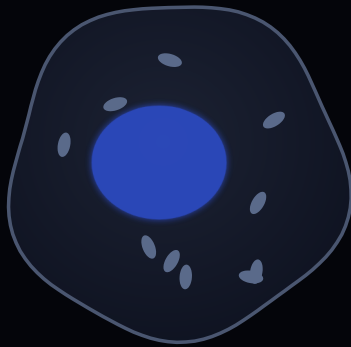
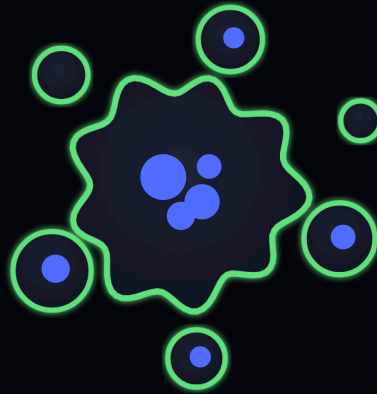
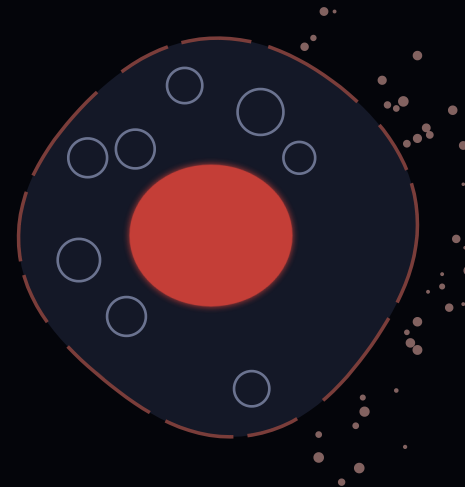




Healthy
annexin V-, PI-



Apoptosis
annexin V+ (green), PI- early



Necrosis
membrane ruptured, PI+ (red)

How the two ways of dying look in a flow-cytometry stain. Annexin V (green) binds phosphatidylserine flipped to the surface of apoptotic cells; propidium iodide (red) enters only when the membrane has broken, as in necrosis.

Cell signalling, apoptosis and necrosis

How cells receive and relay messages, and the two very different ways they die: quietly by programme, or messily by injury.

■ Signalling
 ■ Messengers and caspases
 ■ Apoptosis
 ■ Necrosis

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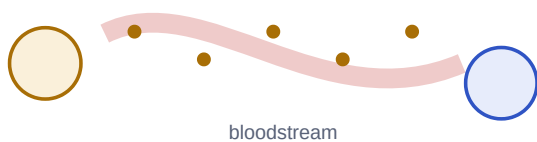
1. Principles of cell signalling

Cell signalling is the process by which cells detect signals from their surroundings or from other cells and convert them into a response. Every pathway has the same parts:

1. **Signal (ligand, first messenger):** a hormone, growth factor, neurotransmitter, cytokine, gas (NO), or a physical stimulus such as light.
2. **Receptor:** a protein that binds the signal specifically, with high affinity, and changes shape.
3. **Transduction:** relay proteins and **second messengers** (cAMP, Ca^{2+} , IP_3) carry and **amplify** the signal.
4. **Response:** changes in enzyme activity, ion flow, the cytoskeleton, or gene expression.
5. **Termination:** the signal is switched off so the cell can respond again.

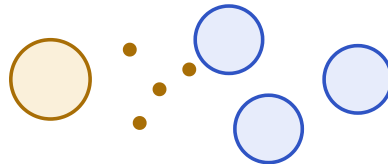
Endocrine

hormone in blood, distant targets



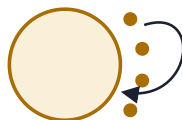
Paracrine

local mediators, nearby cells



Autocrine

cell responds to its own signal



Contact-dependent

membrane-bound ligand (Notch–Delta)

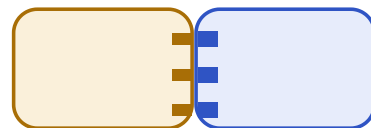


Figure 1. Modes of signalling, classified by the distance the signal travels. Synaptic signalling, where a neurotransmitter crosses a narrow synaptic cleft, is a specialised form of paracrine signalling.

Amplification

One adrenaline molecule bound to one β -adrenergic receptor can activate many G proteins; each adenylyl cyclase makes many cAMP molecules; each PKA phosphorylates many phosphorylase kinases, and so on. By the end of the glycogen-breakdown cascade, a single hormone molecule can release millions of glucose molecules.

The same signal can mean different things in different cells. Acetylcholine makes skeletal muscle contract (nicotinic receptor), slows heart muscle (muscarinic M2 receptor) and makes salivary glands secrete (M3). The response depends on the receptor and the machinery the cell has, not on the signal alone.

2. Receptor types

Receptor class	How it works	Examples
Intracellular (nuclear) receptors	Small hydrophobic ligands cross the membrane; the receptor–ligand complex binds DNA response elements and acts as a transcription factor	Steroid hormones (cortisol, oestrogen), thyroid hormone, vitamin D, retinoic acid
Ion-channel-linked	Binding opens or closes a channel; fastest response (milliseconds)	Nicotinic acetylcholine receptor, GABA _A , NMDA
G protein-coupled (GPCR)	Seven-transmembrane receptor activates a heterotrimeric G protein	β-adrenergic, muscarinic, rhodopsin, olfactory, glucagon receptors
Enzyme-linked	Receptor is itself an enzyme, or is associated with one	Receptor tyrosine kinases (insulin, EGF), cytokine receptors (JAK-STAT), TGF-β receptors (Ser/Thr kinases), guanylyl cyclase (ANP)
Other surface receptors	Proteolysis or degradation-controlled pathways	Notch, Wnt/Frizzled, Hedgehog/Patched

Nitric oxide is a special case: a dissolved gas that diffuses into neighbouring cells and directly activates soluble guanylyl cyclase, raising cGMP and relaxing smooth muscle (the basis of nitroglycerin's action in angina).

3. G protein-coupled receptors

GPCRs are the largest family of cell-surface receptors (about 800 genes in humans) and the target of roughly a third of all approved drugs. Each is a single polypeptide crossing the membrane seven times.

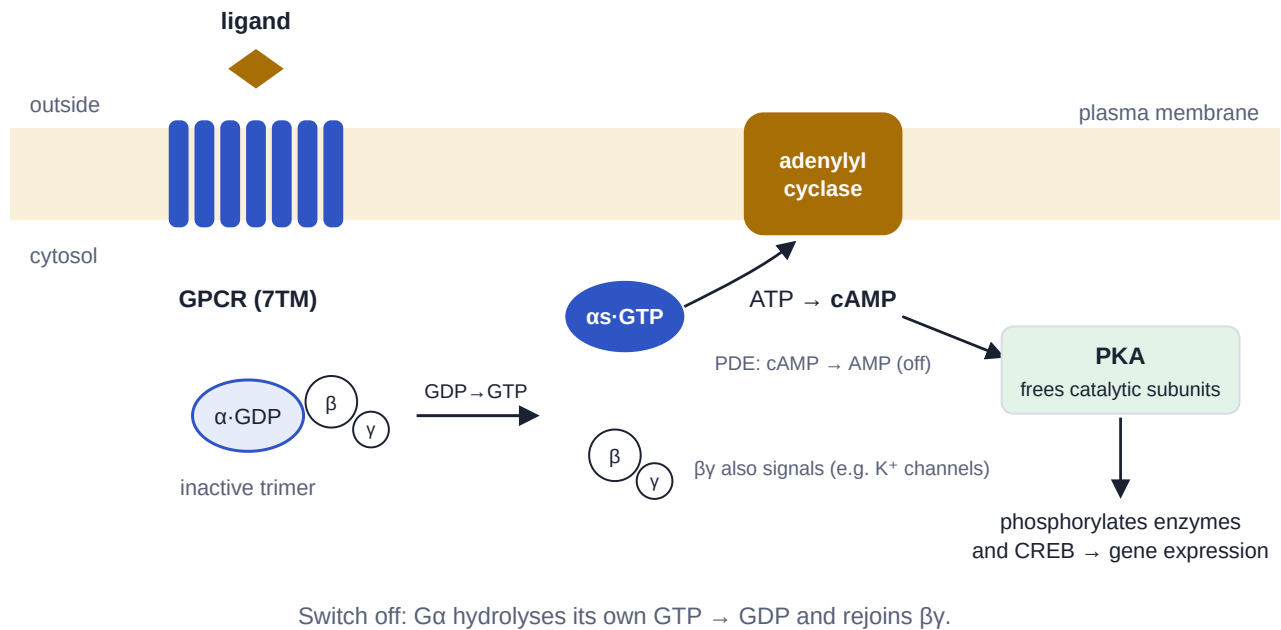


Figure 2. The G_s pathway. The G protein acts as a molecular switch: "on" when G α holds GTP, "off" when it has hydrolysed it to GDP.

The G protein cycle

1. At rest, the **heterotrimeric G protein** (α , β , γ) has GDP bound to its α subunit.
2. The ligand-bound receptor acts as a **guanine nucleotide exchange factor (GEF)**: G α releases GDP and binds GTP.
3. G α -GTP separates from $\beta\gamma$. Both can regulate target proteins.
4. G α 's intrinsic GTPase activity hydrolyses GTP (accelerated by RGS proteins); G α -GDP rejoins $\beta\gamma$ and the system resets.

G protein	Effector	Second messenger	Example receptor
G _s	Activates adenylyl cyclase	↑ cAMP → PKA	β-adrenergic, glucagon, TSH
G _i	Inhibits adenylyl cyclase; $\beta\gamma$ opens K ⁺ channels	↓ cAMP	α ₂ -adrenergic, M2 muscarinic, opioid
G _q	Activates phospholipase C-β	IP ₃ → Ca ²⁺ ; DAG → PKC	α ₁ -adrenergic, M1/M3, histamine H1

G protein	Effector	Second messenger	Example receptor
G_t (transducin)	Activates cGMP phosphodiesterase	↓ cGMP	Rhodopsin (vision)
G_{12/13}	Rho GEFs	Rho → cytoskeleton	Thrombin receptors

The phosphoinositide pathway

Phospholipase C- β cleaves the membrane lipid **PIP₂** into two messengers. **IP₃** diffuses to IP₃-gated channels on the ER and releases Ca²⁺. **DAG** stays in the membrane and, with Ca²⁺, activates **protein kinase C**. Ca²⁺ itself acts through **calmodulin**, which activates CaM-kinases, myosin light-chain kinase and other targets.

Desensitisation

Continued stimulation leads **GPCR kinases (GRKs)** to phosphorylate the receptor, which then binds **β -arrestin**. Arrestin blocks G protein coupling and triggers endocytosis of the receptor. This is why β -agonist inhalers lose effectiveness with overuse.

Toxins that jam the switch

Cholera toxin ADP-ribosylates G_s α so it cannot hydrolyse GTP; it stays on, cAMP rises in intestinal cells, the CFTR channel pumps out Cl⁻, and water follows, causing massive watery diarrhoea. **Pertussis toxin** ADP-ribosylates G_i α so it cannot be activated, which also raises cAMP by removing the brake.

4. Enzyme-linked receptors

Receptor tyrosine kinases (RTKs)

RTKs have an extracellular ligand-binding domain, a single transmembrane helix, and a cytoplasmic tyrosine kinase domain. About 58 exist in humans, including receptors for insulin, EGF, PDGF, VEGF and NGF.

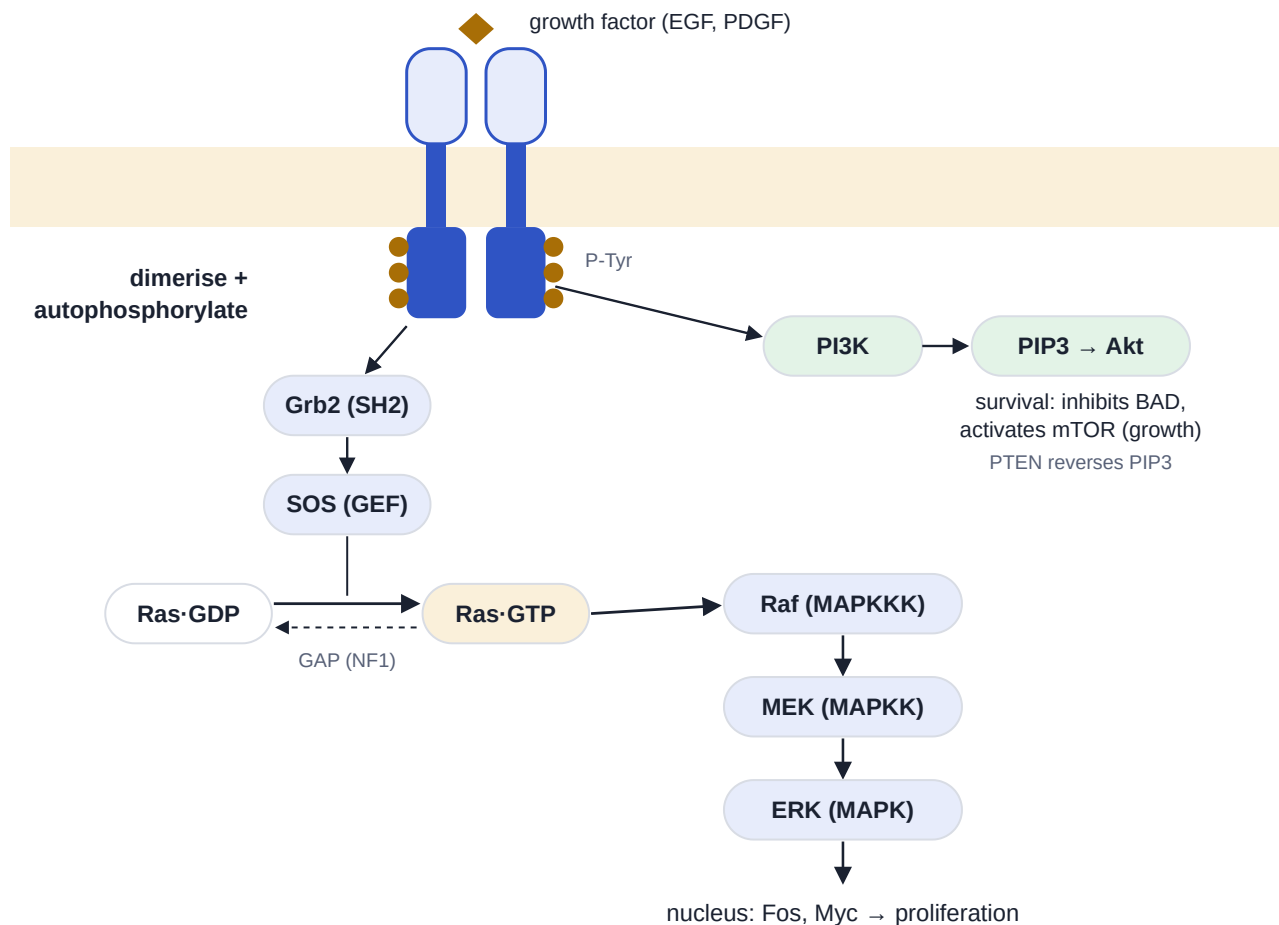


Figure 3. RTK signalling. Ligand binding brings two receptors together; each phosphorylates tyrosines on the other. Phosphotyrosines are docking sites for proteins with SH2 domains, which start the Ras–MAPK and PI3K–Akt pathways.

1. **Dimerisation** of receptors on ligand binding (the insulin receptor is already a disulfide-linked dimer and changes conformation instead).
2. **Trans-autophosphorylation** of tyrosines in the kinase domains and tails.
3. **Adaptors** dock via SH2 domains. Grb2 binds the receptor and brings SOS, a Ras-GEF, to the membrane.
4. **Ras**, a small monomeric GTPase anchored to the membrane by a lipid tail, swaps GDP for GTP and becomes active. GAPs such as NF1 speed its GTP hydrolysis to switch it off.
5. **MAP kinase cascade:** Ras-GTP activates Raf, which phosphorylates MEK, which phosphorylates ERK. ERK enters the nucleus and activates transcription factors for cell proliferation.
6. **PI3K–Akt branch:** PI3K makes PIP₃, which recruits and activates Akt (protein kinase B). Akt promotes survival (it phosphorylates and inactivates the pro-apoptotic protein BAD) and growth through mTOR. The phosphatase **PTEN** removes PIP₃ and opposes this branch.

Signalling and cancer

About 30% of human cancers carry activating **Ras** mutations (e.g. G12V), which cripple its GTPase so it stays on. Overactive RTKs (HER2 in breast cancer, EGFR in lung cancer), activated *BRAF* (V600E in melanoma) and loss of PTEN do the same further along. Drugs target each step: trastuzumab (anti-HER2), gefitinib and erlotinib (EGFR kinase), vemurafenib (BRAF), and imatinib, which blocks the non-receptor tyrosine kinase BCR-ABL in chronic myeloid leukaemia.

Other enzyme-linked receptors

- **Cytokine receptors (JAK-STAT):** the receptors have no kinase of their own but are bound to **Janus kinases (JAKs)**. Ligand binding activates JAKs, which phosphorylate the receptor; **STAT** proteins dock, are phosphorylated, dimerise, and move to the nucleus as transcription factors. Used by interferons, interleukins, erythropoietin and growth hormone.
- **TGF- β receptors:** serine/threonine kinases. Type II receptor phosphorylates type I, which phosphorylates **R-SMADs** (SMAD2/3); these join SMAD4 and enter the nucleus.
- **Receptor guanylyl cyclases:** the ANP receptor makes cGMP directly, leading to vasodilation and sodium excretion.

5. Other pathways and signal termination

Pathway	Key steps	Roles
Notch	Delta on a neighbouring cell binds Notch; two cleavages (the last by γ -secretase) release the Notch intracellular domain, which enters the nucleus	Lateral inhibition, neural and T-cell fate
Wnt/β-catenin	Without Wnt, a destruction complex (APC, axin, GSK-3) marks β -catenin for degradation. Wnt binding to Frizzled blocks the complex; β -catenin enters the nucleus	Development, stem cells; APC loss in colon cancer
Hedgehog	Hedgehog binds Patched, relieving inhibition of Smoothened; Gli transcription factors are activated	Limb patterning; basal cell carcinoma
NF-κB	Signals activate IKK, which phosphorylates I κ B for degradation, freeing NF- κ B	Inflammation, immunity, survival

Switching signals off matters as much as switching them on:

- Ligand removal: reuptake or breakdown (acetylcholinesterase).

- GTP hydrolysis by G proteins and Ras.
- Second messenger breakdown: **phosphodiesterases** destroy cAMP and cGMP. Caffeine inhibits cAMP phosphodiesterase; **sildenafil** inhibits PDE5, prolonging cGMP.
- Protein phosphatases reverse kinase action.
- Receptor desensitisation, internalisation and degradation.

6. Ways a cell can die

Cell death is classified by mechanism (Nomenclature Committee on Cell Death). The two classic forms are:

- **Apoptosis**: a regulated, energy-requiring programme of self-destruction. Cells shrink, break into membrane-bound pieces and are eaten without inflammation. It is physiological as well as pathological.
- **Necrosis**: accidental death after severe injury. Cells swell and burst, spilling their contents and provoking inflammation. It is always pathological.

We now also recognise **regulated forms of necrosis** (necroptosis, pyroptosis, ferroptosis), which look like necrosis but are driven by specific molecular pathways, and **autophagy-dependent death**.

7. Apoptosis

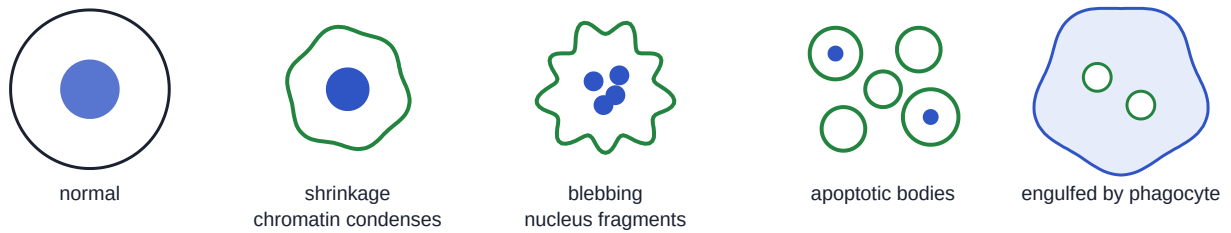
The term, from the Greek for "falling off" (like leaves from a tree), was introduced by **Kerr, Wyllie and Currie in 1972**. The genetic programme was worked out in the nematode *Caenorhabditis elegans*, where exactly 131 of the 1,090 somatic cells formed during development die. **H. Robert Horvitz** identified the genes: *egl-1* (BH3-only), *ced-9* (BCL-2), *ced-4* (Apaf-1) and *ced-3* (caspase). He shared the 2002 Nobel Prize with Sydney Brenner and John Sulston.

Physiological roles

- **Development**: sculpting fingers and toes by removing webbing; removing the tadpole tail; eliminating excess neurons (half of those formed die for lack of trophic factor).
- **Tissue homeostasis**: balancing cell division in gut epithelium and bone marrow; the menstrual shedding of endometrium after hormone withdrawal.
- **Immune system**: deleting self-reactive lymphocytes in the thymus; removing lymphocytes after an infection; killing infected cells by cytotoxic T cells and NK cells.
- **Defence**: removing cells with irreparable DNA damage, preventing cancer.

Morphology and biochemistry

Apoptosis



Necrosis

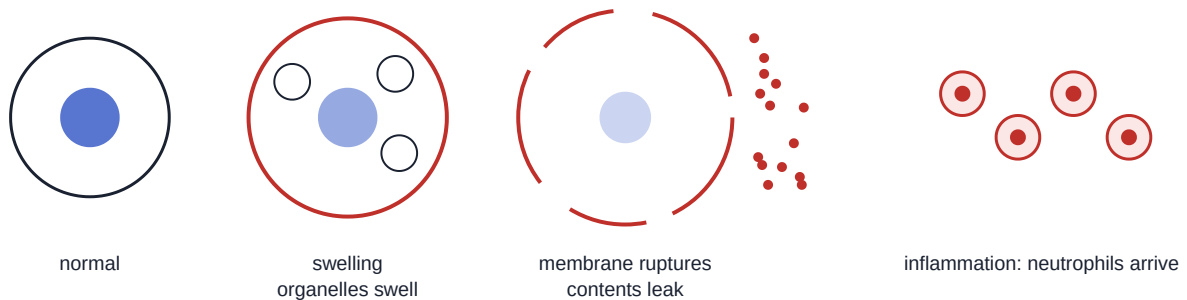


Figure 4. The morphological sequence of apoptosis (top) and necrosis (bottom). Apoptosis keeps the membrane intact until the fragments are eaten, so nothing leaks out and there is no inflammation.

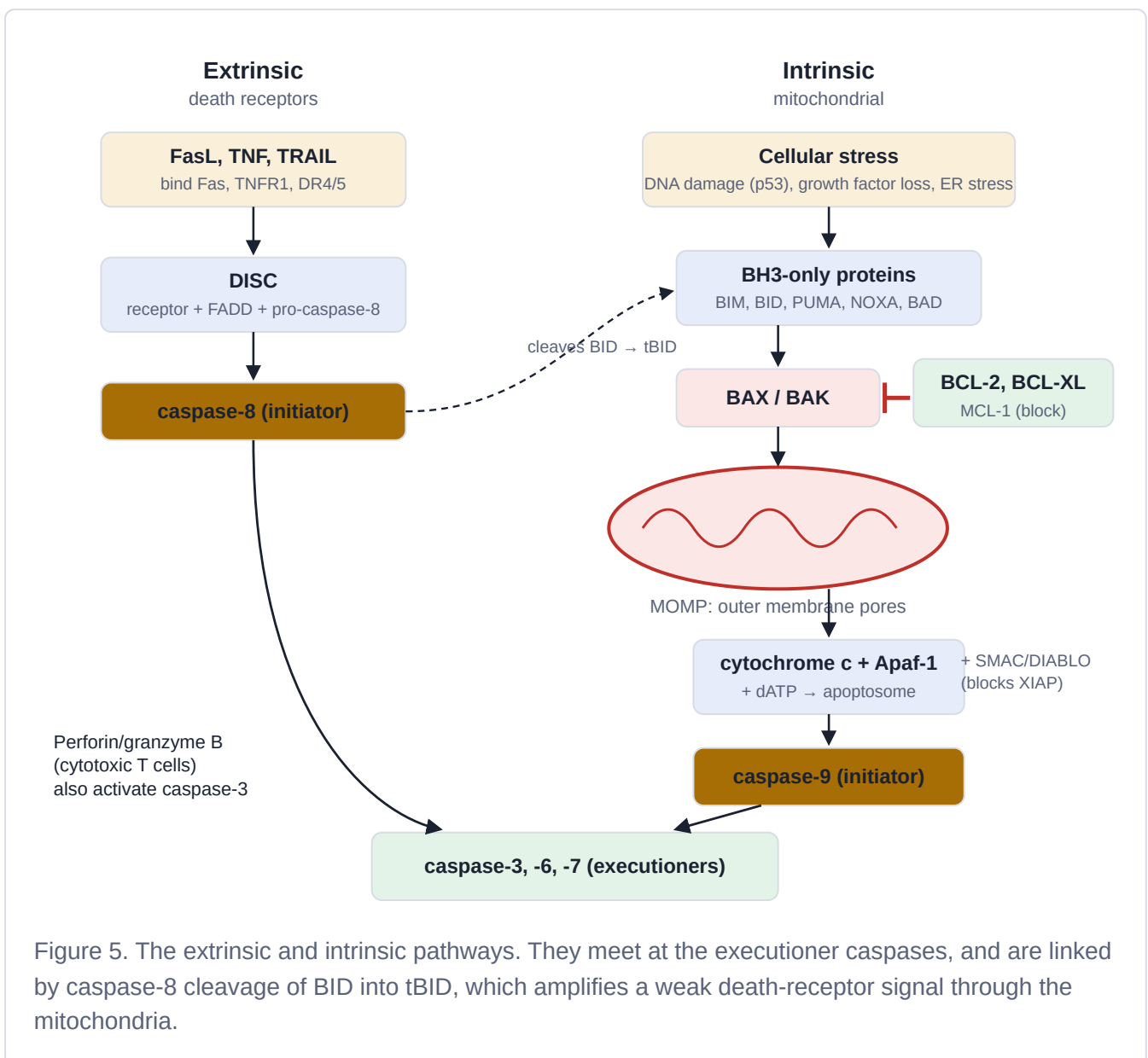
- **Cell shrinkage** with dense cytoplasm and tightly packed organelles.
- **Chromatin condensation** against the nuclear envelope (**pyknosis**), then nuclear fragmentation (**karyorrhexis**).
- **Membrane blebbing** and formation of **apoptotic bodies**, membrane-bound fragments containing organelles and nuclear pieces.
- **"Eat-me" signal**: phosphatidylserine, normally confined to the inner leaflet, flips to the outer leaflet (scramblase activated, flippase cleaved). Macrophages recognise it and engulf the cell (efferocytosis).
- **DNA ladder**: caspase-activated DNase (CAD), freed when caspase-3 cleaves its inhibitor ICAD, cuts DNA between nucleosomes into multiples of about 180–200 bp, giving a "ladder" on gel electrophoresis.
- Cleavage of key proteins by caspases: nuclear lamins, PARP, cytoskeletal proteins.

Caspases: the executioners

Caspases are **cysteine** proteases that cleave after **aspartate** residues. They are made as inactive zymogens (pro-caspases) and activated by cleavage or by being gathered into large complexes, where they activate each other.

Group	Caspases	Activation and role
Initiators	2, 8, 9, 10	Long prodomains; activated by dimerisation in platforms (DISC for 8 and 10, apoptosome for 9); cleave executioners
Executioners	3, 6, 7	Short prodomains; cleaved by initiators; cut hundreds of cellular proteins
Inflammatory	1, 4, 5, 11	Activated in inflammasomes; process IL-1 β and IL-18 and cause pyroptosis

The two pathways



Extrinsic (death receptor) pathway

Death ligands (Fas ligand, TNF, TRAIL) bind death receptors of the TNF receptor family, which carry a cytoplasmic **death domain**. The receptors cluster and recruit the adaptor **FADD**, which recruits pro-caspase-8 through death effector domains. This **death-inducing signalling complex (DISC)** activates caspase-8, which cleaves caspase-3 directly. The protein **c-FLIP** can block this step. Cytotoxic T cells use Fas ligand, and also deliver **granzyme B** through **perforin** pores, which activates caspases and BID directly.

Intrinsic (mitochondrial) pathway

Triggered from inside the cell: DNA damage, loss of growth factors, ER stress, hypoxia, oncogene activation. It is controlled by the **BCL-2 family**, which share BCL-2 homology (BH) domains:

Subgroup	Members	Role
Anti-apoptotic	BCL-2, BCL-XL, MCL-1	Bind and restrain BAX/BAK and BH3-only proteins
Pro-apoptotic effectors	BAX, BAK	Oligomerise to form pores in the outer mitochondrial membrane
BH3-only sensors	BIM, BID, PUMA, NOXA, BAD	Sense stress; neutralise anti-apoptotic proteins or directly activate BAX/BAK

1. Stress activates BH3-only proteins. p53, for example, induces PUMA and NOXA after DNA damage; growth factor withdrawal releases BAD from its Akt-dependent inactivation.
2. BAX and BAK are freed, oligomerise and cause **mitochondrial outer membrane permeabilisation (MOMP)**, the point of no return.
3. **Cytochrome c** leaves the intermembrane space and binds **Apaf-1**. With dATP/ATP, seven Apaf-1 molecules assemble into a wheel-shaped **apoptosome**, which activates caspase-9.
4. Caspase-9 activates caspases-3 and -7.
5. **SMAC/DIABLO**, also released, neutralises **XIAP**, an inhibitor of caspases, so the cascade can proceed.

Detecting apoptosis

Method	What it detects
Annexin V + propidium iodide (flow cytometry)	Annexin V binds exposed phosphatidylserine. Annexin V+/PI- = early apoptosis; +/+ = late apoptosis or necrosis; -/+ = necrosis
TUNEL assay	Labels free 3'-OH ends of fragmented DNA in tissue sections

Method	What it detects
DNA laddering	Internucleosomal fragments of $\approx 180\text{--}200$ bp on agarose gel (necrosis gives a smear)
Caspase activity / cleaved caspase-3 antibodies	Caspase activation
PARP cleavage (Western blot)	116 kDa PARP cut to an 89 kDa fragment by caspase-3
Cytochrome c release, mitochondrial membrane potential dyes	MOMP

8. Necrosis

Necrosis is the death of cells or tissue in a living organism after irreversible injury, typically affecting sheets of contiguous cells. Causes include ischaemia (loss of blood supply), toxins, physical trauma, extreme temperature, and infection.

Mechanism

1. **ATP depletion:** failure of oxidative phosphorylation stops the Na^+/K^+ -ATPase. Na^+ and water enter and the cell swells (hydropic change, "oncosis").
2. **Anaerobic glycolysis** produces lactic acid, lowering pH and clumping chromatin.
3. **Ca^{2+} influx** activates phospholipases, proteases and endonucleases, and damages mitochondria (mitochondrial permeability transition).
4. **Reactive oxygen species**, especially on reperfusion, peroxidise membrane lipids.
5. **Membrane rupture** releases enzymes and **DAMPs** (HMGB1, ATP, uric acid), activating inflammation.

Nuclear changes seen under the microscope: **pyknosis** (shrinkage, dark), **karyorrhexis** (fragmentation) and **karyolysis** (fading as DNase digests the DNA). The cytoplasm becomes intensely eosinophilic (pink).

Patterns of tissue necrosis

Type	Feature	Typical setting
Coagulative	Tissue architecture preserved for days; "ghost" outlines of cells; proteins denatured, enzymes inactivated	Infarcts of heart, kidney, spleen

Type	Feature	Typical setting
Liquefactive	Tissue digested into a liquid mass	Brain infarcts; bacterial abscesses (pus)
Caseous	Cheese-like, friable white material within a granuloma	Tuberculosis
Fat	Lipases release fatty acids that combine with Ca^{2+} (saponification), giving chalky white spots	Acute pancreatitis; breast trauma
Fibrinoid	Immune complexes and fibrin deposited in vessel walls, bright pink	Vasculitis, malignant hypertension
Gangrenous	Dry: coagulative, in limbs with arterial occlusion. Wet: with bacterial infection and liquefaction	Diabetic foot, bowel

Enzymes leaking from necrotic cells are used as diagnostic markers: **cardiac troponins** and CK-MB in myocardial infarction, **ALT and AST** in liver injury, amylase and lipase in pancreatitis.

9. Regulated necrosis

Form	Key molecules	Features
Necroptosis	RIPK1, RIPK3, MLKL	Triggered by TNF when caspase-8 is inhibited (e.g. by viral proteins). RIPK3 phosphorylates MLKL, which punctures the plasma membrane. A back-up defence against viruses that block apoptosis. Blocked by necrostatin-1
Pyroptosis	Inflammasome, caspase-1 (or 4/5/11), gasdermin D	Cleaved gasdermin D forms membrane pores; IL-1 β and IL-18 released. Fiery, inflammatory death of infected macrophages
Ferroptosis	Iron, lipid peroxides, GPX4, glutathione	Iron-dependent lipid peroxidation when GPX4 fails; blocked by ferrostatin-1 and iron chelators (Dixon, 2012)
NETosis	Neutrophil elastase, PAD4	Neutrophils expel chromatin nets that trap microbes

Form	Key molecules	Features
Parthanatos	PARP-1 overactivation	Excessive DNA repair consumes NAD ⁺ and ATP; AIF moves to the nucleus

Autophagy ("self-eating") is primarily a survival process: cells enclose damaged organelles or proteins in double-membrane autophagosomes and digest them in lysosomes, especially during starvation (mTOR inhibition releases ULK1). Excessive autophagy can contribute to death in some settings. Yoshinori Ohsumi received the 2016 Nobel Prize for discovering its genes in yeast.

10. Apoptosis vs necrosis

Feature	Apoptosis	Necrosis
Nature	Programmed, regulated	Accidental (except regulated forms)
Stimuli	Physiological and mild pathological (DNA damage, loss of survival signals)	Always pathological, severe (ischaemia, toxins, trauma)
Extent	Single, scattered cells	Groups or sheets of cells
Energy	Requires ATP	Results from ATP loss
Cell size	Shrinks	Swells
Nucleus	Condensation and fragmentation	Pyknosis, karyorrhexis, karyolysis
Plasma membrane	Intact; blebs; phosphatidylserine exposed	Ruptured
Organelles	Intact until late	Swollen, damaged
DNA on gel	Ladder (≈180–200 bp steps)	Smear
Cellular contents	Contained in apoptotic bodies	Leak out
Inflammation	None; phagocytes release anti-inflammatory signals	Yes

Feature	Apoptosis	Necrosis
Fate	Engulfed by macrophages or neighbours	Cleared by inflammatory cells; may calcify (dystrophic calcification)

11. Clinical correlations

Too little apoptosis	Too much apoptosis
Follicular lymphoma: t(14;18) places <i>BCL2</i> next to the immunoglobulin heavy-chain enhancer, over-expressing BCL-2 so B cells fail to die	Neurodegeneration: Alzheimer, Parkinson, Huntington disease, ALS
Cancers with p53 loss: damaged cells survive (about half of all human tumours)	Ischaemic injury: the zone around a stroke or infarct
Autoimmune lymphoproliferative syndrome (ALPS): Fas or FasL mutations; self-reactive lymphocytes persist	AIDS: loss of CD4 T cells
Viral persistence: EBV, HPV (E6 degrades p53), adenovirus (E1B-19K is a BCL-2 mimic)	Toxic hepatitis, graft-versus-host disease

Venetoclax, a BH3-mimetic drug that blocks BCL-2, frees BIM and restarts apoptosis in chronic lymphocytic leukaemia and acute myeloid leukaemia. Most chemotherapy and radiotherapy kill tumour cells largely by triggering the intrinsic pathway through DNA damage and p53.

12. Quick revision

Remember	Fact
Second messengers	cAMP, cGMP, IP ₃ , DAG, Ca ²⁺ , NO
G proteins	G _s ↑cAMP; G _i ↓cAMP; G _q → PLC → IP ₃ /DAG
Toxins	Cholera: G _s stuck on. Pertussis: G _i stuck off

Remember	Fact
RTK cascade	RTK → Grb2 → SOS → Ras → Raf → MEK → ERK
JAK-STAT	Cytokine receptors; STAT dimers enter nucleus
Apoptosis term	Kerr, Wyllie and Currie, 1972
<i>C. elegans</i> genes	<i>egl-1</i> → $\neg$ <i>ced-9</i> $\neg$ <i>ced-4</i> → <i>ced-3</i> ; 131 cells die
Caspases	Initiators 2, 8, 9, 10; executioners 3, 6, 7; inflammatory 1, 4, 5, 11
Extrinsic	FasL/TNF → FADD → DISC → caspase-8
Intrinsic	BH3-only → BAX/BAK → cytochrome c → Apaf-1 apoptosome → caspase-9
Markers	Annexin V (PS), TUNEL, DNA ladder, cleaved PARP
Necrosis types	Coagulative (heart), liquefactive (brain), caseous (TB), fat (pancreas), fibrinoid, gangrenous
Regulated necrosis	Necroptosis (RIPK3/MLKL), pyroptosis (gasdermin D), ferroptosis (GPX4)

13. End-semester question bank

Long answer (10–15 marks)

1. What is cell signalling? Describe the different types of signalling and classify cell-surface receptors with examples.
2. Describe the structure and mechanism of G protein-coupled receptors. Explain the cAMP and phosphoinositide second-messenger pathways.
3. Describe signal transduction through receptor tyrosine kinases, including the Ras-MAP kinase cascade. How do mutations in this pathway lead to cancer?
4. Define apoptosis. Describe its morphological and biochemical features and its physiological significance.
5. Explain the intrinsic and extrinsic pathways of apoptosis and the role of the BCL-2 family and caspases.
6. What is necrosis? Describe its mechanism and the different morphological types, with examples.

7. Compare apoptosis and necrosis. Add a note on necroptosis and pyroptosis.
8. Discuss the role of apoptosis in development, immunity and disease, with reference to cancer and neurodegeneration.

Short notes (5 marks)

1. Second messengers
2. Heterotrimeric G proteins
3. Ras as a molecular switch
4. JAK-STAT pathway
5. Cholera and pertussis toxins
6. Caspases
7. Apoptosome
8. BCL-2 family
9. Caseous necrosis
10. Programmed cell death in *C. elegans*

Differentiate between

1. Endocrine and paracrine signalling
2. G_s and G_i proteins
3. Apoptosis and necrosis
4. Intrinsic and extrinsic pathways of apoptosis
5. Coagulative and liquefactive necrosis
6. Initiator and executioner caspases

Draw and label

1. The G protein cycle and cAMP pathway
2. RTK-Ras-MAPK signalling
3. Intrinsic and extrinsic apoptotic pathways
4. Morphological stages of apoptosis and necrosis

▼ Very short answer (2 marks), with answers

1. Enzyme that makes cAMP? **Adenylyl cyclase**

2. Second messenger releasing Ca^{2+} from the ER? **IP_3**
3. Protein that desensitises GPCRs after GRK phosphorylation? **β -arrestin**
4. GEF that activates Ras downstream of RTKs? **SOS**
5. Domain that binds phosphotyrosine? **SH2 domain**
6. Who coined the term apoptosis? **Kerr, Wyllie and Currie (1972)**
7. Protein that binds cytochrome c to form the apoptosome? **Apaf-1**
8. Adaptor linking Fas to caspase-8? **FADD**
9. Size of DNA ladder fragments? **Multiples of $\approx 180\text{-}200$ bp**
10. Type of necrosis in tuberculosis? **Caseous**
11. Protein that forms pores in pyroptosis? **Gasdermin D**
12. Translocation causing BCL-2 over-expression in follicular lymphoma? **t(14;18)**

14. Case-based MCQs

Ten hard questions that test application rather than recall. Choose an answer before opening the explanation.

Q1. A traveller returning from Bangladesh has profuse 'rice-water' diarrhoea. Intestinal cells show very high cAMP. Adding a non-hydrolysable GTP analogue to normal intestinal membranes would reproduce which feature of the toxin's action, and why?

- A. Inactivation of adenylyl cyclase, because GTP is required to switch it off
- B. Persistent activation of $\text{G}_s\alpha$, because the toxin, like the analogue, prevents the 'off' step of GTP hydrolysis
- C. Blockade of $\text{G}_i\alpha$ activation
- D. Direct opening of CFTR without cAMP

▼ Show answer

Answer: B. Cholera toxin ADP-ribosylates $\text{G}_s\alpha$ and abolishes its GTPase activity, so once GTP is bound it stays on. A non-hydrolysable GTP analogue has the same effect: $\text{G}\alpha$ cannot turn itself off. Sustained cAMP activates PKA, which opens CFTR, causing Cl^- and water loss. Blocking G_i activation describes pertussis toxin.

Q2. A child with whooping cough has a markedly raised lymphocyte count. The pertussis toxin ADP-ribosylates a G protein α subunit. The result, and its effect on cAMP in target cells, is:

- A. $G_i\alpha$ is locked in the GDP-bound state and cannot inhibit adenylyl cyclase, so cAMP rises
- B. $G_s\alpha$ is locked on, so cAMP rises
- C. $G_q\alpha$ is inactivated, so IP_3 falls
- D. $G_i\alpha$ is locked on, so cAMP falls

▼ Show answer

Answer: A. Pertussis toxin modifies $G_i\alpha$ so that it cannot couple to its receptor and exchange GDP for GTP. The inhibitory brake on adenylyl cyclase is lost, and cAMP rises. Lymphocytes whose chemokine (G_i -coupled) receptors are blocked cannot leave the blood, causing lymphocytosis.

Q3. A tumour cell line carries Ras G12V, which cannot hydrolyse GTP. Which drug would be expected to be **least** effective at blocking its proliferation signal?

- A. An inhibitor of MEK
- B. An antibody against the EGF receptor
- C. An inhibitor of ERK
- D. An inhibitor of Raf

▼ Show answer

Answer: B. Blocking a step upstream of a constitutively active component is useless: Ras G12V is on regardless of whether the EGF receptor is signalling. Inhibitors of Raf, MEK or ERK act downstream of Ras and can still block the pathway. This is why KRAS-mutant colorectal cancers do not respond to anti-EGFR antibodies such as cetuximab.

Q4. A lymph node biopsy shows a follicular lymphoma with the translocation t(14;18). The lymphoma cells accumulate mainly because they:

- A. Proliferate faster because BCL-2 activates cyclin D1
- B. Resist the intrinsic pathway of apoptosis because over-expressed BCL-2 restrains BAX and BAK
- C. Lack Fas receptors, so cytotoxic T cells cannot kill them
- D. Have constitutively active caspase-8

▼ **Show answer**

Answer: B. The translocation places *BCL2* under the immunoglobulin heavy-chain enhancer. BCL-2 binds and neutralises BH3-only proteins and BAX/BAK, preventing mitochondrial outer membrane permeabilisation. The tumour grows mainly because cells fail to die, not because they divide fast, which is why follicular lymphoma is typically slow-growing. Venetoclax targets exactly this defect.

Q5. Fibroblasts from a patient are treated with a DNA-damaging drug. Cytochrome c is released from mitochondria normally, but caspase-9 is not activated and the cells survive. Which protein is most likely defective?

- A. BAX
- B. Apaf-1
- C. FADD
- D. Caspase-8

▼ **Show answer**

Answer: B. Cytochrome c release shows that everything up to MOMP works, so BAX/BAK are functional. The next step is binding of cytochrome c to Apaf-1 to build the apoptosome, which activates caspase-9. FADD and caspase-8 belong to the extrinsic pathway and are not needed for DNA-damage-induced death.

Q6. Flow cytometry of treated leukaemia cells shows a large population that stains with annexin V but excludes propidium iodide. These cells are most likely:

- A. Viable cells with high phosphatidylserine synthesis
- B. In early apoptosis, with phosphatidylserine exposed but membranes still intact
- C. Necrotic, with ruptured membranes
- D. In late apoptosis, with ruptured membranes

▼ **Show answer**

Answer: B. Annexin V binds phosphatidylserine on the outer leaflet, an early apoptotic change. Propidium iodide exclusion shows the membrane is still intact. Late apoptotic and necrotic cells are PI-positive because their membranes have been breached.

Q7. A 12-year-old has enlarged lymph nodes and spleen and autoimmune haemolytic anaemia. His blood contains many T cells lacking both CD4 and CD8. Genetic testing shows a heterozygous mutation in the death domain of *FAS*. The main defect is:

- A. Failure of positive selection in the thymus
- B. Failure of activation-induced death of lymphocytes after immune responses
- C. Excessive apoptosis of T cells
- D. Defective perforin release by cytotoxic T cells

▼ **Show answer**

Answer: B. This is autoimmune lymphoproliferative syndrome. Fas-FasL interaction normally kills repeatedly stimulated lymphocytes, contracting the response and removing self-reactive cells. With defective Fas, lymphocytes accumulate (including unusual double-negative T cells) and autoimmunity follows. Death-domain mutations act dominantly because Fas must trimerise.

Q8. A patient dies 3 days after an occlusion of the middle cerebral artery. Another patient dies 3 days after occlusion of a coronary artery. At autopsy, the infarcted tissues will most likely show:

- A. Coagulative necrosis in both
- B. Liquefactive necrosis in the brain and coagulative necrosis in the heart
- C. Coagulative necrosis in the brain and liquefactive necrosis in the heart
- D. Caseous necrosis in both

▼ **Show answer**

Answer: B. Ischaemic infarcts in solid organs like the heart show coagulative necrosis, with cell outlines preserved for days because injury also denatures the enzymes that would digest them. The brain, rich in lipids and with little supporting connective tissue, digests itself, and ischaemic brain infarcts undergo liquefactive necrosis, later forming a cyst.

Q9. Cells are infected with a virus that encodes a caspase-8 inhibitor. When the cells are stimulated with TNF, they still die, their membranes rupture and inflammatory molecules are released. This death would be blocked most specifically by:

- A. A pan-caspase inhibitor
- B. An inhibitor of RIPK1 or RIPK3 kinase activity (e.g. necrostatin-1)
- C. Over-expression of BCL-2
- D. Knockout of gasdermin D

▼ **Show answer**

Answer: B. When caspase-8 is inhibited, TNF signalling switches to necroptosis: RIPK1 and RIPK3 form the necrosome and phosphorylate MLKL, which ruptures the membrane. A caspase inhibitor would not help (caspases are already blocked, and caspase-8 normally suppresses necroptosis). BCL-2 blocks only the intrinsic pathway; gasdermin D mediates pyroptosis.

Q10. In *C. elegans*, loss of *ced-9* causes many extra cells to die and kills the embryo. In a double mutant lacking both *ced-9* and *ced-3*, which of the 131 cells that normally die during development will die?

- A. All 131, plus many others
- B. None of them
- C. About half of them
- D. Only the neurons among them

▼ **Show answer**

Answer: B. The pathway runs *egl-1* $\rightarrow$ *ced-9* $\rightarrow$ *ced-4* $\rightarrow$ *ced-3*. Loss of *ced-9* alone kills many extra cells because the brake is removed. But *ced-3* encodes the executioner caspase at the end of the pathway, and its loss prevents all cell death. In an epistasis test, the downstream gene's phenotype wins, so no cells die.

For further reading: Alberts, *Molecular Biology of the Cell*; Lodish, *Molecular Cell Biology*; Robbins and Cotran, *Pathologic Basis of Disease*; Karp, *Cell and Molecular Biology*.