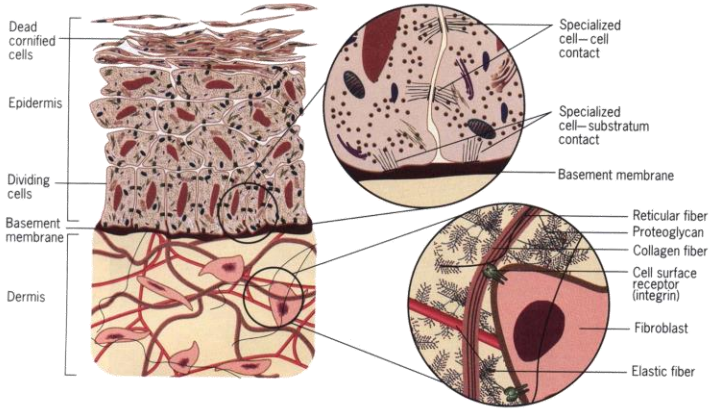
# ROLE OF THE SUBSTRATE IN REGENERATION

- relationship between the regenerating cells and their substrate is critical for:
  - maintenance of normal tissue functions and
  - the migrations or changes in state that are part of healing and regenerative processes
- cell adhesion molecules: membrane-bound glycoprotein molecules that mediate attachments between cells and other cells or components of the ECM



# ECM function

- *Mechanical support* for cell anchorage and cell migration, and maintenance of cell polarity
- *Control of cell growth.* ECM components can regulate cell proliferation by signaling through cellular receptors of the integrin family.
- *Maintenance of cell differentiation.* The type of ECM proteins can affect the degree of differentiation of the cells in the tissue, also acting largely via cell surface integrins.

# ECM function

- *Scaffolding for tissue renewal.* The maintenance of normal tissue structure requires a basement membrane or stromal scaffold. The integrity of the basement membrane or the stroma of the parenchymal cells is critical for the organized regeneration of tissues.
- *Establishment of tissue microenvironments.* Basement membrane acts as a boundary between epithelium and underlying connective tissue and also forms part of the filtration apparatus in the kidney.
- *Storage and presentation of regulatory molecules.*.

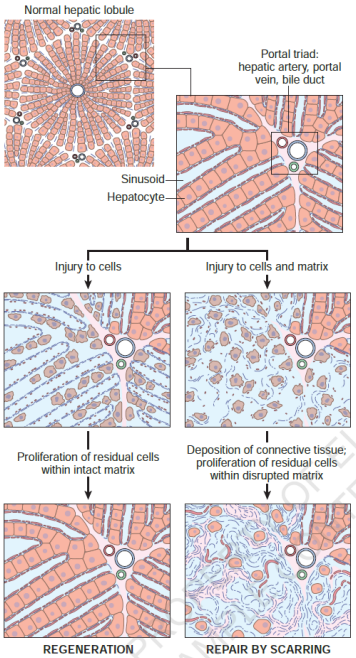
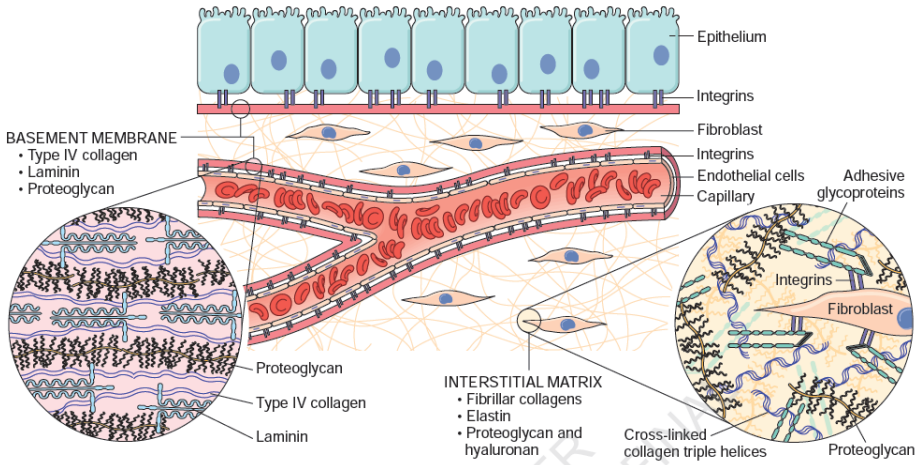



FIGURE 3–2 Role of the extracellular matrix in regeneration and repair. Liver regeneration with restoration of normal tissue after injury requires an intact cellular matrix. If the matrix is damaged the injury is repaired by fibrous tissue deposition and scar formation.

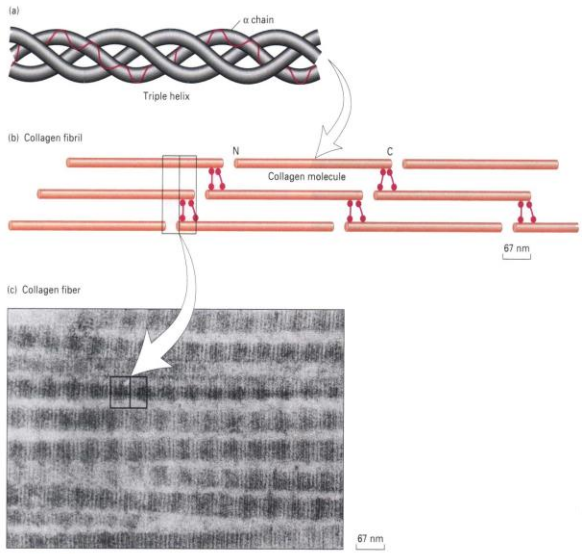
- The ECM is composed of three groups of macromolecules:
  - *fibrous structural proteins*, such as collagens and elastins that provide tensile strength and recoil;
  - *adhesive glycoproteins* that connect the matrix elements to one another and to cells; and
  - *proteoglycans and hyaluronan* that provide resilience and lubrication.

- Two basic forms of ECM:
  - *interstitial matrix*
    - is found in spaces between epithelial, endothelial, and smooth muscle cells, as well as in connective tissue.
    - It consists mostly of fibrillar and nonfibrillar collagen, elastin, fibronectin, proteoglycans, and hyaluronan.
  - *basement membranes*.
    - closely associated with cell surfaces,
    - consist of nonfibrillar collagen (mostly type IV), laminin, heparin sulfate, and proteoglycans.



# Kolagen

- merupakan glikoprotein
- disintesis terutama oleh fibroblast, sel otot polos dan sel epitel
- struktur kolagen memiliki kesamaan pada :
  - semua molekul kolagen memiliki trimer yang terdiri dari rantai polipeptida → rantai a
  - 3 rantai polipeptida kolagen berikatan membentuk struktur yang unik – *rodlike triple helix*

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# ECM

## collagen

**Table 2.1** Classification and types of collagens based on the collagen chain encoding genes, their distribution in tissues and disorders caused by their mutations

| Classification | Type | Own chains | Gene | Exons | Chromosome[a] | Distribution in tissues | Disorders caused by mutation in genes |
|---|---|---|---|---|---|---|---|
| Fibrillar collagens | I | α1 | *COL1A1* | 51 | 17q21.3–q22 | Bone, tendon, ligament, skin | Osteogenesis imperfecta, osteoporosis |
| | | α2 | *COL1A2* | 52 | 7q22.1 | | |
| | II | α1 | *COL2A1* | 54 | 12q13.11 | Cartilage, intervertebral disc, vitreous humor | Several chondrodysplasias, osteoarthritis |
| | | | *Col2a1* | 54 | | | |
| | III | α1 | *COL3A1* | 51 | 2q24.3–q31 | Co-expressed with collagen I in vasculature and skin | Ehlers-Danlos syndrome (type IV), arterial aneurysms |
| | | | *Col3a1* | 51 | 1 | | |
| | V | α1 | *COL5A1* | 66 | 9q34.2–q34.3 | Co-expressed with collagen I in lung, cornea and bone | Ehlers-Danlos syndrome (types I and II) |
| | | α2 | *COL5A2* | 54 | 2q14–q32 | | |
| | | α3 | *COL5A3* | 66 | 19p13.2 | | |
| | XI | α1 | *COL11A1* | 68 | 1p21 | Co-expressed with collagen II | Chondrodispasias, non-systematic heating loss, osteoarthritis |
| | | α2 | *COL11A2* | 66 | 6p21.2 | | |
| | | α1(II) | | | | | |
| | XXIV | α1 | *COL24A1* | 57 | 1p22.3 | Co-expressed with collagen I in bone and cornea | Not known |
| | XXVII | α1 | *COL27A1* | 61 | 9q32 | Co-expressed with collagen II in cartilage and epithelia | Not known |
| | XXVIII | α1 | *COL28A1* | 32 | 7p21.3 | Peripheral nerves | Not known |
| 3D network (BM-collagens) | IV | α1 | *COL4A1* | 52 | 13q34 | Most basement membranes | Alport syndrome |
| | | α2 | *COL4A2* | 47 | 13q34 | Glomerular and alveolar BM | (COL4A3, COL4A4, COL4A5) |
| | | | *Col4a2* | 47 | 8 | | Alport syndrome with diffuse oesophageal leiomyomatosis (COL4A5, COL4A6) |
| | | α3, | *COL4A3* | 52 | 2q34–q37 | | |
| | | α4 | *COL4A4* | 48 | 2q35–q37 | | |
| | | α5 | *COL4A5* | 51 | Xq22 | | Lethality at 14 weeks, progressive glomerulonephritis, renal failure[b] |
| | | α6 | *COL4A6* | 46 | Xq22 | | |

Table 2.1 (continued)

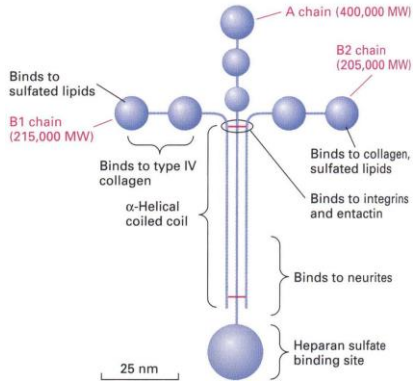
| Classification | Type | Own chains | Gene | Exons | Chromosome[a] | Distribution in tissues | Disorders caused by mutation in genes |
|---|---|---|---|---|---|---|---|
| Microfibril (Beaded-filaments) | VI | α1 | *COL6A1* | 36 | 21q22.3 | Wide tissue distribution, not bone | Bethlem myopathy |
| | | α2 | *COL6A2* | 36 | 21q22.3 | | |
| | | α3 | *COL6A3* | 41 | 2q37 | | |
| Anchoring fibril | VII | α1 | *COL7A1* | 118 | 3p21 | Squamous epithelium BM zone | Epidermolysis bullosa |
| | | | *Col7a1* | 118 | 9 | | |
| Hexagonal lattice | VIII | α1 | *COL8A1* | 5 | 3q12.3 | Many tissues, descement's membrane of cornea | Corneal endothelial dystrophy |
| | | α2 | *COL8A2* | 2 | 1p34.2 | | |
| | X | α1 | *COL10A1* | 3 | 6q21–q22 | Hypertrophic cartilage | Schmid metaphyseal chondrodisplasia |
| | | | *Col10a1* | 3 | 10 | | |
| FACITs | IX | α1 | *COL9A1* | 38 | 6q12–q14 | Associated with type II fibrils in cartilage and cornea | Epiphyseal dysplasia, intervertebral disc disease, osteoarthrosis |
| | | α2 | *COL9A2* | 32 | 1p32 | | |
| | | | *Col9a2* | 32 | 4 | | |
| | | α3 | *COL9A3* | 32 | 20q13.3 | | |
| | XII | α1 | *COL12A1* | 65 | 6q12–q13 | Associated with type I fibrils in perichondrium, ligament, and tendon | Disruption of periodontal and skin matrix structure[b] |
| | XIV | α1 | *COL14A1* | 44 | 8q23 | Associated with type I fibrils in many tissues | Not known |
| | XVI | α1 | *COL16A1* | 67 | 1p35–p34 | Associated with type II fibrils in hyaline cartilage and with microfibrils in skin | Not known |
| | XX | α1 | *COL20A1* | 35 | 20q13.33 | Associated with type I fibrils in sternal cartilage, cornea, and tendon | Not known |
| | XXI | α1 | *COL21A1* | 28 | 6p12.3–p11.2 | Associated with type I fibrils in vessel walls | Not known |
| FACIT-like | XIX | α1 | *COL19A1* | 51 | 6q12–q14 | Rare BM zones, in developing muscle | Abnormal muscle layer in the oesophagus[b] |
| | XXII | α1 | *COL22A1* | 63 | 8q24.23 | Associated with microfibrils at tissue junctions | Not known |
| | XXVI | α1 | *EMID2* | 13 | 7q22.1 | Testis and ovary | Not known |
| Trans-membrane collagens | XIII | α1 | *COL13A1* | 41/42 | 10q22 | Many tissues at a low level | Fetal lethal, cardiovascular and placental defects, tumor formation[b] |
| | | | *Col13a1* | 42 | 10 | | Progressive muscular atrophy[b] |
| | XVII | α1 | *COL17A1* | 56 | 10q24.3 | Skin and intestinal epithelia | Epidermolysis bullosa |
| | XXIII | α1 | *COL23A1* | 20 | 5q35.3 | Heart, lung and brain, metastatic tumor cells | Not known |
| | XXV | α1 | *COL25A1* | 35 | 4q25 | Neurons | Not known |
| Multiplexins | XV | α1 | *COL15A1* | 42 | 9q21–q22 | Many BM zones | Mild myopathy, cardiovascular defects[b] |
| | | | *Col15a1* | 40 | 4 | | |
| | XVIII | α1 | *COL18A1* | 43 | 21q22.3 | Endothelial and epithelial BM zones | Knobloch syndrome |
| | | | *Col18a1* | 43 | 10 | | Vascular abnormalities in the eye[b] |

[a] The cromosomal locations and the exones were collected from the Entrez Gene data base

[b] In transgenic mouse models; BM – basement membrane; Modified from Jäälinoja et al. 2007, Cosgrove et al. 1996, Reichenberger et al. 2000, Myllyharju et al. 2001, Fukai et al. 2002, Sund et al. 2001, Kvist et al. 2001 and Eklund et al. 2001.

# ECM: Non collagen
# **Laminin**

- Terdiri dari 3 rantai polipeptida yang berikatan melalui rantai disulfida
- Berperan dalam migrasi sel, diferensiasi, pertumbuhan
- Berperan dalam migrasi PGC
- ECM lain : tenascin, entactin, trombospondin

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# Laminin

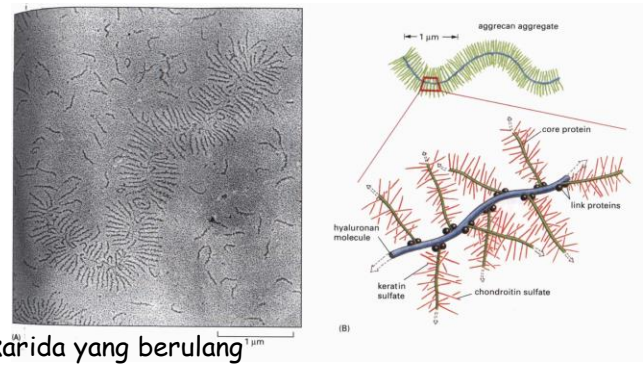
**Table 2.2** Classification laminins, their abbreviations according to current nomenclature with some alternative names and the genes encoding them

| Laminin (LM) | Abbreviation and alternative names | Genes encoding the laminin chains |
|---|---|---|
| LM-α1β1γ1 | LM-111, Ln-1 | *LAMA1, LAMB1, LMAC1* |
| LM-α2β1γ1 | LM-211, Ln-2 | *LAMA2, LAMB1, LAMC1* |
| LM-α1β2γ1 | LM-121, Ln-3 | *LAMA1, LAMB2, LAMC1* |
| LM-α2β2γ1 | LM-221, Ln-4 | *LAMA2, LAMB2, LAMC1* |
| LM-α3Aβ3γ2 | LM-332/LM-3A32, Ln-5/5A | *LAMA3A, LAMB3, LAMC2* |
| LM-α3Bβ3γ2 | LM-3B32, Ln-5B | *LAMA3B, LAMB3, LAMC2* |
| LM-α3Aβ1γ1 | LM-311/LM-3A11, Ln-6 | *LAMA3A, LAMB1, LAMC1* |
| LM-α3Aβ2γ1 | LM-321/LM-3A21, Ln-7 | *LAMA3A, LAMB2, LAMC1* |
| LM-α4β1γ1 | LM-411, Ln-8 | *LAMA4, LAMB1, LAMC1* |
| LM-α4β2γ1 | LM-421, Ln-9 | *LAMA4, LAMB2, LAMC1* |
| LM-α5β1γ1 | LM-511, Ln-10 | *LAMA5, LAMB1, LAMC1* |
| LM-α5β2γ1 | LM-521, Ln-11 | *LAMA5, LAMB2, LAMC1* |
| LM-α2β1γ3 | LM-213, Ln-12 | *LAMA2, LAMB1, LAMC3* |
| LM-α3β2γ3 | LM-323, Ln-13 | *LAMA3, LAMB2, LAMC3* |
| LM-α4β2γ3 | LM-423, Ln-14 | *LAMA4, LAMB2, LAMC3* |
| LM-α5β2γ3 | LM-523, Ln-15 | *LAMA5,LAMB2, LAMC3* |
| LM-α5β2γ2 | LM-522 | *LAMA5, LAMB2, LAMC2* |

Modified from Patarroyo et al. 2002, Aumailley et al. 2005, Tzu et al. 2008 and Egles et al. 2007.

# Proteoglikan

- Kompleks protein polisakarida
- Terdiri atas :
  - core protein berikatan dengan rantai glikosamino-glikans (GAG) → terdiri atas disakarida yang berulang
- mengikat banyak molekul air
- membentuk gel yang porous

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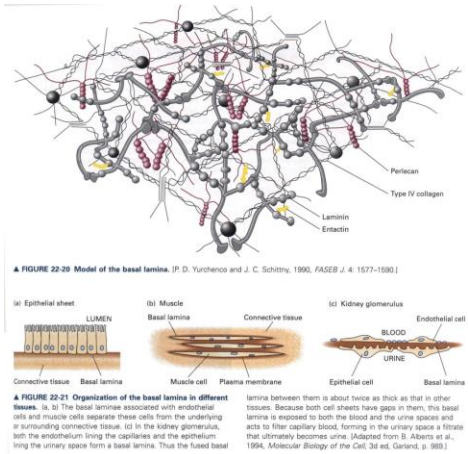
# Basal membran

- Mengelilingi sel otot dan sel lemak
- Di bawah jaringan epitel, sel-sel endotelium
- tempat pelekatan sel;
- substrat untuk migrasi sel;
- membatasi jaringan dalam suatu organ,
- sebagai suatu barier makromolekul. BM mencegah lalunya protein dari darah → pada dinding kapiler Dalam ginjal double layer yang memisahkan kapiler pada glomerulus dari dinding tubulus ginjal.
- sebagai barier untuk invasi sel ke suatu jaringan




▲ **FIGURE 22-20 Model of the basal lamina.** [P. D. Yurchenco and J. C. Schittny, 1990, *FASEB J.* 4: 1577–1590.]

▲ **FIGURE 22-21 Organization of the basal lamina in different tissues.** (a, b) The basal laminae associated with endothelial cells and muscle cells separate these cells from the underlying or surrounding connective tissue. (c) In the kidney glomerulus, both the endothelium lining the capillaries and the epithelium lining the urinary space form a basal lamina. Thus the fused basal lamina between them is about twice as thick as that in other tissues. Because both cell sheets have gaps in them, this basal lamina is exposed to both the blood and the urine spaces and acts to filter capillary blood, forming in the urinary space a filtrate that ultimately becomes urine. [Adapted from B. Alberts et al., 1994, *Molecular Biology of the Cell*, 3d ed, Garland, p. 989.]

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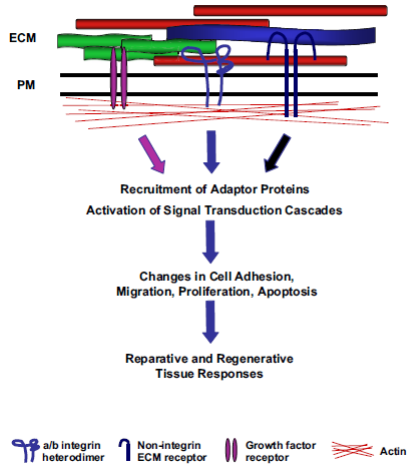
# Cell-ECM interaction



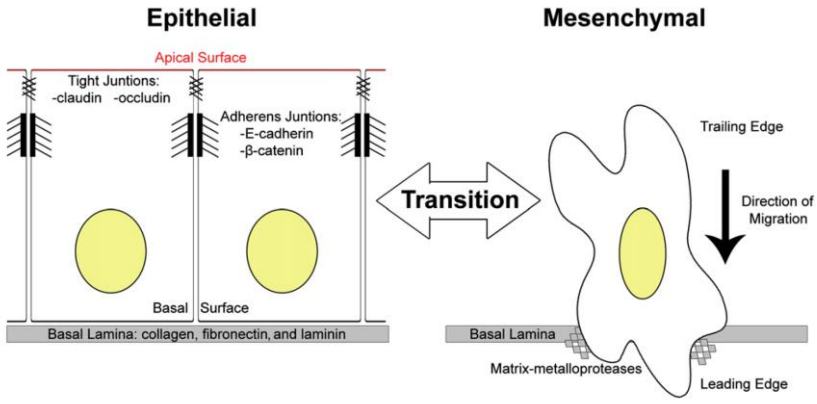
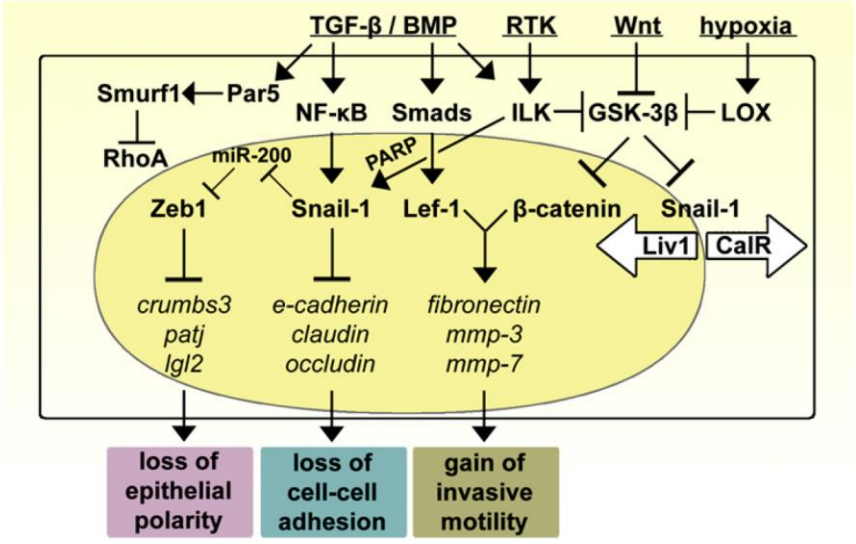




**FIGURE 1.1**

**Epithelial versus mesenchymal.** Epithelial cells adhere tightly together by tight junctions and adherens junctions localized near the apical surface. Epithelial cells also have a basal surface that rests on a basal lamina. Mesenchymal cells in contrast do not have well-defined cell-cell adhesion complexes, have front-end/back-end polarity instead of apical/basal polarity, and are characterized by their ability to invade the basal lamina.

- EMT pathway




## Components of invasion

a) Matrix degrading enzymes

b) Cell adhesion

c) Cell motility

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# Matrix degrading enzymes

- Required for a controlled degradation of components of the extracellular matrix (ECM)
- The proteases involved in this process are classified into serine-, cysteine-, aspartyl-, and metalloproteinase.

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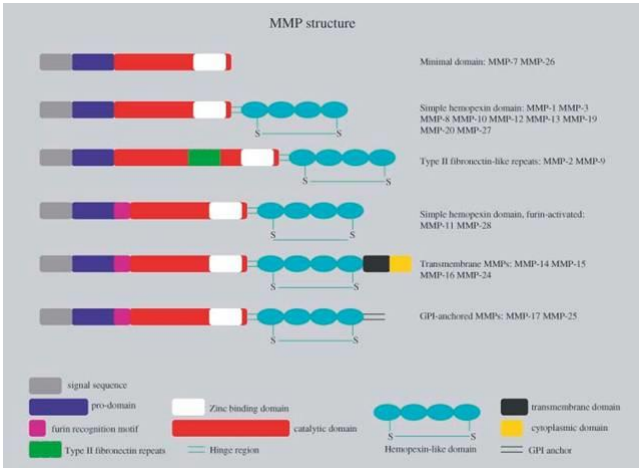
**Table 2.6** Classification of matrix metalloproteinases (MMPs) based on their domain arrangement and their alternative names, molecular weights and their collagenous, non-collagenous and other substrates

| Classification | MMP | Alternative names | Mw[a] | Collagen substrates | Non-collagenous substrates | Other substrates |
|---|---|---|---|---|---|---|
| **Archetypal MMPs** | | | | | | |
| Collagenase | 1 | Collagenase-1 | 52/41 | I, II, III, VII, VIII, X, XI, gelatin | Dntactin, fibronectin, laminin, perlecan, proteoglycans, tenascin, vitronectin | $\alpha_1$-antiprotease, α1PI, α2M, casein, C1q, fibrinogen, IL-1α and -β, proMMP-1, -2, pro-TNF-α, SDF-1 |
| | 8 | Collagenase-2 | 75/58;54/42 | I, II, III, V, VII, VIII, X, gelatin | Entactin, fibronectin, laminin, proteoglycans, tenascin | ADAMTS-1, α2M, α1PI, fibrinogen, Ln-5, proMMP-8, substance P, tissue factor pathway inhibitor |
| | 13 | Collagenase-3 | 60/48 | I, II, III, IV,V, VII, IX, X, XIV, XVII, gelatin | Fibronectin, laminin, proteoglycans, tenascin | C1q, fibrinogen, MCP-3, proMMP-9, -13, SDF-1 |
| Stromelysin | 3 | Stromelysin-1 | 54/43, 28 | IIII, IV, V, VII, IX, X, XI, gelatin | Decorin, elastin, fibronectin, laminin, proteoglycans, tenascin, vitronectin | α1PI, α2M, E-cadherin, casein, fibrin, fibrinogen, L-selectin, proHB-EGF, proMMPs, proTNF-α |
| | 10 | Stromelysin-2 | 54/43, 24 | I, III, IV, V, IX, X, XI, gelatin | Aggrecan, elastin, fibronectin, integrin, laminin, vitronectin | α1PI, α2M, casein, fibrin/fibrinogen, proαdefensin, proMMPs, proTNF-α |

# MMP family

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## Matrix metalloproteinases (MMP)

- 16 members, subdivided into 4 groups, based on their structural characteristics and substrate specificities
- Soluble and secreted groups; collagenase, gelatinase and stromelysins
- Membrane type (MT-MMP) group are anchored in the plasma membrane
- A zinc ion in the active centre of the protease is required for their catalytic activities.

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# Regulation of MMP

- MMP is controlled by an increased expression on a transcriptional level.
- MMPs are calcium-dependent proteases, which are synthesized as a inactive proenzymes and are activated by the cleavage of a propeptide.
- MMP activity is regulated by specific inhibitors, the tissue inhibitors of MMP (TIMPs). Binding TIMP to MMP is in a 1:1 stoichiometry.
- MMP2 and MMP9, which cleave type IV collagen the major constituent of basement membrane, are believed to be of special importance

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# Serine proteases

- Serine protease involved in ECM degradation are plasmin, plasminogen activators and cathepsin G.
- Plasmin is believed to be the most important serine protease, firstly because its ability to degrade several matrix components like gelatin, fibronectin or laminin, and secondly by the possible activation of numerous proforms of MMPs by propeptide cleavage.
- Plasmin is synthesized in its inactive proform, plasminogen, which can be converted to plasmin by plasminogen activator.

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# Plasminogen activator

- Two main types : urokinase (uPA) and tissue (tPA).
- There are specific inhibitors (PAI-1 and PAI-2) for the PA.

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**Examples of Growth Factors and Cytokines That Are Bound to Components of the Extracellular Matrix**

| **Bound to heparin/heparan sulfate proteoglycans** | **Bound to chondroitin sulfate chains** |
|---|---|
| EGF | Platelet factor 4 |
| FGF-1 to FGF-9 | |
| GM-CSF | **Bound to matrix proteins** |
| HB-EGF | Collagen type IV: TGF-β, BMP-2, BMP-7 |
| HGF/SF | Decorin: TGF-β |
| IL-2, -3, -4, -6, -8 | Fibrin: TGF-β |
| IP-10 | Fibronectin: TGF-β, TNF-α |
| KGF | IGF-binding proteins: IGF-1 |
| MCP-1 | Proteoglycan core proteins: TGF-β |
| MK | SPARC: PDGF-AB, PDGF-BB |
| NT-6 | Thrombospondin: TGF-β |
| PDGF-A, -B | 160-kDa protein: LIF |
| Platelet factor-4 | |
| Purpurin | |
| Schwann cell growth factor | |
| TGF-β | |
| VEGF | |

# Cell attachment

1. Integrin: cell-matrix adhesion

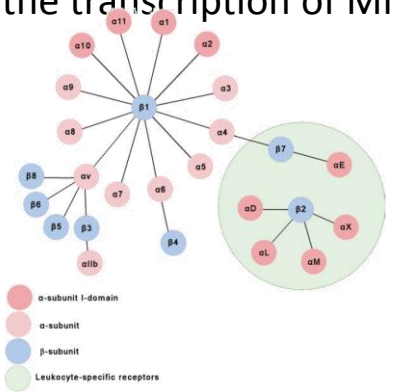
2. E-cadherin/catenin adhesion complex: cell-cell adhesion

**Classes of Cell-Surface Adhesion Molecules**

| Class | Interaction type | $Ca^{++}$ or $Mg^{++}$ involvement | Ligand |
|---|---|---|---|
| Cell adhesion molecules (CAMs) | Homophilic/ heterophilic | No | Other CAMs |
| Cadherins | Homophilic | Yes | Identical cadherins |
| Selectins | Heterophilic | Yes | Sialyl structures, in blood vessels only |
| Integrins | Heterophilic | No | Various: collagen, fibronectin, vitronectin, laminin, thrombospondin, complement, fibrinogen, von Willebrand factor |

# Integrin

- Heterodimeric transmembrane receptors consists of $\alpha$ and $\beta$ subunits
- Function to provide interactions between cells and macromolecules in the ECM
- Integrin can affect the transcription of MMP genes

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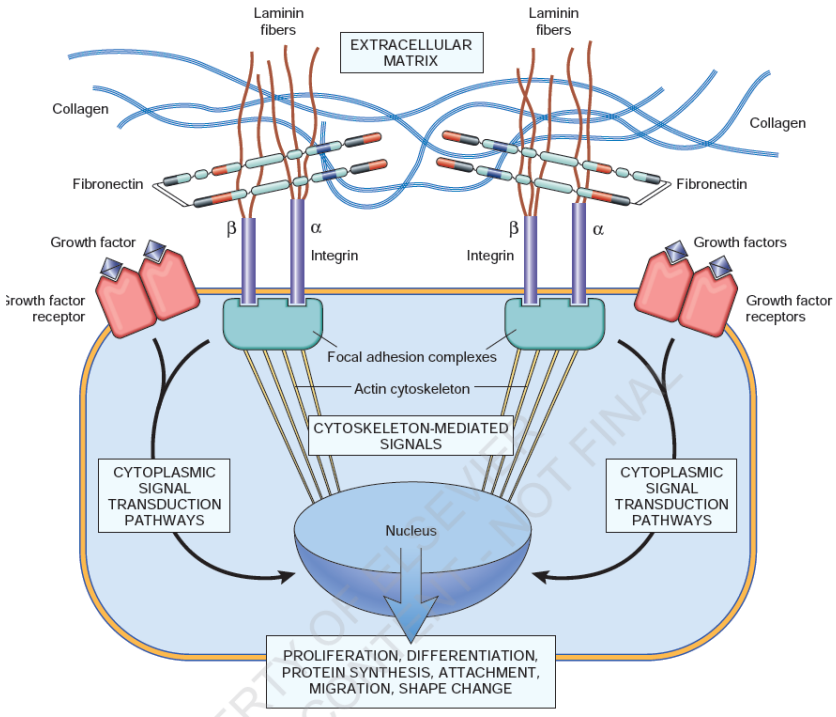
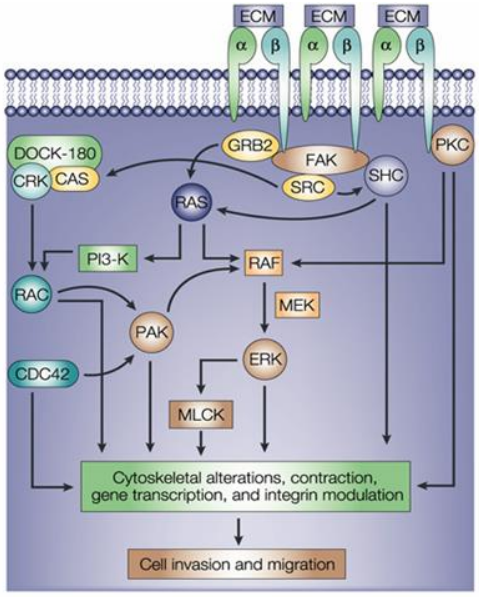



# Integrin signaling

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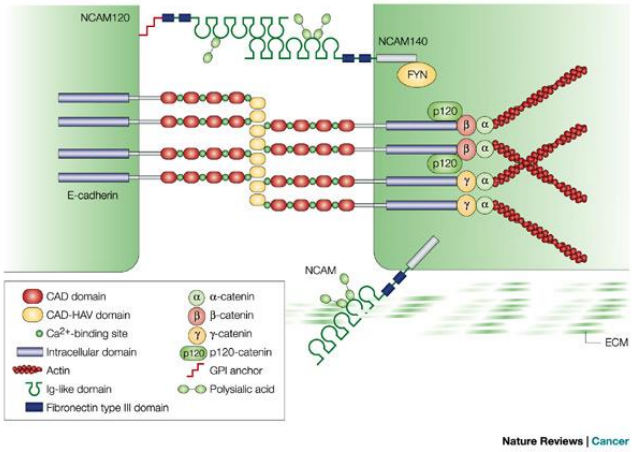
# 2) E-cadherin and catenin complex

- Most important cell-cell adhesion molecules




Cadherin-mediated cell-cell adhesion

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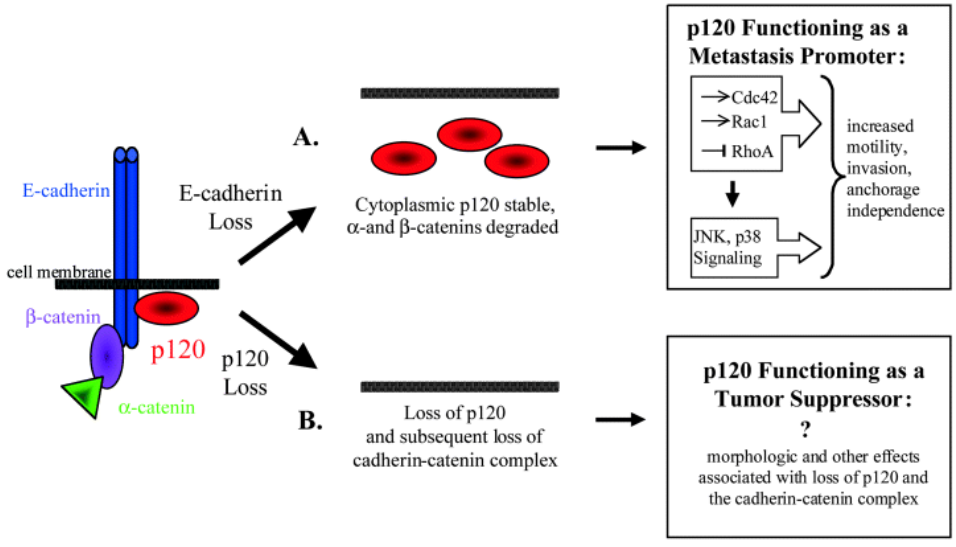
# p120 catenin

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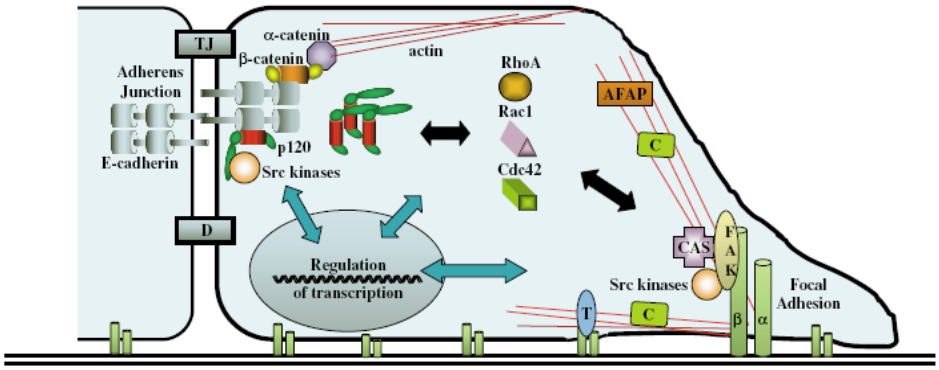



T Tensin (p210)

CAS Crk associated substrate (CAS p130)

FAK Focal adhesion kinase (FAK p125)

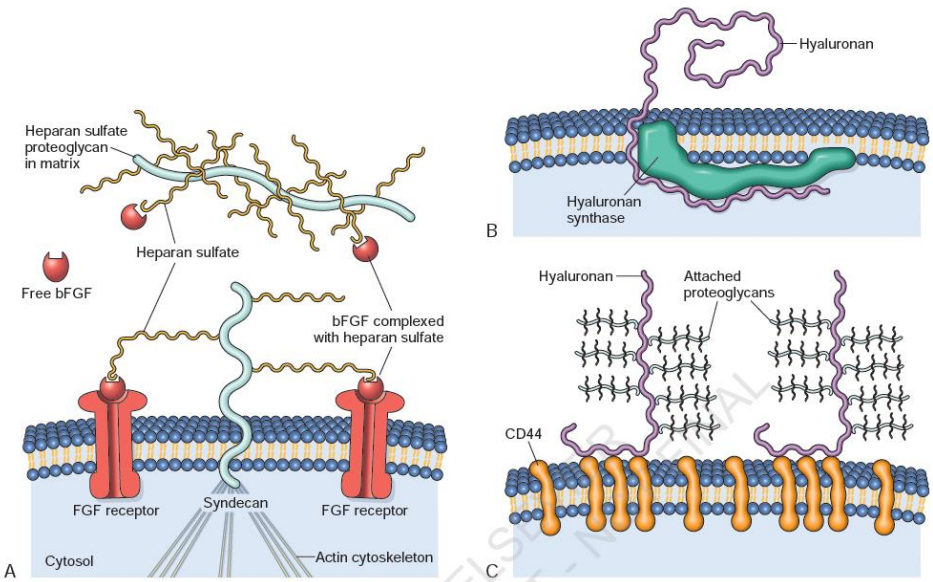
p120-catenin (p120$^{ctn}$)

AFAP Actin filament associated protein (AFAP p110)

C Cortactin (p85)

Src (and Src-like) kinases: Fyn, Yes, Fer

TJ: tight junction
D: desmosome



# c) Cell migration

1. Small Rho GTPase family

2. Motility promoting factors

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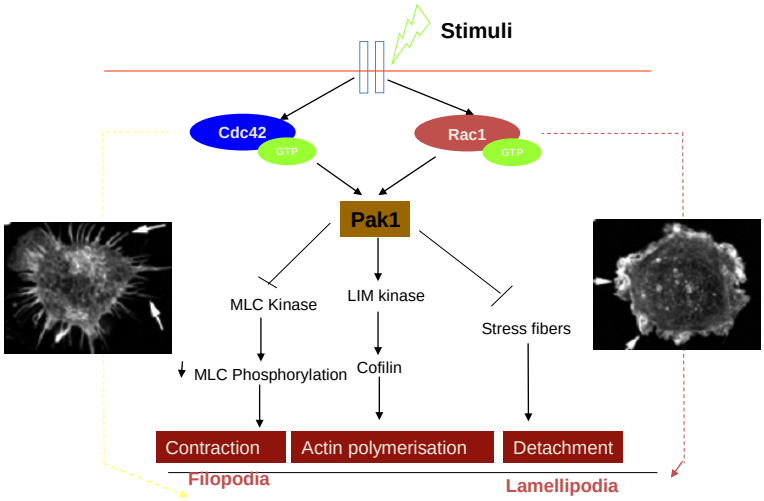
# Small Rho GTPase

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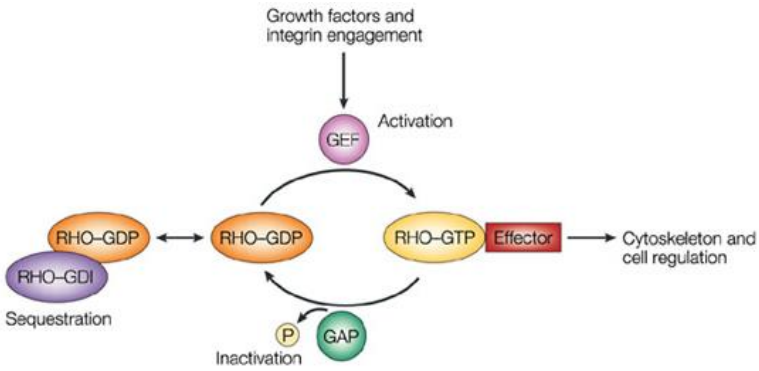
# Model of Rho GTPase regulation

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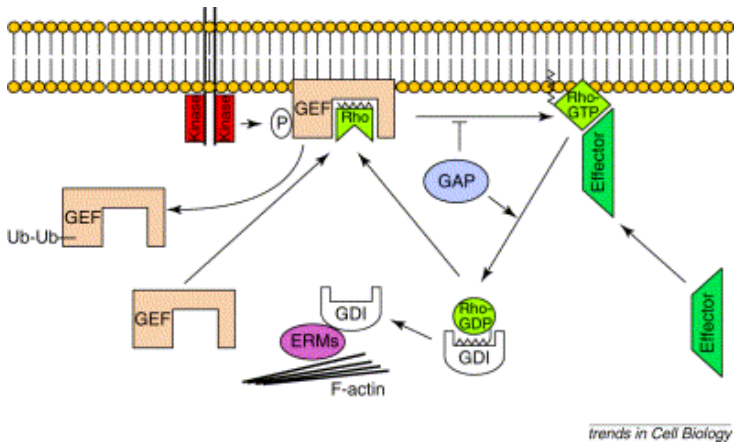
# Regulation of Rho GTPase

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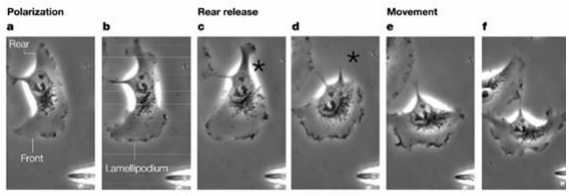
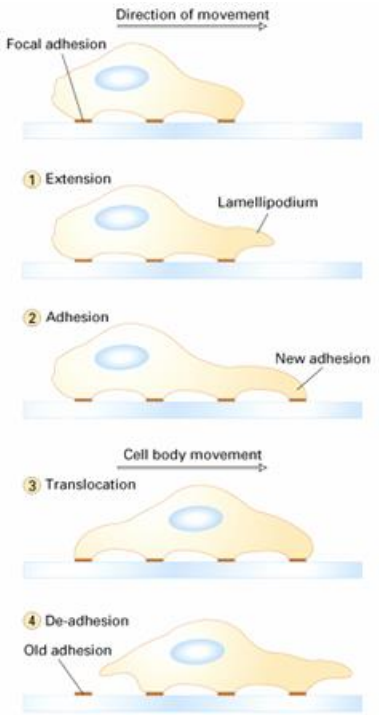
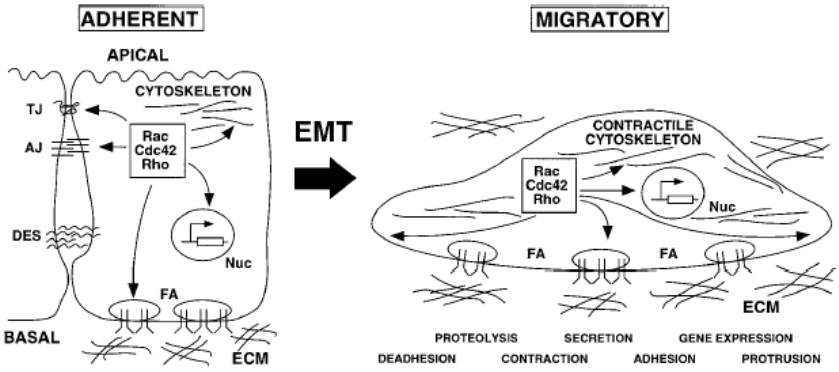
# Cell movement

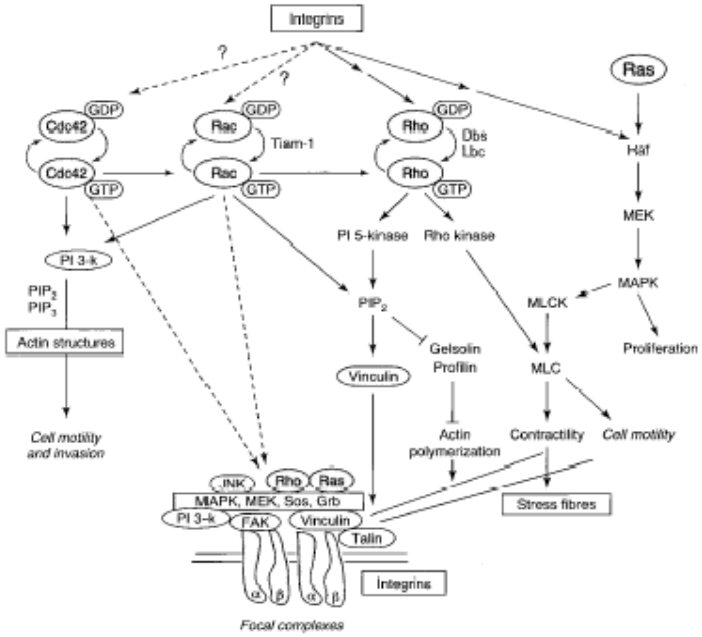



Rho GTPase is required for the transition of invasive phenotype



**Signaling pathways related to integrin and small GTPase**

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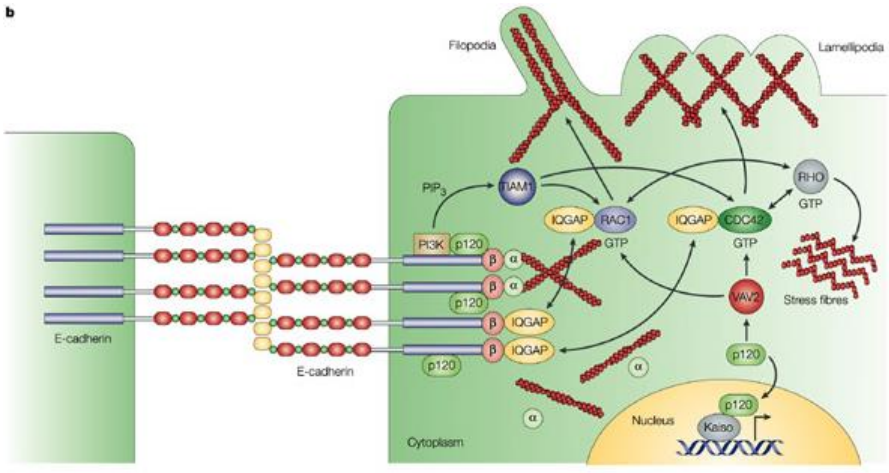
# E-cadherin and Rho GTPase signaling

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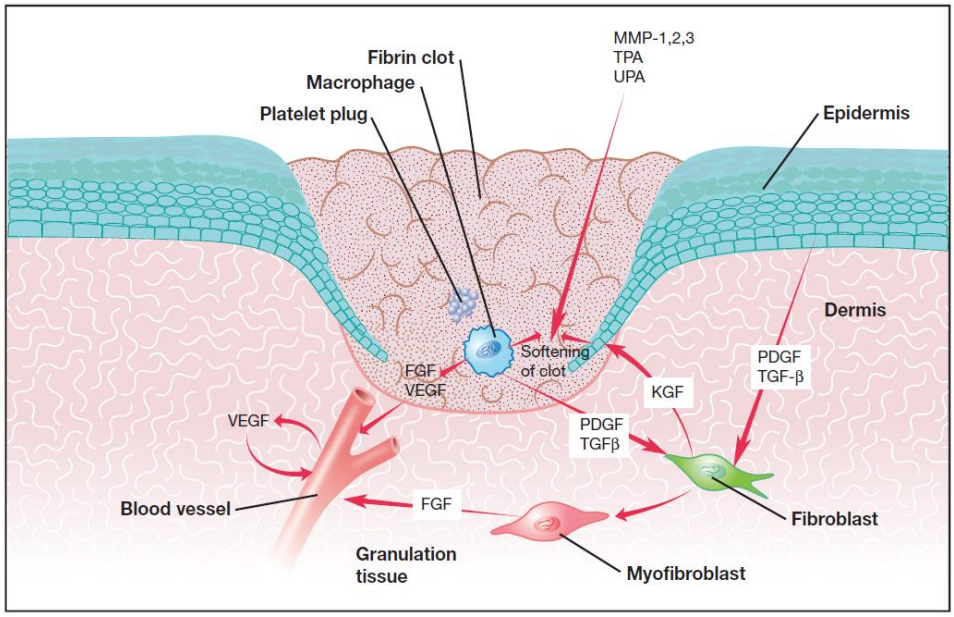
MMP-1,2,3
TPA
UPA
Fibrin clot
Macrophage
Platelet plug
Epidermis
Dermis
Softening
of clot
FGF
VEGF
KGF
PDGF
TGF-β
VEGF
PDGF
TGFβ
Blood vessel
FGF
Fibroblast
Granulation
tissue
Myofibroblast