Molecular biology of the cell
BIO 207

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CHAPTER 14

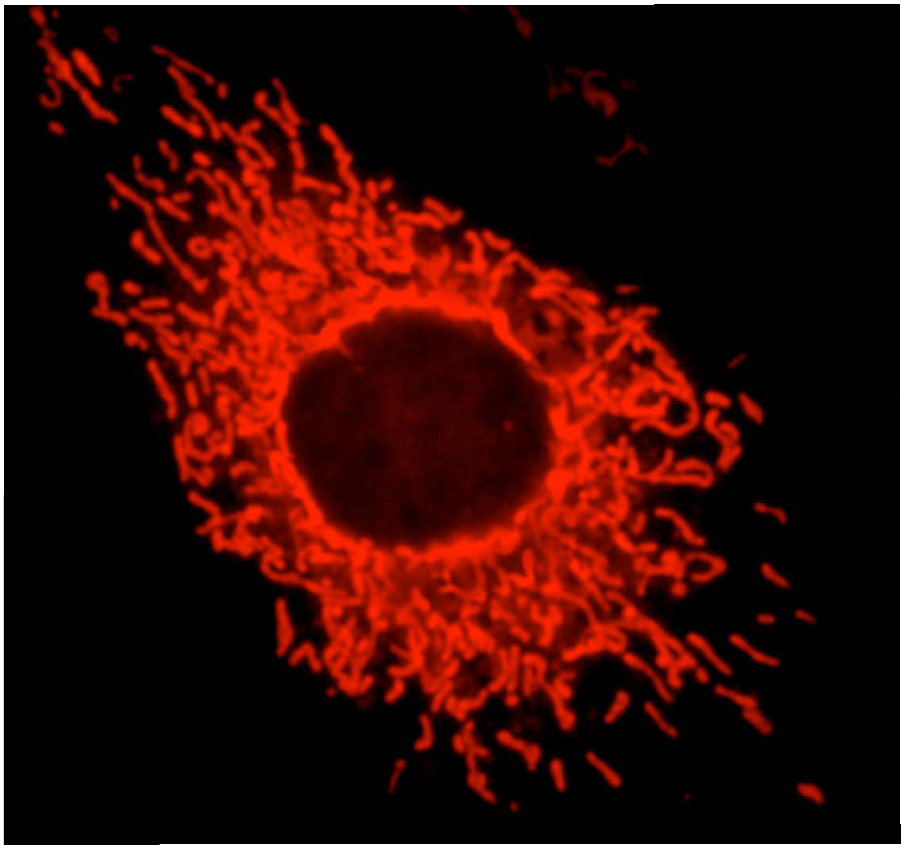
Energy Conversion: Mitochondria and Chloroplasts

IN THIS CHAPTER

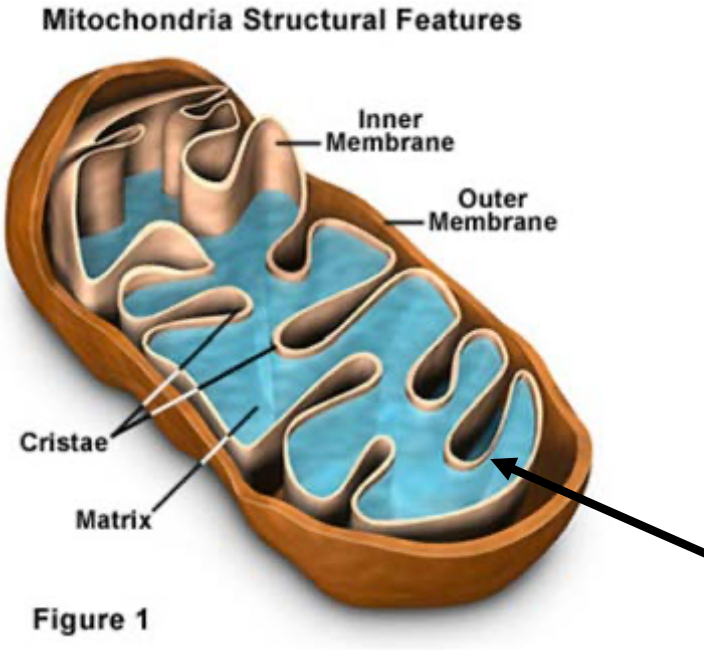
CHAPTER 18

Cell Death

Mitochondrion



ATP generation
Double membrane structure
Network-like but not continuous



Location of mitochondria

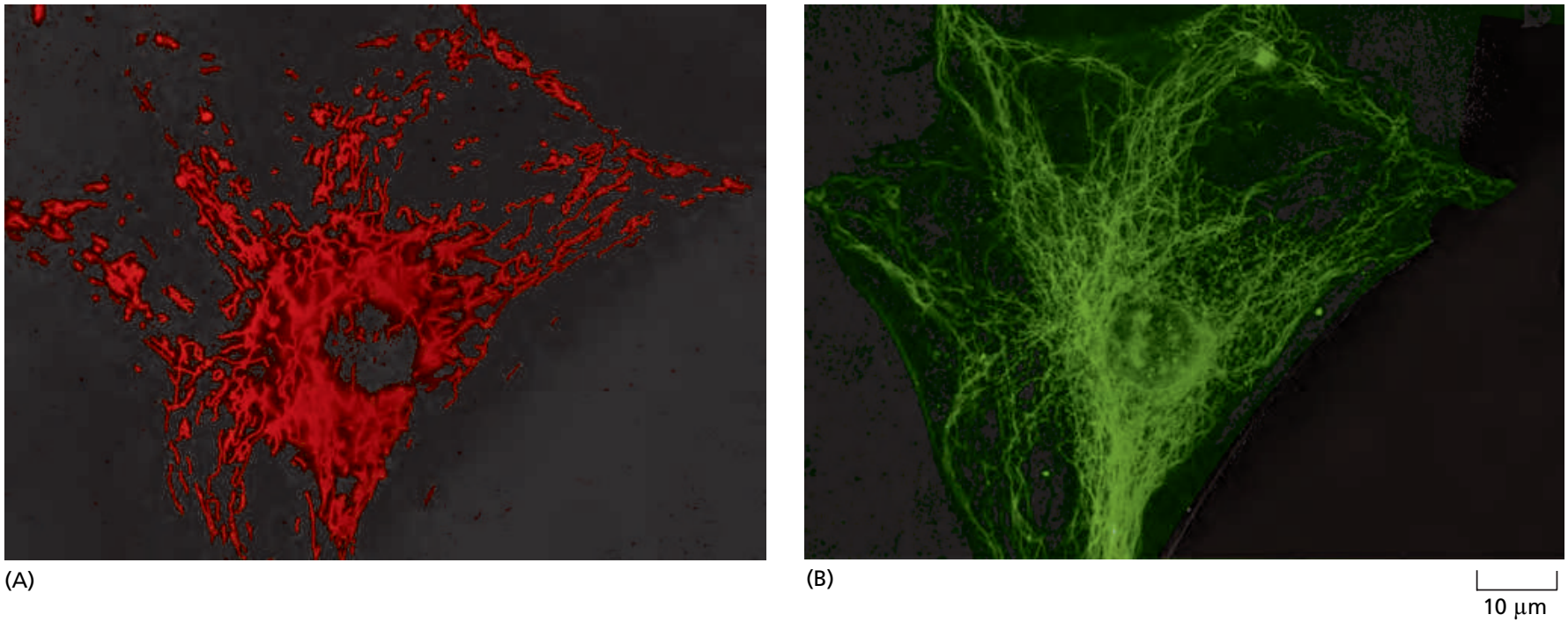


Figure 14–5 The relationship between mitochondria and microtubules. (A) A light micrograph of chains of elongated mitochondria in a living mammalian cell in culture. The cell was stained with a fluorescent dye (rhodamine 123) that specifically labels mitochondria in living cells. (B) An immunofluorescence micrograph of the same cell stained (after fixation) with fluorescent antibodies that bind to microtubules. Note that the mitochondria tend to be aligned along microtubules. (Courtesy of Lan Bo Chen.)

Location of mitochondria near site of large ATP use

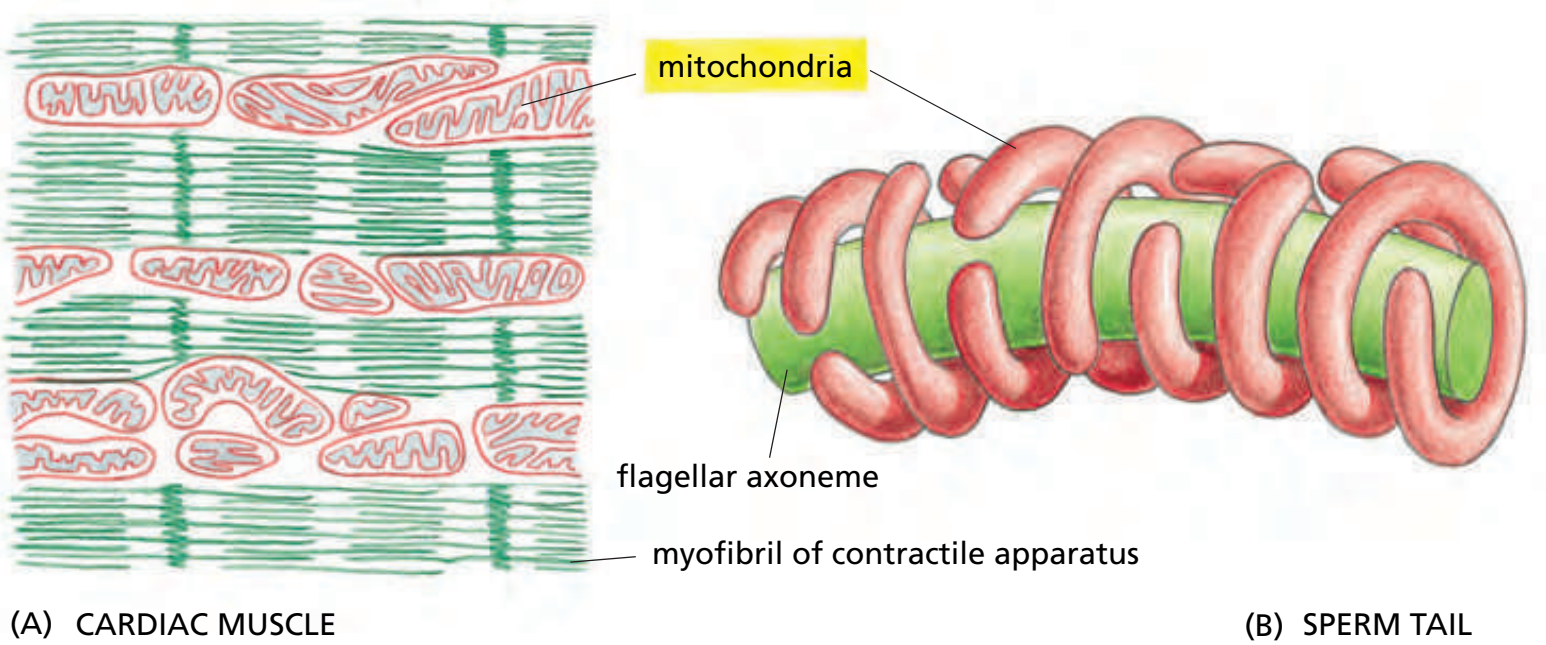
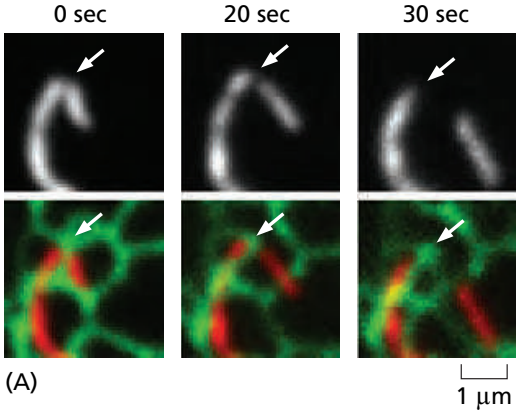


Figure 14–6 Localization of mitochondria near sites of high ATP demand in cardiac muscle and a sperm tail. (A) Cardiac muscle in the wall of the heart is the most heavily used muscle in the body, and its continual contractions require a reliable energy supply. It has limited built-in energy stores and has to depend on a steady supply of ATP from the copious mitochondria aligned close to the contractile myofibrils (see Figure 16–32). (B) During sperm development, microtubules wind helically around the flagellar axoneme, where they are thought to help localize the mitochondria in the tail to produce the structure shown.

Association with the ER



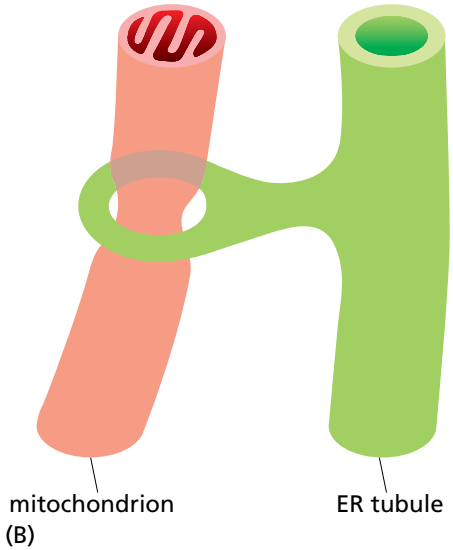


Figure 14–7 Interaction of mitochondria with the endoplasmic reticulum. (A) Fluorescence light microscopy shows that tubules of the ER *(green)* wrap around parts of the mitochondrial network *(red)* in mammalian cells. The mitochondria then divide at the contact sites. After contact is established, fission occurs within less than a minute, as indicated by time-lapse microscopy. (B) Schematic drawing of an ER tubule wrapped around part of the mitochondrial reticulum. It is thought that ER–mitochondrial contacts also mediate the exchange of lipids between the two membrane systems. (A, adapted from J.R. Friedman et al., *Science* 334:358–362, 2011.)

Structure of mitochondria

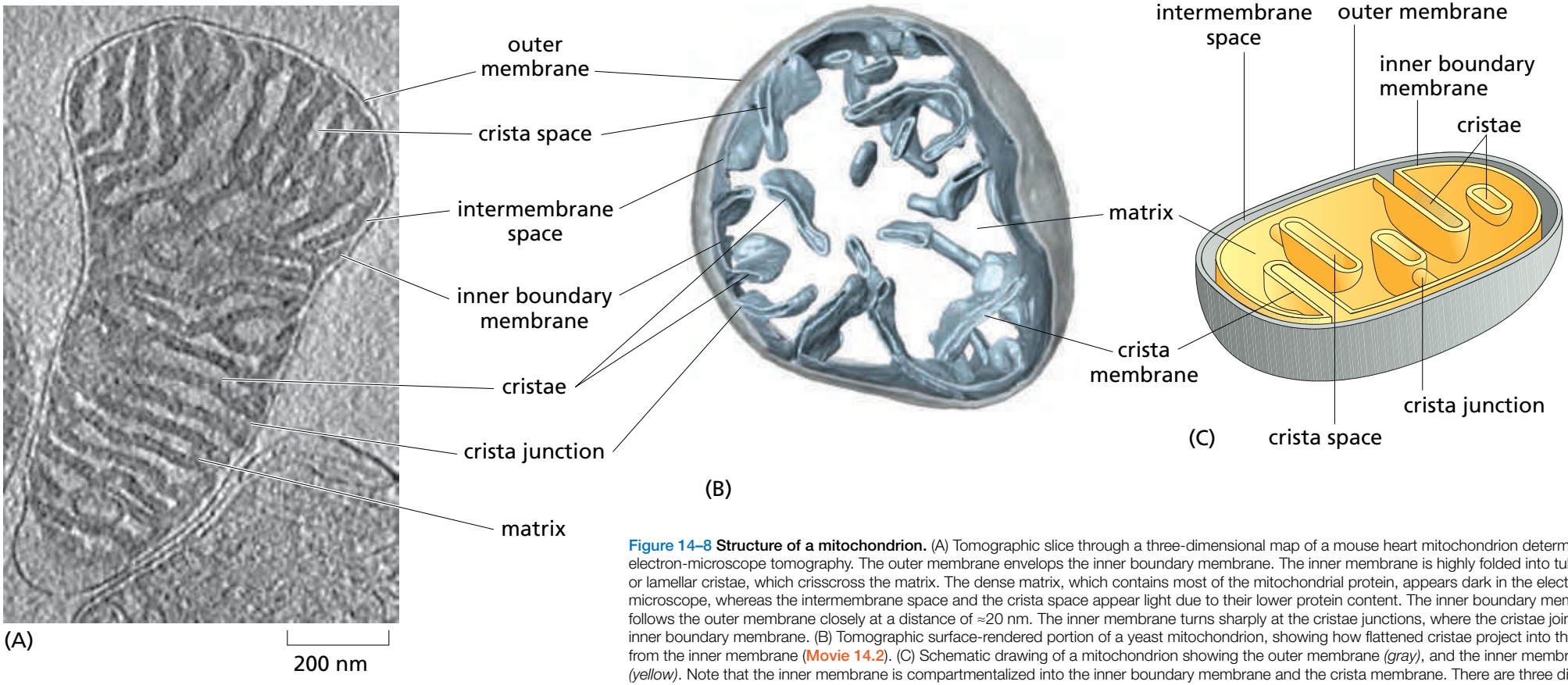


Figure 14–8 Structure of a mitochondrion. (A) Tomographic slice through a three-dimensional map of a mouse heart mitochondrion determined by electron-microscope tomography. The outer membrane envelops the inner boundary membrane. The inner membrane is highly folded into tubular or lamellar cristae, which crisscross the matrix. The dense matrix, which contains most of the mitochondrial protein, appears dark in the electron microscope, whereas the intermembrane space and the crista space appear light due to their lower protein content. The inner boundary membrane follows the outer membrane closely at a distance of ≈20 nm. The inner membrane turns sharply at the cristae junctions, where the cristae join the inner boundary membrane. (B) Tomographic surface-rendered portion of a yeast mitochondrion, showing how flattened cristae project into the matrix from the inner membrane (Movie 14.2). (C) Schematic drawing of a mitochondrion showing the outer membrane *(gray)*, and the inner membrane *(yellow)*. Note that the inner membrane is compartmentalized into the inner boundary membrane and the crista membrane. There are three distinct spaces: the inner membrane space, the crista space, and the matrix. (A, courtesy of Tobias Brandt; B, from K. Davies et al., *Proc. Natl Acad. Sci. USA* 109:13602–13607, 2012. With permission from the National Academy of Sciences.)

Energy generation in the mitochondrion (Recap BIO 110)

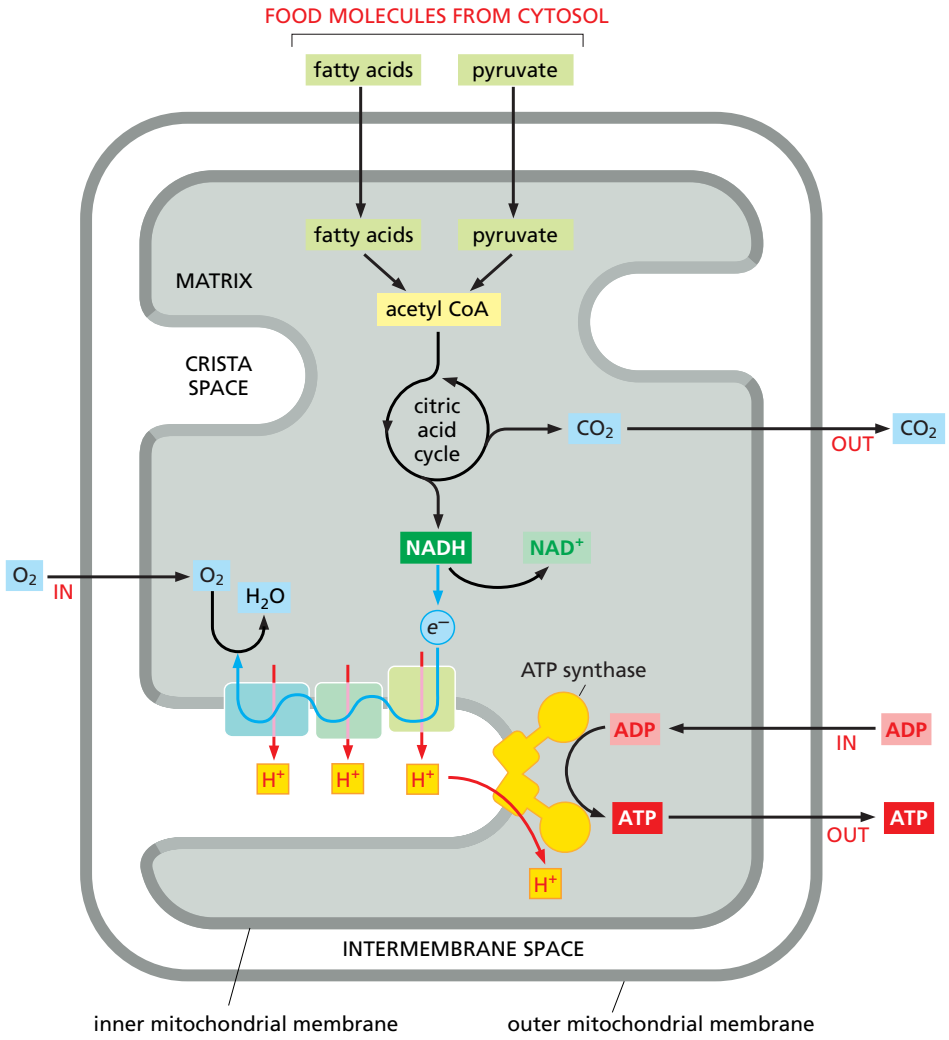


Figure 14–10 A summary of the energy-converting metabolism in mitochondria. Pyruvate and fatty acids enter the mitochondrion *(top of the figure)* and are broken down to acetyl CoA. The acetyl CoA is metabolized by the citric acid cycle, which reduces NAD^+ to NADH, which then passes its high-energy electrons to the first complex in the electron-transport chain. In the process of oxidative phosphorylation, these electrons pass along the electron-transport chain in the inner membrane cristae to oxygen (O_2). This electron transport generates a proton gradient, which drives the production of ATP by the ATP synthase (see Figure 14–3). Electrons from the oxidation of succinate, a reaction intermediate in the citric acid cycle (see Panel 2–9, pp. 106–107), take a separate path to enter this electron-transport chain (not shown, see p. 772).

The membranes that comprise the mitochondrial inner membrane—the inner boundary membrane and the crista membrane—contain different mixtures of proteins and they are therefore shaded differently in this diagram.

Electron transfer through the respiratory-chain

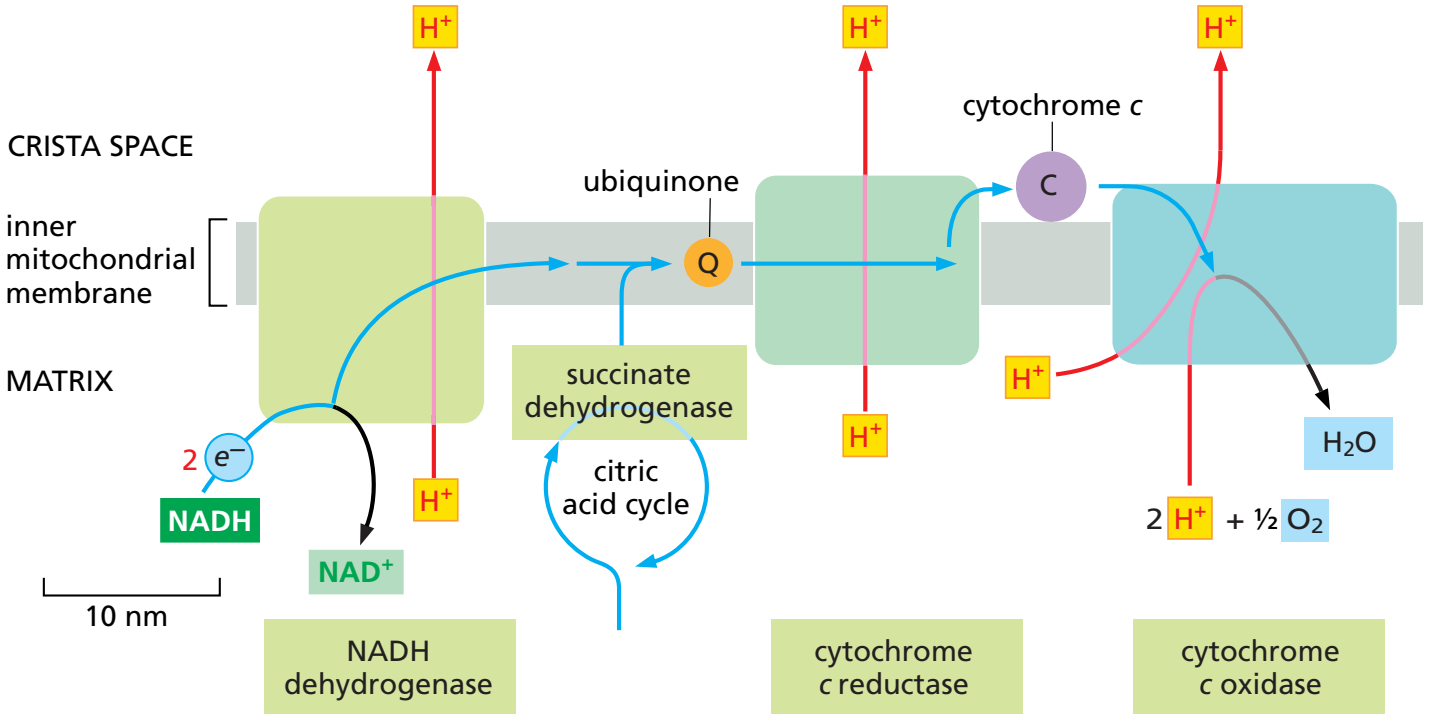


Figure 14–18 The path of electrons through the three respiratory-chain proton pumps. (Movie 14.3) The approximate size and shape of each complex is shown. During the transfer of electrons from NADH to oxygen *(blue arrows)*, ubiquinone and cytochrome *c* serve as mobile carriers that ferry electrons from one complex to the next. During the electron-transfer reactions, protons are pumped across the membrane by each of the respiratory enzyme complexes, as indicated *(red arrows)*.

For historical reasons, the three proton pumps in the respiratory chain are sometimes denoted as Complex I, Complex III, and Complex IV, according to the order in which electrons pass through them from NADH. Electrons from the oxidation of succinate by succinate dehydrogenase (designated as Complex II) are fed into the electron-transport chain in the form of reduced ubiquinone. Although embedded in the crista membrane, succinate dehydrogenase does not pump protons and thus does not contribute to the proton-motive force; it is therefore not considered to be an integral part of the respiratory chain.

Electron transfer through the respiratory-chain

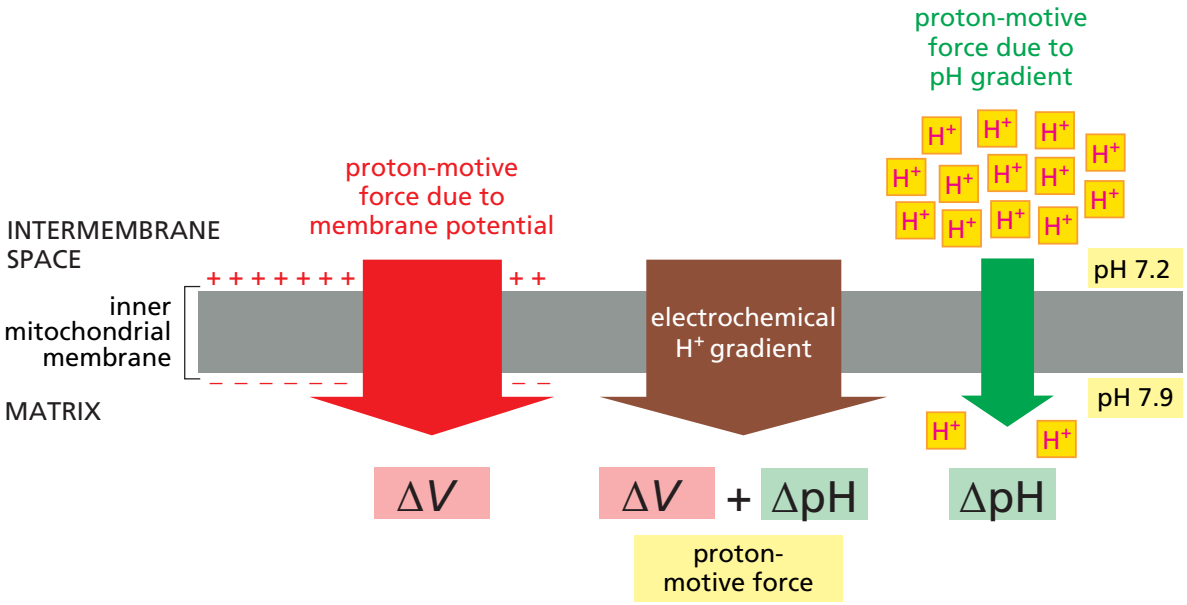


Figure 14–14 The electrochemical proton gradient across the inner mitochondrial membrane. This gradient is composed of a large force due to the membrane potential (ΔV) and a smaller force due to the H^+ concentration gradient—that is, the pH gradient (ΔpH). Both forces combine to generate the proton-motive force, which pulls H^+ back into the mitochondrial matrix. The exact relationship between these forces is expressed by the Nernst equation (see Panel 11–1, p. 616).

ATP synthase structure

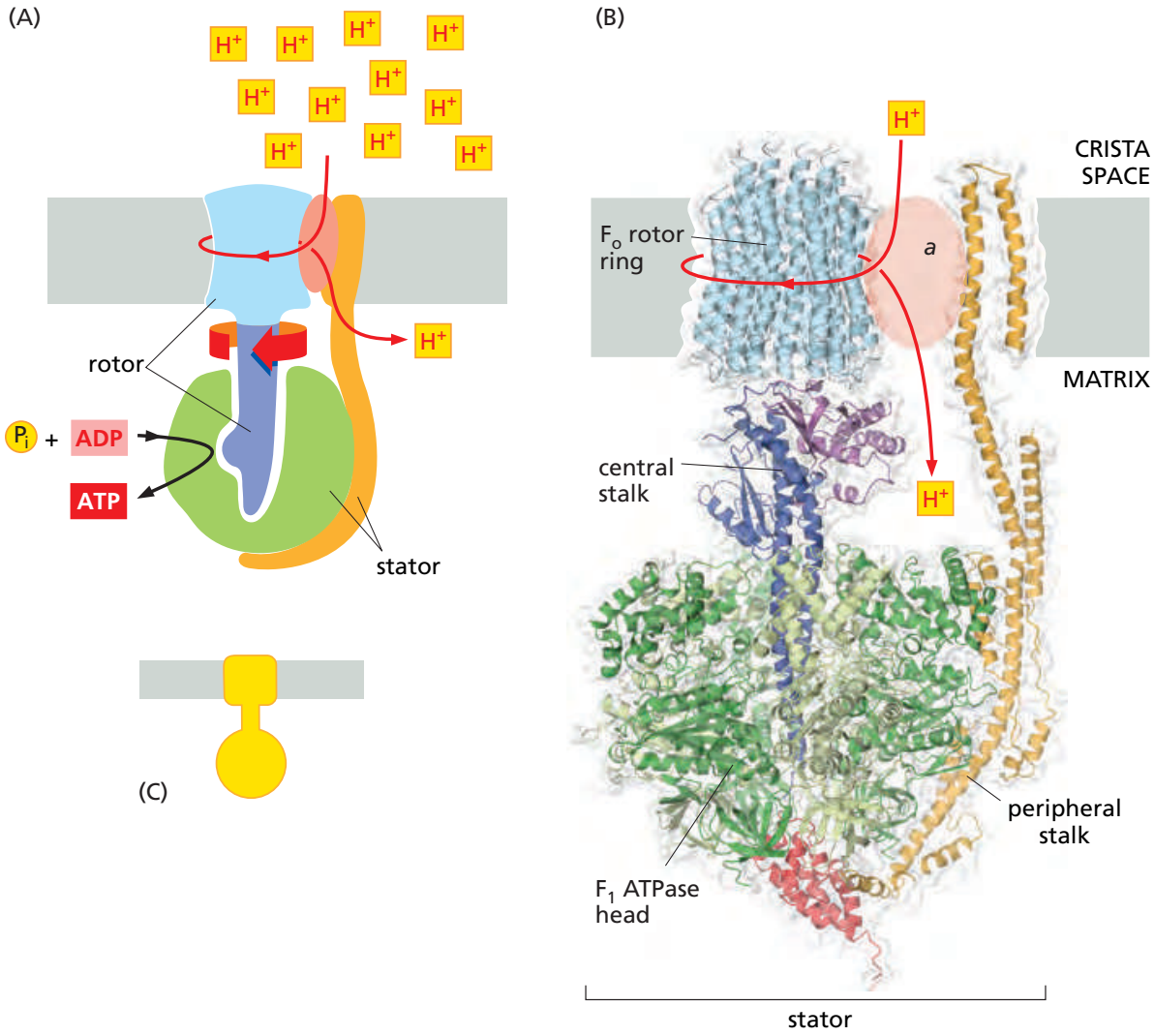


Figure 14–30 ATP synthase. The three-dimensional structure of the F_1F_o ATP synthase, determined by x-ray crystallography. Also known as an F-type ATPase, it consists of an F_o part (from "oligomycin-sensitive factor") in the membrane and the large, catalytic F_1 head in the matrix. Under mild dissociation conditions, this complex separates into its F_1 and F_o components, which can be isolated and studied individually. (A) Diagram of the enzyme complex showing how its globular head portion *(green)* is kept stationary as proton-flow across the membrane drives a rotor *(blue)* that turns inside it. (B) In bovine heart mitochondria, the F_o rotor ring in the membrane *(light blue)* has eight *c* subunits. It is attached to the γ subunit of the central stalk *(dark blue)* by the ε subunit *(purple)*. The catalytic F_1 head consists of a ring of three α and three β subunits *(light and dark green)*, and it directly converts mechanical energy into chemical-bond energy in ATP, as described in the text. The elongated peripheral stalk of the stator *(orange)* is connected to the F_1 head by the small δ subunit *(red)* at one end, and to the *a* subunit in the membrane *(pink oval)* at the other. Together with the *c* subunits of the ring rotating past it, the *a* subunit creates a path for protons through the membrane. (C) The symbol for ATP synthase used throughout this book.

The closely related ATP synthases of mitochondria, chloroplasts, and bacteria synthesize ATP by harnessing the proton-motive force across a membrane. This powers the rotation of the rotor against the stator in a counterclockwise direction, as seen from the F_1 head. The same enzyme complex can also pump protons against their electrochemical gradient by hydrolyzing ATP, which then drives the clockwise rotation of the rotor. The direction of operation depends on the net free-energy change (ΔG) for the coupled processes of H^+ translocation across the membrane and the synthesis of ATP from ADP and P_i (Movie 14.5 and Movie 14.6).

Measurement of the torque that the ATP synthase can produce by ATP hydrolysis reveals that the ATP synthase is 60 times more powerful than a diesel engine of equal dimensions. (B, courtesy of K. Davies. PDB codes: 2WPD, 2CLY, 2WSS, 2BO5.)

Location of the ATP synthase

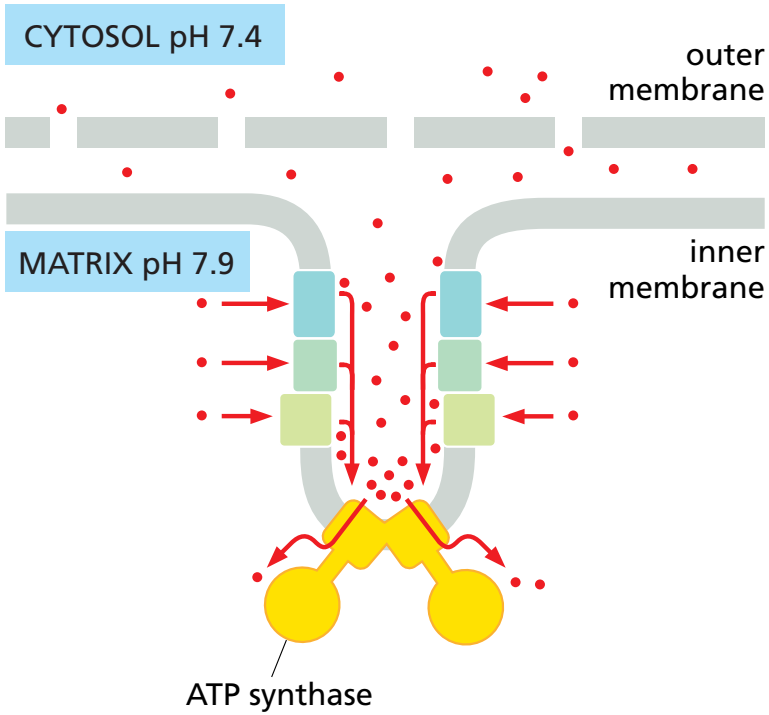


Figure 14–33 ATP synthase dimers at cristae ridges and ATP production. At the crista ridges, the ATP synthases *(yellow)* form a sink for protons *(red)*. The proton pumps of the electron-transport chain *(green)* are located in the membrane regions on either side of the crista. As illustrated, protons tend to diffuse along the membrane from their source to the proton sink created by the ATP synthase. This allows efficient ATP production despite the small H^+ gradient between the cytosol and matrix. *Red arrows* show the direction of the proton flow.

ATP/ADP exchange through a carrier

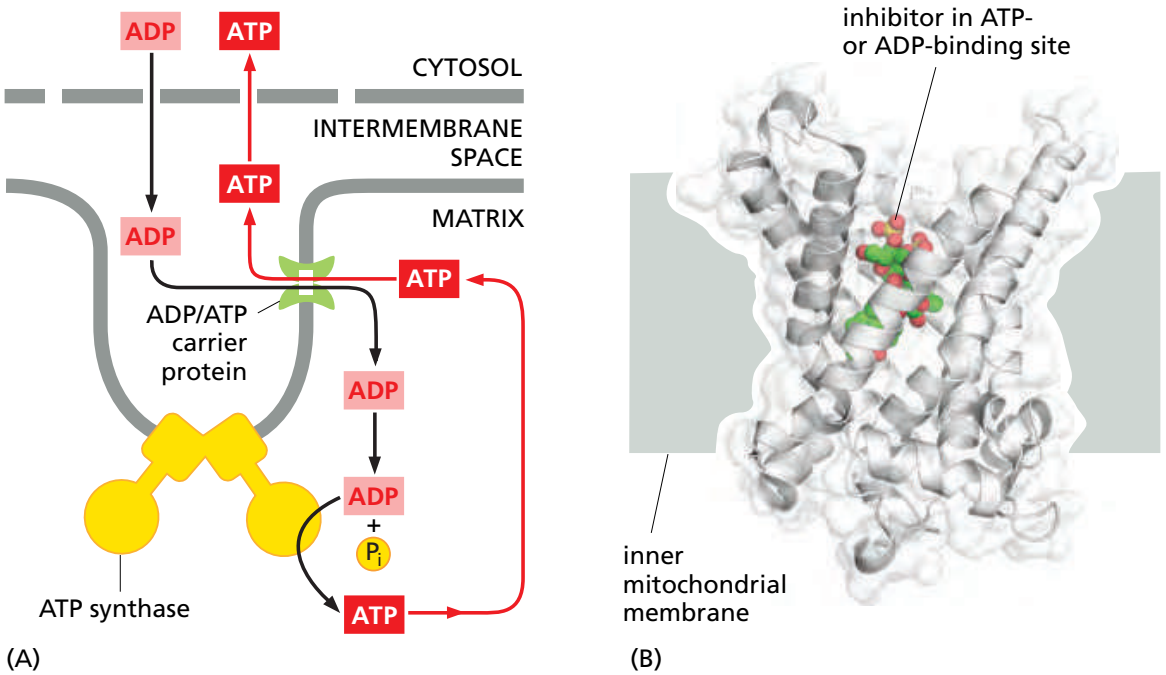


Figure 14–34 The ADP/ATP carrier protein. (A) The ADP/ATP carrier protein is a small membrane protein that carries the ATP produced on the matrix side of the inner membrane to the intermembrane space, and the ADP that is needed for ATP synthesis into the matrix. (B) In the ADP/ATP carrier, six transmembrane α helices define a cavity that binds either ADP or ATP. In this x-ray structure, the substrate is replaced by a tightly bound inhibitor instead *(colored)*. When ADP binds from outside the inner membrane, it triggers a conformational change and is released into the matrix. In exchange, a molecule of ATP quickly binds to the matrix side of the carrier and is transported to the intermembrane space. From there the ATP diffuses through the outer mitochondrial membrane to the cytoplasm, where it powers the energy-requiring processes in the cell. (B, PDB code: 1OKC.)

The mitochondrial genome

The size of the mitochondrial genomes varies between organisms

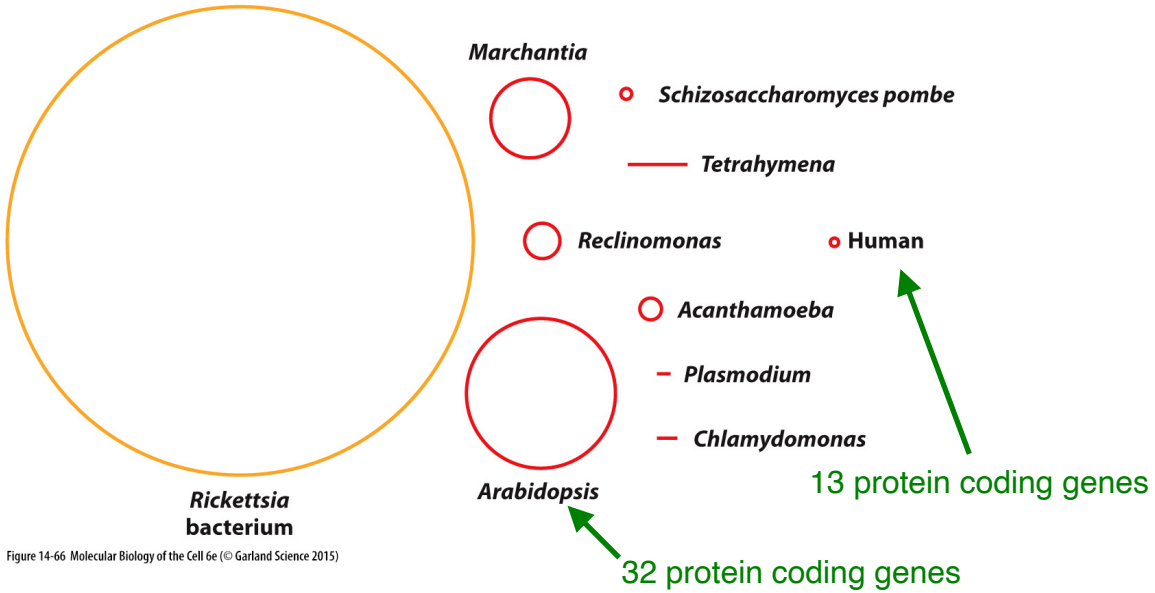


Figure 14-66 Molecular Biology of the Cell 6e (© Garland Science 2015)

Size of the mitochondrial genome in animals: 16500 bp, circular

DNA replication occurs throughout the cell cycle (not in phase with nuclear DNA replication, confined to S phase)

In humans, the genes encode: 2 ribosomal RNAs, 22 transfer RNAs, 13 proteins

The mitochondrial genome varies between organisms

Only five genes are invariably found in mitochondrial DNA
- ribosomal RNA
- cytochrome b (cob)
- cytochrome oxidase (cox)

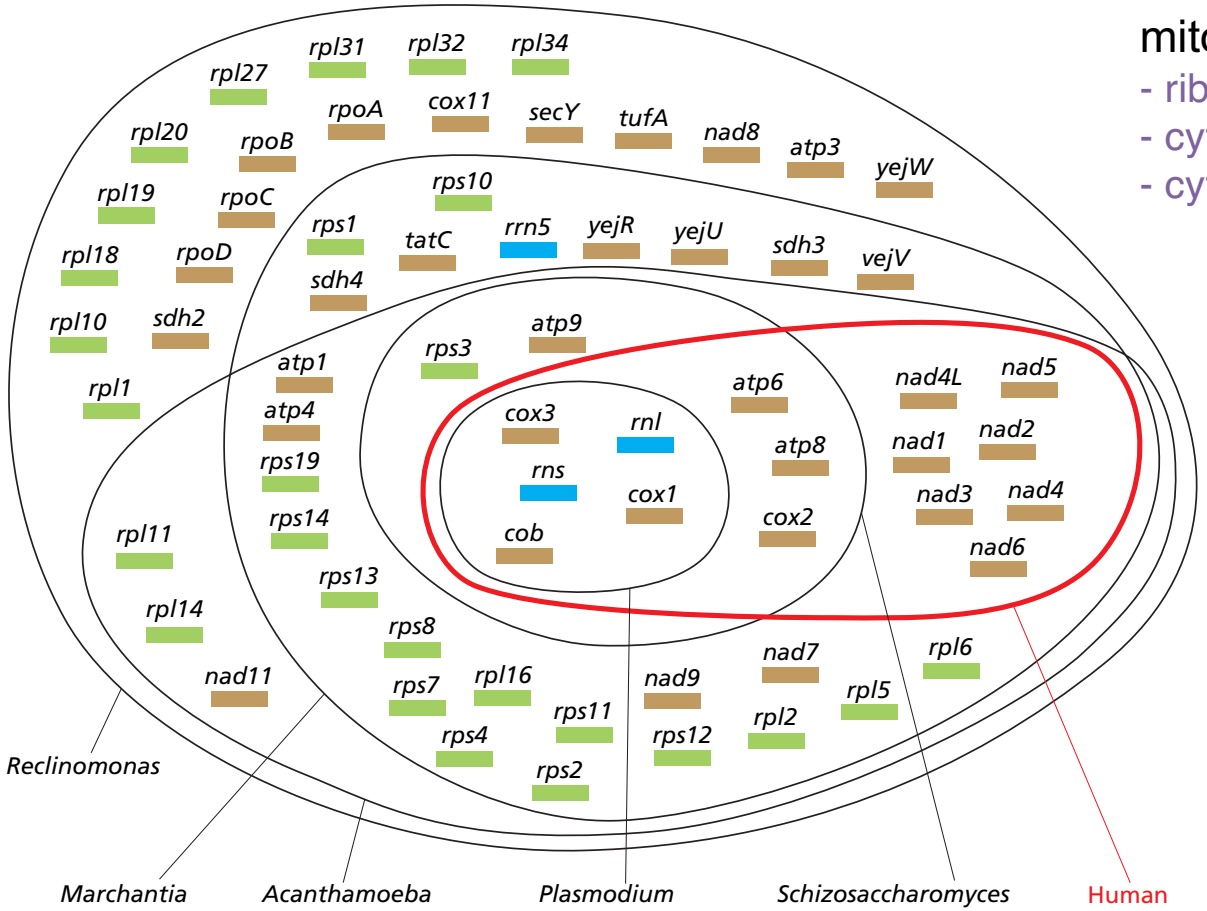


Figure 14–60 Comparison of mitochondrial genomes. Less complex mitochondrial genomes encode subsets of the proteins and ribosomal RNAs that are encoded by larger mitochondrial genomes. In this comparison, there are only five genes that are shared by the six mitochondrial genomes; these encode ribosomal RNAs (*rns* and *rnl*), cytochrome *b* (*cob*), and two cytochrome oxidase subunits (*cox1* and *cox3*). *Blue* indicates ribosomal RNAs; *green*, ribosomal proteins; and *brown*, components of the respiratory chain and other proteins. (Adapted from M.W. Gray, G. Burger and B.F. Lang, *Science* 283:1476–1481, 1999.)

The human mitochondrial genome

Protein-coding genes are all implicated in the oxidative phosphorylation
- NADH dehydrogenase: complex I
- Cytochrome b: complex III
- Cytochrome oxidase: complex IV
- ATP synthase

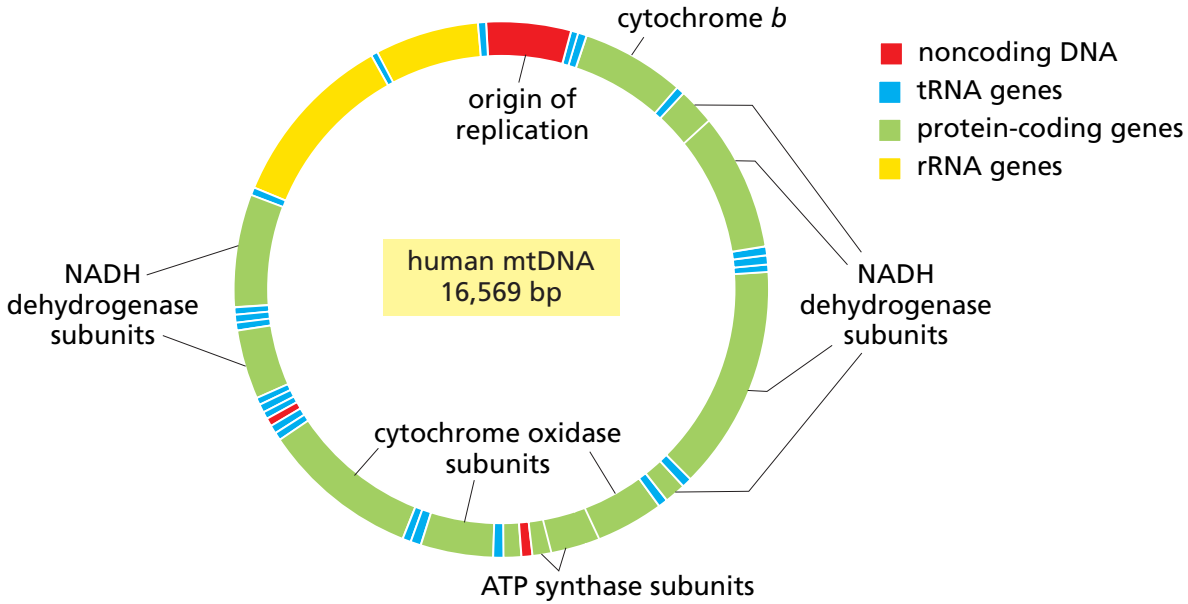


Figure 14–65 The organization of the human mitochondrial genome. The human mitochondrial genome of ≈16,600 nucleotide pairs contains 2 rRNA genes, 22 tRNA genes, and 13 protein-coding sequences. There are two transcriptional promoters, one for each strand of the mitochondrial DNA (mtDNA). The DNAs of many other animal mitochondrial genomes have been completely sequenced. Most of these animal mitochondrial DNAs encode precisely the same genes as humans, with the gene order being identical for animals ranging from fish to mammals.

Mitochondrial DNA is read differently!

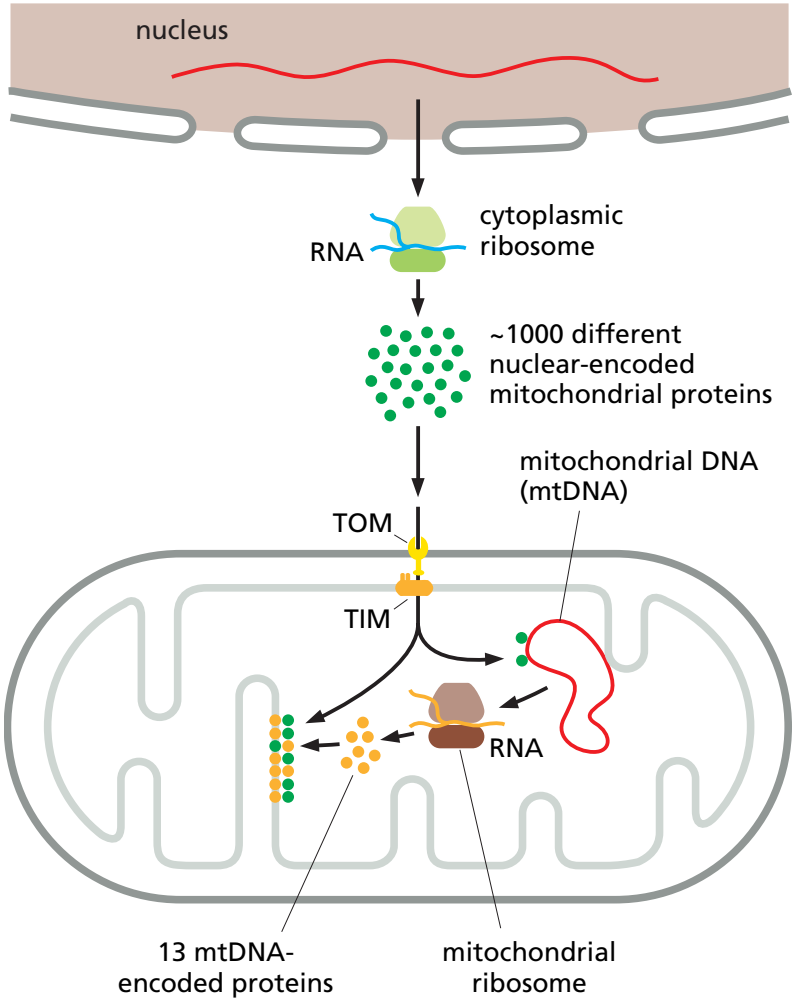
TABLE 14–3 Some Differences Between the "Universal" Code and Mitochondrial Genetic Codes*

Codon	"Universal" code	Mitochondrial codes			
		Mammals	Invertebrates	Yeasts	Plants
UGA	STOP	*Trp*	*Trp*	*Trp*	STOP
AUA	Ile	*Met*	*Met*	*Met*	Ile
CUA	Leu	Leu	Leu	*Thr*	Leu
AGA AGG	Arg	*STOP*	*Ser*	Arg	Arg

**Red* italics indicate that the code differs from the "Universal" code.

Protein localization to the mitochondrion

Mitochondrial protein import and local production



Most mitochondrial proteins are imported from the cytosol

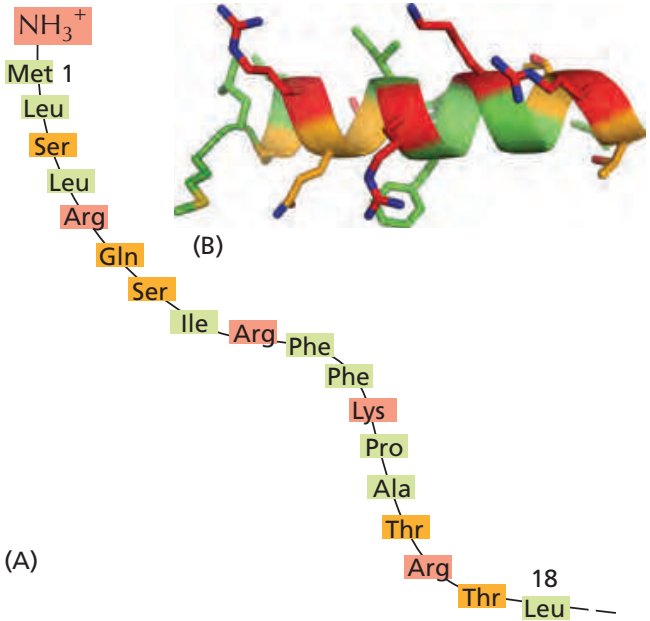
- Proteins are made in the cytosol, then translocated

- Proteins imported into the matrix space usually possess an N-terminal signal sequence, which is removed by a signal peptidase after import

- The other proteins (destined to outer or inner membrane, or intermembrane space) have an internal signal sequence that is usually not removed

translation of mitochondrial protein precursor in cytosol → post-translational import into mitochondria

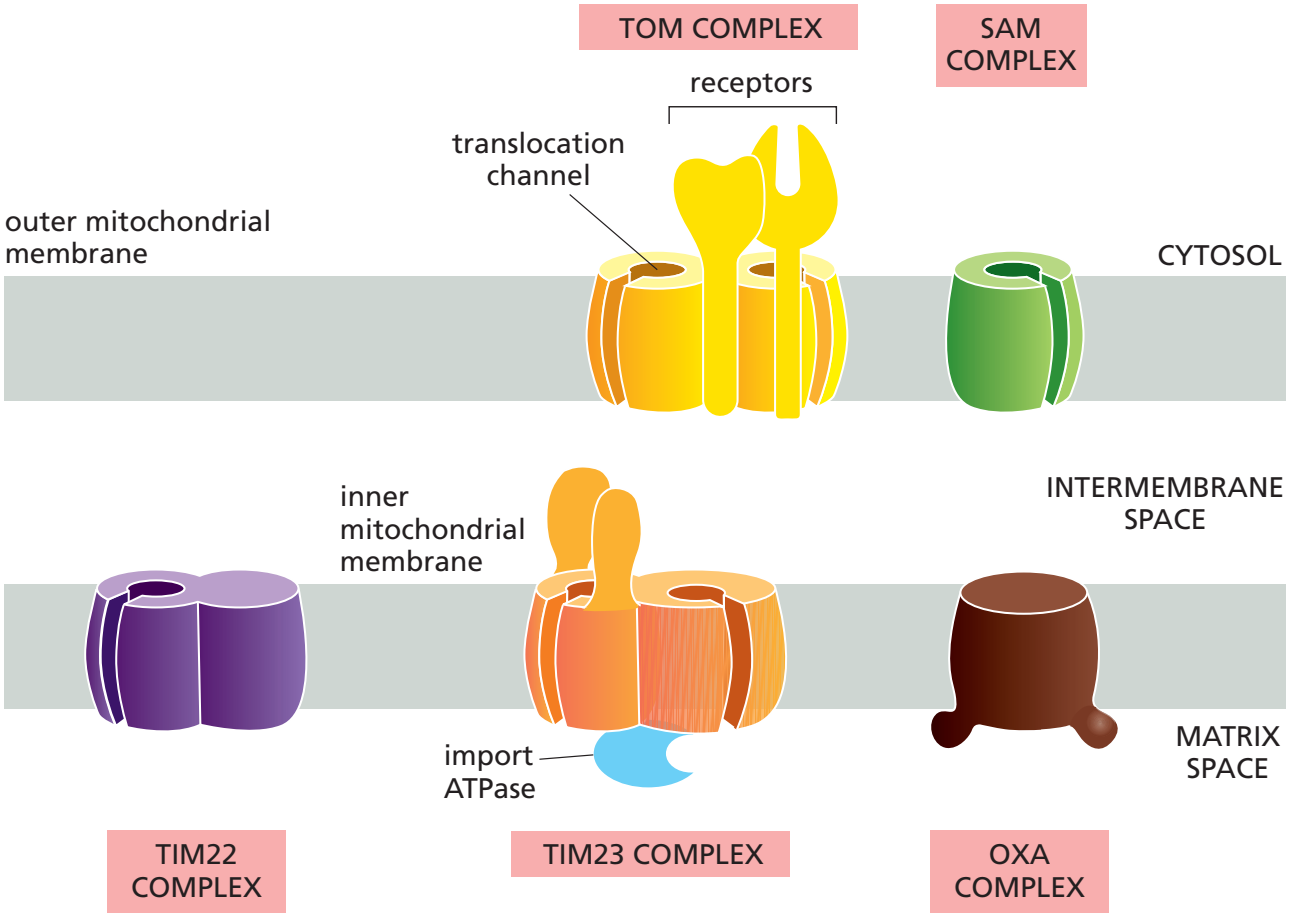
A signal sequence for mitochondrial protein import



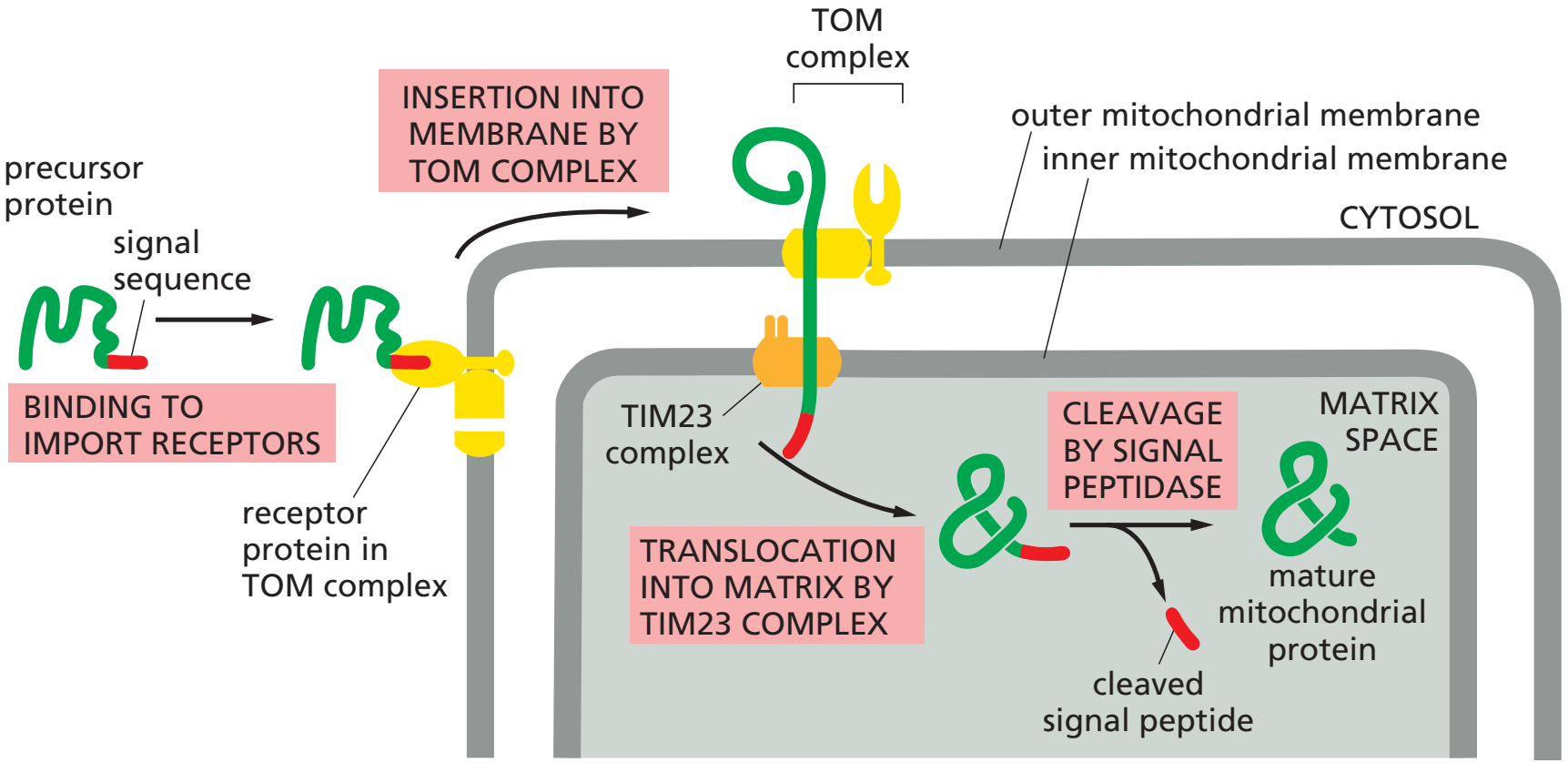
Cytochrome Oxidase Multi protein complex located in inner mitochondrial membrane. Terminal enzyme electron transport chain (Chapter 14 MCB)

First 18 a.a. are the signal locating to protein the mitochondrion

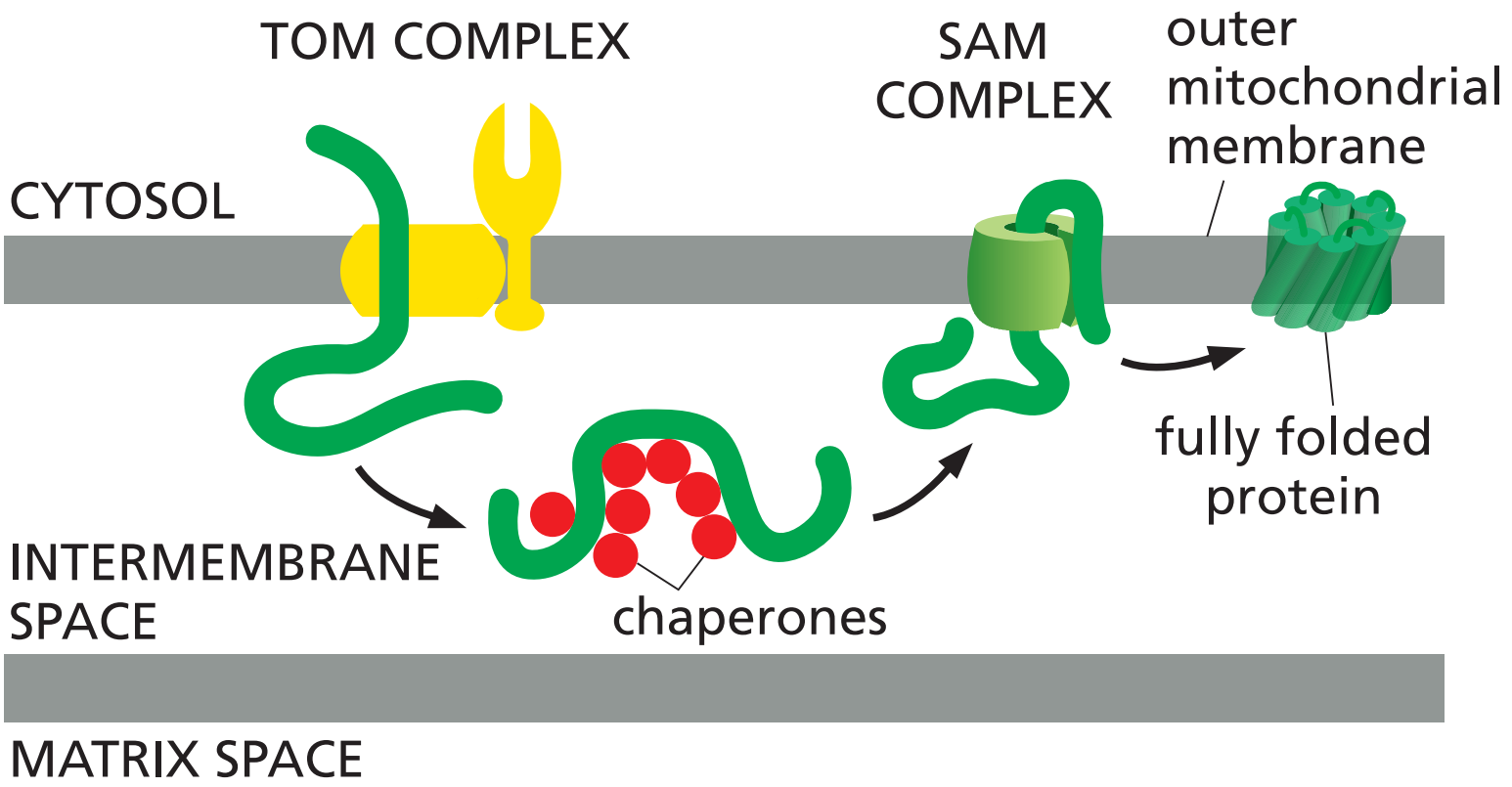
The protein translocators in the mitochondrial membranes



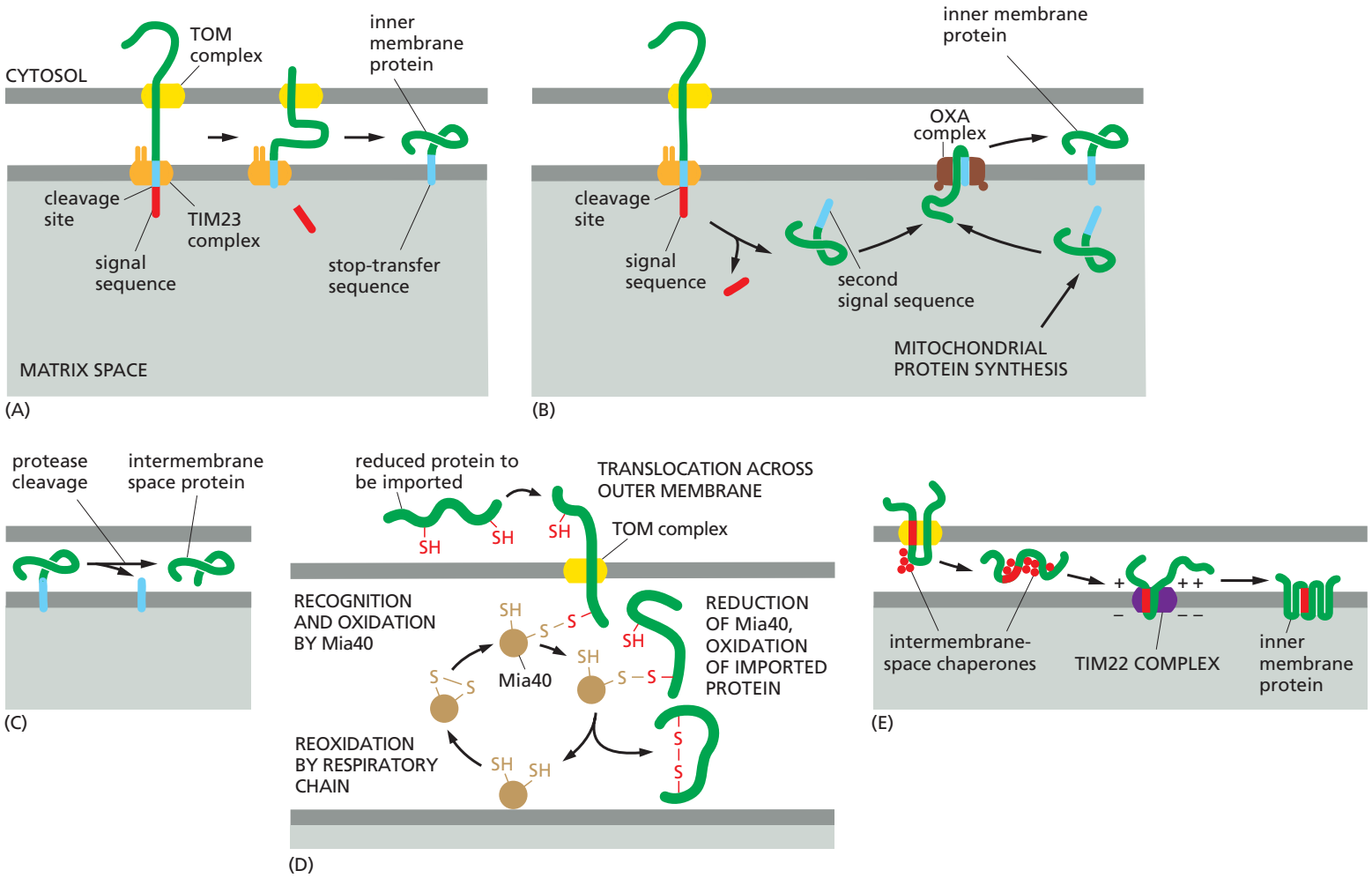
Protein import by mitochondria



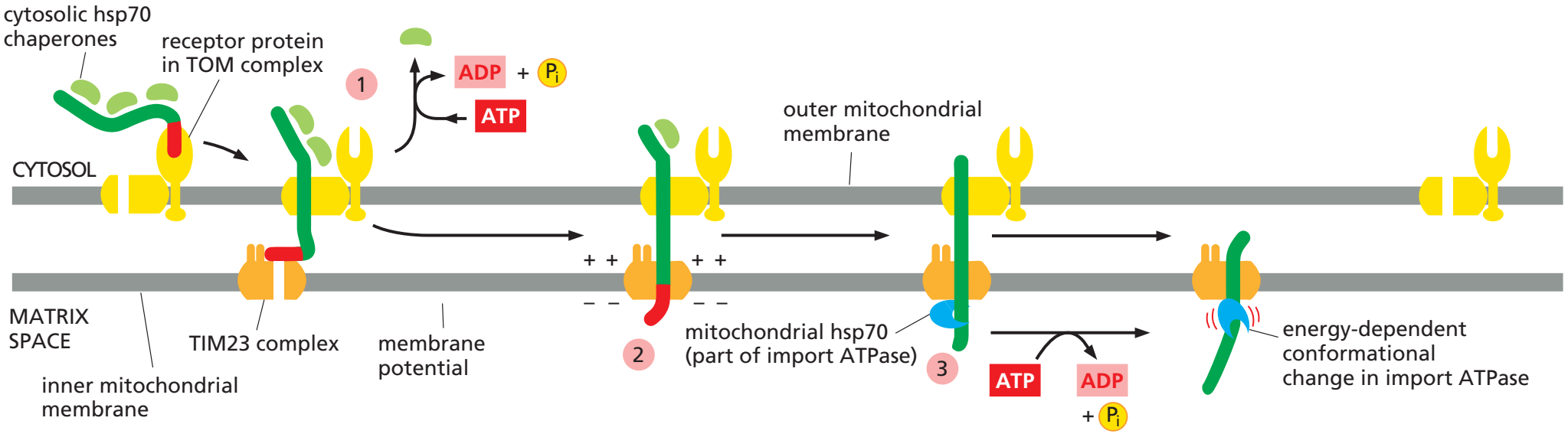
Integration of porins into the outer mitochondrial membrane



Protein import from the cytosol into the inner mitochondrial membrane and intermembrane space



The role of energy in protein import into the mitochondrial matrix space



Mitochondrion fission and fusion

Mitochondrial dynamics

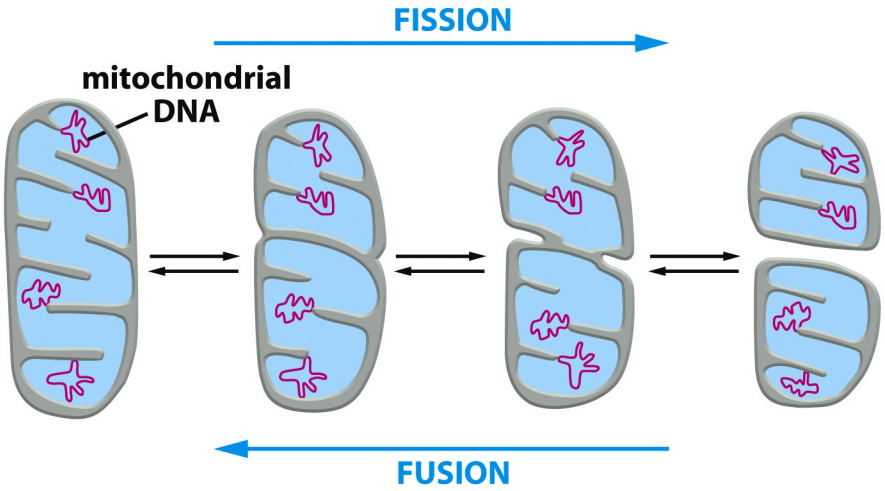


Figure 14-56a *Molecular Biology of the Cell* (© Garland Science 2008)

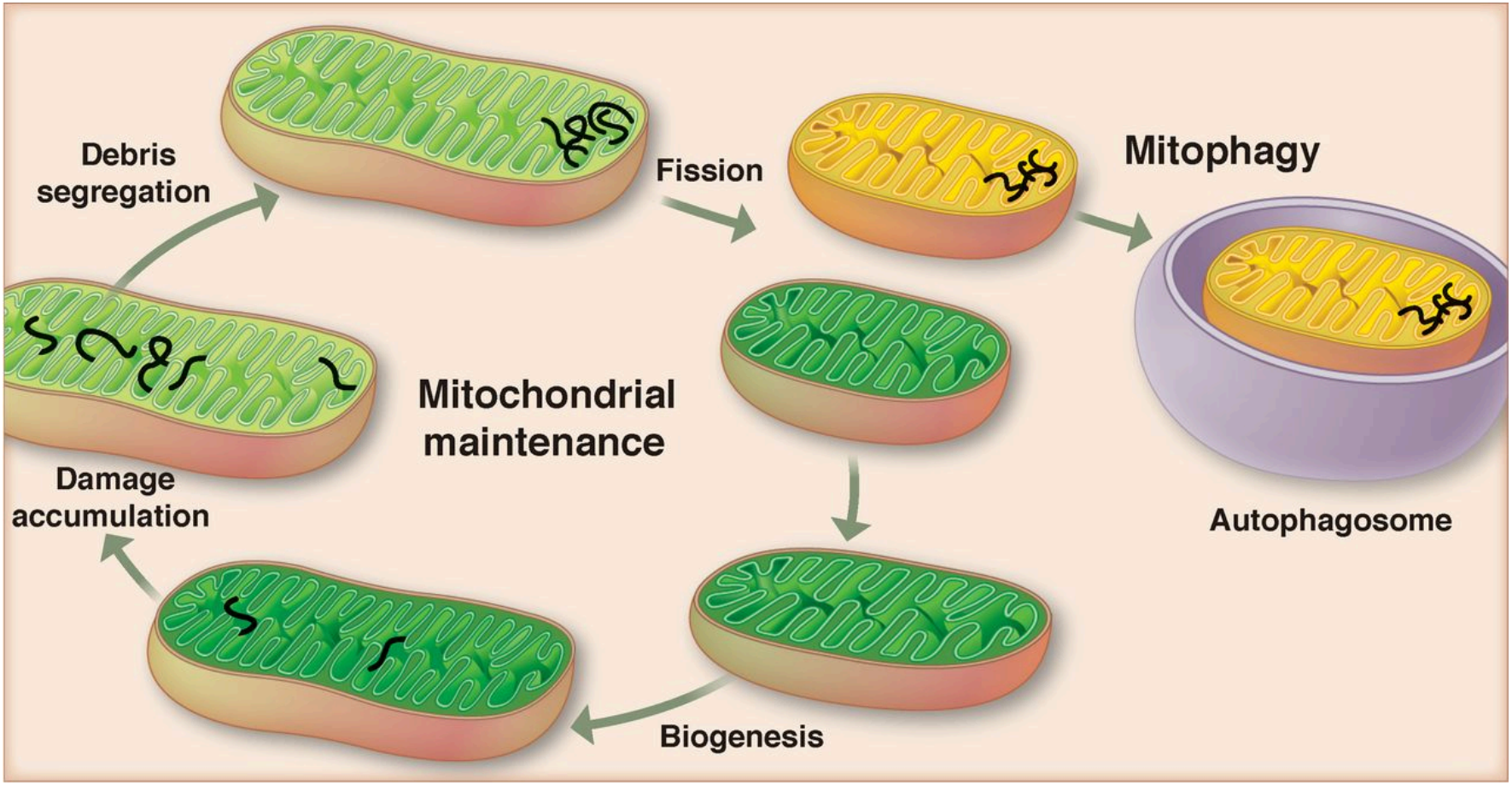
- Mitochondria arise from the division of an existing mitochondrion

- Mitochondrial transport is required to distribute mitochondria where they are needed. Ex: in neurons, they are recruited to regions with high energy demands.

- Mitochondrial length, shape, size and number are controlled by fusion and fission

- Fission and fusion are complex, because of the double membrane, and because integrity of the separate mitochondrial compartments must be maintained

Fission followed by autophagy gets rid of damaged mitochondria



R J Youle, A M van der Bliek Science 2012;337:1062-1065

Article

Distinct fission signatures predict mitochondrial degradation or biogenesis

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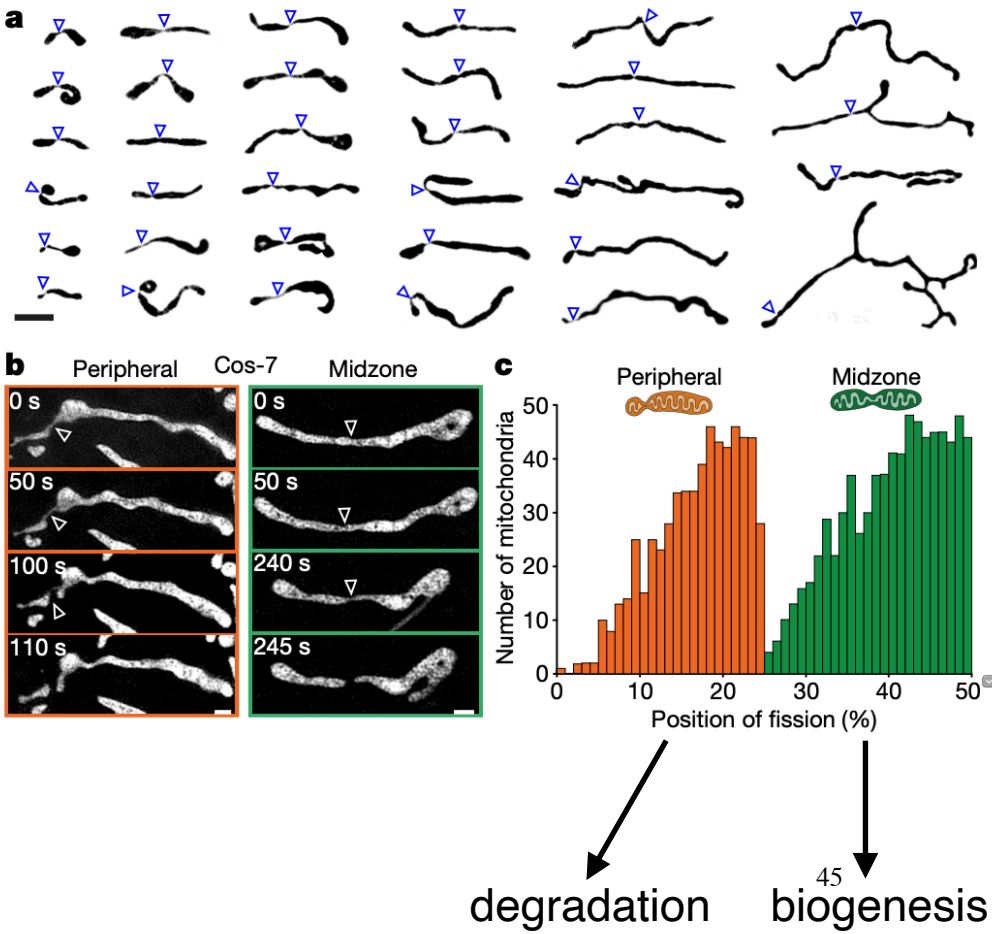
Tatjana Kleele[1✉], Timo Rey[1], Julius Winter[1], Sofia Zaganelli[1], Dora Mahecic[1], Hélène Perreten Lambert[1], Francesco Paolo Ruberto[2], Mohamed Nemir[2], Timothy Wai[3], Thierry Pedrazzini[2] & Suliana Manley[1✉]

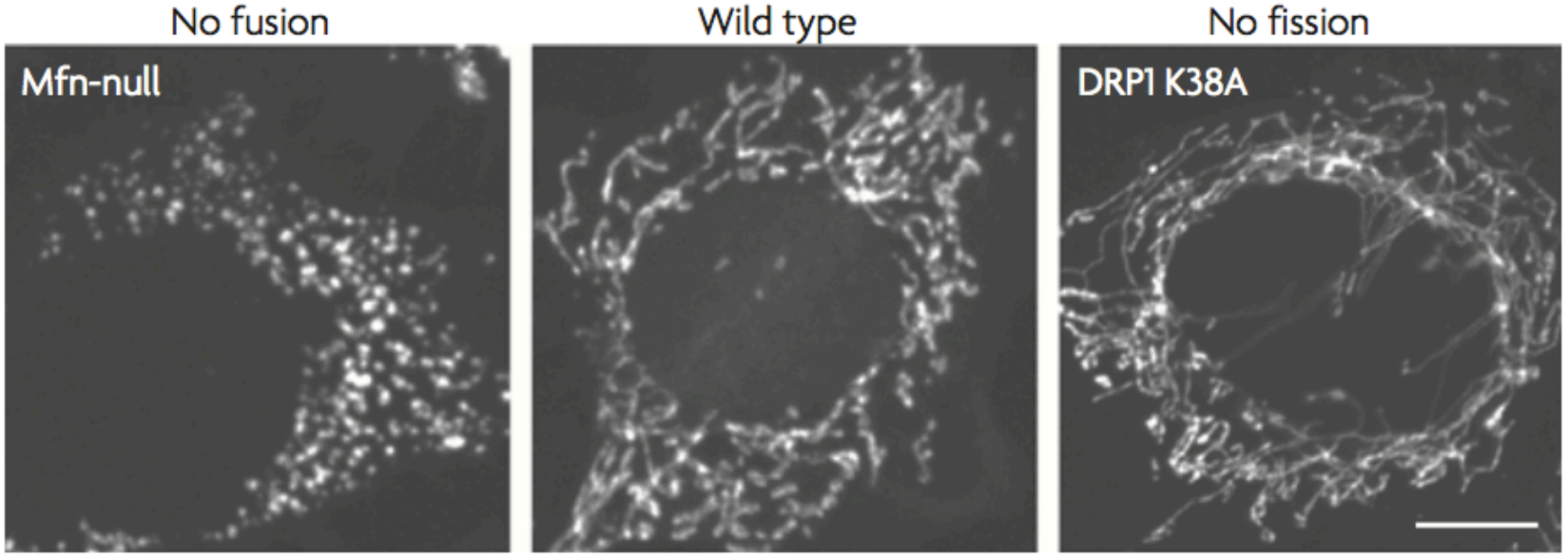


Phenotypes resulting from unbalanced fission-fusion

Mammalian cells

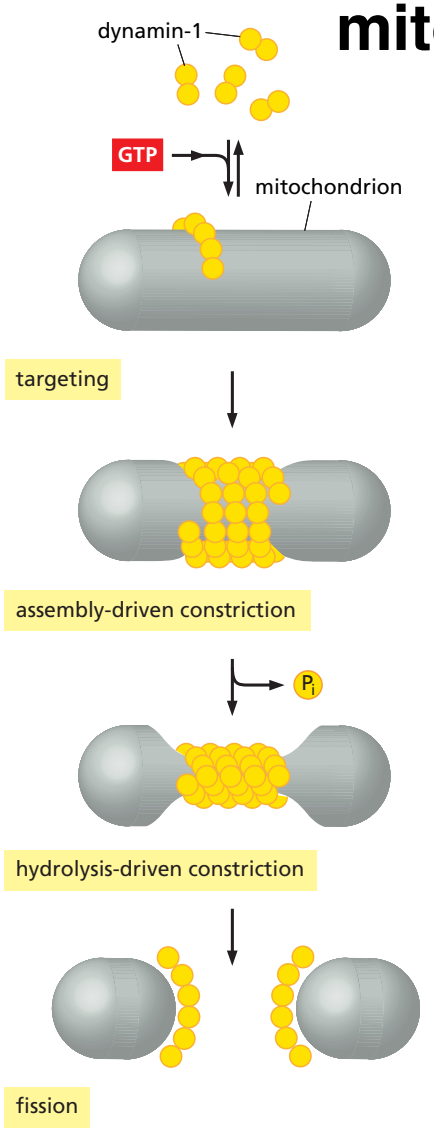
Mitofusin (Mfn) controls fusion

dynamin-related protein 1 (DRP1) controls fission



Functions and dysfunctions of mitochondrial dynamics. Scott A. Detmer & David C. Chan. Nature Reviews Molecular Cell Biology 8, 870-879 (2007)

mitochondrial fission driven by Dynamin-1



Recruitment of dynamin-1 (DRP1) from cytosol to mitochondrial outer membrane

oligomerization into ring- and spiral-like structures, which wrap around the scission site – this process is GTP- hydrolysis dependent.
This constricts the mitochondria

The fission of other organelles ex. peroxisomes is similar

Figure 14–63 A model for mitochondrial division. Dynamin-1 *(yellow)* exists as dimers in the cytosol, which form larger oligomeric structures in a process that requires GTP hydrolysis. Dynamin assemblies interact with the outer mitochondrial membrane through special adaptor proteins, forming a spiral of GTP-dynamin around the mitochondrion that causes a constriction. A concerted GTP-hydrolysis event in the dynamin subunits is then thought to produce the conformational changes that result in fission. (Adapted from S. Hoppins, L. Lackner and J. Nunnari, *Annu. Rev. Biochem.* 76:751–780, 20

mitochondrial fusion

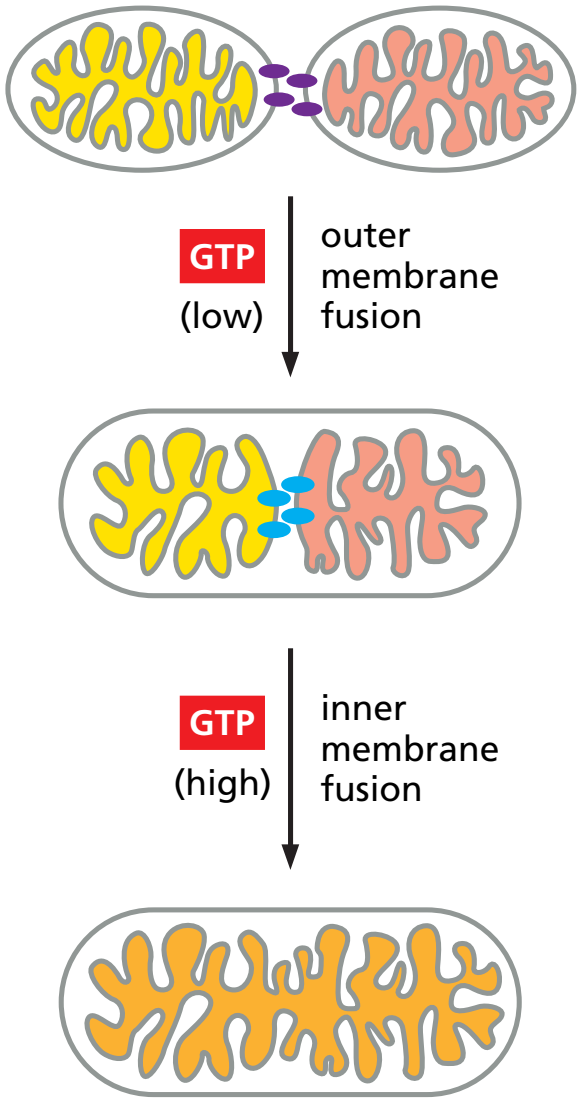
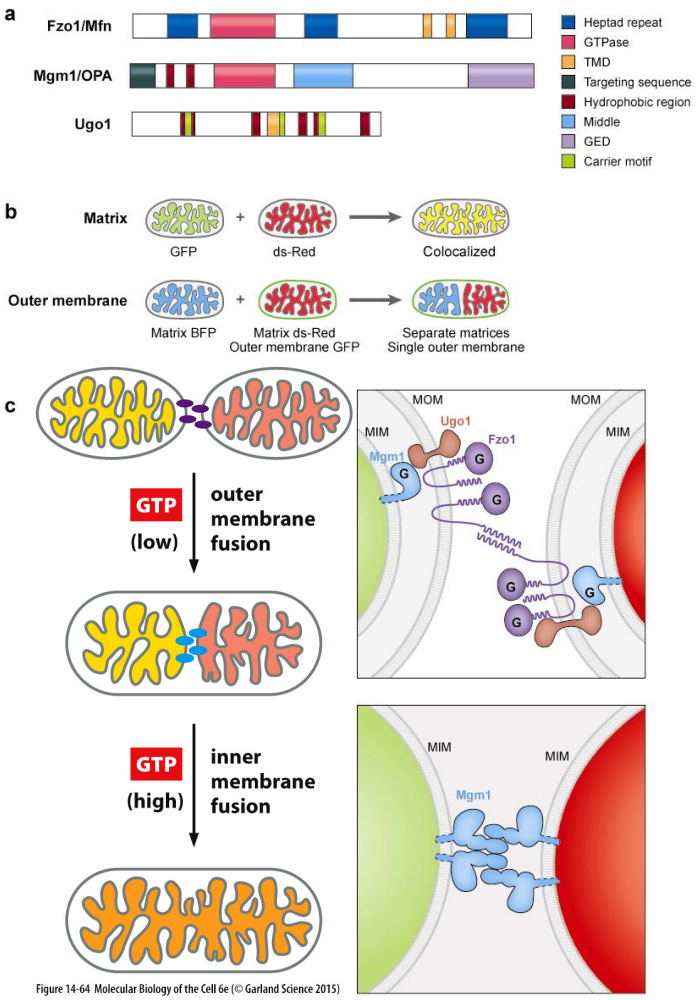


Figure 14–64 A model for mitochondrial fusion. The fusions of the outer and inner mitochondrial membranes are coordinated sequential events, each of which requires a separate set of protein factors. Outer membrane fusion is brought about by an outer-membrane GTPase *(purple)*, which forms an oligomeric complex that includes subunits anchored in the two membranes to be fused. Fusion of outer membranes requires GTP and an H^+ gradient across the inner membrane. For fusion of the inner membrane, a dynamin-related protein forms an oligomeric tethering complex *(blue)* that includes subunits anchored in the two inner membranes to be fused. Fusion of the inner membranes requires GTP and the electrical component of the potential across the inner membrane. (Adapted from S. Hoppins, L. Lackner and J. Nunnari, *Annu. Rev. Biochem*. 76:751–780, 2007.)

Mechanisms of mitochondrial fusion

Mfn = mitofusin
Opa = optic atrophy protein

MOM: mitochondrial outer membrane
MIM: mitochondrial inner membrane

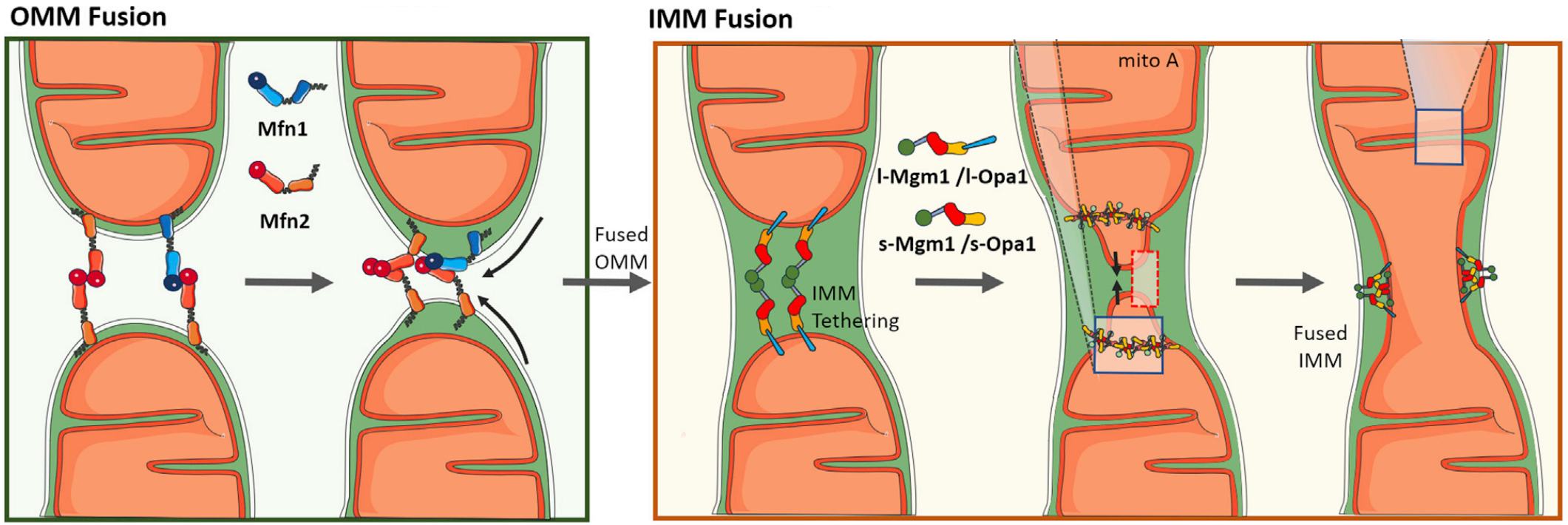


Hoppins S, et al. 2007.
Annu. Rev. Biochem. 76:751–80

Fusion of MOM is mediated by both Mfn1 and Mfn2; they form oligomers between mitochondria (in *trans*) to initiate fusion.

Fusion of MIM is mediated by Opa1

mitochondrial fusion



Mitochondrion

- Shape & Location
- ATP production
- Mitochondrial genome
- Protein translocation
- Fusion and Fission

Cell death

Cell death

Programmed cell death

- Apoptosis ->
  - Intrinsic regulation: Mitochondria
  - Extrinsic regulation: cell signalling
- Phagocytosis
- *Ferroptosis*
- *Necroptosis (Phenotypically similar to necrosis, but the induction is controlled)*
- *Pyroptosis (during infection)*
- *Cornification (Skin keratinization)*
- *…*

Unprogrammed

- Necrosis

nature reviews molecular cell biology
https://doi.org/10.1038/s41580-023-00689-6

Review article

Check for updates

A guide to cell death pathways

Junying Yuan [1,2] & Dimitry Ofengeim [3]

Distinct forms of cell death

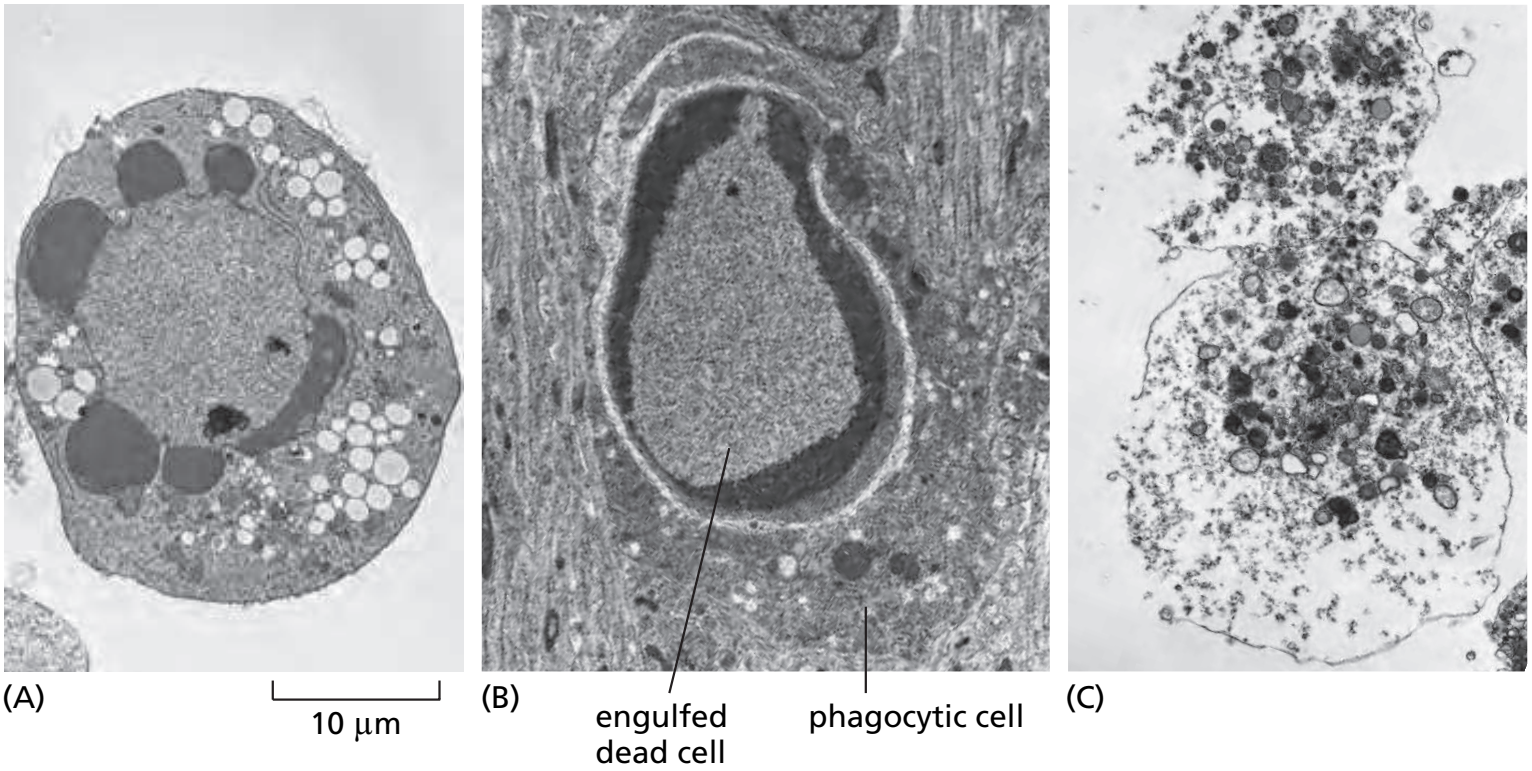
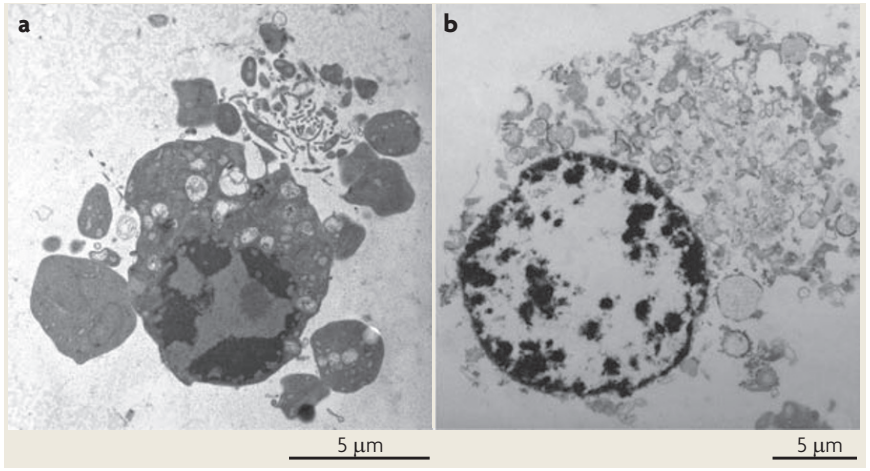


Figure 18–1 Two distinct forms of cell death. These electron micrographs show cells that have died by apoptosis (A and B) or by necrosis (C). The cells in (A) and (C) died in a culture dish, whereas the cell in (B) died in a developing tissue and has been engulfed by a phagocytic cell. Note that the cells in (A) and (B) have condensed but seem relatively intact, whereas the cell in (C) seems to have exploded. The large vacuoles visible in the cytoplasm of the cell in (A) are a variable feature of apoptosis. (Courtesy of Julia Burne.)

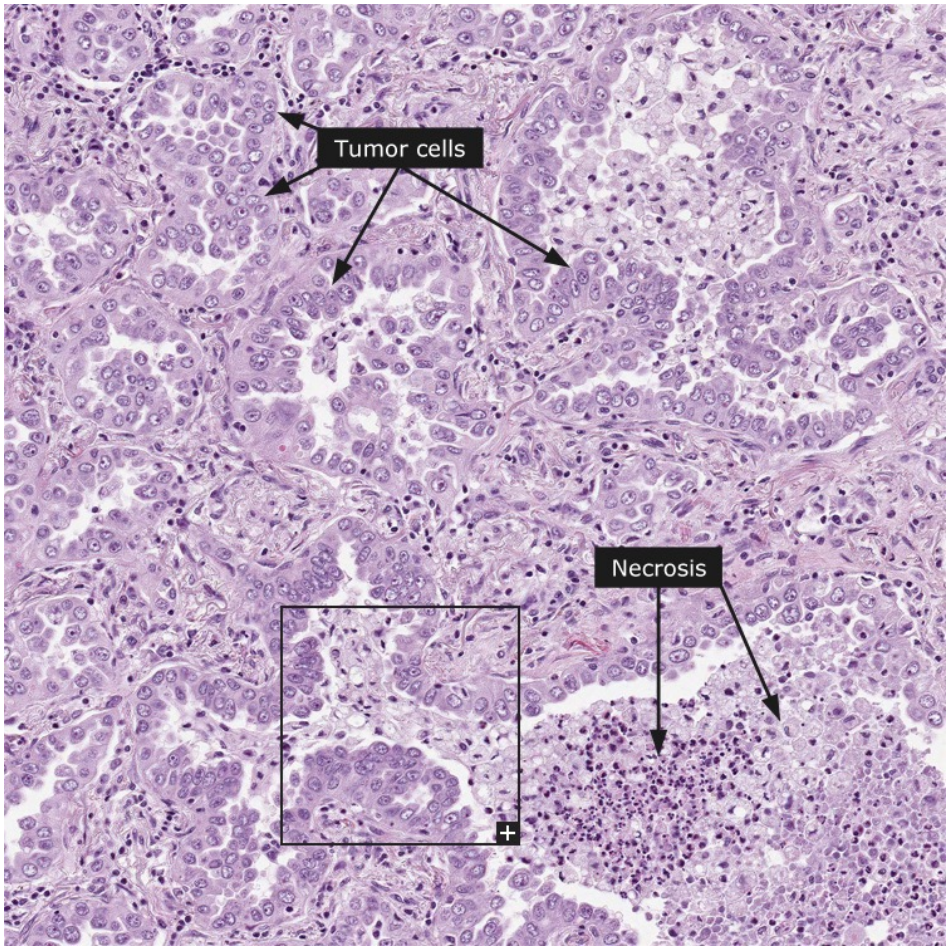
Necrosis (Accidental cell death)

Apoptosis Necrosis



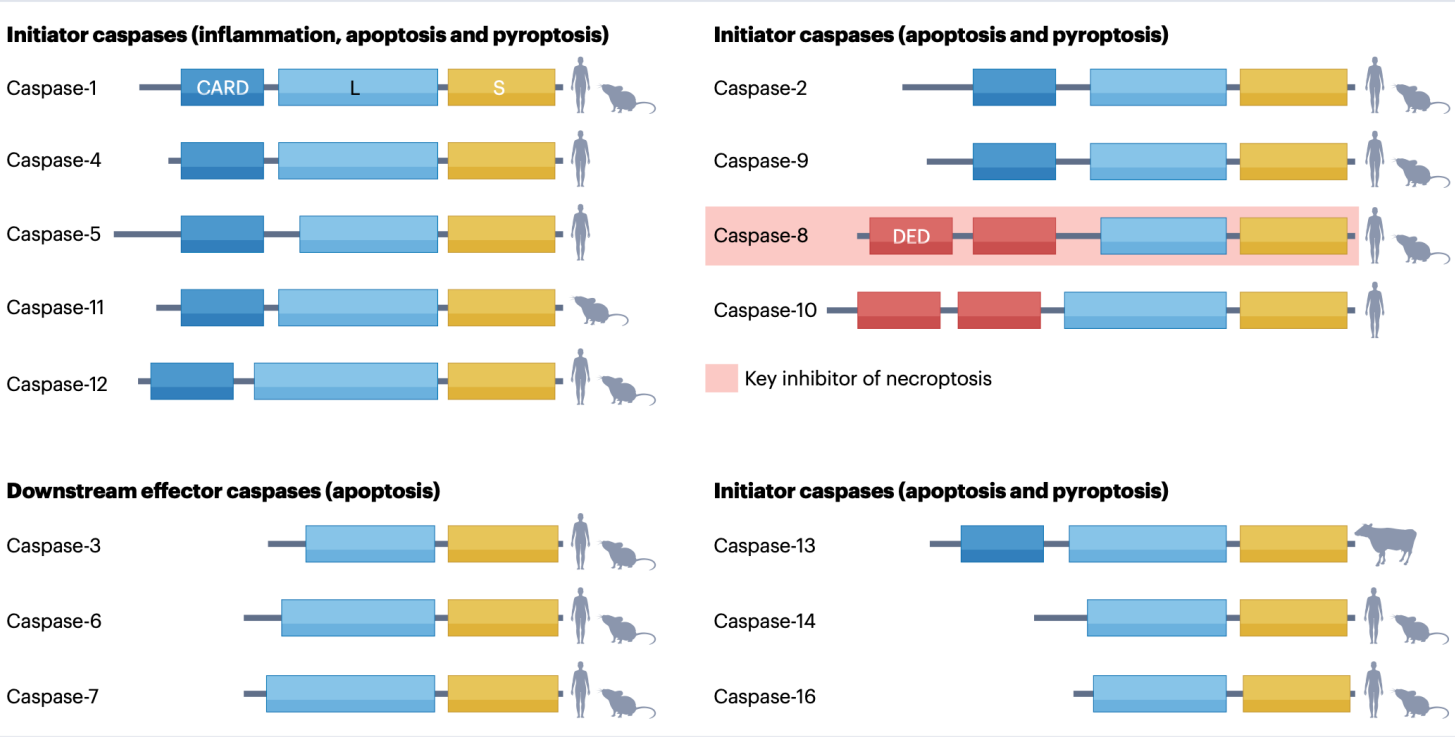
- (Sudden) loss of nutrient supply
- Wounds
- Infections
- Venom

Explosion of the cell and release of cellular contents into extracellular milieu



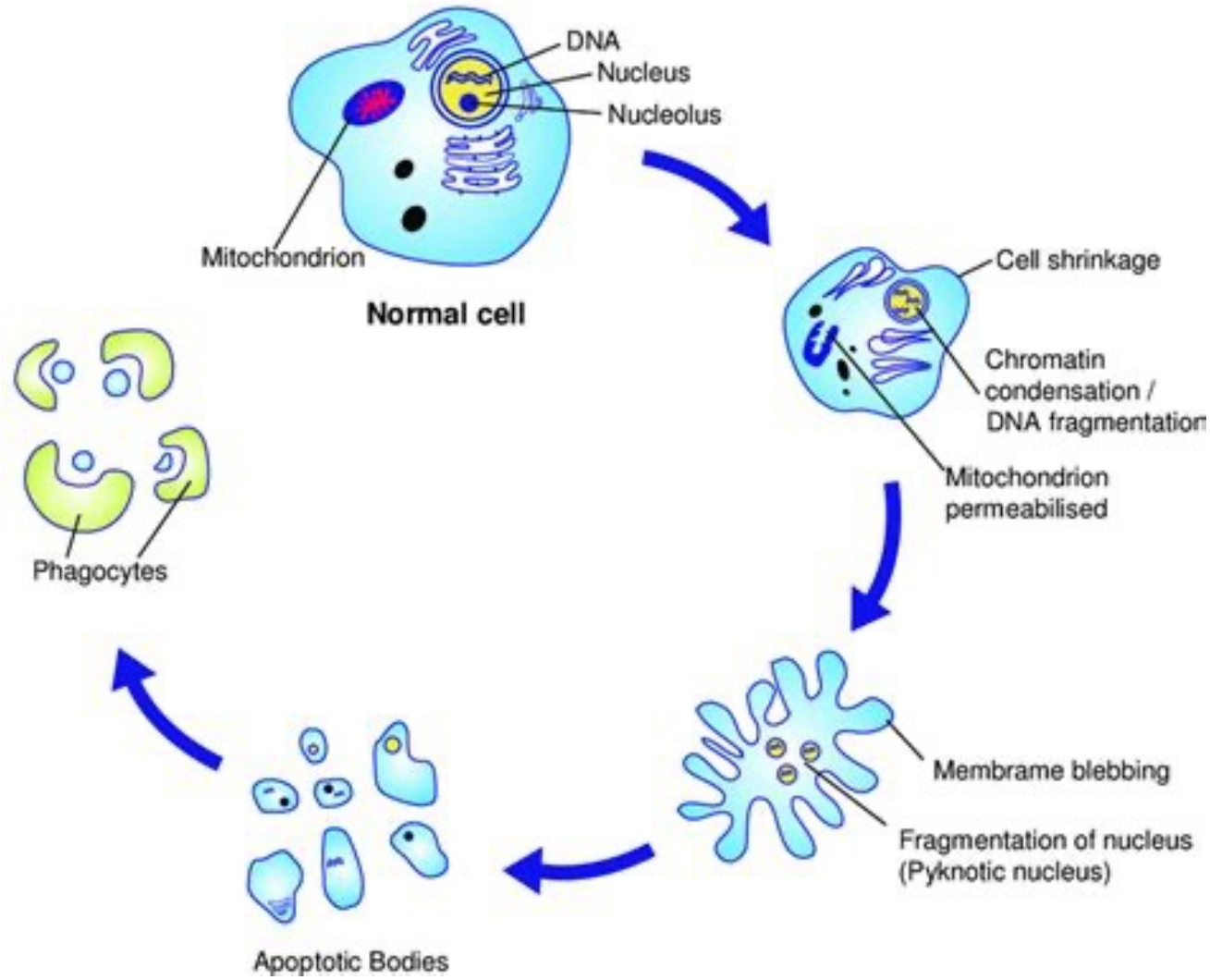
Mammalian caspase families

The first mammalian caspase, caspase-1, was identified as the IL-1β-converting enzyme involved in cleaving pre-IL-1β to generate mature IL-1β[218,219]. The molecular mechanism of programmed cell death in the nematode *Caenorhabditis elegans*, mediated by *Egl-1*, *Ced-9*, *Ced-3* and *Ced-4*, provides a prototypic example of apoptosis[220]. Mammalian caspases (see the figure) were found to be the sequence and functional homologues of *C. elegans Ced-3* in mediating apoptosis[221,222]. The genomes of mammals encode multiple caspases (the mouse genome encodes 11 caspases; the human genome encodes 13 caspases), which mediate cell death as well as inflammation[99]. Caspases are classified based on the prodomain length (short or long) and sequence features (CARD or DED) as well as the functions (initiator caspase or downstream caspase). Caspase-8 is an important inhibitor of necroptosis.



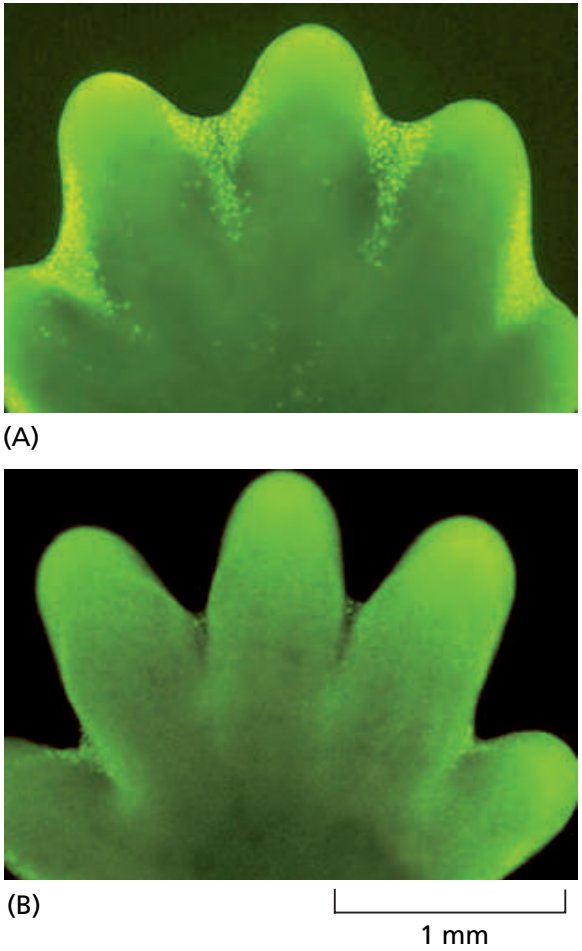
Programmed cell death
Apoptosis

Apoptosis


DNA
Nucleus
Nucleolus
Mitochondrion
Normal cell
Cell shrinkage
Chromatin condensation / DNA fragmentation
Mitochondrion permeabilised
Membrame blebbing
Fragmentation of nucleus (Pyknotic nucleus)
Apoptotic Bodies
Phagocytes

Roles of apoptosis

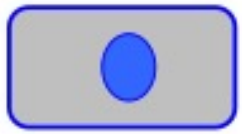
Clearing of defective cells
Example: Cells with DNA damage



Apoptosis plays an important role during development of many tissues

Figure 18–2 Sculpting the digits in the developing mouse paw by apoptosis. (A) The paw in this mouse fetus has been stained with a dye that specifically labels cells that have undergone apoptosis. The apoptotic cells appear as *bright green dots* between the developing digits. (B) The interdigital cell death has eliminated the tissue between the developing digits, as seen one day later, when there are very few apoptotic cells. (From W. Wood et al., *Development* 127:5245–5252, 2000. With permission from The Company of Biologists.)

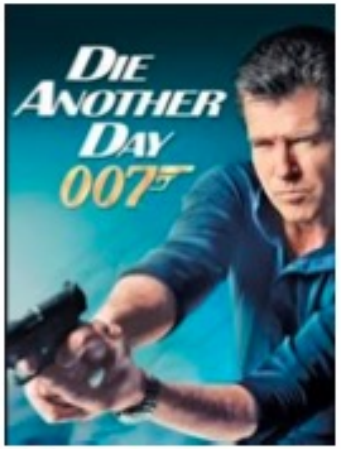
STEPS OF APOPTOSIS



Healthy cell

↓ DEATH SIGNAL (extrinsic or intrinsic)

Commitment to die (reversible) → ex: DNA repair occurs



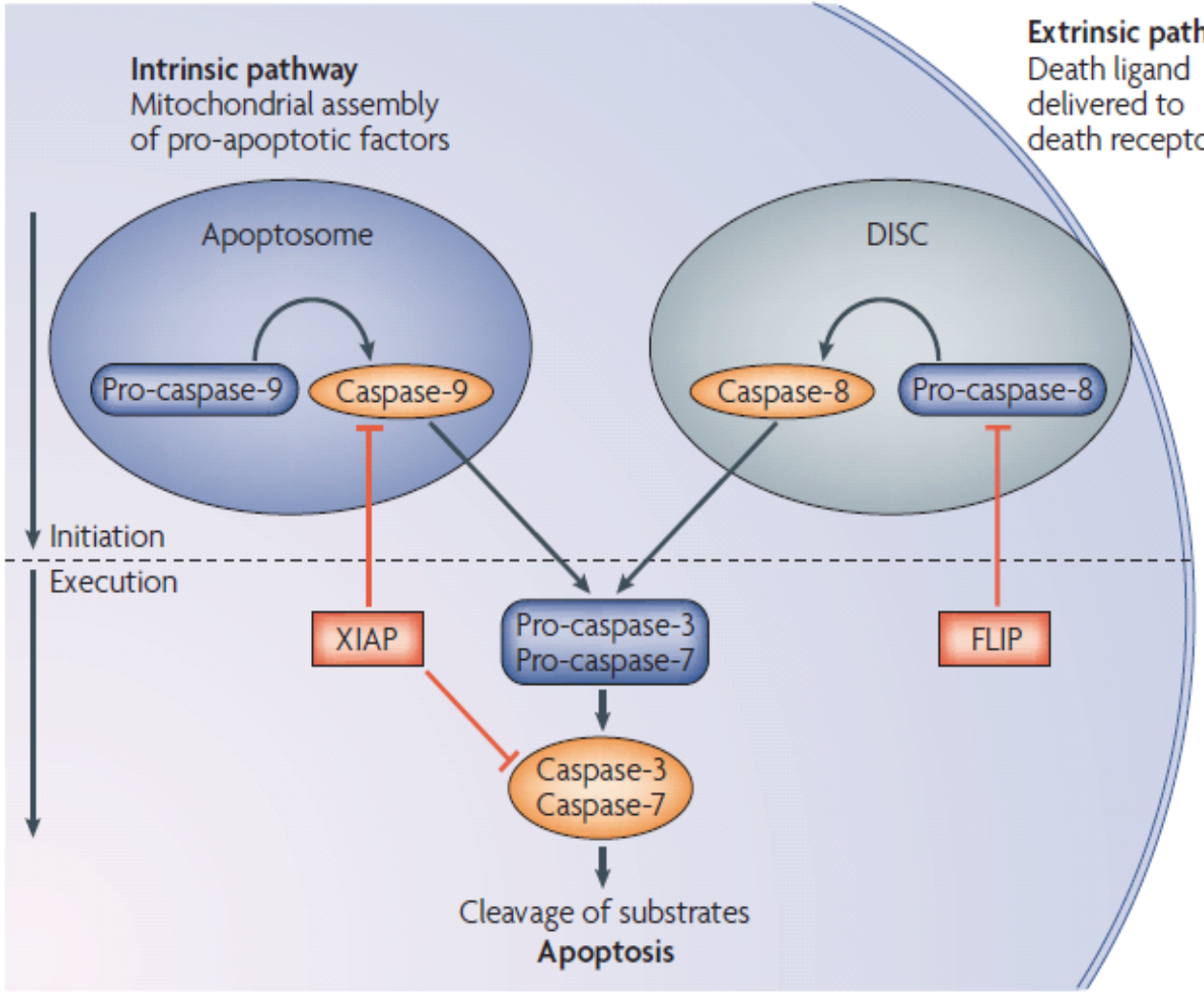
↓ EXECUTION (irreversible)

Dead cell (condensed)

↓ ENGULFMENT (macrophages, neighboring cells)

DEGRADATION

Intrinsic and extrinsic activation of apoptosis



The initiator caspase (9 or 8) differs, but the pathways converge on the executioner caspases (3, 7)

XIAP and FLIP block caspases and are essential to ensure tight, all-or-nothing regulation

Caspase activation during apoptosis

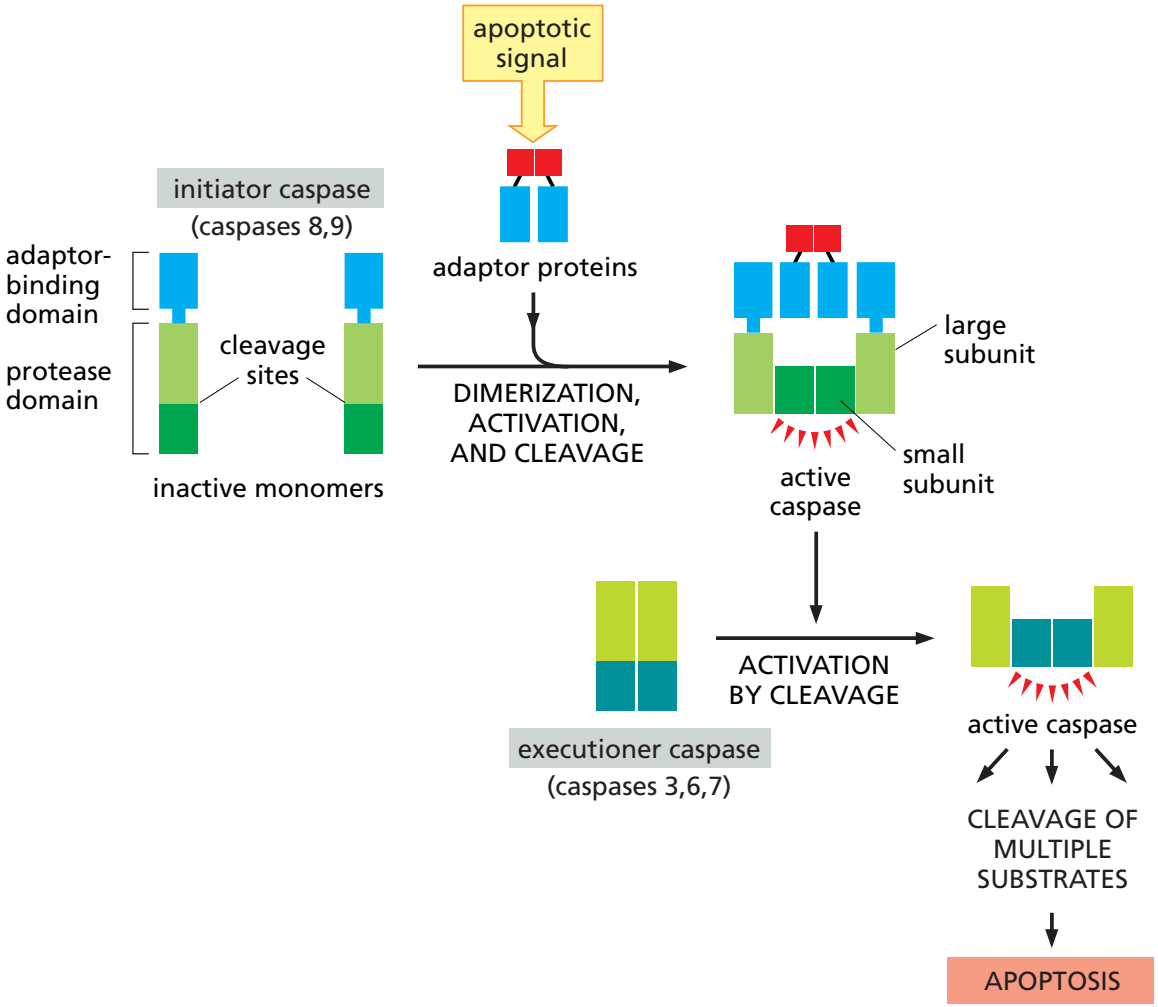
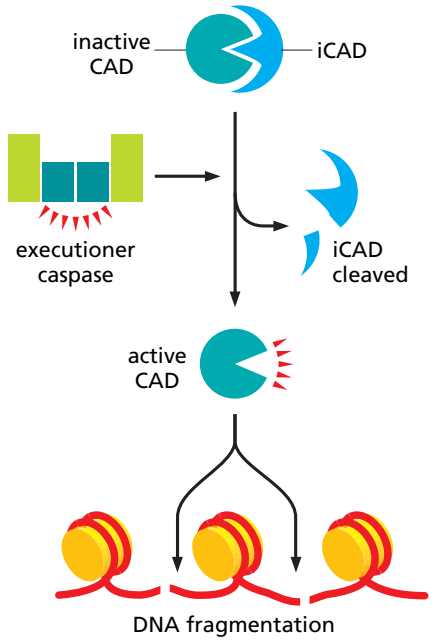
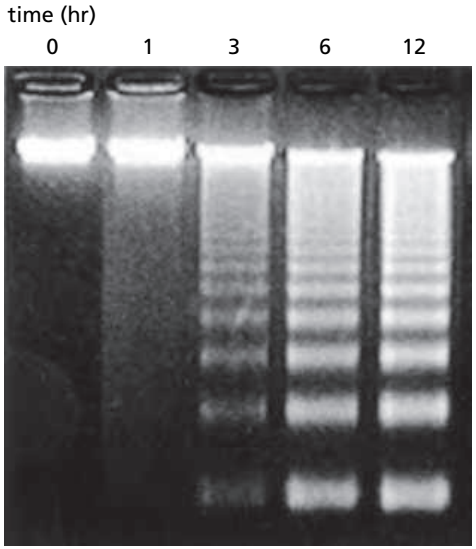


Figure 18–3 Caspase activation during apoptosis. An initiator caspase contains a protease domain in its carboxy-terminal region and a small protein interaction domain near its amino terminus. It is initially made in an inactive, monomeric form, sometimes called procaspase. Apoptotic signals trigger the assembly of adaptor proteins carrying multiple binding sites for the caspase amino-terminal domain. Upon binding to the adaptor proteins, the initiator caspases dimerize and are thereby activated, leading to cleavage of a specific site in their protease domains. Each protease domain is then rearranged into a large and small subunit. In some cases (not shown), the adaptor-binding domain of the initiator caspase is also cleaved (see Figure 18–5). Executioner caspases are initially formed as inactive dimers. Upon cleavage at a site in the protease domain by an initiator caspase, the executioner caspase dimer undergoes an activating conformational change. The executioner caspases then cleave a variety of key proteins, leading to the controlled death of the cell.

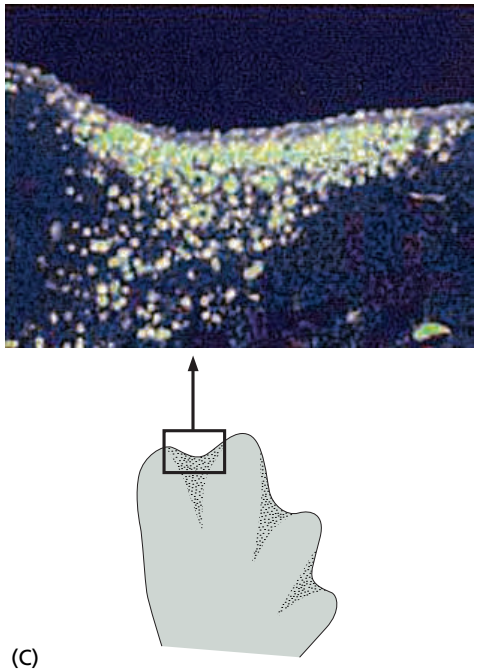
DNA fragmentation during apoptosis by an endonuclease CAD



(A)



(B)



(C)

Figure 18–4 DNA fragmentation during apoptosis. (A) In healthy cells, the endonuclease CAD associates with its inhibitor, iCAD. Activation of executioner caspases in the cell leads to cleavage of iCAD, which unleashes the nuclease. Activated CAD cuts the chromosomal DNA between nucleosomes, resulting in the production of DNA fragments that form a ladder pattern (see B) upon gel electrophoresis. (B) Mouse thymus lymphocytes were treated with an antibody against the cell-surface death receptor Fas (discussed in the text), inducing the cells to undergo apoptosis. DNA was extracted at the times indicated above the figure, and the fragments were separated by size by electrophoresis in an agarose gel and stained with ethidium bromide. Because the cleavages occur in the linker regions between nucleosomes, the fragments separate into a characteristic ladder pattern on these gels. Note that in gel electrophoresis, smaller molecules are more widely separated in the lower part of the gel, so that removal of a single nucleosome has a greater apparent effect on their gel mobility. (C) Apoptotic nuclei can be detected using a technique that adds a fluorescent label to DNA ends. In the image shown here, this technique was used in a tissue section of a developing chick leg bud; this cross section through the skin and underlying tissue is from a region between two developing digits, as indicated in the underlying drawing. The procedure is called the TUNEL (TdT-mediated dUTP nick end labeling) technique because the enzyme terminal deoxynucleotidyl transferase (TdT) adds chains of labeled deoxynucleotide (dUTP) to the 3′-OH ends of DNA fragments. The presence of large numbers of DNA fragments therefore results in bright fluorescent dots in apoptotic cells. (B, from D. McIlroy et al., *Genes Dev.* 14:549–558, 2000. With permission from Cold Spring Harbor Laboratory Press; C, from V. Zuzarte-Luís and J.M. Hurlé, *Int. J. Dev. Biol.* 46:871–876, 2002. With permission from UBC Press.)

The extrinsic pathway of apoptosis activated through Fas death receptors

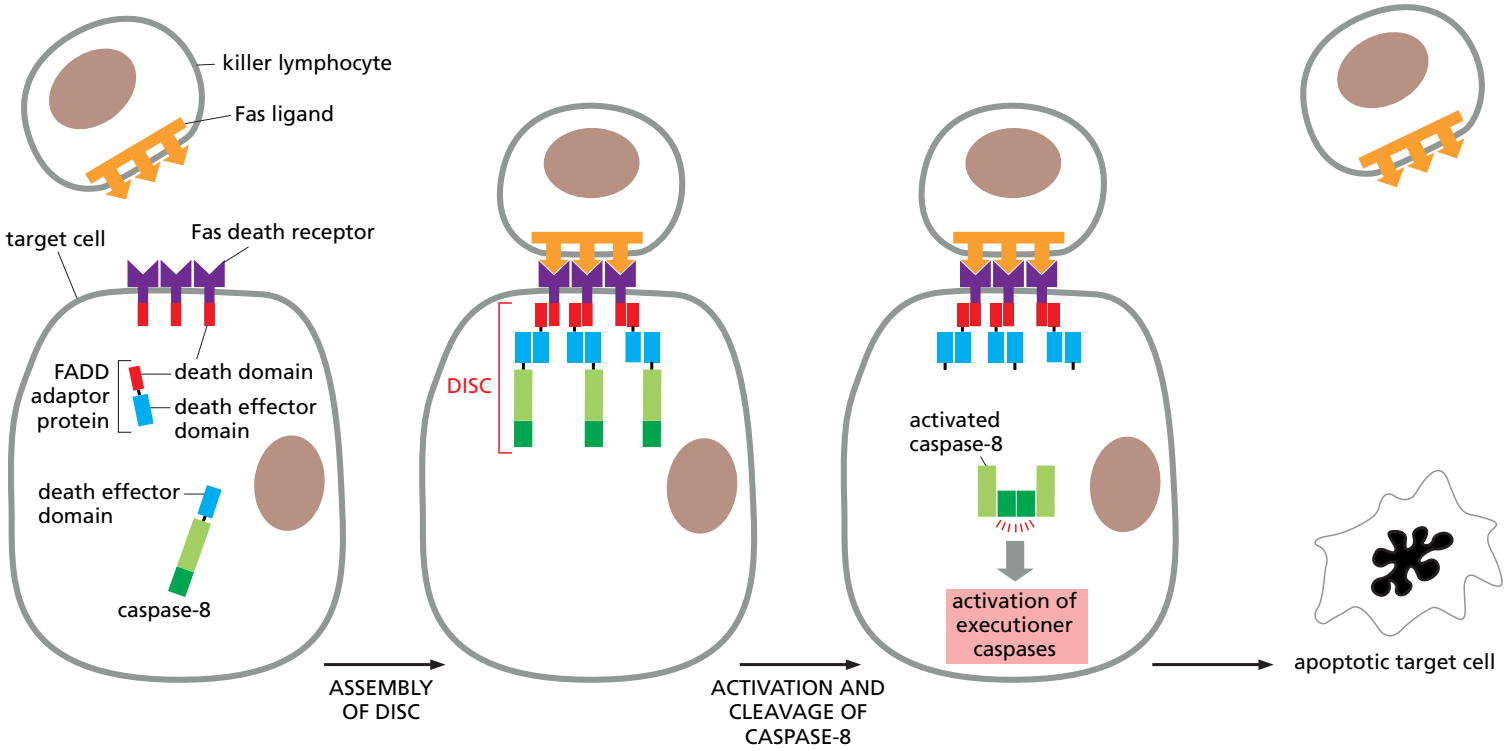


Figure 18–5 The extrinsic pathway of apoptosis activated through Fas death receptors. Trimeric Fas ligands on the surface of a killer lymphocyte interact with trimeric Fas receptors on the surface of the target cell, leading to clustering of several ligand-bound receptor trimers (only one trimer is shown here for clarity). Receptor clustering activates death domains on the receptor tails, which interact with similar domains on the adaptor protein FADD (FADD stands for Fas-associated death domain). Each FADD protein then recruits an initiator caspase (caspase-8) via a death effector domain on both FADD and the caspase, forming a death-inducing signaling complex (DISC). Within the DISC, two adjacent initiator caspases interact and cleave one another to form an activated protease dimer, which then cleaves itself in the region linking the protease to the death effector domain. This stabilizes and releases the active caspase dimer into the cytosol, where it activates executioner caspases by cleaving them.

Release of cytochrome *c* from mitochondria in the intrinsic pathway of apoptosis

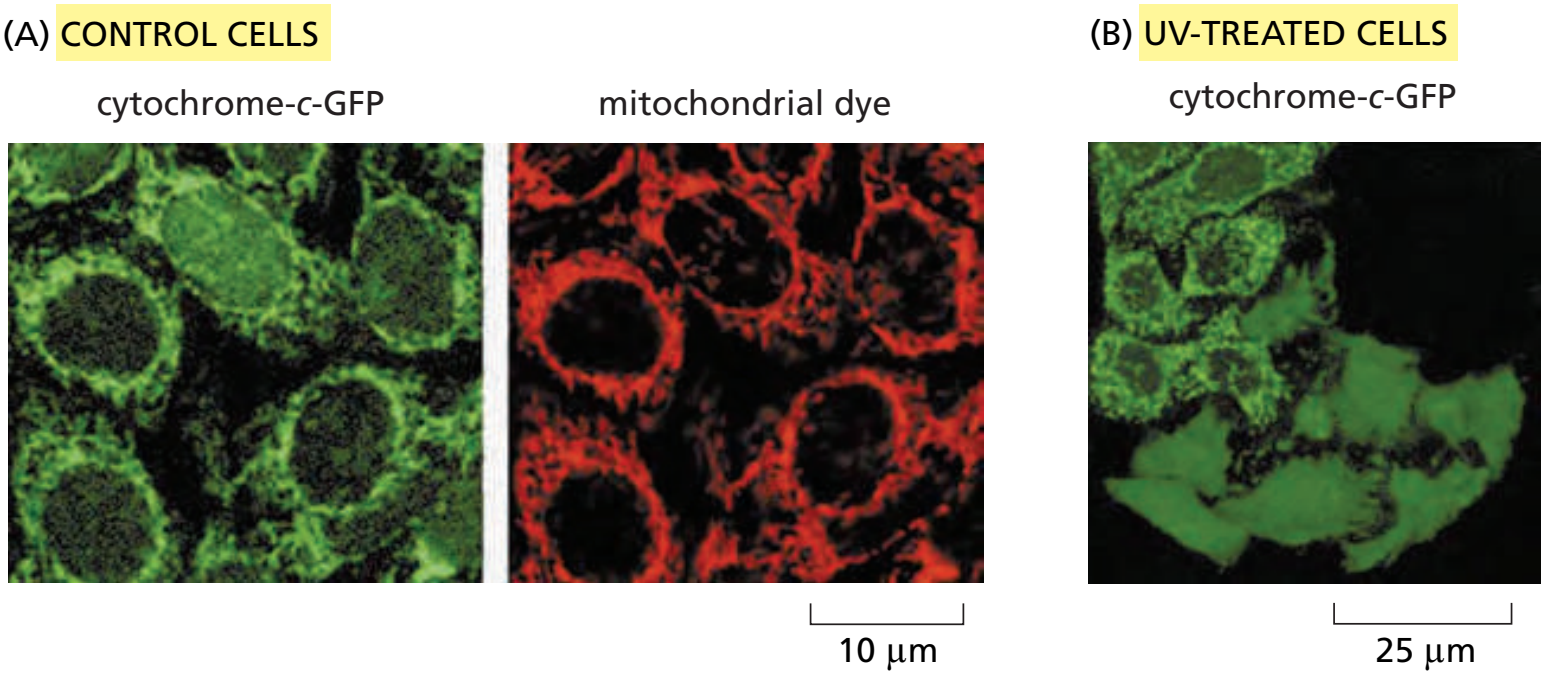


Figure 18–6 Release of cytochrome *c* from mitochondria in the intrinsic pathway of apoptosis. Fluorescence micrographs of human cancer cells in culture. (A) The control cells were transfected with a gene encoding a fusion protein consisting of cytochrome *c* linked to green fluorescent protein (cytochrome-*c*-GFP); they were also treated with a red dye that accumulates in mitochondria. The overlapping distribution of the *green* and *red* indicates that the cytochrome-*c*-GFP is located in mitochondria. (B) Cells expressing cytochrome-*c*-GFP were irradiated with ultraviolet (UV) light to induce the intrinsic pathway of apoptosis and were photographed 5 hours later. The six cells in the bottom half of this micrograph have released their cytochrome *c* from mitochondria into the cytosol, whereas the cells in the upper half of the micrograph have not yet done so (**Movie 18.1**). (From J.C. Goldstein et al., *Nat. Cell Biol*. 2:156–162, 2000. With permission from Macmillan Publishers Ltd.)

The intrinsic pathway of apoptosis

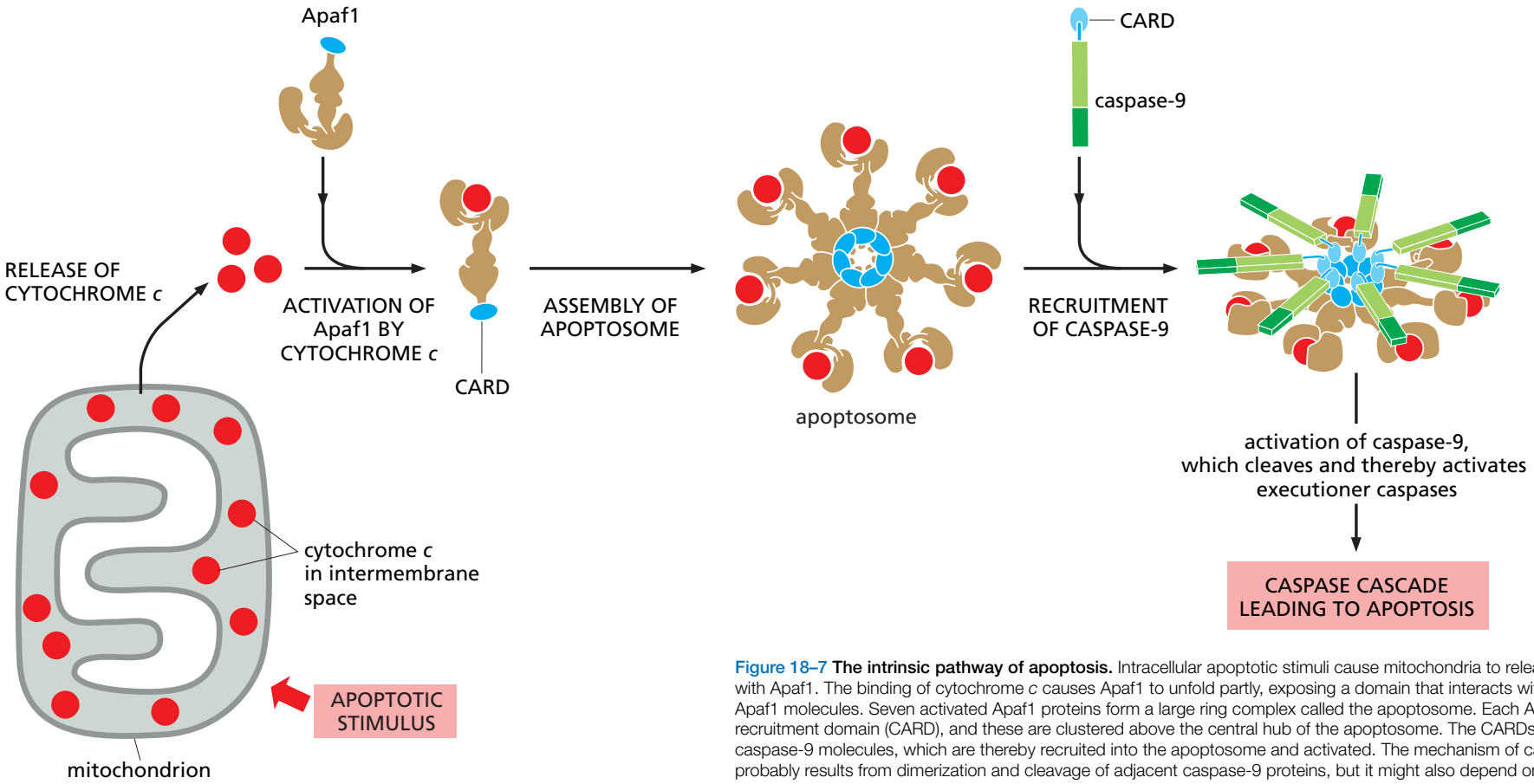


Figure 18–7 The intrinsic pathway of apoptosis. Intracellular apoptotic stimuli cause mitochondria to release cytochrome *c*, which interacts with Apaf1. The binding of cytochrome *c* causes Apaf1 to unfold partly, exposing a domain that interacts with the same domain in other activated Apaf1 molecules. Seven activated Apaf1 proteins form a large ring complex called the apoptosome. Each Apaf1 protein contains a caspase recruitment domain (CARD), and these are clustered above the central hub of the apoptosome. The CARDs bind similar domains in multiple caspase-9 molecules, which are thereby recruited into the apoptosome and activated. The mechanism of caspase-9 activation is not clear: it probably results from dimerization and cleavage of adjacent caspase-9 proteins, but it might also depend on interactions between caspase-9 and Apaf1. Once activated, caspase-9 cleaves and thereby activates downstream executioner caspases. Note that the CARD is related in structure and function to the death effector domain of caspase-8 (see Figure 18–5). Some scientists use the term "apoptosome" to refer to the complex containing caspase-9.

The three classes of Bcl2 family proteins

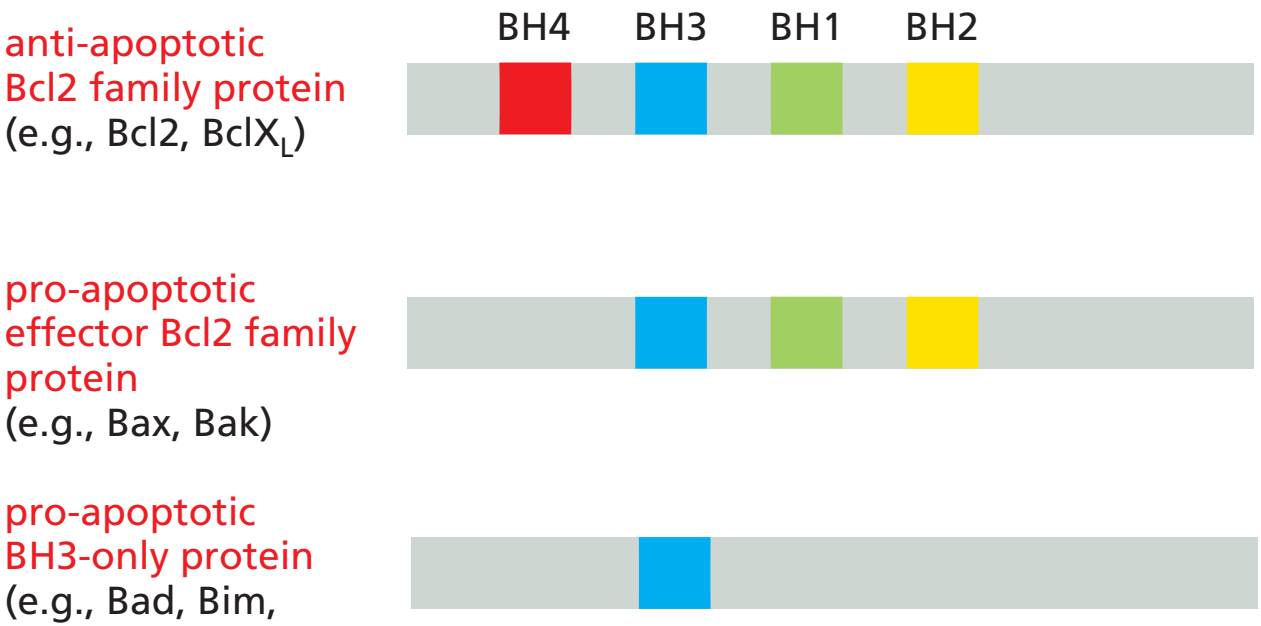


Figure 18–8 The three classes of Bcl2 family proteins. Note that the BH3 domain is the only BH domain shared by all Bcl2 family members; it mediates the direct interactions between pro-apoptotic and anti-apoptotic family members.

The role of pro-apoptotic effector Bcl2 family proteins (mainly Bax and Bak) in the release of mitochondrial intermembrane proteins in the intrinsic pathway of apoptosis

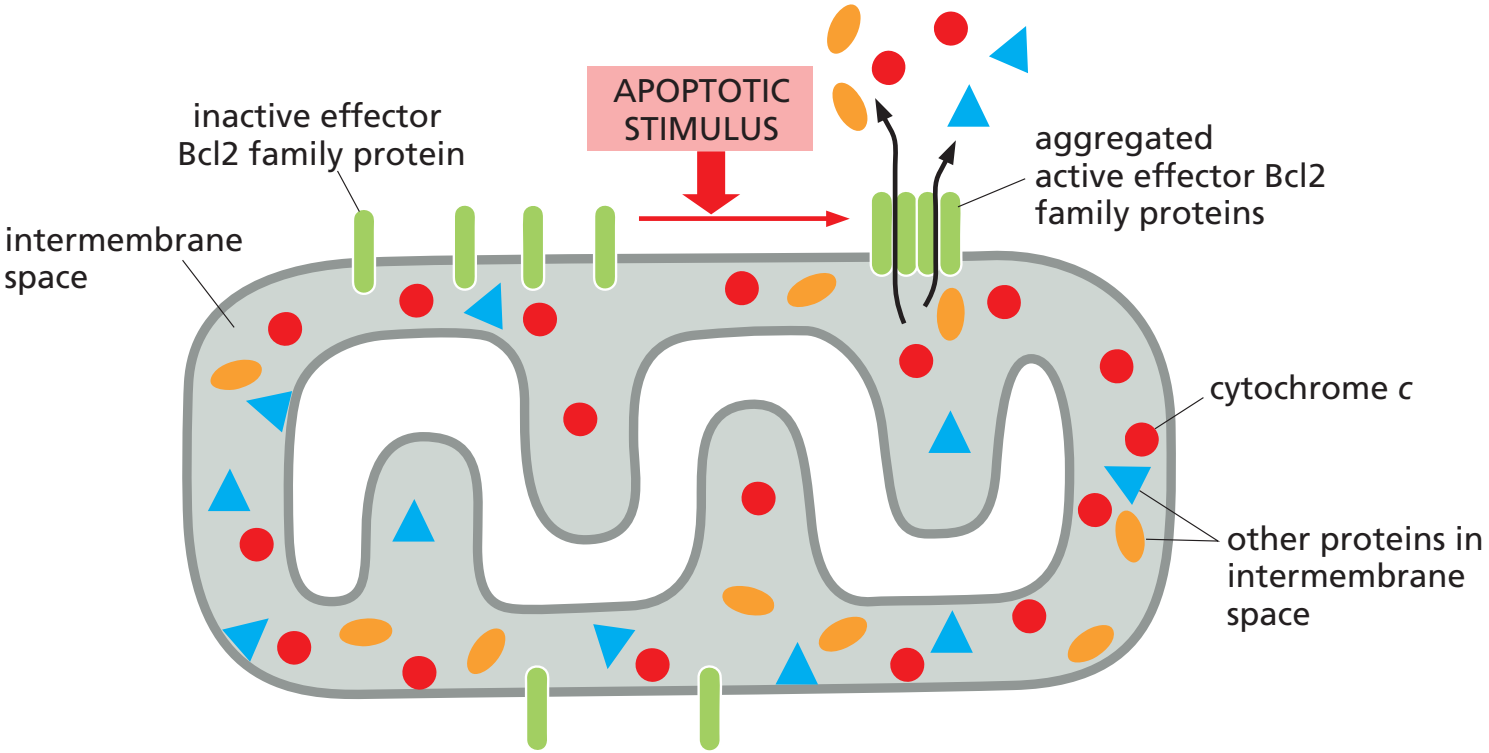


Figure 18–9 The role of pro-apoptotic effector Bcl2 family proteins (mainly Bax and Bak) in the release of mitochondrial intermembrane proteins in the intrinsic pathway of apoptosis. When activated by an apoptotic stimulus, the effector Bcl2 family proteins aggregate on the outer mitochondrial membrane and release cytochrome *c* and other proteins from the intermembrane space into the cytosol by an unknown mechanism.

How pro-apoptotic BH3- only and anti-apoptotic Bcl2 family proteins regulate the intrinsic pathway of apoptosis

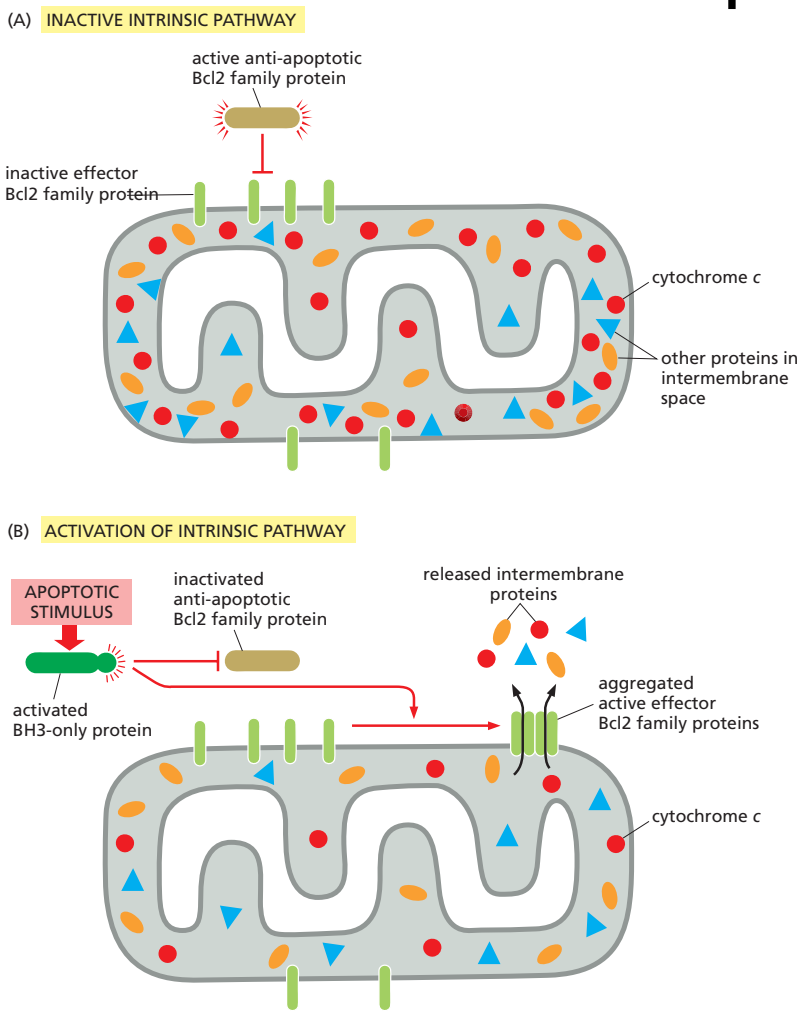
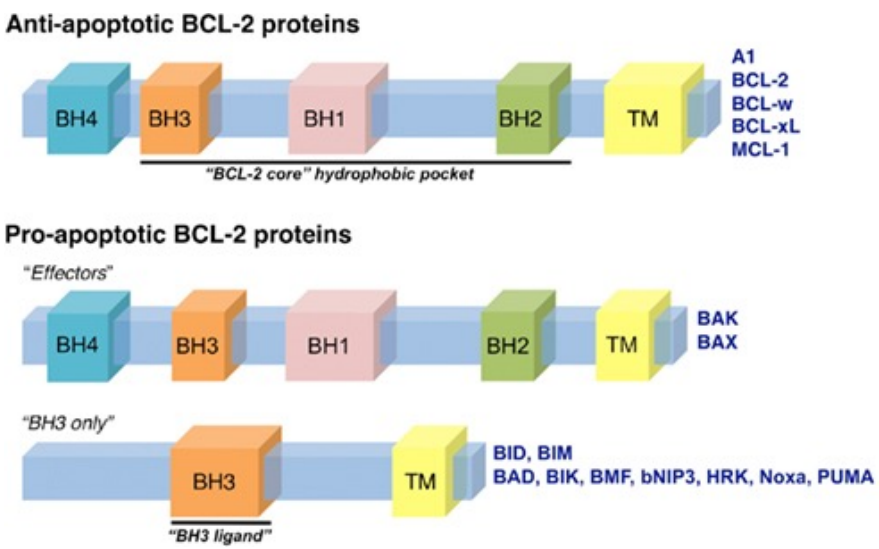
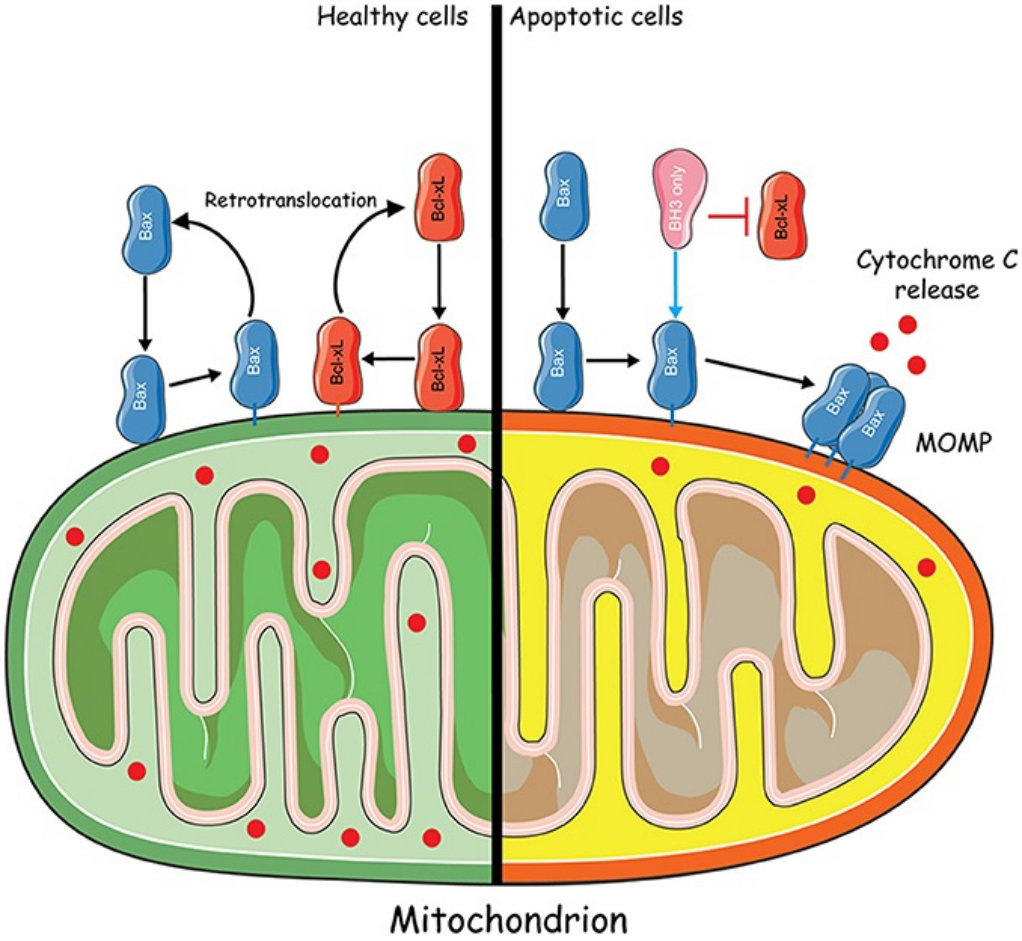


Figure 18–10 How pro-apoptotic BH3-only and anti-apoptotic Bcl2 family proteins regulate the intrinsic pathway of apoptosis. (A) In the absence of an apoptotic stimulus, anti-apoptotic Bcl2 family proteins bind to and inhibit the effector Bcl2 family proteins on the mitochondrial outer membrane (and in the cytosol—not shown). (B) In the presence of an apoptotic stimulus, BH3-only proteins are activated and bind to the anti-apoptotic Bcl2 family proteins so that they can no longer inhibit the effector Bcl2 family proteins; the latter then become activated, aggregate in the outer mitochondrial membrane, and promote the release of intermembrane mitochondrial proteins into the cytosol. Some activated BH3-only proteins may stimulate mitochondrial protein release more directly by binding to and activating the effector Bcl2 family proteins. Although not shown, the anti-apoptotic Bcl2 family proteins are bound to the mitochondrial surface.


Anti-apoptotic BCL-2 proteins
BH4
BH3
BH1
BH2
TM
A1
BCL-2
BCL-w
BCL-xL
MCL-1
"BCL-2 core" hydrophobic pocket
Pro-apoptotic BCL-2 proteins
"Effectors"
BH4
BH3
BH1
BH2
TM
BAK
BAX
"BH3 only"
BH3
TM
BID, BIM
BAD, BIK, BMF, bNIP3, HRK, Noxa, PUMA
"BH3 ligand"


Healthy cells
Apoptotic cells
Retrotranslocation
Bax
Bcl-xL
BH3 only
Cytochrome C release
MOMP
Mitochondrion

Extracellular survival factors can inhibit apoptosis

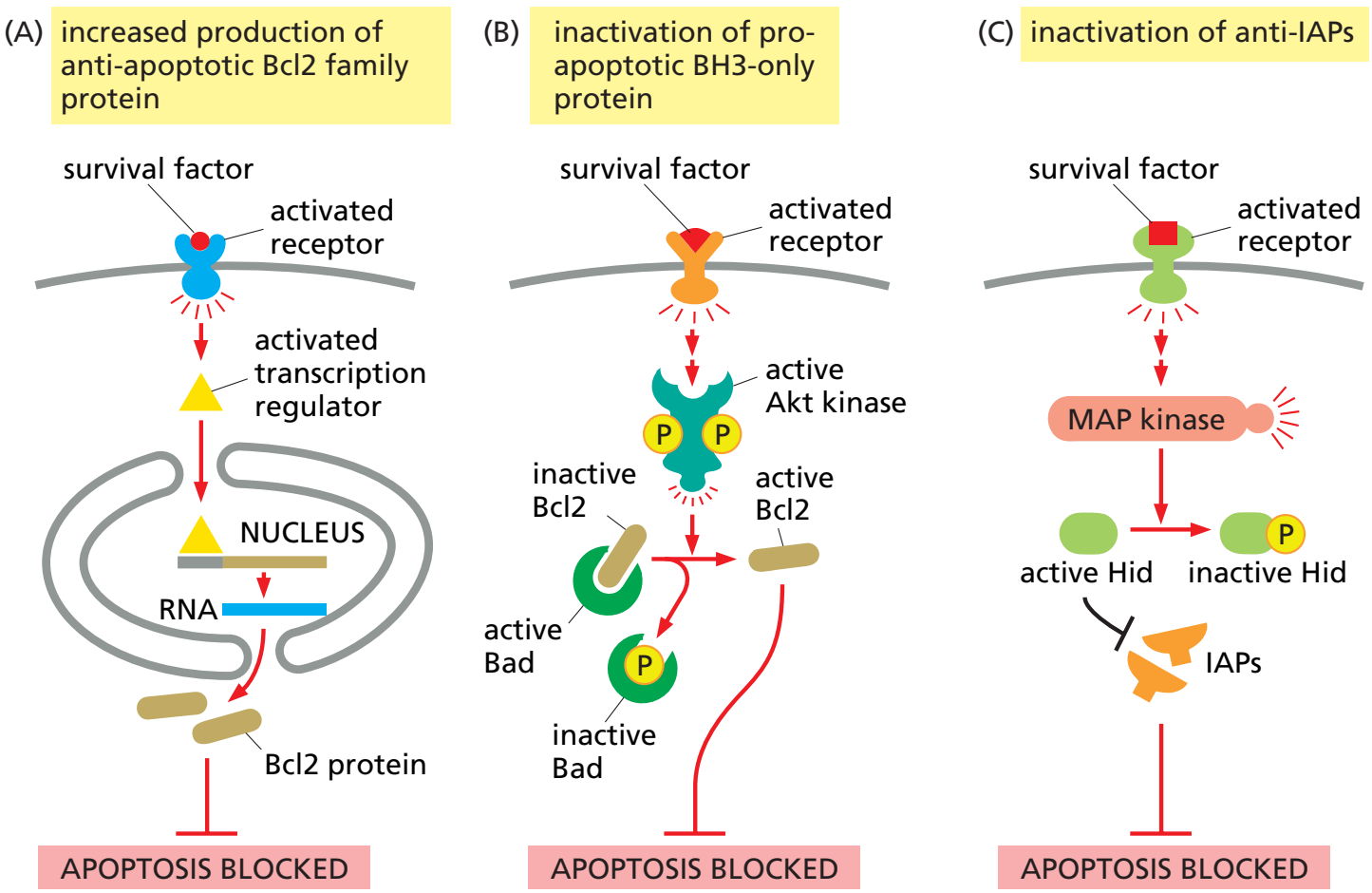


Figure 18–12 Three ways that extracellular survival factors can inhibit apoptosis. (A) Some survival factors suppress apoptosis by stimulating the transcription of genes that encode anti-apoptotic Bcl2 family proteins such as Bcl2 itself or $Bcl-X_L$. (B) Many others activate the serine/threonine protein kinase Akt, which, among many other targets, phosphorylates and inactivates the pro-apoptotic BH3-only protein Bad (see Figure 15–53). When not phosphorylated, Bad promotes apoptosis by binding to and inhibiting Bcl2; once phosphorylated, Bad dissociates, freeing Bcl2 to suppress apoptosis. Akt also suppresses apoptosis by phosphorylating and inactivating transcription regulatory proteins that stimulate the transcription of genes encoding proteins that promote apoptosis (not shown). (C) In *Drosophila*, some survival factors inhibit apoptosis by stimulating the phosphorylation of the anti-IAP protein Hid. When not phosphorylated, Hid promotes cell death by inhibiting IAPs. Once phosphorylated, Hid no longer inhibits IAPs, which become active and block apoptosis. MAP kinase, mitogen-activated protein kinase.

The role of survival factors and cell death in adjusting the number of developing nerve cells to the amount of target tissue

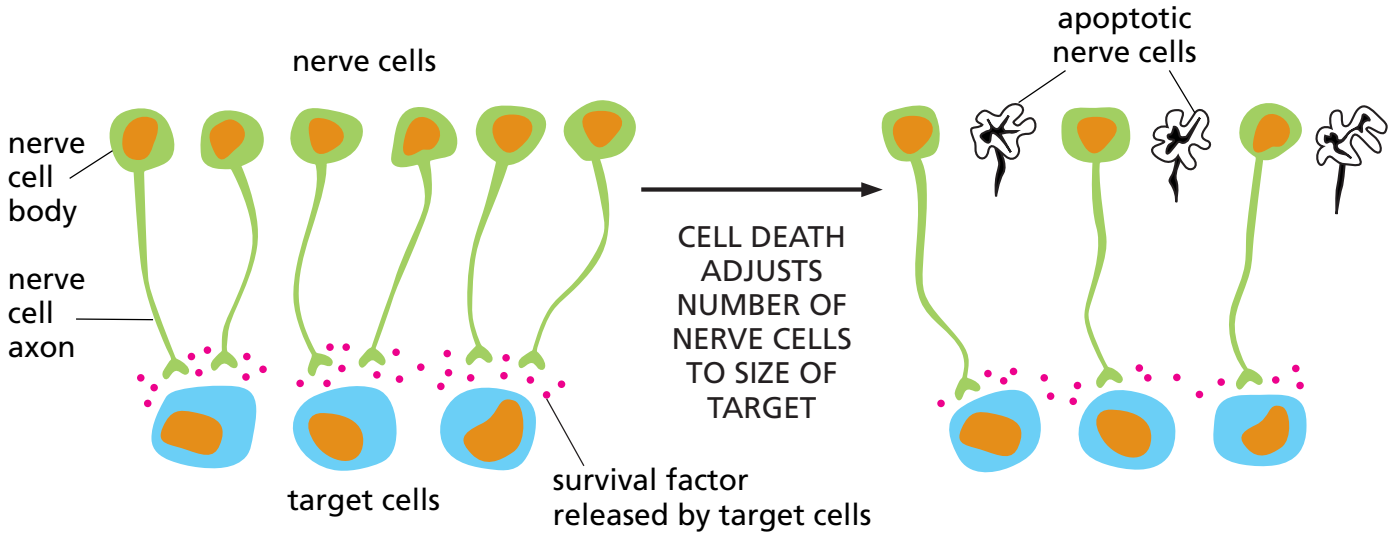
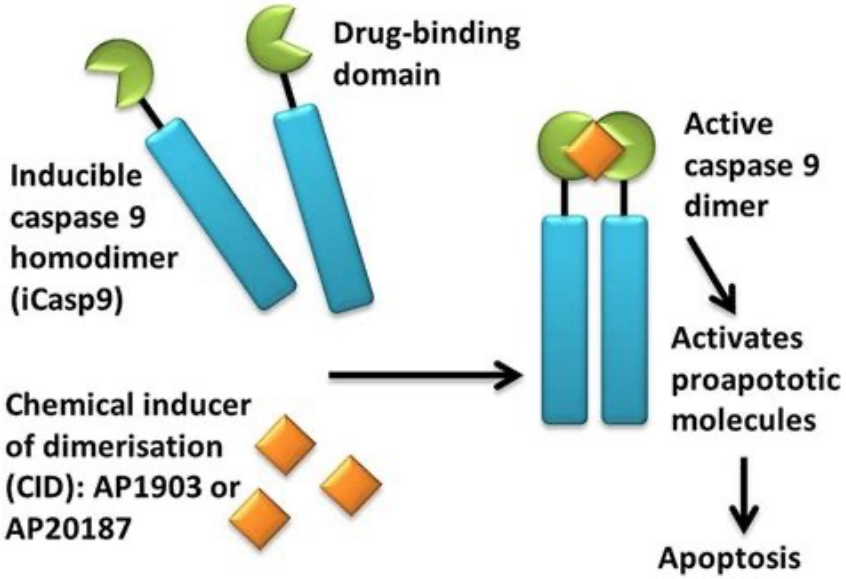
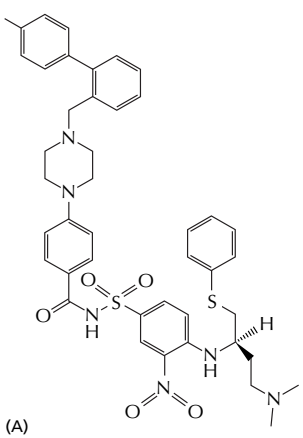


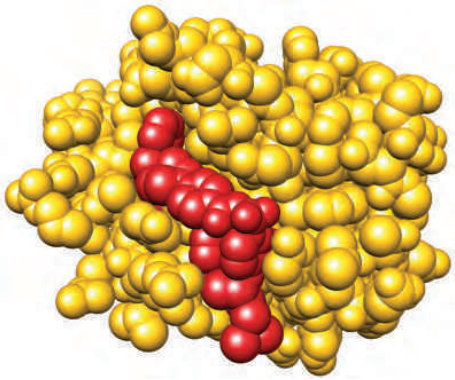
Figure 18–11 The role of survival factors and cell death in adjusting the number of developing nerve cells to the amount of target tissue. More nerve cells are produced than can be supported by the limited amount of survival factors released by the target cells. Therefore, some nerve cells receive an insufficient amount of survival factors to avoid apoptosis. This strategy of overproduction followed by culling helps ensure that all target cells are contacted by nerve cells and that the extra nerve cells are automatically eliminated.

Control over apoptosis





(A)



(B)

Figure 18–13 How the chemical ABT-737 inhibits anti-apoptotic Bcl2 family proteins. As shown in Figure 18–10B, an apoptotic signal results in activation of BH3-only proteins, which interact with a long hydrophobic groove in anti-apoptotic Bcl2 family proteins, thereby preventing them from blocking apoptosis. Using the crystal structure of the groove, the drug shown in (A), called ABT-737, was designed and synthesized to bind tightly in the groove, as shown for the anti-apoptotic Bcl2 family protein, $BclX_L$, in (B). By inhibiting the activity of these proteins, the drug promotes apoptosis in any cell that depends on them for survival. (PDB code: 2YXJ.)

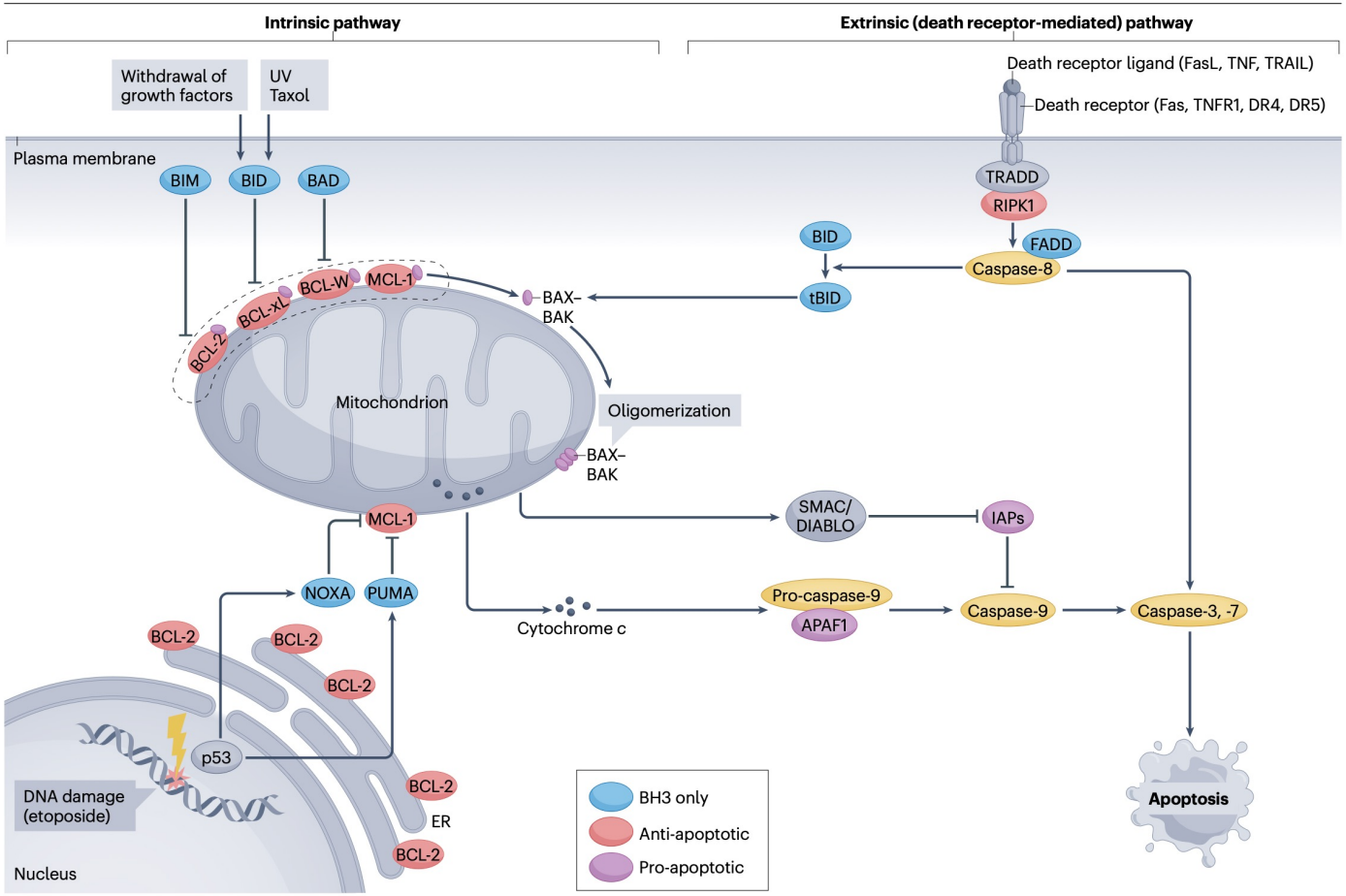
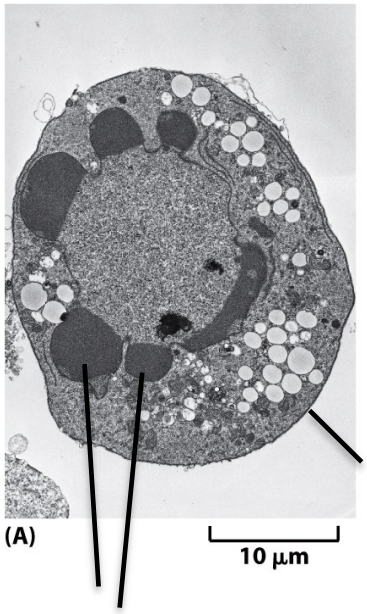


Fig. 1 | Intrinsic and extrinsic apoptosis. Extrinsic apoptosis (right) is mediated by the activation of plasma membrane-localized death receptors (such as TNFR1, Fas and TRAIL receptors DR4 and DR5) by their cognate ligands (such as TNF, FasL and TRAIL, respectively). Intrinsic apoptosis (left) can be activated by growth factor withdrawal, mitochondrial damage, DNA damage and chemotherapeutic drugs such as taxol. Activation of BH3-only members of the BCL-2 family, such as transcriptional induction of NOXA and PUMA by p53, post-translational modification of BAD and BIM, and cleavage of BID by caspase-8, induces mitochondrial damage by inactivating pro-survival BCL-2 family members, such as BCL-2, BCL-xL and MCL-1, and activating oligomerization of pro-death BCL-2 family members BAX and BAK. Mitochondrial damage leads to the release of cytochrome c and second mitochondria-derived activator of caspase (SMAC; also known as DIABLO) to promote the activation of caspase-9 mediated by APAF1. Activated caspase-9 in turn cleaves downstream caspases, caspase-3 and caspase-7, to mediate the execution of intrinsic apoptosis. Activated caspase-3 and caspase-7 can also exert feedback activation of upstream caspases to allow amplification of caspase cascades. ER, endoplasmic reticulum; UV, ultraviolet.

Identifying apoptotic cells

- Cytochrome C release from the mitochondria to the cytoplasm
- Transmission electron microscopy
- DNA fragmentation : laddering
- Caspase activity assays: cleavage of targets, colorimetric assays
- Recognition of phosphatidylserine

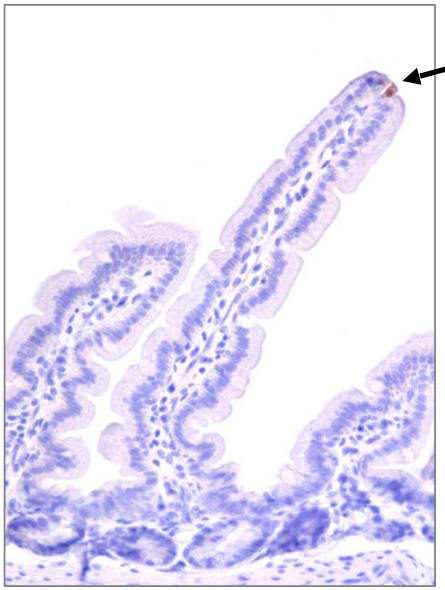
Transmission electron microscopy



Intact plasma membrane

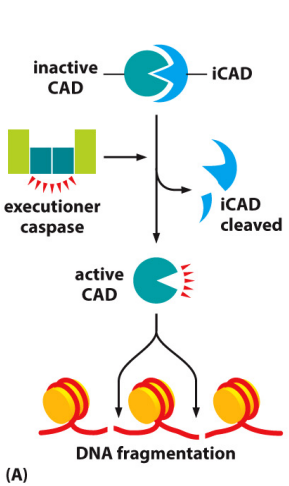
condensed chromatin

Immunohistochemistry



cleaved caspase3

DNA fragmentation assay



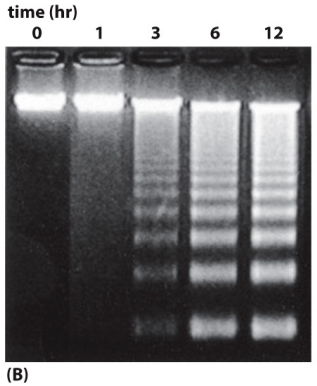
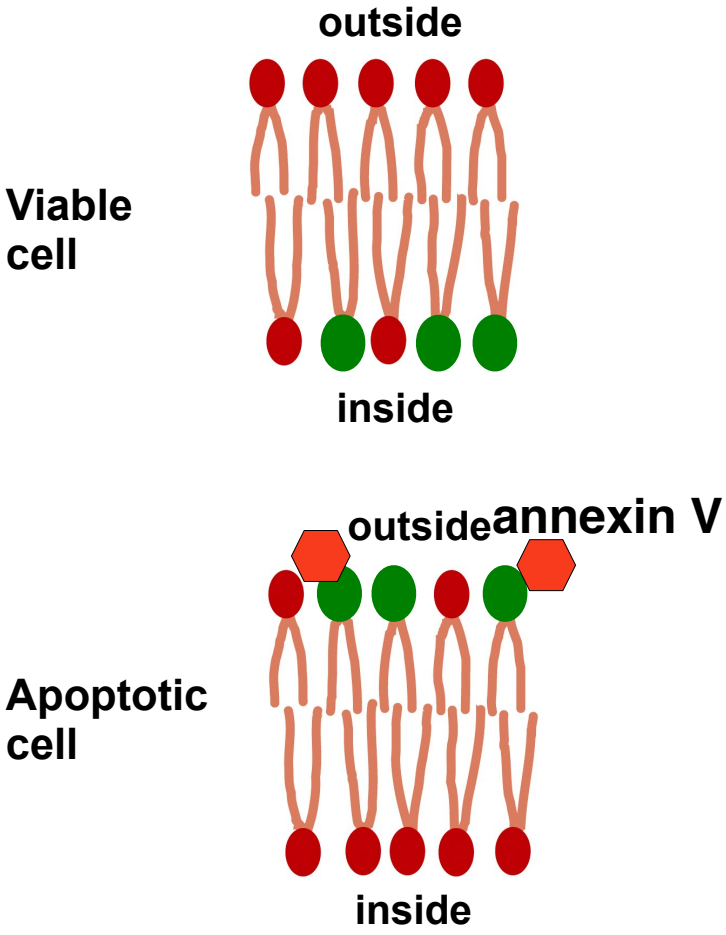


Figure 18-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

≈ 180 nt

Phosphatidylserine (PS): a marker of apoptotic cells

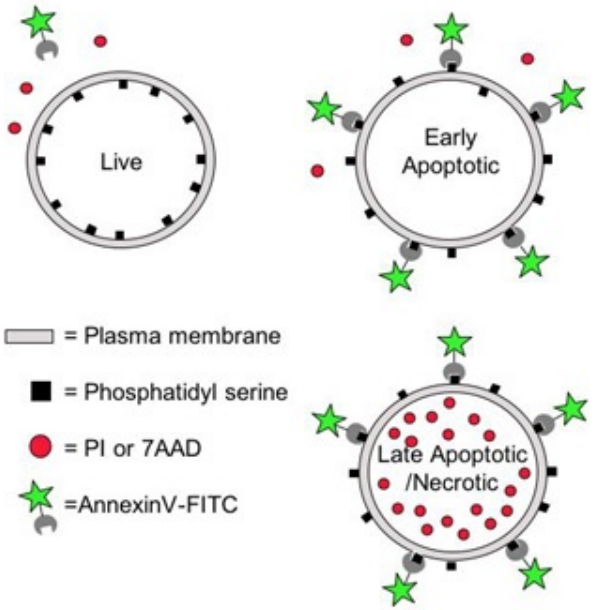


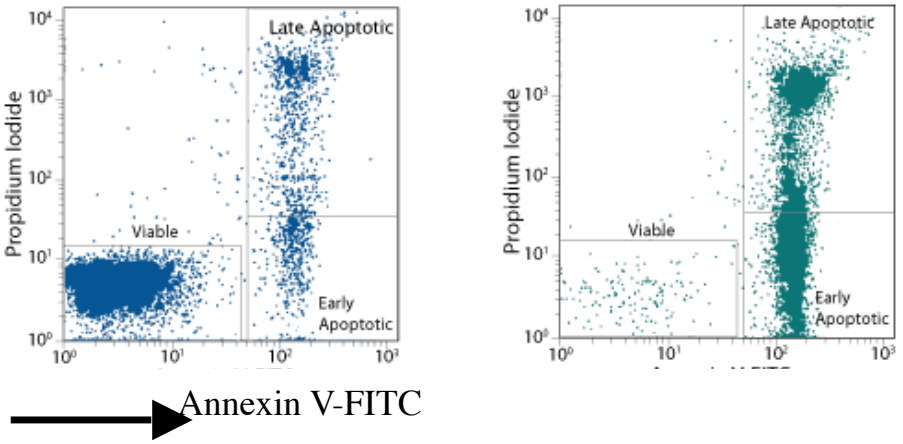
PS is translocated to the outer membrane during apoptosis

Externalisation of PS from the inner to the outer plasma membrane can be detected by annexin V that specifically binds PS

Apoptosis detection by flow cytometry

Fluorescent labeling of annexin V enables the detection of apoptotic cells by flow cytometry

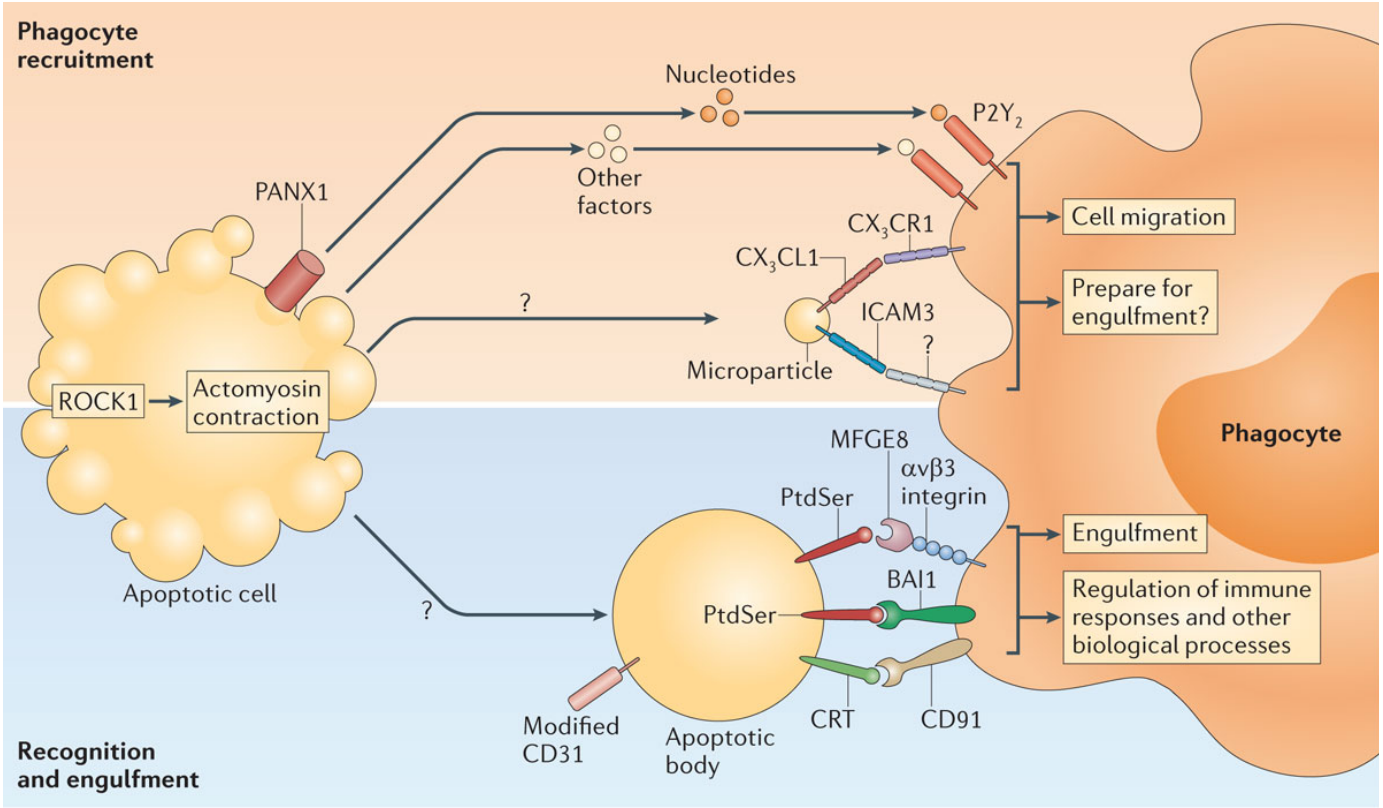




Why is PS externalized during apoptosis?

External PS is an "eat-me" signal

Phagocytosis of apoptotic cells prevents inflammation and autoimmunity



Nature Reviews | Immunology

Poon et al., Apoptotic cell clearance: basic biology and therapeutic potential, volume 14, pages166–180(2014)

Cell death

- Several varieties of cell death
- Cell death is regulated by both extrinsic as intrinsic factors
- Apoptosis is the most common and most studied variation
- Apoptosis is important during development
- Apoptosis is tightly regulated
  - Extrinsic: Caspases
  - Intrinsic: by BH domain containing proteins
- Apoptosis evasion is common in cancer