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

- **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

Table 1. The 42-suite conformers for RNA backbone

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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.
- ...
“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.
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.
RNA interference

RNA interference (RNAi) is a mechanism in molecular biology where the presence of certain fragments of double-stranded RNA (dsRNA) interferes with the expression 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 expression 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 helix 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 (RNAases) 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 miRNAs 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.
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.

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. LæBEAN²,⁴

- 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

Central Dogma

DNA --> RNA --> Protein