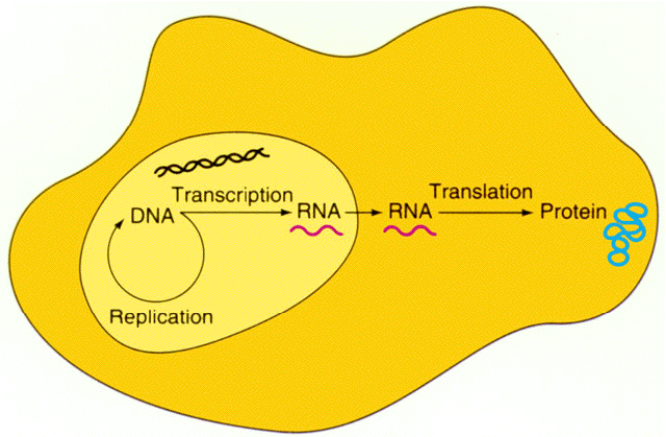
Information Transfer within a Cell and the Three Critical Processes that do this.



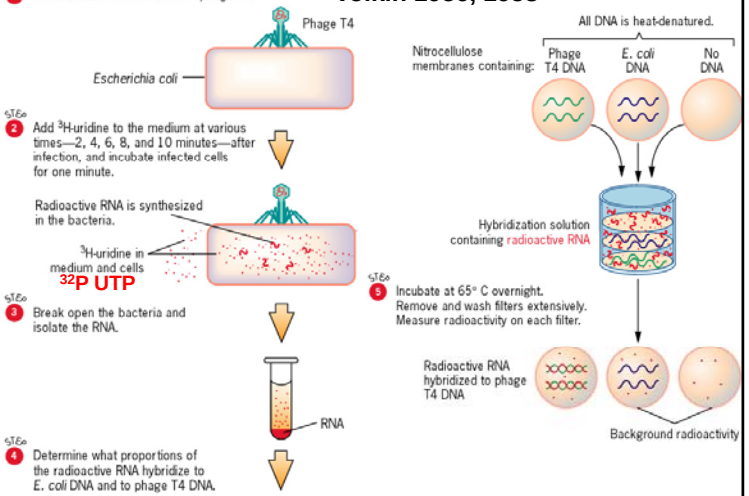
A. Crick postulated an intermediate that would have hydrogen bonding to the bases in the DNA and covalent bonding to amino acids (actually two intermediates necessary, mRNA and tRNA).

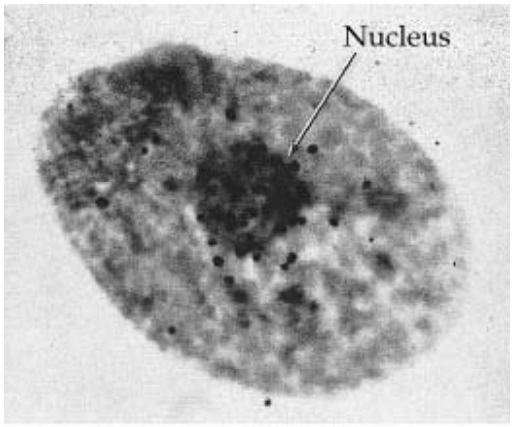
B. Volkin's Expt (base composition of RNA was similar to that of the DNA phage) shows intermediacy of RNA.

C. Pulse chase experiments and the movement of mRNA

D. Classes of RNA in prokaryotes; mRNA, tRNA, rRNA (23S, 16S, 5S in prokaryotes), snRNA, miRNA (RNAi).

Volkin 1956, 1958

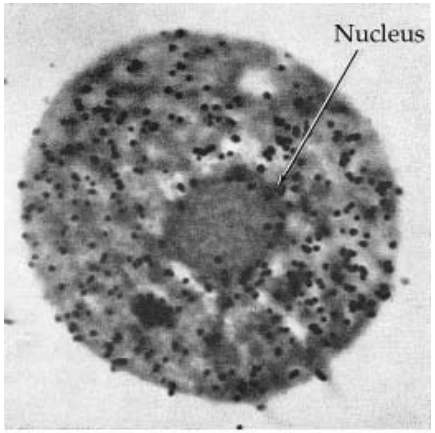




Credit: From D. Prescott, "Cellular Sites of RNA Synthesis, " Prog. Nucleic Acid Res. Mol. Biol. 3:33-57, 1964.

³H-Uridine in UTP

Fig 12.6a Autoradiographs demonstrating the synthesis of RNA in the nucleus and its subsequent transport to the cytoplasm.



Credit: From D. Prescott, "Cellular Sites of RNA Synthesis, " Prog. Nucleic Acid Res. Mol. Biol. 3:33-57, 1964.

Fig 12.6b Autoradiographs demonstrating the synthesis of RNA in the nucleus and its subsequent transport to the cytoplasm.

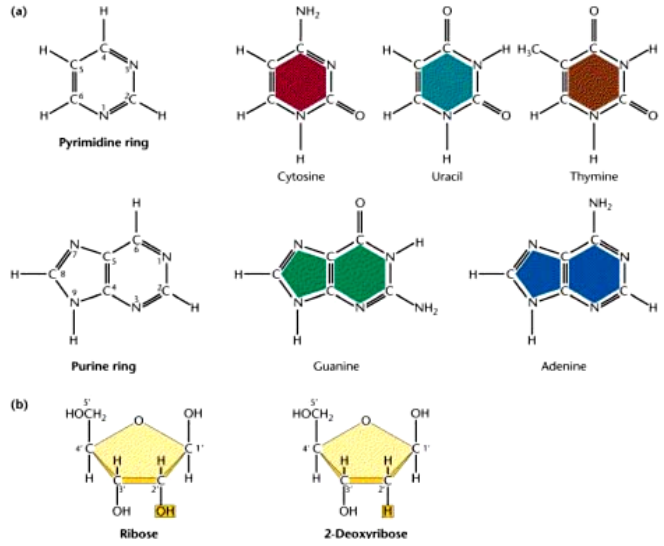


TABLE 10.4 RNA CHARACTERIZATION

RNA Class	% Total RNA*	Components (Svedberg Coefficient)	Eukaryotic (E) or Prokaryotic (P)	Number of Nucleotides
Ribosomal (rRNA)	80	5S	P and E	120
		5.8S	E	160
		16S	P	1542
		18S	E	1874
		23S	P	2904
		28S	E	4718
Transfer (tRNA)	15	4S	P and E	75–90
Messenger (mRNA)	5	varies	P and E	100–10,000

*In *E. coli*

Also

snRNAs (small nuclear RNAs)

miRNAs (microRNA & interfering RNA, RNAi)

Transcription

The Players:

RNA polymerase Synthesizes 5' to 3'

uses NTPs (ribose) not dNTP (deoxyribose)

uses UTP and not TTP

does not need primer, Why?

DNA template (Promoter)

How is the information of the genes converted into the production of different proteins?

RNA was shown to be an intermediate (Crick's prediction). What are the characteristics of this code?

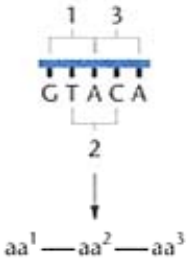
Since mRNA is made of 4 bases, how many bases would be needed to code for the 20 amino acids?

-- If each base coded for one amino acid, how many amino acids could be encoded in mRNA?
Answer: 4

-- If two bases coded for each amino acid?
Answer: Doublet code 4^2=16 words (still not enough)

1) Deductive reasoning also lead to speculation of triplet code: 4^3=64 words.

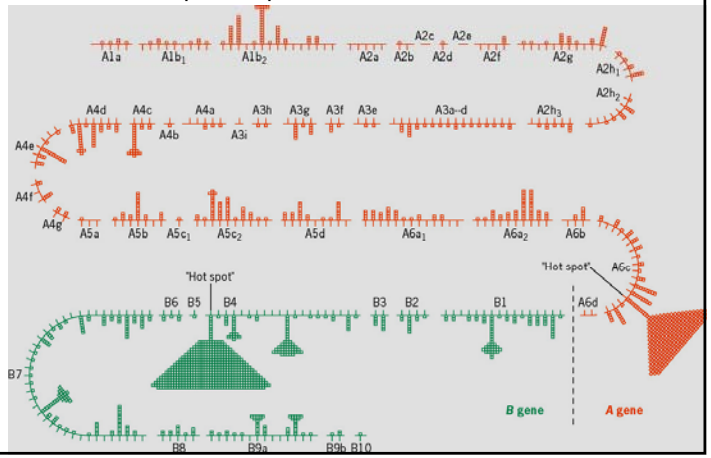
2) Single base pair changes generally changed one amino acid in a protein.
Conclusion: Code probably <u>not overlapping</u>.



A base substitution would often change 3 aa at once if code was overlapping.

What else can we deduce?

With **Benzer's** map, researchers could look to see what each mutation did to the protein product!!

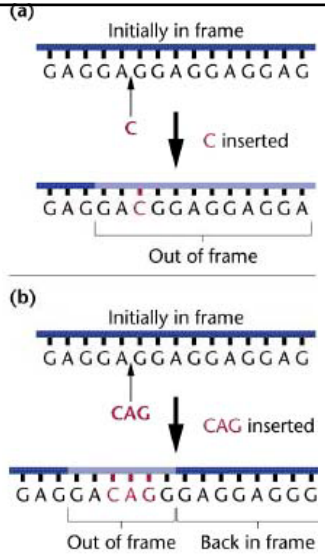


Benzer had used Proflavin as a mutagen that induces insertions and deletions

How do insertions and deletions affect the message?

1 or 2 bp insertions or deletions puts message out of frame.

Multiples of 3 bp changes keeps the message in frame.



What is happening?

	THE FAT CAT ATE THE BIG RAT
Delete C	THE FAT ATA TET HEB IGR AT
Insert A	THE FAT ATA ATE THE BIG RAT
	*Suppressor Mutation
Delete 3 bases	THE FAT ATE THE BIG RAT

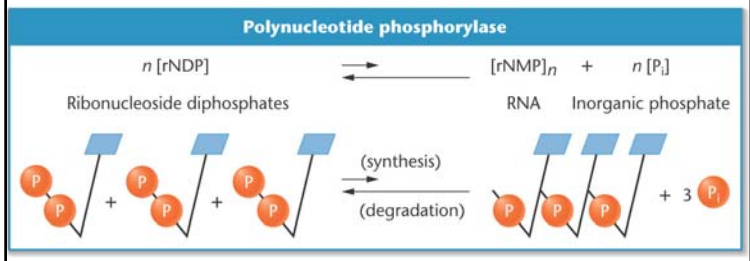
We "know"
1) triplets – deductive reasoning and experimental 3 bp deletions
2) non overlapping – 1 base substitution → 1 aa change

3) no punctuation – 1 base insertion/deletion affects all subsequent aa in the message (frameshift)

What are all the extra triplets for?
Degeneracy?

OK, now we know some of the characteristics of the code, but…What is the code?????

Race was on to decipher it. One method was to create synthetic mRNA and see what polypeptide it would make.



Cracking the Code

1) Nierenberg and Matthaei 1962: Homopolymers

Results:		
	UUU	phenylalanine
	CCC	proline
	AAA	lysine
	GGG	----------

2) Mixed/Random Copolymers

3) Repeating Copolymers. Gobind Khorana 1967 (Nobel Prize) he generated short nucleotides (di, tri tetra) and then linked them.

Mixture of 5 parts C: 1 part A

Possible compositions	Probability of occurrence of any triplet	Possible triplets	Final %
3A	$(1/6)^3 = 1/216 = 0.4\%$	AAA	0.4
1C:2A	$(5/6)(1/6)^2 = 5/216 = 2.3\%$	AAC ACA CAA	3 x 2.3 = 6.9
2C:1A	$(5/6)^2(1/6) = 25/216 = 11.6\%$	ACC CAC CCA	3 x 11.6 = 34.8
3C	$(5/6)^3 = 125/216 = 57.9\%$	CCC	57.9
			100.0

Chemical synthesis of message

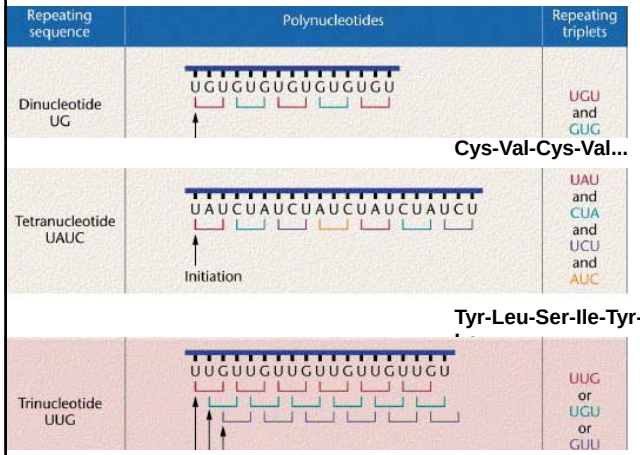
RNA

CCCCCCCCCACCCCCCAACCACCCCCACCCCCACCCAA

Translation of message

Percentage of amino acids in protein		Probable base-composition assignments
Lysine	<1	AAA
Glutamine	2	1C:2A
Asparagine	2	1C:2A
Threonine	12	2C:1A
Histidine	14	2C:1A, 1C:2A
Proline	69	CCC, 2C:1A

Repeating Copolymers



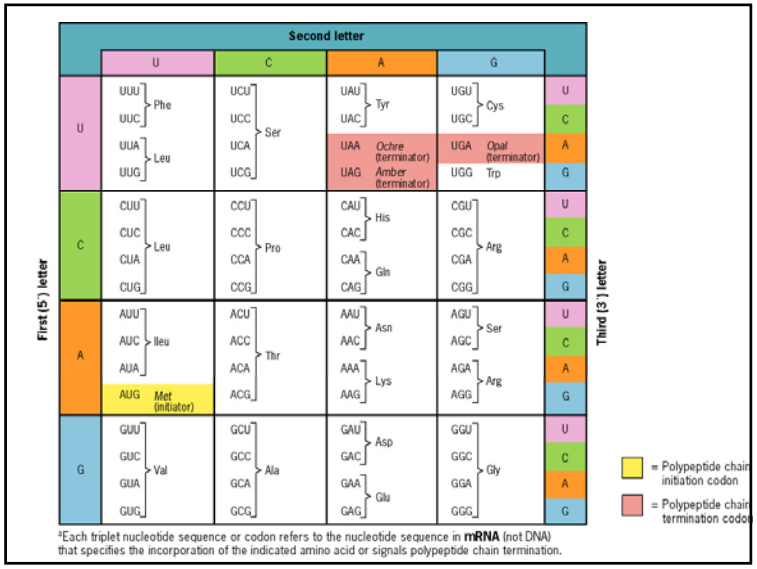
Repeating Copolymers (continued)

$(GAUA)_n$
GAUAGAUAGAUAGAUAGAUAGAUA…..

What polypeptide was translated from this message?

ZIPPO, NADA, NONE!!!

CONCLUSION: One of the triplets codes for a STOP



Differences Between Human and Yeast Mitochondrial Genetic Codes

	Amino Acid		
		Mitochondrial Code	
Codon[a]	Nuclear Code	Mammal	Yeast
UGA	Termination	Tryptophan	Tryptophan
AUA	Isoleucine	Methionine	Isoleucine
CUN[b]	Leucine	Leucine	Threonine
AGG, AGA	Arginine	Termination	Arginine
CGN[b]	Arginine	Arginine	Termination?

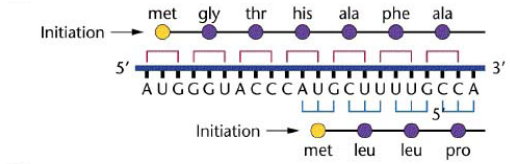
[a]All sequences read 5′ to 3′.
[b]N = any one of the four bases A, G, U, and C.

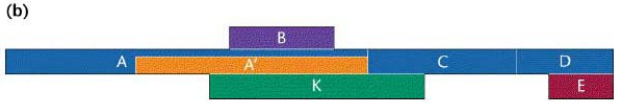
Similar to Table 14.5

Once the Code was cracked, small genome organisms were examined to count the genes and identify the spaces between genes.

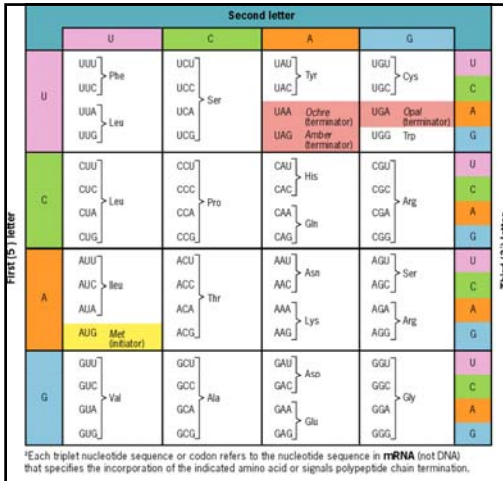
ϕX174 5386 nucleotides 1795 aa?

Adding up the length of its proteins 2300+ aa





Genes can be overlapping!! (The code is NOT overlapping).



Repeating Copolymer

5’- GUGUGUGUG...

A) his-his-his…

B) thr-his-thr-his…

C) cys-cys-cys…

D) cys-val-cys-val…

E) val-val-val…