16. Cell Death and Senescence

Cell Death

- Apoptosis
- Necrosis
- Ferroptosis 铁死亡
- Necroptosis
- Pyrotosis
- NETosis

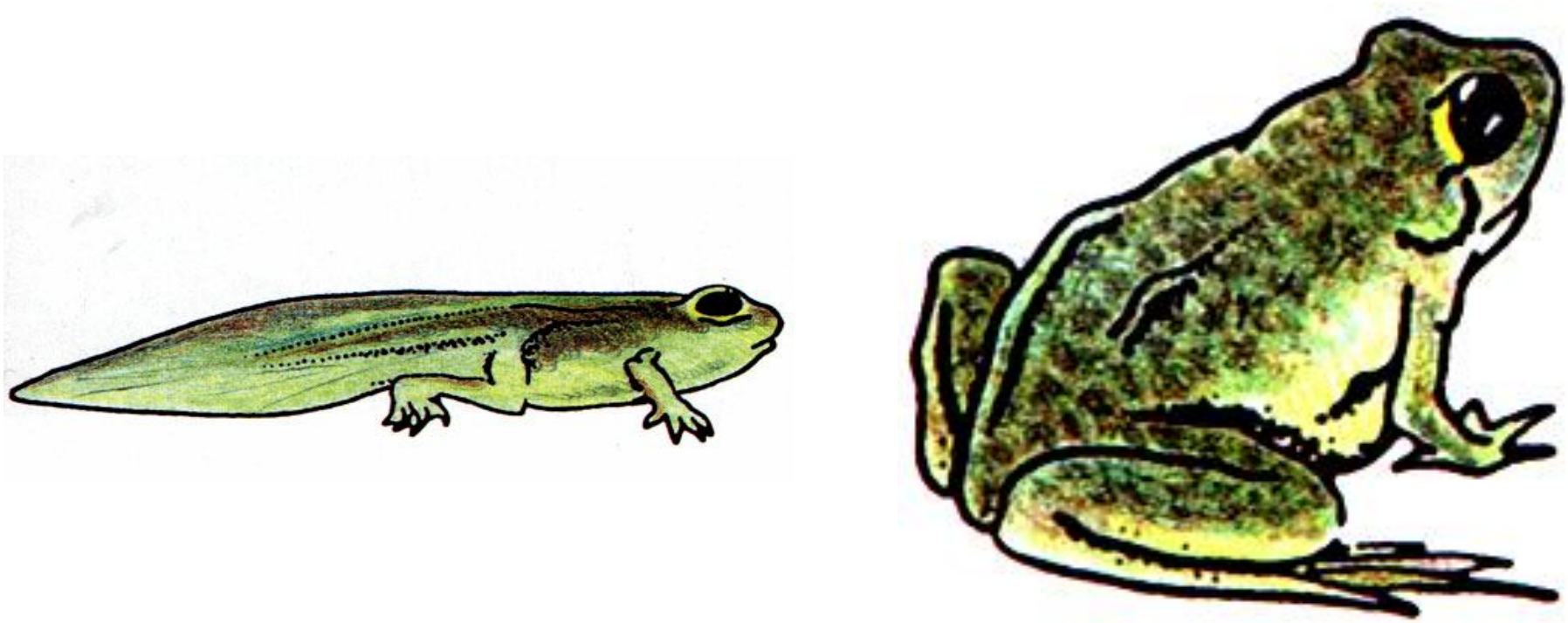
16-1 Apoptosis

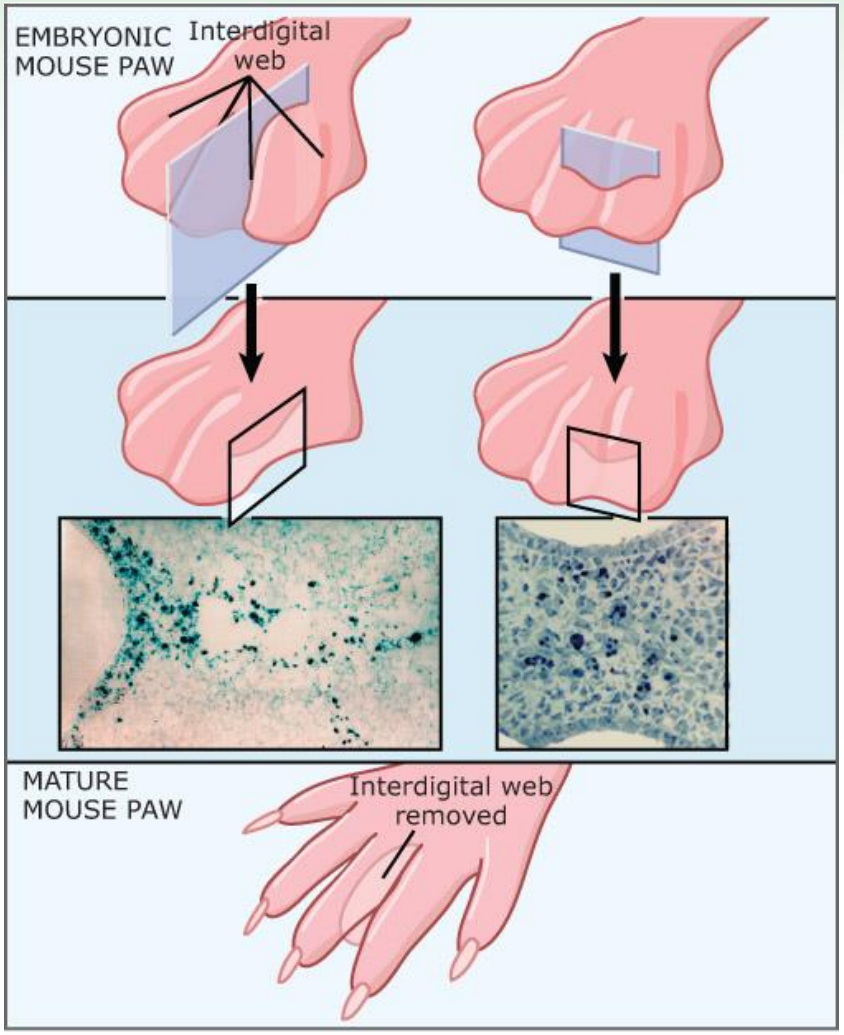
- **Programmed cell death** is a developmental process that usually proceeds by apoptosis.

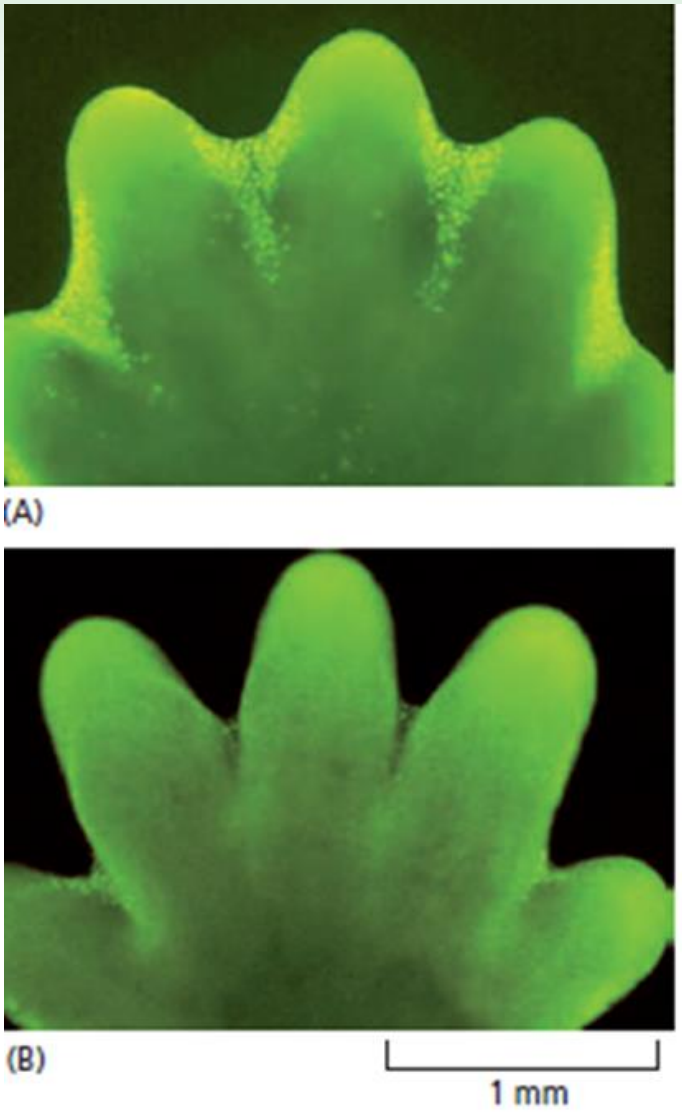
- **Apoptosis** is also the mode of cell death occurring in a variety of other settings and has roles in normal homeostasis, inhibition of cancer, and disease processes.

- Most animal cells possess the molecules comprising the pathways that can cause death by apoptosis, and these pathways are activated by appropriate stimuli.

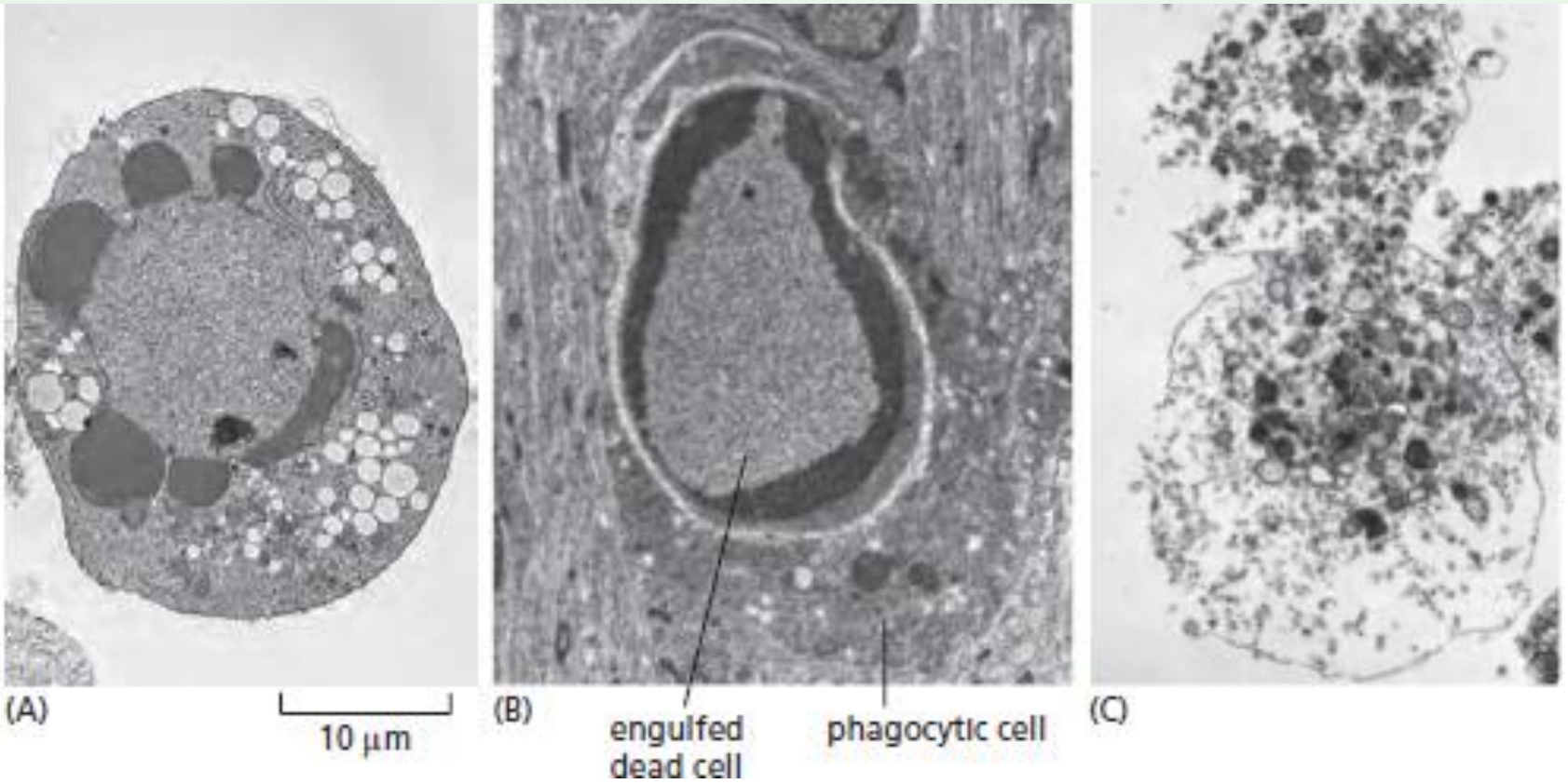
Programmed cell death



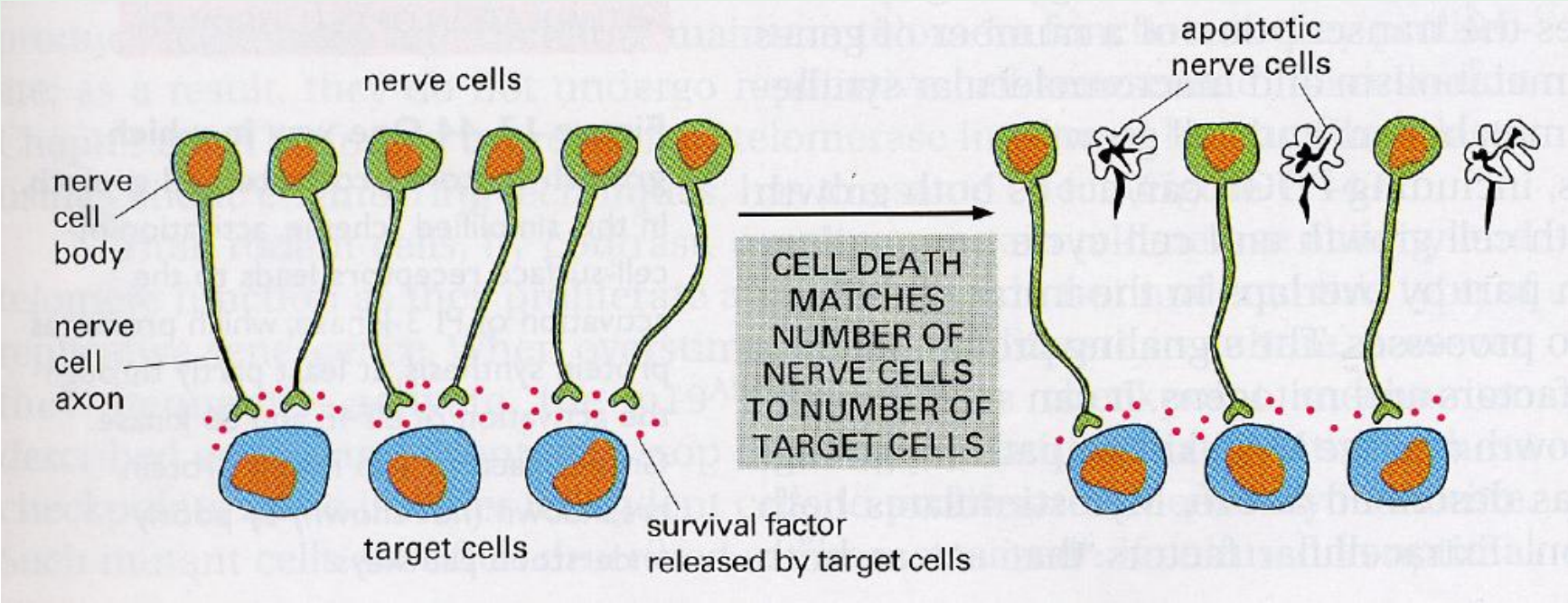




Programmed cell death in developing mammalian limb.

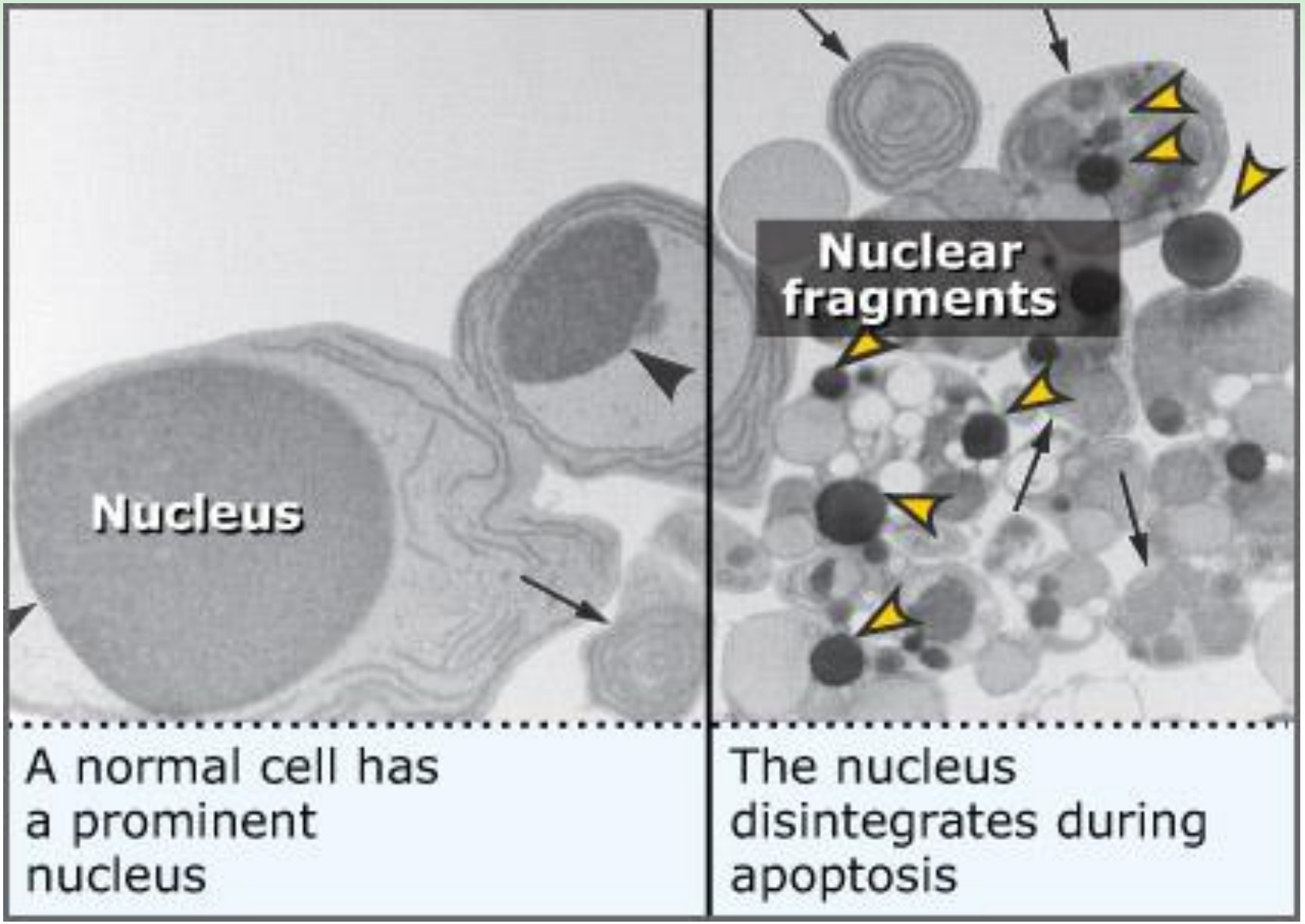


Two distinct forms of cell death

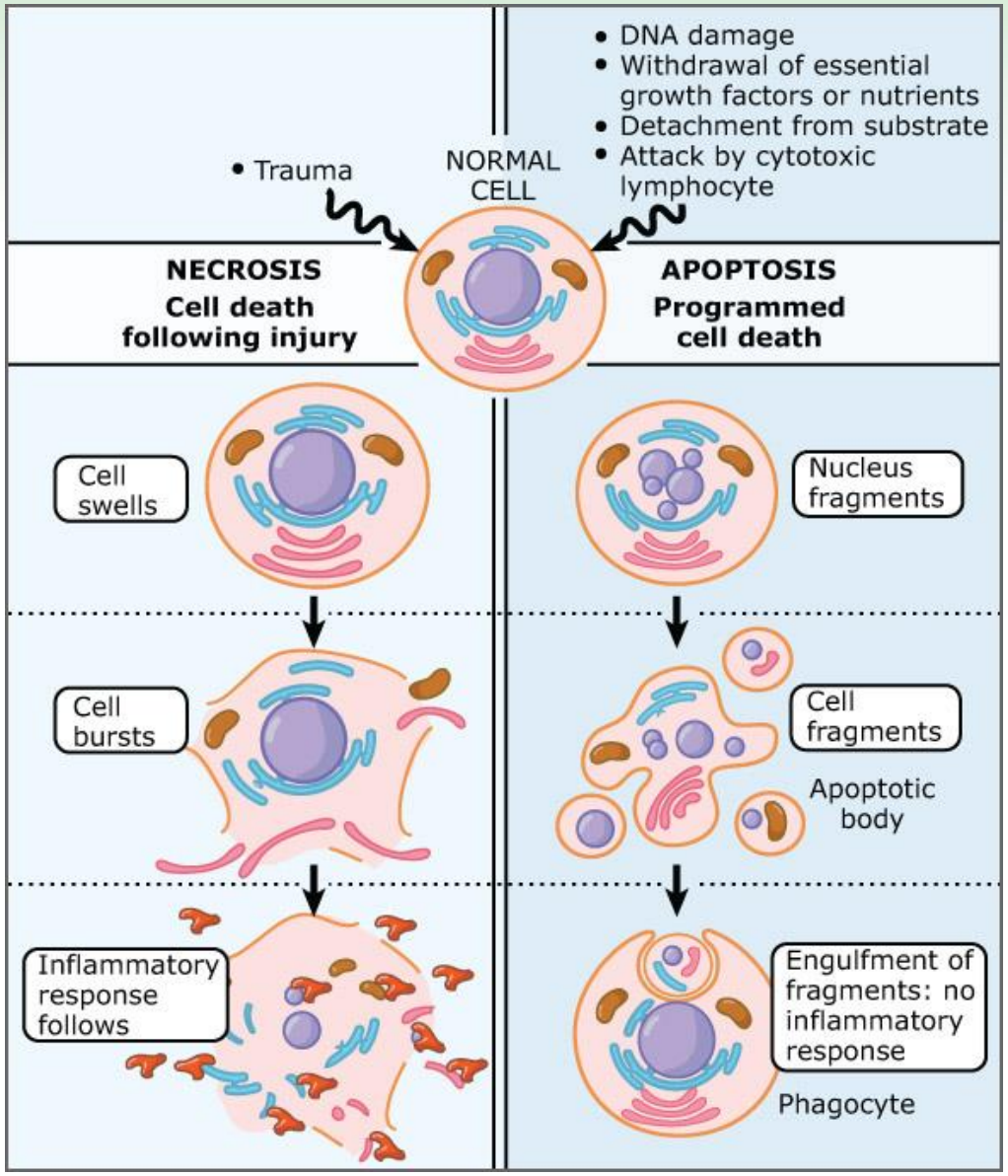


Programmed cell death refers specifically to cell death occurring at a defined point in development.

Apoptosis is defined by the morphologic features of the cell death.



An apoptotic cell is destroyed from within. Cell structure changes during apoptosis. The left panel shows a normal cell. The right panel shows an apoptosing cell; gold arrows indicate condensed nuclear fragments.



Apoptosis versus necrosis. Cellular damage can result in necrosis, which has a different appearance than apoptosis, as organelles swell and the plasma membrane ruptures, without chromatin condensation.

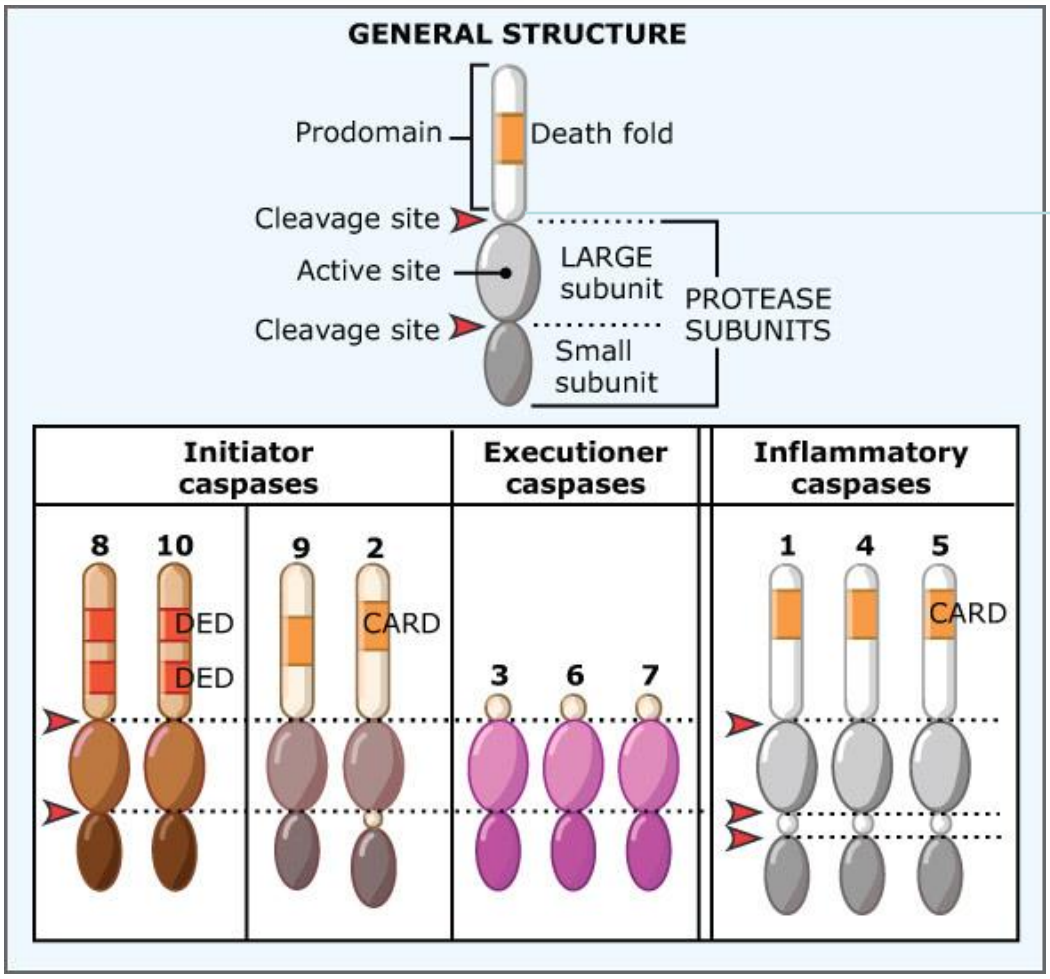
Caspases orchestrate apoptosis by cleaving specific substrates

- Proteases called “caspases” fall into three types: initiator, executioner, and inflammatory. The first two types function in apoptosis.

- The morphologic and biochemical features of cells undergoing apoptosis are caused by the action of the executioner caspases on their substrates.

Caspases：Cysteine aspartate–specific proteases，胱天蛋白酶

- Many caspase substrates are known, as well as the effects of their cleavage on cells in some cases.



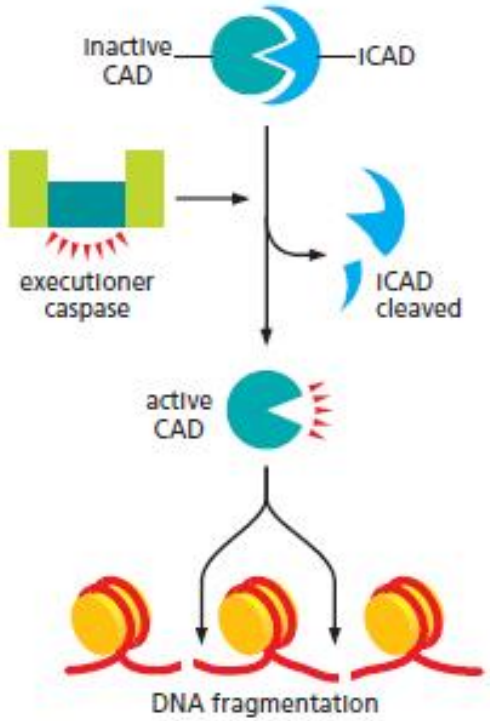
Asp-Xaa-Xaa-Asp/Gly

Asp-Xaa-Xaa-Asp/Ser

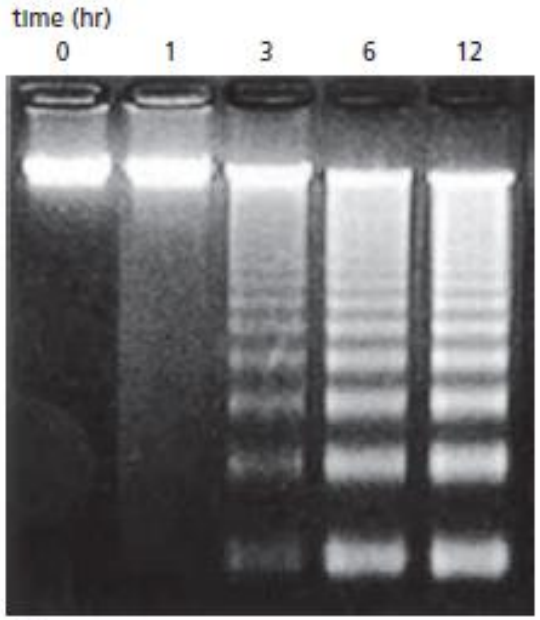
Asp-Xaa-Xaa-Asp/Asp

- Executioner caspases are activated by cleavage, whereas initiator caspases are activated by dimerization.
- Cleavage of executioner caspases at specific sites is necessary and sufficient for their activation.
- This cleavage is usually mediated by the initiator caspases.

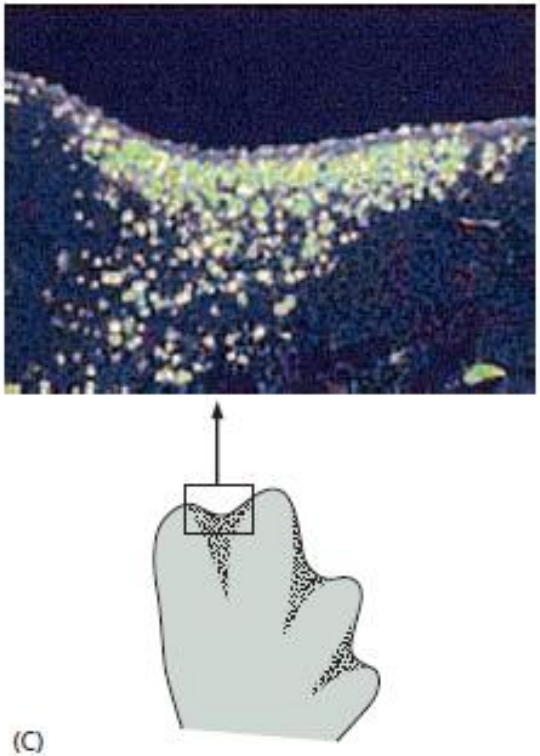
Different types of vertebrate caspases are shown schematically. Note the prodomains and protein–protein interaction regions of the initiator and inflammatory caspases.



(A)



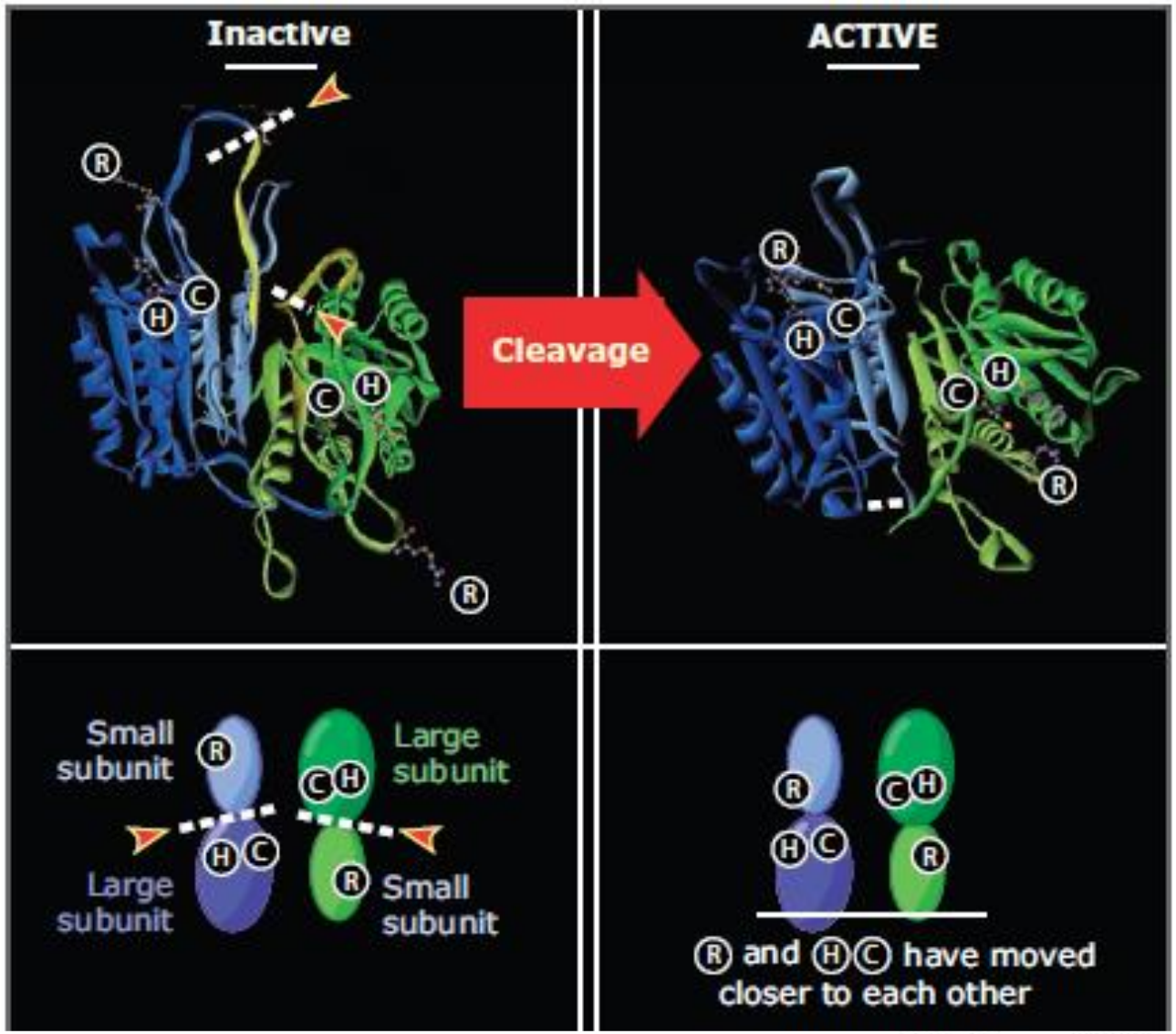
(B)



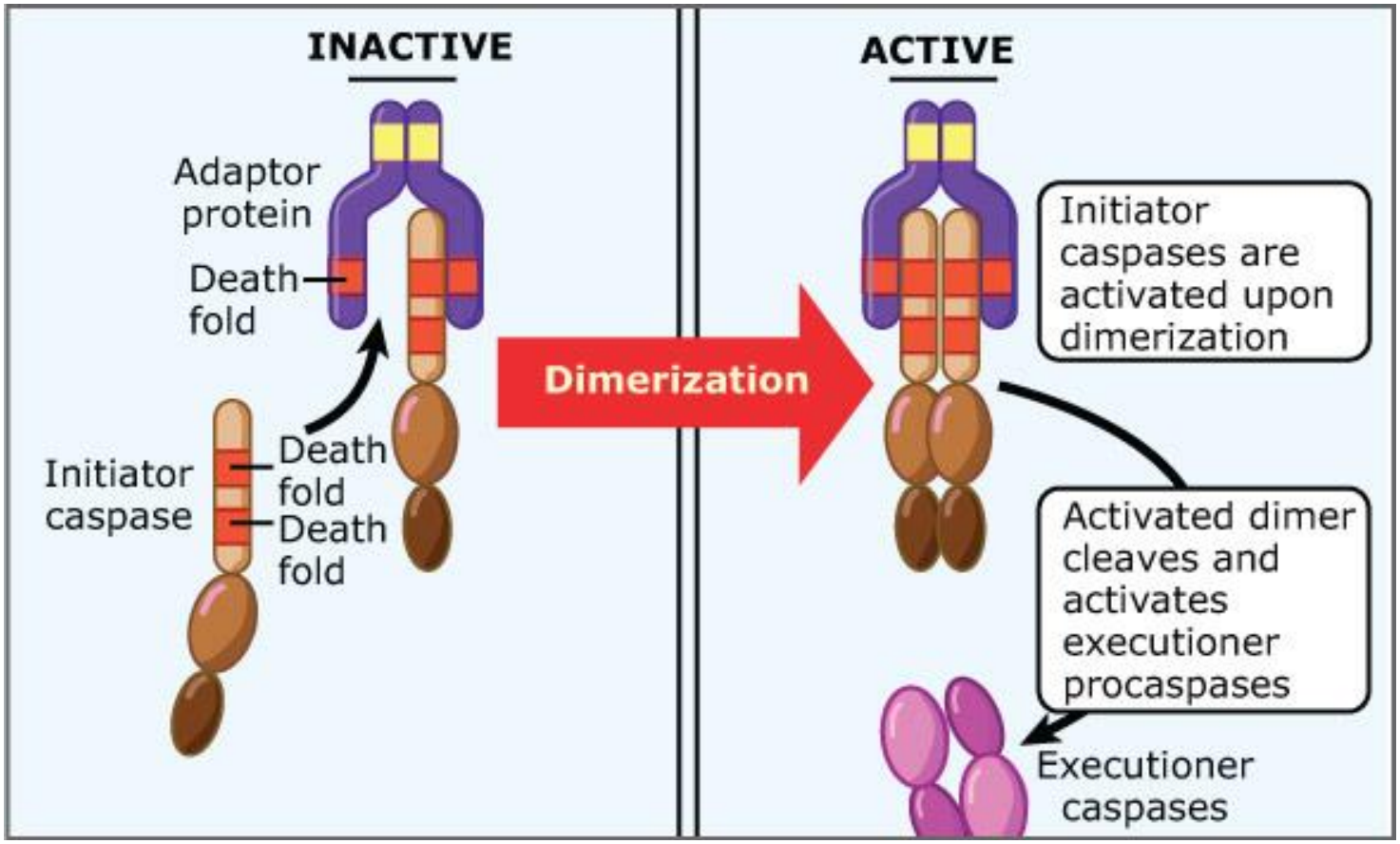
(C)

DNA is degraded during apoptosis.

Executioner caspases are activated by cleavage, whereas initiator caspases are activated by dimerization



- Initiator caspases are activated by adaptor molecules that contain protein-protein interaction domains called death folds.

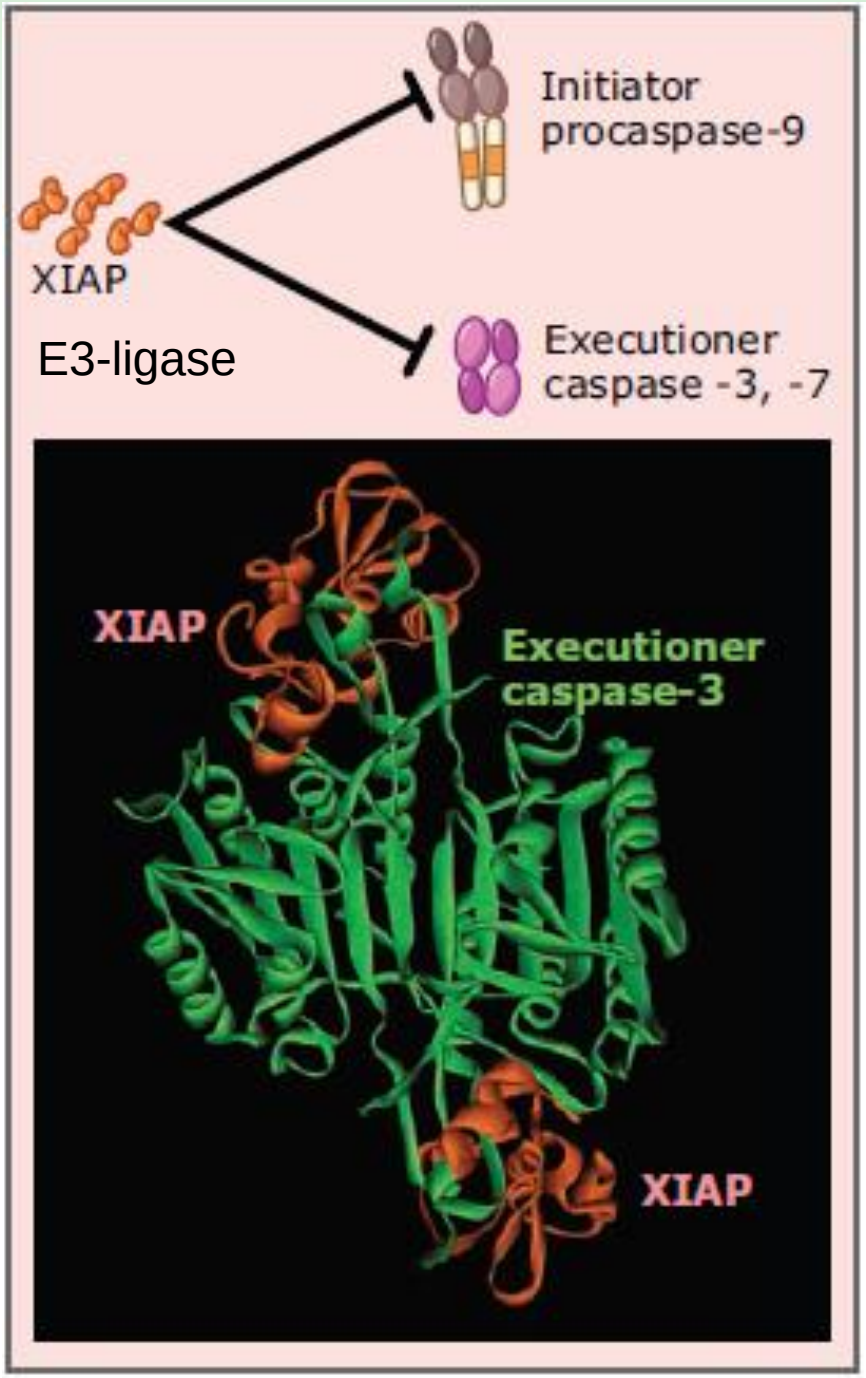


Initiator caspases are activated by dimerization.

Some inhibitors of apoptosis proteins block caspases

- Since executioner **caspases are activated by cleavage**, and since these caspases can cleave and activate each other, any proteolytic activity of the caspases will be rapidly amplified in cells, resulting in their death by apoptosis. It is, therefore, important that there be **mechanisms** present to **limit potential "accidental" activation of caspases** in cells that are not signaled to die.

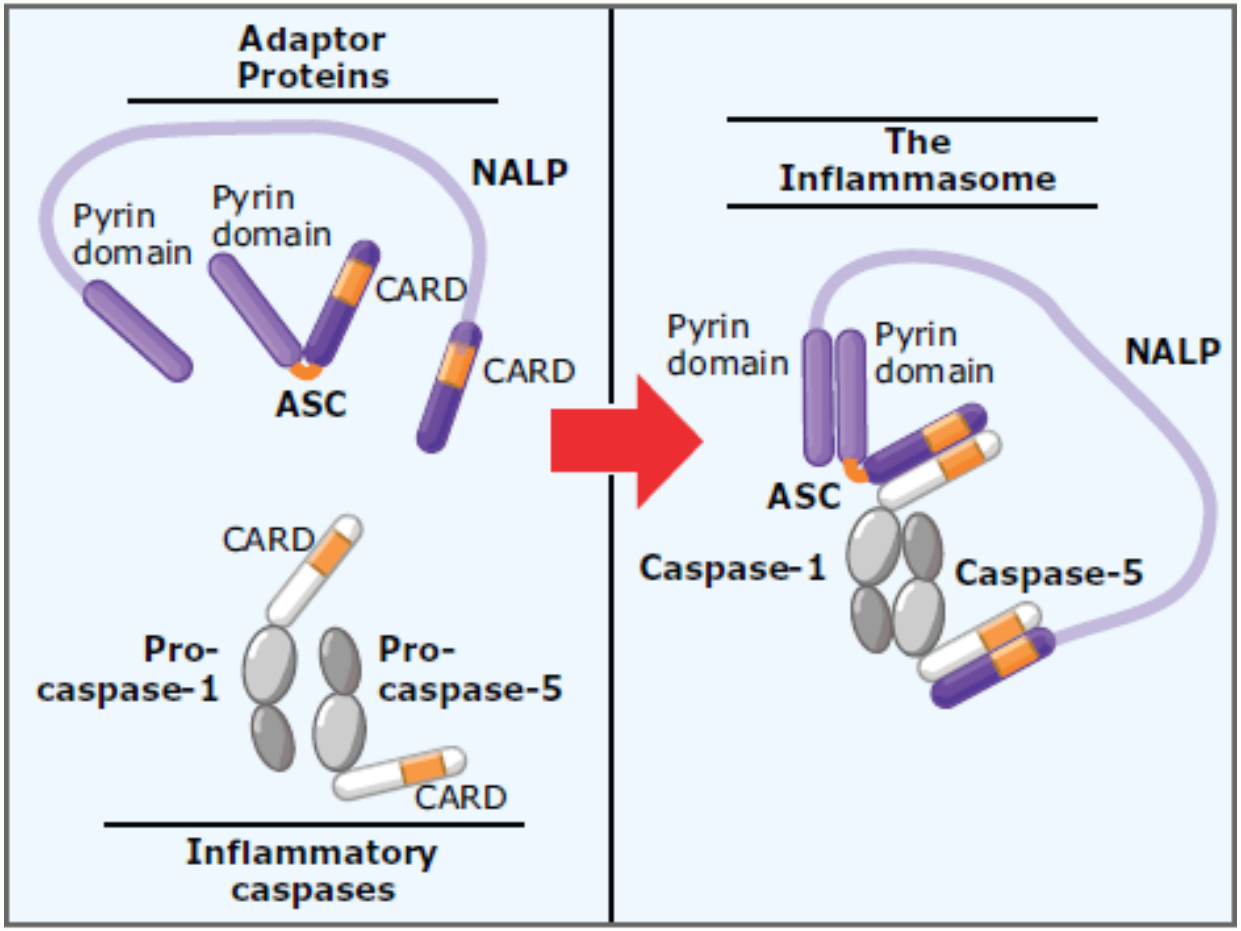
- The inhibitors of apoptosis proteins (IAPs) comprise a family of proteins with different functions; some of these proteins bind to and inhibit caspases and induce their degradation by the proteasome.



Those IAPs with RING domains, **function as ubiquitin E3-ligases**, and can effectively target both itself and its target caspases for ubiquitination and degradation by the proteasome.

XIAP binds to and inhibits caspases-9, -3, and -7. The structure of the interaction between caspase-3 and XIAP （X-linked IAP） is shown. (Structure from Protein Data Bank 1I30. M. A. Seefeld, et al., Bioorg. Med. Chem. Lett. 11 (2001): 2241?244.)

Some caspases have functions in inflammation



Another set of proteases in this family acts to process cytokines rather than to regulate apoptosis.

caspase-1

↓

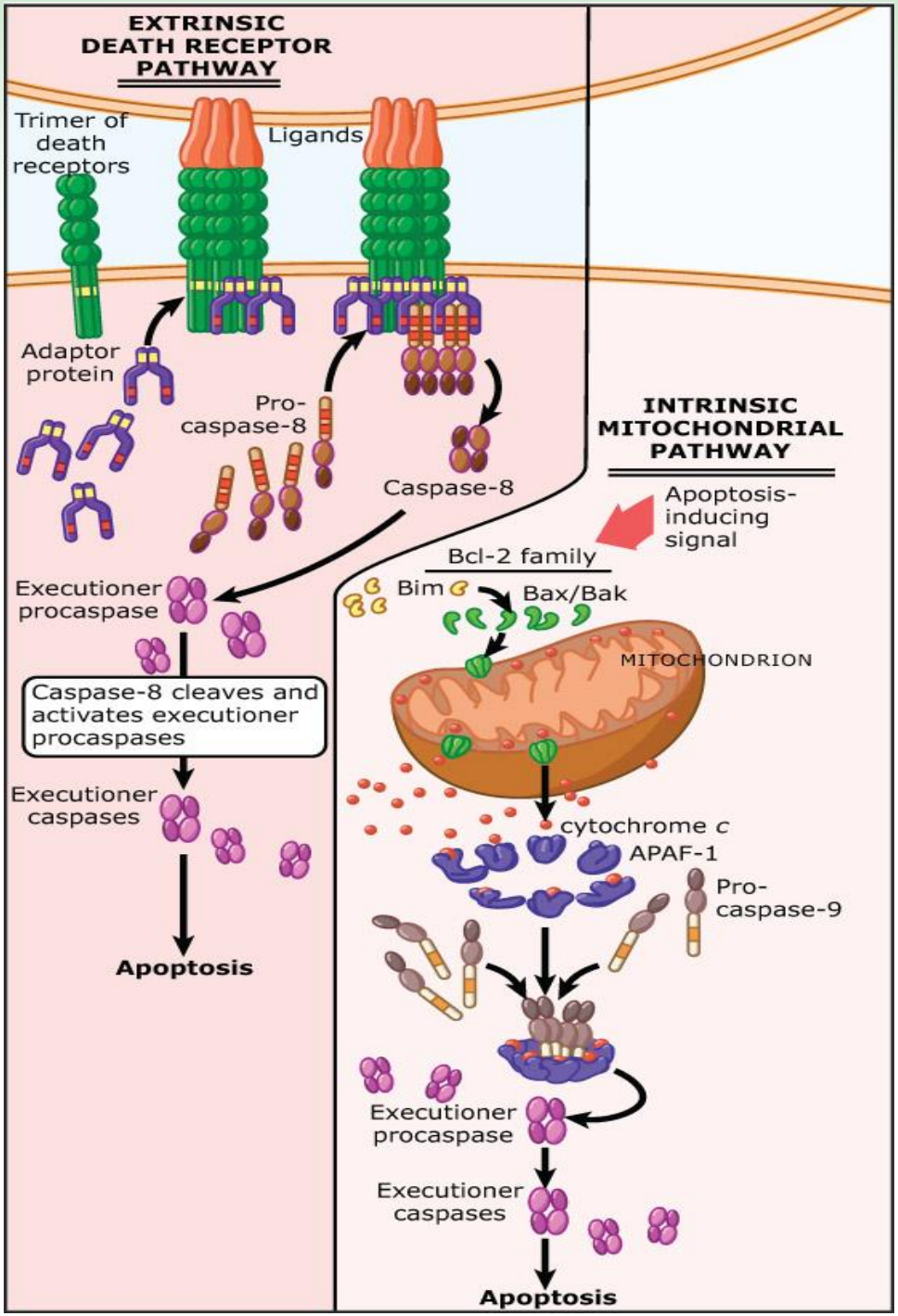
interleukin-1b

Mice lacking caspase-1, caspase-11, or ASC fail to secrete interleukins-1 or -18 but show no obvious defects in development or apoptosis. In contrast, humans with activating mutations in NALP-1 have inflammatory syndromes relating to elevated cytokine secretion.

Death receptor pathway of apoptosis

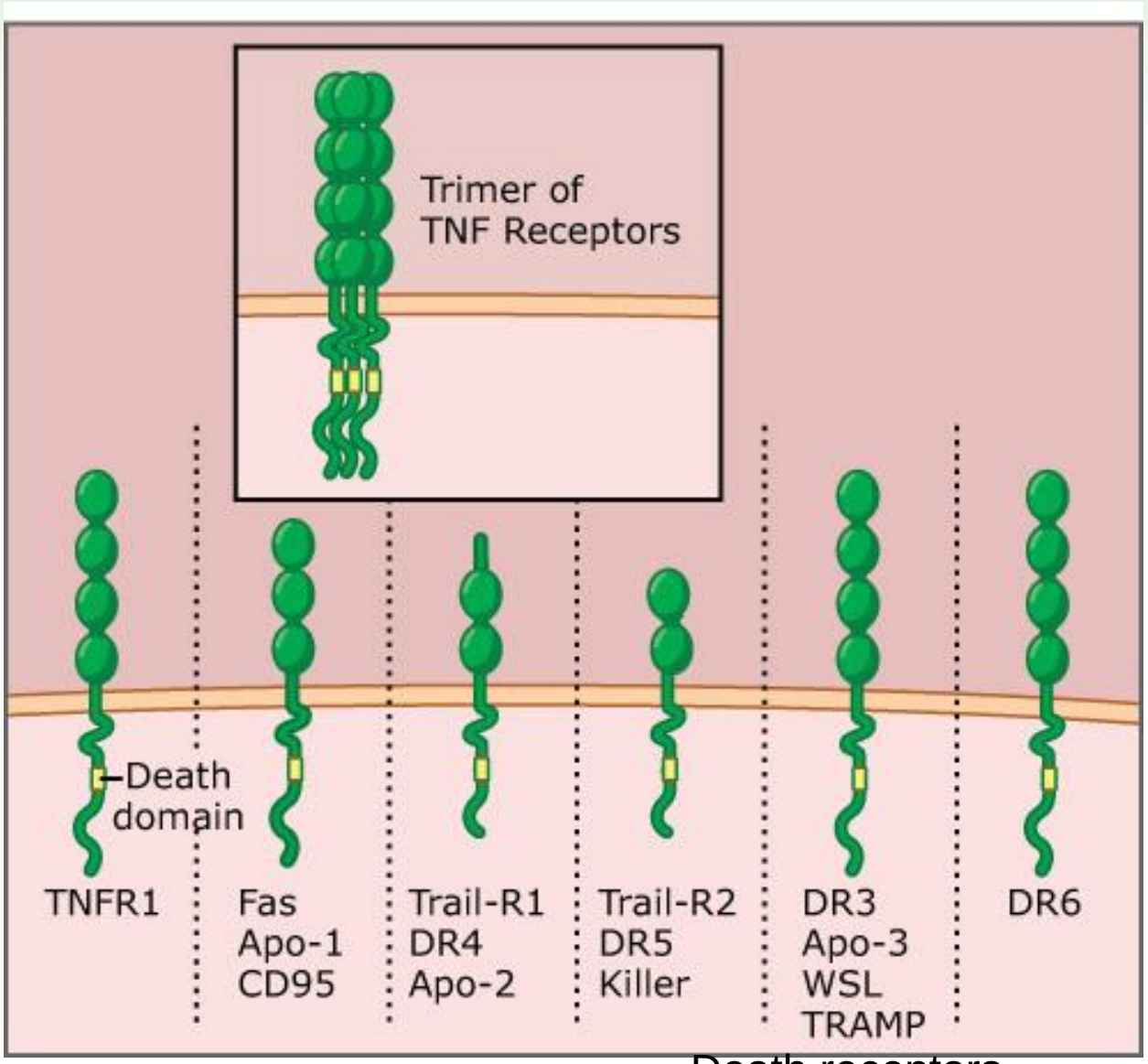
- Two well-characterized pathways of apoptosis are the **death receptor (extrinsic) pathway** and the **mitochondrial (intrinsic) pathway**.

- Caspase activation and apoptosis are induced by the binding of specialized ligands in the tumor necrosis factor (TNF) family to their receptors (death receptors).



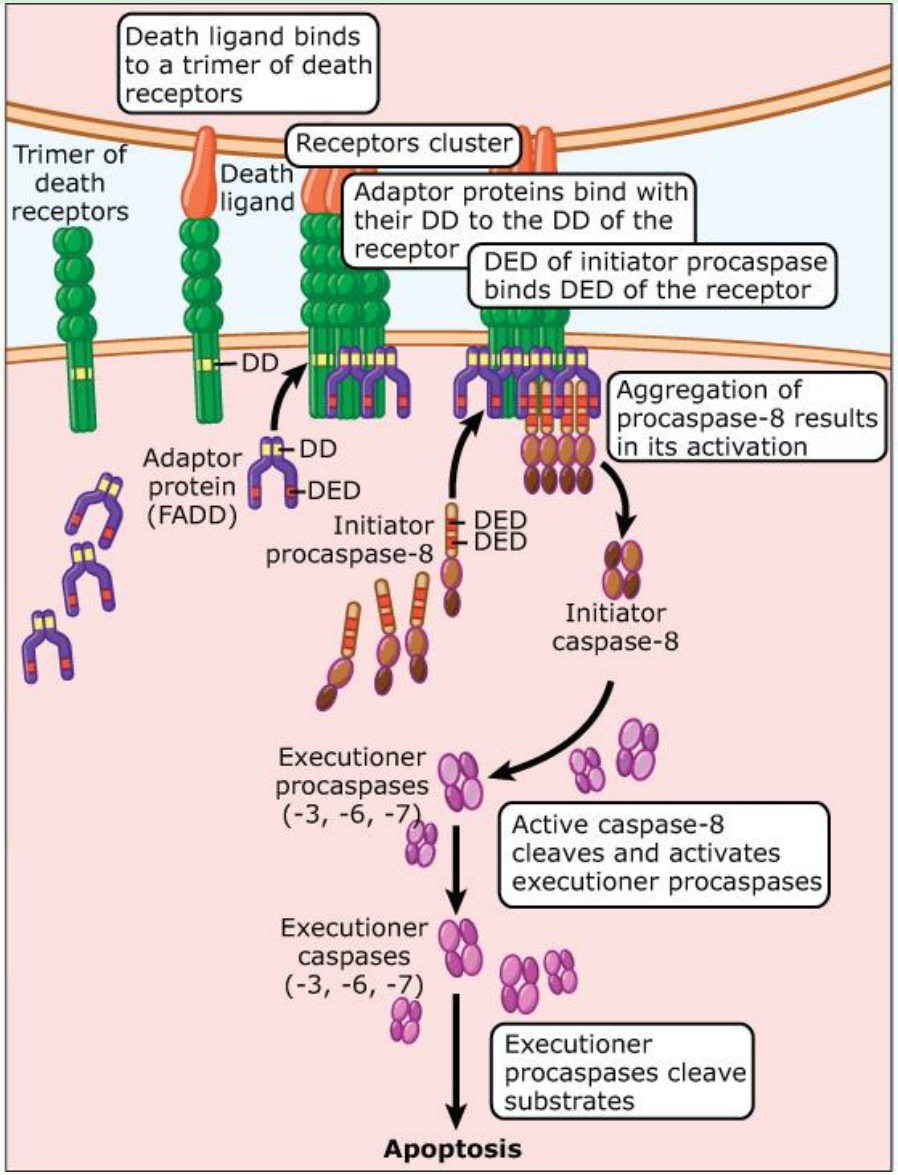
Caspase activation and apoptosis are induced by the binding of specialized ligands in the TNF family to their receptors (death receptors).

Overview of two apoptotic pathways



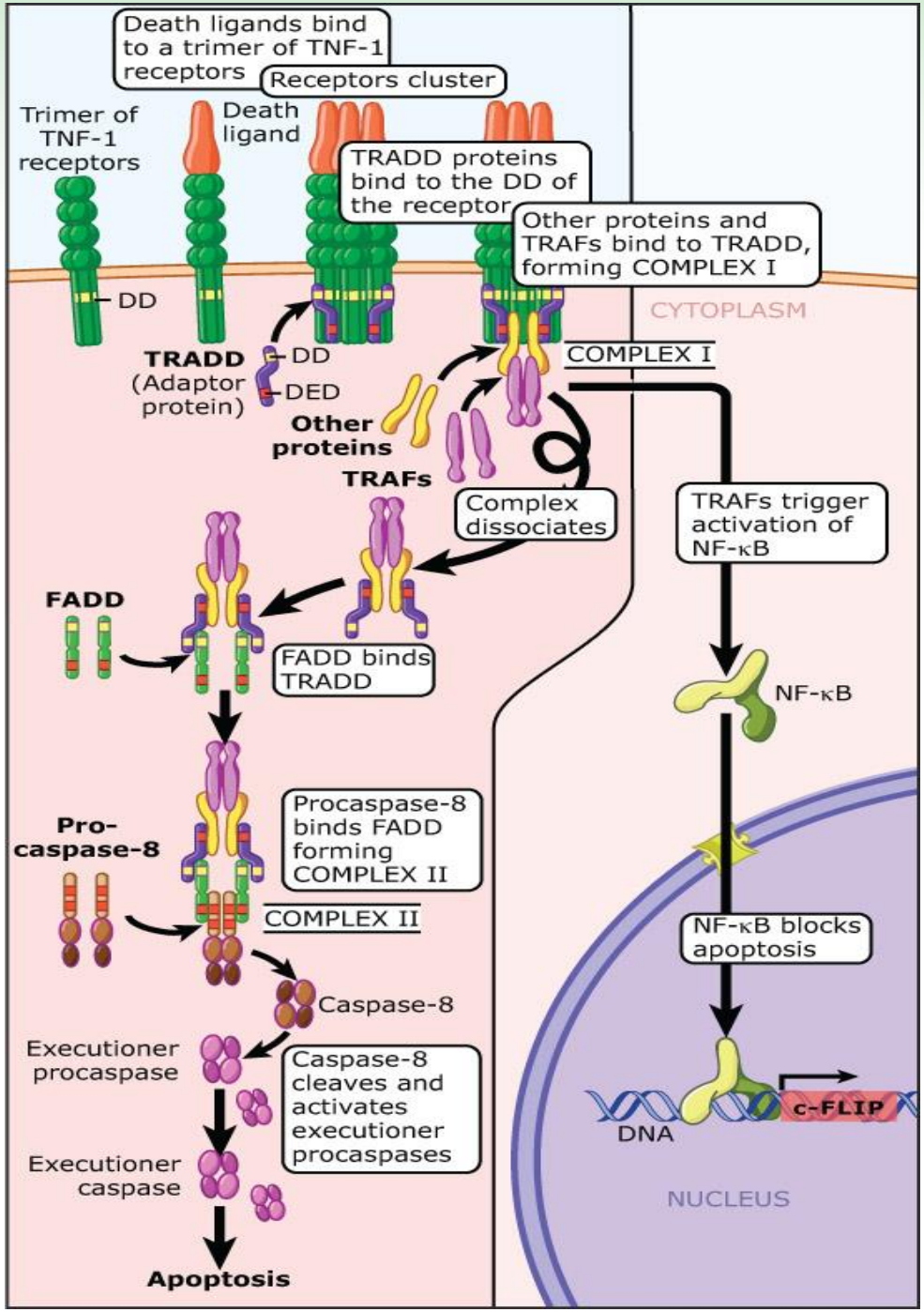
Death receptors.

The death receptors are members of the TNFR family that carry death domains in their intracellular regions. These exist as trimers on the cell surface of many cell types in vertebrates.



Ligation of death receptors causes the recruitment of the adaptor protein FADD to the intracellular region of the death receptor, via death domain (DD)–DD interactions. Caspase-8 is then recruited to FADD via DED–DED interactions. The dimerization of caspase-8 activates it through induced proximity. The active caspase-8 can cleave and activate executioner caspases to cause apoptosis. The complex of death receptor, FADD, and caspase-8 is called a death-inducing signaling complex (DISC).

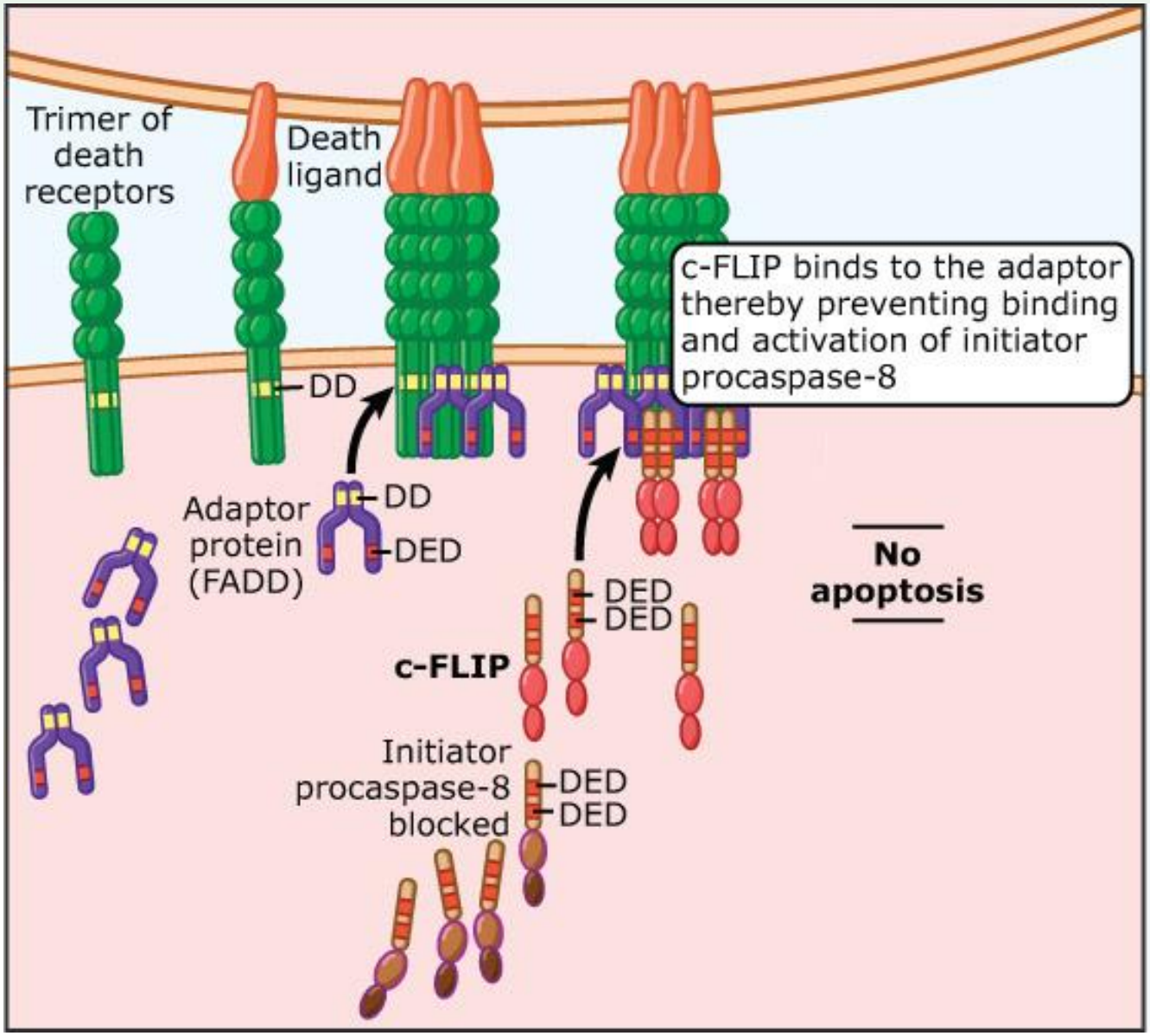
The death receptor pathway of apoptosis.



Model for signaling via TNFR1.

Binding of TNF to TNFR1 can trigger NF-kB activation, blocking apoptosis, or caspase activation leading to apoptosis. The ligation leads to the formation of a complex (Complex I) composed of the intracellular region of the receptor, an adaptor protein (TNFR-associated death domain), and other proteins. This complex dissociates and in the cytosol binds to FADD, which in turn can bind and activate **caspase-8** (Complex II). The active caspase-8 cleaves and activates executioner caspases to cause apoptosis. **The activation of NF-kB**, however, can also induce antiapoptotic molecules that prevent caspase activation and death.

c-FLIP can block death receptor-induced apoptosis.



One important protein expressed after NF-kB activation is called c-FLIP. This protein is interesting in that it is closely related to caspase-8 but lacks elements required for protease activity

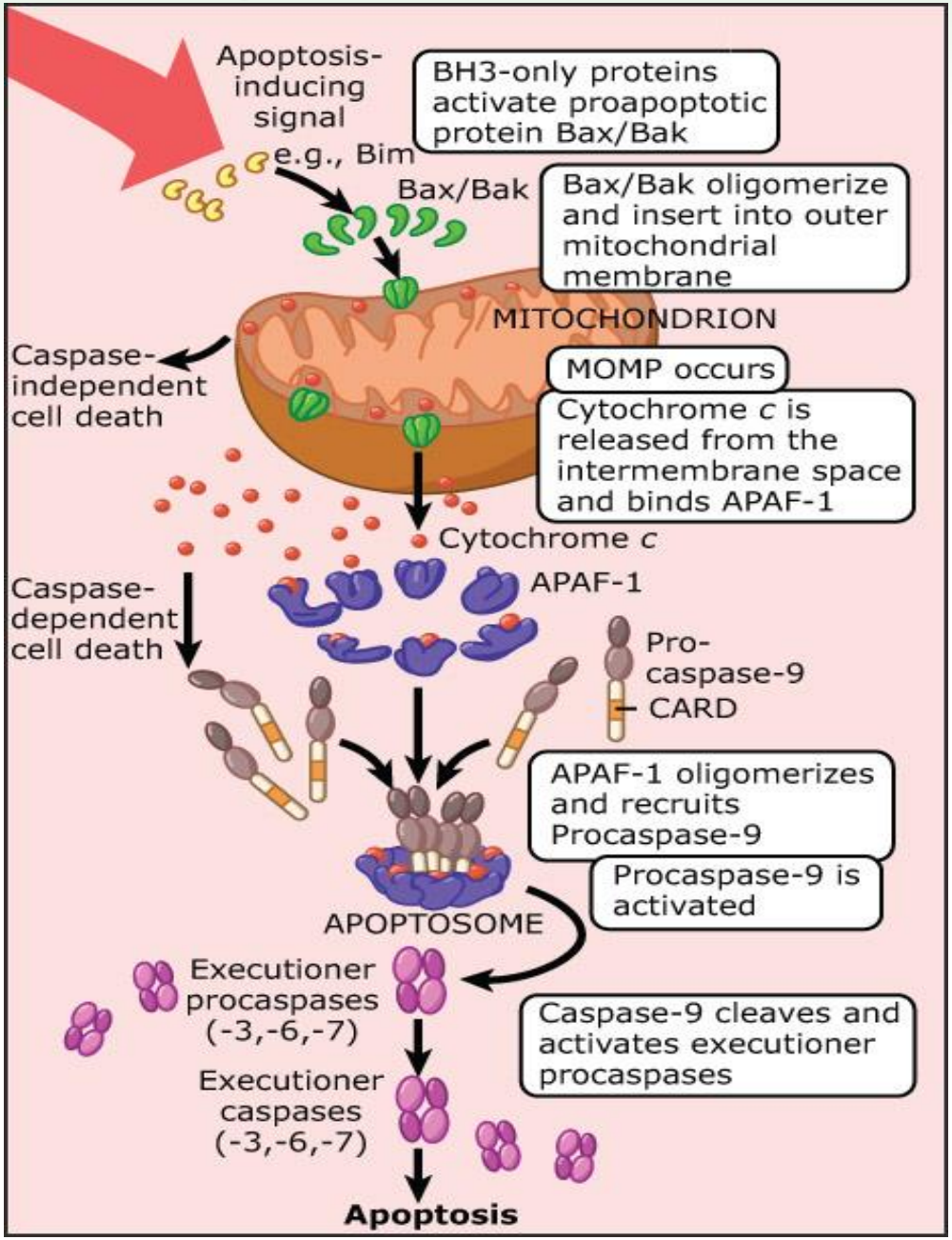
Question：

Knockouts for **FADD, c-FLIP, or caspase-8** showed a striking phenotype distinct from that of any death receptor–deficient animals. Each of these mutant mice dies at the same early stage of embryonic life, and this death is clearly not due to defects in apoptosis. Instead, it appears that these molecules are required for signaling events that participate in cell survival at a critical time in embryogenesis. Exactly what these signals might be remains obscure.

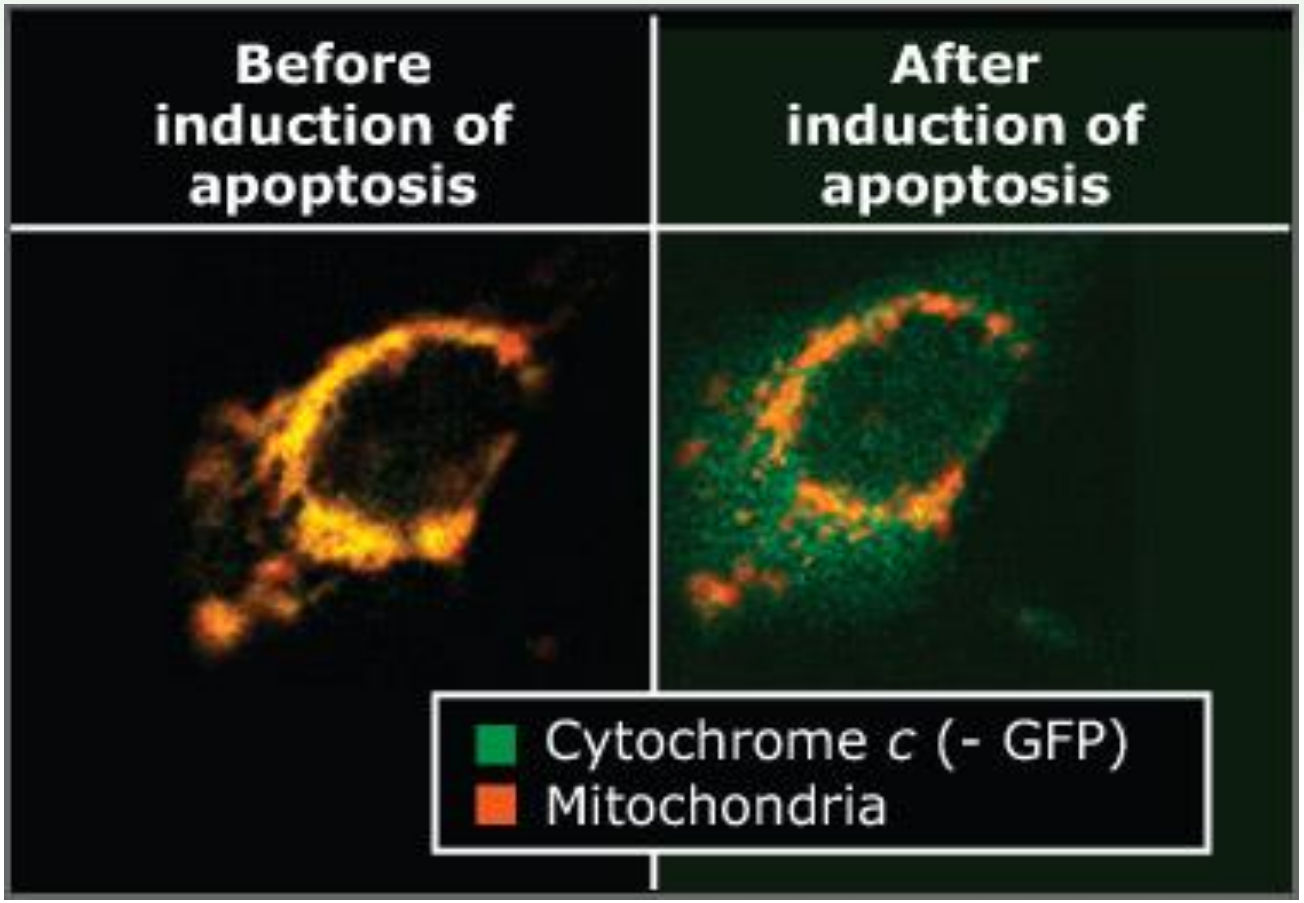
The mitochondrial pathway of apoptosis

- Most apoptosis in mammalian cells proceeds via a pathway in which the mitochondrial outer membranes are disrupted, releasing the contents of the mitochondrial intermembrane space into the cytosol.

- Mitochondrial outer membrane permeabilization (MOMP) is a key feature of this pathway.

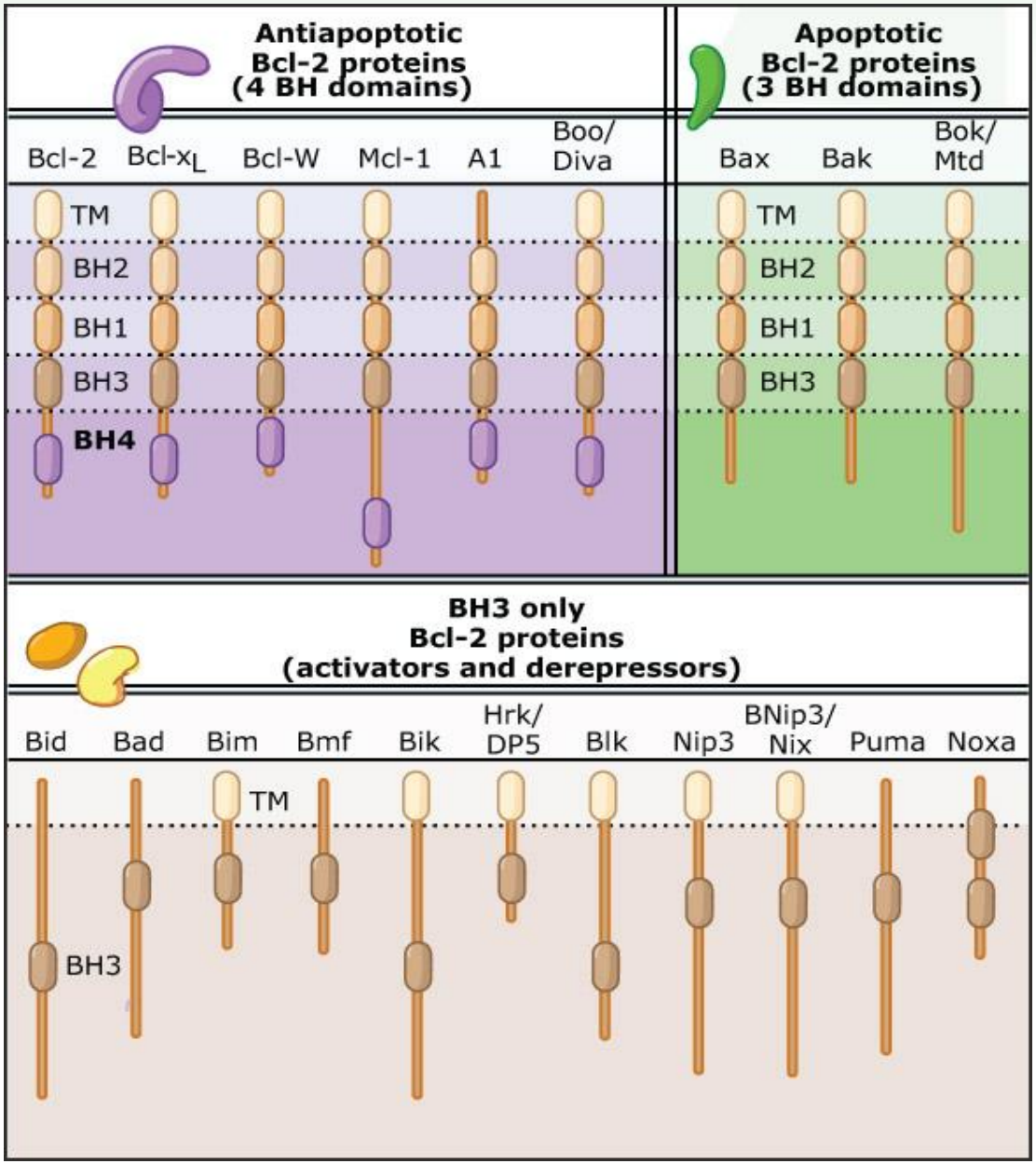


Cytochrome c release during apoptosis



Cells expressing cytochrome c bound to green fluorescent protein (cytochrome c-GFP) were also stained with tetramethylrhodamine ethyl ester to identify mitochondria (red) (left image). Upon induction of apoptosis, cytochrome c-GFP suddenly diffused from the mitochondria into the cytosol (right image, several hours after induction). Evidence of caspase activation followed within minutes.

Bcl-2 family proteins mediate and regulate MOMP and apoptosis



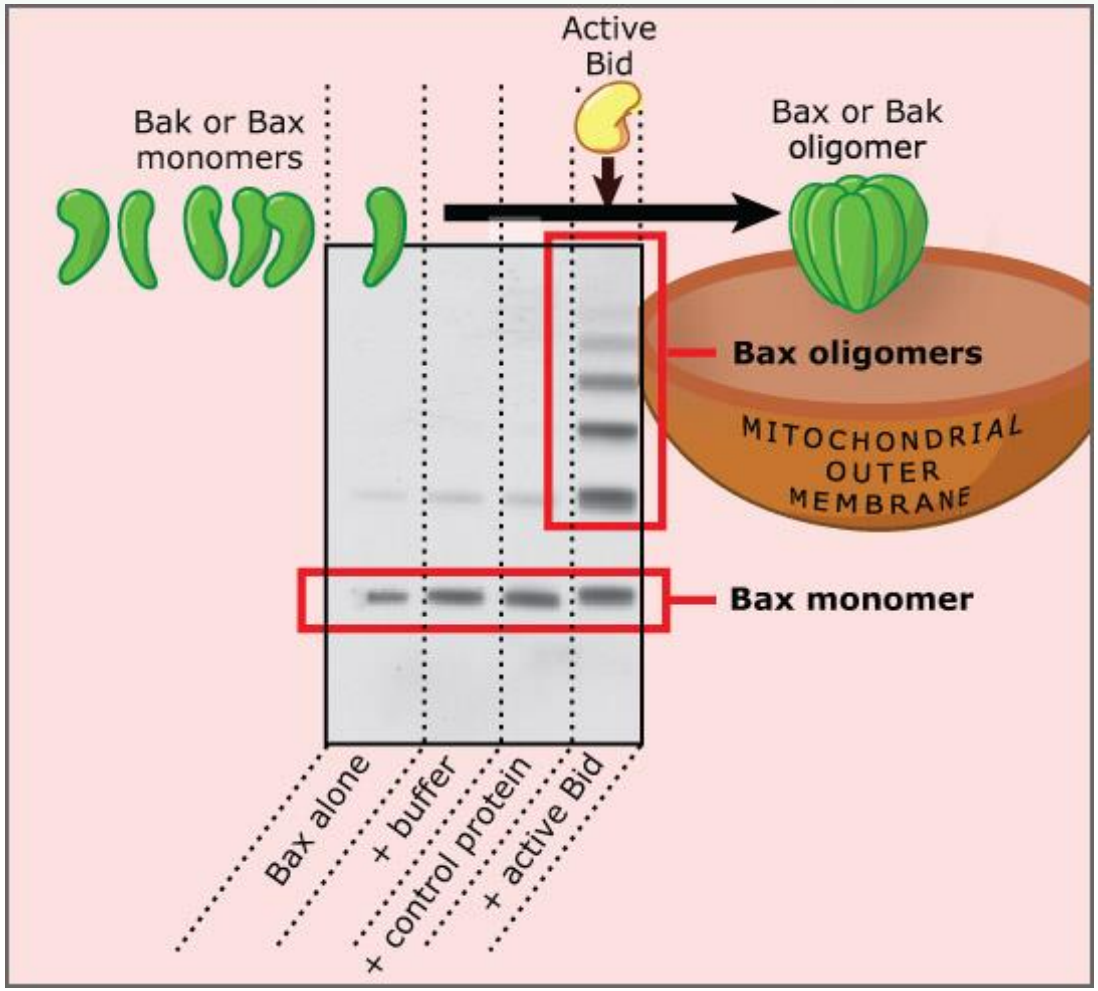
- The Bcl-2 family proteins are central to the mitochondrial pathway of apoptosis.
- There are 3 classes of Bcl-2 proteins that induce, directly cause, or inhibit MOMP.
- Pore-forming protein(细菌), and diphtheria toxin B chain(白喉毒素B链）

Bax and Bak

- Bax and Bak are essential for the **permeabilization** of the mitochondrial outer membrane and are required for the **mitochondrial pathway** of apoptosis.

- Bax and Bak probably directly cause the membrane disruption associated with MOMP.

Bax or Bak, when activated by BH3-only proteins



Activation of BAK/BAX by Bid results in oligomerization and MOMP.

$BaK^{-/-}$: Proundly normal

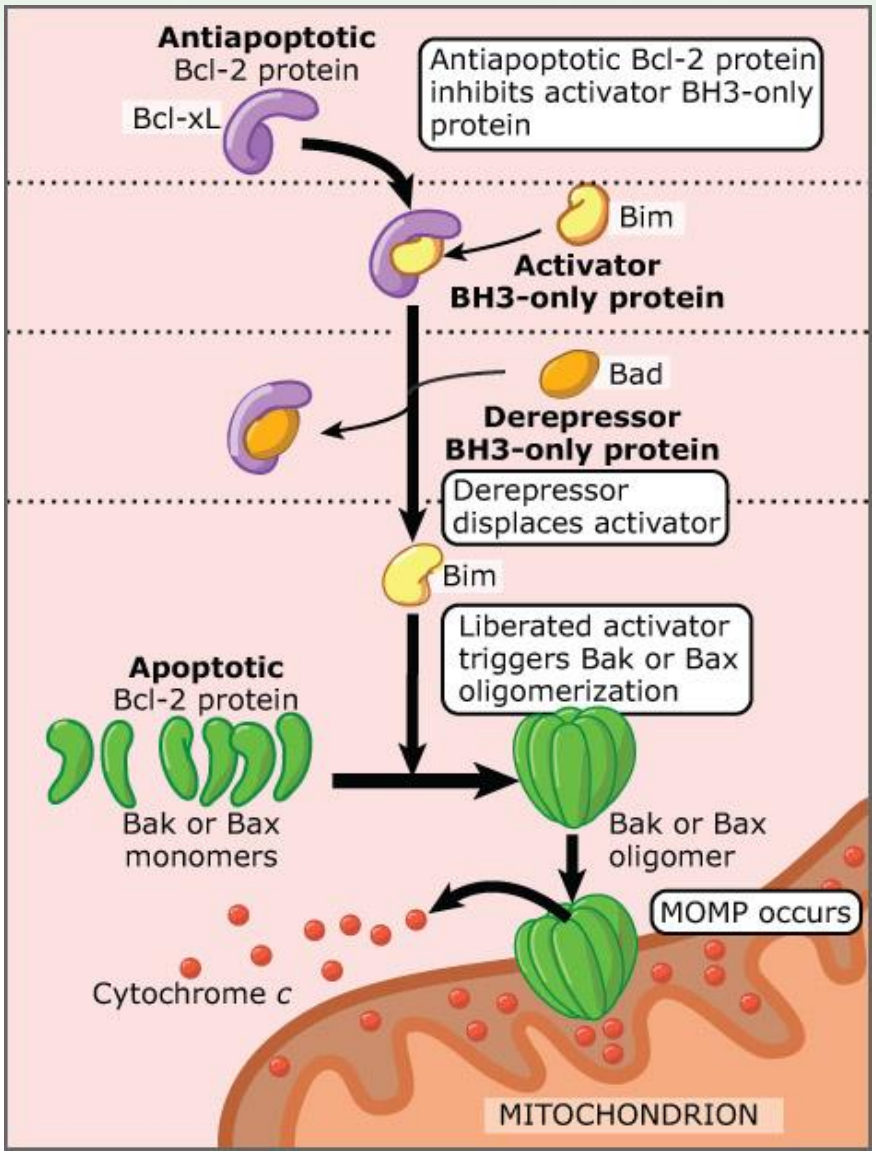
$BaX^{-/-}$: Showing some developmental defects, were also fairly normal

BaK, BaX^{DKO}: No MOMP, death

Growth factor and cell fate, autophagy

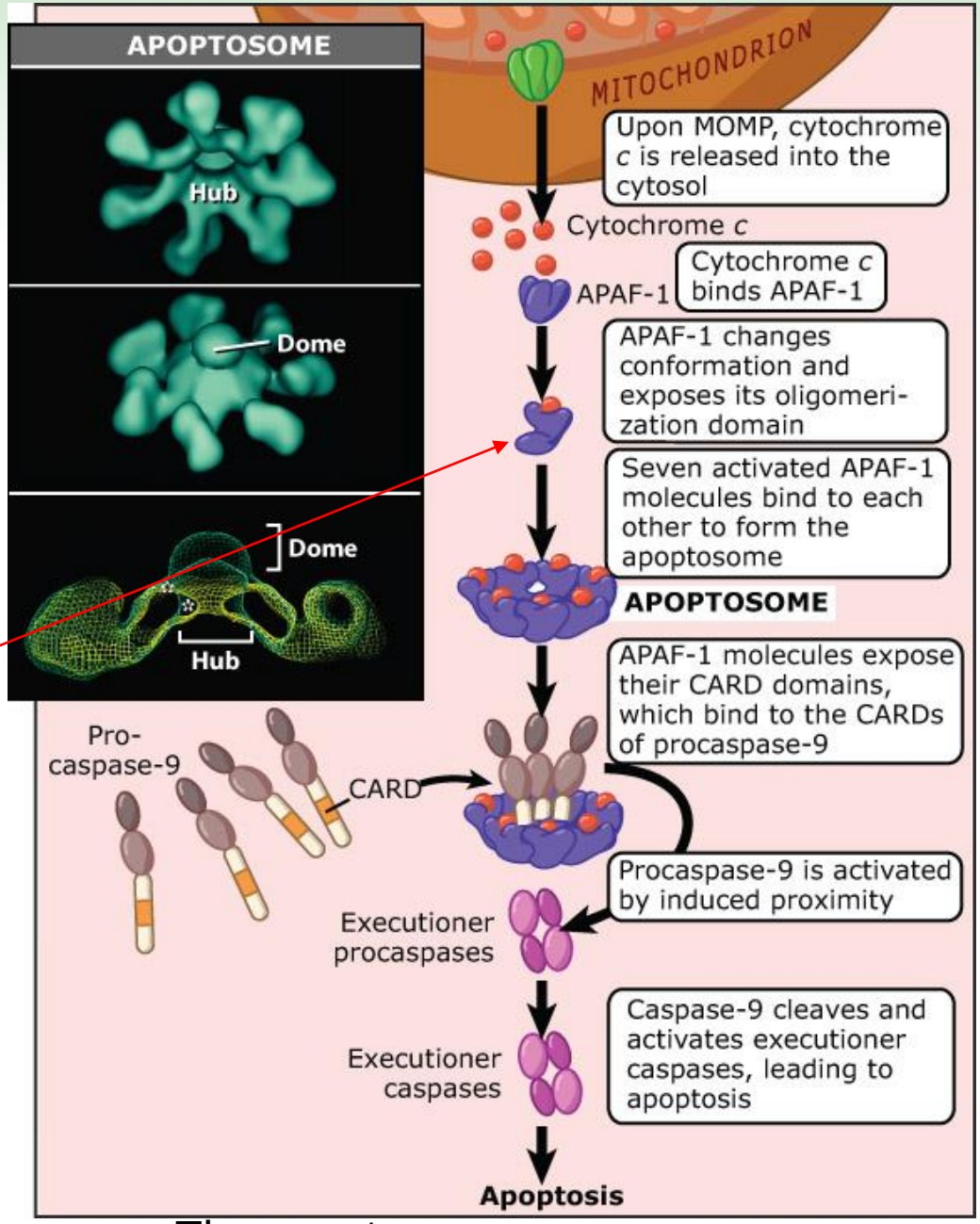
When Bax-Bak double-knockout cells were deprived of growth factors, however, they did not die but instead underwent **autophagy** (“self-eating”), sustaining themselves for several weeks by metabolizing intracellular components

Activation of Bax and Bak is controlled by other Bcl-2 family proteins



- The antiapoptotic members of the Bcl-2 family block the permeabilization of the mitochondrial outer membrane by Bax and Bak.
- The BH3-only proteins of the Bcl-2 family either directly activate Bax and Bak or interfere with the antiapoptotic Bcl-2 protein functions.

Proapoptotic functions of “BH3-only” Bcl-2 proteins.



The apoptosome.

Cytochrome *c*, released upon mitochondrial outer membrane permeabilization, induces caspase activation.

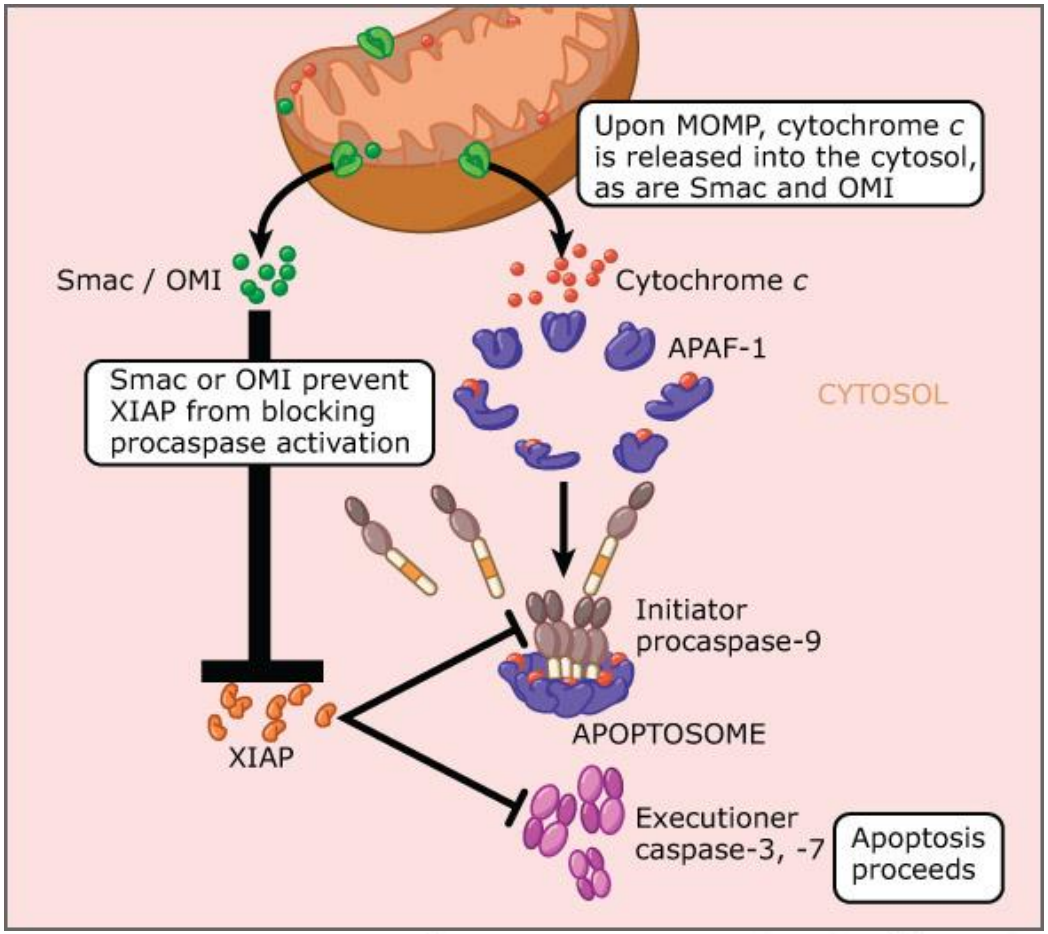
- Holocytochrome *c* triggers the activation of cytosolic **apoptotic protease activating factor-1 (APAF-1),** which binds and activates caspase-9.
- APAF-1, caspases-9, ncaspases-3 nockout mice
- Cytochrome C mutation

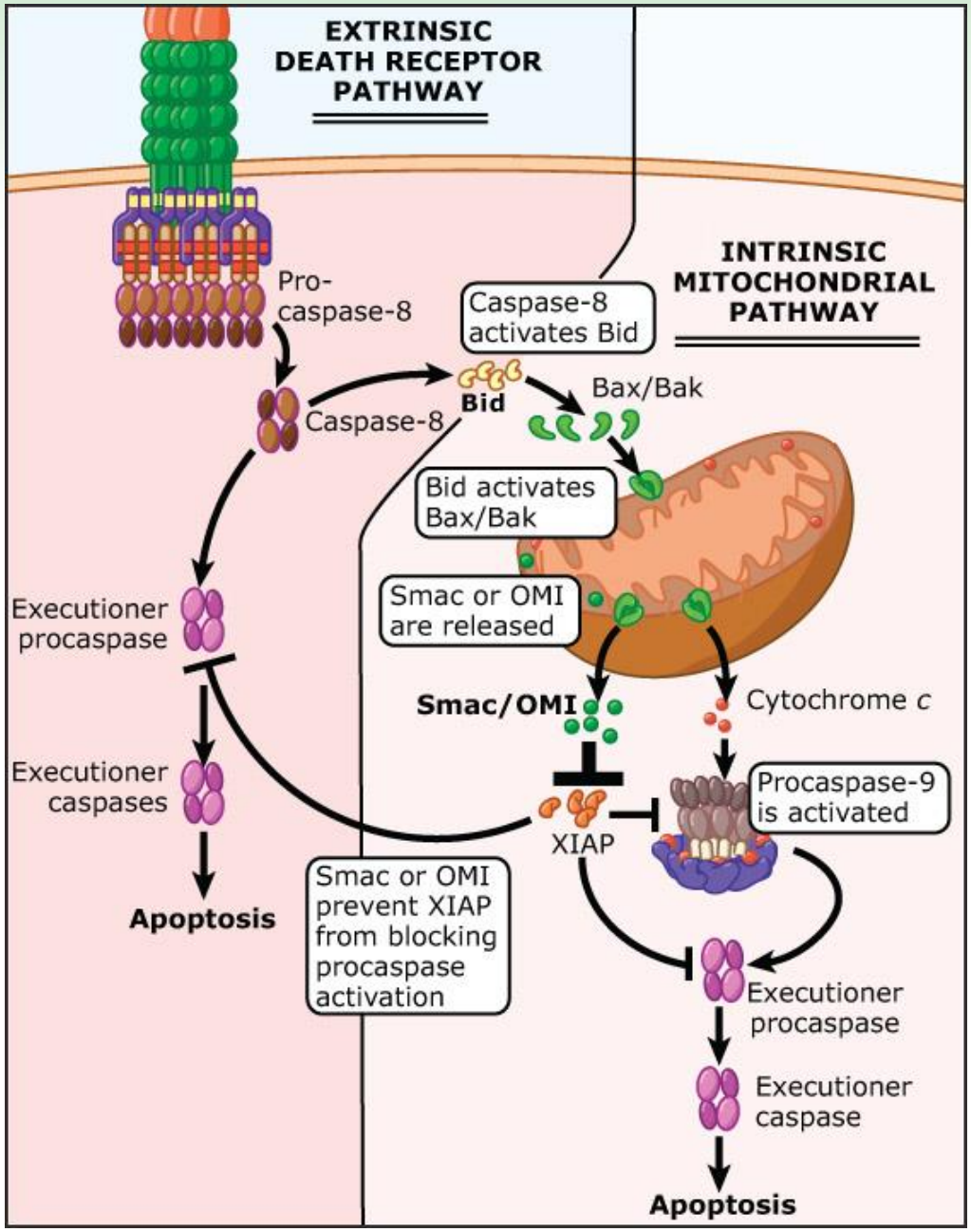
Some proteins released upon mitochondrial outer membrane permeabilization block inhibitors of apoptosis proteins.

- The mitochondrial intermembrane space proteins Smac and Omi antagonize the caspase-inhibitory activity of IAPs.

The **knockouts of XIAP and Smac** have little or no phenotypes, whereas that of **Omi** shows neurologic defects, most likely due to loss of an important mitochondrial activity unrelated to its IAP-inhibitory function.

Protein interference with IAP-mediated inhibition of caspases.



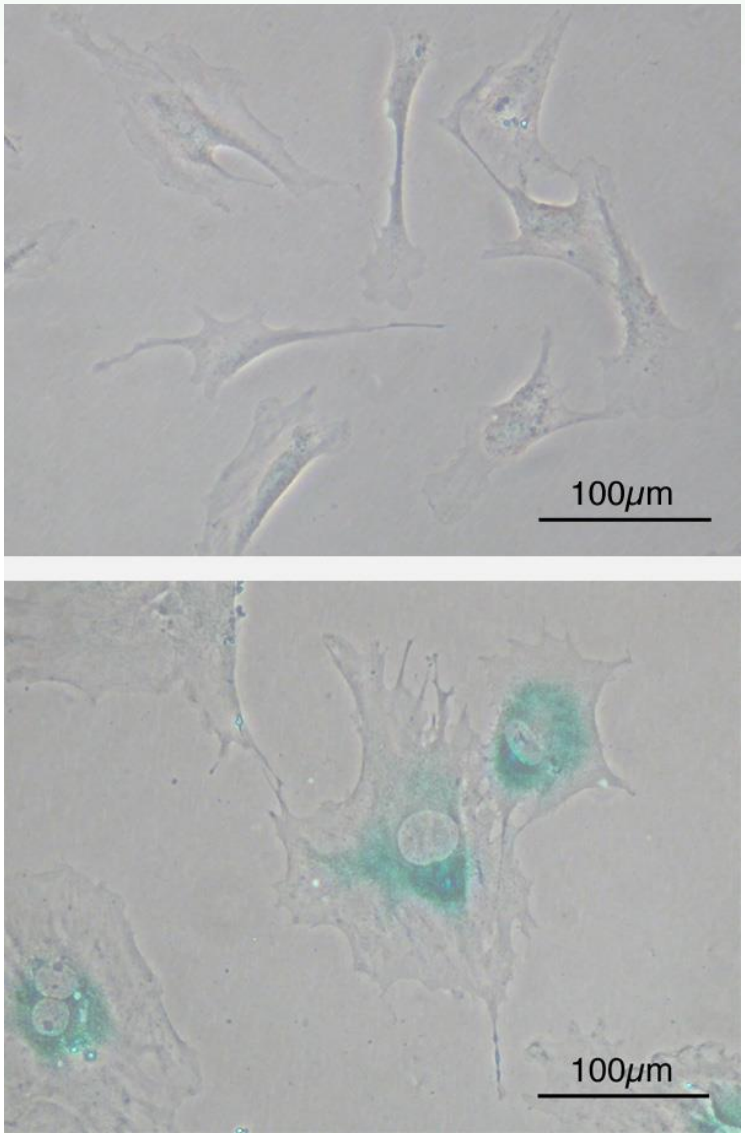


The death receptor pathway of apoptosis can engage mitochondrial outer membrane permeabilization through the cleavage of the BH3-only protein Bid.

- Caspase-8, activated upon ligation of death receptors, cleaves the BH3-only protein Bid, resulting in its activation.

Bid bridges the death receptor and mitochondrial pathways.

16-2. Cellular senescence



β- 半乳糖苷酶染色

Cell ageing or cell senescence

Replicative senescence

- August Weismann.
- Alexis Carrel: Chicken embryo cells (34 years)
- HeLa Cell (1951~)
- Leonard Hayflick and Paul Moorhead
- Elizabeth Blackburm,端粒：四膜虫（TTGGGG）
  人（TTAGGG）
- Telomerase

P53↑ → p16, p21 ⊣ cyclin 2,4,6

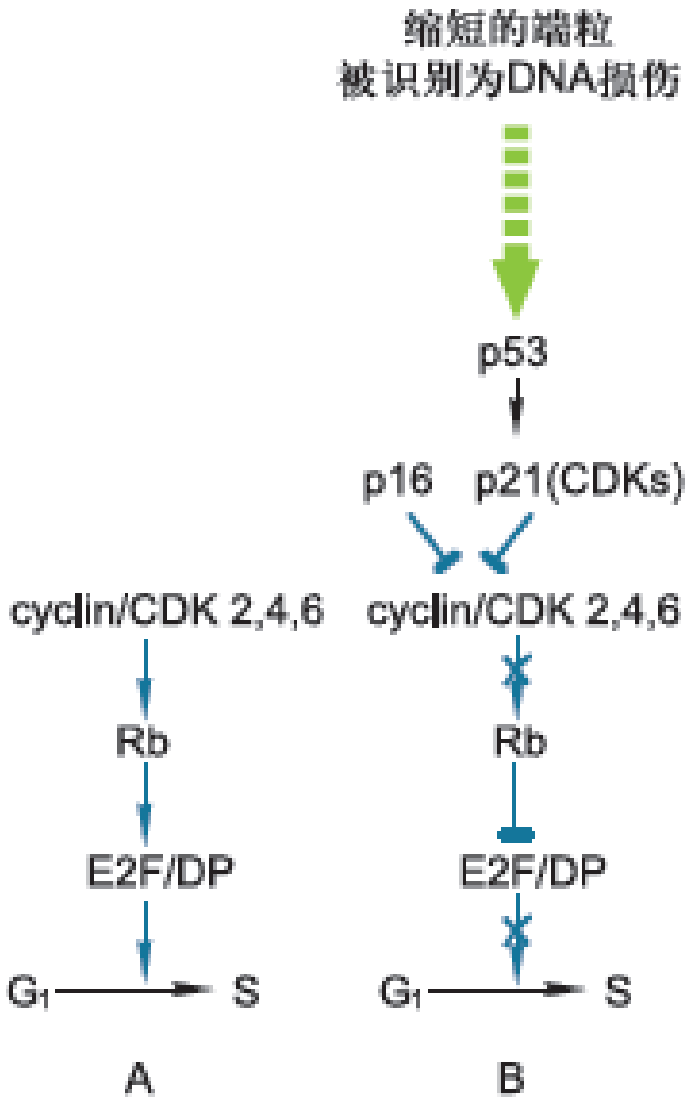


图 15-2　细胞衰老的信号通路

- 希望课堂内容对大家有用。
- 祝愿大家都取得好成绩！
- 许多问题还是谜一样的存在，需要大家共同努力。