Cell Biology

Unit 4 · Cell Cycle, Division and Cell Death

A cell grows, copies its DNA, divides, and sometimes chooses to die. This unit explains the four phases of the cell cycle, mitosis and meiosis, the cyclin–CDK engine and its checkpoints, apoptosis, how broken controls cause cancer, stem cells, and how cells are grown and immortalized in culture. After it you can draw and explain each stage, predict what a mutation or drug does to the cycle, and work the cell-count and doubling-time sums examiners set.

Taught at 7 of 10 | Usually Sem 3 | 9 chapters

About this unit

This unit follows a cell through its life: how it grows and copies its DNA (Chapter 4.1), how it splits its chromosomes equally in mitosis (4.2) or halves them in meiosis to make gametes (4.3), which molecules drive the cycle forward (4.4) and which stop it when something is wrong (4.5). It then asks what happens when a cell must die (4.6), when the brakes fail and the cell turns cancerous (4.7), how stem cells renew our tissues (4.8), and how we grow and immortalize cells in the lab (4.9). Read the chapters in order: each one uses the words of the one before.

Chapters

1. Phases of the cell cycle
2. Mitosis
3. Meiosis
4. Regulation of the cell cycle: cyclins and CDKs
5. Cell cycle checkpoints
6. Apoptosis (programmed cell death)
7. Cancer and oncogenes such as Ras and Raf
8. Stem cells and their therapeutic uses
9. Cell culture and immortalization of cells ADVANCED

How to use this book

- Read the lead paragraph and the figure of a chapter first; they carry the one idea.
- Cover the solution of the worked example and do it yourself on paper, then compare step by step.

- Answer each Check yourself question aloud or on paper before you open it.

4.1 Phases of the cell cycle

The cell cycle is the ordered series of events by which one cell grows, copies its DNA exactly once, and divides into two daughter cells. It exists because a human body of roughly 30 trillion cells has to replace worn-out cells every day, from skin to the lining of the gut to blood, without ever losing or doubling a chromosome. The cycle has four phases, G1, S, G2 and M, and a resting state, G0, outside the cycle. Every later chapter of this unit hangs on these names.

> You need: the nucleus and chromatin (Unit 3, Nucleus, nuclear envelope, nuclear pore and nucleolus) and the idea that DNA is packed into chromosomes.

The four phases and G0

The cycle is split into **interphase** (G1, S and G2), when the cell grows and prepares, and the **M phase**, when it divides. Interphase is not a "resting" stage, though old books call it that; the cell is busiest then.

- **G1 (gap 1)**: the cell grows, makes proteins and organelles, and checks whether the outside signals (growth factors, nutrients, space) say "divide". This is the most variable phase. Late in G1 the cell passes the **restriction point**; after it, the cell goes on to divide even if growth factors are removed.
- **S (synthesis)**: the cell replicates its DNA. Each chromosome becomes two identical **sister chromatids** held together by a protein ring called **cohesin**. The centrosome is also duplicated in this window.
- **G2 (gap 2)**: the cell checks that replication is complete and the DNA is undamaged, makes the proteins of the spindle, and keeps growing.
- **M (mitosis)**: the nucleus divides (mitosis, Chapter 4.2) and then the cytoplasm divides (**cytokinesis**).
- **G0**: a cell that leaves G1 and stops cycling. Some cells stay in G0 for life (most neurons, cardiac muscle cells); others, such as liver cells (hepatocytes) and resting lymphocytes, can return to G1 when stimulated.



Figure 4.1.1 Left: the cycle of a typical proliferating human cell in culture drawn as a 24 hour clock; M is the thin slice at the top. Right: DNA per cell stays at 2C through G1, climbs during S, and stays at 4C until the cell divides. Notice that DNA doubles in S, not in mitosis.

How long each phase takes

A typical proliferating human cell in culture takes about 24 hours per cycle: roughly 11 hours in G1, 8 hours in S, 4 hours in G2 and 1 hour in M (from memory of the standard textbook values; check). Two things matter for the exam. First, M is short, so in any field of view under the microscope most cells are in interphase. Second, the length of the whole cycle is set mainly by G1. Early frog embryo cells skip the gaps and alternate S and M in about 30 minutes, while some adult cells sit in G0 for years.

The **DNA content** per cell is written in units of C, where 1C is the DNA of one haploid set. A diploid human cell in G1 is 2C (46 chromosomes, one chromatid each); after S it is 4C (still 46 chromosomes, now two chromatids each). The chromosome number does not change in S; only the chromatid number and the DNA amount do. Flow cytometry measures this DNA content cell by cell and gives the familiar 2C peak, 4C peak and the S-phase valley between them (see Unit 6, Flow cytometry).

Measuring the phases in the lab

In an asynchronous culture (cells at every stage, growing steadily), the fraction of cells you see in a phase is close to the fraction of the cycle that phase takes. So if T is the cycle time:

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duration of a phase ≈ (fraction of cells in that phase) × T
mitotic index (MI) = cells in mitosis ÷ total cells counted
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This is an approximation: a dividing culture has slightly more young cells (in G1) than old ones, so the exact formula has a correction factor, but university exams use the simple form. Other methods: labelling with a thymidine analogue such as BrdU or EdU marks cells in S; staining with propidium iodide gives DNA content; and a protein such as Ki-67 marks any cell in the cycle, not in G0.

WORKED EXAMPLE

Question (6 marks). A culture of human cells doubles every 20 h. Of 1,000 cells counted, 600 are in G1, 280 have intermediate DNA content (between 2C and 4C), 90 have 4C DNA and are not condensed, and 30 are in mitosis. (a) Find the mitotic index. (b) Estimate the length of each phase. (c) What is the DNA content and chromosome number of a G2 cell?

1. Mitotic index: MI = 30 ÷ 1,000 = 0.03, or 3%.
2. Assign the groups: 2C cells are in G1, intermediate cells are in S, 4C uncondensed cells are in G2, and the 30 condensed cells are in M. Check: 600 + 280 + 90 + 30 = 1,000.
3. G1 ≈ 0.60 × 20 h = 12 h. S ≈ 0.28 × 20 h = 5.6 h. G2 ≈ 0.09 × 20 h = 1.8 h. M ≈ 0.03 × 20 h = 0.6 h (36 min).
4. Check the sum: 12 + 5.6 + 1.8 + 0.6 = 20 h.
5. A G2 cell has finished replication, so it holds 4C DNA but still 46 chromosomes, each made of two sister chromatids (92 chromatids).

Answer: MI = 3%; G1 ≈ 12 h, S ≈ 5.6 h, G2 ≈ 1.8 h, M ≈ 0.6 h; a G2 cell is 4C with 46 chromosomes.

EXAM TRAPS

- "Interphase is the resting phase" is wrong. G0 is the resting state; interphase is when growth and DNA synthesis happen.
- Chromosome number stays 46 through S and G2. DNA content goes from 2C to 4C. Do not write "92 chromosomes" for a G2 cell; write "46 chromosomes, 92 chromatids".
- G0 is outside the cycle, not a fifth phase between G2 and M. Cells enter G0 from G1.
- Cytokinesis is part of the M phase but is not the same as mitosis; mitosis is the division of the nucleus.
- Favourite question: "With a neat diagram, describe the phases of the cell cycle." Draw the circle with all four phases, G0, and mark the restriction point in late G1.

CHECK YOURSELF

- REMEMBER **Name the four phases of the eukaryotic cell cycle in order.** 2 marks

  G1, S, G2 and M (M = mitosis followed by cytokinesis). G1, S and G2 together form interphase.

- REMEMBER **What is G0? Give one example of a cell that stays in it.** 2 marks

  A non-dividing state entered from G1, outside the cycle. Mature neurons and cardiac muscle cells stay in G0; hepatocytes can return to G1 after injury.

- UNDERSTAND **Why are most cells in a root-tip squash seen in interphase?** 2 marks

  Because interphase takes most of the cycle time (about 23 of 24 hours in a typical human cell) and mitosis is short, so at any moment most cells are in interphase.

- UNDERSTAND **What changes and what stays the same in S phase?** 3 marks

  DNA content doubles (2C to 4C) and each chromosome becomes two sister chromatids held by cohesin; the centrosome duplicates. The chromosome number (46 in humans) stays the same.

- APPLY **A culture has T = 18 h and a mitotic index of 5%. How long is M?** 2 marks

  M ≈ 0.05 × 18 h = 0.9 h, about 54 minutes.

- APPLY **A flow cytometry plot shows 70% of cells at 2C, 10% at 4C and 20% in between. Which phases are these?** 3 marks

  2C = G1 (and any G0 cells); in between = S; 4C = G2 plus M, which DNA content alone cannot separate.

- ANALYZE **Two cell types have the same S, G2 and M times but different cycle times. Which phase differs, and why is that expected?** 4 marks

  G1 differs. G1 is the phase where the cell reads growth signals and decides whether to divide, so it absorbs most of the variation between cell types; S, G2 and M are relatively fixed.

BEYOND THE SYLLABUS

Live-cell imaging now follows single cells through many cycles using fluorescent reporters such as FUCCI, which colour cells red in G1 and green in S/G2/M. Single-cell RNA sequencing assigns a cycle phase to every cell in a tissue from its gene expression, and analysis tools routinely "regress out" this cell-cycle signal. Cancer pathologists use Ki-67 staining to grade how fast a tumour grows. None of this is for the exam; search "FUCCI reporter" and "Ki-67 proliferation index" to read further.

4.2 Mitosis

Mitosis is the division of a nucleus into two nuclei that each receive one copy of every chromosome. It follows S phase, where every chromosome was copied into two sister chromatids, and its whole job is to pull those sisters apart to opposite ends of the cell before the cytoplasm splits. The result is two daughter cells genetically identical to the parent. Mitosis builds the body from a zygote, heals wounds, and renews skin and blood; when it goes wrong, cells gain or lose chromosomes, which is common in cancer.

You need: 4.1 Phases of the cell cycle (sister chromatids, 2C and 4C) and microtubules (Unit 3, Cytoskeleton: microtubules, actin and intermediate filaments).

The machinery: chromatids, kinetochores and the spindle

Each replicated chromosome has two **sister chromatids** joined along their length by **cohesin** and most tightly at the **centromere**. On each centromere sits a protein plate, the **kinetochore**, one per chromatid, which grips microtubules. The **mitotic spindle** is built from microtubules that grow out of two **centrosomes** (in animal cells each holds a pair of centrioles). There are three kinds of spindle microtubules: **kinetochore microtubules** that attach to chromosomes, **polar (interpolar) microtubules** that overlap in the middle and push the poles apart, and **astral microtubules** that anchor the poles to the cell cortex.

The stages, step by step

1. **Prophase.** Chromatin condenses into visible chromosomes, helped by the protein complex **condensin**. The two centrosomes move apart and the spindle begins to form. The nucleolus fades.

2. **Prometaphase.** The nuclear envelope breaks down when CDK1 phosphorylates the nuclear lamins. Spindle microtubules can now reach the chromosomes and capture kinetochores. Chromosomes jerk back and forth as attachments are made and corrected.
3. **Metaphase.** All chromosomes line up on the **metaphase plate**, the plane midway between the poles, with the two sister kinetochores attached to opposite poles (bi-orientation). The cell waits here until every kinetochore is attached (the spindle checkpoint, Chapter 4.5). Chromosomes are most condensed now, which is why karyotypes are made from metaphase cells.
4. **Anaphase.** The enzyme **separase** cuts cohesin, so the sisters separate at once; each chromatid is now called a chromosome. In **anaphase A** the kinetochore microtubules shorten and pull chromosomes to the poles. In **anaphase B** the polar microtubules slide apart and push the poles further away.
5. **Telophase.** The chromosomes reach the poles and decondense. A nuclear envelope re-forms around each set, and the nucleoli reappear. There are now two nuclei in one cell.
6. **Cytokinesis.** In animal cells a **contractile ring** of actin and myosin II pinches the cell in two along a **cleavage furrow**. In plant cells, which have a rigid wall, vesicles from the Golgi gather at the middle on a structure called the **phragmoplast** and fuse into a **cell plate**, which becomes the new wall.

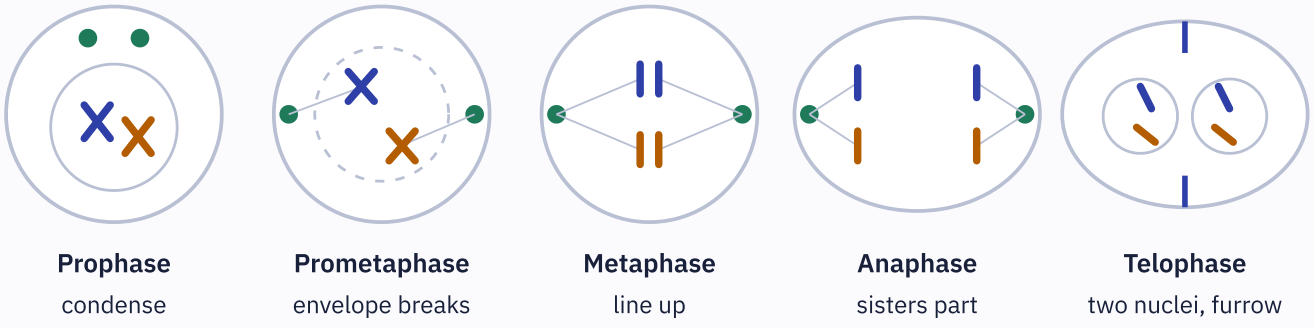


Figure 4.2.1 Mitosis with two chromosomes (blue and orange); green dots are centrosomes. In metaphase the two sister chromatids of each chromosome face opposite poles; in anaphase they move apart, so each daughter nucleus gets one blue and one orange chromosome. The short bars in telophase mark the cleavage furrow.

Why mitosis matters, and how it is studied

Mitosis keeps the chromosome number constant (2n → 2n) and the genetic content identical, which is what growth, repair and asexual reproduction need. The classic practical is the **onion root-tip squash**: the tip of an *Allium cepa* root (2n = 16) is fixed, softened in acid, stained

(acetocarmine or orcein) and squashed, and cells at each stage are counted. Drugs that bind tubulin, such as **colchicine**, stop spindle formation and trap cells in metaphase; this is used to collect metaphase chromosomes for karyotyping. The anticancer drug **paclitaxel (taxol)** does the opposite, freezing microtubules so they cannot shorten, and it also blocks mitosis.

WORKED EXAMPLE

Question (6 marks). An onion root cell has 2n = 16. (a) Give the number of chromosomes, chromatids and the DNA content (in C) of one cell in G1, metaphase, and anaphase, and of one daughter cell after cytokinesis. (b) In a squash, 52 of 400 cells are in some stage of mitosis. Find the mitotic index.

STAGE	CHROMOSOMES	CHROMATIDS	DNA
G1	16	16	2C
Metaphase	16	32	4C
Anaphase (whole cell)	32	32	4C
Daughter cell	16	16	2C

1. After S phase each of the 16 chromosomes has two chromatids, so metaphase has 16 chromosomes, 32 chromatids, 4C.
2. In anaphase the sisters separate and each is counted as a chromosome: 32 chromosomes in one cell, still 4C, until the cell divides.
3. Each daughter receives 16 chromosomes of one chromatid each: 2C, like the parent in G1.
4. Mitotic index = 52 ÷ 400 = 0.13 = 13%.

EXAM TRAPS

- In anaphase a human cell briefly has 92 chromosomes (an onion cell 32). Examiners love this; count separated chromatids as chromosomes.
- Mitosis is nuclear division; cytokinesis is cytoplasmic division. Some cells (osteoclasts, early insect embryos) do mitosis without cytokinesis and become multinucleate.
- Plant cells have no centrioles and no cleavage furrow; they build a cell plate from the inside out.
- Colchicine arrests cells in metaphase by preventing spindle assembly; it does not "break chromosomes".
- Do not write "chromosomes replicate in prophase". Replication happened earlier, in S phase.

CHECK YOURSELF

– REMEMBER **List the stages of mitosis in order.** 2 marks

Prophase, prometaphase, metaphase, anaphase, telophase; cytokinesis follows. Some books merge prometaphase into prophase.

– REMEMBER **Which enzyme cuts cohesin at anaphase?** 1 mark

Separase.

– UNDERSTAND **Distinguish anaphase A from anaphase B.** 3 marks

Anaphase A: kinetochore microtubules shorten and pull chromosomes to the poles. Anaphase B: polar microtubules slide apart (and astral microtubules pull) so the poles move further apart.

– UNDERSTAND **How does cytokinesis differ in plant and animal cells?** 4 marks

Animal: an actin–myosin contractile ring forms a cleavage furrow that pinches the cell from outside in. Plant: Golgi vesicles collect at the phragmoplast and fuse into a cell plate that grows from the centre out to the old wall.

– APPLY **A cell with 2n = 8 is in metaphase of mitosis. How many kinetochores does it have?** 2 marks

One kinetochore per chromatid: 8 chromosomes × 2 chromatids = 16 kinetochores.

– APPLY **Why would you treat cells with colchicine before making a karyotype?** 2 marks

Colchicine blocks spindle assembly, so cells pile up in metaphase, when chromosomes are most condensed and easiest to count and band.

– ANALYZE **A drug lets cells enter mitosis but chromosomes never separate, although the spindle looks normal. Suggest two targets.** 4 marks

Separase (cannot cut cohesin) or the APC/C (cannot destroy securin, which keeps separase inactive). A non-cleavable cohesin would give the same result.

BEYOND THE SYLLABUS

Errors in mitosis produce aneuploid cells, and most solid tumours are aneuploid. Live imaging of fluorescent histones and tubulin lets researchers measure how long each cell spends in each stage, and drug screens look for compounds that stop mitosis only in cancer cells, for example inhibitors of the mitotic kinases Aurora and Polo-like kinase 1. This is not for the exam; search "chromosomal instability" and "PLK1 inhibitor" to go further.

4.3 Meiosis

Meiosis is the special division that makes gametes (sperm and eggs in animals, spores in plants). One round of DNA replication is followed by two divisions, so one diploid cell (2n) gives four haploid cells (n). Halving the chromosome number keeps it constant from generation to generation when two gametes fuse at fertilization. On the way, meiosis shuffles the parents' chromosomes and swaps pieces between them, so every gamete is genetically unique. This is the cellular basis of Mendel's laws and of most variation in a population.

You need: 4.2 Mitosis (chromatids, kinetochores, the spindle). Meiosis reuses the same machinery with a few key changes.

Homologues, bivalents and crossing over

A diploid cell has two copies of each chromosome, one from the mother and one from the father. These two are **homologous chromosomes** (homologues): same genes in the same order, possibly different alleles. In mitosis homologues act independently. In meiosis I they find each other and pair along their length (**synapsis**), held by a protein zipper called the **synaptonemal complex**. A paired set is a **bivalent**, also called a **tetrad** because it has four chromatids. Inside the bivalent, non-sister chromatids break and rejoin with each other: this is **crossing over**, and the visible cross-shaped point where they remain linked is a **chiasma** (plural chiasmata).

Chiasmata also hold the homologues together until anaphase I, so they are needed for correct separation.

Meiosis I: the reductional division

Prophase I is long and is split into five substages, a favourite exam list:

1. **Leptotene**: chromosomes condense into thin threads.
2. **Zygotene**: homologues pair; the synaptonemal complex starts to form (synapsis).
3. **Pachytene**: synapsis is complete; crossing over takes place at recombination nodules.
4. **Diplotene**: the synaptonemal complex dissolves and homologues pull apart except at chiasmata, which become visible. Human oocytes pause here (the dictyotene stage) from before birth until ovulation, years later.
5. **Diakinesis**: chromosomes condense fully, chiasmata move toward the chromosome ends (terminalization), the nucleolus and nuclear envelope disappear.

In **metaphase I** the bivalents, not single chromosomes, line up on the plate. Both sister kinetochores of one homologue face the same pole, and the two homologues face opposite poles. Each bivalent orients independently of the others: this is **independent assortment**. In **anaphase I** the homologues separate and move to opposite poles; the sister chromatids stay joined because the cohesin at the centromere is protected (by a protein called shugoshin). **Telophase I** and cytokinesis give two cells, each with n chromosomes, each chromosome still made of two chromatids. Meiosis I is therefore the division that halves the number.

Meiosis II: the equational division

After a short pause (**interkinesis**) with **no DNA replication**, each cell divides again. Meiosis II works like mitosis: chromosomes line up singly at metaphase II, the centromeric cohesin is cut, and sister chromatids go to opposite poles at anaphase II. The result is four haploid cells, each with n single-chromatid chromosomes (1C DNA). Because of crossing over, the two sisters were no longer identical, so all four products differ.

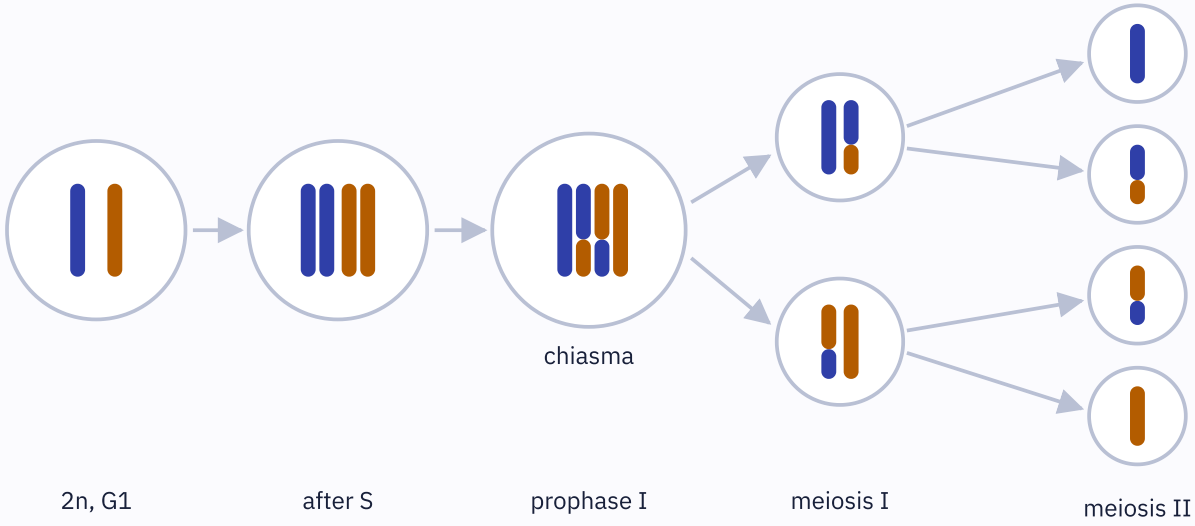


Figure 4.3.1 One homologous pair (blue from the mother, orange from the father). After S each homologue has two chromatids; in prophase I they pair and two non-sister chromatids exchange their lower ends (the chiasma). Meiosis I separates the homologues; meiosis II separates the sisters. Notice that the four haploid cells are all different.

Why it matters: variation and errors

Three sources of variation follow from meiosis and fertilization. **Independent assortment**: with n pairs, a parent can make 2^n chromosome combinations; for humans (n = 23) that is 2^{23} = 8,388,608, before any crossing over. **Crossing over** creates new allele combinations within each chromosome. **Random fertilization** pairs any sperm with any egg: $2^{23} \times 2^{23} \approx 7 \times 10^{13}$ possible zygotes from one couple, still ignoring crossing over.

When homologues (in meiosis I) or sisters (in meiosis II) fail to separate, a gamete gets an extra or missing chromosome. This is **nondisjunction**. A gamete with two copies of chromosome 21 fertilized by a normal one gives trisomy 21 (Down syndrome); the risk rises with maternal age, partly because oocytes stay arrested in prophase I for decades. In males one spermatocyte gives four sperm; in females one oocyte gives one egg and two or three small polar bodies.

FEATURE	MITOSIS	MEIOSIS
Divisions after one S phase	one	two
Daughter cells	2, diploid, identical	4, haploid, all different
Pairing of homologues	no	yes (prophase I)
Crossing over	no (rare)	yes
At metaphase plate	single chromosomes	bivalents (I), single chromosomes (II)

FEATURE	MITOSIS	MEIOSIS
Where	somatic cells	germ cells in gonads, plant sporangia

WORKED EXAMPLE

Question (6 marks). A cell has 2n = 8. Give the chromosome number, chromatid number and DNA content (C) per cell at: G1, metaphase I, end of telophase I, and end of telophase II. How many chromosome combinations can its gametes have without crossing over?

STAGE	CHROMOSOMES	CHROMATIDS	DNA
G1 (before S)	8	8	2C
Metaphase I	8 (as 4 bivalents)	16	4C
After telophase I (each cell)	4	8	2C
After telophase II (each cell)	4	4	1C

1. S doubles DNA (2C → 4C) and chromatids (8 → 16) but not chromosomes.
2. Meiosis I separates homologues: chromosome number halves to 4; each still has two chromatids, so 8 chromatids and 2C.
3. Meiosis II separates sisters: 4 chromosomes, 4 chromatids, 1C.
4. Combinations: n = 4 pairs, so $2^4 = 16$.

EXAM TRAPS

- The chromosome number halves in meiosis I, not meiosis II. Meiosis II halves the DNA content (2C → 1C) but not the number.
- There is no S phase between meiosis I and II. Interkinesis is not interphase.
- Crossing over happens in pachytene; chiasmata become visible in diplotene. Do not swap these.
- Crossing over is between non-sister chromatids of homologues, not between sister chromatids.
- 2^n uses the haploid number n: humans give 2^{23}, not 2^{46}.

CHECK YOURSELF

- REMEMBER **Name the five substages of prophase I in order.** 2 marks

  Leptotene, zygotene, pachytene, diplotene, diakinesis.

- REMEMBER **What is the synaptonemal complex?** 2 marks

  A protein structure that zips homologous chromosomes together along their length during zygotene and pachytene.

- UNDERSTAND **Why is meiosis I called reductional and meiosis II equational?** 3 marks

  Meiosis I separates homologues, halving the chromosome number (2n → n). Meiosis II separates sister chromatids; the chromosome number stays n in each product, like mitosis.

- UNDERSTAND **Give two roles of chiasmata.** 2 marks

  They are where crossing over has joined non-sister chromatids (recombination), and they hold homologues together so the bivalent orients correctly and separates properly at anaphase I.

- APPLY **A plant has 2n = 24. How many bivalents form, and how many chromosomes are in each pollen grain nucleus?** 2 marks

  12 bivalents; each haploid product has 12 chromosomes.

- APPLY **Nondisjunction of chromosome 21 happens in meiosis I in a mother. What fraction of her gametes from that cell carry two copies?** 3 marks

  In meiosis I both homologues go to one cell; after meiosis II that cell's two products each carry two copies of chromosome 21, and the other two carry none. So 2 of 4 have two copies (though in oogenesis only one becomes the egg).

- ANALYZE **A mutant lacks the protection of centromeric cohesin in meiosis I. What goes wrong?** 4 marks

  Sister chromatids separate too early, at anaphase I, along with the homologues. In meiosis II there are single chromatids with nothing to pair them, so they segregate randomly and many gametes become aneuploid.

BEYOND THE SYLLABUS

Recombination in meiosis starts with deliberate DNA double-strand breaks made by an enzyme called Spo11, which are then repaired using the homologue; this links meiosis to DNA repair biology. Geneticists use crossover frequencies to build linkage maps, and fertility clinics screen embryos for aneuploidy. Not for the exam; search "Spo11 double-strand break" and "maternal age aneuploidy" to read further.

4.4 Regulation of the cell cycle: cyclins and CDKs

The cell cycle is driven by a family of enzymes called **cyclin-dependent kinases (CDKs)**. A CDK is a protein kinase: it adds phosphate groups to serine or threonine residues of target proteins and so switches them on or off. A CDK is inactive on its own; it works only when bound to a partner protein, a **cyclin**, whose level rises and falls in each cycle. Different cyclin–CDK pairs appear at different times and push the cell through G1, into S, and into mitosis.

You need: 4.1 Phases of the cell cycle and the idea of a kinase (Unit 5, Kinase pathways).

How the engine was found

Three lines of work met in the 1980s. In 1971 Yoshio Masui and Clement Markert showed that cytoplasm from a mature frog egg, injected into an immature oocyte, made it enter M phase; they called the unknown agent **maturation-promoting factor (MPF)**, later read as M-phase promoting factor. Leland Hartwell found *cdc* (cell division cycle) mutants in budding yeast that stopped at fixed points, and named the commitment point in G1 **Start**. Paul Nurse found the fission yeast gene *cdc2*, a kinase, and its human equivalent. Tim Hunt saw a protein in sea urchin eggs that built up and then vanished at every division and named it cyclin. MPF turned out to be cyclin B bound to Cdc2 (now called CDK1). Hartwell, Hunt and Nurse shared the 2001 Nobel Prize in Physiology or Medicine.

Which cyclin with which CDK, and when

CDK levels stay roughly constant; cyclin levels oscillate. In mammals the main pairs are:

COMPLEX	ACTIVE IN	WHAT IT DOES
Cyclin D–CDK4 or CDK6	G1 (as long as growth factors are present)	Starts phosphorylating Rb; links outside signals to the cycle
Cyclin E–CDK2	Late G1 to G1/S	Completes Rb phosphorylation; commits the cell to S; starts DNA replication
Cyclin A–CDK2 (later CDK1)	S and G2	Drives replication and stops it from being repeated; prepares mitosis
Cyclin B–CDK1 (MPF)	G2/M to metaphase	Condenses chromosomes, breaks down the nuclear envelope (lamin phosphorylation), builds the spindle

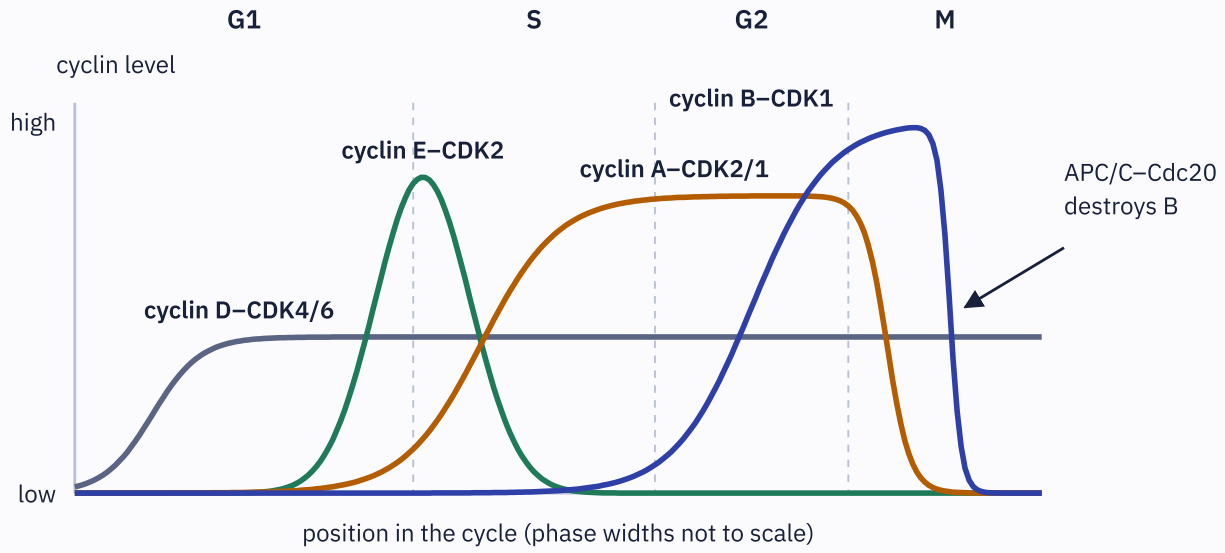


Figure 4.4.1 Relative levels of the four cyclins across one cycle, drawn as smooth model curves (not measured data). Cyclin D tracks growth-factor signals; cyclin E peaks at the G1/S transition; cyclin A rises in S and falls early in M; cyclin B peaks in mitosis and is destroyed at the metaphase–anaphase transition by the APC/C.

Four ways the cell controls a CDK

1. **Cyclin supply and destruction.** Cyclin genes are transcribed at set times, and cyclins are destroyed by ubiquitin tagging followed by the proteasome. Two ubiquitin ligases do this: **SCF** (active from late G1, destroys cyclin E and CDK inhibitors) and the **anaphase-promoting complex/cyclosome (APC/C)** (active from mid-mitosis to G1, with its partner Cdc20 or Cdh1; destroys securin and cyclins A and B).
2. **Activating phosphorylation.** A cyclin–CDK pair becomes fully active only when **CDK-activating kinase (CAK)**, itself a complex of CDK7 and cyclin H, phosphorylates a threonine in the CDK's activation loop.

3. **Inhibitory phosphorylation.** The kinase **Wee1** adds a phosphate to tyrosine 15 near the CDK's ATP site, holding cyclin B–CDK1 off during G2. The phosphatase **Cdc25** removes it to start mitosis. Active CDK1 then activates more Cdc25 and inhibits Wee1, a **positive feedback** loop that makes the entry into mitosis sudden and complete, like a switch.
4. **CDK inhibitor proteins (CKIs).** The **INK4** family (p15, p16, p18, p19) blocks CDK4/6. The **Cip/Kip** family (p21, p27, p57) blocks CDK2 and CDK1 complexes. p21 is the protein that p53 switches on after DNA damage (Chapter 4.5).

The Rb–E2F switch at the restriction point

In early G1 the **retinoblastoma protein (Rb)** binds the transcription factor **E2F** and keeps it silent. Growth factors activate the Ras–MAPK pathway (Chapter 4.7), which raises cyclin D. Cyclin D–CDK4/6 partly phosphorylates Rb, freeing some E2F. E2F turns on the genes for cyclin E, cyclin A and replication enzymes. Cyclin E–CDK2 then phosphorylates Rb fully, freeing more E2F, which makes more cyclin E. This positive feedback is the **restriction point**: once passed, the cell no longer needs growth factors to finish the cycle. Rb loss removes the switch, which is why *RB1* is a tumour suppressor.

WORKED EXAMPLE

Question (8 marks). Predict what happens to cells in each case, and explain why. (a) Fission yeast lacking Wee1. (b) Cells lacking Cdc25. (c) Cells expressing a cyclin B that cannot be ubiquitinated. (d) Cells over-expressing p16.

1. **(a) No Wee1.** Nothing holds CDK1 off with the tyrosine 15 phosphate, so cyclin B–CDK1 turns on early and cells enter mitosis before growing to normal size. They divide small: this "wee" phenotype gave the gene its name.
2. **(b) No Cdc25.** The inhibitory phosphate cannot be removed, so CDK1 stays off. Cells keep growing but arrest in G2; in yeast they become very long.
3. **(c) Non-degradable cyclin B.** The APC/C still destroys securin, so separase cuts cohesin and sisters separate. But CDK1 stays active, so cells cannot carry out telophase and cytokinesis; they arrest late in mitosis with separated chromosomes.
4. **(d) Too much p16.** CDK4/6 is blocked, Rb stays unphosphorylated, E2F stays bound, and cells arrest in G1.

Rule used: follow the chain CKI or kinase → CDK activity → target (Rb, lamins, securin) → phase. Name the phase where the cell stops.

EXAM TRAPS

- CDK levels are roughly constant; it is the cyclin level that oscillates. Write "cyclin levels rise and fall", not "CDKs are made and destroyed".
- MPF = cyclin B + CDK1 (Cdc2). Do not pair cyclin B with CDK4.
- Wee1 inhibits and Cdc25 activates CDK1. A common slip is to swap them.
- Phosphorylated Rb is inactive (releases E2F). Hypophosphorylated Rb is the active brake.
- The APC/C is a ubiquitin ligase, not a kinase or protease; the proteasome does the destruction.

CHECK YOURSELF

– REMEMBER **What is MPF made of?** 1 mark

Cyclin B bound to CDK1 (Cdc2).

– REMEMBER **Name the two families of CDK inhibitors with one member each.** 2 marks

INK4 (for example p16) and Cip/Kip (for example p21 or p27).

– UNDERSTAND **Why is a CDK called "cyclin-dependent"?** 2 marks

It has no useful kinase activity alone; binding a cyclin opens its active site and directs it to targets, so its activity depends on cyclin presence.

– UNDERSTAND **Explain how phosphorylation of Rb pushes a cell from G1 into S.** 4 marks

Unphosphorylated Rb binds E2F. Cyclin D–CDK4/6 and then cyclin E–CDK2 phosphorylate Rb, which releases E2F; E2F switches on S-phase genes (cyclin E, cyclin A, DNA polymerase). Positive feedback through cyclin E makes the step irreversible.

– APPLY **Palbociclib is a CDK4/6 inhibitor used in breast cancer. In which phase do treated cells stop, and why?** 3 marks

G1. Without CDK4/6 activity Rb is not phosphorylated, E2F stays bound, and the cell cannot pass the restriction point. It works only if the tumour still has functional Rb.

– APPLY **A mutant CDK1 has phenylalanine instead of tyrosine at position 15. Predict its phenotype.** 3 marks

Wee1 cannot phosphorylate residue 15, so CDK1 cannot be held off; cells enter mitosis early, often before replication or repair is finished, like the wee1 mutant.

– ANALYZE **Why does the cell use positive feedback at both the restriction point and the G2/M transition?** 4 marks

Positive feedback turns a gradual rise in a signal into an all-or-none, irreversible switch. A cell must not be half in S or half in mitosis, and it must not slip back; feedback through E2F–cyclin E and through CDK1–Cdc25–Wee1 gives this.

BEYOND THE SYLLABUS

CDK4/6 inhibitors (palbociclib, ribociclib, abemaciclib) are now standard drugs for hormone-receptor-positive breast cancer, and inhibitors of CDK2, Wee1 and CDK7 are in clinical trials. Systems biologists model the cycle as a set of coupled feedback loops that behave like an oscillator. Not for the exam; search "CDK4/6 inhibitor mechanism" and "cell cycle bistability" to read further.

4.5 Cell cycle checkpoints

A checkpoint is a control mechanism that stops the cell cycle until a required event has finished correctly: DNA must be undamaged before it is copied, fully copied before it is divided, and every chromosome attached to the spindle before the sisters are pulled apart. Checkpoints act on the cyclin–CDK engine of Chapter 4.4, holding it off until the problem is fixed or, if it cannot be fixed, sending the cell to permanent arrest or death. When checkpoints fail, mutations and chromosome errors pile up, which is a key step towards cancer.

You need: 4.4 Cyclins and CDKs (CDK inhibitors, Wee1 and Cdc25, the APC/C).

How a checkpoint is built

Every checkpoint has three parts, which makes answers easy to structure: **sensors** that detect the problem (a broken DNA end, a stalled replication fork, an unattached kinetochore), **transducers** (usually protein kinases) that amplify the signal, and **effectors** that block a specific cyclin–CDK or the APC/C. The arrest gives the cell time to repair; it is not itself a repair process.

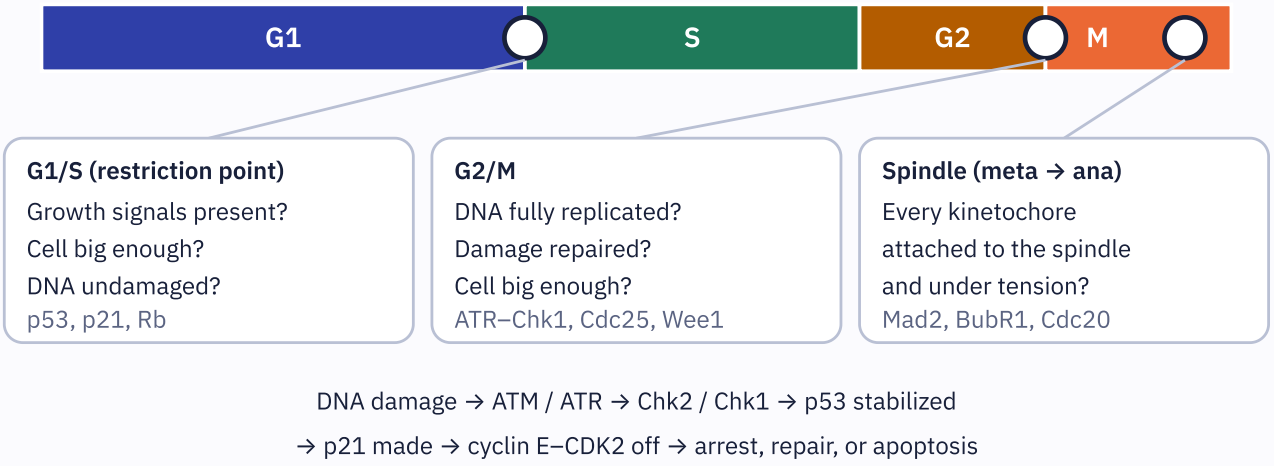


Figure 4.5.1 The three main checkpoints (open circles) on the cycle, what each one asks, and its key proteins in grey. The two lines at the bottom trace the DNA damage pathway that arrests cells in G1. Notice that the spindle checkpoint sits inside M, before anaphase.

The DNA damage checkpoints (G1/S, intra-S and G2/M)

1. **Sensing.** The kinase **ATM** is activated by DNA double-strand breaks (for example from X-rays). The kinase **ATR** is activated by single-stranded DNA, as at stalled replication forks or UV damage being processed.
2. **Transducing.** ATM activates the kinase **Chk2**; ATR activates **Chk1**. Both phosphorylate **p53** and the phosphatases of the Cdc25 family.
3. **G1 arrest through p53.** Normally p53 is kept low because the protein **MDM2** tags it for destruction. Phosphorylated p53 escapes MDM2, builds up, and acts as a transcription factor. It switches on **p21**, which inhibits cyclin E–CDK2 (and cyclin A–CDK2), so Rb stays unphosphorylated and the cell stays in G1. Chk1 and Chk2 also cause degradation of Cdc25A, which gives a fast, p53-independent brake.
4. **G2 arrest.** Chk1 phosphorylates **Cdc25C**, which is then held in the cytoplasm by 14-3-3 proteins. Without Cdc25C, CDK1 keeps its inhibitory tyrosine 15 phosphate from Wee1, and the cell cannot enter mitosis.
5. **Outcome.** If repair succeeds, p53 falls and the cycle resumes. If damage is too great, p53 switches on pro-apoptotic genes such as *PUMA*, *NOXA* and *BAX* (Chapter 4.6), or drives permanent arrest (senescence).

Because p53 stops damaged cells from dividing it is called the "guardian of the genome". The *TP53* gene is mutated in about half of all human cancers, more often than any other gene (from memory; check). Inherited loss of one copy causes Li-Fraumeni syndrome, with early cancers of many types; inherited loss of ATM causes ataxia telangiectasia, with radiation sensitivity.

A related guard stops DNA from being copied twice in one cycle. Origins are "licensed" in G1 when the MCM helicase is loaded on them, and fired in S; high CDK activity and the protein geminin then block new licensing until the next G1. This is why each origin fires only once per cycle.

The spindle assembly checkpoint (SAC)

Anaphase is irreversible: once cohesin is cut, chromatids cannot be rejoined. So the cell waits in metaphase until every kinetochore is attached. Each **unattached kinetochore** catalyses the formation of the **mitotic checkpoint complex (MCC)**, made of Mad2, BubR1, Bub3 and Cdc20. The MCC holds Cdc20 so the APC/C cannot use it. Securin and cyclin B therefore survive, separase stays inhibited, and anaphase is blocked. A single unattached kinetochore is enough to hold the whole cell. When the last kinetochore attaches, MCC production stops, APC/C–Cdc20 turns on, and anaphase starts within minutes. The kinase Aurora B detects wrong attachments that lack tension (both sisters tied to the same pole) and releases them so they can be corrected. Drugs that disturb microtubules, such as nocodazole and paclitaxel, keep the SAC on and arrest cells in mitosis.

WORKED EXAMPLE

Question (8 marks). Two human cell lines, one with normal p53 and one lacking p53, are given a dose of X-rays. The table gives the percentage of cells in each phase, measured by DNA content, before and 16 h after (illustrative data). Explain the results.

LINE	TIME	G1	S	G2/M
Normal p53	before	55	30	15
Normal p53	16 h	70	8	22
No p53	before	55	30	15
No p53	16 h	25	20	55

1. X-rays make double-strand breaks, which activate ATM and Chk2.
2. Normal line: p53 is stabilized, p21 is made, cyclin E–CDK2 is blocked. Cells in G1 cannot enter S, so G1 rises from 55% to 70% and S falls from 30% to 8%. Cells already past G1 stop at G2/M (22%) through Chk1/Chk2 acting on Cdc25.
3. No-p53 line: the G1 checkpoint is lost. Damaged G1 cells enter S and move on, so G1 falls to 25%. They pile up at G2/M (55%) because the G2 checkpoint works through Chk1–Cdc25C–CDK1 and does not need p53.
4. Conclusion: p53 is needed for G1 arrest after DNA damage but not for G2 arrest. The p53-null cells replicate damaged DNA, which raises the chance of mutations.

EXAM TRAPS

- A checkpoint stops the cycle; it does not repair DNA. Repair is done by separate repair pathways while the cycle is paused.
- p21 is a CDK inhibitor made because of p53; p53 itself does not bind CDKs.
- The spindle checkpoint targets the APC/C (through Cdc20), not a CDK directly.
- ATM responds to double-strand breaks; ATR to single-stranded DNA and stalled forks. Do not reverse them.
- The restriction point (growth-factor dependence) and the G1 DNA-damage checkpoint both act in late G1 but sense different things. Name both when asked about "the G1 checkpoint".

CHECK YOURSELF

- REMEMBER **Name the three major cell cycle checkpoints.** 3 marks

  G1/S (restriction point and DNA damage), G2/M (replication complete and DNA intact), and the spindle assembly checkpoint at the metaphase–anaphase transition.

- REMEMBER **Which protein keeps p53 levels low in an undamaged cell?** 1 mark

  MDM2, a ubiquitin ligase that targets p53 for destruction.

- UNDERSTAND **Explain how one unattached kinetochore can stop anaphase.** 4 marks

  It catalyses formation of the mitotic checkpoint complex (Mad2, BubR1, Bub3, Cdc20), which inhibits APC/C–Cdc20. Securin and cyclin B are not destroyed, separase stays inactive, cohesin stays intact, so no anaphase.

- UNDERSTAND **Why is p53 called the guardian of the genome?** 2 marks

  It senses DNA damage signals and stops the cycle (through p21) or triggers apoptosis, so cells with damaged DNA do not pass mutations on.

- APPLY **Cells are treated with nocodazole for 12 h. What happens to the mitotic index, and why?** 3 marks

  It rises sharply. Nocodazole stops microtubules forming, so kinetochores stay unattached, the SAC stays on, and cells accumulate in prometaphase/metaphase.

- APPLY **A tumour lacks Chk1 activity. Which arrest after UV damage is weakened?** 2 marks

  The intra-S and G2/M arrests that depend on ATR–Chk1 acting on Cdc25; cells may enter mitosis with damaged or unreplicated DNA.

- ANALYZE **Many cancers lack p53. Why might Chk1 or Wee1 inhibitors kill such cancer cells more than normal cells?** 4 marks

  p53-null cells cannot arrest in G1 and depend on the G2/M checkpoint to repair damage before mitosis. Blocking Chk1 or Wee1 removes that last brake, so they enter mitosis with damaged DNA and die. Normal cells still have the p53-driven G1 arrest.

BEYOND THE SYLLABUS

The idea in the last question, killing a cancer cell by removing the backup of a pathway it has already lost, is called synthetic lethality. Its best-known use is PARP inhibitors (such as olaparib) in BRCA-mutant breast and ovarian cancers. Chk1, ATR and Wee1 inhibitors are in clinical trials on the same logic. Not for the exam; search "synthetic lethality PARP BRCA" to read further.

4.6 Apoptosis (programmed cell death)

Apoptosis is a tidy, energy-requiring form of cell suicide carried out by a set of proteases called caspases. The cell shrinks, its chromatin condenses, its DNA is cut into regular pieces, and it breaks into membrane-wrapped fragments that neighbouring cells or macrophages swallow, with no inflammation. The body uses it to sculpt organs (removing the webbing between fingers), to remove unwanted immune cells, and to kill cells whose DNA is beyond repair. Too little apoptosis helps cancer survive; too much destroys neurons in degenerative disease.

You need: 4.5 Cell cycle checkpoints (p53) and mitochondria (Unit 3, Mitochondria: structure and function).

Apoptosis versus necrosis

The word apoptosis (Greek, "falling off", as leaves fall from a tree) was introduced in 1972 by John Kerr, Andrew Wyllie and Alastair Currie to separate this orderly death from **necrosis**, the accidental death caused by injury.

FEATURE	APOPTOSIS	NECROSIS
Cause	Programmed: signals, DNA damage, development	Injury: toxins, heat, loss of blood supply
Cell size	Shrinks	Swells
Membrane	Stays intact; blebs; forms apoptotic bodies	Bursts; contents leak out
DNA	Cut between nucleosomes: "ladder" on a gel	Degraded at random: "smear"
Energy	Needs ATP	Does not
Inflammation	None	Yes

FEATURE	APOPTOSIS	NECROSIS
Cells affected	Single cells	Groups of cells

The genes were found in the nematode worm *Caenorhabditis elegans*, where exactly 131 of the 1,090 somatic cells made during development die. The worm genes *ced-3* (a caspase), *ced-4* (the equivalent of human Apaf-1) and *ced-9* (the equivalent of Bcl-2) have human counterparts. Sydney Brenner, Robert Horvitz and John Sulston shared the 2002 Nobel Prize in Physiology or Medicine for this work.

The executioners: caspases

Caspases are cysteine proteases that cut their targets just after an aspartate residue (cysteine-aspartate protease). They are made as inactive **procaspases**. **Initiator caspases** (caspase-8, -9 and -10) are activated by being brought close together on a protein platform; they then cut and activate the **executioner caspases** (caspase-3, -6 and -7). Executioners cleave hundreds of proteins: nuclear lamins (the nucleus collapses), cytoskeletal proteins (the cell blebs), and ICAD, the inhibitor of a nuclease called CAD. Freed CAD cuts DNA in the linker regions between nucleosomes, giving fragments in multiples of about 180–200 base pairs, the **DNA ladder**. The membrane lipid **phosphatidylserine (PS)**, normally on the inner face, flips to the outer face and acts as an "eat me" signal for phagocytes.

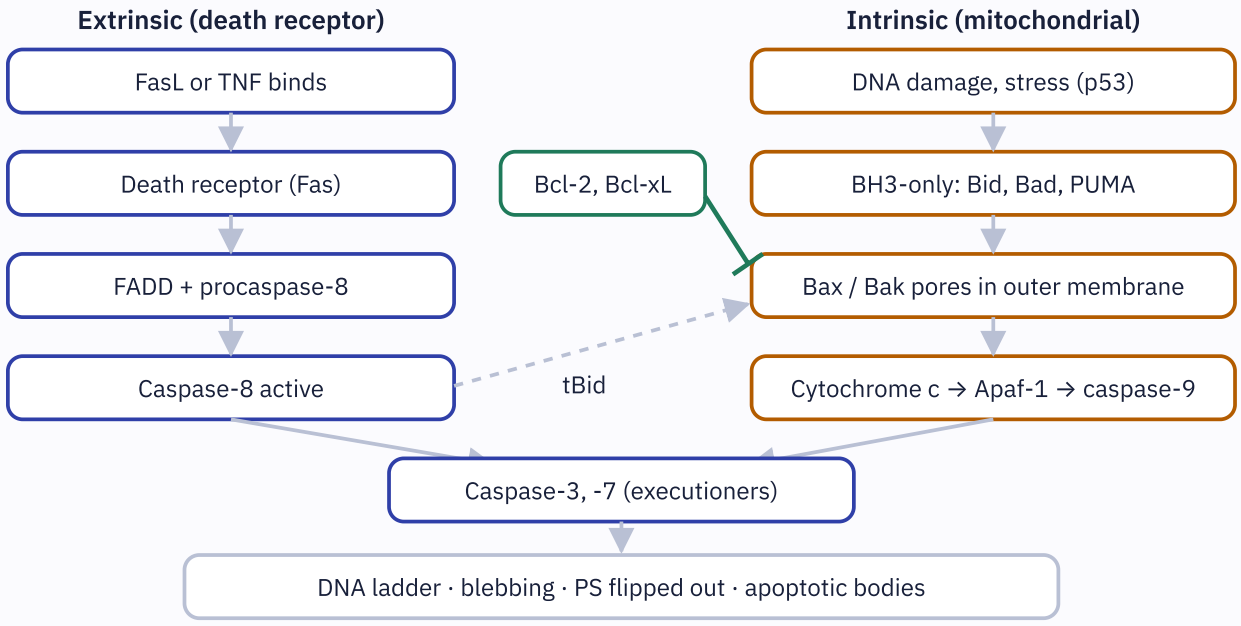


Figure 4.6.1 The two main routes to apoptosis. Left: a death ligand on the outside activates caspase-8. Right: internal stress makes BH3-only proteins switch on Bax and Bak, which release cytochrome c from mitochondria to build the apoptosome and activate caspase-9. Bcl-2 (green) blocks Bax and Bak. The dashed tBid arrow shows how caspase-8 can recruit the mitochondrial route. Both routes end at caspase-3 and -7.

Two pathways in

1. **Extrinsic (death receptor) pathway.** A ligand such as Fas ligand or TNF binds its receptor (Fas, TNF receptor 1) on the target cell. The receptor's cytoplasmic death domain recruits the adaptor **FADD** and procaspase-8, forming the **death-inducing signalling complex (DISC)**. Procaspase-8 molecules activate each other. Cytotoxic T cells use Fas ligand to kill infected cells this way.
2. **Intrinsic (mitochondrial) pathway.** DNA damage (through p53), growth-factor withdrawal or stress activate **BH3-only proteins** (Bid, Bad, Bim, PUMA, Noxa). These activate **Bax** and **Bak**, which form pores in the outer mitochondrial membrane (mitochondrial outer membrane permeabilization, MOMP). **Cytochrome c** leaks out and, with **Apaf-1** and dATP, builds a wheel-shaped complex, the **apoptosome**, which activates caspase-9. Mitochondria also release Smac (DIABLO), which neutralizes the caspase blockers called IAPs (inhibitors of apoptosis, such as XIAP).
3. **Brakes and crosstalk.** Anti-apoptotic members of the same family, **Bcl-2**, Bcl-xL and Mcl-1, bind and hold Bax and Bak. The balance between pro- and anti-apoptotic Bcl-2 proteins decides whether a cell dies. Caspase-8 can cut Bid into tBid, which triggers the mitochondrial route and amplifies a weak extrinsic signal.

Ras and Raf: survival signals

Growth factors keep cells alive as well as dividing, and much of this goes through Ras (Chapter 4.7). Ras activates PI3-kinase, which activates the kinase **Akt**; Akt phosphorylates **Bad**, which is then held by 14-3-3 proteins and cannot release Bcl-2's brake. The Ras → Raf → MEK → ERK cascade adds a second shield: ERK phosphorylates **Bim** and marks it for destruction, and raises Mcl-1. In normal cells, though, a sudden strong oncogenic Ras signal can trigger p53 and stop the cell, a safety net that must also be lost before cancer forms.

WORKED EXAMPLE

Question (6 marks). Cancer cells treated with a drug for 24 h are stained with annexin V–FITC (binds exposed PS) and propidium iodide (PI, enters only cells with broken membranes) and 10,000 cells are read on a flow cytometer: annexin$^-$PI$^-$ 6,200; annexin$^+$PI$^-$ 2,300; annexin$^+$PI$^+$ 1,100; annexin$^-$PI$^+$ 400. (a) Classify each group and give percentages. (b) What fraction is apoptotic? (c) DNA from the treated cells gives bands at about 200, 400 and 600 bp. What does this show?

1. Annexin$^-$PI$^-$: live cells, 6,200 ÷ 10,000 = 62%.
2. Annexin$^+$PI$^-$: PS is exposed but the membrane is intact: early apoptotic, 23%.
3. Annexin$^+$PI$^+$: PS exposed and membrane broken: late apoptotic (secondary necrosis), 11%.
4. Annexin$^-$PI$^+$: membrane broken without PS exposure: necrotic or damaged, 4%.
5. Apoptotic = early + late = 23% + 11% = 34%.
6. Bands at multiples of about 200 bp are the DNA ladder made when CAD cuts linker DNA between nucleosomes: the cells die by apoptosis, not necrosis, which would give a smear.

EXAM TRAPS

- Bcl-2 is anti-apoptotic; Bax, Bak and the BH3-only proteins are pro-apoptotic. They belong to the same family, so read the name carefully.
- Cytochrome c triggers apoptosis only when it leaves the mitochondrion; inside, it is part of the electron transport chain.
- Caspase-8 belongs to the extrinsic route and caspase-9 to the intrinsic route; caspase-3 is shared.
- Apoptosis needs ATP and makes no inflammation. Writing "inflammation" or "swelling" describes necrosis.
- Favourite question: "Compare the intrinsic and extrinsic pathways of apoptosis with a diagram." Draw both, show where they meet, and name Bcl-2.

CHECK YOURSELF

– REMEMBER **Name two initiator and two executioner caspases.** 2 marks

Initiators: caspase-8, caspase-9 (also -10). Executioners: caspase-3, caspase-7 (also -6).

– REMEMBER **What is the apoptosome made of?** 2 marks

Cytochrome c, Apaf-1 and dATP, which recruit and activate procaspase-9.

– UNDERSTAND **Why does apoptosis not cause inflammation?** 2 marks

The membrane stays intact, contents are packed into apoptotic bodies, and exposed phosphatidylserine lets phagocytes eat them quickly, so no cell contents leak to alarm the immune system.

– UNDERSTAND **How does Akt keep a cell alive?** 3 marks

Akt phosphorylates the BH3-only protein Bad, which is then bound by 14-3-3 proteins and cannot inhibit Bcl-2 and Bcl-xL; Bax and Bak stay held and mitochondria stay intact.

– APPLY **Follicular lymphoma cells over-express Bcl-2. Predict their response to DNA-damaging chemotherapy, and suggest a drug class.** 3 marks

They resist apoptosis: p53 signals still reach BH3-only proteins, but excess Bcl-2 holds Bax and Bak. A BH3-mimetic such as venetoclax, which blocks Bcl-2, can restore death.

– APPLY **Of 8,000 cells, 960 are annexin$^+$PI$^-$ and 320 are annexin$^+$PI$^+$. What percentage is apoptotic?** 2 marks

(960 + 320) ÷ 8,000 = 1,280 ÷ 8,000 = 16%.

– ANALYZE **A cell line lacks Apaf-1. Which death signals can still kill it, and why?** 4 marks

Death-receptor signals can still kill it if caspase-8 activates enough caspase-3 directly (in so-called type I cells). Signals that rely on mitochondria (DNA damage, growth-factor loss, and death receptors in type II cells that need tBid) fail, because no apoptosome forms and caspase-9 is not activated.

BEYOND THE SYLLABUS

Venetoclax, a BH3-mimetic that blocks Bcl-2, is approved for chronic lymphocytic leukaemia and acute myeloid leukaemia. Research now recognizes several other regulated death programmes: necroptosis (through the kinases RIPK1 and RIPK3 and the protein MLKL), pyroptosis (through inflammatory caspases and gasdermins), and ferroptosis (iron-dependent lipid peroxidation). Not for the exam; search "regulated cell death nomenclature" to read further.

4.7 Cancer and oncogenes such as Ras and Raf

Cancer is a disease of cells that divide without control, ignore the signals that should stop or kill them, and eventually invade other tissues. It arises from mutations that accumulate in one cell lineage over years: some mutations turn on growth-promoting genes (**oncogenes**) and others switch off brake genes (**tumour suppressors**). The Ras–Raf pathway is the classic example of an oncogenic signal, because a single amino acid change in Ras or Raf can lock it permanently "on".

You need: 4.4 Cyclins and CDKs, 4.5 Checkpoints and 4.6 Apoptosis. Receptor tyrosine kinases are covered in Unit 5, Cell-surface and intracellular receptors.

What makes a cell cancerous

A **tumour** (neoplasm) is a mass of cells growing without normal control. A **benign** tumour stays in place inside a capsule; a **malignant** tumour (cancer) invades nearby tissue and can spread through blood or lymph to form secondary tumours, which is **metastasis**. Cancers are named by tissue: **carcinomas** from epithelia (most cancers), **sarcomas** from connective tissue and muscle, **leukaemias** and **lymphomas** from blood-forming and immune cells.

Douglas Hanahan and Robert Weinberg summarized what cancer cells acquire as the **hallmarks of cancer** (2000, updated 2011): sustained growth signalling, escape from growth suppressors, resistance to cell death, unlimited replication (telomere maintenance), new blood vessel growth (angiogenesis), and invasion and metastasis; the 2011 update added reprogrammed energy metabolism and escape from the immune system, made possible by genome instability and inflammation. Each hallmark matches a chapter of this unit: a checkpoint lost (4.5), apoptosis escaped (4.6), telomerase switched on (4.9).

Oncogenes and tumour suppressors

A **proto-oncogene** is a normal gene that promotes growth, such as a growth factor receptor or Ras. A mutation that makes it overactive turns it into an **oncogene**. Because the mutant protein works even when the other copy is normal, the change is **dominant** at the cell level: one hit is enough. A proto-oncogene becomes an oncogene in four ways:

- **Point mutation** that locks the protein on: *KRAS* G12V, *BRAF* V600E.
- **Gene amplification**: many copies, too much protein, for example *ERBB2* (HER2) in some breast cancers.

- **Chromosome translocation**: the Philadelphia chromosome, t(9;22), fuses *BCR* to *ABL* and makes an always-active kinase in chronic myeloid leukaemia; in Burkitt lymphoma, t(8;14) places *MYC* next to an antibody gene enhancer.
- **Viral insertion or capture**: the *src* gene of Rous sarcoma virus was shown by J. Michael Bishop and Harold Varmus to be a captured cellular gene (Nobel Prize 1989).

A **tumour suppressor** restrains growth or triggers death: *RB1*, *TP53*, *APC*, *BRCA1*. Loss of function is **recessive** at the cell level, so both copies must be lost. Alfred Knudson's **two-hit hypothesis** (1971), from childhood retinoblastoma, explains why: children who inherit one bad copy of *RB1* need only one more hit in any retinal cell, so they get tumours early and often in both eyes; in sporadic cases both hits must happen in the same cell, so the tumour is single and later. Viruses can do the same job: the human papillomavirus E6 protein targets p53 for destruction and E7 binds Rb, which is why HPV causes cervical cancer.

Ras: a molecular switch that gets stuck

Ras is a small GTPase of about 21 kDa, anchored to the inner face of the plasma membrane by a lipid (farnesyl) group. Humans have three genes: *HRAS*, *KRAS* and *NRAS*. Ras is **on** when it holds GTP and **off** when it holds GDP.

1. A growth factor such as EGF binds its receptor tyrosine kinase (RTK); the receptor pairs and phosphorylates its own tyrosines.
2. The adaptor **Grb2** binds the phosphotyrosines and brings **SOS**, a guanine nucleotide exchange factor (GEF), to the membrane.
3. SOS makes Ras release GDP; Ras picks up GTP, which is more plentiful in the cell, and turns on.
4. Ras–GTP recruits and activates **Raf**, a serine/threonine kinase (A-Raf, B-Raf, C-Raf or Raf-1).
5. Raf phosphorylates **MEK**; MEK phosphorylates **ERK** (a MAP kinase). This three-kinase chain is the MAPK cascade and amplifies the signal.
6. ERK enters the nucleus and activates transcription factors (Elk-1, Fos, Myc), which switch on genes such as cyclin D. The cell passes the restriction point (Chapter 4.4).
7. Ras switches itself off by hydrolysing GTP to GDP, but slowly; **GTPase-activating proteins (GAPs)** such as neurofibromin (NF1) speed this up a great deal.

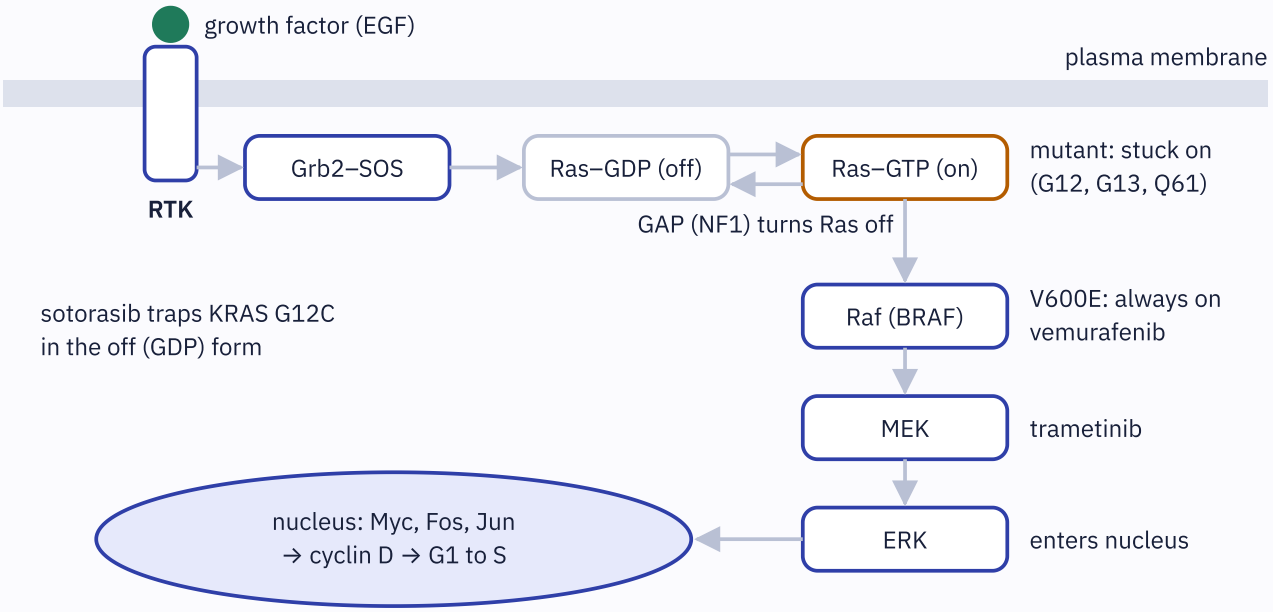


Figure 4.7.1 The Ras–Raf–MEK–ERK pathway from a growth factor to the nucleus. SOS turns Ras on and a GAP turns it off. Mutations at glycine 12, glycine 13 or glutamine 61 stop GAPs from switching Ras off; the BRAF V600E mutation makes Raf active without Ras. Drug names show where each class acts.

Oncogenic Ras mutations, mostly at codons 12, 13 and 61, stop GAPs from triggering GTP hydrolysis, so Ras stays bound to GTP and signals all the time, with or without growth factor. *KRAS* is the most often mutated of the three Ras genes and is mutated in most pancreatic cancers and in many colorectal and lung adenocarcinomas. In the *BRAF* gene, the V600E change (valine 600 to glutamate) mimics the activating phosphorylation, so B-Raf is active without Ras; it is found in roughly half of melanomas. Loss of the GAP *NF1* has a similar effect to a Ras mutation. As Chapter 4.6 showed, the same pathway also blocks apoptosis, so the cell both divides and refuses to die.

Cancer usually needs several such changes. In colorectal cancer, the common order is loss of *APC* (a small polyp), then a *KRAS* mutation (a larger adenoma), then loss of *TP53* (carcinoma). Carcinogens supply the mutations: tobacco (oral cancer from chewing tobacco is common in India), UV light, and aflatoxin from mouldy grains and groundnuts, which causes liver cancer.

WORKED EXAMPLE

Question (8 marks). Predict whether each tumour will respond to the drug, and explain using the pathway. (a) A colorectal tumour with *KRAS* G12V treated with cetuximab, an antibody that blocks EGFR. (b) A melanoma with *BRAF* V600E treated with vemurafenib, a B-Raf V600E inhibitor. (c) The same melanoma treated with cetuximab. (d) A tumour with normal Ras but loss of *NF1*, treated with a MEK inhibitor.

1. **(a) No response.** The block is upstream (at the receptor), but the mutant Ras is already GTP-bound and signals to Raf without the receptor. This is why *KRAS* testing is done before anti-EGFR antibodies in colorectal cancer.
2. **(b) Response expected.** The drug blocks the mutant kinase that drives the signal; MEK and ERK activity fall. Resistance often appears in months, which is why it is combined with a MEK inhibitor.
3. **(c) No response.** B-Raf V600E is active without Ras or the receptor; blocking EGFR is upstream of the lesion.
4. **(d) Response possible.** Without NF1, Ras stays on longer, but the signal still passes through MEK; a MEK inhibitor acts downstream of the defect.

Rule: a drug works only if it acts at or below the mutated step in the pathway.

EXAM TRAPS

- Ras is called p21ras because it weighs about 21 kDa. This is not the p21 CDK inhibitor of Chapters 4.4 and 4.5.
- Oncogenic Ras is stuck on because it cannot hydrolyse GTP efficiently, not because it binds GTP more strongly.
- Oncogenes are dominant (one mutant copy is enough); tumour suppressors are recessive at the cell level (both copies lost). Inherited predisposition is still dominant in families because the second hit is very likely.
- Proto-oncogene is the normal gene; oncogene is the mutated, overactive form. Do not call the normal gene an oncogene.
- Favourite question: "Explain the role of Ras and Raf in oncogenesis." Draw the pathway, name the switch (GTP/GDP, SOS, GAP), name the mutations, and add the survival (anti-apoptotic) effect.

CHECK YOURSELF

– REMEMBER **Name the three kinases of the MAPK cascade downstream of Ras.** 2 marks

Raf (MAPKKK) → MEK (MAPKK) → ERK (MAPK).

– REMEMBER **Give two examples of tumour suppressor genes.** 1 mark

TP53 and *RB1* (also *APC*, *BRCA1*, *NF1*).

– UNDERSTAND **Distinguish a proto-oncogene from an oncogene, with an example.** 3 marks

A proto-oncogene is a normal growth-promoting gene (*KRAS*); an oncogene is its mutated, overactive form (*KRAS* G12V), which drives growth without a signal.

– UNDERSTAND **Explain Knudson's two-hit hypothesis.** 4 marks

Both copies of a tumour suppressor must be lost for a tumour. In hereditary retinoblastoma one bad copy is inherited, so one somatic hit suffices: early, bilateral, multiple tumours. In sporadic cases both hits occur in one cell: later, single tumour.

– APPLY **A cell has a Ras mutant that cannot release GDP. Predict its effect.** 3 marks

SOS cannot load GTP, so Ras stays off even when growth factor is present; ERK is not activated and cells fail to enter S. This is a dominant-negative Ras, used in research to block the pathway.

– APPLY **Imatinib treats chronic myeloid leukaemia. What is its target, and how did the target arise?** 3 marks

It inhibits the BCR–ABL tyrosine kinase, made by the Philadelphia chromosome translocation t(9;22), which fuses *BCR* to *ABL* and gives an always-active kinase.

– ANALYZE **Why does a single *KRAS* mutation rarely cause cancer on its own in a normal cell?** 4 marks

Strong oncogenic signals trigger safety responses: p53 activation and senescence or apoptosis. Checkpoints and telomere shortening also limit division. Further hits (loss of *TP53* or *CDKN2A*, telomerase activation) are needed to remove these brakes.

BEYOND THE SYLLABUS

Ras was long called "undruggable" until covalent inhibitors of KRAS G12C (sotorasib, adagrasib) reached the clinic for lung cancer; inhibitors for other KRAS mutants are in trials. Tumours are now routinely sequenced to choose targeted drugs, and immunotherapy (checkpoint antibodies against PD-1, CAR-T cells) has added a second route. Not for the exam; search "precision oncology" and "KRAS G12C inhibitor".

4.8 Stem cells and their therapeutic uses

A stem cell is a cell that can do two things: copy itself (**self-renewal**) and give rise to specialized cells (**differentiation**). Our blood, skin and gut lining are replaced every few days to weeks only because small pools of stem cells keep dividing in them. Because stem cells can rebuild tissue, they are used to treat blood cancers and thalassaemia today, and are being tested for eye, nerve and heart repair.

You need: 4.2 Mitosis and 4.4 cell cycle control; differentiation is gene control (studied with gene expression in your molecular biology course).

Potency: how many cell types a stem cell can make

- **Totipotent**: can form the whole organism plus the placenta and membranes. Only the zygote and the cells of the first few divisions.
- **Pluripotent**: can form every cell of the body (all three germ layers: ectoderm, mesoderm, endoderm) but not the placenta. The **inner cell mass (ICM)** of the blastocyst, and **embryonic stem (ES) cells** grown from it.
- **Multipotent**: can form several cell types of one tissue family. Haematopoietic stem cells (all blood cells), neural stem cells, mesenchymal stromal cells.
- **Unipotent**: make one cell type but still self-renew, for example spermatogonial stem cells.

Mouse ES cells were first grown in 1981 (Martin Evans and Matthew Kaufman; Gail Martin) and human ES cells in 1998 (James Thomson). In 2006 Kazutoshi Takahashi and Shinya Yamanaka showed that four transcription factors, **Oct4, Sox2, Klf4 and c-Myc** (OSKM), can reprogram mouse skin fibroblasts into **induced pluripotent stem (iPS) cells**; human iPS cells followed in 2007. John Gurdon, who had shown in 1962 that a frog gut-cell nucleus could make a whole tadpole, shared the 2012 Nobel Prize with Yamanaka.

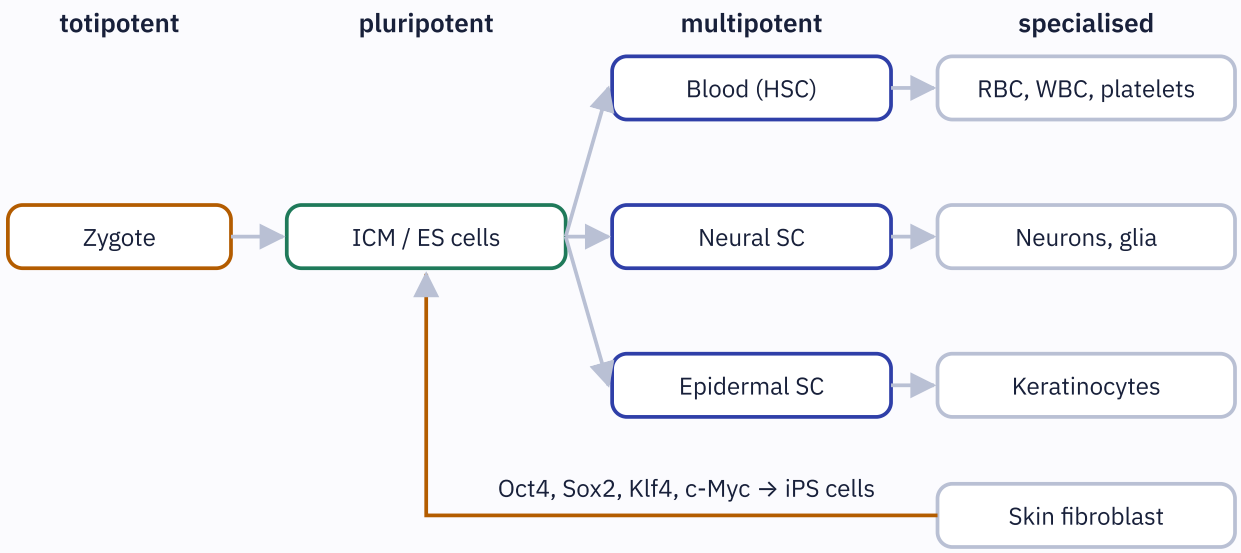


Figure 4.8.1 Stem cells ranked by potency, from left to right. A zygote is totipotent; the inner cell mass and ES cells are pluripotent; adult stem cells such as haematopoietic, neural and epidermal stem cells are multipotent and make the specialized cells on the right. The orange loop shows reprogramming: four factors turn a skin fibroblast back into a pluripotent iPS cell.

Adult stem cells and tissue renewal

Adult stem cells live in a **niche**, a local environment of neighbouring cells, matrix and signals (Wnt, Notch, BMP) that keeps them undifferentiated. A stem cell can divide **asymmetrically**, giving one stem cell and one committed daughter, so the stem cell number stays constant. The committed daughter is often a **transit-amplifying cell** that divides rapidly a few times before differentiating, which multiplies the output without using up stem cells.

1. **Epidermis.** Stem cells in the basal layer of the skin divide; daughters move upward, stop dividing, fill with keratin and die to form the protective outer layer, which is shed. Hair follicles have their own stem cells in a region called the bulge, which also help heal wounds.
2. **Blood (haematopoiesis). Haematopoietic stem cells (HSCs)** in the bone marrow make two branches: the myeloid line (red cells, platelets, granulocytes, monocytes) and the lymphoid line (B, T and NK cells). They carry the surface marker CD34, which is used to count and collect them. The adult body makes billions of new blood cells every day from this small pool.
3. **Gut lining.** Stem cells at the base of intestinal crypts, marked by the gene *Lgr5*, renew the epithelium every few days.
4. **Skeletal muscle. Satellite cells**, which sit under the basal lamina of muscle fibres and express the transcription factor Pax7, stay quiet until injury, then divide; their progeny fuse with each other or with damaged fibres to rebuild muscle.

5 **Blood vessels.** Endothelial cells lining vessels and lymphatics are not stem cells, but they can divide when needed; new capillaries sprout from existing vessels in response to signals such as VEGF (vascular endothelial growth factor), the angiogenesis that tumours also exploit (Chapter 4.7).

Therapeutic uses

- **Haematopoietic stem cell transplantation (HSCT)**, the established therapy, for leukaemia, lymphoma, aplastic anaemia and thalassaemia major. Sources: bone marrow, peripheral blood after mobilizing HSCs with G-CSF, and umbilical cord blood. **Autologous** transplants use the patient's own cells (after high-dose chemotherapy); **allogeneic** transplants use a donor matched for HLA type and carry the risk of graft-versus-host disease.
- **Skin and eye.** Sheets of cultured epidermal cells cover large burns; limbal stem cells from the edge of the cornea restore the corneal surface after chemical burns. A simplified limbal transplant method was developed at L V Prasad Eye Institute, Hyderabad (from memory; check).
- **iPS-cell therapies in trials**: retinal pigment cells for macular degeneration (first transplanted in Japan in 2014), dopamine neurons for Parkinson's disease, heart muscle and insulin-producing cells.
- **Disease models and drug testing**: iPS cells from a patient carry the patient's genes, so their neurons or heart cells can be used to study the disease and screen drugs; organoids (mini-organs grown from stem cells) extend this.

Issues. ES cells need the destruction of an early embryo, which raises ethical objections; iPS cells avoid this. Pluripotent cells left undifferentiated can form **teratomas**, and c-Myc is itself an oncogene. In India, stem cell research and therapy fall under the ICMR National Guidelines for Stem Cell Research (2017), which treat uses other than established HSCT as research to be done only in approved clinical trials (from memory; check); clinics that sell unproven "stem cell therapy" fall outside these rules.

WORKED EXAMPLE

Question (6 marks). A 50 kg thalassaemia patient is to receive an allogeneic transplant. The transplant team wants at least 2×10^6 $CD34^+$ cells per kg body weight (a commonly used minimum; check your source). The donor's mobilized blood collection holds 3.0×10^{10} nucleated cells, of which 0.40% are $CD34^+$. (a) Is the dose enough? (b) If a cord blood unit has 1.5×10^9 nucleated cells with 0.30% $CD34^+$, what is its dose for this patient?

1. $CD34^+$ cells in the collection = $3.0 \times 10^{10} \times 0.0040 = 1.2 \times 10^8$ cells.
2. Dose = $1.2 \times 10^8 \div 50$ kg = 2.4×10^6 cells/kg. This is above 2×10^6 cells/kg, so the dose is enough.
3. Cord blood $CD34^+ = 1.5 \times 10^9 \times 0.0030 = 4.5 \times 10^6$ cells.
4. Dose = $4.5 \times 10^6 \div 50 = 9.0 \times 10^4$ cells/kg, about 25 times lower. This is why cord blood is used mostly for children, or two units are combined for adults.

EXAM TRAPS

- Totipotent includes extra-embryonic tissues (placenta); pluripotent does not. ES cells are pluripotent, not totipotent.
- iPS cells are made from adult cells by reprogramming; they are not "adult stem cells".
- Self-renewal and differentiation are both needed in the definition. A progenitor that only differentiates is not a stem cell.
- Mesenchymal stromal cells are often marketed widely, but HSCT is the only routinely established stem cell therapy. Write "established" versus "experimental".
- Autologous = own cells; allogeneic = donor cells; syngeneic = identical twin.

CHECK YOURSELF

– REMEMBER **Name the four Yamanaka factors.** 2 marks

Oct4, Sox2, Klf4 and c-Myc.

– REMEMBER **What are satellite cells?** 2 marks

Pax7-expressing muscle stem cells under the basal lamina of skeletal muscle fibres; they divide after injury and fuse to regenerate fibres.

– UNDERSTAND **Differentiate totipotent, pluripotent and multipotent cells with one example each.** 3 marks

Totipotent: forms embryo and placenta (zygote). Pluripotent: all body cells, not placenta (ES cells). Multipotent: several types of one family (HSCs make all blood cells).

– UNDERSTAND **How does asymmetric division keep a stem cell pool constant?** 2 marks

Each division gives one stem cell (self-renewal) and one differentiating daughter, so stem cell number stays the same while output continues.

– APPLY **A 70 kg patient needs 2×10^6 $CD34^+$ cells/kg. How many $CD34^+$ cells must be collected?** 2 marks

$2 \times 10^6 \times 70 = 1.4 \times 10^8$ $CD34^+$ cells.

– APPLY **Suggest why iPS cells from a patient with an inherited heart rhythm disorder are useful to a drug company.** 3 marks

They can be turned into heart muscle cells carrying the patient's mutation, showing the disorder in a dish; candidate drugs can be screened on them for benefit and for heart toxicity before human trials.

– ANALYZE **Compare ES cells and iPS cells for therapy.** 4 marks

Both pluripotent. ES: from embryos, ethical concerns, not patient-matched (rejection). iPS: from the patient's own cells, no embryo, immune-matched, but reprogramming can leave genetic or epigenetic errors and the factors (c-Myc) raise tumour risk; both can form teratomas if undifferentiated cells remain.

BEYOND THE SYLLABUS

Gene-edited HSCs are now a therapy: in 2023 the first CRISPR-based treatment (exa-cel) was approved for sickle cell disease and beta-thalassaemia, editing the patient's own HSCs to restore fetal haemoglobin. Organoids grown from Lgr5 stem cells are used to test cancer drugs on a patient's own tumour. Not for the exam; search "exa-cel CRISPR thalassaemia" and "intestinal organoids".

4.9 Cell culture and immortalization of cells

Cell culture is growing cells outside the body in a sterile vessel with a nutrient medium, at body temperature. Normal cells taken from a tissue divide only a limited number of times and then stop; **immortalization** is any change that lets a line divide indefinitely. Cultured cells are the workhorses of biotechnology: they make vaccines and antibody drugs, test toxicity, and model cancer. Others teach it in a separate animal cell culture course.

You need: 4.7 Cancer and oncogenes (p53, Rb) and 4.8 Stem cells.

Primary cultures, cell lines and the Hayflick limit

A **primary culture** is made directly from tissue (often by trypsin digestion). When the cells are transferred (**subcultured** or **passaged**) to new flasks they become a **cell line**. Most animal cells are **adherent** (they grow attached to the plastic, in one layer, and stop when they touch: contact inhibition); blood cells and many tumour lines grow in **suspension**. Typical conditions: DMEM or RPMI-1640 medium with about 10% fetal bovine serum, 37 °C, and 5% CO_2 to buffer the bicarbonate.

In 1961 Leonard Hayflick and Paul Moorhead showed that normal human fibroblasts divide about 40 to 60 times and then enter **replicative senescence**: alive but permanently arrested. This is the **Hayflick limit**. Its main cause is telomere shortening. Every round of replication loses some DNA from chromosome ends, because DNA polymerase cannot finish the end of the lagging strand. Short telomeres look like broken DNA and trigger the p53 and Rb brakes of Chapter 4.5.

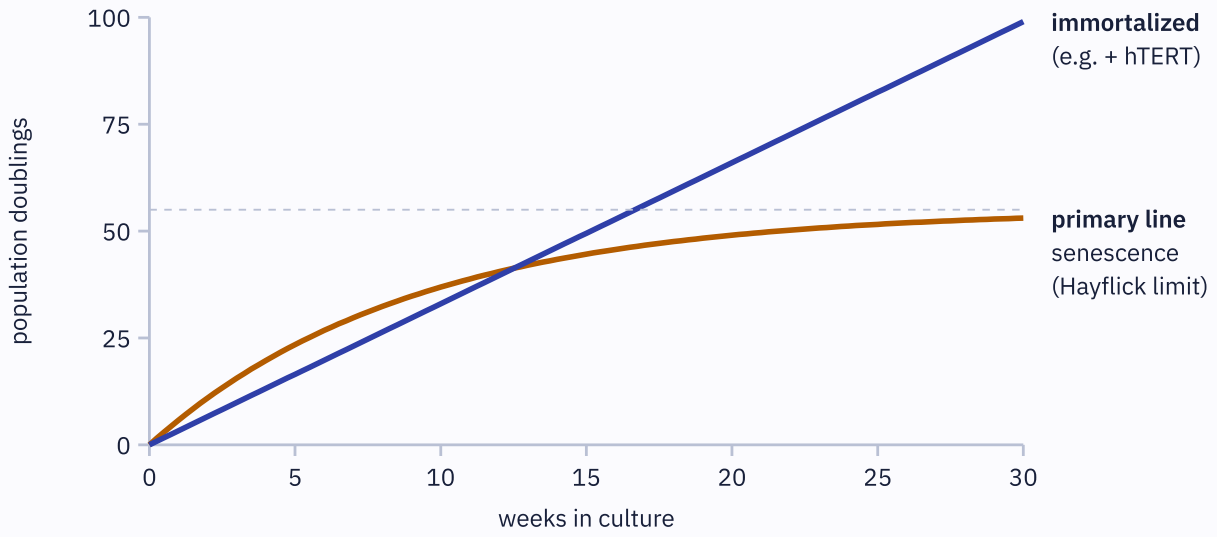


Figure 4.9.1 Cumulative population doublings against time for a normal (primary) fibroblast line and the same cells immortalized with hTERT. These are model curves, not measured data. The primary line slows and stops near its Hayflick limit; the immortalized line keeps doubling at a steady rate.

How cells are immortalized

1. **Telomerase.** Introducing the gene for the catalytic subunit of telomerase, **hTERT**, rebuilds telomeres; many cell types then grow indefinitely with a near-normal karyotype.
2. **Viral oncogenes.** SV40 large T antigen, or HPV E6 and E7, inactivate p53 and Rb, so the cells ignore the senescence signal. Full immortality usually also needs telomerase.
3. **Epstein–Barr virus** immortalizes human B lymphocytes into lymphoblastoid lines.
4. **Tumour cells** are often already immortal. HeLa, from the cervical cancer of Henrietta Lacks in 1951, was the first continuous human line; it carries HPV-18 and expresses telomerase.
5. **Cell fusion.** A hybridoma fuses an antibody-making B cell with an immortal myeloma cell (Köhler and Milstein, 1975), giving an immortal line that secretes one monoclonal antibody.

Applications. Vero (monkey kidney) cells for polio and rabies vaccines; Chinese hamster ovary (CHO) cells for most therapeutic antibodies and proteins; HEK293 cells for viral vectors; MTT and similar assays for drug toxicity, which reduce animal testing. Lines must be checked for mycoplasma and cross-contamination.

WORKED EXAMPLE

Question (6 marks). A suspension of cells is diluted 1:2 with trypan blue and loaded into a haemocytometer. The four large corner squares (each 1 mm × 1 mm × 0.1 mm deep) hold 52, 48, 55 and 45 unstained cells. (a) Find the viable cell concentration. (b) 2.0×10^5 cells seeded at time zero grow to 1.6×10^6 cells after 72 h in log phase. Find the doubling time.

1. Mean per square = (52 + 48 + 55 + 45) ÷ 4 = 200 ÷ 4 = 50 cells.
2. Each square holds 0.1 mm^3 = 10^{-4} mL, so multiply by 10^4, then by the dilution factor 2: 50 × 2 × 10^4 = 1.0×10^6 viable cells/mL.
3. Number of doublings $n = \log_2(N \div N_0) = \log_2(1.6 \times 10^6 \div 2.0 \times 10^5) = \log_2 8 = 3$.
4. Doubling time $t_d = t \div n = 72\ h \div 3 = 24\ h$. In general $t_d = t \times \ln 2 \div \ln(N \div N_0)$.

EXAM TRAPS

- A cell line is not automatically immortal. "Finite cell line" (normal cells, will senesce) versus "continuous cell line" (immortal).
- Trypan blue stains dead cells (they cannot exclude the dye). Count the unstained cells for viability.
- Immortal is not the same as transformed. hTERT cells are immortal but usually still contact-inhibited and do not form tumours; transformed cells lose these controls.
- The 5% CO_2 is for pH buffering with bicarbonate, not for "respiration".

CHECK YOURSELF

– REMEMBER **What is the Hayflick limit?** 1 mark

The limited number of divisions (about 40 to 60) a normal human cell makes in culture before senescence.

– UNDERSTAND **How does SV40 large T antigen immortalize cells?** 3 marks

It binds and inactivates p53 and Rb, removing the checkpoints that cause senescence; telomerase activation is often also needed for full immortality.

– APPLY **Cells go from 5×10^4 to 8×10^5 in 96 h. Find the doubling time.** 2 marks

N/N_0 = 16, so n = 4 doublings; t_d = 96 ÷ 4 = 24 h.

– APPLY **A haemocytometer count averages 30 cells per large square with no dilution. Give the concentration.** 1 mark

$30 \times 10^4 = 3 \times 10^5$ cells/mL.

– ANALYZE **Why do drug companies prefer hTERT-immortalized lines to tumour lines for some toxicity tests?** 3 marks

hTERT lines keep near-normal chromosomes and checkpoints, so they respond more like normal tissue; tumour lines carry many mutations that change drug responses.

BEYOND THE SYLLABUS

Industry grows CHO cells serum-free in bioreactors of thousands of litres, and 3D cultures and organ-on-chip devices are replacing flat flasks. Not for the exam; search "CHO cell bioprocessing" and "organ-on-a-chip".

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
Cell cycle	G1 → S → G2 → M; G0 = resting, entered from G1	4.1
Phase length	duration ≈ (fraction of cells in phase) × cycle time T	4.1
Mitotic index	cells in mitosis ÷ total cells counted	4.1, 4.2
DNA content	2C in G1, 4C in G2/M, 1C in a gamete	4.1, 4.3
Mitosis	prophase, prometaphase, metaphase, anaphase, telophase; 2n → 2n	4.2
Meiosis	meiosis I separates homologues (2n → n); meiosis II separates sisters	4.3
Gamete combinations	2^n (n = haploid number); humans 2^{23} = 8,388,608	4.3
MPF	cyclin B + CDK1	4.4
Rb–E2F	CDK4/6 and CDK2 phosphorylate Rb → E2F free → S-phase genes	4.4
DNA damage arrest	ATM/ATR → Chk2/Chk1 → p53 → p21 → CDK2 off	4.5
Spindle checkpoint	unattached kinetochore → MCC → APC/C–Cdc20 off → no anaphase	4.5
Apoptosis	extrinsic (Fas, caspase-8) and intrinsic (Bax/Bak, cytochrome c, caspase-9) → caspase-3/7	4.6
Ras pathway	RTK → Grb2–SOS → Ras–GTP → Raf → MEK → ERK → cyclin D	4.7
Potency	totipotent > pluripotent > multipotent > unipotent	4.8
Cell count	cells/mL = mean per large square × dilution × 10^4	4.9
Doubling time	$t_d = t \times \ln 2 \div \ln(N \div N_o)$	4.9

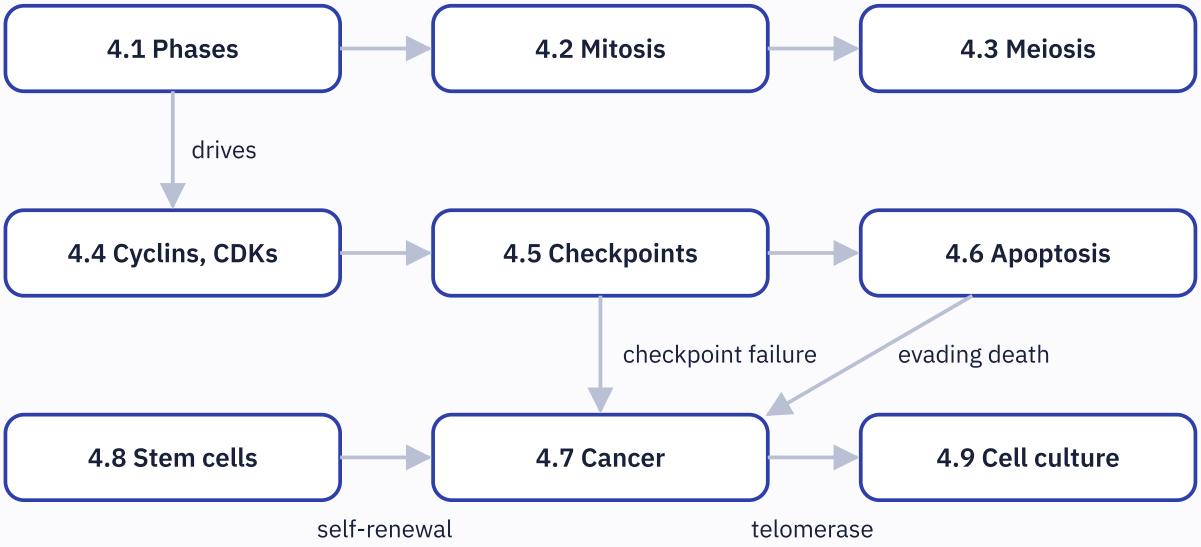


Figure 4.S.1 How the chapters connect. The phases (4.1) are run by cyclins and CDKs (4.4) and lead to mitosis or meiosis. Checkpoints (4.5) either pause the cycle, send the cell to apoptosis (4.6), or, when they fail, let cancer start (4.7). Stem cells share self-renewal with cancer cells, and cancer cells share telomerase-driven immortality with cultured cell lines.

1. The cell cycle has four phases; DNA doubles only in S, and G1 sets the overall cycle length.
2. Mitosis gives two identical diploid cells; anaphase is the moment sister chromatids part.
3. Meiosis has two divisions after one S phase: meiosis I halves the chromosome number and creates variation by crossing over and independent assortment.
4. Cyclin–CDK pairs drive each transition; cyclins rise and fall while CDK levels stay steady.
5. CDKs are controlled by cyclin supply and destruction, activating and inhibitory phosphorylation, and CDK inhibitors.
6. Checkpoints sense DNA damage, incomplete replication and unattached kinetochores, and stop the cycle through p53–p21, Chk1–Cdc25 and the MCC.
7. Apoptosis is orderly, caspase-driven death through death receptors or mitochondria, balanced by the Bcl-2 family.
8. Cancer needs gain of oncogenes (Ras, Raf) and loss of tumour suppressors (p53, Rb); Ras stuck in the GTP form signals non-stop.
9. Stem cells self-renew and differentiate; HSC transplantation is the established therapy, iPS cells the growing frontier.
10. Normal cells stop at the Hayflick limit; telomerase or viral oncogenes immortalize cells for culture.

Exam question bank

Remember and understand

- UNDERSTAND **With a neat diagram, describe the phases of the eukaryotic cell cycle and the events in each.** 10 marks

  Circle diagram with G1, S, G2, M and G0. G1: growth, restriction point. S: DNA replication, sister chromatids, centrosome duplication, 2C → 4C. G2: checks, spindle proteins. M: mitosis and cytokinesis. G0: quiescence, examples. Typical times (about 24 h total). Chromosome number unchanged in S.

- UNDERSTAND **Describe the stages of mitosis with diagrams. Add a note on cytokinesis in plant and animal cells.** 12 marks

  Prophase (condensation, spindle), prometaphase (envelope breakdown, kinetochore capture), metaphase (plate, bi-orientation), anaphase A and B (separase, cohesin), telophase (envelope re-forms). Cytokinesis: contractile ring and cleavage furrow versus phragmoplast and cell plate. Diagrams of each stage.

- UNDERSTAND **Explain prophase I of meiosis and the significance of meiosis.** 10 marks

  Leptotene, zygotene (synapsis, synaptonemal complex), pachytene (crossing over), diplotene (chiasmata, oocyte arrest), diakinesis (terminalization). Significance: halving of chromosome number, variation through crossing over, independent assortment (2^n), random fertilization; nondisjunction consequences.

- REMEMBER **Compare apoptosis and necrosis, and describe the intrinsic and extrinsic pathways of apoptosis.** 12 marks

  Comparison table (cause, size, membrane, DNA ladder versus smear, ATP, inflammation). Extrinsic: FasL/TNF, death receptor, FADD, DISC, caspase-8. Intrinsic: BH3-only, Bax/Bak, MOMP, cytochrome c, Apaf-1, apoptosome, caspase-9. Convergence on caspase-3/7; Bcl-2 regulation; tBid crosstalk.

- UNDERSTAND **What are stem cells? Classify them by potency and describe their therapeutic applications.** 10 marks

  Definition (self-renewal and differentiation); toti-, pluri-, multi-, unipotent with examples; ES cells and iPS cells (OSKM); adult stem cells and niches; HSCT (sources, autologous/allogeneic, GvHD), skin and limbal grafts, iPS trials, disease models; ethics and tumour risk.

Apply

– APPLY **Of 2,000 cells in a culture with a 22 h cycle, 1,100 have 2C DNA, 500 have intermediate DNA, 340 have 4C DNA without condensed chromosomes and 60 are in mitosis. Calculate the mitotic index and the length of each phase, and sketch the expected DNA-content histogram.** 8 marks

MI = 60 ÷ 2,000 = 3%. G1 = 0.55 × 22 = 12.1 h; S = 0.25 × 22 = 5.5 h; G2 = 0.17 × 22 = 3.74 h; M = 0.03 × 22 = 0.66 h. Histogram: tall 2C peak, lower 4C peak (G2 + M), S cells between. State the asynchronous-culture assumption.

– APPLY **Explain how cyclin–CDK complexes are regulated, and predict the effect of losing Wee1, losing Cdc25, and over-expressing p27.** 12 marks

Cyclin synthesis and SCF/APC/C destruction; CAK activation; Wee1/Cdc25 on tyrosine 15; INK4 and Cip/Kip inhibitors. Wee1 loss: early mitosis, small cells. Cdc25 loss: G2 arrest, large cells. Excess p27: CDK2 blocked, G1 arrest.

– APPLY **Describe the role of Ras and Raf in oncogenesis and in protection from apoptosis. Predict the response of a KRAS-mutant tumour to an EGFR antibody.** 12 marks

RTK → Grb2–SOS → Ras–GTP → Raf → MEK → ERK → cyclin D; GAP switch-off; G12/G13/Q61 and BRAF V600E mutations; survival via PI3K–Akt–Bad and ERK–Bim; no response to EGFR antibody because the lesion is downstream; drugs at or below the lesion (KRAS G12C, BRAF, MEK inhibitors).

– APPLY **Explain the Hayflick limit and three methods of immortalizing cells. A culture grows from 1×10^5 to 3.2×10^6 cells in 100 h; find the doubling time.** 10 marks

Senescence after about 40–60 doublings from telomere shortening and p53/Rb activation. Methods: hTERT, SV40 large T or HPV E6/E7, EBV, hybridoma fusion, tumour origin. $N/N_o = 32$, $n = 5$, $t_d = 100 \div 5 = 20$ h. Applications: vaccines, CHO proteins, toxicity tests.

Analyze and evaluate

– ANALYZE **Explain the three major cell cycle checkpoints. Discuss how loss of p53 changes the response of a cell to DNA damage, and how this can be used in cancer therapy.** 14 marks

G1/S, G2/M and spindle checkpoints with sensors, transducers and effectors (ATM/ATR, Chk1/Chk2, p53–p21, Cdc25C, MCC). p53 loss: no G1 arrest, reliance on G2/M; less apoptosis; mutation accumulation. Therapy: Chk1/Wee1/ATR inhibitors and synthetic lethality (PARP in BRCA).

– ANALYZE **"Cancer is a disease of the cell cycle." Evaluate this statement using cyclins, checkpoints, apoptosis and telomeres.** 16 marks

Spans Chapters 4.4–4.9. Supporting: cyclin D amplification, Rb and p16 loss, p53 loss, checkpoint failure and aneuploidy, Bcl-2 over-expression, telomerase activation. Qualifying: invasion, metastasis, angiogenesis, metabolism and immune escape go beyond the cycle. Conclusion: cycle control is central but not the whole disease (hallmarks).

– ANALYZE **Compare mitosis and meiosis in mechanism and purpose, and explain how errors in each lead to disease.** 12 marks

Spans 4.2, 4.3 and 4.5. Table of differences (divisions, pairing, crossing over, products). Errors: mitotic nondisjunction and chromosome instability in tumours; meiotic nondisjunction giving trisomy 21; role of the spindle checkpoint and of centromeric cohesin protection; maternal-age effect.

– ANALYZE **Stem cells and cancer cells both self-renew. Compare them, and evaluate the safety risks of iPS-cell therapies.** 12 marks

Spans 4.7–4.9. Shared: self-renewal, telomerase, active Wnt/Myc programmes. Differences: niche control, normal checkpoints, differentiation. Risks: teratoma from residual pluripotent cells, c-Myc oncogene, reprogramming mutations, culture-acquired changes; safeguards (non-integrating methods, purity testing).

Glossary

Anaphase-promoting complex (APC/C) Ubiquitin ligase that, with Cdc20 or Cdh1, tags securin and mitotic cyclins for destruction.

Apoptosome Wheel-like complex of cytochrome c, Apaf-1 and dATP that activates caspase-9.

Bivalent (tetrad)	A pair of synapsed homologous chromosomes in meiosis I, with four chromatids.
Caspase	Cysteine protease that cuts after aspartate; initiators (8, 9, 10) and executioners (3, 6, 7) carry out apoptosis.
CDK	Cyclin-dependent kinase: serine/threonine kinase active only with a cyclin partner.
Checkpoint	Control that halts the cycle until a required step is complete and correct.
Chiasma	Visible point where non-sister chromatids of homologues remain joined after crossing over.
Cohesin	Protein ring holding sister chromatids together until separase cuts it at anaphase.
Crossing over	Exchange of segments between non-sister chromatids of homologues in pachytene.
Cyclin	Regulatory protein whose level rises and falls in the cycle and which activates a CDK.
E2F	Transcription factor for S-phase genes, held inactive by unphosphorylated Rb.
G0	Non-dividing state entered from G1; some cells can return to the cycle.
Hayflick limit	The finite number of divisions (about 40–60) of normal human cells in culture.
Immortalization	Change that lets a cell line divide indefinitely, for example by telomerase or viral oncogenes.
iPS cell	Induced pluripotent stem cell made by reprogramming an adult cell with Oct4, Sox2, Klf4 and c-Myc.
Kinetochore	Protein structure on each chromatid's centromere that binds spindle microtubules.
Mitotic index	Fraction of cells in a population that are in mitosis.
MPF	Maturation- or M-phase-promoting factor: cyclin B with CDK1.
Nondisjunction	Failure of homologues or sister chromatids to separate, giving aneuploid cells.
Oncogene	Mutated, overactive form of a growth-promoting proto-oncogene; dominant at the cell level.

p53	Tumour suppressor transcription factor that arrests the cycle (via p21) or triggers apoptosis after DNA damage.
Pluripotent	Able to form every cell type of the body but not the placenta.
Ras	Small membrane GTPase, on with GTP and off with GDP, relaying growth-factor signals to Raf.
Restriction point	Point in late G1 after which a cell completes the cycle without further growth factors.
Senescence	Permanent cell cycle arrest of a living cell, for example at short telomeres.
Spindle assembly checkpoint	Mechanism that blocks anaphase until all kinetochores are attached to the spindle.
Stem cell niche	Local environment of cells, matrix and signals that maintains stem cells.
Synaptonemal complex	Protein zipper that holds homologues together during synapsis.
Telomerase	Reverse transcriptase enzyme that extends telomeres; its catalytic subunit is TERT.
Tumour suppressor	Gene whose loss of both copies removes a brake on growth, for example *RB1* or *TP53*.

Sources and further reading

Textbooks

- Bruce Alberts and others, *Molecular Biology of the Cell* (Garland Science / W. W. Norton): the chapters on the cell cycle, cell death, cancer, and stem cells and tissue renewal (from memory; check the edition you are prescribed).
- Harvey Lodish and others, *Molecular Cell Biology* (W. H. Freeman): chapters on regulating the eukaryotic cell cycle and on cancer (from memory; check).
- Geoffrey M. Cooper, *The Cell: A Molecular Approach* (Sinauer / Oxford University Press): chapters on the cell cycle, cell death and cell renewal, and cancer (from memory; check).
- Gerald Karp, *Cell and Molecular Biology: Concepts and Experiments* (Wiley) (from memory; check).
- R. Ian Freshney, *Culture of Animal Cells: A Manual of Basic Technique* (Wiley), for Chapter 4.9 (from memory; check).

Open resources

- The 2nd edition (2000) of Cooper's *The Cell: A Molecular Approach* is free to read on the NCBI Bookshelf; its cell cycle section: ncbi.nlm.nih.gov/books/NBK9887 (found by search; the page did not load from the writing environment, so check).
- National Cancer Institute, RAS Initiative, frederick.cancer.gov/initiatives/ras-initiative, for current Ras biology and drugs (found by search; not opened).

Values given as typical (phase lengths, the Hayflick range, the $CD34^+$ dose) are standard textbook figures written from memory and marked where they appear; check them against your prescribed book.

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