Cell Biology

Unit 4 · Lesson 3 · Cancer, stem cells, culture

The notes for Lesson 3 of Unit 4: chapters 4.7 to 4.9 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 3 of 3 | 3 chapters | Chapters 4.7 to 4.9

About these notes

These are the notes for **Lesson 3 · Cancer, stem cells, culture** of the University free n easy video lessons on Cell Biology Unit 4, Cell Cycle, Division and Cell Death. They are chapters 4.7 to 4.9 of the unit book, unchanged, so a page here is the same page in the full book.

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.

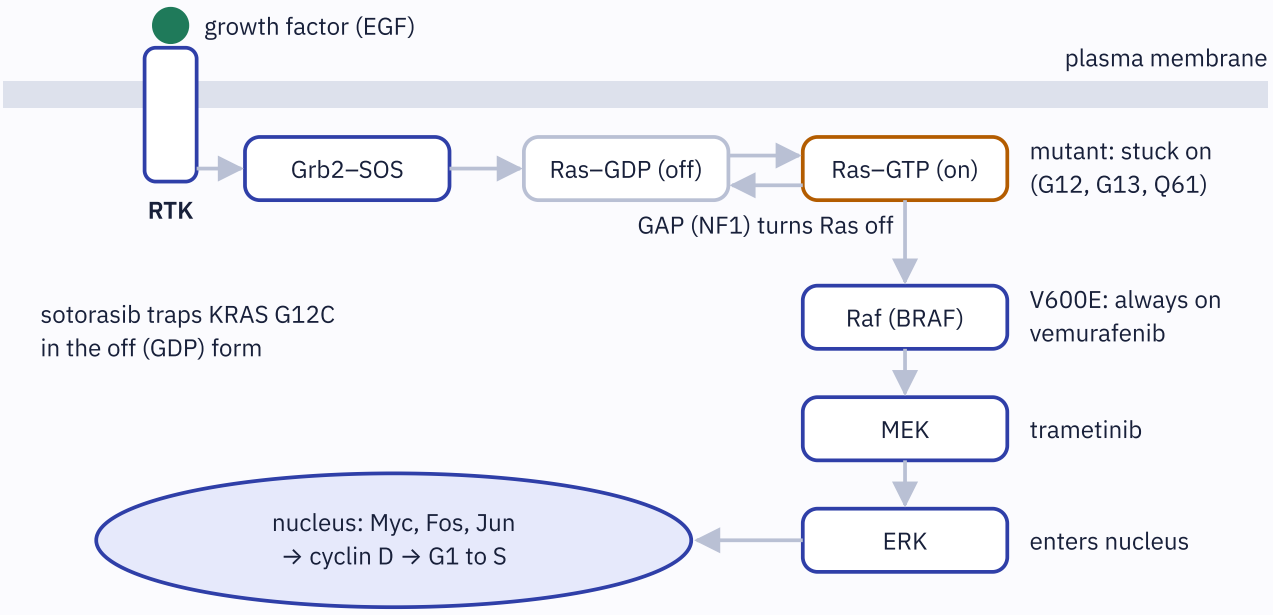


Figure 4.7.1 The Ras–Raf–MEK–ERK pathway from a growth factor to the nucleus. SOS turns Ras on and a GAP turns it off. Mutations at glycine 12, glycine 13 or glutamine 61 stop GAPs from switching Ras off; the BRAF V600E mutation makes Raf active without Ras. Drug names show where each class acts.

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.

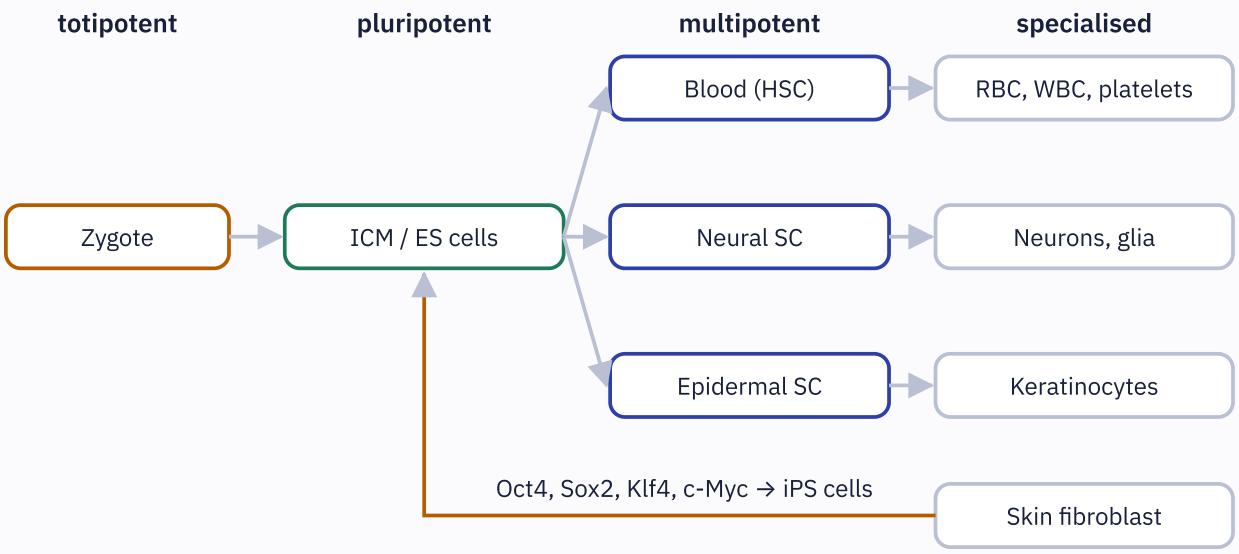


Figure 4.8.1 Stem cells ranked by potency, from left to right. A zygote is totipotent; the inner cell mass and ES cells are pluripotent; adult stem cells such as haematopoietic, neural and epidermal stem cells are multipotent and make the specialized cells on the right. The orange loop shows reprogramming: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

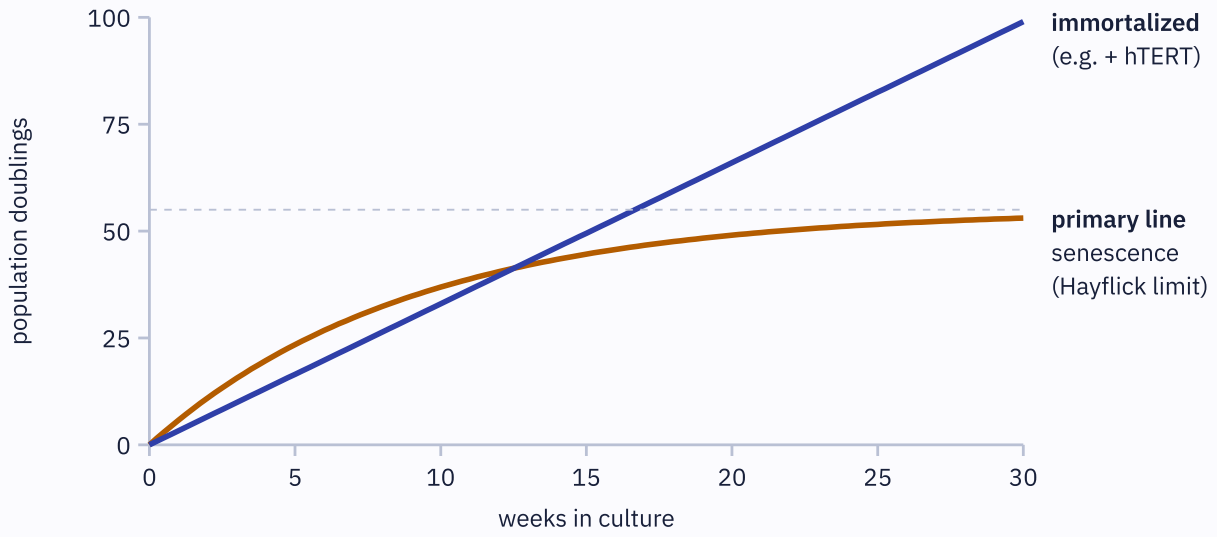


Figure 4.9.1 Cumulative population doublings against time for a normal (primary) fibroblast line and the same cells immortalized with hTERT. These are model curves, not measured data. The primary line slows and stops near its Hayflick limit; the immortalized line keeps doubling at a steady rate.

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; $t_d = 96 \div 4 = 24$ 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".

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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

Glossary (terms used in this lesson)

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.

Cyclin Regulatory protein whose level rises and falls in the cycle and which activates a CDK.

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.
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.
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*.

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.

Syllabus: the University free n easy universal syllabus, built from top universities' syllabi.

Built for the University free n easy YouTube channel from the universal syllabus of Cell Biology (Oct 2026). Written with top universities' syllabi and standard textbooks; check a formula against your prescribed book before an exam, and report mistakes on the channel.