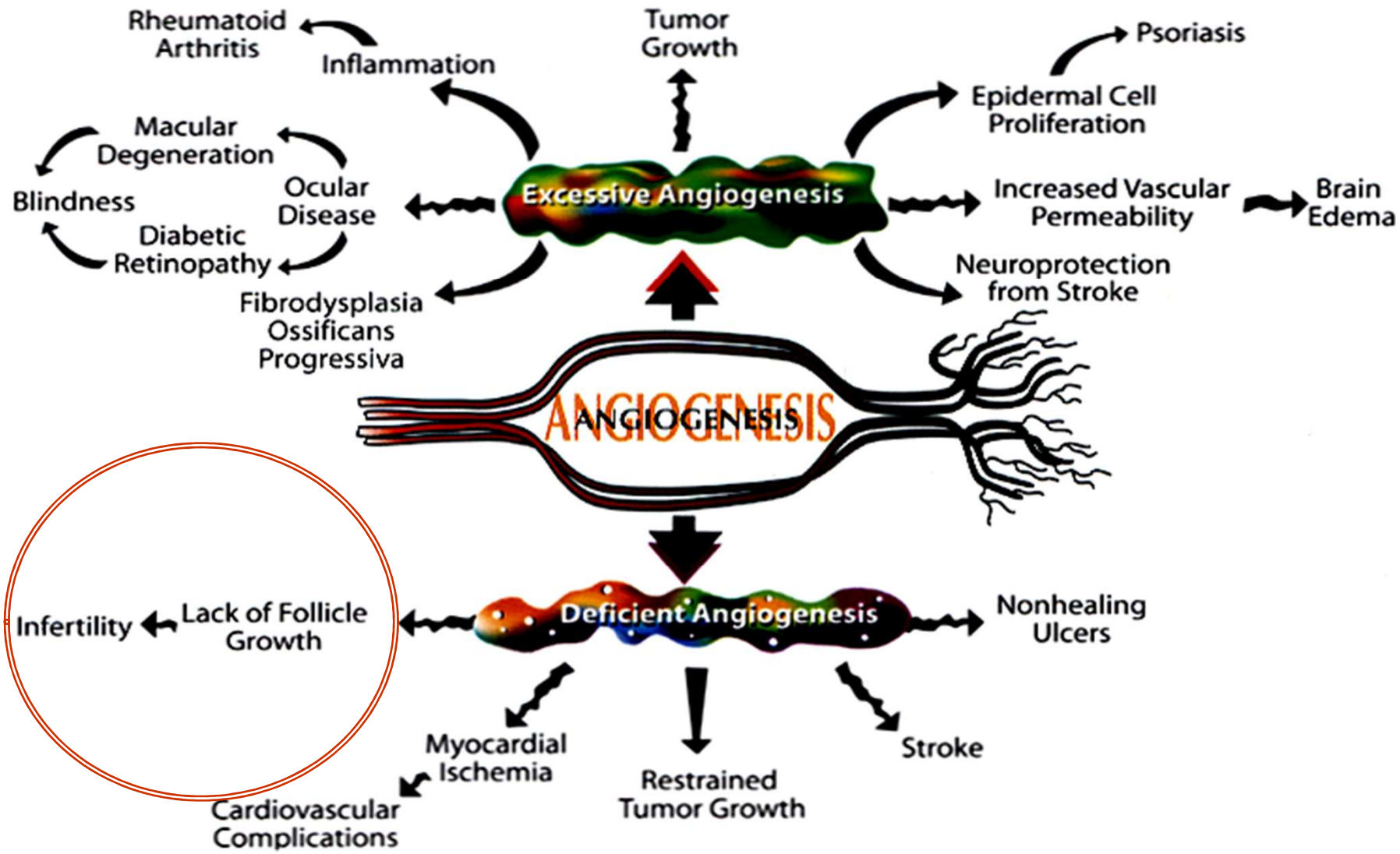
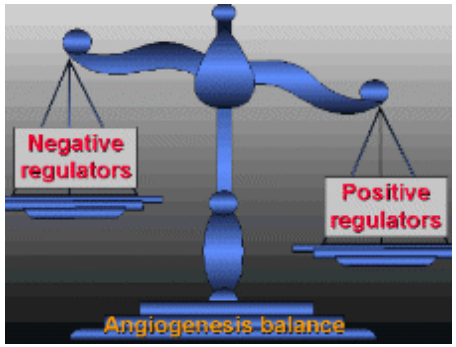
VASCULOGENESIS is the ex novo formation of blood vessels from differentiating angioblasts and their following organization into a primordial vascular network

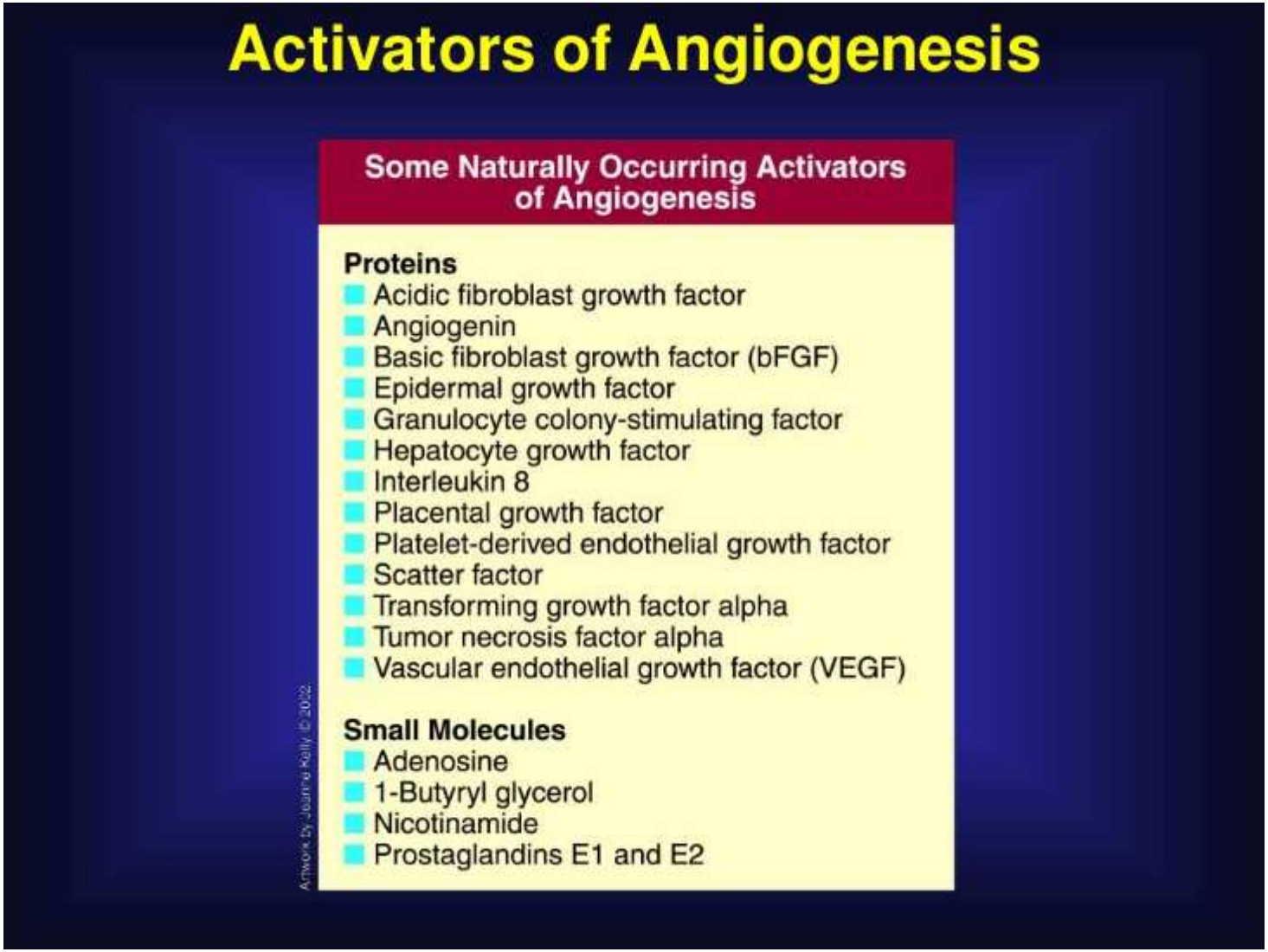
ANGIOGENESIS is the formation of new capillaries from pre-existing vessels





POSITIVE REGULATORS	NEGATIVE REGULATORS
Fibroblast growth factors	Thrombospondin-1
Placental growth factor	Angiostatin
Vascular endothelial growth factor	Interferon alpha
Transforming growth factors	Prolactin 16-kd fragment
Angiogenin	Metallo-proteinase inhibitors
Interleukin-8	Platelet factor 4
Hepatocyte growth factor	Genistein
Granulocyte colony-stimulating factor	Placental proliferin-related protein
Platelet-derived endothelial cell growth factor	Transforming growth factor beta
Angiopoietin 1	Endostatin

Activators of Angiogenesis



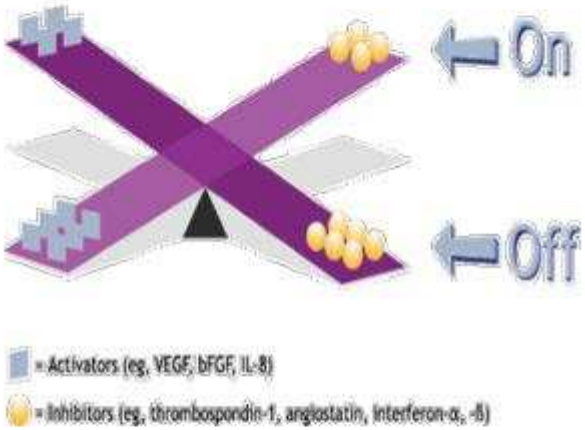
Inhibitors of Angiogenesis

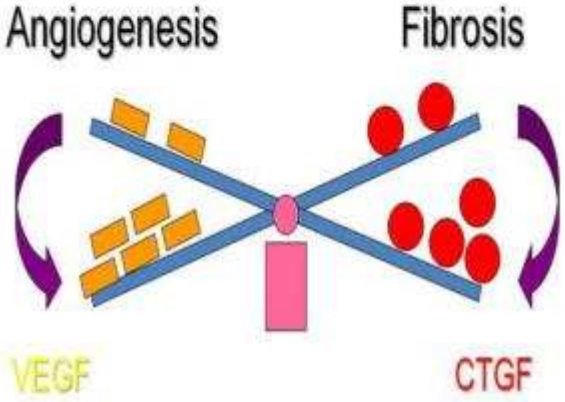
Angiogenesis Inhibitors

Proteins

- Angiostatin
- Endostatin
- Interferons
- Platelet factor 4
- Prolactin 16Kd fragment
- Thrombospondin
- TIMP-1 (tissue inhibitor of metalloproteinase-1)
- TIMP-2 (tissue inhibitor of metalloproteinase-2)
- TIMP-3 (tissue inhibitor of metalloproteinase-3)

The balance hypothesis of the 'angiogenic switch'.





Angiogenesis is tightly controlled by the balance of two sets of counteracting factors – angiogenic activators and inhibitors.

VEGF

- VEGFs are dimeric glycoproteins with many isoforms
- Several agents can induce VEGFs, *the most important being* ***hypoxia***. Other inducers are platelet-derived growth factor (**PDGF**), **TGF-β**, and **TGF-α**

FGFs

- constitute a family of factors with more than 20 members, the best characterized are *FGF-1 (**acidic FGF**)* and *FGF-2 (**basic FGF**)*.
- are produced by many cell types
- FGF-2 participates in angiogenesis mostly by stimulating the proliferation of endothelial cells.
- It also promotes the migration of macrophages and fibroblasts to the damaged area, and stimulates epithelial cell migration to cover epidermal wounds.

TGF-β functions

1- is a potent fibrogenic agent.

- It stimulates the production of collagen, fibronectin, and proteoglycans
- It inhibits collagen degradation by both decreasing proteinase activity and increasing the activity of tissue inhibitors of proteinases known as TIMPs .
- TGF-β is involved not only in scar formation after injury but also in the development of fibrosis in lung, liver, and kidneys that follows chronic inflammation.

2- TGF-β inhibits lymphocyte proliferation and can have a strong anti-inflammatory effect.

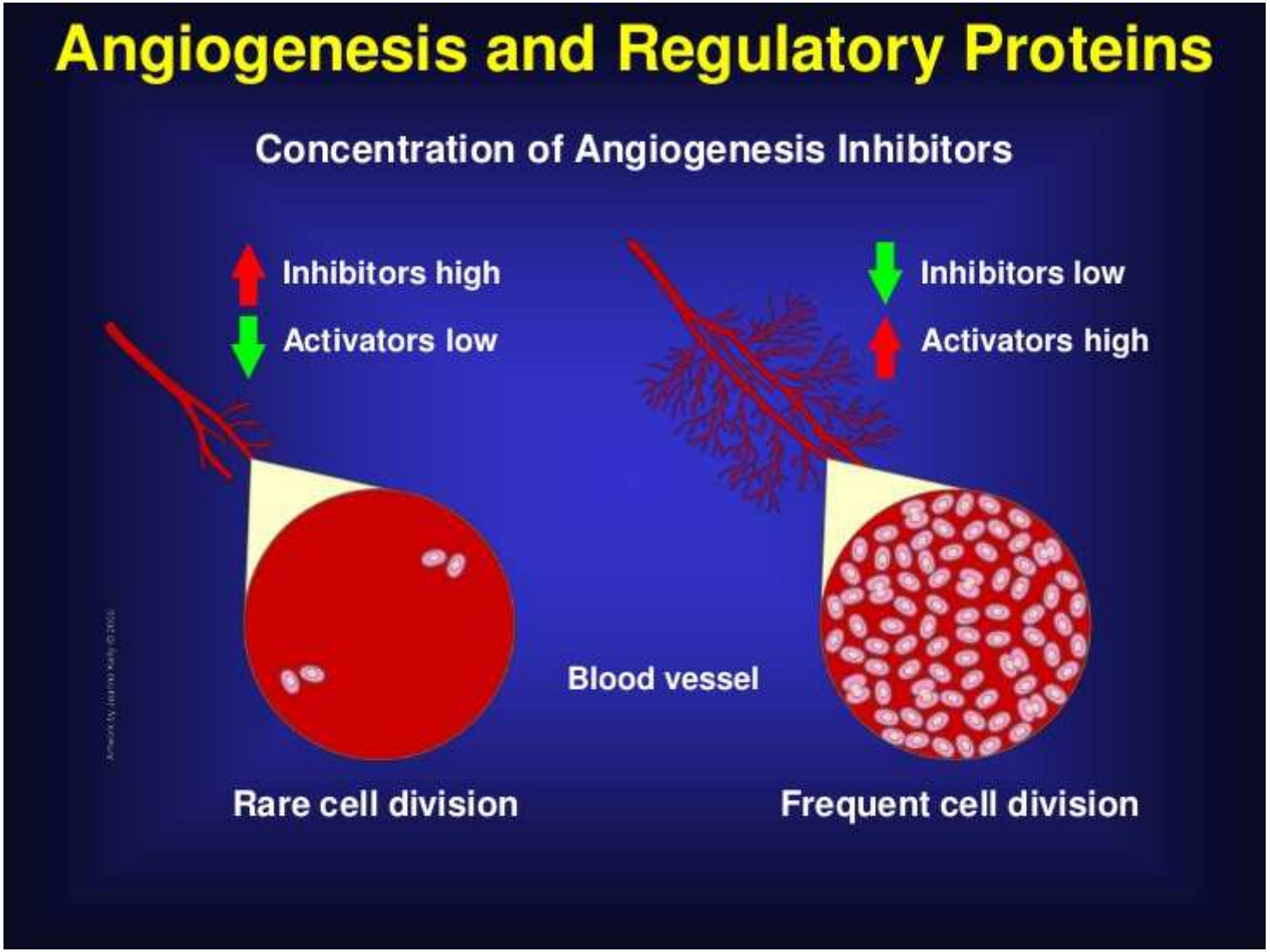
ECM and Tissue Remodeling

- even after its synthesis and deposition, scar ECM continues to be modified and remodeled.
- *The outcome of the repair process is, in part, a balance between ECM synthesis and degradation*
- The *degradation* of collagens and other ECM components is accomplished by a family of matrix metalloproteinases (**MMPs),** which are dependent on *zinc ions* for their activity.
- MMPs include:

- *interstitial collagenases,*
- *gelatinases*
- *stromelysins*

which degrade a variety of ECM constituents

Function	Growth factors
Epithelial proliferation	EGF, TGF-α, KGF, HGF
Monocyte chemotaxis	PDGF, FGF, TGF-β
Fibroblast migration	PDGF, FGF, TGF-β
Fibroblast proliferation	PDGF, EGF, FGF, TNF
Angiogenesis	VEGF, Ang, FGF
Collagen synthesis	TGF-β, PDGF
Collagenase secretion	PDGF, FGF, EGF, TNF; TGF-βinhibits

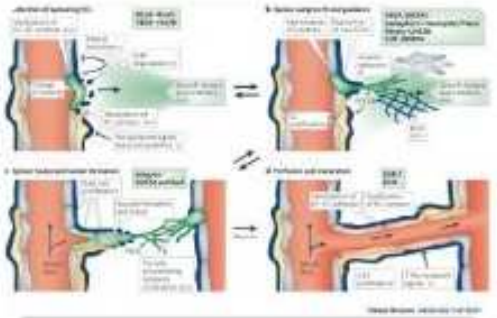

Angiogenesis and Regulatory Proteins
Concentration of Angiogenesis Inhibitors
Inhibitors high
Activators low
Inhibitors low
Activators high
Blood vessel
Rare cell division
Frequent cell division

The Angiogenic Process

TYPE

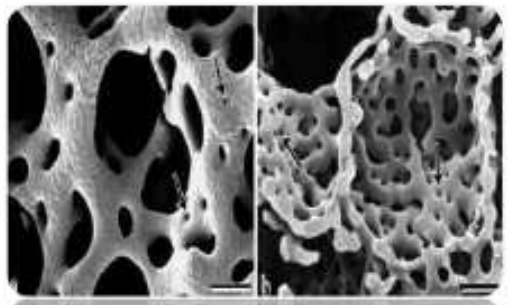
Types of Angiogenesis

Sprouting Angiogenesis

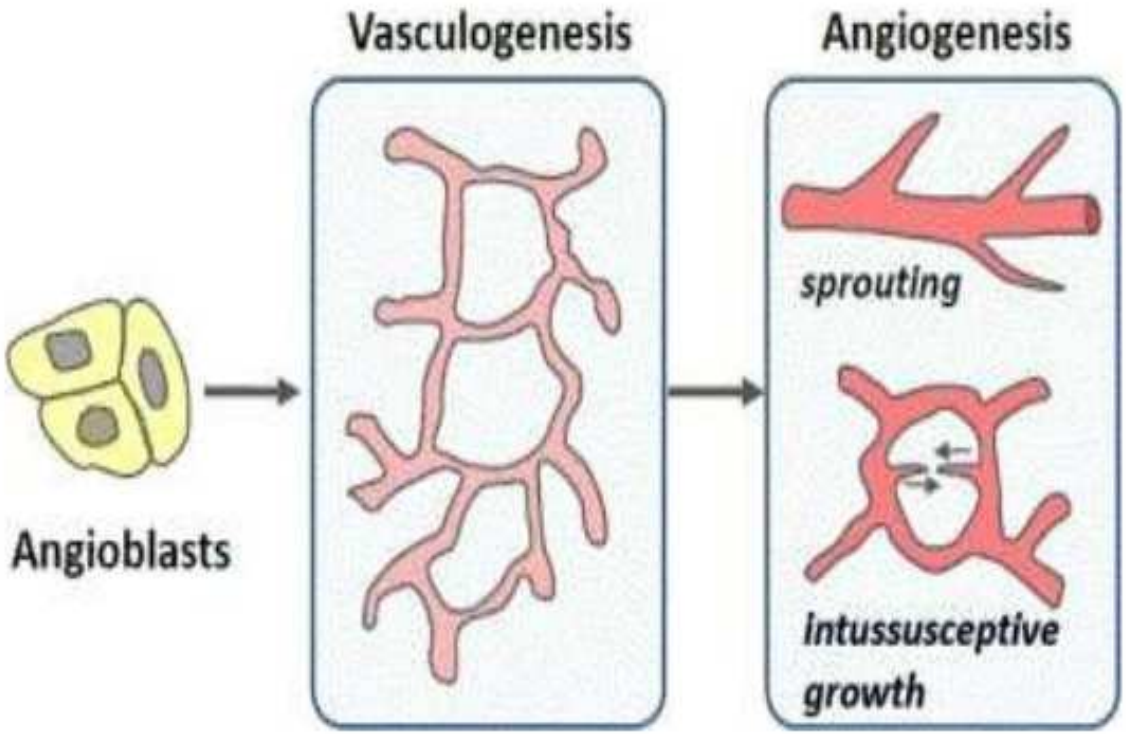


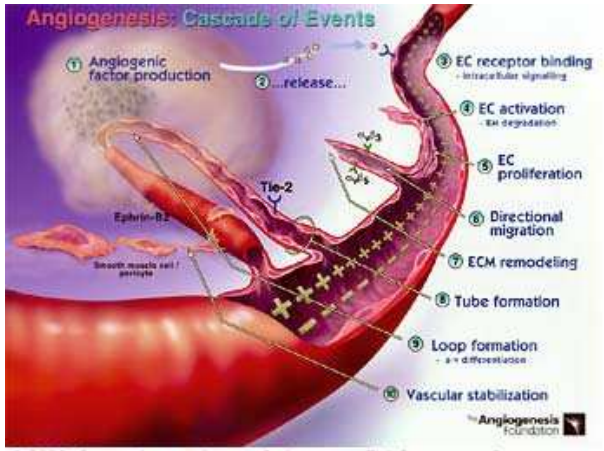
Sprouting angiogenesis

Intussusceptive Angiogenesis



Intussusceptive angiogenesis

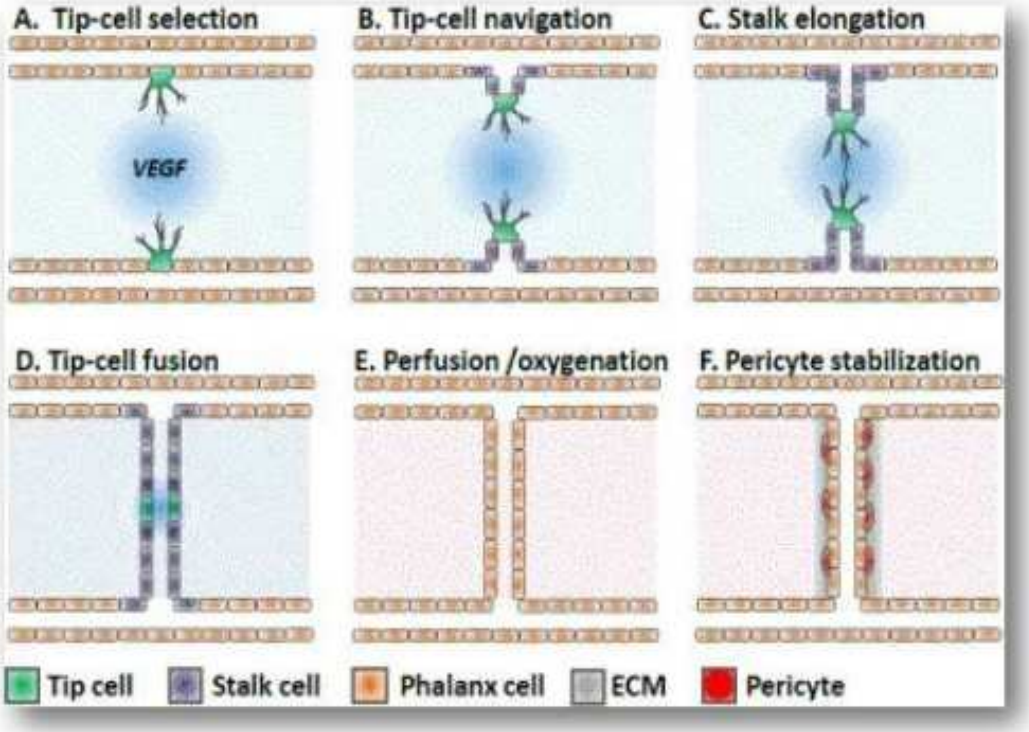

Vasculogenesis
Angiogenesis
sprouting
Angioblasts
intussusceptive
growth



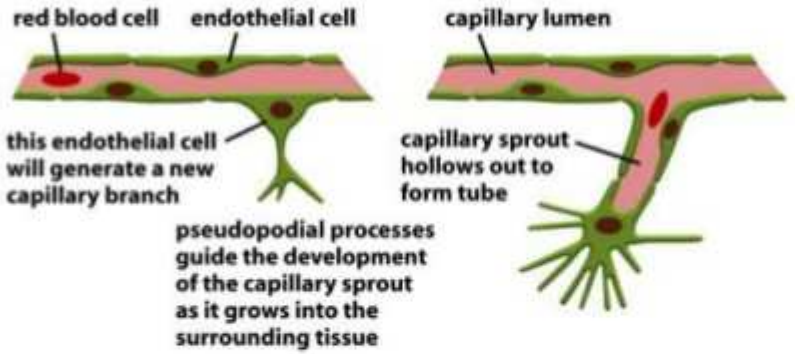
Sprouting Angiogenesis

1. Due to diseased or injured tissue angiogenesis growth factors (proteins) are released to nearby tissues.
2. These proteins bind to receptors on endothelial cells of neighboring blood vessels.
3. EC's are activated and signals are sent for the production of new molecules (enzymes).
4. Tiny holes are made in the basement membrane of the preexisting blood vessel by the enzymes.
5. The EC's divide and travel to the diseased tissue (tumor).
6. New blood vessel tubes are formed by sprouting EC's that roll up.
7. These tubes connect to form blood vessel loops that circulate blood.
8. Blood flow begins once the tubes are stabilized by special muscle cells.

Sprouting Angiogenesis

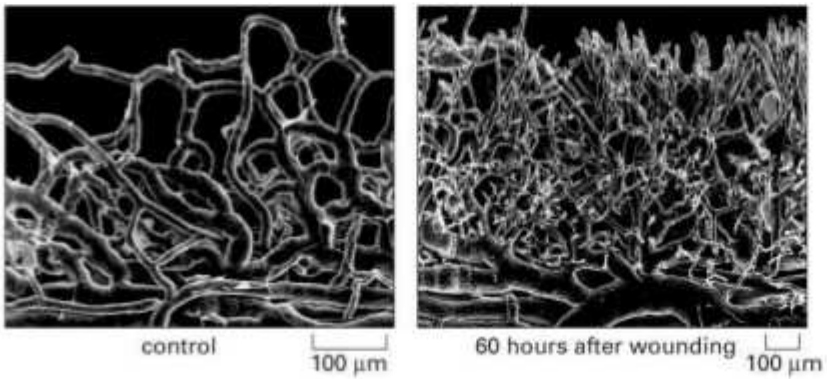


Angiogenesis:
Sprouting of cells from mature endothelial cells of the vessel wall



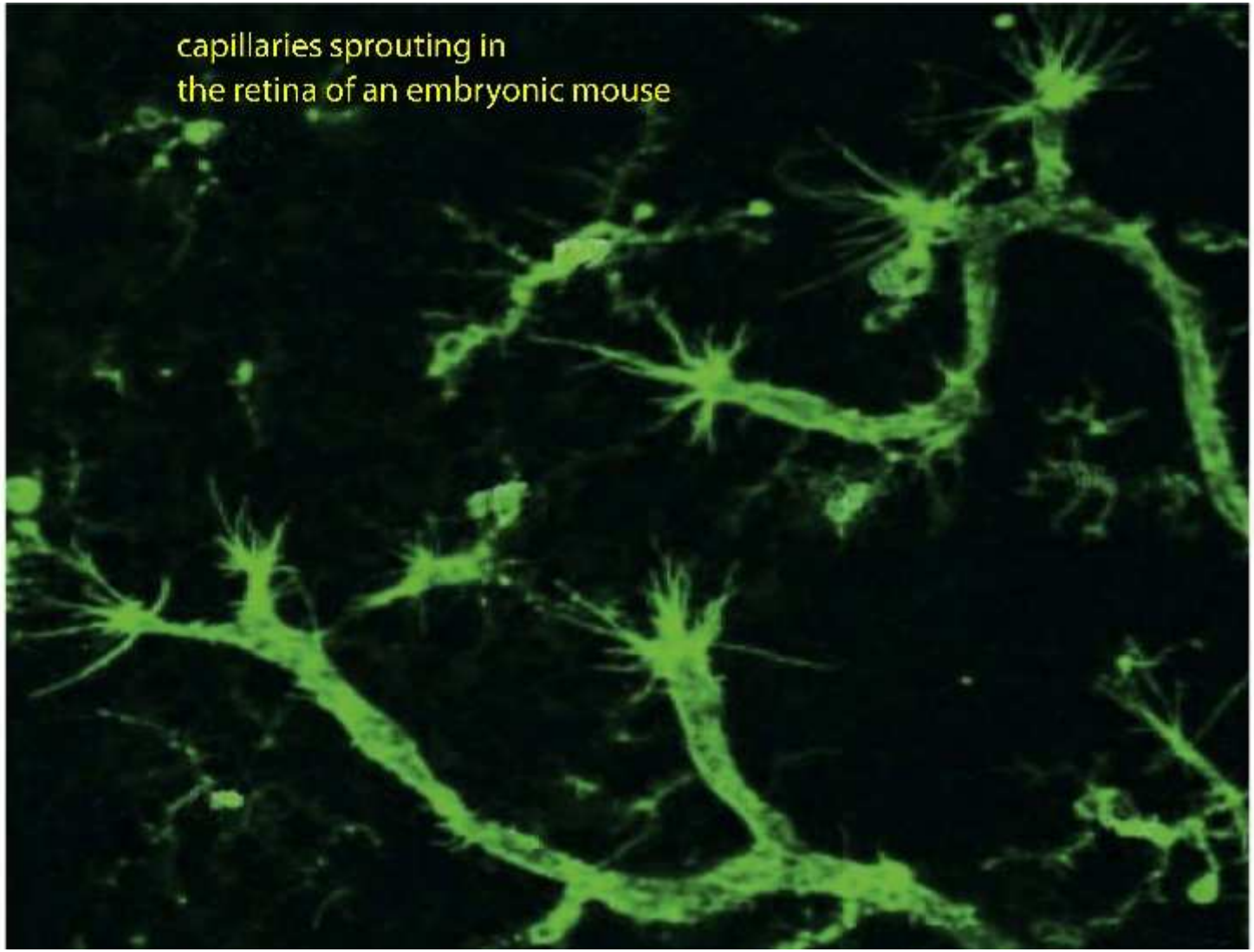
(secretion of proteases, resolution of Basal lamina, migration towards Chemotactic gradient, proliferation, Tube formation)

VEGF is factor largely specific for endothelial cells,
bFGF can also induce,
not specific for EC)

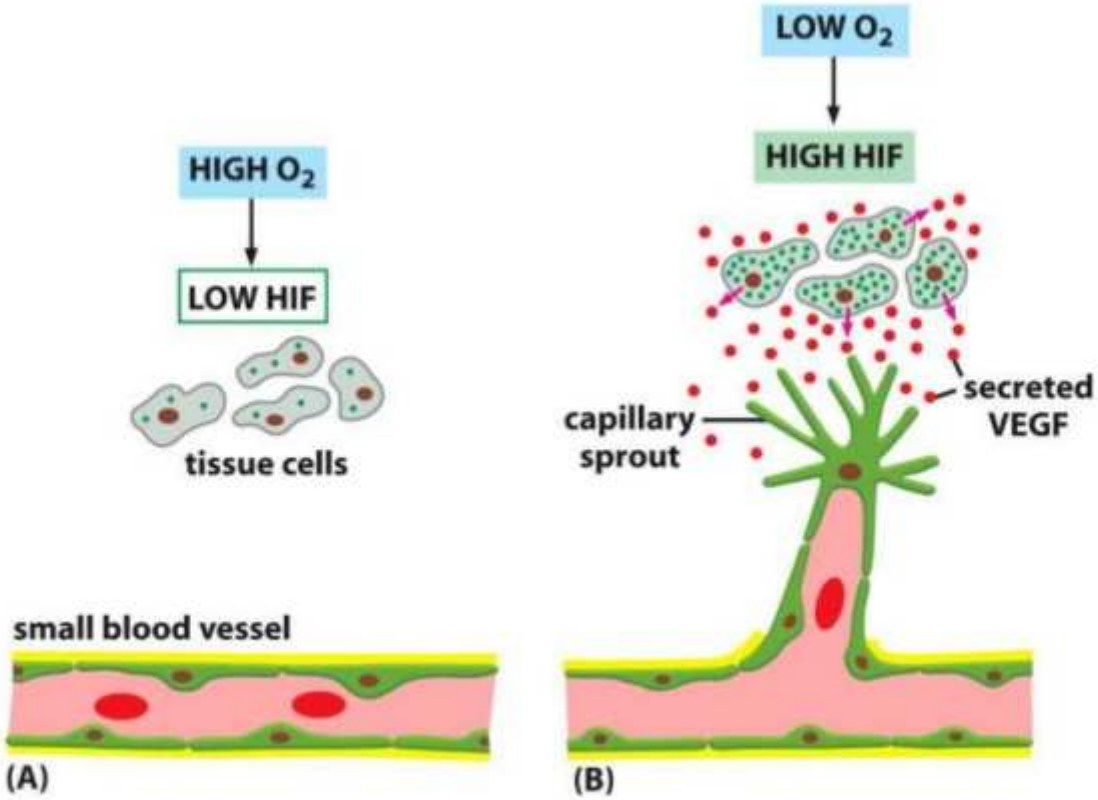


Mouse cornea: wounding induces angiogenesis, chemotactic response to angiogenic factors

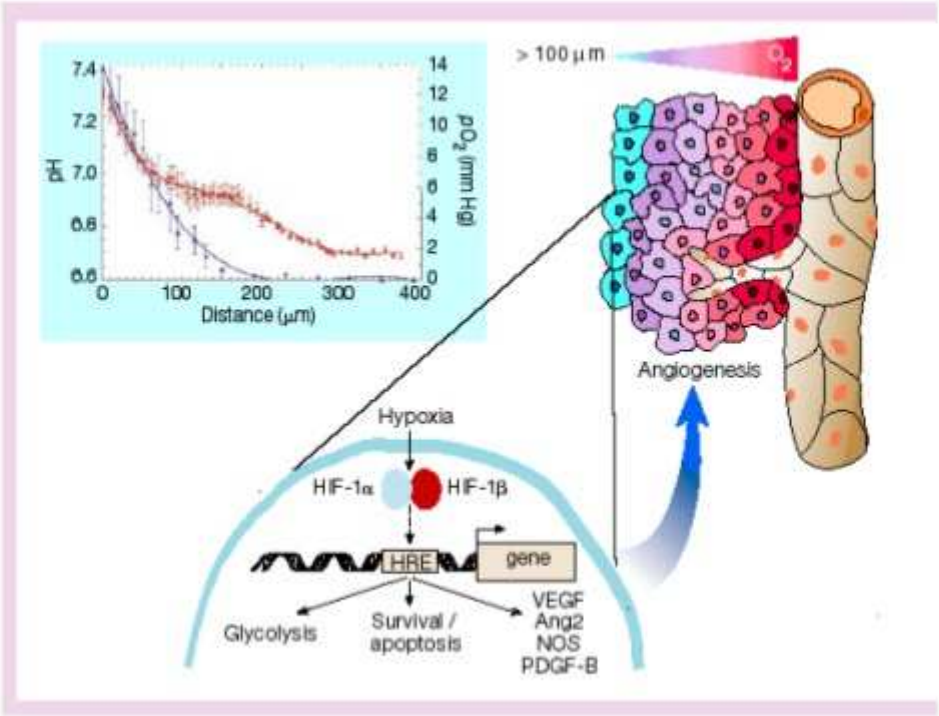
Figure 22–27. Molecular Biology of the Cell, 4th Edition.


capillaries sprouting in
the retina of an embryonic mouse

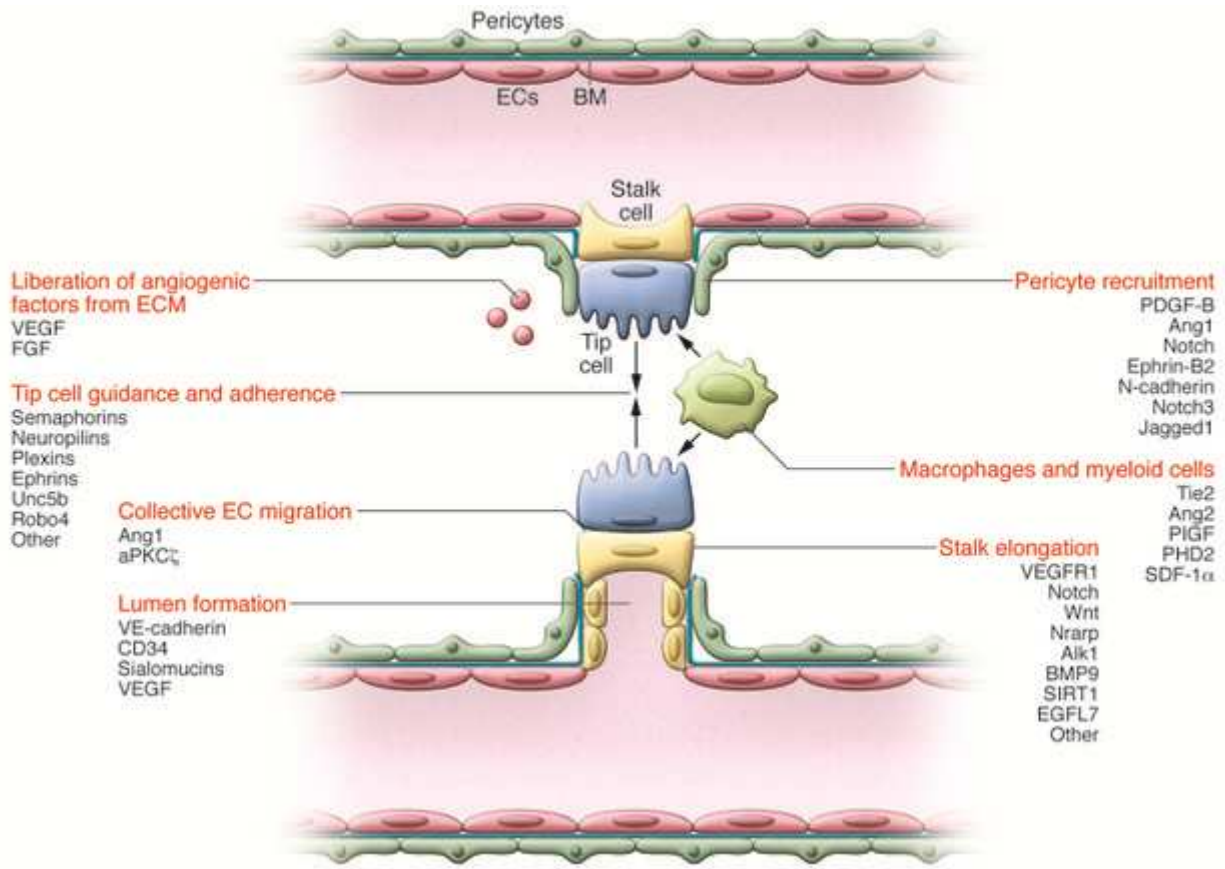
Sprouting towards chemotactic gradient: VEGF



Hypoxia - HIF - VEGF
every cell must be within 50 to 100 μm of a capillary

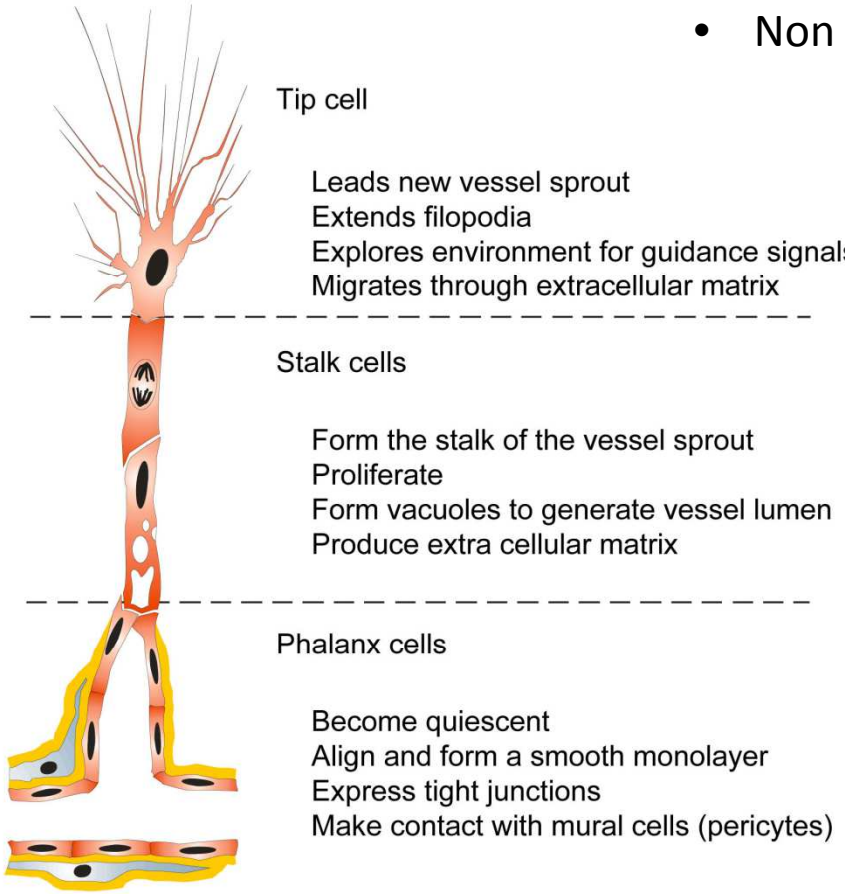


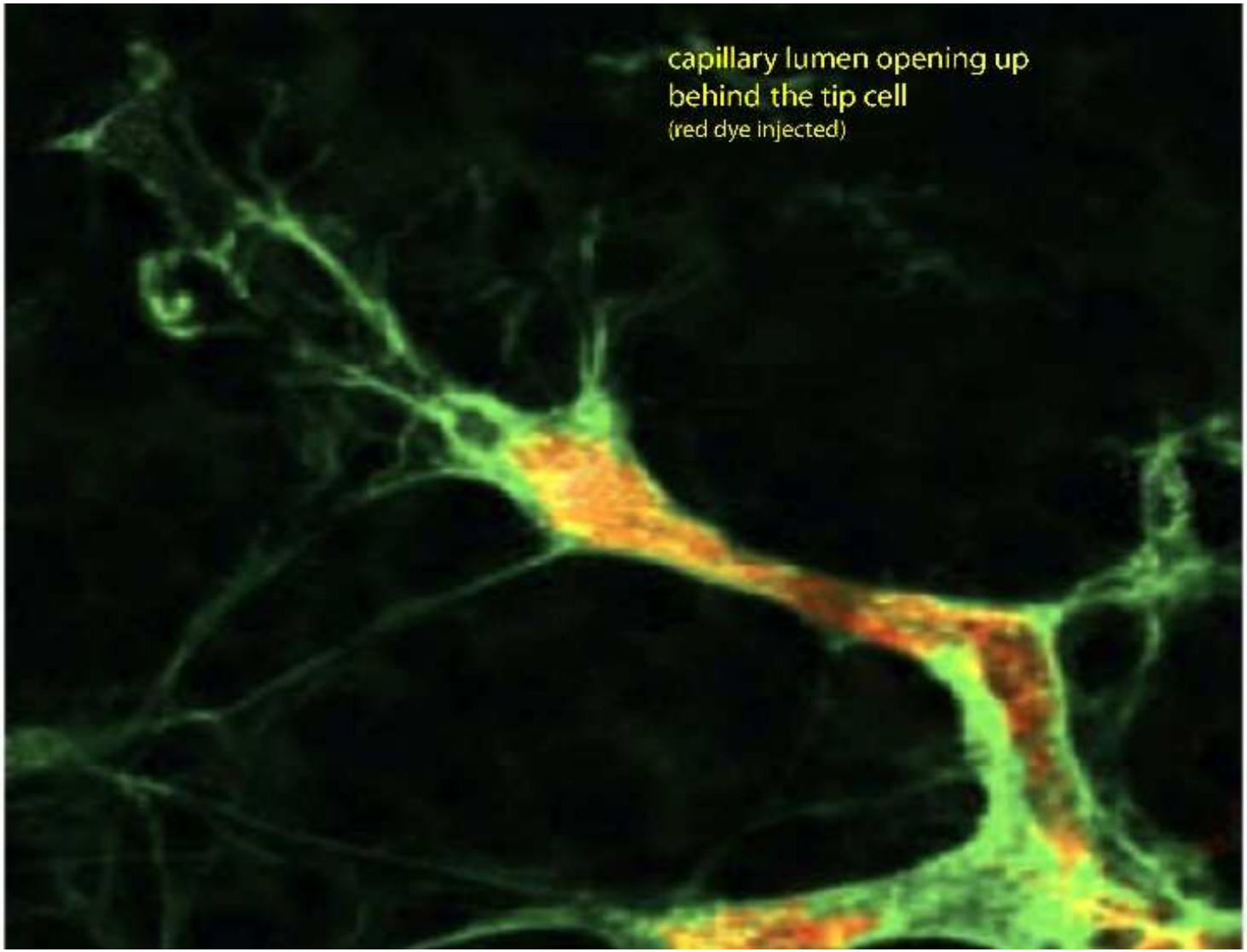
HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor

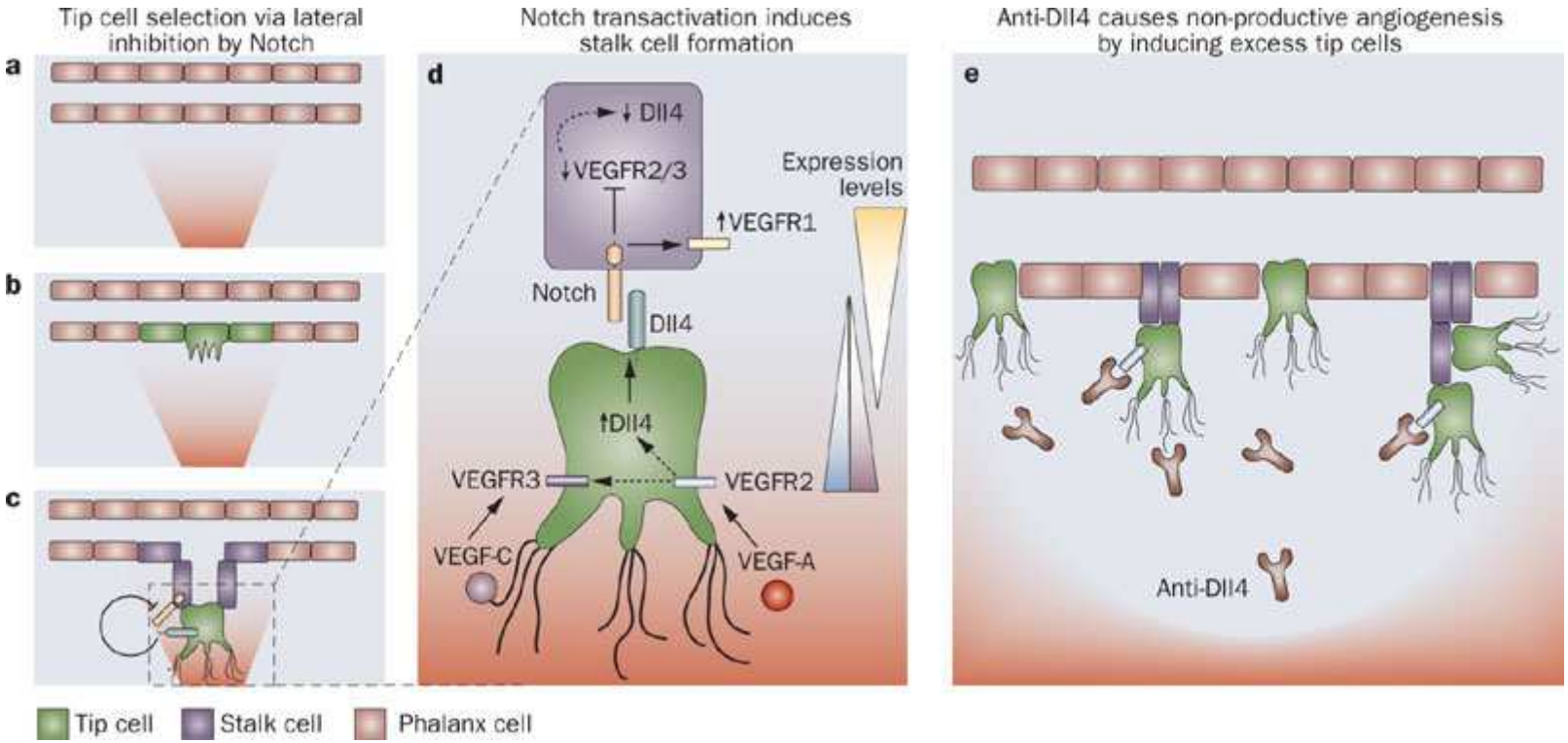

Pericytes
ECs
BM
Stalk cell
Tip cell
Liberation of angiogenic factors from ECM
VEGF
FGF
Tip cell guidance and adherence
Semaphorins
Neuropilins
Plexins
Ephrins
Unc5b
Robo4
Other
Collective EC migration
Ang1
aPKCζ
Lumen formation
VE-cadherin
CD34
Sialomucins
VEGF
Pericyte recruitment
PDGF-B
Ang1
Notch
Ephrin-B2
N-cadherin
Notch3
Jagged1
Macrophages and myeloid cells
Tie2
Ang2
PlGF
PHD2
SDF-1α
Stalk elongation
VEGFR1
Notch
Wnt
Nrarp
Alk1
BMP9
SIRT1
EGFL7
Other

Tip cells

- Apex of sprout
- Hightly motile
- Exend numerous filopodia
- Tubeless
- Non proliferative phenotypes




capillary lumen opening up
behind the tip cell
(red dye injected)

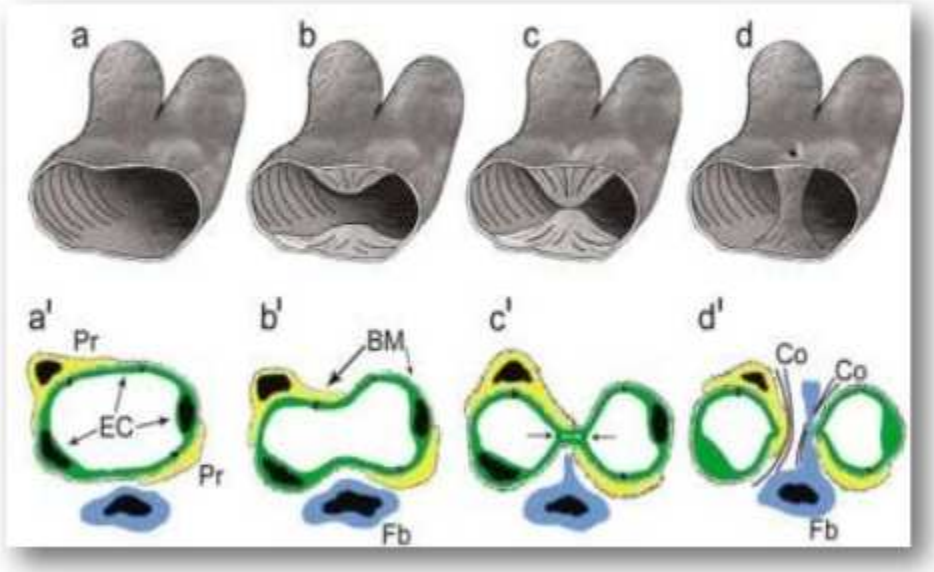


Upen activationf Notch signalling stalk cell inhibit excess sprout formation through down-regulation of expression of VEGF receptor

The angiogenic process, as currently understood, can be summarized as follows:

- A cell activated by a lack of oxygen releases angiogenic molecules that attract inflammatory and endothelial cells and promote their proliferation.
- During their migration, inflammatory cells also secrete molecules that intensify the angiogenic stimuli.
- The endothelial cells that form the blood vessels respond to the angiogenic call by differentiating and by secreting matrix metalloproteases (MMP), which digest the blood-vessel walls to enable them to escape and migrate toward the site of the angiogenic stimuli.
- Several protein fragments produced by the digestion of the blood-vessel walls intensify the proliferative and migratory activity of endothelial cells, which then form a capillary tube by altering the arrangement of their adherence-membrane proteins.
- Finally, the capillaries emanating from the arterioles and the venules will join, thus resulting in a continuous blood flow.

Intussusceptive Angiogenesis



Intussusceptive Angiogenesis

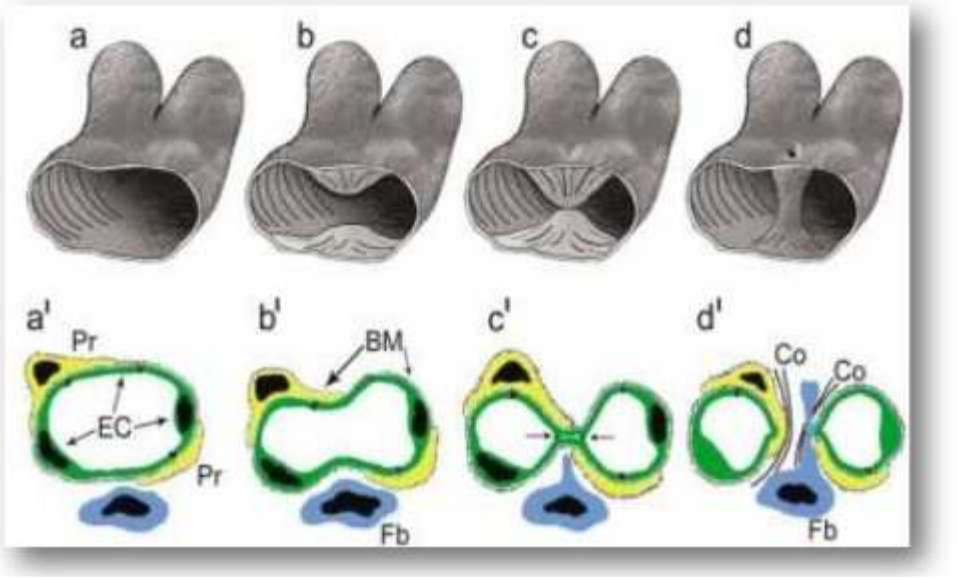
Intussusception, also known as splitting angiogenesis, was first observed in neonatal rats. In this type of vessel formation, the capillary wall extends into the lumen to split a single vessel in two. There are four phases of intussusceptive angiogenesis.

First, the two opposing capillary walls establish a zone of contact.

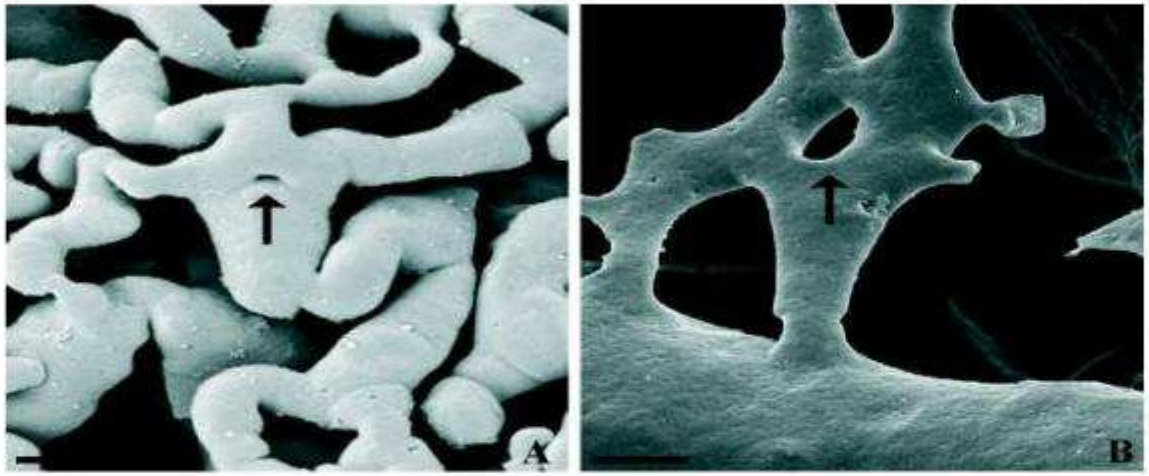
Second, the endothelial cell junctions are reorganized and the vessel bilayer is perforated to allow growth factors and cells to penetrate into the lumen.

Third, a core is formed between the two new vessels at the zone of contact that is filled with pericytes and myofibroblasts. These cells begin laying collagen fibers into the core to provide an extracellular matrix for growth of the vessel lumen.

Finally, the core is fleshed out with no alterations to the basic structure.



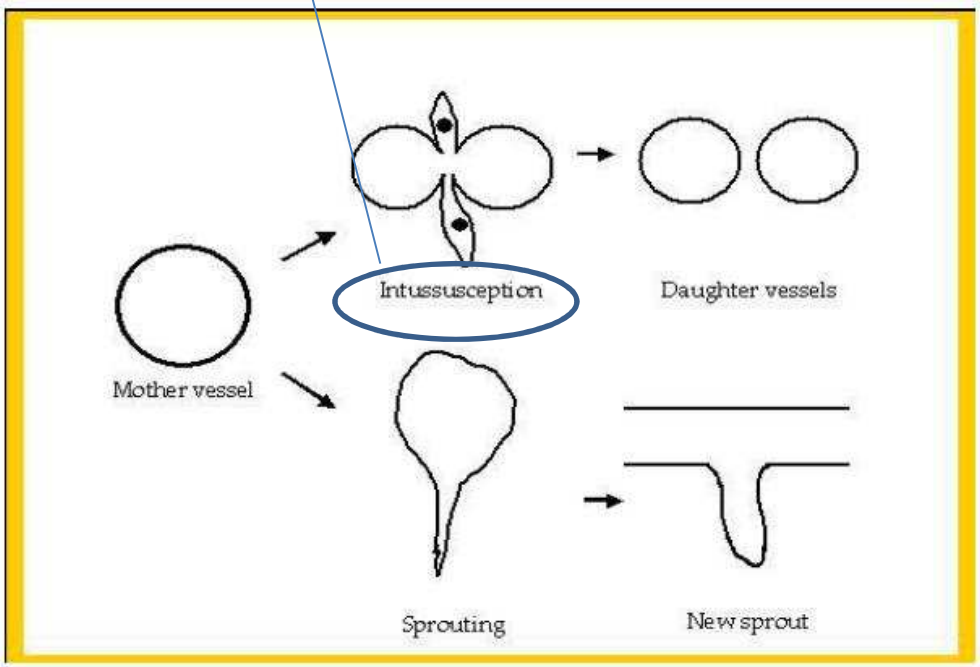
Intussusceptive Angiogenesis



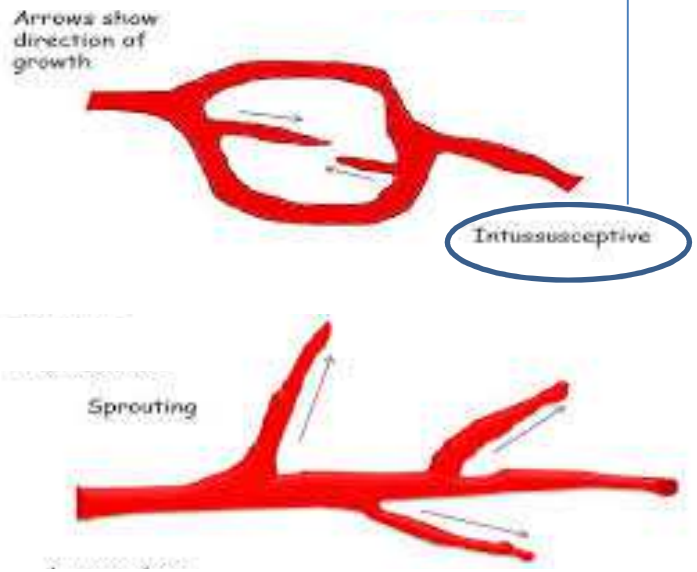
Intussusception is important because it is a reorganization of existing cells. It allows a vast increase in the number of capillaries without a corresponding increase in the number of endothelial cells. This is especially important in embryonic development as there are not enough resources to create a rich microvasculature with new cells every time a new vessel develops.

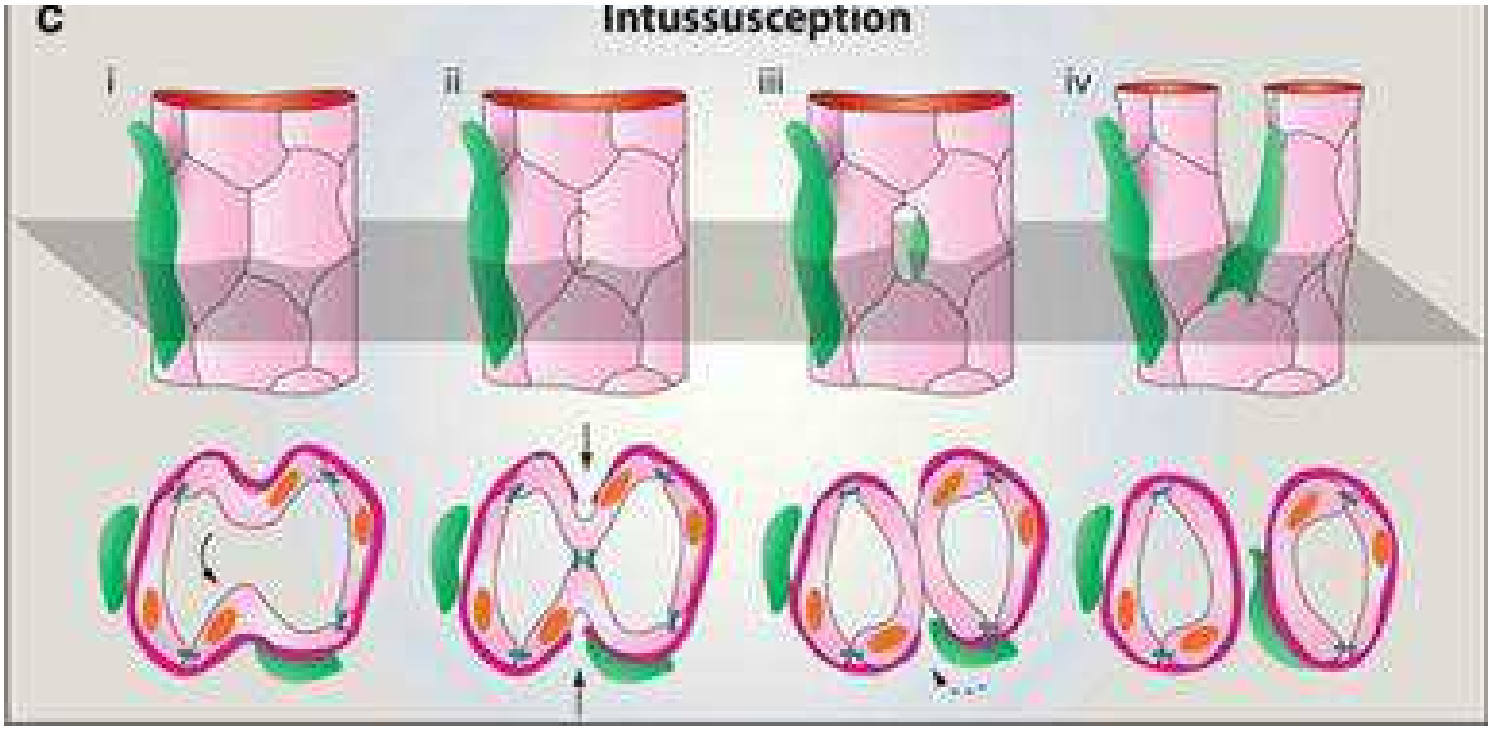
Intravascular process (unseen by standard light microscopy)

The diagram of the difference between two kinds of angiogenesis

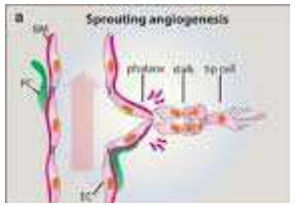


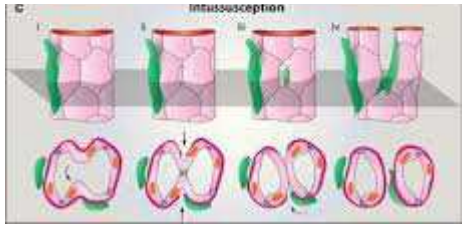
Capillary replication and remodeling





Intussusceptive pillar is compose of endothelial membrane, occasional myofibroblast and rare pericytes





Endothelial cells projections are believed to contein contractile and shape consistent with filopodia

Basement membrane is degraded and tip cells project into the extracellular matrix

Basement membrane is not degraded. Endothelial cell projections are not oriented into the extracellular matrix (across the vessel lumen)

gradient to guide endothelial cell projection

No gradient to guide endothelial cell projection