Felix Loosli
ITG/KIT (Karlsruhe)
Developmental neurobiology of vertebrates

Introduction to Genetics

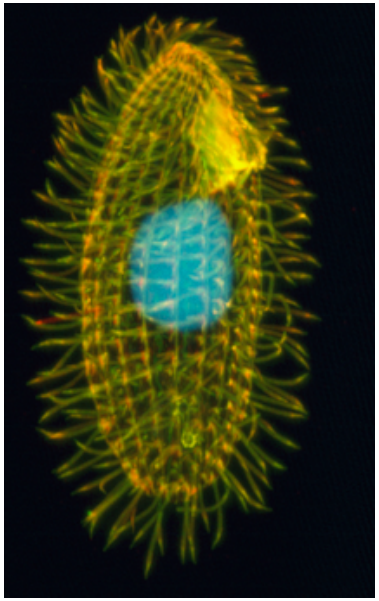
Catalytic RNAs

Lewin‘s GeneX Chapter 23
Albert‘s Molecular Biology of the Cell 5th ed. Chapter 6

Ribozyme : a RNA that has catalytic activity

Group I and II self splicing introns
Ribonuclease P (RNase P: t-RNA maturation)
Viroids
Ribosome
Evolutionary considerations

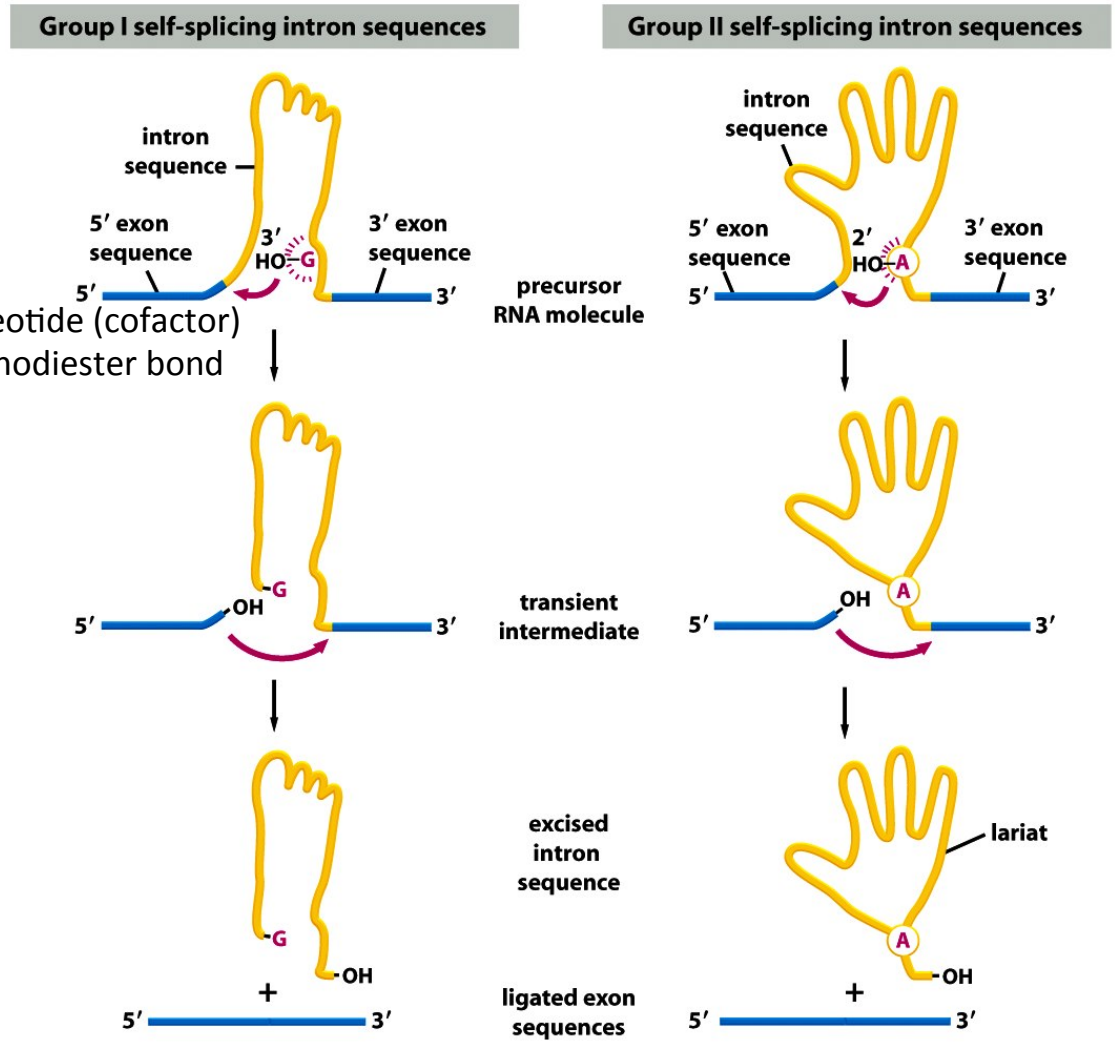
Tetrahymena thermophilus



Thomas R. Cech
rRNA self-splicing
Splicing occurred without added cell extract

Sidney Altman
Catalytic RNA of RNase P which contains a RNA molecule essential for catalysis

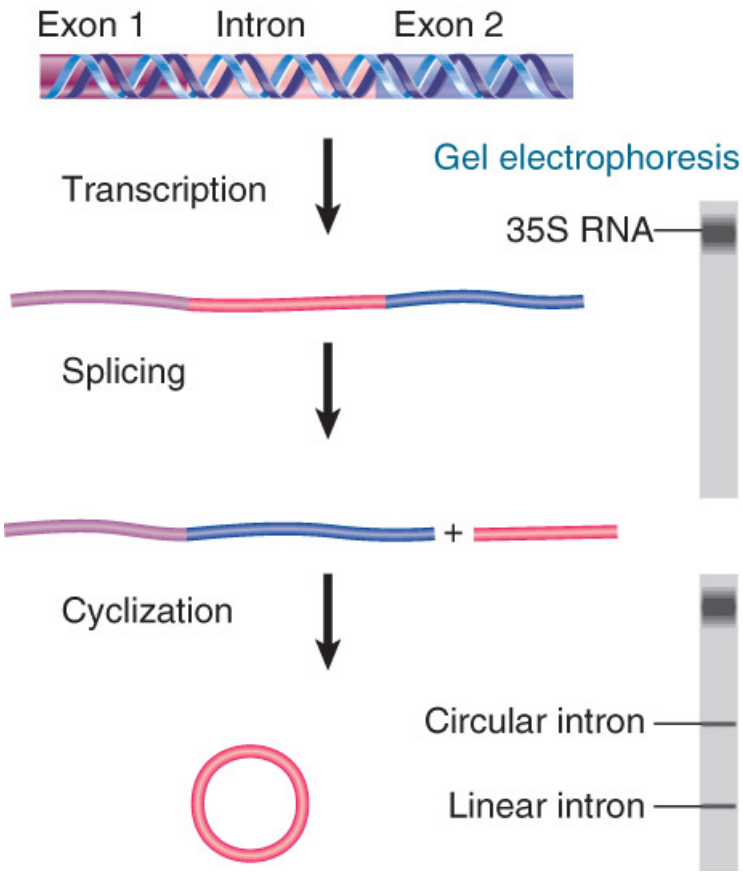
rRNA genes Mitochondrial/Chloroplast genes

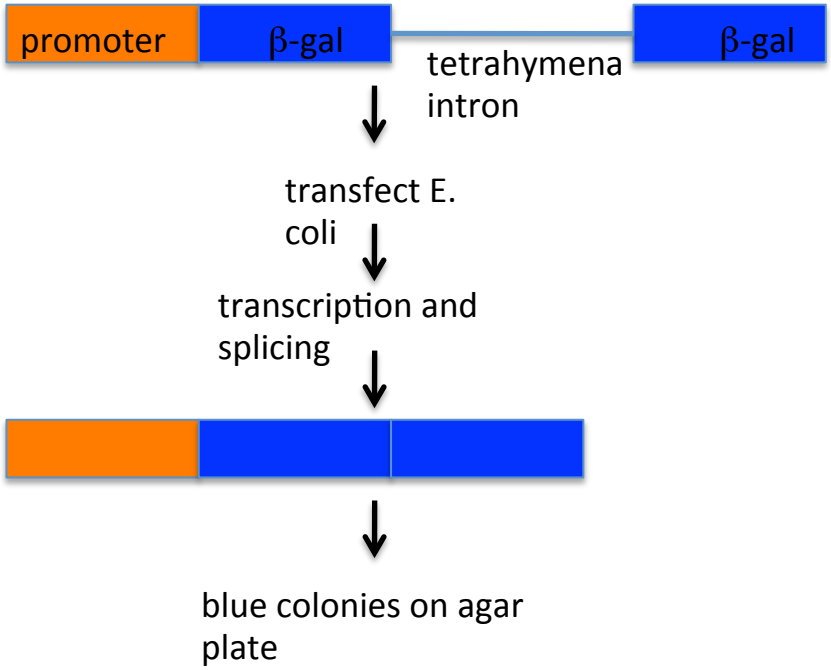


Bound G nucleotide (cofactor) attacks phosphodiester bond

Two consecutive transesterifications; no ATP required

2nd structure of RNA is essential


Exon 1
Intron
Exon 2
Transcription
Gel electrophoresis
35S RNA
Splicing
+
Cyclization
Circular intron
Linear intron


promoter
β-gal
β-gal
tetrahymena intron
transfect E. coli
transcription and splicing
blue colonies on agar plate

GTP conc. >rRNA conc.
change of 2nd structure of products (products are 'removed' from the reaction)
-> reaction proceeds to completion

In vitro self splicing is very slow, *in vivo* proteins have accessory functions

Group I introns share a common 2nd structure: core structure is the active site

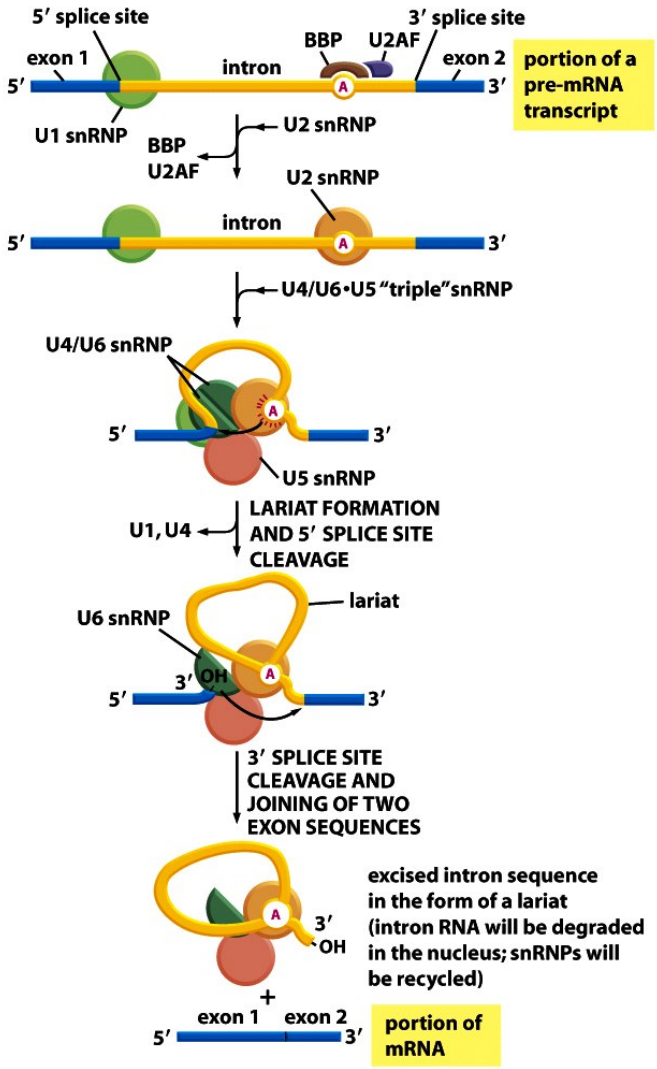
Group I introns can also catalyse:

- Sequence specific RNA cleavage (ribo endonuclease)
- RNA ligase
- Phospatase (not related to self-splicing activity)

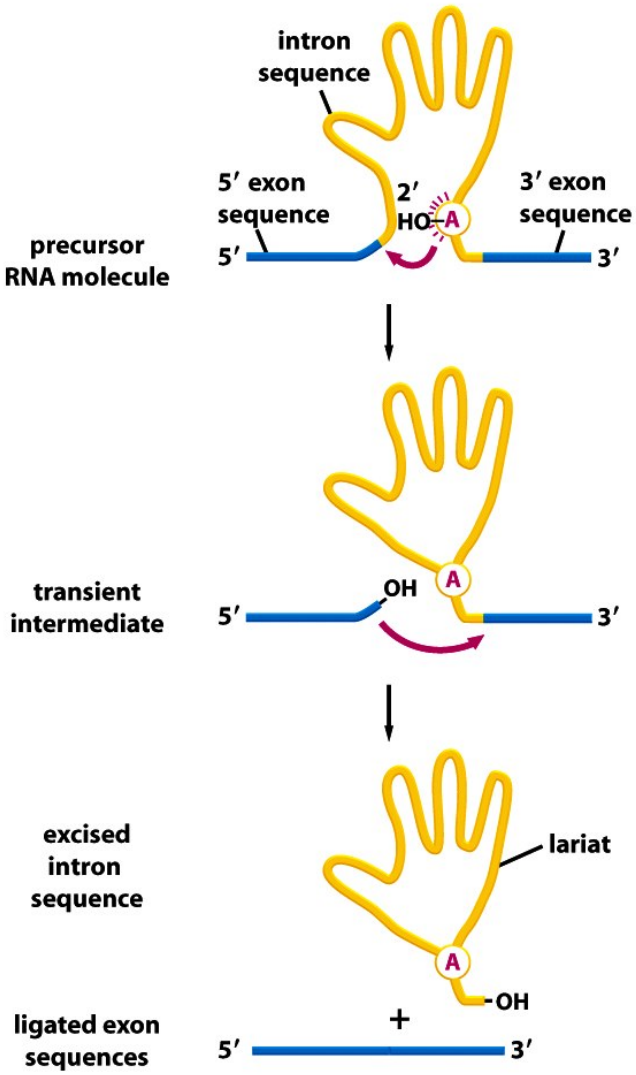
Group II Introns May Code for Multifunction Proteins

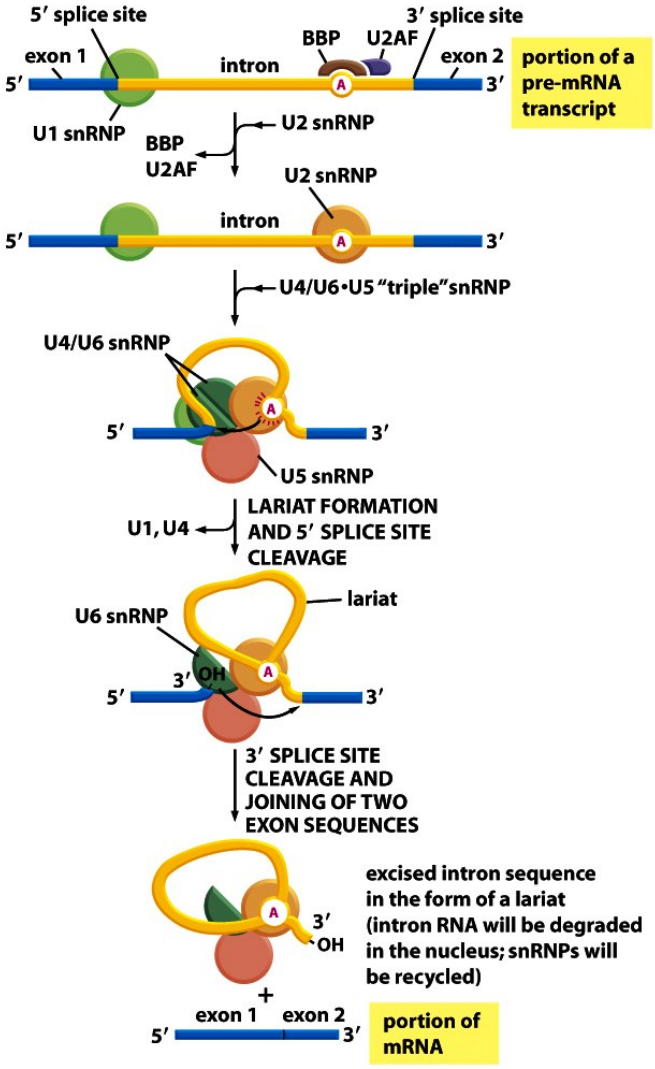
- Group II introns can autosplice *in vitro*, but are usually assisted by protein activities encoded by the intron
- A single open reading frame codes for a protein with reverse transcriptase activity, maturase activity, a DNA-binding motif, and a DNA endonuclease
- The mature helps the intron to fold into the active structure required for splicing
- The endonuclease activity mediates ‘homing’ (insertion of reverse transcribed intron at a specific target site)
- Product of evolution?

Major spliceosome



Group II self-splicing intron sequences

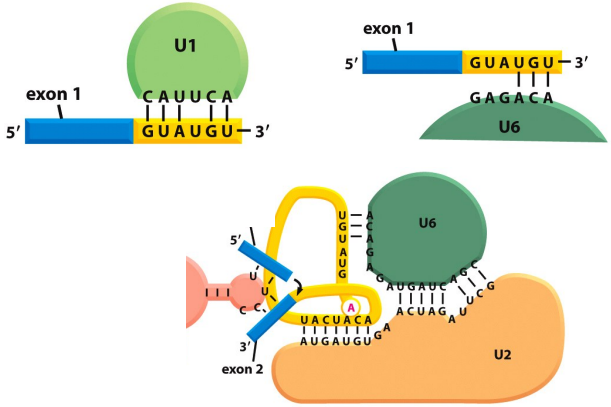




mRNA splicing: removal of intervening introns from primary transcript

snRNAs and proteins form snRNPs

snRNAs position reactants and provide catalytic site for intron-exon cleavage and exon-exon fusion



Catalytic RNA

Evolutionary hypothesis:

Pre-mRNA splicing mechanism of splicesome evolved from group II self splicing because of their similarity

Evolutionary advantage

The intron sequence constraints are overcome by the snRNP (Structure provided by proteins)

This provides more flexibility in intron splicing (sequence of intron is not relevant)

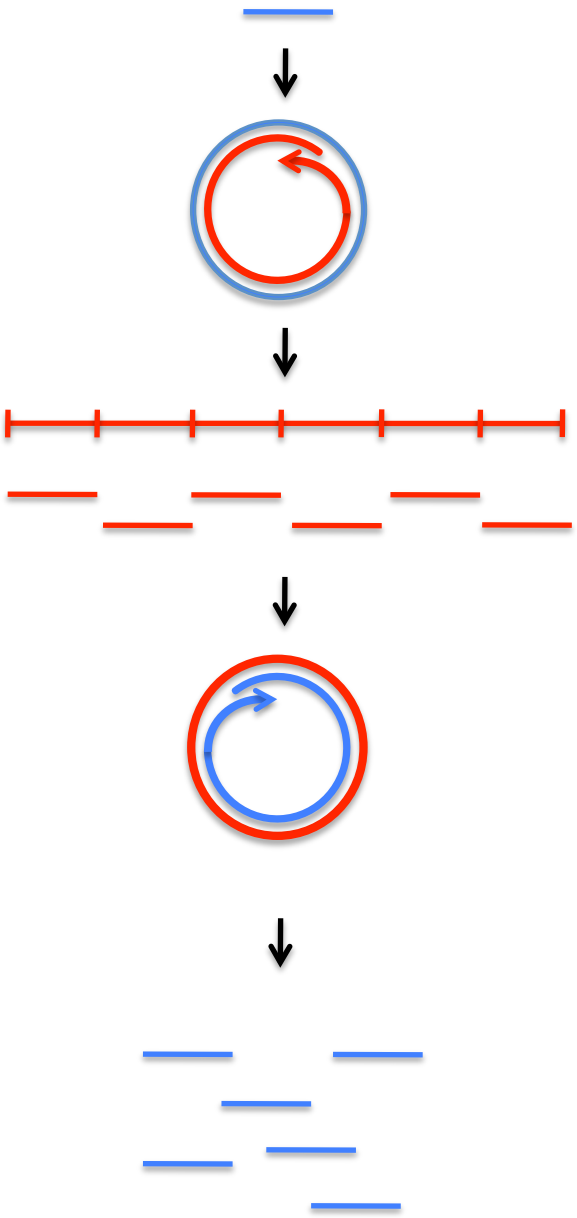
Ribonuclease P (RNase P): endonuclease involved in tRNA processing (5’ processing)

- RNase P is a ribonucleoprotein in which a RNA molecule has the catalytic activity
- RNase P is essential for bacteria, archaea, and eukaryotes
- RNase MRP in eukaryotes is related to RNase P and is involved in rRNA processing and degradation of cyclin B mRNA
- Both proteins and RNA are essential for the function of RNase P

Viroids
small plant pathogens consisting of cytoplasmic ssRNA (< 1kb) molecules with
self-cleaving activity (hammer-head ribozyme)
RNA is highly self-complementary, circular
RNA does not encode a protein (no open reading frame)
RNA molecules are replicated by RNApol II (rolling circle)

Virusoids
small ssRNAs (> 1kb) depend on viruses for replication and encapsulation
Genome encodes structural proteins
RNA is highly complementary, circular with self-cleaving activity (hammer-head ribozyme)
-> Hepatitis D
Coinfection of Hepatitis B virus and Hepatitis D virusoid (Hepatitis delta virus HDV),
virusoid uses Hepatitis B coat protein -> same mode of infection
->Hepatitis B vaccination protects also against HDV

Replication of viroids by rolling circle



Circularization (host)

RNA pol II
rolling circle ‘replication’
of minus strand

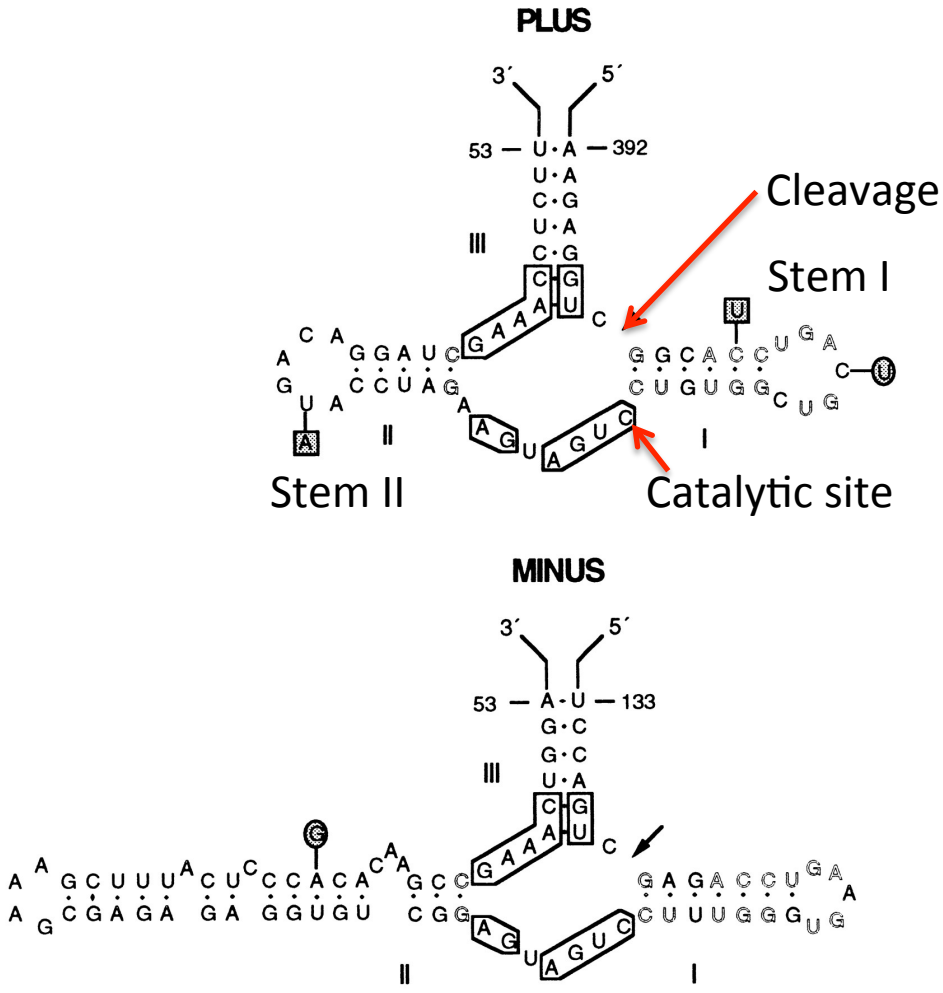
autocleavage of concatemers

ligation of minus strand (host)
rolling circle replication
of plus strand

autocleavage of concatemers

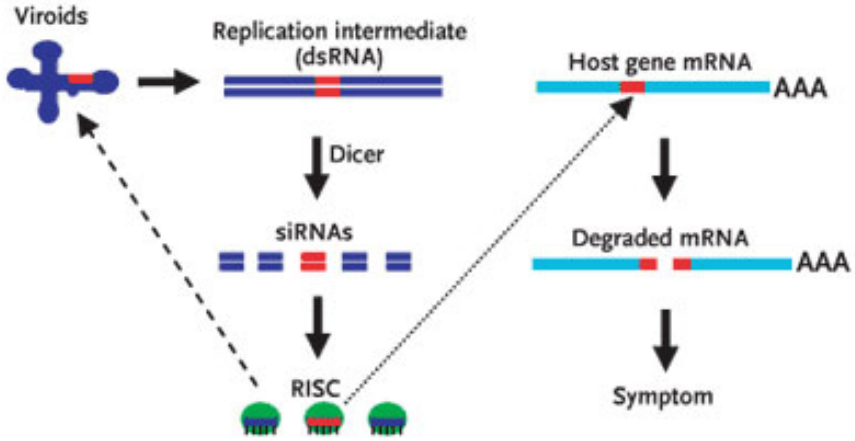
‘+’ and ‘-’ strand have
autocleavage activity

Conserved hammerhead ribozyme structure of viroids



Navarro B , Flores R PNAS 1997;94:11262-11267

Viroids can elicit RNA interference (RNAi) and by that silence gene activity



- RNA endonuclease Dicer cleaves dsRNA (including hairpin RNA) into 20-25bd fragments -> siRNA
- Guide strand of siRNA is loaded into RISC complex, passenger strand is degraded
  (5' stability of siRNA determines which is the guide strand)
- Argonaute RNAses of RISCcomplex degrade RNA target that is complementary to guide strand

-> host mRNAs with complementarity to viroid genome are degraded

The ribosome is a ribozyme

The ribosome is composed of two-thirds RNA and one-third protein

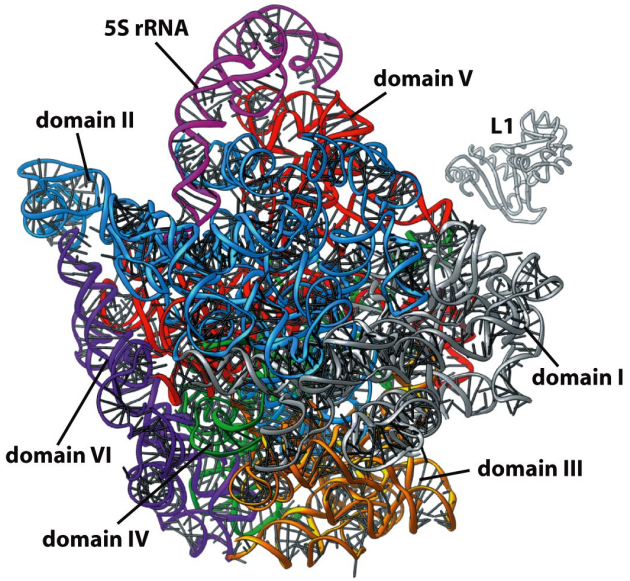
Ribosomal RNAs (rRNAs):
- make up the overall ribosomal structure
- form the central core
- position tRNAs on the mRNA
- have catalytic activity to form peptide bonds

rRNAs catalyse polypetide synthesis!!

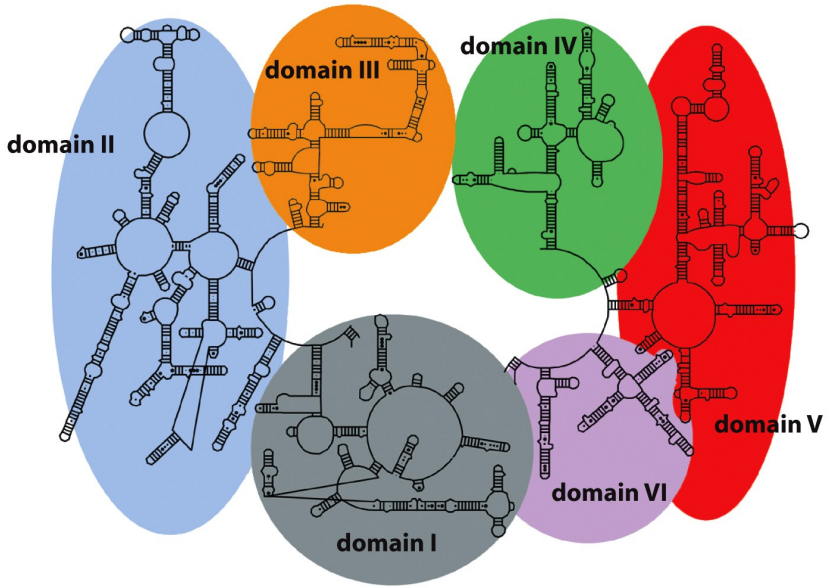
Ribosomal proteins:
- located on the surface of ribosome
- aid initial assembly of rRNAs to form the core
- stabilise RNA core
- permit conformational rRNA changes during protein synthesis

Ribosomal RNAs form the structural core of the ribosome

Type	Size	Large subunit	Small subunit
prokaryotic	70S	50S: 5S+23S	30S: 16S
eukaryotic	80S	60S: 5S+5.8S+28S	40S: 18S



5S+23S rRNAs of large subunit



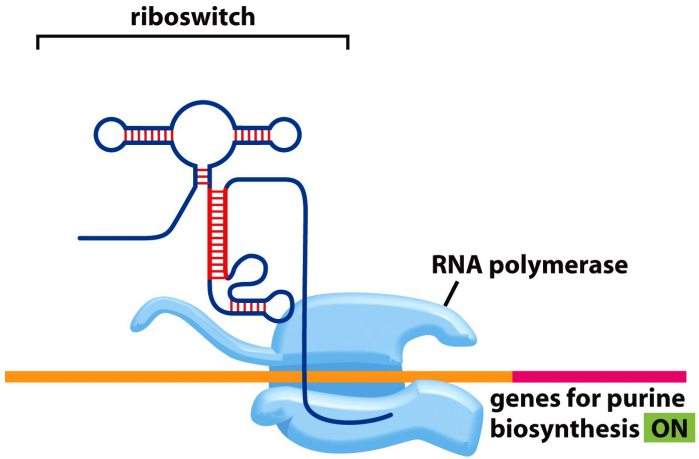
23S rRNA folds into pocket where peptide chain and aminoacyl-tRNA undergo catalysis

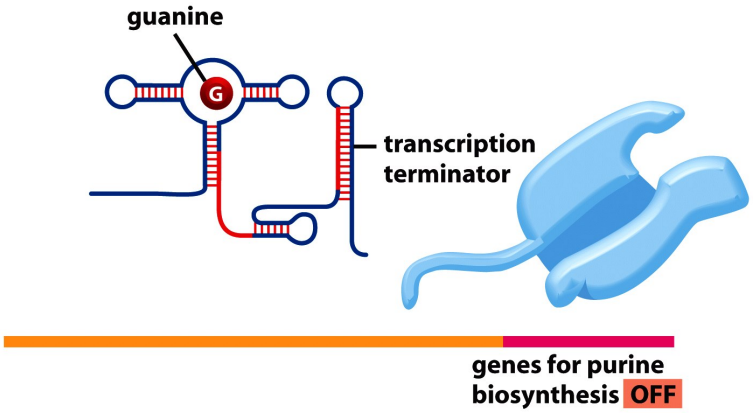
Extreme structural constraints for rRNAs: very high sequence conservation

Science 16 December 2011: Vol. 334 no. 6062 pp. 1524-1529 DOI: 10.1126/science.1212642
RESEARCH ARTICLE
The Structure of the Eukaryotic Ribosome at 3.0 Å Resolution
A Ben-Shem et al.

RNA editing
changes in the ORF of mRNA by base-modifications (A->I) or nucleotide insertion
apolipoportein-B, glutamate receptor
multienzyme complexes (ADAR, APOBEC) catalyze editing by sequence specific RNA binding
Trypanosoma: RNA editing of mitochondrial cytochrome oxidase II mRNA
insertion/deletion of several uridines is mediated by guide RNAs (short non-coding RNAs)

Riboswitches:
short sequence of RNA that changes conformation upon binding of small molecule (metabolite), conformational change results in transcription termination





Central Dogma of biology

The flow of information is from DNA to protein (with RNA as an intermediate)

DNA → RNA → Protein

There is a sequential flow of residue-by-residue transfer of information. This information cannot be transferred from protein to protein or from protein to DNA (irreversible flow).

How can such a system arise when DNA is required to make proteins and proteins are required to make DNA?

Hypothesis: RNA world predates DNA/RNA/protein world

-> catalytic RNAs, cofactors (ATP, Acetyl-Co-A, NADH)

Primordial soup: ‘origins of life’ experiments
→Nucleotide formation in prebiotically plausible conditions

Meteorite studies
→RNA building blocks may have been formed extraterrestrially

but:
• nucleotide 5’->3’ polymerization require activation of the phosphate group and the only effective activation by triphosphates is not plausible under prebiotic conditions
• non-catalyzed polymerization leads mostly to 5’-5’ polymers

RNA world
• nucleotides in primordial soup form polymers that may have catalytic properties that lower the energy of their chains being created-> promote their formation
• by molecular evolution eventually self replicating RNA molecules developed
• RNA-catalyzed peptide bonding

compartmentalization to keep cooperative systems together and exclude parasitic molecules -> vesicle formation by amphiphilic molecules