



Week 14

Cellular reproduction: mitosis and cytokinesis

Read: (Karp, 9th ed.)

Chapter 14.

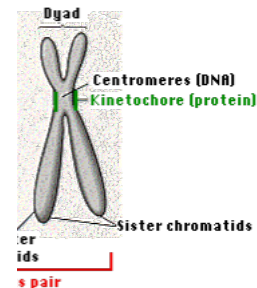
Section M phase: Mitosis and cytokinesis

Meiosis

1

Mitosis review

- **Mitosis** is a process of nuclear division in which two nuclei with identical genetic content are produced.
- It is usually accompanied by cytokinesis.
- Mitosis maintains the chromosome number.
- Mitosis can occur in either haploid or diploid cells.
- The phases of mitosis are: *prophase, prometaphase, metaphase, anaphase, and telophase.*



2

Mitosis review

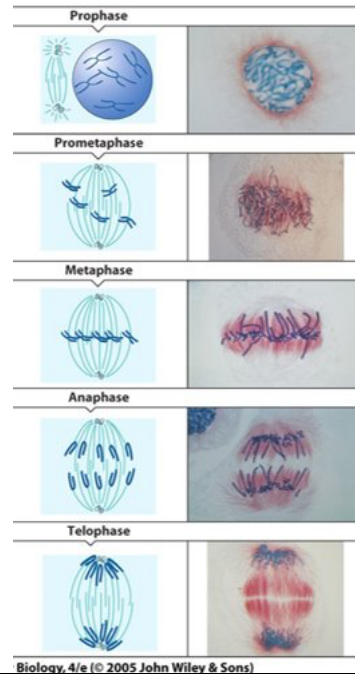
Prophase – nuclear envelope disappears;
chromosomes condense; mitotic spindle forms

Prometaphase – chromosomes attach to spindle;
chromosomes start moving to the equator

Metaphase – chromosomes aligned
along equator

Anaphase – chromatids separate;
chromosomes migrate to poles; spindle poles move farther
apart

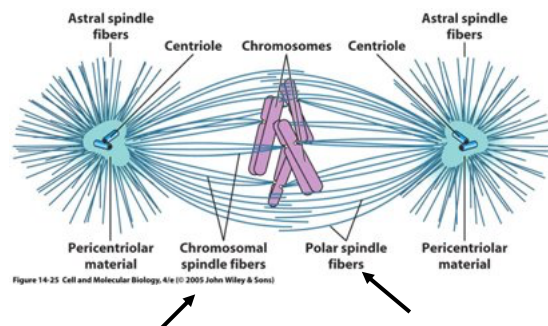
Telophase – chromosomes decondense;
Nuclear envelope forms



3

The mitotic spindle contains 3 sets of microtubules

- (1) Astral microtubules: Radiate from centrosome, form aster
- help position mitotic apparatus, determine cleavage plane

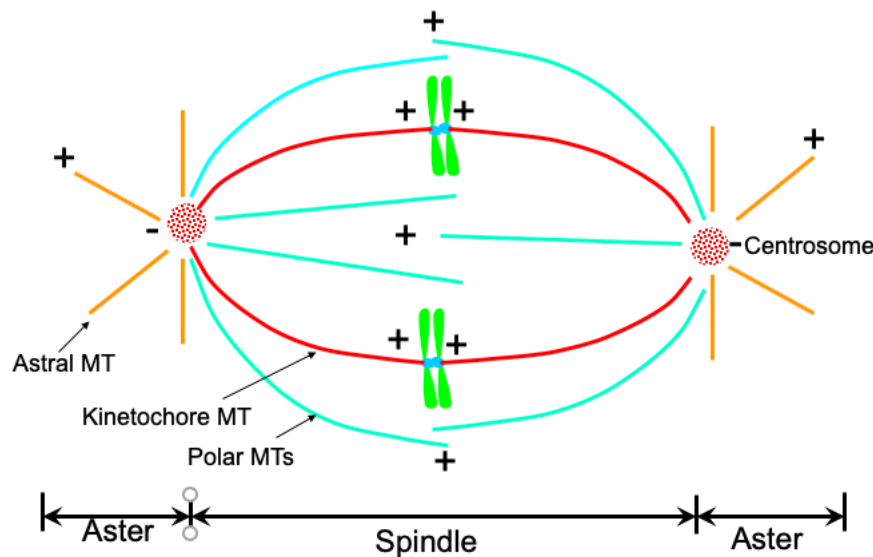


- (2) Kinetochore microtubules attach
to chromosomes at kinetochore; pull
chromosomes to different poles

- (3) Polar microtubules interdigitate
with opposing pair; support
framework; help push centrosomes
apart

4

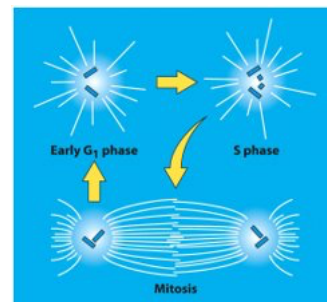
Orientation of the mitotic spindle (at metaphase)



5

Formation of the mitotic spindle

- The **centrosome** is a microtubule-organizing structure.
- The *centrosome cycle*, in which centrioles are duplicated, progresses along with the cell cycle.
- Note that certain types of animal cells and most plants lack centrosomes, but mitosis still works just fine!

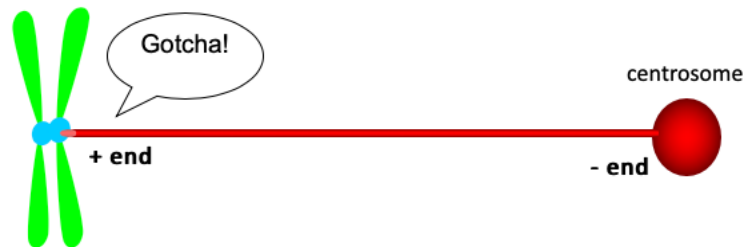


- The centrosome cycle of an animal cell.
- At S-phase daughter procentrioles form.
- Daughter pericentrioles elongate during G2 phase.
- Centrosome pairs split at mitosis.
- Each centriole pair becomes part of a centrosome.

6

Prometaphase: attachment of chromosomes to the spindle

- Rapid fluctuations in spindle MTs length
 - Occurs during prometaphase once nuclear envelope is broken down
 - Dynamic MT acts as a “poker”:



7

Microtubules attach to chromosomes at the kinetochore

Two proteins act as linkers of kinetochores to dynamic MTs:

1. motor proteins and
2. Ndc80.

Ndc80 is essential and binds to the adjacent MT.

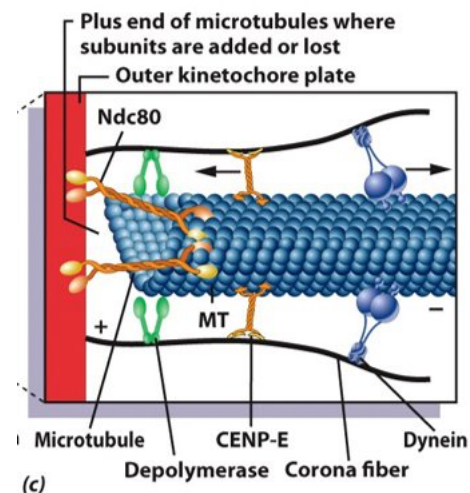
CENP-E, a kinesin motor protein associated with the kinetochore and acts as a MT coupler.

a) MTs terminate at the kinetochore of a mammalian cell.

b) Several proteins found at the outer surface of the kinetochore

1. Dynein
2. CENP-E

3. Depolymerase (also a kinesin, but it doesn't move)

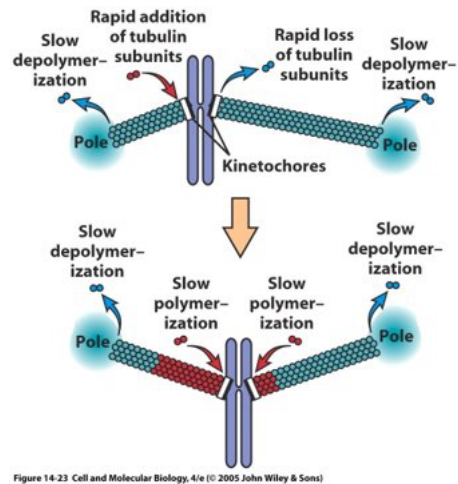


8

Chromosome movement to the mitotic plate during prometaphase

A combination of microtubular dynamics and motors moves the chromosomes:

- Rapid polymerization & depolymerization occurs at the (+) end of microtubules
- Microtubule motors assist in:
 - i) maintaining flow of tubulin subunits
 - ii) tethering the kinetochore



9

Chromosome movement to the equator during prometaphase

- B. Depolymerization



- In this scenario, there is a net LOSS at the (+) end, and the chromosome
- moves to the right

- C. Polymerization

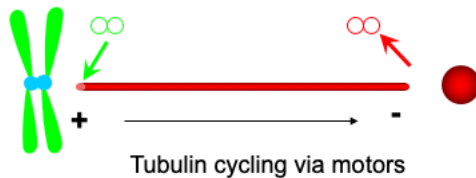


- In this scenario, there is a net gain at the (+) end, and the chromosome moves away

10

Microtubules are still dynamic at metaphase!!

A. Treadmilling



In this scenario, there is no net elongation / shrinking of the MT

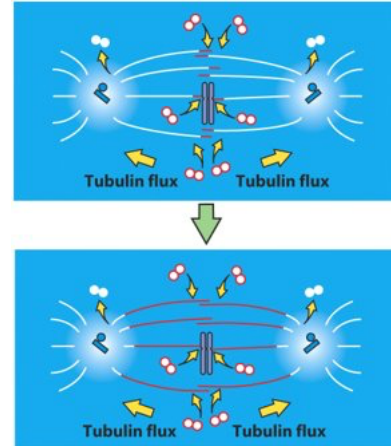


Figure 14-26 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

In this experiment, red-labeled tubulin subunits were added to cell;

In top panel, red dye is first seen only at kinetochore, proving it is site of addition of subunits;

In bottom panel, after several minutes, the red-labeled subunits are being pushed to the minus end

11

The spindle assembly checkpoint

Cell monitors the status of events in metaphase before going on to anaphase

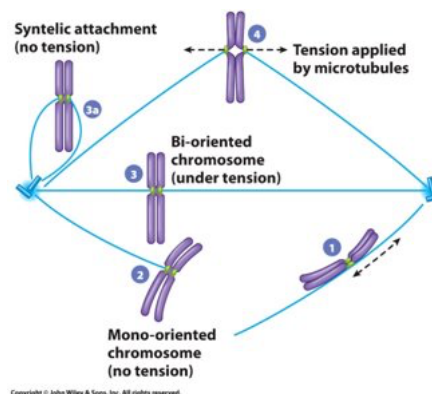
- It is important that the chromosomes are all evenly lined up as the cell goes on to anaphase, otherwise aneuploidy in the daughter cells can easily occur

If one of the chromosomes is not at the metaphase plate it waits, to give it a chance to get there.

#3 is on its way to becoming #4 (i.e. attached to two microtubules, aligned at the metaphase plane)

3a is not good... if this occurred when the cell went into anaphase the daughter cell on the left would get both chromosomes!

#2 hasn't yet been attached by the other chromosome

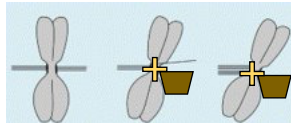


Copyright © John Wiley & Sons, Inc. All rights reserved.

12

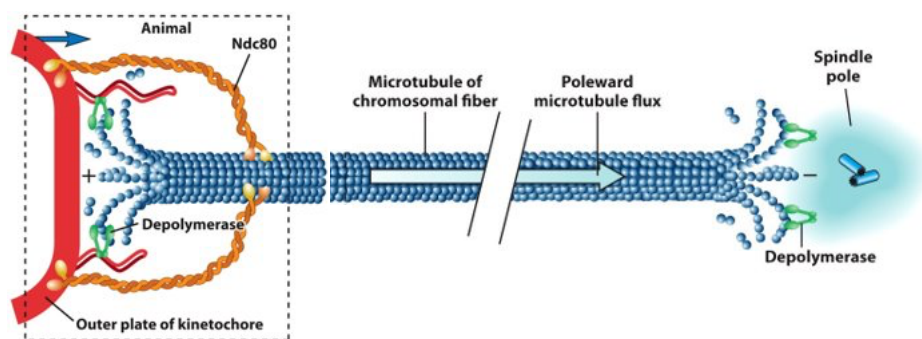
Mechanism of the spindle assembly checkpoint

- Sister chromatids are held together by protein called **securin**
- The **Anaphase Promoting Complex (APC)** causes ubiquitination of securin, thereby allowing chromatids to split apart
- Cdc20 is an activator of the Anaphase Promoting Complex (APC)
- **MAD2 protein** is bound to the **kinetochore** of un-aligned or improperly attached metaphase chromosomes
- Mad2 binds to **and sequesters cdc20**
- thus, if improperly aligned chromosomes, the APC isn't activated and the securin isn't destroyed



13

Anaphase chromatid movement is driven (mostly) by microtubule depolymerization

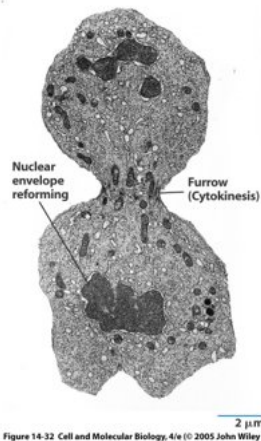


- Ncd80 appears to anchor the kinetochore to the + end of the MT.
- MT are depolymerized at both the + and – ends through the action of a depolymerase (again, that weird kinesin).
 - Causes MT unwinding and movement.
- Dynein function may play a role too... inhibiting dyneins slow down chromatid movement

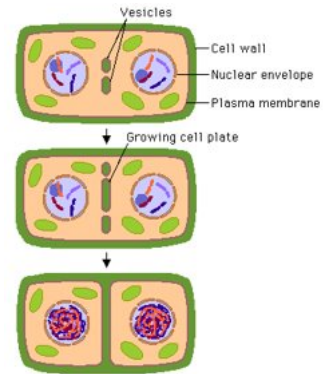
14

Cytokinesis is different between plant and animal cells

Animal cells: cleavage furrow

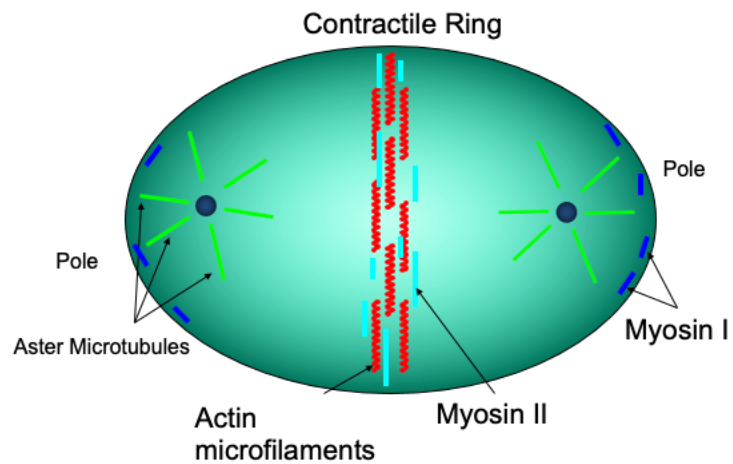


Plant cells: Formation of cell plate



15

Cytokinesis



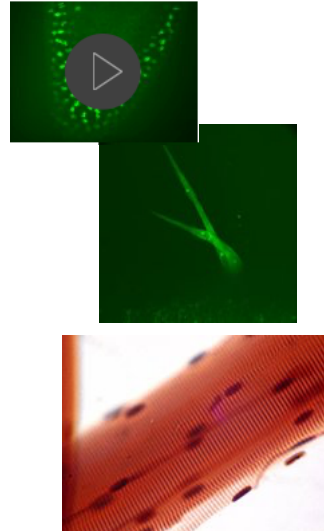
16

Mitosis can sometimes occur without cell division

- Early insect embryos
 - E.g.: the developing *Drosophila* embryo
 - During early fly development, nuclear divisions occur without cellular division
- Some plant trichomes (leaf hairs) have many nuclei per cell
- Not all multinucleated cells arise in this manner
e.g. multinucleated striated muscle cells arise by cell fusion

What is a benefit of being multinucleated?

Rapid gene expression – lots of RNA & protein



17

And... cell division can occur without mitosis

- **Meiosis:**
- 2 sequential cell divisions without an intervening phase of DNA replication
- Results in 4 haploid cells
 - ($n=1$ – each cell has only one copy of every chromosome, not 2, as in a diploid organism)
- In animal cells, meiosis produces the gametes (sperm and eggs)
- In plant cells, this produces gametophytes, which in turn make sperm and eggs

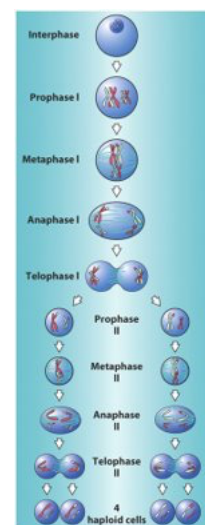


Figure 14-39 Cell and Molecular Biology, 4/e © 2001

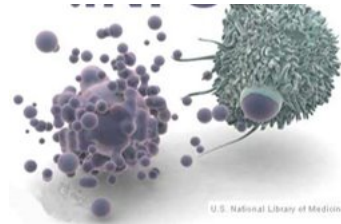
18

WYSKFTS

- What occurs in each phase of mitosis
- Parts and functions of mitotic spindle
- How microtubules attach at the kinetochore
- Components and regulation of spindle checkpoint
- Distinction of mitosis vs cytokinesis
- Animal vs plant cytokinesis
- Mitosis without cytokinesis, and vice versa

19

Week 14 Apoptosis



U.S. National Library of Medicine

Suggested readings: Karp 9th edition Ch. 15 :



20

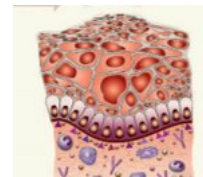
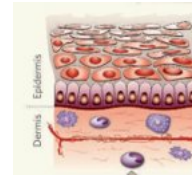
Cell death!

There are two ways in which cells die:

1. They are killed by injurious agents.
2. They are induced to commit suicide.

1. Death by injury (aka 'Necrosis')

- Cells can be damaged by injury
 - mechanical damage
 - exposure to toxic chemicals
- They undergo a characteristic series of changes:
 - The cell and their organelles can swell (lose the ability to control the passage of ions and water).
 - The cell contents leak out, leading to inflammation of surrounding tissues.

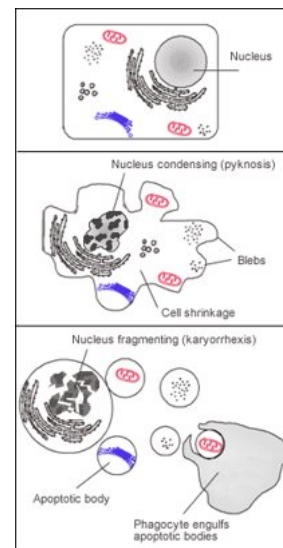


21

Cell death! (cont.)

2. Death by suicide

- Cells that are induced to commit suicide:
 - shrink
 - develop bubble-like blebs on their surface
 - have their DNA degraded
 - have their mitochondria break down
 - break into small, membrane-wrapped fragments
- The phospholipid phosphatidylserine, which is normally hidden within the plasma membrane, is exposed on the surface.
- This is bound by receptors on phagocytic cells which then engulf the cell fragments.
- The phagocytic cells secrete cytokines that inhibit inflammation



22

Apoptosis for development

Cell death by suicide is so orderly that the process is often called **programmed cell death** or more frequently **apoptosis**.

Why should a cell commit suicide? There are 2 reasons.

1. Programmed cell death is needed for proper development.

Examples of apoptosis in development:

- The resorption of the tadpole tail
- The formation of the fingers and toes requires removal of the tissue between them.
- Menstruation - the sloughing off of the inner lining of the uterus
- Neural innervations in tissues requires removal of surplus neurons during development – some neurons die once the tissue has enough innervations



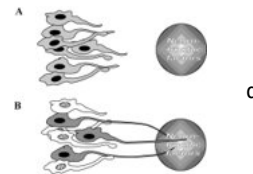
a



b



c



d

23

Apoptosis for protection

2. Programmed cell death is needed to destroy cells that represent a threat to the integrity of the organism.

Examples:

a. Cells infected with viruses

- Cytotoxic T lymphocytes (CTLs) kill virus- infected cells by inducing apoptosis

b. Cells of the immune system

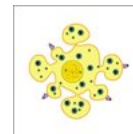
- At the end of an infection, CTLs induce apoptosis in each other and even in themselves. Defects in the apoptotic machinery is associated with autoimmune diseases

c. Cells with DNA damage

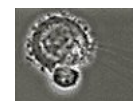
- Cells respond to DNA damage by increasing their production of p53 protein. p53 is a potent **inducer** of apoptosis. Mutations in the *p53* gene are often found in cancer cells.

d. Cancer cells

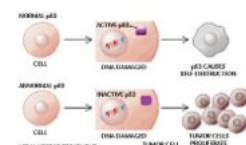
- Radiation and chemicals used in cancer therapy induce apoptosis in some types of cancer cells.



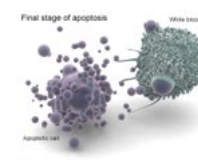
a



b



c

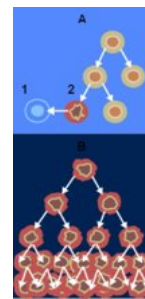


d

24

How important is apoptosis?

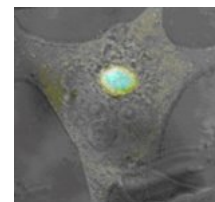
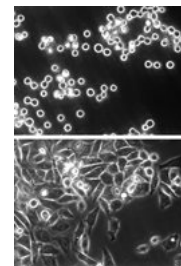
- In the average human adult, 50 - 70 billion cells die each day due to apoptosis
- In the average child between 8 to 14 yrs, 20 - 30 billion cells die a day.
- Research on apoptosis has increased substantially since the early 1990s.
 - It's an important biological phenomenon
 - Defects in apoptosis lead to diseases:
 - excessive apoptosis causes atrophy (tissue wasting)
 - insufficient apoptosis results in uncontrolled cell proliferation, such as cancer



25

What makes a cell decide to commit suicide?

- **The balance between:**
- the withdrawal of positive signals; i.e. signals needed for continued survival, and
- the receipt of negative signals.
-
- **Withdrawal of positive signals**
- continued survival of many cells requires continuous stimulation from other cells and usually continued adhesion to other cells or substrate.
- growth factors
- various cytokines stimulate mitosis
-
- **Receipt of negative signals**
- increased levels of oxidants within the cell
- damage to DNA by oxidants, UV light, X-rays, cytotoxic compounds
- High intracellular $[Ca^{2+}]$
- accumulation of mis-folded proteins
- Death activators - molecules that bind to specific receptors on the cell surface and signal the cell to begin the apoptosis program.



26

What signals induce a cell to undergo apoptosis?

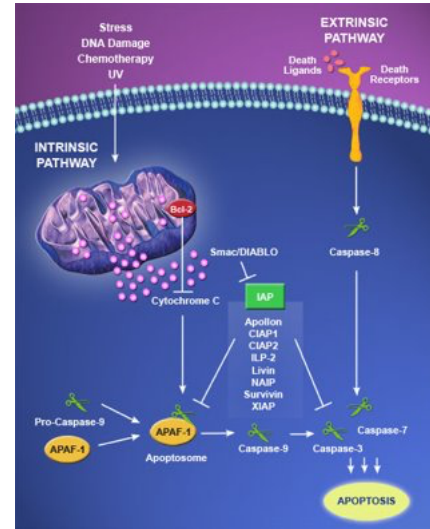
There are 2 general mechanisms to induce apoptosis.

1. Intrinsic factors:

- signals arising from within the cell itself often as a result of some damage to key organelles, like mitochondria

2. Extrinsic factors:

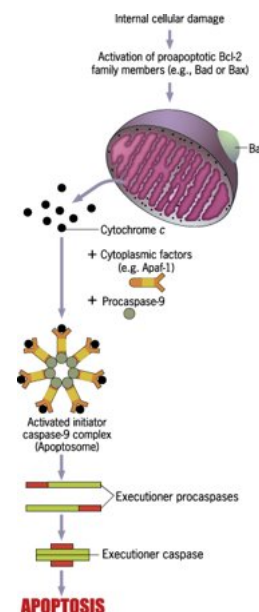
- death activators** binding to receptors at the cell surface (e.g. Tumour necrosis factor =TNF)



27

An intrinsic pathway of apoptosis

- The outer membranes of mitochondria display the protein Bcl-2 on their surface.
- Internal **damage** to the cell (e.g., from reactive oxygen species) causes Bcl-2 to activate a related protein, **Bax**, which punches holes in the outer mitochondrial membrane, causing cytochrome c to leak out.
- The released cytochrome c binds to apoptosis-promoting proteins (**Apaf-1** and **procaspase-9**)
- These proteins aggregate to form **apoptosomes**.
- The apoptosomes activate executioner **caspases** (which are proteases).
- There is an expanding cascade of many caspases, which digest structural proteins in the cytoplasm, degrade DNA, etc.
- Phagocytes are recruited to destroy the dying cell.

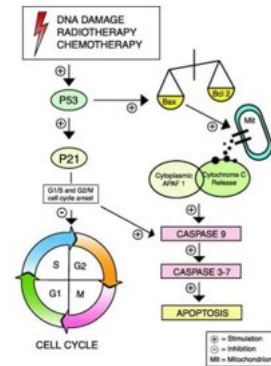


28

What internal factors can induce the intrinsic apoptosis pathway?

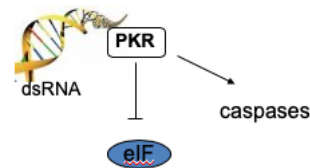
p53

- Recall that p53 is a protein that stops cells from dividing when they have suffered from DNA damage (in this case, it acts as a transcription factor)
- p53 also appears to directly activate Bax proteins, causing them to punch holes in the mitochondria, thereby inducing the caspases to begin apoptosis.



Viral double-stranded RNA (dsRNA)

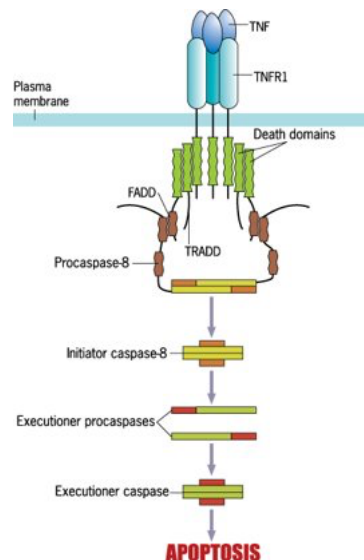
- dsRNA is detected by PKR, a protein that first stops the cell's translational machinery (by inhibiting an eIF), and then by inducing caspases (this pathway may also be considered an extrinsic pathway)



29

Extrinsic (receptor-mediated) pathway of apoptosis

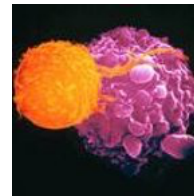
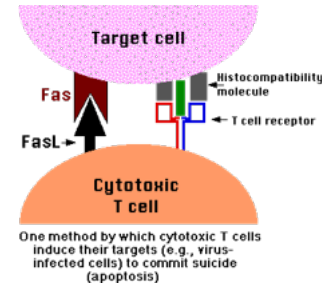
- TNF receptors** (e.g. TNFR1) are integral proteins that detect signals like Tumour Necrosis Factor, which is a type of **death activator**
- TNFR1 cytoplasmic domains then bind to other proteins, which recruit **Procaspase 8**
- This leads to activation of **caspase 8**
- caspase 8 (like caspase 9) initiates a cascade of caspase activation
- This leads to phagocytosis of the cell.



30

Immune cells give cells the extrinsic death activator

- A natural defense mechanism of virally-infected cells is to transport some viral proteins to the cell surface
- The viral proteins are coupled to MHC proteins on the surface of the infected cell
- MHC proteins allow binding with immune system cells such as Natural Killer and cytotoxic T cells
- Cytotoxic T cells signal to the infected cell through the interaction of their FasL protein with the infected cell's Fas protein (a cell surface receptor)
- Fas induces the infected cell to undergo apoptosis.
- Unfortunately, many viruses encode proteins that can inhibit apoptosis.



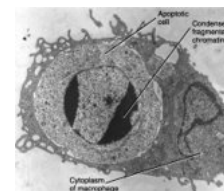
FasL is a TNF

MHC = major histocompatibility complex

31

The visible signs of apoptosis (at the cellular level)

- A cell undergoing apoptosis shows a characteristic morphology that can be observed with a microscope:
- Cell shrinkage and rounding due to the breakdown of the cytoskeleton by caspases.
- Chromatin undergoes condensation
- The nuclear membrane becomes broken and the DNA inside it is fragmented. The nucleus breaks into several discrete pieces
- The cell membrane shows irregular buds known as blebs
- The cell breaks apart into several vesicles called *apoptotic bodies*, which are then phagocytosed.
- Apoptosis progresses quickly and its products are quickly removed, making it difficult to detect or visualize.



<http://www.youtube.com/watch?v=witLM--V2v8>

32

What you should know after this section

- How does cell death due to injury differ from cell death due to apoptosis?
- Provide examples of when apoptosis is used during development.
- Provide examples of when apoptosis is used for protection.
- Describe the key molecules involved in the intrinsic pathway of apoptosis. What factors induce this pathway?
- Describe the key molecules involved in the extrinsic pathway of apoptosis. What factors induce this pathway?