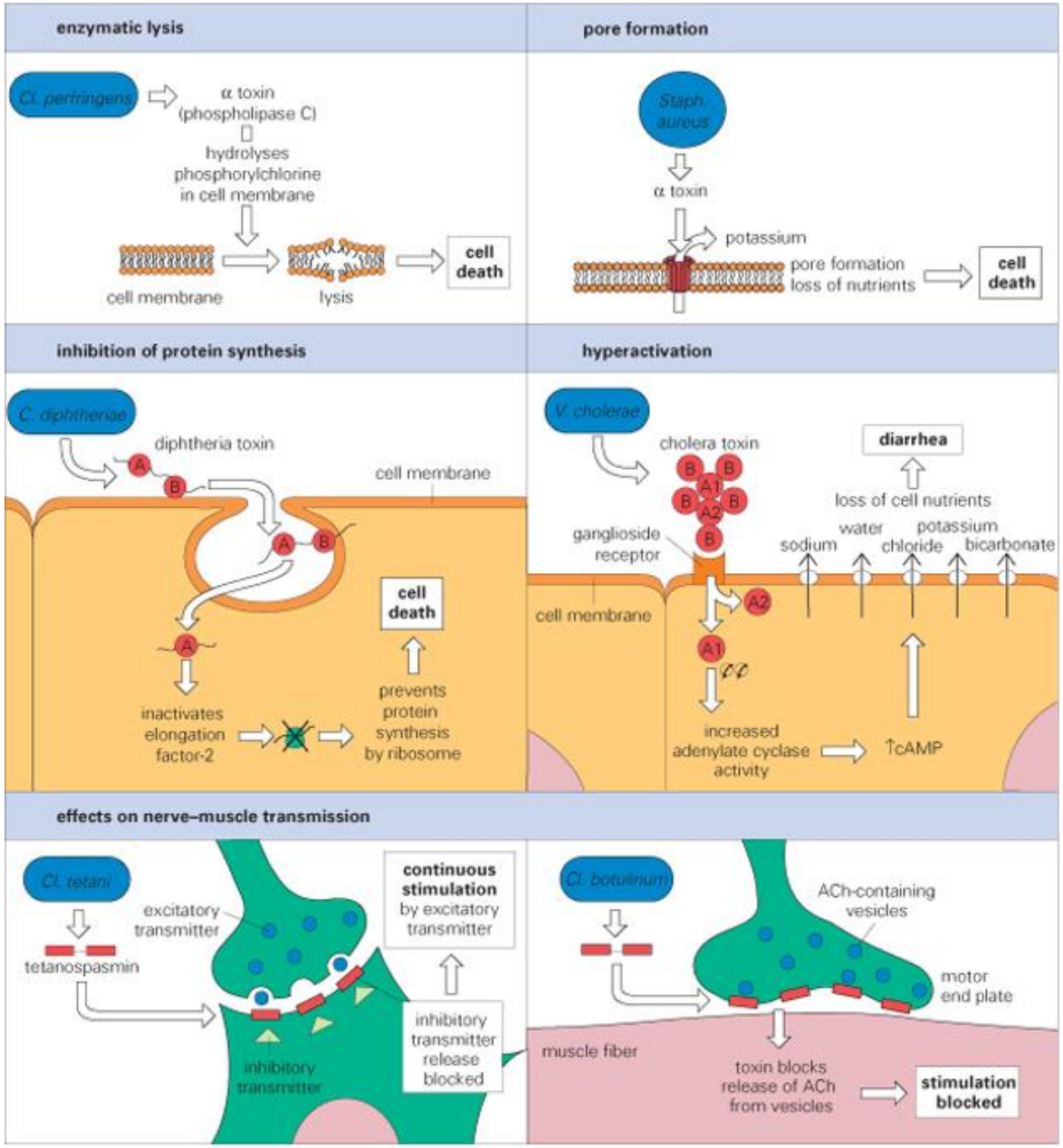
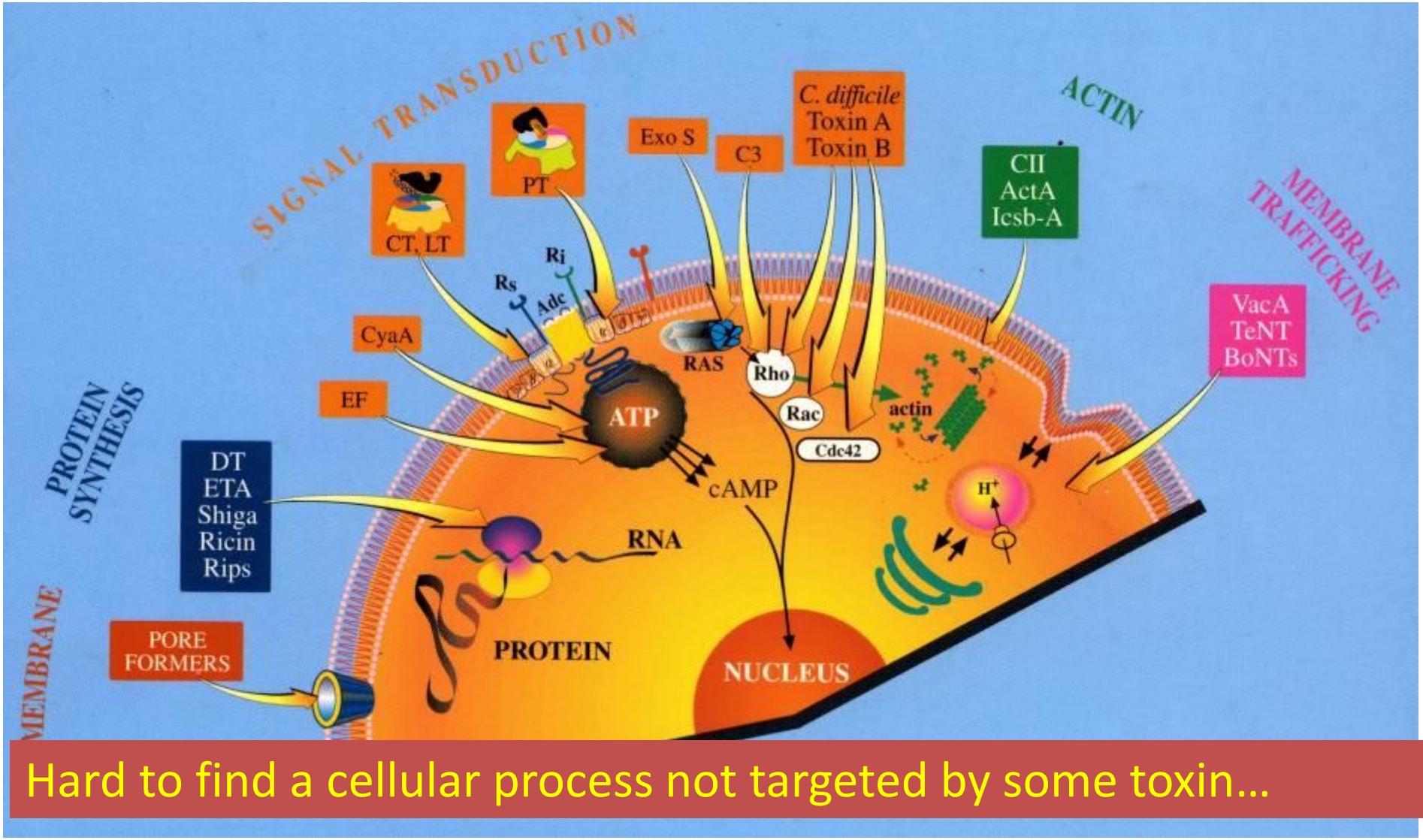
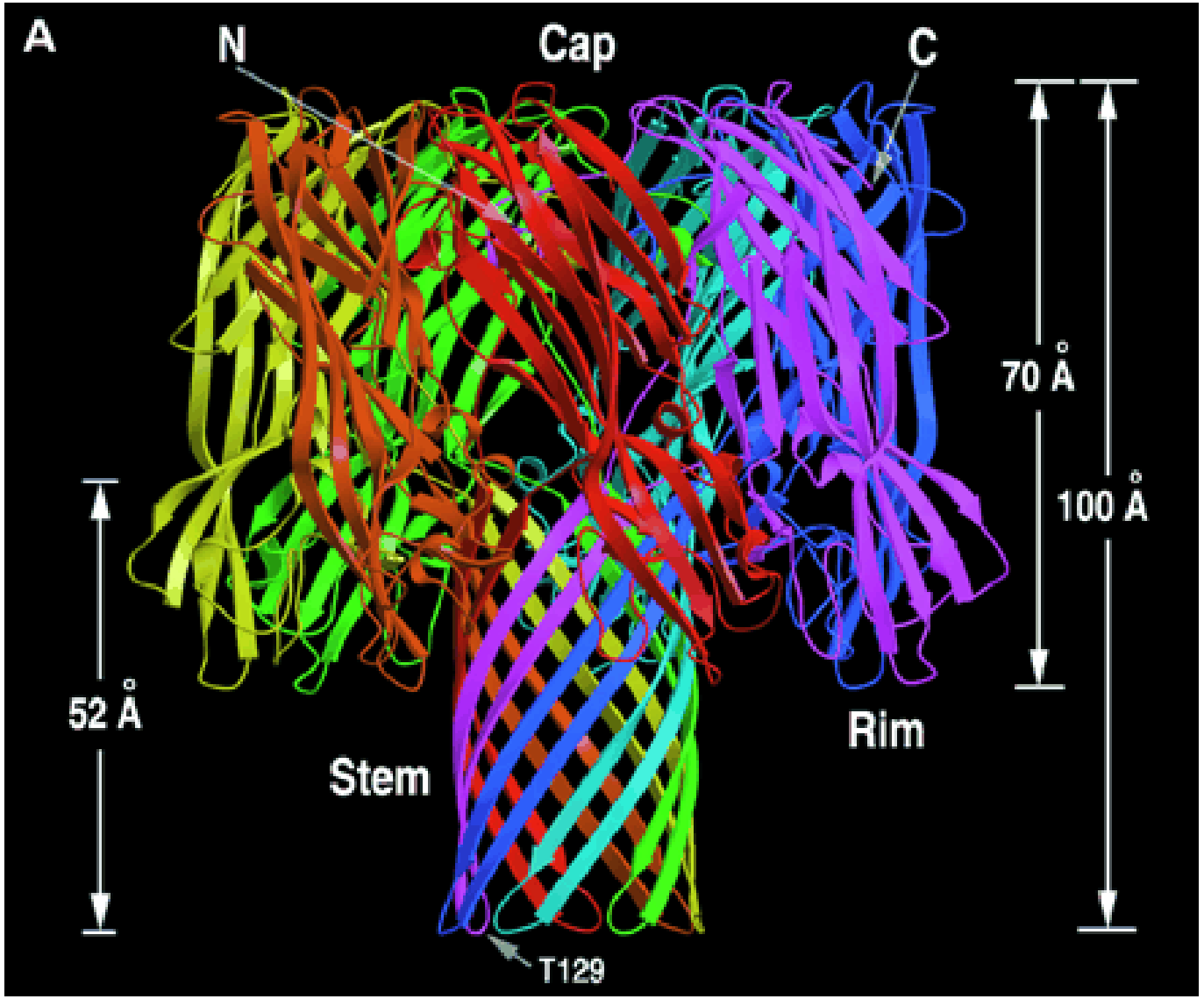
Principles of toxin action



Bacterial toxins are “smart, pretty and usefull”

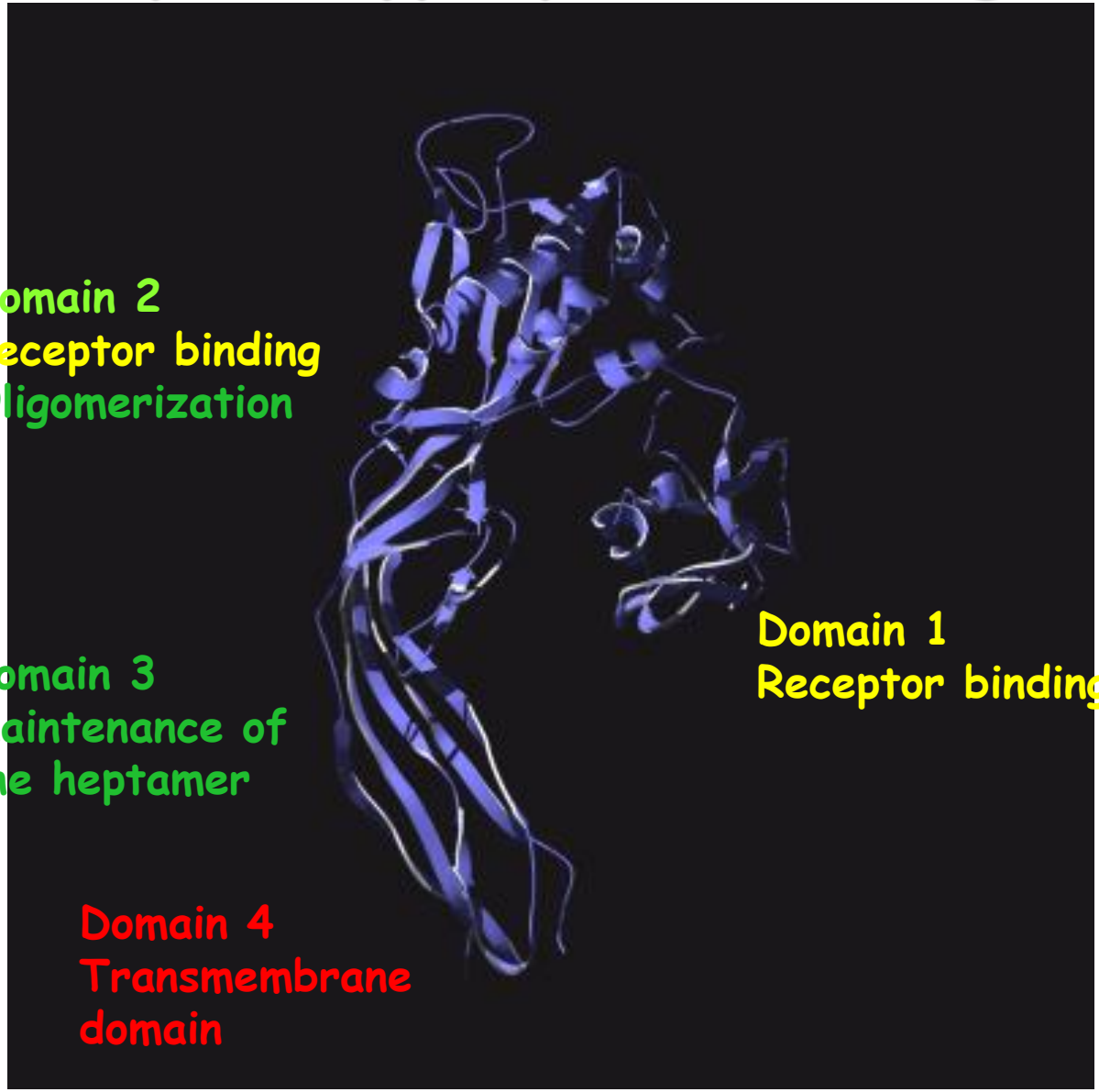


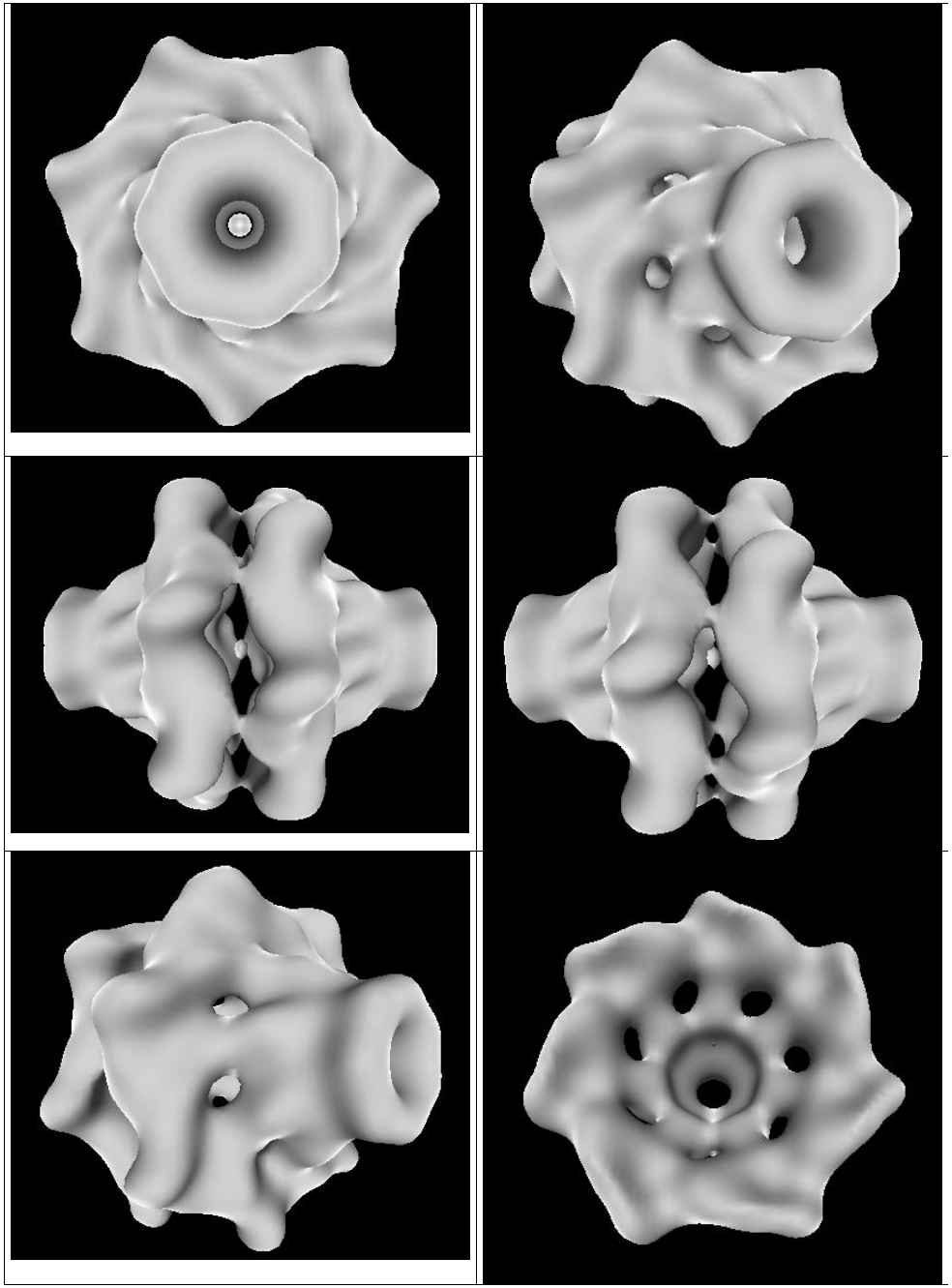
Hard to find a cellular process not targeted by some toxin…



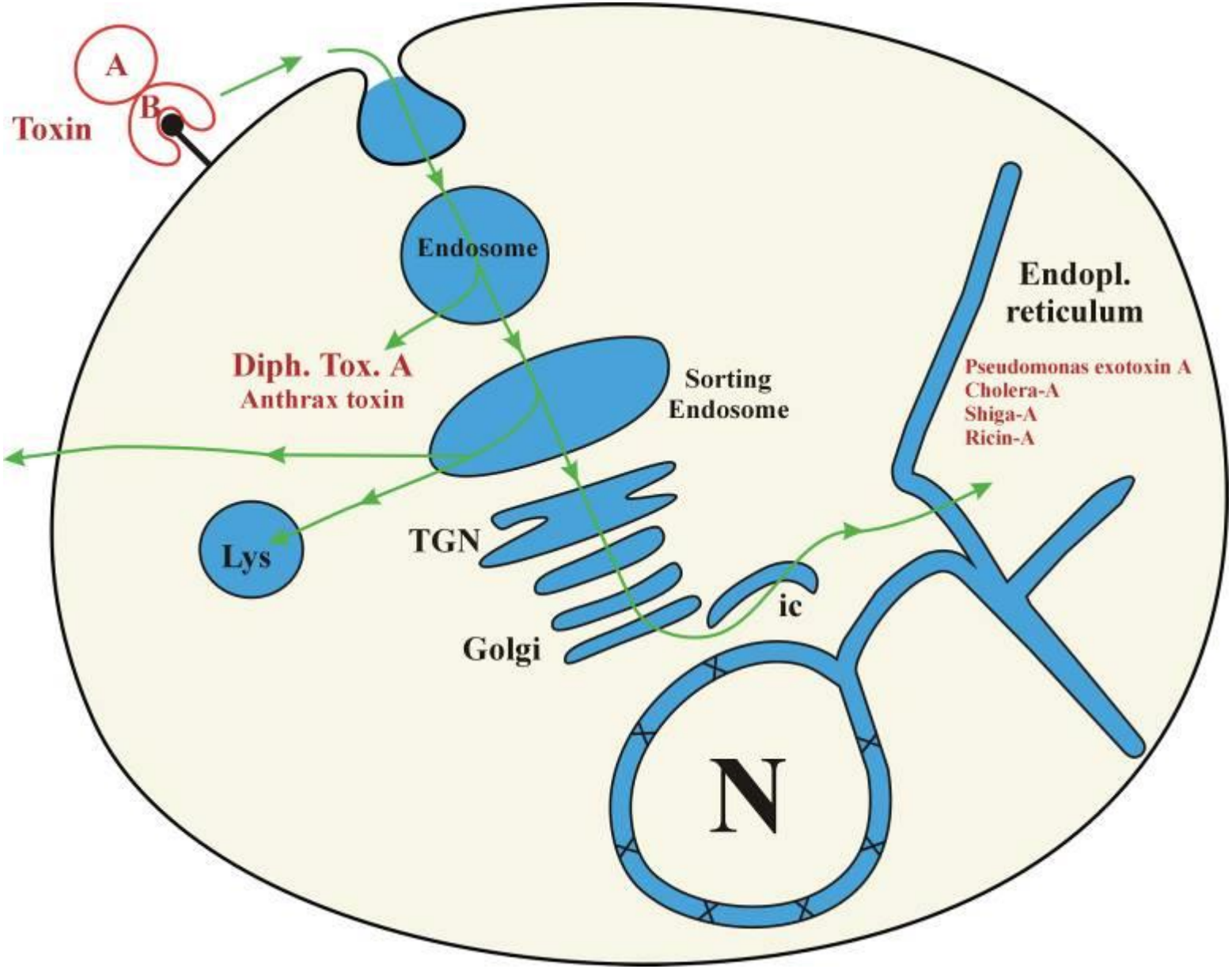
heptamer of alpha-toxin

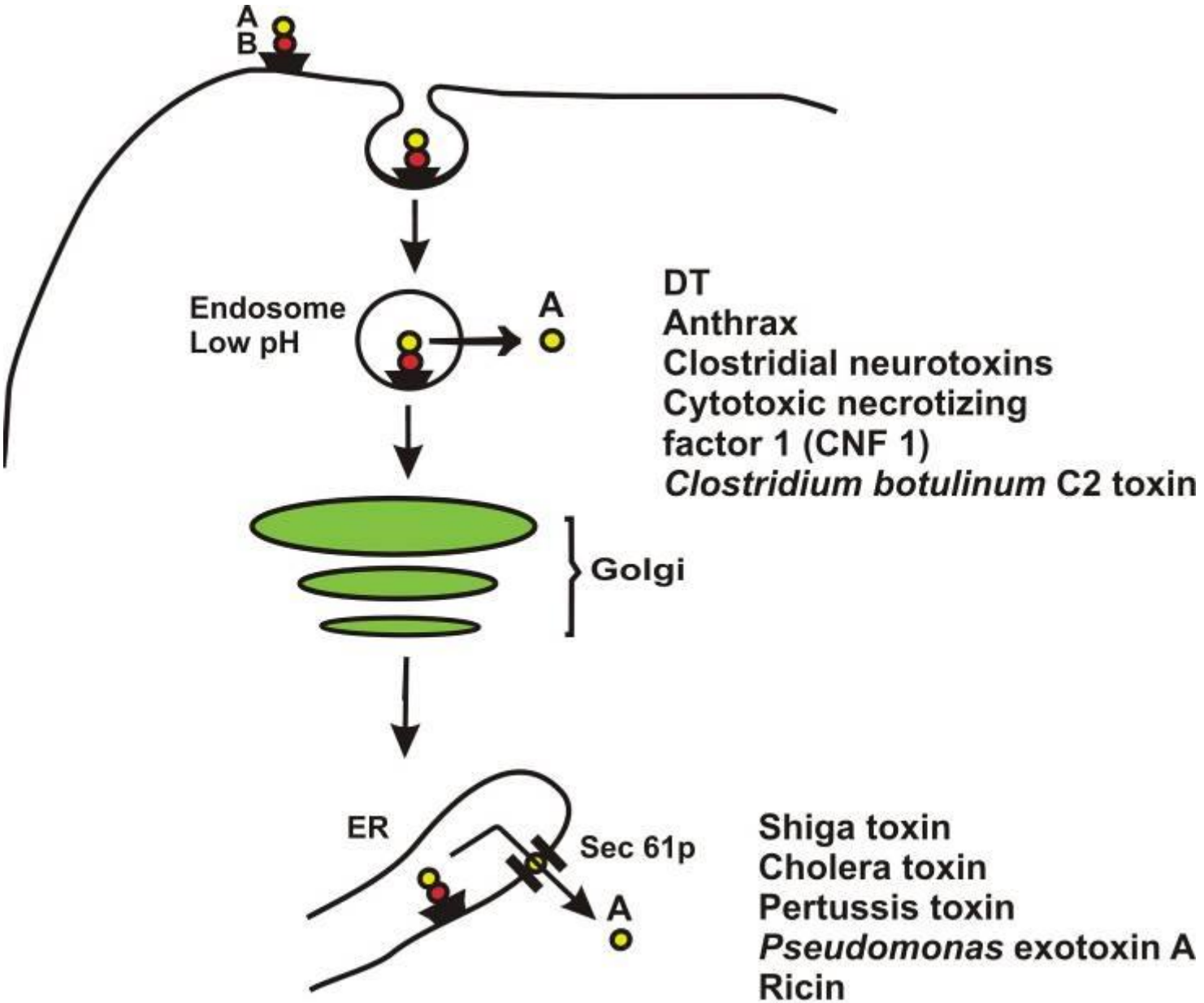
Aerolysin a prototypic pore-forming toxin



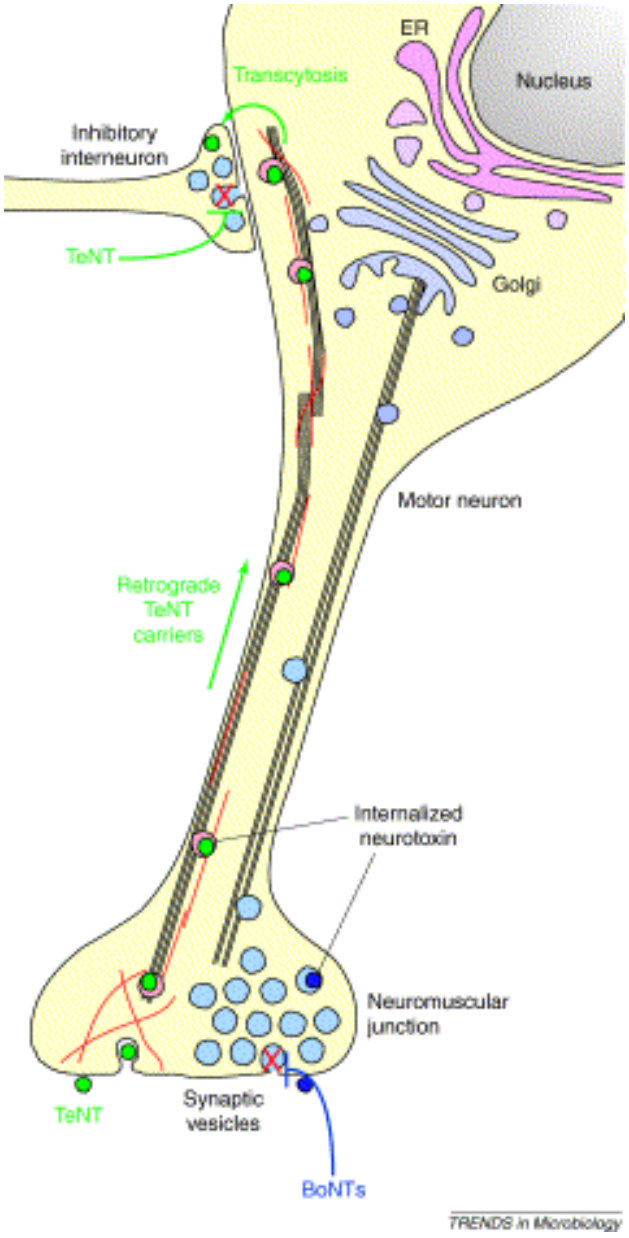


Intracellular transport and translocation of toxins to the cytosol




A
B
Endosome
Low pH
A
DT
Anthrax
Clostridial neurotoxins
Cytotoxic necrotizing
factor 1 (CNF 1)
Clostridium botulinum C2 toxin
Golgi
ER
Sec 61p
A
Shiga toxin
Cholera toxin
Pertussis toxin
Pseudomonas exotoxin A
Ricin

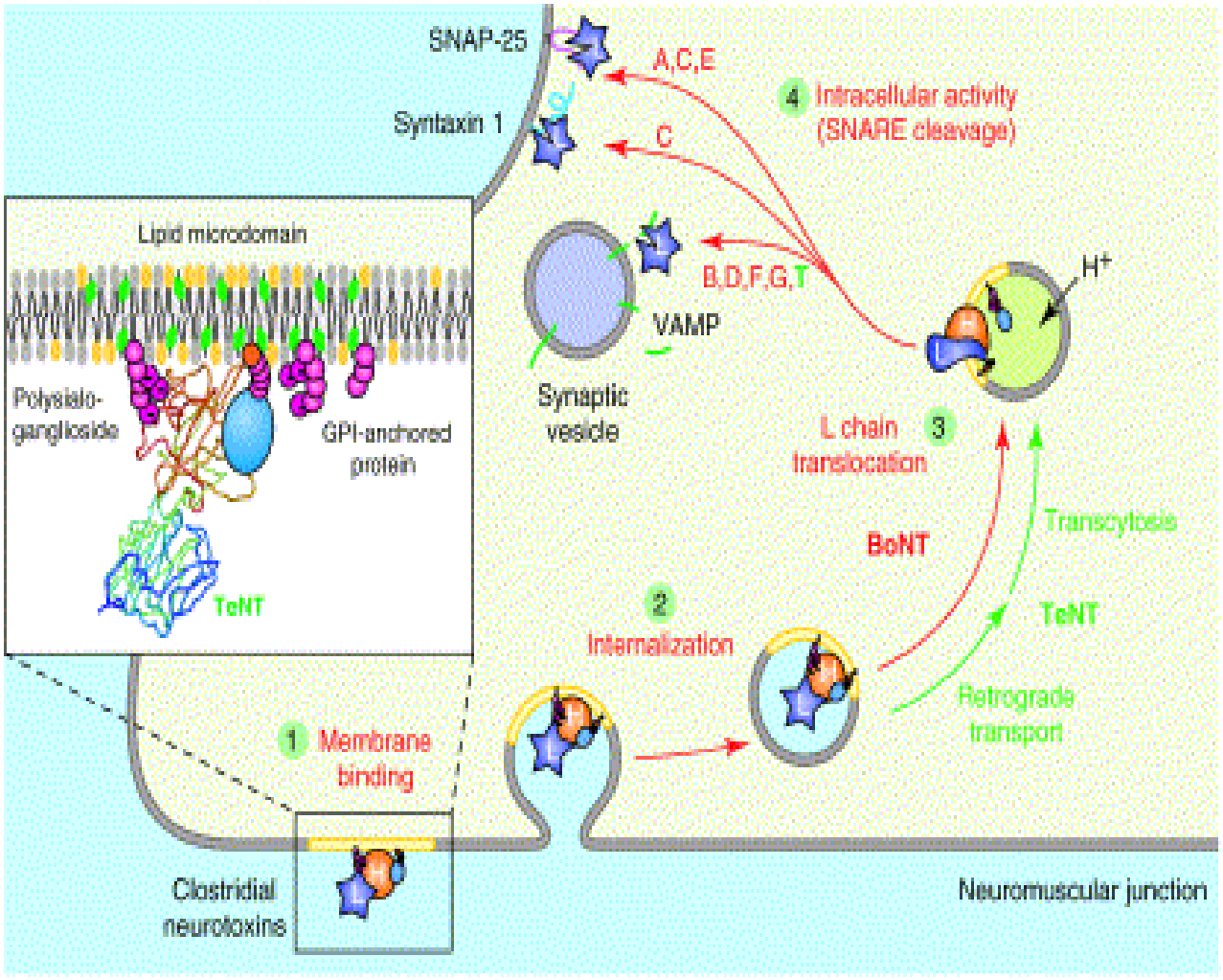
Clostridial neurotoxin trafficking



The sites of action of tetanus (TeNT; green) and botulinum neurotoxins (BoNTs; blue) on mammalian motor neuron and an interacting spinal inhibitory interneurons.

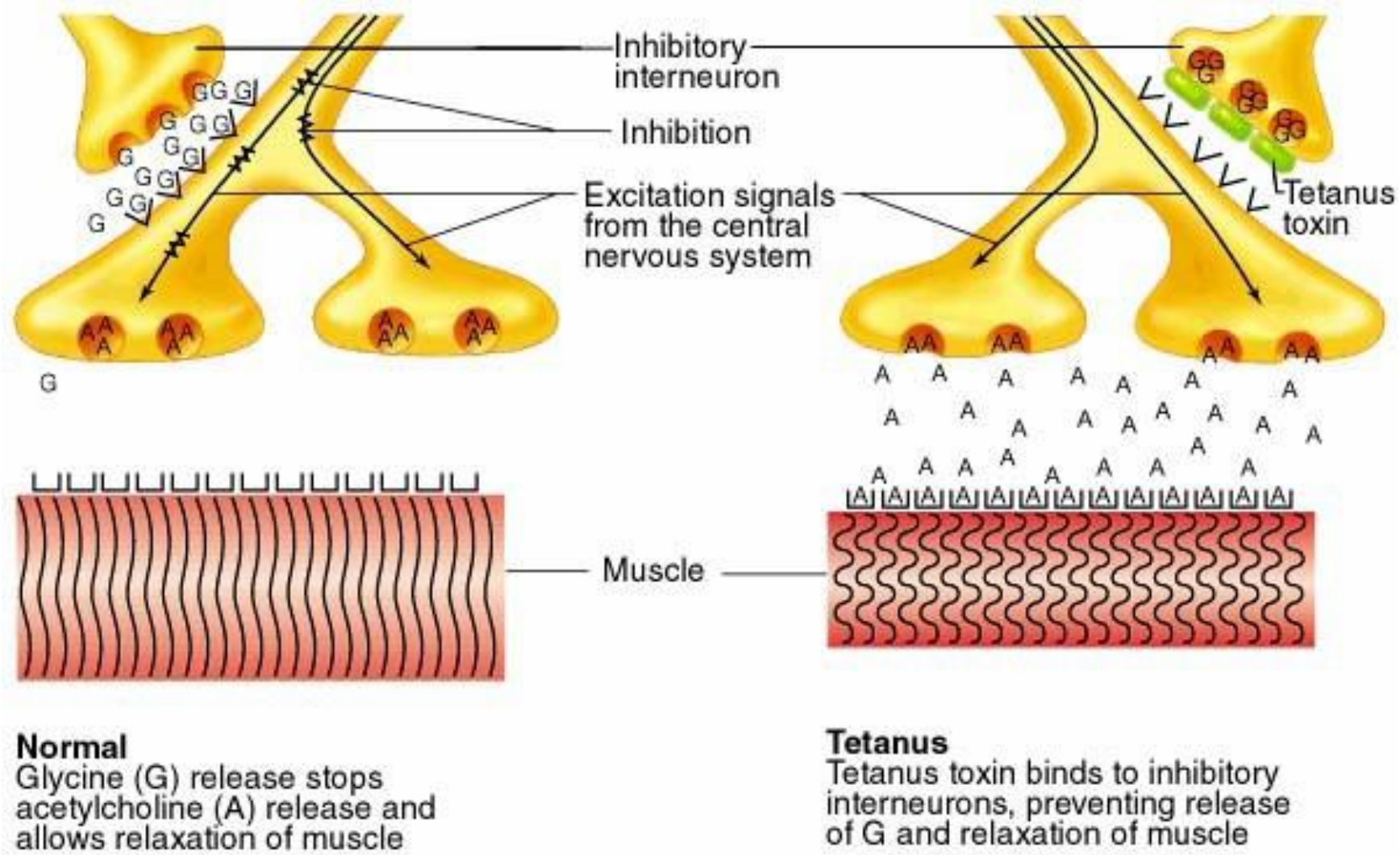
At the neuromuscular junction (NMJ), BoNTs are internalized in synaptic endosomal compartments, one of which might coincide with synaptic vesicles. By contrast, TeNT is sorted to the retrograde transport pathway. Microtubule tracks are shown in dark brown, whereas actin microfilaments are in red. Both cytoskeletal elements are required for fast retrograde transport of TeNT in motor neurons. Red crosses indicate the preferential sites of neurotransmitter release inhibition caused by BoNTs (NMJ) and TeNT (inhibitory interneuron synapse of the spinal cord).

Mechanism of action



TeNT binds polysialogangliosides (in magenta) and GPI-anchored proteins (in blue) within lipid rafts. (2) Neurospecific binding is followed by internalization and sorting to specific intracellular routes which differ for BoNTs and TeNT. TeNT enters non-acidified carriers that are recruited to the fast retrograde transport pathway and then reaches adjacent inhibitory interneurons via transcytosis. BoNT-containing endocytic structures instead remain at the neuromuscular junction. (3) The light (L) chains cross the endocytic membrane to reach the cytoplasm. This is assisted by the N-terminal portion of the heavy chain (H_N (4) **Different L chains specifically cleave distinct members of the SNARE family**. **TeNT (T) and BoNT serotype B, D, F and G act on VAMP/synaptobrevin (in green) on synaptic vesicles.** BoNT-A and E cleave SNAP-25 (in pink), whereas BoNT-C cleaves both syntaxin 1 (in cyan) and SNAP-25, two proteins of the pre-synaptic plasma membrane.

Clostridium tetani toxin (tetanospasmin)



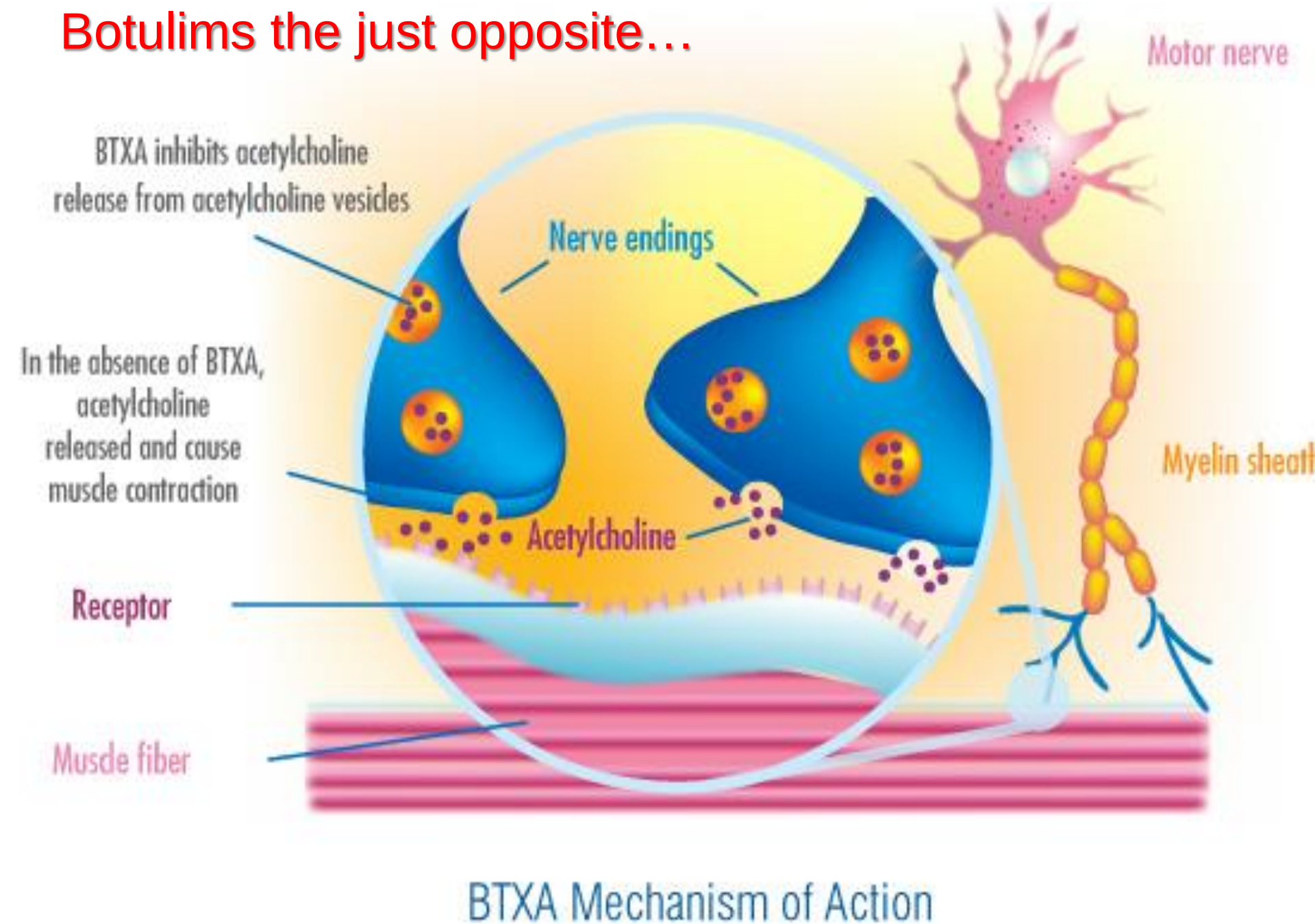
Clostridium tetani toxin (tetanospasmin)

soldier dying of tetanus - spasms of respiratory muscles

Normal
Glycine (G) release stops acetylcholine (A) release and allows relaxation of muscle

Tetanus
Tetanus toxin binds to inhibitory interneurons, preventing release of G and relaxation of muscle

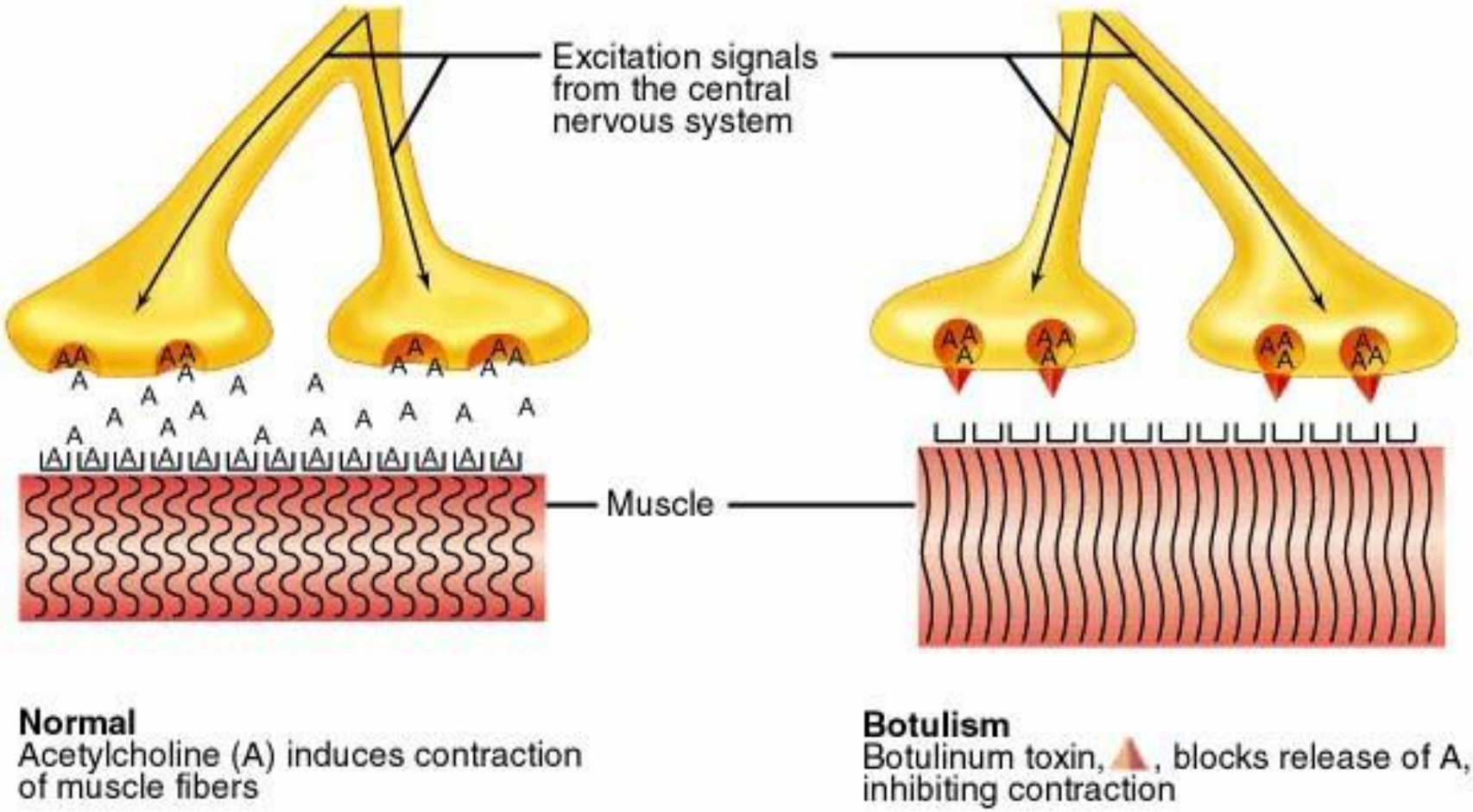
Botulims the just opposite…



BTXA Mechanism of Action

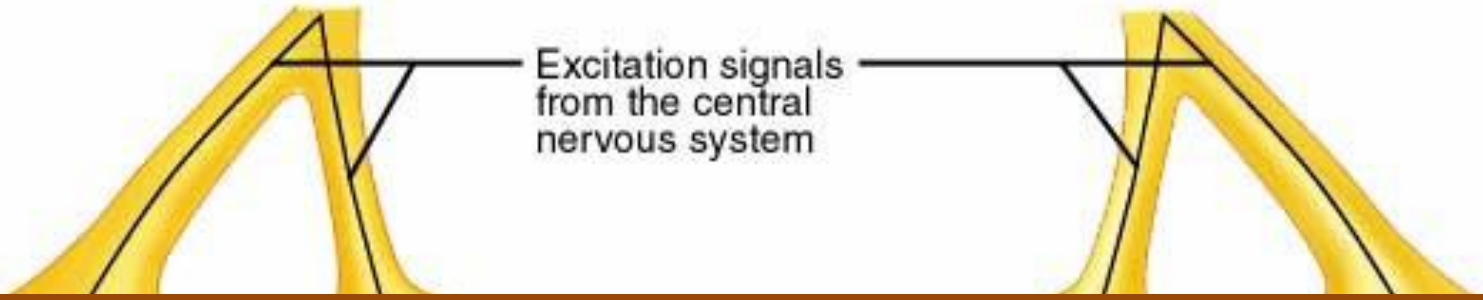
Clostridium botulinum toxin (botulismus)

blocking acetylcholine release causes descending weakness of skeletal muscles and death from respiratory paralysis due to interferences with muscle contraction



Clostridium botulinum toxin (botulismus)

blocking acetylcholine release causes descending weakness of skeletal muscles and death from respiratory paralysis due to interferences with muscle contraction



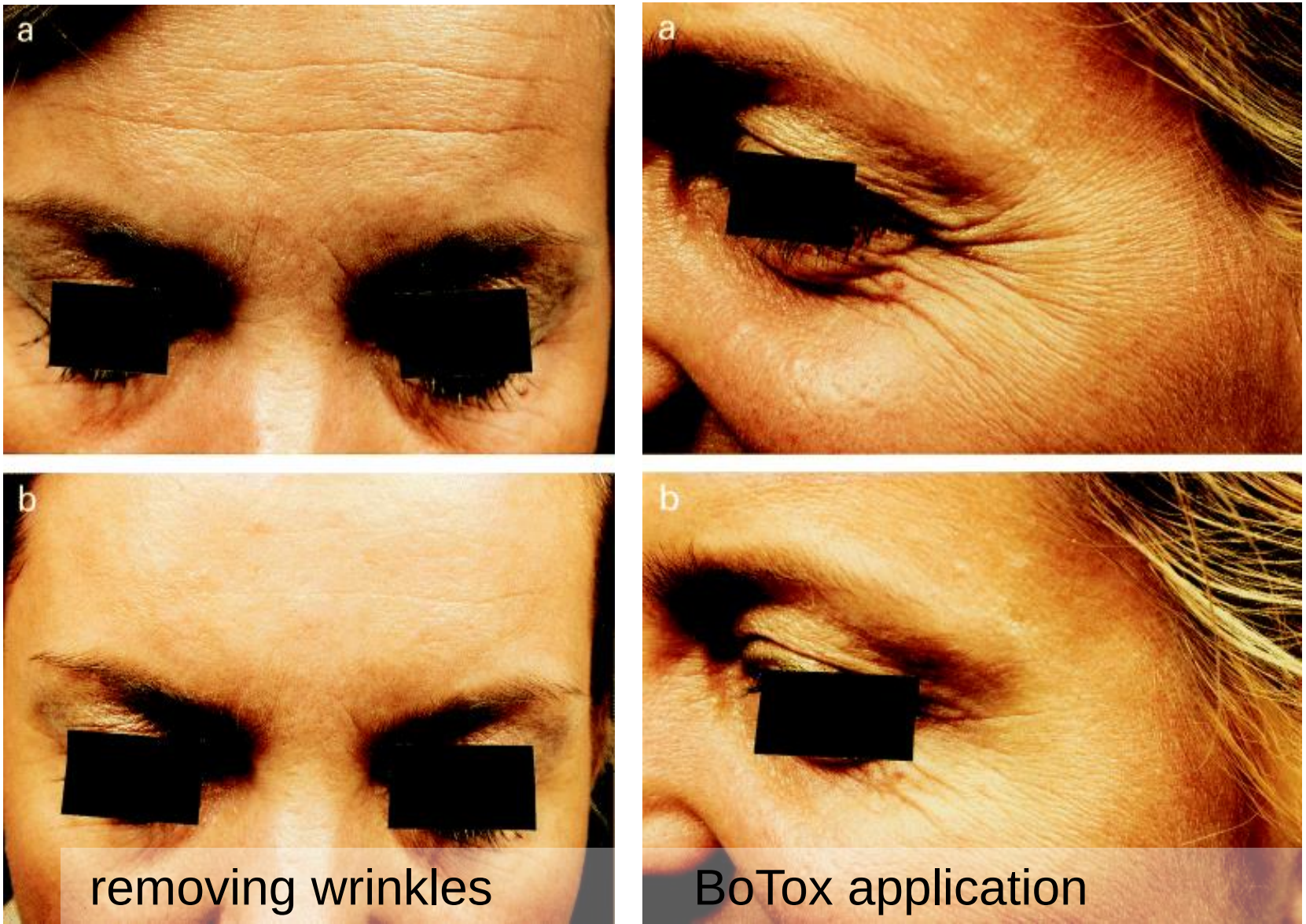
The estimated human dose LD50, based on animal studies, is approximately 0.09 to 0.15 μg by intravenous administration
0.7 to 0.9 μg by inhalation and 70 μg by oral administration

Death is usually the result of respiratory failure or secondary infection associated with prolonged mechanical ventilation.

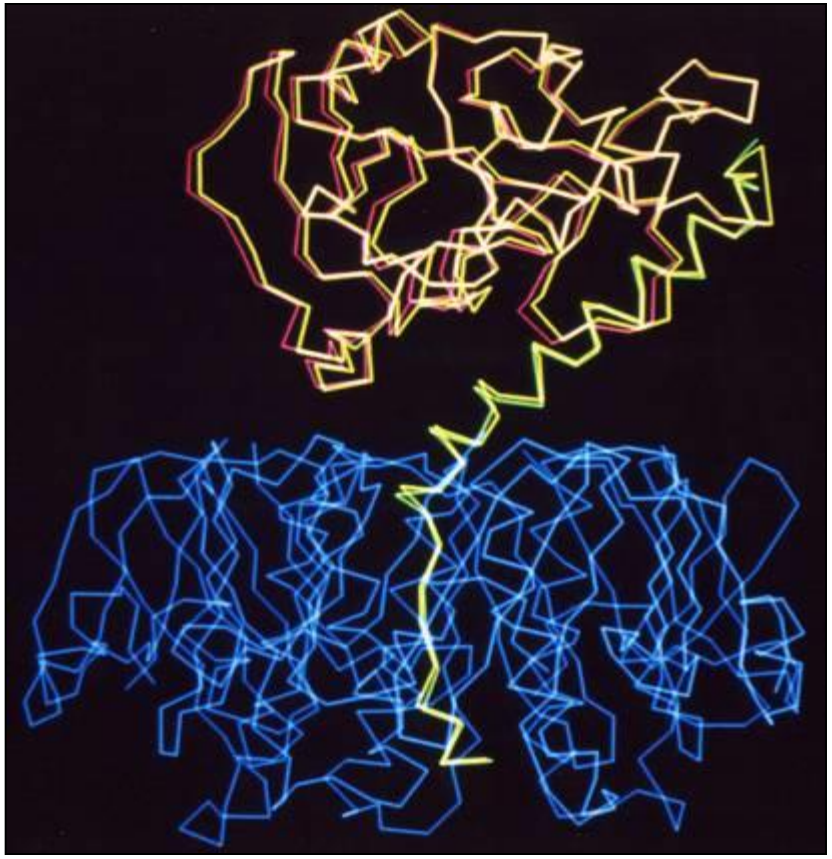
Normal
Acetylcholine (A) induces contraction of muscle fibers

Botulism
Botulinum toxin, ▲, blocks release of A, inhibiting contraction

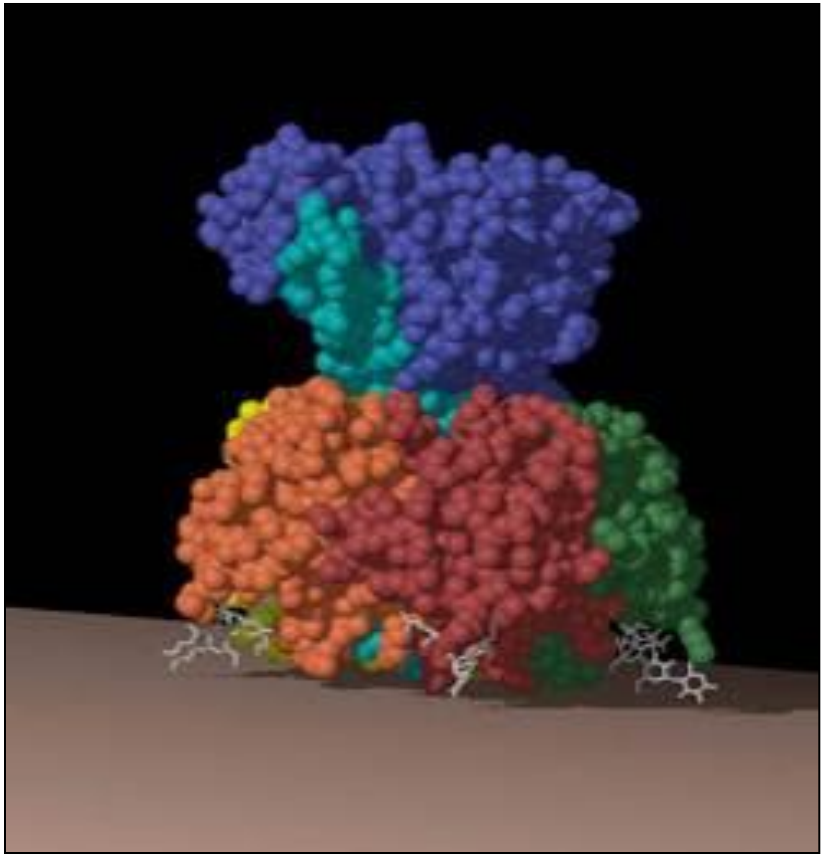
Botulinum toxin can make you pretty…



Cholera and *E. coli* heat-labile toxin: 3D structure

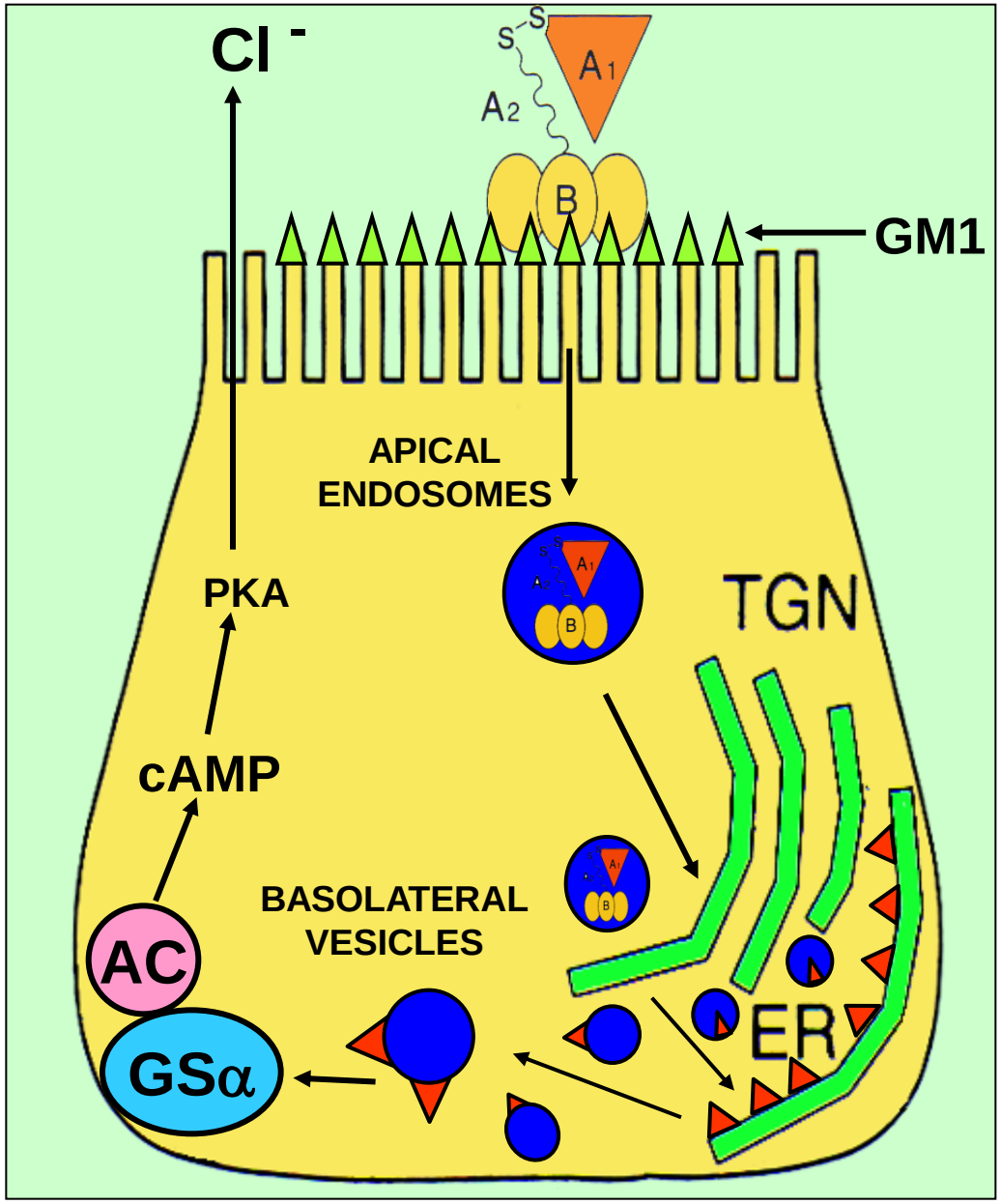


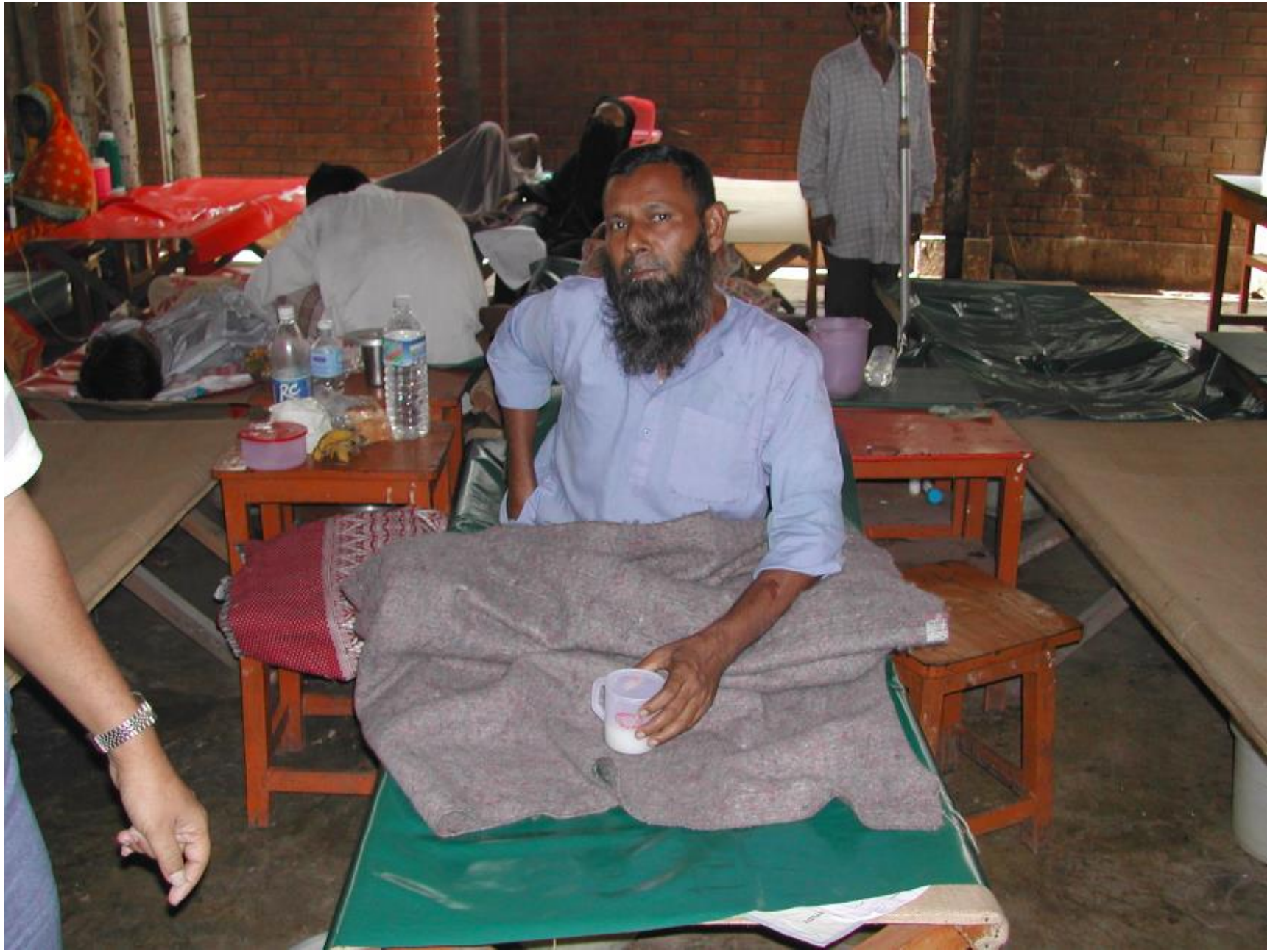
Sixma *et al.*, Nature 351: 371-377, 1991



Merritt *et al.*, Prot. Sci. 3: 166-175, 1994

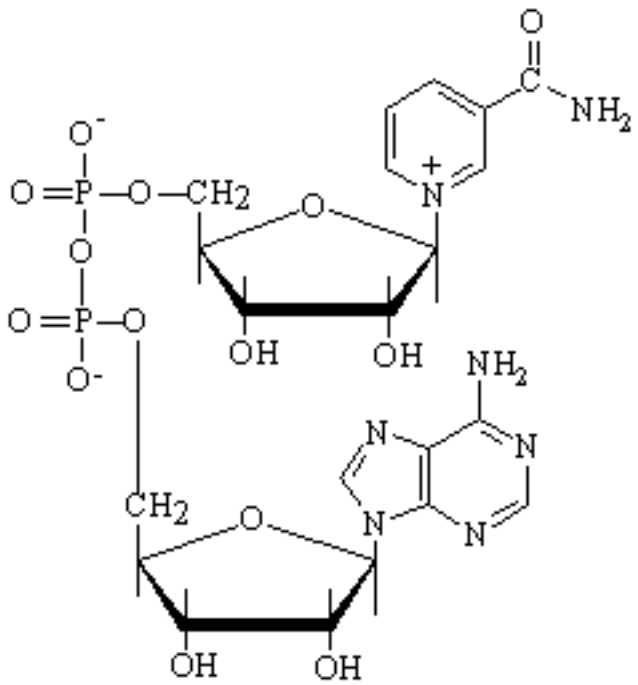
Action of cholera toxin and related enterotoxins





ADP-ribosyltransferase toxins

NAD + TARGET ⇌ **ADP-Ribose-TARGET + Nic**



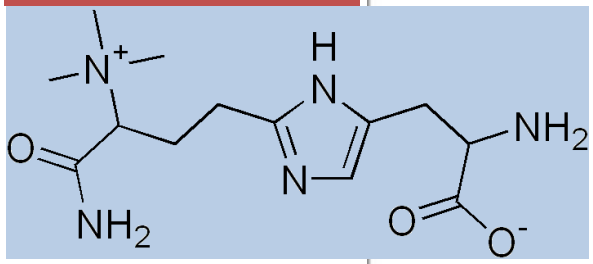
Cholera toxin
Pertussis toxin
Diphteria toxin
Exotoxin A of Pseodomonas
T3SS effectors ExoS and ExoT of *Pseudomonas*

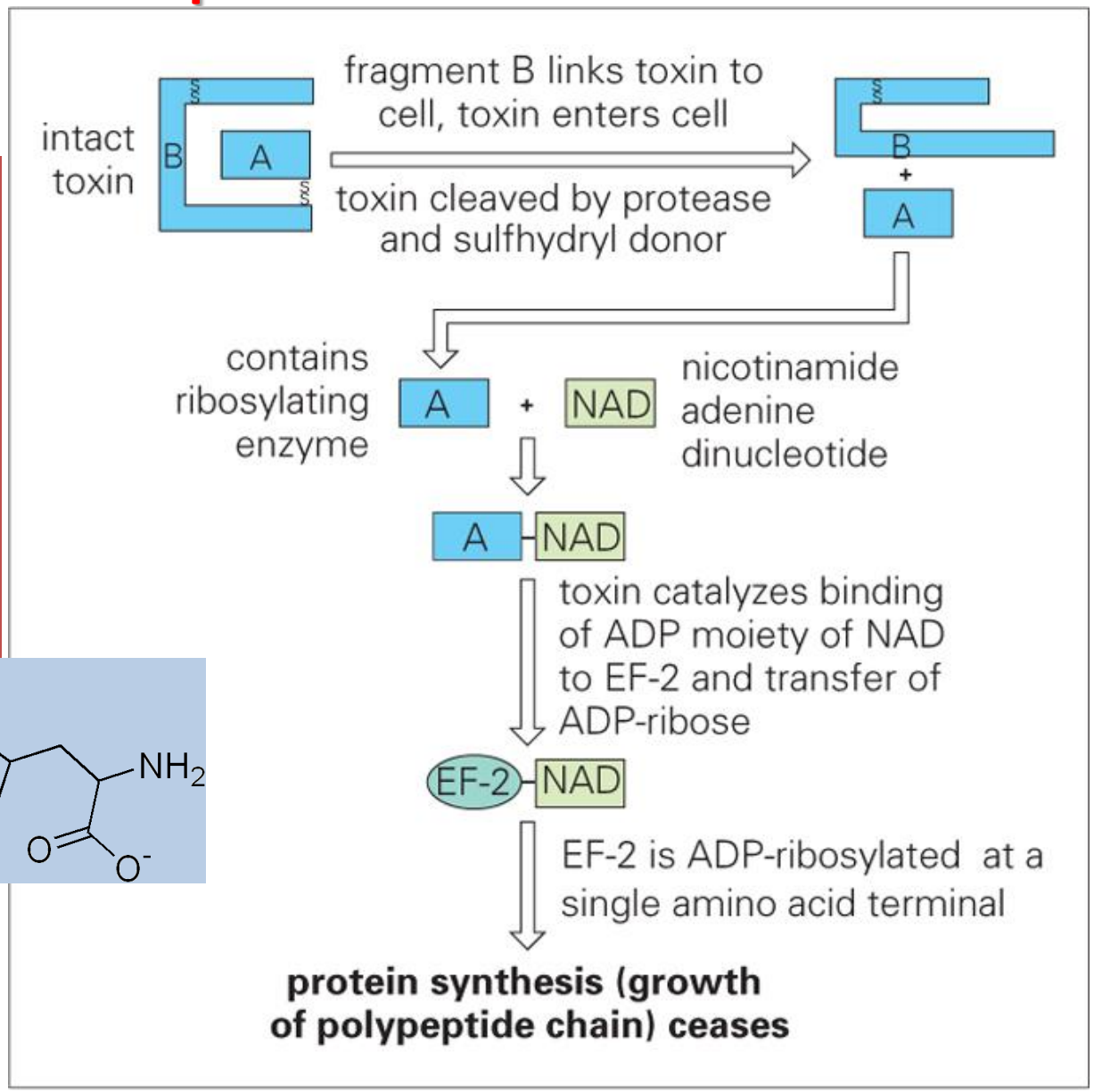
Clostridial C3-like toxins

etc…

Diphteria toxin action

Diphthamide is a modified histidine amino acid found in eukaryotic elongation factor 2 (eEF-2). It is ADP-ribosylated by diphtheria toxin, which renders the elongation factor inactive.

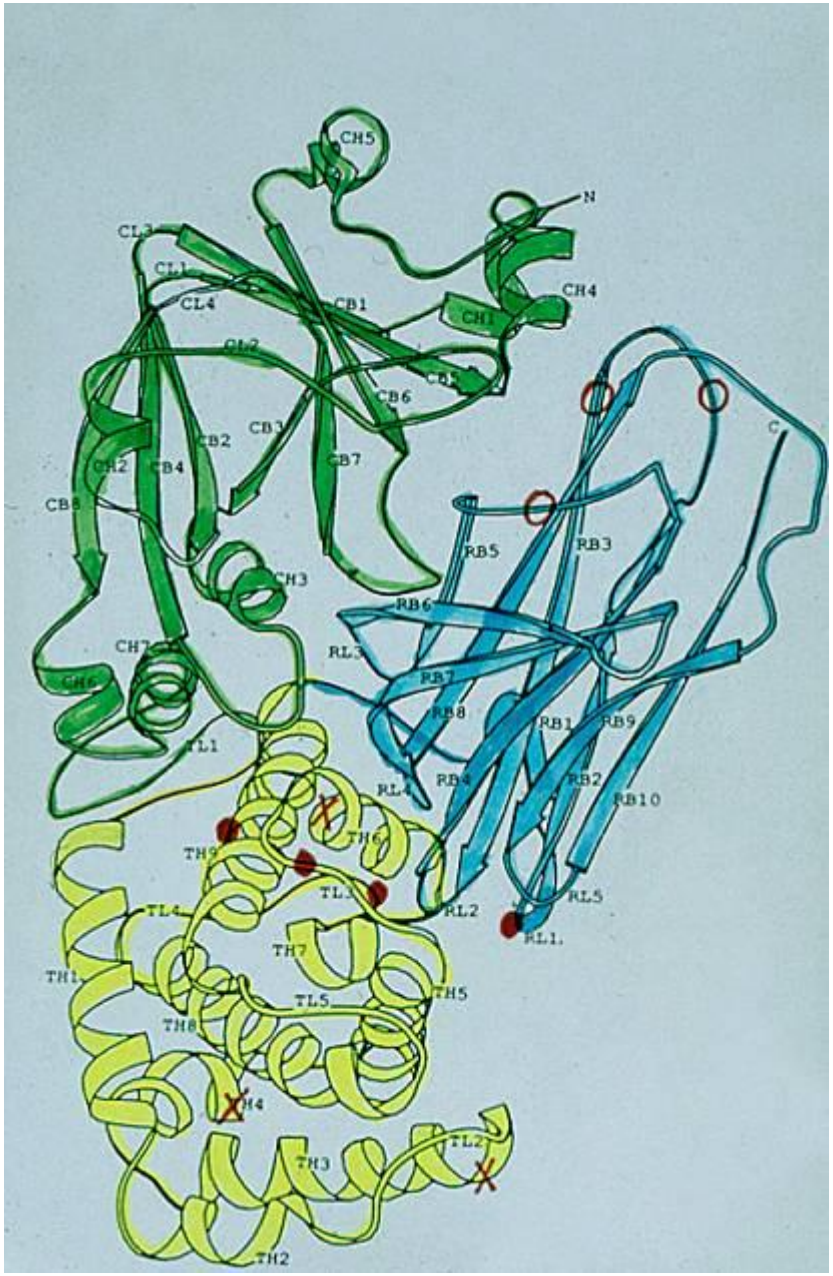




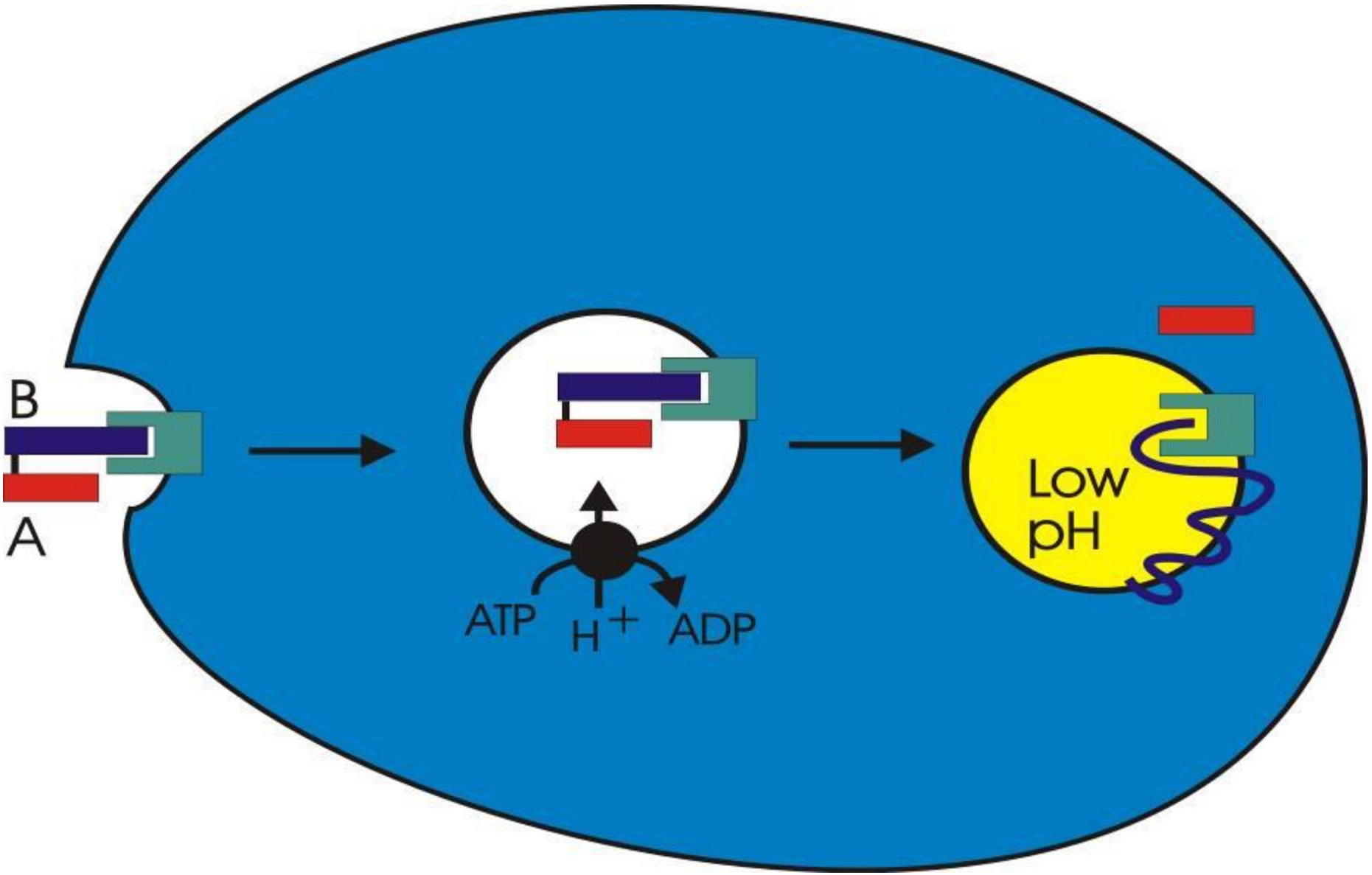
Structure of diphtheria toxin

A-fragment

T-domain



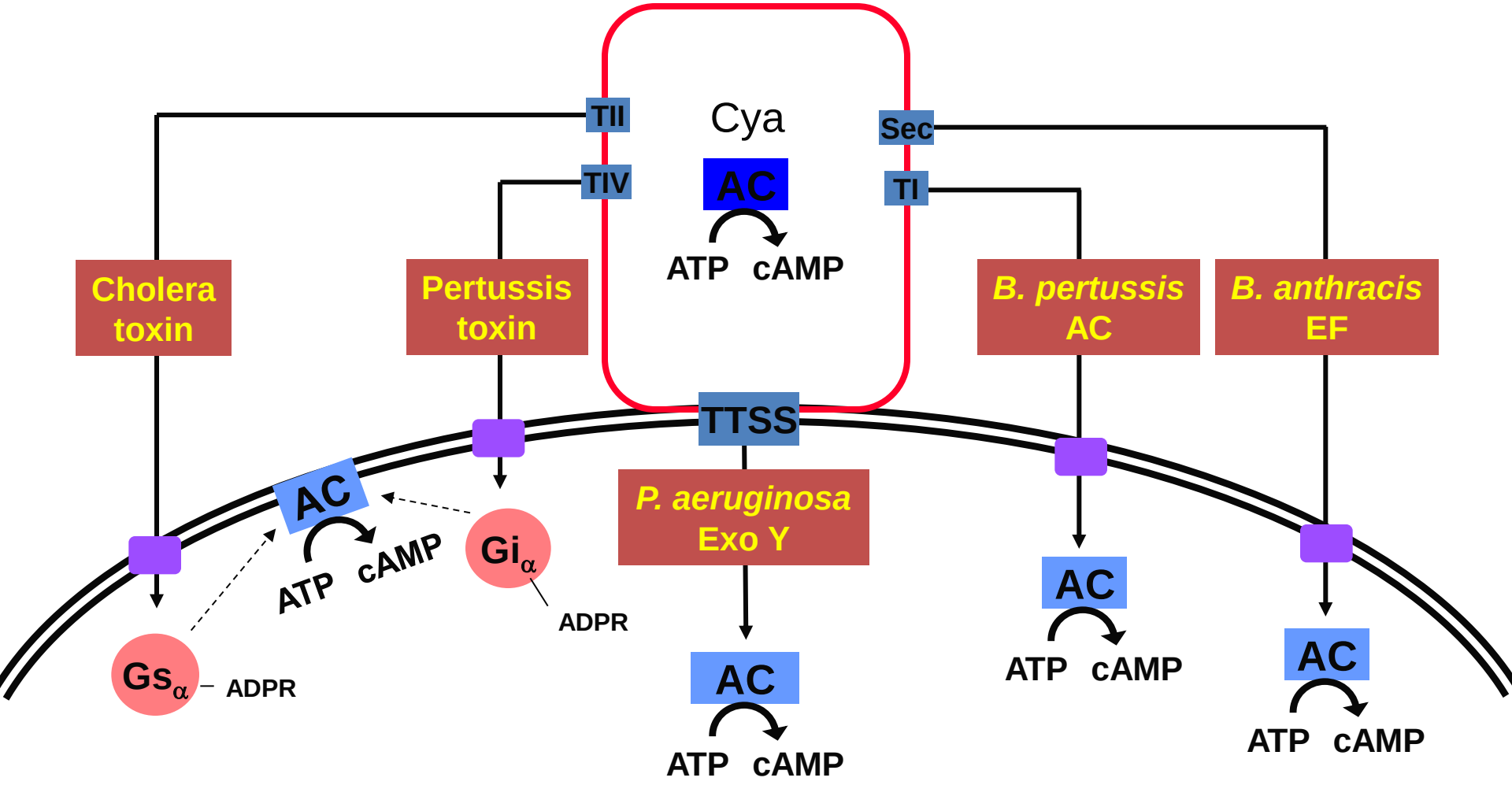
R-domain


B
A
ATP
H+
ADP
Low
pH

Toxins interfering with cellular signaling

The ‘smartest’ toxins subvert cell signaling

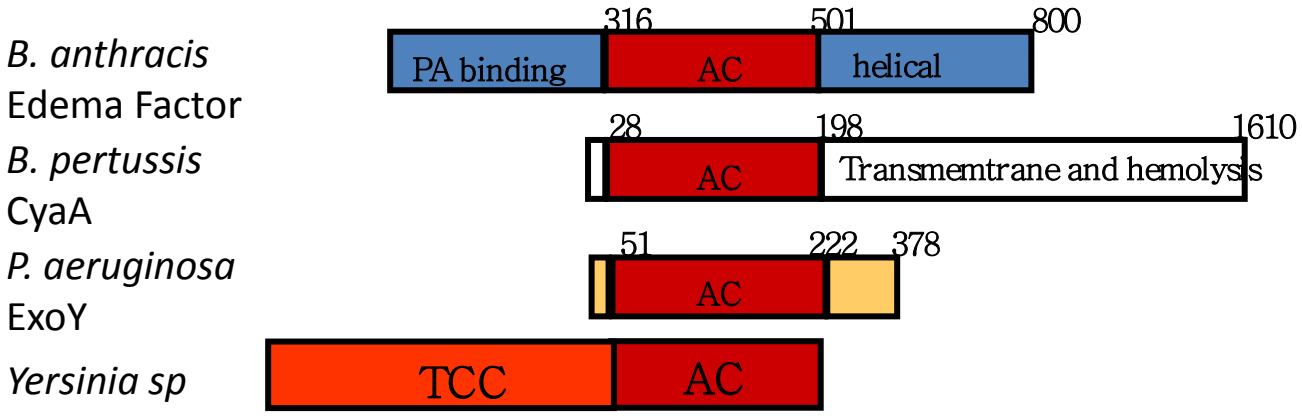
Such as fooling cells by cAMP – the second messenger…!



Bacterial Toxins foolling cell signaling through elevating intracellular cAMP Levels

1. **Adenylyl cyclase toxins:**

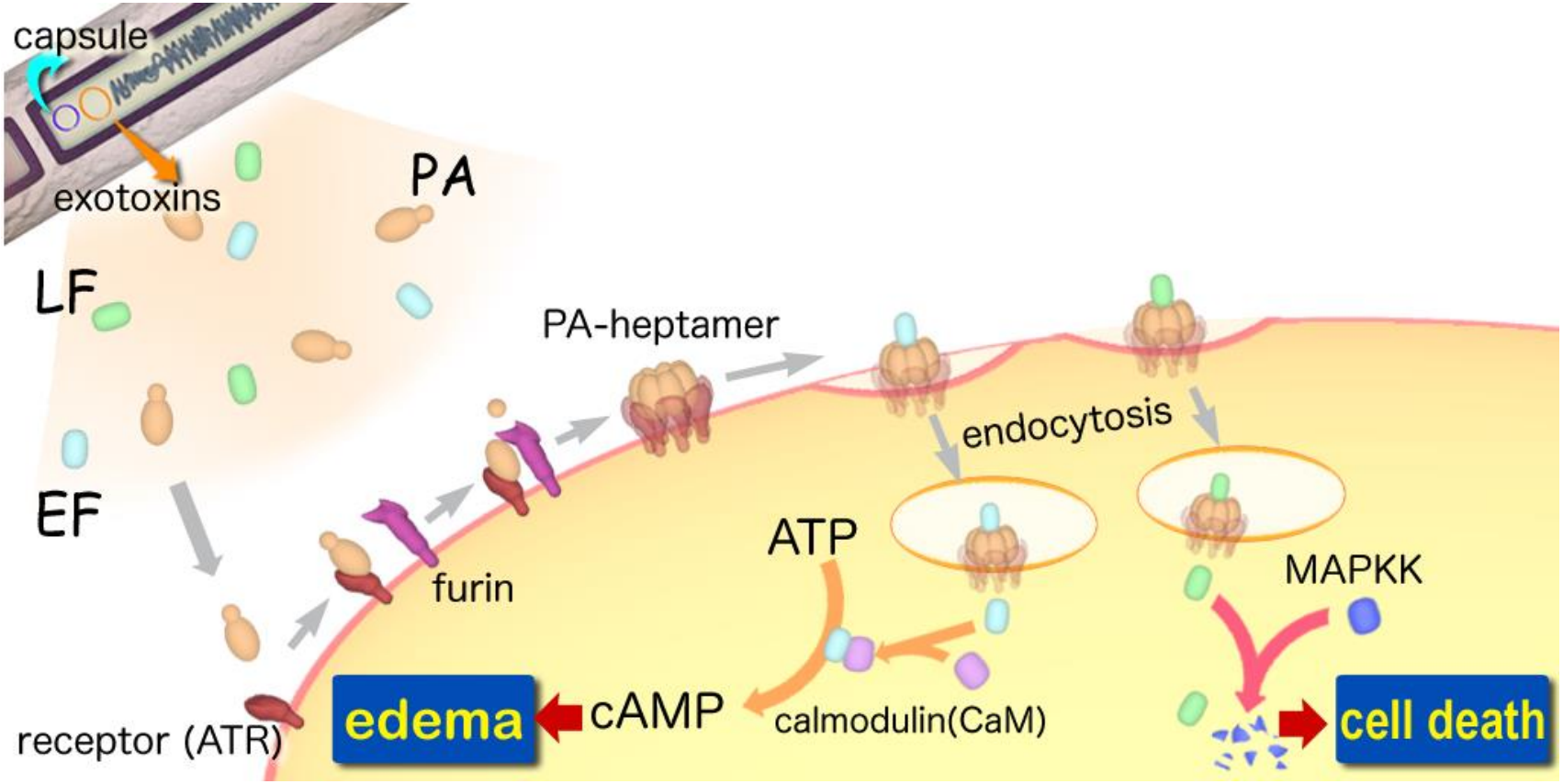
Exotoxin	Activator	Disease
Edema factor	calmodulin	Anthrax
CyaA	calmodulin	Whooping cough
ExoY	?	10-20% nosocomial infections
Tcc-AC	?	Plague



2. **ADP ribosylation factors:**

Exotoxin	Target	Disease
Cholera toxin	a subunit of Gs	Massive diarrhea
Pertussis toxin	a subunit of Gi	Whooping cough

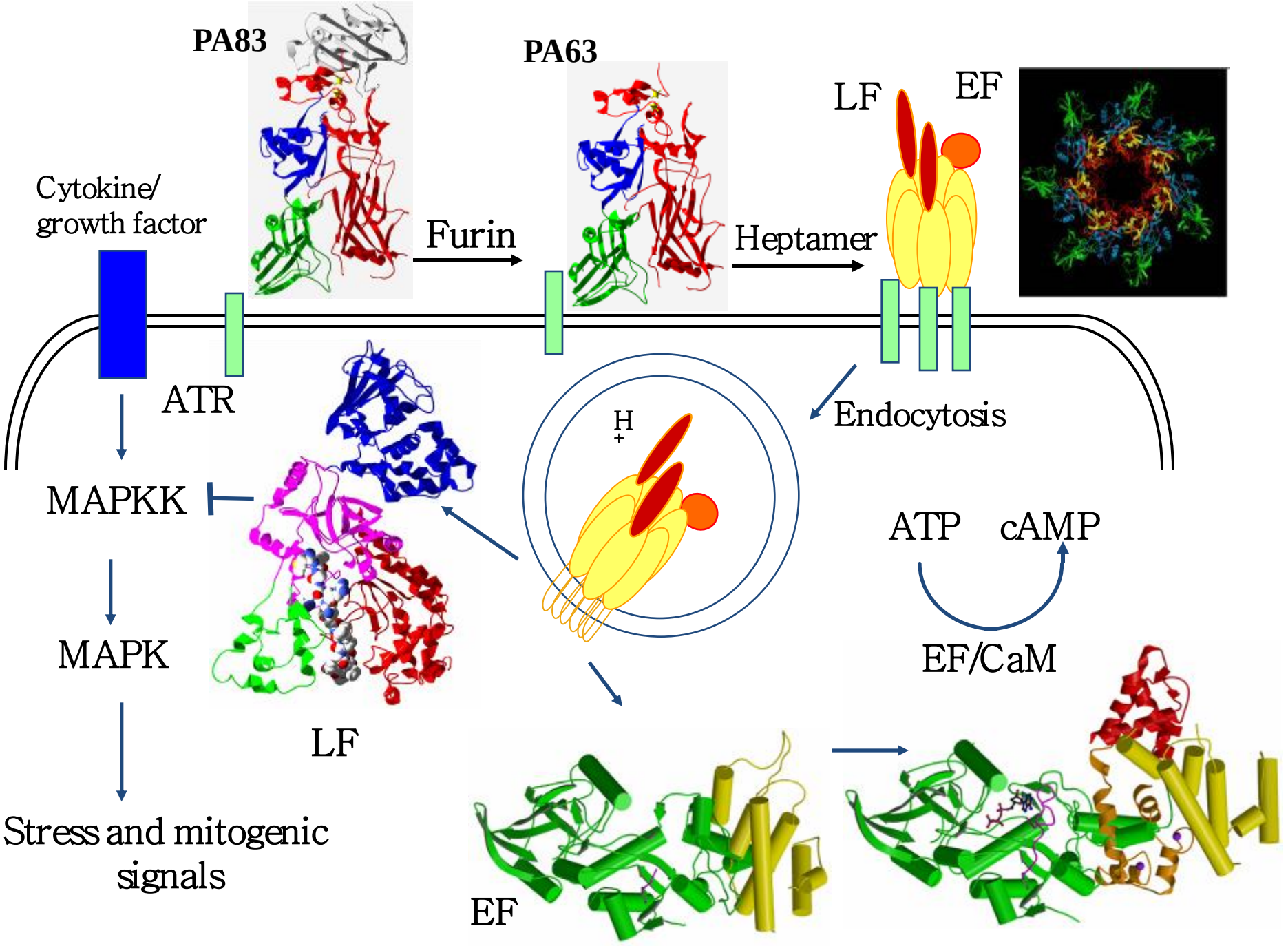
Mechanism of action of anthrax toxins



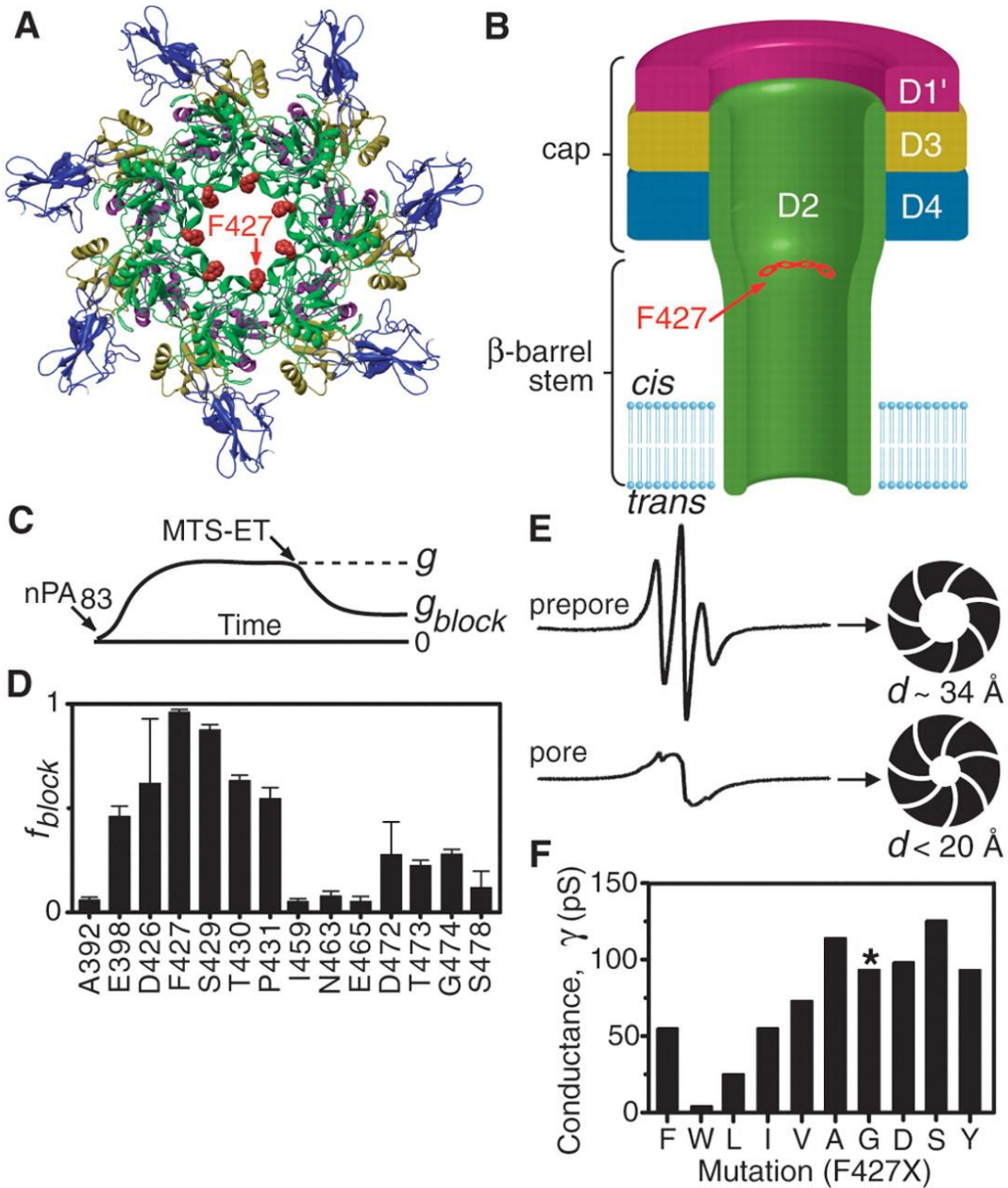
EF acts as a Ca^{2+} and calmodulin dependent adenylate cyclase that greatly increases the level of cAMP in the cell. This increase in cAMP upsets water homeostasis, severely throws the intracellular signaling pathways off balance, and impairs macrophage function, allowing the bacteria to further evade the immune system.

LF also helps the bacteria evade the immune system through killing macrophages. Once in these cells, LF acts as a Zn^{2+}-dependent endoprotease that snips off the N-terminus of mitogen-activated protein kinase kinases (MAPKK). This inhibits these kinases by not allowing them to efficiently bind to their substrates, which leads to altered signaling pathways and ultimately to apoptosis.

Thus, the synergistic effect of these three proteins leads to cellular death through a cascade of events that allow the proteins to enter the cell and disrupt cellular function.

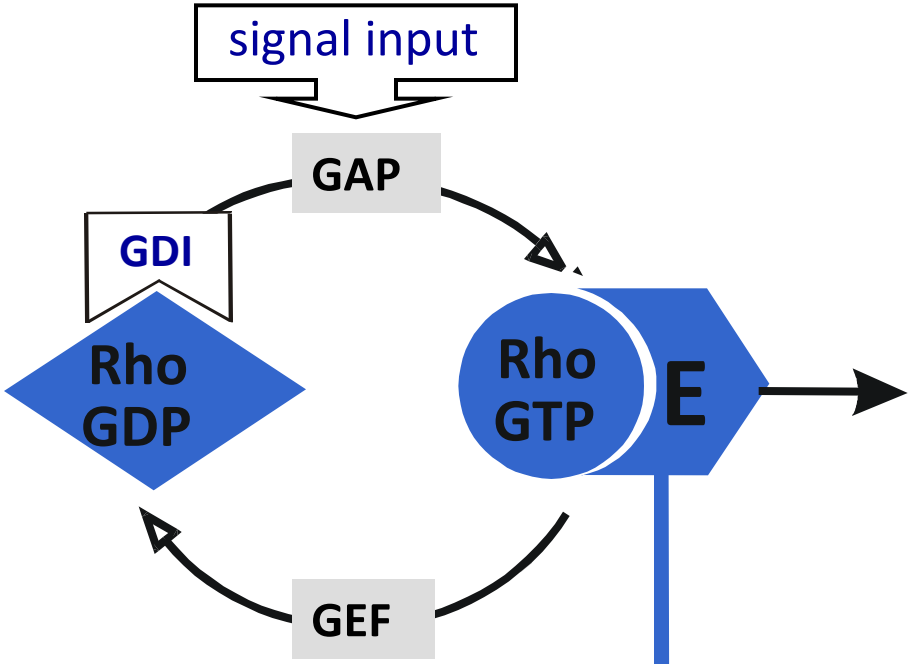

PA83
PA63
LF
EF
Cytokine/
growth factor
Furin
Heptamer
ATR
Endocytosis
H+
MAPKK
MAPK
ATP
cAMP
EF/CaM
LF
Stress and mitogenic
signals
EF

A Phenylalanine Clamp Catalyzes Protein Translocation Through the Anthrax Toxin Pore



Structural models of a lumen-facing phenylalanine heptad. (A) A ribbons rendering of the PA63 prepore (27), viewed axially, where domain 4 is proximal. Domains are colored: D1' (magenta), D2 (green), D3 (gold), and D4 (blue). F427 (red, space filling) is modeled into the structure. (B) Hypothetical cross section of the PA63 channel, or pore, colored as in (A). The membrane-spanning tube is the 14-stranded ß barrel from domain 2 (5, 6). (C) Illustration of the effect of MTS-ET modification on Cys-substituted mutants of PA63 in macroscopic conductance studies. Conductance, g, is determined from the current, I, and as g = I/. (D) Fraction of conductance blocked (fblock) by MTS-ET modification (28) in domain 2 cap residues [as in (C), where fblock = 1 - gblock/g] (table S2). Error bars show means + SE (n = 3). (E) EPR spectra of PA63 heptamers uniformly labeled at F427C with a Cys-reactive nitroxide spin label in the prepore state at pH 8.5 (upper spectrum) and the pore state at pH 6 (lower spectrum). Approximate luminal diameters, d, are based on the observed spin-spin interactions. (F) Unitary conductance, , of single PA63 channels, with indicated substitutions at F427. Channels formed by F427G PA63 (*) initially opened to a conductance of 90 pS, but, unlike any of the other channels, flickered to 60 and 30 pS substates. values are accurate to at least ±10%, except for F427L and F427W, which are accurate to ±20%.

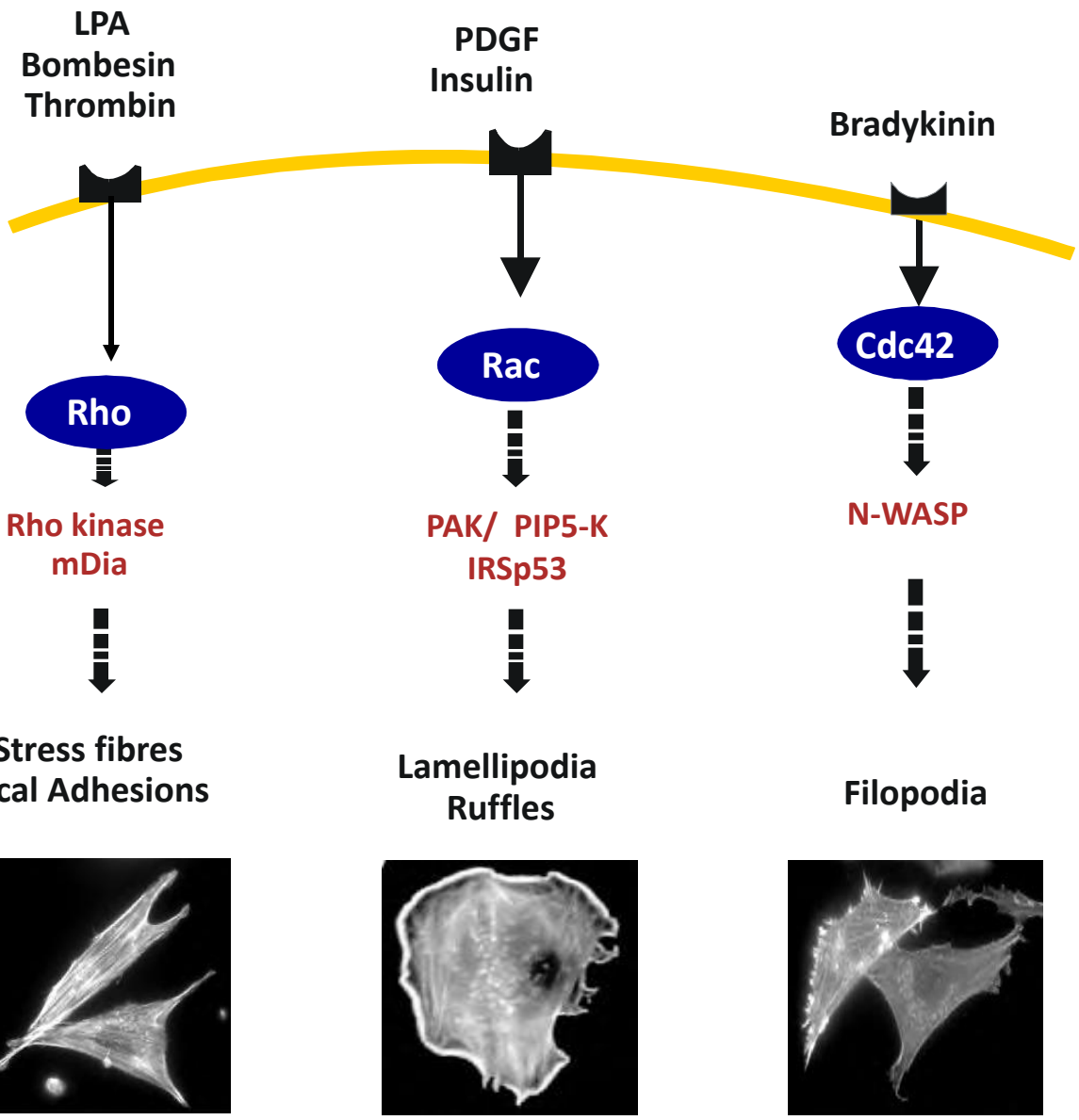
Targeting the Rho-GTPase family cycle…



- **actin cytoskeleton** — cell shape, cell movement, cell-cell interactions, axonal guidance
- **gene transcription**
- **cell cycle progression**
- **apoptosis**
- **oncogenic transformation** with Ras

Ser/Thr-kinases: *ROK, PAK*
Lipid kinases: *PI3K, PIP-5K*
Lipases: *PLD, PLCβ2*
Scaffold proteins: *Dia, Rhotekin*

Rho-GTPase cascade



RhoA,B,C

Rac1,2,3

Cdc42

RhoG

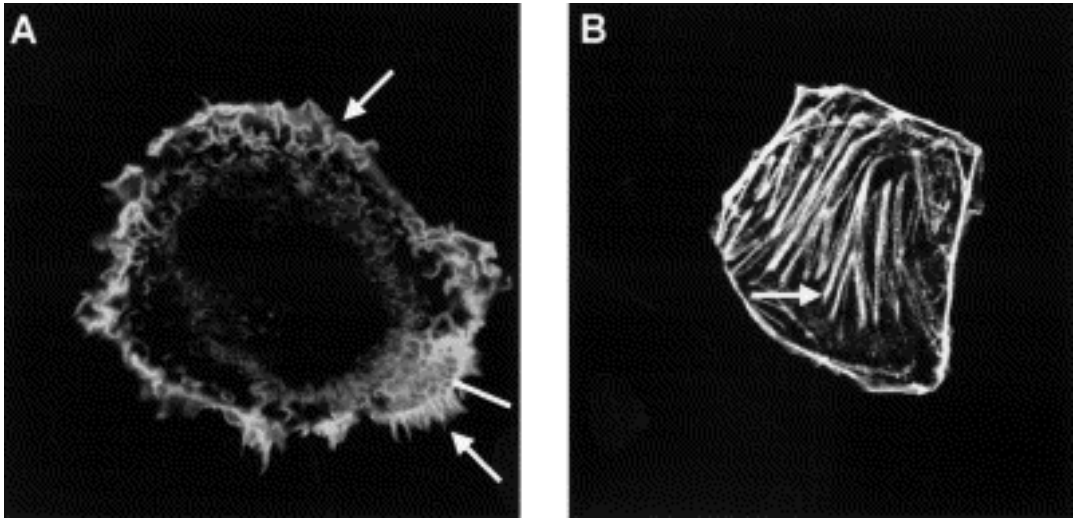
TC10

Rnd1,2,3 (RhoE)

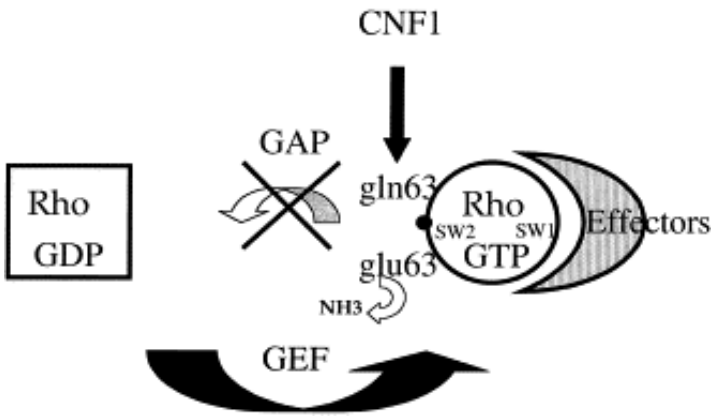
RhoD

RhoH (TTF)

Action of *E. coli* CNF1



Effects of CNF1 on the actin cytoskeleton of HEP-2 and Vero cells The actin cytoskeleton is stained by fluorescein isothycyanate-phalloidin. (A) HEp-2 cell treated with 10^{-10} M CNF1 for 12 h. Thin arrow: lamellipodia; thick arrow: filopodia; line: pinocytic vacuoles. (B) Vero cells treated withe 10^{-10} M CNF1 for 12 h. Arrowhead: stress fiber.



Molecular mechanism of CNF1 on Rho GTP-binding proteins This figure depicts the current status concerning the mechanism of action *E. coli* CNF1/CNF2 on the small GTPases activation/deactivation cycle. GEF, guanine exchange factor; GAP, GTPase activating protein; SW1 switch 1 domain, SW2 switch 2 domain. CNF1/CNF2 modify by deamidation glutamine 63 of Rho (61of Rac, Cdc42) and thus inhibit GAP activity toward the GTPases which remain in their active state bound to GTP thus able to permanently activate their downstream effectors.

Deaminating Rho-family GTPAses as mechanism of altering actin cytoskeleton homeostasis

the case of *E. coli cytotoxic necrotizing factor 1 (CNF1)*

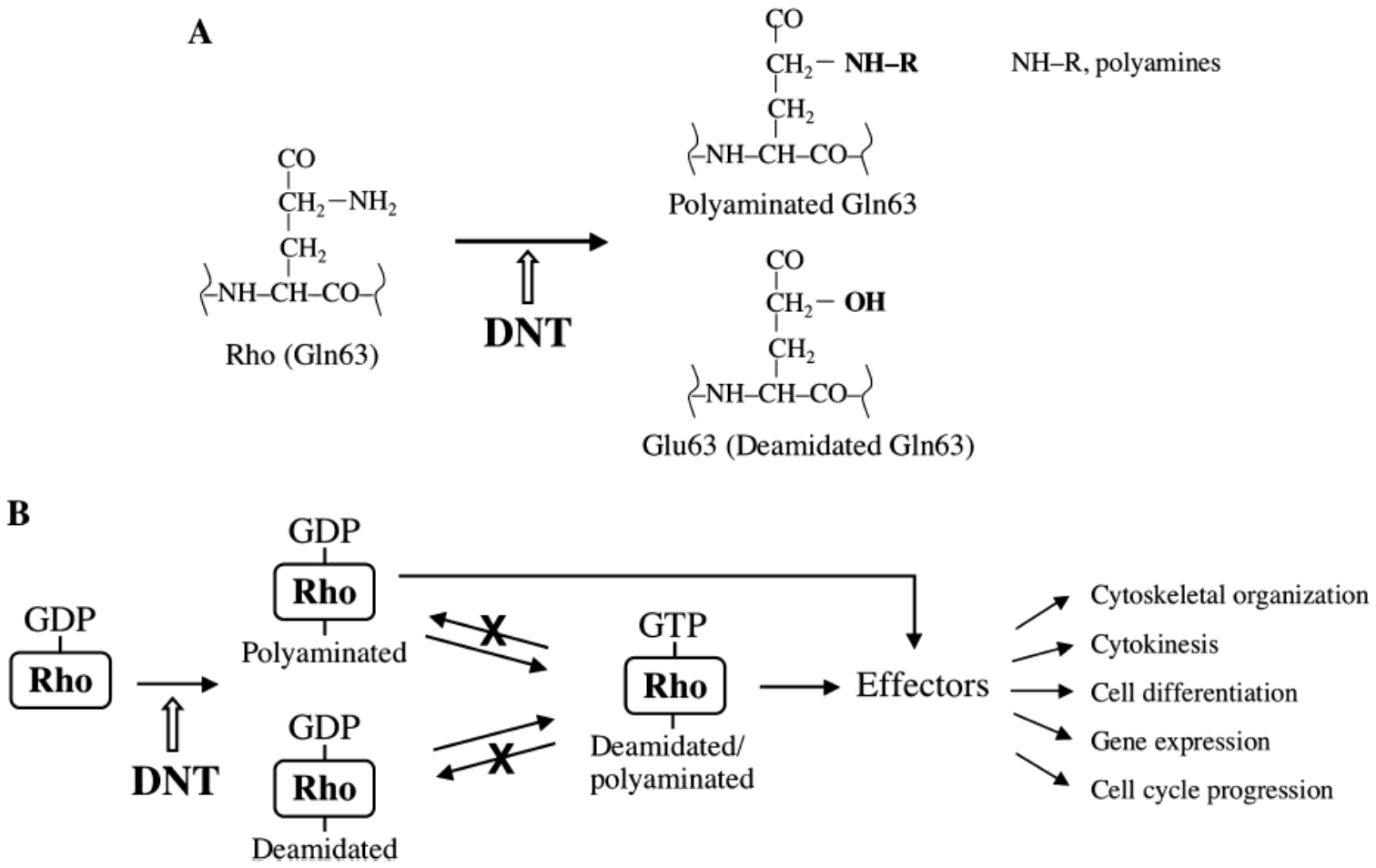
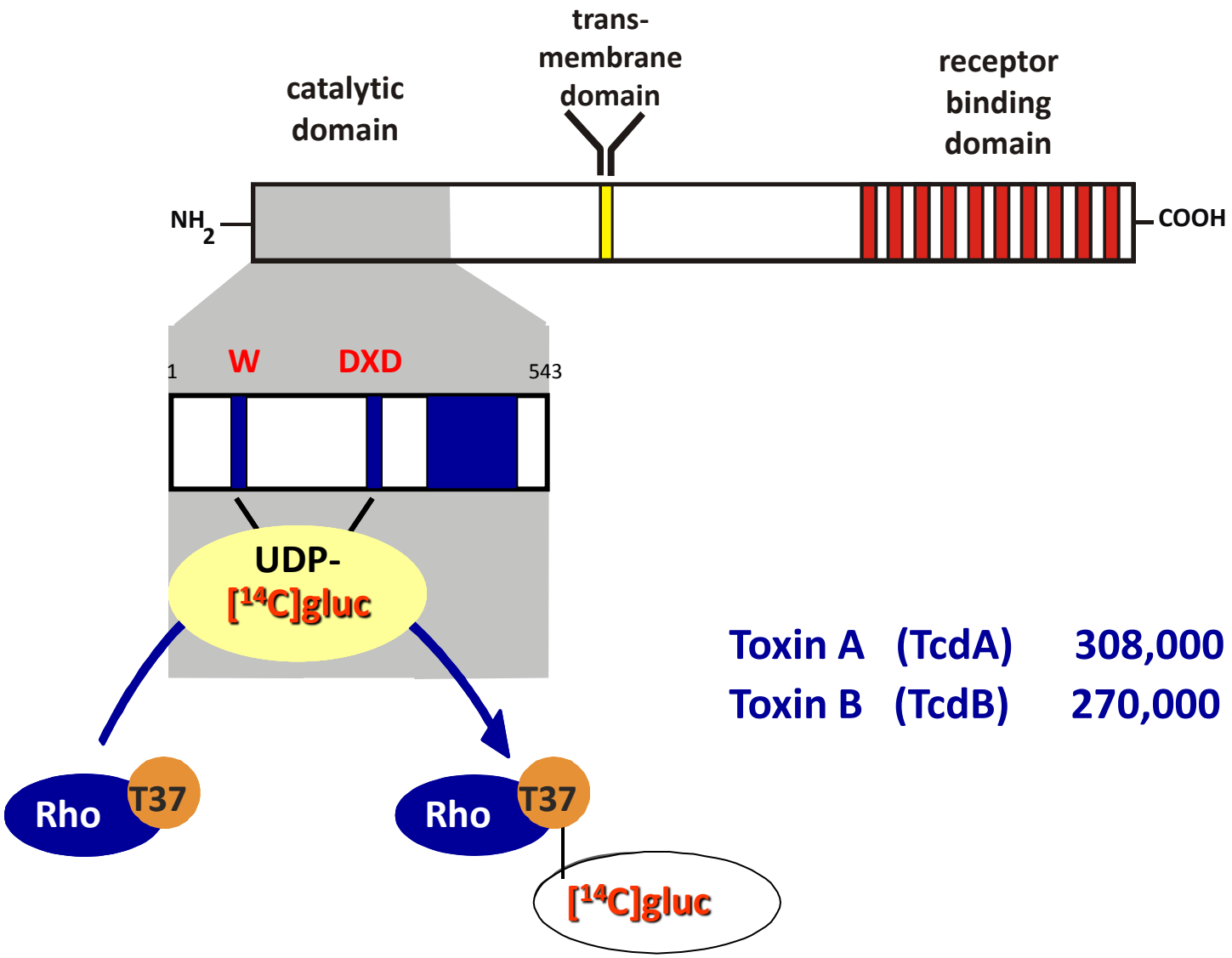


Fig. 2. **Modification of Rho GTPase by DNT.** (A) Transglutaminase activity of DNT. DNT catalyzes polyamination or deamidation at Gln63 of RhoA. See the text for details. (B) The modifications abrogate the GTP-hydrolyzing activity of the GTPases. Furthermore, in the case of the polyamination, the GTPases gain the ability to interact with downstream effectors (especially ROCK) in a GTP-independent manner. As a result, the modified GTPases function as constitutive analogues and induce anomalous-cellular events.

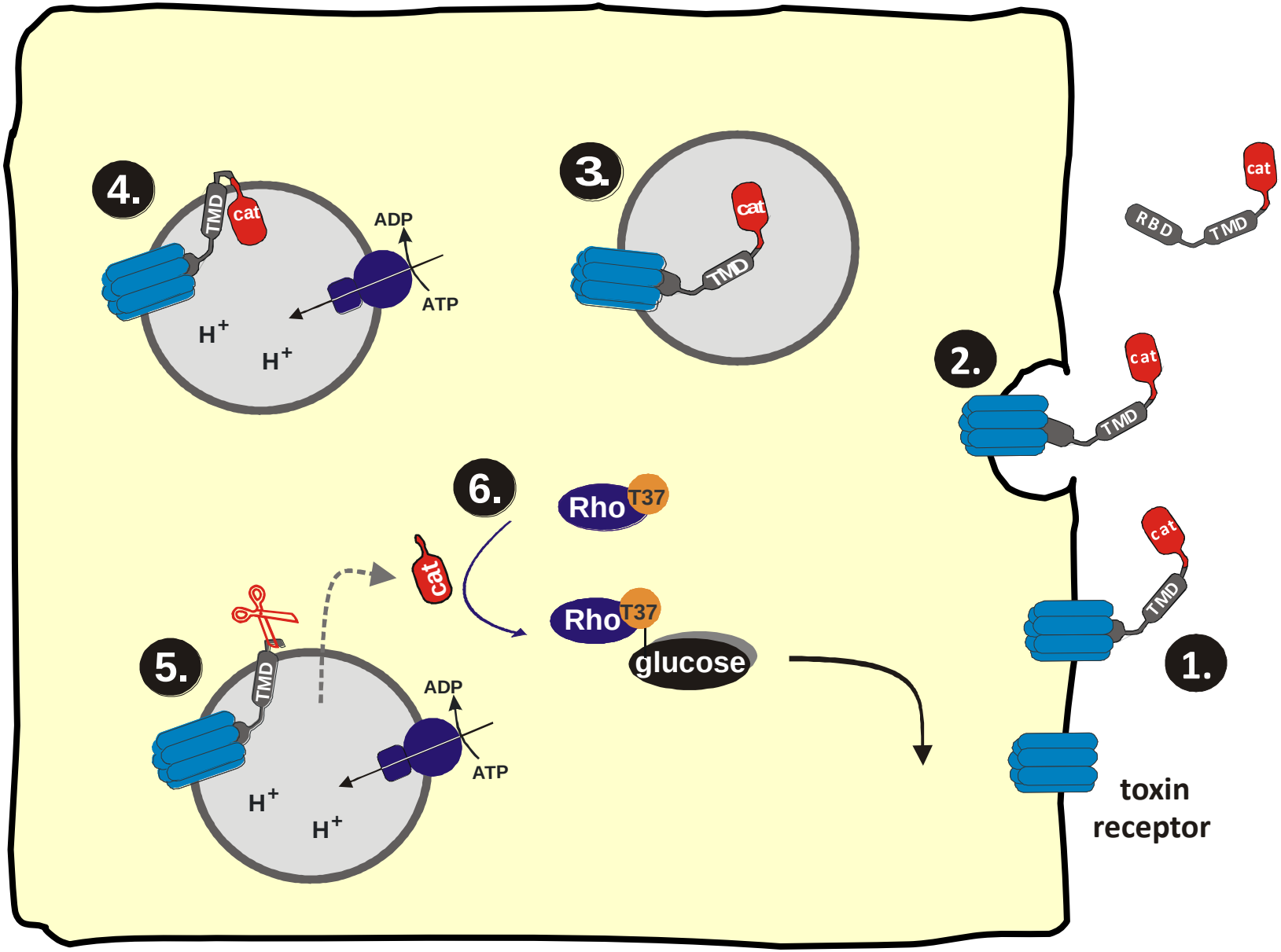
Clostridium difficile glucosylating toxin



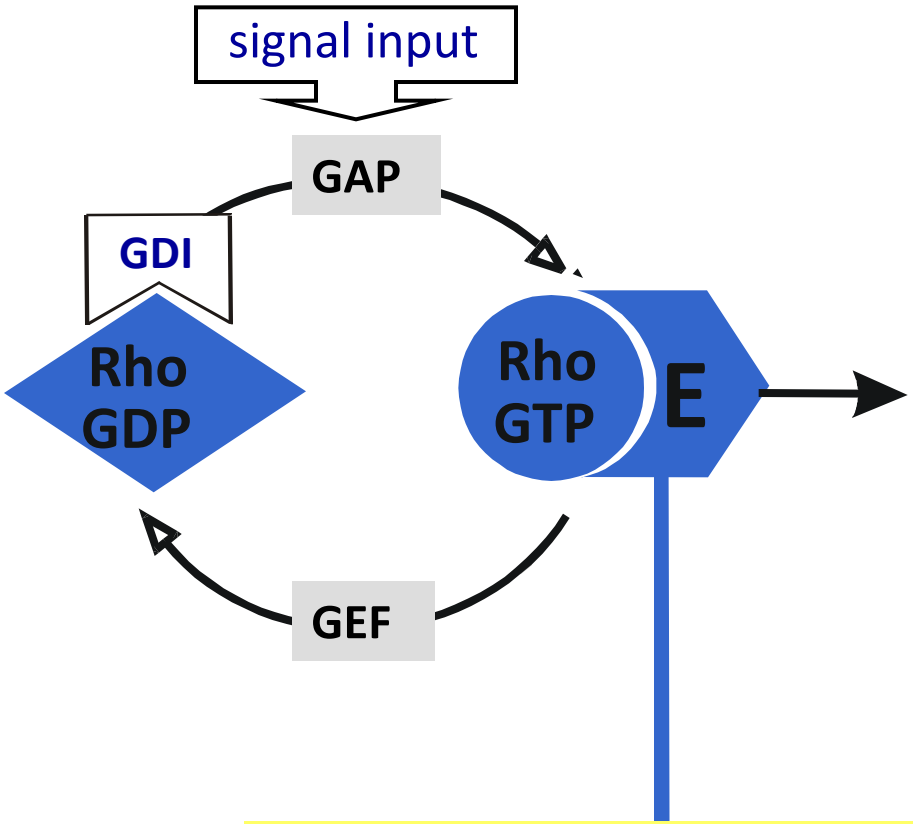
Toxin A (TcdA) 308,000 Da

Toxin B (TcdB) 270,000 Da

Cell entry of *C. difficile* Toxin A and Toxin B



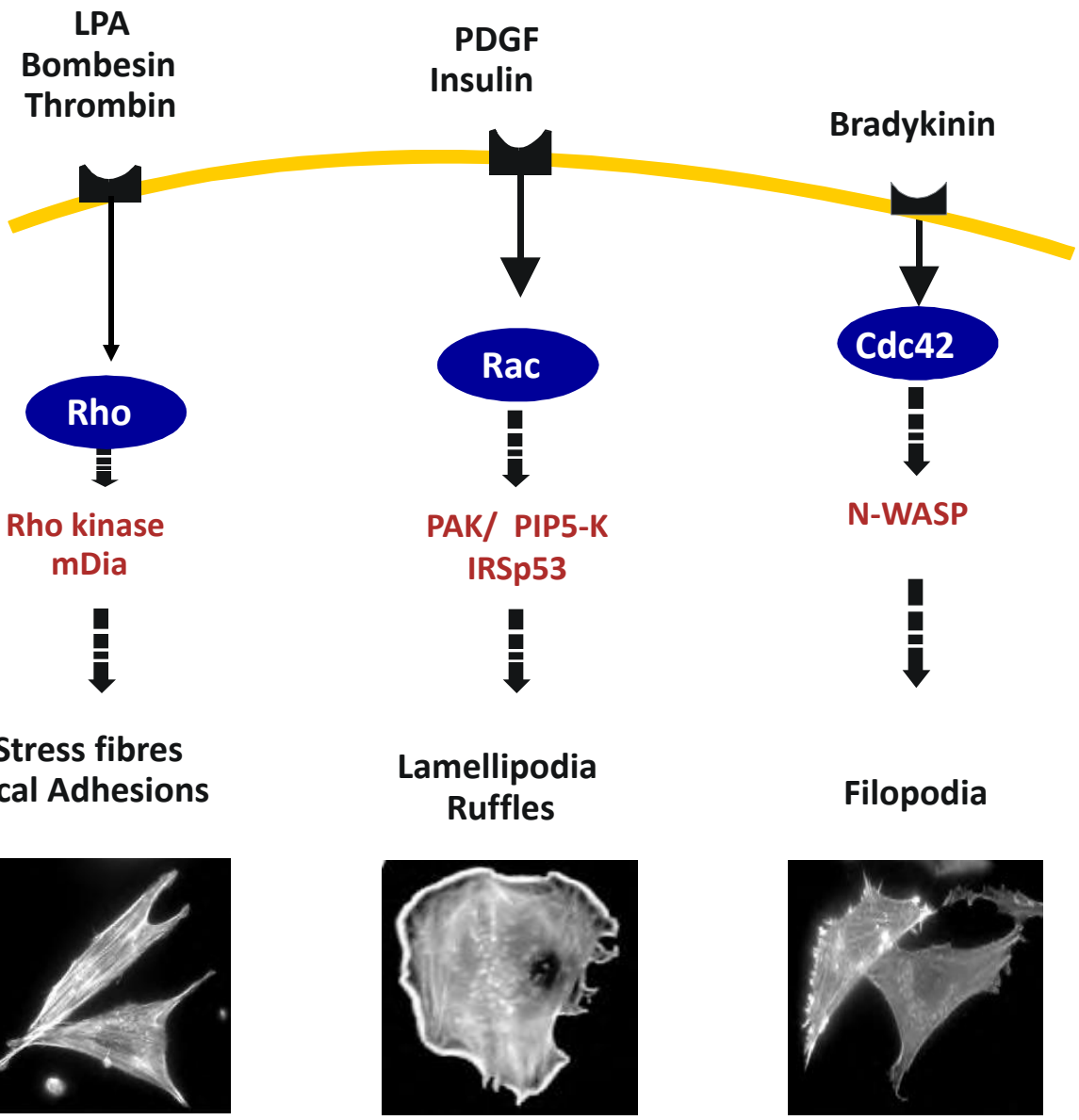
Rho-GTPase cycle



- **actin cytoskeleton** — cell shape, cell movement, cell-cell interactions, axonal guidance
- **gene transcription**
- **cell cycle progression**
- **apoptosis**
- **oncogenic transformation** with Ras

Ser/Thr-kinases: *ROK, PAK*
Lipid kinases: *PI3K, PIP-5K*
Lipases: *PLD, PLCβ2*
Scaffold proteins: *Dia, Rhotekin*

Rho-GTPase cascade



RhoA,B,C

Rac1,2,3

Cdc42

RhoG

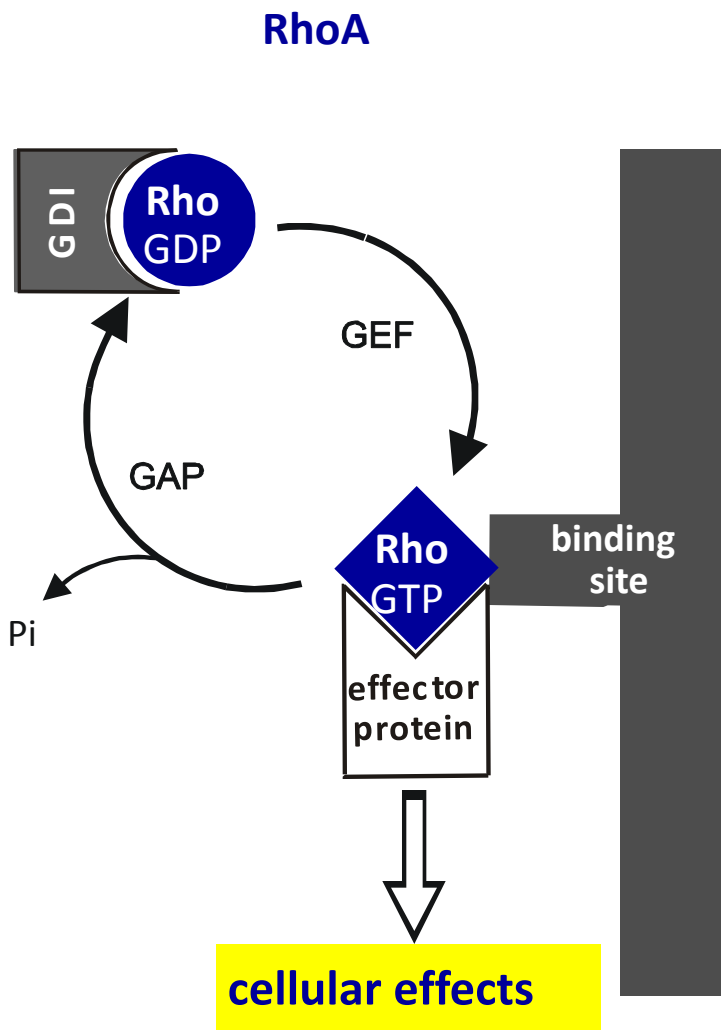
TC10

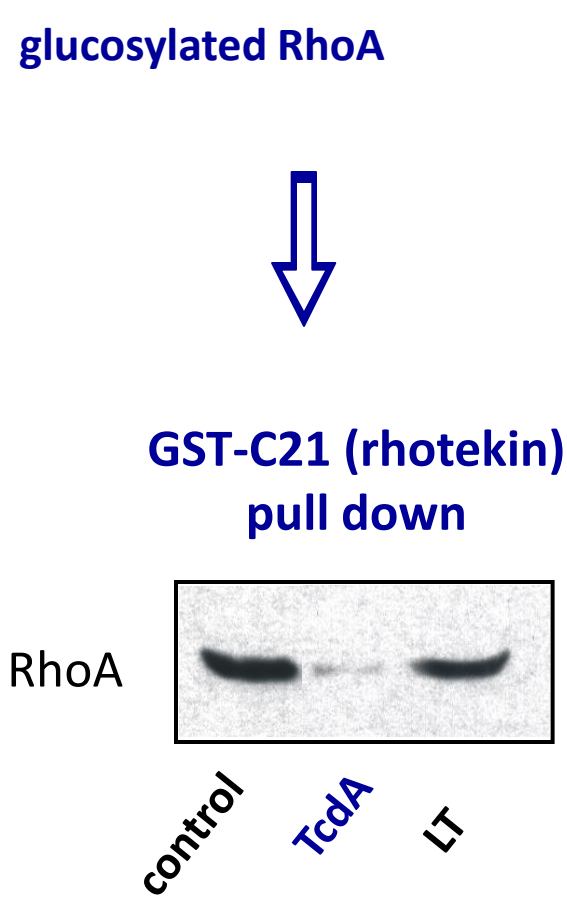
Rnd1,2,3 (RhoE)

RhoD

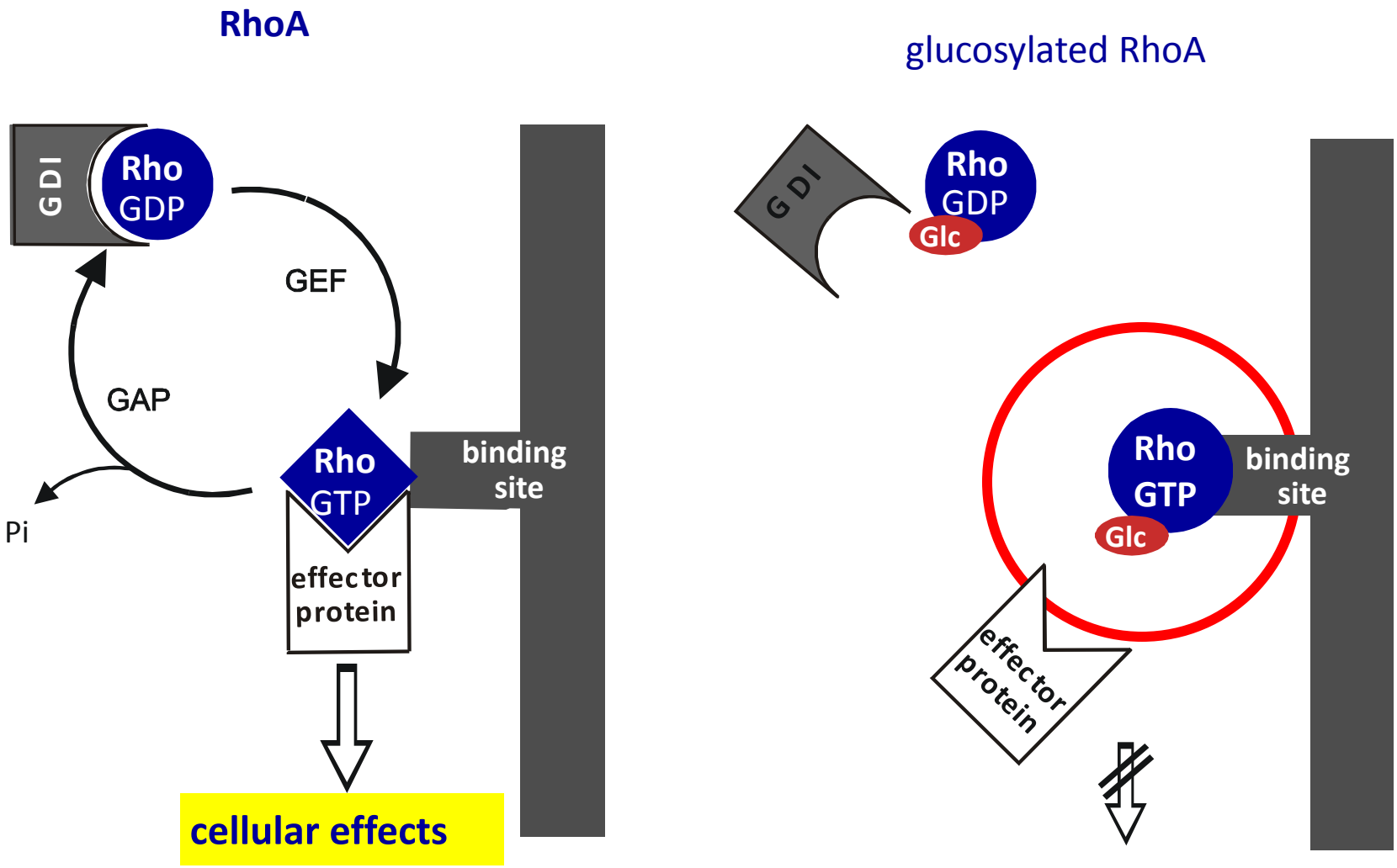
RhoH (TTF)

Cytosol – membrane cycling of Rho GTPases

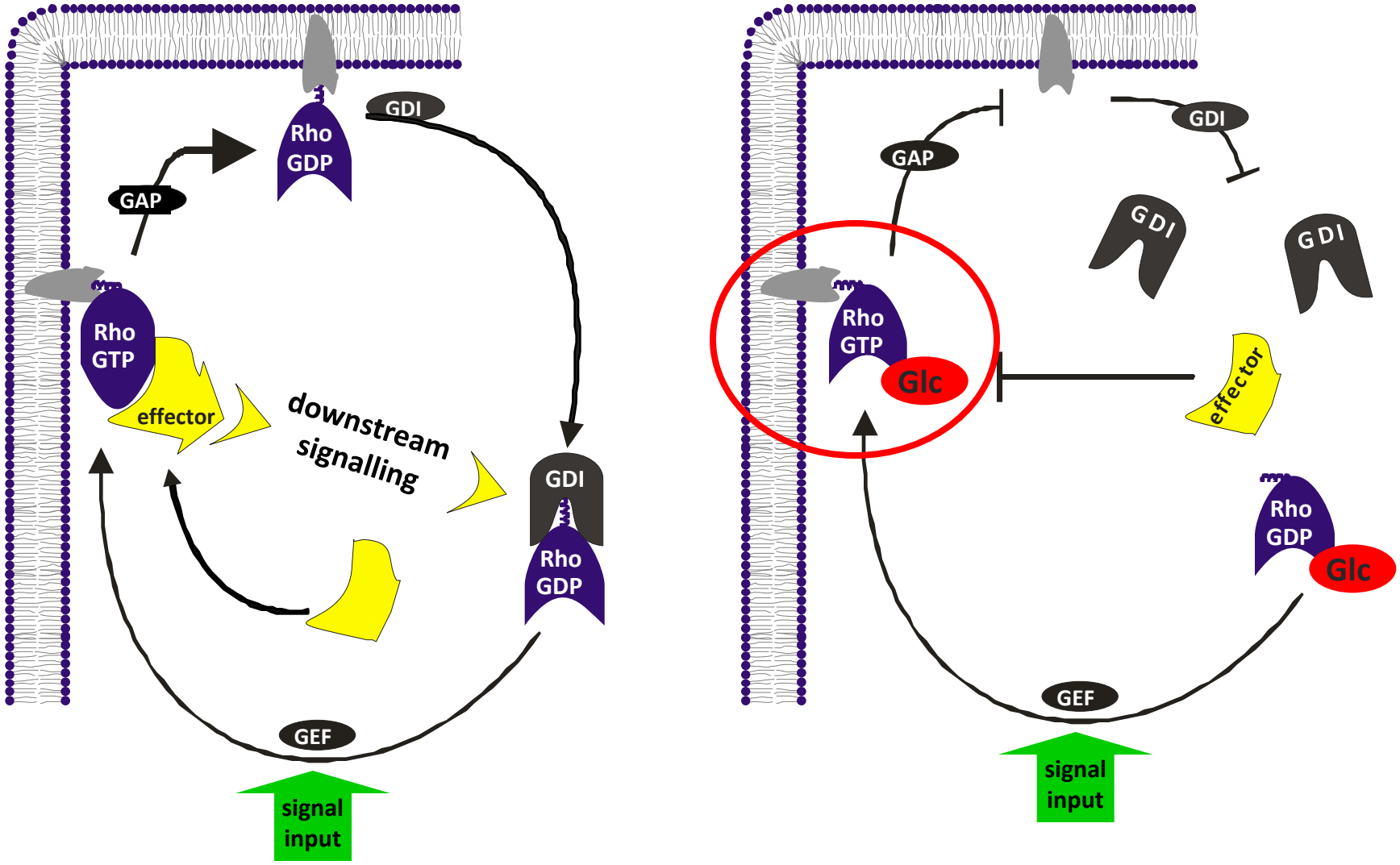




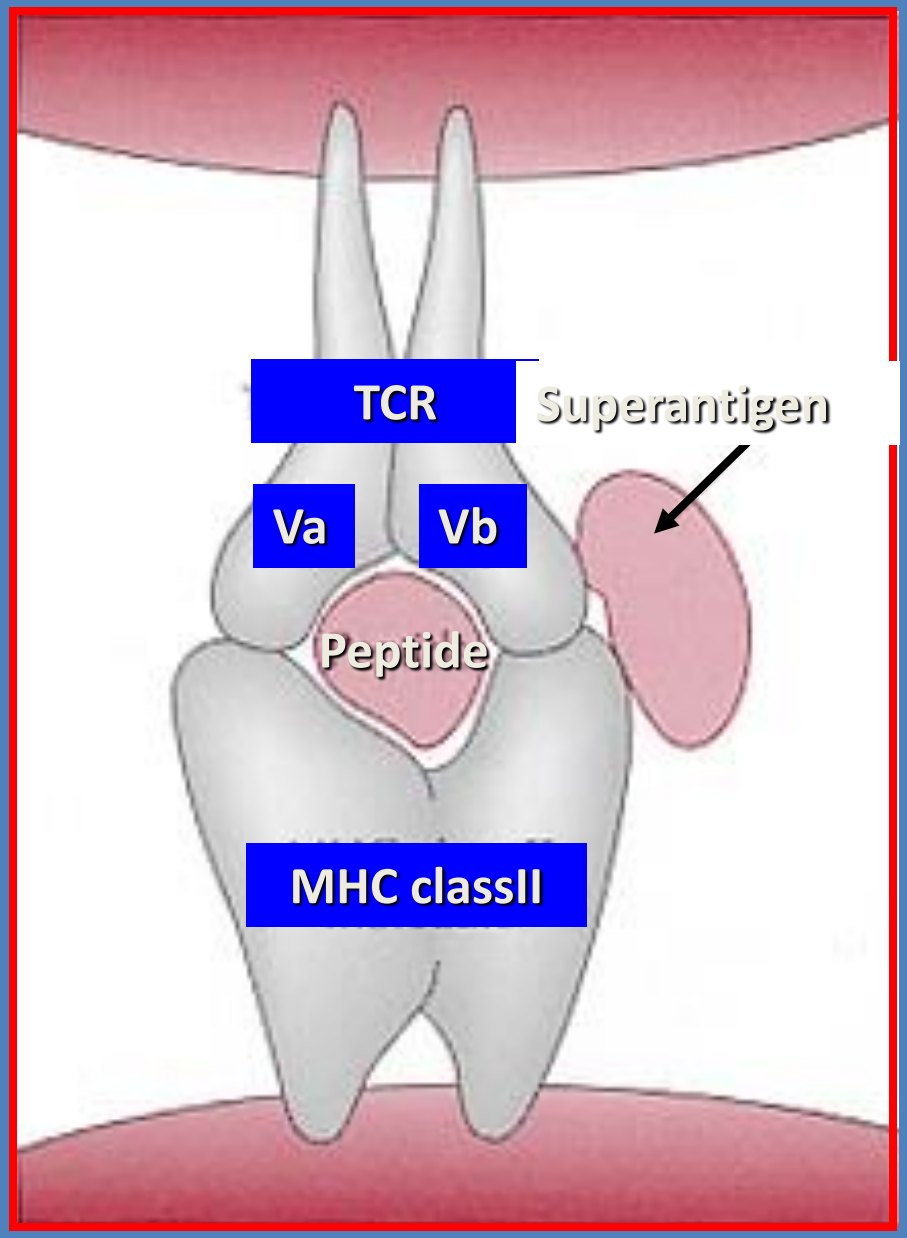
cellular mode of action



cellular mode of action



Superantigens



Superantigen		Disease
S.aureus exotoxins		
- Enterotoxins SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, SEA G-L		Food poisoning & TSS
- TSST-1		Toxic shock
- ETA, ETB	Exfoliatins	(SSSS)
S.pyogenes exotoxins		
- Erythrogenic toxins SPE A-C	Scarlet fever,	Toxic shock
- Exotoxines mitogènes SPEF, SSA, SPM, SPM-2, SMEZ, SPEG SPEH, SPEJ, SMEZ-2		Toxic shock
Y.pseudotuberculosis mitogen (YPM)		
C.perfringens enterotoxin		
M.arthritidis supernatant		

Results of superantigen activation of T cells

- Increased IL-2 and TNF-α production; edema; hypotension, multiorgan failure, rash; possible death
- Expansion of, followed by depletion of specific T cell populations
- T cell anergy

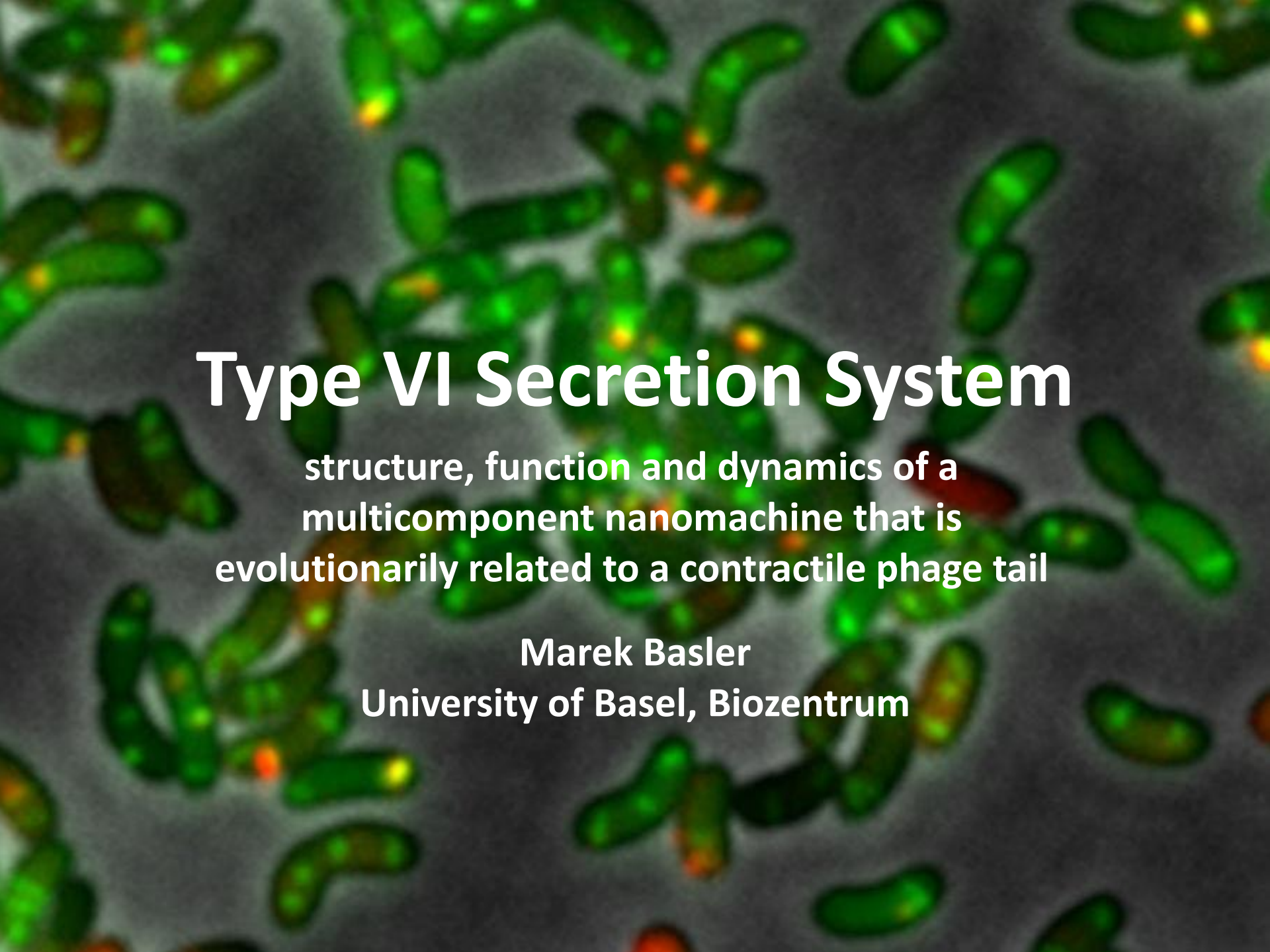
Staphylococcal Toxic Shock Syndrome

Age	Primarily 15-35 yrs
Sex	Greatest in women
Severe pain	Rare
Hypotension	100%
Erythroderma rash	Very common
Renal failure	Common
Tissue necrosis	Rare
Predisposing factors	Tampons, packing
Mortality	<3%

Streptococcal Superantigens

SpeA	Scarlet fever, shock, necrotising fasciitis
SpeB	Cellulitis, invasive infections, cysteine protease, SAg??
SpeC	Scarlet fever
SpeF	DNaseB, streptodornase, SAg??
SSA	

SpeG, SpeH, SpeJ, SMEZ identified from genome sequence

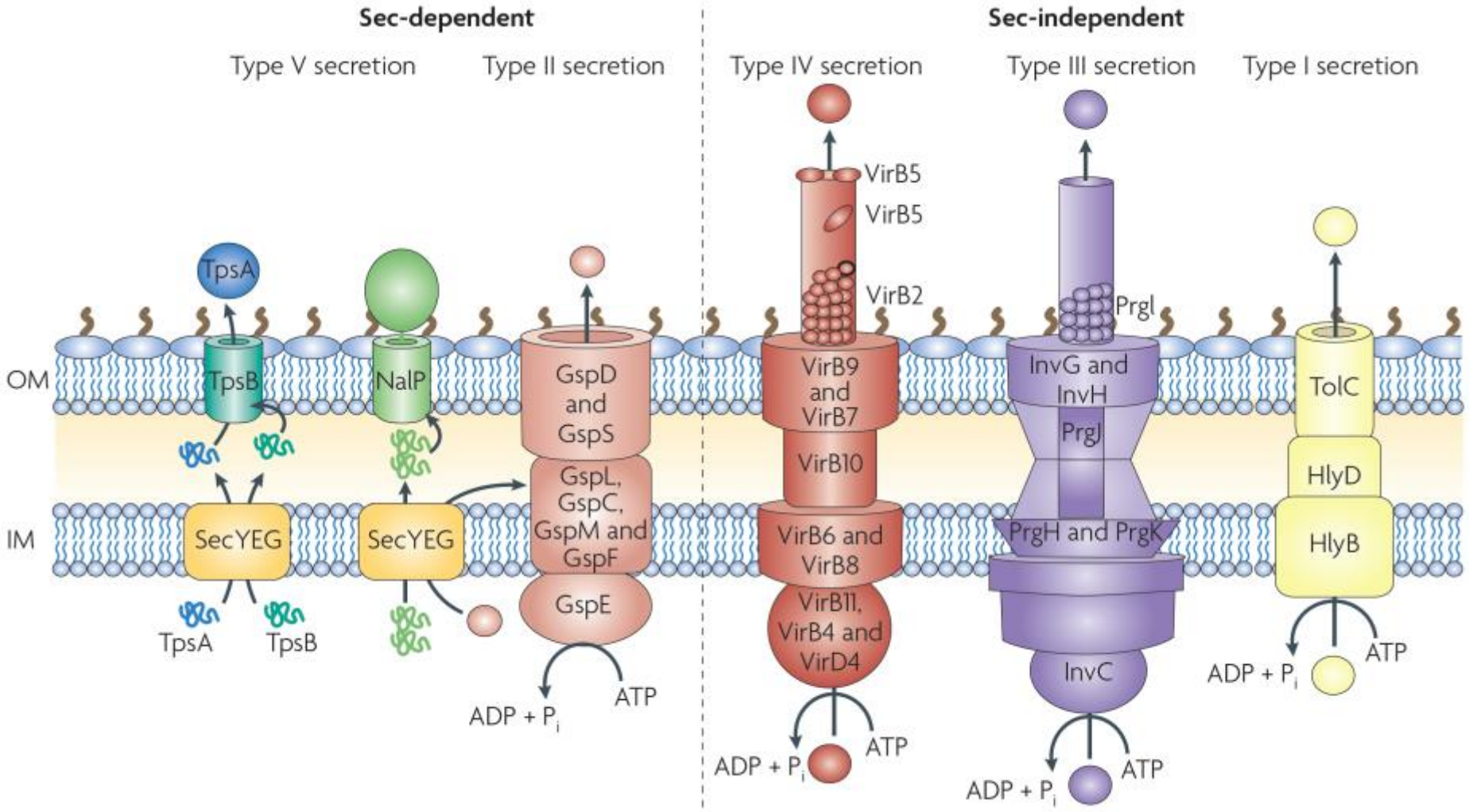


Type VI Secretion System

structure, function and dynamics of a multicomponent nanomachine that is evolutionarily related to a contractile phage tail

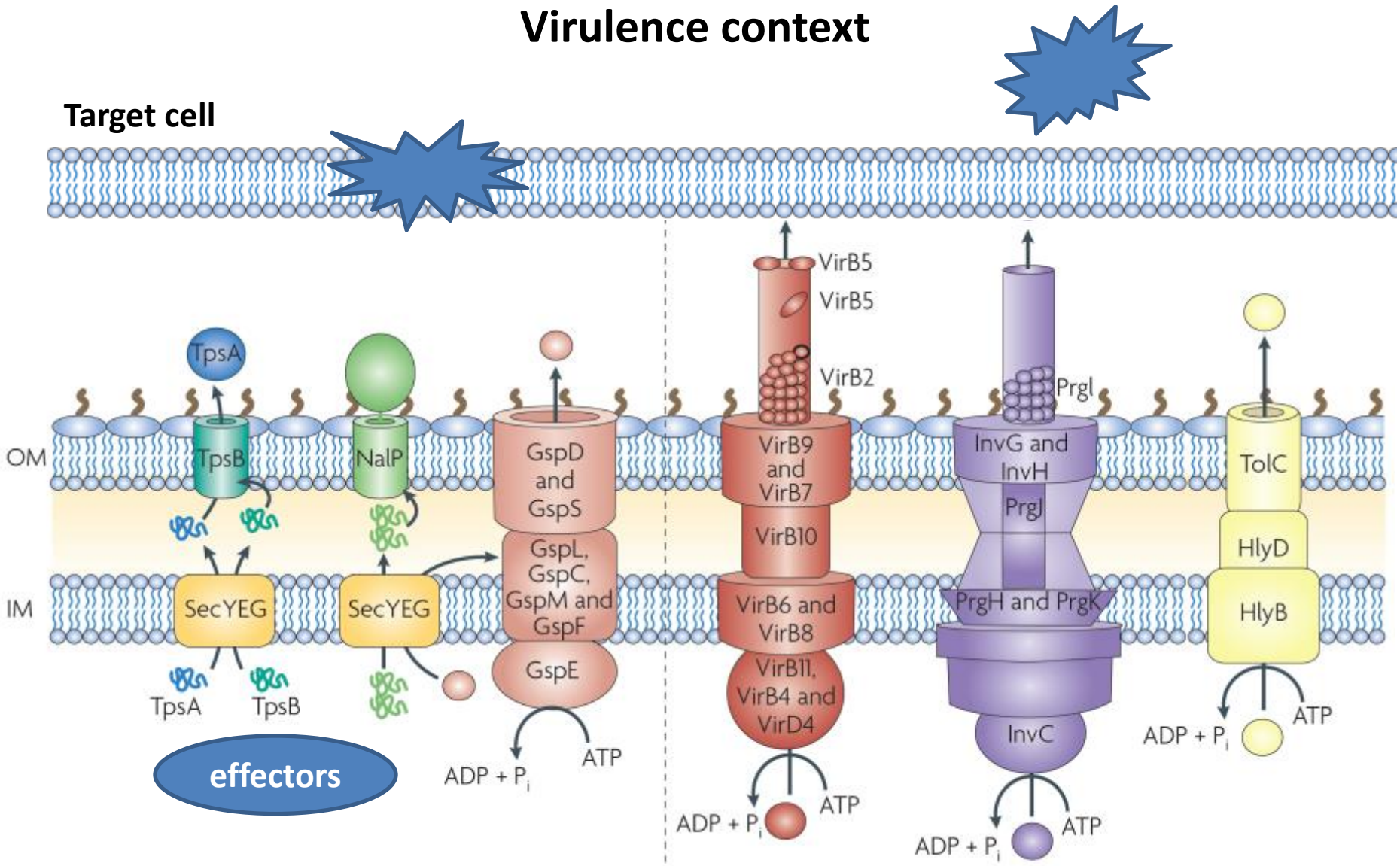
Marek Basler
University of Basel, Biozentrum

Secretion systems of G- bacteria



Fronzes et al., Nature Rev. Microbiol. 2009

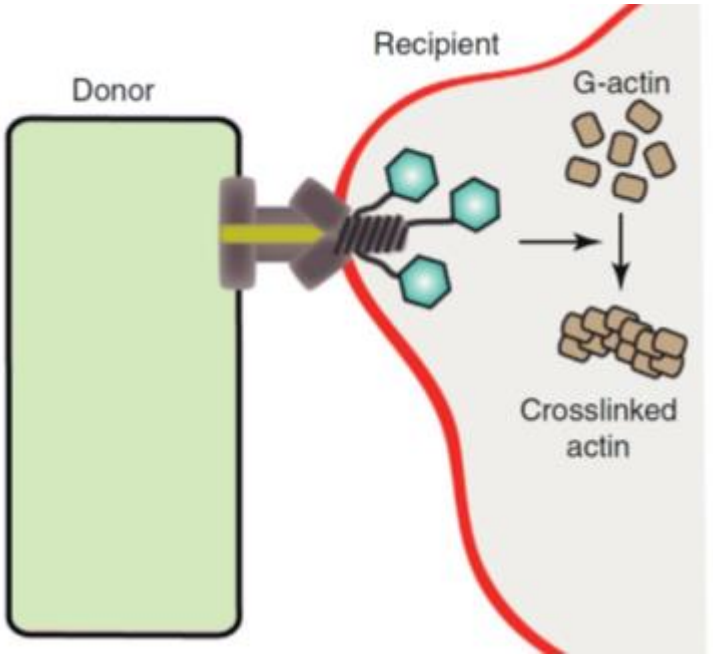
Secretion systems of G- bacteria



Fronzes et al., Nature Rev. Microbiol. 2009

T6SS is a virulence factor

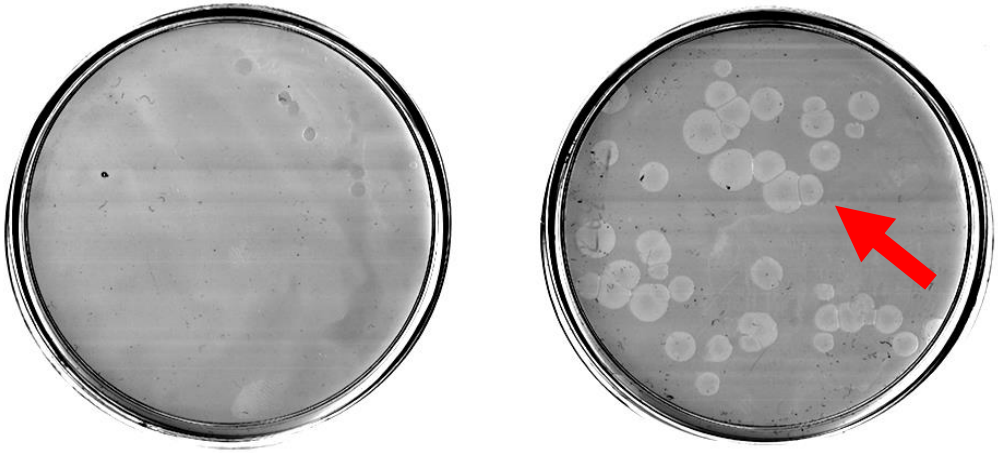
- *Dictyostelium* model to screen for novel virulence factors of *Vibrio cholerae*.
- Secretes **Hcp and three VgrG** proteins
- VgrG1-ACD effector delivered into target cells



Schwarz *et al.*, Trends 2010

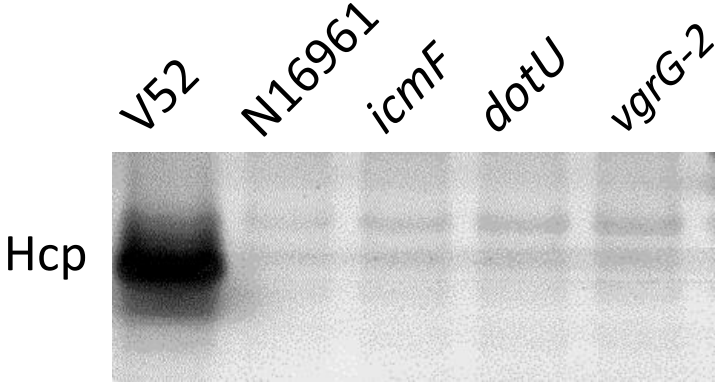
V52
virulent

N16961
avirulent



Pukatzki *et al.*, PNAS 2006

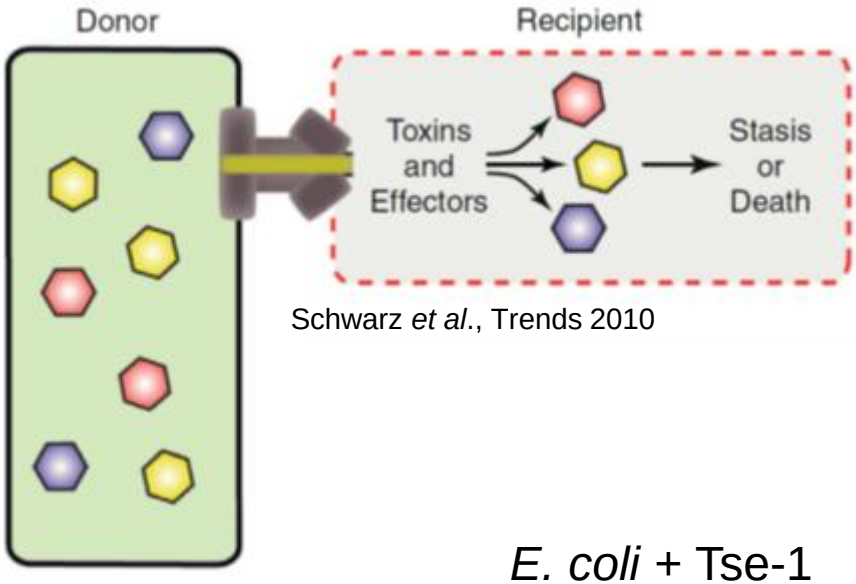
Culture supernatants analysis



T6SS targets bacterial cells

P. aeruginosa T6SS-1:

- secretes cell wall-targeting effectors
- inhibits bacteria

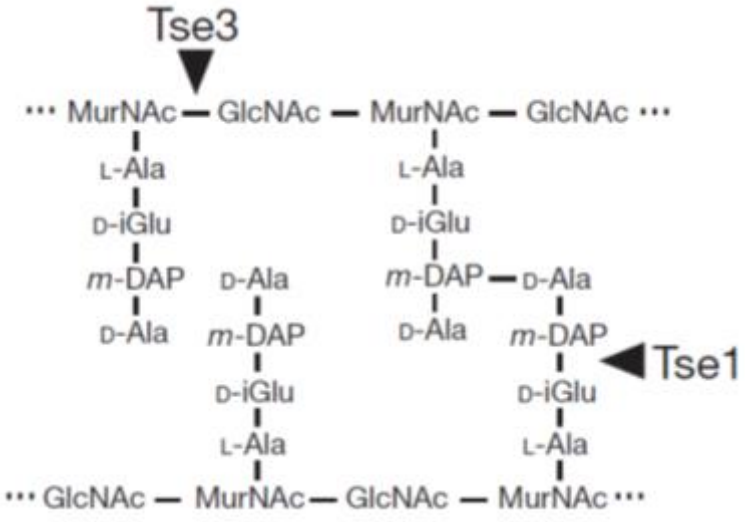


Schwarz *et al*., Trends 2010

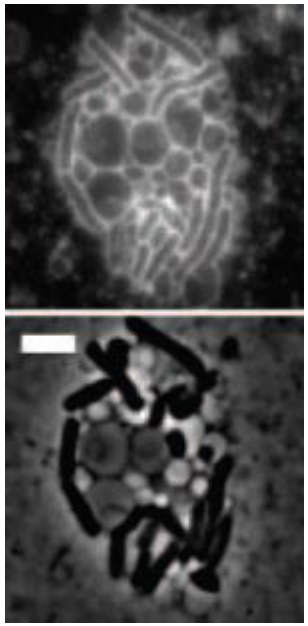
V. cholerae T6SS:

- kills *E. coli* quickly and efficiently
  MacIntyre *et al*., PNAS 2010

- secretes lipase
- lysozyme activity of VgrG3
  Dong *et al*, PNAS 2013



E. coli + Tse-1



Hood *et al*., Cell Host & Microbe 2010, Russell *et al*., Nature 2011

T6SS is present in ~25% of all G- bacteria

Pseudomonas

Vibrio

Escherichia coli

Acinetobacter

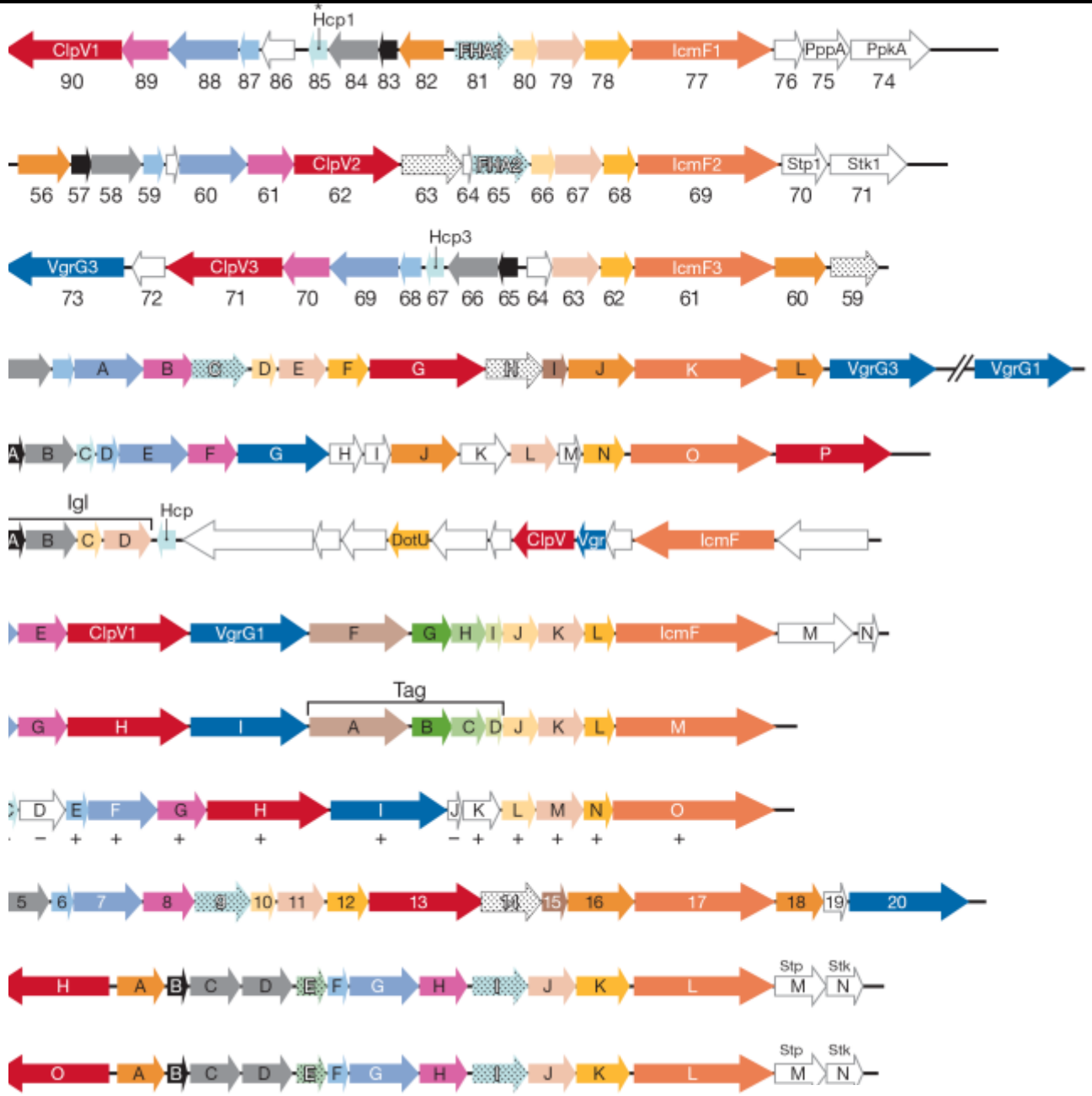
Burkholderia

Klebsiella

Photorhabdus

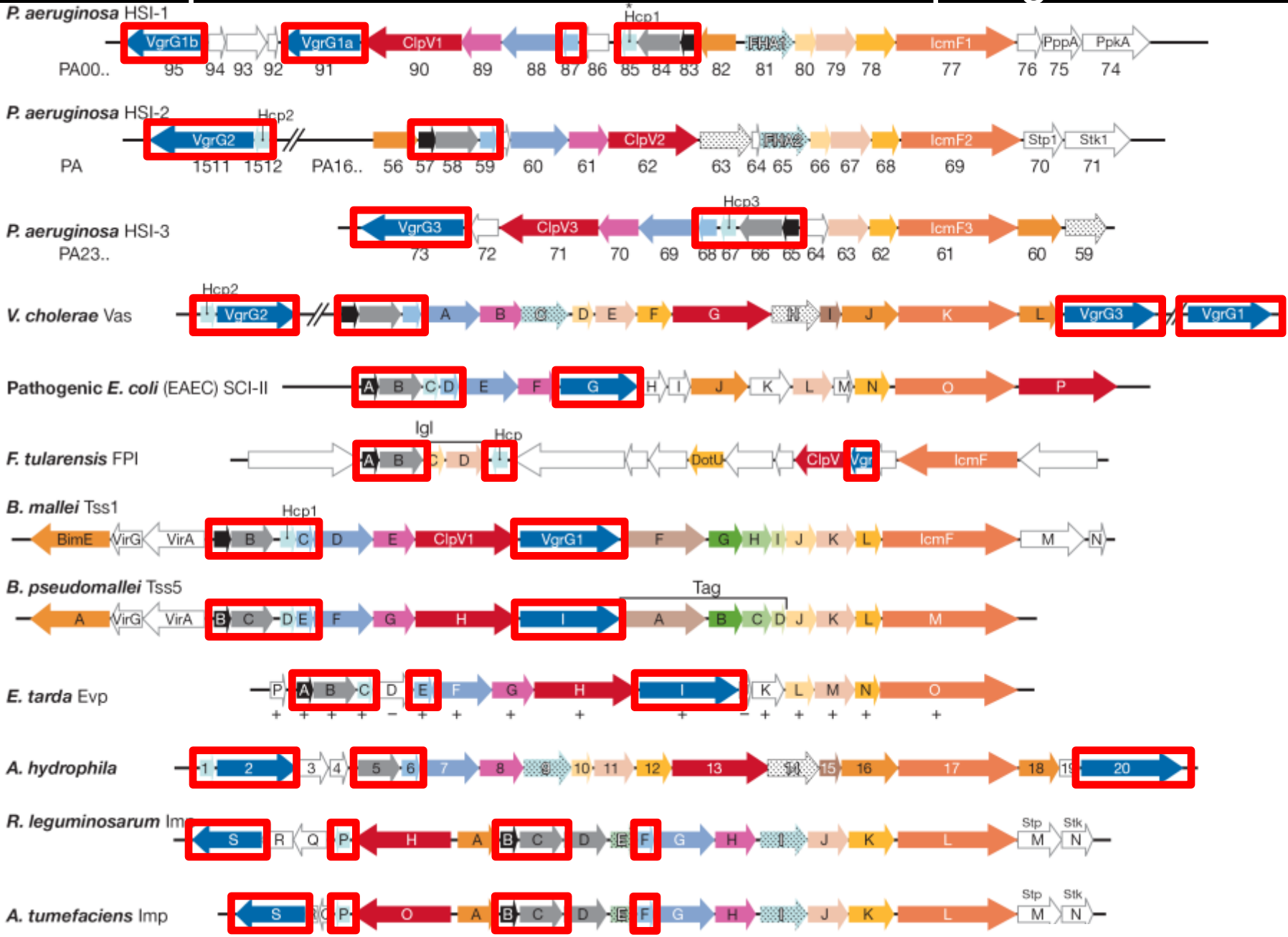
Salmonella

Yersinia



Cascales E. EMBO 2008

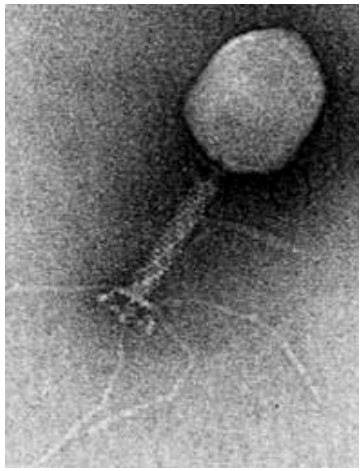
Components of T6SS are related to phage tail



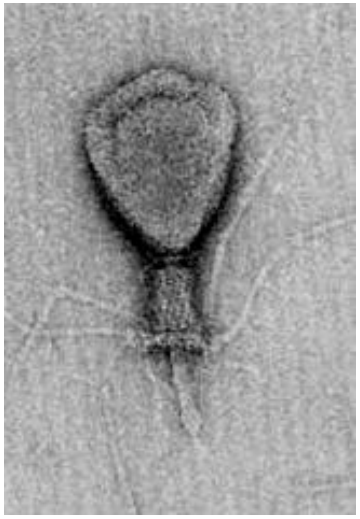
Cascales E. EMBO 2008

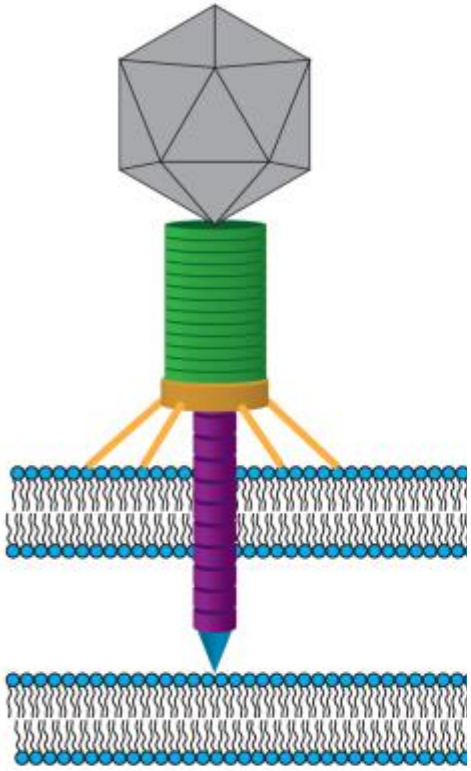
Simple model of T6SS based on phage homology

T4
extended tail



contracted tail

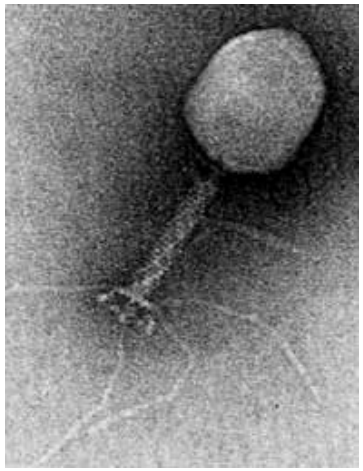




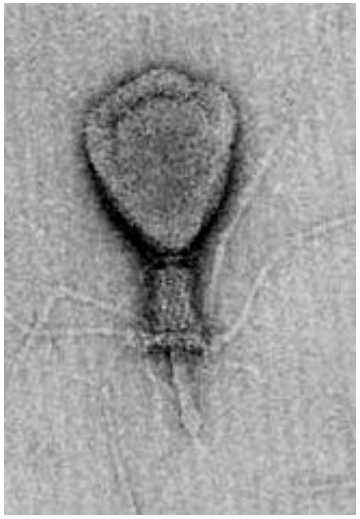
Protein translocation by sheath contraction

Simple model of T6SS based on phage homology

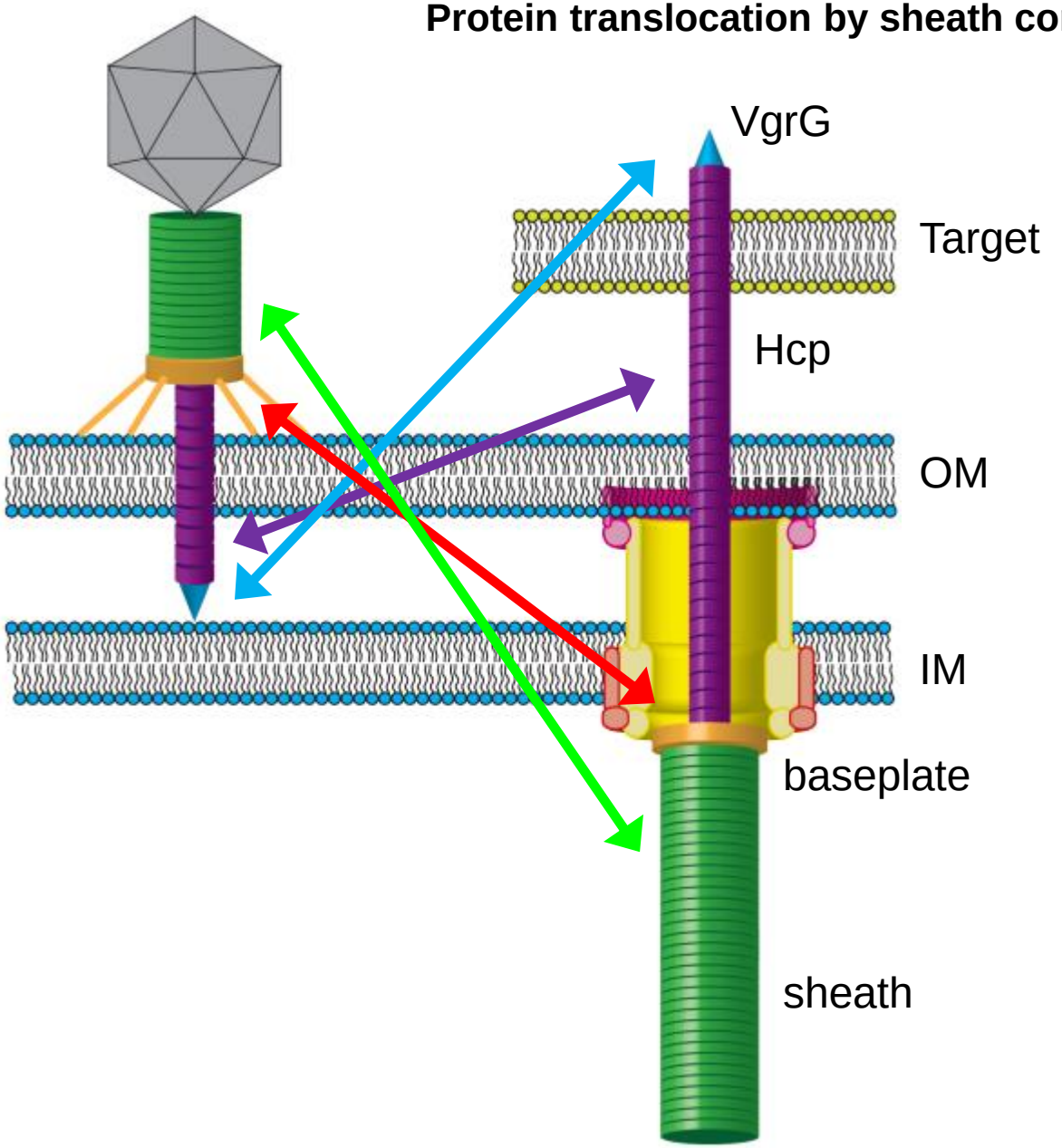
T4
extended tail



contracted tail

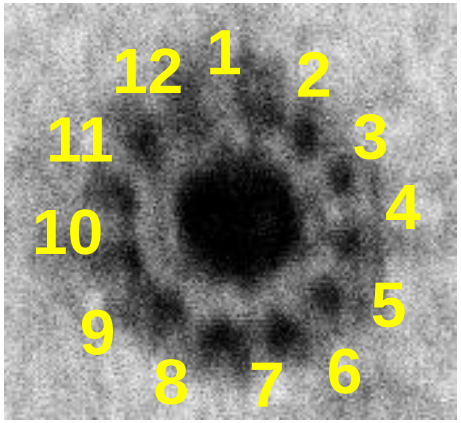


Protein translocation by sheath contraction

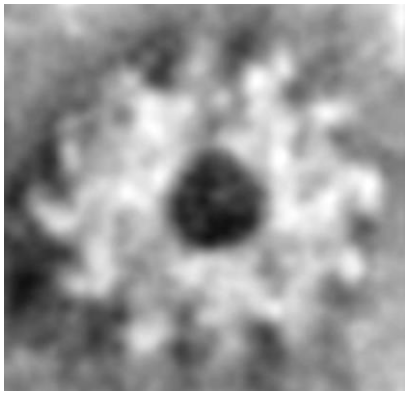


Purification of VipA/VipB sheath-like structures from *V. cholerae*

T6SS sheath

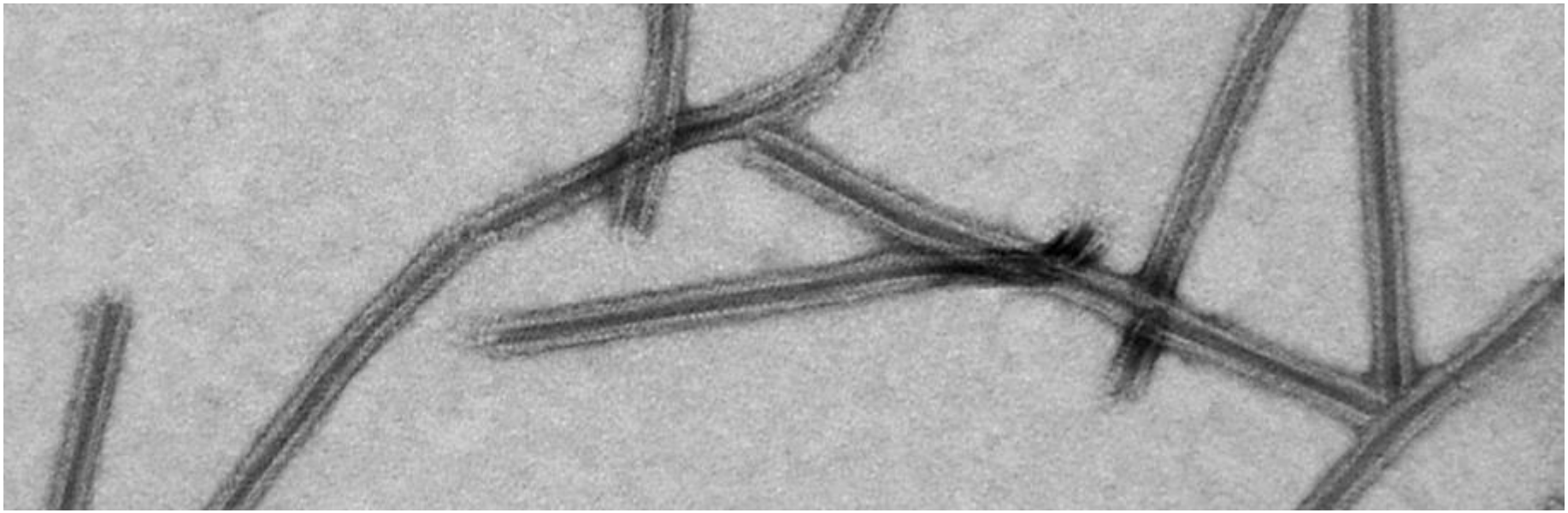


T4 sheath



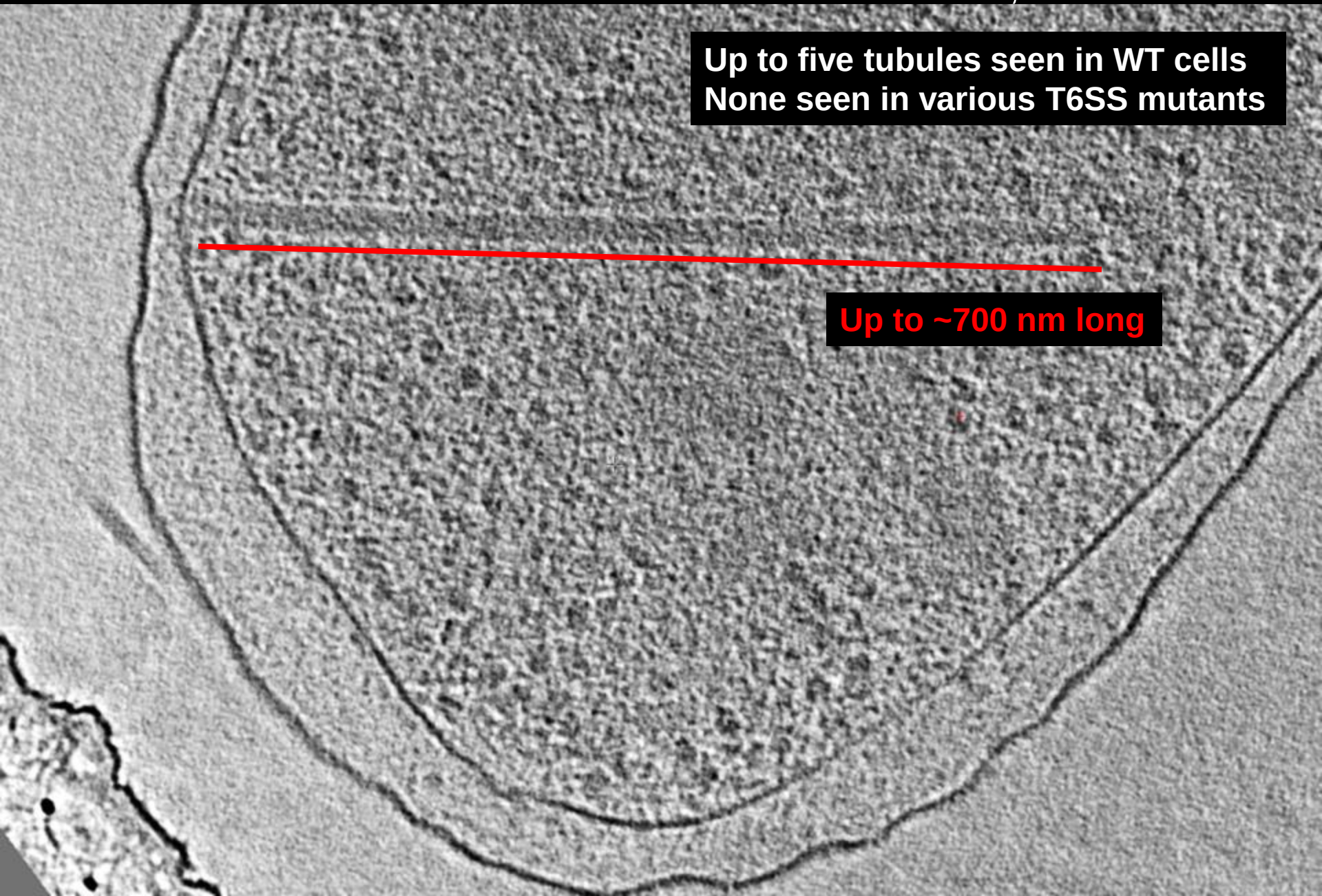
Moody M.F., JMolBiol 1967

~25nm wide, 10 nm inner diameter
up to 500nm long!



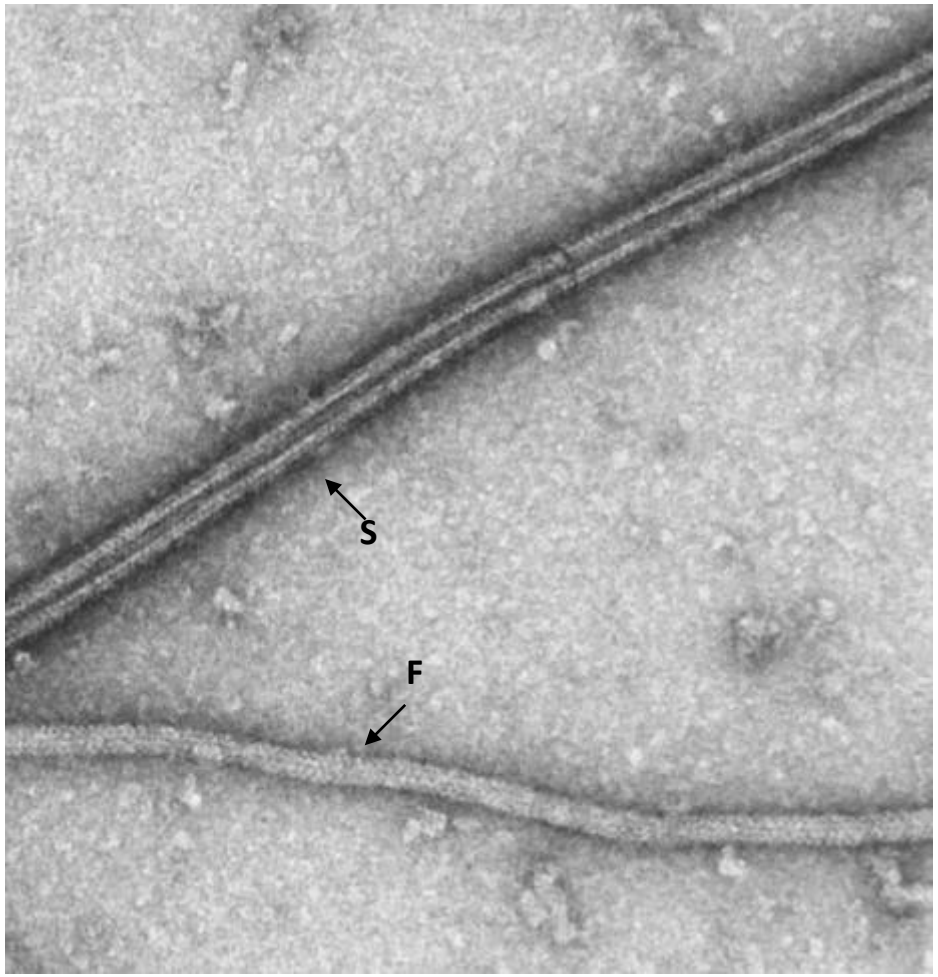
Electron Cryo Tomography (ECT) of T6S+ *V. cholerae*

Collaboration with Martin Pilhofer and Grant Jensen, Caltech

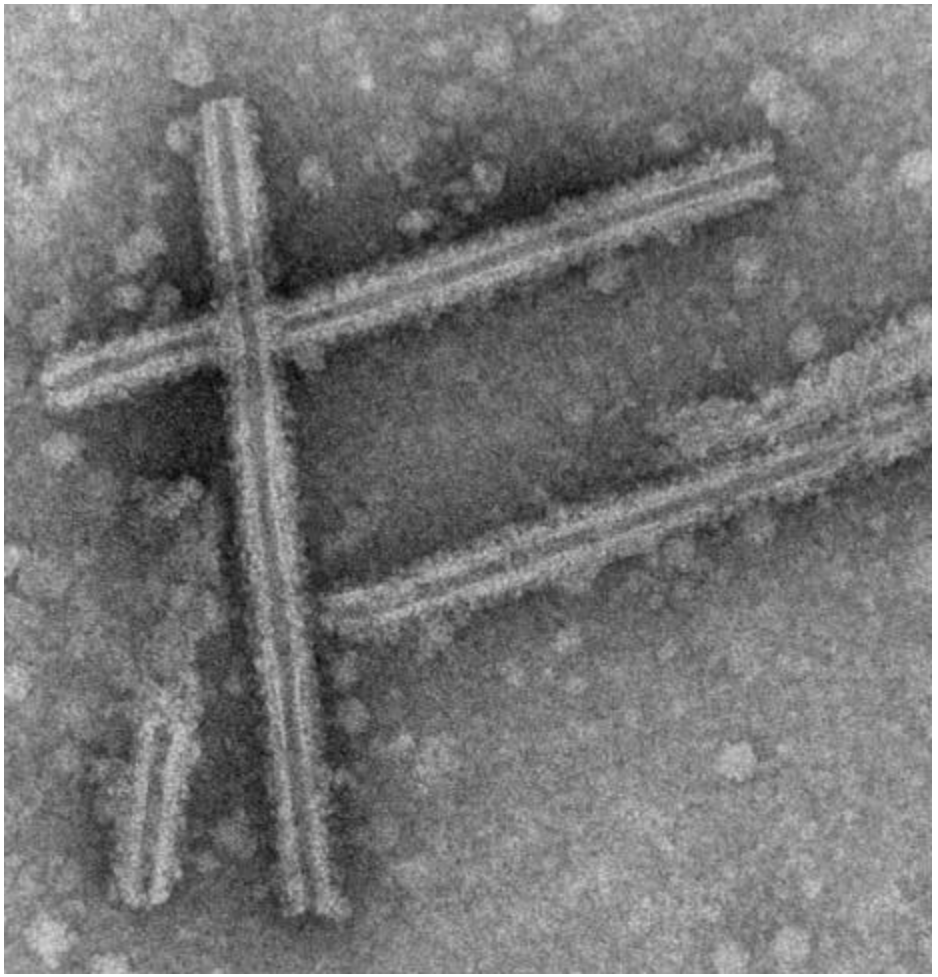


VipA-sfGFP assembles into a long sheath

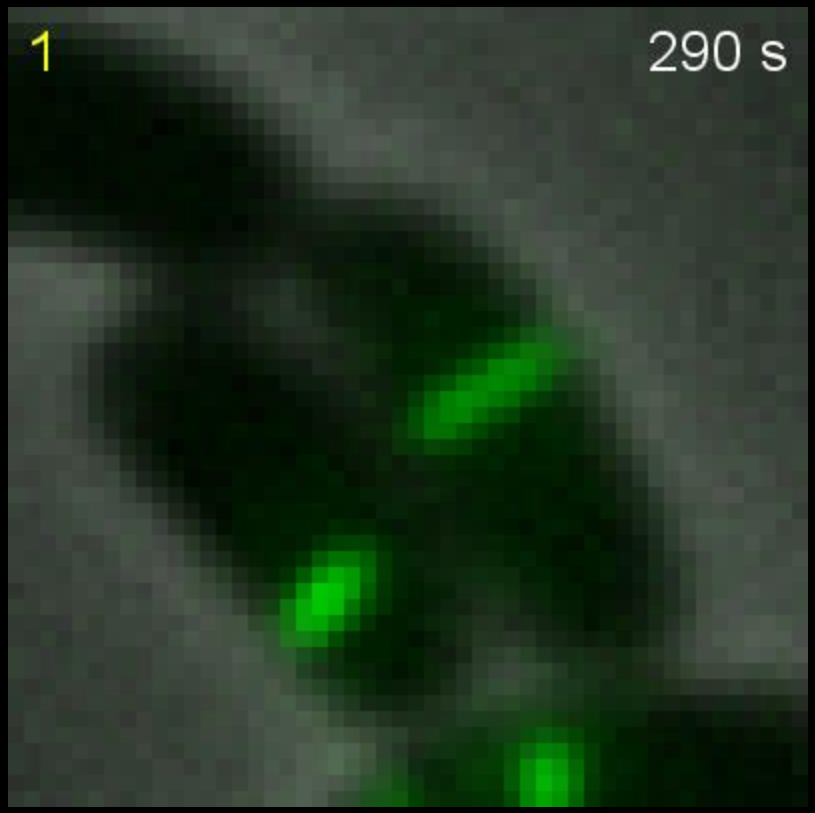
WT



ΔVipA + VipA-sfGFP



Dynamics of T6SS sheath in *V. cholerae*

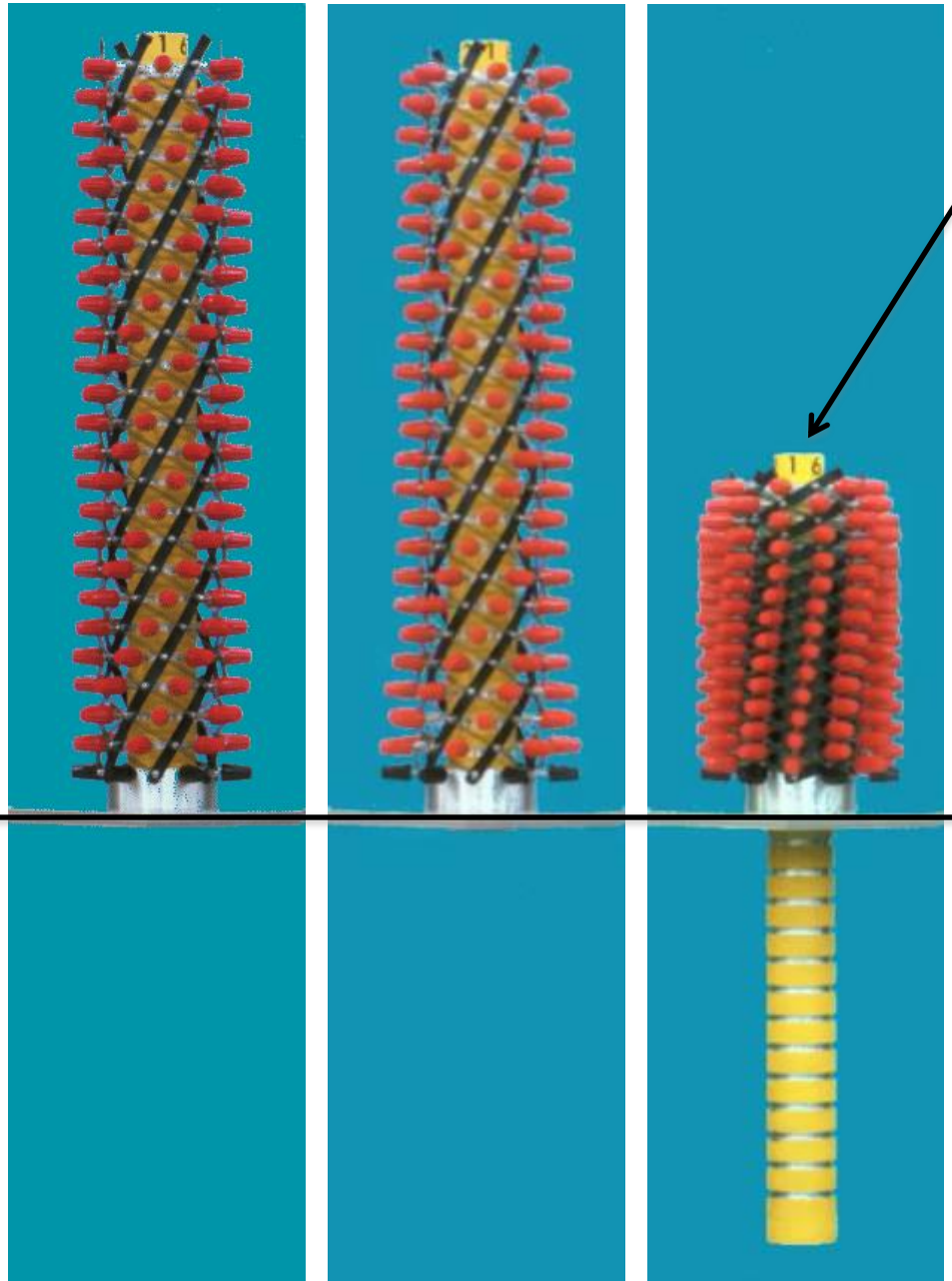


V. cholerae **VipA-sfGFP, 10s/frame, 50x speed**

- Polymerizes from the membrane out in ~30s
- Contracts to ~50% in less than 5ms!
- Disassembles in ~30s
- Whole cycle restarts at apparently random location

Basler *et al.*, Nature 2012

T4 phage tail sheath contraction



Inside the T6SS+ cell

Outside the T6SS+ cell

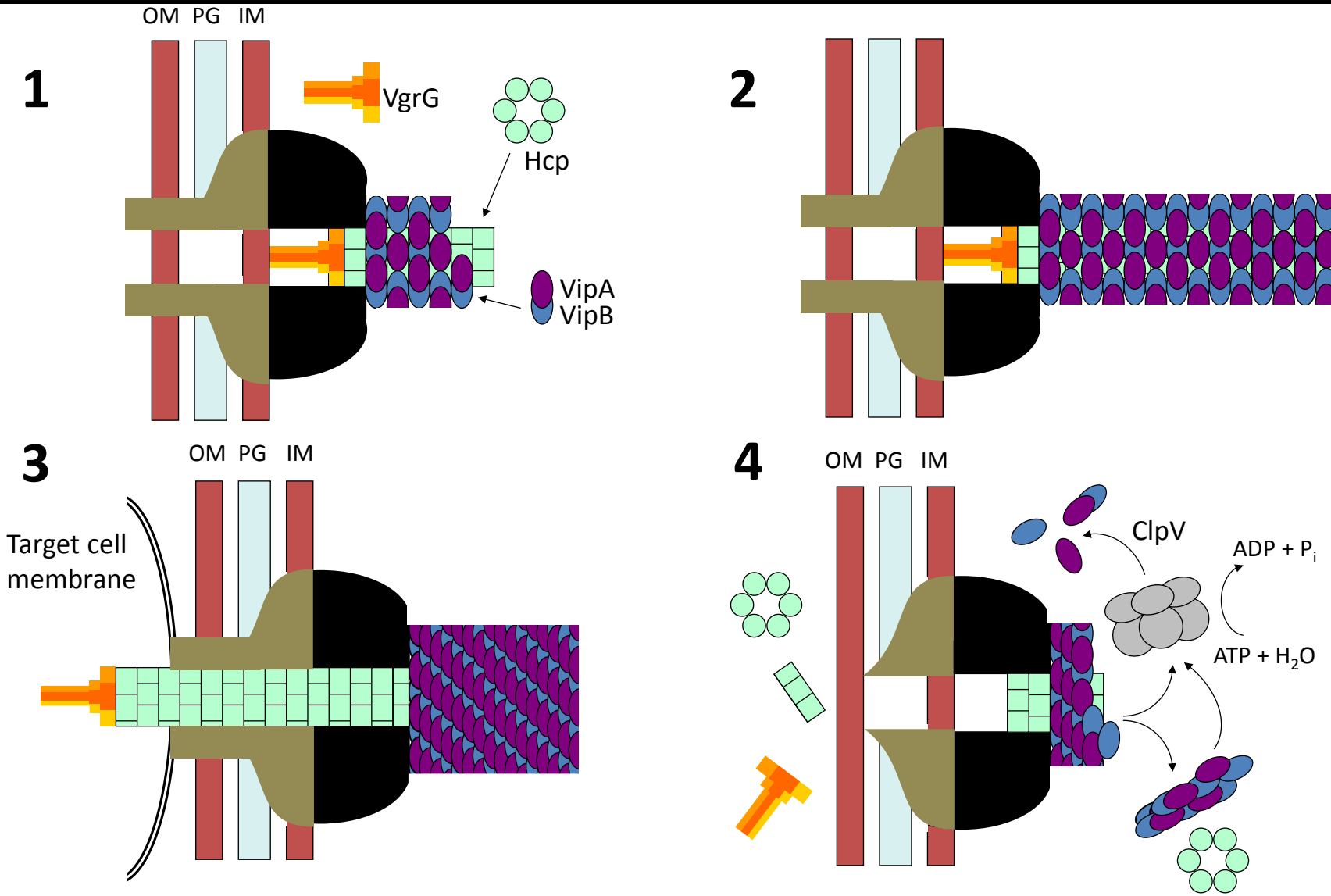
One full turn of Hcp tube per 100 nm length of uncontracted sheath, full sheath could be 5-10 turns per less than 5ms!

Red - VipA/VipB helical sheath

Yellow - Hcp tube

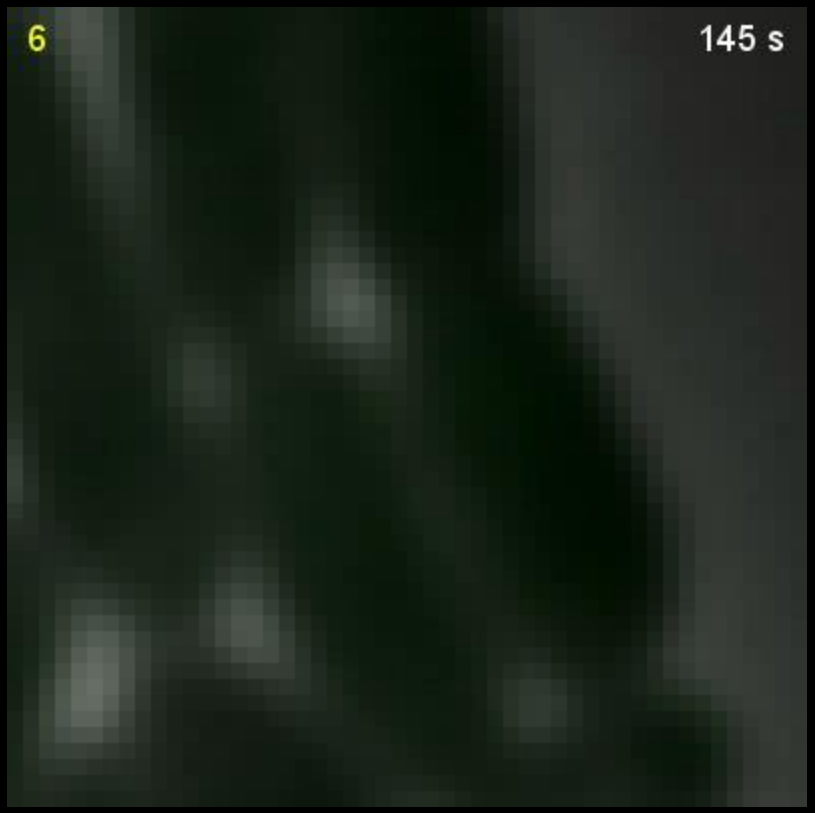
Animation by Don Casper

Model of T6SS dynamics



Basler *et al.*, Nature 2012

Cells respond to T6SS activity in a neighboring cell



P. aeruginosa **ClpV1-GFP - 5s/frame, 50x speed**

What is the signal for "dueling"?

Can heterologous organisms trigger the dueling response?

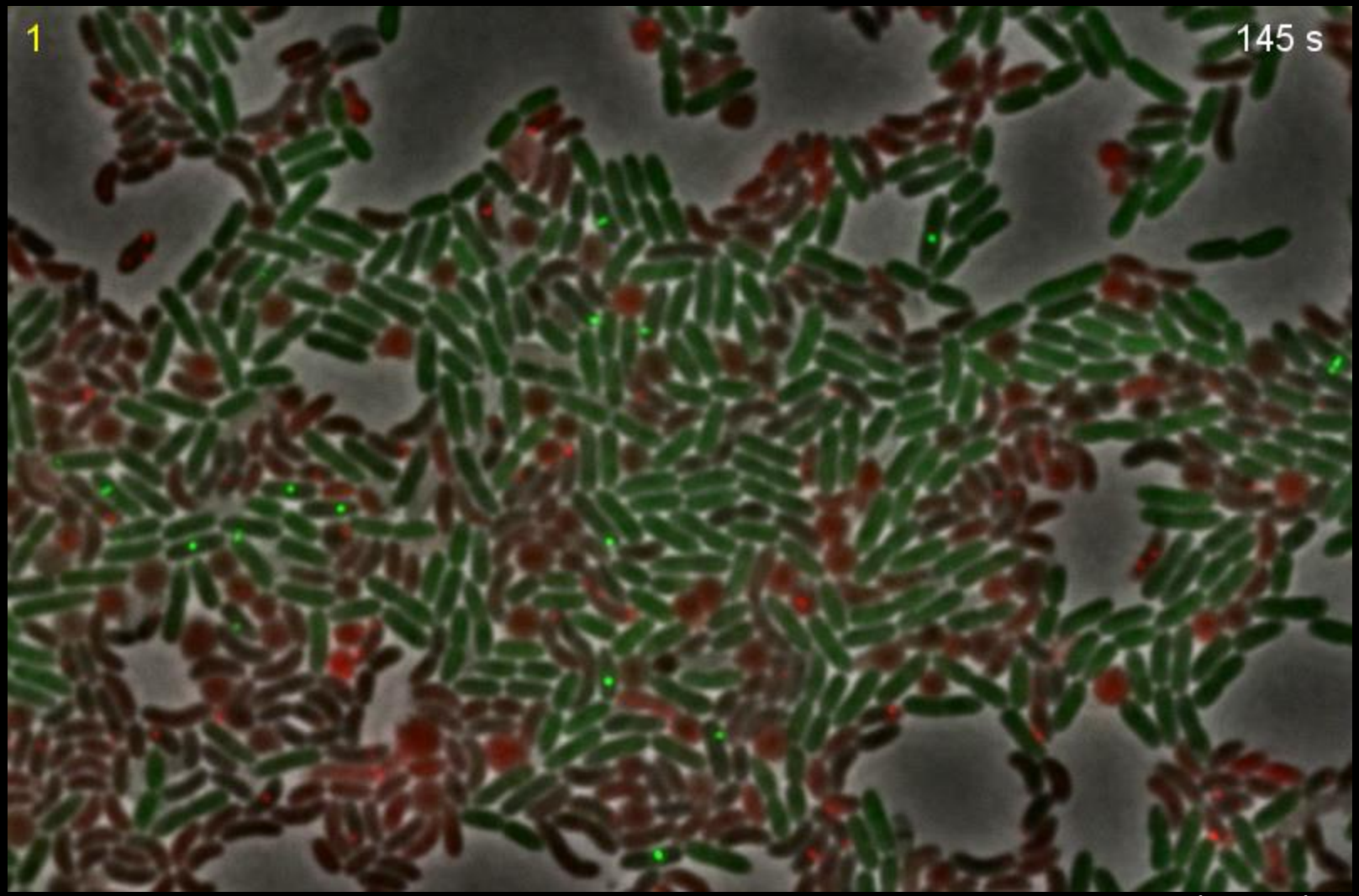
Basler and Mekalanos, Science 2012

P. aeruginosa delivers cell wall-targeting effectors to *V. cholerae*

Delivery is contact dependent

P. aeruginosa ClpV

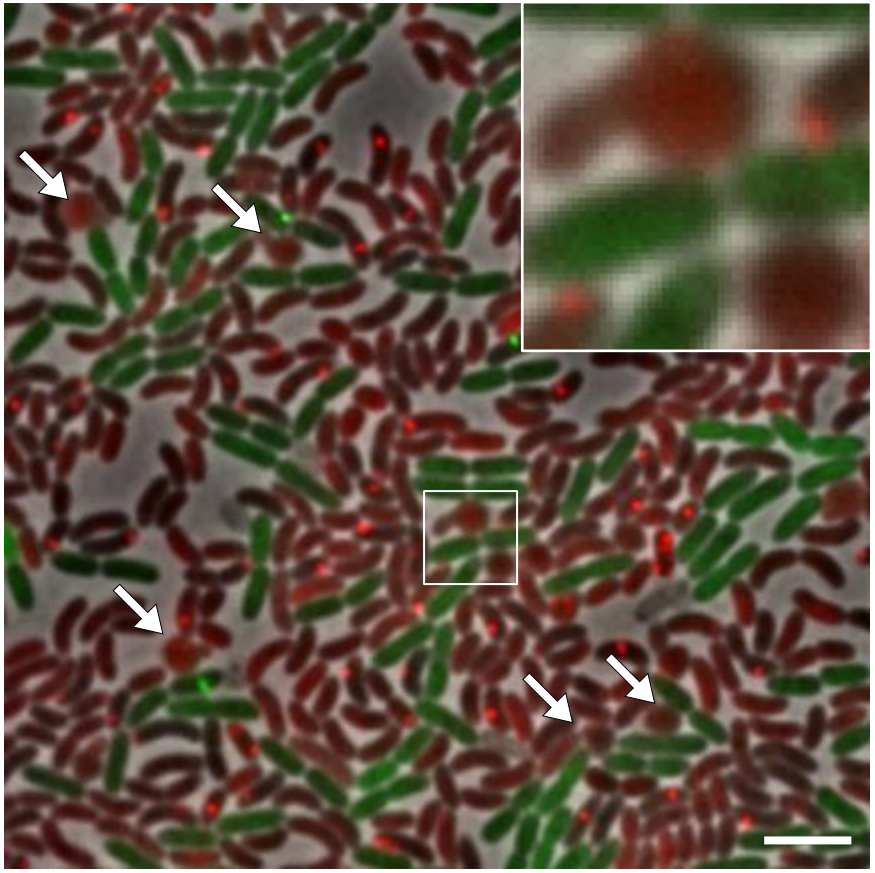
V. cholerae ClpV



Basler *et al.*, Cell 2013

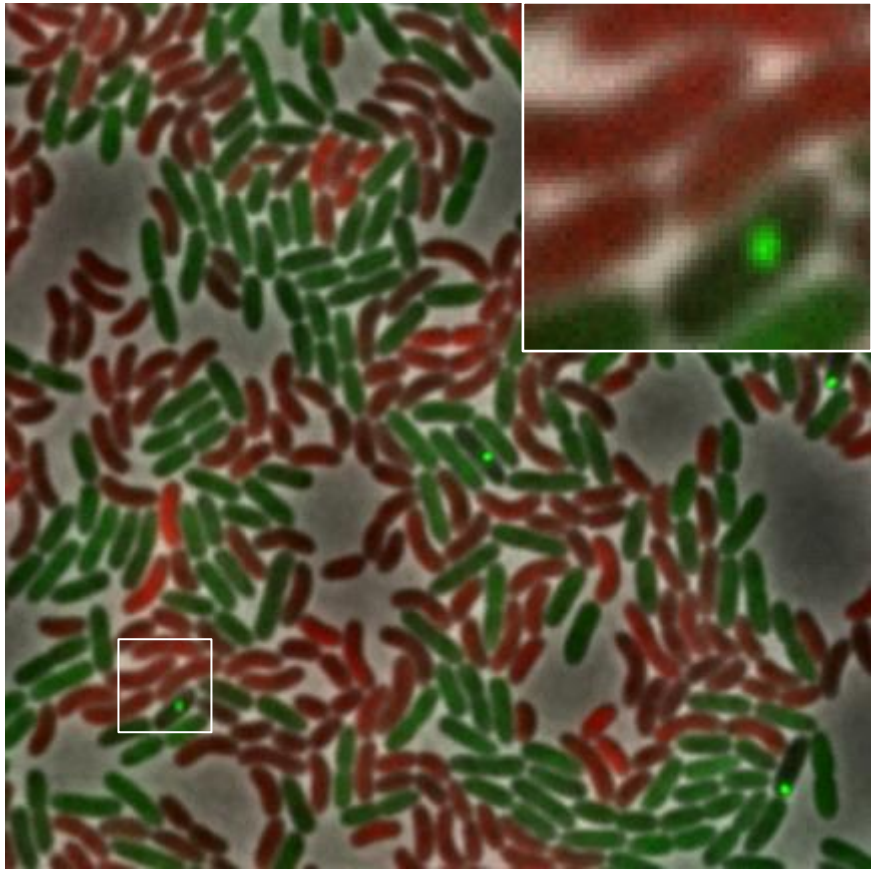
Pseudomonas targets only T6SS+ *Vibrio*

V. cholerae T6SS+



30 ± 14 round cells/field (n = 60 fields)

V. cholerae T6SS-

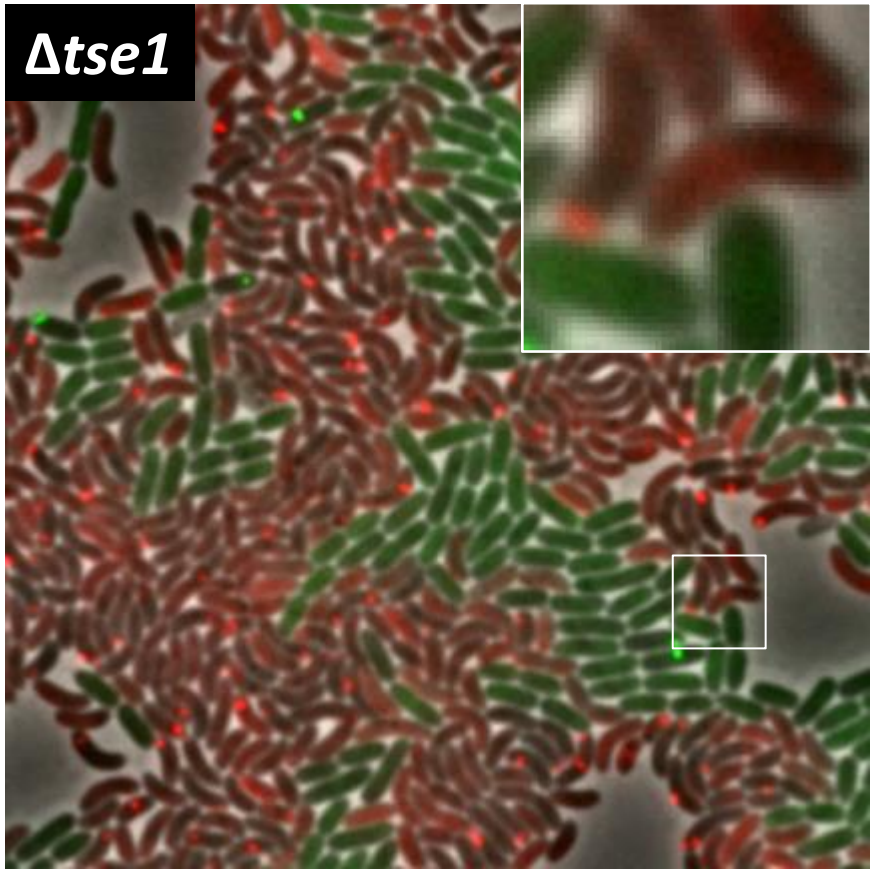


1.1 ± 1.2 (n = 30, p-val < 10^{-22})

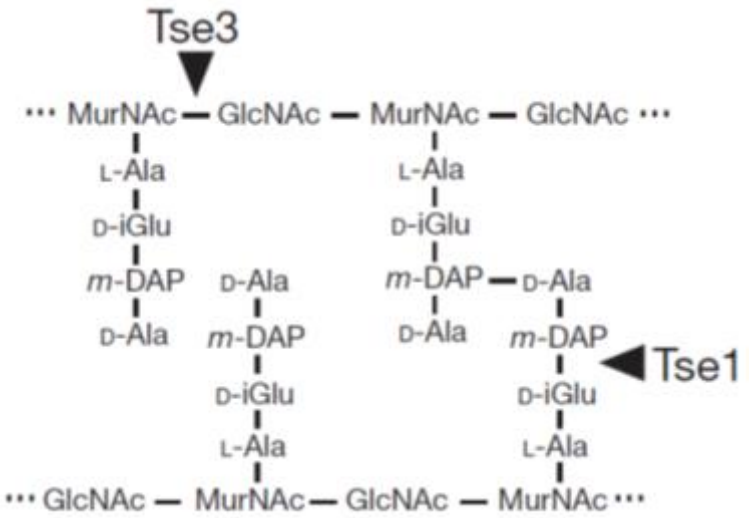
Pseudomonas Tse1 effector is responsible for rounding

P. aeruginosa T6SS-1:

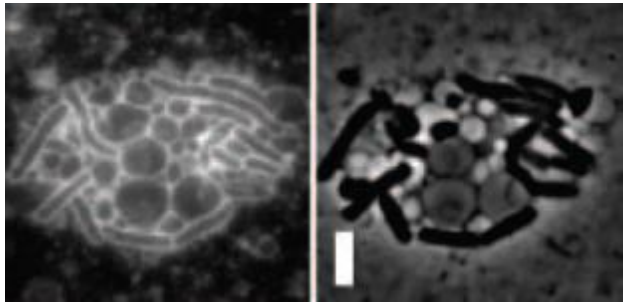
- secretes cell wall-targeting effectors
- inhibits bacteria



0.03 ± 0.18 (n = 30, p-val < 10^{-22})

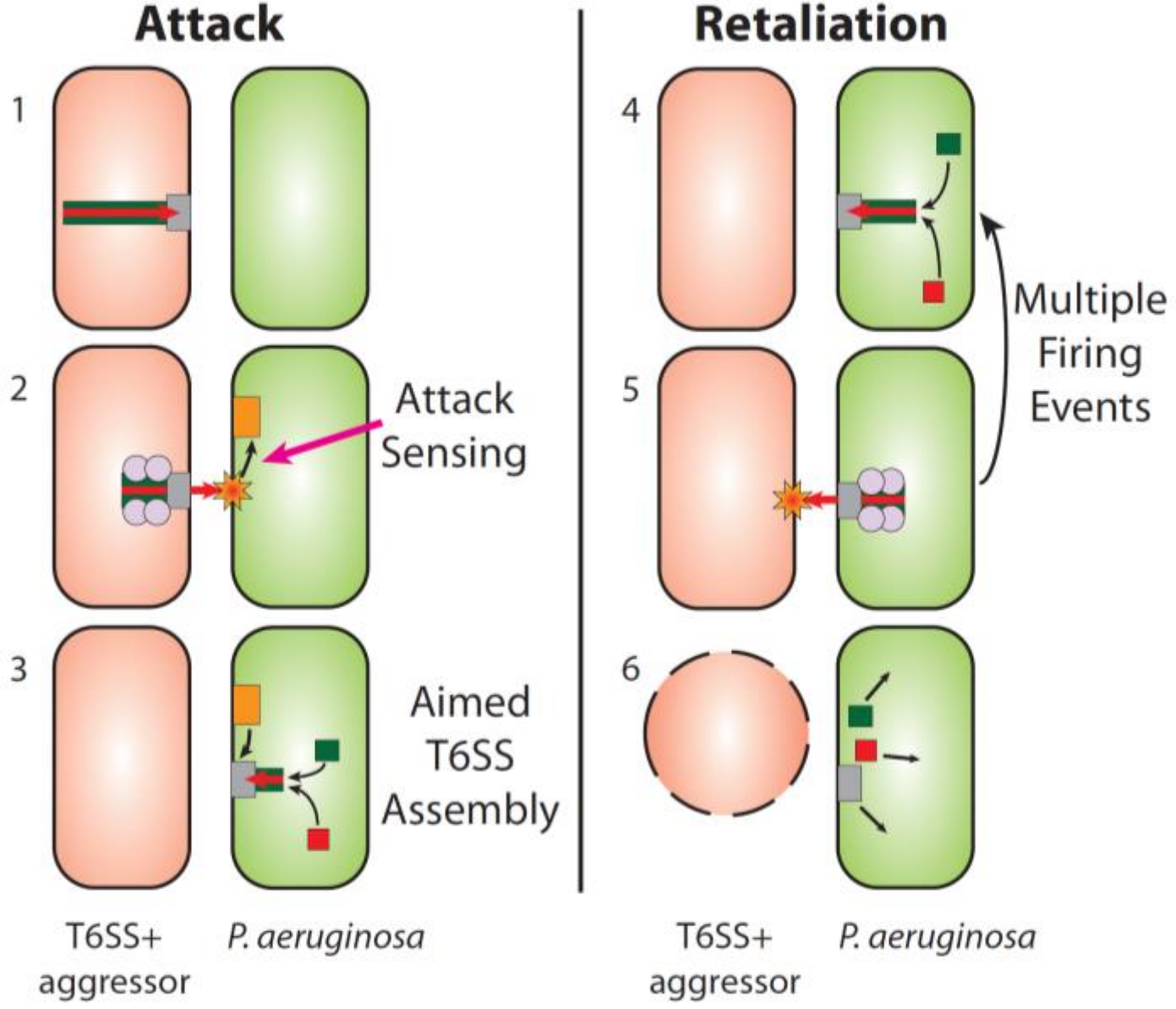


E. coli + Tse-1

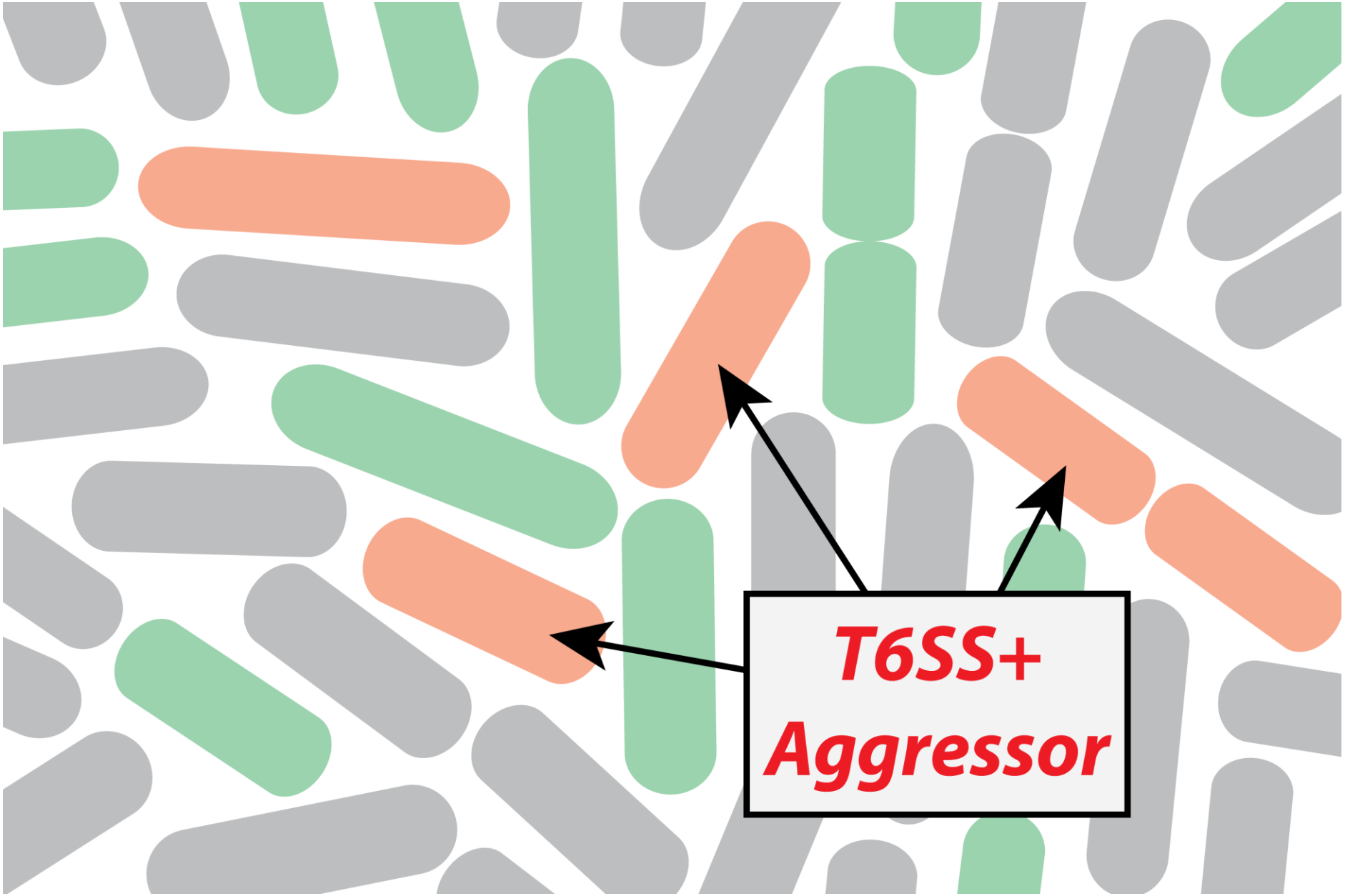


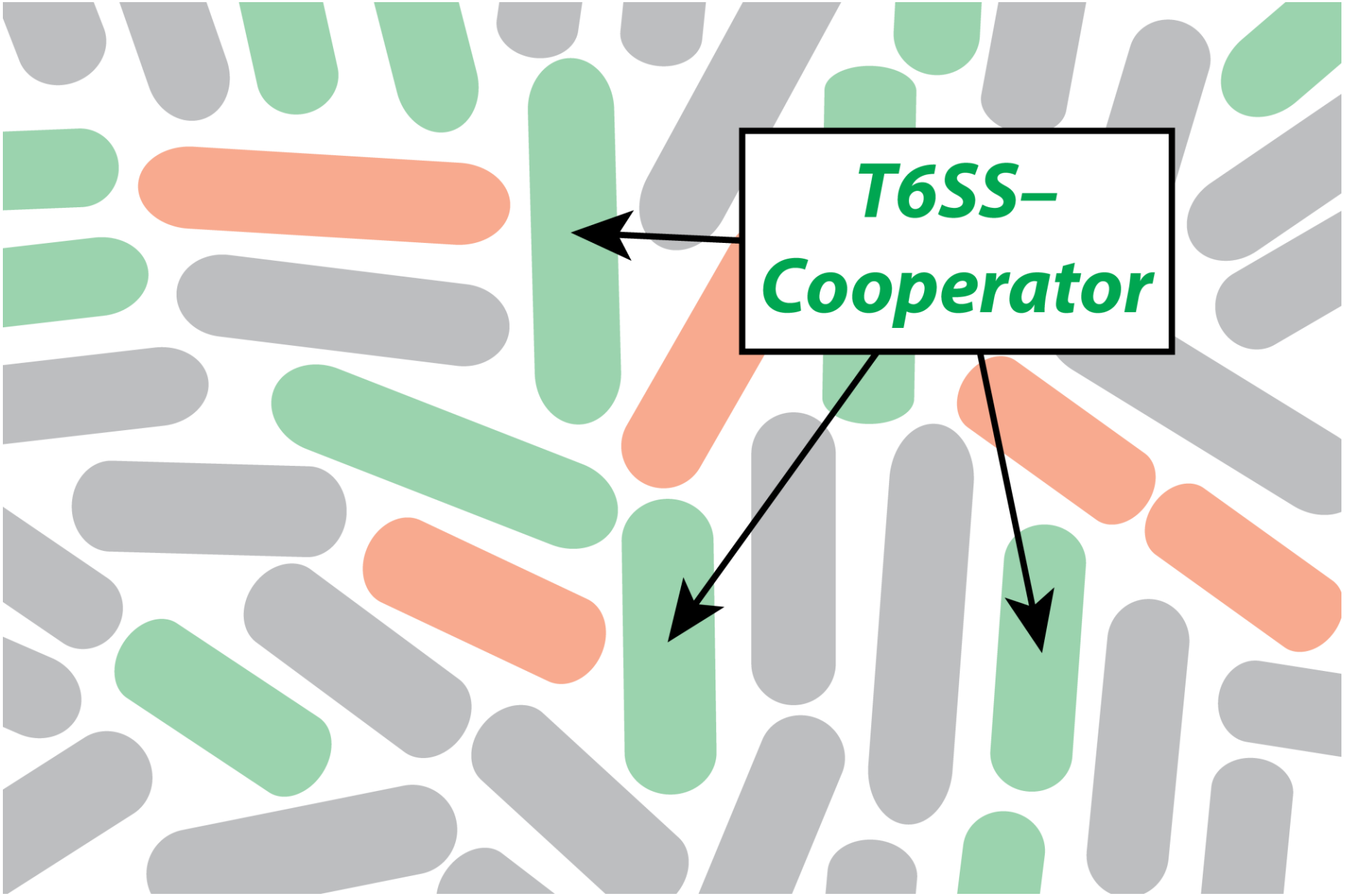
Hood *et al*., Cell Host & Microbe 2010,
Russell *et al*., Nature 2011

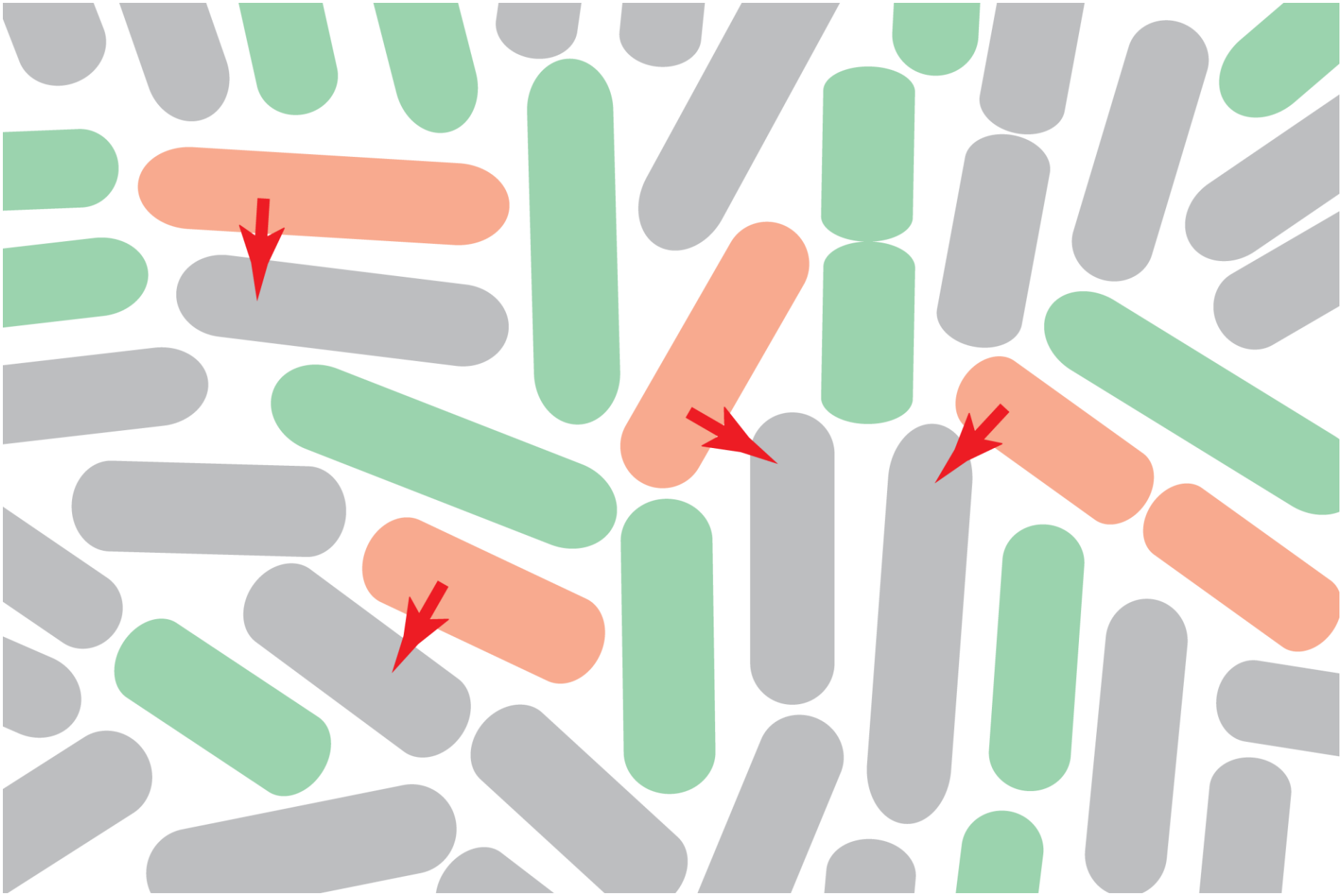
Tit-for-tat: *P. aeruginosa* kills in self defense

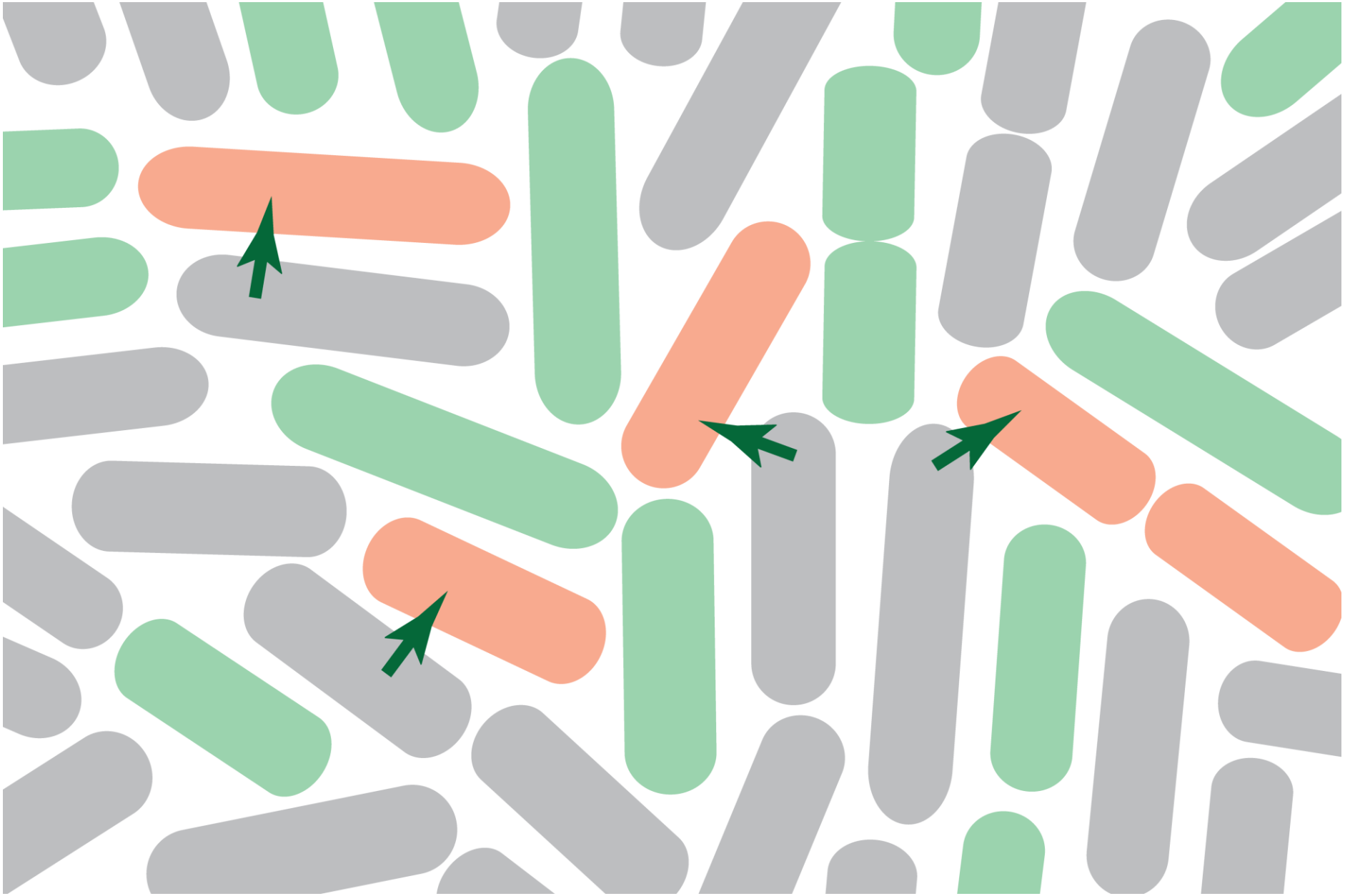


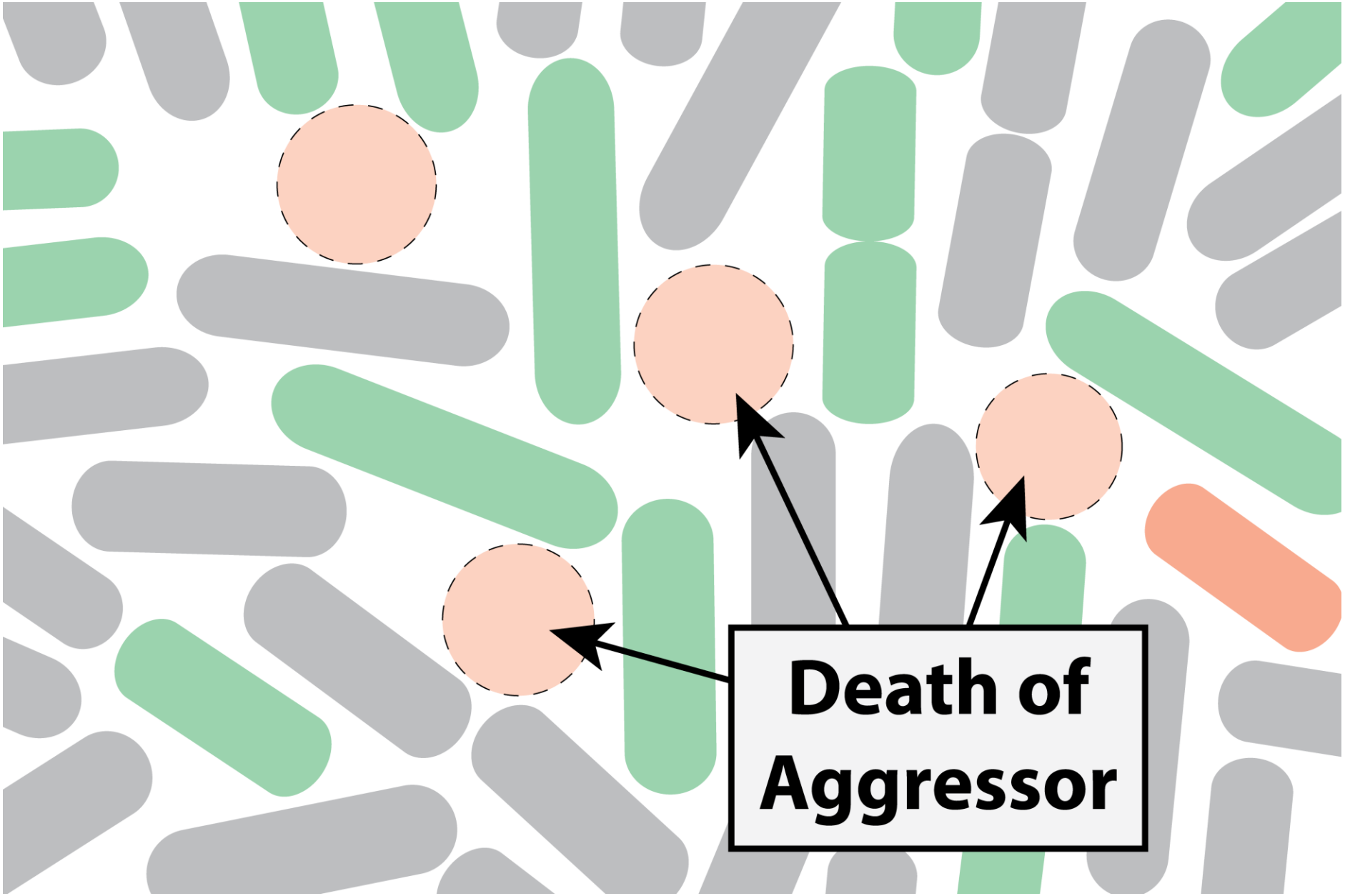
Basler *et al.*, Cell 2013


T6SS+
Aggressor


T6SS–
Cooperator





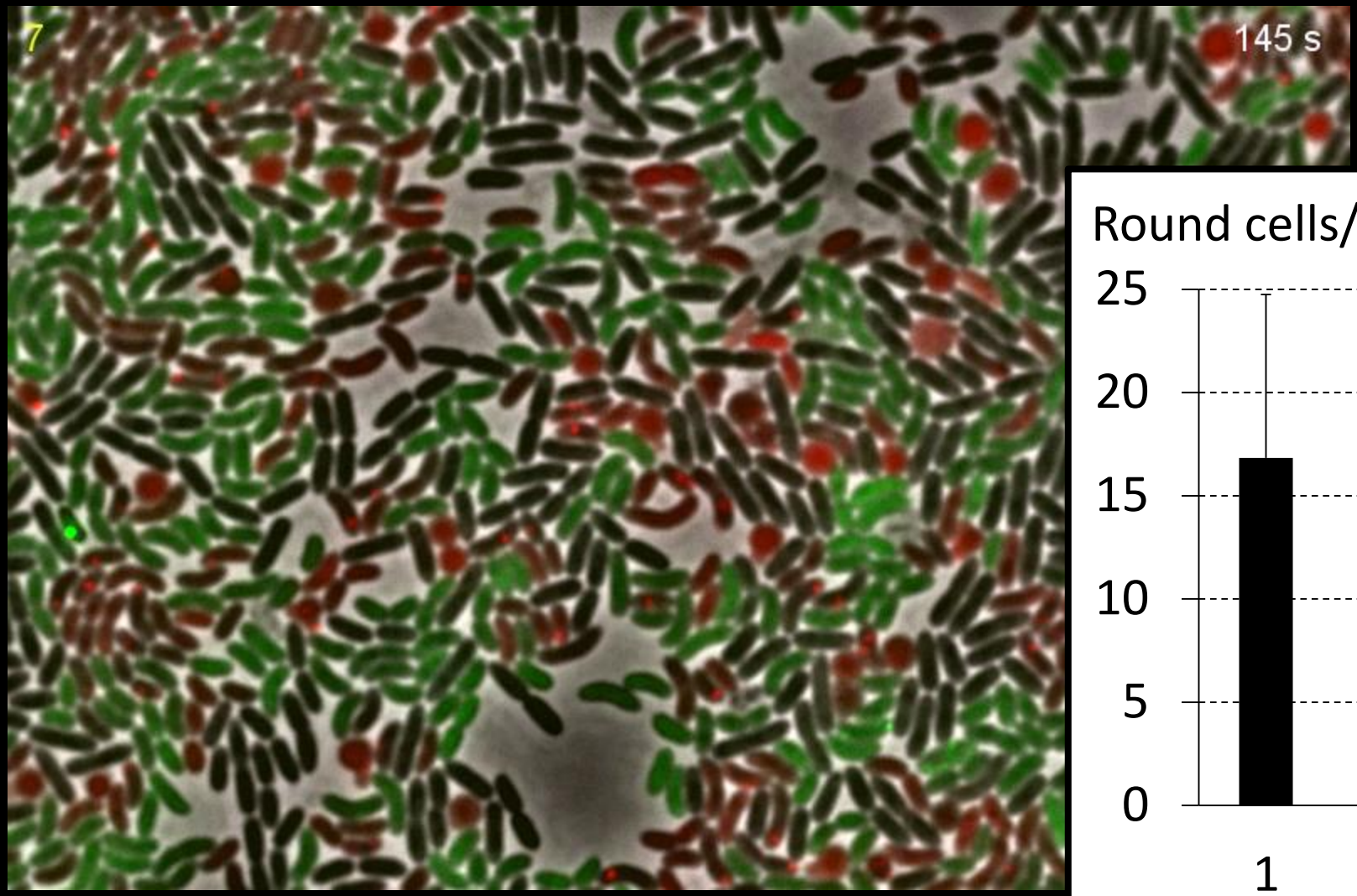

Death of
Aggressor

T6SS+ *Vibrio* is targeted with high precision

Peaceful bystanders are fine

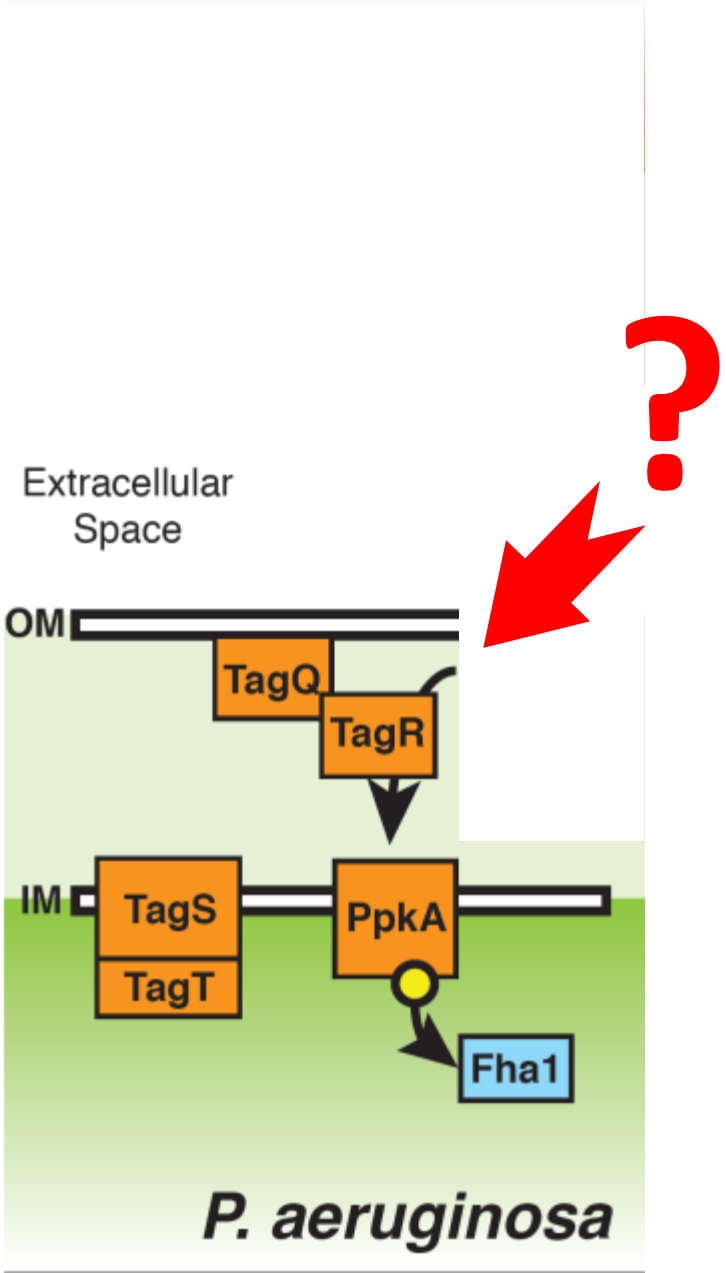
V. cholerae T6SS-

V. cholerae T6SS+



How is the dueling response regulated?

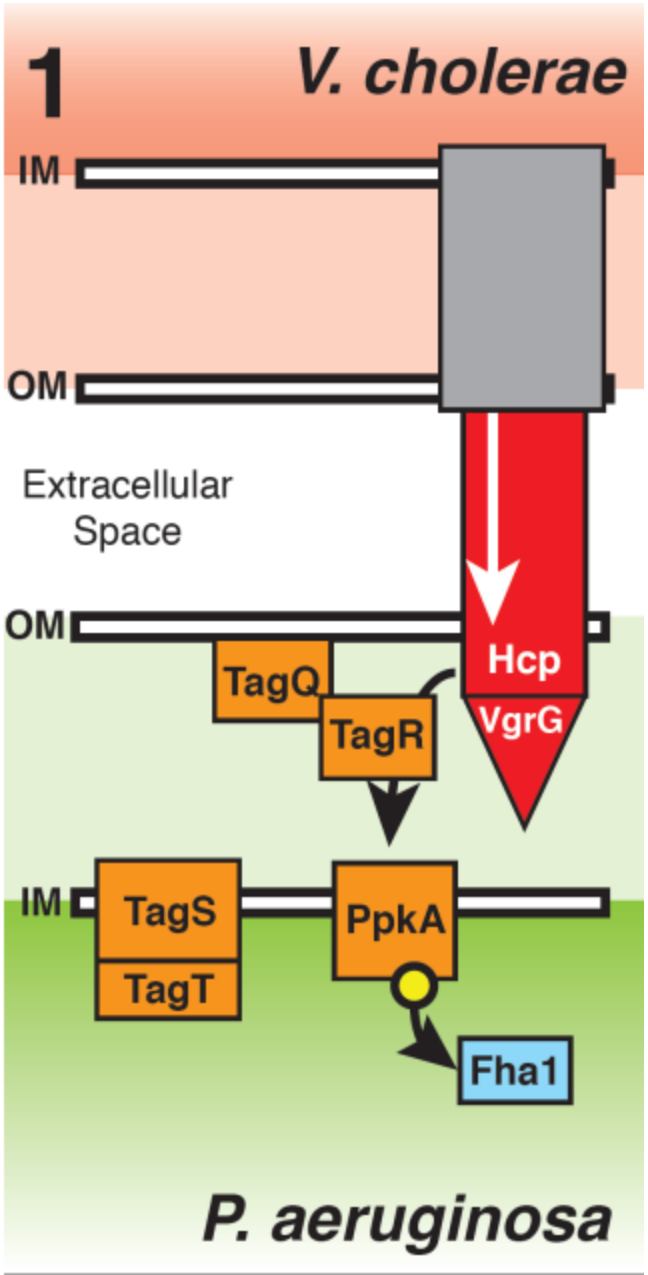
Regulation of T6SS dueling in *Pseudomonas*



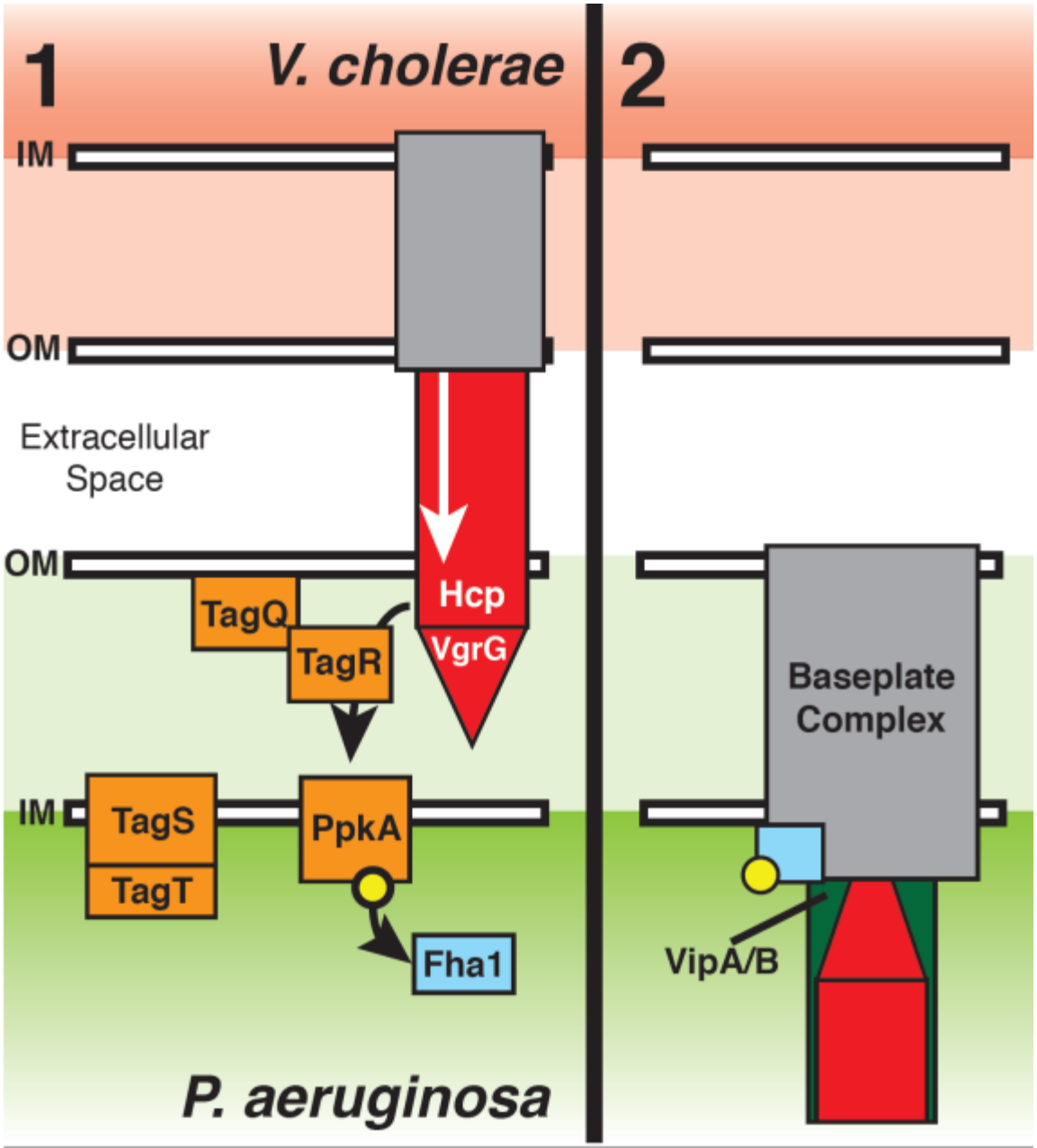
Mougous *et al.*, Nature Cell Biol. 2007

Casabona *et al*. Env Microbiol, 2012

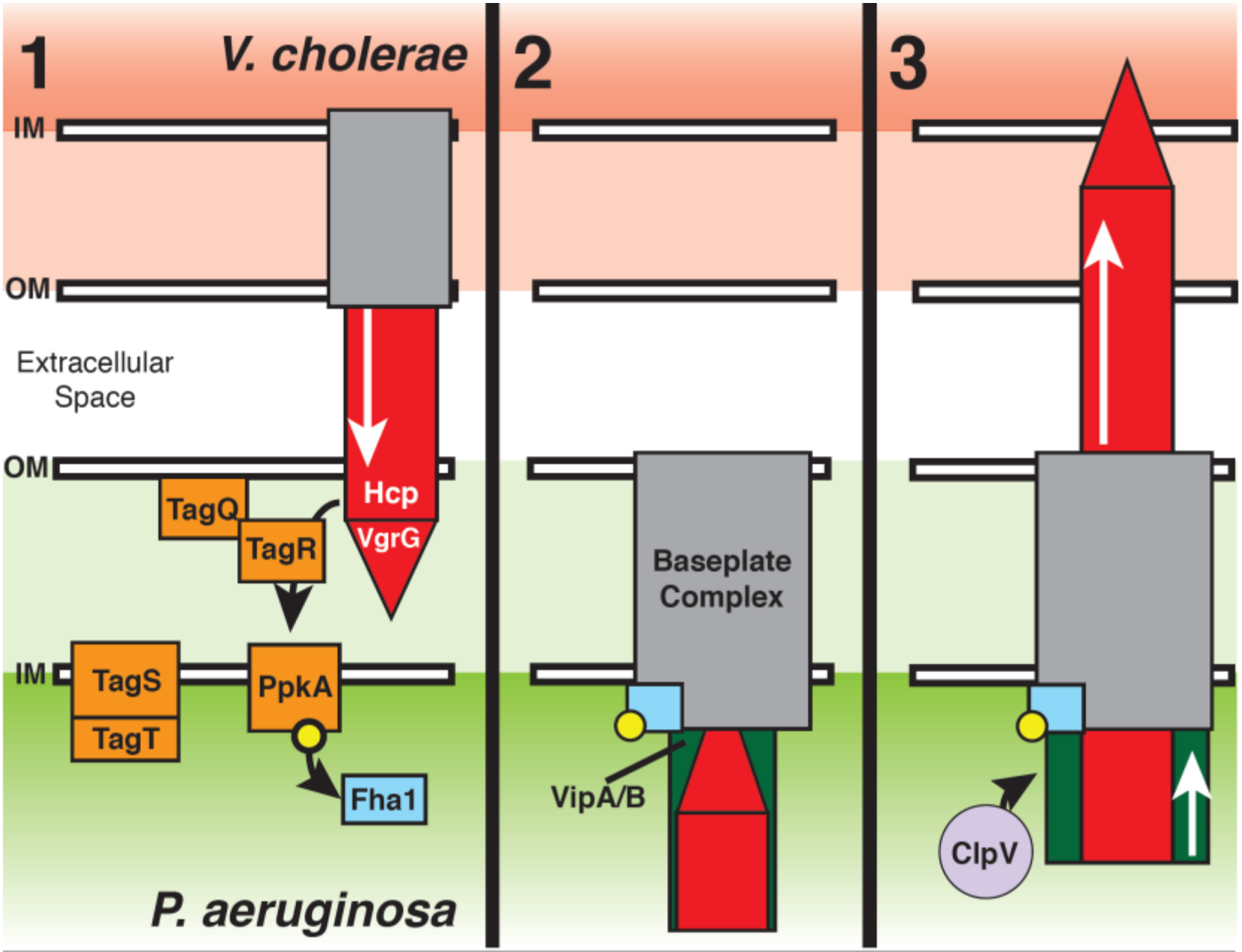
Regulation of T6SS dueling in *Pseudomonas*



Regulation of T6SS dueling in *Pseudomonas*



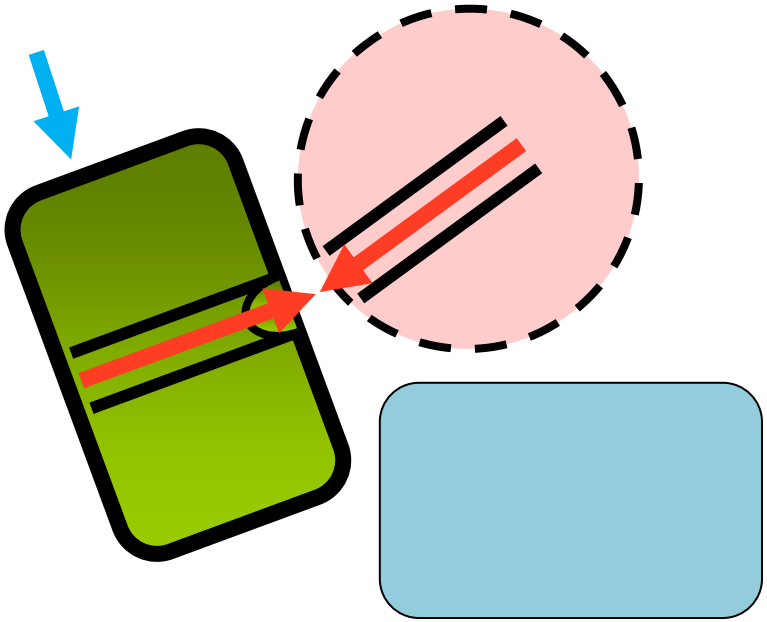
Regulation of T6SS dueling in *Pseudomonas*



Summary of different T6SS killing strategies

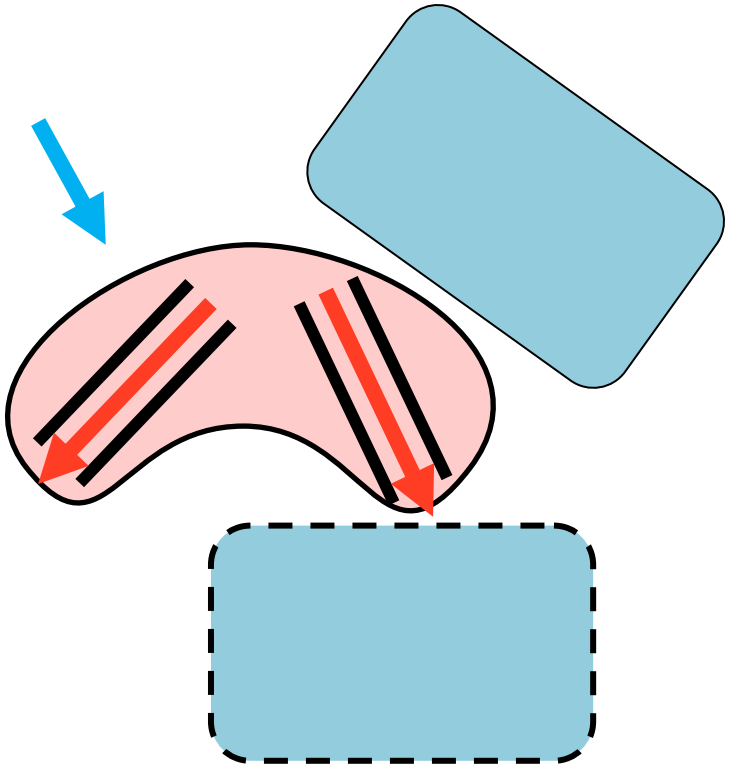
Pseudomonas aeruginosa

- highly regulated
- kills in self defense
- sensing membrane damage
- precise aiming



Vibrio cholerae

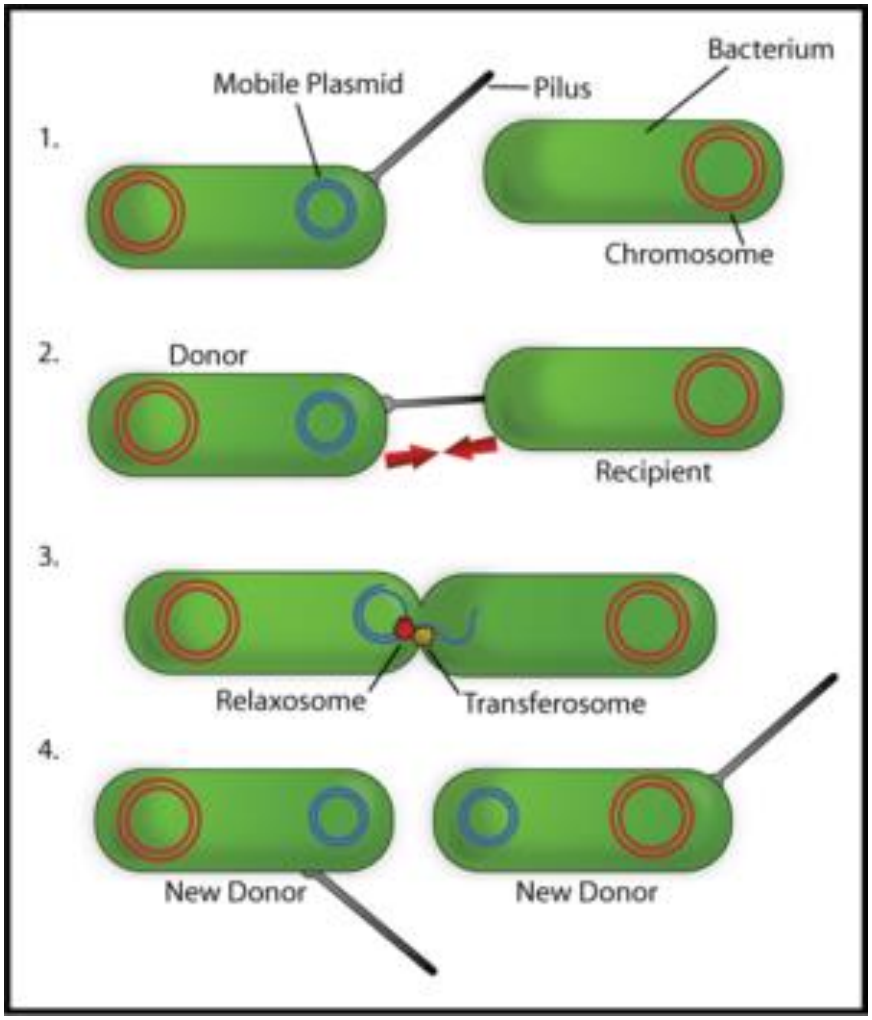
- highly active
- no aiming, random firing
- kills without provocation

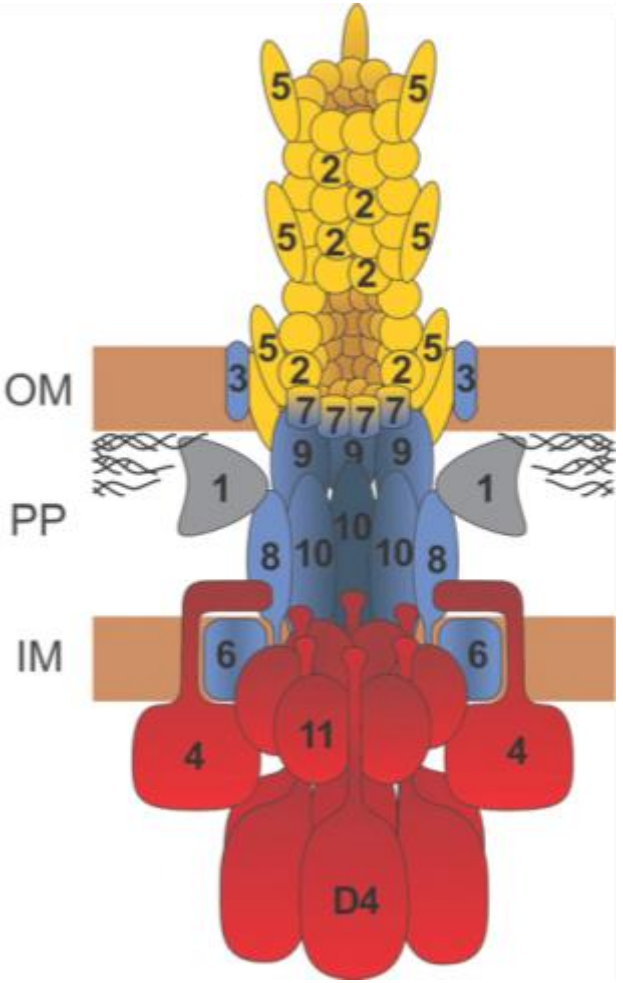


T6SS-mediated immunity to T4SS-mediated gene transfer

Ho, Basler and Mekalanos, Science, Oct 11 2013

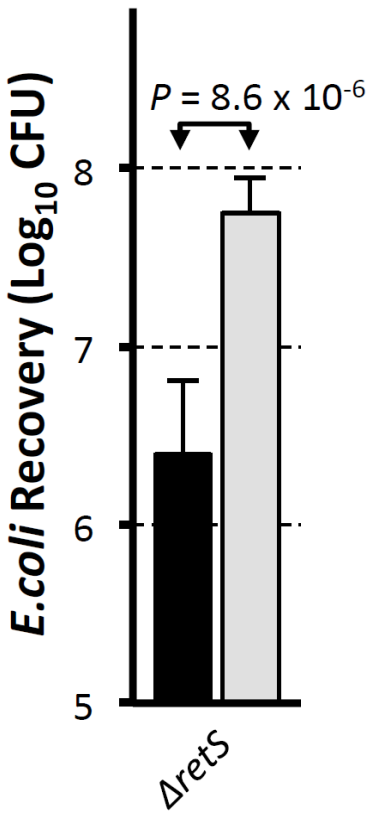
Conjugation mediated by T4SS





Schröder and Lanka. 2005. *Plasmid*.

P. aeruginosa specifically kills *E. coli* carrying RP4 plasmid

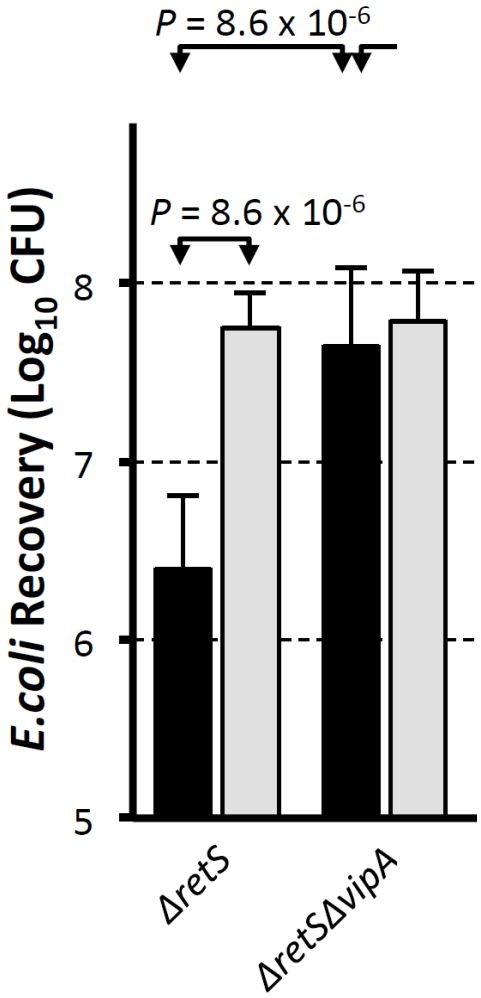


MC1061 RP4

MC1061

- Killing of *E.coli* is dependent on presence of RP4 plasmid

P. aeruginosa specifically kills *E. coli* carrying RP4 plasmid

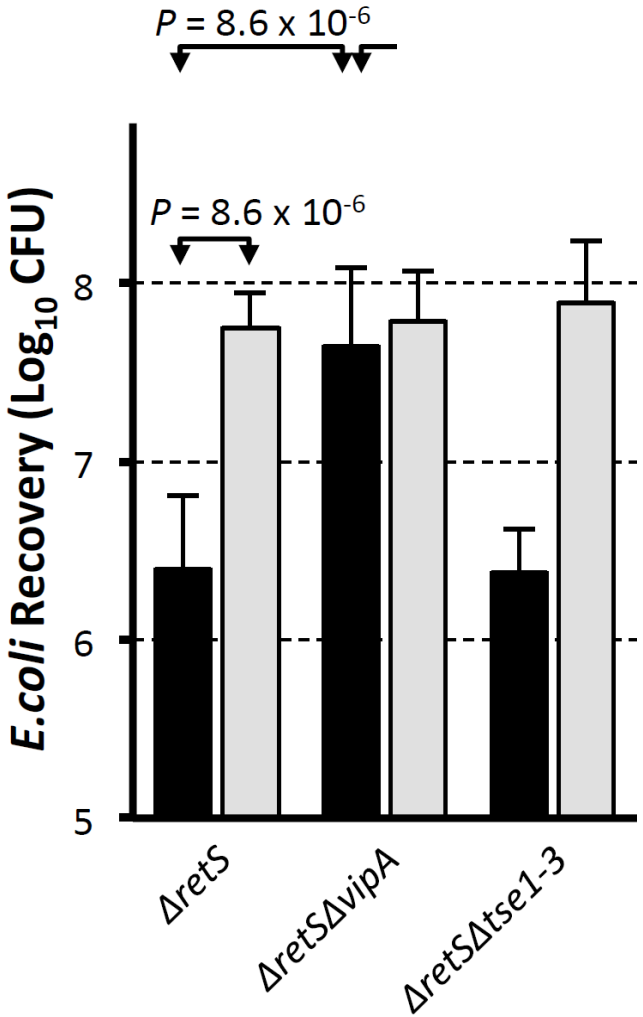


MC1061 RP4

MC1061

- Killing of *E.coli* is dependent on presence of RP4 plasmid
- T6SS dependent

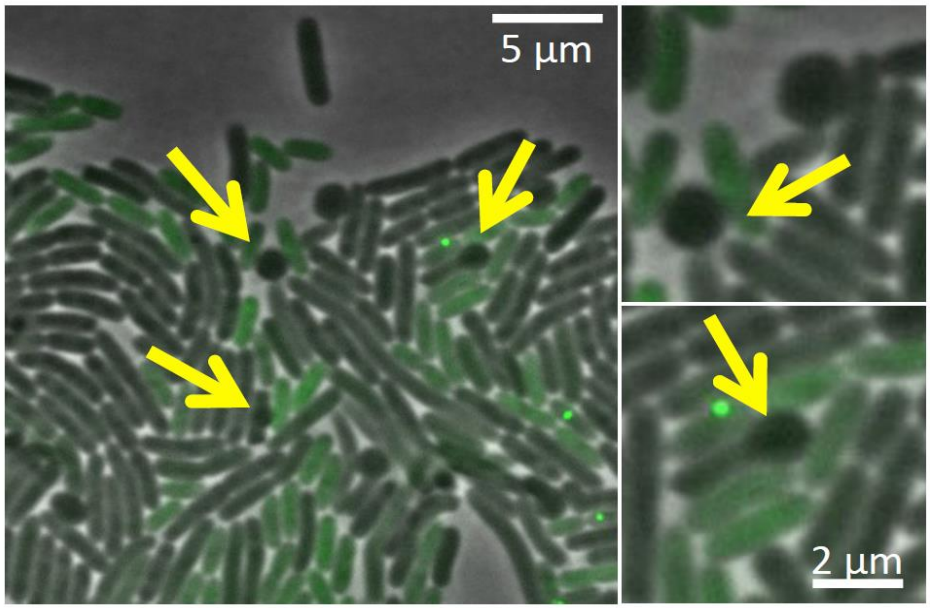
P. aeruginosa specifically kills *E. coli* carrying RP4 plasmid



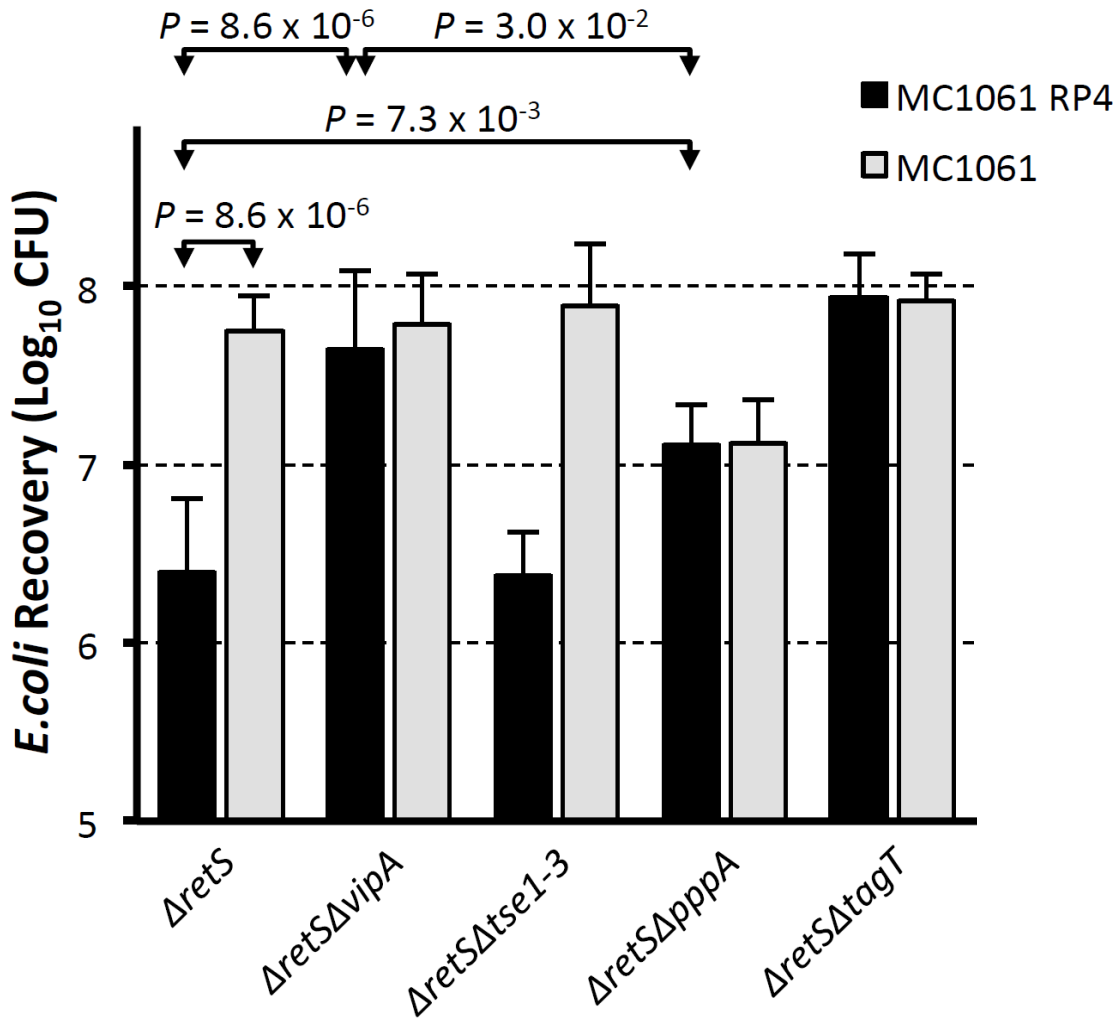
MC1061 RP4

MC1061

- Killing of *E.coli* is dependent on presence of RP4 plasmid
- T6SS dependent
- Tse effectors are translocated but not involved in killing

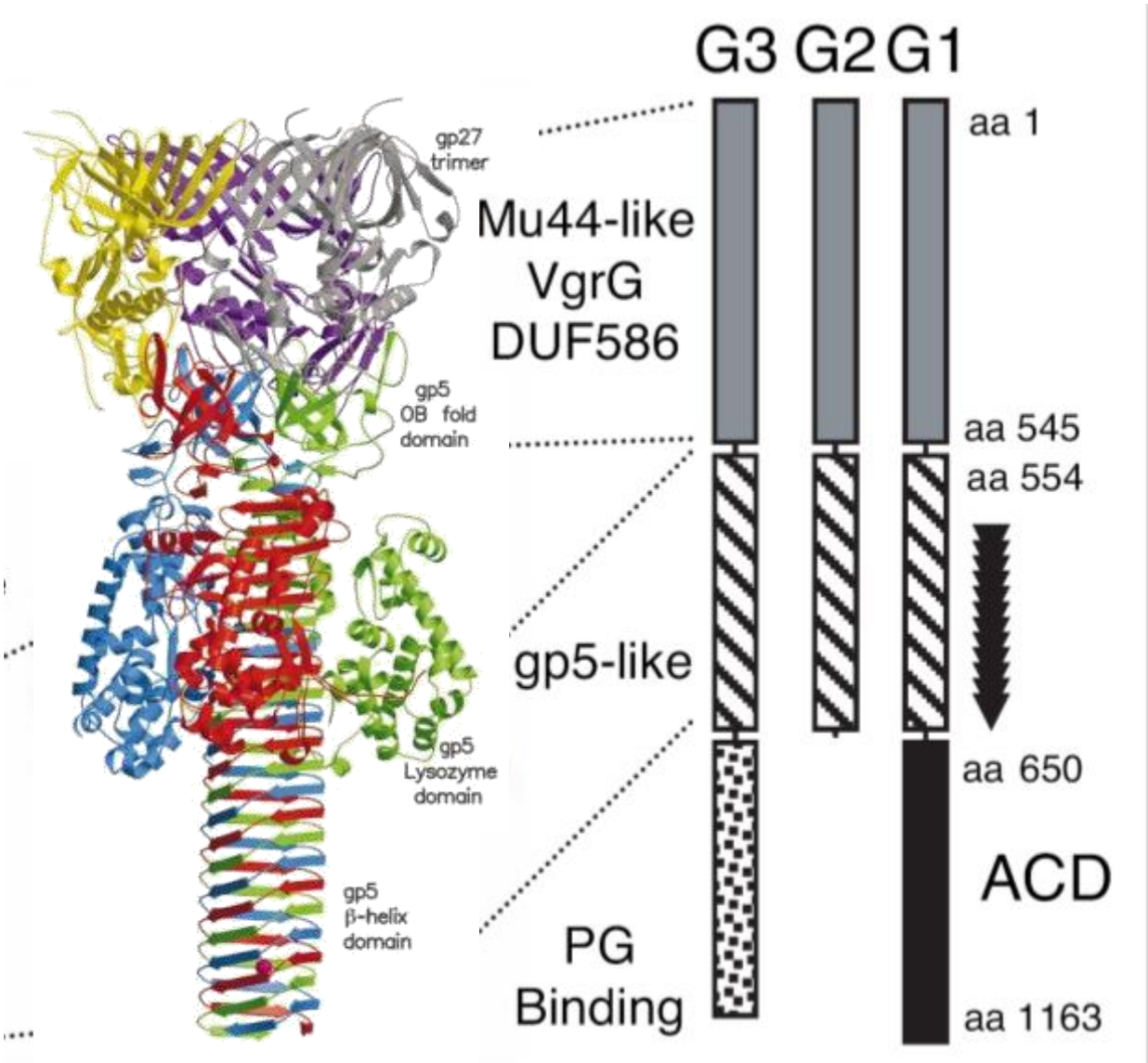


P. aeruginosa specifically kills *E. coli* carrying RP4 plasmid



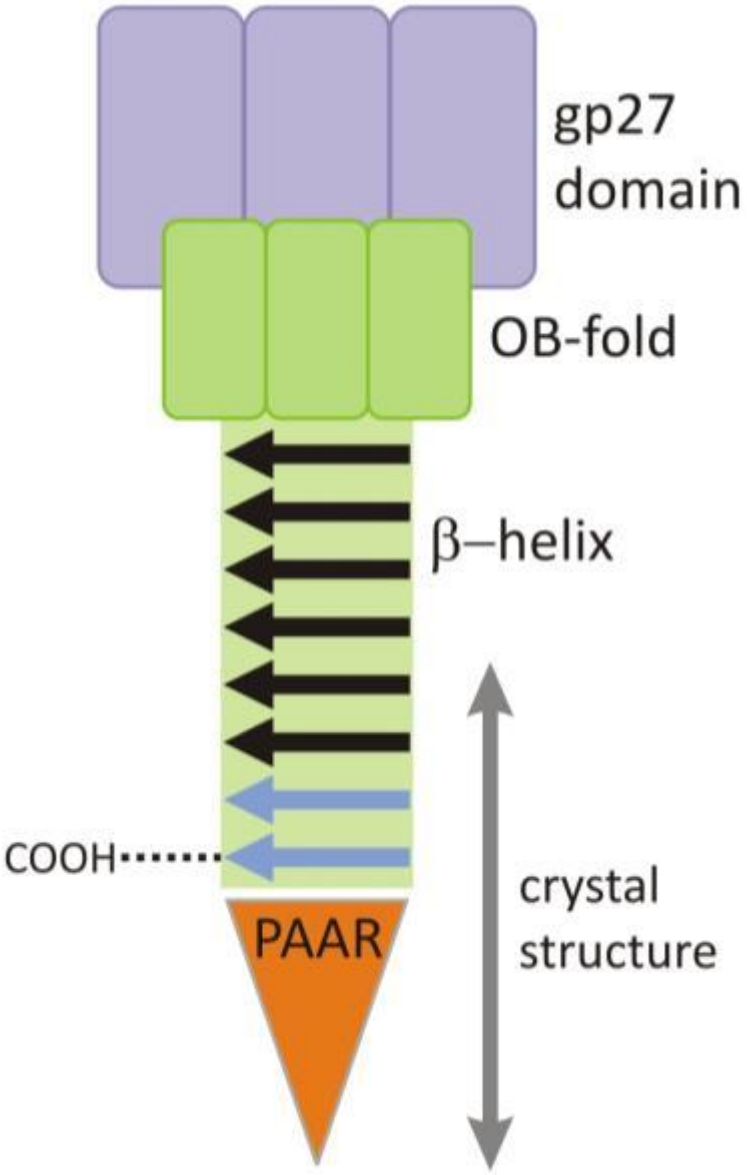
- Killing of *E.coli* is dependent on presence of RP4 plasmid
- T6SS dependent
- Tse effectors are translocated but not involved in killing
- Dependent on TagQRST signaling cascade
- RP4 plasmid does not make *E. coli* more sensitive

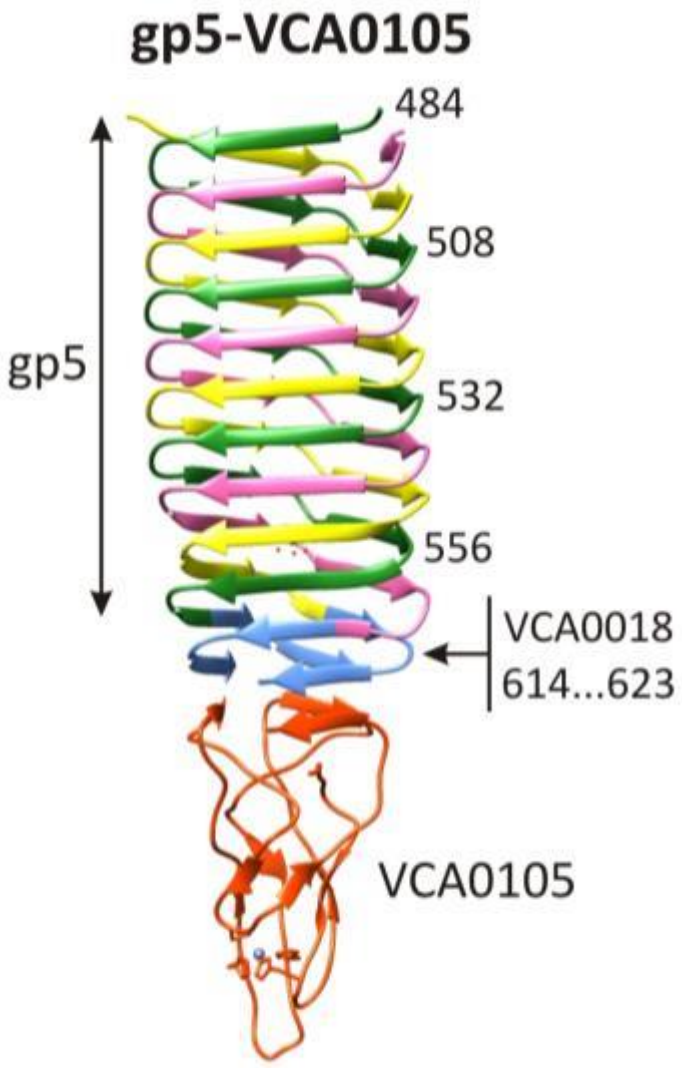
Some VgrGs are T6SS effectors



Pukatzki *et al.* PNAS 2007

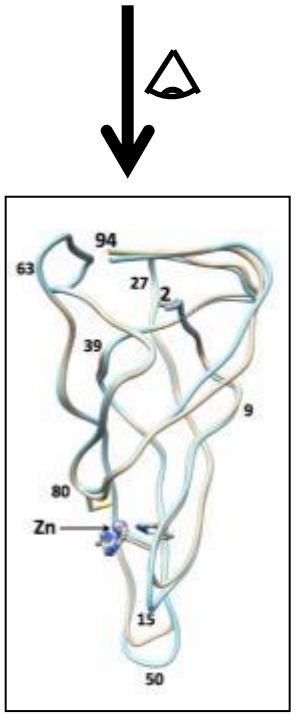
PAAR-protein sharpens the tip of VgrG

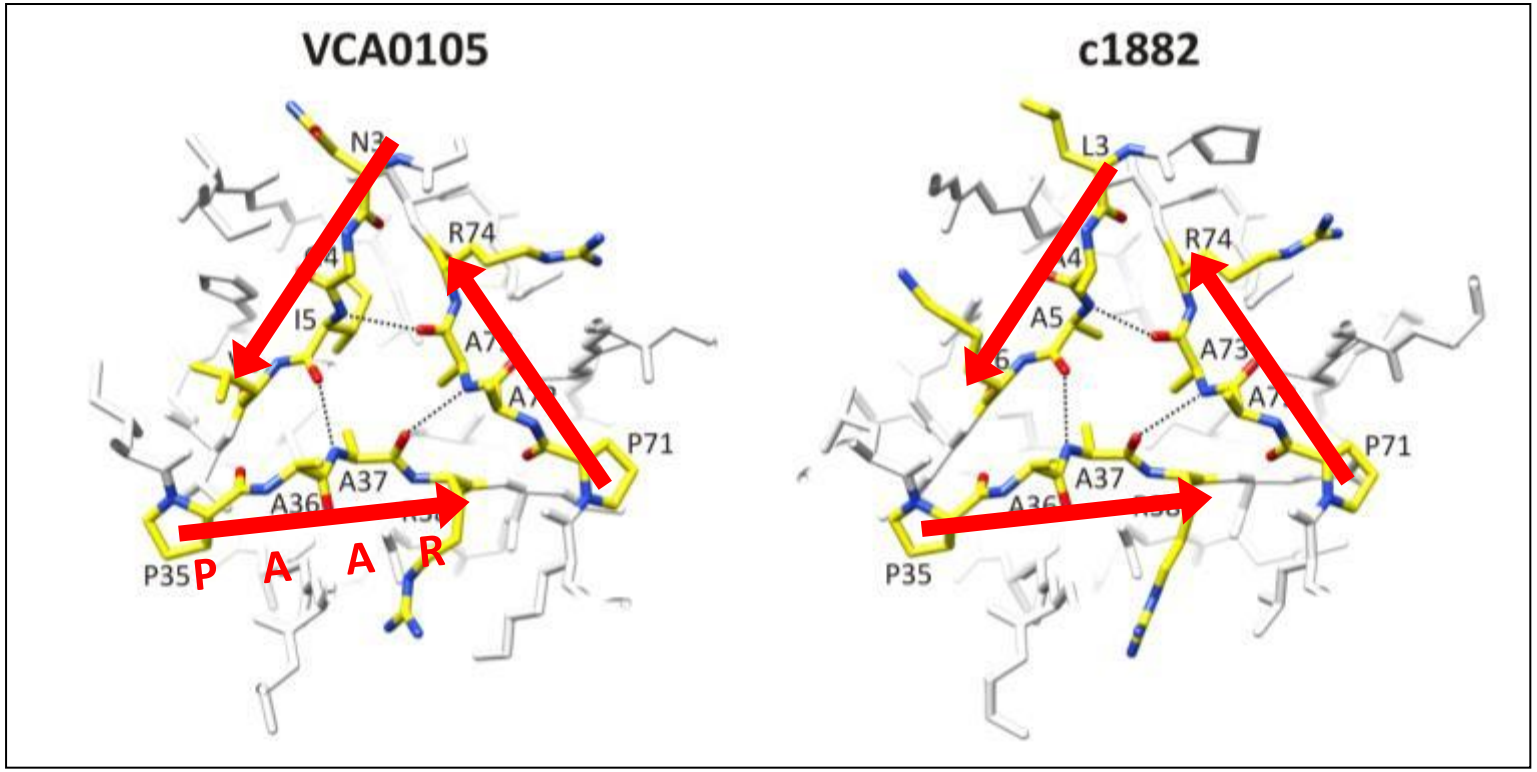




Shneider, *et al*. Nature 2013

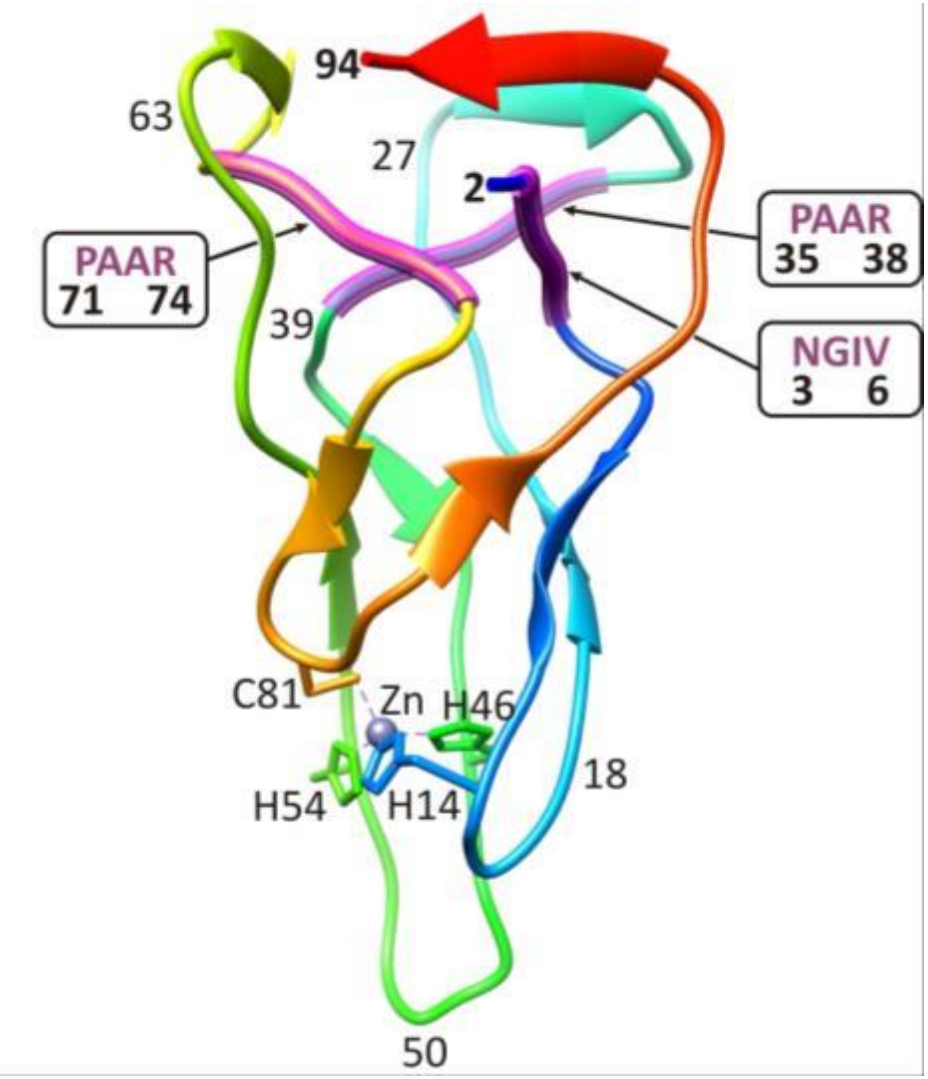
Pseudotrimeric structure of PAAR

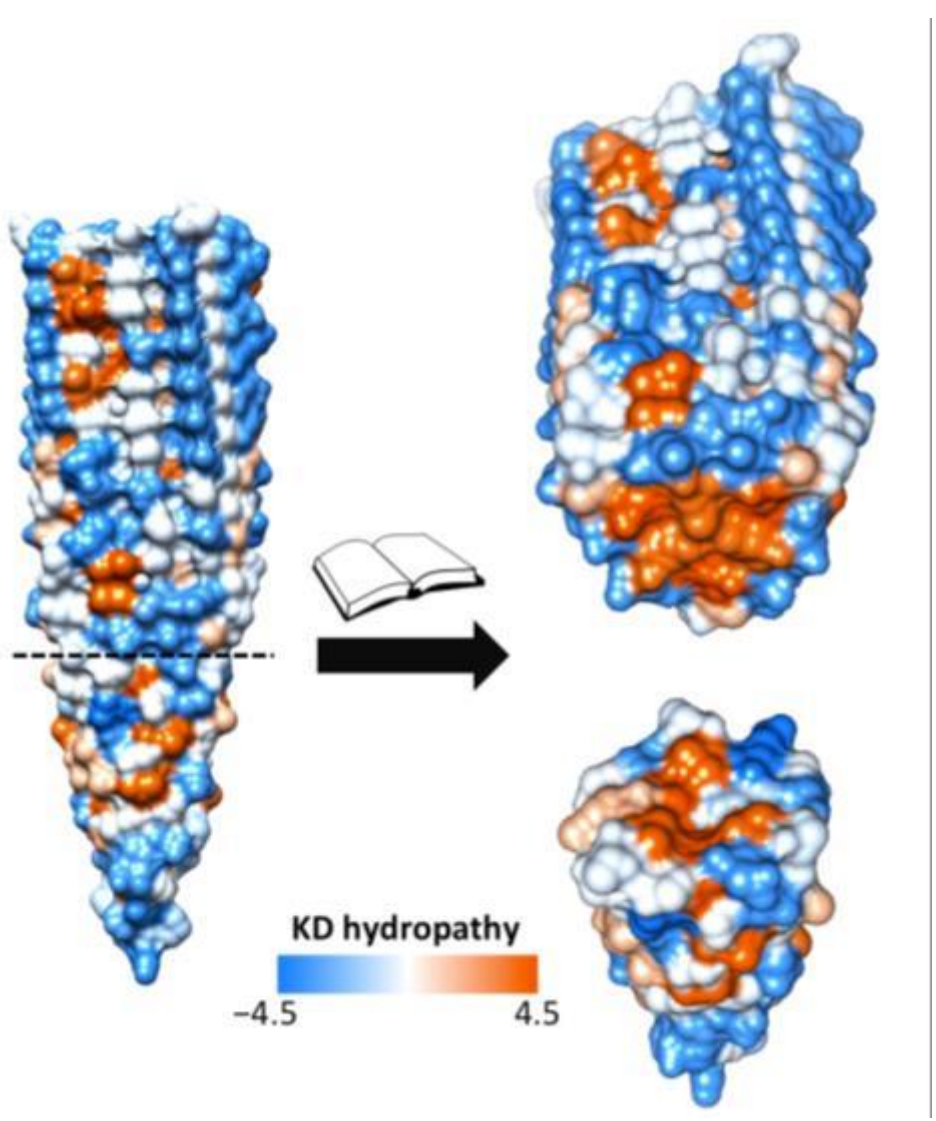




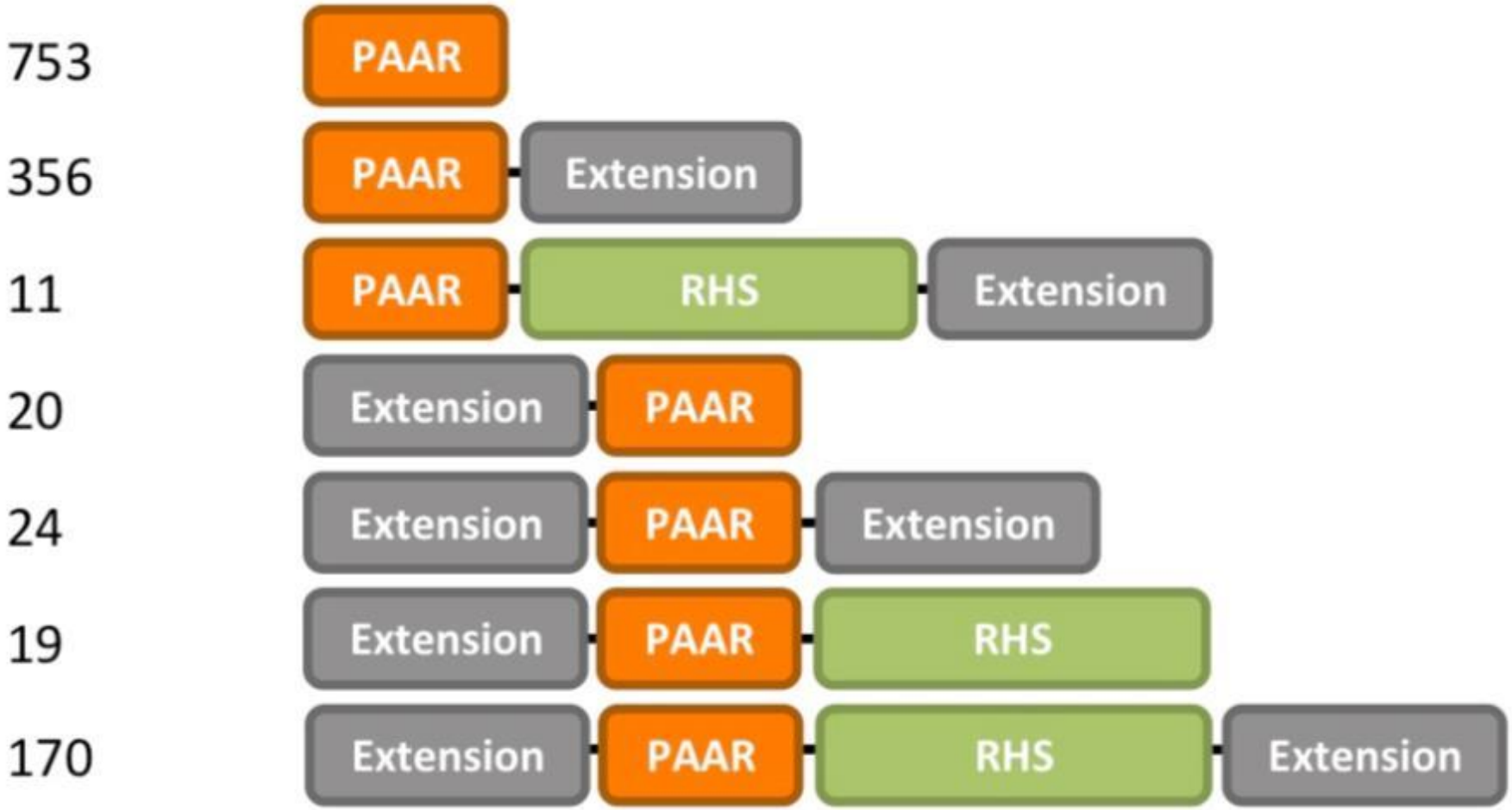
Shneider, *et al*. Nature 2013

PAAR-protein sharpens the tip of VgrG





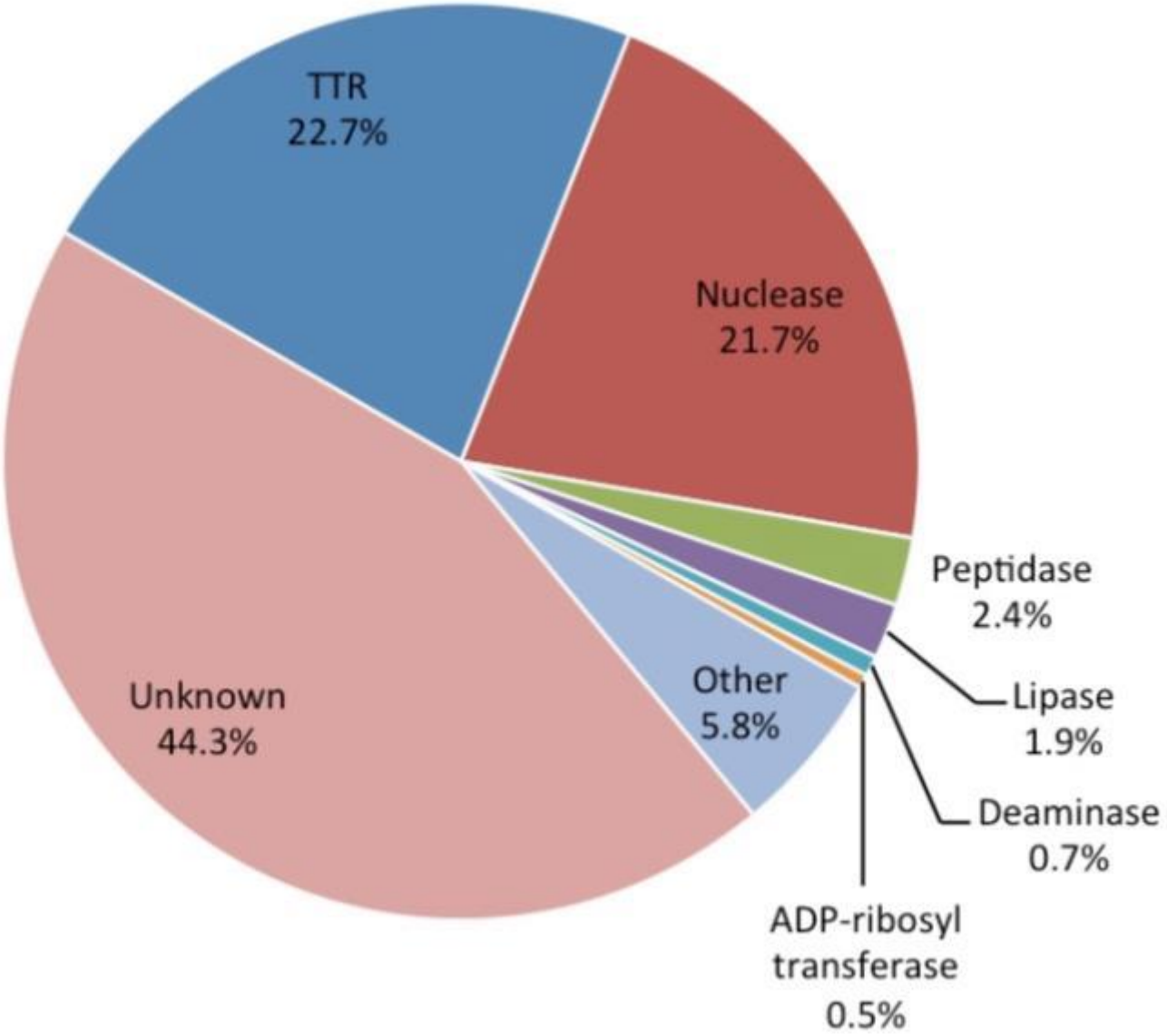
Domain architectures of PAAR proteins



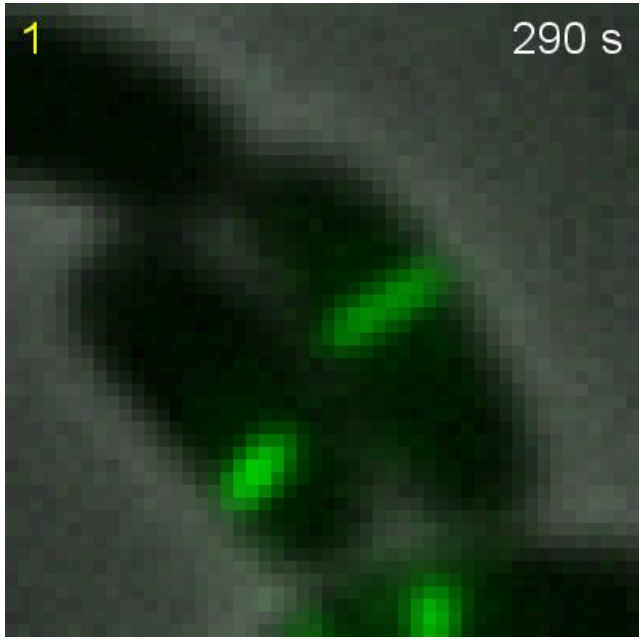
# of occurrences

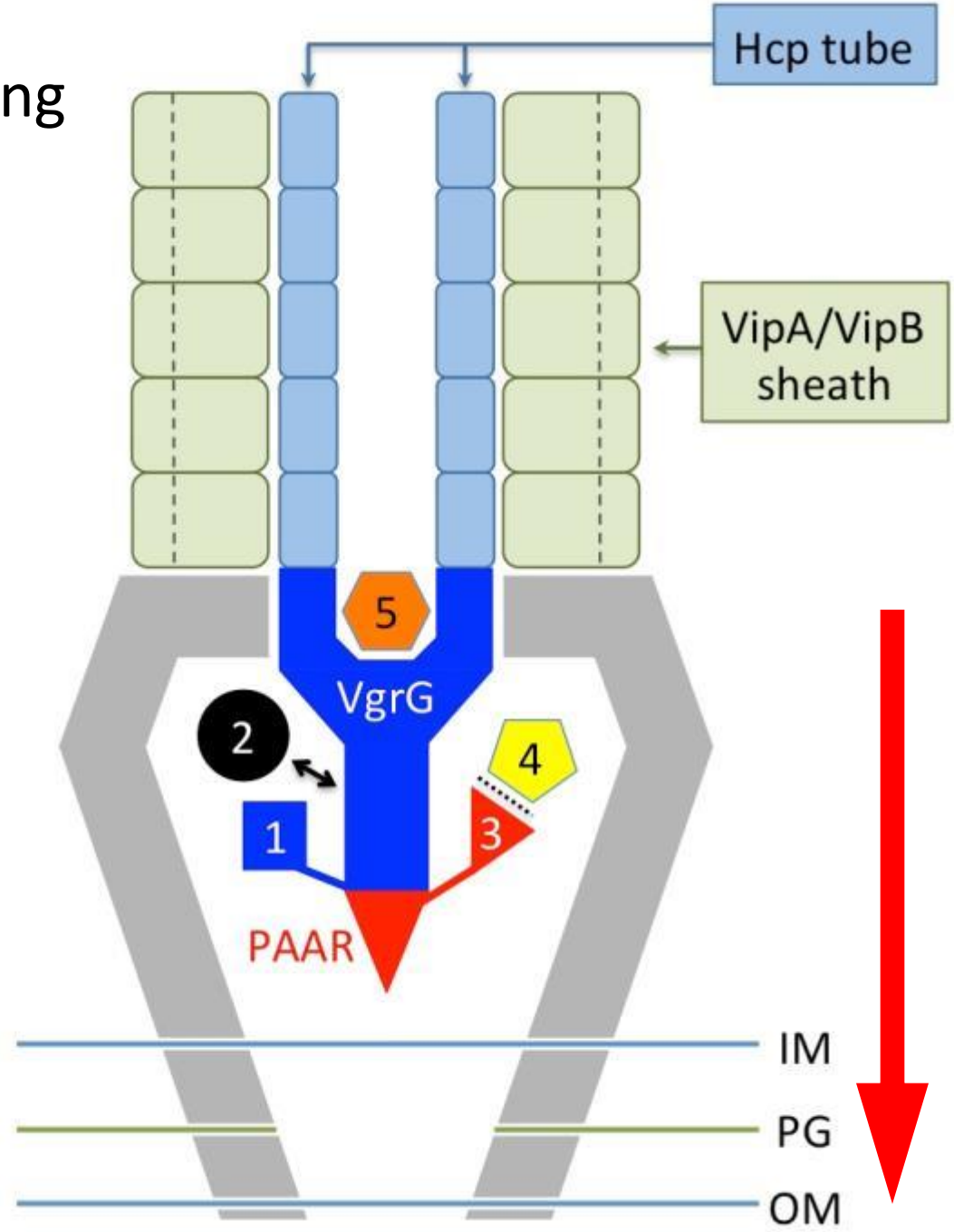
Shneider, *et al*. Nature 2013

Predicted functions of PAAR proteins



All cargo proteins interacting with the VgrG spike are translocated into a nearby target cell upon a single T6SS sheath contraction.





Shneider, *et al*. Nature 2013

Acknowledgements

CryoEM

Martin Pilhofer, Greg Henderson, Grant Jensen - Caltech

Fluorescence Microscopy

Nick Peters & Tom Bernhardt – Harvard Medical School

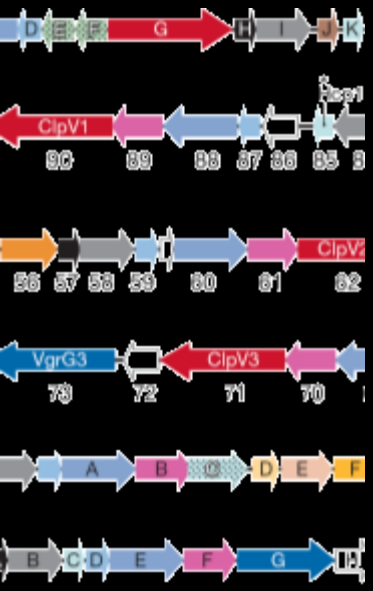
Jennifer Waters – Harvard Nikon Imaging Center

Structural biology

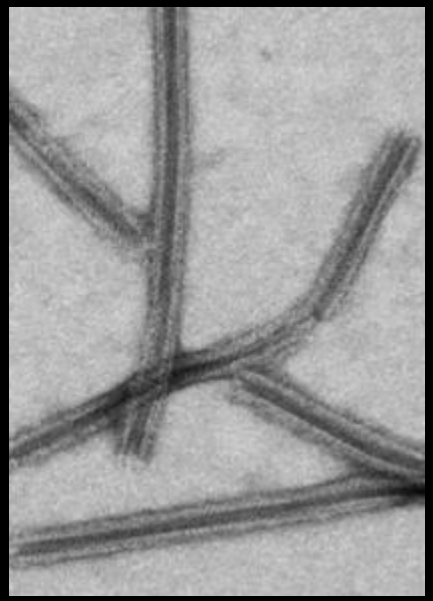
Petr Leiman - EPFL

Summary

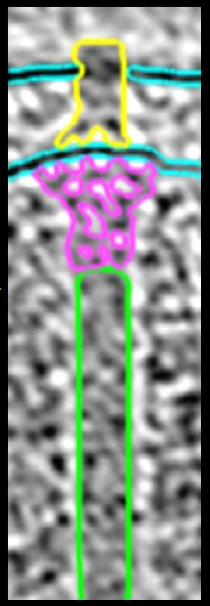
Bioinformatics



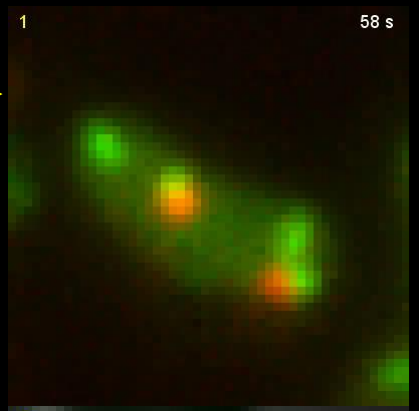
Sheath identification

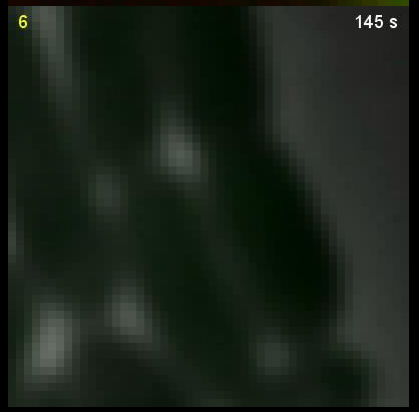


ECT



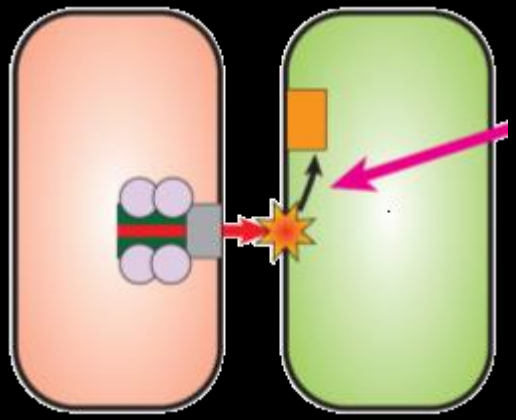
Live cell imaging





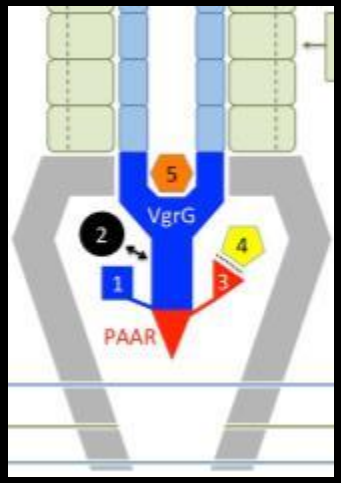
Dueling

Sensing, prey selection



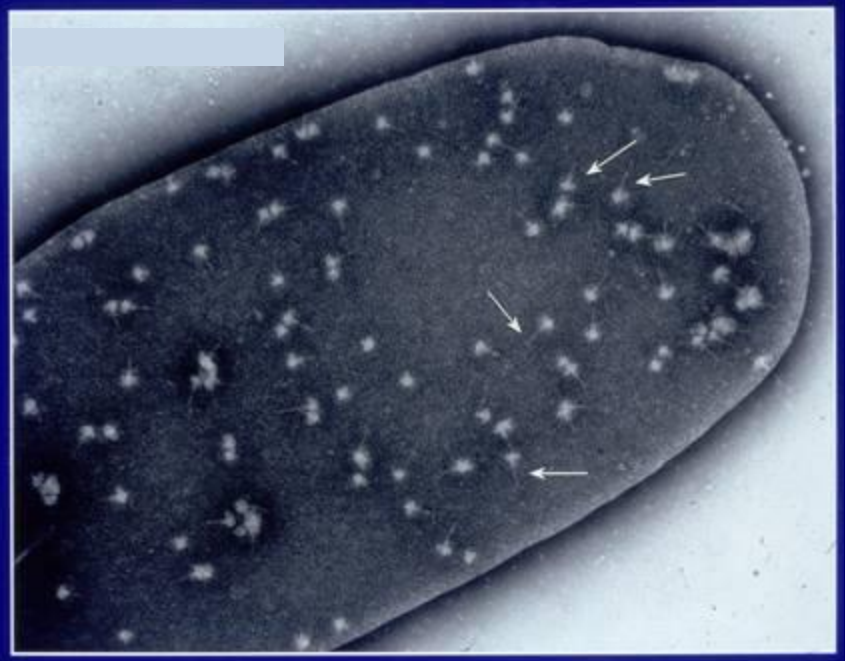
Immunity to T4SS-med. gene transfer

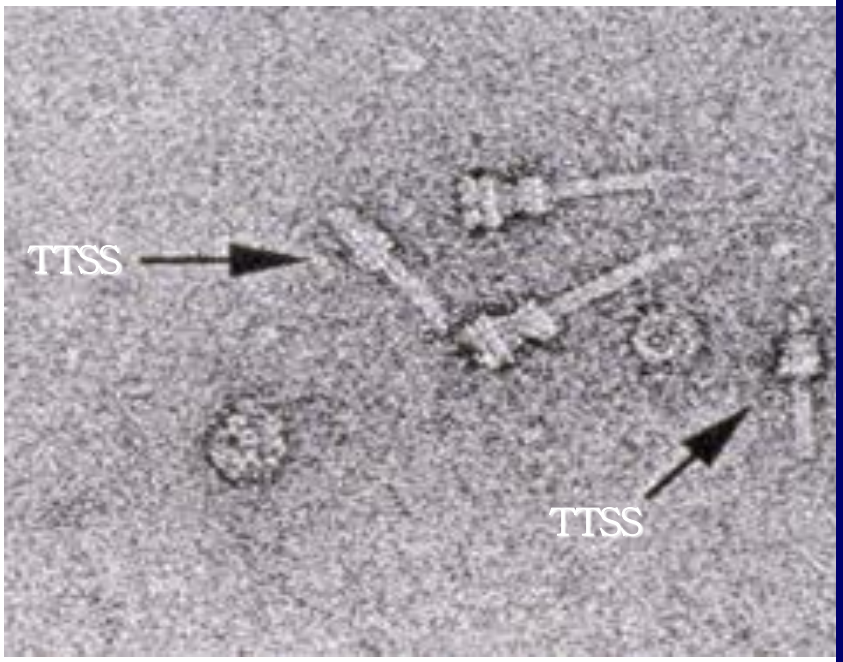
Substrate translocation

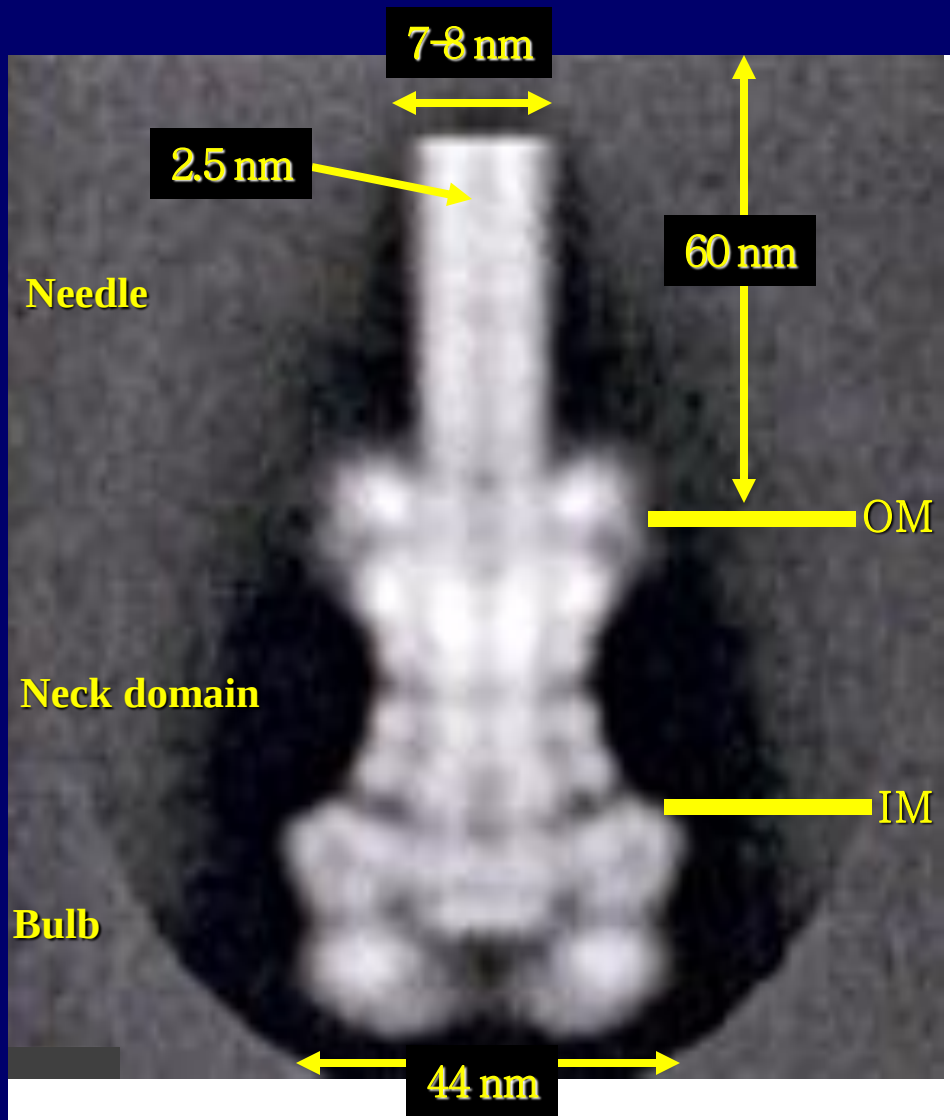


The Type III secretion system effectors

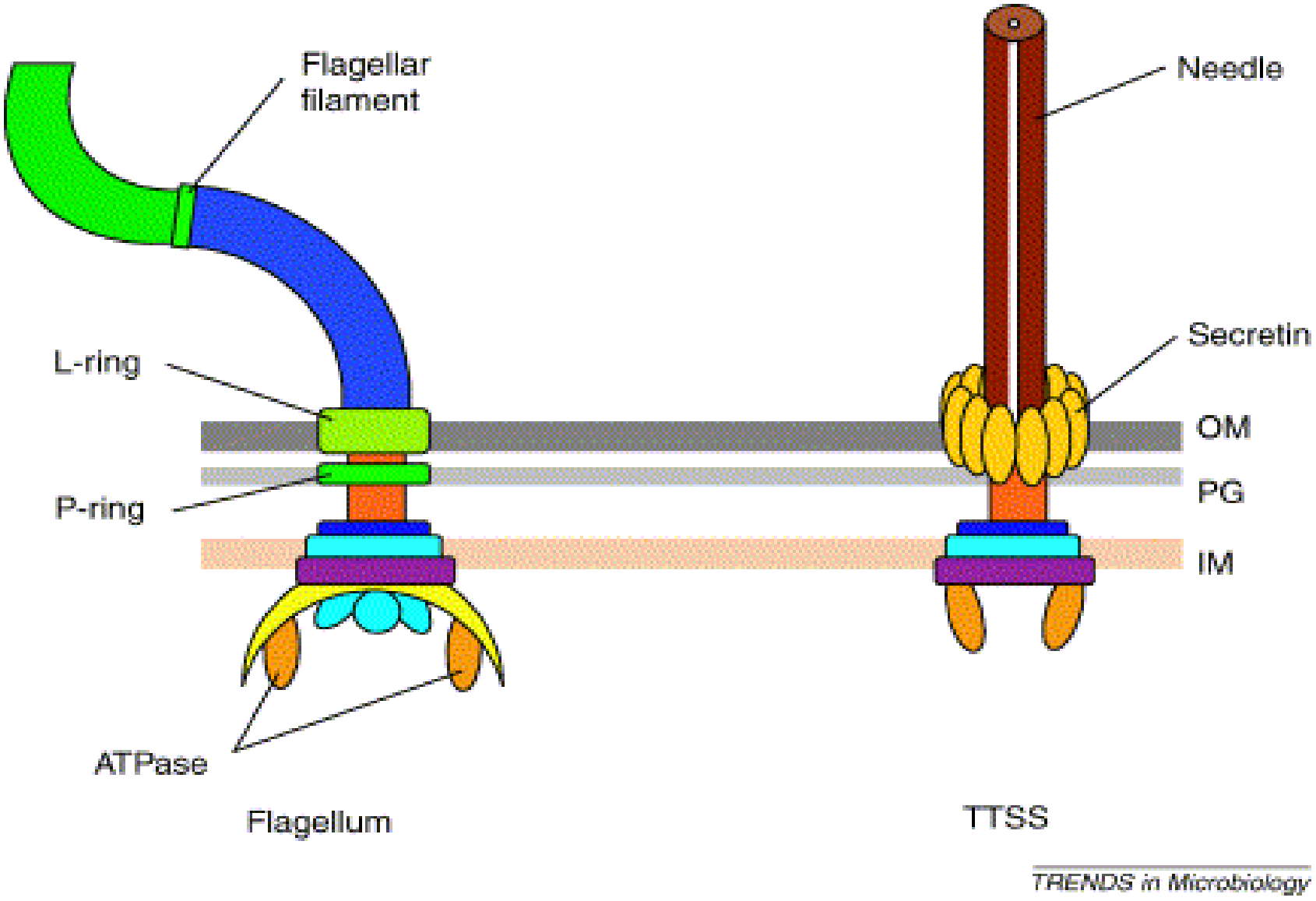
Shigella Type Three Secretory System TTSS



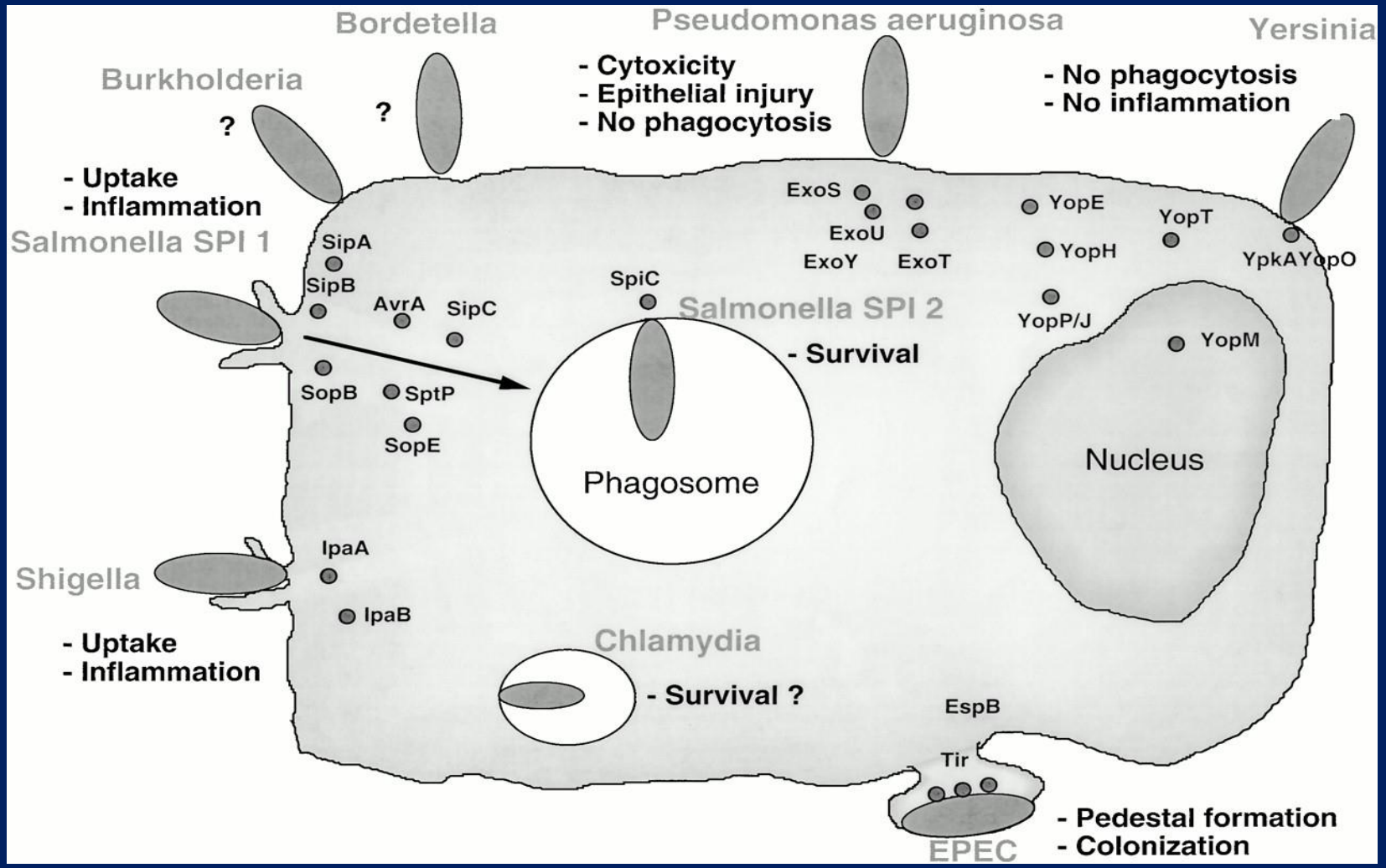




Resemblance between flagella and type III injectosomes

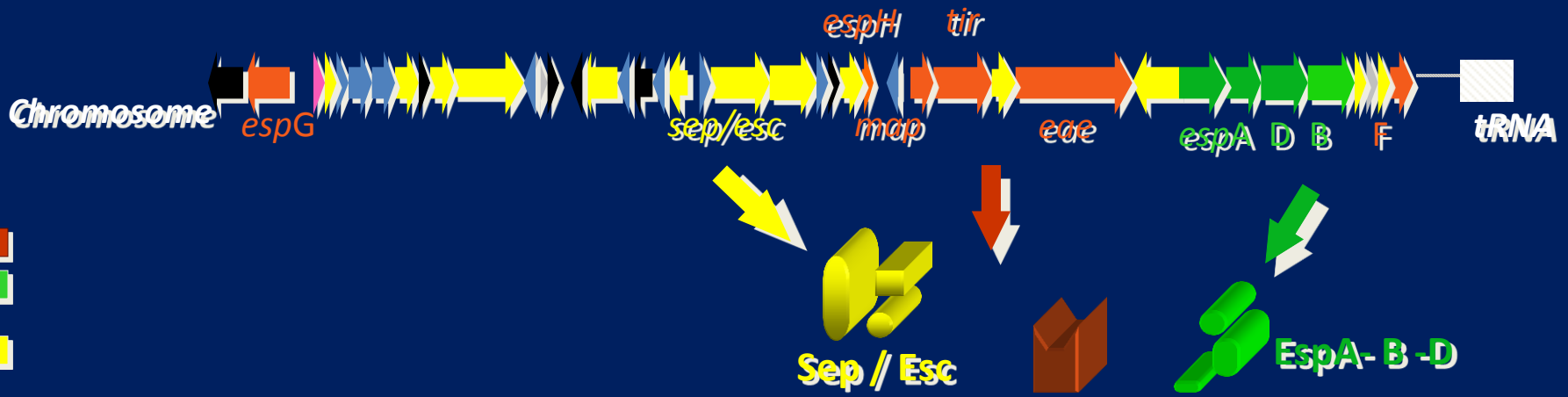


Type III secretion systems in animal pathogens

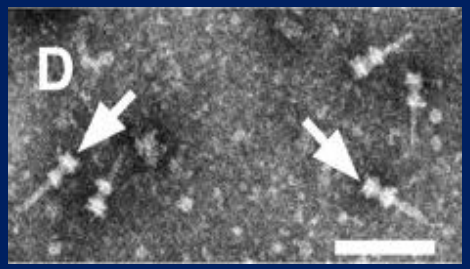


Adapted from GR Cornelis and F Van Gijsegem Annu. Rev. Microbiol. 2000. 54:735-774.

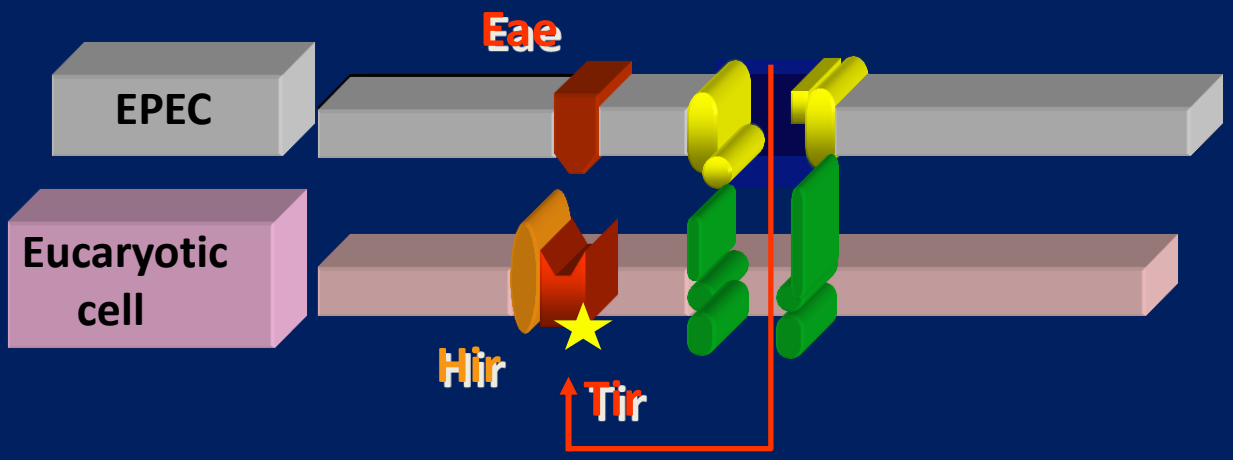
The LEE codes for a TTSS



« The needle »



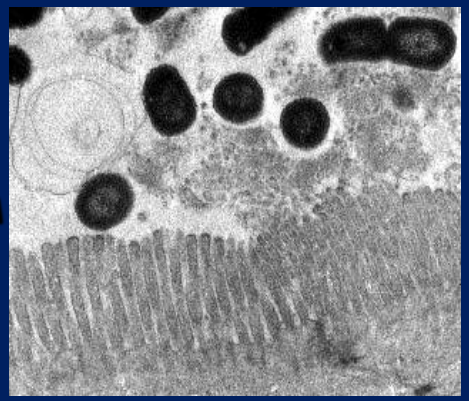
Abe *et al*

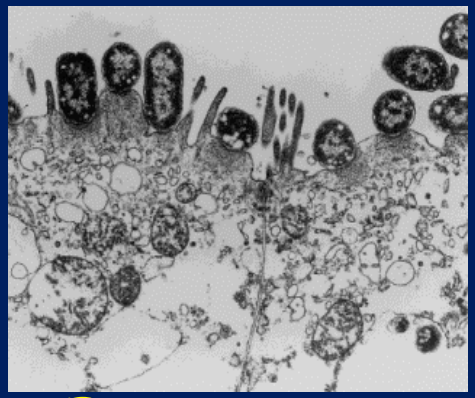


A/E Lesions , FAS, diarrhea

Phenotypes associated with AEEC infection

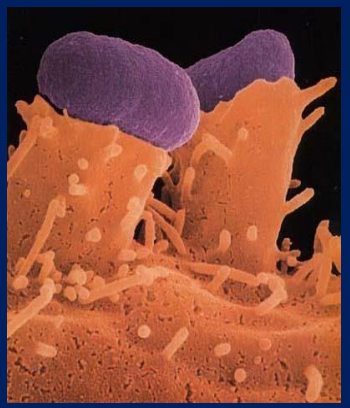
In vivo intestinal Epithelium





Transmission EM

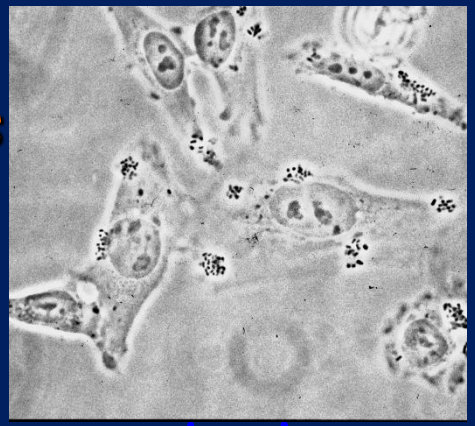
Attaching-Effacing lesion(A/E)



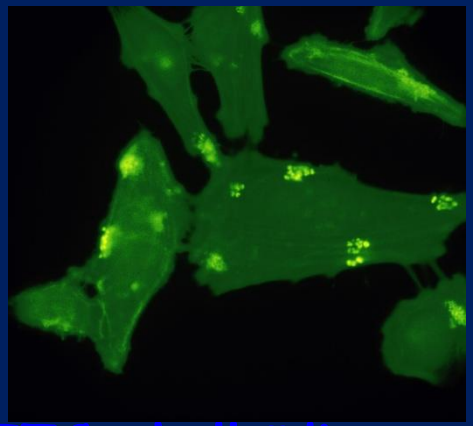
Finlay *et al.*

Fluorescent Actin Staining test (FAS)

In vitro Hela cells



Phase contrast



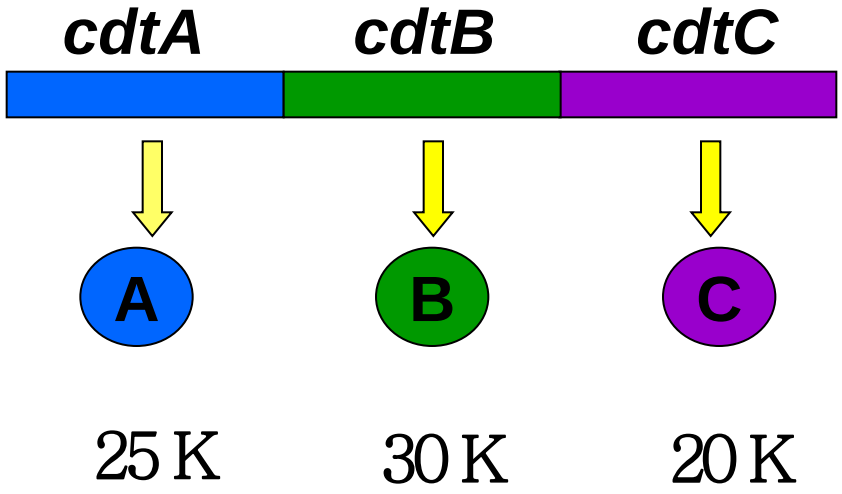
FITC-phalloidine

Cytolethal distending toxins

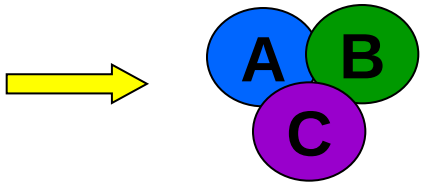
- Newly discovered family of bacterial toxins
- Cell distention followed by cell death (1987)
- Produced by several Gram negative bacterial species
- CDT from *Haemophilus ducreyi* (HdCDT)
- Cause of CHANCROID, a sexually transmitted disease, characterised by slowly healing genital ulcers

1994: CDTs are encoded by 3 linked genes

Expression of all the three genes is required to produce an active toxin



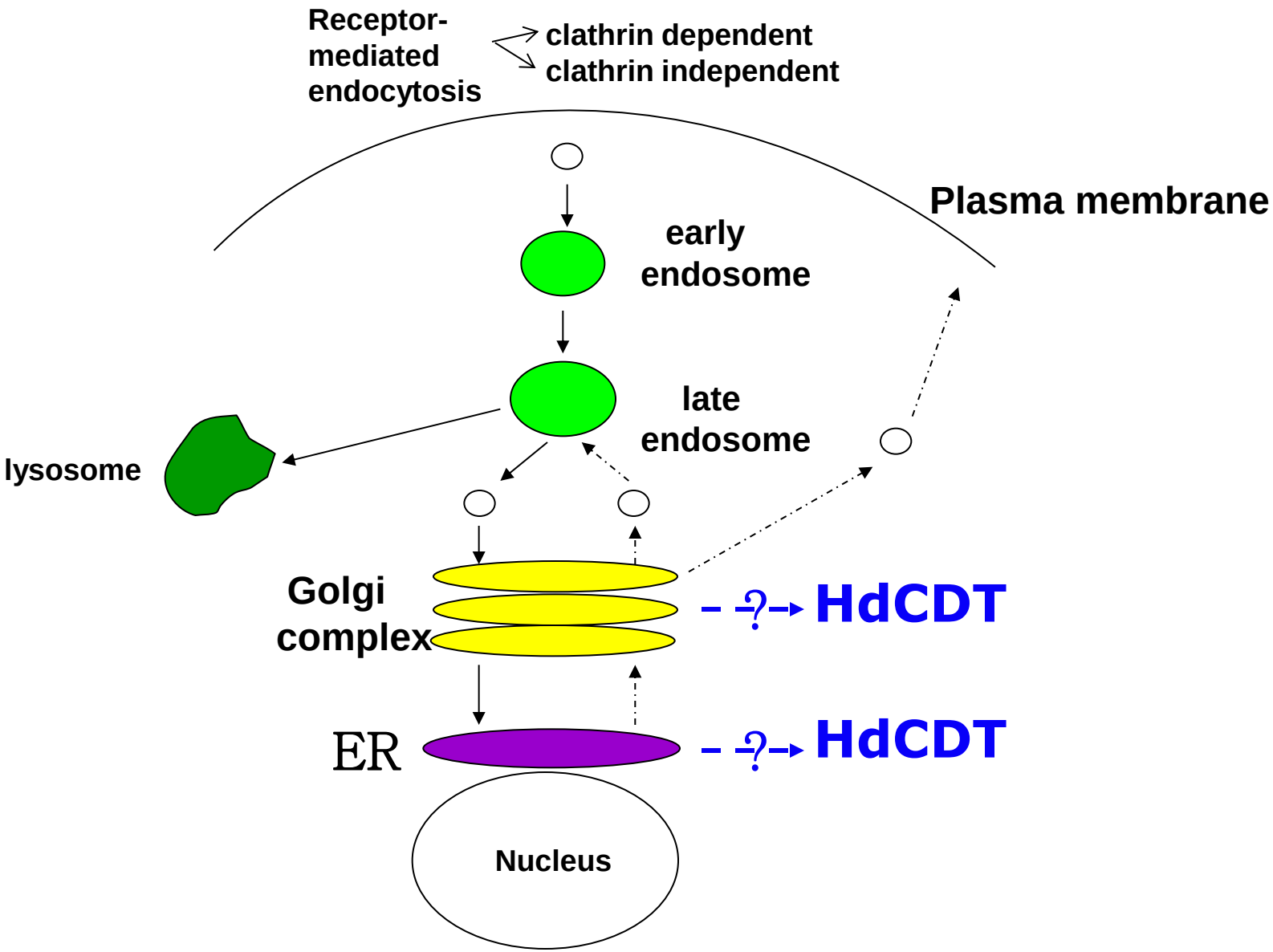
1997: CDTs induce G2 arrest



2000:

- **B is active subunit**
- **A & C for binding??**
- **Receptor structure??**

Cellular internalization of HdCDT



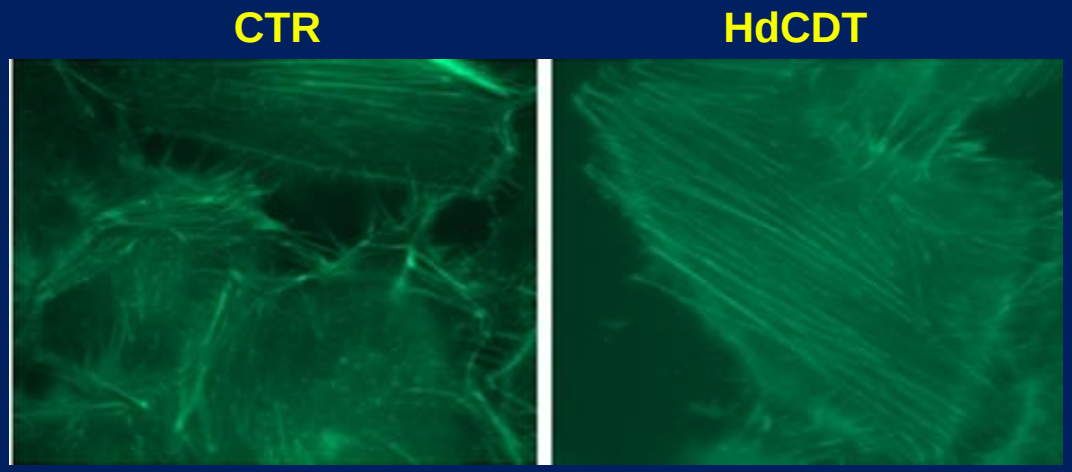
Structural and functional homology between CdtB and mammalian DNase I

1. Plasmid digestion in vitro by the *E. coli* cdtB
Elwell & Dreyfus Mol. Microbiol. 37, 952 (2000)

2. Nuclear fragmentation and chromatin collapse by transfection of *C. jejuni* cdtB
Lara-Tejero and Galan Science 290, 354 (2000)

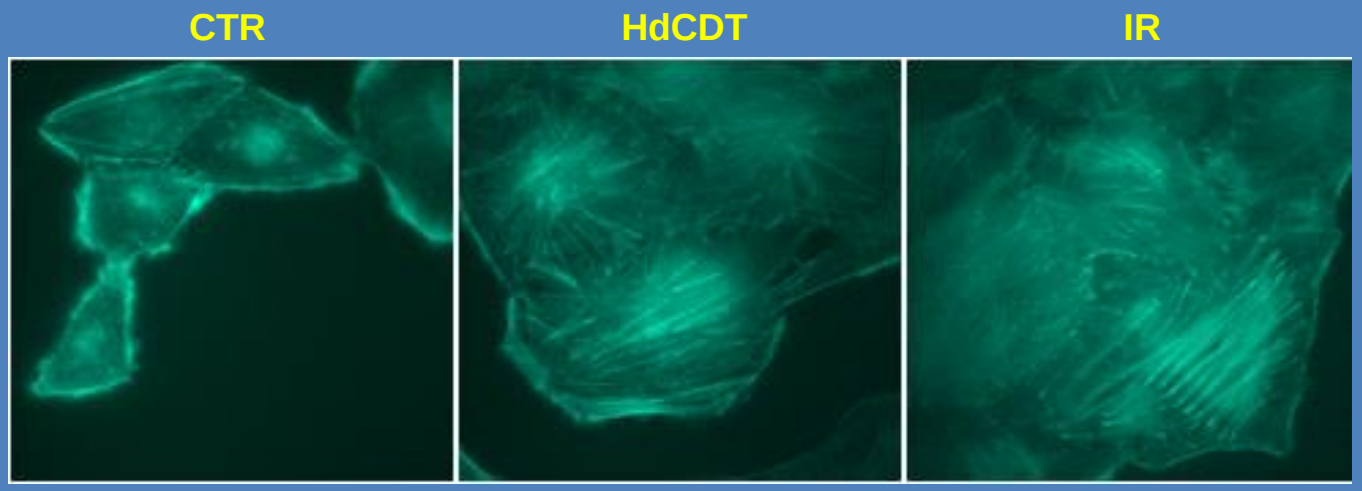
In both studies, mutations in residues critical for DNase activity abolished cell intoxication

Actin stress fibers are promoted in CDT-treated cells



What is the molecular mechanism(s)?

Ionizing radiation also induces stress fibers and RhoA activation

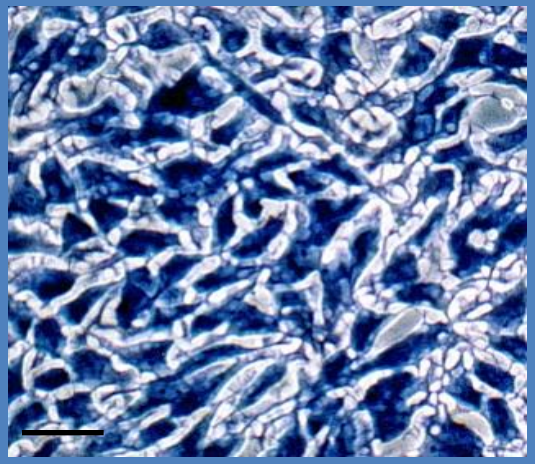


Take home message

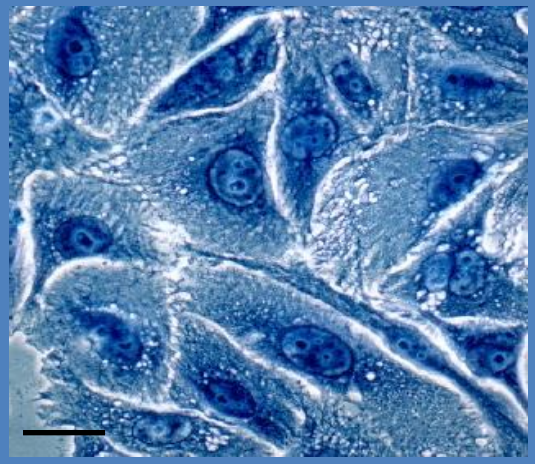
CDTs induce DNA damage evoking:

- check point responses
- cell cycle arrest
- cell distention
- stress fiber promotion

Certain EPEC and EHEC strains are able to induce an original CytoPathic Effect (CPE)



control
(72 h)



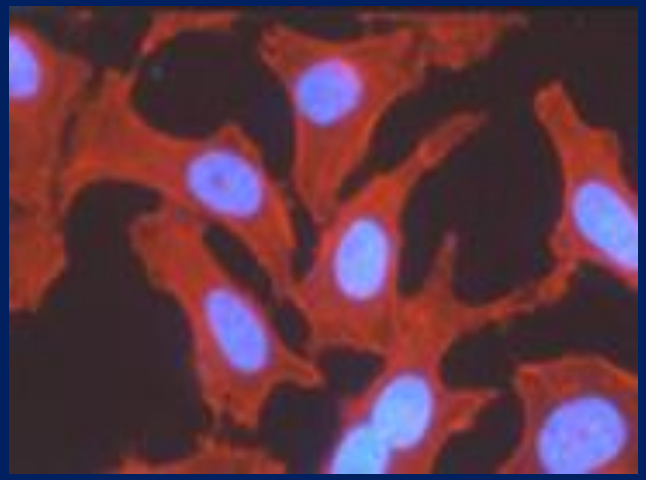
EPECwt
(72 h)

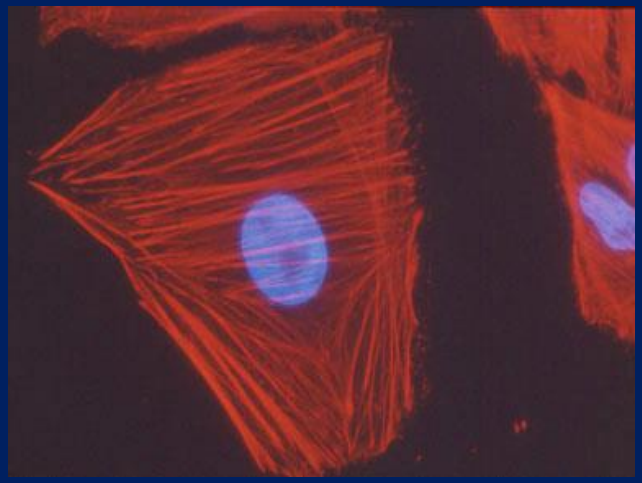
HeLa cells

CPE is a progressive and irreversible effect characterised by the induction of large mononucleated cells

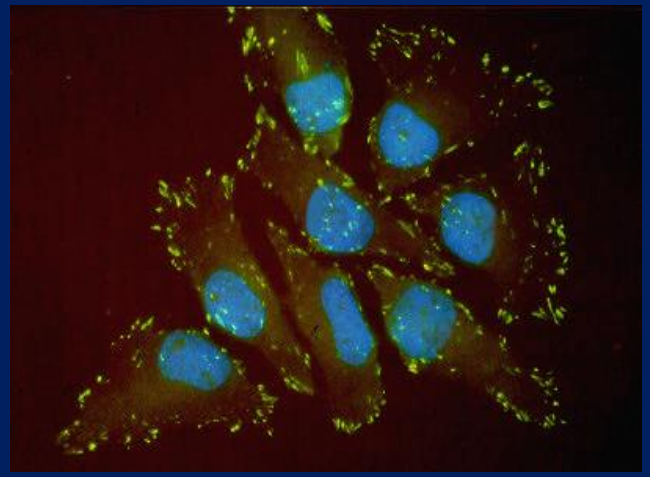
CPE: alterations of cytoskeleton

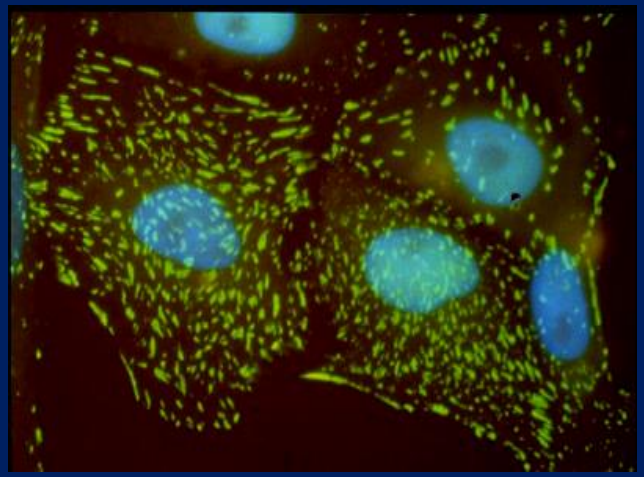
HeLa cells





Stress Fiber
(F-actin)



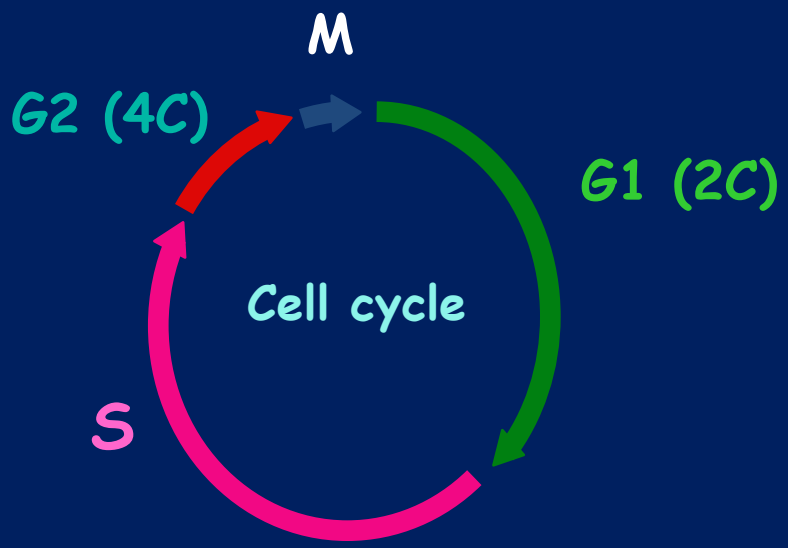


focal adhesions
(vinculin)

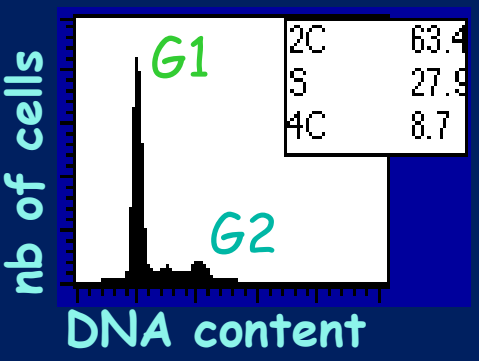
control
(72h)

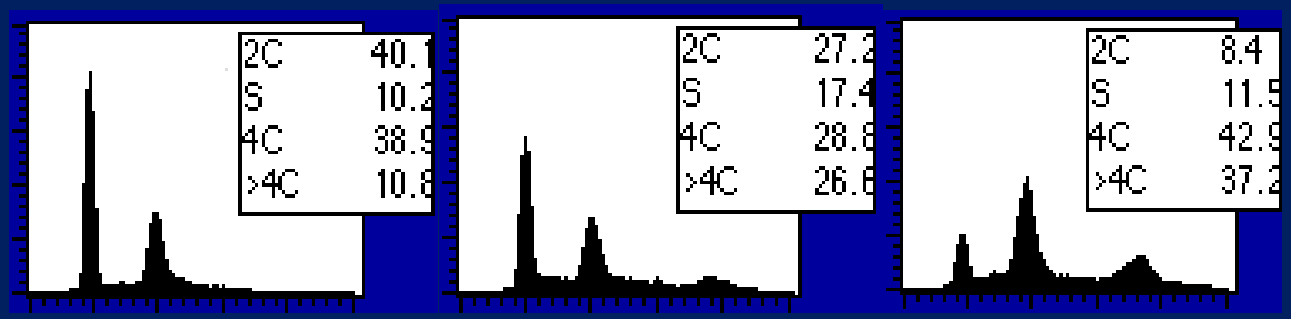
EPEC
(72h)

CPE: inhibition of mitosis



control (72h)

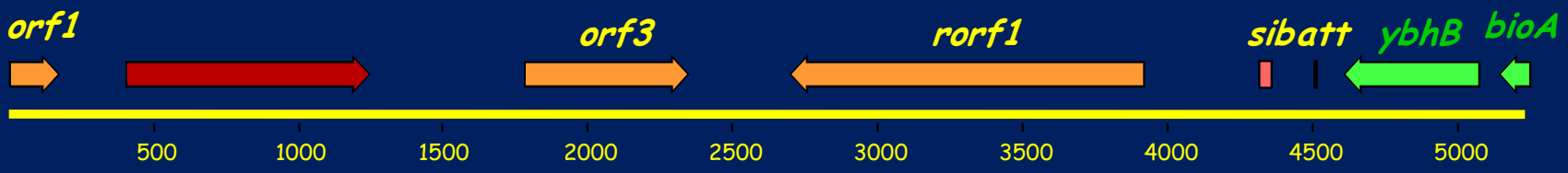




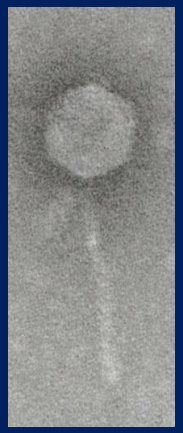
EPEC (24h) (48h) (72h) HeLa cells

Cif is encoded by a lambdoid prophage present in EPEC and EHEC

(Cycle Inhibiting Factor)
CIF

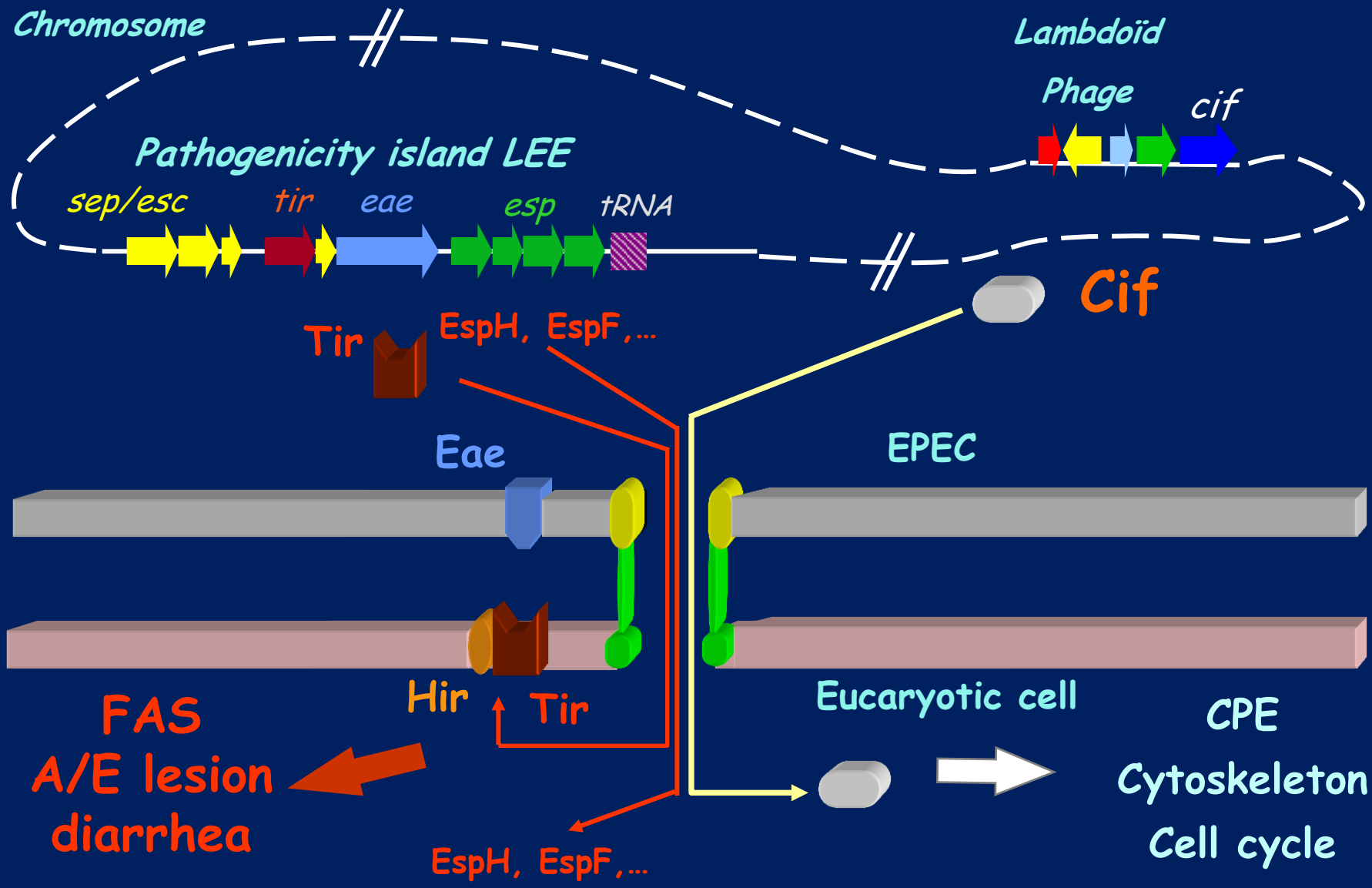


orf1, orf3 and rorf1 are similar to lambda-phages genes present in EHEC O157:H7 strains EDL933 and Sakai.



Genes ybhB and bioA are part of the bio operon located at 17.3 min in the K12 chromosome

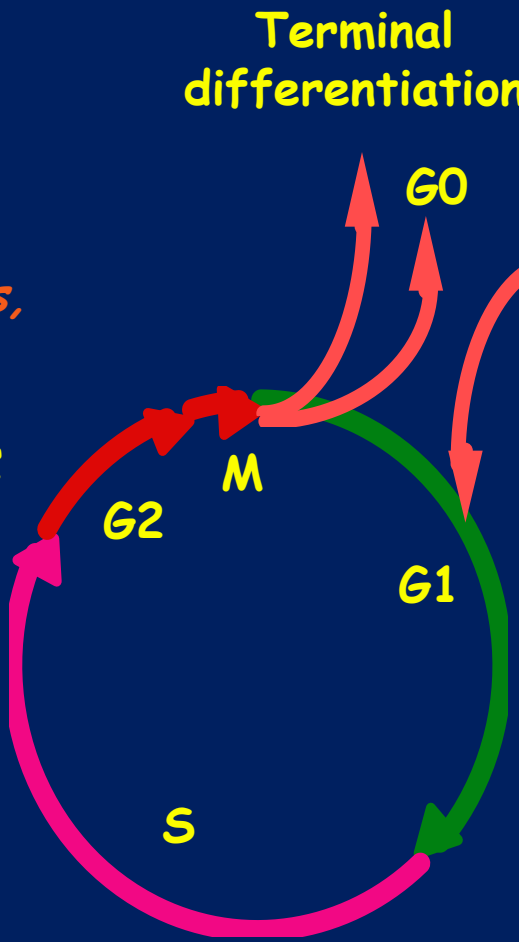
Cif a novel AEEC type III effector not coded by the LEE



Cif belongs to the expending family of virulence factors subverting the host cell cycle

CDT produced by *E. coli, C. jejuni, A. actinomycetemcomitens, H. ducreyi* etc.

CIF produced by AEEC



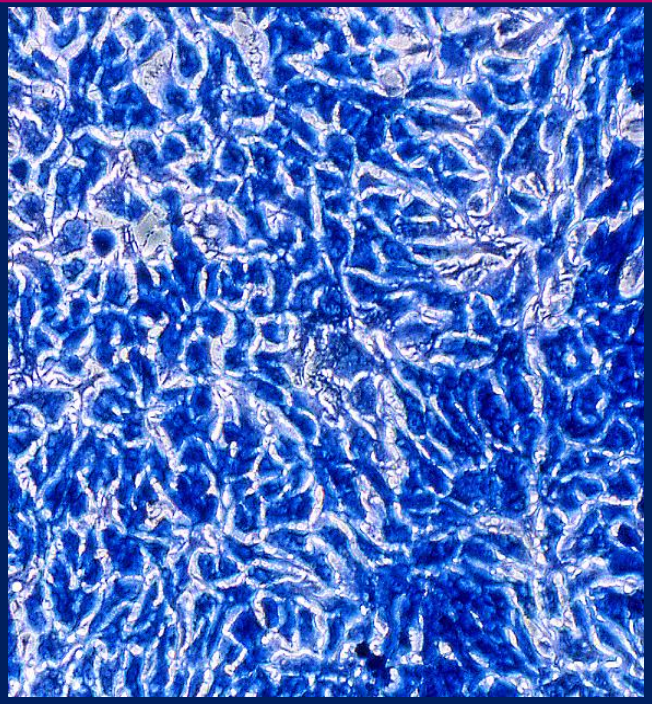
CNF produced by *E. coli*
DNT produced by *Bordetella*
PMT produced by *P. multocida*

Fip produced by *F. nucleatum*

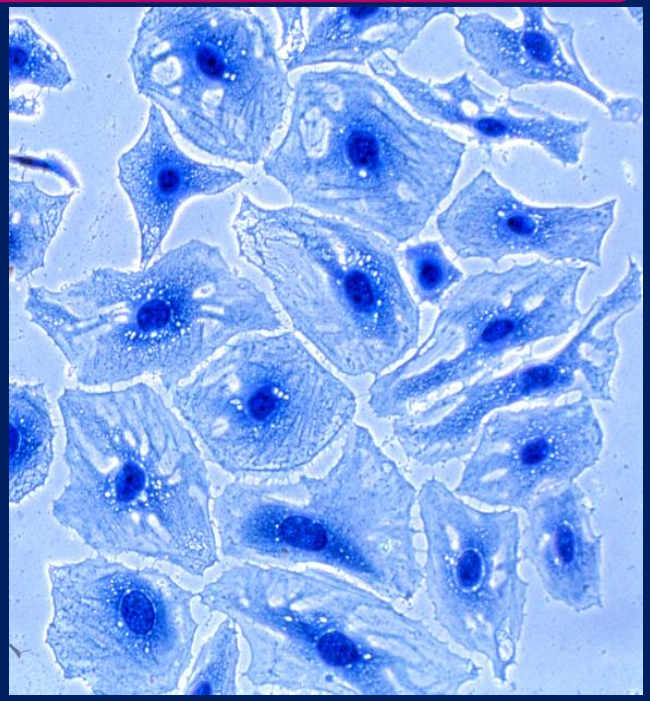
H. pylori

Colibactin, a hybrid peptide-polyketide genotoxin produced by *Escherichia coli*

CytoPathic Effect (CPE) produced by NewX 72h after the interaction

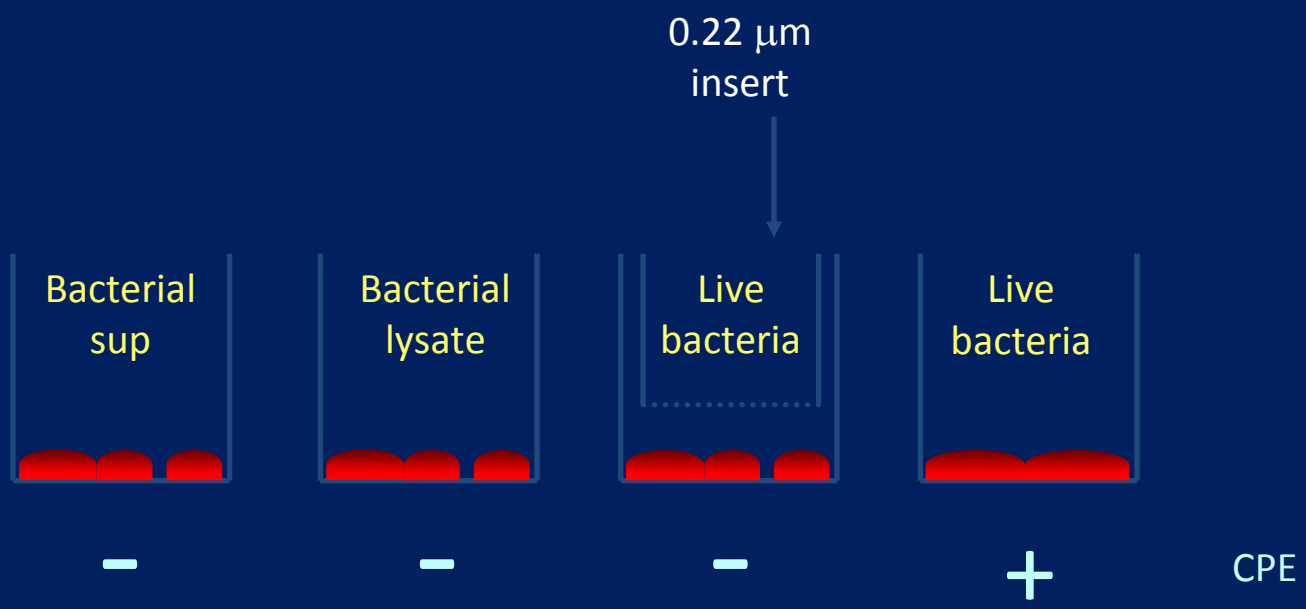


HeLa cells previously infected with *E. coli* MG1655

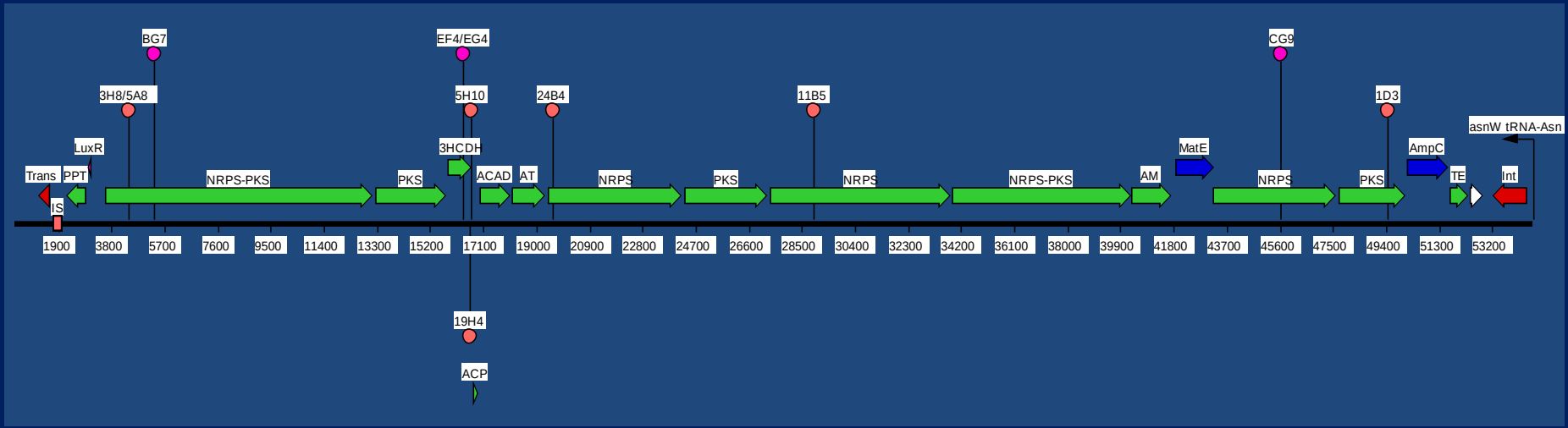


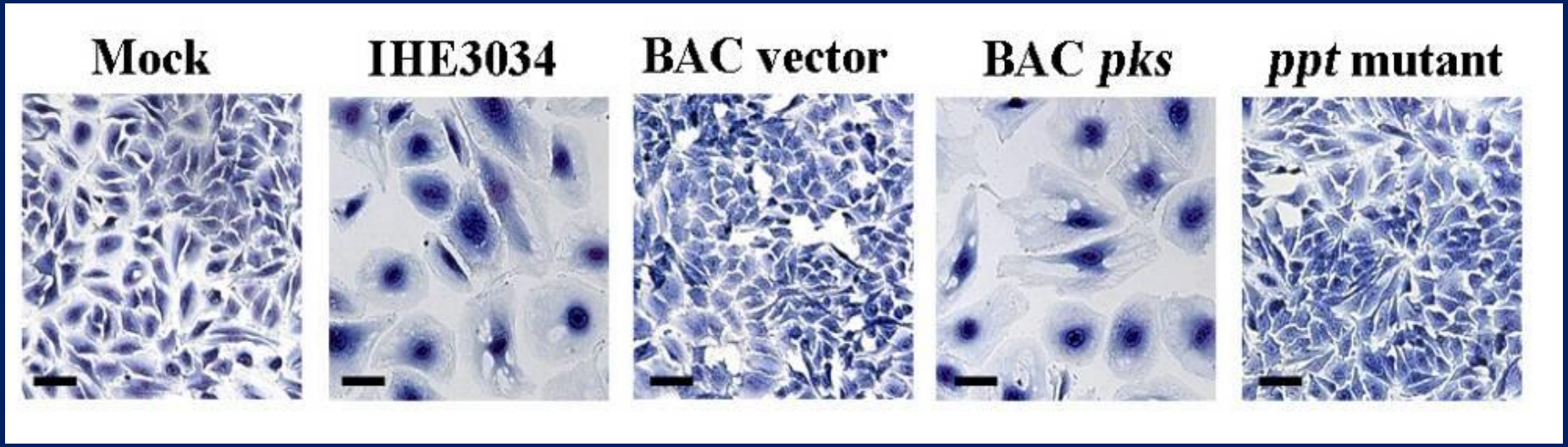
HeLa cells previously infected with ExPEC strain IHE3034

CPE-triggering is “contact-dependent”

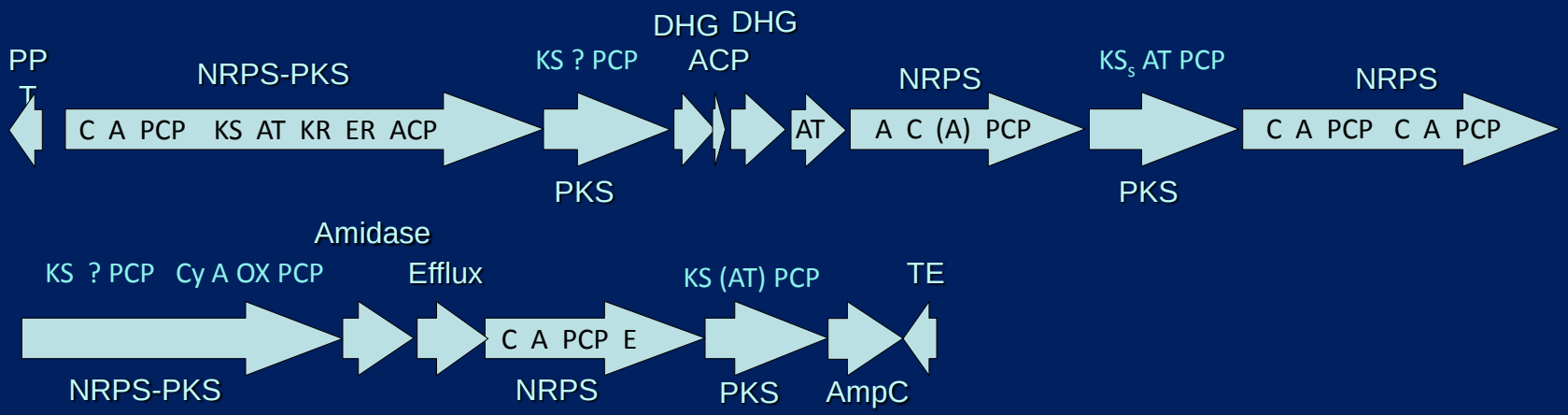


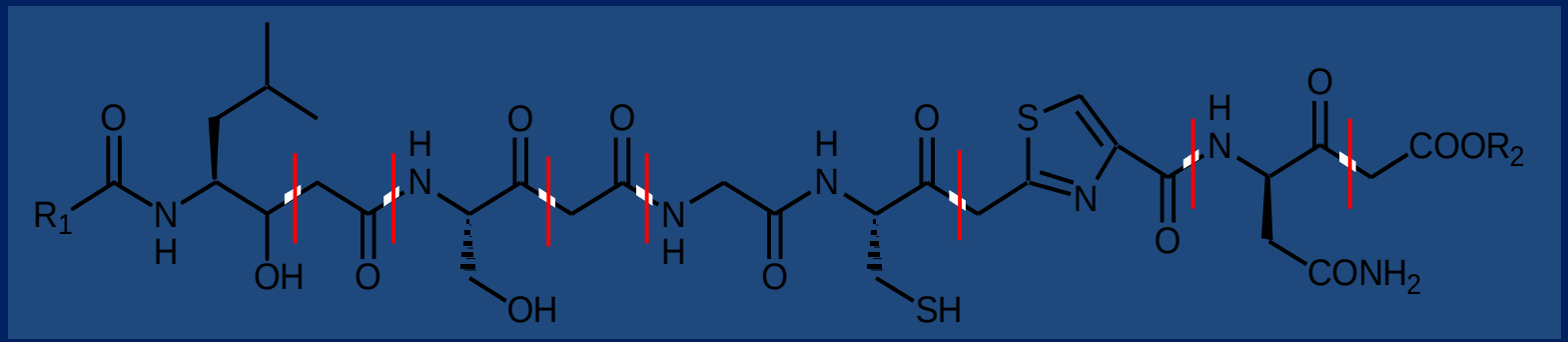
A genomic island confer cytopathic activity





This cluster allows synthesis of a secondary metabolite: A polyketide-peptide hybrid compound

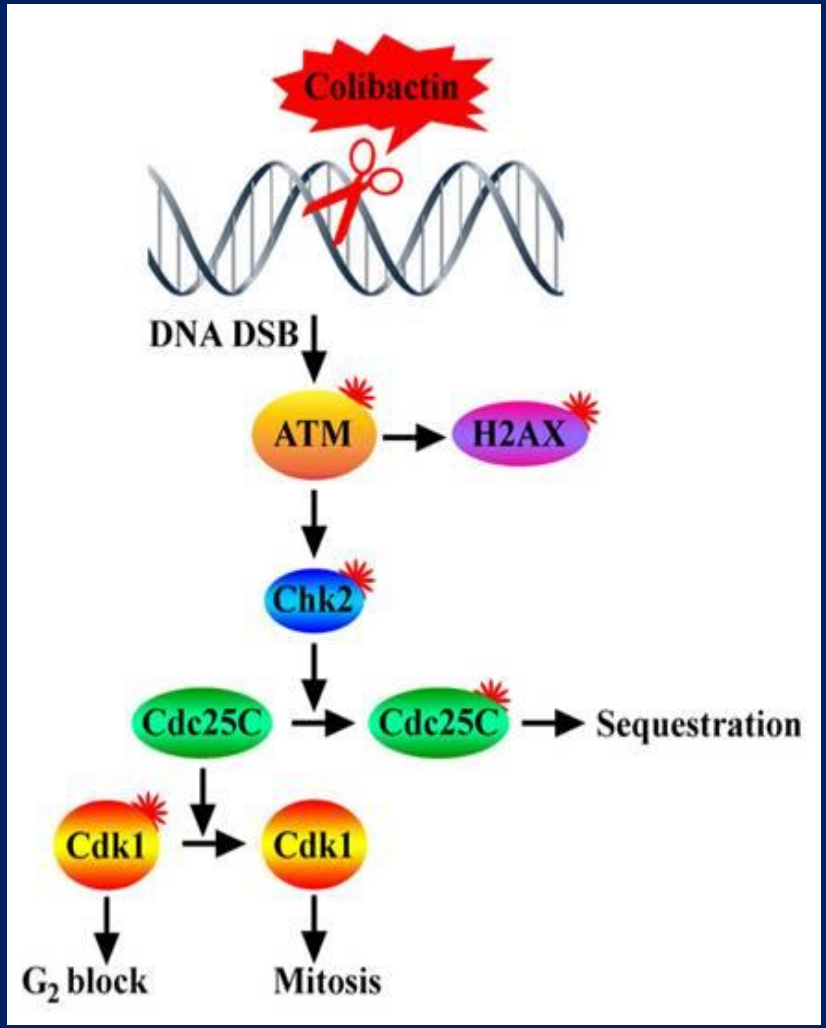




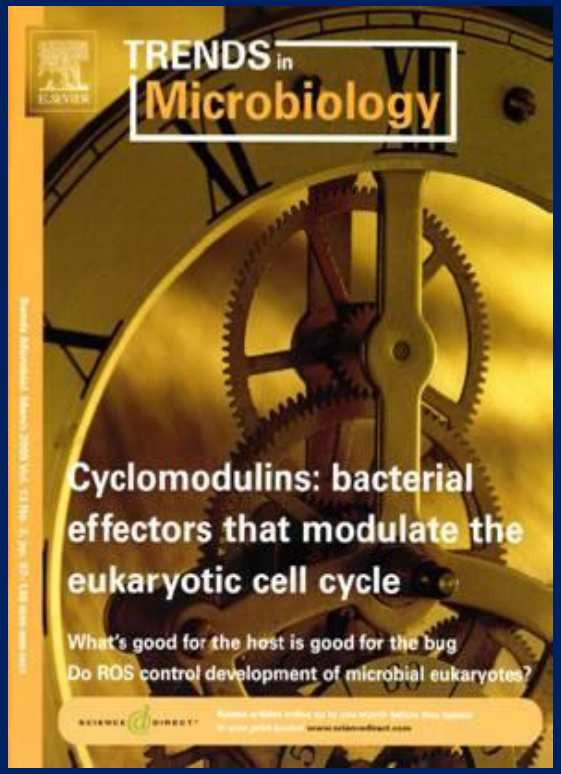
Predicted structure

Colibactin

Colibactin mode of action



The cyclomodulins



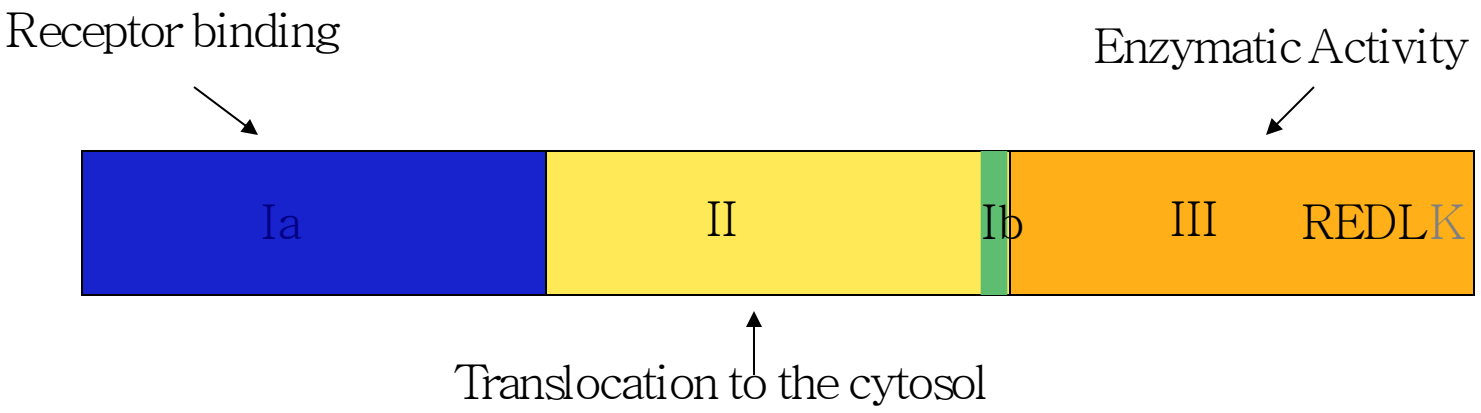
Inhibitors

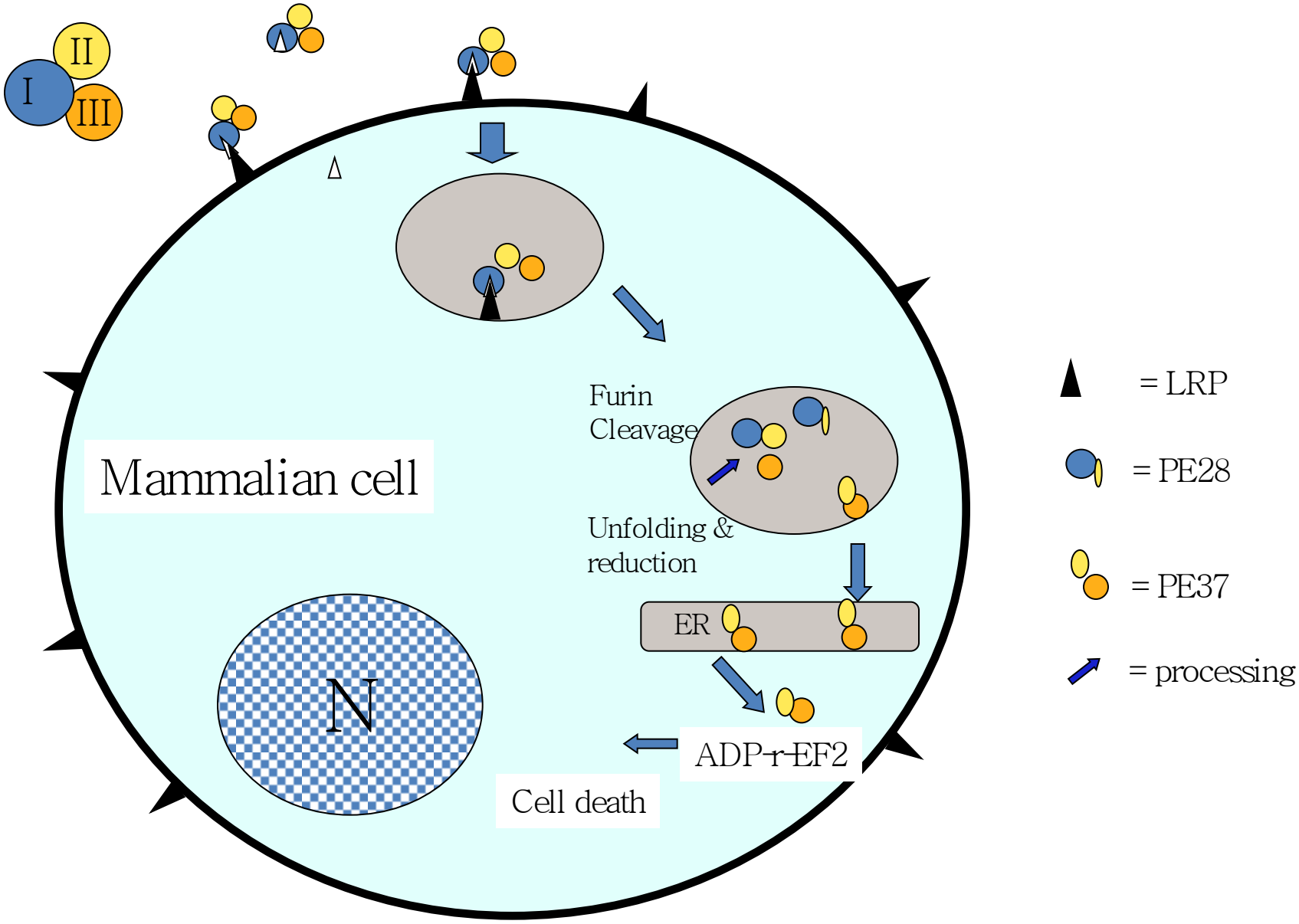
Activators

Cif
p21
Cdc25C
Chk2
ATM
DNA damage
CDT-B
A
C
Coli bactine
G2 checkpoint (CyclinB Cdk1-P)
Terminal differentiation
G_0
M
G_2
G_1
S
HOST CELL CYCLE
G1 checkpoint (Cyclin Cdk)
VacA
p21
Myco lactone
Cif
antiproliferative
CagA
Grb2
SHP2
Ras
MAPK
PMT
Gq
Rho-GTP
Deamidation
CNF
DNT
prioliferative

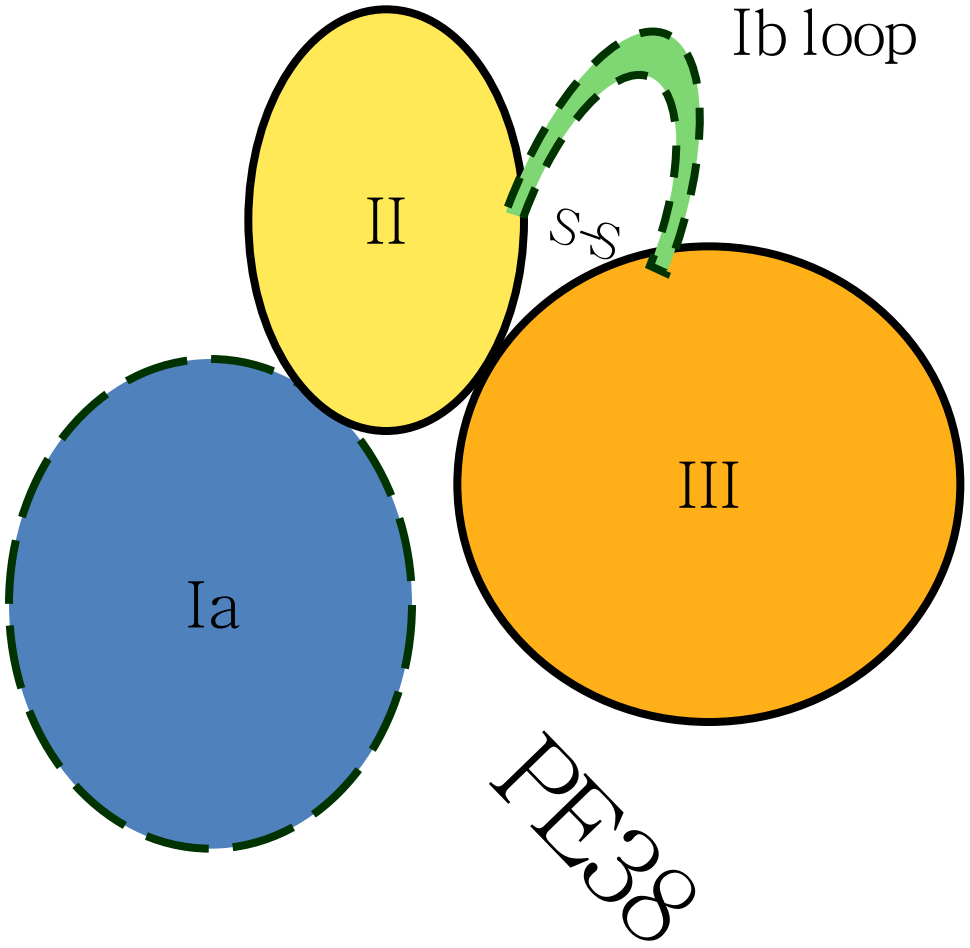
Recombinant Immunotoxins for the Treatment of Cancer.

Functional domains of Pseudomonas Exotoxin A




II
I
III
Mammalian cell
Furin
Cleavage
Unfolding &
reduction
ER
N
ADP-r-EF2
Cell death
= LRP
= PE28
= PE37
= processing

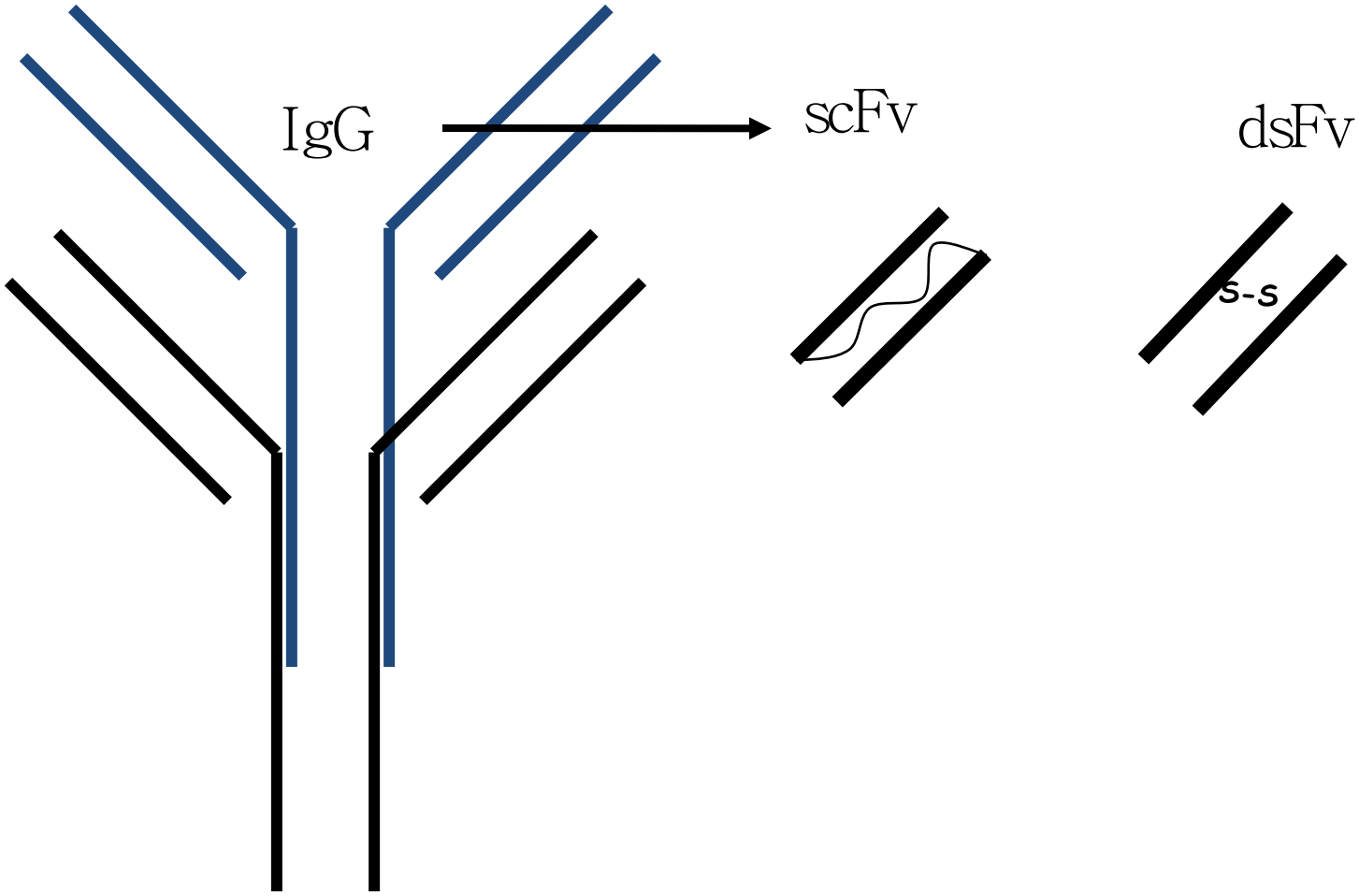
Structure of Pseudomonas Exotoxin



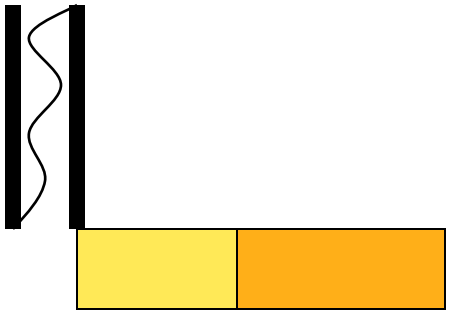
This is a 3-part problem.

- Antibody - recombinant fragment.
- Toxin - bacterial.
- Target - cell surface -allow internalization, not shed and expressed on every cell.

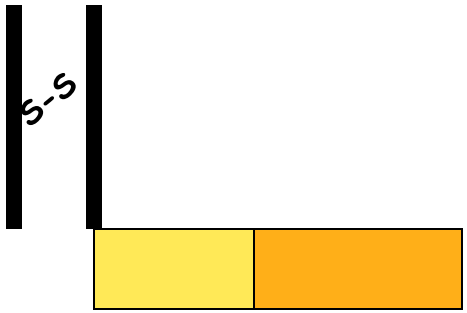
Generation of recombinant antibody fragments.



Antibody-toxin fusions - recombinant Immunotoxins



scFv-PE38



dsFv-PE38

Surface Target	Recombinant Immunotoxin	Cancer type
Lewis y	LMB1, LMB7 & LMB9	Epithelial cell Cancers (colon, stomach, breast etc)
CD25	LMB2	Leukemias (ATL, CLL etc)
CD22	BL22	Leukemias & Lyphomas (HCL, CLL, ALL, NHL etc)
Mesothelin	SS1	Ovarian and Mesothelioma
EGF-R (mut)	MR1	Glioblastoma multiforme
CD30	???	Hodgkin's Disease

Surface Target	Toxin Agent	Cancer-related Target.
EGF-R	TGFα-PE38	brain tumors
IL4/IL13	IL4/13-PE38	brain and kidney tumors
NK1-R	SubP-PE35	Neurons CNS - pain control

Clinical development of the recombinant Immunotoxin, BL22.

CD22 as a surface target.

- Primarily a B-cell marker.
- Expressed on mature B-cells and B-cell malignancies but not on precursors/stem cells.
- Lectin - involved in adhesion of activated B-cells.
- Co-receptor with BCR.
- Internalized efficiently - not shed.

Phase I trial - patient eligibility.

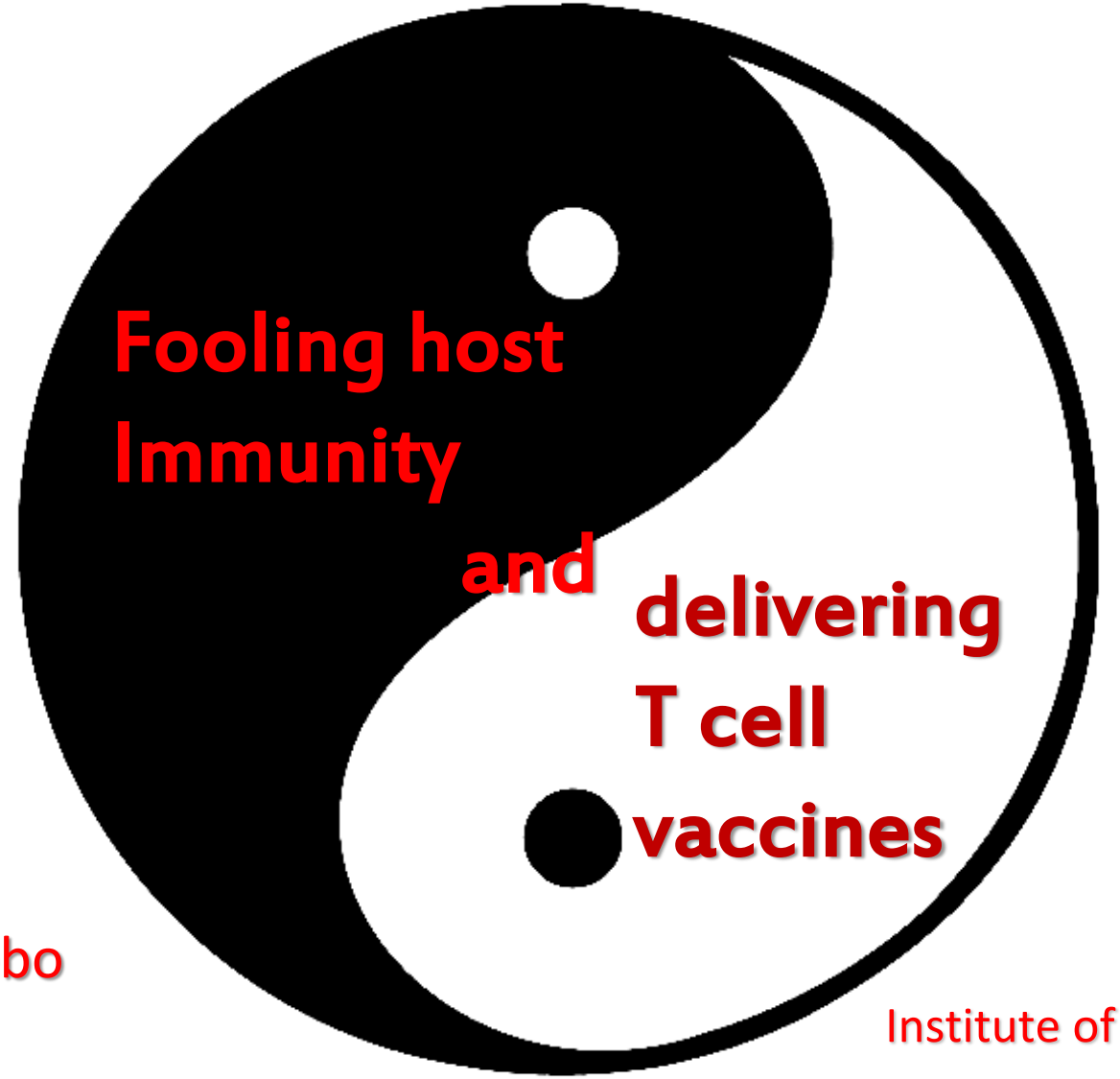
- Diagnosis of B-cell leukemia/lymphoma
- Failed standard treatments
- CD22-positive malignant cells
- Adequate hepatic, renal and pulmonary function
- Absence of CNS disease
- No pre-existing neutralizing antbodies

BL22 - summary.

Complete remissions were obtained in the majority of patients with HCL.

Stable disease and partial remissions were seen with CLL patients. CLL - fewer CD22 sites on their leukemic cells.

THE YIN AND YANG OF A BACTERIAL TOXIN



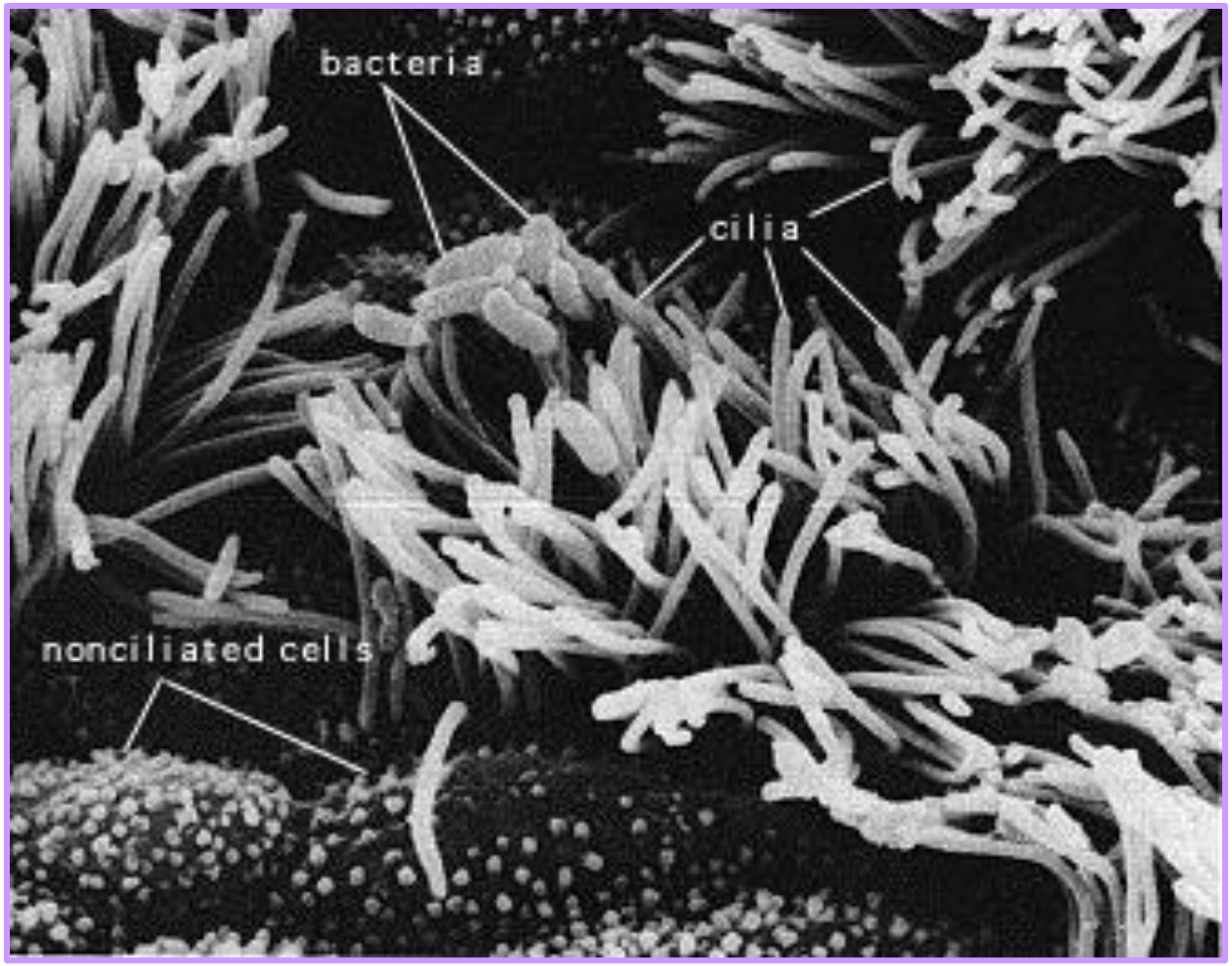
P. Šebo

PRAGUE
Institute of Microbiology

or:

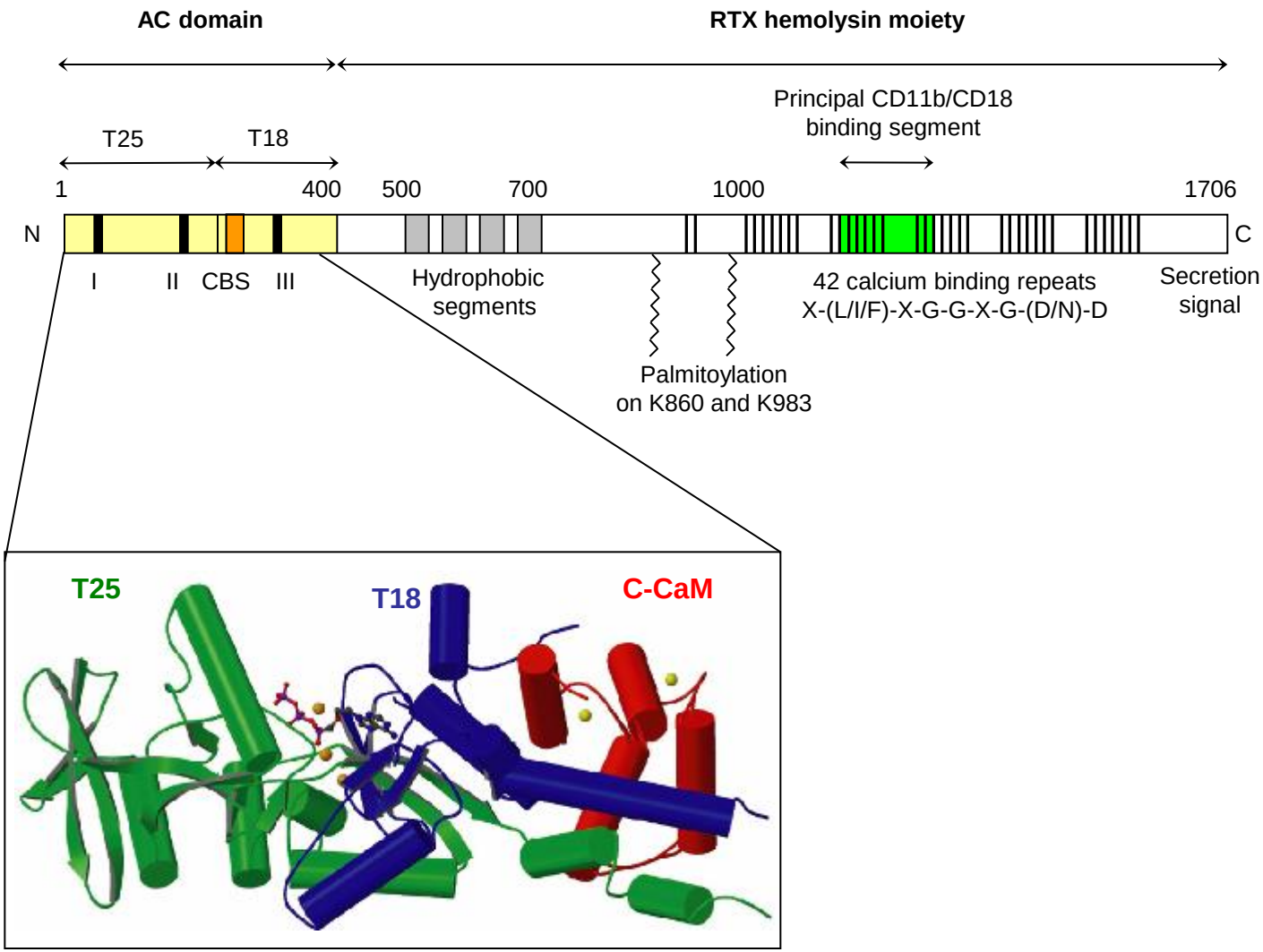
Drugs from Bugs…

Without Adenylate cyclase toxin *Bordetella pertussis* is avirulent



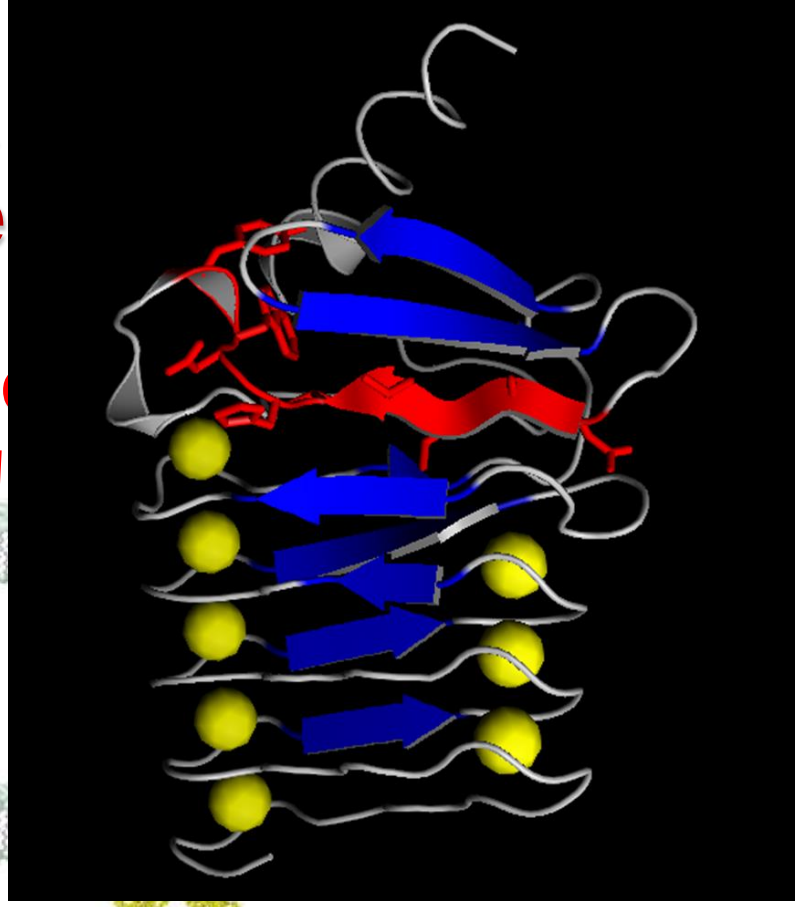
Colonisation of respiratory epithelium by *B. pertussis*
www.textbookofbacteriology.net

Adenylate cyclase toxin - cytolysin

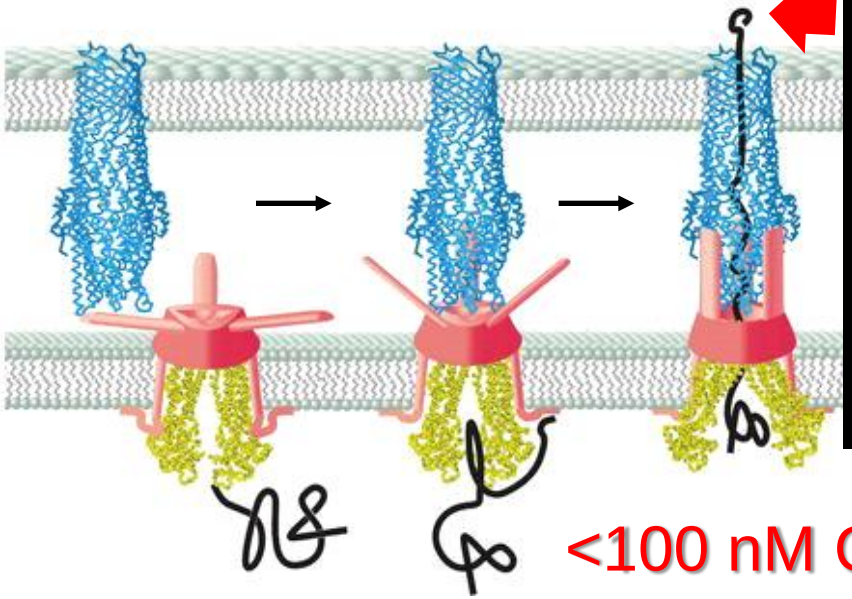


Guo Q. *et al.* (20005) EMBO J. 24, 3190–3201

ACT is an RTX
secreted by a type



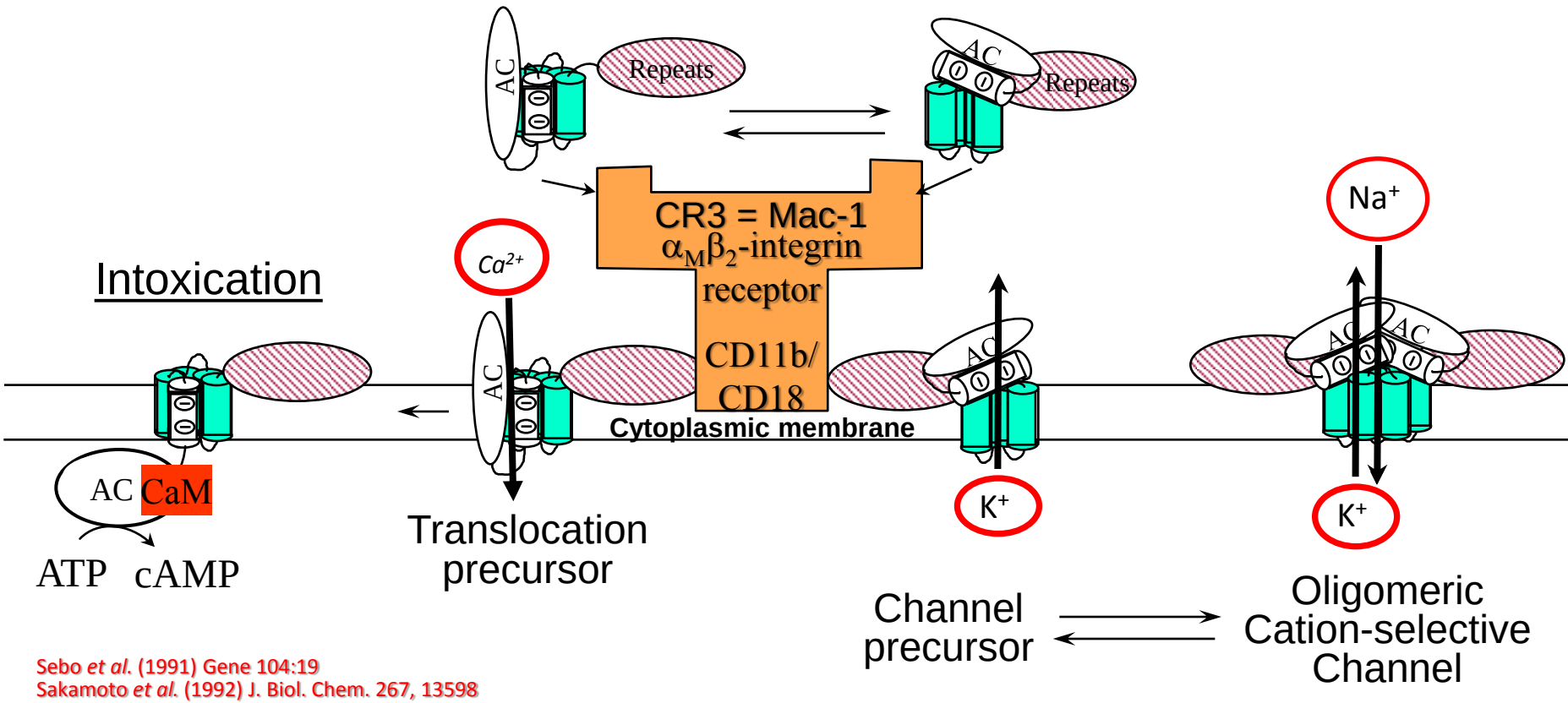
>100 μM



<100 nM Ca^{2+}

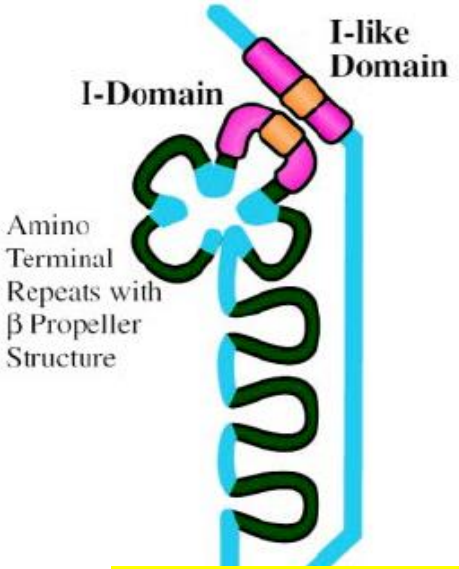
Need to unfold and refold
on the way to target…

The three cytotoxic activities of ACT adenylate cyclase toxin & pore-forming hemolysin/Cytolysin



Sebo *et al.* (1991) Gene 104:19
Sakamoto *et al.* (1992) J. Biol. Chem. 267, 13598
Benz *et al.* (1994) J. Biol. Chem. 269, 27231
Hackett *et al.* (1995) J. Biol. Chem. 270, 20250
Gray *et al.* (1998) J. Biol. Chem. 273, 18260
Osickova *et al.* (1999) J. Biol. Chem. 274, 37644
Basler *et al.* (2007) J. Biol. Chem. 282, 12419
Fiser R. *et al.* (2007) *J. Biol. Chem. 282, 2808*
Osickova *et al.* (2010) *Mol. Microbiol.* 75:15450-1562

ACT targets myeloid phagocytes bearing $\alpha_M\beta_2$ integrin CD11b/CD18



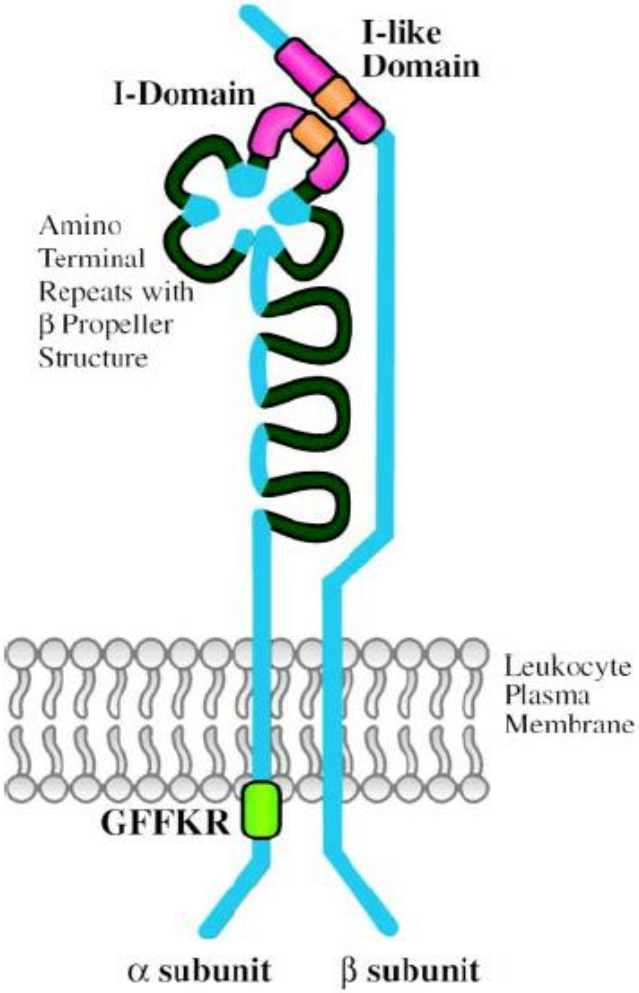
- β_2 subfamily
- **complement receptor 3** (CR3), Mac-1, Mo-1, $\alpha_M\beta_2$
- monocytes, granulocytes, macrophages, NK cells, neutrophils and **dendritic cells**, certain B cell subtypes

ACT first recognizes N-linked glycans of CD11b/CD18

Morova et al. (2008) PNAS 105, 5355

Guermonprez et al. 2001, J Exp. Med.

ACT targets myeloid phagocytes bearing $\alpha_M\beta_2$ integrin CD11b/CD18



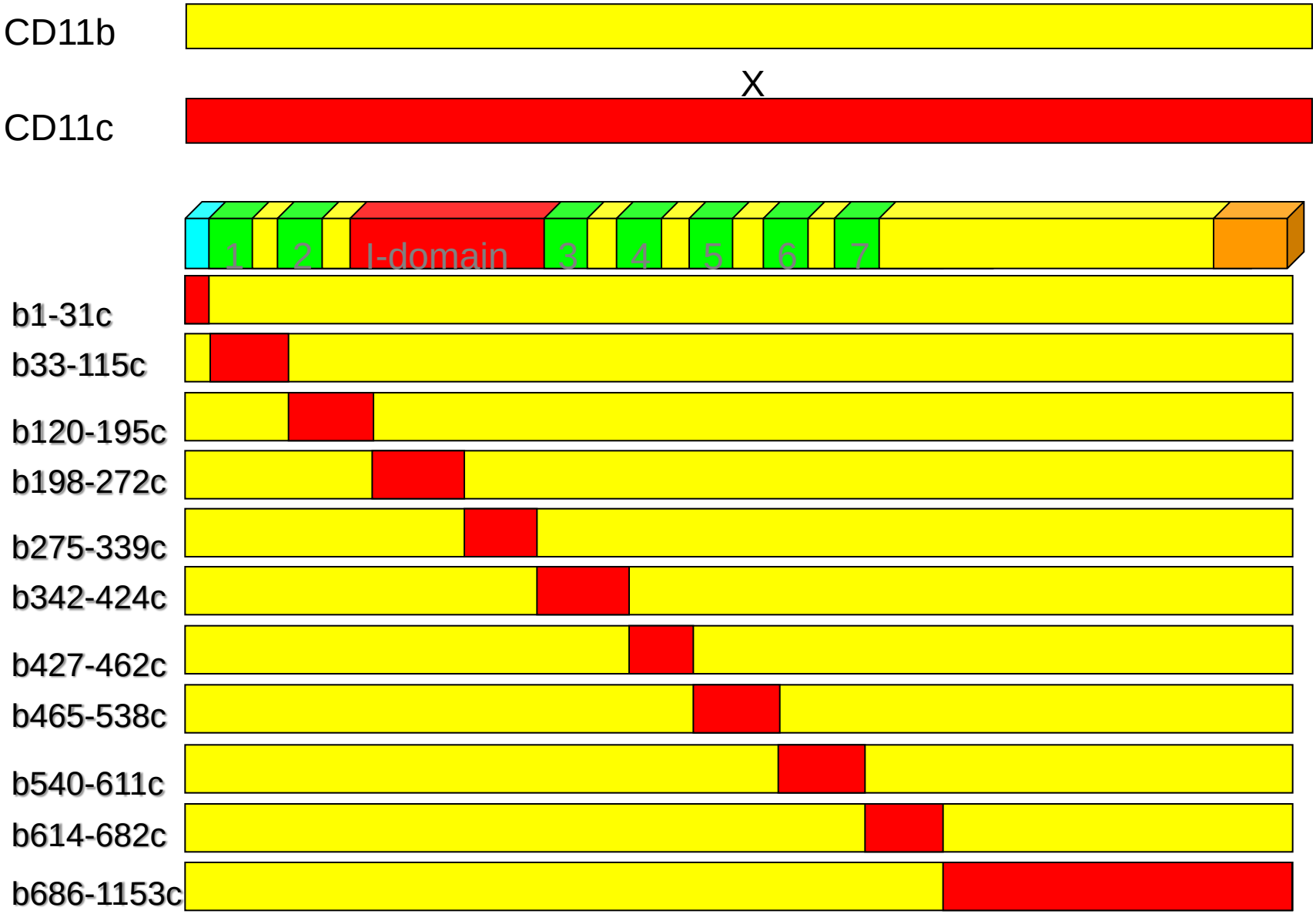
- β_2 subfamily
- **complement receptor 3** (CR3), Mac-1, Mo-1, $\alpha_M\beta_2$
- monocytes, granulocytes, macrophages, NK cells, neutrophils and **dendritic cells**, certain B cell subtypes

Guermonprez et al. 2001, J Exp. Med.

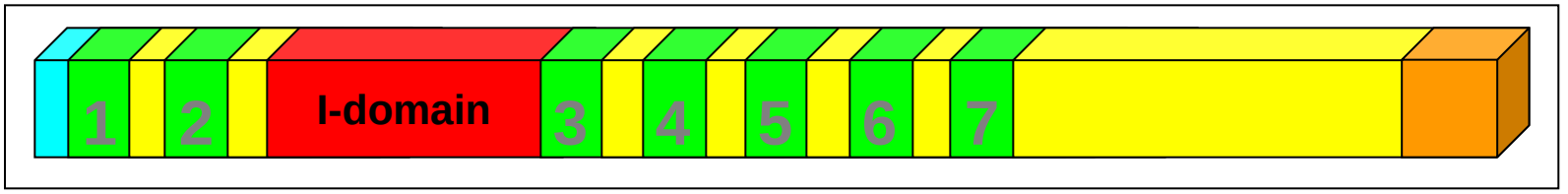
Selectivity of ACT for the given $\alpha_M\beta_2$ integrin is, however, dictated by a CD11b-specific segment …

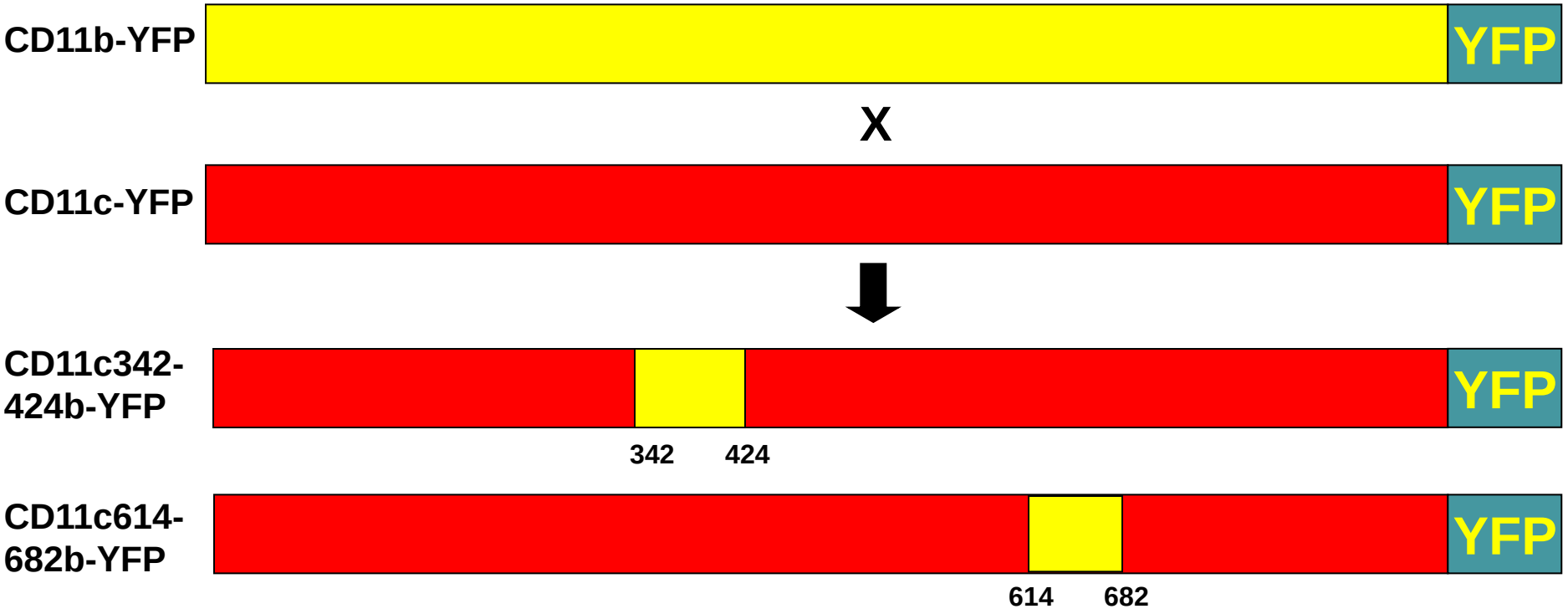
Radim Osicka – Poster No. 39

Mapping ACT binding site using CD11b/CD11c chimaeras

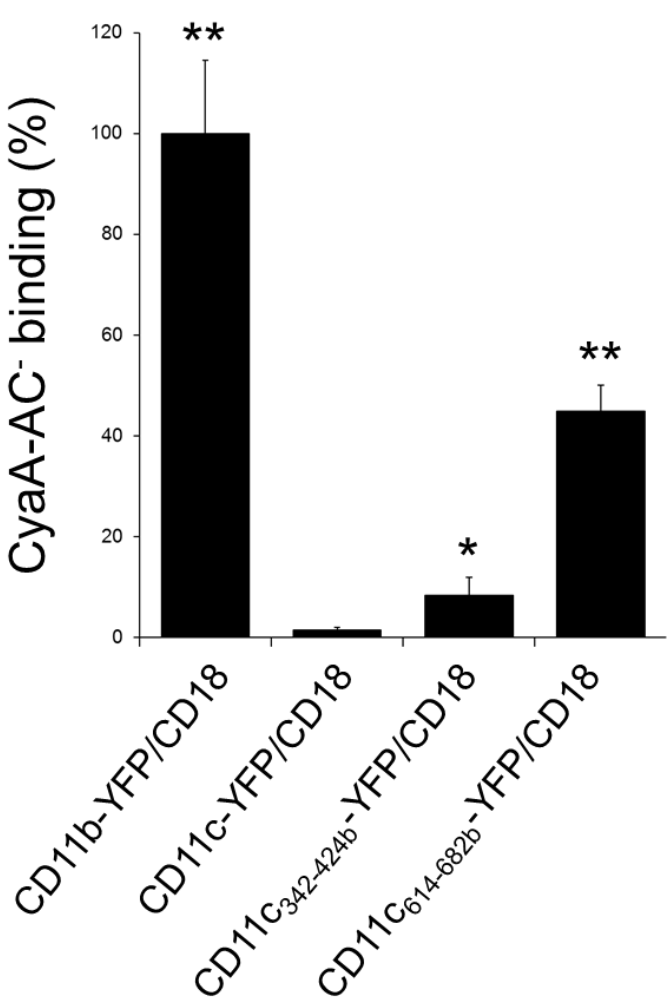


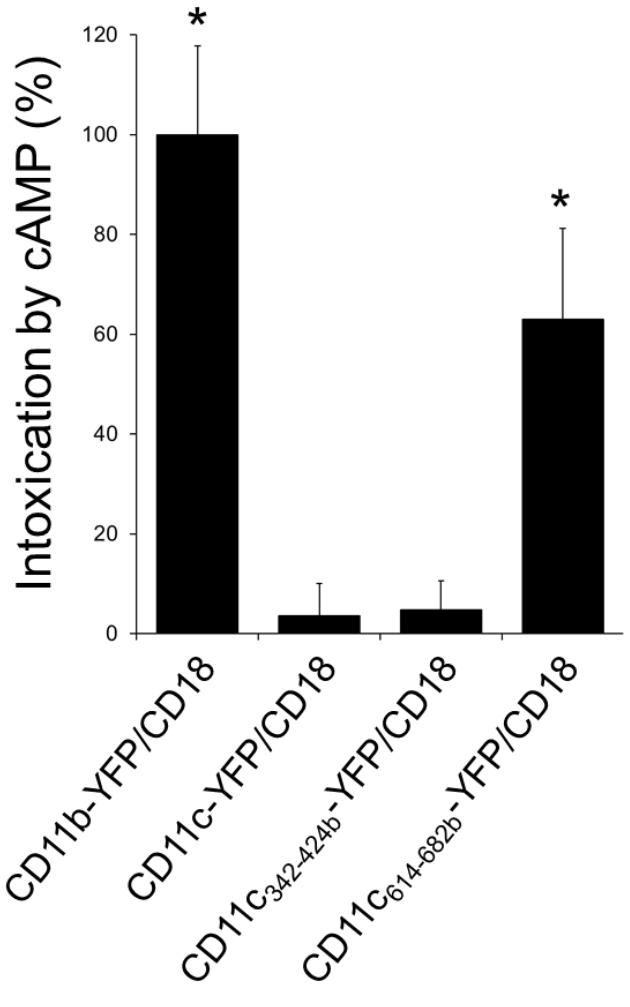
Construction of CD11c harboring residues 342-424 and/or 614-682 replaced with homologous segments of CD11b



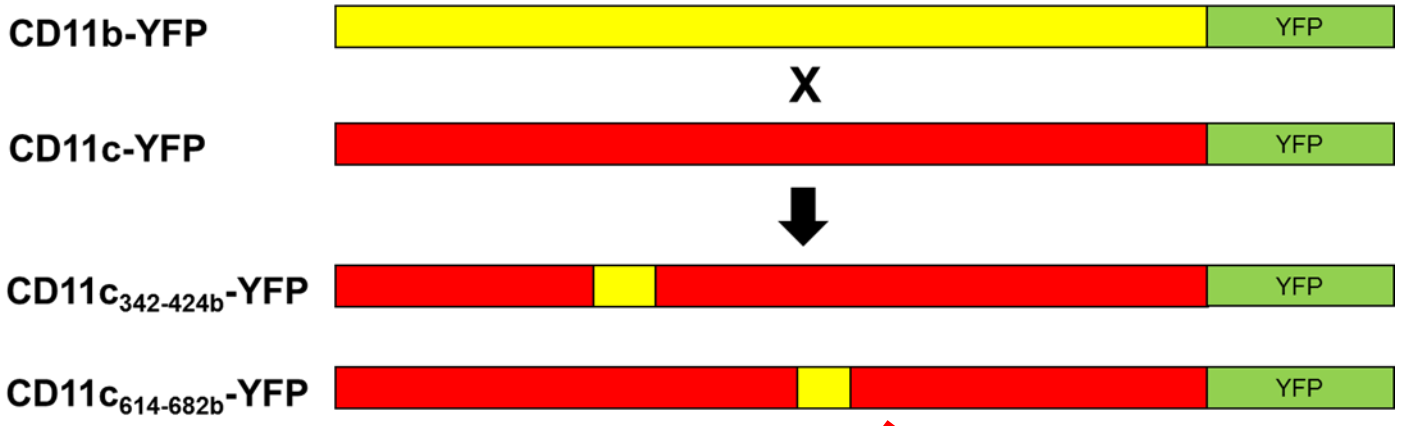


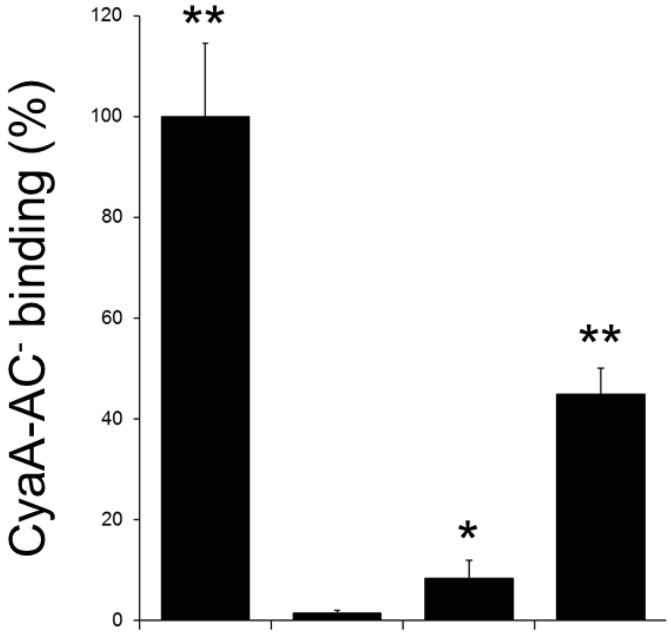
The CD11c integrin with 'transplanted' residues 614-682 of CD11b productively binds CyaA

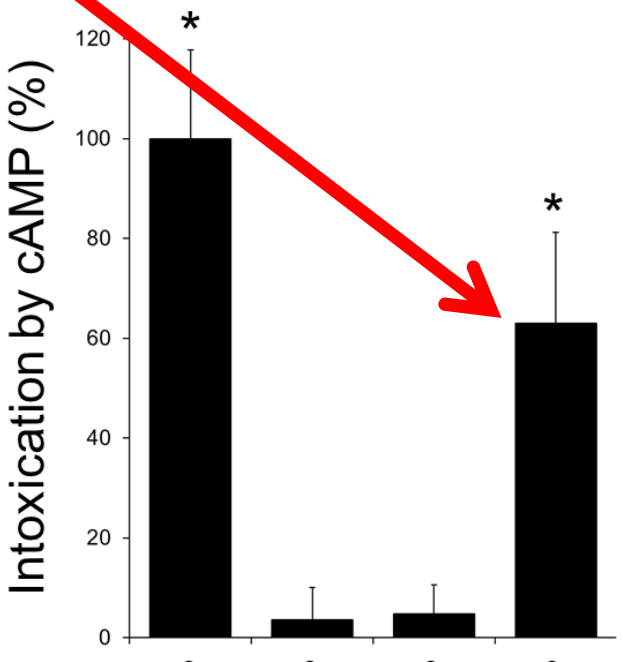




The CD11c integrin with ‘transplanted’ residues 614-682 of CD11b productively binds CyaA



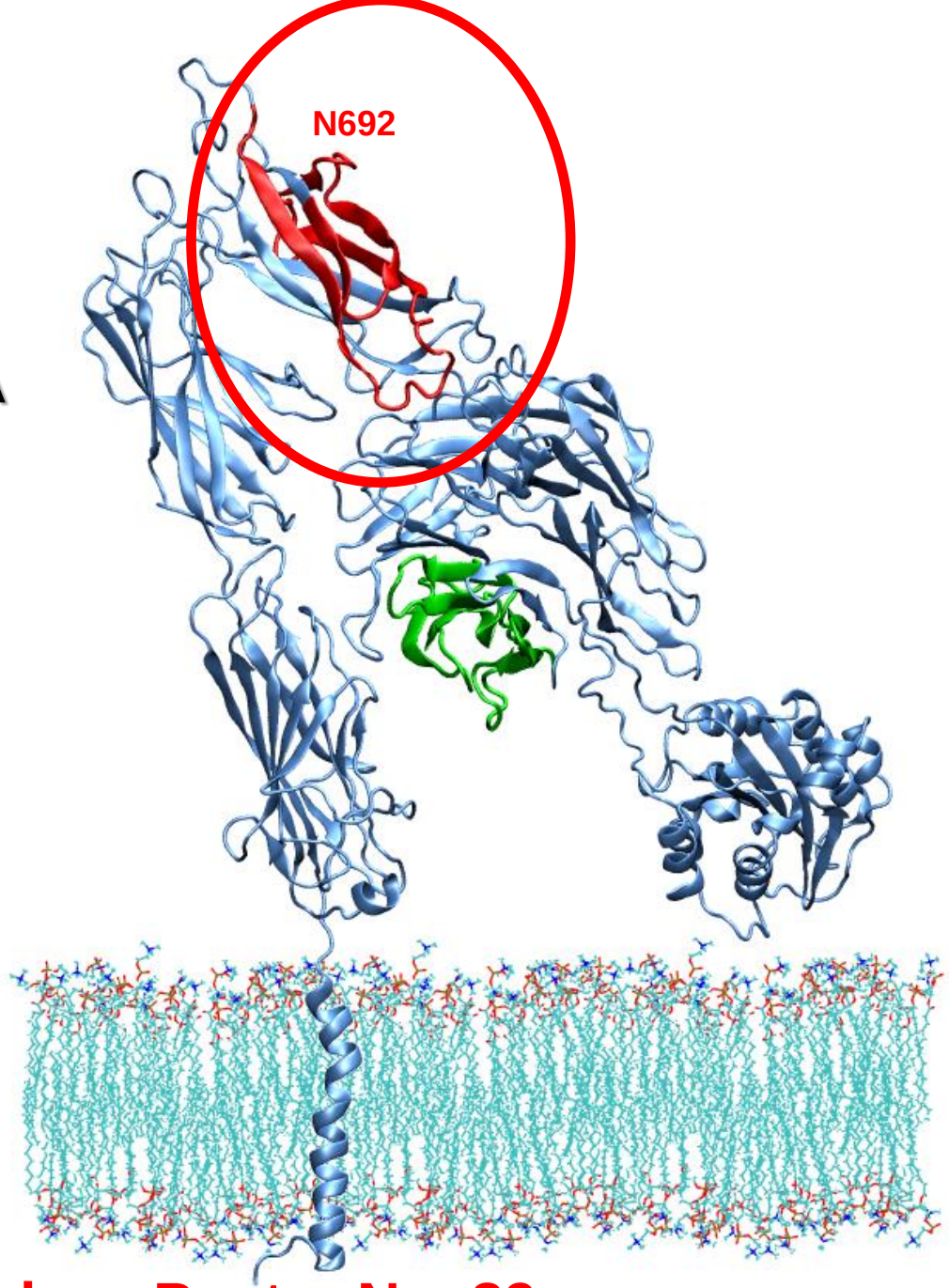




Proteinaceous segments Specifically involved in CyaA binding:

CD11b – residues 614-682

CD11b - residues 342-424

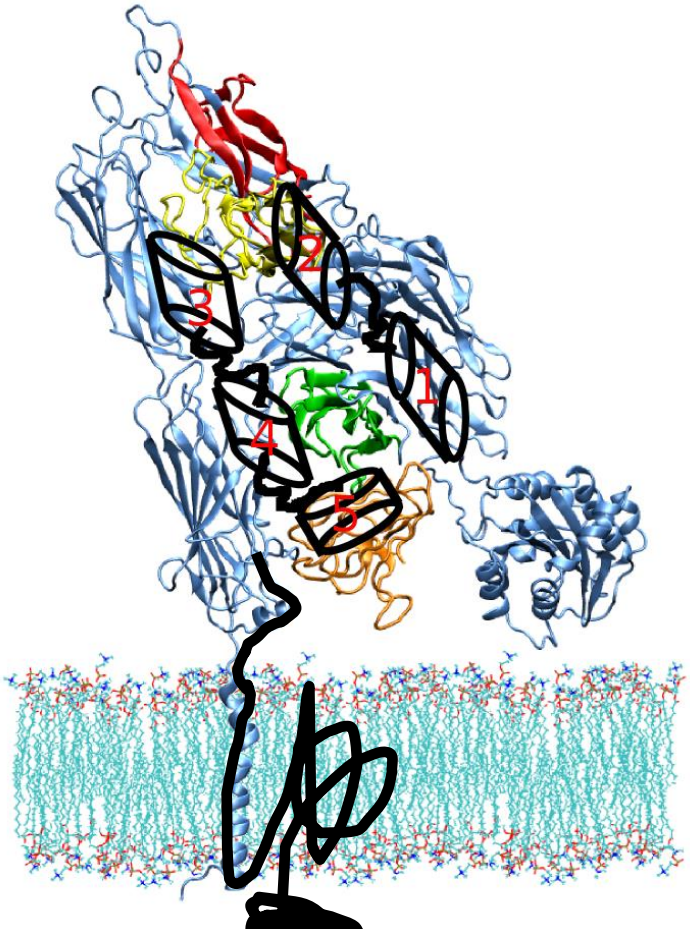


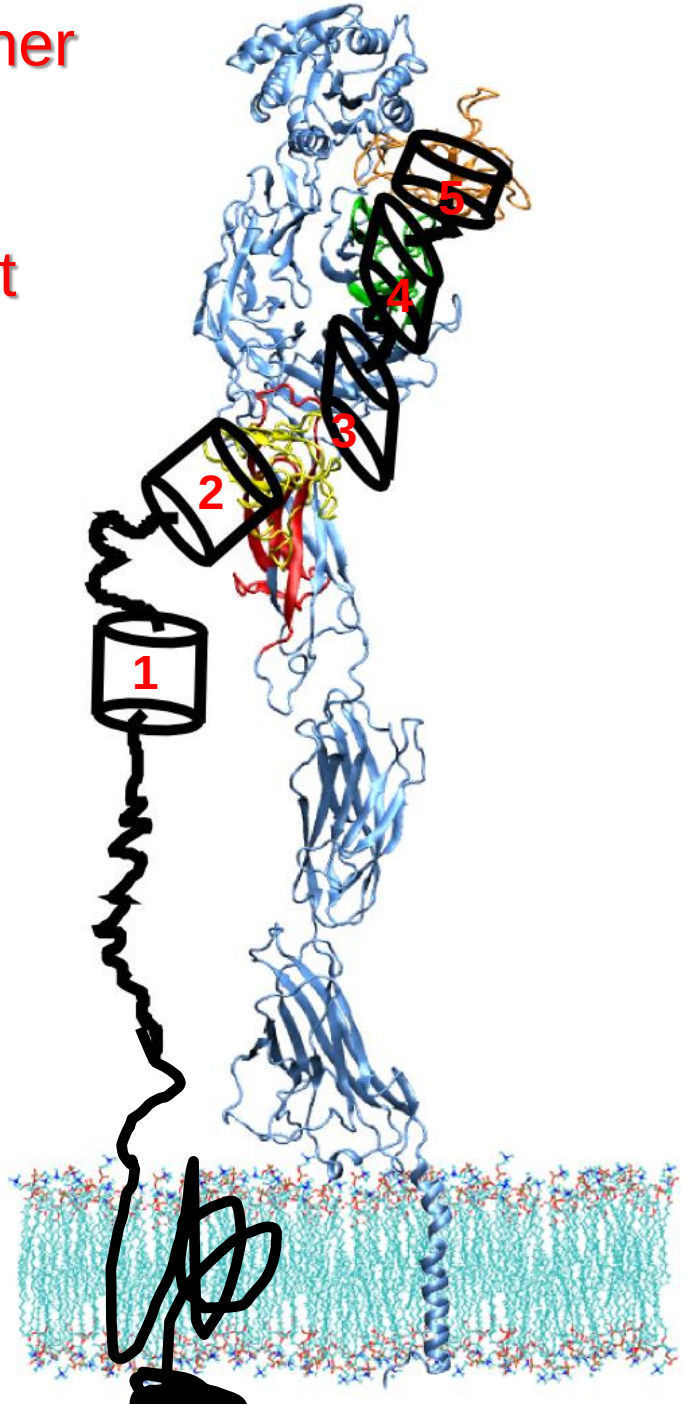
Radim Osicka – Poster No. 39

CyaA RTX domain – binds with 3 x higher affinity to ‘closed’ CD11b

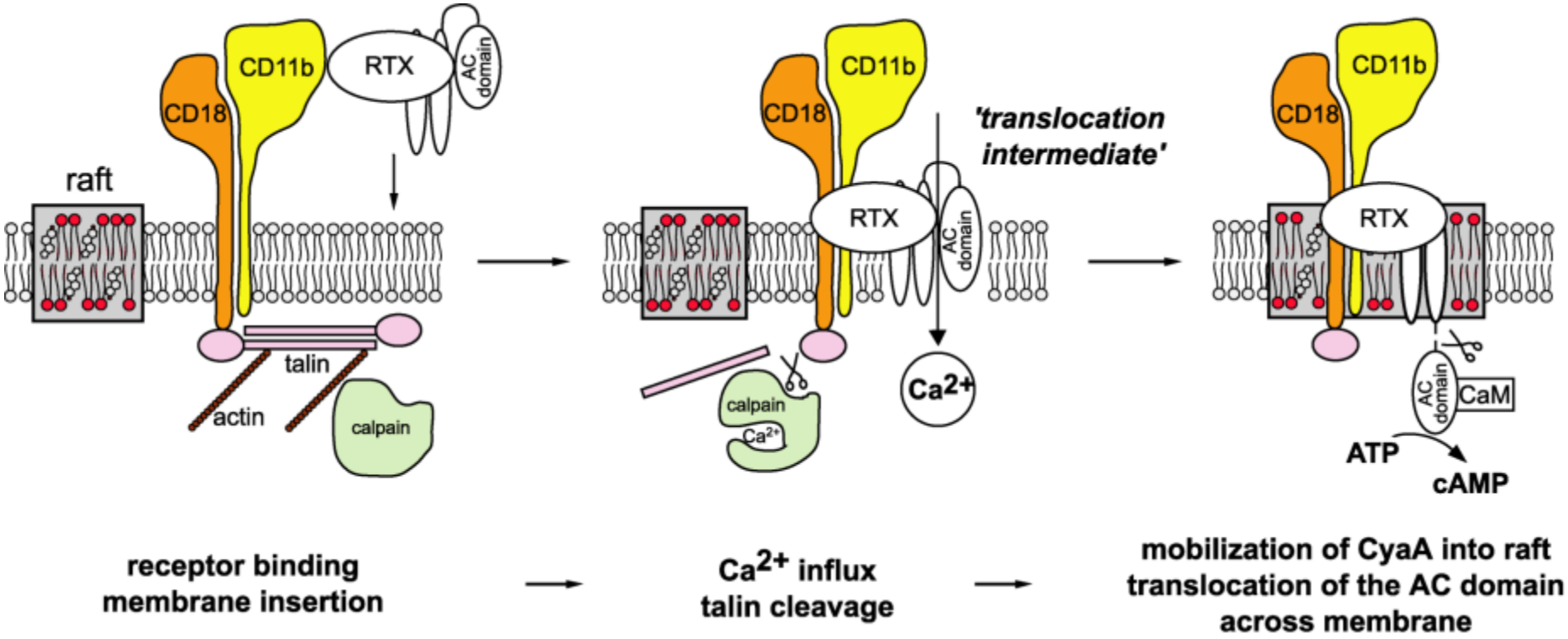
Subversive toxin binding occurs without integrin Syk/Src signaling activation…

(**Radim Osicka – Poster No. 39)**



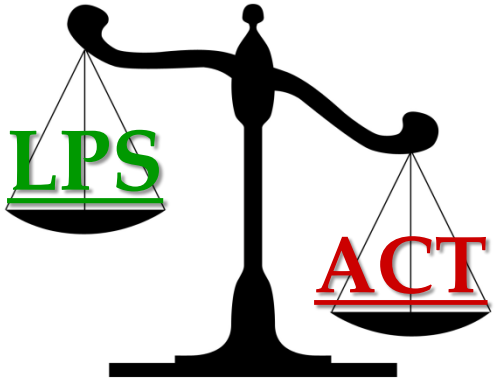


*A*denylate cyclase toxin hijacks the β_2 integrin receptor into lipid rafts to accomplish membrane translocation in two steps



Bumba et al. (2010). PLoS Pathog 6(5): e1000901.

ACT/cAMP signaling breakes the hell loose… and supresses TLR signaling of the bug…



↓ ciliary beating

defensins and other antimicrobial peptides:
hβdefensin2 ,
↓ βdefensin1,
↓ cathelicidin

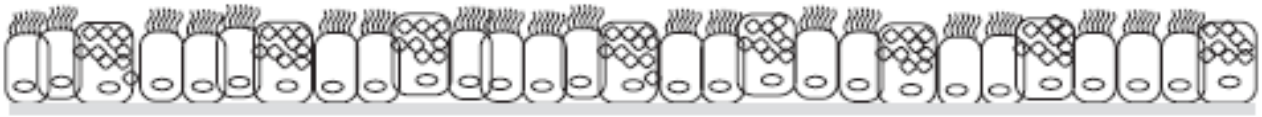
signal transduction events:
NF-κB, ↓ MAPK – p38, ERK, JNK

expression and upregulation of TLR:
TLR1-6, 9, TLR4, TLR2

mucin: MUC2, MUC5AC ↑

other soluble factors:
↓ O_2^-, NO, ↑ PGE2

AEC



cAMP

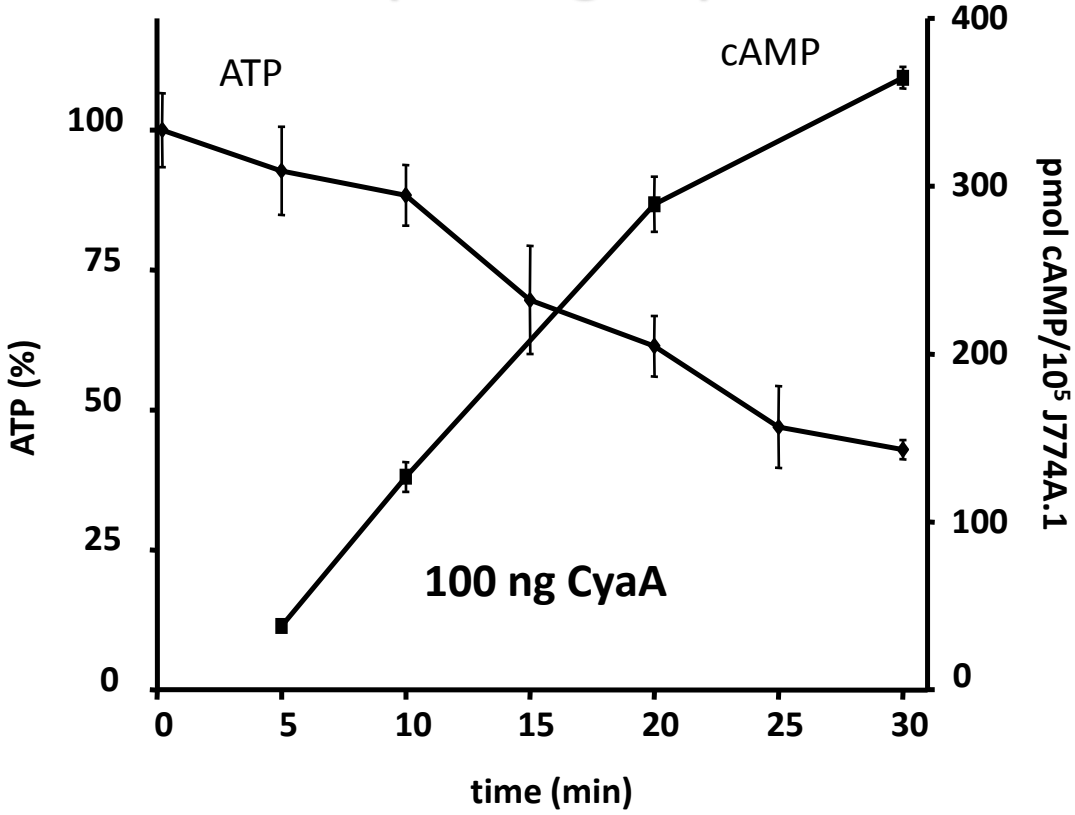
other cells

cytokine and chemokines:
IL-1α, ↑ IL-1β, ↑ IL-6, ↑ IL-8, ↑ IL-10, ↓ TNFα, ↓ IFNβ, TGF-β, ↓ GM-CSF, MCP-1, ↓ MIP-1α, RANTES,..

expression of costimulatory x inhibitory molecules: ↑ CD80, CD86, ↓ CD40, ↓ CD54, B7-H2, B7-H3 x ↑ FasL, PD-L1, PD-L2

Death of CD11b$^+$ cells induced by 'high' ACT doses (>50 ng/ml)

Cell lysis = **ATP depletion** + **pore-forming activity**



ATP depletion and pore-forming activity synergize in killing of CD11b$^+$ cells

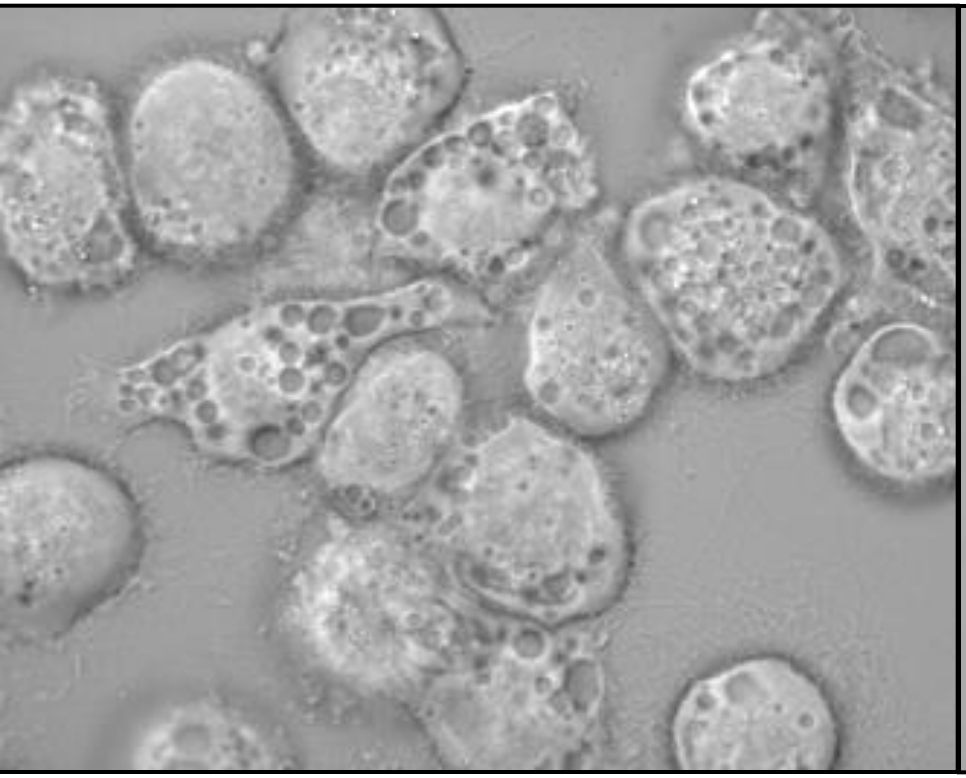
Basler *et al*., 2006, *Infect. Immun.*, 74, 2207-2214. .

High toxin dose - Emmentaler…

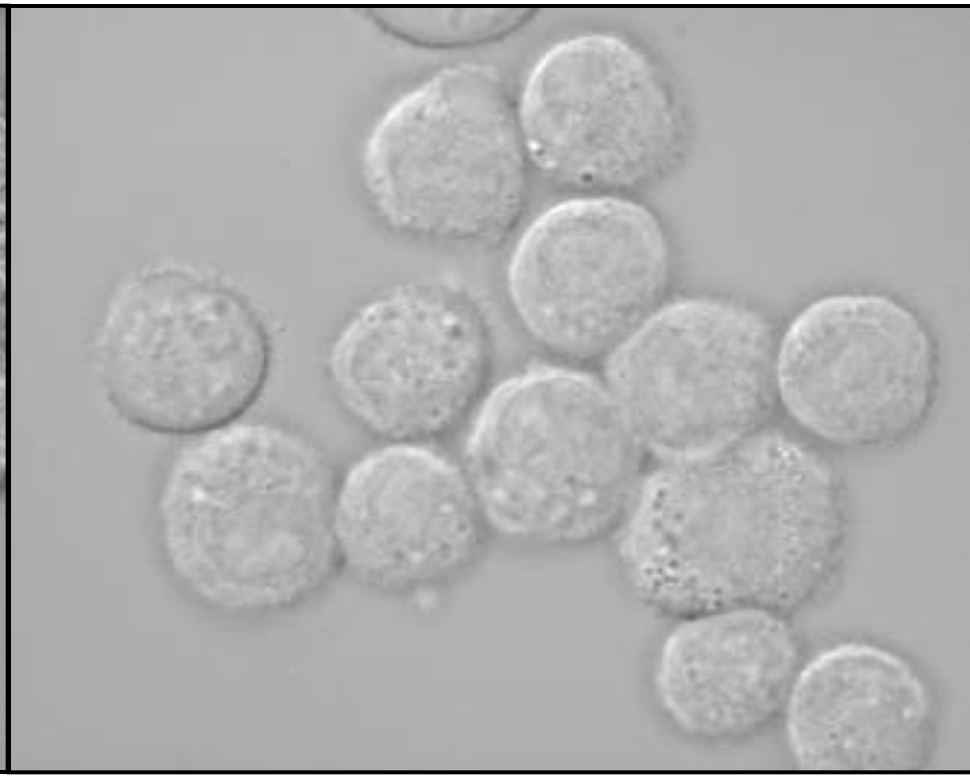
(>100 ng/ml of LPS-free ACT)

induces vacuolization of J774A.1 cells

AC^+ 200 ng/ml – 90 min



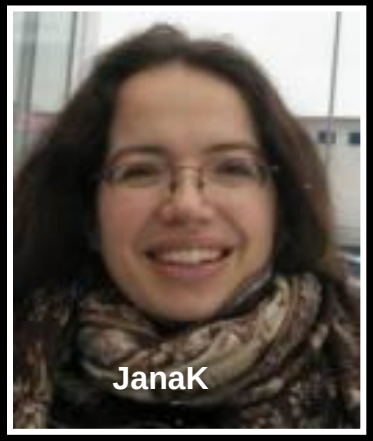
AC^- 200 ng/ml – 90 min



Basler *et al.*, 2006, *Infect. Immun.*, 74, 2207-2214. .

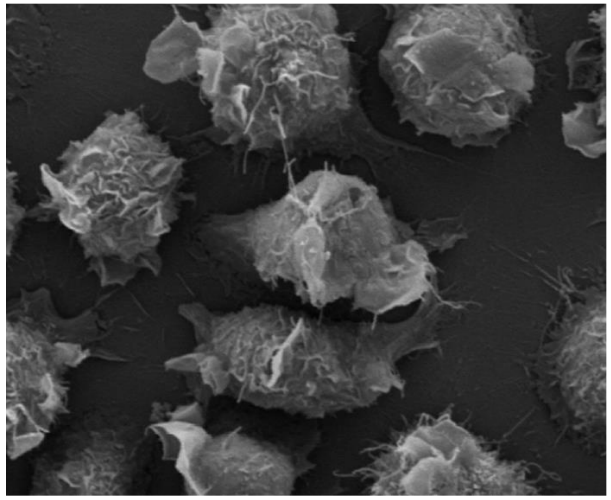
CyaA-induced morphological rearrangements

Mouse macrophage-like cell line J774 A.1:

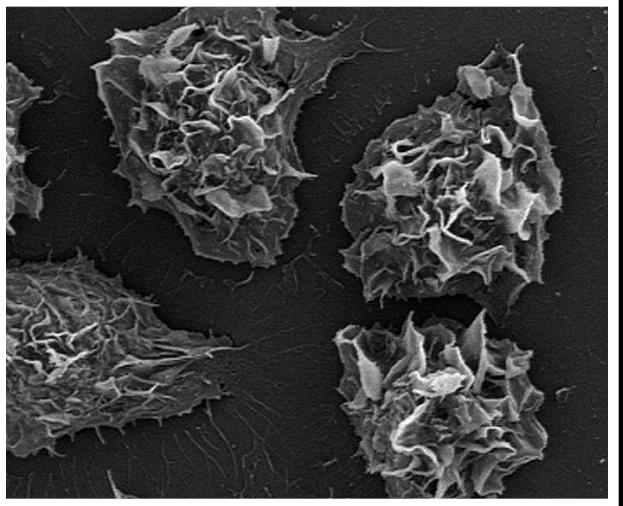


Kamanova et al. (2008)
J. Immunol. 181, 5587-97

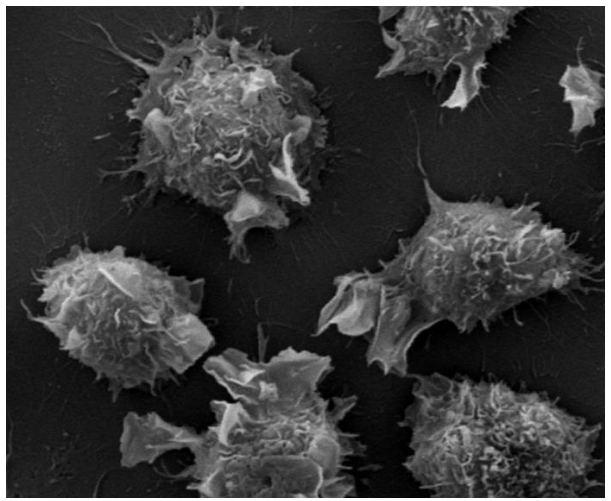
Buffer, 5 min



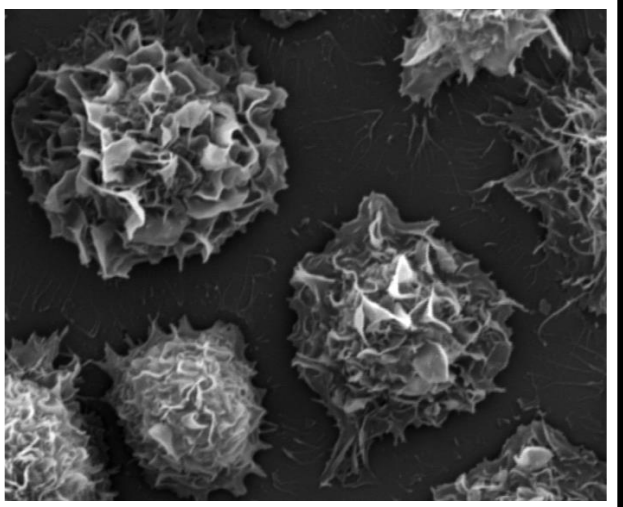
CyaA, **10 ng/ml,** 5 min

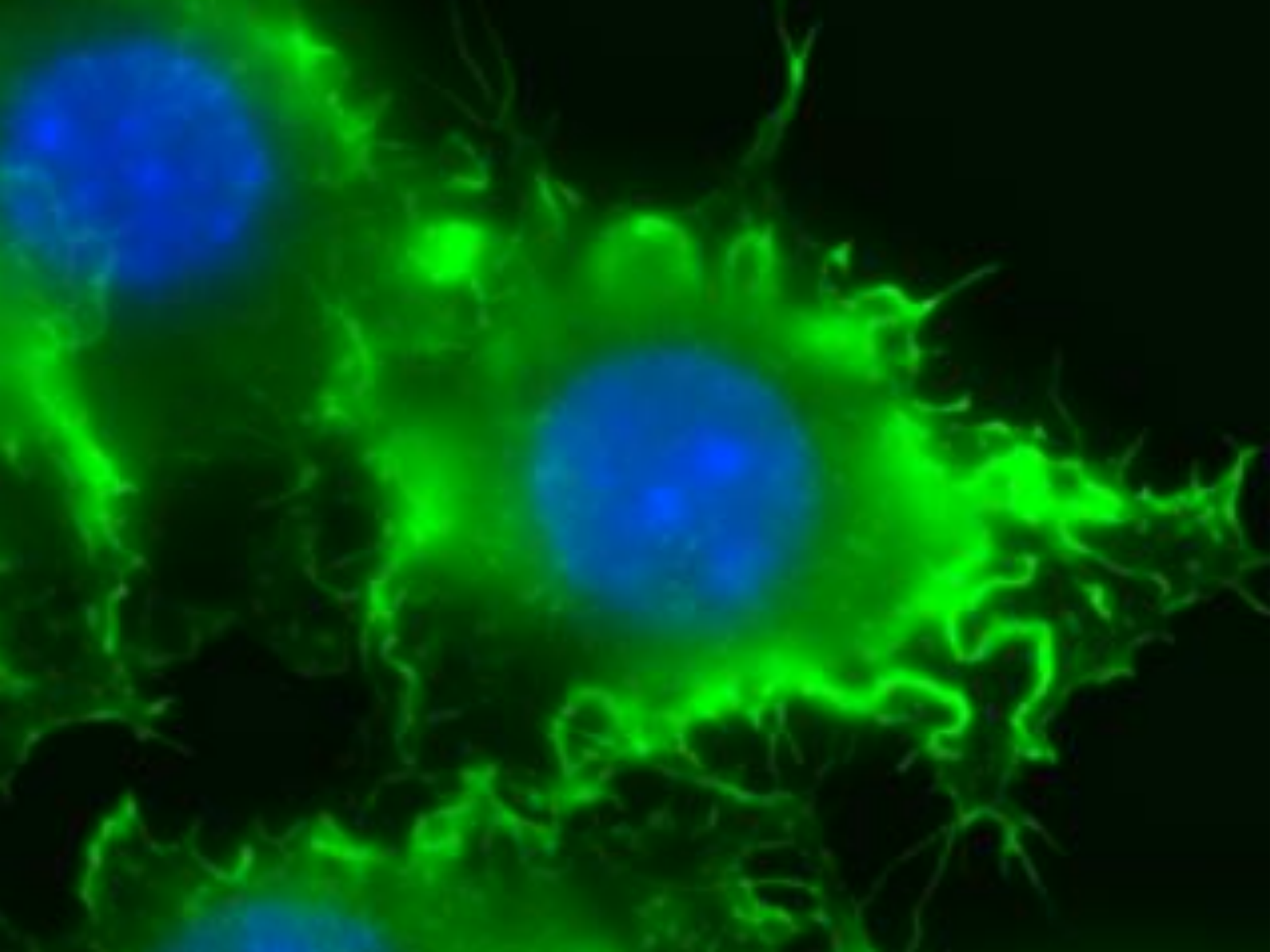


CyaA-AC$^-$, 10 ng/ml, 5 min



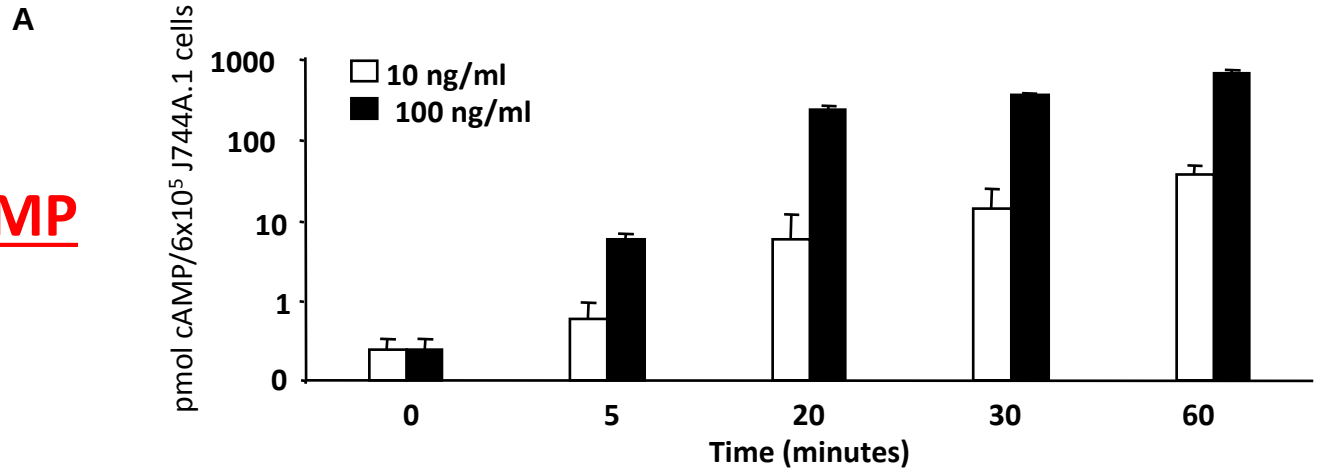
db-cAMP, 2mM, 10 min



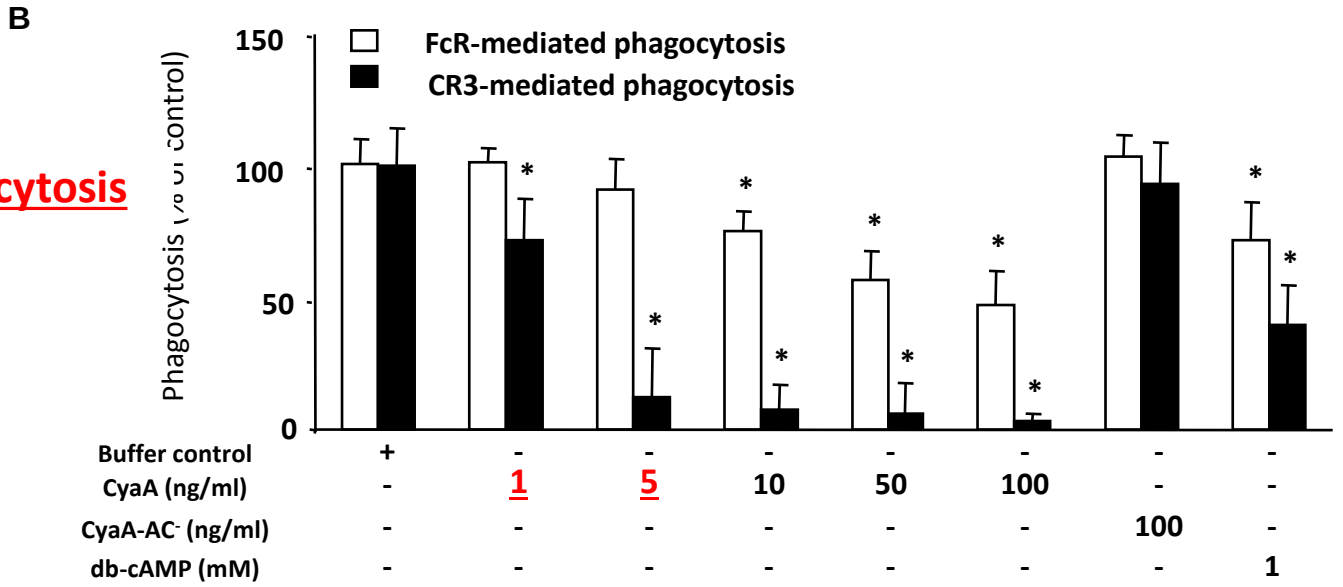


ACT at low doses ablates complement-mediated phagocytosis

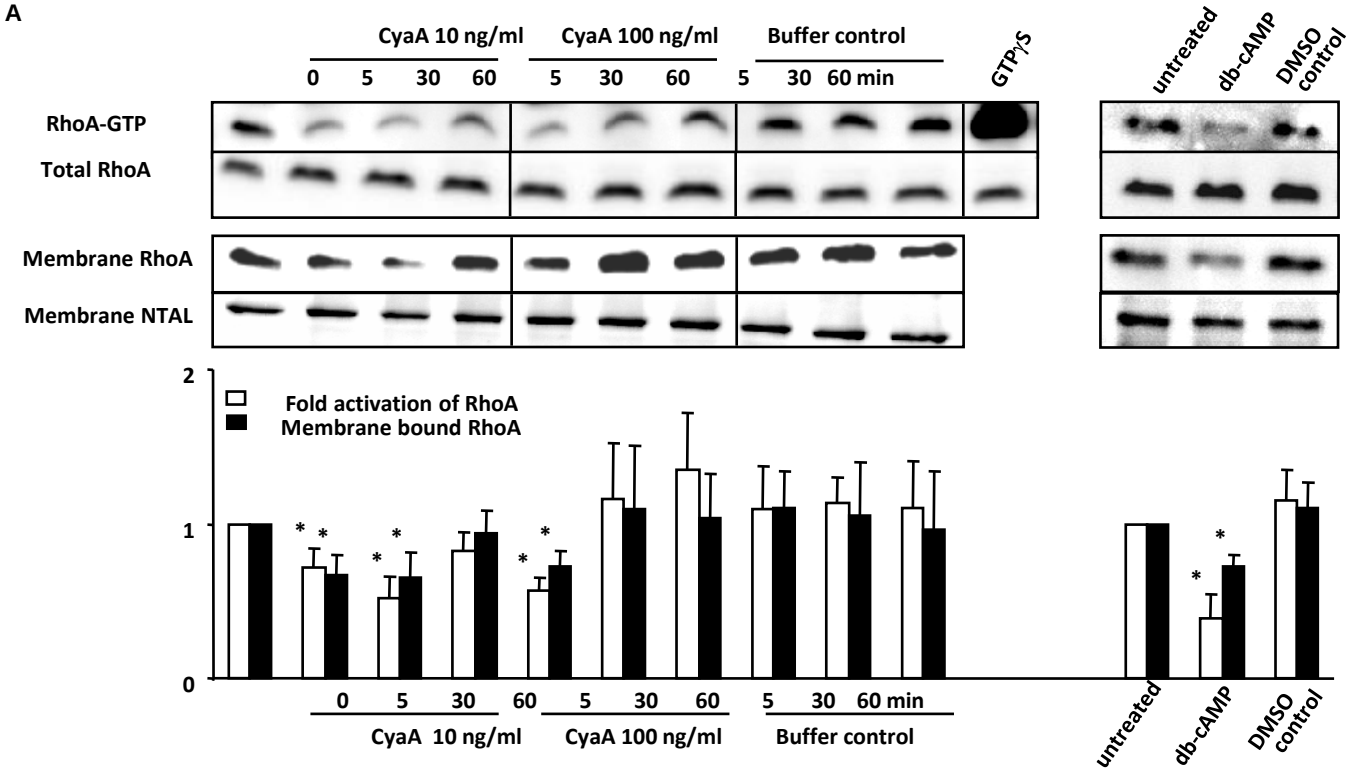
cAMP

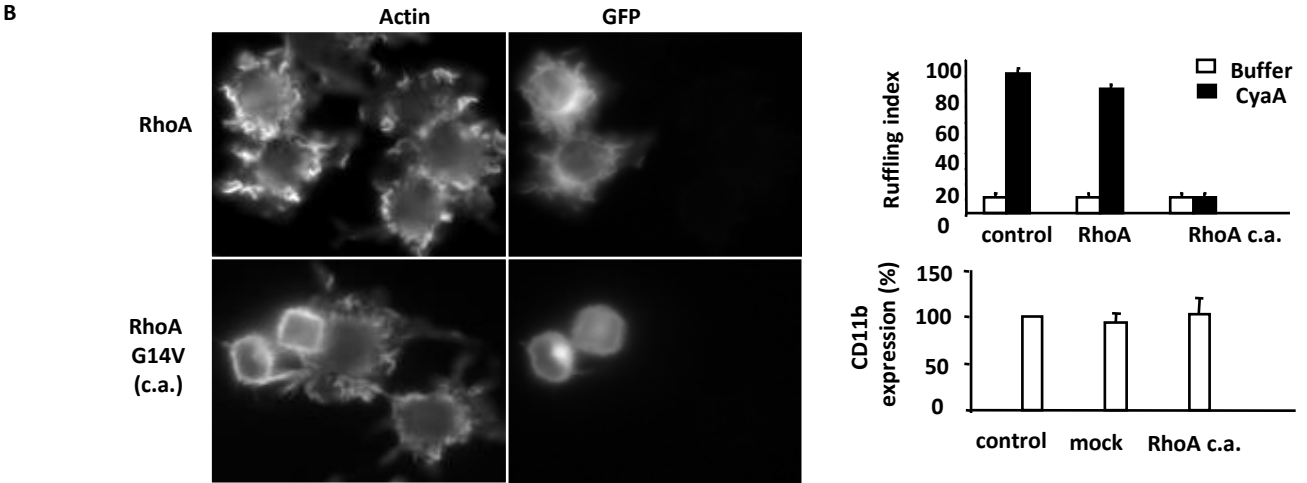


phagocytosis



ACT transiently inactivates RhoA





The bug really needs ACT for knocking down innate immunity:

- All *Bordetellae* pathogenic to mammals produce adenylate cyclase toxin-hemolysin (Except for certain *B. bronchispetica* lineages)
- ACT sequences are highly conserved in *B. pertussis* isolates
- Strains not producing ACT have not been isolated from patients, so far (in contrast to PT, FHA or pertactin, which all are dispensable)
- ACT is an extremely potent toxin that **knocks-down phagocytes in 30-60 seconds** (PT needs > 30 min (12 h) for that)
- ACT **instantaneously blocks oxidative burst of neutrophils** at pM conc. in 30 seconds
- ACT **blocks uptake of complement-opsonized particles** at pM conc.

Quantification of AC Toxin from *Bordetella pertussis in vitro* and during infection of Baboon/Human Infants

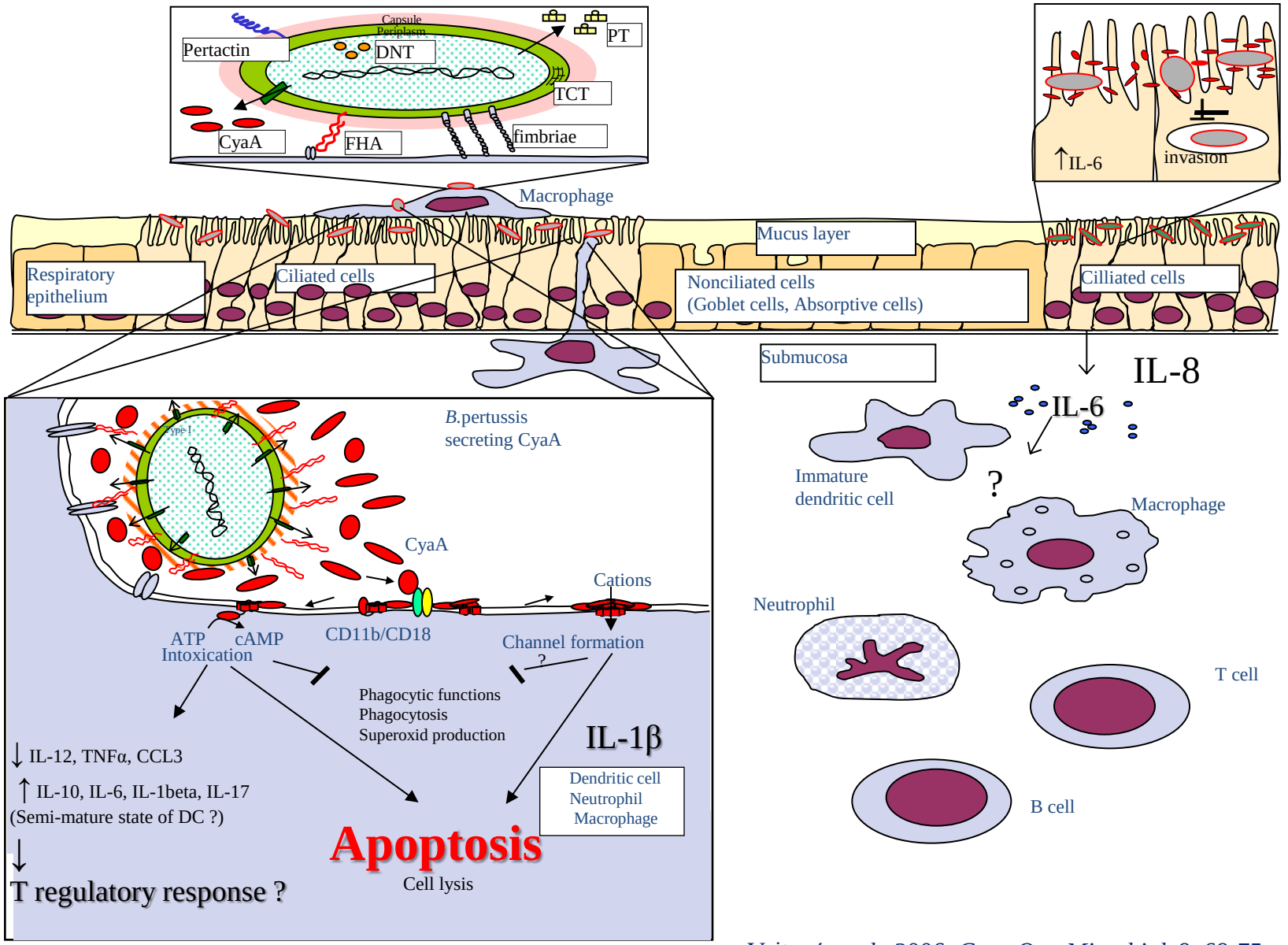
Eby, Gray, Warfel, Paddock, Jones, Day, Bowden, Poulter, Donato and Hewlett

- **NP secretions with *B. pertussis* (10^8 /ml) –**
  infant baboons 1-5 ng/ml ACT
  human infants - 12-20 ng/ml ACT
- ***B. pertussis* cultured to 10^8 /ml *in vitro* produces ~60 ng/ml in 6 hours and centrifugation onto host cells increases intoxication 4-fold**

Infection and Immunity, 2013

ACT is a SWIFT SABOTEUR of immune responses

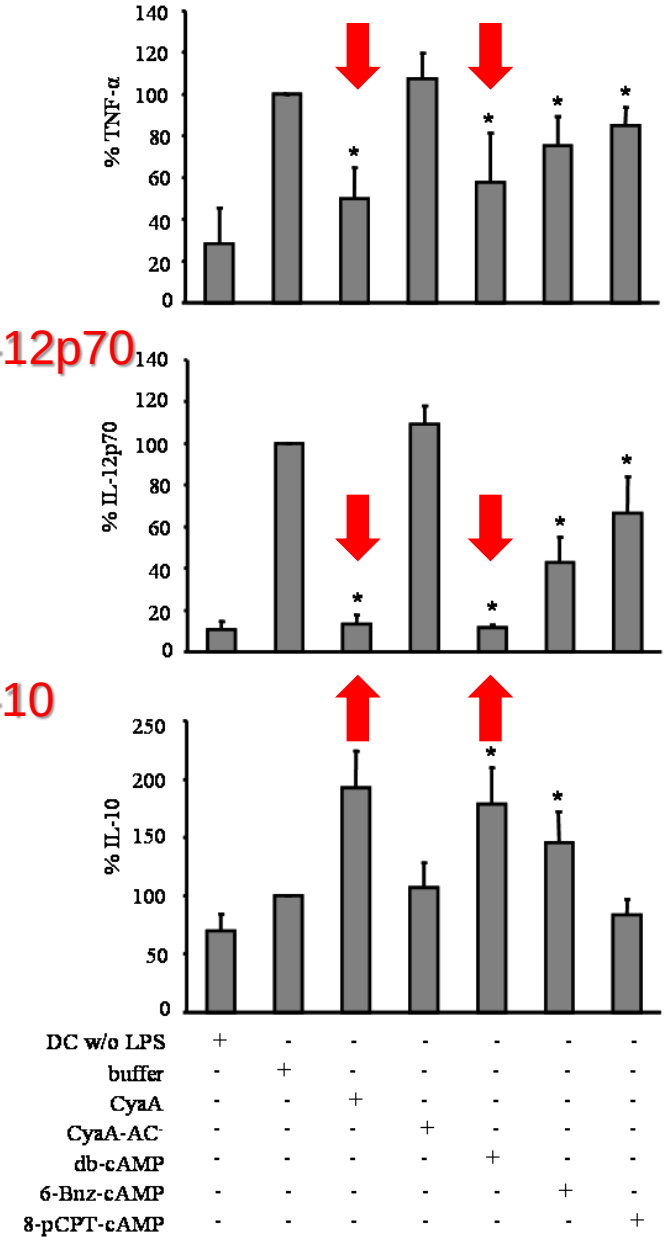
low ACT (CyaA) concentrations make a difference on respiratory mucosa…



Osičková *et al.*, (1999) J. Biol. Chem. 274, 37644)

Vojtová *et al.*, 2006, *Curr. Op.. Microbiol.* 9, 69-75

TNFα

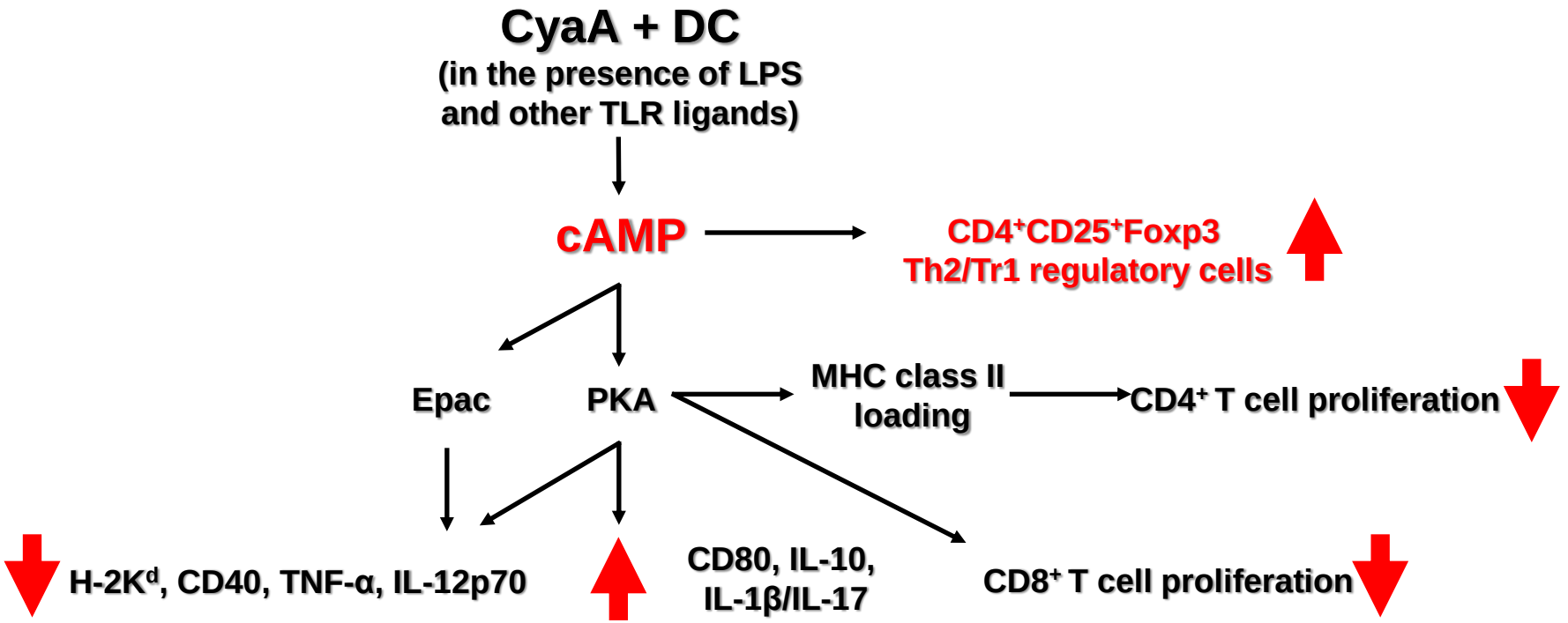


ACT (CyaA) skewes TLR-stimulated cytokine production in DC towards tolerance?

n = 4, * P< 0,05; 100 % urea + LPS (buffer)

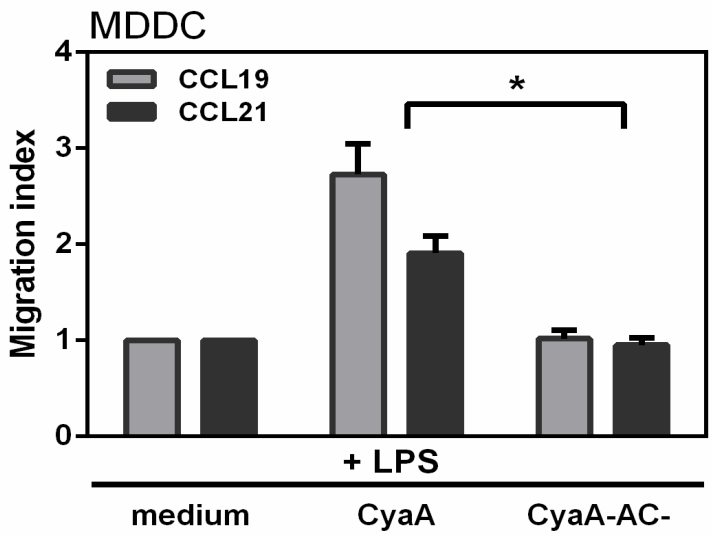
Irena Adkins

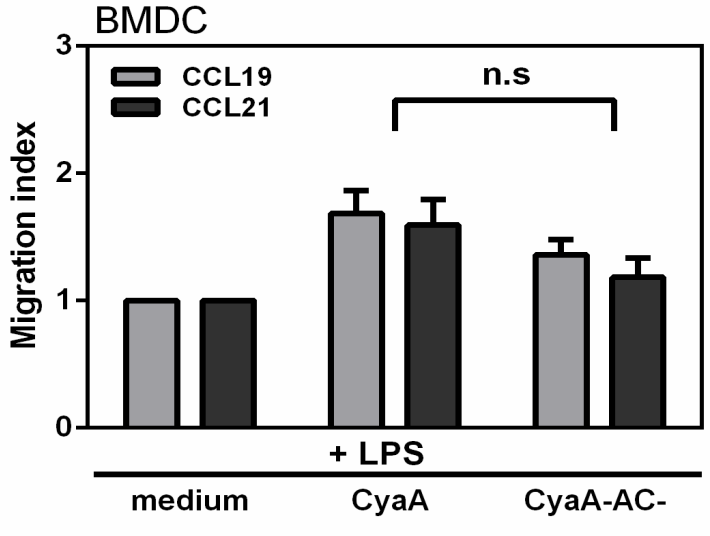
cAMP signaling of ACT dampens and skews adaptive immunity towards initial T_{h2}/T_{r1}-mediated tolerance of colonizing *Bordetellae* followed by delayed T_{h1}/T_{h17} mediated clearance?

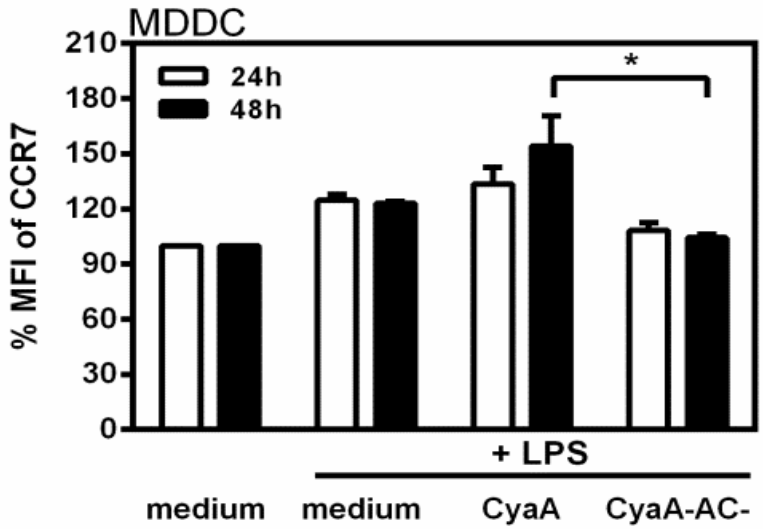


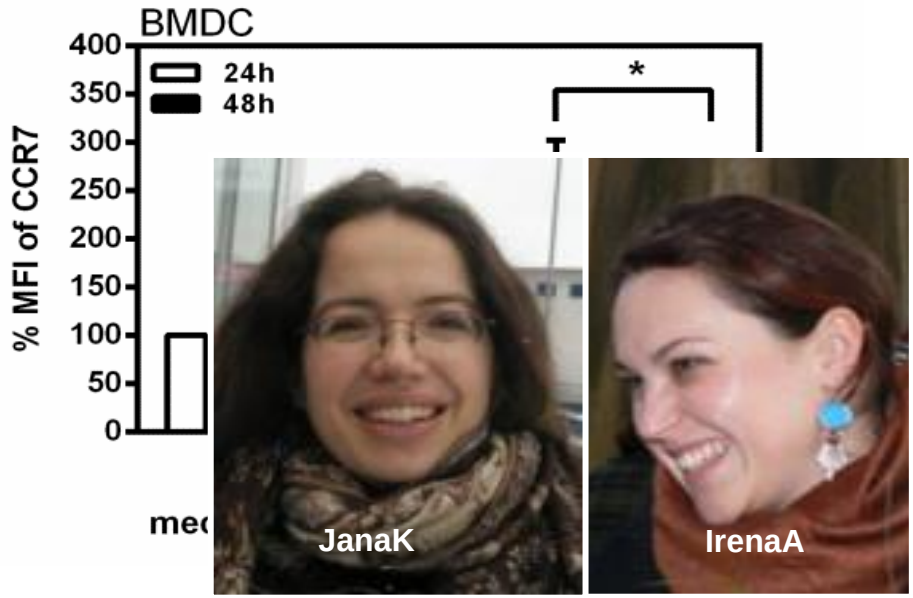
Relman lab:	Boschwitz *et al.* (1997) *JID* 176:678	Human MoDCs
Guiso lab:	Njamkempo *et al.* (2000) *J. Cell. Physiol.* 183:91	Human Monocytes
Lewis lab:	Bagley *et al.* (2002): *J. Leukoc. Biol.* 72:962	Human MoDCs
Mills lab:	Ross *et al.* (2004) *Infect. Immun.* 72:1568	Mouse BMDCs
Mills lab:	Boyd *et al.* (2005) *J. Immunol.* 175: 730	Mouse BMDCs
Ausiello lab:	Spensieri *et al.* (2006) *Infect. Immun.* 74:2831	Human MoDC
Mills lab:	Hickey *et al.* (2008) *J. Leukoc. Biol.* 84:234	Mouse BMDCs
Ausiello lab:	Fedele *et al.* (2010) *PLoS One.* 5(1): e8734	Human MoDC
Sebo lab:	Adkins et al. unpublished	BMDC and Human MoDC

cAMP signaling of ACT promotes migration of DC



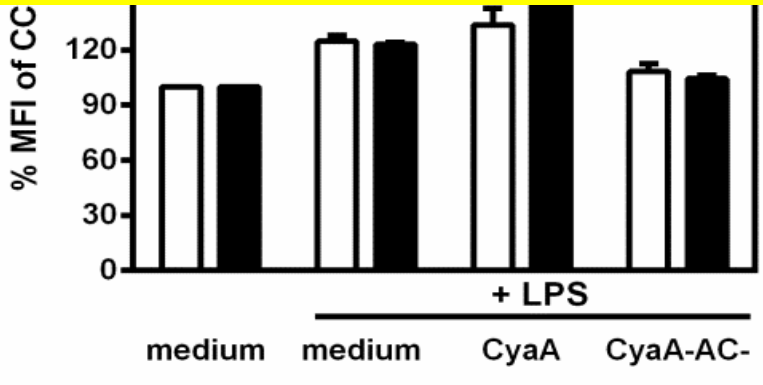


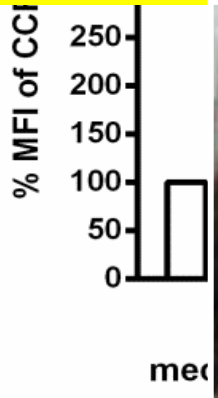


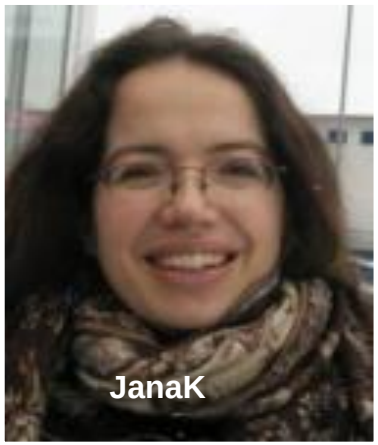


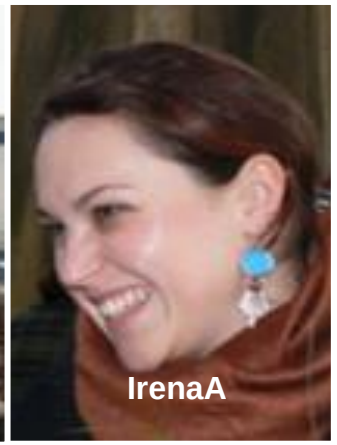
cAMP signaling of ACT promotes migration of DC

Outmigration of tolerogenic DC from mucosa into lymph nodes to hamper antigen-specific T cell responses???

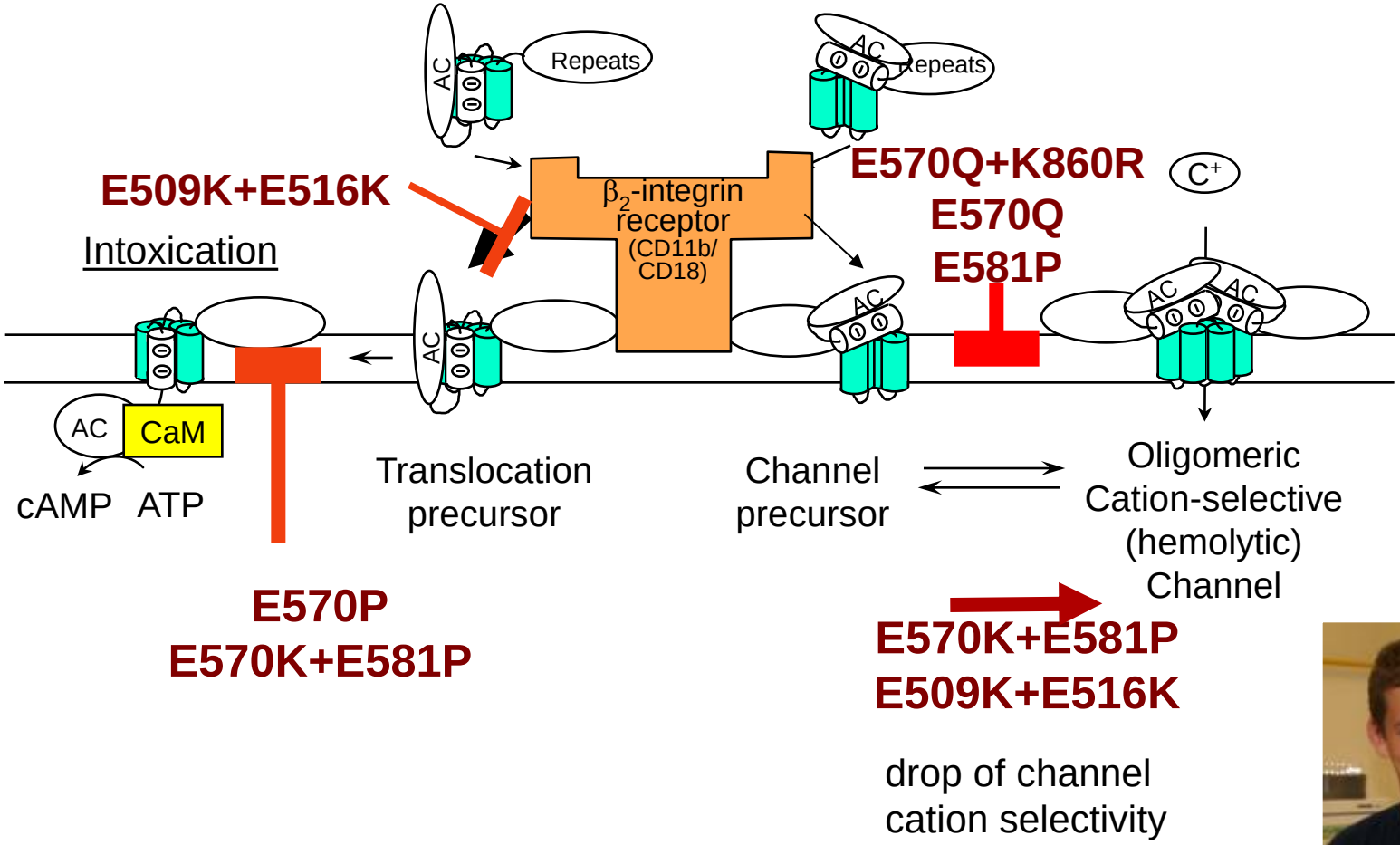






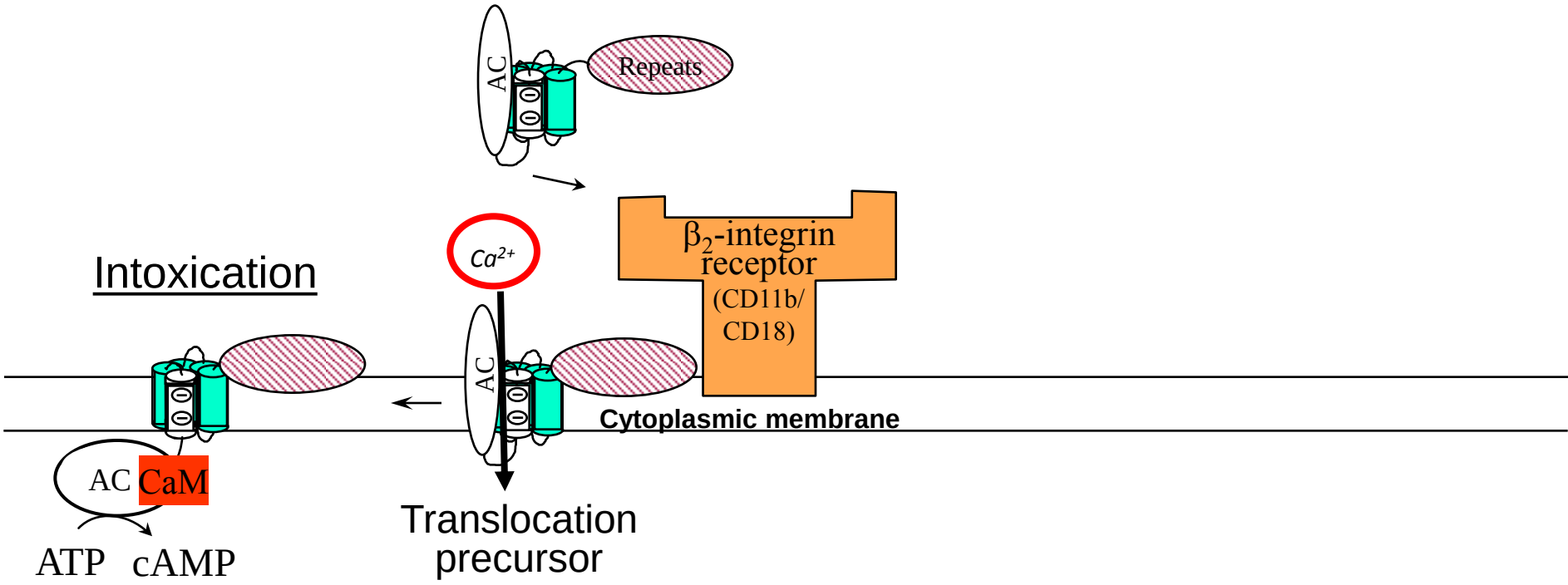


The toolbox: **A panel of mutations characterized that block ACT activity at each individual step of toxin action**



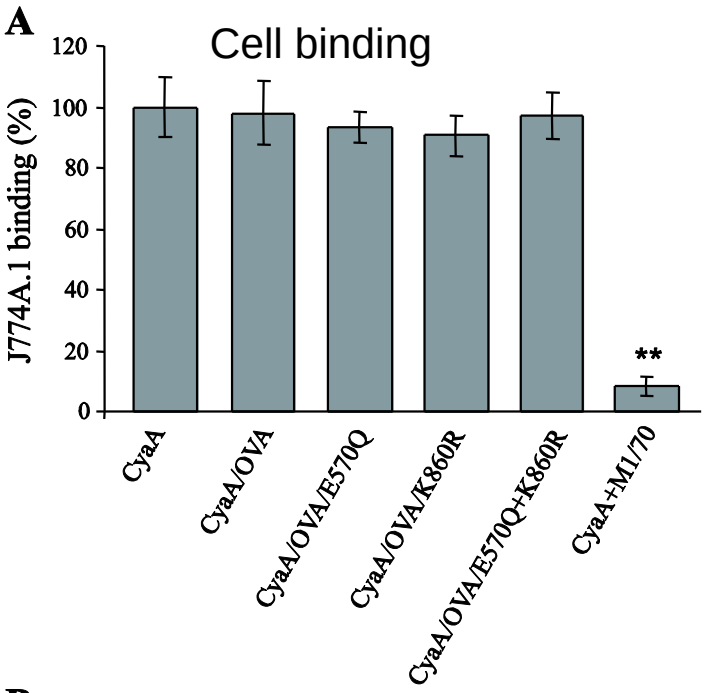
Basler *et al.*, (2007) *J. Biol. Chem.* **282**, 12419

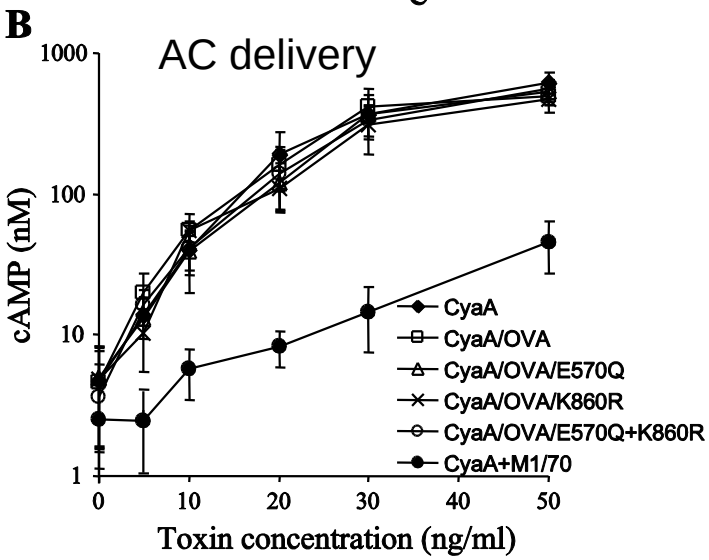
So, this appears to be real…

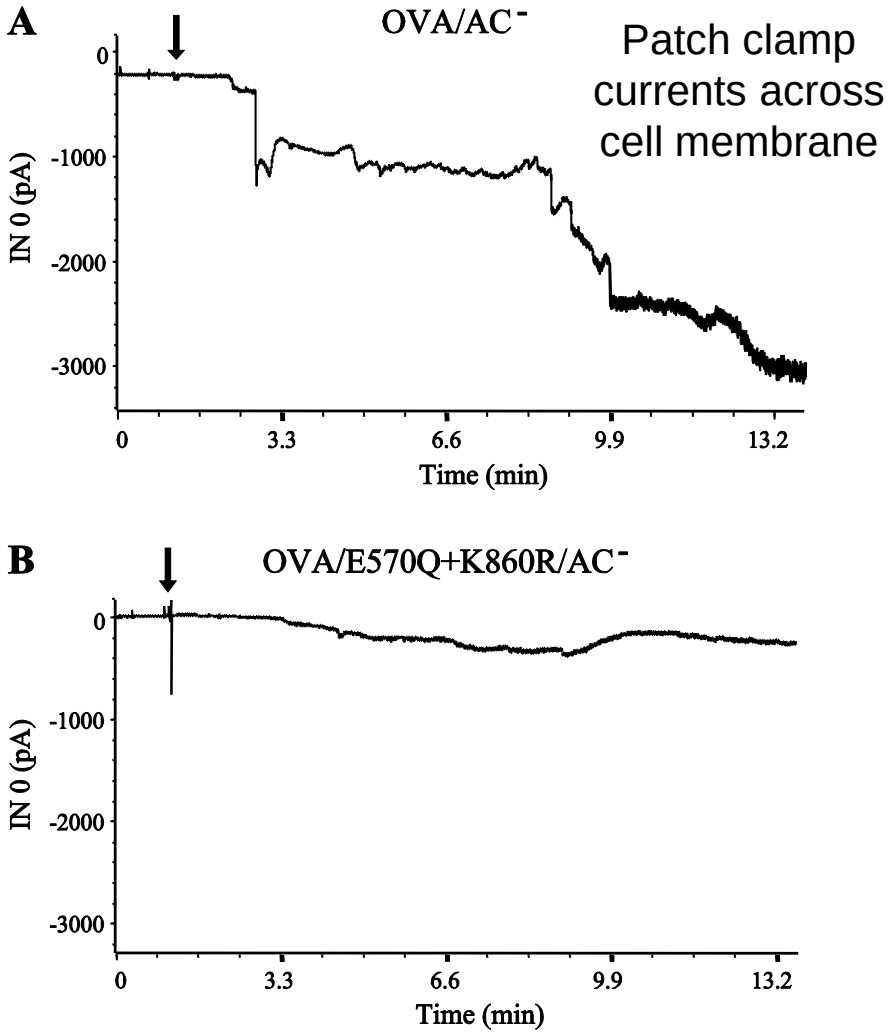


Osickova *et al.*, (1999) J. Biol. Chem. 274, 37644
Basler et al., (2007) J. Biol. Chem. 282, 12419
Fiser R. *et al.*(2007) *J. Biol. Chem. 282, 2808*

AC domain is not translocated across the CyaA pore





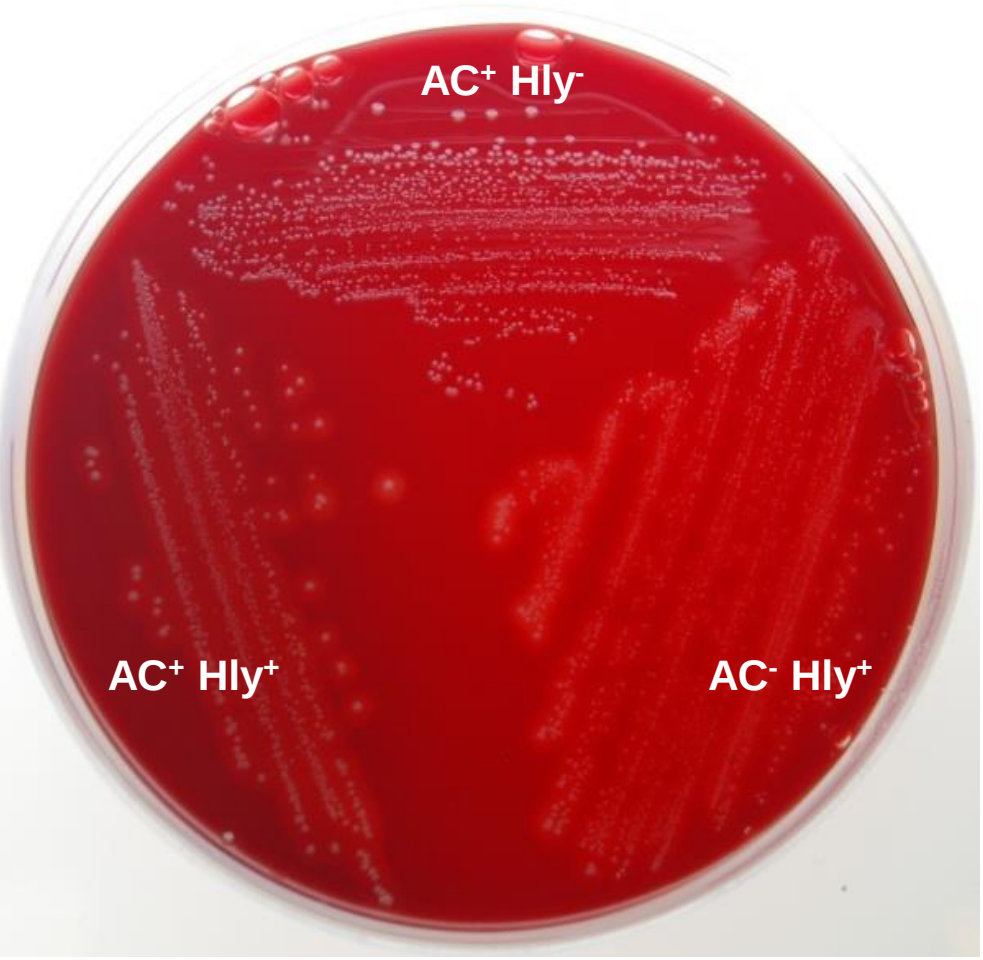


Patch clamp currents across cell membrane

The E570Q+K860R toxin delivers the AC domain into cells without permabilizing he cell membrane

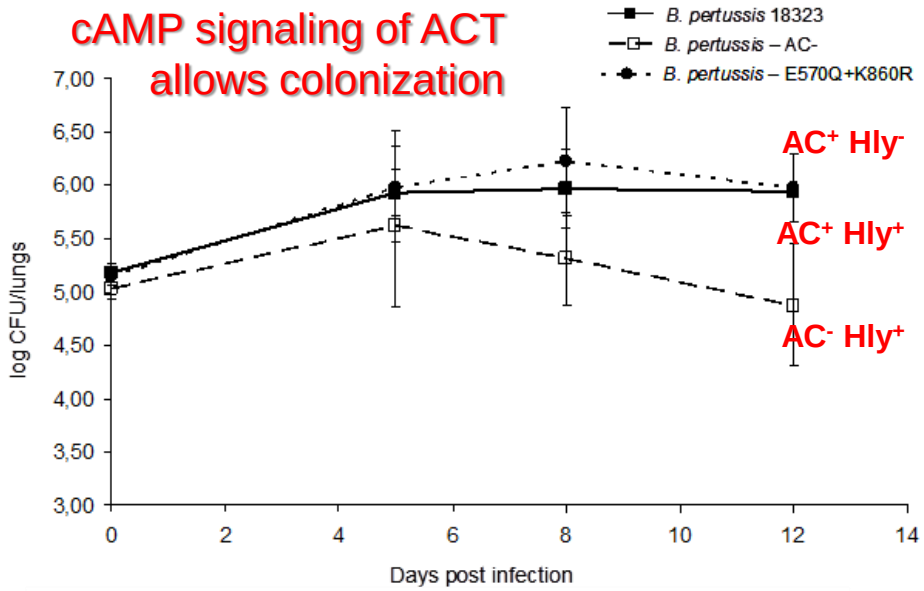
Osickova *et al.* (2010) *Mol. Microbiol. 75:15450-1562*

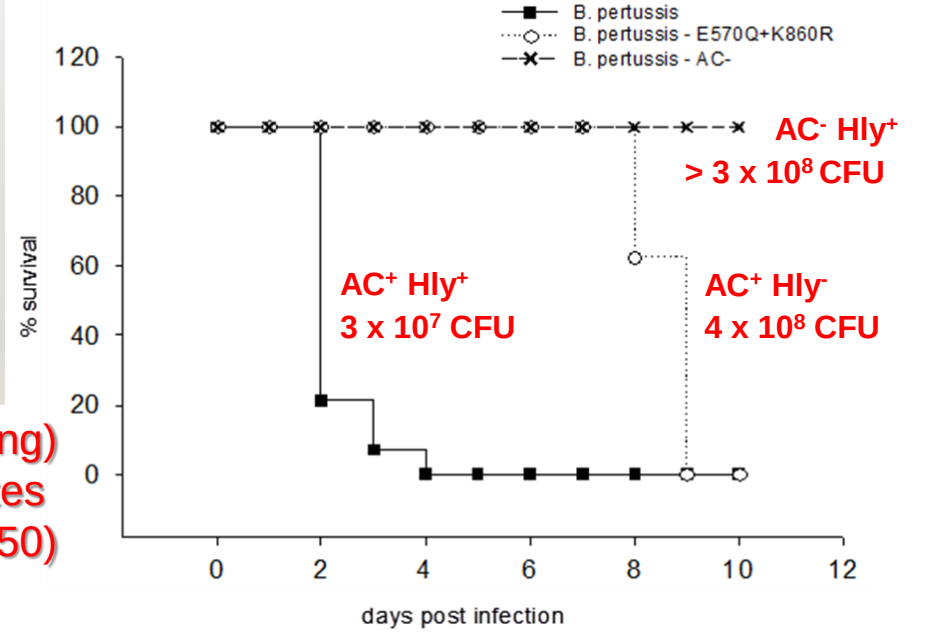
AC⁻ *B. pertussis* is avirulent and pore-forming (hemolytic) activity contributes virulence

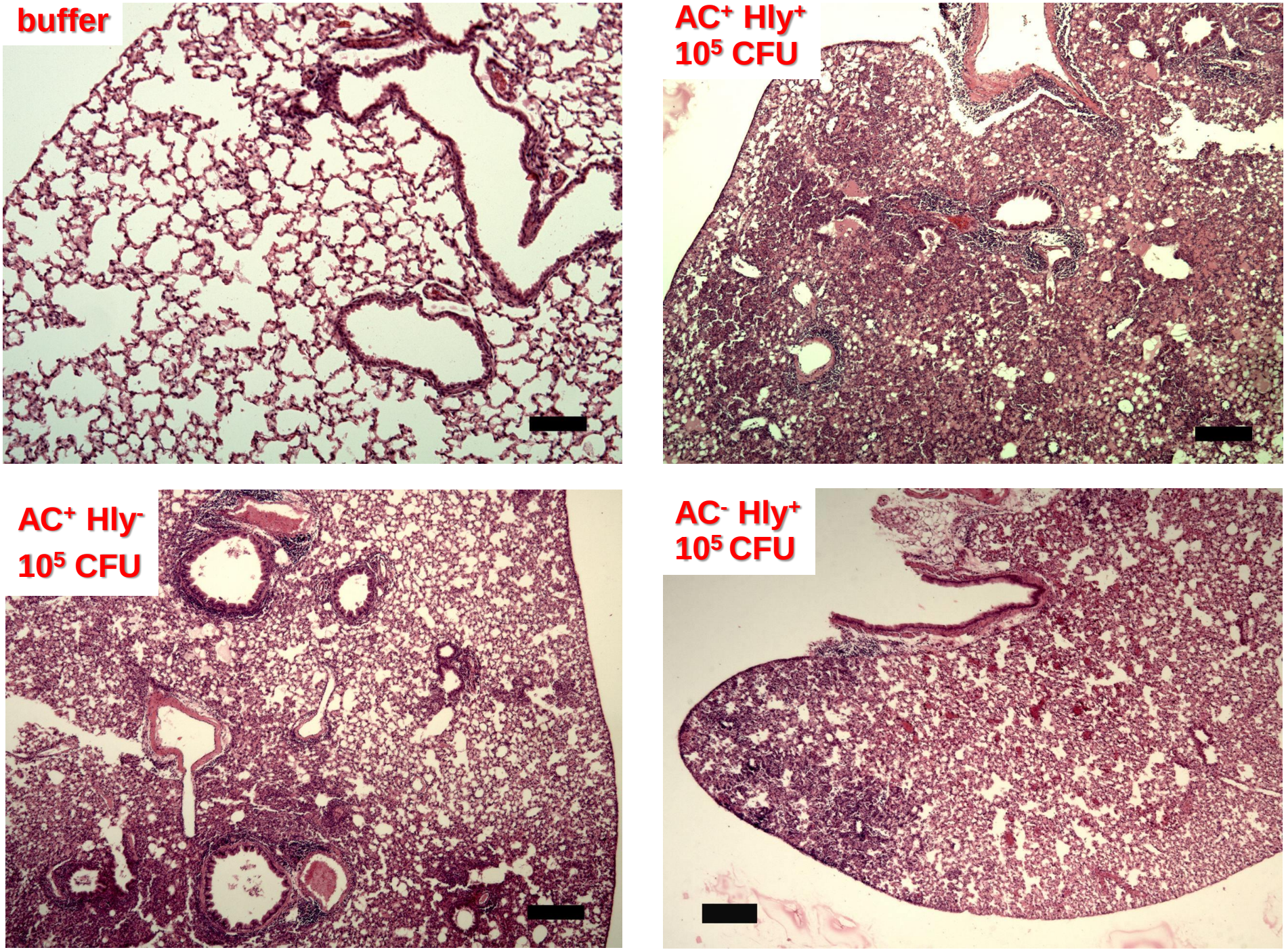


Hemolytic (pore-forming) activity contributes to pathology (LD50)

cAMP signaling of ACT allows colonization






buffer
AC+ Hly+
10^5 CFU
AC+ Hly-
10^5 CFU
AC- Hly+
10^5 CFU

In conclusion:

The pore-forming (hemolytic) activity of ACT is important for *B. pertussis* virulence, contributing to inflammation that will eventually help to clear the infection (in mice)

The **cAMP signaling of ACT,**

however, prevails and

knocks down the innate immunity

and

dampens the adaptive immune response

in order

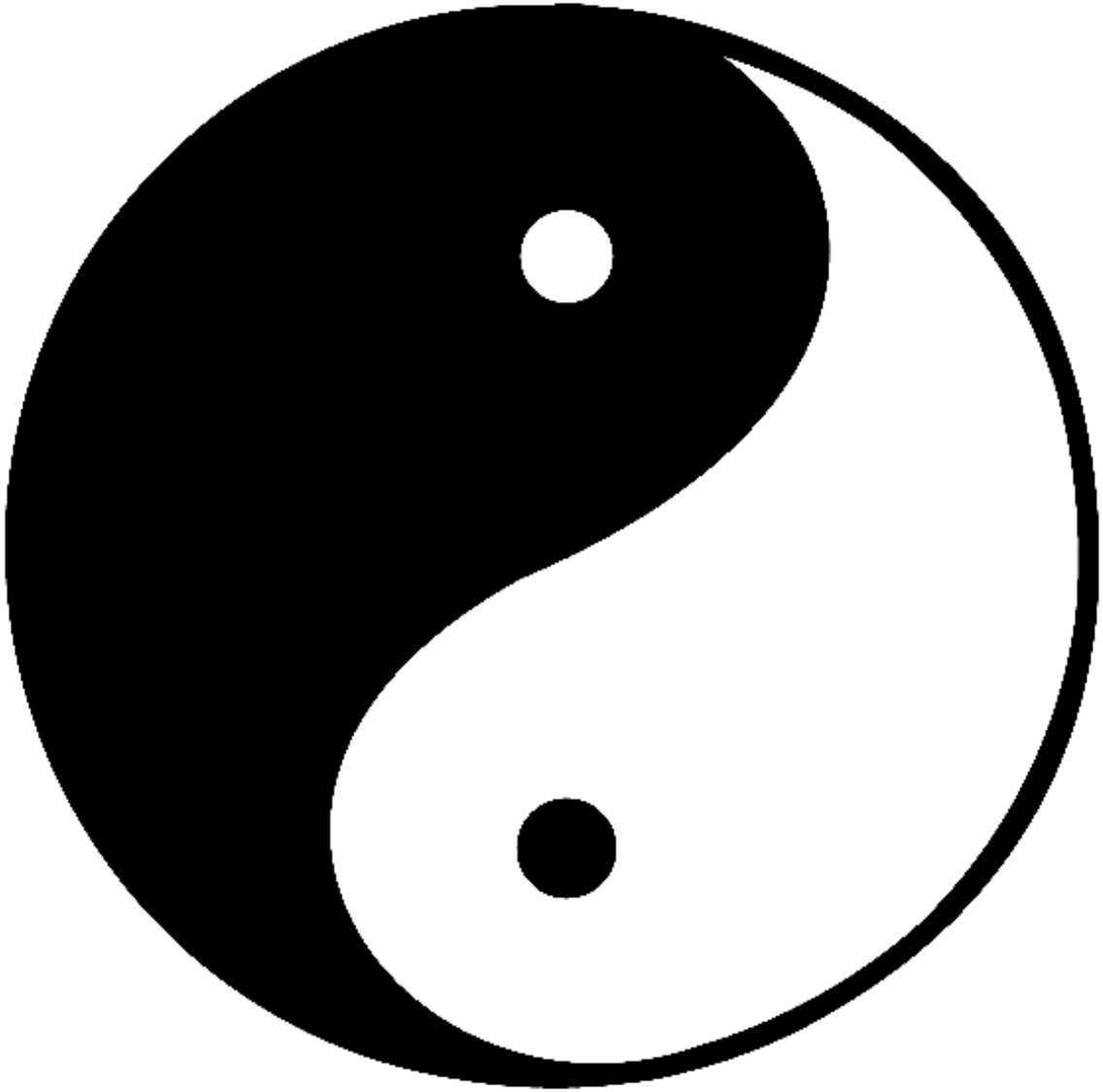
to enable host colonization

Explaining why is it so important
to add the AC toxoid
into the aP vaccine

if we are serious about

breaking the vicious circle
of epidemic whooping cough
spread in the most developed
countries …

THE YIN
OF A BACTERIAL TOXIN"



Not suprisingly, hence, ACT is a protective antigen

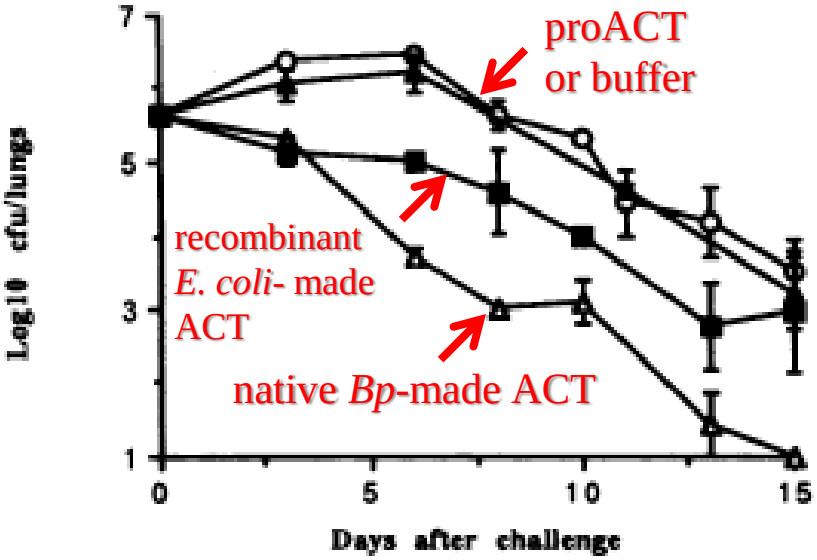
INFECTION AND IMMUNITY, Sept. 1993, p. 3583–3589
0019-9567/93/093583-07$02.00/0

Vol. 61, No. 9

CyaC-Mediated Activation Is Important Not Only for Toxic but Also for Protective Activities of *Bordetella pertussis* Adenylate Cyclase-Hemolysin

FOTINI BETSOU,[1] PETER ŠEBO,[2] AND NICOLE GUISO[1]*
Unité de Bactériologie Moléculaire et Médicale[1] and Unité de Biochimie des Régulations Cellulaires,[2] Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

Received 8 April 1993/Returned for modification 7 May 1993/Accepted 1 June 1993



(at that time ACT samples contained LPS)

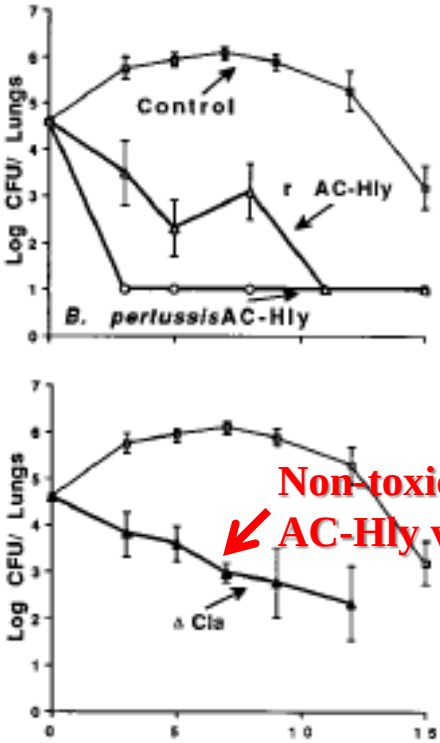
INFECTION AND IMMUNITY, Sept. 1995, p. 3309–3315
0019-9567/95/$04.00+0

Vol. 63, No. 9

The C-Terminal Domain Is Essential for Protective Activity of the *Bordetella pertussis* Adenylate Cyclase-Hemolysin

FOTINI BETSOU,[1] PETER SEBO,[2]† AND NICOLE GUISO[1]*
Unité de Bactériologie Moléculaire et Médicale[1] and Unité de Biochimie des Régulations Cellulaires,[2] Institut Pasteur, 75724 Paris Cedex 15, France

Received [illegible] 1995



Highly purified CyaA-AC$^-$ protects on its own

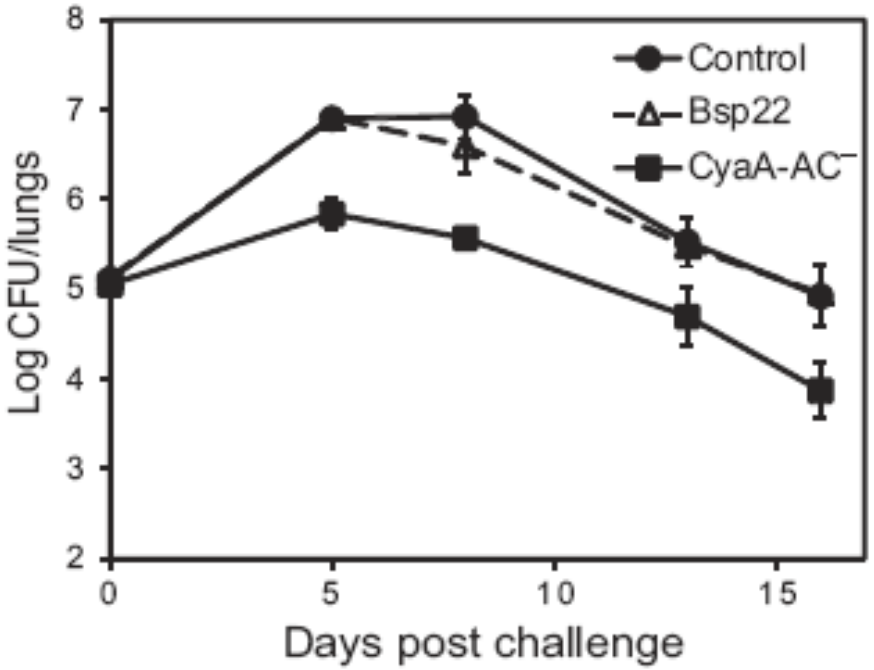
Infection and Immunity **81:** 2761–2767 (2013)

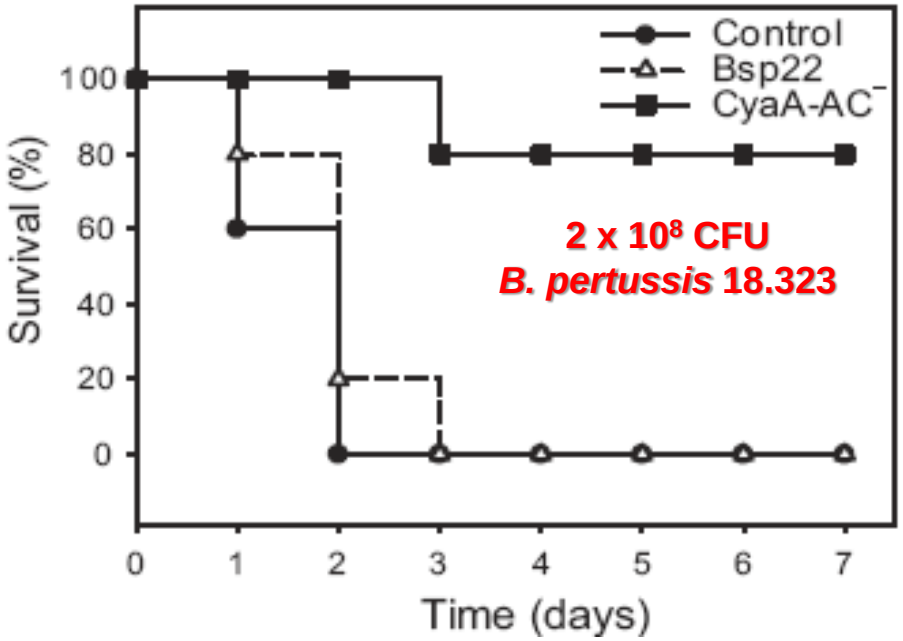
The *Bordetella pertussis* Type III Secretion System Tip Complex Protein Bsp22 Is Not a Protective Antigen and Fails To Elicit Serum Antibody Responses during Infection of Humans and Mice

Rodrigo Villarino Romero,[a] Ilona Bibova,[a] Ondrej Cerny,[a] Branislav Vecerek,[a] Tomas Wald,[a] Oldrich Benada,[a] Jana Zavadilova,[b] Radim Osicka,[a] Peter Sebo[a]

Institute of Microbiology of the ASCR, Prague, Czech Republic[a]; National Institute of Public Health, Prague, Czech Republic[b]

Poster: Villarino, Bibova *et al.*





Addition of CyaA-AC$^-$ improves performance of the aP vaccine

INFECTION AND IMMUNITY, Dec. 2006, p. 6797–6805 Vol. 74, No. 12
0019-9567/06/$08.00+0 doi:10.1128/IAI.01104-06

Effect of Different Forms of Adenylate Cyclase Toxin of *Bordetella pertussis* on Protection Afforded by an Acellular Pertussis Vaccine in a Murine Model[▽]

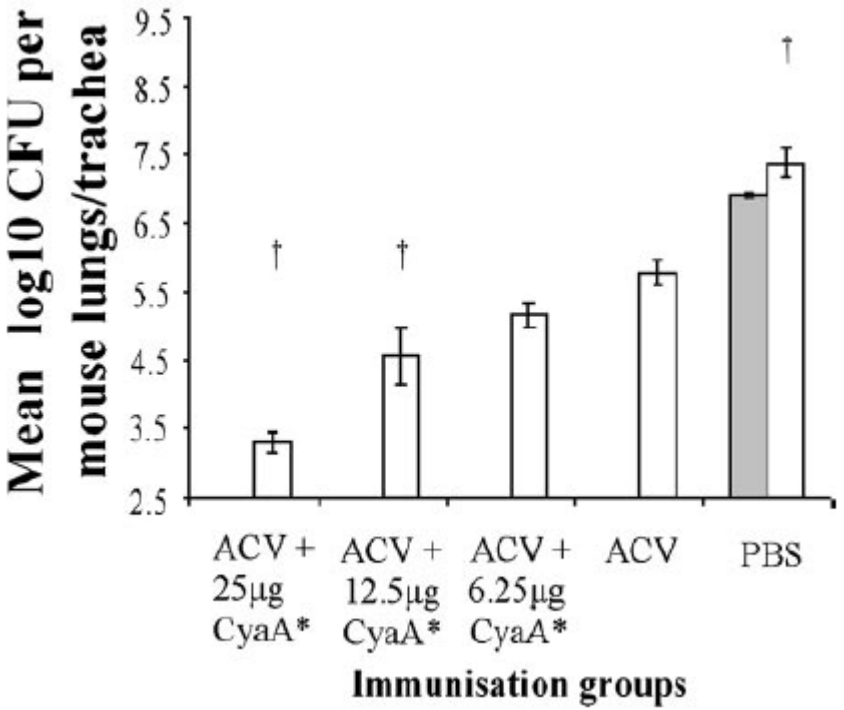
Gordon Y. C. Cheung,[1] Dorothy Xing,[2] Sandra Prior,[2] Michael J. Corbel,[2] Roger Parton,[1] and John G. Coote[1]*

Division of Infection and Immunity, Glasgow Biomedical Research Centre, University of Glasgow, Glasgow,[1] and Division of Bacteriology, National Institute of Biological Standards and Control, South Mimms, Hertfordshire,[2] United Kingdom

Received 14 July 2006/Accepted 12 September 2006

Four recombinant forms of the cell-invasive adenylate cyclase toxin (CyaA) of *Bordetella pertussis* were compared for the ability to enhance protection against *B. pertussis* in mice when coadministered with an acellular pertussis vaccine (ACV). The four forms were as follows: fully functional CyaA, a CyaA form lacking adenylate cyclase enzymatic activity (CyaA*), and the nonacylated forms of these toxins, i.e., proCyaA and proCyaA*, respectively. None of these forms alone conferred significant ($P > 0.05$) protection against *B. pertussis* in a murine intranasal challenge model. Mice immunized with ACV alone showed significant ($P < 0.05$) reductions in bacterial numbers in the lungs after intranasal challenge compared with those for control mice. When administered with ACV, both CyaA and CyaA* further reduced bacterial numbers in the lungs of mice after intranasal challenge compared with those for ACV-immunized mice, but the enhanced protection was only significant ($P < 0.05$) with CyaA*. Coadministration of CyaA* with ACV caused a significant ($P < 0.05$) increase in immunoglobulin G2a antibody levels against pertactin compared with those in mice immunized with ACV alone. Spleen cells from mice immunized with ACV plus CyaA* secreted larger amounts of interleukin-5 (IL-5), IL-6, gamma interferon (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) than did cells from mice immunized with ACV plus CyaA or ACV alone after stimulation in vitro with a mixture of *B. pertussis* antigens. Spleen cells from mice immunized with ACV plus CyaA* also secreted larger amounts of IFN-γ and GM-CSF than did cells from mice immunized with CyaA* alone after stimulation in vitro with CyaA*. Macrophages from mice immunized with ACV plus CyaA* produced significantly ($P < 0.05$) higher levels of nitric oxide than did macrophages from mice immunized with CyaA* alone, ACV alone, or ACV plus CyaA after stimulation in vitro with a mixture of *B. pertussis* antigens or heat-killed *B. pertussis* cells. These data suggest that the enhancement of protection provided by CyaA* was due to an augmentation of both Th1 and Th2 immune responses to *B. pertussis* antigens.

one-eighth of human dose of DTaP/ACV
(Infanrix, GSK) **+ CyaA-AC$^-$** 2 x i.p.
challenged with 4 x 10^6 B. pertussis 18.323 i.n.



Make ACT to a tool of the immunologist:

- ACT targets the $\alpha_M\beta_2$ integrin CD11b/CD18

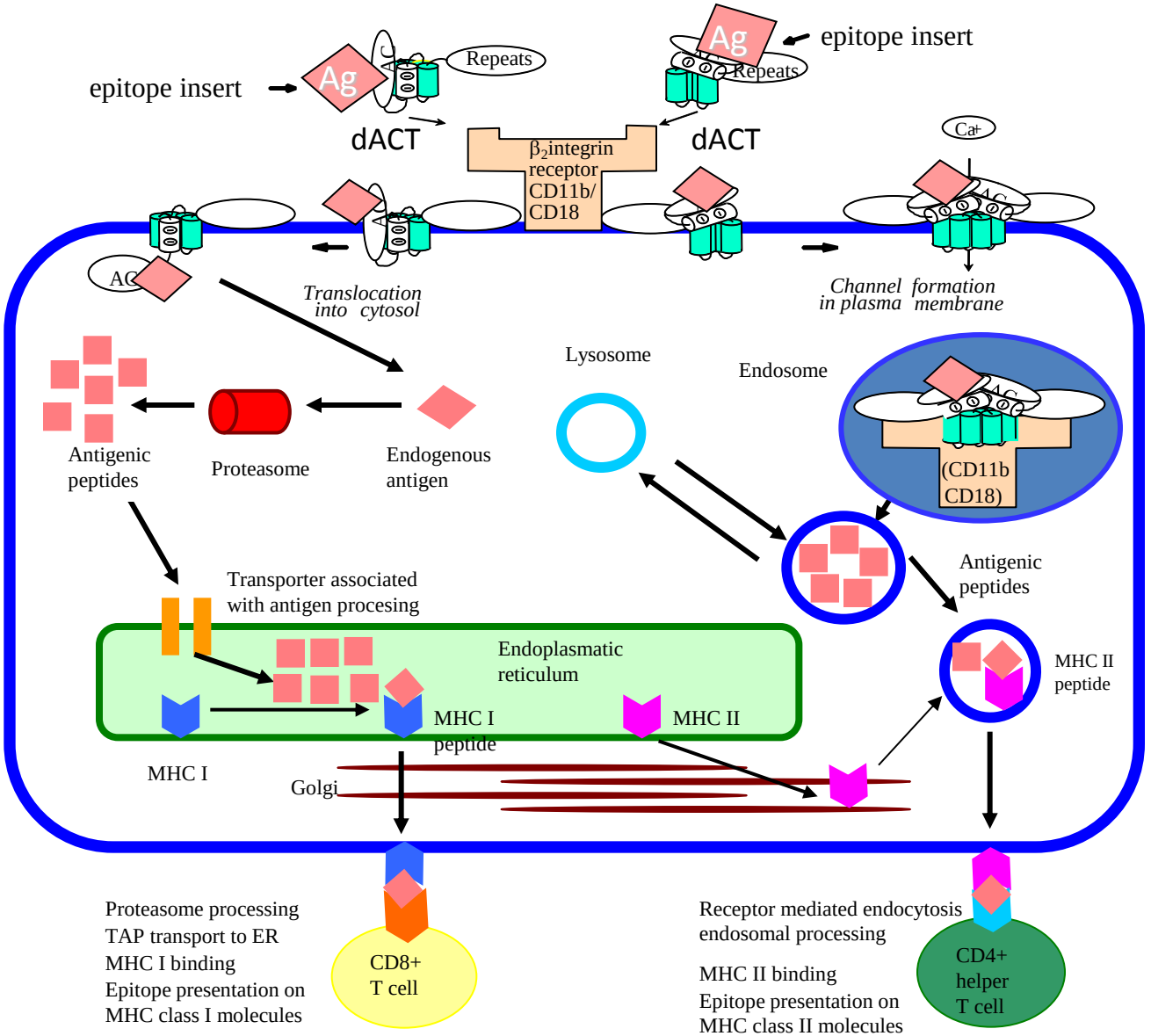
  specifically present on professional antigen presenting cells:

  - dendritic cells
  - macrophages

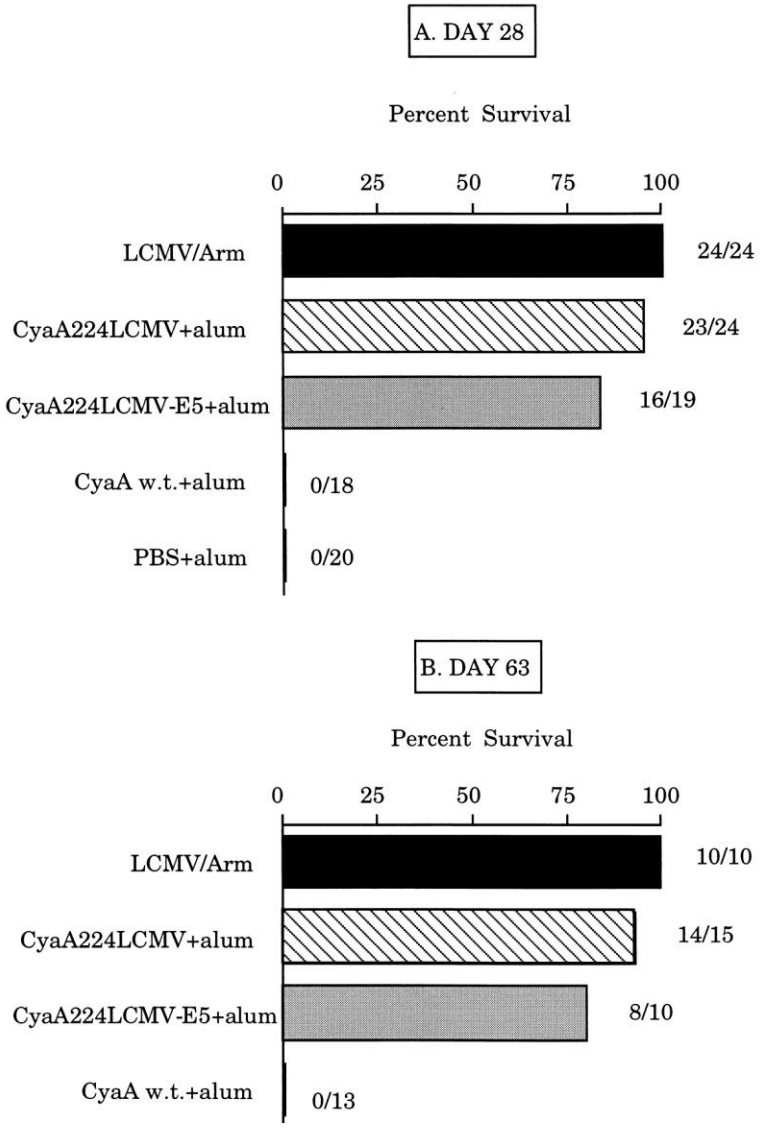
- Use ***DETOXIFIED*** **dACT- AC^-** to a novel tool for antigen delivery to dendritic cells:
  - –for vaccination against infections
  - –Immunotherapy of certain tumors
  - –diagnostics of infections and cancer

Exploit for antigen delivery to DCs…

dACT as a novel antigen delivery tool



Immunization with CyaA-LCMV affords protection against a lethal challenge by LCMV



Saron, M. F. et al. (1997*) Proc. Natl. Acad. Sci. USA* 94, 3314-3319

dCyaA constructs allow induction of

POLYVALENT

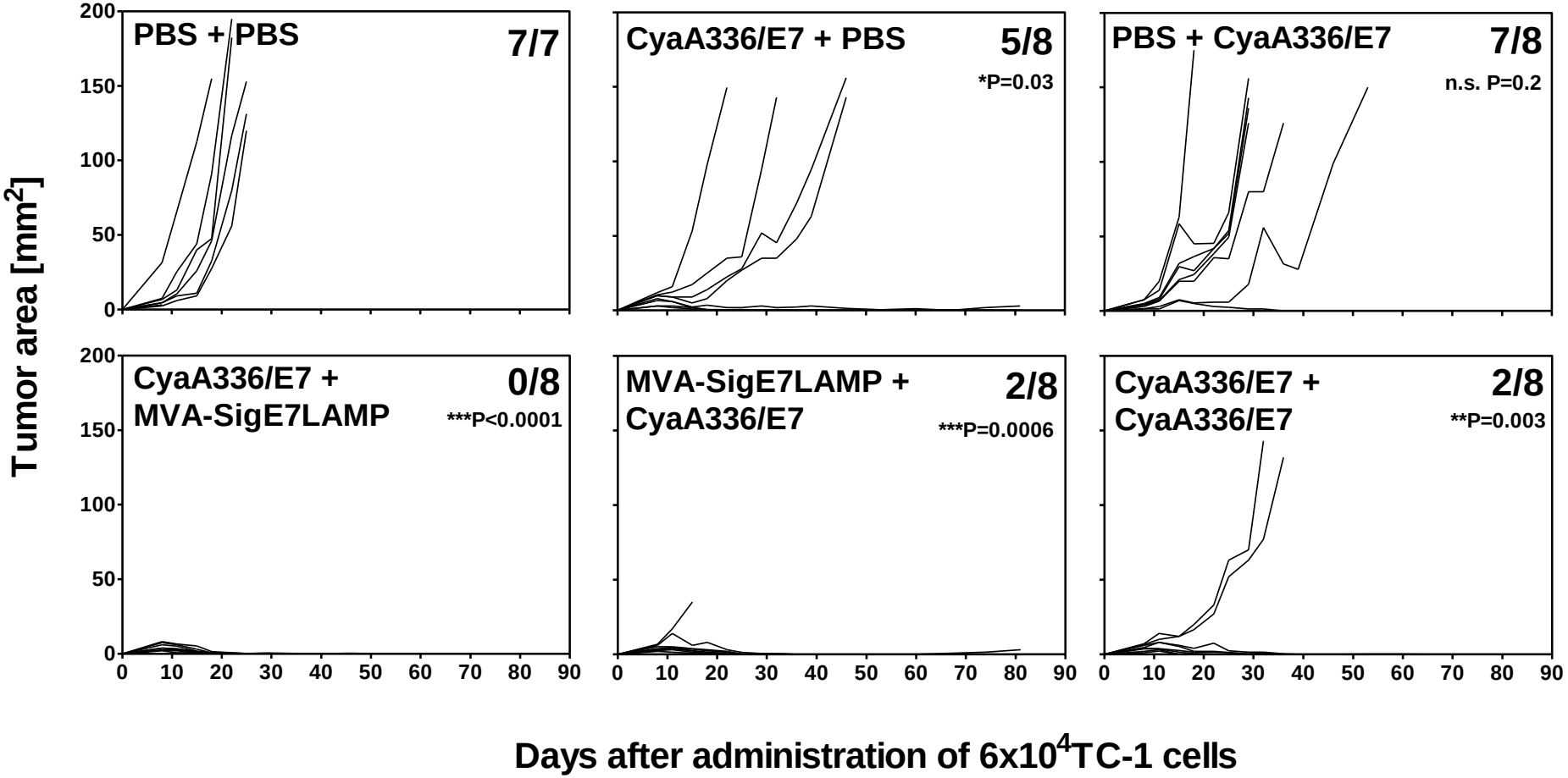
CD8^{+} CTL responses

Insertion point	CTL induction
SSLAHG^{107} VR-*V3-LCMV-OVA*-VH ^{108}HTAVDL	+++
LKEYIG^{335} VR-*V3-LCMV-OVA*-VH ^{336}QQRGEG	+++
SEATGG^{232} VR-*V3-LCMV-OVA*-VH ^{233}LDRERI	+++

Mice are protected against an LCMV challenge

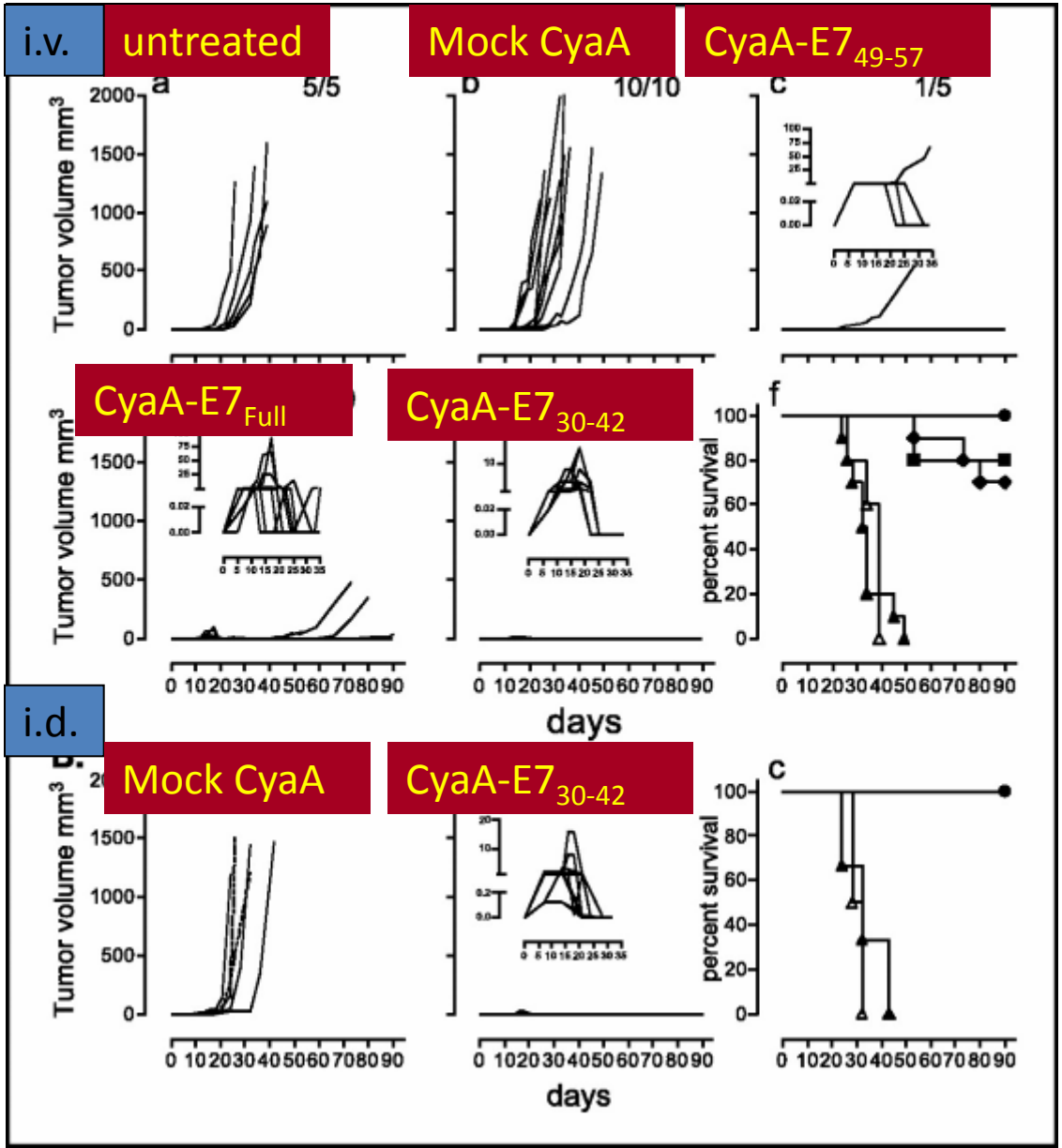
Fayolle *et al.* (2001) *J. Virol.* 75, 7330-8

Prime/Boost Immunotherapy of HPV16-induced tumors
by combimations of CyaA-E7 and MVA-E7 vaccines
(higher challenge dose)



Mackova *et al.* (2006) Cancer Immun. Immunother.55, 39-46

Therapeutic vaccination with recombinant HPV16-E7 CyaAs allows eradication of established tumors and mice survival



Preville *et al.(2005) Cancer Res. 65, 641-649*

Induction of protective immunity against mouse malaria

Mice immunized with:	infected	% protection
PBS	10/10	0%
α-CTLA-4	5/5	0%
ACT-CSP	9/9	0%
ACT-CSP + α-CTLA-4	4/10	**60%**

→ prime/boost immunisation with ACT-CSP does not induce protective immunity

→ blockade of CTLA-4 during boost immunisation leads to significantly enhanced protection against P. berghei challenge

Tartz *et al*., 2006, *Infect. Immun.*

dACT allows induction of antigen-specific T cell responses

$CD8^+$ Antigens	$CD4^+$ Antigens		
OVA	MalE	Sebo *et al*., 1995, *Infect. Immun*.	HIV, LCMV *in vitro*
LCMV	MAGE	Fayolle *et al*., 1996, *J. Immunol*.	HIV
Apa, Cfp	ESAT-6, CFP10	Saron *et al*., 1997, *Proc. Natl. Acad. Sci. U.S.A.*	LCMV *in vivo*
gp120	TB-10.4	Osicka *et al*., 2000, *Infect. Immun*.	permisive sites
E7	Ag85A	Fayolle *et al*., 2001, *J. Virol*.	polyvalent CTL response
CSP		Loucka *et al*., 2002, *Infect. Immun*.	$CD4^+$ T cell response
Melanoma tyrosinase		Schlecht *et al.*, 2004, *J. Immunol.*	Mechanisms
ESAT-6, CFP10		Mackova *et al*., 2006, *Cancer Immunol. Immunother.*	Tumor immunotherapy
TB-10.4		Tartz *et al*., 2006, *Infect. Immun*.,	protection against malaria
		Wilkinson *et al.* 2005 *Infect Immun.*	Improvement of LTBI detection
		Anderson *et al.* 2006 *Am. J. Crit. Care Resp. Med.*	Improvement of LTBI detection
		Majlessi *et al*., 2006, *Infect. Immun*.,	IFNγ and immunity against MTB
		Hervas-Stubs *et al.*, 2006, *Infect Immun*	IFNγ and immunity against MTB

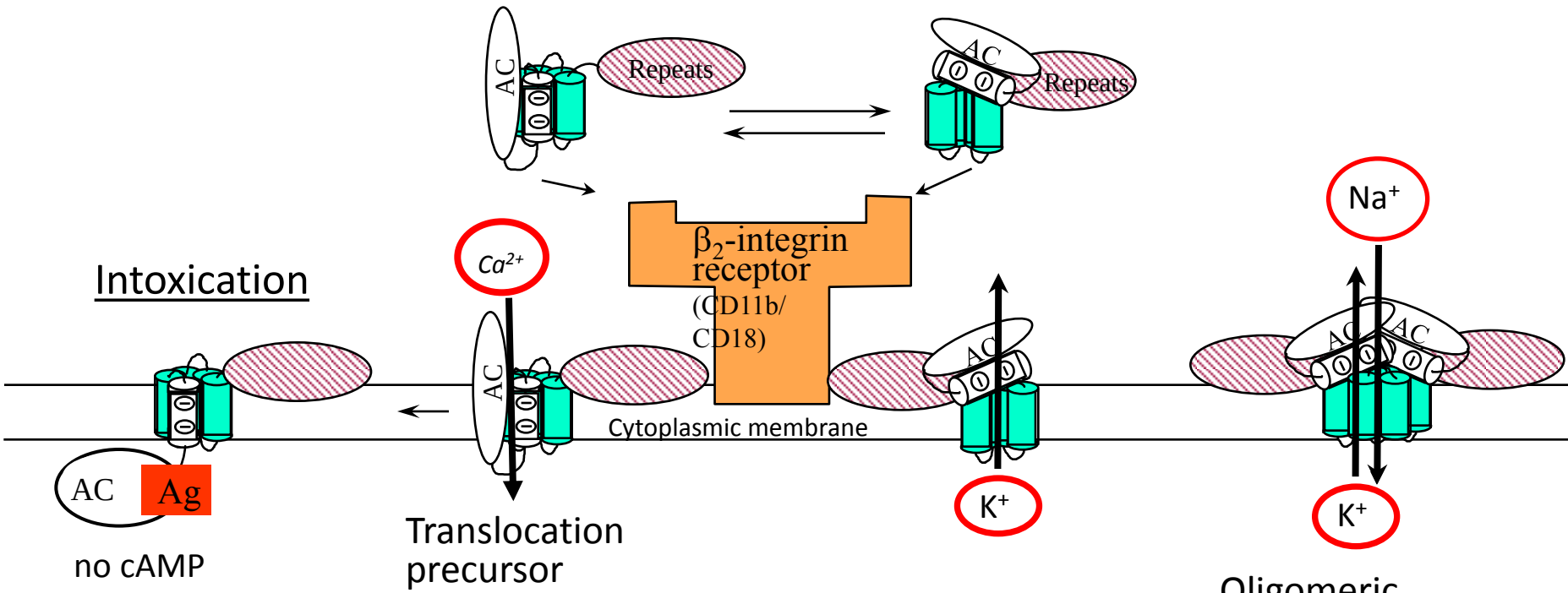
September 2012 - Genticel S.A. completed Phase I clinical trial for HPV16/18-induced cervical carcinoma

Using a cGMP batch of the adenylate cyclase (CyaA-AC$^-$) toxoid for delivery of HPV E7 antigen as immunotherapeutic vaccine

safe, immunogenic, inducing CD8$^+$ CTLs and HPV 16/18 virus load reduction demonstrated

…Heading for phase II trial = will be of interest to see pertussis incidence in CyaA-E7 toxoid treated woman…

Even the AC^{-} toxoid can exhibit immunomodultory activity through calcium signaling, cell permabilization and inflammasome activation and other?...



Osickova *et al.*, (1999) J. Biol. Chem. 274, 37644
Osickova *et al.* (2010) *Mol. Microbiol. 75:15450-1562*

Fiser R. *et al.*(2007) *J. Biol. Chem. 282, 2808*

Dunne *et al.* (2010) *J. Immunol. 2010,* 185: : 1711–1719

Current status of dACT-antigen delivery technology

1997 - Protective immunity against a virus (LCMV)
1999 - Immunotherapy of transplanted tumors in mice
2004 - Enhanced detection of latent tuberculosis
2005 - Protective immunity against *Plasmodium* (mouse malaria model)
2005 - immunotherapy of experimental tumors (such as HPV16 – induced)

(US Patent No. 5,503,829, No. 5,679,784, No. 5,935,580, *EU Patent application* No. 03291486.3, *US Prov 03495, 6094 (2003)*)

It flies or it dies???

tell you next time…

Phase I/II clinical trial in melanoma patients starts soon (EU 6.FP consortium THERAVAC)

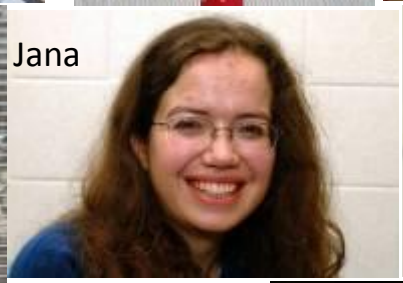

Peter


Ivo


Irena


Marek
Jirka
Radim


Marcela


Jana


Jana


Zuzana


Jana


Lada


Adriana


Radek


Lenka


Ondra


Jana H

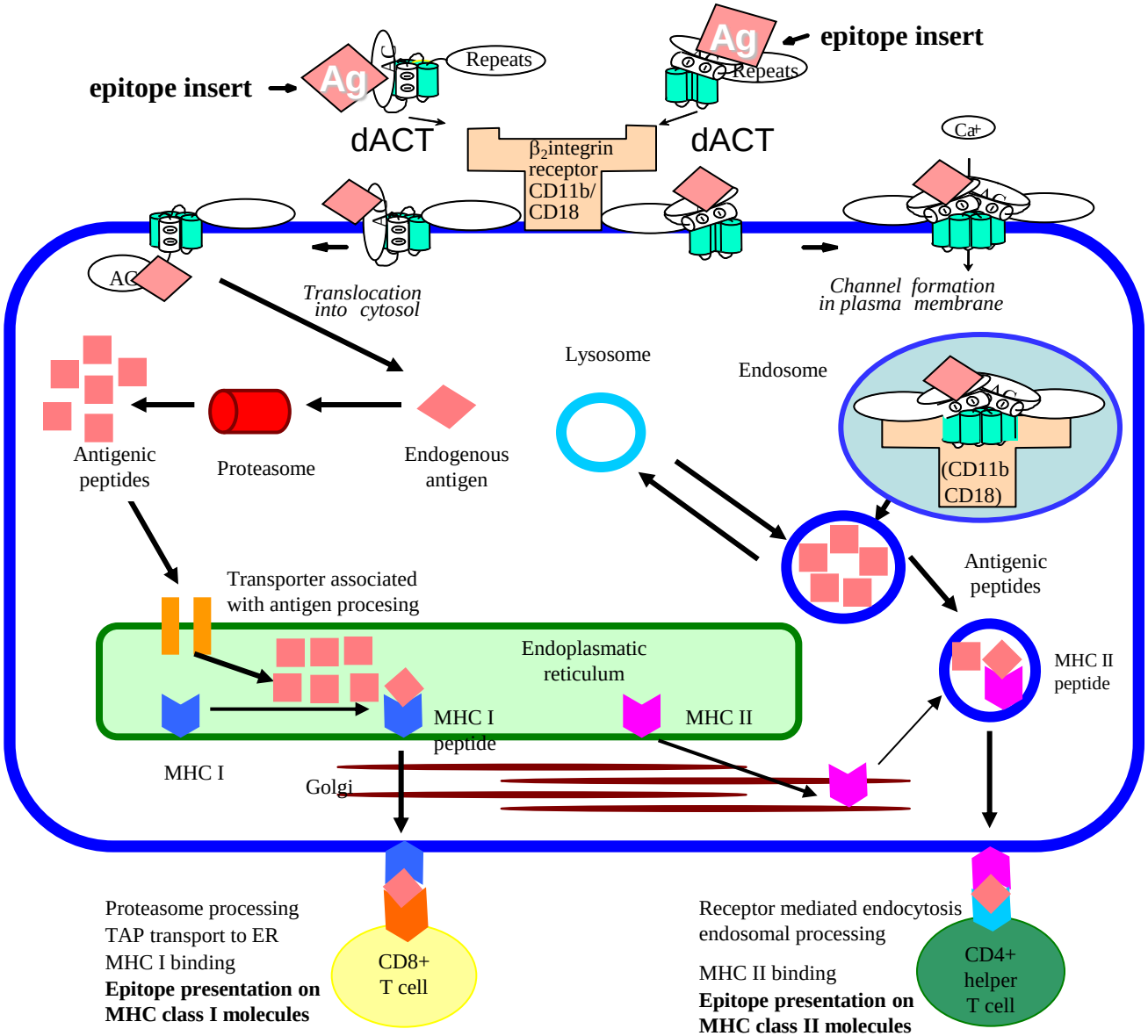

Katka


Ilona


Hanka


Soňa

dACT as a novel antigen delivery tool



Thanks to the team and you for patience…

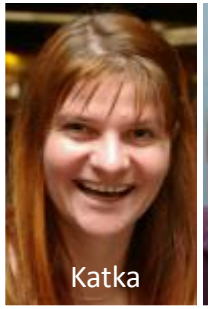

Peter
the big boss...


Radim


Jirka


Marek


Radek


Katka


Irena L.


JanaV


Jiřina

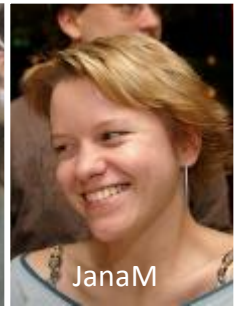

JanaK


OndraČ


Kája


MartinaK


JanaH


JanaM


PavelMi


PavelMa


Tomáš


Jawid


MartinaS


Eva


Zuzana


HanaJ


Láďa


IrenaA


Lenka


OndraS


Delphine


IlonaB


Adriana


Marcela


HankaK


Soňa


IlonaK