CELL CYCLE

❑ CELL DIVISION

Features

- Number of cells in human: ~1 billion per gram of tissue – all derived from single cell (fertilised egg)
- Large numbers of cells must replicate often to produce adult human (or replace damaged cells)
- Essential that replication precisely regulated
- Process different in prokaryotic and eukaryotic cells

❑ CELL CYCLE

Features

- Essential that cell division precisely regulated
- Phases of cell cycle must follow in correct order – one phase must be completed before next phase begins
- Cell cycle times vary for different cell types – for most human cells: between 10 and 30 hours, for E. coli: 20 minutes

STUDYING CELL CYCLE

- Normal human cells grown in culture (in vitro) will not divide indefinitely:
  - Human fibroblasts go through 25 – 40 cell divisions and stop – enter replicative cell senescence
  - Immortalise human cells undergo mutations that allow to continue to grow indefinitely in culture (cell lines)
  - Cells derived from human cancers also grow indefinitely in culture
    - Most commonly used: human cell line derived from cervical cancer biopsy HeLa (from patient name)

❑ PHASES OF CELL CYCLE

INTERPHASE

Features

- Appears inactivate microscopically
- Longest part of cell cycle

G1 (Gap or Growth 1) Phase	• Most variable in length • First phase after cell division • Cells mature and make more cytoplasm and organelles • Normal metabolic activities occur • Preparation for S-phase • Cell requires external signal to exit phase
S (Synthesis) Phase	• Replication of DNA and histones
G2 (Gap or Growth 2) Phase	• Cell prepares for division • Synthesis of materials needed for mitosis (not DNA) – proteins, organelles, microtubules, and centrioles

G_0 PHASE OF CYCLE

- Cells may exit cycle and enter G_0 stage

Quiescent/Senescent: Still metabolically active, but may be terminally differentiated (never divide again)

- G_0 can followed by re-entry into cell cycle
  - Most lymphocytes in human blood in G_0
  - With proper stimulation, such as encountering appropriate antigen, stimulated to re-enter cell cycle (at G_1)

- Represents active repression of genes needed for mitosis

❑ MITOSIS

- Proceeds through series of stages
  - Stages characterised by location and behaviour of chromosomes
  - Some conversions between stages irreversible transitions

- Requires formation of spindle
  - Chromosomes separated by mitotic spindle

Spindle: Symmetrical, bipolar structure composed of microtubules that extend between two poles
→ At each pole: centrosome

- Spindle formation and function depend on dynamic behaviour of microtubules and associated motor proteins
  - Spindle complex assembly of microtubules and microtubule-dependent proteins
  - Microtubules highly organised with respect to polarity

❑ CELL PROLIFERATION IN ADULTS

- Cells vary in capacity to divide

Never Divide	• Lens, nerve, and cardiac muscle cells
Normally do not Divide, can be Stimulated to do so	• Skin fibroblasts, smooth muscles, endothelial cells (line blood vessels), epithelial cells (lung, liver, kidney)
Frequent Turnover	• Embryonal, haematopoietic, epithelial cells of skin and digestive tract • Cells do not replicate themselves – replaced by proliferative stem cells

CELL CYCLE REGULATION I

❑ MATURATION PROMOTING FACTOR

Key Subunits of MPF

- Cyclin: protein whose levels vary (cycle) with cell cycle
- Cyclin-dependent kinase: enzyme that phosphorylates substrates only when bound by cyclin

- Fluctuating levels of MPF drives cell into and out of mitosis

MPF ACTIVITY

- Many studies on regulation of cell cycle conducted in yeast
  - Main cyclin-dependent kinase: Cdc2

- MPF regulates entry into mitosis by phosphorylating different proteins

MPF Activities that will Induce Mitosis

Change Induced	Direct Effect of MPF
Chromatin condensation	Phosphorylation of histone H1
Spindle formation	Phosphorylation of microtubule associated proteins (MAPs)
Nuclear envelope between fragmentation of Golgi and ER	Phosphorylation of lamins

- MPF directly phosphorylates lamin filaments
  - Breakdown of lamin filaments required for mitosis

❑ CDK

CDK: Core of cell-cycle control system

→ CDK activity terminated by cyclin degradation

Regulation of CDK Activity

Cyclin Binding	• Primary mechanism of regulation • Causes conformational change in CDK to allow protein kinase activity • Breakdown of cyclins regulates CDK activity
Phosphorylation	• Can activate or inhibit kinase activity • Target amino acid residues lie adjacent to substrate binding site
CKIs	• Specific CDK inhibitors (CKIs) inhibit by binding to CDK-cyclin and masking substrate site

CELL CYCLE REGULATION II

❑ REGULATION 1: CYCLIN DEGRADATION

Features

- Short-lived proteins (cyclins A and B) degraded by ubiquitin/proteasome pathway
- Targeted for destruction by being ubiquitinated
- Involves covalent attachment of ubiquitin by set of enzymes (E1, E2, E3)
- Process requires energy

Ubiquitin

- Highly conserved and essential for life
- Protein has 76 amino acids (8.5 kD) and essentially same in all species

UBIQUITINATION

- Involves multiple rounds of addition of Ub to target protein
  - E1: Ub activating enzyme
  - E2: Ub conjugating enzyme
  - E3: Ub ligase

SIGNALS FOR UBIQUITINATION

- Some proteins (such as cyclins) contain destruction box – targets proteins for ubiquitination (cyclin destruction box)

- Amino acid at NH-terminal of box acts as signal for ubiquitination

NH-Terminal Amino Acid	Half Life
Stabilising: • Met, Gly, Ala, Ser, Thr, Val	> 20 hours
Destabilising: • Ile, Gln • Tyr, Glu, Pro • Leu, Phe, Asp, Lys, Arg	 ~ 30 minutes ~ 10 minutes ~ 3 minutes

Anaphase Promoting Complex (APC)

- Ubiquitin ligase
- Can add ubiquitin to different proteins, depending on partner protein – either Cdc20 or Cdh1

- APC also ubiquitinates mitotic cyclin → targets it for destruction

❑ PHOSPHORYLATION

- MPF activity regulated by phosphorylation
  - Y15: Tyrosine at position 15 in CDK
  - T161: Threonine at position 161 in CDK

❑ CKIs

CDK INHIBITORS (CKIs)

INK4: Inhibitor of CDK 4

CIP/KIP: Cyclin/kinase inhibitor

p15: Protein that has molecular weight of 15 kDa (p16: 16 kDa)

→ Proteins initially only known by size and now retained designations

Ink4 Family (Inhibitors of CDK4)	• Bind CDK and exclude cyclin from activating site • p15, p16, p18, p19 specific for G1 CDK
CIPs and KIPs (Cyclin/Kinase Inhibitory Proteins)	• p21, p27, p57 act on S-phase CDK/cyclin complexes • Binding partly masks ATP binding site

p27: CDK Inhibitor that Arrests Cell Cycle Progression

➢ Binds CDK2/cyclin A (S phase) complex