CSEP 590 A
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

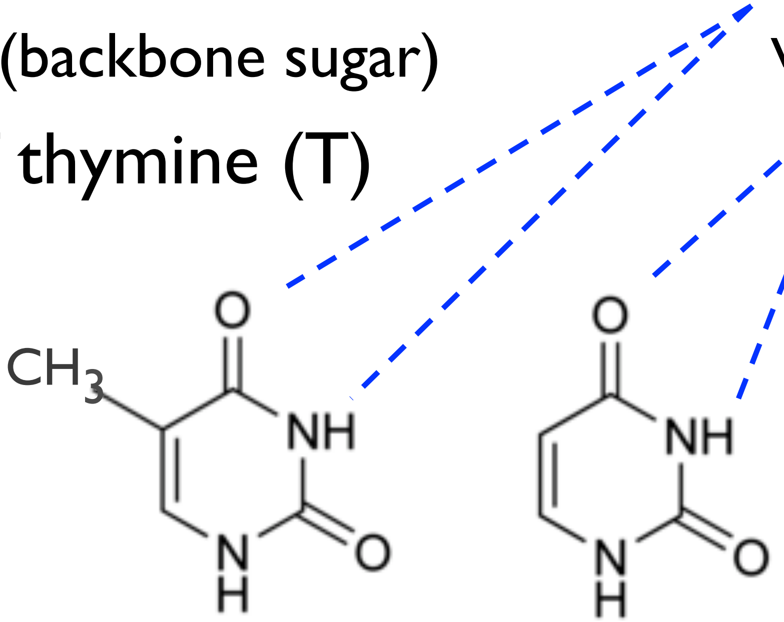
- Today
    - Noncoding RNA Examples
    - RNA structure prediction
- Next Time
    - RNA “motif” models
    - Search
    - Motif discovery

RNA

DNA: DeoxyriboNucleic Acid

RNA: RiboNucleic Acid

- Like DNA, except:
- Lacks OH on ribose (backbone sugar)
- Uracil (U) in place of thymine (T)
- A, G, C as before



pairs with A

thymine

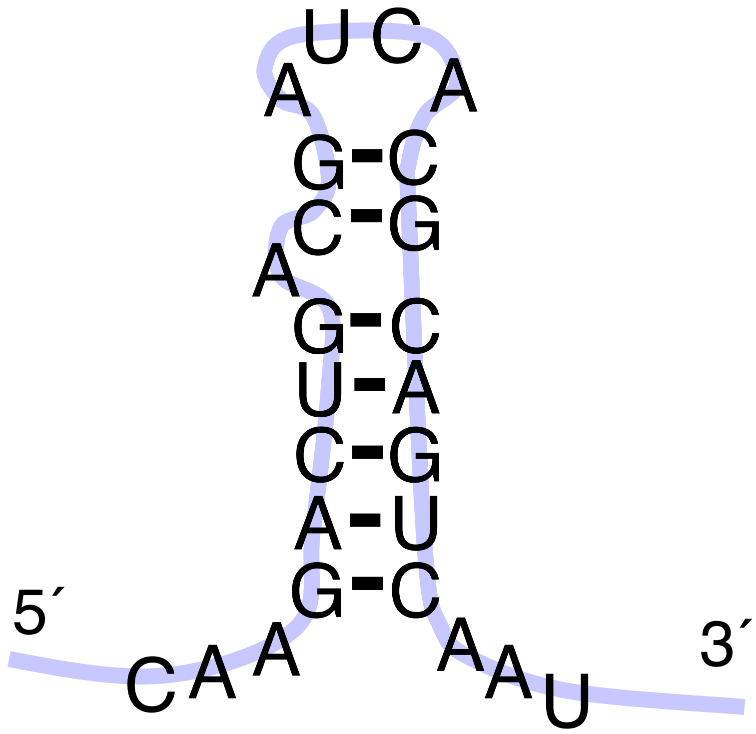
uracil

RNA Secondary Structure:
RNA makes helices too

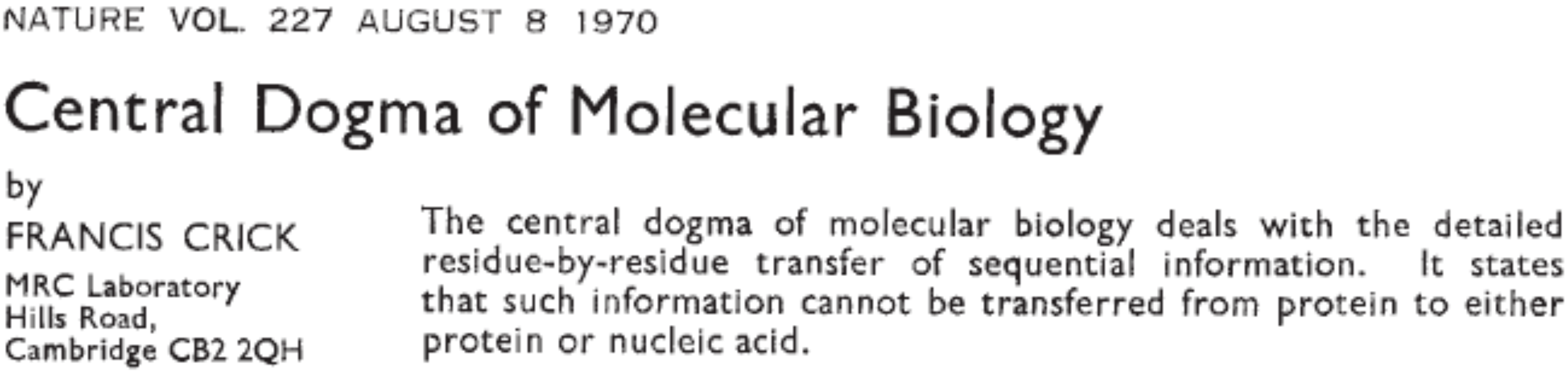
Base pairs

A-U
C-G

U-G



Usually *single* stranded

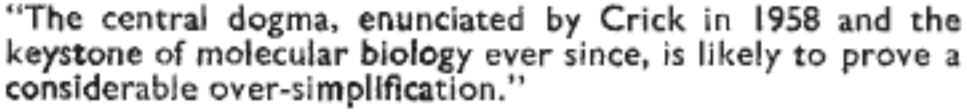


NATURE VOL. 227 AUGUST 8 1970

Central Dogma of Molecular Biology

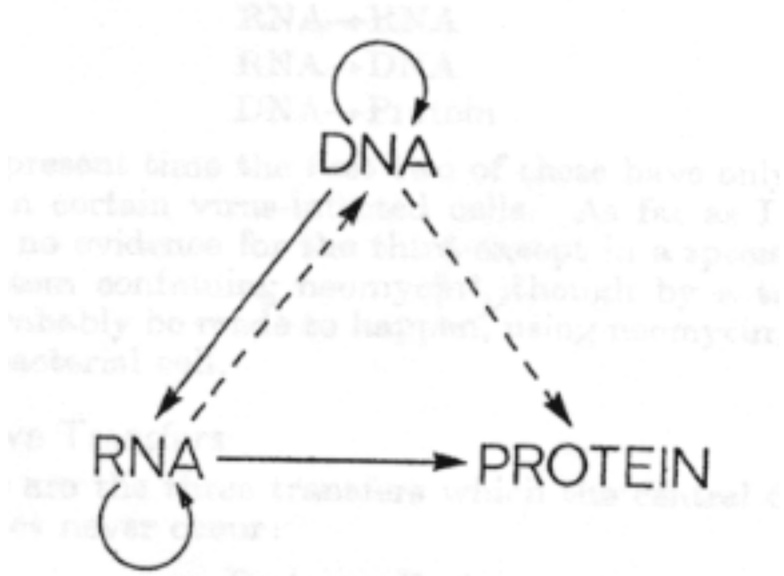
by
FRANCIS CRICK
MRC Laboratory
Hills Road,
Cambridge CB2 2QH

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

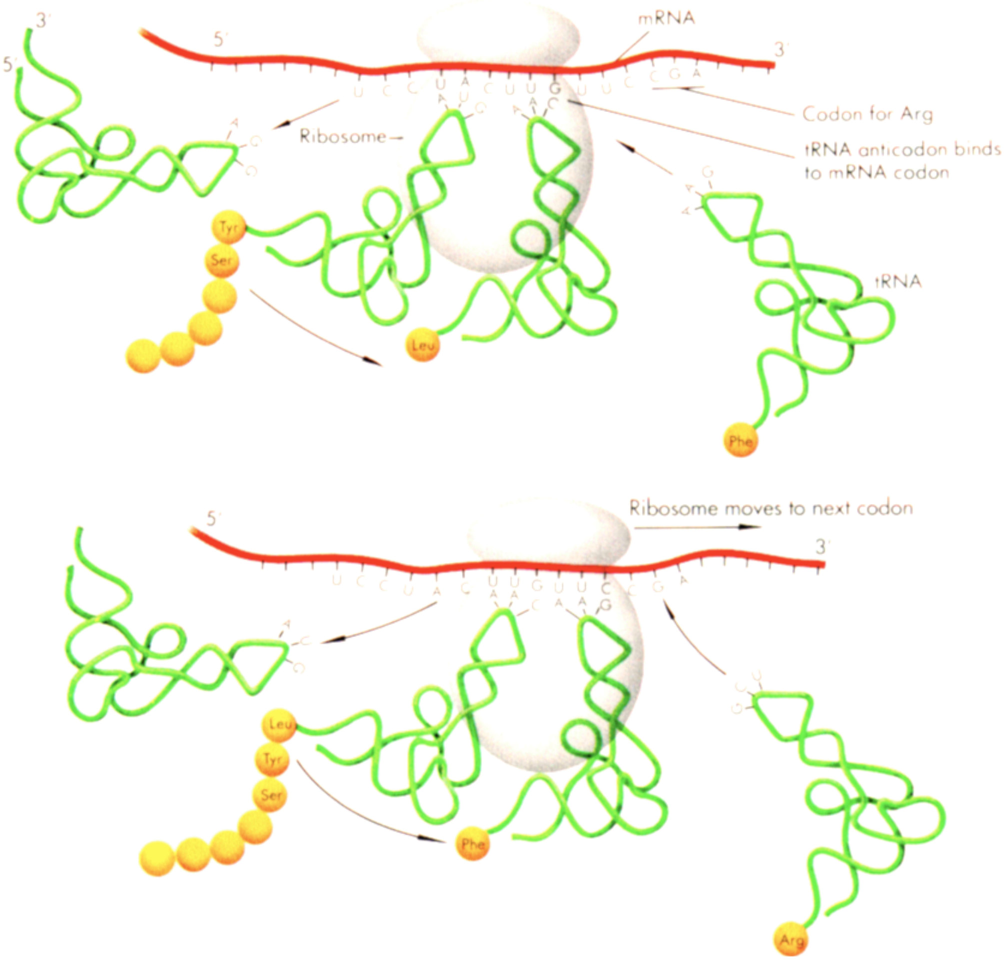


"The central dogma, enunciated by Crick in 1958 and the keystone of molecular biology ever since, is likely to prove a considerable over-simplification."

Fig. 2. The arrows show the situation as it 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.



Ribosomes



Watson, Gilman, Witkowski, & Zoller, 1992

Ribosomes

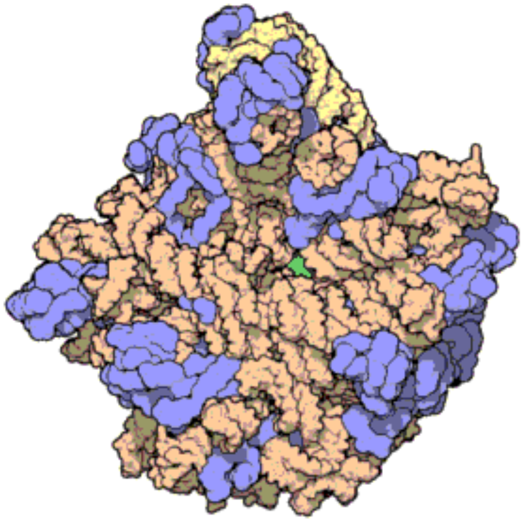
1974 Nobel prize to Romanian biologist George Palade (1912-2008) for discovery in mid 50's

50-80 proteins

3-4 RNAs (half the mass)

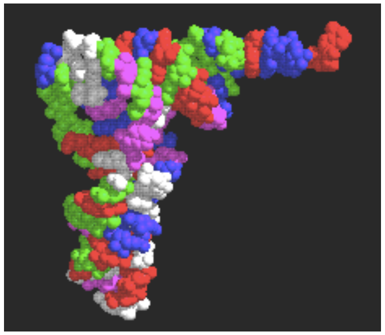
Catalytic core is RNA

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



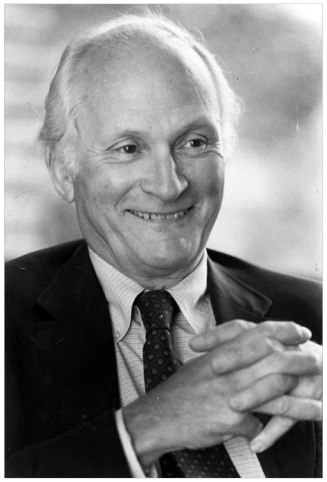
Atomic structure of the 50S Subunit from *Haloarcula marismortui*. Proteins are shown in blue and the two RNA strands in orange and yellow. The small patch of green in the center of the subunit is the active site.
- Wikipedia

Transfer RNA

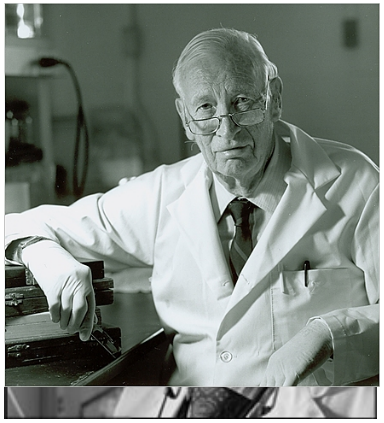


The “adapter” coupling mRNA to protein synthesis.

Discovered in the mid-1950s by



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



Bacteria

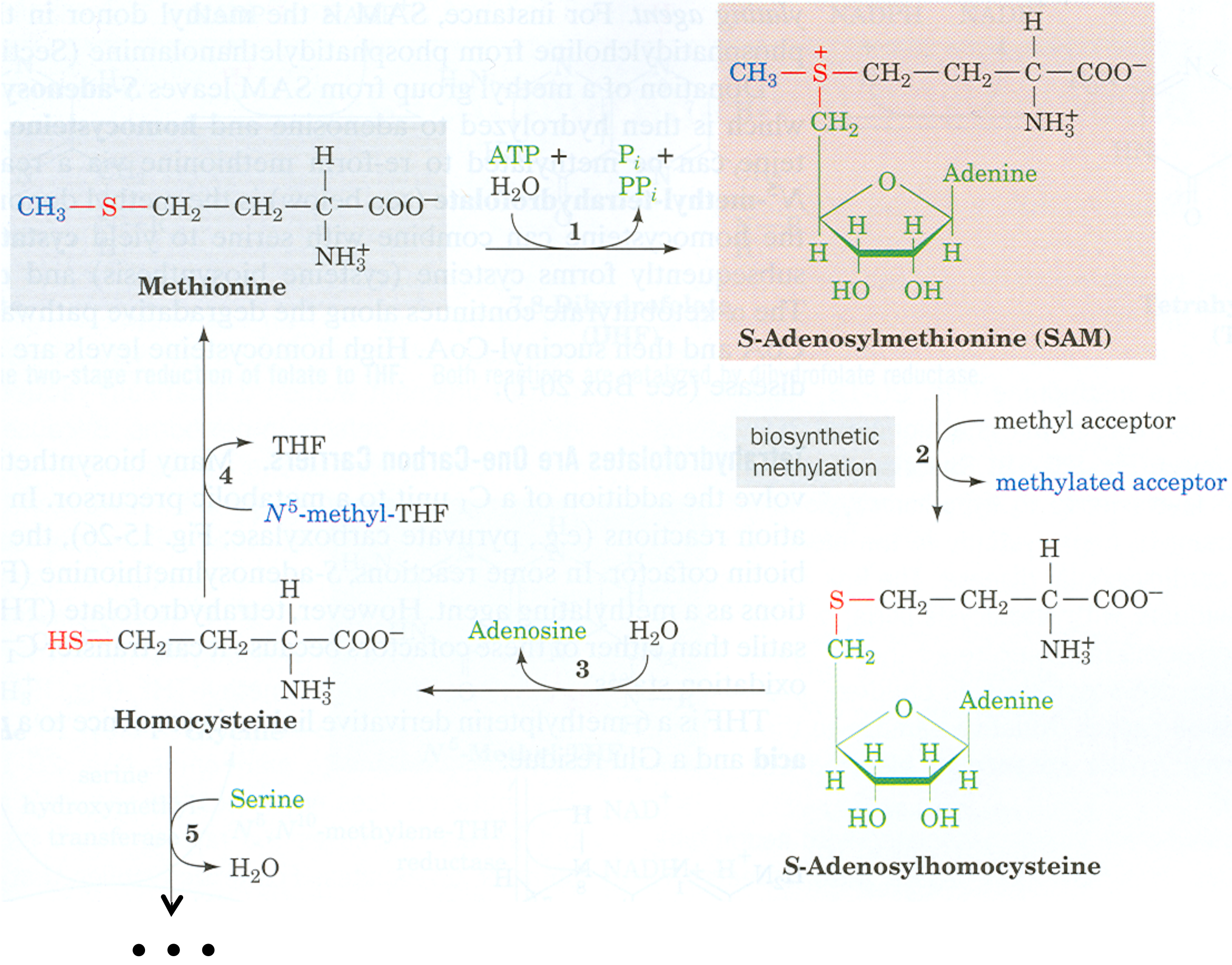
Triumph of proteins

80% of genome is coding DNA

Functionally diverse

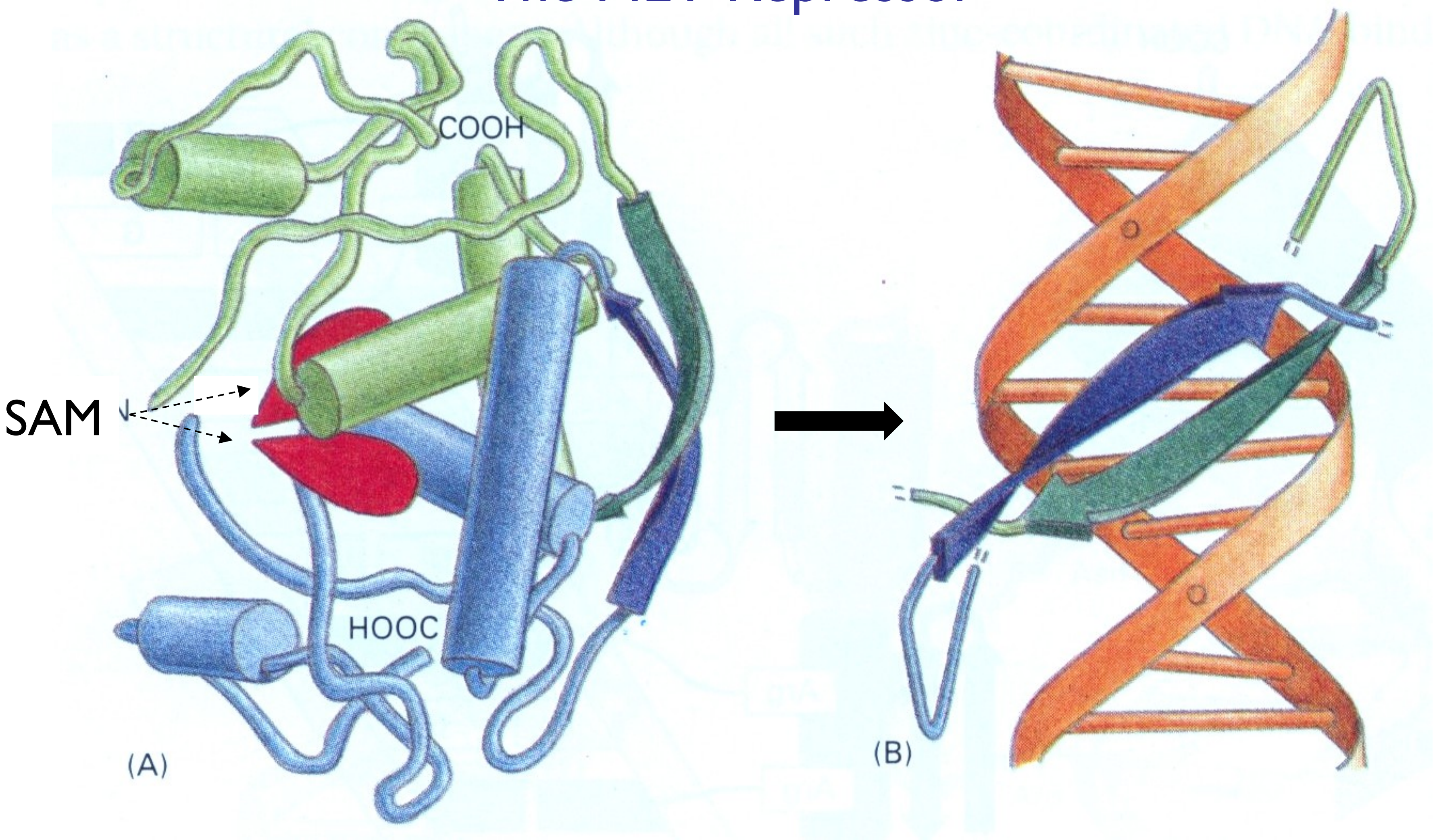
- receptors
- motors
- catalysts
- regulators (Monod & Jakob, Nobel prize 1965)
- …

Proteins Catalyze Biochemistry: Met Pathways



Proteins Regulate Biochemistry:

The MET Repressor



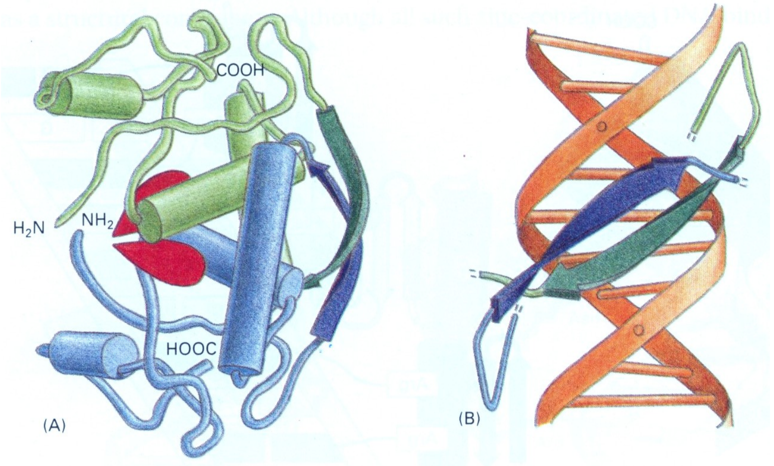
SAM

Protein

DNA

Alberts, et al, 3e.

Not the only way!

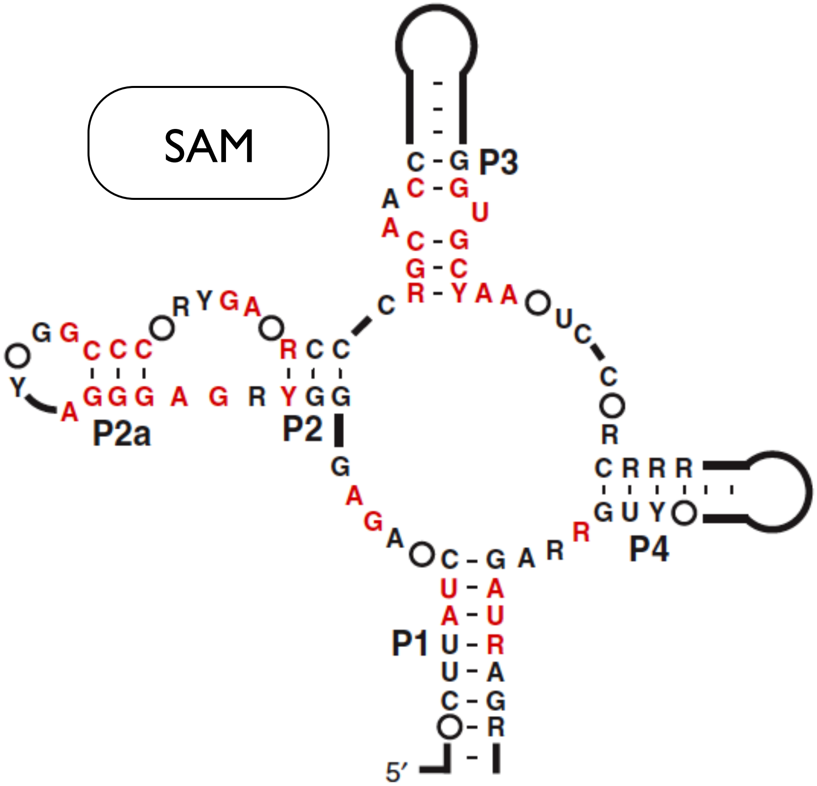


Alberts, et al, 3e.

← Protein way

Riboswitch alternative

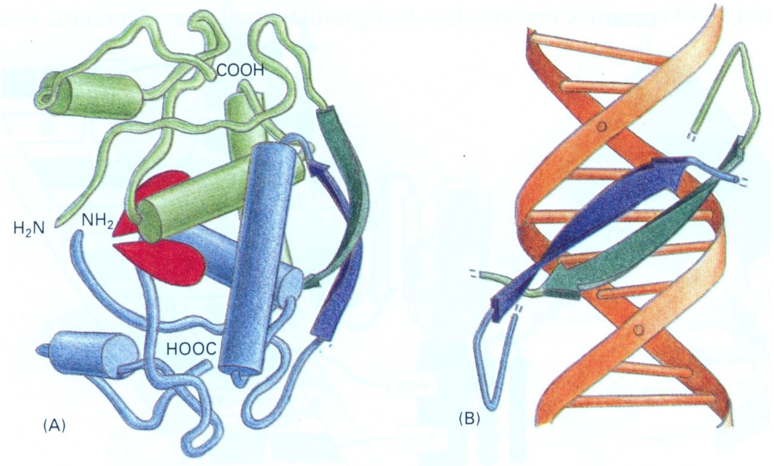
SAM



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.

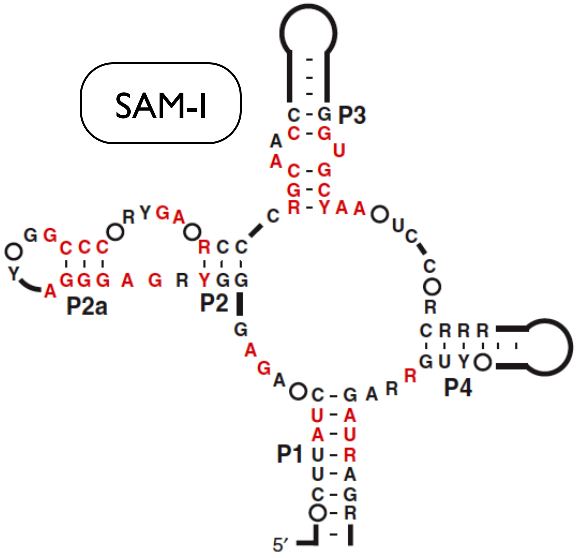


← Protein way

Riboswitch alternatives

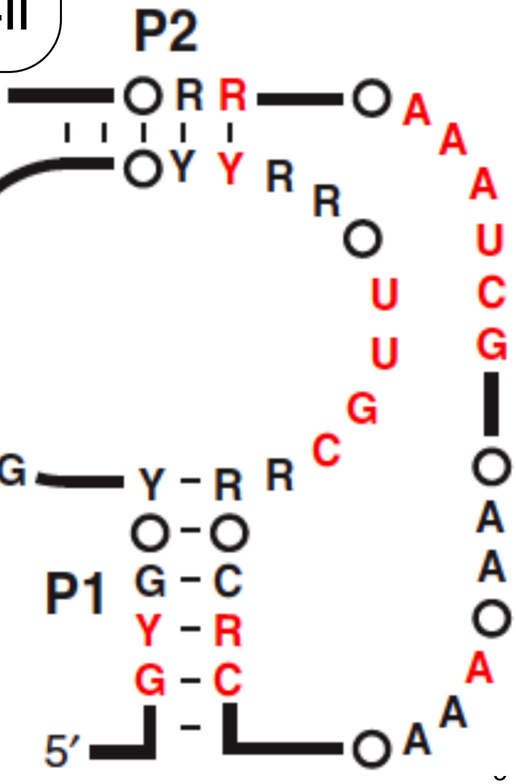
↓

SAM-I



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

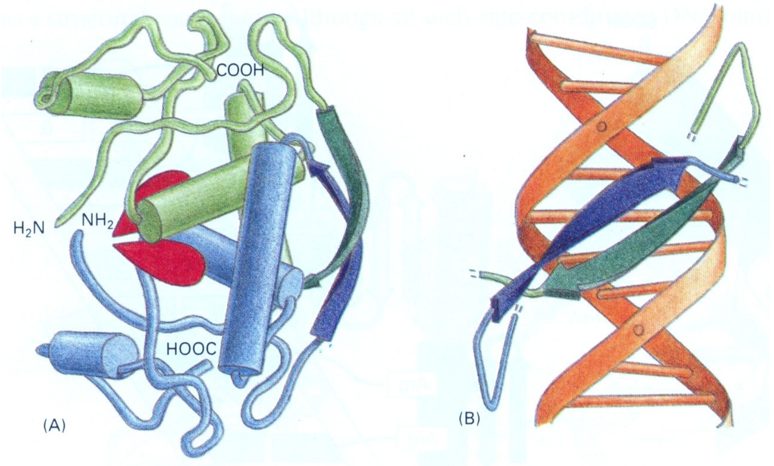
SAM-II



Corbino et al., Genome Biol. 2005

Not the only way!

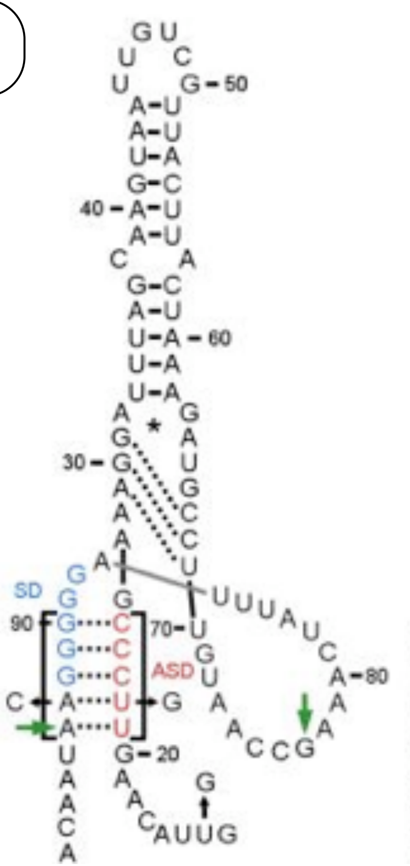
Alberts, et al, 3e.



Protein
way

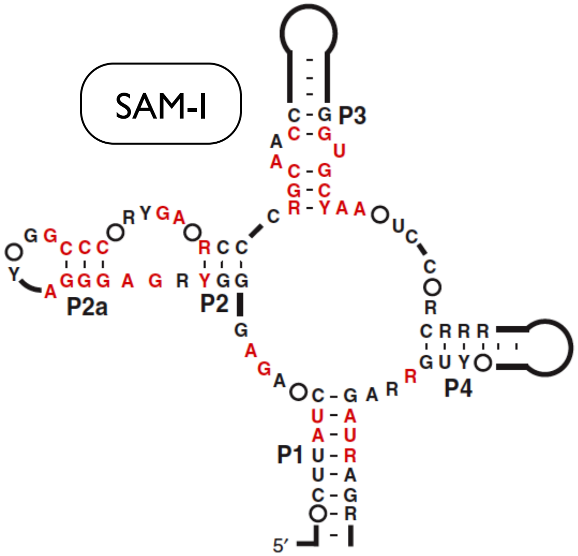
Riboswitch
alternatives

SAM-III



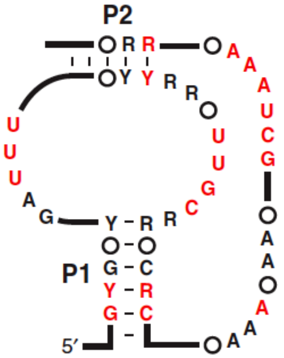
Fuchs et al.,
NSMB 2006

SAM-I



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

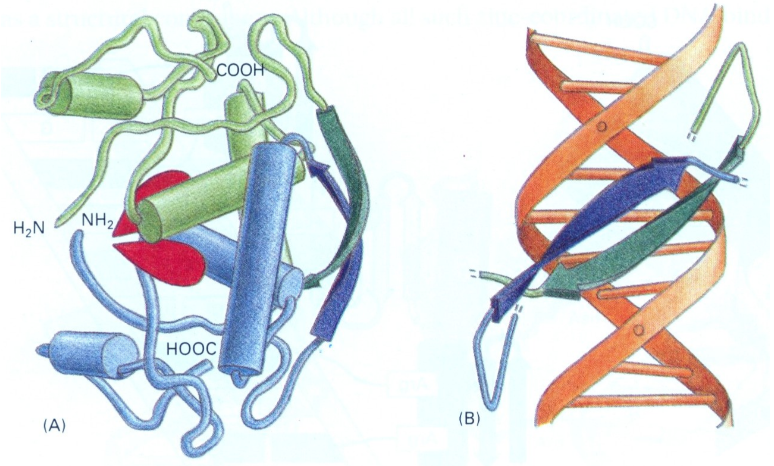
SAM-II



Corbino et al.,
Genome Biol. 2005

Not the only way!

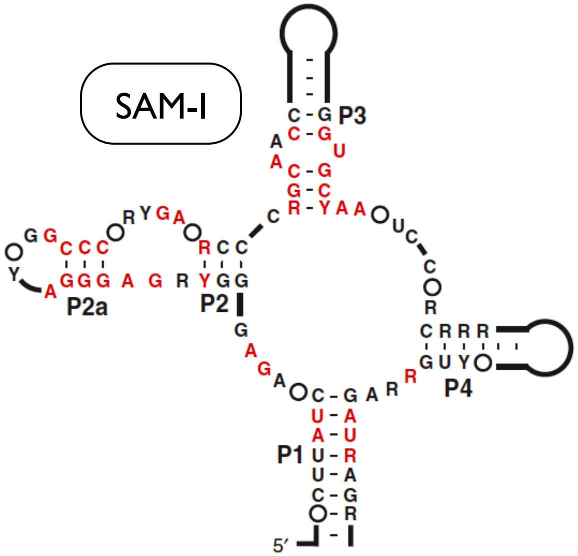
Alberts, et al, 3e.



Protein way

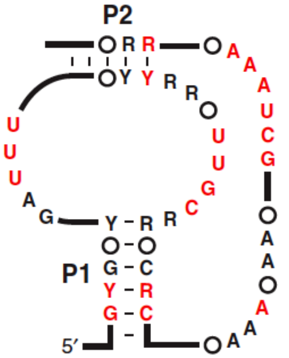
Riboswitch alternatives

SAM-I



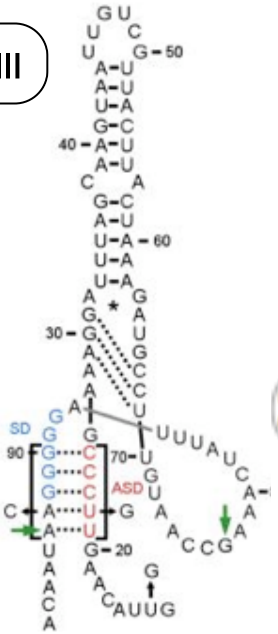
Grundy, Epshtein, Winkler et al., 1998, 2003

SAM-II



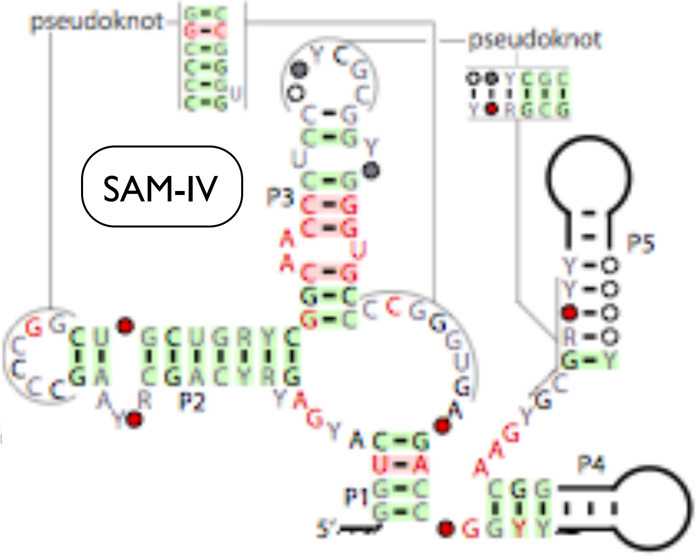
Corbino et al., Genome Biol. 2005

SAM-III



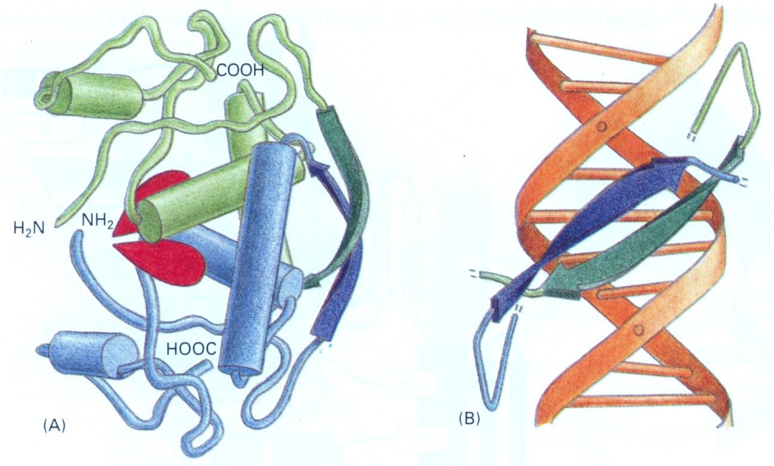
Fuchs et al., NSMB 2006

SAM-IV



Weinberg et al., RNA 2008

Not the only way!

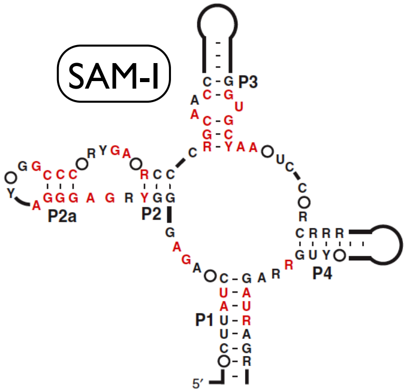


Alberts, et al, 3e.

← Protein way

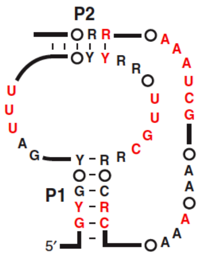
Riboswitch alternatives ↓

SAM-I



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

SAM-II

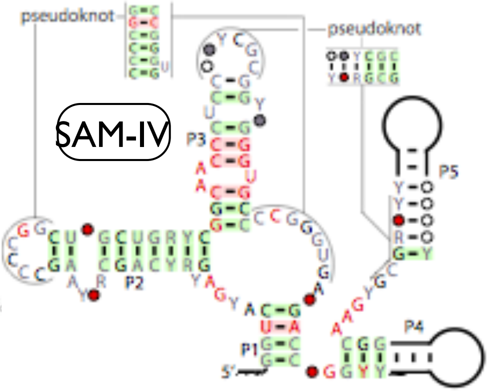


Corbino et al., Genome Biol. 2005

SAM-III

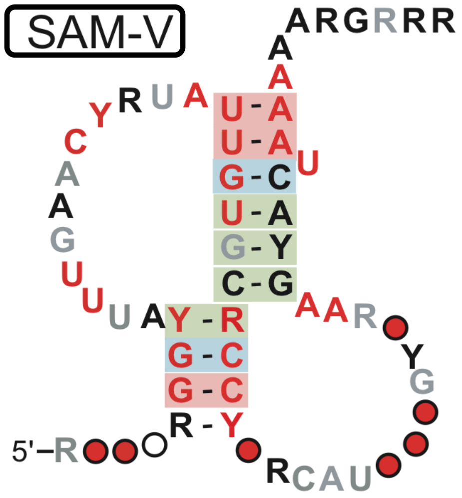
Fuchs et al., NSMB 2006

SAM-IV



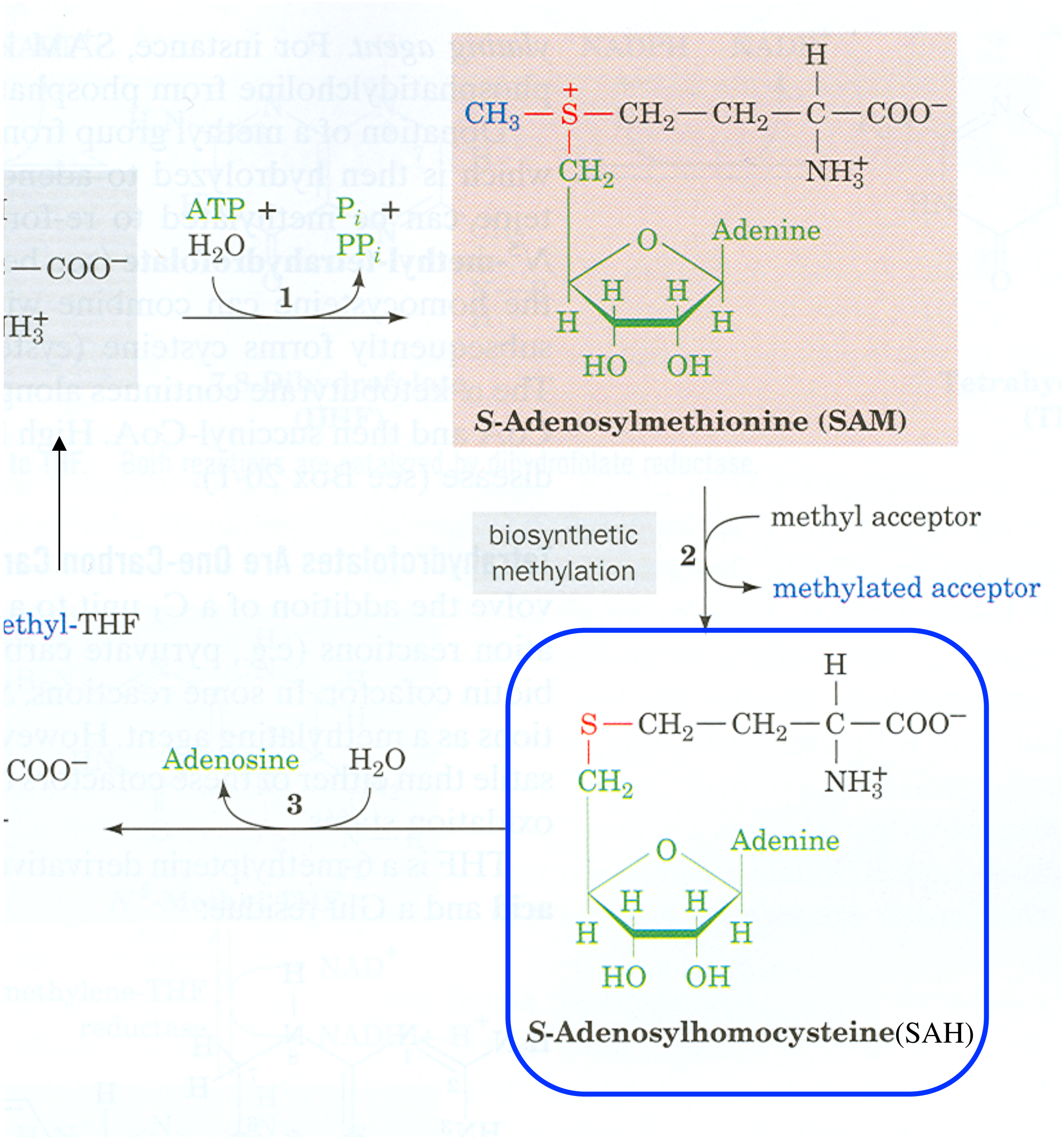
Weinberg et al., RNA 2008

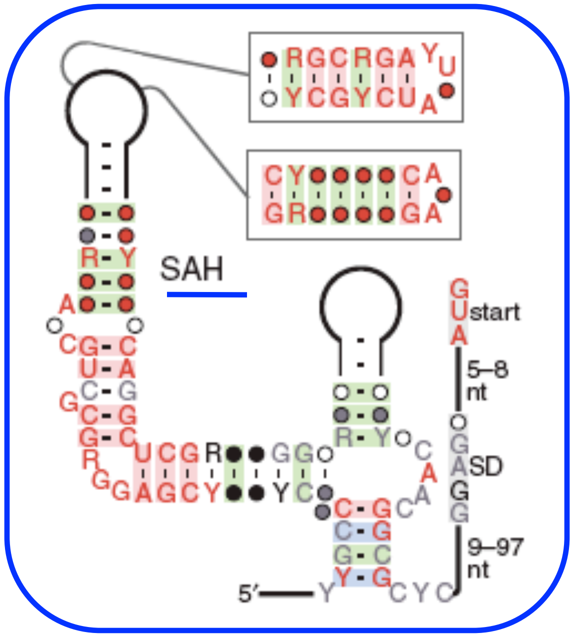
SAM-V

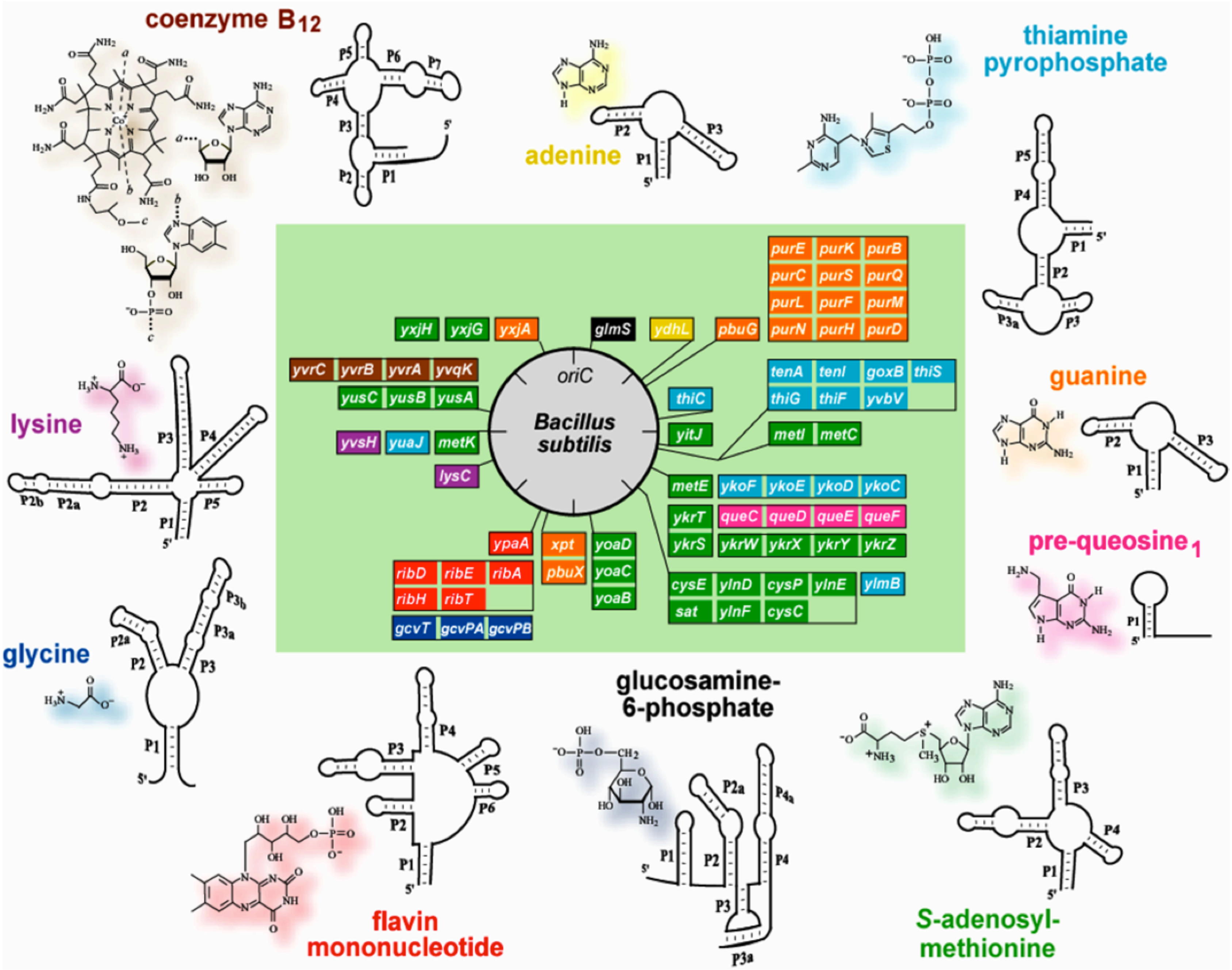


Meyer, etal., BMC Genomics 2009

And in other bacteria, a riboswitch senses SAH



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Co
a
b
c
P5
P6
P7
P4
P3
P2
P1
5'
adenine
P2
P3
P1
5'
thiamine pyrophosphate
P5
P4
P1
5'
P2
P3a
P3
lysine
P3
P4
P2b
P2a
P2
P5
P1
5'
glycine
P3b
P3a
P2a
P2
P3
P1
5'
guanine
P2
P3
P1
5'
pre-queosine1
P1
5'
oriC
Bacillus subtilis
purE
purK
purB
purC
purS
purQ
purL
purF
purM
purN
purH
purD
yxjH
yxjG
yxjA
glmS
ydhL
pbuG
yvrC
yvrB
yvrA
yvqK
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yusB
yusA
yvsH
yuaJ
metK
lysC
thiC
yitJ
tenA
tenI
goxB
thiS
thiG
thiF
yvbV
metI
metC
metE
ykoF
ykoE
ykoD
ykoC
ykrT
queC
queD
queE
queF
ykrS
ykrW
ykrX
ykrY
ykrZ
ypaA
xpt
yoaD
ribD
ribE
ribA
pbuX
yoaC
ribH
ribT
yoaB
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gcvPA
gcvPB
cysE
ylnD
cysP
ylnE
ylmB
sat
ylnF
cysC
flavin mononucleotide
P4
P3
P5
P6
P2
P1
5'
glucosamine-6-phosphate
P2a
P4a
P1
P2
P4
P3
P3a
5'
S-adenosyl-methionine
P3
P2
P4
P1
5'

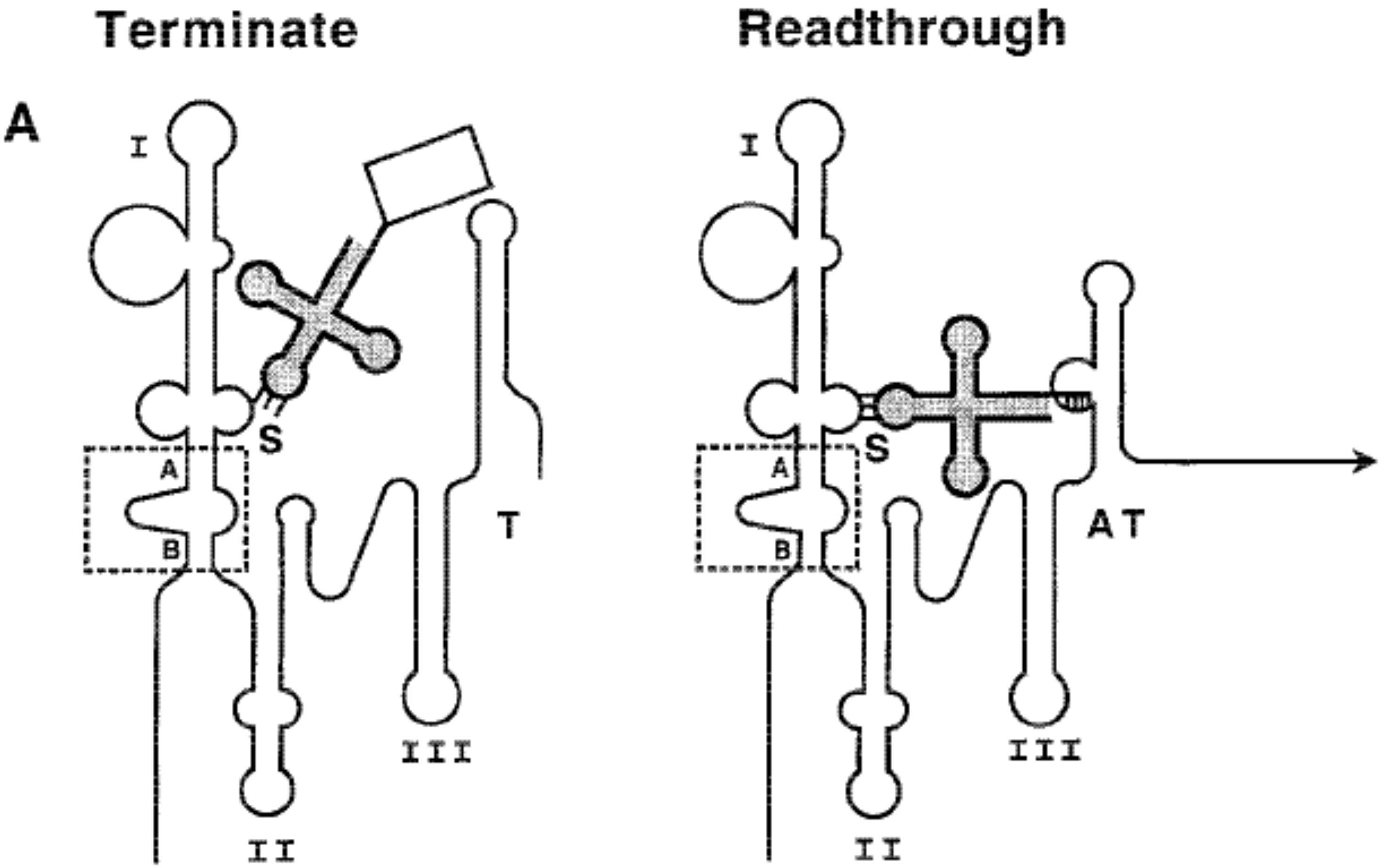
New Antibiotic Targets?

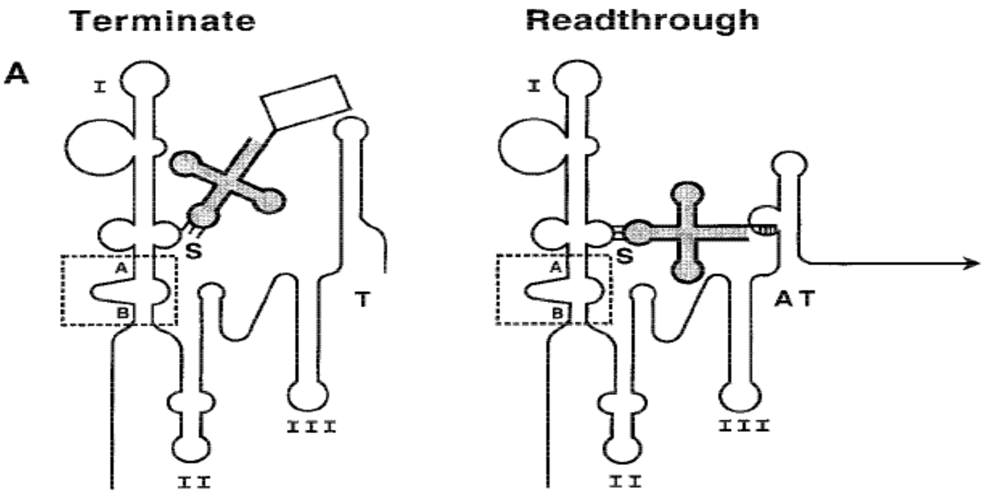
Old drugs, new understanding:

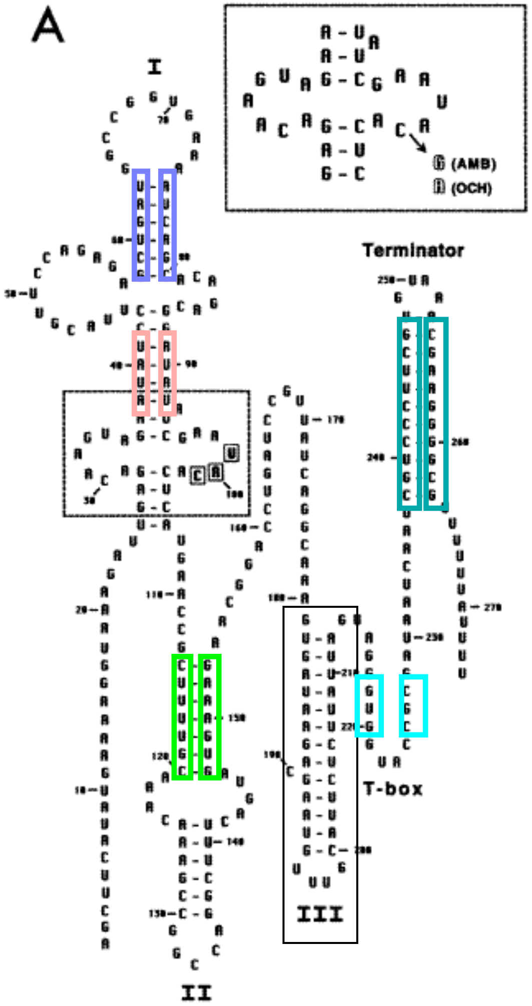
- TPP riboswitch ~ pyrithiamine
- lysine riboswitch ~ L-aminoethylcysteine, DL-4-oxalysine
- FMN riboswitch ~ roseoflavin

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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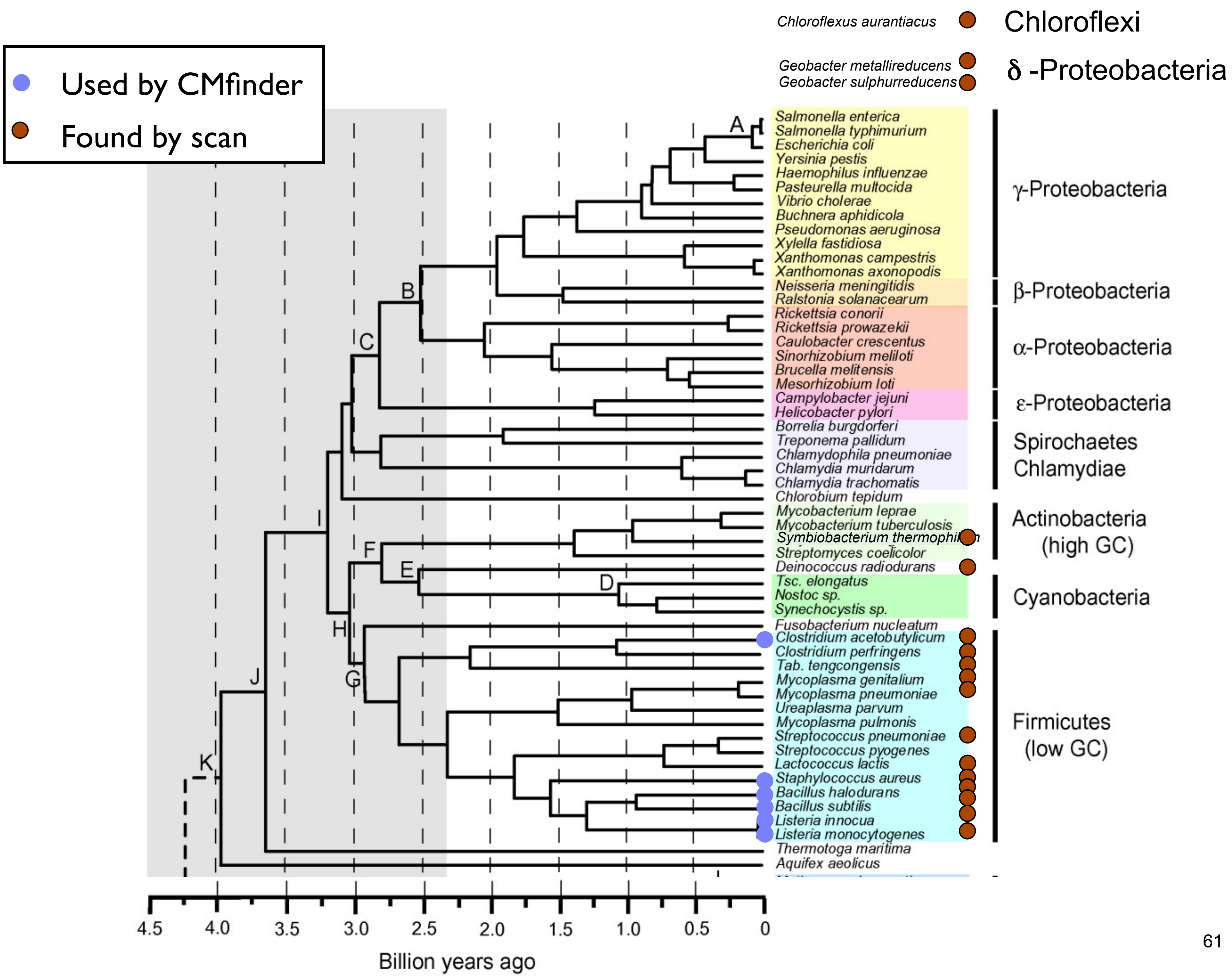
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● Found by scan
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Geobacter metallireducens
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δ -Proteobacteria
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Salmonella typhimurium
Escherichia 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
Chlorobium tepidum
Mycobacterium leprae
Mycobacterium tuberculosis
Symbiobacterium thermophilum
Streptomyces coelicolor
Actinobacteria (high GC)
Deinococcus radiodurans
Tsc. elongatus
Nostoc sp.
Synechocystis sp.
Cyanobacteria
Fusobacterium nucleatum
Clostridium acetobutylicum
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

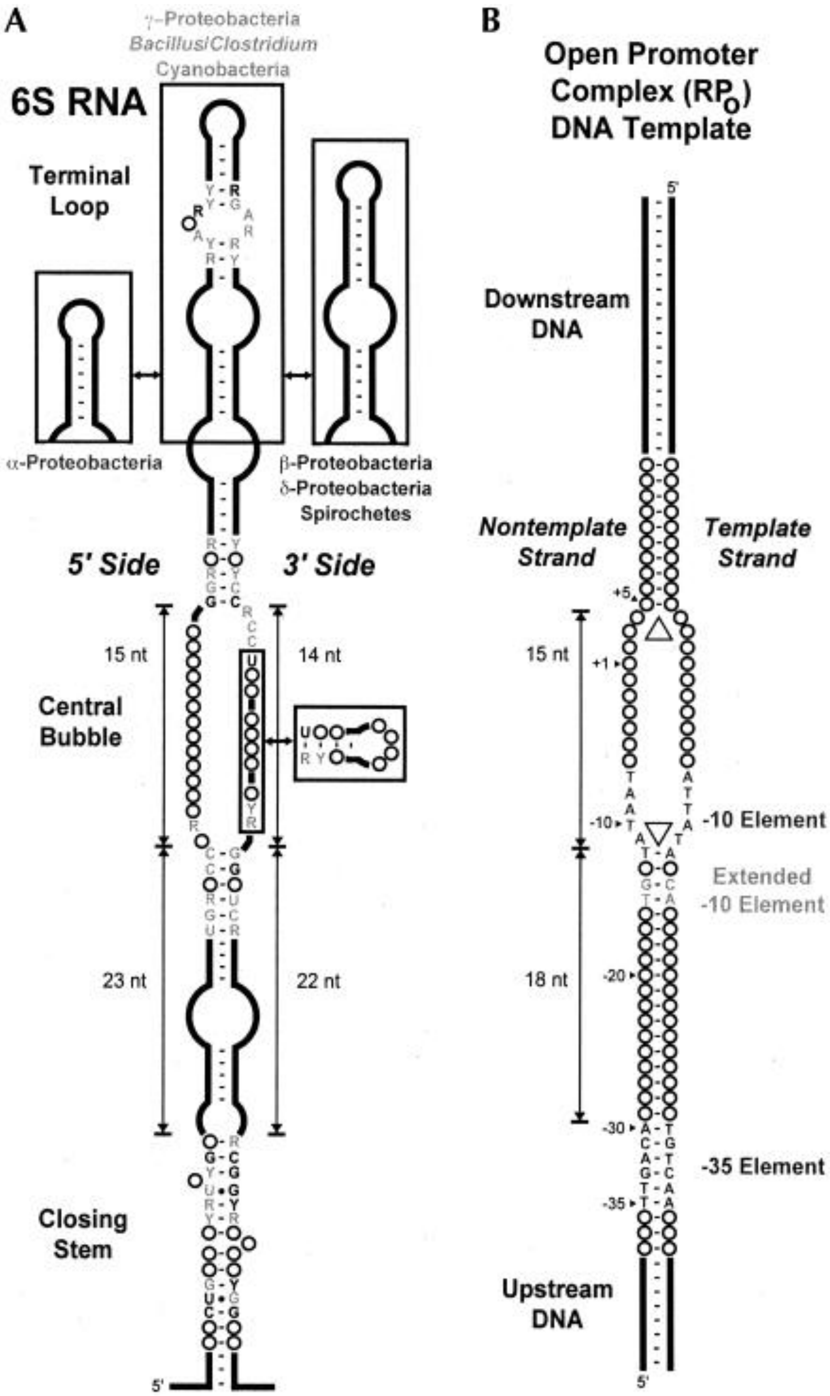
medium size (175nt)

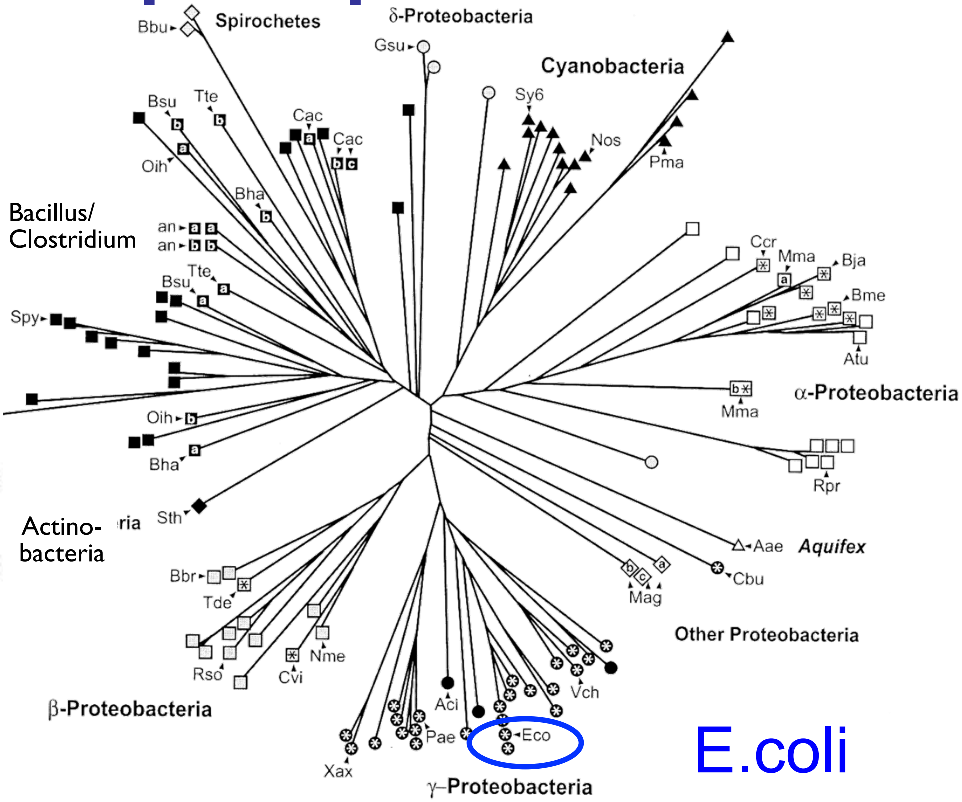
structured

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 last 5-10 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
- Hundreds 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

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?)

RNAz

S Washietl, IL Hofacker, M Lukasser, A Huttenhofer, 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

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." *Genome Res., 16, #7 (2006) 885-9.*
1800 candidates from 36970 (of 100,000) pairs

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
6500 candidates in ENCODE alone (better FDR, but still high)

Thousands of Predictions

Bottom line?

A significant number of “one-off” examples

Extremely wise-spread ncRNA expression

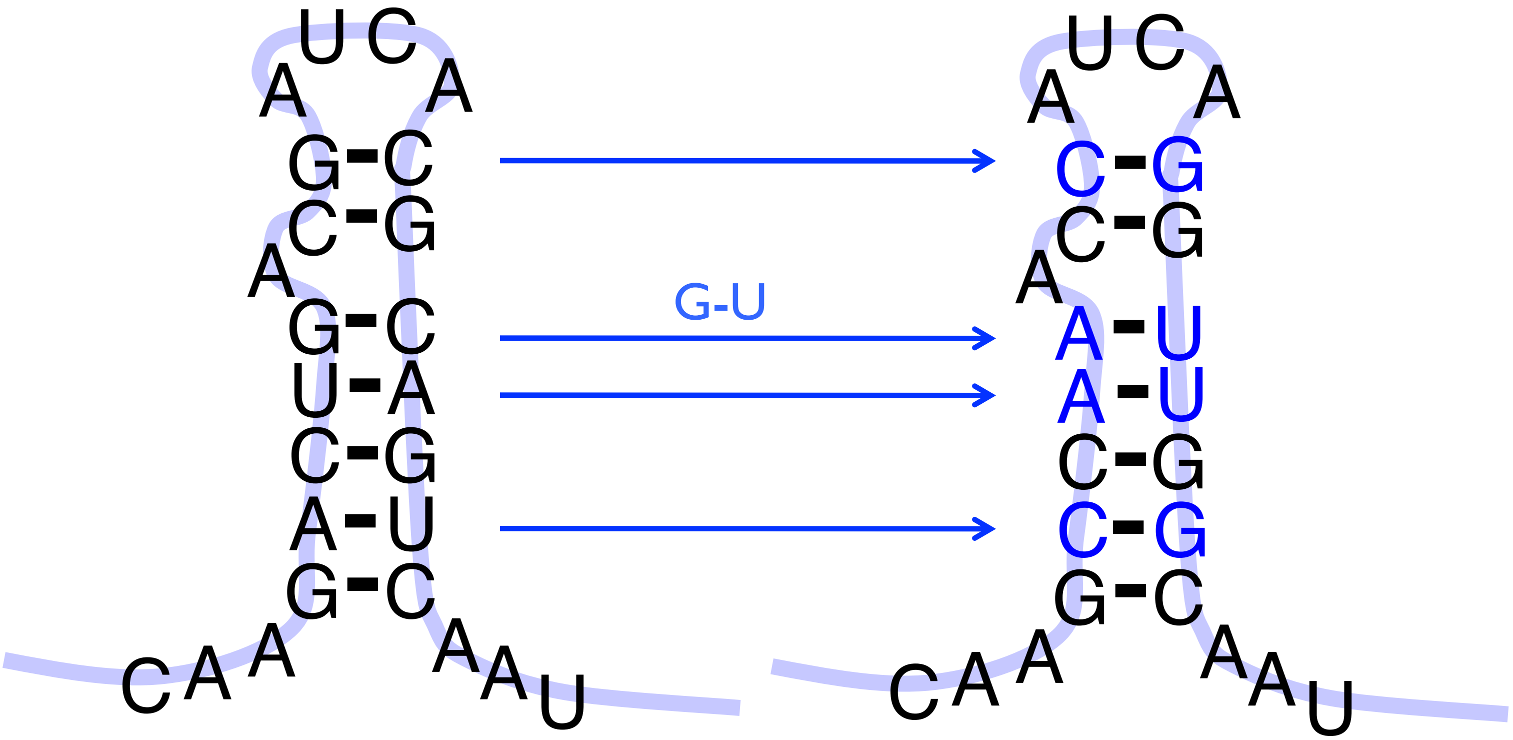
At a minimum, a vast evolutionary substrate

New technology (e.g. RNAseq) exposing more

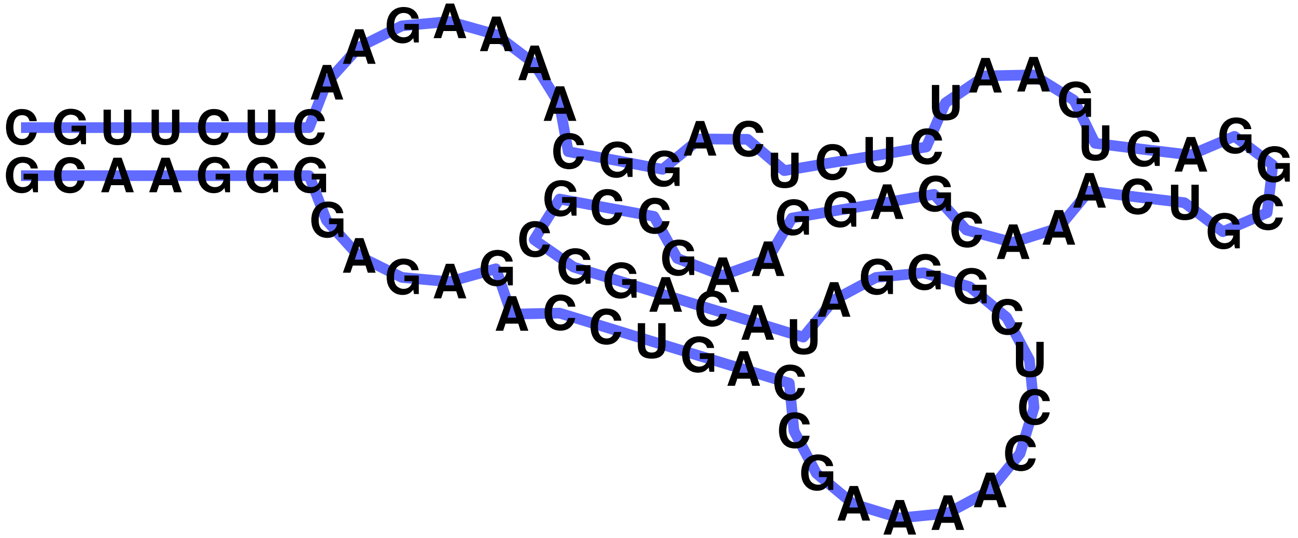
How do you recognize an interesting one?

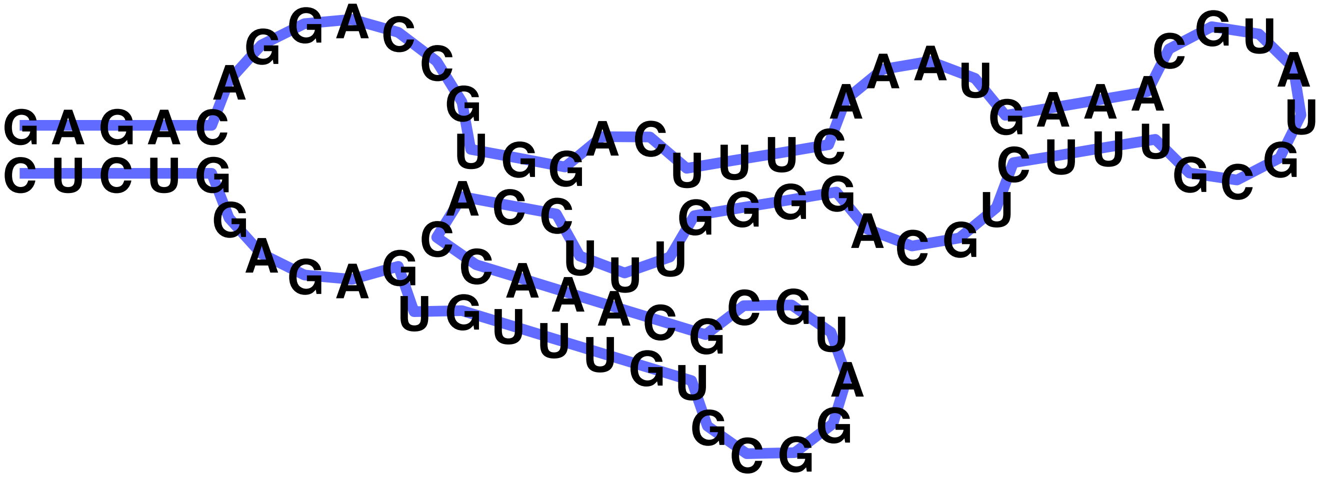
Conserved secondary structure

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

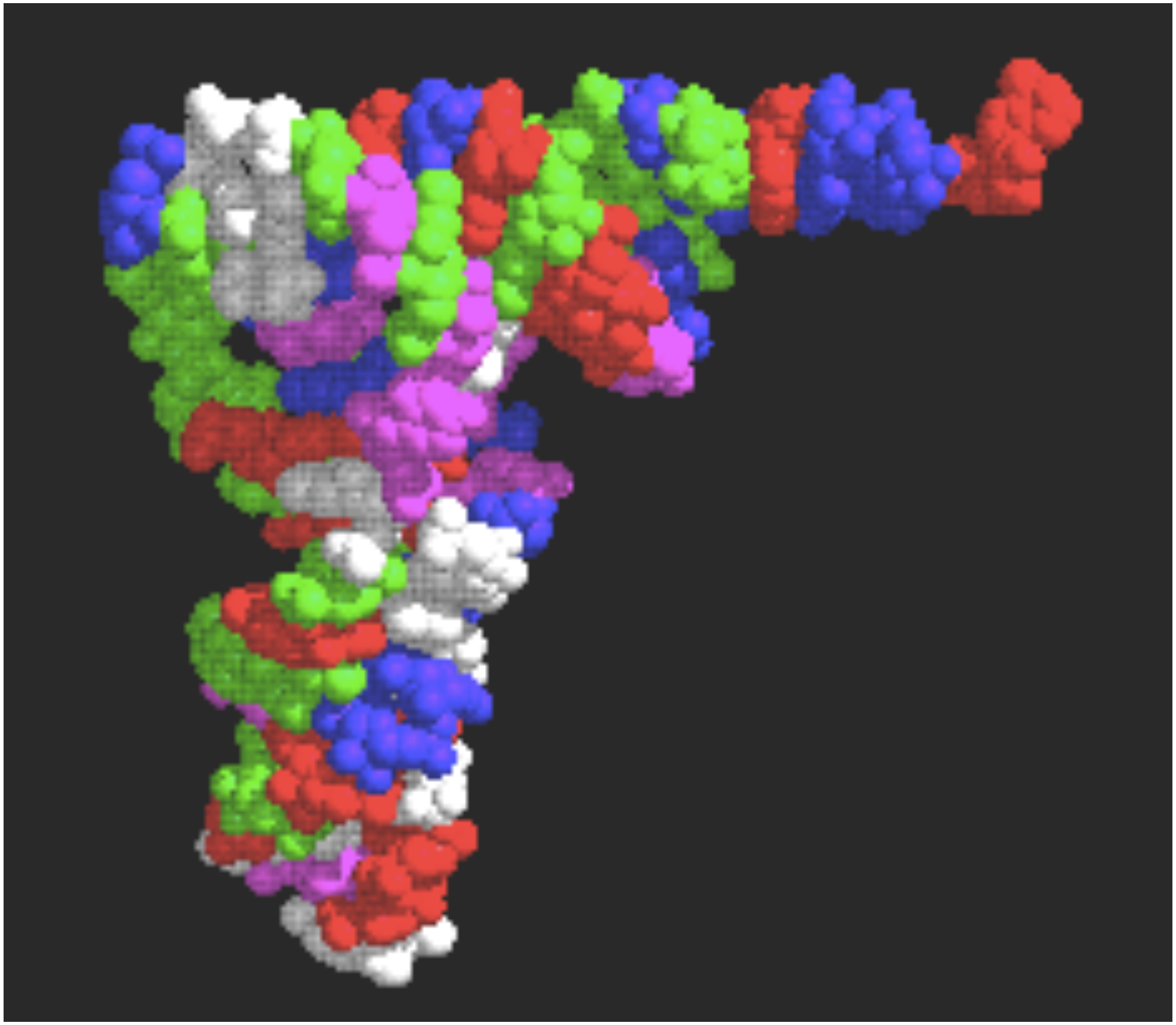
Secondary Structure: Pairing

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

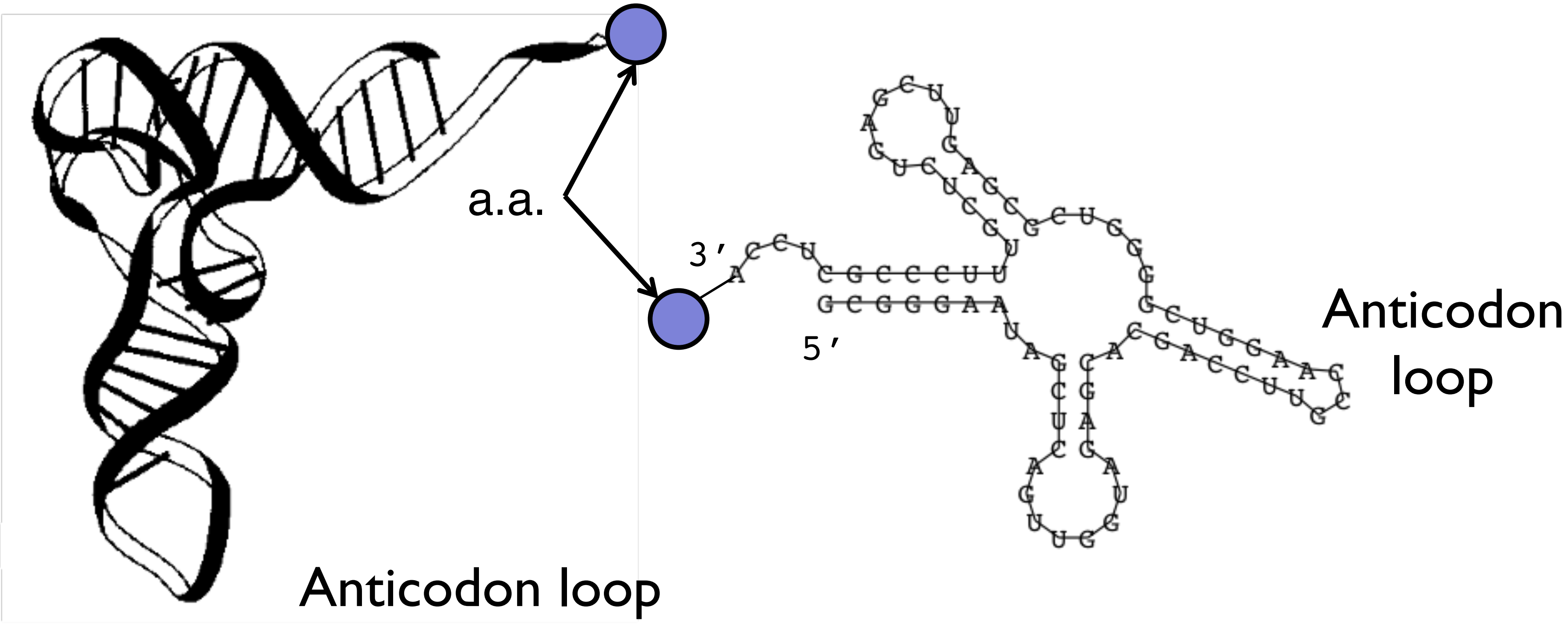
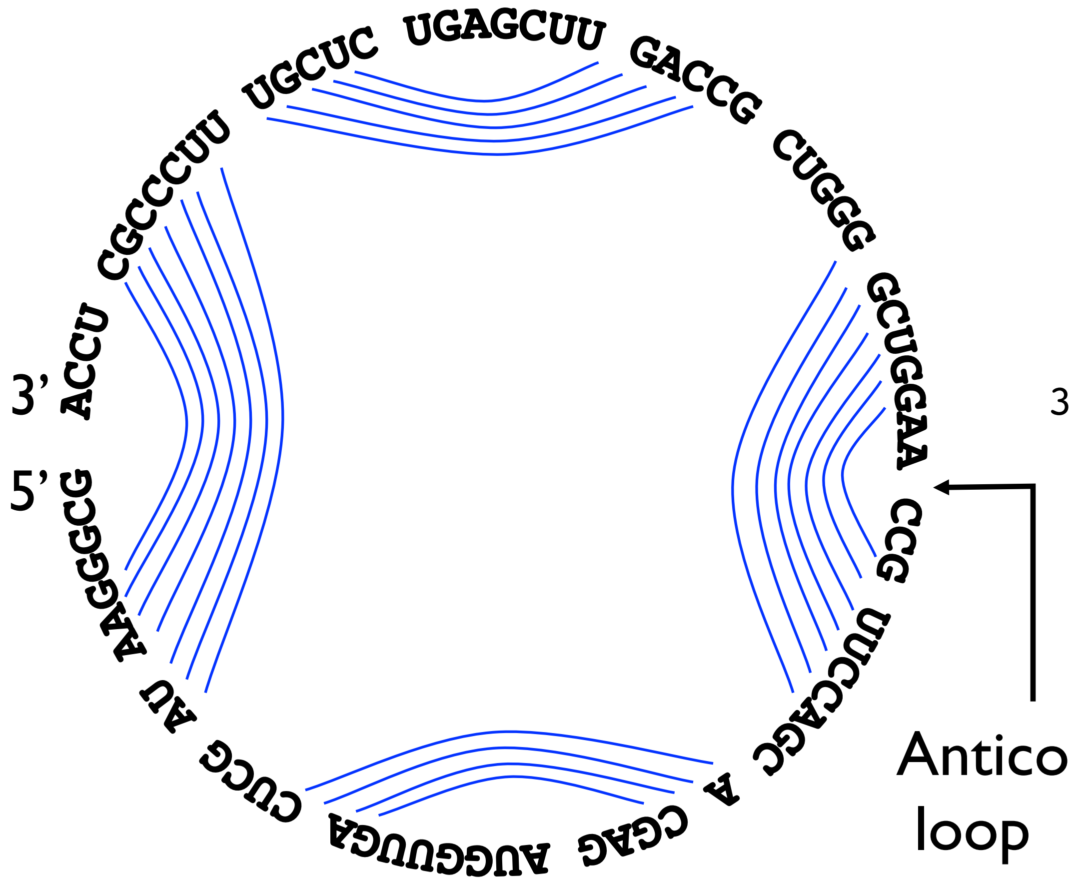


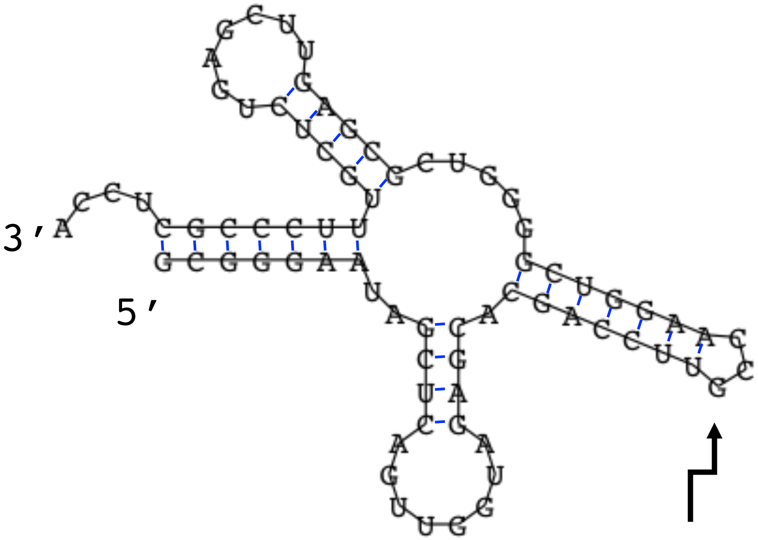
Figure 1: a) The spatial structure of the phenylalanine tRNA form yeast

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

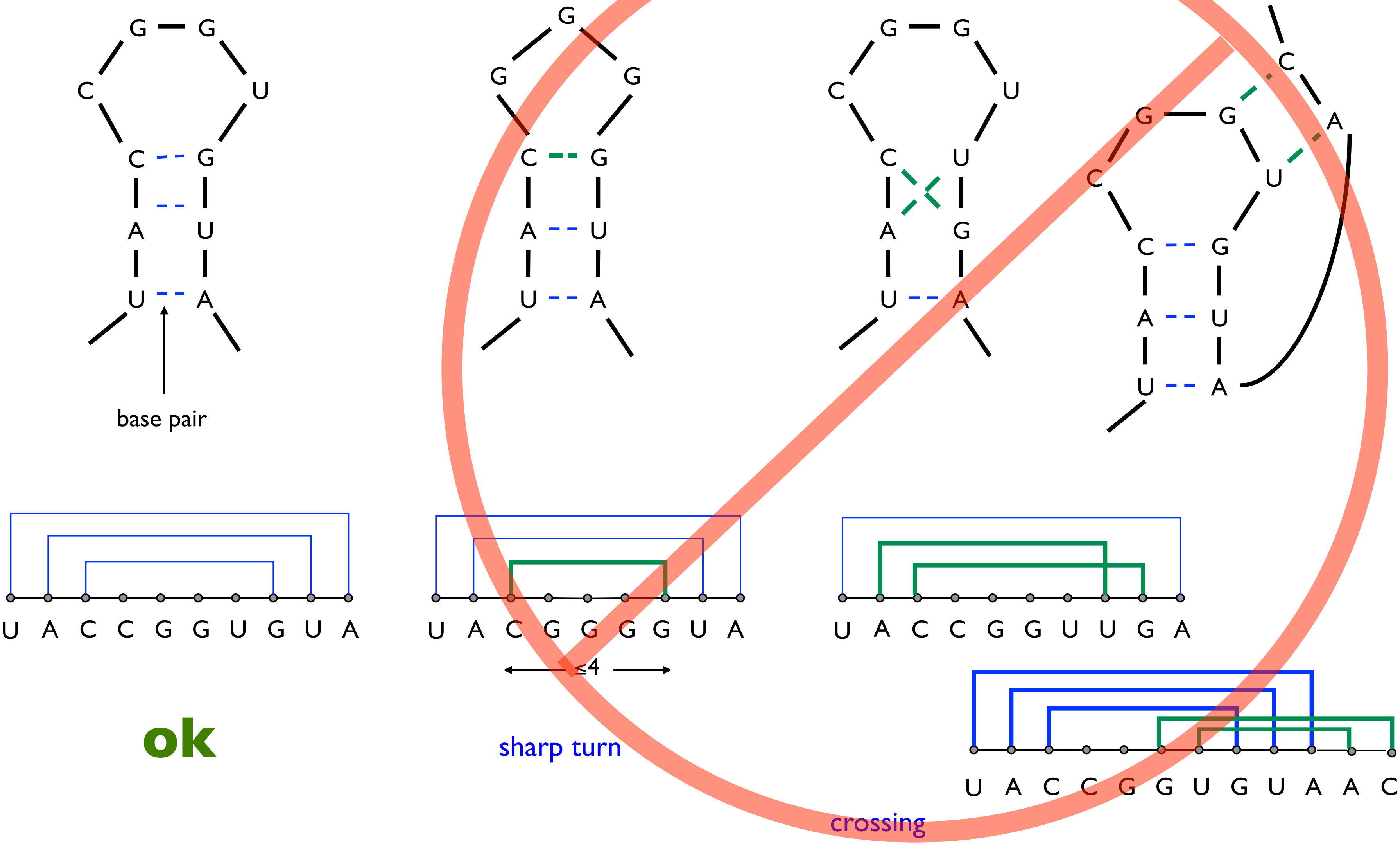
if i•j & i'•j' are two different pairs with $i \le i'$, then

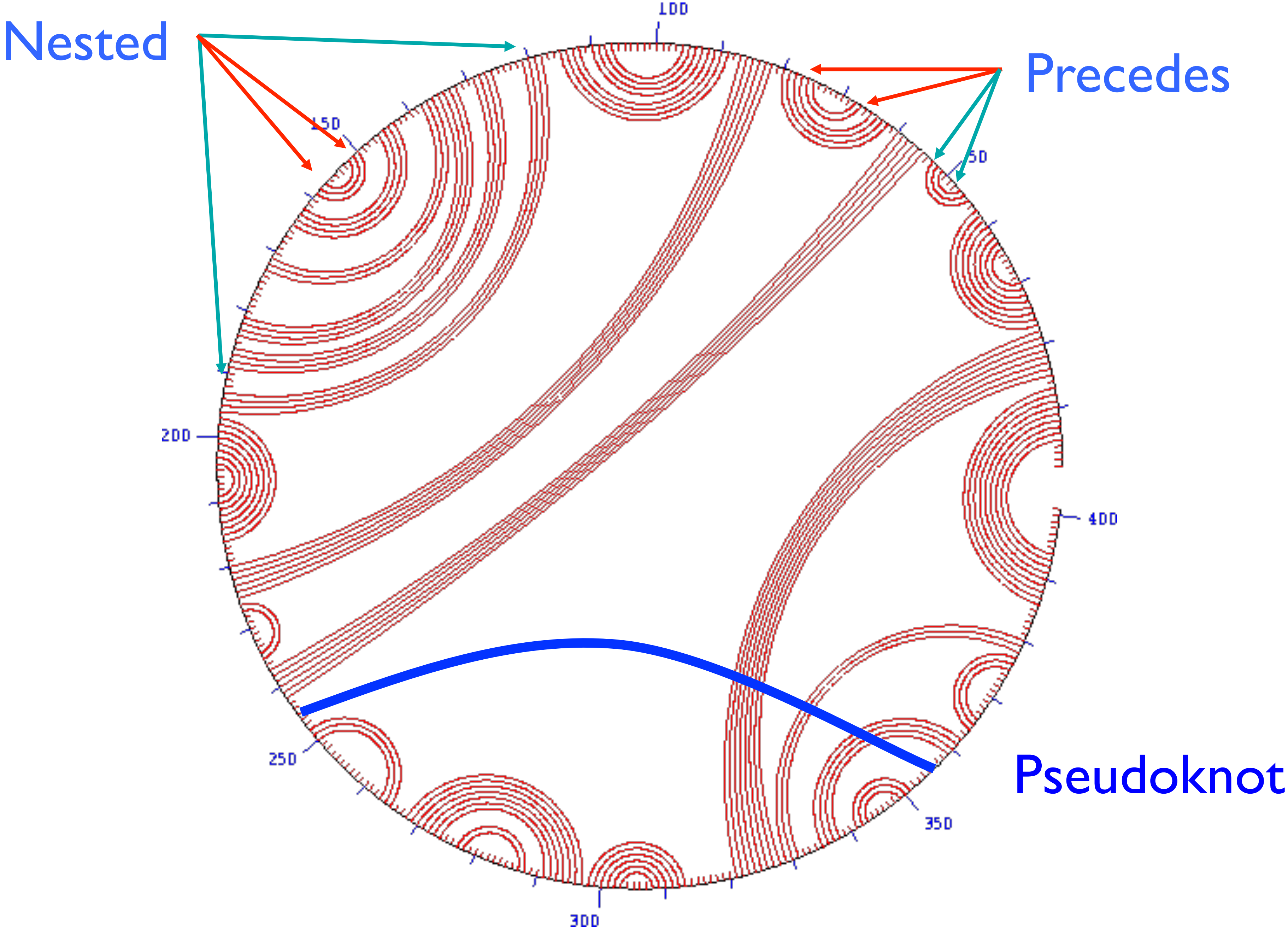
$j < i'$, or

$i < i' < j' < j$

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

RNA Secondary Structure: Examples




Nested
Precedes
100
150
50
200
400
250
350
300
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

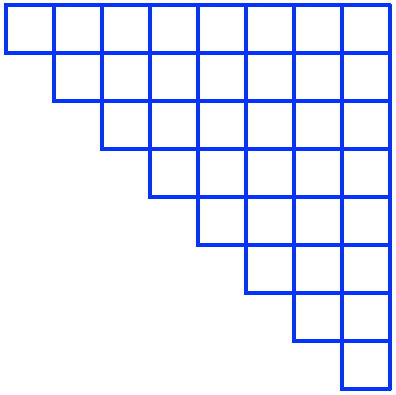
Nussinov: Max Pairing

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:

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



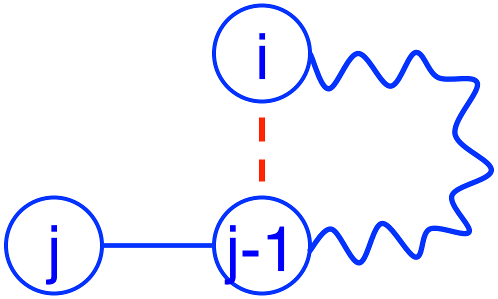
R Nussinov, AB Jacobson, "Fast algorithm for predicting the secondary structure of single-stranded RNA." PNAS 1980.

"Optimal pairing of r_i … r_j"

Two possibilities

j Unpaired:

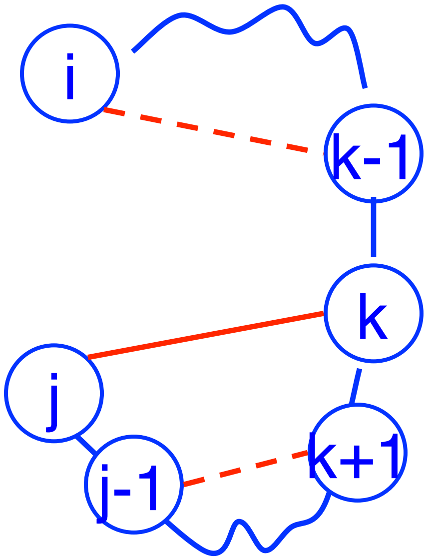
Find best pairing of r_i … r_{j-1}



j Paired (with some k):

Find best r_i … r_{k-1} +

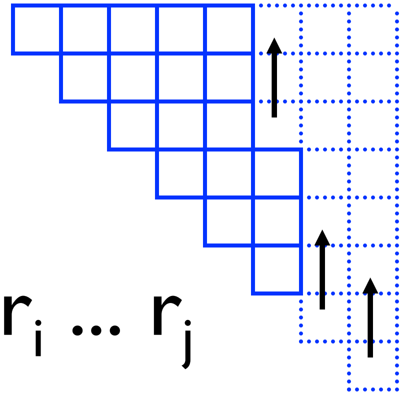
best r_{k+1} … r_{j-1} plus 1



Why is it slow?

Why do pseudoknots matter?

Nussinov:
A Computation Order

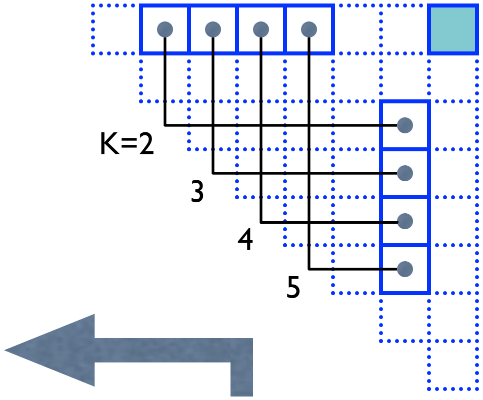


B(i,j) = # pairs in optimal pairing of r_i ... r_j

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

B(i,j) = max of:

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



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
- \+ works on single sequences
- \- ignores pseudoknots
- \- only finds “optimal” fold

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

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:

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

energy of k-j *pair*

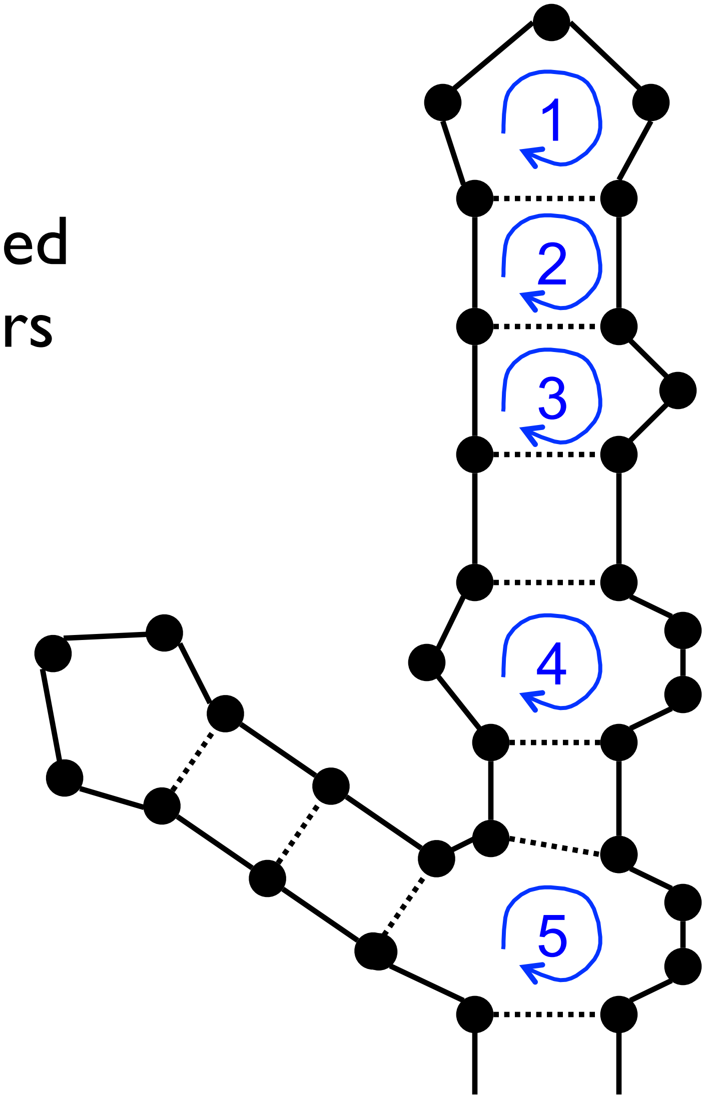
Time: $O(n^3)$

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•j

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

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

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, 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

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”