COVENANT UNIVERSITY
NIGERIA

TUTORIAL KIT
OMEGA SEMESTER

PROGRAMME: BIOCHEMISTRY

COURSE: BCH 224

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BCH 224: INTRODUCTORY MOLECULAR BIOLOGY

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1. Describe the process by which plasmids serve as important tools in DNA technology.
2. How does Biochemistry relate to Molecular Biology?
3. Compare and contrast the prokaryotic and the eukaryotic genome and cell.
4. Describe in detail the following terms with appropriate diagrammatic illustrations: genes, genome, chromosome, transposons and viruses.
5. Classify histone proteins and describe in detail how they function in packaging DNA into chromosomes of eukaryotic cells.
6. Describe the replication cycle of Ebola virus (a retrovirus); why is it a retro virus?
7. Discuss in detail five (5) experiments that led to the discovery and establishment of deoxyribonucleic acid (DNA) as the genetic material of living organisms.
8. Describe the processes involved in lytic and lysogenic cycles of bacteriophages; differentiate between these two cycles.
9. Classify plasmids based on their function and enumerate five (5) characteristics that make them suitable vectors in DNA technology.
10. Compare and contrast mitochondrial and chloroplast DNA; what class of DNA are they by location?

ANSWERS

1. Describe the process by which plasmids serve as important tools in DNA technology.

A plasmid is an independent, circular, self-replicating DNA molecule that carries only a few genes.

Plasmids serve as vectors (vehicle/carrier) in DNA manipulation. They are cut open at specific sites (genes that code for antibiotic resistance) by specific restriction enzymes used in cutting the genomic DNA containing the gene of interest. The gene of interest to be cloned is then incubated with the plasmids for the formation of recombinant DNA to take place. The sticky ends of the genes anneal with complimentary strands on the sticky ends of plasmids and DNA ligase catalyzes the formation of the phosphoester bond. Not all plasmids pick up DNA. After the reaction, the mixture is transferred from test tube to be incubated with a selected host organism e.g. *Escherichia coli.* After incubation, many copies of the organisms are made in order to multiply copies of genes. The bacterial molecules are then grown on media containing antibiotics to identify the bacteria that picked up DNA (transformed bacteria). These are then further isolated and cultured for multiple copies to attain the purpose for which the gene is being cloned.

NB**:** This is only a summary; explore textbooks and journal articles for more details.

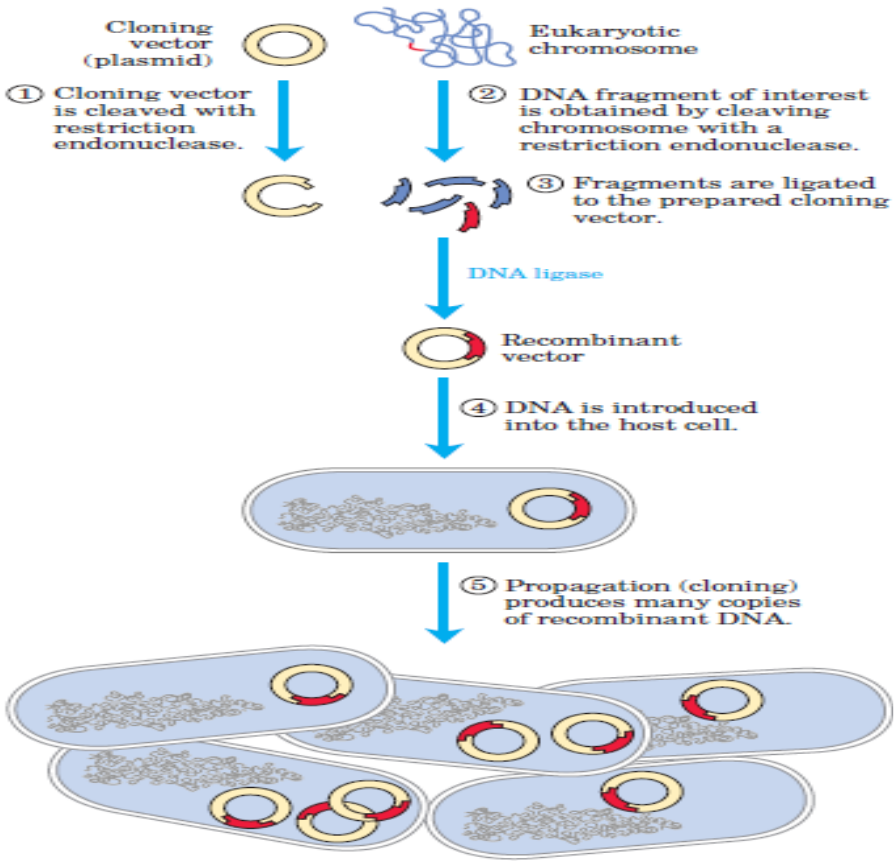

Figure 1: Schematic illustration of DNA cloning

2. Compare and contrast the prokaryotic and the eukaryotic genome and cell.

Both prokaryotic and eukaryotic DNA are double stranded and supercoiled or compacted into chromosome. Both contain functional genes which code for vital products in the cell. Both are negatively supercoiled (twisted upon itself).

Table 1: Differences between prokaryotic and eukaryotic genome

Prokaryotic genome	**Eukaryotic genome**
Exists as a single bacterial chromosome	Mostly packaged into several chromosomes
Simple genome organization	Complex genome organization
Smaller genome size	Large genome size
Between 500 and 4000 genes	Between 6,000 and 30,000 genes
Packaged in nucleoid (not enveloped in nucleus)	Packaged in a double-membraned nuclear envelope
Circular outside the nucleoid	Linear
Complexed to several histone-like DNA-binding proteins mostly HU, HLP-1 and H-NS	Complexed with histone and nonhistone proteins
Genes usually do not have introns	Genes have introns
Few regulatory DNA	Large amount of regulatory DNA
Little or no genetic redundancy	High degree of genetic redundancy

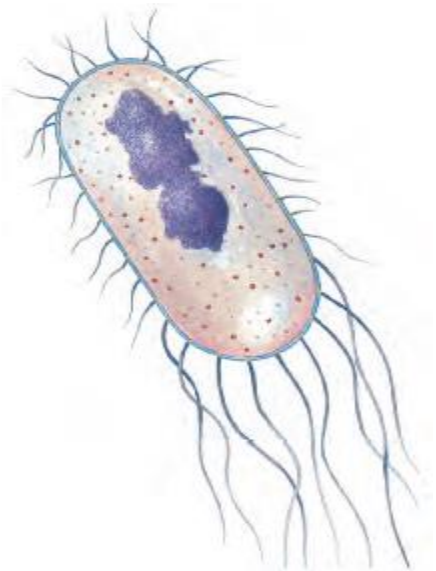

Figure 1: Nucleoid region in (a) *E. coli* cells and (b) a bacterial cell.

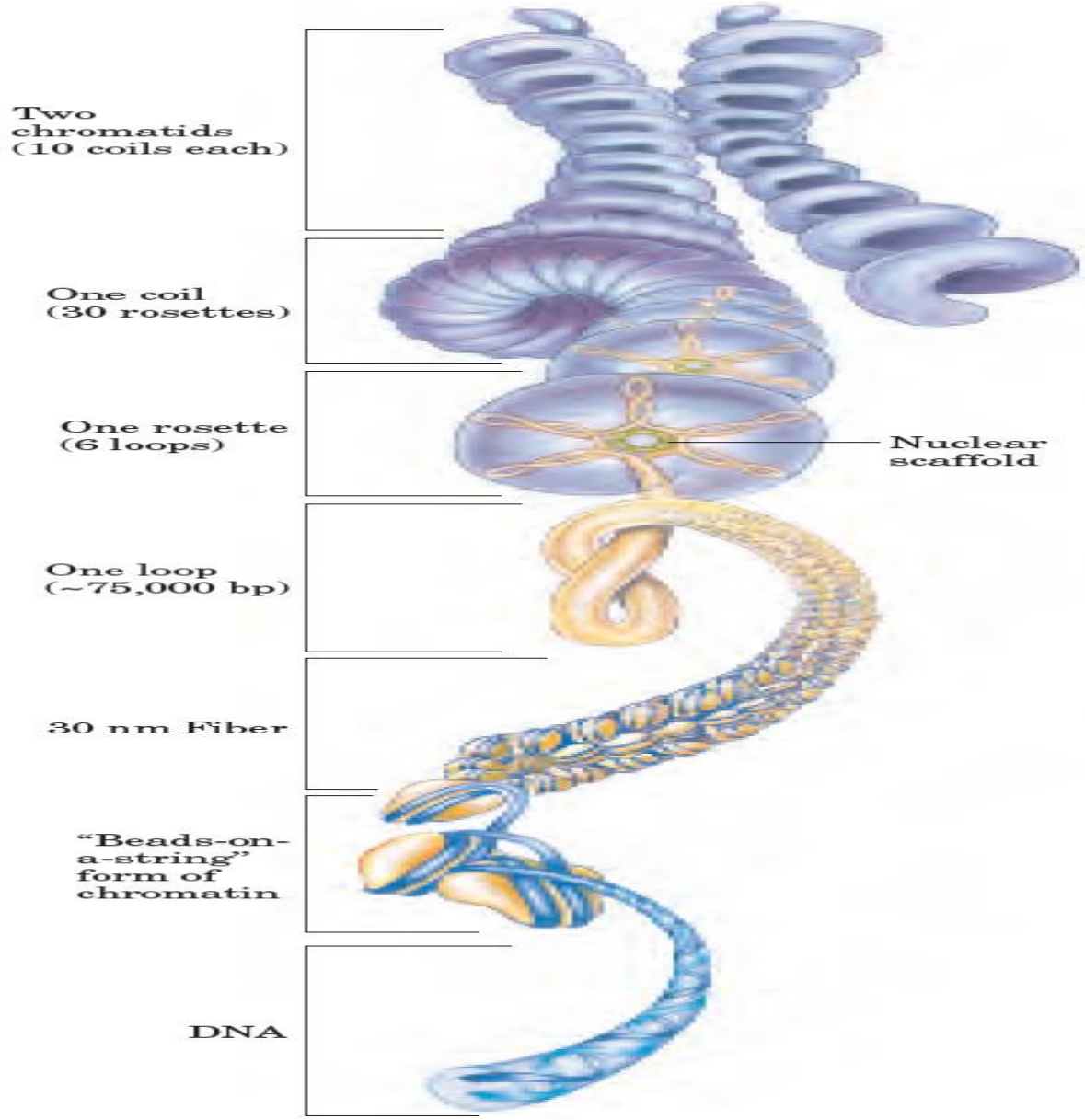

Figure 2: Compaction of DNA in eukaryotic chromosome

Prokaryotic versus eukaryotic cell

Similarities: Both are cells of organisms. They are composed of genetic material (DNA). They possess cell membrane, cytosol and enzymatic machinery for DNA replication, transcription and translation.

Differences

Prokaryotic cell	**Eukaryotic cell**
No true nucleus	Possess true nucleus
Strictly unicellular	Unicellular and multicellular

No membrane-bound organelles	Possess membrane-bound organelles
Flagella lack 9+2 array of microtubules	Possess 9+2 array of microtubules
Smaller in size	Larger in size

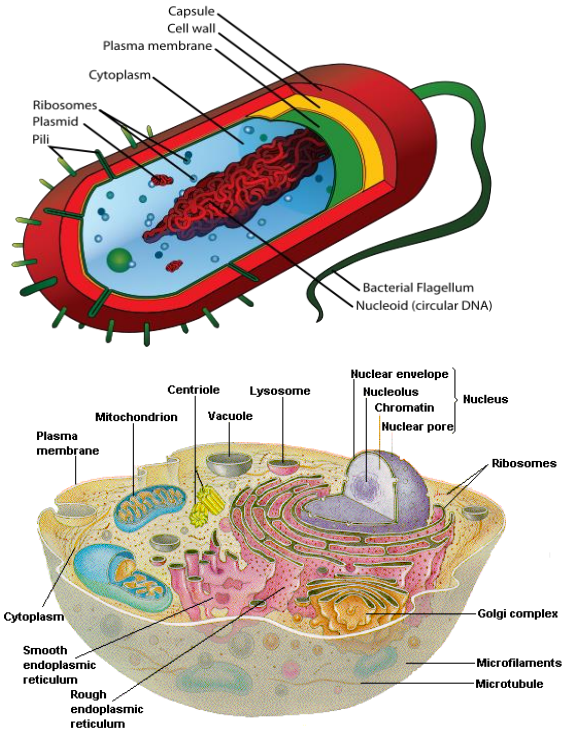

Figure 3: Prokaryotic and eukaryotic cell

5. Classify histone proteins and describe in detail how they function in packaging DNA into chromosomes of eukaryotic cells.

Histone proteins are of five types: H1/H5, H2A, H2B, H3 and H4. H1/H5 are linker histones while the remaining 4 are core histone proteins; 2 molecules each of these 4 form the histone octamer in a nucleosome.

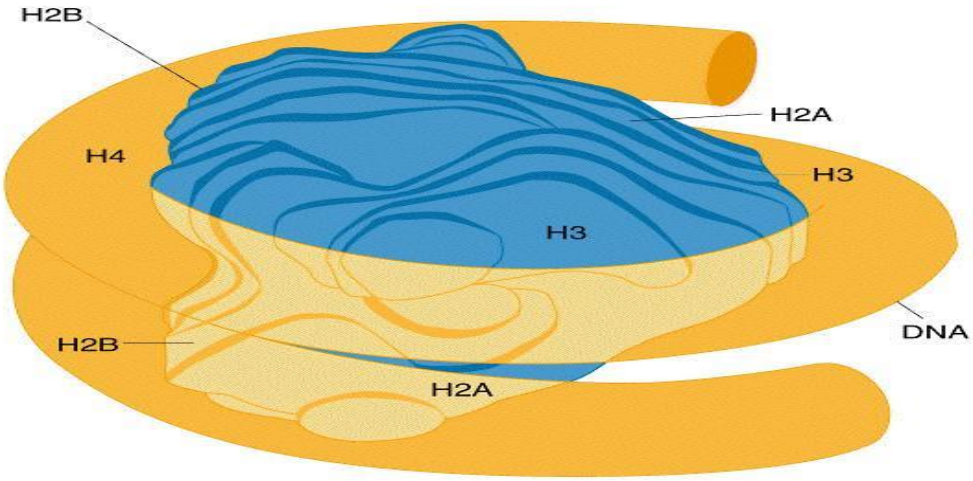

Figure 3: A nucleosome

7. Discuss in detail five (7) experiments that led to the discovery and establishment of deoxyribonucleic acid (DNA) as the genetic material of living organisms.

i. Friedrich Miescher (1868): first to isolate a phosphorus-containing substance the nuclei of pus cells (leukocytes) obtained from discarded surgical bandages, which he called "nuclein".

ii. Erwin Chargaff (1940s): demonstrated that there is a relationship between the number of Adenine and Thymine; and Guanine and Cytosine in various species of organisms (A≈T and G≈C).

iii. Avery, McCleod and McCarty (1944): found that DNA extracted from a virulent (disease-causing) strain of the bacterium *Streptococcus pneumoniae*, also known as pneumococcus, genetically transformed a nonvirulent strain of this organism into a virulent form.

iv. Rosalind Franklin and Maurice Wilkins (1950s): showed that DNA produces a characteristic x-ray diffraction pattern and deduced from this that DNA molecules are helical with two periodicities along their long axis,a primary one of 3.4 Å and a secondary one of 34 Å.

v. Hershey and Chase (1952): show that when the bacterial virus (bacteriophage) T_2 infects its host cell, *Escherichia coli*, it is the phosphorus-containing DNA of the viral particle, not the sulfur-containing protein of the viral coat, that enters the host cell and furnishes the genetic information for viral replication.

vi. James Watson and Francis Crick (1953): postulated a three-dimensional model of DNA structure that accounted for all the available data. It consists of two helical DNA chains wound around the same axis to form a right-handed double helix

9. Classify plasmids based on their function and enumerate five (5) characteristics that make them suitable vectors in DNA technology.

Classification of plasmids based on their function

i. Fertility F-plasmids, which contain *tra* genes, which are capable of conjugation and result in the expression of sex pilli.

ii. Resistance (R) plasmids, which contain genes that provide resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
iii. Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
iv. Degradative plasmids, which enable the digestion of unusual substances, e.g. toluene and salicylic acid.
v. Virulence plasmids, which turn the bacterium into a pathogen.

Five (5) characteristics of a plasmid. Plasmids are

i. Small (1-200 kb)

ii. Circular

iii. Self replicating

iv. Capable of independent existence

v. Carry only a few genes

vi. Extrachromosomal

vii. Easily moved out of and into bacterial cells

viii. Carry functions advantageous to the host

11. Compare and contrast DNA replication and transcription

Replication	Transcription
• both strands of DNA are copied • The non-template DNA strand, although not directly involved in transcription is by convention called the coding strand • Coding strand is similar in sequence to the single-stranded mRNA molecules that carry the coded message. • dNTPs (deoxyribonucleotide triphosphate molecules) are used= dATP, dGTP, dCTP, and dTTP • DNA polymerase binds, undergoes initiation, elongation and termination stages	• only one of the two DNA strands is copied—the template strand—serves as a template for mRNA • base uracil (U) is employed in RNA whereas the base thymine (T) is incorporated into DNA • NTPs (ribonucleoside triphosphate molecules) are used= ATP, GTP, CTP, and UTP • RNA polymerase binds, undergoes initiation, elongation and termination stages

DNA replication and transcription both utilize the base pairing mechanism to copy sequence information from a DNA template strand to produce a complementary strand using polymerase enzymes

12. Describe the process of translation and RNA modification

13. What is RNA processing in eukaryotes?

- In eukaryotes, chemical modification both during and, after transcription are made to the RNA before it can function in the cell. These transcripts are often cleaved at a specific site before transcription is actually terminated. The cleavage site is 10–35 nucleotides downstream from a special AAUAAA sequence in the growing RNA chain. Capping is addition of the poly(A) tail. Splicing is done to remove the introns from within the gene leaving the exons (the coding regions) of the sequence. Spliceosomes Remove Introns from Pre-mRNA and splice together the remaining RNA segments (exons).

14. What are transcription factors, how can they be used to control transcription?

15. What is gene expression, how can this be used to control activities of an organism

Gene expression is the process by which a gene's coded information is converted into the structures operating in cells. It is the interpreting of DNA information i.e. from RNA to protein which produces a particular trait is called gene expression. Regulation of gene expression, protein synthesis and protein levels are controlled during transcription, this is a mechanism used by all organisms to produce biomolecules used to adapt for developmental and environmental changes.

16. CUU, CUC, CUA, CUG codes for Leucine, what is this phenomenon called.

17. **What is genetic code**: It is the genetic information stored on the RNA read as a set of **three** bases that codes for a protein. E.g. in an amino acid sequence, ACC directs addition of threonine and CCC of proline.

18. What is mutation, describe the different types of mutations

19. What are mutagens: a mutagen is a physical or chemical agent that changes the genetic material, usually DNA. Mutagens can be environmental factors.

Chemicals e.g. Hydroxylamine NH_2OH, Base analogs, reactive oxygen species

Radiation e.g. Ultraviolet radiation (non-ionizing radiation)which can alter cytosine and thymine in DNA

Alkylating agents e.g., *N*-ethyl-*N*-nitrosourea which intercalates DNA

Oxidative reagents e.g. Nitrous acid converts amine groups on Adenine and Cytosine to diazo groups.

20. What are recombinant DNA techniques, discuss how one of such techniques can be used to detect an infective pathogen in a host.