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HANDOUT

MB503T

Cell and Molecular Biology (Theory)

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MB503T

Cell and Molecular Biology (Theory)

Lecture-Wise Study Handout
Virtual University of Pakistan

12 lectures • prepared for exam study

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About This Course

Cell and Molecular Biology studies life at two connected scales: the cell as the unit of life, and the molecules — DNA, RNA and proteins — that carry information and do the work inside it. The central dogma (DNA → RNA → protein) links the two scales and organizes the entire course.

This handout covers cell structure in condensed form, then DNA structure and replication, transcription, translation, gene regulation, mutations, and the core molecular techniques (PCR, electrophoresis, cloning, sequencing) that define modern biotechnology. Every lecture carries definitions, examples and exam pointers.

Lecture 1: The Cell Revisited: Stage for Molecular Events

Learning Objectives

- Recall cell structure essentials
- State the central dogma

The cell theory states that all organisms consist of cells, the cell is the basic unit of life, and all cells arise from pre-existing cells. Cells come in two architectures: prokaryotic (bacteria, archaea — no nucleus, DNA in a nucleoid, small, dividing by binary fission) and eukaryotic (animals, plants, fungi — true nucleus, membrane-bound organelles, larger, dividing by mitosis).

The plasma membrane — a phospholipid bilayer with embedded proteins (fluid mosaic model) — controls traffic: passive transport (diffusion, osmosis) down gradients, active transport (e.g. the sodium–potassium pump) against them using ATP. Organelles divide labour: nucleus (genome), mitochondria (ATP), ER and Golgi (protein processing), lysosomes (recycling).

Because this course's heart is molecular, remember the cell as the stage on which molecular events play: replication in the nucleus, transcription in the nucleus, translation on ribosomes, energy from mitochondria. Each molecular process you will study happens in a specific cellular location — a favourite exam framing.

Key Definitions

Central dogma is defined as the information flow $\text{DNA} \rightarrow \text{RNA} \rightarrow \text{protein}$, the organizing principle of molecular biology

Key Points — very important for exams

Prokaryotic vs eukaryotic architecture; fluid-mosaic membrane; organelle division of labour.

Central dogma: $\text{DNA} \rightarrow \text{RNA} \rightarrow \text{protein}$ — the course's organizing principle.

Map each molecular process to its cellular location (nucleus, ribosome, mitochondrion).

Review Questions

- ? Where do replication, transcription and translation occur in a eukaryotic cell?
- ? Why is the central dogma called a dogma?

Section Summary

Cells provide the compartments; the central dogma describes the information flow between them.

Exam Pointers

- Summarize cell architecture briefly and state the central dogma.

Lecture 2: DNA Structure: The Double Helix

Learning Objectives

- Describe DNA structure
- Relate structure to function

DNA's structure — the double helix (Watson and Crick, 1953, building on Franklin's X-ray data and Chargaff's rules) — explains its function. Two antiparallel sugar-phosphate backbones wind around paired bases: A with T (two hydrogen bonds), G with C (three). The sequence of bases encodes information; the complementary pairing enables exact copying.

Chargaff's rules (A=T, G=C) and the antiparallel, right-handed helix with major and minor grooves are exam staples. DNA is packaged with histones into nucleosomes, coiling into chromosomes — packaging that must be unwound for replication and transcription, linking structure to the processes ahead.

DNA is chemically stable (deoxyribose, double-stranded, with repair systems) — suited to long-term storage — while RNA is single-stranded and reactive — suited to transient messages. This stability/reactivity contrast explains why DNA stores and RNA carries, a very important conceptual pairing.

Key Definitions

Chargaff's rules is defined as the base-pairing regularities (A=T, G=C) that revealed DNA's complementary structure

Key Points — very important for exams

Antiparallel backbones; A–T (2 H-bonds), G–C (3 H-bonds); sequence = information.

Chargaff's rules + Franklin's X-ray → Watson–Crick model (1953).

DNA stable for storage; RNA reactive for messaging — structure matches role.

Review Questions

- ? Why does G–C pairing bind more strongly than A–T?
- ? How does complementarity enable replication?

Section Summary

The double helix stores information in sequence and copies it through complementarity.

Exam Pointers

- Describe the double helix and explain how structure enables copying and storage.

Lecture 3: DNA Replication

Learning Objectives

- Describe replication enzymes and steps

- Explain fidelity mechanisms

DNA replication is semiconservative: each daughter molecule keeps one parental strand and builds one new one — proved by the Meselson–Stahl experiment. It begins at origins of replication, where helicase unwinds the helix and single-strand binding proteins stabilize it; topoisomerase relieves supercoiling ahead of the fork.

DNA polymerase III synthesizes the new strand 5'→3', continuously on the leading strand and in Okazaki fragments on the lagging strand; polymerase I replaces RNA primers (laid by primase) with DNA, and ligase seals the nicks. The enzymes' 5'→3' directionality explains the leading/lagging asymmetry — a classic exam explanation.

Fidelity is extraordinary — about one error per billion bases — thanks to polymerase proofreading (3'→5' exonuclease) and mismatch repair. Telomeres (chromosome-end repeats, maintained by telomerase) solve the end-replication problem; their shortening limits cell divisions and links replication to ageing and cancer.

Key Definitions

Semiconservative replication is defined as replication in which each daughter DNA keeps one parental and one new strand

Okazaki fragments is defined as short DNA segments synthesized on the lagging strand, later joined by ligase

Key Points — very important for exams

Semiconservative (Meselson–Stahl); origins; helicase, SSB proteins, topoisomerase.

Pol III synthesizes 5'→3': leading strand continuous, lagging strand in Okazaki fragments; primase, Pol I, ligase.

Proofreading + mismatch repair → extreme fidelity; telomeres/telomerase and the end problem.

Review Questions

- ? Why is the lagging strand synthesized discontinuously?
- ? How do cells achieve one-in-a-billion accuracy?

Section Summary

Unwind, prime, synthesize 5'→3', proofread — the fork copies three billion bases with near-perfect fidelity.

Exam Pointers

- Describe the replication fork enzymes and explain leading/lagging strand synthesis.

Lecture 4: Transcription: DNA to RNA

Learning Objectives

- Describe transcription
- Explain mRNA processing

Transcription copies a gene's DNA into messenger RNA. RNA polymerase binds the promoter, unwinds DNA, and synthesizes RNA 5'→3' using the template strand — no primer needed, unlike replication. In bacteria, transcription and translation are coupled in the cytoplasm; in eukaryotes, transcription occurs in the nucleus and the mRNA is processed before export.

Eukaryotic processing creates mature mRNA: a 5' cap (for ribosome binding and protection), a 3' poly-A tail (stability), and splicing — introns removed, exons joined by the spliceosome. Alternative splicing lets one gene produce multiple proteins, multiplying the proteome beyond gene count — a very important modern insight.

Transcription factors control initiation by binding promoters and enhancers; the TATA box positions RNA polymerase II. Regulation at initiation is the main control point of gene expression — the subject of the regulation lecture ahead.

Key Definitions

RNA splicing is defined as removal of introns and joining of exons from pre-mRNA by the spliceosome

Transcription factors is defined as proteins that bind promoters/enhancers to control transcription initiation

Key Points — very important for exams

RNA polymerase: promoter → unwind → synthesize 5'→3', no primer; bacteria couple it to translation.

Eukaryotic processing: 5' cap, poly-A tail, splicing (introns out, exons joined).

Alternative splicing: one gene → many proteins; transcription factors control initiation.

Review Questions

- ? How does eukaryotic transcription differ from bacterial?
- ? What is alternative splicing and why does it matter?

Section Summary

Transcription makes the RNA copy; eukaryotic processing edits it into export-ready messages.

Exam Pointers

- Describe transcription and eukaryotic mRNA processing; explain alternative splicing's significance.

Lecture 5: Translation: RNA to Protein

Learning Objectives

- Describe translation
- Explain the genetic code

Translation decodes mRNA into protein on ribosomes. Transfer RNAs are the adaptors: each carries an amino acid and an anticodon pairing with an mRNA codon. The genetic code — triplet, redundant (64 codons, 20 amino acids), unambiguous and nearly universal — is read 5'→3'; AUG (methionine) starts, UAA/UAG/UGA stop.

The ribosome cycles through three stages: initiation (small subunit + initiator tRNA find the start codon; large subunit joins), elongation (codons read; peptide bonds formed by peptidyl transferase; the ribosome translocates), termination (release factor at a stop codon; polypeptide released). Energy comes from GTP.

Post-translational modification then matures the protein: folding (chaperones assist), cleavage, and chemical additions (phosphorylation, glycosylation) that control activity and targeting. Protein synthesis is antibiotic territory — many antibiotics (streptomycin, tetracycline) target bacterial ribosomes specifically, sparing ours.

Key Definitions

Genetic code is defined as the triplet, redundant, near-universal mapping of 64 codons to 20 amino acids and stop signals

Key Points — very important for exams

tRNA adaptors link codons to amino acids; code is triplet, redundant, universal; AUG start, UAA/UAG/UGA stop.

Ribosome cycle: initiation → elongation (peptide bonds, translocation) → termination.

Post-translational modification matures proteins; bacterial ribosomes are antibiotic targets.

Review Questions

- ? Why is the code called redundant but unambiguous?
- ? How do antibiotics exploit translation?

Section Summary

Ribosomes read codons and build polypeptides — the central dogma's final step.

Exam Pointers

- Describe translation stages and the genetic code's properties.

Lecture 6: Gene Regulation and Operons

Learning Objectives

- Explain operon logic
- Compare regulatory strategies

Gene regulation answers: which genes, when, how much? Bacteria use operons — clustered genes under one promoter. The lac operon (lactose metabolism) is the classic: without lactose, the repressor blocks transcription; with lactose (converted to allolactose), the repressor releases — inducible, negative control. The trp operon works in reverse: tryptophan activates the repressor — repressible control for biosynthesis.

Eukaryotes regulate at multiple levels: chromatin remodelling (acetylation opens, methylation silences), transcription factors and enhancers, alternative splicing, mRNA stability, and post-translational control. Combinatorial control — many factors per gene — enables cell-type-specific expression from one genome.

Epigenetics (heritable expression changes without DNA alteration — DNA methylation, histone modification) explains cellular memory: how a liver cell stays a liver cell through divisions. It also links environment to genes — nutrition and stress leaving molecular marks — a frontier examiners increasingly probe.

Key Definitions

Operon is defined as a bacterial gene cluster (e.g. lac, trp) transcribed from a single promoter under shared control

Epigenetics is defined as heritable changes in gene expression without altering the DNA sequence

Key Points — very important for exams

lac operon: inducible (lactose removes repressor); trp operon: repressible (tryptophan activates repressor).

Eukaryotes: chromatin, transcription factors, splicing, mRNA stability — combinatorial control.

Epigenetics: methylation/histone marks give cellular memory without DNA change.

Review Questions

- ? Why is the lac operon called inducible?
- ? How does one genome produce 200+ cell types?

Section Summary

From bacterial operons to epigenetic memory, regulation decides which genes speak, when.

Exam Pointers

- Explain the lac and trp operons; compare bacterial and eukaryotic regulation.

Lecture 7: Mutations and DNA Repair

Learning Objectives

- Classify mutations
- Describe DNA repair pathways

Mutations are heritable DNA changes. By scale: point mutations (substitutions — silent, missense, nonsense), insertions/deletions (frameshifts if not in multiples of three), and chromosomal (deletions, duplications, inversions, translocations). By cause: spontaneous (replication errors, depurination) versus induced (radiation, chemicals, viruses).

Effects range from neutral to lethal: sickle-cell disease (a single missense mutation, Glu→Val in haemoglobin) shows one base changing a life; nonsense mutations truncate proteins; frameshifts scramble everything downstream. Mutagens are tested by the Ames test (bacterial reversion assay) — a rapid screen for carcinogens.

DNA repair defends the genome: direct reversal, base excision, nucleotide excision (defective in xeroderma pigmentosum — extreme sun sensitivity), and mismatch repair (defective in some hereditary cancers). Mutation + repair balance determines genetic stability — and when repair fails, cancer follows.

Key Definitions

Missense mutation is defined as a point mutation changing one amino acid to another (e.g. sickle-cell Glu→Val)

Nonsense mutation is defined as a point mutation converting an amino-acid codon into a stop codon, truncating the protein

Key Points — very important for exams

Point (silent/missense/nonsense), indels (frameshift), chromosomal; spontaneous vs induced.

Sickle-cell: one missense mutation, systemic disease; Ames test screens mutagens.

Repair: direct, base/nucleotide excision, mismatch — failures cause XP and hereditary cancers.

Review Questions

- ? Why are frameshifts usually worse than missense mutations?
- ? How does defective nucleotide excision repair cause disease?

Section Summary

Mutations fuel evolution and cause disease; layered repair systems hold the line.

Exam Pointers

- Classify mutations and explain repair systems with disease examples.

Lecture 8: Recombinant DNA and Cloning

Learning Objectives

- Explain recombinant DNA tools
- Describe a cloning workflow

Recombinant DNA technology cuts and pastes DNA using restriction enzymes (which cut at specific palindromic sequences, leaving sticky ends) and DNA ligase (which seals the joints). Vectors carry the construct into hosts: plasmids (small, high-copy bacterial circles), phages, and for eukaryotes, cosmids, YACs and viral vectors.

Cloning workflow: cut vector and insert with the same enzyme → ligate → transform into E. coli (heat shock or electroporation) → select with antibiotic-resistance markers and screen (blue-white screening via lacZ disruption). The result: bacteria manufacturing human insulin, growth hormone or vaccines — the founding application of biotechnology.

Genomic versus cDNA libraries: genomic libraries contain all DNA (introns included); cDNA libraries (made by reverse transcriptase from mRNA) contain only expressed sequences — the right choice for finding expressed genes. Very important: match the library to the question.

Key Definitions

Restriction enzymes is defined as bacterial enzymes cutting DNA at specific sequences, producing sticky ends for cloning

Key Points — very important for exams

Cut (restriction enzymes) → paste (ligase) → deliver (vectors: plasmids, phages, YACs).

Transform E. coli; select by antibiotic resistance; screen blue-white via lacZ.

Products: insulin, vaccines; libraries: genomic (all DNA) vs cDNA (expressed genes).

Review Questions

- ? Why must vector and insert be cut with the same enzyme?
- ? When would you use a cDNA library instead of genomic?

Section Summary

Cut, paste, deliver, select — recombinant DNA turned bacteria into drug factories.

Exam Pointers

→ Describe the recombinant DNA workflow: enzymes, vectors, transformation, selection.

Lecture 9: PCR and Gel Electrophoresis

Learning Objectives

- Explain PCR
- Describe electrophoretic separation

The polymerase chain reaction amplifies DNA exponentially: each cycle doubles the target — denaturation (~94°C, strands separate), annealing (~55°C, primers bind), extension (72°C, Taq polymerase synthesizes). After 30 cycles, one molecule becomes a billion. Primers define the target; Taq (from a hot-spring bacterium) survives the heat.

PCR's power is sensitivity and specificity: forensics (a hair's DNA), diagnostics (pathogen detection, including COVID testing), and research all depend on it. Variants: RT-PCR (from RNA via reverse transcriptase), qPCR/real-time (quantifies starting amount), multiplex (several targets at once). Primer design — length, melting temperature, avoiding dimers — decides success or failure.

Gel electrophoresis separates the products: DNA, uniformly negative, migrates toward the positive electrode through agarose, small fragments fastest; ethidium bromide or safer dyes visualize bands against a ladder of known sizes. Together, PCR + electrophoresis answer: is the sequence present, and how big is it?

Key Definitions

Polymerase chain reaction (PCR) is defined as cyclical in-vitro DNA amplification: denature → anneal → extend, doubling each round

Key Points — very important for exams

PCR: denature (94°C) → anneal primers (~55°C) → extend (72°C, Taq); 30 cycles ≈ billion-fold.

Uses: forensics, diagnostics, research; variants RT-PCR, qPCR, multiplex.

Electrophoresis: size-separates DNA in agarose; bands read against a ladder.

Review Questions

- ? What do primers determine in PCR?
- ? Why does smaller DNA migrate faster in a gel?

Section Summary

PCR amplifies the needle; electrophoresis finds it in the haystack.

Exam Pointers

→ Describe PCR cycles and applications; explain gel electrophoresis.

Lecture 10: DNA Sequencing Technologies

Learning Objectives

- Describe sequencing methods
- Choose technologies for applications

Sequencing reads the base order. Sanger sequencing (dideoxy chain termination): DNA polymerase extends primers with a mix of normal dNTPs and chain-terminating ddNTPs, each labelled; fragments

ending at each position separate by size, revealing the sequence. It sequenced the first human genome — accurate but slow and costly.

Next-generation sequencing parallelizes massively: millions of fragments sequenced simultaneously (sequencing by synthesis, ion torrent), collapsing cost and time — the \$1,000 genome. Third-generation (PacBio, nanopore) reads single long molecules in real time, resolving repeats and structural variants Sanger and short reads miss.

Applications define modern biology: variant discovery for disease, cancer mutation profiling for targeted therapy, pathogen surveillance, and personalized medicine — treatment matched to the patient's genome. The exam theme: match the technology to the question (accuracy vs throughput vs read length).

Key Definitions

Sanger sequencing is defined as the dideoxy chain-termination method that first made DNA sequencing practical

Key Points — very important for exams

Sanger: ddNTP termination — accurate, slow; sequenced the first human genome.

NGS: millions of parallel short reads — cheap, fast; third-gen: long single-molecule reads.

Uses: disease variants, cancer profiling, pathogen surveillance, personalized medicine.

Review Questions

? How does dideoxy termination reveal sequence?

? When are long reads essential?

Section Summary

From Sanger to nanopore: reading DNA went from years and billions to hours and hundreds.

Exam Pointers

→ Compare Sanger, next-generation and third-generation sequencing with applications.

Lecture 11: Genomics, CRISPR and Bioinformatics

Learning Objectives

- Describe genome science
- Explain CRISPR and bioinformatics

Genomics studies whole genomes: sequencing, assembly, annotation (finding genes), and comparison. The Human Genome Project (2003) revealed ~20,000 protein-coding genes — far fewer than expected — and that 99.9 percent of human DNA is identical between individuals; the 0.1 percent (SNPs) underlies diversity and disease risk.

Functional genomics asks what genes do: transcriptomics (RNA-seq measures expression), proteomics (mass spectrometry identifies proteins), and CRISPR screens (systematic gene knockouts revealing function). CRISPR-Cas9 — guided RNA directing the Cas9 nuclease to cut specific DNA — is the landmark tool: precise, cheap genome editing, from curing sickle-cell in trials to engineering crops.

Bioinformatics is the computational backbone: sequence alignment (BLAST), genome browsers, phylogenetic trees, protein structure prediction (AlphaFold). Very important: modern molecular biology is inseparable from computation — the sequence is data before it is biology.

Key Definitions

CRISPR-Cas9 is defined as RNA-guided nuclease system enabling precise, programmable genome editing

Key Points — very important for exams

Human genome: ~20,000 genes; 99.9% identical; SNPs carry diversity and risk.

Functional genomics: RNA-seq, proteomics, CRISPR screens — from sequence to function.

CRISPR-Cas9: RNA-guided cutting for editing; bioinformatics (BLAST, AlphaFold) computes biology.

Review Questions

- ? How does CRISPR-Cas9 achieve specificity?
- ? What does RNA-seq measure?

Section Summary

Genomes are read, edited and computed — the molecular biology of the data age.

Exam Pointers

→ Describe genomics, functional genomics and bioinformatics with the CRISPR mechanism.

Lecture 12: Applications: Medicine, Forensics and Biotechnology

Learning Objectives

- Describe molecular medicine
- Survey biotech applications

Molecular techniques are now medical routine. Diagnostics: PCR detects pathogens and mutations; FISH visualizes chromosomal abnormalities; microarrays and NGS panels profile cancers. Recombinant therapeutics: insulin, clotting factors, monoclonal antibodies (e.g. trastuzumab for HER2 breast cancer) — all manufactured by engineered cells.

Gene therapy delivers working genes: ex vivo (edit cells outside, e.g. CAR-T immune cells for leukaemia) or in vivo (viral vectors delivering genes, as in spinal muscular atrophy treatment). CRISPR trials now edit patients' own cells for sickle-cell disease — cures, not lifelong treatment.

Forensics and agriculture complete the picture: DNA fingerprinting (STR profiling) identifies individuals with near-certainty; genetically modified crops (Bt cotton, golden rice) use the same recombinant logic for yield and nutrition. The unifying exam point: one toolbox — cut, copy, read, edit DNA — serving medicine, justice and agriculture.

Key Definitions

CAR-T cell therapy is defined as therapy using engineered immune cells expressing chimeric antigen receptors, e.g. for leukaemia

Key Points — very important for exams

Diagnostics: PCR, FISH, NGS panels; therapeutics: recombinant proteins, monoclonal antibodies.

Gene therapy: ex vivo (CAR-T) and in vivo vectors; CRISPR cures in trials (sickle-cell).

Forensics: STR fingerprinting; agriculture: Bt crops — one DNA toolbox, many fields.

Review Questions

- ? How does CAR-T therapy work?
- ? Give two agricultural applications of recombinant DNA.

Section Summary

Reading and editing DNA now diagnoses disease, convicts criminals, cures patients and improves crops.

Exam Pointers

- Survey molecular biology's applications in medicine, forensics and agriculture.

Glossary of Key Terms

Central dogma — the information flow DNA → RNA → protein, the organizing principle of molecular biology

Chargaff's rules — the base-pairing regularities (A=T, G=C) that revealed DNA's complementary structure

Semiconservative replication — replication in which each daughter DNA keeps one parental and one new strand

Okazaki fragments — short DNA segments synthesized on the lagging strand, later joined by ligase

RNA splicing — removal of introns and joining of exons from pre-mRNA by the spliceosome

Transcription factors — proteins that bind promoters/enhancers to control transcription initiation

Genetic code — the triplet, redundant, near-universal mapping of 64 codons to 20 amino acids and stop signals

Operon — a bacterial gene cluster (e.g. lac, trp) transcribed from a single promoter under shared control

Epigenetics — heritable changes in gene expression without altering the DNA sequence

Missense mutation — a point mutation changing one amino acid to another (e.g. sickle-cell Glu→Val)

Nonsense mutation — a point mutation converting an amino-acid codon into a stop codon, truncating the protein

Restriction enzymes — bacterial enzymes cutting DNA at specific sequences, producing sticky ends for cloning

Polymerase chain reaction (PCR) — cyclical in-vitro DNA amplification: denature → anneal → extend, doubling each round

Sanger sequencing — the dideoxy chain-termination method that first made DNA sequencing practical

CRISPR-Cas9 — RNA-guided nuclease system enabling precise, programmable genome editing

CAR-T cell therapy — therapy using engineered immune cells expressing chimeric antigen receptors, e.g. for leukaemia