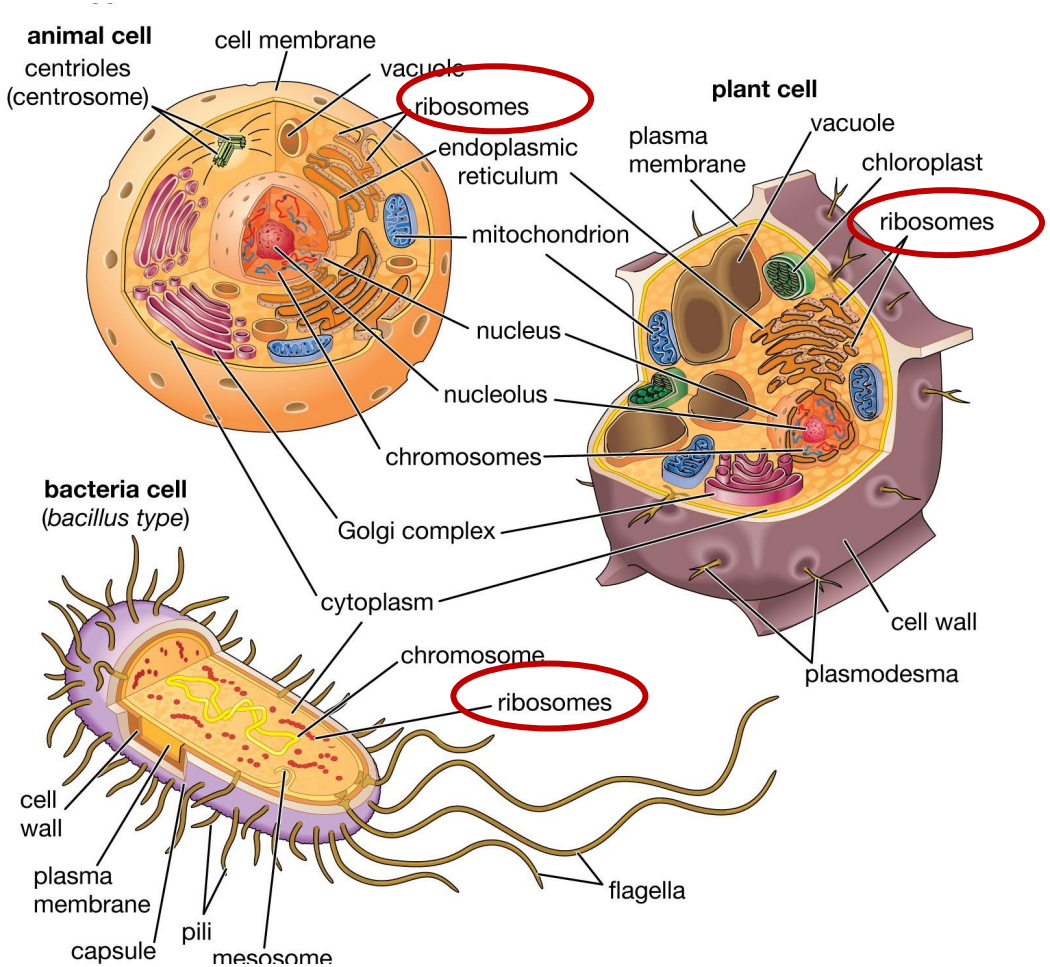
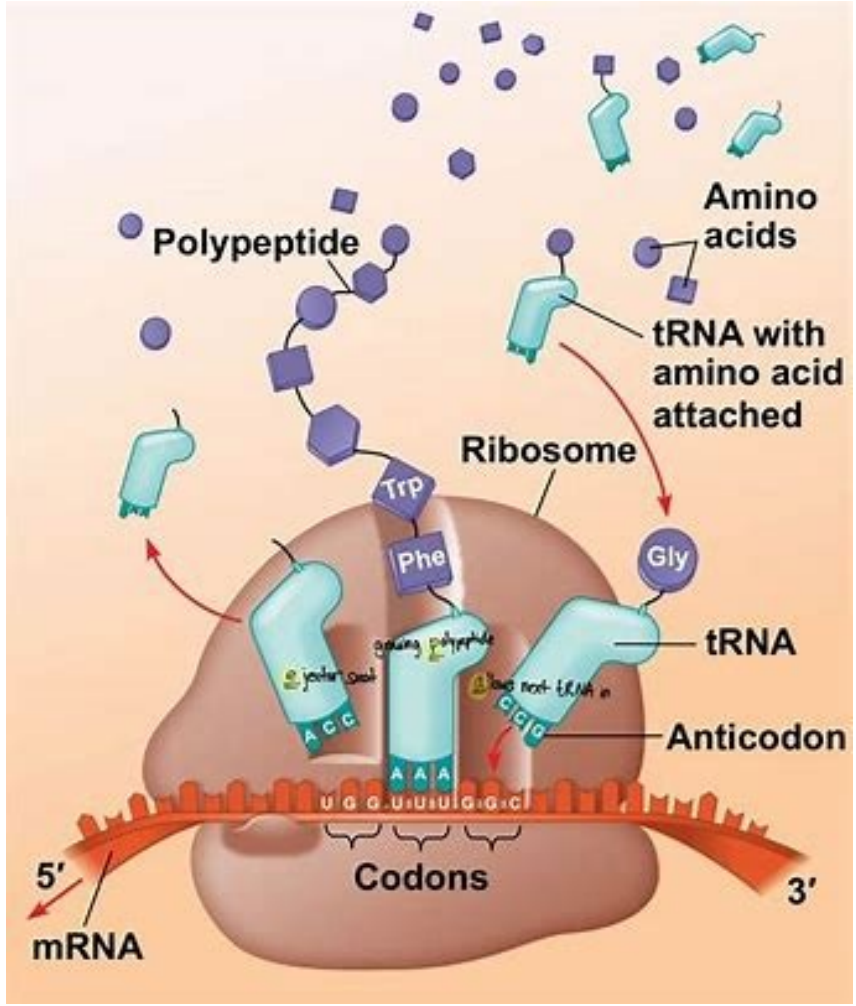
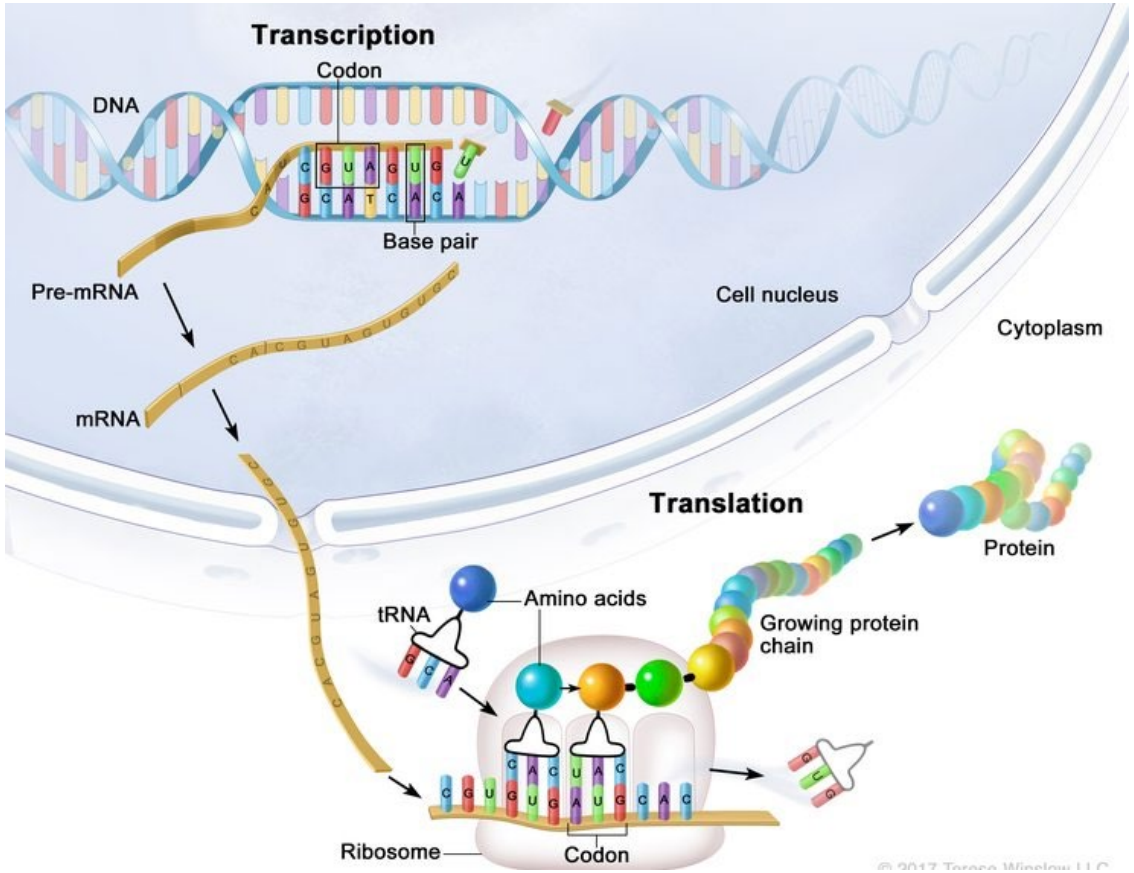
Ribosome: cellular machinery responsible for making proteins

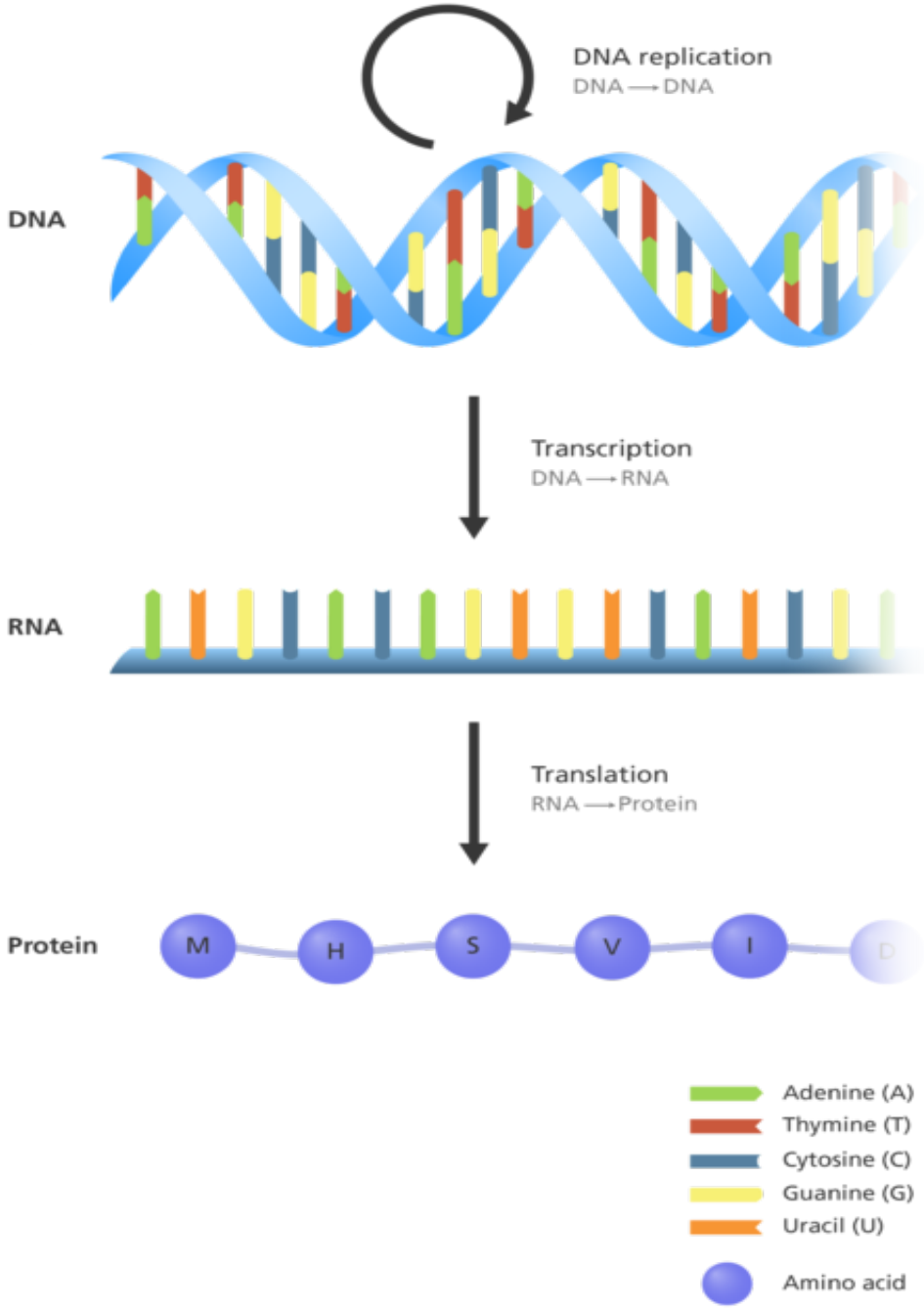




The central dogma of molecular biology is a theory which states that genetic information flows from DNA, to RNA, to protein.

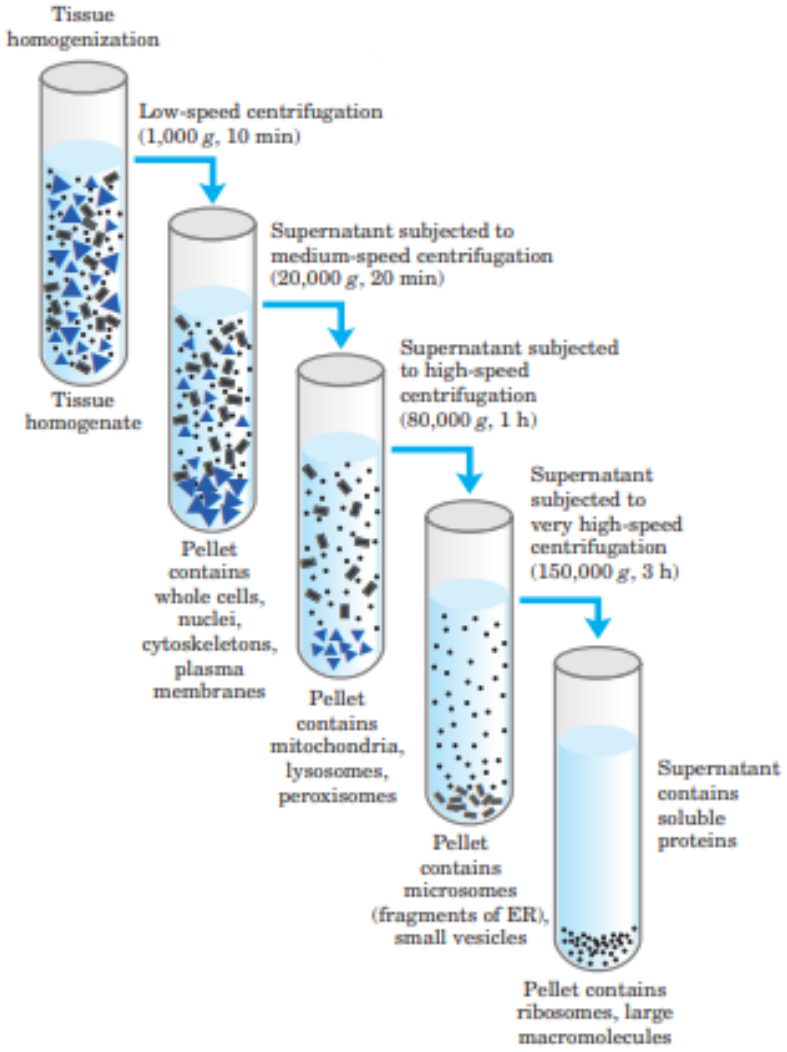
- **Experimental basis for site of protein synthesis (Ribosome), Aminoacyl-tRNA synthetases for activation of amino acids and tRNA.**
- **Experimental basis to establish relationships between codons and amino acids**





Three major advances set the stage for our present knowledge of protein biosynthesis

Differential centrifugation



First, in the early 1950s, Paul Zamecnik and his colleagues designed a set of experiments to investigate where in the cell proteins are synthesized. They injected radioactive amino acids into rats and, at different time intervals after the injection, removed the liver, homogenized it, fractionated the homogenate by centrifugation, and examined the subcellular fractions for the presence of radioactive protein.

Second key advance was made by Mahlon Hoagland and Zamecnik when they found that amino acids were "activated" for protein synthesis when incubated with ATP and the cytosolic fraction of liver cells. The amino acids became attached to a heat-stable soluble RNA of the type that had been discovered and characterized by Robert Holley, and later called transfer RNA (tRNA), to form aminoacyl-tRNAs. The enzymes that catalyze this process are the aminoacyl-tRNA synthetases.

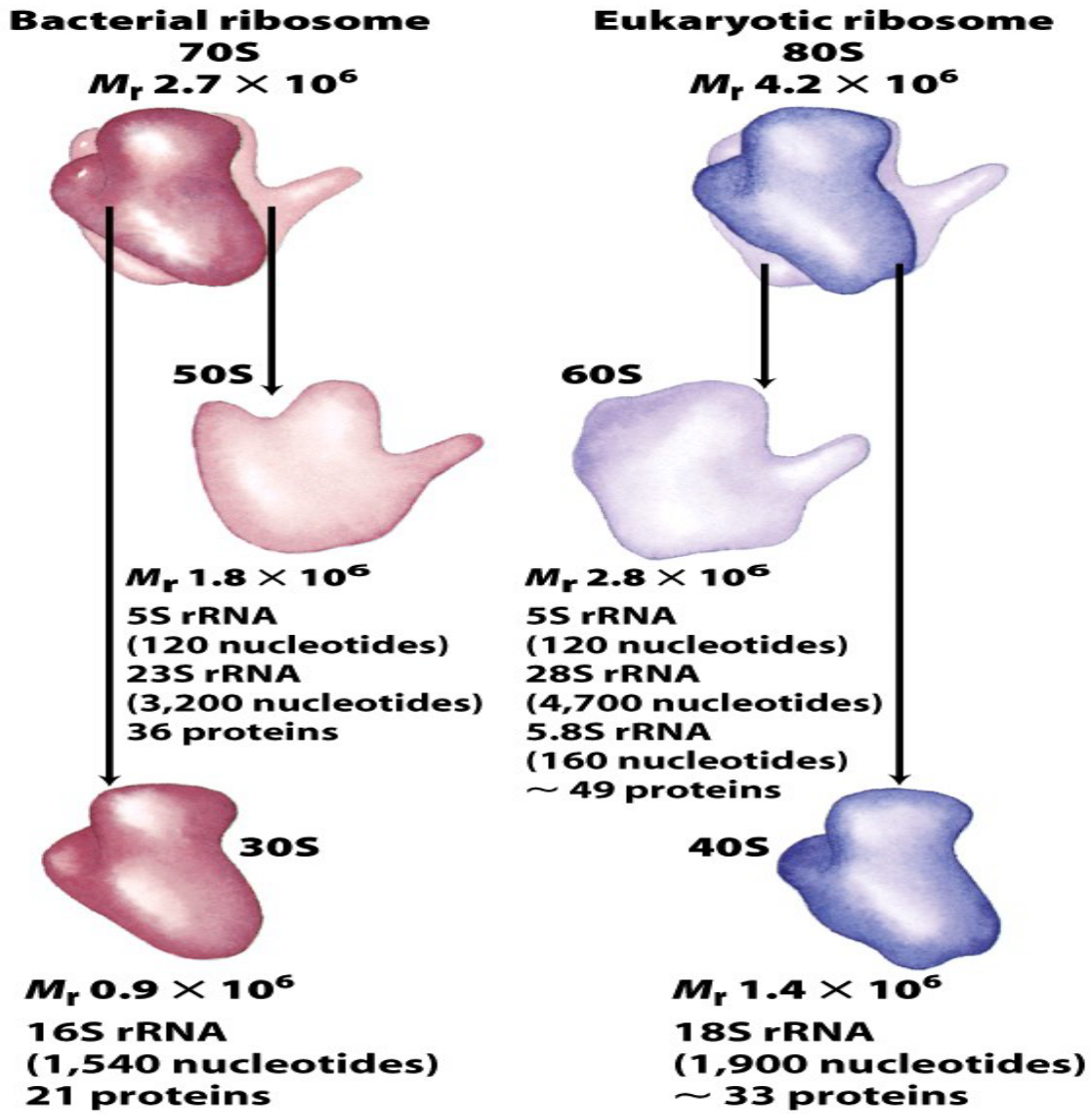
Third advance was how the genetic information encoded in the 4-letter language of nucleic acids could be translated into the 20-letter language of proteins. The tRNA adaptor, the same molecule that activates the amino acid for peptide bond formation, also "translates" the nucleotide sequence of an mRNA into the amino acid sequence of a polypeptide. The overall process of mRNA-guided protein synthesis is often referred to simply as translation.

Composition of bacterial and eukaryotic ribosomes: a large complex of RNAs, ribosomal RNAs (rRNAs) and many small proteins.

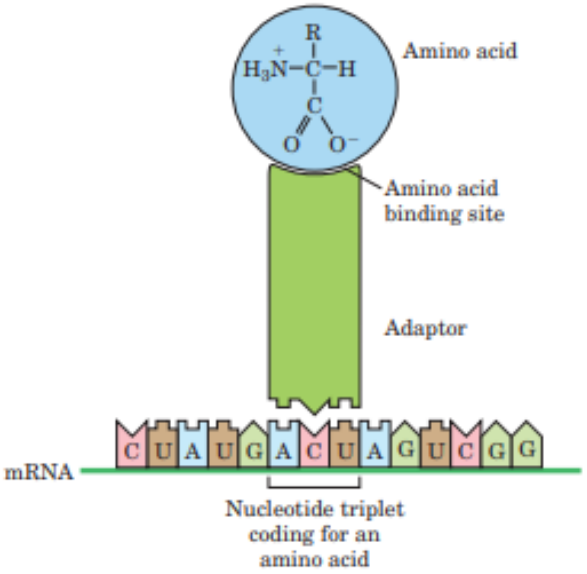
- Overall, very similar
- Two subunits
- In eukaryotes, larger (80S), more complex than bacterial 70S
- Chloroplasts and mitochondria have ribosomes similar to bacteria.

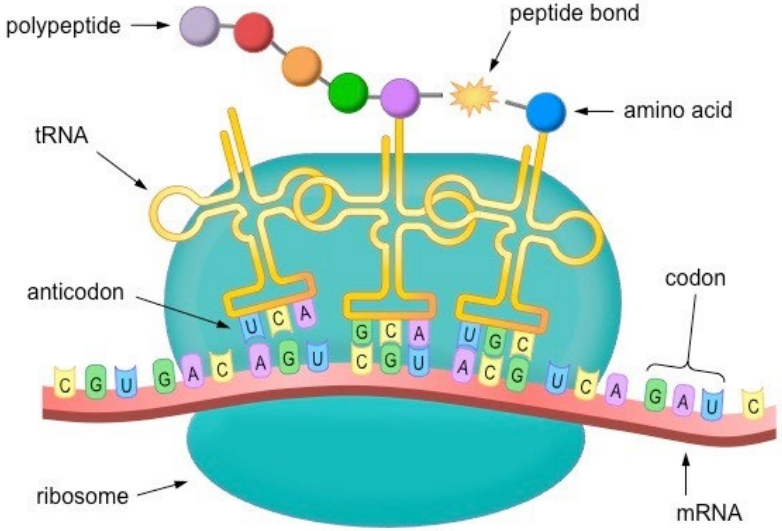
In 1960s, Masayasu Nomura demonstrated that both ribosomal subunits can be broken down into RNA and protein components and reconstituted *in vitro* under appropriate experimental conditions.

The first high resolution structures of bacterial ribosomal subunits was determined by Venki Ramakrishnan, Thomas Steitz, Ada Yonath, Harry Noller and others.



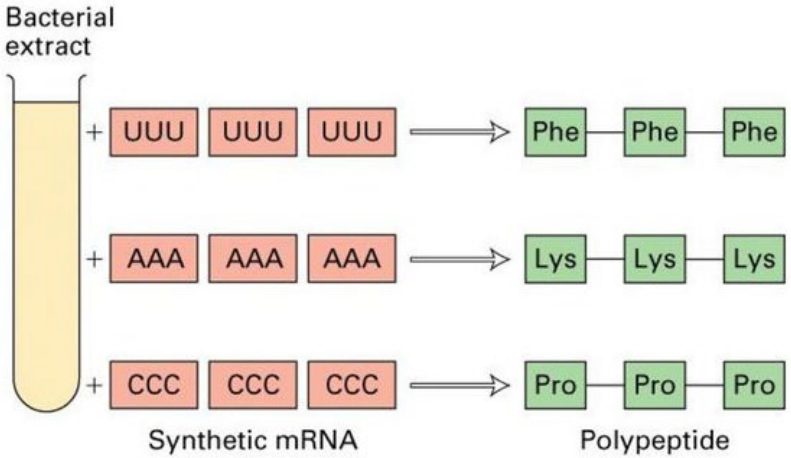
Requirements of protein translation: ribosome, mRNA, tRNA, amino acids, aminoacyl tRNA synthetases





Amino acid	*Three-letter code*	*Symbol*
Leucine	Leu	L
Isoleucine	Ile	I
Threonine	Thr	T
Serine	Ser	S
Phenylalanine	Phe	F
Alanine	Ala	A
Asparagine	Asn	N
Methionine	Met	M
Proline	Pro	P
Glycine	Gly	G
Lysine	Lys	K
Glutamine	Gln	Q
Tyrosine	Tyr	Y
Valine	Val	V
Histidine	His	H
Tryptophan	Trp	W
Aspartic acid	Asp	D
Glutamic acid	Glu	E
Arginine	Arg	R
Cysteine	Cys	C

Nirenberg and Heinrich Matthaei, a postdoctoral fellow conducted the experiments by studying the long linear molecule synthetic RNA.



The Genetic Code for Proteins Consists of triplets of Nucleotides

- There are 20 common amino acids.
- A four-letter code in groups of two (4^2) is insufficient (16).
- A four-letter code in groups of three (4^3) is sufficient (64).

- Random polymers of 3 nucleotides based on the base composition, triplet base for Asn, Gln, His, Lys, Pro and Thr was revealed although the order or sequence of bases was not known
- Hargovind Khorana: developed chemical methods to synthesize polyribonucleotides with defined repeating sequences of two to four bases. The polypeptide produced from these mRNAs had one or few amino acids in repeating patterns.

First letter of codon (5′ end)	Second letter of codon: U		C		A		G	
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
	UUA	Leu	UCA	Ser	UAA	Stop	UGA	Stop
	UUG	Leu	UCG	Ser	UAG	Stop	UGG	Trp
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
	AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

Amino acid	Number of codons	Amino acid	Number of codons
Met	1	Tyr	2
Trp	1	Ile	3
Asn	2	Ala	4
Asp	2	Gly	4
Cys	2	Pro	4
Gln	2	Thr	4
Glu	2	Val	4
His	2	Arg	6
Lys	2	Leu	6
Phe	2	Ser	6