Cell Biology

Unit 4 · Lesson 2 · Cyclins, checkpoints, apoptosis

The notes for Lesson 2 of Unit 4: chapters 4.4 to 4.6 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 2 of 3 | 3 chapters | Chapters 4.4 to 4.6

About these notes

These are the notes for **Lesson 2 · Cyclins, checkpoints, apoptosis** of the University free n easy video lessons on Cell Biology Unit 4, Cell Cycle, Division and Cell Death. They are chapters 4.4 to 4.6 of the unit book, unchanged, so a page here is the same page in the full book.

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

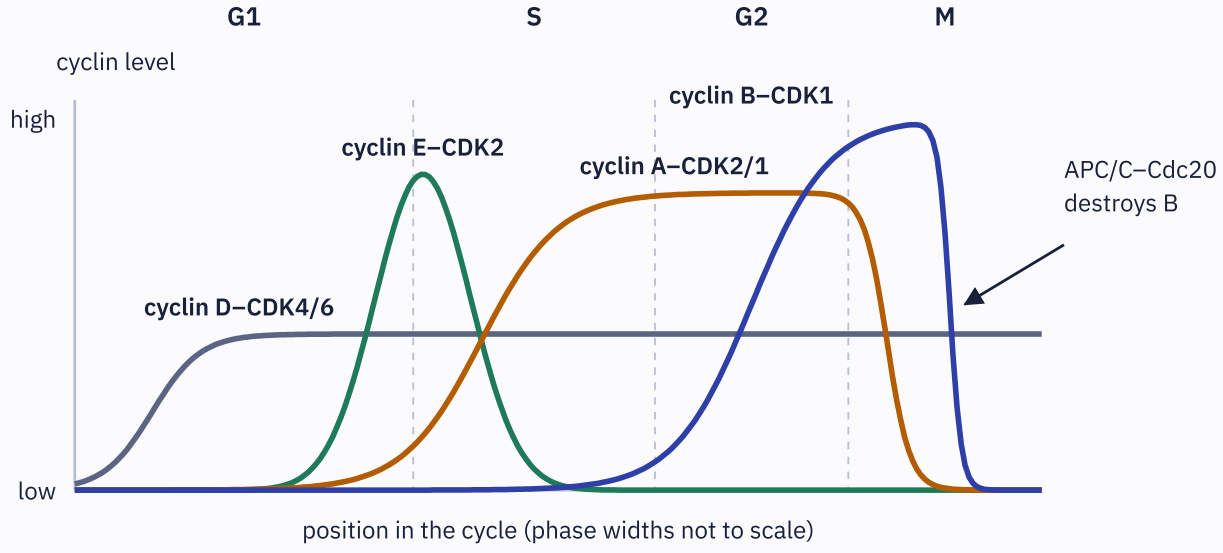


Figure 4.4.1 Relative levels of the four cyclins across one cycle, drawn as smooth model curves (not measured data). Cyclin D tracks growth-factor signals; cyclin E peaks at the G1/S transition; cyclin A rises in S and falls early in M; cyclin B peaks in mitosis and is destroyed at the metaphase–anaphase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

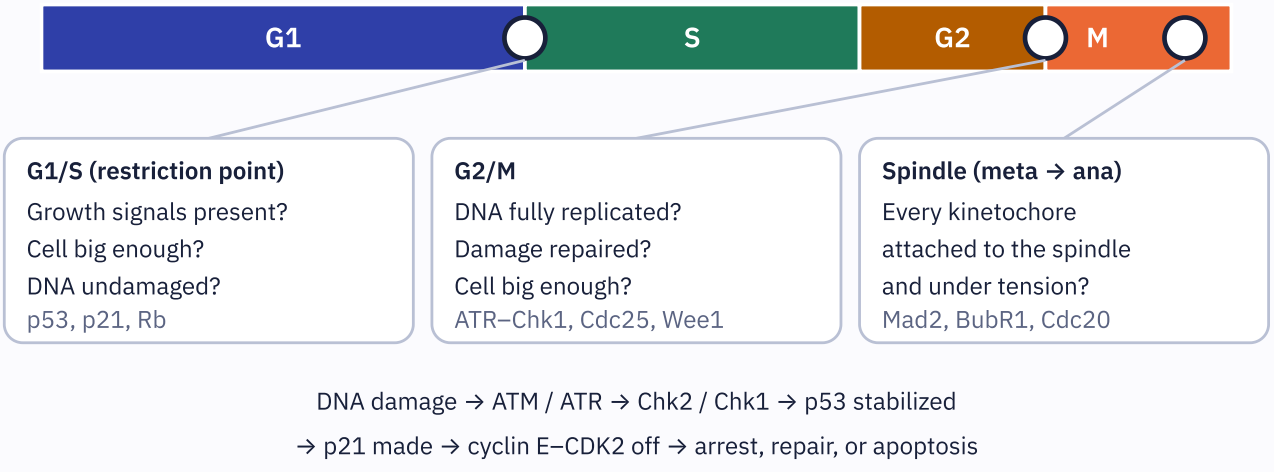


Figure 4.5.1 The three main checkpoints (open circles) on the cycle, what each one asks, and its key proteins in grey. The two lines at the bottom trace the DNA damage pathway that arrests cells in G1. Notice that the spindle checkpoint sits inside M, before anaphase.

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.

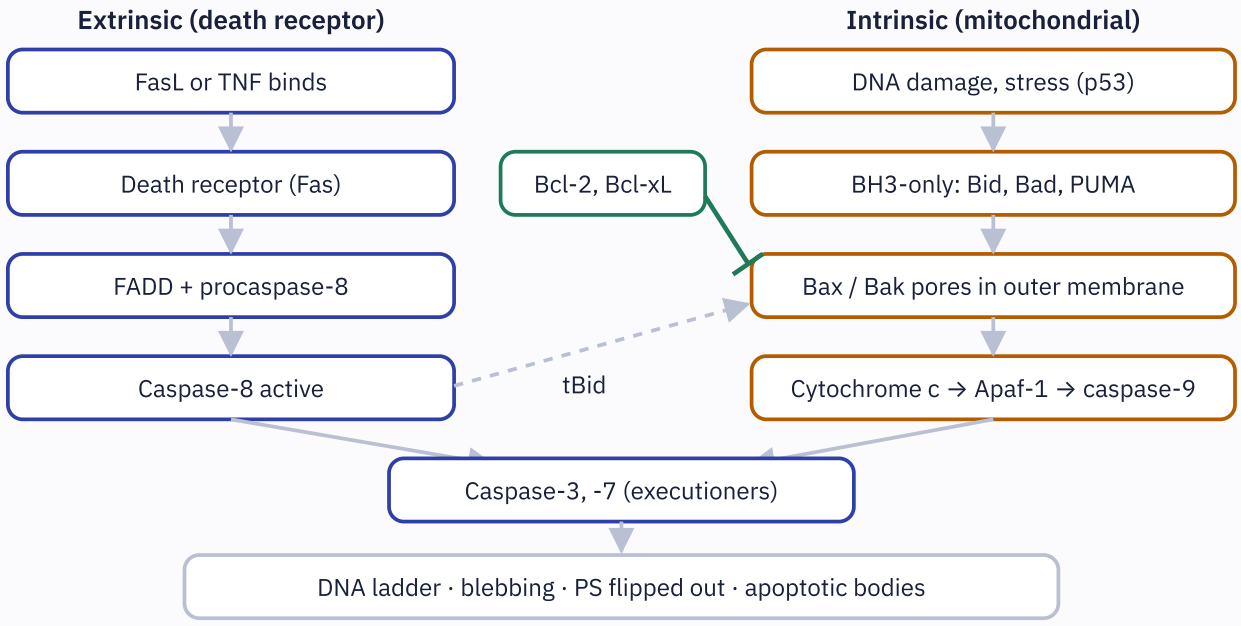


Figure 4.6.1 The two main routes to apoptosis. Left: a death ligand on the outside activates caspase-8. Right: internal stress makes BH3-only proteins switch on Bax and Bak, which release cytochrome c from mitochondria to build the apoptosome and activate caspase-9. Bcl-2 (green) blocks Bax and Bak. 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.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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

Glossary (terms used in this lesson)

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

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.

Cohesin Protein ring holding sister chromatids together until separase cuts it at anaphase.

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.

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.
p53	Tumour suppressor transcription factor that arrests the cycle (via p21) or triggers apoptosis after DNA damage.
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.
Tumour suppressor	Gene whose loss of both copies removes a brake on growth, for example *RB1* or *TP53*.

The full unit book

Every chapter of Unit 4, the unit summary, the exam question bank with model answers and the full glossary: Cell Biology · Unit 4 · Cell Cycle, Division and Cell Death (one file, works offline).

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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