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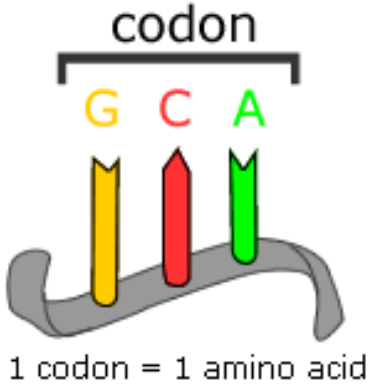
DAYTONA STATE COLLEGE

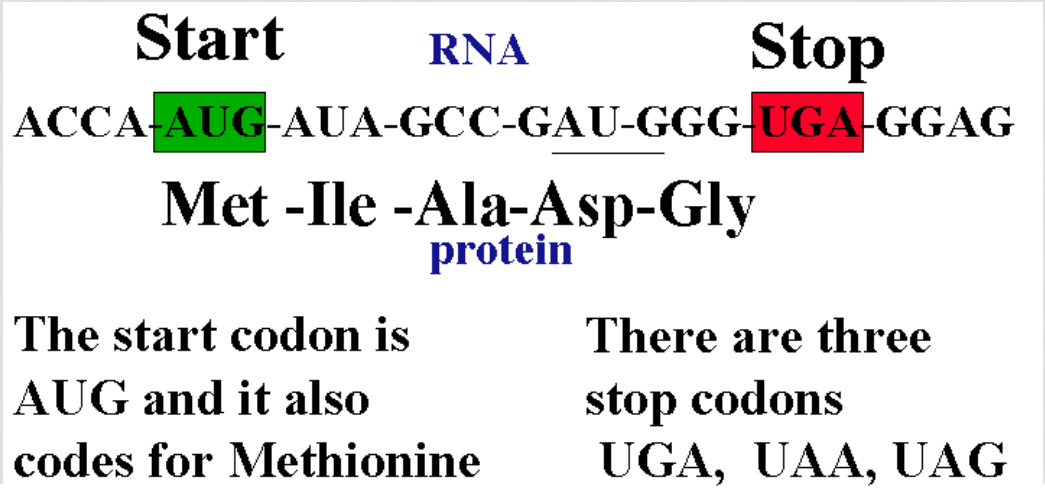
GENETICS TEST IV

REVIEW

BE ABLE TO DEFINE



- Codons
  - A triplet of nucleotides that specifies a particular amino acid or a start or stop signal in the genetic code. Sixty-one codons specify the amino acids used in proteins, and three codons, called stop codons, signal termination of growth of the polypeptide chain. One codon acts as a start codon in addition to specifying an amino acid.
  - In the mRNA, **triplet codons** specify **one amino acid**
  - The code contains **"start"** signals that are necessary to **initiate** and translation
  - The code contains "**stop**" signals, certain codons (**nonsense codons**) that are necessary to **terminate** translation



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- Genetic code
  - The deoxynucleotide triplets that encode the 20 amino acids or specify termination of translation.
  - The genetic code is
    - Unambigous
      - Each codon codes for only one amino acid
    - Degenerate
      - More than one codon per amino acid
      - Some amino acids are specified by more than one codon.
    - Commaless
      - No punctuation or extra bases between codons
      - The genetic code reads three nucleotides at a time in a continuous linear manner, thus the code is comma-less (no extra bases between codons)
    - Nonoverlapping
      - After initiation of translation each nucleotide is part of only one codon
      - Sequence of amino acids correlates linearly with sequences of mutations.
      - If nucleotide overlaps, each single nucleotide change would yield more than one amino acid change, however protein sequencing finds only one amino acid change.
    - Nearly Universal
      - Same in all prokaryotes and eukaryotes
        - Few minor exceptions such as mitochondria.

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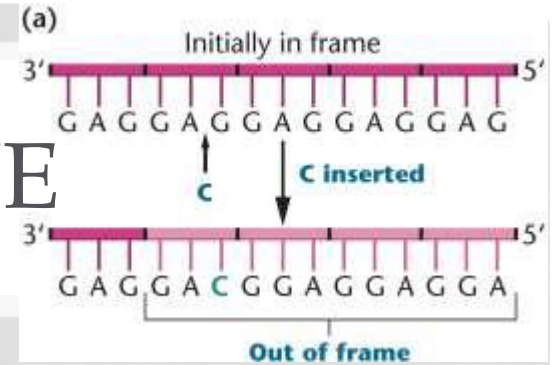

- Frameshift mutation
  - Frameshift mutations result from insertions or deletions of a base pair.
  - A mutational event leading to the insertion of one or more base pairs in a gene, shifting the codon reading frame in all codons that follow the mutational site.
  - A frameshift mutation occurs when any number of bases are added or deleted, except multiples of three, which would reestablish the initial frame of reading.
  - The triplet nature of the genetic code was revealed by frameshift mutations.
  - +1 base = mutant (changes all amino acids down stream from insertion
  - +1+1 double mutant is still mutant (changes all amino acids down stream from insertion
- Nonsense mutation
  - A mutation that changes a codon specifying an amino acid into a termination codon, leading to premature termination during translation of mRNA.
  - A nonsense mutation changes a codon into a stop codon and results in premature termination of translation
- Nonsense suppression
  - When a change in tRNA anticodon places an amino acid in the stop codon.

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- Nirenberg and Matthaei
  - Cracked the genetic code by using nucleic acid homopolymers to translate specific amino acids.
  - Added RNA homopolymers to the in vitro translation system to decipher which amino acids were encoded by the first few codons based on which amino acids were incorporated into the polypeptide.
- RNA homopolymers
  - RNA nucleotides with only one type of ribonucleoside
- RNA heteropolymers
  - RNA nucleotides with two or more different ribonucleosides

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TABLE 13.2

Amino Acid Assignments to Specific Trinucleotides Derived from the Triplet-Binding Assay

Trinucleotides	Amino Acid
AAA AAG	Lysine
AUG	Methionine
AUU AUG AUA	Isoleucine
CCU CCC CCG CCA	Proline
CUC CUA CUG CUU	Leucine
GAA GAG	Glutamic acid
UCU UCC UCA UCG	Serine
UGU UGC	Cysteine
UUA UUG	Leucine
UUU UUC	Phenylalanine

- Nirenberg and Leder
  - Developed the triplet binding assay to determine other specific codon assignments.
- Triplet binding essay
  - In this technique ribosomes bind to a single codon of three nucleotides and the complementary amino acid charged tRNA will be able to bind.

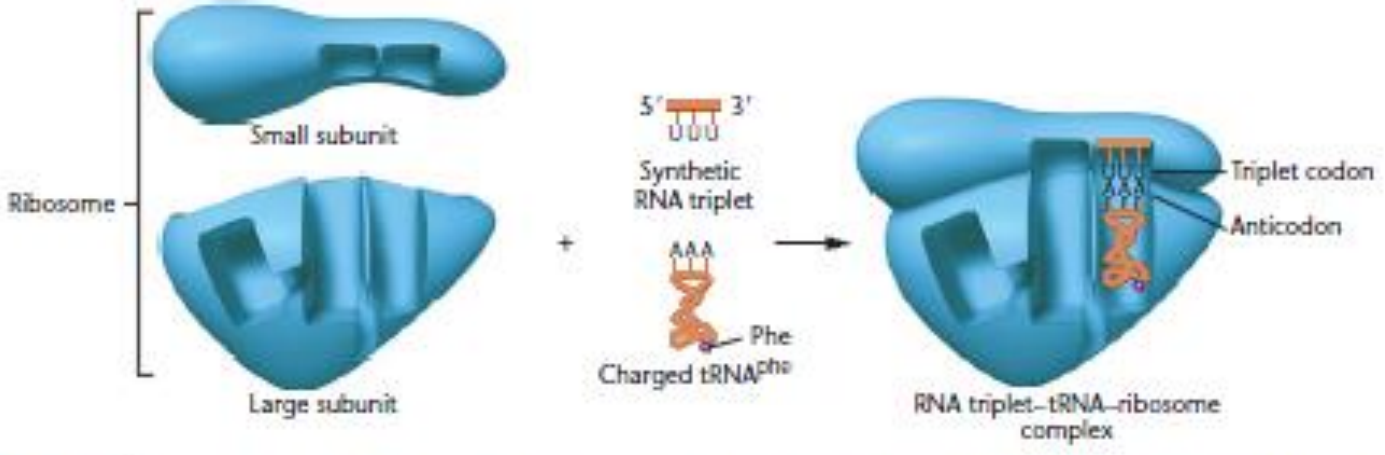

FIGURE 13–5 The behavior of the components during the triplet-binding assay. When the UUU triplet is positioned in the ribosome, it acts as a codon, attracting the complementary AAA anticodon of the charged tRNA^{phe}.

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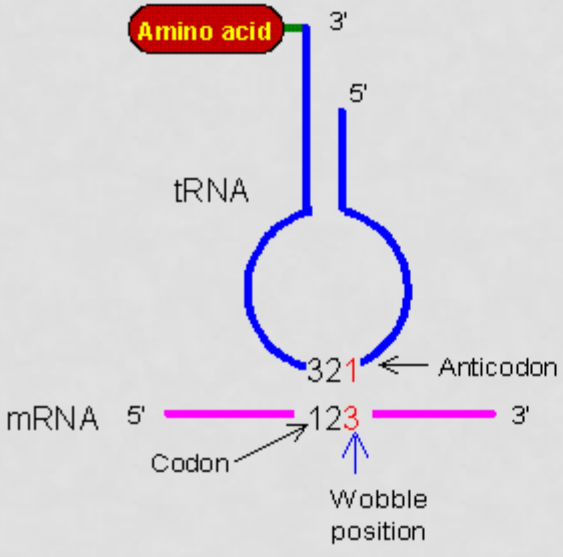
- Wobble hypothesis
  - Predicts that the initial two ribonucleotides of triplet codes are often more critical than the third. The third position of the codon-anticodon interaction would be less spatially constrained and need not adhere as strictly to the established base-pairing rules at the third position of the codon.
  - An idea proposed by Francis Crick, stating that the third base in an anticodon can align in several ways to allow it to recognize more than one base in the codons of mRNA.



Wobble bases

tRNA	C	A	G	U	I
mRNA	G	U	C U	A G	C A U

Wobble bases

mRNA	C	A	G	U
tRNA	G I	U I	C U	A G I

TABLE 13.4

Anticodon–Codon Base-Pairing Rules

Base at first position (5′ end) of tRNA	*Base at third position (3′ end) of mRNA*
A	U
C	G
G	C or U
U	A or G
I	A, U, or C

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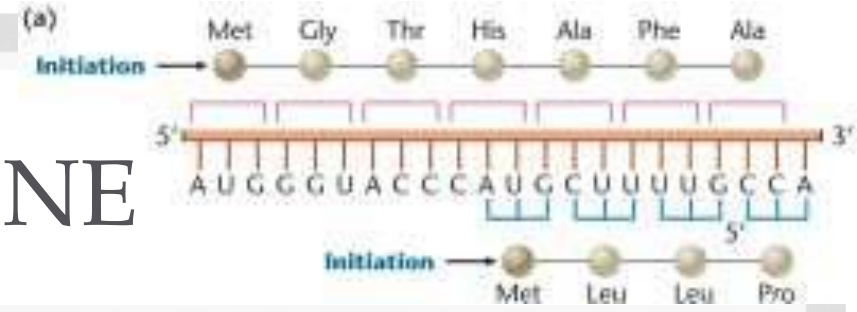

- Promoter
  - An upstream region of a gene serving a regulatory function and to which RNA polymerase binds prior to the initiation of transcription.
  - Transcription begins with template binding by RNA polymerase at a promoter.
- Open reading frame (ORF)
  - A nucleotide sequence organized as triplets that encodes the amino acid sequence of a polypeptide, including an initiation codon and a termination codon.
  - In some viruses, overlapping genes have been identified in which initiation at different AUG positions out of frame with one another leads to distinct polypeptides
- Chromatin remodeling
  - A process in which the structure of chromatin is altered by a protein complex, resulting in changes in the transcriptional state of genes in the altered region.
  - Eukaryotic transcription requires chromatin to become uncoiled, making DNA accessible to RNA polymerase and other regulatory proteins. This transition is referred to as chromatin remodeling.

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- Enhancers
  - A DNA sequence that enhances transcription and the expression of structural genes. Enhancers can act over a distance of thousands of base pairs and can be located upstream, downstream, or internal to the gene they affect, differentiating them from promoters.
- Silencers
  - Sequence that has many of the properties possessed by an enhancer but represses transcription.

- Enhancers and silencers
  - can be upstream, within, or downstream of the gene
  - can modulate transcription from a distance
  - act to increase or decrease transcription in response to cell's requirement for a gene product or at a particular time during development or place within an organism

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- TATA box
  - A short nucleotide sequence 20–30 bp upstream from the initiation site of eukaryotic genes to which RNA polymerase II binds. The consensus sequence is TATAAAA. Also known as a Goldberg–Hogness box.
  - The TATA box is a core promoter element that binds the TATA-binding protein (TBP) of transcription factor TFIID and determines the start site of transcription
- Heterogeneous nuclear RNA (hnRNA)
  - The collection of RNA transcripts in the nucleus, consisting of precursors to and processing intermediates for rRNA, mRNA, and tRNA. Also includes RNA transcripts that will not be transported to the cytoplasm, such as snRNA.
  - **Heterogeneous nuclear RNA (hnRNA)** is posttranscriptionally processed before it can leave the nucleus

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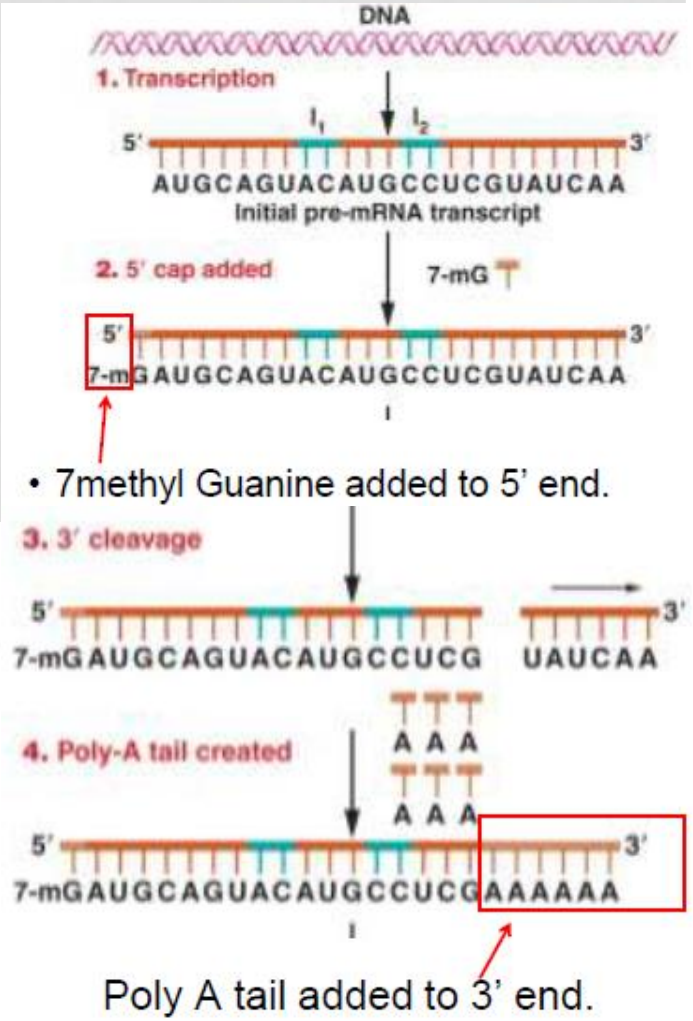
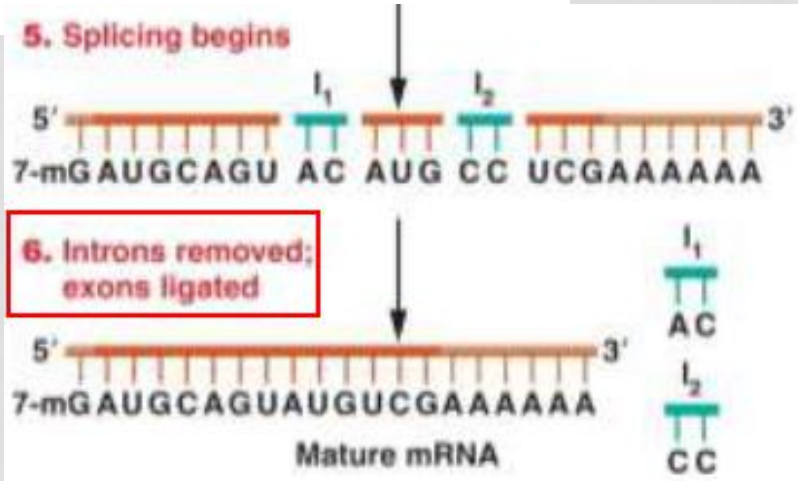
- Eukaryotic mRNA processing: 5' cap, 3' poly A tail, remove introns
  - **Heterogeneous nuclear RNA (hnRNA)** is posttranscriptionally processed before it can leave the nucleus
    - Addition of a 5' cap that protects from nuclease attack and may be involved in the transport of the transcript across the nucleus
    - poly-A tail added to aid transport to cytoplasm
    - Introns are removed by splicing (**Figure 13.10**)
      - Almost all have introns except histone gene and interferon





BE ABLE TO DEFINE

- Introns
  - Intervening sequence in split genes; removed from the RNA after transcription.
  - Regions of the initial RNA transcript that are not expressed in the amino acid sequence of the protein.
- Exon
  - Coding region of a split gene (a gene that is interrupted by introns). After processing, the exons remain in messenger RNA.
- Group I introns
  - Belongs to a class of introns in some ribosomal RNA genes that are capable of self-splicing.
  - Removed via a process of self-excision using ribozyme.

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- Group II introns
  - *Belongs to a class of introns in some protein-encoding genes that are capable of self-splicing and are found in mitochondria, chloroplasts, and a few eubacteria.*
  - Type II introns are more complex and more common, they require a spliceosome, to splice out the intron in a reaction involving the formation of a lariat structure.
- Ribozyme
  - RNA molecule that can act as a biological catalyst.
  - RNA acts as its own catalytic activity
- Spliceosome
  - Large complex consisting of several RNAs and many proteins that splices protein-encoding pre-mRNA; contains five small ribonucleoprotein particles (U1, U2, U4, U5, and U6).

BE ABLE TO DEFINE

- Translation
  - Process by which a protein is assembled from information contained in messenger RNA.
  - The biological polymerization of amino acids into polypeptide chains.
  - Translation requires
    - Amino acids
    - Messenger RNA (mRNA)
    - Ribosomes
    - Transfer RNA (tRNA)
- rRNA
  - RNA molecule that is a structural component of the ribosome.
  - rRNA perform important catalytic functions associated with translation
    - They promote the binding of the various molecules involved in translation and fine-tune the process.
- rDNA
  - The rRNA genes that are part of a moderately repetitive DNA fraction and are present in clusters at various chromosomal sites.
    - Each cluster contains tandem repeats separated by noncoding spacer DNA

BE ABLE TO DEFINE

- Posttranscriptionally modified bases.
  - tRNA are composed of 75-90 nucleotides that contain posttranscriptionally modified bases
    - Modified bases enhance hydrogen bonding efficiency during translation.

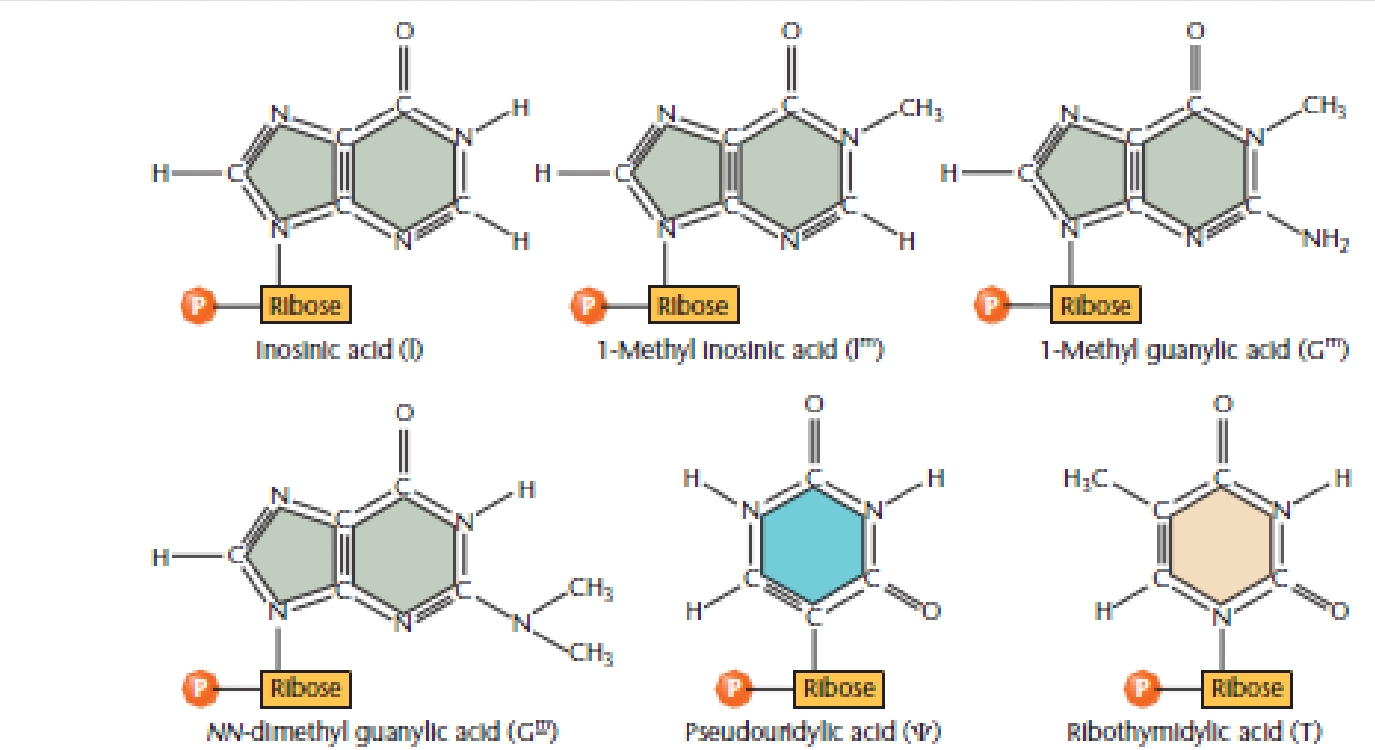
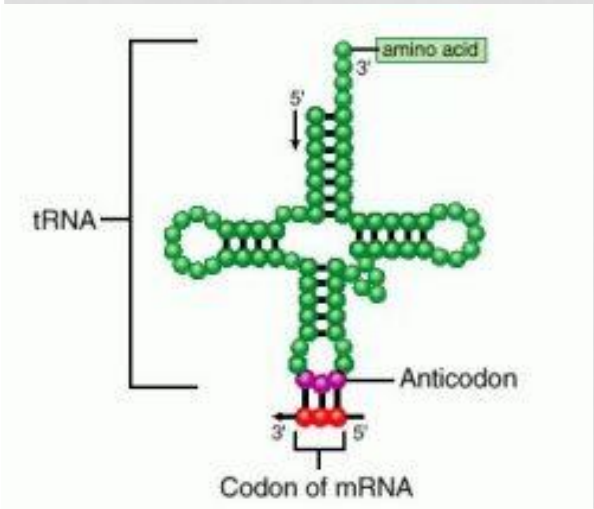
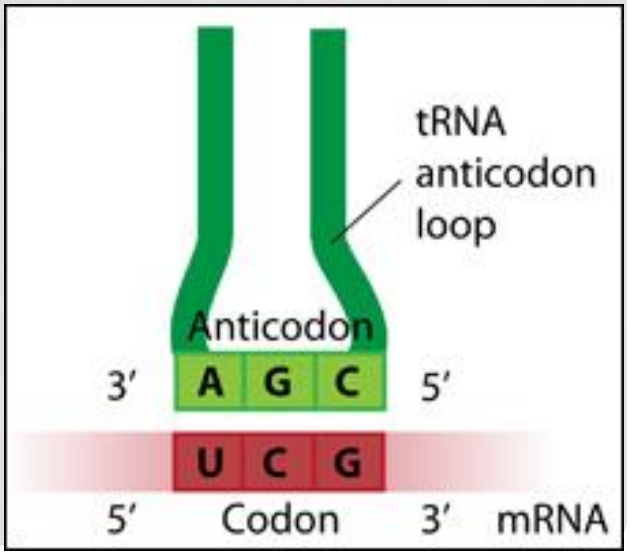


FIGURE 14–2 Ribonucleotides containing unusual nitrogenous bases are found in transfer RNA.

BE ABLE TO DEFINE

- Codon
  - Sequence of three nucleotides that codes for one amino acid in a protein.
  - See slide one.
- Anticodon
  - Sequence of three nucleotides in tRNA that pair with the corresponding codon in mRNA in translation.
  - In a tRNA molecule, the nucleotide triplet that binds to its complementary codon triplet in an mRNA molecule.
  - tRNA has an anticodon that complementarily base-pairs with the codon in the mRNA.





BE ABLE TO DEFINE

- tRNA functional domains
  - The two-dimensional structure of tRNAs is a cloverleaf (Figure 14.3 and Figure 14.4)
  - tRNA has four sites

1. **an anticodon** that binds to the codon on mRNA
2. **Amino acid attachment site**
3. **Site that binds to the enzyme that adds the amino acid** (aminoacyl tRNA synthetase).
4. **Site binds to the ribosome**

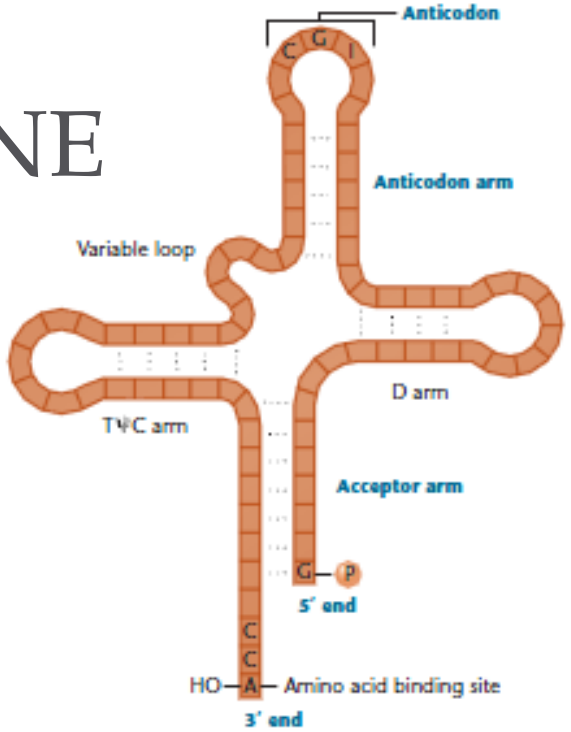
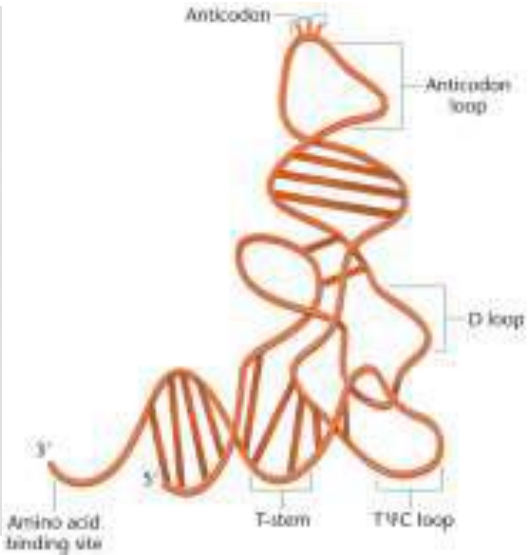


FIGURE 14–3 Holley's two-dimensional cloverleaf model of transfer RNA. Hydrogen bonds are designated by dots (···).

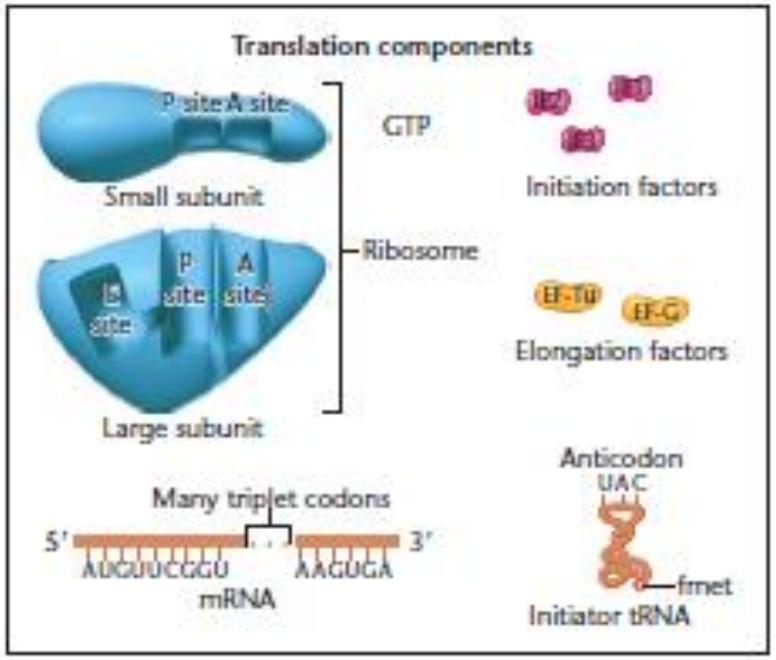


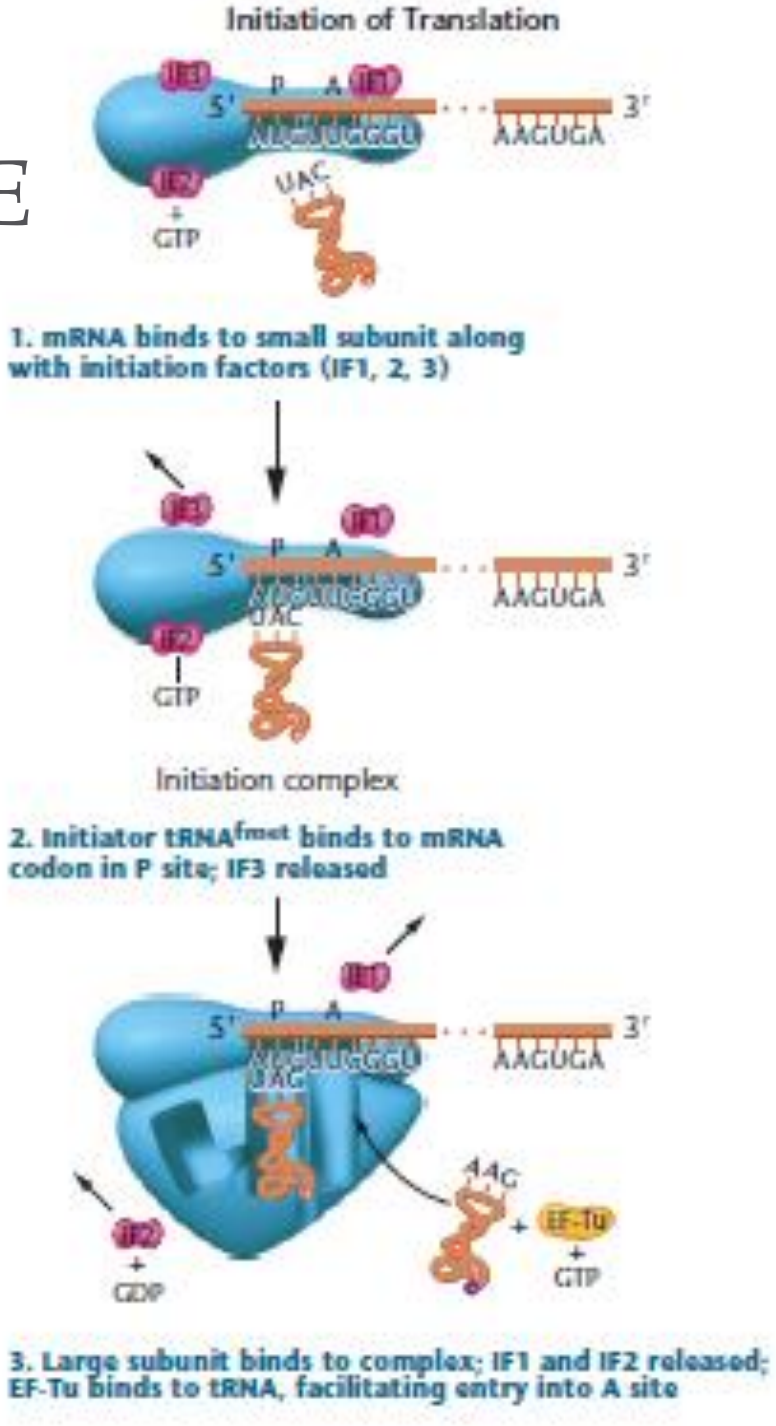
BE ABLE TO DEFINE

- Amino acid binding site
  - The corresponding amino acid is covalently linked to the CCA sequence at the 3' end of all tRNAs
- tRNA binding site
  - Sites where tRNA binds to the large ribosomal subunit.
- Aminoacyl tRNA synthetase
  - Before translation can proceed, tRNA molecules must be chemically linked to their respective amino acids
    - Activation (charging or aminoacylation) done by aminoacyl tRNA synthetase
    - There are 20 different synthetases, one for each amino acid, and they are highly specific since they recognize only one amino acid

BE ABLE TO DEFINE

- Initiation of translation
  - Requires the following.
    - The small ribosomal subunit
    - The large ribosomal subunit
    - GTP
    - Charged initiator tRNA
    - Mg^{+2}
    - Initiation factors





BE ABLE TO DEFINE

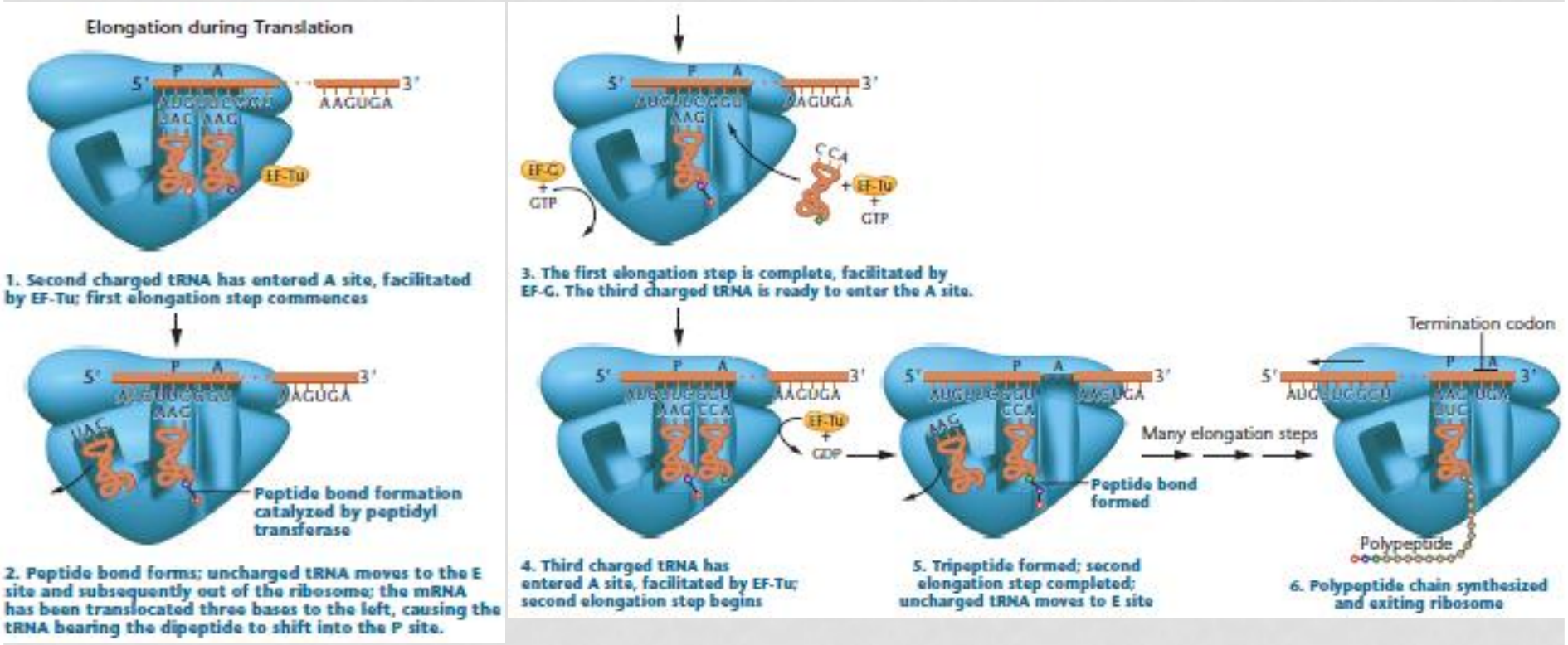
- Initiator tRNA
  - Binds to the P site
  - Carries the amino acid **f-met** in prokaryotes, and **met** in eukaryotes.
- AUG start codon
  - Codon that codes for **f-met** in prokaryotes and **met** in eukaryotes and designates the start of translation.
- Shine-Dalgarno sequence
  - The nucleotides AGGAGG that serve as a ribosome-binding site in the leader sequence of prokaryotic genes. The 16S RNA of the small ribosomal subunit contains a complementary sequence to which the mRNA binds.
  - In bacteria, the AUG start codon is preceded by a Shine-Dalgarno sequence (AGGAGG) (only purines), which base-pairs with a region of the 16S rRNA of the 30S small subunit, facilitating initiation

BE ABLE TO DEFINE

- Elongation
  - The second phase of translation.
  - The lengthening of growing polypeptide chain by one amino acid is called elongation.
  - Requires both ribosomal subunits assembled with the mRNA to form the P and A site.
- E site
  - Exit site in large ribosomal subunit
- P site
  - Peptidyl site in large ribosomal subunit
  - The site holding the tRNA with the peptide chain attached
- A site
  - Aminoacyl site in large ribosomal subunit
  - Site holding the tRNA with the amino acid attached.

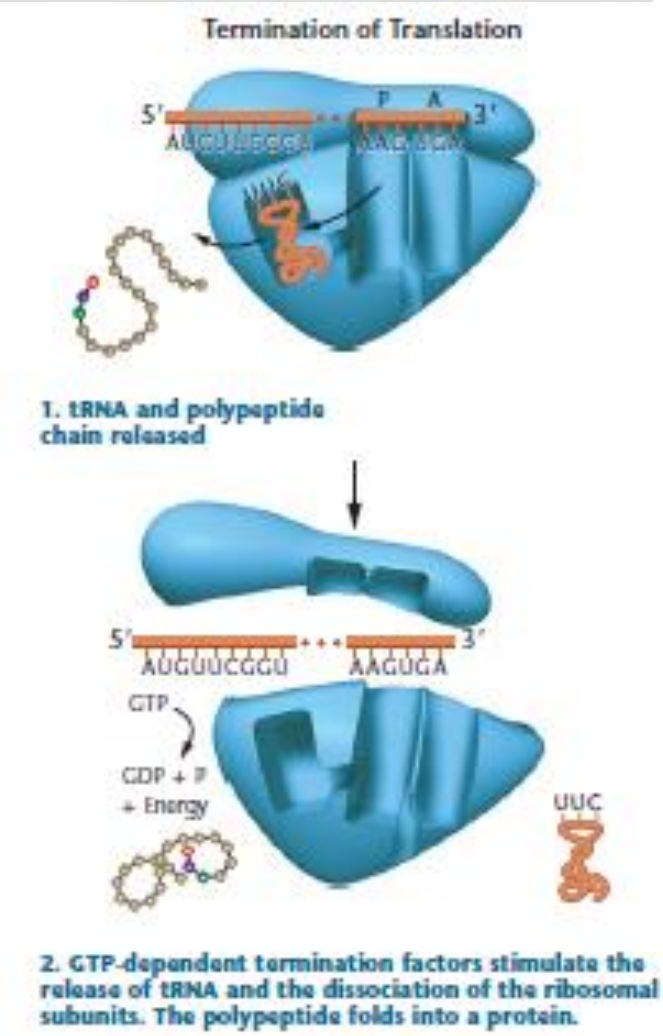
BE ABLE TO DEFINE

- Elongation of translation



BE ABLE TO DEFINE

- Peptidyl transferase
  - Peptidyl transferase catalyzes peptide bond formation between the amino acid on the tRNA at the A site and the growing peptide chain bound to the tRNA in the P site. Is a riboenzyme not protein.
- Termination of translation
  - Termination is signaled by a stop codon (UAG, UAA, UGA) in the A site
  - GTP-dependent release factors cleave the polypeptide chain from the tRNA and release it from the translation complex.



BE ABLE TO DEFINE

- Posttranslational modification of proteins
  - Some proteins may be modified after they have been synthesized. This is called **posttranslational modification**
  - These modifications are crucial to the functional capability of the final protein product

- Several examples of posttranscriptional modification are given below:
  - *The N-terminus amino acid is usually removed or modified*
  - *Individual amino acid residues are sometimes modified*
  - *Carbohydrate side chains are sometimes attached*
  - *Polypeptide chains may be trimmed*
  - *Signal sequences are removed*
  - *Polypeptide chains are often complexed with metals*

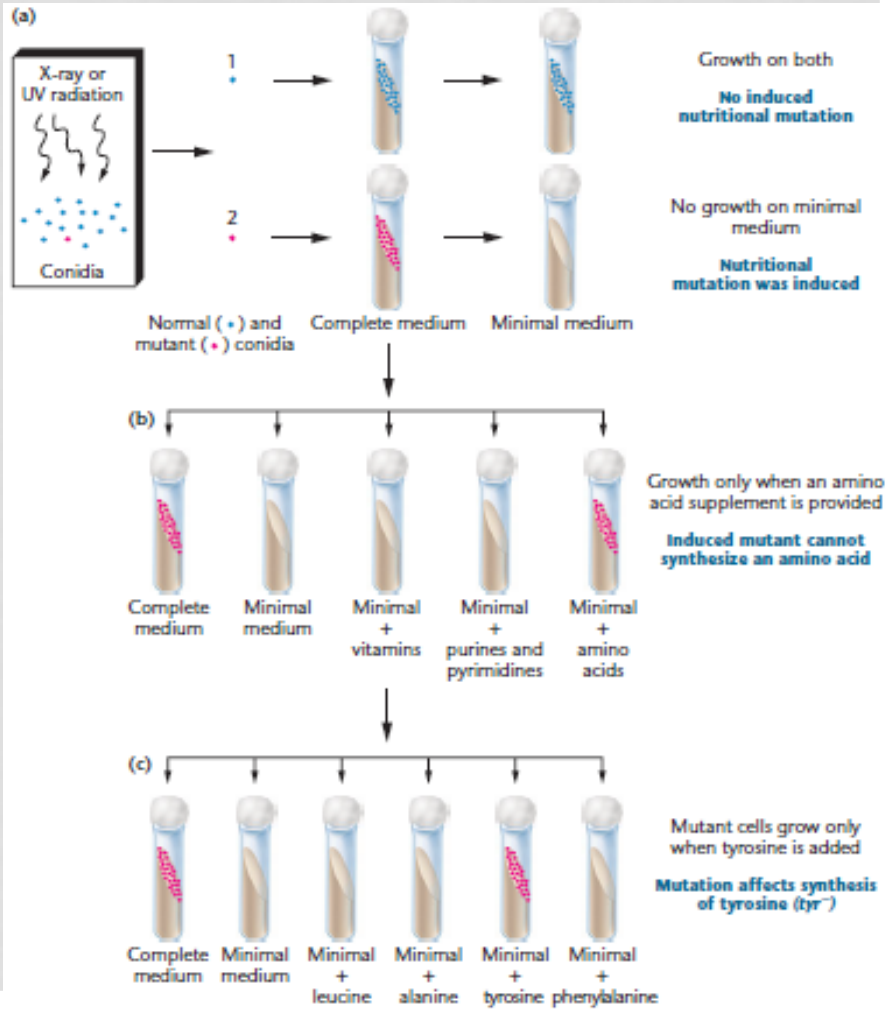
BE ABLE TO DEFINE

- Polysomes or polyribosomes
  - mRNAs with several ribosomes translating at once.
- Kozak sequence
  - A short nucleotide sequence adjacent to the initiation codon that is recognized as the translational start site in eukaryotic mRNA.

- Many eukaryotic mRNAs contain a purine (A or G) three bases upstream from the **AUG** initiator codon, which is followed by a G …((A/G)$_1$N$_2$N$_3$**AUG**G)
- This **Kozak sequence** is considered to increase the efficiency of translation by interacting with the initiator tRNA

BE ABLE TO DEFINE

- Beadle and Tatum
  - Beadle and Tatum showed that nutritional mutations in the bread mold *Neurospora* caused the loss of an enzymatic activity that catalyzes an essential reaction in wild-type organisms



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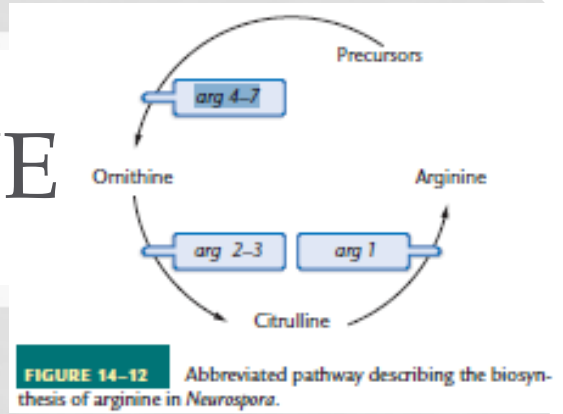


FIGURE 14–12 Abbreviated pathway describing the biosynthesis of arginine in *Neurospora*.

- One gene one enzyme hypothesis
  - Beadle and Tatum's studies supported the one-gene one enzyme hypothesis.
- One gene one polypeptide
  - Not all proteins are enzymes, and some proteins have more than one subunit; therefore the one gene one enzyme hypothesis was modified to the one gene one polypeptide chain hypothesis
  - Studies of human hemoglobin established that one gene encodes for one polypeptide.
- Telomere
  - The heterochromatic terminal region of a chromosome.
  - Maintains chromosome integrity.

BE ABLE TO DEFINE

- Colinearity
  - The linear relationship between the nucleotide sequence in a gene (or the RNA transcribed from it) and the order of amino acids in the polypeptide chain specified by the gene.
  - In colinearity, the order of nucleotides in a gene correlates directly with the order of amino acids in the corresponding polypeptide

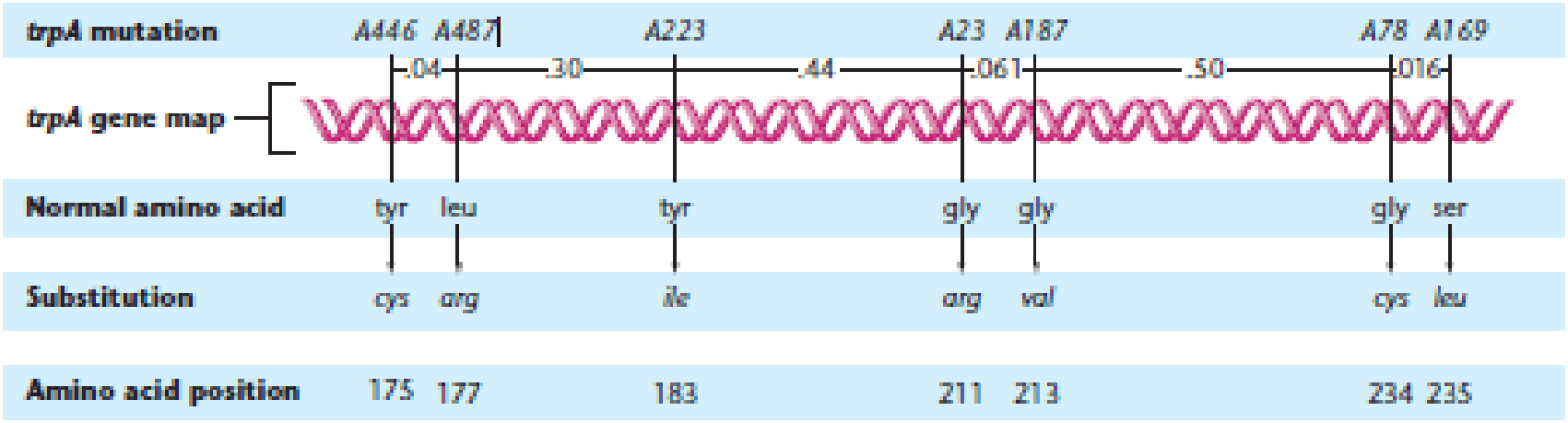
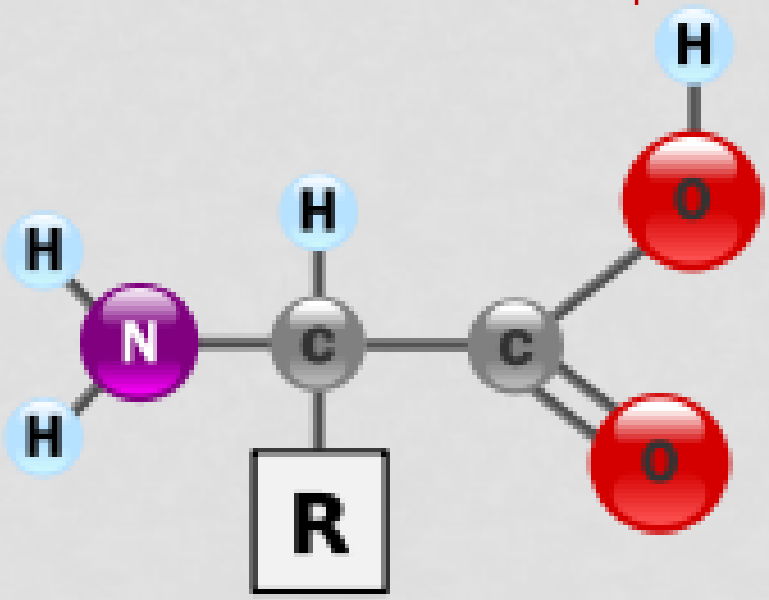


FIGURE 14–15 Demonstration of colinearity between the genetic map of various *trpA* mutations in *E. coli* and the affected amino acids in the protein product. The numbers shown between mutations represent linkage distances.

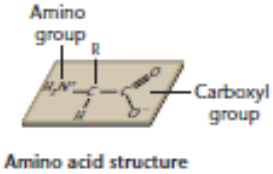
BE ABLE TO DEFINE

- Amino acids
  - Aminocarboxylic acids comprising the subunits that are covalently linked to form proteins.
  - Amino acids all have
    - A carboxyl group
    - An amino group
    - A R (radical) group bound to a central carbon atom
  - The R group of an amino acid confers specific chemical properties.

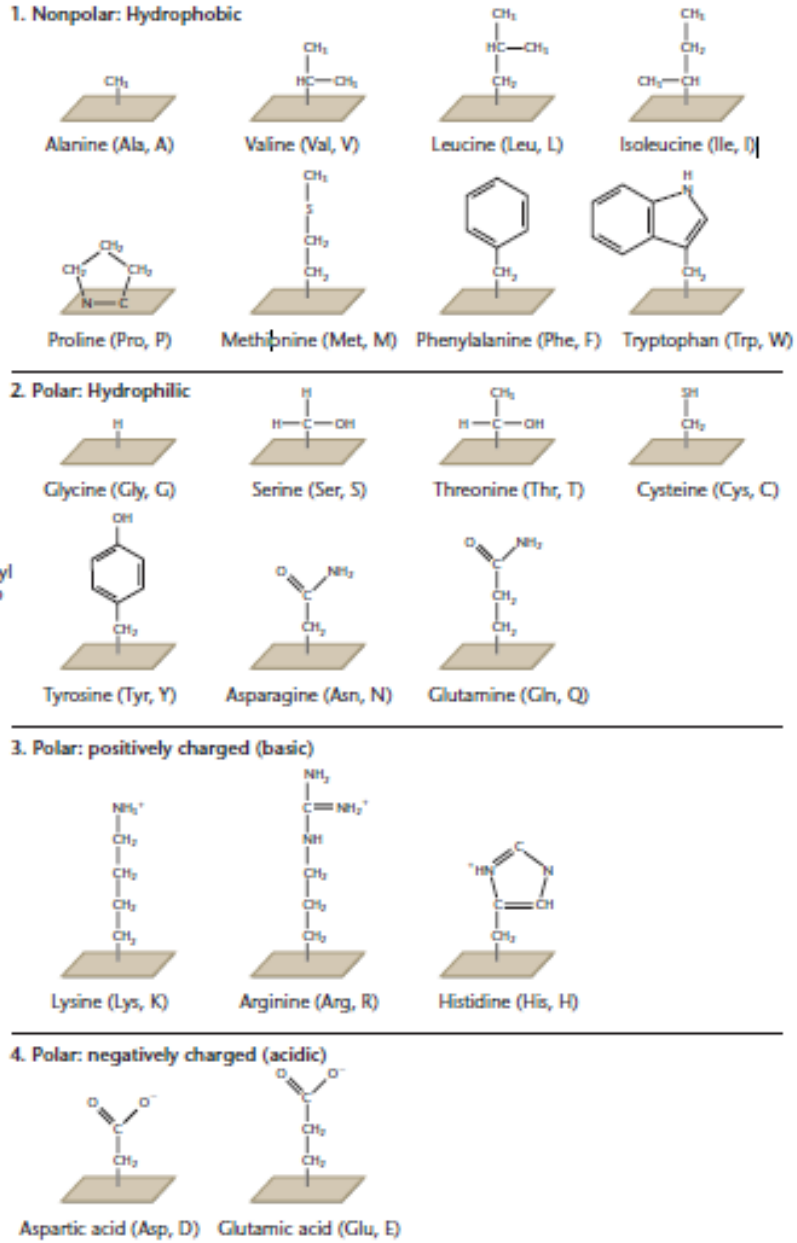


AMINO ACIDS

- Chemical structures and designations of the 20 amino acids encoded by living organisms, divided into four major categories. Each amino acid has two abbreviations in universal use; for example, alanine is designated either Ala or A.



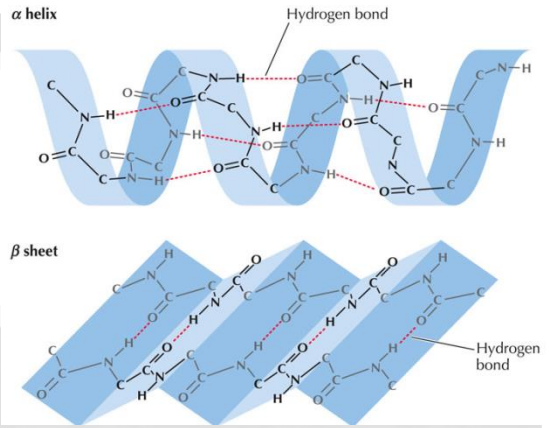
Amino acid structure



BE ABLE TO DEFINE

- Peptide bond
  - The covalent bond between the amino group of one amino acid and the carboxyl group of another amino acid.
  - A peptide bond forms by a dehydration reaction between the carboxyl group of one amino acid and the amino group of another.
- Primary level of protein structure
  - Linear sequence of amino acids from first to last.
- Secondary level of protein structure
  - Localized folding of part of a polypeptide chain (for example alpha helix and beta pleated sheet).
- Tertiary level of protein structure
  - Complete folding of one polypeptide chain

BE ABLE TO DEFINE



- Quaternary level of protein structure
  - Complete folding of two or more polypeptide chains. .
- Alpha helix
  - A common secondary structure of proteins, characterized by a single, spiral chain of amino acids stabilized by hydrogen bonds.
- Beta pleated sheet
  - A secondary structure that occurs in many proteins and consists of two or more parallel adjacent polypeptide chains arranged in such a way that hydrogen bonds can form between the chains. Unlike in an alpha helix where the amino acids form a coil, in beta sheets the amino acids are arranged in a zigzag pattern that forms a straight chain. The proteins in silk have a beta-sheet structure.

BE ABLE TO DEFINE

- Examples of protein function:
  histones, regulation, receptor, transport, enzymes, structural, etc

- Proteins play diverse roles in the body
- **Hemoglobin** and **myoglobin** transport oxygen, which is essential for cellular metabolism
- **Collagen** and **keratin** are structural proteins associated with the skin, connective tissue, and hair of organisms
- **Actin** and **myosin** are contractile proteins found in abundance in muscle tissue
- **Tubulin** is the basis of function of microtubules in mitotic and meiotic spindle fibers

- **Enzymes**, the largest group of proteins, are involved in catabolism or anabolism (make or break bonds or speed up other reactions), a process whereby the energy of activation for a given reaction is lowered (**Figure 14.21**)
- Other examples are
  - the **immunoglobulins**, which function in the immune system of vertebrates
  - **transport proteins**, involved in movement of molecules across membranes
  - some of the **hormones** and their **receptors**, which regulate various types of chemical activity
  - **histones**, which bind to DNA in eukaryotic organisms
  - **Transcription factors**, which regulate gene expression

BE ABLE TO DEFINE

- Domains of protein
  - Proteins are made up of one or more functional Domains.

- Distinct regions made up of specific amino acid sequences are associated with unique functions in proteins
- These sequences constitute protein domains that fold into stable, unique conformations
- Different **protein domains** impart different functional capabilities

- Exon shuffling

- Exons have been proposed to encode protein domains, and **exon shuffling** may be a kind of evolution to form unique genes in eukaryotes

- 18 exons of the LDL receptor protein.
- The exons are organized into five functional domains and one signal sequence….
- For example; EGF region in LDL receptor protein is similar to part of epidermal growth factor and three blood clotting proteins.

BE ABLE TO DEFINE

- Constitutive
  - Constitutive - always on-many at low levels
- Inducible
  - Inducible (adaptive) - turns on when needed
- Repressible
  - On until it is turned

inducible enzyme system An enzyme system under the control of an inducer, a regulatory molecule that acts to block a repressor and allow transcription.

repressible enzyme system An enzyme or group of enzymes whose synthesis is regulated by the intracellular concentration of certain metabolites.

- Enzymes may be:
  - **Constitutive** (always on-many at low levels)
  - **Inducible** (adaptive) (turns on when needed)
  - **Repressible** – On until it is turned off

BE ABLE TO DEFINE

- Positive control
  - transcription occurs only if a regulator molecule directly stimulates RNA production
- Negative control
  - genetic expression occurs unless it is shut off by some form of a regulator molecule

- Regulation of the inducible or repressible type may be under **positive control** or **negative control**
  - Negative control: genetic expression occurs *unless it is shut off by some form of a regulator molecule*
  - Positive control: transcription occurs *only if a regulator molecule directly stimulates RNA production*

BE ABLE TO DEFINE

- Lactose Metabolism in E. coli Inducible System
  - In the presence of lactose, the concentration of the enzymes responsible for its metabolism increases rapidly from a few molecules to thousands per cell
  - The enzymes responsible for lactose metabolism are inducible
  - **Lactose is the inducer.** (Do not confuse with product of the I gene which is the repressor).
- Inducer
  - An effector molecule that activates transcription.
- Polycistronic
  - More then one gene is coded for by one mRNA.

polycistronic mRNA A messenger RNA molecule that encodes the amino acid sequence of two or more polypeptide chains in adjacent structural genes.

BE ABLE TO DEFINE

- Cis-acting sites vs. trans-acting elements
  - Regulatory regions are almost always located upstream of the gene cluster they control and are ***cis*-acting**
  - The molecules that bind these *cis*-acting sites are called ***trans*-acting elements**
  - Binding of a *trans-acting* element at a *cis-acting* site can regulate the gene cluster either negatively (by turning off transcription) or positively (by turning on transcription of the genes in the cluster)

***cis*-acting sequence** A DNA sequence that regulates the expression of a gene located on the same chromosome. This contrasts with a trans-acting element where regulation is under the control of a sequence on the homologous chromosome. See also *trans-acting element*.

***trans*-acting element** A gene product (usually a diffusible protein or an RNA molecule) that acts to regulate the expression of a target gene.

BE ABLE TO DEFINE

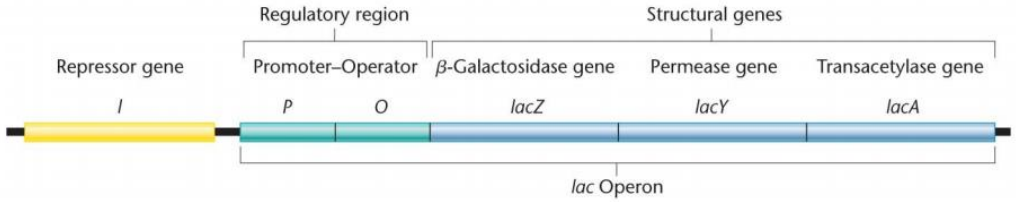
- Operon
  - A genetic unit consisting of one or more structural genes encoding polypeptides, and an adjacent operator gene that regulates the transcriptional activity of the structural gene or genes.
  - Jacob and Monod proposed the operon model, in which a group of genes is regulated and expressed together as a unit.

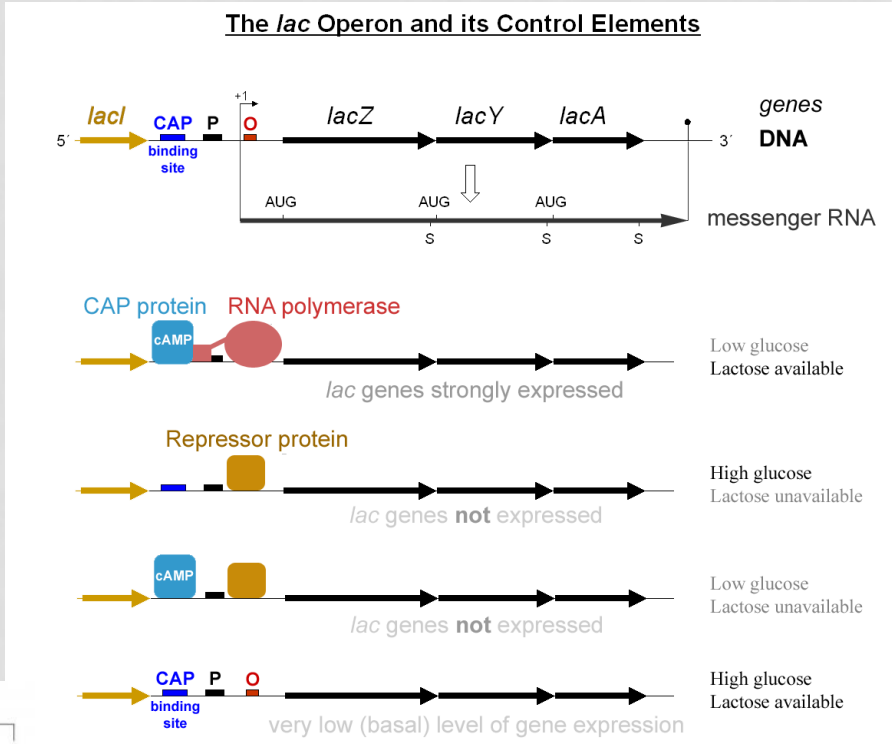
BE ABLE TO DEFINE

- Lac operon
  - Analysis of lac expression in the absence or presence of lactose in partial diploid merozygotes was used to prove the operon model for the lac operon

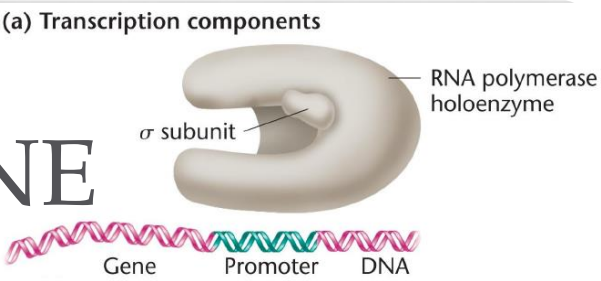
- The *lac* operon is subject to negative control because transcription occurs only when the repressor fails to bind to the operator region

The ***lac* operon** has three structural genes, *lacZ*, *lacY*, and *lacA*, with an upstream regulatory region consisting of an operator and a promoter (**Figure 16.1**)



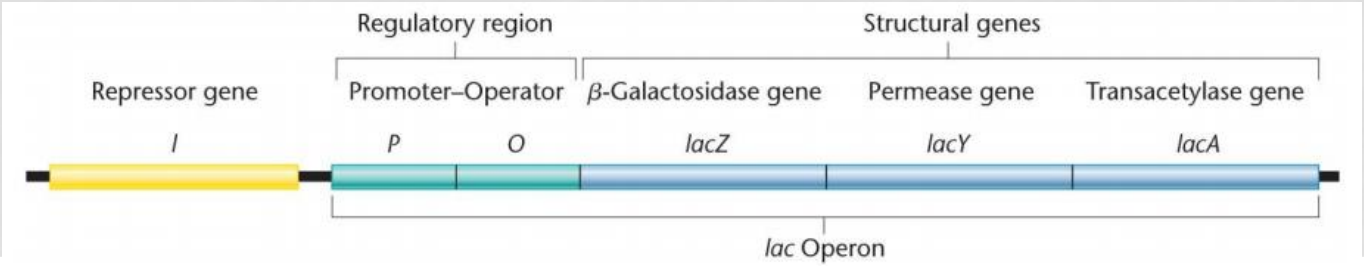


BE ABLE TO DEFINE



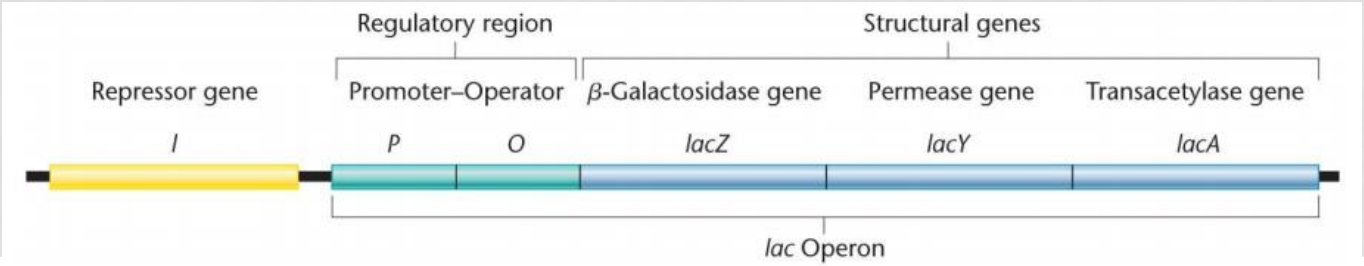
- The σ subunit is responsible for promoter recognition (initiation of transcription)

- Promoter
  - promoter element - An upstream regulatory region of a gene to which RNA polymerase binds prior to the initiation of transcription.
- Operator
  - operator region - In bacterial DNA, a region that interacts with a specific repressor protein to regulate the expression of an adjacent gene or gene set.
- Regulatory site
  - A DNA sequence that functions in the control of expression of other genes, usually by interaction with another molecule.



BE ABLE TO DEFINE

- Structural genes of the lac operon
  - Genes coding for the primary structure of an enzyme are called structural genes.
  - The lac operon has three structural genes
    - *lacZ* – encodes for β-galactosidase, an enzyme that converts the disaccharide lactose to its monosaccharides glucose and galactose.
    - *lacY* – specifies the primary structure of permease, an enzyme that facilitates the entry of lactose into the bacteria cell.
    - *lacA* – codes for the enzyme transacetylase, which may be involved in the removal of toxic by products of lactose digestion from the cell.



BE ABLE TO DEFINE

- Constitutive mutants of the lac operon

A Comparison of Gene Activity (+ or −) in the Presence or Absence of Lactose for Various *E. coli* Genotypes

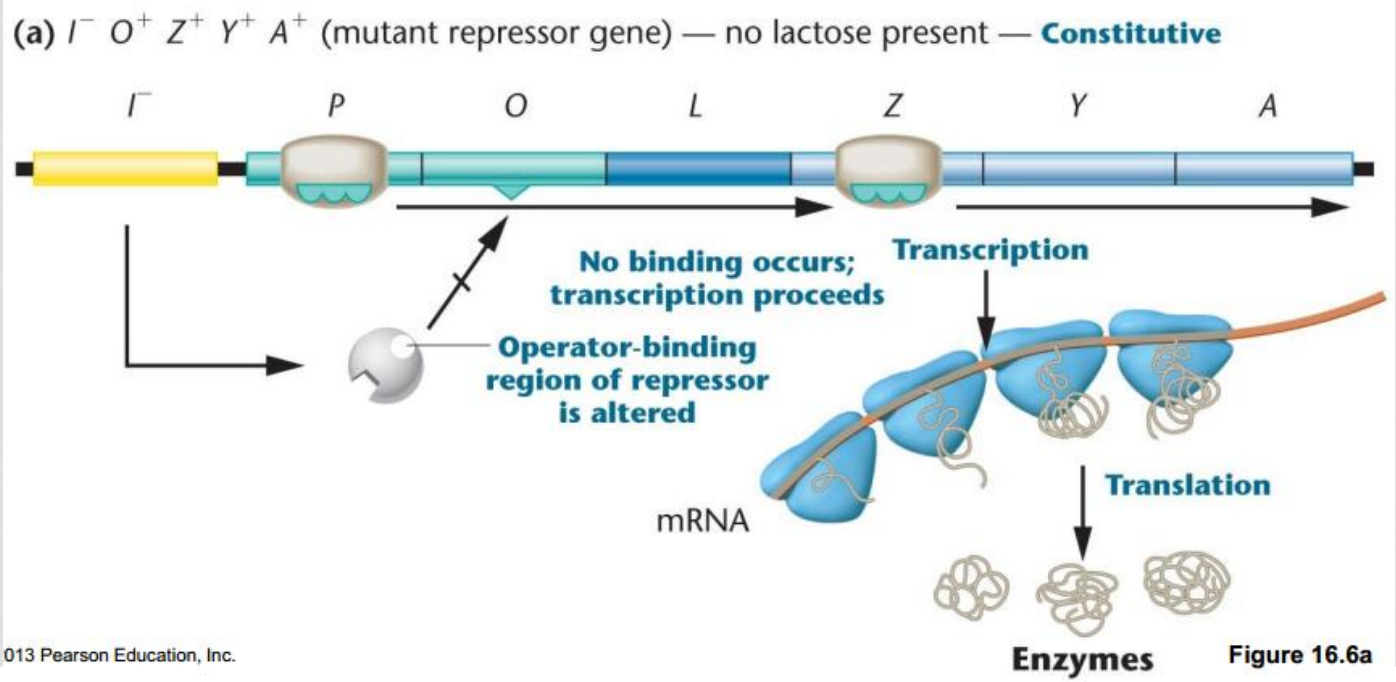
	Genotype	Presence of β-Galactosidase Activity: Lactose Present	Lactose Absent
	$I^+O^+Z^+$	+	−
A.	$I^+O^+Z^-$	−	−
	$I^-O^+Z^+$	+	+
	$I^+O^CZ^+$	+	+
B.	$I^-O^+Z^+/F'I^+$	+	−
	$I^+O^CZ^+/F'O^+$	+	+
C.	$I^+O^+Z^+/F'I^-$	+	−
	$I^+O^+Z^+/F'O^C$	+	−
D.	$I^SO^+Z^+$	−	−
	$I^SO^+Z^+/F'I^+$	−	−

Note: In parts B to D, most genotypes are partially diploid, containing an F factor plus attached genes (F′).

BE ABLE TO DEFINE

- Constitutive mutants of the lac operon

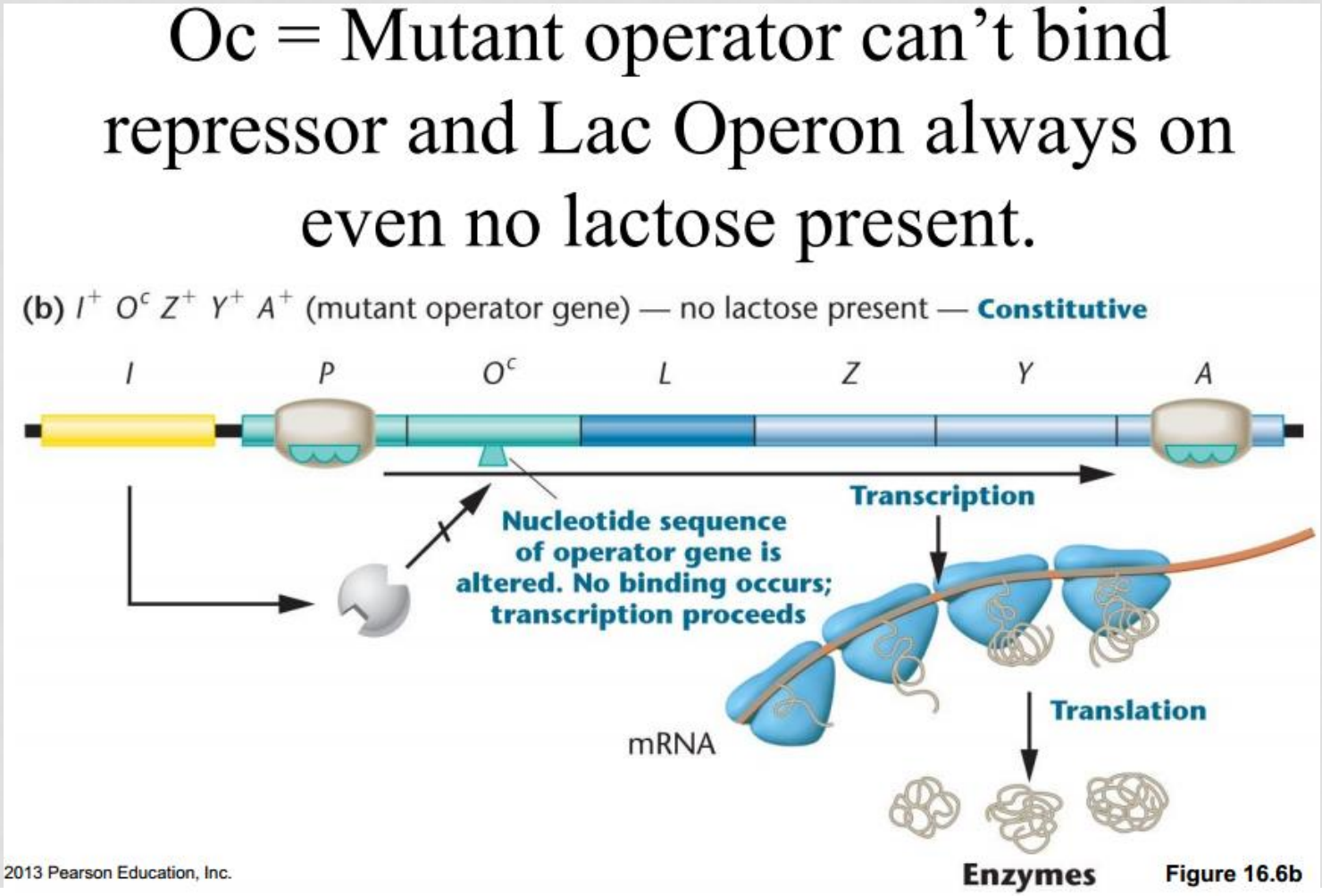
I- = Mutant repressor can't bind to operator and Lac Operon always on even no lactose present.



BE ABLE TO DEFINE

- Constitutive mutants of the lac operon

Oc = Mutant operator can't bind repressor and Lac Operon always on even no lactose present.



BE ABLE TO DEFINE

- Constitutive mutants of the lac operon

IS = Mutant repressor can't bind lactose and Lac Operon always off even when lactose is present.

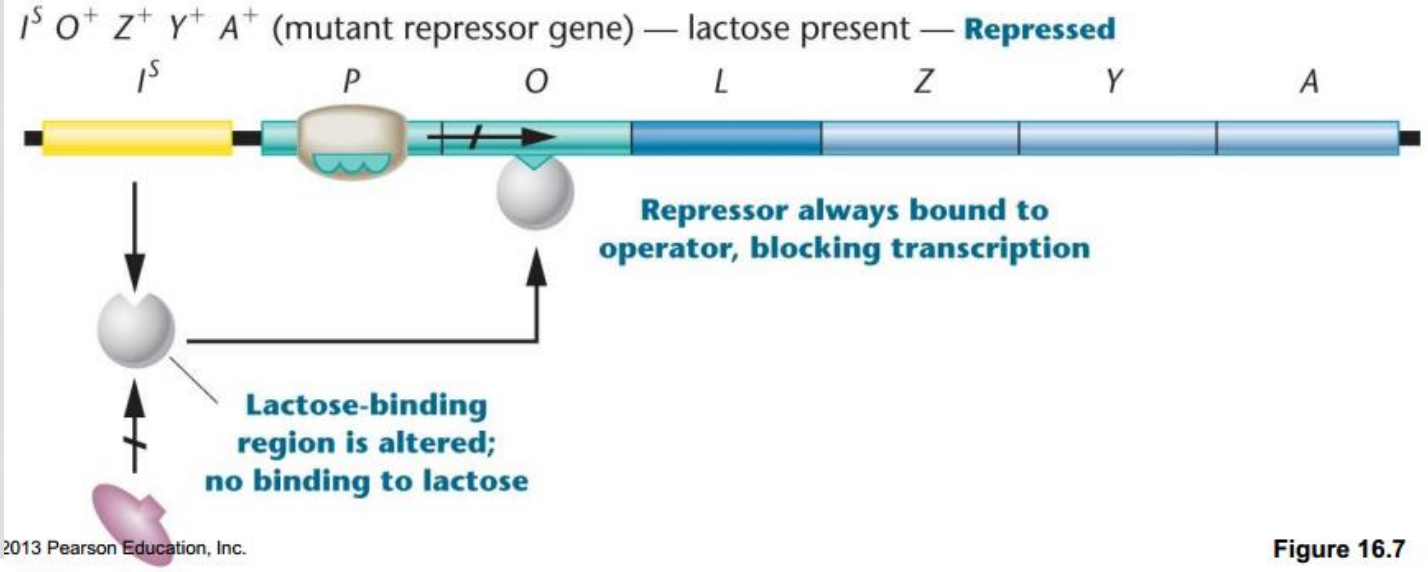


Figure 16.7

BE ABLE TO DEFINE

- Jacob and Monod
  - Proposed the operon model, in which a group of genes is regulated and expressed together as a unit.
- Diploid merozygotes
  - merozygote - A partially diploid bacterial cell containing, in addition to its own chromosome, a chromosome fragment introduced into the cell by transformation, transduction, or conjugation.
  - Analysis of lac expression in the absence or presence of lactose in partial diploid merozygotes was used to prove the operon model for the lac operon
- Catabolite- activating protein (CAP)
  - The catabolite-activating protein (CAP) is involved in repressing expression of the lac operon when glucose is present.

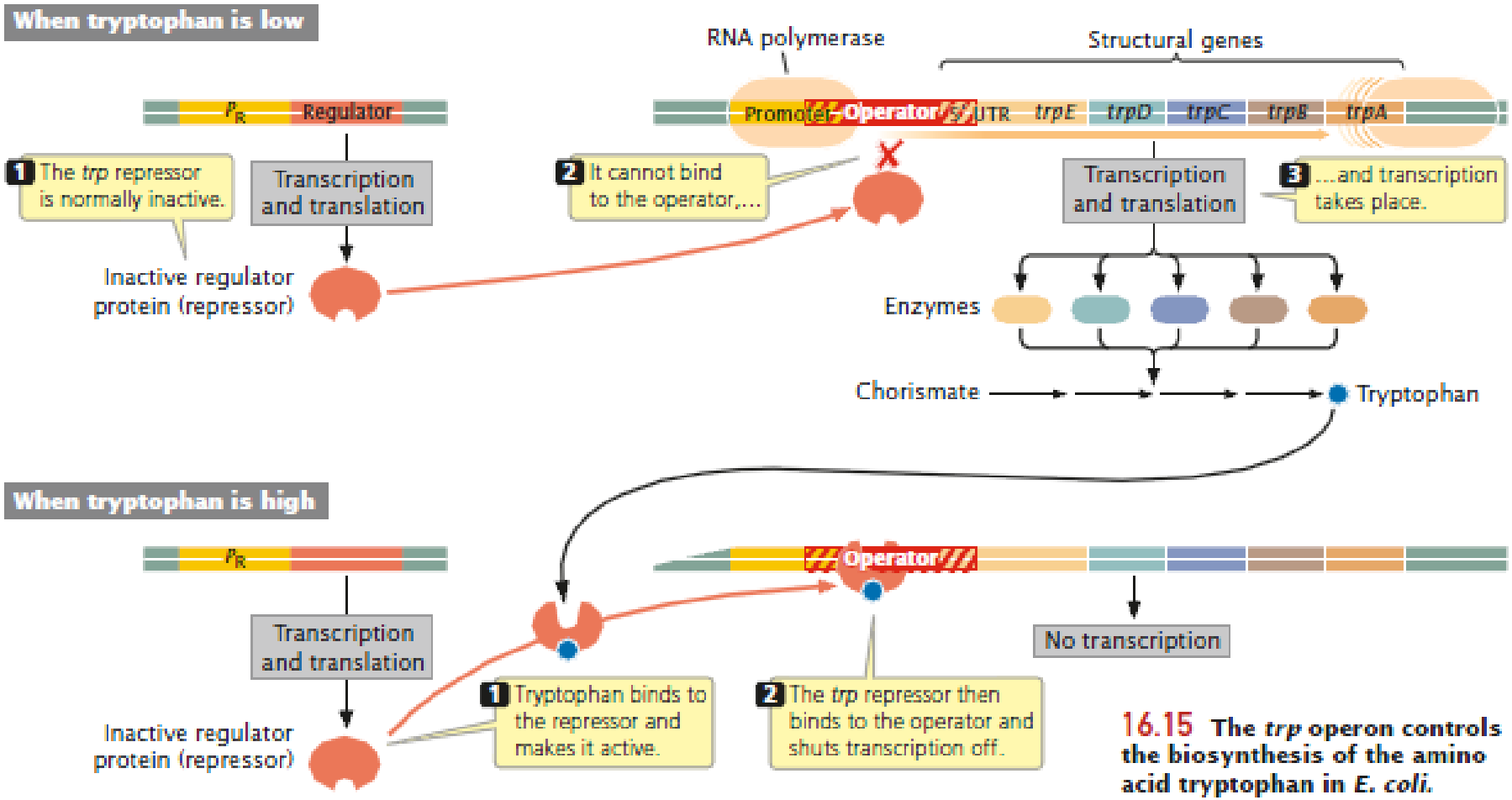
BE ABLE TO DEFINE

- Catabolite repression
  - Presence of glucose inhibiting the Lac operon (and others) is called catabolite repression.
- Repressor
  - A protein that binds to a regulatory sequence adjacent to a gene and blocks transcription of the gene.
- Corepressor
  - Substance that inhibits transcription in a repressible system of gene regulation; usually a small molecule that binds to a repressor protein and alters it so that the repressor is able to bind to DNA and inhibit transcription.
- Attenuator
  - A nucleotide sequence between the promoter and the structural gene of some bacterial operons that regulates the transit of RNA polymerase, reducing transcription of the related structural gene.
- Attenuation
  - In attenuation, transcription begins at the start site, but termination takes place prematurely, before the RNA polymerase even reaches the structural genes.

BE ABLE TO DEFINE

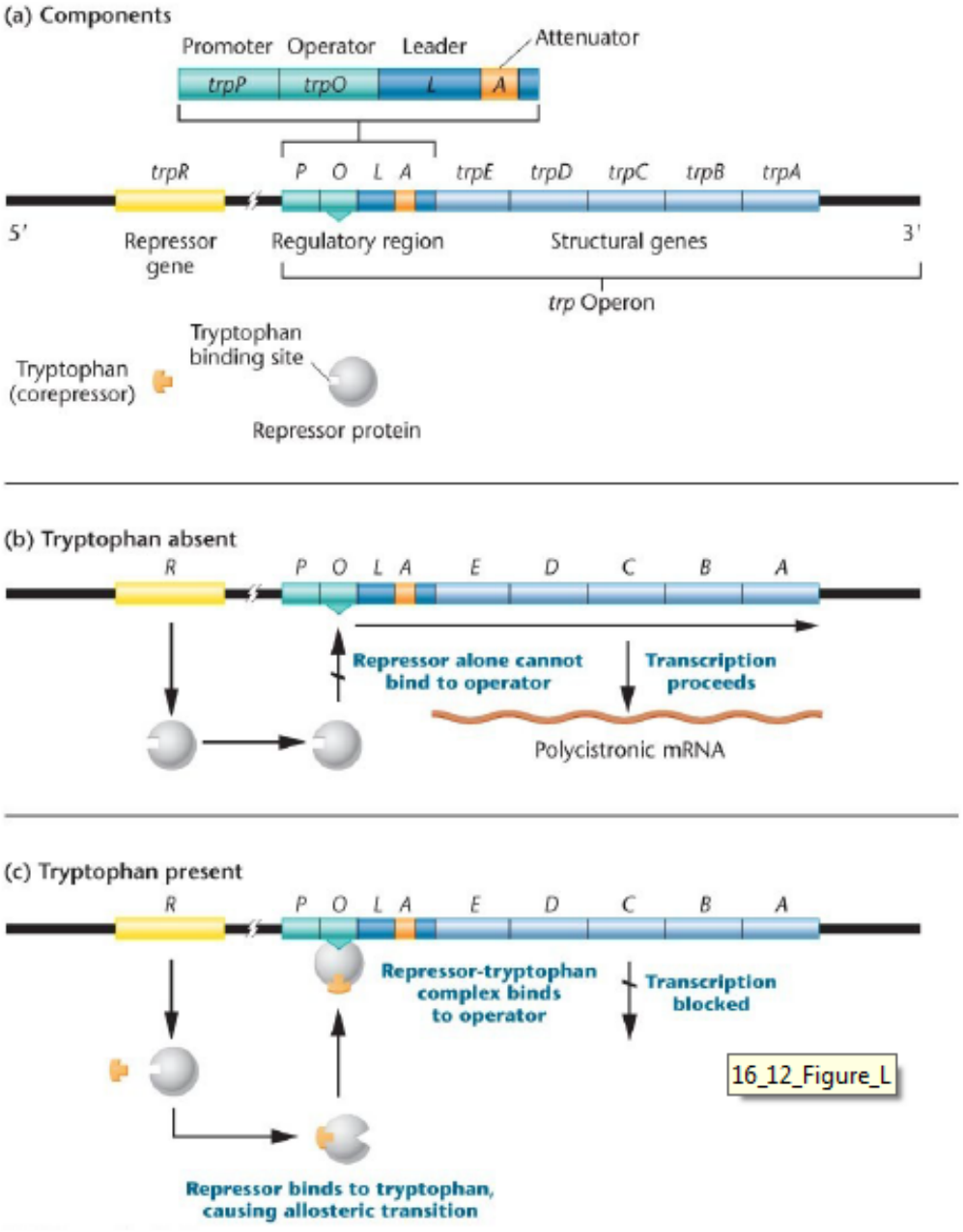
- Tryptophan (trp) Operon in E. coli – repressible
  - There are five enzymes involved in tryptophan production, and they are part of an operon.
  - In the presence of tryptophan, the operon is repressed and none of the enzymes are produced.
  - When tryptophan (corepressor) is present, the system is repressed and enzymes are not made.
  - In the absence of tryptophan, an inactive repressor is made that cannot bind to the operator (O), thus allowing transcription to proceed.
  - In the presence of tryptophan, it binds to the repressor causing allosteric transition to occur. This complex binds to the operator region, leading to repression of the operon.
  - In the addition to operator regulation of transcription there is additional regulation called attenuation.
  - Transcription of the leader region of the *trp* operon can occur even when the operon is repressed in the presence of tryptophan (attentuation).
  - In the presence of tryptophan, the hairpin structure called a transcriptional terminator forms. In the absence of tryptophan a different hairpin forms and acts as an antiterminator.

TRYPTOPHAN (TRP) OPERON IN E. COLI – REPRESSIBLE



16.15 The *trp* operon controls the biosynthesis of the amino acid tryptophan in *E. coli*.

TRYPTOPHAN (TRP) OPERON IN E. COLI – REPRESSIBLE



TRYPTOPHAN (TRP) OPERON IN E. COLI – REPRESSIBLE

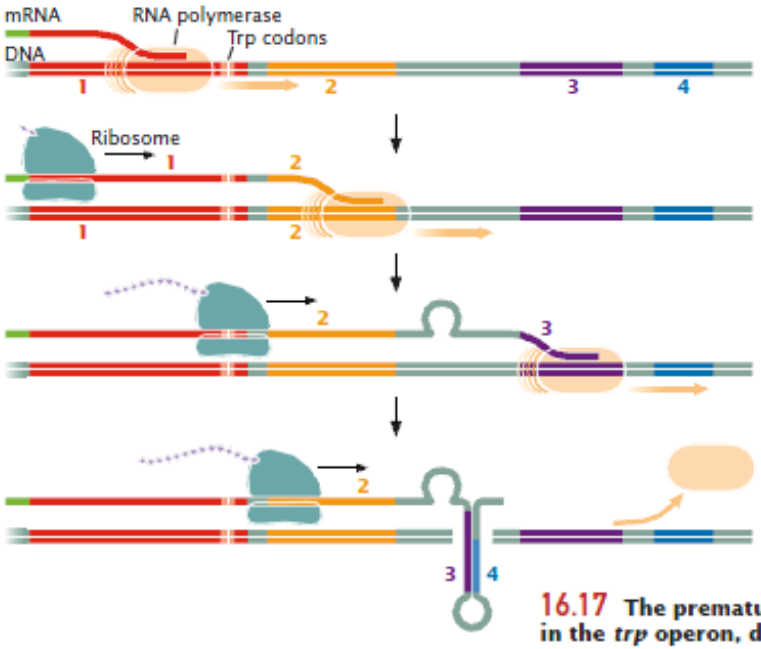
When tryptophan is high

(a) RNA polymerase begins transcribing DNA, producing region 1 of the 5′ UTR.

(b) A ribosome binds to the 5′ end of the 5′ UTR and translates region 1, while region 2 is being transcribed.

(c) RNA polymerase transcribes region 3. The ribosome does not stall at the Trp codons, because tryptophan is abundant.

(d) The ribosome covers part of region 2, preventing it from pairing with region 3. Region 4 is transcribed and pairs with region 3, producing the attenuator that terminates transcription.



16.17 **The premature termination of transcription (attenuation) takes place in the *trp* operon, depending on the cellular level of tryptophan.**

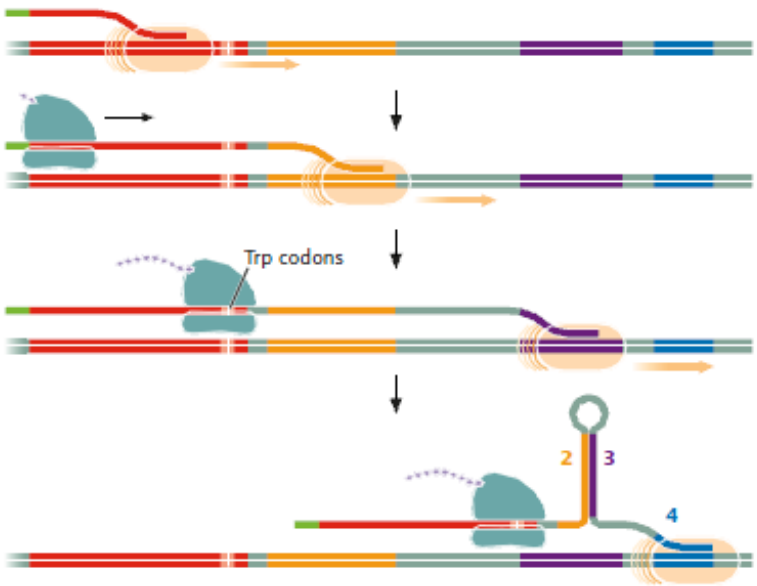
When tryptophan is low

(e) RNA polymerase begins transcribing the DNA, producing region 1 of the 5′ UTR.

(f) A ribosome attaches to the 5′ end of the 5′ UTR and translates region 1 while region 2 is being transcribed.

(g) The ribosome stalls at the Trp codons in region 1 because tryptophan is low. Because the ribosome is stalled, region 2 is not covered by the ribosome when region 3 is transcribed.

(h) When region 3 is transcribed, it pairs with region 2. When region 4 is transcribed, it cannot pair with region 3, because region 3 is already paired with region 2; the attenuator never forms, and transcription continues.



BE ABLE TO DEFINE

- Terminator
  - Terminator - Sequence of DNA nucleotides that causes the termination of transcription.
  - Hairpin structures formed in the presence of tryptophan that act as a transcriptional terminator and causes transcription to stop.
- Antiterminator
  - Antiterminator - Protein or DNA sequence that inhibits the termination of transcription.
  - Hairpin structured formed in the absence of tryptophan that act as antiterminator and allow transcription to proceed.
- Leader region of mRNA and trp regulation
  - The leader region (part of the mRNA at beginning) can form two different conformations depending on the presence or absence of tryptophan.
  - The leader region contains two tryptophan codons.
  - The antiterminator hairpin structure forms in the absence of tryptophan because the ribosome stalls at the trp codons because there is not adequate charged $tRNA^{trp}$
  - In the presence of tryptophan, the ribosome proceeds through this sequence and the terminator hairpin can form.

BE ABLE TO DEFINE

5' – ^{m}CG – 3'
3' – GC^{m} – 5'

- DNA methylation
  - DNA methylation is associated with decreased gene expression.
  - Methylation can repress transcription by binding to transcription factors of DNA
  - Methylation occurs most often on the cytosine of CG doubled in DNA.
- CAAT
  - CAAT box - A highly conserved DNA sequence found in the untranslated promoter region of eukaryotic genes. This sequence is recognized by transcription factors.
  - promoter region – mutations in promoter region CAAT lower transcription
- TATA
  - Goldberg–Hogness box - A short nucleotide sequence 20–30 bp upstream from the initiation site of eukaryotic genes to which RNA polymerase II binds. The consensus sequence is TATAAAA. Also known as a TATA box.
  - promoter region – mutations in promoter region TATA lower transcription

BE ABLE TO DEFINE

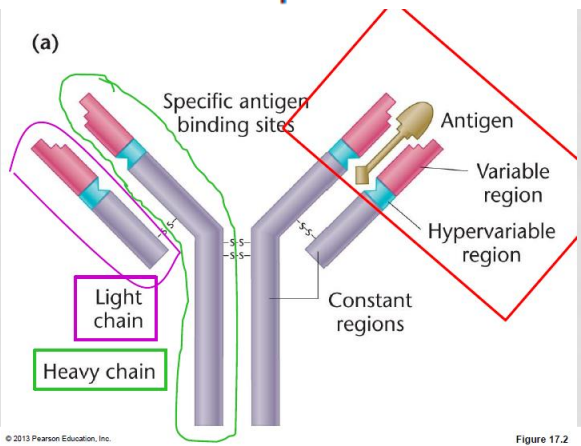
- Genomic rearrangement
  - DNA amplification
    - Tandemly duplicated genes for amplification
  - DNA rearrangement
    - New genes are made by rearrangement
      - Example White blood cells - antibodies

- Genomic DNA in most organisms is stable; however, some gene regulation by DNA rearrangement exists
  - DNA (gene) amplification
  - DNA rearrangements during developmental regulation
    - Creation of new gene from gene fragments
    - Switch in expression of genes due to recombination
    - Loss of DNA sequences in somatic cells

BE ABLE TO DEFINE

- DNA rearrangement
  - New genes are made by rearrangement
    - Example White blood cells - antibodies

– DNA rearrangements during developmental regulation
  - Creation of new gene from gene fragments
  - Switch in expression of genes due to recombination
  - Loss of DNA sequences in somatic cells



- **Antigen recognition** allows the immune system to bind to foreign substances (**antigens**)
- **Humoral immunity** involves production of **immunoglobins** (antibodies) that directly bind to antigens
  - Synthesized by blood cells called **B lymphocytes** (B cells) that are made up of four variable regions allowing recognition of a specific antigen (**Figure 17.2**)
- Two other mechanisms further increase antibody diversity
  - Imprecise recombination between any particular pair of LV and J regions shows considerable variation
  - High **hypermutation** (random somatic mutation) introduces more variation into the LVJ region's sequence

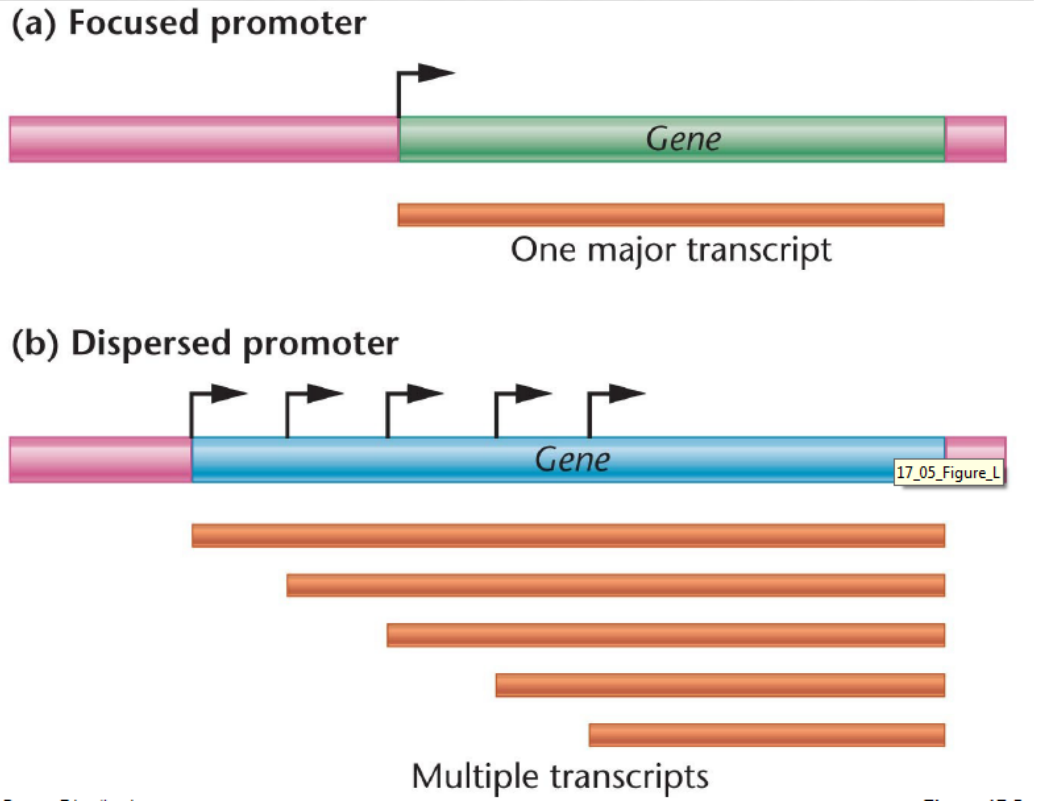
BE ABLE TO DEFINE

- Chromosome territory
  - During interphase each chromosome occupies a discrete area called chromosome territory.
- Chromatin remodeling
  - Chromatin remodeling is an important step in gene regulation and involves changes to either the nucleosome or DNA.
  - chromatin remodeling - A process in which the structure of chromatin is altered by a protein complex, resulting in changes in the transcriptional state of genes in the altered region.
- Histone acetyltransferase enzymes (HATs)
  - Histone acetylation of the nucleosome is catalyzed by histone acetyltransferase enzymes (HATs) and is associated with increased transcription

BE ABLE TO DEFINE

- Focused promoters and dispersed promoters

- **Promoters** are nucleotide sequences that serve as recognition sites for the transcription machinery
  - –Critical for initiation of transcription
  - –Located adjacent to genes regulated
- Great diversity exits in promoters in terms of structure and function (Figure 17.5)
  - **–Focused promoters**
    - Specific transcription start site
  - **–Dispersed promoters**
    - Several start sites

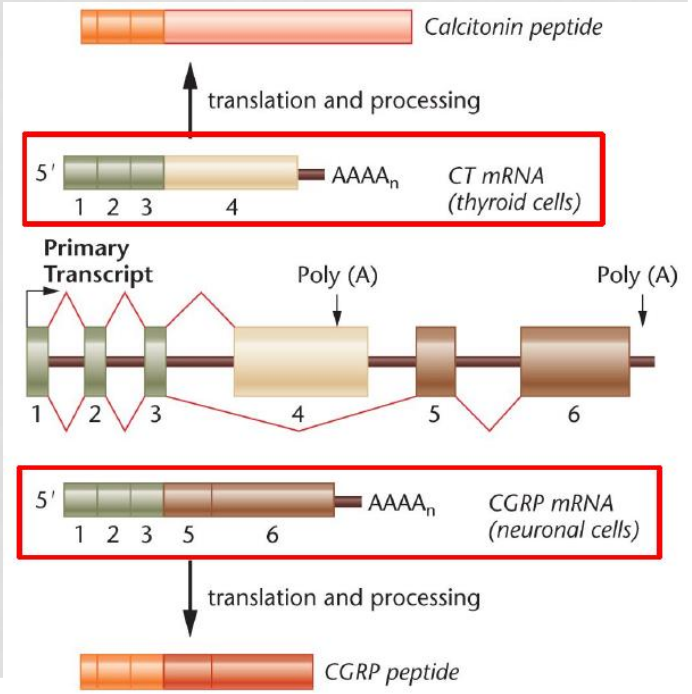


BE ABLE TO DEFINE

- Posttranscriptional regulation
  - Posttranscriptional gene regulation occurs at all the steps from RNA processing to protein modification
  - Transcriptional control plays a major role of regulation in eukaryotes.
  - Posttranscriptional regulation also plays significant role
    - Removal of introns and splicing together of exons
    - Addition of a cap and poly-A tail
    - Translation
    - Stability.
  - Includes
    - Alternative splicing
    - mRNA stability
    - Translational regulation
    - Protein stabilty.

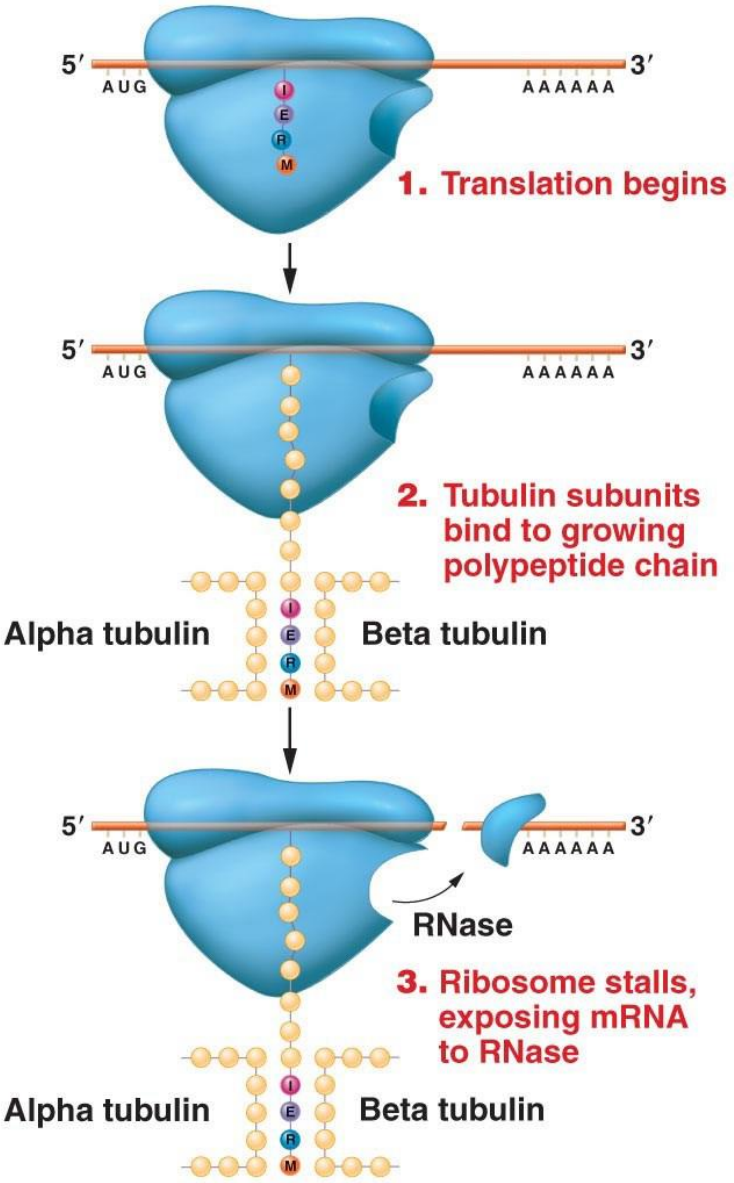
BE ABLE TO DEFINE

- Alternative splicing
  - Alternative splicing can generate different forms of rRNA from identical pre-mRNA, giving rise to a number of proteins from one gene.
  - As a result the number of proteins that a cell can make (its proteome) is not directly related to the number of genes in the genome.
  - Approximately two-thirds of human genes undergo alternative splicing.
  - Changes in splicing patterns can have many different effects on the translated protein. Even small changes can alter the proteins enzymatic activity, receptor binding capacity, or protein localization in the cell.



BE ABLE TO DEFINE

- mRNA stabilty
  - A way to control mRNA stability is through translation level control – translation of the message controls its stability.
  - The steady-state level of an mRNA is its amount in the cell. This is determined by a combination of the transcription rate and the rate of mRNA degradation.
  - mRNA may be degraded along three general pathways.
    - Shortening of the poly-A tail
    - Decapping enzymes removing the 7'methylguanosine cap.
    - Internal cleavage by endonuclease.



BE ABLE TO DEFINE

- translational regulation
  - Translation can be regulated to produce the correct quantity of a protein

- Translation plays a role in mRNA stability for the tubulin genes and has been proposed as a regulatory mechanism for other genes as well **(9th edition…Figure 18.21).**
- This type of translational regulation is known as **autoregulation**.

BE ABLE TO DEFINE

- protein stability
  - Posttranslational stability of the protein can be modulated
    - P53 protein
  - A protein can be modified after translation to change its structure and hence its activity

BE ABLE TO DEFINE

- RNA interference (RNAi), Dicer, small interfering RNAs (siRNAs), RNA-induced silencing complex (RISC complex)
  - Short RNA molecules regulate gene expression in the cytoplasm of plants, animals, and fungi by repressing translation and triggering mRNA degradation
  - This form of sequence-specific posttranscriptional regulation is known as **RNA interference (RNAi)**
  - Together, these phenomena are known as **RNA-induced gene silencing**

RNA interference (RNAi) Inhibition of gene expression in which a protein complex (RNA-induced silencing complex, or RISC), containing a partially complementary RNA strand binds to an mRNA, leading to degradation or reduced translation of the mRNA.

- RNA interference uses a protein called **Dicer** to cleave double-stranded RNA molecules into **small interfering RNAs (siRNAs)** and **micro RNAs** (miRNAs)
  - siRNAs and miRNAs repress mRNA translation and trigger degradation
    - Inhibit transcription of specific genes by associating with **RNA-induced initiation of transcription (RITS)** and **RNA-induced silencing complex (RISC complex)** or **RISC**

BE ABLE TO DEFINE

- Rho (ρ) termination factor – Rho dependent vs rho independent.
  - The enzyme traverses the entire gene until a termination nucleotide sequence is encountered
  - In bacteria this termination is transcribed into RNA and causes the newly formed transcript to fold back on itself, forming what is called a hairpin structure held together by hydrogen bonds.
  - In some cases, termination depends on the rho (ρ) termination factor

BE ABLE TO DEFINE

- Rho (ρ) termination factor – Rho dependent vs rho independent.
  - (ρ) -independent transcription termination proceeds in the absence of any soluble factors other than the DNA template, RNAP and the adjacent RNA chain. Termination usually occurs within a sequence which would generate a stretch of U residues in the RNA product at least 4 or 5 long, 15 to 20 nucleotides downstream from a sequence of DNA which is GC rich and shows dyad symmetry.
  - (ρ) -dependent transcription termination - These termination events are catalyzed by a protein factor called ρ which interacts in an unknown manner with RNAP and the ρ -dependent termination site to cause termination of transcription.

CROSSWORD

Across

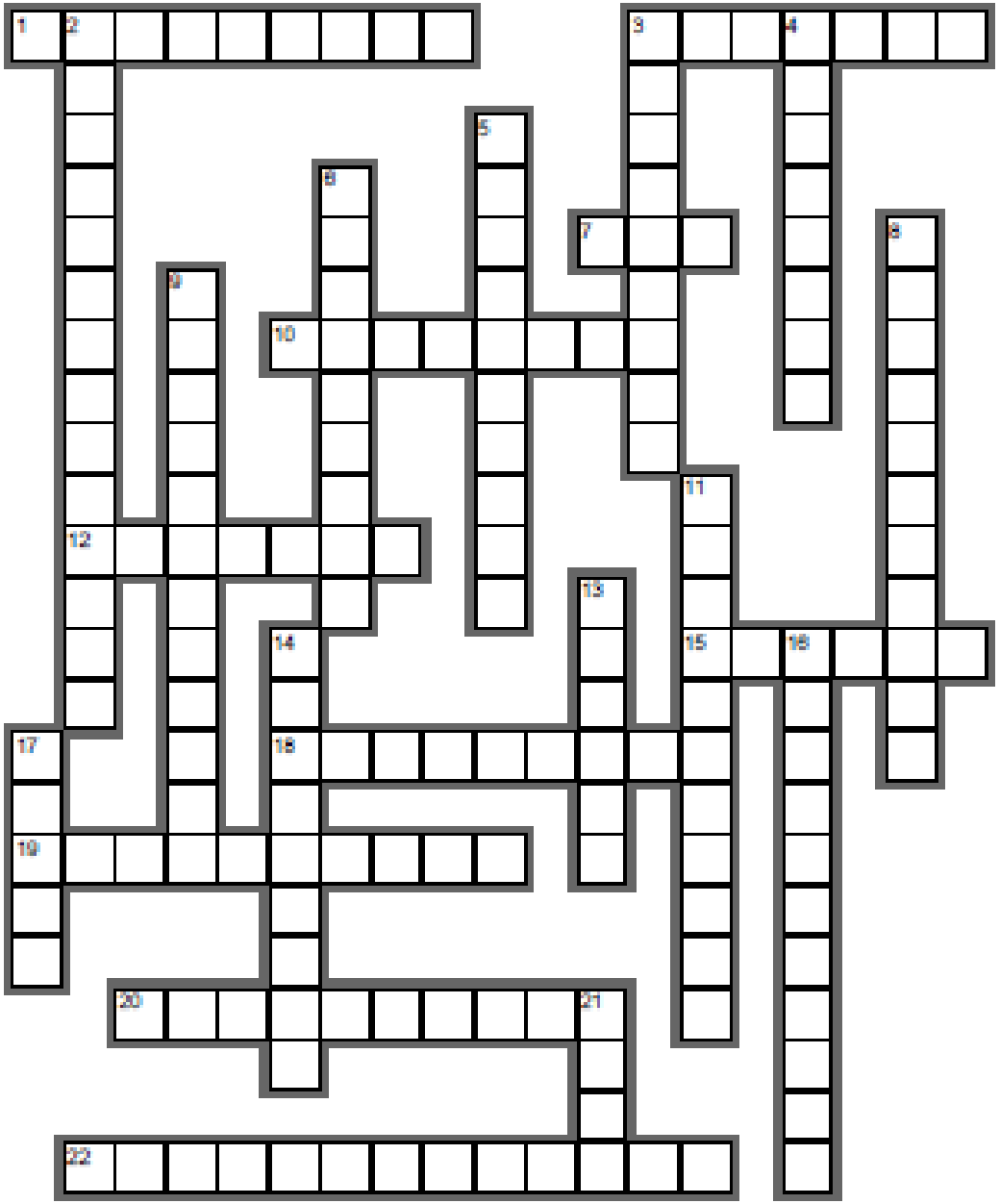
1. Part of tRNA that binds to the codon of mRNA.
3. ___ is the part of a nucleotide sequence coding for a single gene.
7. At the 5' end of the eukaryotic mRNA is a modified G, that is called a 5' _____
10. Change in one base of DNA that creates a premature stop codon.
12. The __ structure of protein is the linear sequence of amino acids from first to last.
15. This part of the primary RNA transcript is removed in processing to a mature RNA transcript (in eukaryotes it is removed mainly from mRNA).
18. __ enzymes are enzymes whose transcription is turned on when needed.
19. The genetic code has more codons than amino acids (64 codons code for 20 amino acids)
20. Presence of glucose inhibiting the Lac operon (and others) is called ___ repression
22. The Lac operon has __ mRNA, where one mRNA codes for three gene products.

CROSSWORD

Down

2. After initiation of translation each nucleotide is part only one codon means the genetic code is __.
3. No extra bases separating codons in the genetic code
4. The __ structure is the complete folding of one polypeptide chain.
5. A change in DNA sequence that yields an addition or subtraction of 1 or 2 (or multiples of 1 or 2 bases) excluding multiples of 3 bases
6. The __ structure of protein is the localized folding of part of a polypeptide chain (like alpha helix or beta pleated sheet).
8. Each codon for only one aa means the code is __.
9. __ proteins are those proteins whose transcription is always on in a cell...many at low levels.
11. The order of nucleotides in a gene correlates directly with the order of amino acids in the corresponding polypeptide.
13. The __ hypothesis concerns the specificity of the anticodon for first two nucleotides of the codon.
14. The genetic code is the same in all species means the genetic code is __.
16. Four syllable word for stop codon is the __ codon.
17. Three bases that code for one amino acid
21. Codes for amino acid sequence in mRNA of eukaryotes

CROSSWORD



CONCEPT QUESTIONS

- Part of tRNA that binds to the codon of mRNA.
  - Anticodon
- ___ is the part of a nucleotide sequence coding for a single gene.
  - Cistron
- At the 5' end of the eukaryotic mRNA is a modified G, that is called a 5' _____.
  - Cap
- Change in one base of DNA that creates a premature stop codon.
  - Nonsense

CONCEPT QUESTIONS

- The __ structure of protein is the linear sequence of amino acids from first to last.
  - Primary
- This part of the primary RNA transcript is removed in processing to a mature RNA transcript (in eukaryotes it is removed mainly from mRNA).
  - Intron
- __ enzymes are enzymes whose transcription is turned on when needed.
  - Inducible
- The genetic code has more codons than amino acids (64 codons code for 20 amino acids)
  - Degenerate

CONCEPT QUESTIONS

- Presence of glucose inhibiting the Lac operon (and others) is called ___ repression
  - Catabolite
- The Lac operon has __ mRNA, where one mRNA codes for three gene products.
  - Polycistronic
- After initiation of translation each nucleotide is part of only one codon means the genetic code is __.
  - Nonoverlapping
- No extra bases separating codons in the genetic code
  - Commaless

CONCEPT QUESTIONS

- The __ structure is the complete folding of one polypeptide chain.
  - Tertiary
- A change in DNA sequence that yields an addition or subtraction of 1 or 2 (or multiples of 1 or 2 bases) excluding multiples of 3 bases
  - Frameshift
- The __ structure of protein is the localized folding of part of a polypeptide chain (like alpha helix or beta pleated sheet).
  - Secondary
- Each codon for only one aa means the code is __.
  - Unambiguous

CONCEPT QUESTIONS

- __ proteins are those proteins whose transcription is always on in a cell...many at low levels.
  - Constitutive
- The order of nucleotides in a gene correlates directly with the order of amino acids in the corresponding polypeptide.
  - Colinearity
- The __ hypothesis concerns the specificity of the anticodon for first two nucleotides of the codon.
  - Wobble
- The genetic code is the same in all species means the genetic code is __.
  - Universal

CONCEPT QUESTIONS

- Four syllable word for stop codon is the __ codon.
  - Termination
- Three bases that code for one amino acid
  - Codon
- Codes for amino acid sequence in mRNA of eukaryotes.
  - Exo

- An XX *Drosophila* fertilized egg is a homozygous mutant for sex lethal. What affect do you predict this will have on phenotype.
  - The phenotype will be male

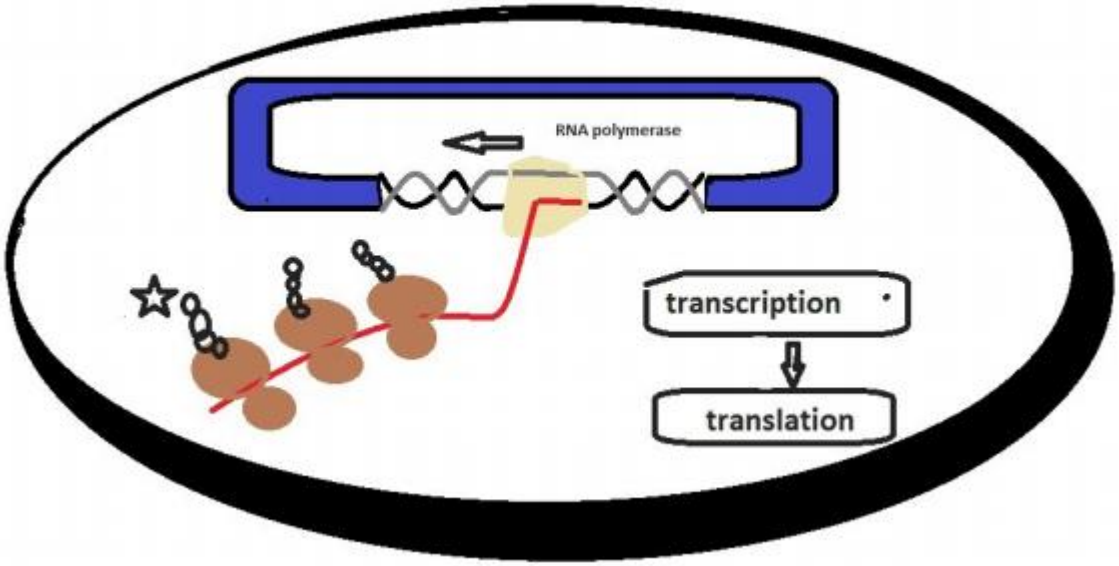
CONCEPT QUESTIONS

- Constitutive genes are expressed _____
  - Continously
- Lac operon is optionally expressed _____
  - In the presence of lactose and the absence of glucose
- A mutation in the O region of the lac operon would most likely result in _____.
  - Constitutive expression of lac operon, whether or not lactose was present as the repressor cannot bind.
- Attenuation in the E. coli *trp* operon _____
  - Results from formation of trp mRNA secondary structures.

CONCEPT QUESTIONS

- Eurkaryotic regulation of gene expression occurs at the level of _____
  - All levels, including transcription, splicing and processing and mRNA degradation
- An enchancer sequence _____
  - Can be inverted without altering its functional ability.
- A protein causes the acetylation of histones. What affect do you think this will have on a gene in that region.
  - It will cause an increase in expression.
- The advantage of RNAi over other functional genomic techniques is that _____
  - RNAi introduces no mutations to the organism.

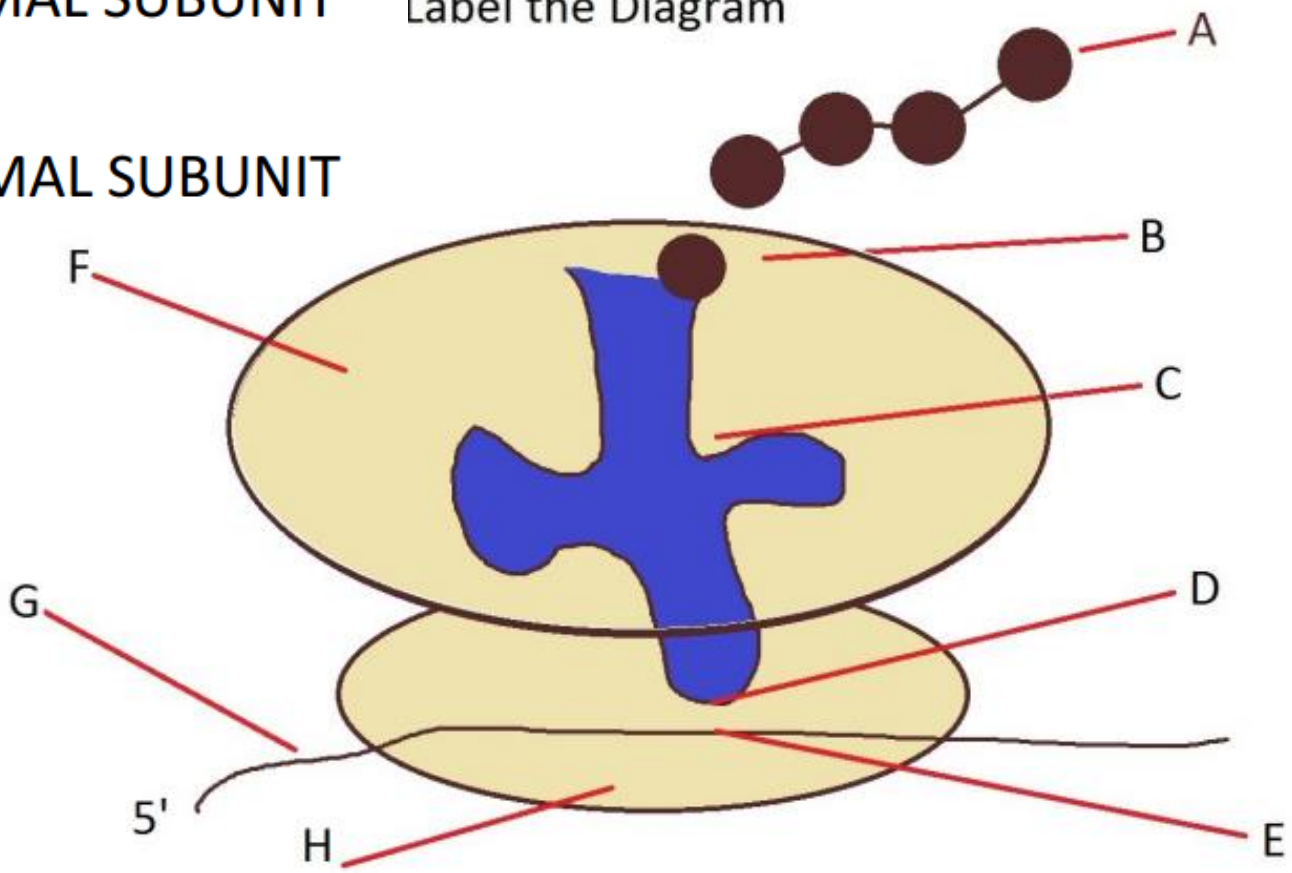
PROKARYOTIC CELL



- In a prokaryotic cell, transcription and translation are ___
  - Coupled
- What type of ribosomes are presented on the drawing?
  - Polyribosomes.
- What terminus (N or C) of the polypeptide chain is shown on the drawing. (Marked by the star)
  - N Terminus
- In prokayotes a small 30 S ribosomal subunit contains the ____ S rRNA
  - 16

A. GROWING POLYPEPTIDE CHAIN
B. AMINO ACID
C. tRNA
D. ANTICODON
E. CODON
F. LARGE RIBOSOMAL SUBUNIT
G. mRNA
H. SMALL RIBOSOMAL SUBUNIT

Label the Diagram



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