

Cracking the Secret of Genetic Code

Biological Life Sciences
By Ashutosh Upadhye



DNA vs RNA

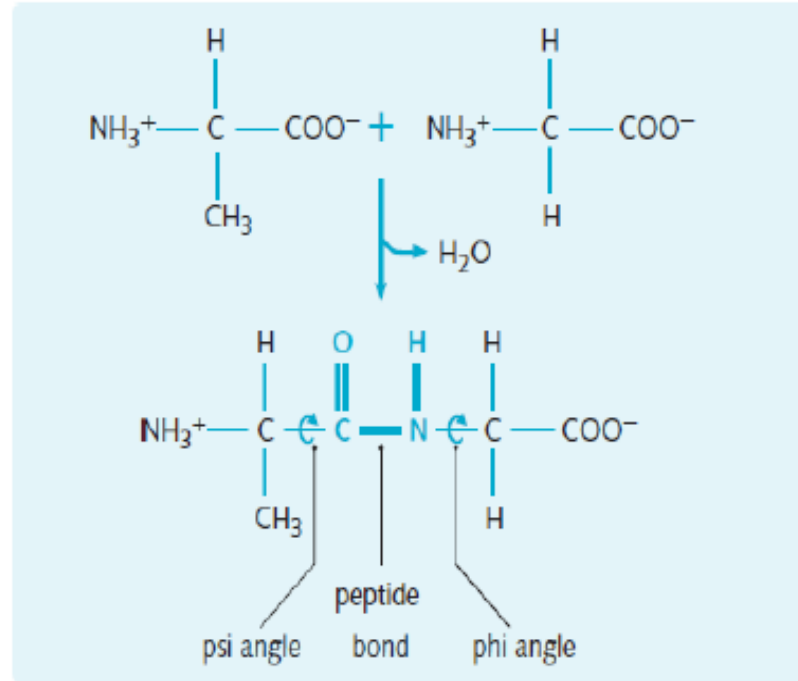
Bases

- The nucleotide bases in nucleic acids contain nitrogen derived from either purines or pyrimidines.
- Purines (Double ring)
 - Adenine
 - Guanine
- Pyrimidines (Single ring)
 - Cytosine
 - Thymine*
 - Uracil*



AMINO ACIDS

- There are 20 naturally occurring amino acids, which differ in their side group.
- All amino acids, except glycine, have an asymmetrical α -carbon atom, giving rise to D or L stereoisomer forms; however, only the L form is found in humans.
- Amino acids form proteins by joining together through peptide bonds.



Amino acids code

Nonpolar and uncharged

A	Ala	Alanine
F	Phe	Phenylalanine
G	Gly	Glycine
I	Ile	Isoleucine
L	Leu	Leucine
M	Met	Methionine
P	Pro	Proline
V	Val	Valine
W	Trp	Tryptophan

Polar and uncharged

C	Cys	Cysteine
N	Asn	Asparagine
Q	Gln	Glutamine
S	Ser	Serine
T	Thr	Threonine
Y	Tyr	Tyrosine

Positively charged (basic)

H	His	Histidine
K	Lys	Lysine
R	Arg	Arginine

Negatively charged (acidic)

D	Asp	Aspartic acid
E	Glu	Glutamic acid

Ambiguous codes

B	Asx	Asparagine or aspartic acid
Z	Glx	Glutamine or glutamic acid



Deciphering the Code

➤ **Marshall Nirenberg and Heinrich Matthaei**

at the National Institutes of Health used a precise and logical series of experiments to “crack the code”.

➤ **They were among the first to characterize specific coding sequences.**

➤ **Made possible by advancements that:**

- * Allowed protein synthesis in vitro**

- * Synthesizing RNA strands in vitro**



Deciphering the genetic code

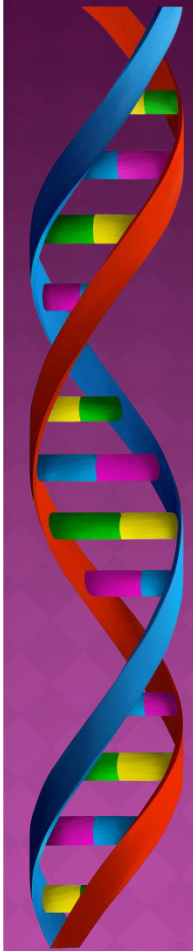
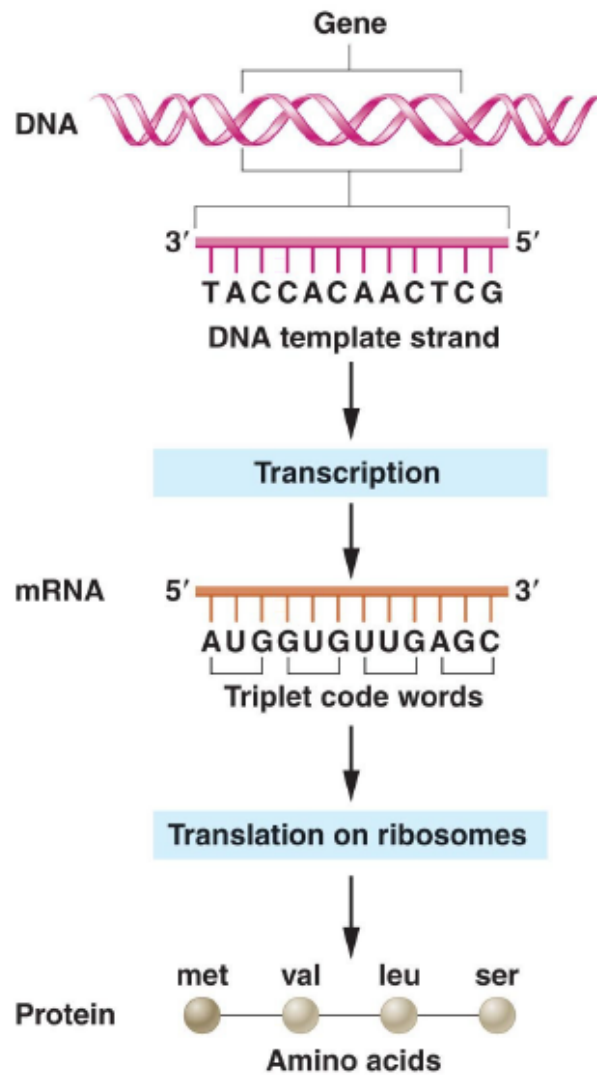
The genetic code is cracked by two different ways:

- Theoretical approach → triplet hypothesis
- practical approach



Codon length (# of nucleotides)	# of possible codons
1	4^1
2	$4^2 = 16$
3	$4^3 = 64$
4	$4^4 = 256$

- Even though genetic information is stored in DNA, the code that is translated into proteins resides in RNA.
- How only four nucleotides could specify 20 the amino acids?



Evidence for the Triplet Code

- How Many RNA Bases Specify One amino acid code; 20 amino acids code?
- If a codon consisted of only one mRNA base?
- Two base, for example, provides only 16 unique code words (4²). Not enough..!
- A triplet code yields 64 words (4³) and therefore is sufficient for the 20 amino acids.
- A four-letter code (4⁴), which would specify 256 words.

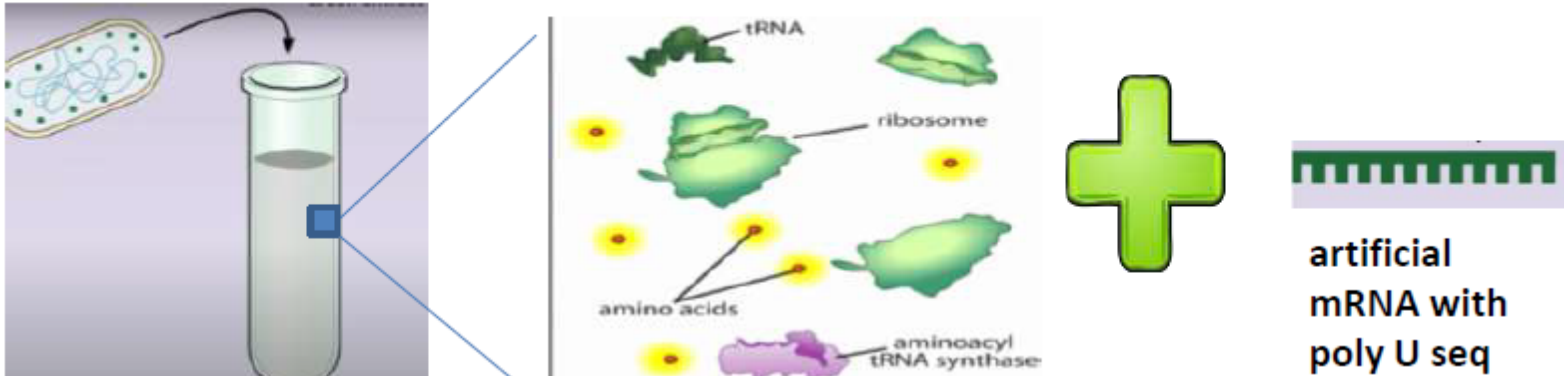


Practical approach

- The cracking of the genetic code began in 1961, with work from the American biochemist [Marshall Nirenberg](#) and his post-doctoral fellow, [J. Heinrich Matthaei](#) at the [National Institutes of Health](#) (NIH)

Their success relied on two experimental innovations:

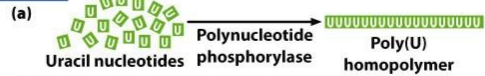
- A way to make artificial mRNA molecules with specific, known sequences.
- A system to translate mRNAs into polypeptides outside of a cell (a "cell-free" system). Nirenberg's system consisted of cytoplasm from burst *E. coli* cells, which contains all of the materials needed for translation.



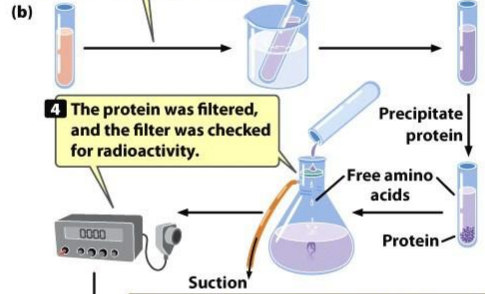
Experiment

Question: What amino acids are specified by codons composed of only one type of base?

Methods

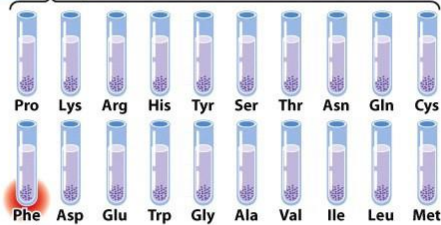


- 1 A homopolymer—in this case, poly(U) mRNA—was added to a test tube containing a cell-free translation system, 1 radioactively labeled amino acid, and 19 unlabeled amino acids.
- 2 The tube was incubated at 37°C.
- 3 Translation took place.



Results

- 5 The procedure was repeated in 20 tubes, with each tube containing a different labeled amino acid.



- 6 The tube in which the protein was radioactively labeled contained newly synthesized protein with the amino acid specified by the homopolymer. In this case, UUU specified the amino acid phenylalanine.

Conclusion: UUU encodes phenylalanine; in other experiments, AAA encoded lysine, and CCC encoded proline.



Marshall Nirenberg and Heinrich Matthaei experiments

- Synthesized simple mRNA molecules were added to test tubes that contained all the chemicals and structures needed for translation, extracted from *E. coli*.
- The 1st synthetic mRNA they made had the sequence *UUUUUU*.... Result: Peptides of phenylalanine.
- Conclusion: The codon UUU specifies the amino acid phenylalanine



Marshall Nirenberg and Heinrich Matthaei experiments

- The 2nd experiments AAAAAA..... Result: Peptide of lysine
- The 3rd experiments CCCCCC.... Result: Peptide for Proline.
- GGGGGG.... was unstable, so this part of the experiment could not be done.
- Next to prove other codon-amino acid pairs hence researchers synthesized chains of alternating bases



Marshall Nirenberg and Heinrich Matthaei experiments

- Synthetic mRNA of sequence *AUAUAU . . .* introduced codons AUA and UAU alternating **Isoleucines** and **Tyrosines**, but which one is which?
- Another experiment with a more complex sequence answered the question.
- The mRNA *UUUAUAUUUAUA*, *UUU* codes for phenylalanine, *AUA* code for isoleucine.
- Hence if *AUA* codes for isoleucine, then *UAU* must code for tyrosine... right?



The code is unambiguous

- Sixty of the possible 64 codons specify particular amino acids
- Three indicate “stop,” and one encodes both the amino acid methionine and “start.”
- This means that some amino acids are specified by more than one codon.
- For example, both UUU and UUC encode phenylalanine.



- By 1965, using the cell-free system and other techniques, Nirenberg, Khorana, and their colleagues had deciphered the entire genetic code.
- It was found that all the 64 triplets codons
- [The remaining three codons do not code for any amino acid They are called nonsense codons /stop codons
- The nonsense codons also have a function They act as stop signals (chain termination signals)
- Nirenberg and Khorana (along with another genetic code researcher, Robert Holley) received the Nobel Prize in 1968.



The Genetic code

Second Letter

		Second Letter					
		U	C	A	G		
1st letter	U	UUU Phe UUC UUA Leu UUG	UCU UCC Ser UCA UCG	UAU Tyr UAC UAA Stop UAG Stop	UGU Cys UGC UGA Stop UGG Trp	U C A G	
	C	CUU CUC Leu CUA CUG	CCU CCC Pro CCA CCG	CAU His CAC CAA Gln CAG	CGU CGC Arg CGA CGG	U C A G	
	A	AUU AUC Ile AUA AUG Met	ACU ACC Thr ACA ACG	AAU Asn AAC AAA Lys AAG	AGU Ser AGC AGA Arg AGG	U C A G	
	G	GUU GUC Val GUA GUG	GCU GCC Ala GCA GCG	GAU Asp GAC GAA Glu GAG	GGU GGC Gly GGA GGG	U C A G	



The Genetic code

- Almost all amino acids are specified by two, three, or four different codons.
- Three amino acids (arginine, serine, and leucine) are specified by six codons.
- Methionine and tryptophan are encoded by single codons.
- In many codons specifying the same amino acid (synonymous codons), the first two positions are the same but the third position differs (Proline); degenerate



Wobble hypothesis

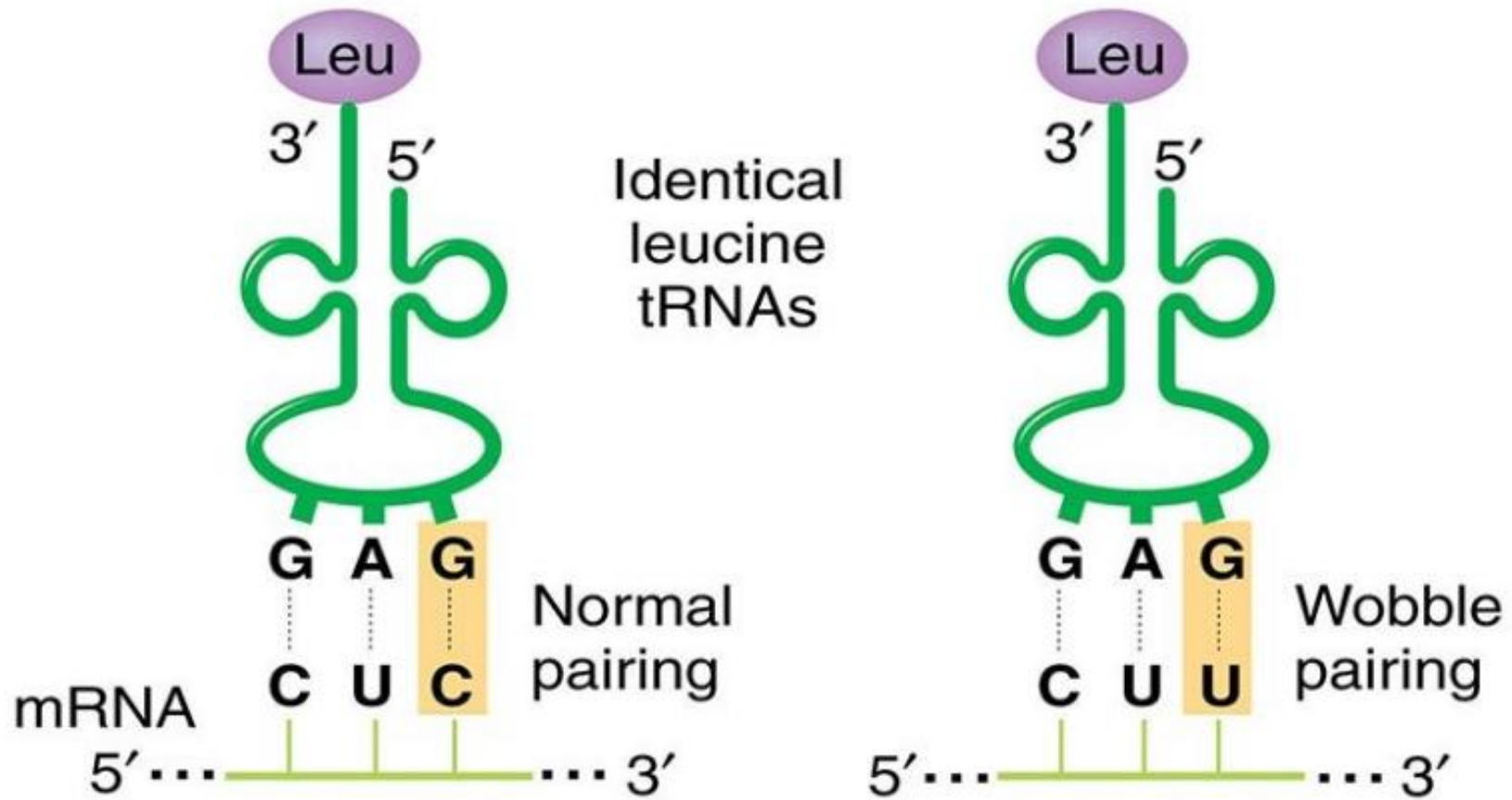
- Crick postulated the **wobble hypothesis**.
- He predicted that the initial two ribonucleotides of triplet codes are often more critical than the third member in attracting the correct tRNA.
- He hypothesized that hydrogen bonding at the third position of the codon-anticodon interaction would be less spatially constrained.
- In addition, it may also need not to adhere strictly to the base-pairing rules.



Wobble hypothesis

- 61 different tRNAs could theoretically exist, one for each codon that specifies an amino acid, however only 49 different genes encode tRNAs.
- This is because the same type of tRNA can detect synonymous codons that differ only in whether the wobble (third) position is U or C.
- The same type of tRNA, for example, binds to both UUU and UUC codons, which specify the amino acid phenylalanine.
- Synonymous codons ending in A or G use different tRNAs

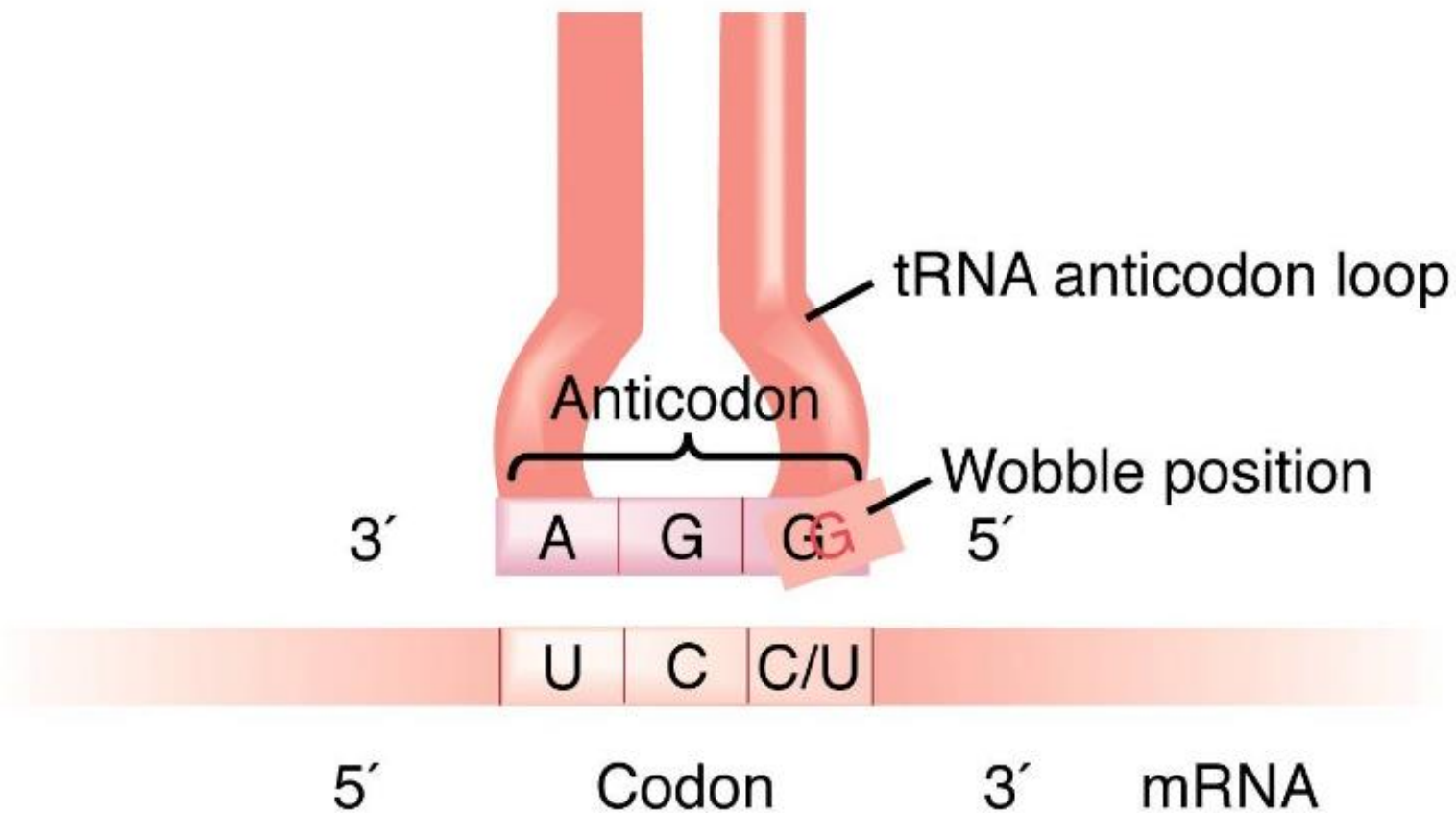




Crick's hypothesis hence predicts that the initial two ribonucleotides of triplet codes are often more critical than the third member in attracting the correct tRNA.



Wobble base pairs



**Thank You
For Watching
If you like the Video please press
like button
Share, Comment and Subscribe**

