Chapter 19

The Cell Cycle, DNA Replication, and Mitosis
Figure 19-1B

(b) The cell cycle

INTERPHASE

G1

G2

S (DNA synthesis)

M
Figure 19-1A

(a) The M (mitotic) phase

MITOSIS

Chromosomes condensing
Centromere
Mitotic spindle
Sister chromatids separating
Daughter cells forming

CYTOKINESIS
(a) Prophase

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Figure 19-20B

PROMETAPHASE

- Astral MT
- Fragments of nuclear envelope
- Spindle pole
- Kinetochore
Figure 19-21B

(b) Prometaphase

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(d) **Anaphase**

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Anaphase A (chromosome-to-pole movement)

Anaphase B (pole-pole separation)
Video: Spindle formation during mitosis
Figure 19-20E
(e) Telophase

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Figure 19-26

Chromosome

Kinetochore MTs

Kinetochore

1 μm
Figure 19-27A

(a) Three roles played by motor proteins

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(c) Polar microtubules during anaphase
Figure 19-28

Cleavage furrow

Contractile ring (actin)

Daughter cells
Figure 19-29A

(a) Myosin and tubulin during cytokinesis

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Figure 19-29B

(b) Activated Rho during cytokinesis
Figure 19-30

Daughter nucleus

Cell plate forming → Original cell wall → Daughter cells

5 μm
(a) Unequal cleavage in a frog embryo

(b) Asymmetric spindles in a sea urchin embryo
Progression Through the Cell Cycle Is Controlled at Several Key Transition Points

- Control of the cell cycle must
  - 1. Ensure that events of each phase are carried out in the correct order and at the appropriate time
  - 2. Ensure that each phase is completed before the next one begins
  - 3. Respond to external conditions
Figure 19-32

G2-M Transition
Influenced by:
- Cell size
- DNA damage
- DNA replication

Metaphase-Anaphase Transition
Influenced by:
- Chromosome attachments to spindle

Restriction Point (Start)
Influenced by:
- Growth factors
- Nutrients
- Cell size
- DNA damage

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Mitotic Cdk-Cyclin Drives Progression Through the G2-M Transition by Phosphorylation Key Proteins Involved in the Early Stages of Mitosis

- Evidence of a control molecule triggering mitosis came from experiments involving frog eggs (Masui)

- Mature eggs develop from oocytes through meiosis; the oocyte arrests shortly after meiosis begins until a hormone signal is received

- Injecting the cytoplasm of a mature egg into an immature oocyte causes it to immediately proceed through meiosis
Figure 19-34

1. Extract cytoplasm from mature egg cell.
2. Inject extracted cytoplasm into an oocyte.

Micropipette

Meiosis

Mature egg cell
Activation of mitotic Cdk

- Activation of mitotic Cdk involves phosphorylation and dephosphorylation

- The binding of mitotic cyclin to mitotic Cdk forms a cyclin-Cdk complex that is initially inactive (1)

- *Inhibiting* kinases phosphorylate two sites on the Cdk, blocking the active site (2)
Figure 19-36

1. When mitotic Cdk and mitotic cyclin first bind together, they form an inactive complex.

2. Two inhibitory phosphate groups are attached to the Cdk molecule by enzymes called “inhibiting kinases.”

3. An activating phosphate group (yellow) is added by an “activating kinase,” but the Cdk remains inactive as long as the inhibitory phosphate groups (white) are present.

4. A phosphatase removes the inhibiting phosphates, thereby activating the mitotic Cdk-cyclin complex.
Active mitotic Cdk-cyclin stimulates:
1. Nuclear envelope breakdown
2. Chromosome condensation
3. Mitotic spindle formation
4. Targeted protein degradation

Figure 19-37
(a) The anaphase-promoting complex targets securin and mitotic cyclin for degradation. 1 The destruction of securin allows separase to cleave the cohesins that hold sister chromatids together, thereby initiating anaphase. 2 The degradation of mitotic cyclin depresses mitotic Cdk activity, leading to cytokinesis, chromosome decondensation, and nuclear envelope reassembly.
Figure 19-39

Growth factor → Ras pathway → Cdk-cyclin

Phosphorylated Rb protein

DNA

Rb

E2F

Genes needed for S phase are NOT transcribed

ATP → ADP

Gene transcription

mRNA translation

Enzymes and other proteins required for S phase
(b) The mitotic spindle checkpoint prevents anaphase from starting until all chromosomes are attached to the spindle. Unattached chromosomes keep the “checkpoint on” by organizing Mad and Bub proteins into a complex that prevents Cdc20 from activating the anaphase-promoting complex. After all chromosomes are attached, the Mad-Bub complex is not formed (“checkpoint off”) and the anaphase-promoting complex is free to initiate anaphase.
Figure 19-40

Damaged DNA

Activates

ATM or ATR

Activates

Checkpoint kinases

ATP

ADP

P3

p53

Degradation

Mdm2

p53

p53

p53

p53

1

p21 (Cdk inhibitor)

Inhibits

Cdk-cyclin

Cannot phosphorylate Rb protein

CELL CYCLE ARREST

2

Puma

Inhibits

Bcl-2

Cannot inhibit apoptosis

APOPTOSIS (cell death)
Inhibitory Growth Factors Act Through Cdk Inhibitors

- Some growth factors inhibit cell proliferation, e.g., transforming growth factor $\beta$ (TGF$\beta$)

- TGF$\beta$ binding to its receptor phosphorylates Smad proteins that move into the nucleus and activate expression of genes coding for proteins that inhibit proliferation

- Two Cdk inhibitors that block cell cycle progression are p15 and p21
Apoptosis

- Damaged or diseased cells need to be eliminated
- In such cases, the process must not damage surrounding cells
- Multicellular organisms accomplish this through a programmed cell death—apoptosis
Apoptosis and necrosis

- Cell death called necrosis sometimes follows tissue injury

- Necrosis involves swelling and rupture of injured cells, whereas apoptosis involves a specific series of events that lead to dismantling of the cell contents
Steps of apoptosis

• The cell’s DNA segregates near the periphery of the nucleus and the cytoplasm decreases (1)

• The cell produces small cytoplasmic extensions and the nucleus begins to fragment (2)

• DNA is cleaved by an apoptosis-specific endonuclease and the cell is dismantled into small pieces called *apoptotic bodies*
Steps of apoptosis (continued)

- Inactivation of a phospholipid translocator (flippase) causes accumulation of phosphatidylserine in the outer leaflet of the plasma membrane.

- This serves as a signal for the remnants of the affected cell to be engulfed by nearby cells via phagocytosis (3).
As a cell begins to undergo apoptosis, its chromosomes condense and its cytoplasm shrinks.

Eventually the nucleus becomes fragmented, its DNA is digested at regular intervals ("laddering"), the cytoplasm becomes fragmented, and the cell extends numerous blebs.

Ultimately the remnants of the dead cell (apoptotic bodies) are ingested by phagocytic cells.
Figure 19-44B,C,D
Caspases

- Apoptosis proceeds through the activation of a series of enzymes called **caspases**

- They are produced as inactive precursors called **procaspases** and are cleaved to create active enzymes
Apoptosis Is Triggered by Death Signals or Withdrawal of Survival Factors

- There are two main routes by which cells can activate caspases and enter apoptosis

- Activation can occur directly, e.g., when human cells are infected by viruses, *cytotoxic T lymphocytes* are activated and induce apoptosis

- This is triggered when cells receive *cell death signals*
Apoptosis in cell infected by viruses

- Two death signals are *tumor necrosis factor* and *CD95/Fas*

- CD95 is a protein on the surface of infected cells; lymphocytes have a protein on their surfaces that binds CD95, causing it to aggregate

- Adaptor proteins attach to the CD95, which recruits *procaspase-8* to the sites of clustering
Initiator and executioner caspases

• When the procaspase is activated it acts as an *initiator caspase*, initiating the cascade

• Initiator caspases also activate an executioner caspase, *caspase-3*, which is important for activating many steps in apoptosis
The second type of apoptosis

• One of the best-studied cases of the second type of apoptosis involves *survival factors*

• When survival factors are withdrawn, a cell may enter apoptosis

• The site of action is the mitochondrion

• Healthy cells have several *anti-apoptotic* proteins in the outer mitochondrial membrane
The second type of apoptosis (continued)

- The proteins are structurally related to a protein called Bcl-2 which, together with other proteins, counteracts proteins that promote apoptosis (pro-apoptotic proteins)

- When cellular signals shift in balance toward pro-apoptotic proteins, the cell is more likely to undergo apoptosis

- One pro-apoptotic protein is called Bad (Bcl-2-associated death promoter)
Mitochondria trigger apoptosis

- Mitochondria trigger apoptosis by releasing cytochrome c into the cytosol after accumulation of pro-apoptotic proteins lead to formation of channels in the outer mitochondrial membrane.

- Cytochrome c stimulates calcium release from mitochondria and ER, where it binds IP$_3$ receptors.

- It also activates an initiator procaspase, procaspase-9, which then activates caspase-3.
Damaged cells can trigger their own apoptosis

- If a cell suffers such damage that it can’t repair itself, it may trigger its own demise

- It can enter apoptosis through the activity of p53, which acts through the protein Puma, which binds and inhibits Bcl-2
Figure 19-45

Cytotoxic T lymphocyte

1. Death receptor
2. Initiator caspase
3. Executioner caspase
4. Absence of survival factors
5. Inhibits (Bcl-2)
6. Cytochrome c
7. Initiator procaspase (procaspase-9)
8. Executioner caspase
9. DNA damage