Angiogenesis in Human Development

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BACKGROUND READING:

Vascular Development

"Signaling Vascular Morphogenesis and Maintenance"
Douglas Hanahan. *Science* 277: 48-50. in Perspectives. (1997)

Notch and arterial specification

"Notch Function in the Vasculature: insights from zebrafish, mouse and man"
Carrie Shawber and Jan Kitajewski. *BioEssays 3*: 225-34 (2004)

Wnts and retinal angiogenesis

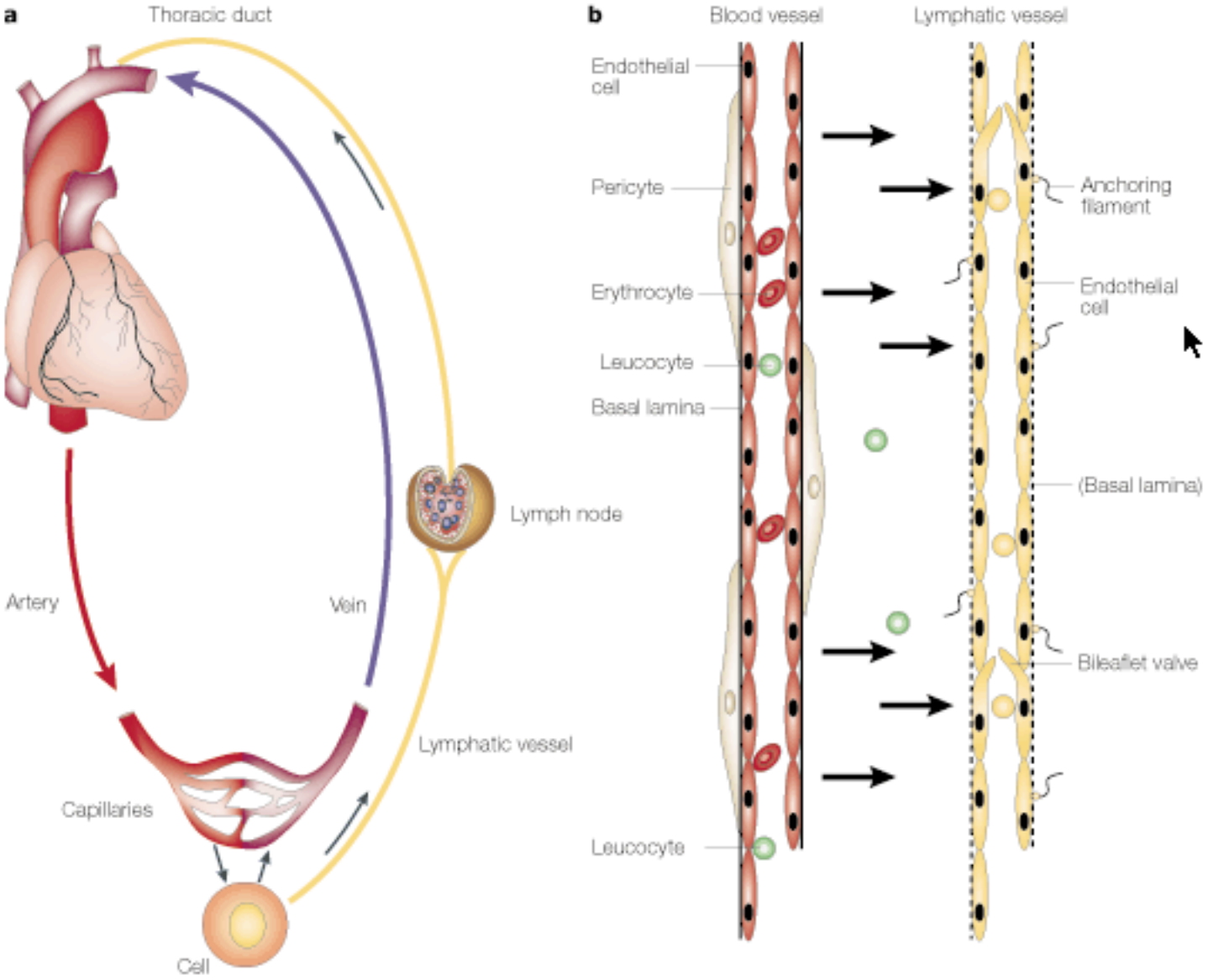
"Wnt/Frizzled Signaling in the Vasculature: New Angiogenic Factors in Sight"
Nestor Masckauchan and Jan Kitajewski. *Physiology 21*: 181-188 (2006)

Vascular Development

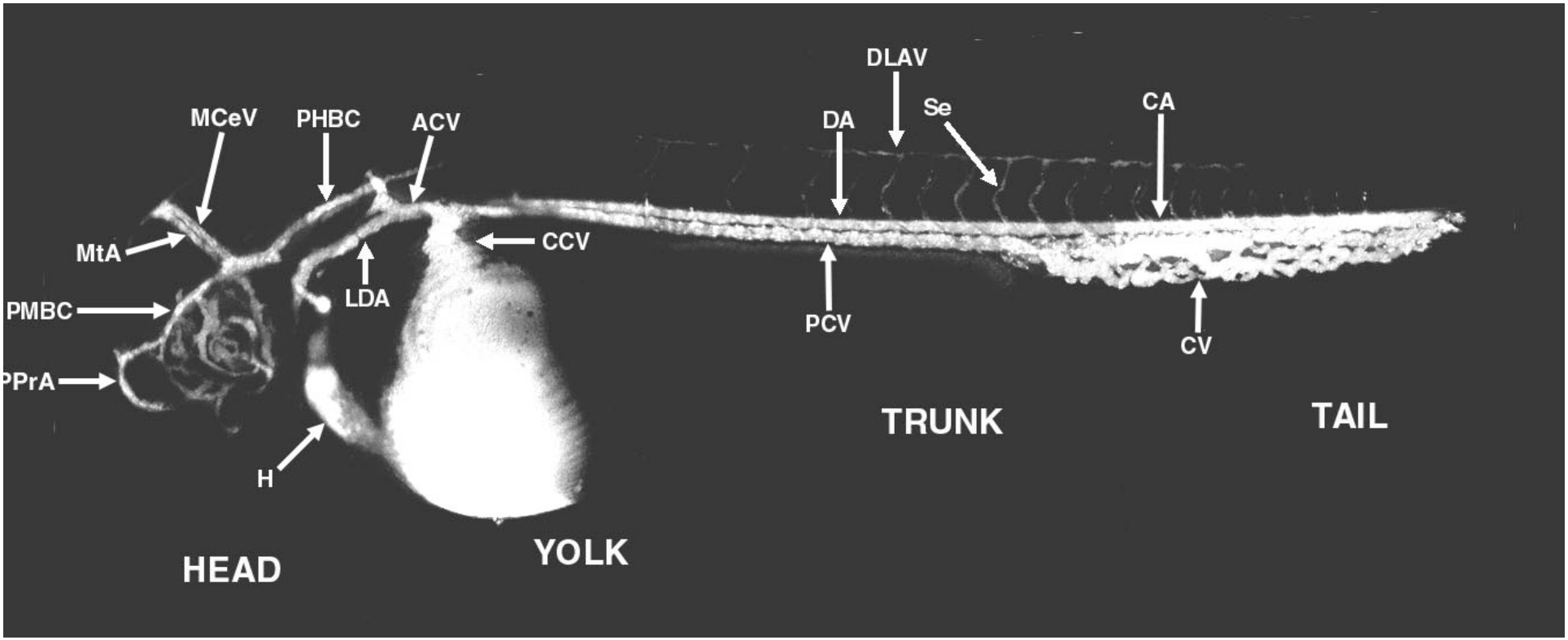
- Vasculogenesis = de novo tube formation
- Angiogenesis = sprouting of new tubes off of pre-existing tubes

- Endothelial Cell = cell type that makes up and lines blood vessels
- Mural Cells = specialized cells that surround blood vessels
  - Pericytes
  - Smooth muscle cells

- Angiogenic Factors
  - Vascular Endothelial Growth Factor (VEGF-A, VEGF-B, PlGF, VEGF-C, VEGF-D
  - Angiopoietins (Ang 1, Ang2, ……..)
  - Notch ligands (Jagged1, Delta4)


a
Thoracic duct
Lymph node
Artery
Vein
Lymphatic vessel
Capillaries
Cell
b
Blood vessel
Lymphatic vessel
Endothelial cell
Pericyte
Erythrocyte
Leucocyte
Basal lamina
Leucocyte
Anchoring filament
Endothelial cell
(Basal lamina)
Bileaflet valve

2.5 day


MCeV
PHBC
ACV
DLAV
DA
Se
CA
MtA
CCV
LDA
PMBC
PCV
CV
PPrA
TRUNK
TAIL
H
HEAD
YOLK

Nature Biotechnology **22,** 595 - 599 (2004)

Chemical suppression of a genetic mutation in a zebrafish model of aortic coarctation

Randall T Peterson1, Stanley Y Shaw1, 2, Travis A Peterson1, David J Milan1, Tao P Zhong1, 3, Stuart L Schreiber2, Calum A MacRae1 & Mark C Fishman1, 4

1 Developmental Biology Laboratory, Cardiovascular Research Center, Massachusetts General Hospital

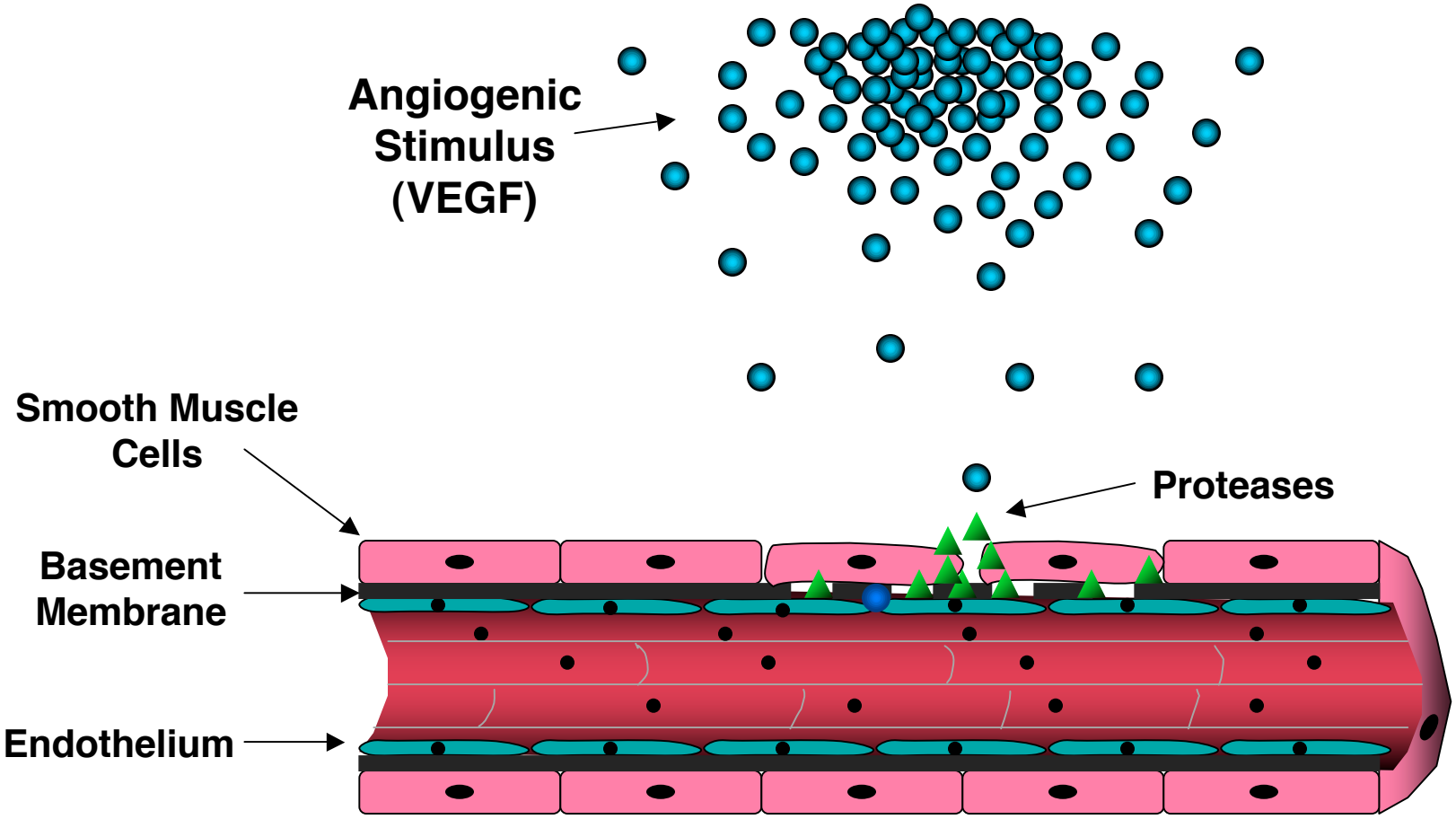
Nature Chemical Biology **1**, 263-264 (2005)

High-throughput assay for small molecules that modulate zebrafish embryonic heart rate.

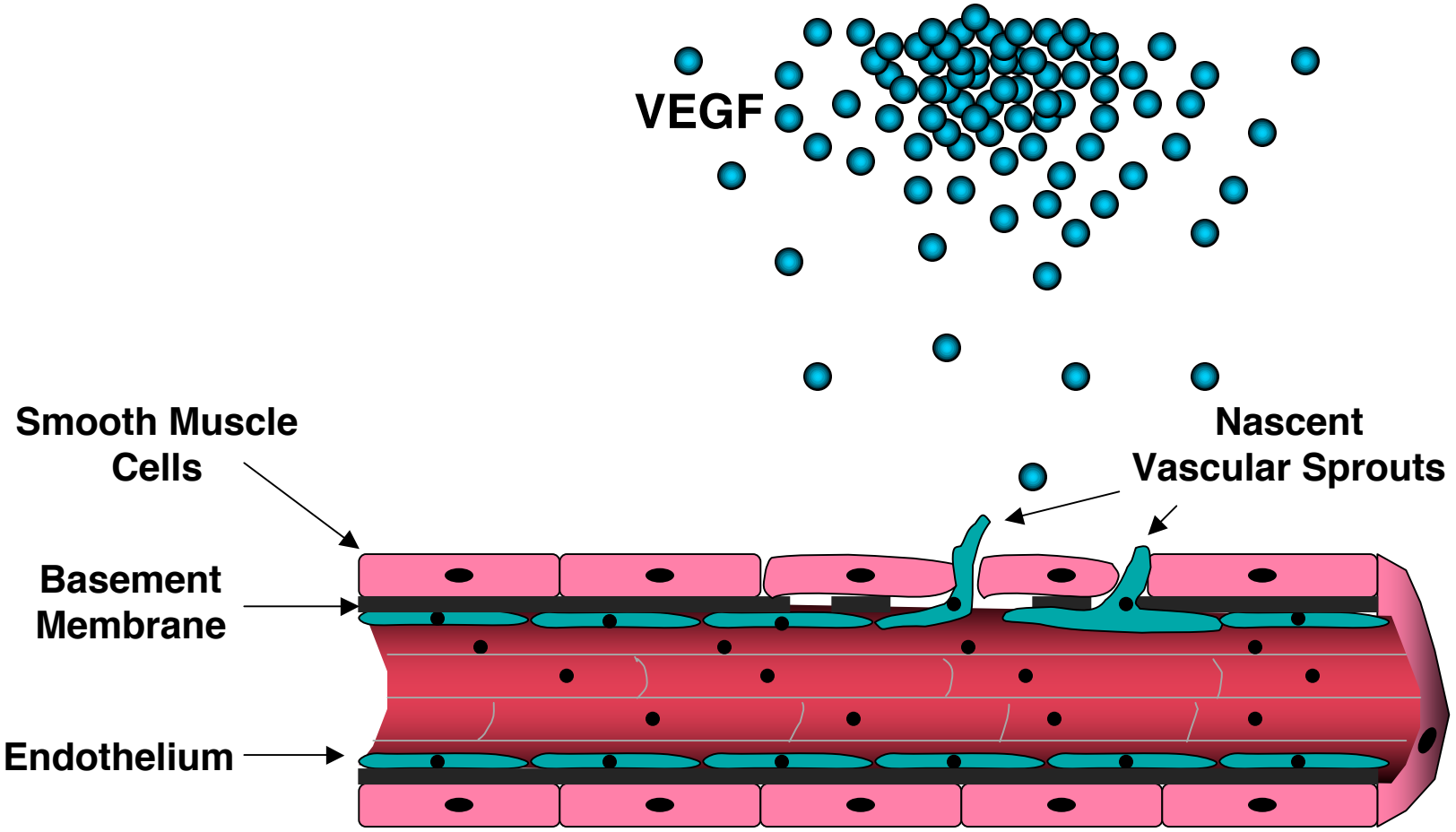
Burns CG, David J Milan, Grande DJ, Rottbauer W, Calum A MacRae & Mark C Fishman

Developmental Biology Laboratory, Cardiovascular Research Center, Massachusetts General Hospital

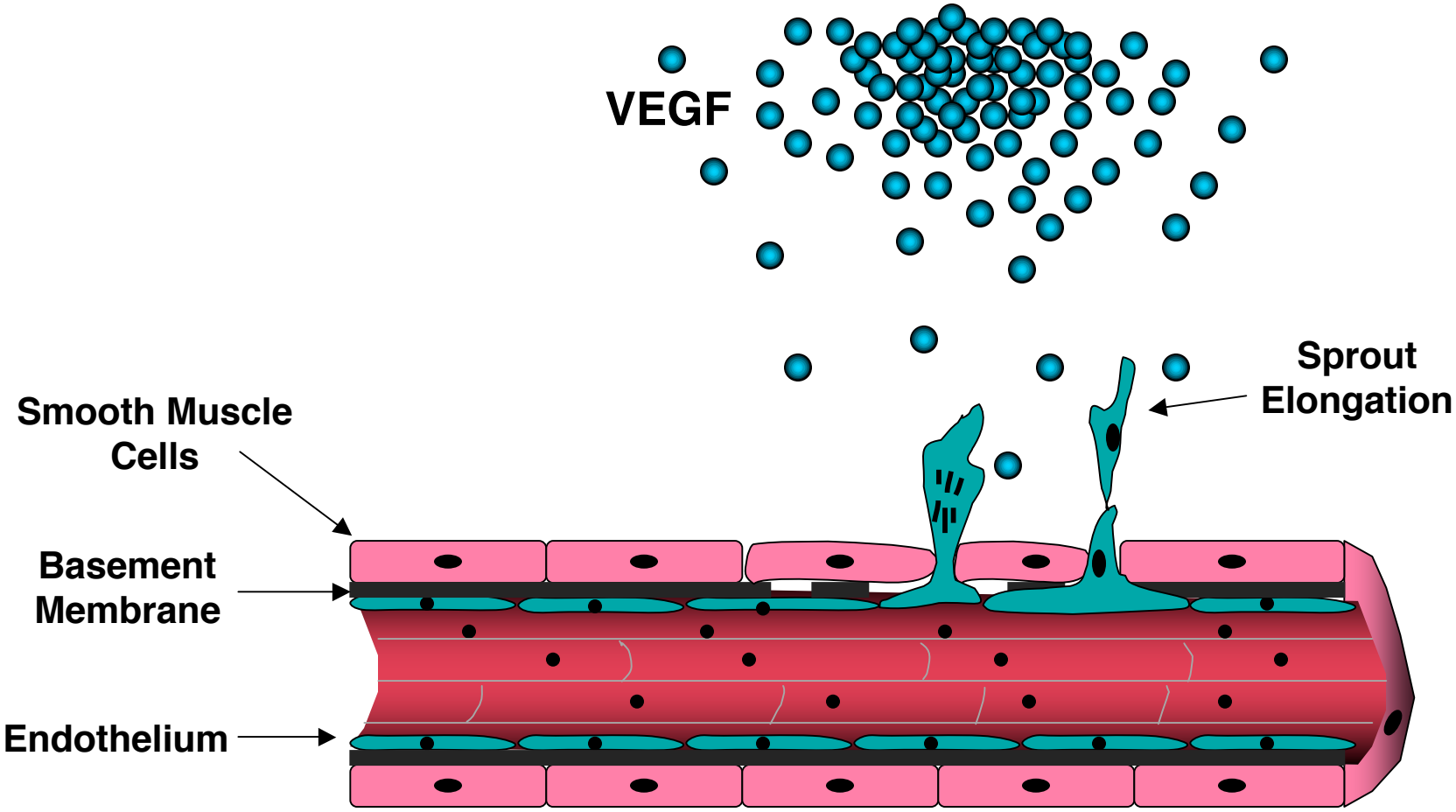
Angiogenesis - Basement Membrane Breakdown



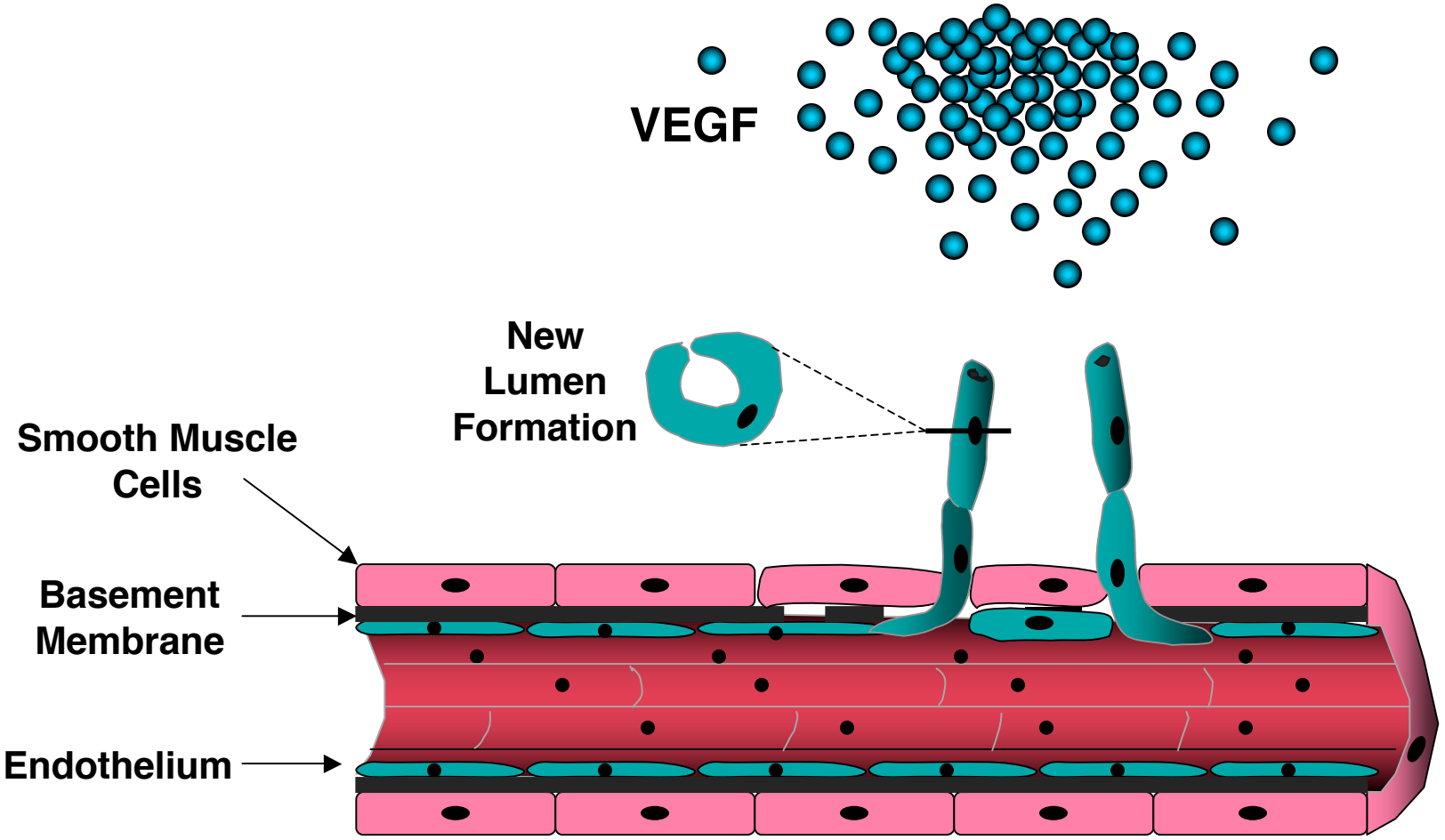
Angiogenesis - Endothelial Cell Migration



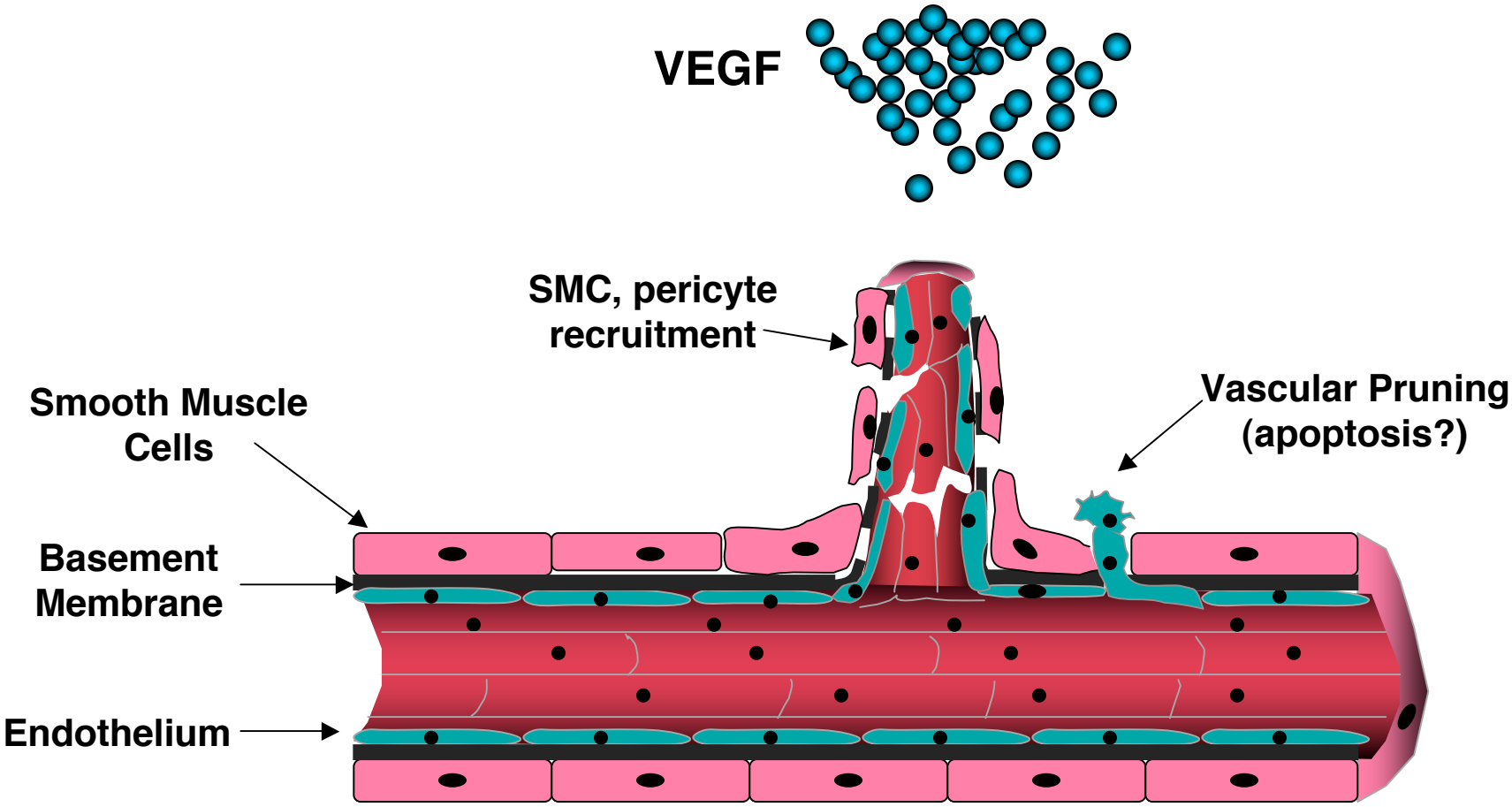
Angiogenesis - Endothelial Cell Proliferation



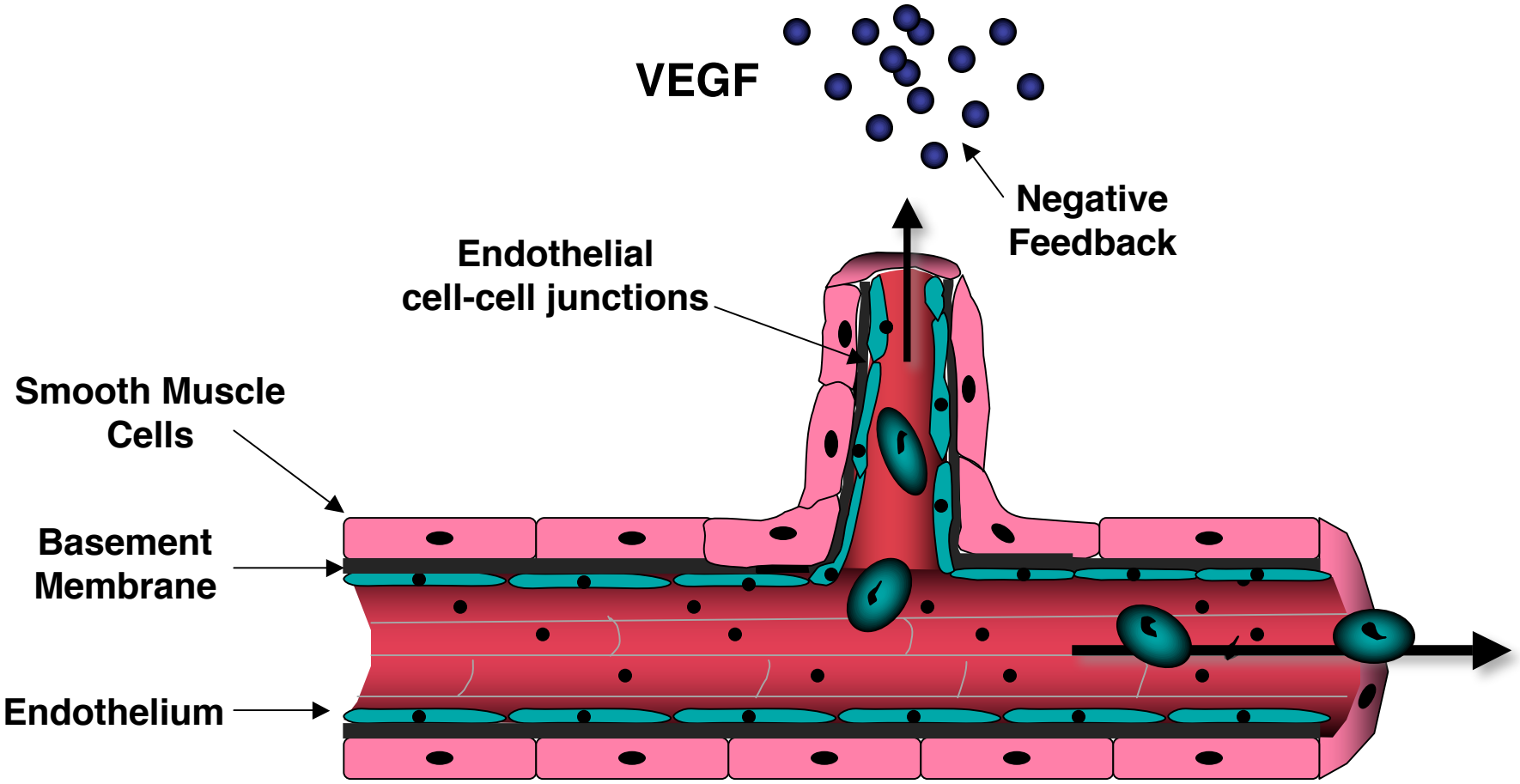
Angiogenesis - Capillary Morphogenesis



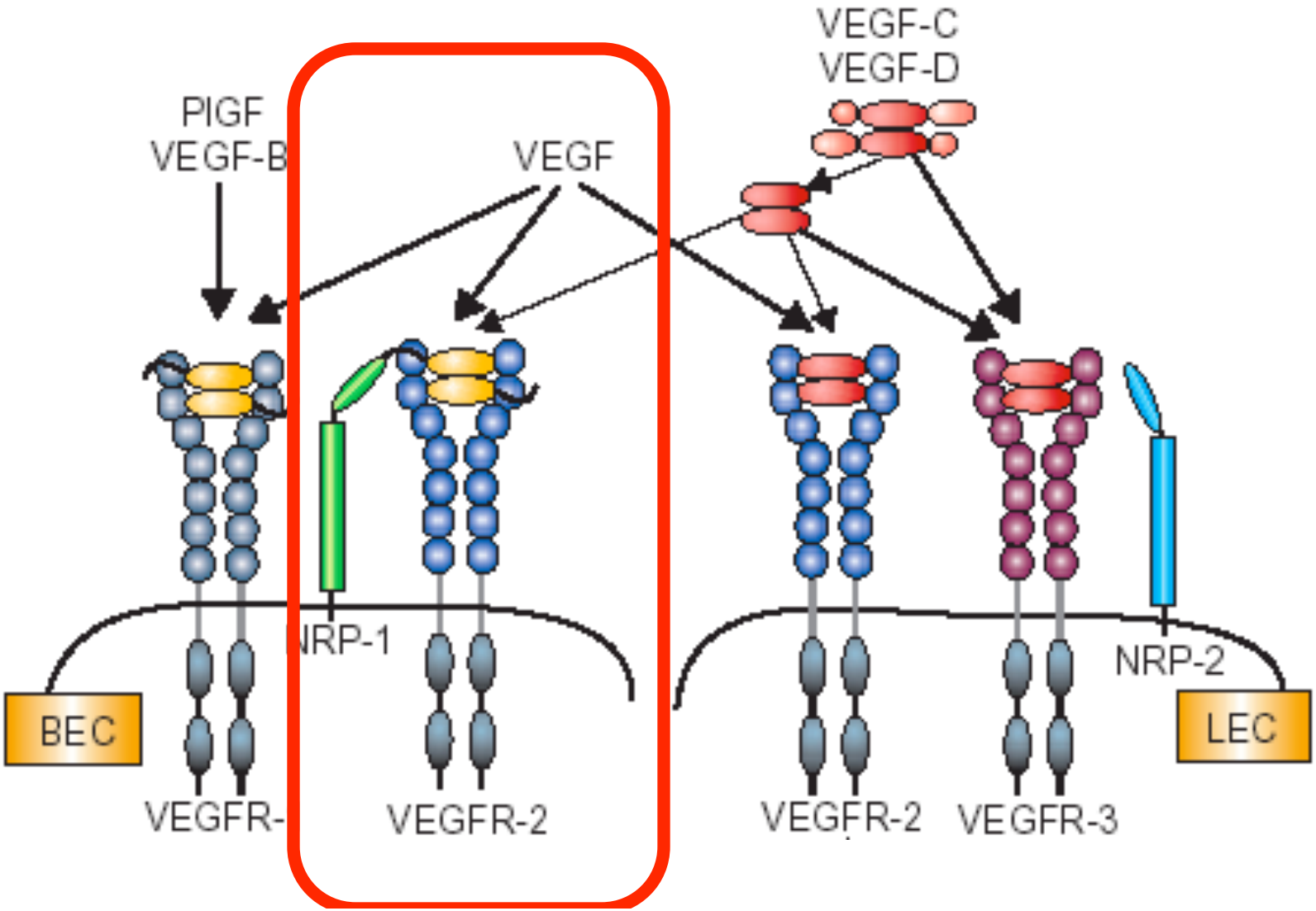
Angiogenesis - Vascular Maturation

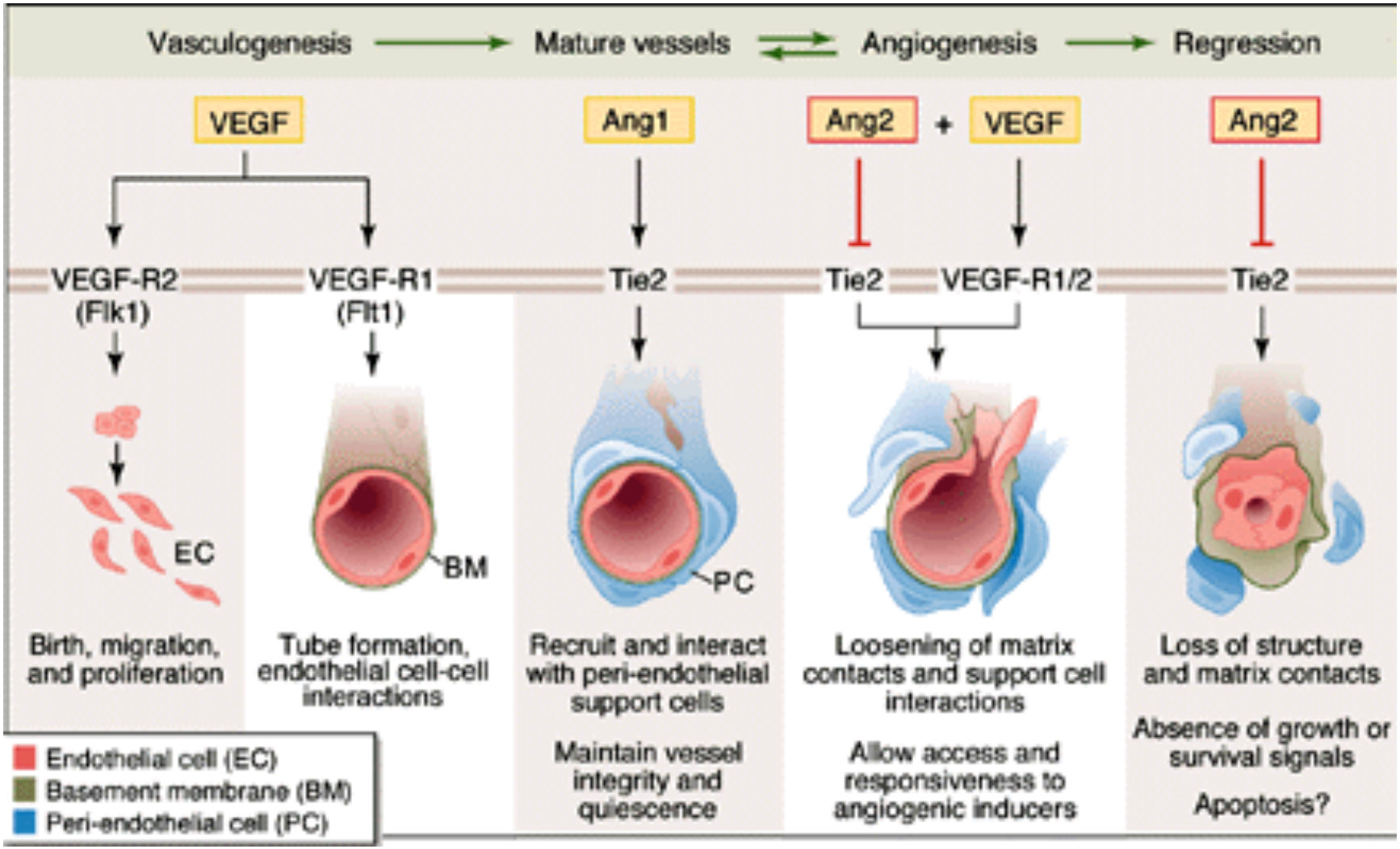


Angiogenesis - Vascular Maturation

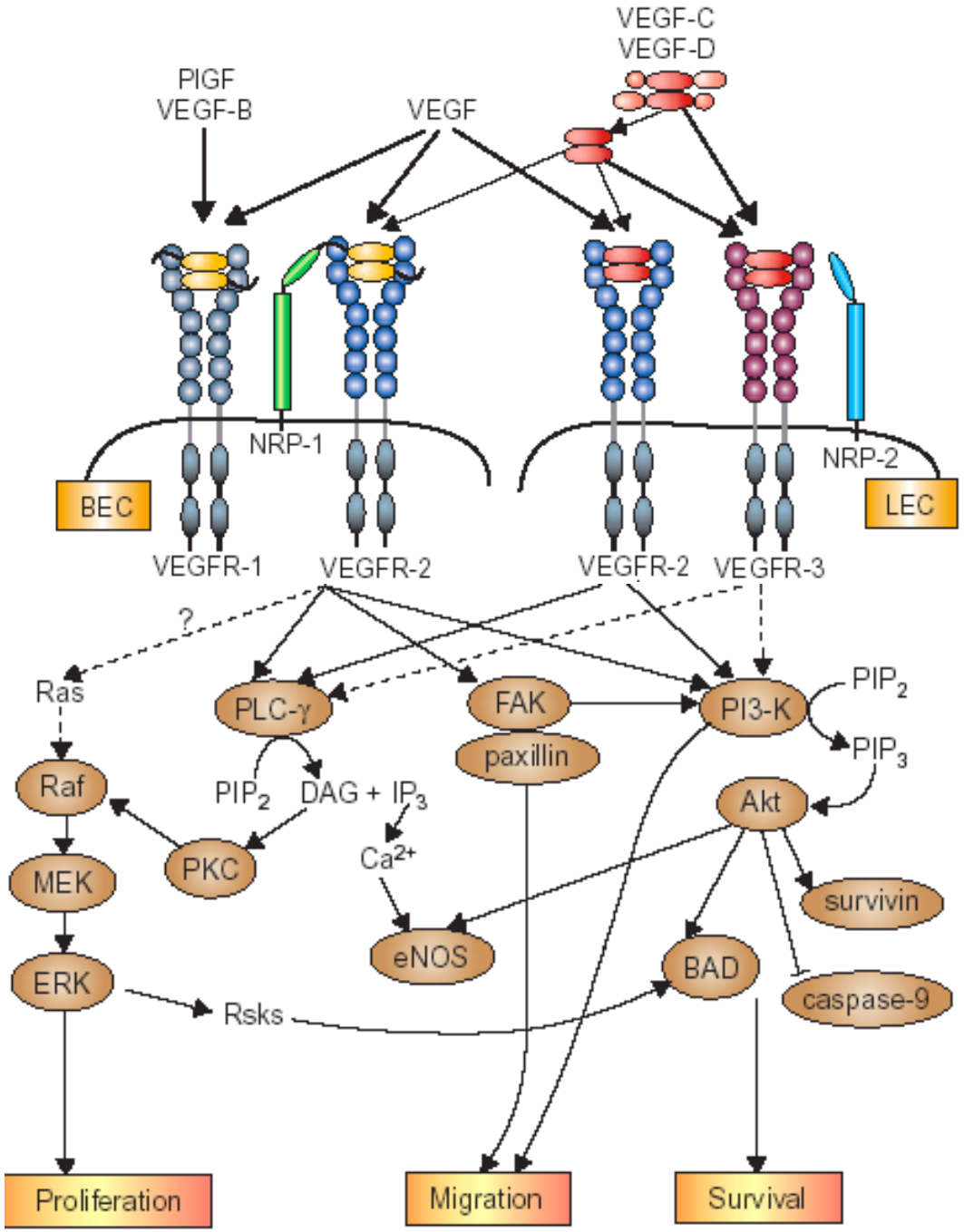


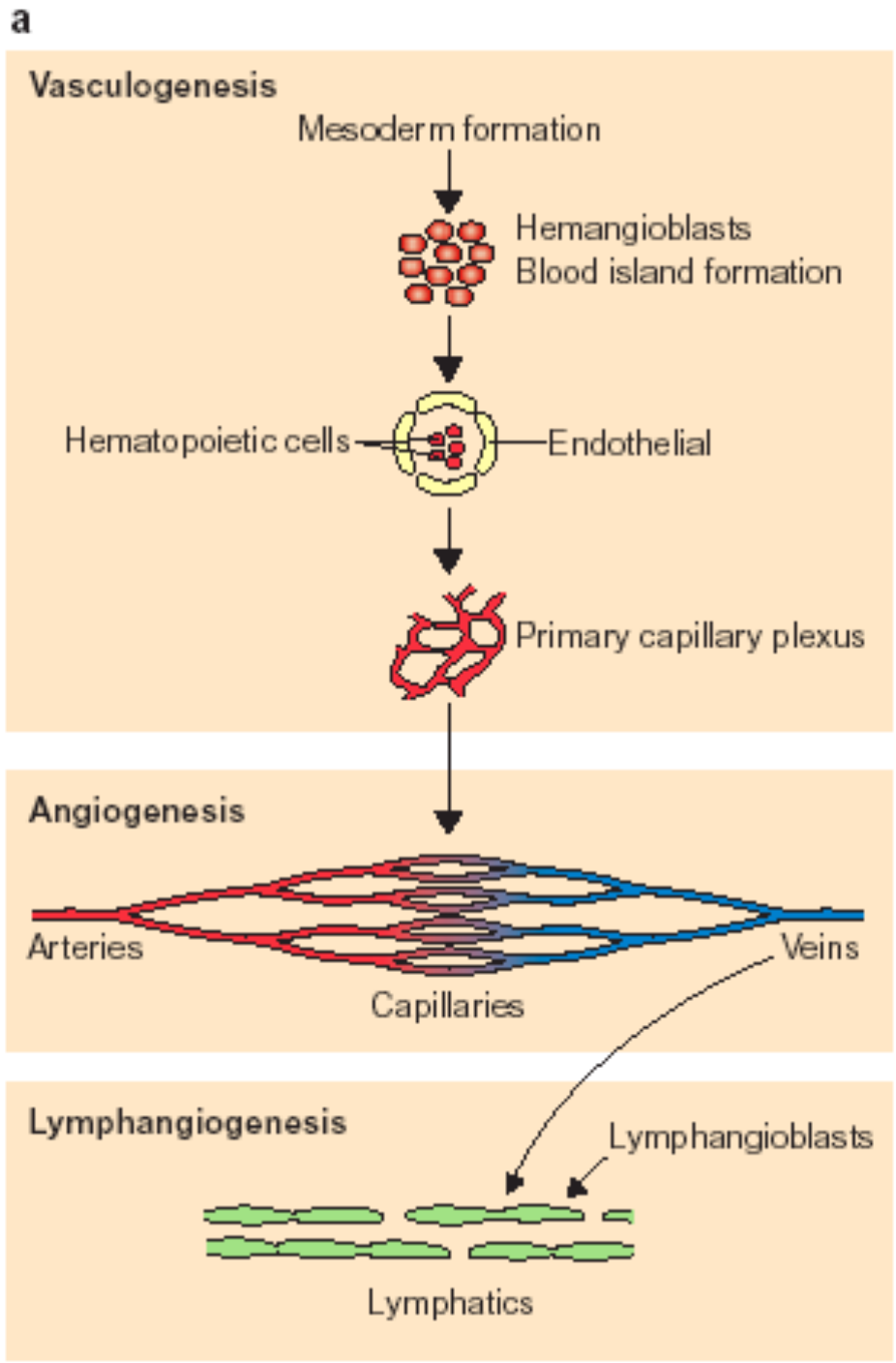
VEGF and VEGF Receptors



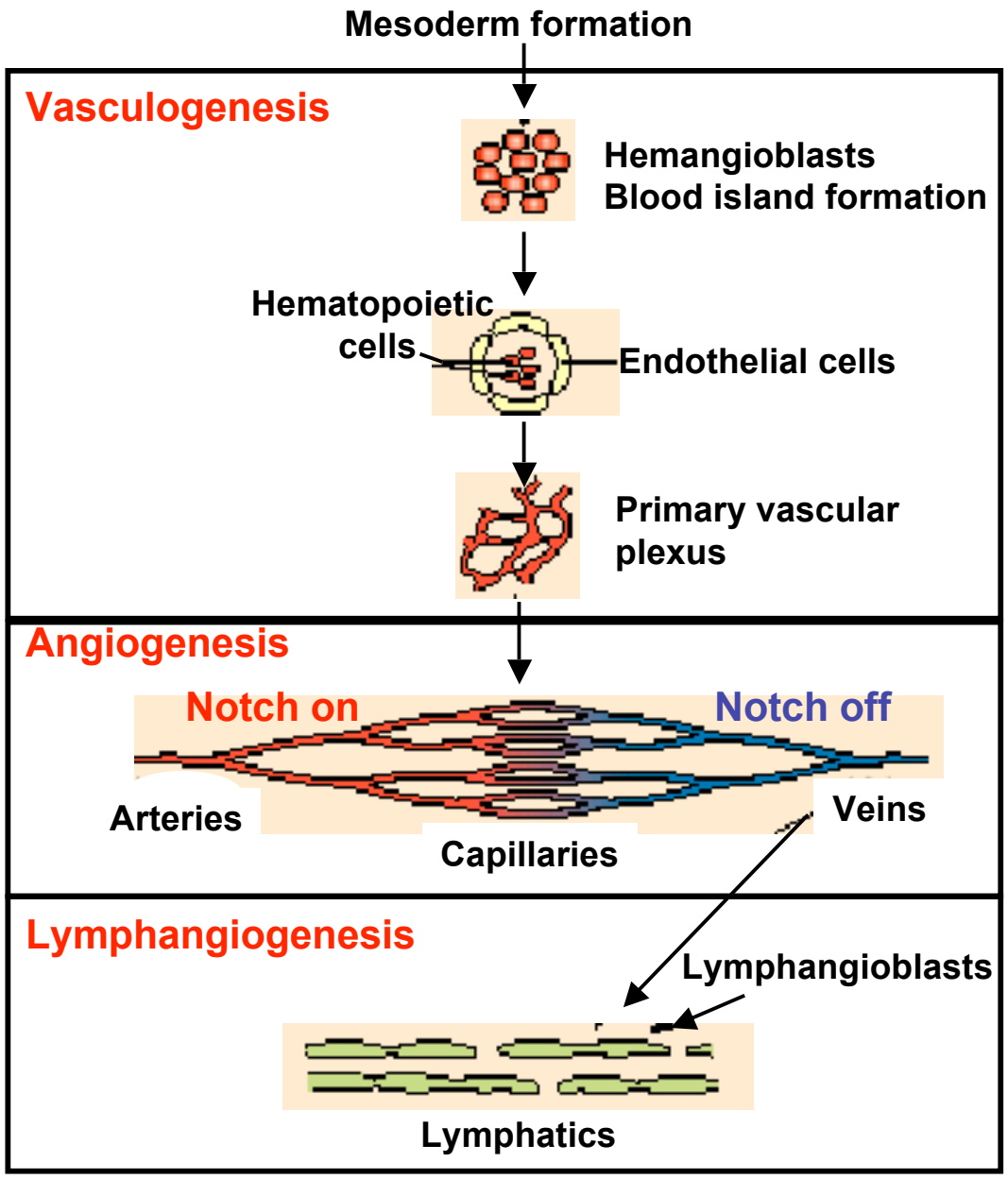

Vasculogenesis
Mature vessels
Angiogenesis
Regression
VEGF
Ang1
Ang2
+
VEGF
Ang2
VEGF-R2
(Flk1)
VEGF-R1
(Flt1)
Tie2
Tie2
VEGF-R1/2
Tie2
EC
BM
PC
Birth, migration, and proliferation
Tube formation, endothelial cell-cell interactions
Recruit and interact with peri-endothelial support cells
Maintain vessel integrity and quiescence
Loosening of matrix contacts and support cell interactions
Allow access and responsiveness to angiogenic inducers
Loss of structure and matrix contacts
Absence of growth or survival signals
Apoptosis?
Endothelial cell (EC)
Basement membrane (BM)
Peri-endothelial cell (PC)

VEGF-receptor signaling




a
Vasculogenesis
Mesoderm formation
Hemangioblasts
Blood island formation
Hematopoietic cells
Endothelial
Primary capillary plexus
Angiogenesis
Arteries
Veins
Capillaries
Lymphangiogenesis
Lymphangioblasts
Lymphatics

Vascular Development



Tal1/scl
Gata 1

Tal1/scl
Gata 1

VEGF A
VEGFR-2
TGFβ

VEGF A
VEGFR-1/2/3
TGFβ
EphrinB2/EphB4
Endoglin

VEGF C/D
VEGFR-3

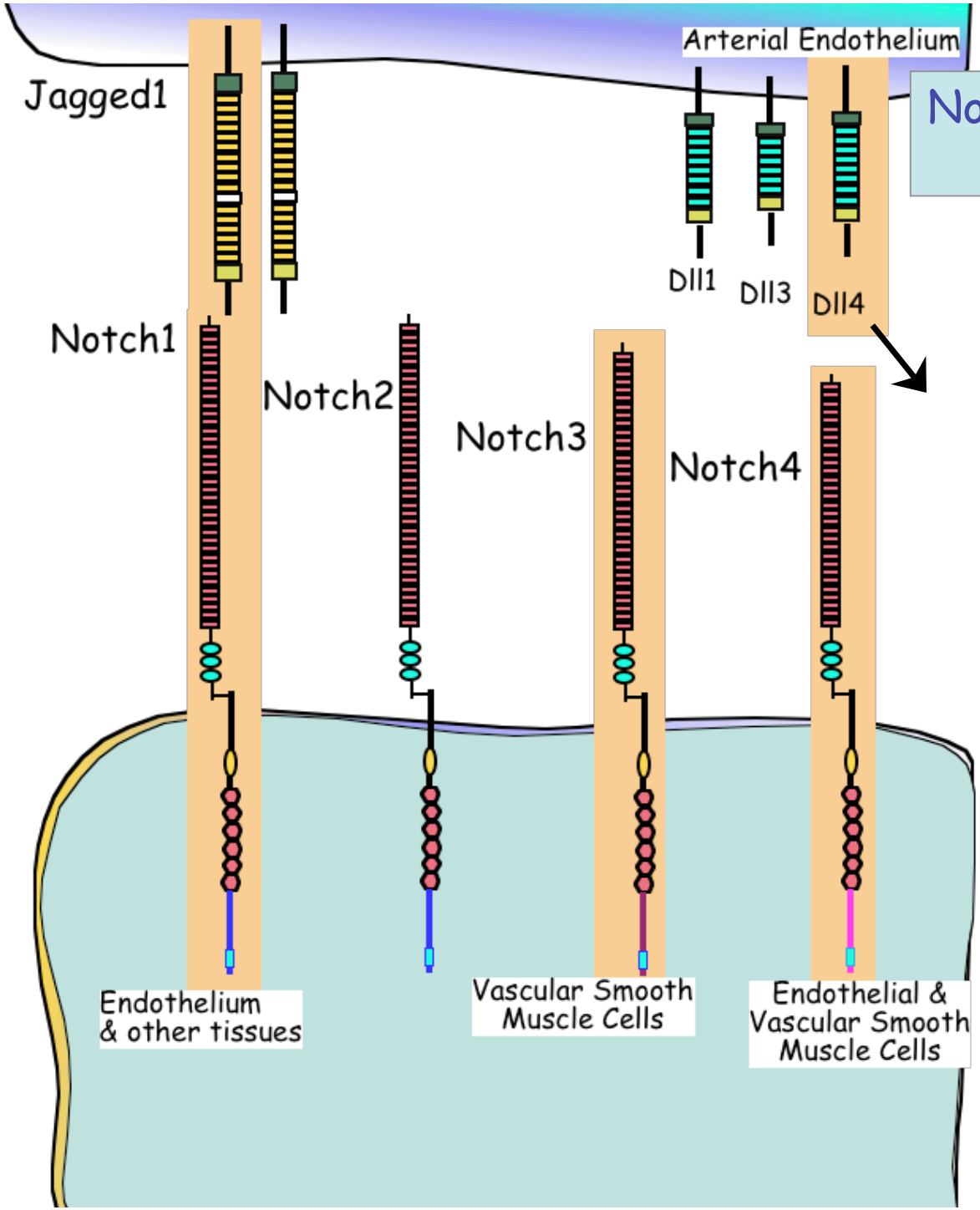
GM-CSF

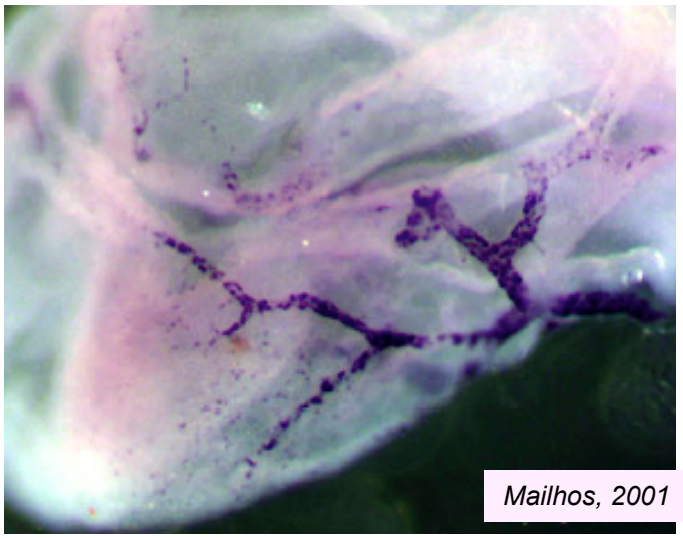
VE-Cadherin
bFGF

Ang1/2
Tie2
PDGF B
Id1/3
Notch

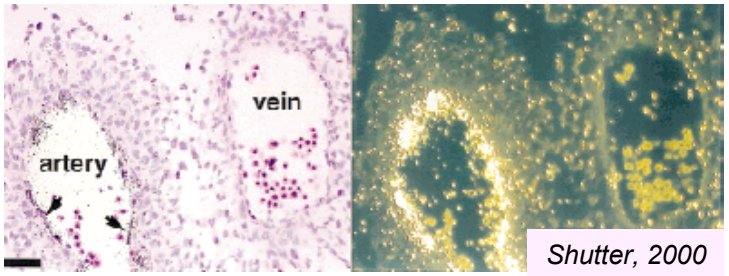
Karkkarnin et al., 2002 Nature Cell Biology

Notch/Notch ligands expressed in arterial endothelium





Mailhos, 2001

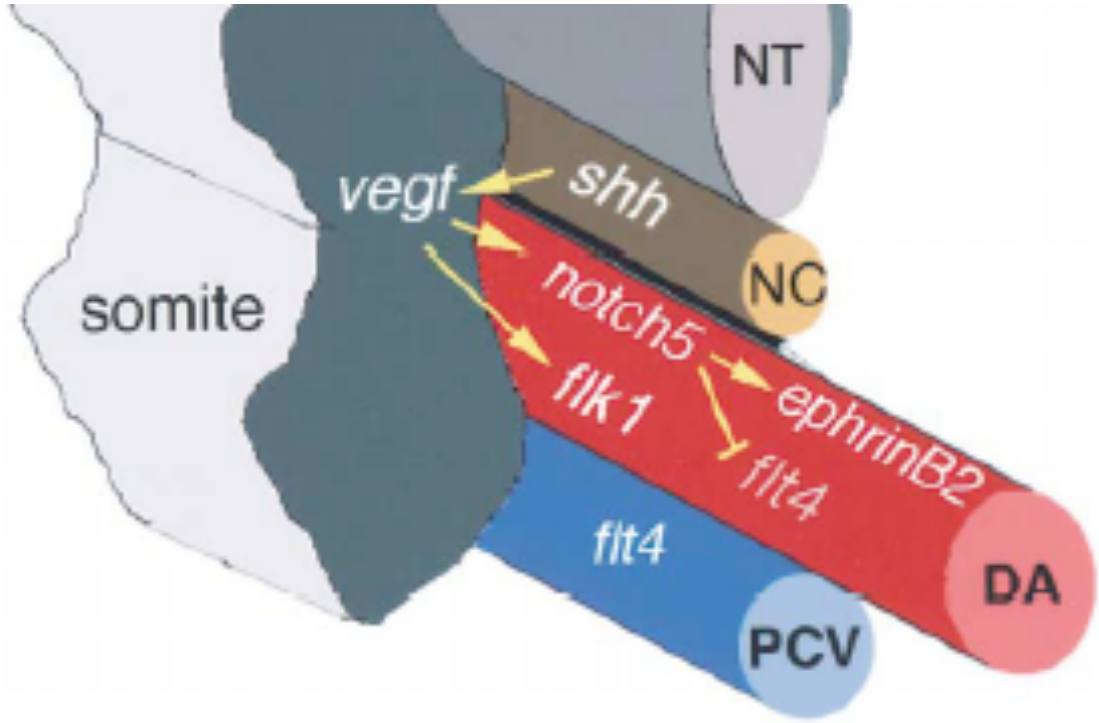


Shutter, 2000

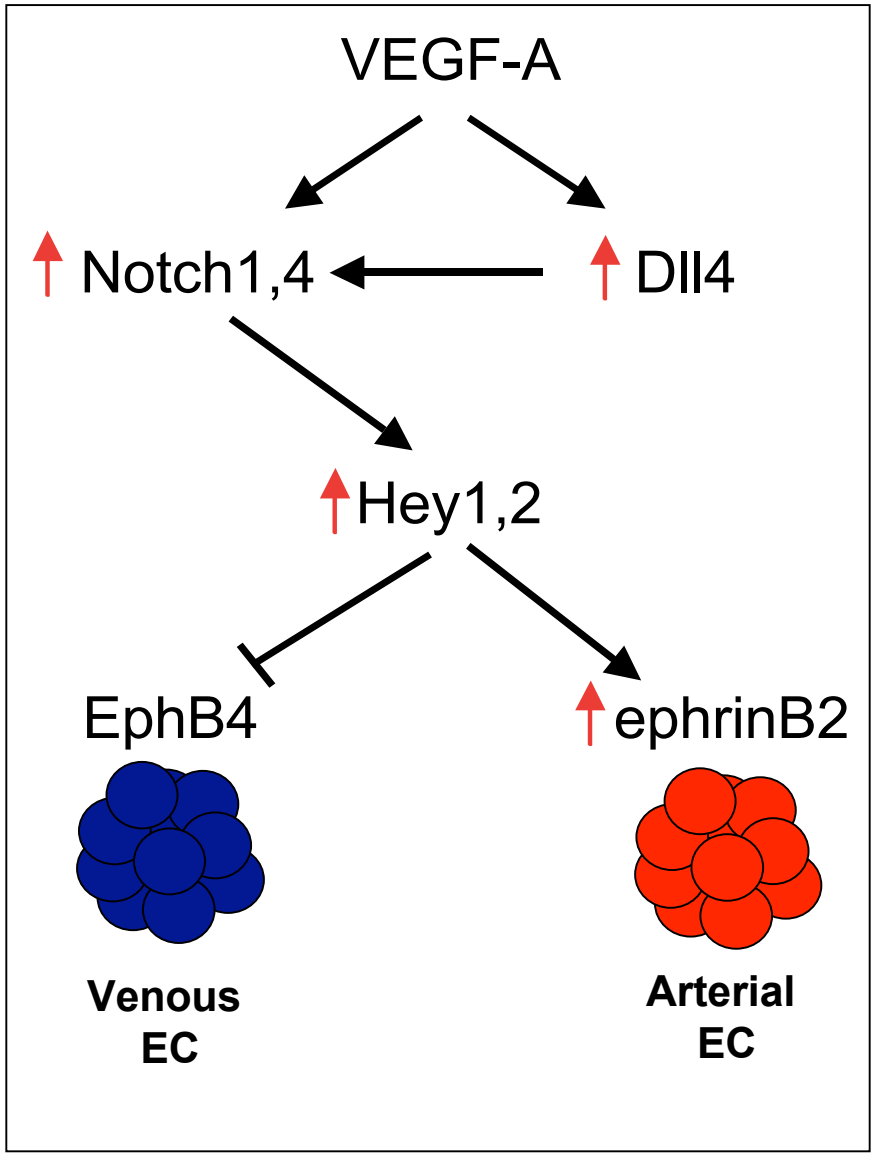
Developmental Cell, Vol. 3, 127–136, July, 2002, Copyright ©2002 by Cell Press

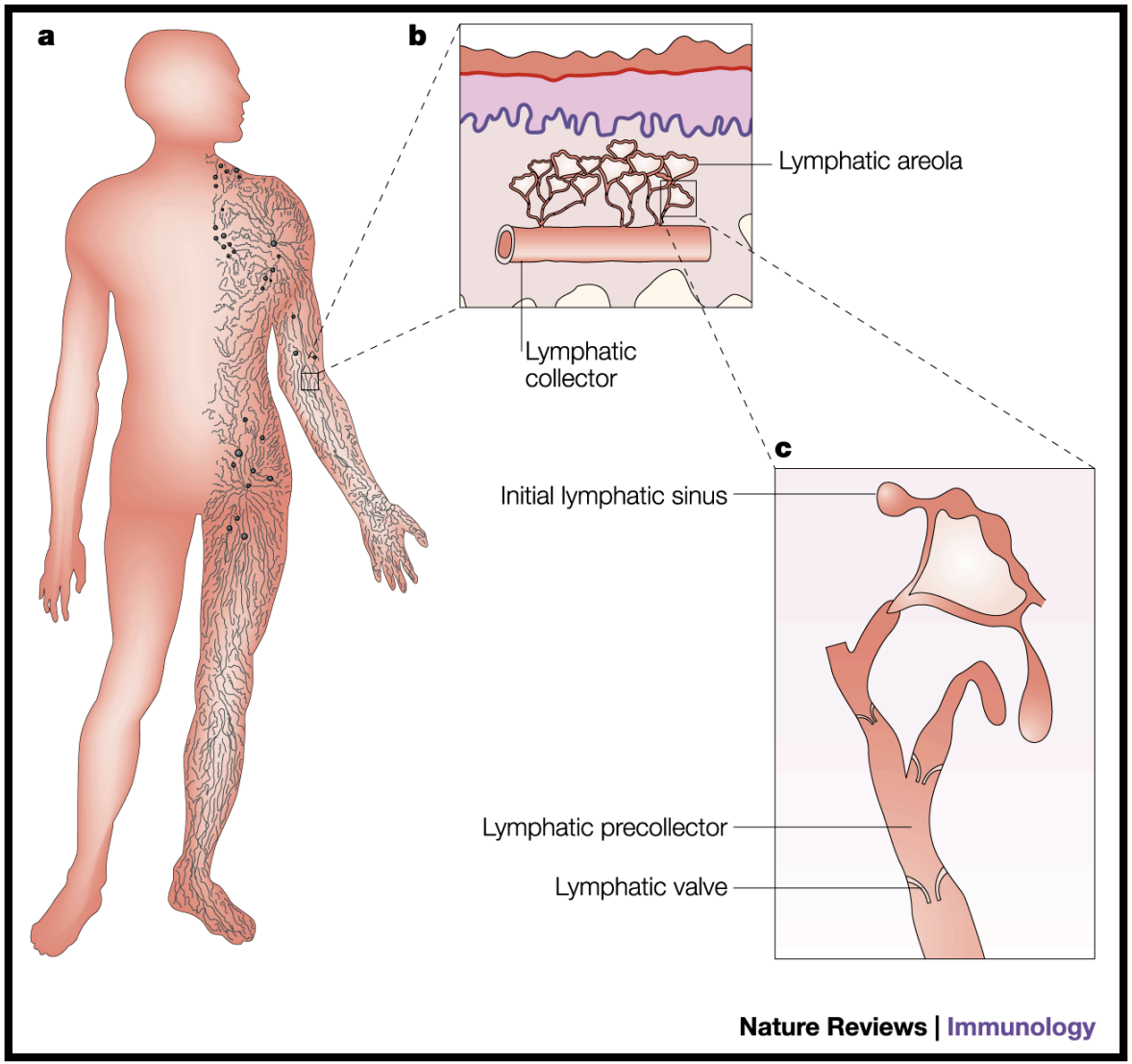
sonic hedgehog and *vascular endothelial growth factor* Act Upstream of the Notch Pathway during Arterial Endothelial Differentiation

Nathan D. Lawson, Andreas M. Vogel,[2] and Brant M. Weinstein[1]



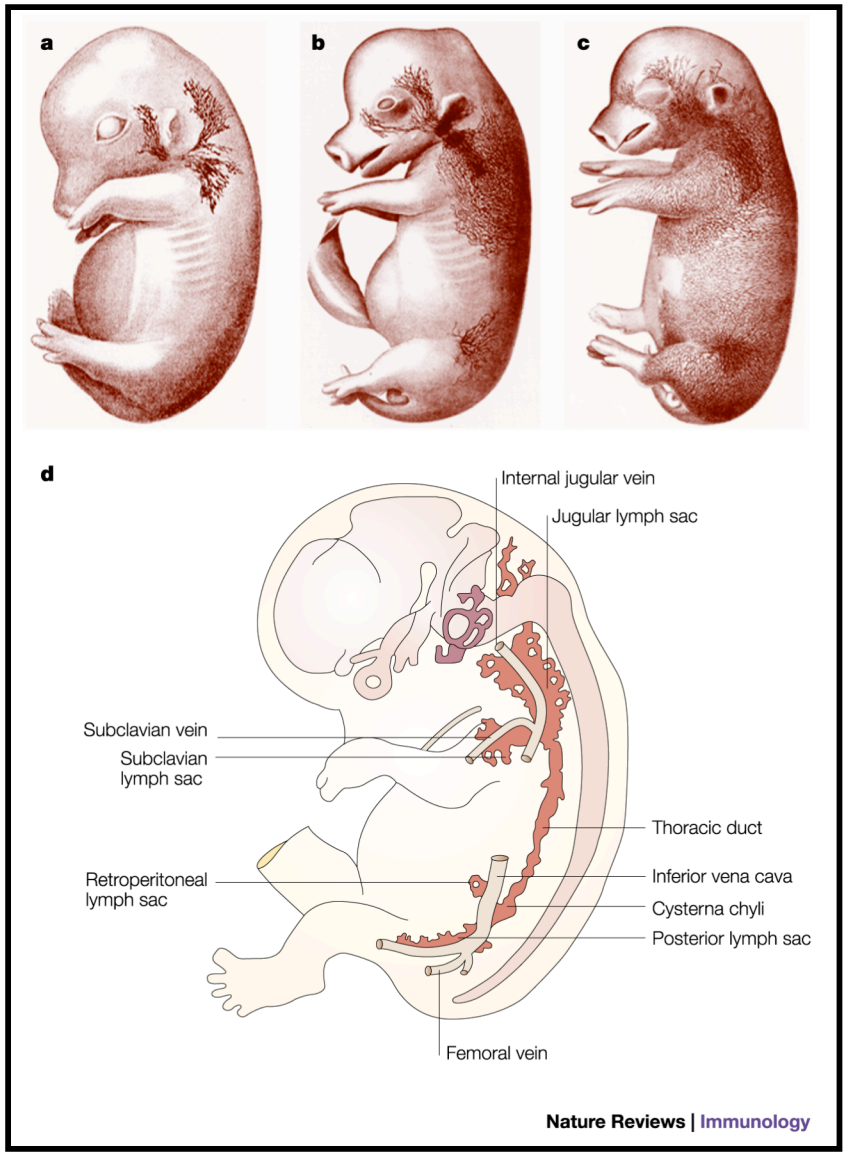
Arterial/Venous Specification





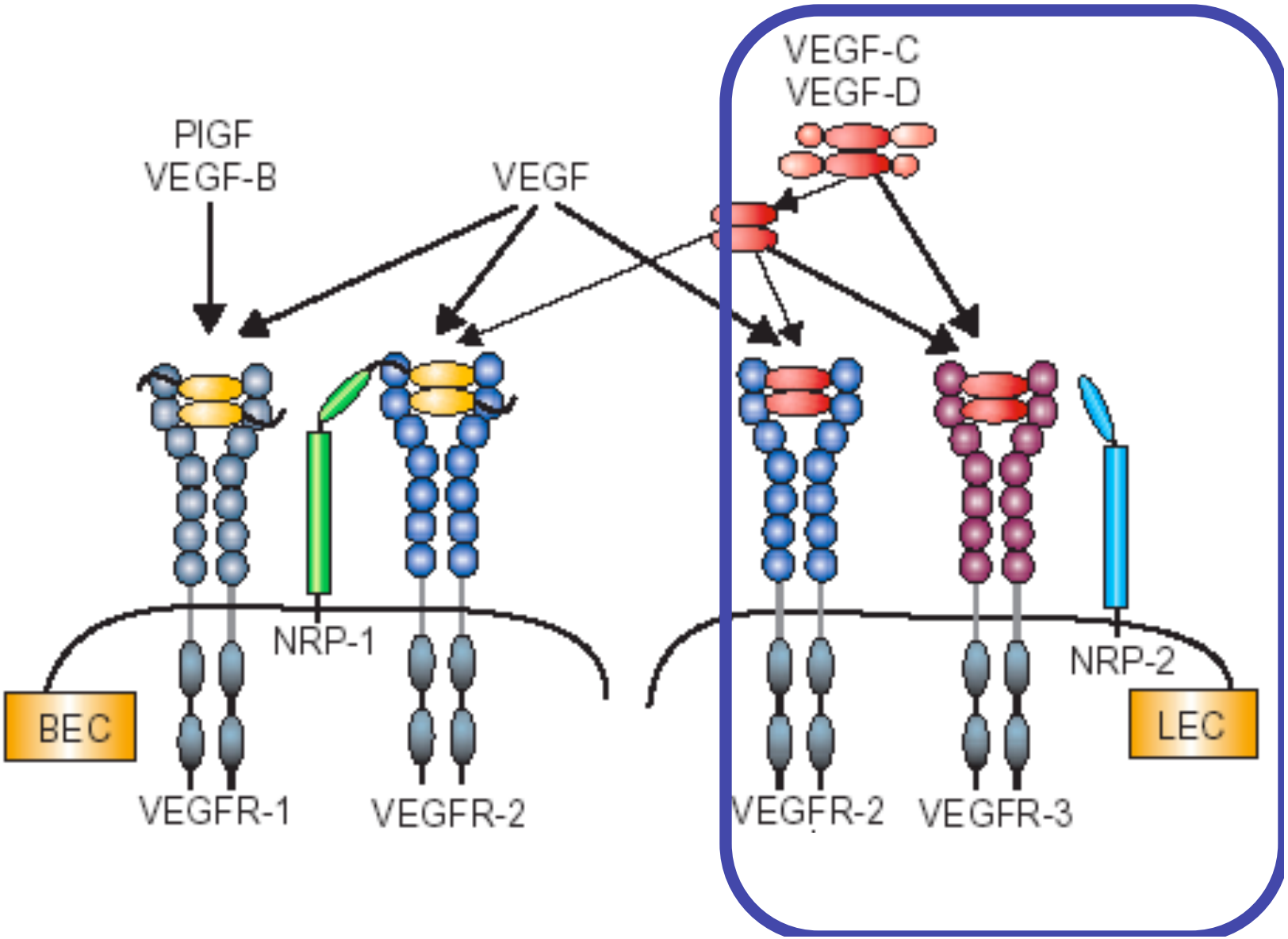
Oliver, G., 2004

F.Sabin Model of Lymphatic Vasculature Development (venous origin)

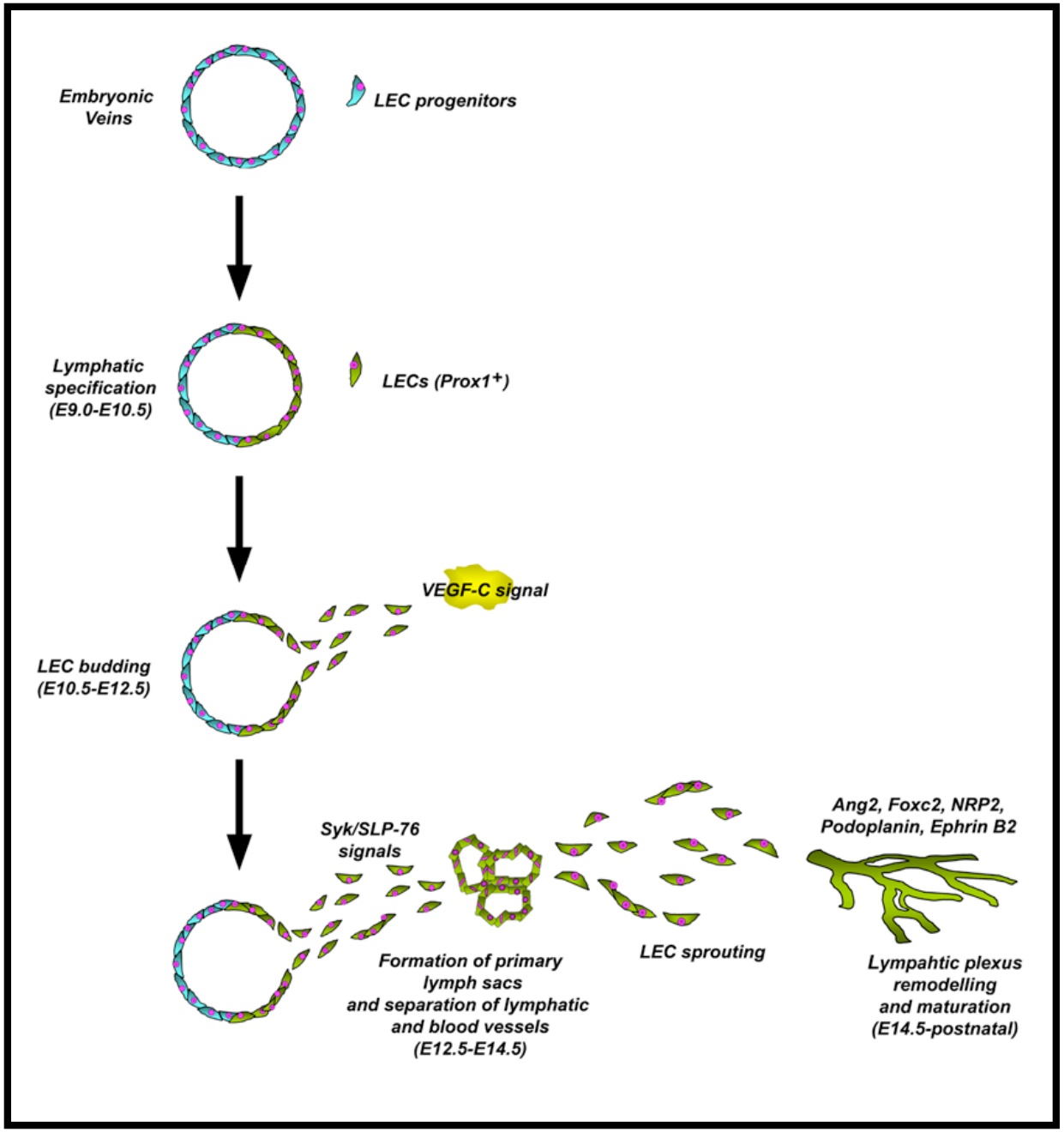


Oliver, G., 2004

VEGF and VEGF Receptors

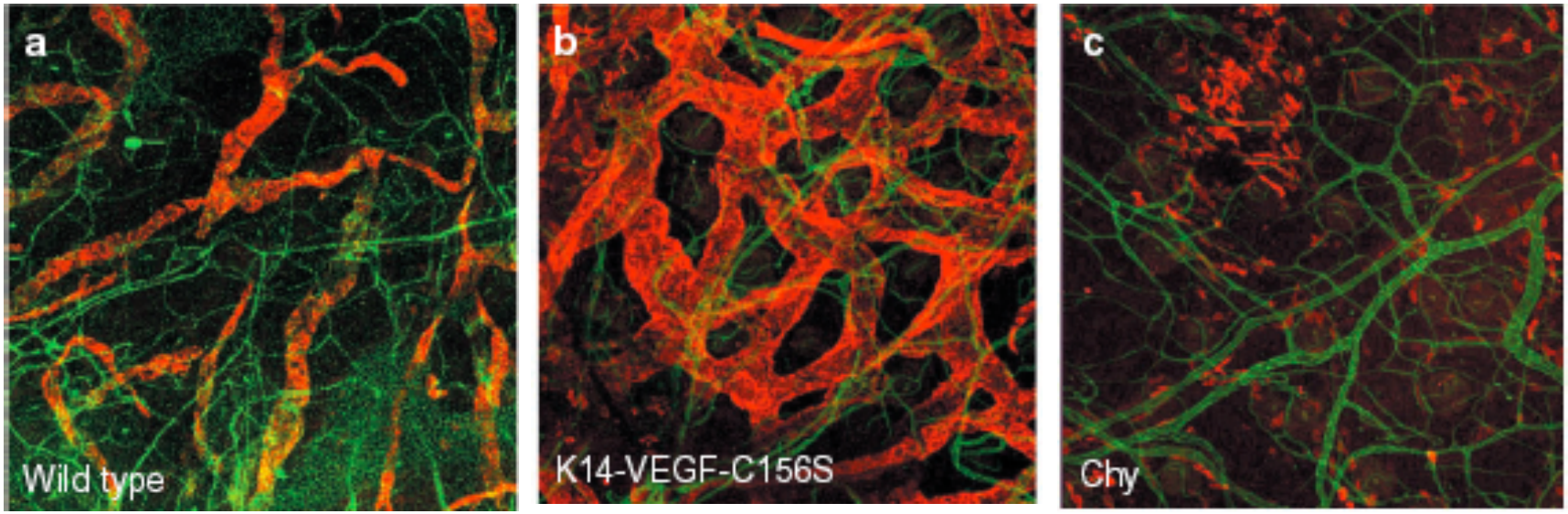


Lymphatic Vasculature Development



Structure of blood and lymphatic vasculature in dermis

normal + lymphatics - lymphatics



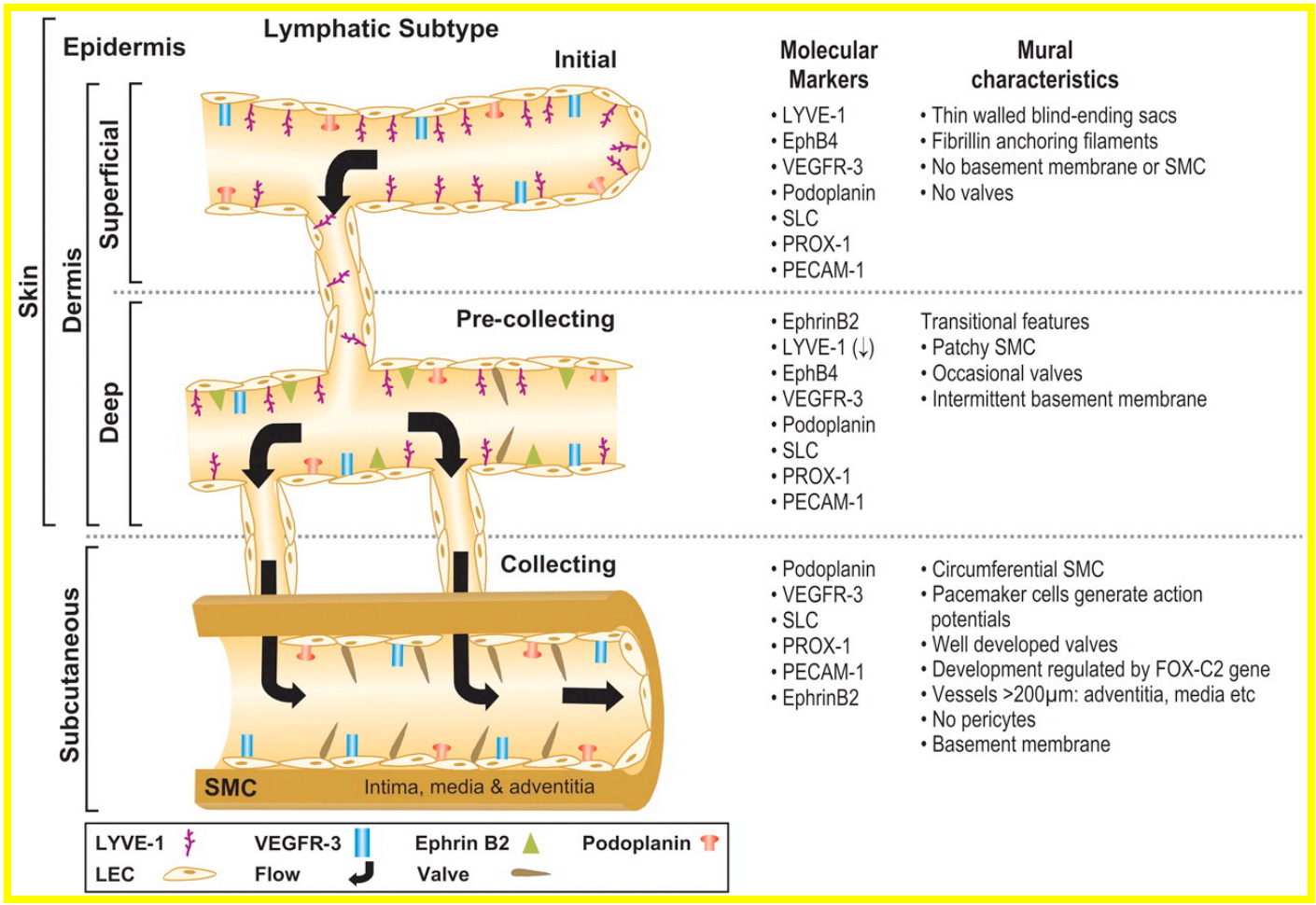
Mouse with mutation in VEGFR-3

blood vessel

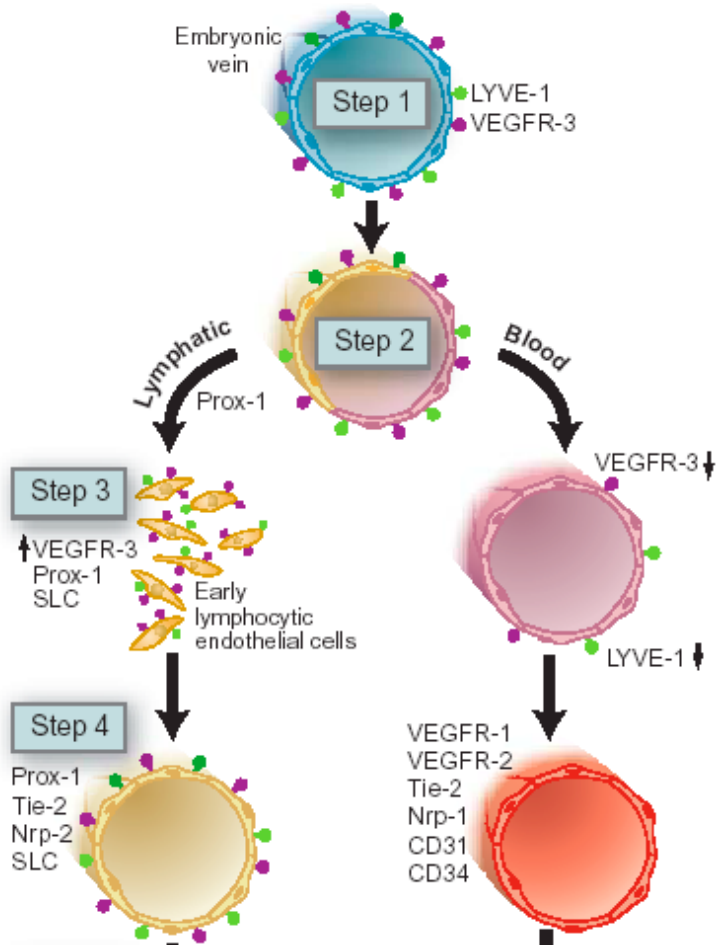
lymphatic vessel

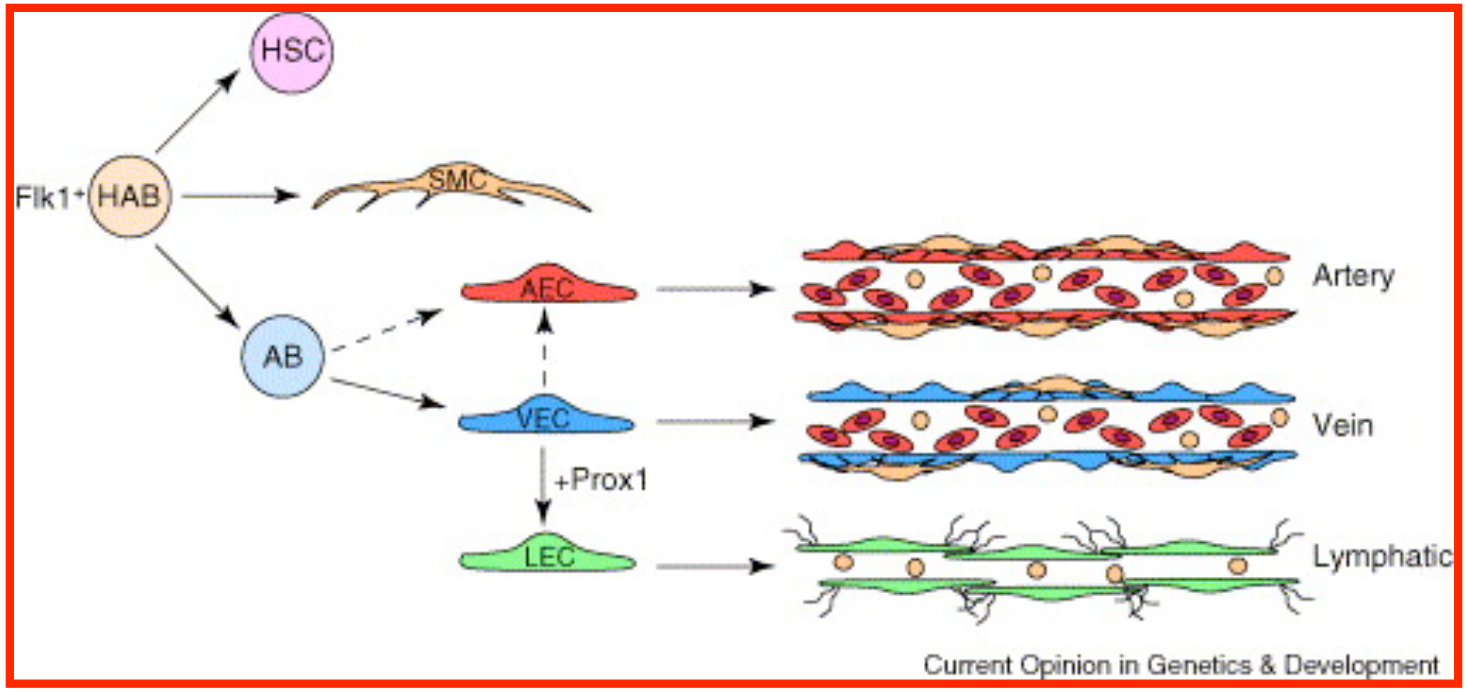
Figure 2 **Blood and lymphatic vessels in mouse ear.** a, **Whole mount immunohistochemical staining of lymphatic vessels (LYVE-1; red) and blood vessels (PECAM-1; green) in mouse ear.** b, **Transgenic overexpression of the VEGF-C mutant C156S in the skin leads to enlargement of the lymphatic vessels, whereas only a few lymphatic ECs are present in the ear of a Chy lymphoedema mouse**[12] (c). **We thank G. Thurston for the stainings and D. Jackson for the LYVE-1 antibodies.**

Molecular characteristics of lymphatic vessel subtypes found in the dermal and subcutaneous layers of normal mammalian skin

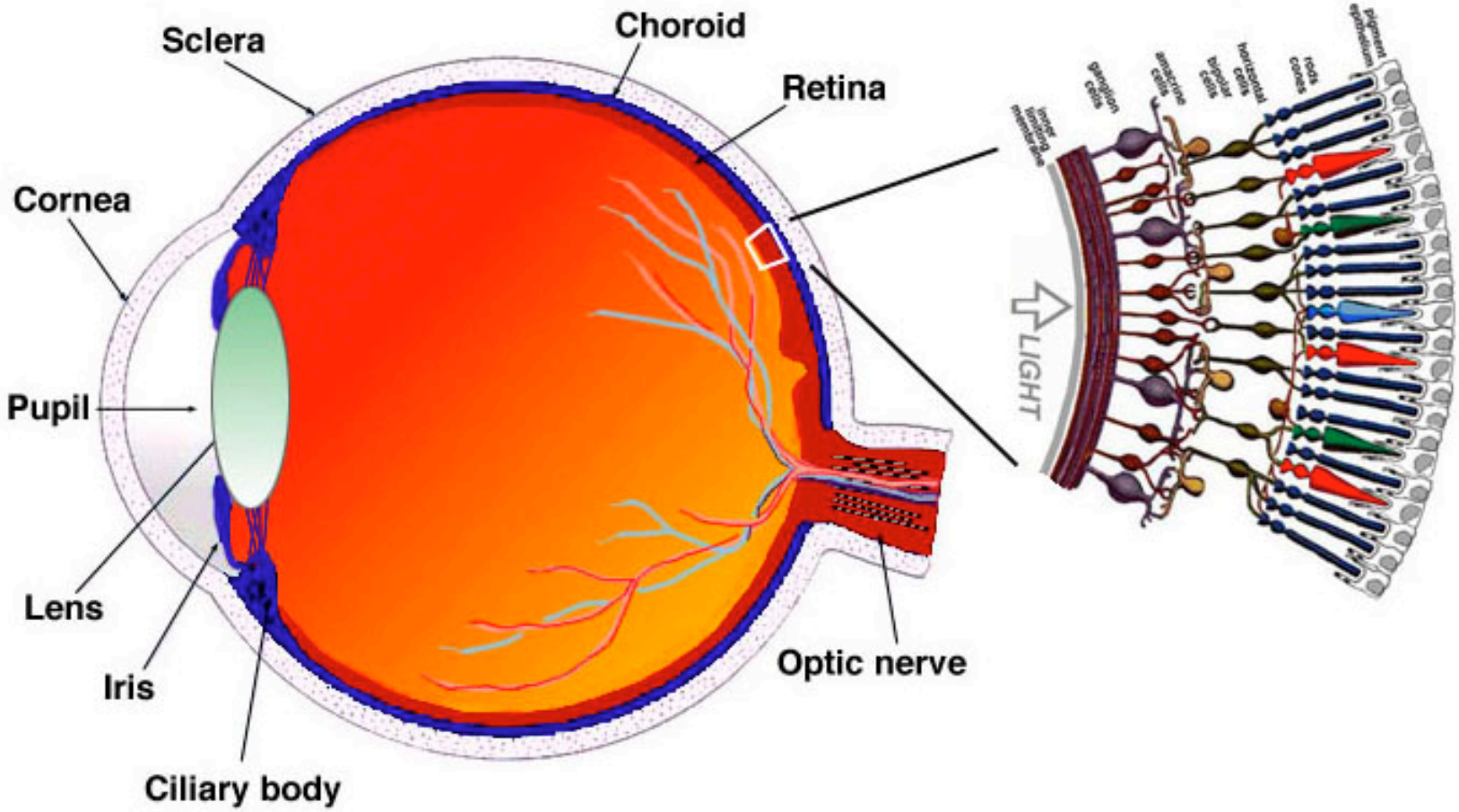


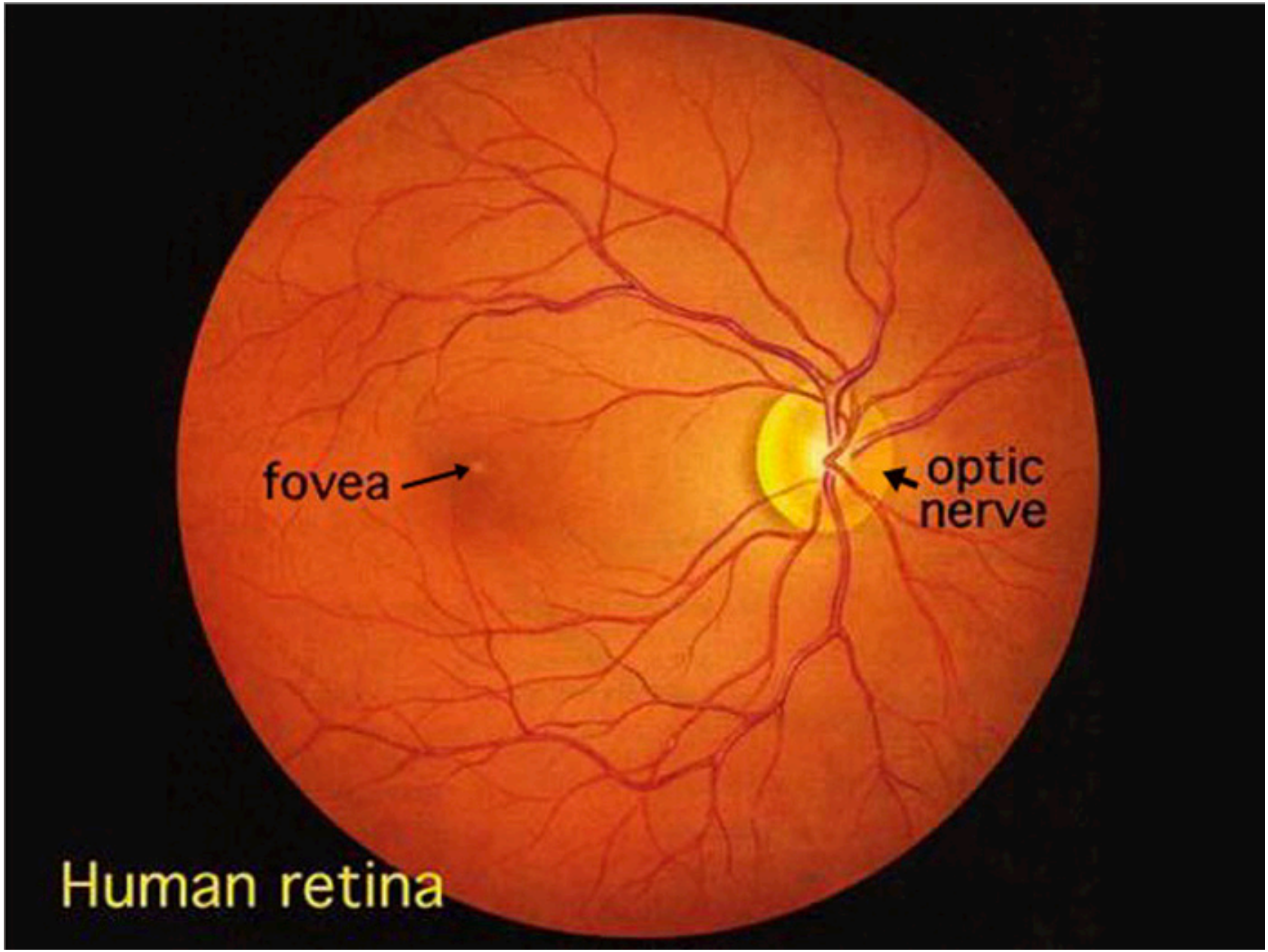
Shayan, R. et al. Carcinogenesis 2006 27:1729-1738


Embryonic vein
Step 1
LYVE-1
VEGFR-3
Step 2
Lymphatic
Prox-1
Blood
Step 3
VEGFR-3
Prox-1
SLC
Early lymphocytic endothelial cells
VEGFR-3
LYVE-1
Step 4
Prox-1
Tie-2
Nrp-2
SLC
VEGFR-1
VEGFR-2
Tie-2
Nrp-1
CD31
CD34



Harvey and Oliver, Curr.Opin Genet Dev, 2004


Sclera
Choroid
Retina
Cornea
Pupil
Lens
Iris
Ciliary body
Optic nerve
LIGHT


fovea
optic nerve
Human retina

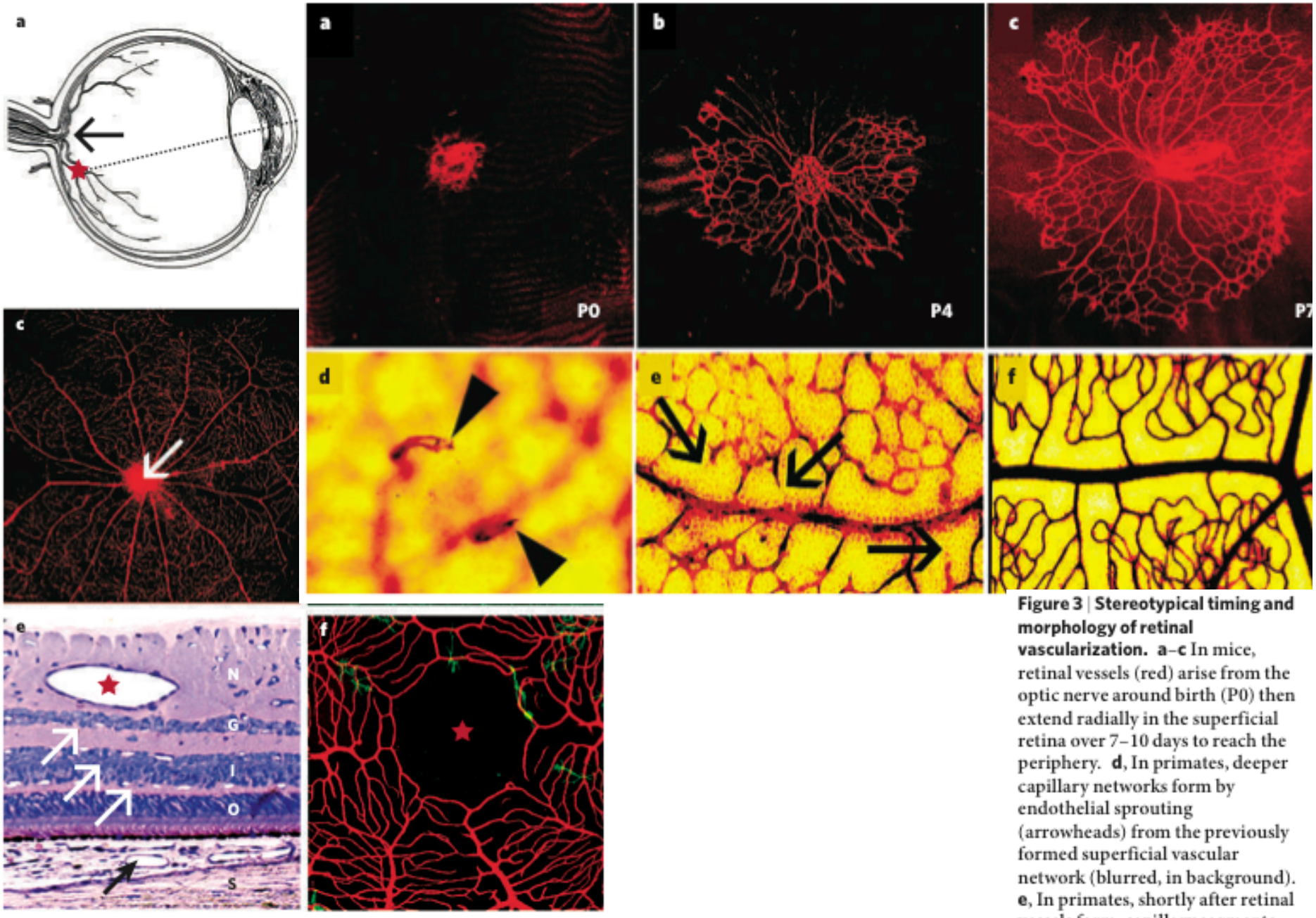
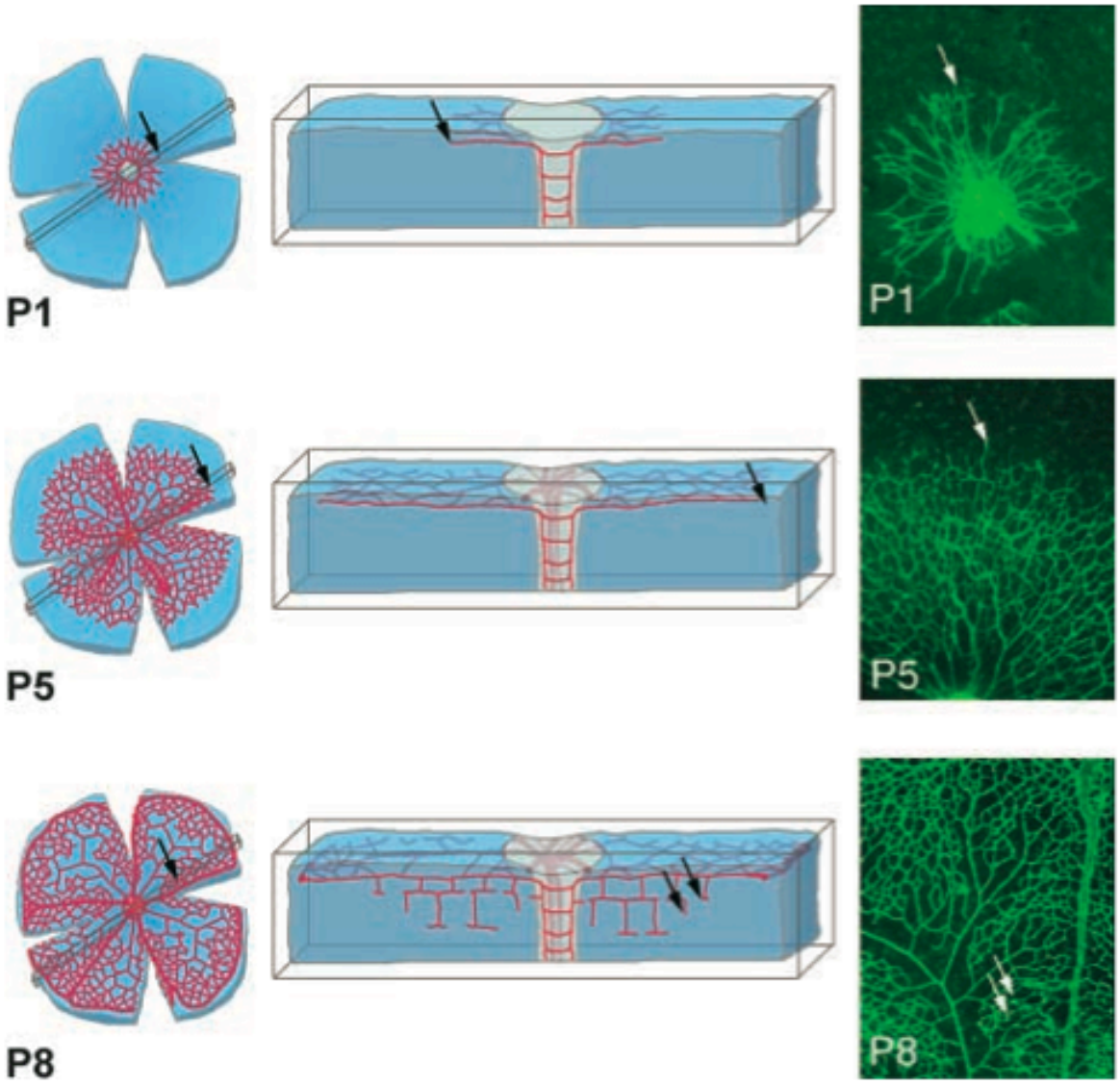
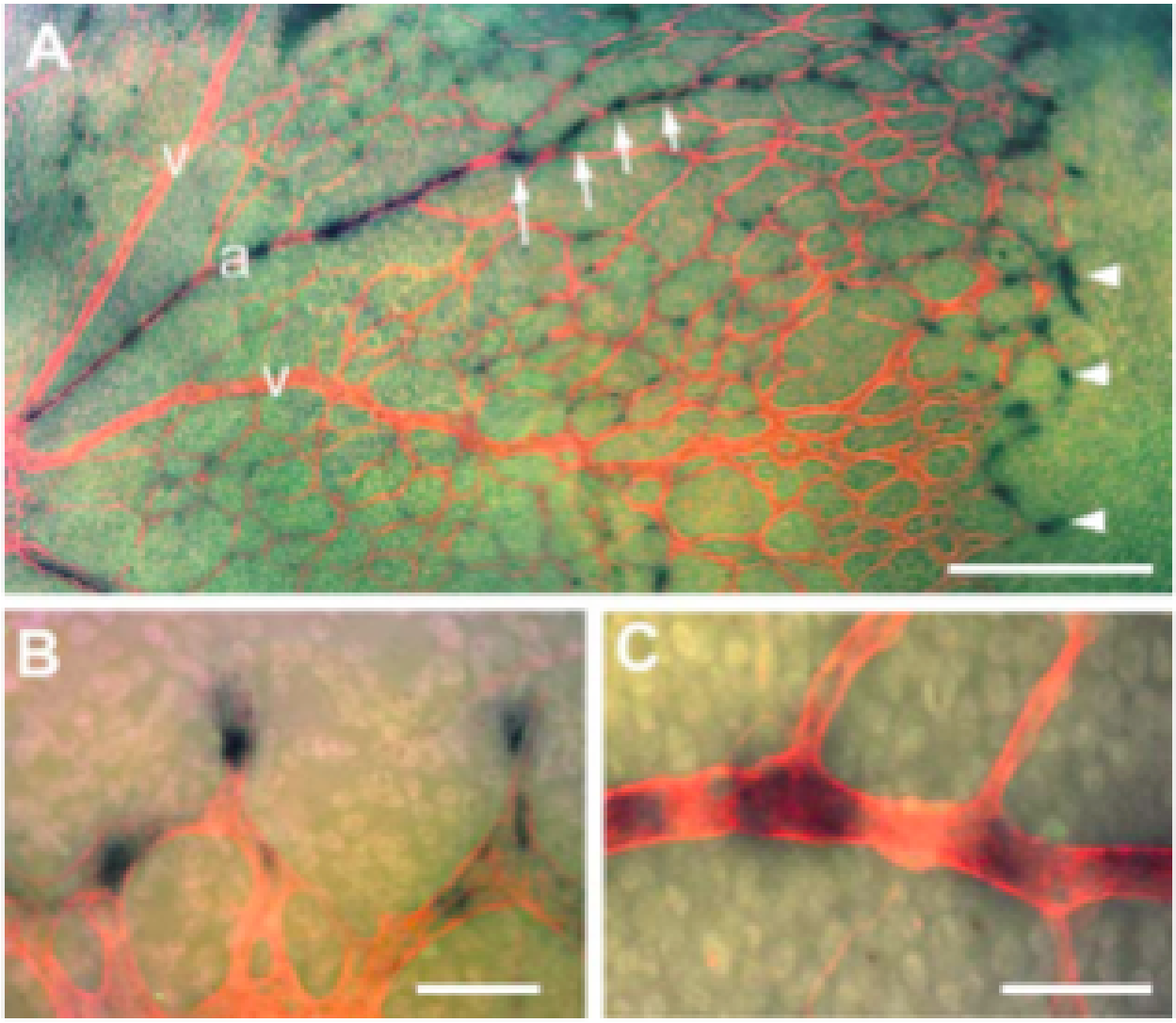


Figure 3 | Stereotypical timing and morphology of retinal vascularization. **a**–**c** In mice, retinal vessels (red) arise from the optic nerve around birth (P0) then extend radially in the superficial retina over 7–10 days to reach the periphery. **d**, In primates, deeper capillary networks form by endothelial sprouting (arrowheads) from the previously formed superficial vascular network (blurred, in background). **e**, In primates, shortly after retinal vessels form, capillary segments adjacent to nascent arteries (arrows) retract, to yield a periarterial capillary-free zone.


P1
P5
P8
P1
P5
P8

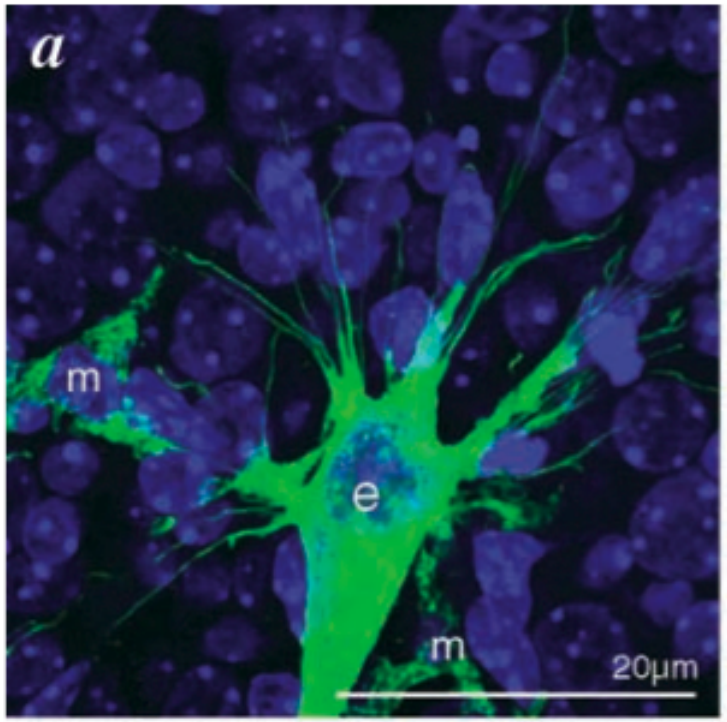
Delta4 expressed in retinal vasculature

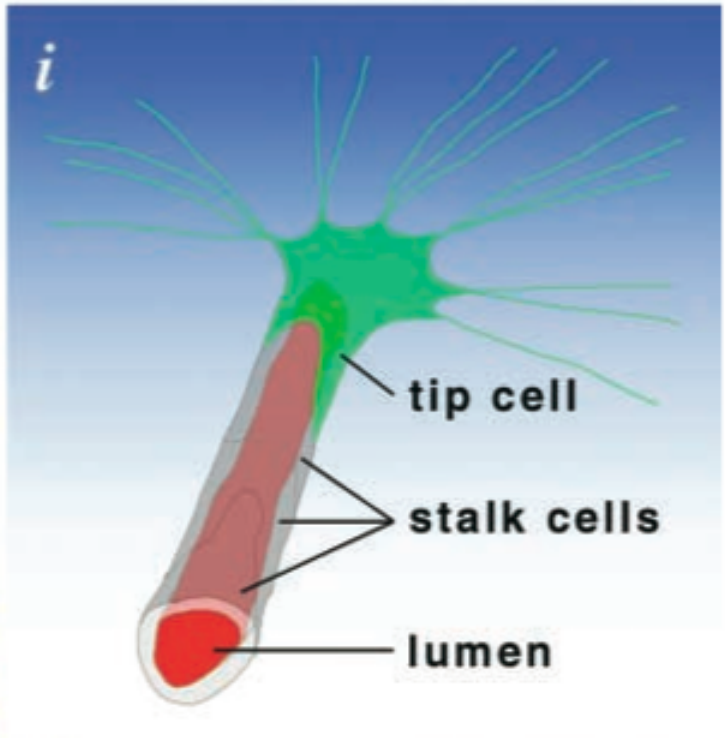
arterial expression *tip cell expression*



in situ hybridization

Fruttinger, 2004


a
m
e
m
20µm


i
tip cell
stalk cells
lumen

Normal intraretinal vasculature

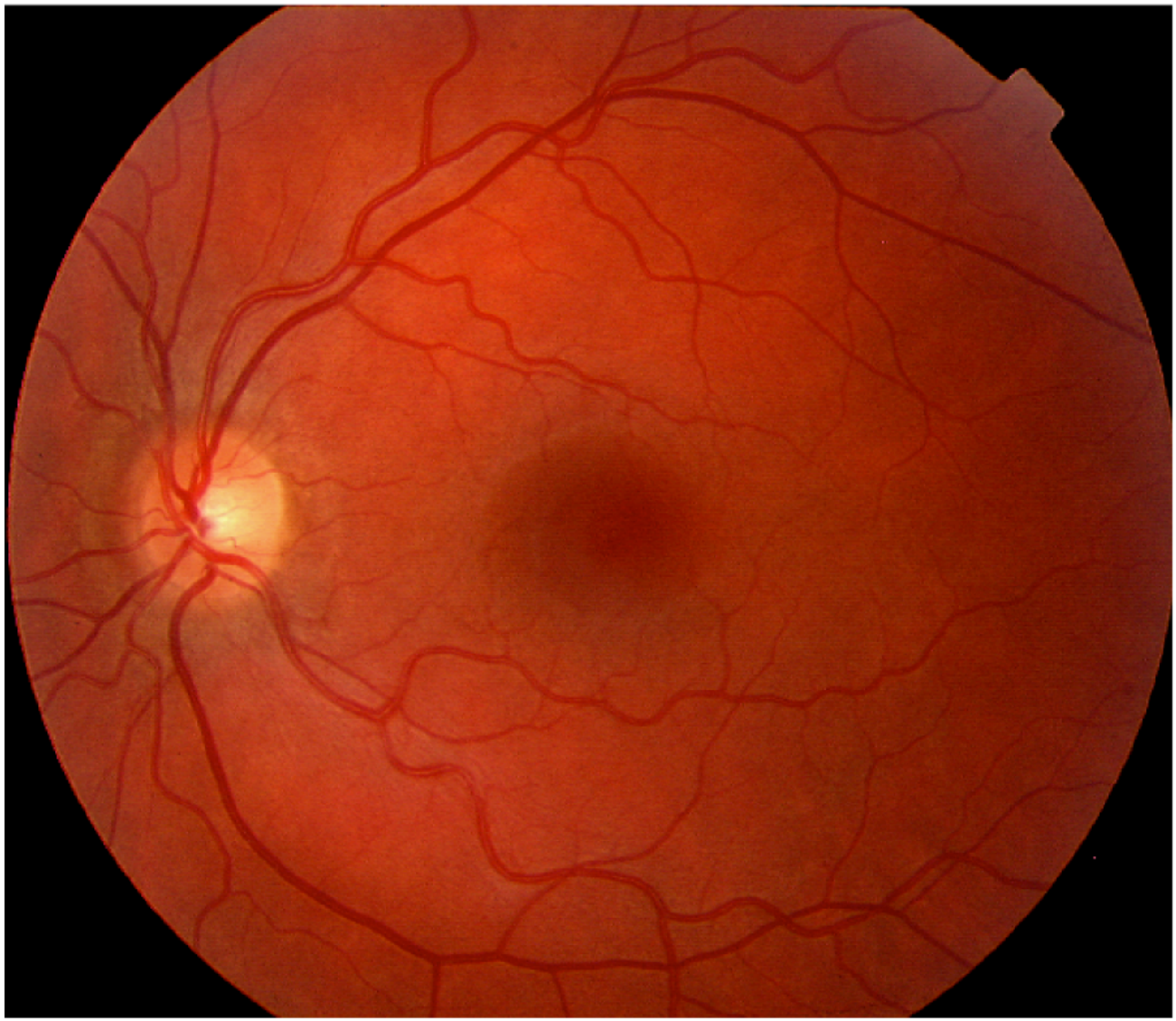
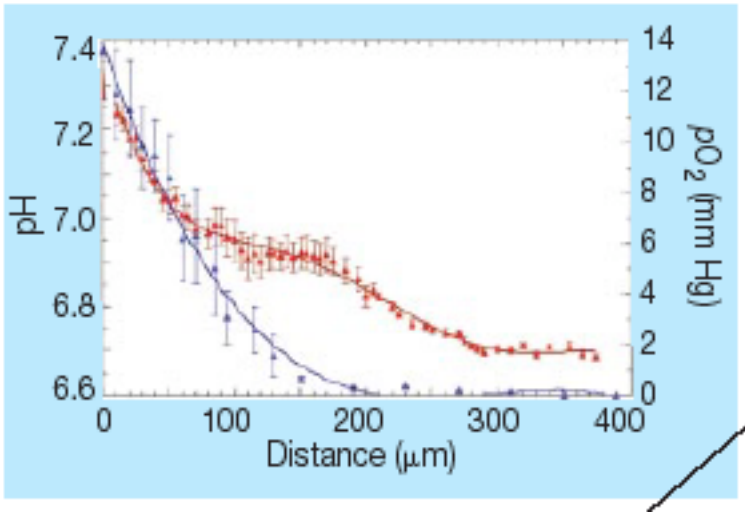
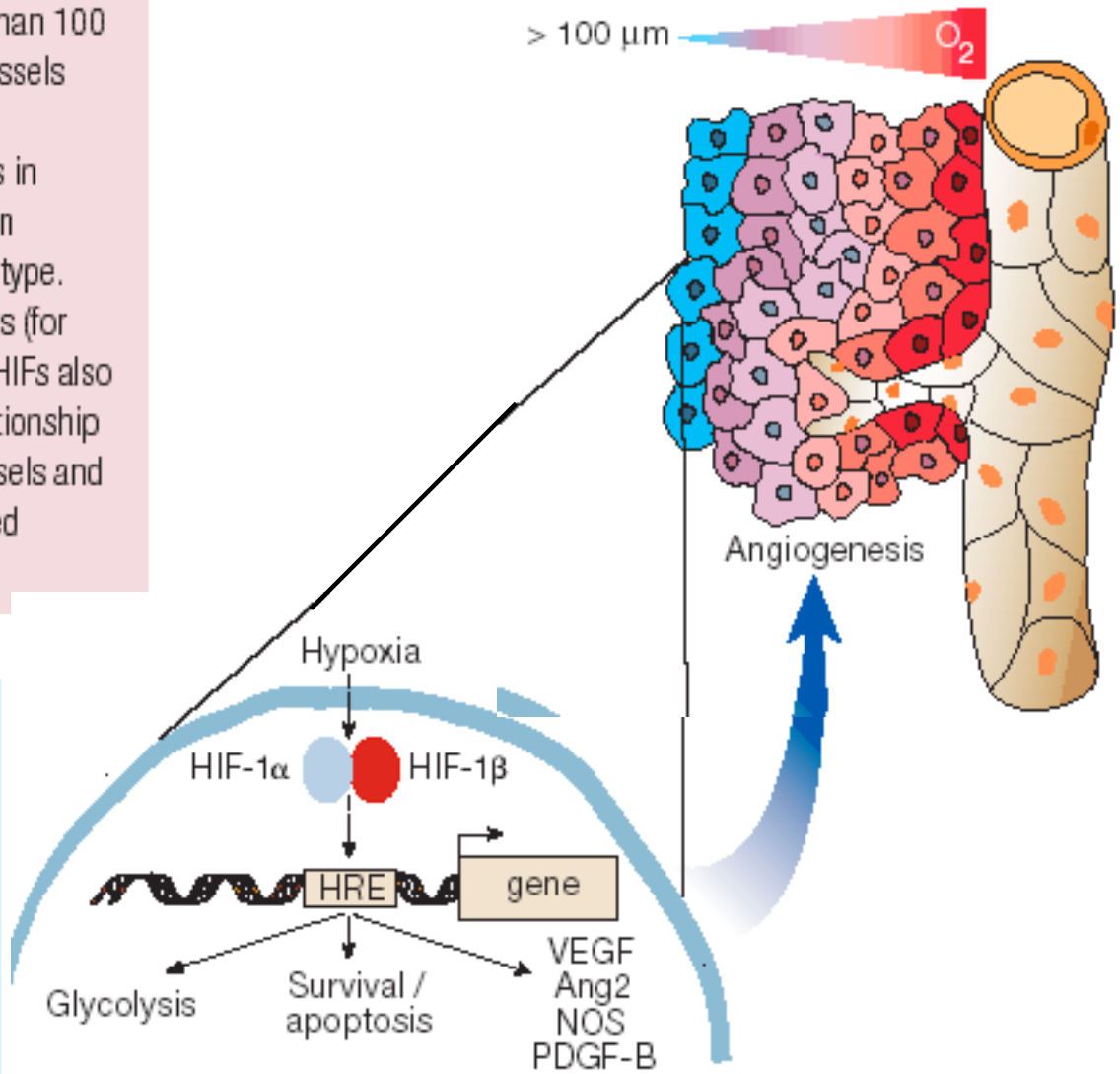


Figure 2 Role of hypoxia in tumour angiogenesis. Because of the irregular pattern and organization of the tumour vasculature, some cells in tumours are located more than 100 μm (the diffusion limit for oxygen) away from blood vessels and become hypoxic (red-to-blue gradient indicates progressive hypoxia). Tumour cells survive fluctuations in oxygen tensions, in part because clones are selected in hypoxic tumours that switch to a proangiogenic phenotype. HIFs increase transcription of several angiogenic genes (for example, genes encoding VEGF, PDGF-BB and NOS). HIFs also affect cellular survival/apoptosis pathways. Inset: relationship between the distance of tumour cells from nearby vessels and their degree of hypoxia (blue symbols) and acidosis (red symbols)[24].





Retinopathy of Prematurity

L.E.H. Smith / Growth Hormone & IGF Research 14 (2004) S140–S144

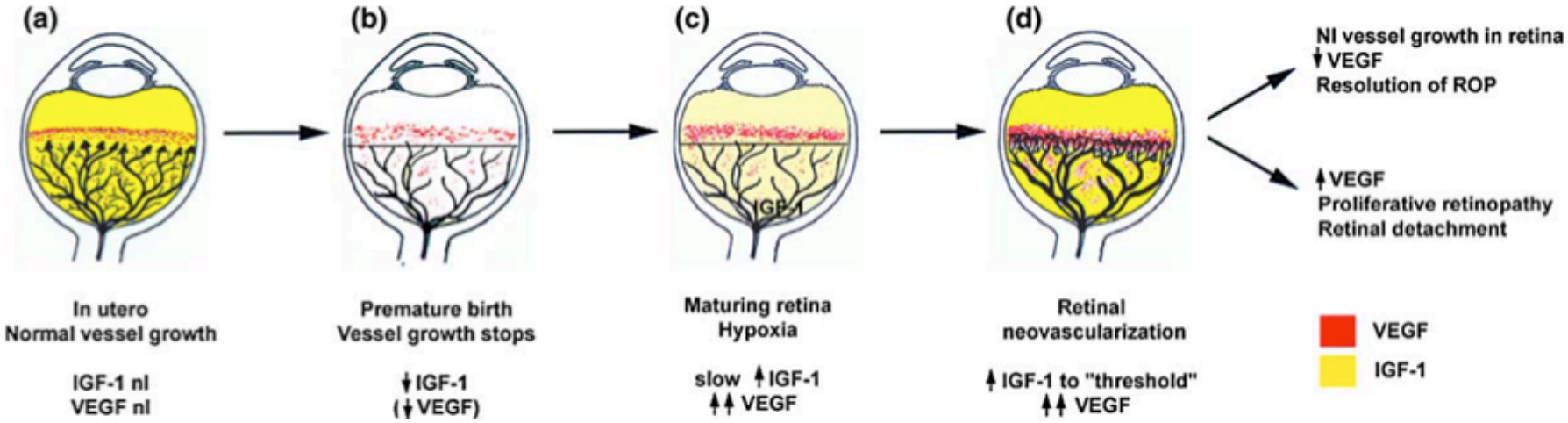
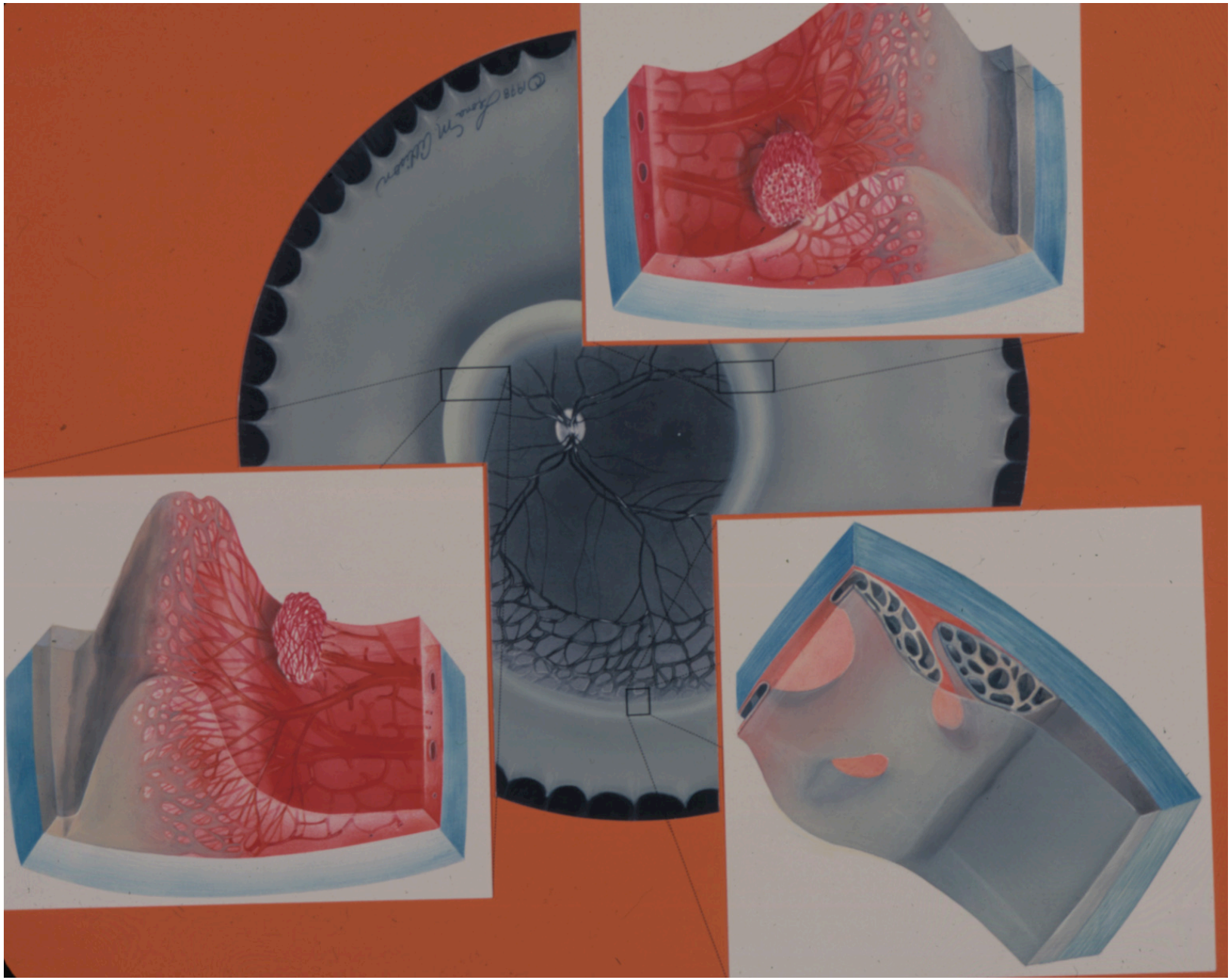


Fig. 1. Mechanism for retinopathy of prematurity (ROP) (Reprinted, with permission, from [37]).



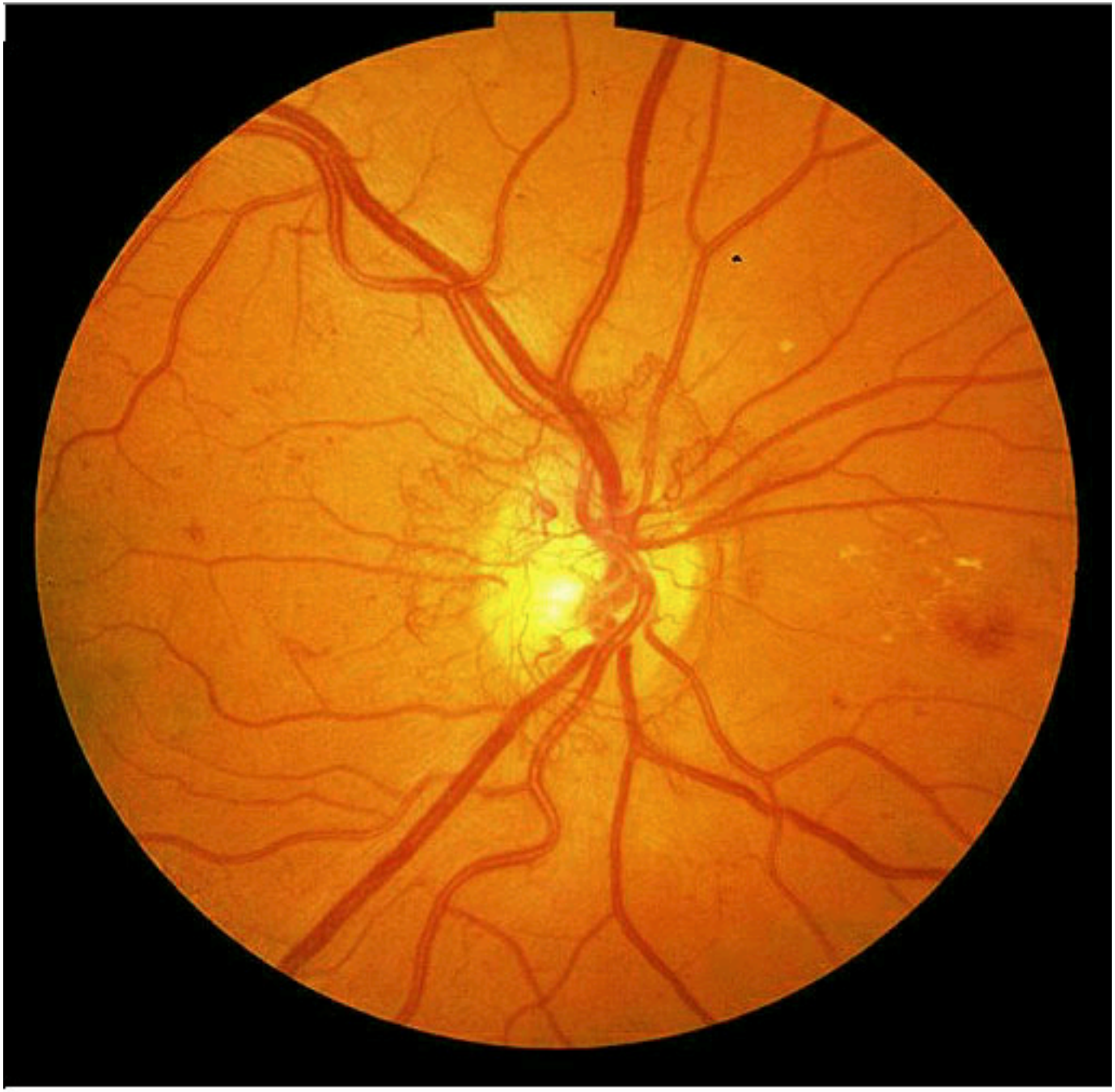


Fig. 25. A view of the fundus of the eye and of the retina in a patient who has advanced diabetic retinopathy.

Familial Exudative Vitreoretinopathy

- First described by Criswick and Schepens [Am. J. Ophthalmol. 68: 578-594 (1969)]
- Autosomal dominant, recessive, and X-linked forms; variable phenotype
- Clinical characteristics

  mild to severe vision loss

  retina: avascular peripheral retina, exudates,

  neovascularization, fibrovascular masses,

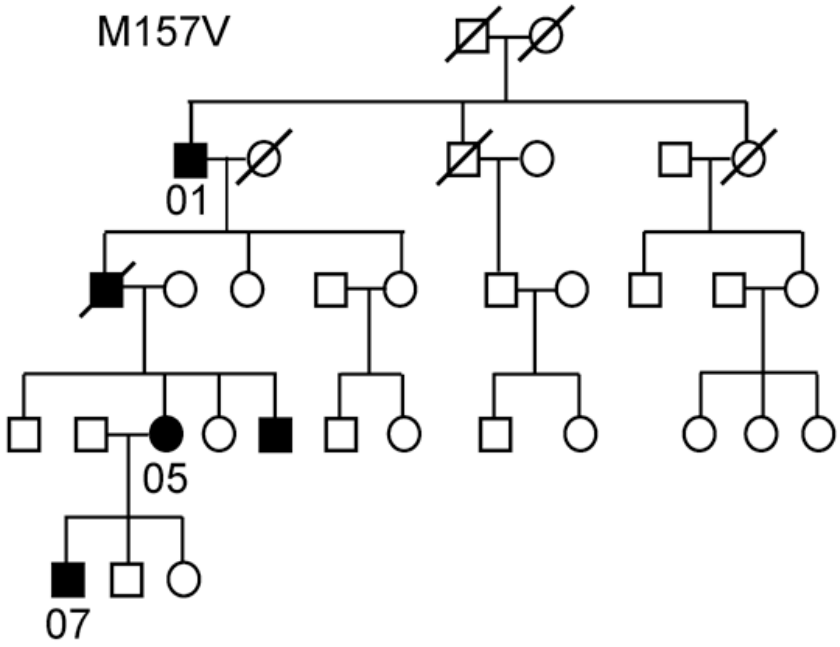
  traction or rhegmatogenous retinal detachment

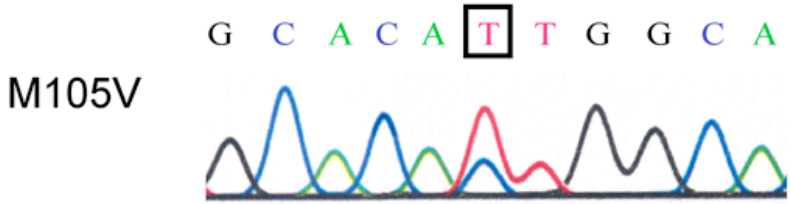
  vitreous: posterior vitreous detachment,

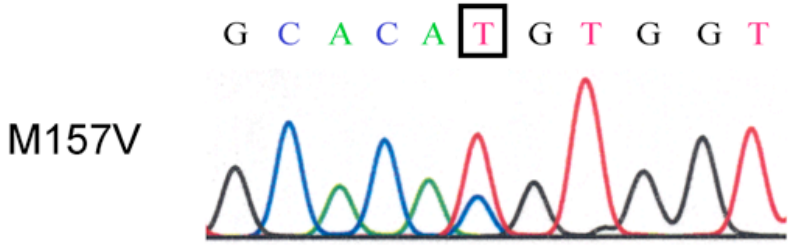
  fibrovascular membranes, hemorrhage

  other: cataract, neovascular glaucoma

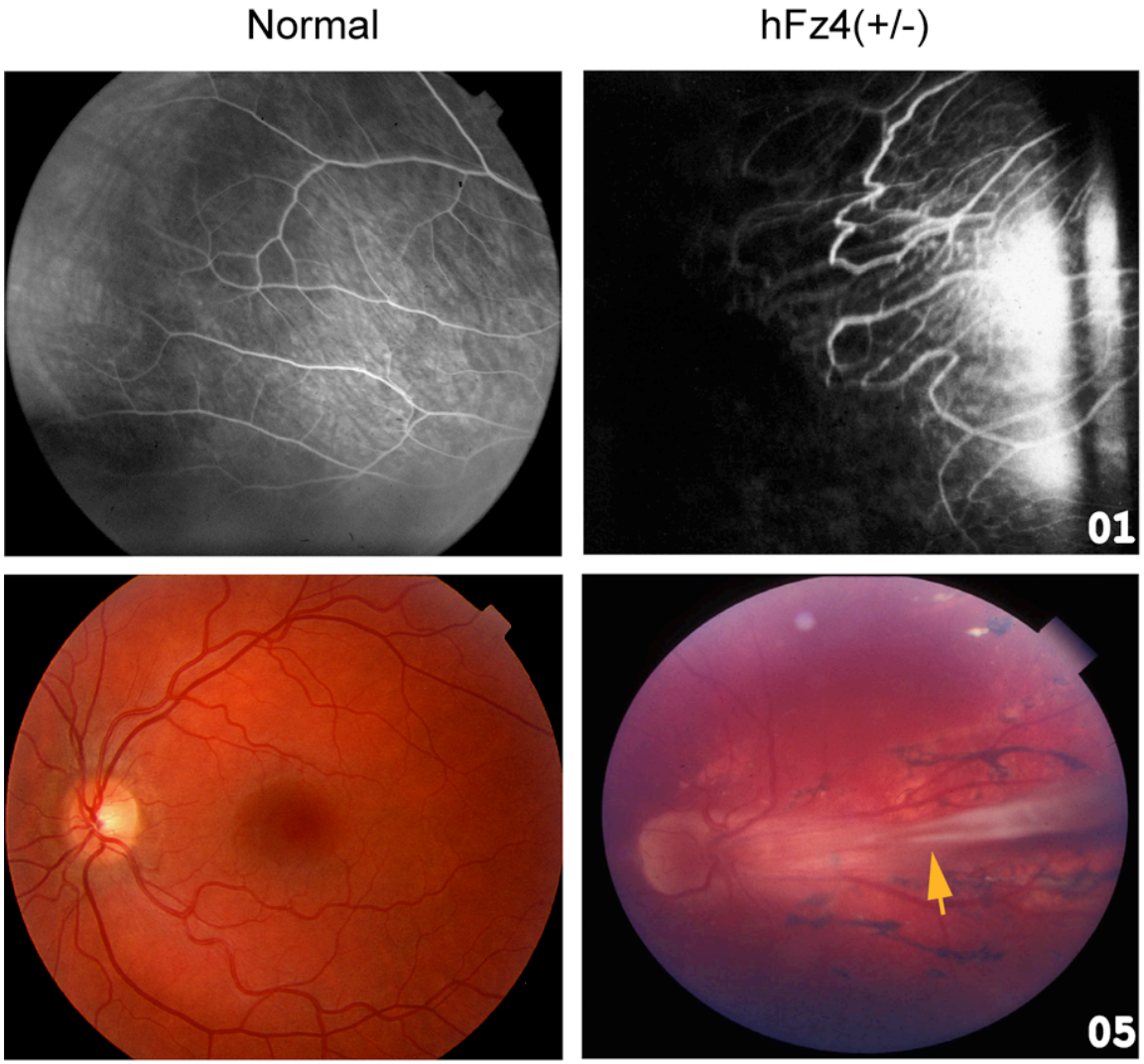
Autosomal dominant
FEVR mutations
In the cysteine-rich
domain (CRD) of Fz4



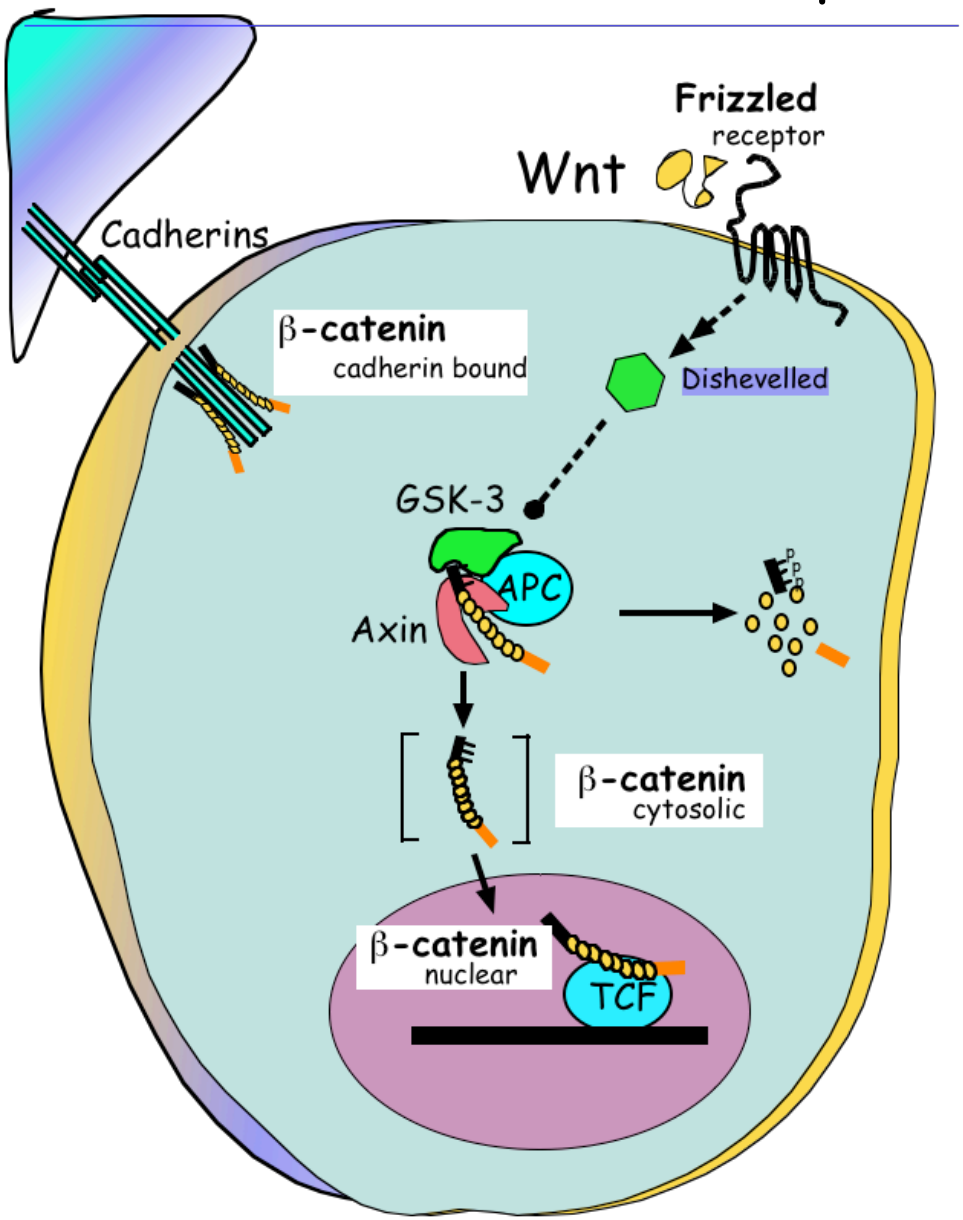




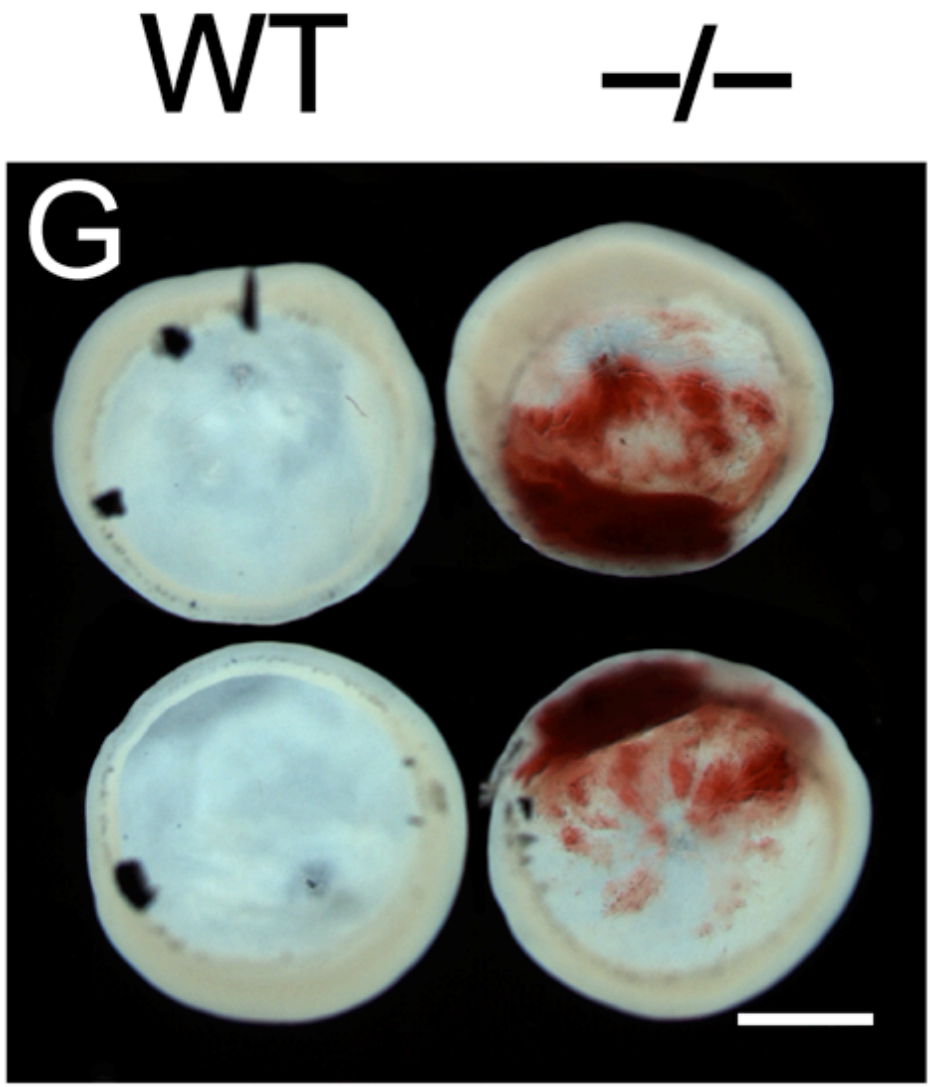
Retinal defects in FEVR patients heterozygous for Fz4 M157V



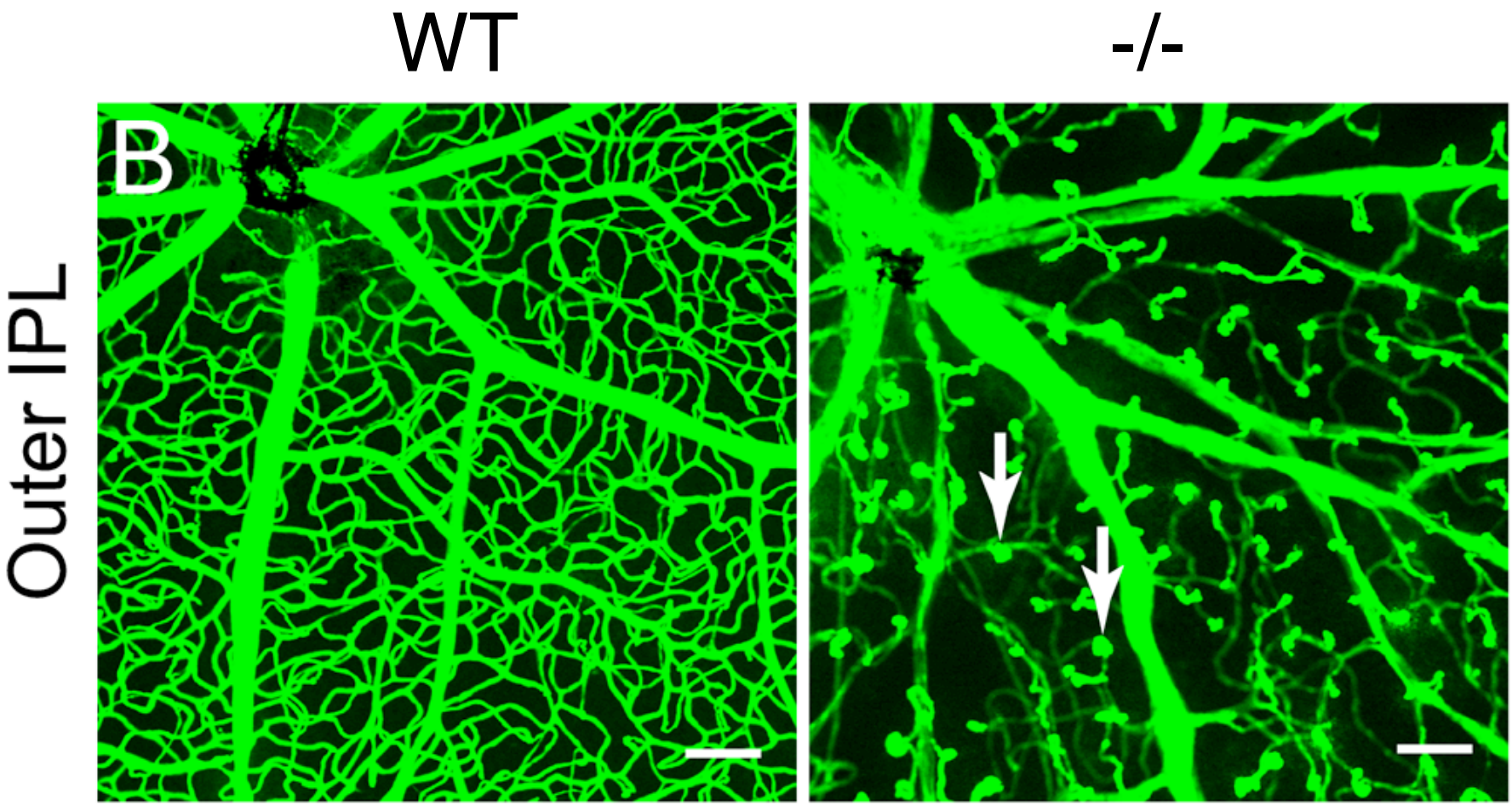
Frizzled-4 is a Wnt receptor



Intraocular hemorrhage in Fz4(-/-) mice



Absence of intra-retinal capillaries in Fz4(-/-) mice



Norrie Disease

- First described by Norrie (1927) and analyzed systematically by Mette Warburg [Acta Ophthalmologica 39: 757-772 (1961); 41: 134-146 (1963); 89: 1-147 (1966)]
- X-linked recessive with variable phenotype
- Clinical characteristics

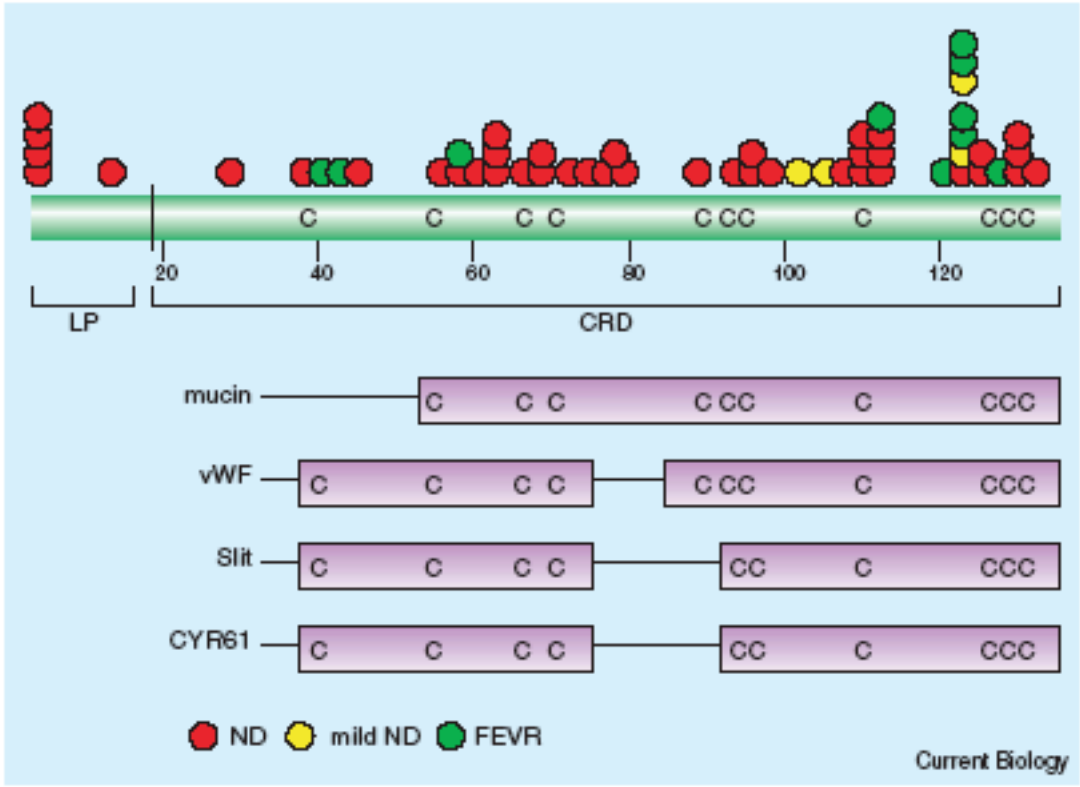
  moderate vision loss to congenital blindness

  retina: retinal folding and detachment, retinal degeneration, fibrovascualar masses, vitreoretinal hemorrhage

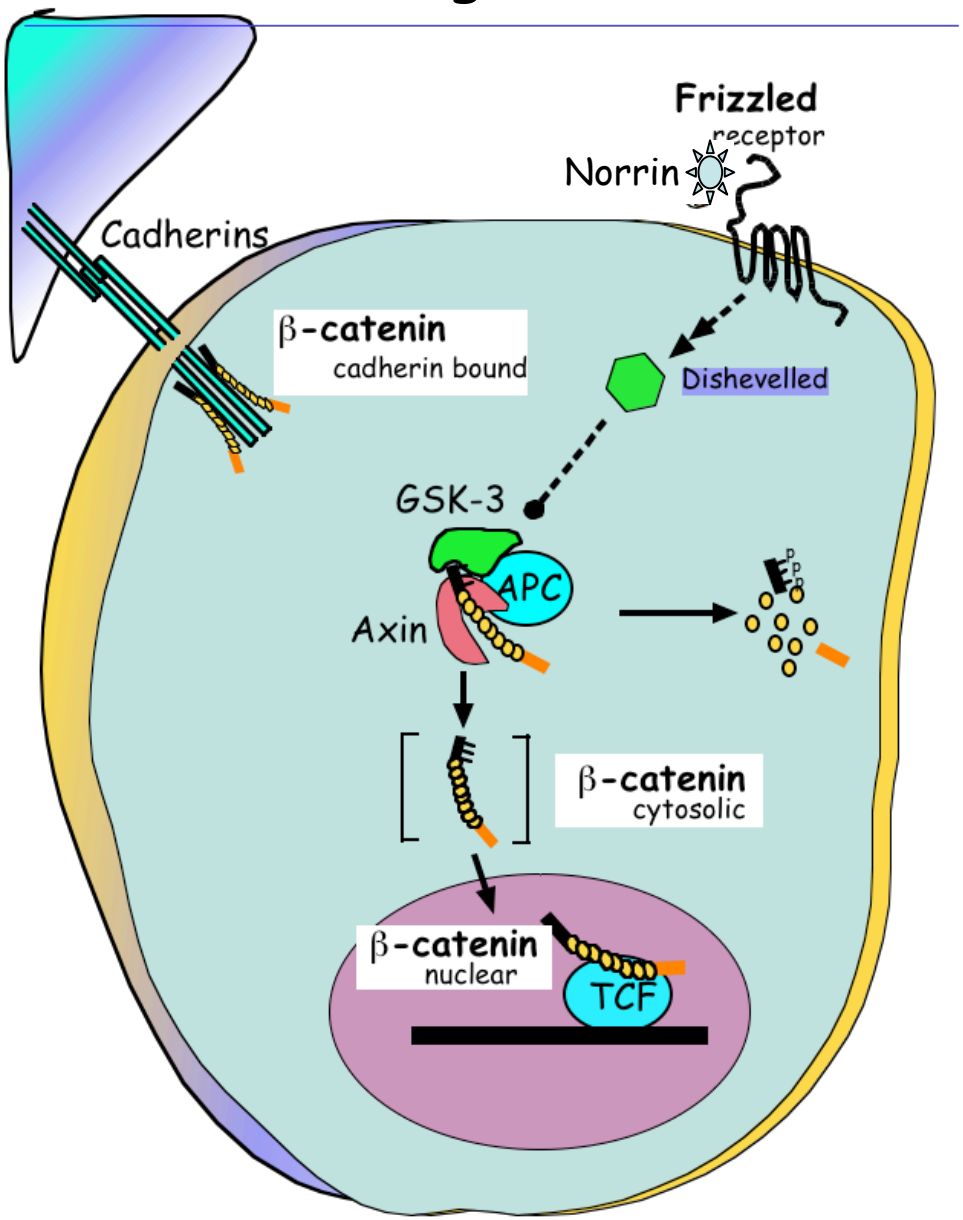
  vitreous: persistent primary vitreous

  other: progressive sensorineural deafness

Figure 1. The Norrin protein.
Missense and nonsense mutations in Norrin leading to classic or mild Norrie disease (ND) and to FEVR. Amino acid numbering is given on the scale below the protein. LP, leader peptide; CRD, cysteine-rich domain. The CRD is homologous to domains in the proteins indicated below (adapted from [1]).



Norrin is a ligand for Frizzled-4



Molecular genetics of Norrie Disease and FEVR

- FEVR

  One autosomal dominant FEVR gene identified by Robaitaille et al [Nature Genetics 32: 326-330 (2002)] encodes Frizzled4, a putative Wnt receptor. A second autosomal dominant FEVR locus encodes the Wnt co-receptor Lrp5 [Toomes et al [IOVS 45: 2083-2090 (2004)]; Jiao et al [Am J Hum Genet 75: 878-884 (2004)].

- Norrie disease

  Gene identified by Berger et al and Chen et al [Nature Genetics 1: 199-203 and 204-208 (1992)]

  The encoded protein is small (133 amino acids in length), has the same pattern of cysteines as seen in transforming growth factor beta, and begins with a signal sequence (i.e. it looks like a secreted protein). No known biochemical function.

Vessel component to human disease

- Tumor angiogenesis
- Diabetic vascular complication
  - Diabetic retinopathy
  - Stroke
  - Ischemia
  - Wound repair
- Heart disease
- Obesity
- Blindness
  - Wet Macular Degeneration
  - Retinopathy of Prematurity

Research opportunity

Summer research on tumor angiogenesis
at Eisai Pharmaceuticals, Japan
with Dr. Yasuhiro Funahashi