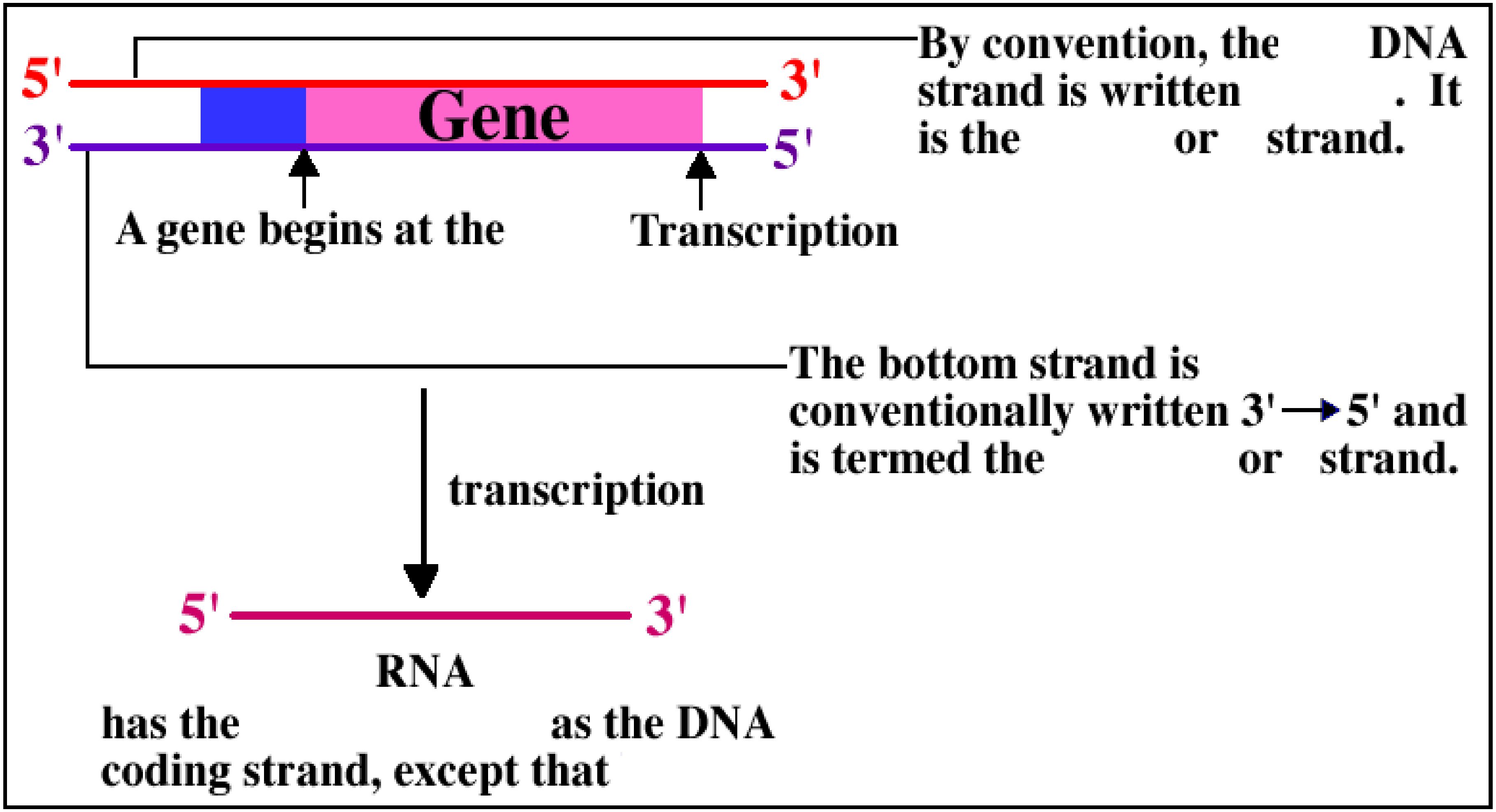
III. Transcription



By convention, the DNA strand is written . It is the or strand.

A gene begins at the

Transcription

The bottom strand is conventionally written 3' → 5' and is termed the or strand.

has the as the DNA coding strand, except that

-an at which the transcripition complex assembles

-In bacteria, may be under control of the same promoter. This is called an . mRNAs formed will carry the information for more than one gene and are thus called mRNAs.

-In eukaryotes, generally has its own promoter. mRNAs carry the information for only one gene and are termed .

Promoters have (hypothetical sequences made up of nuceotides found most often in each position). For prokaryotic promoters:

$$5'...\underbrace{\text{TTGACA}}..................\underbrace{\text{TATAAT}}........\text{Start site}$$

If a promoter very the consensus sequence (a), then the gene/genes downstream are very transcribed. If the match is quite poor then the promoter is and the gene/genes are transcribed quite .

Genes downstream of which promoter would be transcribed the most frequently?

5' TTCACA......................TATTTT..............start site

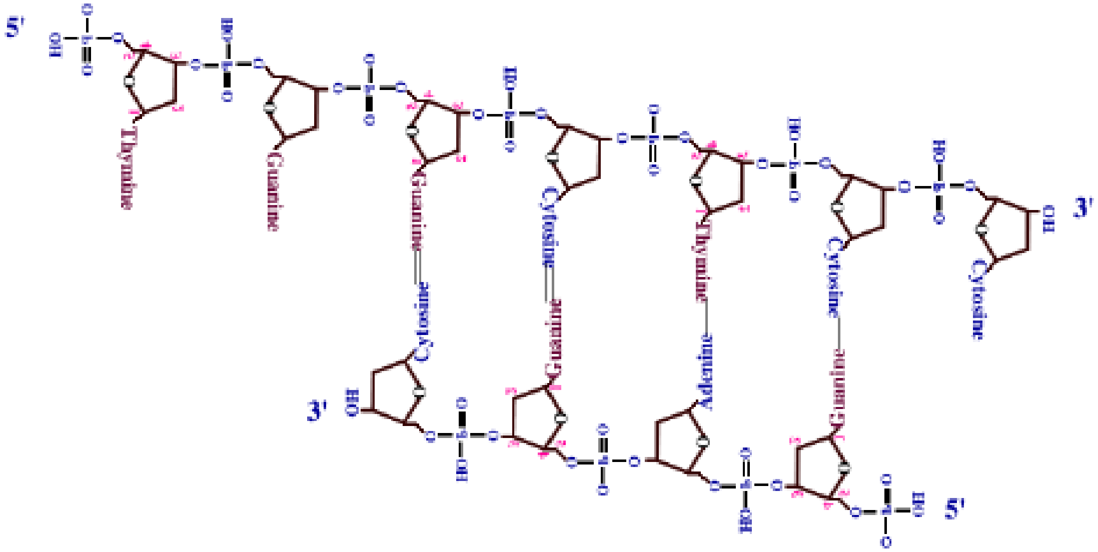
5' TACACA.....................TAATTT.............start site

5' TTGAGA.....................TATATT.............start site

All of the following accurately describe either prokaryotic replication or transcription EXCEPT?

a. During replication, Okazaki fragments are formed during the synthesis of the lagging strand of DNA but not during the synthesis of the leading strand.

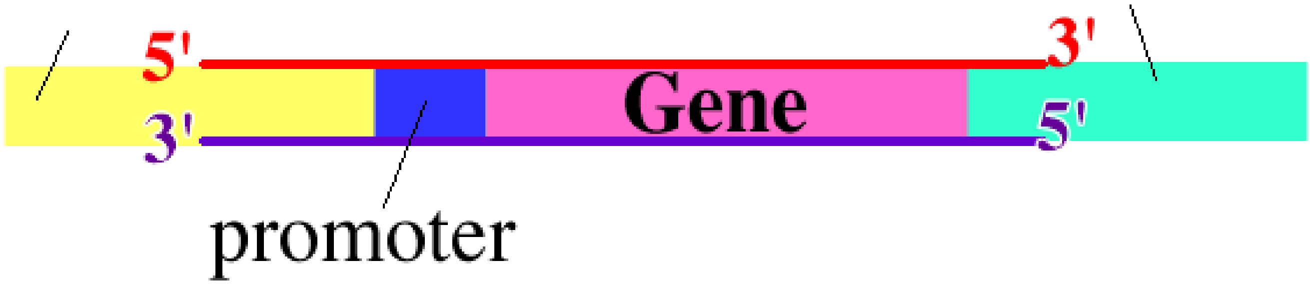
b. The next deoxynucleoside triphosphate that DNA polymerase would add to the currently synthesizing strand would be dCTP.



c. A promoter with the -35 sequence of TGCAGA and a -10 sequence of TATTGT is stronger than a promoter with a -35 sequence of TTGAGA and a -10 sequence of TATATT.

d. The coding strand of DNA has the same sequence as the RNA transcript only Us replace Ts in the RNA.

A promoter is of a gene



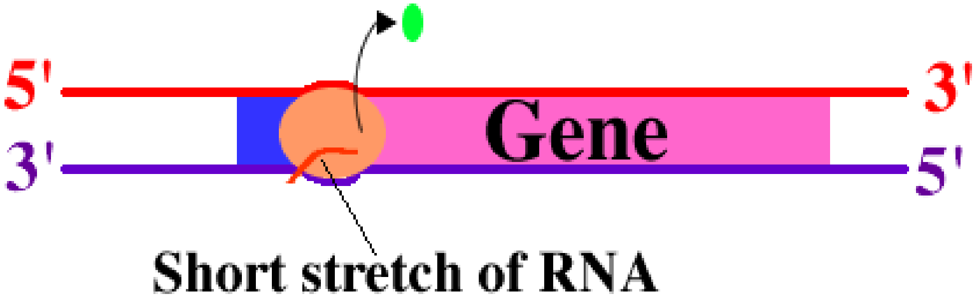
A. Initiation

-The of RNA polymerase allows the holoenzyme to bind tightly to the promoter.

-Binding of RNA polymerase and a short stretch (16-20 bp) of DNA called the .

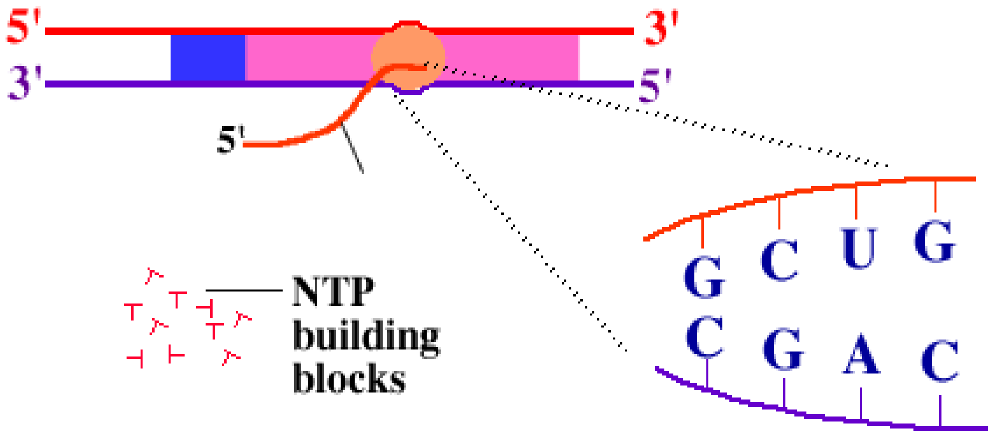
-A short stretch of complementary RNA (~ 9 nucleotides) is

-The   from the core enzyme.



B.

1. RNA polymerase moves along the template strand   at a time.



b. As RNA polymerase moves, the previous stretch of DNA and the current stretch is


5'
3'
Gene
3'
5'

2. As soon as RNA polymerase has left the promoter region, a new RNA polymerase enzyme can bind and start a

C.

Transcription termination

Termination due to formation

A DNA sequence encodes for two complementary regions of RNA. These regions hydrogen-bond and form a loop that causes RNA polymerase to leave the template.

termination

The rho enzyme

destabilizing the RNA-DNA hybrid and causing termination.

http://en.wikipedia.org/wiki/Stem-loop

Using the DNA strand depicted below as a template, write the sequence of the first portion of the RNA transcript for:

```
           -10      +1
            |         |
5'.......TATAATGCATTACAACGGCTTACGATTAATCCAGGAT...3'
3'.......ATATTACGTAATGTTGCCGAATGCTAATTAGGTCCTA...5'
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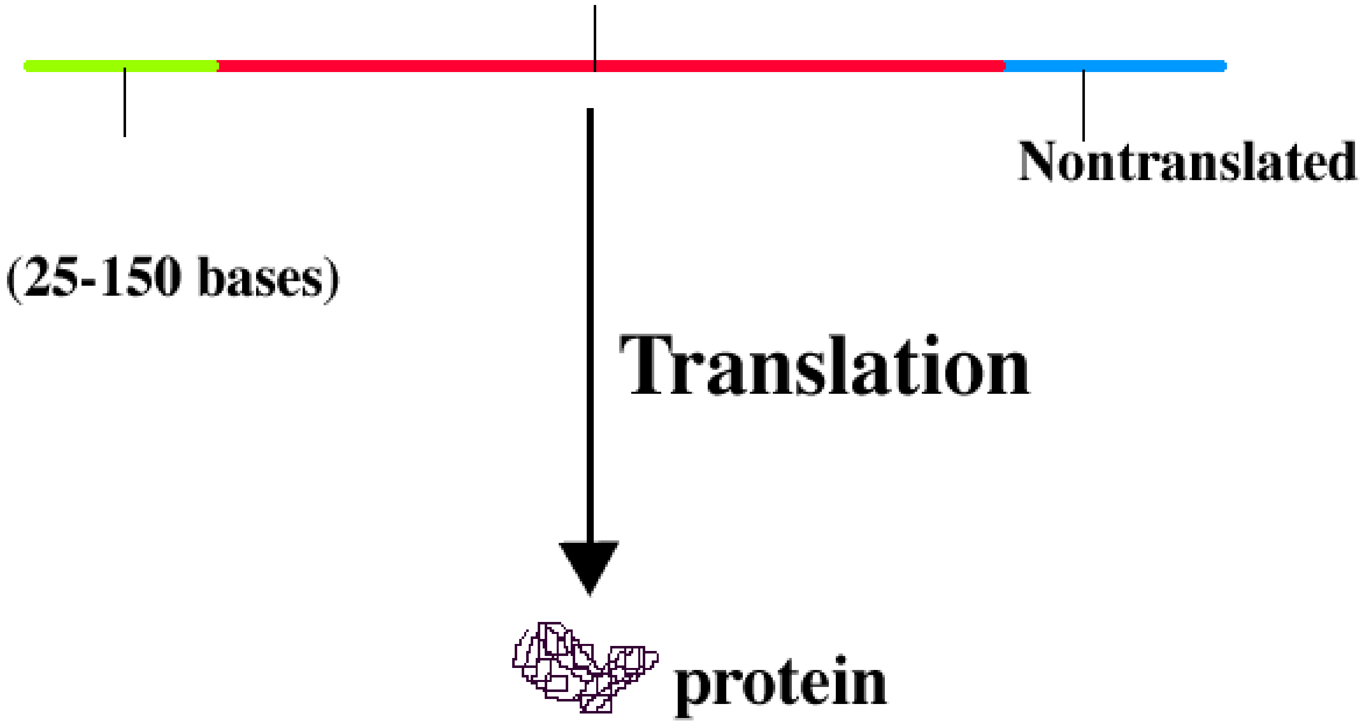
IV. Translation

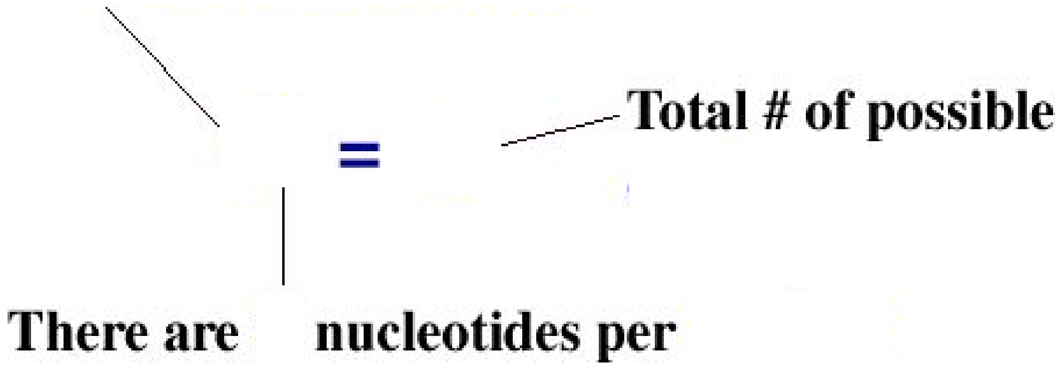


Recall the structure of an amino acid:

- During translation, amino acids are added to the

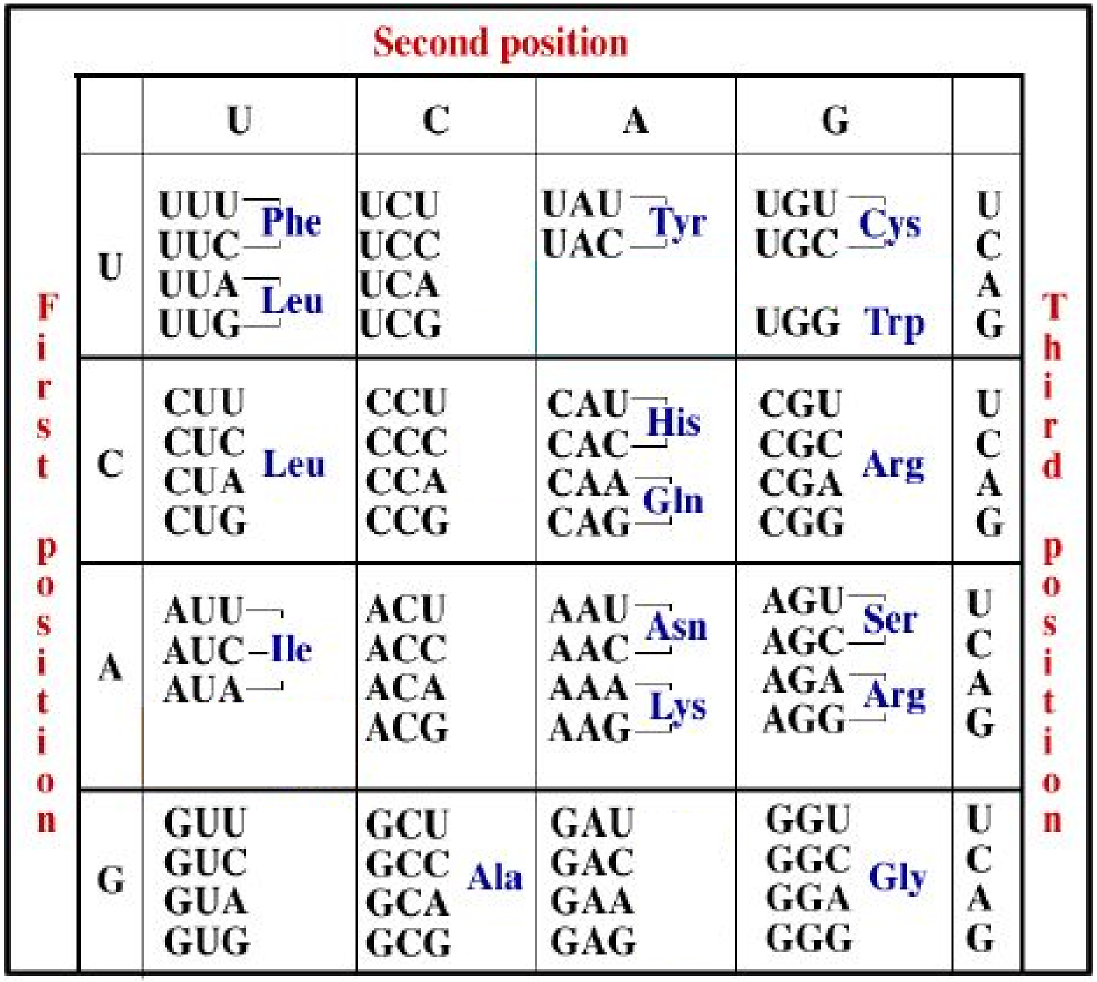
- Each amino acid is specified by a 3-nucleotide sequence (called a) on the mRNA.

The Players:
  1. mRNA
  The codons:



a. Three of the codons are

b. The other 61 codons each specify a particular . Since there are only 20 amino acids, this means that



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

c. The mRNA also carries the information that indicates

That is, it specifies

What is the correct reading frame for the following mRNA sequence?

5'CGAUCAUGCGUUCGGGA...

2. Ribosomes

a. Translation

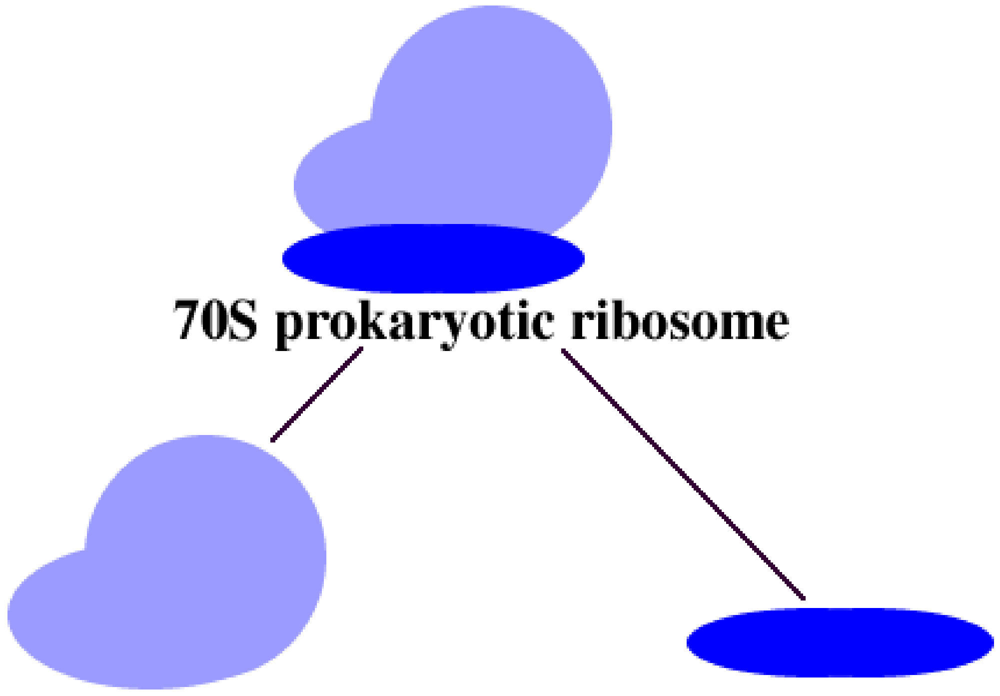
b. Moves along the mRNA in the

that specifies a single amino acid.

c. of amino acids.

d. There may be as many as ribosomes in a growing *E. coli* cell.

http://www.hhmi.org/biointeractive/rna/lectures.html



70S prokaryotic ribosome

50 S subunit Composed of the and

30 S subunit Composed of a and

3. tRNA has a cloverleaf structure

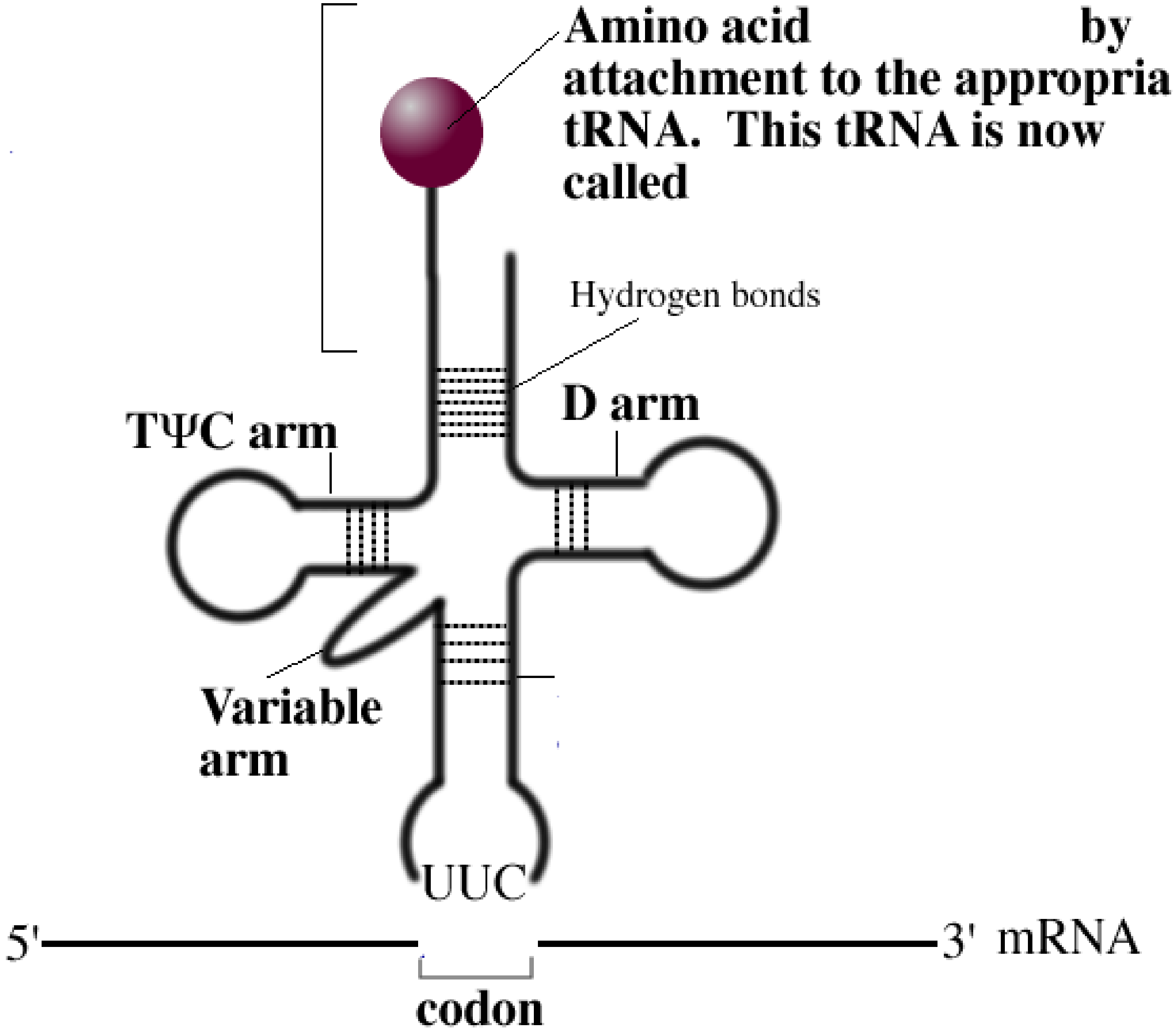


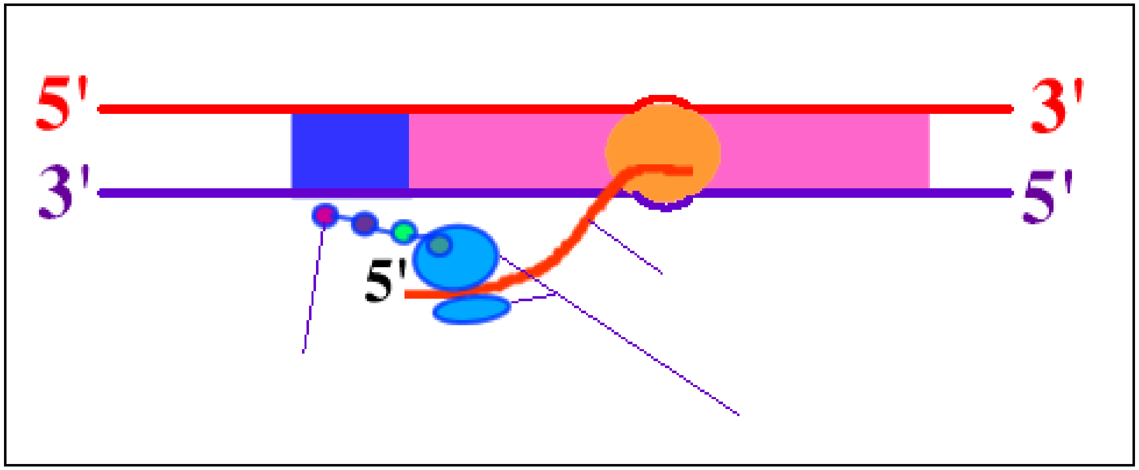
Fig 12.35 (8th) or 13.34 (9th)

b. Although there are 64 codons, there are because have no corresponding tRNA and the anticodon of some tRNAs can . (e.g. The anticodon 3'CCG5' can recognize both) This is called

4. Initiation of translation

a. In prokaryotes, translation begins while mRNA is still being .

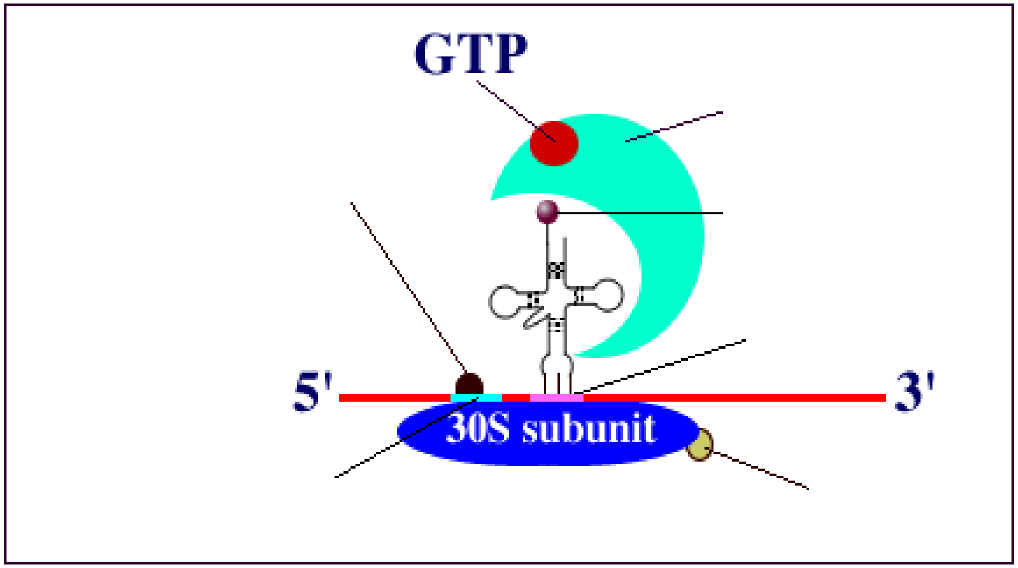
Fig 12.34 (8th) or 13.33 (9th)



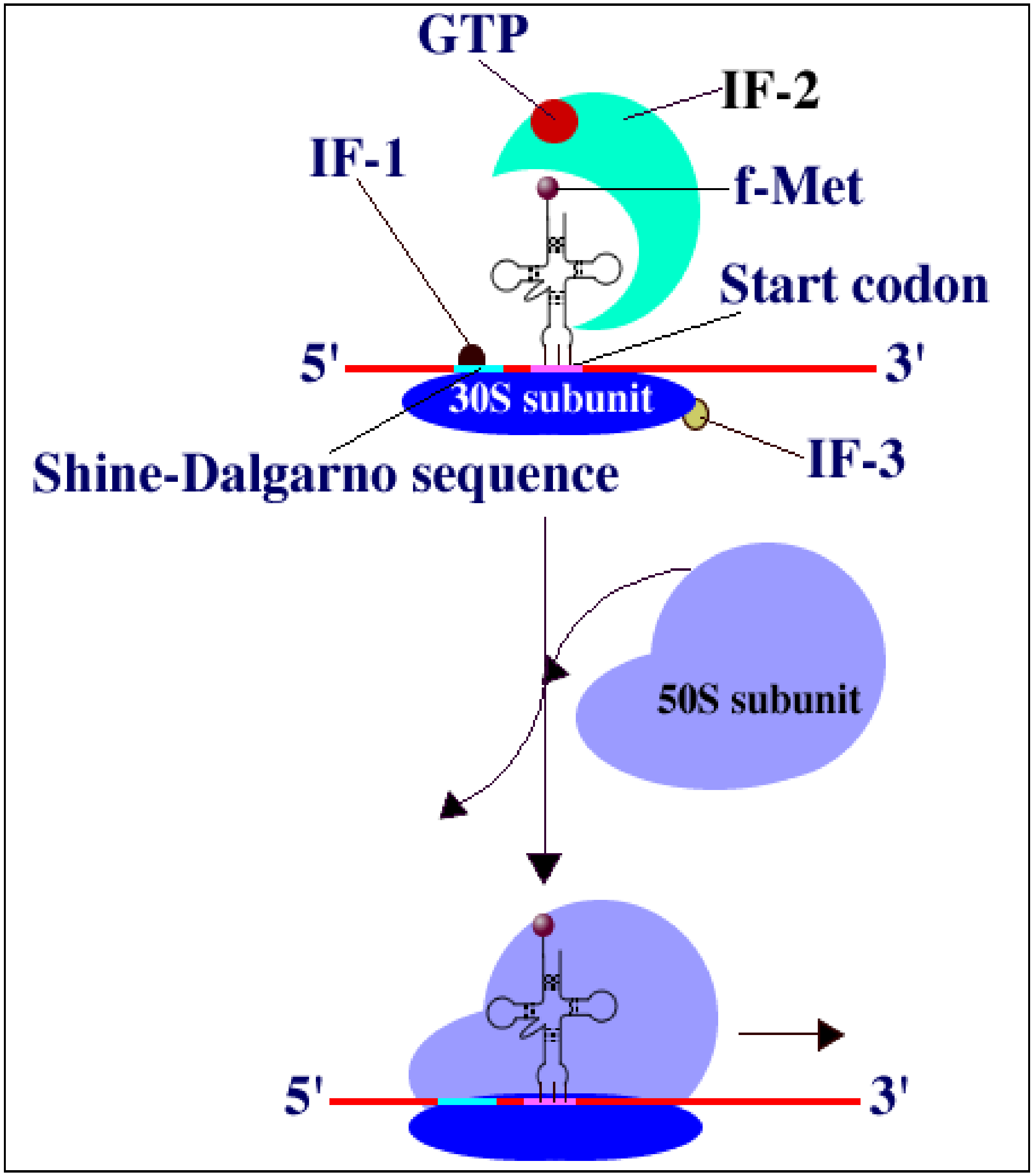
*Not to scale!

b. The 30S subunit of the ribosome binds to a sequence on the mRNA called the

c. At the first beyond the ribosome-binding site, an consisting of the 30S ribosomal subunit, a tRNA carrying an and proteins called come together.



The subunit then joins the complex, IF-2-GTP hydrolyzes GTP and the initiation factors dissociate from the complex.

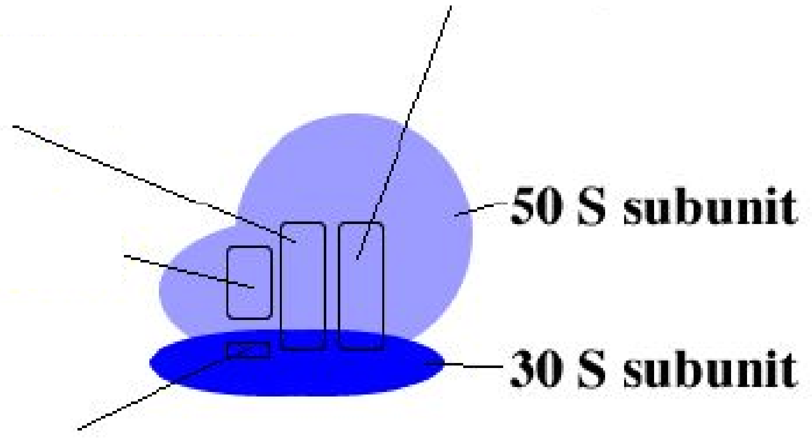


The only time AUG serves as the start codon is when it is downstream from a ribosome-binding site (Shine-Dalgarno sequence). Otherwise it just encodes for methionine.
A ribosome binds to the following mRNA molecule at the site indicated by the dark box (Shine-Dalgarno sequence). Determine where translation will start and what are the first three amino acids that will be incorporated into the polypeptide?

5’ ■GCCGGAAUGGUCAUGGGAAACC… 3’

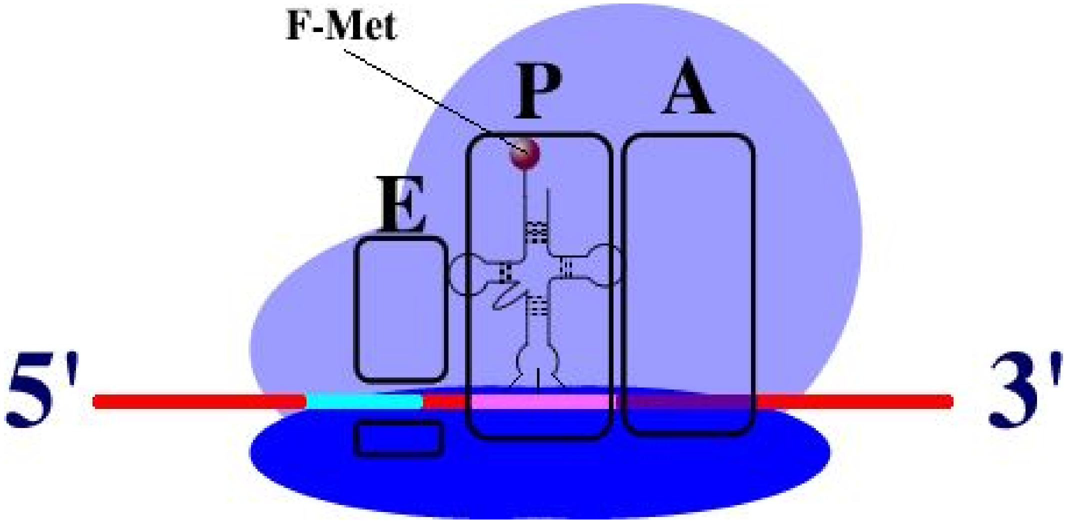
5.

a. Several binding sites on the 70S ribosome play a role in the elongation process:



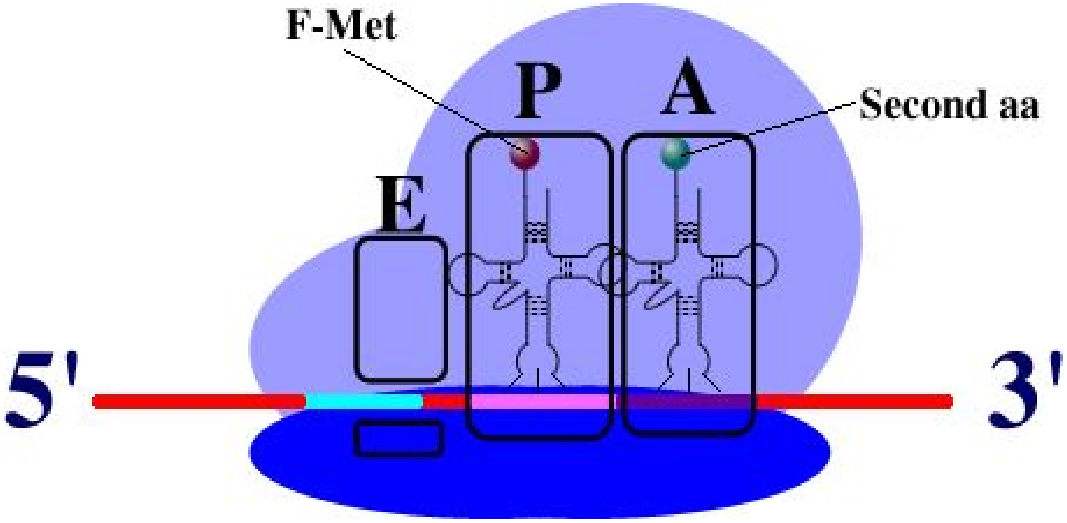
b. The Process:

1.) The initiating tRNA and its f-Met residue .

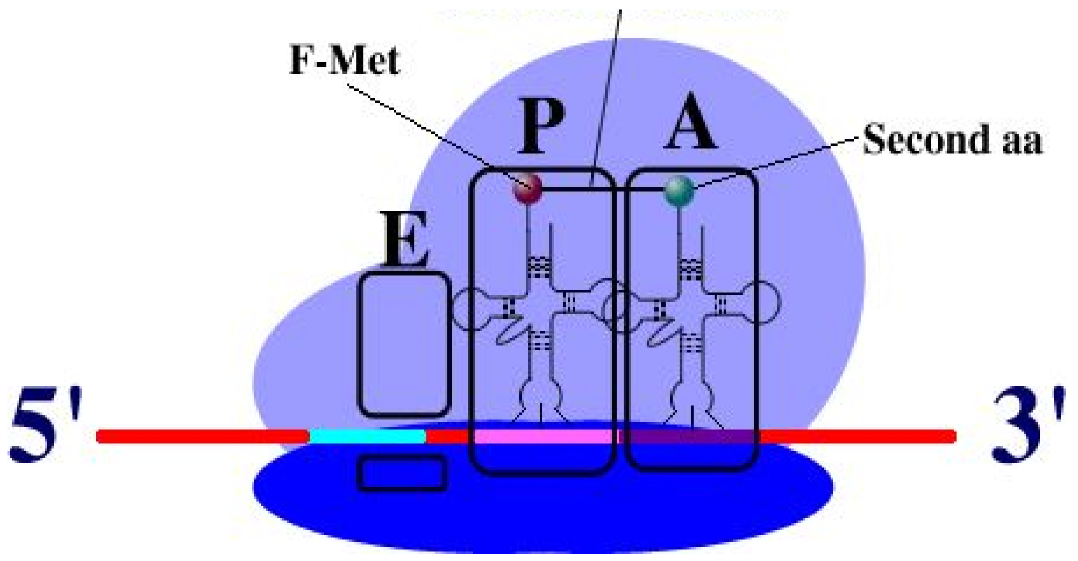


2.) A tRNA carrying the amino acid that corresponds to the

.

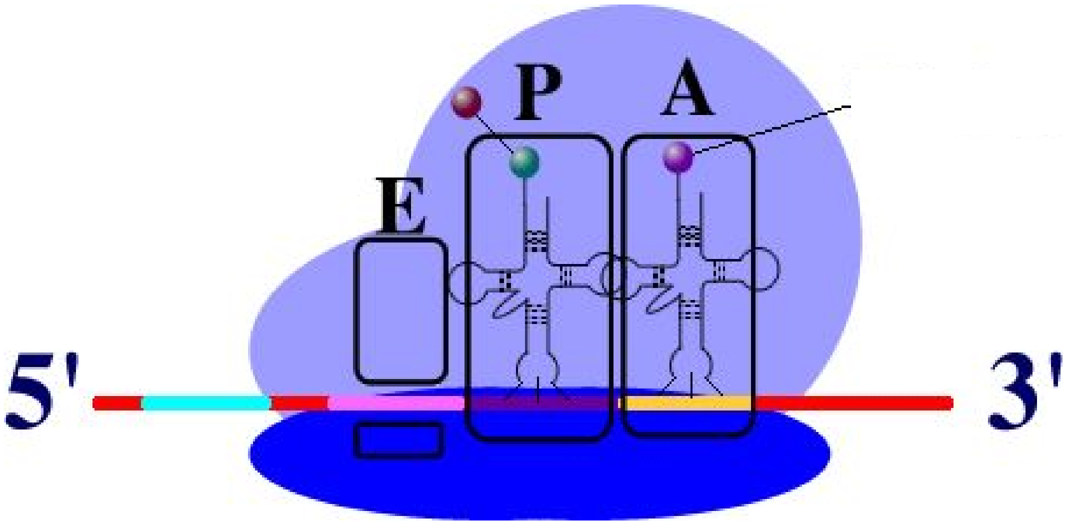


3.) The f-Met bound to the tRNA in the P-site is   to the amino acid carried by the tRNA in the A-site.



4.)   = the ribosome advances the distance of one codon and the tRNA and f-Met

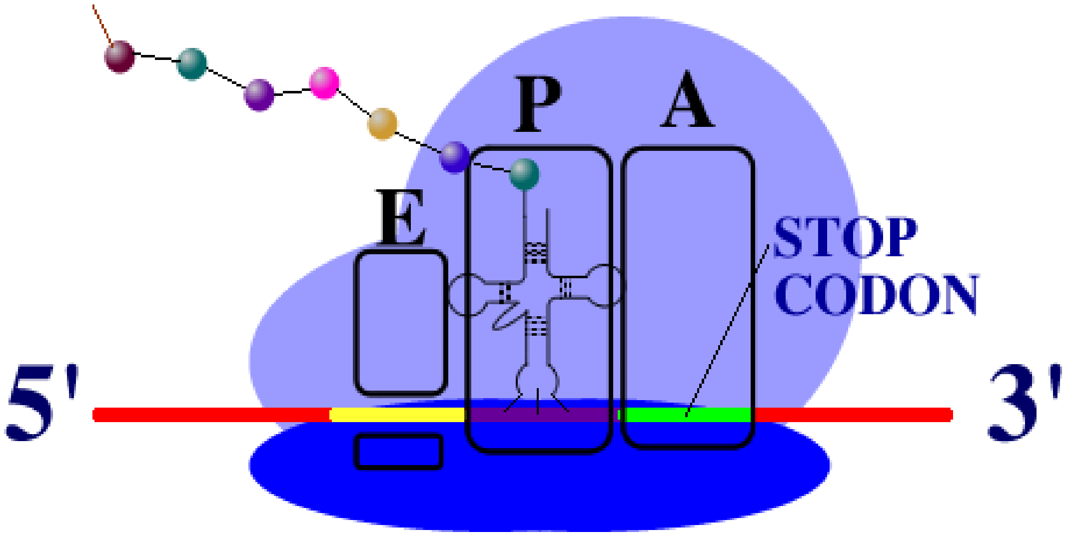
5.) The tRNA and amino acid that had been in the A-site now occupy the . The A-site is momentarily vacant but is quickly filled by a



c. NOTE: The same mRNA can be translated by several ribosomes at once. Many ribosomes on an mRNA = or just

6. Termination

a. Elongation is terminated when the ribosome reaches a



b. Proteins called   free the polypeptide by breaking the bonds between the last amino acid and its tRNA.

c. The ribosome   of the mRNA and   into its 30S and 50S components.

7. Post-translational Modification

Proteins may be modified by the addition of a or a may help some proteins to fold into their final, functional shape.

II. Eukaryotic Gene Expression

A. Eukaryotes have RNA polymerase.

B. The in eukaryotes differ.

C. Eukaryotic mRNAs are more often after transcription.

1.

Shortly after transcription begins, the 5' end of the transcript is "capped" by the .

2.

Before transcription has been terminated, the mRNA is cleaved at a particular sequence and a series of

3. Modifications likely help to and

B. The of eukaryotic genes are commonly interrupted by .

1. Both exons and introns are transcribed to generate the
.

2. The introns are removed by .