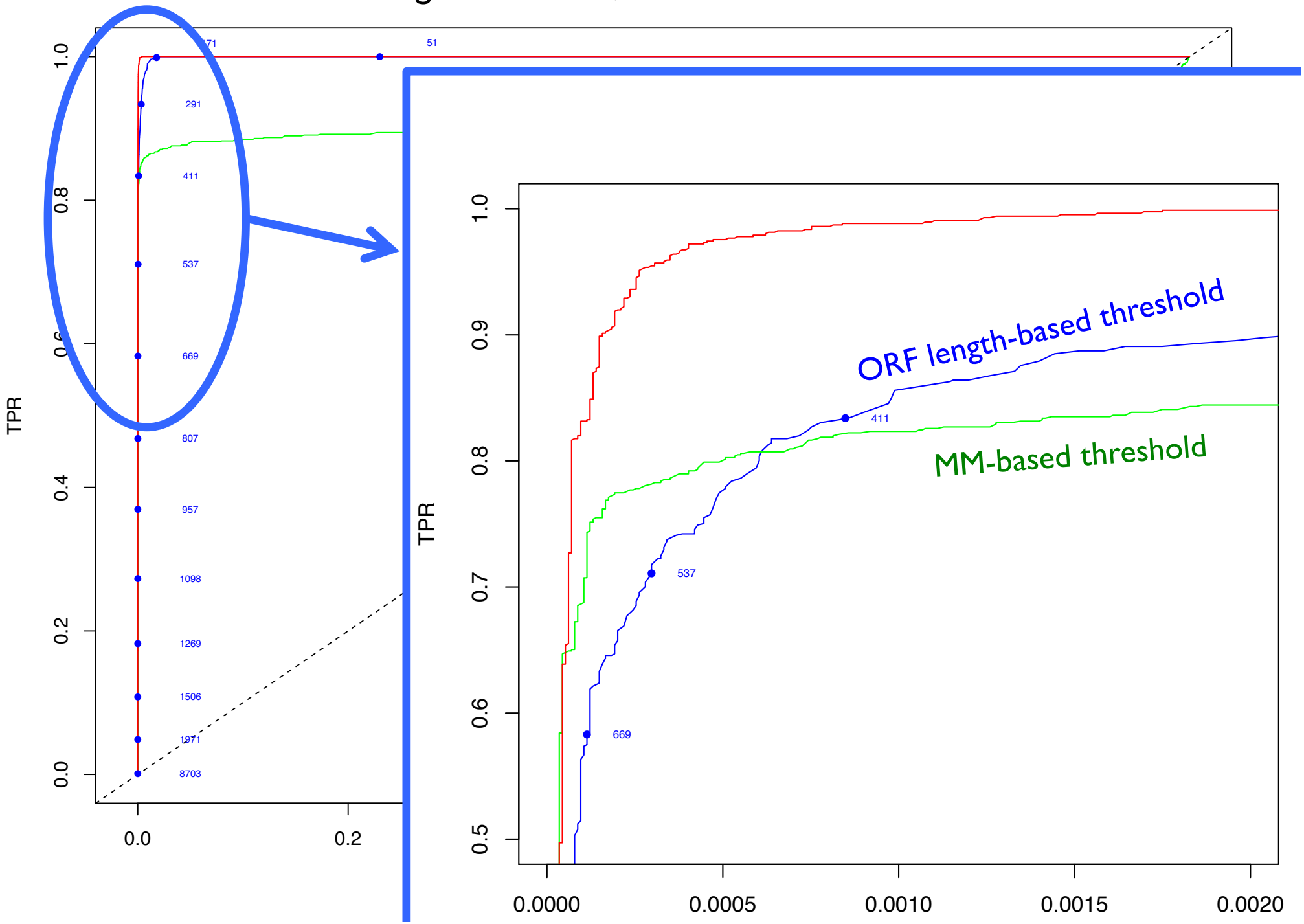
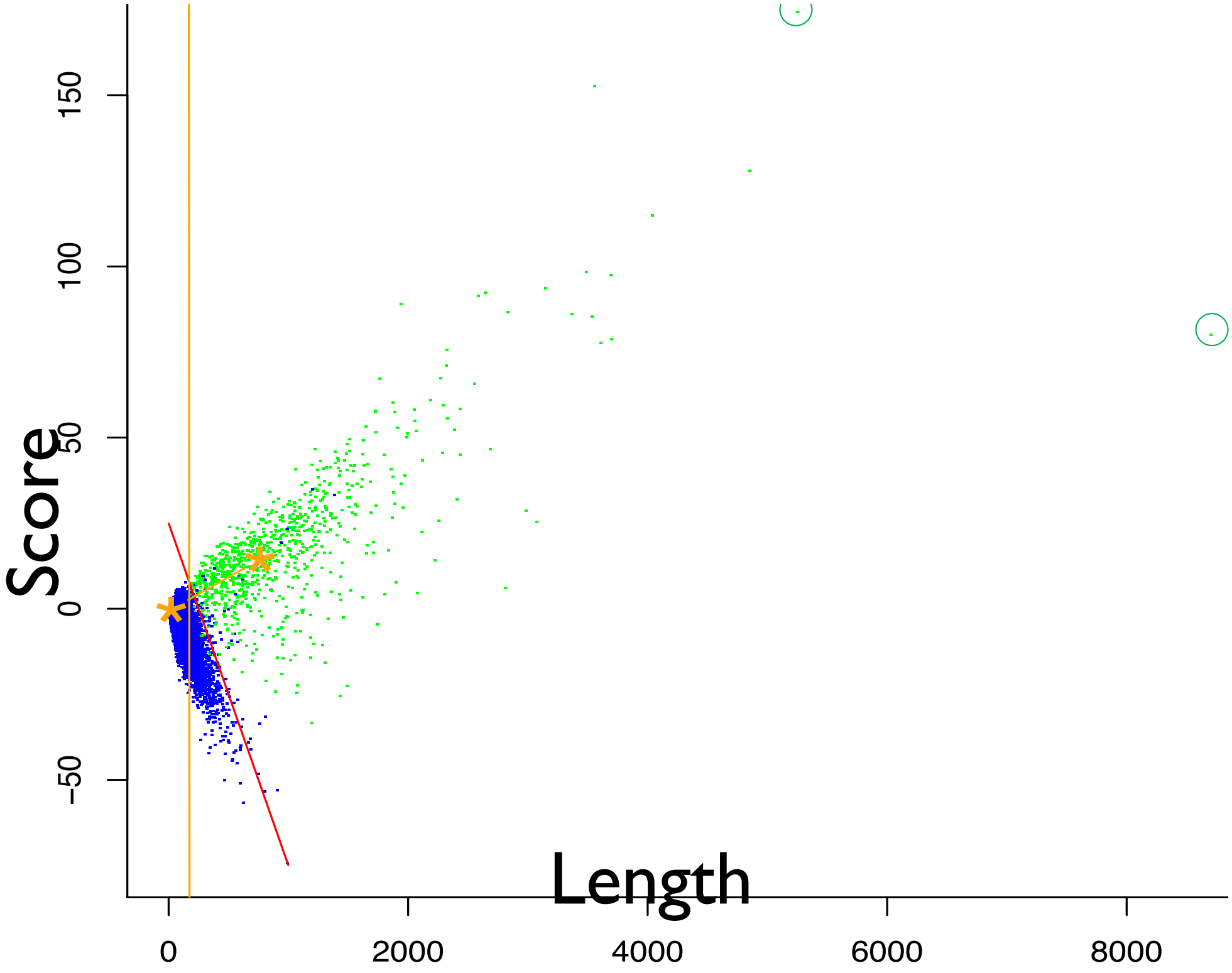
CSEP 527
Computational Biology

RNA: Function, Secondary Structure
Prediction, Search, Discovery

Blue = ORF length threshold; Green = Markov Model threshold




Score
150
100
50
0
−50
Length
0
2000
4000
6000
8000

CSEP 527
Computational Biology

RNA: Function, Secondary Structure
Prediction, Search, Discovery

The Message

Cells make lots of ~~RNA~~ *noncoding* RNA

Functionally important, functionally diverse

Structurally complex

New tools required

alignment, discovery, search, scoring, etc.

Rough Outline

- Today
  - Noncoding RNA Examples
  - RNA structure prediction
- Next Time
  - RNA “motif” models
  - Search
  - Motif discovery

RNA

DNA: Deoxyribo Nucleic Acid

RNA: RiboNucleic Acid

Like DNA, except:

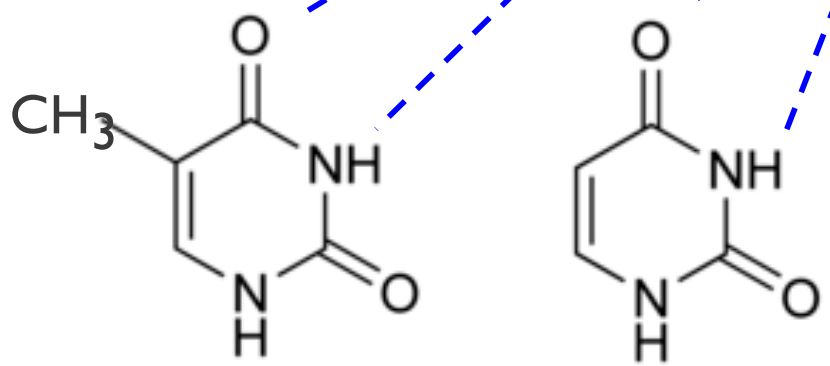
Adds an OH on ribose (backbone sugar)

Uracil (U) in place of thymine (T)

A, G, C as before

pairs with A

thymine



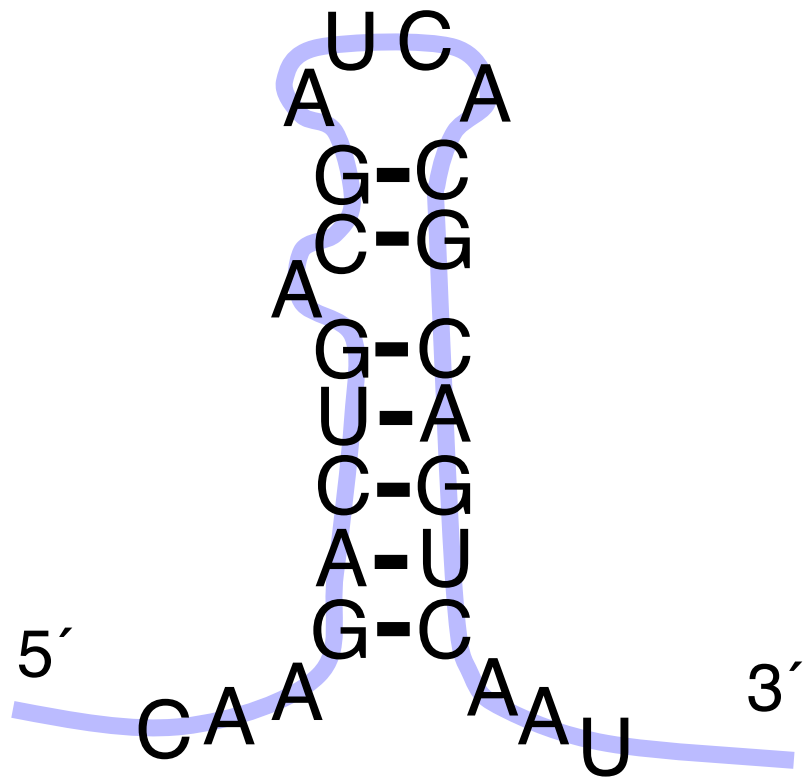
uracil

RNA Secondary Structure:
RNA makes helices too

Base pairs

A-U
C-G

U-G



Usually *single* stranded

NATURE VOL. 227 AUGUST 8 1970

Central Dogma of Molecular Biology

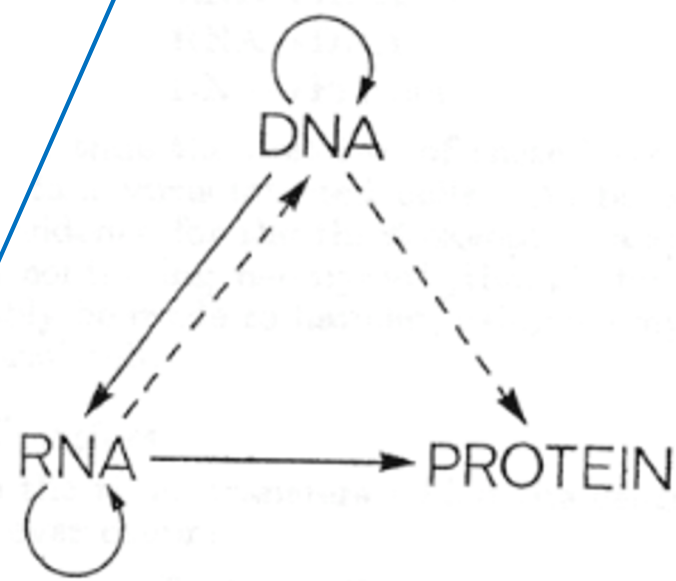
by
FRANCIS CRICK
MRC Laboratory
Hills Road,
Cambridge CB2 2QH

The central dogma of molecular biology deals with the detailed residue-by-residue transfer of sequential information. It states such information cannot be transferred from protein to either nucleic acid.

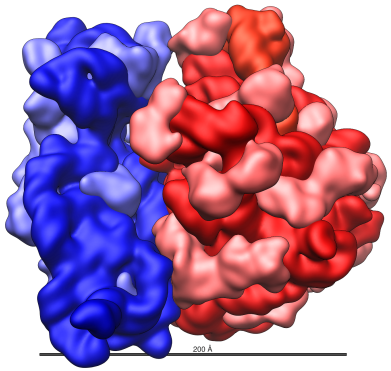
"The central dogma, the keystone of molecula considerable over-si

Re: Origins – Chicken & Egg Problem

Fig. 2. The arrows show the situa seemed in 1958. Solid arrows represent probable transfers, dotted arrows possible transfers. The absent arrows (compare Fig. 1) represent the impossible transfers postulated by the central dogma. They are the three possible arrows starting from protein.



“Classical” RNAs



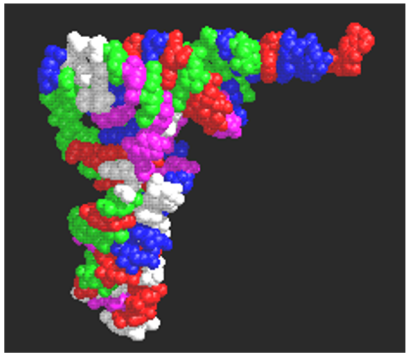
rRNA - ribosomal RNA (~4 kinds, 120-5k nt)

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

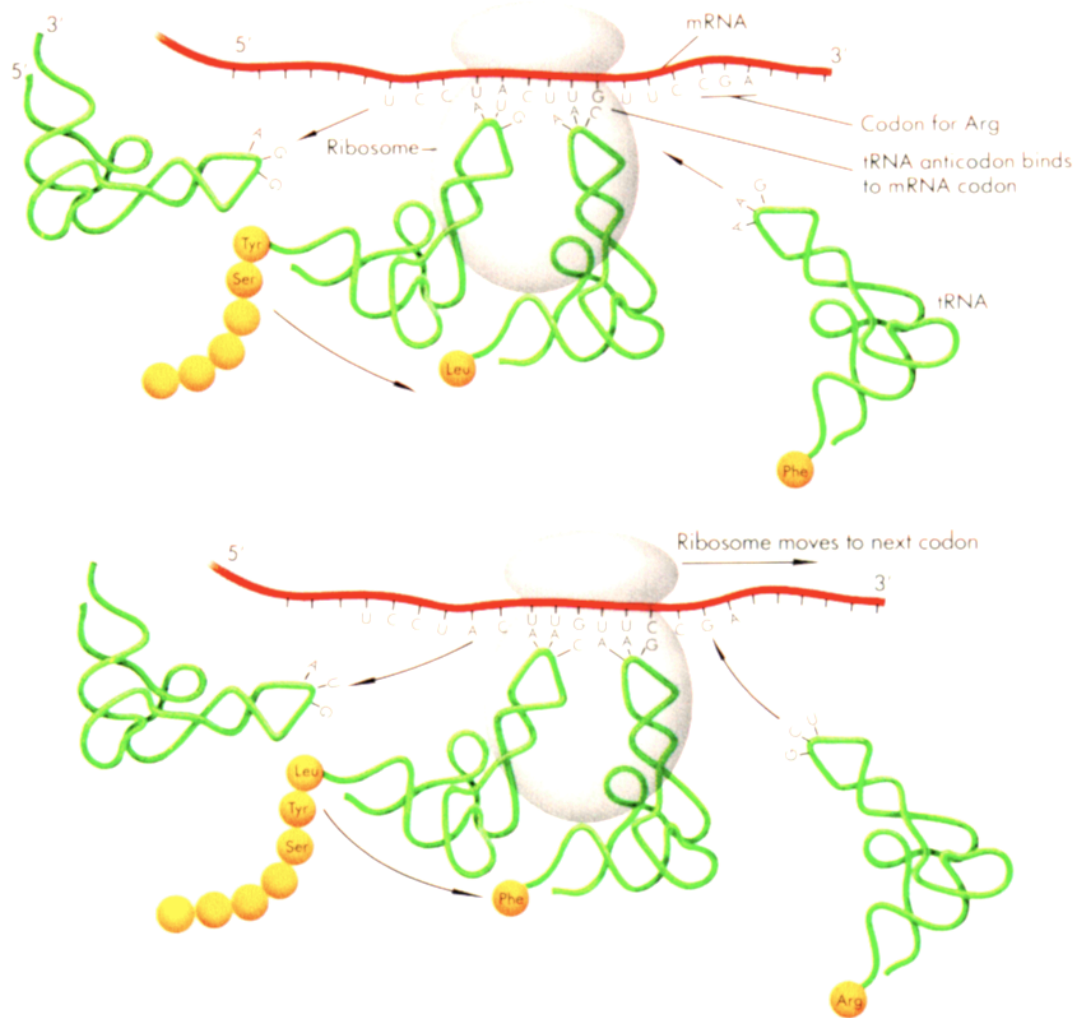
RNaseP - tRNA processing (~300 nt)

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

a handful of others



Ribosomes



Watson, Gilman, Witkowski, & Zoller, 1992

Ribosomes

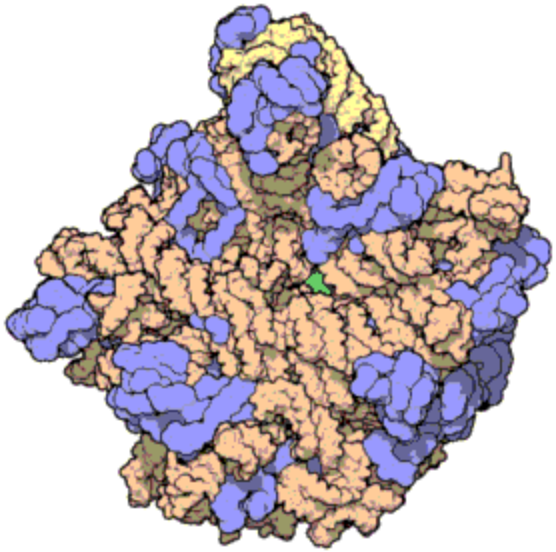
1974 Nobel prize to Romanian biologist George Palade (1912-2008) for discovery in mid 50's

50-80 proteins

3-4 RNAs (half the mass)

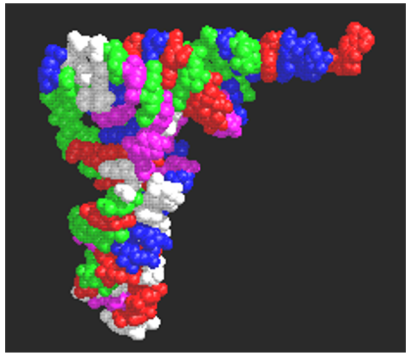
Catalytic core is RNA

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



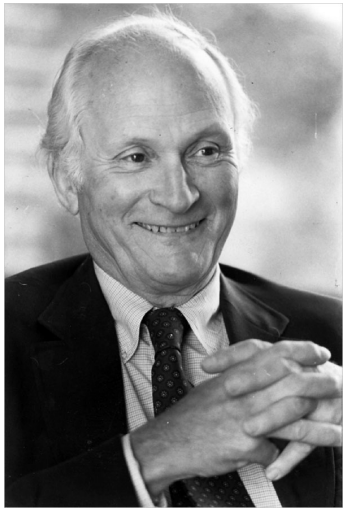
Atomic structure of the 50S Subunit from *Haloarcula marismortui*. Proteins are shown in blue and the two RNA strands in orange and yellow. The small patch of green in the center of the subunit is the active site.
- Wikipedia

Transfer RNA

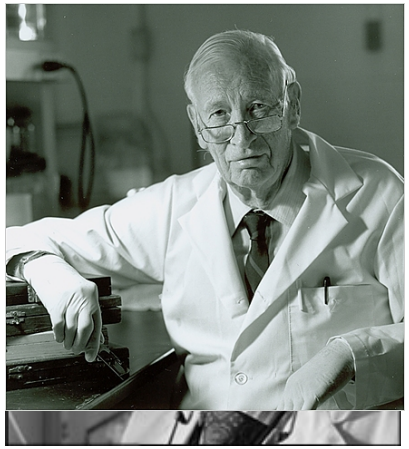


The "adapter" coupling mRNA to protein synthesis.

Discovered in the mid-1950s by



Mahlon Hoagland (1921-2009, left), Mary Stephenson, and Paul Zamecnik (1912-2009; Lasker award winner, right).



Bacteria

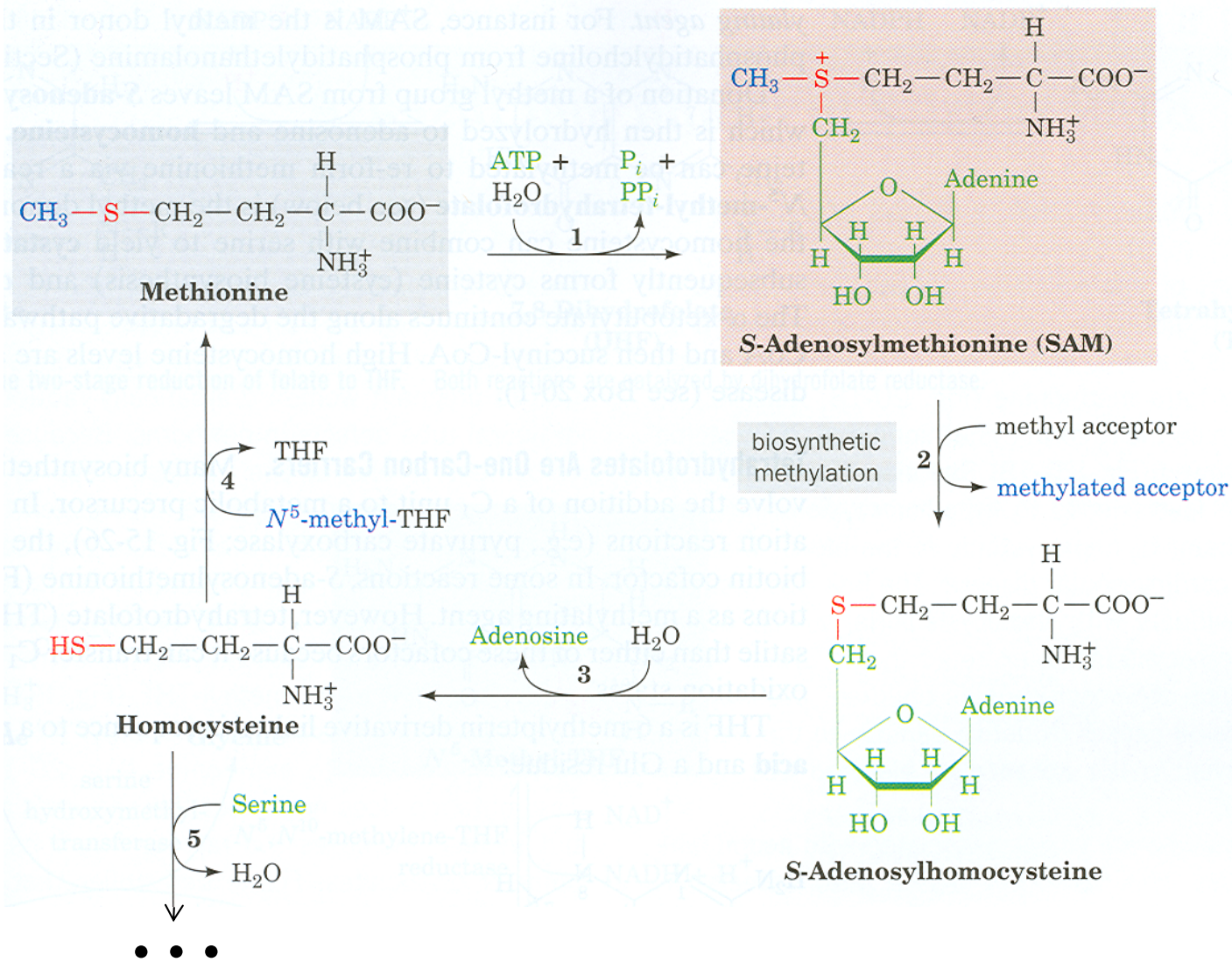
Triumph of proteins

50-80% of genome is coding DNA

Functionally diverse

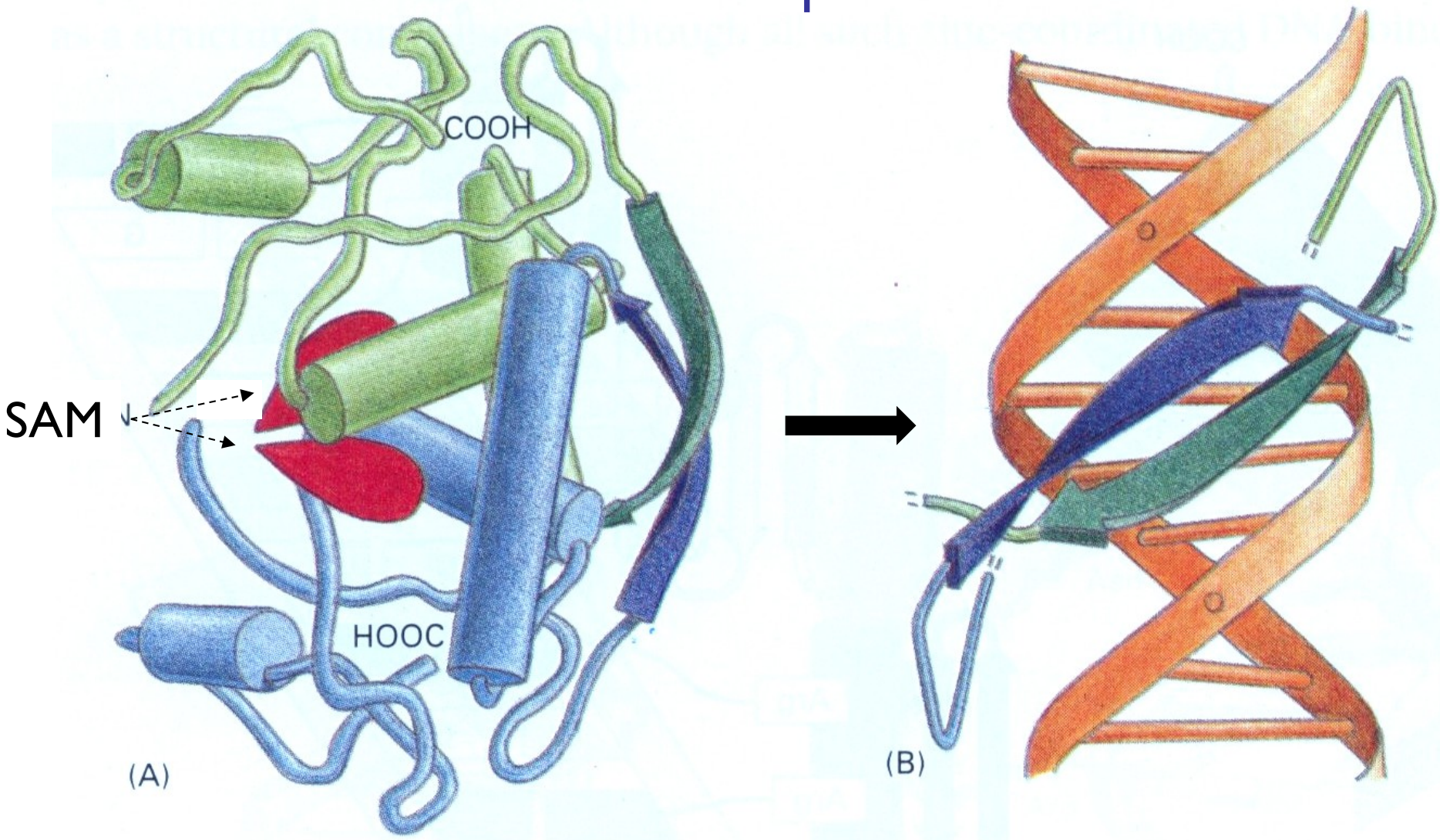
- receptors
- motors
- catalysts
- regulators (Monod & Jakob, Nobel prize 1965)
- …

Proteins Catalyze Biochemistry: Met Pathways



Proteins Regulate Biochemistry:

The MET Repressor



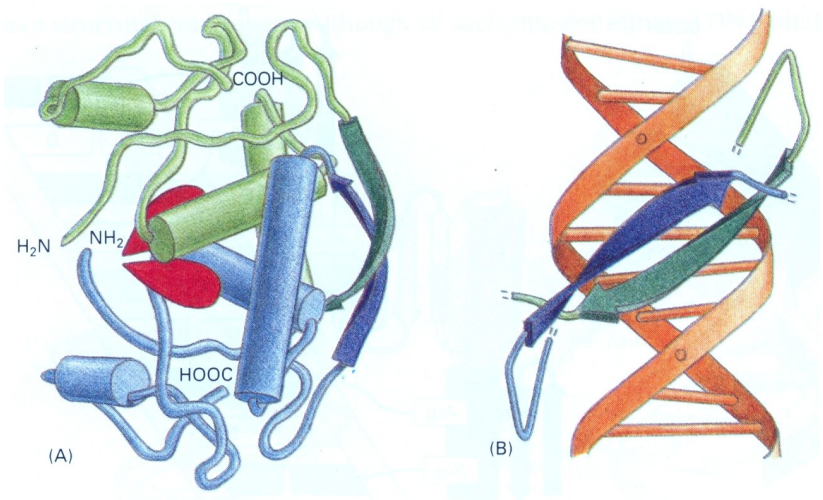
Protein

Alberts, et al, 3e.

DNA

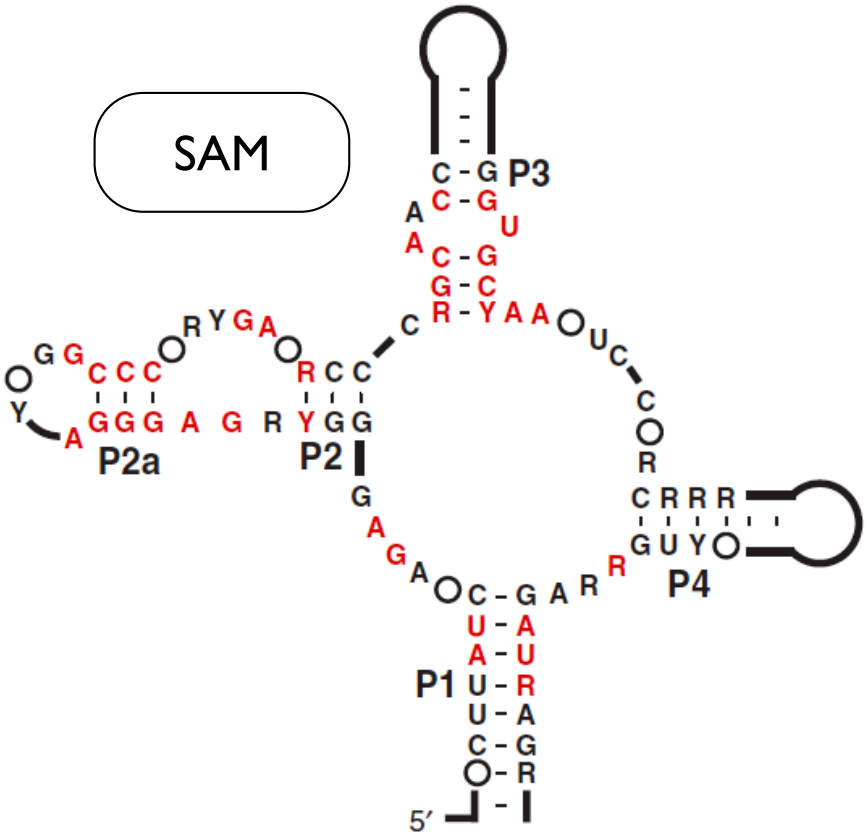
Not the only way!

Alberts, et al, 3e.



Protein way

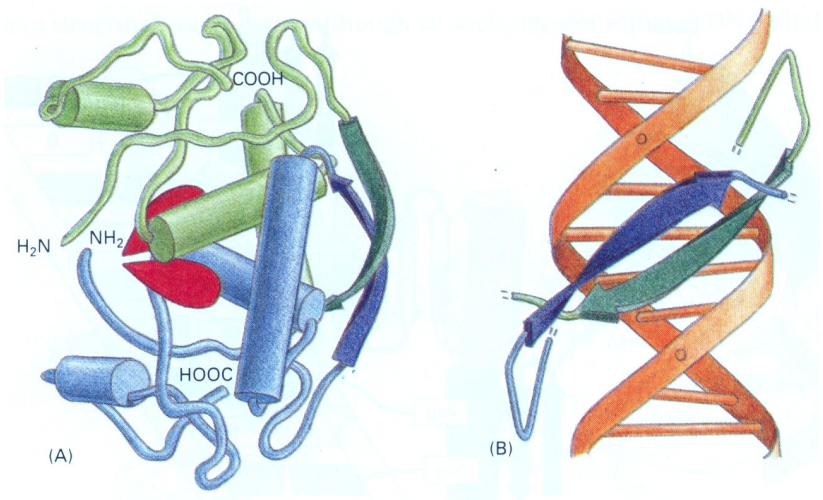
Riboswitch alternative



Grundy & Henkin, Mol. Microbiol 1998
Epshtein, et al., PNAS 2003
Winkler et al., Nat. Struct. Biol. 2003

Not the only way!

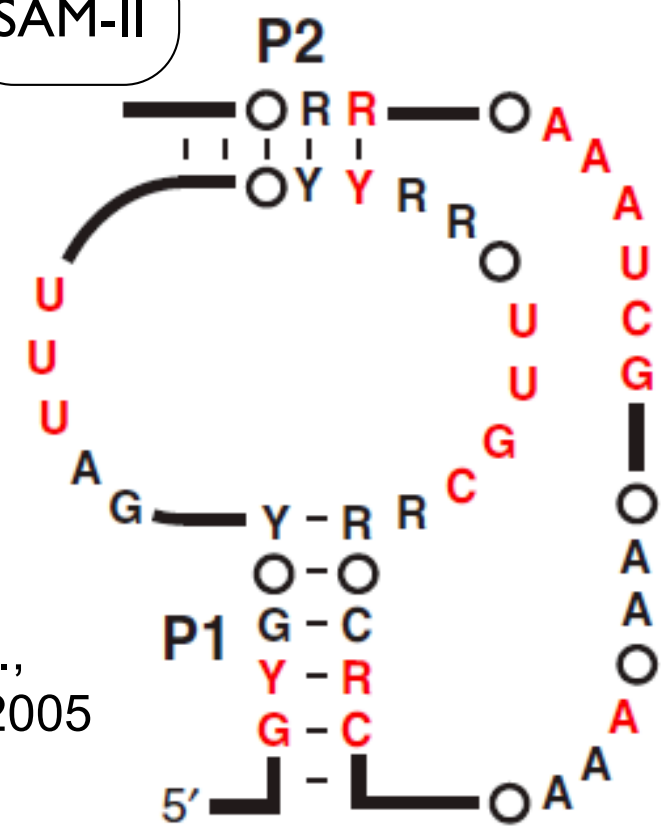
Alberts, et al, 3e.



Protein way

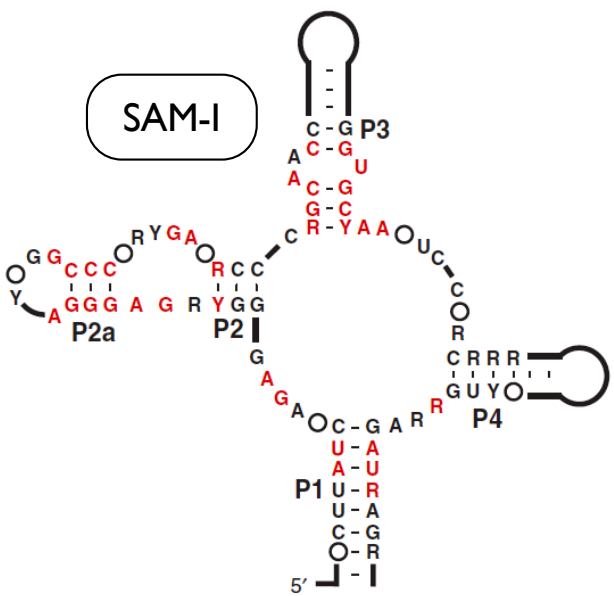
Riboswitch alternatives

SAM-II



Corbino et al., Genome Biol. 2005

SAM-I



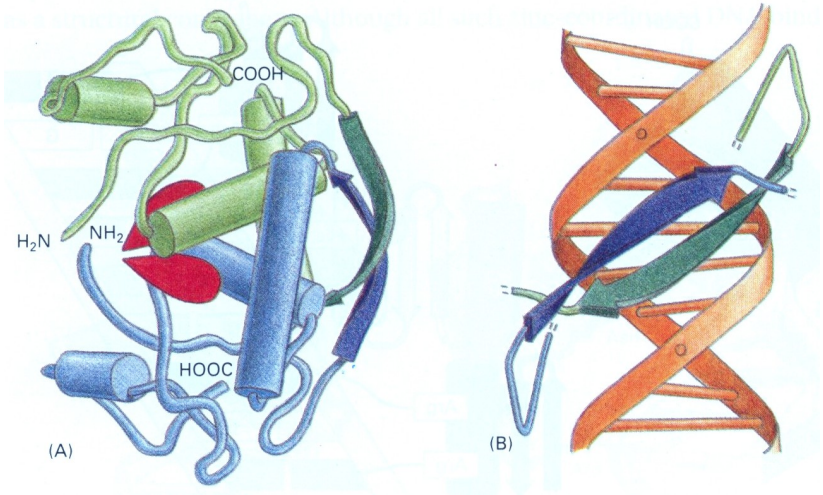
Grundy, Epshtein, Winkler et al., 1998, 2003

Not the only way!

Protein way

Riboswitch alternatives

Alberts, et al, 3e.



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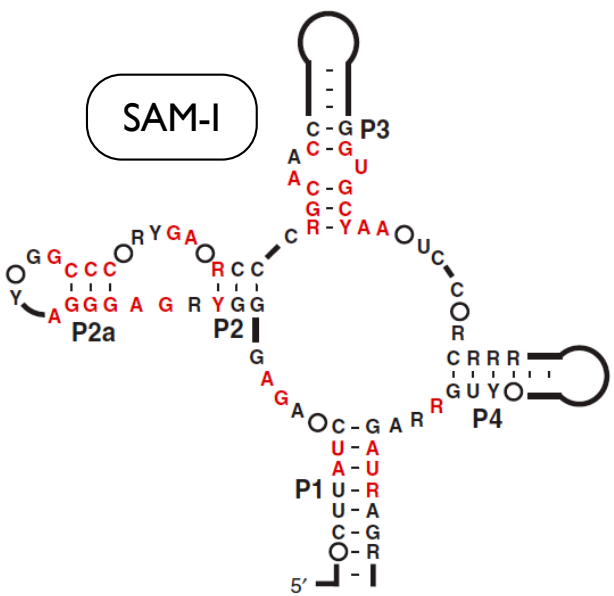
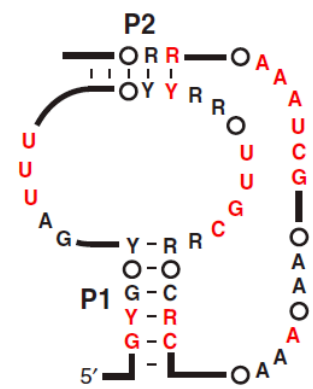


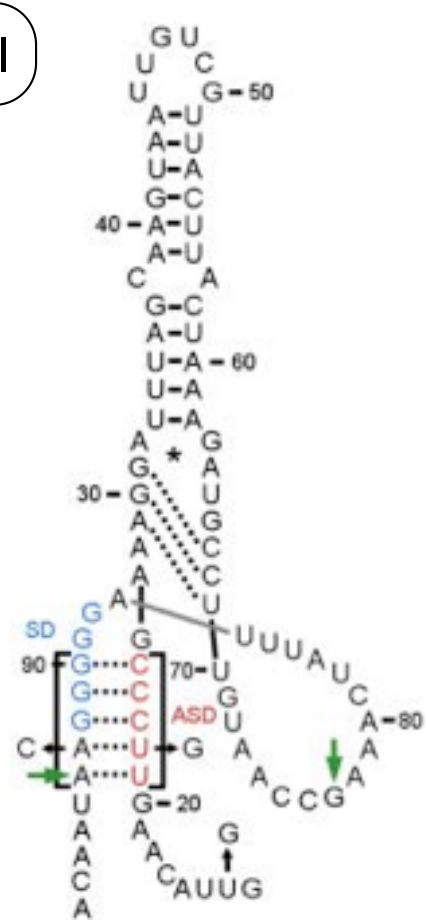
Grundy, Epshtein, Winkler et al., 1998, 2003

SAM-II



Corbino et al., Genome Biol. 2005

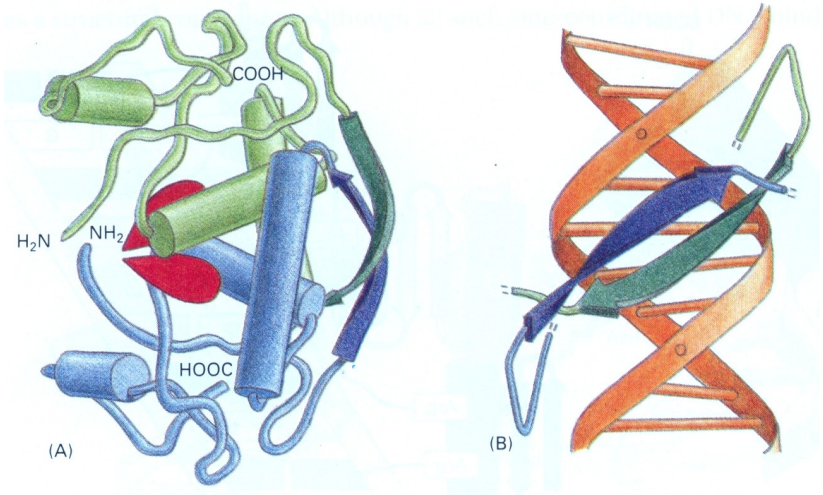
SAM-III



Fuchs et al., NSMB 2006

Not the only way!

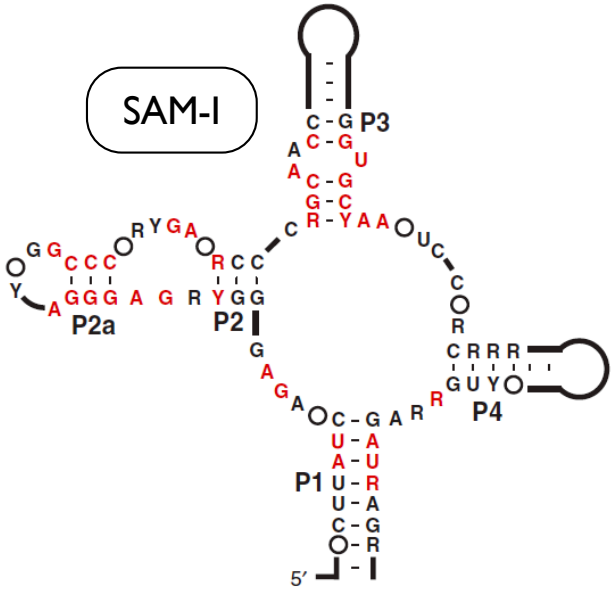
Alberts, et al, 3e.



Protein way

Riboswitch alternatives

SAM-I



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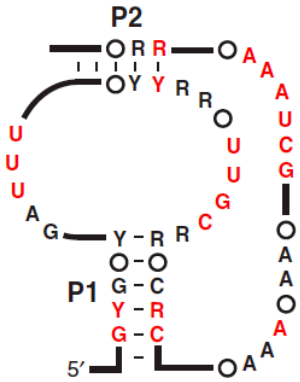

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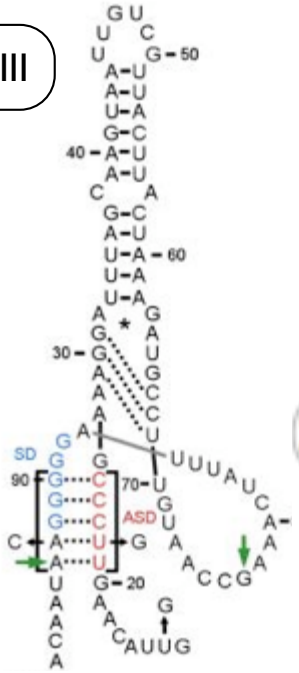

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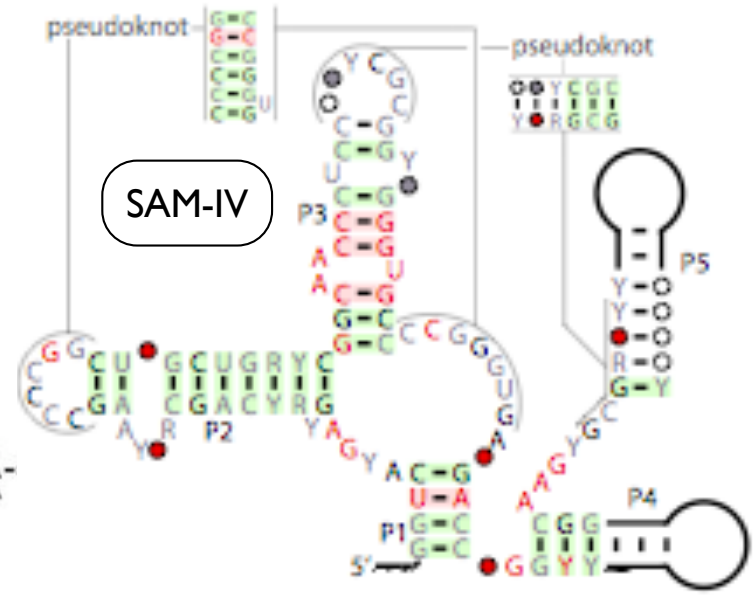


Grundy, Epshtein, Winkler et al., 1998, 2003

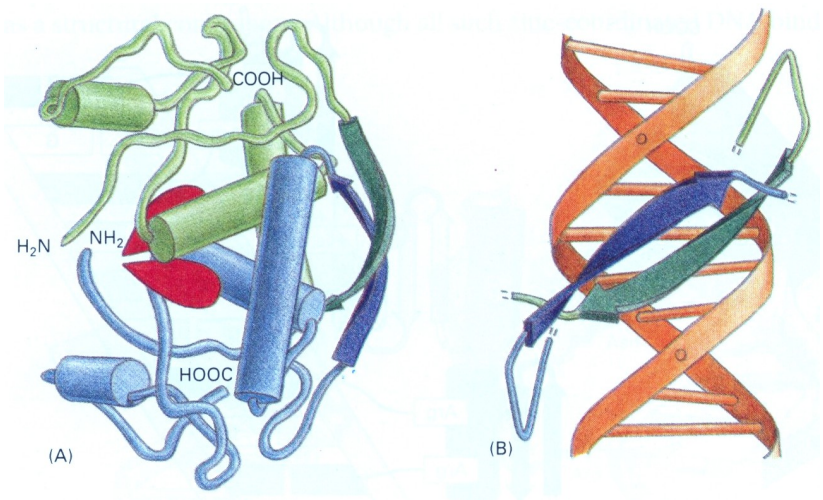
Corbino et al., Genome Biol. 2005

Fuchs et al., NSMB 2006

Weinberg et al., RNA 2008

Not the only way!

Alberts, et al, 3e.



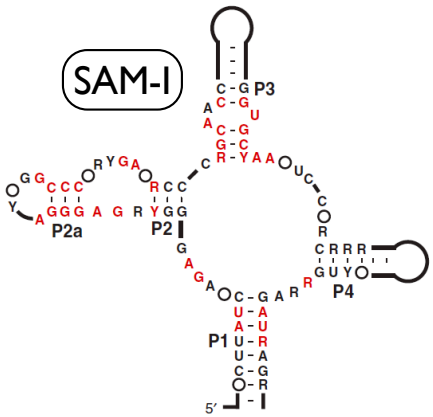
Protein way

Riboswitch alternatives

SAM-VI

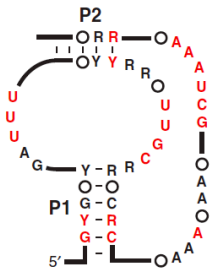
...

SAM-I



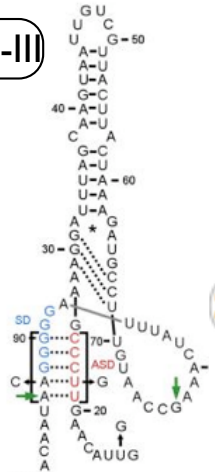
Grundy, Epshtein, Winkler et al., 1998, 2003

SAM-II



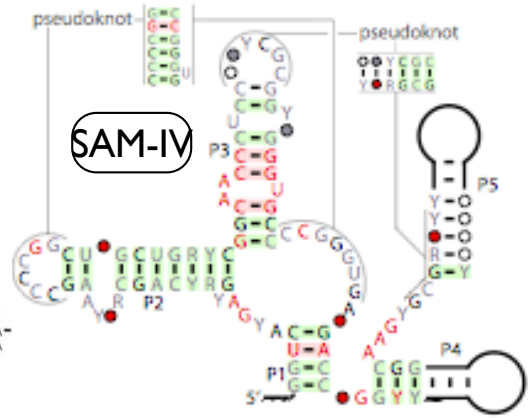
Corbino et al., Genome Biol. 2005

SAM-III



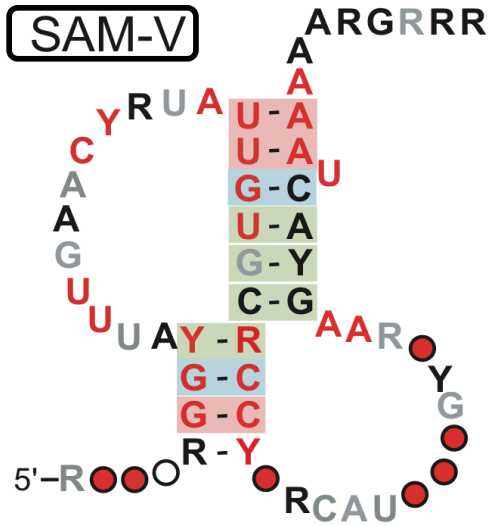
Fuchs et al., NSMB 2006

SAM-IV



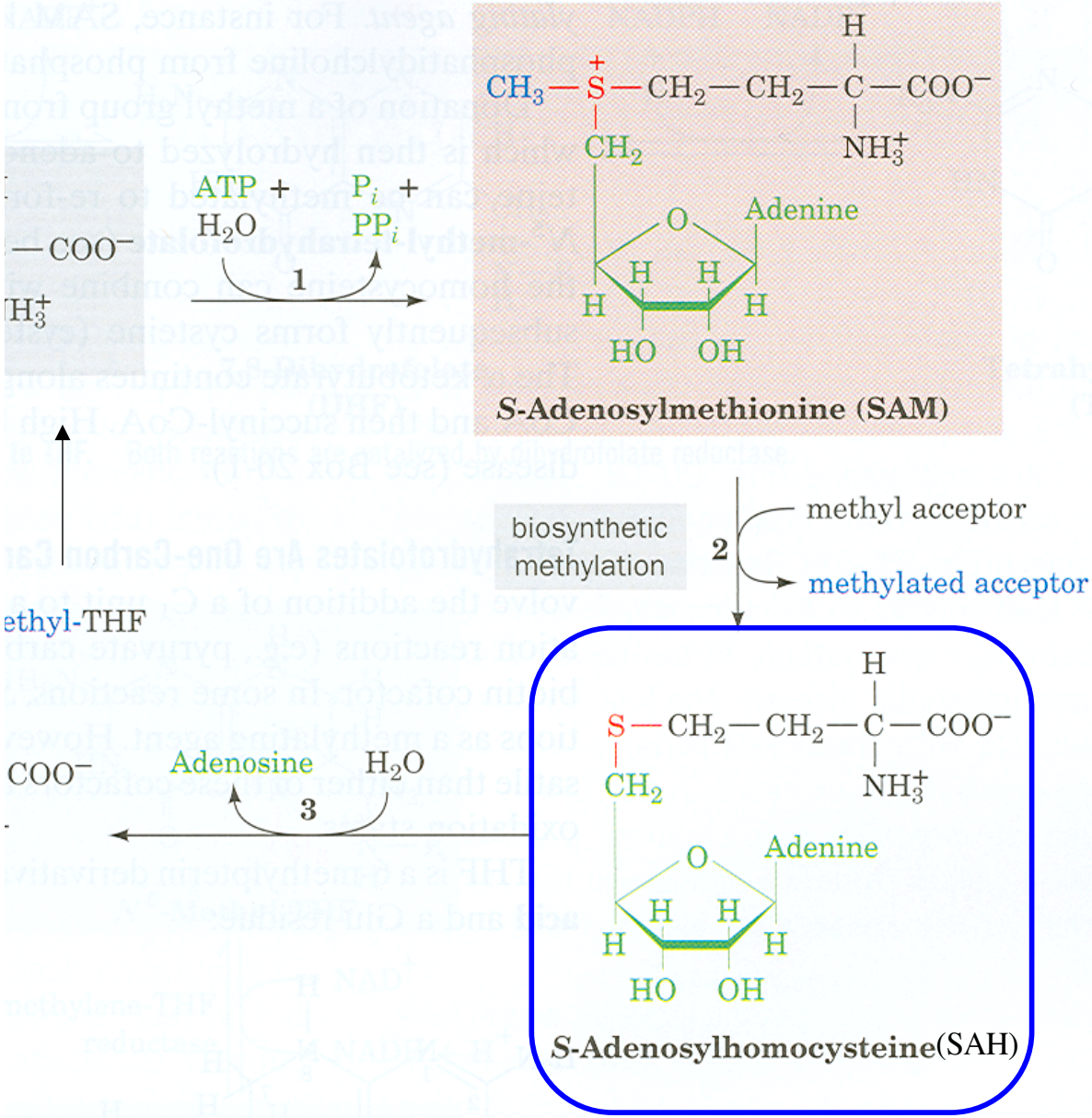
Weinberg et al., RNA 2008

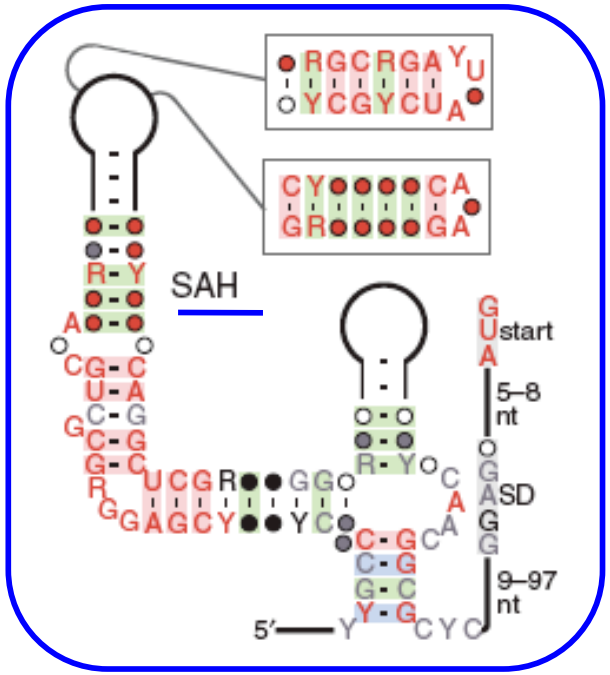
SAM-V



Meyer, etal., BMC Genomics 2009

And in other bacteria, a riboswitch senses SAH





ncRNA Example: Riboswitches

UTR structure that directly senses/binds small molecules & regulates mRNA

widespread in prokaryotes

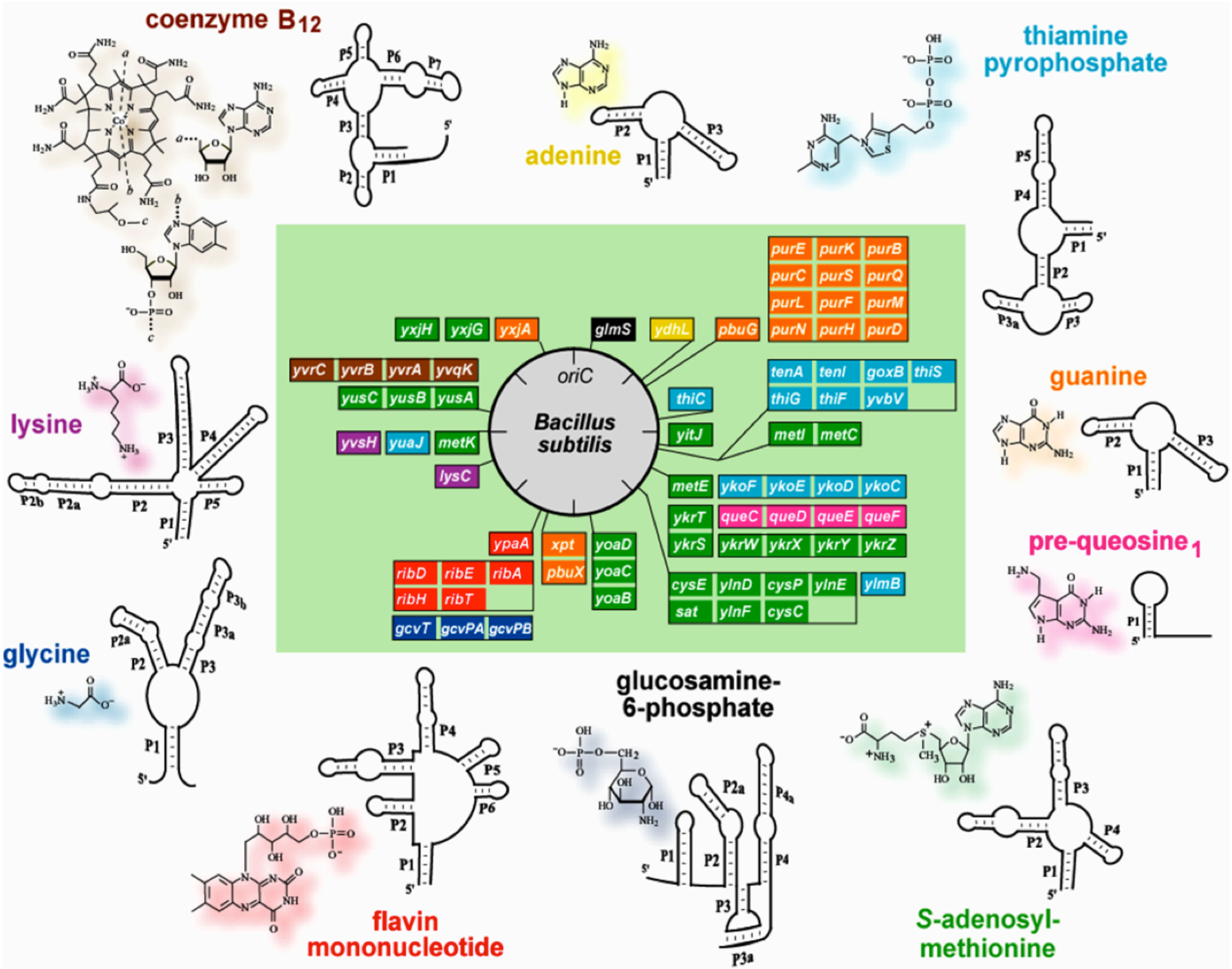
some in eukaryotes & archaea, one in a phage

~ 20 ligands known; multiple nonhomologous solutions for some

dozens to hundreds of instances of each

on/off; transcription/translation; splicing; combinatorial control

all found since ~2003; most via bioinformatics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thiamine pyrophosphate
guanine
pre-queosine1
lysine
glycine
glucosamine-6-phosphate
flavin mononucleotide
S-adenosyl-methionine
Bacillus subtilis
oriC
yxjH yxjG yxjA glmS ydhL pbuG
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thiC
yvsH yuaJ metK
yitJ
metI metC
lysC
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ykrT queC queD queE queF
ykrS ykrW ykrX ykrY ykrZ
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ribD ribE ribA pbuX yoaC
ribH ribT yoaB
cysE ylnD cysP ylnE ylmB
sat ylnF cysC
gcvT gcvPA gcvPB

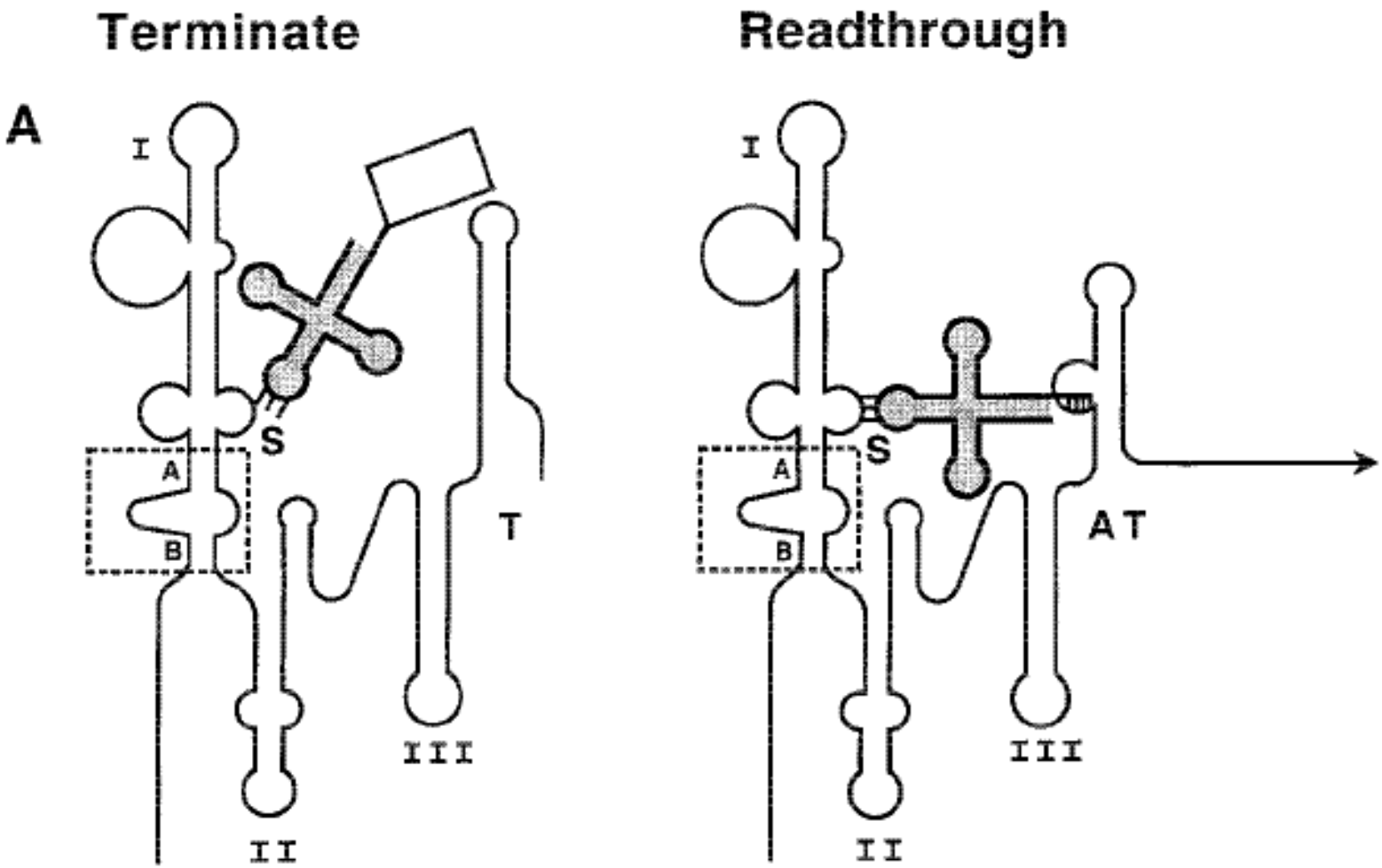
New Antibiotic Targets?

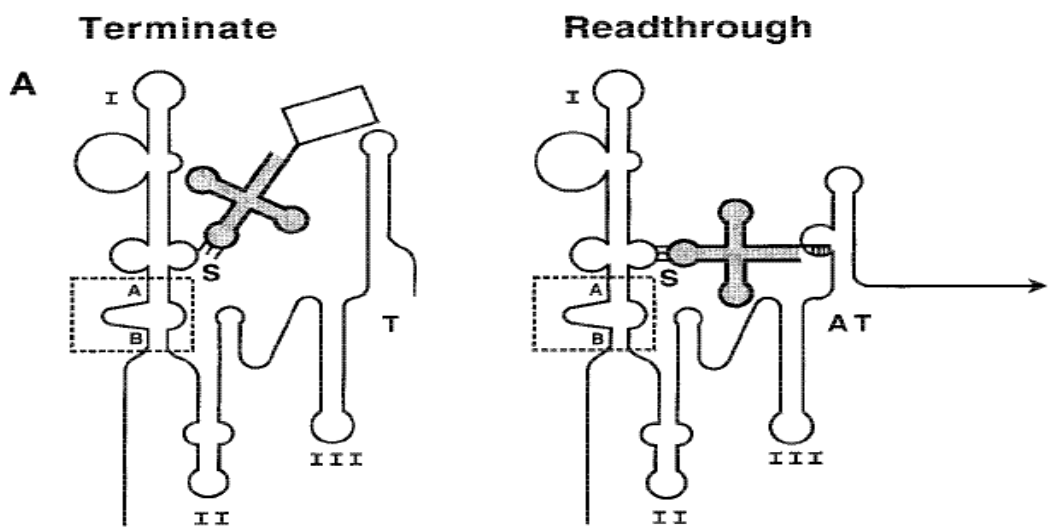
Old drugs, new understanding:

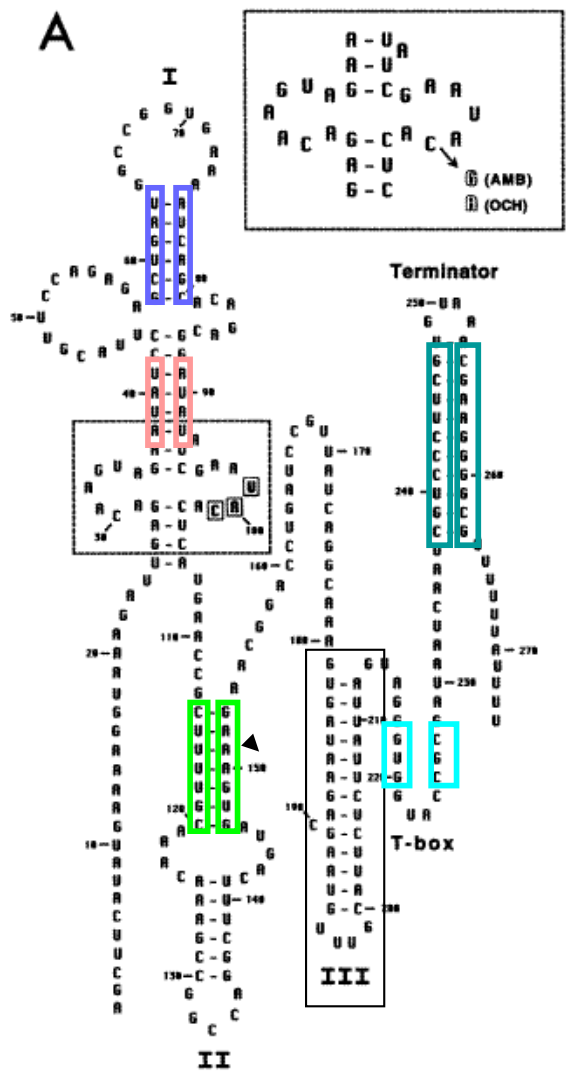
- TPP riboswitch ~ pyrithiamine
- lysine riboswitch ~ L-aminoethylcysteine, DL-4-oxalysine
- FMN riboswitch ~ roseoflavin

Potential advantages - no (known) human riboswitches, but often multiple copies in bacteria, so potentially efficacious with few side effects?

ncRNA Example: T-boxes





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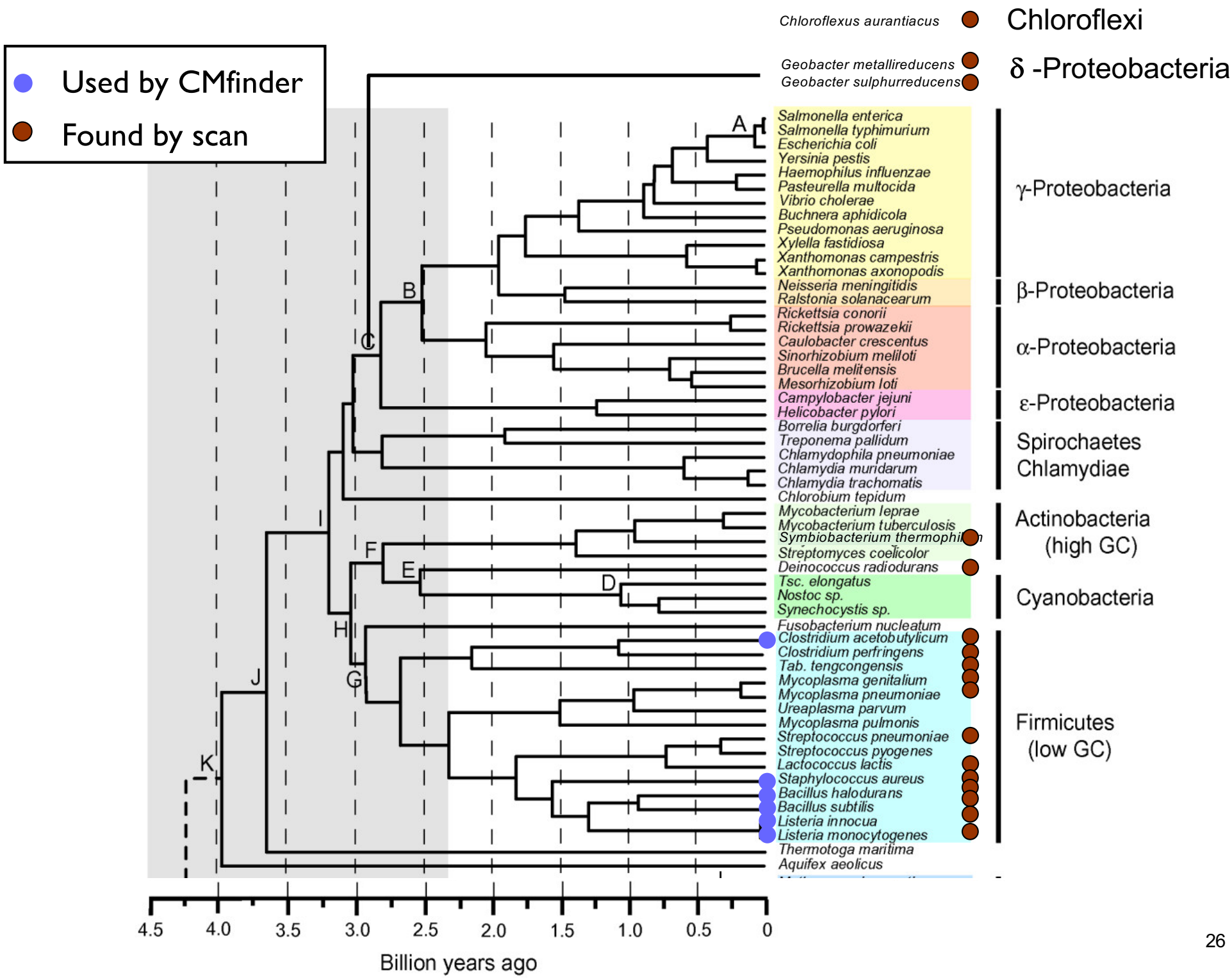
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Geobacter sulphurreducens
δ -Proteobacteria
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Escherichia coli
Yersinia pestis
Haemophilus influenzae
Pasteurella multocida
Vibrio cholerae
Buchnera aphidicola
Pseudomonas aeruginosa
Xylella fastidiosa
Xanthomonas campestris
Xanthomonas axonopodis
γ-Proteobacteria
Neisseria meningitidis
Ralstonia solanacearum
β-Proteobacteria
Rickettsia conorii
Rickettsia prowazekii
Caulobacter crescentus
Sinorhizobium meliloti
Brucella melitensis
Mesorhizobium loti
α-Proteobacteria
Campylobacter jejuni
Helicobacter pylori
ε-Proteobacteria
Borrelia burgdorferi
Treponema pallidum
Chlamydophila pneumoniae
Chlamydia muridarum
Chlamydia trachomatis
Spirochaetes
Chlamydiae
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Mycobacterium tuberculosis
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Deinococcus radiodurans
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Clostridium perfringens
Tab. tengcongensis
Mycoplasma genitalium
Mycoplasma pneumoniae
Ureaplasma parvum
Mycoplasma pulmonis
Streptococcus pneumoniae
Streptococcus pyogenes
Lactococcus lactis
Staphylococcus aureus
Bacillus halodurans
Bacillus subtilis
Listeria innocua
Listeria monocytogenes
Firmicutes
(low GC)
Thermotoga maritima
Aquifex aeolicus
A
B
C
D
E
F
G
H
I
J
K
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Billion years ago

ncRNA Example: 6S

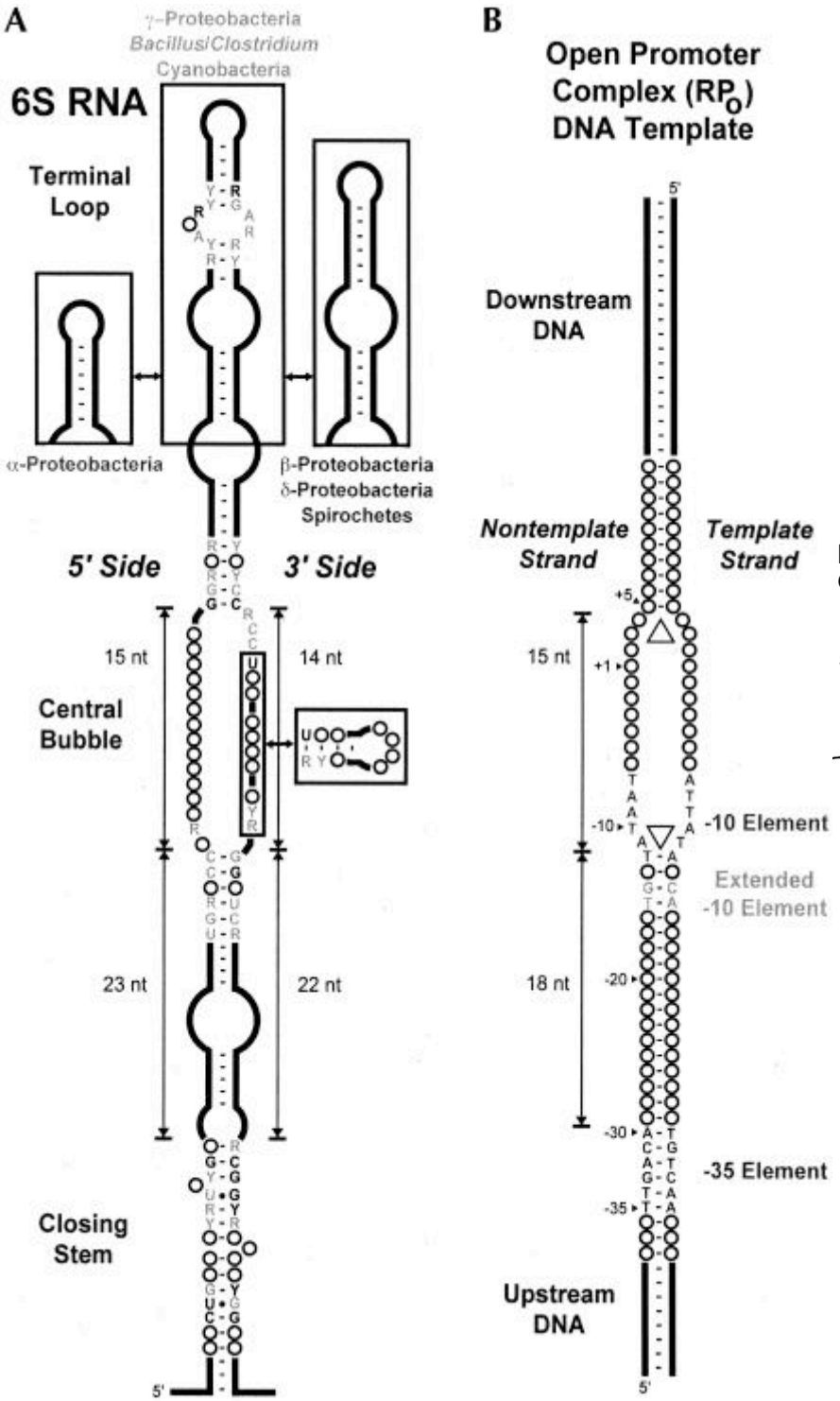
medium size (175nt)

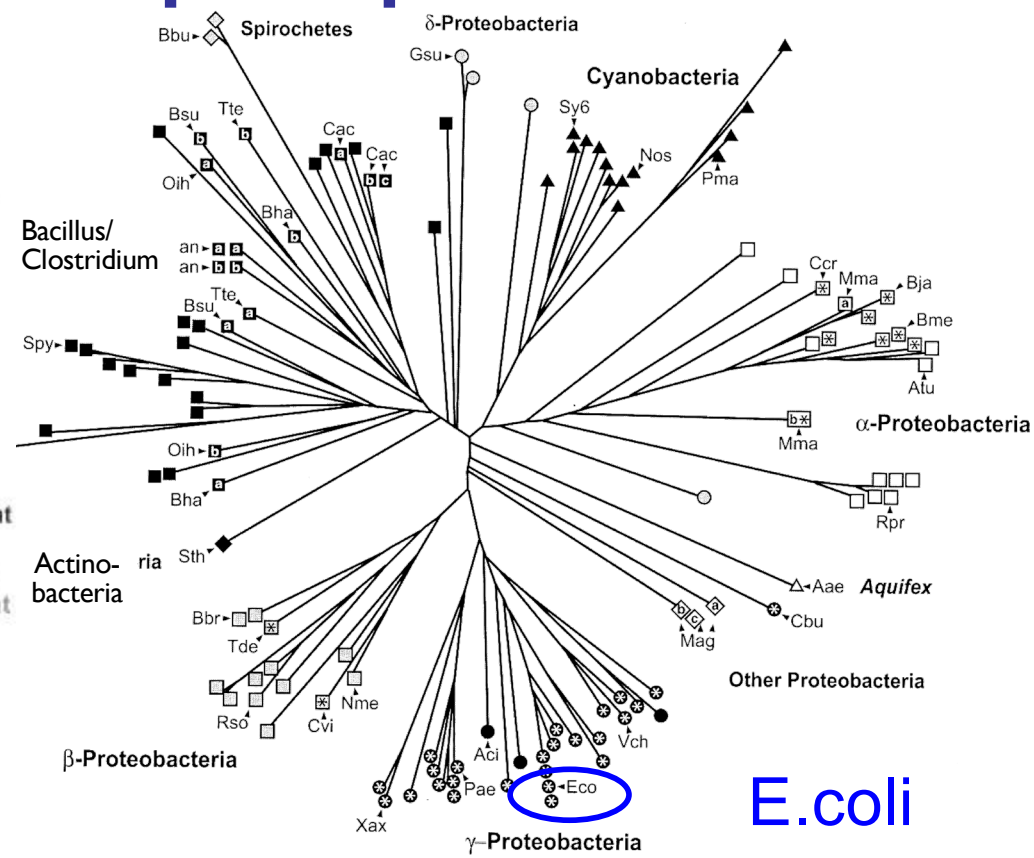
structured

highly expressed in E. coli in certain growth conditions

sequenced in 1971; function unknown for 30 years

6S mimics an open promoter





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

Summary: RNA in Bacteria

Widespread, deeply conserved, structurally sophisticated, functionally diverse, biologically important uses for ncRNA throughout prokaryotic world.

Regulation of MANY genes involves RNA

- In some species, we know identities of more ribo-regulators than protein regulators

Dozens of classes & thousands of new examples in just the last ~10-15 years

Vertebrates

Bigger, more complex genomes

<2% coding

But >5% conserved in sequence?

And 50-90% transcribed?

And *structural* conservation, if any, invisible

(without proper alignments, etc.)

What's going on?

Vertebrate ncRNAs

mRNA, tRNA, rRNA, … of course

PLUS:

snRNA, spliceosome, snoRNA, teleomerase, microRNA, RNAi, SECIS, IRE, piwi-RNA, XIST (X-inactivation), ribozymes, …

MicroRNA

1st discovered 1992 in C. elegans

2nd discovered 2000, also C. elegans

and human, fly, everything between – basically all multi-celled plants & animals

21-23 nucleotides

literally fell off ends of gels

100s – 1000s now known in human

may regulate 1/3-1/2 of all genes

development, stem cells, cancer, infectious disease,…

siRNA

2006 Nobel Prize
Fire & Mello

"Short Interfering RNA"

Also discovered in *C. elegans*

Possibly an antiviral defense, shares machinery with miRNA pathways

Allows artificial repression of most genes in most higher organisms

Huge tool for biology & biotech

ncRNA Example: Xist

large (≈12kb)

largely unstructured RNA

required for X-inactivation in mammals

(Remember calico cats?)

One of many thousands of "Long NonCoding RNAs" (lncRNAs) now recognized, tho most others are of completely unknown significance

Human Predictions

Evofold

S Pedersen, G Bejerano, A Siepel, K Rosenbloom, K Lindblad-Toh, ES Lander, J Kent, W Miller, D Haussler, "Identification and classification of conserved RNA secondary structures in the human genome." PLoS Comput. Biol., 2, #4 (2006) e33.

48,479 candidates (~70% FDR?)

FOLDALIGN

E Torarinsson, M Sawera, JH Havgaard, M Fredholm, J Gorodkin, "Thousands of corresponding human and mouse genomic regions unalignable in primary sequence contain common RNA structure." *Genom. Res.*, 16, #7 (2006) 885-9.

1800 candidates from 369700 (of 100,000) pairs

RNAz

S Washietl, IL Hofacker, M Lukasser, A Hutenhofer, PF Stadler, "Mapping of conserved RNA secondary structures predicts thousands of functional noncoding RNAs in the human genome." Nat. Biotechnol., 23, #11 (2005) 1383-90.

30,000 structured RNA elements

1,000 conserved across all vertebrates.

~1/3 in introns of known genes, ~1/6 in UTRs

~1/2 located far from any known gene

CMfinder

Torarinsson, Yao, Wiklund, Bramsen, Hansen, Kjems, Tommerup, Ruzzo and Gorodkin. Comparative genomics beyond sequence based alignments: RNA structures in the ENCODE regions. *Genome Research*, Feb 2008, 18(2):242-251 PMID: 18096747

Seemann, Mirza, Hansen, Bang-Berthelsen, Garde, Christensen-Dalsgaard, Torarinsson, Yao, Workman, Pociot, Nielsen, Tommerup, Ruzzo, Gorodkin. The identification and functional annotation of RNA structures conserved in vertebrates. Genome Res, Aug 2017, 27(8):1371-1383 PMID: 28487280.

Thousands of Predictions

Bottom line?

A significant number of "one-off" examples

Extremely wide-spread ncRNA expression

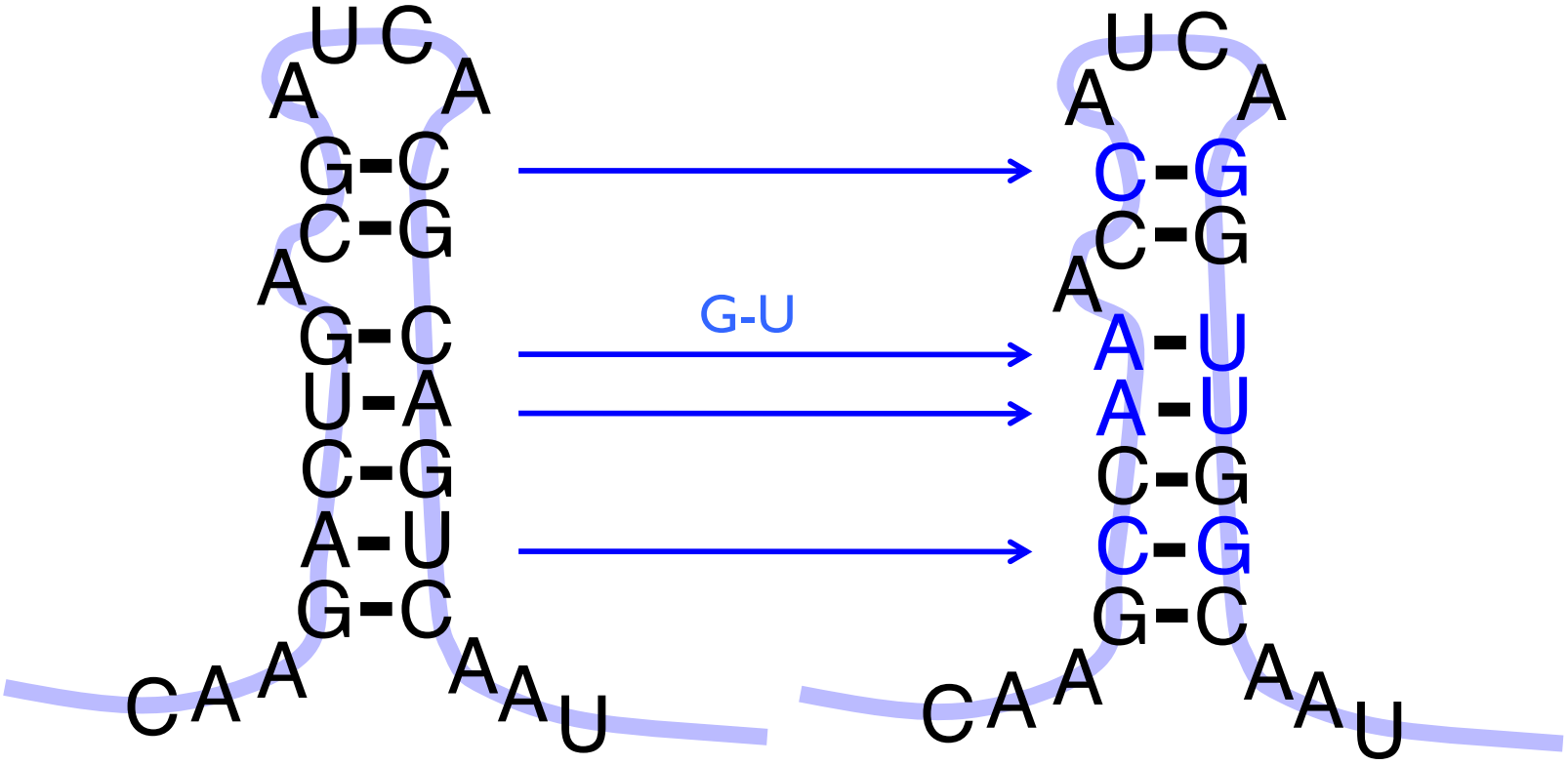
At a minimum, a vast evolutionary substrate

New technology (e.g., RNAseq) exposing more

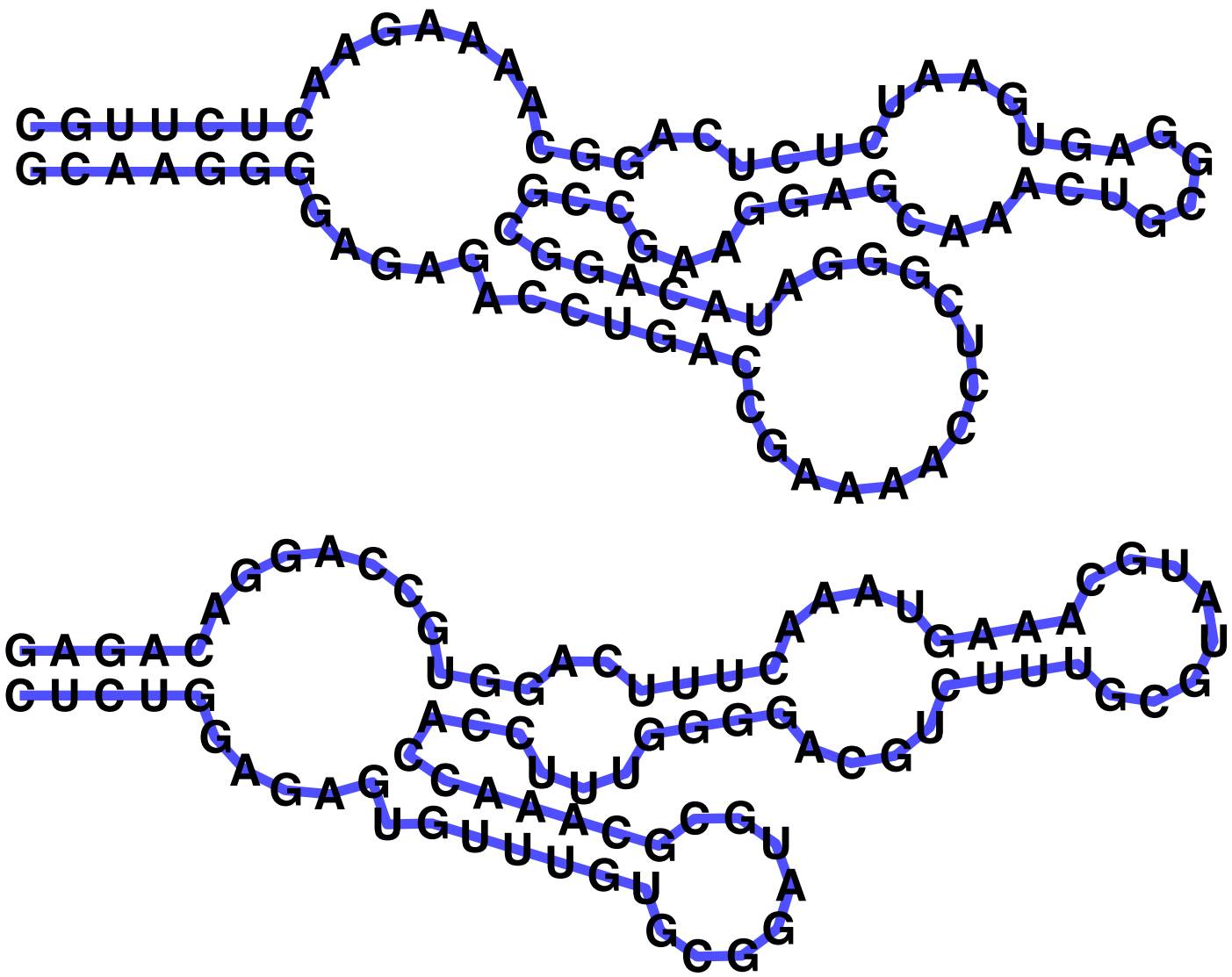
How do you recognize an interesting one?

A Clue: Conserved secondary structure

RNA Secondary Structure: can be fixed while sequence evolves



Why is RNA hard to deal with?



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

Structure Prediction

RNA Structure

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

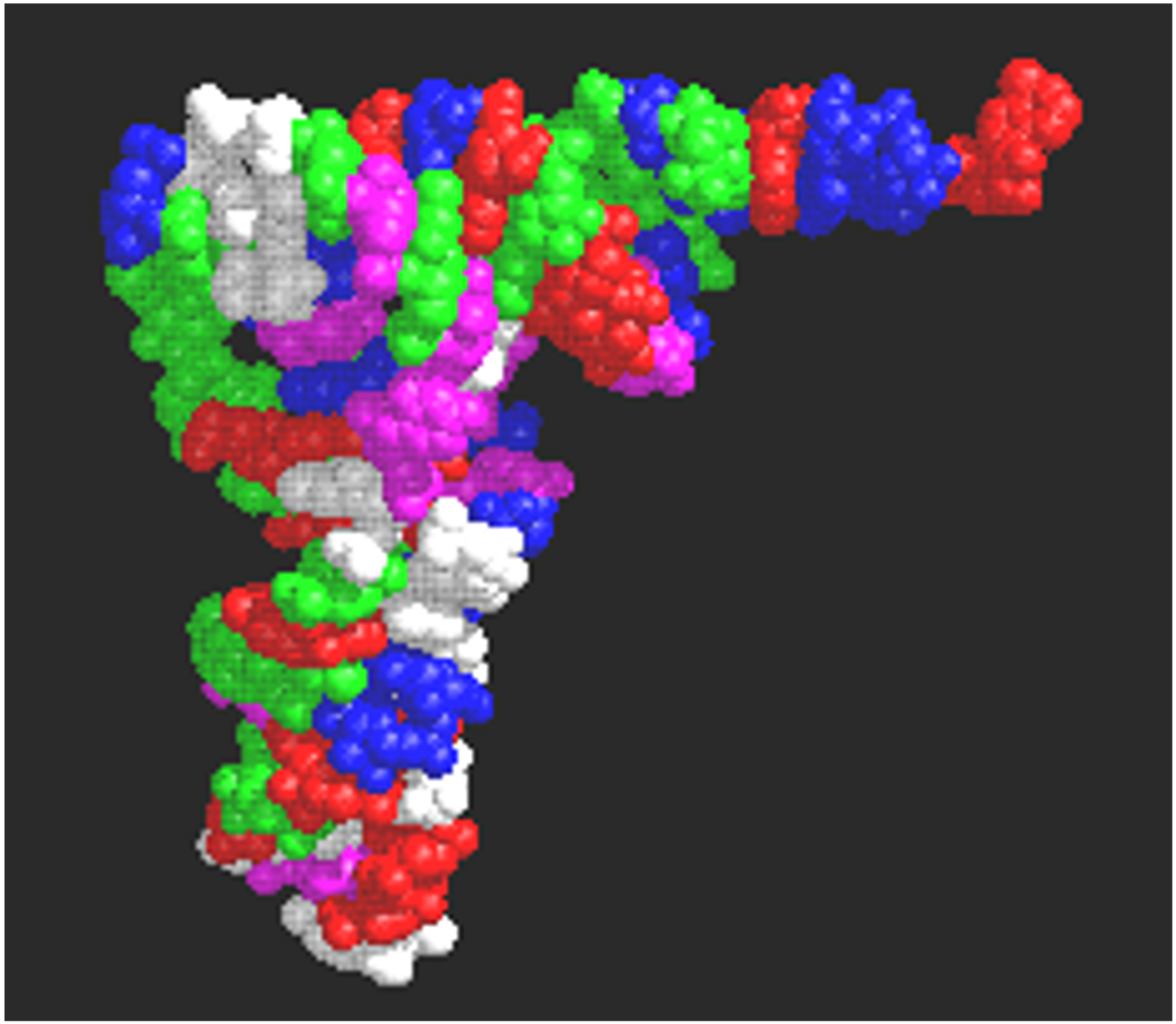
RNA Pairing

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)

tRNA 3d Structure



tRNA - Alt. Representations

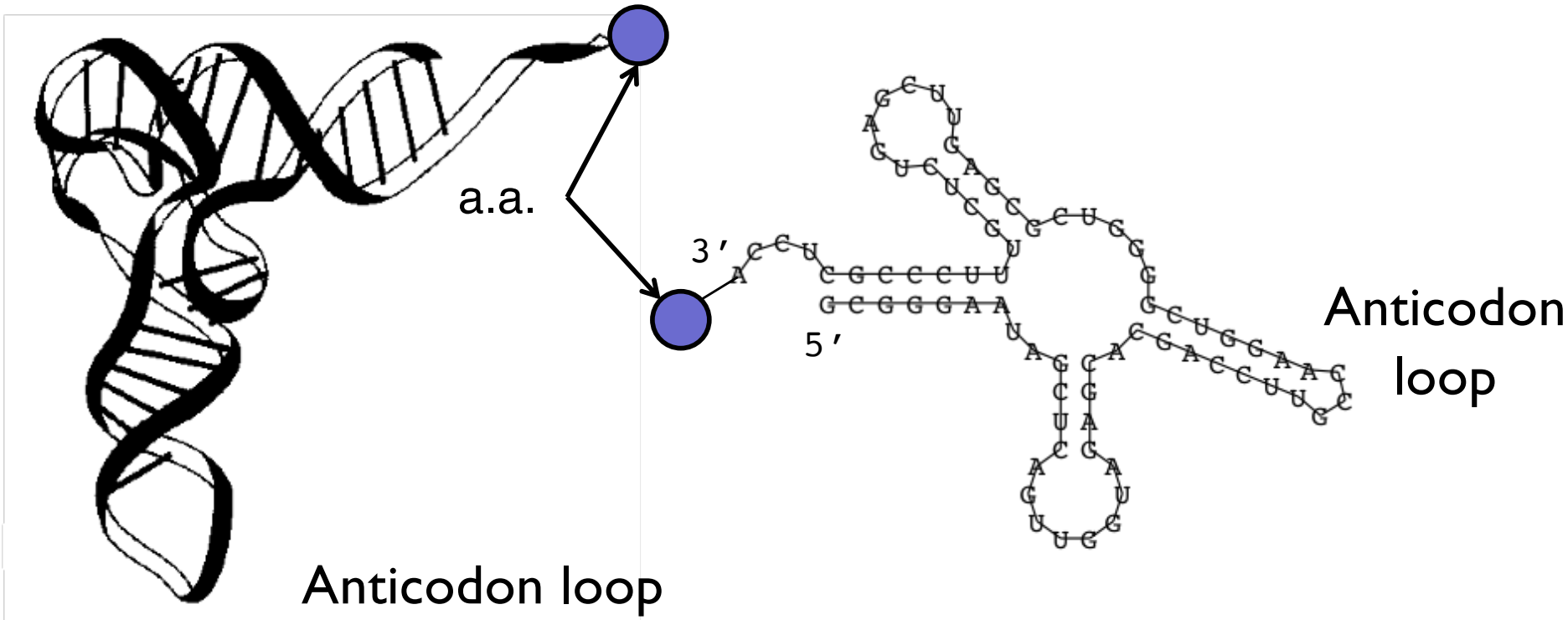
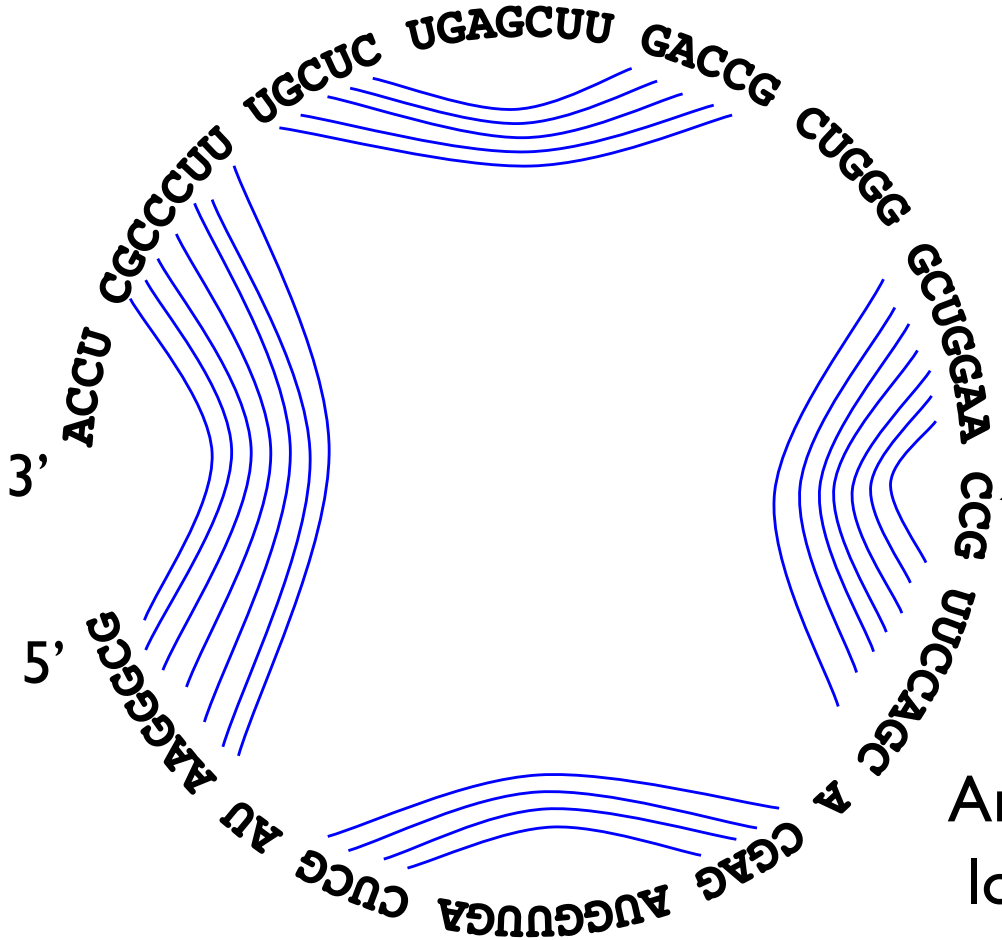


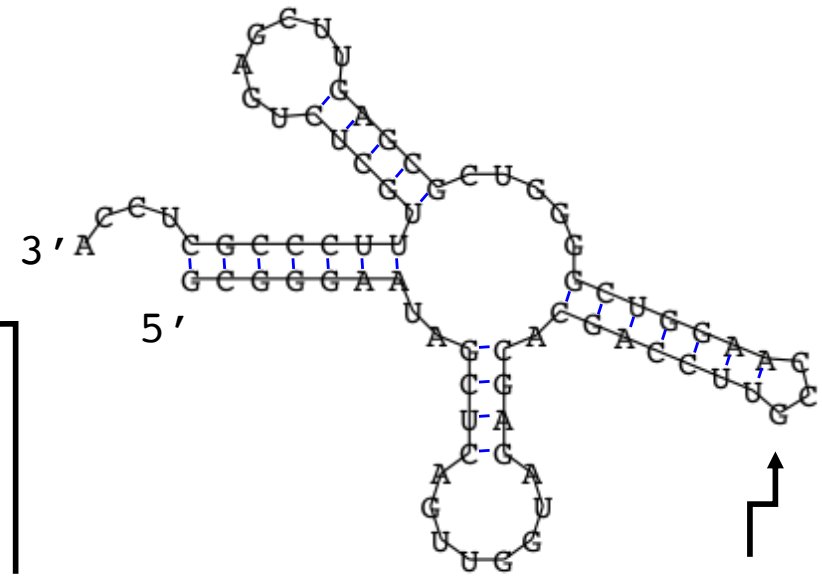
Figure 1: a) The spatial structure of the phenylalanine tRNA form yeast

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

tRNA - Alt. Representations



Anticodon loop



Anticodon loop

Definitions

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

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

$i < j-4$, and } no sharp turns

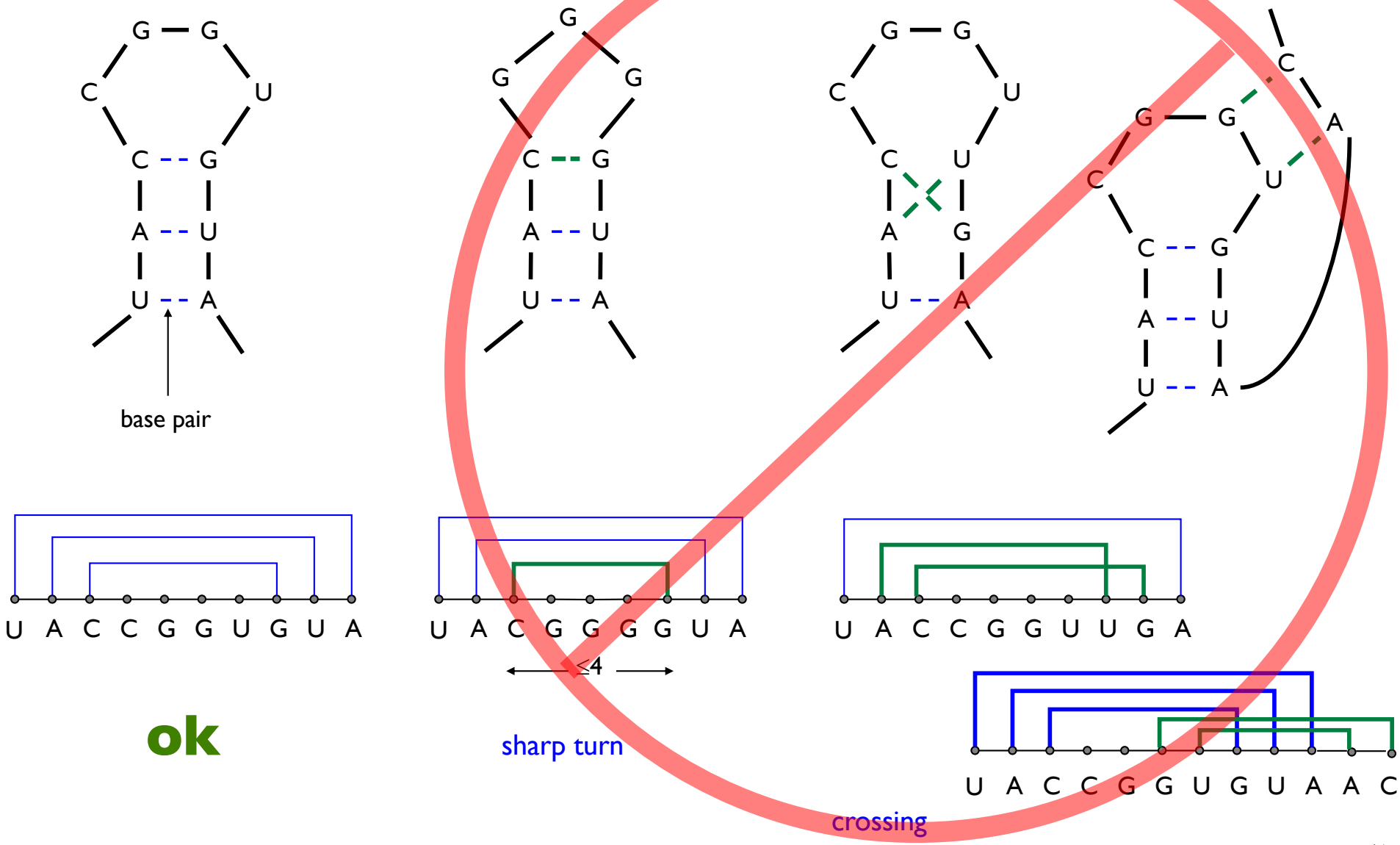
if i•j & i'•j' are two different pairs with $i \leq i'$, then

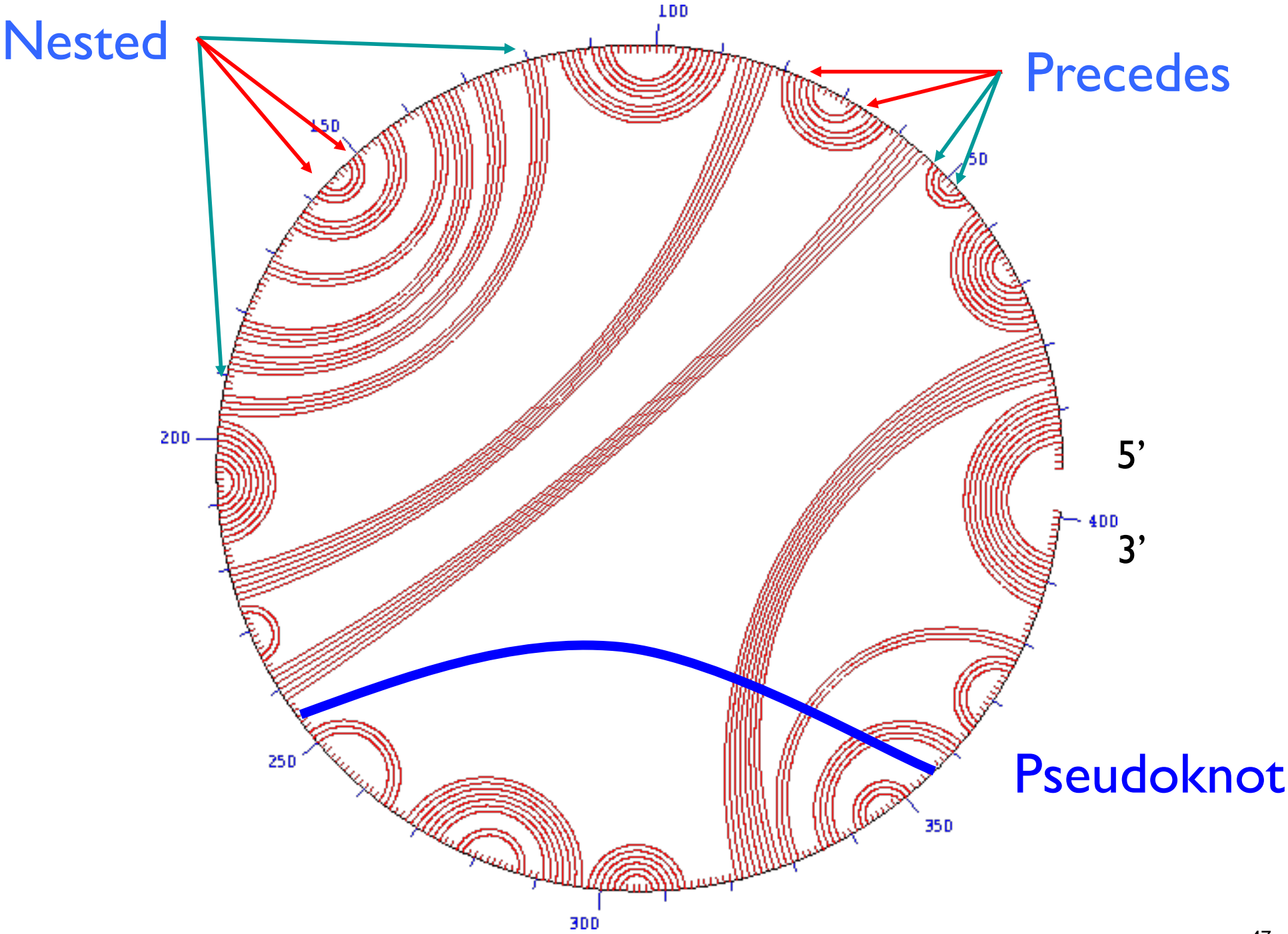
$j < i'$, or

$i < i' < j' < j$

} 2nd pair follows 1st, or is nested within it; no "pseudoknots" And pairs, not triples, etc.

RNA Secondary Structure: Examples




Nested
Precedes
100
150
50
200
400
250
350
300
5'
3'
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
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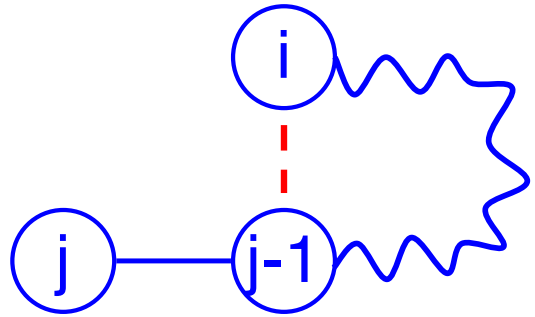
"Optimal pairing of r_i ... r_j"
Two possibilities

j Unpaired:

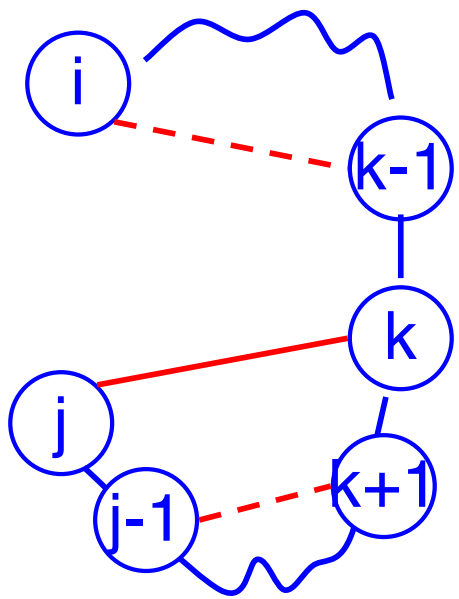
Find best pairing of r_i ... r_{j-1}



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?

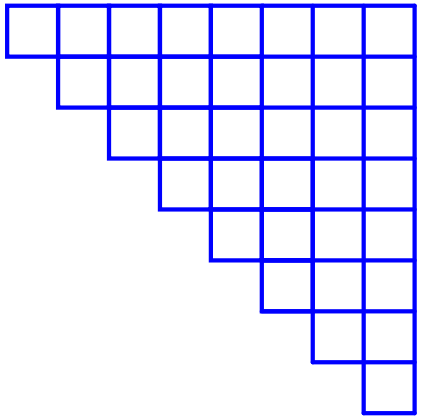
Nussinov: Max Pairing

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

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

Otherwise

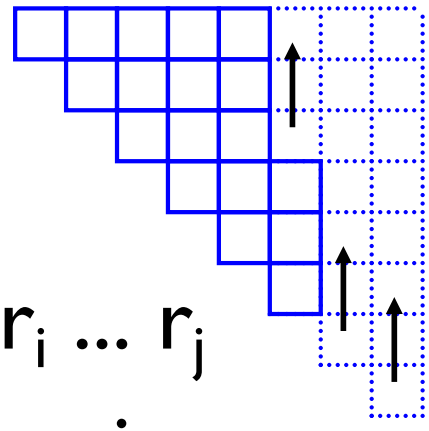
$B(i,j)$ = max of:



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

R Nussinov, AB Jacobson, "Fast algorithm for predicting the secondary structure of single-stranded RNA." PNAS 1980.

Nussinov: A Computation Order



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

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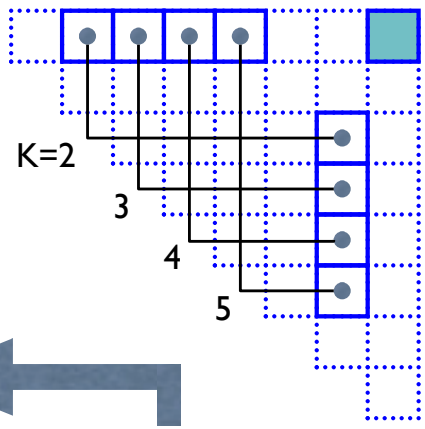
$B(i,j)$ = max of:

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



Time: $O(n^3)$

Which Pairs?

Usual dynamic programming “trace-back” tells you *which* base pairs are in the optimal solution, not just how many

Approaches to Structure Prediction

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

Minimum Energy
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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:

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

energy of k-j *pair*

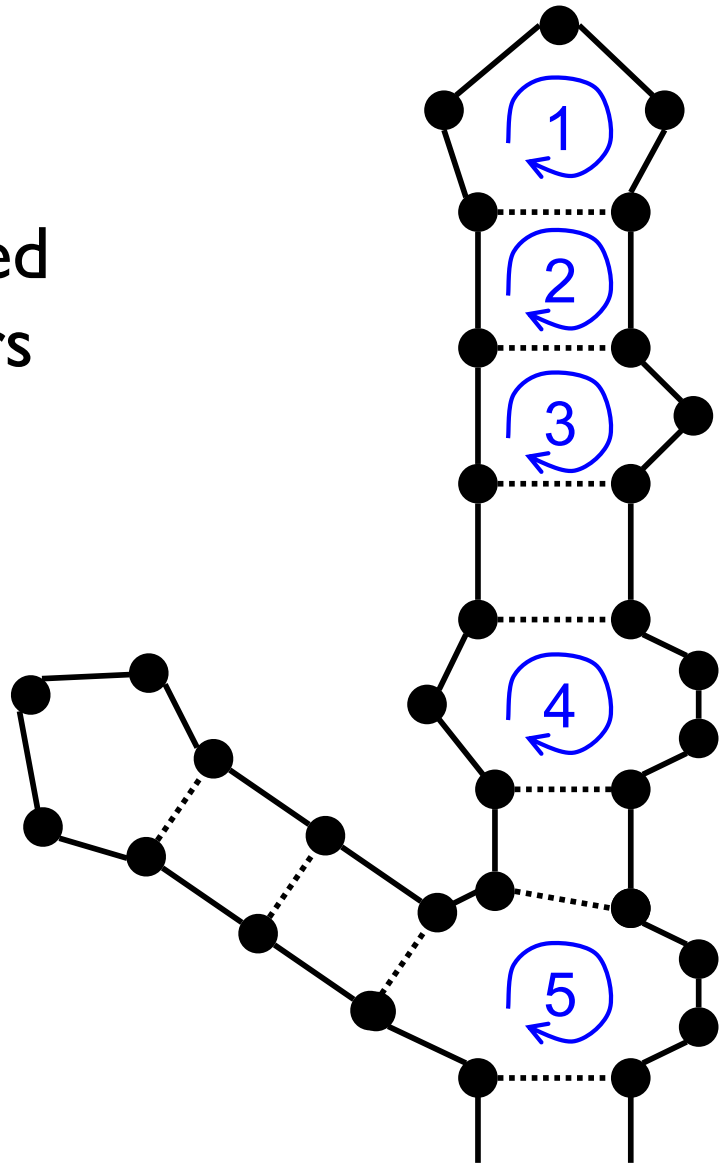
Time: $O(n^3)$

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



Zuker: Loop-based Energy, I

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

$V(i,j)$ = as above, but forcing pair $i \bullet j$

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

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

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 [Andronescu et al., 2007]

Single Seq Prediction Accuracy

Mfold, Vienna,... [Nussinov, Zuker, Hofacker, McCaskill]

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

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- + simple
- - too inaccurate

Minimum Energy
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Partition Function
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- - ignores pseudoknots

Approaches, II

Comparative sequence analysis

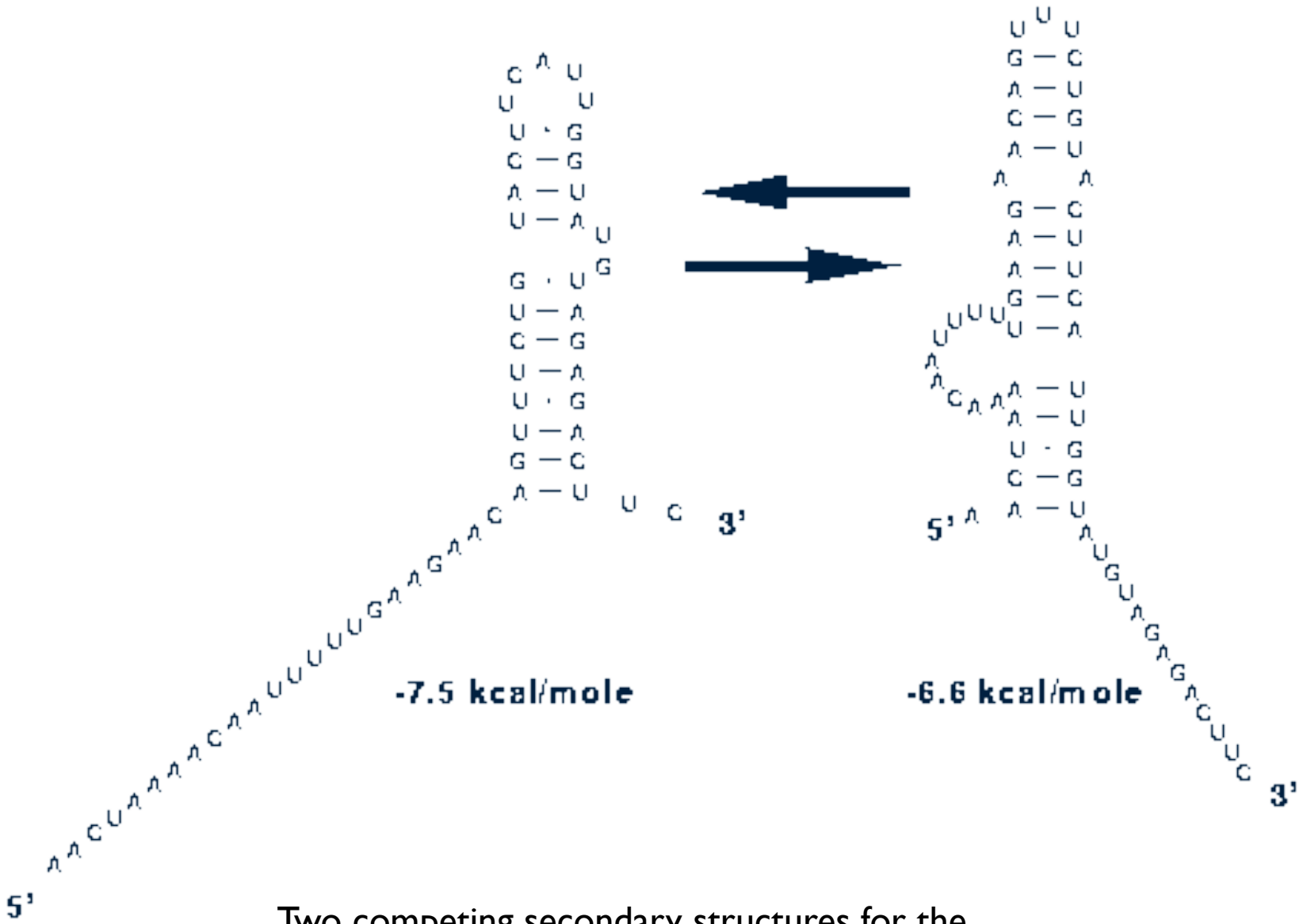
+ handles all pairings (potentially incl. pseudoknots)
- requires several (many?) aligned, appropriately diverged sequences

Stochastic Context-free Grammars

Roughly combines min energy & comparative, but no pseudoknots

Physical experiments (x-ray crystallography, NMR)

Next Lecture



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

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 from sequence alone due, e.g., to codon structure, which RNAs lack

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

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