Mechanobiologyand infectious diseases

How bacterial virulence factors and toxins hijack the eucaryotic sysyem in the aethernal host-pathogen fight

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Outline

Part 0: introduction

Part I

Virulence factors hijacking host mechanics

- Adhesion
  - *Y.pseudotuberculosis*
  - UPEC
- FA dynamics
  - *H.pylori*
  - *S.flexneri*
  - EPEC
- RhoGTPases
  - Overview
  - CNF

Part II

Mechanical forces at host-pathogen interface

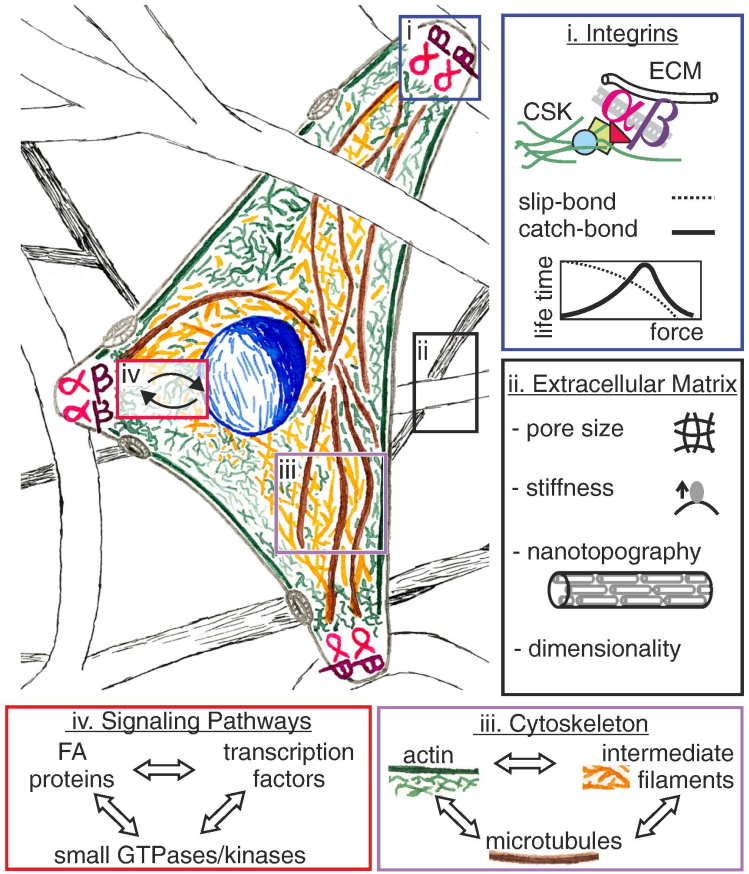
- Chatch bond vs Slip bond
  - FimH
- How mechanics influences bacterial virulence
  - EHEC
  - *P.aeruginosa*

Part III

My work

Part 0:
Introduction to mechanobiology

What mechanobiology is?

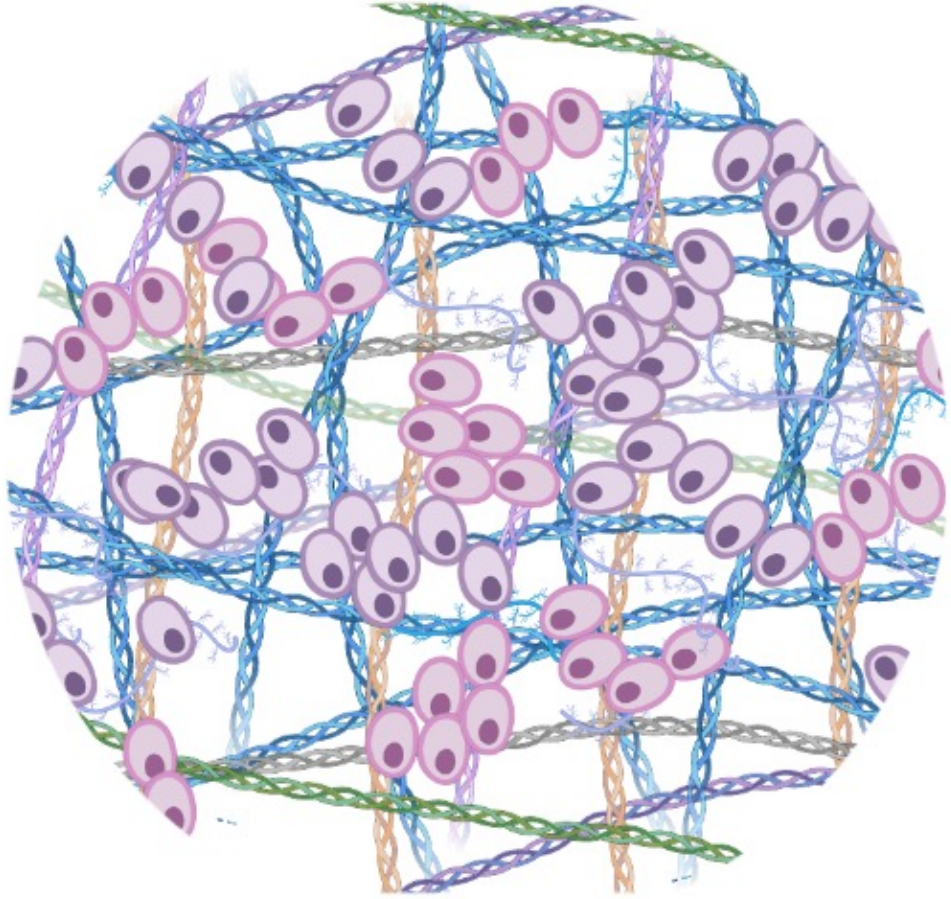


- Multidisciplinar field
- How cells can sense and respond to the physical environment
- Development, stem cells, physiology, disease

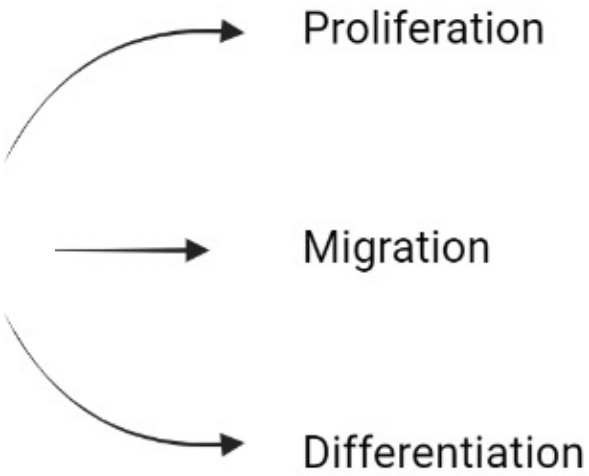
Infection and Host-Pathogen interaction

Jansen et al. BBA 2015

The extracellular matrix



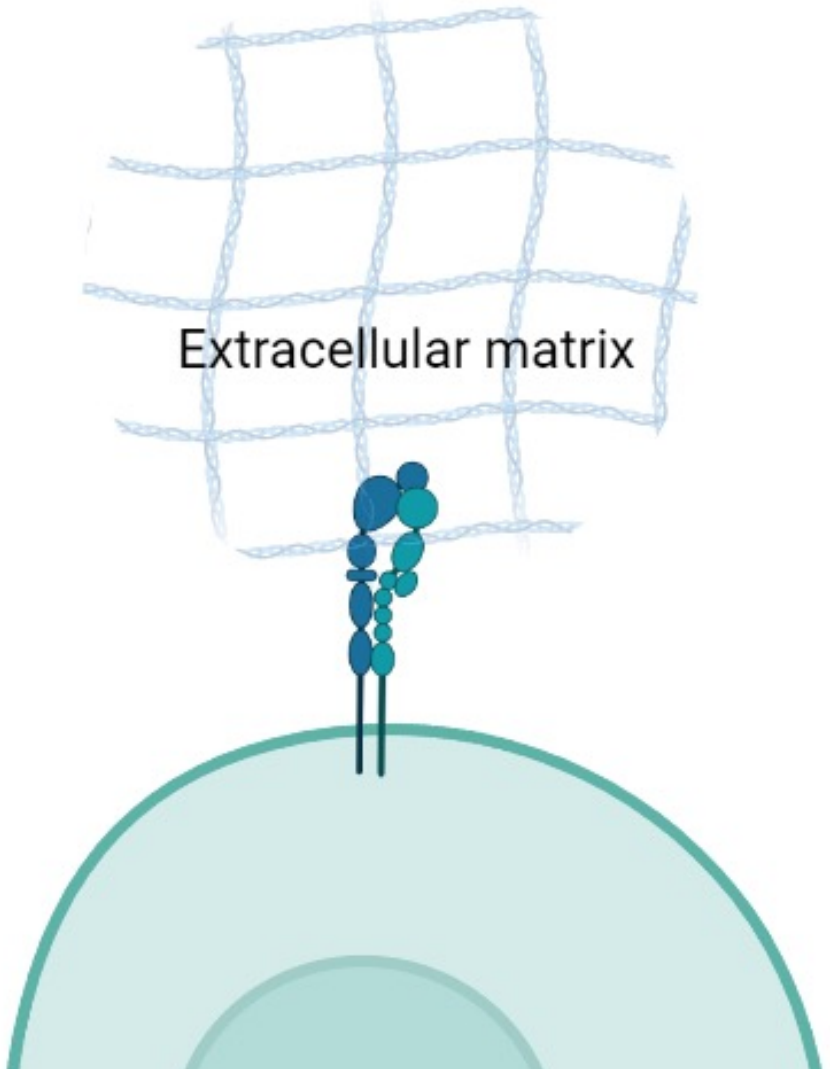
Extracellular matrix



A complex mixture of macromolecules

Sustains chemically and physically the cells

How?



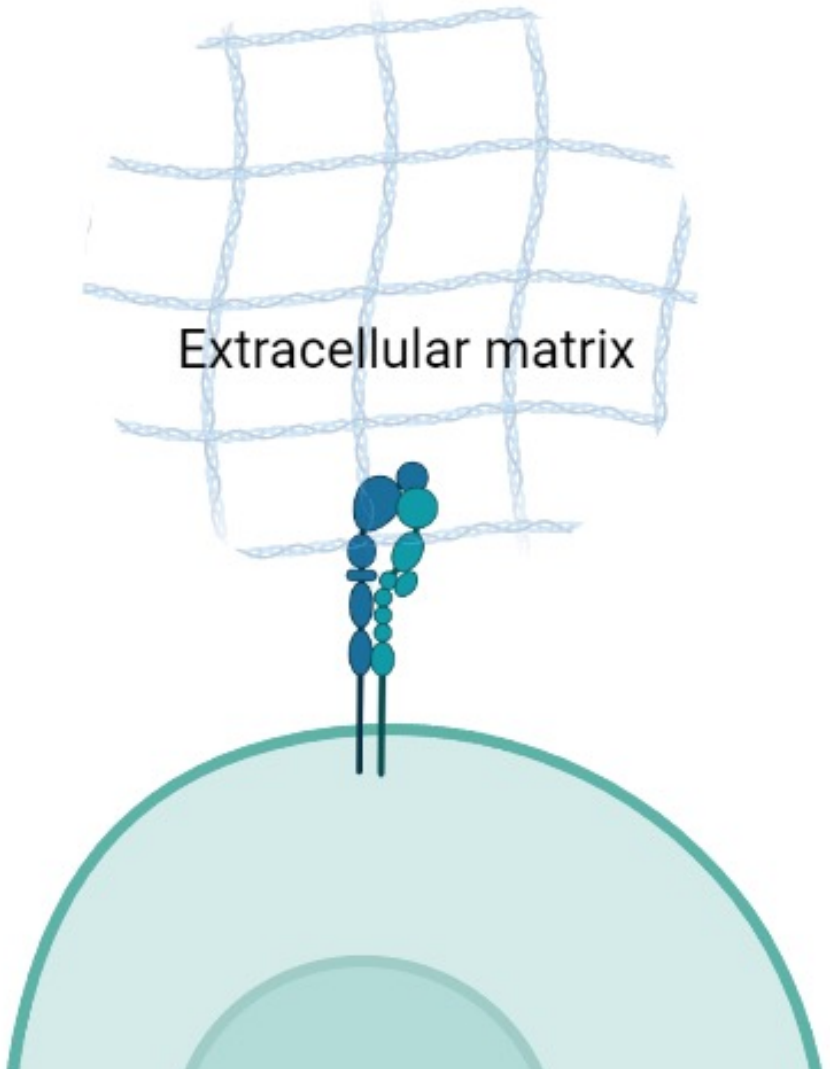
- Adhesion by surface proteins represents the first direct way for the cell to establish an interaction with the extracellular environment, in which mechanical stresses are constantly everywhere.

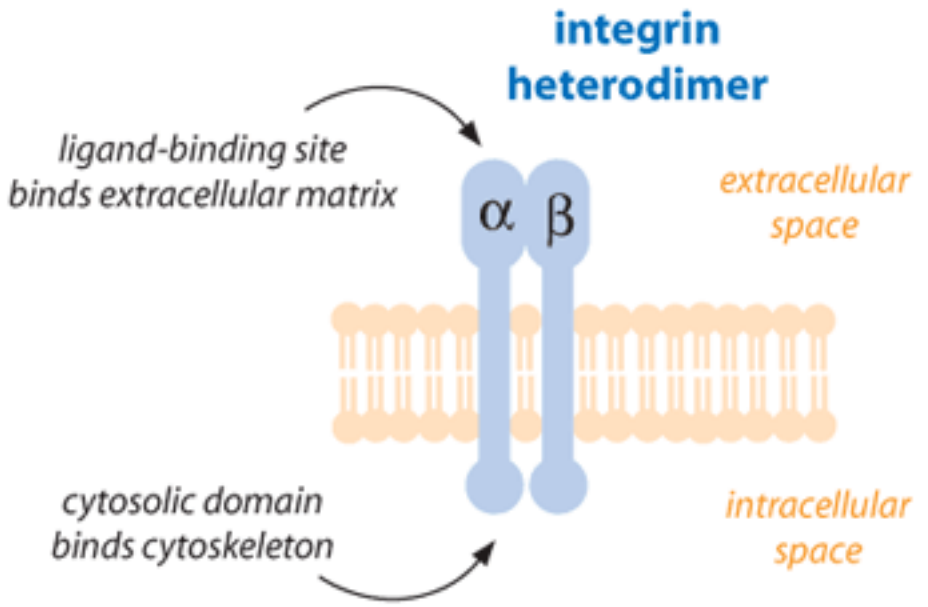
- Maintains tissue integrity

- fundamental for cell life, regulating every step from differentiation to senescence

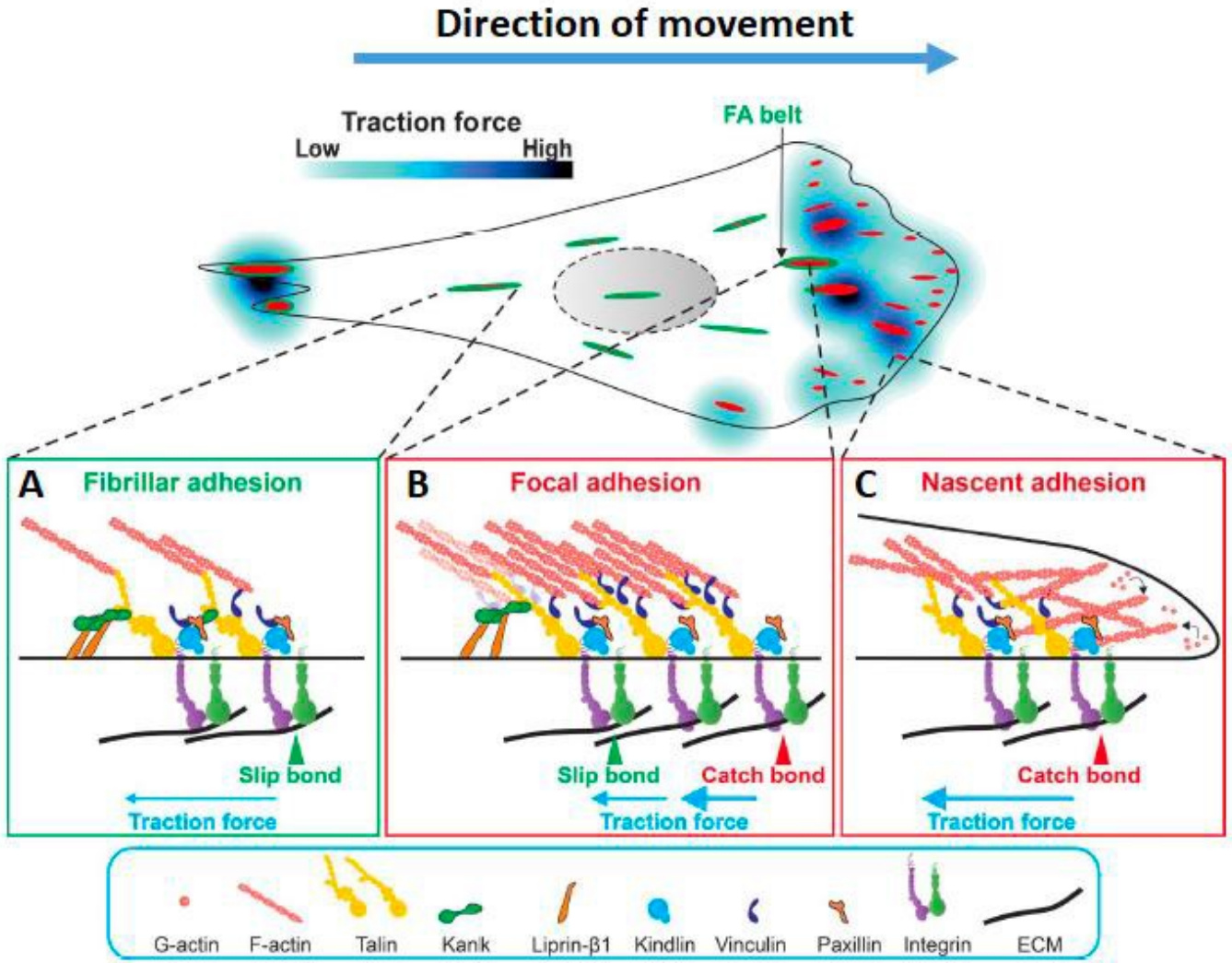
- Mechanical forces are transduced (or mechanotransduced) from the environment to the cell and back by complex signaling systems studied in the field of mechanobiology.

How?

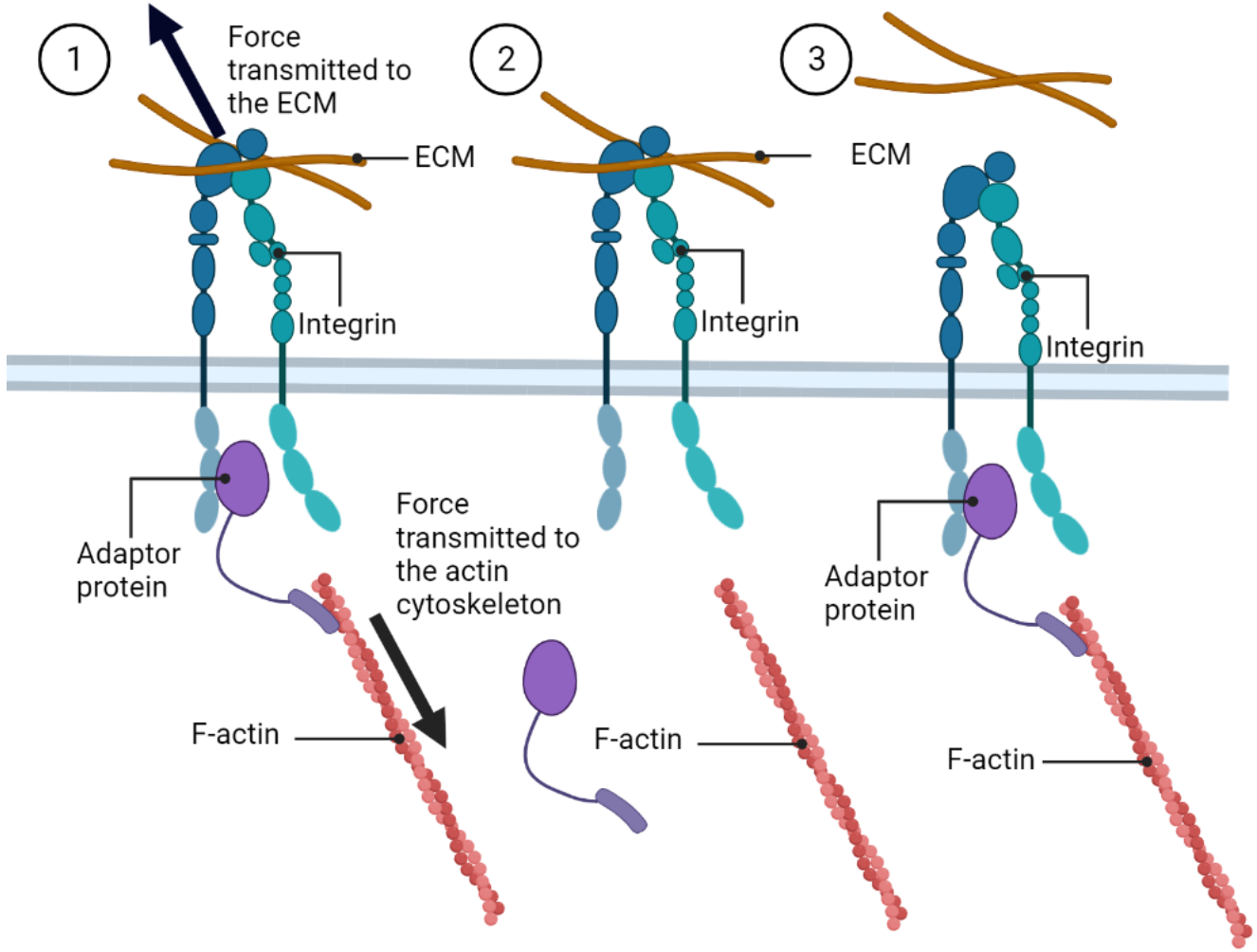

Extracellular matrix


integrin
heterodimer
ligand-binding site
binds extracellular matrix
α
β
extracellular
space
cytosolic domain
binds cytoskeleton
intracellular
space

Adhesive structures in the eucaryotic cell

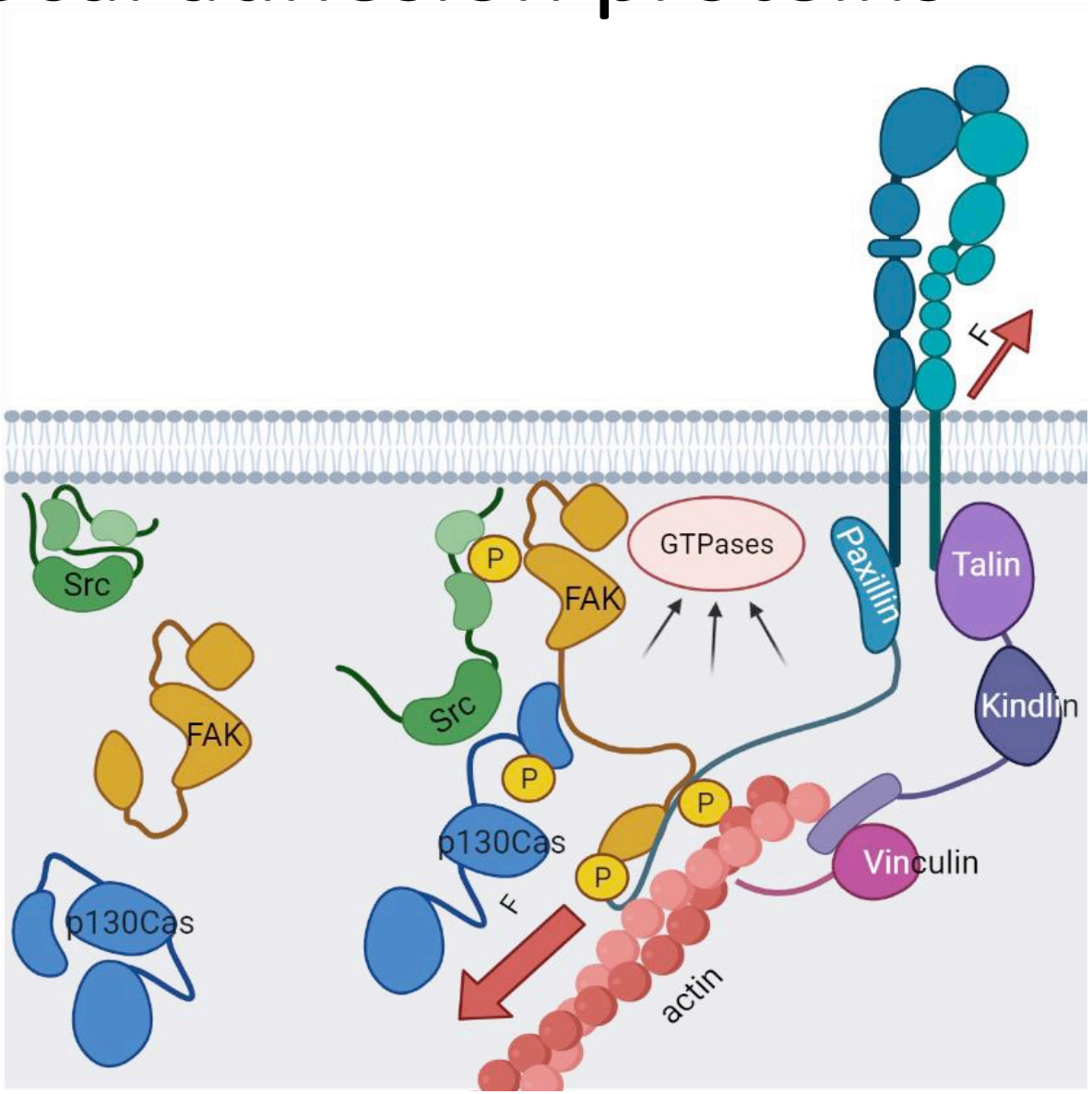


How does it work: the molecular clutch



The result of mechanotransduction is the activation of small GTPases which ultimately adapt the cytoskeleton and the cell behaviour to the environment

Focal adhesion proteins

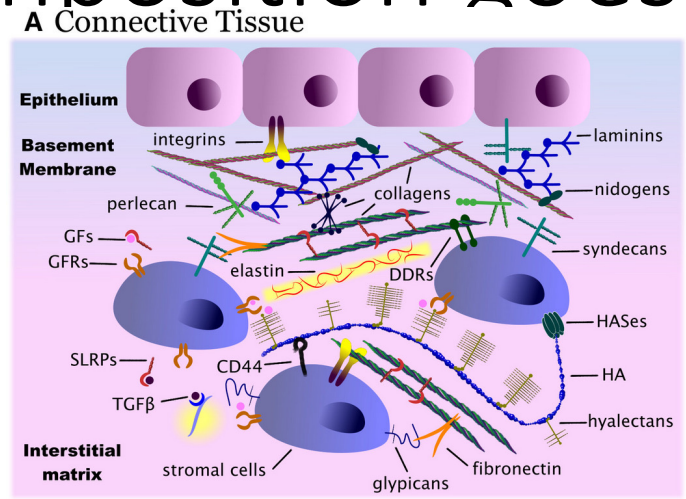


Selection of proteins localized at the adhesion site relevant for this study

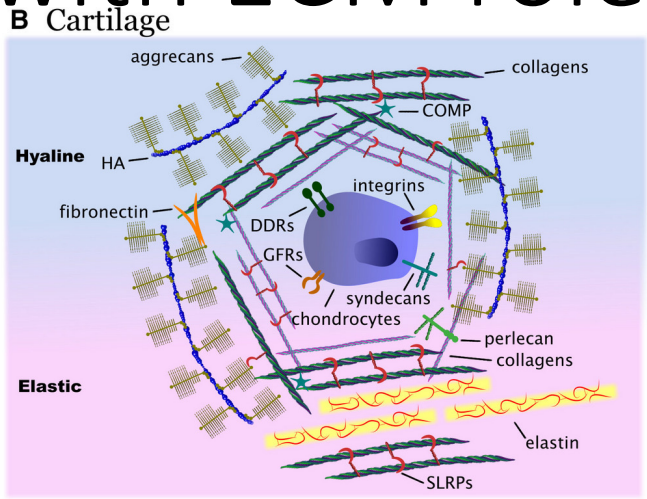
Name	interactions	Function	Mechanosensor?
Talin	Integrin cytosolic domain Actin	Start the FA complex, links integrin to the cytoskeleton	Yes, unfolding domains upon force load
Vinculin	With Talin and F-actin	Strengthen the FA connection with the cytoskeleton, force build-up	Yes
Paxillin	With FAK, integrin, vinculin	FAK substrate	Suggested, no formal evidence
FAK	With talin, paxillin, Src, p130Cas	Autophosphorylates at tyrosine 397, create a complex with Src, phosphorylates GEFs for Rac1 ans RhoA, integrin activation	Suggested, still to be demonstrated
Src	With FAK, p130Cas	Src-FAK complex binds and activates p130Cas	na
P130Cas	With FAK		Yes
Arp2/3	With vinculin, with Rac1	Actin branching Rac1 dependent, recruitment by vinculin at FAs	na
Rac1	Arp2/3, GEFs and GAPs	Nucleation of NAs, FA turnover, actin branching	na
RhoA	ROCK, GEFs and GAPs	Maturation of FAs, indirect phosphorylation of myosin II and actomyosin contraction, stress fiber formation	na

Table 1 List of proteins localized at the adhesion site relevant for this study, with relative function.

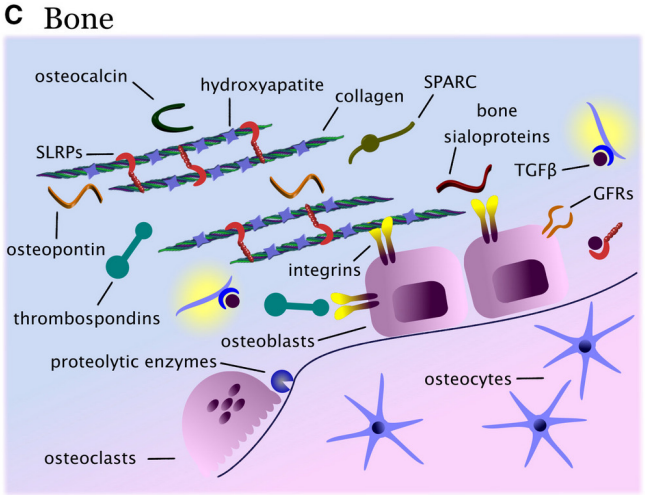
ECM composition goes with ECM roles



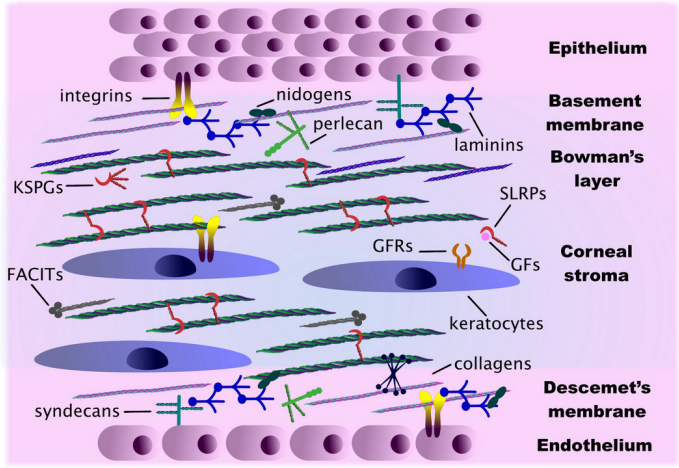
Connection, support, nourishion



Mechanical support, viscoelasticity, lubrication



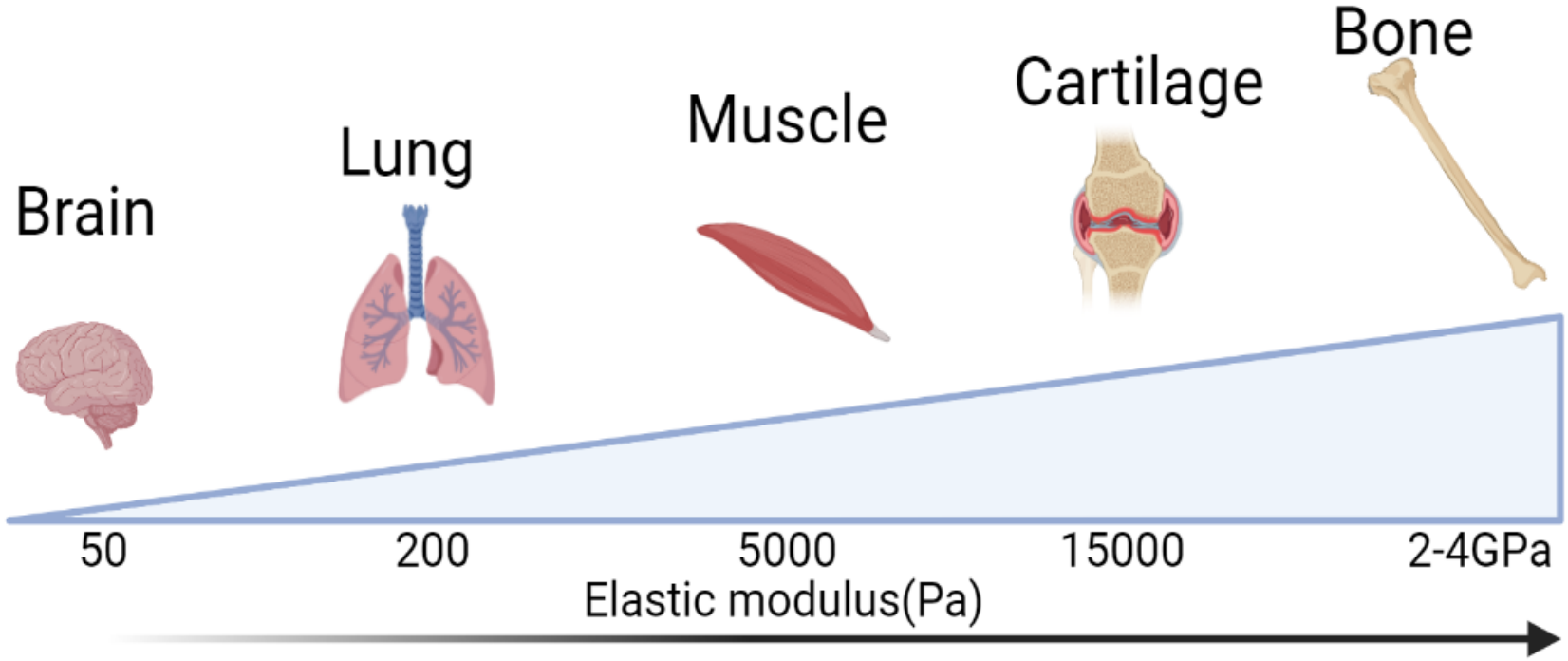
Durable, strong, stiff sctucture with low elasticity for shock absorption

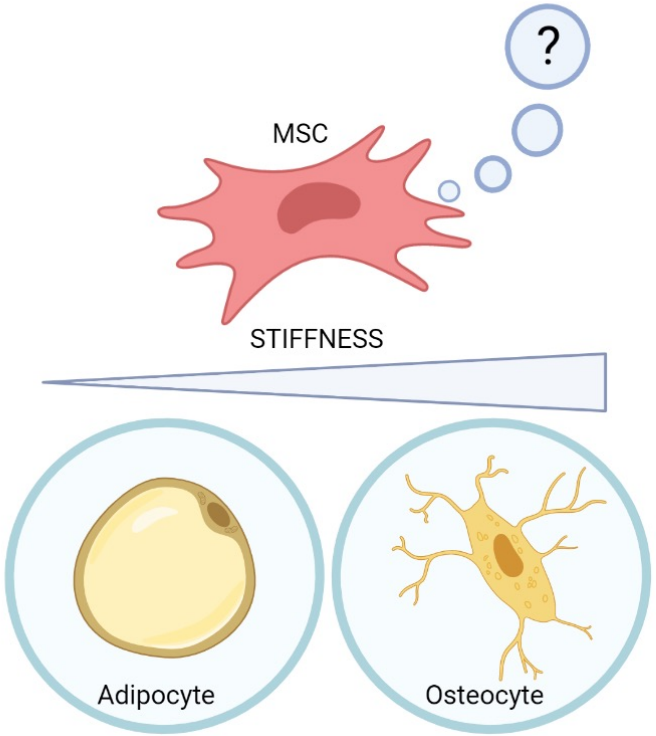


Transparency, refraction

Karamanos et al. FEBS 2021

ECM stiffness





Stiffness =Force load/deformation occurring

Stiffness = cross-sectional area * **Elastic modulus**/length of the sample

Engler *et al.* 2006

.ECM stiffness

Stiffness of the organ is related to the tissue function.

Static environment > low stiffness
high mechanical loads > high elastic modulus.

Organ stiffness can vary for different reasons.

- physiological situation

ECM stiffness

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- within an organ in function of the region

.ECM stiffness

Stiffness of the organ is related to the tissue function.

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Organ stiffness can vary for different reasons.

- physiological situation
- within an organ in function of the region
- Time

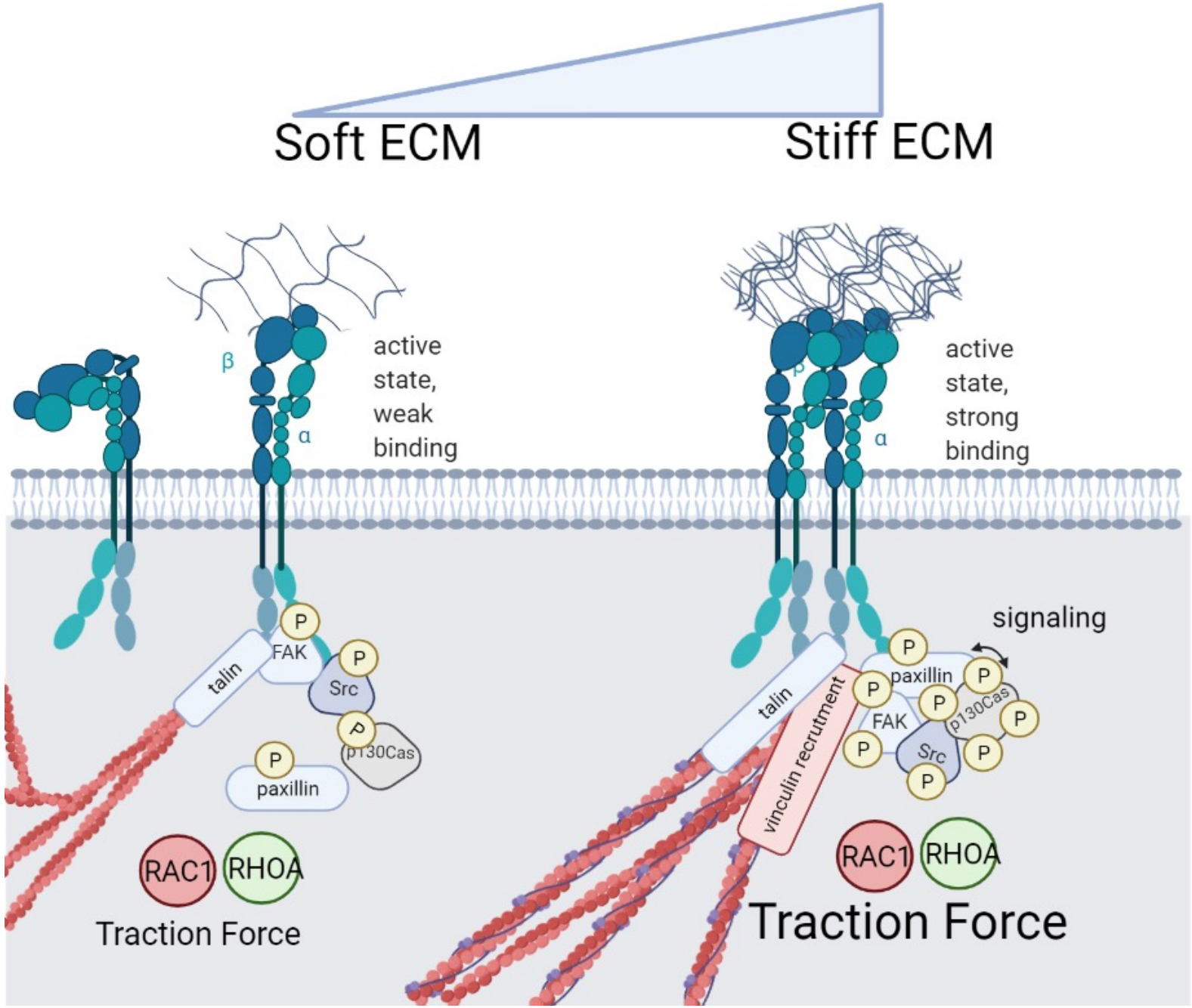
.ECM stiffness

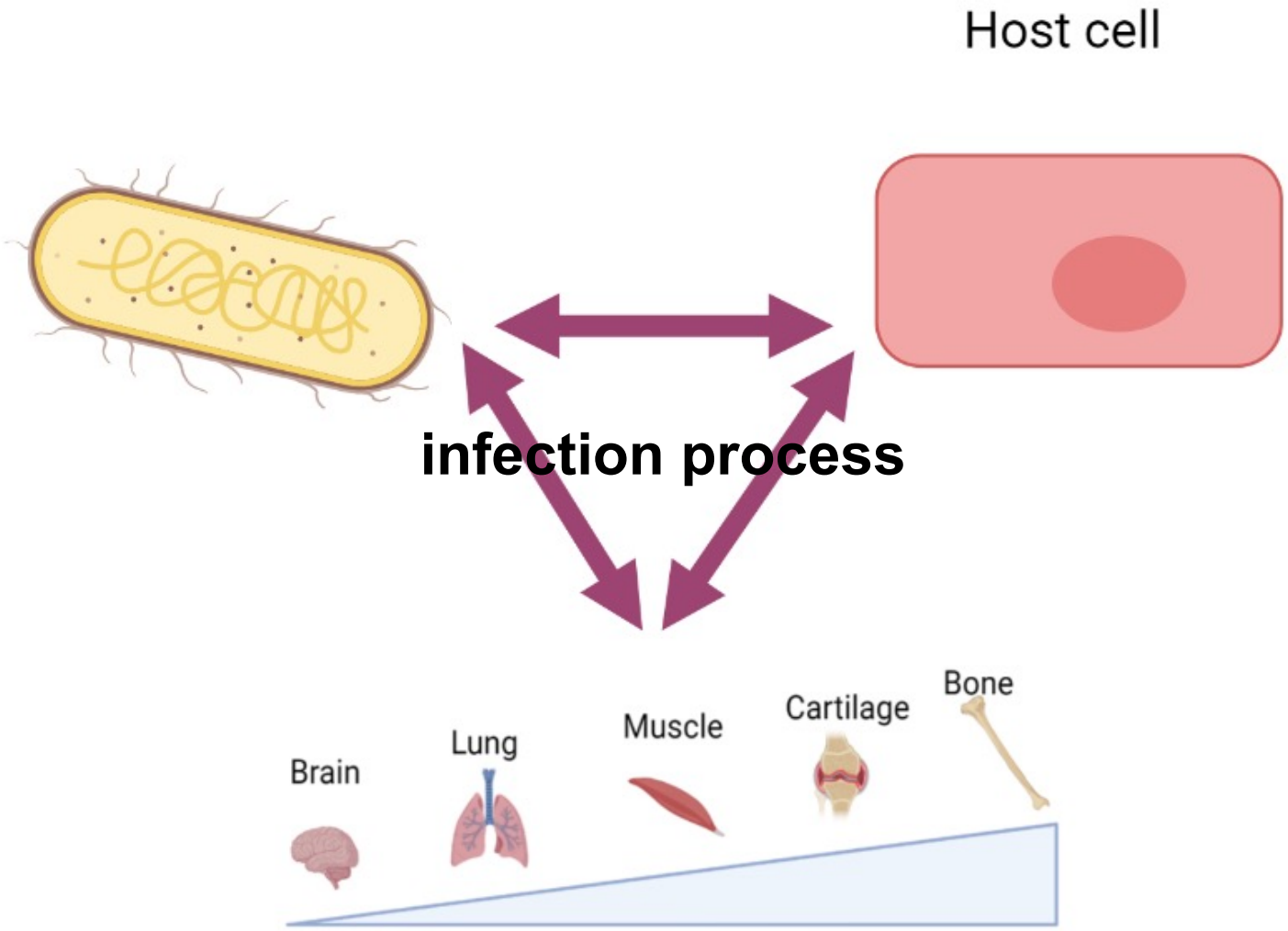
Stiffness of the organ is related to the tissue function.

Static environment > low stiffness
high mechanical loads > high elastic modulus.

Organ stiffness can vary for different reasons.

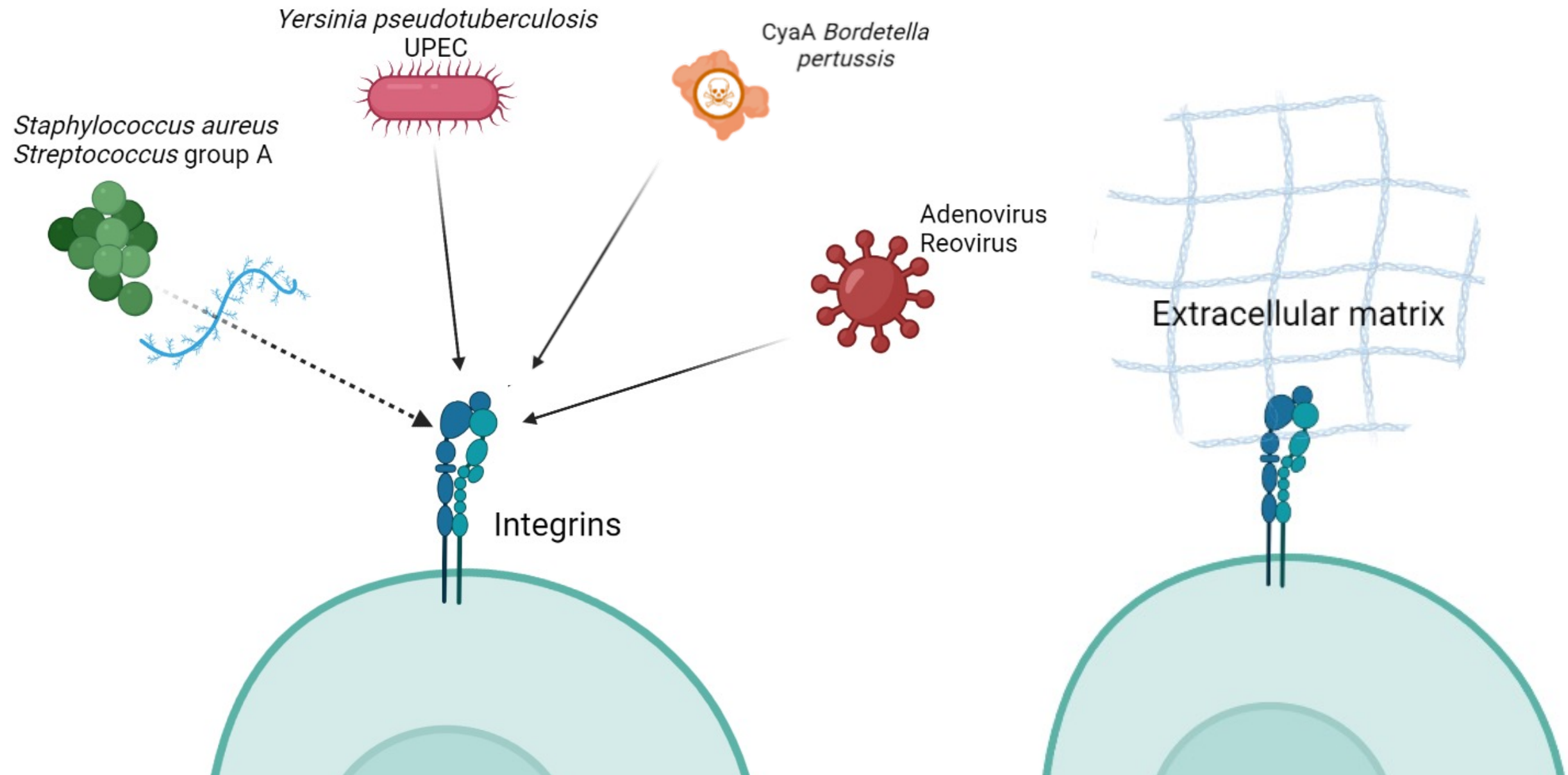
- physiological situation
- within an organ in function of the region
- Time
- Pathology l conditions such as fibrosis and tumorigenesis


Soft ECM
Stiff ECM
β
α
active state, weak binding
active state, strong binding
P
FAK
Src
p130Cas
paxillin
talin
RAC1
RHOA
Traction Force
signaling
vinculin recrutment


Host cell
infection process
Brain
Lung
Muscle
Cartilage
Bone
Mechanical features of the tissue

Part i: virulence factors hijacking the host mechanics

Integrins are hijacked by pathogens



Integrins as receptors for bacteria

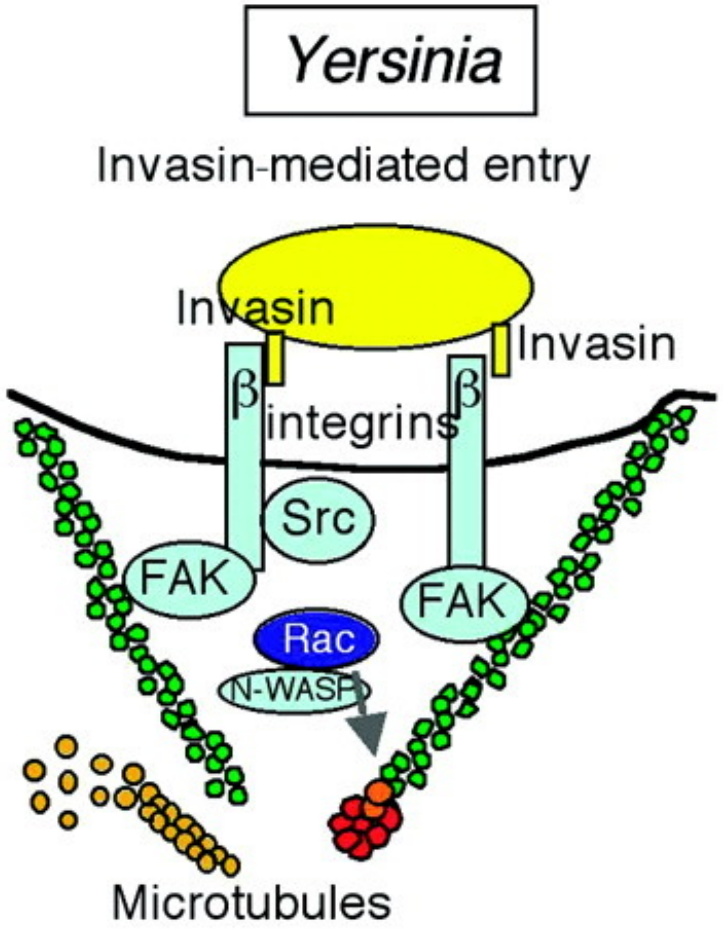
Pathogen	Surface protein	Integrin Involved	Reference
Yersinia pseudotuberculosis and *enterocolitica*	Invasin	α3β1 α4β1 α5β1 α6β1	Isberg *et al.* 2000 ; Isberg and Leong, 1990
Pseudomonas aeruginosa	PilY1	αVβ5	Johnson *et al.* 2011
Shigella flexneri	IpaB IpaC IpaD	α5β1	Watarai *et al.* 1996
Neisseria gonorrhoeae and *N. meningitidis*	Opa_{50}	Indirect, αVβ3 αVβ5	Dehio *et al.* 1998;
Streptococcus group A	F1	Indirect, α5β1 αvβ3	Ozeri *et al.* 2001
Staphylococcus aureus	FnBpA FnBpB	Indirect, β1 integrins	Fowler *et al.* 2000
Uropathogenic *E.coli*	FimH	β1 and α3 integrins	Eto *et al.* 2007
Helicobacter pylori	CagL	α5β1 α6β1	Kwok *et al.* 2007 Buß *et al.* 2019

Integrins are receptors for exotoxins

Toxin	Integrin involved	Reference
Adenylate cyclase toxin from *Bordetella*	α_Mβ2 integrin (CD11b/CD18, Mac-1, or CR3)	Bumba *et al.* 2010
RTX toxins (leukotoxin and α-hemolysin)	LFA-1 β2 integrin (CD11a/CD18)	Lally *et al.* 1997
Anthrax toxin	α4β1and α5β1 *via* PA (carrier protein)	Martchenko *et al.* 2010
FHA toxin of *Bordetella*	α_Mβ2 integrin (CD11b/CD18, Mac-1, or CR3)	Relman *et al.* 1990
Vacuolating toxin from *H. pylori*	CD18 (LFA-1 or Mac-1)	Sewald *et al.* 2008

Table 2. Integrins are receptors for bacteria and exotoxins. A non-exhaustive list of bacterial species and toxin types interacting with integrin receptors at the host cell surface.

Yersinia pseudotuberculosis



Most estensively studied mechanism of invasion

Isberg and Leon 1990: receptor on a non-phagocytic cell allows for bacterial invasion

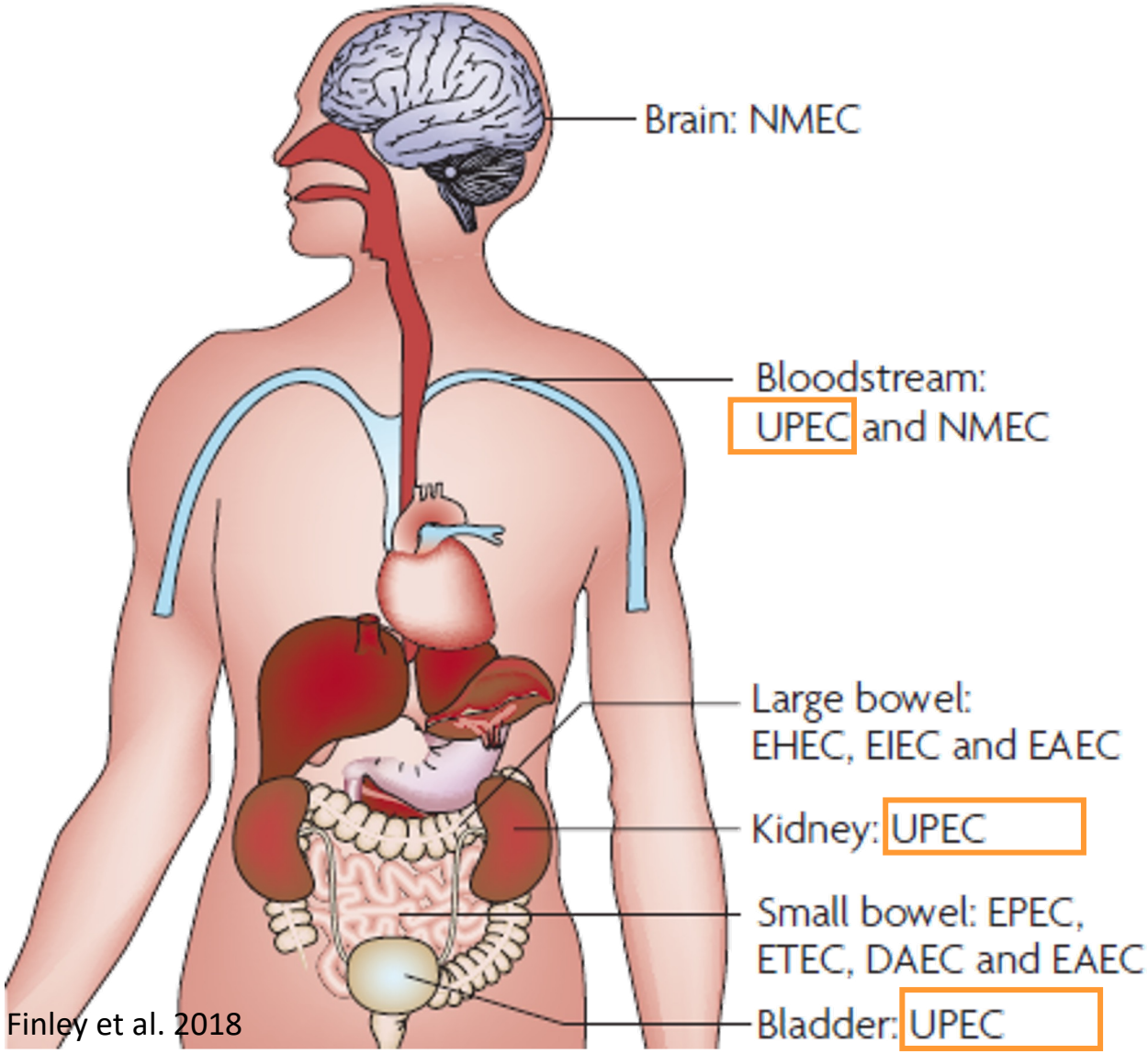
Invasin: 103kDa outer membrane protein binds to multiple integrin heterodimers α3β1, α4β1, α5β1 and α6β1

Use of blocking antibodies against α5β1

Cossart and Sansonetti, Science 2004

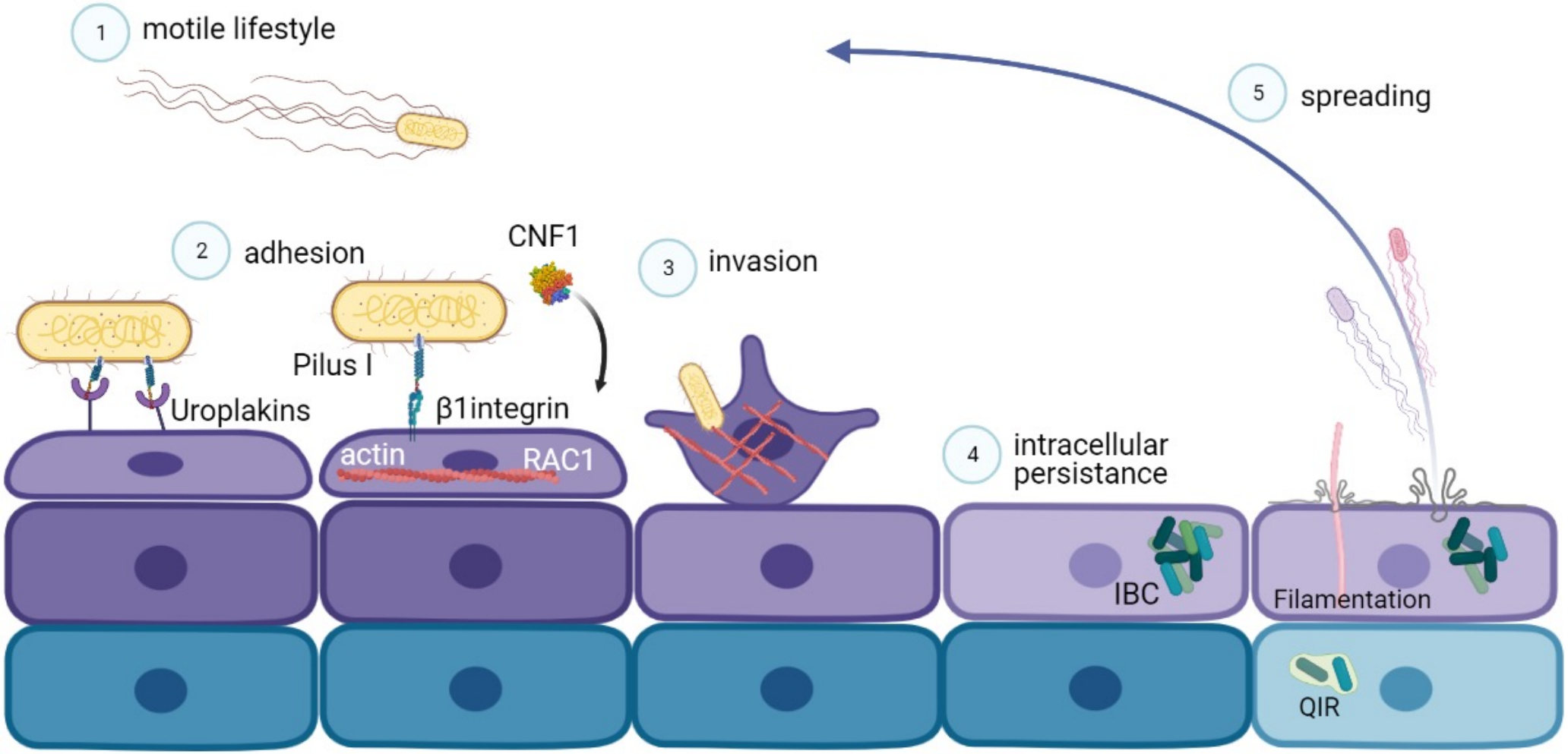
Uropathogenic *E. coli*

- Extraintestinal *E. coli* (ExPEC)
- Phylogenetic groups B2/D
- 80% of urinary tract infections in healthy patients
- Now MDR, global burden

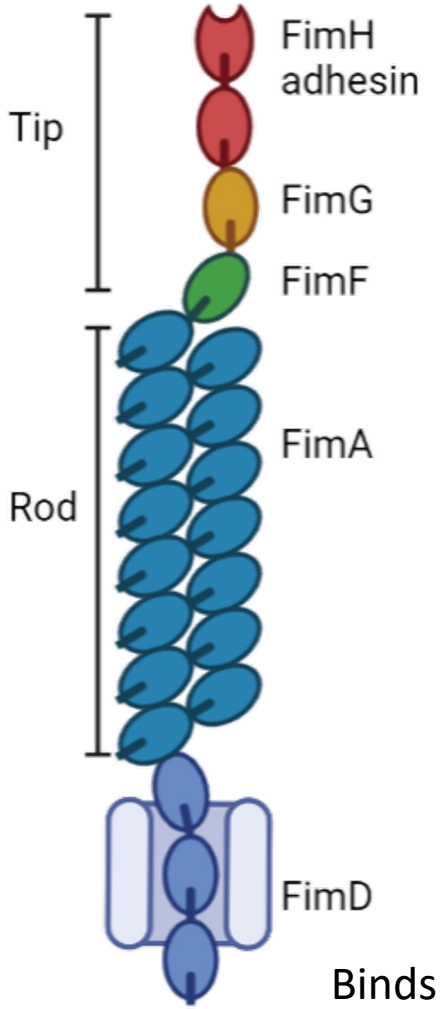


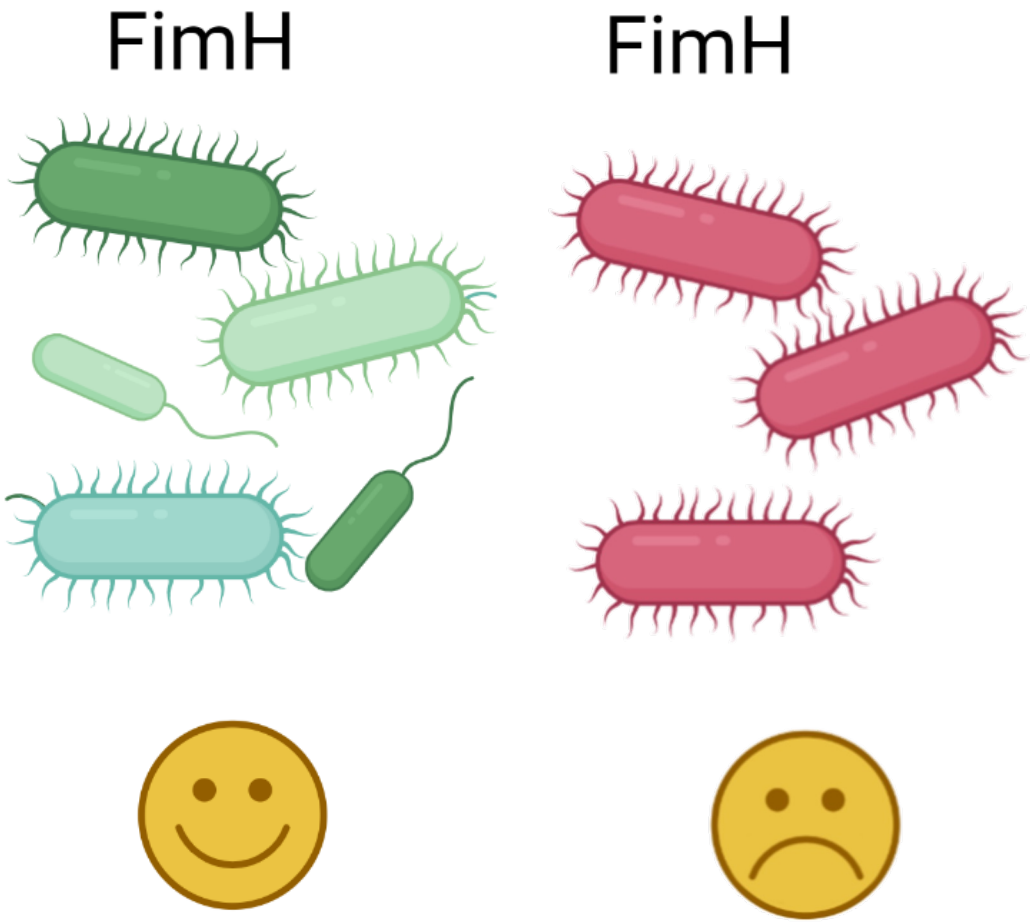
Finley et al. 2018

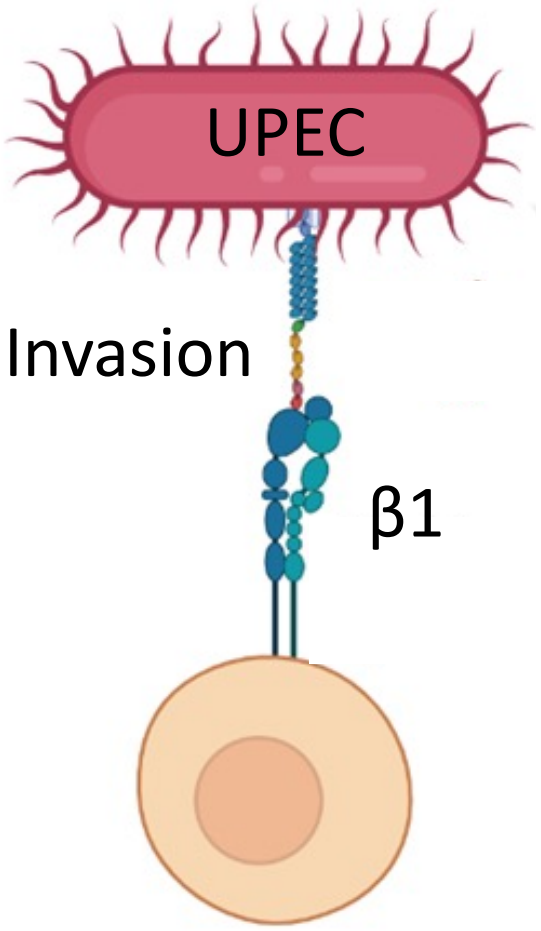
Uropathogenic *E. coli*



Uropathogenic E.coli



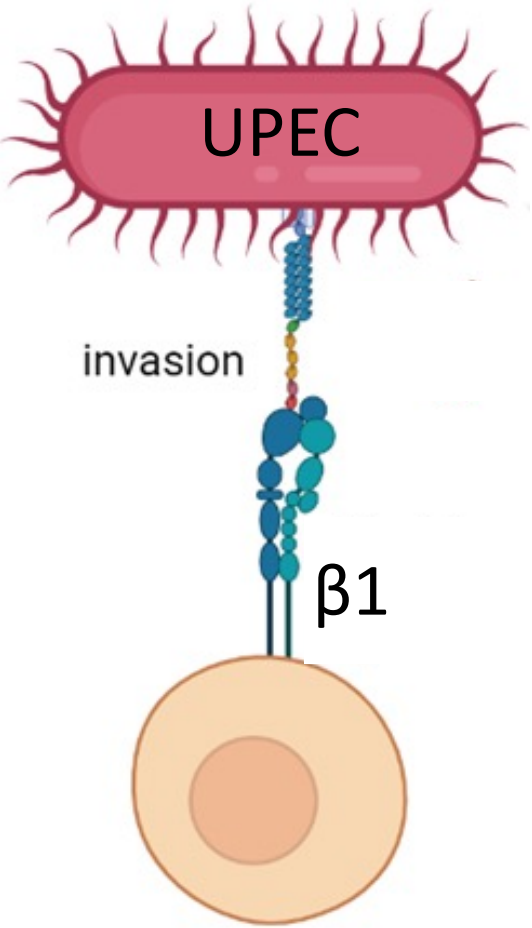




Binds to mannosylated integrins

Eto *et al.* 2007

Uropathogenic E.coli

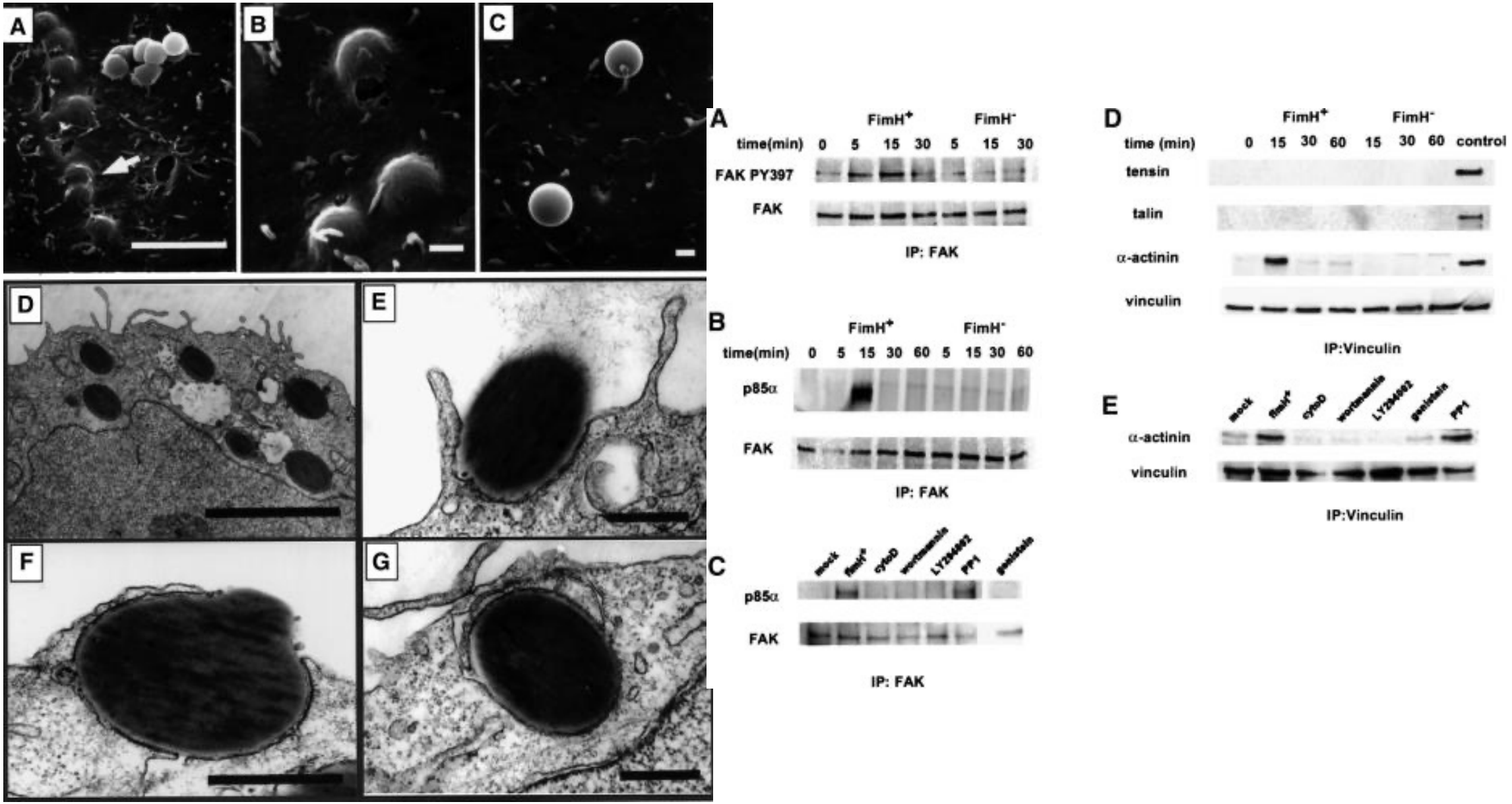


FimH was known as a surface adherence factor

First FimH role in bacterial invasion into mammalian cells (Martinez et al. 2000) making *de facto* UPEC an intracellular *E. coli* pathotype, validating the phenotypic observation in animal infection models made in ‘90s (McTaggart et al. 1990).

Binds to mannosylated integrins

Eto *et al.* 2007



Internalization of FimCH coated beads by 5367 cells. Martinez et al. 2000

Hijacking FA dynamics

- Although adhesion corresponds to the first interaction between the host and the pathogen, it is not sufficient to establish a process of infection. (ex FimH)
- Bacteria have evolved an entire arsenal of virulence factors able to hijack cell responses, starting from adhesion.
- Focal adhesion signaling pathways are manipulated by bacteria in multiple ways (Murphy et al. 2021):

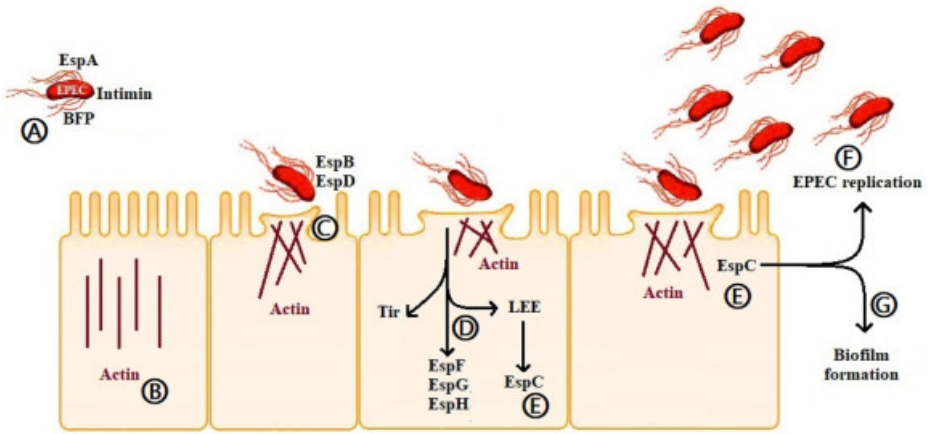
● proteolytic cleavage of FA proteins

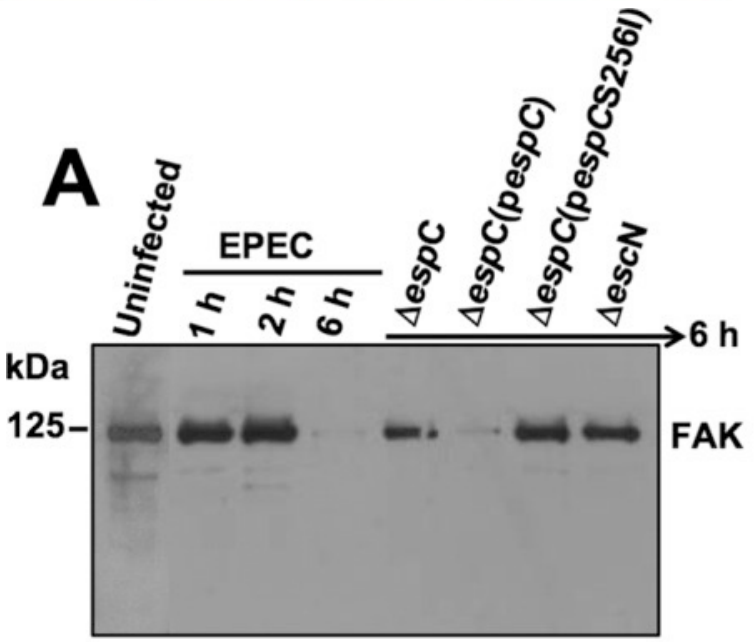
● phosphorylation of FA proteins

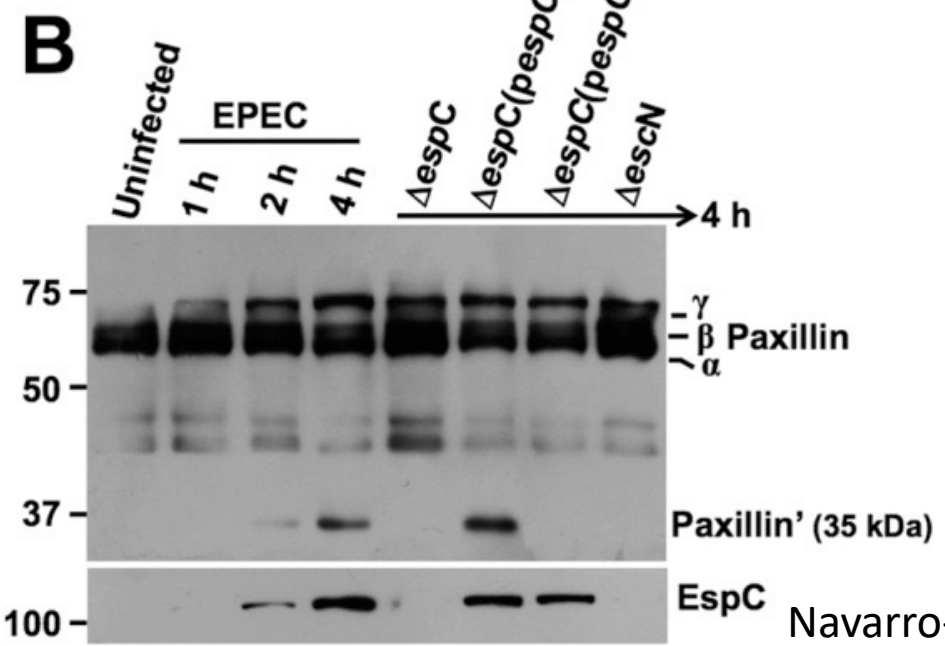
● molecular mimicry of motifs commonly found in FA proteins

Enteropathogenic *E. coli* EspC

proteolytic cleavage of FA proteins







Navarro-Garcia 2014

Helicobacter pylori CagA

Cellular Microbiology (2007) **9**(5), 1148–1161

doi:10.1111/j.1462-5822.2006.00856.x
First published online 9 January 2007

The *Helicobacter pylori* CagA protein disrupts matrix adhesion of gastric epithelial cells by dephosphorylation of vinculin

CagA is phosphorylated by Src on a tyrosine residue

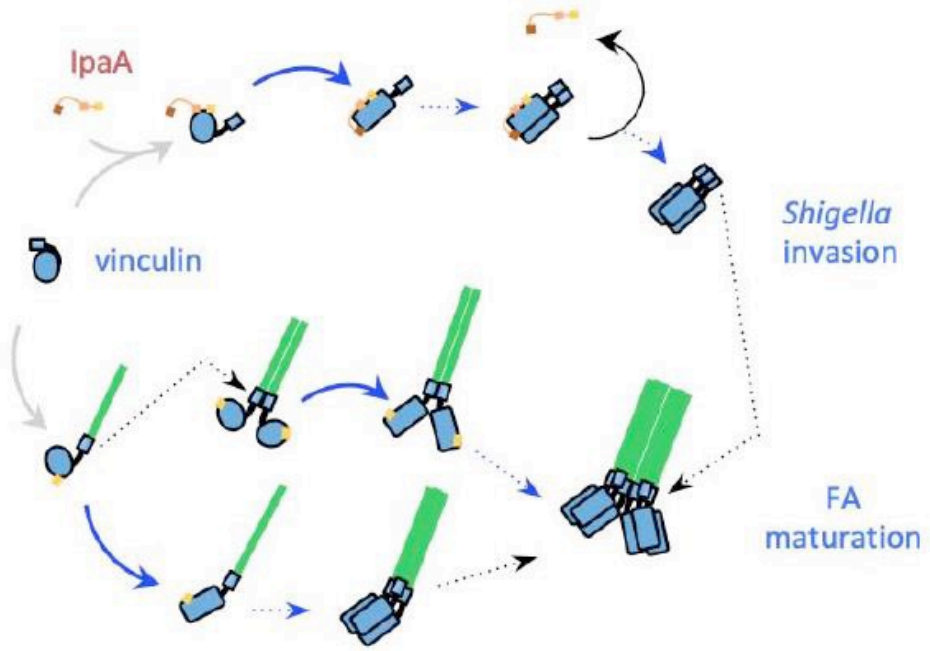
P-CagA inactivates Src and dephosphorylates Vinculin

Tyrosine dephosphorylation of vinculin results in severe cellular deficiencies in cell-matrix adhesion, cell spreading and wound repair.

Moese et al. 2007

Shigella flexneri IpaA

molecular mimicry



Type III secretion system effector

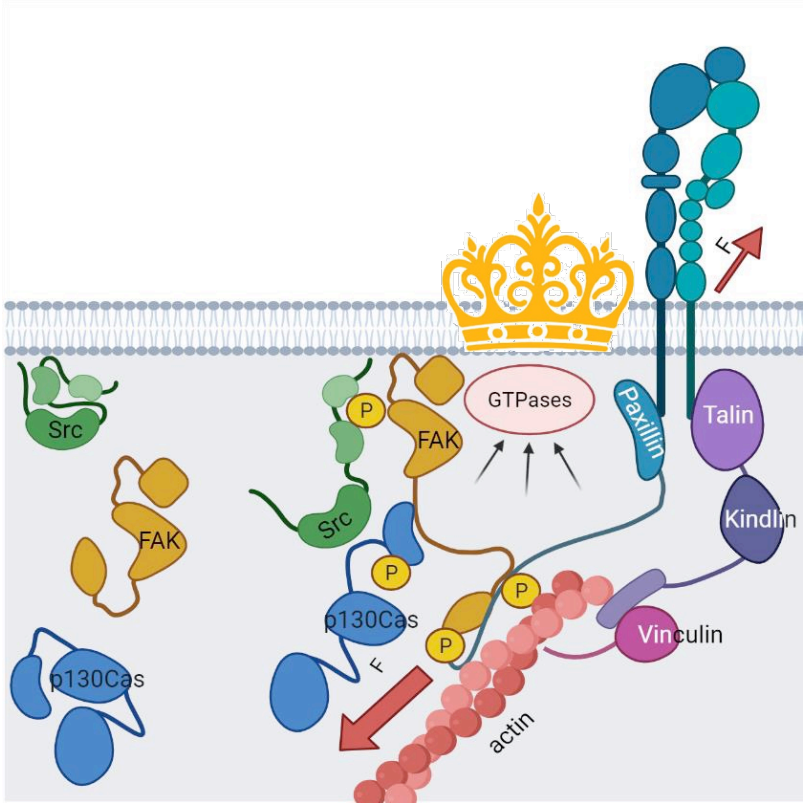
shares with talin the Vinculin binding sites (VBSs) domain. This domain is responsible for the strengthening of the cytoskeletal association via viculin recruitment at the adhesion site.

During infection, IpaA induces vinculin supra-activation and oligomerization without any upstream mechanotransduction events (clockwise events in the figure).

As a result, the bacterium is able to **mimic the process of FA maturation** (counterclockwise events in the figure) to **strengthen cell adhesion**. In green: actin fibers.

Valencia-Gallardo et al. 2020

Hijacking FA downstream key effectors: the RhoGTP-ases

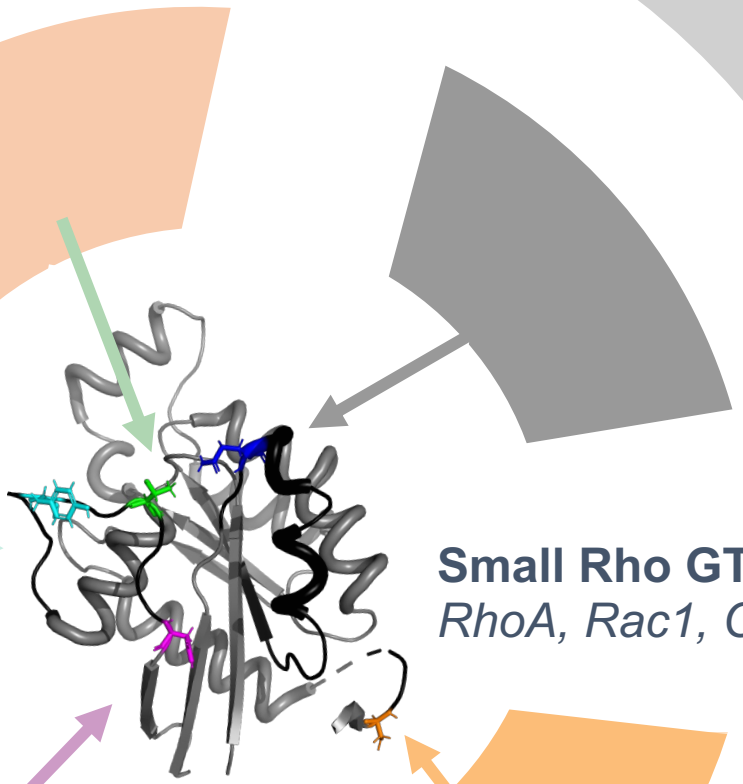


RhoGTPase, a common target of bacterial species

Glycosylation/ AMPylation
C. difficile *V. parahaemolyticus*
C. Sordellii *C. perfringens*

Adenylylation / Glycosylation
H. somni
P. asymbiotica
Y. ruckeri

ADP-ribosylation
C. botulinum
S. aureus
Deamidation
B. cenocepacia



Small Rho GTPases
RhoA, Rac1, Cdc42

→ ***Master regulator of actin cytoskeleton***

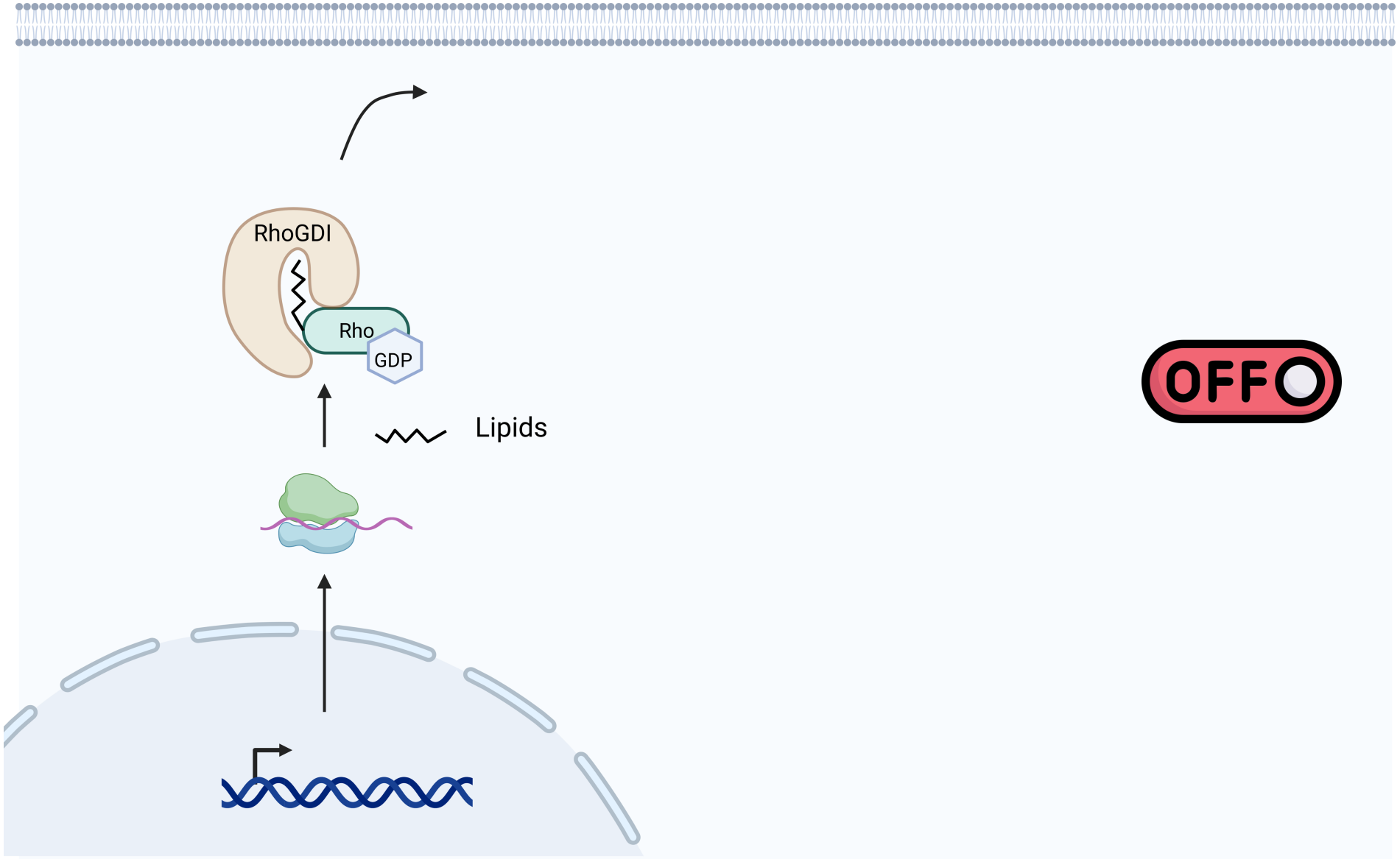
Deamidation
Transglutamination
ADP-ribosylation
P. luminescens
E. coli
Y. pseudotuberculosis
B. pertussis and parapertussis
Vibrio parahaemolyticus

Proteolysis
Yersinia spp.
P. luminescens

N^E-Fatty acylation
V. vulnifucus, Vibrio spp.
S. flexneri

RhoGTPases, important molecular switches for cells

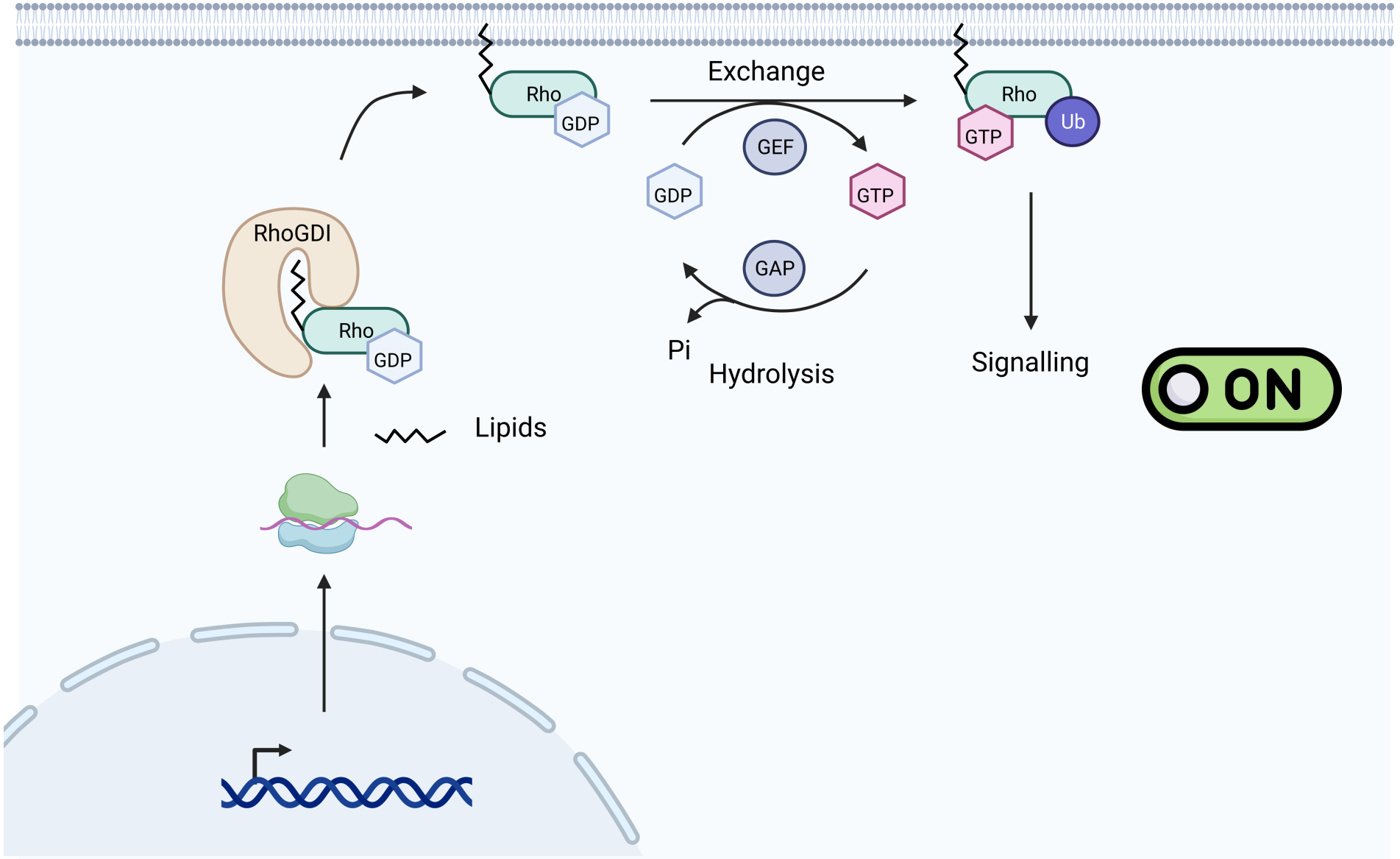
Spatio-temporal regulation



Adapted from Hodge, Ridley 2016 by Paillares E.

RhoGTPases, important molecular switches for cells

Spatio-temporal regulation



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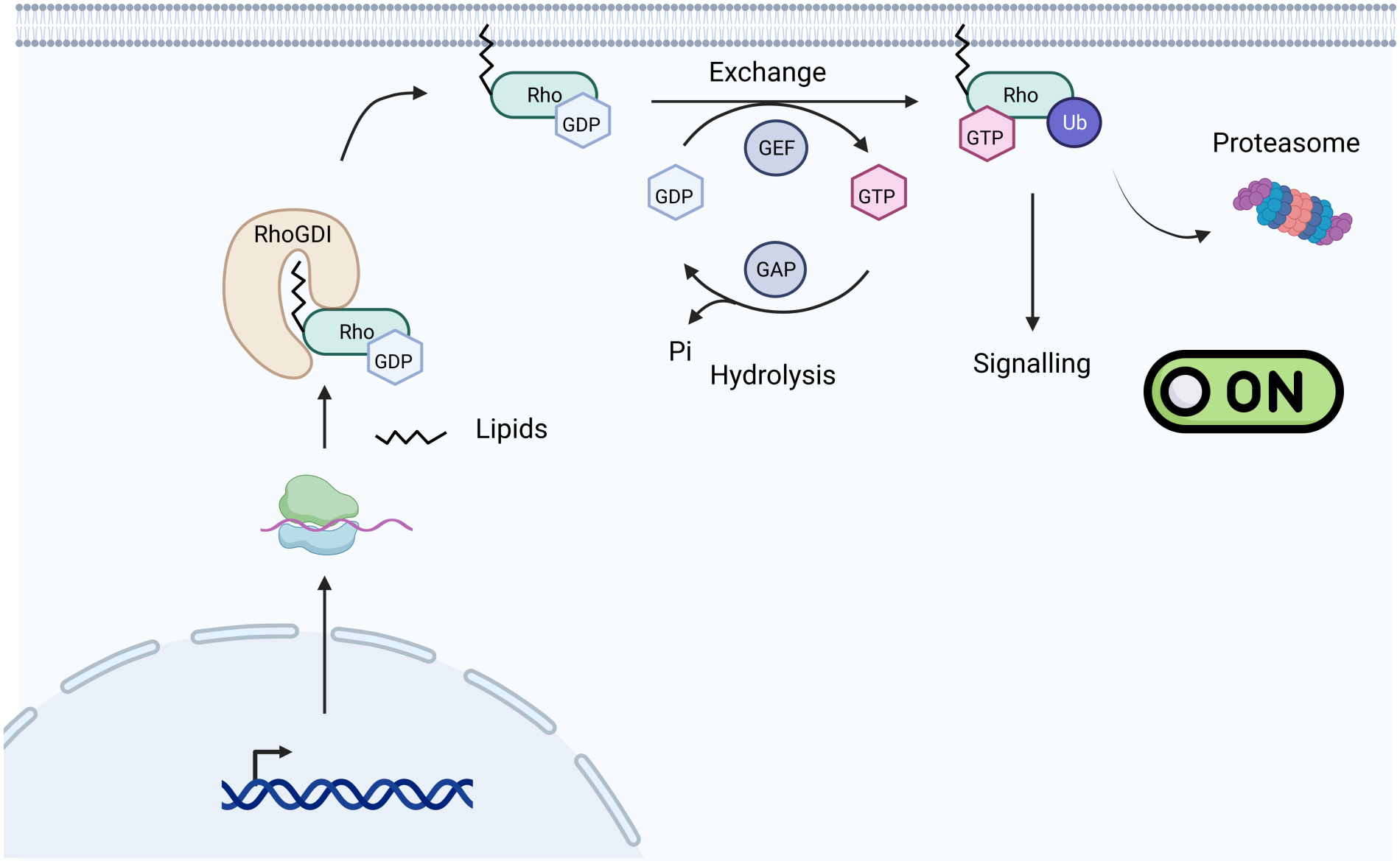
RhoGTPases, important molecular switches for cells

Spatio-temporal regulation



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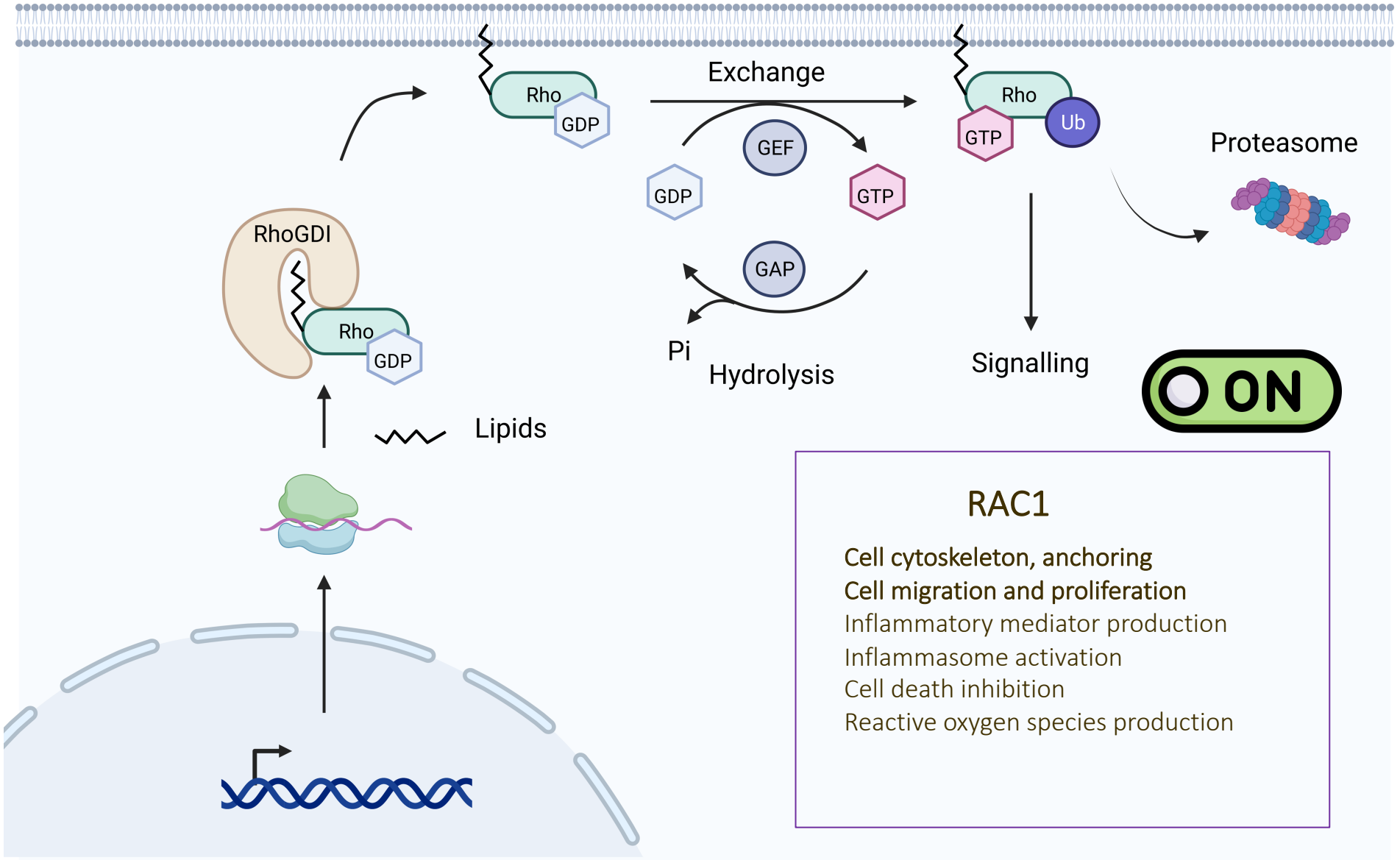
RhoGTPases, important molecular switches for cells

Spatio-temporal regulation



RAC1

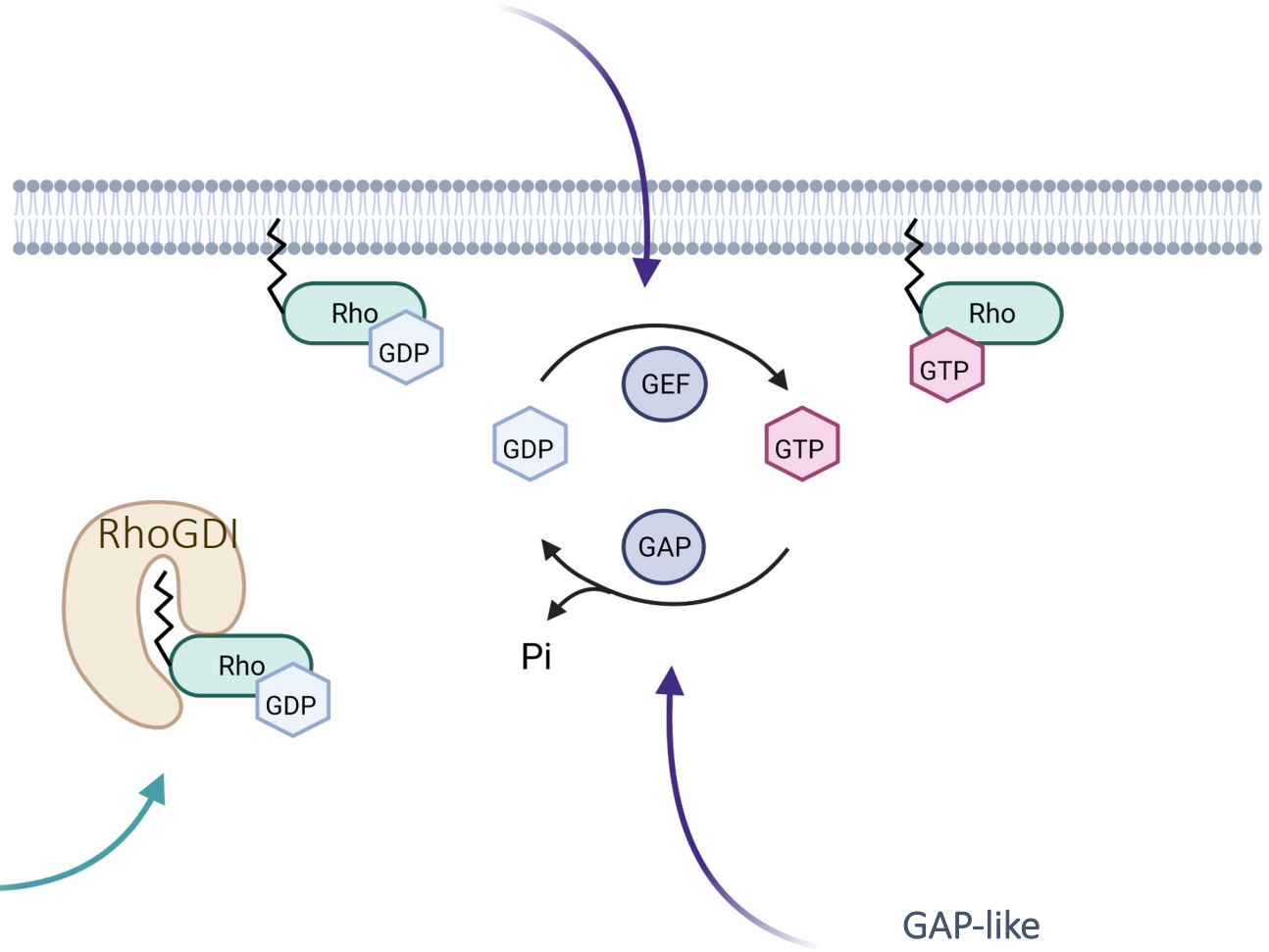
Cell cytoskeleton, anchoring
Cell migration and proliferation
Inflammatory mediator production
Inflammasome activation
Cell death inhibition
Reactive oxygen species production

Adapted from Hodge, Ridley 2016 by Paillares E.

Toxins target actin cytoskeleton regulators : small GTPases

GEF-like
SopE, Salmonella

Reversible modification
by mimicry of host factors



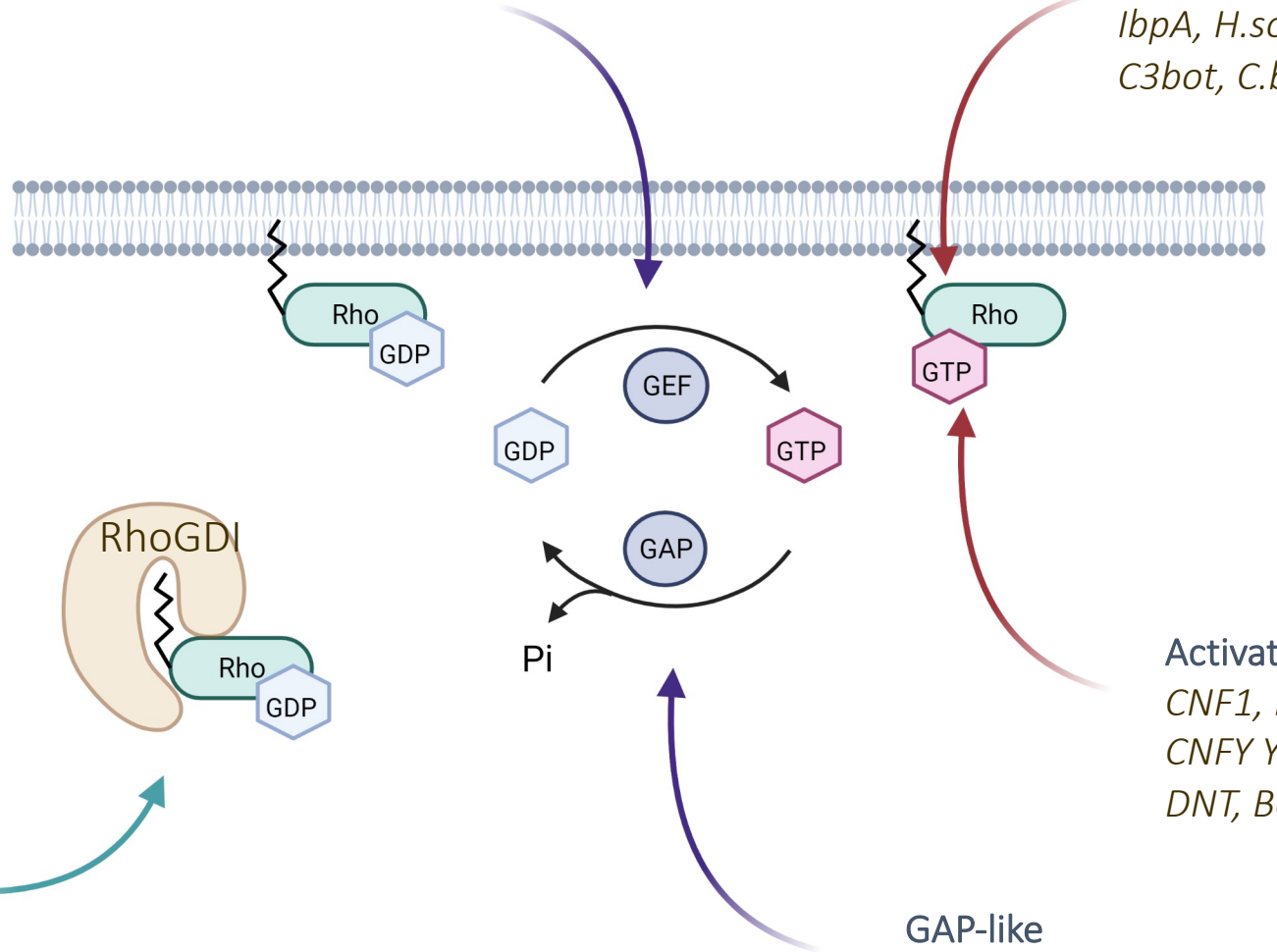
GDI-like
YpkA (YopO), Yersinia

GAP-like
SptP, Salmonella
YopE, Yersinia

Toxins target actin cytoskeleton regulators : small GTPases

GEF-like
SopE, Salmonella

Inactivate RhoGTPases
TcdB/TcdA, C. difficile, glucosyltransferase
IbpA, H.somni, AMPyltransferase
C3bot, C.botulinum, ADP-ribosyltransferase

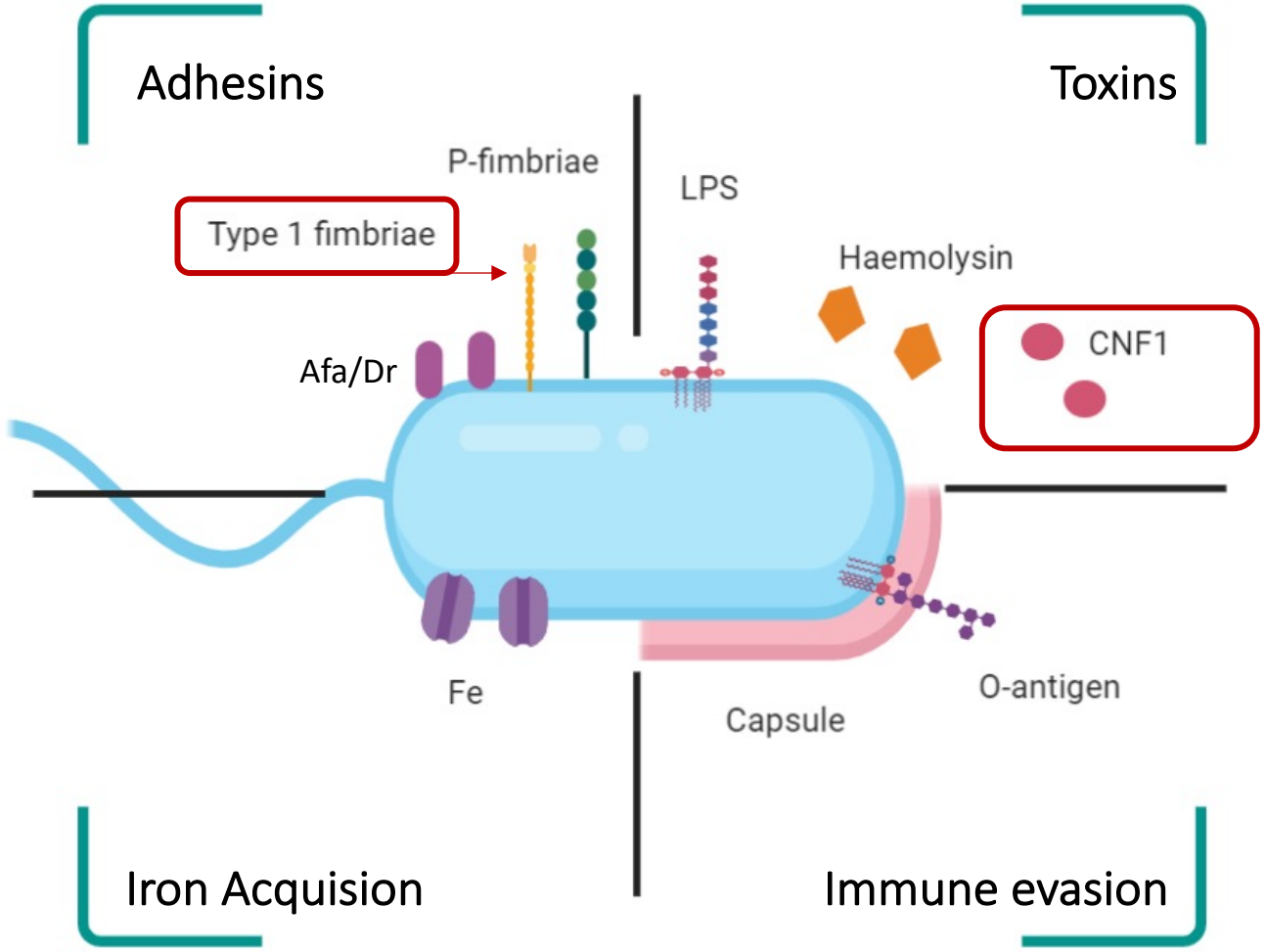


Activate RhoGTPases
CNF1, E.coli, deamidase
CNFY Y. pseudotuberculosis
DNT, Bordetella, transglutaminase

GDI-like
YpkA (YopO), Yersinia

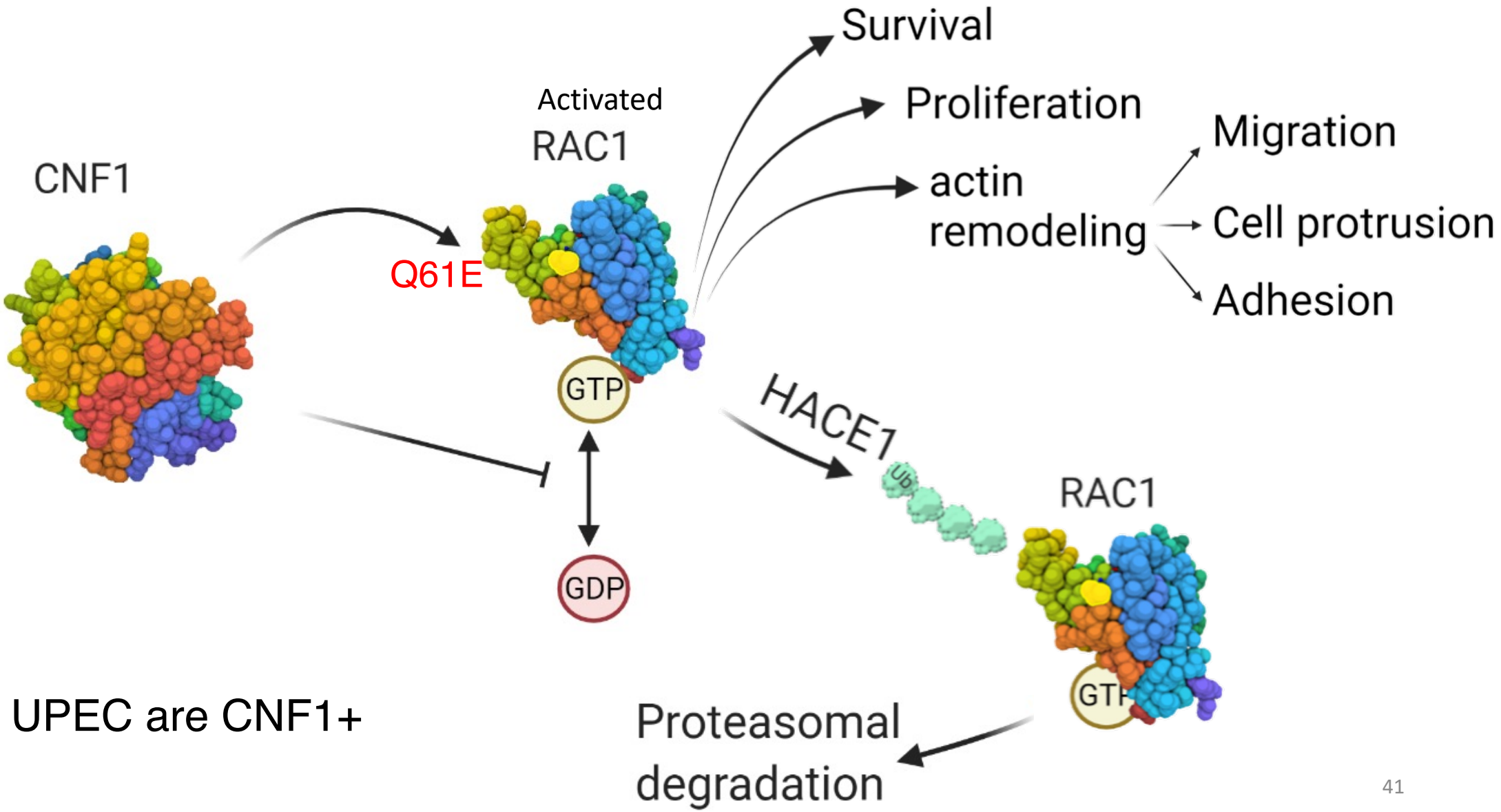
GAP-like
SptP, Salmonella
YopE, Yersinia

UPEC virulence factors

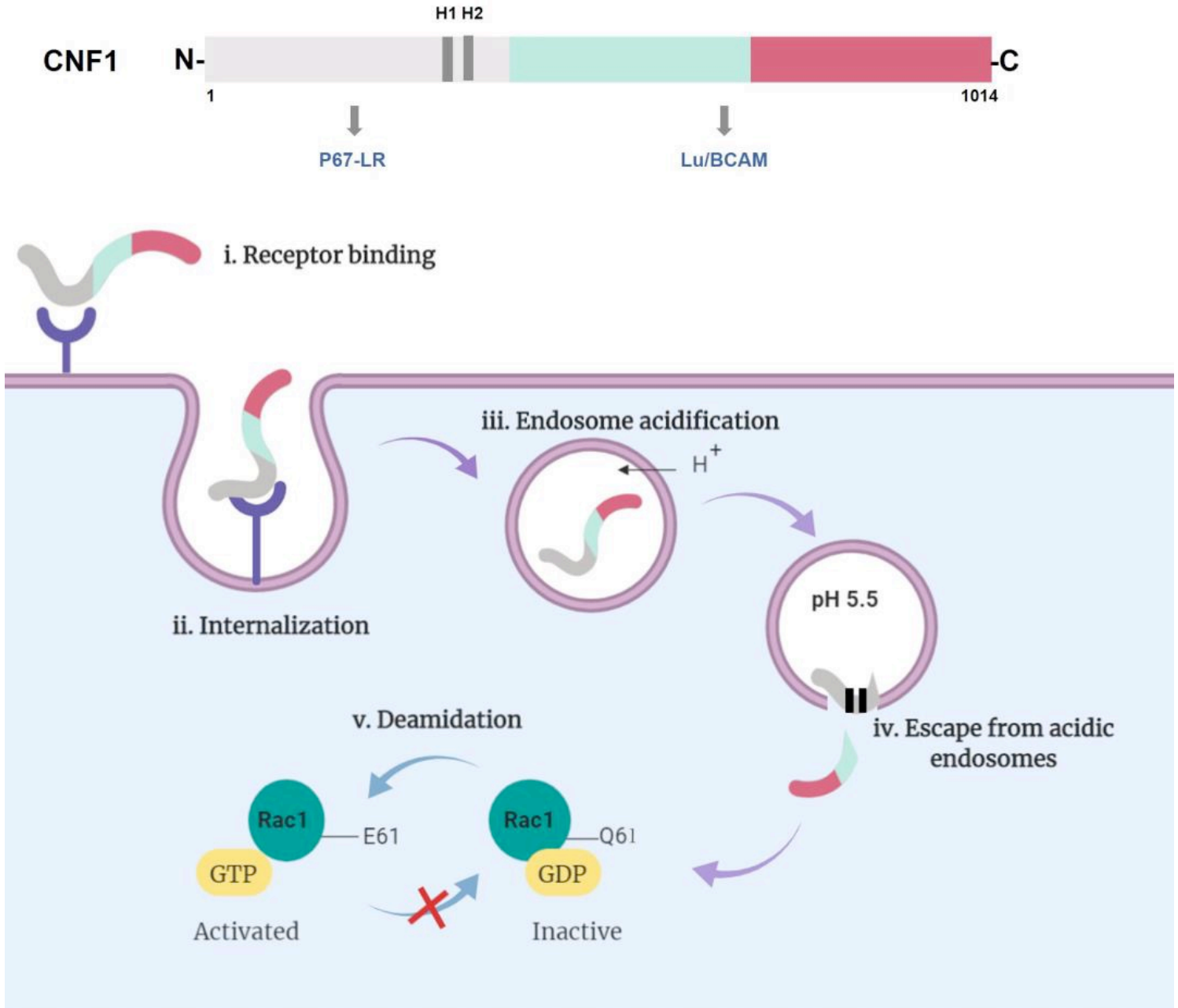


Adapted from Lüthje, P., and Brauner, A. 2014 by Paillares, E.

CNF1 toxin



30% of UPEC are CNF1+



Scheme by Paillares E.