Genome 559: Introduction to Statistical and Computational Genomics

Winter 2010

Lecture 20a:

RNA

Function, Search, Discovery

The Message

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

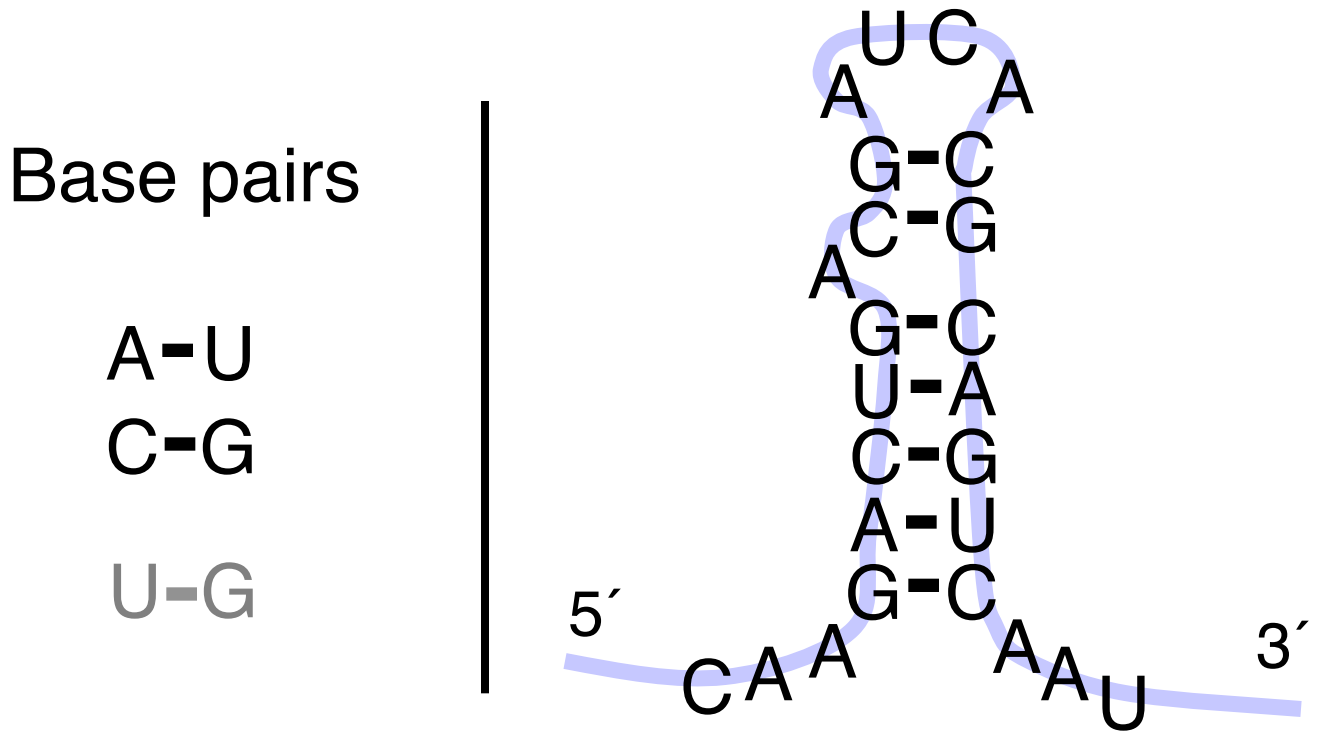
- Functionally important, functionally diverse, structurally complex

Computational tools needed

- Algorithms for alignment, discovery, search, scoring, etc.
- *Blended with* knowledge of the biology

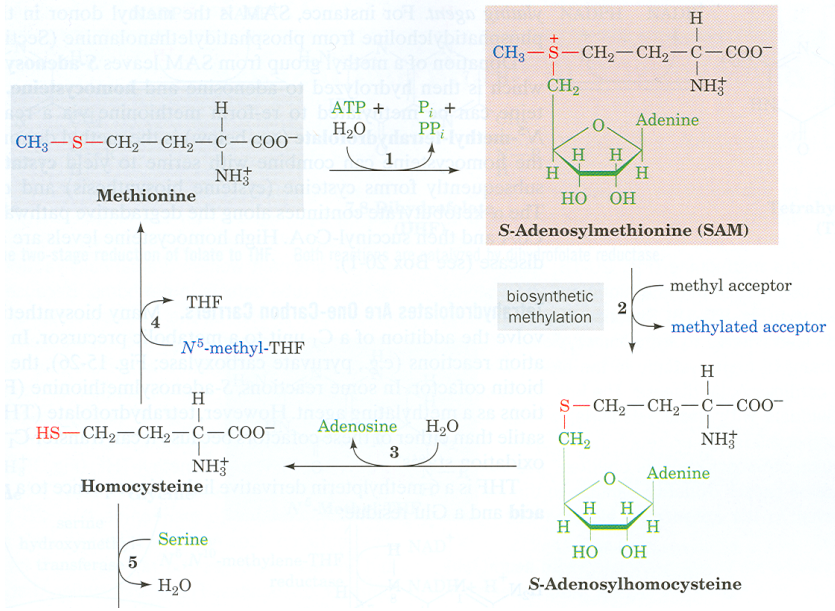
RNA

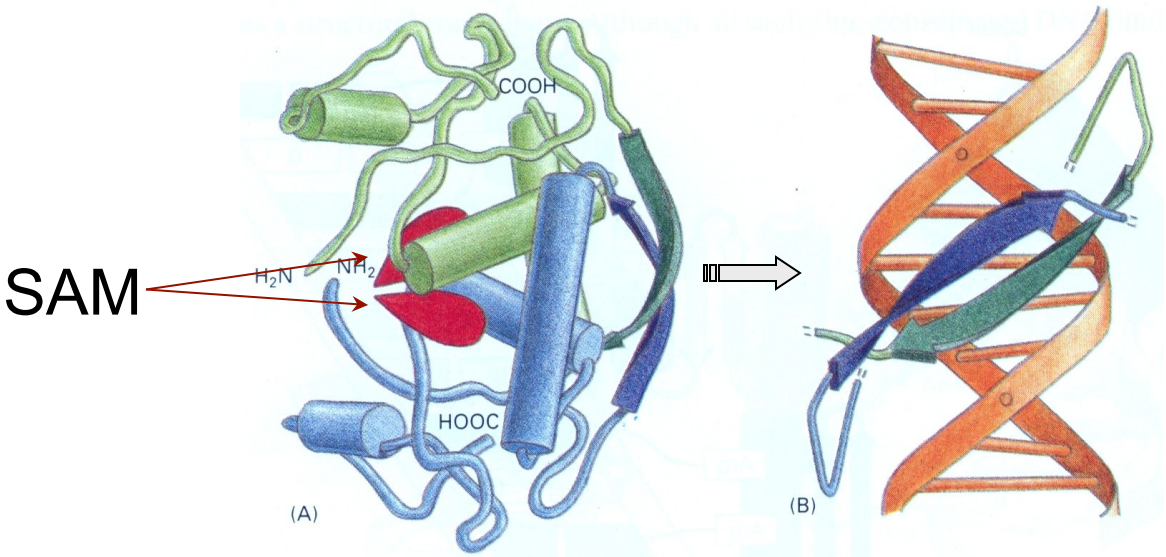
RNA Secondary Structure:
RNA makes helices too



Usually *single* stranded

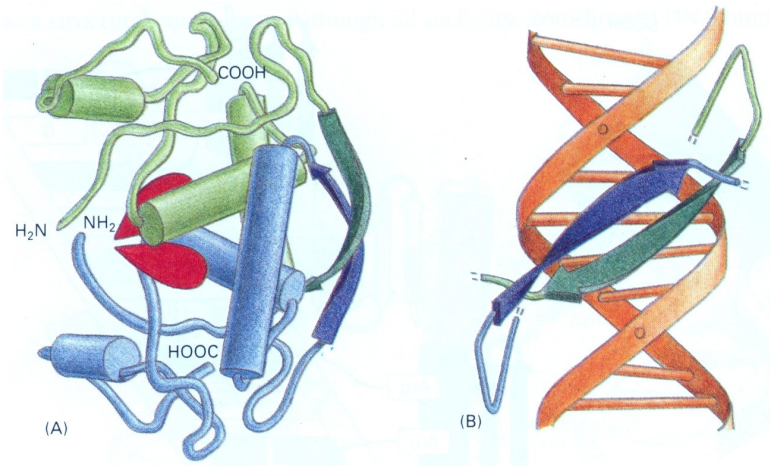
Central Dogma & Conventional Wisdom: Proteins catalyze & regulate biochemistry





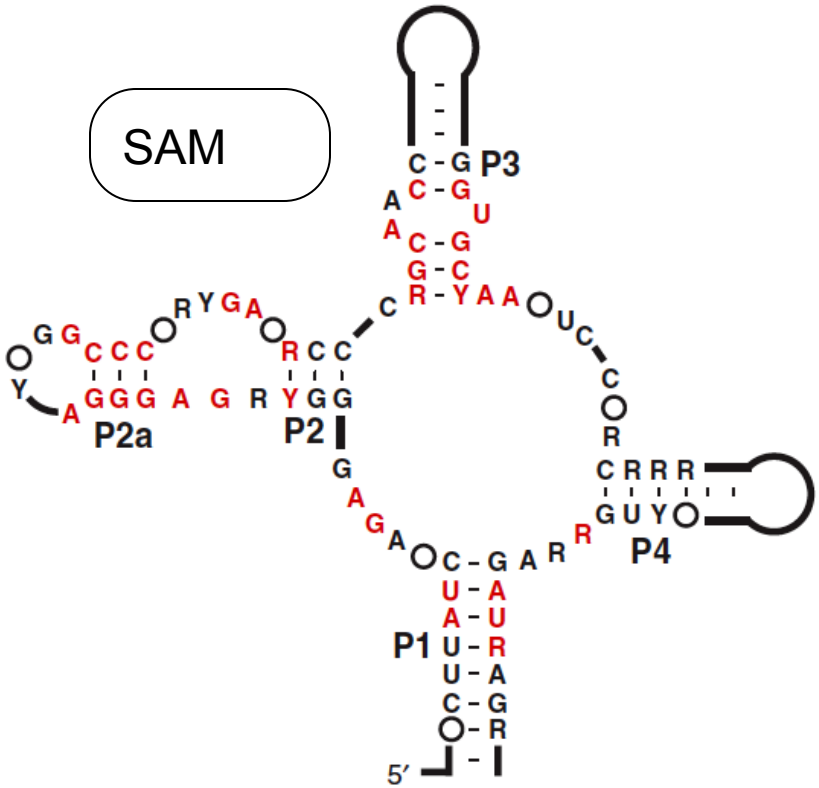
The Met Repressor

Alberts, et al, 3e.



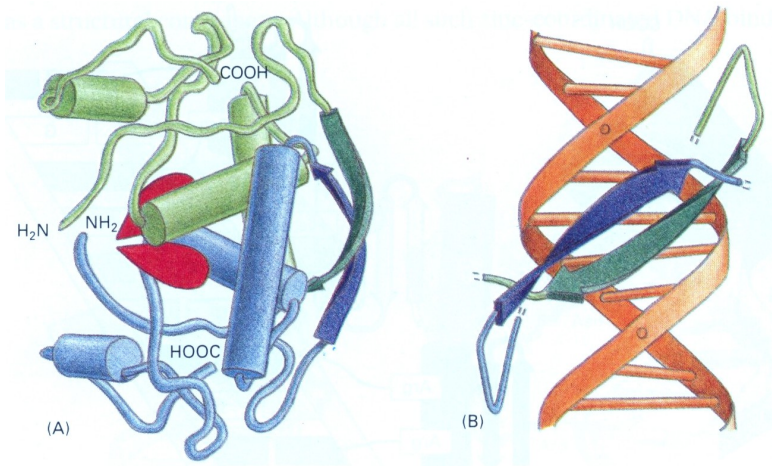
The protein way

Riboswitch alternative



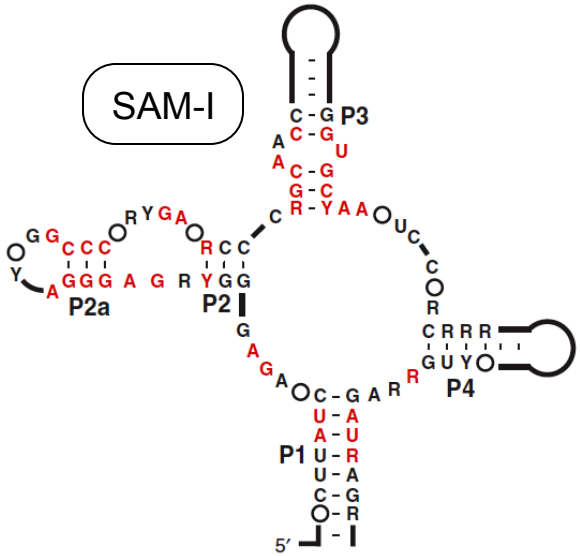
Grundy & Henkin, Mol. Microbiol 1998
Epshtein, et al., PNAS 2003
Winkler et al., Nat. Struct. Biol. 2003

Alberts, et al, 3e.

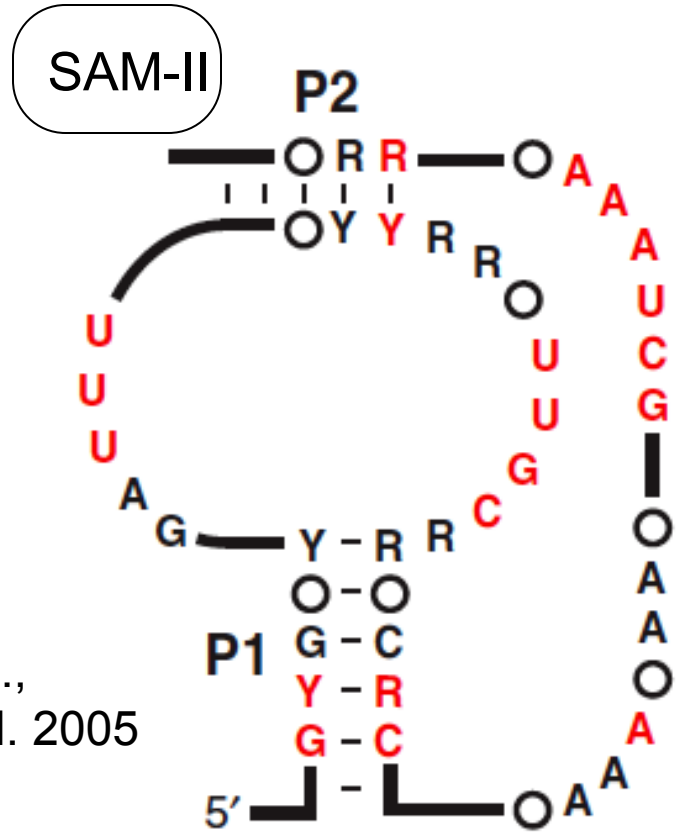


The protein way

Riboswitch alternatives

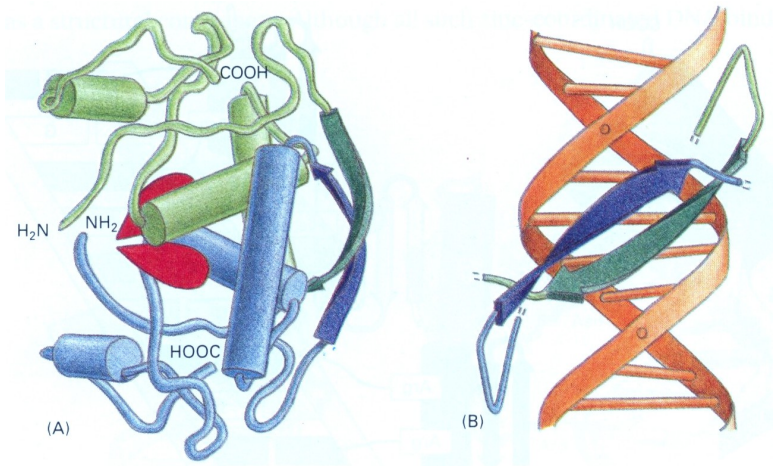


Grundy, Epshtein, Winkler et al., 1998, 2003



Corbino et al., Genome Biol. 2005

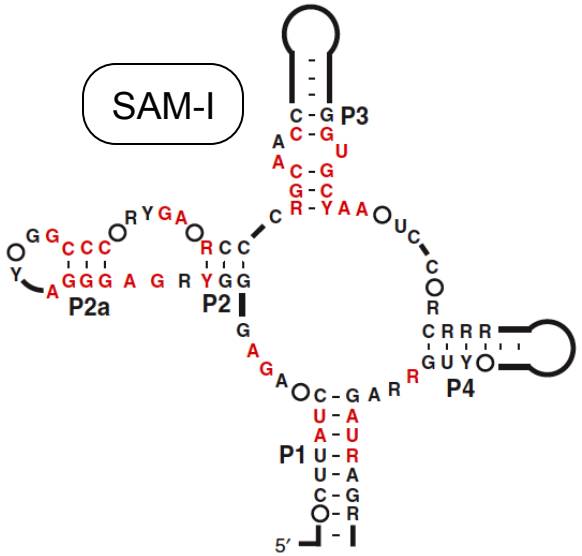
Alberts, et al, 3e.



The protein way

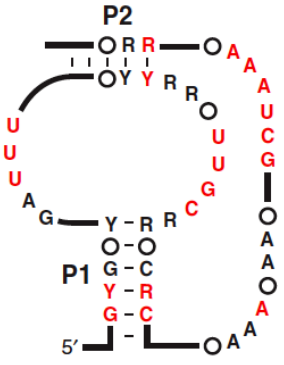
Riboswitch alternatives

SAM-I



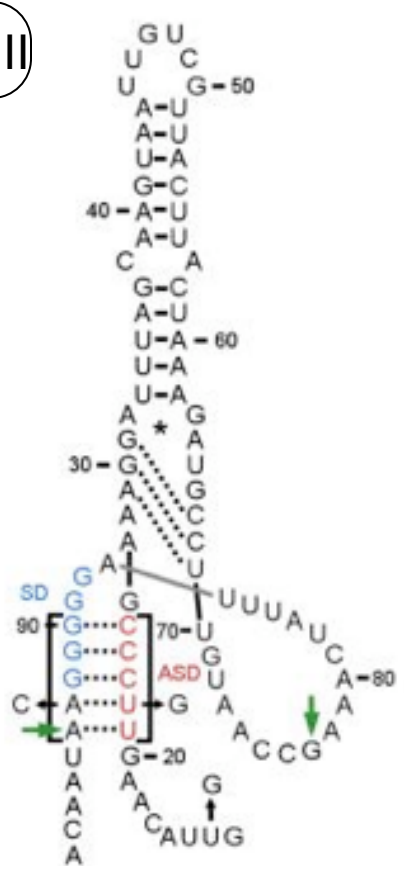
Grundy, Epshtein, Winkler et al., 1998, 2003

SAM-II



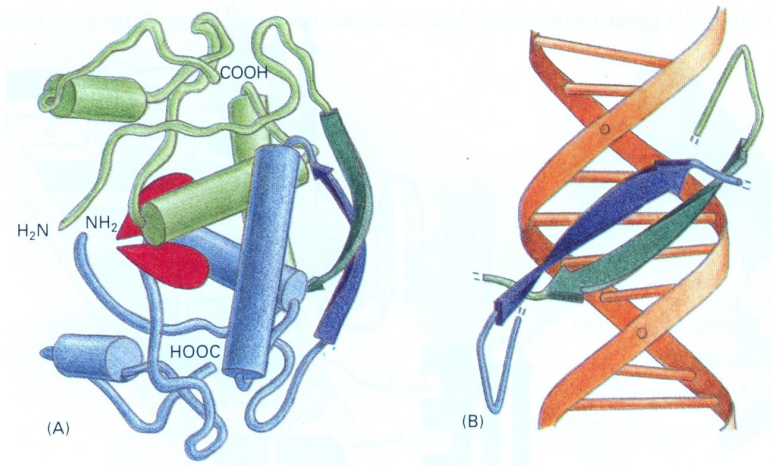
Corbino et al., Genome Biol. 2005

SAM-III



Fuchs et al., NSMB 2006

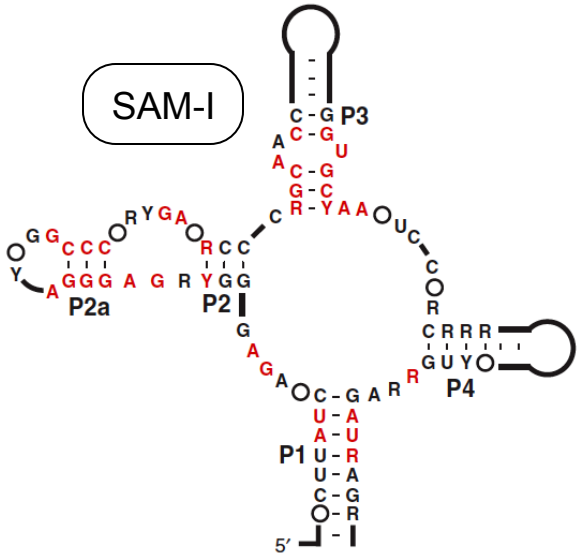
Alberts, et al, 3e.



The protein way

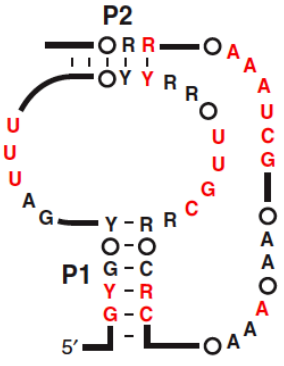
Riboswitch alternatives

SAM-I



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

SAM-II

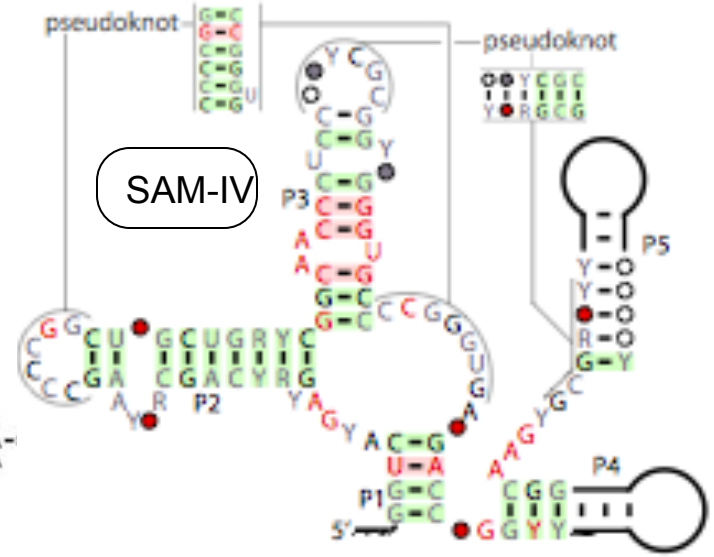


Corbino et al., Genome Biol. 2005

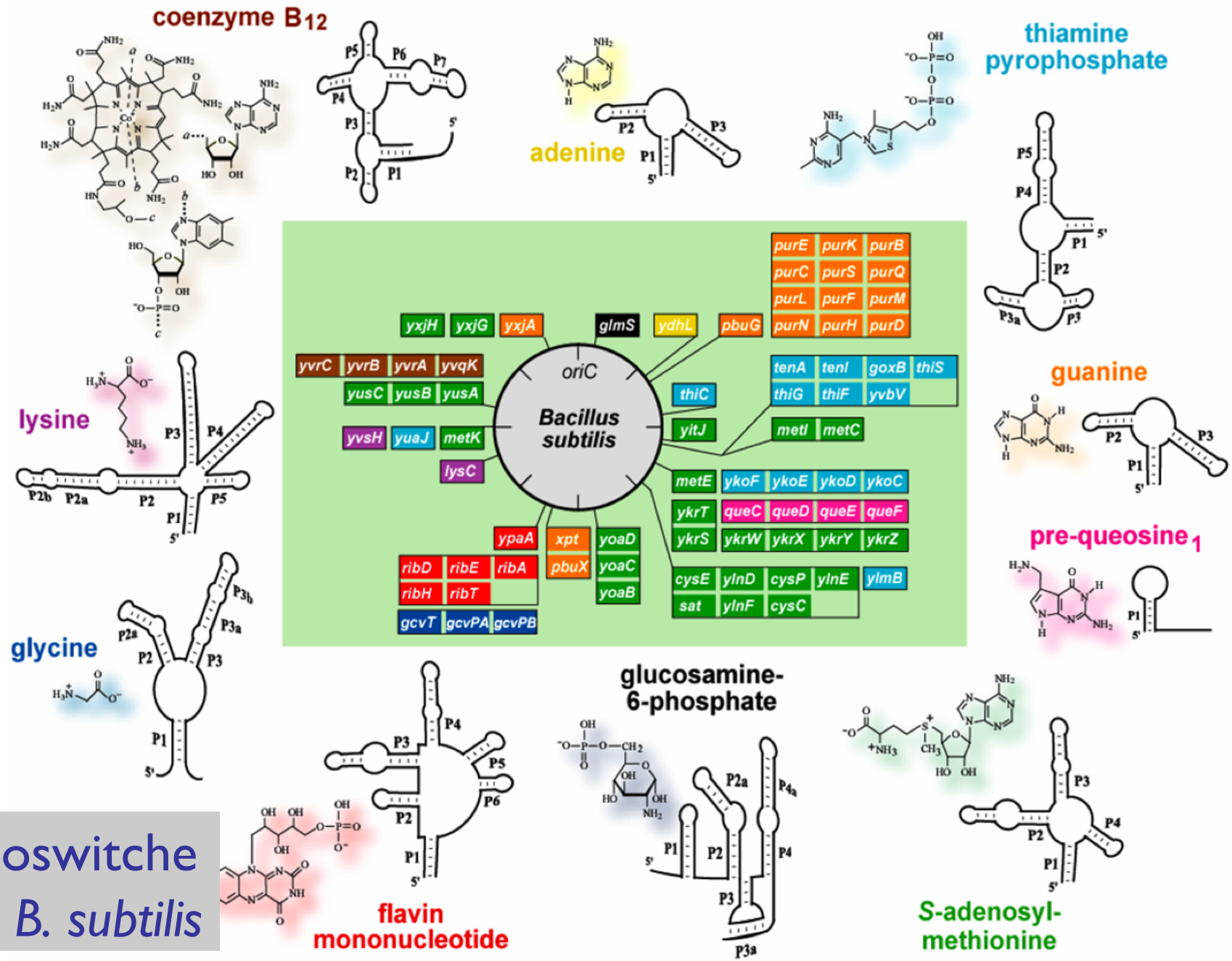
SAM-III

Fuchs et al., NSMB 2006

SAM-IV



Weinberg et al., RNA 2008



Riboswitches in *B. subtilis*

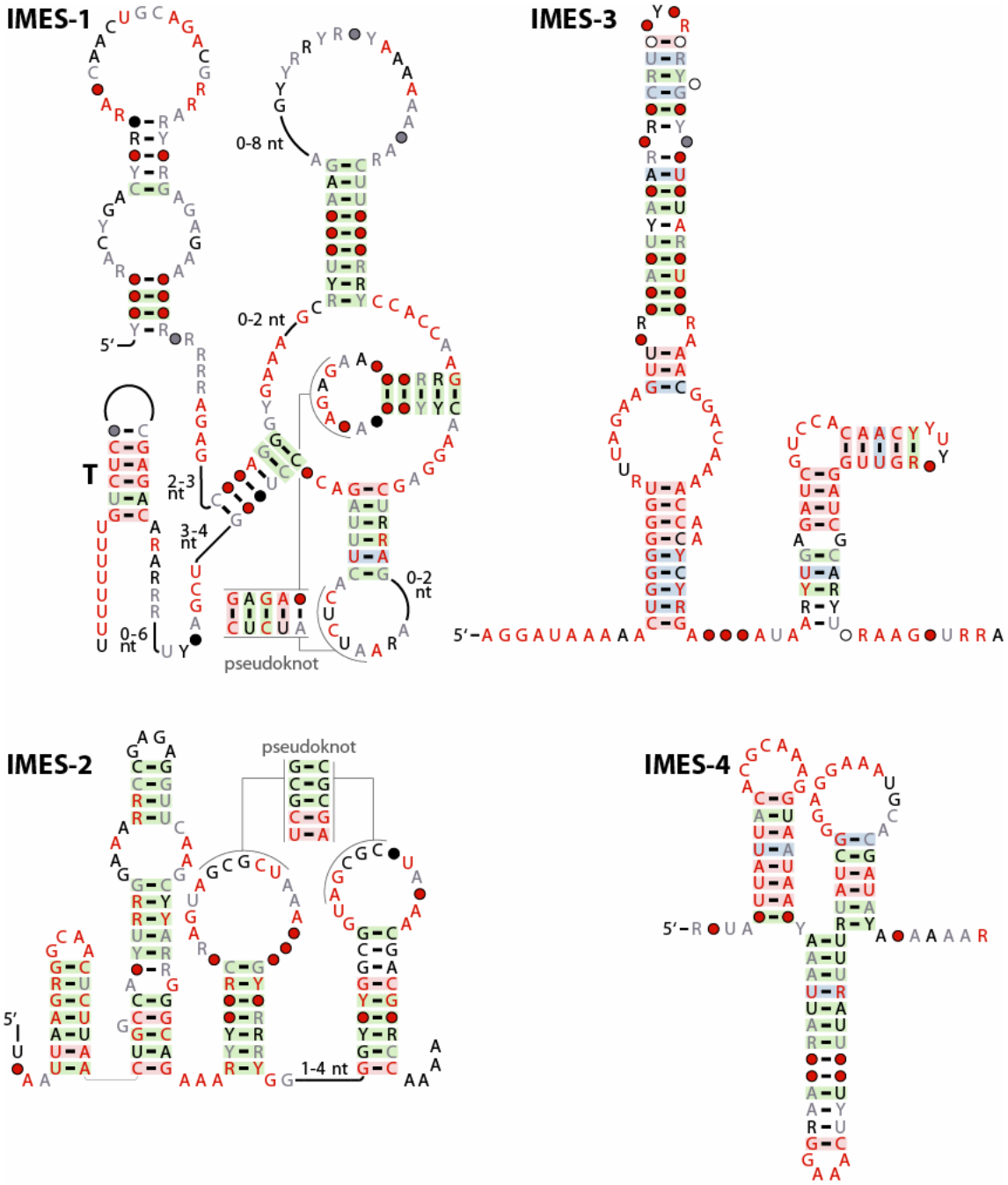
RNAs of unusual abundance

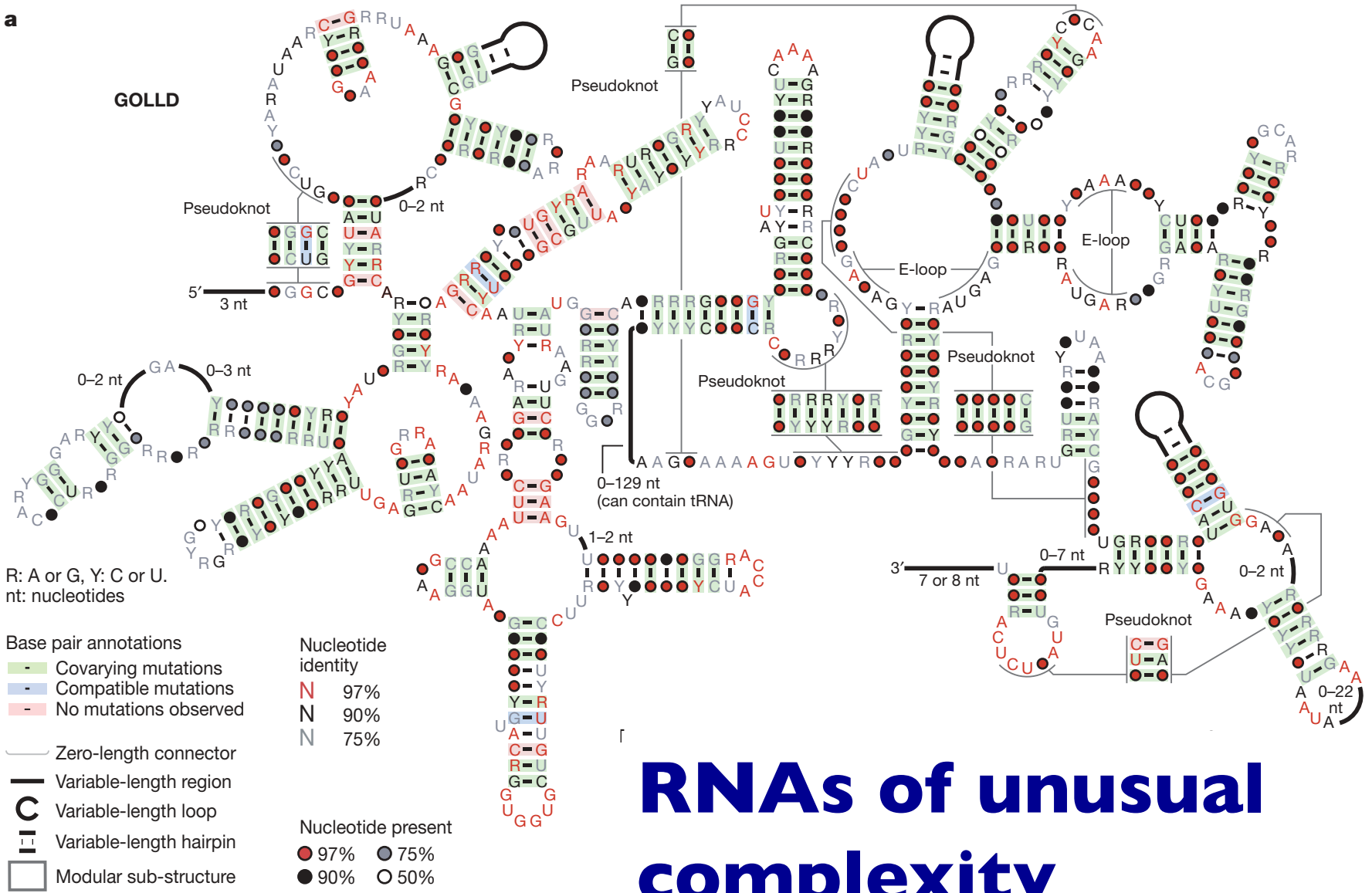
Still being doscovered.

More abundant than 5S rRNA

From unknown marine organisms

Weinberg et al.,
Nature, Dec 2009

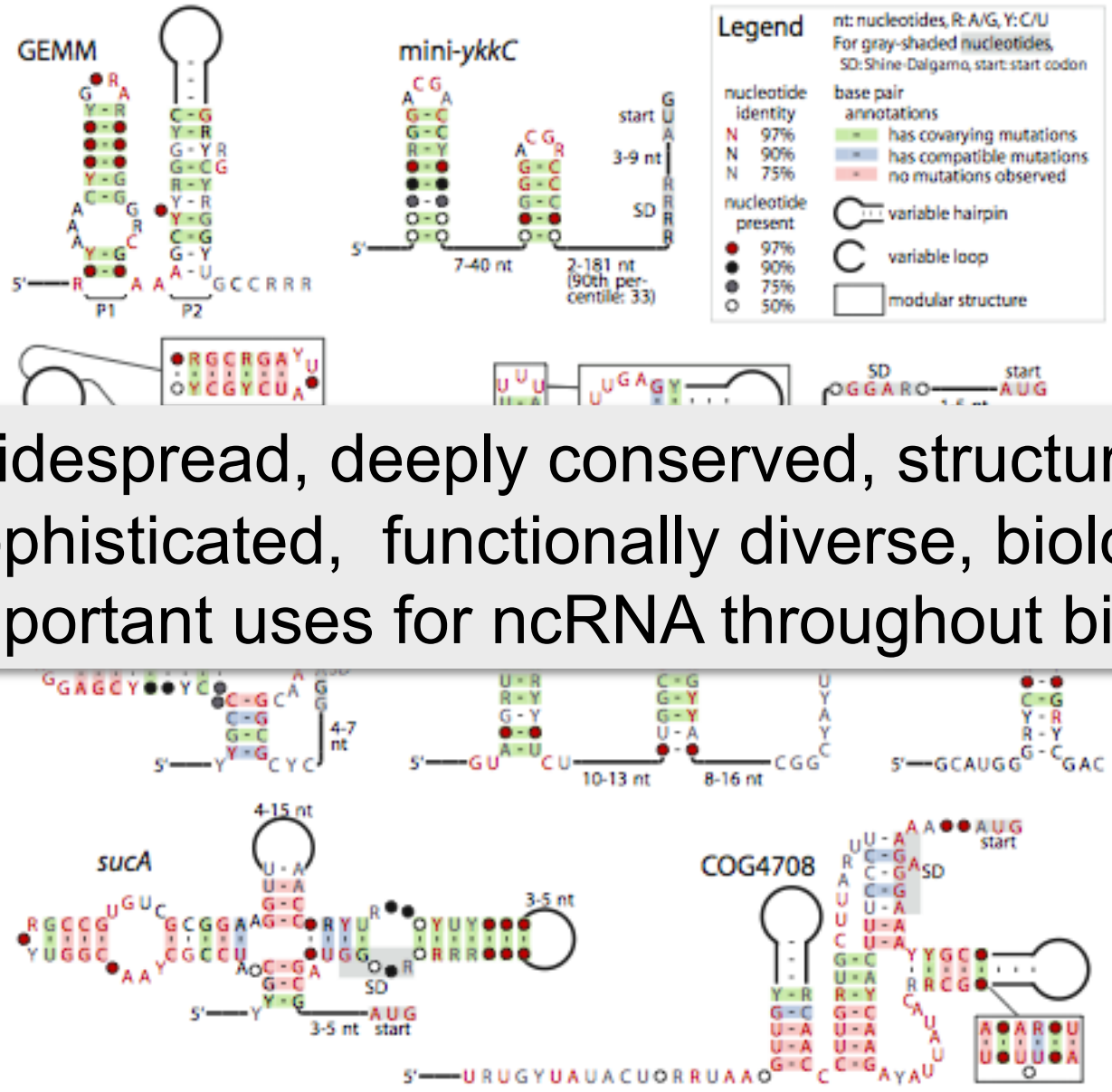




RNAs of unusual complexity

still being discovered

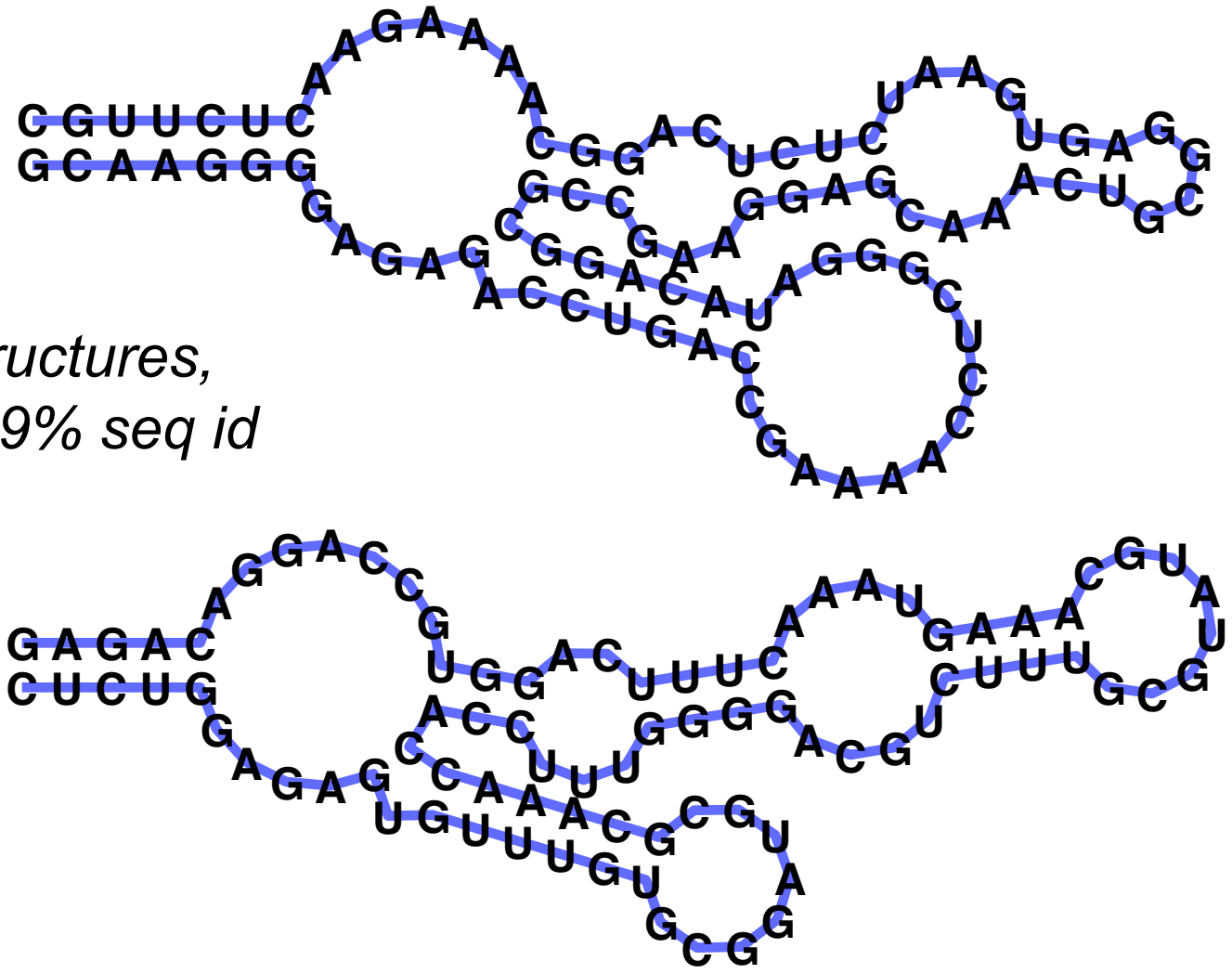
Weinberg et al., *Nature*, Dec 2009



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

Weinberg, et al. Nucl. Acids Res., July 2007 35: 4809-4819.

Why is RNA hard to deal with?

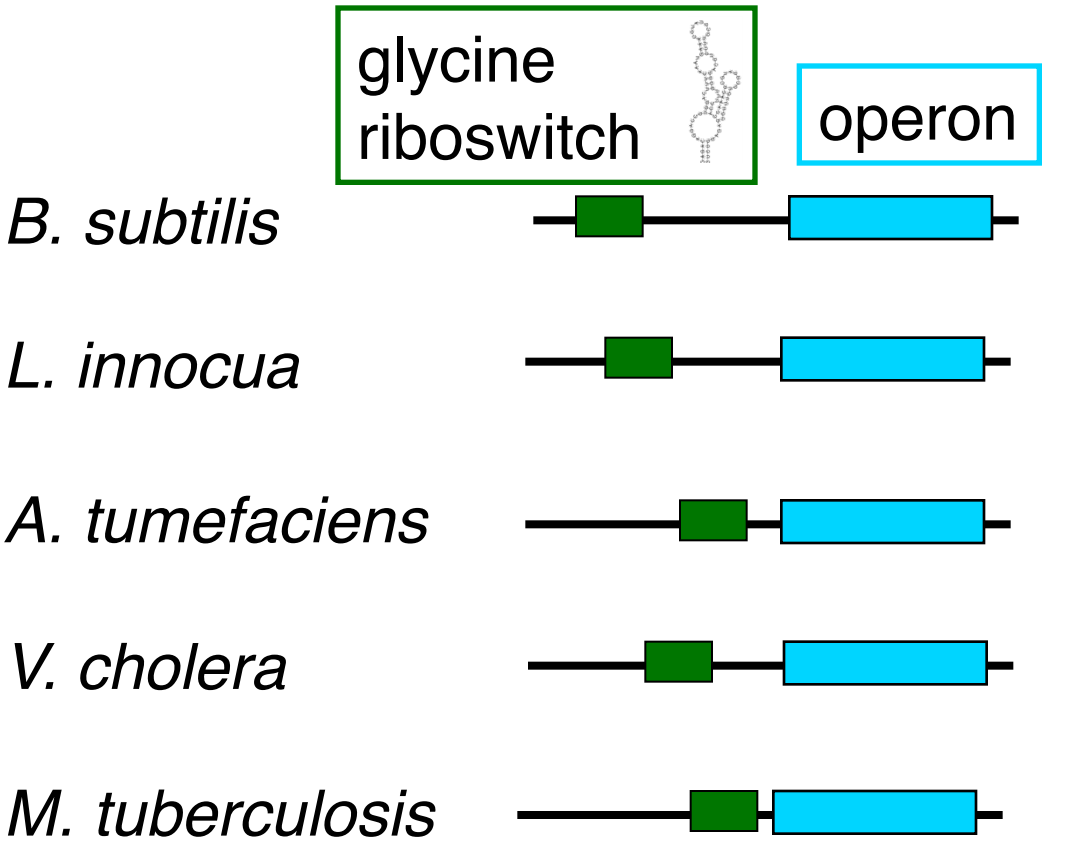


Similar structures,
but only 29% seq id

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

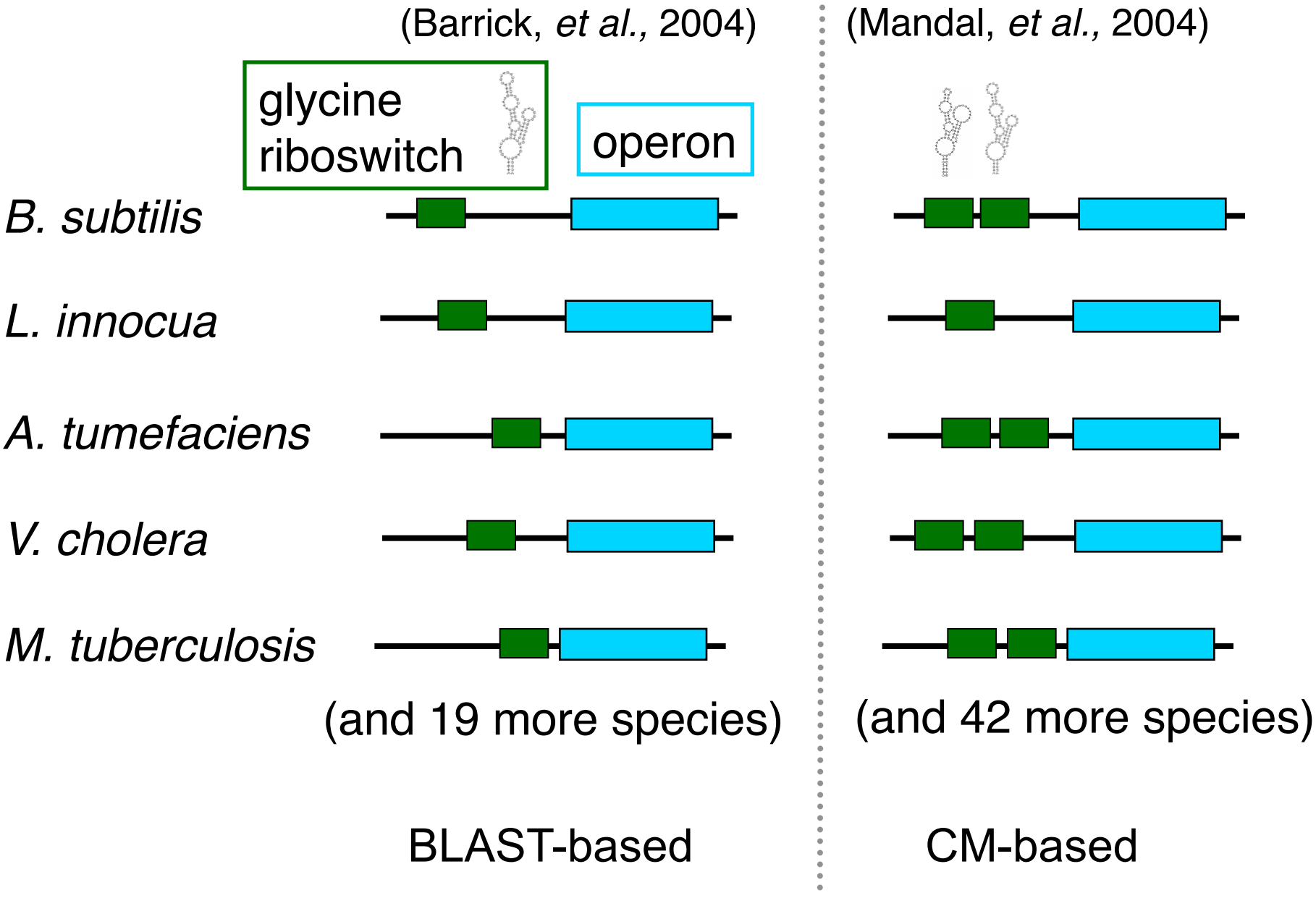
Impact of RNA homology search

(Barrick, *et al.,* 2004)

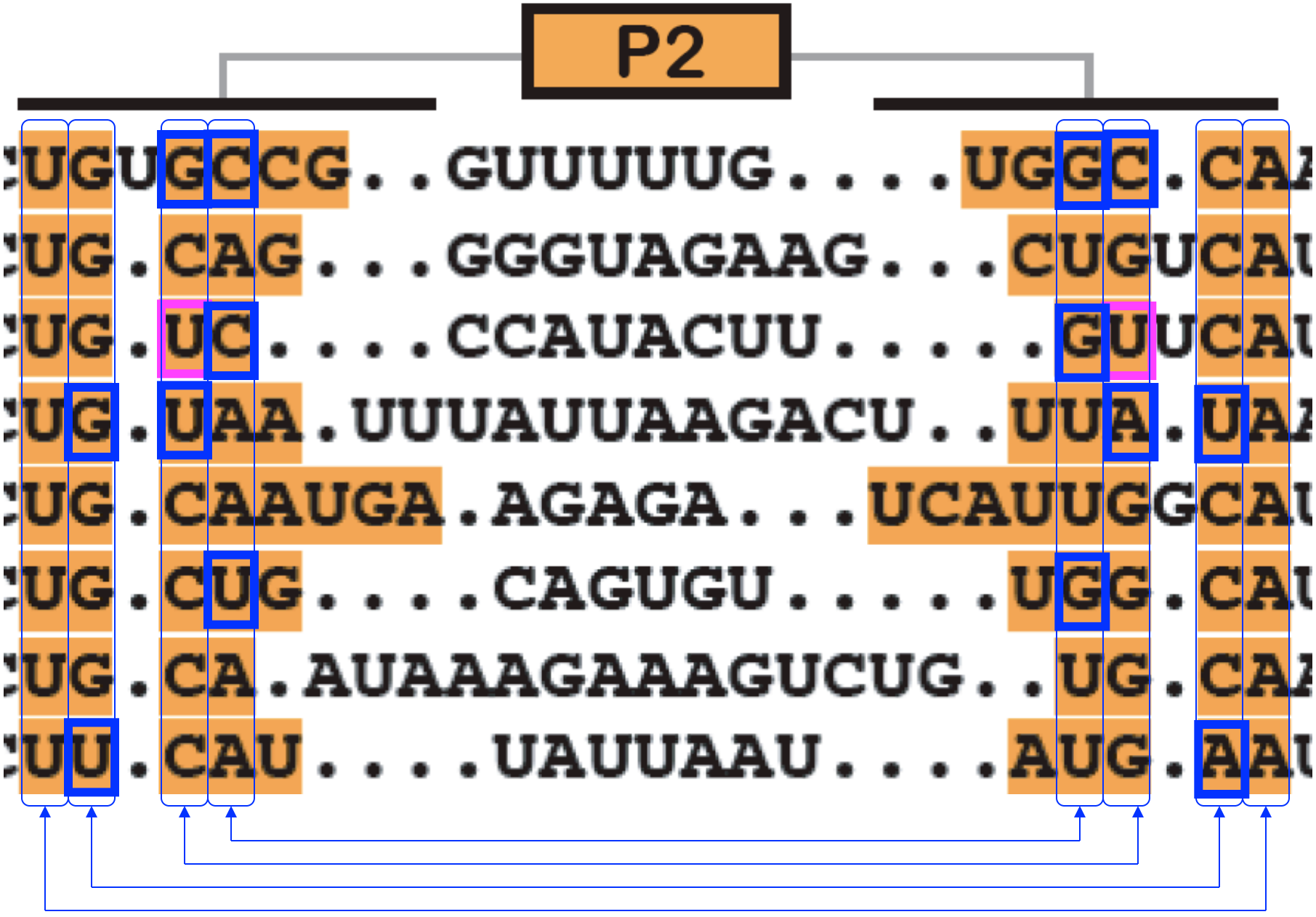


(and 19 more species)

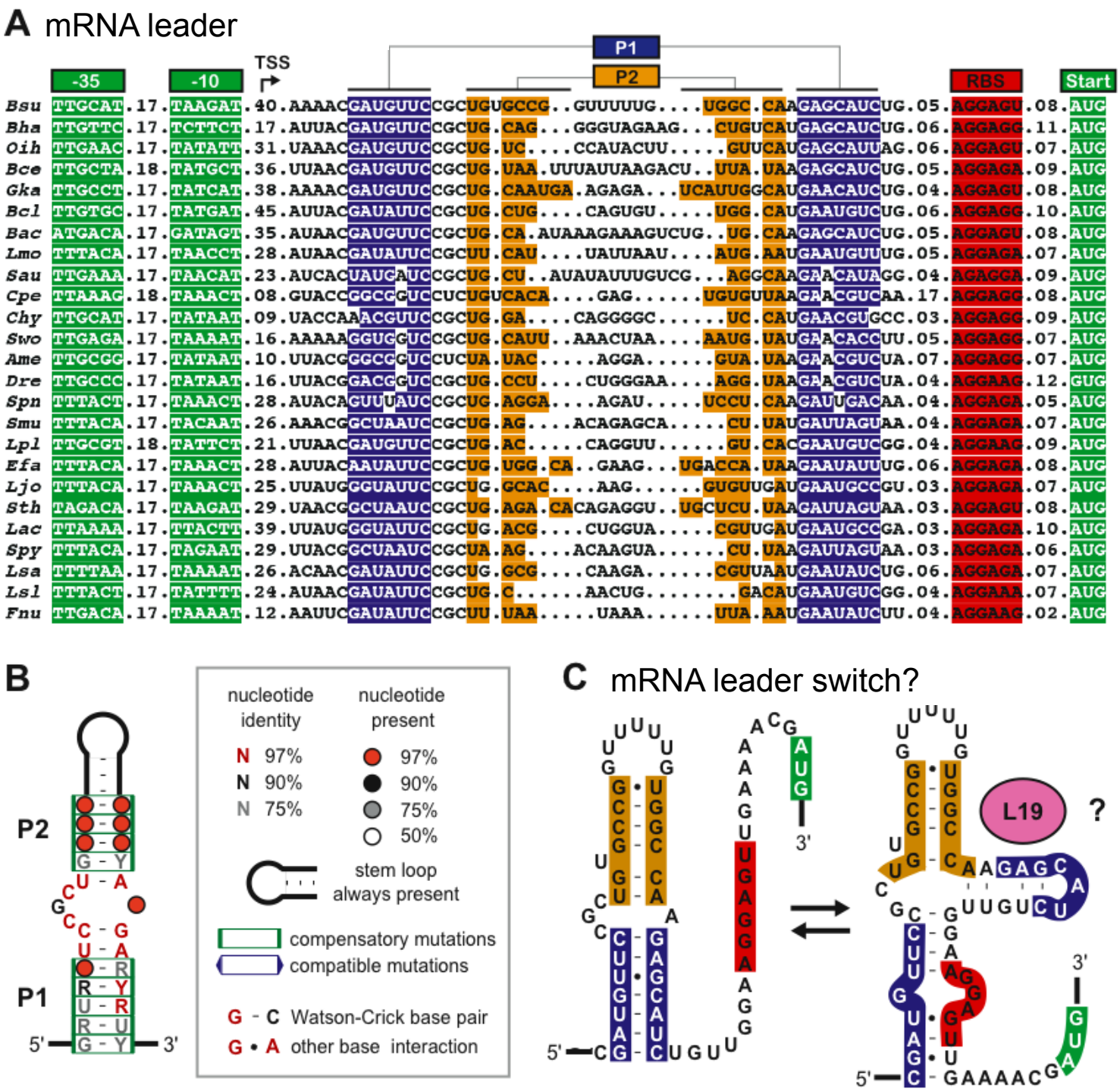
Impact of RNA homology search



Motif Description & Inference



Covariation is strong evidence for base pairing


A mRNA leader
P1
P2
TSS
-35
-10
RBS
Start
Bsu TTGCAT.17.TAAGAT.40.AAAACGAUGUUCCGCUGUGCCG..GUUUUUG....UGGC.CAAGAGCAUCUG.05.AGGAGU.08.AUG
Bha TTGTTC.17.TCTTCT.17.AUUACGAUGUUCCGCUG.CAG...GGGUAGAAG...CUGUCAUGAGCAUCUG.06.AGGAGG.11.AUG
Oih TTGAAC.17.TATATT.31.UAAACGAUGUUCCGCUG.UC....CCAUACUU.....GUUCAUGAGCAUUAG.06.AGGAGU.07.AUG
Bce TTGCTA.18.TATGCT.36.UUAACGAUGUUCCGCUG.UAA.UUUAUUAAGACU..UUA.UAAGAGCAUCUG.05.AGGAGA.09.AUG
Gka TTGCCT.17.TATCAT.38.AAAACGAUGUUCCGCUG.CAAUGA.AGAGA...UCAUUGGCAUGAACAUCUG.04.AGGAGU.08.AUG
Bcl TTGTGC.17.TATGAT.45.AUUACGAUAUUCCGCUG.CUG....CAGUGU.....UGG.CAUGAAUGUCUG.06.AGGAGG.10.AUG
Bac ATGACA.17.GATAGT.35.AUAACGAUGUUCCGCUG.CA.AUAAAGAAAGUCUG..UG.CAAGAGCAUCUG.05.AGGAGU.08.AUG
Lmo TTTACA.17.TAACCT.28.AUAACGAUAUUCCGCUU.CAU....UAUUAAU....AUG.AAUGAAUGUUUG.05.AGGAGA.07.AUG
Sau TTGAAA.17.TAACAT.23.AUCACUAUGAUCCGCUG.CU..AUAUAUUUGUCG...AGGCAAGAACAUAGG.04.AGAGGA.09.AUG
Cpe TTAAAG.18.TAAACT.08.GUACCGGCGGUCCUCUGUCACA....GAG......UGUGUUAAGAACGUCAA.17.AGGAGG.08.AUG
Chy TTGCAT.17.TATAAT.09.UACCAAACGUUCCGCUG.GA....CAGGGGC.....UC.CAUGAACGUGCC.03.AGGAGG.09.AUG
Swo TTGAGA.17.TAAAAT.16.AAAAAGGUGGUCCGCUG.CAUU..AAACUAA....AAUG.UAUGAACACCUU.05.AGGAGG.07.AUG
Ame TTGCGG.17.TATAAT.10.UUACGGGCGGUCCUCUA.UAC.....AGGA.....GUA.UAAGAACGUCUA.07.AGGAGG.07.AUG
Dre TTGCCC.17.TATAAT.16.UUACGGACGGUCCGCUG.CCU....CUGGGAA....AGG.UAAGAACGUCUA.04.AGGAAG.12.GUG
Spn TTTACT.17.TAAACT.28.AUACAGUUUAUCCGCUG.AGGA....AGAU.....UCCU.CAAGAUUGACAA.04.AGGAGA.05.AUG
Smu TTTACA.17.TACAAT.26.AAACGGCUAAUCCGCUG.AG....ACAGAGCA.....CU.UAUGAUUAGUAA.04.AGGAGA.07.AUG
Lpl TTGCGT.18.TATTCT.21.UUAACGAUGUUCCGCUG.AC.....CAGGUU.....GU.CACGAAUGUCGG.04.AGGAAG.09.AUG
Efa TTTACA.17.TAAACT.28.AUUACAAUAUUCCGCUG.UGG.CA..GAAG...UGACCA.UAAGAAUAUUUG.06.AGGAGA.08.AUG
Ljo TTTACA.17.TAAACT.25.UUAUGGGUAUUCCGCUG.GCAC....AAG.....GUGUUGAUGAAUGCCGU.03.AGGAGA.07.AUG
Sth TAGACA.17.TAAGAT.29.UAACGGCUAAUCCGCUG.AGA.CACAGAGGU..UGCUCU.UAAGAUUAGUAA.03.AGGAGU.08.AUG
Lac TTAAAA.17.TTACTT.39.UUAUGGGUAUUCCGCUG.ACG....CUGGUA.....CGUUGAUGAAUGCCGA.03.AGGAGA.10.AUG
Spy TTTACA.17.TAGAAT.29.UUACGGCUAAUCCGCUA.AG....ACAAGUA.....CU.UAAGAUUAGUAA.03.AGGAGA.06.AUG
Lsa TTTTAA.17.TAAAAT.26.ACAACGAUAUUCCGCUG.GCG....CAAGA.....CGUUAAUGAAUAUCUG.06.AGGAGA.07.AUG
Lsl TTTACT.17.TATTTT.24.AUAACGAUAUUCCGCUG.C.....AACUG.....GACAUGAAUGUCGG.04.AGGAAA.07.AUG
Fnu TTGACA.17.TAAAAT.12.AAUUCGAUAUUCCGCUU.UAA.....UAAA.....UUA.AAUGAAUAUCUU.04.AGGAAG.02.AUG
B
P2
P1
5'
3'
nucleotide identity
nucleotide present
N 97%
N 90%
N 75%
97%
90%
75%
50%
stem loop always present
compensatory mutations
compatible mutations
G - C Watson-Crick base pair
G • A other base interaction
C mRNA leader switch?
L19
?
5'
3'

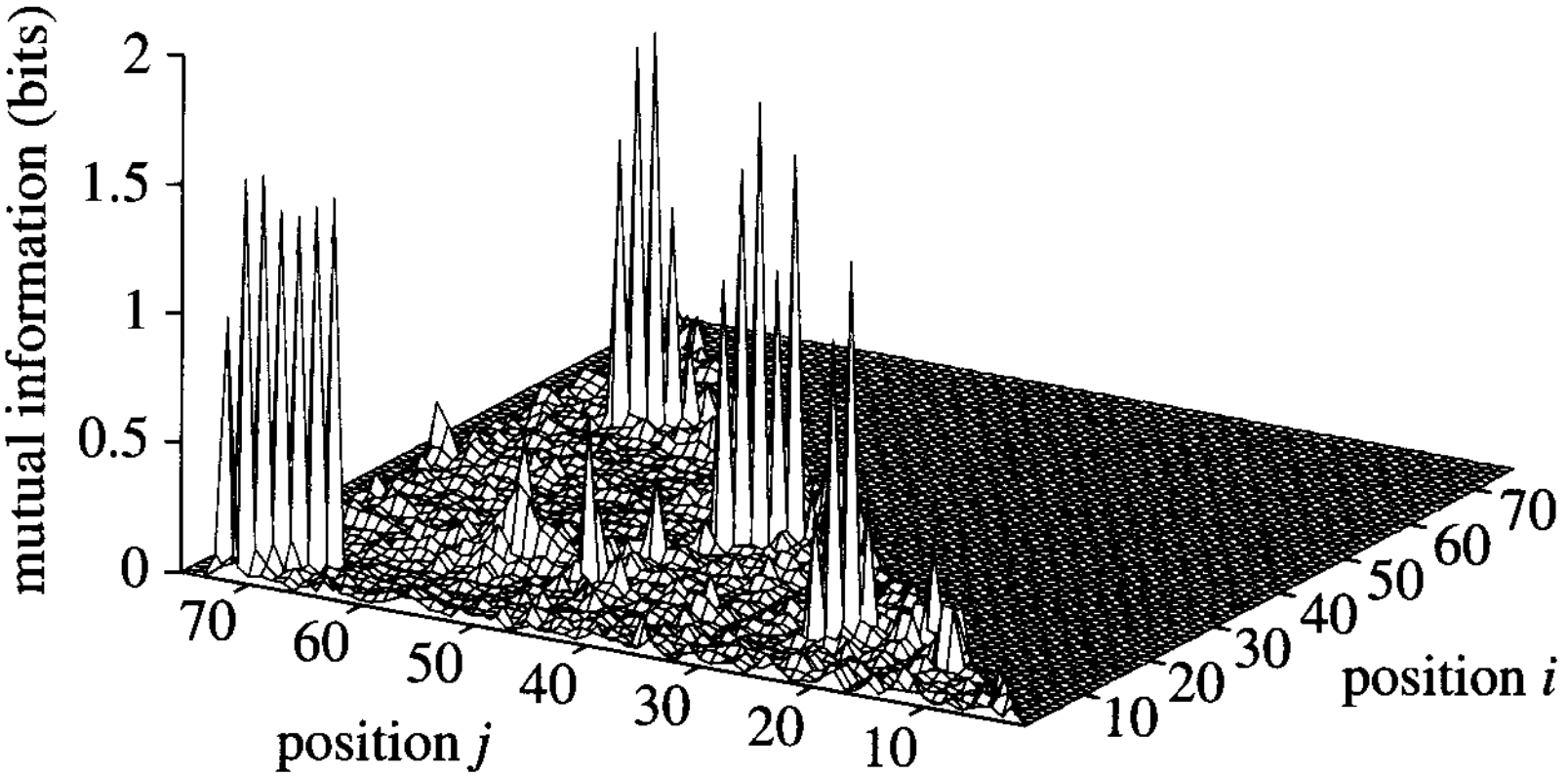
Mutual Information

$$M_{ij} = \sum_{i,j} f_{i,j} \log_2 \frac{f_{i,j}}{f_i f_j}; \quad 0 \le M_{ij} \le 2$$

Max when *no* seq conservation but perfect pairing;

Expected score gain from modeling i & j as paired.

Given columns, finding optimal pairing *without pseudoknots* can be done by dynamic programming



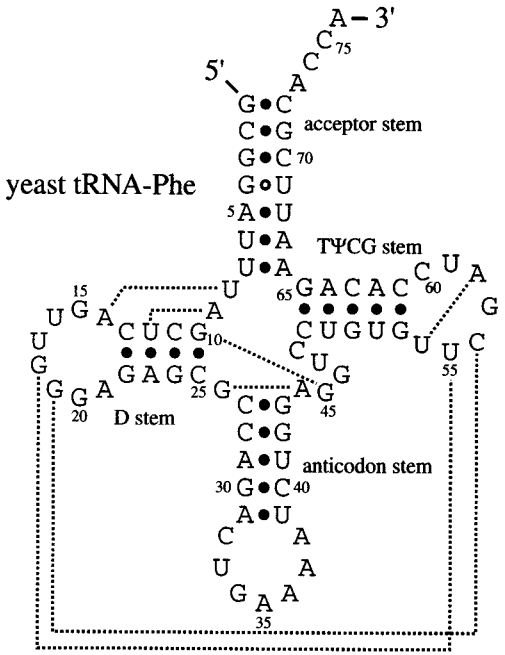


Figure 10.6 *A mutual information plot of a tRNA alignment (top) shows four strong diagonals of covarying positions, corresponding to the four stems of the tRNA cloverleaf structure (bottom; the secondary structure of yeast phenylalanine tRNA is shown). Dashed lines indicate some of the additional tertiary contacts observed in the yeast tRNA-Phe crystal structure. Some of these tertiary contacts produce correlated pairs which can be seen weakly in the mutual information plot.*

RNA Motif Models

"Covariance Models" (Eddy & Durbin 1994)

- aka profile stochastic context-free grammars
- aka hidden Markov models on steroids

Model position-specific nucleotide preferences *and* base-pair preferences

Pro: accurate

Con: model building hard, search sloooow

Profile Hmm Structure

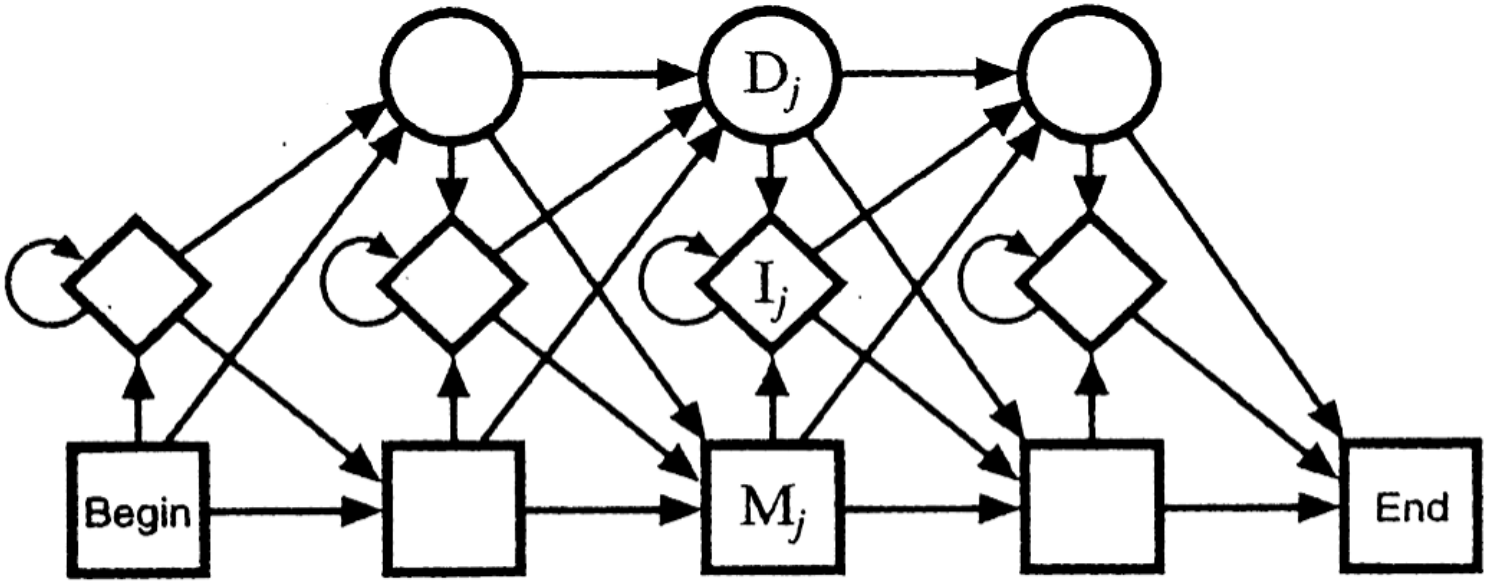


Figure 5.2 *The transition structure of a profile HMM.*

M_j: Match states (20 emission probabilities)

I_j: Insert states (Background emission probabilities)

D_j: Delete states (silent - no emission)

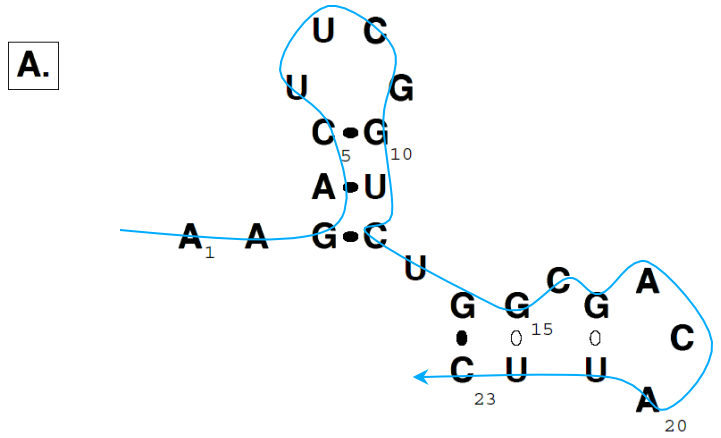
CM Structure

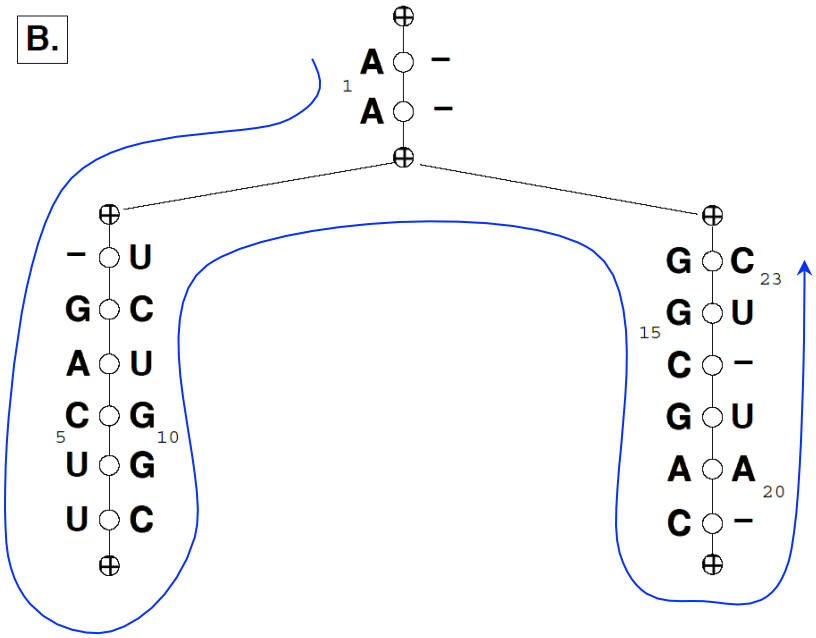
A: Sequence + structure

B: the CM "guide tree"

C: probabilities of letters/ pairs & of indels

Think of each branch being an HMM emitting both sides of a helix (but 3' side emitted in reverse order)





CM Viterbi Alignment
(the "inside" algorithm)

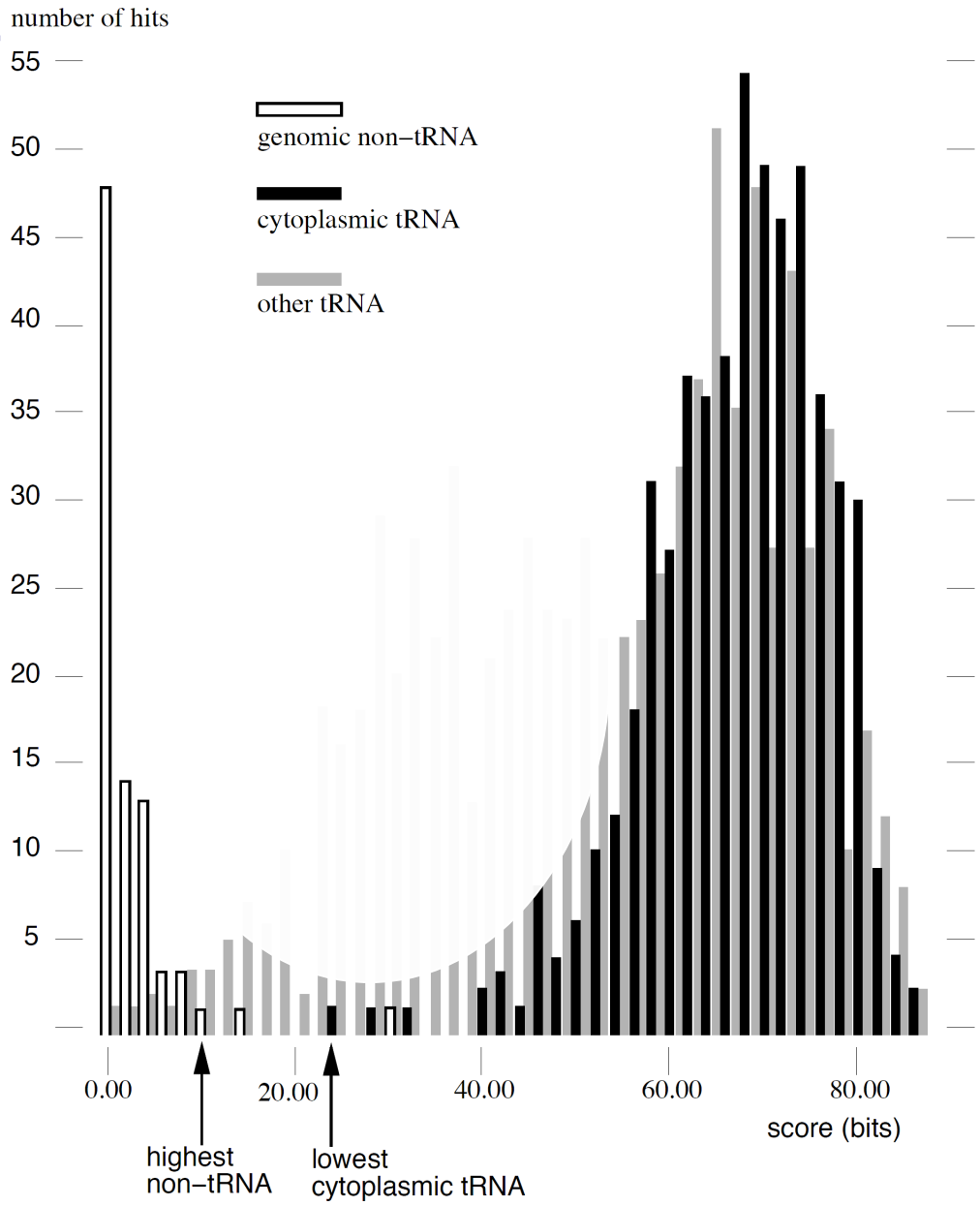
$$S_{ij}^{y} = \max_{\pi} \log P(x_{ij} \text{ generated starting in state } y \text{ via path } \pi)$$

$$S_{ij}^{y} = \begin{cases} \max_z [S_{i+1,j-1}^{z} + \log T_{yz} + \log E_{x_i,x_j}^{y}] & \text{match pair} \\ \max_z [S_{i+1,j}^{z} + \log T_{yz} + \log E_{x_i}^{y}] & \text{match/insert left} \\ \max_z [S_{i,j-1}^{z} + \log T_{yz} + \log E_{x_j}^{y}] & \text{match/insert right} \\ \max_z [S_{i,j}^{z} + \log T_{yz}] & \text{delete} \\ \max_{i<k\le j} [S_{i,k}^{y_{left}} + S_{k+1,j}^{y_{right}}] & \text{bifurcation} \end{cases}$$

Time $O(qn^3)$, q states, seq len n

compare: O(qn) for profile HMM, or pairwise alignment

Example: searching for tRNAs



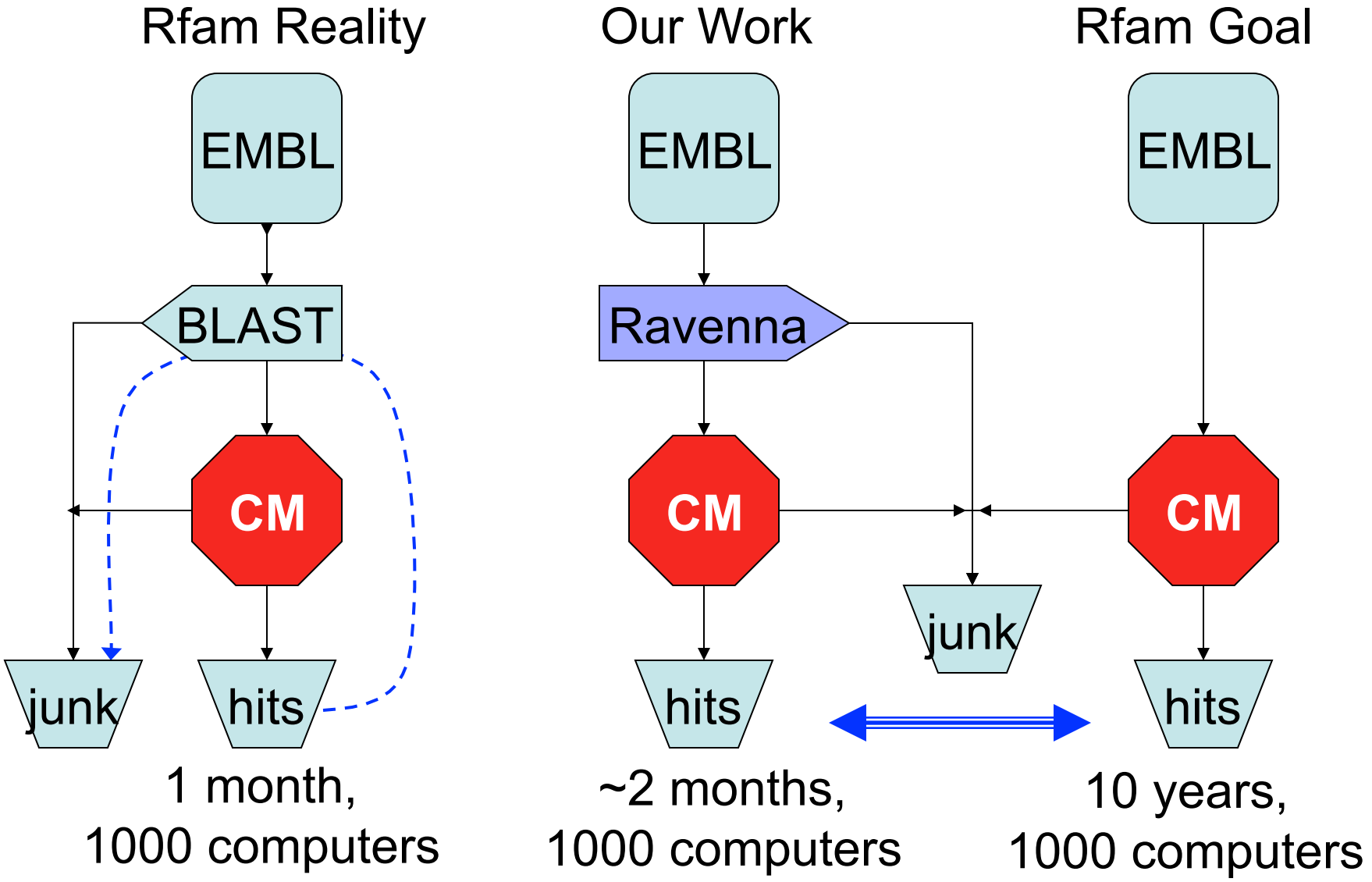
Fast Motif Search

Faster Genome Annotation
of Non-coding RNAs
Without Loss of Accuracy

Weinberg & Ruzzo

Recomb '04, ISMB '04, Bioinformatics '06

CM's are good, but slow



Results: New ncRNA's?

Name	# found BLAST + CM	# found rigorous filter + CM	# new
Pyrococcus snoRNA	57	180	123
Iron response element	201	322	121
Histone 3' element	1004	1106	102
Purine riboswitch	69	123	54
Retron msr	11	59	48
Hammerhead I	167	193	26
Hammerhead III	251	264	13
U4 snRNA	283	290	7
S-box	128	131	3
U6 snRNA	1462	1464	2
U5 snRNA	199	200	1
U7 snRNA	312	313	1

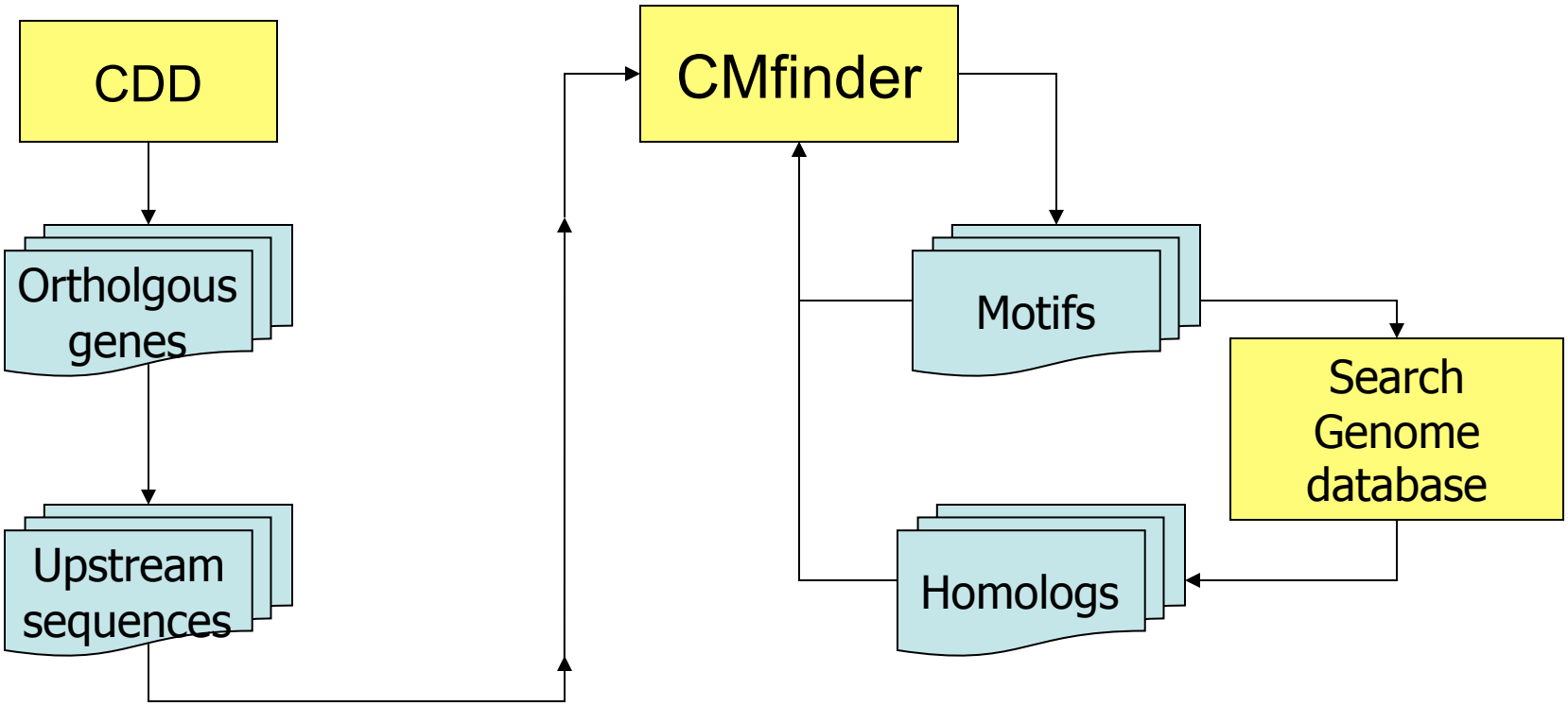
Motif Discovery In Prokaryotes

(Vertebrates too, but no time today…

see, e.g., Torarinsson, et al.

Genome Research, Jan 2008)

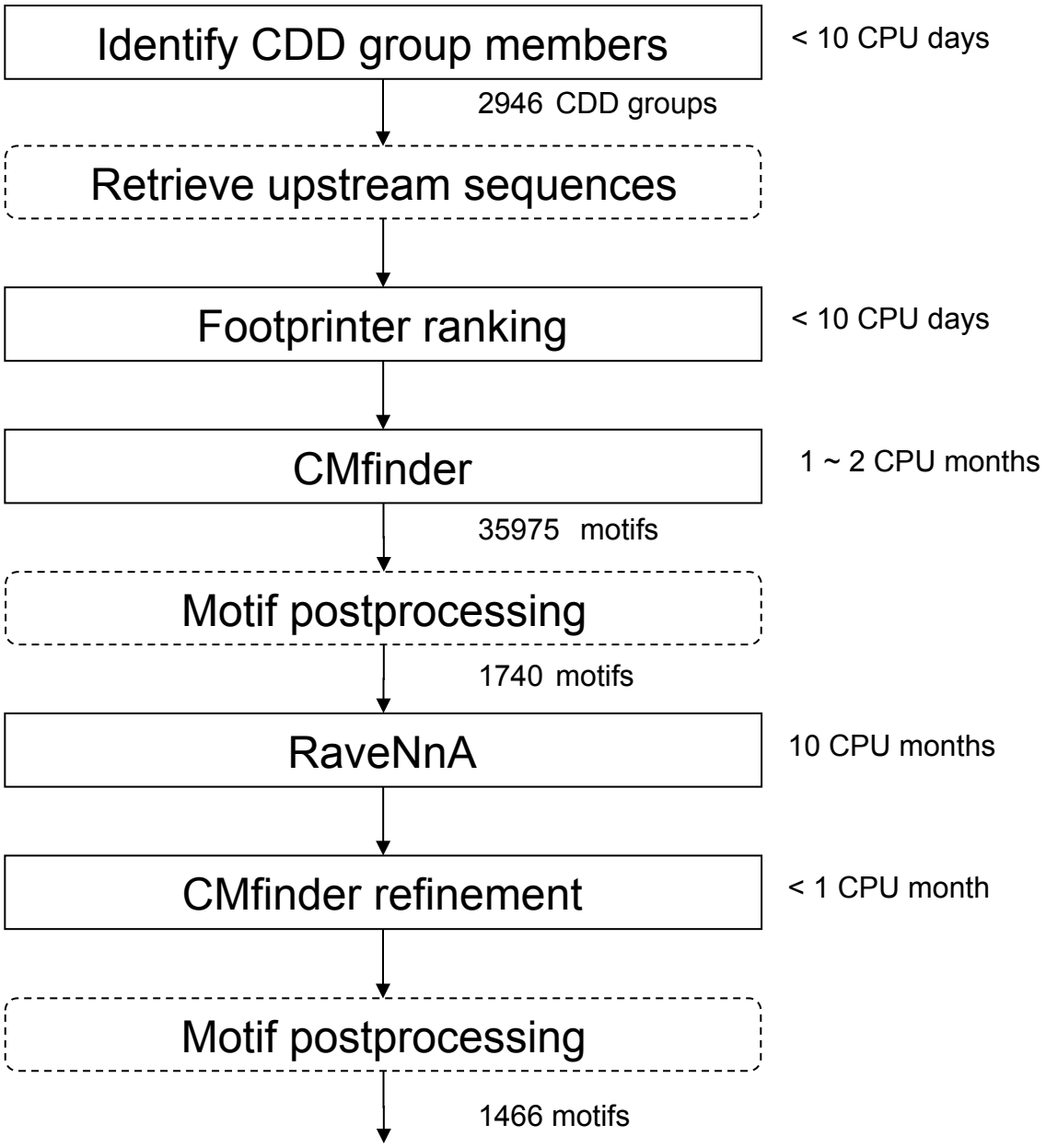
A pipeline for RNA motif genome scans

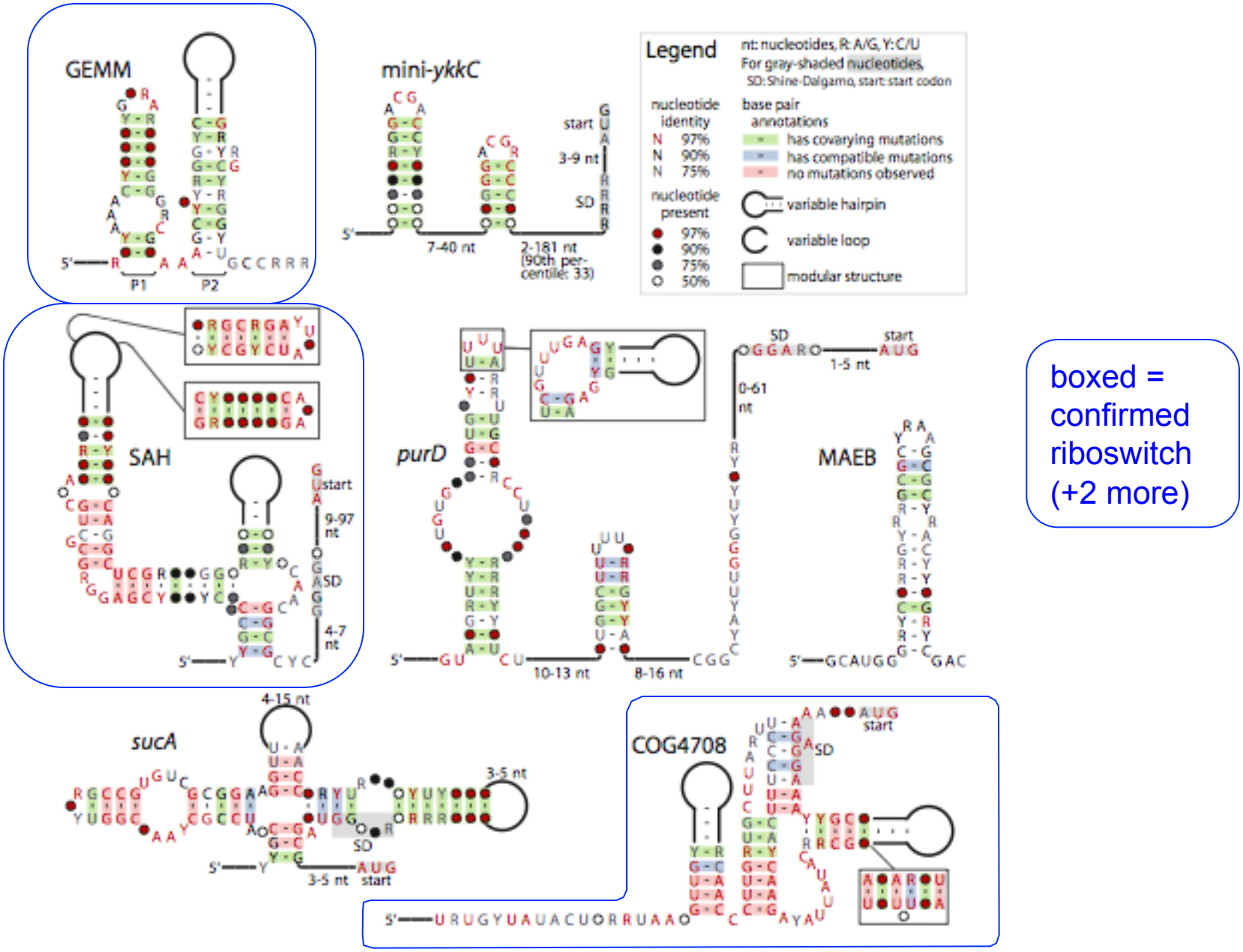


Yao, Barrick, Weinberg, Neph, Breaker, Tompa and Ruzzo. A Computational Pipeline for High Throughput Discovery of cis-Regulatory Noncoding RNA in Prokaryotes. *PLoS Computational Biology*. 3(7): e126, July 6, 2007.

Analysis Pipeline and Processing Times

Input from ~70 complete Firmicute genomes available in late 2005-early 2006, totaling ~200 megabases





Weinberg, et al. Nucl. Acids Res., July 2007 35: 4809-4819.

New Riboswitches

(all lab-verified)

SAM – IV	(S-adenosyl methionine)
SAH	(S-adenosyl homocystein)
MOCO	(Molybdenum Cofactor)
PreQ1 – II	(queuosine precursor)
GEMM	(cyclic di-GMP)

Summary

ncRNA - apparently widespread, much interest

Covariance Models - powerful but expensive

RaveNnA filtering - search ~100x faster with no/little loss

CMfinder - CM-based motif discovery in unaligned sequences

Pipelines integrating comp and bio for ncRNA discovery

Many vertebrate ncRNAs? *structural,* not seq conservation; functional significance unclear

BIG CPU demands…

Still need for further methods development & application

Final Exam

Thursday 3/18, 4:30-6:20, this lab

2 parts:

A. 60-80%: pencil + paper, computers off,
   closed book, but one 8.5x11 sheet of notes
   covers theory and Python both

B. 40-20%: computers on,
   2-3 small programming problems

Course Wrap Up

Modern biology is suddenly very data-rich

Mathematical & computational tools needed

We showed: sequence modeling, alignment & search, phylogeny, linkage mapping, some data bases

Python is a good tool for doing much of this

There's lots more!

Check out, e.g., GENOME 540/1, CSE 527…

We hope you enjoyed it.

Thanks!