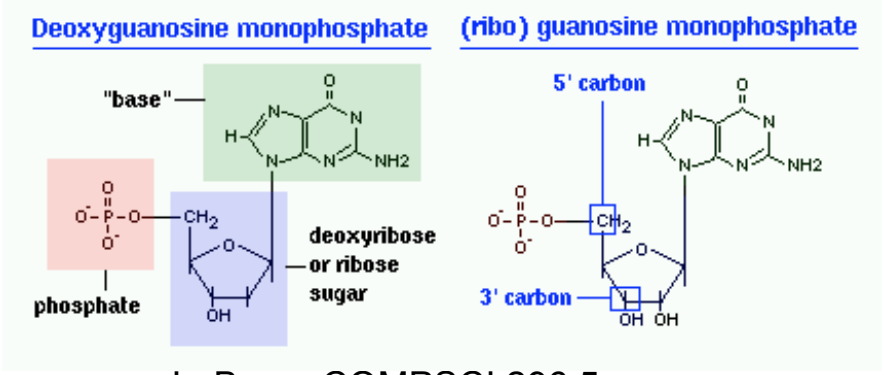
# RNA structure

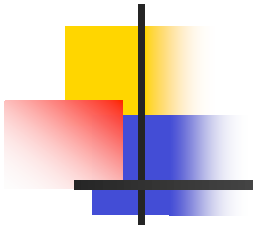
- Single strand (usually)
- A,C,G,U  (no T)
- Main types:
  - mRNA, tRNA, rRNA, snRNA
- Substructures:
  - duplex, ss, hairpins, bulges, internal loops, junctions, tertiary base-pairing, base-triplets

Tetrahymena group I intron

RNase P



# RNA structural hierarchy



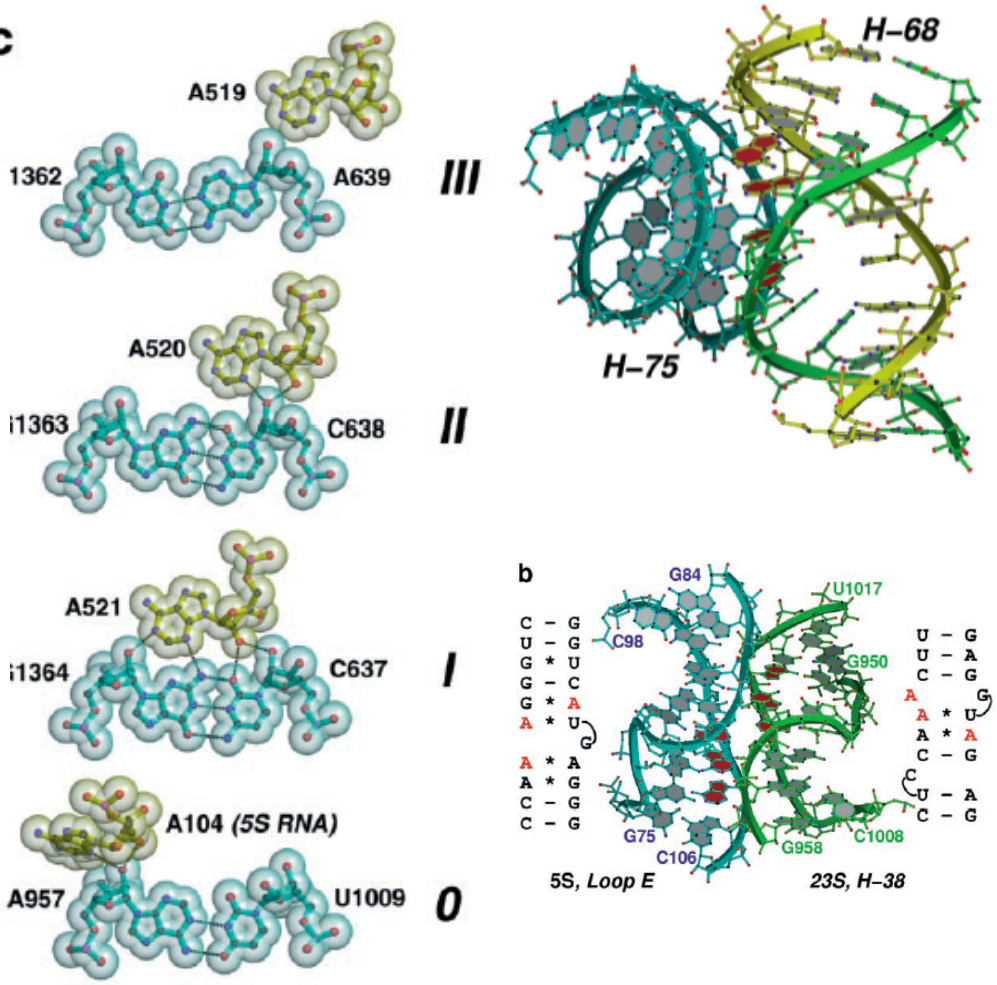
- Primary structure - covalent chemical struct.
  - Nucleotide base sequence
- Secondary structure - “local” chain folding
  - Base-pairing pattern (helix, bulged-G, tetraloops, etc.)
  - 2D prediction (mFold)
- Tertiary structure - “global” chain folding
  - 3D interactions
  - http://scor.lbl.gov/
- Quaternary structure - associations between well-folded units

# RNA tertiary interactions in the large ribosomal subunit: The A-minor motif

Poul Nissen*†, Joseph A. Ippolito*, Nenad Ban*‡, Peter B. Moore*§¶, and Thomas A. Steitz*§‖

PNAS | April 24, 2001 | vol. 98 | no. 9 | 4899–4903

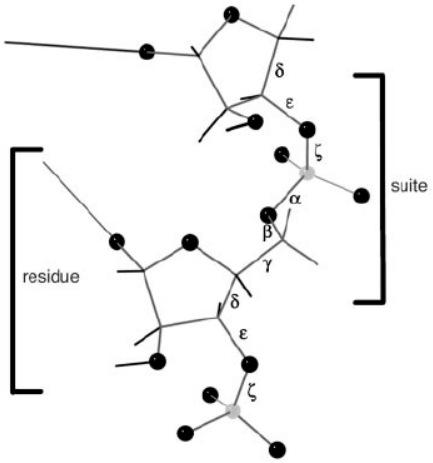
Analysis of the 2.4-Å resolution crystal structure of the large ribosomal subunit from *Haloarcula marismortui* reveals the existence of an abundant and ubiquitous structural motif that stabilizes RNA tertiary and quaternary structures. This motif is termed the A-minor motif, because it involves the insertion of the smooth, minor groove edges of adenines into the minor groove of neighboring helices, preferentially at C-G base pairs, where they form hydrogen bonds with one or both of the 2′ OHs of those pairs. A-minor motifs stabilize contacts between RNA helices, interactions between loops and helices, and the conformations of junctions and tight turns. The interactions between the 3′ terminal adenine of tRNAs bound in either the A site or the P site with 23S rRNA are examples of functionally significant A-minor interactions. The A-minor motif is by far the most abundant tertiary structure interaction in the large ribosomal subunit; 186 adenines in 23S and 5S rRNA participate, 68 of which are conserved. It may prove to be the universally most important long-range interaction in large RNA structures.

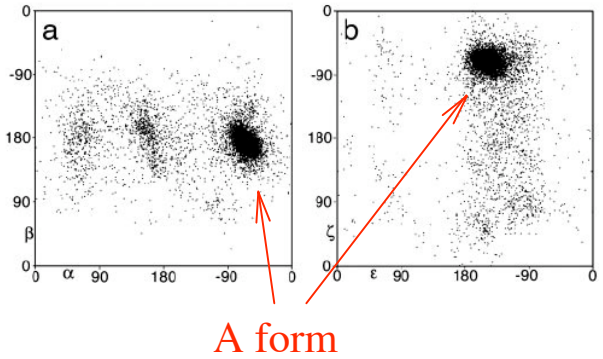


# RNA backbone is rotameric

Laura J. W. Murray, W. Bryan Arendall III, David C. Richardson, and Jane S. Richardson*

13904–13909 | PNAS | November 25, 2003 | vol. 100 | no. 24




A form

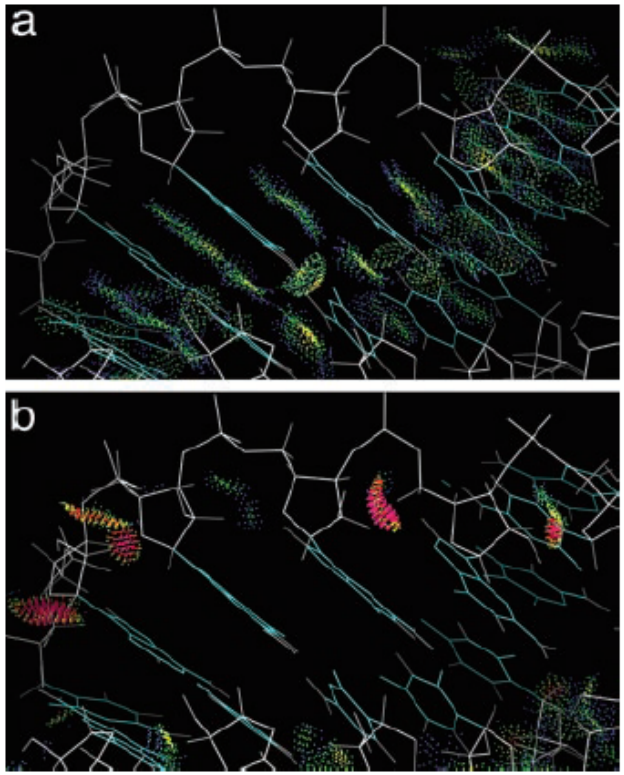



Table 1. The 42 suite conformers for RNA backbone

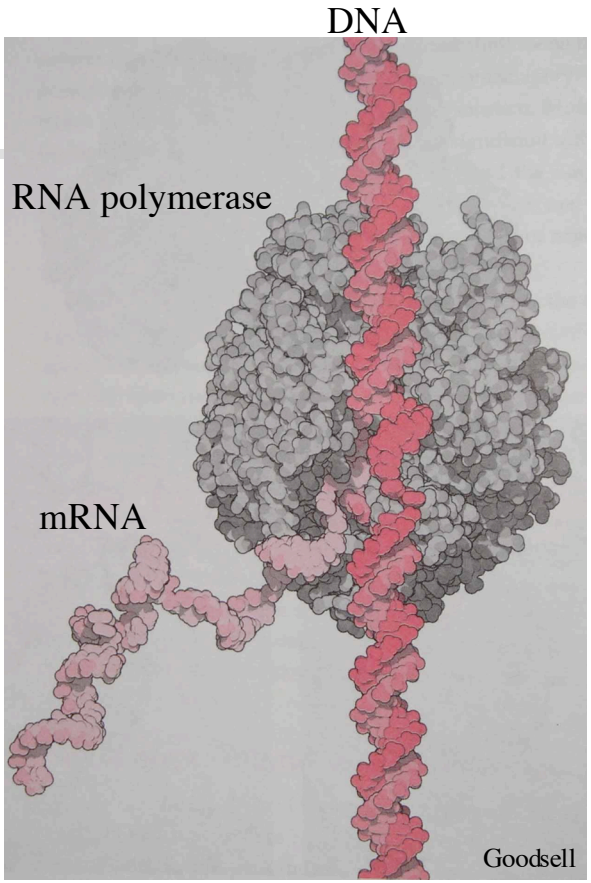
| C3' (2nd) | α δ / β ε / γ ζ | C3' e p | C3' e t | C3' e -140 | C3' e m | C2' e p | C2' e t | C2' e m |
|---|---|---|---|---|---|---|---|---|
| | p t p | * | | * | * | ** | * | * |
| | p 110 t | * | | | | | | |
| | p t t | | | | | *[a] | | |
| | t t p | | | | ***[b] | * | ** | * |
| | t 135 t | | | | * | | | |
| | t t t | | | | ***[c] | | | |
| | m t p | | ** | ** | **A**[d] | | **[e] | *** |
| | m -135 p | | | | * | | | |
| | -110 80 t | | | | **[f] | | | |
| | m t t | | | | | | | * |
| C2' (2nd) | p t p | * | * | * | | ** | * | |
| | p 110 t | * | | | | | | |
| | p t m | | | * | | | | |
| | t t p | **[g] | | | *[h] | | | |
| | t t t | | | | * | | | |
| | m t p | | * | * | *** | * | * | *[i] |
| | m -135 p | | | | ***[j] | | | * |
| | m t m | | | | * | | | * |

Conformer frequencies:
* ~1% of non-A
** 2-3% of non-A
*** 4-20% of non-A

Angle codes:
t trans
m gauche minus
p gauche plus
e eclipsed

# DNA is transcribed to mRNA

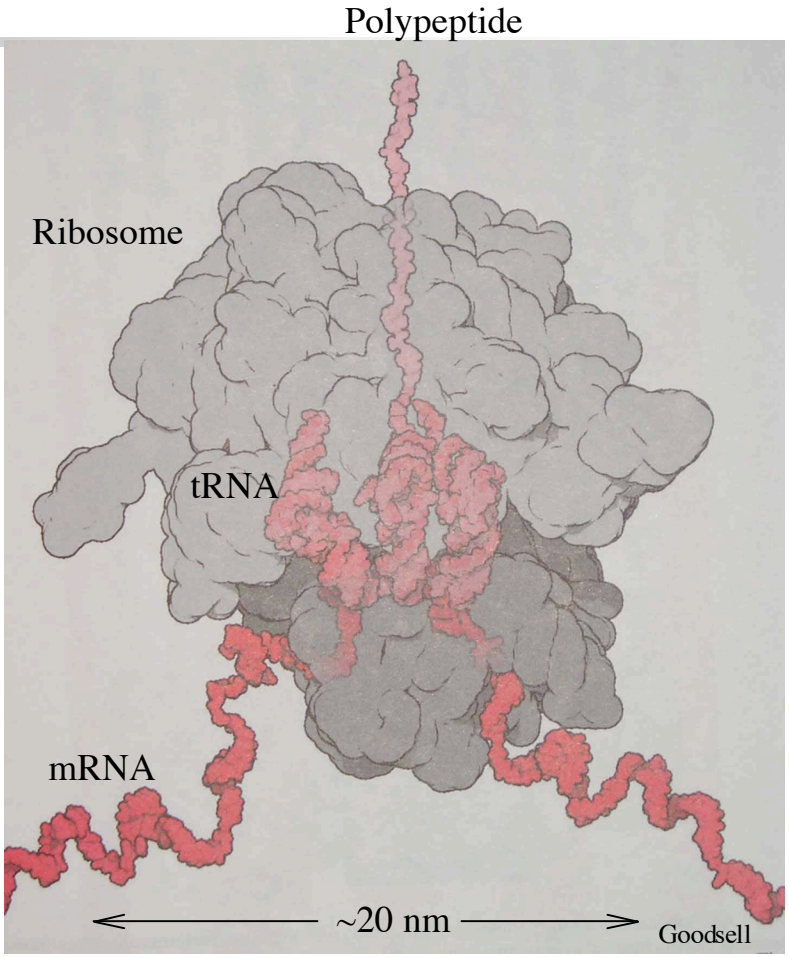
- Single strand.
- Temporary.
- Secondary structure undesirable.
- Poly-A tail.



# mRNA is translated to protein by ribosomes

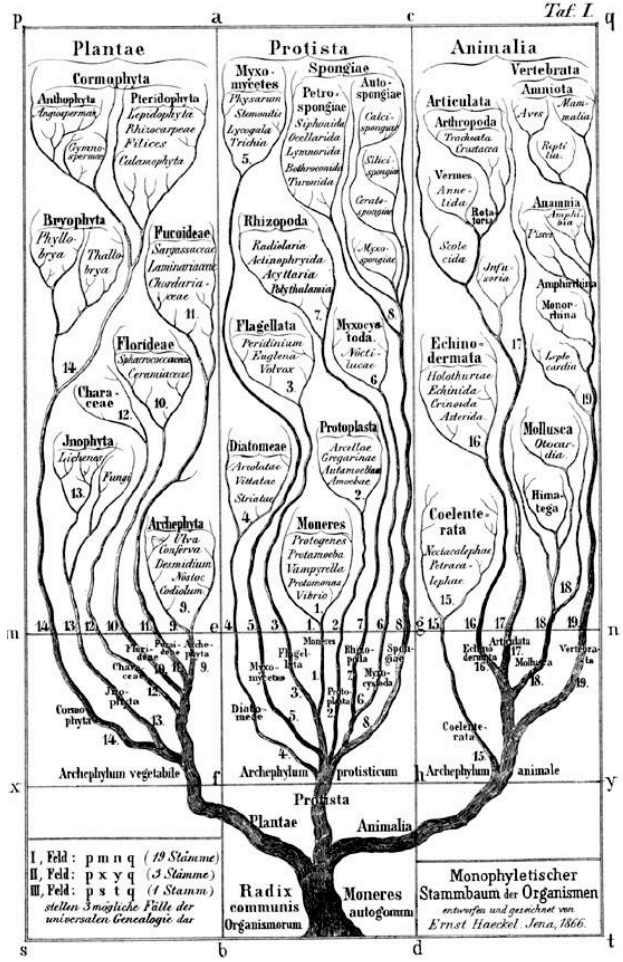
- Ribosome
  - Large subunit
  - Small subunit
- Make up 25% of the dry weight of pancreas cells (specialize in protein synthesis). 1 cell can synthesize 5 million molecules of protein per minute.
- …

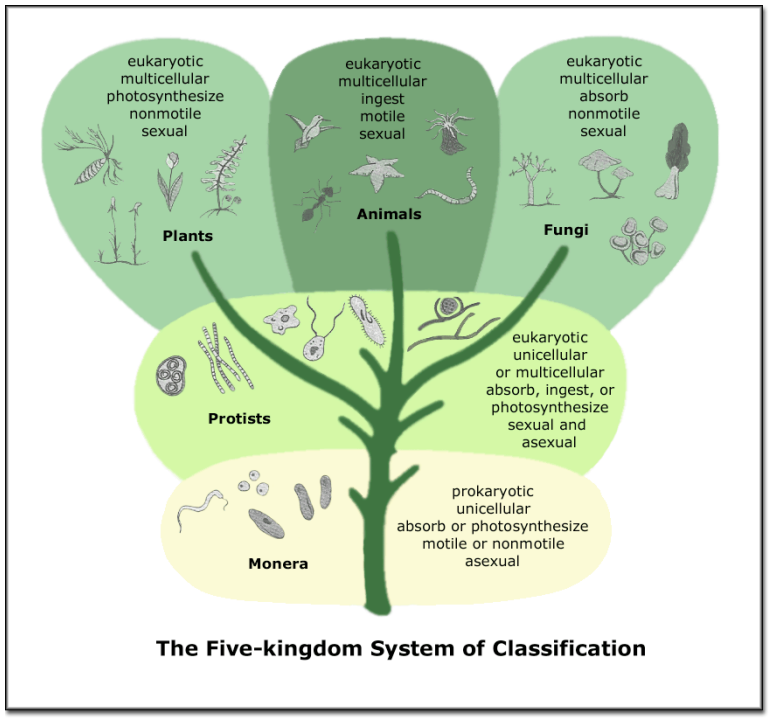
| Comparison of Ribosome Structure in Prokaryotes, Eukaryotes, and Mitochondria | | | |
|---|---|---|---|
| | **Bacterial (70S)** | **Eukaryotic (80S)** | **Mitochondrial (55S)** |
| **Large Subunit** | **50S** | **60S** | **39S** |
| **rRNAs** (1 of each) | 23S (2904 nts) | 28S (4700 nts) | 16S (1560 nts) |
| | 5S (120 nts) | 5S (120 nts) | |
| | | 5.8S (160 nts) | |
| **Proteins** | 33 | ~49 | 48 |
| | | | |
| **Small Subunit** | **30S** | **40S** | **28S** |
| **rRNA** | 16S (1542 nts) | 18S (1900 nts) | 12S (950 nts) |
| **Proteins** | 20 | ~33 | 29 |

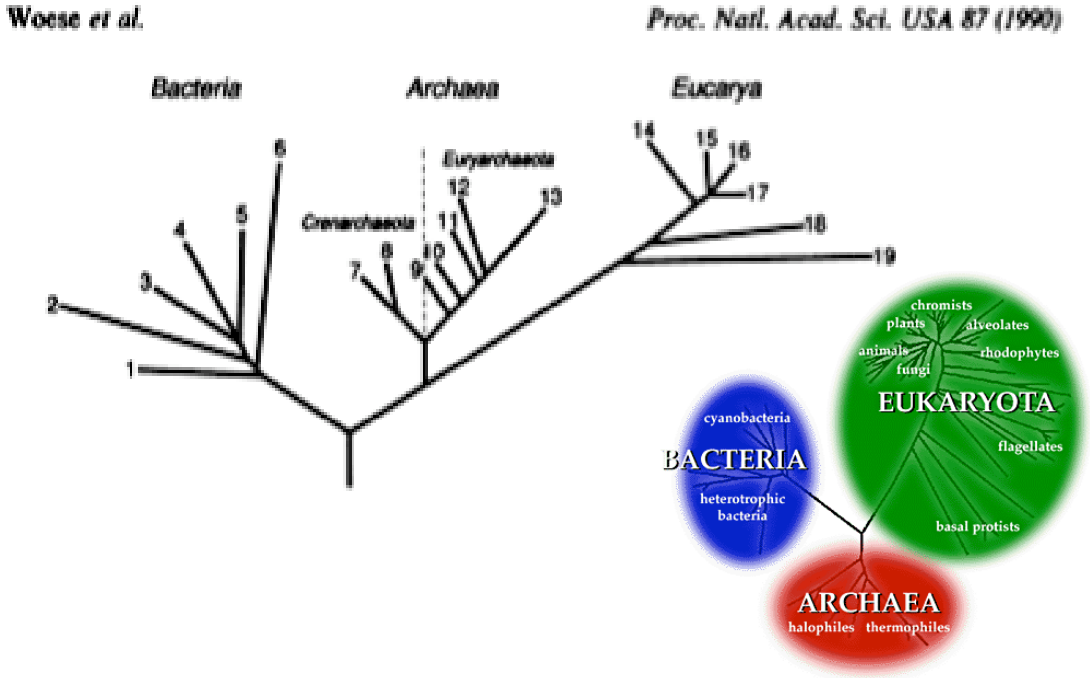


# “The Tree of Life”

- Anatomical systematics
- Haeckel’s line of descent
- 5 Kingdom Model
- 3 Domain (Woese 1990, Pace)

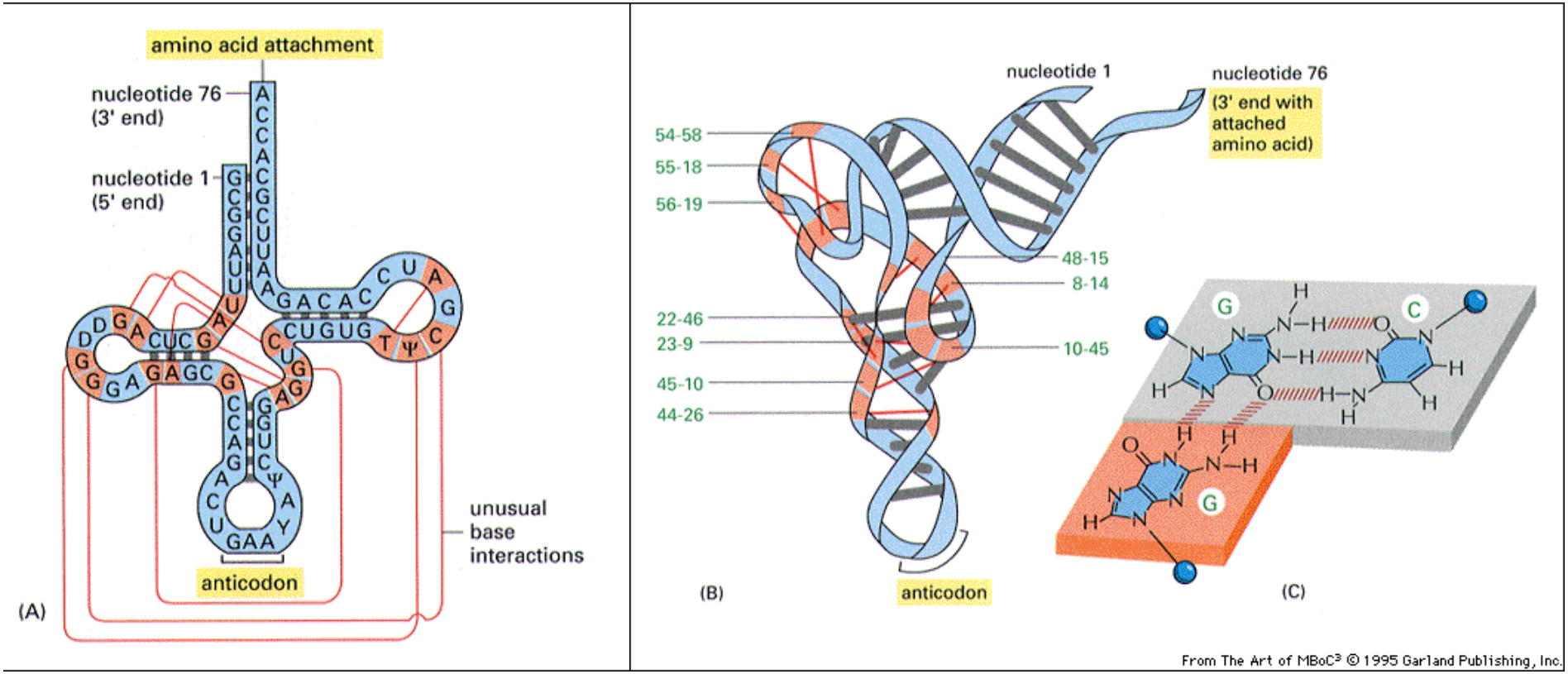


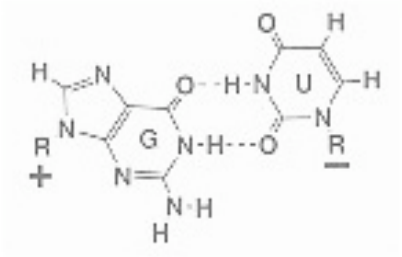
# Secondary Structure

## Example: tRNA

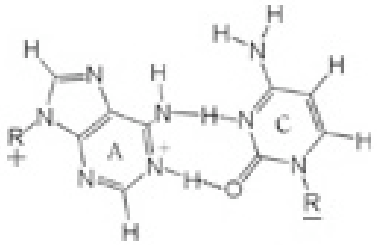


# Alternative Base Pairing

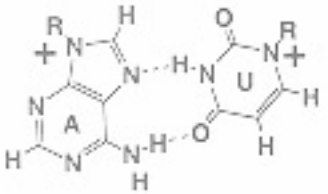
- Watson-Crick
  - G-C, A-U
- Wobble
  - G-U, A-C
- Hoogsteen
  - A-U
- Others: triplets, single H-bonds, pH effects.



GU Wobble

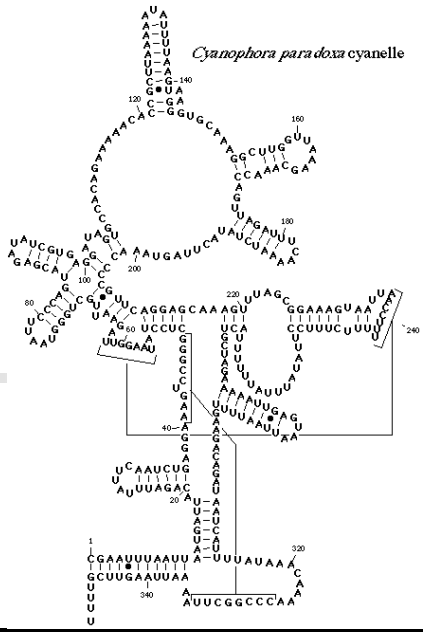


AC Wobble



AU Hoogsteen

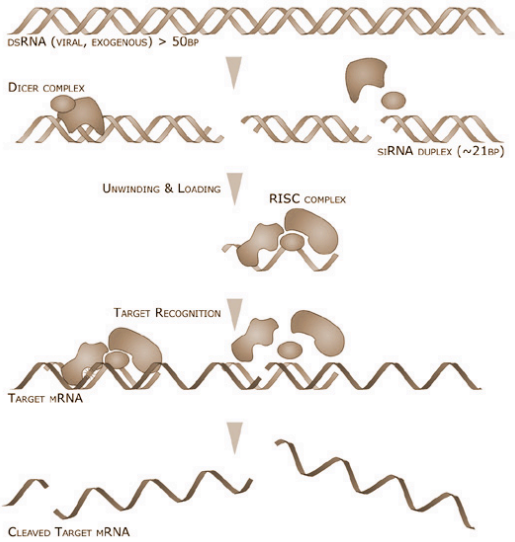
# RNase P RNA






- Rnase P enzyme is involved in tRNA processing that removes the 5' leader sequences from tRNA precursors.
- Rnase P contains RNA and protein but some Rnase P RNAs are enzymatically active without protein cofactors.
- Model molecule for modeling by sequence comparison.

# RNAi




## RNA interference

From Wikipedia, the free encyclopedia.
(Redirected from RNAi)

**RNA interference (RNAi)** is a mechanism in molecular biology where the presence of certain fragments of double-stranded RNA (dsRNA) interferes with the **ex**pression of a particular gene which shares a homologous sequence with the dsRNA.

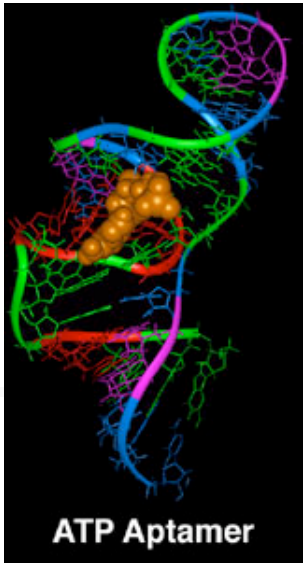
Before RNAi was well characterized, it was called by other names, including **post transcriptional gene silencing** and **transgene silencing**. Only after these phenomena were characterized at the molecular level was it obvious that they were the same phenomenon.

The use of RNA to reduce **ex**pression in plants has been a common procedure for many years. Single-stranded antisense RNA was introduced into plant cells that hybridized to the cognate, single-stranded, sense messenger RNA. While scientists first believed that the resulting dsRNA heli**x** could not be translated into a protein, it is now clear that the dsRNA triggered the RNAi response. The use of dsRNA became more widespread after the discovery of the RNAi machinery, first in petunias and later in roundworms (*C. elegans*).

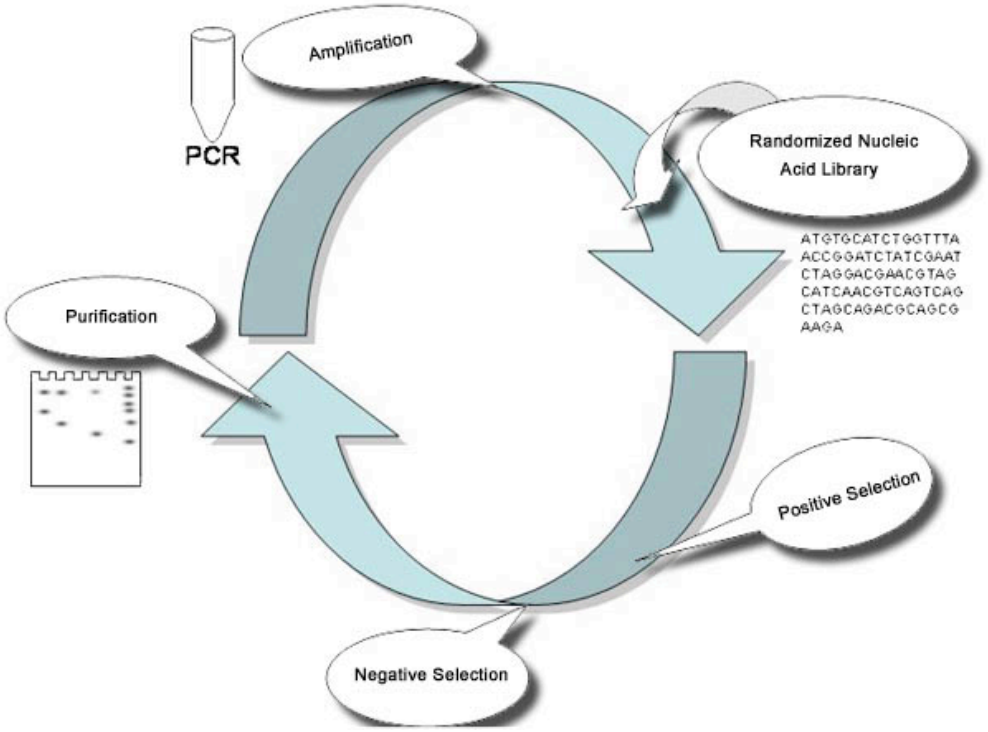
RNAi appears to be a highly potent and specific process which is actively carried out by special mechanisms in the cell, known as the RNA interference machinery. While the complete details of how it works are still unknown, it appears that the machinery, once it finds a double-stranded RNA molecule, cuts it up, separates the two strands, and then proceeds to destroy other single-stranded RNA molecules that are complementary to one of those segments. dsRNAs direct the creation of small interfering RNAs (siRNAs) which target RNA-degrading enzymes (RNAses) to destroy transcripts complementary to the siRNAs.

The life cycle and replication of many viruses involves a double-stranded RNA stage, so it is likely that the RNA interference machinery evolved as a defense against these viruses. The machinery is however also used by the cell itself to regulate gene activity: certain parts of the genome are transcribed into microRNA, short RNA molecules that fold back on themselves in a hairpin shape to create a double strand. When the RNA interference machinery detects these double strands, it will also destroy all mRNAs that match the microRNA, thus preventing their translation and lowering

# *In vitro* evolution of functional RNA



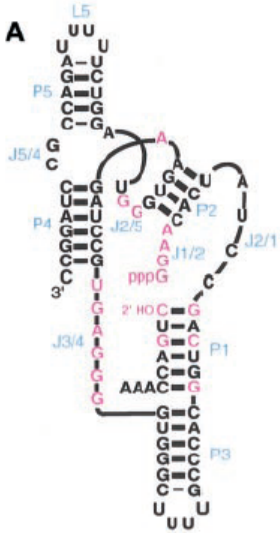

- Start with random sequence library and select functional molecules. Repeat.
- Groups: Szostak, Ellington, Bartel, Breaker, Eaton, Feldheim, etc.



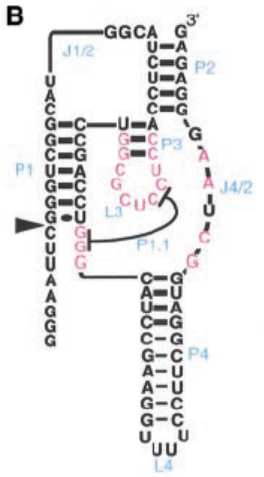
# One Sequence, Two Ribozymes: Implications for the Emergence of New Ribozyme Folds

Erik A. Schultes and David P. Bartel*

*Science* (2000) **289,** 448-452

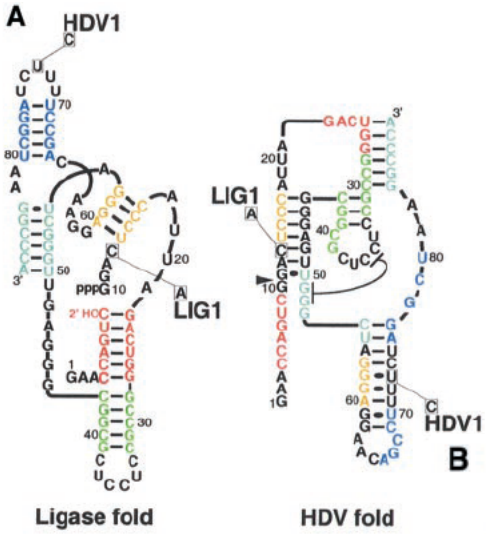


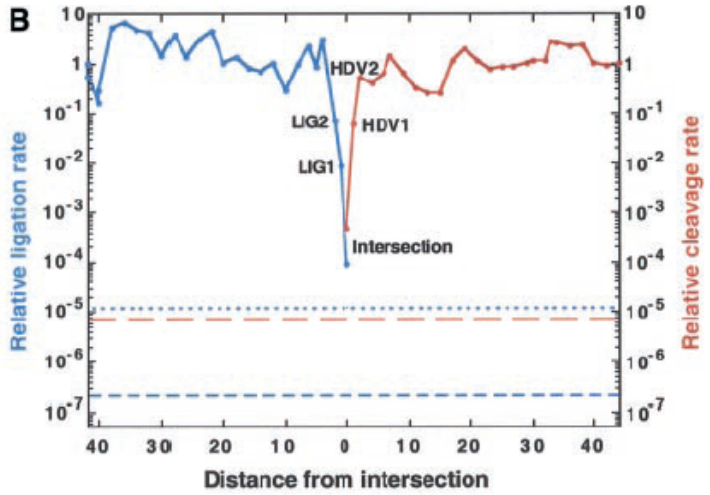

Class III ligase




HDV self-cleaving ribozyme

- Hepatitis delta virus & class III ligase (*in vitro* evolved).
- "Intersection" sequence has both activities.
- Neutral paths and networks.
- Implications for evolution of new folds.
- See also: Folded order for free from random RNA libraries.
  - Schultes *et al* (2005) Nature Struct Mol Biol **12**, 1130



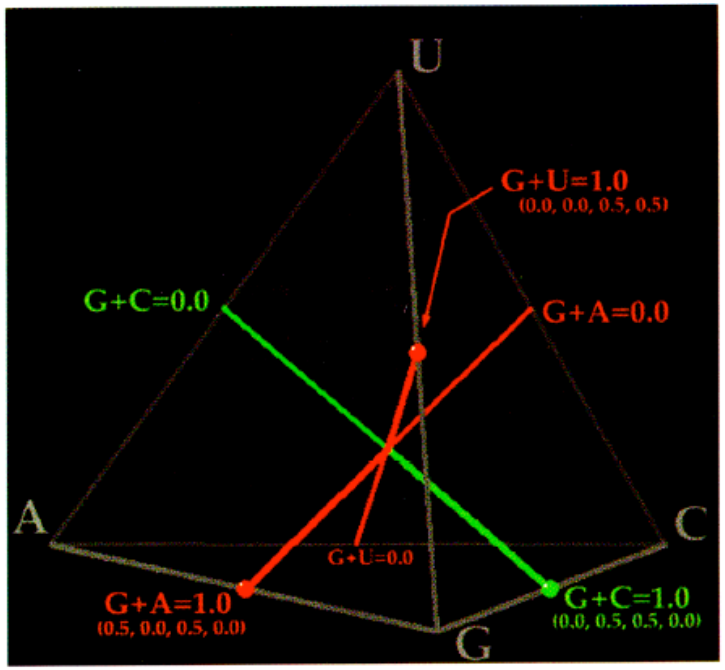



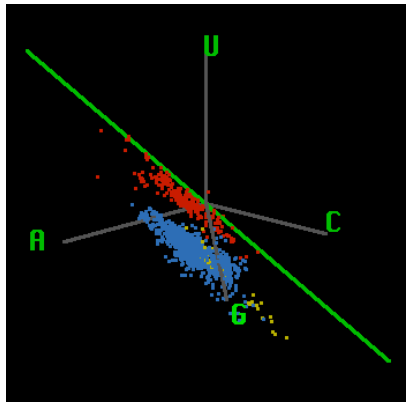
*RNA* (1997), 3:792–806. Cambridge University Press. Printed in the USA.

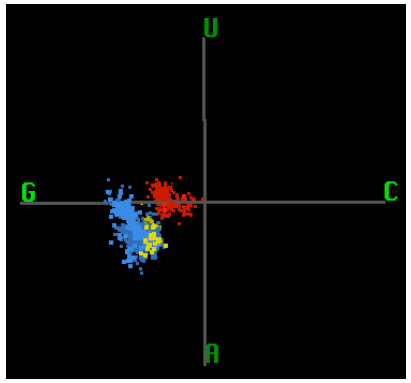

# Global similarities in nucleotide base composition among disparate functional classes of single-stranded RNA imply adaptive evolutionary convergence

ERIK SCHULTES,[1,2] PETER T. HRABER,[3] and THOMAS H. LABEAN[2,4]

- RNA composition space visualization.
- Interesting clustering for functional RNA from biological and in vitro sources.

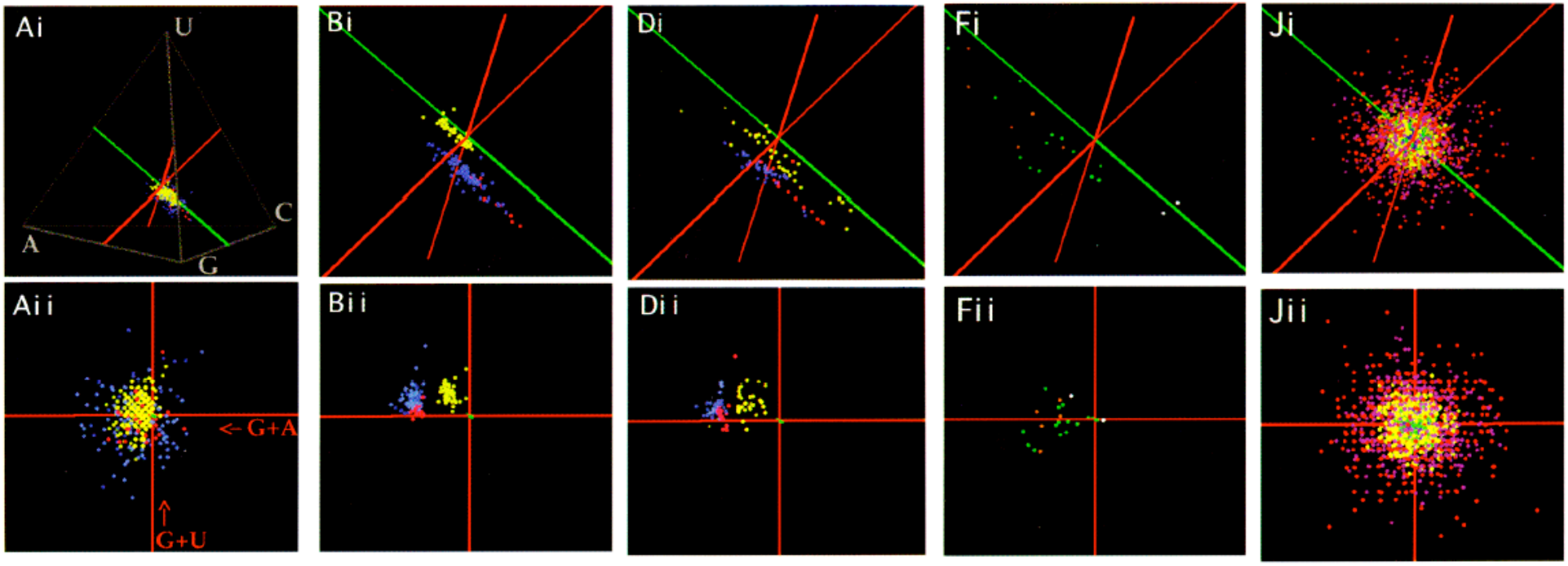


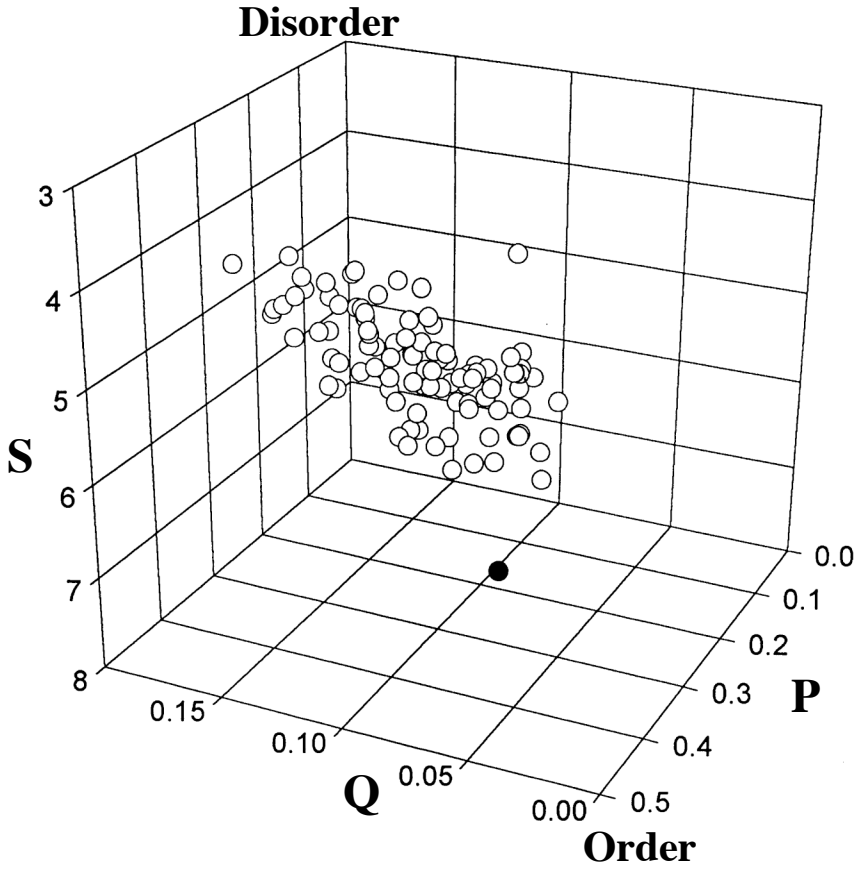
RNA (1997) **3**, 792-806

Archea (red)
Bacteria (blue)
Eucarya (yellow)

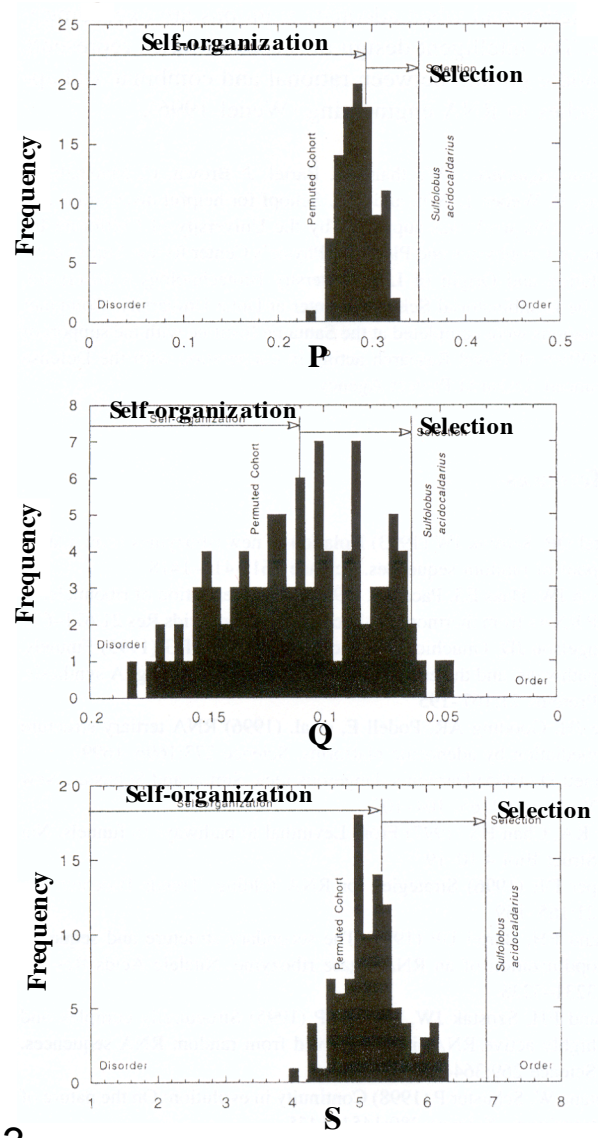



5S rRNA | 16S rRNA | 23S rRNA | Introns & HDV | Uniform random

# Self-organization vs. Selection in RNA Secondary Structure




S = mean length of helical stems
Q = uniqueness of fold
P = base-pairing propensity




*J.Mol.Evol.* (1999) **49**:76-83