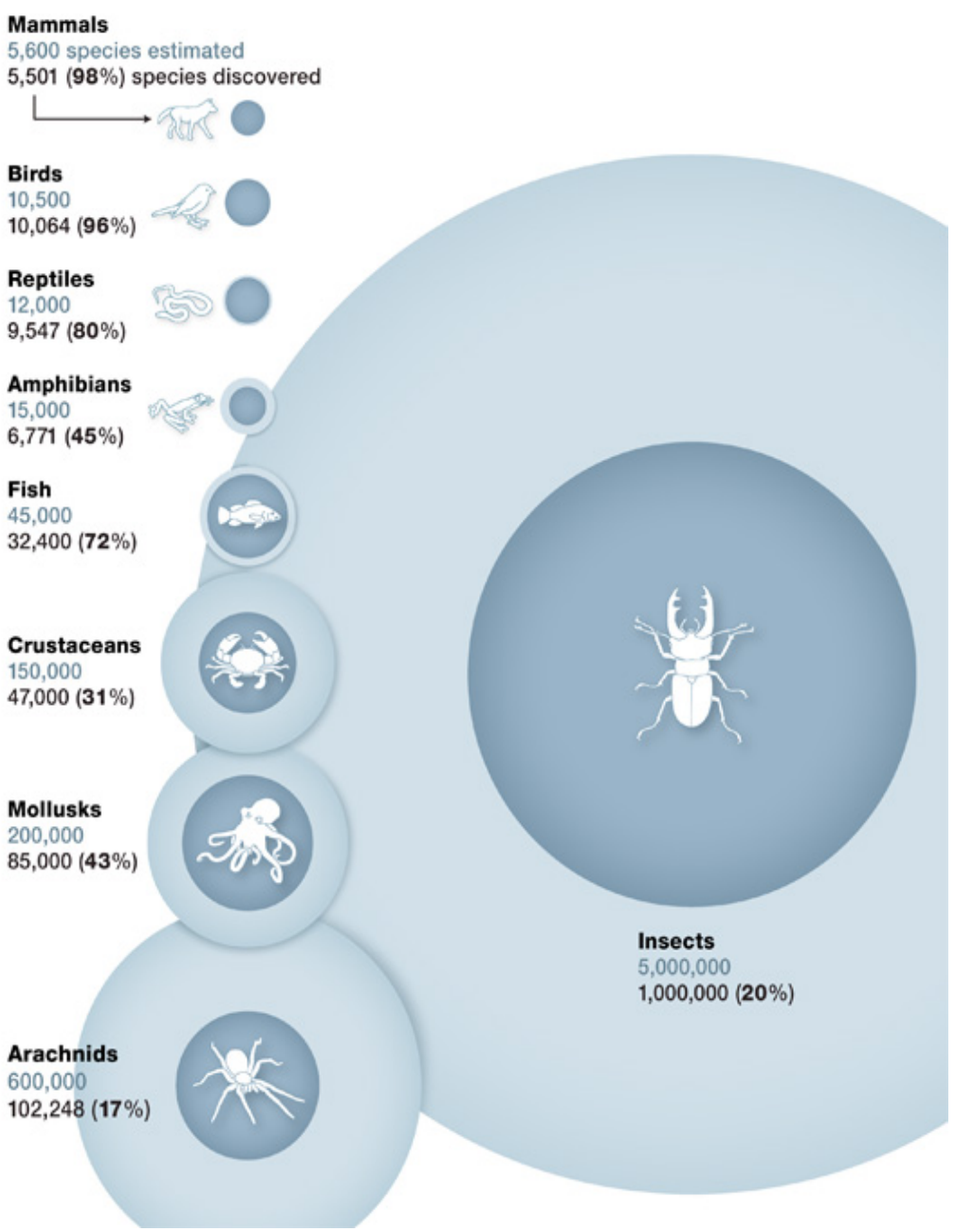
EL PODOCITO

HERNÁN TRIMARCHI

HOSPITAL BRITÁNICO DE BUENOS AIRES
ARGENTINA

2015


Peak Fitz Roy & Cerro Torre Santa Cruz Argentina


Mammals
5,600 species estimated
5,501 (98%) species discovered
Birds
10,500
10,064 (96%)
Reptiles
12,000
9,547 (80%)
Amphibians
15,000
6,771 (45%)
Fish
45,000
32,400 (72%)
Crustaceans
150,000
47,000 (31%)
Mollusks
200,000
85,000 (43%)
Arachnids
600,000
102,248 (17%)
Insects
5,000,000
1,000,000 (20%)

Pertea and Salzberg *Genome Biology* 2010, **11**:206
http://genomebiology.com/2010/11/5/206

Genome Biology

REVIEW

Between a chicken and a grape: estimating the number of human genes

Mihaela Pertea and Steven L Salzberg*

Abstract

Many people expected the question 'How many genes in the human genome?' to be resolved with the publication of the genome sequence in 2001, but estimates continue to fluctuate.

Ever since the discovery of the genetic code, scientists have been trying to catalog all the genes in the human genome. Over the years, the best estimate of the number of human genes has grown steadily smaller, but we still do not have an accurate count. Here we review the history of efforts to establish the human gene count and present the current best estimates.

The first attempt to estimate the number of genes in the human genome appeared more than 45 years ago, while the genetic code was still being deciphered. Friedrich Vogel published his 'preliminary estimate' in 1964 [1], based on the number of amino acids in the alpha- and beta-chains of hemoglobin (141 and 146, respectively). Knowing that three nucleotides corresponded to each amino acid, he extrapolated to compute the molecular weight of the DNA comprising these genes. He then made several assumptions in order to produce his estimate: that these proteins were typical in size (they are actually smaller than average); that nucleotide sequences were uninterrupted on the chromosomes (introns were discovered more than 10 years later [2,3]); and that the entire genome was protein coding. All these assumptions were reasonable at the time, but later discoveries would reveal that none of them was correct. Vogel then used the molecular weight of the human haploid chromosomes to correctly calculate the genome size as 3×10^9 nucleotides, and dividing that by the size of a 'typical' gene, came up with an estimate of 6.7 million genes.

*Correspondence: salzberg@umd.edu
Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD 20742, USA

Even at the time, Vogel found this number 'disturbingly high', but no one suspected in 1964 that most human genes were interrupted by multiple introns, nor did anyone know that vast regions of the human genome would turn out to contain seemingly meaningless repetitive sequences. Since Vogel's initial attempt, many scientists have tried to estimate the number of genes in the human genome, using increasingly sophisticated molecular tools. Over the years, the number has gradually come down, in a process that has been humbling at times, as we realized that many other species - even plants - are predicted to have more genes than we do (Figure 1). An estimate of 100,000 genes appeared in the 1990 joint National Institutes of Health (NIH)/Department of Energy (DOE) report on the Human Genome Project [4]; this was apparently based on a very rough (and incorrect) calculation that typical human genes are 30,000 bases long, and that genes cover the entire 3-gigabase genome.

Many people, including many geneticists, expected that we would have a definitive gene count when the human genome was finally completed, and indeed one of the main surprises upon the initial publication of the human genome in February 2001 [5,6] was that the number had again dropped, quite precipitously. However, as we shall see, the publication of the human genome did not come anywhere close to producing a precise gene list or even a gene count, and in the years since the number has continued to fluctuate. As a result, even today's best estimates still have a large amount of uncertainty associated with them.

In order to count genes, we need to define what we mean by a 'gene', a term whose meaning has changed dramatically over the past century. For our discussion, we will restrict the definition of gene to a region of the genome that is transcribed into messenger RNA and translated into one or more proteins. When multiple proteins are translated from the same region due to alternative mRNA splicing, we will consider this collection of alternative isoforms to be a single gene. In this respect, our definition of a gene is equivalent to what may also be called a chromosomal locus. We will exclude non-protein-coding RNA genes (such as microRNAs (miRNAs) and small nuclear RNAs (snRNAs)), in part

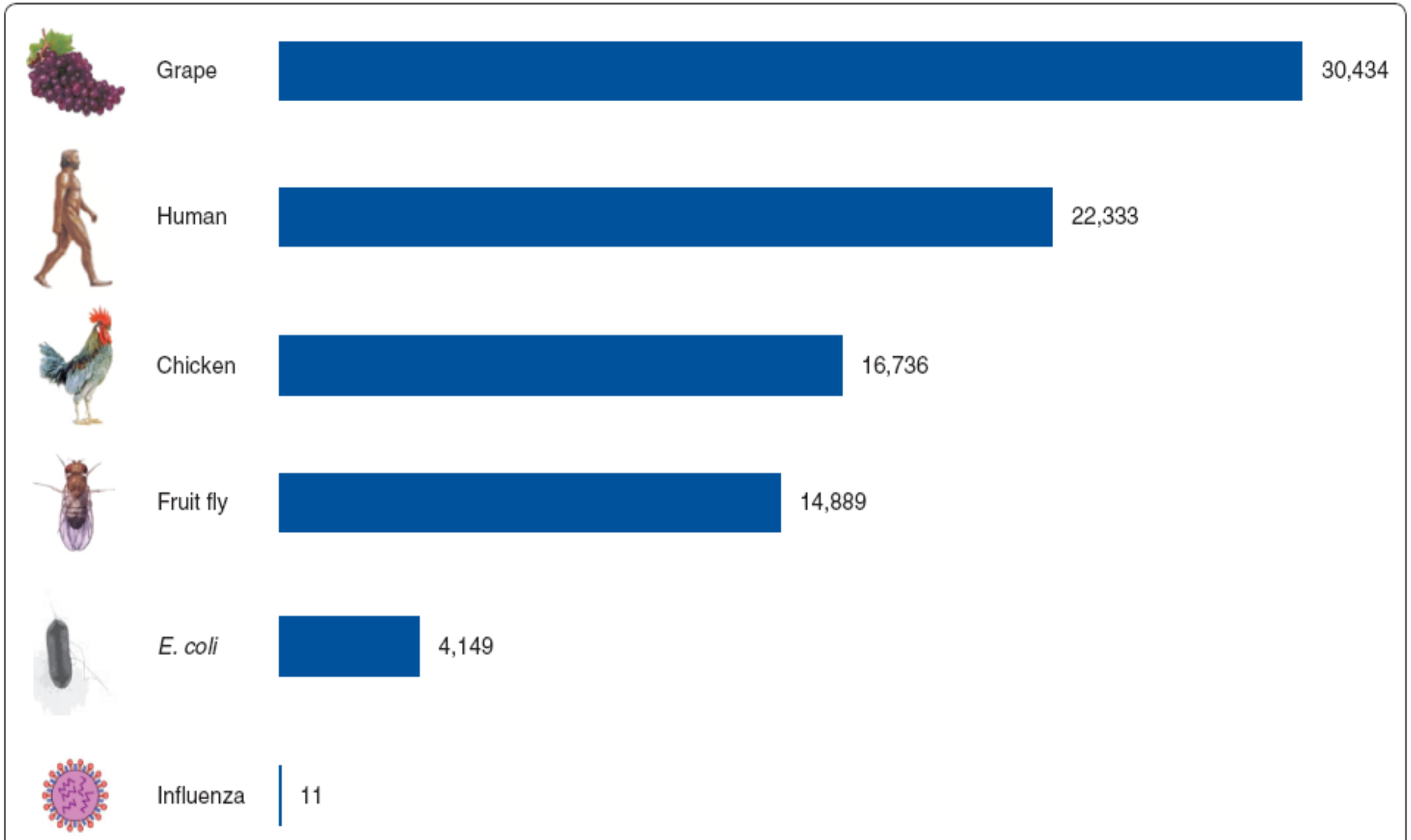
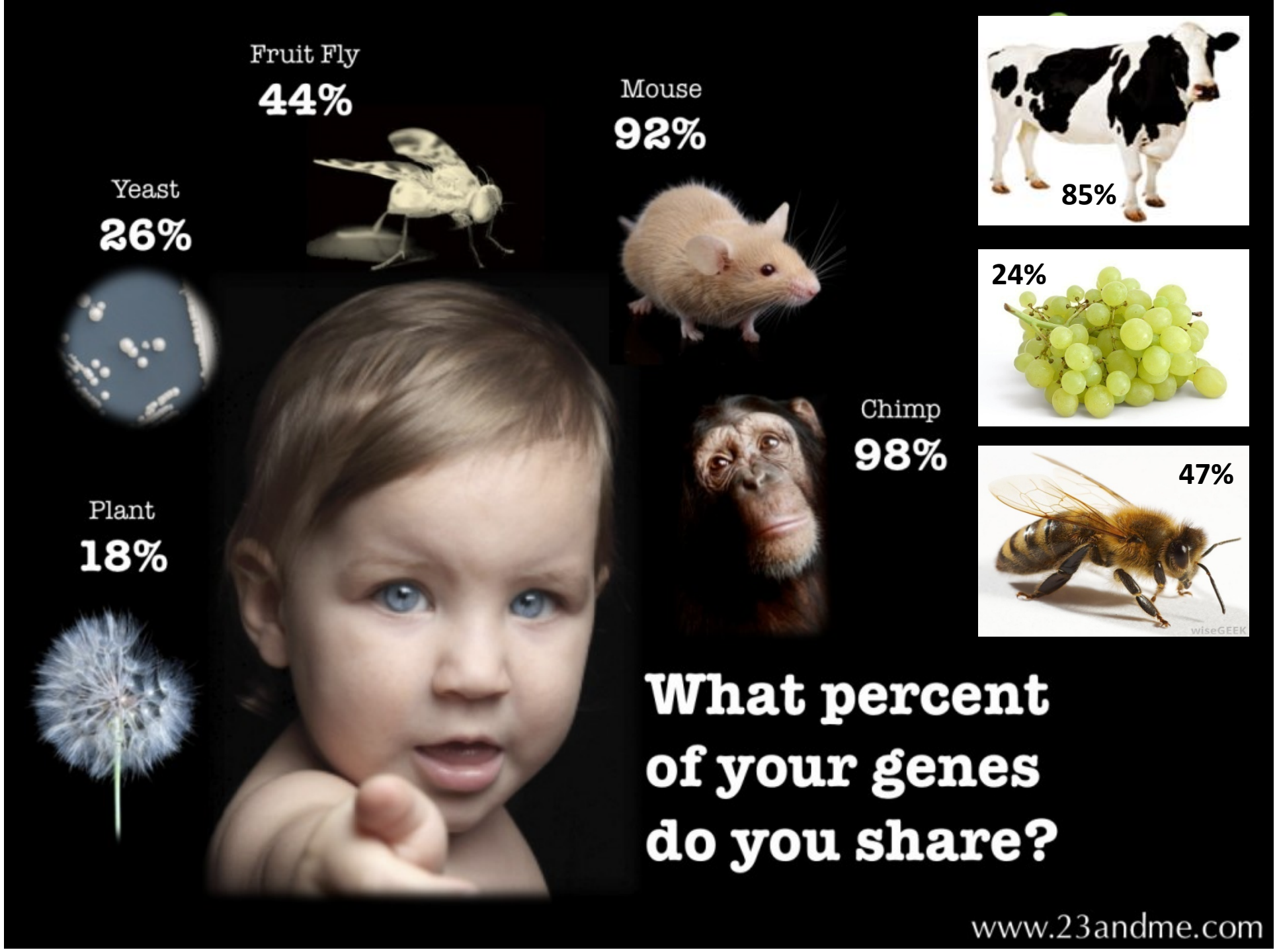
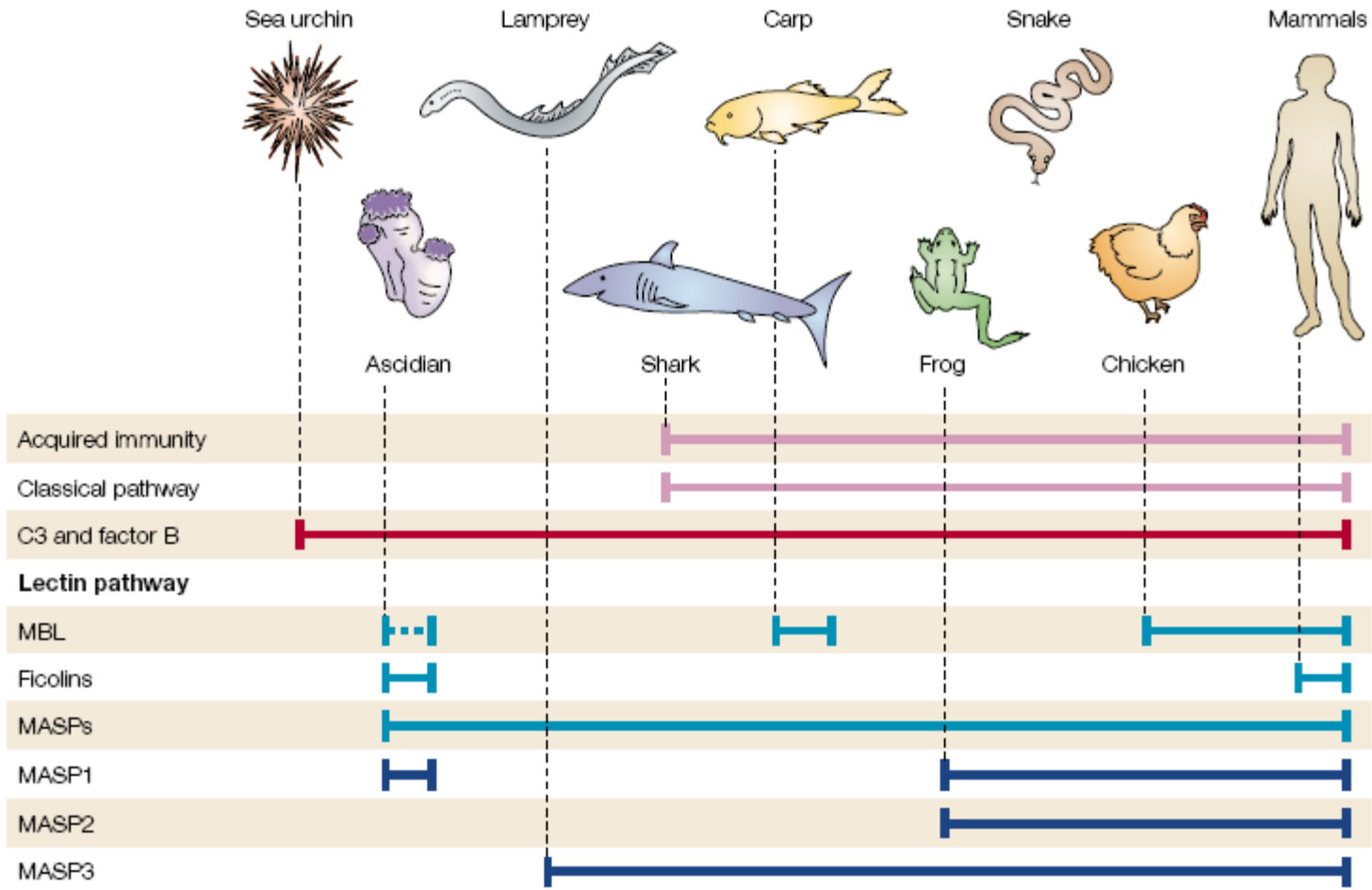
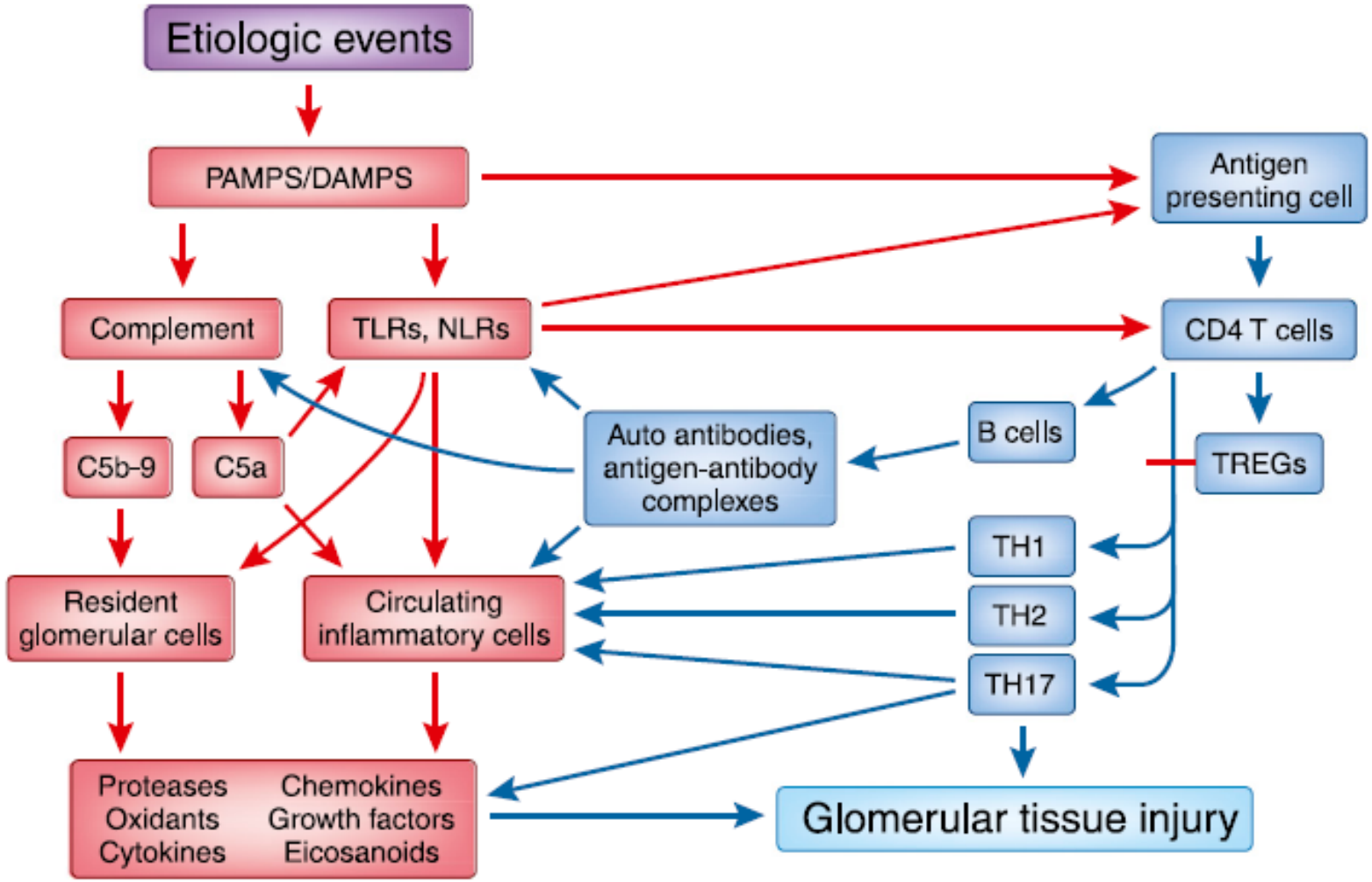
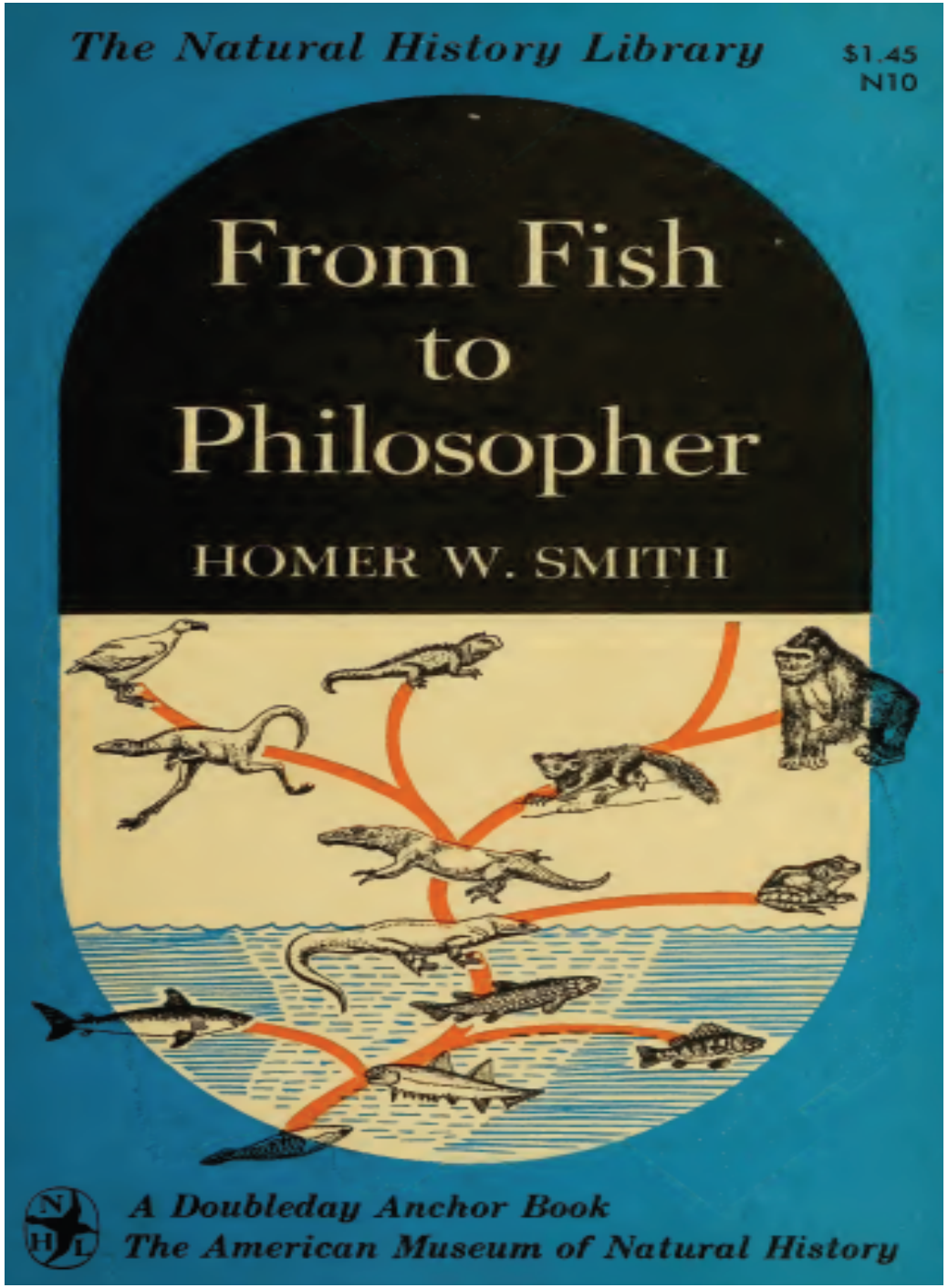


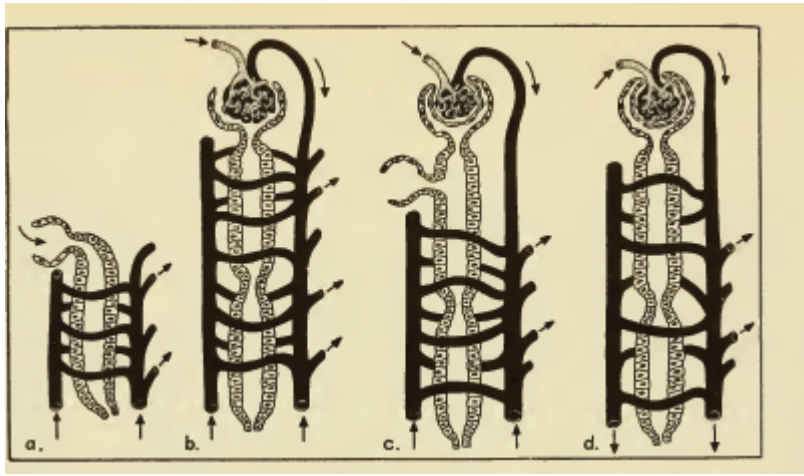
Figure 1. Gene counts in a variety of species. Viruses, the simplest living entities, have only a handful of genes but are exquisitely well adapted to their environments. Bacteria such as *Escherichia coli* have a few thousand genes, and multicellular plants and animals have two to ten times more. Beyond these simple divisions, the number of genes in a species bears little relation to its size or to intuitive measures of complexity. The chicken and grape gene counts shown here are based on draft genomes [50,51] and may be revised substantially in the future.


Fruit Fly
44%
Mouse
92%
Yeast
26%
Plant
18%
Chimp
98%
85%
24%
47%
wiseGEEK
What percent of your genes do you share?
www.23andme.com


Sea urchin
Lamprey
Carp
Snake
Mammals
Ascidian
Shark
Frog
Chicken
Acquired immunity
Classical pathway
C3 and factor B
Lectin pathway
MBL
Ficolins
MASPs
MASP1
MASP2
MASP3


Etiologic events
PAMPS/DAMPS
Antigen presenting cell
Complement
TLRs, NLRs
CD4 T cells
C5b-9
C5a
Auto antibodies, antigen-antibody complexes
B cells
TREGs
TH1
TH2
TH17
Resident glomerular cells
Circulating inflammatory cells
Proteases
Oxidants
Cytokines
Chemokines
Growth factors
Eicosanoids
Glomerular tissue injury


The Natural History Library
$1.45
N10
From Fish to Philosopher
HOMER W. SMITH
A Doubleday Anchor Book
The American Museum of Natural History



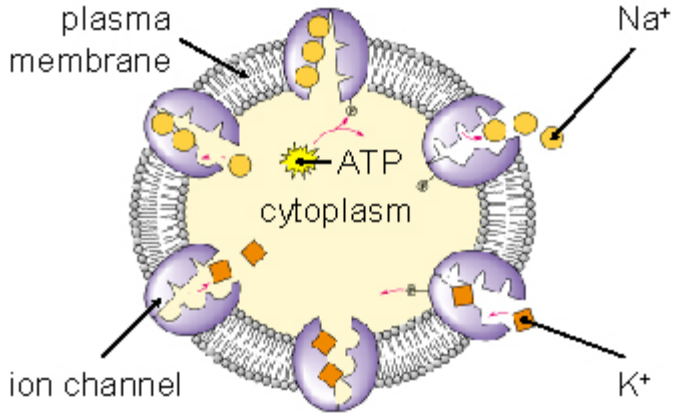


FIGURE 5. Four Stages in the Evolution of the Vertebrate Nephron—*(a) In the protovertebrate the renal tubule drained the coelom or body cavity by means of an open mouth or coelomostome. (b) The glomerulus was evolved in the earliest vertebrates as a device to excrete water, and was at first only loosely related to the coelomostome. (c) Later the glomerulus became sealed within the end of the tubule, the coelomostome persisting in some species. (d) In the higher vertebrates, the coelomostome has disappeared entirely, leaving the typical vertebrate nephron. The primitive blood supply to the protovertebrate tubule persists as the "renal-portal system" in the fishes, Amphibia, reptiles and birds (a to c), but disappears in the mammals (d), leaving the tubules supplied only by post-glomerular blood.*

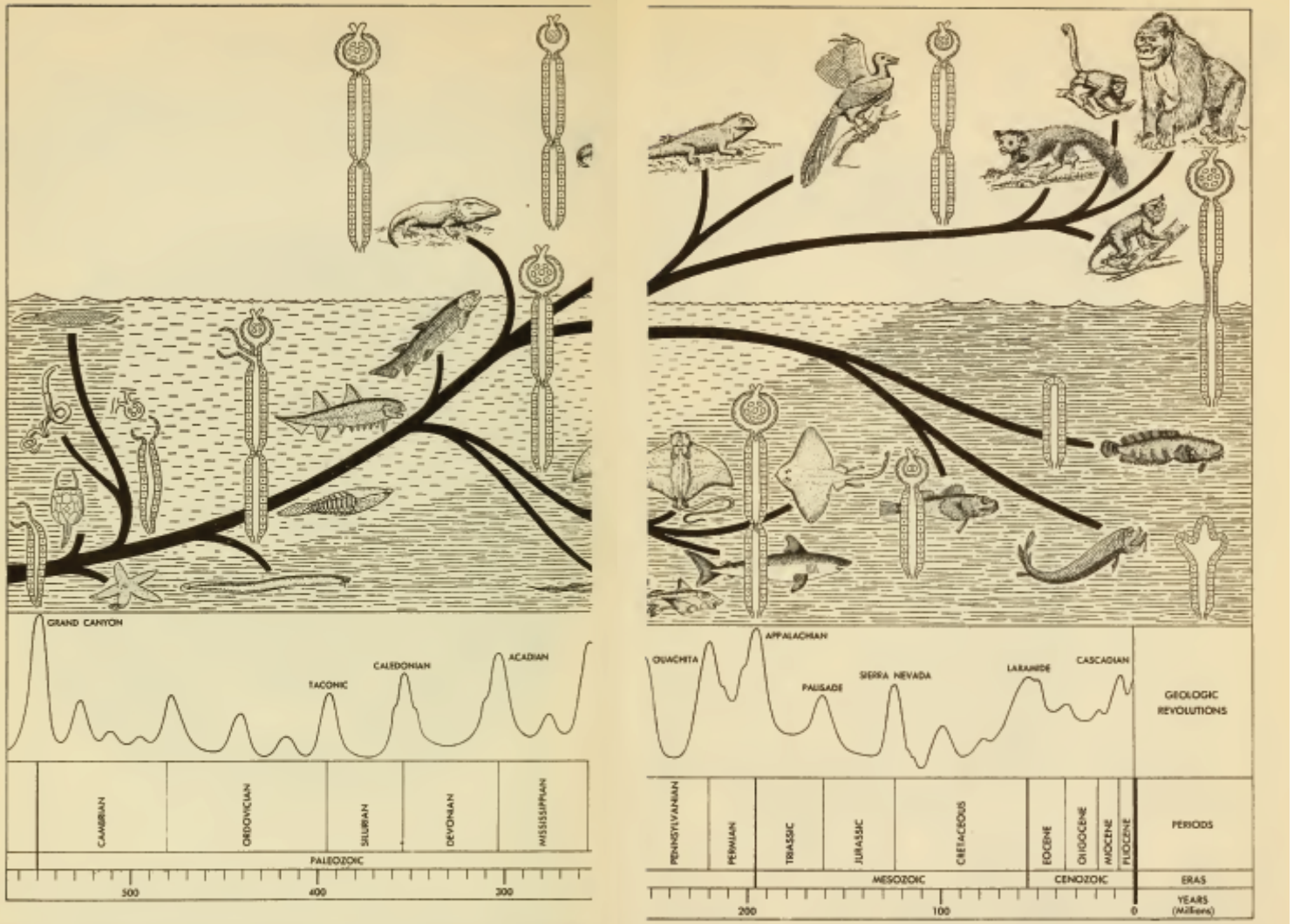
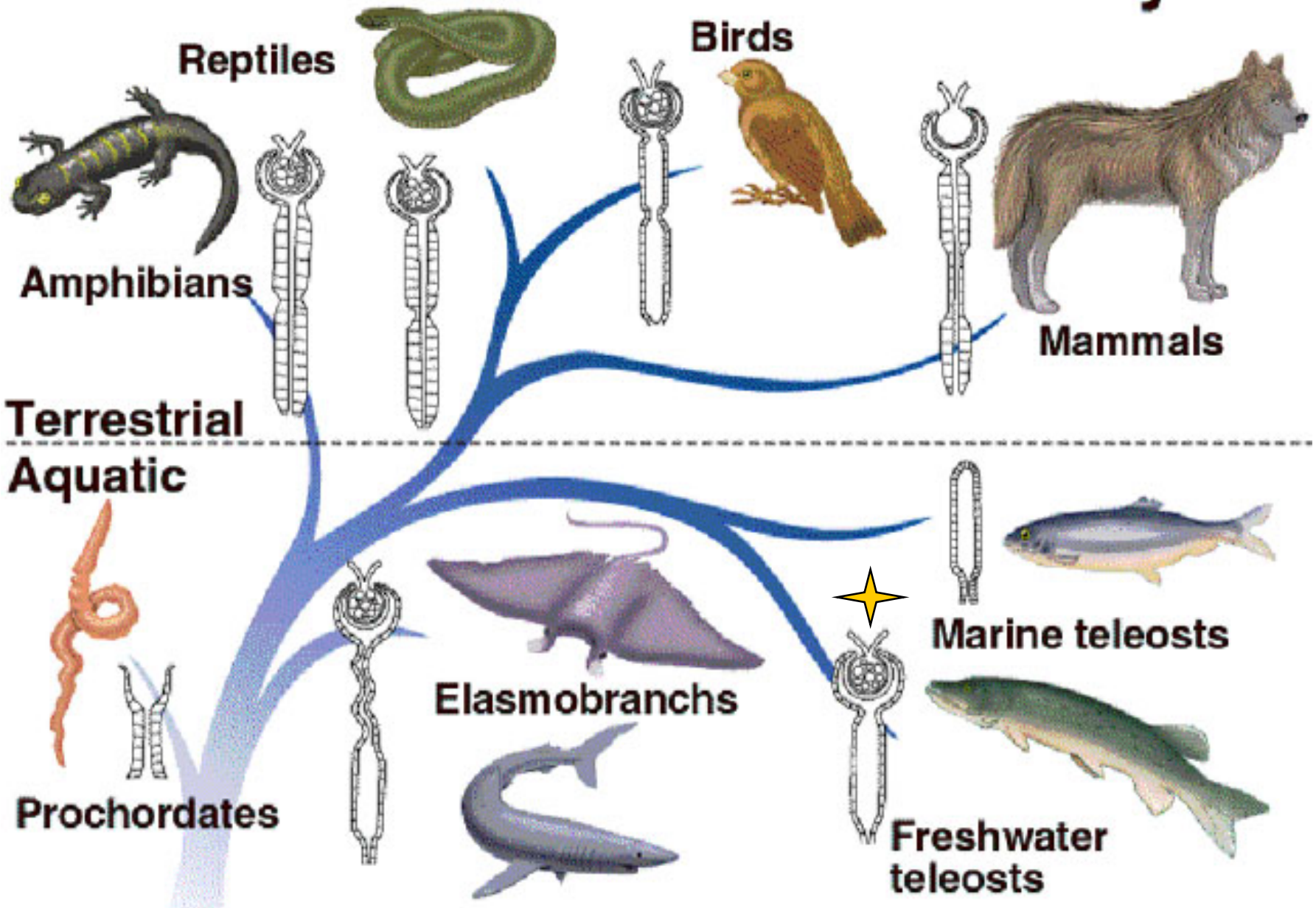


FIGURE 2. Synopsis of the Evolution of Vertebrates—*showing the evolution of the vertebrates in relation to a salt-water (darkly shaded) and fresh-water (lightly shaded) habitat. The irregular curve illustrates mountain-building episodes (geologic revolutions) which have importantly influenced this evolutionary history.*

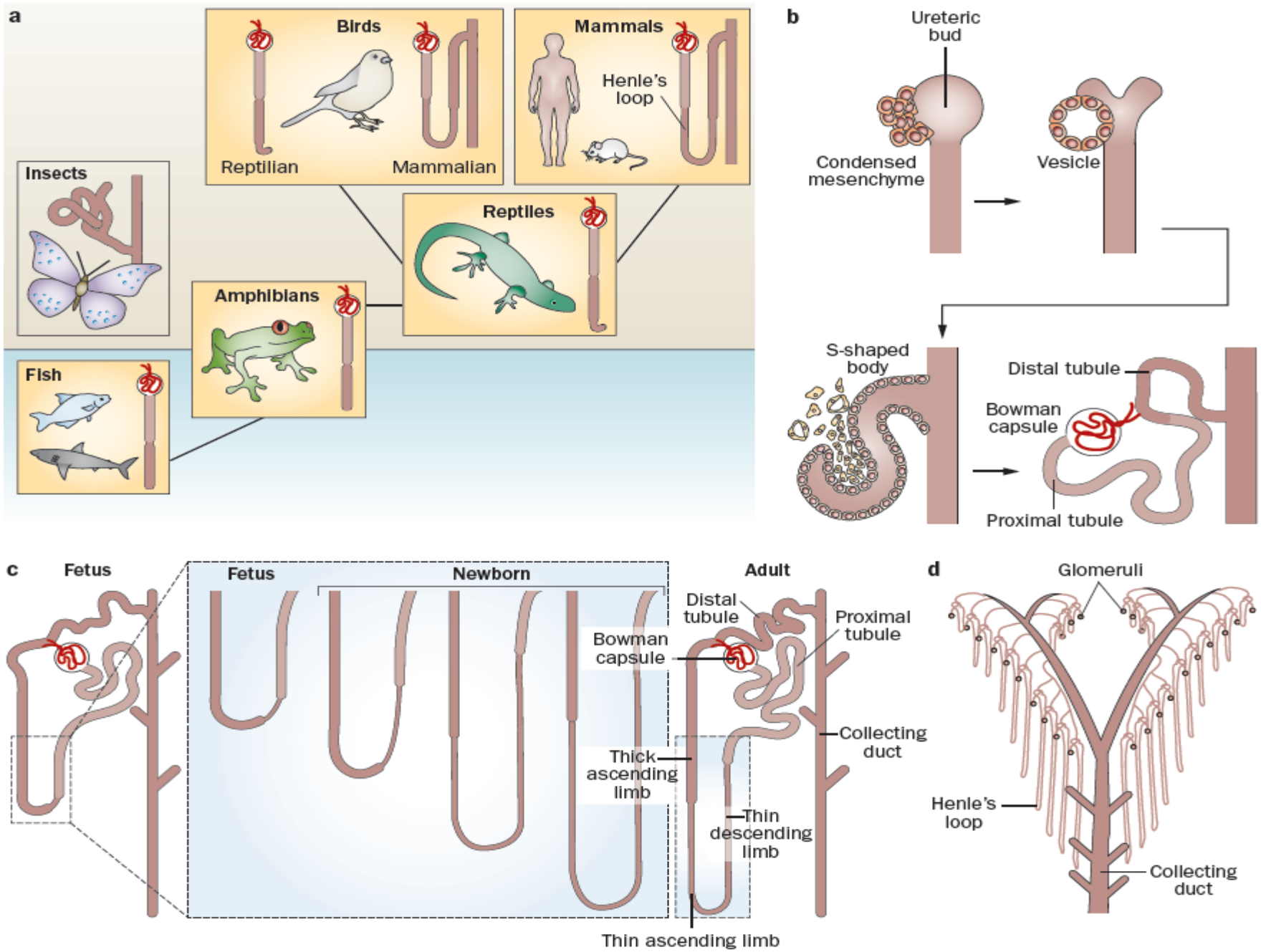
The time scale is such that the Pleistocene era (one million years in length) and Recent Time (about twenty-five thousand years) could not be included, and these are merely suggested by the heavy line at zero time.

The entire period encompassed by documented history is only about six thousand years, or one hundred-thousandth of the interval elapsing since the opening of the Paleozoic era (Cambrian period), when fossilized animals first begin to appear in the sedimentary rocks.

Evolution of Vertebrate Kidneys

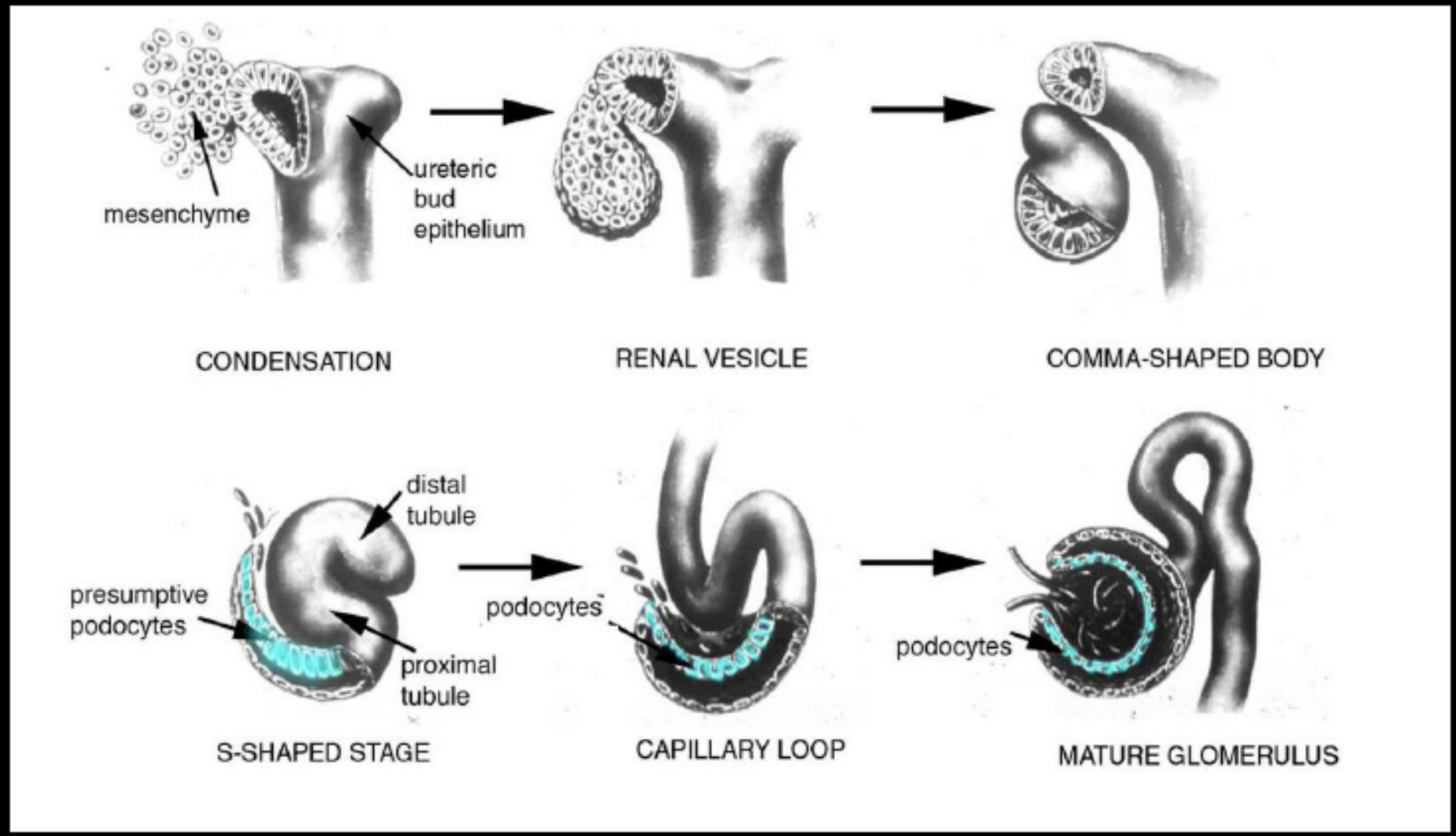


teleostos de agua dulce

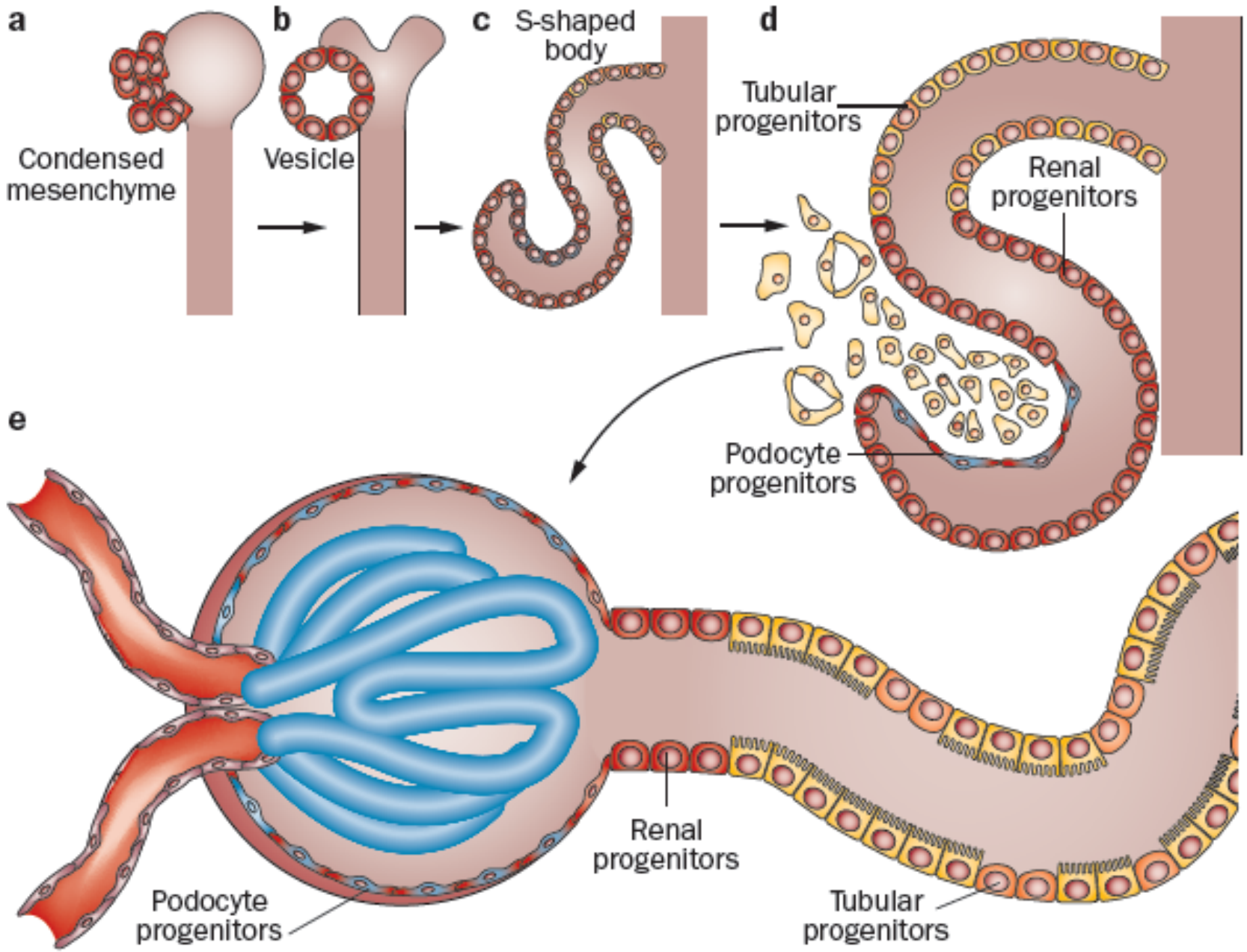


Romagnani, P. *et al. Nat. Rev. Nephrol.* **9**, 137–146 (2013)

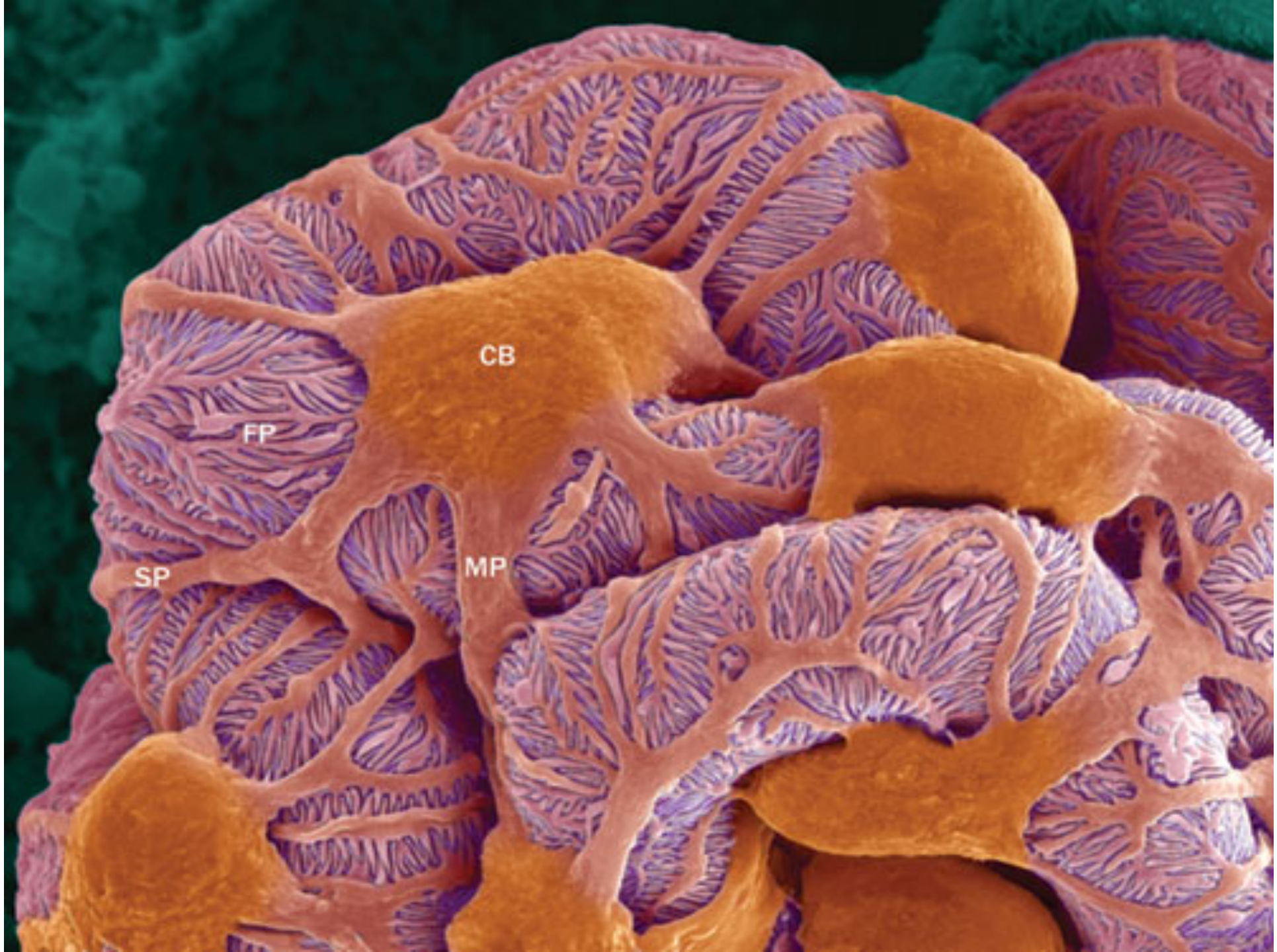
Development of the glomerular tuft



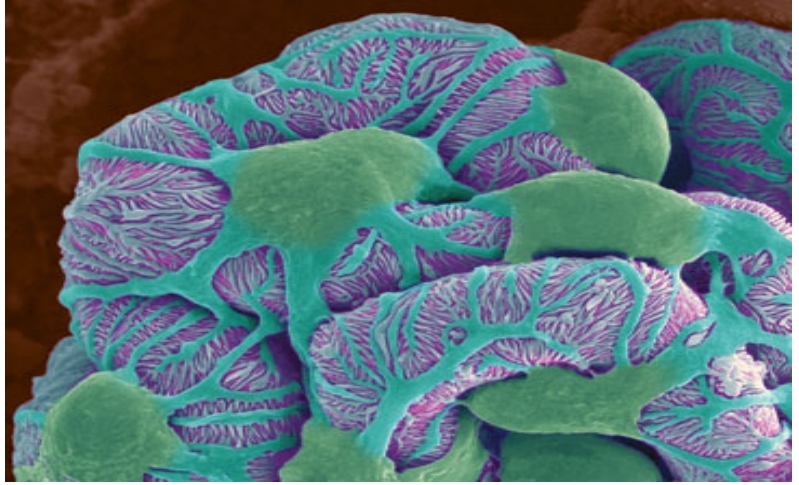
Adapted from Saxen

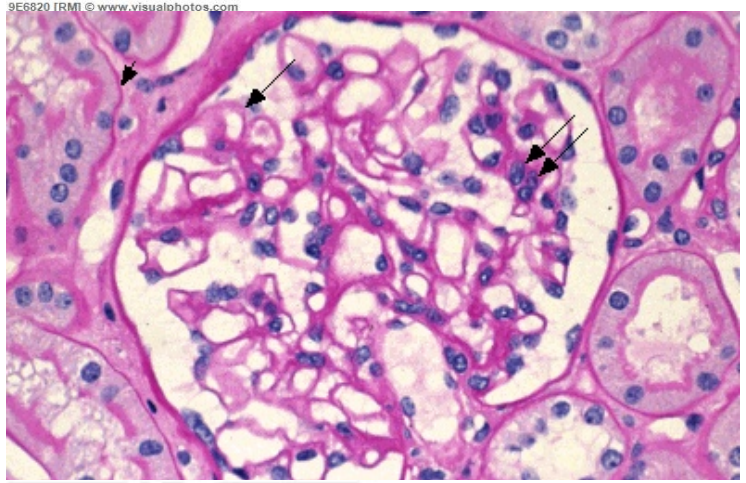


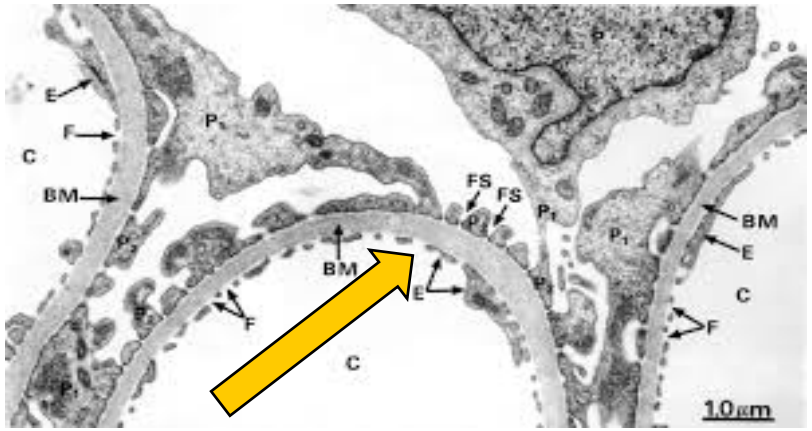
Romagnani, P. *et al. Nat. Rev. Nephrol.* **9**, 137–146 (2013)

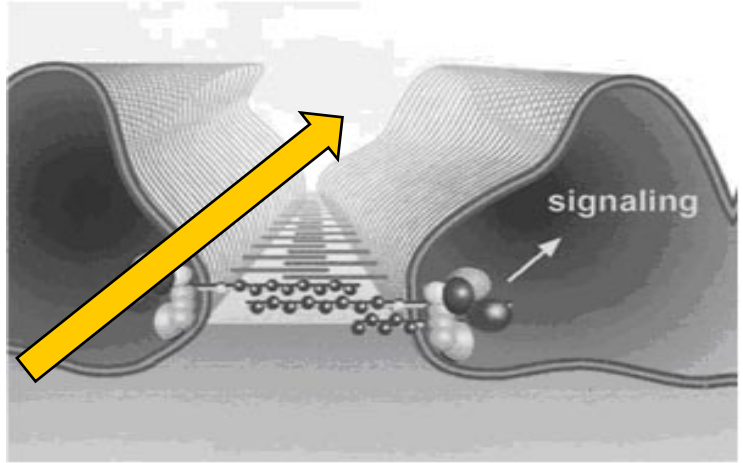

CB
FP
SP
MP

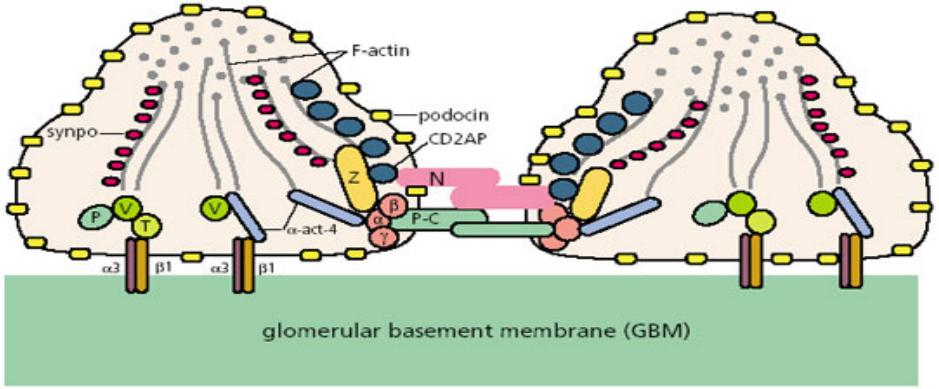

visualphotos.com

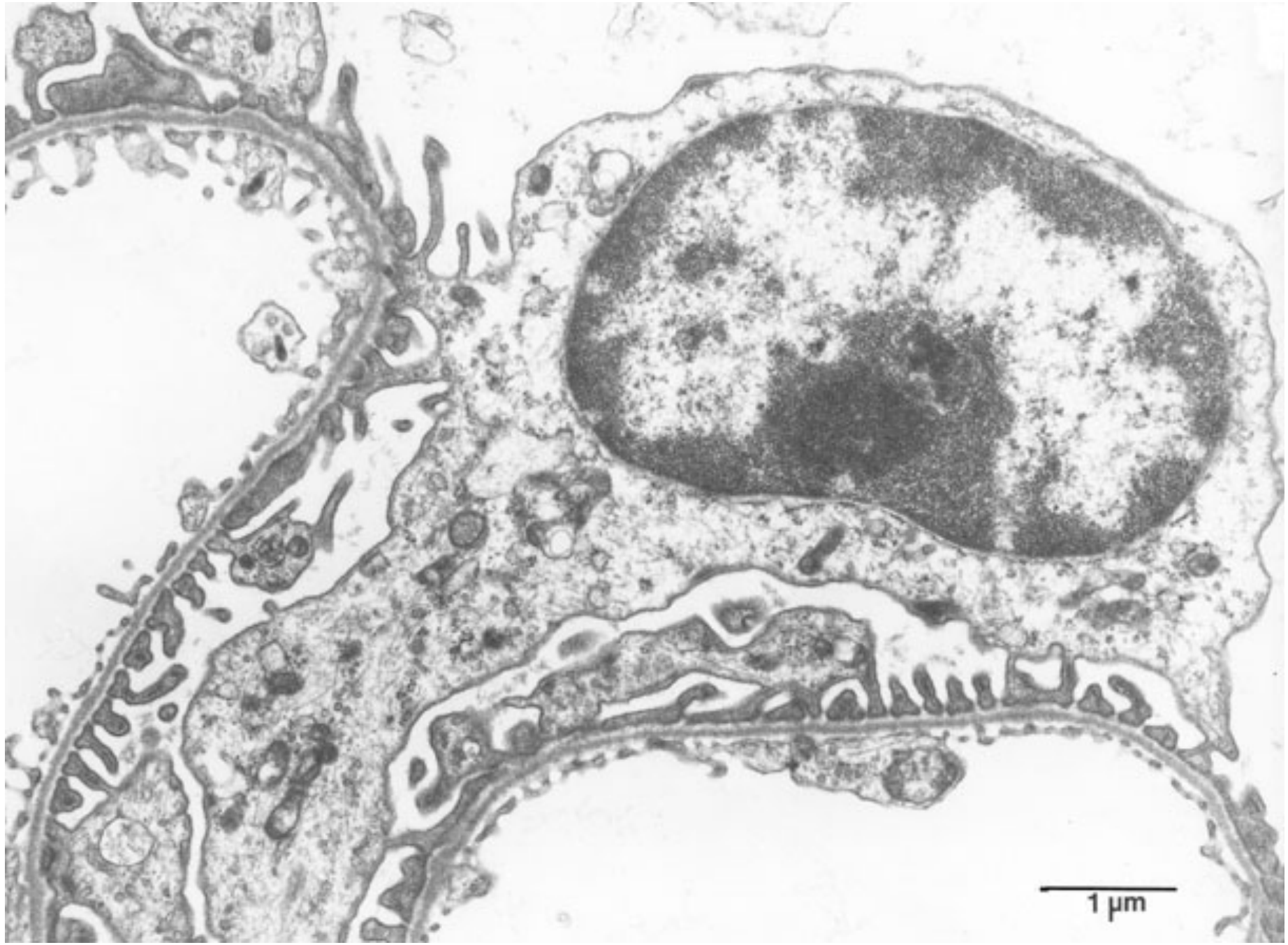


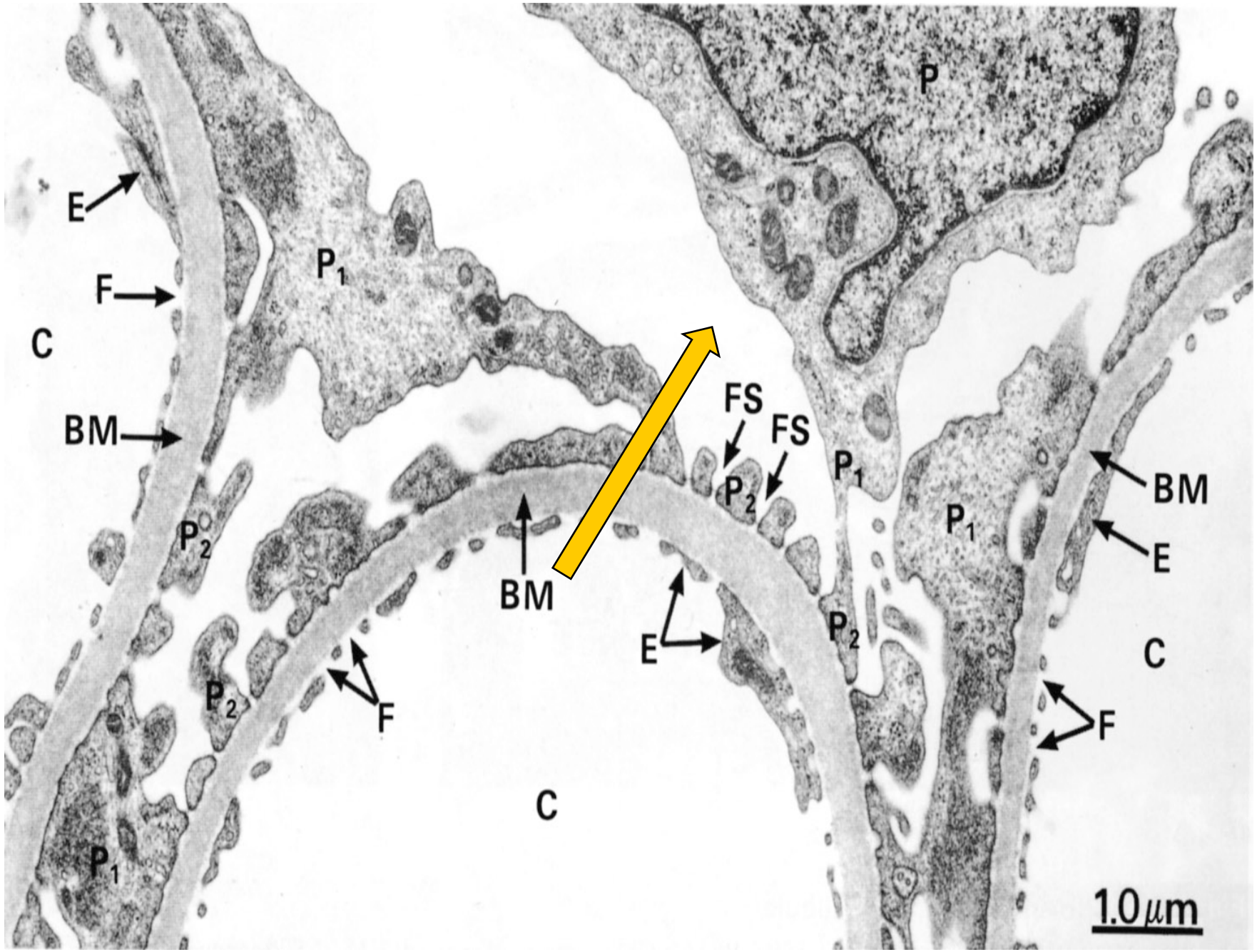

9E6820 [RM] © www.visualphotos.com


E
F
C
BM
P
FS
1.0 µm


signaling

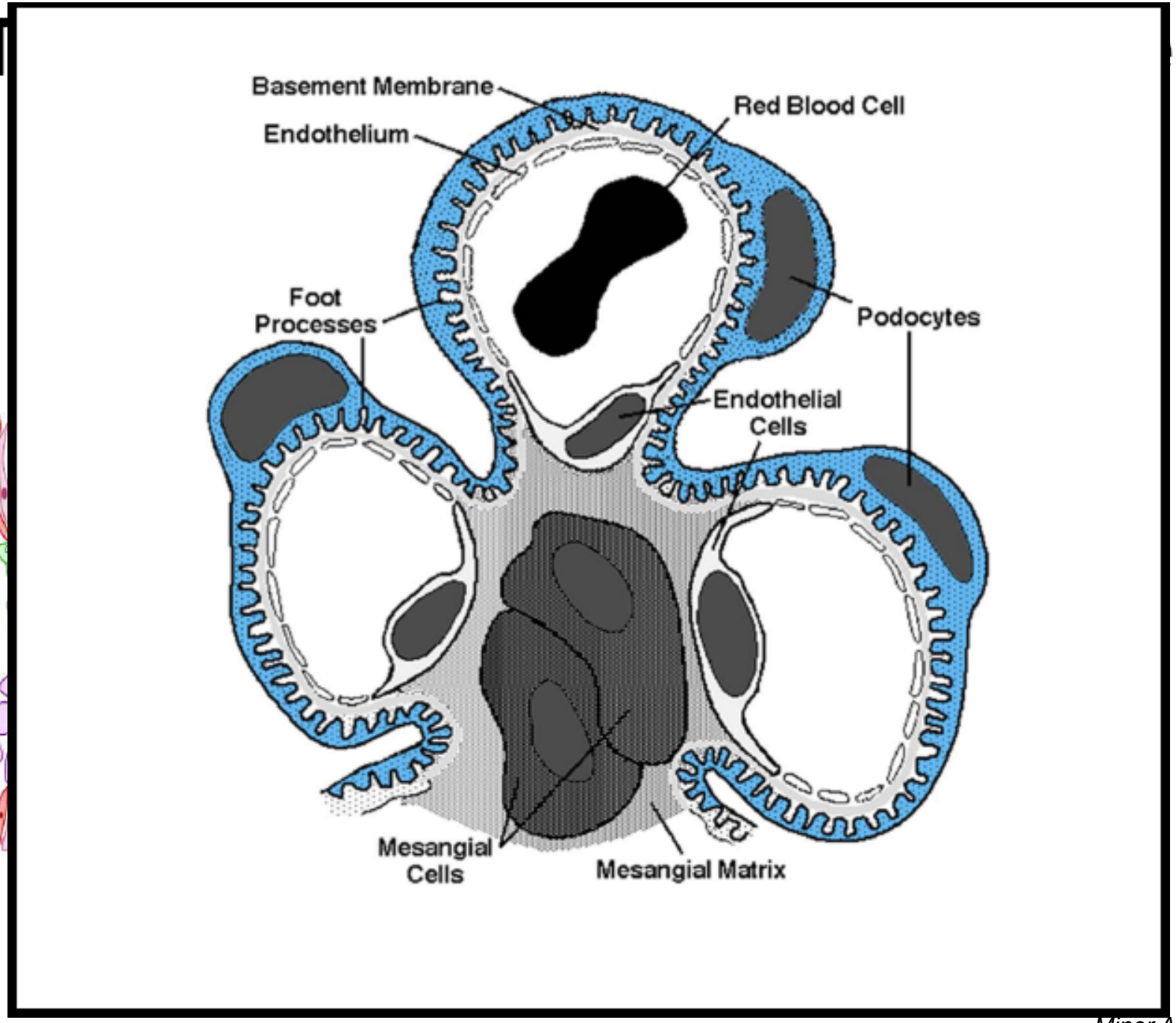

F-actin
podocin
synpo
CD2AP
Z
N
α-act-4
P-C
α3
β1
glomerular basement membrane (GBM)

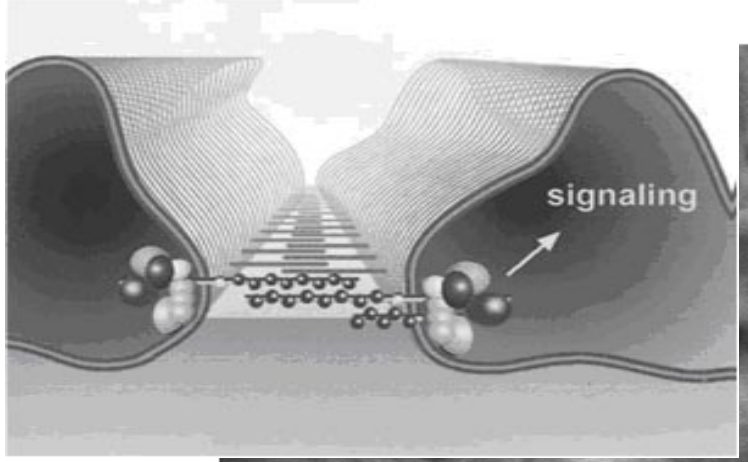

1 µm


P
E
F
C
BM
P_1
P_2
FS
FS
BM
E
F
C
P_1
P_2
BM
E
C
F
1.0 μm

T…r

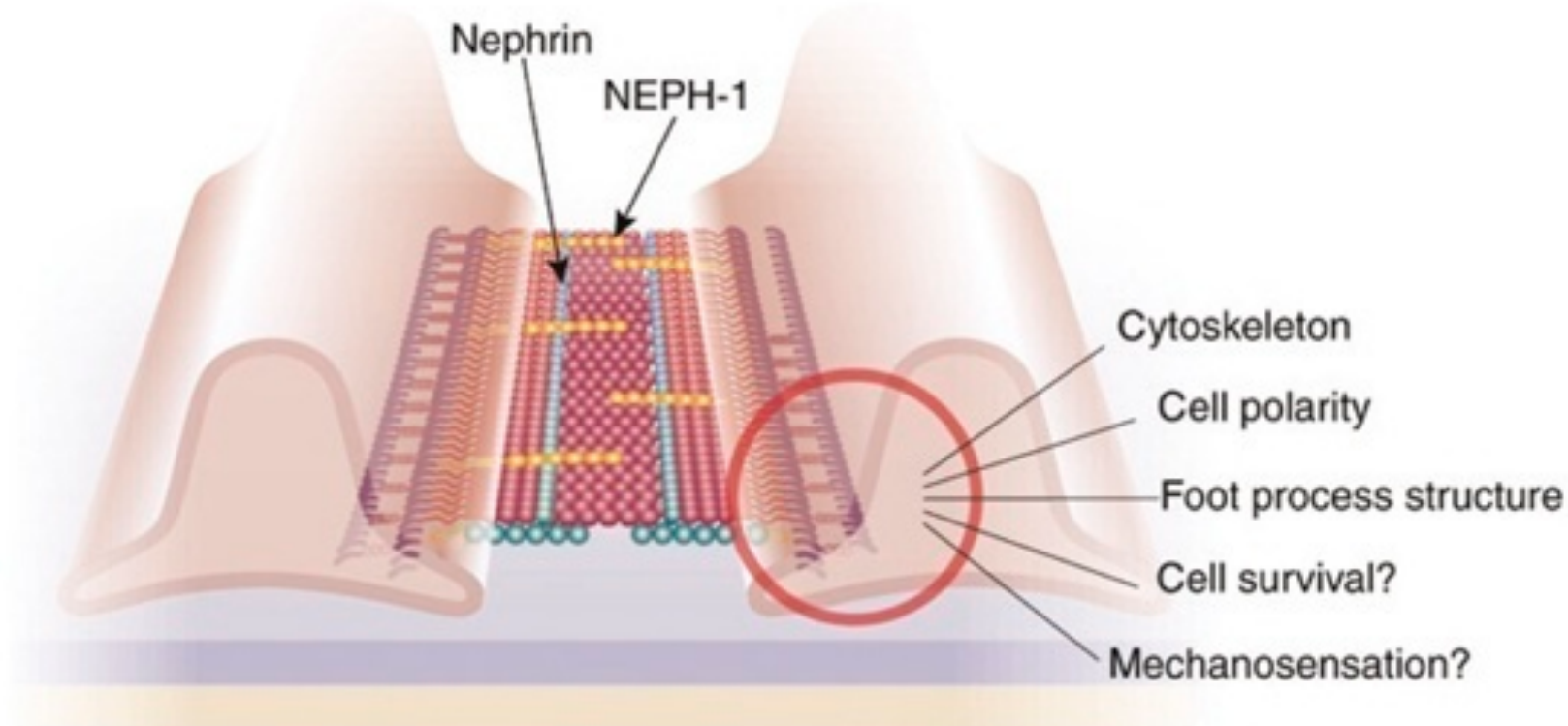
- …

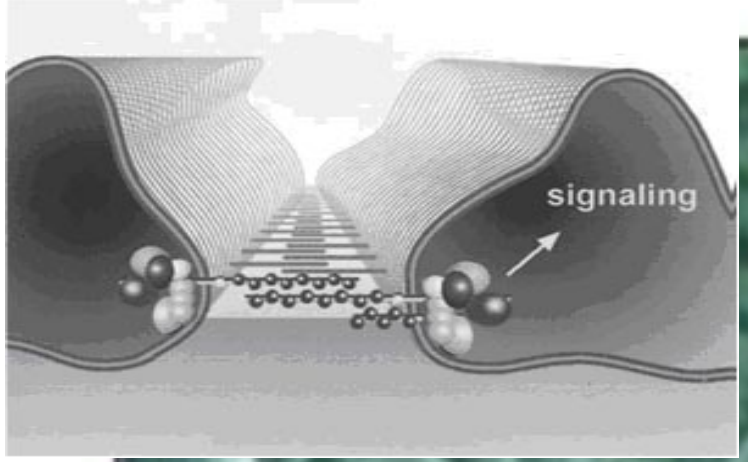

Basement Membrane
Endothelium
Red Blood Cell
Foot Processes
Podocytes
Endothelial Cells
Mesangial Cells
Mesangial Matrix

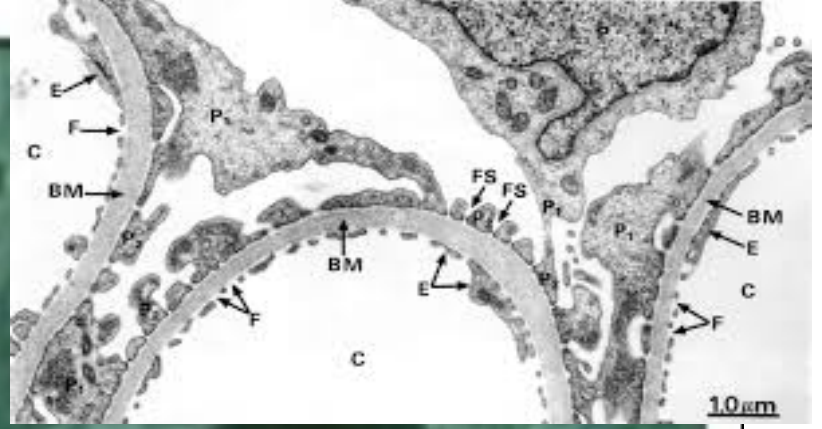


Scanning electron photomicrographs from Wistar rat, taken at high magnification (x140,000). Visualization of epithelial filtration pores by an innovative SEM technique based on a new detector, called in-lens, more sensitive than conventional one, that captures more efficiently the secondary electrons from the sample surface, due to its position closer to the sample.
This approach allowed us to observe the deepest regions of the filtration slits.

Gagliardini et al., *J Am Soc Nephrol,* 2010


Nephrin
NEPH-1
Cytoskeleton
Cell polarity
Foot process structure
Cell survival?
Mechanosensation?



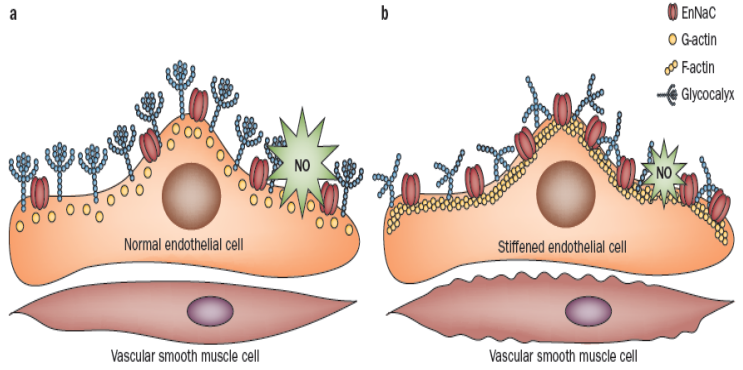


S Quaggin Aahrus 2011

The glomerular barrier – an integrative view

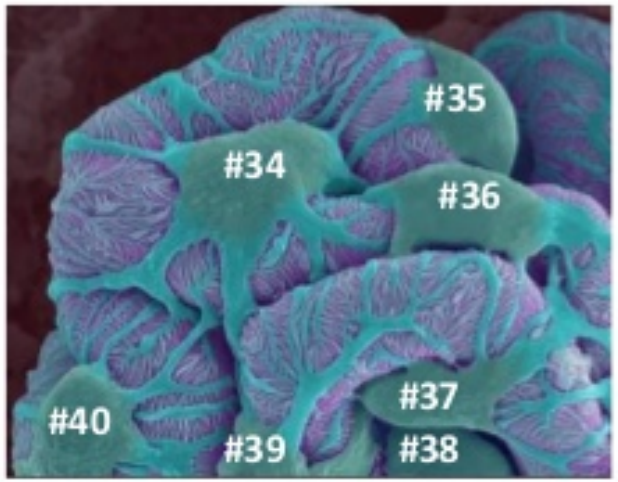
The podocytes, GBM and the endothelium

- are all important for an intact barrier
- They represent barriers in series
- There is intense communication between the podocyte, the mesangial and the endothelial cells
- The **endothelium** is of paramount importance to prevent albuminuria since it is the *'first line of defense'*



Each Podocyte Counts!

2%
Tryggvason 2011



1,200,000,000 de podocitos

Each glomerulus has 500-600 podocytes.

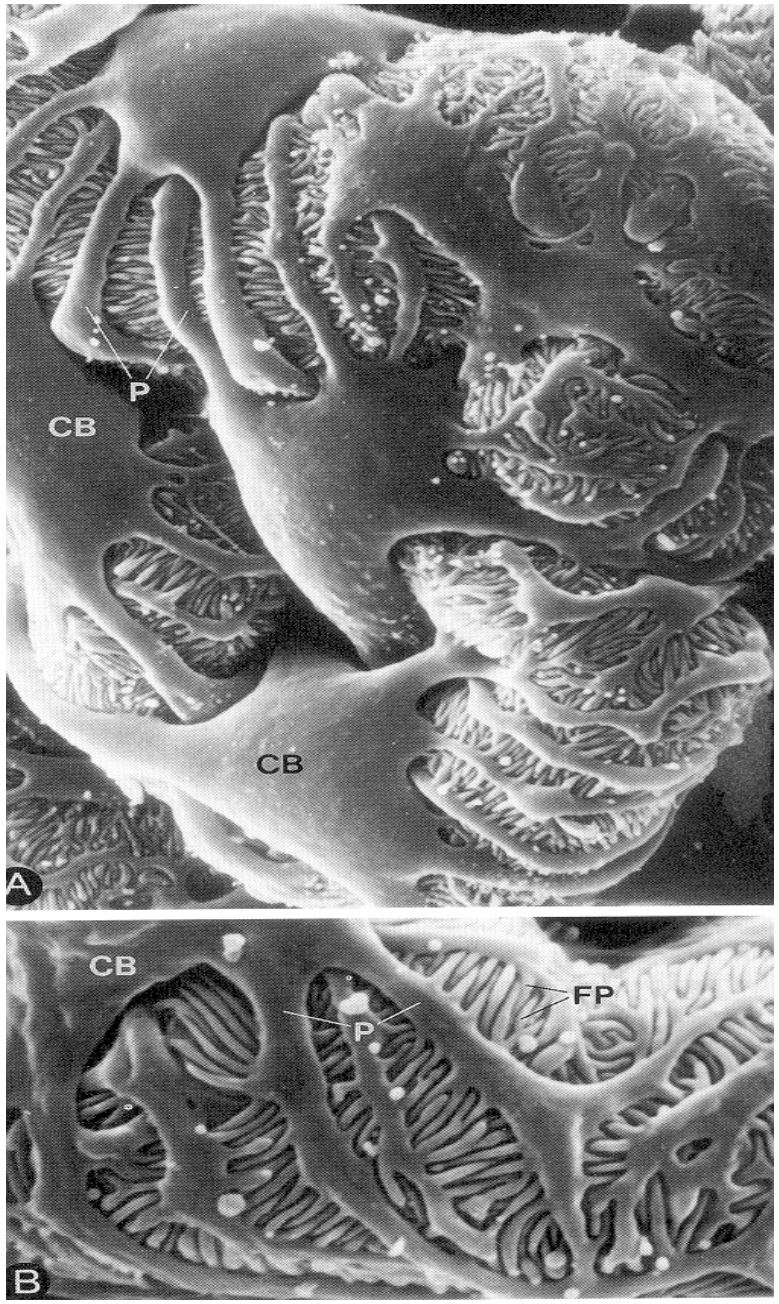
Podocytes do not efficiently proliferate.

Podocyte loss is cumulative in time

Once a glomerulus loses more than ~20% of its podocytes, it scars down. This injury is irreversible.

120 podocitos/glomérulo

200,000,000 de podocitos



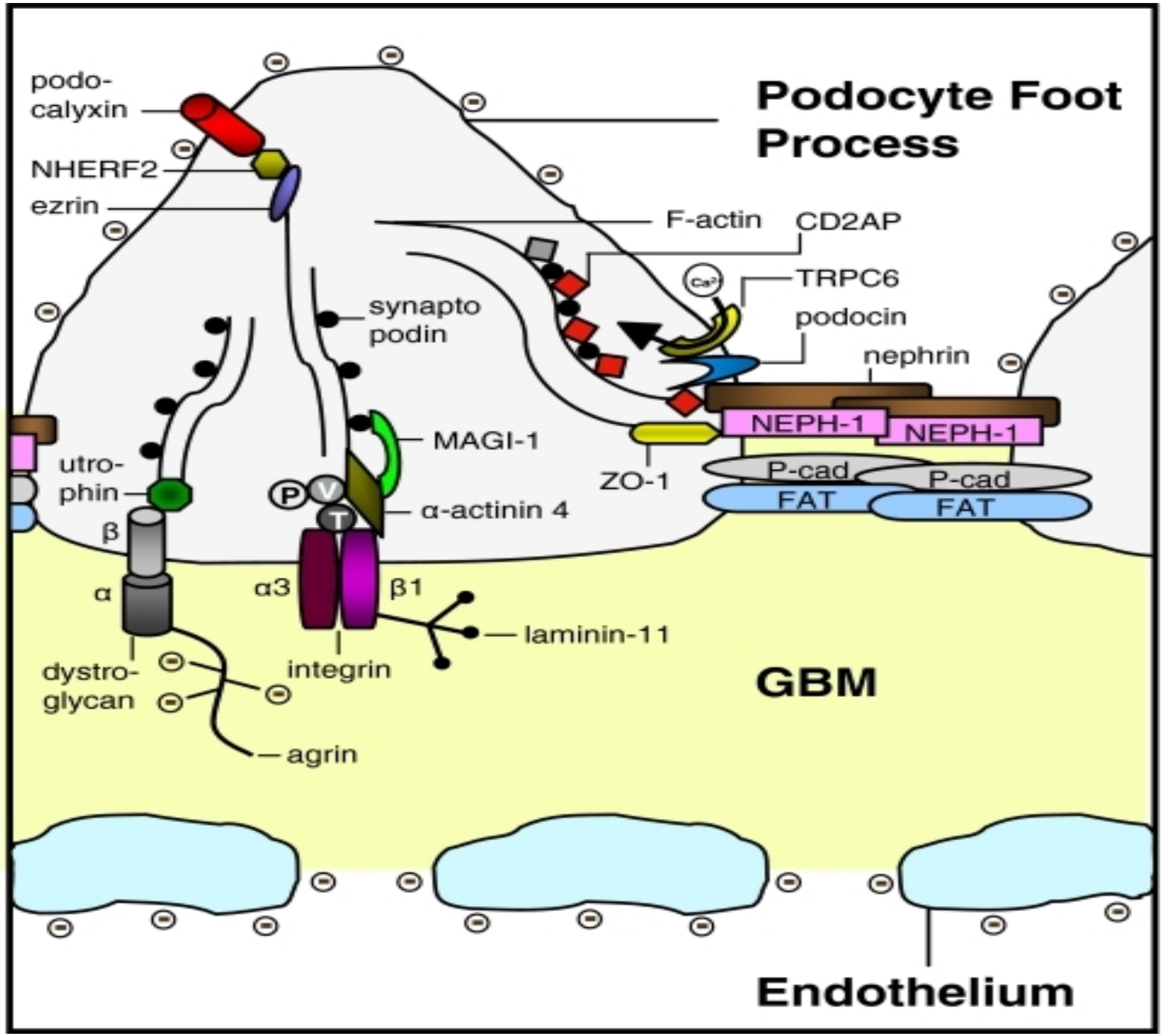
PODOCITO

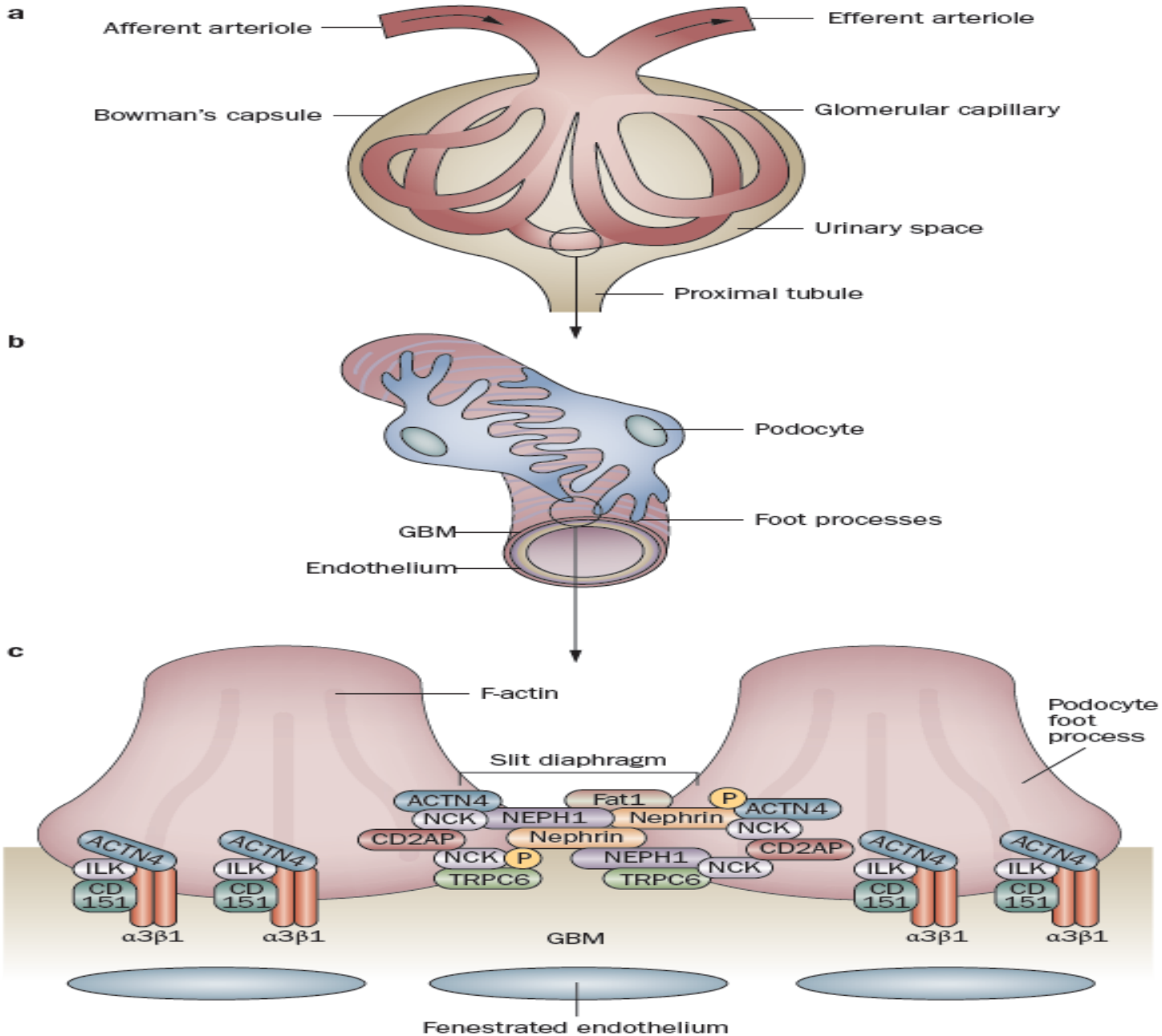
LA CÉLULA MÁS GRANDE DEL GLOMÉRULO

ALTAMENTE DIFERENCIADA

INCAPAZ DE DIVIDIRSE

MANTENIMIENTO
(síntesis y degradación)
DE LA MEMBRANA BASAL


Podocyte Foot Process
podo-calyxin
NHERF2
ezrin
F-actin
CD2AP
TRPC6
podocin
nephrin
synapto podin
MAGI-1
α-actinin 4
utro-phin
β
α
α3
β1
laminin-11
integrin
dystro-glycan
agrin
ZO-1
NEPH-1
NEPH-1
P-cad
P-cad
FAT
FAT
GBM
Endothelium


a
Afferent arteriole
Efferent arteriole
Bowman's capsule
Glomerular capillary
Urinary space
Proximal tubule
b
Podocyte
Foot processes
GBM
Endothelium
c
F-actin
Podocyte foot process
Slit diaphragm
ACTN4
Fat1
P
ACTN4
NCK
NEPH1
Nephrin
NCK
CD2AP
Nephrin
CD2AP
NCK
P
NEPH1
NCK
TRPC6
TRPC6
ACTN4
ILK
CD 151
α3β1
GBM
Fenestrated endothelium

PODOCITO

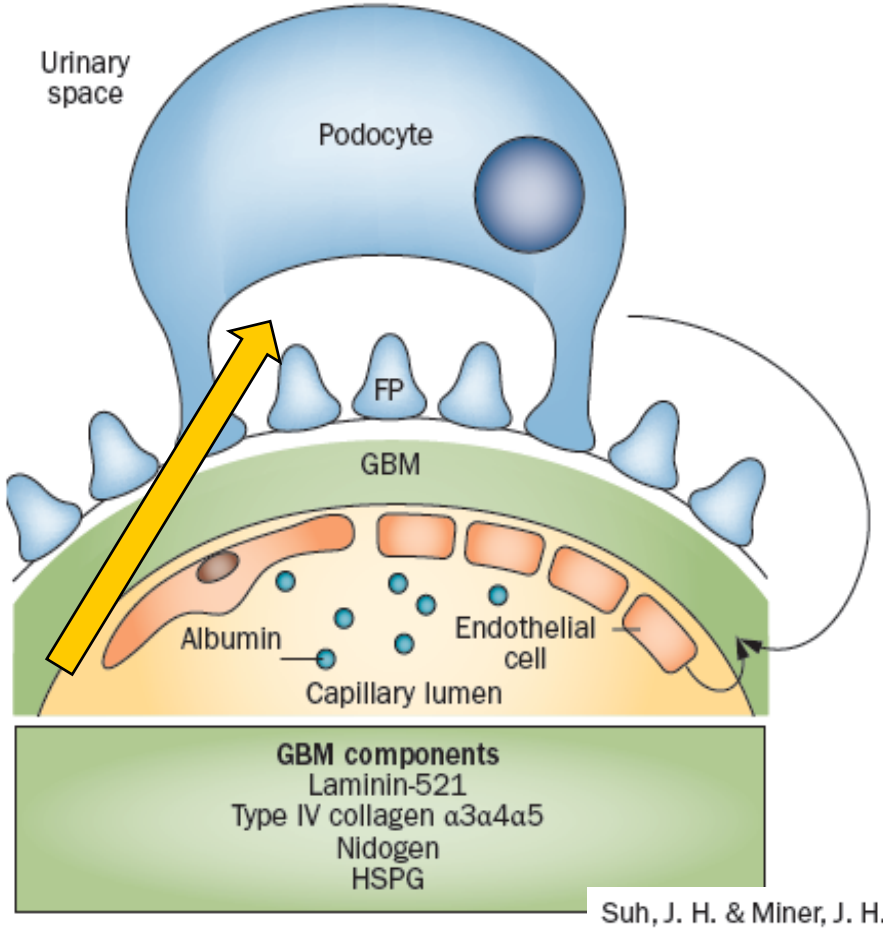
- **1. Barrera de permeabilidad**
- **2. Mantenimiento de la arquitectura glomerular**
- **3. Biosíntesis**
- **4. Interacción con el medio**

1. BARRERA DE PERMEABILIDAD

Es una barrera a la filtración de macromoléculas al cubrir la membrana basal con citoplasma.

Las abundantes cargas negativas del glicocálix actúan como una barrera electrostática.

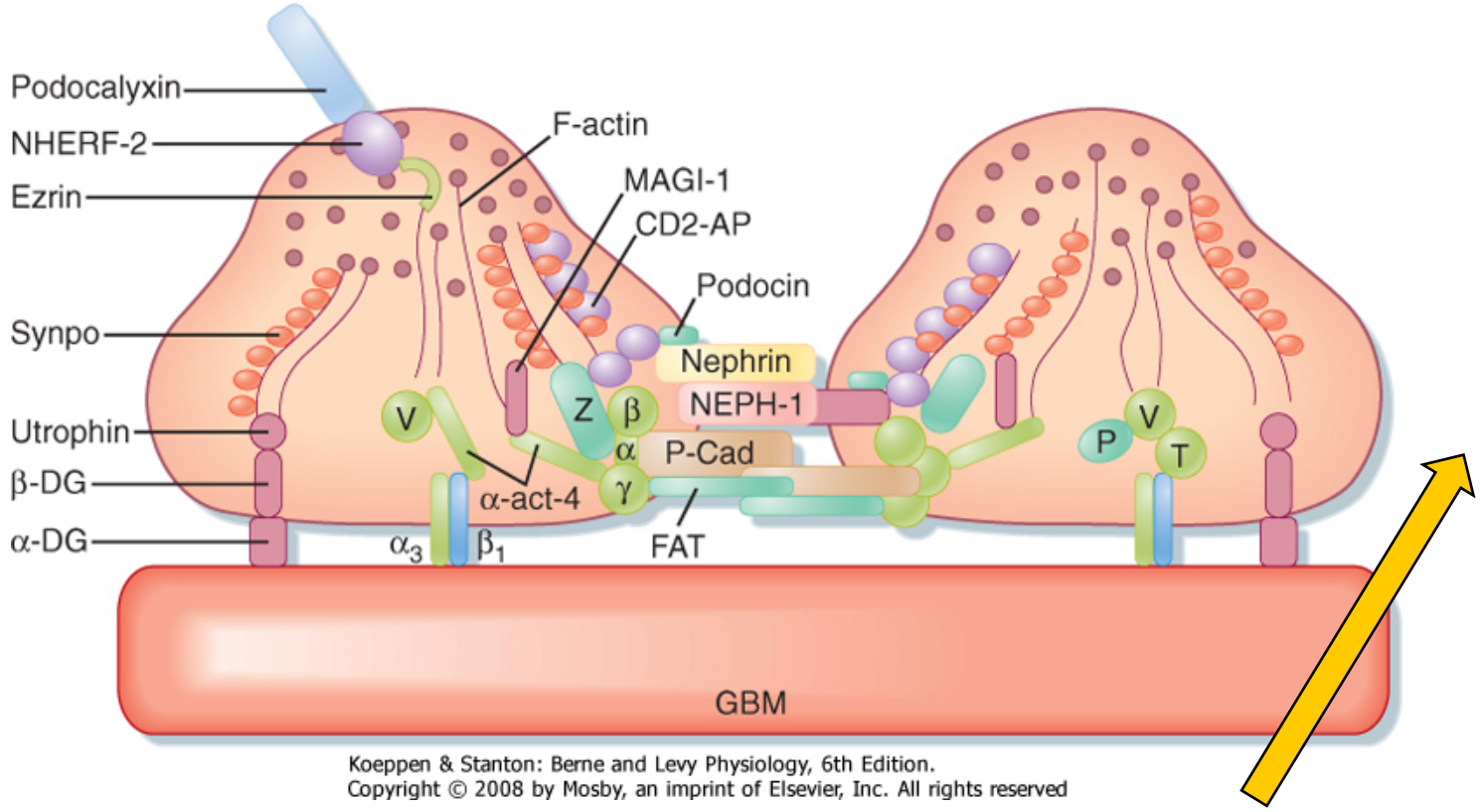
Los residuos filtrados y atrapados en la membrana basal son fagocitados por el podocito



Suh, J. H. & Miner, J. H. *Nat. Rev. Nephrol.* **9**, 470–477 (2013)

2. MANTENIMIENTO DE LA ARQUITECTURA GLOMERULAR

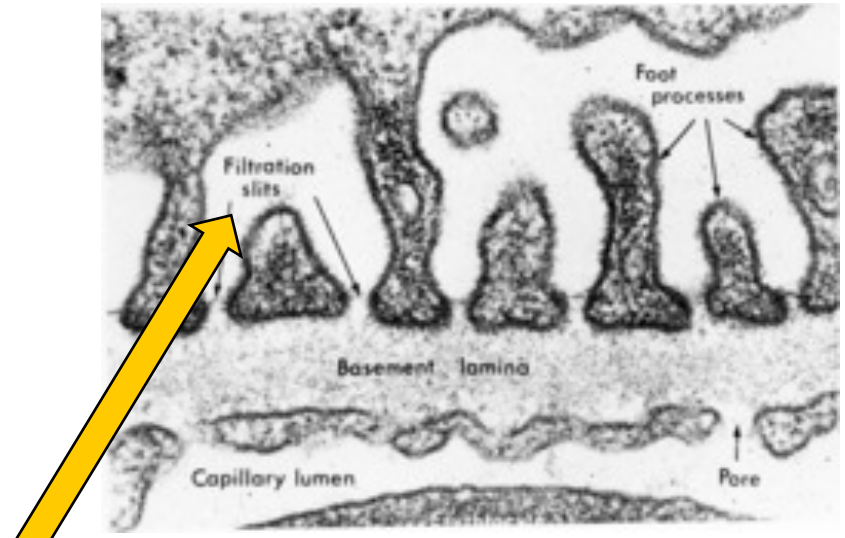
Contrarrestan las fuerzas hidrostáticas propias de los capilares glomerulares valiéndose de integrinas y distroglicanos.



3. BIOSÍNTESIS

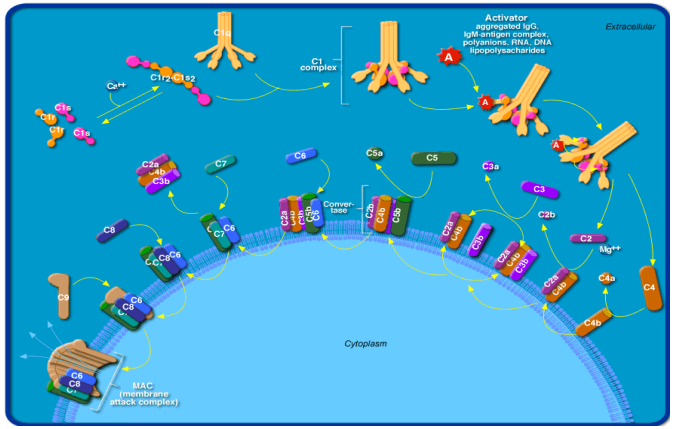
Síntesis y degradación de la membrana basal:

Colágeno tipo IV
Fibronectina
Laminina
Heparán sulfato
Prostaglandinas
Factores de crecimiento
VEGF
Epoetinas



4. INTERACCIÓN CON EL MEDIO

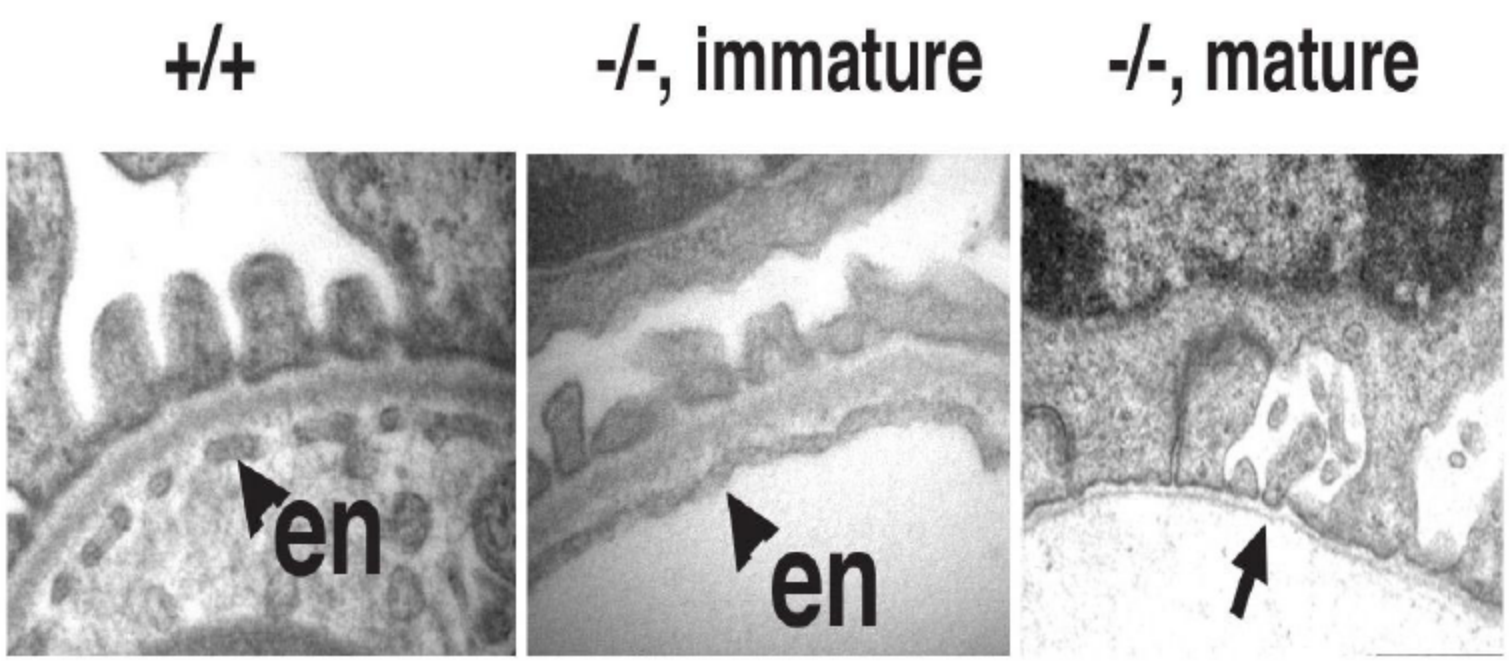
Con proteínas reguladoras del complemento,
Receptores IgG-Fc
Receptores de las LDL
Interacción con el sistema plasminógeno-plasmina



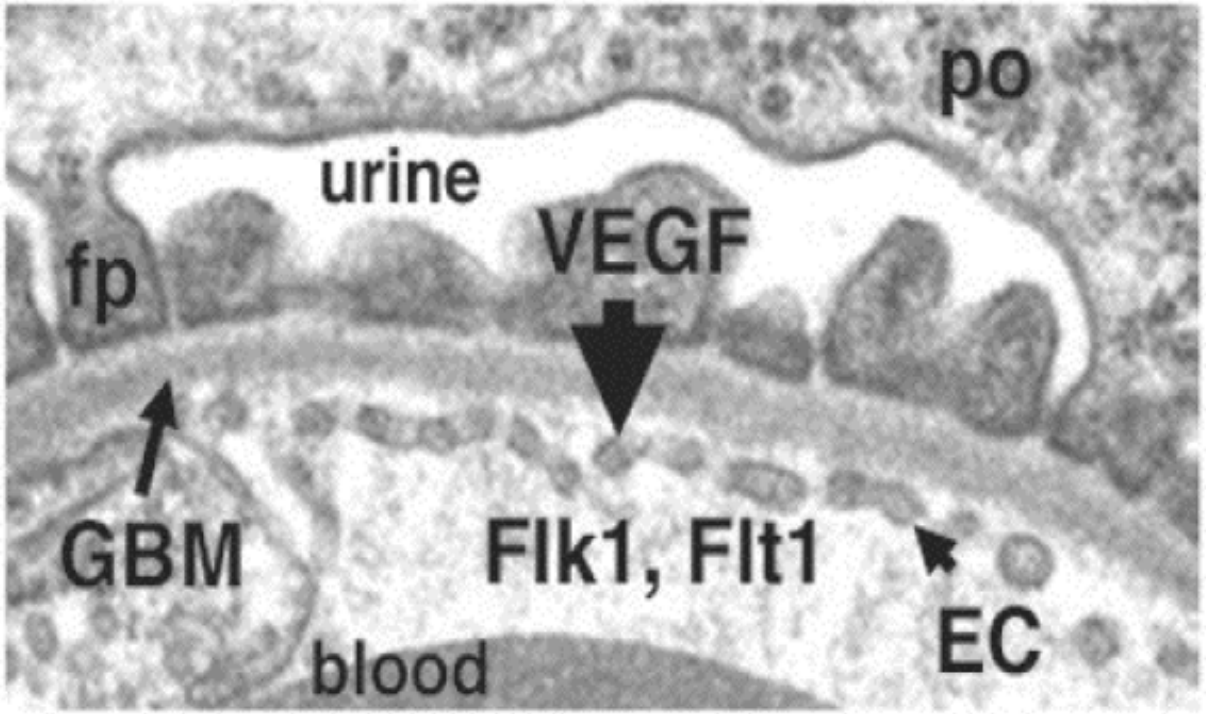
- Exquisite sensitivity of glomerular barrier to VEGF levels – dysregulation = proteinuria
- Crosstalk between glomerular cells critical for barrier integrity (endothelium!)
- Multiple angiogenic factors are expressed in glomerulus (Ang1 and VEGF) –play very different roles

S Quaggin Aahrus 2011

VEGF-A is required for fenestration & survival of ECs



S Quaggin Aahrus 2011

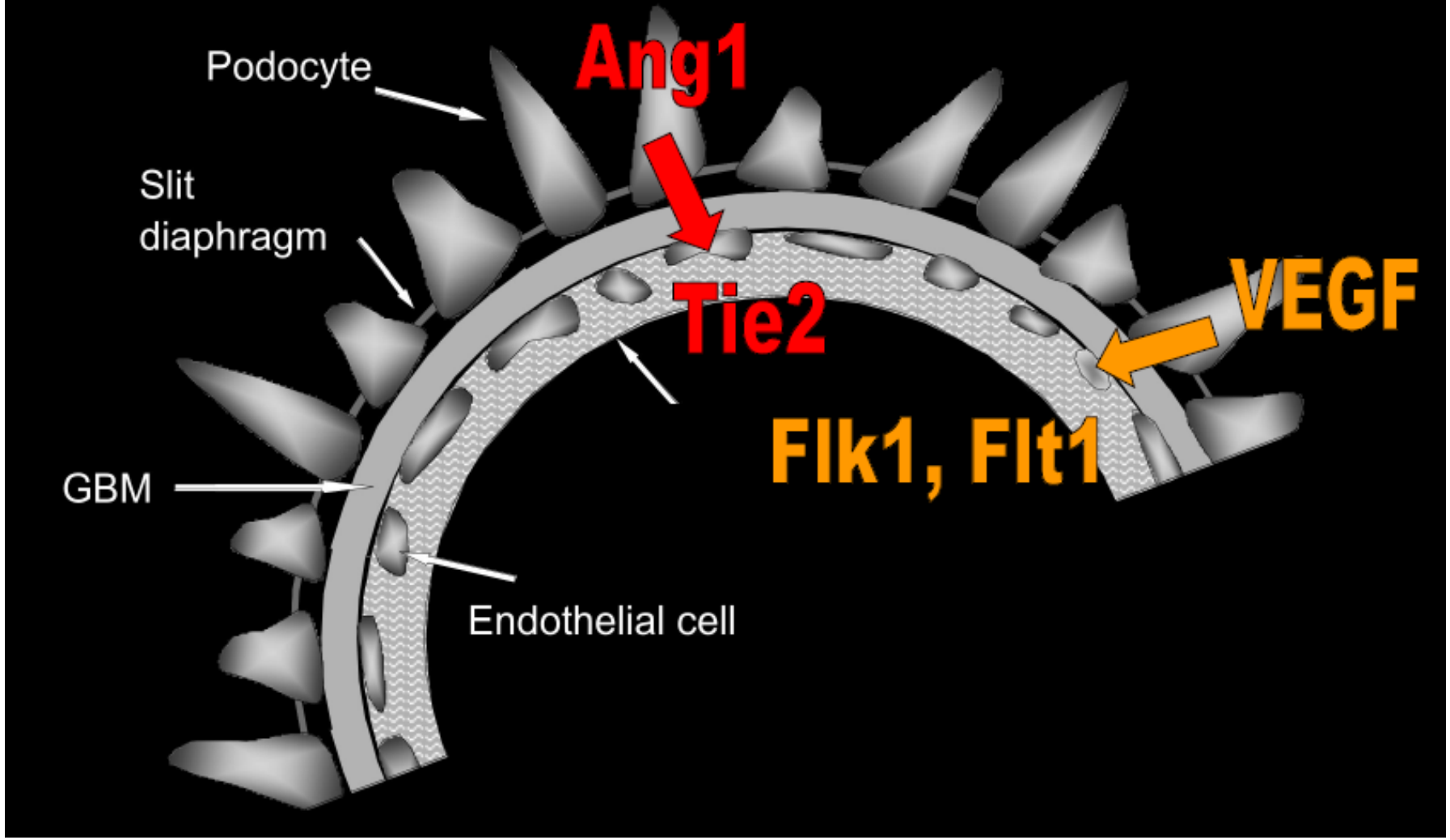


Hypothesis

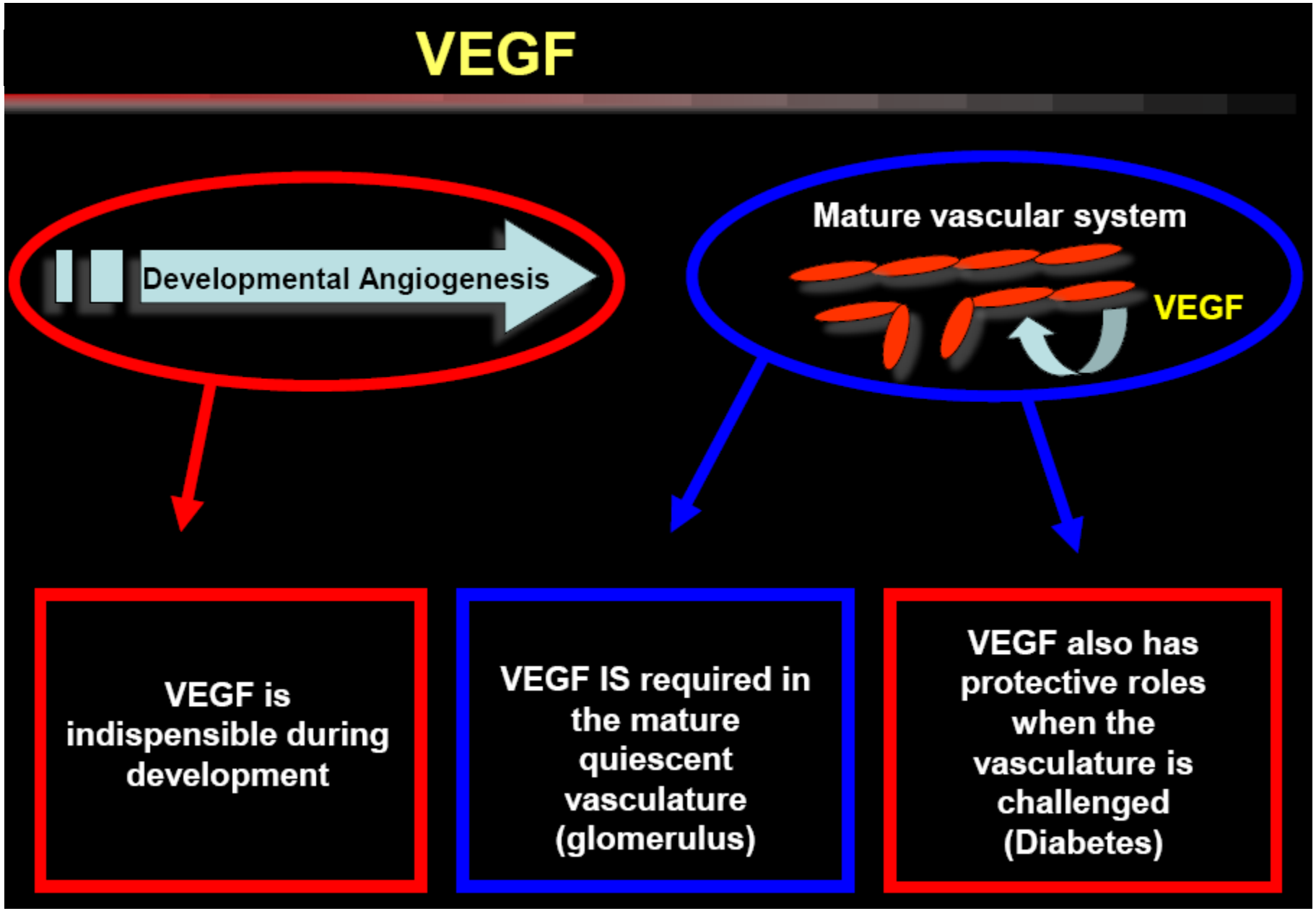
VEGF inhibitors cause proteinuria and glomerular injury by local reduction of VEGF ("on target effect")

S Quaggin Aahrus 2011

Ang1 and VEGF in the glomerulus



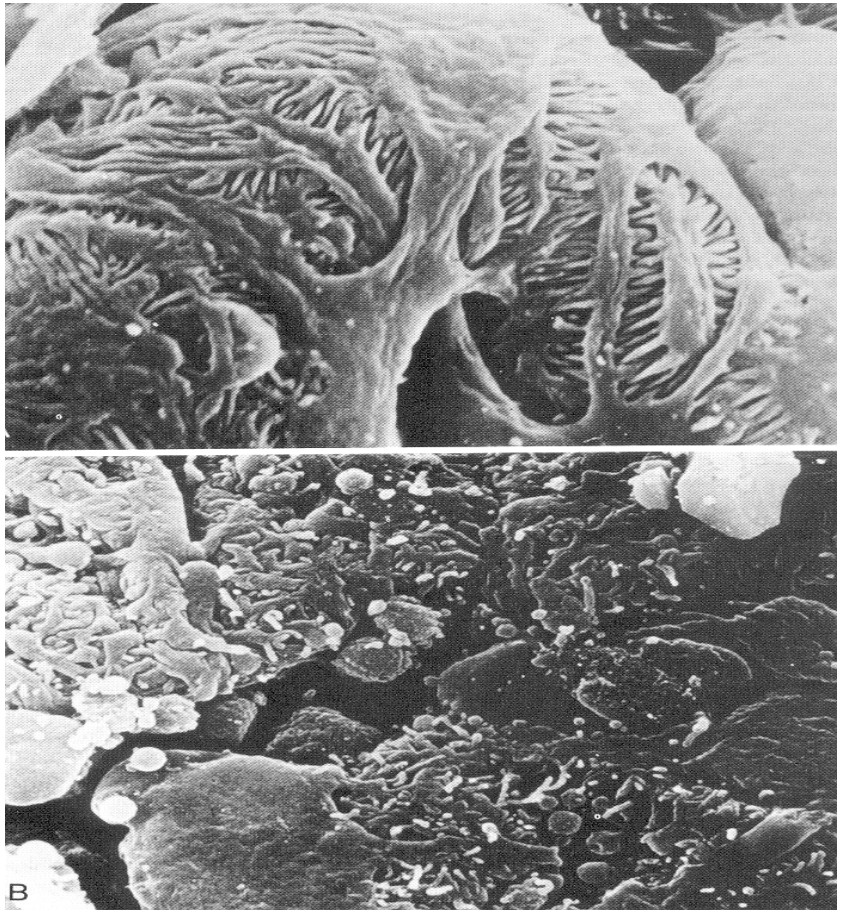
S Quaggin Aahrus 2011

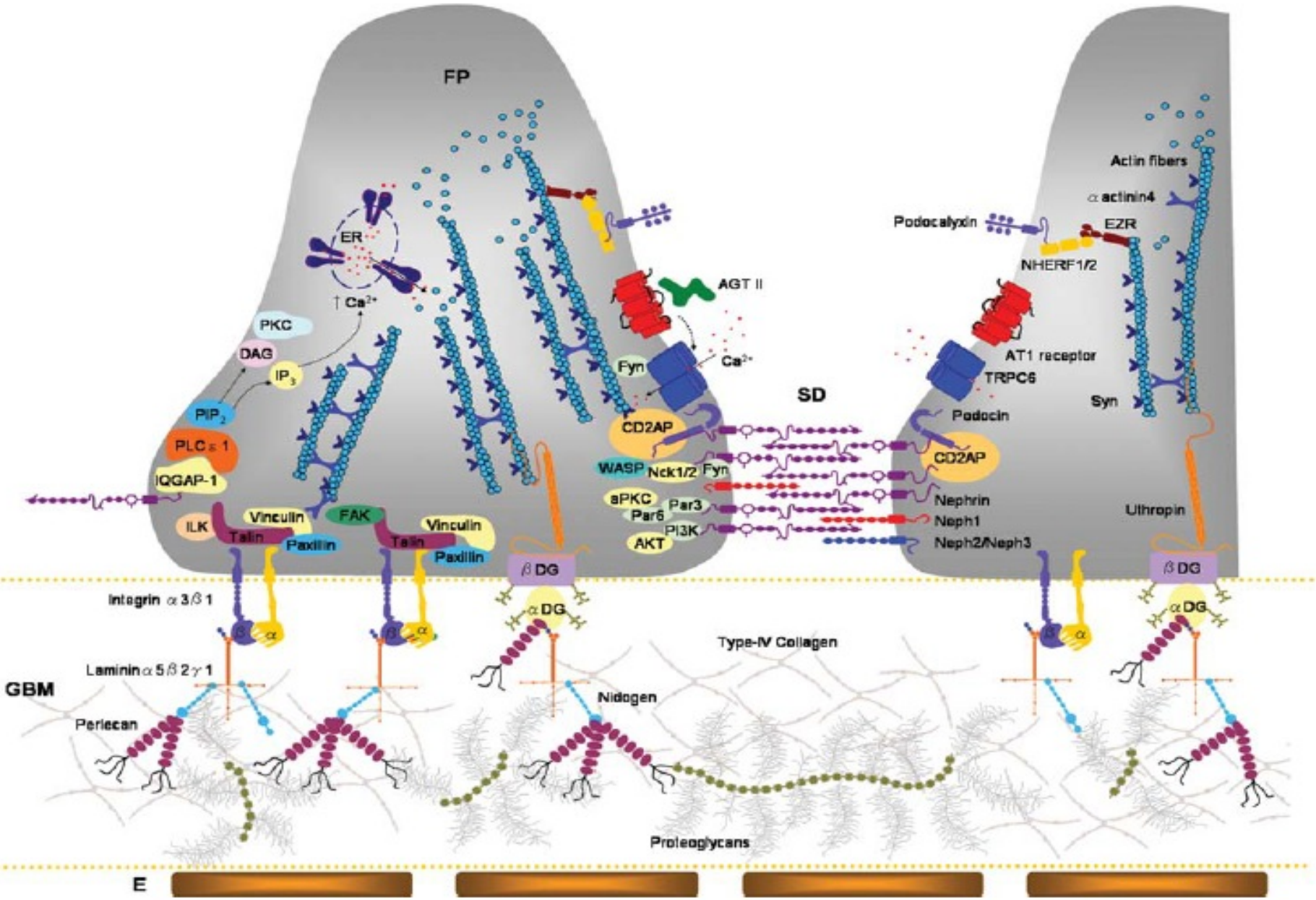


S Quaggin Aahrus 2011

PODOCITO: Injuria

- **La injuria y la disfunción del podocito se manifiestan clínicamente por sólo dos signos principales:**
- **PROTEINURIA POR DESDIBUJAMIENTO DE LOS PEDICELOS, VACUOLIZACIÓN Y/O DESPRENDIMIENTO DE LOS PODOCITOS**
- **DISMINUCIÓN DE LA FILTRACIÓN GLOMERULAR POR ESCLEROSIS**




FP
SD
ER
↑ Ca2+
PKC
DAG
IP3
PIP2
PLCε1
IQGAP-1
ILK
Vinculin
Talin
Paxillin
FAK
Vinculin
Talin
Paxillin
AGT II
Ca2+
Fyn
CD2AP
WASP
Nck1/2
Fyn
aPKC
Par3
Par6
PI3K
AKT
β DG
α DG
Integrin α3β1
Laminin α5β2γ1
GBM
Perlecan
Nidogen
Type-IV Collagen
Proteoglycans
E
Actin fibers
α actinin4
EZR
Podocalyxin
NHERF1/2
AT1 receptor
TRPC6
Podocin
CD2AP
Nephrin
Neph1
Neph2/Neph3
Syn
Uthropin
β DG
α DG

Nephrin-interacting protein *nePH1*

Esta proteína también conecta al diafragma a la actina.

Su fosforilación aumenta la polimerización de la actina luego de la fosforilación de la nefrina.

Tanto las proteínas CD2aP y nCK son fundamentales en la conexión funcional de la actina al diafragma.

La interacción con CD2aP predominaría en estados estables, mientras que con las proteínas nCK lo sería en el desarrollo y en la injuria podocitaria.

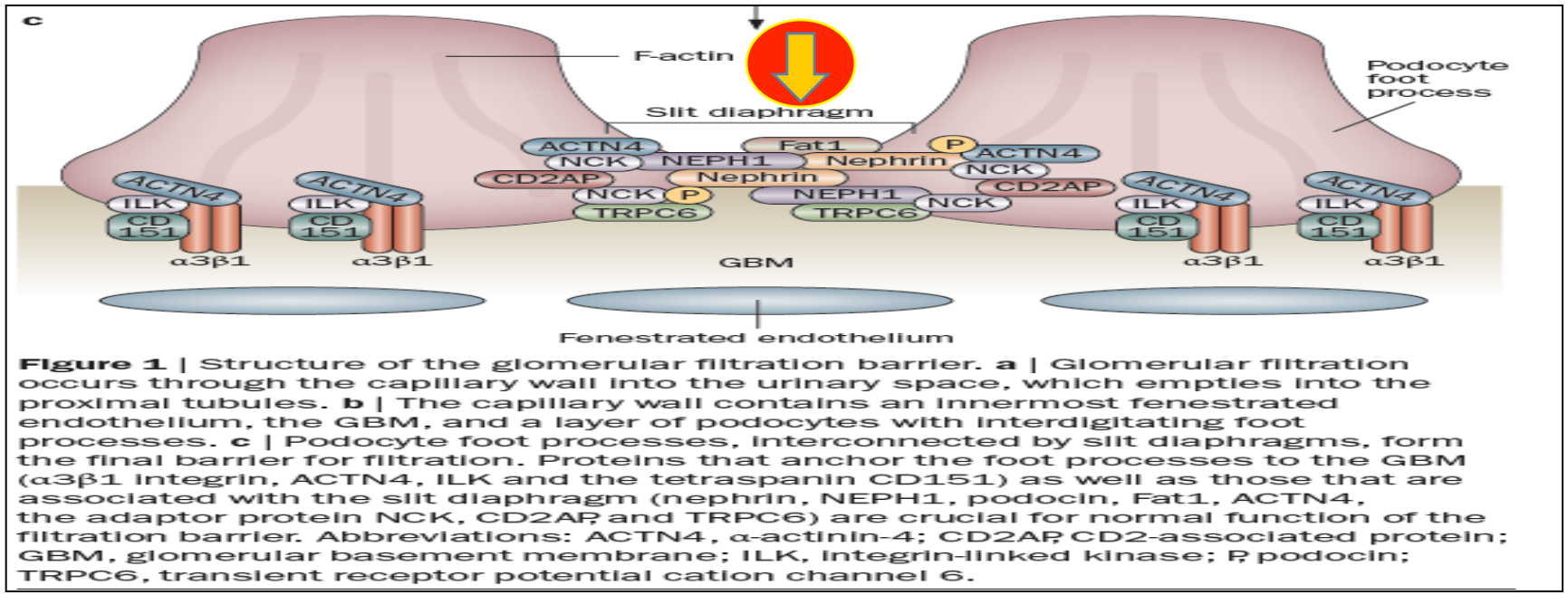


Figure 1 | Structure of the glomerular filtration barrier. **a** | Glomerular filtration occurs through the capillary wall into the urinary space, which empties into the proximal tubules. **b** | The capillary wall contains an innermost fenestrated endothelium, the GBM, and a layer of podocytes with interdigitating foot processes. **c** | Podocyte foot processes, interconnected by slit diaphragms, form the final barrier for filtration. Proteins that anchor the foot processes to the GBM (α3β1 integrin, ACTN4, ILK and the tetraspanin CD151) as well as those that are associated with the slit diaphragm (nephrin, NEPH1, podocin, Fat1, ACTN4, the adaptor protein NCK, CD2AP, and TRPC6) are crucial for normal function of the filtration barrier. Abbreviations: ACTN4, α-actinin-4; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; ILK, integrin-linked kinase; P, podocin; TRPC6, transient receptor potential cation channel 6.

Rol del TRPC6 Transient receptor potential cation channel 6 (trPC6)

Sobreexpresada en familias con FsGs autosómica-dominante.
Estos canales regulan la entrada de calcio intracelular.

En los podocitos, el trPC6 se localiza en la hendidura del diafragma, y participa en la señalización.

Su sobreexpresión resulta en proteinuria.

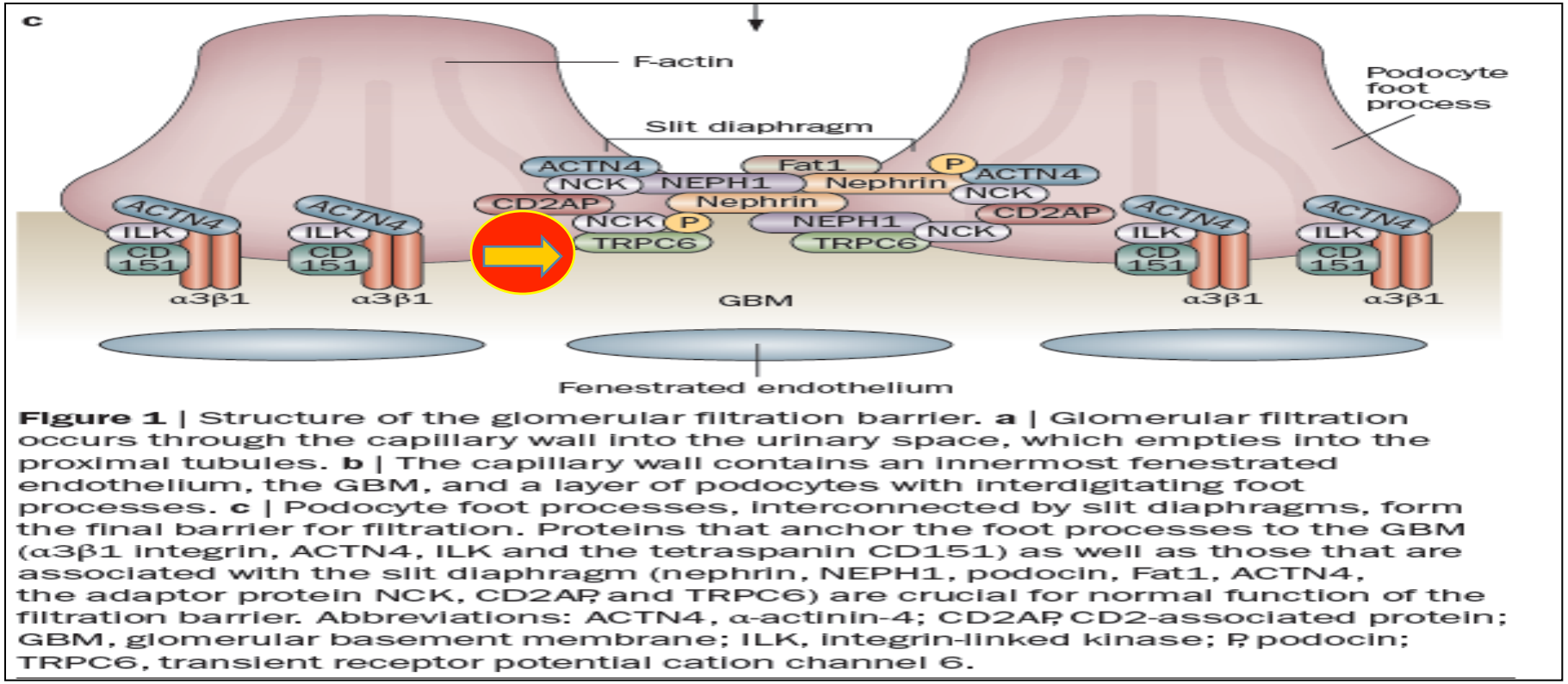


Figure 1 | Structure of the glomerular filtration barrier. **a** | Glomerular filtration occurs through the capillary wall into the urinary space, which empties into the proximal tubules. **b** | The capillary wall contains an innermost fenestrated endothelium, the GBM, and a layer of podocytes with interdigitating foot processes. **c** | Podocyte foot processes, interconnected by slit diaphragms, form the final barrier for filtration. Proteins that anchor the foot processes to the GBM (α3β1 integrin, ACTN4, ILK and the tetraspanin CD151) as well as those that are associated with the slit diaphragm (nephrin, NEPH1, podocin, Fat1, ACTN4, the adaptor protein NCK, CD2AP, and TRPC6) are crucial for normal function of the filtration barrier. Abbreviations: ACTN4, α-actinin-4; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; ILK, integrin-linked kinase; P, podocin; TRPC6, transient receptor potential cation channel 6.

LA VIA NOTCH

La vía notch ha sido involucrada en la patogenenia de la proteinuria.

Las moléculas notch son proteínas de transmembrana que al activarse por ligandos extracelulares, sufren clivaje proteolítico y liberan el dominio notch intracelular.

Este dominio luego se transloca al núcleo, donde estimula la transcripción de diversos genes.

Su activación se ve en podocitos dañados, y la expresión de notch1 resulta en apoptosis podocitaria, albuminuria, y glomeruloesclerosis.

La supresión de la vía notch atenúa la proteinuria.

Kidney International (2010) **77**, 662–668

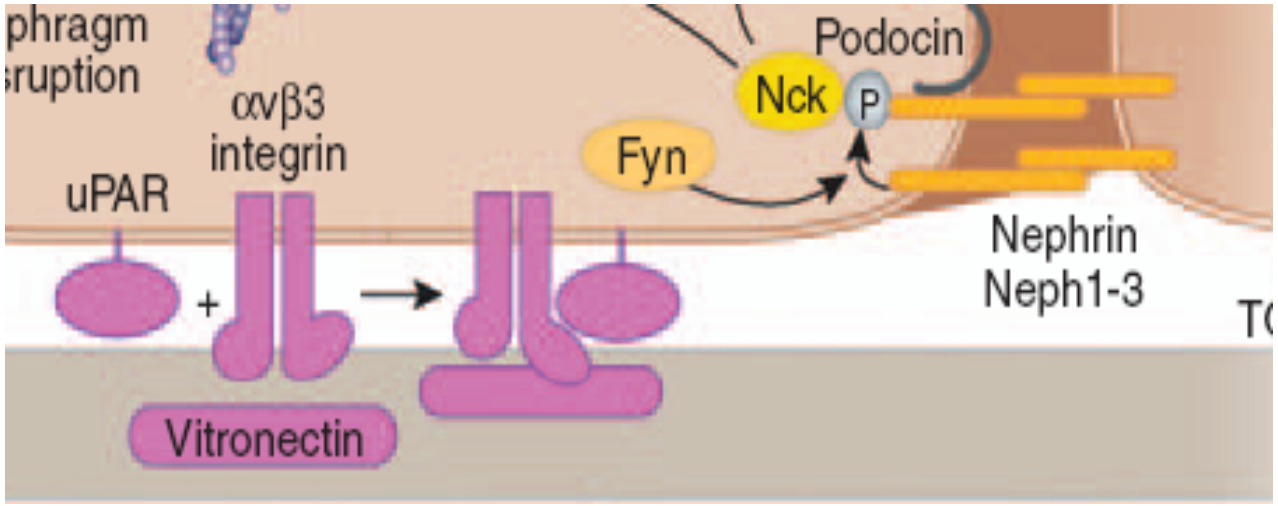
Receptor de Urokinasa

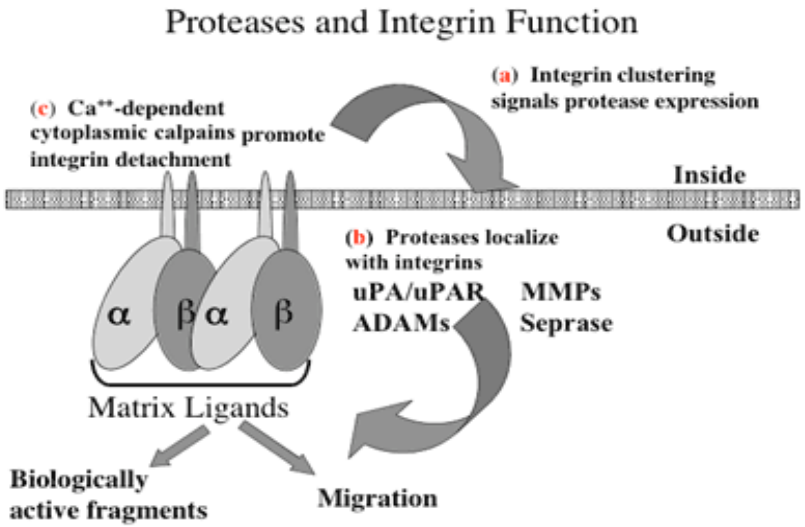
Este receptor ha sido implicado en la patogenia de la proteinuria.

Es una proteinasa, pero también presenta interacciones con otras proteínas de membrana como las integrinas.

Durante la injuria podocitaria, el receptor (uPar) promueve el desdibujamiento de los pedicelos por su interacción con la integrina αvβ3.

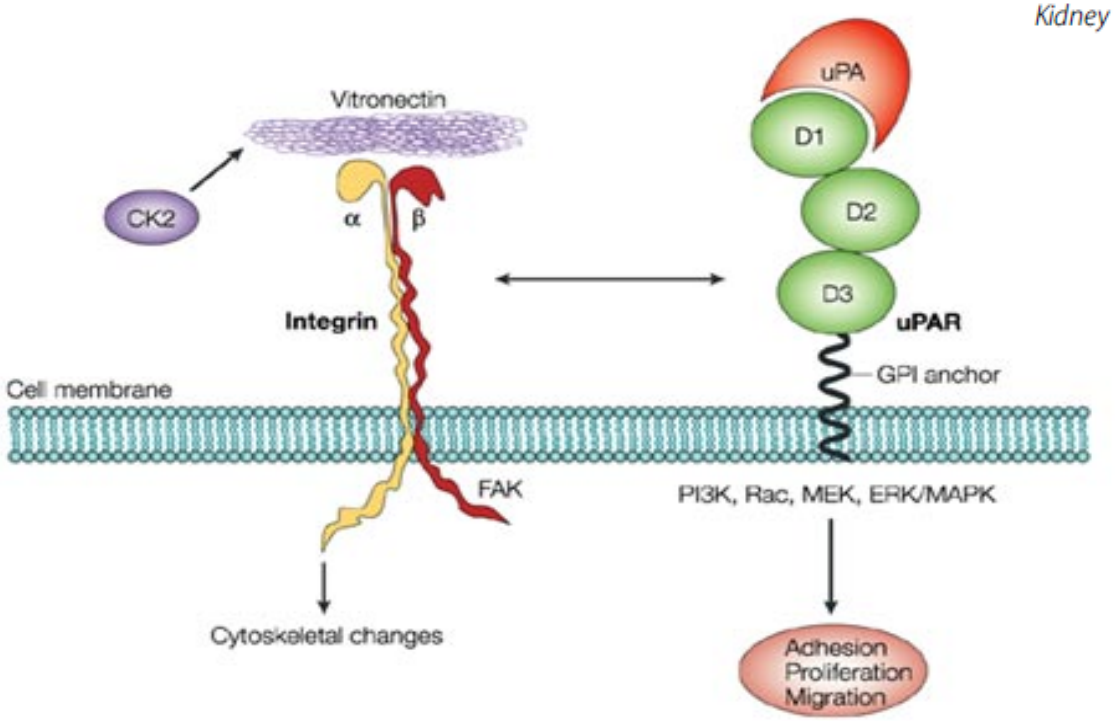
La expresión de la vitronectina, el ligando extracelular de la integrina αvβ3, está estimulado en la proteinuria.





Kidney International (2010) **77**, 662–668

Kidney International (2010) **77**, 662–668



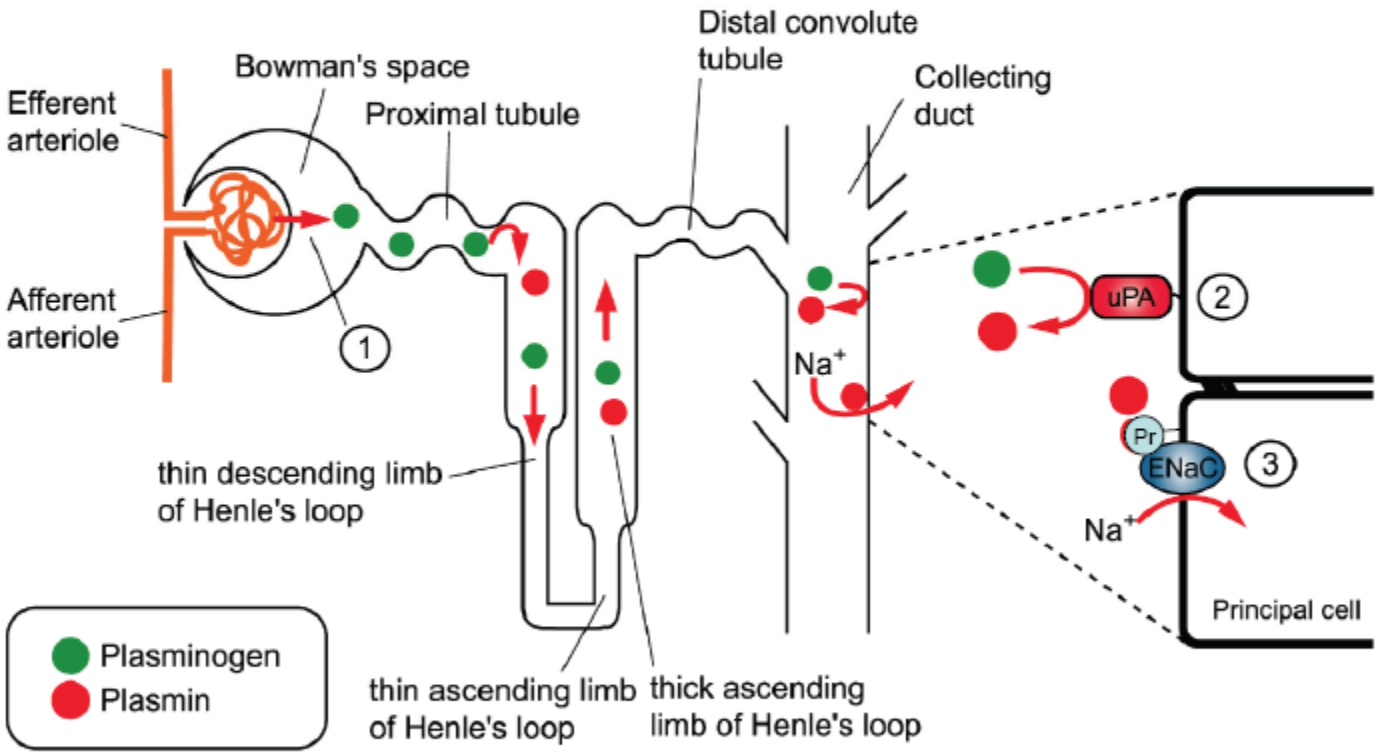
El uPAR se une tanto a la urokinasa (uPA) como a la vitronectina, que es a su vez el receptor del PAI-1.

LaProtein kinasa CK2 fosforila a la vitronectina y regula la adhesión celular uPA-dependiente a la vitronectina.

El uPAR carece de un dominio citosólico pero transmite señales intracelulares por su asociación con las integrinas de transmembrana.

ERK, extracellular-signal-regulated kinase FAK, focal adhesion kinase MAPK, mitogen-activated protein kinase.

A novel model for stimulation of sodium reabsorption in nephrotic syndrome



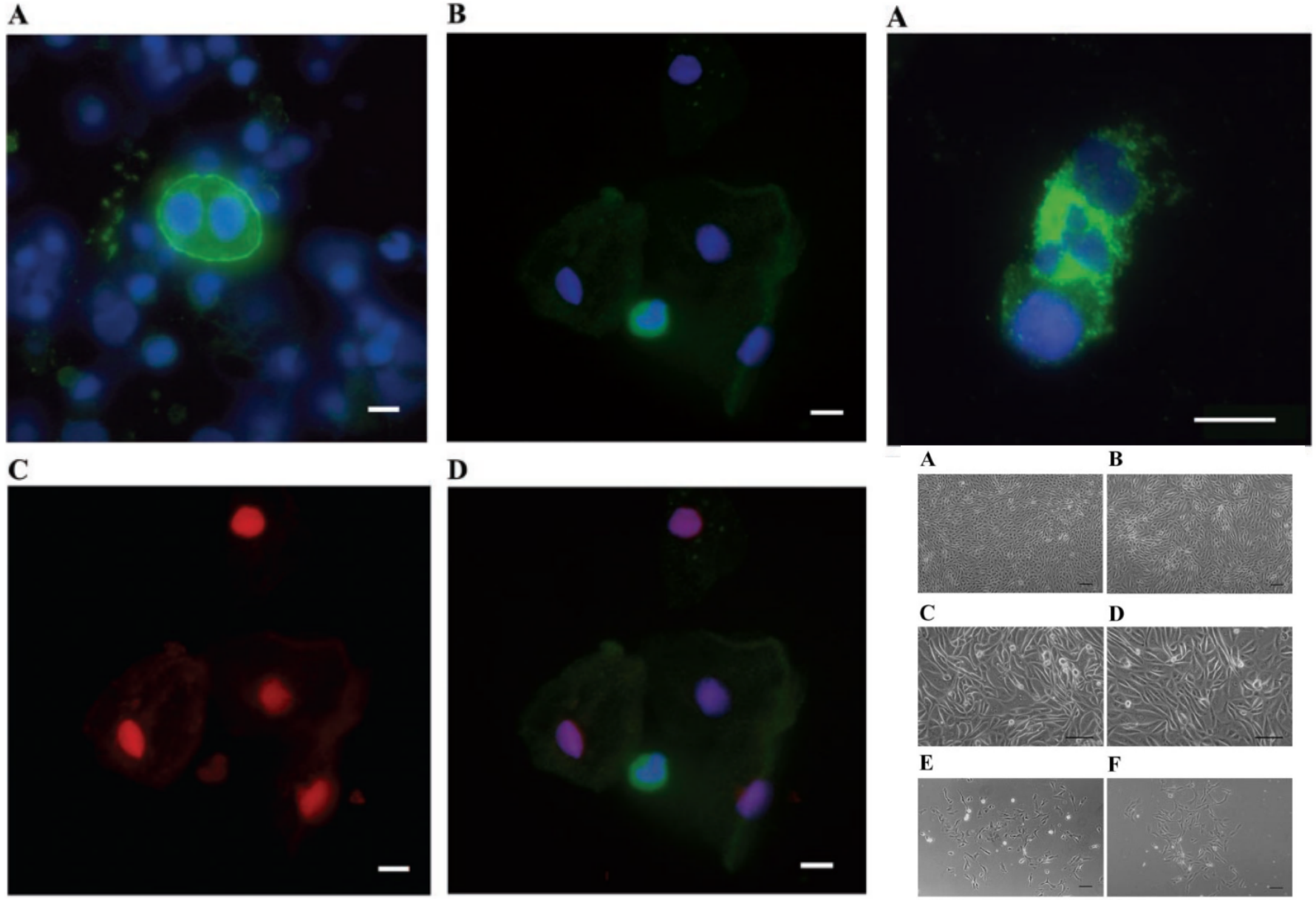
Proteinuria may promote distal sodium reabsorption

Table 1 | Pathways involved in acquired glomerular diseases

Molecular pathways	Human diseases
Transmembrane receptors	
Nephrin	DNP, MCD
B7-1	LN
uPAR	FSGS, DNP
Notch	FSGS, DNP
PLA_2R	MN
Ion channels	
TRPC6	MCD, MN
Growth factors	
VEGF-A	Preeclampsia
TGF-β	DNP
Proteases	
Cathepsin L → dynamin, synaptopodin	MN, FSGS, DNP

Abbreviations: DNP, diabetic nephropathy; FSGS, focal segmental glomerulosclerosis; LN, lupus nephritis; MCD, minimal change disease; MN, membranous nephropathy; TGF-β, transforming growth factor-β; TRPC6, transient receptor potential cation channel-6; uPAR, urokinase plasminogen-activator receptor; VEGF-A, vascular endothelial growth factor A.
Only those pathways that have been shown to be involved in acquired human glomerular diseases are listed.

ACT 2: GOING, GOING, GONE… THE ROLE OF PODOCYTE DEPLETION

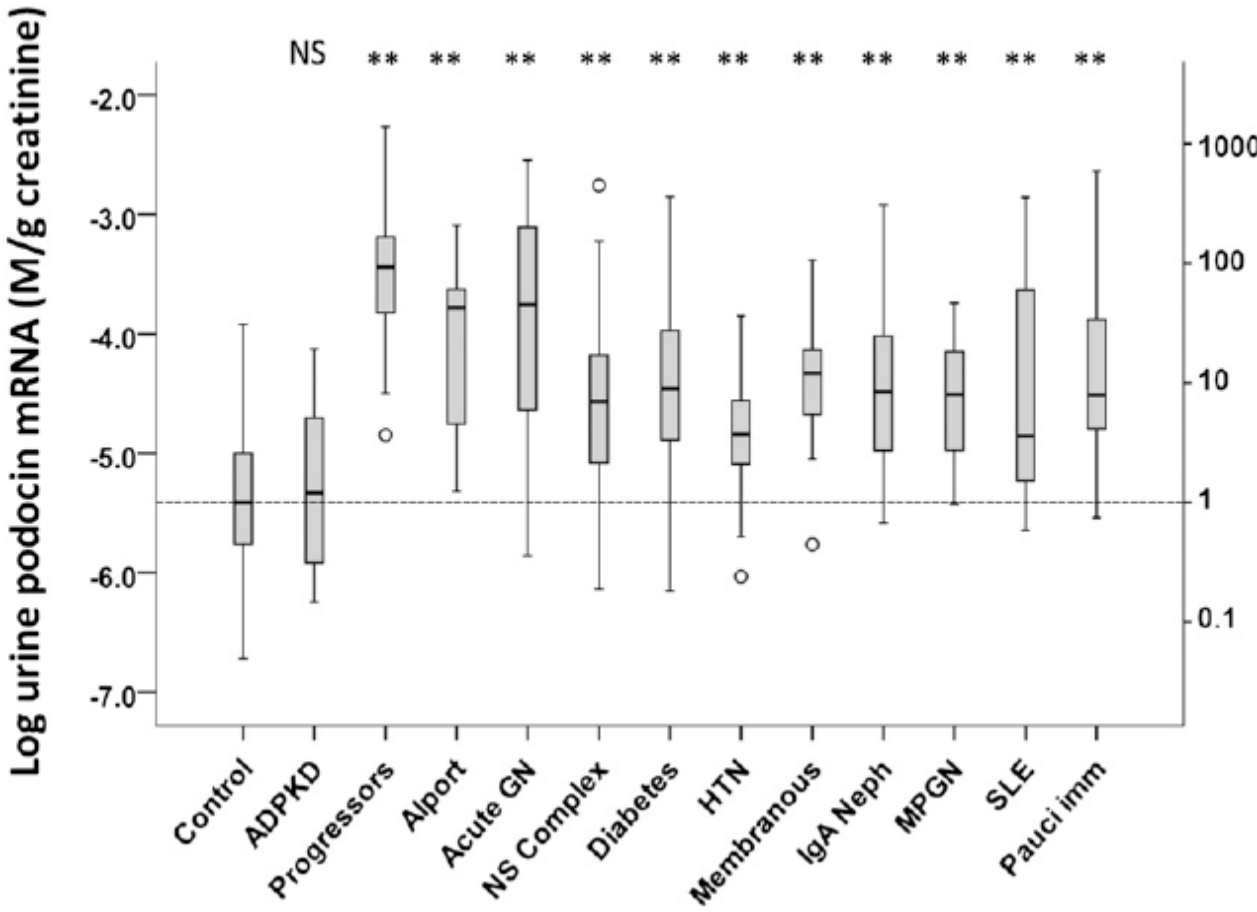


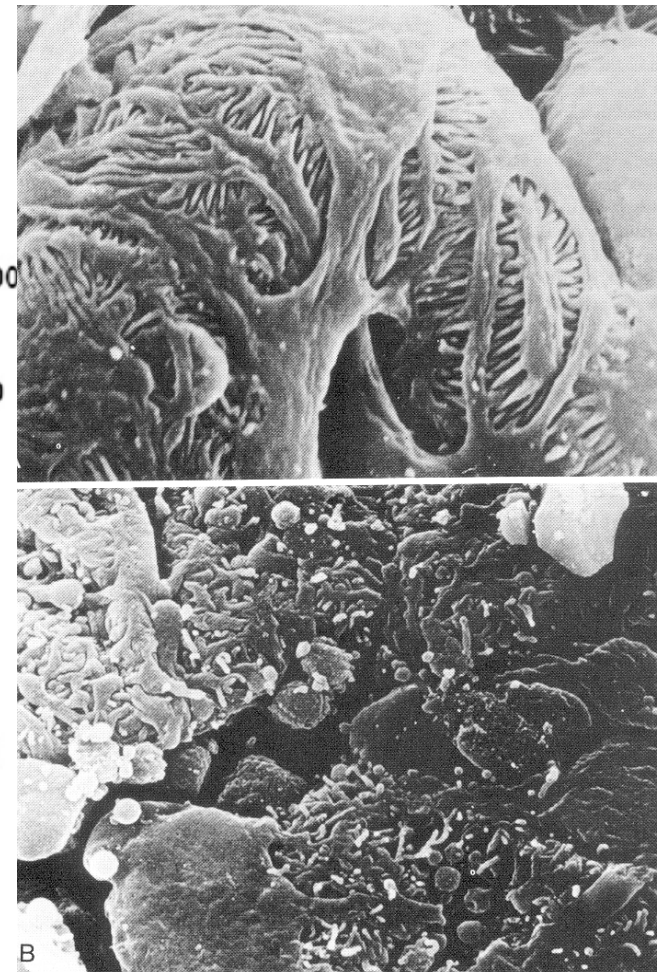
Vogelmann et al.

Am J Physiol Renal Physiol. 2003 July ; 285(1): F40–F48.

Urine Podocyte mRNAs, Proteinuria, and Progression in Human Glomerular Diseases

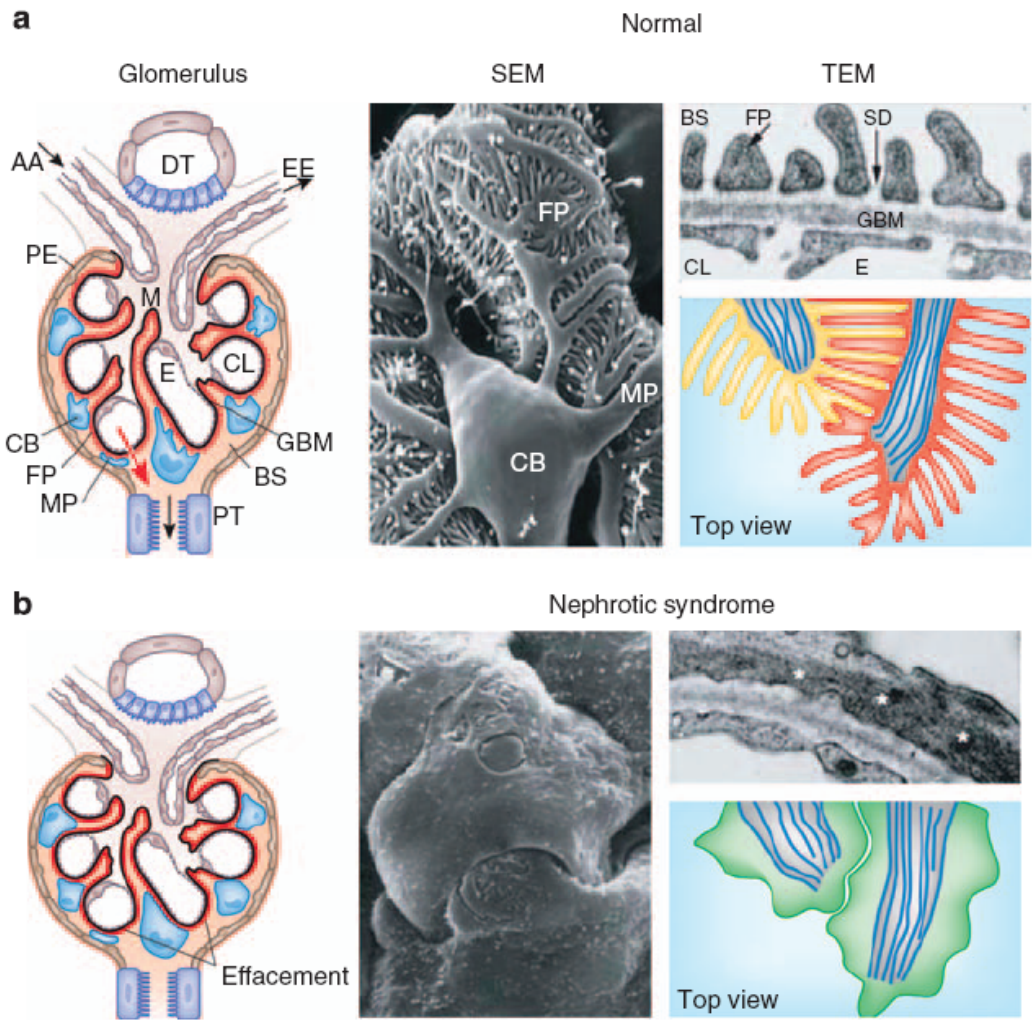
Larysa Wickman,* Farsad Afshinnia,† Su Q. Wang,† Yan Yang,† Fei Wang,‡ Mahboob Chowdhury,† Delia Graham,* Jennifer Hawkins,† Ryuzoh Nishizono,† Marie Tanzer,* Jocelyn Wiggins,† Guillermo A. Escobar,§ Bradley Rovin,‖ Peter Song,‡ Debbie Gipson,* David Kershaw,* and Roger C. Wiggins†





J Am Soc Nephrol 24: 2081–2095, 2013

ACT 5: THE MISSING LINK: HOW IRREVERSIBLE FOOT PROCESS EFFACEMENT AND PODOCYTE LOSS PROMOTE GLOMERULOSCLEROSIS



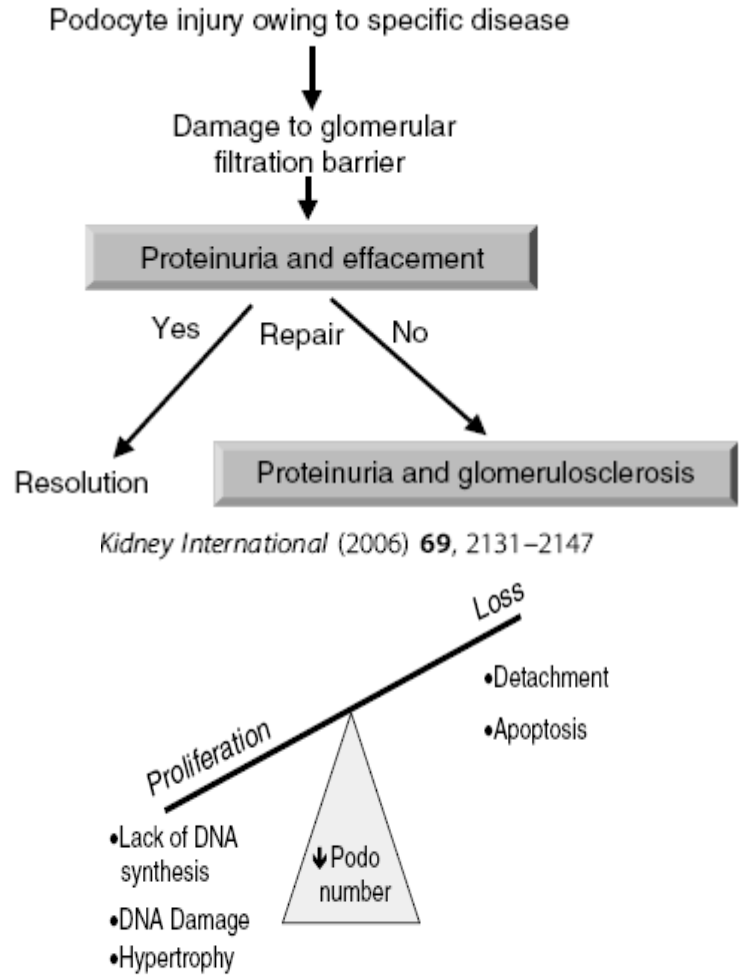
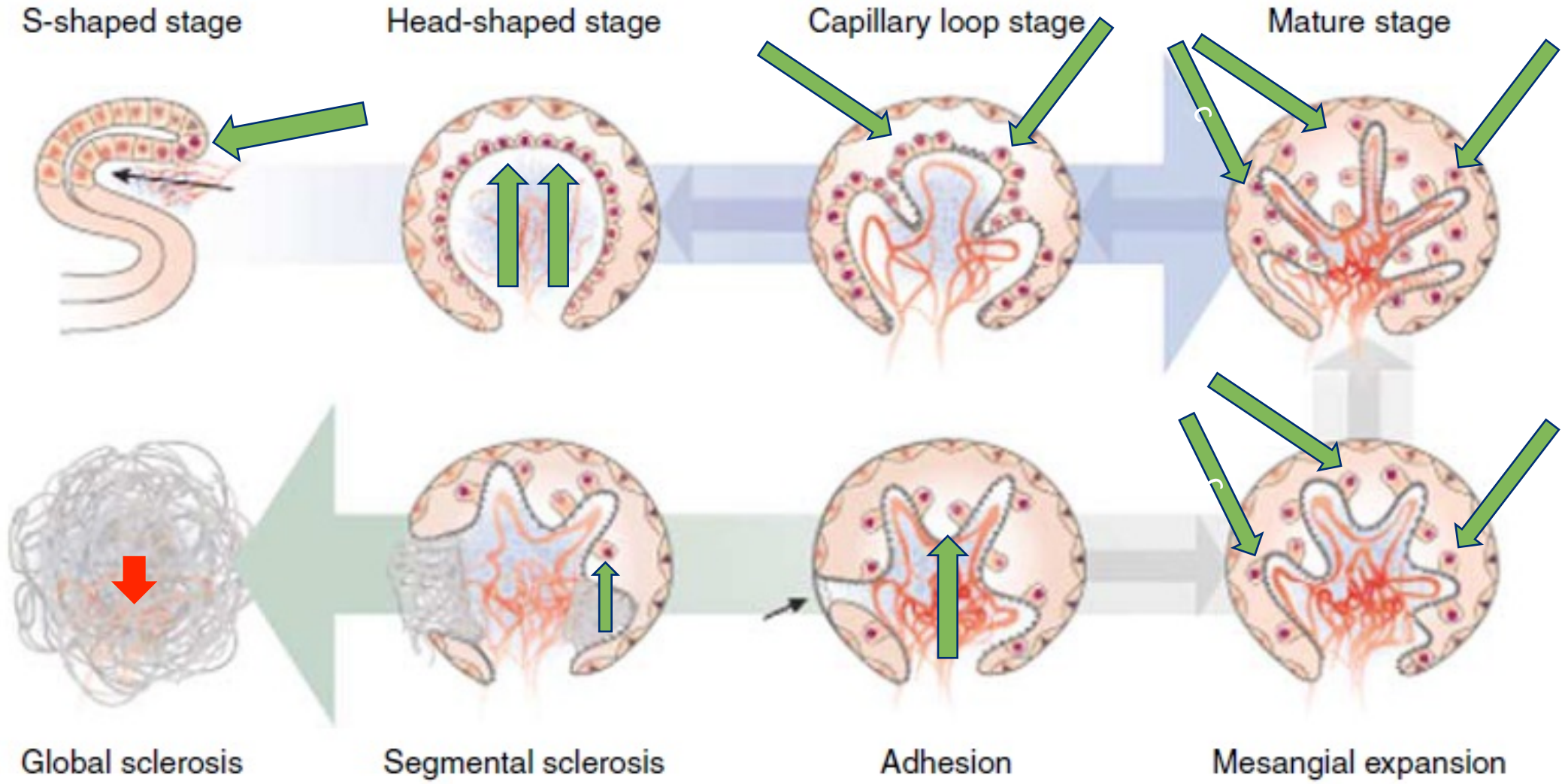


Figure 2 | Factors governning podocyte number. Total podocyte (podo) number is a balance between proliferation and loss. Podocyte number is reduced by either a decrease in proliferation owing to lack of DNA synthesis, DNA damage or hypertrophy, and/or an in crease in podocyte loss owing to detachment and apoptosis.



Loss of podocytes beyond a critical level results in a fibrotic glomerular response in that part of the glomerulus

Loss of some podocytes (20%) is associated with mesangial expansion possibly as an attempt to reduce the filtration surface area

Loss of podocytes beyond a critical level results in widespread scarring of that glomerulus

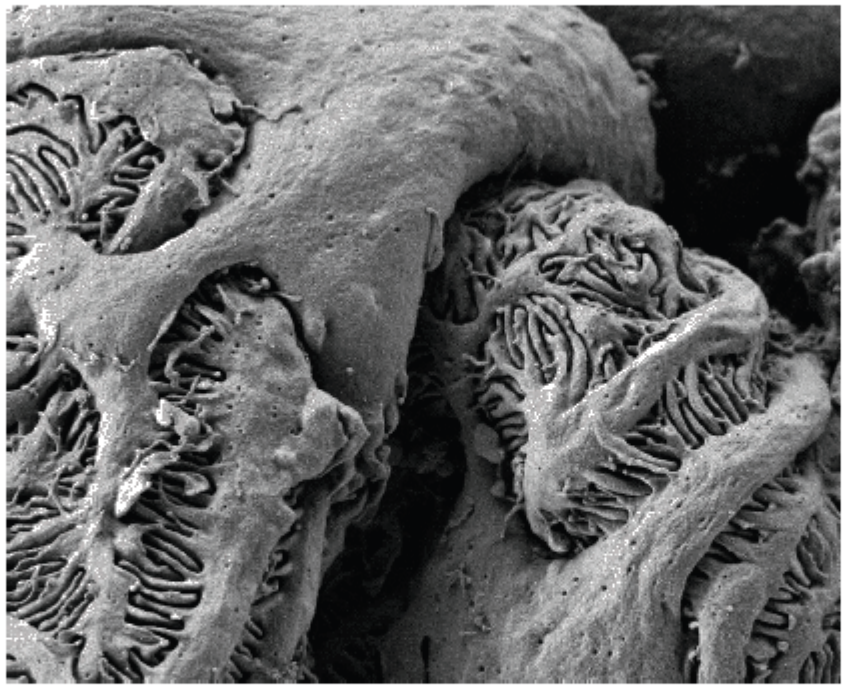
Loss of podocytes resulting in appearance of bare areas of filtration surface results in adhesion of the bare surface to Bowman's capsule (synechia)

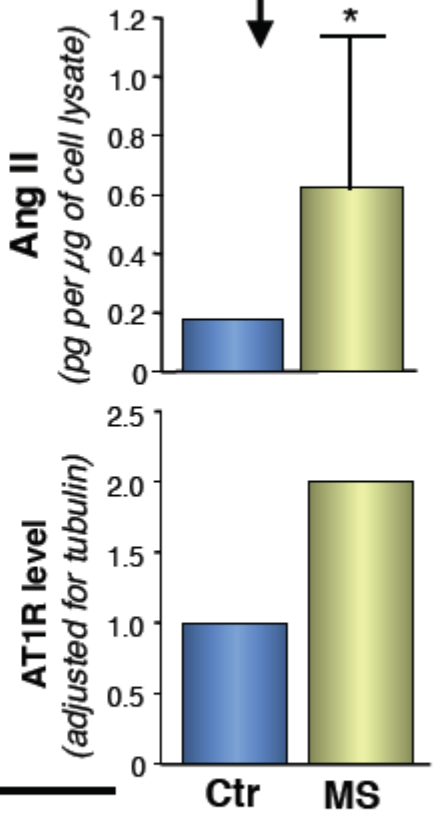
RC Wiggins: Spectrum of podocytopathies *Kidney International* (2007) **71**, 1205–1214

Glomerular hypertension

Mechanical strain

Podocyte number





Durvasula et al., *Kidney Int*, 2004

Pore dimension

Proteinuria

Disease progression

TIPOS DE PROTEINURIA

En condiciones normales, la excreción urinaria de proteínas no excede los 150 mg/día y consiste principalmente de proteínas filtradas (60%) y de la proteína tubular de Tamm-Horsfall (40%).

La proteína urinaria más importante es la albúmina, constituyendo el 20% de la proteinuria diaria, a razón de hasta 20 mg/día (13.8 mg/min).

La proteinuria usualmente refleja un aumento en la permeabilidad glomerular a la albúmina y otras macromoléculas plasmáticas

Hay varios tipos básicos de proteinuria:

Glomerular

Tubular

Sobreflujo

Dinámica (inducida por ejercicio)

Algunos conceptos

La variante glomerular la forma más frecuente de proteinuria (alrededor del 90%).

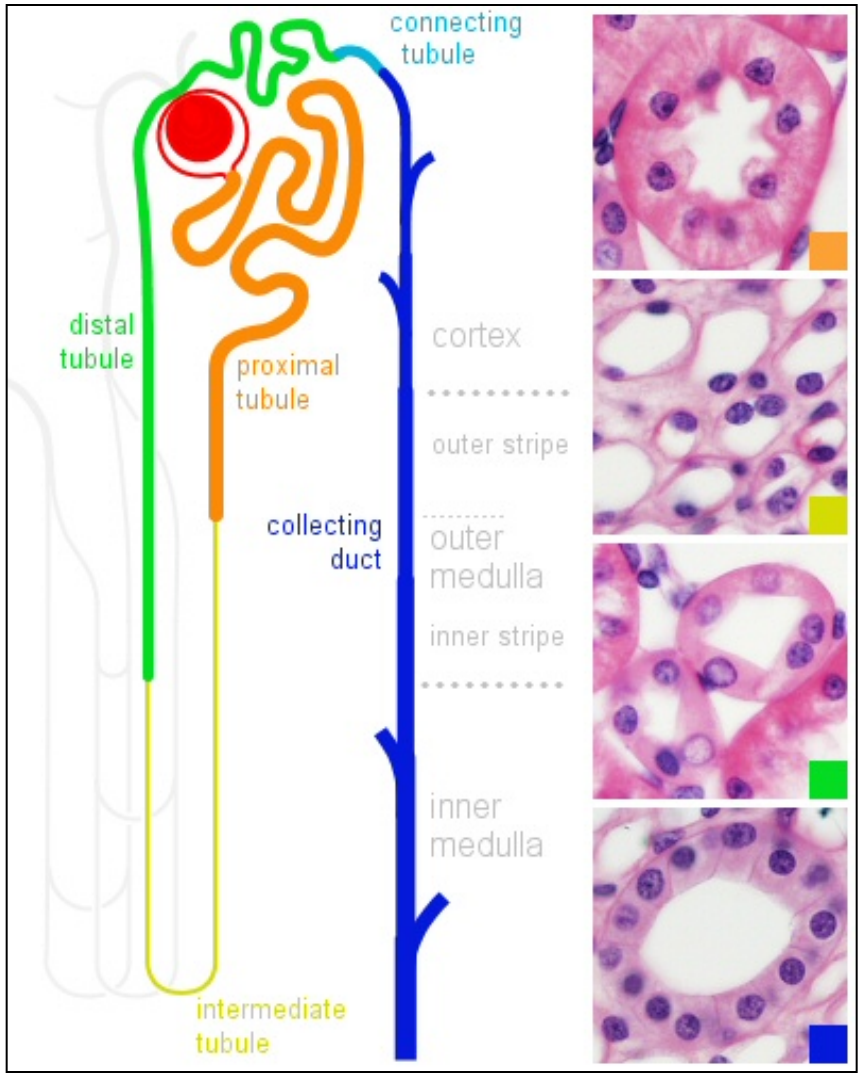
Proteínas de bajo peso, como la β2-microglobulina, aminoácidos, y cadenas livianas, tienen un peso molecular de hasta 25 kDa (albúmina: 69 kDa).

Estas proteínas cruzan libremente la membrana basal glomerular y luego son completamente reabsorbidas por las células proximales tubulares.

Recordar que la enfermedades glomerulares, al progresar, se compañan de injuria tubular y proteinuria tubular.

Table 1 | Types of proteinuria

Types	Characteristics
Glomerular	Most common form, up to 90% Feature of chronic kidney disease Loss of albumin and higher molecular weight proteins
Tubular	Low molecular weight proteins, such as β2-microglobulin
Overflow	Increased production, that is, light chains in multiple myeloma
Post-exercise	Transient benign Can be up to 10 g/day
Post-prandial	Transient physiological proteinuria Possibly through insulin action in podocytes
Infection-associated	Physiological response Mediated by toll-receptors Possibly involved in clearing pathogens from the circulation



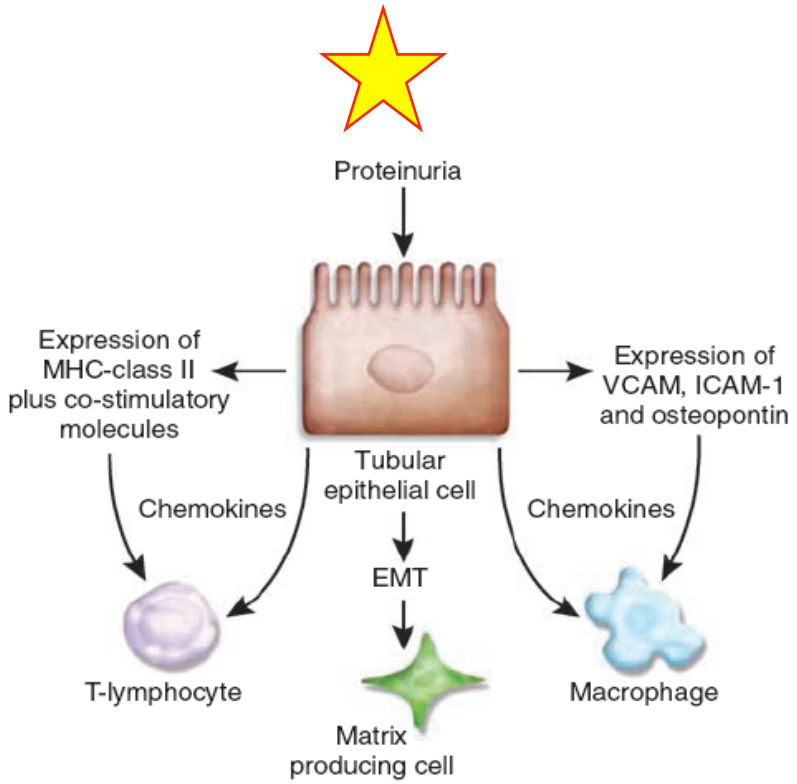
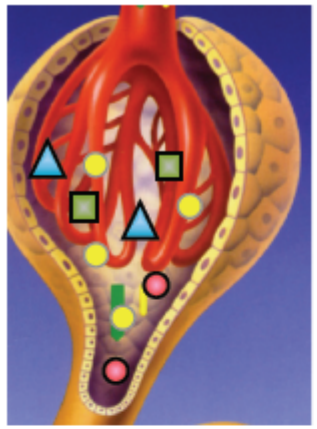
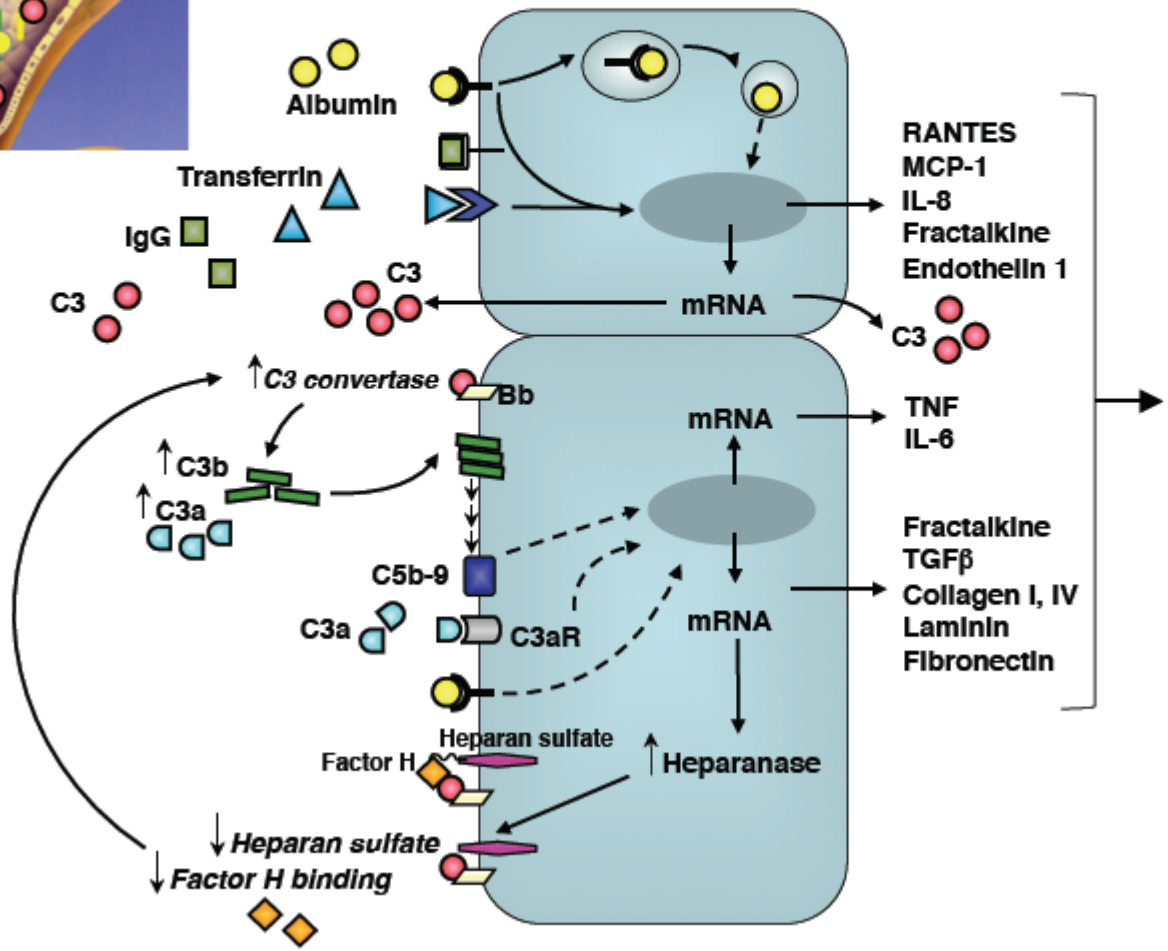


Figure 1 | Effects of proteinuria on tubular epithelial cells. Increased protein absorption by tubular cells may result in direct tubular toxicity, release of chemokines and cytokines, increased expression of adhesion and MHC class II molecules along with co-stimulatory molecules. The net effect is an increased influx of mononuclear inflammatory cells. The evidence for direct proteinuria induced EMT is weak.





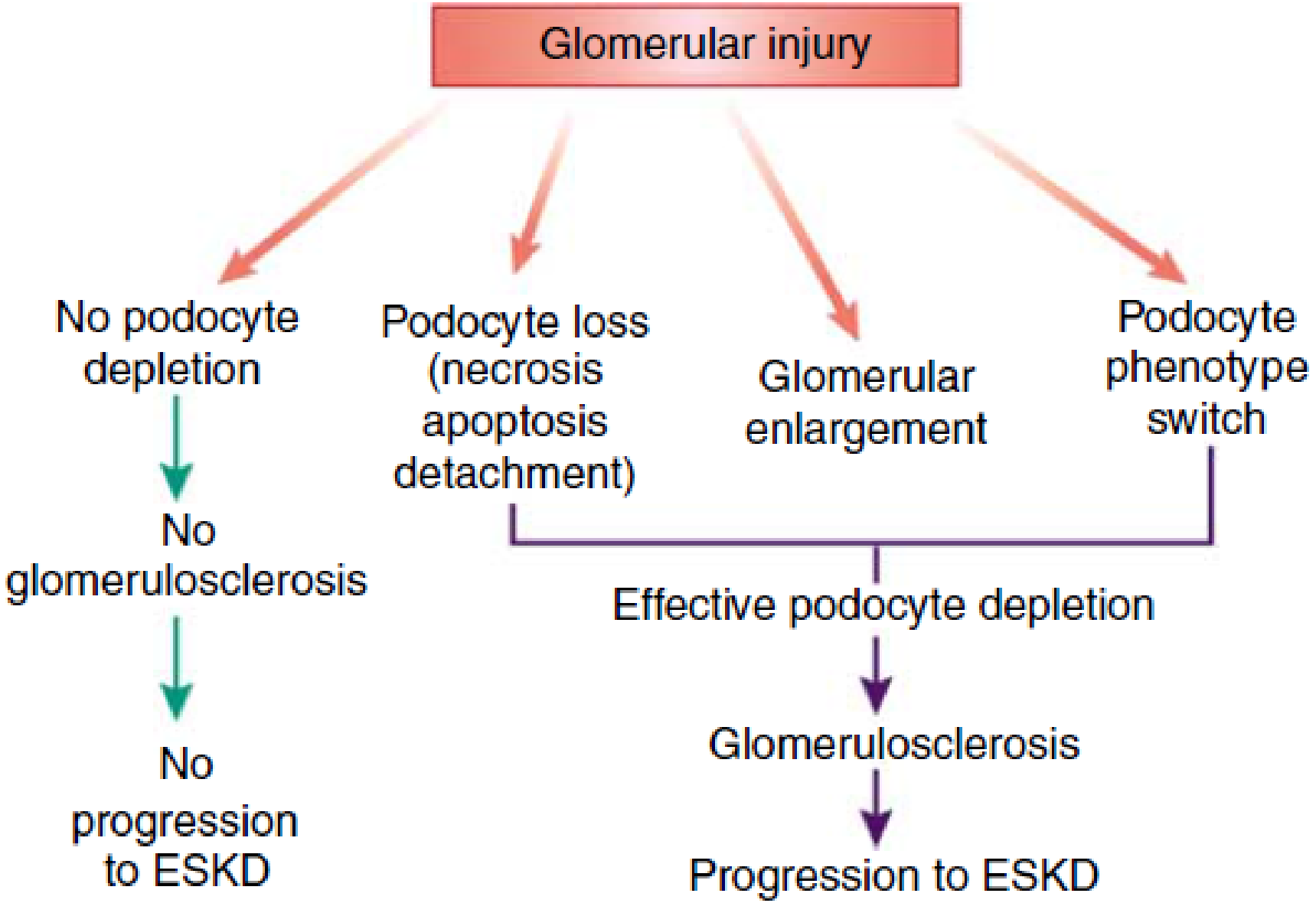
Inflammation and T cell recruitment

↓

Accumulation of fibroblasts

↓

Fibrosis



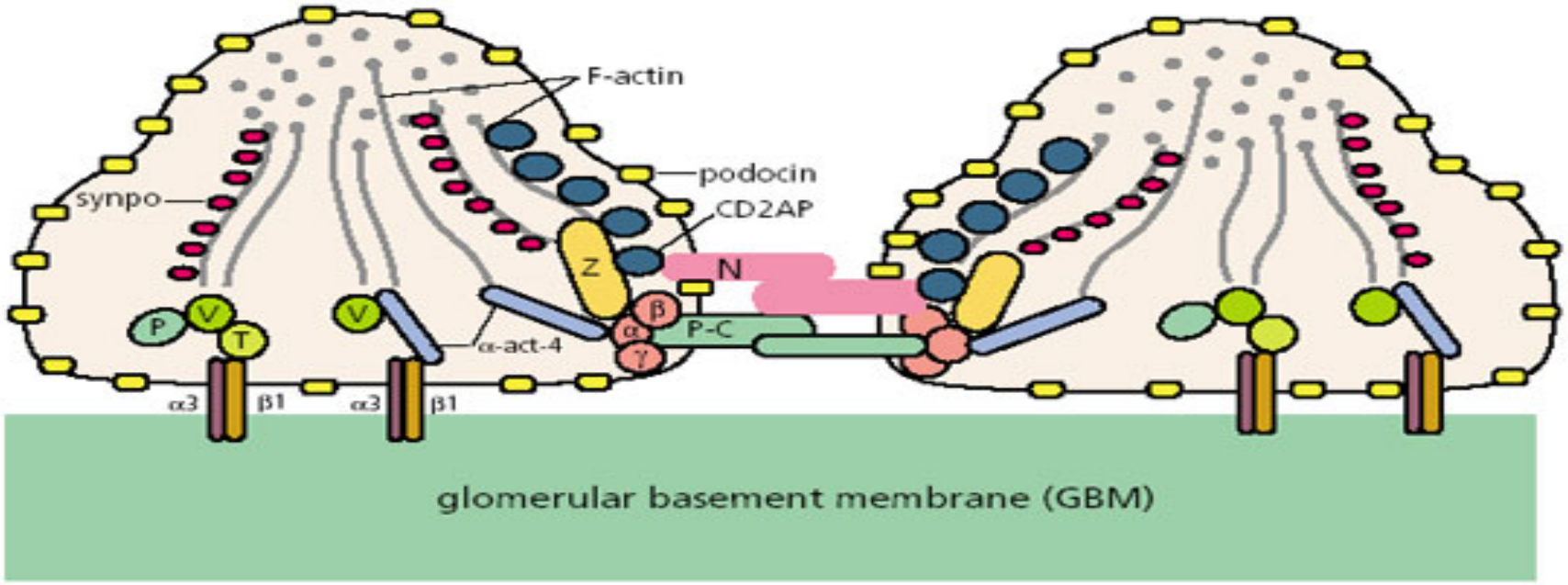
RC Wiggins: Spectrum of podocytopathies *Kidney International* (2007) **71,** 1205–1214;

Los IECASs y los ARA II producen cambios reversibles en la estructura y función de la pared capilar glomerular y de las células mesangiales y de la matriz, al inhibir la acción de la angiotensina II:

Reordenan y estabilizan las hendiduras diafragmáticas, al polimerizar la actina en el citoesqueleto de los podocitos

Reducen la síntesis del TGF-β, colágenos I y III

Reducen la hipertrofia celular inducida por la angiotensina II

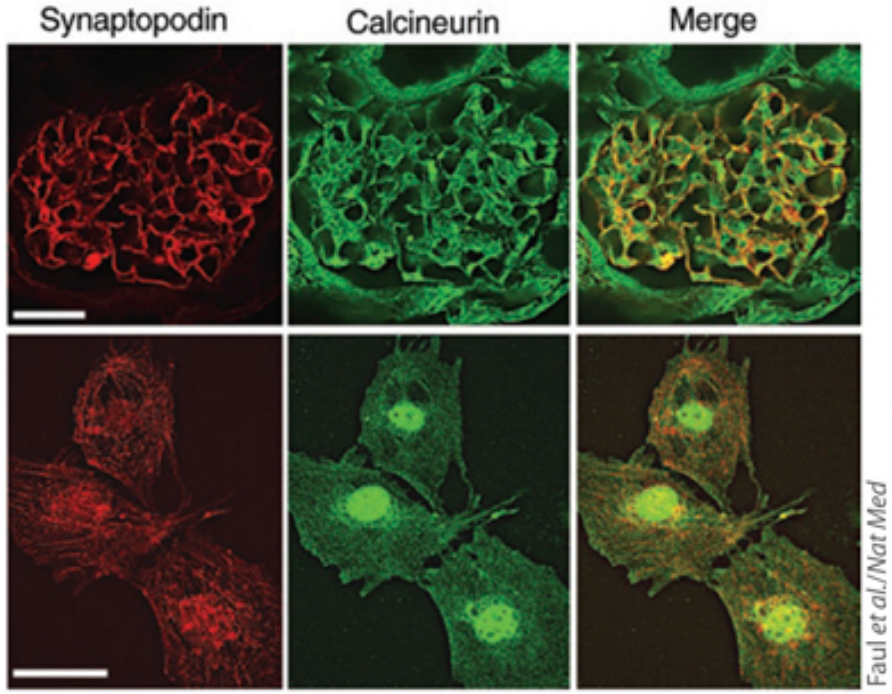


CICLOSPORINA

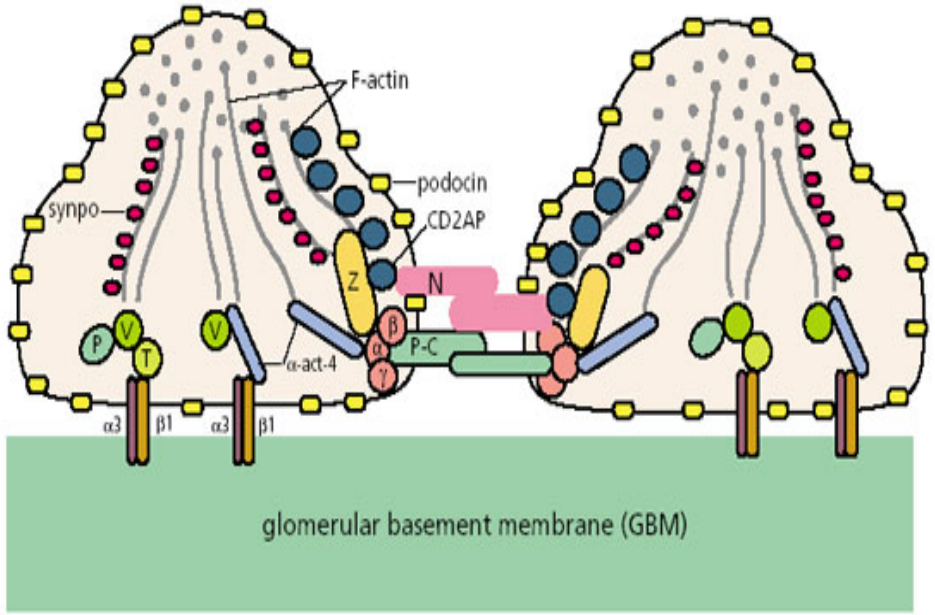
La CsA bloquea la defosforilación de la sinaptopodina, una proteína organizadora de la actina del podocito. Este bloqueo inhibe la proteólisis de la sinaptopodina, estabilizando las hendiduras diafragmáticas y la contracción-relajación normal del podocito.

Este efecto es independiente de la acción sobre las células B y T.

Interesante: La expresión de calcineurinas en el podocito resulta en la degradación de la sinaptopodina y el desarrollo de proteinuria.



Faul *et al./Nat Med*



Rol del TRPC6 Transient receptor potential cation channel 6 (trPC6)

Sobreexpresada en familias con FsGs autosómica-dominante.
Estos canales regulan la entrada de calcio intracelular.

En los podocitos, el trPC6 se localiza en la hendidura del diafragma, y participa en la señalización.

Su sobreexpresión resulta en proteinuria. Es blanco del Tacrolimus

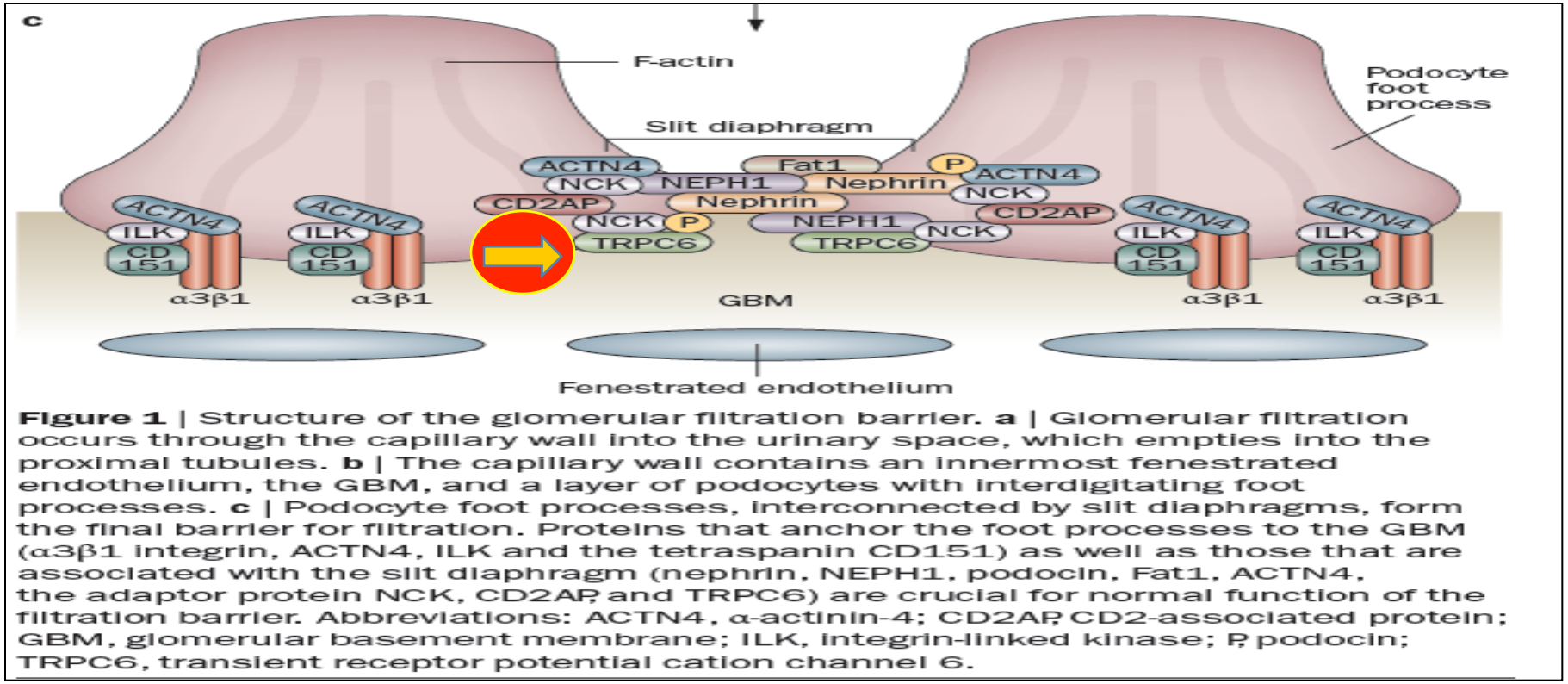


Figure 1 | Structure of the glomerular filtration barrier. **a** | Glomerular filtration occurs through the capillary wall into the urinary space, which empties into the proximal tubules. **b** | The capillary wall contains an innermost fenestrated endothelium, the GBM, and a layer of podocytes with interdigitating foot processes. **c** | Podocyte foot processes, interconnected by slit diaphragms, form the final barrier for filtration. Proteins that anchor the foot processes to the GBM (α3β1 integrin, ACTN4, ILK and the tetraspanin CD151) as well as those that are associated with the slit diaphragm (nephrin, NEPH1, podocin, Fat1, ACTN4, the adaptor protein NCK, CD2AP, and TRPC6) are crucial for normal function of the filtration barrier. Abbreviations: ACTN4, α-actinin-4; CD2AP, CD2-associated protein; GBM, glomerular basement membrane; ILK, integrin-linked kinase; P, podocin; TRPC6, transient receptor potential cation channel 6.

Table 2 Effects and target structures of new anti-proteinuric strategies on podocytes

New anti-proteinuric strategies	Target structures/pathways on podocytes
Abatacept (CTLA-4–Ig)	B7-1, integrin signalling [4, 93, 96]
Adalimumab (Anti-TGF-β)	TGF-β/SMAD, apoptosis [111]
Amiloride (suPAR blocker)	β3-integrin signalling [99, 100]
Oral ManNAc	Angptl 4 [116]
Ruboxistaurin (selective PKC-β inhibitor)	(Among others) extracellular matrix synthesis/ turnover [120, 121]
Saquinavir (protease inhibitor)	NF-κB/IκBα [122, 123]
TRPC6 siRNA	Ca^{2+} levels, actin cytoskeleton rearrangement [107–110]

Ig, Immunoglobulin; TGF-β, transforming growth factor beta, MAnNAc, *N*-acetyl-D-mannosamine; protein kinase C beta; TRPC6, transient receptor potential cation channel, subfamily C, member 6; siRNA, small interfering RNA; Angptl 4, angiopoietin-like 4; NF-κB/IκBα, nuclear factor kappa-light-chain-enhancer of activated B cells/inhibitor of kappa B

Abatacept and Glomerular Diseases: The Open Road for the Second Signal as a New Target is Settled Down

Hernán Trimarchi*

Plasma levels and urinary excretion of fibrinolytic and protease inhibitory proteins in nephrotic syndrome

N. D. VAZIRI, E. C. GONZALES, B. SHAYESTEHFAR, and C. H. BARTON

IRVINE, CALIFORNIA

J Lab Clin Med
July 1994

Protease Crosstalk with Integrins: the Urokinase Receptor Paradigm

Harold A. Chapman, Ying Wei

Thromb Haemost 2001; 86: 124–9

Nephrol Dial Transplant (2012) 27: 1746–1755
doi: 10.1093/ndt/gfr612
Advance Access publication 9 November 2011

Amiloride off-target effect inhibits podocyte urokinase receptor expression and reduces proteinuria

Bin Zhang[1,2,*], Shaoting Xie[1,2,*], Wei Shi[1] and Yun Yang[1]

Pediatr Nephrol
DOI 10.1007/s00467-015-3116-4

EDUCATIONAL REVIEW

Podocyte directed therapy of nephrotic syndrome—can we bring the inside out?

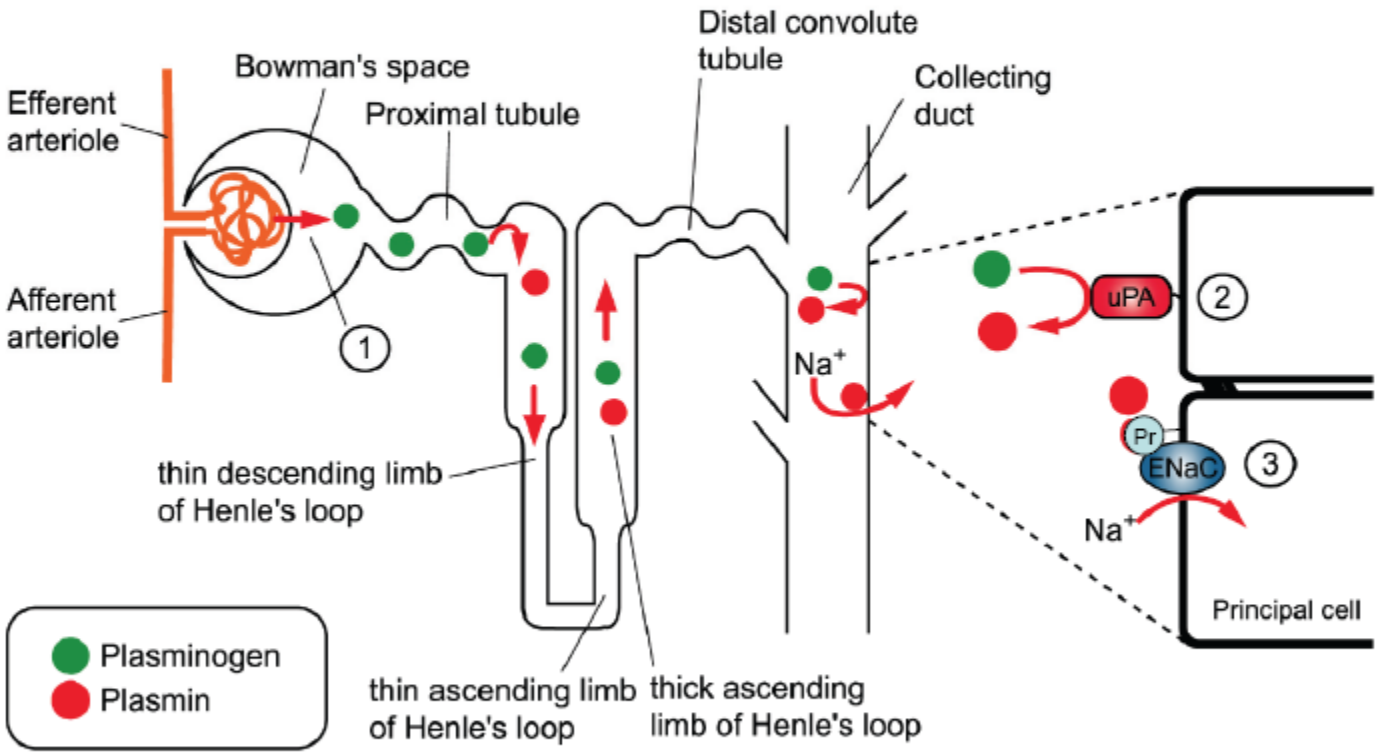
Janina Müller-Deile[1] · Mario Schiffer[1]

Case Report

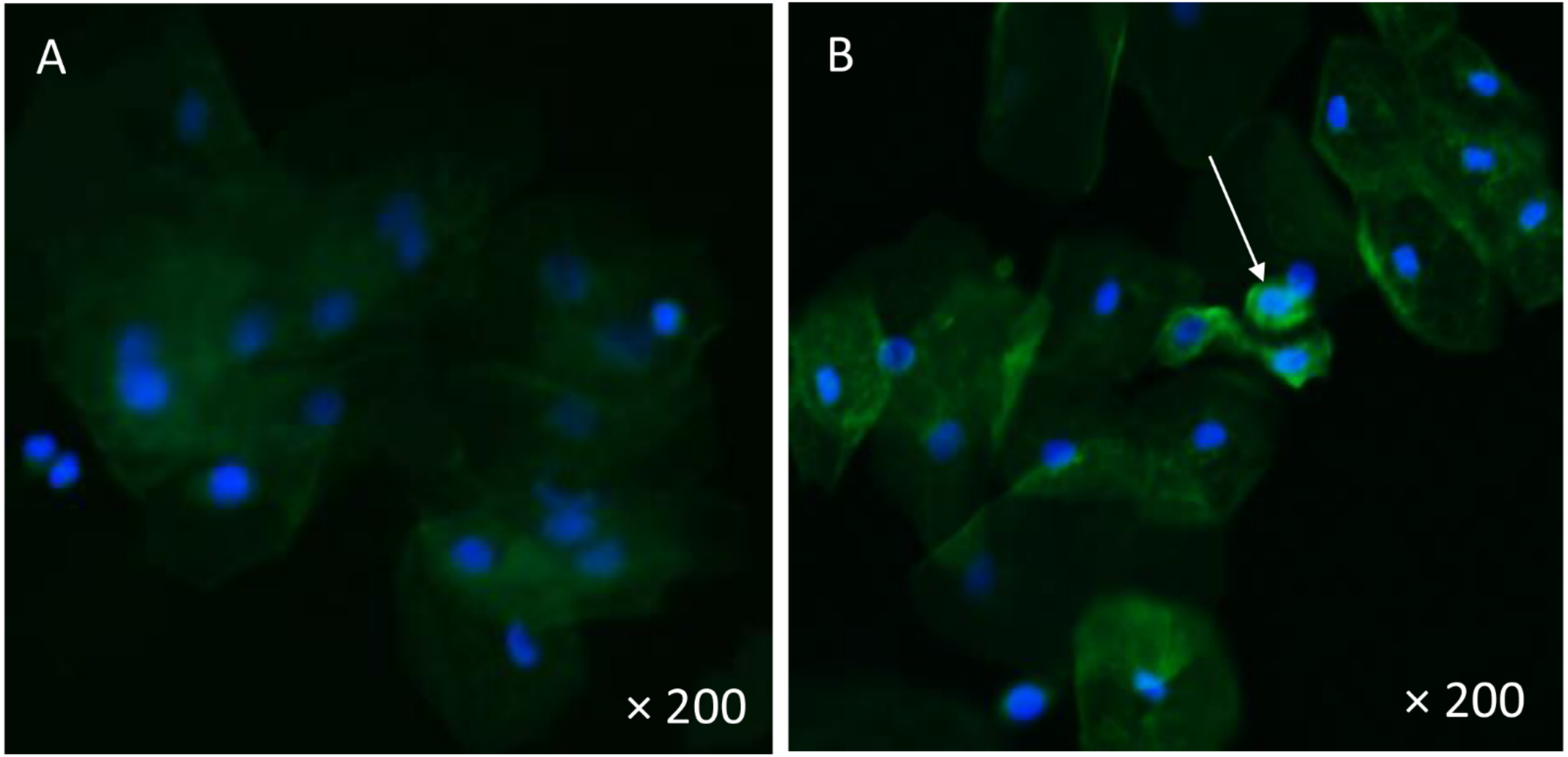
Amiloride as an Alternate Adjuvant Antiproteinuric Agent in Fabry Disease: The Potential Roles of Plasmin and uPAR

H. Trimarchi, M. Forrester, F. Lombi, V. Pomeranz, M. S. Raña, A. Karl, and J. Andrews

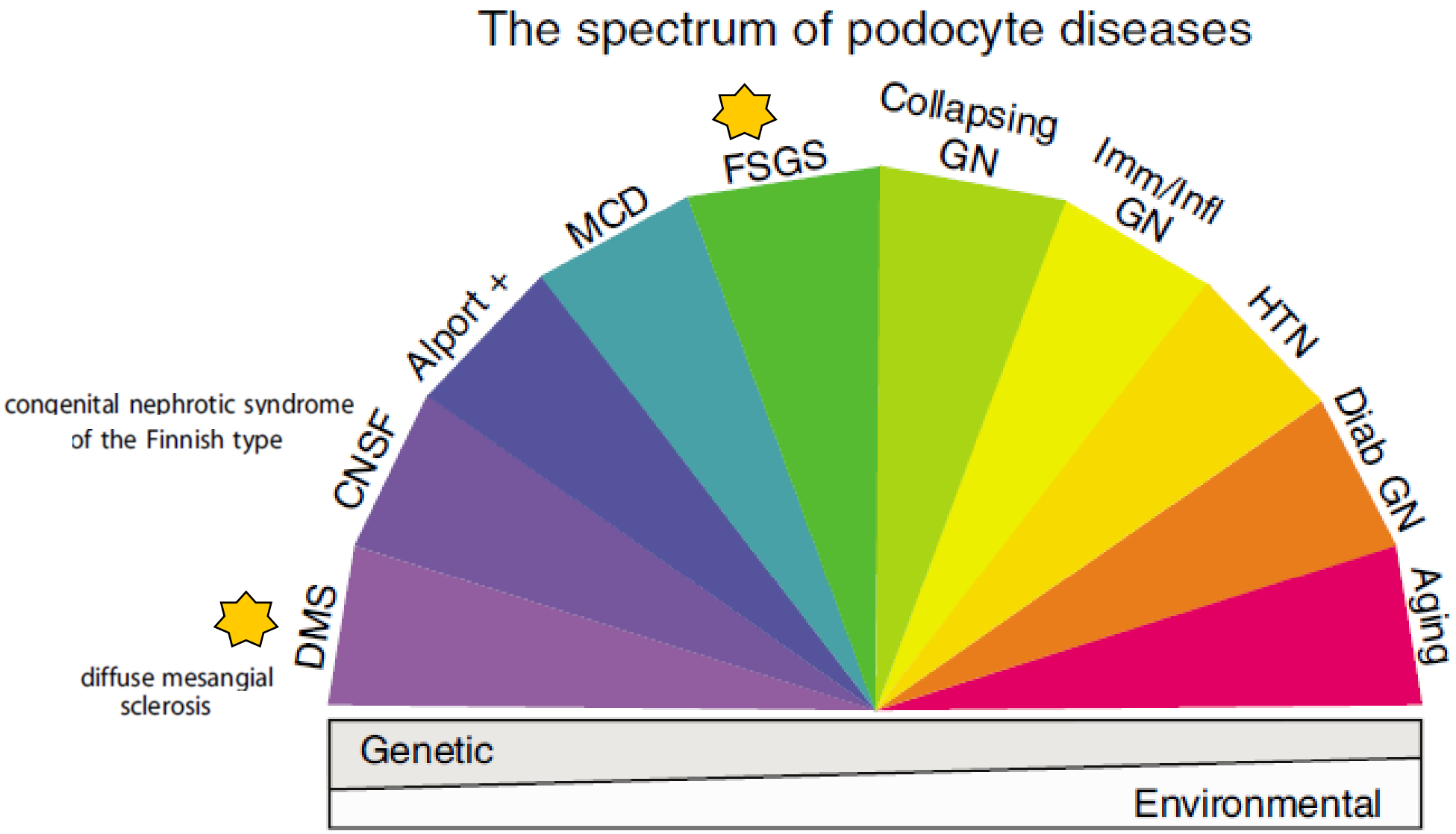
A novel model for stimulation of sodium reabsorption in nephrotic syndrome



Proteinuria may promote distal sodium reabsorption


A
× 200
B
× 200

Cerro Torre, glacier and lagoon, Santa Cruz Argentina


The spectrum of podocyte diseases
FSGS
Collapsing GN
Imm/Infl GN
HTN
Diab GN
Aging
MCD
Alport +
CNSF
congenital nephrotic syndrome of the Finnish type
DMS
diffuse mesangial sclerosis
Genetic
Environmental

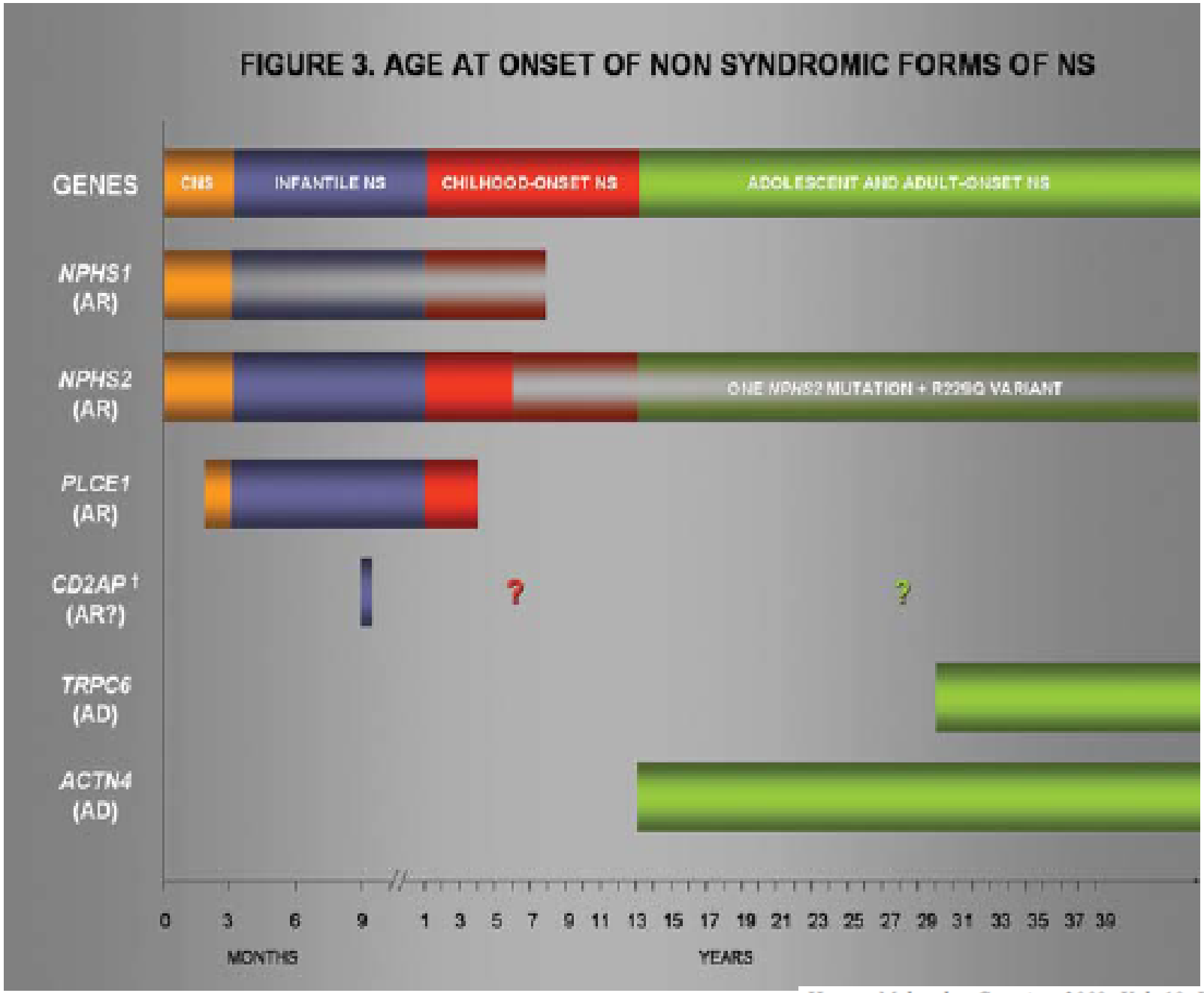
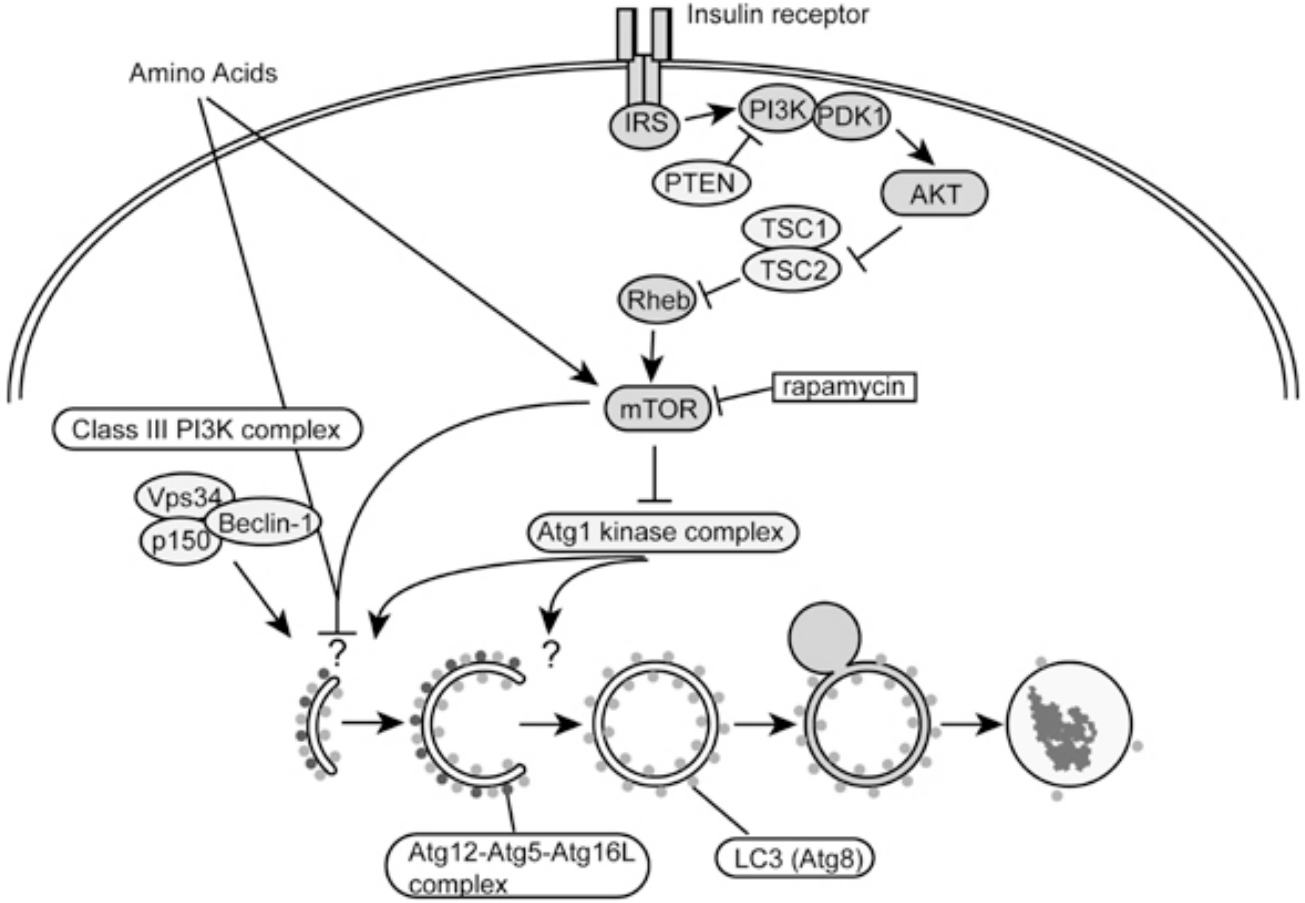


FIGURE 3. AGE AT ONSET OF NON SYNDROMIC FORMS OF NS
GENES
CNS
INFANTILE NS
CHILHOOD-ONSET NS
ADOLESCENT AND ADULT-ONSET NS
NPHS1 (AR)
NPHS2 (AR)
ONE NPHS2 MUTATION + R229Q VARIANT
PLCE1 (AR)
CD2AP † (AR?)
?
?
TRPC6 (AD)
ACTN4 (AD)
0 3 6 9 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
MONTHS
YEARS

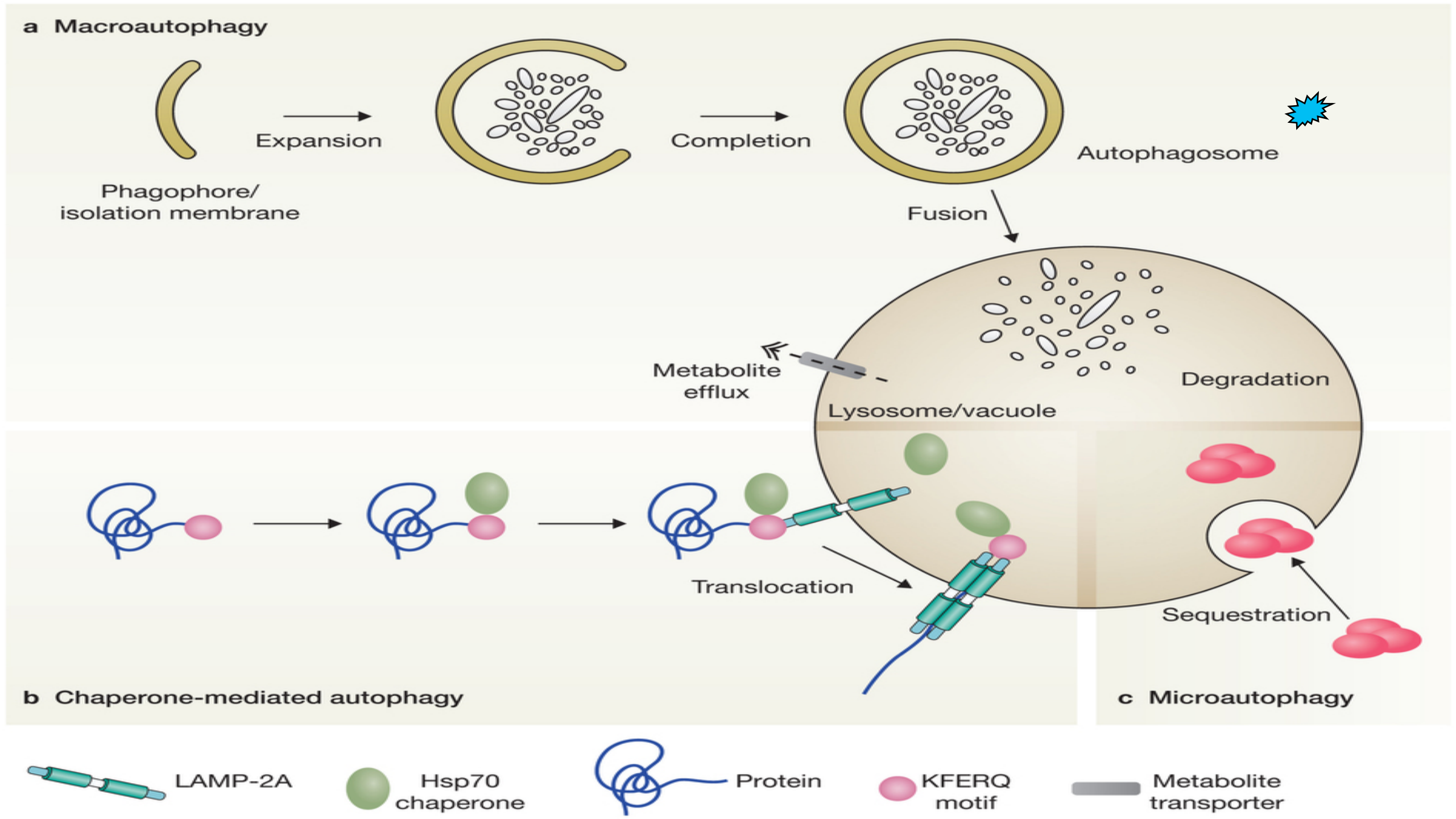
Accumulation of Gb_3 is accompanied by an increase in autophagosomes, suggesting that deregulated autophagy pathways have some involvement in the pathogenesis of glomerular damage in Fabry disease.

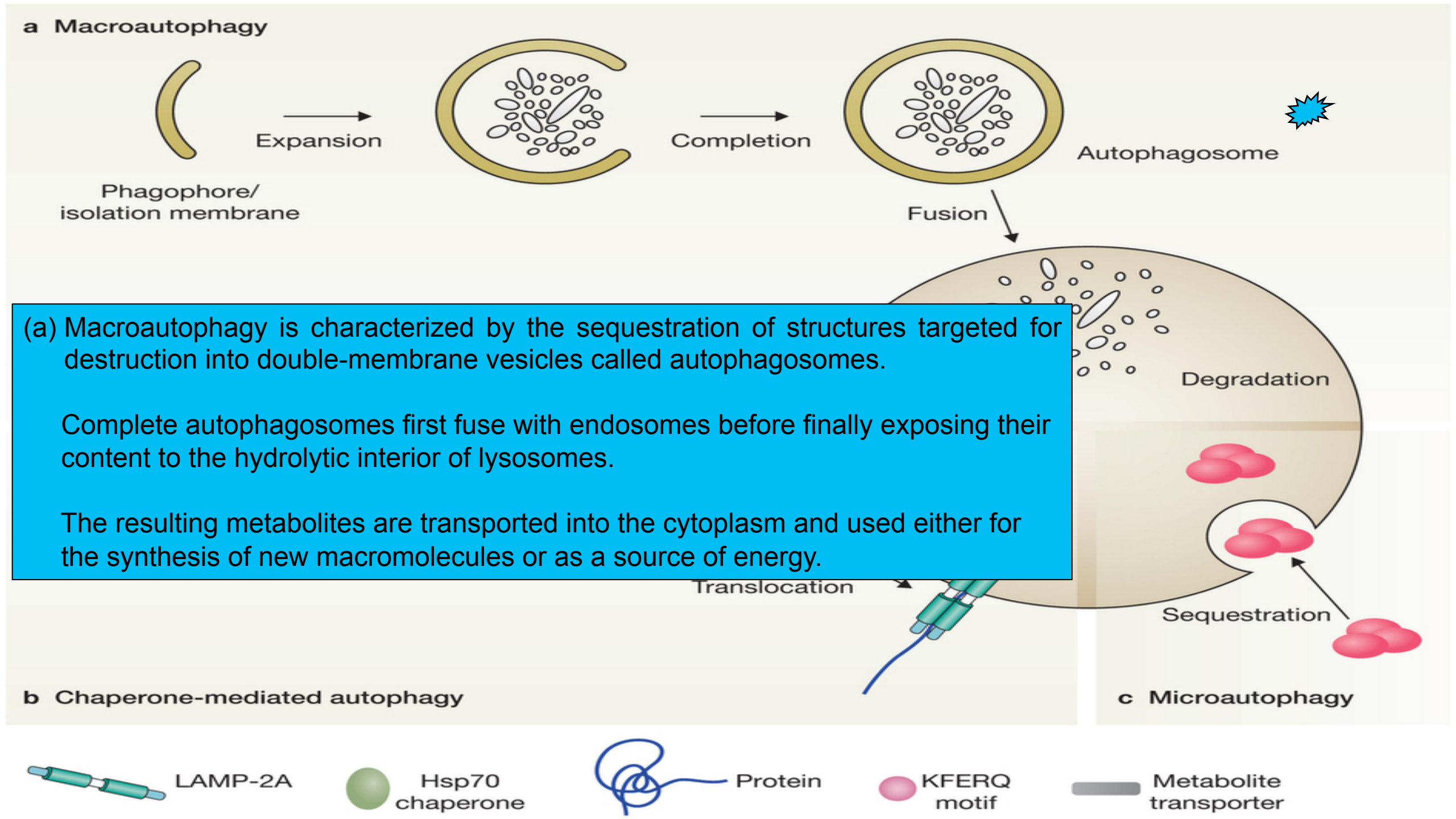
AUTOPHAGY

Autophagy enables the cell to have access to nutrients in situations of stress or starvation.
Intracellular material is degraded in a lysosome dependent mechanism, and autophagy serves as an intracellular recycling system.



An isolation membrane engulfs intracellular targets to become an autophagosome containing the LC3-II isoform of the **essential autophagy protein LC3**. The autophagosome then fuses with a lysosome to form a so called autophagolysosome, which contains damaged and dysfunctional organelles like mitochondria.


a Macroautophagy
Phagophore/
isolation membrane
Expansion
Completion
Autophagosome
Fusion
Metabolite
efflux
Degradation
Lysosome/vacuole
Translocation
Sequestration
b Chaperone-mediated autophagy
c Microautophagy
LAMP-2A
Hsp70
chaperone
Protein
KFERQ
motif
Metabolite
transporter



(a) Macroautophagy is characterized by the sequestration of structures targeted for destruction into double-membrane vesicles called autophagosomes.

Complete autophagosomes first fuse with endosomes before finally exposing their content to the hydrolytic interior of lysosomes.

The resulting metabolites are transported into the cytoplasm and used either for the synthesis of new macromolecules or as a source of energy.

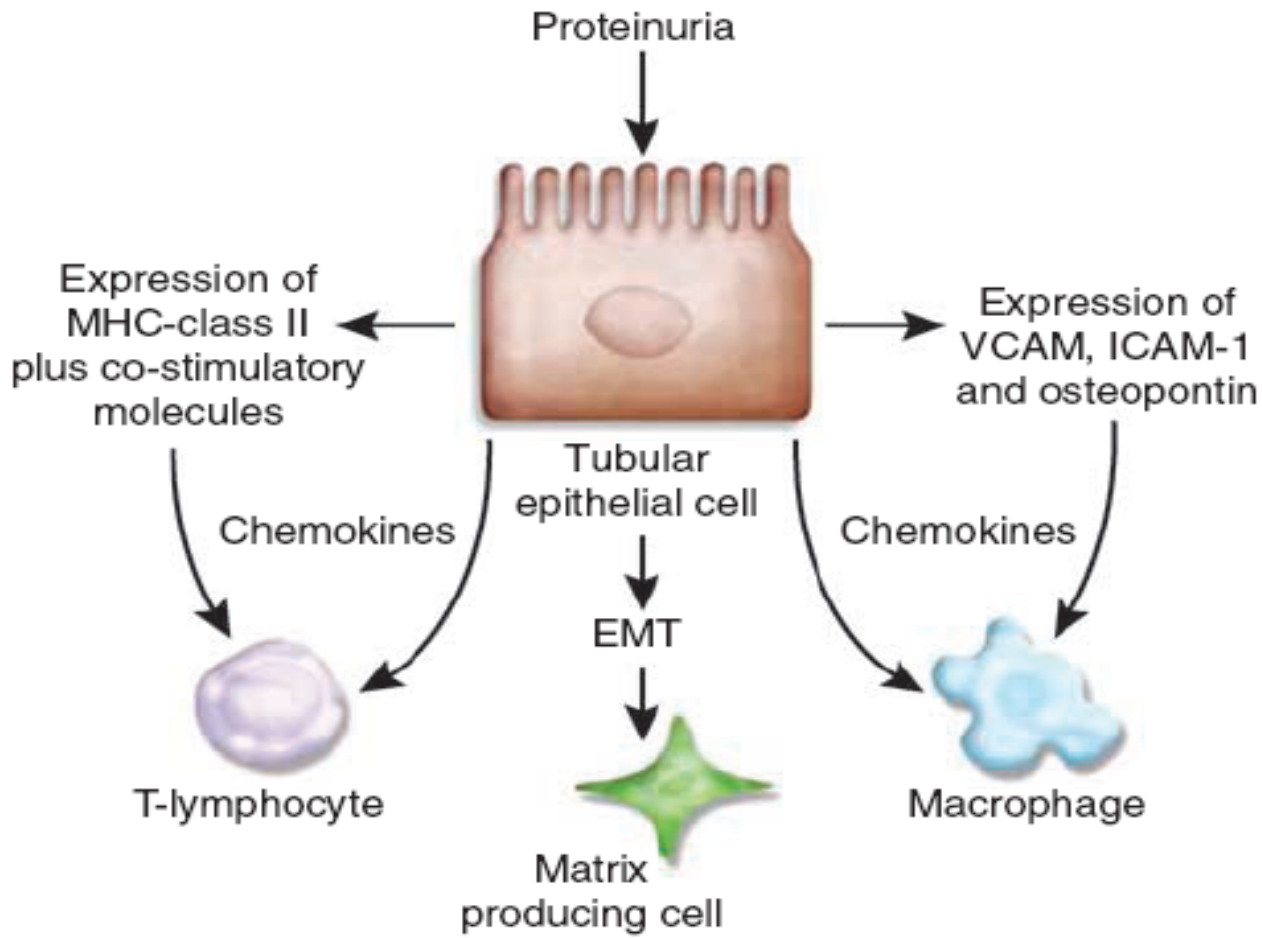
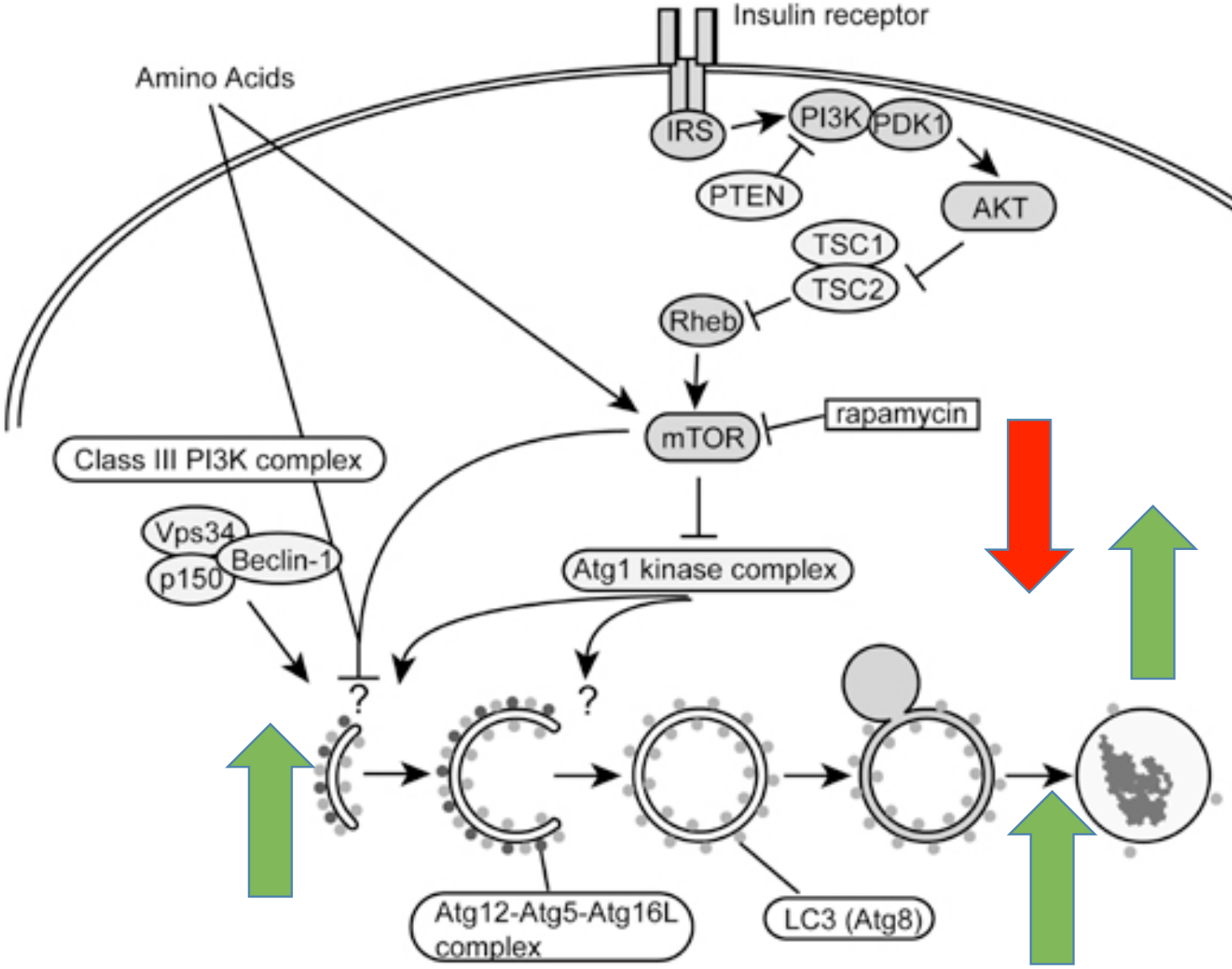
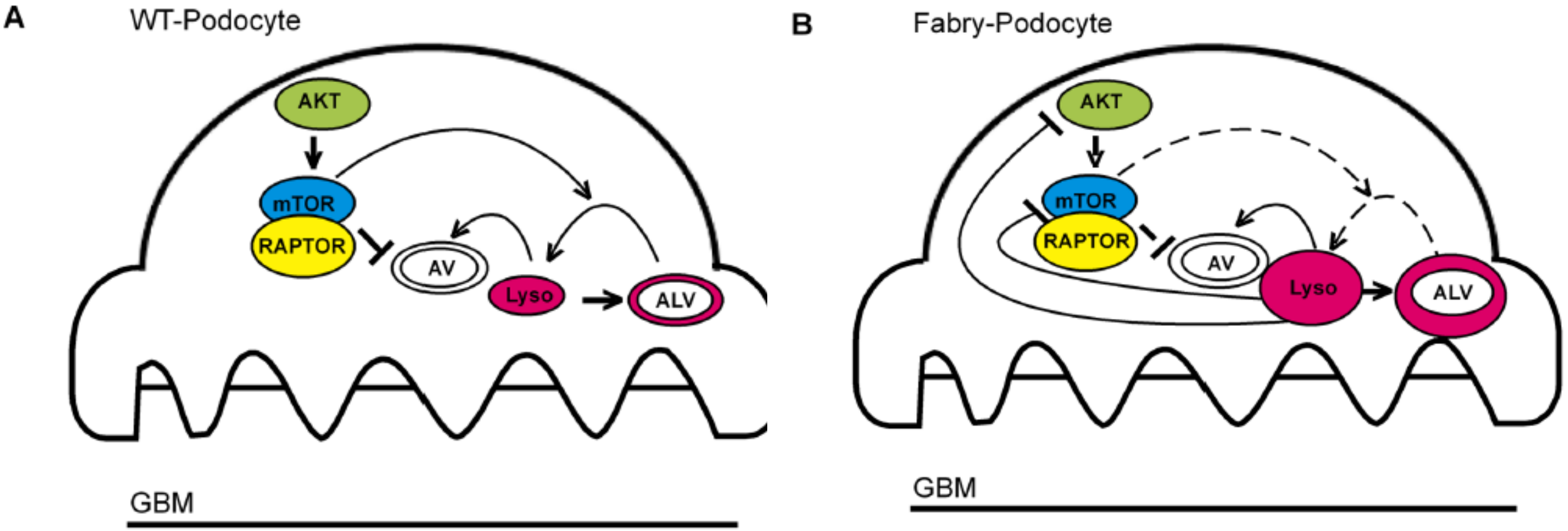


Figure 1 | Effects of proteinuria on tubular epithelial cells. Increased protein absorption by tubular cells may result in direct tubular toxicity, release of chemokines and cytokines, increased expression of adhesion and MHC class II molecules along with co-stimulatory molecules. The net effect is an increased influx of mononuclear inflammatory cells. The evidence for direct proteinuria induced EMT is weak.

Interestingly, these changes were accompanied by an increase in autophagosomes as indicated by an increased abundance of LC3-II and a loss of mTOR kinase activity, a negative regulator of autophagy.



Dysregulated autophagy in a-galactosidase A-deficient podocytes may be the result of deficient mTOR kinase activity.

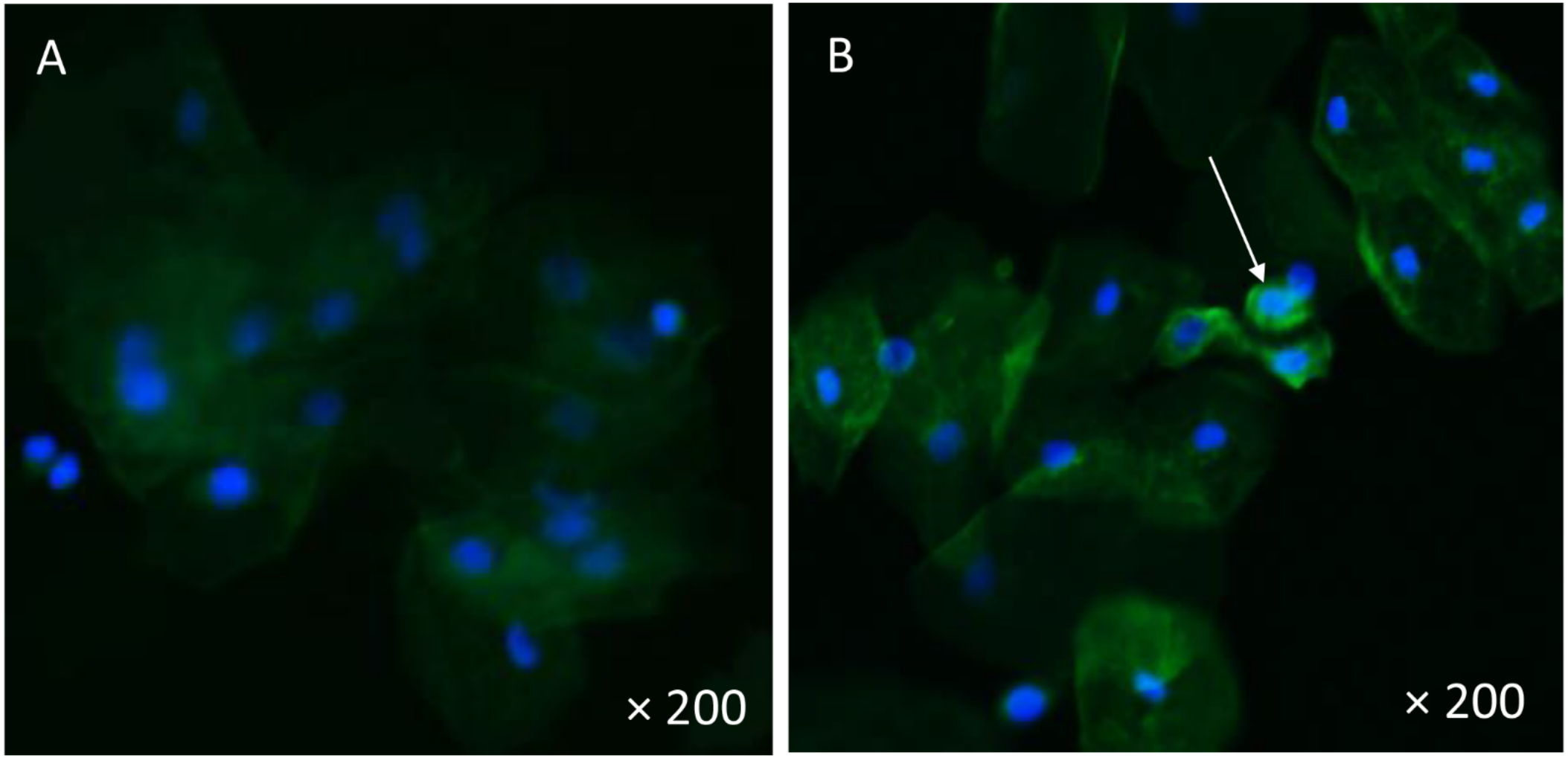


Model on the effect of a-Gal depletion in podocytes

mTOR negatively regulates the formation of autophagy vesicles and promotes the recovery of autophagosomes (AV) and lysosomes (Lys) from autophagolysosomes (ALV) in podocytes (continuous lines).

In Fabry disease a-Gal A dysfunction leads to an accumulation of Gb3 in lysosomes, an increase in autophagosomes and furthermore dysregulates autophagy signaling (dashed lines) by an inhibition of mTOR and its upstream regulator AKT (continuous line).

PODOCYTURIA IN FABRY DISEASE IS ELEVATED IN UNTREATED VS TREATED ADULT PATIENTS AND DOES NOT CORRELATE WITH PROTEINURIA OR RENAL FUNCTION

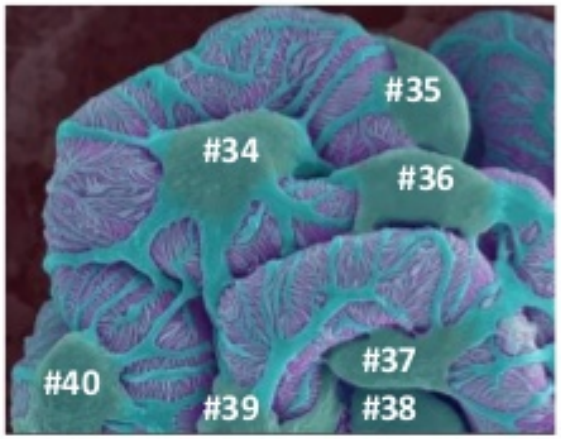


Trimarchi H et al. To be presented at ERA-EDTA London 2015

Each Podocyte Counts!

PODOCYTES 2%
Tryggvason 2011

1,500,000 **– 2,000,000 glomeruli**



Each glomerulus has 500-600 podocytes.

Podocytes do not efficiently proliferate.

Podocyte loss is cumulative in time

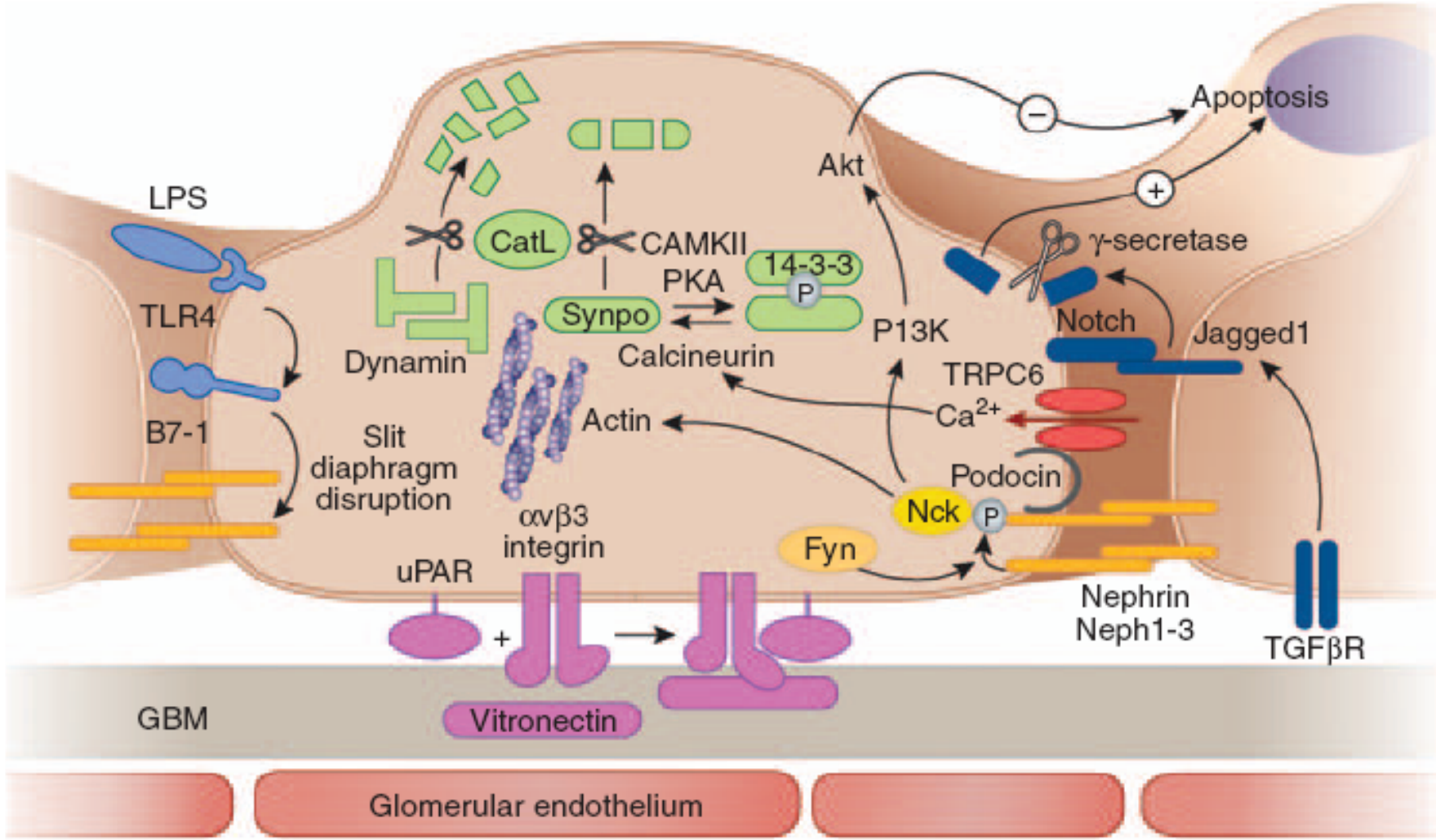
1,000,000,000 podocytes

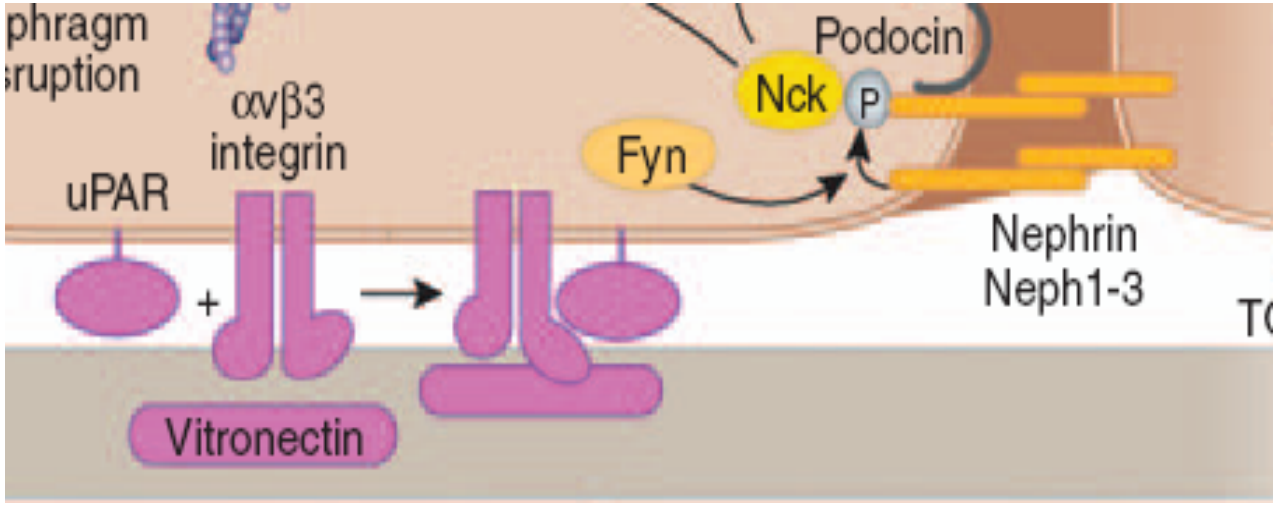
Once a glomerulus loses more than ~20% of its podocytes, it scars down. This injury is irreversible.

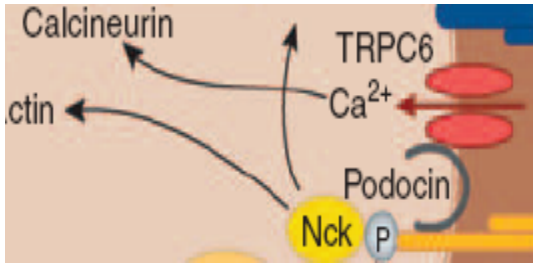
100 podocytes/glomerulus loss of 1 nephron

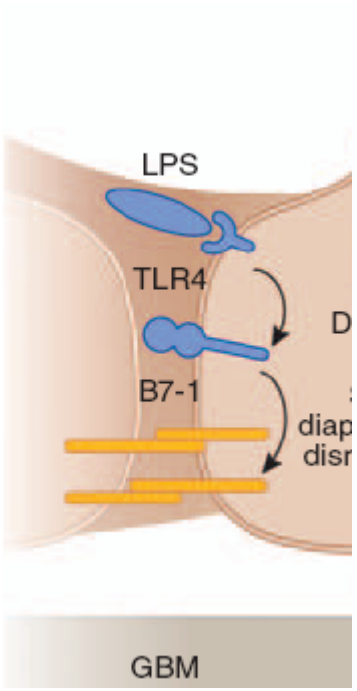
200,000,000 of podocyte losses leads to ESRD

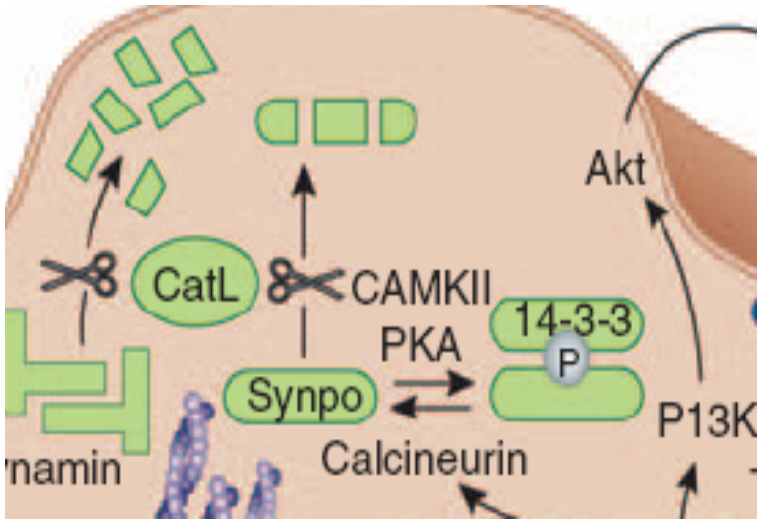
With permission B Najafian FSIG Expert Fabry Conference San Diego 2014

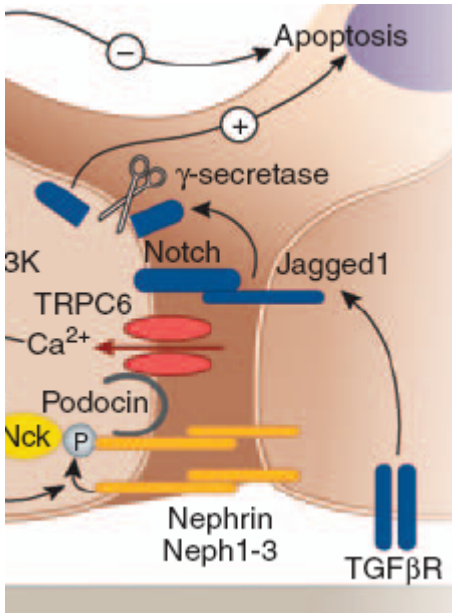

LPS
TLR4
B7-1
Slit
diaphragm
disruption
CatL
CAMKII
PKA
14-3-3
P
Synpo
Dynamin
Calcineurin
Actin
αvβ3
integrin
uPAR
Vitronectin
GBM
Glomerular endothelium
Akt
P13K
Apoptosis
γ-secretase
Notch
Jagged1
TRPC6
Ca2+
Podocin
Nck
Fyn
Nephrin
Neph1-3
TGFβR

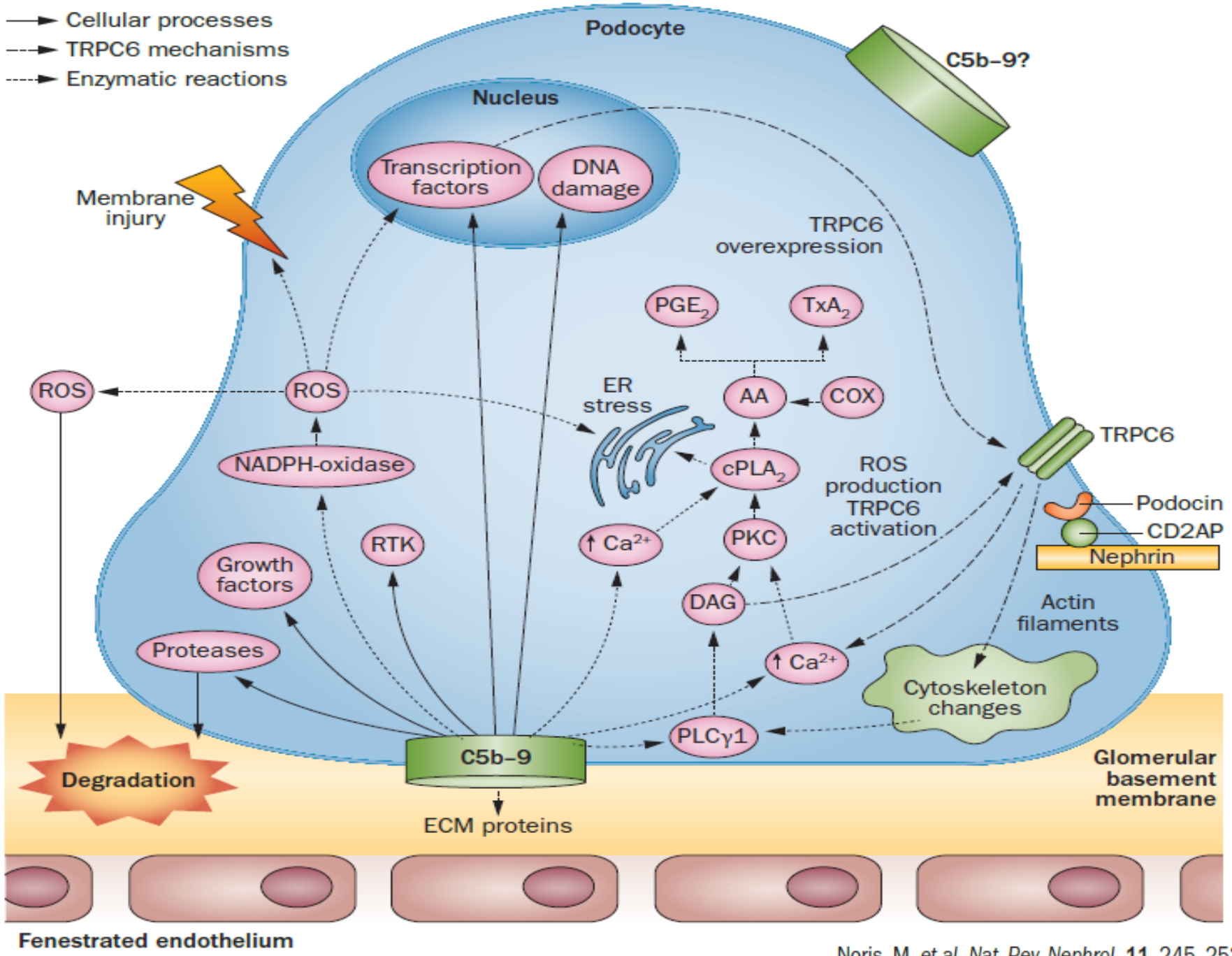

phragm
ruption
αvβ3
integrin
uPAR
Vitronectin
Fyn
Nck
P
Podocin
Nephrin
Neph1-3


Calcineurin
ctin
TRPC6
Ca2+
Podocin
Nck
P

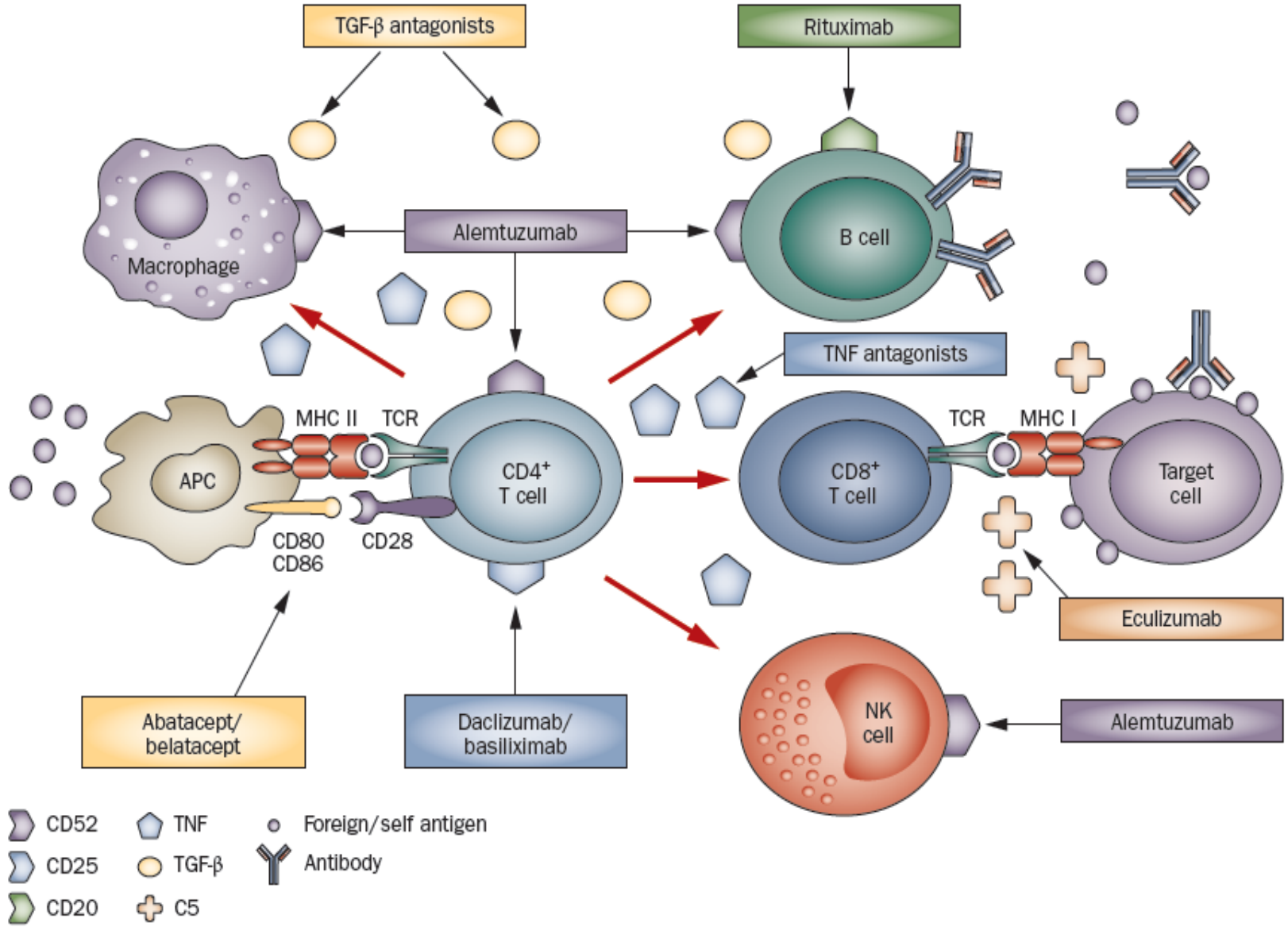

LPS
TLR4
B7-1
GBM

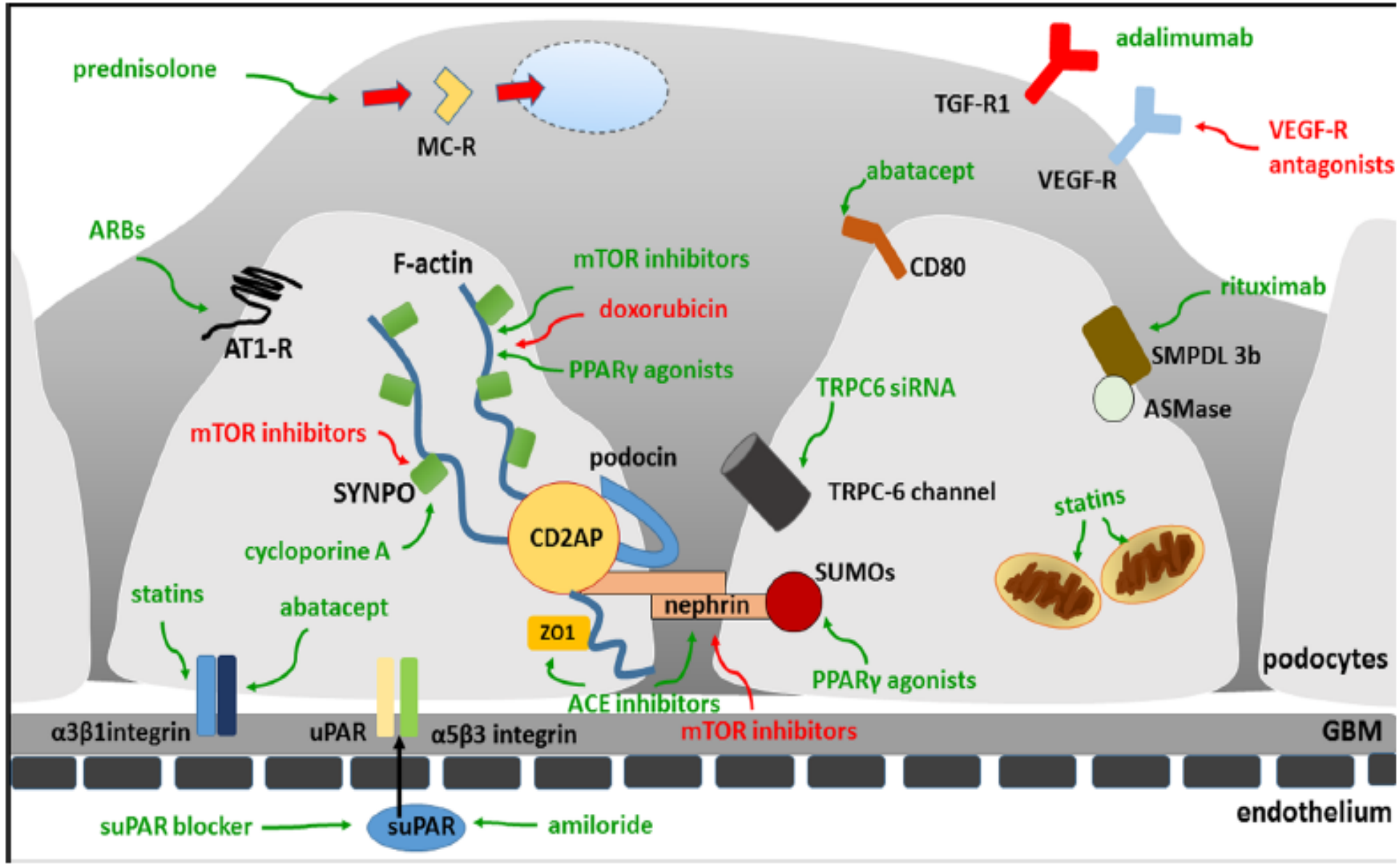

CatL
CAMKII
PKA
14-3-3
P
Synpo
Calcineurin
Akt
P13K

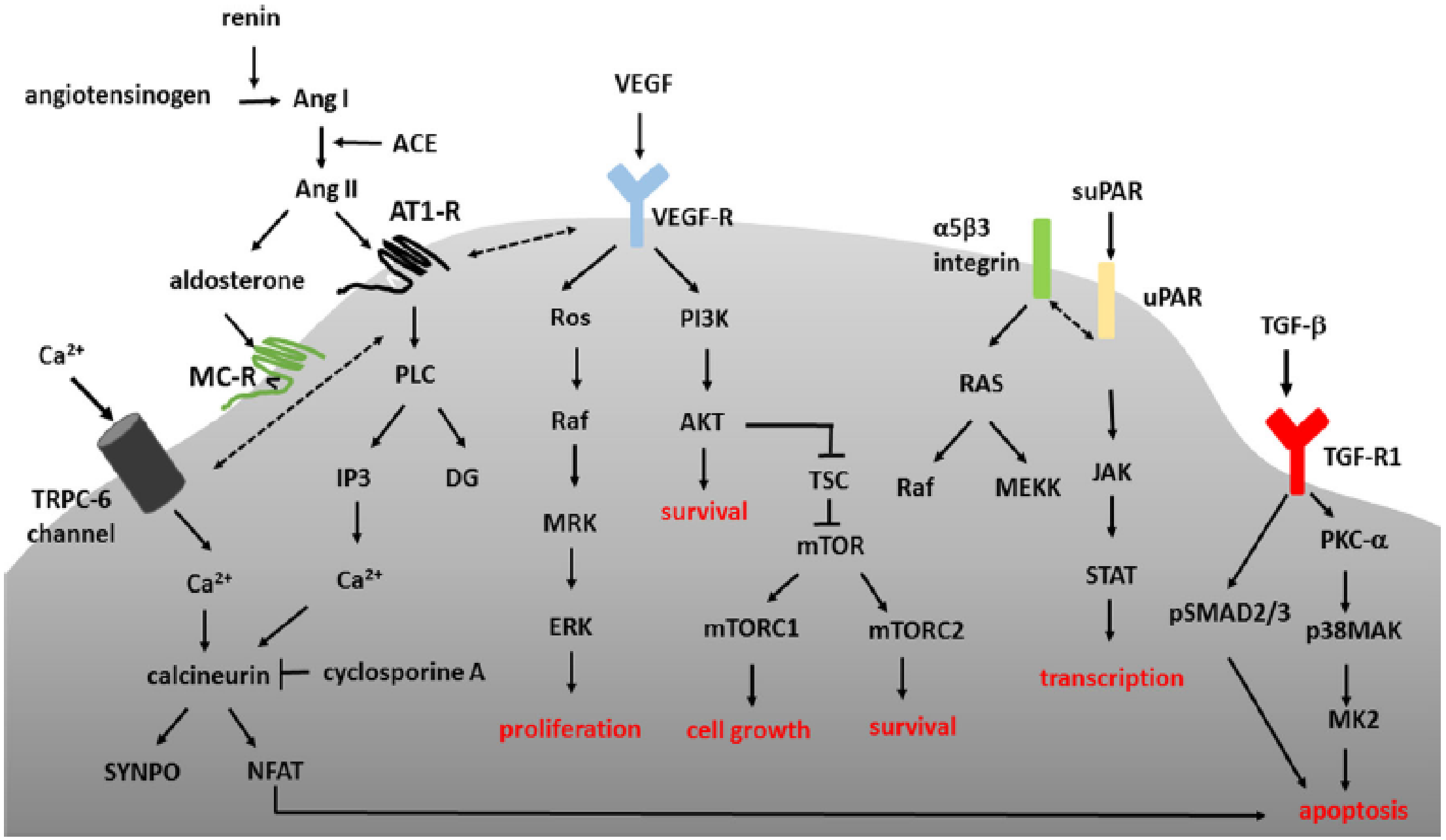

Apoptosis
γ-secretase
Notch
Jagged1
TRPC6
Ca2+
Podocin
Nck
P
Nephrin
Neph1-3
TGFβR



Noris, M. *et al. Nat. Rev. Nephrol.* **11**, 245–252 (2015)


TGF-β antagonists
Rituximab
Macrophage
Alemtuzumab
B cell
TNF antagonists
MHC II
TCR
APC
CD4+
T cell
CD80
CD86
CD28
CD8+
T cell
TCR
MHC I
Target
cell
Eculizumab
Abatacept/
belatacept
Daclizumab/
basiliximab
NK
cell
Alemtuzumab
CD52
CD25
CD20
TNF
TGF-β
C5
Foreign/self antigen
Antibody


prednisolone
MC-R
adalimumab
TGF-R1
VEGF-R
VEGF-R antagonists
abatacept
CD80
ARBs
AT1-R
F-actin
mTOR inhibitors
doxorubicin
PPARγ agonists
rituximab
SMPDL 3b
ASMase
TRPC6 siRNA
TRPC-6 channel
mTOR inhibitors
SYNPO
podocin
CD2AP
cycloporine A
statins
SUMOs
nephrin
statins
abatacept
ZO1
PPARγ agonists
ACE inhibitors
podocytes
α3β1integrin
uPAR
α5β3 integrin
mTOR inhibitors
GBM
endothelium
suPAR blocker
suPAR
amiloride

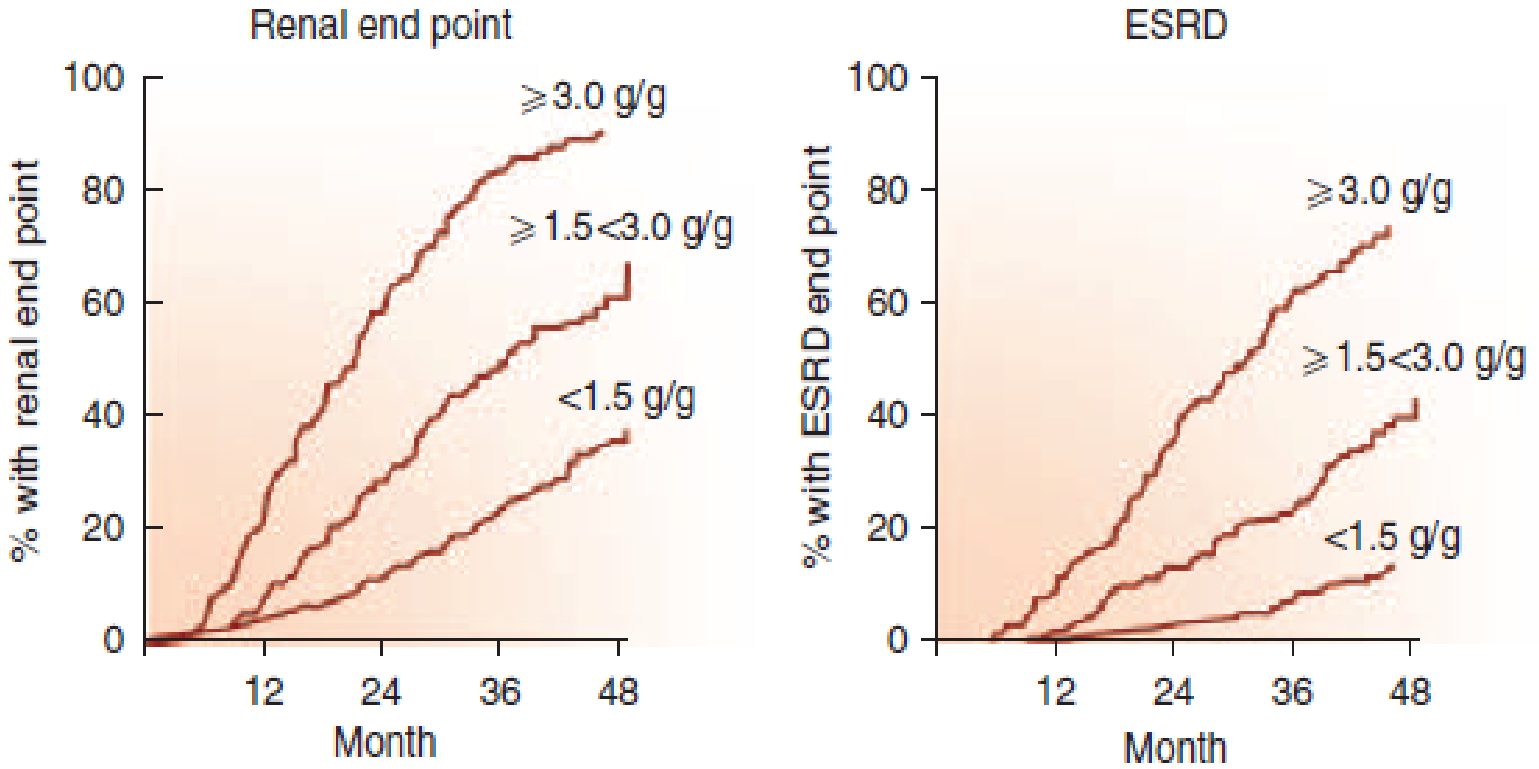

renin
angiotensinogen
Ang I
ACE
Ang II
AT1-R
aldosterone
MC-R
Ca2+
TRPC-6
channel
PLC
IP3
DG
Ca2+
Ca2+
calcineurin
cyclosporine A
SYNPO
NFAT
VEGF
VEGF-R
Ros
Raf
MRK
ERK
proliferation
PI3K
AKT
survival
TSC
mTOR
mTORC1
mTORC2
cell growth
survival
α5β3
integrin
suPAR
uPAR
RAS
Raf
MEKK
JAK
STAT
transcription
TGF-β
TGF-R1
PKC-α
pSMAD2/3
p38MAK
MK2
apoptosis

It is an early sign of Fabry nephropathy

Often the most frequent clinical manifestation

Proteinuria is an independent risk factor affecting the extent of renal decline in treated and untreated patients, and in determining the success of ERT.

Proteinuria



Data from 1,262 adult patients (585 males, 677 females) in the Fabry Registry demonstrated overt proteinuria (>300 mg/day) in 43% and 26% of males and females with CKD stage 1, respectively, with higher proportions in patients with more advanced kidney involvement.

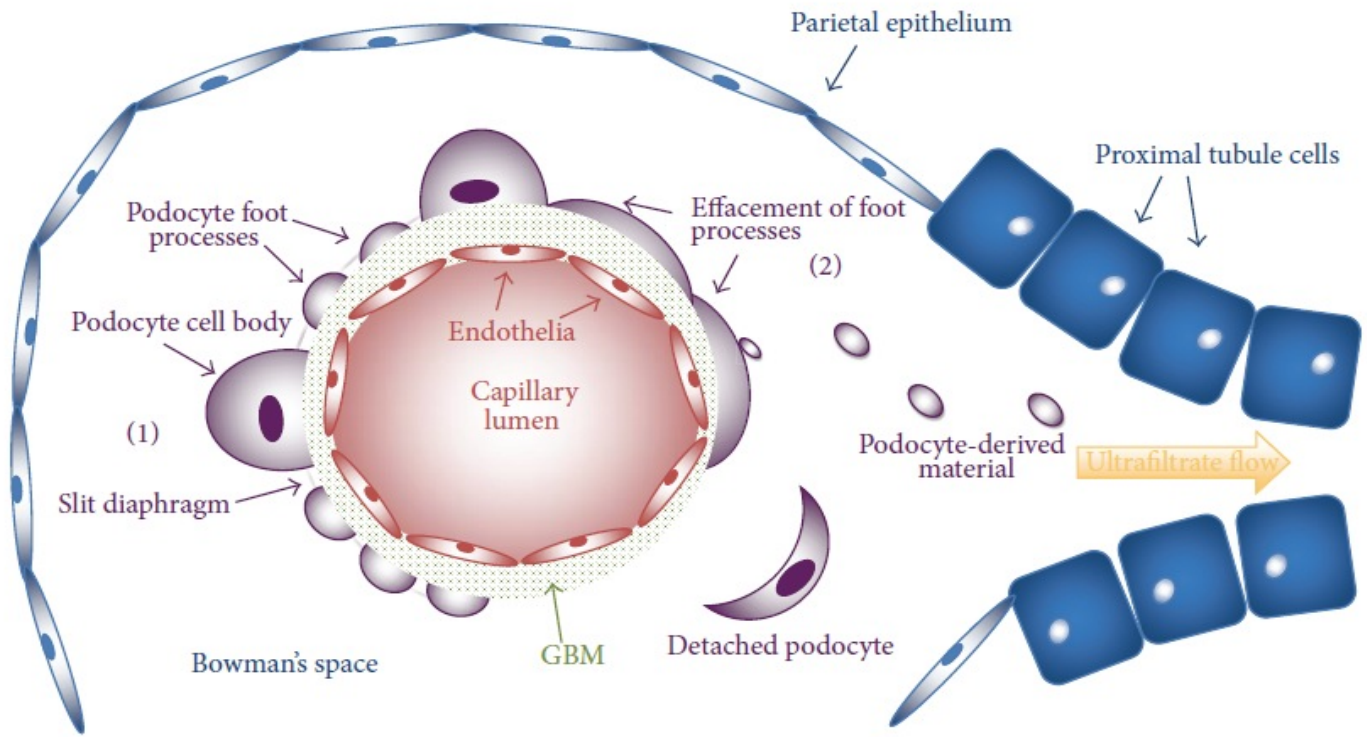
Proteinuria should be monitored regularly and treated appropriately.

Waldek and Feriozzi *BMC Nephrology* 2014, **15**:72

Podocyturia: What is in a name?

Hernán Trimarchi

Nephrology and Kidney Transplant Unit, Department of Medicine, Hospital Británico de Buenos Aires, Buenos Aires, Argentina



Hindawi Publishing Corporation
Case Reports in Nephrology
Volume 2015, Article ID 257628, 4 pages
http://dx.doi.org/10.1155/2015/257628

Case Report

Copious Podocyturia without Proteinuria and with Normal Renal Function in a Young Adult with Fabry Disease

H. Trimarchi,[1] R. Canzonieri,[2] A. Muryan,[2] A. Schiel,[2] A. Araoz,[3] M. Forrester,[1] A. Karl,[1] F. Lombi,[1] J. Andrews,[1] V. Pomeranz,[1] T. Rengel,[1] and E. Zotta[3]

2. Case Presentation

The diagnosis of Fabry disease was made in an 18-year-old male who suffered from acroparesthesias, decreased sweating and frequent episodes of diarrhea. The test for α-galactosidase disclosed decreased enzyme activity, 0.1 nmol/hour/liter (normal > 4 nmol/hour/liter). A novel mutation [c.100A>G (p.N34D)] was identified in the gene of α-galactosidase A, diagnosed by sequential analysis. The laboratory results were unremarkable, with a creatinine clearance of 115 ml/min, a negative 24-hour urinary protein excretion; microalbuminuria: 28 µg/min. A renal ultrasound was normal.

?

podocyte count was assessed by counting in urinary smears the number of cells in 10 microscopy fields of x20. The podocyte count was 1.6 cells per x20 field; the number of podocytes per gram of urinary creatinine was 133, and the number of podocytes/100 mL of urine was 8. Podocytes were identified by tagging synaptopodin (ab109560 Alexa Fluor®, Abcam, Cambridge United Kingdom) an specific marker of podocytes, to establish their identity by immunofluorescence techniques using a secondary antibody (Alexa Fluor® 488, Abcam, Cambridge United Kingdom). The smears were analyzed employing an epifluoresence microscopy, Nikon Eclipse E 200. This result was compared with a control

COPIOUS PODOCYTURIA WITHOUT PROTEINURIA AND WITH NORMAL RENAL FUNCTION IN A YOUNG ADULT WITH FABRY DISEASE

H. Trimarchi[1], R. Canzonieri[2], A. Muryan[2], A. Schiel[2], A. Araoz[3], M. Forrester[1], A. Karl[1], F. Lombi[1], J. Andrews[1], V. Pomeranz[1], T. Rengel[1], E. Zotta[3].

[1] Nephrology and [2] Laboratory Services, Hospital Británico de Buenos Aires, Buenos Aires Argentina, and [3] IFIBIO Houssay- UBA CONICET, Facutad de Medicina, Universidad de Buenos Aires, Argentina

Submitted to Case Reports in Nephrology

?

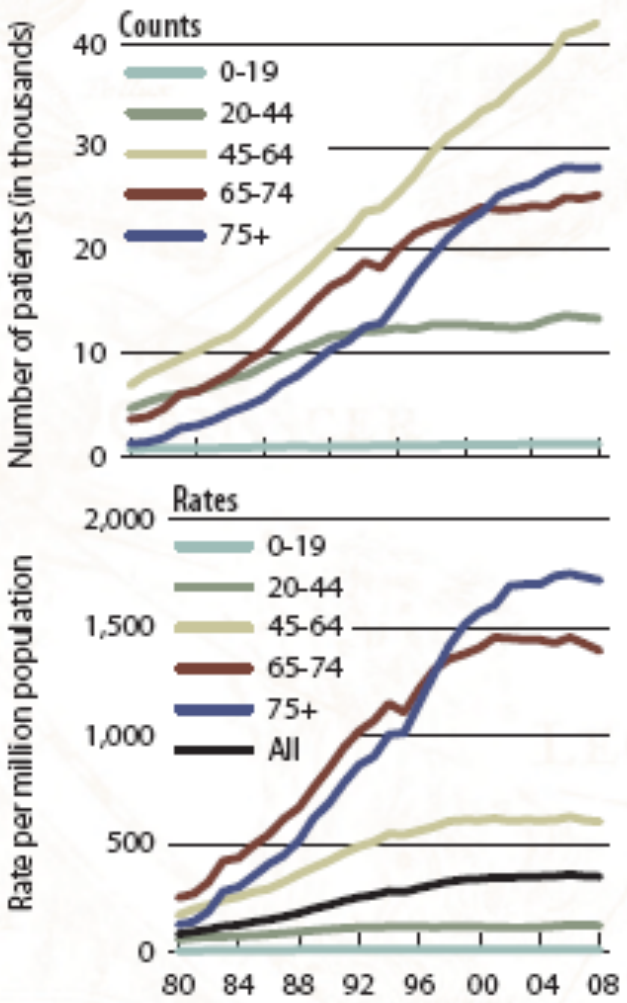
The patient declined to undergo a kidney biopsy, but accepted enzyme replacement therapy intravenously with agalasidase beta 1mg/kg every fortnight (Fabrazyme, Genzyme Corp, Cambridge MA USA) and enalapril 5 mg/day orally.

52nd ERA-EDTA CONGRESS
UNITED KINGDOM LONDON 2015 MAY 28TH\31ST
Held jointly with THE RENAL ASSOCIATION founded 1950
www.era-edta2015.org

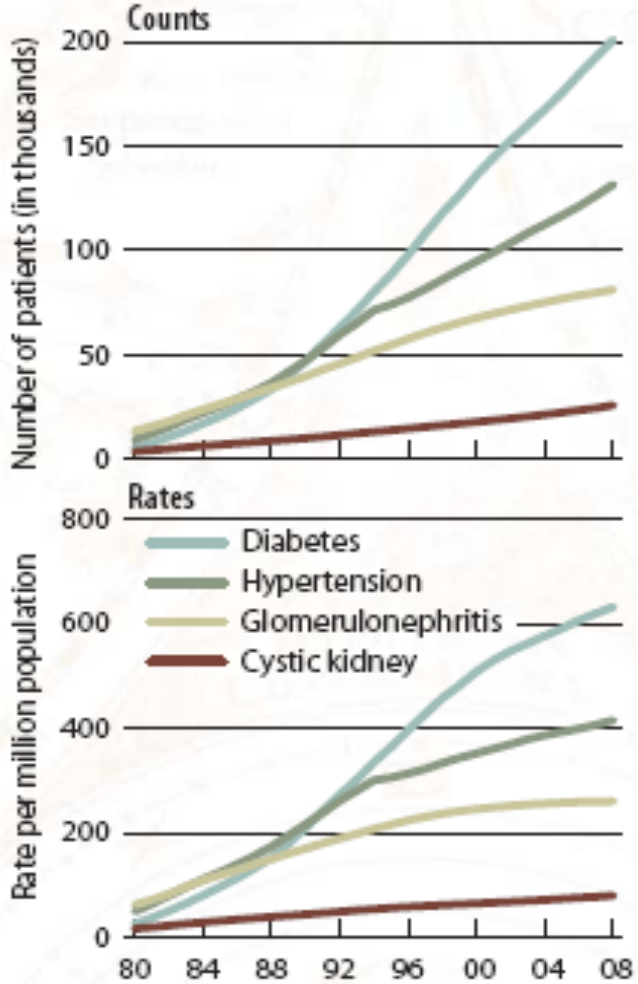
Dear Dr TRIMARCHI,

We have the pleasure of informing you that your abstract entitled **“PODOCYTURIA IN FABRY DISEASE IS ELEVATED IN UNTREATED VS TREATED ADULT PATIENTS AND DOES NOT CORRELATE WITH PROTEINURIA OR RENAL FUNCTION” has been accepted as a Poster Presentation** at the 52nd ERA-EDTA Congress, which will be organised in collaboration with the Renal Association in London, United Kingdom (May 28-31, 2015).

2 4 ii Incident counts & adjusted rates of ESRD, by age



2 15 ii Prevalent counts & adjusted rates of ESRD, by primary diagnosis




Glaciar Perito Moreno Santa Cruz Argentina