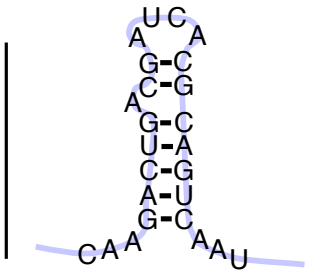
CSE 427
Winter 2008

RNA
Secondary Structure Prediction

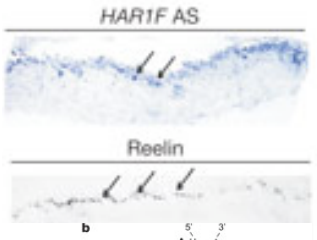
RNA Secondary Structure:
RNA makes helices too

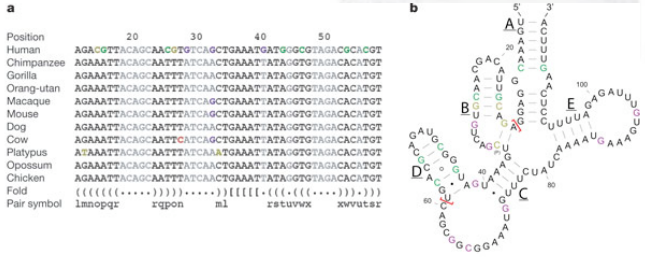
Base pairs

A-U
C-G



Fastest Human Gene?





Origin of Life?

Life needs

- information carrier: DNA
- molecular machines, like enzymes: Protein
- making proteins needs DNA + RNA + proteins
- making (duplicating) DNA needs proteins

Horrible circularities! How could it have arisen in an abiotic environment?

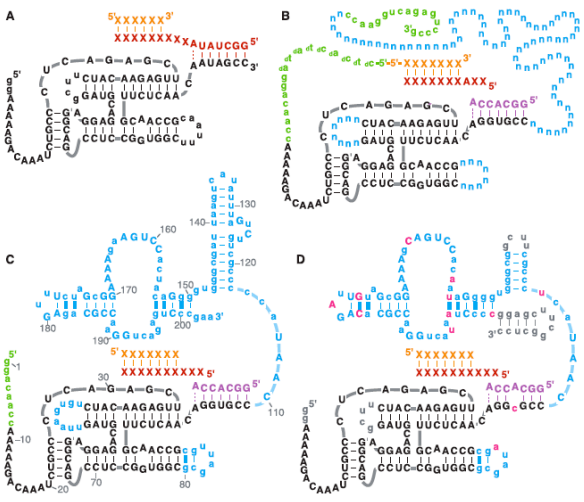
Origin of Life?

RNA can carry information too
(RNA double helix)

RNA can form complex structures

RNA enzymes exist (ribozymes)

The "RNA world" hypothesis:
1st life was RNA-based



Round	Mutagenesis	Template RNA	NTPs	Time (hour)	Selection criteria
1	Synthesis	3′-GGUCAGAUU	^{4S}UTP (2 mM)	36	^{4S}U
2	None	3′-GGUCAGAACC	^{4S}UTP (2 mM)	20	^{4S}U
3	None	3′-GGUCAGAA	^{4S}UTP (2 mM)	20	^{4S}U
4	None	3′-CUUAGUUCAUU	^{4S}UTP (2 mM)	19	^{4S}U
5	None	3′-CUUAGUUCAUU	^{4S}UTP (2 mM)	1	^{4S}U
6	None	3′-GGUCAGAUU	^{4S}UTP, ^{B}ATP (1 mM each)	14	^{B}A, ^{4S}U
7	None	3′-CUUAGUUCAUU	^{4S}UTP, ^{B}ATP (1 mM each)	17	^{B}A, ^{4S}U
8	None	3′-GGUCAGAUU	^{4S}UTP, ^{B}ATP (1 mM each)	17	^{B}A, ^{4S}U
9	None	3′-GGUCAGAUU	^{4S}UTP, ^{B}ATP (1 mM each)	4	^{B}A, ^{4S}U
10	None	3′-CUUAGUUCAUU	^{4S}UTP (1 mM)	20	^{4S}U
11	Synthesis	3′-UCGACGGAACC	^{4S}UTP (1 mM)	4	2 ^{4S}U
12	None	3′-ACCUGAGAAGG	^{4S}UTP (1 mM)	4	2 ^{4S}U
13	None	3′-CAAGUCCAACC	^{4S}UTP (1 mM)	0.2	2 ^{4S}U
14	None	3′-UCGACGGAACC	^{4S}UTP (1 mM)	0.2	2 ^{4S}U
15	PCR	3′-UCGACGG^{2N}p^{2N}PCCUGCGUC	^{4S}UTP (0.1 mM), Comp. NTPs	20	2 ^{4S}U
16	PCR	3′-CAAGUCC^{2N}p^{2N}PUGAUCGUA	^{4S}UTP (0.1 mM), Comp. NTPs	4	2 ^{4S}U
17	PCR	3′-ACCUGAG^{2N}p^{2N}PGUGUAUGU	^{4S}UTP (0.1 mM), Comp. NTPs	2	2 ^{4S}U
18	None	3′-UCGACGG^{2N}p^{2N}PCCUGCGUC	^{4S}UTP (0.1 mM), Comp. NTPs	0.1	2 ^{4S}U

Outline

Biological roles for RNA

What is "secondary structure?

How is it represented?

Why is it important?

Examples

Approaches

RNA Structure

Primary Structure: Sequence

Secondary Structure: Pairing

Tertiary Structure: 3D shape

RNA Pairing

Watson-Crick Pairing

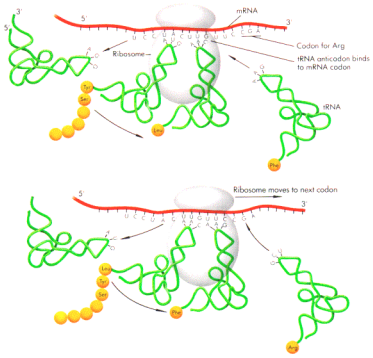
C - G ~ 3 kcal/mole

A - U ~ 2 kcal/mole

"Wobble Pair" G - U ~1 kcal/mole

Non-canonical Pairs (esp. if modified)

Ribosomes



Watson, Gilman, Witkowski, & Zoller, 1992

Ribosomes

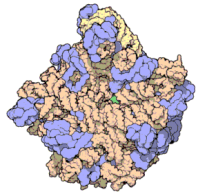
1974 Nobel prize to Romanian biologist George Palade for discovery in mid 50's

50-80 proteins

3-4 RNAs (half the mass)

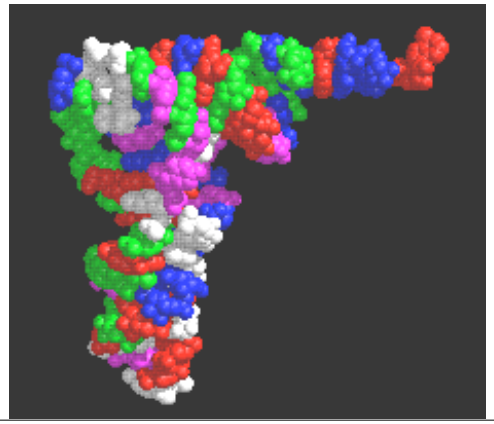
Catalytic core is RNA

Of course, mRNAs and tRNAs (messenger & transfer RNAs) are critical too

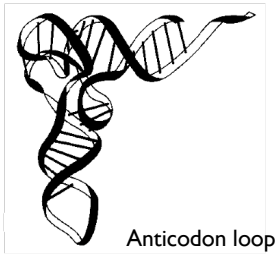


Atomic structure of the 50S Subunit from *Haloarcula marismortui*. Proteins are shown in blue and the two RNA strands in orange and yellow. The small patch of green in the center of the subunit is the active site.
- Wikipedia

tRNA 3d Structure



tRNA - Alt. Representations



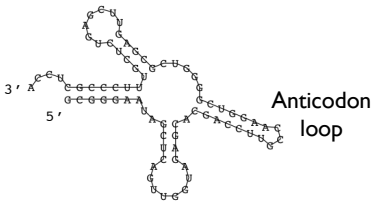
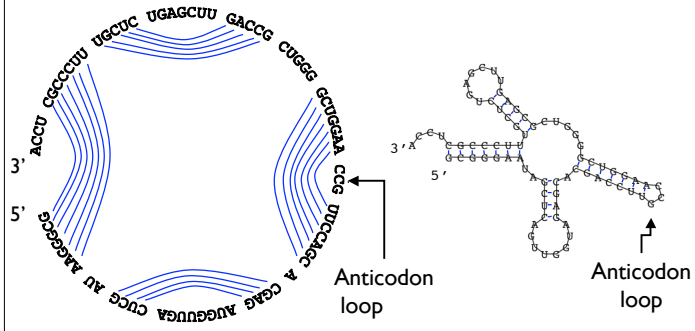


Figure 1: a) The spatial structure of the phenylalanine tRNA form yeast

b) The secondary structure extracts the most important information about the structure, namely the pattern of base pairings.

tRNA - Alt. Representations



"Classical" RNAs

tRNA - transfer RNA (~61 kinds, ~ 75 nt)

rRNA - ribosomal RNA (~4 kinds, 120-5k nt)

snRNA - small nuclear RNA (splicing: U1, etc, 60-300nt)

RNaseP - tRNA processing (~300 nt)

RNase MRP - rRNA processing; mito. rep. (~225 nt)

SRP - signal recognition particle; membrane targeting (~100-300 nt)

SECIS - selenocysteine insertion element (~65nt)

6S - ? (~175 nt)

Semi-classical RNAs
(discovery in mid 90's)

tmRNA - resetting stalled ribosomes

Telomerase - (200-400nt)

snoRNA - small nucleolar RNA (many varieties; 80-200nt)

Recent discoveries

microRNAs (Nobel prize 2006, Fire & Mello)

riboswitches

many ribozymes

regulatory elements

…

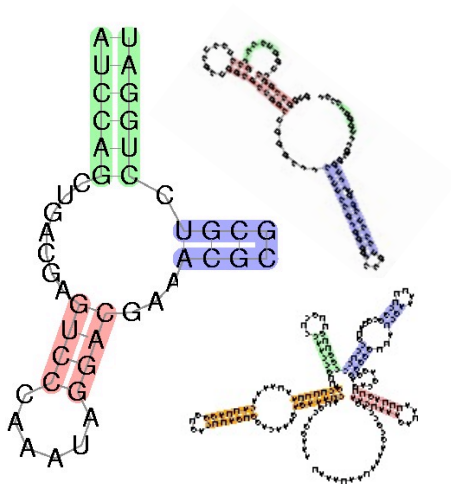
Hundreds of families

Rfam release 1, 1/2003: 25 families, 55k instances
Rfam release 7, 3/2005: 503 families, 300k instances

Why?

RNA's fold, and function

Nature uses what works



Noncoding RNAs

Dramatic discoveries in last 5 years

100s of new families

Many roles: Regulation, transport, stability, catalysis, …

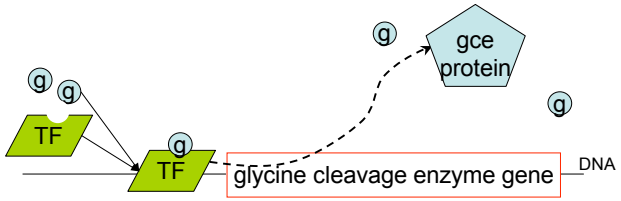
1% of DNA codes for protein, but 90% of it is copied into RNA, i.e. ncRNA >> mRNA

Significance unclear, controversial

Example: Glycine Regulation

How is glycine level regulated?

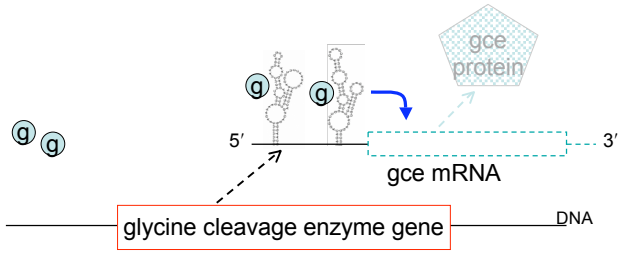
Plausible answer:



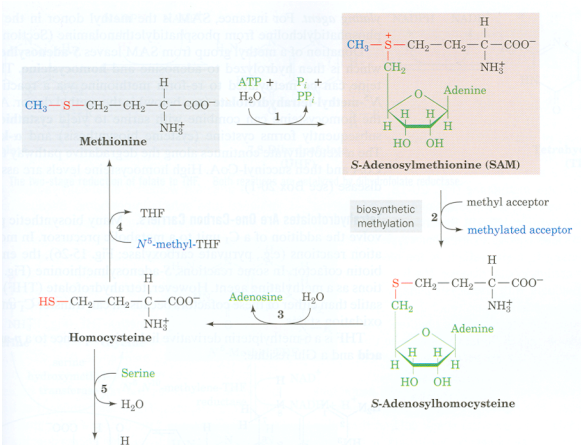
transcription factors (proteins) bind to DNA to turn nearby genes on or off

The Glycine Riboswitch

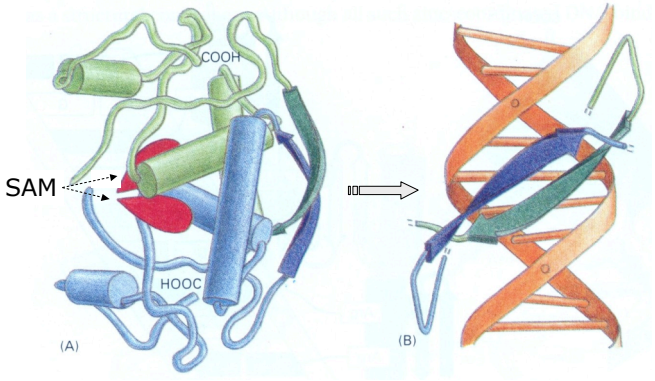
Actual answer (in many bacteria):



Mandal et al. Science 2004



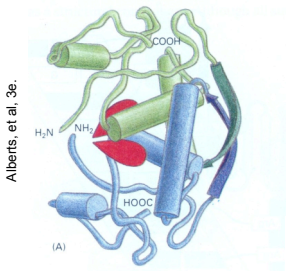
Gene Regulation: The MET Repressor



Protein

Alberts, et al, 3e.

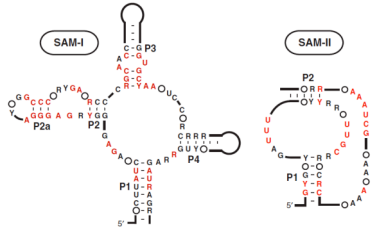
DNA



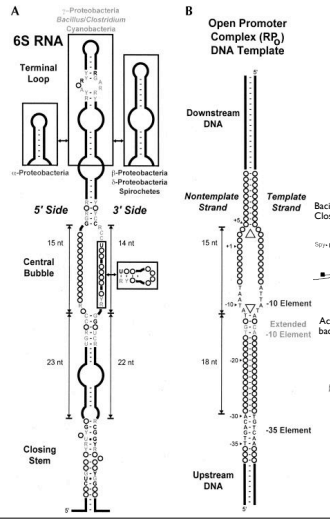
Alberts, et al, 3e.

The protein way

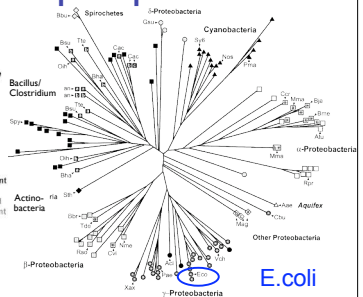
Riboswitch alternatives



Corbino et al., Genome Biol. 2005



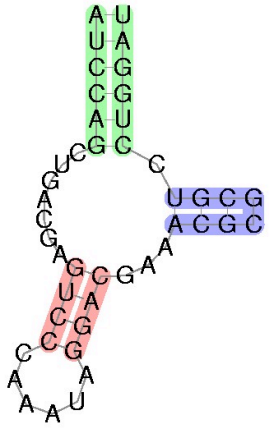
6S mimics an open promoter



Barrick et al. *RNA* 2005
Trotochaud et al. *NSMB* 2005
Willkomm et al. NAR 2005

The Hammerhead Ribozyme

Involved in "rolling circle replication" of viruses.

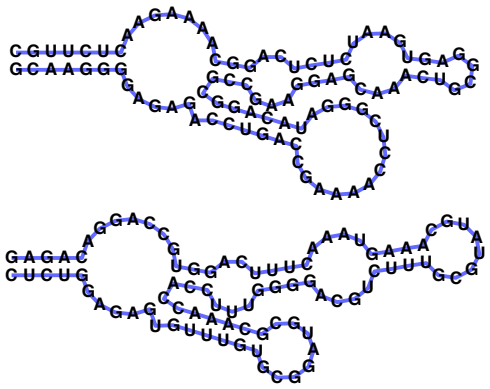


Wanted

Good structure prediction tools
Good motif descriptions/models
Good, fast search tools
("RNA BLAST", etc.)
Good, fast motif discovery tools
("RNA MEME", etc.)

Importance of structure makes last 3 hard

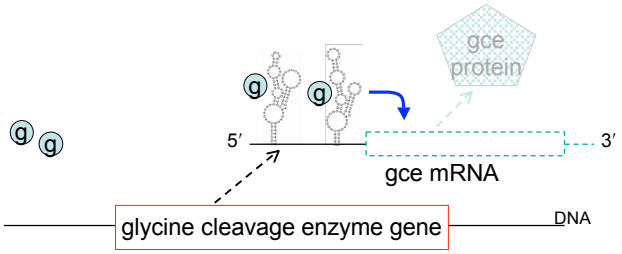
Why is RNA hard to deal with?



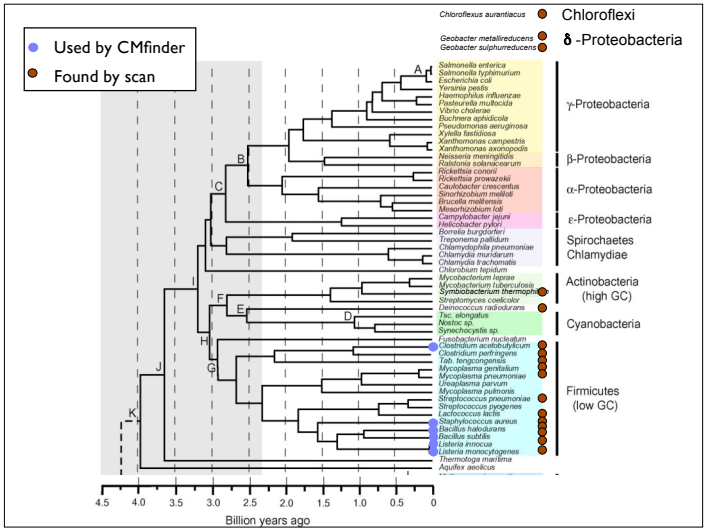
A: *Structure* often more important than *sequence*

The Glycine Riboswitch

Actual answer (in many bacteria):



Mandal et al. Science 2004



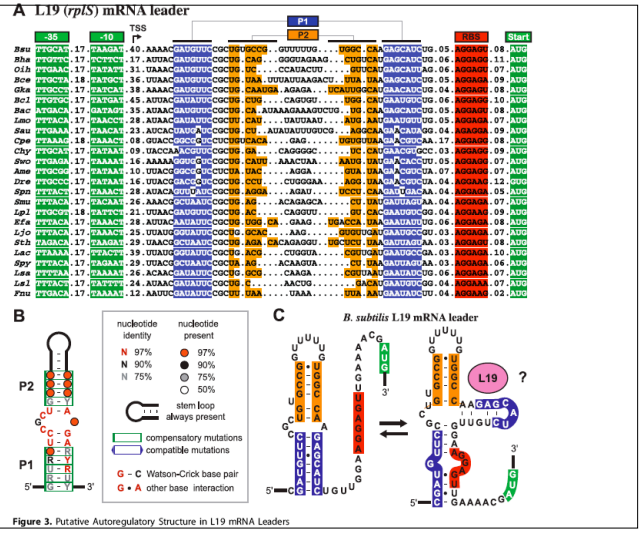
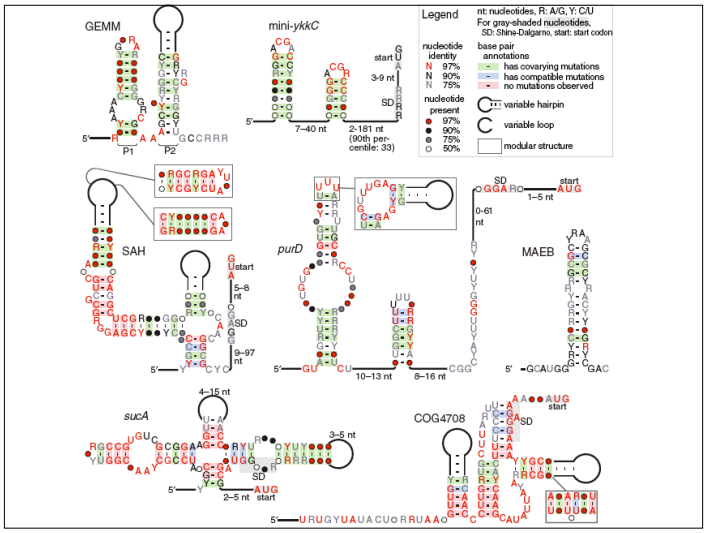


Figure 3. Putative Autoregulatory Structure in L19 mRNA Leaders



Task 1:
Structure Prediction

RNA Pairing

Watson-Crick Pairing

C - G ~ 3 kcal/mole

A - U ~ 2 kcal/mole

"Wobble Pair" G - U ~ 1 kcal/mole

Non-canonical Pairs (esp. if modified)

Definitions

Sequence $^{5'}\ r_1\ r_2\ r_3 \ldots r_n\ ^{3'}$ in {A, C, G, T}

A Secondary Structure is a set of pairs i•j s.t.

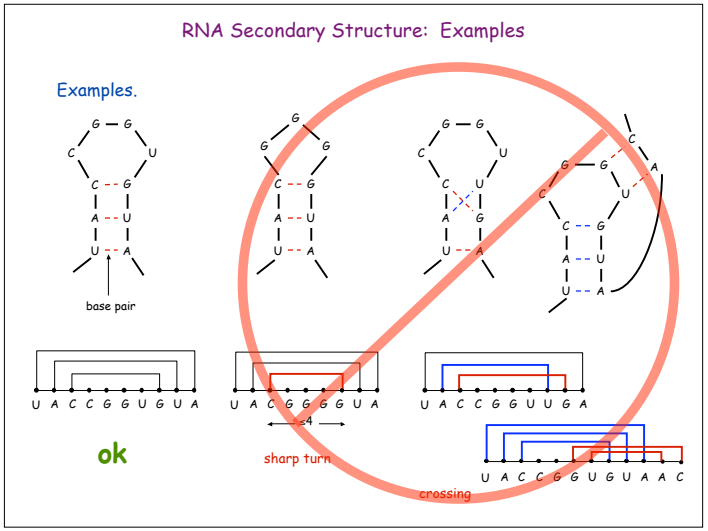
i < j-4, and } no sharp turns

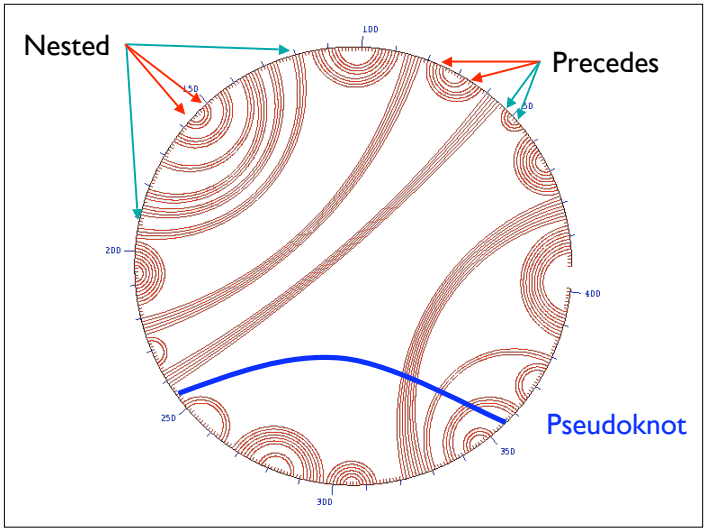
if i•j & i'•j' are two different pairs with i ≤ i', then

j < i', or

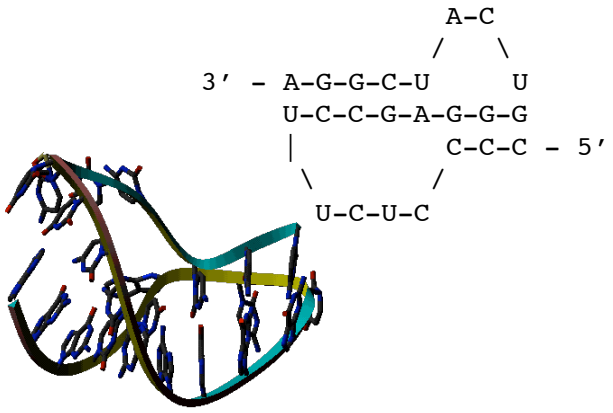
i < i' < j' < j

} 2nd pair follows 1st, or is nested within it; no "pseudoknots."





A Pseudoknot



Approaches to Structure Prediction

Maximum Pairing
- \+ works on single sequences
- \+ simple
- \- too inaccurate

Minimum Energy
- \+ works on single sequences
- \- ignores pseudoknots
- \- only finds "optimal" fold

Partition Function
- \+ finds all folds
- \- ignores pseudoknots

RNA Secondary Structure (somewhat oversimplified)

Secondary structure. A set of pairs $S = \{ (b_i, b_j) \}$ that satisfy:

- [Watson-Crick.]
  - S is a *matching* , i.e. each base pairs with at most one other, and
  - each pair in S is a Watson-Crick pair: A-U, U-A, C-G, or G-C.
- [No sharp turns.] The ends of each pair are separated by at least 4 intervening bases. If $(b_i, b_j) \in S$, then $i < j - 4$.
- [Non-crossing.] If (b_i, b_j) and (b_k, b_l) are two pairs in S, then we cannot have $i < k < j < l$. (Violation of this is called a *pseudoknot*.)

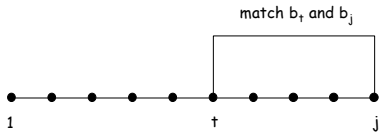
Free energy. Usual hypothesis is that an RNA molecule will form the secondary structure with the optimum total free energy.

approximate by number of base pairs

Goal. Given an RNA molecule $B = b_1 b_2 \ldots b_n$, find a secondary structure S that maximizes the number of base pairs.

RNA Secondary Structure: Subproblems

First attempt. OPT[j] = maximum number of base pairs in a secondary structure of the substring $b_1 b_2 \ldots b_j$.



Difficulty. Results in two sub-problems.

- Finding secondary structure in: $b_1 b_2 \ldots b_{t-1}$. ← OPT(t-1)
- Finding secondary structure in: $b_{t+1} b_{t+2} \ldots b_{j-1}$. ← not OPT of anything; need more sub-problems

Dynamic Programming Over Intervals: (R. Nussinov's algorithm)

Notation. OPT[i, j] = maximum number of base pairs in a secondary structure of the substring $b_i b_{i+1} \ldots b_j$.

- Case 1. If $i \geq j - 4$.
  - OPT[i, j] = 0 by no-sharp turns condition.
- Case 2. Base b_j is not involved in a pair.
  - OPT[i, j] = OPT[i, j-1]
- Case 3. Base b_j pairs with b_t for some $i \leq t < j - 4$.
  - non-crossing constraint decouples resulting sub-problems
  - $OPT[i, j] = 1 + \max_t \{ OPT[i, t-1] + OPT[t+1, j-1] \}$

take max over t such that $i \leq t < j-4$ and b_t and b_j are Watson-Crick complements

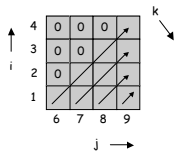
Key point: Either last base is unpaired (case 1,2) or paired (case 3)

Remark. Same core idea in CKY algorithm to parse context-free grammars.

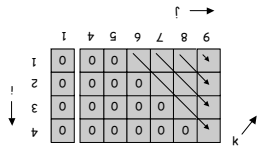
Bottom Up Dynamic Programming Over Intervals

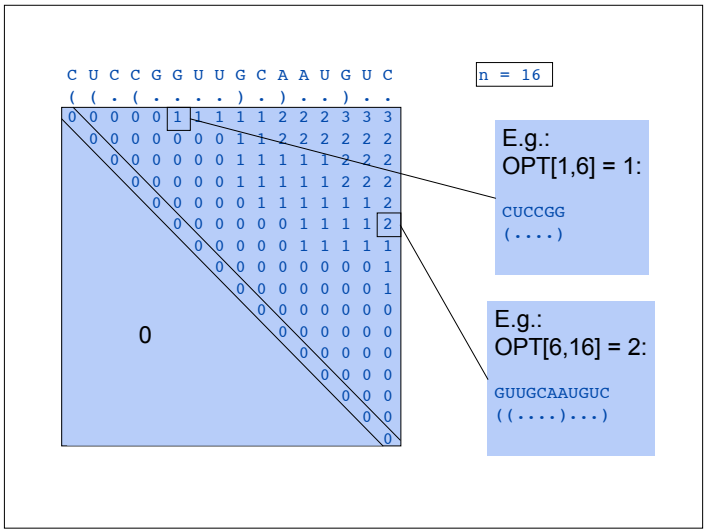
Q. What order to solve the sub-problems?
A. Do shortest intervals first.

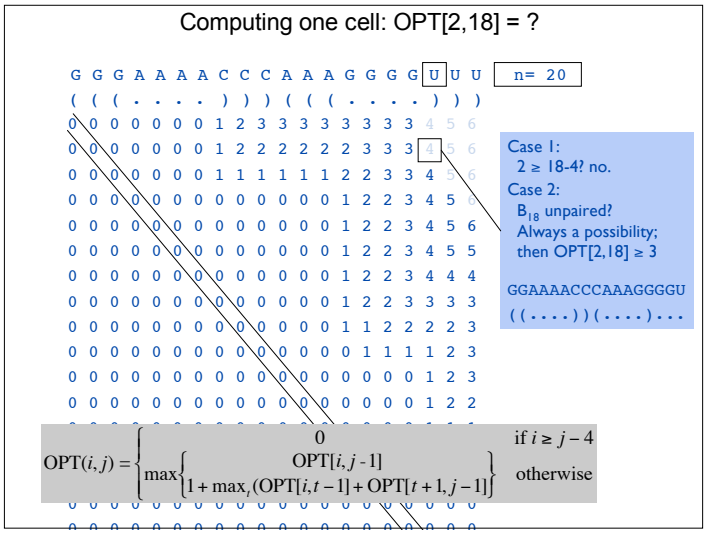
```
RNA(b1,…,bn) {
   for k = 5, 6, …, n-1
      for i = 1, 2, …, n-k
         j = i + k
         Compute OPT[i, j]
                          ↖ using recurrence
   return OPT[1, n]
}
```

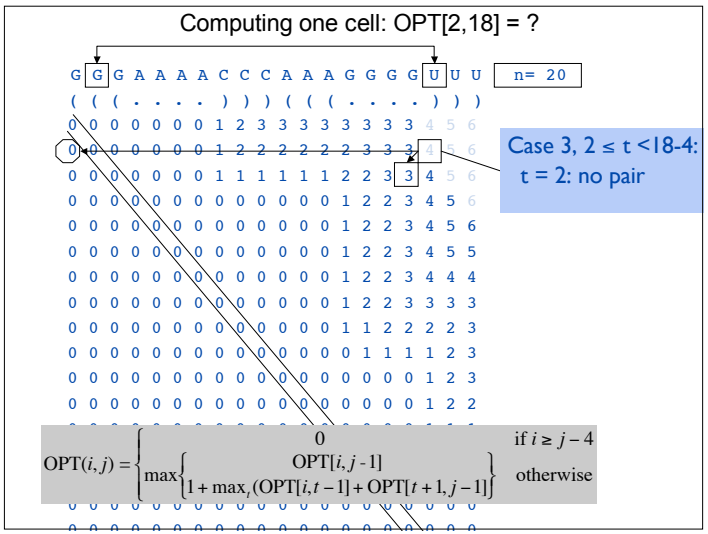


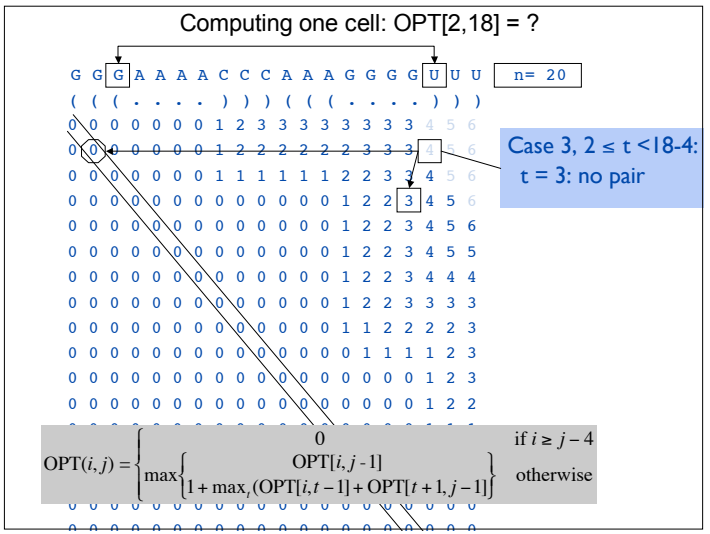
Running time. $O(n^3)$.



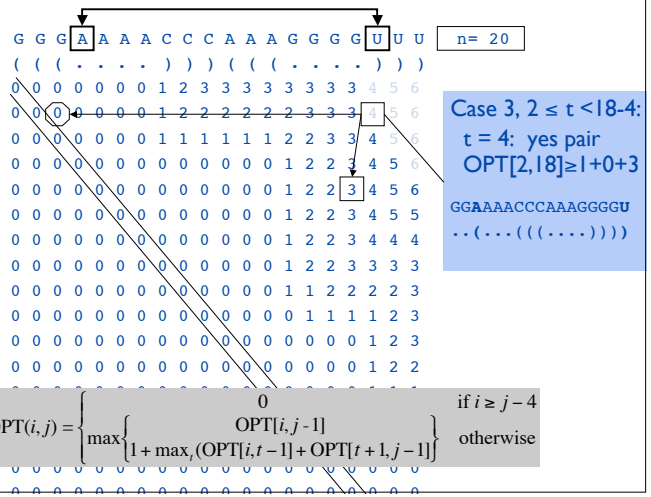

C U C C G G U U G C A A U G U C
((. (. . . .) .) . .) . .
n = 16
0
E.g.:
OPT[1,6] = 1:
CUCCGG
(....)
E.g.:
OPT[6,16] = 2:
GUUGCAAUGUC
((....)...)


Computing one cell: OPT[2,18] = ?
G G G A A A A C C C A A A G G G G U U U
n= 20
(((. . . .))) (((. . . .)))
Case 1:
2 ≥ 18-4? no.
Case 2:
B18 unpaired?
Always a possibility;
then OPT[2,18] ≥ 3
GGAAAACCCAAAGGGGU
((....))(....)...
OPT(i, j) = { 0 if i ≥ j − 4
max{ OPT[i, j-1] ; 1 + max_t (OPT[i, t−1] + OPT[t+1, j−1]) } otherwise

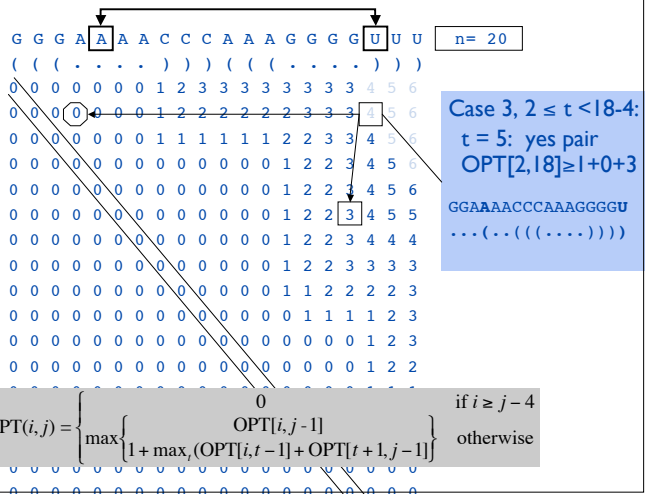

Computing one cell: OPT[2,18] = ?
G G G A A A A C C C A A A G G G G U U U
n= 20
(((. . . .))) (((. . . .)))
Case 3, 2 ≤ t <18-4:
t = 2: no pair
OPT(i, j) = { 0 if i ≥ j − 4
max{ OPT[i, j-1] ; 1 + max_t (OPT[i, t−1] + OPT[t+1, j−1]) } otherwise


Computing one cell: OPT[2,18] = ?
G G G A A A A C C C A A A G G G G U U U
n= 20
(((. . . .))) (((. . . .)))
Case 3, 2 ≤ t <18-4:
t = 3: no pair
OPT(i, j) = { 0 if i ≥ j − 4
max{ OPT[i, j-1] ; 1 + max_t (OPT[i, t−1] + OPT[t+1, j−1]) } otherwise

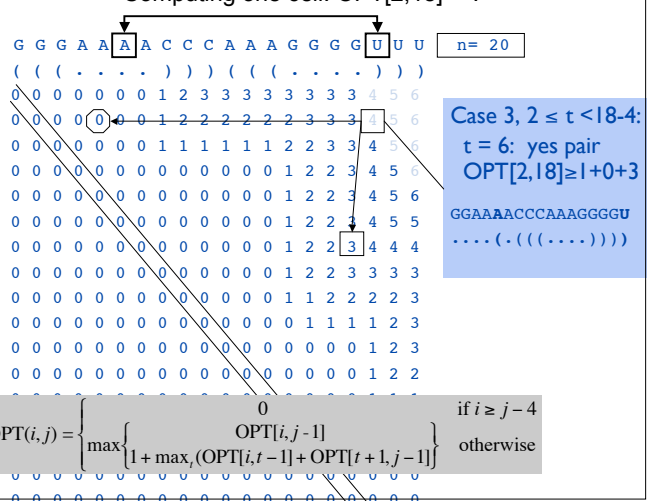
Computing one cell: OPT[2,18] = ?



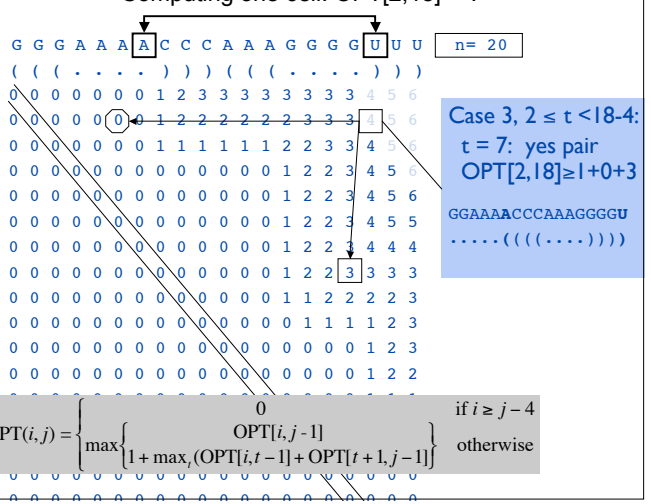
Computing one cell: OPT[2,18] = ?



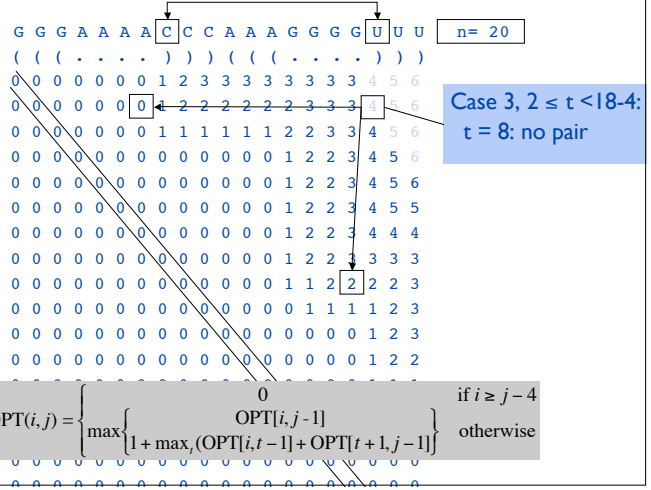
Computing one cell: OPT[2,18] = ?



Computing one cell: OPT[2,18] = ?



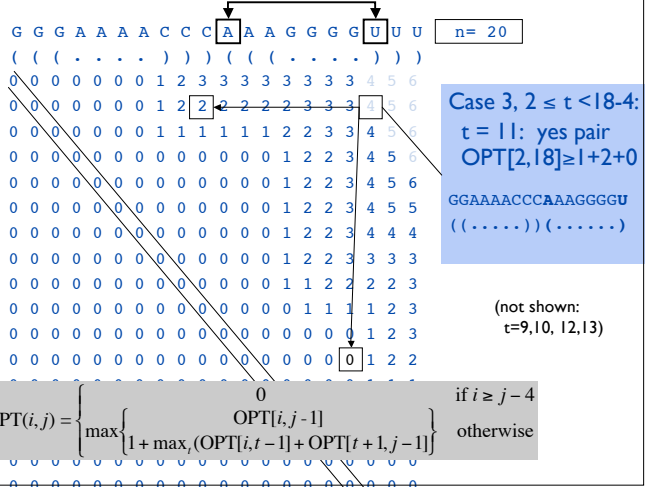
Computing one cell: OPT[2,18] = ?



Case 3, 2 ≤ t <18-4:
t = 8: no pair

$$OPT(i,j) = \begin{cases} 0 & \text{if } i \geq j-4 \\ \max\left\{ \begin{array}{c} OPT[i,j-1] \\ 1+\max_t(OPT[i,t-1]+OPT[t+1,j-1]) \end{array} \right\} & \text{otherwise} \end{cases}$$

Computing one cell: OPT[2,18] = ?



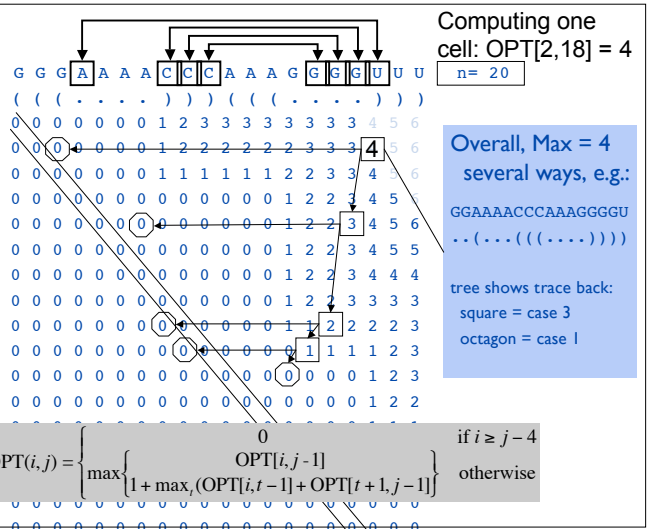
Case 3, 2 ≤ t <18-4:
t = 11: yes pair
OPT[2,18]≥1+2+0

GGAAAACCCAAAGGGGU
((.....))(.....)

(not shown:
t=9,10, 12,13)

$$OPT(i,j) = \begin{cases} 0 & \text{if } i \geq j-4 \\ \max\left\{ \begin{array}{c} OPT[i,j-1] \\ 1+\max_t(OPT[i,t-1]+OPT[t+1,j-1]) \end{array} \right\} & \text{otherwise} \end{cases}$$

Computing one cell: OPT[2,18] = 4



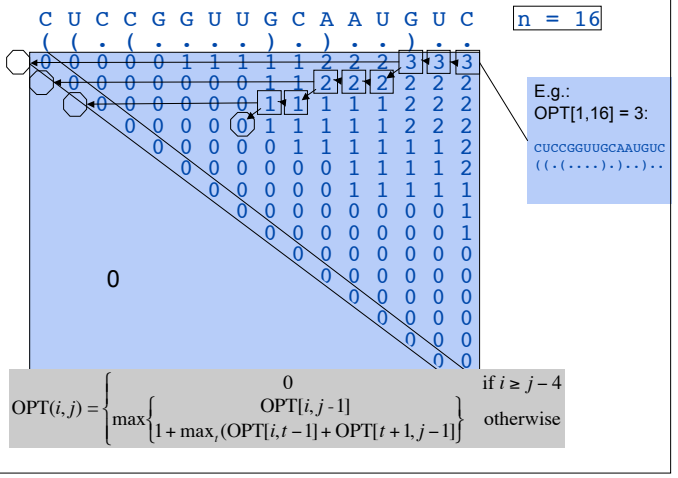
Overall, Max = 4
several ways, e.g.:

GGAAAACCCAAAGGGGU
..(...(((....)))).

tree shows trace back:
square = case 3
octagon = case 1

$$OPT(i,j) = \begin{cases} 0 & \text{if } i \geq j-4 \\ \max\left\{ \begin{array}{c} OPT[i,j-1] \\ 1+\max_t(OPT[i,t-1]+OPT[t+1,j-1]) \end{array} \right\} & \text{otherwise} \end{cases}$$

Another Trace Back Example



E.g.:
OPT[1,16] = 3:

CUCCGGUUGCAAUGUC
((.(....).)..)..

$$OPT(i,j) = \begin{cases} 0 & \text{if } i \geq j-4 \\ \max\left\{ \begin{array}{c} OPT[i,j-1] \\ 1+\max_t(OPT[i,t-1]+OPT[t+1,j-1]) \end{array} \right\} & \text{otherwise} \end{cases}$$

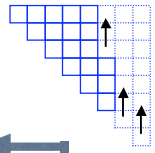
Nussinov: Max Pairing

B(i,j) = # pairs in optimal pairing of r_i ... r_j

B(i,j) = 0 for all i, j with i ≥ j-4; otherwise

B(i,j) = max of:

- B(i,j-1)
- max { B(i,k-1)+1+B(k+1,j-1) | i ≤ k < j-4 and r_k-r_j may pair}

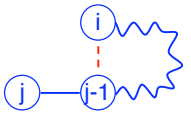


Time: $O(n^3)$

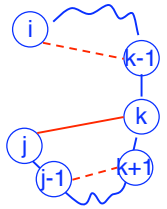
"Optimal pairing of r_i ... r_j"

Two possibilities

J Unpaired:
Find best pairing of r_i ... r_{j-1}



J Paired (with some k):
Find best r_i ... r_{k-1} +
best r_{k+1} ... r_{j-1} plus 1



Why is it slow?
Why do pseudoknots matter?

Pair-based Energy Minimization

E(i,j) = energy of pairs in optimal pairing of r_i ... r_j

E(i,j) = ∞ for all i, j with i ≥ j-4; otherwise

E(i,j) = min of:

- E(i,j-1)
- min { E(i,k-1) + e(r_k, r_j) + E(k+1,j-1) | i ≤ k < j-4 }

energy of j-k pair

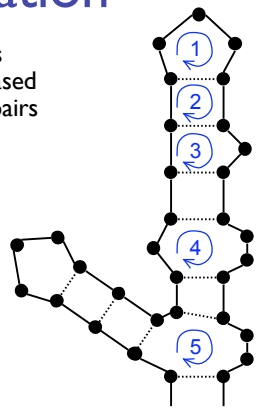
Time: $O(n^3)$

Loop-based Energy Minimization

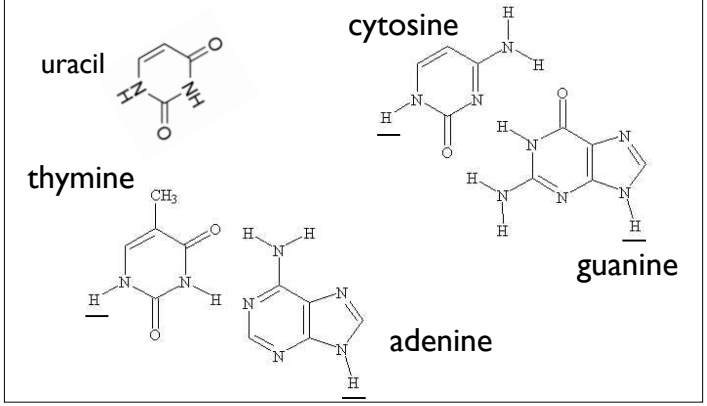
Detailed experiments show it's more accurate to model based on loops, rather than just pairs

Loop types

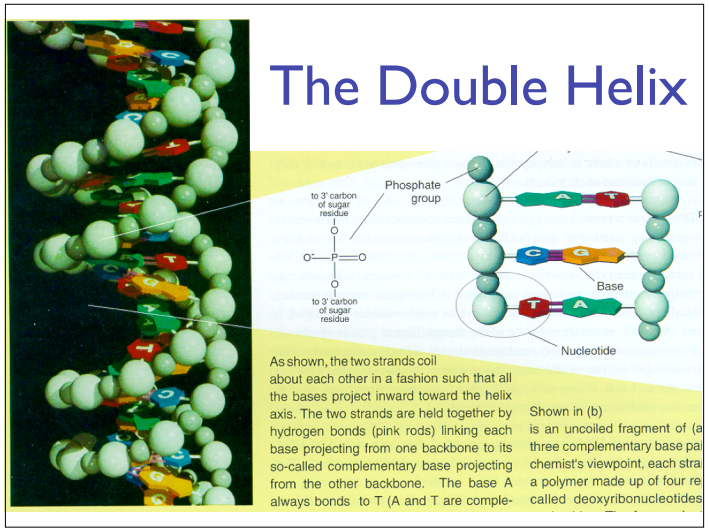
1. Hairpin loop
2. Stack
3. Bulge
4. Interior loop
5. Multiloop



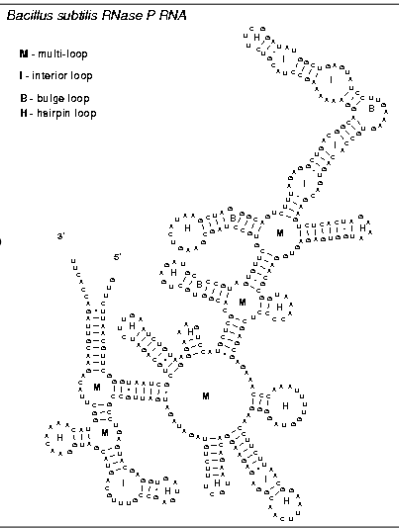
Base Pairs and Stacking



The Double Helix



Loop Examples



Zuker: Loop-based Energy, I

W(i,j) = energy of optimal pairing of r_i ... r_j

V(i,j) = as above, but forcing pair i•j

W(i,j) = V(i,j) = ∞ for all i, j with i ≥ j-4

W(i,j) = min(W(i,j-1),
min { W(i,k-1)+V(k,j) | i ≤ k < j-4 }
)

Zuker: Loop-based Energy, II

hairpin stack bulge/ interior multi- loop

$V(i,j) = \min(eh(i,j), es(i,j)+V(i+1,j-1), VBI(i,j), VM(i,j))$

$VM(i,j) = \min \{ W(i,k)+W(k+1,j) \mid i < k < j \}$

$VBI(i,j) = \min \{ ebi(i,j,i',j') + V(i', j') \mid i < i' < j' < j \ \& \ i'-i+j-j' > 2 \}$

bulge/ interior

Time: $O(n^4)$

$O(n^3)$ possible if ebi(.) is "nice"

Energy Parameters

Q. Where do they come from?

A1. Experiments with carefully selected synthetic RNAs

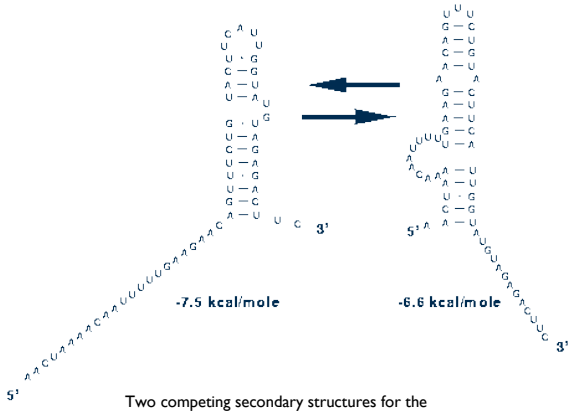
A2. Learned algorithmically from trusted alignments/structures

Suboptimal Energy

There are always alternate folds with near-optimal energies. Thermodynamics: populations of identical molecules will exist in different folds; individual molecules even flicker among different folds

Mod to Zuker's algorithm finds subopt folds

McCaskill: more elaborate dyn. prog. algorithm calculates the "partition function," which defines the probability distribution over all these states.
(Key addition: recurrence must count each possibility exactly once.)



Two competing secondary structures for the Leptomonas collosoma spliced leader mRNA.

Accuracy

Latest estimates suggest ~50-75% of base pairs predicted correctly in sequences of up to ~300nt

Definitely useful, but obviously imperfect

Approaches to Structure Prediction

Maximum Pairing
- \+ works on single sequences
- \+ simple
- \- too inaccurate

Minimum Energy
- \+ works on single sequences
- \- ignores pseudoknots
- \- only finds "optimal" fold

Partition Function
- \+ finds all folds
- \- ignores pseudoknots

Approaches, II

Comparative sequence analysis
- \+ handles all pairings (incl. pseudoknots)
- \- requires several (many?) aligned, appropriately diverged sequences

Stochastic Context-free Grammars
Roughly combines min energy & comparative, but no pseudoknots

Physical experiments (x-ray crystalography, NMR)

Summary

RNA has important roles beyond mRNA
Many unexpected recent discoveries

Structure is critical to function
True of proteins, too, but they're easier to find, due, e.g., to codon structure, which RNAs lack

RNA secondary structure can be predicted (to useful accuracy) by dynamic programming

Next time: RNA "motifs" (seq + 2-ary struct) well-captured by "covariance models"