

Cell injury, necrosis and  
apoptosis.

Wound healing

September 27, 2011



# Cell survival and death

- ✔ The balance between cell survival and death is under tight genetic control. A multiplicity of extracellular signals and intracellular mediators is involved in maintaining this balance.
- ✔ When the cell is exposed to physical, biochemical or biological injury, or deprived of necessary substances, it activates a series of stress-response genes.
- ✔ With minimal insults, the cell may recover.
- ✔ With greater insults, single cell death, or , results; the cell dies and is recycled to its neighbours.
- ✔ If the insult overwhelms a large number of cells then necrosis ensues, with an accompanying inflammatory response.
- ✔ Dysregulation of the controlling of this system results in disease.
- ✔ Deficient is associated with cancer, auto-immunity and viral infections.
- ✔ Excessive is associated with ischaemic heart disease, stroke, neurodegenerative disease, sepsis and multiple organ dysfunction syndrome. There are myriad therapeutic options unfolding as understanding is gained of and its control.



# Cell death

- ✔ Cell death, a tightly controlled, finely orchestrated event, may be described either as apoptotic or nonapoptotic cell death, traditionally called 'necrosis'.
- ✔ Apoptosis is a process of cell suicide, the genes of which are encoded in the chromosomes of all nucleated cells. Physiological cell death that removes unwanted cells plays an important role in development, tissue homeostasis and defence against viral infection and mutation.
- ✔ Apoptosis is regulated by complex molecular signalling systems. Tissue ischaemia and reperfusion activate these molecular systems, which therefore represent a therapeutic target for novel treatment to preserve cellular integrity in critical organs such as the brain and heart.
- ✔ Apoptotic cells undergo orderly, energy-dependent enzymatic breakdown into characteristic molecular fragments, deoxyribonucleic acid (DNA), lipids and other macromolecules, which are packaged into small vesicles that may be phagocytosed and reused. The cells involute and die with minimal harm to nearby cells.
- ✔ In contrast 'necrotic' cell death is characterised by inflammation and widespread damage.



# Mechanisms of cell death

- ✓ Following an appropriate stimulus, the first stage or 'decision phase' of is the genetic control point of cell death. This is followed by the second stage or 'execution' phase, which is responsible for the morphological changes of cells.
- ✓ There are four main groups of stimuli for apoptosis.
- ✓ The **first** group of stimuli causes DNA damage and include ionising radiation and alkylating anticancer drugs.
- ✓ The **second** group induces via receptor, either by receptor activation mediated by glucocorticoids (acting on the thymus), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), or by withdrawal of growth factors (nerve growth factor and interleukin (IL)-3).
- ✓ The **third** group comprises biochemical agents that enhance the downstream components of the apoptotic pathway and includes phosphatases and kinase inhibitors (e.g. calphostin C, staurosporine).
- ✓ The **fourth** group comprises agents that cause direct cell membrane damage and includes heat, ultraviolet light and oxidising agents (superoxide anion, hydrogen peroxide). Excessive production of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and the hydroxyl radicals, produces free radicals that damage lipid membranes, proteins, nucleic acids and extracellular matrix glycosaminoglycans. Many of these stimuli cause necrosis in larger doses.
- ✓ Injury to cell membranes induces by activating acid sphingomyelinase that generates the second messenger ceramide from membrane lipids.



# Cell death

- ✔ is clearly an important factor in development, homeostasis, pathology and in aging, but medical efforts based on controlling cell death have not become major aspects of medicine. Most effort has focused on the machinery of cell death, or the proximate effectors of apoptosis and their closely associated and interacting proteins. But cells have many options other than apoptosis. These include autophagy, necrosis, atrophy and stepwise or other alternate means of self-disassembly.
- ✔ The response of a cell to a noxious or otherwise intimidating signal will depend heavily on the history, lineage and current status of the cell.
- ✔ Many metabolic and other processes adjust the sensitivity of cells to signals, and viruses aggressively attempt to regulate the death of their host cells.
- ✔ Another complicating factor is that many death-associated proteins may have functions totally unrelated to their role in cell death, generating the possibility of undesirable side effects if one interferes with them.



# Necrosis or apoptosis?

- ✔ Cells usually die either by necrosis or apoptosis. The characteristics of apoptotic death are more clearly understood when compared to the characteristics of necrotic death.
- ✔ Necrosis is a pathological death of cells resulting from irreversible damage and is a term commonly used in pulpal diagnosis.
- ✔ The earliest irreversible changes are mitochondrial, consisting of swelling and granular calcium deposits. After such changes, the outlines of individual cells are indistinct and affected cells may become merged, sometimes forming a focus of coarsely granular, amorphous or hyaline material.
- ✔ These features include
  - cell swelling
  - membrane lysis
  - inflammatory response



# Difference between apoptosis and necrosis

<b>Apoptosis</b>	<b>Necrosis</b>
Physiological or pathological	Always pathological
Asynchronous process in single cells	Occurs synchronously in multiple cells
Genetically controlled	Caused by overwhelming noxious stimuli
Late loss of membrane integrity	Early loss of membrane integrity
Cell shrinkage	Generalised cell and nucleus swelling
Condensation of nuclear contents ('ladder' formation of chromatin)	Nuclear chromatin disintegration
No inflammatory reaction	Inflammatory reaction

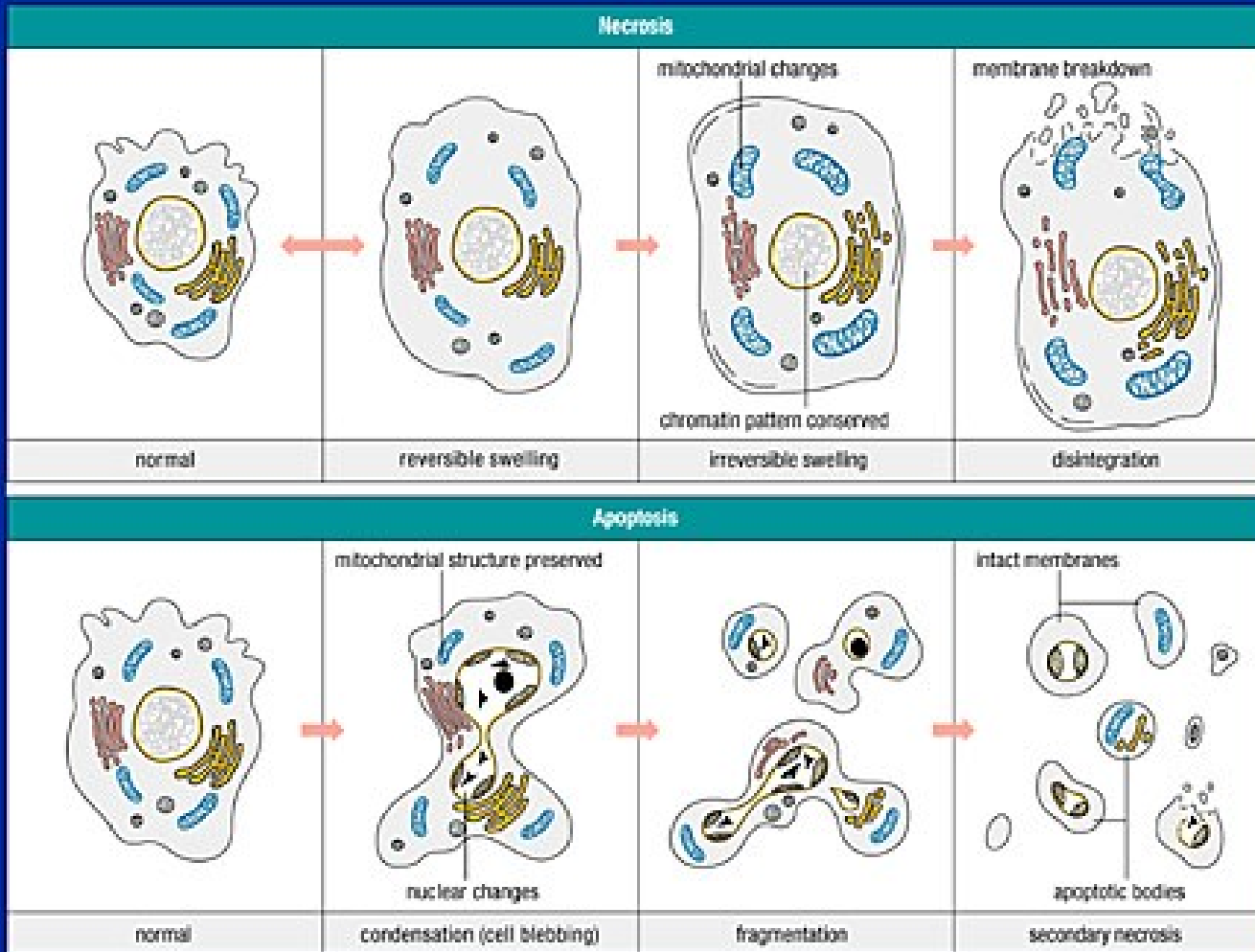


# Necrosis

- ✓ has been defined as a type of cell death that lacks the features of apoptosis and autophagy, and is usually considered to be uncontrolled.
- ✓ Recent research suggests, however, that its occurrence and course might be tightly regulated. After signaling- or damage-induced lesions, necrosis can include signs of controlled processes such
- ✓ as mitochondrial dysfunction,
- ✓ enhanced generation of reactive oxygen species,
- ✓ ATP depletion,
- ✓ proteolysis by calpains and cathepsins,
- ✓ early plasma membrane rupture.
- ✓ In addition, the inhibition of specific proteins involved in regulating apoptosis or autophagy can change the type of cell death to necrosis. Because necrosis is prominent in ischemia, trauma and possibly some forms of neurodegeneration, further biochemical comprehension and molecular definition of this process could have important clinical implications.



# Two Different Types of Cell Death





# Apoptosis-description I

- ✔ is a fundamental process that is essential for development and homeostasis, but also contributes to diverse pathologic processes, ranging from cancer and atherosclerosis to rheumatic and neurodegenerative diseases.
- ✔ The endothelium senses and transduces signals between blood and tissue, orchestrates the trafficking of hematopoietic cells, maintains a non-thrombogenic surface permitting the flow of blood, and initiates and amplifies the inflammatory response. Situated at the interface between blood and tissue, the endothelium is exposed to stimuli with the potential to promote or prevent.
- ✔ Physiological endothelial function involves a balance between pro- and anti-apoptotic signals, and perturbation of this balance may contribute to the pathogenesis of diverse vascular diseases.



# Apoptosis-description II

- In this particular mechanism, and there are many, procaspase (inactive form) is activated to the protease caspase. An amplification cascade then ensues with caspases activating other caspases, eventually cleaving the host cell by acting on a variety of cell structures such as the nuclear membrane. The cell shrinks in the process and there is a loss of cell-cell junctions resulting in detachment from adjacent cells. The chromatin condenses, the cytoplasm 'blebs' (forms so-called 'pseudopods') and the cell breaks up into fragments known as 'apoptotic bodies'. Indirectly activated endonucleases lead to breakdown of the DNA into multiples of 180-200 base pair fragments. Finally, either macrophages or adjacent cells phagocytose the apoptotic bodies.



# Apoptosis-description III

- ✓ The entire process of takes about 1 h from initiation The initiating triggers are many and varied, and are grouped broadly as physiological or nonphysiological.
- ✓ These include, but are not limited to, the following: Fas ligands (Fas), tumour necrosis factor (TNF), nerve growth factor (NGF), nitric oxide (NO), lipopolysaccharide (LPS), host immune reactions, kinins and glucocorticoids.
- ✓ The best characterised apoptotic trigger is the Fas ligand, a member of the TNF super-family. The Fas receptor is a cell surface glycoprotein that mediates apoptotic signals from the cell surface into the cytoplasm. When the Fas ligand binds to the Fas receptor on the cell membrane, the newly formed Fas complex is allowed to associate with intracellular proteins. The morphological changes of specific intracellular proteins induced by this complex result in the activation of other substances such as IL-1 $\beta$  converting enzyme (ICE).



# Definition

- Apoptosis is now recognized as an important process in different biological systems, including embryonic development, cell turnover, and immune response against tumorigenic or virus-infected cells.
- Under either physiological or pathological conditions, apoptosis is mostly driven by interactions among several families of protein, i.e. caspases, Bcl-2 family proteins, and inhibitor of apoptosis proteins (IAP). Other proteases such as granzyme B, calpain and cathepsin have also been demonstrated to play a vital role in apoptosis occurring under certain physiological states.



# Decision phase (genetic control)

- ✔ is controlled genetically and two genes, *Bcl-2* and *p53* are important. The first, *Bcl-2*, is a family of genes that regulates apoptosis; found on the mitochondrial membrane, endoplasmic reticulum it may control calcium channels. It is now recognised that there is a family of mammalian proteins similar to *Bcl-2* that promotes or inhibits apoptosis. Proteins such as *Bcl-2* and *Bcl-xL* prevent, whereas *Bcl-2* associated x proteins (*Bax*) such as *Bax*, *Bad*, *Bak* and *Bcl-xS* promote apoptotic processes.
- ✔ The gene *p53* is a 53-kDa nuclear phospho-protein that binds to DNA to act as a transcription factor, and controls cell proliferation and DNA repair. Mutations of *p53* have been found in > 50% of human cancers (e.g. colon carcinoma) and are associated with resistance to treatment. The gene *c-myc* is a proto-oncogene that encodes a sequence-specific DNA-binding protein that acts as a transcription factor and induces in the presence of *p53*. The *c-myc* protein is elevated in many tumours.



# Execution phase

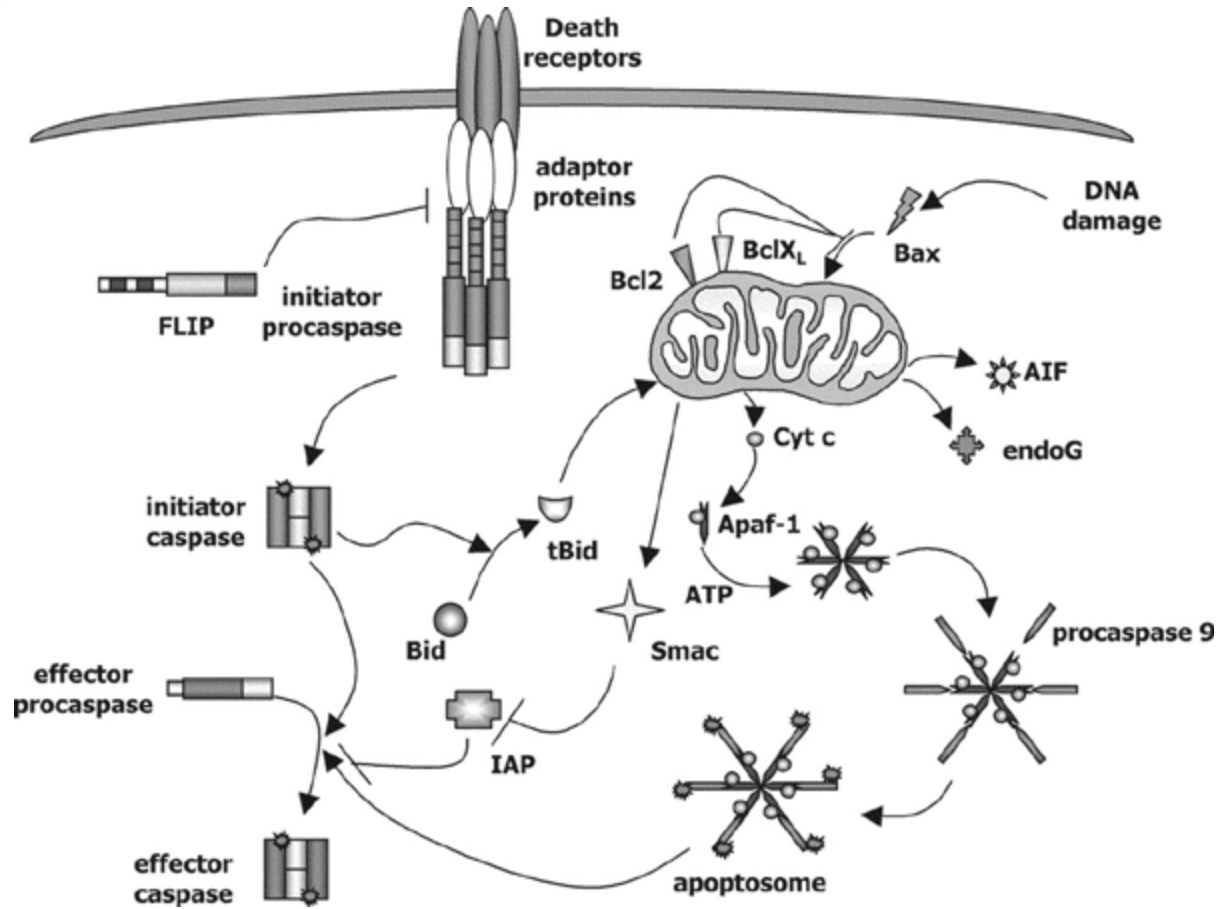
- ✔ The central events in are proteolysis and mitochondrial inactivation. Cellular disruption results from activation of a family of cysteine proteases called caspases (CASP).
- ✔ Caspases are proenzymes that have been conserved from nematodes to humans. Ten human caspases (CASP 1-10) have been described. There are two subfamilies of caspases, the *ced-3* subfamily (produced by *ced-3* gene) and the ICE (IL-1 $\beta$ -converting enzyme) subfamily. Caspase 1, which is related to ICE, is mainly involved in inflammation. The *ced-3* caspases are important effectors of apoptosis. Caspase 8 or FADD-like interleukin converting enzyme (FLICE) is the most important enzyme of the *ced-3* subfamily. The actions of the caspases are varied; some are endonucleases that cleave DNA, some cleave cytoskeletal proteins and others cause a loss of cell adhesion.
- ✔ The integrity of the plasma membrane of the apoptotic cell is maintained initially, although 'budding' of the cell membrane can occur later. There is no leakage of lysosomal enzymes that can damage nearby cells or elicit immune responses. The apoptotic cell expresses membrane signals that induce phagocytosis. Macrophages can recognise neutrophils undergoing via complexes involving thrombospondin receptors (CD36) and the  $\alpha\text{v}\beta 3$  integrin.



# Mechanisms of apoptosis

- ✓ Apoptosis can be initiated by two pathways. The death-receptor pathway (**extrinsic pathway**) is induced by ligand binding to TNFR superfamily members. Receptors then recruit adaptor proteins through DD homophilic interactions. Adaptor proteins in turn recruit initiator pro-caspases by DED interaction, leading to DISC formation. Initiator procaspases are then converted in active caspases able to cleave substrates such as Bid or effector pro-caspases. The death receptor-induced triggering of apoptosis is impaired by recruitment of FLIP, an enzymatically inactive initiator caspase homologue.
- ✓ The second pathway (**intrinsic pathway**) is triggered by mitochondria in response to intracellular injuries such as DNA damage. Pro-apoptotic members of the Bcl-2 family of proteins induce mitochondrial release of various molecules such as cytochrome *c*, which can be counteracted by anti-apoptotic Bcl-2 family members. Cytochrome *c* binds to Apaf-1, which upon ATP-dependent conformational change and oligomerization associates with pro-caspase 9, forming the apoptosome, which is able to cleave effector pro-caspases. Effector caspase cleavage can be inhibited through activity of IAP proteins, which are themselves antagonized by the Smac/DIABLO protein released from mitochondria. Cleavage of Bid ensures the cross-talk between both apoptosis signaling pathways, since truncated Bid inserts itself in the mitochondrial membranes and induces cytochrome *c* release.







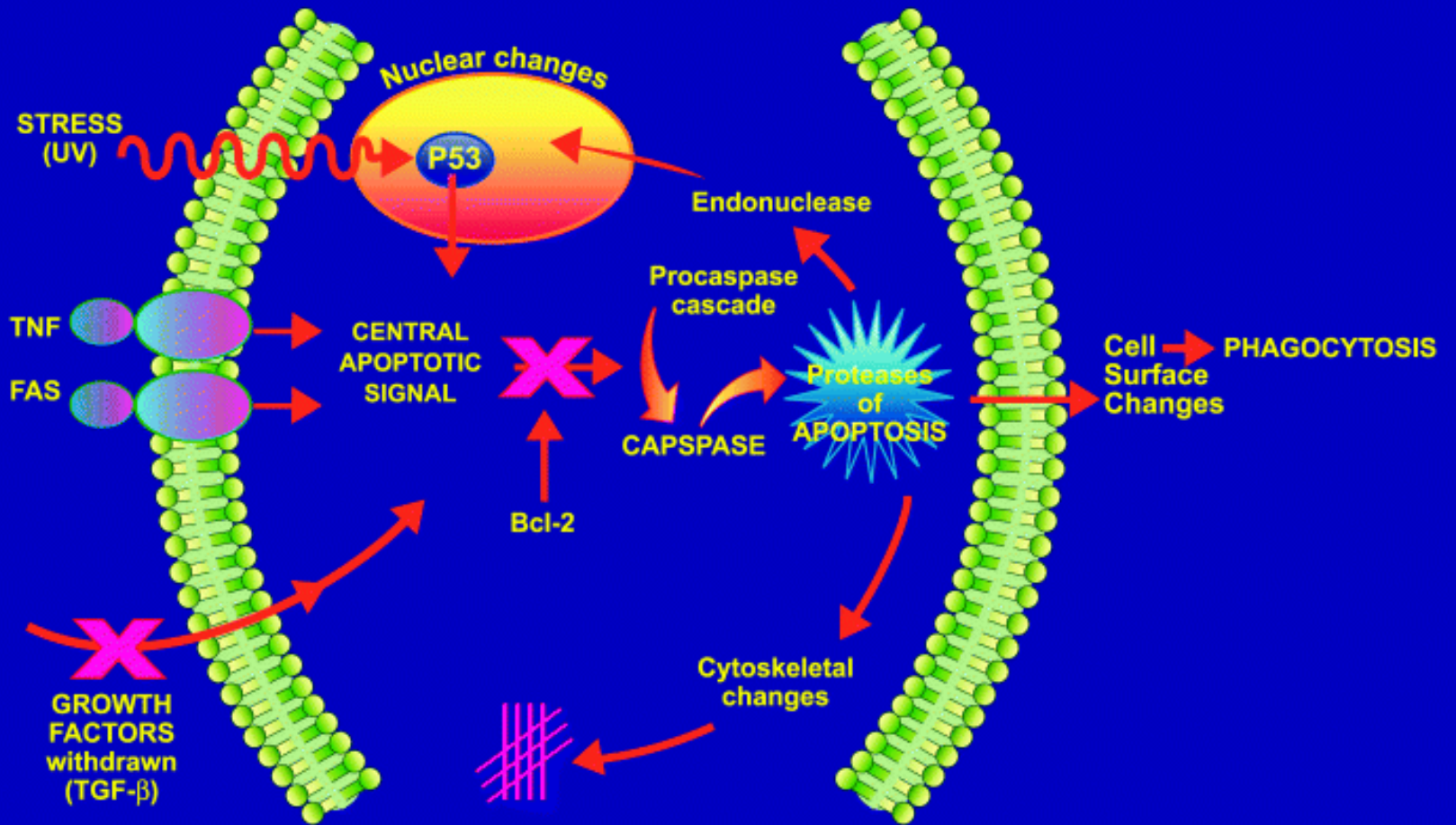
# Apoptosis pathways

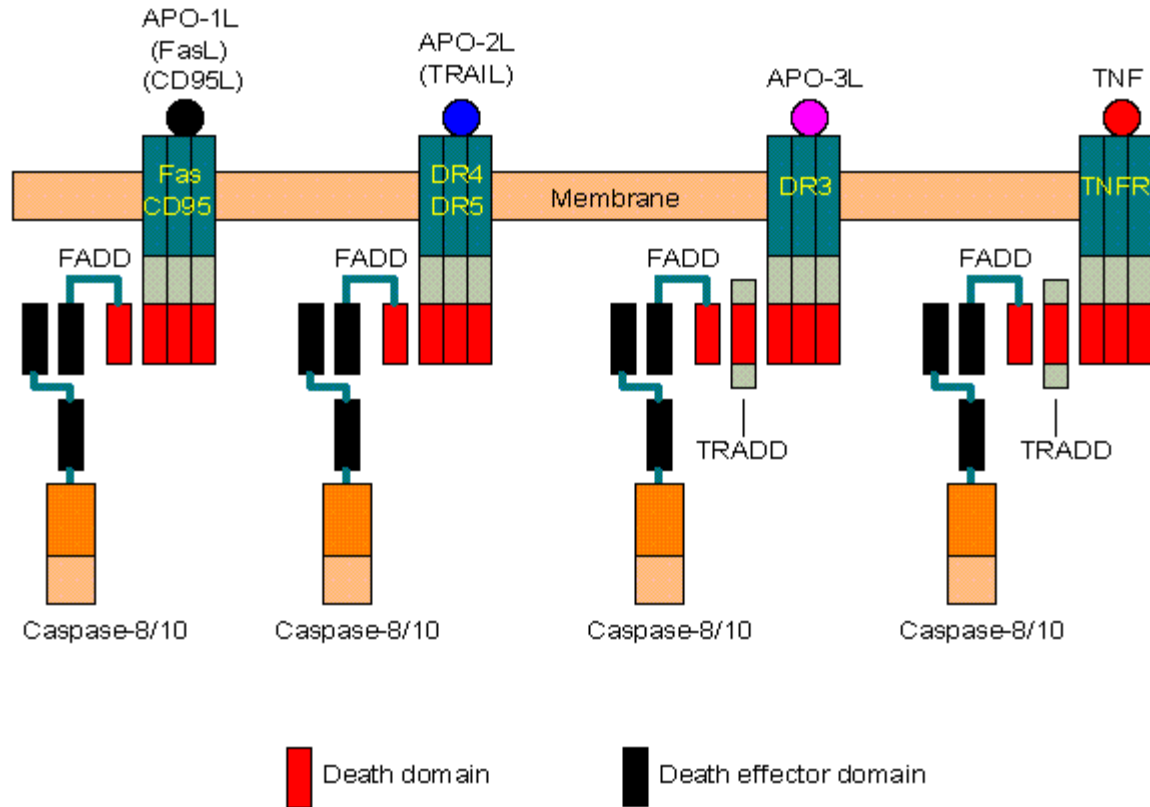
- ✔ In the past two decades there has been remarkable progress in defining the genes and pathways that regulate.
- ✔ Several gene families play a pivotal role in regulation of in endothelial cells as in other cell types. The caspase family of cysteine proteases includes proteases that initiate and proteases that act as executioners, ultimately resulting in the dismantling of the apoptotic cell.
- ✔ The Bcl-2 family includes both pro-apoptotic proteins and anti-apoptotic proteins that largely determine whether a cell lives or dies.
- ✔ Inhibitors of proteins (IAPs) directly bind and inhibit caspases, and are also important determinants of cell fate during.
- ✔ Operationally, one can define 'apoptotic' death as a process mediated by caspases and/or inhibited by anti-apoptotic Bcl-2 proteins or IAPs. However, it is important to recognize that there are caspase-independent mechanisms of apoptotic cell death, non-apoptotic functions of caspases, and functions of Bcl-2 proteins apart from.



# Apoptosis-signal for

- ✓ The signal that initiates may result from binding of a cell-surface 'death' receptor or from damage to the genome. Death receptors that initiate include the Fas receptor and the TNF receptor system.
- ✓ The Fas receptor is a transmembrane glycoprotein death receptor that is activated by binding of Fas ligand (Fas-L) to cell membranes. Intracellular molecules known as Fas-associated death domain (FADD) are produced. Fas receptors are found in epithelial tissues, tumours and haemopoietic tissues, and may be induced in other tissues that do not express them. The Fas pathway is important in controlling the immune response. Cytotoxic T lymphocytes expressing Fas ligands activate cells bearing Fas receptors and induce apoptosis.







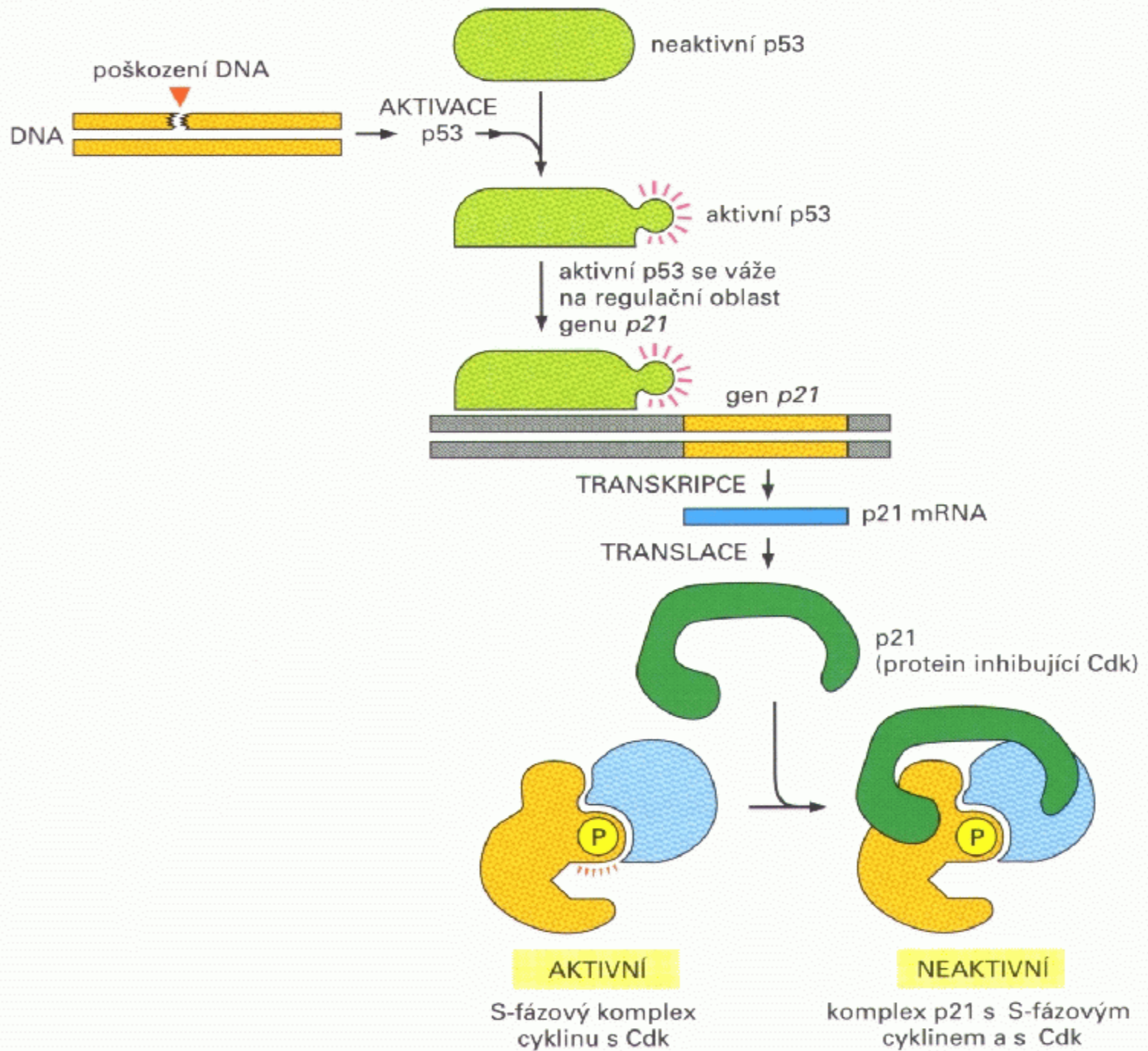
# To the previous picture

- ✓ **Death receptors:** Fas/CD95, DR4/DR5, DR3, and **TNFR** (Tumor Necrosis Factor Receptor).
- ✓ **Adaptors:** **FADD** (Fas-associated death domain protein) and **TRADD** (TNFR-associated death domain protein).
- ✓ **Activation:** Binding of death ligands (FasL/CD95L, TRAIL/APO-2L, APO-3L and TNF) induces trimerization of their receptors, which then recruit adaptors and activate caspases.
- ✓ **Note:** TRADD is involved only in the coupling between caspases and DR3 or TNFR. This adaptor can also recruit other proteins to inhibit apoptosis through the NF- $\kappa$ B pathway.

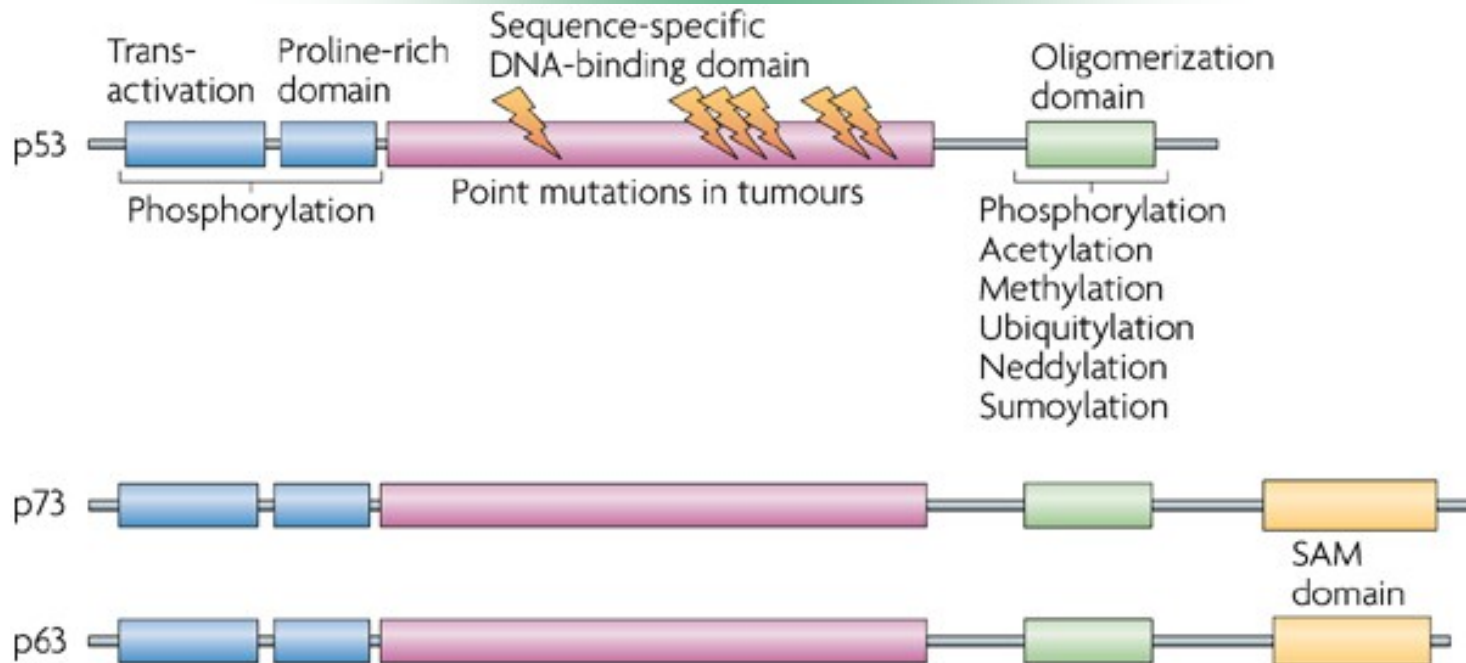


# TNF receptor system

- ✓ The TNF receptor system mediates different biochemical pathways. A TNF-related -inducing ligand (TRAIL) has been discovered. Cancer cells are susceptible to TRAIL-induced apoptosis. Following binding of the TNF receptor, intracellular molecules called 'death domains' are produced. A TNF receptor associated death domain (TRADD) has been identified. Tumour necrosis factor may suppress by binding to the receptor, TNFR2, which activates a protein known as nuclear factor  $\kappa$ B (NF- $\kappa$ B), classed as an inhibitor of protein (IAP) that prevents the execution phase of apoptosis. It is a DNA binding protein that regulates many pro-inflammatory genes for the production of cytokines and other pro-inflammatory molecules.



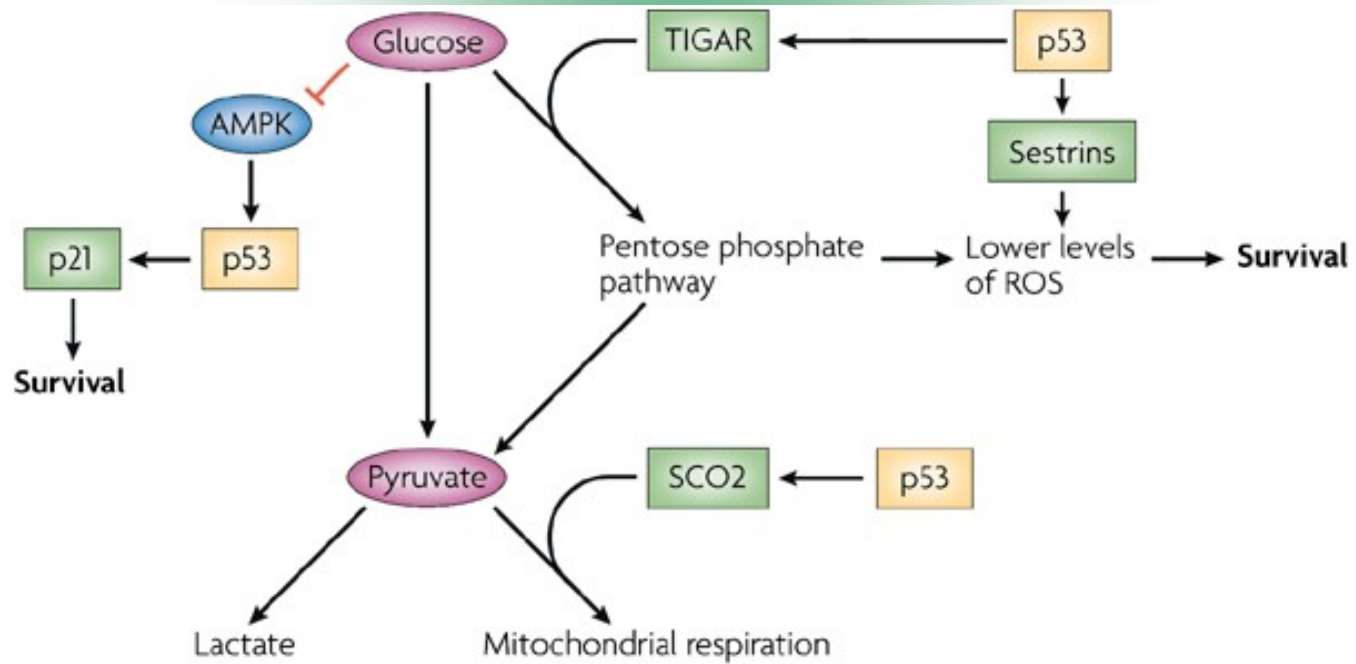






# P53-to the previous figure

- ✓ The major functional domains of the p53 protein are shown, including the N-terminal transactivation domains, the central sequence-specific DNA-binding domain and the C-terminal regulatory domain.
- ✓ p53 is subject to numerous post-transcriptional modifications, including phosphorylation, acetylation, methylation and modification with ubiquitin-like proteins, that can affect the function and stability of p53. Phosphatases, de-acetylases and de-ubiquitylating enzymes have been identified that can reverse most of these modifications.
- ✓ Most of the point mutations found in naturally occurring cancers occur in the central DNA-binding domain, and the position of the hotspots for these mutations are indicated by the orange lightning bolts. The p53-related proteins p63 and p73 show a similar overall structure, although some isoforms of these p53 relatives also contain a C-terminal sterile -motif (SAM) domain.

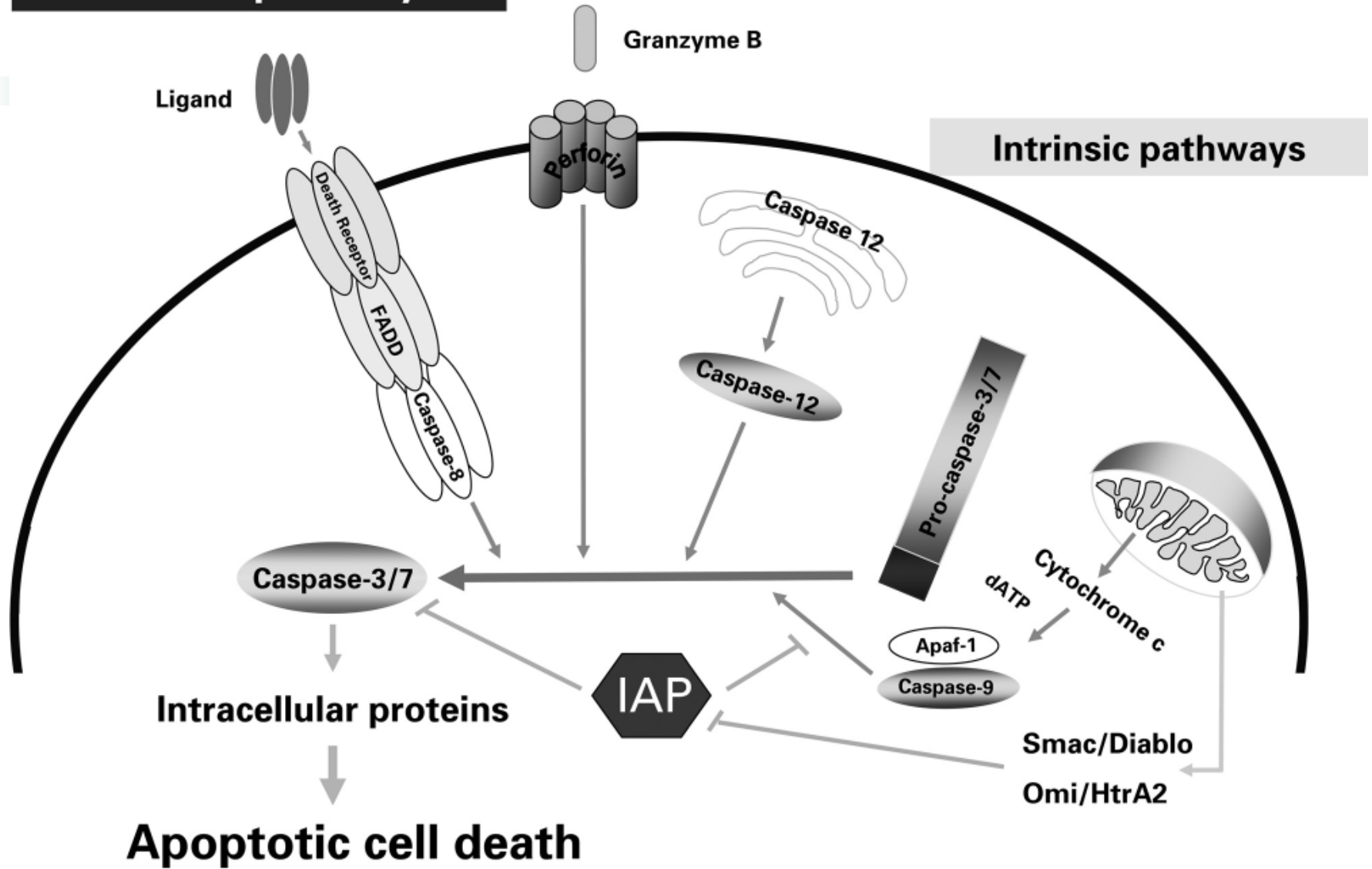




# P53-metabolic pathway

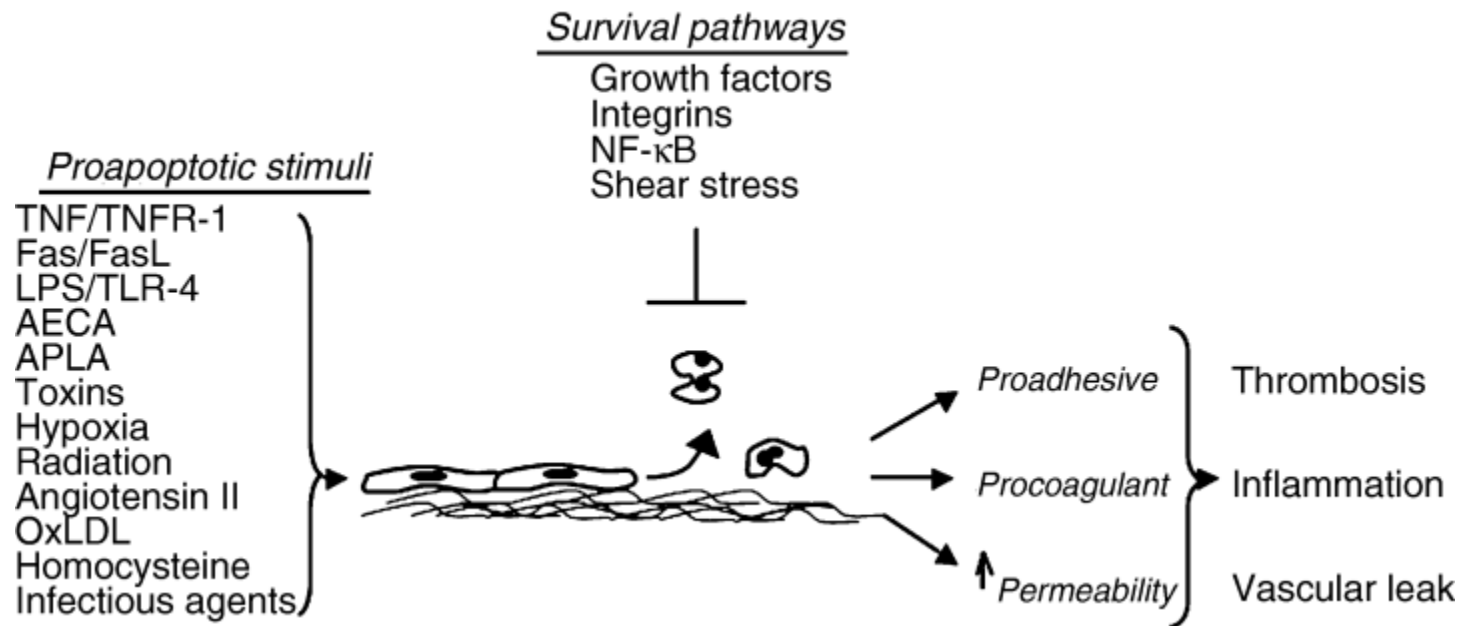
- Some of the points at which p53 can affect metabolic pathways. This is a new and rapidly moving area of research, and the influence of p53 on metabolism is likely to be much broader than illustrated here. In response to nutrient stress, p53 can become activated by AMP kinase (AMPK), promoting cell survival through an activation of the cyclin-dependent kinase inhibitor p21. Other functions of p53 include regulating respiration, through the action of SCO2, or in decreasing the levels of reactive oxygen species (ROS), through the actions of TIGAR (Tp53-inducible glycolysis and apoptosis regulator) or sestrins.

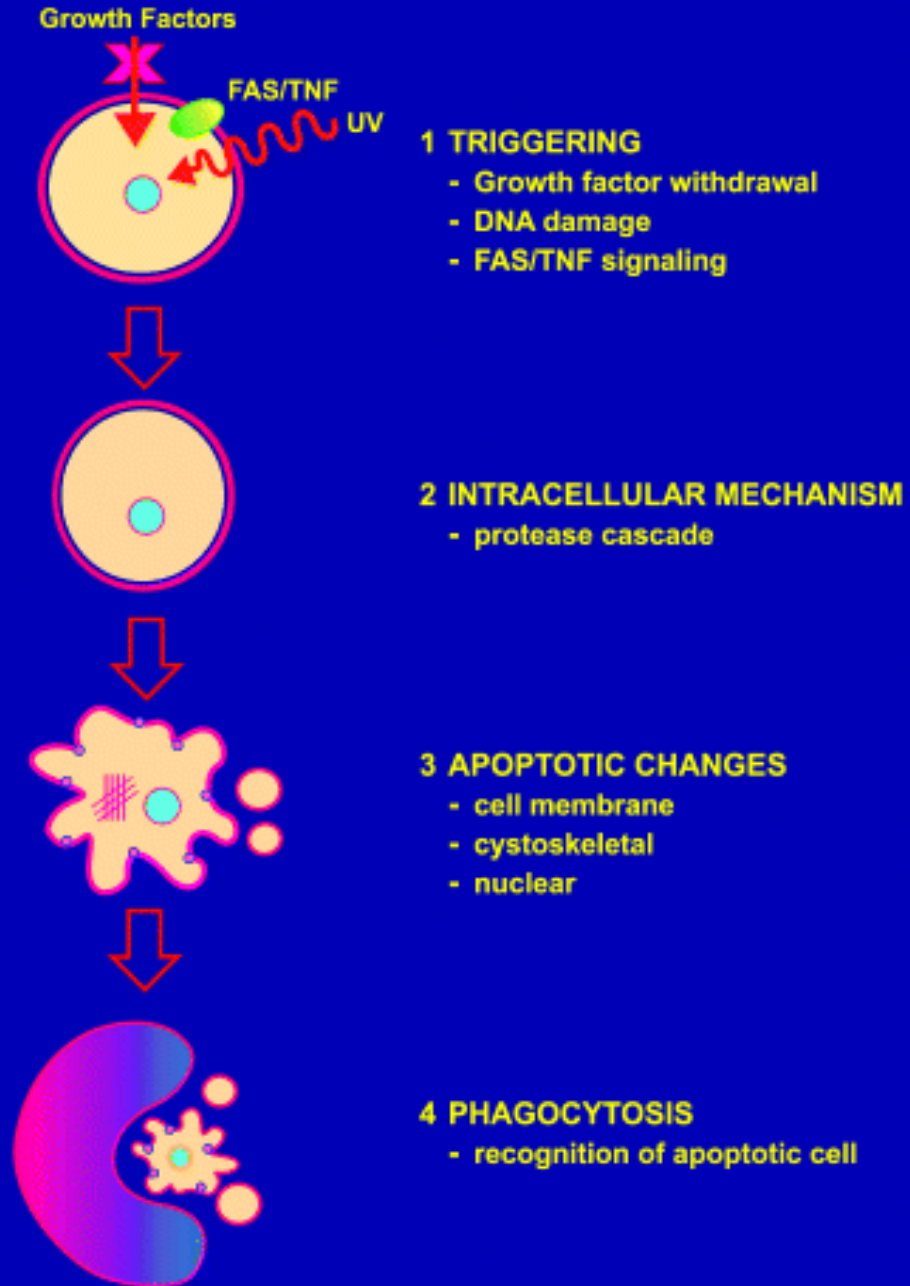
# Extrinsic pathways





Endothelial cell apoptosis can lead to disruption of the endothelial barrier with vascular leak, extravasation of plasma proteins, and exposure of a prothrombotic subendothelial matrix. Apoptotic endothelial cells are themselves procoagulant and proadhesive *in vitro*. Endothelial apoptosis thus has the potential to be an important mechanism of vascular injury and dysfunction







# Caspase family

- ✓ Caspase stands for cysteine-dependent aspartate-specific protease. To date, at least 14 members of this family have been identified in mammals although not all of them function during apoptosis.
- ✓ Members of the caspase family can be divided into three subgroups
- ✓ initiators in apoptosis (caspase-2, 8, 9 and 10)
- ✓ Executioners in apoptosis (caspase-3, 6 and 7)
- ✓ participants in cytokine activation (caspase-1, 4, 5, 11, 12, 13 and 14).





# Initiator caspases and adaptor proteins

- ✓ Initiator caspases play a role in initiating the apoptotic pathway. A different combination of initiator caspases, adaptors and regulatory proteins are required for the control and execution of different death stimuli.
- ✓ Caspase-2 is known to mediate stress-induced apoptotic death such as
- ✓  $\beta$ -amyloid toxicity and trophic factor deprivation.
- ✓ Activation of caspase-2 involves a large protein complex containing the death domain-containing protein PIDD and the adaptor protein RAIDD.
- ✓ Caspase-8 and its adaptor, FADD, are needed for Fas- and TNF-R1-transduced apoptosis although they are dispensable for other cell death pathways
- ✓ In thymocytes and embryonic fibroblasts, caspase-9 and its adaptor Apaf-1 are required for DNA damage, corticosteroid and staurosporine-induced cell death but not Fas- and TNF-R1-transduced apoptosis



# Initiator caspases and adaptor proteins

- ✔ Caspase-10 recruitment during TRAIL and Fas mediated apoptosis also requires FADD.
- ✔ Both caspase-8 and caspase-10 contain death effector domain and they appear to play a major role in Fas-mediated apoptosis in human T cells.
- ✔ Caspase-12, which is involved in the maturation of the cytokines, was activated in endoplasmic reticulum by stress-induced apoptotic signals and, in turn, processed downstream executioner caspases. In this aspect, caspase-12 may be considered as an initiator caspase.



# Executioner caspases

- ✔ Subsequent to the recruitment and autocatalytic cleavage of caspase-8 and caspase-9, a second subpopulation of caspases, caspase-3, 6 and 7, are activated.
- ✔ These are known as the executioner caspases because they play a key role in the enzymatic cleavage of a variety of cellular proteins.



# Stimuli for apoptosis

DNA (genome) damage	Ionising radiation
	Anti-cancer drugs (e.g. alkylating agents)
Activation of death receptors	Binding of 'death receptors' (e.g. Fas receptor, TNF receptor)
	Withdrawal of growth factors (e.g. nerve growth factor, IL-3)
Stimulation of apoptotic pathway	Phosphatases, kinase inhibitors
Direct physical cell damage	Heat, ultraviolet light, oxygen free radicals, hydrogen peroxide
DNA, deoxyribonucleic acid; TNF, tumour necrosis factor; IL-3, interleukin-3.	



# Proapoptotic stimuli

## ✓ **Death receptors**

- ✓ The death receptor family of cell surface receptors, including Fas, tumor necrosis factor receptor (TNFR-1) and TRAIL-R, can initiate in multiple cell types. Ligation of the death receptor recruits the adaptor molecule Fas-associated death domain (FADD) and leads to the aggregation of pro-caspase-8, which auto-activates and then activates downstream effector caspases such as caspase-3.
- ✓ The death receptor ligands, TNF $\alpha$  and TRAIL, can directly kill endothelial cells *in vitro*. TNF $\alpha$ -mediated killing is exaggerated by inhibitors of protein synthesis, due in part to inhibition of NF- $\kappa$ B-dependent anti-apoptotic proteins.



# Proapoptotic stimuli

## ✔ **Toll-like receptors**

- ✔ Toll-like receptors (TLRs) are pattern recognition receptors that are a significant part of the innate immune system and share signaling pathways with interleukin-1 (IL-1)/IL-1receptor (IL-1-R).
- ✔ TLRs and IL-1-R interact with the intracellular adaptor molecules. Signaling induced by ligation of TLRs and IL-1-R is mostly pro-inflammatory.



# Proapoptotic stimuli

## ✔ **Other stimuli**

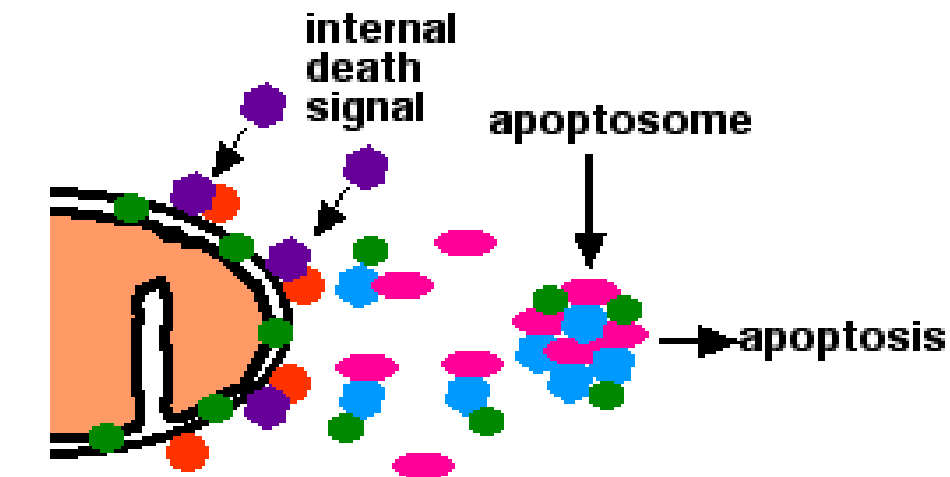
- ✔ In addition to death receptor and TLR signaling, a wide variety of stimuli have been reported to induce apoptosis of endothelial cells *in vitro*.
- ✔ These include antiendothelial cell antibodies (AECA) and antiphospholipid antibodies, infectious organisms and toxins, hypoxia and hyperoxia, angiotensin II, homocysteine, radiation, and oxidants such as oxidized low-density lipoprotein (oxLDL).



# Apoptosis triggered by internal signals: the intrinsic or mitochondrial pathway

- ✓ In a healthy cell, the outer membranes of its mitochondria display the protein [Bcl-2](#) on their surface. Bcl-2 **inhibits** apoptosis.
- ✓ Internal damage to the cell (e.g., from [reactive oxygen species](#)) causes
  - related proteins, **Bad** and **Bax**, to migrate to the surface of the mitochondrion where they bind to Bcl-2 — blocking its protective effect — and punch holes in the outer mitochondrial membrane, causing
    - [cytochrome c](#) to leak out.
- ✓ The released cytochrome c binds to the protein **Apaf-1** ("apoptotic protease activating factor-1"). Using the energy provided by [ATP](#), these complexes aggregate to form **apoptosomes**. The apoptosomes bind to and activate **caspase-9**.
- ✓ Caspase-9 is one of a family of over a dozen caspases. They are all [proteases](#). They get their name because they cleave proteins — mostly each other — at aspartic acid ([Asp](#)) residues). Caspase-9 cleaves and, in so doing, activates other caspases (caspase-3 and -7).
- ✓ The activation of these "executioner" caspases creates an expanding cascade of proteolytic activity (rather like that in [blood clotting](#) and [complement](#) activation) which leads to
  - digestion of structural proteins in the cytoplasm,
  - degradation of chromosomal DNA,
  - and phagocytosis of the cell.

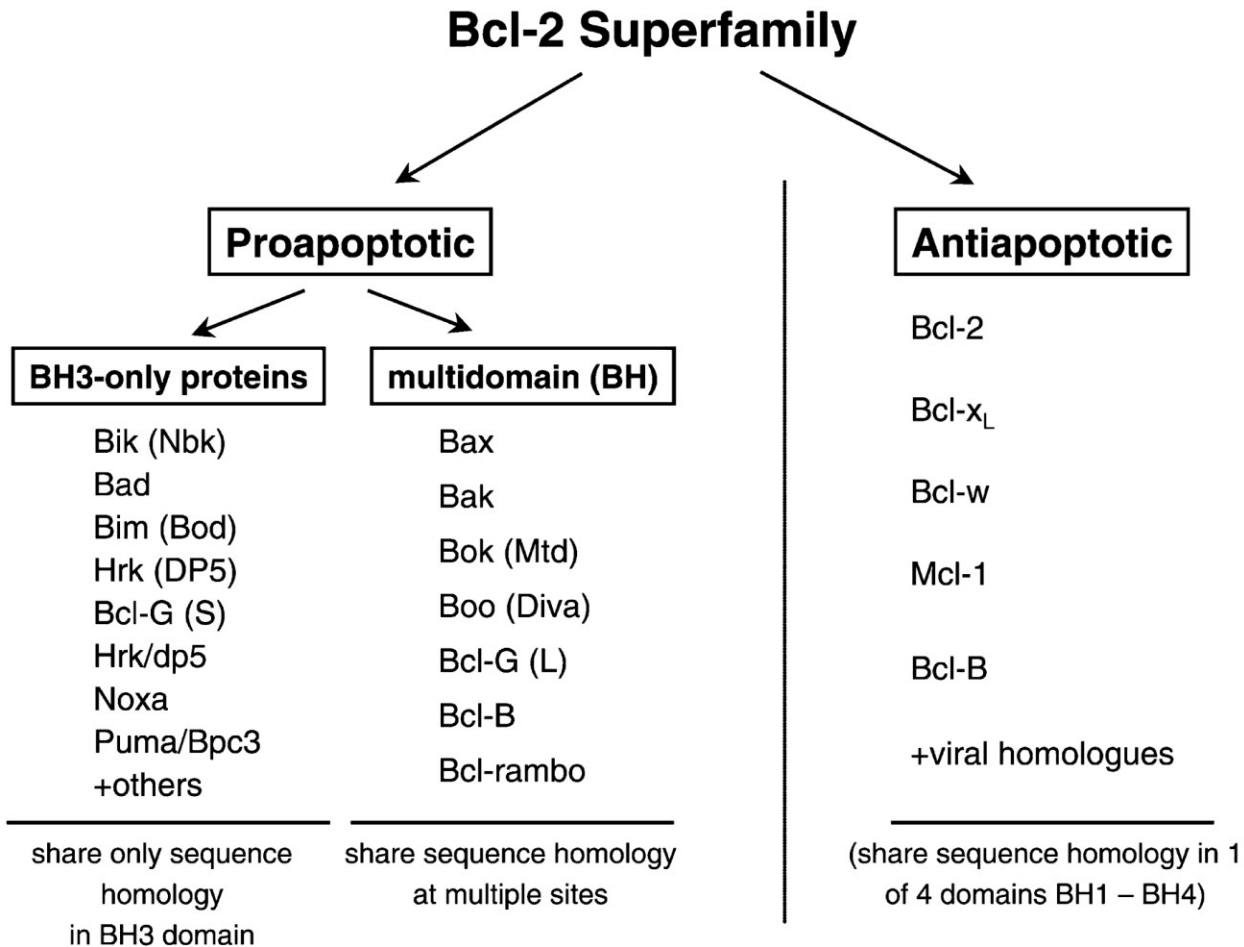




- Bcl-2
- Bad, Bax
- Apaf-1
- caspase 9
- cytochrome c



Members of the Bcl-2 superfamily are key regulators of mitochondrial apoptosis  
The Bcl-2 superfamily can be subdivided into pro- and antiapoptotic family members

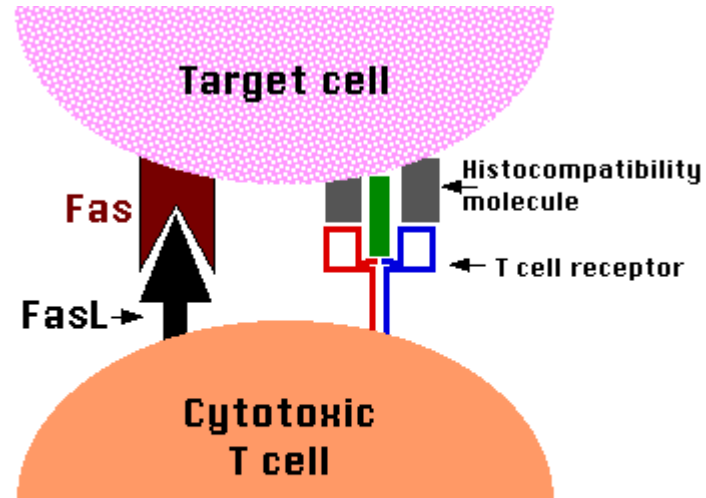


Mayer, B. et al. *News Physiol Sci* 18: 89-94 2003;  
doi:10.1152/nips.01433.2002



## Apoptosis triggered by external signals: the extrinsic or death receptor pathway

- ✓ **Fas** and the **TNF receptor** are [integral membrane proteins](#) with their receptor domains exposed at the surface of the cell
- ✓ binding of the complementary **death activator** (**FasL** and **TNF** respectively) transmits a signal to the cytoplasm that leads to
- ✓ activation of **caspase 8**
- ✓ caspase 8 (like caspase 9) initiates a cascade of caspase activation leading to
- ✓ phagocytosis of the cell.

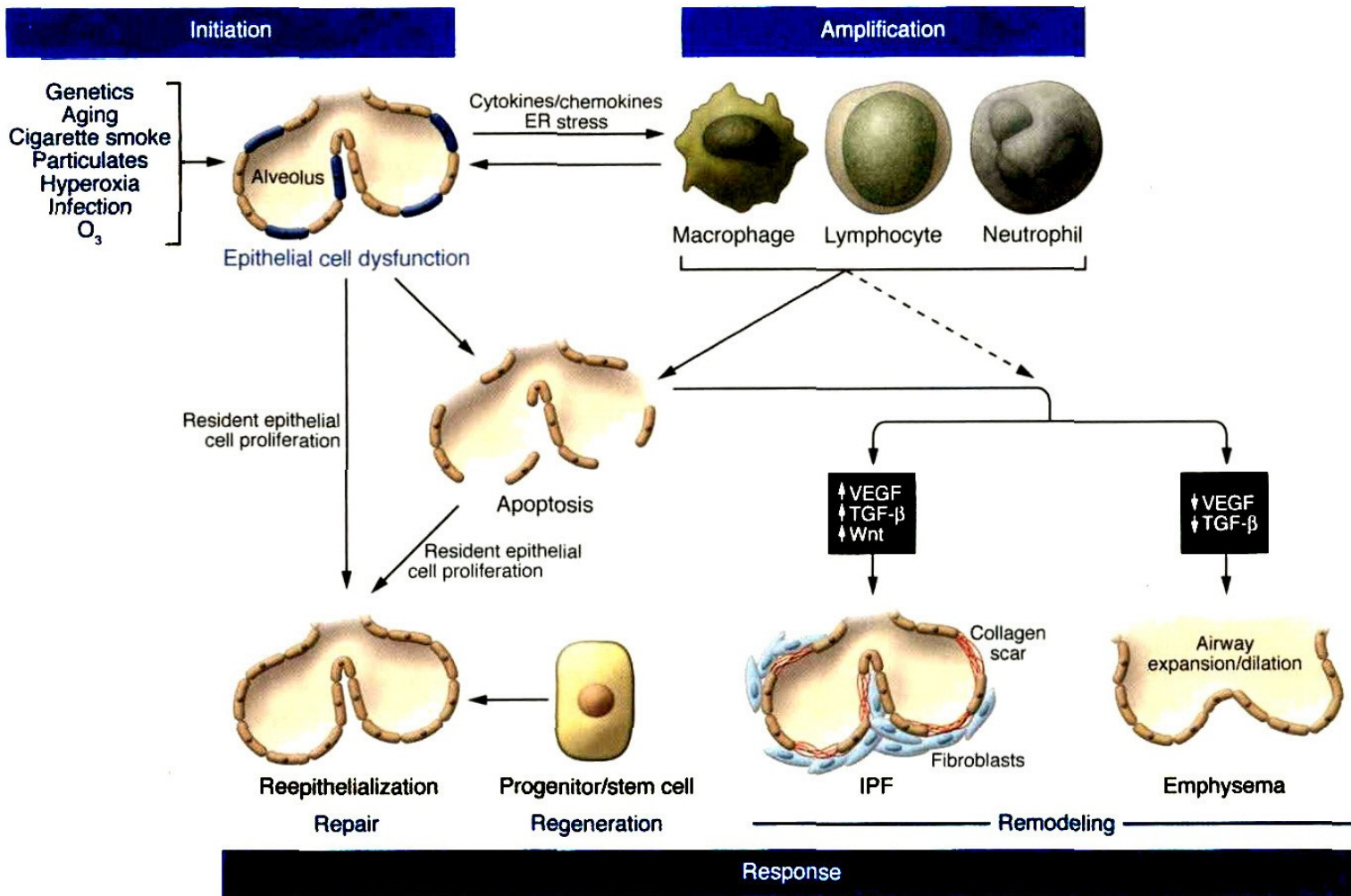


One method by which cytotoxic T cells induce their targets (e.g., virus-infected cells) to commit suicide (apoptosis)



# Human diseases associated with disordered apoptosis

Increased apoptosis	Decreased apoptosis
Central nervous system	Degenerative diseases (Alzheimer's and Parkinson's disease)
	Cerebral ischaemia
Myocardium	Peri-infarct border zones
Lymphocytes	Lymphocyte depletion in sepsis and HIV infection
Macrophages	Bacillary dysentery ( <i>Shigella dysenteriae</i> )
Decreased apoptosis	
Epithelial tissues	Carcinogenesis
Blood vessels	Intimal hyperplasia
Lymphocytes	Autoimmune disorders
Haemopoietic system	Leukaemia, lymphoma

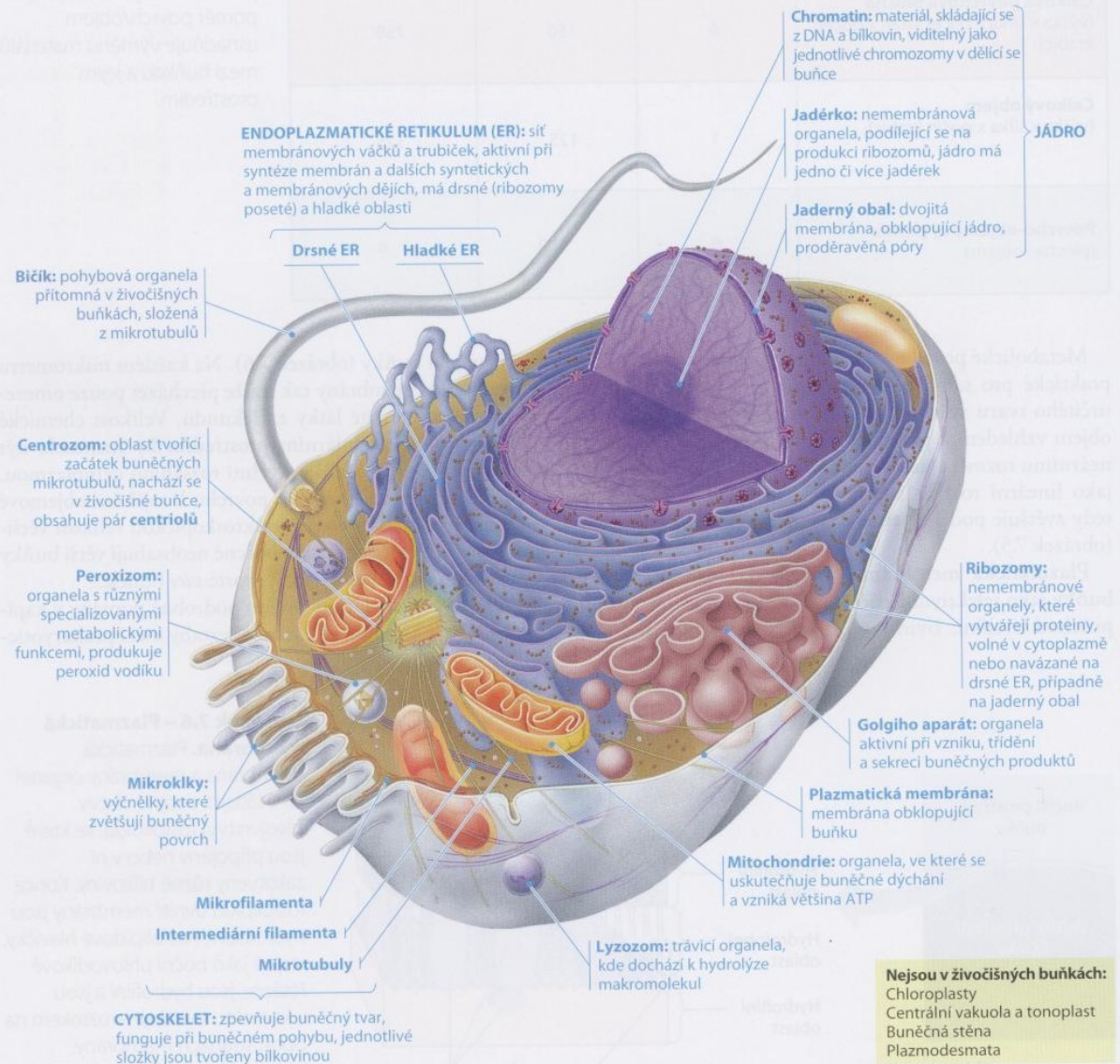


**Figure 3**

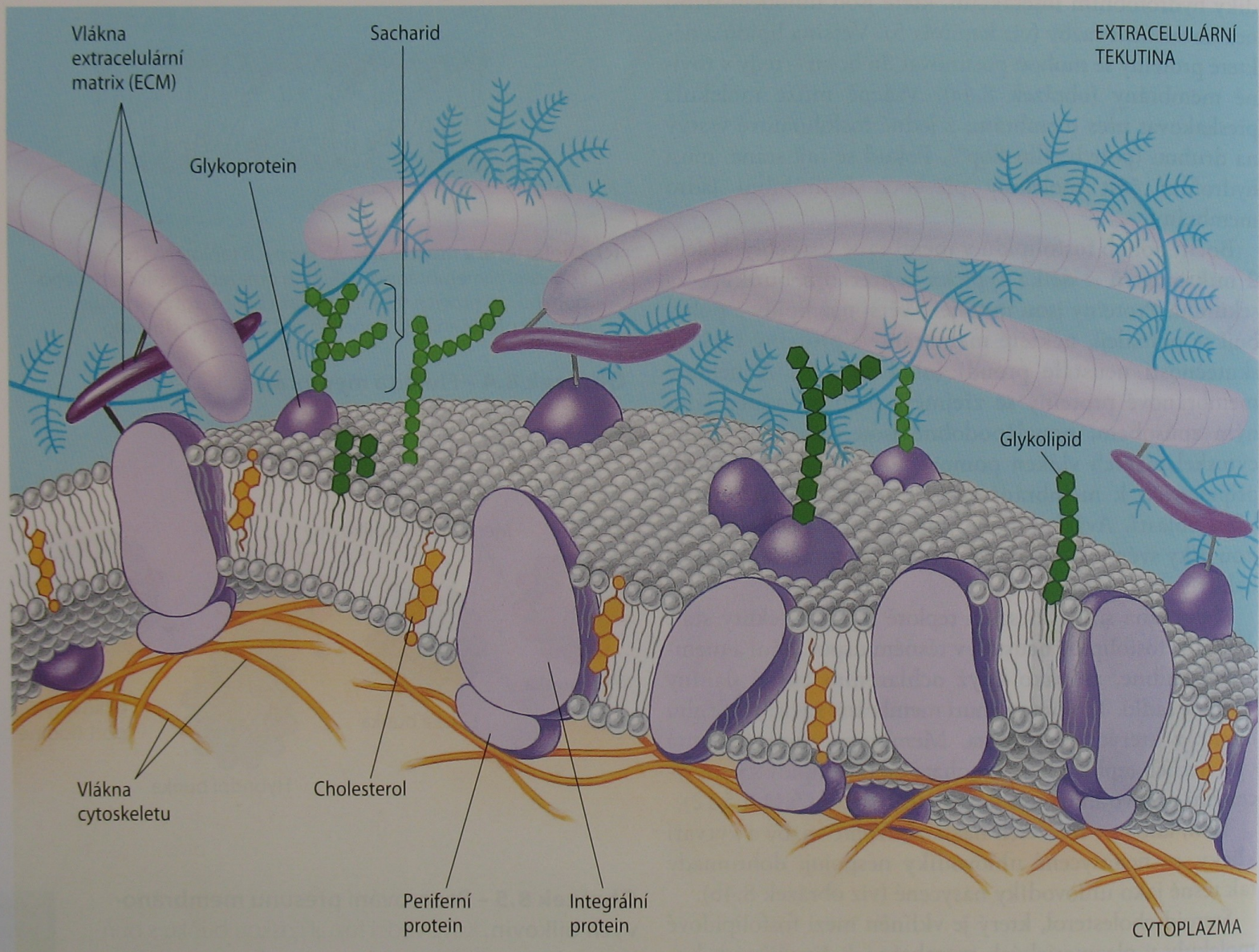
The epithelial-mesenchymal wound model for lung injury and repair. In the initiation stage, multiple microinjuries damage and activate alveolar epithelial cells. In the amplification stage, the resulting ER stress and other activating signals in AEC cells can promote release of chemokines/cytokines, promoting migration of inflammatory effector cells into the distal lung. Persistent activation and/or ongoing ER stress can result in apoptosis and AEC cell drop-out. In the response stage, in order to restore the denuded epithelial surface, repair via reepithelialization is attempted using either local proliferation/transdifferentiation of AT2 cells or regional expansion of progenitor cell populations such as BASCs (regeneration). In the absence of successful reepithelialization (repair or regeneration), remodeling is initiated. In a fibrotic phenotype (e.g., IPF), the local milieu (including high TGF-β and high VEGF levels) promotes proliferation of fibroblasts, activation of myofibroblasts, increases in basement membrane disruption, and neovascularization, with resulting formation of a collagen scar. In emphysema, under different local conditions (including low VEGF and low TGF-β levels), airspace enlargement takes place.



# Cell

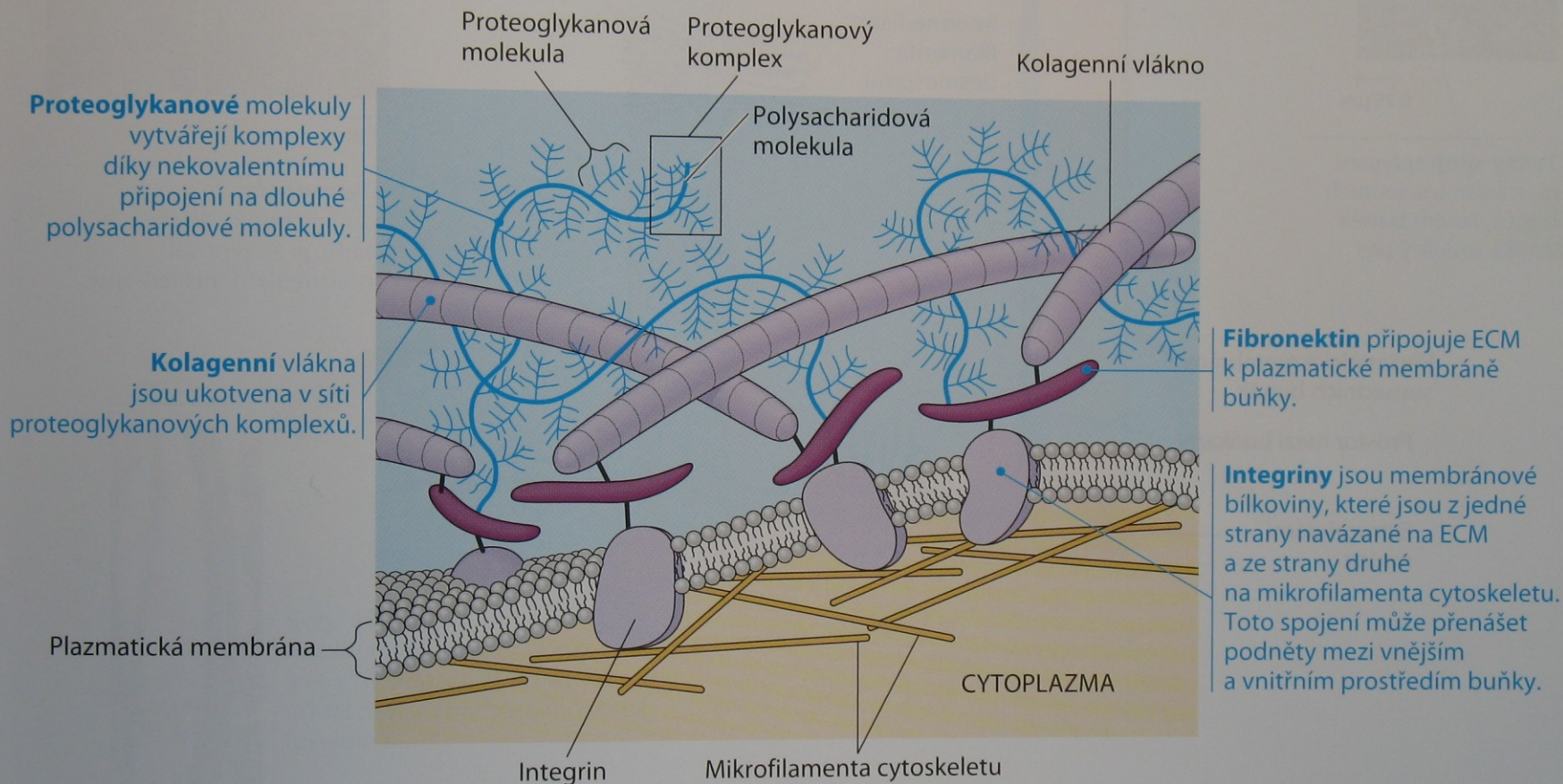


**Obrázek 7.7 – Schéma živočišné buňky.** V tomto náčrtu živočišné buňky jsou zahrnuty nejčastější struktury živočišných buněk (ve skutečnosti takhle nevypadá žádná buňka). Buňka má množství organel („malých orgánů“), mnoho z nich je ohraničeno membránami. Nejnápadnější organelou živočišné buňky je obvykle jádro. K většině metabolických aktivit dochází v cytoplazmě, oblastí mezi jádrem a plazmatickou membránou. Cytoplazma obsahuje mnoho organel, nacházejících se v polotekutém médiu, v cytosolu. Velkou částí cytoplazmy prostupuje membránový labyrint, nazývaný endoplazmatické retikulum (ER).



**Obrázek 8.6 – Podrobná struktura plazmatické membrány živočišné buňky, příčný řez.** Viz obrázek 7.29 – detaily ECM.





**Obrázek 7.29 – Extracelulární matrix (ECM) živočišné buňky.** Molekulární složení a struktura ECM se mezi jednotlivými buněčnými typy liší. Zde se nachází tři typy glykoproteinů: proteoglykan, kolagen a fibronektin. Kolagenová vlákna jsou ukotvena v proteoglykanových komplexech, které se skládají z molekul proteoglykanů, rozvětřujících se z dlouhých polysacharidových molekul jako malé stromečky. Fibronektinové molekuly jsou přílnavé, takže připojují ECM k plazmatické membráně buňky, a to díky svému spojení s membránovými bílkovinami, nazývanými integriny.



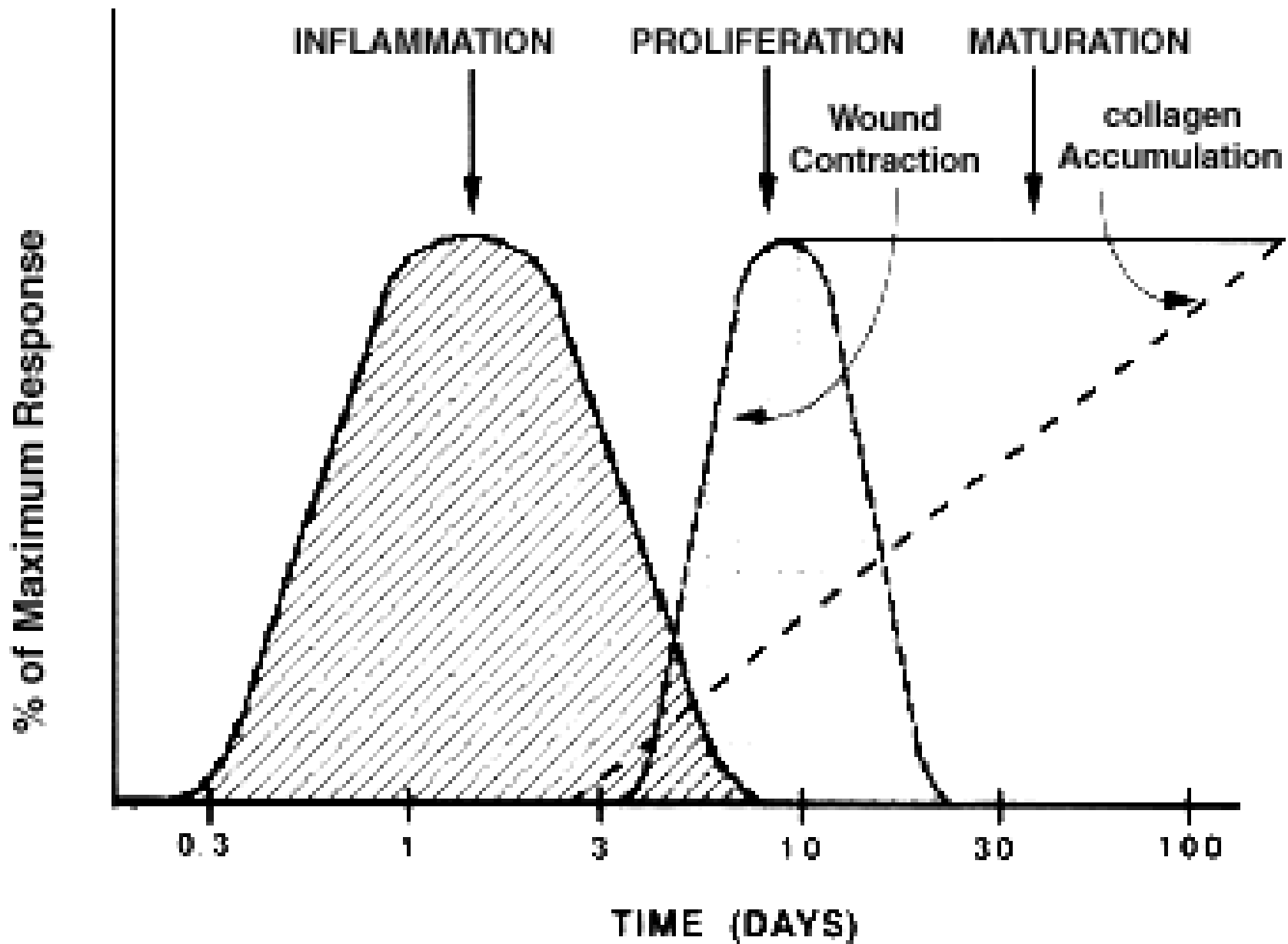
# Wound healing

- ✔ is a natural restorative response to tissue injury.
- ✔ Healing is the interaction of a complex cascade of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured skin.
- ✔ Under the most ideal circumstances, healing is a systematic process, traditionally explained in terms of 3 classic phases: **inflammation, proliferation, and maturation.**



# Wound healing

- **The inflammatory phase:** a clot forms and cells of inflammation debride injured tissue during.
- **The proliferative phase:** epithelialization, fibroplasia, and angiogenesis occur; additionally, granulation tissue forms and the wound begins to contract.
- **The maturation phase:** Collagen forms tight cross-links to other collagen and with protein molecules, increasing the tensile strength of the scar.





# Inflammatory Phase

- ✓ The body responds quickly to any disruption of the skin's surface.
- ✓ Within seconds of the injury, **blood vessels constrict** to control bleeding at the site.
- ✓ **Platelets** coalesce within minutes to stop the bleeding and begin clot formation.

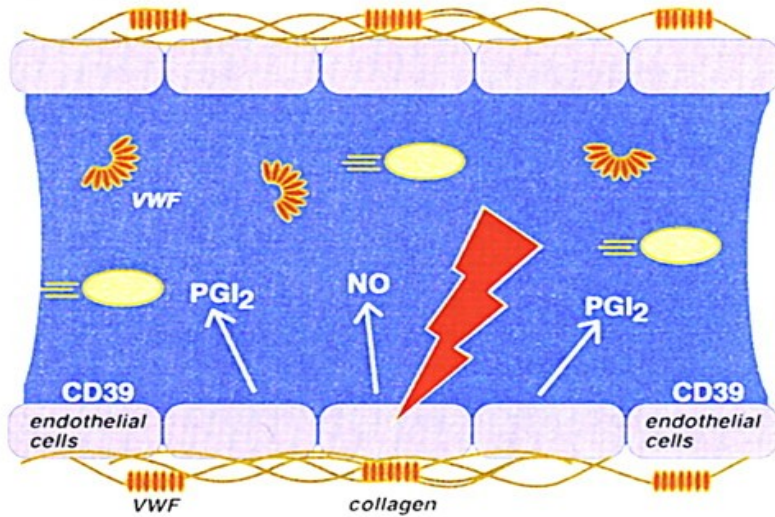


# Inflammatory Phase

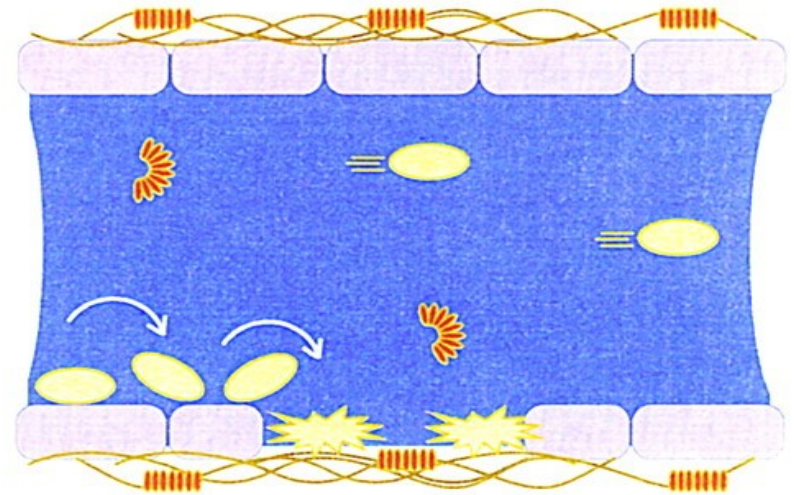
- ✔ **Endothelial cells** *retract* to expose the subendothelial collagen surfaces;
- ✔ **platelets attach** to these surfaces.
- ✔ **Adherence** to exposed collagen surfaces and to other platelets occurs through **adhesive glycoproteins**: fibrinogen, fibronectin, thrombospondin, and von Willebrand factor.

# Blood clot formation

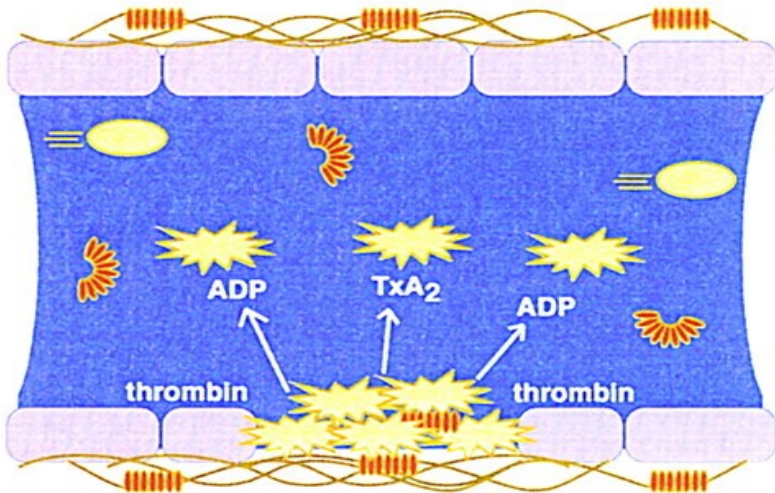
A. Injury



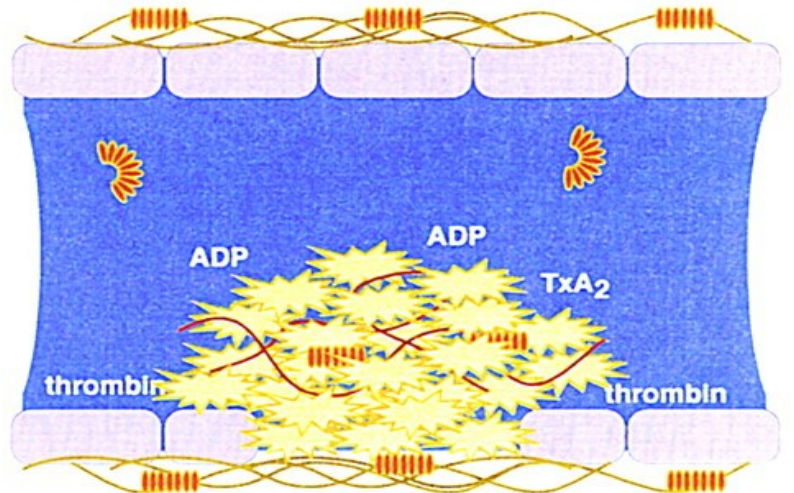
B. Initiation



C. Extension (recruitment)



D. Perpetuation (stabilization)



# Thrombus formation

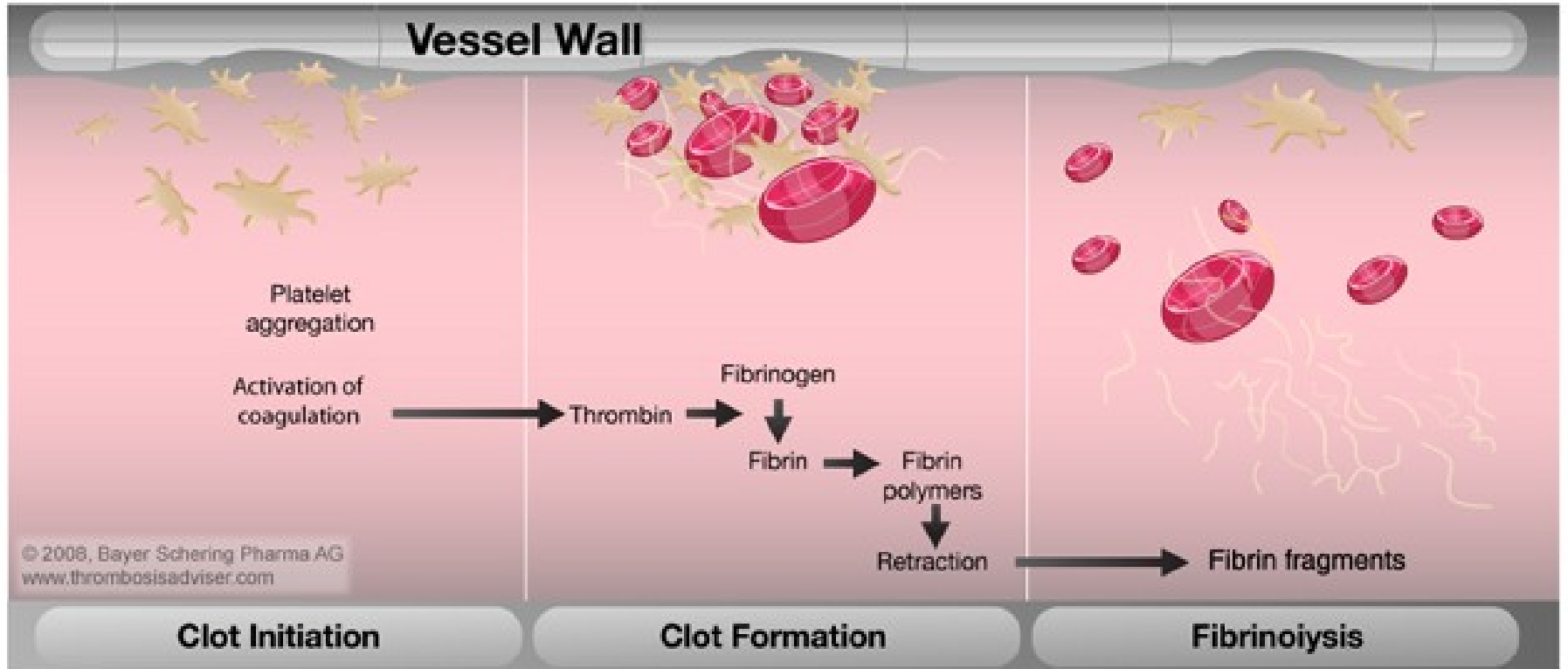
**Platelets**

**Erythrocytes**

**Fibrin**

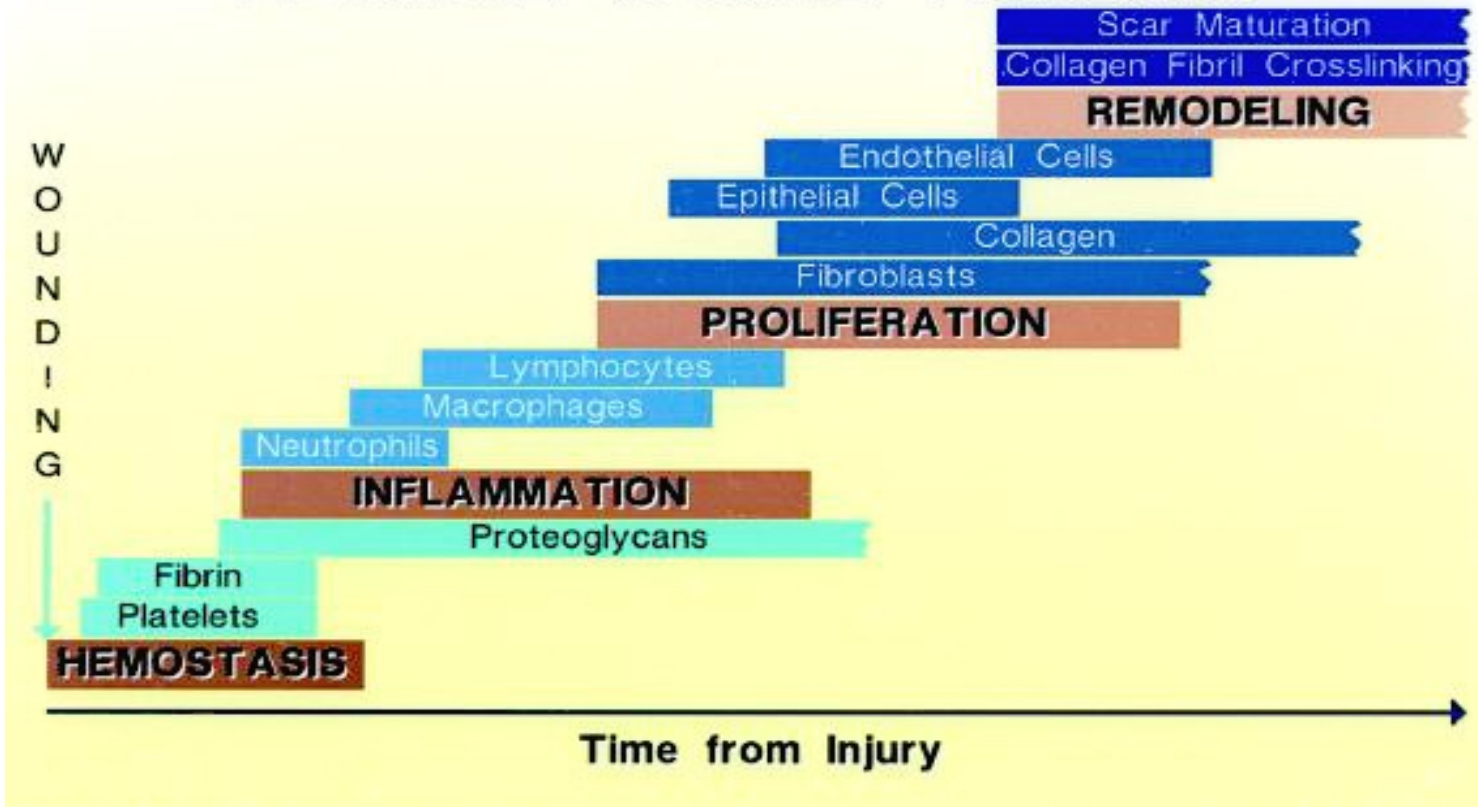








# NORMAL WOUND HEALING





## Regeneration

Exact replacement of the tissue by the same tissue type cells

## Normal reparation

New balance in the tissue



## Insufficient healing

Chronic ulcers

## Excessive healing

Fibrosis and contractures

According to Diegelmann and Evans, 2004



# Inflammatory Phase

- The **aggregation of platelets** results in the formation of the **primary platelet plug**. Aggregation and attachment to exposed collagen surfaces activates the platelets.
- Activation enables platelets to **degranulate and release chemotactic and growth factors**, such as platelet-derived growth factor (PDGF), proteases, and **vasoactive agents** (eg, serotonin, histamine).



# Inflammatory Phase

- ✓ The result of platelet aggregation and the coagulation cascade is **clot formation**.
- ✓ Clot formation is limited in duration and to the site of injury.
- ✓ Clot formation dissipates as its stimuli dissipate. Plasminogen is converted to plasmin, a potent enzyme aiding in cell lysis.
- ✓ Clot formation is limited to the site of injury because uninjured nearby endothelial cells produce prostacyclin, an inhibitor of platelet aggregation. In the uninjured nearby areas, antithrombin III binds thrombin, and protein C binds factors of the coagulation cascade, namely, factors V and VII.



# Inflammatory phase

- Both pathways proceed to the activation of thrombin, which converts fibrinogen to fibrin.
- **The fibrin product is essential to wound healing and is the primary component of the wound matrix into which inflammatory cells, platelets, and plasma proteins migrate.**
- Removal of the fibrin matrix impedes wound healing.



# Inflammatory Phase

- ✓ In addition to activation of fibrin, **thrombin** facilitates migration of inflammatory cells to the site of injury by **increasing vascular permeability**. By this mechanism, factors and cells necessary to healing flow from the intravascular space and into the extravascular space.



# Inflammatory Phase

- **Platelets** also release factors that attract other important cells to the injury.
- **Neutrophils** enter the wound to fight infection and to attract macrophages.
- **Macrophages** break down necrotic debris and activate the fibroblast response.
- The inflammatory phase lasts about **24 hours** and leads to the proliferation phase of the healing process.





# Proliferation Phase

- On the surface of the wound, **epidermal cells** burst into mitotic activity within **24 to 72 hours**. These cells begin their migration across the surface of the wound.
- **Fibroblasts** proliferate in the deeper parts of the wound. These fibroblasts begin to synthesize small amounts of collagen which acts as a scaffold for migration and further fibroblast proliferation.



# Proliferation Phase

- ✓ **Granulation tissue**, which consists of *capillary loops* supported in this developing *collagen matrix*, also appears in the deeper layers of the wound.
- ✓ The proliferation phase lasts from **24 to 72 hours** and leads to the **maturation phase** of wound healing.



# Proliferation Phase

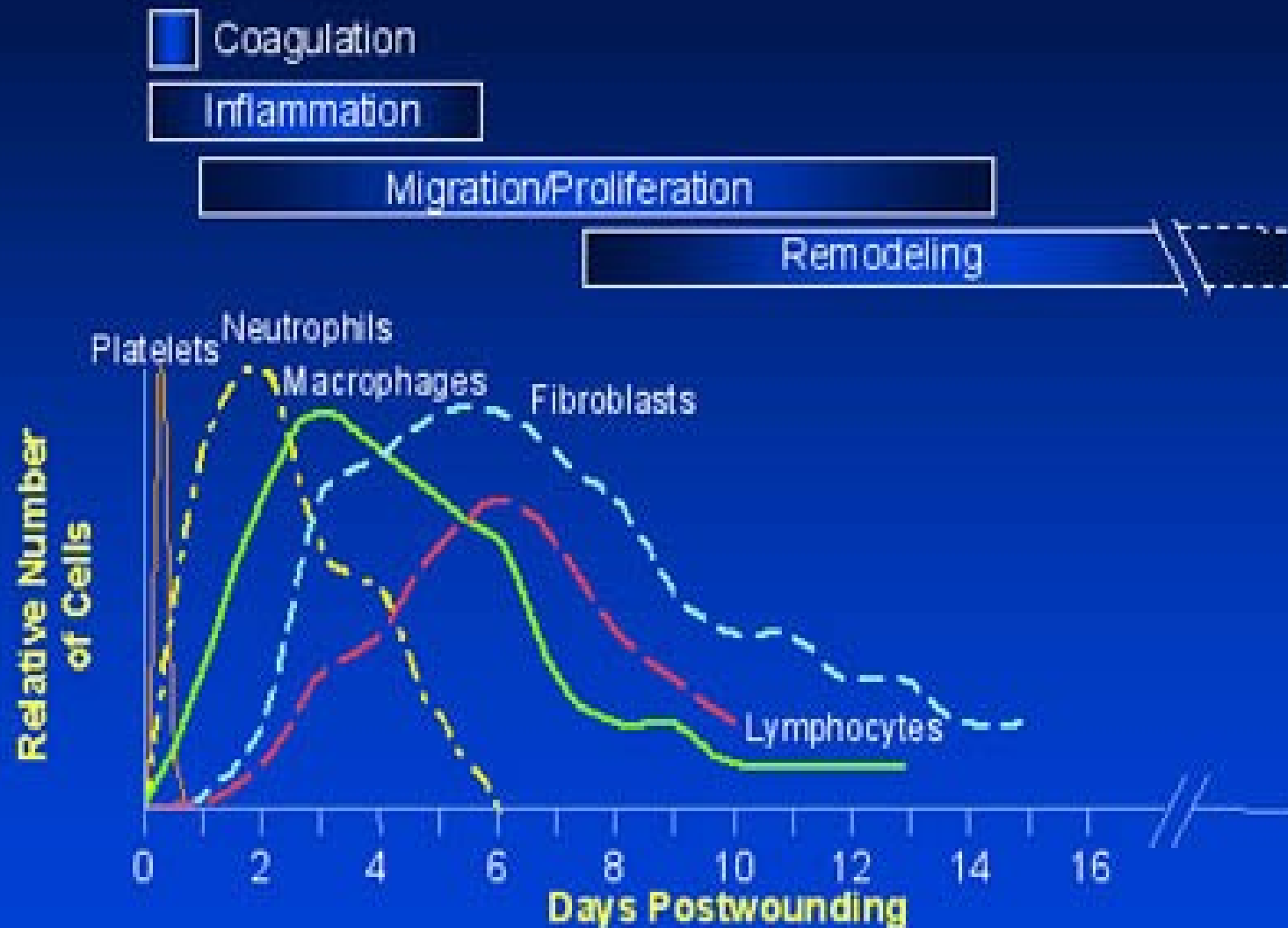
- ✔ **Four to five days** after the injury occurs, fibroblasts begin producing large amounts of **collagen and proteoglycans**.
- ✔ Proteoglycans appear to enhance the formation of collagen fibers, but their exact role is not completely understood.
- ✔ Collagen fibers are laid down randomly and are cross-linked into large, closely packed bundles.



# Proliferation Phase

- ✓ Within two to three weeks, the wound can resist normal stresses, but wound strength continues to build for several months.
- ✓ The proliferation phase lasts from **15 to 20 days** and then wound healing enters the maturation phase.

# CELL RECRUITMENT IN THE WOUND



Adapted with permission from Witte MB and Barbul A. *Surg Clin North Am.* 1997;77:512.



# Maturation Phase

- ✓ During the maturation phase, **fibroblasts** leave the wound and **collagen** is remodelled into a more organized matrix.
- ✓ **Tensile strength** increases for up to one year following the injury. While healed wounds never regain the full strength of uninjured skin, they can regain up to 70 to 80% of its original strength.

# REMODELING

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- Changes in matrix composition over time

Extracellular matrix



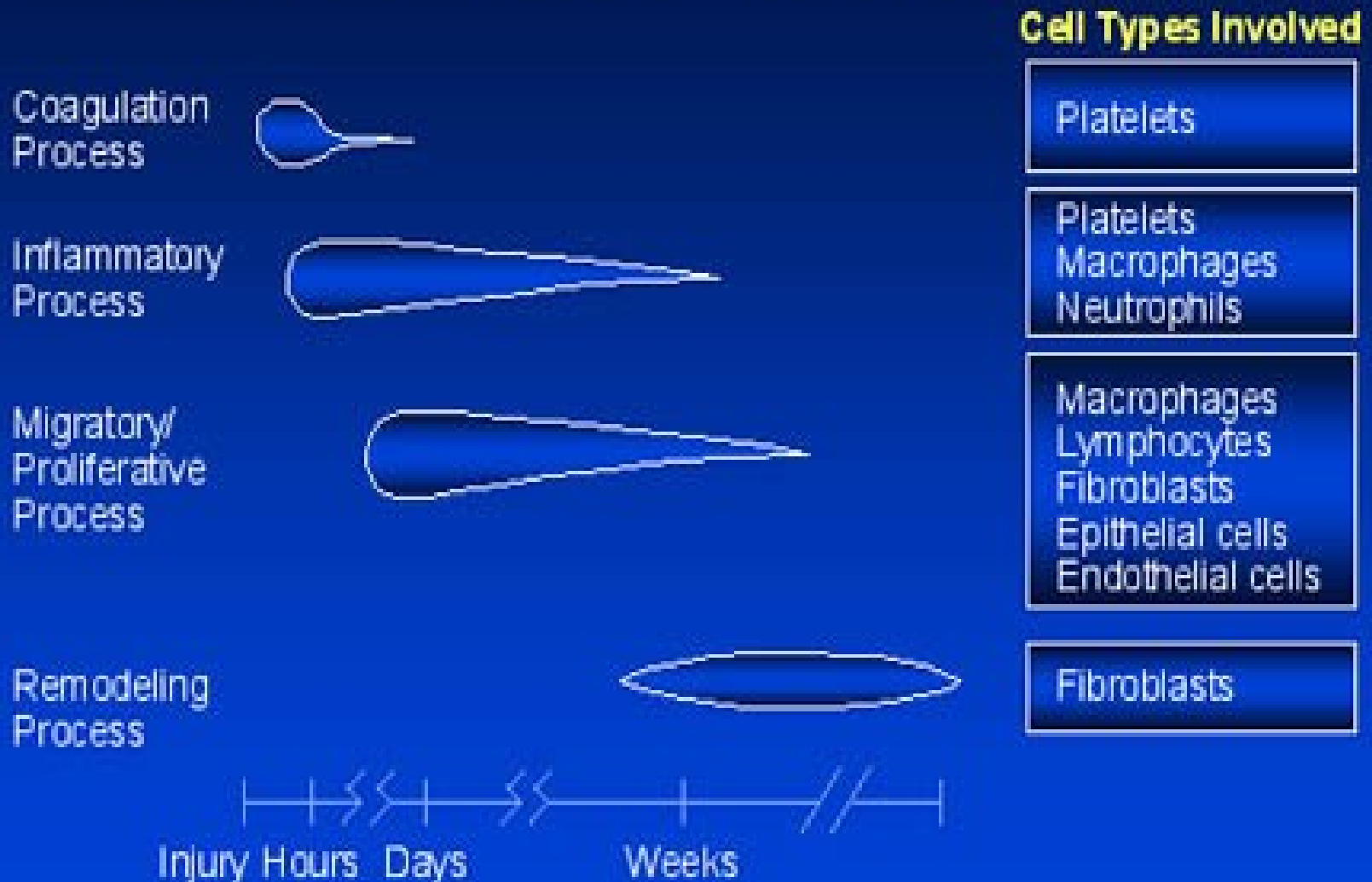
Collagen



Scar



# COMPONENTS OF WOUND HEALING







# Chronic Wounds

- ✔ Failure or delay of healing components
- ✔ Unresponsiveness to normal growth regulatory signals
- ✔ Associated with repeated trauma, poor perfusion/oxygenation and/or excessive inflammation
- ✔ Systemic disease
- ✔ Genetic factors



# Factors affecting wound healing

- ✓ Local
- ✓ Regional
- ✓ Systemic



# Local factors affecting wound healing

- ✓ Mechanical injury
- ✓ Infection edema
- ✓ Ischemia/hypoxia/necrosis
- ✓ Topical factors
- ✓ Ionizing radiation
- ✓ Foreign bodies



# Regional factors affecting wound healing

- ✔ Arterial insufficiency
- ✔ Venous insufficiency
- ✔ Neuropathy



# Systemic factors affecting wound healing

- ✔ Hypoperfusion
- ✔ Inflammation
- ✔ Nutrition
- ✔ Metabolic diseases
- ✔ Immunodeficiency/  
immunosuppression
- ✔ Connective tissue disorders
- ✔ Smoking



# Scar formation

- ✔ There are three variable parameters responsible for the pathological evolution of a scar:
- ✔ the cellular population, the fundamental matrix, and the fibers. The pathological evolution is produced by:
- ✔ deviations in the continuity of the healing
- ✔ deviations in the reactivity of the organism
- ✔ deviations produced by the traumatic agent



# Keloid scar





# Keloid scarring







Thank you for your attention

