

Wikipedia (Today's Weirdness)



An odd-eyed cat is a cat with one blue eye (the "odd" eye) and one green, yellow, or brown eye. This is a feline form of complete heterochromia, a condition also present in humans and some other animals (including horses and huskies). It can occur in a cat of any fur color, provided that it possesses the white spotting gene. Animals affected by the condition can have partial heterochromia, in which only part of the iris is a different color.

Photo: Keith Kissel

Announcements

HW #3, due next Wednesday, although the qualitative questions you should know by Monday (for quiz).

Protein Folding, next week (a bit delayed) because Klaus Schulten's people are going to give a special lecture Feb 13

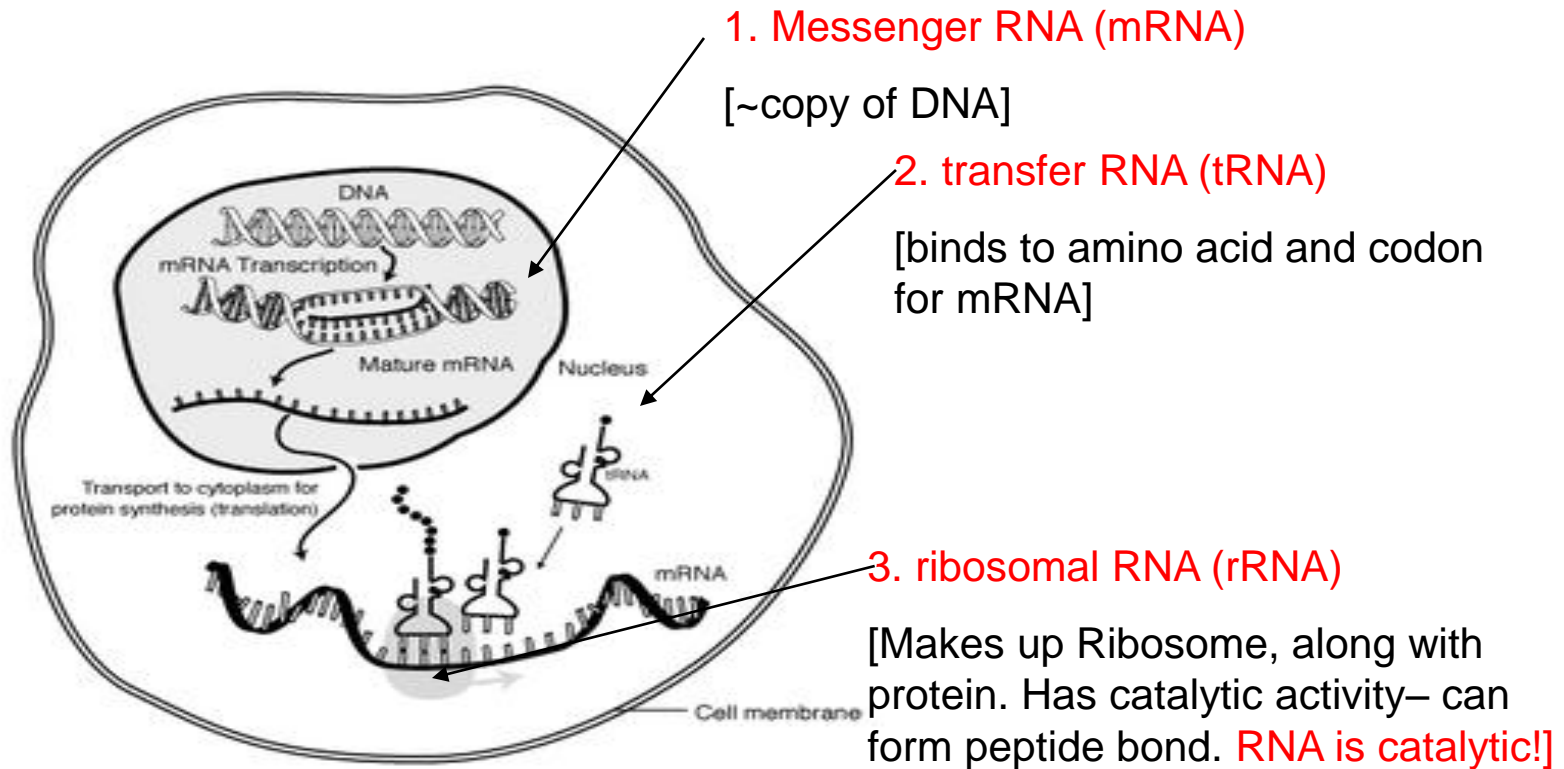
Today's Main Points

- Know 3 types of RNA
- Ribosome—Ribozyme
- How Ribosome makes RNA → Protein
- Interfering RNA—important biologically & possibly medically
- Beginning of Magnetic Tweezers?

RNA has 3 different uses

3 different names, (mRNA, tRNA, rRNA)

A key element of the central dogma of molecular biology:
that DNA makes RNA makes protein



3 types of RNA con't

mRNA— transcribed from DNA (a copy, except ribonucleic instead of deoxyribonucleic acid, U instead of T)

Transfer RNA— small piece of RNA that has an anti-codon corresponding to one amino acid.

Ribosome—a huge complex that is a combination of protein and RNA called Ribonucleoproteins. The ribosome is also a ribozyme, a enzyme creating a polypeptide from amino acids: active site is made of RNA.

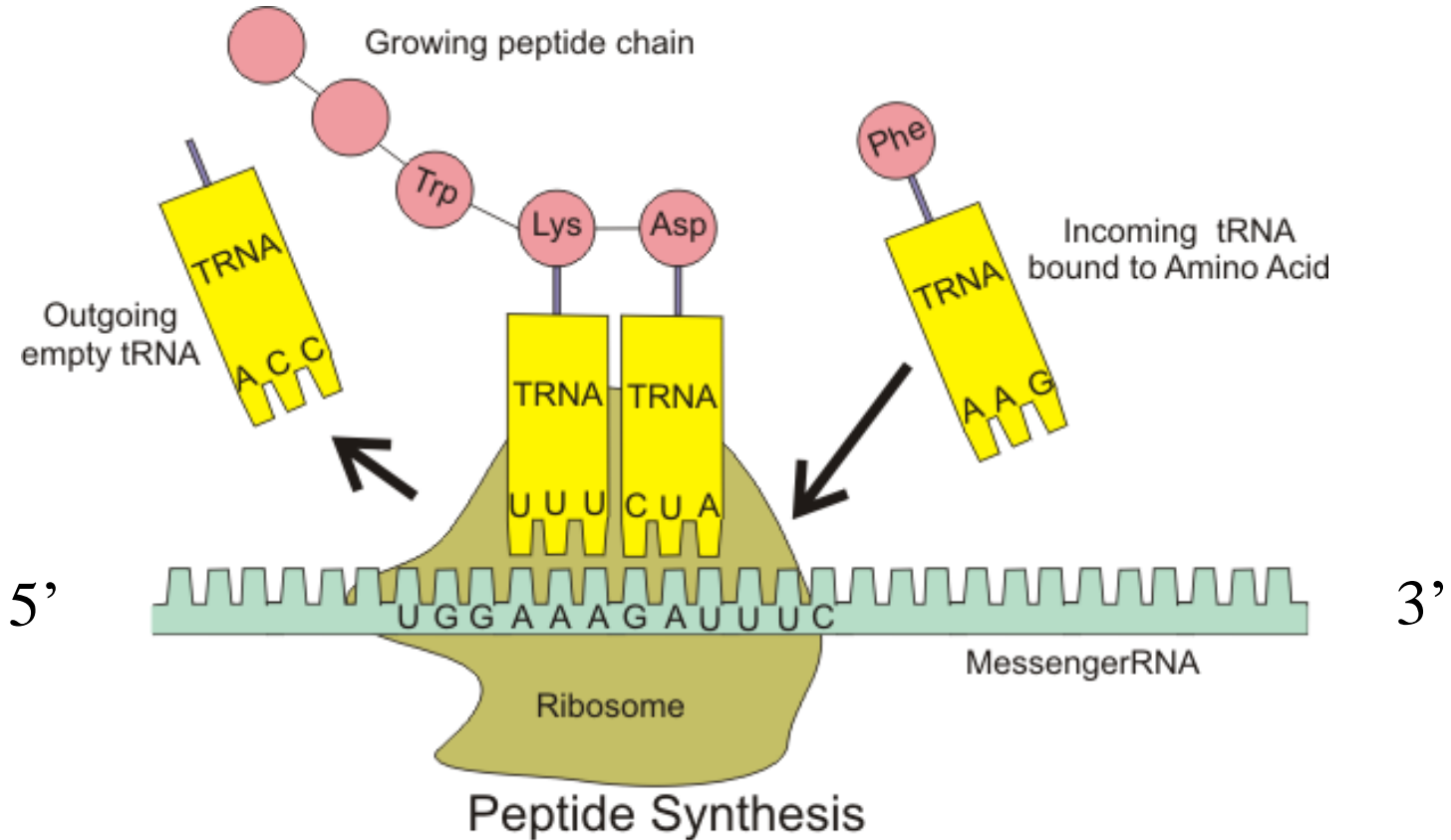


30S ribosome

http://www.mrc-lmb.cam.ac.uk/ribo/homepage/images/30s_overview_front.jpg

Ribosome

A key element of the central dogma of molecular biology:
that DNA makes RNA makes protein

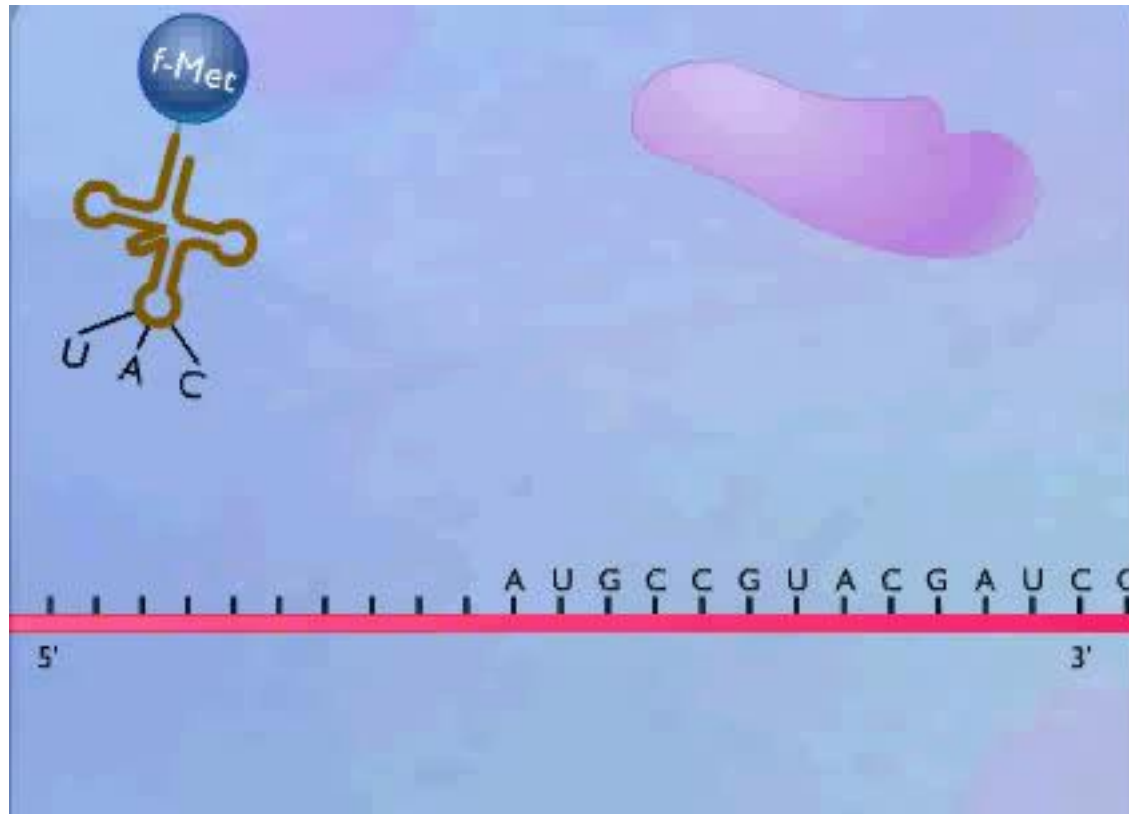


Takes the sequence in mRNA
and produces a polypeptide (protein) from it

Stages in translation

- Initiation
- Elongation
- Termination
- Recycling

Watch Video of action of Ribosome



<http://www.youtube.com/watch?feature=endscreen&NR=1&v=Ikq9AcBcohA>

Initiation

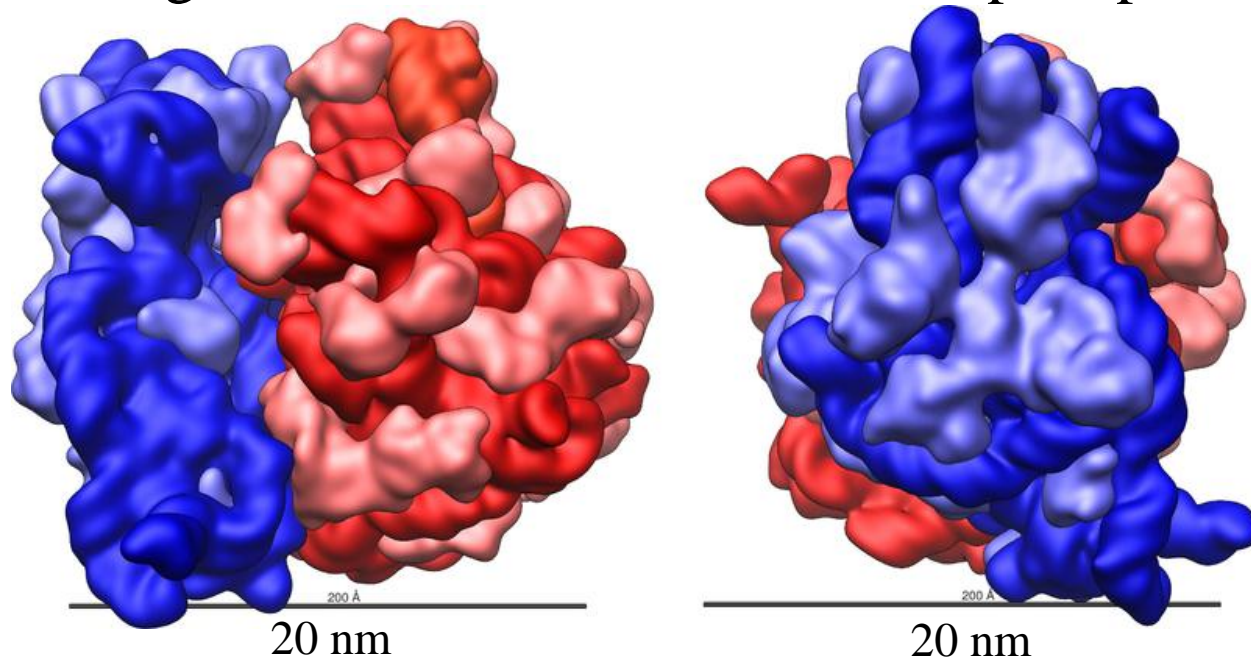
- mRNA binds to small subunit of the ribosome (30S).
- fMet-tRNA (methionine) binds to the AUG codon at the P site (peptidyl) of the ribosome.
- Initiation factors (IF1, IF2 and IF3) help to recruit large ribosomal sub-unit and assemble the initiation complex.

<http://www.youtube.com/watch?feature=endscreen&NR=1&v=Ikq9AcBcohA>

Ribosome is made of two subunits

30S and 50S sub-units

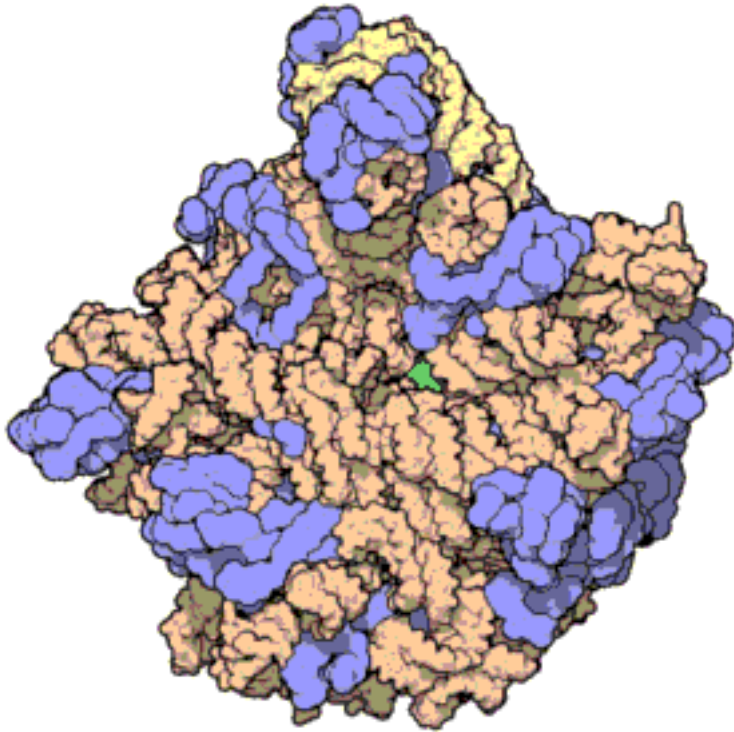
The smaller subunit binds to the mRNA, while the larger subunit binds to the tRNA and the amino acids. When a ribosome finishes reading a mRNA, these two subunits split apart.



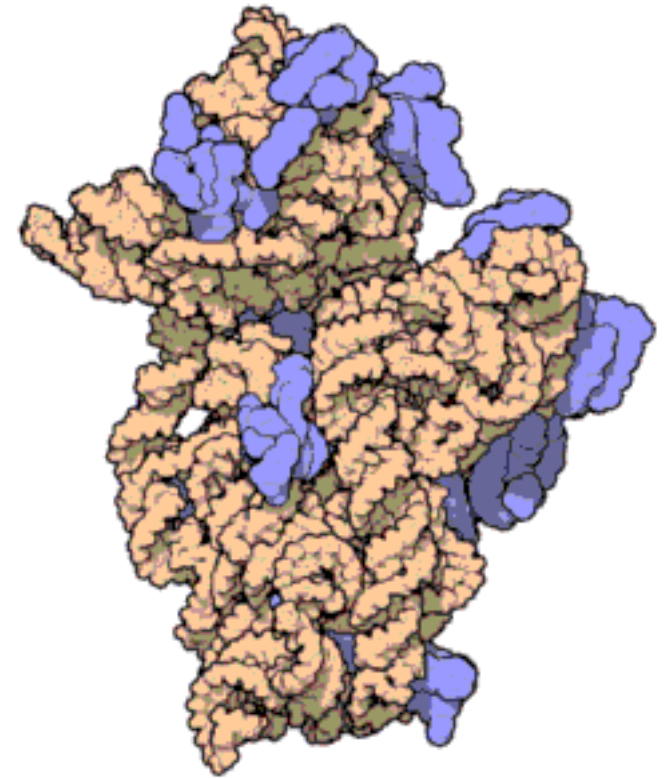
The assembly process involves the coordinated function of over 200 proteins in the synthesis and processing of the four rRNAs, as well as assembly of those rRNAs with the ribosomal proteins.

Ribosome:

mixture of RNA and Proteins



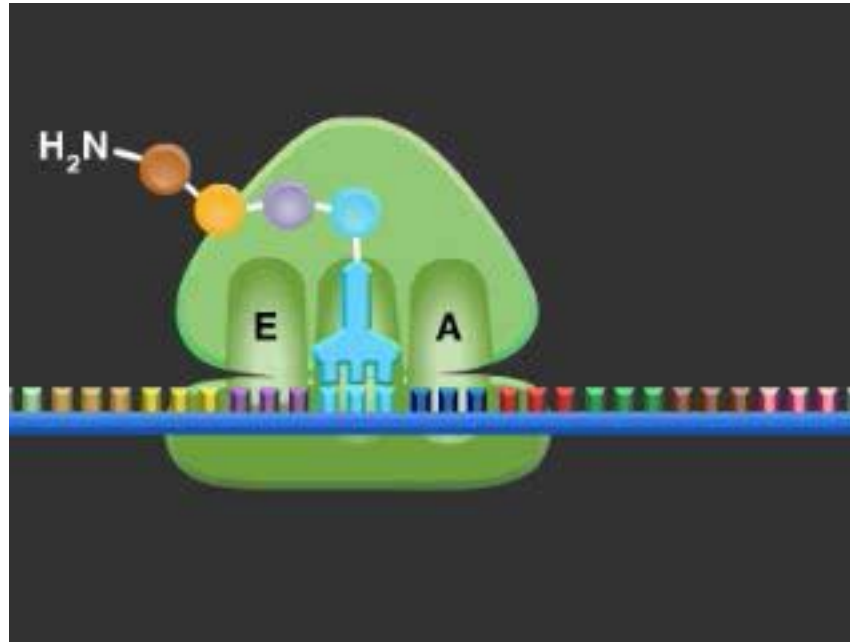
50 S



30 S

Watch Video of Ribosome in Action:

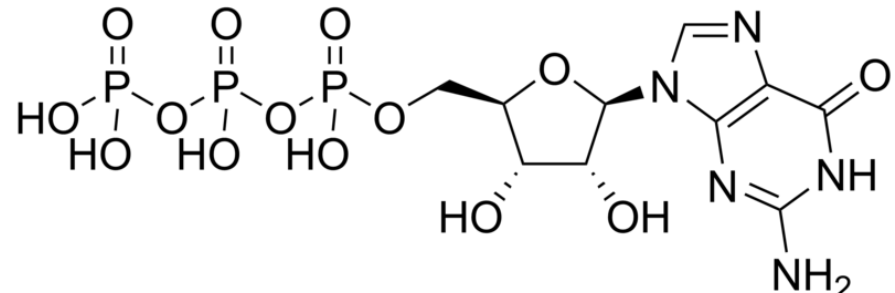
Notice that there are some extra Elongation Factors (EFs) which utilize GTP



<http://www.youtube.com/watch?v=1PSwhTGFMxs&feature=endscreen&NR=1>

Assortment of Initiation, Elongation, Recycling and Release factors and GTP

- IF1, IF2 and IF3: initiation factors
- EF-Tu and EF-G: elongation factors
- RF1, RF2 and RF3: release factors
- RRF: ribosome recycling/release factor
- GTP: Guanosine triphosphate

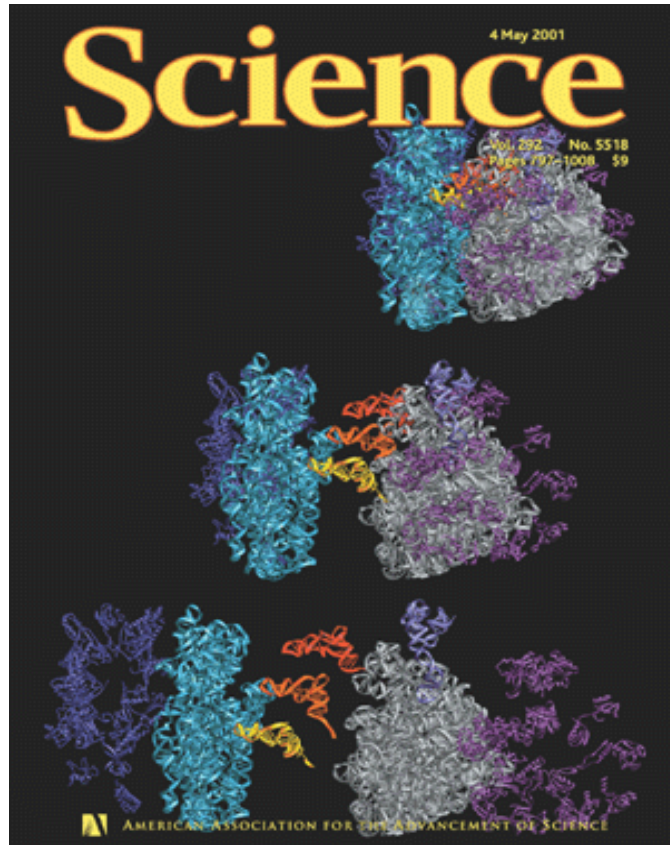


Discussion of movie on translation: (Venki Ramakrishnan's, 2009 Nobel Prize Winner) home page. http://www.mrc-lmb.cam.ac.uk/ribo/homepage/mov_and_overview.html

The movie is actually pretty long including the initiation, elongation, termination and recycling stage.

Evidence that RNA have these properties?

The Ribosome is an RNA-based catalytic machine.



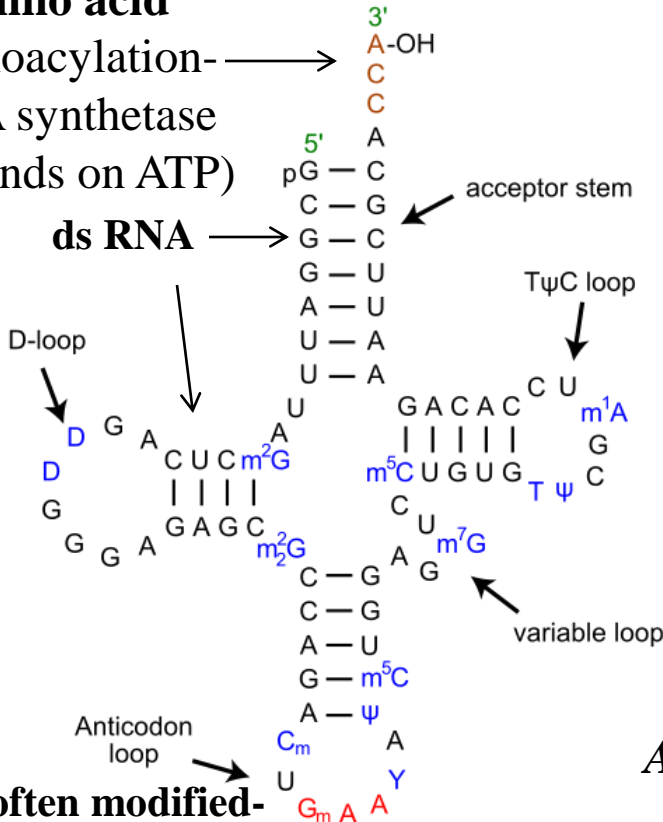
The ribosome has **three** binding sites for tRNA molecules that span the space between the two ribosomal subunits: the A (aminoacyl), P (peptidyl), and E (exit) sites. In addition, the ribosome has **two** other sites for tRNA binding that are used during mRNA decoding or during the initiation of protein synthesis. These are the T site (named elongation factor Tu) and I site (initiation).

Transfer RNA (tRNA)

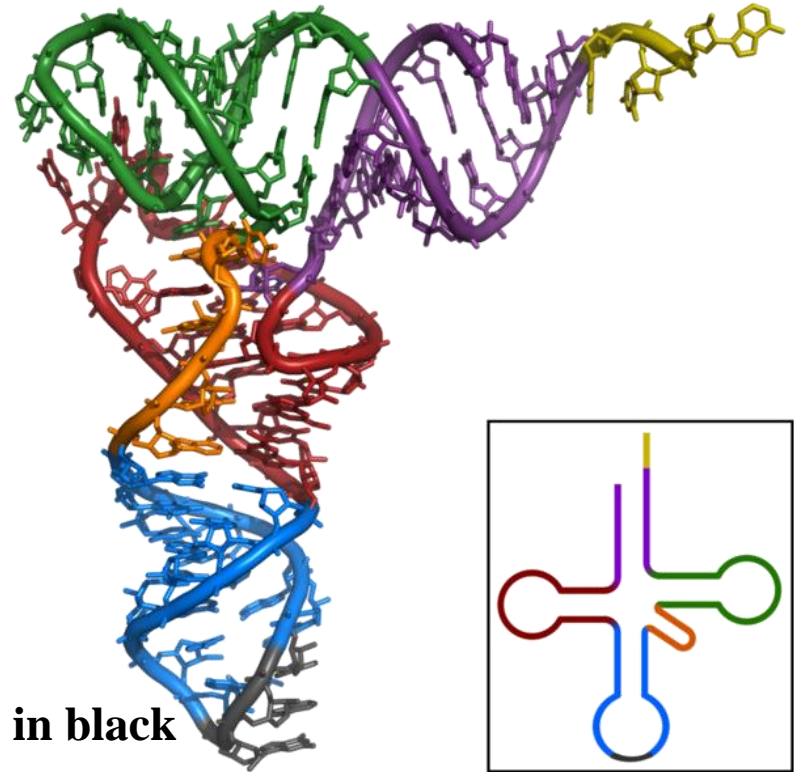
CCA tail binds to amino acid

Aminoacylation-tRNA synthetase (depends on ATP)

ds RNA



Tertiary structure of tRNA.



Anticodon in black

Its primary structure (including bases which have been methylated), its secondary structure (usually visualized as the *cloverleaf structure*), and its tertiary structure (all tRNAs have a similar L-shaped 3D structure that allows them to fit into the P and A sites of the ribosome).

A lot of tRNA genes

In the human genome, which according to current estimates has about 27,161 genes in **total, there are about 4,421 non-coding RNA genes, which include tRNA genes. There are 22 mitochondrial tRNA genes;** 497 nuclear genes encoding cytoplasmic tRNA molecules and there are 324 tRNA-derived putative pseudogenes.

Cytoplasmic tRNA genes can be grouped into 49 families according to their anticodon features. These genes are **found on all chromosomes, except 22 and Y chromosome.**

Ribosomes differ across kingdoms

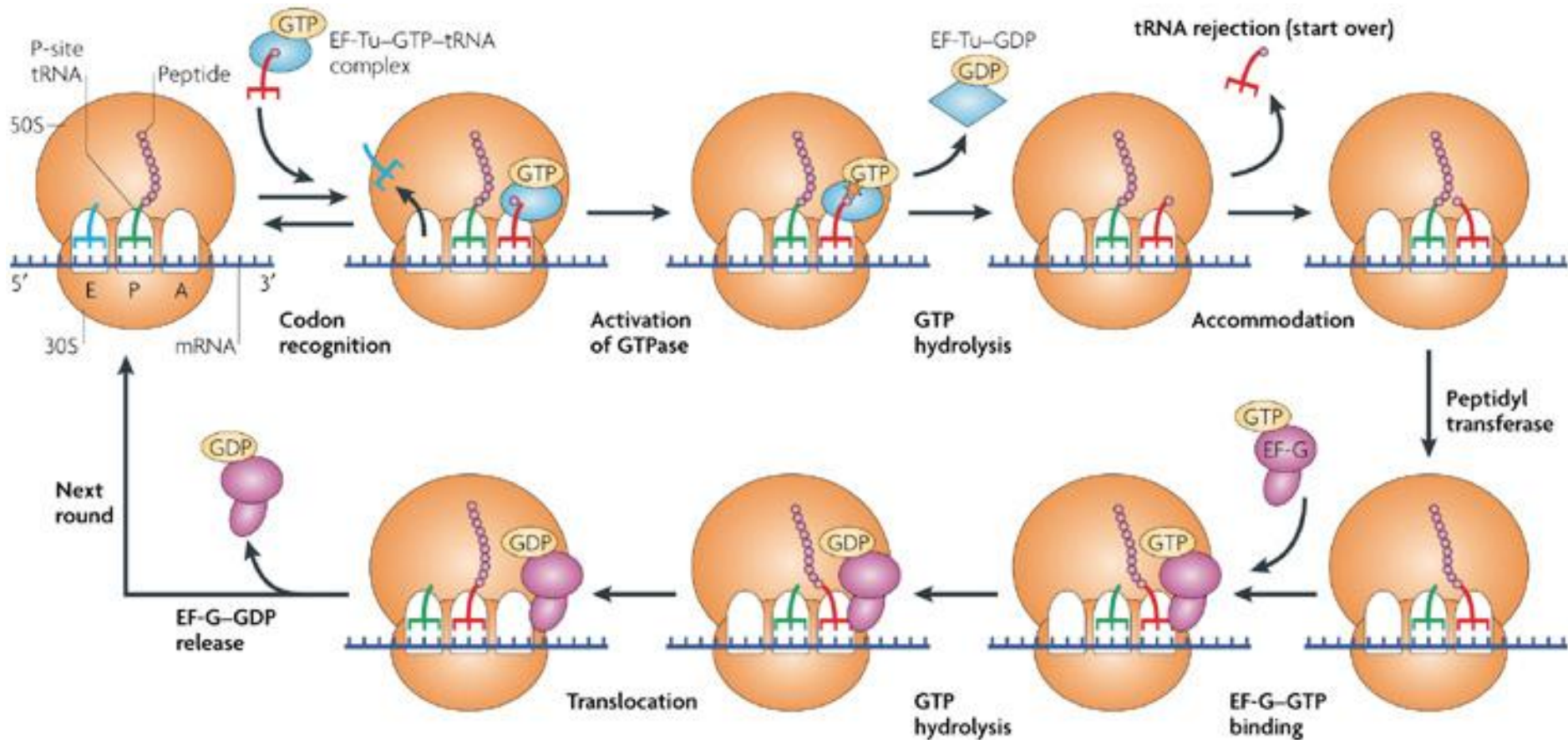
Antibiotics are possible because of this!

- Archaeal, eubacterial and eukaryotic ribosomes differ in their size, composition and the ratio of protein to RNA. Because they are formed from two subunits of non-equal size, they are slightly longer in the axis than in diameter. **Prokaryotic ribosomes** are around 20 nm (200 Å) in diameter and are composed of 65% ribosomal RNA and 35% ribosomal proteins. **Eukaryotic ribosomes** are between 25 and 30 nm (250–300 Å) in diameter and the ratio of rRNA to protein is close to 1.
- By comparing rRNA sequences, Carl Woese got idea 3 branches of life.
- These differences in ribosome structure allow some antibiotics to kill bacteria by inhibiting their ribosomes, while leaving human ribosomes unaffected. The ribosomes in the mitochondria of eukaryotic cells functionally resemble in many features those in bacteria, reflecting the likely evolutionary origin of mitochondria.

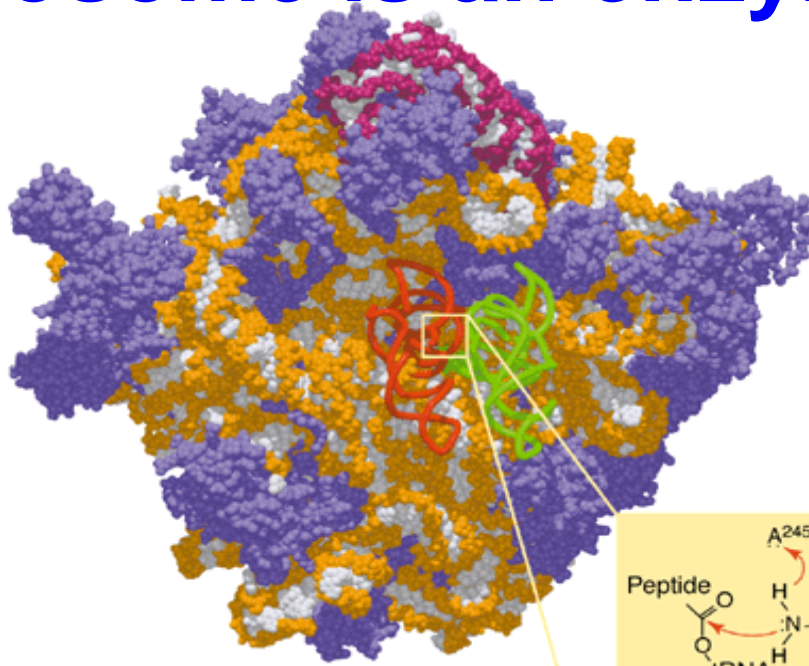
Nobel Prize in Physiology or Medicine, awarded to 3 professors in 1974, for the discovery of the ribosomes.

Elongation

(just showed this briefly— refer to video)

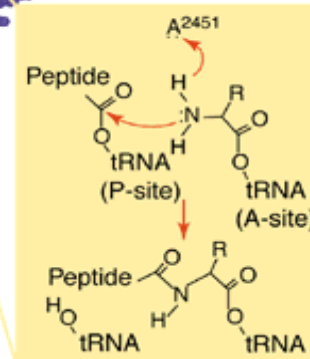


Ribosome is an enzyme (a ribozyme)



Proteins;
23S rRNA;
5S rRNA (at the top)
A-site tRNA
P-site tRNA

T. Steitz, 2000, Science

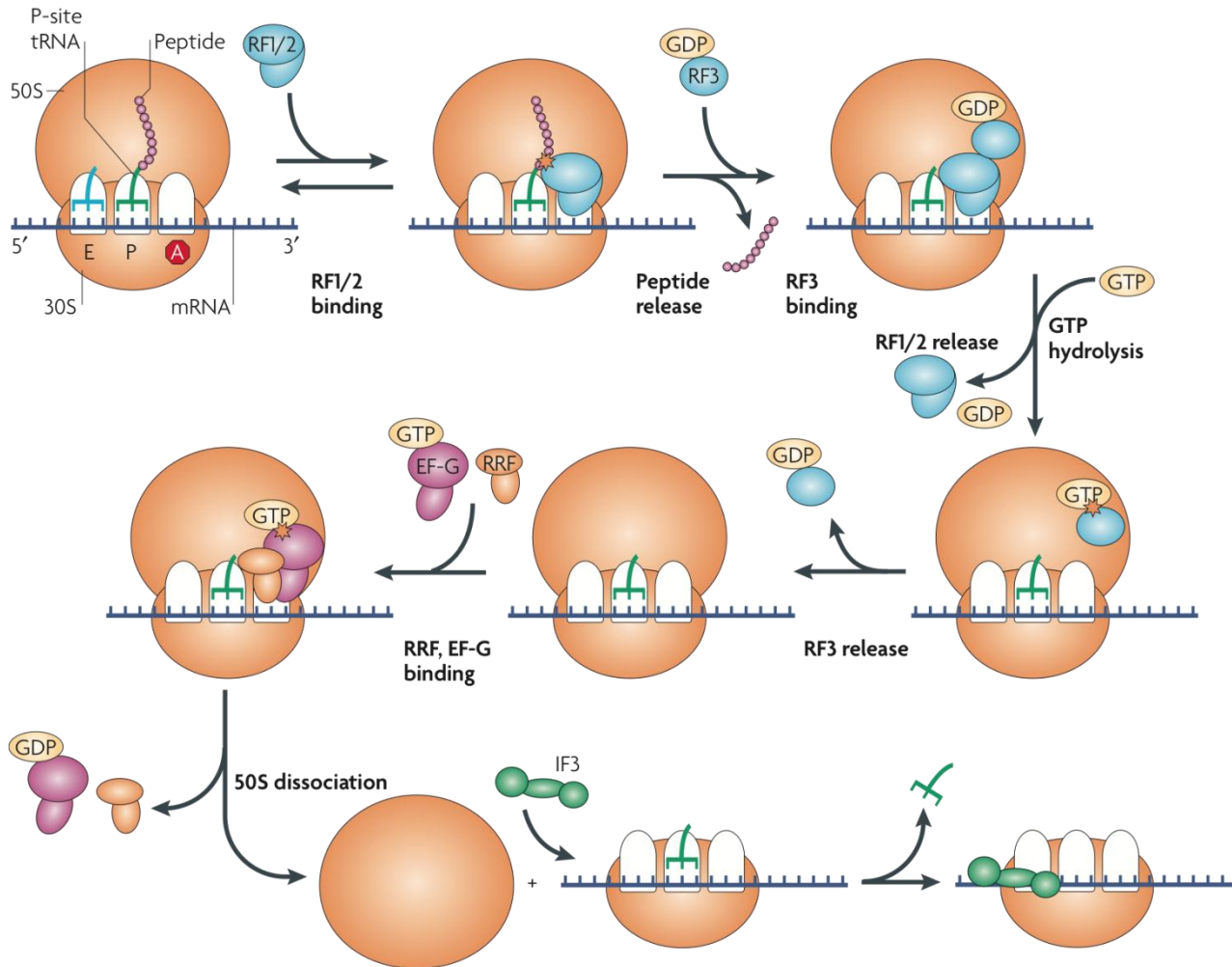


(Top) The large subunit of the ribosome seen from the viewpoint of the small subunit. **(Bottom)** The peptidyl transfer mechanism catalyzed by RNA. The general base (adenine 2451 in *Escherichia coli* 23S rRNA) is rendered unusually basic by its environment within the folded structure; it could abstract the proton at any of several steps, one of which is shown here.

Nobel Prize 2009: Atomic Structure of Ribosome
(Steitz, Ramakrishnan, Yonath)

Termination and Recycling

(just showed this very briefly— refer to video)



Ref: Thomas A. Steitz, A structural understanding of the dynamic ribosome machine. *Nature Reviews Molecular cell biology*, Volume 9, 243. (2008)

Watch Video

http://www.mrc-lmb.cam.ac.uk/ribo/homepage/mov_and_overview.html

The whole thing

RNA Interference (RNAi)

RNA may do more than produce proteins!

(Does it have to do with junk DNA??)

- Two types of small ribonucleic acid (RNA) molecules – microRNA (miRNA) and small interfering RNA (siRNA) – are central to RNA interference.
- These small RNAs can bind to other specific messenger RNA (mRNA) molecules and either increase or decrease their activity, for example by preventing an mRNA from producing a protein.
- RNA interference has an important role in defending cells against parasitic genes – viruses and transposons – but also in directing development as well as gene expression in general.

DNA → RNA → Proteins

RNAi continued

- The RNAi pathway is found in many eukaryotes including animals and is initiated by the enzyme Dicer, which cleaves long double-stranded RNA (dsRNA) molecules into short fragments of ~20 nucleotides that are called siRNAs. Each siRNA is unwound into two single-stranded (ss) ssRNAs, namely the passenger strand and the guide strand. The passenger strand will be degraded, and the guide strand is incorporated into the RNA-induced silencing complex (RISC). The most well-studied outcome is post-transcriptional gene silencing, which occurs when the guide strand base pairs with a complementary sequence of a messenger RNA molecule and induces cleavage by Argonaute, the catalytic component of the RISC complex.
- A valuable research tool, both in cell culture and in living organisms and promising tool in biotechnology and medicine. Synthetic dsRNA introduced into cells can induce suppression of specific genes of interest.

2006 Nobel Prize on RNA interference
Fire and Mello

Possible therapeutic applications and challenges

- Given the ability to knock down, in essence, any gene of interest, RNAi via siRNAs has generated a great deal of interest in both basic and applied biology. There are an increasing number of large-scale RNAi screens that are designed to identify the important genes in various biological pathways. Because disease processes also depend on the activity of multiple genes, it is expected that in some situations turning off the activity of a gene with an siRNA could produce a therapeutic benefit.
- However, applying RNAi via siRNAs to living animals, especially humans, poses many challenges. Under experiments, siRNAs show different effectiveness in different cell types in a manner as yet poorly understood: Some cells respond well to siRNAs and show a robust knockdown, whereas others show no such knockdown (even despite efficient transfection).
- Phase I results of the first two therapeutic RNAi trials (indicated for age-related macular degeneration, aka AMD) reported at the end of 2005 that siRNAs are well tolerated and have suitable pharmacokinetic properties.[7]
- Proof of concept trials have indicated that Ebola-targeted siRNAs may be effective as post-exposure prophylaxis in humans, with 100% of non-human primates surviving a lethal dose of Zaire Ebolavirus, the most lethal strain.[8]

Next: Beginning of Magnetic Traps...