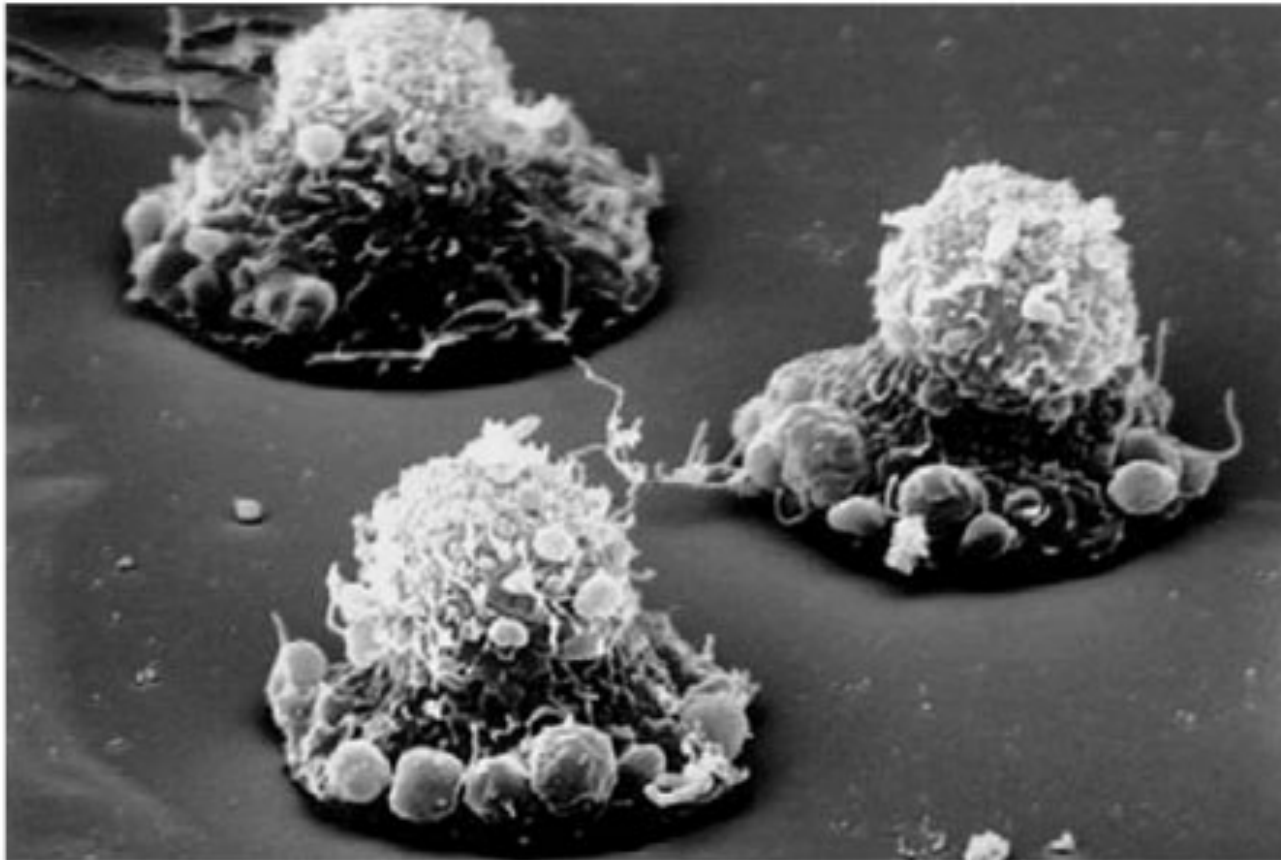


# Cell Cycle III: Apoptosis



# Apoptosis



- “Programmed cell death”.
- Number of cells is, in part, controlled by regulation of cell death.
- Differs from necrosis: trauma or cytotoxicity leading to  $\downarrow$ ATP and  $\downarrow$ Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, followed by lysis.
- Why does apoptosis occur?

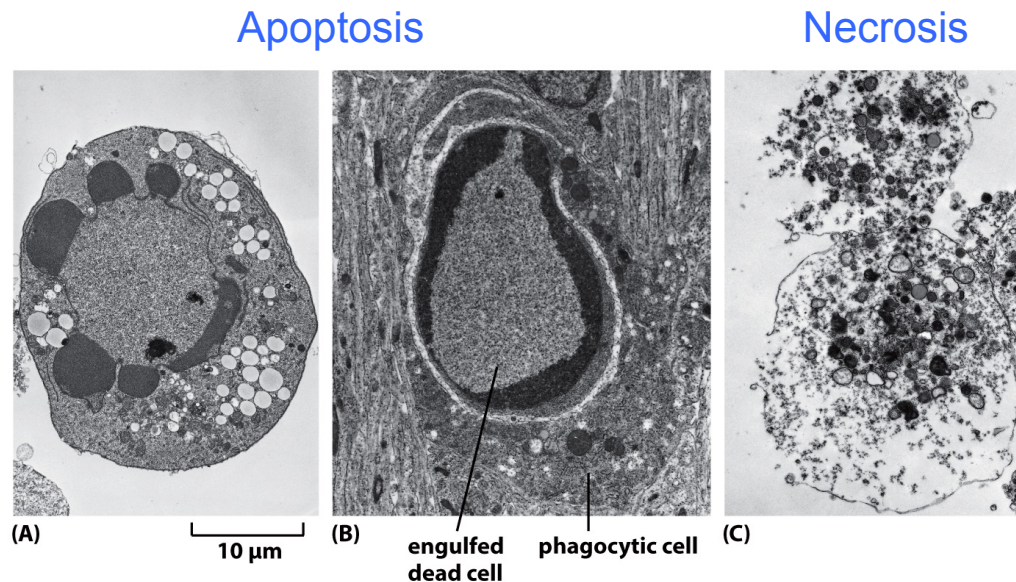


Figure 18-1 Molecular Biology of the Cell 6e (© Garland Science 2015)

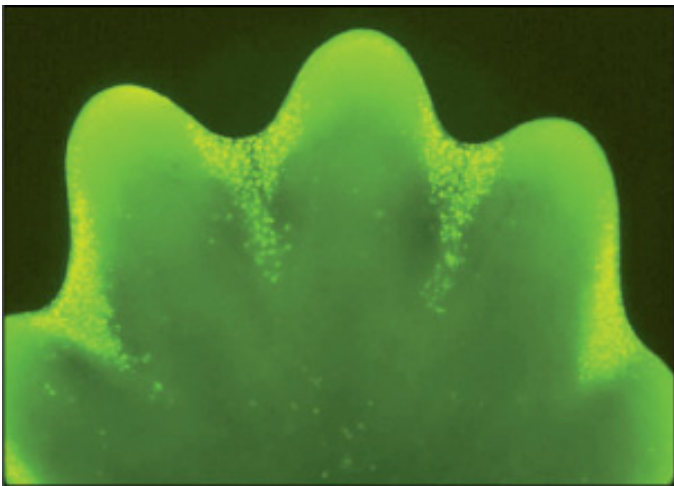


# Apoptosis

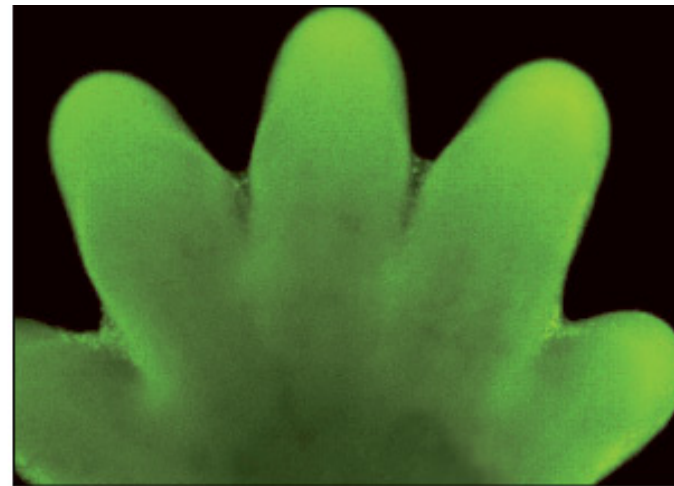
- Apoptosis in development.
- Balance of cell division with death in adult tissues.

*Mouse paw*

Cells undergoing apoptosis



Loss of cells between digits



1 mm

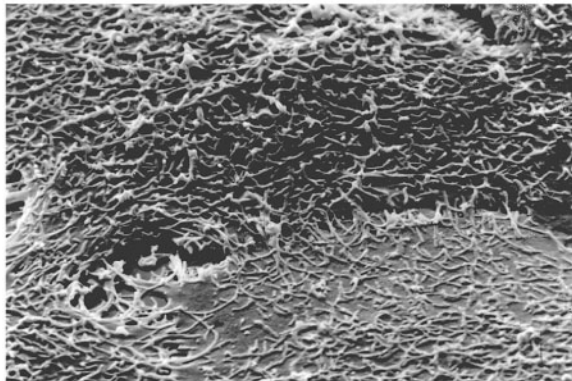
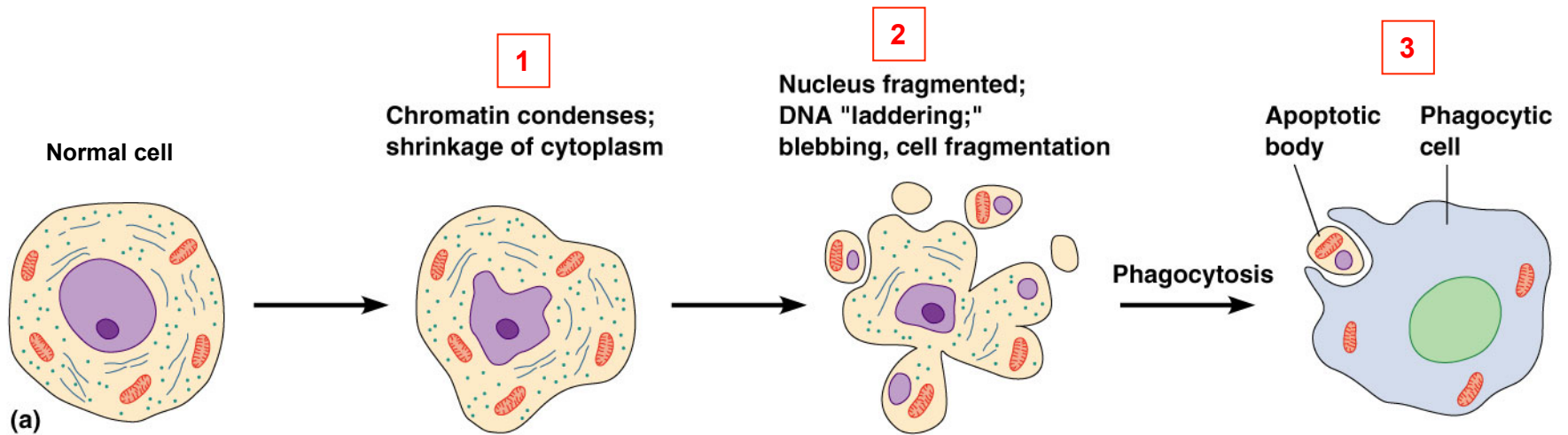
Figure 17–35. Molecular Biology of the Cell, 4th Edition.

# General Characteristics

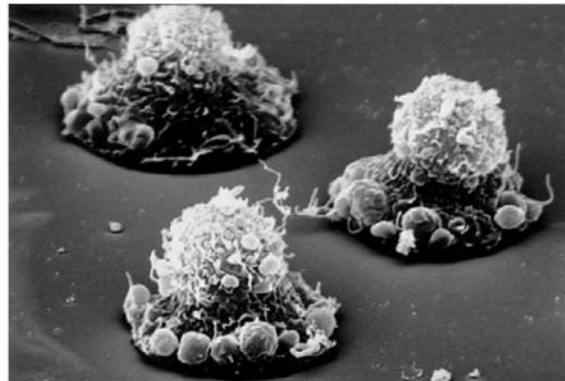
- In order for apoptosis to occur, a cell must undergo significant biochemical and morphological change.
- Initiation by intracellular or extracellular signal.
- Activation of a series of proteins involved in promoting apoptosis (or inhibition of those that prevent it).
- Important intracellular proteins necessary for survival are cleaved during apoptosis.
- Orderly disposal of dead cell.



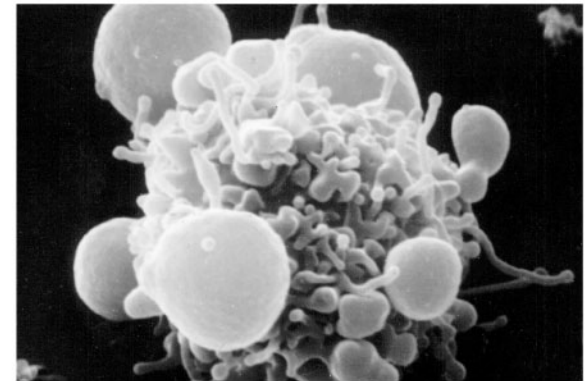
# Structural Changes During Apoptosis



(b) Normal



(c) Loss of adhesion



(d) "Blebbing"

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# Phagocytosis

- Asymmetric distribution of plasma membrane is lost.
- Negatively charged *phosphatidylserine* then becomes exposed on the outside of cell.
- The cell is then marked for *phagocytosis* by a *macrophage*.

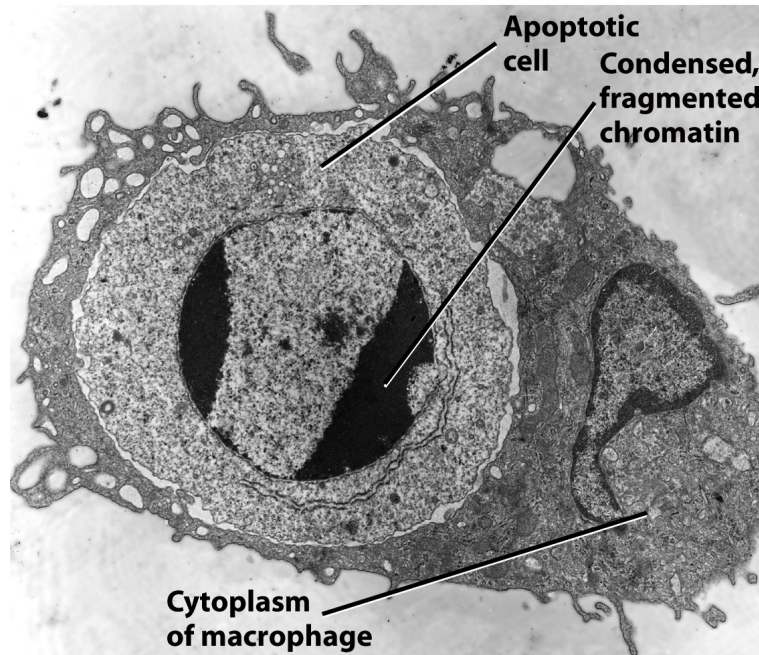


Figure 15-38 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

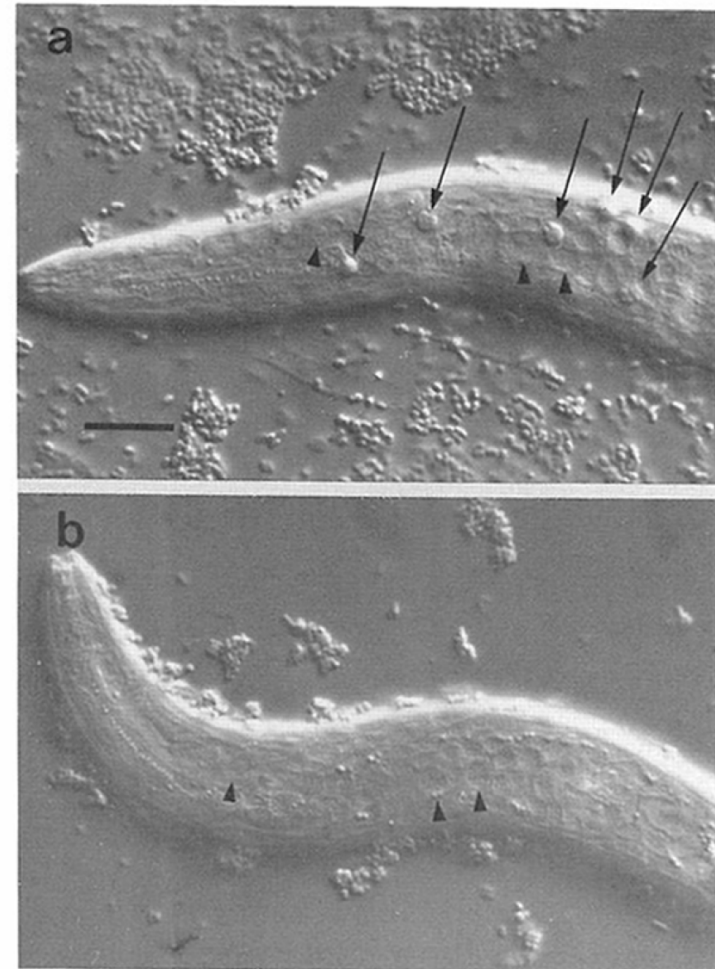


# Cell-death Mutants

- *Caenorhabditis elegans* is a nematode.
- 959 somatic cells.
- 131 apoptose every time.
- Discovery of *ced-3* gene that encodes proteins similar to mammalian protease.
- “caspases”.
- *ced-4* encodes Apaf-1; *ced-9* encodes Bcl-2.

*ced-3* mutation prevents apoptosis in all 131 cells

Normal



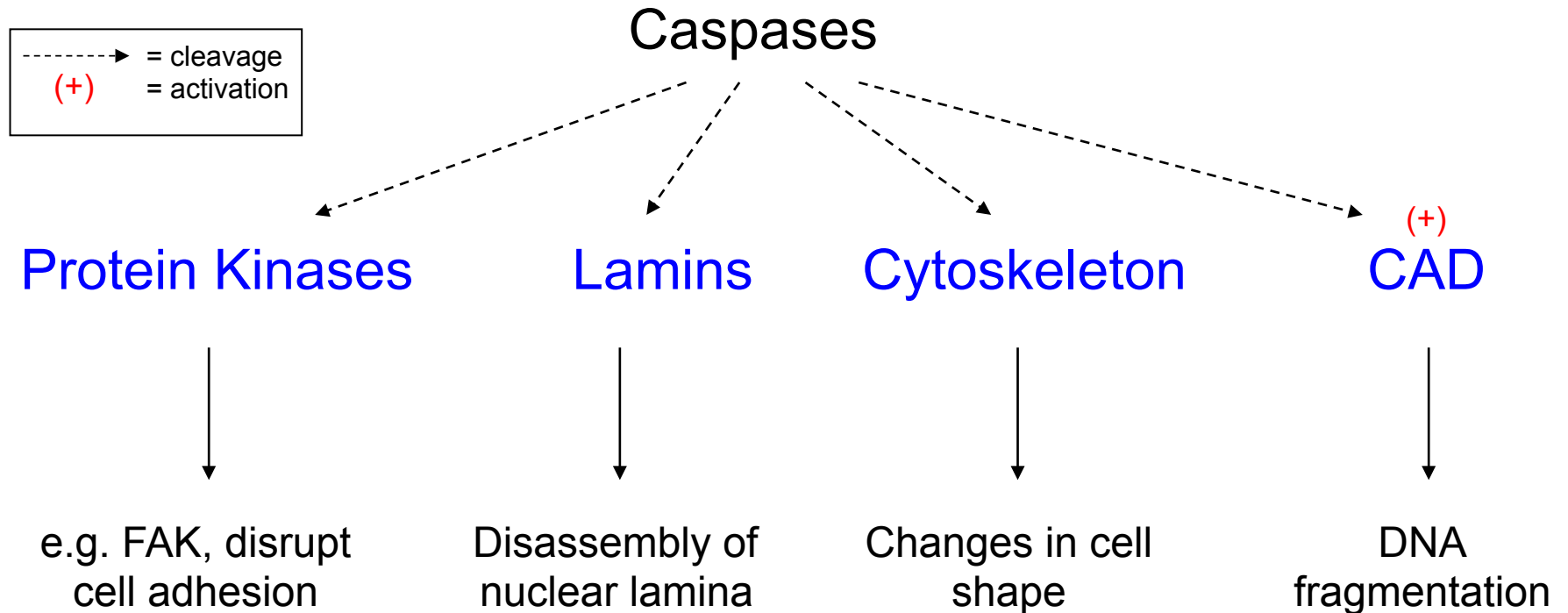
*ced-3* mutant



# Caspases

- Identification of *ced-3* gene led to the discovery of homologous family of proteins, called “caspases”, in mammals.
- They are proteases that cleave essential proteins.
- Involved in *most* changes observed during cell death.
- This enzyme has a cysteine residue at its catalytic site and cleaves other proteins at an aspartate site.
- Caspases may also cleave each other, leading to their activation.

# Caspases Cleave Essential Proteins



From Karp 2008. Cell and Molecular Biology.

CAD = caspase-activated DNase; FAK = focal adhesion kinase

# Procaspase Activation



- Initiator (or “pro”) caspases are inactive.
- An apoptotic signal triggers assembly of adaptor proteins that activate caspases.
- These caspases go on to activate executioner caspases by proteolytic interactions (cleavage).

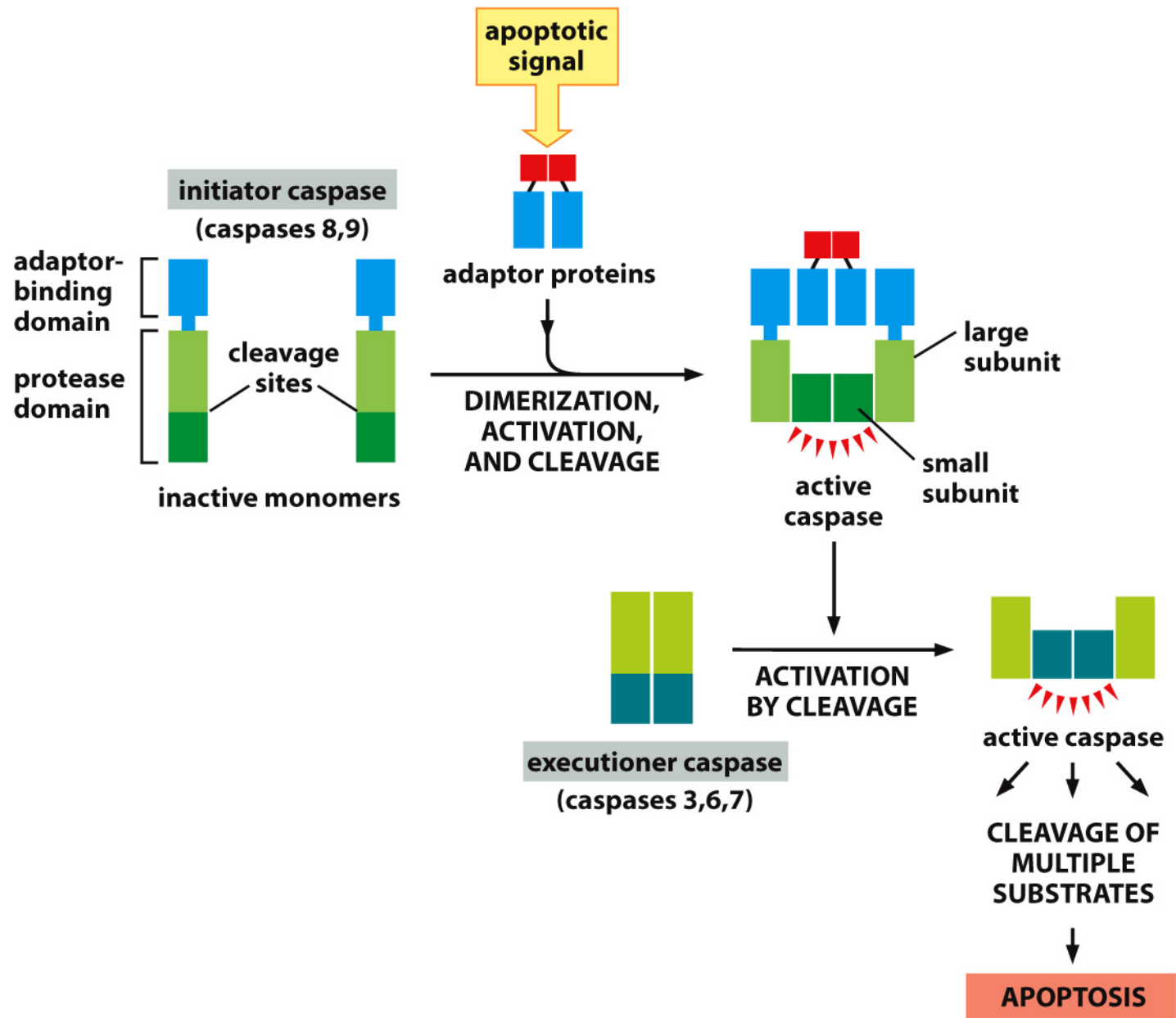


Figure 18-3 Molecular Biology of the Cell 6e (© Garland Science 2015)

# The Caspase Cascade



- An active caspase activates another caspase...
- Irreversible, “all-or-none”.
- Amplification.
- Result: intracellular proteins are cleaved.

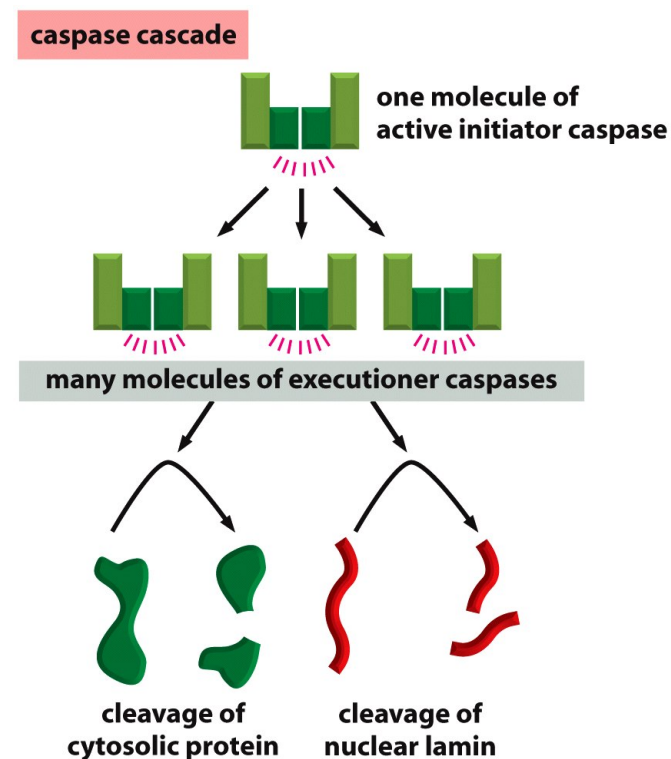


Figure 18-5b *Molecular Biology of the Cell* (© Garland Science 2008)

# 2 Major Apoptotic Pathways



## Extrinsic pathway

- **Procaspase activation** triggered from *outside* the cell.
- “Cell death” receptors.

## Intrinsic pathway

- **Procaspase activation** triggered from *inside* the cell.
- e.g. intracellular damage.
- e.g. via Bcl-2 family of proteins (*note*: this may involve an extracellular survival factor).

# Extrinsic Pathway



- Extracellular signal (Fas ligand) activates death receptor (Fas protein).
- Recruitment of adaptor proteins and procaspase activation.
- Caspase cascade.

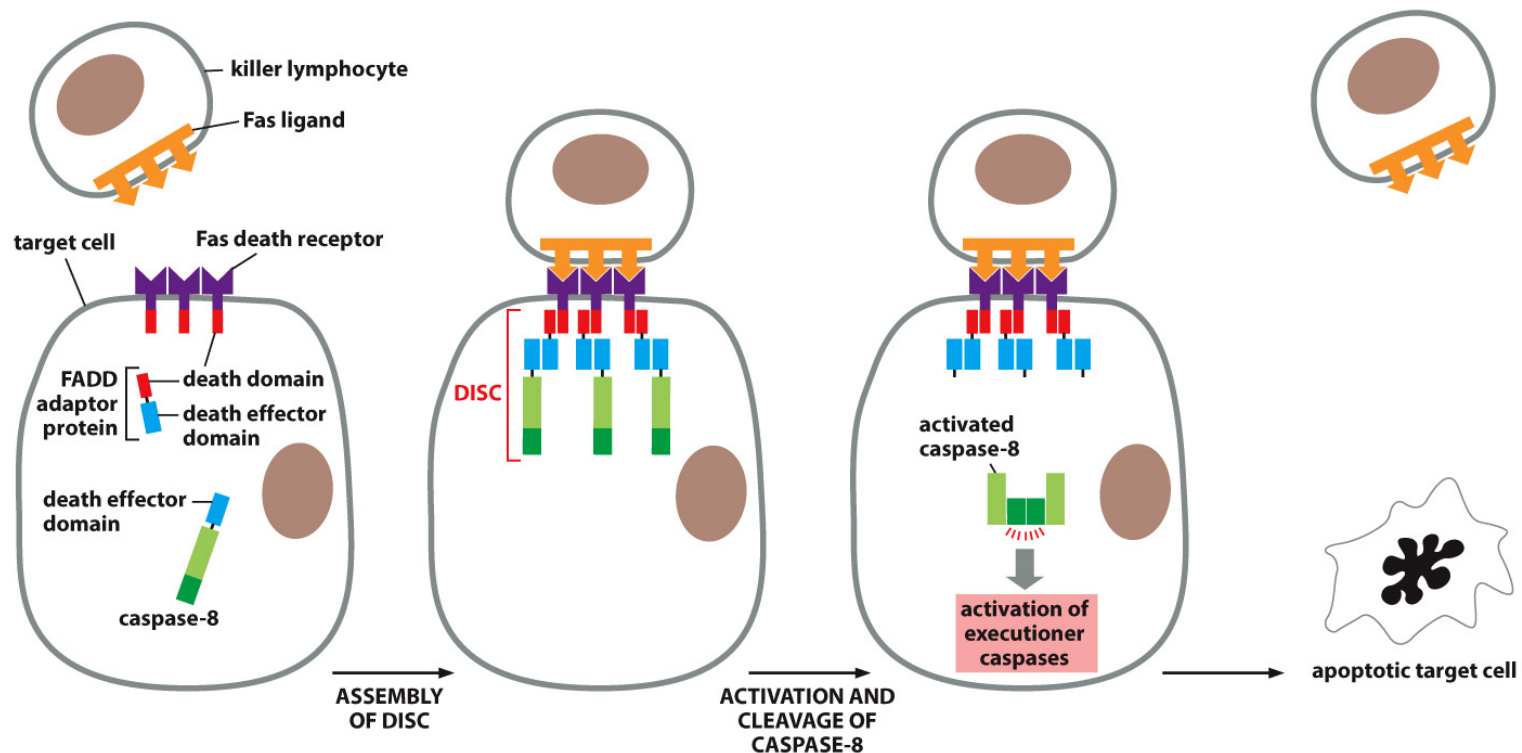


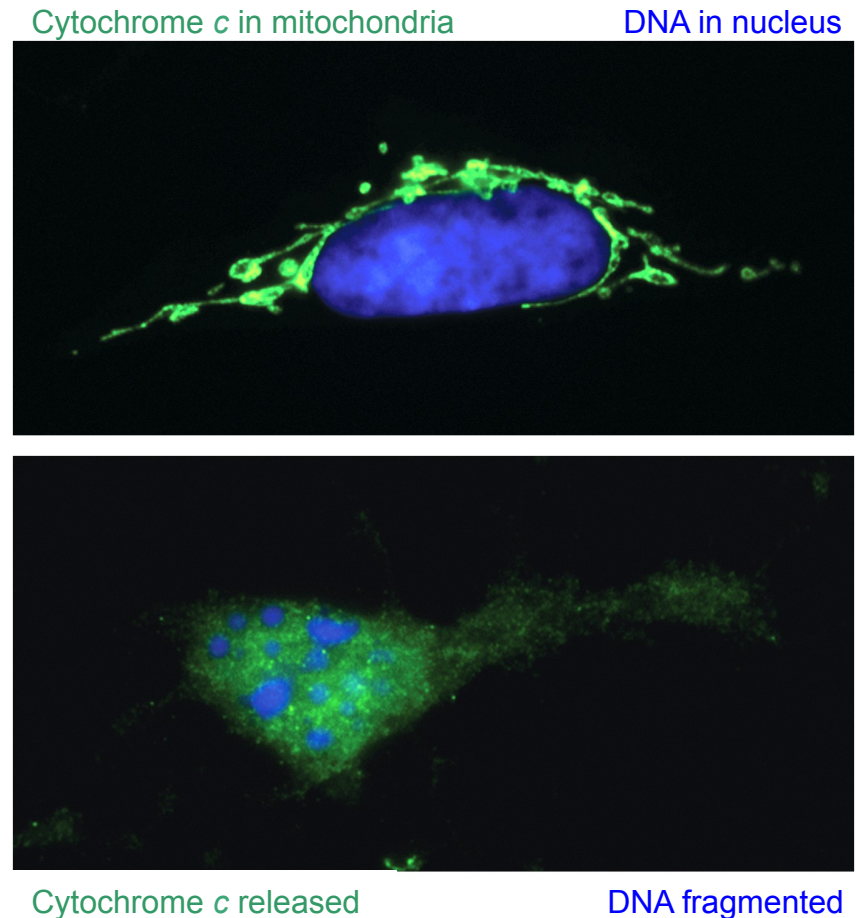
Figure 18-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

FADD = Fas-associated death domain; DISC = death-inducing signalling complex

# Intrinsic Pathway



- An intracellular death signal initiates caspase cascade and cell death.
- Initiated by e.g. DNA damage or loss of survival factor.
- Bcl-2 protein family
  - Bcl-2 inhibits apoptosis
  - Bax and Bak act on mitochondria and release **cytochrome c**.
- Balance of these determines fate (i.e. life or death) of cell.



# Intrinsic Pathway

- Apoptotic signal activates **Bcl2** family proteins (e.g. **Bax**) to form aggregates in outer mitochondrial membrane.
- Induces cytochrome *c* release from intermembrane space.

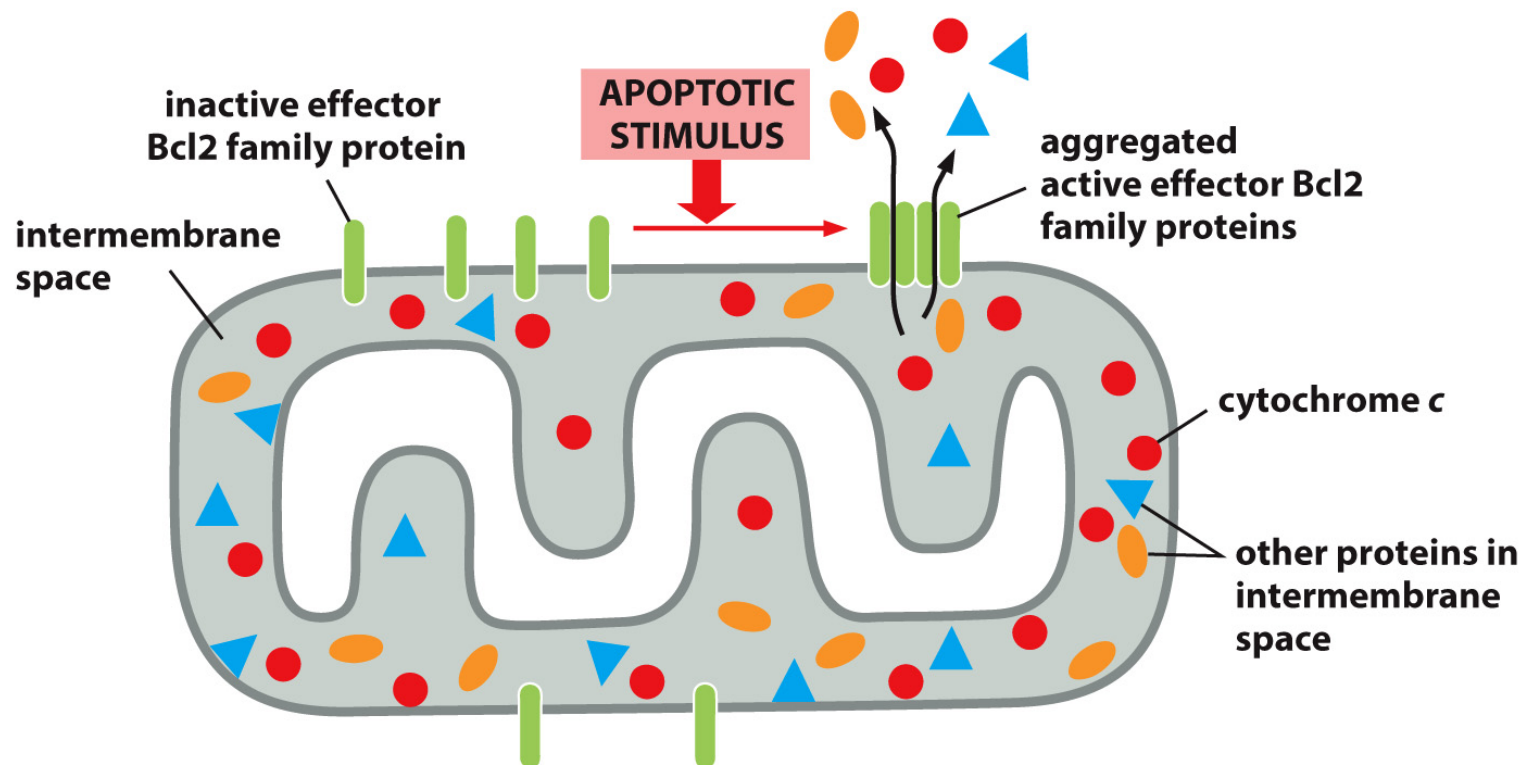


Figure 18-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Intrinsic Pathway



- Formation of “apoptosome”.
- Caspase cascade.

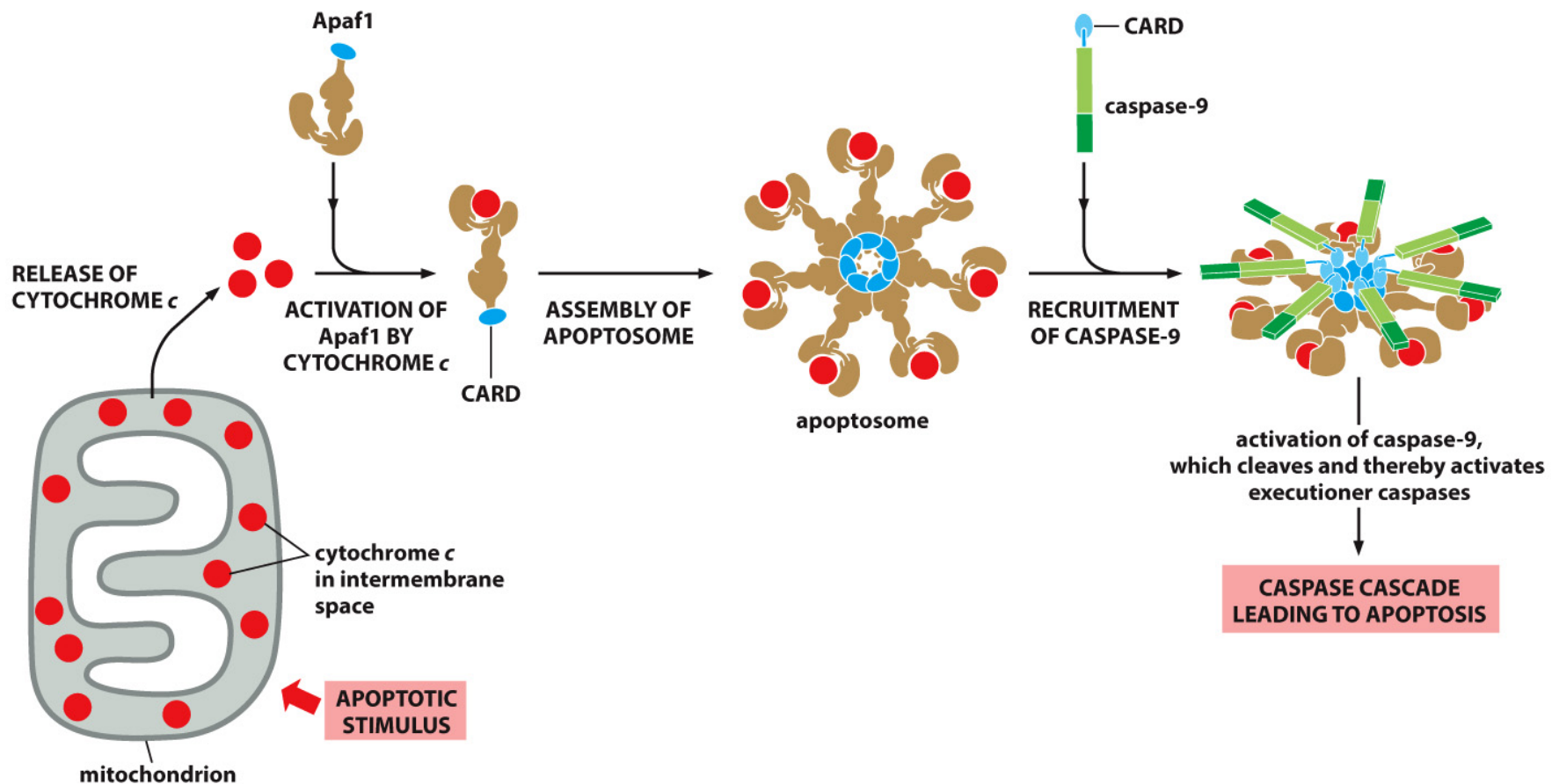
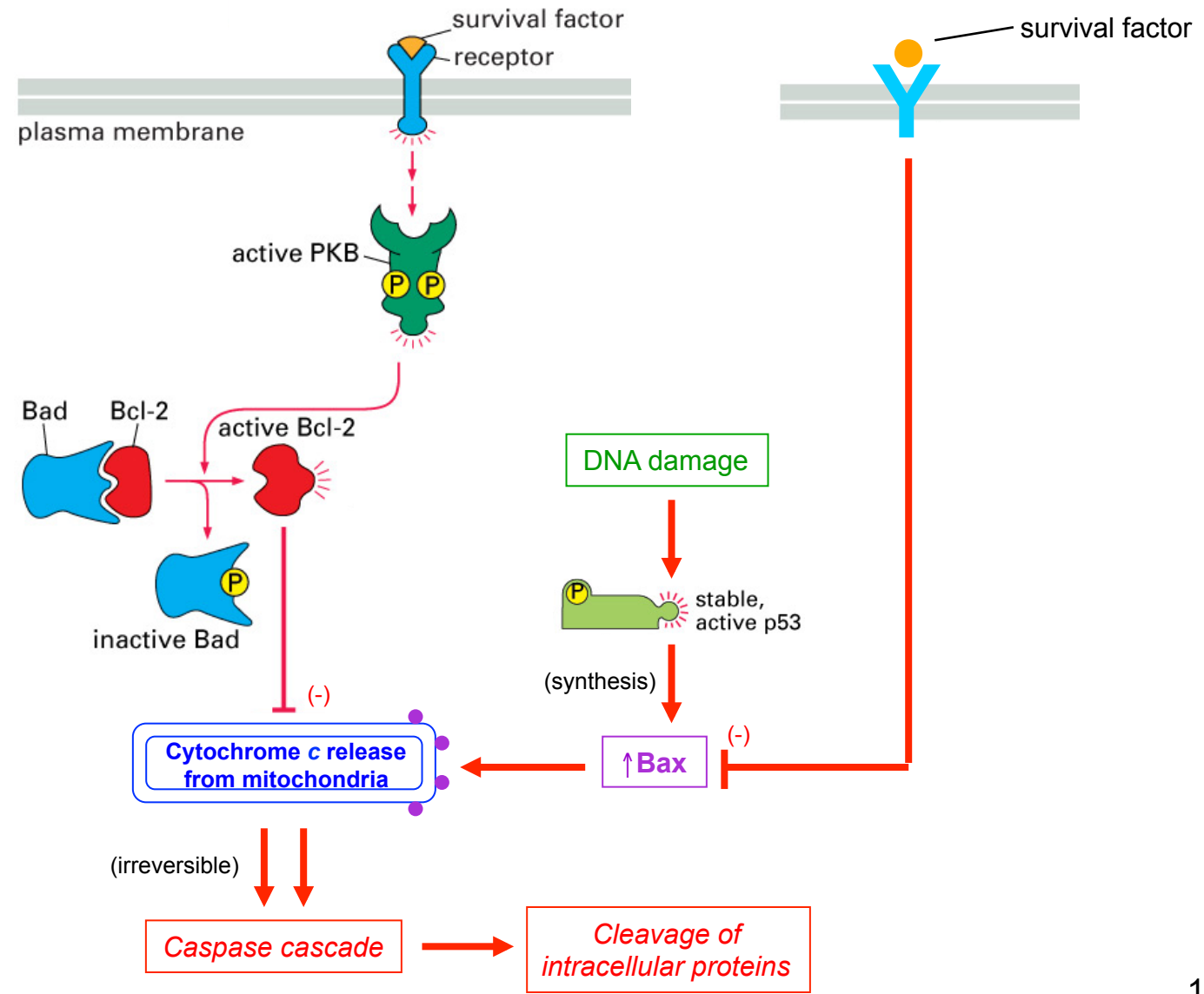


Figure 18-7 Molecular Biology of the Cell 6e (© Garland Science 2015)

Apaf-1 = apoptotic protease activating factor-1; CARD = caspase recruitment domain

# Some Mechanisms of the Intrinsic Pathway



# Apoptosis in the Developing Nervous System

- Recall NGF.
- A growth factor, but *also* a survival factor.
- Released by “target” cells.
- Bax is an important mediator\*.

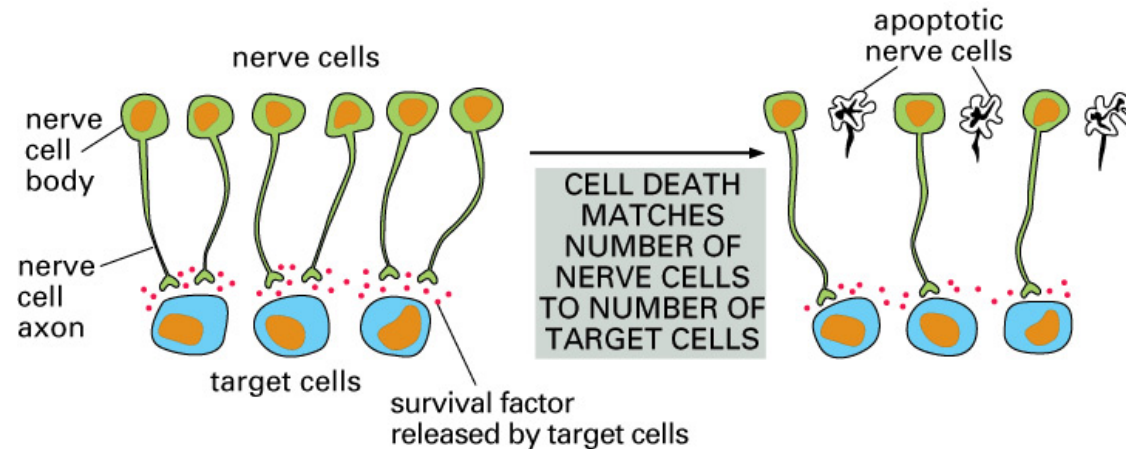
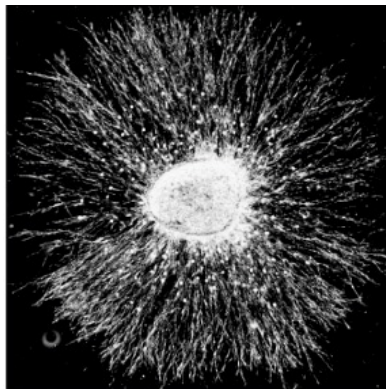


Figure 17-46. Molecular Biology of the Cell, 4th Edition.

\*Lindsten et al. 2005. *Neuroscientist* 11:10-15

# Apoptosis in the Developing Nervous System

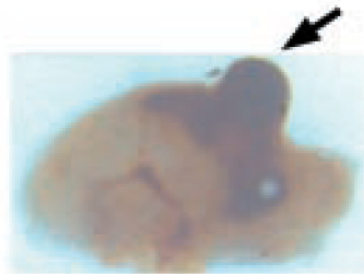


- Mutations in genes encoding important proteins in the apoptotic pathway have drastic consequences.
- In developing mouse embryo, forebrain protrusion and excessive mitosis and differentiation result.

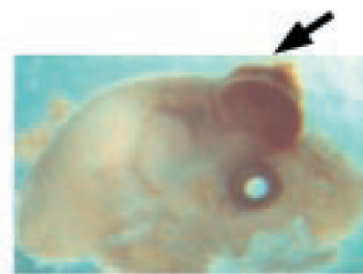
**Wt**



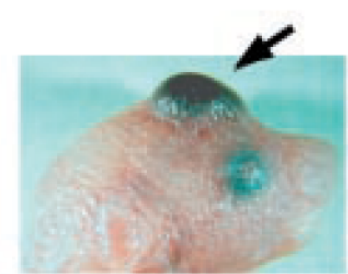
**Casp3 mt**



**Casp9 mt**



**Apaf1 mt**



# A Summary

*Apoptosis in vitro*



# Things to Consider...

How do extrinsic and intrinsic pathways of apoptosis differ? For example, think about how the caspase cascade is initiated in each case.