



**Dept: Genetics and Genetic engineering Program: Biotechnology (English)**

**Subject: Molecular Biology Level: Three**

**First Term: 2016/2017 Time: 2 Hours**

**Answer the following questions:**

***First Question*: *(20 marks*)**

***: Explain 3 point only:***

 (1): Enzymes using in Genetic Engineering.

(2) : Prokaryotic & Eukaryotic Ribosome and tRNA

(3): Advantages and disadvantages for cloning vectors.

(4) : Genetic code.

***Second Question (20 marks )***

***Discuss briefly 2 point only:***

(1): General Recombination and Site Specific Recombination.

(2): Types of DNA mutations.

(3) : Types of DNA damage and Repair.

***Third Question (20 marks)***

***Write briefly about:***

1. : Gene Regulation in Prokaryotes and in Eukaryotes.
2. : Transcription and Translation in Prokaryotes and in Eukaryotes.

 ***With my best wishes***

***Prof .Dr/ Mohamed Serag El-din***





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**Model Answer**

 ***First Question (20 marks):***

(1**): there are five groups of enzymes**:

Nucleases & Ligase & polymerase & Modifying enzymes and Topisomerase. **(please explain these groups briefly**).

 **(2):** : **Prokaryotic & Eukaryotic Ribosome and tRNA**

 **tRNAs** carry amino acids to ribosome to be linked together by the ribosome. One by one, they bind to the ribosome, add their amino acid to the chain, and then depart 50S and 30S made primarily of RNA and are both larger than the average protein. 50S has rather rigid structure, while 30S has fairly flexible one. Not all about them has been discovered yet, but significant progress has been made in that respect

 tRNA molecules carry amino acids to the ribosome for incorporation into a polypeptide aminoacyl-tRNA synthetases add amino acids to the acceptor arm of tRNA the anticodon loop contains 3 nucleotides complementary to mRNA codons

**The ribosome has multiple tRNA binding sites:**

**P s**ite : binds the tRNA attached to the growing peptide chain

**A** site : binds the tRNA carrying the next amino acid

**E** site : binds the tRNA that carried the last amino acid

 **(3): Advantages and disadvantages for cloning vectors**.

***Natural Vectors:***

 **(1): Plasmid vectors:** ( are double-stranded, circular, self-replicating, extra-chromosomal DNA molecules)

 **(2): Bacteriophage Vectors:** ( viruses which infected bacterial host)

**COSMID Vectors** : ( Vector which contain Plasmid vector with cos site from bacteriophage )

***Artificial Vectors* :**

***BACs*** : Bacterial Artificial Chromosomes.***YACs* :** Yeast Artificial Chromosomes

**Please mention advantages and disadvantages for all these groups.**

 **(4): Genetic Code:**

The genetic code is responsible for building all the proteins in the body using 20 different amino acids.

**Codon**: three consecutive nucleotides that specify a single amino acid.

Amino acids are attached to make a polypeptide, protein.

**Anticodon**: the complimentary RNA bases of a codon. A three letter “word” that specifies an amino acid. There are “start” codons & “stop” codons

Tells ribosome where to start reading the RNA strand. There is a message, but one must start at the right place to read it Code written in three letter words - codon .There are three reading frames, but only one gives an intelligible message – frame.A start codon (NOW) and a stop codon (END) define the frame to use

Each amino acid in a protein is specified by 3 nucleotides of codon. Each codon specifies only ONE amimo acid. There are 64 possible codons but only 20 amino acids. Degeneracy ,An amino acid can be specified by multiple codons

***Special* codons:**

 AUG (which specifies methionine) = start codon

 AUG specifies internal methionines

 UAA, UAG and UGA = termination, or stop, codons

The code is degenerate. More than one codon can specify the same amino acid

**For example**: GGU, GGC, GGA and GGG all code for lysine

The code is nearly universal . Only a few rare exceptions have been noted

 Deciphering the genetic code required determining how 4 nucleotides (A, T, G, and C) could encode more than 20 amino acids.

***Second Question (20 marks )***

(1): **General Recombination and Site Specific Recombination.**

**General recombination** allows large fraction of genetic information to move from one chromosome to another.

General recombination requires the breakage of double helices, beginning with a single strand breakage. General recombination is facilitated by Rec A in bacteria and its homolog in eukaryotes.Holiday junction is the intermediate state of general recombination.

**Site Specific Recombination**

Moves specialized nucleotide sequence (mobile genetic elements) between non-homologous sites within a genome.Transpositional site-specific recombination

Conservative site-specific recombination .DNA site-specific recombination

transpositional; conservative. Transposons: mobile genetic elements

Transpositional: DNA only transposons, retroviral-like retrotransposons, nonretroviral retrotransposons site .

**(2): Types of DNA mutations**

**\* Point mutations** alter a single base substitution mutations – substitute one base for another- transitions or transversions, also called missense mutations.

**nonsense mutations** – create stop codon

**frameshift mutations** – caused by insertion or deletion of a single base

triplet repeat expansion mutations involve a sequence of 3 DNA nucleotides that are repeated many times. triplet repeats are associated with some human genetic diseases the abnormal allele causing the disease contains these repeats whereas the normal allele does not

**\* Chromosomal mutations** change the structure of a chromosome.

**deletions** – part of chromosome is lost

**duplication** – part of chromosome is copied

**inversion** – part of chromosome in reverse order

**translocation** – part of chromosome is moved to a new location

**Types of DNA Damage**

 Deamination & Depurination &T-T and T-C dimmers& Alkylation& Oxidative damage & Replication errors & Double-strand breaks (DSB and Spontaneous DNA damage

**Types of DNA Repair**

**Pathways to remove DNA damage**: base excision repair, nucleotide excision repair

**Damage detection**: base flipping

**The repair of Double-strand break**: nonhomologus end joining, homologous end joining

**DNA repair enzymes**: heat shock proteins

Repair of UV-induced dimers in the light

**Photoreactivation**

1. Light-dependent, UV-A 🡪 blue light (360-420 nm)
2. Catalyzed by Photolyases:

Enzymes that convert the dimers to monomers .Use FAD as chromophore and electron donor also have another chromophore that acts as antenna

3 classes: CPD I and II for T-T dimers, and a 6-4 \photolyase for T-C dimers

**Base Excision Repair (BER)**

Not much known about this pathway in plants. Probably important though, based on the existence of 16 genes homologous to *DNA glycosylases*, and 3 homologous to *AP endonucleases* in the *Arabidopsis* genome.

**Mismatch Repair**

problem: how do cells know which is the right template strand?

In *E. coli*, new DNA not methylated right away. Mismatch recognized by *mutS*, then *mutL* binds and attracts *mutH* (endonuclease that cleaves mismatch and nearest CTAG that is not methylated). Eucaryotes (including *Arabidopsis*) have *mutS* and *mutL* homologues, but no *mutH* . Also have the requisite exonucleases, but not clear how the strand specificity is determined .

 **Repair of Double-strand breaks (DSBs)**

 general ways to repair DSBs:

1. Homologous recombination (HR) - repair of broken DNA using the intact homologue. Very accurate.
2. Non-homologous end joining (NHEJ) - ligating non-homologous ends. Prone to errors, ends can be damaged before ligation (genetic material lost), or get translocations.

 Usage: NHEJ >> HR in plants and animals (in the cells’ nucleus).

***Third Question (20 marks)***

1. **: Gene Regulation in Prokaryotes and in Eukaryotes.**

**Gene Regulation in Prokaryotes :**

 Genetic information always goes from DNA to RNA to protein

 Gene regulation has been well studied in *E. coli*. When a bacterial cell encounters a potential food source it will manufacture the enzymes necessary to metabolize that food addition to sugars like glucose and lactose *E. coli* cells also require amino acids. One essential is tryptophan. When *E. coli* is swimming in tryptophan (milk & poultry) it will absorb the amino acids from the media. When tryptophan is not present in the media then the cell must manufacture its’ own amino acids

***Trp* Operon**

*E. coli* uses several proteins encoded by a cluster of 5 genes to manufacture the amino acid tryptophan. All 5 genes are transcribed together as a unit called an operon, which produces a single long piece of mRNA for all the genes. RNA polymerase binds to a promoter located at the beginning of the first gene and proceeds down the DNA transcribing the genes in sequence*.* In addition to amino acids, *E. coli* cells also metabolize sugars in their environment.In 1959 Jacques Monod and François Jacob looked at the ability of *E. coli* cells to digest the sugar lactose.

 **(Lac Operon )**

 consists of three structural genes and one Operator gene . In the presence of the sugar lactose, *E. coli* makes an enzyme called *beta galactosidase* .*Beta galactosidase* breaks down the sugar lactose so the *E. coli* can digest it for food. It is the LAC Z gene in *E coli* that codes for the enzyme *beta galactosidase.* The tryptophan gene is turned on when there is no tryptophan in the media. That is when the cell wants to make its’ own tryptophan. *E. coli* cells can not make the sugar lactose. They can only have lactose when it is present in their environment .Then they turn on genes to beak down lactose

The *E. coli* bacteria only needs *beta galactosidase* if there is lactose in the environment to digest. There is no point in making the enzyme if there is no lactose sugar to break down. It is the combination of the promoter and the DNA that regulate when a gene will be transcribed

This combination of a promoter and a gene is called an (OPERON)

**Operon** is a cluster of genes encoding related enzymes that are regulated together

**Operon** consists of : - A promoter site where RNA polymerase binds and begins transcribing the message.- A region that makes a repressor

- Repressor sits on the DNA at a spot between the promoter and the gene to be transcribed . This site is called the operator.

**Gene Regulation in Eukaryotes :**

 In eukaryotic organisms like our selves there are several methods of regulating protein production. Most regulatory sequences are found upstream from the promoter. Genes are controlled by regulatory elements in the promoter region that act like one/off switches or dimmer switches . Specific transcription factors bind to these regulatory elements and regulate transcription. Regulatory elements may be tissue specific and will activate their gene only in one kind of tissue. Sometimes the expression of a gene requires the function of two or more different regulatory elements

 **Eukaryotic DNA differs from prokaryotic DNA** it that the coding sequences along the gene are interspersed with non coding sequences

 The coding sequences are called Exon. The non coding sequences are called introne. After the initial transcript is produced the introns are spliced out to form the completed message ready for translation. Introns can be very large and numerous, so some genes are much bigger than the final processed mRNA. Muscular dystrophy. DMD gene is about 2.5 million base pairs long Has more than 70 introns. The final mRNA is only about 17,000 base pairs long

**(2) Transcription and Translation in Prokaryotes and in Eukaryotes.**

important stages in protein synthesis: The coding by triplets of bases to produce mRNA (**Transcription).** The linking of mRNA to tRNA at ribosomes **(Translation)**

**Prokaryotic Transcription:**

Prokaryotic cells contain a single type of RNA polymerase found in 2 forms: core polymerase is capable of RNA elongation but not initiation

holo enzyme is composed of the core enzyme and the sigma factor which is required for transcription initiation. A transcriptional unit extends from the promoter to the terminator. The promoter is composed of a DNA sequence for the binding of RNA polymerase the start site (+1) – the first base to be transcribed. During elongation, the transcription bubble moves down the DNA template at a rate of 50 nucleotides/sec.The transcription bubble consists of:RNA polymerase.DNA template growing RNA transcript Transcription stops when the transcription bubble encounters terminator sequencesthis often includes a series of A-T base pairs,

**In prokaryotes**, transcription and translation are often coupled – occurring at the same time

**Eukaryotic Transcription :**

RNA polymerase I transcribes rRNA. RNA polymerase II transcribes mRNA and some snRNA. RNA polymerase III transcribes tRNA and some other small RNAs. Each RNA polymerase recognizes its own promoter. Initiation of transcription of mRNA requires a series of transcription factors. Transcription factors – proteins that act to bind RNA polymerase to the promoter and initiate transcription .

**In eukaryotes**, the primary transcript must be modified by:

addition of a 5’ cap, addition of a 3’ poly-A tail, removal of non-coding sequences (introns) . The spliceosome is the organelle responsible for removing introns and splicing exons together. Small ribonucleoprotein particles (snRNPs) within the spliceosome recognize the intron-exon boundaries.

**Translation In prokaryotes**, initiation of translation requires the formation of the initiation complex including an initiator tRNA charged with N-formylmethionine the small ribosomal subunit

mRNA strand. The ribosome binding sequence of mRNA is complementary to part of rRNA , Elongation of translation involves the addition of amino acids a charged tRNA binds to the A site if its anticodon is complementary to the codon at the A site. peptidyl transferase forms a peptide bond the ribosome moves down the mRNA in a 5’ to 3’ direction

There are fewer tRNAs than codons. Wobble pairing allows less stringent pairing between the 3’ base of the codon and the 5’ base of the anticodon.

This allows fewer tRNAs to accommodate all codons. Elongation continues until the ribosome encounters a stop codon. Stop codons are recognized by release factors which release the polypeptide from the ribosome.

**Translation In Eukaryotes**,

May occur on ribosomes in the cytoplasm or on ribosomes of the RER.

Signal sequences at the beginning of the polypeptide sequence bind to the signal recognition particle (SRP) . The signal sequence and SRP are recognized by RER receptor proteins. The signal sequence/SRP holds the ribosome on the RER.As the polypeptide is synthesized it passes through a pour into the interior of the endoplasmic reticulum.

 ***With my best wishes***

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