Transcription & Translation

Transcription
- Overview-mRNA structure
- Process
- RNA Polymerase
- Fidelity

Translation
- Genetic Code
  - triplet
  - decyphering
- tRNA
  - Structure
  - Anticodon
  - Acylation (charging)
    - Aminoacyl-tRNA Synthetases
    - Mechanism
    - Fidelity
- Protein Biosynthesis
  - Overview
  - Process
  - Ribosome review
  - Peptidyl Transferase
  - Fidelity

Lecture 25 (11/16/20)

TODAY

- Reading: Ch25; 990-995, 1005-1012
  Ch26; 1035-1038
  Ch27; 1077-1085, 1092-1096

- Problems: Ch25 (text); 1-3,5-7,10,13-16,12
  Ch25 (study-guide: applying); 1,4
  Ch25 (study-guide: facts); 3,4,6
  Ch26 (text); 1,2,5,6,12
  Ch26 (study-guide: applying); 1
  Ch26 (study-guide: facts); 1,3,5
  Ch27 (text); 6,7,9
  Ch27 (study-guide: applying); 1,3,5

NEXT

- Reading: Ch27; 1088-1091, 1096-1108

- Problems: Ch27 (text); 5,8,10,11,13,16,17
  Ch27 (study-guide: applying); 2,3
  Ch27 (study-guide: facts); 4,6

Nucleic Acids

A. The 4 S's of Nucleotides & NA
B. Structure of the Information
C. Recombinant DNA: Biochemical Basis of Biotechnology
   1. Restriction enzymes, DNA ligase
   2. Vectors and Inserts to make recombinant DNA (rDNA)
   3. Transformation of hosts
   4. Selection of transformants
   5. Expression
   6. Site-directed mutagenesis

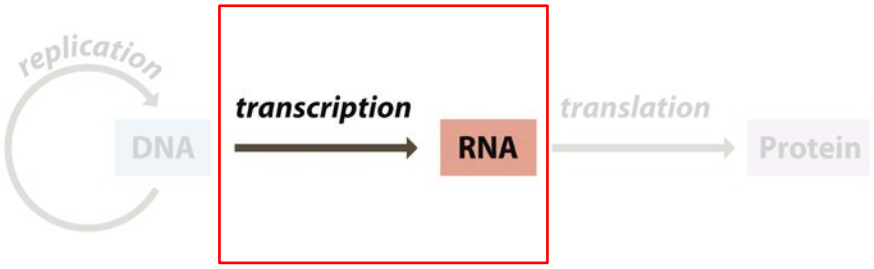
D. Replication
   1. Polymerases
   2. Fidelity
      a. Polymerase recognition
      b. Exonuclease
      c. Mis-match repair
      d. Post-replication repair
         i. Direct reversal
         ii. Base excision
         iii. Nucleotide excision
   3. Sequence determination
   4. PCR

E. Transcription
   1. RNA polymerase
   2. fidelity

F. Translation
   1. Genetic code
   2. tRNA

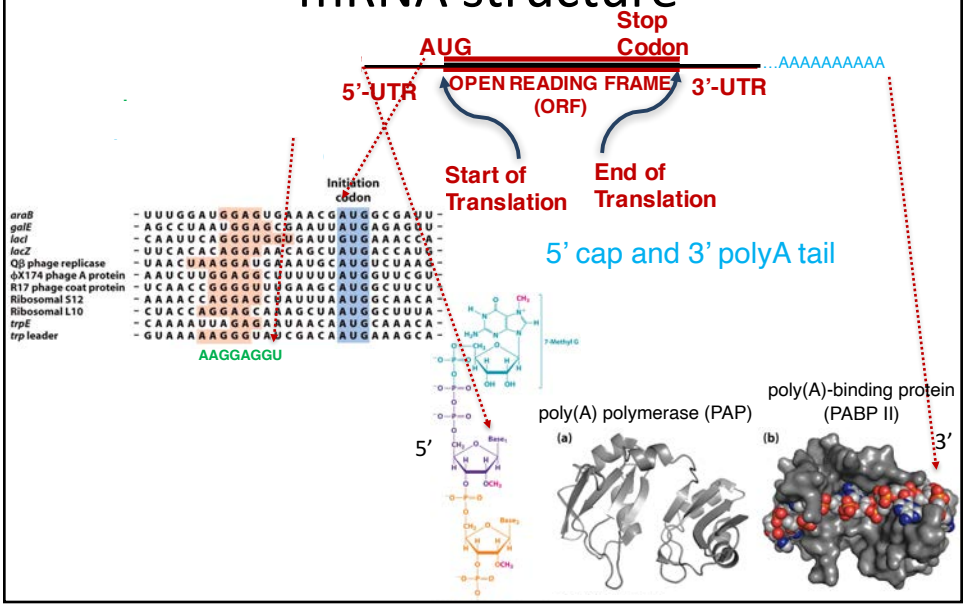
Transcription

Nucleic acid function: Central Dogma



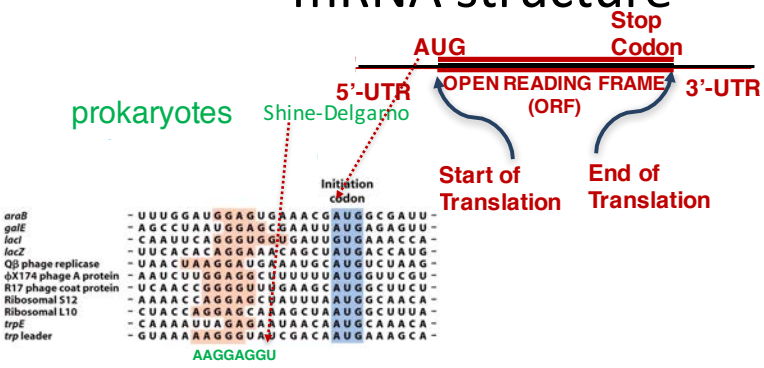
Transcription

mRNA structure



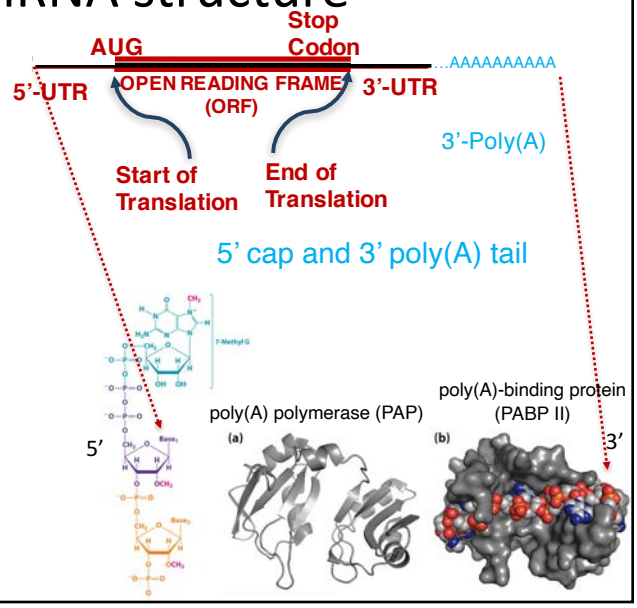
Transcription
mRNA structure

prokaryotes



Transcription
mRNA structure

eukaryotes 5'-cap



Transcription

Process:
- **Initiation**
- **Elongation**
- **Termination**

RNA Polymerases need 3 things:
- NTPs
- Template
- Place to start (NO primer needed)

$$(RNA)_{n\ residues} + NTP \rightleftharpoons (RNA)_{n+1\ residues} + PP_i$$

H_2O

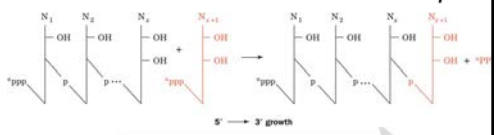
$2\ P_i$

(5′) CGCTATAGCGTTT (3′) DNA nontemplate (coding) strand

(3′) GCGATATCGCAAA (5′) DNA template strand

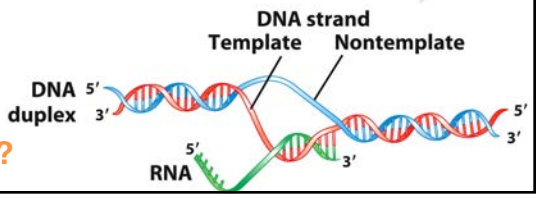
What is the sequence of the newly synthesized RNA strand?

5′-CGCUAUAGCGUUU-3′



5′ → 3′ growth

Direction of transcription

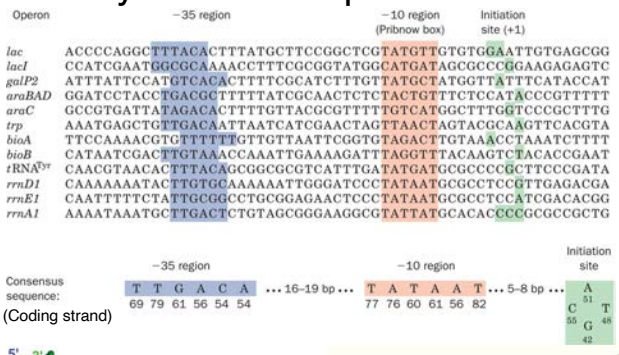


What about a place to start?

Transcription

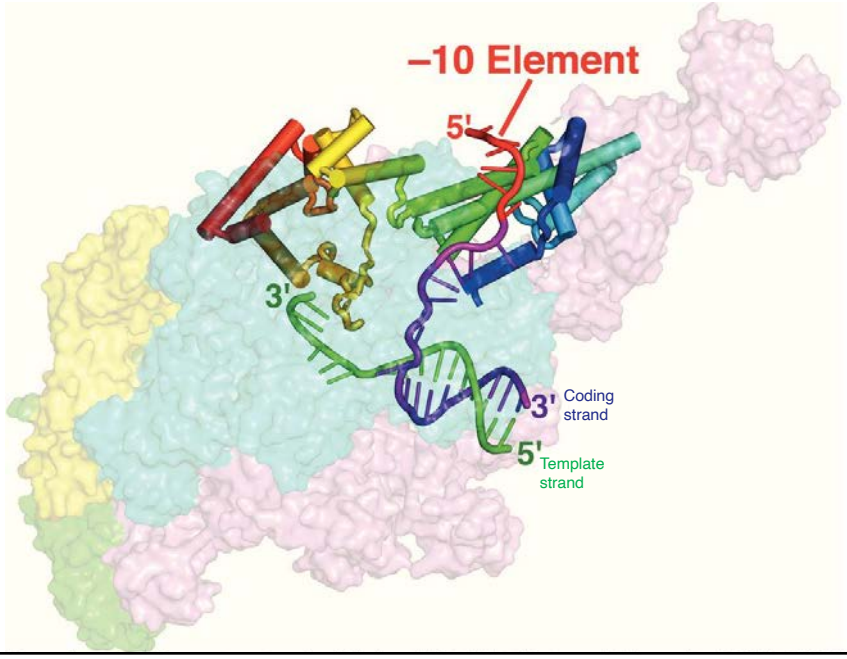
Promoters

Prokaryotic Transcription – Initiation (a place to start)



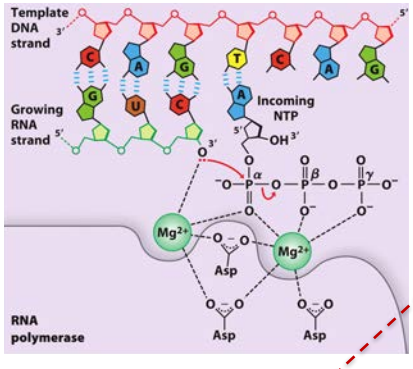
- RNA pol requires signal to begin transcription
  - ***why?***
- σ factor recognizes **promoter** region of a gene/operon (–10 & –35 regions)
  - σ factor-RNA polmerase complex work together to transcribe DNA at specific start sites.
  - Once σ factor interacts with –10 element, the complex unwinds DNA ~2 turns (open complex).
- Called the "Holoenzyme"

RNAP Complexed With Promoter

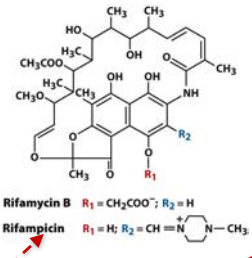


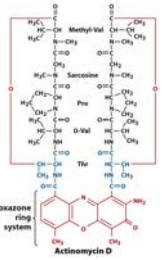
Transcription

Mechanism



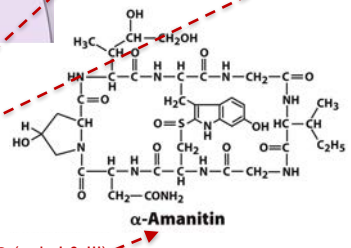
Inhibitors of Transcription



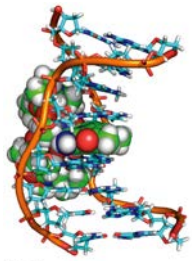


Inhibits elongation at first phosphodiester bond

Inhibits elongation by intercalating



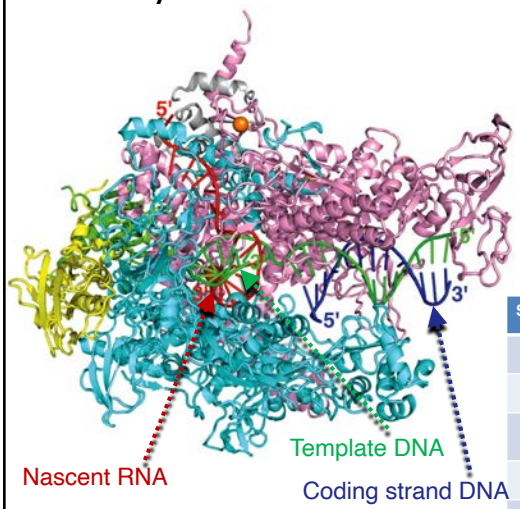
Inhibits EUKARYOTIC RNAPs (only I & III)



Actinomycin D-DNA complex
PDBid 1DSC

Transcription

RNA Polymerase Structure



RNA pol is a multi-subunit enzyme
$(\alpha_2\beta\beta'\omega\sigma)$

These subunits make up the **core complex:**

- two **α** subunits make non-specific contacts with DNA for positioning
- the **β** and **β'** subunits catalyze the addition of ribonucleotides to the growing chain
- the **ω** subunit acts to stabilize the complex

Subunit	Gene	MW	#	Role
α_2	*rpoA*	34	2	Non-specific DNA binding
β	*rpoB*	150	1	Polymerase
β'	*rpoC*	155	1	Non-specific DNA binding & polymerase
ω	*rpoZ*	10	1	Zn^{2+} binding
σ	*rpoD*	70	1	Promoter recognition

RNA•DNA duplex is in the A-form
It uses the same mechanism for correct W-C bp and fidelity
Therefore, error rate is the same 1/10,000

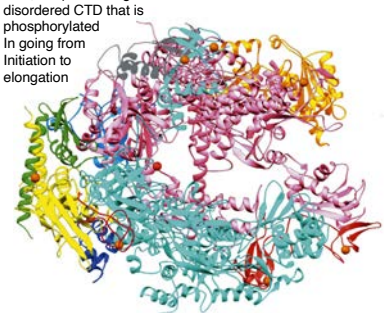
Rate is ~ 50 base/sec
Processivity is ~2000 bp

No proofreading

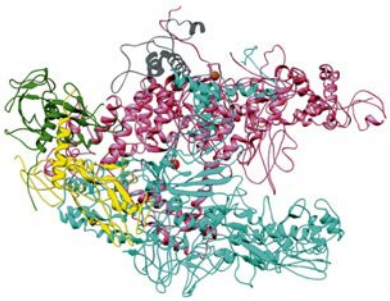
Transcription

Eukaryotic RNA Polymerase II Conformations

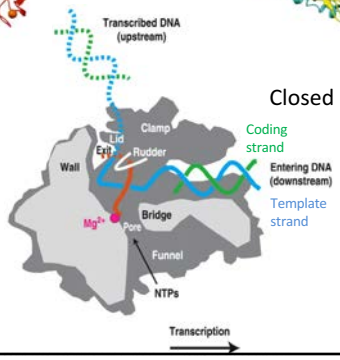
Also, the β' homolog has a disordered CTD that is phosphorylated In going from Initiation to elongation



Open conformation



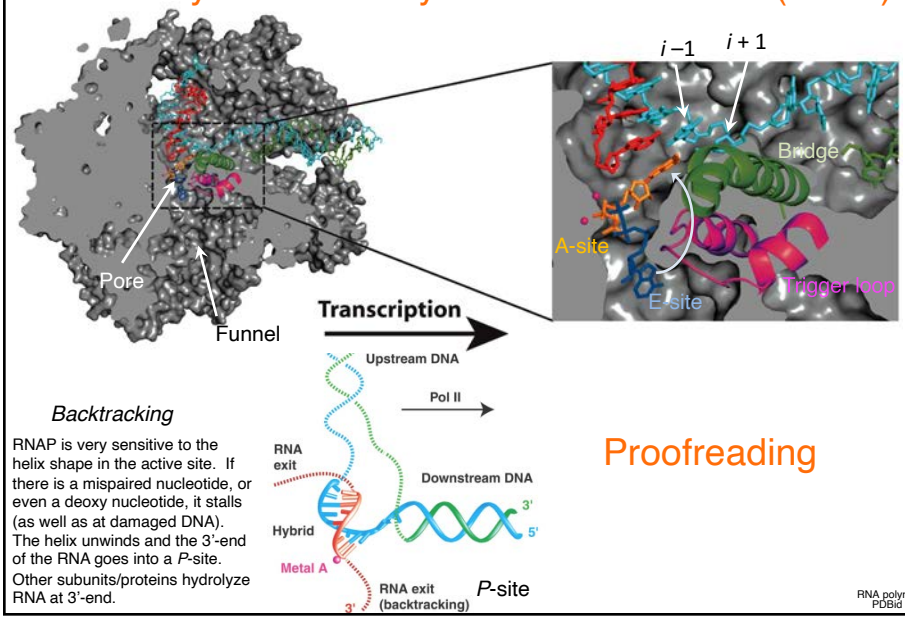
Closed conformation



Yeast RNA polymerase
PDBid 1I50

Transcription

Eukaryotic RNA Polymerase II NTP sites (A & E)



Backtracking

RNAP is very sensitive to the helix shape in the active site. If there is a mispaired nucleotide, or even a deoxy nucleotide, it stalls (as well as at damaged DNA). The helix unwinds and the 3'-end of the RNA goes into a *P*-site. Other subunits/proteins hydrolyze RNA at 3'-end.

Proofreading

RNA polymerase II PDBid 2E2H

Transcription & Translation

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Transcription & Translation

Nucleic acid function: Central Dogma

TABLE 26-1 **Some Noncoding RNAs**

Type	Size (nt)	Function
Ribosomal RNA (rRNA)	120–4718	Translation (ribosome structure and catalytic activity)
Transfer RNA (tRNA)	54–100	Delivery of amino acids to ribosomes during translation
Small interfering RNA (siRNA)	20–25	Sequence-specific inactivation of mRNA
Micro RNA (miRNA)	20–25	Sequence-specific inactivation of mRNA
Large intergenic noncoding RNA (lincRNA)	Up to 17,200	Transcriptional control
Small nuclear RNA (snRNA)	60–300	RNA splicing
Small nucleolar RNA (snoRNA)	70–100	Sequence-specific methylation of rRNA

Translation: The Genetic Code

Recall: Genetic Code is Degenerate & Nonrandom

Gold = hydrophobic amino acids; pyrimidine at second position

Polar amino acids (blue = basic; red = acidic; purple = uncharged polar) have purine at second position

How was the Triplet code discovered?

TABLE 27-1 **The "Standard" Genetic Code***

First position (5′ end)	Second position U	Second position C	Second position A	Second position G	Third position (3′ end)
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA STOP	UGA STOP	A
	UUG Leu	UCG Ser	UAG STOP	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met†	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

*Nonpolar amino acid residues are gold, basic residues are blue, acidic residues are red, and polar uncharged residues are purple.
†AUG forms part of the initiation signal as well as coding for internal Met residues.

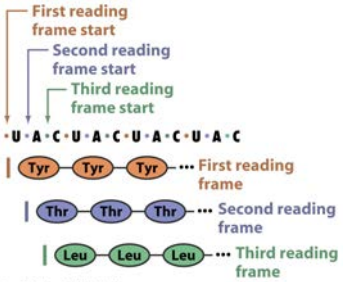
Translation: The Genetic Code

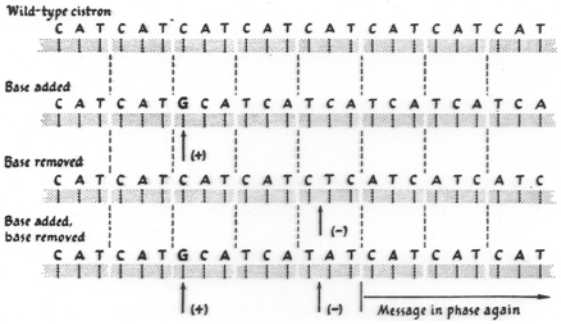
ORFs

THE XIG RED FOX ATE THE EGG

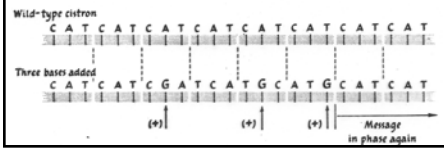
If one base is deleted: THE IGR EDF OXA TET HEE GG

If one base is then inserted: THE IGR EDX FOX ATE THE EGG





Brenner & Crick Experiment:



The regain of function for the triple mutant told Brenner and Crick that it was a triplet code, uninterrupted.

How was the code deciphered?

Translation: The Genetic Code

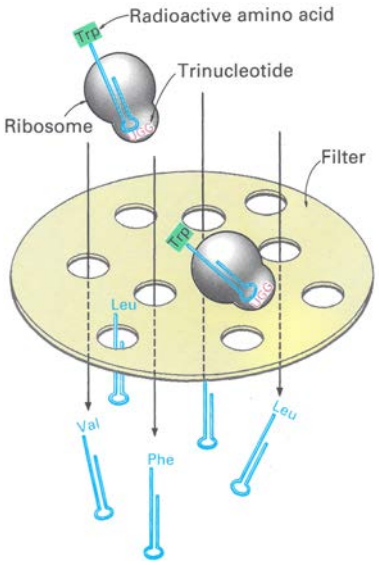
Key Developments:

1. **Chemical synthesis of nucleic acids**
2. **<u>in vitro</u> protein synthesis**

Nirenberg (NIH)

1. First codons used Polynucleotide Phosphorylase (NDP ⇌ RNA + P_i) to make RNA in vitro: poly-A, poly-C, etc.

Result:
UUU=Phe, AAA=Lys,
CCC=Pro, GGG=Gly



2. Chemical synthesis of defined triplets.
   - Use ribosomes and charged tRNA with different radioactive amino acids
   - Mix and filtrate - only those amino acids with correct tRNA to complementary "mRNA" will complex with the ribosome

Result: 50/64 determined

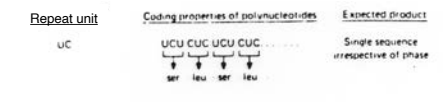
Translation: The Genetic Code

Key Developments:
1. **Chemical synthesis of nucleic acids**
2. **<u>in vitro</u> protein synthesis**

<u>Khorana (MIT)</u>

- Chemical synthesis of repetitive RNAs by first making small overlapping complementary DNAs, ligating, and using RNA polymerase to make corresponding repetitive RNAs.
- Add synthetic RNAs to in vitro protein synthesis cocktail with radioactive amino acids.
- Analyze sequences of the radioactive protein produced.

Result: nearly all codons determined, but some remained ambiguous.
Combined data from Nirenberg established the CODE.
This method was only one able to determine the stop codons.



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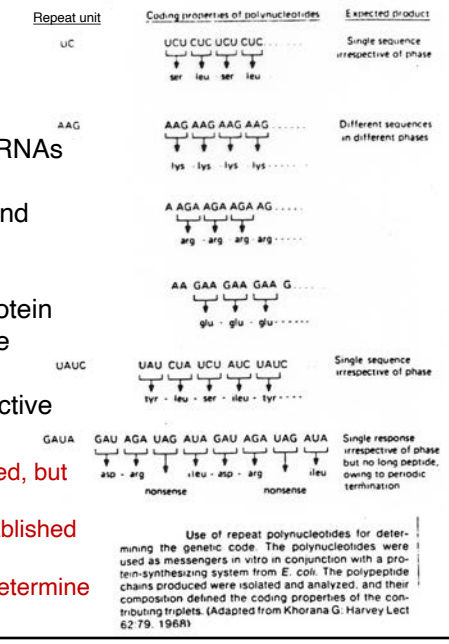

Use of repeat polynucleotides for determining the genetic code. The polynucleotides were used as messengers in vitro in conjunction with a protein-synthesizing system from *E. coli*. The polypeptide chains produced were isolated and analyzed, and their composition defined the coding properties of the contributing triplets. (Adapted from Khorana G: Harvey Lect 62:79, 1968)

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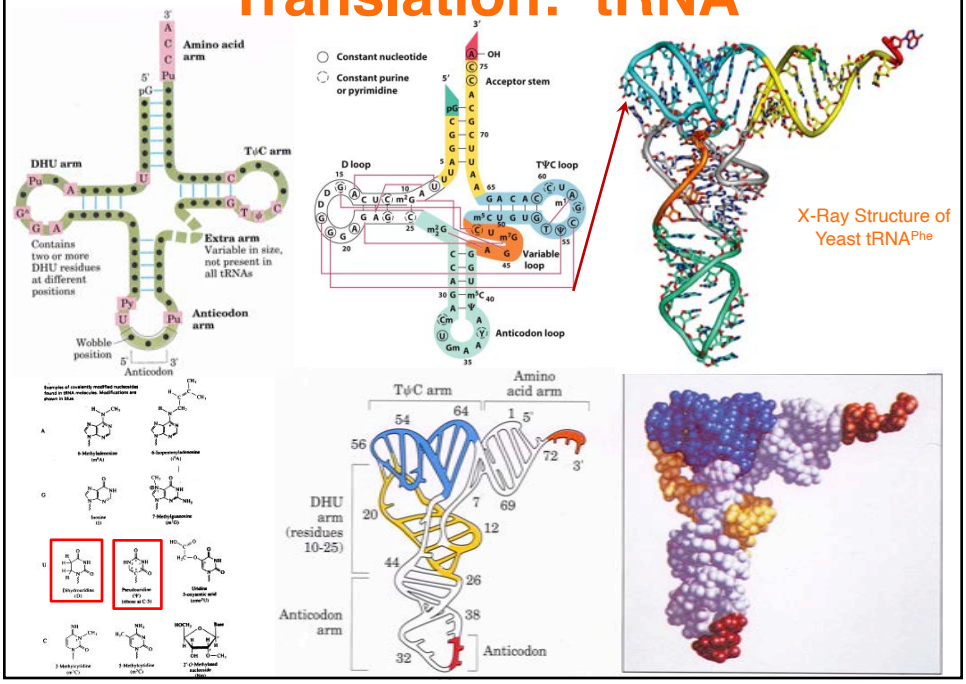
Translation: tRNA



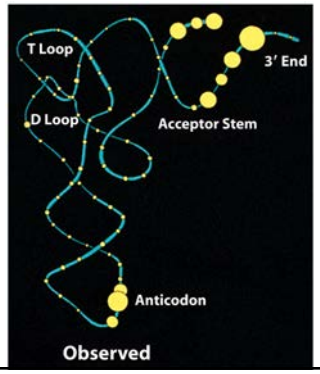
Translation: tRNA

Acylation = Charging of tRNA

THE KEY ENZYMES FOR THIS ARE THE Aminoacyl-tRNA Synthetases

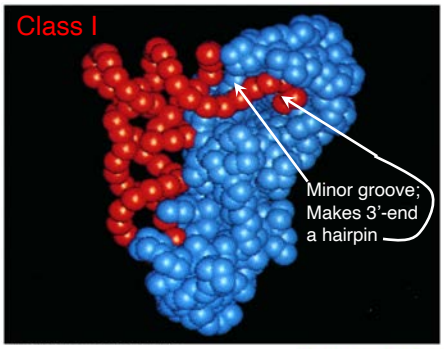
How do they recognize the correct tRNA?

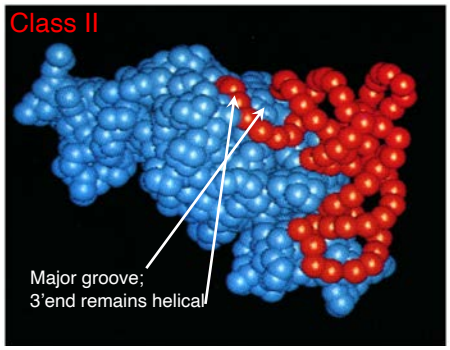
E. coli GlnRS-tRNA^{Gln}-ATP
PDBids 1GTR

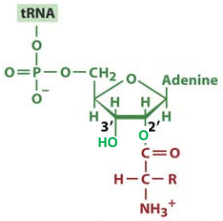


Translation: tRNA

Class I and II Aminoacyl-tRNA synthetases

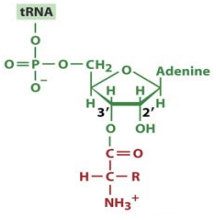






Classes of Aminoacyl–tRNA Synthetases

	Amino Acids	
Class I	Arg	Leu
	Cys	Met
	Gln	Trp
	Glu	Tyr
	Ile	Val
Class II	Ala	Lys
	Asn	Pro
	Asp	Phe
	Gly	Ser
	His	Thr



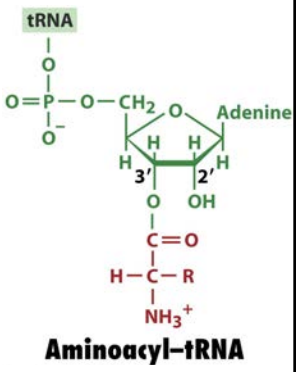
Translation: tRNA

Aminoacylation: 2 steps

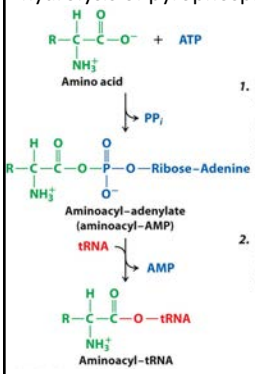
2 step reaction:

1) A.A. + ATP → AA-AMP + PP_i
2) AA-AMP + tRNA → AA-tRNA + AMP

(AA-tRNA is shown at right = "charged" tRNA)



Again, just like with DNA and RNA synthesis, subsequent hydrolysis of pyrophosphate provides driving force for reaction



1. *The amino acid reacts with ATP to form an aminoacyl–adenylate (aminoacyl–AMP). The subsequent hydrolysis of the PP_i product makes this step irreversible in vivo.*

2. *The amino acid, which has been "activated" by its adenylation, reacts with tRNA to form an aminoacyl–tRNA and AMP.*

First ½ reaction is sequential random bi uni