Repair

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FRCPath

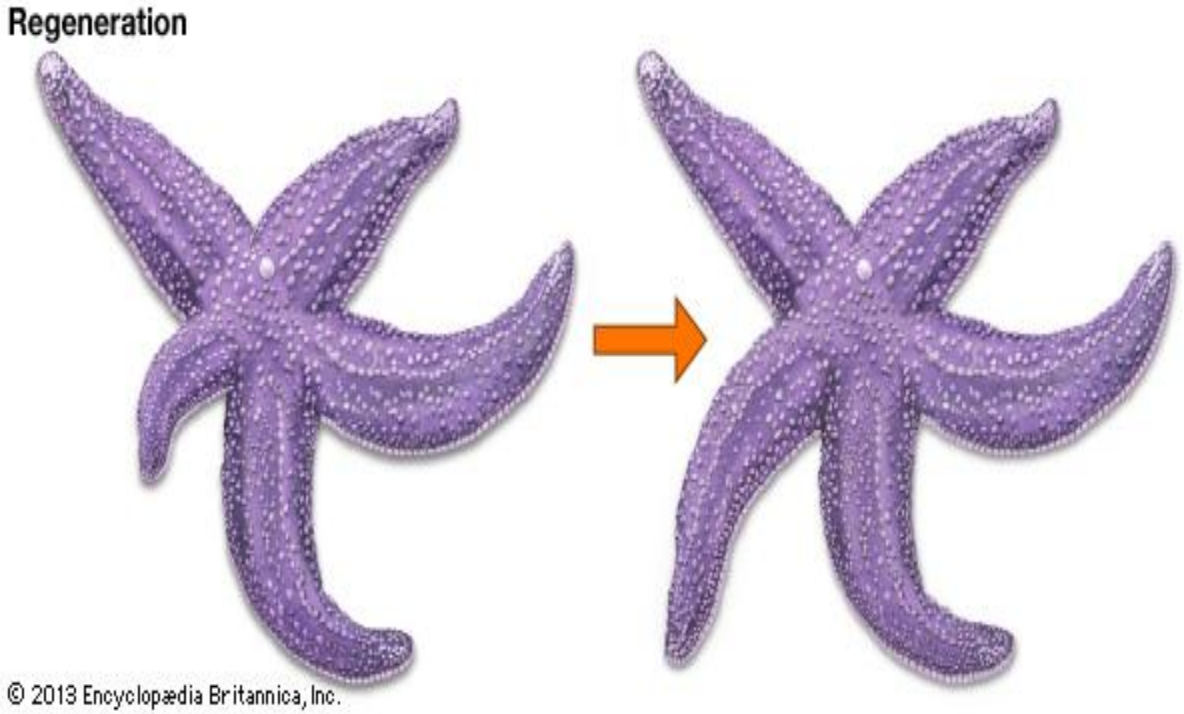
Tissue repair

- Restoration of tissue architecture and function after injury.

Two types :

- 1) regeneration.
- 2) scar formation.

regeneration



Regeneration

- Replacement of damaged cells and restoration of normal function.

- Happens by 1.proliferation of residual, uninjured cells that can replicate and 2. tissue replacement from stem cells.

Scar formation

- = repair by fibrous tissue, resulting in a scar.
- Happens if the injured tissue unable to replicate or the supportive structures of the tissue are severely injured.
- The scar cannot perform the lost function but it gives structural support.

BOB THE BUILDER RULE!!

- If it can be fixed: regeneration
- If not: scarring

- In many situation … both, regeneration and scar formation contribute to repair.

terminology

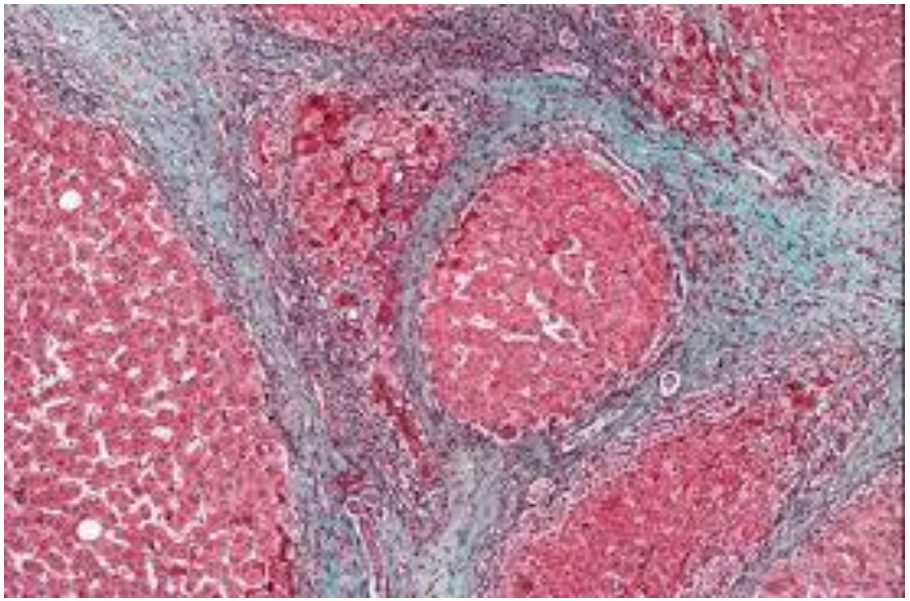
- Fibrosis: collagen deposition after inflammation or in the heart after ischemia.

- Organization= fibrosis in tissue space occupied by inflammatory exudate.(e:g :organizing pneumonia)

- Repair= healing

fibrosis

- Fibrosis in the liver… blue color



Cell and tissue regeneration

Cells that replicate during repair:

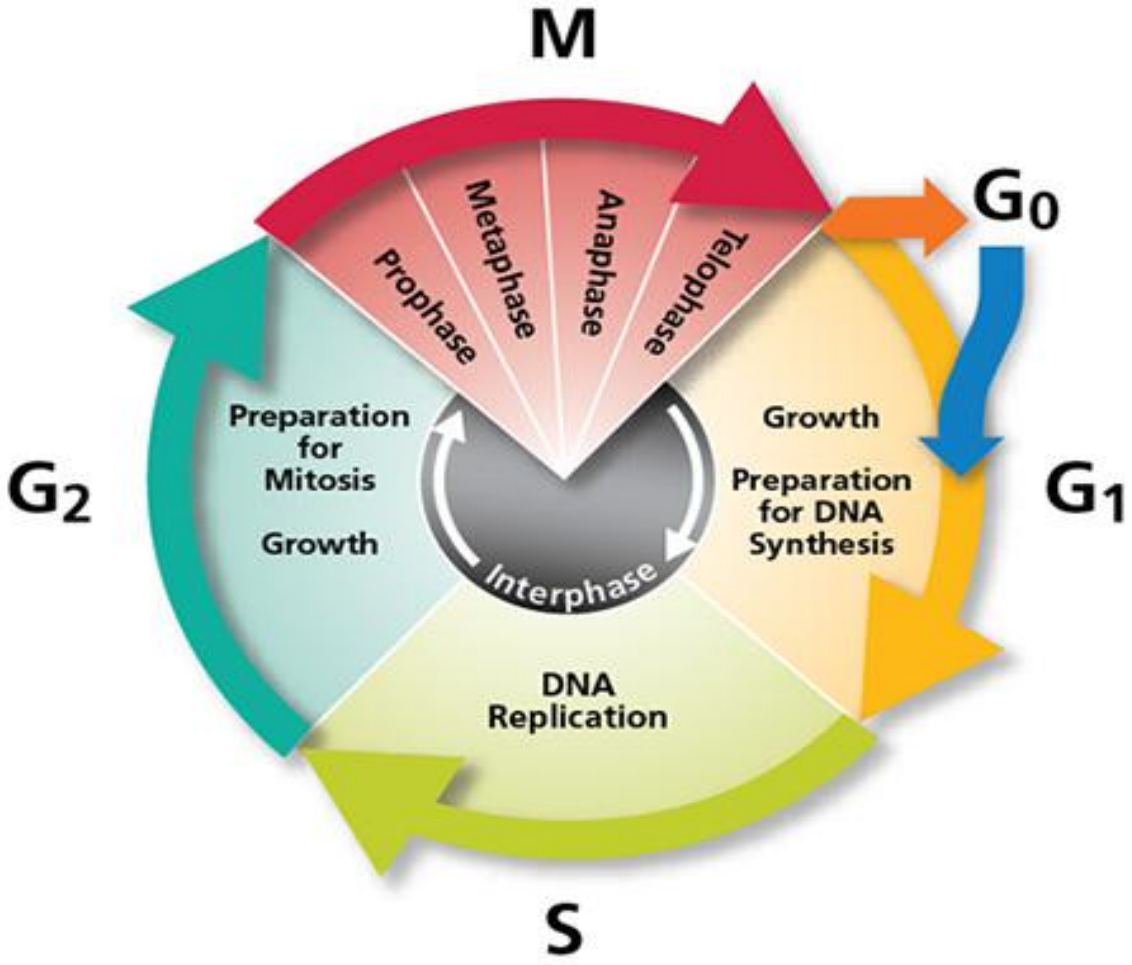
- 1. Remnant of injured tissue.
- 2. Endothelial cells.
- 3. Fibroblasts.

- The proliferation of all these cells is controlled by growth factors.

Normal size of cell population is controlled by a balance between:

- Cell proliferation
- Cell death by apoptosis
- Formation of new differentiated cells from stem cells.

Cell cycle


M
Prophase
Metaphase
Anaphase
Telophase
G0
G1
S
G2
Growth
Preparation for DNA Synthesis
DNA Replication
Preparation for Mitosis
Growth
Interphase

Cell cycle

- Non dividing cells are arrested in G1 phase or exited the cell cycle at G0 phase.
- Growth factors .. Stimulate transition from G0 to G1 and beyond into S phase, G2 and M phase.
- This progression is regulated by **cyclins** that are regulated by cyclin dependent kinases.

Proliferation capacities of tissues

- Labile cells
- Stable cells
- Permeant cells

LABILE TISSUES ARE ALWAYS ON THE MOVE

Labile tissue

- Labile tissue= continuously dividing tissue. continuously lost and replaced by proliferation of mature cells and by maturation from stem cells.
- Examples: Hematopietic cells, skin and surface epithelium

STABLE (QUIESENT) ARE RESTING FOR NOW BUT CAN BE AROUSED

Stable tissue

- Quiescent , inactive cells
- Minimal replicative activity in the normal state.
- Can proliferate in response to injury
- Examples: Parenchyma of solid organs, Endothelial cells, Fibroblasts, Smooth muscle cells.

- **Stable tissue ,except the liver, has limited capacity to regenerate.**

The exception: liver

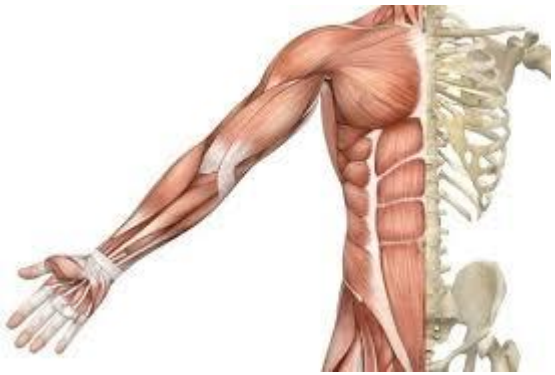
- Up to 60% of liver can be removed for donation.. TNF and IL6 cause transition of hepatocytes from G0 to G1, the cell cycle progresses by HGF and EGF
- Note EGF include TGF alpha
- IMPORTANT NOTE: liver regeneration happens if connective tissue framework preserved, if not (in abscess, severe inflammation..) causes scarring even though hepatocytes can regenerate.

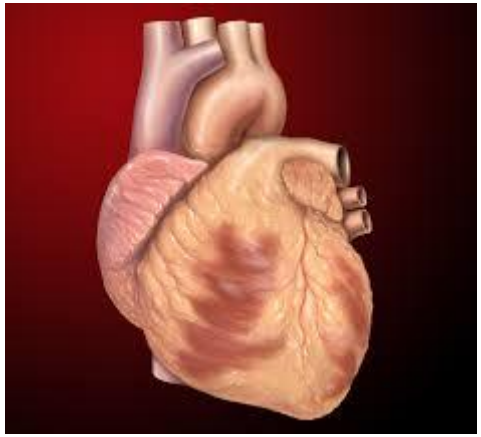
PERMANENT TISSUE GENERALLY CANNOT ENTER THE CELL CYCLE

Permanent tissue

- Permanent tissue: terminally differentiated and non proliferative.
- Neurons and cardiac muscle
- Limited stem cell replication and differentiation occur in some areas of the adult brain
- Cardiac stem cells may proliferate.
- Skeletal muscle usually classified as permanent but stellate cells provide some regeneration.

Permanent tissue

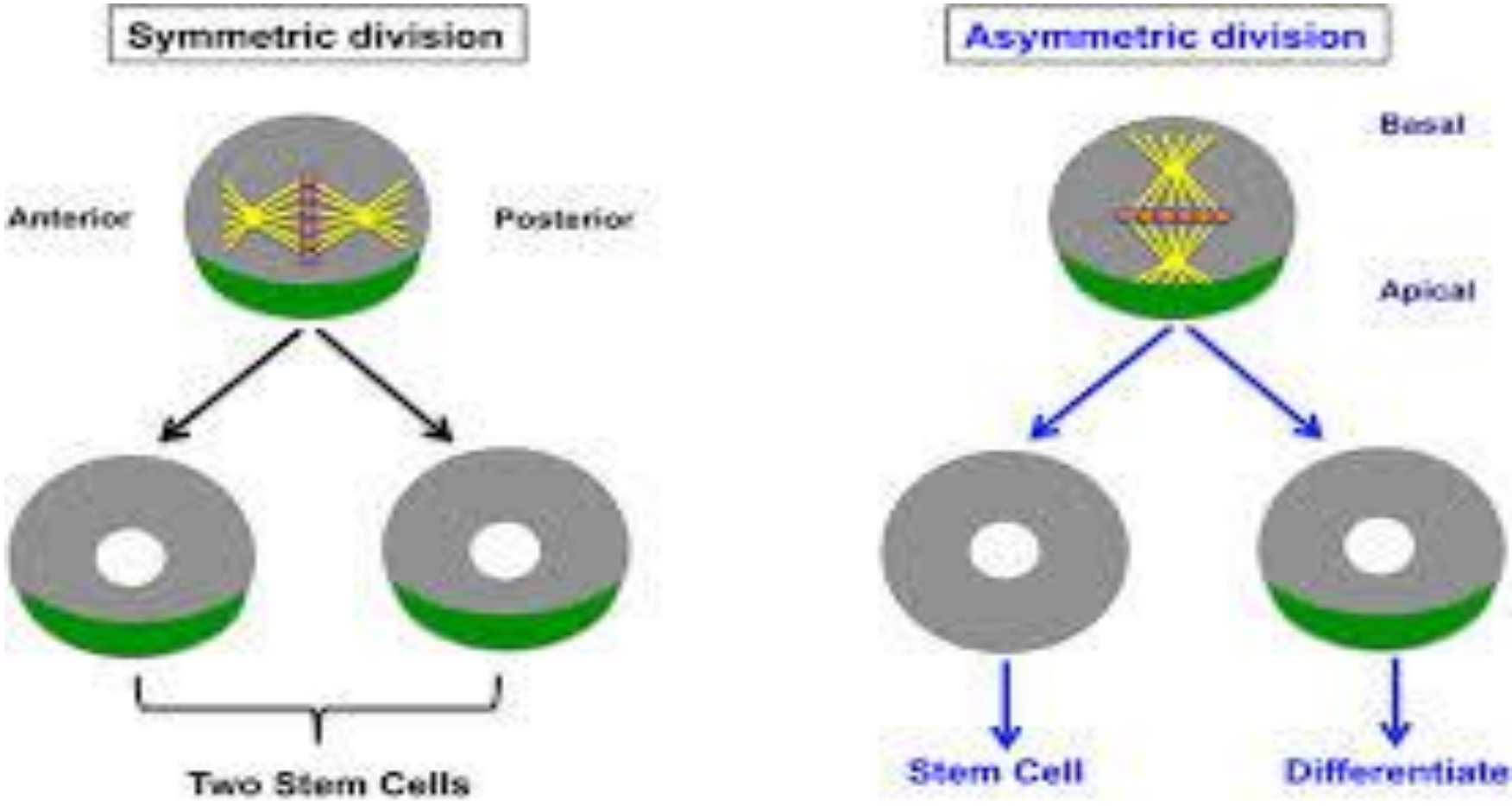


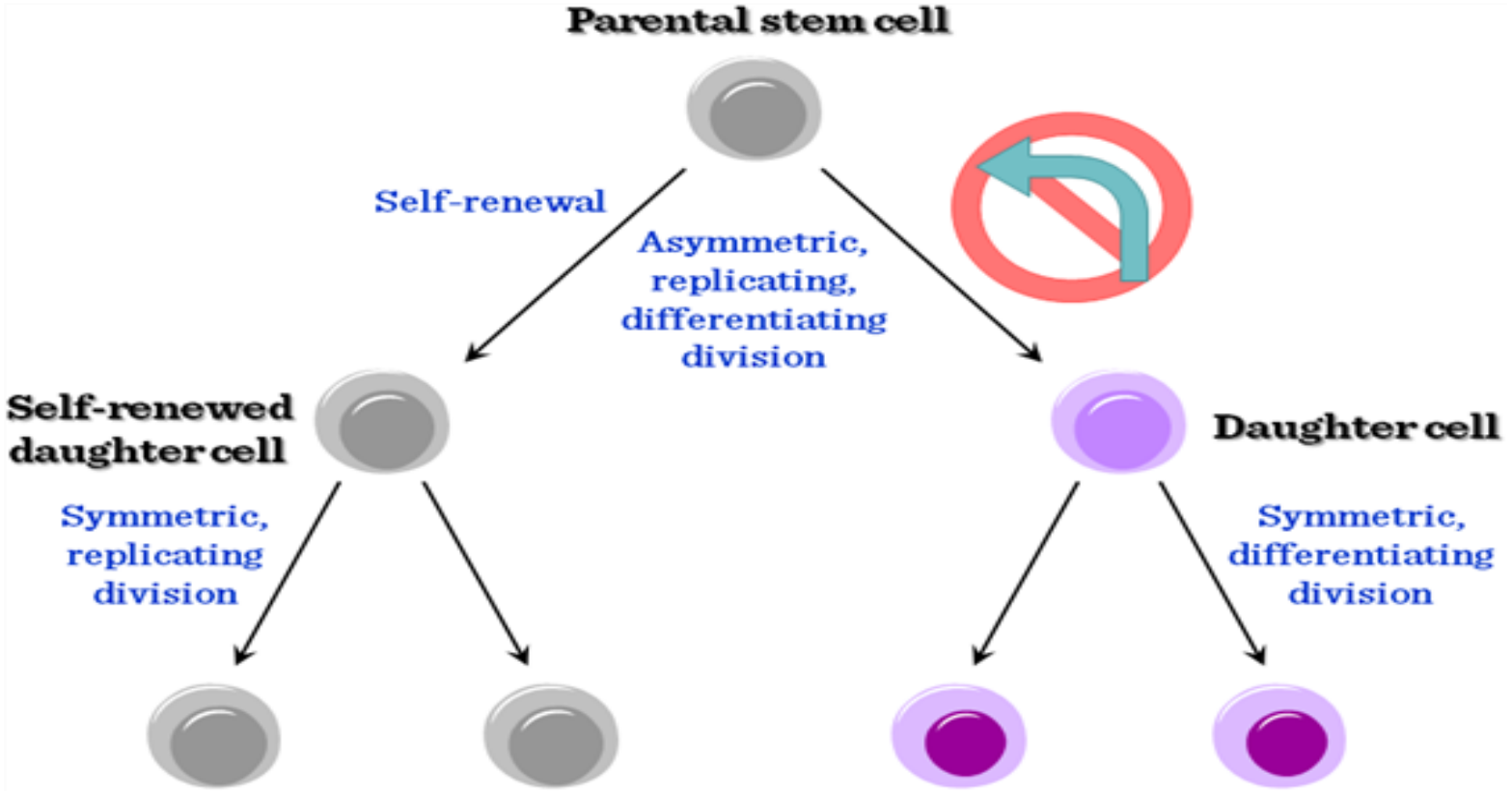


Stem cells

- Self renewal capacity
- Asymmetric replication

Asymmetric replication




Parental stem cell
Self-renewal
Asymmetric, replicating, differentiating division
Self-renewed daughter cell
Daughter cell
Symmetric, replicating division
Symmetric, differentiating division

Stem cells

- 1. embryonic stem cells.
- 2. adult stem cells.

Embryonic stem cells (ES)

- Undifferentiated.
- Extensive cell renewal capacity.
- Present in the inner cell mass of the blastocyst

ES

- Can be maintained in culture for mare more than a year without differentiation
- Under appropriate conditions: Can differentiate to the three germ cell layers.

Adult stem cells

- Less undifferentiated.
- Their lineage potential restricted to the differentiated cells in the organ they are found in.
- Important in maintaining tissue size and in repair.

Tissue (adult) stem cell use in medicine

Restricted by:

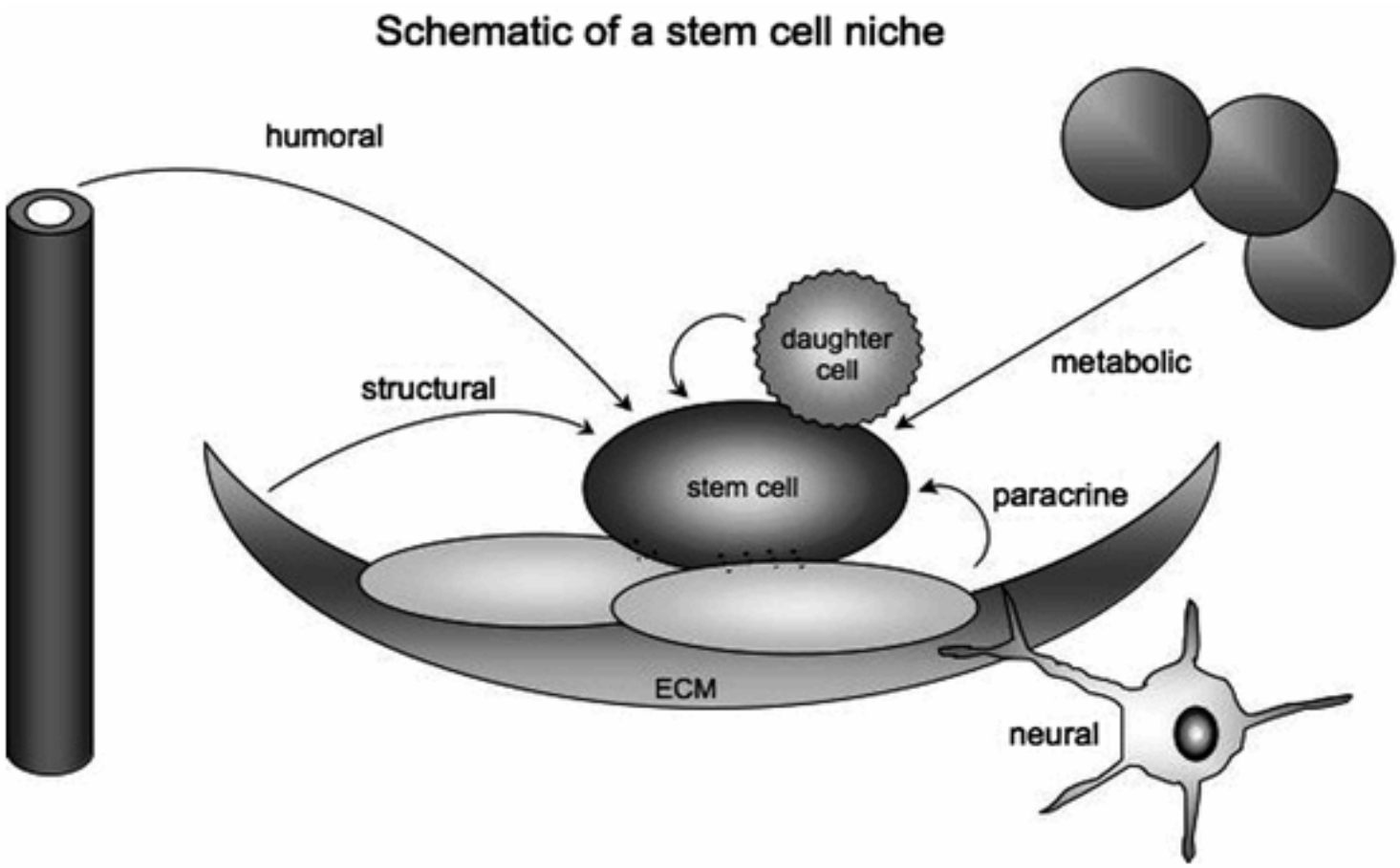
1. Difficulty in isolating them to purity

2. They are present in stem cell niches… without which they cannot function properly.

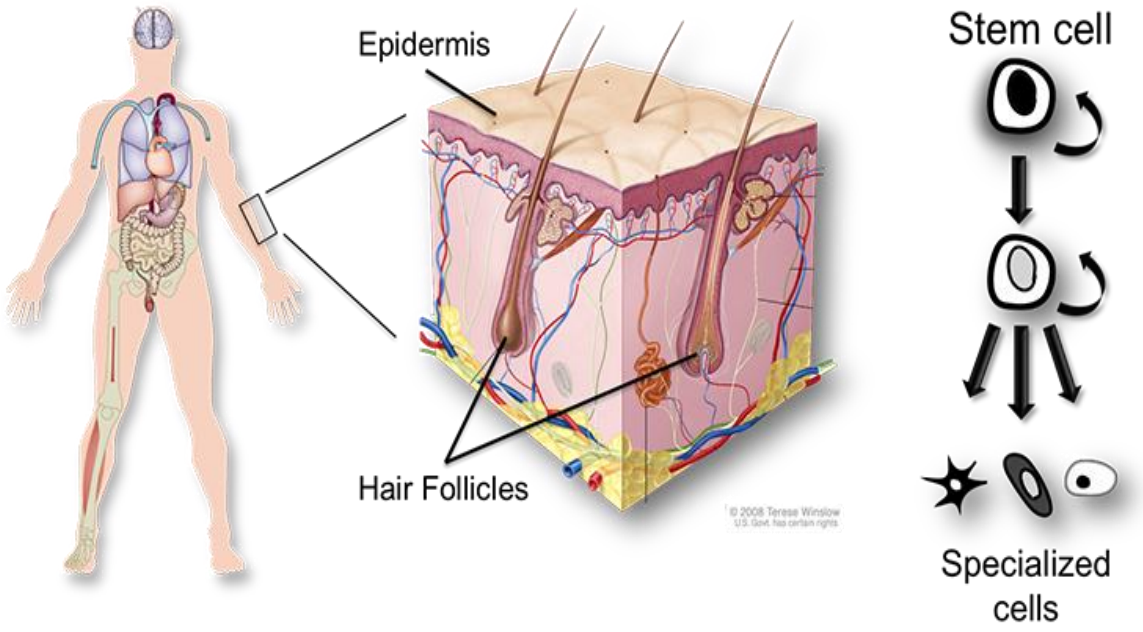
Stem cell niches

- microenvironment, within the specific anatomic location where stem cells are found, which interacts with stem cells to regulate cell fate.

Stem cell niches



Skin stem cells



Tissue stem cells uses

- 1.Regenerative medicine… to regenerate damaged tissues…….. difficult because of the problems mentioned previously!

- 2.Treatment of certain diseases…. Leukaemia and lymphoma

Treating leukemia and lymphoma by stem cells

- hematopoietic stem cells arerare but can be purified according to cell surface markers)
- Can be isolated from bone marrow and peripheral blood (need to be mobilized by cytokine= granulocyte colony stimulating factor

NOTE

- Bone marrow also contains mesenchymal stem cells that can differentiate to mesenchymal cells like chondroblasts, osteoblasts..

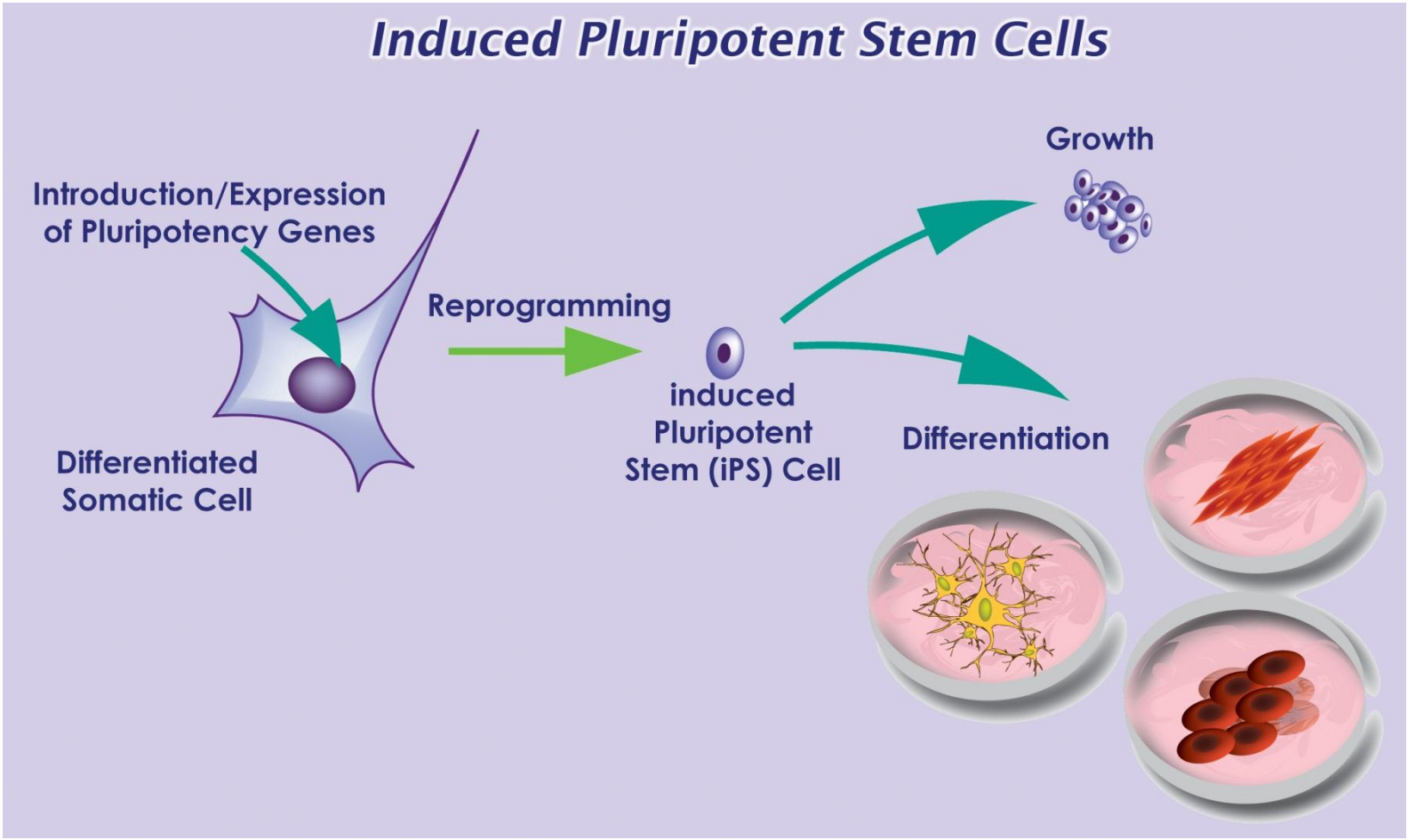
Embryonic stem cells uses

- Regenerative medicine..
- Problem: immunologic rejection.
- Solution: tried to generate stem cells from patients' own cells = iPS.

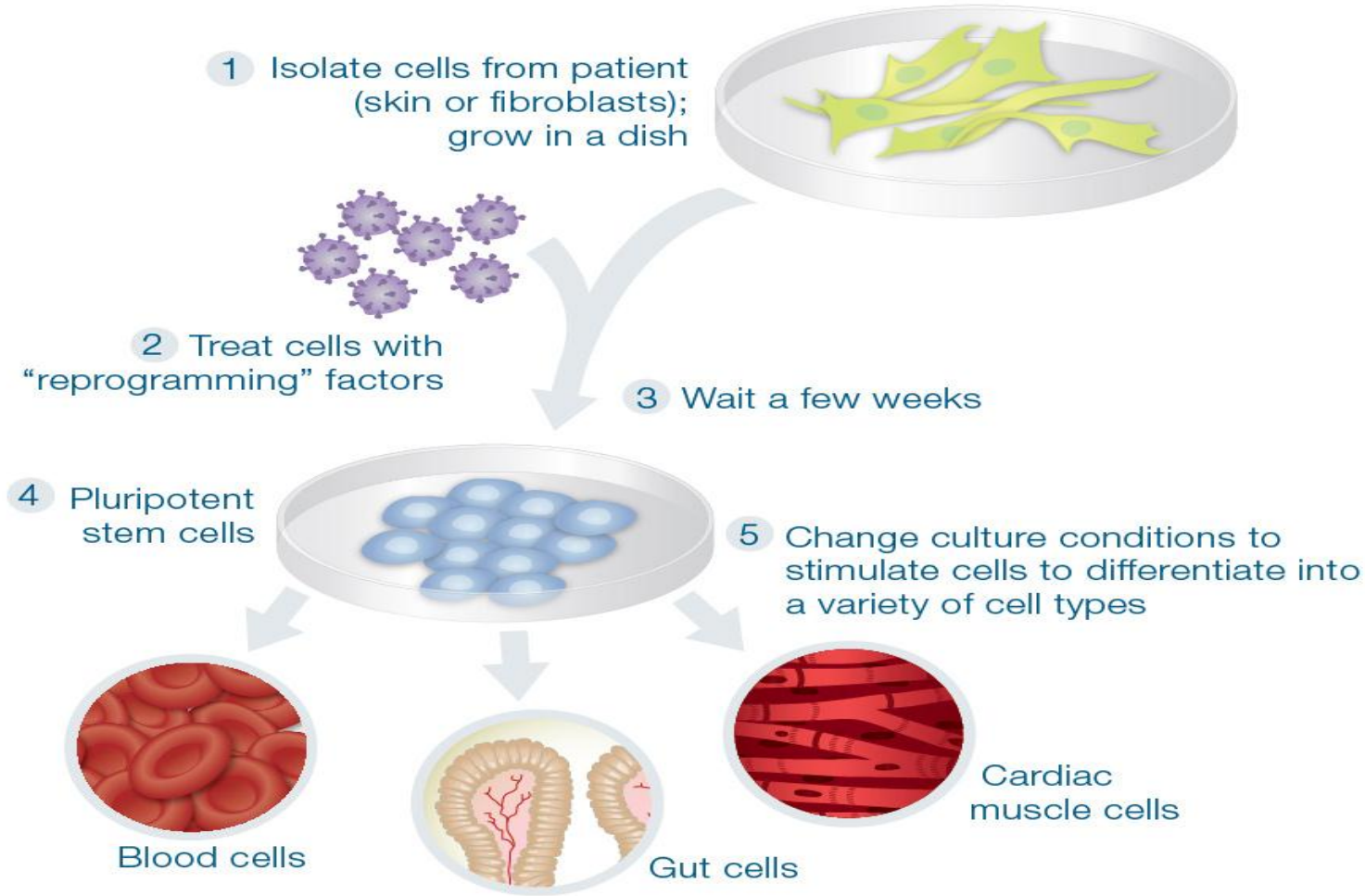
iPS

- = induced pleuripotent stem cells.
- How? By identifying certain genes needed for stem-cell-ness
- These genes are introduced in differentiated cells… this causes reprogramming of somatic cell nucleus.. It acquires properties of embryonic stem cells .
- Clinical usefulness???

iPS


Induced Pluripotent Stem Cells
Introduction/Expression of Pluripotency Genes
Differentiated Somatic Cell
Reprogramming
induced Pluripotent Stem (iPS) Cell
Growth
Differentiation

Creating **iPS cells**



Growth factors

- Proteins that stimulate cell survival and proliferation.
- They can also promote migration, differentiation and other cellular responses.
- derived from macrophages, endothelial cells, mesenchymal and many other cells.

Growth factors

Cause growth by:

- 1.promote entry of cells into cell cycle
- 2.relieve blocks on cell cycle progression
- 3.prevent apoptosis
- 4.increase protein synthesis (in G phase)
- 5. stimulate the function of growth control genes = proto-oncogenes

- The binding of growth factors to specific receptors on the plasma membrane is usually necessary for cell division

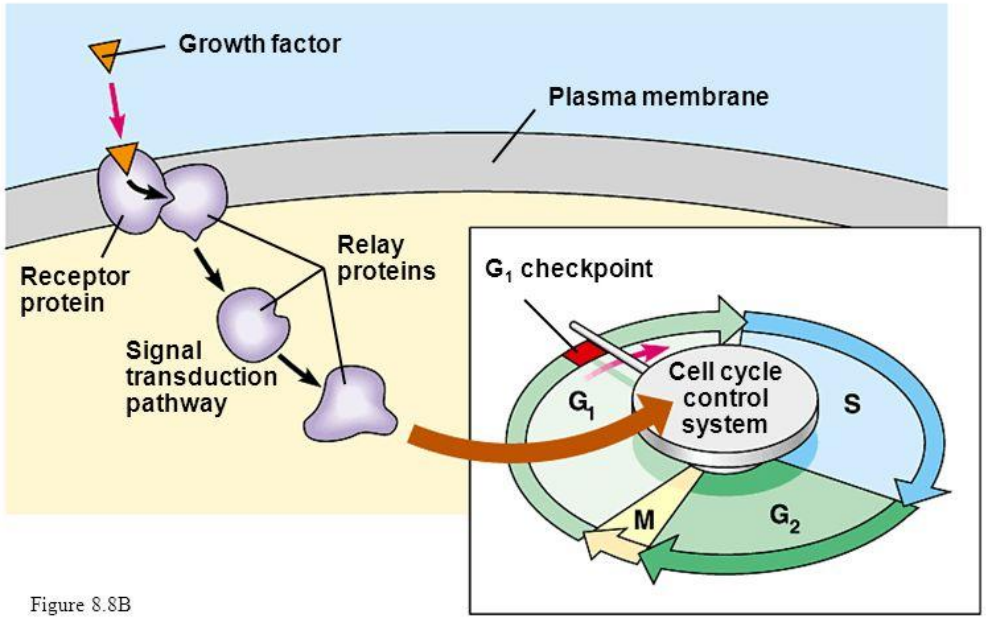


Figure 8.8B

GFs

- EPIDERMAL GROWTH FACTOR
- TGF ALPHA
- TGF BETA
- HEPATOCYTE GF
- PDGF
- KERATINOCYTE GF
- VEGF

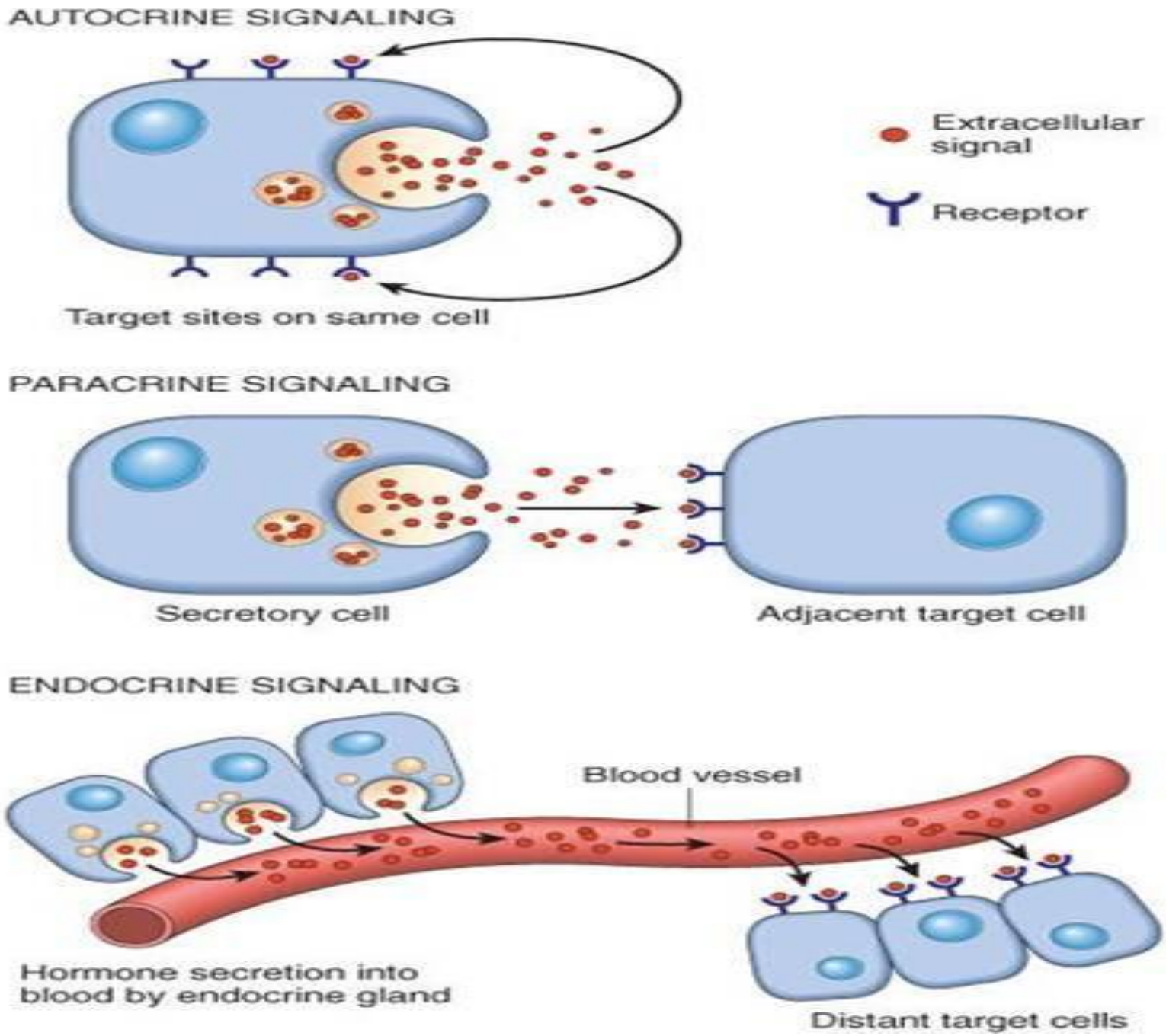
Signalling mechanisms of GFs

- GFs function through receptors…. And trigger biochemical signals … which stimulate or repress gene expression

GF signalling

Can be

- Autocrine.. On the same cell that produced the factor
- Paracrine… between adjacent cells
- Endocrine.. Through blood.

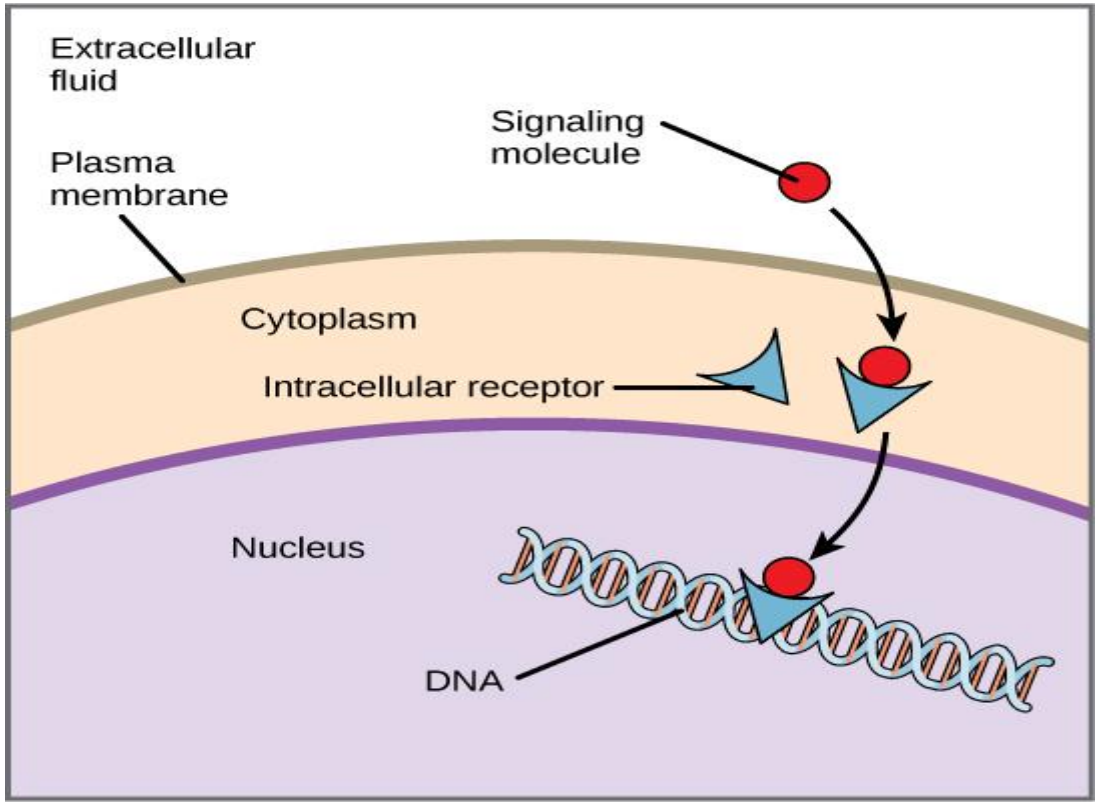

AUTOCRINE SIGNALING
Extracellular signal
Receptor
Target sites on same cell
PARACRINE SIGNALING
Secretory cell
Adjacent target cell
ENDOCRINE SIGNALING
Blood vessel
Hormone secretion into blood by endocrine gland
Distant target cells

NOTE

- RECEPTORS ARE USUALLY ON CELL SURFACE BUT SOME CAN BE INITRACELLULAR

- Ligands of intracellular receptors need to be hydrophobic so they can enter the cell: vit D, steroids, thyroid hormones.

Intracellular receptors



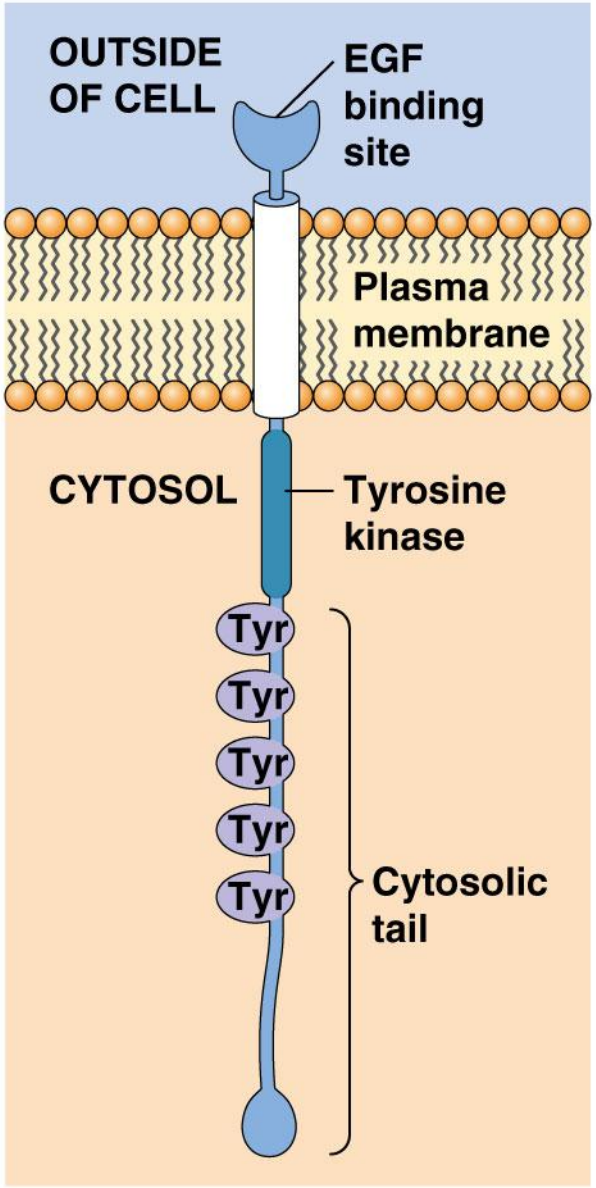
GF receptors

THREE TYPES:

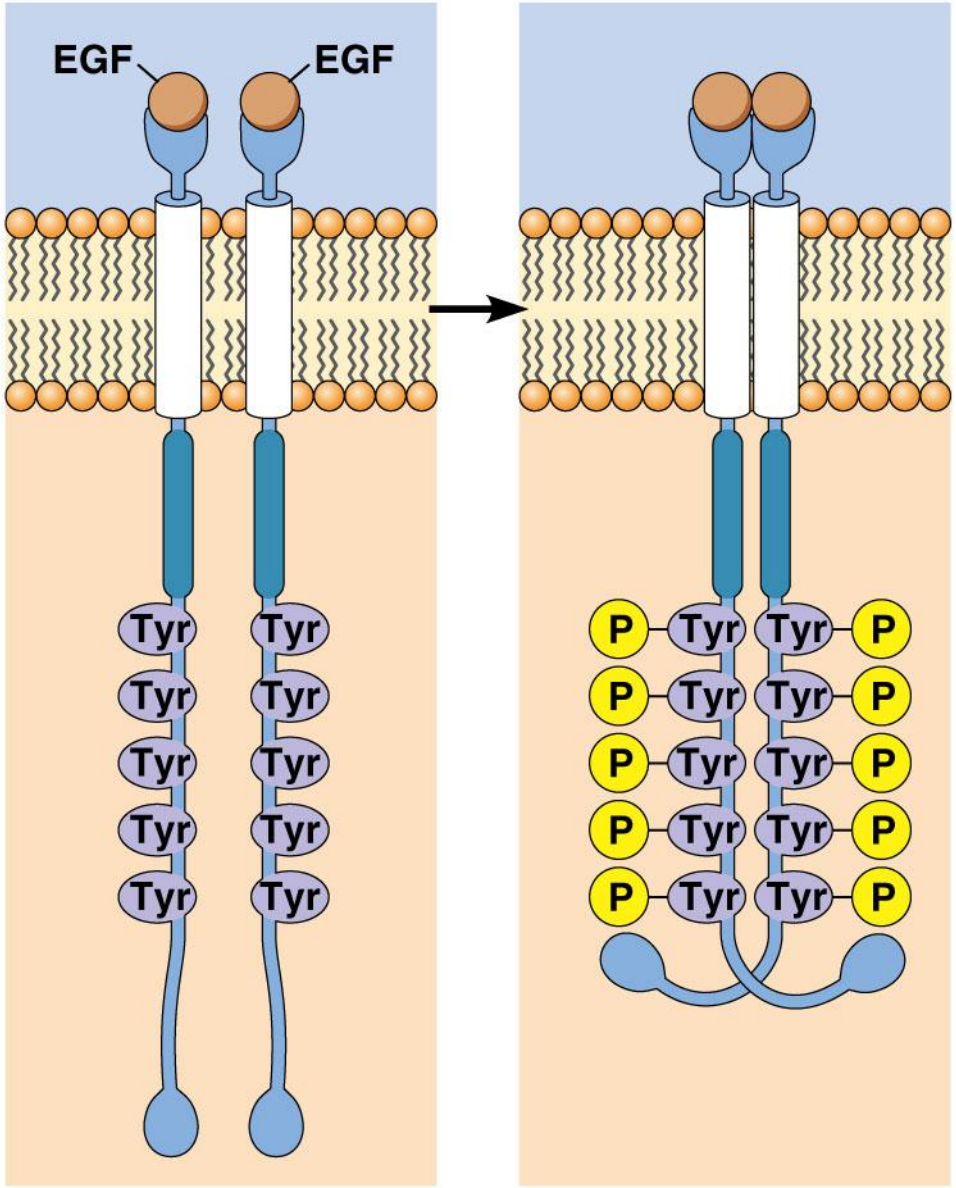
- Receptors with intrinsic kinase activity.
- G protein coupled receptors
- Receptors without intrinsic enzymatic activity.

Receptors with intrinsic kinase activity

- Ligand binds to receptor… dimerization and phosphorylation of the receptor subunits….
- Phosphorylated receptor… bind and activate intracellular proteins (RAS, phosphastidylinositol 3 kinase (PI3 kinase) and phospholipase C sigma…..cell proliferation .



(a) Structure of the epidermal growth factor (EGF) receptor

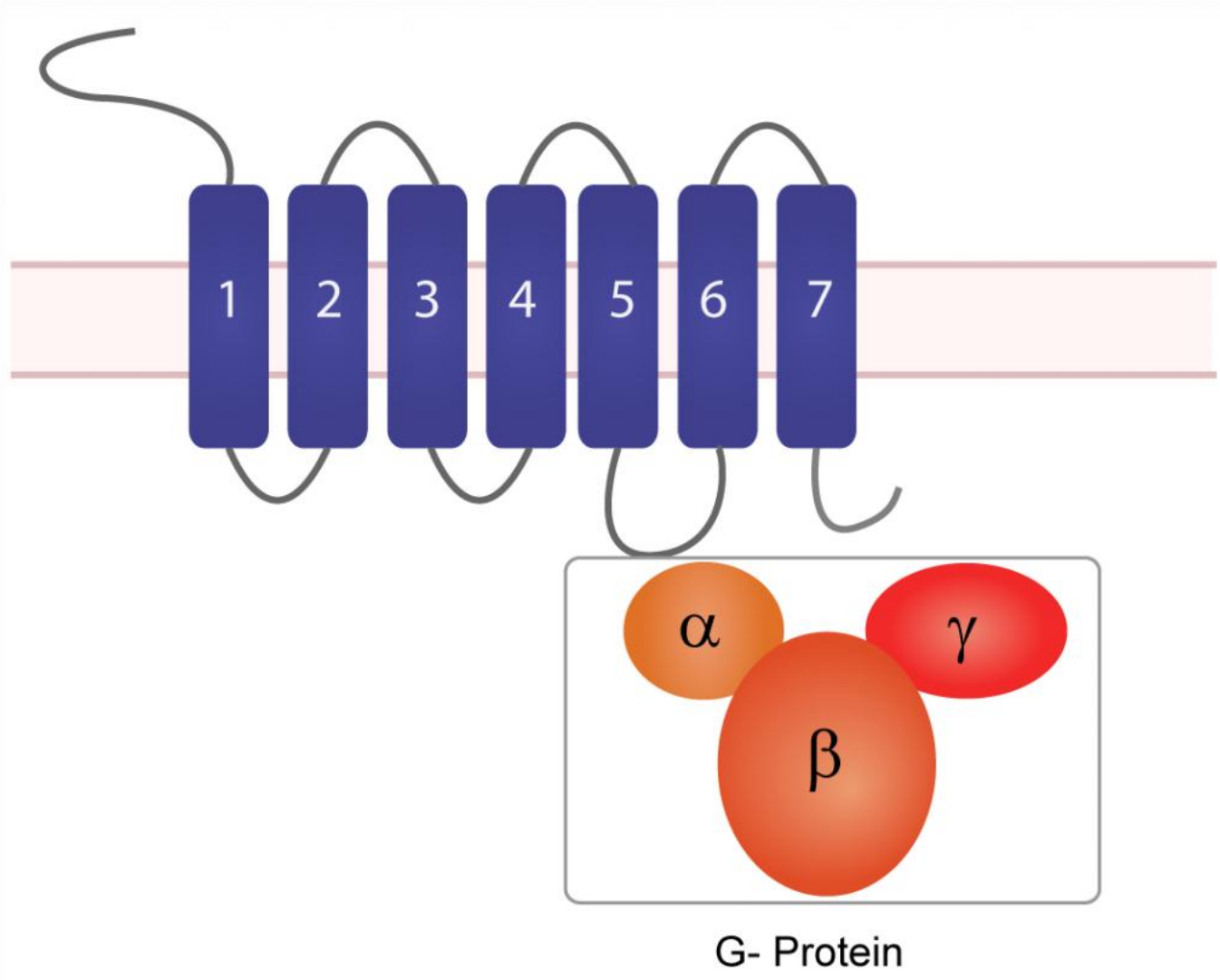


(b) Activation of the EGF receptor

- Receptors with intrinsic kinase activity: used by EGF (epidermal growth factor) and HGF (hepatocyte GF)

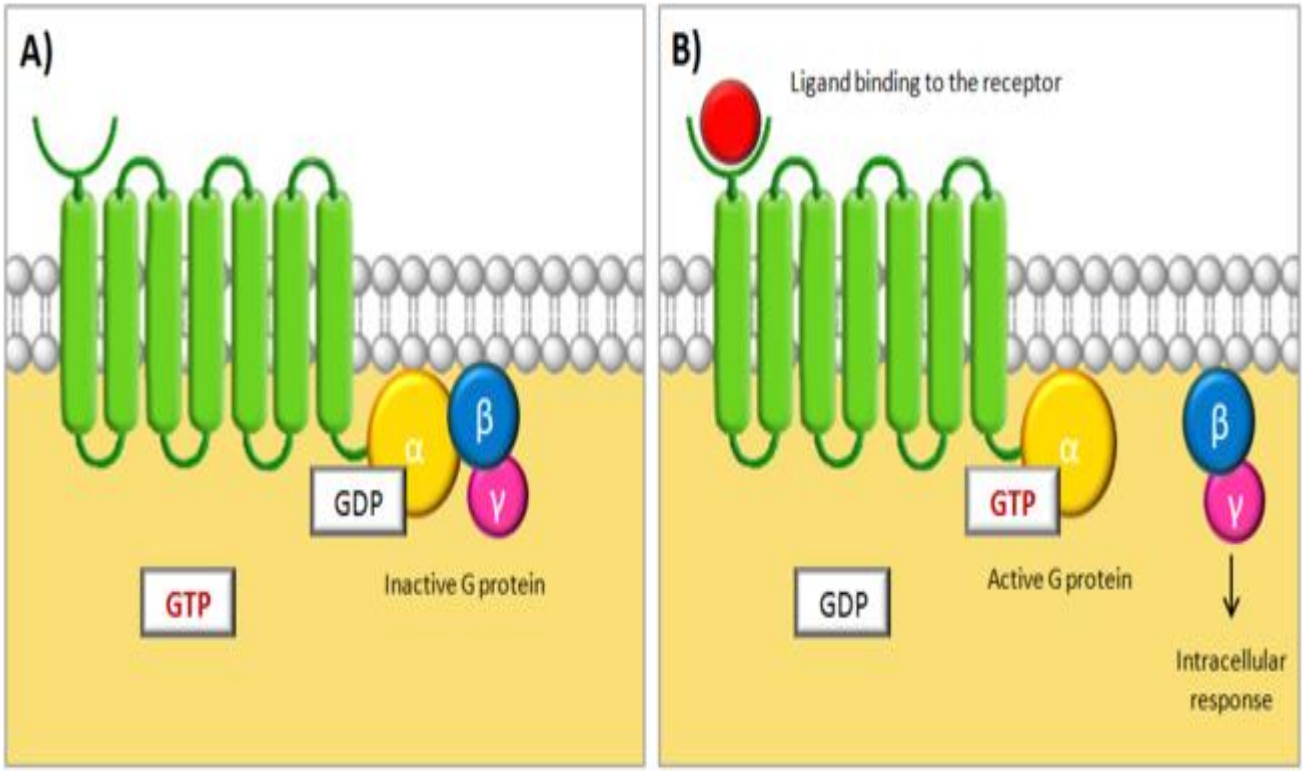
G protein coupled receptors

- Seven transmembrane alpha helices, coupled with G protein (GTP binding protein).
- Ligand binding: GDP in the G protein changes to GTP… receptor activated.
- Signal transduction through second messengers including c AMP and inositol triphosphate (IP3) .. Calcium influx
- This is the largest family of receptors.
- Used by chemokines



G- Protein

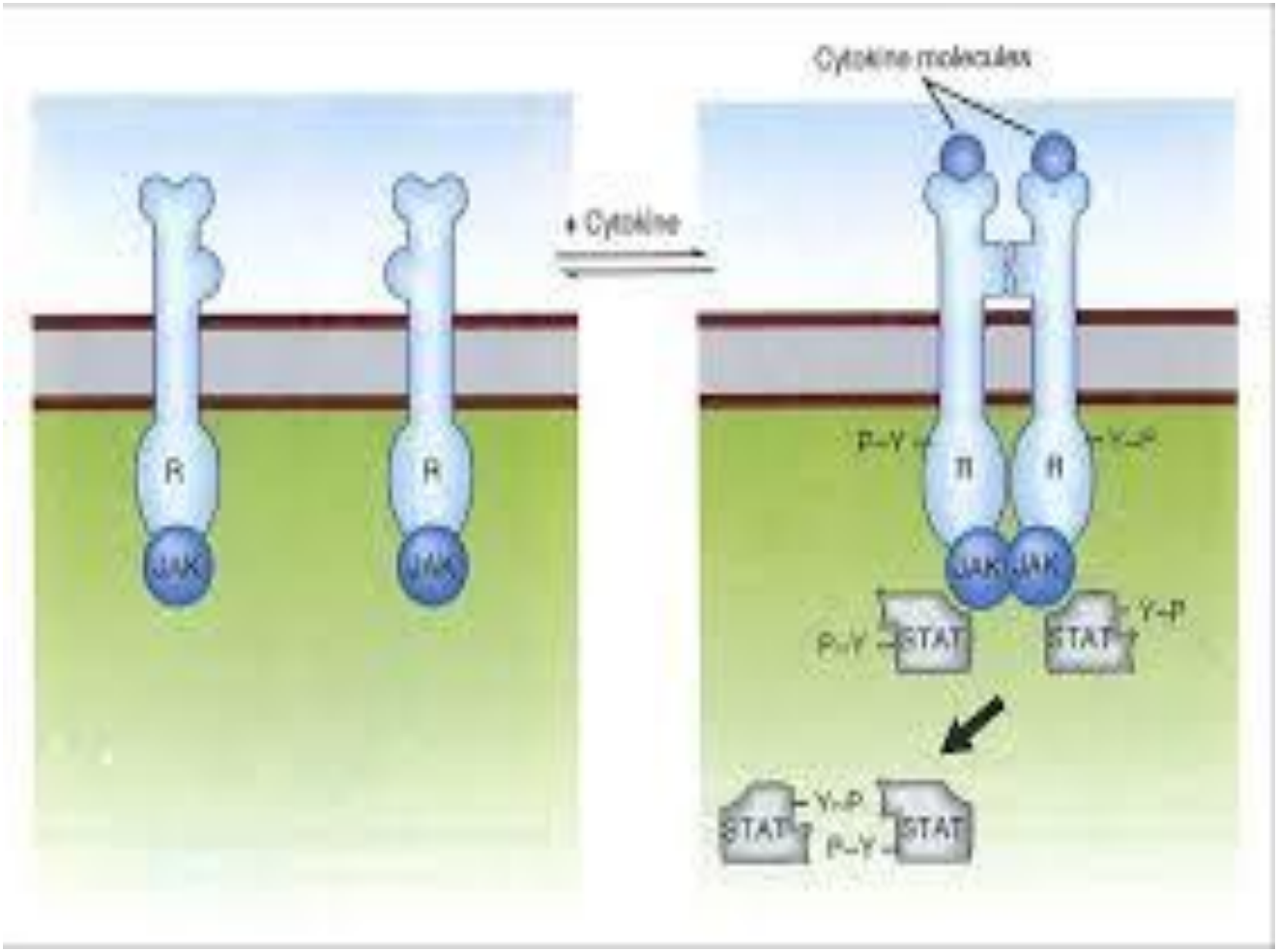
G protein- coupled receptors

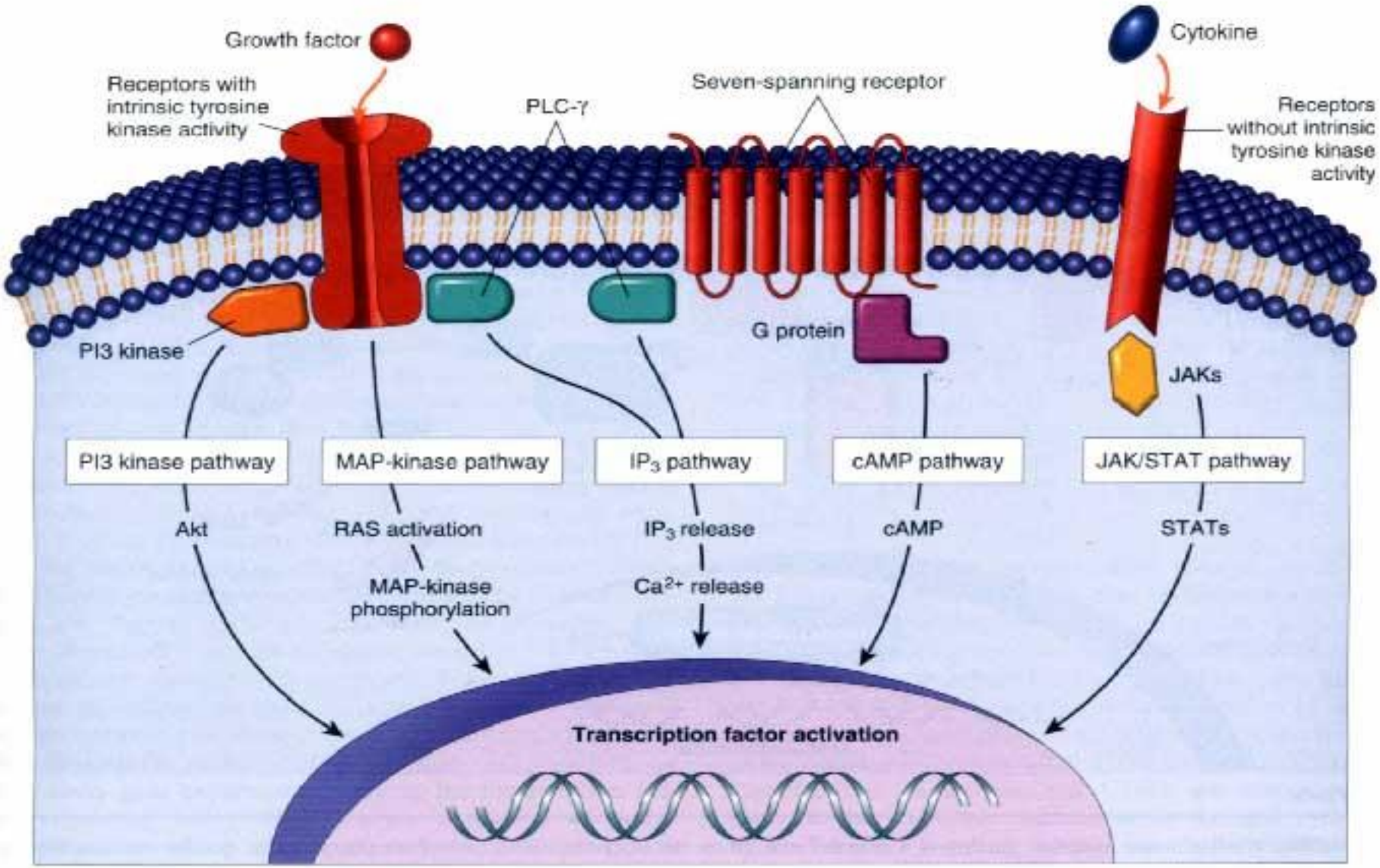


Receptors without intrinsic enzymatic activity

- Ligand.. Conformational change of receptor intracellular domain…. So it can bind to intracytoplasmic kinase (Janus kinases = JAKs).

- Now receptor activated… stimulation of STATs (signal transducers and activators of transcription)…. goes to nucleus… induces transcription
- Used by cytokines.


Cytokine molecules
+ Cytokine
R
R
JAK
JAK
P–Y
Y–P
JAK
JAK
STAT
STAT
P–Y
Y–P
STAT
STAT
Y–P
P–Y



Simplified overview of signal transduction pathway
(from Kumar: Robbins Pathology. 7th. edition.2005)

Role of extracellular matrix in tissue repair

- ECM is composed of several proteins that assemble into a network which surrounds cells.
- Intact extracellular matrix is essential for regeneration… if it is damaged, repair occurs by scarring (bob the builder can’t fix it !)

ECM functions

- Mechanical support.
- Sequesters water.
- Provides turgor for soft tissue.
- sequesters minerals .. Important in bone rigidity.
- Regulates proliferation, movement and differentiation of cells.
- Reservoir of growth factors.

ECM

Two basic forms:

- 1. interstitial matrix.
- 2. basement membrane

Interstitial matrix

- Present in spaces between cells in connective tissue.
- Also between epithelium and supportive vascular and smooth muscle structures.
- It is synthesized by mesenchymal cells, like fibroblasts.
- Forms 3D amorphous gel.

Basement membrane

- Highly organised matrix around epithelial cells, endothelial and smooth muscle cells.
- Synthesized from overlying epithelium and underlying mesenchymal cells.

- Composed of 1) Amorphous nonfibrillar type IV collagen and

  2) laminin.

ECM

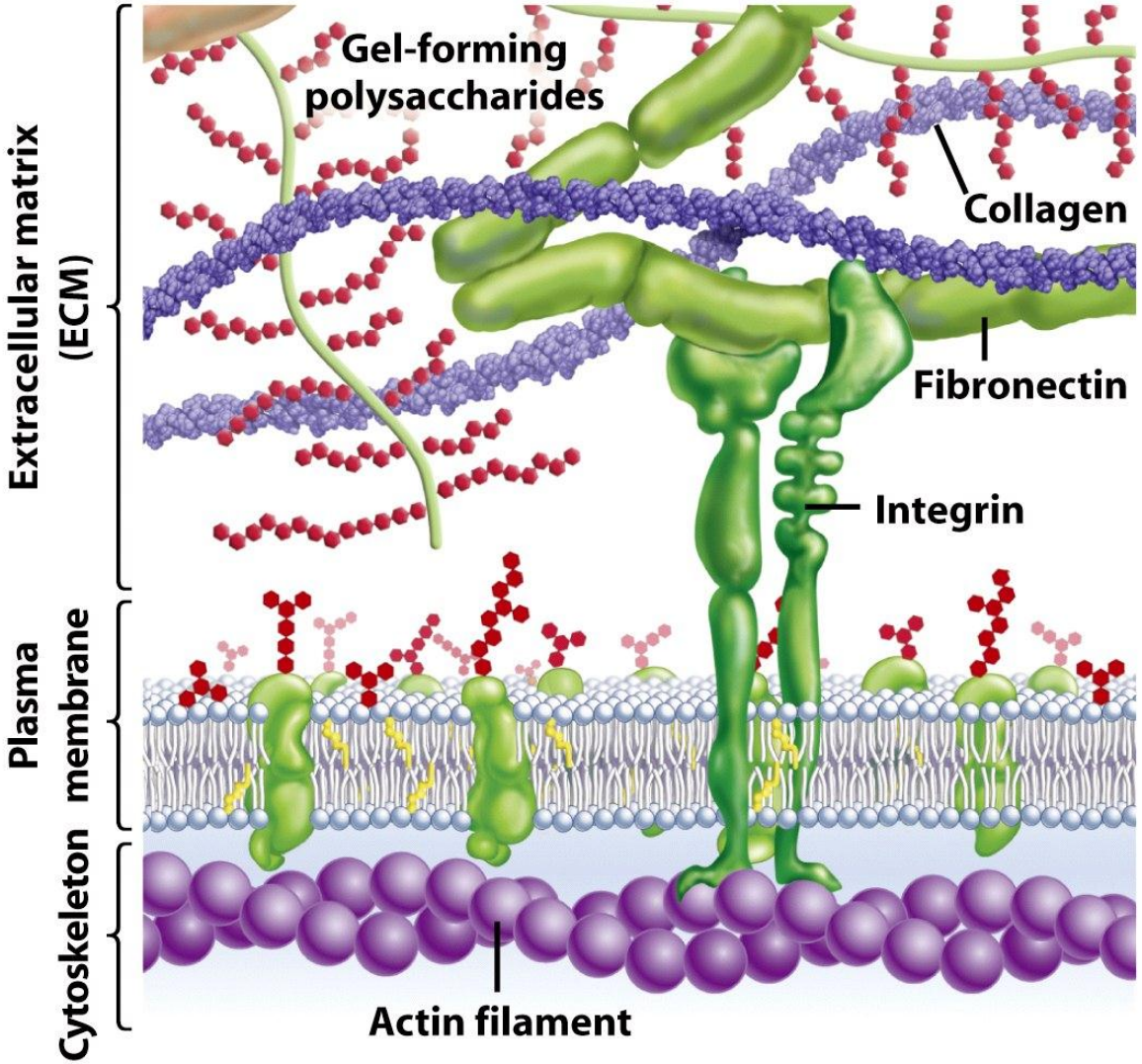
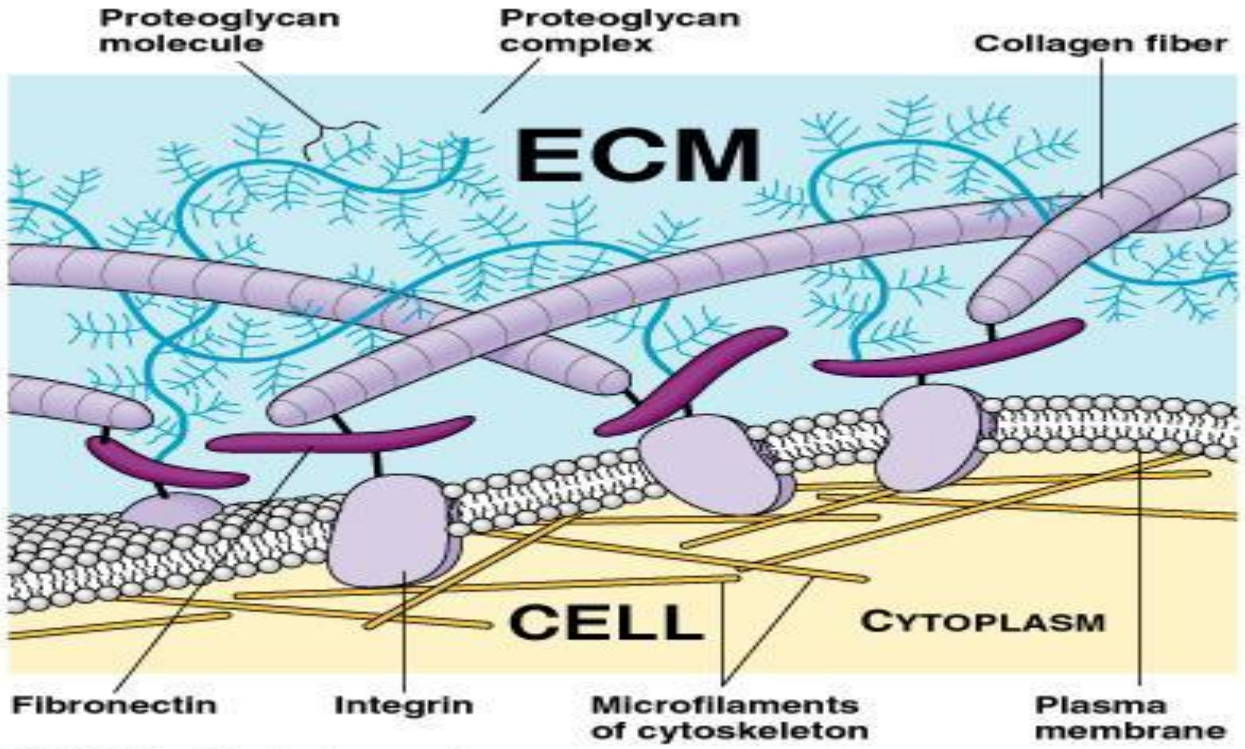


Figure 8-4 Biological Science, 2/e

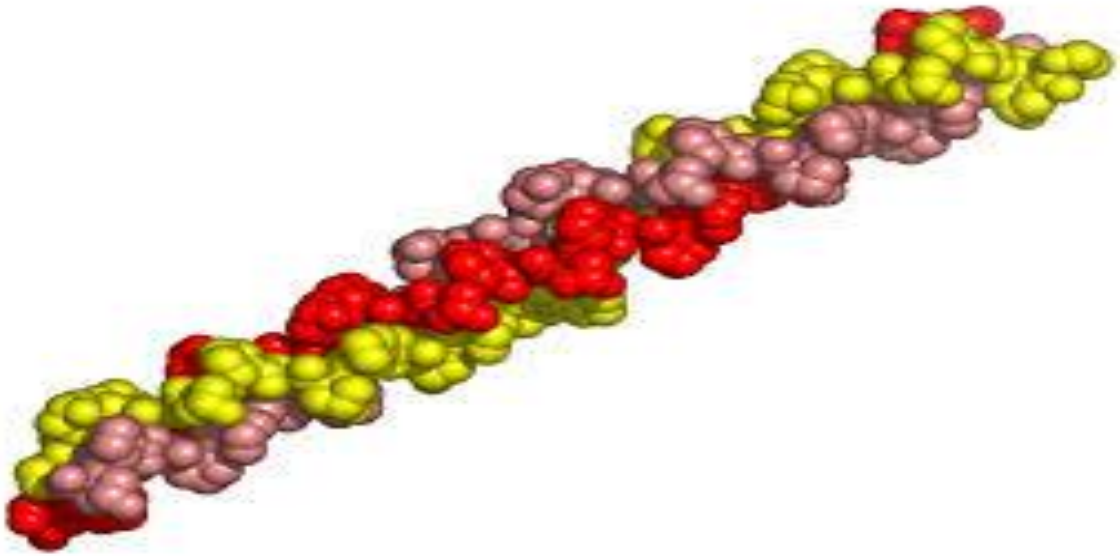


Components of extracellular matrix

- Fibrous structural proteins: Collagen and Elastin
- Water hydrated gel :Proteoglycans and hyaluronon
- Adhesive glycoproteins and adhesion receptors

collagen

- Triple helix structure



collagen

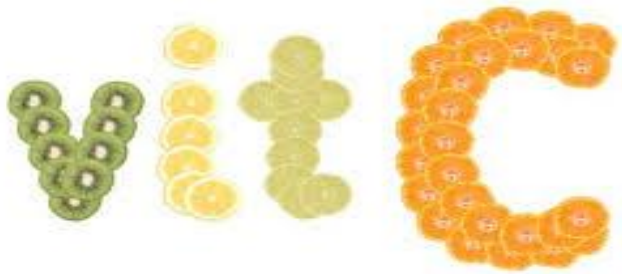
- Fibrillar collagen (types I, II, III, and V)
- Important for tensile strength.
- Forms a major proportion of connective tissue in healing.
- Strength is vitamin C dependant. (important for cross linking)

Nonfibrillar collagens

1. type IV present in basement membranes
2. type IX present in intervertebral disks
3. type VII present in dermal-epidermal junctions

Vitamin C deficiency

- Poor cross linking of collagen
- Skeletal deformity
- Easy bleeding due to week vascular basement membrane
- Poor wound healing

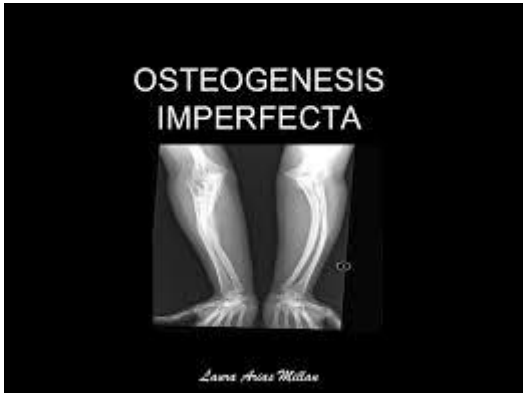


NOTE

*genetic defects in collagen

1. Ehlers- Danlos syndrome

2. Osteogenesis imperfecta



elastin

- Important for recoil and returning to a baseline structure after stress .
- Important in large blood vessels, skin and ligaments.
- Defect: Marfan syndrome: skeletal abnormalities and week aortic wall.

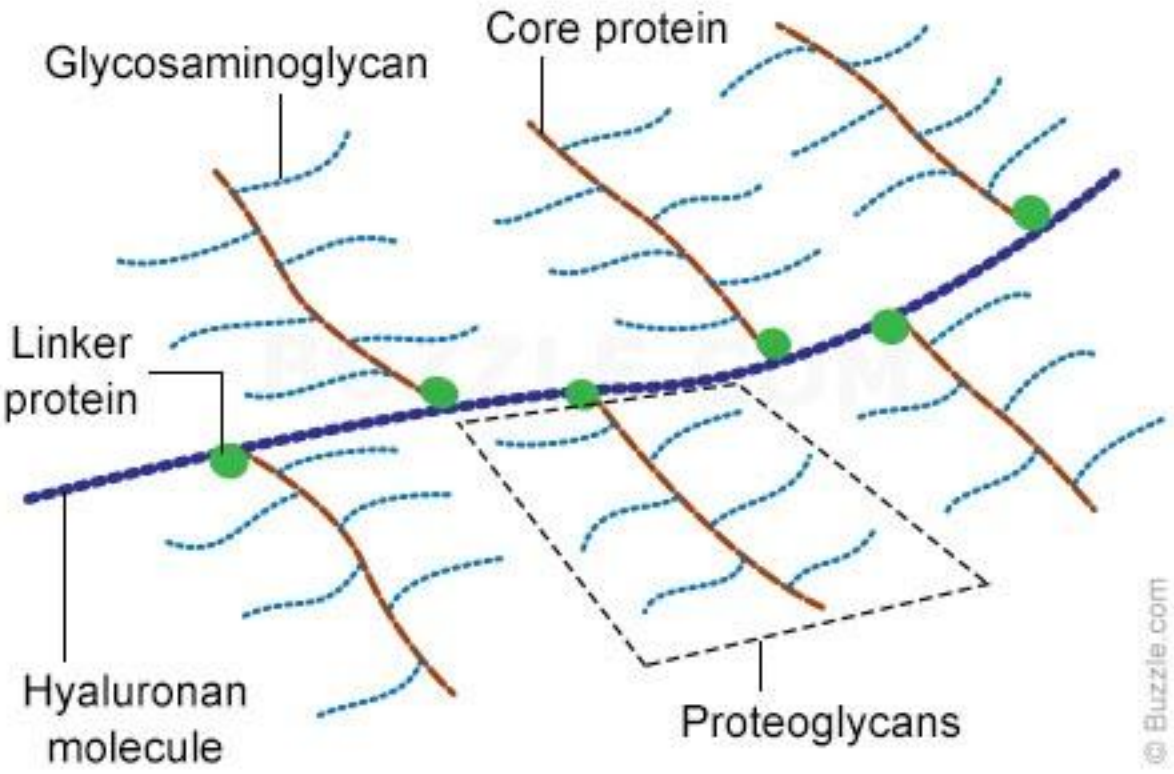
Proteoglycans

- Form compressible gel important for lubrication (e:g in joints)
- Composed of polysaccharide chain (glycosaminoglycans or polysaccharide) linked to a protein backbone
- Note proteo.. Refers to the protein, glycan to the saccharide

Hyaluronic acid

- = hyaluronan
- Mucopolysaccharide without protein core
- Important for: compressibility and reservoir of growth factors

Structure of Proteoglycans



Adhesive Glycoproteins

- Involved in cell-to-cell adhesion, the linkage of cells to the ECM.
- include: Fibronectin, laminin and integrins

fibronectin

- In tissues it forms fibrillar aggregates at wound healing sites
- In plasma it binds to fibrin within blood clotting sites

laminin

- Important for attachment of cells to underlying basement membrane
- Also modulates cell proliferation differentiation and motility

integrins

- Adhesion molecule
- Present on plasma membranes of almost all cells except RBC
- Important for cell movement, proliferation and differentiation

Scar formation

Scar formation

Three steps:

1. angiogenesis.
2. Migration and proliferation of fibroblasts.
3. Remodeling.

STEP 1: angiogenesis

- Development of new blood vessels from existing vessels , mainly venules.

NOTE: Angiogenesis is important in

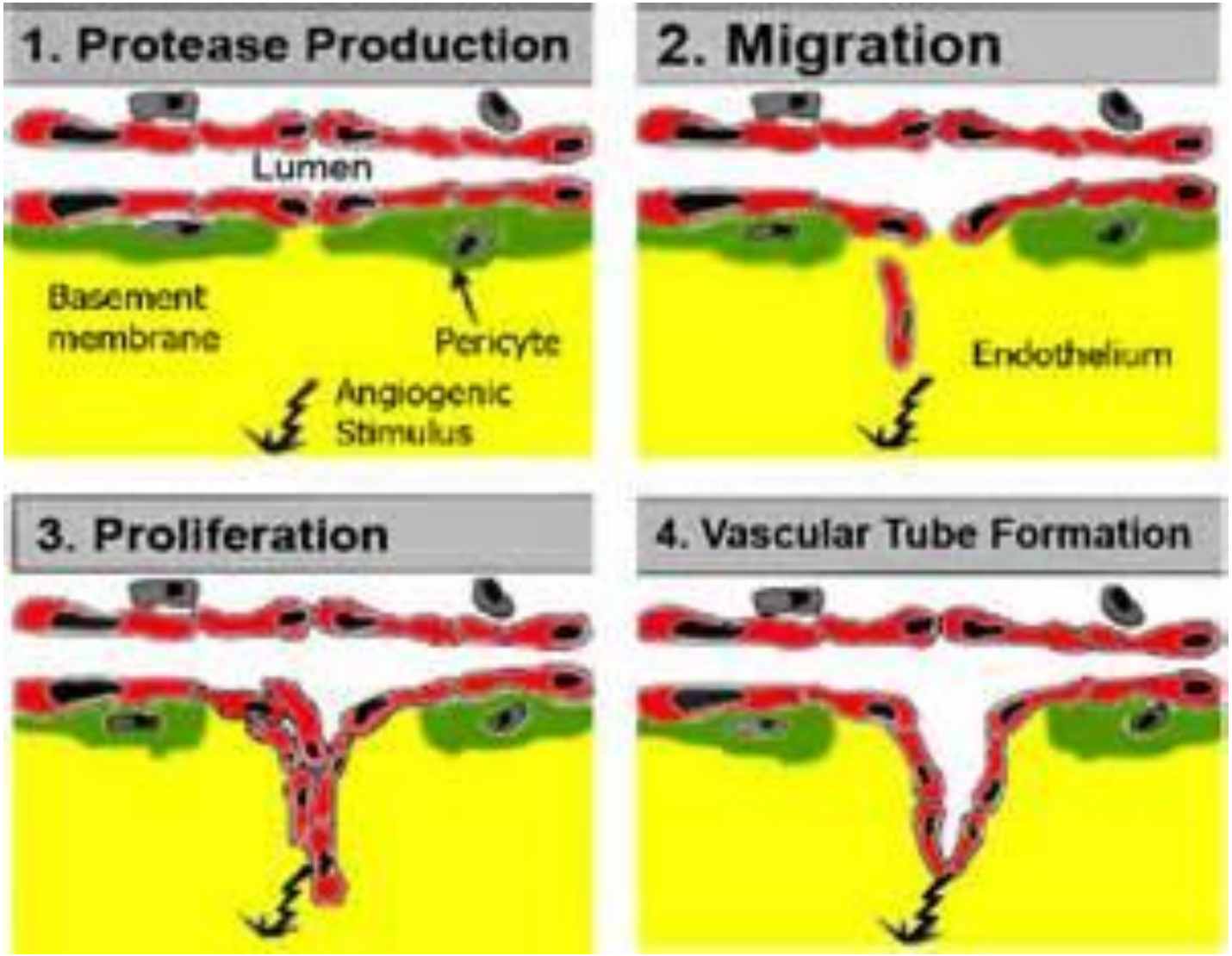
A. healing

B. collateral circulation in ischemia

C. neoplasia

Angiogenesis.. steps

- 1. vasodilation (due to NO) and increased permeability (VEGF)
- 2. Separation of pericytes from abluminal surface.
- 3. endothelial cell migration
- 4. endothelial cell prolifeartion
- 5. remodeling into capillary tubes
- 6. recruitment of periendothelial cells: pericytes and smooth muscle cells.
- 7.Supression of endothelial proliferation
- 8. Deposition of basement membrane


1. Protease Production
Lumen
Basement membrane
Pericyte
Angiogenic Stimulus
2. Migration
Endothelium
3. Proliferation
4. Vascular Tube Formation

GF in angiogenesis

- VEGF family.. Stimulate migration and proliferation of endothelial cells.

- Antibodies against VEGF can be used in treating some tumors and in age related macular degeneration

- FGF = fibroblast growth factor.
- Angiopoietins ANG 1 AND ANG2 … especially important for ECM production

VEGF

- Stimulate migration @ proliferation of endothelial cells.
- Vasodilation and production of NO

- Act by receptors which are stimulated by hypoxia and PDGF and TGFs

FGF

- Causes proliferation and migration of endothelial cells.

angiopoietins

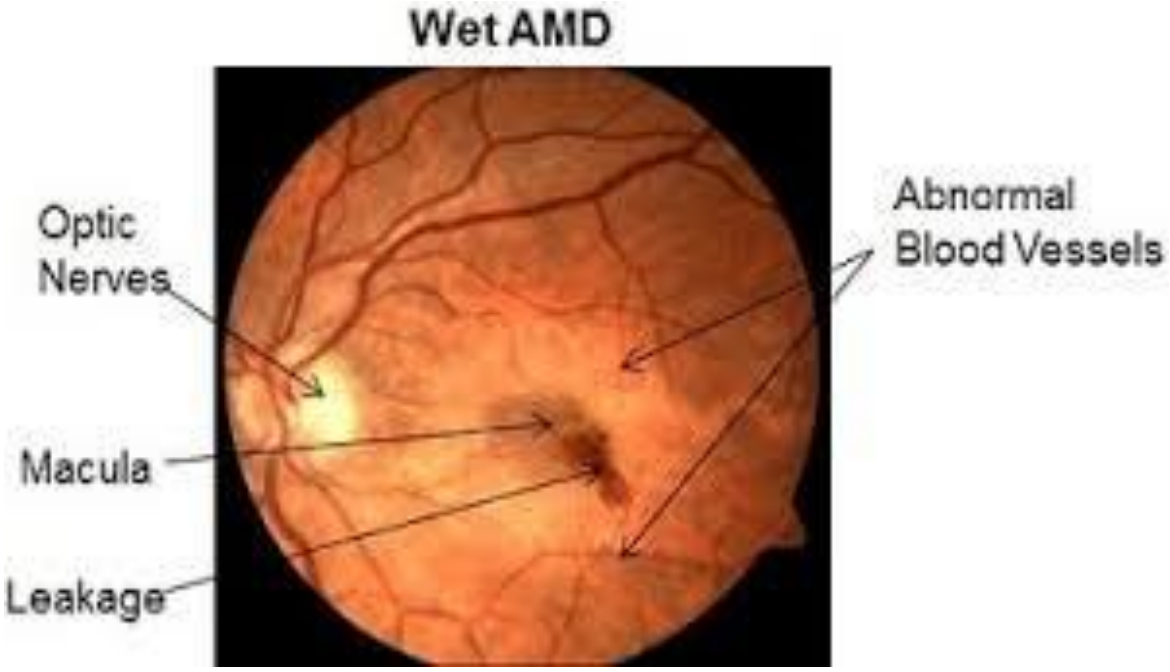
- Stabilization of newly formed blood vessels by pericytes, smooth muscle cells and deposition of connective tissue.

NOTE

- Newly formed vessels are leaky because: junctions not well-formed and VEGF increases permeability.

- People with wet macular degeneration have new, leaky vessels, that's why they have increased intraocular pressure.

Wet macular degeneration



Step 2: Deposition of connective tissue

A. Migration and proliferation of fibroblasts into the site of injury and

B. Deposition of ECM proteins produced by these cells.

Fibroblast migration and activation

- PDGF, FGF and TGF BETA.

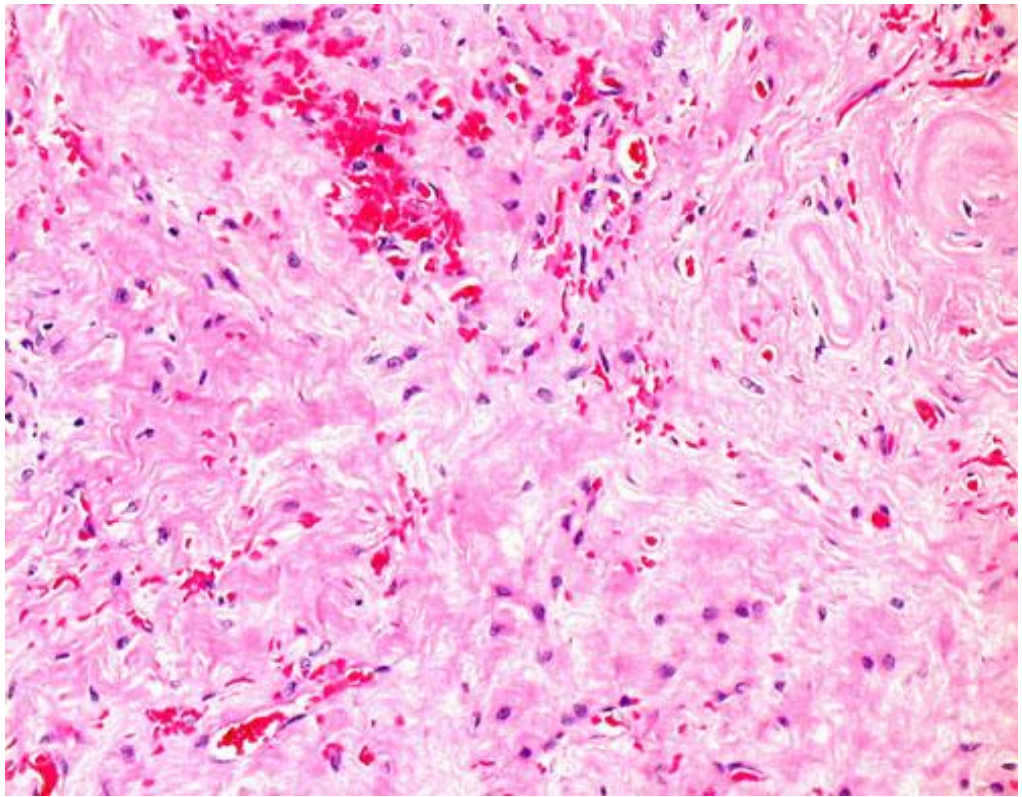
- Stimulated fobroblasts: synthetic activity.
- Synthesize ECM components.

Note:

- Collagen synthesis, is critical to the development of strength .
- Collagen synthesis by fibroblasts begins early in wound healing and continues for several weeks, depending on the size of injury.

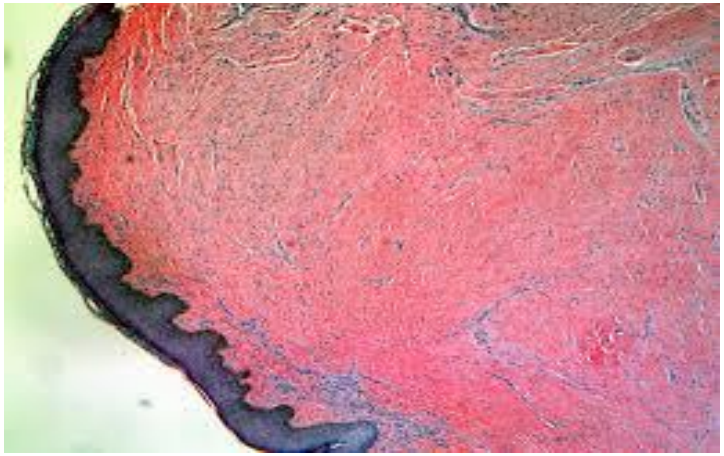
Granulation tissue

- The presence of active fibroblasts senthesyzing collagen + the angiogenesis.. = granulation tissue



scar

- With time the granulation tissue becomes a scar…. Mainly collagen with minimal fibrblasts and decreased vesseles



GF important for ECM deposition and scar formation

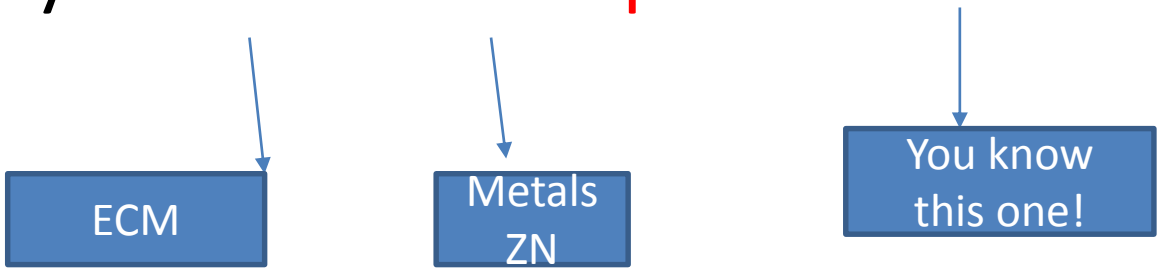
- TGF beta :1. increased collagen and other ECM component production. 2. inhibit collagen degradation

Note that TGF beta also limits inflammation.

- PDGF : migration & proliferation of fibroblasts.
- Cytokines: migration & proliferation of fibroblasts and ECM deposition

Step 3: remodeling

- Net scar: balance between collagen synthesis and degradation
- Degradation: by matrix metallo-proteinases MMP



- NOTE: Degradation of ECM can also happen by other enzymes like elastase and other proteiases

MMP

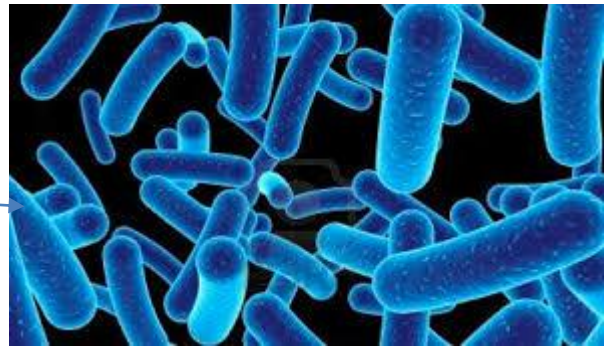
- Interstitial collagenase.. Degrades fibrillar collagen
- Gelatinase: degrades amorphous collagen and fibronectin
- Stromelysin: can degrade almost all ECM component except fibrillar collagen

MMP

- MMP are dangerous! They can degrade ECM so they need to be tightly regulated
- Secreted as zymogenes (inactive enzymes)
- Activated when needed
- GF and cytokines regulate secretion and synthesis
- Inhibited by tissue inhibitors of metalloproteinases TIMPs

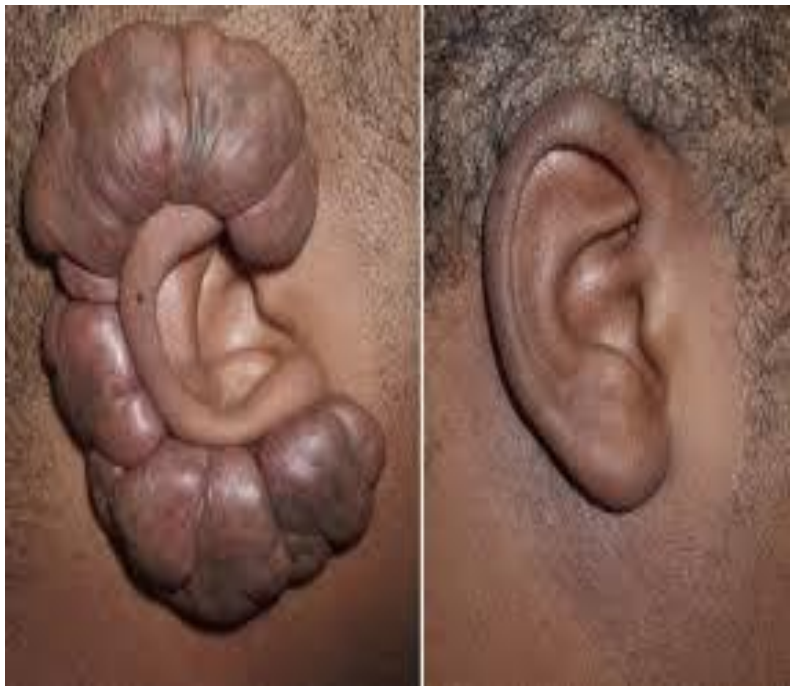
Factors affecting wound healing

- 1.Infection: most common cause of delayed healing because it increases inflammation and tissue damage



- 2. nutritional deficiency: protein and vit C
- 3. steroids: inhibit TGF beta
- 4. mechanical : pressure at the site of wound
- 5. poor perfusion: due to DM or vascular diseases
- 6.foreign bodies.. BUT not sutures (at leaset in the early stages)
- 7.cell growth aberrations: eloid and proud flesh

keloid



Wound healing

Healing by first intention (primary union)

- healing of a clean, uninfected surgical incision approximated by surgical sutures
- focal disruption of epithelial basement membrane continuity so.. A small scar is formed, with minimal wound contraction
- *epithelial regeneration is the principal mechanism of repair*

Healing by second intention or second Union

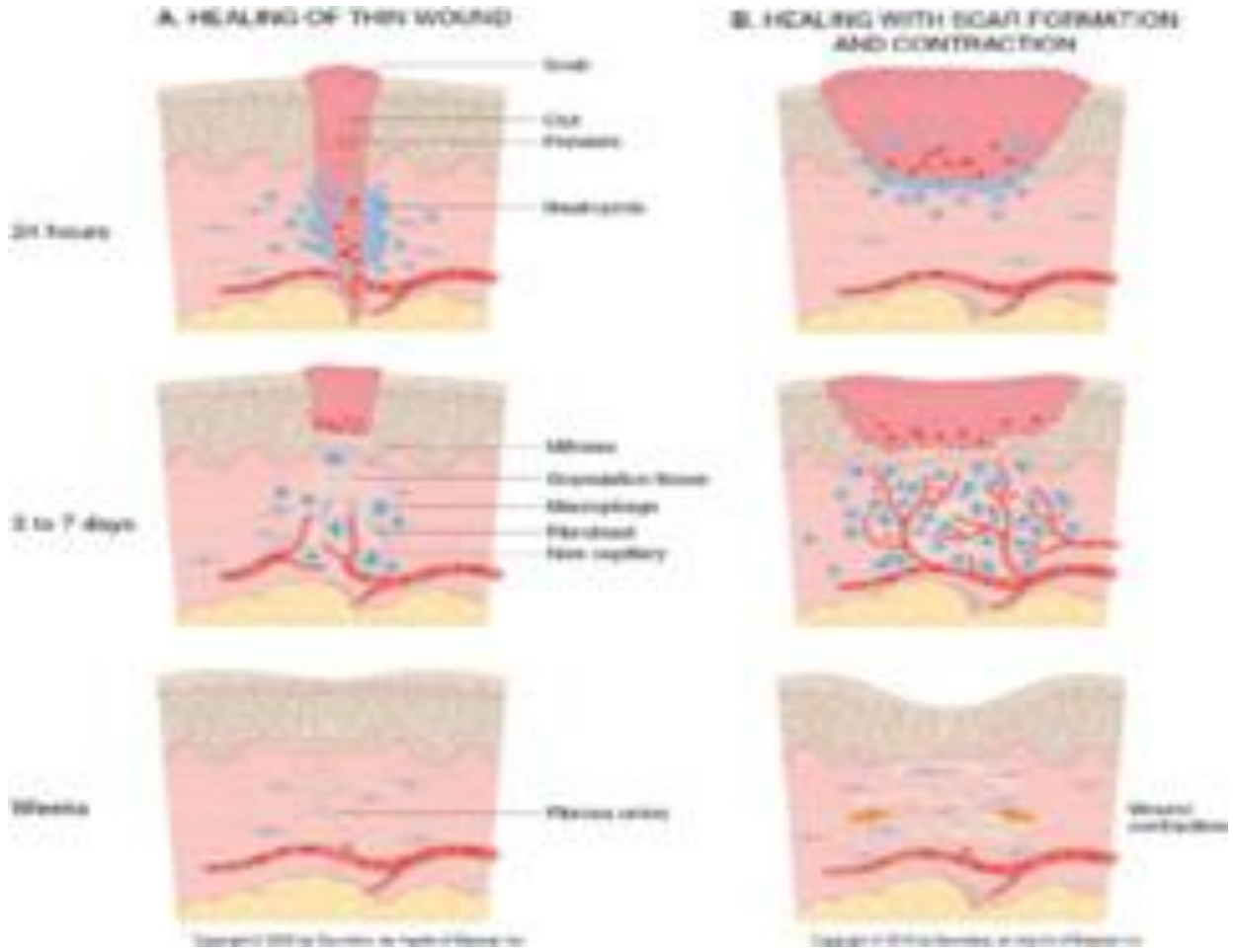
- tissue loss is extensive, e:g in large wounds, abscess , or ulceration
- The repair process is more complex and involves a combination of regeneration and scarring.

- Healing by secondary union is characterized by

1. Intense inflammation
2. Abundant granulation tissue,
3. Formation of a large scar,
4. wound contraction mediated by myofibroblasts

Wound contraction

- Within 6 weeks, large skin defects may be reduced to 5% to 10% of their original size.

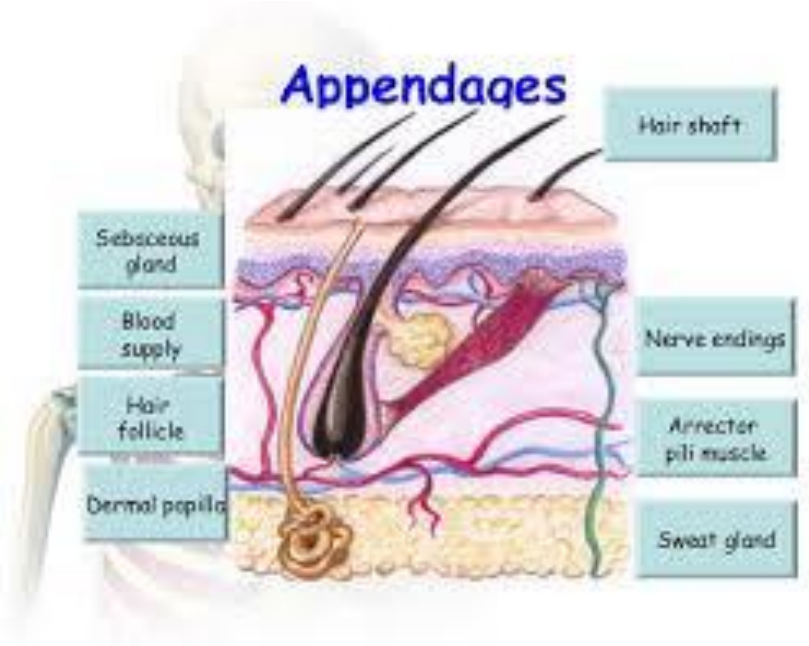

A. HEALING OF THIN WOUND
B. HEALING WITH SCAR FORMATION AND CONTRACTION
24 hours
3 to 7 days
Weeks

Healing by first intention

time	Inflammatory cell	Granulation tissue	collagen	Epithelial. Skin cells 9epidermis)
24 hours	neutrophils	starts		Mitotically active And start depositing basement membrane material
Day 3	macrophages	increases	Vertical fibers , Still week	Thickened epidermis
Day 5		Peak angiogenesis	Bridge incision	Normal thickness, maturation
Week 2	decrease	Replaced by scar	increased	

NOTE

- Dermal appendages lost in the incision cannot be replaced



Healing by second intention, differences from primary union:

- Large clot in the wound
- More inflammation
- Large defect: more granulation tissue needed
- So: more scar
- Wound contraction more

Wound strength

-sutured wounds= 70% of normal skin strngth due to the sutures.

-At 1 week (sutures removed) .. 10% of normal.

- strength then increases as a result of collagen synthesis exceeding degradation during the first 2 months and by structural modification of collagen.

- maximum strength reached: 70% to 80% of normal by 3 months .