CSE 527

Autumn 2006

Lectures 15-16

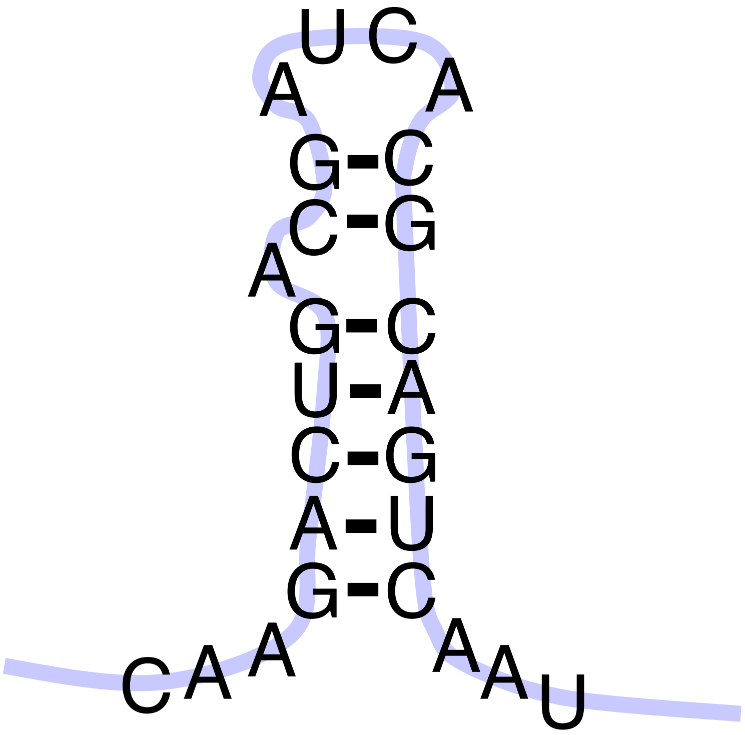
RNA

Secondary Structure Prediction

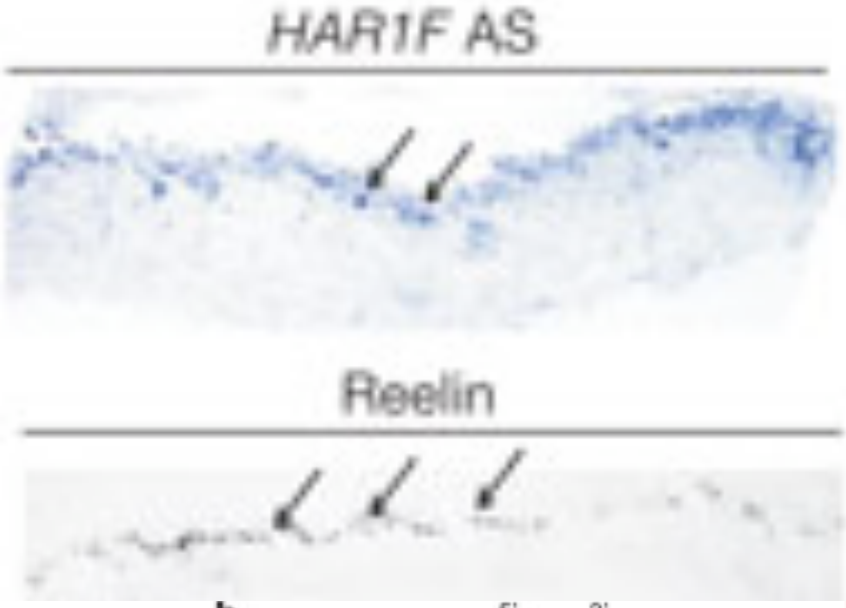
RNA Secondary Structure:
RNA makes helices too

Base pairs

A-U
C-G



Fastest Human Gene?



a

```
Position                20        30        40        50
Human         AGACGTTACAGCAACGTGTCAGCTGAAATGATGGGCGTAGACGCACGT
Chimpanzee    AGAAATTACAGCAATTTATCAACTGAAATTATAGGTGTAGACACATGT
Gorilla       AGAAATTACAGCAATTTATCAACTGAAATTATAGGTGTAGACACATGT
Orang-utan    AGAAATTACAGCAATTTATCAACTGAAATTATAGGTGTAGACACATGT
Macaque       AGAAATTACAGCAATTTATCAGCTGAAATTATAGGTGTAGACACATGT
Mouse         AGAAATTACAGCAATTTATCAGCTGAAATTATAGGTGTAGACACATGT
Dog           AGAAATTACAGCAATTTATCAACTGAAATTATAGGTGTAGACACATGT
Cow           AGAAATTACAGCAATTCATCAGCTGAAATTATAGGTGTAGACACATGT
Platypus      ATAAATTACAGCAATTTATCAAATGAAATTATAGGTGTAGACACATGT
Opossum       AGAAATTACAGCAATTTATCAACTGAAATTATAGGTGTAGACACATGT
Chicken       AGAAATTACAGCAATTTATCAACTGAAATTATAGGTGTAGACACATGT
Fold          (((((((.....)))))).....))[[[[[.(((.(((....))).)))
Pair symbol   lmnopqr     rqpon     ml      rstuvwx    xwvutsr
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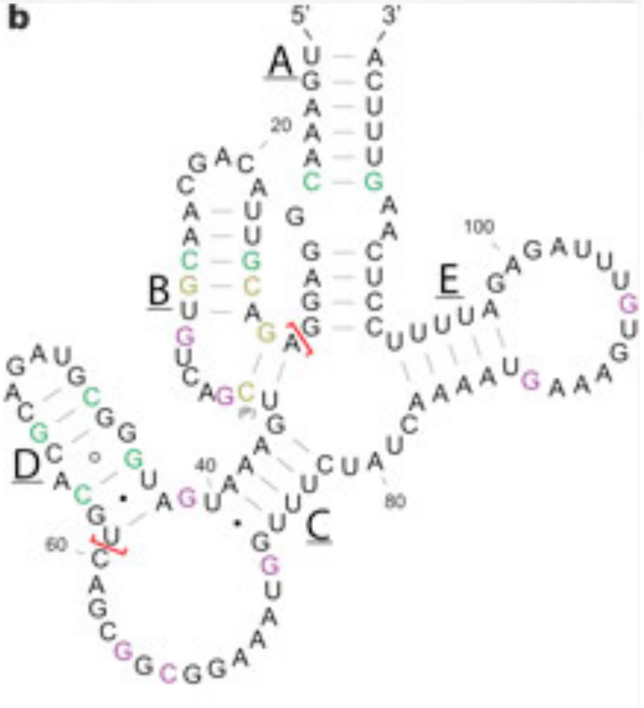


Origin of Life?

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

Outline

Biological roles for RNA

What is “secondary structure?

How is it represented?

Why is it important?

Examples

Approaches

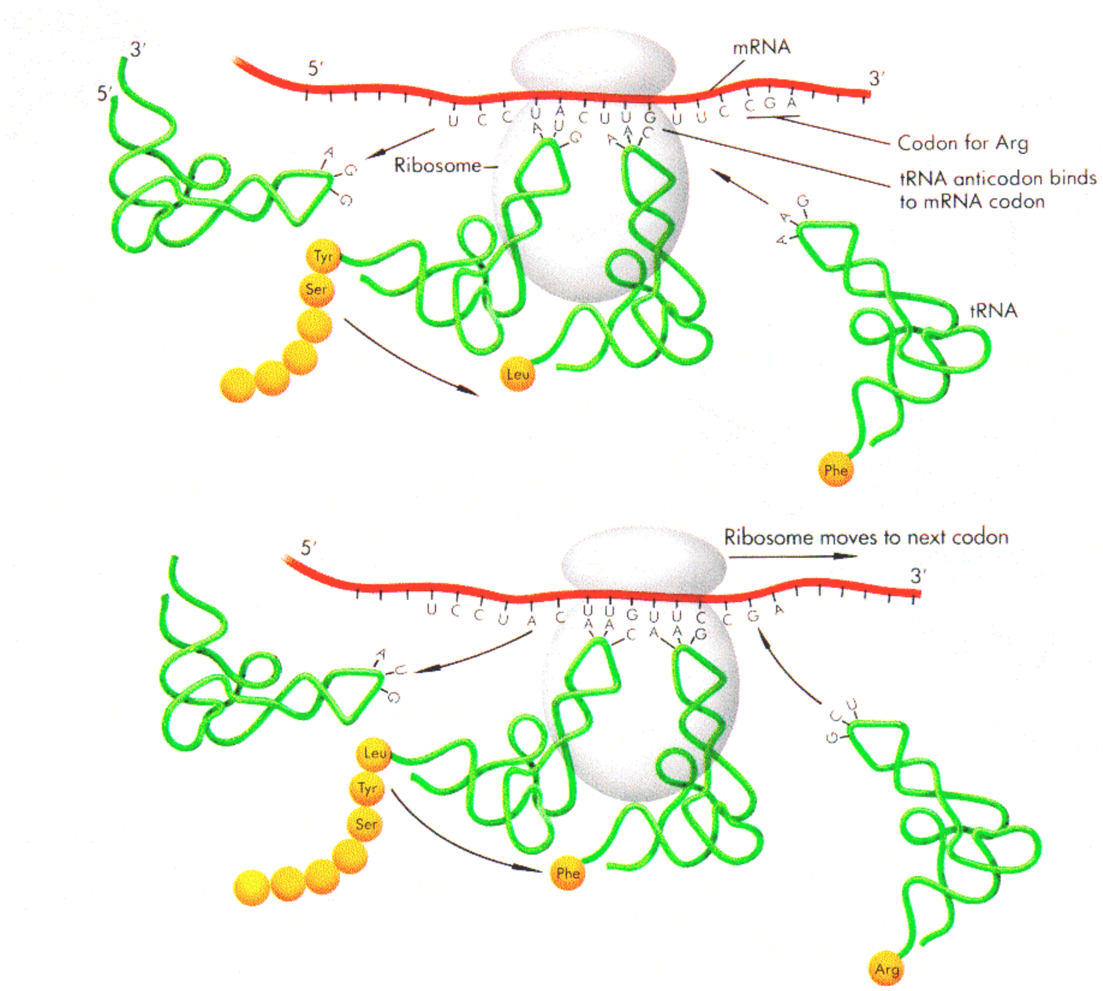
RNA Structure

Primary Structure:	Sequence
Secondary Structure:	Pairing
Tertiary Structure:	3D shape

RNA Pairing

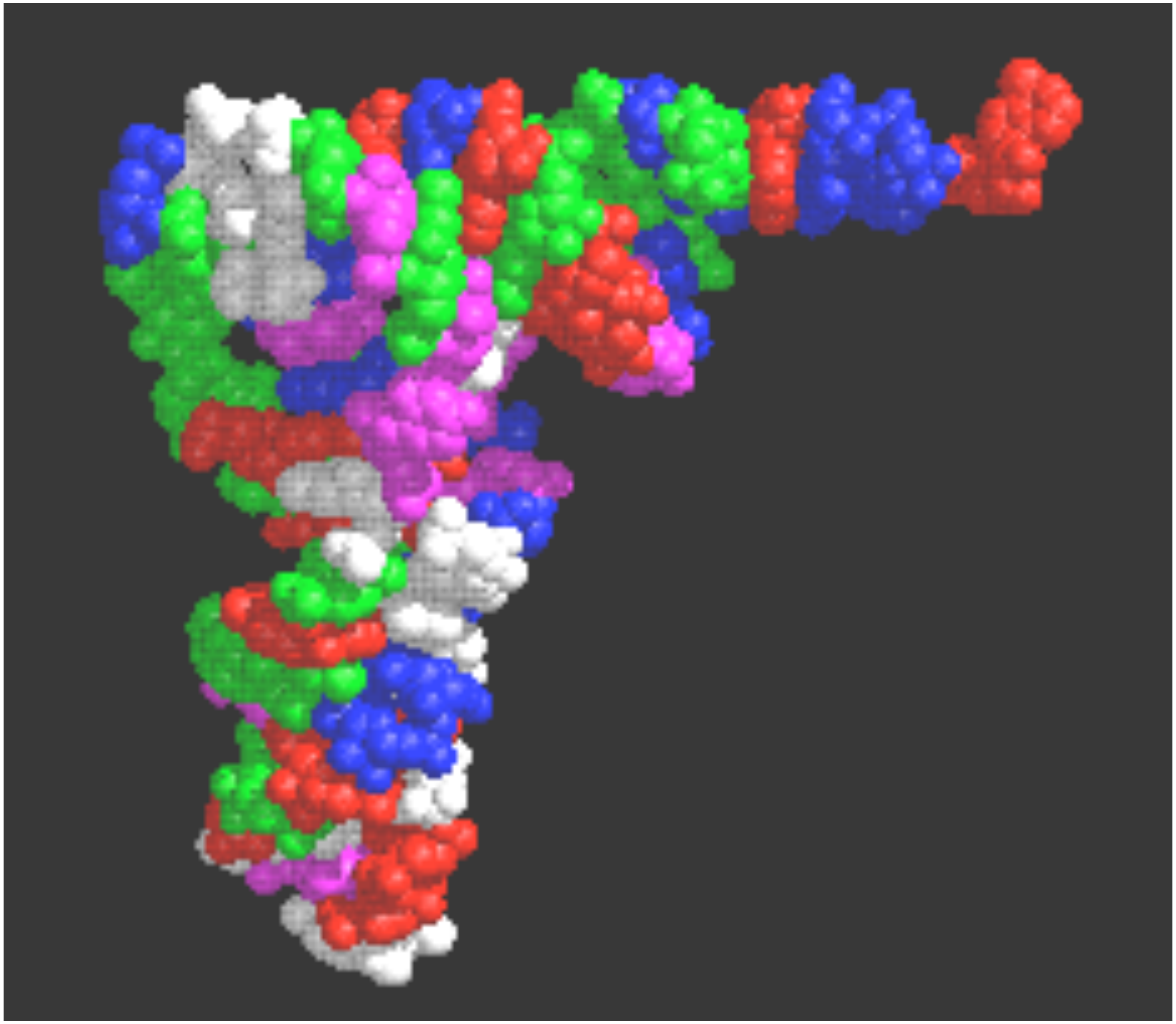
Watson-Crick Pairing	
C - G	~ 3 kcal/mole
A - U	~ 2 kcal/mole
“Wobble Pair” G - U	~1 kcal/mole
Non-canonical Pairs (esp. if modified)	

Ribosomes

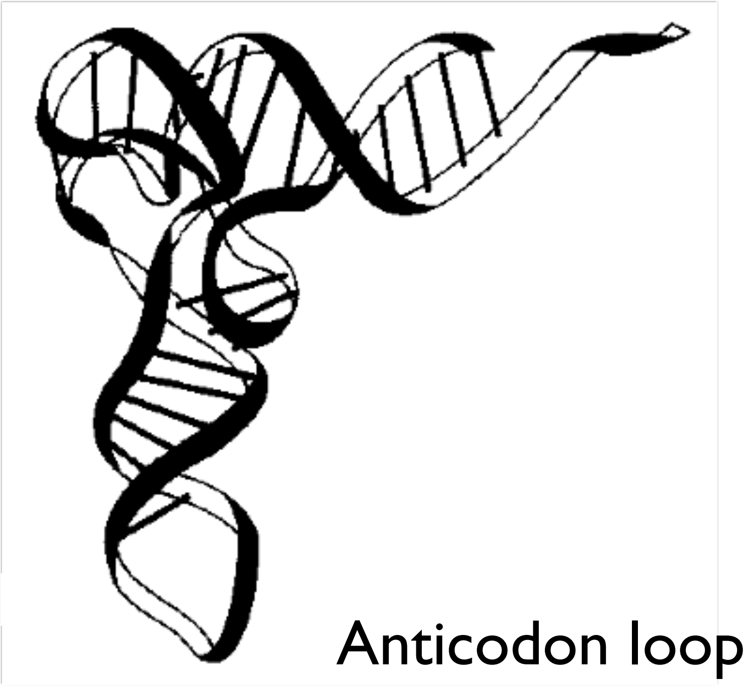

3′
5′
5′
mRNA
3′
U C C U A C U U G U U C C G A
A U G
A A C
A G G
Ribosome
Codon for Arg
tRNA anticodon binds to mRNA codon
G A A
tRNA
Tyr
Ser
Leu
Phe
5′
Ribosome moves to next codon
3′
U C C U A C U U G U U C C G A
A A C
A A G
A C G
U C G
Leu
Tyr
Ser
Phe
Arg

Watson, Gilman, Witkowski, & Zoller, 1992

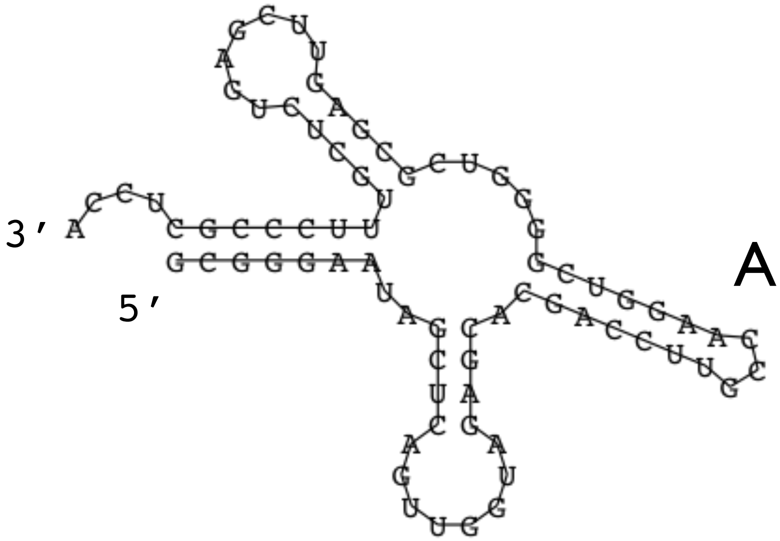
tRNA 3d Structure



tRNA - Alt. Representations



Anticodon loop

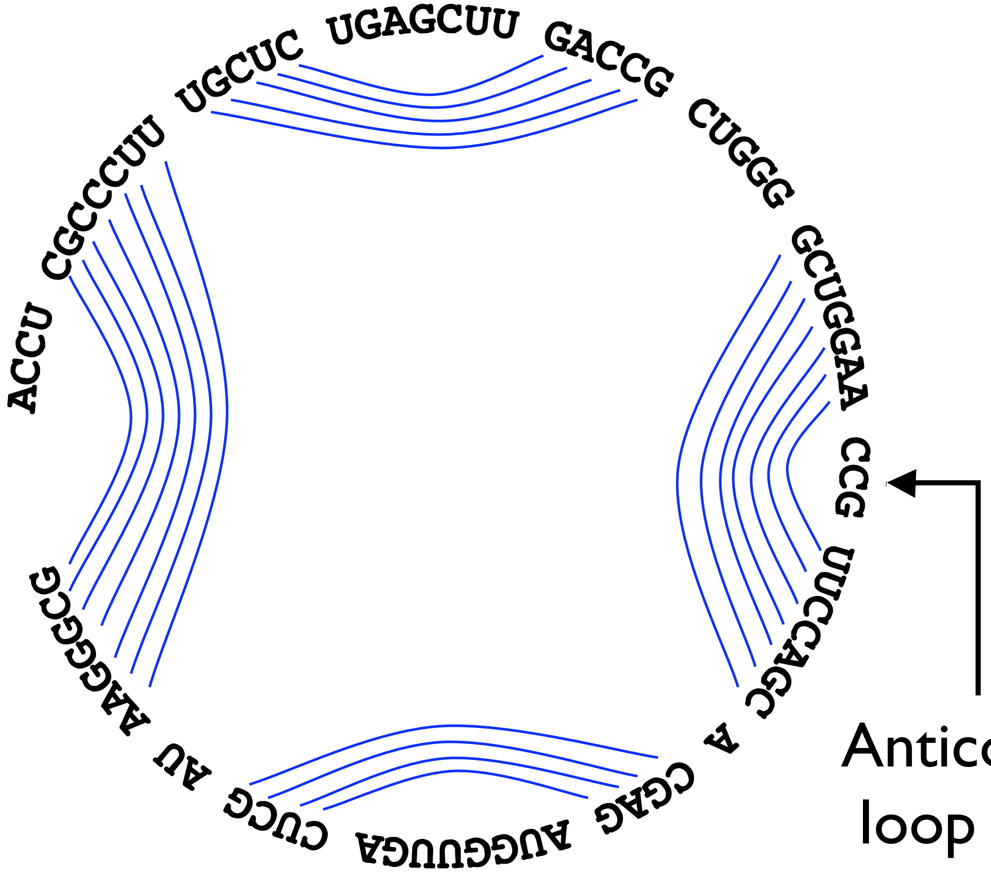


Anticodon loop

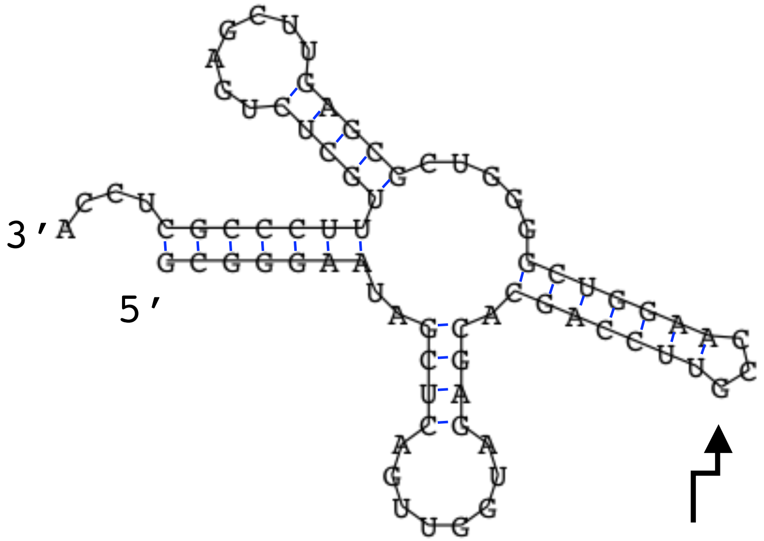
Figure 1: a) The spatial structure of the phenylalanine tRNA form yeast

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

tRNA - Alt. Representations



Anticodon loop



Anticodon loop

“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

…

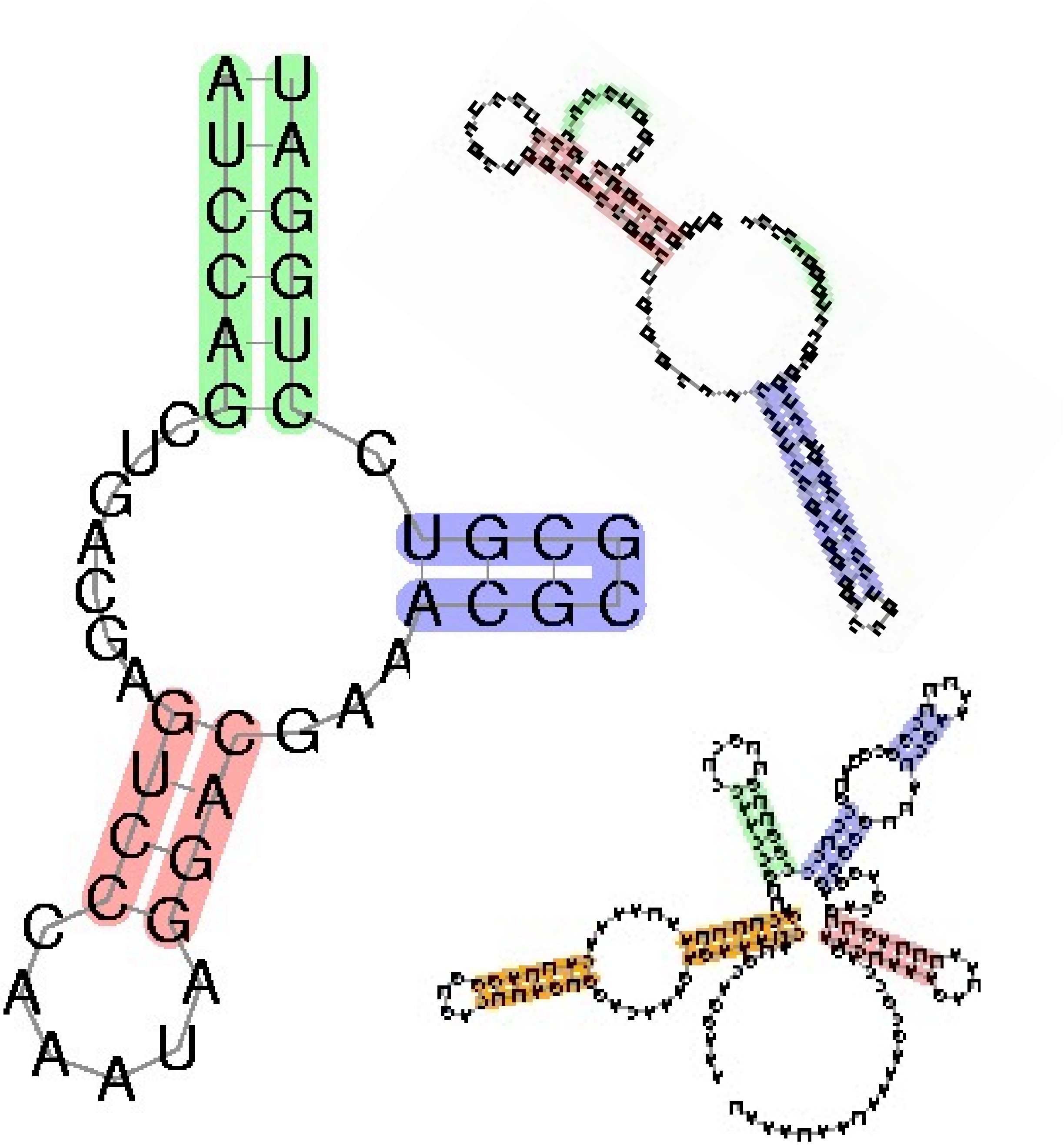
Hundreds of families

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



Breakthrough of the Year

Noncoding RNAs

Dramatic discoveries in last 5 years

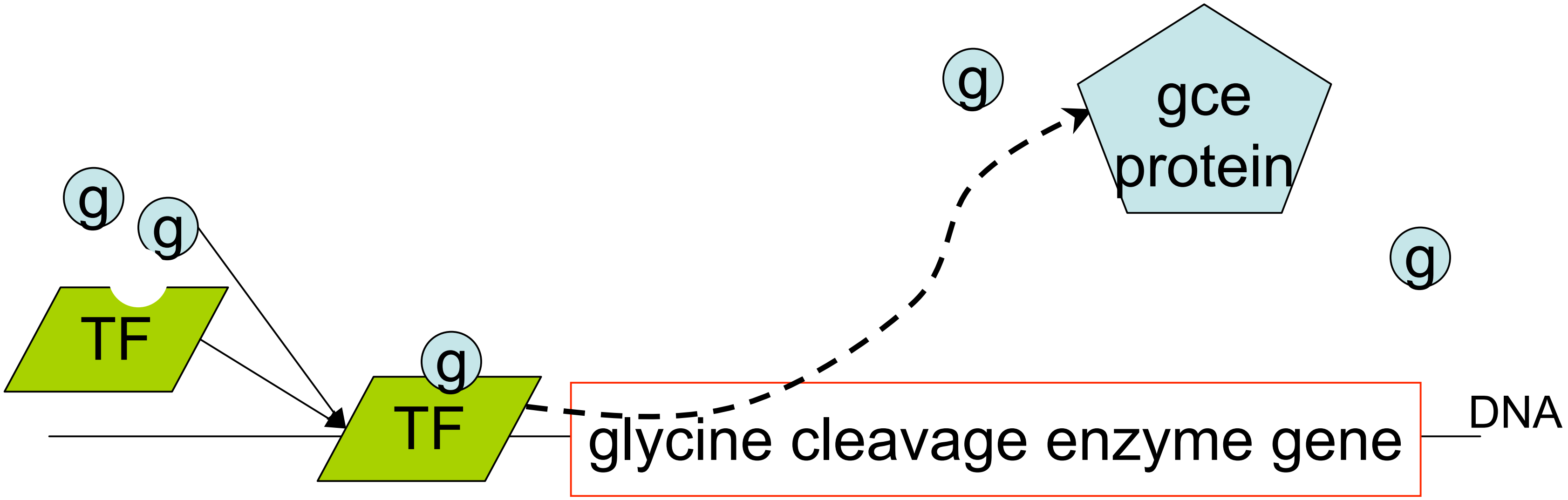
- *100s* of new families
- Many roles: Regulation, transport, stability, catalysis, …

1% of DNA codes for protein, but 30% of it is copied into RNA, i.e. ncRNA >> mRNA

Example: Glycine Regulation

How is glycine level regulated?

Plausible answer:



g

gce protein

g

g

g

TF

g

TF

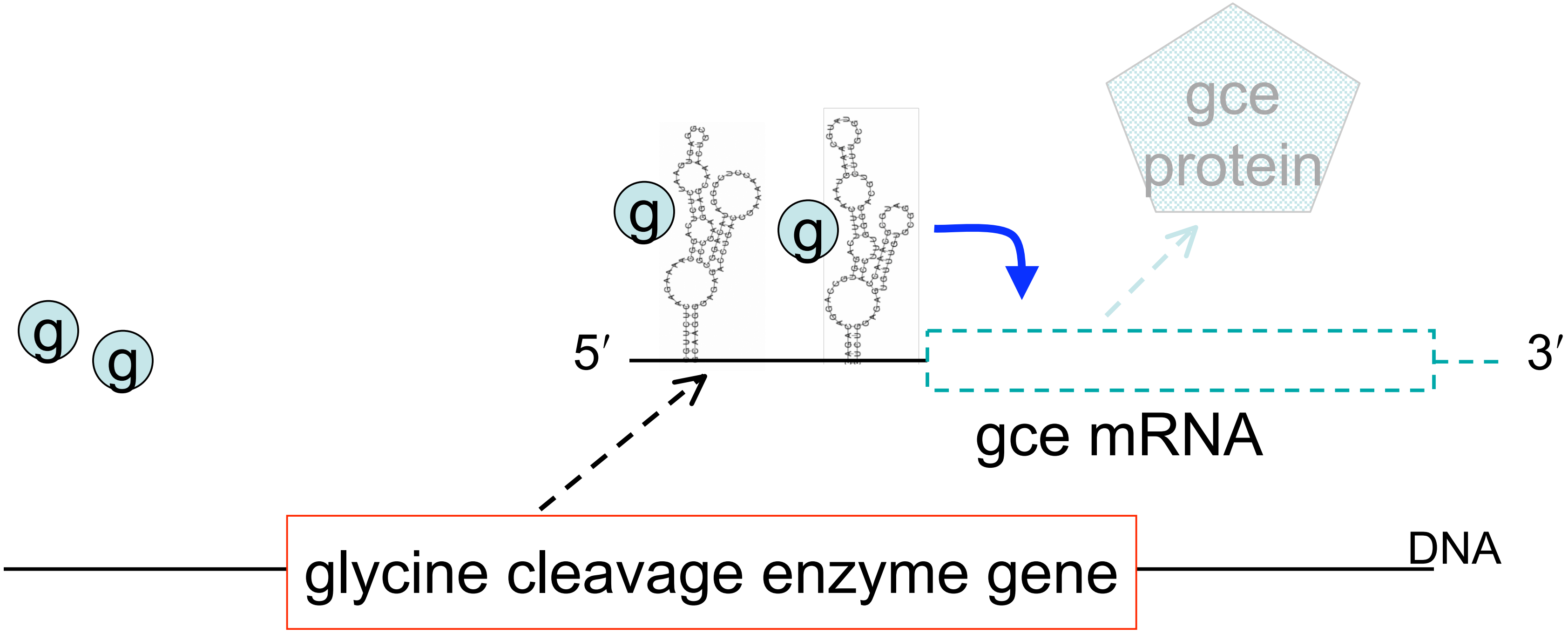
glycine cleavage enzyme gene

DNA

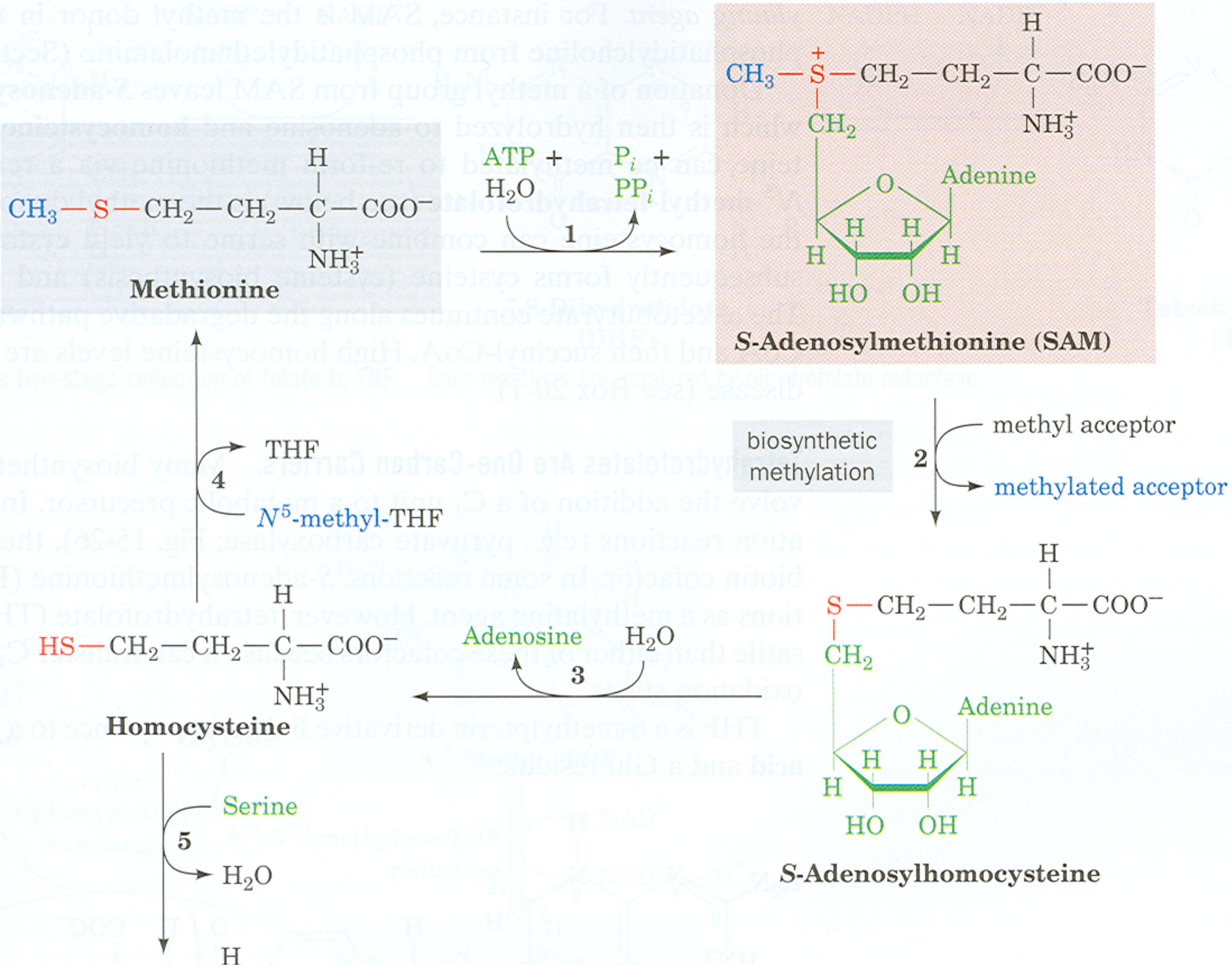
transcription factors (proteins) bind to DNA to turn nearby genes on or off

The Glycine Riboswitch

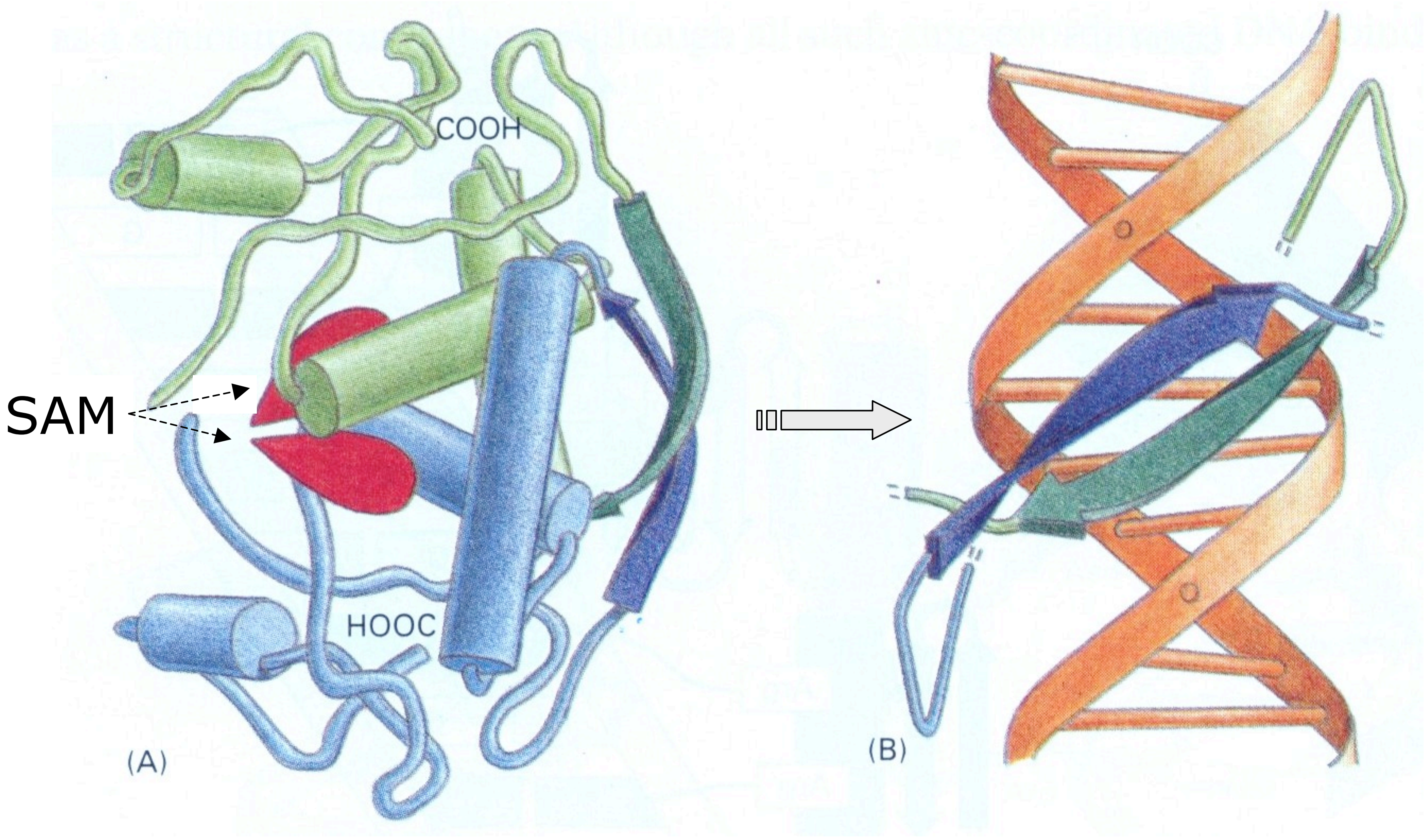
Actual answer (in many bacteria):



Mandal et al. Science 2004


Methionine
ATP + H₂O
Pᵢ + PPᵢ
1
S-Adenosylmethionine (SAM)
Adenine
biosynthetic methylation
2
methyl acceptor
methylated acceptor
S-Adenosylhomocysteine
Adenine
Adenosine
H₂O
3
Homocysteine
THF
4
N⁵-methyl-THF
Serine
5
H₂O

Gene Regulation: The MET Repressor



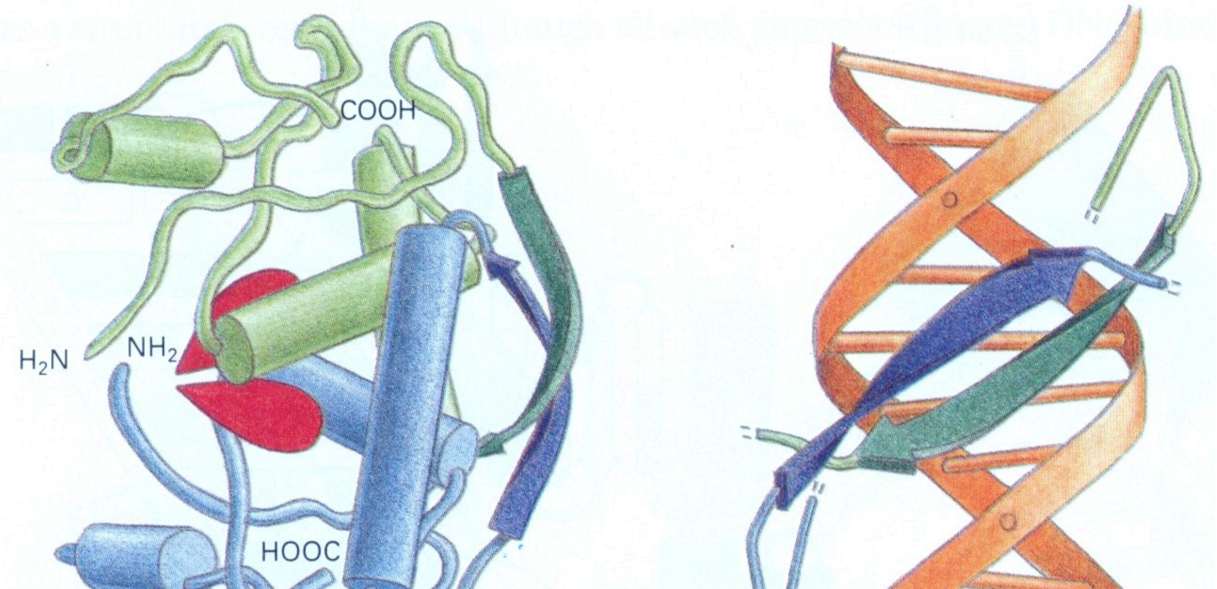
SAM

Protein

Alberts, et al, 3e.

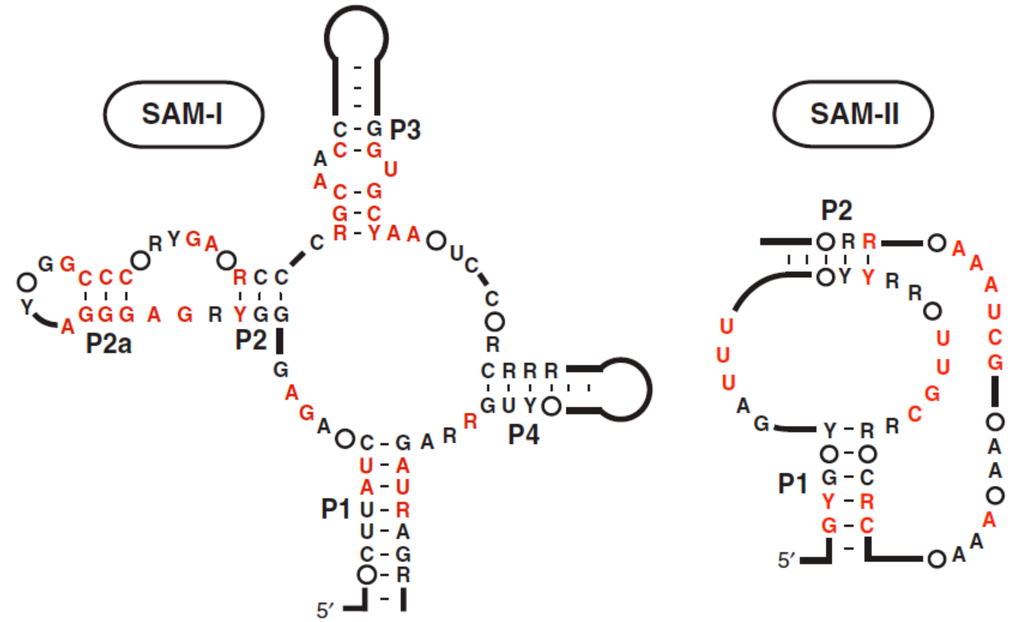
DNA

Alberts, et al, 3e.



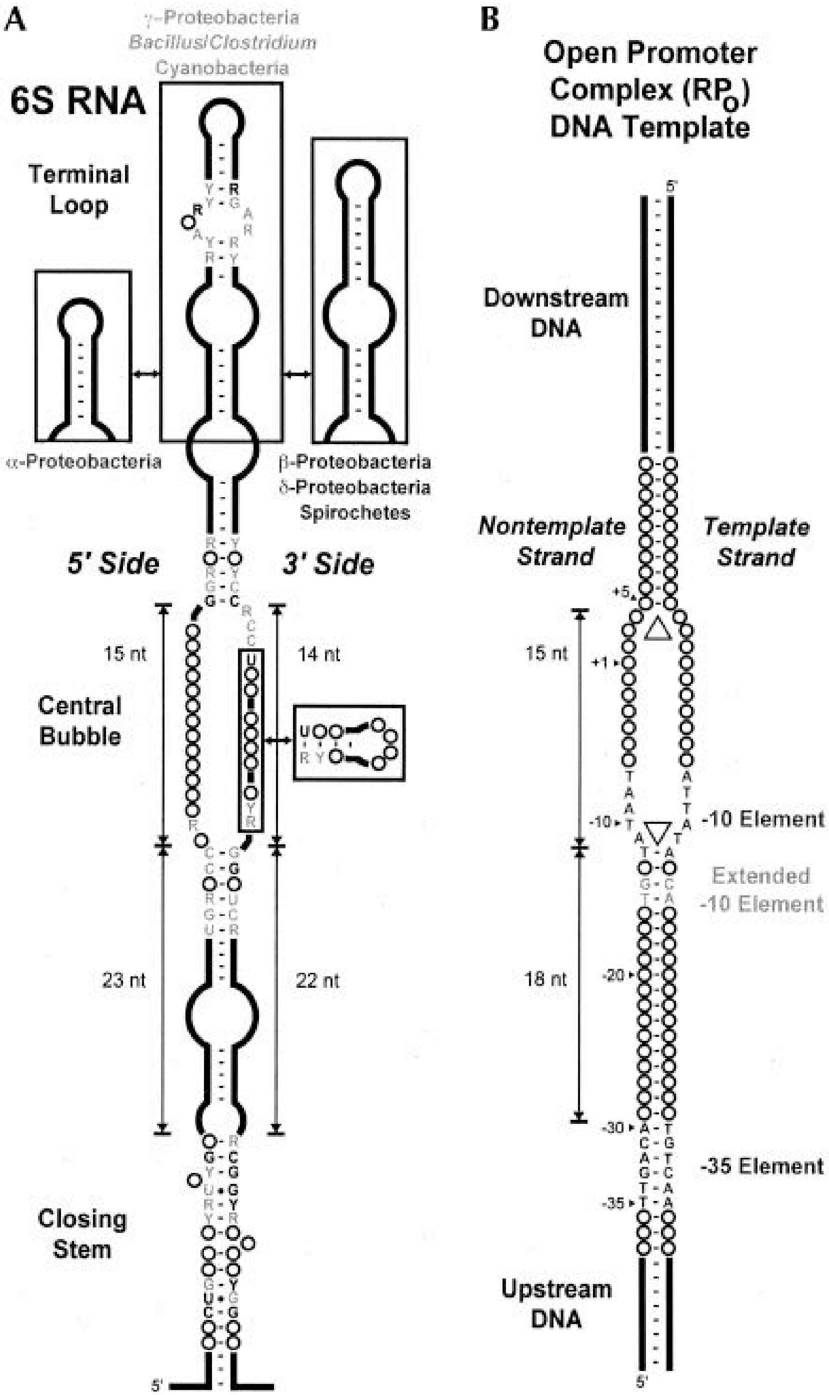
The protein way

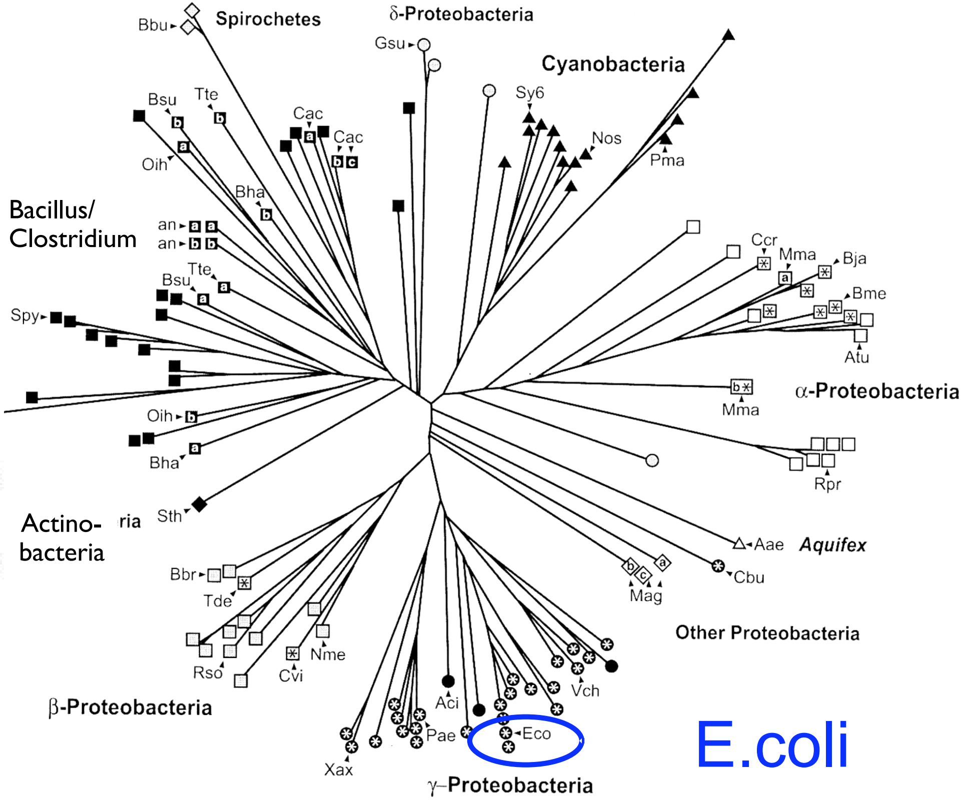
Riboswitch alternatives



Corbino et al., Genome Biol. 2005

6S mimics an open promoter

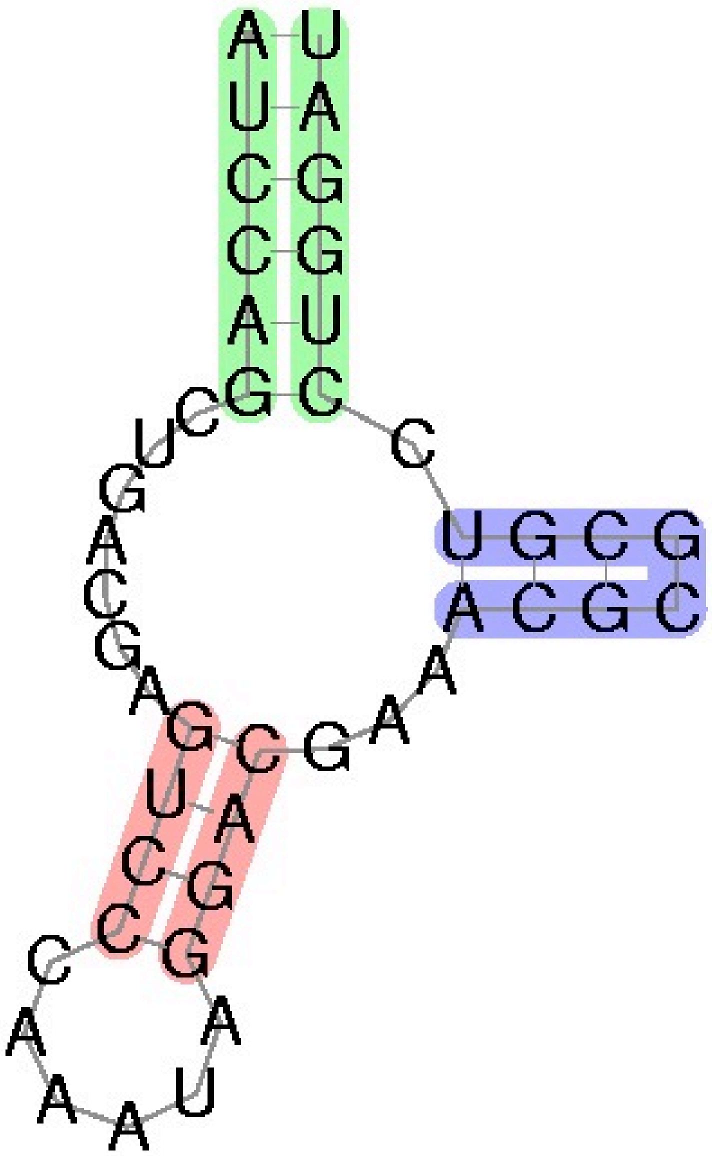




Barrick et al. *RNA* 2005
Trotochaud et al. *NSMB* 2005
Willkomm et al. NAR 2005

The Hammerhead Ribozyme

Involved in “rolling circle replication” of viruses.



Wanted

Good structure prediction tools

Good motif descriptions/models

Good, fast search tools

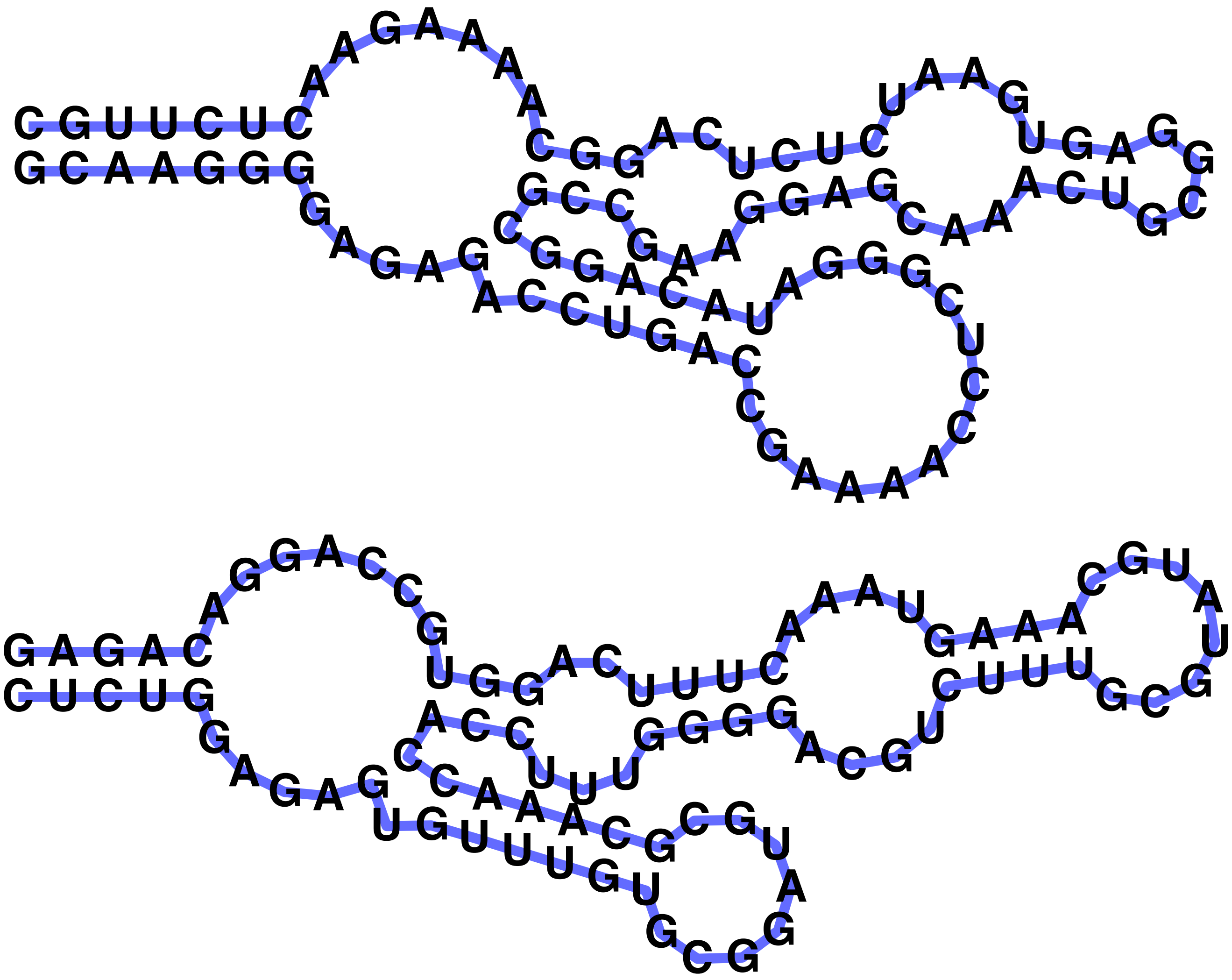
("RNA BLAST", etc.)

Good, fast motif discovery tools

("RNA MEME", etc.)

Importance of structure makes last 3 hard

Why is RNA hard to deal with?


CGUUCUCAAGAAAACGGACUCUCUAAGUGAGGCGUCAAACGAGGAAGCCGCGGACAUAGGGCUCCAAAAGCCAGUCCAGAGAGGGGAACG
GAGACAGGACCGUGGACUUUCAAAUGAAACGUAUGCGUUUCUGCAGGGGUUUCCACCAAACGCGUAGGCGUGUUUGUGAGAGGUCUC

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

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}

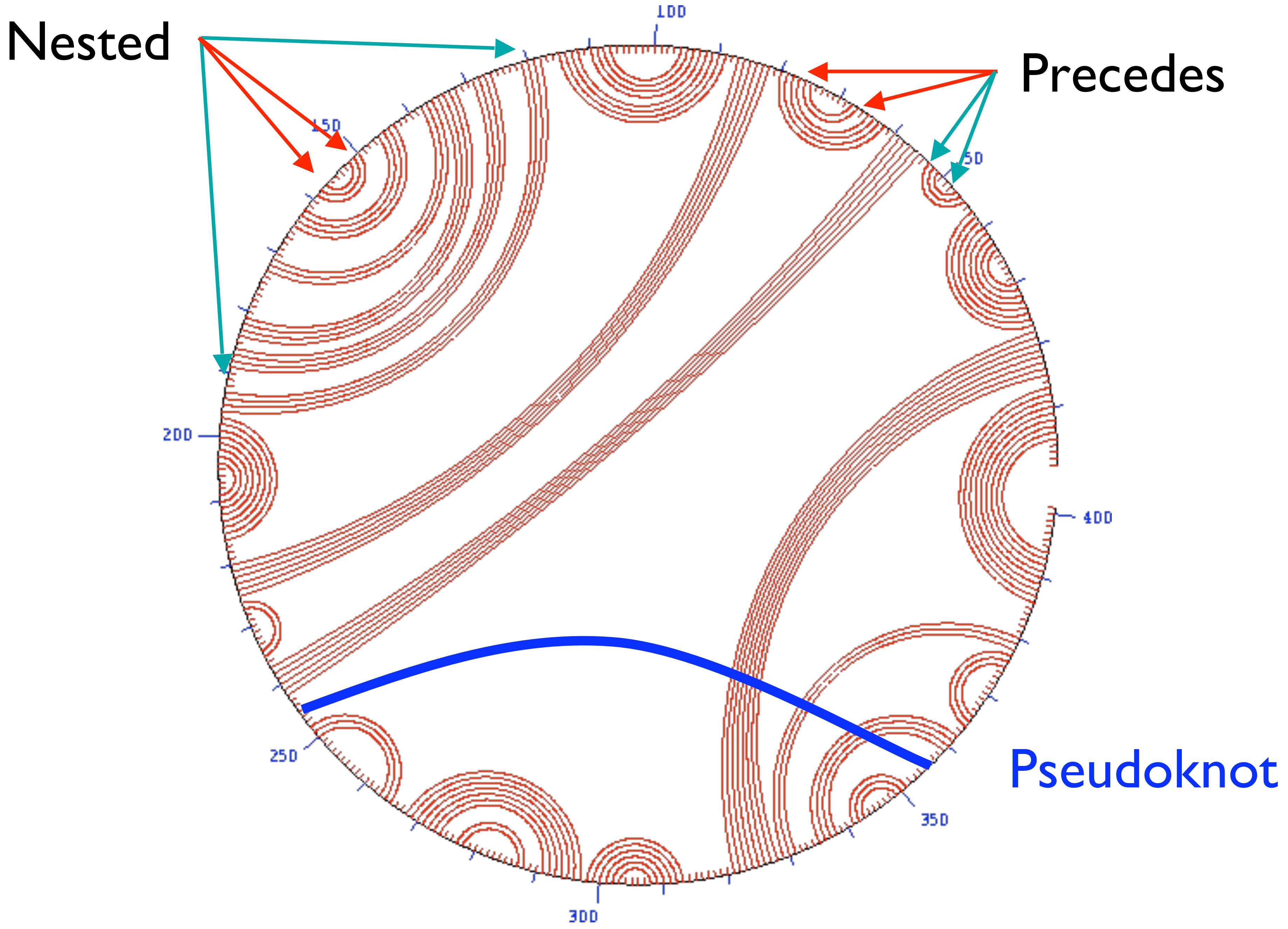
A Secondary Structure is a set of pairs i•j s.t.

$$\left.i < j-4, \text{ and}\right\} \quad \text{no sharp turns}$$

if i•j & i'•j' are two different pairs with $i \le i'$, then

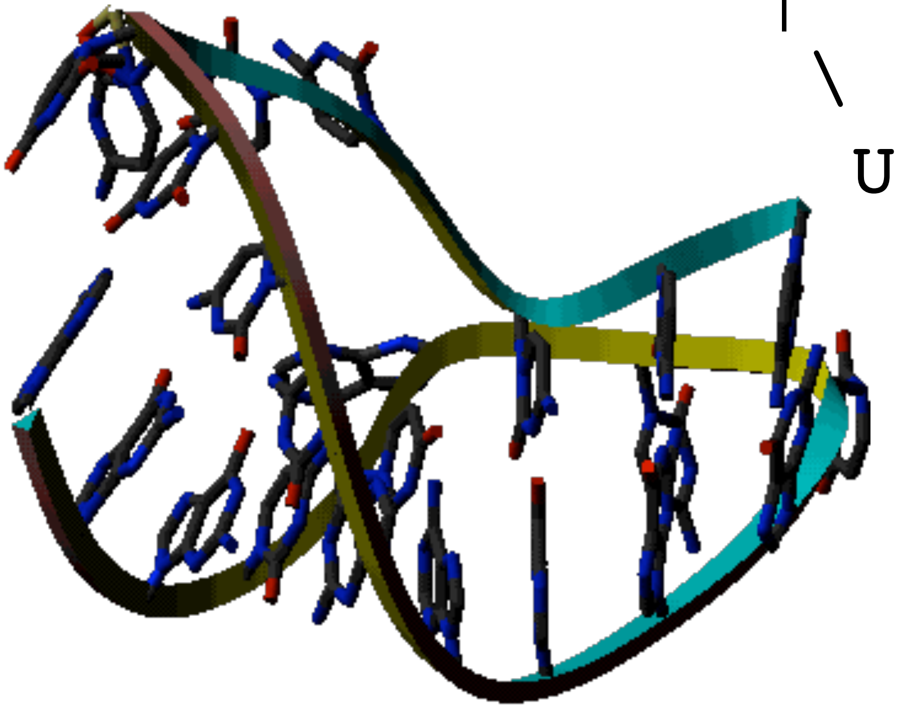
$$\left.\begin{array}{l} j < i', \text{ or} \\ i < i' < j' < j \end{array}\right\}$$

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


Nested
Precedes
Pseudoknot
100
150
50
200
400
250
300
350

A Pseudoknot

```
               A-C
              /   \
3' - A-G-G-C-U     U
     U-C-C-G-A-G-G-G
     |         C-C-C - 5'
      \       /
       U-C-U-C
```



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

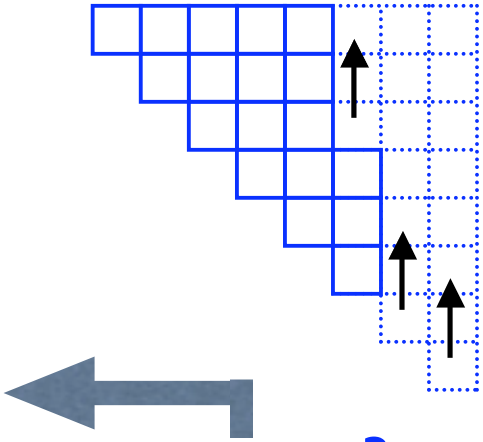
Nussinov: Max Pairing

B(i,j) = # pairs in optimal pairing of $r_i \ldots r_j$

B(i,j) = 0 for all i, j with $i \geq j-4$; otherwise

B(i,j) = max of:

$$\begin{cases} B(i,j-1) \\ \max \{ B(i,k-1)+1+B(k+1,j-1) \mid \\ \qquad i \leq k < j-4 \text{ and } r_k\text{-}r_j \text{ may pair}\} \end{cases}$$



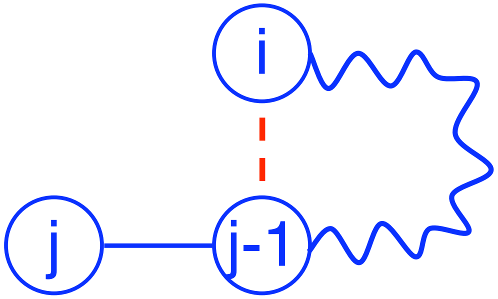
Time: $O(n^3)$

“Optimal pairing of r_i … r_j”

Two possibilities

J Unpaired:

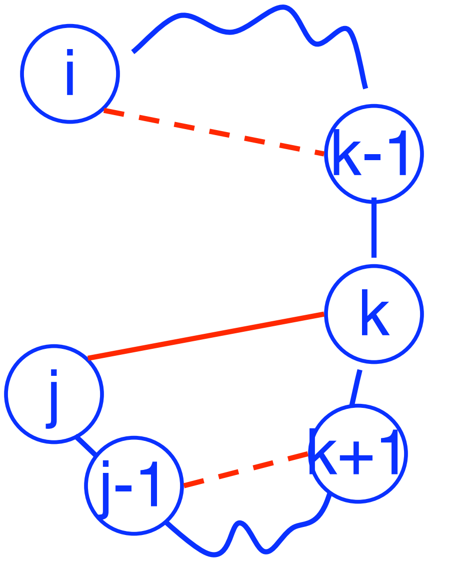
Find best pairing of r_i … r_{j-1}



J Paired:

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 $r_i \ldots r_j$

$E(i,j) = \infty$ for all i, j with $i \geq j-4$; otherwise

E(i,j) = min of:

energy of j-k pair

$$\begin{cases} E(i,j-1) \\ \min \{\, E(i,k-1) + e(r_k, r_j) + E(k+1,j-1) \mid i \leq k < j-4 \,\} \end{cases}$$

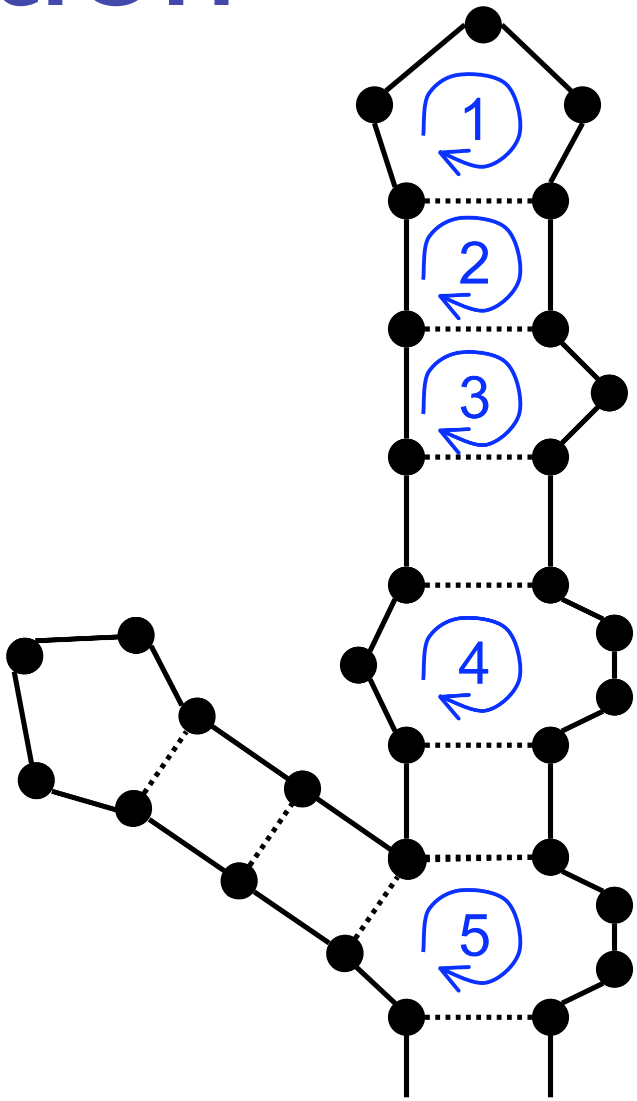
Time: $O(n^3)$

Loop-based Energy Minimization

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

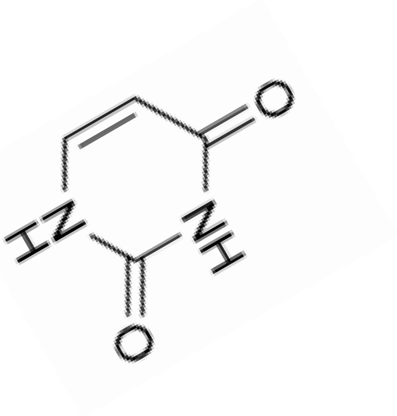
Loop types

- Hairpin loop
- Stack
- Bulge
- Interior loop
- Multiloop

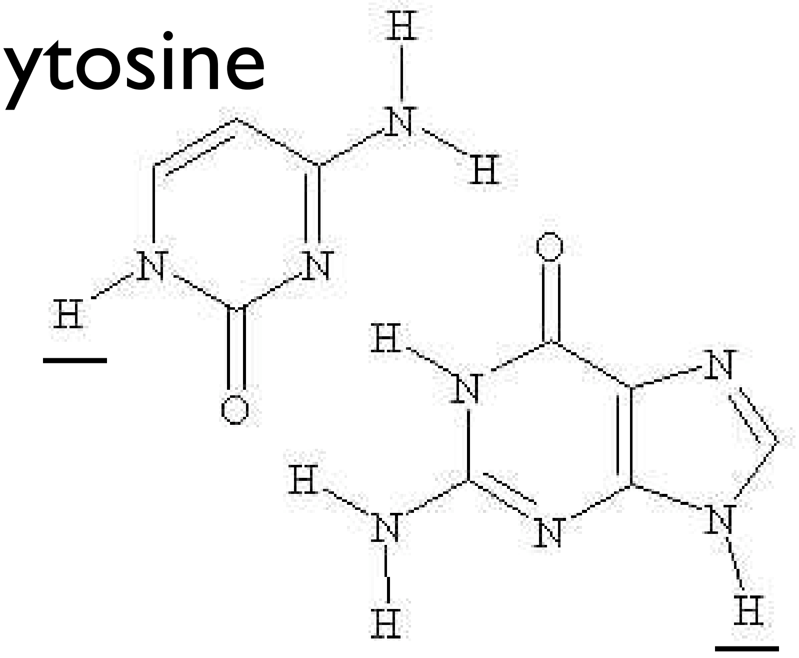


Base Pairs and Stacking

uracil

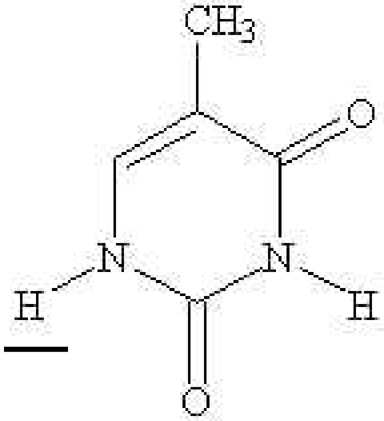


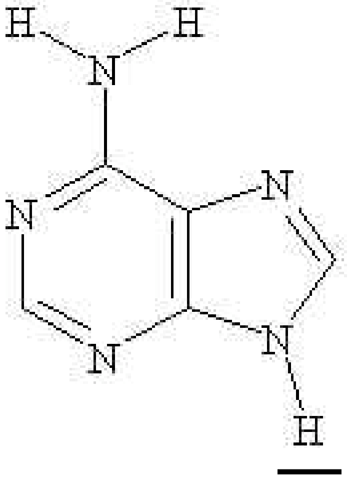
cytosine



guanine

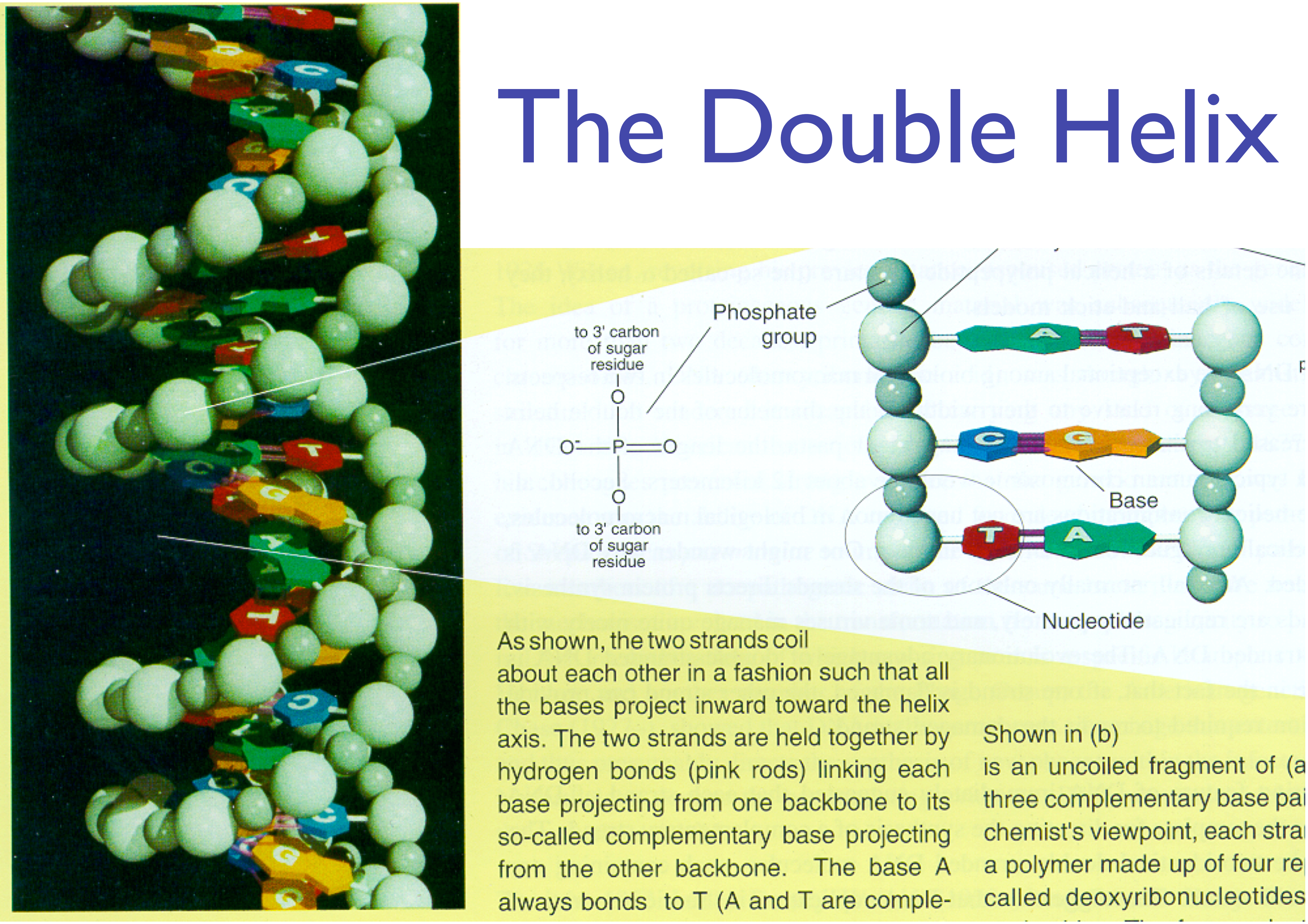
thymine





adenine

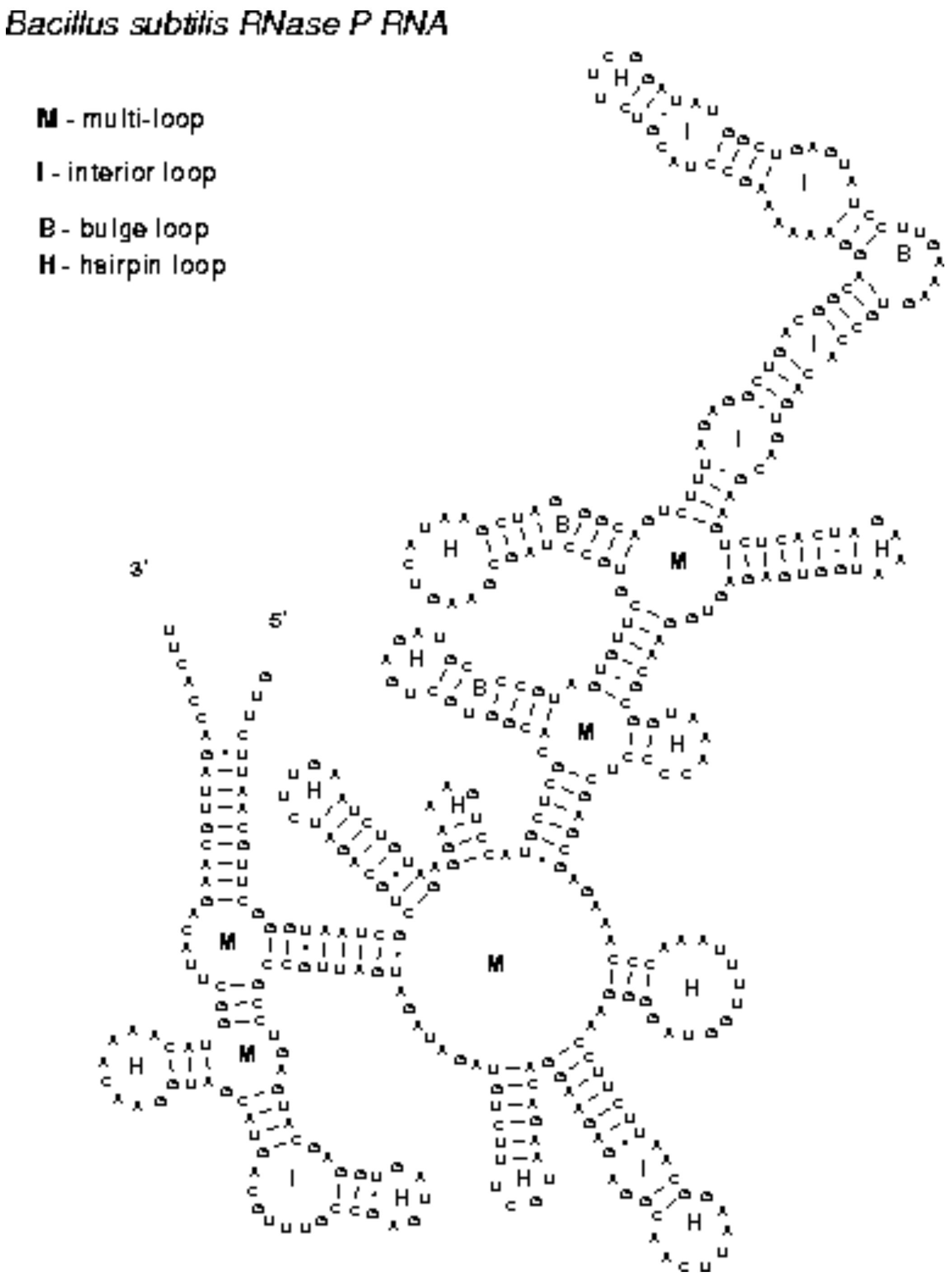
The Double Helix



As shown, the two strands coil about each other in a fashion such that all the bases project inward toward the helix axis. The two strands are held together by hydrogen bonds (pink rods) linking each base projecting from one backbone to its so-called complementary base projecting from the other backbone. The base A always bonds to T (A and T are comple-

Shown in (b) is an uncoiled fragment of (a three complementary base pai chemist's viewpoint, each stra a polymer made up of four re called deoxyribonucleotides

Loop Examples



Bacillus subtilis RNase P RNA

Zuker: Loop-based Energy, I

W(i,j) = energy of optimal pairing of $r_i \dots r_j$

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

$W(i,j) = V(i,j) = \infty$ for all i, j with $i \geq j-4$

$$W(i,j) = \min(W(i,j-1),$$
$$\min \{ W(i,k-1)+V(k,j) \mid i \leq k < j-4 \}$$
$$)$$

Zuker: Loop-based Energy, II

hairpin stack bulge/interior multi-loop

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

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

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

bulge/interior

Time: $O(n^4)$

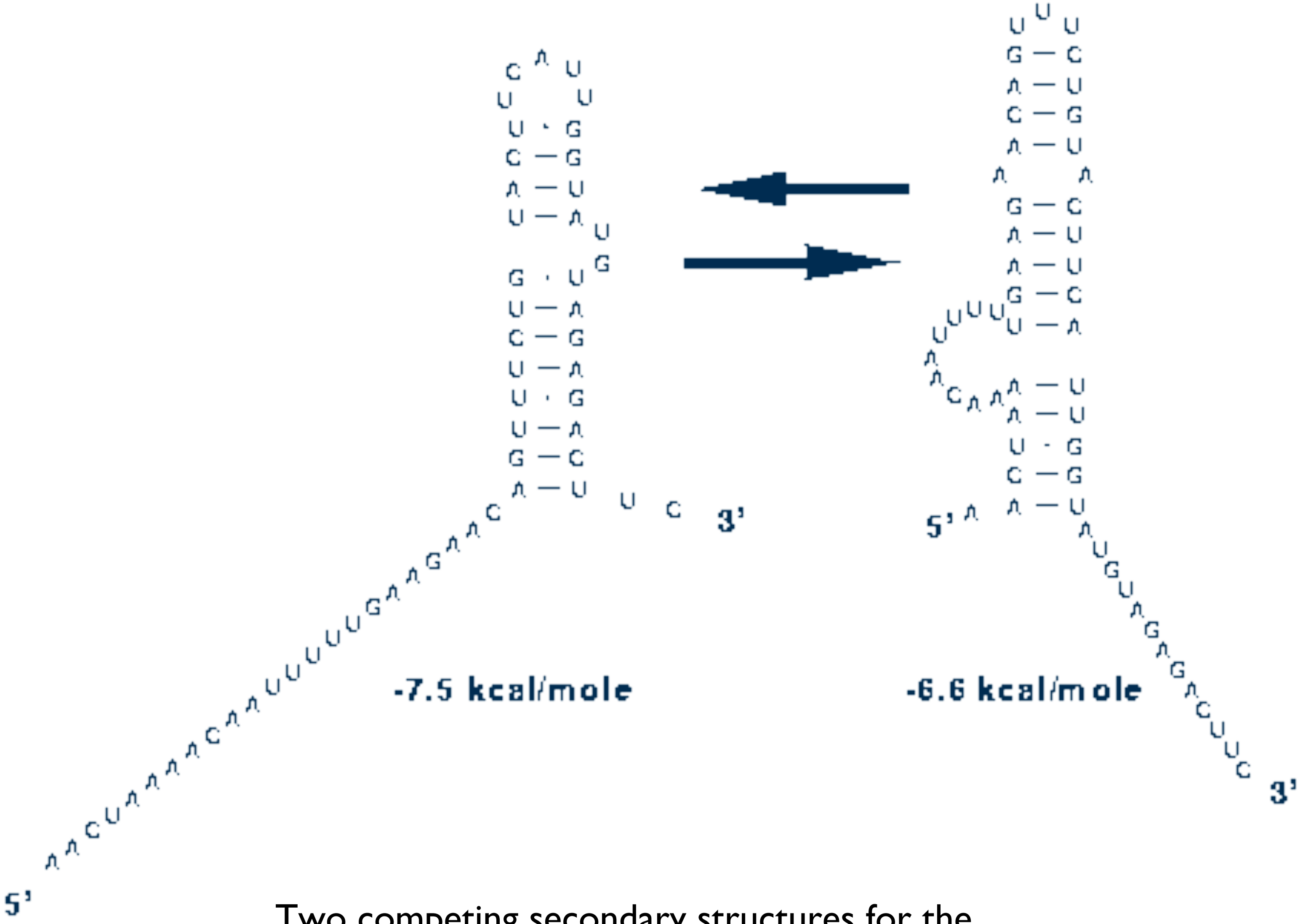
$O(n^3)$ possible if ebi(.) is “nice”

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.)

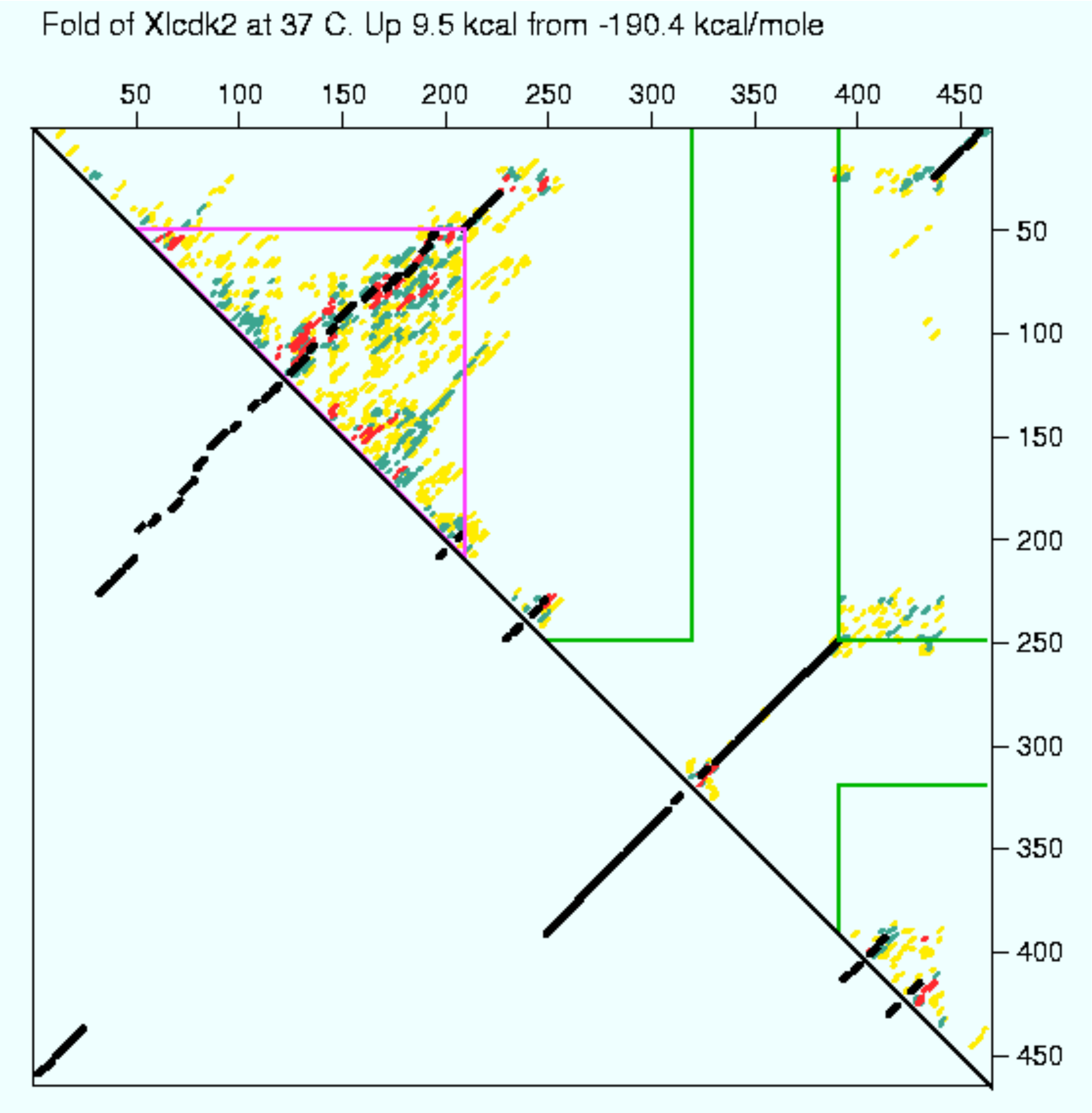


Two competing secondary structures for the Leptomonas collosoma spliced leader mRNA.

Example of suboptimal folding

Black dots: pairs in opt fold

Colored dots: pairs in folds 2-5% worse than optimal fold



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"