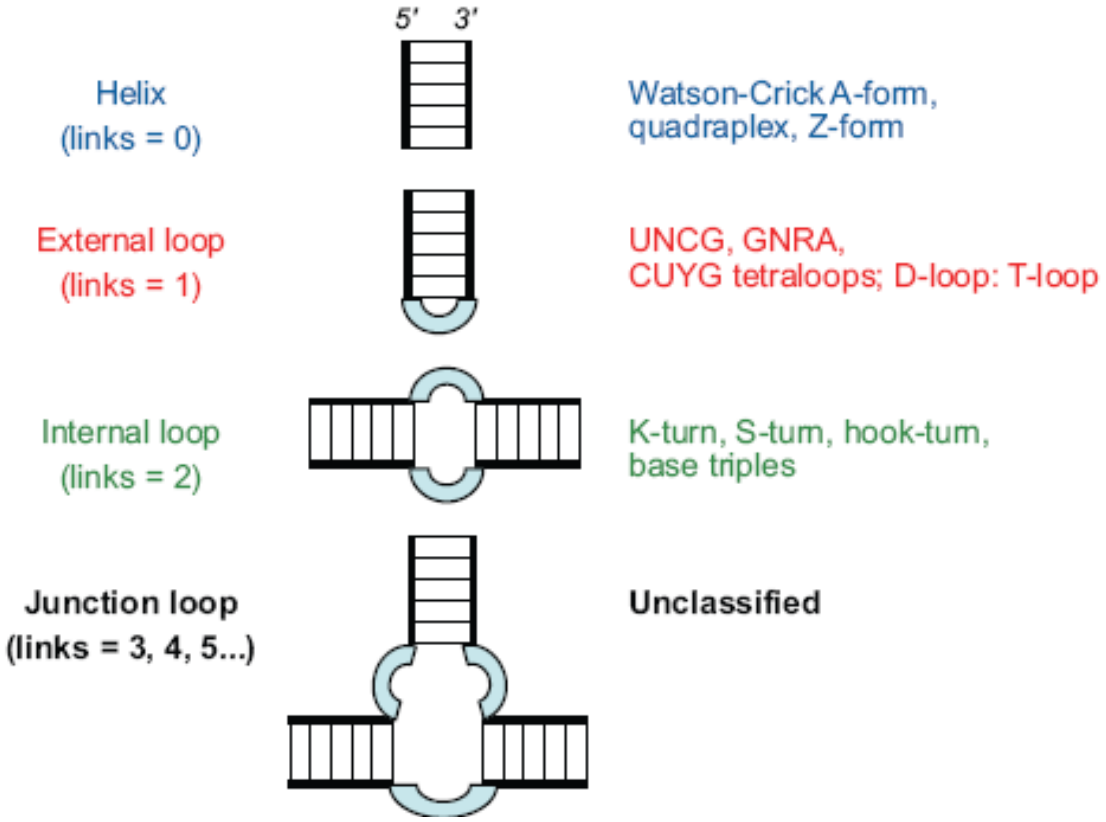


Coaxial Stacking

End-to-end stacking of two helices

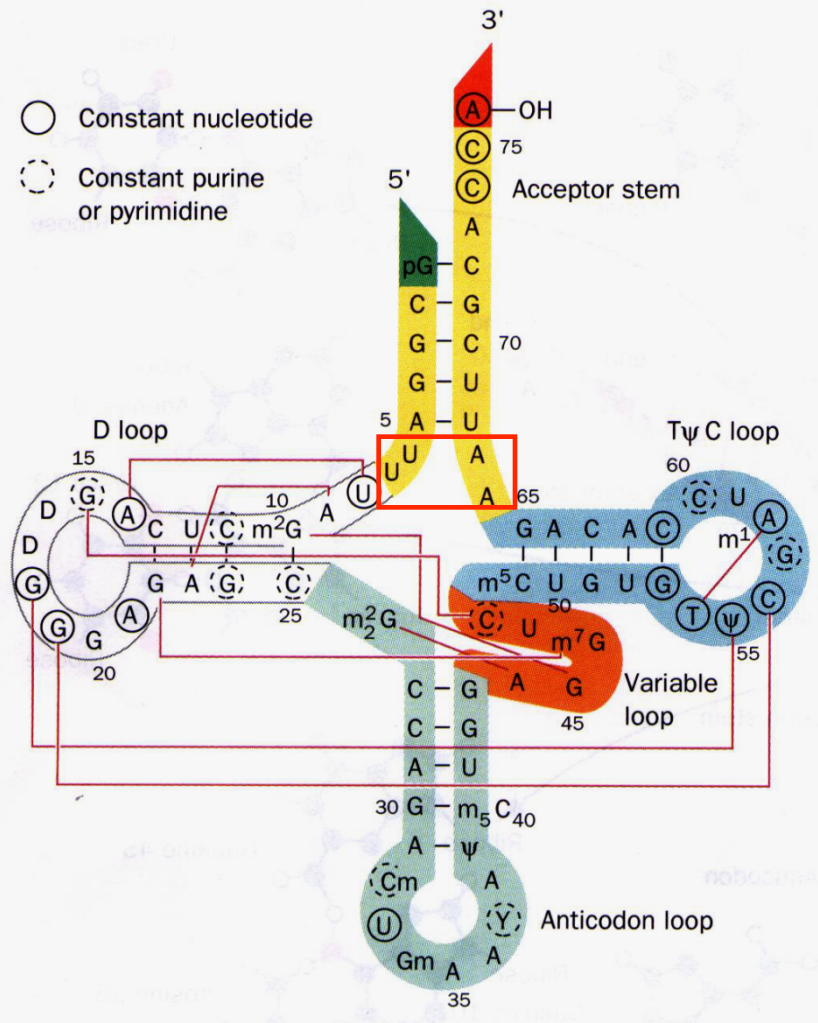
Major determinant for alignment of helices in large RNAs

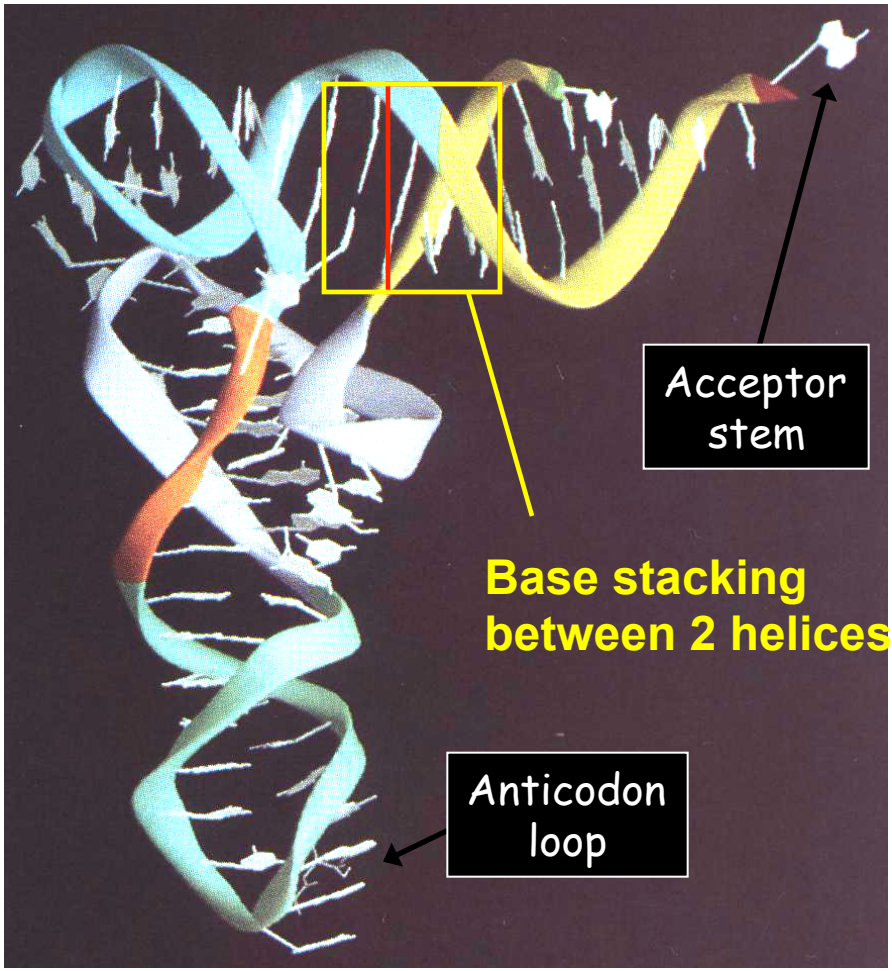
Most prevalent tertiary structure

Stabilized by WC bp, van der Waals and hydrophobic effects

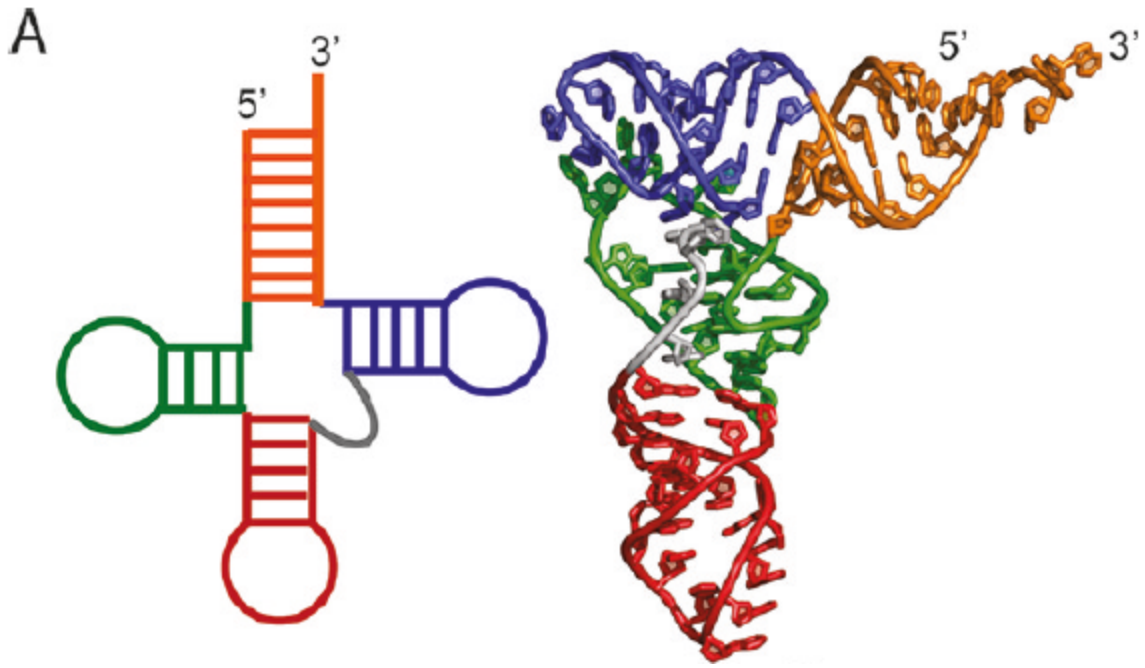
$tRNA^{Phe}$ Structure

Quigley and Rich, 1976





Coaxial interactions important for tRNA structure (continuous helix)
Mediated by stacking interactions, van der Waals, hydrophobic interactions



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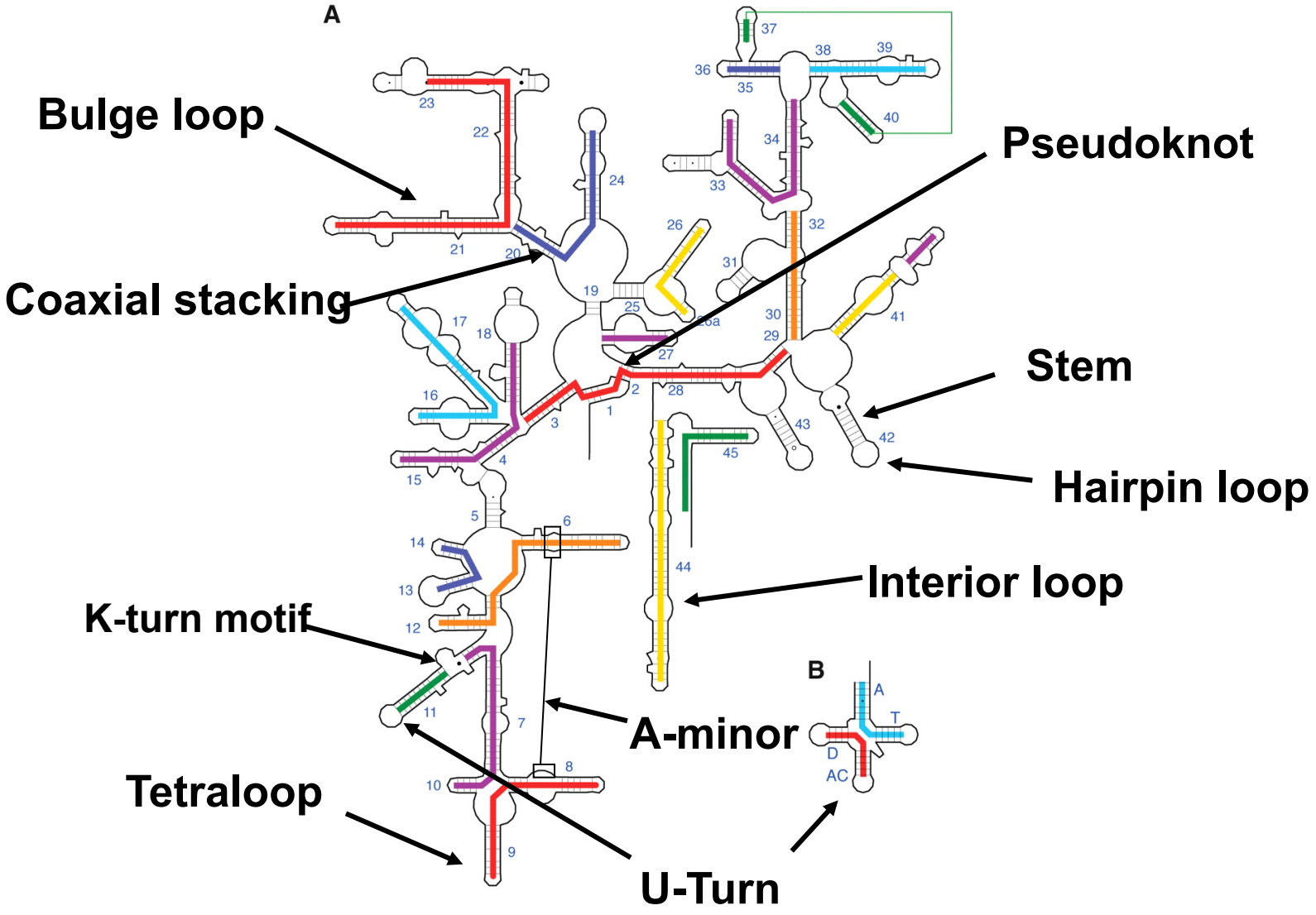
RNA Structural Motifs



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