INTRODUCTION TO CELL BIOLOGY

<table>
<tr><td rowspan="5">Biomolecules</td><td colspan="2">

Human cell contains > 20,000 unique proteins and 1000’s of distinct metabolites (small molecules including lipids)
A key component of understanding how a protein works is realising where it is within the cell
Much is known about some genes/proteins, nothing about others

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<tr><td rowspan="3">Gene ontology → a project that seeks to describe biology using a controlled vocabulary, and in a discrete way, such that a computer can understand what a protein does. This covers three domains:</td><td>i) Cellular Component: the parts of a cell with which the protein associates</td></tr>
<tr><td>ii) Molecular Function: the elemental activities of a protein at the molecular level, such as binding or catalyst</td></tr>
<tr><td>iii) Biological Process: operations or sets of molecular events with a defined beginning and end, pertinent to the functioning of integrated living units (cells, tissues, organs, organisms)</td></tr>
<tr><td colspan="2">Protein location within the cell → important because:

Eukaryotic cells are divided into various morphologically and functionally distinct compartments

These compartments are made up of 100’s to 1000’s of proteins which provide its structure and function

Proteins must be targeted to the appropriate compartment to ensure proper function
Knowing a proteins subcellular localisation is critical to understanding the function of individual proteins

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<tr><td colspan="2">Detecting individual proteins in cells → cannot be observed with electron microscopy, so identification techniques have to be developed:

Utilising the immune system

Body recognises foreign material and combats it (fight, kill, remove)
Develop antigen-specific antibodies to combat foreign material
By putting a human protein in another mammal, that mammal will develop antibodies specific to that protein
These antibodies can then be used to identify the location of that protein within the human cell

Indirect immunolabelling → the primary antibody developed in another mammal is directed against an immobilised antigen, antigen A. It binds, and then marker-coupled secondary antibodies directed at the non-human antibody bind, and mark the proteins location (as they are easily visible)
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Immunofluorescence Microscopy	**i)** **Cultivation:** *culture* cells (mammalian cell culture is the process of growing animal cells in vitro in a flask or dish) **ii)** **Fixation:** in living cells, organelles move around. Thus, to stabilise the cells internal structures, the organelles are cross-linked together, making them static **iii)** **Permeabilization:** the lipid bilayer does not allow antibodies to pass through, and thus holes must be punched in it **iv)** **Blocking:** helps get specificity **v)** ***Primary*** **antibody incubation:** antigen-specific antibodies developed from a non-human mammal enter the cell and bind to their antigen **vi)** ***Secondary*** **antibody incubation:** these antibodies bind to the non-human antibody, and are marked with dyes that *fluoresce*, meaning they *absorb* light at one set of wavelengths and *emit* light at another set of wavelengths (process called *fluorescence*) **vii)** **Nucleus staining:** by staining the nucleus and other particular regions of the cell, a reference point is made, which can be used to identify fluorescent regions **viii)** **Mounting:** tissue is 'mounted' onto a 12mm x 1mm coverslip **ix)** **Microscopy:** tissue is observed
	Fluorescence Spectra • Fluorescent dyes absorb and emit light best at certain wavelengths - We can show this by plotting absorbance or emission versus wavelength on a graph - Since the light that gets absorbed by the dye excites the dye molecules to a more energetic state, it is called absorption *excitation*
	Stoke Shift → the difference between the peak emission and peak absorption wavelengths • Typically, excitation (absorption) light is many times brighter than the emission light - If we shone the excitation light onto the sample and looked for fluorescence, we might have a hard time seeing our emission - This is solved by using filters that allow the excitation light to get to the sample, but only the emitted light gets to our eyes/camera

The Airy Disk & Resolution	• When light from the various points of a specimen passes through the objective lens and is reconstituted as an image, the various points of the specimen appear in the image as small patterns (not points) known as *Airy patterns* - In Airy Disks, there is a maximum point of light intensity which is surrounded by rings of fluorescence - This scattering prevents us from seeing objects close together (i.e.- objects whose Airy Disks overlap) • **Effective Resolution (d_0)** → the smallest distance between two objects that still allows for them to be seen as separate entities - For light microscopes, this is about 0.2µm (200nm) - E.g.- if two objects were less than 200nm apart, we could not resolve each one • **Super Resolution Microscopy** → allows for greater resolutions because it can resolve more points of light - Resolution of 10-30 nm • **Confocal Fluorescence Microscopy** → captures the light from one focal plane and removes any other sources of light - Allows the visualisation of thick specimens - Removes blur that conventional microscopy can't • **Live Cell Imaging** → uses naturally occurring fluorescent proteins to observe living cells - Often able to be genetically coded into animals

MODULE 1- CELL CYCLE

Cell → a membrane-bound structure containing biomolecules that acts as the structural, functional and biological unit of all organisms	
Central Dogma of Molecular Biology	Gene expression dictates cell identity and function: DNA → RNA → Protein Organisms genome encoded in DNA, which is *transcribed* into RNA by RNA polymerase. RNA is then translated into protein, which performs a function.
Gene structure and nomenclature	**Promotor** → transcription factors and polymerase bind here to begin transcribing the gene
	Exon → protein coding region that is translated into mRNA
	Introns → non-coding regions that are spliced out of mRNA (can have regulatory functions)
	Diploid organisms → have two copies of every gene. If both copies are identical, the organism is ***homozygous*** at that locus.
	Alleles → different versions of the same gene (differences in DNA sequences of the same gene). If the two copies of a gene are different, the organism is ***heterozygous*** at that locus.
DNA Transcription into mRNA	**Capping** → after transcription, the primary RNA transcript is capped at the 5' end with a special nucleotide; important for stability and translation
	Cleavage → primary RNA transcript is cleaved at the 3' end
	Polyadenylation → 3' end is polyadenylated by an enzyme called a polyadenylase; mRNA will have long tails of 'A's', important for stability and translation (binding site for proteins)
	RNA splicing → introns are removed by splicing factors that bind to acceptor and donor sites within introns. Results in mature mRNA
mRNA Translation into Protein	Single-stranded mRNA is translated into a protein within the ribosomes (after exiting the nucleus and travelling through the cytoplasm)
	Codons → exist within mRNA as nucleotide triplets; they specify what amino acid goes at their point in the sequence
	tRNA → have **anticodons** and **amino acids** attached; the anticodon is matched appropriately to the codon, such that the correct amino acid is retrieved
Proteins	**Proteins** → the workhorse of the cell: • Structural • Sensors • Transporters • Enzymes • Transcription Factors • Cellular Communication • Signal Transduction

	Amino Acids → amino acid sequences make up proteins, though there are only 20 amino acids
	Protein (amino acid) sequences → determine protein *structure*
	Protein structure → determines protein *function*
	Prion → infectious protein with *normal* DNA sequence; infectious nature allows it to convert normal proteins into prion form Goes against Central Dogma because while the prion is a mutated protein, its DNA is normal (i.e.- protein structure/function is not determined by DNA sequence, but by something else)
Approaches for Studying Cells	**Cell Biology** → through direct observation (microscopy); labelling cell structures of interest and observing them
	Biochemistry → isolating and describing proteins
	Genetics → looking at mutant genes, their mutant proteins, and the effects on the cell or animal
	Genomics and Proteomics → looking at all genes (or proteins) at the same time
	Developmental Biology → studying differential gene expression and the signals that lead to the mature organism
The Cell Cycle	**Interphase** → everything except Mitosis
	G1 Phase (Gap 1) → recovery from mitosis, growth
	S Phase (Synthesis) → DNA is duplicated
	G2 Phase (Gap 2) → pre-mitosis checkpoints
	M Phase (Mitosis) → chromosome segregation and cell division
	G0 Phase (Gap 0) → temporary or permanent exit from the cell cycle
Chromosome Structure and Movements	**Chromosome** → the structural unit of genetic material consisting of genetic material consisting of double stranded DNA and proteins
	Chromatid → one copy of a duplicated chromosome (still a chromosome)
	Sister Chromatids → identical copies of a chromosome joined by a centromere
	Homologous Chromosomes → chromosome pair that includes one from each parent (maternal and paternal). Different alleles.
	Cohesins → proteins that hold the sister chromatids together
	Centromere → repetitive DNA sequence that serves as a landing pad for mitotic machinery
	Kinetochore → protein complex that binds to the centromere, linking the centromere to microtubules
	Chromosome Segregation in Mitosis → • Duplicated chromosomes line up independently of one another • Each pair of sister chromatids (identical) separate • Each daughter cell gets *all* of the genetic information

<table>
<tr><td rowspan="7">Stages of Mitosis</td><td colspan="2">Interphase → chromosome duplication and cohesion; centrosome duplication, one for each daughter cell (both occur in S Phase)</td></tr>
<tr><td colspan="2">Prophase → breakdown of interphase microtubule and its replacement by two mitotic asters (centrosome + emerging microtubules); mitotic aster separation; chromosome condensation for movement</td></tr>
<tr><td colspan="2">Prometaphase → nuclear envelope breakdown; condensed chromosomes captured, bi-oriented and brought to spindle equator by microtubules</td></tr>
<tr><td colspan="2">Metaphase → chromosomes aligned independently at the metaphase plate</td></tr>
<tr><td rowspan="2">Anaphase → Anaphase Promoting Complex (APC/C) activated, and cohesins degraded</td><td>Anaphase A → chromosome movement to poles</td></tr>
<tr><td>Anaphase B → spindle pole separation</td></tr>
<tr><td colspan="2">Telophase → nuclear envelope reassembly; assembly of contractile ring</td></tr>
<tr><td colspan="2">Cytokinesis → reformation of interphase microtubule array; contractile ring forms cleavage furrow</td></tr>
<tr><td rowspan="3">Mitotic Machinery</td><td>Centrosomes (Spindle Poles) → microtubule organising centres</td><td>Centrioles → contained within the centrosomes and are composed of bundles of microtubules (source of microtubules)</td></tr>
<tr><td colspan="2">Microtubules → capture and move chromosomes; anchor to the plasma membrane</td></tr>
<tr><td colspan="2">Molecular Motors → drive chromosome movement</td></tr>
<tr><td rowspan="4">Mitosis vs Meiosis</td><td colspan="2">Meiosis → cell division that only occurs in the germline cells; the goal of meiosis is to produce four unique gametes that are appropriate for sexual reproduction (i.e.- shuffle alleles and reduce to 1n)</td></tr>
<tr><td colspan="2">Mitosis → cell division that occurs in the somatic cells; the goal of mitosis is to produce two identical daughter cells (i.e.- keep all alleles the same)</td></tr>
<tr><td colspan="2">n → number of chromosomes of each type</td></tr>
<tr><td colspan="2">Differences Between Mitosis and Meiosis →

• Mitosis
- In a mitotic cell, the number of chromosomes goes from 2n to 4n after S-phase
- Most cells are somatic, and have either 2n or 4n

• Meiosis
- A reductive process that produces gametes viable for sexual reproduction
- Reduces the number of chromosomes down to 1n
- In sexual reproduction, new combinations of existing alleles create new phenotypes (this drives evolution)</td></tr>
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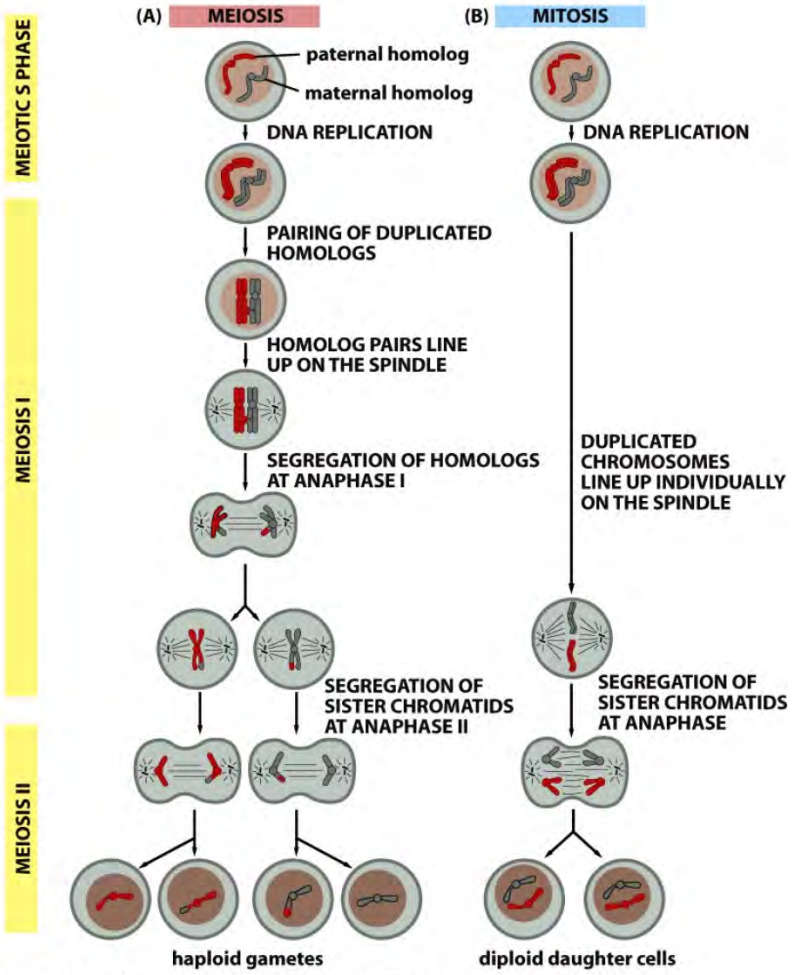
<tr><td></td><td> 

Figure 17-47 Molecular Biology of the Cell 5/e (© Garland Science 2008)</td></tr>
<tr><td rowspan="2">Stages of Meiosis</td><td>Meiosis I →

i) In meiotic S-phase, DNA replication occurs
ii) Homologous chromosomes pair and line up on the mitotic spindle; recombination occurs between homologous chromosomes
iii) Segregation of homologous chromosomes at Anaphase I; sister chromatids remain intact; each daughter cell gets a different set of chromosomes; each daughter cell is 2n, but contains either a maternal or paternal version of the chromosome</td></tr>
<tr><td>Meiosis II →

i) Chromosomes are lined up independently on the metaphase plate
ii) Segregation of sister chromatids in Anaphase II, forming four unique haploid gametes (1n)</td></tr>
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The Cell Cycle: Mechanisms of Regulation	**Phosphorylation** → the enzymatic process of adding phosphate groups to target substrates to activate or inactivate them; temporary and reversible • Kinase - An enzyme which adds phosphate groups to their targets - Phosphate group taken from ATP (making it ADP) • Phosphatase - An enzyme that removes a phosphate from its target - Requires a water molecule to remove phosphate group • Phosphate groups are attached to the side chains of specific amino acids - Only Ser, Thr, and Tyr can be phosphorylated, because they have hydroxyl as their side chain - Specific kinases phosphorylate specific residues on specific proteins
	Phosphorylation: Kinases → heterodimeric protein kinases drive the cell cycle • Cyclin dependent kinases (CDKs) are a catalytic subunit present throughout the cell - The cyclin-regulatory subunit is cyclical, and expressed at specific cell cycle stages - Cyclin recognises the substrate and determines the CDK's specificity - CDK cannot exert kinase activity without being bound to cyclin • Distinct CDK's regulate different cell cycle transitions - G0: CDK's are inactive - CDK's are essential for progressing through the cell cycle • A kinase assay can be used to test the activity of a CDK - Pull down cyclin/CDK complex using antibodies - Add substrate (histone, H1, protein) and radioactive ATP - Quantify the amount of labelled phosphate transferred to substrate on an SDS PAGE gel
	Ubiquitination → a mechanism which degrades a protein; permanent and irreversible • Ubiquitin-protein ligases attach ubiquitin to a target protein - Repeats multiple times, resulting in polyubiquitination - Proteasome recognises *polyubiquitination*, and destroys the protein

	Ubiquitin-Protein Ligases → • SCF Complex - Involved in the G1-S phase transition • Anaphase Promoting Complex or Cyclosome (APC/C) - Involved in metaphase-anaphase and anaphase-telophase transitions
The G1/S Phase Transition	**G1 Cyclin/CDK Complexes** → promote S-phase entry: • G1 Cyclin-CDKs - Phosphorylate transcription factors - Transcription factors drive the expression of genes that code for tools of DNA replication - Transcribed genes include enzymes to make deoxynucleotides, DNA polymerases, replication proteins and **S-phase cyclins** • SCF Ubiquitin-Protein Ligases - The boundary between G1 and S phase is defined by an inhibitor of S-phase cyclin/CDKs (Sic1) - During G1, S-phase cyclins are created and bind to their CDK's, but the complexes action is prevented by these inhibitors - However, the inhibitor serves as a substrate for the binding of G1/S cyclin-CDK's, which phosphorylates the inhibitor, making it a substrate for the ubiquitinating SCF Ubiquitin-Protein Ligase - Once the inhibitor has been ubiquitinated, the S-phase cyclin-CDK's become active - The cell is then abruptly pushed into S-phase • S-phase cyclin-CDKs promote DNA replication - Phosphorylates and activates numerous proteins that go onto replicate the DNA - The onset of DNA replication means that S-phase has begun - S-phase cyclin/CDKs also prepare the cell for mitosis in a similar way that G1/S CDKs play in G1 • The G1/S transition is **abrupt** because the S-phase cyclin-CDK inhibitor is a poor substrate - Therefore, requires high levels of G1/S kinase to become phosphorylated (G1/S kinase peaks mid G1 phase) - Needs to be phosphorylated on multiple sites - Makes it one of the last substrates to get phosphorylated in G1

Identifying Cell Cycle Genes	**Experimental Process →** • Yeast mutants identified crucial players in the G2/M transition, including activators and inhibitors of the mitotic cyclin/CDK • Screen for temperature-sensitive mutants - Mutagenize, and grow up cells at permissive temperature - Then shift them to restrictive temperature - Characterise lines that fail to grow after the temperature shift • Cell growth and cell division are uncoupled in S. Pombe - Mitosis-defective mutants thus form long rod-shaped cells - Mutants that enter mitosis prematurely show a phenotype of very small cells **Identified Genes →** • Cdc2 is a cyclin dependent kinase (CDK) - cdc2, when lost, gives a long phenotype - cdc2, when dominant, gives a small phenotype • Cdc13 is ac cyclin that forms heterodimers with cdc2 - Cdc13 mutants also give a long phenotype - Cdc13 = mitotic cyclin • Cdc25 drives mitosis (is a phosphatase) - Deficit of Cdc25 results in a long cell phenotype (increased G2) - Excess of Cdc25 results in a small cells phenotype (decreased G2) • Wee1 inhibits mitosis (is a kinase) - Deficit of Wee1 results in a small cell phenotype (decreased G2) - Excess of Wee1 results in a long cell phenotype (increased G2)
Entry into Mitosis	**Entry into Mitosis** → controlled by a cascade of kinase and phosphatase activity • Mitotic cyclin and CDK subunits are assembled - Wee1 phosphorylates Tyrosine Y15 of the CDK subunit, inactivating it - CAK phosphorylates Thymine T161, activating it (however, the inhibition of Y15 means the CDK remains inactive overall) - Cdc25, a phosphatase, reverses the phosphorylation of Y15, creating an active mitotic kinase and allowing mitosis to begin • If Wee1 is lost, there is less regulation, and the cell will enter mitosis early, making it shorter - If Cdc25 is lost, the cell can never enter mitosis, making it long