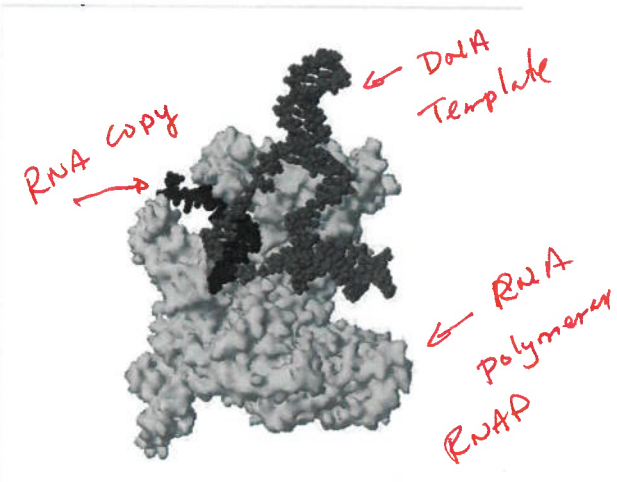
Lecture 35 : Review of Central Dogma :

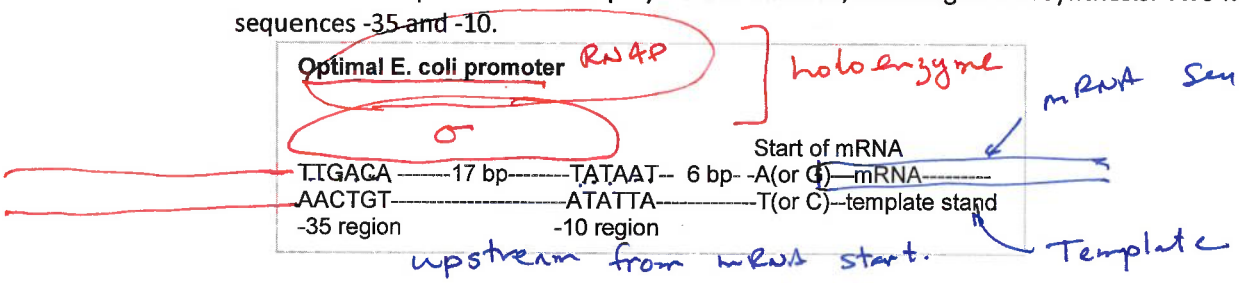
I. mRNA Synthesis (DNA Transcription):



RNA polymerase:

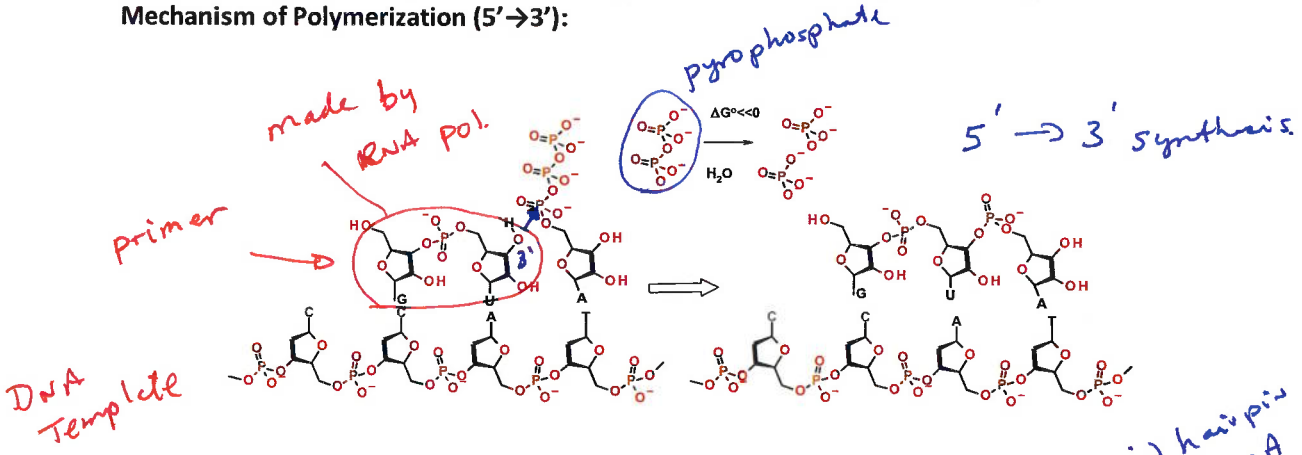
- Holoenzyme: $\sigma + \alpha_2\beta\beta'$
- Core polymerase = $\alpha_2\beta\beta'$ – sufficient for elongation.
- Binds to promoter (P) sequence in a base specific manner **via σ subunit.**
- Uses DNA as a template, making a copy of the template (lower) strand.
- Generates an RNA copy of the DNA template.
- NTPs are polymerized in the 5'→3' direction.
- Does not require an external primer (makes its own!).
- No error checking, mRNA is 'disposable'.

Promoter: DNA sequence that RNA polymerase binds to, initiating mRNA synthesis. Two key sequences -35 and -10.



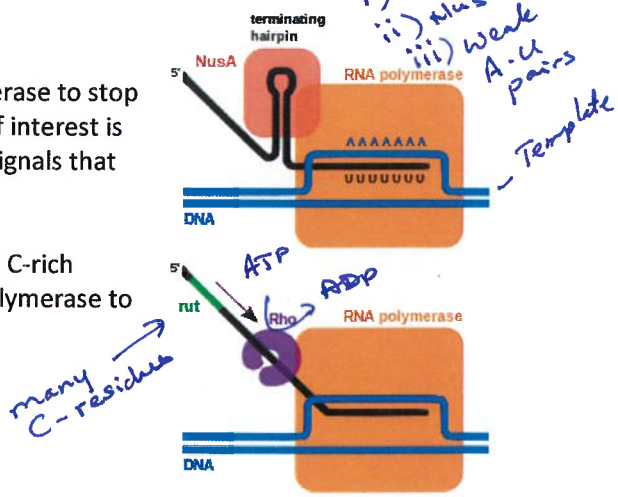
The rate of transcription depends on the sequence of the -35 and -10 regions. Strong promoters lead to frequent mRNA production, weak promotors cause infrequent mRNA production.

Mechanism of Polymerization (5'→3'):

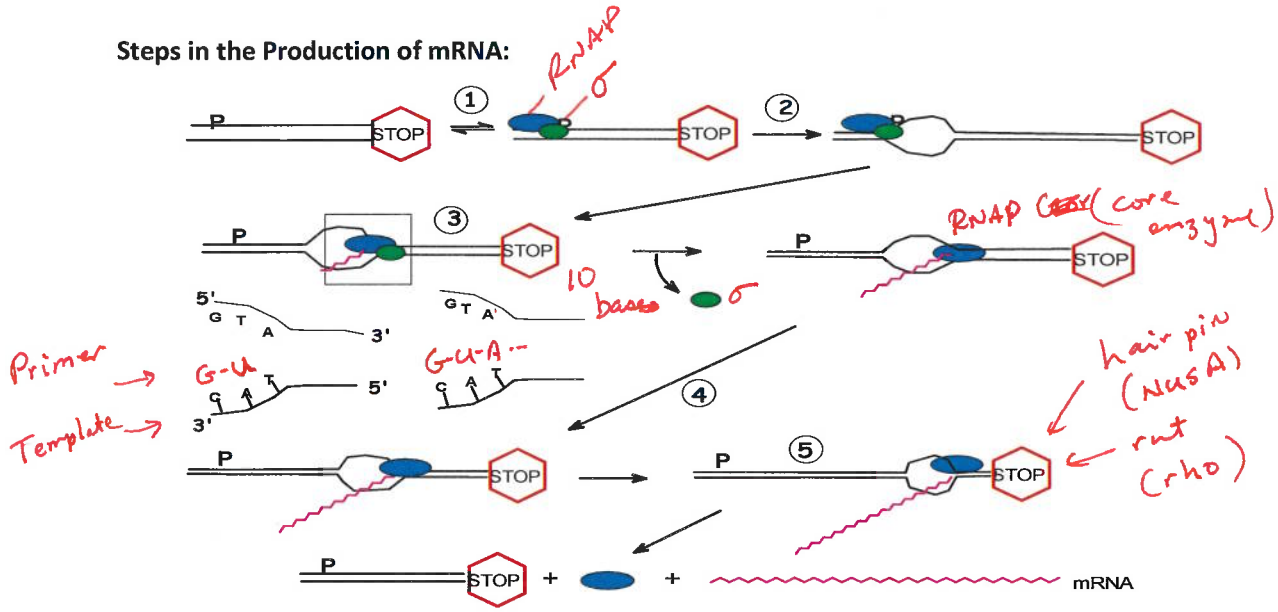


* **Transcriptional termination signal**: Causes RNA polymerase to stop and leave the DNA template so that only the gene of interest is transcribed into mRNA. There are two termination signals that are used in E.coli:

1. RNA Hairpin formation at 3' end of mRNA:
2. Rho dependent termination, rho protein binds to C-rich regions and moves to the 3' end, causing RNA polymerase to dissociate.



Steps in the Production of mRNA:



1. **Template binding**: Holoenzyme (R) binds to promoter site (P) on DNA, initially reversibly.
2. **"Open complex" formation**: A irreversible, committed step, DNA is melted (from bases -9 to +2).
3. **Primer generation & initiation:** A dinucleotide is generated by the polymerase – which then acts as a primer. When the RNA chain is about 10 nucleotides long, σ-subunit dissociates, leaving the core enzyme to elongate the RNA.
4. **Chain elongation:** RNA chain growth is from 5' to 3', and elongation is rapid: about 50 nucleotides/sec, **processively**.
5. **Chain termination:** Termination occurs at specific DNA sequences, causing release of mRNA.

II. Protein Synthesis :

A. RNA molecules involved in protein synthesis:

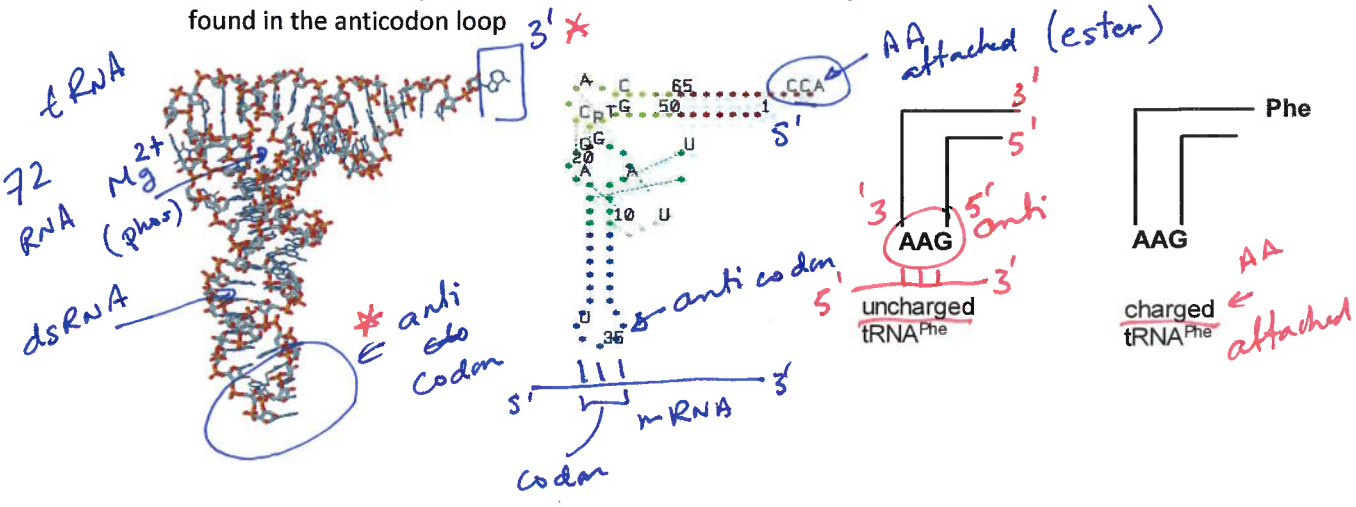
mRNA – messenger RNA is copy of the DNA that encodes a gene. This was made by RNA polymerase. The mRNA specifies the order of amino acids to be used in making the protein.

tRNA – transfer RNA is the dictionary the converts the codon to a specific amino acid. One part of the tRNA recognizes the codon, the other part contains the amino acid to add.

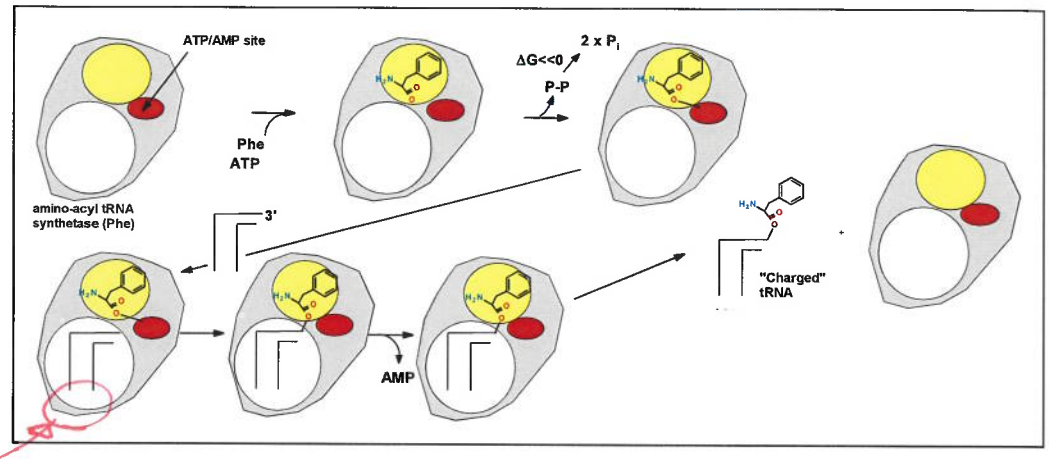
rRNA – ribosomal RNA is found in the ribosome and is responsible for most of the function in protein synthesis.

tRNA Structure and Function: There are generally 25-45 different tRNAs/organism. The structure of this single chain RNA molecule is stabilized by W-C H-bonds, non-W-C H-bonds, and phosphate-metal (Mg^{2+}) interactions.

- **Acceptor stem:** amino acids are attached to the 3' terminus of the tRNA by enzymes called **aminoacyl-tRNA Synthetases (aaRS).** These enzymes attach the *correct* amino acid to the *correct* tRNA. There are ~25-30 of these enzymes, essentially one for each amino acid. This process is often referred to as "charging" the tRNA.
- **Anti-codon arm**: contains the anticodon triplet that translates the codon in mRNA to an amino acid. Watson-Crick H-bonds + other basepairing are used to recognize codons.
- Modified bases: many tRNAs contain modified bases, in particular the base inosine is often found in the anticodon loop



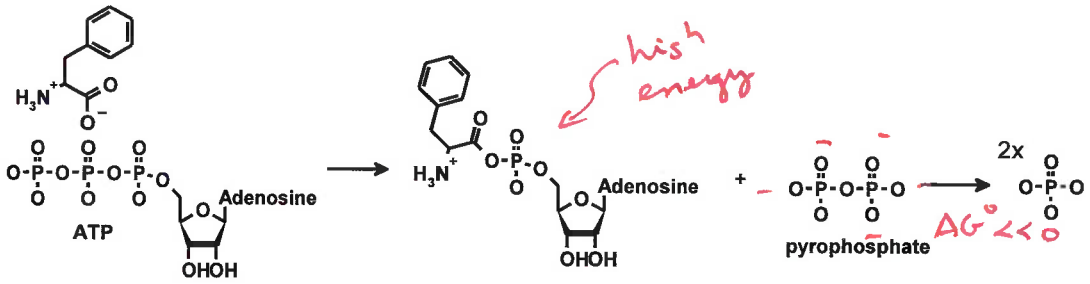
B. "Charging" of tRNA: amino acyl tRNA synthetases (aa tRNA syn) – a two step process.
[AA_X + ATP → AA_X-AMP + 2 P_i] + $tRNA^X$ → $tRNA^X$-AA_X + (AMP +2P_i)



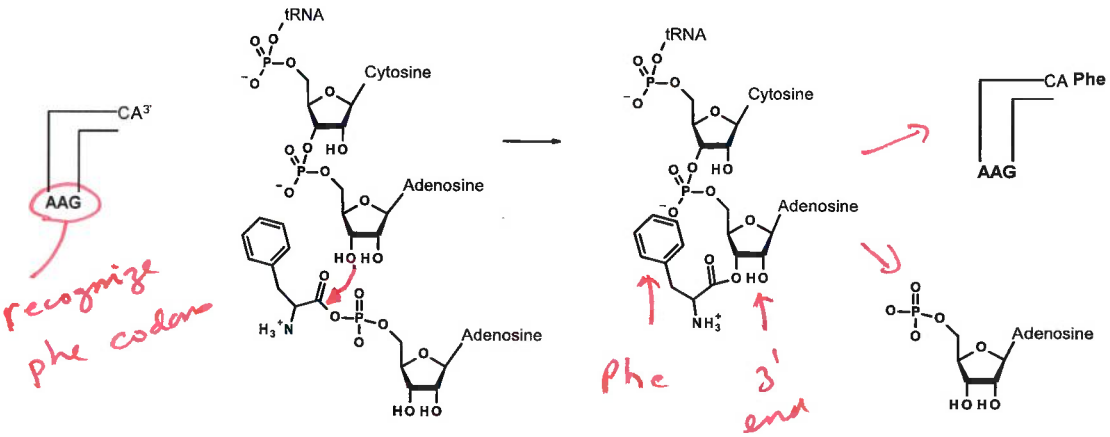
recognize phe anti codon

There are separate aminoacyl tRNA synthases for each amino acid/tRNA combination. They add the correct amino acid to the correct tRNA. The aminoacyl tRNA synthase shown below will only bind the tRNA for phe, with the anti-codon 3'-AAG-5', and will only add Phe to the bound tRNA. No other tRNA will be bound and no other amino acid will be added to that tRNA.

Step 1. Activation of the amino acid (2 ATP equivalents required)



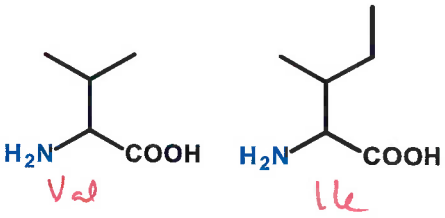
Step 2. Transfer of activated AA to tRNA

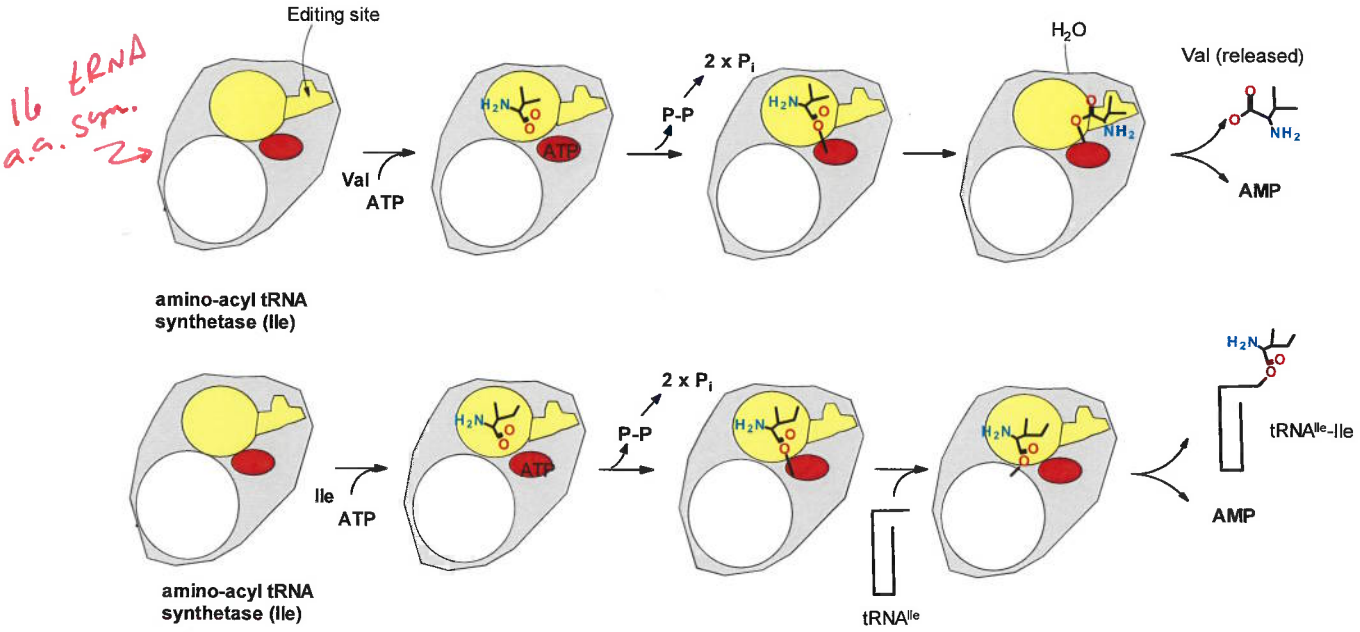


Editing Charged tRNAs:

Many aminoacyl tRNA synthases have editing capacity, e.g. aa-tRNA^{Ile}, will remove valine that has been incorrectly activated to Val-AMP, preventing the attachment of valine to tRNA^{Ile}.

Only the valine sidechain can fit in the editing site, isoleucine cannot, so the charged tRNA^{Leu}-Leu is released.



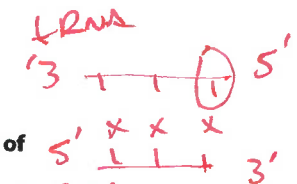


Reflection: What other pairs of amino acids might also require an editing step for tRNA charging?

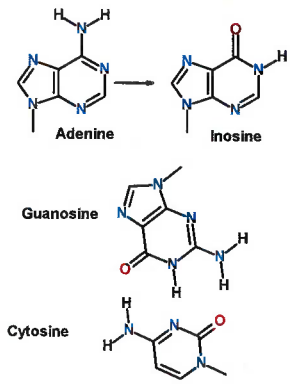
Ser & Thr.

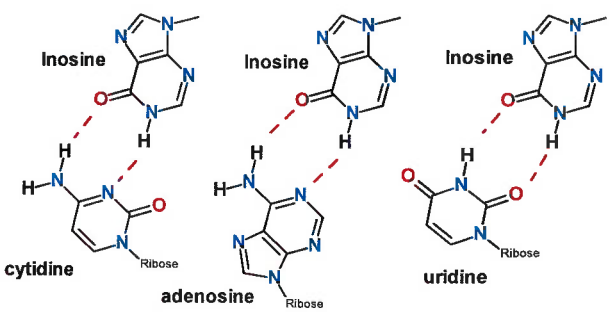
C. Codon-Anticodon Interactions + Wobble pairing:

1. There are usually ~30 tRNAs in an organism, yet all 61 codons can be translated. Therefore, one tRNA must be able to read more than one codon.
2. Degeneracy at the third position of codon-anticodon pairing allows multiple codons that can be interpreted by a single tRNA.
3. Codon-anticodon pairing can involve the base inosine in the anti-codon loop. Inosine can pair with C, U, or A. Inosine is produced by deamination of adenine:



If these bases are in first, or wobble, position of anticodon					
C	A	G	U	I	then the tRNA may recognize codons in mRNA having these bases in third position
G	U	C U	A G	C A U	





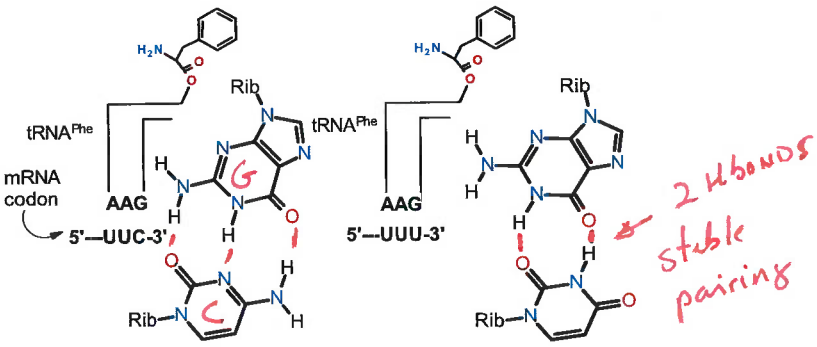
Example: Translating Phe Codons.

5' Base	Middle Base				3'
	U	C	A	G	
U (=T)	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C

i) The codon-anticodon pairing is **anti-parallel**, as are most pairings of nucleic acid strands:a)

ii) The anticodon on the tRNA is 5'-GAA-3'.

iii) The complementary codons in the mRNA are 5'-UUC-3' and 5'-UUU-3'.



C-G 'Watson-Crick' Base Pair U-G Wobble Base Pair

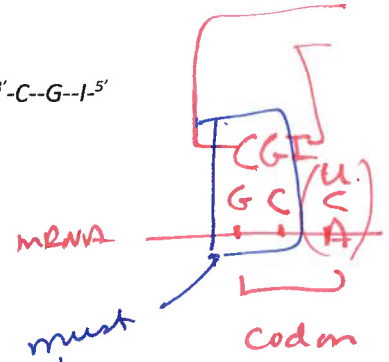
iv) The anticodon GAA can pair with either codon due to degeneracy at the third position (wobble basepair).

***Example**: Which codon(s) will this tRNA anti-codon sequence translate:* $^{3'}$-C--G--I-$^{5'}$

i) What are the possible codons that could be recognized by this tRNA?

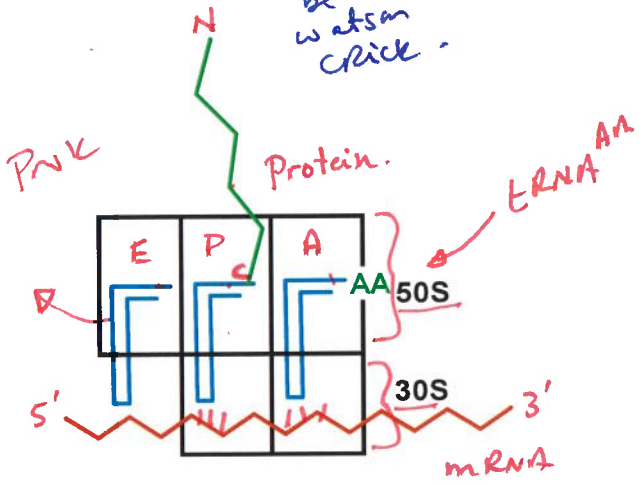
ii) What amino acid do these codons code for?

	T	C	A	G	
G	Val	Ala	Asp	Gly	T
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G



D. Ribosome: The ribosomal subunits and their RNA components are named for their sedimentation coefficients, S, which is a measure of how rapidly they move when a centrifugal force is applied. 50s+30s=70s

- 30S – mRNA tRNA interactions.
- 50S – peptide bond formation.
- Exit tunnel – where growing protein chain exits the ribosome, amino term first.



tRNA binding sites:

A site binds the **aminoacyl-tRNA** (charged tRNA) containing the next amino acid to be added.

P site holds the **peptidyl-tRNA** at the start of each cycle - the growing chain. The peptide shifts to the A site, temporarily, during each addition of an amino acid.

E site is the **exit** site for the uncharged tRNA, after peptide bond formation.

tRNA path through the ribosome: A (charged) → P (holds protein) → E (no amino acid)

E. Features of the mRNA (Example - Synthesis of Met-Lys-Ala-Met).

Beginning with the DNA:

```
                                    mRNA Start
TTGACATTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAGGAACAGCTATGAAAGCTATGTAATTTATG.
AACTGTAAATACGAAGGCCGAGCATATTACACACCTTAACACTCGCCTATTGTTCCCGTGTGTCCTCCTTGTCGATACTTTCGATACATTAAATAC.
 -35                    -10              Lac operator
        Lac UV5 Promoter
```

The mRNA:

Without punctuation:

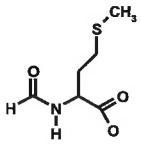
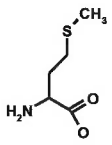
GAAUUGUGAGCGGAUAACAAUUUCACACAGGAGGAACAGCUAUGAAAGCUAUGUAAUUUAUG . . . . .

With punctuation:

RBS 6 based

GAAUUGUGAGCGGAUAACAAUUUCACACAGGAGGAACAGCUAUG , AAA , GCU , AUG , UAA , UUU , AUG . . .

fMet-Lys-Ala-Met-STOP

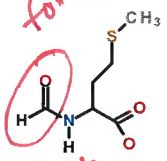
Ribosome Binding Site (RBS): (Shine-Dalgarno [SD] sequence-AGGAGG.) *Positions mRNA on the ribosome so that the correct start codon is used.* *Complementary to 16s rRNA in 30s subunit.* The optimal spacing between the SD sequence and the AUG is 6 or 7 bases.	**Start codon:** AUG codes for the 1st amino acid, always a modified methionine (N-formyl methionine, fMet) in proks. Normal Met in eukaryotic cells. *This codon sets the reading frame.*  N-formylMet  Met	**Codons:** Each triplet of bases following the start codon codes for one amino acid. *Translation performed by appropriately charged tRNAs.*	**Stop codon:** Signals end of the protein (UAG, UAA, UGA) Protein is hydrolyzed from last tRNA.

F. Protein Synthesis (Prokaryotic)

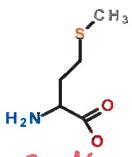
Step I - Chain Initiation

N-formylmethionine (fMet) and its charged tRNA function uniquely to initiate chains in prokaryotic (e.g. bacteria). The N-formyl group mimics a peptide bond, allowing the first methionine to bind to the site on the ribosome that normally holds the growing peptide.

formyl



prok



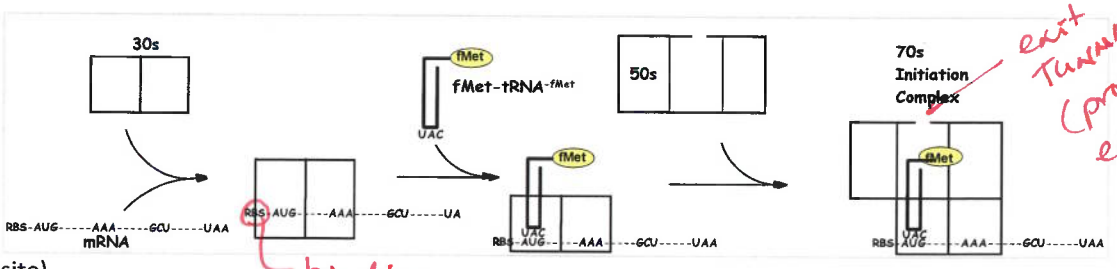
euk.

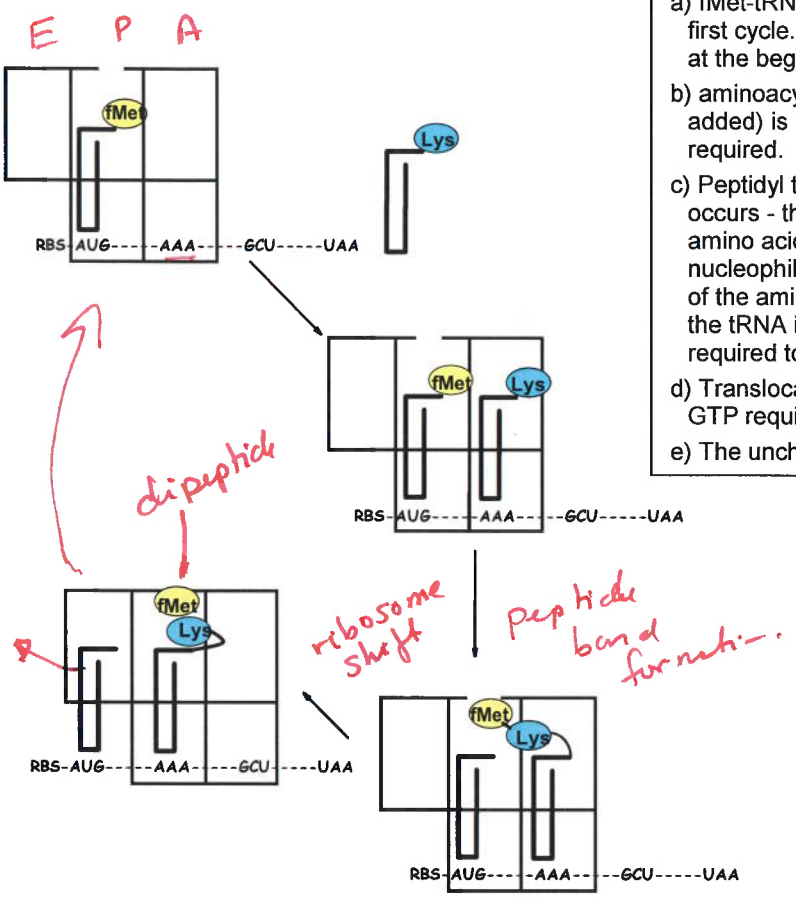
Steps in formation of **Initiation Complex**:

i) mRNA first associates with the 30S subunit – the ribosome binding site (also called the Shine-Dalgarno sequence) forms Watson-Crick hydrogen bonds to a region at the 3' end of 16S rRNA in the 30S subunit of the ribosome.

ii) fMet-tRNA^{fMet} and the 50S subunit combine to form the 70S initiation complex.

Step II - Chain Elongation: (Met-Lys-Ala; mRNA = SD-AUG-AAA-GCU-UAA) RBS=ribosome binding site)





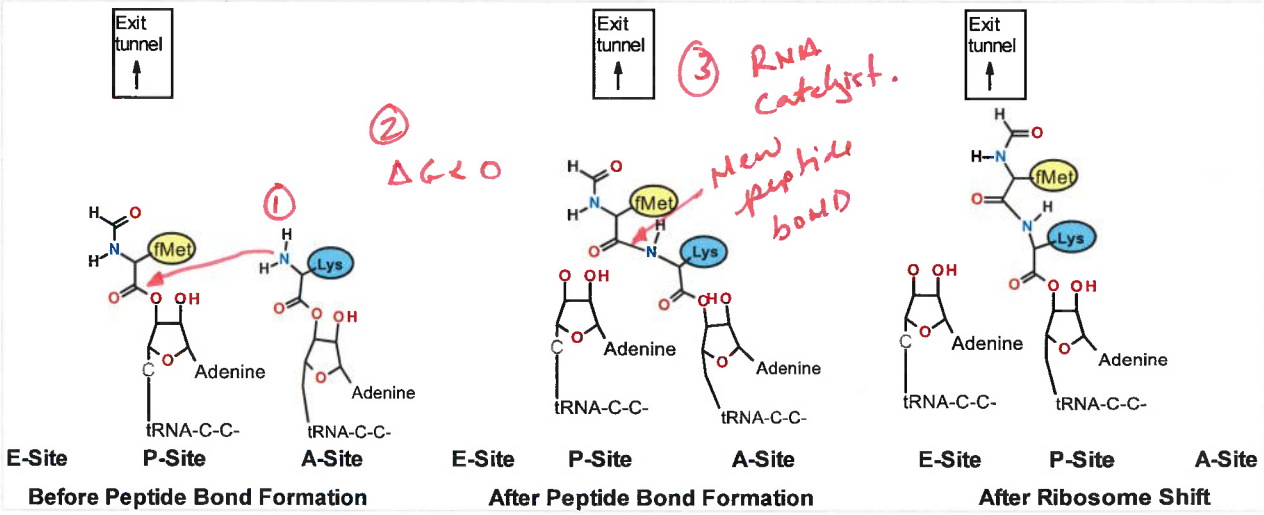
a) fMet-tRNA occupies the P site in the first cycle. Growing protein is found here at the beginning of subsequent cycles.

b) aminoacyl-tRNA-Lys (next AA to be added) is brought to the A site. GTP required.

c) Peptidyl transfer, or **transpeptidation** occurs - the free amino group of the amino acid in the A-site performs a nucleophilic attack on the carbonyl group of the amino acid (or peptide) attached to the tRNA in the P-site. No energy is required to form the peptide bond.

d) Translocation of the ribosome occurs. GTP required.

e) The uncharged tRNA leaves the E site.



Step III - Chain Termination:

Protein release factor (which is a protein that looks like a tRNA) and a stop codon are required to finish the polypeptide chain. Water hydrolyzes the peptide from the P-site, releasing the complete tri-peptide. The mRNA is released and the ribosome 30s and 50s subunits dissociate.