



La-Net

BIO 205

INTRODUCTORY DEVELOPMENTAL / CELL BIOLOGY



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Course code: BIO 205

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Series 1: History and Trends in Cell Biology

- **Part 1.1: Early History of Cell Biology**

- Sub Part 1.1.1: Pre-microscopic understanding of life
- Sub Part 1.1.2: The invention of the microscope and early cell observations
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- **Part 1.3: Milestones in Molecular and Cellular Biology**

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Introduction

Cell biology is the branch of biology that studies the structure, function, and behaviour of cells, the fundamental units of life. All living organisms, from the simplest bacteria to the most complex multicellular plants and animals, are composed of cells; understanding the cell is therefore central to understanding all of biology. The history of cell biology is closely tied to the development of technologies that allow scientists to visualise and manipulate cells and their components, from the first primitive light microscopes of the seventeenth century to the cryo-electron microscopes and super-resolution fluorescence systems of the twenty-first century.

This Series traces the intellectual and technological history of cell biology from pre-scientific ideas about the organisation of living matter through the formulation of the cell theory in the nineteenth century, the ultrastructural revolution brought about by electron microscopy in the twentieth century, and the molecular revolution that followed the discovery of the DNA double helix; it concludes with a survey of current frontiers, including live-cell imaging, cell signalling networks, stem cell biology, and synthetic biology. This historical context is essential for understanding why modern cell biology is structured the way it is and how today's experimental tools were developed.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 1.1** describe the pre-microscopic understanding of life, explain the key observations made with early microscopes, and state the three tenets of the cell theory (Linked to Part 1.1),
- **SLO 1.2** describe the development of light microscopy, electron microscopy, and cell fractionation as tools for studying cells, and explain what each technique revealed about cell structure (Linked to Part 1.2),
- **SLO 1.3** describe the key milestones of the molecular revolution in cell biology, from the discovery of DNA structure to the development of genomics and proteomics (Linked to Part 1.3), and



- **SLO 1.4** describe current frontiers in cell biology, including super-resolution microscopy, cell signalling, stem cell research, and synthetic biology, and their medical applications (Linked to Part 1.4).

1.1 Early History of Cell Biology

1.1.1 Pre-microscopic understanding of life

Before the invention of the microscope, ancient Greek, Roman, and Arab scholars speculated extensively about the organisation and composition of living matter, but without the tools to observe cells directly, their ideas remained largely philosophical. Aristotle (384 to 322 BC) proposed that the body was composed of four humours (blood, phlegm, yellow bile, and black bile) and that living matter was organised into hierarchical levels (organs, tissues), but he had no concept of the cell as the fundamental structural unit. Galen (129 to 216 AD) extended Aristotelian anatomy through animal dissection and recognised that different tissues had different textures and compositions, but again without knowledge of cellular organisation. Islamic scholar Ibn Sina (Avicenna, 980 to 1037 AD) produced encyclopaedic works on medicine and natural history but similarly lacked the technology to observe cells. The dominant pre-microscopic view was that living matter was fundamentally different from non-living matter (vitalism) and was organised as a continuum of tissue types rather than as discrete structural units.

The concept of spontaneous generation also pervaded pre-microscopic biology: many philosophers and naturalists believed that living organisms could arise spontaneously from non-living matter (maggots from meat; mice from grain; algae from muddy water). This idea persisted until Francesco Redi (1668) demonstrated experimentally that maggots did not arise on meat protected from flies, and was finally disproved for microorganisms by Louis Pasteur's swan-neck flask experiments (1859 to 1861). The eventual refutation of spontaneous generation was closely tied to microscopic observations of microorganisms and laid intellectual groundwork for the germ theory of disease and the understanding that all cells come from pre-existing cells (the third tenet of cell theory).



Fill in the blank: Before the microscope, the dominant view of living matter was _____ (the idea that life is fundamentally different from non-living matter and cannot be reduced to simple physical components); the pre-microscopic era lacked the technology to observe cells but generated important anatomical knowledge through dissection and philosophical reasoning.

1.1.2 The invention of the microscope and early cell observations

The invention of the compound microscope in the late sixteenth century opened the first window into the microscopic world. The earliest microscopes are attributed to Dutch spectacle makers, including Hans Janssen and his son Zacharias Janssen (around 1590), who combined two lenses in a tube to produce a magnified image. Galileo Galilei also constructed a compound microscope around 1624. However, the first major biological discoveries using the microscope are credited to Robert Hooke (1635 to 1703), an English natural philosopher, who published his observations in *Micrographia* (1665). Hooke examined thin slices of cork under his compound microscope and observed that the cork was divided into tiny box-like compartments, which he named cells (from the Latin *cella*, meaning a small room), because they reminded him of the cells of a monastery. Hooke's cells were in fact the empty cell walls of dead plant cells, as cork is composed of dead cells whose cytoplasmic contents have long since disappeared.

The Dutch draper and self-taught microscopist Antonie van Leeuwenhoek (1632 to 1723) made even more remarkable observations. Leeuwenhoek constructed single-lens microscopes of exceptional quality (magnifying up to approximately 270×), far superior to the compound microscopes of his day. Using these instruments, he was the first to observe and describe bacteria (which he called animalcules) in dental scrapings and pond water (1674 to 1683), protozoa, red blood cells, spermatozoa, and the capillary circulation of blood in the tail of a fish. His observations, communicated to the Royal Society of London in a series of letters, established that a rich microscopic world of living organisms existed that was completely invisible to the naked eye. Subsequent microscopists of the seventeenth and eighteenth centuries, including Marcello Malpighi (1628 to 1694; first to describe capillaries in frog lungs, 1661) and Nehemiah Grew (1641 to 1712; described plant cell walls and pores), extended microscopic knowledge of tissues and cells, though the overarching framework of cell theory had not yet been formulated.



Fill in the blank: Robert Hooke named the small box-like compartments he observed in cork _____ (from the Latin for small room) in his 1665 publication *Micrographia*; Antonie van Leeuwenhoek was the first to observe bacteria, protozoa, and spermatozoa using single-lens microscopes of his own construction.

1.1.3 Formulation of the cell theory

The cell theory, one of the great unifying principles of biology, was formulated in the mid-nineteenth century through the work of Matthias Schleiden, Theodor Schwann, and Rudolf Virchow. Matthias Schleiden (1804 to 1881), a German botanist, concluded in 1838 that all plant tissues are composed of cells and that the cell is the basic unit of plant structure. Theodor Schwann (1810 to 1882), a German zoologist and Schleiden's colleague, extended this conclusion to animal tissues in 1839, proposing that all animals are also made of cells and that the cell is the elementary unit of life. Together, Schleiden and Schwann formulated the first two tenets of the cell theory: (1) All living organisms are composed of one or more cells; (2) The cell is the basic unit of structure and function in all living organisms. Rudolf Virchow (1821 to 1902), a German physician and pathologist, added the third and crucial tenet in 1855: (3) All cells arise from pre-existing cells (*Omnis cellula e cellula*), rejecting spontaneous generation and establishing that life is a continuous process of cell division from existing cells.

The cell theory transformed biology by providing a unifying structural framework: just as atoms are the fundamental units of matter, cells are the fundamental units of life. The theory also had profound practical implications for medicine: Virchow applied cell theory to pathology, arguing that disease is fundamentally a disorder of cells (cellular pathology), not of humours or vital forces. This shift from humoral to cellular concepts of disease laid the foundation for modern medicine. The cell theory as currently stated has three core tenets (as above) and is sometimes extended with two additional statements: (4) Cells contain hereditary information (DNA) that is passed from parent cell to daughter cell during cell division; (5) All cells are basically similar in chemical composition and metabolic activities. These extensions reflect the insights of the twentieth-century molecular revolution and complete the modern formulation of cell theory.

Fill in the blank: The three tenets of the cell theory are: (1) all living organisms are composed of one or more _____; (2) the cell is the basic unit of structure and function; (3) all cells arise



from pre-existing cells (Virchow, 1855); Virchow's contribution rejected spontaneous generation and established that cell division is the only source of new cells.

Pilot questions 1.1

1. Describe the pre-microscopic understanding of living matter and state one limitation of Aristotle's view of biological organisation.
2. State two key observations made by Robert Hooke and explain what he actually saw when he observed cork cells.
3. Who was Antonie van Leeuwenhoek and what was his most important contribution to cell biology?
4. State the three tenets of the cell theory and name the scientist who contributed each tenet.
5. Explain how the refutation of spontaneous generation supported the development of the cell theory.

1.2 Development of Microscopy and Cell Study Techniques

1.2.1 Light microscopy and histological methods

Light (optical) microscopy uses visible light and glass lenses to magnify specimens. The development of high-quality light microscopes in the nineteenth century, combined with the introduction of staining techniques, opened the era of systematic cell and tissue study (histology). Key developments in light microscopy and histology: (1) Achromatic lenses (1820s to 1830s): the combination of crown glass and flint glass lenses eliminated chromatic aberration (colour fringing), dramatically improving image quality; this made the systematic study of cells practical for the first time. (2) Synthetic dyes (1850s onwards): the development of aniline dyes (mauveine by William Perkin, 1856; haematoxylin and eosin, the H&E stain) provided differential staining of cell components; haematoxylin stains nuclei blue-purple; eosin stains cytoplasm and extracellular matrix pink; the H&E stain remains the most widely used histological stain in diagnostic pathology today. (3) Immersion oil objectives (1870s): Carl Zeiss and Ernst Abbe introduced oil-immersion lenses that increased resolution by using immersion oil (refractive index matching the glass lens) between the objective and the coverslip; this allowed resolution of structures as small as approximately 200 nm, approaching the theoretical limit of



light microscopy (the Abbe diffraction limit: resolution limit $d = \lambda/2NA$, where λ is the wavelength of light and NA is the numerical aperture of the objective).

(4) Phase contrast microscopy (Frits Zernike, 1930s; Nobel Prize 1953): converts differences in refractive index within the cell (invisible to the eye in bright-field illumination) into differences in light intensity (contrast), allowing the observation of living, unstained cells without fixation; this was the first technique to allow long-term observation of living cells in culture. (5) Differential interference contrast (DIC) microscopy (Georges Nomarski, 1950s): uses polarised light to produce a pseudo-three-dimensional image of living, unstained cells with excellent contrast. (6) Fluorescence microscopy (1940s to present): uses fluorescent dyes or genetically encoded fluorescent proteins (e.g. green fluorescent protein, GFP, 1994; Nobel Prize 2008 to Chalfie, Shimomura, Tsien) to label specific molecules within living cells; fluorescence microscopy revolutionised cell biology by allowing specific proteins, organelles, and DNA sequences to be visualised in their cellular context. (7) Confocal microscopy (Marvin Minsky, 1957; commercially available from mid-1980s): uses a pinhole aperture to exclude out-of-focus light, producing optically thin sections ('optical sectioning') of fluorescently labelled specimens; a series of optical sections can be reconstructed into a three-dimensional image.

Fill in the blank: The theoretical resolution limit of light microscopy is set by the Abbe _____ limit ($d = \lambda/2NA$); phase contrast microscopy (Zernike) allows observation of living unstained cells; fluorescence microscopy using GFP labels specific molecules; confocal microscopy produces optical sections by excluding out-of-focus light.

1.2.2 Electron microscopy and ultrastructural studies

The electron microscope (EM) uses a beam of electrons rather than light to form an image, achieving resolution more than 1,000 times greater than light microscopy. The wavelength of electrons is approximately 10,000 times shorter than visible light, so electron microscopes are not subject to the same diffraction limit as light microscopes; modern transmission electron microscopes (TEMs) can resolve structures down to approximately 0.1 nm (atomic resolution). The first practical transmission electron microscope was built by Ernst Ruska and Max Knoll in Germany in 1931 to 1932 (Ruska received the Nobel Prize in Physics in 1986). The first biological application of the TEM was made by Marton in 1934. Two main types of electron



microscope: (1) Transmission electron microscope (TEM): electrons pass through an ultrathin section (approximately 50 to 100 nm thick, cut with an ultramicrotome from a resin-embedded specimen) and are differentially absorbed or scattered by the specimen; dense structures (stained with heavy metal salts such as osmium tetroxide, lead citrate, and uranyl acetate) appear dark in the image; the TEM revealed the ultrastructure (fine structure) of cell organelles for the first time.

Key ultrastructural discoveries made with the TEM: the double-membrane structure of the nuclear envelope (1950s); the unit membrane model of the plasma membrane (Robertson, 1959); the internal membrane system of mitochondria (cristae) and chloroplasts (thylakoids); the endoplasmic reticulum (ER) and the Golgi apparatus as membrane systems; ribosomes as electron-dense particles on the rough ER; the microtubule structure of the mitotic spindle and flagella/cilia (the 9+2 axoneme structure). (2) Scanning electron microscope (SEM): electrons are scanned across the surface of a specimen coated with a thin metal film (usually gold or gold-palladium); secondary electrons emitted from the surface are detected to produce a three-dimensional image of the specimen surface; the SEM provides spectacular images of cell surfaces, pollen grains, insect compound eyes, and other biological surfaces; resolution is lower than TEM (approximately 5 to 10 nm) but the images are intuitively interpretable. (3) Cryo-electron microscopy (cryo-EM): specimens are rapidly frozen (vitrified) in a thin layer of ice without chemical fixation or staining; this preserves native molecular structures; computational averaging of many images of the same molecule allows near-atomic-resolution structures to be determined; cryo-EM has revolutionised structural biology and earned the Nobel Prize in Chemistry in 2017 (Jacques Dubochet, Joachim Frank, Richard Henderson).

Fill in the blank: The transmission electron microscope (TEM) achieves resolution to approximately 0.1 nm by using _____ rather than light; key ultrastructural discoveries with the TEM include the double membrane of the nuclear envelope, the cristae of mitochondria, and the 9+2 axoneme of cilia; cryo-EM (Nobel 2017) determines near-atomic resolution structures without staining.



1.2.3 Cell fractionation and biochemical approaches

While microscopy reveals where structures are located within the cell, cell fractionation allows the isolation of specific organelles in bulk quantities so their biochemical composition and function can be studied. Cell fractionation is based on differential centrifugation: cells are disrupted (homogenised) in an isotonic buffer (to keep organelles intact), and the resulting homogenate is centrifuged at progressively increasing speeds to separate organelles by their size and density. Standard differential centrifugation protocol: (1) Low speed ($600 \times g$; 10 minutes): unbroken cells, cell debris, and nuclei sediment in the pellet; the supernatant is retained for further fractionation. (2) Medium speed ($10,000 \times g$; 20 minutes): mitochondria, chloroplasts, lysosomes, and peroxisomes sediment; the supernatant is retained. (3) High speed ($100,000 \times g$; 60 minutes): microsomes (fragments of ER and Golgi membranes) and small vesicles sediment; the supernatant contains soluble cytoplasmic proteins (the cytosol fraction). Each pellet fraction can then be resuspended and subjected to further fractionation using density gradient centrifugation (equilibrium sedimentation in a sucrose or CsCl gradient) to obtain highly purified organelle preparations.

Biochemical analysis of fractionated organelles: protein composition (SDS-PAGE; Western blotting; mass spectrometry); lipid composition (thin-layer chromatography; mass spectrometry); enzymatic activities (biochemical assays for marker enzymes: succinate dehydrogenase for inner mitochondrial membrane; acid phosphatase for lysosomes; catalase for peroxisomes; glucose-6-phosphatase for ER); DNA/RNA content. Cell fractionation combined with enzyme cytochemistry, immunofluorescence, and genetic approaches has provided a comprehensive picture of the molecular composition of each organelle. Other biochemical cell biology methods: subcellular proteomics (mass spectrometry-based identification of all proteins in a specific organelle or compartment); proximity labelling (BioID; TurboID: enzymes fused to a protein of interest that biotinylate all proteins within approximately 10 nm, allowing proteomic identification of interaction partners in living cells); chromatin immunoprecipitation (ChIP) for studying protein-DNA interactions.

Fill in the blank: Cell fractionation separates organelles by _____ centrifugation: nuclei and cell debris pellet at low speed ($600 \times g$); mitochondria and chloroplasts at medium speed ($10,000$



×g); microsomes at high speed (100,000 ×g); marker enzymes (e.g. succinate dehydrogenase for mitochondria; catalase for peroxisomes) verify the purity of each fraction.

Pilot questions 1.2

1. Explain the Abbe diffraction limit and state approximately what resolution is achievable with the best light microscopes.
2. Explain how phase contrast microscopy differs from bright-field microscopy and state its main advantage.
3. Compare TEM and SEM in terms of specimen preparation, type of information obtained, and resolution.
4. Describe the principle of differential centrifugation and state what organelles are recovered at each centrifugation step.
5. Name two marker enzymes used to verify organelle purity in cell fractionation experiments and state which organelle each marks.

1.3 Milestones in Molecular and Cellular Biology

1.3.1 Discovery of DNA structure and the molecular revolution

The molecular revolution in cell biology was triggered by the elucidation of the structure of DNA by James Watson and Francis Crick in 1953, building on X-ray crystallography data from Rosalind Franklin and Maurice Wilkins. The double-helix model immediately suggested the mechanism for DNA replication (complementary base pairing) and for the encoding of genetic information (the sequence of bases). The central dogma of molecular biology (Francis Crick, 1957; published 1958) provided the framework: information flows from DNA to RNA to protein (DNA → RNA → protein); with the additional routes of DNA replication (DNA → DNA) and reverse transcription (RNA → DNA, discovered by Temin and Baltimore 1970). Key milestones of the molecular revolution: (1) The genetic code (1961 to 1966): Nirenberg, Khorana, and colleagues deciphered the triplet genetic code, showing that codons of three nucleotides specify each of the 20 amino acids. (2) Restriction enzymes and DNA cloning (1970 to 1973): discovery of restriction endonucleases (Arber, Smith, Nathans; Nobel 1978) and DNA ligase enabled the



cutting and joining of DNA molecules; recombinant DNA technology (Boyer and Cohen, 1973) allowed specific genes to be cloned in bacterial plasmids.

(3) DNA sequencing (Sanger, 1977; Nobel 1980): Frederick Sanger developed the chain-termination (dideoxy) method for sequencing DNA, making it possible to read the nucleotide sequence of genes; the Maxam-Gilbert chemical cleavage method was developed simultaneously. (4) Polymerase chain reaction (PCR; Kary Mullis, 1983; Nobel 1993): exponential amplification of specific DNA sequences in vitro using thermostable DNA polymerase (Taq polymerase from *Thermus aquaticus*); PCR transformed molecular biology, forensic science, diagnostics, and evolutionary biology. (5) The first recombinant protein: human insulin produced in *Escherichia coli* by Genentech (1982) was the first recombinant pharmaceutical, demonstrating the commercial power of genetic engineering. (6) Transgenic animals: the first transgenic mice were produced by Jaenisch (1974) and by Gordon and Ruddle (1980); transgenic technology provided powerful tools for studying gene function in vivo and for producing animal models of human diseases.

Fill in the blank: The central dogma of molecular biology states that genetic information flows from _____ to RNA to protein; restriction enzymes and DNA ligase (early 1970s) enabled recombinant DNA technology; PCR (Mullis, 1983; Nobel 1993) allows exponential in vitro amplification of specific DNA sequences.

1.3.2 Development of cell culture and cloning technologies

Cell culture is the technique of growing cells outside the body in an artificial medium under controlled conditions. The development of reliable cell culture methods was essential for modern cell biology because it allowed cells to be studied individually, manipulated genetically, and used in large numbers for biochemical experiments. History of cell culture: Ross Harrison (1907) was the first to demonstrate that cells could survive and grow outside the body, maintaining nerve fibres from frog embryos in hanging drops of frog lymph for several weeks; this was the first tissue culture experiment. Alexis Carrel (1873 to 1944) at the Rockefeller Institute developed long-term culture methods using aseptic technique and nutrient media, and maintained a culture of chicken heart fibroblasts for over 20 years (though this result was later questioned). Enders, Weller, and Robbins (Nobel 1954) grew poliovirus in human tissue cultures, enabling



the development of the polio vaccine by Jonas Salk (1955). HeLa cells: in 1951, cells from the cervical carcinoma of Henrietta Lacks were cultured by George Gey at Johns Hopkins University; HeLa cells were the first human cell line to be maintained indefinitely in culture; they have been used in thousands of research studies and played a key role in the development of the polio vaccine; HeLa cells continue to grow in laboratories worldwide.

Primary culture: cells taken directly from tissues and cultured for the first time; primary cultures have limited lifespan (the Hayflick limit; approximately 50 doublings for normal human somatic cells before senescence, related to telomere shortening). Cell lines: cells that have been adapted to grow indefinitely in culture (immortalised); most cell lines are derived from tumour cells (which have bypassed senescence) or are artificially immortalised (by transfection with viral oncogenes such as SV40 T antigen or telomerase). Cloning of organisms: following the nuclear transfer experiments of Briggs and King with frog embryo nuclei (1952), Gurdon (Nobel 2012) demonstrated that somatic cell nuclei could direct normal development (somatic cell nuclear transfer, SCNT). Dolly the sheep (Wilmut et al., Roslin Institute, 1996) was the first mammal cloned from an adult somatic cell (a mammary gland cell), demonstrating that the nucleus of a differentiated adult cell retains the complete genome and can be reprogrammed to a totipotent state; this proof-of-concept profoundly impacted developmental biology and bioethics.

Fill in the blank: Ross Harrison (1907) demonstrated that cells could survive _____ the body (in vitro) in the first tissue culture experiment; HeLa cells (established 1951) were the first immortal human cell line; Dolly the sheep (1996) was the first mammal cloned from an adult somatic cell by somatic cell nuclear transfer.

1.3.3 Genomics, proteomics, and systems biology

Genomics is the large-scale study of genomes (the complete set of genetic information of an organism). The Human Genome Project (HGP; 1990 to 2003): an international consortium of research centres coordinated by Francis Collins (NIH) and a competing private effort by Celera Genomics (Craig Venter); together they produced a reference sequence of the approximately 3.2 billion base pairs of the human genome; the project found approximately 20,000 to 25,000 protein-coding genes (far fewer than many had predicted, since much of the genome consists of non-coding RNA genes, regulatory sequences, and repetitive elements); the HGP sequence was



deposited in public databases (GenBank, EMBL, DDBJ) under Bermuda principles of immediate data release. Next-generation sequencing (NGS): from 2005, massively parallel (next-generation) sequencing platforms (Roche 454, Illumina, ABI SOLiD; followed by third-generation platforms such as Oxford Nanopore and PacBio) reduced the cost of sequencing a human genome from approximately 3 billion USD (HGP) to below 1,000 USD (by 2014), democratising genomics and enabling population-scale sequencing, cancer genomics, metagenomics, and personalised medicine. RNA-seq (transcriptomics): genome-wide sequencing of cDNA made from cellular mRNA, providing a snapshot of gene expression levels across the entire transcriptome.

Proteomics: the large-scale study of the complete protein complement (proteome) of a cell, tissue, or organism; unlike the genome (essentially fixed), the proteome is highly dynamic and changes with cell type, developmental stage, and environmental conditions; key proteomics technologies: two-dimensional gel electrophoresis (2D-PAGE; separates proteins by isoelectric point in one dimension and molecular weight in the second) and mass spectrometry (identifies and quantifies proteins from peptide fragments produced by proteolytic digestion; tandem mass spectrometry, MS/MS; label-free and isotope-labelling quantification, SILAC). Systems biology: integrates genomic, transcriptomic, proteomic, metabolomic, and other large-scale data using computational and mathematical modelling to understand cells and organisms as integrated systems; rather than studying individual genes or proteins in isolation, systems biology seeks to understand emergent properties (behaviours that arise from networks of interacting components that cannot be predicted from the behaviour of individual components); examples include protein interaction networks (interactome), gene regulatory networks, metabolic networks, and signal transduction networks.

Fill in the blank: The Human Genome Project (1990 to 2003) sequenced the approximately 3.2 billion base pairs of the human genome and found approximately 20,000 to 25,000 protein-coding _____; next-generation sequencing reduced the cost of a human genome sequence from approximately 3 billion USD to below 1,000 USD; systems biology integrates multi-omics data to understand cells as integrated networks.

Pilot questions 1.3



1. State the central dogma of molecular biology and name the scientist who formulated it.
2. Describe how recombinant DNA technology was developed and state two practical applications.
3. What was the Human Genome Project and what were its two main findings about the human genome?
4. Distinguish between genomics and proteomics in terms of what is studied and why the proteome is more complex than the genome.
5. Explain why systems biology requires computational and mathematical modelling, rather than traditional reductionist approaches.

1.4 Current Trends and Frontiers in Cell Biology

1.4.1 Live-cell imaging and super-resolution microscopy

One of the most important frontiers in cell biology is the ability to observe molecular events in real time in living cells. Live-cell imaging overcomes a fundamental limitation of classical microscopy: conventional fixation and staining kills cells and provides only a static snapshot, whereas live-cell imaging allows dynamic processes (vesicle trafficking, cytoskeletal remodelling, cell division, signal transduction) to be followed over time. Key live-cell imaging technologies: (1) Fluorescent protein tagging: genes encoding fluorescent proteins (GFP and its variants, including red fluorescent protein, mCherry, YFP, CFP; Nobel 2008 to Chalfie, Shimomura, Tsien) can be fused to any gene of interest and expressed in living cells; the fluorescent fusion protein reports the location and movements of the tagged protein in real time. (2) Total internal reflection fluorescence microscopy (TIRF): illuminates only molecules within approximately 100 nm of the coverglass (the evanescent field), dramatically reducing background fluorescence; ideal for imaging single molecules at the plasma membrane. (3) Fluorescence recovery after photobleaching (FRAP): a region of fluorescent protein is bleached by a high-intensity laser pulse, and the recovery of fluorescence is monitored as unbleached molecules diffuse back in; provides quantitative information on molecular mobility and exchange rates.

Super-resolution microscopy breaks the Abbe diffraction limit (approximately 200 nm for visible light), allowing structures 10 to 20 times smaller to be resolved. The Nobel Prize in Chemistry



2014 was awarded to Eric Betzig, Stefan Hell, and William Moerner for the development of super-resolution fluorescence microscopy. Main super-resolution techniques: (1) STED microscopy (Stimulated Emission Depletion; Stefan Hell): uses a doughnut-shaped depletion laser to quench fluorescence at the periphery of the excitation spot, restricting fluorescent emission to a region smaller than the diffraction limit; achieves lateral resolution of approximately 20 to 50 nm. (2) PALM and STORM (Photo-Activated Localisation Microscopy; STochastic Optical Reconstruction Microscopy; Betzig, Moerner, Zhuang): use photoswitchable or photoactivatable fluorophores that are stochastically activated and imaged one at a time; by fitting the point spread function of each single molecule, its position can be localised to approximately 10 to 30 nm; accumulating thousands of single-molecule localisations builds up a super-resolution image. These techniques have revealed the nanoscale organisation of the plasma membrane, the architecture of synaptic proteins, and the structure of nuclear pore complexes.

Fill in the blank: Super-resolution microscopy overcomes the Abbe _____ limit (≈ 200 nm for visible light); STED microscopy uses a depletion laser to restrict emission to below the diffraction limit (≈ 20 to 50 nm resolution); PALM/STORM localise individual fluorescent molecules to ≈ 10 to 30 nm; the Nobel Prize in Chemistry 2014 recognised these techniques.

1.4.2 Cell signalling and systems approaches

Cell signalling (signal transduction) is the process by which cells receive, process, and respond to information from their environment and from other cells. The systematic study of cell signalling pathways has become one of the dominant themes of modern cell biology. Key signalling pathways: (1) G-protein coupled receptor (GPCR) signalling: the largest family of cell surface receptors (approximately 800 in humans); ligand binding activates a heterotrimeric G protein that modulates effectors (adenylyl cyclase, phospholipase C) to generate second messengers (cAMP, IP₃, DAG); Nobel Prize 2012 to Lefkowitz and Kobilka for GPCR structure and function. (2) Receptor tyrosine kinase (RTK) signalling: receptors with intrinsic tyrosine kinase activity (epidermal growth factor receptor, EGFR; insulin receptor; FGF receptor); ligand binding induces dimerisation and autophosphorylation; activated receptor recruits and phosphorylates scaffold proteins that activate the RAS-MAPK and PI3K-AKT-mTOR pathways. (3) Wnt signalling: controls cell fate decisions in development; inhibits GSK3 β , stabilising β -



catenin, which enters the nucleus to activate transcription; dysregulation is a major driver of colorectal cancer.

Systems approaches to cell signalling: traditional biochemistry studied one signalling component at a time; systems biology integrates global phosphoproteomic data (all phosphorylation events in the cell at a given time), protein interaction networks, and gene expression data to build computational models of entire signalling networks; phosphoproteomics (mass spectrometry-based global identification of phosphorylated proteins and phosphorylation sites) has revealed that signal transduction involves hundreds of simultaneous phosphorylation events that form complex interconnected networks (crosstalk, feedback loops, feedforward loops). CRISPR-Cas9 screens: genome-wide loss-of-function screens using the CRISPR-Cas9 system to systematically disrupt every gene in a cell line and determine which genes are required for a specific cellular response (e.g. sensitivity or resistance to a drug, migration, survival); these functional genomics screens provide a systems-level view of signalling dependencies. Organoids: three-dimensional self-organising cell cultures derived from stem cells or tumour cells that recapitulate organ architecture and function; used to model disease, test drugs, and study cell signalling in a physiologically relevant context.

Fill in the blank: GPCR signalling is mediated by _____ proteins that generate second messengers (cAMP, IP₃, DAG) after receptor activation; receptor tyrosine kinases (RTKs) activate the RAS-MAPK and PI3K-AKT pathways through autophosphorylation; CRISPR-Cas9 genome-wide screens identify which genes are required for specific cellular responses.

1.4.3 Stem cells, synthetic biology, and medical applications

Stem cell biology has emerged as one of the most clinically significant frontiers of cell biology. Stem cells are defined by two properties: self-renewal (the ability to divide and produce daughter cells with the same developmental potential) and multipotency or pluripotency (the ability to differentiate into multiple or all cell types). Types of stem cells: (1) Embryonic stem cells (ESCs): derived from the inner cell mass of the blastocyst (approximately 5 to 7 days post-fertilisation); pluripotent (can give rise to all three germ layers and all somatic cell types); first derived from mouse embryos by Evans and Kaufman (1981) and from human embryos by Thomson et al. (1998); human ESC research was controversial because it required the



destruction of human embryos. (2) Adult stem cells: tissue-specific stem cells found in most adult tissues (haematopoietic stem cells in bone marrow; neural stem cells in the brain; intestinal epithelial stem cells in the crypts of Lieberkuhn); multipotent (can differentiate into the cell types of their tissue of origin); responsible for tissue maintenance and repair. (3) Induced pluripotent stem cells (iPSCs): Shinya Yamanaka (Nobel 2012) demonstrated in 2006 (mouse) and 2007 (human) that adult somatic cells can be reprogrammed to a pluripotent state by forced expression of four transcription factors (Oct4, Sox2, Klf4, c-Myc; the Yamanaka factors); iPSCs avoid the ethical issues of ESCs and can be made from the patient's own cells, circumventing immune rejection.

Synthetic biology applies engineering principles to biology to design and build novel biological systems or redesign existing ones for useful purposes. Approaches: (1) Metabolic engineering: engineering microorganisms (yeast, E. coli) to produce valuable compounds (biofuels, pharmaceuticals, food ingredients); example: Artemisinin (anti-malarial drug) precursor semi-synthesised in yeast by Jay Keasling's group (2006). (2) Genetic circuits: engineering gene regulatory networks with logic gate behaviour (AND, OR, NOT) for biosensor and therapeutic applications. (3) Genome editing (CRISPR-Cas9): the CRISPR-Cas9 system (Doudna and Charpentier; Nobel 2020) allows precise editing of any genomic locus in virtually any cell type; applications include correction of genetic diseases (e.g. sickle-cell disease, Duchenne muscular dystrophy), cancer immunotherapy (engineered CAR-T cells), and agricultural improvement (disease-resistant crops). (4) Cell therapy and regenerative medicine: autologous iPSC-derived cells are being tested as therapies for macular degeneration (retinal pigment epithelium cells), Parkinson's disease (dopaminergic neurons), and diabetes (beta cells); the first iPSC clinical trial was conducted in Japan by Masayo Takahashi (2014).



CELL BIOLOGY MILESTONES: 1590 – 2024

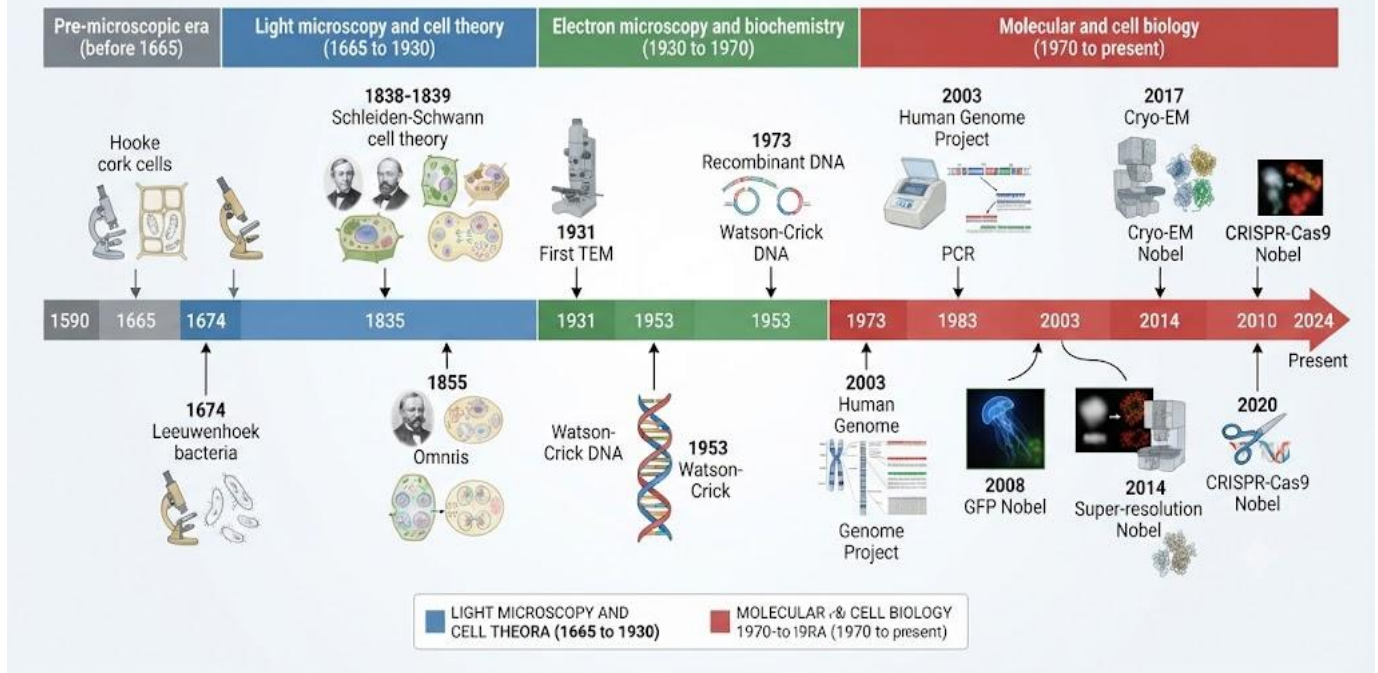


Figure 1.1: Timeline of major milestones in the history of cell biology from the invention of the microscope (1590) to the present day

Pilot questions 1.4

1. Explain the principle of STED microscopy and state the resolution it achieves.
2. Distinguish PALM/STORM from STED microscopy in terms of their underlying physical principles.
3. Define stem cells and distinguish between embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) in terms of origin and ethical considerations.
4. Explain the CRISPR-Cas9 system and state two of its applications in medicine.
5. Define synthetic biology and give one example of a synthetic biology product with medical or industrial significance.



Series Summary

This Series traced the **historical development of cell biology**, from early ideas about living organisms to the emergence of modern cellular and molecular biology. You explored the contributions of pioneering scientists, the development of cell theory, and the evolution of microscopy and cell fractionation techniques that transformed our understanding of cell structure and function.

The Series also examined the molecular revolution, including discoveries in DNA, recombinant DNA technology, PCR, cell culture, genomics, proteomics, and current advances such as super-resolution microscopy, stem cell research, CRISPR gene editing, and synthetic biology. By completing this Series, you should now be able to explain the historical milestones in cell biology, describe the development and applications of key cellular and molecular techniques, evaluate modern advances in the field, and relate these discoveries to contemporary biological research, thereby achieving all four **Series Learning Outcomes (SLOs)** and the **overall course outcomes**.

Glossary of Terms

- **Abbe diffraction limit:** the theoretical resolution limit of light microscopy: $d = \lambda/2NA$, where λ = wavelength of light and NA = numerical aperture of the objective; approximately 200 nm for visible light; sets the upper limit of conventional light microscopy resolution.
- **Cell fractionation:** the isolation of subcellular components by disrupting cells and separating organelles by differential centrifugation; allows the biochemical study of specific organelles in isolation.
- **Cell line:** a population of cells derived from a primary culture that has been adapted to grow indefinitely in culture (immortalised); most cell lines are derived from tumour cells or artificially immortalised by viral oncogenes.
- **Cell theory:** the unifying principle of biology stating that (1) all living organisms are composed of cells; (2) the cell is the basic unit of structure and function; (3) all cells arise from pre-existing cells (Virchow, 1855).



- **Central dogma:** the principle formulated by Crick (1957) that genetic information flows from DNA to RNA to protein (DNA → RNA → protein); with additional routes of DNA replication (DNA → DNA) and reverse transcription (RNA → DNA).
- **Confocal microscopy:** a form of fluorescence microscopy that uses a pinhole aperture to exclude out-of-focus light, producing optical sections of specimens; enables three-dimensional reconstruction of fluorescently labelled cells.
- **CRISPR-Cas9:** a genome editing system derived from a bacterial adaptive immune system; uses a guide RNA to direct the Cas9 nuclease to a specific genomic locus, where it creates a double-strand break; enables precise genome editing in virtually any cell type; Nobel 2020.
- **Cryo-electron microscopy (cryo-EM):** electron microscopy of specimens preserved in vitreous ice (rapidly frozen without chemical fixation); preserves native molecular structures; enables near-atomic resolution structure determination; Nobel 2017.
- **Differential centrifugation:** sequential centrifugation of a cell homogenate at progressively increasing speeds to separate organelles by size and density; nuclei (600g), mitochondria (10,000g), microsomes (100,000g).
- **Electron microscope (EM):** a microscope that uses a beam of electrons rather than light to form an image; resolution approximately 0.1 nm; TEM (transmission) reveals ultrastructure; SEM (scanning) reveals surface topography.
- **Genomics:** the large-scale study of genomes; includes the Human Genome Project (1990 to 2003); next-generation sequencing; comparative genomics; metagenomics; transcriptomics (RNA-seq).
- **HeLa cells:** the first immortal human cell line, established in 1951 from the cervical carcinoma of Henrietta Lacks; widely used in biomedical research; played a key role in polio vaccine development.
- **Induced pluripotent stem cells (iPSCs):** adult somatic cells reprogrammed to pluripotency by forced expression of four transcription factors (Oct4, Sox2, Klf4, c-Myc; Yamanaka factors); Nobel 2012; avoid ethical issues of ESCs; can be patient-specific.
- **Phase contrast microscopy:** a light microscopy technique (Zernike; Nobel 1953) that converts differences in refractive index within a specimen into differences in light intensity; allows observation of living, unstained cells.



- **Polymerase chain reaction (PCR):** in vitro exponential amplification of a specific DNA sequence using thermostable DNA polymerase and specific primers; developed by Kary Mullis (1983; Nobel 1993); fundamental tool of molecular biology.
- **Proteomics:** the large-scale study of the protein complement (proteome) of a cell; uses 2D-PAGE and mass spectrometry; the proteome is more complex than the genome because of post-translational modifications, protein isoforms, and dynamic changes.
- **Scanning electron microscope (SEM):** an electron microscope that scans a focused electron beam across a metal-coated specimen surface and detects secondary electrons to produce a three-dimensional image of the surface; resolution approximately 5 to 10 nm.
- **STED microscopy:** Stimulated Emission Depletion microscopy; a super-resolution technique using a doughnut-shaped depletion laser to restrict fluorescent emission below the diffraction limit; resolution approximately 20 to 50 nm; Nobel 2014.
- **Super-resolution microscopy:** a group of fluorescence microscopy techniques (STED, PALM, STORM) that overcome the Abbe diffraction limit to achieve resolution of 10 to 50 nm; Nobel Prize in Chemistry 2014.
- **Systems biology:** a research approach that integrates genomic, transcriptomic, proteomic, and other large-scale data using computational modelling to understand cells and organisms as integrated networks; seeks to explain emergent properties not predictable from individual components.
- **Transmission electron microscope (TEM):** an electron microscope in which electrons pass through an ultrathin specimen section; resolution approximately 0.1 nm; revealed the ultrastructure of cell organelles (mitochondrial cristae; nuclear pore complexes; ribosome structure).



Concept Check Questions [Series 1]

Objective Questions

1. Robert Hooke named the small compartments he observed in cork _____ (from the Latin for small room) in his 1665 publication *Micrographia*; he was actually seeing the empty cell walls of dead plant cells. (Linked to SLO 1.1)
2. The three tenets of the cell theory are: (1) all living organisms are composed of cells; (2) the cell is the basic unit of structure and function; (3) all cells arise from _____ cells (Virchow, 1855; *Omnis cellula e cellula*). (Linked to SLO 1.1)
3. The theoretical resolution limit of light microscopy is the Abbe _____ limit: $d = \lambda/2NA$; the best light microscopes resolve structures approximately 200 nm apart; electron microscopes achieve resolution to approximately 0.1 nm. (Linked to SLO 1.2)
4. Cell fractionation separates organelles by differential centrifugation: nuclei pellet at 600g; _____ pellet at approximately 10,000g; microsomes (ER fragments) pellet at approximately 100,000g. (Linked to SLO 1.2)
5. The central dogma of molecular biology states that information flows from DNA to _____ to protein; PCR (Mullis, 1983) allows exponential in vitro amplification of specific DNA sequences using thermostable DNA polymerase. (Linked to SLO 1.3)

Short-Answer Questions

6. State the three tenets of the cell theory and name the scientist associated with each. (Linked to SLO 1.1)
7. Compare light microscopy and transmission electron microscopy in terms of the energy source, theoretical resolution, and specimen preparation. (Linked to SLO 1.2)
8. Describe the principle and biological application of phase contrast microscopy. (Linked to SLO 1.2)
9. Explain how differential centrifugation separates cell organelles and state the organelle recovered at each centrifugation step. (Linked to SLO 1.2)
10. Distinguish between embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). (Linked to SLO 1.4)

Long-Answer Questions



11. Describe the history of cell biology from the invention of the microscope to the formulation of the cell theory, including the contributions of Hooke, Leeuwenhoek, Schleiden, Schwann, and Virchow. (Linked to SLOs 1.1, 1.2)
12. Describe the molecular revolution in cell biology, including the discovery of DNA structure, recombinant DNA technology, and PCR. Describe current frontiers including super-resolution microscopy, stem cells, and CRISPR-Cas9. (Linked to SLOs 1.3, 1.4)



Answers to Concept Check Questions [Series 1]

Objective Questions

1. Cells.
2. Pre-existing.
3. Diffraction.
4. Mitochondria.
5. RNA.

Short-Answer Questions

6. (1) All living organisms are composed of cells (Schleiden 1838; Schwann 1839). (2) The cell is the basic unit of structure and function (Schleiden and Schwann). (3) All cells arise from pre-existing cells (Virchow, 1855; *Omnis cellula e cellula*).
7. Light microscopy: energy source = visible light; resolution ≈ 200 nm (Abbe limit); specimens can be living or fixed/stained. TEM: energy source = electrons; resolution ≈ 0.1 nm; specimens must be fixed, embedded in resin, cut into ultrathin sections (50 to 100 nm), and stained with heavy metals.
8. Phase contrast microscopy converts differences in refractive index within the cell into differences in light intensity, creating contrast in unstained living cells. Application: enables long-term observation of living cells in culture without chemical fixation; widely used to observe cell division, migration, and organelle movements.
9. Cells are homogenised in isotonic buffer and the homogenate is centrifuged at increasing speeds: 600g (10 min) = nuclei and cell debris; 10,000g (20 min) = mitochondria, lysosomes, peroxisomes; 100,000g (60 min) = microsomes (ER and Golgi fragments); supernatant = cytosol.
10. ESCs: derived from the inner cell mass of the blastocyst; pluripotent; require destruction of human embryos (ethically controversial). iPSCs: reprogrammed from adult somatic cells by four transcription factors (Yamanaka factors: Oct4, Sox2, Klf4, c-Myc); pluripotent; no ethical issues; can be patient-specific.

Long-Answer Questions

11. Pre-microscopic era: vitalism; no concept of cells; spontaneous generation accepted (refuted by Redi 1668 and Pasteur 1861). Microscope invention: Janssen (≈ 1590); Hooke (1665; cork cells; Micrographia; named cells from empty plant cell walls); Leeuwenhoek (1674; single-lens microscopes to 270x; first observed bacteria, protozoa, spermatozoa). Cell theory: Schleiden



(1838; all plants composed of cells); Schwann (1839; extended to animals; cell = basic unit); Virchow (1855; Omnis cellula e cellula; all cells from pre-existing cells; cellular pathology; rejected spontaneous generation). Microscopy advances: achromatic lenses (1820s); H&E stain; oil-immersion objectives; Abbe diffraction limit ($\lambda/2NA \approx 200$ nm); phase contrast (Zernike; Nobel 1953); TEM (Ruska 1931; ultrastructure of organelles); SEM (surface imaging); cell fractionation (differential centrifugation; 600g nuclei; 10,000g mitochondria; 100,000g microsomes; marker enzymes).

12. Molecular revolution: Watson and Crick double helix (1953; built on Franklin/Wilkins X-ray data); central dogma (DNA→RNA→protein; Crick 1957); genetic code (1961 to 1966); restriction enzymes (Nobel 1978); recombinant DNA technology (Boyer and Cohen 1973); Sanger sequencing (Nobel 1980); PCR (Mullis 1983; Nobel 1993; thermostable Taq polymerase; exponential amplification); Human Genome Project (1990 to 2003; $\approx 20,000$ to 25,000 genes; 3.2 Gb); NGS (below 1,000 USD/genome); systems biology (omics integration; networks). Current frontiers: super-resolution microscopy (STED ≈ 20 to 50 nm; PALM/STORM ≈ 10 to 30 nm; Nobel 2014); cryo-EM (Nobel 2017; near-atomic resolution without staining); stem cells (ESCs pluripotent; iPSCs from somatic cells by Oct4/Sox2/Klf4/c-Myc; Nobel 2012; patient-specific); CRISPR-Cas9 (Nobel 2020; guide RNA directs Cas9; precise genome editing; gene therapy; CAR-T cells); synthetic biology (metabolic engineering; biosensors; organoids).

Answers to Pilot Questions [Series 1]

Part 1.1

1. Pre-microscopic scholars (Aristotle, Galen) studied living matter through dissection and philosophical reasoning; vitalism (the idea that living matter is fundamentally different from non-living matter) dominated. Limitation of Aristotle's view: he organised living matter into humours and organs but had no concept of the cell as the fundamental structural unit; his hierarchical view was anatomical but not cellular.
2. Hooke (1665) observed thin sections of cork under his compound microscope and saw small box-like compartments, which he named cells; he also observed plant tissue sections showing similar compartments. He was actually seeing the empty cell walls of dead plant cells, as cork consists of dead cells whose cytoplasmic contents had long since disappeared.



3. Antonie van Leeuwenhoek (1632 to 1723) was a Dutch draper who constructed single-lens microscopes of exceptional quality (magnifying up to approximately 270x). His most important contribution was the first observation and description of bacteria (animalcules) in dental scrapings and pond water (1674 to 1683), establishing that a rich microscopic world of living organisms existed.
4. (1) All living organisms are composed of cells (Schleiden 1838; Schwann 1839). (2) The cell is the basic unit of structure and function in all living organisms (Schleiden and Schwann). (3) All cells arise from pre-existing cells (Virchow, 1855; *Omnis cellula e cellula*).
5. The refutation of spontaneous generation (Redi 1668; Pasteur 1861) showed that new living organisms can only arise from pre-existing living matter, not from non-living material. This directly supported the third tenet of cell theory (Virchow 1855: all cells arise from pre-existing cells) by establishing that cell division is the only mechanism for producing new cells.

Part 1.2

1. The Abbe diffraction limit states that the minimum resolvable distance between two points in a light microscope is $d = \lambda/2NA$, where λ = wavelength of light and NA = numerical aperture of the objective. For visible light ($\lambda \approx 500$ nm) with the best oil-immersion objectives ($NA \approx 1.4$), the resolution limit is approximately 200 nm.
2. Bright-field microscopy illuminates the specimen directly; unstained cells show very little contrast because they absorb and scatter light similarly. Phase contrast microscopy (Zernike; Nobel 1953) converts differences in refractive index within the cell into differences in light intensity; main advantage: living, unstained cells can be observed without chemical fixation.
3. TEM: electrons pass through an ultrathin resin-embedded section (50 to 100 nm) stained with heavy metals; reveals internal ultrastructure of organelles; resolution approximately 0.1 nm.
SEM: electrons are scanned across a metal-coated specimen surface; detects secondary electrons to produce a three-dimensional image of the surface; resolution approximately 5 to 10 nm; gives surface topography, not internal structure.
4. Cells are homogenised in isotonic buffer, then centrifuged at increasing speeds: 600g (10 min) sediments nuclei and cell debris; 10,000g (20 min) sediments mitochondria, lysosomes, and peroxisomes; 100,000g (60 min) sediments microsomes (ER/Golgi fragments); the final supernatant is the cytosol fraction.
5. Succinate dehydrogenase is a marker for the inner mitochondrial membrane (marks the mitochondrial fraction). Catalase is a marker for peroxisomes (marks the peroxisomal fraction).



Part 1.3

1. The central dogma of molecular biology states that genetic information flows from DNA to RNA to protein (DNA → RNA → protein); formulated by Francis Crick in 1957 and published in 1958.
2. Recombinant DNA technology was developed using restriction endonucleases (which cut DNA at specific sequences; Nobel 1978 to Arber, Smith, Nathans) and DNA ligase (which joins DNA fragments); Boyer and Cohen (1973) combined these tools to clone foreign DNA into bacterial plasmids. Two applications: production of recombinant human insulin in *E. coli* (Genentech 1982); production of transgenic mice for studying gene function in vivo.
3. The Human Genome Project (1990 to 2003) sequenced the approximately 3.2 billion base pairs of the human genome. Two main findings: (1) the human genome contains approximately 20,000 to 25,000 protein-coding genes (far fewer than predicted); (2) the majority of the genome is non-coding (consisting of regulatory sequences, non-coding RNA genes, and repetitive elements).
4. Genomics studies the complete DNA sequence (genome) of an organism; fixed for a given individual. Proteomics studies the complete protein complement (proteome) of a cell or tissue; the proteome is more complex because the same genome produces different proteins in different cell types and conditions, and proteins undergo post-translational modifications (phosphorylation, glycosylation, etc.) that diversify their function.
5. Traditional reductionist approaches study one gene or protein at a time; this cannot capture emergent properties (behaviours arising from networks of interacting components that are not predictable from individual components). Systems biology integrates genome-wide, transcriptome-wide, and proteome-wide data and uses computational modelling to describe and predict the behaviour of entire cellular networks.

Part 1.4

1. STED (Stimulated Emission Depletion) microscopy uses a doughnut-shaped depletion laser beam superimposed on the excitation spot; the depletion laser quenches fluorescence by stimulated emission at the periphery of the spot, restricting fluorescent emission to a region smaller than the Abbe limit. Resolution achieved: approximately 20 to 50 nm lateral resolution.
2. STED uses a continuous depletion laser to physically restrict the emitting region; it is a deterministic method and can image continuously at high frame rates. PALM/STORM use stochastic photoswitching of individual fluorophores (activated and imaged one at a time); each molecule's position is calculated by fitting its point spread function; thousands of single-molecule localisations are accumulated to build the super-resolution image.



3. Stem cells are characterised by self-renewal and the ability to differentiate into specialised cell types. ESCs: derived from the inner cell mass of the blastocyst; pluripotent; require destruction of human embryos (ethically controversial). iPSCs: derived from adult somatic cells reprogrammed by Oct4, Sox2, Klf4, and c-Myc (Yamanaka factors; Nobel 2012); pluripotent; no ethical issues; patient-specific.
4. CRISPR-Cas9 is a genome editing system in which a guide RNA (gRNA) directs the Cas9 nuclease to a specific genomic locus, where it cuts both DNA strands; the break is repaired by error-prone NHEJ (gene disruption) or homology-directed repair (HDR; precise editing). Two medical applications: correction of genetic diseases (e.g. sickle-cell disease; Duchenne muscular dystrophy); engineering of CAR-T cells for cancer immunotherapy.
5. Synthetic biology applies engineering principles to design and build novel biological systems. One example: artemisinin precursor (anti-malarial drug) production by engineered yeast (Keasling group, 2006), which metabolically engineered *S. cerevisiae* to produce artemisinic acid, an industrial-scale source of artemisinin for malaria treatment.



References and Attributions [Series 1]

Textual References (Books and External Sources)

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Figures, Plates, Tables, and Boxes

- Figure 1.1: Timeline of major milestones in the history of cell biology. Attribution: AI generated. Suggested OER search string: “history of cell biology timeline microscope cell theory molecular biology OER”.



Course code: BIO 205

Course title: Introductory Developmental / Cell Biology

Course credit load: 2 Units

Series 2: Plant and Animal Cell Ultra-Structure

- **Part 2.1: General Organisation of Eukaryotic Cells**
 - Sub Part 2.1.1: Prokaryotic versus eukaryotic cell organisation
 - Sub Part 2.1.2: The plasma membrane and cell surface
 - Sub Part 2.1.3: The cytoplasm, cytosol, and cytoskeleton
- **Part 2.2: Ultra-Structure of the Animal Cell**
 - Sub Part 2.2.1: The nucleus and its components
 - Sub Part 2.2.2: Endomembrane system: endoplasmic reticulum and Golgi apparatus
 - Sub Part 2.2.3: Energy-producing organelles and other cytoplasmic structures
- **Part 2.3: Ultra-Structure of the Plant Cell**
 - Sub Part 2.3.1: The plant cell wall
 - Sub Part 2.3.2: Plastids and vacuoles
 - Sub Part 2.3.3: Plasmodesmata and intercellular communication in plants
- **Part 2.4: Comparison of Plant and Animal Cell Ultra-Structure**
 - Sub Part 2.4.1: Shared features of plant and animal cells
 - Sub Part 2.4.2: Structures unique to plant cells
 - Sub Part 2.4.3: Structures unique to animal cells



Introduction

The cell is the fundamental structural and functional unit of all living organisms. With the advent of electron microscopy in the twentieth century, it became possible to examine the internal architecture of cells at the nanometre scale, revealing a highly organised world of membranes, filaments, and organelles that classical light microscopy could not resolve. This fine internal organisation, visible only with the electron microscope, is called the ultra-structure of the cell.

This Series examines the ultra-structure of plant and animal cells, beginning with features shared by all eukaryotic cells, then analysing the distinctive features of each cell type separately before comparing them side by side. Understanding ultra-structure is foundational for all subsequent topics in this course: the organelles introduced here are the subjects of Series 3, and the processes of cell division (Series 4) and differentiation (Series 5) depend on the structural components described here.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 2.1** distinguish between prokaryotic and eukaryotic cell organisation, describe the plasma membrane structure, and explain the roles of the cytoplasm and cytoskeleton (Linked to Part 2.1),
- **SLO 2.2** describe the ultra-structure of the major components of the animal cell, including the nucleus, ER, Golgi apparatus, mitochondria, lysosomes, and centrioles (Linked to Part 2.2),
- **SLO 2.3** describe the ultra-structure of components unique to plant cells, including the cell wall, plastids, central vacuole, and plasmodesmata (Linked to Part 2.3), and
- **SLO 2.4** systematically compare the ultra-structure of plant and animal cells, listing features shared by both and features unique to each (Linked to Part 2.4).

2.1 General Organisation of Eukaryotic Cells

2.1.1 Prokaryotic versus eukaryotic cell organisation



All cellular life is divided into two fundamental types based on the presence or absence of a membrane-bound nucleus. Prokaryotic cells (Bacteria and Archaea): diameter typically 0.5 to 5 μm ; DNA is a single, circular molecule in a region called the nucleoid (no enclosing membrane); no membrane-bound organelles; ribosomes are 70S; most have a peptidoglycan cell wall; reproduce by binary fission. Eukaryotic cells (Protista, Fungi, Plantae, Animalia): diameter typically 10 to 100 μm ; true nucleus bounded by a double membrane (nuclear envelope) with nuclear pore complexes; DNA is organised as multiple linear chromosomes complexed with histone proteins; extensive endomembrane system; 80S cytoplasmic ribosomes (70S in mitochondria and chloroplasts); reproduce by mitosis or meiosis.

Key differences: size (prokaryotes approximately 10 to 100 times smaller); nuclear organisation (nucleoid versus true nucleus with double-membrane envelope); organelles (none membrane-bound in prokaryotes versus extensive endomembrane system in eukaryotes); ribosomes (70S prokaryotic; 80S eukaryotic cytoplasmic); cell wall (peptidoglycan in bacteria; cellulose in plants; chitin in fungi; none in animals); cytoskeleton (simple FtsZ/MreB in prokaryotes; elaborate actin/tubulin/intermediate filaments in eukaryotes). The endosymbiotic theory (Lynn Margulis, 1967) proposes that mitochondria and chloroplasts evolved from free-living prokaryotes engulfed by ancestral eukaryotic cells; evidence: double membranes; 70S ribosomes; circular DNA; binary fission; similar size to bacteria.

Fill in the blank: Prokaryotic cells lack a membrane-bound _____ and have their DNA in a region called the nucleoid; eukaryotic cells have a true nucleus bounded by a double-membrane nuclear envelope; mitochondria and chloroplasts contain 70S ribosomes and circular DNA, supporting the endosymbiotic theory.

2.1.2 The plasma membrane and cell surface

The plasma membrane surrounds the cell, separating the intracellular environment from the extracellular environment and controlling the movement of molecules. Structure is described by the fluid mosaic model (Singer and Nicolson, 1972): a fluid phospholipid bilayer (approximately 7 to 10 nm thick) in which proteins are embedded (integral proteins) or attached to the surface (peripheral proteins). Each phospholipid has a hydrophilic head (faces the aqueous environment) and two hydrophobic fatty acid tails (face the membrane interior). Cholesterol is intercalated



between phospholipids in animal cell membranes, stabilising fluidity. The glycocalyx is a carbohydrate-rich coat on the outer surface (oligosaccharides on glycoproteins and glycolipids) functioning in cell recognition, adhesion, and immune protection.

Membrane proteins: integral (transmembrane) proteins span the bilayer and include channels (aquaporins, ion channels), transporters (Na^+/K^+ -ATPase, GLUT1), receptors (GPCRs, RTKs), and enzymes; they require detergents for removal. Peripheral proteins are attached non-covalently to the membrane surface and are removed by salt washes. Cell surface specialisations in animal cells include microvilli (actin-supported projections increasing absorptive surface area; intestinal brush border), motile cilia (9+2 axoneme; move fluids over the cell surface; respiratory epithelium), and flagella (propulsion; sperm).

Fill in the blank: The fluid mosaic model describes the plasma membrane as a fluid _____ bilayer with integral proteins embedded within it and peripheral proteins attached to the surface; cholesterol stabilises fluidity in animal membranes; the glycocalyx on the outer surface functions in cell recognition and adhesion.

2.1.3 The cytoplasm, cytosol, and cytoskeleton

The cytoplasm comprises everything inside the plasma membrane excluding the nucleus: the cytosol (a concentrated aqueous solution of ions, metabolites, and proteins in which metabolic reactions such as glycolysis occur), all organelles, and the cytoskeleton. The cytoskeleton is a dynamic network of protein filaments providing mechanical support, determining cell shape, enabling motility, and directing intracellular transport. Three main components: (1) Actin filaments (microfilaments): 7 to 9 nm in diameter; double-helical polymers of G-actin; most abundant at the cell cortex; involved in cell shape, division (contractile ring), migration (lamellipodia and filopodia), and vesicle trafficking. (2) Microtubules: 25 nm in diameter; hollow tubes of α/β -tubulin heterodimers in 13 protofilaments; dynamic; nucleated at the centrosome (MTOC); form the mitotic spindle and the 9+2 axoneme of cilia; serve as tracks for kinesin (plus-end directed) and dynein (minus-end directed) motor proteins. (3) Intermediate filaments: 8 to 12 nm in diameter; ropelike polymers (cytokeratins, vimentin, desmin, lamins); provide mechanical strength; form the nuclear lamina.



Fill in the blank: The cytoskeleton has three components: actin filaments (7 to 9 nm; cell shape, division, motility); _____ (25 nm; tubulin; mitotic spindle; cilia 9+2 axoneme; kinesin and dynein transport); and intermediate filaments (8 to 12 nm; mechanical strength; nuclear lamina).

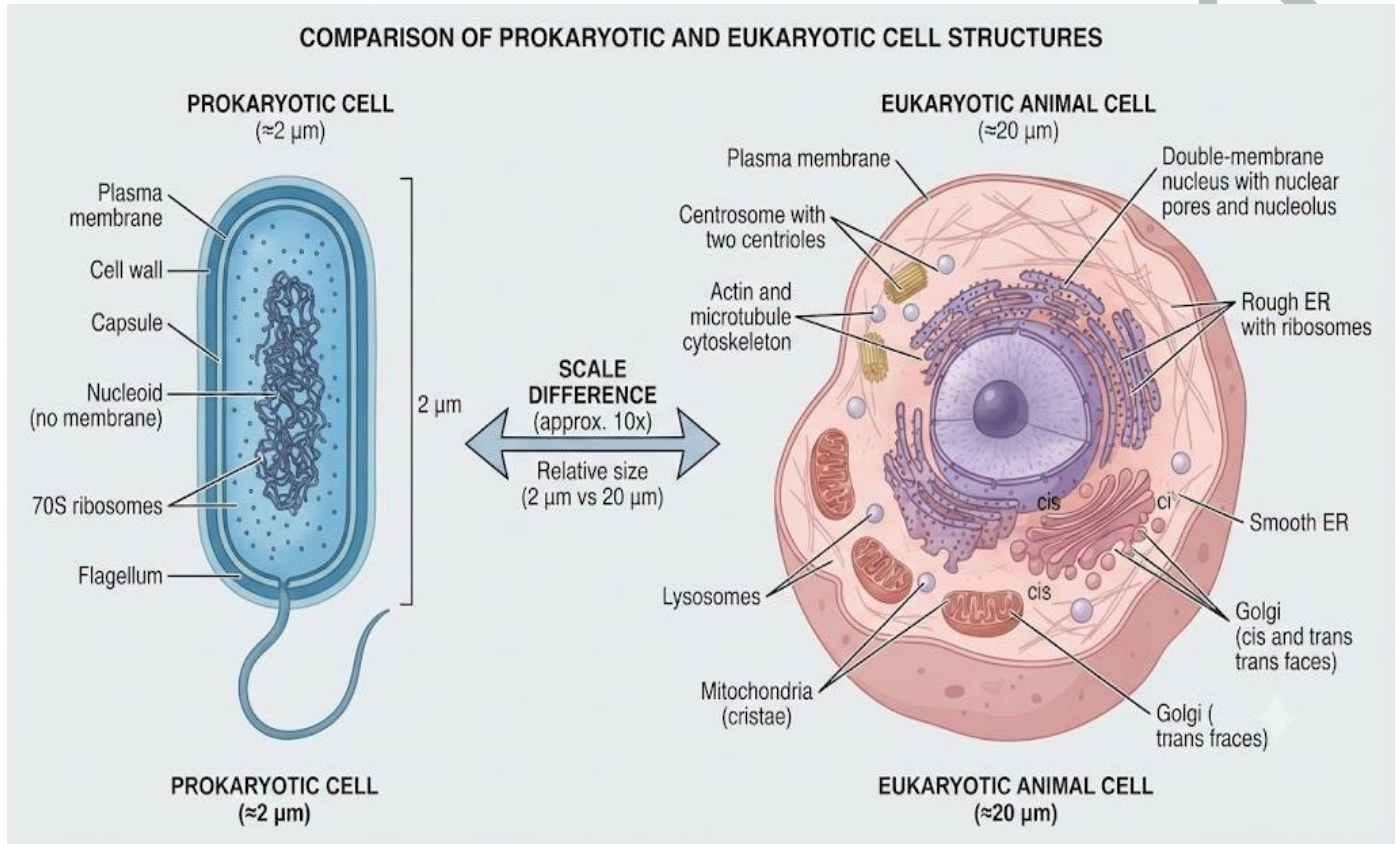


Figure 2.1: Prokaryotic cell (left) versus eukaryotic animal cell (right), illustrating the absence of a nuclear envelope and membrane-bound organelles in the prokaryote

Pilot questions 2.1

1. List five differences between prokaryotic and eukaryotic cells.
2. Describe the fluid mosaic model of the plasma membrane and name its two main structural components.
3. Distinguish between integral and peripheral membrane proteins and give one example of each.
4. State the three components of the eukaryotic cytoskeleton with their diameters and one function each.
5. State the endosymbiotic theory and give three pieces of evidence supporting it.



2.2 Ultra-Structure of the Animal Cell

2.2.1 The nucleus and its components

The nucleus (approximately 5 to 10 μm) is the largest animal cell organelle and the control centre for gene expression. Ultra-structural components: (1) Nuclear envelope: a double membrane (outer membrane continuous with the rough ER; inner membrane lined by the nuclear lamina); separated by the perinuclear space (approximately 20 to 40 nm); the outer membrane bears ribosomes. (2) Nuclear pore complexes (NPCs): approximately 120 nm protein complexes (approximately 30 nucleoporin proteins; 8-fold symmetry; approximately 2,000 to 4,000 per human nucleus) spanning both membranes; regulate bidirectional transport (passive diffusion for small molecules below approximately 40 kDa; active transport via importins/exportins for larger cargo including mRNA). (3) Nucleoplasm: the fluid interior of the nucleus containing transcription factors and DNA replication complexes. (4) Chromatin: DNA complexed with histones; euchromatin (decondensed; transcriptionally active; pale in TEM) and heterochromatin (condensed; inactive; dark in TEM; at the nuclear periphery). (5) Nucleolus: a dense, non-membrane-bound body (1 to 3 per nucleus; 1 to 5 μm); site of rRNA synthesis and ribosome subunit assembly; three sub-regions: fibrillar centre (rDNA); dense fibrillar component (rRNA transcription); granular component (ribosome assembly). (6) Nuclear lamina: meshwork of lamin intermediate filaments (A, B, C) just inside the inner membrane; provides mechanical support; anchors chromatin; disassembles during mitosis.

Fill in the blank: The nuclear envelope is a _____ membrane; nuclear pore complexes (≈ 120 nm; ≈ 30 nucleoporins; 8-fold symmetry) regulate nuclear-cytoplasmic transport; the nucleolus synthesises rRNA and assembles ribosome subunits; the nuclear lamina (lamins A, B, C) supports the inner nuclear membrane and anchors chromatin.

2.2.2 Endomembrane system: endoplasmic reticulum and Golgi apparatus

The endomembrane system is a network of membranes (ER, Golgi, lysosomes, secretory vesicles) that together synthesise, modify, sort, and deliver proteins and lipids. Endoplasmic reticulum (ER): a continuous network of cisternae and tubules continuous with the outer nuclear membrane. Rough ER (RER): studded with ribosomes; synthesises secretory, membrane, and



lysosomal proteins co-translationally; the ER lumen is the site of protein folding (chaperone BiP) and initial N-linked glycosylation; misfolded proteins are retrotranslocated for proteasomal degradation (ERAD). Smooth ER (SER): no ribosomes; tubular morphology; functions in lipid synthesis (phospholipids, cholesterol, steroid hormones), Ca^{2+} storage (sarcoplasmic reticulum in muscle), and drug detoxification by cytochrome P450 enzymes (abundant in liver hepatocytes).

Golgi apparatus: a polarised stack of 4 to 8 flattened cisternae; cis face (cis-Golgi network, CGN) receives COPII vesicles from the ER; trans face (trans-Golgi network, TGN) dispatches cargo; medial cisternae in between. Functions: modifies N-linked oligosaccharides (trimming then addition of sugars); performs O-linked glycosylation; synthesises glycosaminoglycans; phosphorylates mannose residues (mannose-6-phosphate tag) to target proteins to lysosomes; sorts cargo into secretory vesicles (constitutive and regulated secretion) or plasma membrane delivery vesicles.

Fill in the blank: The rough ER is studded with _____ on its cytoplasmic face and synthesises secretory and membrane proteins; the smooth ER synthesises lipids and stores Ca^{2+} ; the Golgi apparatus (cis to trans polarity) modifies oligosaccharides, adds the mannose-6-phosphate lysosomal tag, and sorts cargo for delivery to lysosomes, secretory vesicles, or the plasma membrane.

2.2.3 Energy-producing organelles and other cytoplasmic structures

Mitochondria: principal sites of ATP production by oxidative phosphorylation; typically 1 to 10 μm long; undergo fission and fusion dynamics. Four compartments: (1) Outer mitochondrial membrane (OMM): smooth; contains porins (VDAC); freely permeable to small molecules; TOMM import complex. (2) Intermembrane space (IMS): accumulates H^+ (proton gradient for ATP synthesis); contains cytochrome c (released to initiate apoptosis). (3) Inner mitochondrial membrane (IMM): extensively folded into cristae (greatly increased surface area); contains electron transport chain complexes (I to IV) and ATP synthase (Complex V; $\text{F}_1\text{F}_0\text{-ATPase}$); impermeable to most ions; TIMM import complex. (4) Mitochondrial matrix: circular DNA (≈ 16.6 kb in humans; encodes 13 proteins + 22 tRNAs + 2 rRNAs); 70S mitochondrial ribosomes; enzymes of the citric acid cycle, β -oxidation, and amino acid catabolism.



Lysosomes: membrane-bound organelles (approximately 0.1 to 1.2 μm) containing approximately 60 acid hydrolases (proteases, lipases, nucleases, glycosidases) optimised at pH approximately 4.5; the V-type H^+ -ATPase in the lysosomal membrane maintains the acidic lumen; discovered by Christian de Duve (Nobel 1974). Functions: autophagy (degradation of damaged organelles via autophagosomes; Nobel 2016 Ohsumi); heterophagy (digestion of material taken up by phagocytosis or endocytosis). Lysosomal storage diseases arise from inherited enzyme deficiencies; examples: Pompe disease (acid maltase deficiency; glycogen accumulates); Gaucher disease (glucocerebrosidase deficiency). Peroxisomes: single-membrane organelles containing catalase (destroys H_2O_2) and oxidases; carry out very-long-chain fatty acid β -oxidation. Centrioles/centrosome: two centrioles (9 triplet microtubules; 9×0 arrangement) at right angles, surrounded by pericentriolar material (γ -TuRC; nucleates microtubules); the main MTOC in animal cells; forms the basal bodies of cilia and flagella.

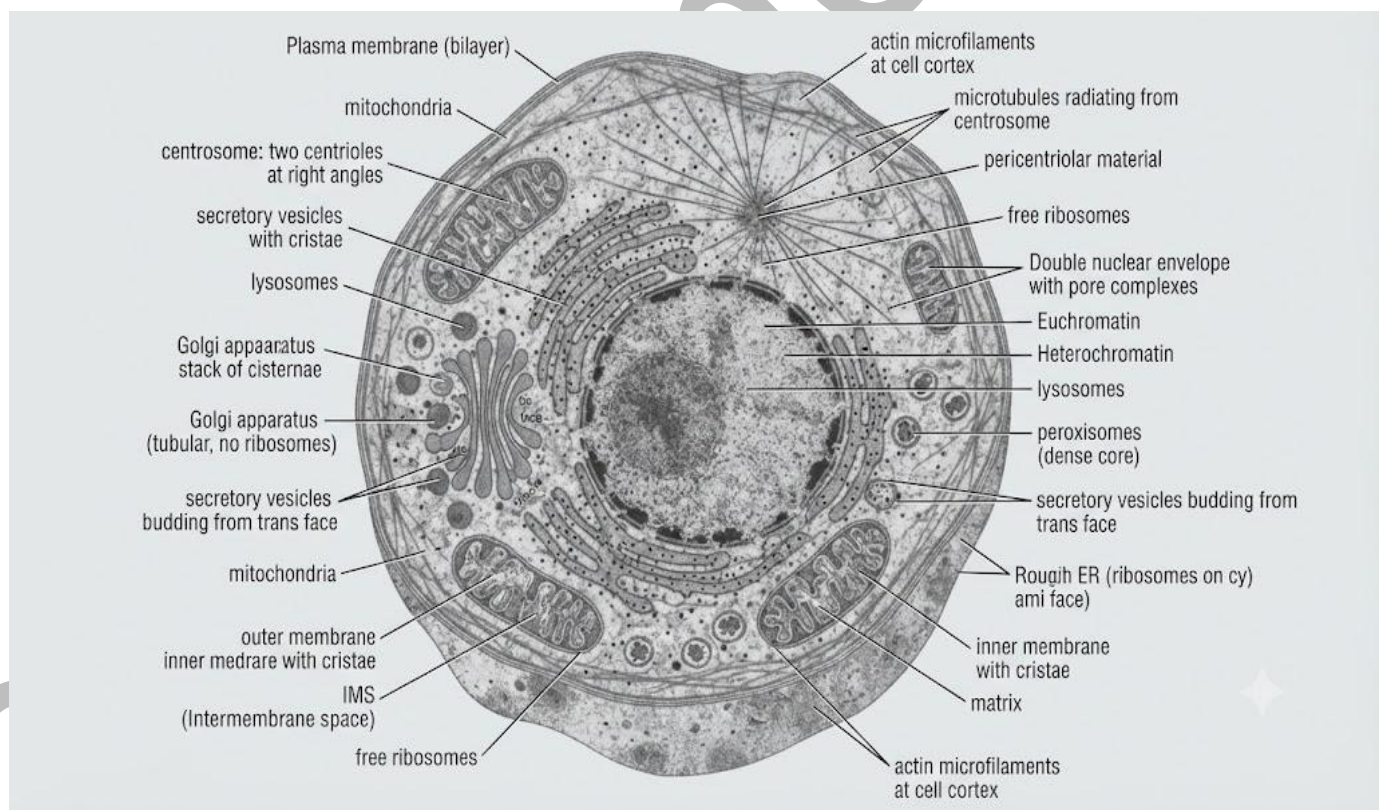


Figure 2.2: Labelled animal cell ultra-structure showing the nucleus, endomembrane system (rough ER, smooth ER, Golgi, lysosomes, secretory vesicles), mitochondria, peroxisomes, centrosome, ribosomes, and cytoskeletal elements



Pilot questions 2.2

1. Describe the ultra-structure of the nuclear envelope, including the number of membranes, the perinuclear space, and the role of nuclear pore complexes.
2. Distinguish between rough ER and smooth ER in terms of ultra-structure and function.
3. Describe the polarity of the Golgi apparatus and the modifications proteins undergo passing from cis to trans.
4. Name and describe the four ultrastructural compartments of the mitochondrion and state the function of each.
5. Explain what lysosomes are, how they maintain their acidic lumen, and name two lysosomal storage diseases.

2.3 Ultra-Structure of the Plant Cell

2.3.1 The plant cell wall

The plant cell wall is a rigid semi-permeable extracellular matrix surrounding the plant cell outside the plasma membrane; it provides structural support, determines cell shape, resists turgor pressure, and protects against pathogens. Layers: (1) Primary cell wall: thin (0.1 to 1.0 μm), extensible; deposited first during growth; three main components: cellulose microfibrils (β -1,4-linked glucose; tensile strength; synthesised by CesA rosette complexes at the plasma membrane, their orientation guided by cortical microtubules); hemicellulose (branched polysaccharides, e.g. xyloglucan; cross-link cellulose microfibrils); pectin (acidic polysaccharides; Ca^{2+} cross-links; wall porosity and flexibility; secreted via Golgi exocytosis). (2) Secondary cell wall: deposited inside the primary wall after growth has ceased; thicker (1 to 10 μm); rigid; composed of highly crystalline cellulose and lignin (aromatic polymer; fills spaces between cellulose; provides compression strength; waterproofing; responsible for mechanical properties of wood); organised in three lamellae (S1, S2, S3) with different microfibril orientations. (3) Middle lamella: shared between adjacent cells; composed mainly of calcium pectate; holds adjacent cells together.

Fill in the blank: The primary cell wall contains _____ microfibrils (β -1,4-linked glucose; tensile strength), hemicellulose (cross-links cellulose), and pectin (acidic polysaccharides; Ca^{2+}



cross-links); the secondary cell wall contains cellulose and lignin (aromatic polymer; compression strength; wood); the middle lamella (calcium pectate) holds adjacent cells together.

2.3.2 Plastids and vacuoles

Plastids are a family of double-membrane organelles found only in plant cells and algae; they evolved from endosymbiotic cyanobacteria (primary endosymbiosis); they contain circular DNA (≈ 150 kb), 70S ribosomes, and replicate by binary fission. Types: (1) Chloroplasts: the site of photosynthesis; approximately 5 to 10 μm long; found in mesophyll cells. Ultra-structure: outer envelope membrane (permeable; TOC import complex); inner envelope membrane (selective; TIC import complex; site of lipid synthesis); intermembrane space; stroma (fluid interior; Calvin cycle enzymes including RuBisCO, the most abundant protein on Earth; circular DNA; 70S ribosomes; starch grains); thylakoids (third membrane system; flattened sacs organised as grana (tightly stacked discs with PS II, LHCII, cytochrome b6f; light reactions; O_2 evolution; ATP and NADPH production) and stroma lamellae (unstacked; PS I; NADPH production)). (2) Leucoplasts: colourless; amyloplasts (starch storage; potato tubers, cereal endosperm); elaioplasts (oil storage); proteinoplasts (protein storage). (3) Chromoplasts: synthesise and store carotenoid pigments (red, orange, yellow; ripe tomatoes, red peppers, carrots); derived from chloroplasts during fruit ripening. (4) Proplastids: undifferentiated; found in meristematic cells; develop into any plastid type.

Central vacuole: one of the most distinctive features of mature plant cells; a single large compartment bounded by the tonoplast membrane; occupies up to 90 percent of cell volume in mature cells; contains cell sap (ions, sugars, organic acids, pigments, secondary metabolites). Functions: maintains turgor pressure (osmotic water uptake into vacuole drives cell expansion); stores ions, sugars, organic acids, and secondary metabolites (alkaloids, tannins, anthocyanins); sequesters toxic compounds; lysosomal digestion (vacuolar hydrolases; functionally analogous to the lysosome of animal cells). Tonoplast: contains V-type H^+ -ATPase (maintains acidic vacuolar lumen) and TIP aquaporins (regulate water flow).

Fill in the blank: Chloroplasts have three membrane systems: outer envelope, inner envelope, and _____ (thylakoid membranes + lumen); grana stacks (PS II, LHCII) carry out the light reactions; the stroma (inside the inner envelope) contains the Calvin cycle enzymes including



RuBisCO; the central vacuole (bounded by the tonoplast) occupies up to 90 percent of mature plant cell volume.

2.3.3 Plasmodesmata and intercellular communication in plants

Plasmodesmata (singular: plasmodesma) are narrow cytoplasmic channels (30 to 60 nm in diameter) traversing the plant cell wall and plasma membrane to directly connect the cytoplasm of adjacent plant cells. Ultra-structure: each plasmodesma is lined by the plasma membrane (continuous between the two connected cells); within the channel runs the desmotubule (a tube of smooth ER approximately 15 nm in diameter, continuous with the ER of both cells); the cytoplasmic sleeve between the desmotubule and the plasma membrane (the annulus) is the main transport pathway; neck regions at each end can be constricted by callose deposition to regulate permeability. Transport: small molecules (ions, sugars, amino acids) pass freely; larger molecules (specific proteins, mRNA, plant viruses via movement proteins) move through when specifically targeted; FLOWERING LOCUS T (FT) protein (the florigen signal for flowering) moves from leaves to the shoot apical meristem through plasmodesmata.

Symplast and apoplast: the symplast is the interconnected network of cytoplasm linked by plasmodesmata; the apoplast is the continuous system of cell walls and intercellular spaces; water and solutes move apoplastically until blocked by the Casparian strip of the root endodermis, which forces all solutes into the symplast. Primary plasmodesmata: formed during cell division when ER tubules are trapped in the developing cell plate. Secondary plasmodesmata: formed de novo through existing cell walls after division. Regulation: callose (β -1,3-glucan) deposition at the neck narrows the channel, reducing the size exclusion limit (SEL) in response to stress, wounding, or pathogen attack.



"Cross-Section Diagram of a Mature Plant Mesophyll Cell."

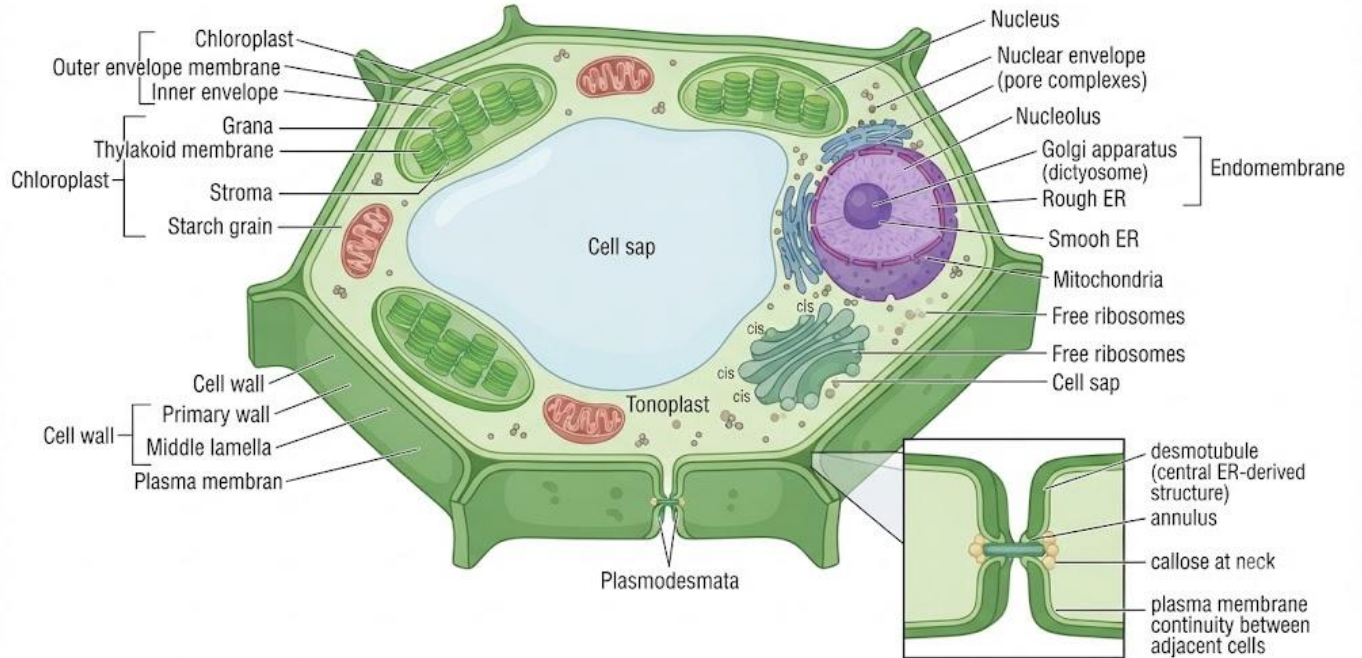


Figure 2.3: Labelled plant cell ultra-structure showing the cell wall, central vacuole, chloroplasts, nucleus, endomembrane system, mitochondria, and plasmodesmata; inset: longitudinal section of a single plasmodesma

Pilot questions 2.3

1. Describe the three main chemical components of the primary plant cell wall and state the function of each.
2. Distinguish between the primary and secondary plant cell wall in terms of composition, thickness, and timing.
3. Describe the ultra-structure of the chloroplast, naming its three membrane systems and stating what occurs at each.
4. List the four types of plastids and state the function of each.
5. Explain the structure of a plasmodesma and distinguish between the symplast and the apoplast.



2.4 Comparison of Plant and Animal Cell Ultra-Structure

2.4.1 Shared features of plant and animal cells

Despite many structural differences, plant and animal cells share a common eukaryotic organisation: (1) Plasma membrane: both have a fluid phospholipid bilayer with integral and peripheral proteins and a glycocalyx. (2) Nucleus: both have a double-membrane nuclear envelope with NPCs; a nucleolus for rRNA synthesis; chromatin; and a nuclear lamina. (3) Endoplasmic reticulum: both have rough ER (protein synthesis and initial glycosylation) and smooth ER (lipid synthesis and Ca^{2+} storage); in plant cells the ER also forms the desmotubule of plasmodesmata. (4) Golgi apparatus: both have a polarised cis-to-trans stack modifying and sorting proteins and lipids; in plant cells (called dictyosomes) the Golgi also produces hemicellulose and pectin for the cell wall. (5) Mitochondria: both have double membranes, cristae, circular DNA, and 70S ribosomes reflecting their endosymbiotic origin. (6) Ribosomes: both have 80S cytoplasmic ribosomes and 70S organellar ribosomes. (7) Cytoskeleton: both have actin filaments, microtubules, and intermediate filaments. (8) Peroxisomes: both contain catalase and oxidases; plant cells additionally have specialised glyoxysomes in germinating seeds.

Fill in the blank: Both plant and animal cells share: plasma membrane (fluid mosaic); nucleus (double-membrane; nucleolus; chromatin); rough and smooth ER; _____ apparatus (cis-to-trans polarity; protein modification and sorting); mitochondria (double membrane; cristae; 70S ribosomes; circular DNA); 80S cytoplasmic ribosomes; and peroxisomes.

2.4.2 Structures unique to plant cells

(1) Cell wall: primary wall (cellulose + hemicellulose + pectin; extensible) and secondary wall (cellulose + lignin; rigid); absent from animal cells. (2) Chloroplasts and other plastids: chloroplasts (photosynthesis), amyloplasts (starch storage), chromoplasts (carotenoids); absent from animal cells; contain 70S ribosomes and circular DNA reflecting endosymbiotic origin. (3) Central vacuole: a single large compartment (tonoplast membrane; up to 90 percent of cell volume); absent from animal cells, which have multiple small lysosomes instead. (4) Plasmodesmata: cytoplasmic channels (30 to 60 nm; desmotubule of ER; annulus) connecting adjacent cells for symplastic communication; animal cells have gap junctions instead (same



function; completely different molecular structure: connexin proteins forming connexons; no ER tubule). (5) Glyoxysomes: specialised peroxisomes in plant seedlings containing the glyoxylate cycle enzymes (isocitrate lyase, malate synthase) for converting fatty acids to sugars; absent from animal cells. (6) Preprophase band: a transient ring of cortical microtubules marking the future cell plate site before mitosis; absent from animal cells. (7) Phragmoplast and cell plate: plant cytokinesis proceeds by phragmoplast (microtubule array guiding Golgi-derived vesicles) building a cell plate outward from the cell centre; animal cells instead divide by an actomyosin contractile ring forming a cleavage furrow.

Fill in the blank: Structures unique to plant cells include: cell wall (cellulose; hemicellulose; pectin; _____ in secondary wall); chloroplasts and other plastids; central vacuole (tonoplast; up to 90 percent of cell volume); plasmodesmata (desmotubule; annulus; symplastic communication); preprophase band; phragmoplast and cell plate (cytokinesis).

2.4.3 Structures unique to animal cells

(1) Centrioles and centrosome: two centrioles (9 triplet microtubules; 9×0 arrangement) in pericentriolar material (PCM; contains γ -TuRC; nucleates microtubules); the main MTOC in animal cells; absent in most plant cells. (2) Lysosomes: membrane-bound organelles (approximately 60 acid hydrolases; pH approximately 4.5; V-type H⁺-ATPase) for autophagy and heterophagy; plant cells have lytic vacuoles that are functionally analogous but structurally distinct. (3) Motile cilia (on vegetative cells): cilia (9+2 axoneme) cover the surface of respiratory, ependymal, and other epithelial cells in animals; plant vegetative cells lack cilia. (4) Specialised cell junctions: tight junctions (seal adjacent epithelial cells; prevent paracellular transport); desmosomes (anchored by keratin intermediate filaments; tensile strength); adherens junctions (actin-anchored; cadherin-catenin); gap junctions (connexin connexons; direct cytoplasmic communication; analogous to plasmodesmata but completely different structure); all absent from plant cells. (5) Extracellular matrix (ECM): collagen, fibronectin, laminin, and proteoglycans secreted by animal cells to form the ECM; provides structural support and signalling; plant cells have a cell wall instead. (6) Glycogen granules: energy stored as glycogen (branched glucose polymer; rosette granules visible in TEM; abundant in hepatocytes and muscle cells); plant cells store starch in amyloplasts instead. (7) Microvilli: actin-supported plasma



membrane projections increasing absorptive surface area (intestinal brush border; kidney proximal tubule); absent from plant cells.

<u>Feature</u>	<u>Plant Cell</u>	<u>Animal Cell</u>
Cell Wall	Present (rigid, cellulose-based) – unique	Absent – unique
Plasma Membrane	Present – shared	Present – shared
Nucleus	Present – shared	Present – shared
Endoplasmic Reticulum (ER)	Present (smooth & rough) – shared	Present (smooth & rough) – shared
Golgi Apparatus	Present – shared	Present – shared
Mitochondria	Present – shared	Present – shared
Chloroplasts/Plastids	Present (photosynthesis, pigment storage) – unique	Absent – unique
Central Vacuole	Large, central, maintains turgor – unique	Small, scattered – unique
Lysosomes	Rare, less prominent	Prominent, numerous – unique
Centrioles/Centrosome	Generally absent (except in lower plants)	Present – unique
Plasmodesmata	Present (cytoplasmic channels) – unique	Absent – unique
Cell Plate/Phragmoplast	Present during cytokinesis – unique	Absent (cleavage furrow instead) – unique
Glycogen vs. Starch Storage	Starch granules – unique	Glycogen granules – unique
Extracellular Matrix (ECM)	Minimal (cell wall provides structure) – unique	Prominent ECM with proteins (collagen, fibronectin) – unique

Box 2.1: Comparison of plant and animal cell ultra-structure, listing shared features and features unique to each cell type

Pilot questions 2.4

1. List five ultra-structural features shared by both plant and animal cells.
2. List five ultra-structural features unique to plant cells not found in animal cells.



3. Explain why animal cells have centrioles but most plant cells do not.
4. Compare plasmodesmata and gap junctions in terms of structure and function.
5. Describe how cytokinesis differs between plant and animal cells at the ultrastructural level.



Series Summary

This Series examined the **structural organisation of prokaryotic and eukaryotic cells**, highlighting the similarities and differences between them and the specialised functions of their organelles. You explored the plasma membrane, cytoskeleton, and the major organelles of animal cells, including the nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, peroxisomes, and centrosomes, together with their roles in cellular organisation and function.

The Series also focused on the distinctive features of plant cells, including the cell wall, chloroplasts, central vacuole, plasmodesmata, and cell plate formation, while reinforcing the organelles shared by both plant and animal cells. By completing this Series, you should now be able to compare prokaryotic and eukaryotic cells, explain the structure and functions of major cellular organelles, distinguish between plant and animal cell features, and relate cell structure to function, thereby achieving all four **Series Learning Outcomes (SLOs)** and the **overall course outcomes**.

Glossary of Terms

- **Apoplast:** the continuous system of plant cell walls and intercellular spaces; movement is blocked at the Casparian strip of the root endodermis, forcing solutes into the symplast.
- **Cell plate:** new cell wall segment built from Golgi-derived vesicles guided by the phragmoplast during plant cytokinesis; expands outward until it reaches the plasma membrane.
- **Cell wall:** rigid semi-permeable extracellular matrix surrounding plant cells; primary wall (cellulose + hemicellulose + pectin); secondary wall (cellulose + lignin); middle lamella (calcium pectate) between adjacent cells.
- **Central vacuole:** large membrane-bound compartment (bounded by tonoplast) in mature plant cells; up to 90 percent of cell volume; maintains turgor; stores secondary metabolites; lytic functions.



- **Centrosome:** main MTOC in animal cells; two centrioles (9 triplet microtubules; 9×0) in pericentriolar material (γ -TuRC); absent in most plant cells; basal bodies of cilia are derived from centrioles.
- **Chloroplast:** plastid responsible for photosynthesis; outer and inner envelope membranes; stroma (Calvin cycle; RuBisCO; circular DNA; 70S ribosomes); thylakoids (grana with light reactions; stroma lamellae with PS I).
- **Cristae:** infoldings of the inner mitochondrial membrane; greatly increase surface area; house electron transport chain (Complexes I to IV) and ATP synthase (Complex V).
- **Desmotubule:** tube of smooth ER running through the centre of each plasmodesma; continuous with ER of both connected cells; part of the symplastic transport pathway.
- **Endosymbiotic theory:** Margulis (1967): mitochondria and chloroplasts evolved from engulfed prokaryotes; evidence: double membranes; 70S ribosomes; circular DNA; binary fission.
- **Fluid mosaic model:** Singer and Nicolson (1972): the plasma membrane is a fluid phospholipid bilayer with embedded integral proteins and surface-attached peripheral proteins; all components can move laterally.
- **Glycocalyx:** carbohydrate coat on the outer plasma membrane surface (oligosaccharides on glycoproteins and glycolipids); cell recognition, adhesion, immune protection.
- **Golgi apparatus:** polarised stack of cisternae (cis face receives from ER; trans face dispatches to lysosomes, secretory vesicles, plasma membrane); modifies glycans; adds mannose-6-phosphate lysosomal tag; called dictyosome in plant cells.
- **Lysosome:** membrane-bound animal cell organelle (0.1 to 1.2 μm ; approximately 60 acid hydrolases; pH approximately 4.5; V-type H^+ -ATPase); functions in autophagy and heterophagy; functionally analogous to plant lytic vacuole.
- **Nuclear pore complex (NPC):** approximately 120 nm protein complex (approximately 30 nucleoporins; 8-fold symmetry) spanning both nuclear membranes; regulates bidirectional nuclear-cytoplasmic transport.
- **Plasmodesmata:** 30 to 60 nm cytoplasmic channels through plant cell walls; lined by plasma membrane; contain desmotubule (ER); annulus is the main transport pathway; regulated by callose; functionally analogous to animal gap junctions.
- **Symplast:** interconnected cytoplasm linked by plasmodesmata; allows direct cell-to-cell transport of small molecules and specific signalling proteins without crossing the plasma membrane.



- **Thylakoid:** flattened membrane sac within chloroplasts; grana (stacked; PS II, LHCII; light reactions) and stroma lamellae (unstacked; PS I); thylakoid lumen accumulates H^+ for ATP synthesis.
- **Tonoplast:** membrane bounding the plant central vacuole; contains V-type H^+ -ATPase (acidic lumen) and TIP aquaporins (water flow regulation).



Concept Check Questions [Series 2]

Objective Questions

6. The fluid mosaic model (Singer and Nicolson, 1972) describes the plasma membrane as a fluid _____ bilayer; integral proteins are embedded within it; cholesterol stabilises fluidity in animal membranes. (Linked to SLO 2.1)
7. The three components of the eukaryotic cytoskeleton are actin filaments (7 to 9 nm), _____ (25 nm; tubulin polymers), and intermediate filaments (8 to 12 nm). (Linked to SLO 2.1)
8. The nucleolus is the site of _____ RNA synthesis and ribosome subunit assembly; it is not bounded by a membrane and is visible in both light and electron micrographs. (Linked to SLO 2.2)
9. The central vacuole of mature plant cells is bounded by the _____ membrane and occupies up to 90 percent of cell volume; it maintains turgor pressure by osmotic water uptake. (Linked to SLO 2.3)
10. Structures unique to plant cells include the cell wall, chloroplasts, central vacuole, _____ (cytoplasmic channels through cell walls), and the phragmoplast with cell plate formed during cytokinesis. (Linked to SLO 2.4)

Short-Answer Questions

11. List five differences between prokaryotic and eukaryotic cells. (Linked to SLO 2.1)
12. Name the four ultrastructural compartments of the mitochondrion and state one function of each. (Linked to SLO 2.2)
13. Describe the ultra-structure of the chloroplast, naming its three membrane systems and stating what occurs at each. (Linked to SLO 2.3)
14. Distinguish between plasmodesmata and gap junctions in terms of structure and function. (Linked to SLO 2.3)
15. List four structures unique to plant cells and four unique to animal cells. (Linked to SLO 2.4)

Long-Answer Questions

13. Describe the ultra-structure of the eukaryotic cell: plasma membrane (fluid mosaic model), cytoskeleton (three components), nucleus, ER, Golgi, mitochondria, and lysosomes. (Linked to SLOs 2.1, 2.2)



14. Compare plant and animal cell ultra-structure: list shared features, describe structures unique to plant cells (cell wall, plastids, central vacuole, plasmodesmata), and structures unique to animal cells (centrosome, lysosomes, ECM, gap junctions). (Linked to SLOs 2.3, 2.4)



Answers to Concept Check Questions [Series 2]

Objective Questions

6. Phospholipid.
7. Microtubules.
8. Ribosomal.
9. Tonoplast.
10. Plasmodesmata.

Short-Answer Questions

11. (1) Size: prokaryotes 0.5 to 5 μm ; eukaryotes 10 to 100 μm . (2) Nucleus: prokaryotes have a nucleoid (no membrane); eukaryotes have a true nucleus. (3) Organelles: none membrane-bound in prokaryotes; extensive endomembrane system in eukaryotes. (4) Ribosomes: 70S in prokaryotes; 80S cytoplasmic in eukaryotes. (5) Cell wall: peptidoglycan in bacteria; cellulose in plants; none in animals.
12. OMM: smooth; porins; freely permeable to small molecules. IMS: accumulates H^+ for ATP synthesis; contains cytochrome c (apoptosis signal). IMM: cristae; electron transport chain (Complexes I to IV); ATP synthase (Complex V). Matrix: circular DNA; 70S ribosomes; citric acid cycle enzymes; β -oxidation.
13. Outer envelope: permeable to small molecules; TOC protein import complex. Inner envelope: selective; TIC import complex; lipid synthesis. Thylakoids: grana (PS II; LHCII; light reactions; O_2 evolution; ATP and NADPH); stroma lamellae (PS I; NADPH production). Stroma: Calvin cycle; RuBisCO; CO_2 fixation; circular DNA; 70S ribosomes.
14. Plasmodesmata (plant): 30 to 60 nm channels through cell wall; lined by plasma membrane; desmotubule of ER; annulus for transport; regulated by callose. Gap junctions (animal): connexin protein channels (connexons); connect adjacent cells; allow small molecule passage; regulated by Ca^{2+} ; no cell wall, no ER tubule.
15. Plant-unique: cell wall; chloroplasts; central vacuole; plasmodesmata. Animal-unique: centrioles/centrosome; lysosomes; glycogen granules; microvilli.

Long-Answer Questions

13. Plasma membrane: fluid mosaic model (Singer and Nicolson 1972); phospholipid bilayer; integral proteins (channels, transporters, receptors); peripheral proteins (surface-attached); cholesterol (fluidity); glycocalyx (recognition). Cytoskeleton: actin filaments (7 to 9 nm; shape,



motility); microtubules (25 nm; spindle, cilia, kinesin/dynein transport); intermediate filaments (8 to 12 nm; mechanical strength; nuclear lamina). Nucleus: double-membrane envelope; NPCs (transport); nucleolus (rRNA; ribosome assembly); chromatin (euchromatin and heterochromatin); nuclear lamina (lamins). Rough ER: ribosomes; protein synthesis, folding, N-glycosylation, ERAD. Smooth ER: lipid synthesis; Ca^{2+} storage; detoxification. Golgi: cis-to-trans; N-glycan modification; mannose-6-phosphate tagging; sorting to lysosomes, secretion, plasma membrane. Mitochondria: OMM (porins); IMS (H^+ ; cytochrome c); IMM (cristae; ETC; ATP synthase); matrix (circular DNA; 70S ribosomes; Krebs cycle). Lysosomes: approximately 60 acid hydrolases; pH 4.5; V-type H^+ -ATPase; autophagy; heterophagy.

14. Shared: plasma membrane; nucleus; rough and smooth ER; Golgi; mitochondria; ribosomes; peroxisomes. Plant-unique: cell wall (cellulose, hemicellulose, pectin; secondary wall adds lignin); chloroplasts (outer envelope, inner envelope, stroma with Calvin cycle/RuBisCO, thylakoids with grana); amyloplasts (starch); chromoplasts (carotenoids); central vacuole (tonoplast; 90 percent of cell volume; turgor; storage; lytic); plasmodesmata (30 to 60 nm; desmotubule; annulus; symplastic transport; callose-regulated); phragmoplast and cell plate (cytokinesis). Animal-unique: centrosome (two centrioles; PCM; MTOC); lysosomes (60 hydrolases; pH 4.5; autophagy); ECM (collagen; fibronectin; laminin); gap junctions (connexins; connexons); tight junctions and desmosomes; glycogen granules; microvilli.

Answers to Pilot Questions [Series 2]

Part 2.1

1. (1) Size: prokaryotes 0.5 to 5 μm ; eukaryotes 10 to 100 μm . (2) Nucleus: nucleoid (no membrane) vs true nucleus (double-membrane envelope). (3) Organelles: none membrane-bound in prokaryotes; extensive endomembrane system in eukaryotes. (4) Ribosomes: 70S vs 80S cytoplasmic. (5) Cell wall: peptidoglycan (bacteria) vs cellulose (plants), chitin (fungi), or none (animals).
2. The fluid mosaic model (Singer and Nicolson, 1972) describes the membrane as a fluid phospholipid bilayer with a mosaic of embedded proteins. Two components: (1) phospholipid bilayer (hydrophilic heads outward; hydrophobic tails inward; approximately 7 to 10 nm; fluid at physiological temperature); (2) proteins (integral: span the bilayer; peripheral: attached to surface).



3. Integral proteins span the bilayer; removed only by detergents; example: Na^+/K^+ -ATPase. Peripheral proteins are attached non-covalently to the surface; removed by salt washes; example: spectrin in erythrocytes.
4. Actin filaments: 7 to 9 nm; cell shape, division, motility. Microtubules: 25 nm; polymerised α/β -tubulin; mitotic spindle, cilia axoneme, kinesin/dynein transport. Intermediate filaments: 8 to 12 nm; mechanical strength; nuclear lamina.
5. The endosymbiotic theory (Margulis, 1967) proposes mitochondria and chloroplasts evolved from engulfed prokaryotes. Three pieces of evidence: double membranes; 70S ribosomes (same as bacteria); circular DNA (like bacterial chromosomes).

Part 2.2

1. The nuclear envelope is a double membrane (outer continuous with rough ER; inner lined by the nuclear lamina); perinuclear space approximately 20 to 40 nm. NPCs (approximately 120 nm; approximately 30 nucleoporins; 8-fold symmetry) allow passive diffusion of small molecules (below approximately 40 kDa) and active transport of proteins and mRNA via importins/exportins.
2. Rough ER: studded with ribosomes; cisternal morphology; synthesises, folds, and N-glycosylates secretory and membrane proteins. Smooth ER: no ribosomes; tubular; synthesises lipids and steroid hormones; stores Ca^{2+} ; detoxifies drugs via cytochrome P450.
3. The Golgi is polarised: cis face (CGN) receives COPII vesicles from the ER; trans face (TGN) dispatches cargo. Modifications: trimming of mannose residues (cis/medial); addition of GlcNAc, galactose, sialic acid, fucose (medial/trans); O-linked glycosylation; mannose-6-phosphate tag for lysosomal targeting (trans).
4. OMM: smooth; porins; freely permeable to small molecules. IMS: H^+ accumulation; cytochrome c (apoptosis). IMM: cristae; ETC (Complexes I to IV); ATP synthase (Complex V). Matrix: circular mtDNA; 70S ribosomes; citric acid cycle; β -oxidation.
5. Lysosomes are membrane-bound organelles (approximately 0.1 to 1.2 μm) containing approximately 60 acid hydrolases at pH approximately 4.5; the acidic lumen is maintained by a V-type H^+ -ATPase. Two storage diseases: Pompe disease (acid maltase deficiency; glycogen accumulates); Gaucher disease (glucocerebrosidase deficiency; glucocerebroside accumulates in macrophages).

Part 2.3

1. Cellulose microfibrils (β -1,4-linked glucose): tensile strength; synthesised by Cesa rosette complexes at the plasma membrane. Hemicellulose (e.g. xyloglucan): cross-links cellulose



microfibrils; provides cohesion. Pectin (acidic polysaccharides): Ca^{2+} cross-links; wall porosity; forms the middle lamella between cells.

2. Primary wall: thin (0.1 to 1.0 μm); extensible; cellulose + hemicellulose + pectin; deposited during growth. Secondary wall: thick (1 to 10 μm); rigid; cellulose + lignin; deposited inside primary wall after growth has ceased; three lamellae (S1, S2, S3).
3. Outer envelope: permeable; TOC import complex. Inner envelope: selective; TIC import complex; lipid synthesis. Thylakoids: grana (PS II; LHCII; light reactions; water splitting; O_2 ; ATP; NADPH); stroma lamellae (PS I; NADPH). Stroma: Calvin cycle (RuBisCO; CO_2 fixation); circular DNA; 70S ribosomes.
4. Chloroplasts: photosynthesis; mesophyll cells. Amyloplasts: starch storage; potato tubers, cereal endosperm. Chromoplasts: carotenoid pigment storage; ripe fruits and flower petals. Proplastids: undifferentiated; meristematic cells; develop into any plastid type.
5. A plasmodesma (30 to 60 nm) is a channel through the plant cell wall lined by plasma membrane; it contains a desmotubule of smooth ER; the annulus between the desmotubule and membrane is the transport pathway; callose at the neck regulates permeability. Symplast: interconnected cytoplasm via plasmodesmata. Apoplast: cell wall and intercellular spaces; blocked at the Casparian strip.

Part 2.4

1. Plasma membrane; nucleus (double-membrane envelope; nucleolus; chromatin; NPCs); rough and smooth ER; Golgi apparatus; mitochondria (double membrane; cristae; 70S ribosomes; circular DNA).
2. Cell wall (cellulose; hemicellulose; pectin; secondary wall + lignin); chloroplasts (photosynthesis); central vacuole (tonoplast; turgor; storage); plasmodesmata (symplastic channels); phragmoplast and cell plate (cytokinesis).
3. Animal cells use the centrosome (two centrioles + PCM) as the main MTOC to organise the mitotic spindle; centrioles also form basal bodies of cilia. Most plant cells lack centrioles and instead organise the mitotic spindle from diffuse acentrosomal MTOCs on the nuclear envelope or in the cytoplasm.
4. Plasmodesmata: 30 to 60 nm plant cell channels; desmotubule of ER; annulus; regulated by callose; allow symplastic flow of ions, small molecules, and specific proteins. Gap junctions: connexin (connexon) channels in animal cells; allow small molecule passage; regulated by Ca^{2+} ; no cell wall, no ER tubule.
5. Plant: the phragmoplast (microtubule array) guides Golgi-derived vesicles to the cell midplane; vesicles fuse to build the cell plate outward until it meets the plasma membrane. Animal: an



actomyosin contractile ring constricts inward at the equator (cleavage furrow) to pinch the cell in two; no new wall is formed.

References and Attributions [Series 2]

Textual References (Books and External Sources)

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Figures, Plates, Tables, and Boxes

- Figure 2.1: Prokaryote vs eukaryote comparison diagram. Attribution: AI generated. Suggested OER search string: “prokaryote eukaryote cell structure comparison labelled diagram OER”.
- Figure 2.2: Animal cell ultra-structure diagram. Attribution: AI generated. Suggested OER search string: “animal cell ultra-structure labelled diagram TEM organelles nucleus mitochondria Golgi ER OER”.
- Figure 2.3: Plant cell ultra-structure with plasmodesma inset. Attribution: AI generated. Suggested OER search string: “plant cell ultra-structure labelled diagram chloroplast central vacuole plasmodesmata cell wall OER”.
- Box 2.1: Plant vs animal cell comparison table. Attribution: AI generated. Suggested OER search string: “plant animal cell ultra-structure comparison table shared unique features OER”.



Course code: BIO 205

Course title: Introductory Developmental / Cell Biology

Course credit load: 2 Units

Series 3: Organelles and Their Functions

- **Part 3.1: The Nucleus and Genetic Control**

- Sub Part 3.1.1: Chromatin organisation and gene regulation
- Sub Part 3.1.2: DNA replication and the cell cycle
- Sub Part 3.1.3: Transcription and RNA processing

- **Part 3.2: The Endomembrane System**

- Sub Part 3.2.1: Endoplasmic reticulum functions
- Sub Part 3.2.2: Golgi apparatus and vesicular sorting
- Sub Part 3.2.3: Lysosomes and cellular degradation

- **Part 3.3: Energy-Converting Organelles**

- Sub Part 3.3.1: Mitochondria and ATP production
- Sub Part 3.3.2: Chloroplasts and photosynthesis
- Sub Part 3.3.3: Peroxisomes and cytoskeletal roles

- **Part 3.4: Membrane Transport**

- Sub Part 3.4.1: Passive transport across membranes
- Sub Part 3.4.2: Active transport and membrane pumps
- Sub Part 3.4.3: Endocytosis and exocytosis



Introduction

An organelle is a specialised compartment within a eukaryotic cell that performs a specific function. Just as the organs of the human body each carry out a distinct role, the organelles of the cell each handle a particular aspect of cellular life. The compartmentalisation of functions into organelles allows the cell to carry out many different and sometimes chemically incompatible reactions at the same time, each in its own controlled environment.

This Series examines what each major organelle actually does. Building on the structural knowledge gained in Series 2, we now ask: how does the nucleus control all cell activities? How are proteins made, modified, and delivered to the right destination? How do mitochondria and chloroplasts produce energy? How do molecules cross the cell membrane? Understanding organelle function is essential for appreciating how cells divide (Series 4) and how cells become specialised during development (Series 5).

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 3.1** describe how DNA is packaged in the nucleus and regulated, explain the stages of the cell cycle and DNA replication, and describe how genes are transcribed and mRNA is processed before leaving the nucleus (Linked to Part 3.1),
- **SLO 3.2** explain how the rough ER synthesises and quality-checks proteins, describe how the Golgi apparatus modifies and sorts proteins, and explain how lysosomes degrade unwanted cellular material (Linked to Part 3.2),
- **SLO 3.3** explain how mitochondria produce ATP, describe the two stages of photosynthesis in chloroplasts, and state the functions of peroxisomes and the cytoskeleton (Linked to Part 3.3), and
- **SLO 3.4** distinguish between passive and active membrane transport, name the major membrane pumps and explain what they do, and describe how cells take in and release material by endocytosis and exocytosis (Linked to Part 3.4).



3.1 The Nucleus and Genetic Control

3.1.1 Chromatin organisation and gene regulation

The nucleus is the command centre of the cell because it contains the DNA that encodes all the instructions needed for life. However, DNA in the nucleus does not exist as a bare, extended strand. It is tightly packaged around protein spools called histones to form a complex known as chromatin. The basic unit of chromatin is the nucleosome, in which a short stretch of DNA (about 147 base pairs) is wound around a core of eight histone proteins. This wrapping allows several metres of DNA to fit inside a nucleus that is only a few micrometres across.

Not all chromatin is equally active. Regions called euchromatin are loosely packed and contain genes that are actively being read and expressed. Regions called heterochromatin are tightly compacted and are generally switched off. The cell can change which regions are active or silent by chemically modifying the histone proteins; for example, adding acetyl groups to histones (a process called acetylation) loosens the chromatin and switches genes on, while removing these groups (deacetylation) tightens chromatin and silences genes. Adding methyl groups to certain positions on histones or to the DNA itself can also silence genes. These changes to gene activity that do not alter the DNA sequence are called epigenetic regulation.

Fill in the blank: The basic unit of chromatin is the _____, in which DNA is wound around a core of eight histone proteins; loosely packed euchromatin contains active genes, while tightly packed heterochromatin is generally silent; histone acetylation loosens chromatin and activates genes.

3.1.2 DNA replication and the cell cycle

For the cell to divide and produce two daughter cells, it must first make a complete copy of all its DNA. This copying process, called DNA replication, takes place during a specific window of the cell cycle. The eukaryotic cell cycle consists of four phases: G1 (the cell grows and prepares for replication); S (DNA synthesis, during which each chromosome is duplicated); G2 (further growth and a check that replication was completed correctly); and M (mitosis, during which the duplicated chromosomes are separated and the cell divides). The progression through these



phases is tightly regulated by proteins called cyclins, which activate enzymes called cyclin-dependent kinases (CDKs) at the correct time.

DNA replication is described as semi-conservative because each new DNA molecule consists of one original (parental) strand and one newly made strand. Replication begins simultaneously at many sites along the chromosomes called origins of replication. At each origin, the two DNA strands are unwound by an enzyme called helicase, and a specialised enzyme called DNA polymerase reads each parental strand and builds the complementary new strand. Because DNA polymerase can only add nucleotides in one direction (5' to 3'), one strand is copied continuously while the other is copied in short fragments that are later joined together. At the ends of chromosomes (telomeres), a special enzyme called telomerase maintains the length of chromosomes in germ cells and stem cells, preventing loss of genetic information with each round of replication.

Fill in the blank: The eukaryotic cell cycle has four phases: G1, _____, G2, and M; DNA replication is semi-conservative (one parental strand + one new strand); helicase unwinds the DNA; DNA polymerase builds the new strand; telomerase maintains chromosome ends in germ cells and stem cells.

3.1.3 Transcription and RNA processing

Genes are expressed through a two-step process: transcription (making an RNA copy of the gene in the nucleus) followed by translation (using that RNA to make a protein in the cytoplasm). Transcription is carried out by an enzyme called RNA polymerase, which reads the DNA template and assembles a matching strand of messenger RNA (mRNA). In eukaryotes, the freshly made mRNA is not yet ready for export; it undergoes three important processing steps while still in the nucleus. First, a protective chemical cap (the 5' cap) is added to the beginning of the mRNA molecule; this cap protects the mRNA from being degraded and is also needed for the ribosome to recognise and begin translating the mRNA. Second, sections of the mRNA called introns (non-coding sequences) are cut out by a large molecular machine called the spliceosome, and the remaining coding sequences (exons) are joined together; this process is called splicing.



Third, a long tail of adenine nucleotides (the poly(A) tail, about 200 bases long) is added to the end of the mRNA; this poly(A) tail protects the mRNA from degradation and helps it to be exported from the nucleus. One of the most significant aspects of splicing is that the same gene can have its exons joined in different combinations to produce different mRNA variants from a single gene, a process called alternative splicing. This greatly expands the number of different proteins a cell can make without increasing the number of genes. Once the mRNA has been capped, spliced, and polyadenylated, it is transported through the nuclear pore complexes into the cytoplasm, where ribosomes translate it into protein.

Fill in the blank: Transcription produces a pre-mRNA copy of a gene; three processing steps prepare it for export: addition of a protective 5' _____, splicing by the spliceosome (introns removed; exons joined), and addition of a 3' poly(A) tail; alternative splicing of the same pre-mRNA can produce multiple different proteins.



EUKARYOTIC GENE EXPRESSION AND REGULATION

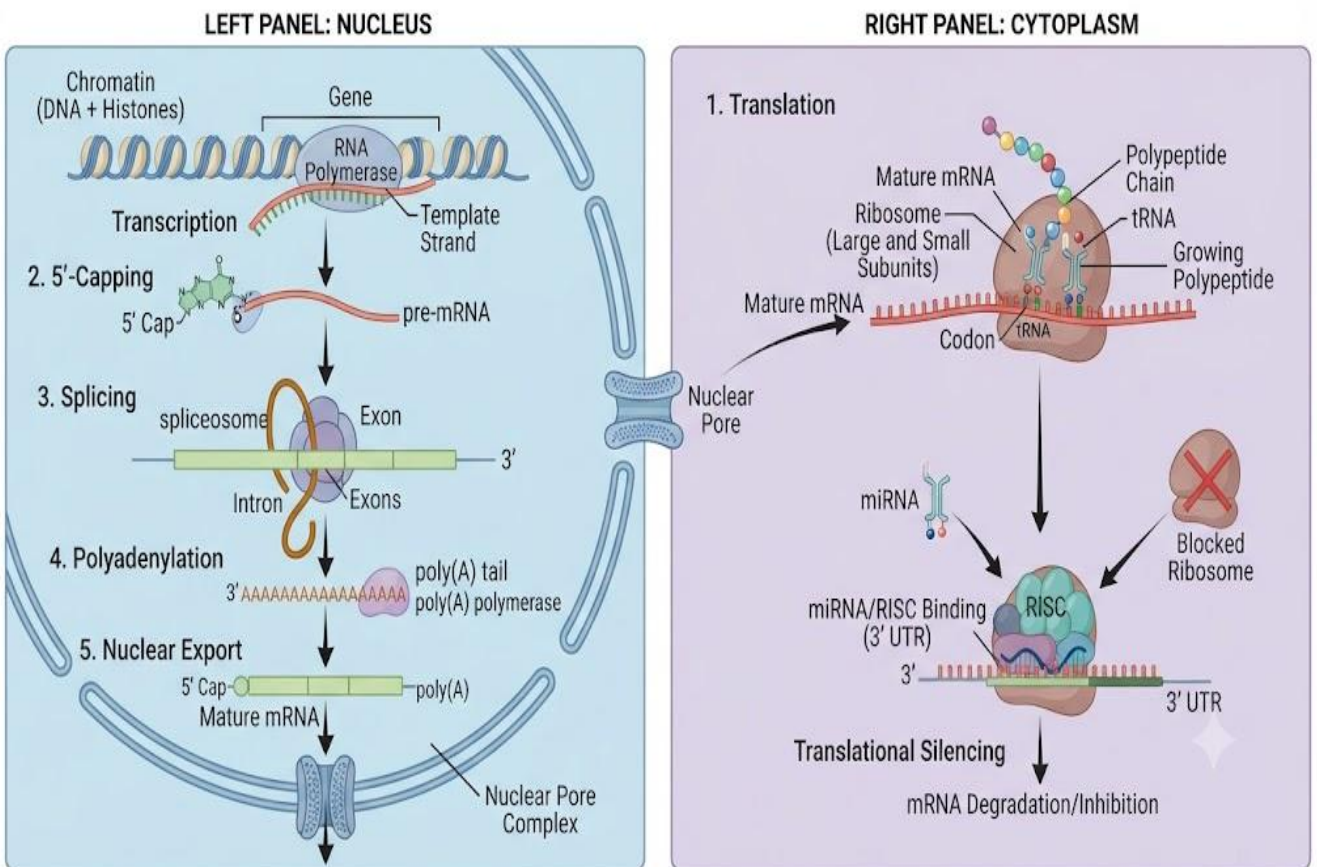


Figure 3.1: Eukaryotic gene expression: transcription in the nucleus, three mRNA processing steps (5' capping; splicing; polyadenylation), export through the nuclear pore complex, and translation in the cytoplasm

Pilot questions 3.1

1. Explain what chromatin is and describe the difference between euchromatin and heterochromatin.
2. Name the four phases of the cell cycle and state what happens in each phase.
3. What is meant by saying DNA replication is semi-conservative?
4. State the three processing steps that a pre-mRNA undergoes in the nucleus before it is exported.
5. What is alternative splicing and why is it important?



3.2 The Endomembrane System

3.2.1 Endoplasmic reticulum functions

The endoplasmic reticulum (ER) is a vast network of membranes that performs different functions depending on its region. The rough ER is covered with ribosomes on its outer surface and is the major site for making proteins that are destined for secretion (release from the cell), for insertion into cell membranes, or for delivery to lysosomes. As the ribosome builds a protein, the growing polypeptide chain is threaded directly into the interior of the rough ER, where it folds into its correct three-dimensional shape with the help of molecular chaperone proteins. During this process, sugar molecules are attached to the protein, a modification known as glycosylation that helps the protein fold correctly and protects it from degradation.

The smooth ER, which lacks ribosomes, serves different purposes. It is the main site where lipids (including phospholipids, cholesterol, and steroid hormones) are synthesised for the cell. In liver cells, the smooth ER also detoxifies drugs, alcohol, and other harmful substances. In muscle cells, a specialised version of the smooth ER called the sarcoplasmic reticulum stores and releases calcium ions, which trigger muscle contraction. If too many proteins fail to fold correctly in the rough ER, a stress response called the Unfolded Protein Response (UPR) is activated, which either restores normal function or, if the situation is unresolvable, triggers cell death.

Fill in the blank: The _____ ER is studded with ribosomes and is the main site for synthesising secretory, membrane, and lysosomal proteins; the smooth ER lacks ribosomes and is responsible for lipid synthesis, Ca^{2+} storage (in muscle), and drug detoxification in liver cells.

3.2.2 Golgi apparatus and vesicular sorting

After proteins are made and initially modified in the rough ER, they are packaged into small membrane-bound sacs called vesicles and transported to the Golgi apparatus, the cell's main sorting and modification centre. The Golgi is organised with a receiving side (called the cis face, which faces the ER) and a dispatching side (called the trans face, which faces the plasma membrane). Proteins enter the Golgi at the cis face and move through a series of flattened membrane compartments (cisternae) to the trans face, during which the sugar chains attached in



the ER are further trimmed and modified. This is similar to a postal sorting office: goods arrive, are labelled, and are packaged for delivery to specific destinations.

At the trans face, proteins are sorted into different types of vesicles depending on where they need to go. Lysosomal proteins are tagged with a special sugar label (mannose-6-phosphate) that directs them into vesicles bound for lysosomes. Secretory proteins are packaged into vesicles that fuse with the plasma membrane, releasing their contents to the outside of the cell (exocytosis). Some secretory vesicles are stored and only released when the cell receives a specific signal, such as a rise in calcium ions; this is called regulated secretion and is how neurons release neurotransmitters or how the pancreas releases insulin. The fusion of vesicles with their target membranes is driven by complementary proteins on the vesicle and the target membrane called SNAREs.

Fill in the blank: Proteins move through the Golgi from the _____ face (receiving, near ER) to the trans face (dispatching); lysosomal proteins are tagged with mannose-6-phosphate at the Golgi and sorted into vesicles bound for lysosomes; regulated secretion is triggered by a signal such as a rise in calcium ions.

3.2.3 Lysosomes and cellular degradation

Lysosomes are small, membrane-bound sacs found in animal cells that function as the cell's main waste-disposal and recycling system. They contain about 60 different digestive enzymes (called acid hydrolases) capable of breaking down all major biological molecules, including proteins, lipids, carbohydrates, and nucleic acids. These enzymes work best in an acidic environment (pH about 4.5), which is maintained inside the lysosome by pumps in the lysosomal membrane that continuously push hydrogen ions into the interior. The acidic pH also protects the rest of the cell: if a lysosome leaks, the enzymes are largely inactive at the neutral cytoplasmic pH.

Lysosomes degrade material through two main routes. In autophagy (meaning 'self-eating'), the cell wraps damaged organelles or accumulated proteins inside a double-membrane structure called an autophagosome, which then fuses with a lysosome so the contents can be digested and the components recycled. This process is essential for maintaining cellular health and was



awarded the Nobel Prize in Physiology or Medicine in 2016. In heterophagy, material taken up from outside the cell (such as bacteria engulfed by a macrophage) is delivered to lysosomes for digestion. When a gene encoding one of the lysosomal enzymes is defective, the substrate that enzyme normally degrades accumulates inside lysosomes and causes a lysosomal storage disease; well-known examples include Pompe disease (glycogen accumulation), Gaucher disease (lipid accumulation in macrophages), and Tay-Sachs disease (lipid accumulation in neurons).

Fill in the blank: Lysosomes contain about 60 _____ enzymes that work at an acidic pH of about 4.5; in autophagy, damaged organelles are enclosed in an autophagosome and delivered to lysosomes for degradation; lysosomal storage diseases occur when a specific lysosomal enzyme is deficient, causing its substrate to accumulate.

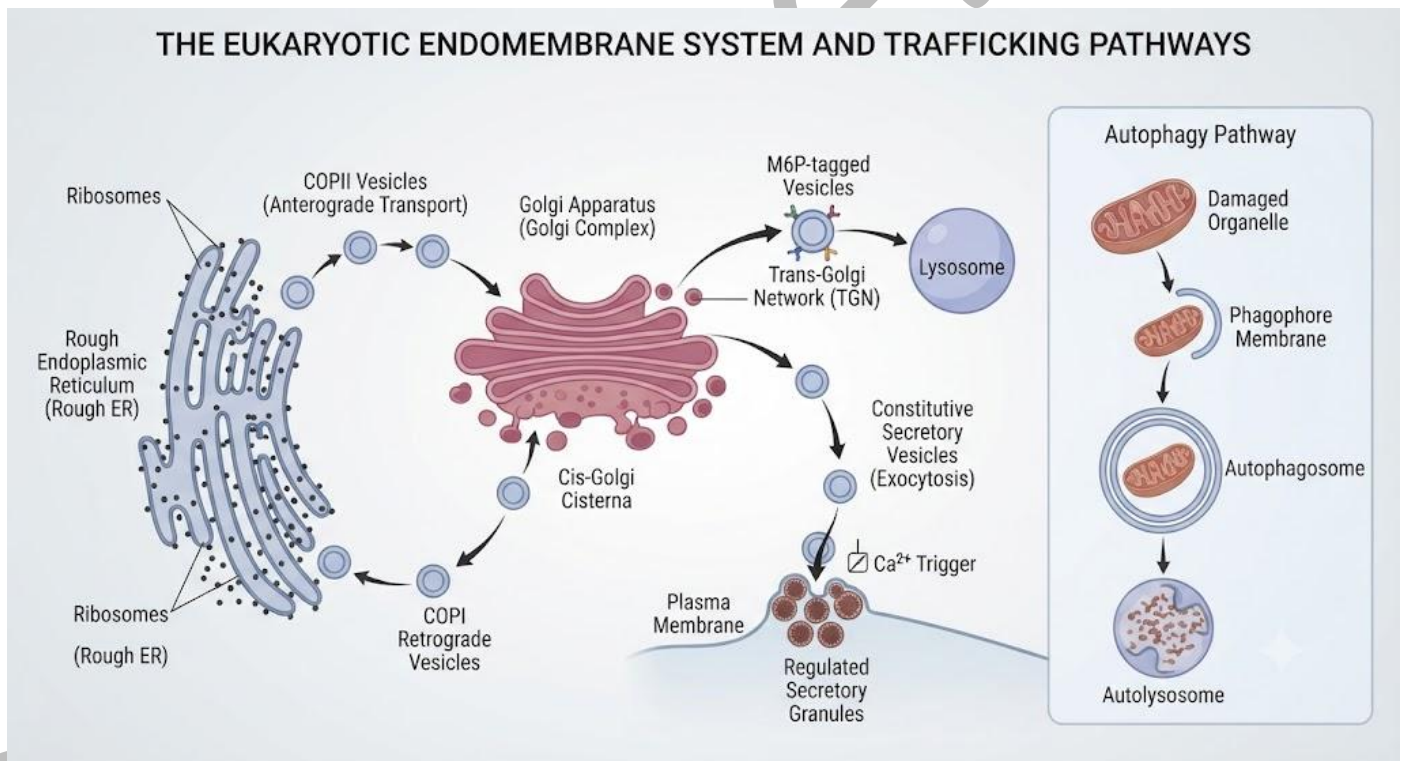


Figure 3.2: The endomembrane system: protein flow from the rough ER through the Golgi (cis to trans) to three destinations – lysosomes (via M6P tag), constitutive secretion, or regulated secretion; and the autophagy pathway (phagophore → autophagosome → autolysosome)



Pilot questions 3.2

1. Explain the difference between the rough ER and the smooth ER in terms of structure and function.
2. Describe the role of the Golgi apparatus in sorting proteins to their correct destinations.
3. What is the role of mannose-6-phosphate in directing proteins to lysosomes?
4. Explain what autophagy is and why it is important for cell health.
5. What are lysosomal storage diseases? Give two examples.

3.3 Energy-Converting Organelles

3.3.1 Mitochondria and ATP production

Mitochondria are often described as the powerhouses of the cell because they produce the majority of the cell's energy in the form of ATP (adenosine triphosphate), the universal energy currency used to drive nearly all cellular processes. Mitochondria generate ATP through a process called oxidative phosphorylation, which takes place at the inner mitochondrial membrane. The process begins when nutrients (particularly glucose and fatty acids) are broken down; this releases high-energy electrons that are passed along a series of protein complexes embedded in the inner membrane, collectively called the electron transport chain. As electrons pass through these complexes, they release energy that is used to pump hydrogen ions (protons) from the inner compartment of the mitochondrion (the matrix) across the inner membrane into the space between the two membranes.

This pumping of protons creates a concentration difference, with many more protons on one side of the inner membrane than the other. Protons then flow back across the membrane through a specialised protein called ATP synthase, much like water flowing through a turbine; this flow drives ATP synthase to produce ATP from ADP and phosphate. This beautifully efficient mechanism, proposed by Peter Mitchell (who received the Nobel Prize in 1978), is called the chemiosmotic theory. Mitochondria also play an important role in apoptosis (programmed cell death): when a cell is damaged beyond repair, a signal protein called cytochrome c is released from between the two mitochondrial membranes into the cytoplasm, triggering a cascade that leads to the orderly dismantling of the cell.



Fill in the blank: Mitochondria produce ATP by oxidative phosphorylation at the inner membrane; the electron transport chain pumps protons across the inner membrane, creating a gradient that drives _____ synthase to produce ATP; this mechanism is called the chemiosmotic theory (Mitchell, Nobel 1978).

3.3.2 Chloroplasts and photosynthesis

Chloroplasts are the organelles responsible for photosynthesis in plant cells and algae. They capture the energy of sunlight and use it to convert carbon dioxide and water into glucose, releasing oxygen as a by-product. Photosynthesis occurs in two stages. The first stage, the light-dependent reactions, takes place in the thylakoid membranes (the stacked disc-like membranes inside the chloroplast). Here, chlorophyll and other pigments in two large protein complexes called Photosystem II and Photosystem I absorb light energy. Photosystem II uses this energy to split water molecules, releasing oxygen into the atmosphere, hydrogen ions, and electrons. These electrons pass along a chain of proteins, releasing energy used to produce ATP, before reaching Photosystem I, which uses additional light energy to produce NADPH, a molecule that carries high-energy electrons.

The second stage, called the Calvin cycle or light-independent reactions, takes place in the fluid-filled stroma of the chloroplast. Here, the ATP and NADPH produced in the light reactions are used to power a cycle of reactions that fix carbon dioxide from the air into organic molecules. The key enzyme driving this process is called RuBisCO, which is considered the most abundant enzyme on Earth. RuBisCO attaches carbon dioxide to an existing five-carbon molecule, and through a series of reactions, this produces a three-carbon sugar called glyceraldehyde-3-phosphate (G3P) that can be used to build glucose, starch, and other organic compounds the plant needs. The Calvin cycle is the foundation of almost all food production on Earth, as virtually all the carbon in living things passed through this cycle at some point.

Fill in the blank: Photosynthesis has two stages: the light-dependent reactions in the _____ membranes (where water is split, O_2 is released, and ATP and NADPH are produced) and the Calvin cycle in the stroma (where RuBisCO fixes CO_2 into organic sugar using the ATP and NADPH from the light reactions).



3.3.3 Peroxisomes and cytoskeletal roles

Peroxisomes are small, single-membrane organelles found in nearly all eukaryotic cells. They take their name from hydrogen peroxide (H_2O_2), a toxic by-product generated when they break down fatty acids and certain amino acids. Peroxisomes contain an enzyme called catalase that rapidly destroys hydrogen peroxide, converting it to harmless water and oxygen, thus protecting the cell from oxidative damage. One of the most important functions of peroxisomes is the breakdown of very-long-chain fatty acids, which are fatty acid molecules too long to be processed by mitochondria; this process only occurs in peroxisomes. In the liver, peroxisomes also contribute to the production of bile acids and the breakdown of alcohol. Defects in peroxisome formation (caused by mutations in genes called PEX genes) lead to a serious disorder called Zellweger syndrome, in which peroxisomes fail to form properly, harmful fatty acids accumulate in the nervous system, and affected infants rarely survive beyond the first year of life.

Although the cytoskeleton is not a membrane-bound organelle, it functions as much more than a passive structural scaffold; it is an active participant in nearly every cellular process. The actin filaments and myosin proteins that form the contractile ring divide the cell in two during cytokinesis. Microtubules form the mitotic spindle that pulls chromosomes to opposite poles during cell division. Motor proteins (kinesin and dynein) use microtubule tracks like roads to carry vesicles, organelles, and other cargo to specific locations within the cell, much like lorries on a motorway. Actin polymerisation at the front of a migrating cell pushes the cell membrane forward, enabling cells to move through tissues. The dynein arms within the shaft of cilia and flagella generate the bending movements that propel fluids across the surface of cells or drive sperm cells through fluid.

Fill in the blank: Peroxisomes contain _____ (which destroys H_2O_2) and carry out the breakdown of very-long-chain fatty acids (a process exclusive to peroxisomes); Zellweger syndrome results from defective peroxisome biogenesis (PEX gene mutations); the cytoskeleton drives cell division, intracellular transport, cell migration, and ciliary movement.



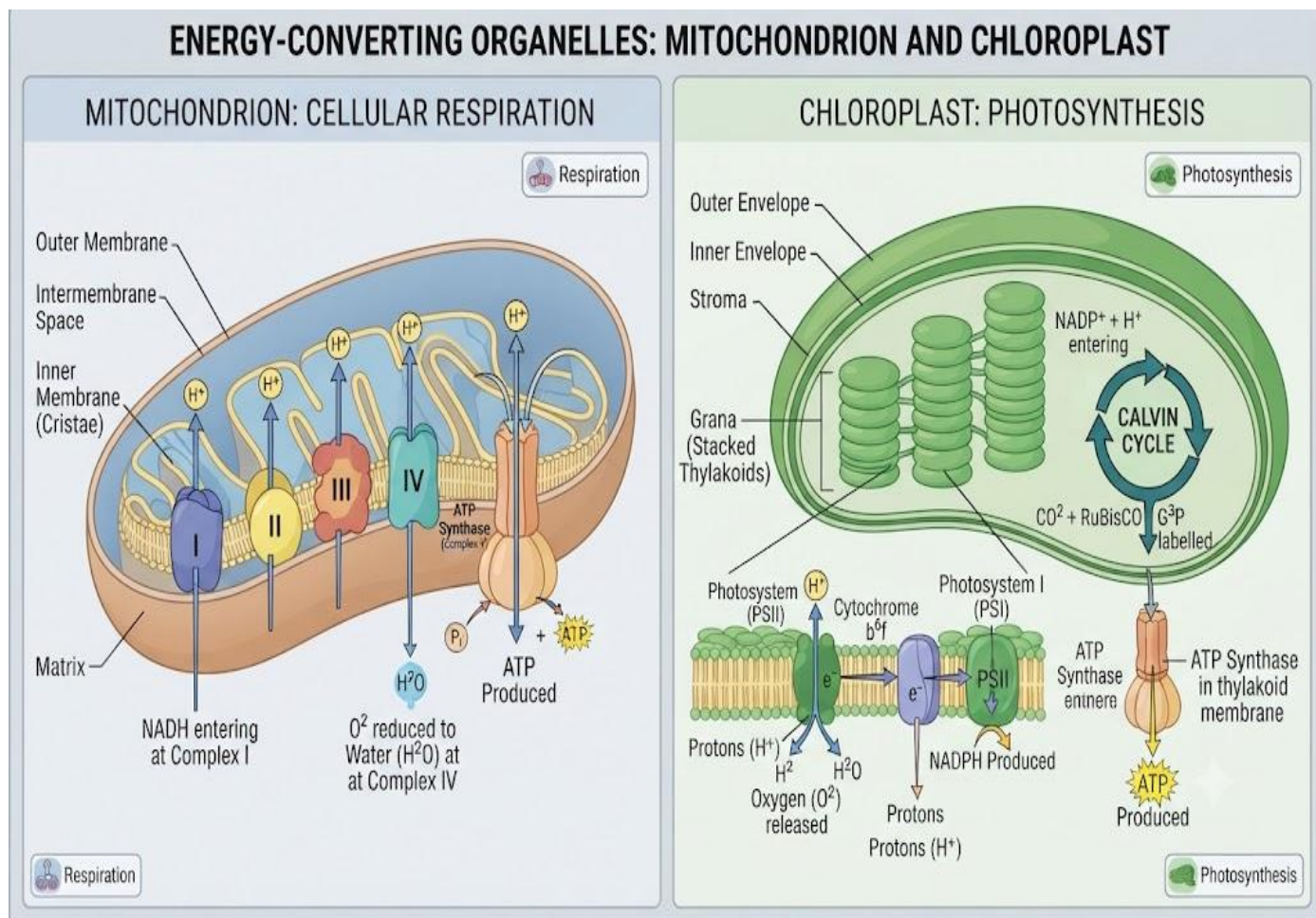


Figure 3.3: Energy conversion in the mitochondrion (left: electron transport chain pumping protons across the inner membrane; ATP synthase driven by proton flow) and the chloroplast (right: light-dependent reactions in the thylakoid membrane; Calvin cycle in the stroma)

Pilot questions 3.3

1. Explain in simple terms how mitochondria produce ATP.
2. What is the chemiosmotic theory and who proposed it?
3. Describe the two stages of photosynthesis and state where each occurs in the chloroplast.
4. What is the role of RuBisCO in the Calvin cycle?
5. State two functions of peroxisomes and explain what Zellweger syndrome is.



3.4 Membrane Transport

3.4.1 Passive transport across membranes

All cells must continuously exchange molecules with their environment, taking in nutrients and oxygen while expelling waste products. The plasma membrane controls what passes in and out. Transport across membranes falls into two broad categories: passive transport (which requires no energy input from the cell) and active transport (which requires the cell to expend energy).

Passive transport moves molecules from a region where they are more concentrated to a region where they are less concentrated – that is, down their concentration gradient. Small, non-polar molecules such as oxygen, carbon dioxide, and steroid hormones can dissolve directly in the lipid bilayer and cross the membrane by simple diffusion without any assistance from proteins. Water also moves passively across membranes by osmosis, and specialised protein channels called aquaporins greatly speed up this water movement.

Many important molecules, including glucose and ions such as sodium, potassium, and calcium, cannot cross the lipid bilayer on their own because they are too polar or too charged. These molecules cross the membrane through specific protein channels or carrier proteins, a process called facilitated diffusion. Ion channels are pores that open and close in response to specific signals (such as a change in voltage or the binding of a ligand) and allow ions to move rapidly down their electrochemical gradient. Carrier proteins such as the glucose transporter GLUT1 bind their specific molecule and undergo a shape change to move it across the membrane. Facilitated diffusion is still passive because molecules move down their concentration gradient without the cell spending energy.

Fill in the blank: Passive transport moves molecules down their _____ gradient without energy; simple diffusion (small non-polar molecules through the lipid bilayer), osmosis (water through aquaporins), and facilitated diffusion (polar molecules or ions through channels or carrier proteins such as GLUT1) are all forms of passive transport.

3.4.2 Active transport and membrane pumps

When a cell needs to move a molecule against its concentration gradient – from a region of low concentration to a region of high concentration – it must use energy. This is called active



transport. The most important active transporter in animal cells is the sodium-potassium pump (Na^+/K^+ -ATPase), which uses the energy from ATP hydrolysis to move three sodium ions out of the cell and two potassium ions into the cell with every pump cycle. This maintains the low sodium concentration inside the cell and creates an electrical charge difference across the membrane (the resting membrane potential) that is essential for nerve and muscle function. Another important pump is the calcium pump (SERCA), which moves calcium ions from the cytoplasm into the endoplasmic reticulum, keeping cytoplasmic calcium at very low levels and allowing calcium to be rapidly released as a signalling molecule when needed.

Cells also use a clever indirect form of active transport called secondary active transport. In this mechanism, the energy stored in the sodium gradient (created by the sodium-potassium pump) is used to drive the uptake of a second molecule against its gradient, without directly using ATP for that second step. For example, the sodium-glucose co-transporter (SGLT1) in the intestinal lining uses the inward flow of sodium ions down their gradient to simultaneously pull glucose into the cell against its gradient, so that glucose is efficiently absorbed even when its concentration inside the cell is already high. This is called a symporter because both molecules move in the same direction. In contrast, antiporters move two molecules in opposite directions; for example, the sodium-hydrogen exchanger (NHE1) uses the inward flow of sodium to push hydrogen ions out of the cell, helping to regulate the pH inside the cell.

Fill in the blank: Active transport moves molecules _____ their concentration gradient and requires energy (ATP); the Na^+/K^+ -ATPase pumps 3 Na^+ out and 2 K^+ in per ATP, maintaining the resting membrane potential; secondary active transport (e.g. SGLT1 symporter) uses the Na^+ gradient to drive glucose uptake without directly consuming ATP.

3.4.3 Endocytosis and exocytosis

Cells can also take in or release large quantities of material in bulk, not through individual protein channels, but by using the membrane itself to form vesicles. Endocytosis is the process by which the plasma membrane folds inward around material outside the cell, pinches off, and brings that material into the cell inside a vesicle. Phagocytosis ('cell eating') is a form of endocytosis used by immune cells such as macrophages to engulf large particles like bacteria or cellular debris; the plasma membrane extends around the particle, forming a large vesicle called



a phagosome that then fuses with a lysosome for digestion. Receptor-mediated endocytosis is a more targeted form in which specific molecules outside the cell bind to receptor proteins on the cell surface; the receptor-molecule complexes cluster in a region of the membrane coated with a protein called clathrin, which pulls the membrane inward to form a vesicle and deliver the cargo to the interior of the cell. The uptake of cholesterol (via LDL receptors) and iron (via transferrin receptors) are well-known examples of this process.

Exocytosis is the reverse process: vesicles inside the cell fuse with the plasma membrane and release their contents to the outside. Constitutive exocytosis occurs continuously in all cell types, constantly delivering newly made membrane proteins and secreted products such as collagen to the cell surface or the extracellular space. Regulated exocytosis, by contrast, occurs only in specialised secretory cells and only when triggered by a specific signal, usually a rise in the concentration of calcium ions inside the cell. When a nerve impulse arrives at a synapse, calcium ions flood into the nerve terminal and trigger synaptic vesicles to fuse with the membrane and release neurotransmitters in a fraction of a millisecond. Similarly, when blood glucose rises after a meal, calcium signalling in the pancreatic beta cells triggers insulin-containing vesicles to release insulin into the bloodstream.

<u>Type</u>	<u>Requires Energy?</u>	<u>Direction of Movement</u>	<u>Examples</u>
Simple Diffusion	No	Down concentration gradient	O ₂ , CO ₂ , steroid hormones through lipid bilayer
Osmosis	No	Water moves from low to high solute concentration	Aquaporin channels
Facilitated Diffusion	No	Down electrochemical gradient	GLUT1 glucose carrier, Na ⁺ channel, K ⁺ channel
Primary Active Transport	Yes (ATP required)	Against concentration gradient	Na ⁺ /K ⁺ -ATPase (3Na ⁺ out, 2K ⁺ in), SERCA Ca ²⁺ pump, V-type H ⁺ -ATPase
Secondary Active Transport	Indirect (uses Na ⁺ gradient)	Against gradient of co-transported solute	SGLT1 symporter, NHE1 antiporter



Box 3.1: Summary of membrane transport mechanisms showing type, energy requirement, direction of movement, and examples

Pilot questions 3.4

1. Explain the difference between simple diffusion and facilitated diffusion.
2. Explain how the Na^+/K^+ -ATPase works and why it is important.
3. What is secondary active transport? Distinguish between a symporter and an antiporter with one example each.
4. Describe receptor-mediated endocytosis and give two examples of molecules taken up by this pathway.
5. Distinguish between constitutive and regulated exocytosis and give one example of regulated exocytosis.



Series Summary

This Series explored the **functions and coordination of the major organelles in eukaryotic cells**, showing how they regulate genetic information, synthesise proteins, recycle cellular components, and maintain normal cell function. You examined the nucleus and cell cycle, RNA processing, the endomembrane system, and the roles of the rough and smooth endoplasmic reticulum, Golgi apparatus, and lysosomes in protein production, modification, transport, and intracellular recycling.

The Series also examined how mitochondria and chloroplasts convert energy to sustain cellular activities, how peroxisomes detoxify harmful substances, and how the cytoskeleton and membrane transport systems support movement, communication, and the exchange of materials. By completing this Series, you should now be able to explain the functions of major eukaryotic organelles, describe how they interact to maintain cellular processes, distinguish the mechanisms of cellular transport and energy conversion, and relate these concepts to normal cell physiology and disease, thereby achieving all four **Series Learning Outcomes (SLOs)** and the **overall course outcomes**.

Glossary of Terms

- **Alternative splicing:** the joining of different combinations of exons from the same pre-mRNA to produce multiple different protein isoforms from a single gene; greatly expands the diversity of the proteome.
- **Autophagy:** a cellular self-cleaning process in which damaged organelles or surplus cytoplasmic material are enclosed in a double-membrane autophagosome and delivered to a lysosome for digestion and recycling.
- **Calvin cycle:** the light-independent reactions of photosynthesis that occur in the chloroplast stroma; carbon dioxide is fixed by the enzyme RuBisCO onto a five-carbon acceptor, and ATP and NADPH from the light reactions drive the production of the sugar G3P.
- **Chemiosmotic theory:** Peter Mitchell's theory (Nobel Prize 1978) that the electron transport chain creates a proton gradient across the inner mitochondrial membrane, and that the flow of protons back through ATP synthase drives the synthesis of ATP.



- **Chromatin:** the complex of DNA and histone proteins that packages DNA inside the nucleus; euchromatin is loosely packed and transcriptionally active; heterochromatin is tightly packed and transcriptionally silent.
- **Endocytosis:** the uptake of material from outside the cell by inward folding of the plasma membrane to form an intracellular vesicle; types include phagocytosis (engulfment of large particles) and receptor-mediated (clathrin-mediated) endocytosis.
- **Epigenetic regulation:** changes in gene activity that do not involve alterations to the DNA sequence itself; achieved through chemical modifications to histone proteins (acetylation, methylation) or to DNA (methylation at CpG sites).
- **Exocytosis:** the fusion of intracellular vesicles with the plasma membrane to release their contents to the extracellular space or to deliver membrane components; constitutive exocytosis is continuous; regulated exocytosis is triggered by a specific signal such as calcium.
- **Facilitated diffusion:** passive transport of polar or charged molecules across a membrane through specific protein channels or carrier proteins, down their electrochemical gradient, without the expenditure of energy.
- **Lysosomal storage disease:** an inherited metabolic disorder caused by a deficiency in a single lysosomal hydrolase enzyme; the enzyme's substrate accumulates inside lysosomes, causing progressive cell damage; examples include Pompe disease, Gaucher disease, and Tay-Sachs disease.
- **Nucleosome:** the basic structural unit of chromatin consisting of approximately 147 base pairs of DNA wound around a core of eight histone proteins (two each of H2A, H2B, H3, and H4); multiple nucleosomes connected by linker DNA give chromatin the appearance of beads on a string.
- **Oxidative phosphorylation:** the process by which ATP is produced in the mitochondrion, using energy released by the passage of electrons through the electron transport chain to pump protons across the inner mitochondrial membrane, creating a gradient that drives ATP synthase.
- **Peroxisome:** a small, single-membrane organelle containing the enzyme catalase (which destroys hydrogen peroxide) and enzymes for the oxidation of very-long-chain fatty acids; defective peroxisome biogenesis causes Zellweger syndrome.
- **Photosystem:** a large protein complex in the thylakoid membrane that captures light energy; Photosystem II splits water to release oxygen and electrons; Photosystem I uses further light energy to produce NADPH.



- **RuBisCO:** ribulose-1,5-bisphosphate carboxylase/oxygenase; the enzyme that catalyses carbon fixation in the Calvin cycle by attaching carbon dioxide to a five-carbon acceptor molecule; the most abundant enzyme on Earth.
- **Secondary active transport:** the use of an electrochemical gradient (usually the sodium gradient maintained by the Na^+/K^+ -ATPase) to drive the uphill transport of another molecule; symporters move both molecules in the same direction; antiporters move them in opposite directions.
- **Signal recognition particle (SRP):** a ribonucleoprotein complex that recognises the signal sequence of a nascent secretory protein, pauses translation, and guides the ribosome to the surface of the rough ER for co-translational protein translocation.
- **Spliceosome:** a large complex of RNA and protein molecules that removes introns from pre-mRNA and joins the exons together; different combinations of exons can be joined (alternative splicing) to produce multiple protein variants from one gene.
- **Unfolded Protein Response (UPR):** a stress response activated when too many misfolded proteins accumulate in the ER; it upregulates chaperone production, reduces overall protein synthesis, and expands the ER to restore normal function; if the stress cannot be resolved, it triggers apoptosis.
- **Zellweger syndrome:** a fatal autosomal recessive disorder caused by mutations in PEX genes that are required for peroxisome biogenesis; peroxisomes fail to form, very-long-chain fatty acids accumulate in the nervous system, causing severe brain malformation and death in infancy.



Concept Check Questions [Series 3]

Objective Questions

1. DNA in the nucleus is packaged into chromatin; loosely packed _____ chromatin contains actively expressed genes, while tightly packed heterochromatin is generally switched off. (Linked to SLO 3.1)
2. Before an mRNA can be exported from the nucleus, it must receive a 5' cap, have its introns removed by the _____, and receive a poly(A) tail at its 3' end. (Linked to SLO 3.1)
3. The rough ER synthesises proteins for secretion and membrane insertion; misfolded proteins that cannot be corrected are retrotranslocated to the cytosol and degraded by the _____ (a large protein-degradation complex). (Linked to SLO 3.2)
4. In the mitochondrion, protons pumped by the electron transport chain flow back across the inner membrane through _____ synthase, whose rotation drives the production of ATP from ADP and phosphate. (Linked to SLO 3.3)
5. The Na^+/K^+ -ATPase is a primary active transporter that moves 3 Na^+ _____ of the cell and 2 K^+ into the cell using one molecule of ATP, maintaining the resting membrane potential. (Linked to SLO 3.4)

Short-Answer Questions

1. Explain what epigenetic regulation is and give two examples of epigenetic modifications. (Linked to SLO 3.1)
2. Describe the role of the Golgi apparatus in protein processing and sorting. (Linked to SLO 3.2)
3. Explain how mitochondria produce ATP, describing the roles of the electron transport chain and the proton gradient. (Linked to SLO 3.3)
4. Describe the two stages of photosynthesis and state where each stage occurs in the chloroplast. (Linked to SLO 3.3)
5. Distinguish between receptor-mediated endocytosis and phagocytosis in terms of the type of material taken up and the cell types involved. (Linked to SLO 3.4)

Long-Answer Questions

1. Describe the organisation and function of the nucleus: how DNA is packaged into chromatin, how gene activity is regulated epigenetically, how the cell cycle controls DNA replication, and how mRNA is processed before export from the nucleus. (Linked to SLO 3.1)
2. Describe the functions of the major organelles involved in protein production and energy conversion: the rough ER and smooth ER, the Golgi apparatus, lysosomes (including autophagy



and lysosomal storage diseases), mitochondria (ATP production by oxidative phosphorylation), and chloroplasts (light-dependent reactions and the Calvin cycle). (Linked to SLOs 3.2, 3.3)

Answers to Concept Check Questions [Series 3]

Objective Questions

1. Eu.
2. Spliceosome.
3. Proteasome.
4. ATP.
5. Out.

Short-Answer Questions

1. Epigenetic regulation refers to changes in gene activity that do not alter the DNA sequence. Two examples: (1) histone acetylation (acetyl groups added to histone proteins loosen chromatin and switch genes on); (2) DNA methylation (methyl groups added to CpG sites on DNA generally silence gene expression).
2. The Golgi apparatus receives proteins from the ER, further modifies their sugar chains, and sorts them into vesicles for three destinations: proteins tagged with mannose-6-phosphate go to lysosomes; secretory proteins are packaged for release from the cell; and membrane proteins are delivered to the plasma membrane.
3. Nutrients are broken down, releasing high-energy electrons that pass through the electron transport chain in the inner mitochondrial membrane; as electrons move through the chain, protons are pumped across the membrane, creating a gradient; protons flow back through ATP synthase, driving the production of ATP (chemiosmotic theory, Mitchell Nobel 1978).
4. Light-dependent reactions: occur in the thylakoid membranes; light energy splits water, releasing oxygen; ATP and NADPH are produced. Calvin cycle (light-independent reactions): occur in the stroma; ATP and NADPH are used by RuBisCO to fix carbon dioxide from the air into the sugar G3P.
5. Receptor-mediated endocytosis: takes up specific molecules (e.g. cholesterol via LDL receptors; iron via transferrin receptors) through receptor-lined clathrin-coated pits; occurs in most cell types. Phagocytosis: engulfs large particles (bacteria, dead cells) using pseudopodia; carried out by specialised immune cells such as macrophages and neutrophils.

Long-Answer Questions



1. Nucleus and gene expression: DNA packaged into chromatin (nucleosome = 147 bp DNA on histone octamer); euchromatin (active; loose) vs heterochromatin (inactive; condensed; nuclear periphery); epigenetic regulation by histone acetylation (HATs; activate), deacetylation (HDACs; repress), and DNA methylation at CpG sites (represses). Cell cycle: G1 (growth) → S (DNA replication; semi-conservative; multiple origins; helicase unwinds; DNA polymerase copies; telomerase maintains chromosome ends) → G2 (repair; checkpoint) → M (mitosis; cytokinesis); regulated by cyclin-CDK complexes. mRNA processing: pre-mRNA receives 5' m7G cap (protects; ribosome binding); spliceosome removes introns and joins exons (alternative splicing diversifies the proteome); poly(A) tail added (protects; facilitates export); mature mRNA exported through nuclear pore complexes to cytoplasm for translation by ribosomes.
2. Rough ER: ribosomes on surface; synthesises secretory, membrane, and lysosomal proteins; proteins folded with chaperone assistance (BiP); N-linked glycosylation added; misfolded proteins degraded by ERAD (retrotranslocation + proteasome); UPR activated by ER stress. Smooth ER: no ribosomes; lipid and steroid hormone synthesis; Ca^{2+} storage; drug detoxification. Golgi: receives protein from ER via COPII vesicles; modifies glycans; tags lysosomal proteins (M6P); sorts to lysosomes, constitutive or regulated secretion; SNARE proteins drive vesicle fusion. Lysosomes: acid hydrolases at pH 4.5; V-type H^{+} -ATPase maintains acidity; autophagy (phagophore → autophagosome → lysosome; Nobel 2016); storage diseases (Pompe: glycogen; Gaucher: glucocerebroside). Mitochondria: ETC pumps protons across inner membrane; proton gradient drives ATP synthase (Complex V); chemiosmosis (Mitchell Nobel 1978); approximately 30–32 ATP per glucose. Chloroplasts: light reactions (thylakoid; PSII splits water, O_2 released; PSI produces NADPH; ATP synthase produces ATP); Calvin cycle (stroma; RuBisCO fixes CO_2 to RuBP → G3P; ATP and NADPH consumed).

Answers to Pilot Questions [Series 3]

Part 3.1

1. Chromatin is the complex of DNA and histone proteins that packages DNA in the nucleus. Euchromatin is loosely packed and contains actively expressed genes (appears pale in TEM). Heterochromatin is tightly compacted and transcriptionally silent (appears dark in TEM; found at the nuclear periphery and at centromeres).



2. G1: cell grows and prepares for replication. S: DNA is replicated (each chromosome duplicated). G2: cell continues to grow and checks that replication was completed correctly. M: mitosis separates the duplicated chromosomes and cytokinesis divides the cell into two daughter cells.
3. Semi-conservative replication means that each new DNA molecule retains one original (parental) strand and one newly synthesised strand; the two strands of the parental helix are separated and each serves as a template for building a new complementary strand, so each daughter molecule is half old and half new.
- (1) 5' cap addition: a modified guanosine cap is added to protect the mRNA and allow ribosome binding. (2) Splicing: the spliceosome removes non-coding introns and joins the coding exons together. (3) Polyadenylation: a poly(A) tail of about 200 adenine nucleotides is added to the 3' end to protect the mRNA and facilitate its export from the nucleus.
4. Alternative splicing is the joining of different combinations of exons from the same pre-mRNA to produce multiple distinct mRNA molecules from a single gene. It is important because it greatly increases protein diversity, allowing approximately 20,000 human genes to produce approximately 90,000 different protein isoforms.

Part 3.2

1. Rough ER has ribosomes on its cytoplasmic surface and synthesises proteins destined for secretion, membranes, or lysosomes; these proteins are folded inside the ER lumen with the help of chaperones. Smooth ER lacks ribosomes and is responsible for lipid and steroid hormone synthesis, calcium ion storage (in muscle), and detoxification of drugs and toxins in the liver.
2. The Golgi receives proteins from the ER, further modifies their sugar chains, and sorts them into vesicles for three destinations: lysosomal proteins (tagged with mannose-6-phosphate) are sent to lysosomes; secretory proteins are packaged into vesicles for release from the cell; and membrane proteins are delivered to the plasma membrane.
3. Lysosomal hydrolases are tagged with mannose-6-phosphate (M6P) in the Golgi; M6P receptors on the TGN bind these tagged proteins and package them into clathrin-coated vesicles; the vesicles fuse with late endosomes, where the acidic pH causes the enzymes to dissociate from the receptors; the enzymes are then delivered to lysosomes and the receptors are recycled back to the Golgi.
4. Autophagy is a process by which a cell degrades and recycles its own damaged or surplus components. A membrane (phagophore) wraps around the material to be degraded, forming a double-membrane autophagosome; this fuses with a lysosome, whose enzymes digest the



contents; the products (amino acids, lipids, sugars) are released back into the cytoplasm for reuse. It is essential for removing damaged organelles and maintaining cellular health.

5. Lysosomal storage diseases are inherited disorders caused by the deficiency of a specific lysosomal enzyme, causing its substrate to accumulate inside lysosomes. Pompe disease: deficiency of acid maltase (alpha-glucosidase); glycogen accumulates in lysosomes, causing muscle weakness and heart disease. Gaucher disease: deficiency of glucocerebrosidase; the lipid glucocerebroside accumulates in macrophages, causing enlarged liver and spleen.

Part 3.3

1. Mitochondria break down nutrients (glucose, fatty acids) to release high-energy electrons. These electrons pass through a series of protein complexes (the electron transport chain) in the inner mitochondrial membrane, and as they do so, protons are pumped across the membrane. The resulting proton gradient drives protons back through ATP synthase, whose rotation generates ATP from ADP and phosphate.
2. The chemiosmotic theory, proposed by Peter Mitchell (Nobel Prize 1978), states that the electron transport chain creates a proton gradient across the inner mitochondrial membrane; the potential energy stored in this gradient (not in any chemical intermediate) drives ATP synthesis as protons flow back through ATP synthase.
3. Light-dependent reactions: occur in the thylakoid membranes; light energy is absorbed by Photosystems II and I; water is split, releasing oxygen; ATP and NADPH are produced. Calvin cycle (light-independent reactions): occur in the chloroplast stroma; RuBisCO uses ATP and NADPH to fix carbon dioxide from the air into the three-carbon sugar G3P.
4. RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is the enzyme that catalyses the first step of carbon fixation in the Calvin cycle; it attaches a molecule of carbon dioxide to a five-carbon acceptor molecule (RuBP), producing two three-carbon molecules (3-PGA) that are then reduced using ATP and NADPH to form G3P.
5. Two functions of peroxisomes: (1) they contain the enzyme catalase, which destroys the toxic molecule hydrogen peroxide; (2) they exclusively break down very-long-chain fatty acids (fatty acids too long to be processed by mitochondria). Zellweger syndrome: a fatal inherited disorder caused by mutations in PEX genes needed to build peroxisomes; without functional peroxisomes, very-long-chain fatty acids accumulate in the brain and other tissues, causing severe neurological damage and death in infancy.

Part 3.4



1. Simple diffusion: small, non-polar molecules (O_2 , CO_2 , steroid hormones) dissolve directly in the lipid bilayer and cross the membrane without any protein assistance, moving from high to low concentration. Facilitated diffusion: polar or charged molecules (glucose, ions) that cannot cross the lipid bilayer are moved down their concentration or electrochemical gradient through specific membrane proteins (channels or carrier proteins such as GLUT1), but without energy input.
2. The Na^+/K^+ -ATPase is a protein pump that uses one ATP molecule to transport three sodium ions out of the cell and two potassium ions into the cell. It maintains the low sodium concentration inside cells, creates the electrical charge difference (resting membrane potential) essential for nerve and muscle function, and generates the sodium gradient used by secondary active transporters.
3. Secondary active transport uses the sodium gradient (created by the Na^+/K^+ -ATPase) to move a second molecule against its own gradient without directly consuming ATP. Symporter: both molecules move in the same direction; example: SGLT1 uses sodium flowing into intestinal cells to simultaneously bring glucose in against its gradient. Antiporter: molecules move in opposite directions; example: NHE1 uses sodium moving in to push hydrogen ions out, regulating intracellular pH.
4. In receptor-mediated endocytosis, specific molecules outside the cell bind to receptor proteins on the cell surface; the receptor-ligand complexes cluster in clathrin-coated pits that pinch inward to form vesicles, delivering the cargo to the cell interior. Two examples: LDL receptors (take up cholesterol-carrying LDL particles from the blood); transferrin receptors (take up the iron-carrying protein transferrin).
5. Constitutive exocytosis occurs continuously in all cell types without any specific trigger, steadily delivering membrane components and secretory products (such as collagen or antibodies) to the cell surface. Regulated exocytosis occurs only in specialised secretory cells and is triggered by a specific signal, usually a rise in intracellular calcium; example: a nerve impulse causes calcium ions to enter the nerve terminal, triggering synaptic vesicles to fuse with the membrane and release neurotransmitters into the synapse.



References and Attributions [Series 3]

Textual References (Books and External Sources)

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Figures, Plates, Tables, and Boxes

- Figure 3.1: Eukaryotic gene expression diagram. Attribution: AI generated. Suggested OER search string: “eukaryotic gene expression transcription RNA processing splicing nuclear export translation diagram OER”.
- Figure 3.2: Endomembrane system and protein sorting diagram. Attribution: AI generated. Suggested OER search string: “endomembrane system protein secretion Golgi lysosome autophagy vesicular transport labelled diagram OER”.
- Figure 3.3: Energy conversion in mitochondria and chloroplasts. Attribution: AI generated. Suggested OER search string: “mitochondria electron transport chain ATP synthase chloroplast photosynthesis light reactions Calvin cycle diagram OER”.
- Box 3.1: Summary table of membrane transport mechanisms. Attribution: AI generated. Suggested OER search string: “membrane transport passive active secondary endocytosis exocytosis comparison summary table OER”.



Course code: BIO 205

Course title: Introductory Developmental / Cell Biology

Course credit load: 2 Units

Series 4: Mitosis and Meiosis

- **Part 4.1: The Cell Cycle and Its Regulation**
 - Sub Part 4.1.1: Overview of the cell cycle
 - Sub Part 4.1.2: Regulation of the cell cycle
 - Sub Part 4.1.3: Cell cycle checkpoints and cancer
- **Part 4.2: Mitosis**
 - Sub Part 4.2.1: Preparation for mitosis
 - Sub Part 4.2.2: The stages of mitosis
 - Sub Part 4.2.3: Cytokinesis in animal and plant cells
- **Part 4.3: Meiosis**
 - Sub Part 4.3.1: Overview and significance of meiosis
 - Sub Part 4.3.2: Meiosis I: the reductive division
 - Sub Part 4.3.3: Meiosis II and the products of meiosis
- **Part 4.4: Comparison of Mitosis and Meiosis**
 - Sub Part 4.4.1: Key similarities
 - Sub Part 4.4.2: Key differences
 - Sub Part 4.4.3: Errors in cell division and their consequences



Introduction

Every living organism grows, repairs damaged tissues, and reproduces. All of these processes depend on cell division – the ability of one cell to produce two daughter cells. There are two fundamentally different types of cell division in eukaryotes, each serving a completely different purpose. Mitosis is the type of cell division used for growth and tissue repair; it produces two daughter cells that are genetically identical to the parent cell. Meiosis, on the other hand, is the specialised form of cell division that produces reproductive cells (gametes such as eggs and sperm); it produces four cells that are genetically unique and contain half the chromosome number of the parent cell.

Understanding these two processes is central to understanding biology. Without mitosis, a fertilised egg could never grow into a complete organism, and wounds could never heal. Without meiosis, sexual reproduction would be impossible, and the chromosome number would double with every generation. This Series explores the cell cycle that controls when and how cells divide, the detailed steps of mitosis and meiosis, how cytokinesis physically divides the cell, and the differences between these two fundamental processes. It also examines what happens when cell division goes wrong.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 4.1** describe the stages of the eukaryotic cell cycle, explain how cyclin-CDK complexes drive cell cycle progression, and describe the roles of checkpoints in preventing errors (Linked to Part 4.1),
- **SLO 4.2** describe in order the stages of mitosis, explain what happens to chromosomes at each stage, and describe how cytokinesis differs between animal and plant cells (Linked to Part 4.2),
- **SLO 4.3** explain the biological significance of meiosis, describe the stages of meiosis I and meiosis II, and explain how genetic diversity is generated during meiosis (Linked to Part 4.3), and



- **SLO 4.4** compare and contrast mitosis and meiosis in terms of purpose, number of divisions, chromosome number, and genetic outcome, and describe the consequences of errors in cell division (Linked to Part 4.4).

4.1 The Cell Cycle and Its Regulation

4.1.1 Overview of the cell cycle

The cell cycle is the ordered series of events a cell goes through from the moment it is formed to the moment it divides into two daughter cells. It can be thought of as the cell's own life cycle, and understanding it is essential because virtually every aspect of growth, development, and disease involves the regulation of this cycle. The cell cycle is divided into two broad phases: interphase and the mitotic phase (M phase). Interphase is by far the longer portion and is when the cell grows and prepares for division. It is itself divided into three sub-phases: G1 (Gap 1), when the cell grows and produces proteins; S phase (Synthesis), when the cell makes a complete copy of all its DNA; and G2 (Gap 2), when the cell continues to grow and checks that replication was completed without errors.

The mitotic phase is the relatively brief period when the cell actually divides. It consists of two events: mitosis (the separation of duplicated chromosomes into two identical sets) and cytokinesis (the physical division of the cytoplasm to produce two daughter cells). Some cells, such as neurons and muscle cells, permanently exit the cell cycle after they differentiate and enter a resting state called G0. Other cells, such as stem cells in the bone marrow and cells lining the intestine, divide frequently throughout life. The total duration of the cell cycle varies enormously: a rapidly dividing human cell completes it in about 24 hours, while some cells cycle far more slowly.

Fill in the blank: The cell cycle consists of interphase (G1, S phase, and G2) and the _____ phase; DNA replication occurs during S phase; cells that permanently stop dividing exit the cell cycle into a resting state called G0.



4.1.2 Regulation of the cell cycle

The cell cycle is tightly regulated to ensure that cell division only occurs when the cell is ready and that each step is completed correctly before the next begins. The key molecular regulators are proteins called cyclins and their partner enzymes called cyclin-dependent kinases (CDKs). CDKs are enzymes that activate target proteins by adding a phosphate group to them, driving the cell into the next phase of the cycle. However, CDKs are only active when bound to their specific cyclin partner, and cyclins are produced and destroyed at specific points in the cycle. This means CDK activity rises and falls in a wave-like pattern that pushes the cell through G1, into S phase, and then into mitosis in the correct order.

Different cyclin-CDK pairs control different transitions: cyclin D with CDK4 and CDK6 drives the cell through G1 and commits it to divide; cyclin E with CDK2 triggers entry into S phase; cyclin B with CDK1 (together known as the Maturation Promoting Factor, MPF) drives the cell into mitosis by causing nuclear envelope breakdown and chromosome condensation. An important target of CDKs is the retinoblastoma protein (Rb), which normally acts as a brake on cell division. When CDK4/cyclin D phosphorylates Rb, it releases this brake, allowing the cell to enter S phase. Mutations that overproduce cyclins or destroy Rb are commonly found in cancer cells.

Fill in the blank: Cyclin-_____ complexes drive the cell cycle; cyclin D/CDK4,6 drives through G1; cyclin B/CDK1 (MPF) triggers entry into mitosis; the retinoblastoma protein Rb normally blocks S phase entry until phosphorylated and inactivated by a CDK-cyclin complex.

4.1.3 Cell cycle checkpoints and cancer

To prevent errors from being passed to daughter cells, the cell cycle has built-in quality control pauses called checkpoints. There are three main checkpoints. The G1 checkpoint (the restriction point or Start) is the most important decision point: the cell assesses whether conditions are favourable for division, whether its DNA is undamaged, and whether it has grown sufficiently; if conditions are not met, the cell pauses in G1 or exits into G0. The G2 checkpoint checks that DNA replication during S phase was completed accurately and that any damage has been repaired before the cell enters mitosis. The spindle assembly checkpoint (SAC), which operates



during mitosis itself, ensures that all chromosomes are properly attached to spindle fibres before the signal is given to separate them, preventing chromosomes from being distributed incorrectly.

The tumour suppressor protein p53 plays a central role at checkpoints. When DNA damage is detected, p53 levels rise and it activates a CDK inhibitor called p21, blocking cell cycle progression so the damage can be repaired; if the damage is irreparable, p53 triggers apoptosis, eliminating the damaged cell. Loss of p53 function is the most common single mutation found in human cancers. Cancer can be understood as a disease of cell cycle dysregulation: mutations that activate accelerator genes (proto-oncogenes) or disable brake genes (tumour suppressors) allow cells to divide without restraint, ignore checkpoints, resist apoptosis, and eventually invade other tissues.

Fill in the blank: The three main cell cycle checkpoints are the G1 checkpoint (readiness for division), the G2 checkpoint (replication complete), and the _____ assembly checkpoint (chromosomes attached to spindle); p53 activates the CDK inhibitor p21 to pause the cycle at checkpoints, and triggers apoptosis if damage is irreparable.

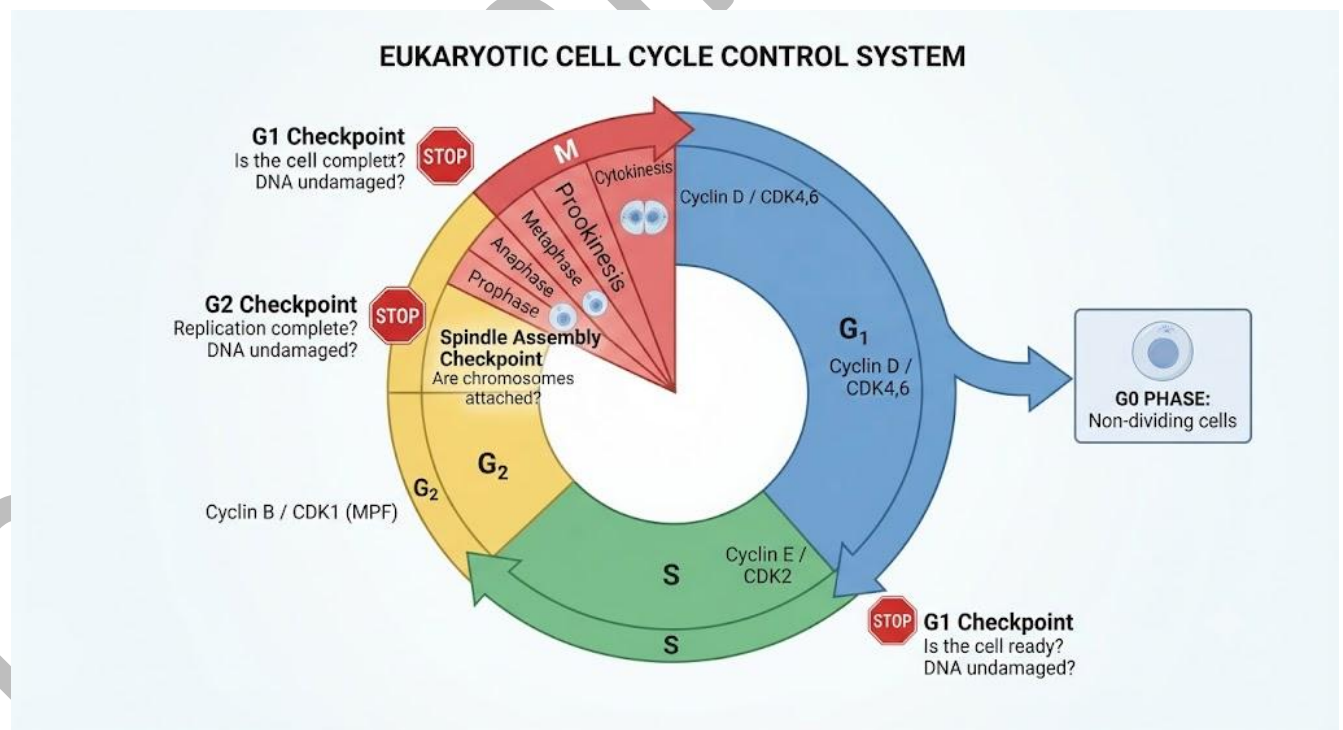


Figure 4.1: The eukaryotic cell cycle showing the four phases (G₁, S, G₂, M), the G₀ resting



state, the three checkpoints (G1, G2, and spindle assembly), and the cyclin-CDK pairs that drive each transition

Pilot questions 4.1

1. Name the four phases of the cell cycle and state what happens during each.
2. Explain what cyclins and CDKs are and why they must work together.
3. What is the G1 checkpoint and why is it the most important decision point in the cell cycle?
4. Explain the role of p53 in protecting cells from becoming cancerous.
5. What is the spindle assembly checkpoint and what happens if it fails?

4.2 Mitosis

4.2.1 Preparation for mitosis

Before a cell can divide its chromosomes, it must make them compact enough to be moved and build the machinery to pull them apart. During S phase, the DNA of each chromosome is duplicated, producing two identical copies called sister chromatids that remain joined together at a region called the centromere by a protein complex called cohesin. As the cell enters mitosis, these sister chromatids condense from their extended interphase form into compact, rod-like structures visible under a light microscope. This condensation is driven by proteins called condensins, which organise the chromatin into tight loops.

Simultaneously, the cell builds the mitotic spindle, the structure that will physically separate the chromosomes. The spindle is assembled from microtubules that grow from two structures called centrosomes (in animal cells), which move to opposite poles of the cell. Kinetochore microtubules attach to a protein complex on the centromere of each chromosome called the kinetochore; polar microtubules from both poles overlap in the middle; and astral microtubules anchor the spindle to the cell cortex. Every chromosome must be properly captured before division proceeds, which is enforced by the spindle assembly checkpoint.



Fill in the blank: During S phase, DNA replication produces two identical sister _____, joined at the centromere by cohesin; condensins drive chromosome condensation as the cell enters mitosis; kinetochore microtubules from opposite poles attach to the kinetochore protein complex on each chromosome.

4.2.2 The stages of mitosis

Mitosis is divided into five stages defined by the behaviour of the chromosomes. In prophase, chromosomes condense and become visible, the nucleolus disappears, and the spindle begins to form. In prometaphase, the nuclear envelope breaks down and spindle microtubules from each pole capture the chromosomes through their kinetochores. In metaphase, all the chromosomes have been captured and align along the equator of the cell, called the metaphase plate, with one kinetochore of each chromosome pair facing one pole and the other facing the opposite pole. This precise alignment is essential to ensure each daughter cell will receive exactly one copy of every chromosome.

Anaphase begins when the anaphase-promoting complex (APC/C) triggers the destruction of a protein called securin, releasing the enzyme separase, which cuts the cohesin holding the sister chromatids together. The sister chromatids are now free and are pulled to opposite poles by shortening of the kinetochore microtubules, while polar microtubules push the poles further apart, elongating the cell. In telophase, the chromosomes arrive at the poles, begin to decondense, the nuclear envelope reforms around each set, and the nucleolus reappears. The cell now contains two nuclei, each with an identical genome.

Fill in the blank: In _____ phase, chromosomes are aligned at the metaphase plate with one kinetochore facing each pole; anaphase begins when APC/C destroys securin, releasing separase, which cleaves cohesin; sister chromatids are pulled to opposite poles; in telophase the nuclear envelope reforms around each set of chromosomes.



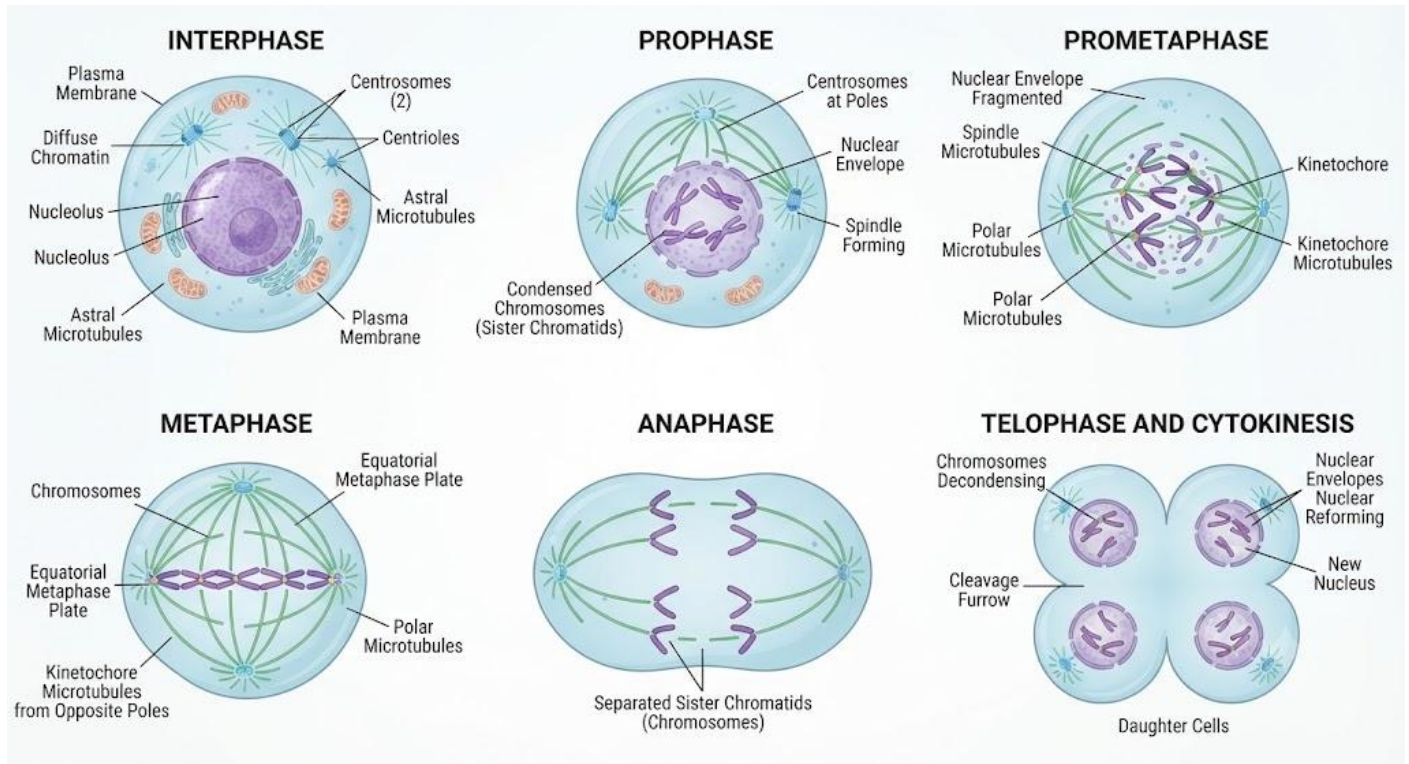


Figure 4.2: The stages of mitosis in an animal cell: interphase; prophase (condensation; spindle formation); prometaphase (nuclear envelope breakdown; kinetochore attachment); metaphase (alignment at metaphase plate); anaphase (sister chromatid separation); telophase and cytokinesis (nuclear envelope reformation; cleavage furrow)

Pilot questions 4.2

1. What are sister chromatids and how are they held together before anaphase?
2. Describe what happens during prophase and prometaphase of mitosis.
3. What is the metaphase plate and why is chromosome alignment there important?
4. Explain what triggers sister chromatid separation at the start of anaphase.
5. Describe how cytokinesis differs between animal cells and plant cells.

4.2.3 Cytokinesis in animal and plant cells

Cytokinesis is the physical division of the cytoplasm to produce two daughter cells. In animal cells, cytokinesis occurs by cleavage. A ring of actin filaments and myosin motor proteins assembles just inside the plasma membrane at the equator of the cell. This contractile ring gradually tightens, like a purse string pulling closed, pinching the plasma membrane inward to



form a cleavage furrow that deepens until the two daughter cells are completely separated. The position of the ring is determined by signals from the overlapping polar microtubules in the middle of the cell.

In plant cells, cytokinesis cannot occur by cleavage because the rigid cell wall prevents the membrane from being pinched inward. Instead, plant cells build a new cell wall between the two daughter cells from the inside outward. A structure called the phragmoplast forms at the site of the former metaphase plate; it consists of microtubules that guide Golgi-derived vesicles containing cell wall materials to the middle of the cell. These vesicles fuse to form a flat disc called the cell plate, which grows outward from the centre to the edges until it fuses with the existing cell wall. The cell plate eventually becomes the new primary cell wall between the two daughter cells.

Fill in the blank: In animal cells, cytokinesis occurs by contraction of an actin-myosin _____ ring that forms the cleavage furrow; in plant cells, cytokinesis occurs by Golgi vesicles fusing to form a cell plate guided by the phragmoplast, which grows outward from the centre to become the new cell wall.

4.3 Meiosis

4.3.1 Overview and significance of meiosis

Meiosis is the specialised type of cell division that produces the reproductive cells (gametes) in sexually reproducing organisms. In animals, it occurs in the gonads to produce sperm and eggs; in plants and fungi, it produces spores. Meiosis differs from mitosis in two fundamental ways: it involves two rounds of cell division (meiosis I and meiosis II), and it reduces the chromosome number by half. Human body cells contain 46 chromosomes (diploid; $2n = 46$), arranged in 23 pairs of homologous chromosomes. The gametes produced by meiosis contain only 23 chromosomes (haploid; $n = 23$), one from each homologous pair. When two gametes fuse at fertilisation, the diploid number is restored in the resulting zygote.

The biological significance of meiosis goes beyond reducing chromosome number. Meiosis is also the engine of genetic diversity. Two mechanisms generate this diversity: crossing over



(recombination), in which homologous chromosomes exchange segments of DNA during meiosis I, producing chromosomes that carry new combinations of alleles; and independent assortment, in which each gamete receives a random mixture of maternal and paternal chromosomes. In humans, independent assortment alone can theoretically produce over 8 million genetically different gametes from a single individual, and crossing over increases this diversity enormously further. This is why every individual in a sexually reproducing species is genetically unique.

Fill in the blank: Meiosis reduces the chromosome number from _____ ($2n$) to haploid (n), producing four genetically unique daughter cells; genetic diversity comes from crossing over (recombination between homologous chromosomes) and independent assortment (random distribution of maternal and paternal chromosomes to gametes).

4.3.2 Meiosis I: the reductive division

Meiosis I is called the reductive division because it is the step that halves the chromosome number by separating the homologous chromosomes. Before meiosis begins, DNA is replicated during S phase, so each chromosome enters meiosis as two sister chromatids. Prophase I is the longest and most distinctive phase of meiosis: homologous chromosomes come together and pair up along their entire length in a process called synapsis, forming structures called bivalents (each containing four chromatids – two from each homologous chromosome). While paired, non-sister chromatids of the two homologous chromosomes physically exchange segments of DNA in crossing over (recombination), facilitated by the synaptonemal complex. The points of exchange are visible as X-shaped structures called chiasmata.

After prophase I, the stages of meiosis I follow a broadly similar pattern to mitosis but with an important difference. In metaphase I, the bivalents align along the metaphase plate, but both kinetochores of each chromosome face the same pole – it is the two homologous chromosomes that face opposite poles, not the sister chromatids. In anaphase I, the homologous chromosomes are pulled apart to opposite poles, while the sister chromatids remain joined. This is the step that halves the chromosome number. In telophase I, the cell divides by cytokinesis to produce two haploid cells, each with chromosomes whose allele composition has been shuffled by crossing over and independent assortment.



Fill in the blank: In prophase I, homologous chromosomes undergo _____ (pairing along their entire length) and crossing over, generating new allele combinations visible as chiasmata; in anaphase I, homologous chromosomes (not sister chromatids) are separated, halving the chromosome number from diploid to haploid.

4.3.3 Meiosis II and the products of meiosis

Meiosis II follows meiosis I with little or no interphase and, crucially, no second round of DNA replication. Meiosis II closely resembles a mitotic division. In prophase II, the spindle reforms in each of the two cells. In metaphase II, the individual chromosomes (each still consisting of two sister chromatids) align along the metaphase plate. In anaphase II, cohesin is cleaved and the sister chromatids are pulled to opposite poles. In telophase II, nuclear envelopes reform and cytokinesis produces a total of four haploid cells from the original diploid parent. All four cells are genetically unique because of crossing over and independent assortment in meiosis I.

The four cells produced differ in animals between the sexes. In males (spermatogenesis), all four cells develop into functional sperm cells of equal size. In females (oogenesis), the meiotic divisions are deliberately unequal in terms of cytoplasm distribution: most of the cytoplasm goes to one large cell (the oocyte) that will become the egg, while the other three cells produced are tiny, non-functional polar bodies that are discarded. This unequal division ensures that the egg has sufficient resources – nutrients, organelles, and regulatory molecules – to support the embryo in its earliest stages of development after fertilisation.

Fill in the blank: Meiosis II separates _____ chromatids (as in mitosis) without any preceding DNA replication; four haploid cells are produced, all genetically unique; in females, only one of the four cells becomes a functional egg, while the other three become non-functional polar bodies.



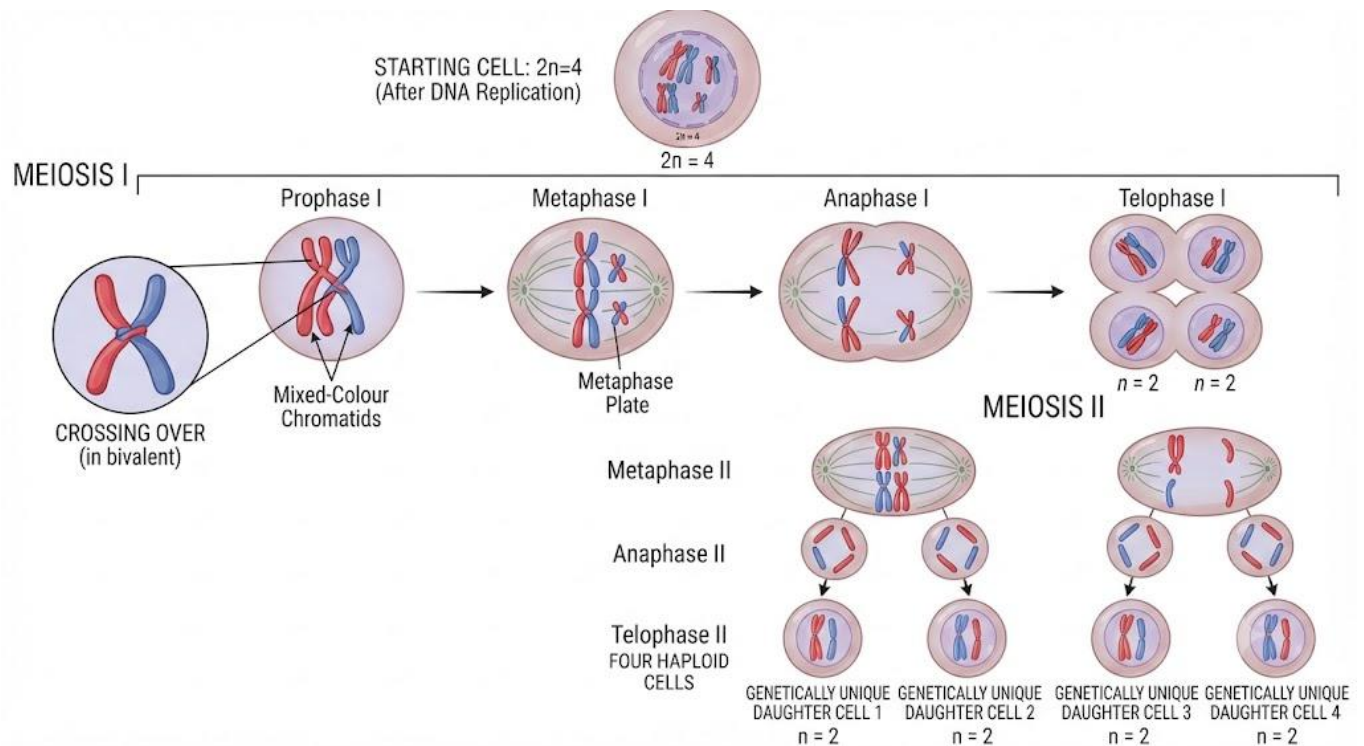


Figure 4.3: Overview of meiosis I (reductive division: homologous chromosomes pair up, undergo crossing over, and separate, halving the chromosome number) and meiosis II (sister chromatids separate, producing four genetically unique haploid cells)

Pilot questions 4.3

1. Explain the biological significance of meiosis in terms of chromosome number and genetic diversity.
2. What is synapsis and when does it occur?
3. Explain crossing over: when does it happen, what does it produce, and why is it significant?
4. What is the key difference between what is separated in anaphase I and in anaphase II?
5. Why does oogenesis in females produce only one functional egg from each meiotic division?

4.4 Comparison of Mitosis and Meiosis

4.4.1 Key similarities



Although mitosis and meiosis serve completely different purposes, they share several fundamental features because they evolved from the same ancestral division process. Both are preceded by DNA replication during S phase, so each chromosome enters division as two joined sister chromatids. Both use a spindle apparatus of microtubules to move chromosomes, attaching to chromosomes at the kinetochore; both involve chromosome condensation, nuclear envelope breakdown, and nuclear envelope reformation in daughter cells. The anaphase-promoting complex (APC/C) triggers chromosome separation in both by destroying securin and activating separase to cleave cohesin. The spindle assembly checkpoint operates in both to ensure chromosomes are properly attached before separation proceeds.

The molecular machinery of the two processes is also largely shared: the same cyclin-CDK complexes regulate entry into both mitosis and meiosis; the same motor proteins drive spindle function; and cytokinesis uses the same mechanisms in both cases – an actin-myosin contractile ring in animal cells and a phragmoplast-guided cell plate in plant cells. These shared features reflect the deep evolutionary relationship between the two types of division; meiosis likely evolved from mitosis by the addition of the pairing and recombination steps of meiosis I and the suppression of DNA replication between the two divisions.

Fill in the blank: Both mitosis and meiosis are preceded by DNA _____ during S phase; both use a microtubule spindle attached to chromosomes at the kinetochore; the APC/C triggers chromosome separation in both; and cytokinesis uses the same mechanisms (contractile ring in animals; cell plate in plants).

4.4.2 Key differences

Despite their similarities, mitosis and meiosis differ fundamentally in purpose, structure, and outcome. Purpose: mitosis produces cells for growth and tissue repair; meiosis produces gametes for sexual reproduction. Number of divisions: mitosis involves one division producing two daughter cells; meiosis involves two divisions producing four daughter cells. Chromosome number: mitosis preserves the diploid chromosome number ($2n \rightarrow 2n$); meiosis halves it ($2n \rightarrow n$). Genetic outcome: mitotic daughters are genetically identical to the parent and to each other; meiotic daughters are all genetically unique because of crossing over and independent assortment.



Another critical difference is in the behaviour of homologous chromosomes. In mitosis, homologous chromosomes never pair up and there is no crossing over or synapsis. In meiosis I, homologous chromosomes pair up, undergo crossing over, and are then separated from each other – this is what reduces the chromosome number and does not happen in mitosis at all. Prophase I of meiosis is also dramatically longer and more complex than prophase of mitosis. A simple rule to remember the key distinction: in mitosis, sister chromatids separate; in meiosis I, homologous chromosomes separate; in meiosis II, sister chromatids separate (like mitosis).

Fill in the blank: Mitosis produces two genetically _____ daughter cells (diploid; $2n$); meiosis produces four genetically unique haploid (n) cells; homologous chromosomes pair and undergo crossing over only in meiosis I; in mitosis, sister chromatids separate, while in meiosis I it is the homologous chromosomes that are separated.

<u>Mitosis</u>	<u>Meiosis</u>
Purpose: Growth, repair, asexual reproduction	Formation of gametes (sexual reproduction)
Where it Occurs: Somatic (body) cells	Germ cells in reproductive organs
Number of Divisions: One	Two (Meiosis I and II)
Number of Daughter Cells: Two	Four
Ploidy of Daughter Cells: Diploid ($2n$), same as parent	Haploid (n), half of parent
Genetic Outcome: Genetically identical cells	Genetically unique cells
Synapsis of Homologous Chromosomes: No	Yes (during Prophase I)
Crossing Over: No	Yes (exchange of genetic material in Prophase I)
Independent Assortment: No	Yes (random distribution of homologues in Metaphase I)
What is Separated in Anaphase: Sister chromatids	Homologous chromosomes (Anaphase I), then sister chromatids (Anaphase II)

Box 4.1: Comparison of mitosis and meiosis: purpose, location, number of divisions, chromosome number, genetic outcome, and key events

4.4.3 Errors in cell division and their consequences



Cell division is remarkably accurate, but errors occasionally occur, and their consequences can be serious. The most common error is non-disjunction, which occurs when chromosomes or sister chromatids fail to separate properly during anaphase. If homologous chromosomes fail to separate in anaphase I of meiosis, one daughter cell receives both copies of that chromosome and the other receives none. The resulting gametes have an abnormal chromosome number, and when fertilised by a normal gamete, the offspring has either too many (trisomy) or too few (monosomy) chromosomes – a condition called aneuploidy. The most familiar example of aneuploidy in humans is Down syndrome (trisomy 21), which results from having three copies of chromosome 21; its frequency increases with maternal age.

Other examples of chromosomal disorders caused by non-disjunction include Turner syndrome (a female with only one X chromosome; 45,X; short stature; infertility) and Klinefelter syndrome (a male with an extra X chromosome; 47,XXY; typically tall; reduced fertility). Polyploidy – having more than two complete sets of chromosomes – is usually lethal in animals but is remarkably common and often beneficial in plants; many important crop plants including wheat, cotton, and oats are polyploid, partly because polyploidy can confer larger size, increased vigour, and higher yield. Errors in mitosis are usually corrected or lead to apoptosis, but in some cases, they contribute to the chromosomal instability seen in cancer cells.

Fill in the blank: Non-disjunction during meiosis produces gametes with an abnormal chromosome number; when fertilised, the offspring is _____ (abnormal chromosome number); Down syndrome (trisomy 21) results from non-disjunction producing a gamete with two copies of chromosome 21; polyploidy (more than two chromosome sets) is usually lethal in animals but common and beneficial in plants.



Series Summary

This Series explored the **processes of mitosis and meiosis**, explaining how eukaryotic cells divide for growth, repair, and reproduction. You examined the cell cycle, the roles of cyclin-CDK complexes and cell cycle checkpoints, and the function of p53 in maintaining genomic integrity. The Series also covered the stages of mitosis and cytokinesis, showing how genetically identical daughter cells are produced to support tissue growth and maintenance.

The Series further examined meiosis as the specialised cell division responsible for producing genetically diverse haploid gametes. You learned how synapsis, crossing over, and independent assortment generate genetic variation, compared the features and outcomes of mitosis and meiosis, and explored how errors such as non-disjunction can lead to chromosomal disorders. By completing this Series, you should now be able to explain the mechanisms and regulation of cell division, distinguish between mitosis and meiosis, describe the sources of genetic variation, and relate abnormalities in chromosome segregation to human genetic disorders, thereby achieving all four **Series Learning Outcomes (SLOs)** and the **overall course outcomes**.

Glossary of Terms

- **Anaphase:** the stage of mitosis and meiosis II when sister chromatids are pulled to opposite poles after cohesin is cleaved; in anaphase I of meiosis, it is the homologous chromosomes that separate.
- **Aneuploidy:** an abnormal chromosome number in a cell resulting from non-disjunction; examples: trisomy 21 (Down syndrome; three copies of chromosome 21); Turner syndrome (45,X; one X chromosome); Klinefelter syndrome (47,XXY).
- **Bivalent (tetrad):** the paired structure formed when two homologous chromosomes (each of two sister chromatids) come together during prophase I of meiosis; contains four chromatids in total; the site of crossing over.
- **Cell cycle:** the ordered sequence of events a cell undergoes from formation to division; consists of interphase (G1, S, G2) and the mitotic phase (M); regulated by cyclin-CDK complexes and three checkpoints.



- **Centromere:** the constricted region of a chromosome where sister chromatids are joined by cohesin and where the kinetochore assembles for spindle attachment.
- **Checkpoint:** a regulatory pause in the cell cycle that prevents progression if conditions are not met or if errors are present; the G1 checkpoint assesses readiness; G2 checks replication completion; the spindle assembly checkpoint ensures all chromosomes are attached to the spindle.
- **Chiasma (pl. chiasmata):** the X-shaped structure visible under the microscope at the site where crossing over has occurred between non-sister chromatids of homologous chromosomes during prophase I of meiosis.
- **Crossing over (recombination):** the physical exchange of corresponding segments of DNA between non-sister chromatids of homologous chromosomes during prophase I of meiosis; produces chromosomes with new combinations of alleles.
- **Cyclin-dependent kinase (CDK):** an enzyme that drives cell cycle transitions by phosphorylating target proteins; only active when bound to its specific cyclin; different cyclin-CDK pairs control different transitions (G1, S phase entry, M phase entry).
- **Cytokinesis:** the physical division of the cytoplasm following nuclear division; in animal cells: actin-myosin contractile ring forms a cleavage furrow; in plant cells: Golgi vesicles fuse to form a cell plate guided by the phragmoplast.
- **Diploid (2n):** having two copies of each chromosome; the normal state of most somatic cells; restored at fertilisation when two haploid gametes fuse.
- **Haploid (n):** having one copy of each chromosome; the state of gametes produced by meiosis; contains half the chromosome number of a diploid cell.
- **Independent assortment:** the random distribution of maternal and paternal homologous chromosomes to daughter cells during metaphase I of meiosis; each chromosome pair orients independently of the others, generating enormous gametic diversity.
- **Kinetochore:** the protein complex that assembles on the centromere of each chromosome and provides the attachment point for spindle microtubules; sends signals to the spindle assembly checkpoint if not properly attached.
- **Maturation Promoting Factor (MPF):** the complex of cyclin B and CDK1 that drives a cell from G2 into mitosis by triggering nuclear envelope breakdown, chromosome condensation, and spindle assembly.



- **Metaphase plate:** the imaginary equatorial plane along which chromosomes are aligned during metaphase; in mitosis and meiosis II, individual chromosomes align; in metaphase I, bivalents (paired homologous chromosomes) align.
- **Mitosis:** nuclear division producing two genetically identical daughter nuclei with the same chromosome number as the parent; used for growth, repair, and asexual reproduction; stages: prophase, prometaphase, metaphase, anaphase, telophase.
- **Non-disjunction:** failure of chromosomes or sister chromatids to separate properly during anaphase; produces gametes or cells with an abnormal chromosome number; can cause aneuploid offspring such as those with Down syndrome.
- **Sister chromatids:** the two identical copies of a chromosome produced by DNA replication; joined at the centromere by cohesin; separated during anaphase of mitosis and anaphase II of meiosis.
- **Spindle assembly checkpoint (SAC):** the checkpoint that halts mitosis and meiosis until all chromosomes are properly attached to spindle microtubules; prevents chromosome mis-segregation.
- **Synapsis:** the precise pairing of homologous chromosomes along their entire length during prophase I of meiosis; facilitated by the synaptonemal complex; a prerequisite for crossing over.
- **Tumour suppressor gene:** a gene whose protein product restrains cell division or promotes apoptosis in damaged cells; loss of function allows uncontrolled division; p53 (TP53) is the most commonly mutated tumour suppressor in human cancers.



Concept Check Questions [Series 4]

Objective Questions

1. The cell cycle consists of interphase (G1, S phase, G2) and the _____ phase; DNA replication occurs during S phase; cells that permanently stop dividing exit into the resting state G0. (Linked to SLO 4.1)
2. During prophase I of meiosis, homologous chromosomes undergo _____ (pairing along their entire length) and then exchange DNA segments by crossing over, generating new combinations of alleles visible as chiasmata. (Linked to SLO 4.3)
3. In mitosis, _____ chromatids are separated during anaphase; in meiosis I, it is homologous chromosomes that are separated; in meiosis II, sister chromatids are separated just as in mitosis. (Linked to SLOs 4.2, 4.4)
4. Mitosis produces two genetically _____ daughter cells (diploid; $2n$), while meiosis produces four genetically unique daughter cells (haploid; n). (Linked to SLO 4.4)
5. Non-disjunction during meiosis produces gametes with an abnormal chromosome number; when fertilised, the offspring is _____ (has an abnormal chromosome number); Down syndrome results from trisomy 21. (Linked to SLO 4.4)

Short-Answer Questions

1. Explain the roles of cyclin D/CDK4,6 and cyclin B/CDK1 in controlling cell cycle progression. (Linked to SLO 4.1)
2. Describe what happens to chromosomes during prophase I of meiosis that does not occur in prophase of mitosis. (Linked to SLO 4.3)
3. Explain why the spindle assembly checkpoint is important and what happens if it fails. (Linked to SLO 4.1)
4. State four key differences between mitosis and meiosis. (Linked to SLO 4.4)
5. What is non-disjunction and what are its consequences? Give one example. (Linked to SLO 4.4)

Long-Answer Questions

1. Describe the eukaryotic cell cycle (phases, cyclin-CDK regulation, and checkpoints), the stages of mitosis from prophase to telophase, and how cytokinesis differs between animal and plant cells. (Linked to SLOs 4.1, 4.2)
2. Describe the stages of meiosis I and meiosis II, explain how genetic diversity is generated, and compare and contrast mitosis and meiosis in terms of purpose, divisions, chromosome number, genetic outcome, and the unique events of meiosis I. (Linked to SLOs 4.3, 4.4)



Answers to Concept Check Questions [Series 4]

Objective Questions

1. Mitotic.
2. Synapsis.
3. Sister.
4. Identical.
5. Aneuploid.

Short-Answer Questions

- Cyclin D/CDK4,6 is active in G1; it phosphorylates and inactivates the retinoblastoma protein (Rb), releasing the brake on S phase entry and committing the cell to divide. Cyclin B/CDK1 (MPF) is active at G2/M; it triggers mitosis by causing nuclear envelope breakdown and chromosome condensation.
- In prophase I of meiosis: homologous chromosomes pair along their entire length (synapsis), forming bivalents; non-sister chromatids exchange DNA segments by crossing over, producing new allele combinations visible as chiasmata. Neither synapsis nor crossing over occurs in prophase of mitosis.
- The spindle assembly checkpoint ensures all chromosomes are properly attached to spindle microtubules before anaphase begins, preventing any chromosome from being left unattached and distributed to the wrong cell. If it fails, chromosomes may be pulled to the wrong pole (non-disjunction), producing aneuploid daughter cells; in cancer cells, a faulty checkpoint contributes to chromosomal instability.
 - Purpose: mitosis = growth and repair; meiosis = gamete production. (2) Divisions: one (mitosis) vs two (meiosis). (3) Daughter cells: two diploid identical cells (mitosis) vs four haploid unique cells (meiosis). (4) Crossing over and synapsis: absent in mitosis; present in prophase I of meiosis.
- Non-disjunction is the failure of chromosomes or sister chromatids to separate during anaphase, producing gametes with too many or too few chromosomes. Example: Down syndrome (trisomy 21) – non-disjunction of chromosome 21 produces a gamete with two copies; after fertilisation, the offspring has three copies of chromosome 21 and 47 chromosomes total.

1. Long-Answer Questions



2. Cell cycle: G1 (growth; protein synthesis) → S (DNA replication; sister chromatids formed) → G2 (preparation; damage repair) → M (mitosis + cytokinesis); cyclin-CDK regulation: cyclin D/CDK4,6 (G1 progression; Rb phosphorylation); cyclin E/CDK2 (S phase entry); cyclin B/CDK1/MPF (G2/M entry; nuclear envelope breakdown); three checkpoints: G1 (readiness; p53 activates p21), G2 (replication complete), SAC (spindle attachment); cancer = checkpoint failure + oncogene activation + tumour suppressor loss. Mitosis: prophase (condensation; spindle forms; nucleolus disappears); prometaphase (nuclear envelope breaks down; kinetochore microtubule capture); metaphase (chromosomes at metaphase plate; one kinetochore per pole); anaphase (APC/C destroys securin; separase cleaves cohesin; sister chromatids to poles); telophase (nuclear envelope reforms; chromosomes decondense; nucleolus returns). Cytokinesis: animal (actin-myosin contractile ring; cleavage furrow); plant (phragmoplast guides Golgi vesicles; cell plate grows outward to become new cell wall).
3. Meiosis I (reductive division): prophase I (synapsis; crossing over; chiasmata; bivalents formed; long and complex); metaphase I (bivalents at metaphase plate; independent assortment of maternal/paternal chromosomes); anaphase I (homologous chromosomes separated; chromosome number halved); telophase I + cytokinesis (two haploid cells, genetically unique). Meiosis II (no DNA replication): sister chromatids separated (as in mitosis); four haploid genetically unique cells produced. Genetic diversity: crossing over (new allele combinations on chromosomes); independent assortment (random maternal/paternal distribution; over 8 million combinations from 23 chromosome pairs in humans). Comparison with mitosis: purpose (repair vs reproduction); divisions (1 vs 2); daughter cells (2 diploid identical vs 4 haploid unique); synapsis (absent vs prophase I); crossing over (absent vs prophase I); what separates in anaphase (sister chromatids in mitosis and meiosis II; homologous chromosomes in meiosis I only); errors: non-disjunction → aneuploidy (Down syndrome trisomy 21; Turner syndrome 45,X; Klinefelter syndrome 47,XXY).

Answers to Pilot Questions [Series 4]

Part 4.1

1. G1: cell grows and produces proteins. S: DNA is replicated; each chromosome becomes two joined sister chromatids. G2: cell continues to grow and checks replication was completed



without errors. M: mitosis (chromosome separation) followed by cytokinesis (cytoplasm division) producing two daughter cells.

2. Cyclins are proteins produced and destroyed at specific times in the cell cycle; they bind to and activate CDKs (kinase enzymes). Because CDKs are only active when bound to a cyclin, and because cyclins rise and fall at specific times, this partnership ensures each CDK is active only at the correct stage and for the correct duration.
3. The G1 checkpoint is where the cell assesses whether it has grown sufficiently, whether conditions are favourable, and whether its DNA is undamaged before committing to divide. Once the cell passes this point, it is committed to completing the cycle; if conditions are not met, the cell pauses in G1 or permanently exits into G0.
4. When DNA damage is detected, p53 levels rise and it activates the CDK inhibitor p21, halting the cell cycle to allow repair. If the damage is too severe, p53 triggers apoptosis, eliminating the cell. Loss of p53 removes this protection and is the most common single mutation in human cancers.
5. The spindle assembly checkpoint pauses cell division until every chromosome has both kinetochores properly attached to microtubules from opposite poles. If even one chromosome is unattached, the checkpoint sends an inhibitory signal to prevent anaphase; if it fails, chromosomes are distributed incorrectly, producing aneuploid daughter cells.

Part 4.2

1. Sister chromatids are the two identical DNA copies of a chromosome produced by replication during S phase. They are held together at the centromere by a protein complex called cohesin, which keeps them joined from their formation until anaphase, when the enzyme separase cleaves cohesin and allows the chromatids to be pulled apart.
2. In prophase, chromosomes condense into visible compact structures, the nucleolus disappears, and the mitotic spindle begins to form as centrosomes move to opposite poles. In prometaphase, the nuclear envelope breaks down and spindle microtubules capture the chromosomes by attaching to their kinetochores.
3. The metaphase plate is the imaginary equatorial plane at the centre of the cell where all chromosomes are aligned during metaphase. This alignment ensures one kinetochore of each chromosome faces one pole and the other faces the opposite pole, guaranteeing that when separation occurs each daughter cell receives exactly one complete set of chromosomes.
4. At the start of anaphase, the APC/C tags the protein securin for destruction; without securin, the enzyme separase is freed and cleaves the cohesin proteins holding sister chromatids



- together at the centromere; the separated chromatids are then pulled to opposite poles by shortening of kinetochore microtubules.
5. Animal cells: an actin-myosin contractile ring assembles at the cell equator and tightens to form a cleavage furrow that pinches the cell in two. Plant cells: a phragmoplast (microtubule array) guides Golgi-derived vesicles to the cell midplane; the vesicles fuse to form the cell plate, which grows outward from the centre to divide the cell and becomes the new primary cell wall.

Part 4.3

1. Meiosis reduces the chromosome number from diploid ($2n$) to haploid (n) so that fertilisation restores the diploid number without doubling it each generation. It also generates genetic diversity through crossing over (new allele combinations on chromosomes) and independent assortment (random distribution of maternal and paternal chromosomes to gametes).
2. Synapsis is the precise pairing of homologous chromosomes along their entire length during prophase I of meiosis, facilitated by the synaptonemal complex protein structure. It produces bivalents (four chromatids) and is a prerequisite for crossing over to occur.
3. Crossing over occurs during prophase I when homologous chromosomes are paired in bivalents; non-sister chromatids break at corresponding points and exchange segments of DNA. This produces chromosomes that carry new combinations of alleles not present in either parental chromosome, greatly increasing the genetic diversity of gametes.
4. In anaphase I, it is the homologous chromosomes that are separated to opposite poles, while the sister chromatids remain joined; this halves the chromosome number. In anaphase II, the sister chromatids of each chromosome are separated, just as in mitotic anaphase, producing the final haploid cells.
5. Meiotic cytokinesis is deliberately unequal in females: nearly all the cytoplasm is given to one cell (the oocyte) to provide the nutrients, organelles, and regulatory molecules needed for early embryonic development. The other three cells (polar bodies) receive minimal cytoplasm and are discarded; this ensures the egg is large enough to sustain the embryo.

Part 4.4

1. Both are preceded by DNA replication in S phase; both use a microtubule spindle attached to chromosomes at the kinetochore; both require APC/C to trigger chromosome separation; both end with cytokinesis using the same mechanisms (contractile ring in animals; cell plate in plants).



- (1) Purpose: mitosis = growth and repair; meiosis = gamete production. (2) Divisions: mitosis = one; meiosis = two. (3) Daughter cells: mitosis = two identical diploid; meiosis = four unique haploid. (4) Crossing over and synapsis: absent in mitosis; present in prophase I of meiosis, generating genetic diversity.
2. Animal cells use a centrosome (two centrioles plus pericentriolar material) as the main microtubule-organising centre for the spindle; plant cells lack centrioles and organise their spindle from diffuse sites around the nuclear envelope and in the cytoplasm. Despite this structural difference, the spindle performs the same function – capturing and separating chromosomes – in both cell types.
 3. Plasmodesmata (plant): cytoplasmic channels (30 to 60 nm) through cell walls containing a desmotubule of ER; allow direct symplastic communication. Gap junctions (animal): protein channels formed by connexin molecules; allow direct cytoplasmic communication. Both allow ions and small molecules to pass between adjacent cells; the key difference is their molecular composition and the presence of an ER tubule in plasmodesmata but not gap junctions.
 4. Non-disjunction is the failure of chromosomes or sister chromatids to separate in anaphase, producing gametes with an abnormal chromosome number. Down syndrome: non-disjunction of chromosome 21 in meiosis I produces a gamete with two copies; after fertilisation with a normal gamete, the offspring has trisomy 21 (47 chromosomes), causing intellectual disability, characteristic facial features, and increased risk of heart defects.



References and Attributions [Series 4]

Textual References (Books and External Sources)

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Figures, Plates, Tables, and Boxes

- Figure 4.1: Eukaryotic cell cycle with checkpoints and cyclin-CDK annotations. Attribution: AI generated. Suggested OER search string: “eukaryotic cell cycle diagram checkpoints cyclin CDK G1 S G2 M phase OER”.
- Figure 4.2: Stages of mitosis in an animal cell. Attribution: AI generated. Suggested OER search string: “stages of mitosis animal cell prophase metaphase anaphase telophase labelled diagram OER”.
- Figure 4.3: Meiosis I and II showing crossing over and independent assortment. Attribution: AI generated. Suggested OER search string: “meiosis stages diagram crossing over independent assortment haploid gametes OER”.
- Box 4.1: Comparison table of mitosis versus meiosis. Attribution: AI generated. Suggested OER search string: “mitosis meiosis comparison table differences similarities OER”.



Course code: BIO 205

Course title: Introductory Developmental / Cell Biology

Course credit load: 2 Units

Series 5: Cell Differentiation and Molecular Basis of Development

- **Part 5.1: Cell Differentiation and Gene Expression**

- Sub Part 5.1.1: What is cell differentiation?
- Sub Part 5.1.2: Transcription factors and cell identity
- Sub Part 5.1.3: Epigenetic control of differentiation

- **Part 5.2: Stem Cells**

- Sub Part 5.2.1: Types of stem cells and their potency
- Sub Part 5.2.2: Embryonic and induced pluripotent stem cells
- Sub Part 5.2.3: Adult stem cells and tissue maintenance

- **Part 5.3: Molecular Signals in Development**

- Sub Part 5.3.1: Induction and cell signalling
- Sub Part 5.3.2: Morphogens and body axis patterning
- Sub Part 5.3.3: Master regulator transcription factors

- **Part 5.4: Cell Death, Cancer, and Regeneration**

- Sub Part 5.4.1: Apoptosis
- Sub Part 5.4.2: Cancer as failed development
- Sub Part 5.4.3: Regeneration and tissue engineering



Introduction

A remarkable fact about multicellular life is that virtually every cell in the human body contains exactly the same DNA, yet cells look and behave in completely different ways. A muscle cell contracts, a neuron transmits electrical signals, a red blood cell carries oxygen, and a liver cell processes nutrients – and all of these differences arise not from different genes but from different patterns of gene activity. The process by which a cell progressively acquires a specialised identity is called cell differentiation, and it is the cellular foundation of all development.

This Series explores how cells become specialised and how that specialisation is maintained and directed. It covers the role of transcription factors and epigenetic mechanisms in controlling gene expression, the nature and medical importance of stem cells, the molecular signals that guide cells along particular developmental pathways, and the roles of programmed cell death (apoptosis) in normal development. It concludes by examining cancer as a breakdown of normal developmental controls and the exciting field of regenerative medicine.

Series Learning Outcomes

Upon completing this Series, you should be able to:

- **SLO 5.1** explain what cell differentiation is, describe how transcription factors control cell identity, and explain how epigenetic mechanisms maintain differentiated states (Linked to Part 5.1),
- **SLO 5.2** describe the different types of stem cells and their potency, distinguish embryonic from induced pluripotent stem cells, and explain how adult stem cells maintain tissues (Linked to Part 5.2),
- **SLO 5.3** explain induction and morphogen gradients in development and describe how master regulator transcription factors direct cell fate (Linked to Part 5.3), and



- **SLO 5.4** describe apoptosis and its role in development, explain how cancer represents a failure of developmental controls, and describe approaches to regeneration and tissue engineering (Linked to Part 5.4).

5.1 Cell Differentiation and Gene Expression

5.1.1 What is cell differentiation?

Cell differentiation is the process by which a less specialised cell progressively acquires a specific identity, with a distinct structure, gene expression pattern, and function. The adult human body contains about 200 different cell types, all descended from the single fertilised egg, yet all carrying exactly the same DNA. Differentiation is therefore not about changing the genetic information but about which genes in that shared DNA are switched on and which are permanently silenced in each cell type. Once a cell has committed to a particular fate – say, becoming a liver cell – it normally remains in that state for life and passes the same identity on to its daughter cells.

Differentiation is important not only for normal development but also for our understanding of disease. Leukaemia, for example, arises when white blood cell precursors fail to differentiate normally and instead continue to proliferate in an immature state. Conversely, cells that have already differentiated can sometimes be experimentally reprogrammed back toward a less specialised state, most famously in the production of induced pluripotent stem cells (iPSCs), which are discussed in Part 5.2. Understanding the molecular mechanisms of differentiation is therefore both a fundamental scientific question and a practical goal for medicine.

Fill in the blank: Cell differentiation is the process by which a cell acquires a specialised _____ with a distinct structure and function; it results from differences in which genes are expressed, not differences in DNA sequence; once a cell has differentiated, it normally maintains that identity and passes it on to its daughter cells.



5.1.2 Transcription factors and cell identity

Transcription factors are proteins that bind to specific DNA sequences near genes and either switch those genes on (activate) or off (repress). Since all cells have the same genes but different transcription factors are active in different cell types, it is the unique combination of transcription factors present in a cell that determines its gene expression profile and therefore its identity.

Think of transcription factors as a combination lock: it is not any single factor but the specific combination that opens the right set of genes for a particular cell type. Most cell types are defined by networks of transcription factors that activate each other's expression and together maintain the cell's identity through positive feedback loops.

Some transcription factors are so powerful that switching a single one on in the wrong cell is enough to convert that cell toward a completely different fate. These are called master regulator transcription factors. The transcription factor MyoD, for example, when expressed in a fibroblast (a connective tissue cell), can convert it into a cell with many characteristics of a muscle cell by activating a whole cascade of muscle-specific genes. Similarly, the transcription factor Pax6 is the master regulator of eye development: when ectopically expressed in the developing leg of a fruit fly, Pax6 can induce the formation of a complete extra eye on the leg. These examples illustrate how a single molecular switch can redirect the entire developmental trajectory of a cell.

Fill in the blank: Transcription factors bind to DNA near genes and activate or repress their _____; the unique combination of transcription factors in each cell type determines its identity; master regulator transcription factors (e.g. MyoD for muscle; Pax6 for eyes) can alone redirect cell fate when expressed in the wrong cell.

5.1.3 Epigenetic control of differentiation

Once a cell has differentiated, it must maintain its identity reliably through many rounds of cell division – a liver cell must produce only liver cells when it divides, not muscle cells or neurons. This is achieved through epigenetic mechanisms: heritable changes in gene activity that do not alter the DNA sequence itself but that are copied to daughter cells when the cell divides. The two main epigenetic mechanisms are histone modification and DNA methylation. Histones are the proteins that DNA is wound around; adding acetyl groups to histones (acetylation) loosens the



chromatin and makes genes more accessible for transcription, while adding methyl groups (methylation) compacts chromatin and silences genes. DNA methylation involves adding methyl groups directly to cytosine bases in the DNA (at CpG sites), which is generally associated with gene silencing.

The key feature of epigenetic marks is that they are faithfully copied to daughter cells during DNA replication, so the gene expression state of a differentiated cell is inherited by all its descendants. This explains how a liver cell, after thousands of rounds of division during a person's lifetime, continues to express liver-specific genes and not muscle or nerve genes: the genes inappropriate for liver function are locked in an epigenetically silenced state that is never reset. Conversely, in cancer cells, these epigenetic patterns are often disrupted: tumour suppressor genes may be silenced by abnormal hypermethylation, while genes that should be permanently off are reactivated.

Fill in the blank: Two main epigenetic mechanisms maintain cell identity: histone _____ (loosens chromatin; activates genes) and histone methylation and DNA methylation (compact chromatin; silence genes); these marks are copied to daughter cells at each division, allowing a differentiated cell to pass its identity reliably to all its descendants.



CELLULAR DIFFERENTIATION AND EPIGENETIC CONTROL OF GENE EXPRESSION

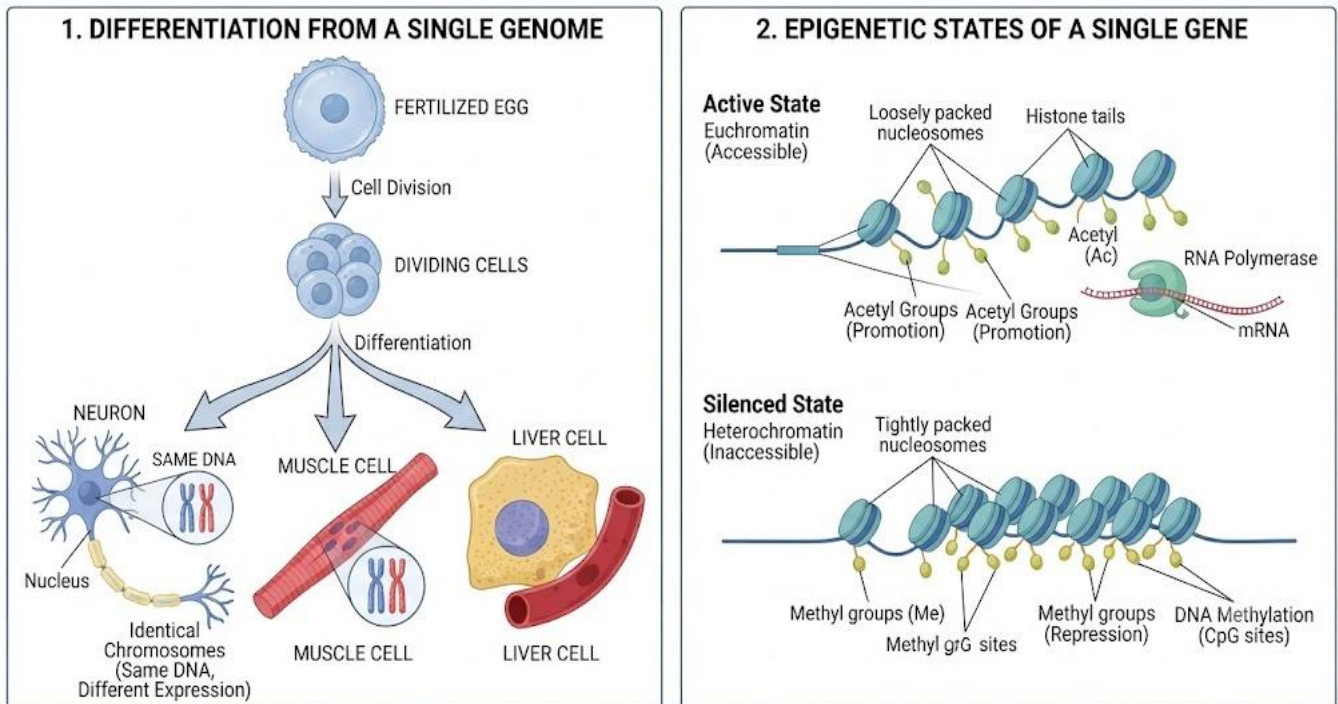


Figure 5.1: Left: cell differentiation from a single fertilised egg to specialised cell types (neuron, muscle, liver), all with identical DNA but different gene expression. Right: epigenetic control – histone acetylation opens chromatin and activates gene expression (top); histone and DNA methylation compact chromatin and silence genes (bottom)

Pilot questions 5.1

1. Explain what cell differentiation is and why all cells in the body are different despite having the same DNA.
2. What is a transcription factor and how do transcription factors control cell identity?
3. What is a master regulator transcription factor? Give one example.
4. Explain the two main epigenetic mechanisms and how each affects gene expression.
5. Why is it important for epigenetic marks to be heritable (passed to daughter cells)?



5.2 Stem Cells

5.2.1 Types of stem cells and their potency

A stem cell is defined by two properties: self-renewal (the ability to divide and produce more stem cells) and potency (the ability to give rise to one or more specialised cell types). Different stem cells vary greatly in their potency. Totipotent cells can give rise to all cell types of the organism including the extra-embryonic tissues such as the placenta; only the fertilised egg and cells of the very first few divisions are truly totipotent. Pluripotent cells can give rise to all cell types of the organism itself (from all three embryonic germ layers) but not to extra-embryonic tissues; embryonic stem cells and iPSCs are pluripotent. Multipotent cells can produce several related cell types but are more restricted; haematopoietic stem cells, for example, can produce all blood cell types but not nerve or muscle cells.

Further along the restriction spectrum are oligopotent stem cells (which can produce only a small number of related types, such as lymphoid progenitors producing T and B cells) and unipotent stem cells (which produce only one cell type, such as spermatogonial stem cells producing only sperm). These categories represent a hierarchy of progressive restriction: as development proceeds, stem cells become more and more committed to particular lineages and lose the flexibility to become other cell types. This progressive restriction is controlled by the transcription factors and epigenetic mechanisms described in Part 5.1.

Fill in the blank: Stem cells are defined by self-renewal and _____; totipotent cells (e.g. fertilised egg) can give rise to all cell types including placenta; pluripotent cells (ESCs; iPSCs) can give rise to all body cell types; multipotent cells (e.g. haematopoietic stem cells) give rise to a limited range of related cell types.

5.2.2 Embryonic and induced pluripotent stem cells

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass (ICM) of the blastocyst, the early embryonic stage reached about 5 to 7 days after fertilisation. ESCs can be grown indefinitely in culture, retain the ability to differentiate into virtually any cell type, and have been invaluable tools for studying early development and testing new therapies. Their main limitation is ethical: obtaining human ESCs requires destroying a human embryo, which



raises serious moral concerns. In 2006, the Japanese scientist Shinya Yamanaka found a way around this problem by showing that adult skin cells could be converted into pluripotent stem cells simply by introducing four transcription factors (Oct4, Sox2, Klf4, and c-Myc), now known as the Yamanaka factors. The resulting cells are called induced pluripotent stem cells (iPSCs).

iPSCs are virtually indistinguishable from ESCs in their gene expression and differentiation potential, but they avoid the ethical concerns of embryo destruction. More importantly for medicine, they can be made from the patient's own cells, which means they will not be rejected by the patient's immune system when used for cell therapy. Yamanaka received the Nobel Prize in Physiology or Medicine in 2012 for this discovery. The practical applications being explored include using patient-derived iPSCs to create replacement cells for conditions such as Parkinson's disease (dopaminergic neurons), diabetes (pancreatic beta cells), and macular degeneration (retinal pigment epithelium cells).

Fill in the blank: ESCs are derived from the _____ cell mass of the blastocyst and are pluripotent; their use raises ethical concerns about embryo destruction. iPSCs are made from adult somatic cells reprogrammed by the Yamanaka factors (Oct4, Sox2, Klf4, c-Myc); they are patient-specific, avoiding immune rejection, and received the Nobel Prize in 2012.

5.2.3 Adult stem cells and tissue maintenance

Adult stem cells are undifferentiated cells found in many tissues throughout the body where they are responsible for maintaining and repairing that tissue throughout life. Different tissues renew at very different rates depending on the activity of their stem cell populations. The intestinal epithelium (gut lining) is among the fastest renewing tissues: it is completely replaced every 3 to 5 days by intestinal stem cells located in specialised pockets called crypts at the base of the intestinal villi. The bone marrow contains haematopoietic stem cells that produce millions of new blood cells every second to replace ageing red blood cells and short-lived white blood cells.

In contrast, tissues with few active stem cells such as the brain and heart muscle have very limited ability to repair themselves. When a person suffers a heart attack or stroke, the dead cells cannot be replaced, resulting in permanent functional loss. Stem cells in tissues are maintained in a specialised microenvironment called the stem cell niche, which provides the physical contacts



and molecular signals that keep stem cells in an undifferentiated, self-renewing state. Examples of niches include the intestinal crypt (where Paneth cells provide key niche signals to intestinal stem cells) and the bone marrow stroma. Disruption of the niche can lead to loss of stem cell function and failure of tissue homeostasis.

Fill in the blank: Adult stem cells in the intestinal _____ replace the gut lining every 3 to 5 days; haematopoietic stem cells in the bone marrow produce all blood cell types; tissues with few stem cells (brain; heart muscle) have limited regeneration; the stem cell niche is the specialised microenvironment that maintains stem cells in a self-renewing state.

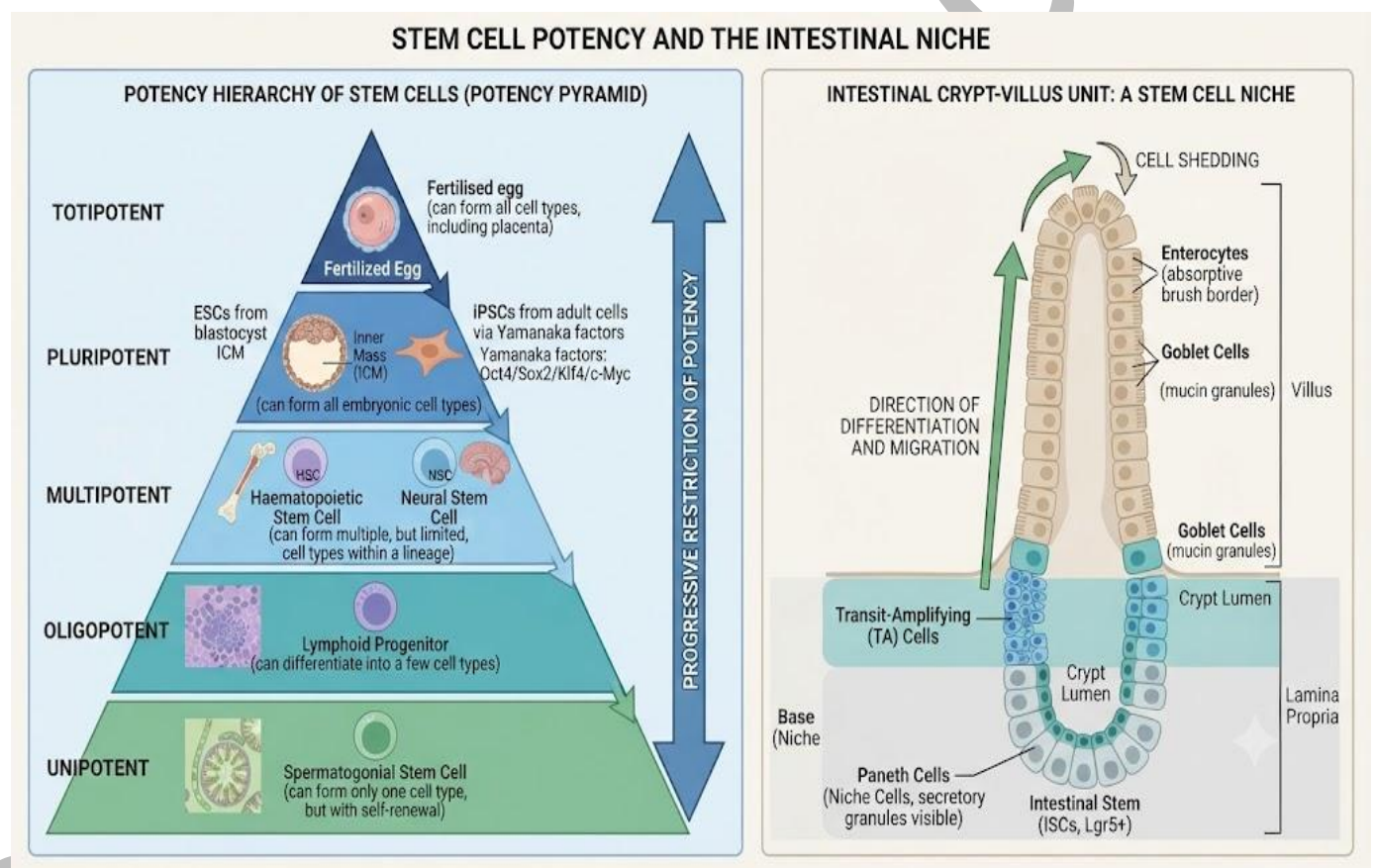


Figure 5.2: Left: stem cell potency hierarchy from totipotent to unipotent, with the origin of ESCs (from ICM of blastocyst) and iPSCs (Yamanaka reprogramming of adult cells) shown at the pluripotent level. Right: the intestinal stem cell niche in the crypt, showing ISCs at the base, Paneth cell niche signals, and differentiated cells migrating up the villus



Pilot questions 5.2

1. Distinguish between totipotent, pluripotent, and multipotent stem cells and give one example of each.
2. Where are ESCs obtained and what is the main ethical concern with their use?
3. How are iPSCs produced and what are two advantages they have over ESCs?
4. What is a stem cell niche and why is it important?
5. Why do the brain and heart have limited regenerative capacity compared to the intestine?

5.3 Molecular Signals in Development

5.3.1 Induction and cell signalling

During embryonic development, the fate of a cell is shaped not only by its own internal gene expression state but also by signals it receives from neighbouring cells. The process by which one group of cells influences the developmental fate of an adjacent group through secreted molecular signals is called induction. This concept was elegantly demonstrated by the German embryologist Hans Spemann and his student Hilde Mangold in 1924. They transplanted a small region of the amphibian embryo (the dorsal lip of the blastopore, now called Spemann's organiser) to the opposite side of a host embryo and observed that a complete second body axis – a second head, notochord, and neural tube – formed at the transplant site, driven by signals from the transplanted tissue.

Induction works through secreted signalling molecules that bind to receptors on target cells and trigger signal transduction cascades that ultimately change gene expression and cell fate. Several major pathways are used repeatedly throughout development, including the Wnt, Notch, Hedgehog (Hh), BMP, and FGF pathways. Remarkably, the same molecular signal can produce different outcomes in different cells or at different times, depending on which genes are already active in the receiving cell. This context-dependence means that a relatively small toolkit of signalling pathways can generate the enormous diversity of cell types seen in a complex animal.

Fill in the blank: Induction is the process by which one group of cells influences the fate of another through secreted _____ signals; first demonstrated by Spemann and Mangold's



organiser transplant in amphibians; the same signalling pathways (Wnt, Notch, Hedgehog, BMP, FGF) are reused in different developmental contexts to produce different outcomes.

5.3.2 Morphogens and body axis patterning

One of the most elegant mechanisms in developmental biology is the morphogen gradient. A morphogen is a signalling molecule that is produced at a specific source and diffuses away from that source through a field of cells, forming a concentration gradient. Cells at different positions in the gradient are exposed to different concentrations of the morphogen and respond by activating different genes, adopting different fates depending on how much morphogen they detect. In this way, a single molecule can pattern an entire tissue just by diffusing away from one end of it. The classic example is Bicoid in the fruit fly *Drosophila*: Bicoid mRNA is deposited at the future head end of the egg by the mother, and the Bicoid protein it encodes diffuses toward the tail end, forming a gradient; high Bicoid concentration activates head-forming genes, while low concentration allows abdominal genes to be expressed.

In vertebrates, similar gradient-based mechanisms pattern the developing body. Sonic Hedgehog (Shh), produced by the floor plate at the base of the developing spinal cord, diffuses upward and specifies different types of neurons at different dorso-ventral positions. Retinoic acid gradients pattern the anterior-posterior body axis. BMP gradients pattern the dorso-ventral axis of the embryo. These gradients do not act in isolation: cells integrate signals from multiple gradients simultaneously, and the combination of signals they receive determines which genes are expressed and therefore what the cell will become.

Fill in the blank: A _____ is a signalling molecule that diffuses from its source to form a concentration gradient; cells at different positions adopt different fates based on the local concentration; Bicoid (*Drosophila* head-tail axis) and Sonic Hedgehog (vertebrate neural tube patterning) are classic examples of morphogens in development.

5.3.3 Master regulator transcription factors

As inductive signals are received, they activate specific transcription factors in target cells that interpret the signal and direct gene expression accordingly. Some of these transcription factors



are master regulators: single factors whose activity alone is sufficient to specify an entire cell fate or body region. The homeotic (Hox) genes, first discovered in *Drosophila*, encode transcription factors that control the identity of body segments along the head-to-tail axis. Loss or gain of Hox gene function produces homeotic transformations, in which one body segment develops the characteristics of a different one; the famous Antennapedia mutation in *Drosophila* causes legs to grow in the place of antennae, revealing that Hox genes specify which structures form in which segment. In vertebrates, Hox genes are arranged in the same linear order on the chromosome as the body regions they control – a phenomenon called collinearity.

Other master regulators control specific organ and tissue types: MyoD drives skeletal muscle differentiation; GATA1 drives red blood cell differentiation; Neurogenin drives neural differentiation; Pax6 drives eye development. These factors do not act in isolation but initiate cascades of gene activation that progressively elaborate the differentiated state, with positive feedback loops reinforcing the commitment. The discovery that just four transcription factors (the Yamanaka factors) can reprogram a fully differentiated adult cell back to pluripotency shows how fundamental transcription factor networks are in controlling cell identity – and how, with the right molecular keys, those locks can be reopened.

Fill in the blank: Hox genes control _____ segment identity along the head-to-tail axis (collinear arrangement; homeotic transformations when mutated); other master regulators include MyoD (muscle), GATA1 (red blood cells), and Pax6 (eyes); they act by initiating gene activation cascades with positive feedback that lock cells into their differentiated state.



MORPHOGEN GRADIENTS AND MASTER REGULATOR CASCADES IN CELL FATE DETERMINATION

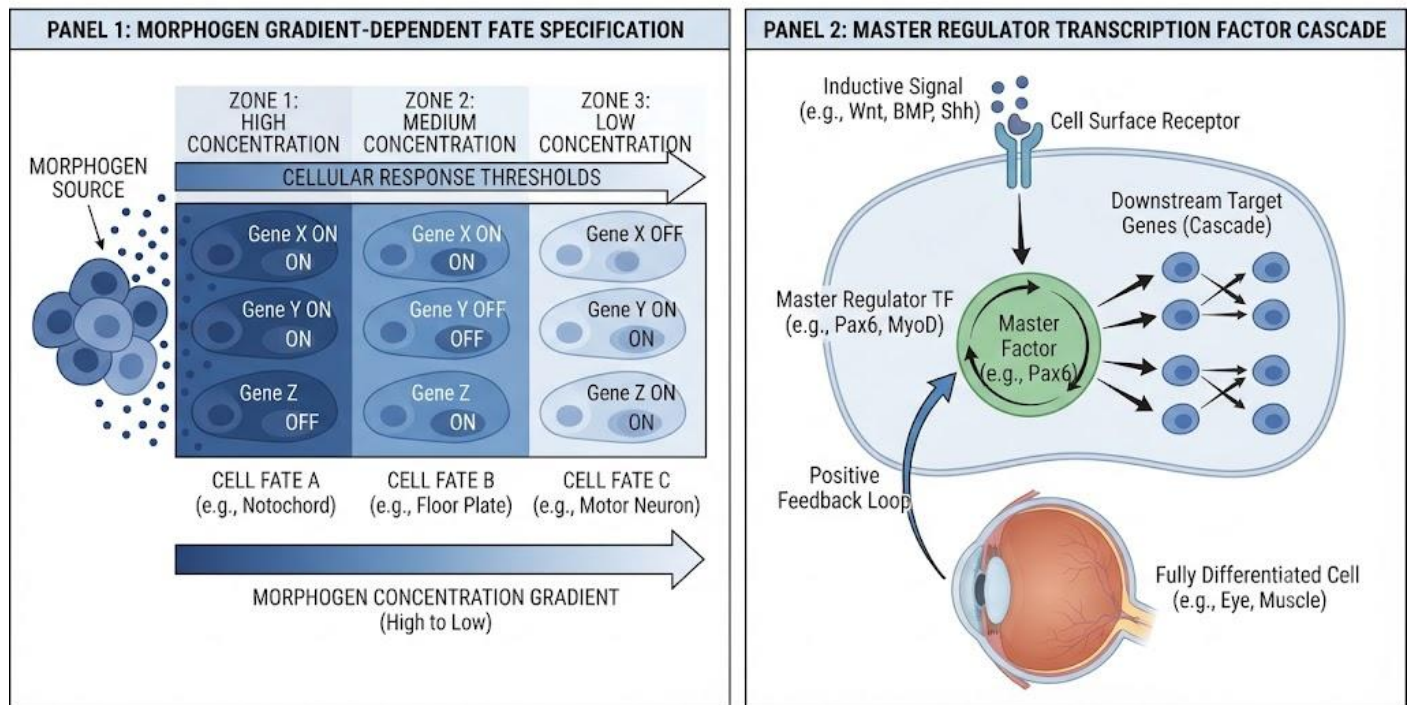


Figure 5.3: Left: a morphogen gradient in which a source cell produces a signalling molecule that diffuses away, specifying three different cell fates at three concentration thresholds. Right: a master regulator transcription factor (activated by an inductive signal) initiates a gene activation cascade that drives differentiation into a specific cell type, reinforced by positive feedback

Pilot questions 5.3

1. What is induction in developmental biology and what did Spemann and Mangold's experiment demonstrate?
2. What is a morphogen? Describe the Bicoid gradient as an example.
3. Why can the same signalling pathway produce different outcomes in different cells?
4. What are Hox genes and what is collinearity?
5. Name three master regulator transcription factors and state what each controls.



5.4 Cell Death, Cancer, and Regeneration

5.4.1 Apoptosis

Apoptosis is the name given to a form of programmed, orderly cell death in which a cell actively dismantles itself in a controlled way. It is distinct from necrosis, which is accidental cell death caused by injury or toxins and which triggers an inflammatory response. Apoptosis is a normal and essential part of development: during the formation of the hand, the cells between the developing fingers die by apoptosis, sculpting the spaces that separate the digits; without this, fingers would remain webbed. During nervous system development, roughly half of all neurons produced die by apoptosis, with only those that successfully connect with target cells surviving. Apoptosis is executed by a family of protein-cleaving enzymes called caspases, which are present in all cells in inactive forms and are activated when a death signal is received.

Two main pathways trigger apoptosis. The intrinsic (mitochondrial) pathway is activated by internal stress signals such as DNA damage: the pro-apoptotic proteins Bax and Bak (members of the Bcl-2 family) cause cytochrome c to be released from the mitochondrial intermembrane space into the cytoplasm; cytochrome c then assembles with other proteins to form a large complex called the apoptosome, which activates caspase-9, initiating the caspase cascade. The extrinsic pathway is activated by external death signals: death ligands (such as FasL or TNF) bind to death receptors on the cell surface, which directly activate caspase-8 and trigger the cascade. Both pathways ultimately lead to the same outcome: activation of executioner caspases that cleave cellular proteins and DNA, causing the cell to shrink, package its contents into membrane-enclosed apoptotic bodies, and be consumed by phagocytic cells without triggering inflammation.

Fill in the blank: Apoptosis is orderly programmed cell death executed by _____ enzymes; the intrinsic pathway (activated by DNA damage) releases cytochrome c from mitochondria and activates caspase-9; the extrinsic pathway (activated by death ligands binding death receptors) activates caspase-8; apoptotic bodies are consumed by phagocytes without inflammation.



5.4.2 Cancer as failed development

Cancer can be understood as a disease in which cells escape the normal developmental controls on growth, division, and differentiation. Normal cells are tightly regulated: they divide only when they receive appropriate growth signals, stop dividing when they contact other cells, and undergo apoptosis when they are damaged beyond repair. A cancer cell has accumulated mutations that disable these controls, allowing it to divide uncontrollably, ignore boundary signals, resist apoptosis, and eventually invade other tissues (metastasis). These mutations fall into two broad categories: mutations that activate proto-oncogenes (normal growth-promoting genes that, when mutated, become permanently active oncogenes driving uncontrolled proliferation – like a stuck accelerator) and mutations that inactivate tumour suppressor genes (which normally act as brakes on cell division; when lost, the brakes are removed).

Cancer is also related to differentiation. Cancer cells frequently dedifferentiate, reverting to a less specialised, more embryonic-like state that makes them more proliferative and invasive. Many tumours contain a subpopulation of cells called cancer stem cells, which share the properties of normal stem cells (self-renewal and the ability to give rise to the different cell types within the tumour) and are thought to be responsible for tumour maintenance and for the regrowth of tumours after treatment. Understanding cancer through the lens of developmental biology – as a failure of normal differentiation, growth control, and apoptosis – has opened new therapeutic strategies, including differentiation therapy (drugs that force cancer cells to re-differentiate and stop proliferating).

Fill in the blank: Cancer arises from mutations in proto-oncogenes (which become _____ and drive uncontrolled proliferation) and tumour suppressor genes (whose loss removes brakes on division); cancer cells often dedifferentiate to a more stem-like state; cancer stem cells are thought to drive tumour maintenance and explain recurrence after treatment.

5.4.3 Regeneration and tissue engineering

Regeneration is the replacement of lost or damaged tissues. The capacity for regeneration varies enormously across organisms. The planarian flatworm can regenerate an entire body from a tiny fragment; the axolotl salamander can regrow complete limbs including bones, muscles, nerves,



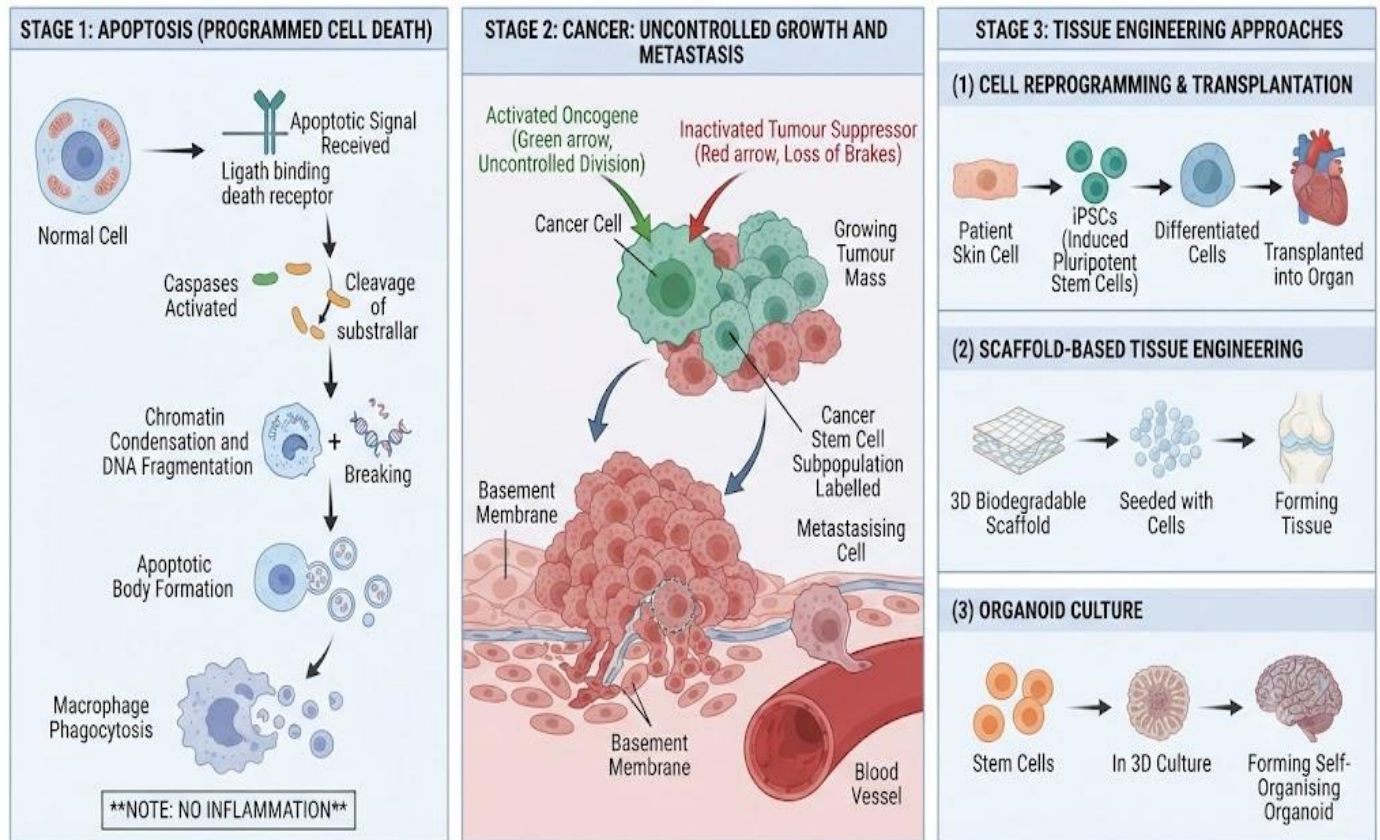
and blood vessels; zebrafish can regenerate their hearts. Mammals, in contrast, have very limited regenerative capacity: we can regenerate skin and partial liver, but cannot regrow a limb or significant amounts of heart or brain tissue. The difference relates to the ability of cells near the wound in highly regenerative animals to dedifferentiate and re-enter the cell cycle, as well as differences in inflammatory responses and the availability of resident stem cells.

Regenerative medicine and tissue engineering aim to use the principles of developmental cell biology to repair or replace tissues that the body cannot regenerate on its own. Three main strategies are being pursued. Cell therapy involves transplanting stem cells or stem cell-derived cells (particularly iPSC-derived cells, which can be made patient-specific to avoid immune rejection) into damaged tissues. Scaffold-based tissue engineering seeds cells onto biodegradable three-dimensional scaffolds that mimic tissue architecture and guide cell organisation and differentiation. Organoids – miniature self-organising three-dimensional tissue cultures derived from stem cells – are being used to model diseases, test drugs, and potentially provide transplantable tissue for conditions including gut disease, Parkinson's disease, and blindness caused by macular degeneration.

Fill in the blank: Regenerative capacity varies: planarians and axolotls have high regeneration; mammals have limited regeneration (skin; partial liver). Tissue engineering strategies include _____ therapy (transplanting iPSC-derived cells), scaffold-based engineering (3D biodegradable scaffolds), and organoids (self-organising 3D stem cell cultures used to model disease and test drugs).



CELLULAR PROCESSES: APOPTOSIS, CANCER, AND TISSUE ENGINEERING



Box 5.1: Left: the stages of apoptosis from signal to phagocytosis of apoptotic bodies. Middle: cancer development through oncogene activation and tumour suppressor loss, leading to invasion and metastasis with cancer stem cells. Right: three tissue engineering strategies – iPSC-based cell therapy, scaffold-based engineering, and organoids

Pilot questions 5.4

1. Explain why apoptosis is important during normal development, giving two examples.
2. Describe the intrinsic and extrinsic pathways of apoptosis.
3. Distinguish between a proto-oncogene and an oncogene and give one example.
4. What are cancer stem cells and why are they clinically important?
5. Describe two strategies used in tissue engineering and regenerative medicine.



Series Summary

This Series explored **how cells become specialised and maintain their identities** throughout development and adult life. You examined the roles of selective gene expression, transcription factors, and epigenetic regulation in cell differentiation, as well as the properties of stem cells, their levels of potency, and the importance of stem cell niches in tissue maintenance and regeneration. The Series also highlighted why different tissues vary in their regenerative capacity.

The Series further examined the molecular mechanisms that guide development, including induction, morphogens, signalling pathways, and Hox genes, alongside the essential role of apoptosis in normal development. It also explained how disruptions to these regulatory processes contribute to cancer and introduced modern approaches in regenerative medicine, such as induced pluripotent stem cells, tissue engineering, and organoids. By completing this Series, you should now be able to explain the mechanisms of cell differentiation and development, evaluate the functions of stem cells and developmental signalling pathways, relate apoptosis and developmental regulation to health and disease, and appreciate the applications of regenerative medicine, thereby achieving all four **Series Learning Outcomes (SLOs)** and the **overall course outcomes**.

Glossary of Terms

- **Apoptosis:** programmed, orderly cell death executed by caspase enzymes; the cell shrinks, its DNA is fragmented, and it breaks into apoptotic bodies consumed by phagocytes without triggering inflammation; essential for development (finger separation; neuron elimination) and for eliminating damaged cells.
- **Cancer stem cells:** a subpopulation of cancer cells with stem cell properties (self-renewal; multipotency) thought to drive tumour maintenance and to explain tumour recurrence after treatment that kills the bulk of the tumour.



- **Caspases:** a family of protease enzymes that are the central executioners of apoptosis; present in cells in inactive forms; activated in a cascade by apoptotic signals; cleave cellular proteins and DNA, leading to orderly cell dismantling.
- **Cell differentiation:** the process by which a less specialised cell progressively acquires a specific identity with distinct structure and function; driven by selective gene expression (not DNA sequence changes); generally irreversible and maintained by epigenetic mechanisms.
- **Embryonic stem cells (ESCs):** pluripotent stem cells from the inner cell mass of the blastocyst; can give rise to all body cell types; grow indefinitely in culture; ethically controversial because their derivation requires embryo destruction.
- **Epigenetic regulation:** heritable changes in gene activity that do not alter DNA sequence; includes histone modification (acetylation activates; methylation silences) and DNA methylation (methyl groups on CpG cytosines; generally silences genes); copied to daughter cells at division.
- **Induced pluripotent stem cells (iPSCs):** pluripotent stem cells produced by reprogramming adult somatic cells with four transcription factors (Oct4, Sox2, Klf4, c-Myc; Yamanaka factors; Nobel 2012); avoid ethical issues of ESC use; can be patient-specific.
- **Induction:** the process by which one group of cells influences the developmental fate of an adjacent group through secreted molecular signals; demonstrated by Spemann-Mangold organiser experiment (1924).
- **Master regulator transcription factor:** a transcription factor whose expression alone can redirect cell fate; examples: MyoD (muscle); Pax6 (eyes; ectopic expression causes extra eyes); GATA1 (red blood cells); Neurogenin (neurons).
- **Morphogen:** a signalling molecule produced at a source that diffuses to form a concentration gradient; cells at different positions adopt different fates based on local concentration; examples: Bicoid (*Drosophila* head-tail axis); Sonic Hedgehog (vertebrate neural tube patterning).
- **Multipotent stem cell:** a stem cell that can give rise to several related cell types; examples: haematopoietic stem cells (all blood cell types); neural stem cells (neurons, astrocytes, oligodendrocytes); intestinal stem cells (all intestinal epithelial cell types).
- **Oncogene:** a mutated or overexpressed proto-oncogene that drives uncontrolled cell proliferation; acts like a stuck accelerator; example: Ras, mutated to a permanently active form in about one-third of all human cancers.



- **Organoid:** a miniature self-organising three-dimensional tissue culture derived from stem cells that mimics the architecture and some functions of a real organ; used to model diseases, test drugs, and develop transplantable tissue.
- **Pluripotent stem cell:** a stem cell that can give rise to all cell types of the organism (all three germ layers) but not to extra-embryonic tissues such as the placenta; examples: ESCs and iPSCs.
- **Stem cell niche:** the specialised microenvironment providing signals and contacts that maintain stem cells in a self-renewing undifferentiated state; examples: intestinal crypt (Paneth cells signal to ISCs); bone marrow stroma (signals haematopoietic stem cells).
- **Totipotent stem cell:** a stem cell capable of giving rise to all cell types including extra-embryonic tissues such as the placenta; only the fertilised egg (zygote) and cells of the first few cleavage divisions are truly totipotent.
- **Transcription factor:** a protein that binds to specific DNA sequences near genes and activates or represses their transcription; the unique combination of transcription factors in each cell type produces its characteristic gene expression profile and identity.
- **Tumour suppressor gene:** a gene whose protein product restrains cell division, promotes apoptosis, or repairs DNA damage; loss of function removes brakes on proliferation; classic examples: p53 (most commonly mutated gene in cancer) and Rb.
- **Yamanaka factors:** the four transcription factors (Oct4, Sox2, Klf4, c-Myc) whose forced expression reprogrammes adult somatic cells into iPSCs; discovered by Shinya Yamanaka (Nobel Prize 2012).

Concept Check Questions [Series 5]

Objective Questions

1. Cell differentiation results not from changes in DNA sequence but from differences in gene _____ – which genes are switched on and which are permanently silenced in each cell type by combinations of transcription factors. (Linked to SLO 5.1)
2. Two main epigenetic mechanisms maintain cell identity: histone _____ (loosens chromatin; activates genes) and DNA methylation (methyl groups on CpG sites; silences genes); both are copied to daughter cells during DNA replication. (Linked to SLO 5.1)



3. iPSCs are produced by reprogramming adult somatic cells with the Yamanaka _____ (Oct4, Sox2, Klf4, c-Myc); they are pluripotent, patient-specific, and avoid the ethical concerns of embryo destruction. (Linked to SLO 5.2)
4. A _____ is a signalling molecule that diffuses from a source to form a concentration gradient; cells at different positions adopt different fates depending on the local concentration; Bicoid and Sonic Hedgehog are classic examples. (Linked to SLO 5.3)
5. Apoptosis is executed by _____ enzymes; the intrinsic pathway releases cytochrome c from mitochondria; the extrinsic pathway is triggered by death ligands binding death receptors on the cell surface; apoptotic bodies are consumed by phagocytes without inflammation. (Linked to SLO 5.4)

Short-Answer Questions

1. Explain what cell differentiation is and why cells have different identities despite sharing the same DNA. (Linked to SLO 5.1)
2. Distinguish ESCs from iPSCs in terms of origin, ethical considerations, and medical advantages. (Linked to SLO 5.2)
3. Explain what induction is in development and describe Spemann and Mangold's experiment. (Linked to SLO 5.3)
4. Distinguish between a proto-oncogene and a tumour suppressor gene and explain how each contributes to cancer when mutated. (Linked to SLO 5.4)
5. Describe two approaches used in tissue engineering and regenerative medicine. (Linked to SLO 5.4)

Long-Answer Questions

1. Describe the molecular basis of cell differentiation (transcription factors; master regulators; epigenetic mechanisms) and the types and properties of stem cells (potency hierarchy; ESCs; iPSCs; adult stem cells; niche). (Linked to SLOs 5.1, 5.2)
2. Describe induction, morphogen gradients, and master regulator transcription factors in development. Explain apoptosis and its developmental roles. Describe how cancer represents a failure of developmental controls. Outline approaches to regeneration and tissue engineering. (Linked to SLOs 5.3, 5.4)



Answers to Concept Check Questions [Series 5]

Objective Questions

1. Expression.
2. Acetylation.
3. Factors.
4. Morphogen.
5. Caspase.

Short-Answer Questions

1. Cell differentiation is the process by which a cell acquires a specialised identity with a distinct structure and function. All cells share the same DNA, but different cell types express different genes; transcription factors present in each cell type bind DNA near genes and switch them on or off, producing the unique gene expression profile that determines each cell's identity.
2. ESCs: derived from the inner cell mass of the blastocyst; pluripotent; require embryo destruction (ethically controversial). iPSCs: produced by reprogramming adult somatic cells with Yamanaka factors; pluripotent; avoid ethical issues of embryo use; can be made from patient's own cells, avoiding immune rejection – a major advantage for cell therapy.
3. Induction is the process by which one group of cells influences the developmental fate of a neighbouring group through secreted molecular signals. Spemann and Mangold (1924) transplanted the dorsal lip of the blastopore (organiser) from one amphibian embryo to the opposite side of a host embryo; a complete second body axis (head, notochord, neural tube) formed at the transplant site, proving that signals from the organiser instruct surrounding cells to change their fate.
4. Proto-oncogene: a normal gene promoting cell growth; when mutated or overexpressed, becomes an oncogene that drives uncontrolled proliferation (stuck accelerator). Example: Ras. Tumour suppressor: normally restrains cell division and promotes apoptosis; when inactivated by mutation, removes the brakes on proliferation. Example: p53. Cancer typically requires mutations in both types: oncogenes drive growth while loss of tumour suppressors removes the safeguards.
5. Cell therapy: iPSC-derived cells are transplanted into damaged tissue; because they come from the patient's own cells, immune rejection is minimised. Organoids: stem cells are cultured in three-dimensional conditions that allow them to self-organise into miniature tissue



structures, which can model diseases, screen drugs, and potentially serve as transplantable tissue.

Long-Answer Questions

1. Differentiation: all cells share the same DNA; different cell types express different genes via unique transcription factor combinations; master regulators (MyoD: muscle; Pax6: eyes; GATA1: red blood cells) alone can redirect fate by activating gene cascades with positive feedback. Epigenetics: histone acetylation (opens chromatin; activates genes); histone/DNA methylation (compacts chromatin; silences genes); both marks copied to daughter cells, maintaining identity through division; cancer often shows disrupted epigenetic patterns. Stem cells: self-renewal and potency; totipotent (zygote; first cleavage cells; all cell types + placenta); pluripotent (ESCs from ICM – ethically controversial; iPSCs from adult cells via Oct4/Sox2/Klf4/c-Myc – Nobel 2012; patient-specific; no embryo destruction); multipotent (haematopoietic stem cells: all blood cells; intestinal stem cells: renew gut every 3 to 5 days); oligopotent; unipotent. Niche (intestinal crypt; bone marrow stroma) maintains stem cell self-renewal; tissues with few stem cells (brain; heart) have poor regeneration.
2. Induction: Spemann-Mangold organiser (1924) transplant induced second body axis; works through secreted signals (Wnt, Notch, Hedgehog, BMP, FGF pathways reused in different contexts; same signal, different outcome depending on receiving cell state). Morphogens: diffuse from source to form gradient; Bicoid (Drosophila head-tail axis; high concentration = head genes); Sonic Hedgehog (vertebrate neural tube; floor plate source; different neuron types at different distances). Hox genes: segment identity; collinear arrangement; homeotic mutations transform one segment into another (Antennapedia: legs from antennae). Apoptosis: caspases execute orderly cell death; intrinsic pathway (DNA damage → cytochrome c from mitochondria → apoptosome → caspase-9); extrinsic pathway (death ligands bind death receptors → caspase-8); apoptotic bodies phagocytosed without inflammation; roles: finger separation; elimination of excess neurons. Cancer: mutations in proto-oncogenes (become oncogenes; Ras; uncontrolled proliferation) and tumour suppressors (p53; Rb; lose brakes); cancer cells dedifferentiate (more stem-like; EMT; invasive); cancer stem cells drive recurrence. Tissue engineering: cell therapy (iPSC-derived cells; patient-specific); scaffold-based (3D biodegradable scaffold + cells → organised tissue); organoids (self-organising 3D stem cell cultures; disease models; drug testing; potential transplant source).



Answers to Pilot Questions [Series 5]

Part 5.1

1. Cell differentiation is the process by which a less specialised cell acquires a specific identity with distinct structure and function. All cells share the same DNA, but each cell type expresses a different subset of genes; it is these differences in gene expression – controlled by transcription factors – that produce the diversity of cell types.
2. Transcription factors are proteins that bind specific DNA sequences near genes and either activate or repress their transcription. The unique combination of transcription factors present in each cell type determines which genes are on and which are off, producing the characteristic gene expression profile that defines that cell's identity.
3. A master regulator transcription factor is one whose expression alone can redirect a cell's developmental fate. Example: MyoD – when experimentally expressed in a fibroblast, it converts the cell to a muscle-like identity by activating the full cascade of muscle-specific genes.
4. Histone modification: adding acetyl groups to histones (acetylation) loosens chromatin and activates gene expression; adding methyl groups (methylation) compacts chromatin and silences genes. DNA methylation: methyl groups added to cytosine bases at CpG sites generally silence the associated gene by making that region of chromatin inaccessible.
5. Epigenetic marks must be heritable so that a differentiated cell passes its identity to all its descendants. Without this, a liver cell dividing during tissue maintenance might produce daughters that lose their liver identity; heritability of epigenetic marks ensures tissues retain the correct cell types throughout the organism's life.

Part 5.2

1. Totipotent: gives rise to all cell types including placenta; example: fertilised egg (zygote). Pluripotent: gives rise to all body cell types but not placenta; example: ESCs (from inner cell mass). Multipotent: gives rise to a limited range of related cell types; example: haematopoietic stem cells (all blood cell types but not nerve or muscle cells).
2. ESCs are obtained from the inner cell mass of the blastocyst (about 5 to 7 days after fertilisation). The main ethical concern is that obtaining them requires destroying a human embryo, raising questions about the moral status of the embryo.



3. iPSCs are made by introducing four transcription factors (Oct4, Sox2, Klf4, c-Myc; Yamanaka factors) into adult somatic cells, reprogramming them to pluripotency. Two advantages over ESCs: (1) no embryo is destroyed; (2) they can be derived from the patient's own cells, so they are genetically matched and will not be rejected by the immune system.
4. The stem cell niche is the specialised microenvironment in a tissue that provides the signals and contacts needed to keep stem cells in a self-renewing undifferentiated state. Without the niche, stem cells would differentiate and be lost; disruption of the niche leads to failure of tissue maintenance.
5. Brain and heart muscle contain very few active stem cells and their cells are largely post-mitotic; they cannot re-enter the cell cycle to replace lost cells. The intestine and bone marrow have abundant, rapidly dividing stem cells that continuously replace cells, giving these tissues high renewal and repair capacity.

Part 5.3

1. Induction is the process by which one group of cells influences the developmental fate of a neighbouring group through secreted signals. Spemann and Mangold (1924) transplanted the dorsal lip of the blastopore (organiser region) from one amphibian embryo onto the opposite side of a host embryo; a complete second body axis formed at the transplant site, demonstrating that the organiser emits signals that instruct surrounding cells to change their fate.
2. A morphogen is a signalling molecule that diffuses from a source to form a concentration gradient; cells adopt different fates depending on the concentration they experience. In *Drosophila*, Bicoid mRNA is deposited at the head end of the egg; the Bicoid protein diffuses toward the tail end; high concentrations activate head-forming genes and low concentrations allow abdominal genes to be expressed instead.
3. The same signalling pathway can produce different outcomes in different cells because the response depends on which transcription factors and genes are already active in the receiving cell at the time the signal arrives. A cell's prior history of signals determines how it interprets a new signal, so the same molecule can activate completely different gene programmes in different cellular contexts.
4. Hox genes encode transcription factors that specify body segment identity along the head-to-tail axis of animals. Collinearity means that the Hox genes are arranged in the same linear order along the chromosome as the body regions they control – genes at one end control head structures, genes at the other end control tail structures.



5. MyoD: master regulator of skeletal muscle differentiation; expression in fibroblasts converts them to muscle-like cells. Pax6: master regulator of eye development; ectopic expression in the leg of a *Drosophila* causes extra eyes to form there. GATA1: master regulator of red blood cell differentiation; required for erythroid lineage commitment.

Part 5.4

1. Two examples: (1) Finger separation – the cells between developing digits die by apoptosis during hand formation, sculpting the spaces between fingers; without this, fingers would remain webbed. (2) Neuron elimination – approximately half of all neurons produced during nervous system development die by apoptosis; only those that successfully connect with target cells survive, ensuring precise neural wiring.
2. Intrinsic pathway: activated by internal stress (e.g. DNA damage); pro-apoptotic Bax/Bak proteins release cytochrome c from mitochondria; cytochrome c forms the apoptosome complex that activates caspase-9. Extrinsic pathway: activated by external death signals; death ligands (FasL, TNF) bind death receptors on the cell surface, directly activating caspase-8.
3. Proto-oncogene: a normal gene promoting cell growth that, when mutated or overexpressed, becomes an oncogene driving uncontrolled proliferation. Example: the Ras gene encodes a normal signalling protein; mutated Ras is permanently active and drives uncontrolled cell division in about one-third of all human cancers.
4. Cancer stem cells are a subpopulation of cancer cells that share the properties of normal stem cells: self-renewal and the ability to produce the different cell types found within the tumour. They are clinically important because they are often resistant to chemotherapy and can regrow the tumour after treatment, explaining why cancers frequently recur.
5. Cell therapy: iPSC-derived cells (made from the patient's own cells) are transplanted into damaged tissue to restore function, avoiding immune rejection. Scaffold-based tissue engineering: stem cells are seeded onto a biodegradable three-dimensional scaffold that mimics tissue architecture, guiding cells to organise and differentiate into functional tissue that can then be transplanted.



References and Attributions [Series 5]

Textual References (Books and External Sources)

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Figures, Plates, Tables, and Boxes

- Figure 5.1: Cell differentiation and epigenetic mechanisms. Attribution: AI generated. Suggested OER search string: “cell differentiation gene expression histone modification DNA methylation epigenetic diagram OER”.
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