Neisseria meningitidis, les secrets de la subversion de l'endothélium vasculaire cérébral

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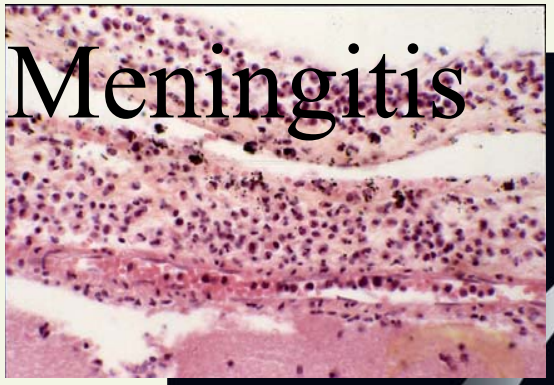
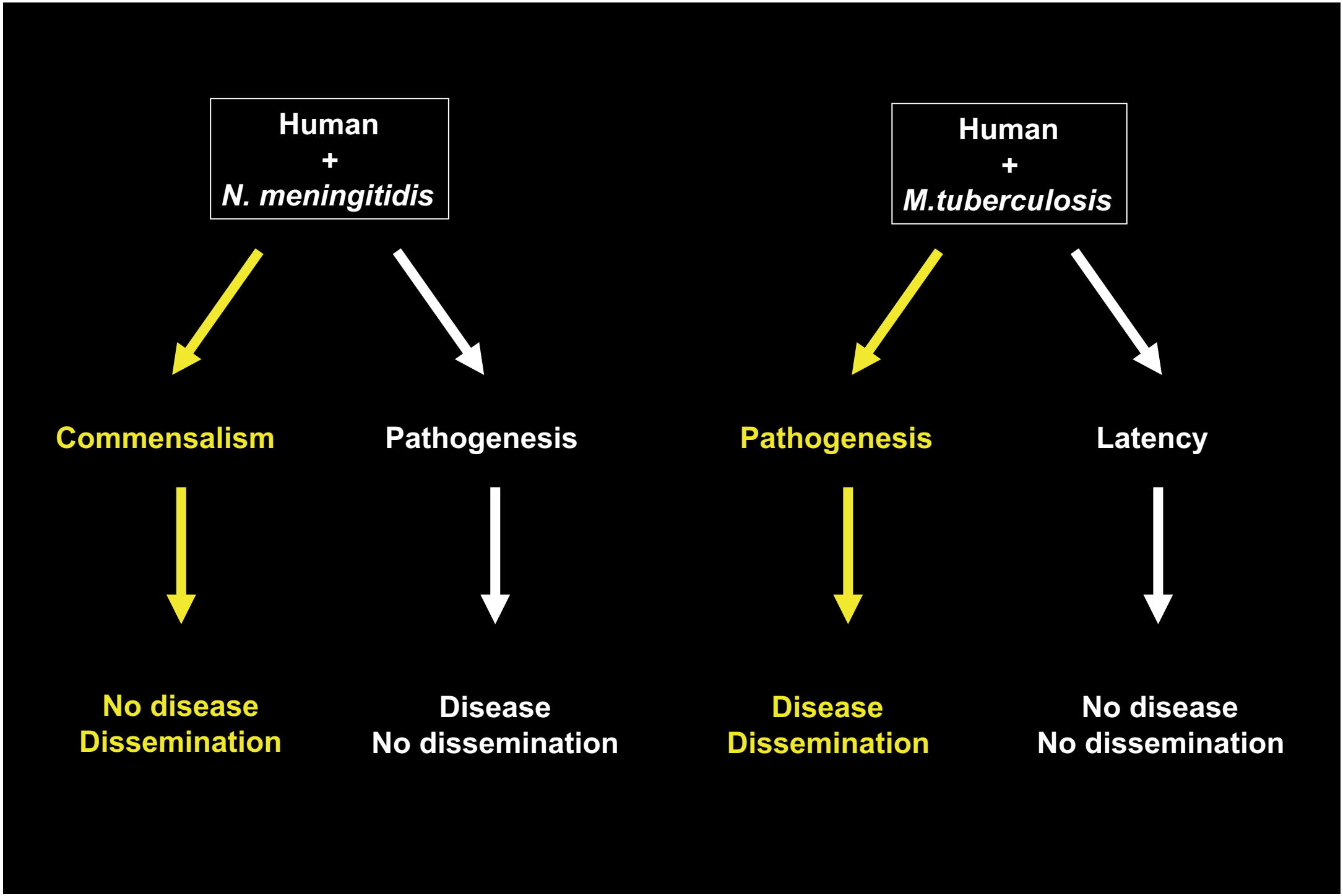


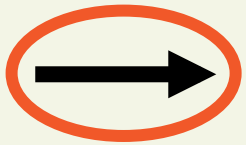
Meningitis

Purpura


Human
+
N. meningitidis
Human
+
M.tuberculosis
Commensalism
Pathogenesis
Pathogenesis
Latency
No disease
Dissemination
Disease
No dissemination
Disease
Dissemination
No disease
No dissemination

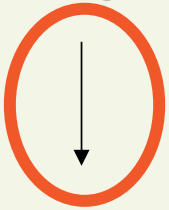
Neisseria meningitidis, a paradigm of extra cellular bacterial pathogen

Human- nasopharynx
+ *N. meningitidis*



Commensalism

Blood
SEPTICEMIA
PURPURA FULMINANS



Cerebrospinal fluid
MENINGITIS

Environment (Influenza virus)

Host factors
- **+ Deficiency in late complement components**
- **+ Deficiency in maltose binding protein**
- **+ Lack of immunity (young children)**

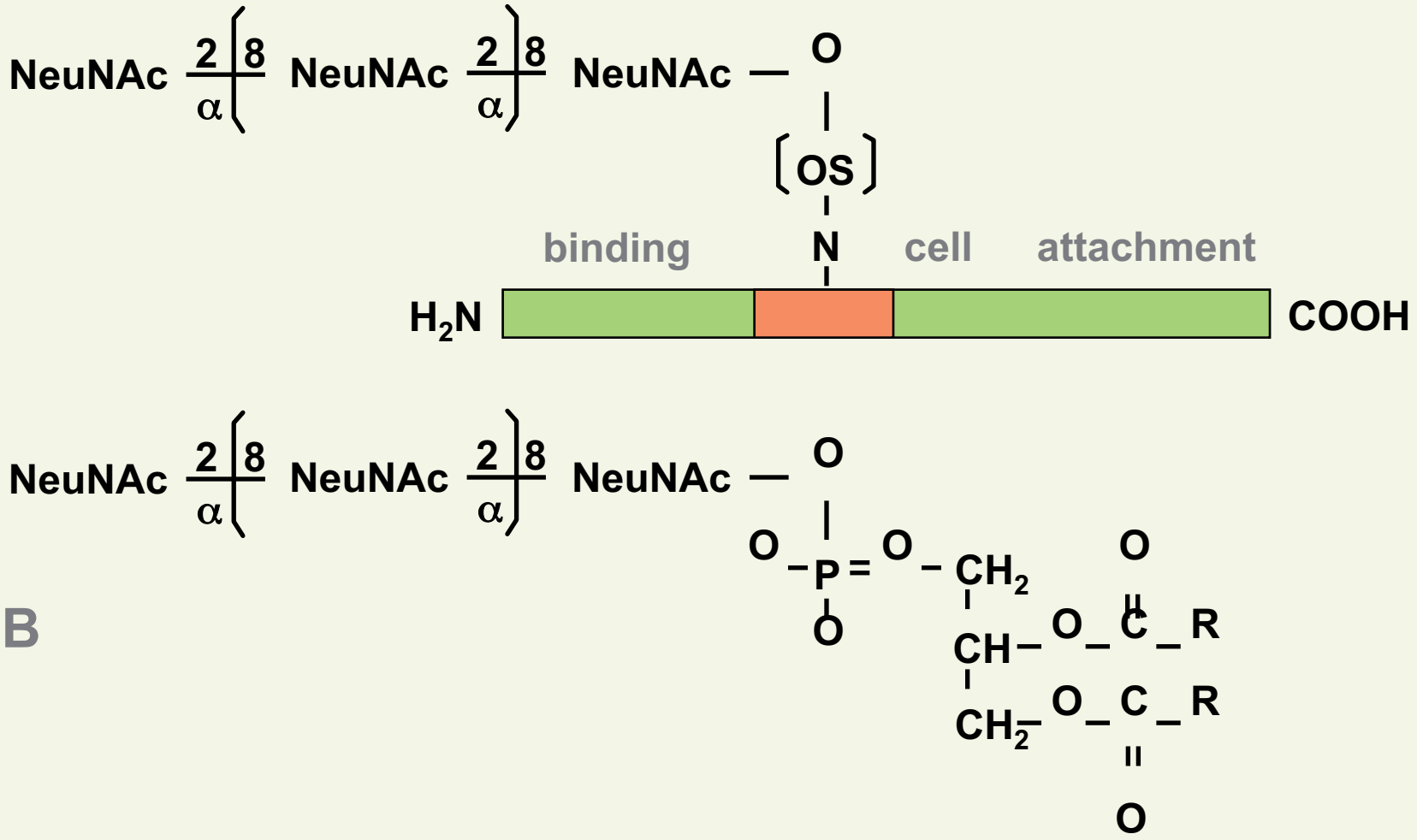
Bacterial factors : capsule (+++), Iron adhesines/pili, Opa.. filamentous phage

Meningococcal infections, a public health burden

- 1 to 1. 5 per 100 000 habitants,
- Two peaks before 2 years and at 15-25 years of age

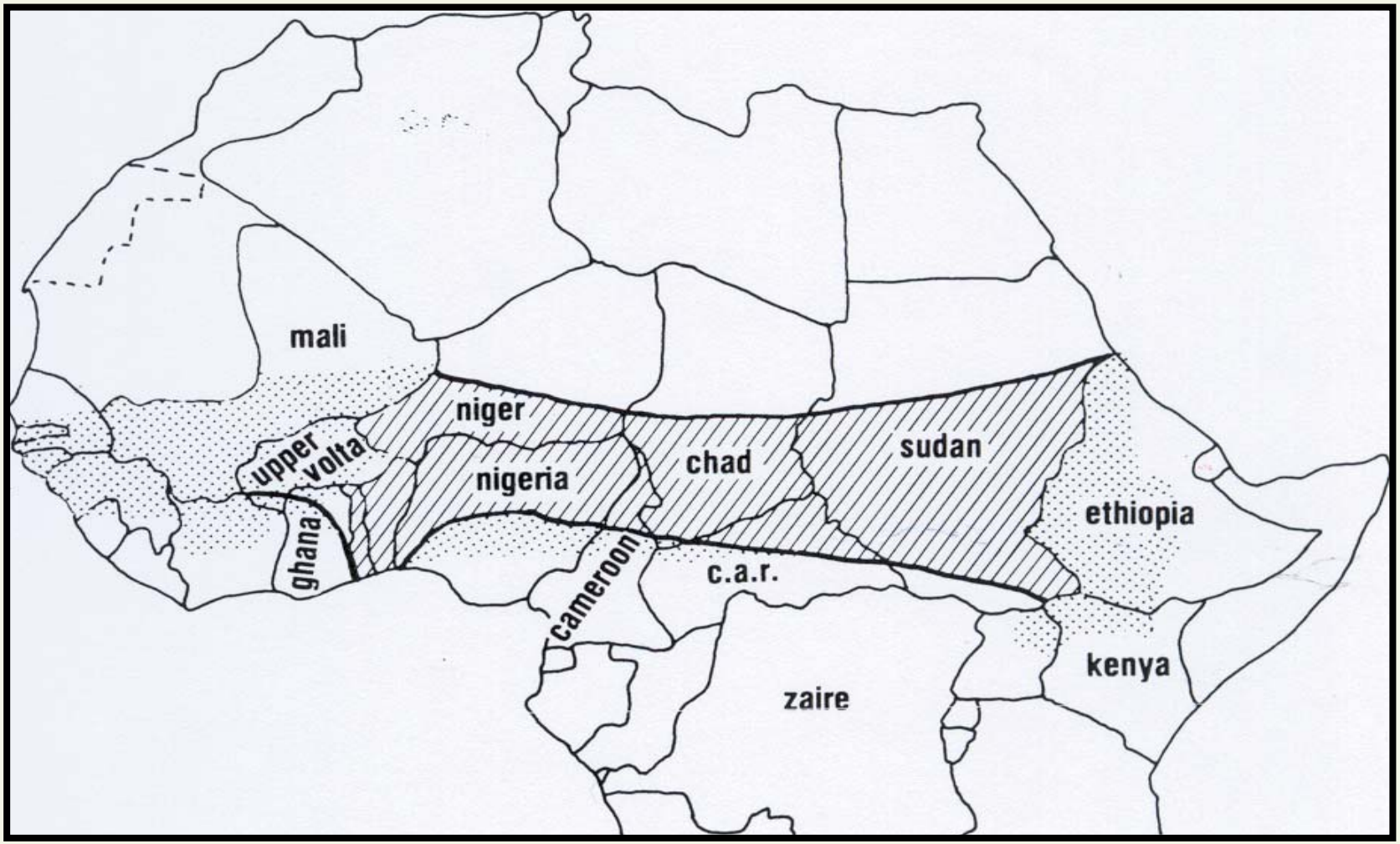
- Fulminant septicemia (30% death) even when treated
- Meningitis (3% death)
-Can be responsible for small epidemics in developed countries and large epidemics in Africa (the meningitis belt)

- Two major serogroups in Europe : B (60%) and C (40%).
- A vaccine against C is available
- No vaccine against B serogroup



Structural relation of the capsular serogroup B of *N.meningitidis* to the carbohydrate terminal of the neonatal neural cell adhesion molecule (n-CAM); NeuNac = *N*-acetylneuraminic acid.

THE AFRICAN MENINGITIS BELT



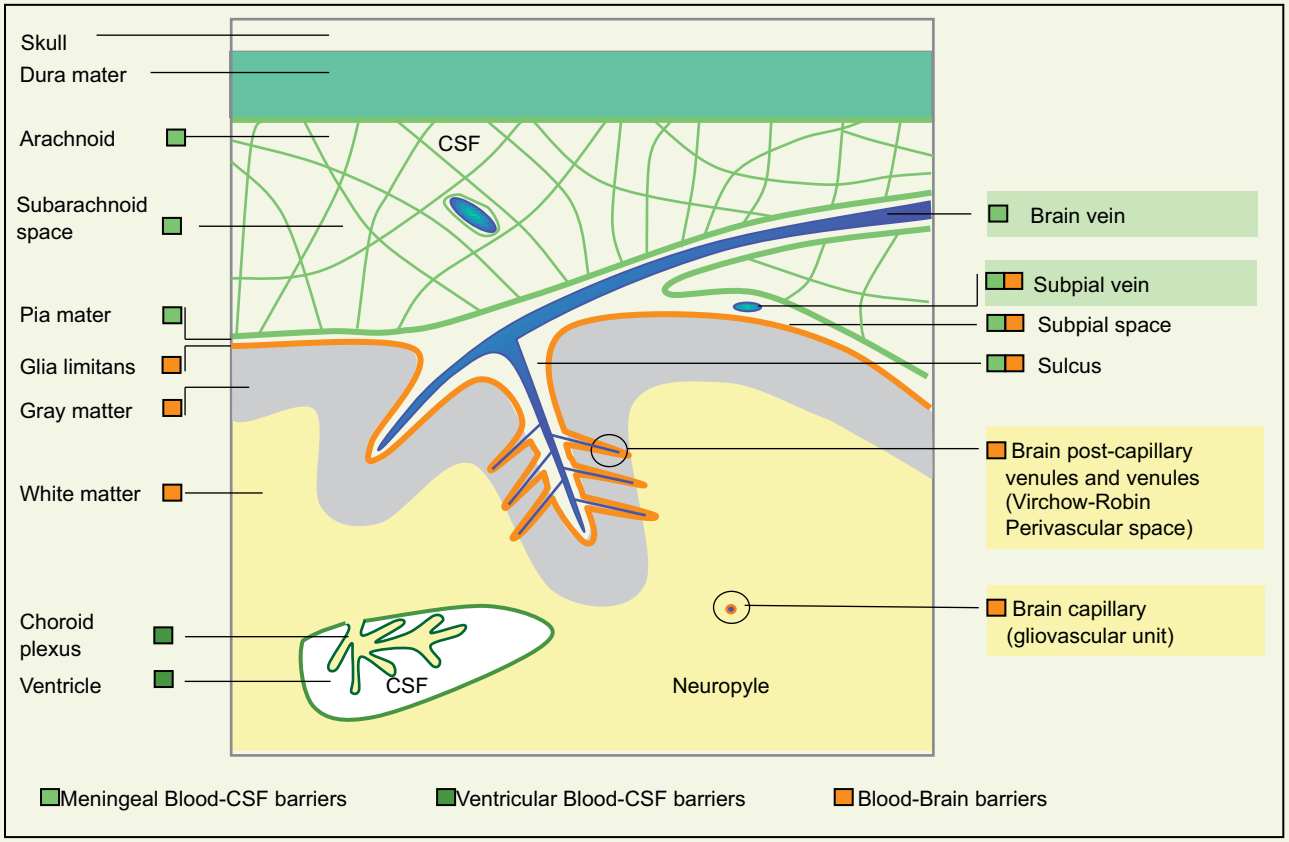
SUPPLÉMENT AU VOL. 28 DU *BULLETIN DE L'ORGANISATION MONDIALE DE LA SANTÉ*

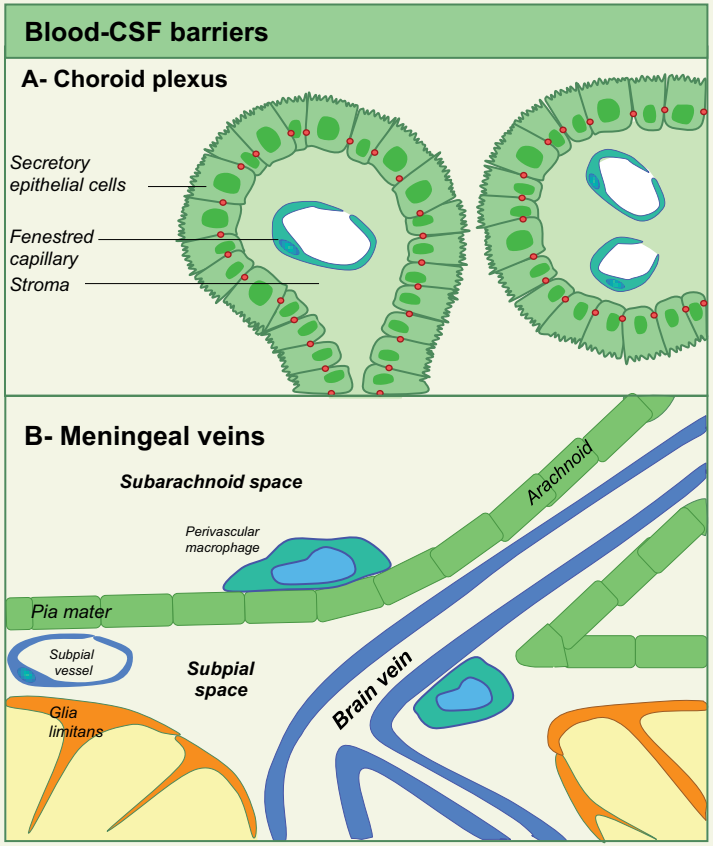
LA MÉNINGITE CÉRÉBRO-SPINALE EN AFRIQUE

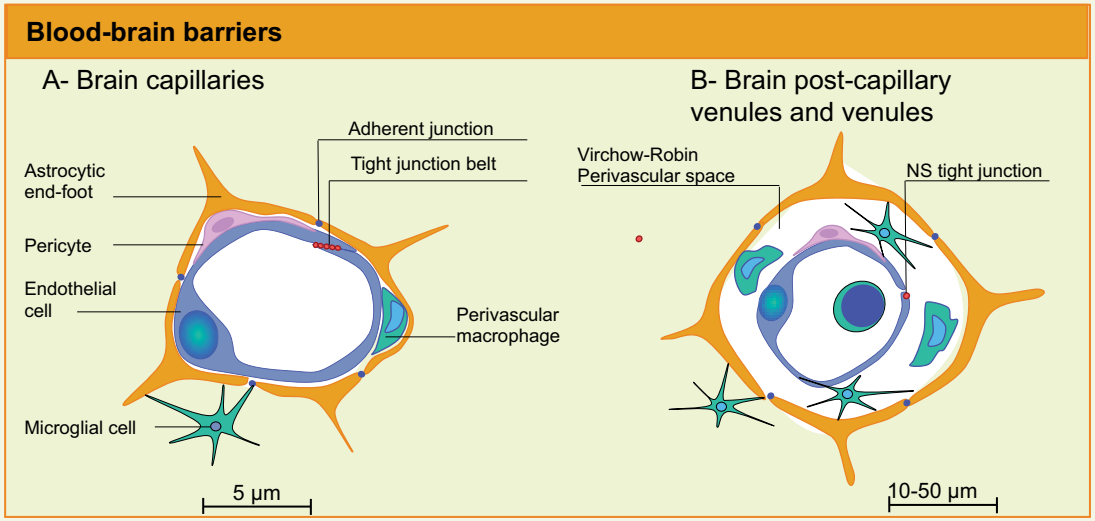
L. Lapeyssonnie

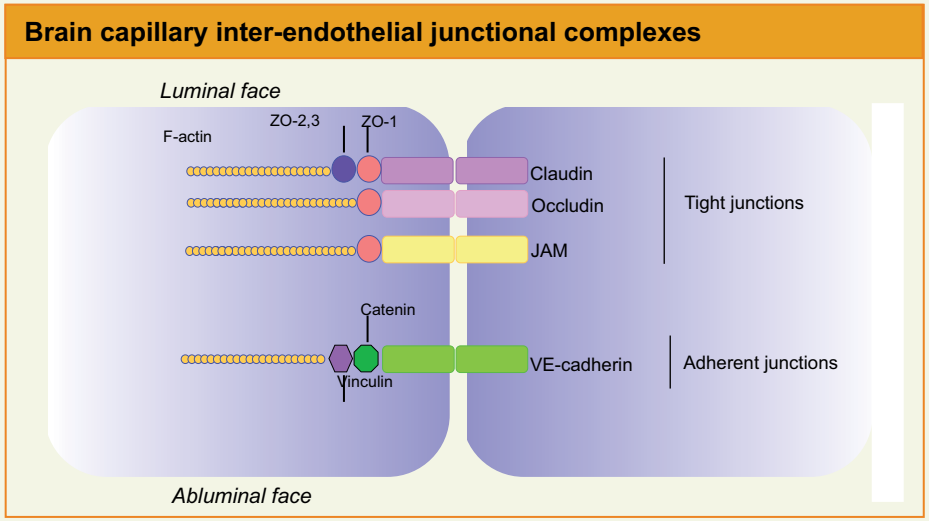
ORGANISATION MONDIALE DE LA SANTÉ
GENÈVE
1963

How *N.meningitidis* once in the bloodstream can cross the blood brain barrier ?

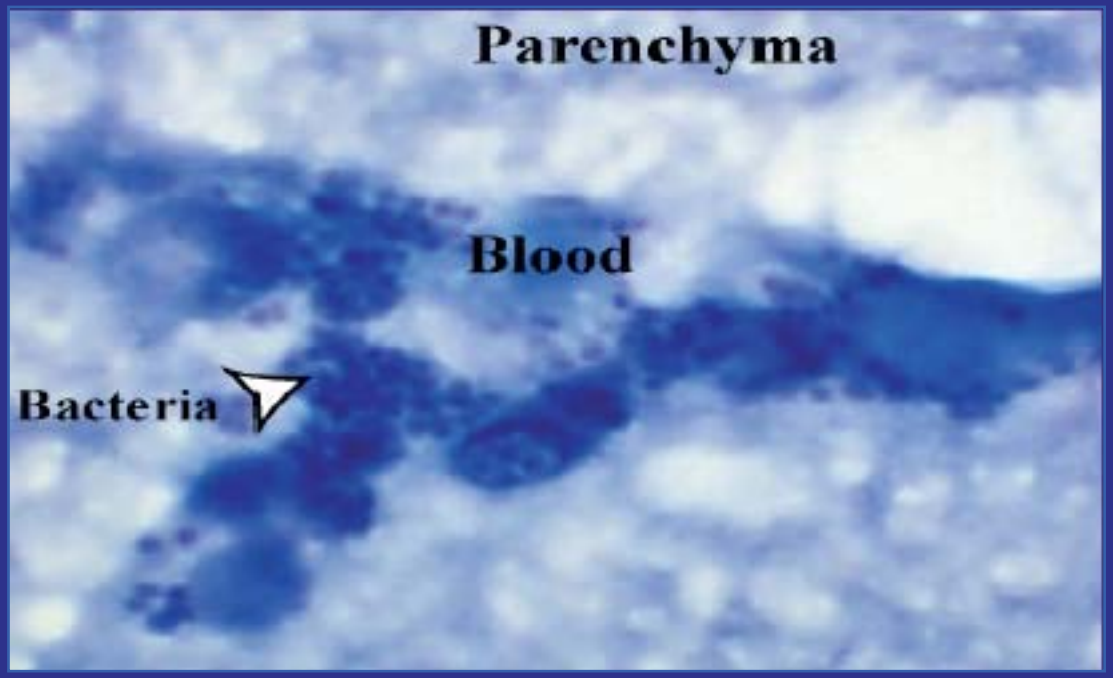

Skull
Dura mater
Arachnoid
Subarachnoid space
Pia mater
Glia limitans
Gray matter
White matter
Choroid plexus
Ventricle
CSF
CSF
Neuropyle
Brain vein
Subpial vein
Subpial space
Sulcus
Brain post-capillary venules and venules (Virchow-Robin Perivascular space)
Brain capillary (gliovascular unit)
Meningeal Blood-CSF barriers
Ventricular Blood-CSF barriers
Blood-Brain barriers

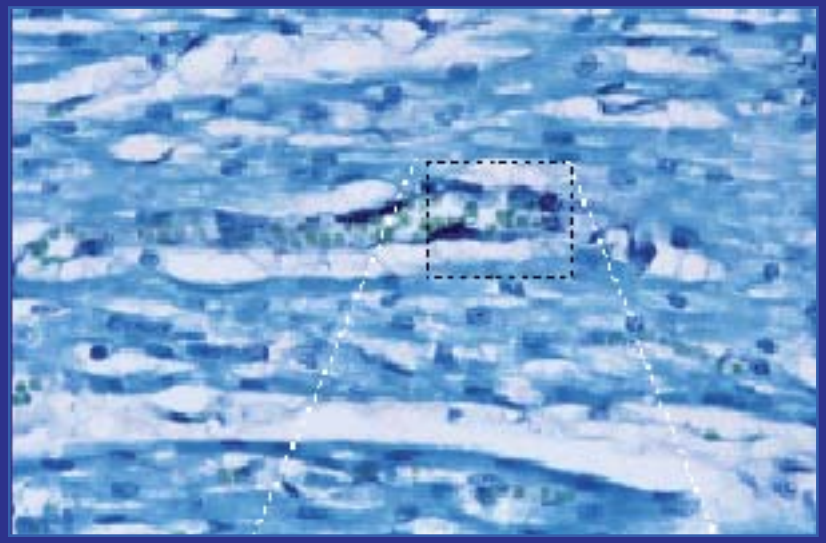

Blood-CSF barriers
A- Choroid plexus
Secretory epithelial cells
Fenestred capillary
Stroma
B- Meningeal veins
Subarachnoid space
Arachnoid
Perivascular macrophage
Pia mater
Subpial vessel
Subpial space
Brain vein
Glia limitans


Blood-brain barriers
A- Brain capillaries
Adherent junction
Tight junction belt
Astrocytic end-foot
Pericyte
Endothelial cell
Perivascular macrophage
Microglial cell
5 µm
B- Brain post-capillary venules and venules
Virchow-Robin Perivascular space
NS tight junction
10-50 µm

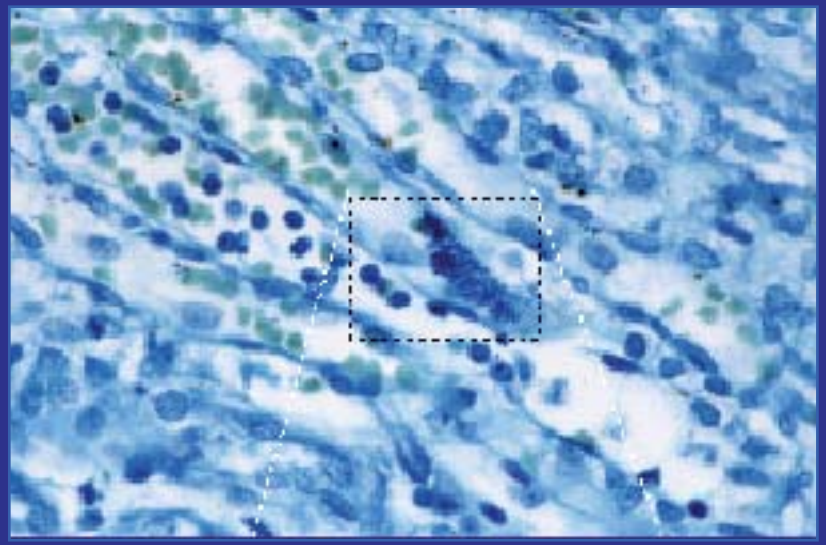

Brain capillary inter-endothelial junctional complexes
Luminal face
F-actin
ZO-2,3
ZO-1
Claudin
Occludin
JAM
Tight junctions
Catenin
VE-cadherin
Vinculin
Adherent junctions
Abluminal face

Brain

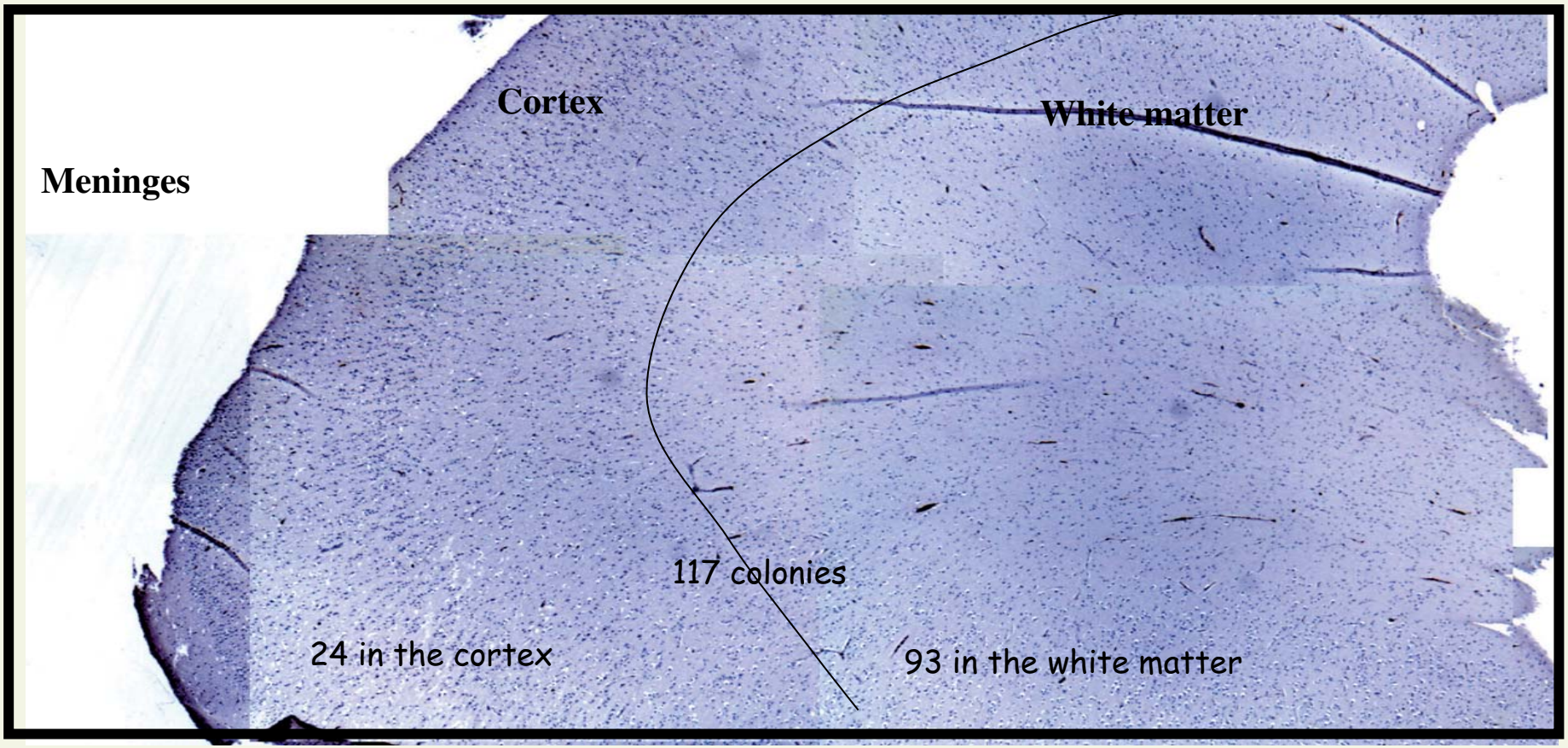


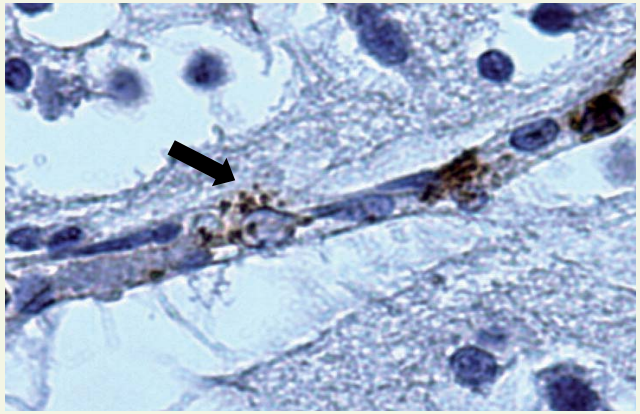


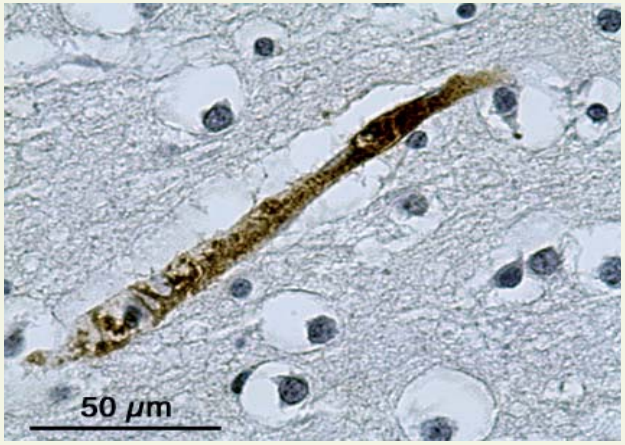
Heart

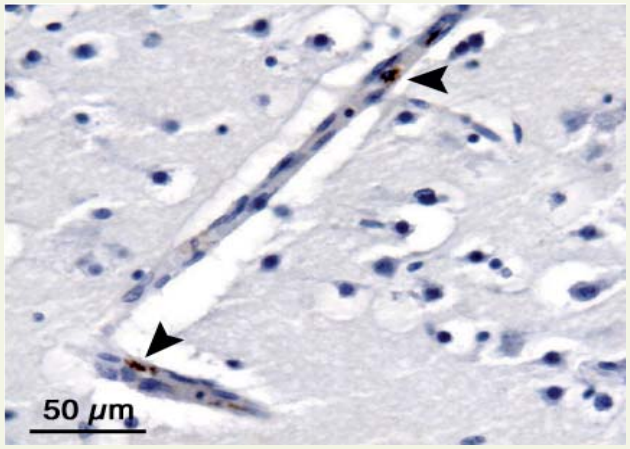


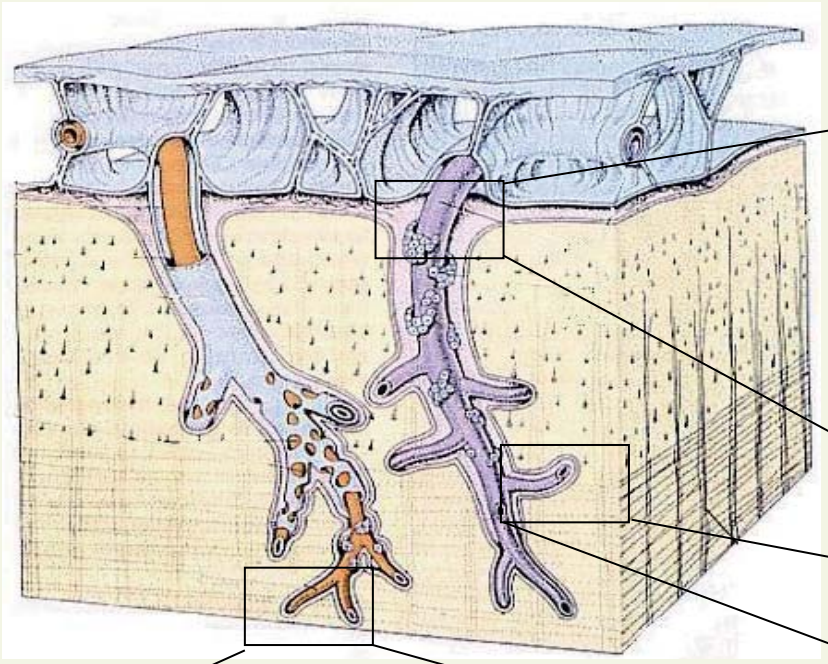
Kidney

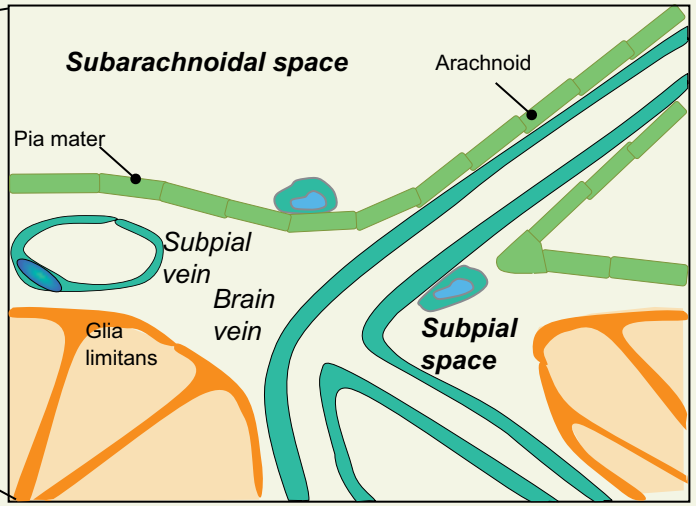

Cortex
White matter
Meninges
117 colonies
24 in the cortex
93 in the white matter



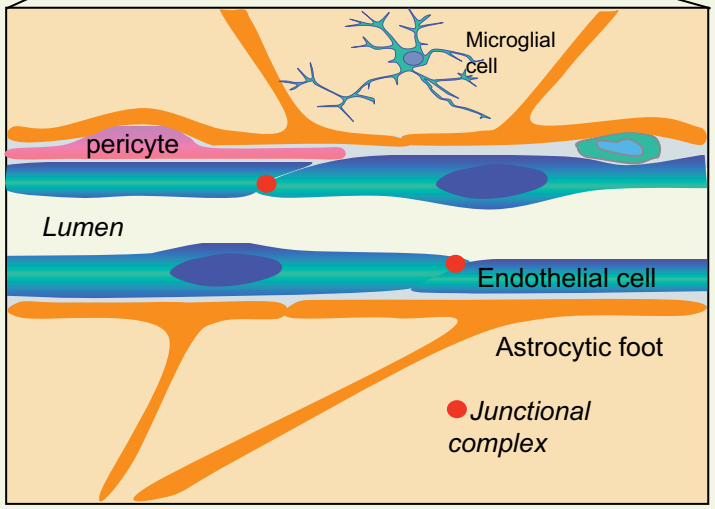

50 µm


50 µm

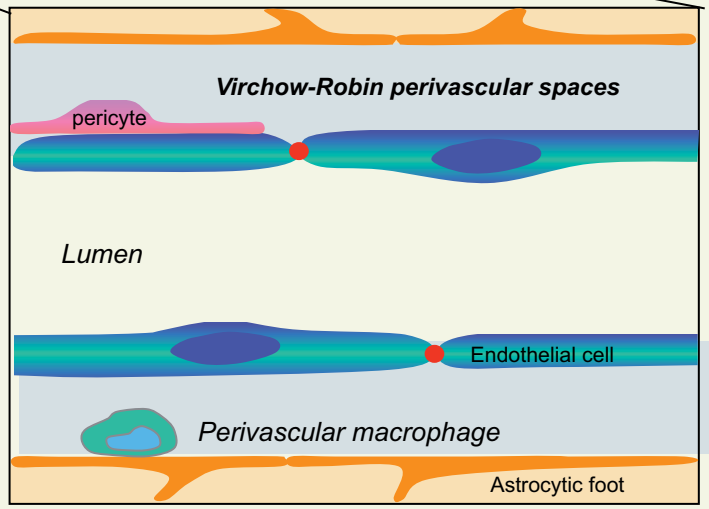




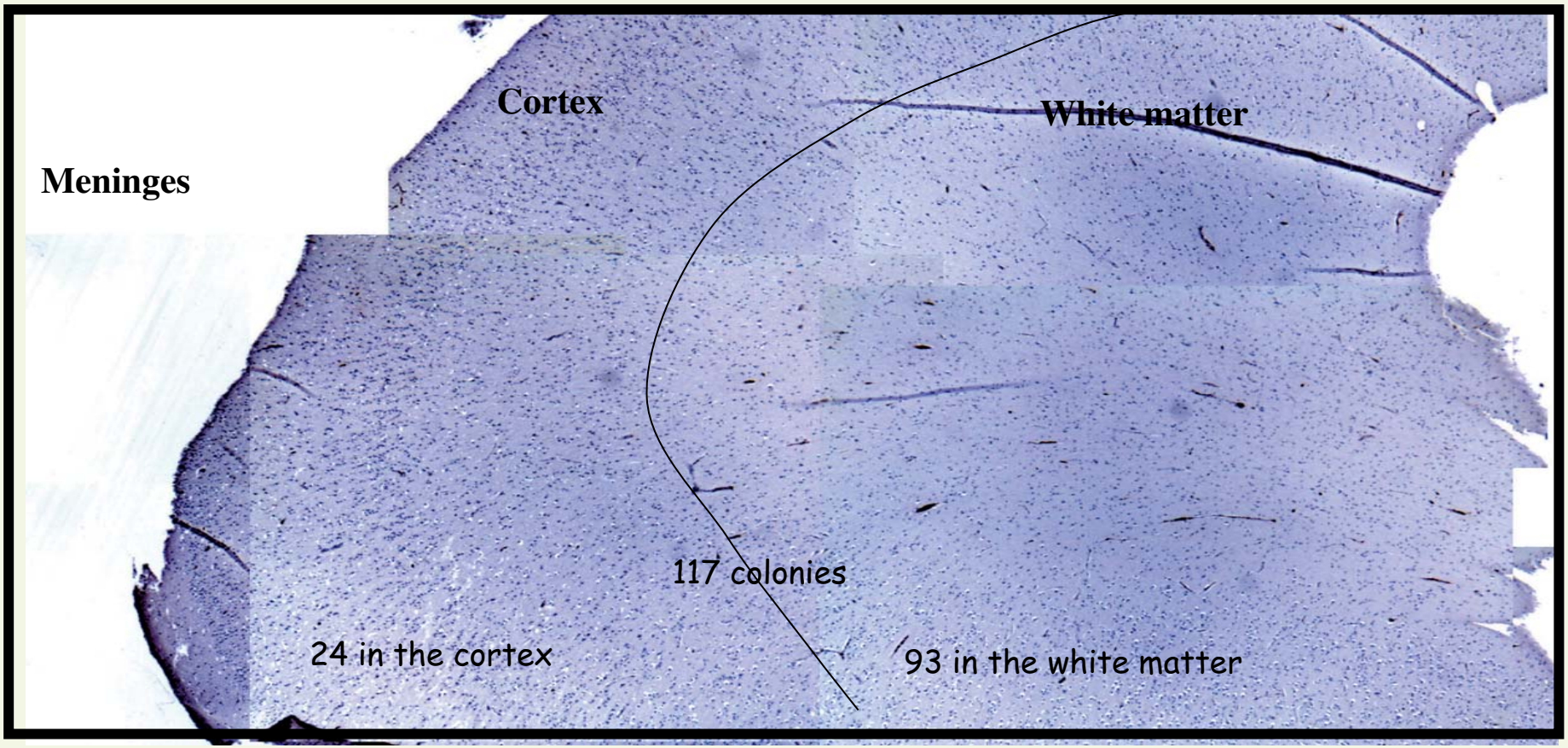
Meningeal spaces



Capillaries
(gliovascular unit)



Post-capillary venule, venules and veins


Cortex
White matter
Meninges
117 colonies
24 in the cortex
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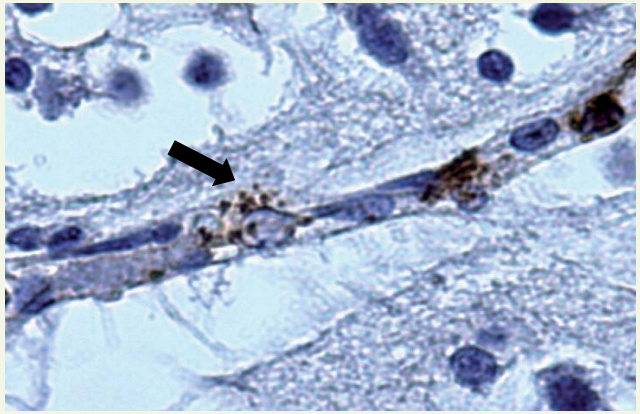
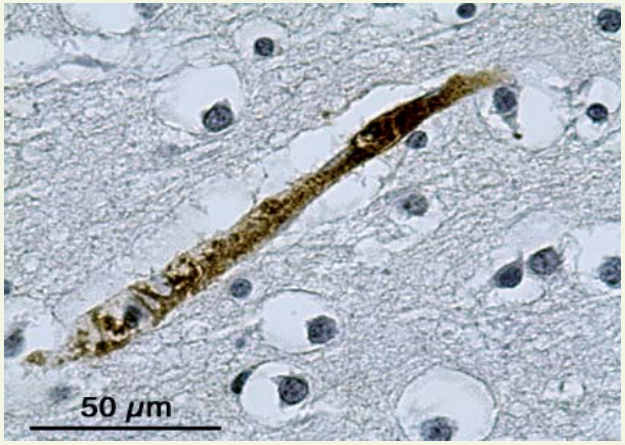
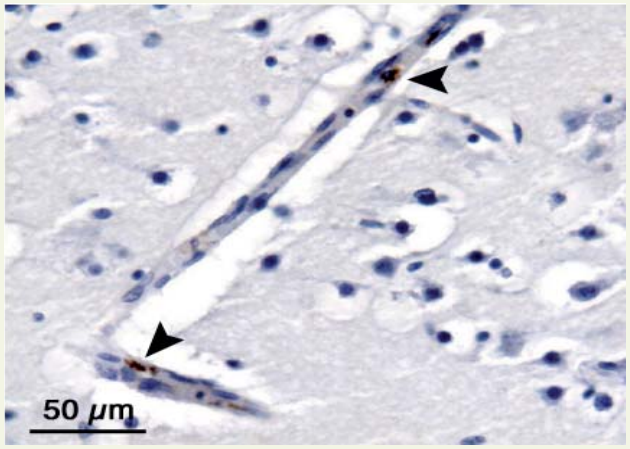
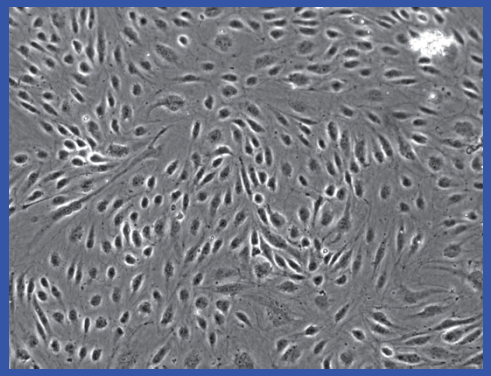


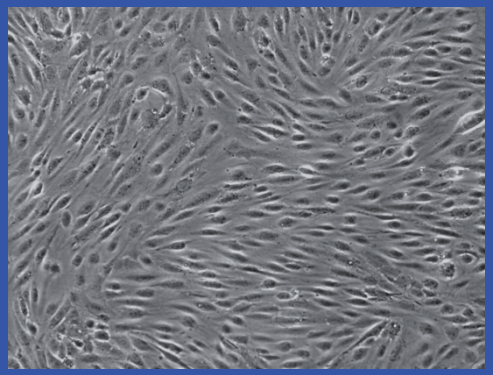

50 µm


50 µm

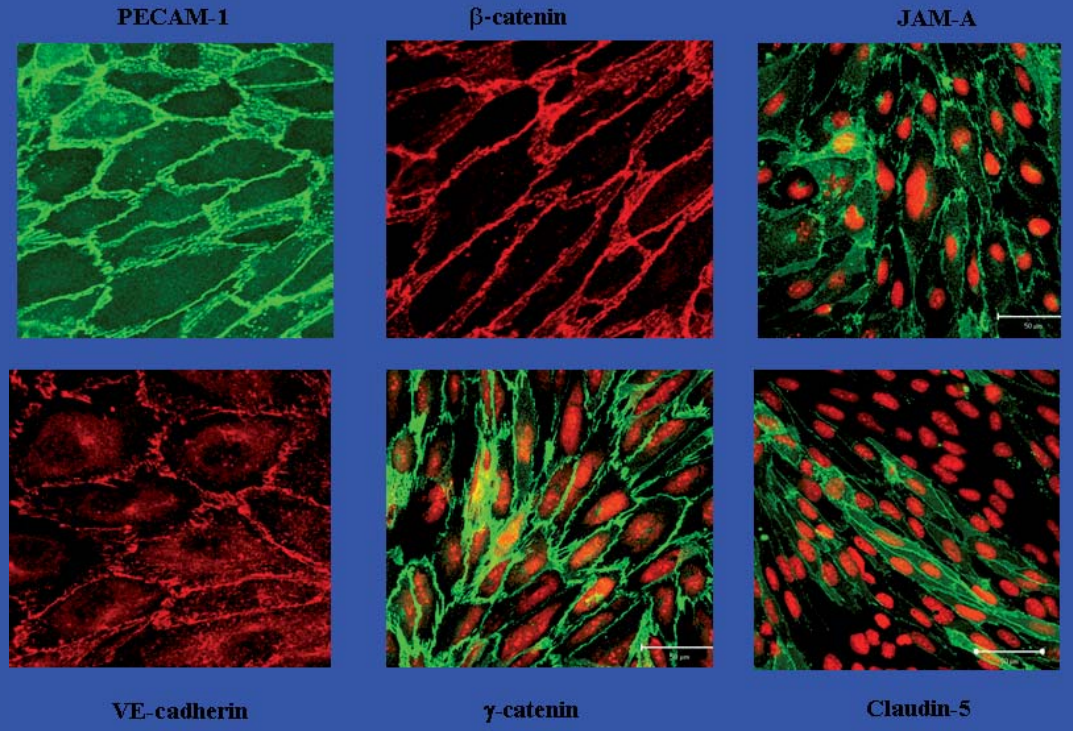
IMMORTALIZATION OF HUMAN BRAIN ENDOTHELIAL CELLS



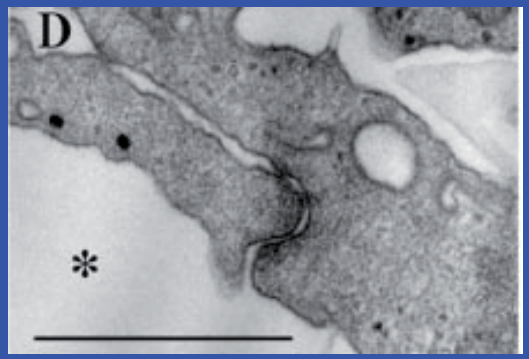
Primary culture



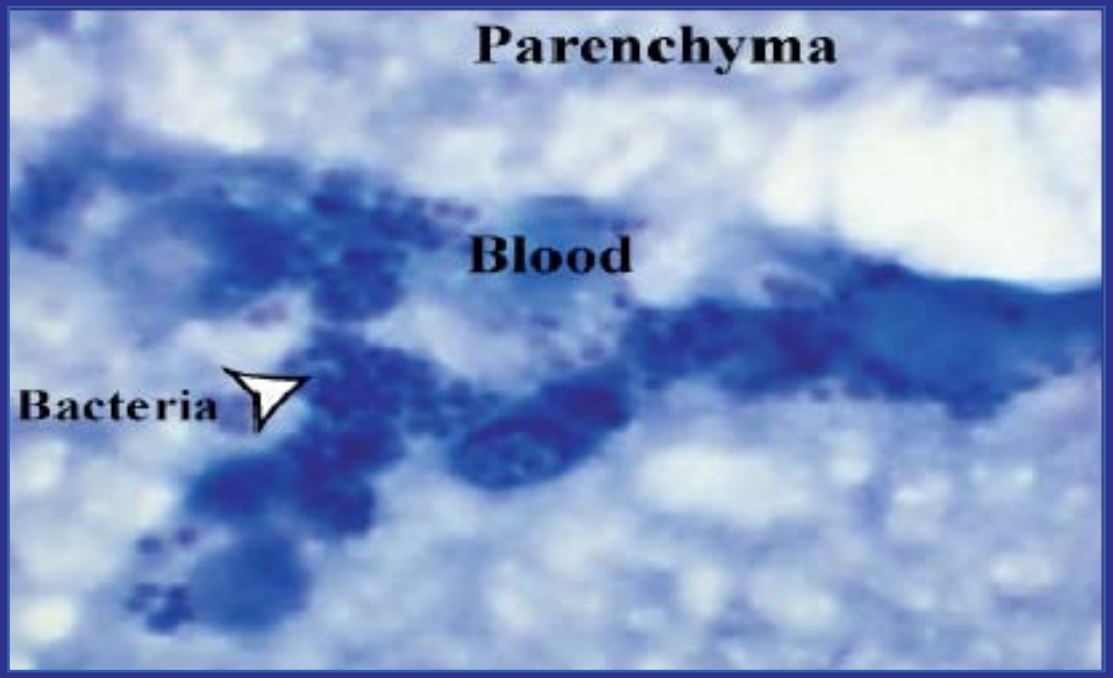
hCMEC/D3 cell line

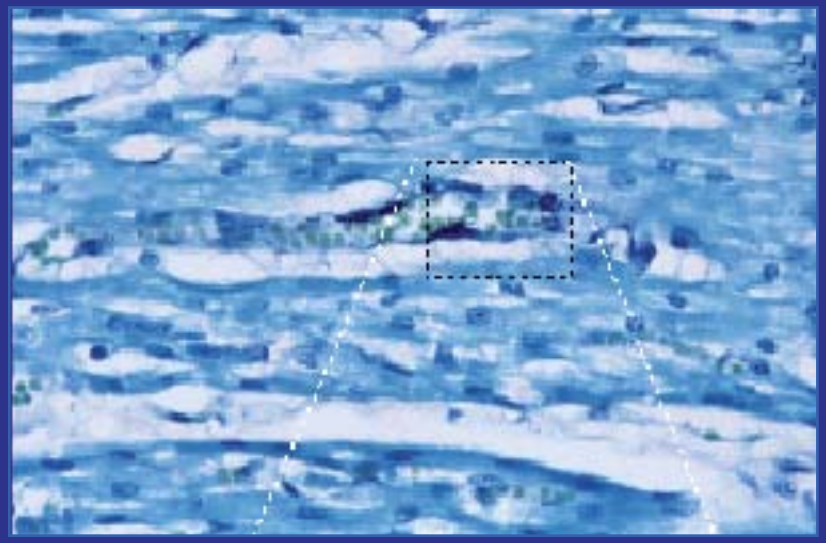


The D3 cell line express junctional proteins and makes tight junctions

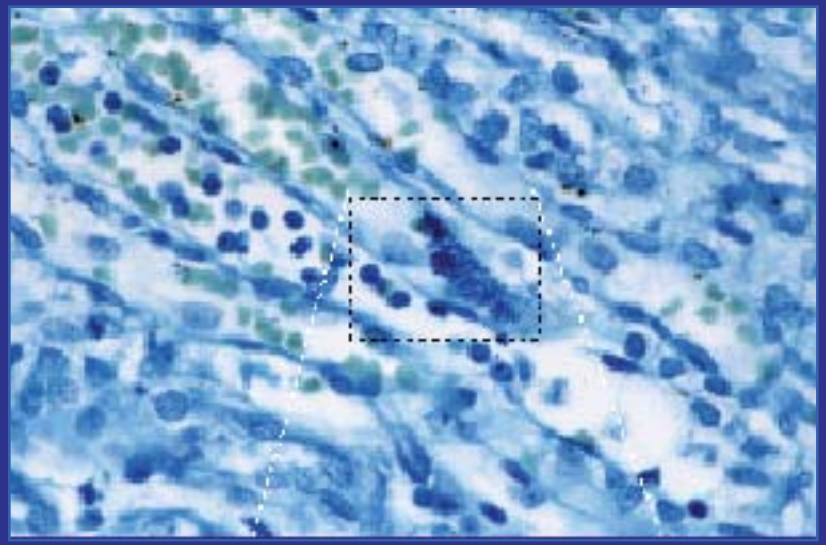


Brain

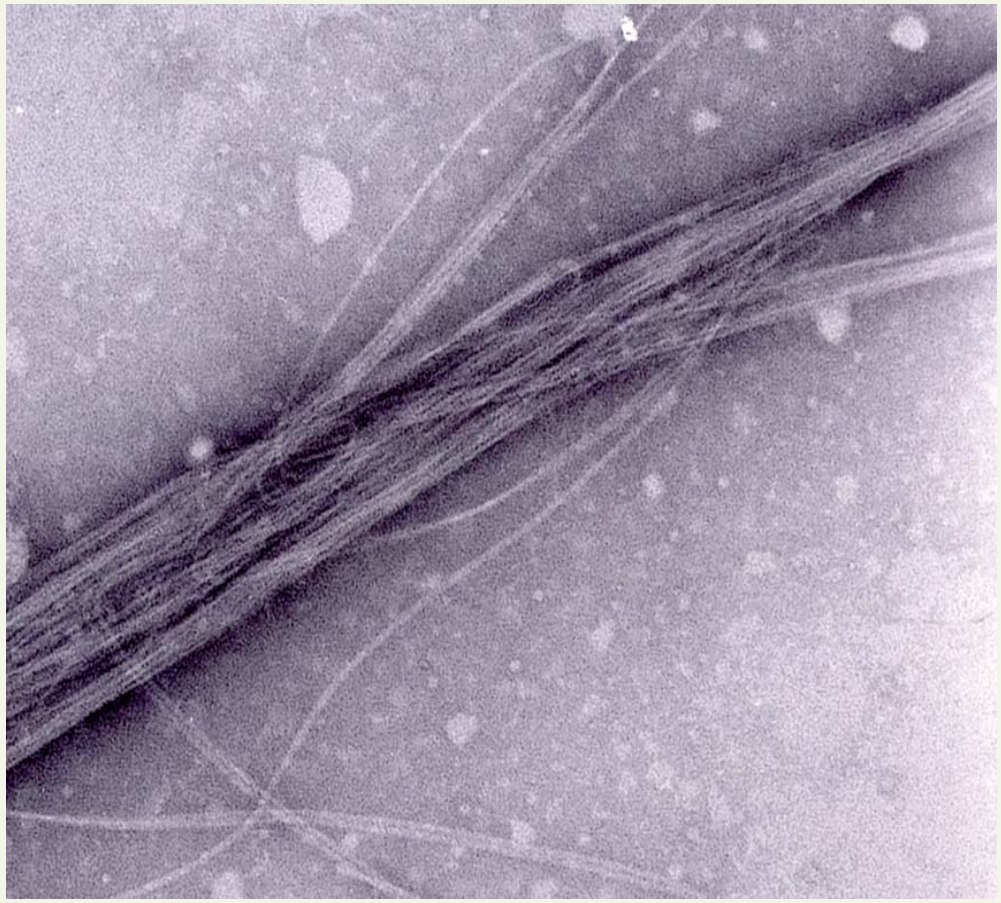




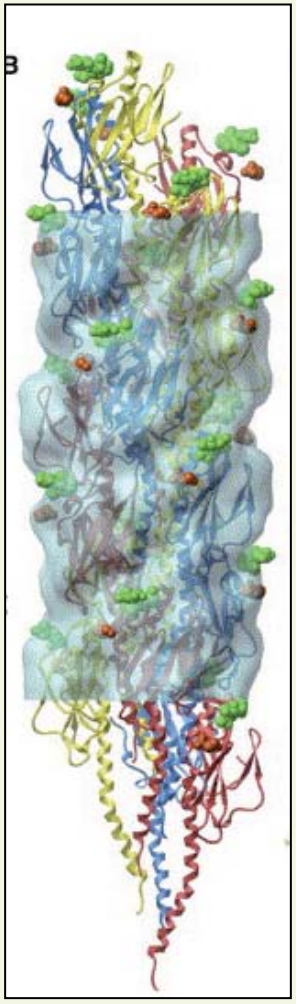
Heart



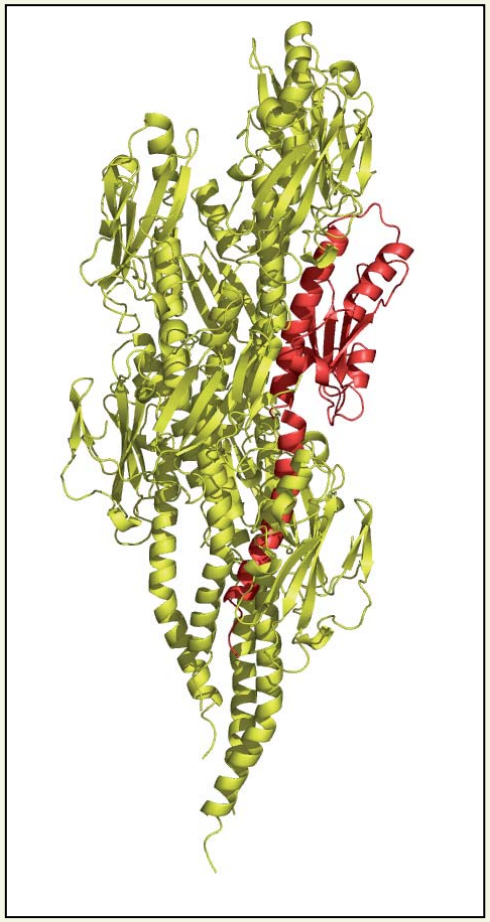
Kidney

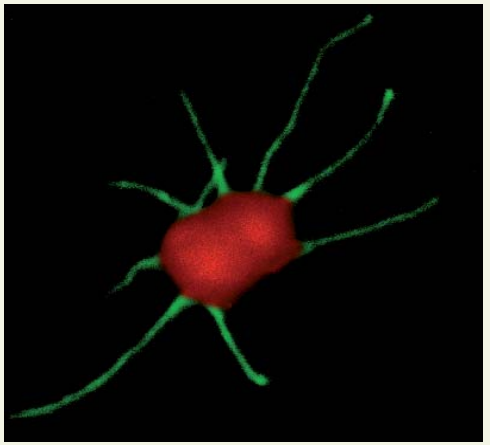


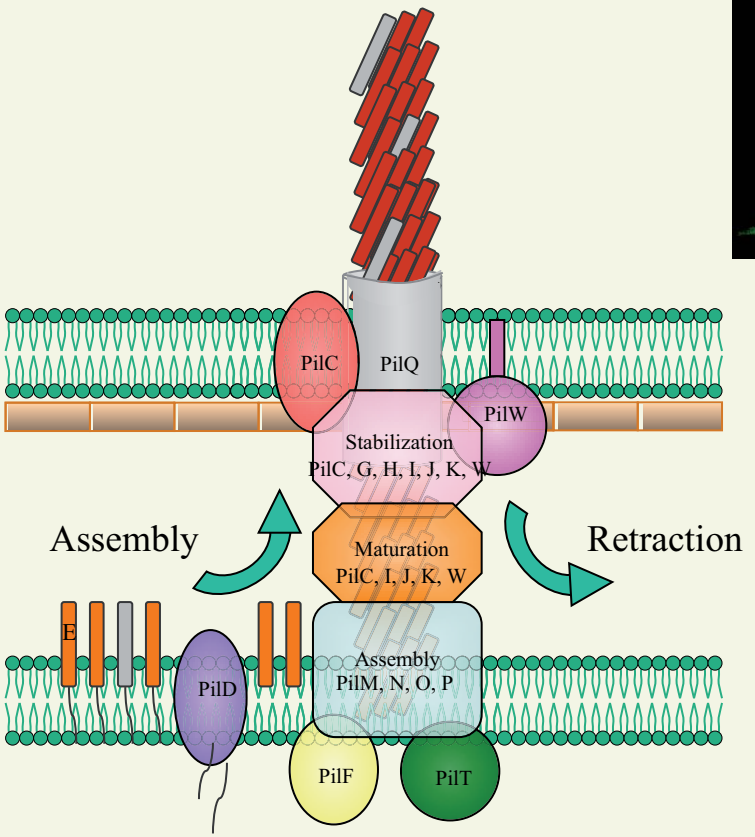
Type IV pili (Tfp)



(Craig *et al*, Molecular Cell 2006)

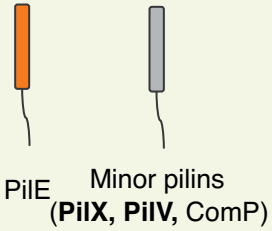






Outer membrane

Inner membrane



Prepilin
Minor pilins

(PilT)
retraction

assembly

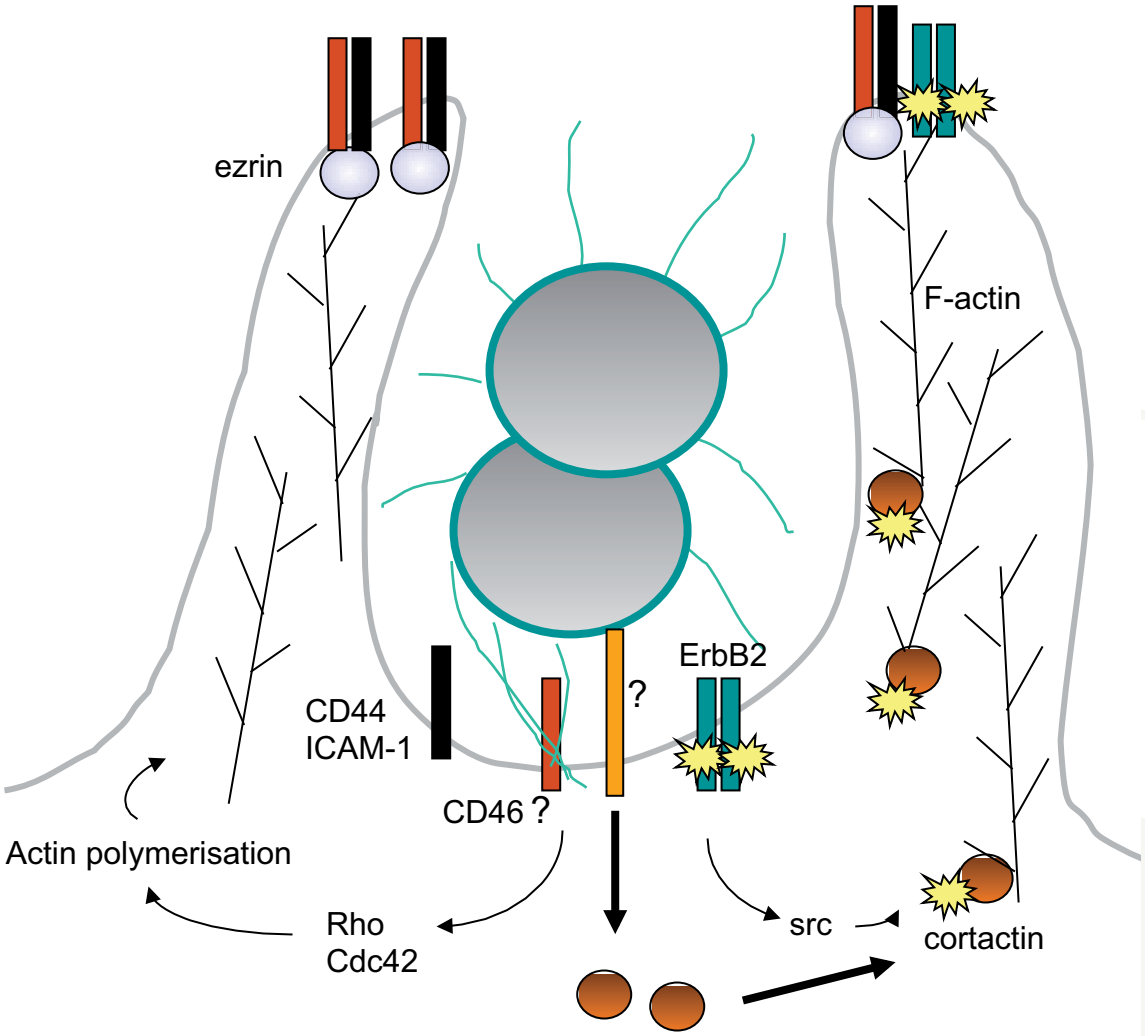
(PilD, F, M, N, O, P)

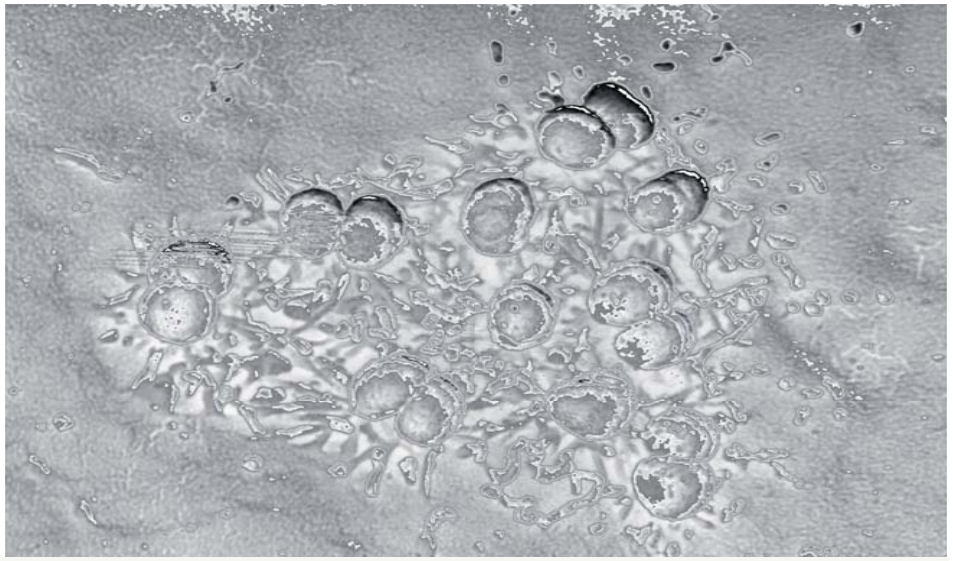
(PilC, G, H, I, J, K, W)

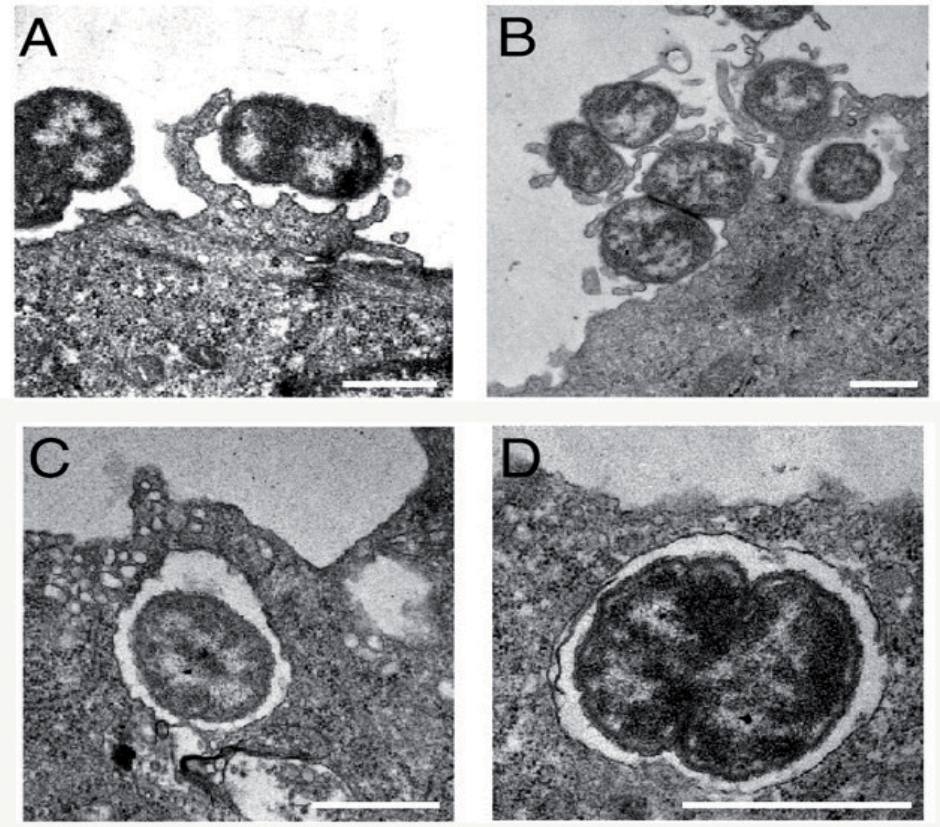
(PilQ)
emergence on the cell surface

Functional Tfp (PilE, **PilX, PilV**, ComP)

Signaling to endothelial cells by *Neisseria meningitidis*



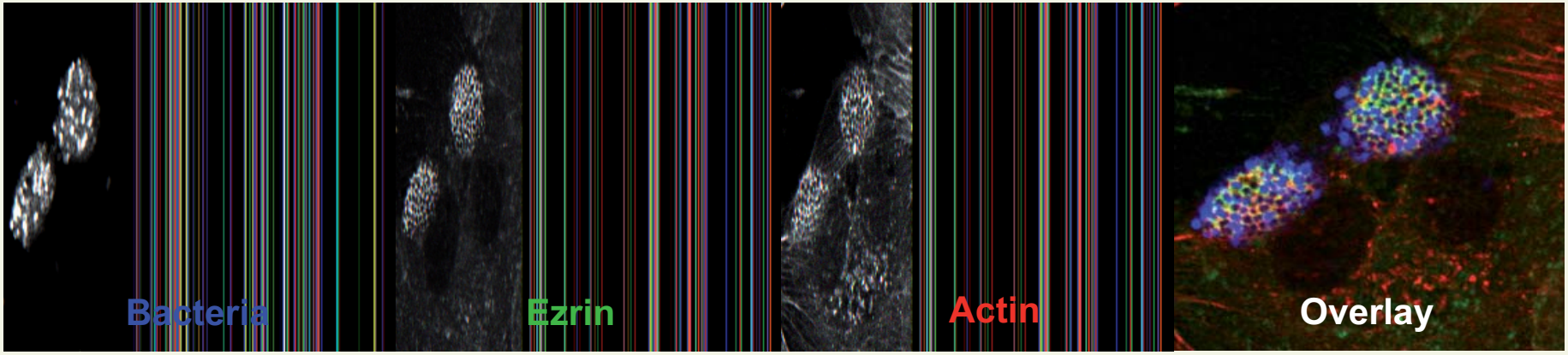




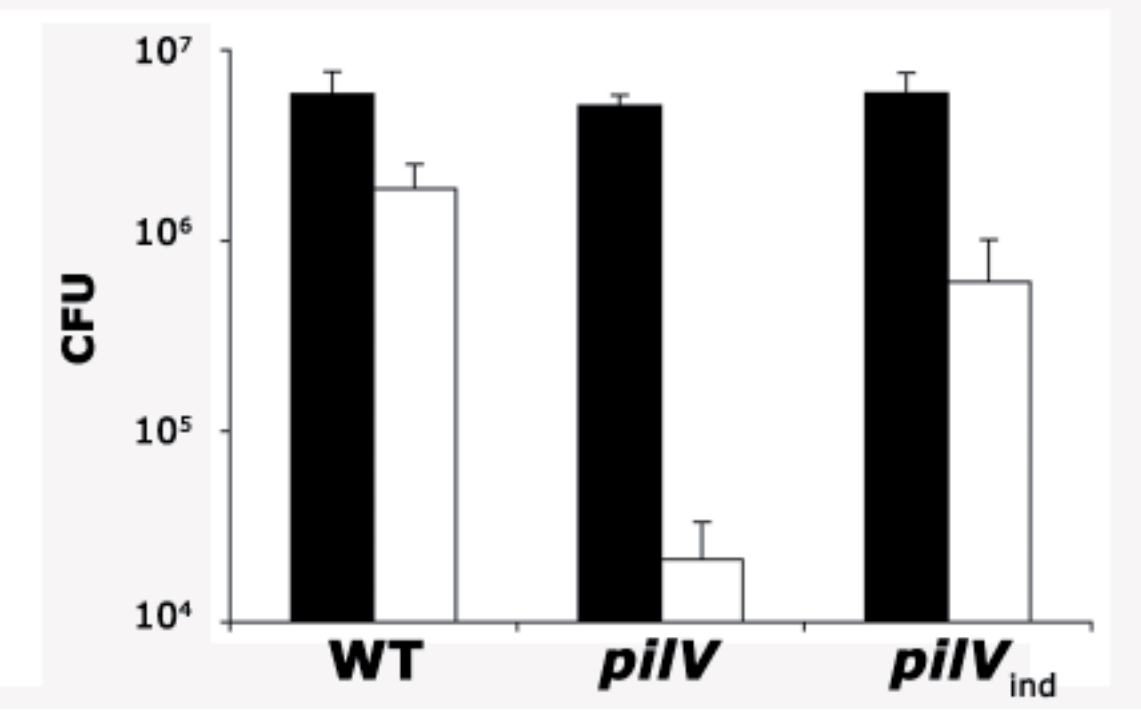
Lambotin et al. J.Cell Science, 2005, 18:3806

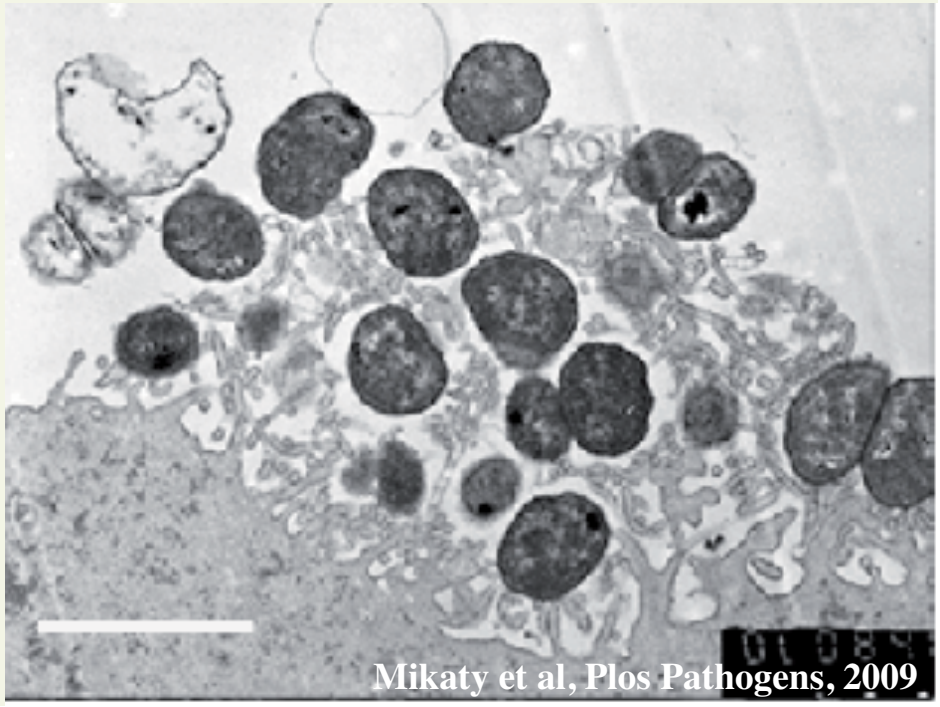
Doulet et al., J.cell.Biol., 2006,173:627

Pili signaling lead to the formation of a « cortical plaque ”



Host cell surface reorganization is responsible for mechanical resistance of Nm colonies growing onto the apical surface





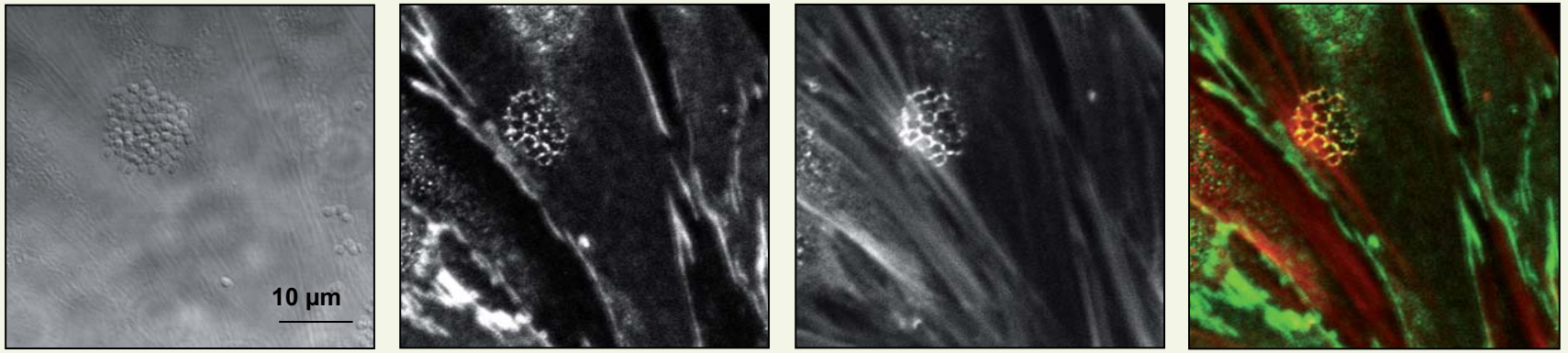
Summary

1. Pili are responsible for the interaction of Nm with endothelial cells and signaling

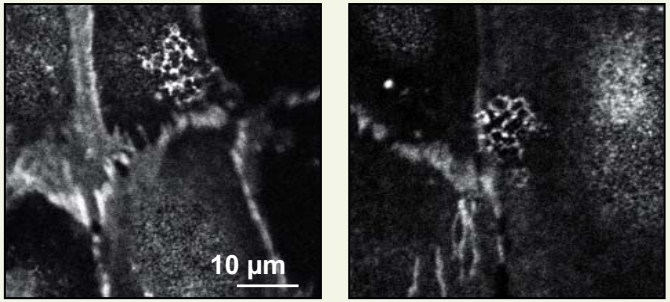
2. How pilus mediated adhesion is responsible for the crossing of the BBB ?

Nm recruit adherens and tight junction proteins

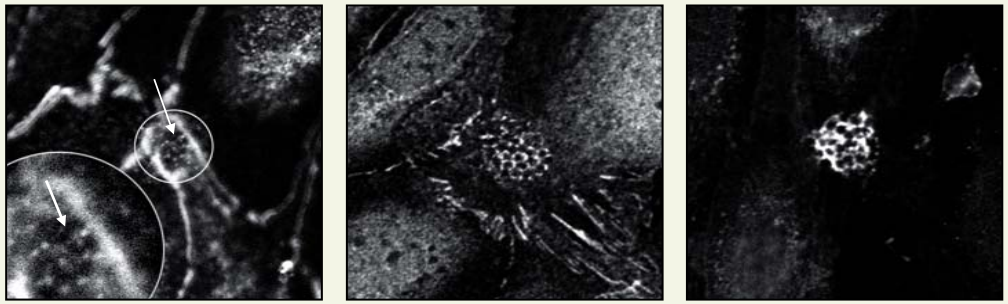
Nm | VE-Cadherin | Actin | Merge

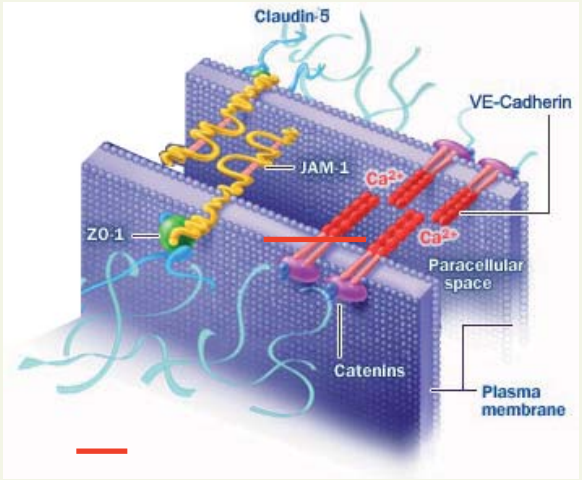


p120-catenin | beta-catenin

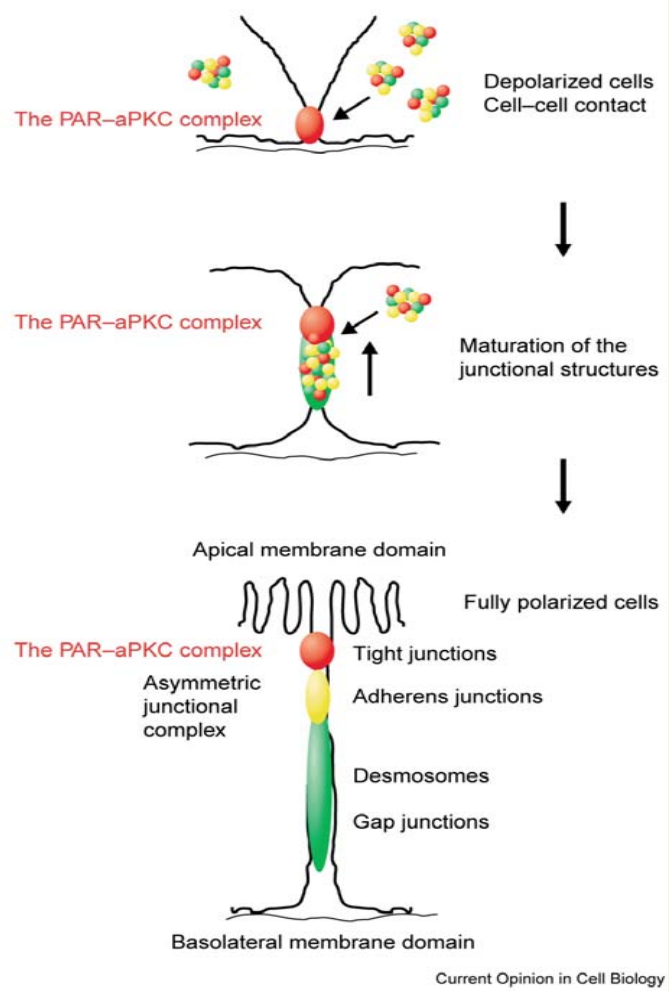


ZO-1 | ZO-2 | claudin-5

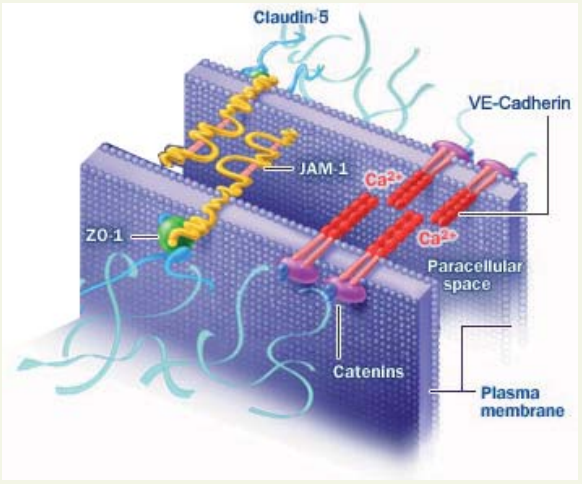




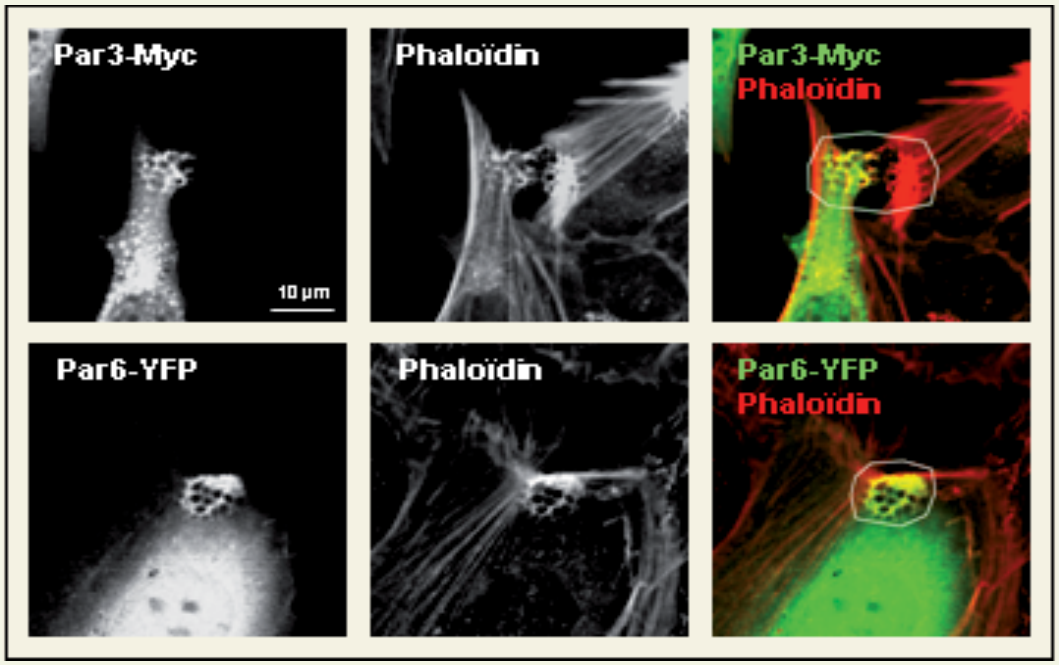
Formation of junctions (epithelial cells)

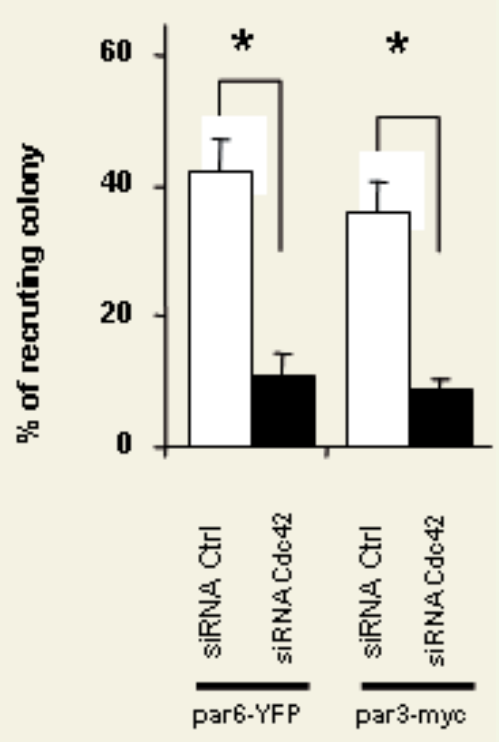


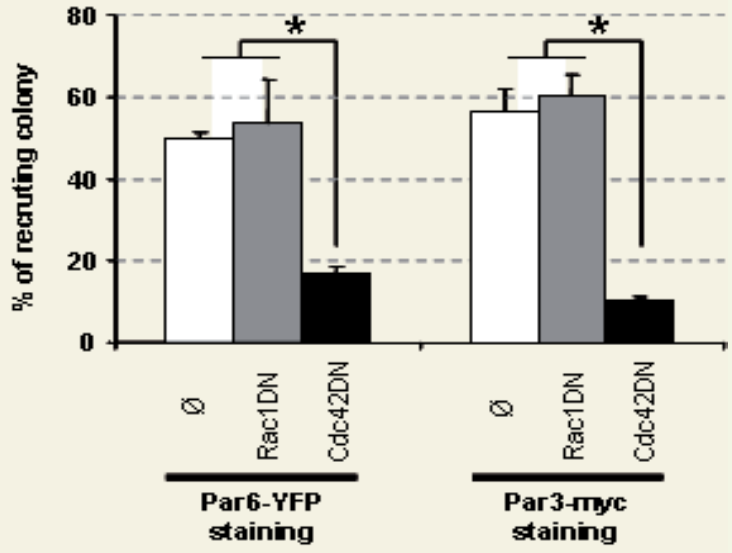
The Par3/Par6/aPKC complex is needed for the recruitment of junctionnal proteins and then segregation of adherens and tight junctions

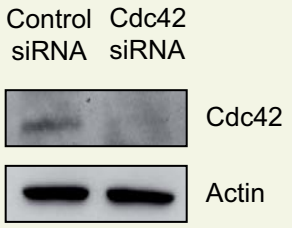


Nm recruits the polarity complex Par3/Par6/PKCζ in a Cdc42 dependent manner

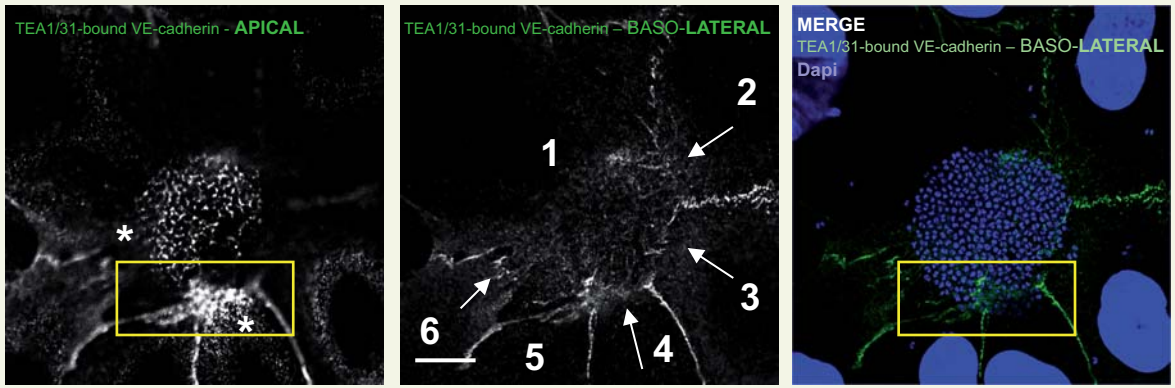


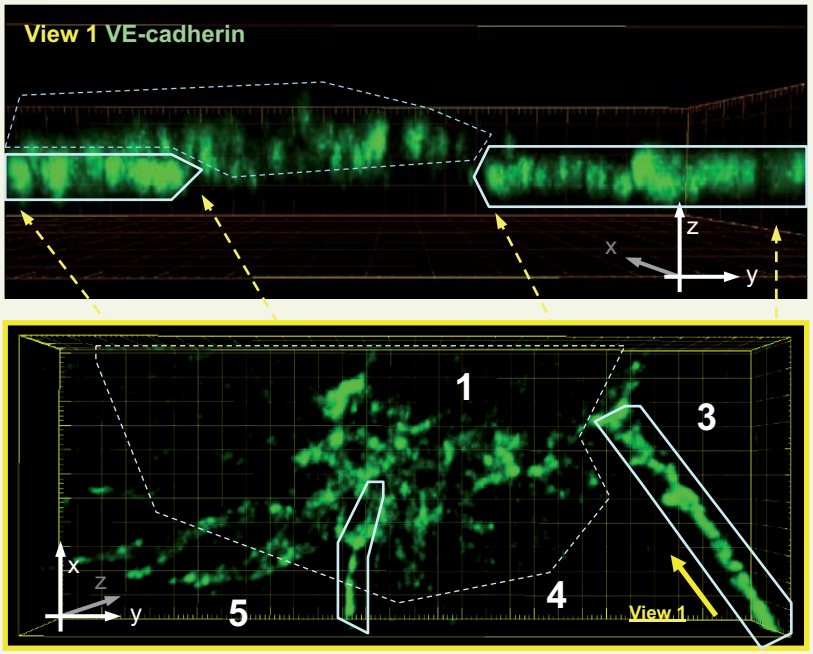


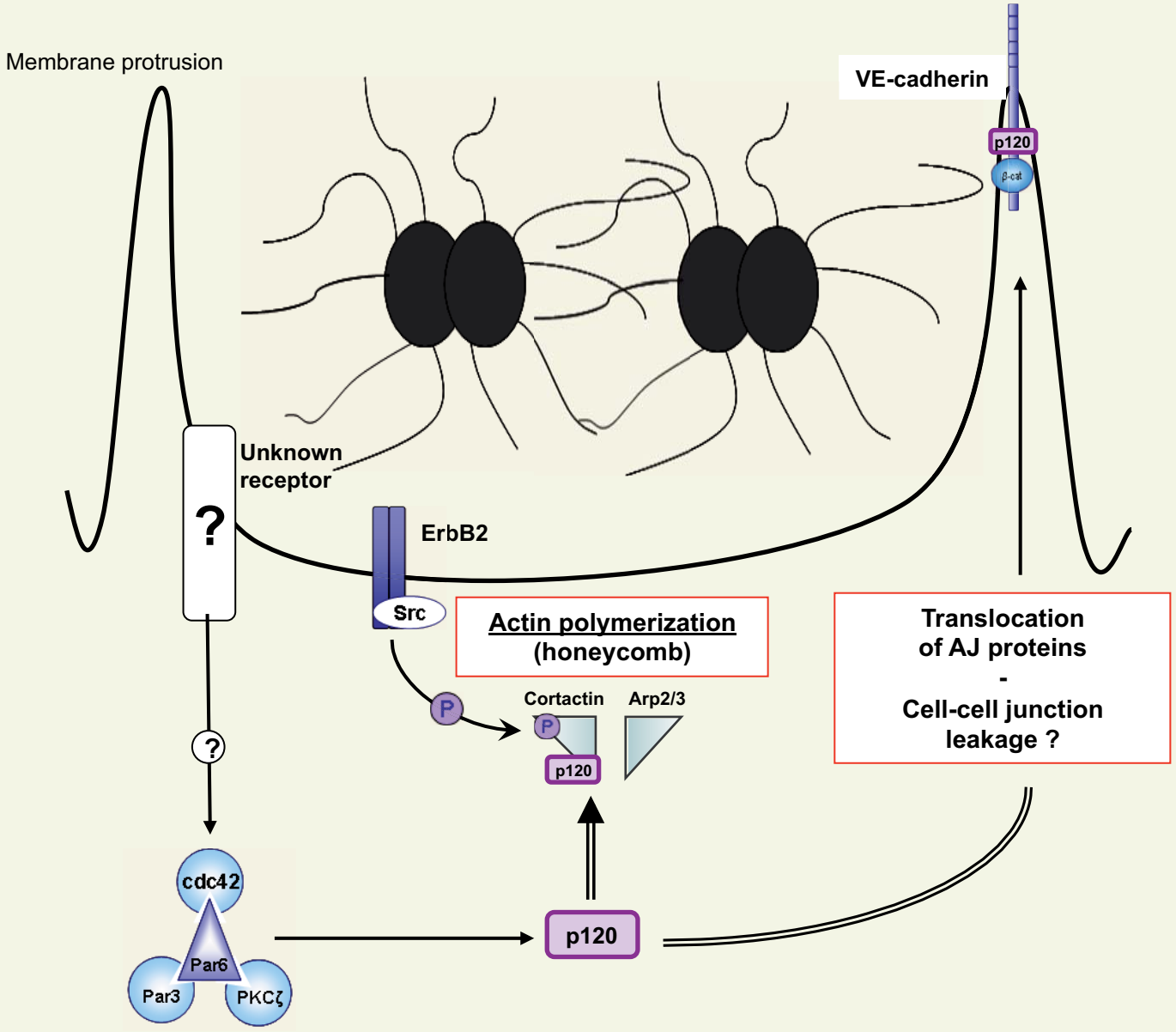




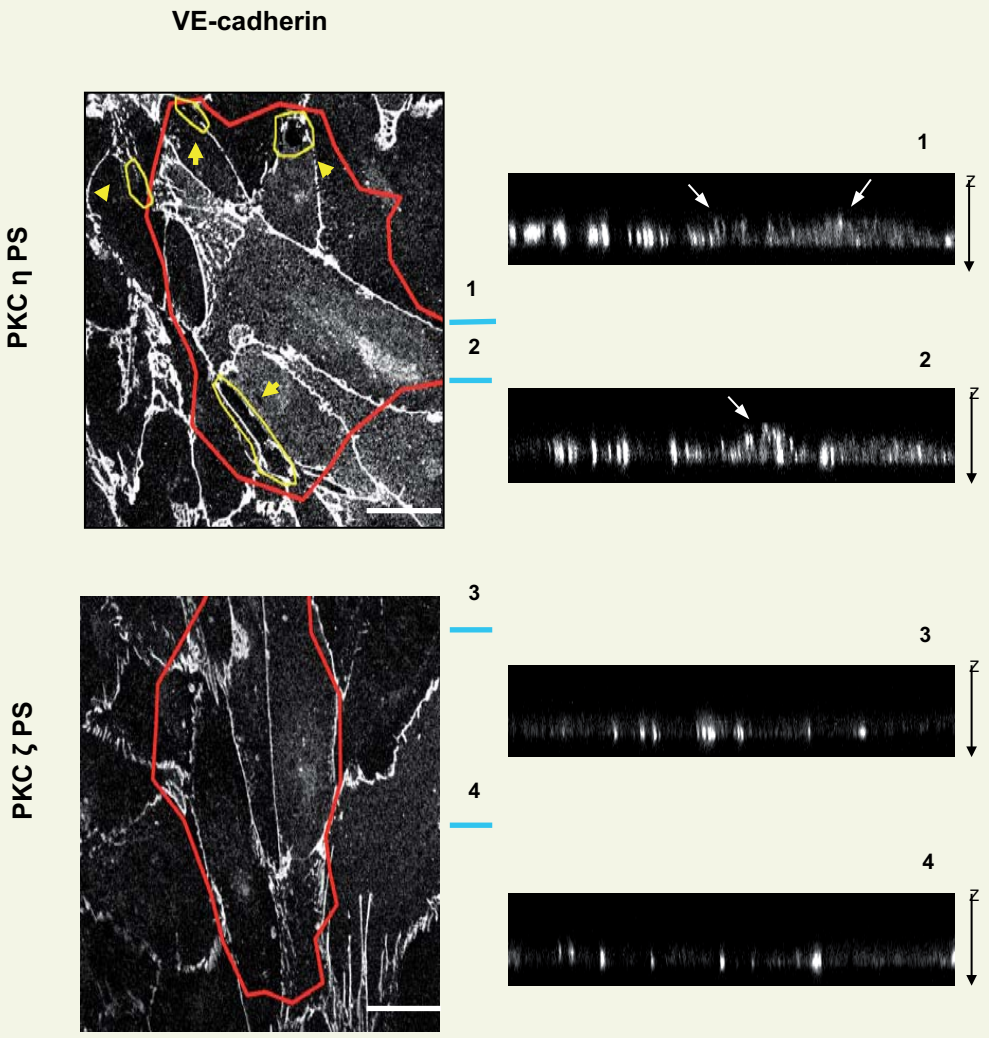
Tagged VE-cadherin is recruited from cell-cell junctions

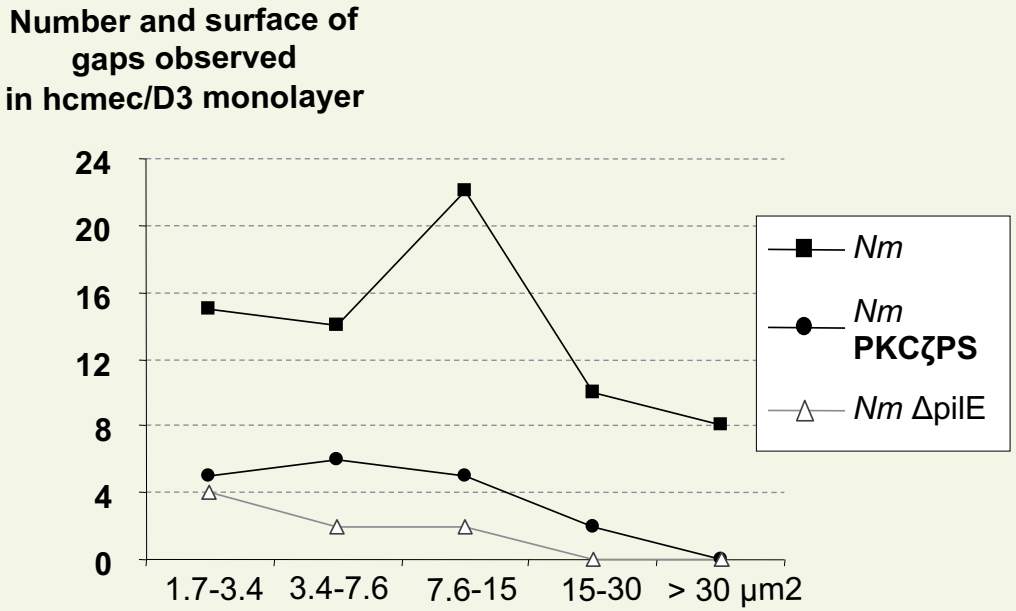





Membrane protrusion
VE-cadherin
p120
β-cat
Unknown receptor
?
ErbB2
Src
Actin polymerization (honeycomb)
Translocation of AJ proteins - Cell-cell junction leakage ?
P
Cortactin
Arp2/3
P
p120
?
cdc42
Par6
Par3
PKCζ
p120

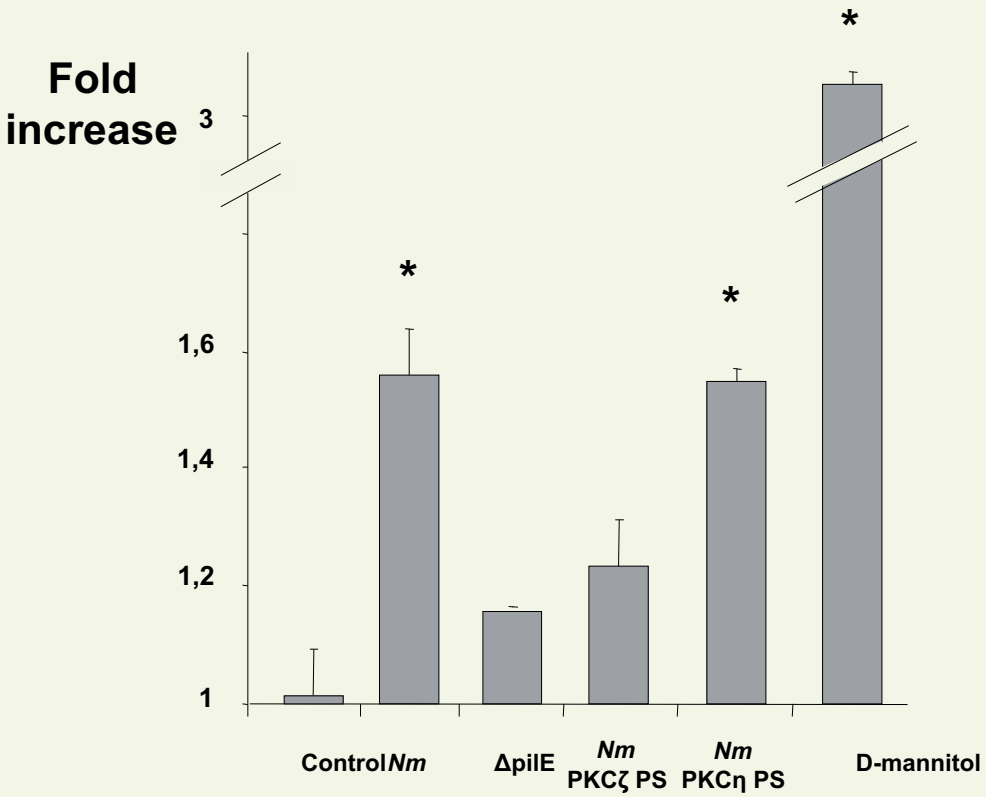
The polarity complex is required to open the paracellular route



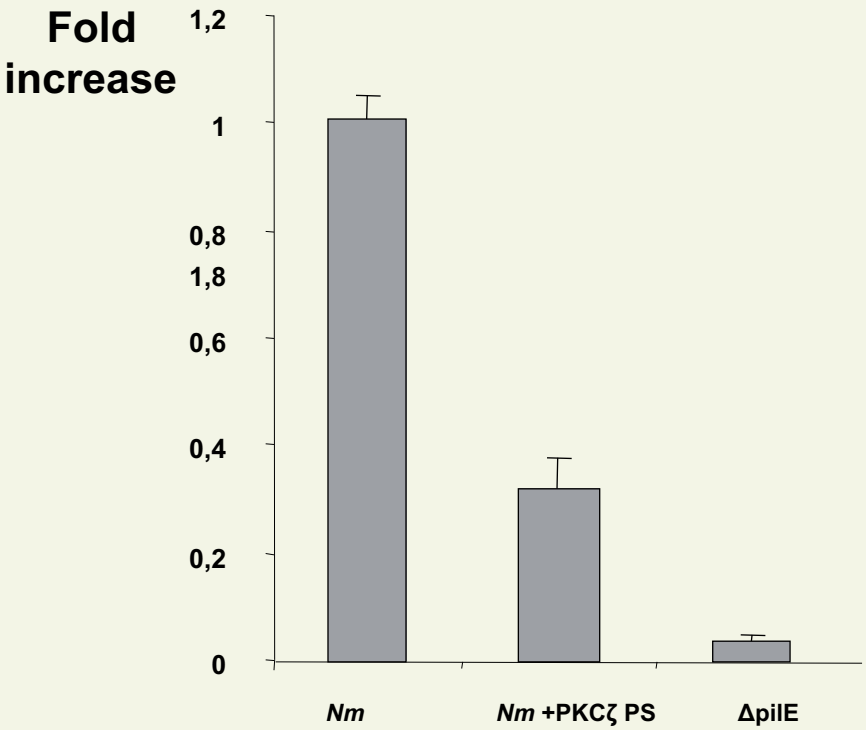


Type IV pilus-mediated signaling induces the opening of the paracellular route

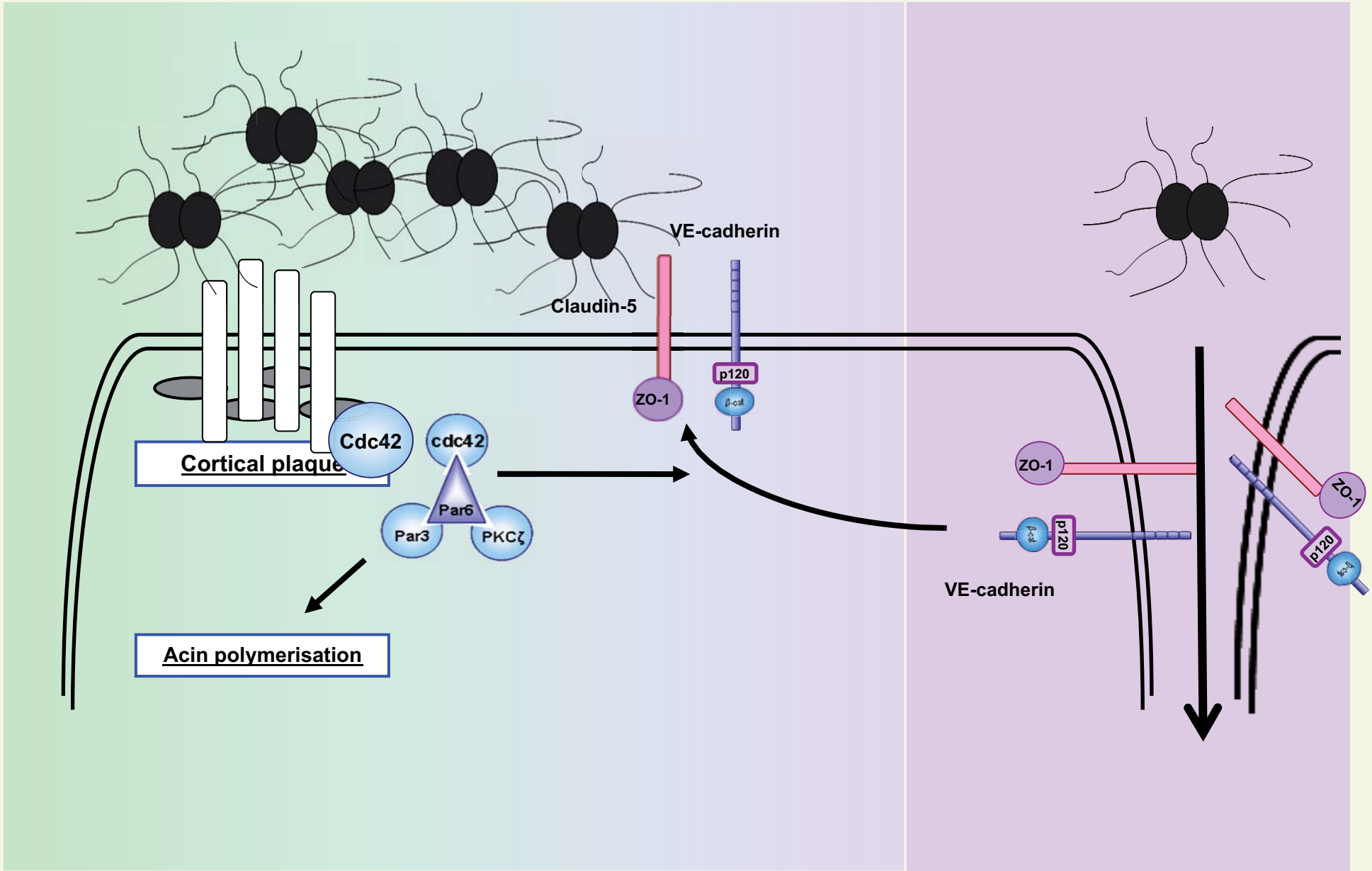
Lucifer Yellow Permeability



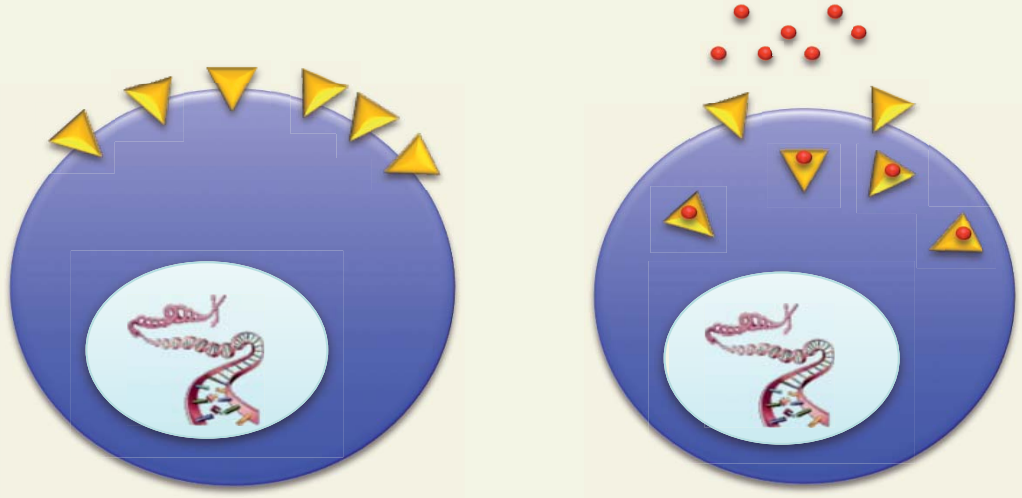
Bacterial diffusion through hCMEC/D3 monolayer



Nm recruit the polarity complex and open the intercellular junctions

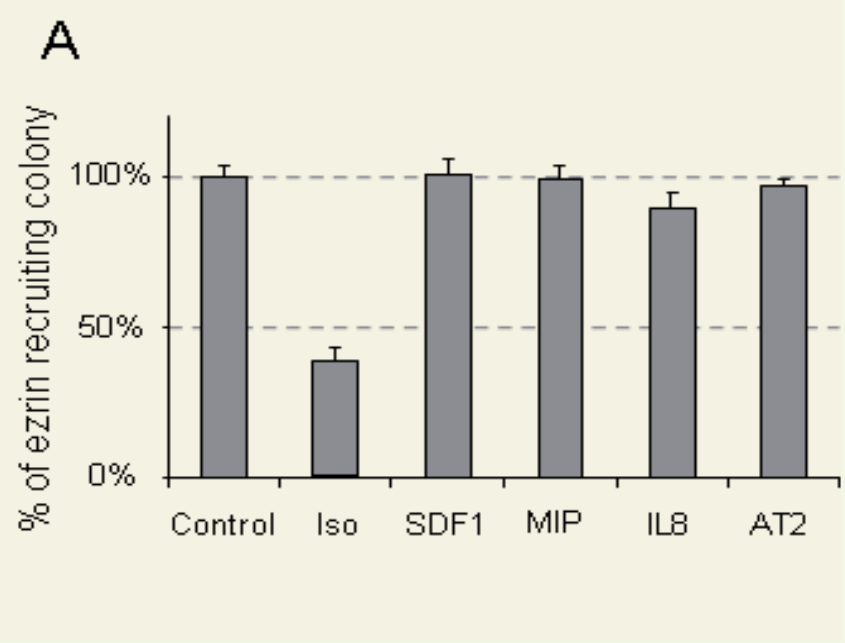


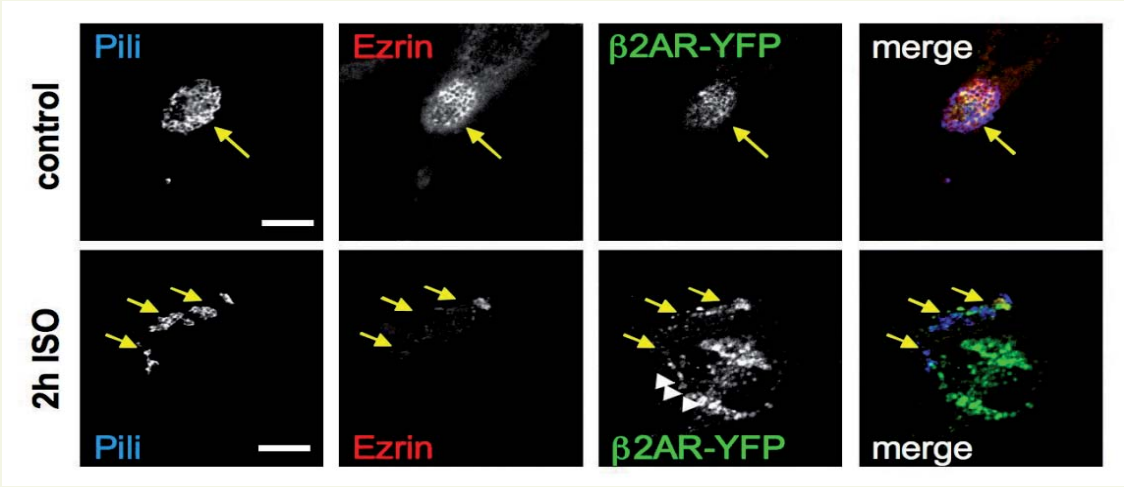
Neisseria meningitidis activate the β2-adrenergic receptor (β2AR)



GPCRs

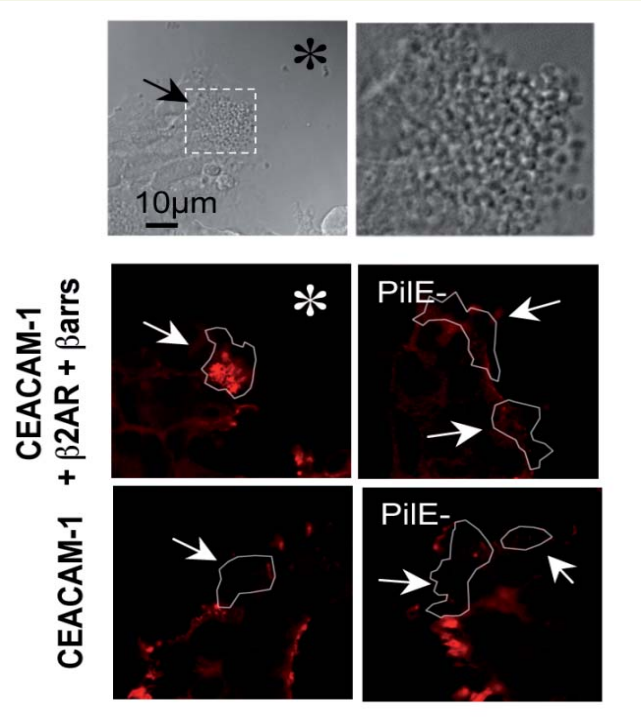
GPCRs + agonist

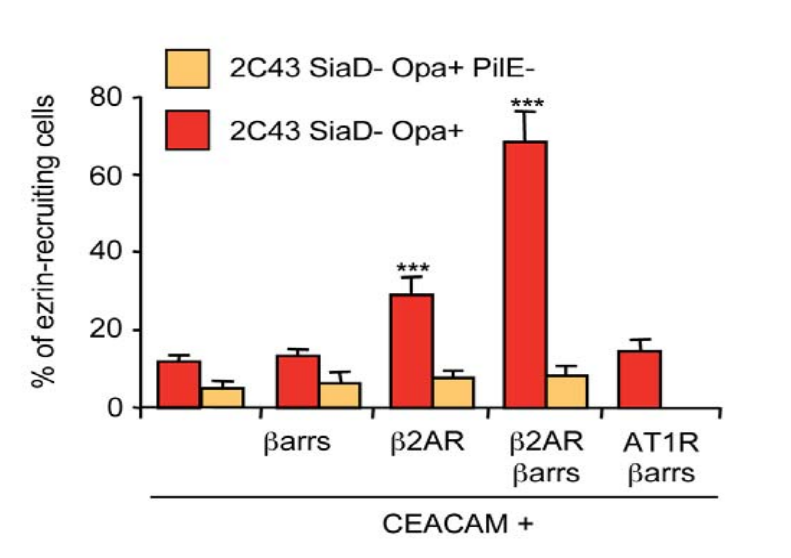




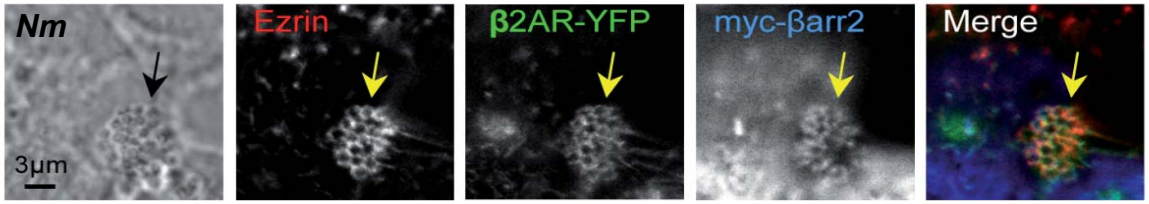
β2-adrenoceptor/βarrestins are sufficient to induce formation of the cortical plaque

Ezrin staining

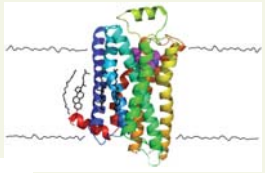


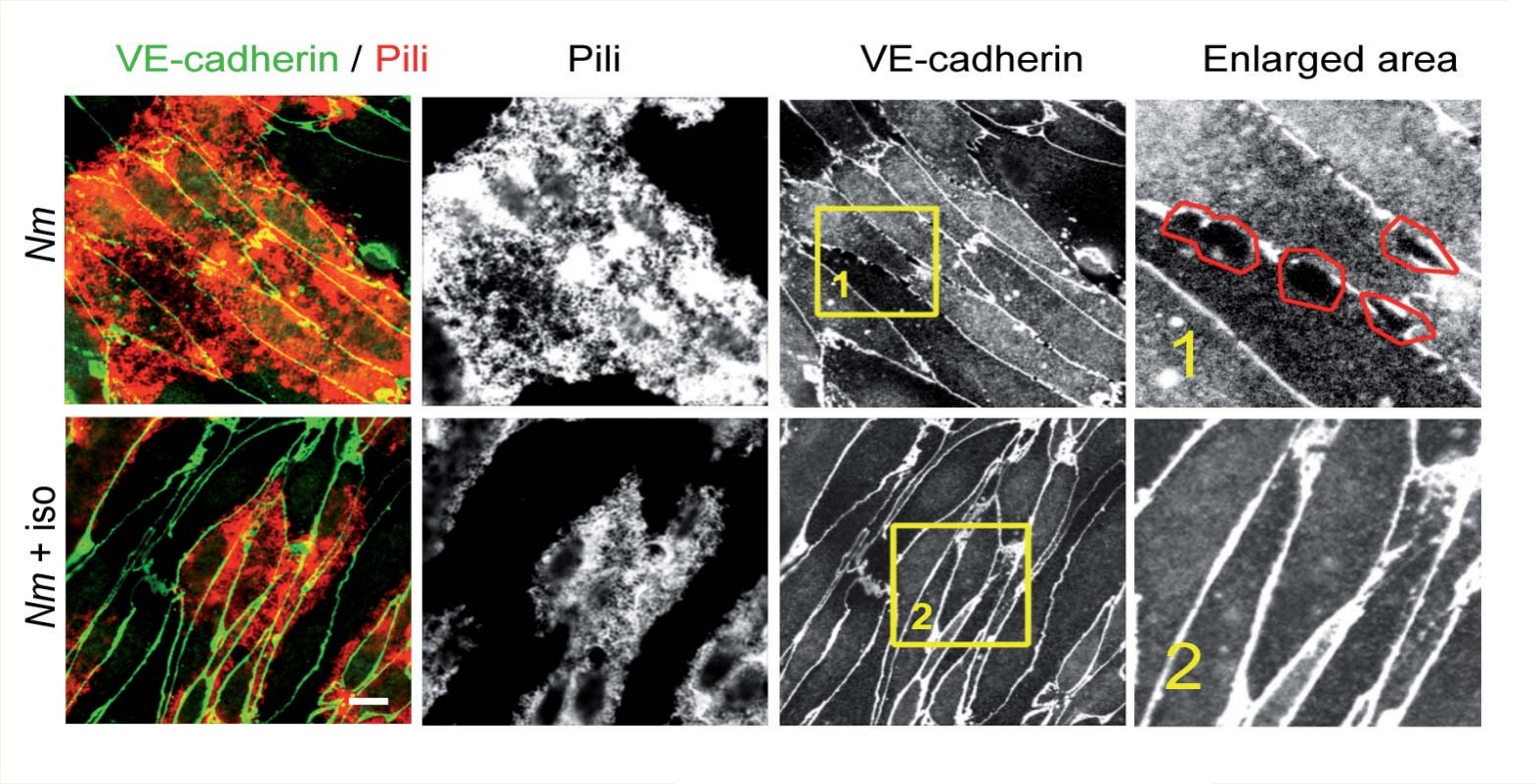


Recruitment of proteins in CEACAM-1/β2AR/βarrs transfected cells

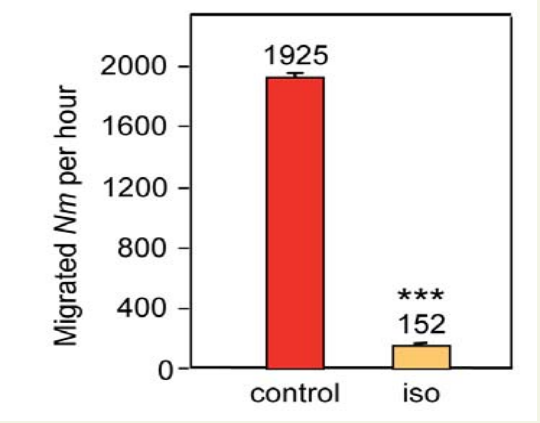


β2-adrenoceptors/barrestins pathway open junctions

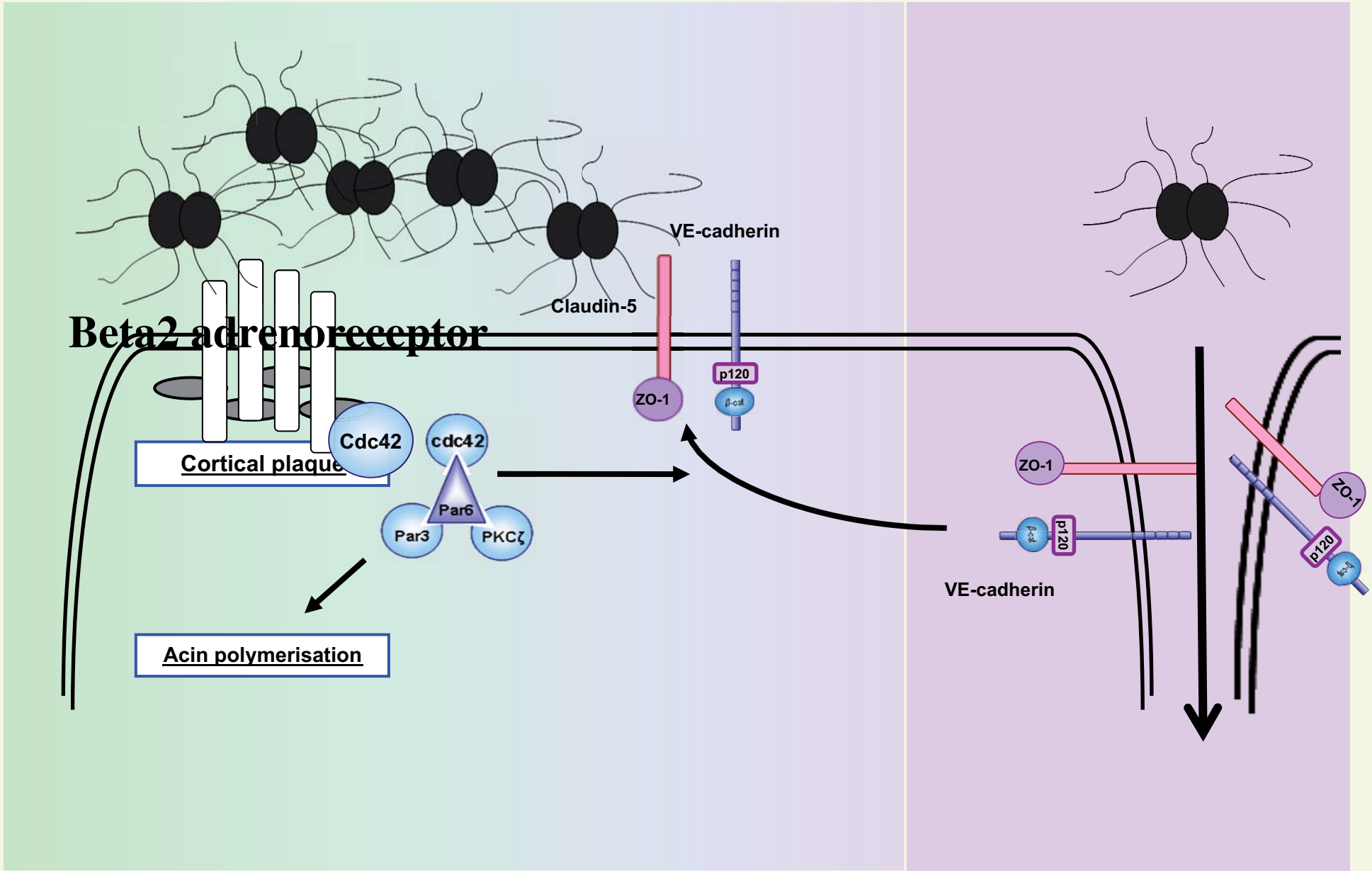




Nm transmigration
5h after infection



Nm recruit the polarity complex and open the intercellular junctions



Conclusions

1. *N.meningitidis* franchit la BHE grâce à ses pili qui permettent adhésion et signalling par deux récepteurs différents

2. Le passage de la BHE est due à l'ouverture des jonctions intercellulaires suite à un recrutement au siège de l'interaction bactérie-cellule des protéines de jonction intercellulaire

3. Le récepteur membranaire induisant la signalisation cellulaire est le récepteur beta2 adrenergique

Applications

1. **Utilisation des composants du pilus interagissant avec le récepteur pour ouvrir la BHE**

2. **Utilisation de ces mêmes épitopes pour une application vaccinale contre *N.meningitis* de sérogroupe B**

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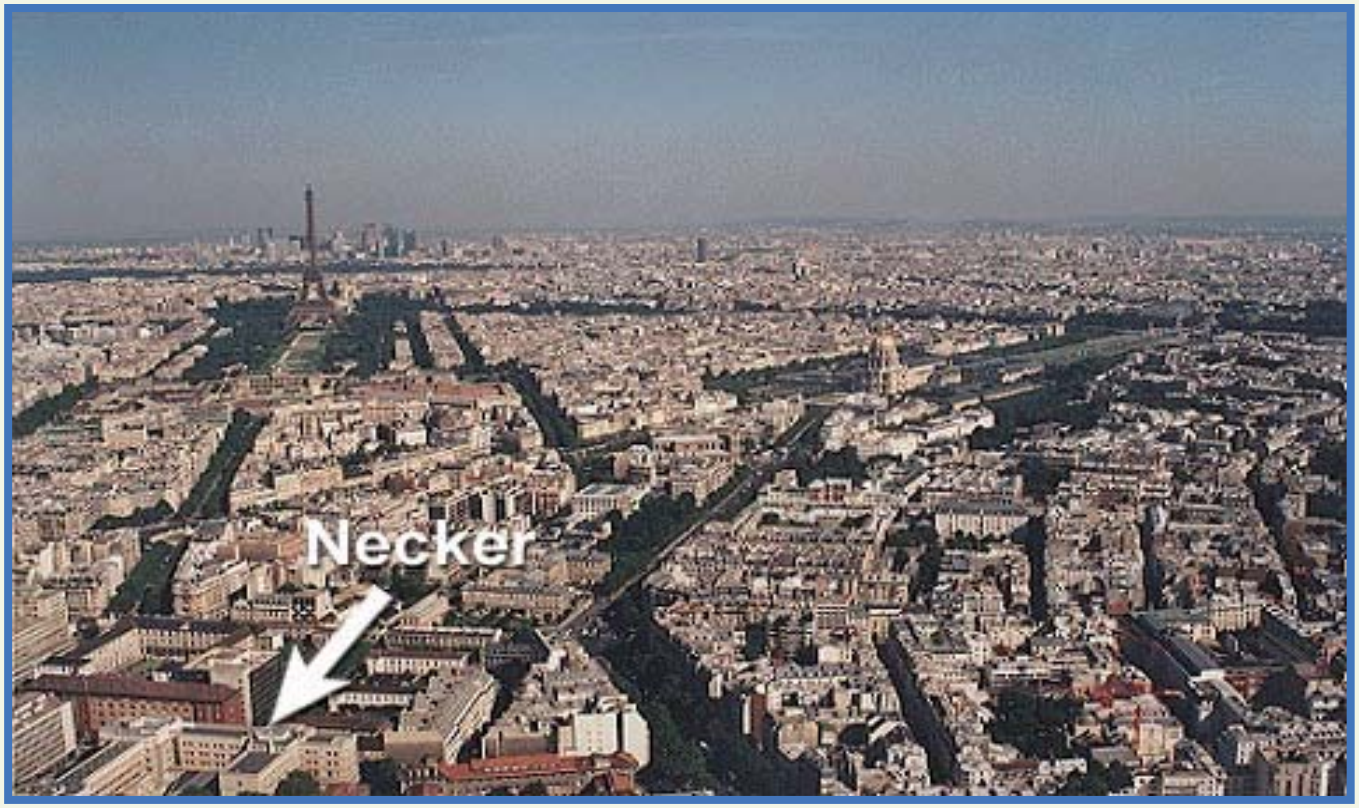
H.Omer
V.Pelicic



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