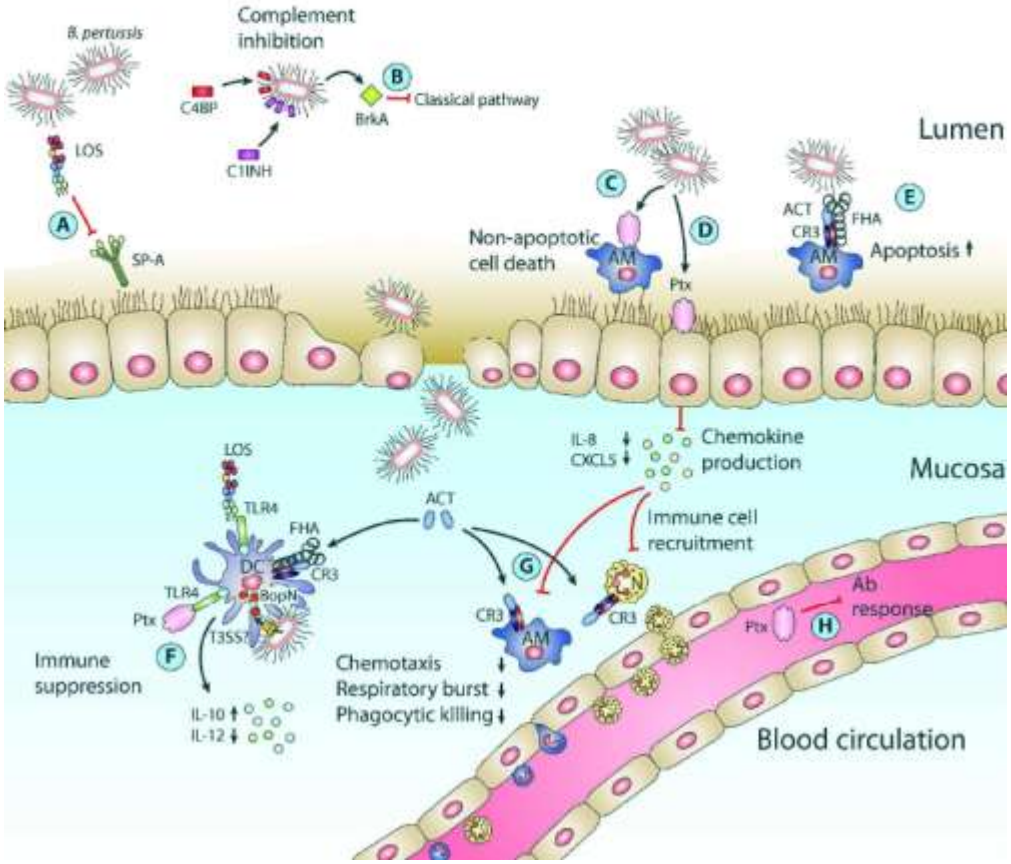
Bordetella pertussis:

One of the few TRUE human pathogens

Virulence as an inherent part of lifestyle

Bordetella pertussis is one of the few REAL human pathogens

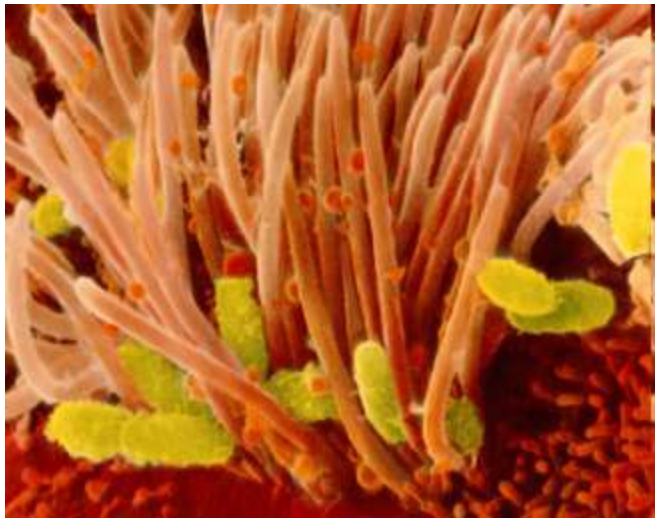


It depends on a human host and is equipped to survive innate immunity, to colonize, multiply and force the host to cough to spread it…

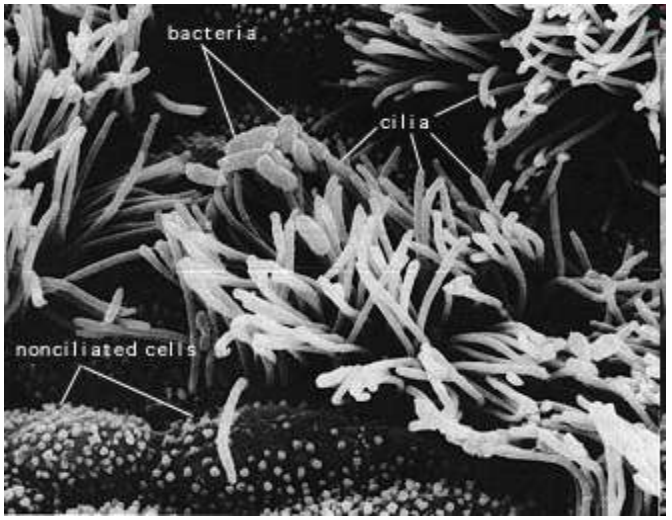
de Gouw *et al.* (2011) FEMS Microbiol. Rev. **35,** 441-474

Bordetella Infection

- colonization of the respiratory tract
- biofilm formation (essential role of adhesins)
- binding to the ciliated epithelial cells in the nasopharynx and trachea and multiplication → death of the cells → bacteria and mucus are not taken out of the airway → persistent coughing



http://children.webmd.com/



http://www.textbookofbacteriology.net

B. pertussis genome : 4,086,186 bp

Tohama I genome sequencing revealed on the top of what was known:

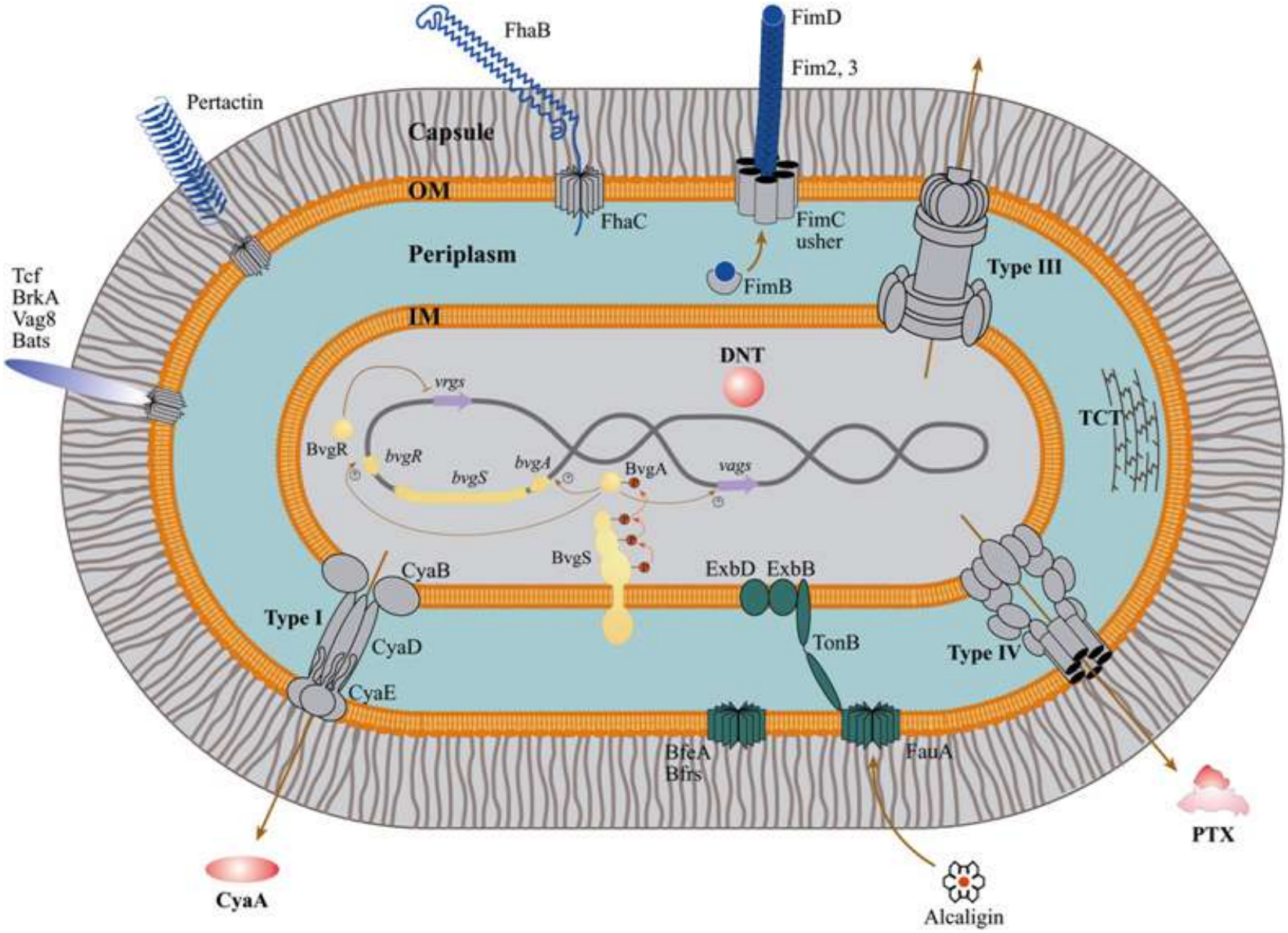
- **200 *IS***
- **130 transcription factors**
- **100 ABC transporters**
- **17 two-component syst.**
- **14 autotransporters**
- **Integrated phage**
- ***Hin*dIII restriction**
- **91 Bug (79 complete)**
- **FHA-like (FhaL, FhaS)**
- **Capsule synth. & export**
- **Intimin-like**
- **Flagellum (65 kb)**
- **Type III secretion syst.**
- **Exported proteases**
- **Siderophore/heme recept.**

King et al. BMC Genomics 2010, 11:64

Changes in the genomic content of circulating *Bordetella pertussis* strains isolated from the Netherlands, Sweden, Japan and Australia: adaptive evolution or drift?

- analyzed 171 *B. pertussis* strains.
- the core genome of B. pertussis, consists of 3,281 conserved CDSs that represent 84.8% of all CDSs found
- genome size of B. pertussis strains is decreasing progressively over the past 60 years.

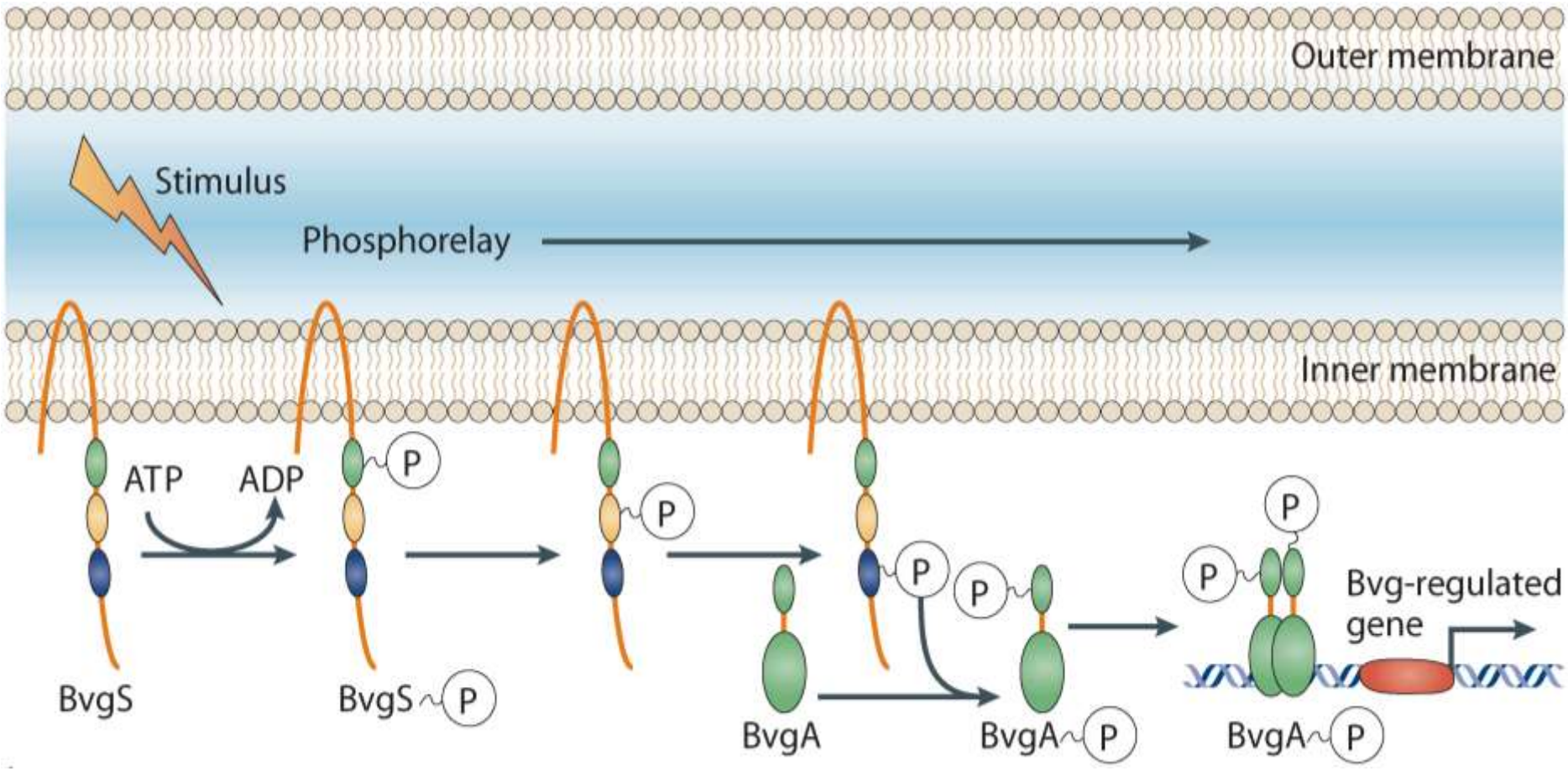
Bordetella virulence factors



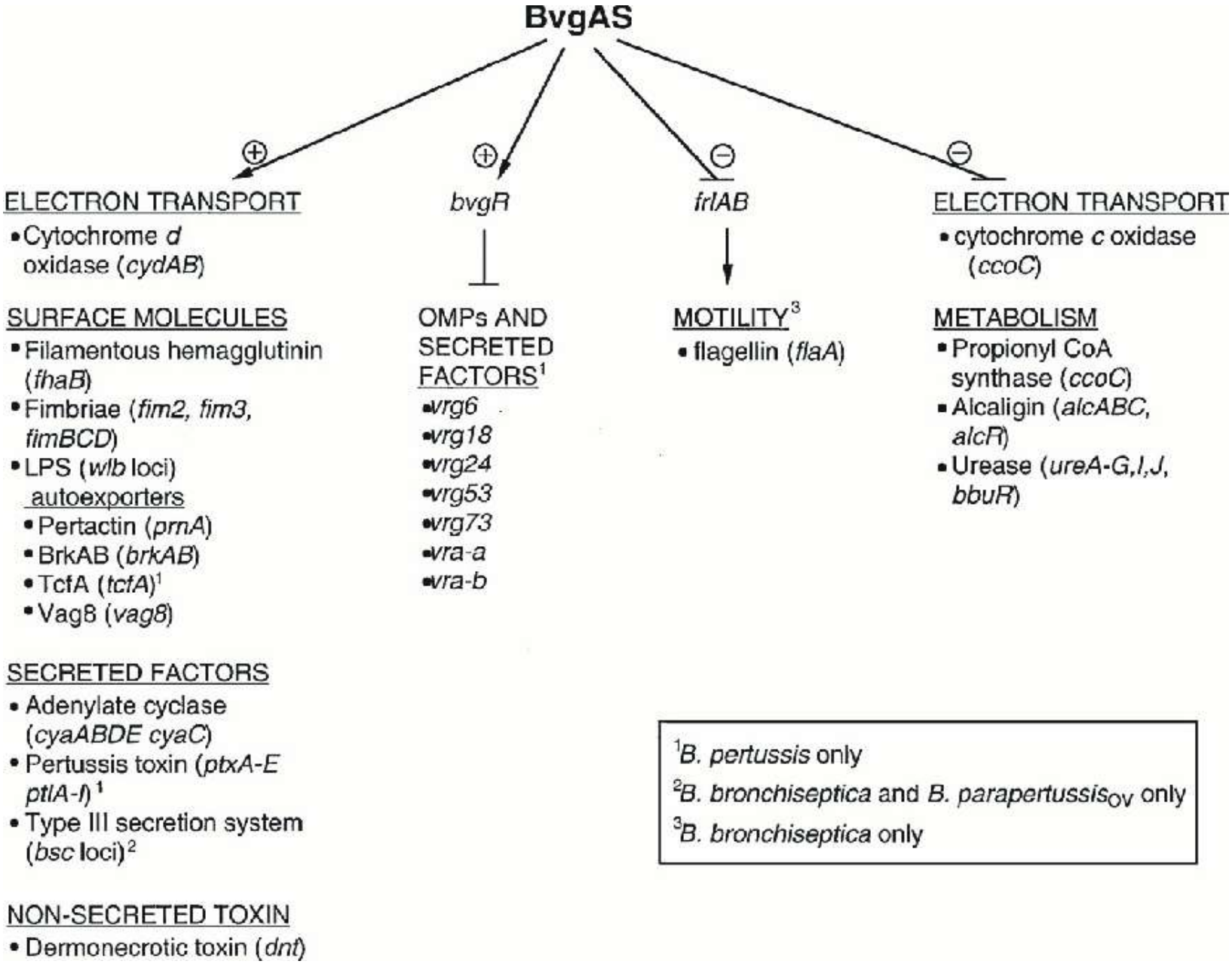
Slide: Courtesy of C. Locht, Institut Pasteur de Lille

BvgAS regulatory system

modulating conditions (sulfate or nicotinic acid or growth temperature below 25°C) → BvgAS phosphorelay is inactivated → no expression of virulence genes → avirulent Bvg⁻ phase



The Bvg-regulon of *Bordetella* species



Bordetella pertussis *in vitro* phenotypic modulation

37 °C

C mode ⇌ **X mode**

25°C
$MgSO_4$
Nicotinic acid

Vrgs

Vags

PTX
FHA
AC
Pertactin
T3SS

What does BvgAS sense in vivo????

INFECTION AND IMMUNITY, June 1998, p. 2762–2768 Vol. 66, No. 6

Neither the Bvg2 Phase nor the *vrg6 Locus of Bordetella pertussis* Is Required for Respiratory Infection in Mice

GUILLERMO MARTINEZ DE TEJADA,1‡ PEGGY A. COTTER,1* ULRICH HEININGER,1§
ANDREW CAMILLI,2 BRIAN J. AKERLEY,3 JOHN J. MEKALANOS,3 AND JEFF F. MILLER1

There is no known environmental or animal reservoir for B. pertussis

it has been assumed that this phenotypic alteration must occur within the human host

constructed Bvg1 and Bvg2 phase-locked mutants *a*

Bvg1 phase of *B. pertussis* is necessary and sufficient for respiratory infection.

a strain with a deletion in the bvgR regulatory locus - ectopic expression of Bvg2 phase phenotypes decreases the efficiency of colonization,

B. pertussis adhesin and toxin confusion….

Adhesins

- **Filamentous haemagglutinin (FHA)**
- **Fimbriae**
- **Pertactin (RGD motif)**
- **Tracheal colonisation factor (TCF)**
- **Bps exopolysacccharide**

Toxins

- **Pertussis toxin (PTX)**
- **Adenylate cyclase (AC)**
- **Dermonecrotic toxin (DNT)**
- **Tracheal cytotoxin (TCT)**
- **Lipopolysaccharide (LOS)**

- Pertactin (neutrophil resistance)
- FHA – immunomodulation through IL-10

Serum resistance

BrkA, capsule, LOS modification, C1ingh binding

Molecular Microbiology (2010) 77(6), 1439–1455

The Bps polysaccharide of Bordetella pertussis promotes colonization and biofilm formation in the nose by functioning as an adhesin

data reveal a biofilm lifestyle for *B. pertussis* in the nose and the requirement of Bps in this developmental process.

Bps functions as an adhesin by promoting adherence of *B. pertussis and Escherichia coli* to human nasal but not lung epithelia.

Marr *et al.* J. Infect Dis. (2010) 202 (12): 1897-1906.

Variability in the Lipooligosaccharide Structure and Endotoxicity among *Bordetella pertussis* Strains

Bordetella endotoxins show remarkable structural variability both among each other and in comparison to other gram-negative bacteria.

Compared to Tohama I derivative BP338, LOS from *Bp* 18323 is a poor inducer of inflammatory cytokines in human and murine macrophages,

18323

1) lacks the ability to modify its lipid A phosphate groups with glucosamine,
2) is distinct in its acylation at the C3′ position of the lipid A diglucosamine backbone,
3) expresses molecular lipooligosaccharide species that lack a terminal heptose.

Adhesins

Filamentous hemagglutinin (FHA)

- mature FHA noncovalently bound to the cell surface → weaker interaction between bacteria and host

- binds galactose residues on sulfated glycolipids (ciliated cells) and CD11b/CD18 cokmplement receptor (neutrophils)

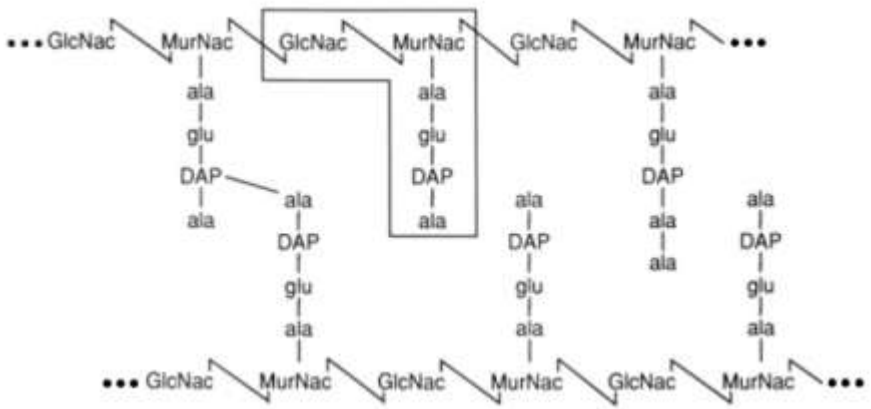
- **Induces immunosuppressive IL-10 …. More of a toxin than an adhesin?**

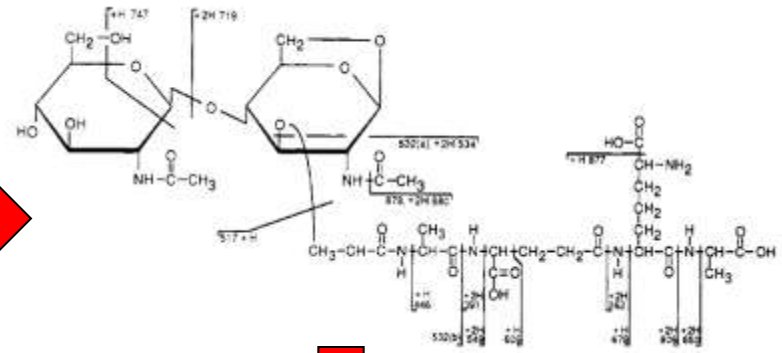
Fimbriae

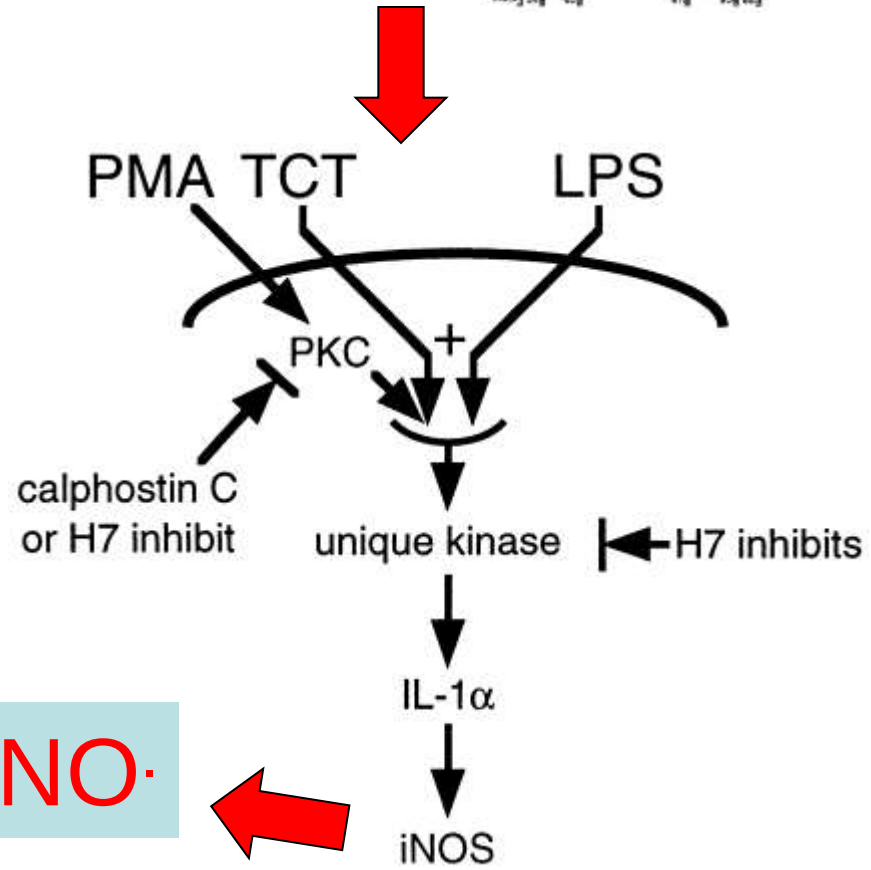
Pertactin (PRN) – involved in resistance to neutrophile clearance

Toxins

Action of tracheal cytotoxin





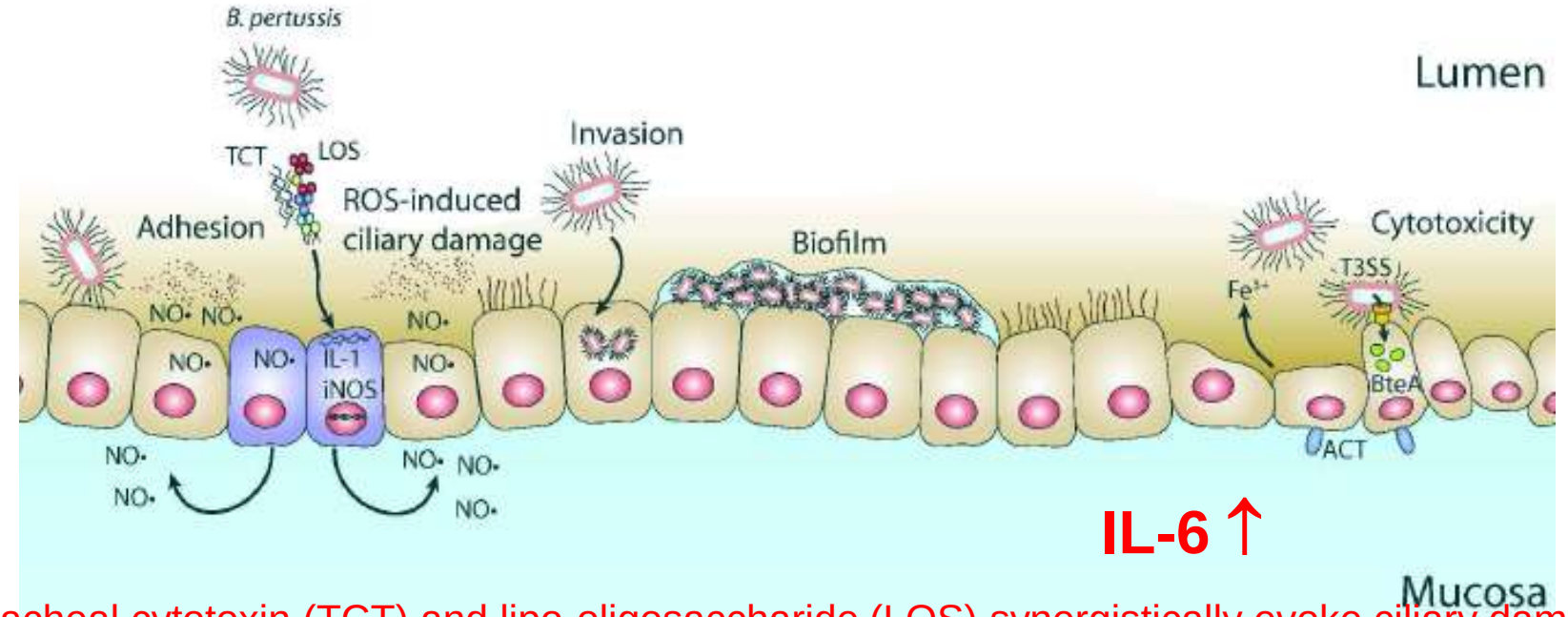


Tracheal cytotoxin (TCT) is released by *B. pertussis.*
This, together with LOS induces IL-1 production in goblet cells, causing induction of iNOS and the production of NO·.
This leads to cytostasis and ciliated epithelial cell damage.

Flak, T. A. et al. 2000. Infect. Immun. 68(3):1235-1242

the Yang:

Pathologic effects of *Bordetella* toxins on respiratory mucosa…



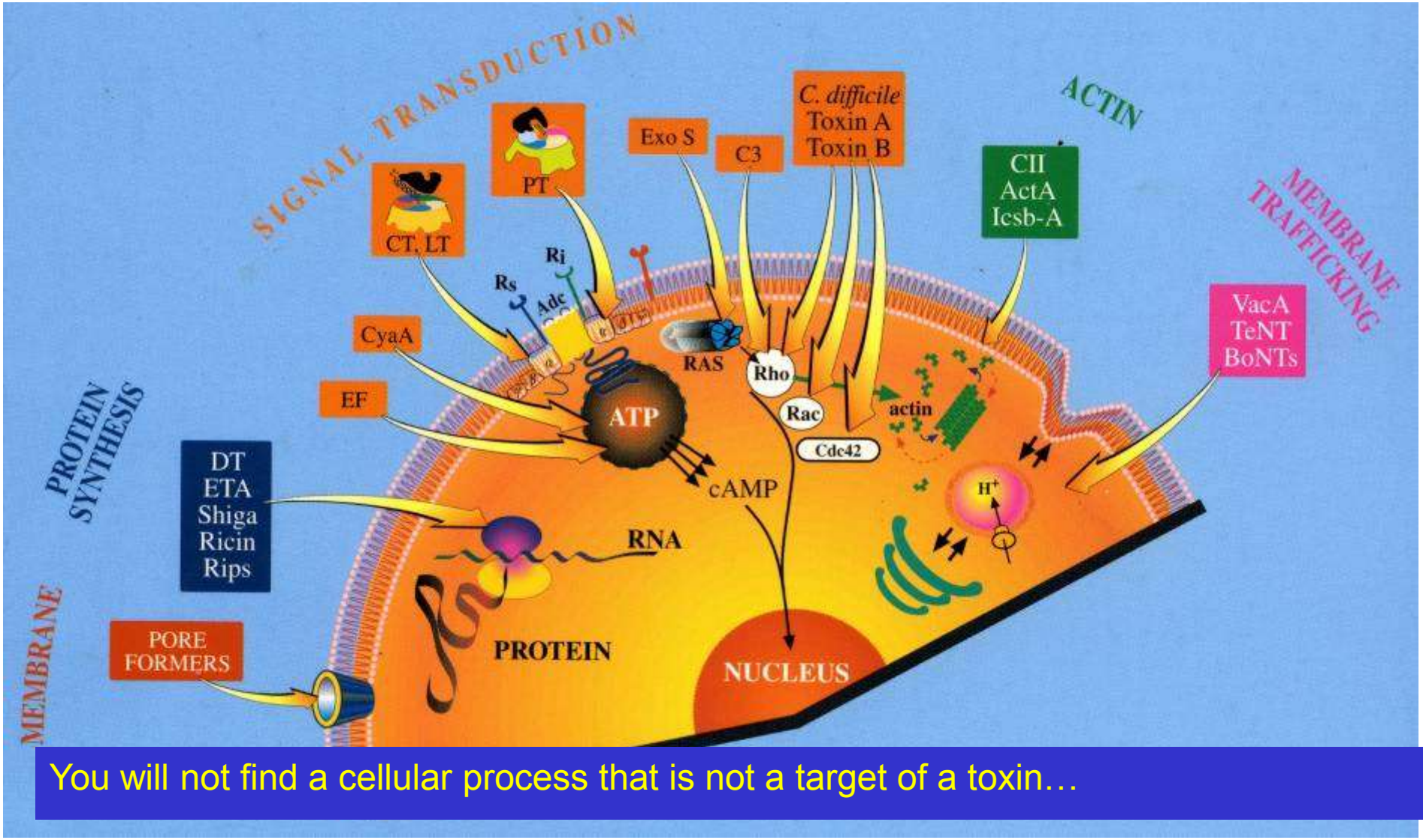
Tracheal cytotoxin (TCT) and lipo-oligosaccharide (LOS) synergistically evoke ciliary damage by initiating the release of destructive reactive oxygen species (ROS), such as nitric oxide (NO) via interleukin 1 (IL-1) induced type II nitric oxide synthases (inducible NOS, or iNOS) activation in mucus-secreting goblet cells

Adenylate cyclase toxin (ACT) and the type III secretion system (T3SS) with its effector protein BteA subvert intraepithelial signaling pathways leading to cytotoxicity.

COX2 activation and PGE_2, cytokines and chemoattractants secretion
Mucus production and lack of ciliary beating provokes COUGH !!!

de Gouw *et al.* (2011) FEMS Microbiol. Rev. **35,** 441-474

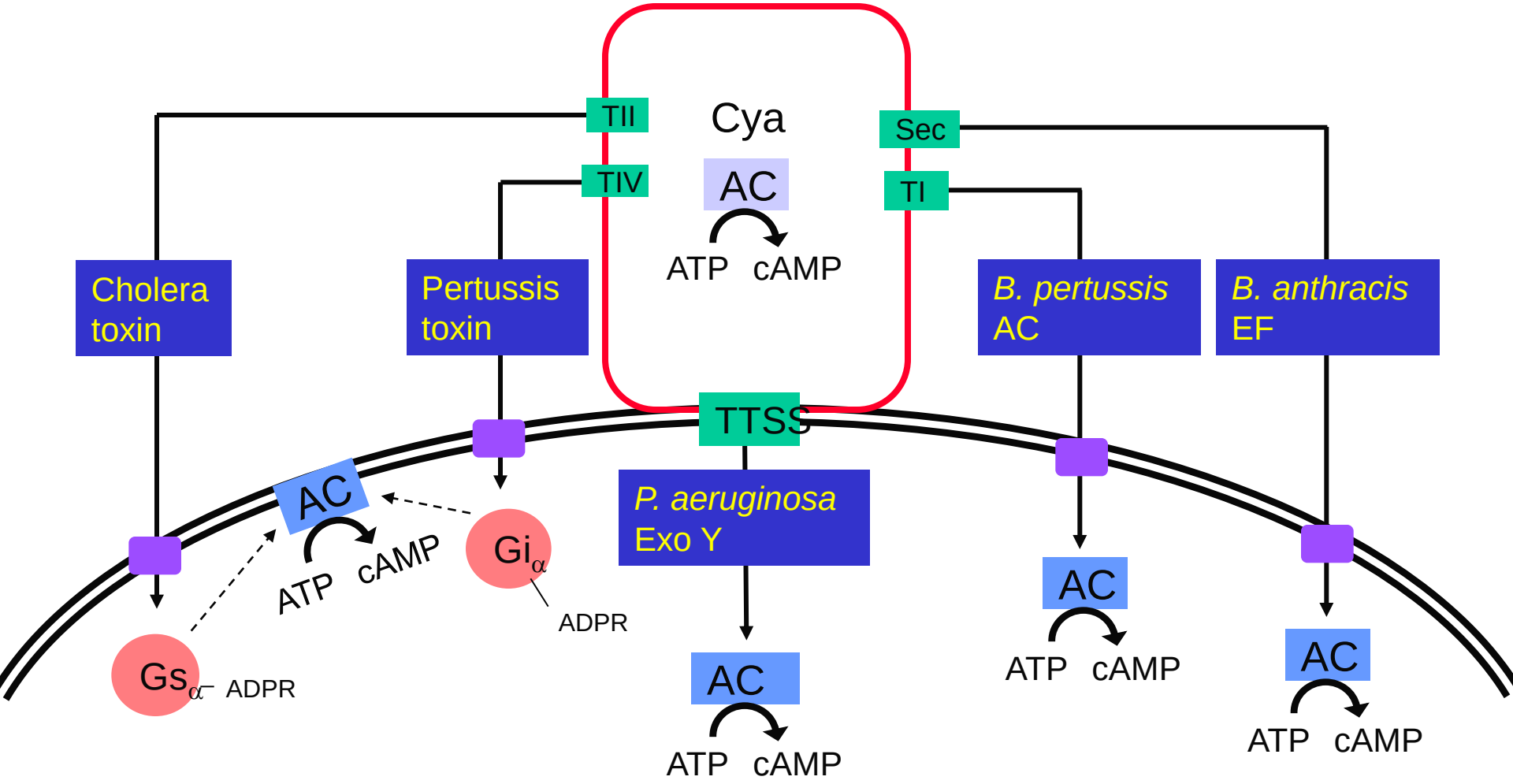
Bacterial protein toxins are “smart, pretty and useful”



You will not find a cellular process that is not a target of a toxin…

The ‘smartest’ toxins subvert cell signaling

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



Slide: courtesy of S. Lory, Hravard Medical School

What all may ACT/cAMP modulate in tracheal epithelial cells during *Bordetella pertussis* infection ?

↓ ciliary beating

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

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

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

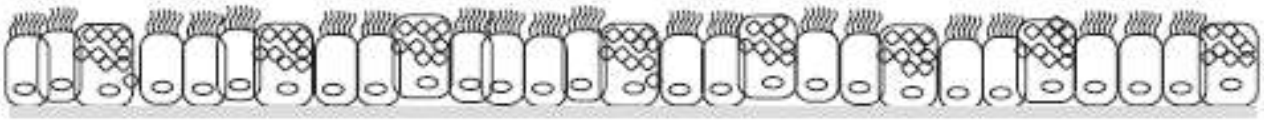
mucin : MUC2, MUC5AC ↑

other soluble factors:
↓ NO, ↑ PGE2

LPS

ACT

cAMP



AEC

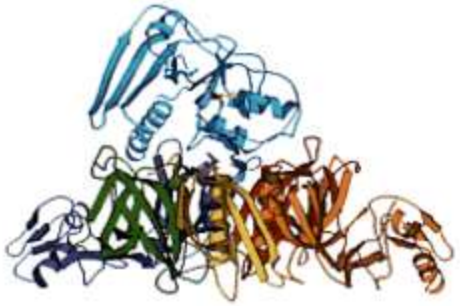
other cells

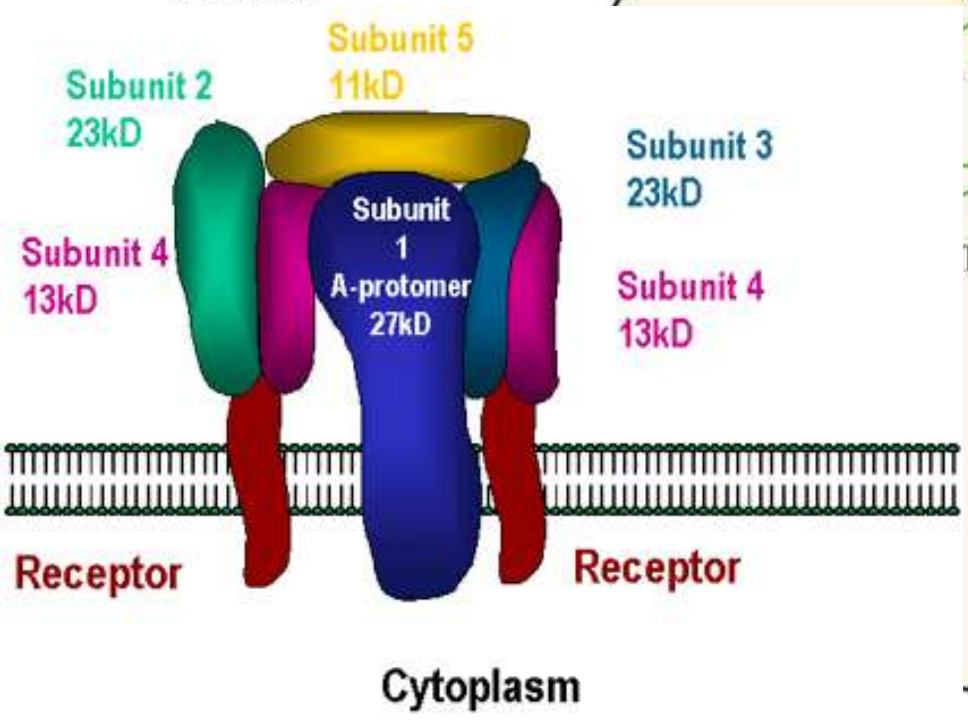
cytokine and chemokine:
IL-1α, ↑ IL-1β, ↑ IL-6, ↑ IL-8,
↑ IL-10, ↓ TNFα, ↓ IFNβ, TGF-β,
↓ GM-CSF, MCP-1, ↓ MIP-1α,
RANTES,..

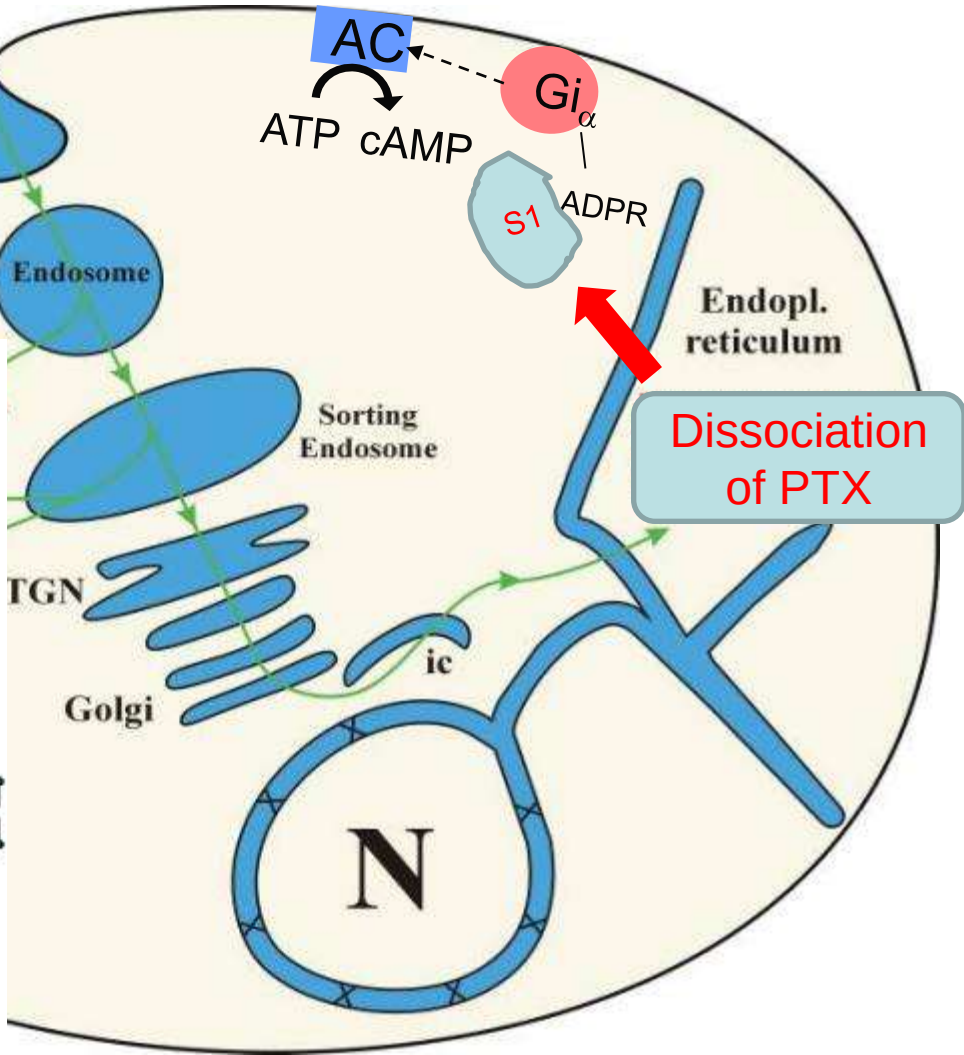
expression of costimulatory x inhibitory molecules: ↑ CD80, CD86, ↓ CD40,
↓ CD54, B7-H2, B7-H3 x ↑ FasL, PD-L1,
PD-L2

Pertussis toxin (PT),

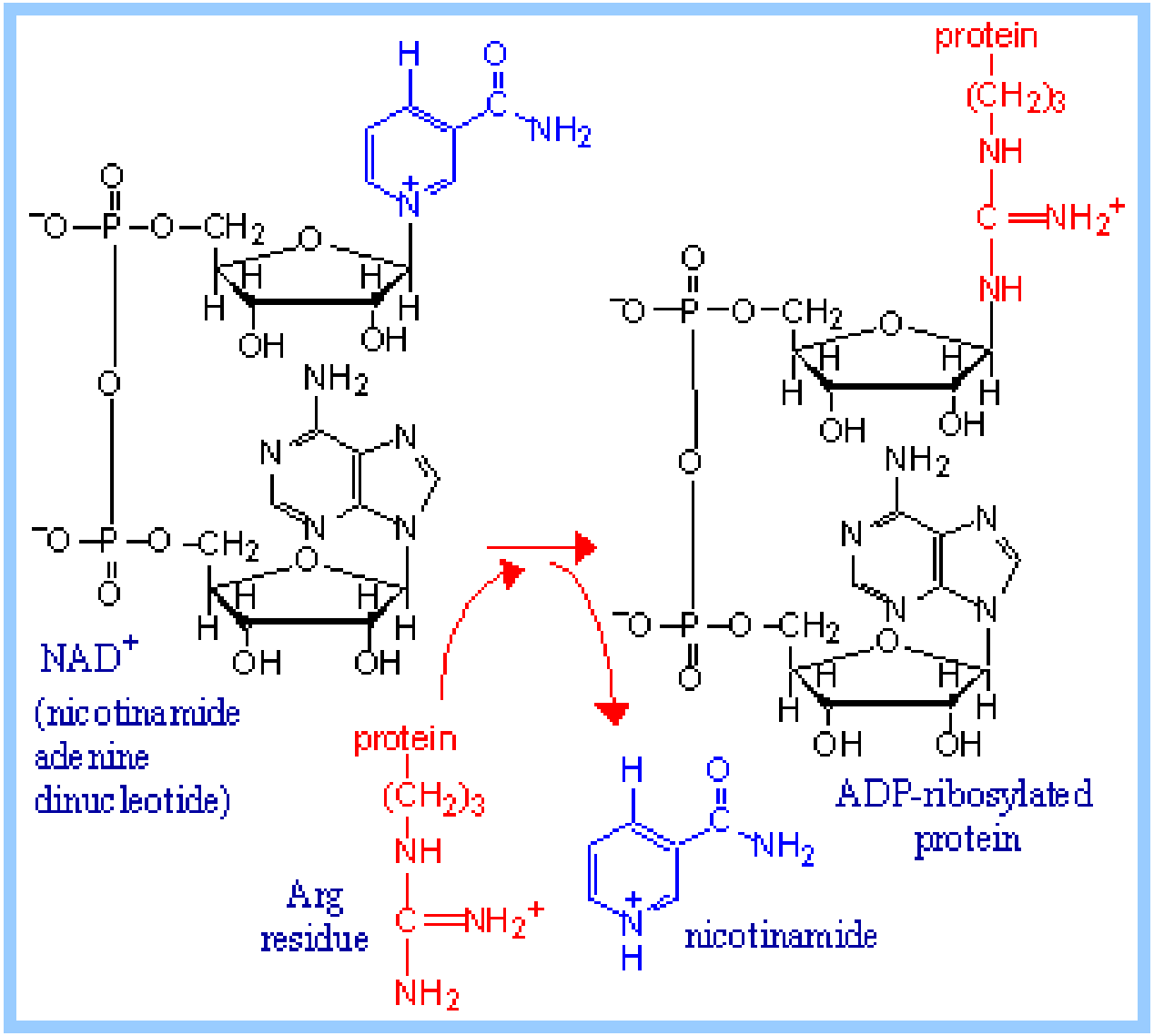
an AB5 exotoxin is trafficked along a retrograde transport pathway , through the Golgi complex to endoplasmic reticulum (ER), where dissociation of the holotoxin I occurs. S1 then trabslocates to cytosol and where it ADP-ribosylates its Gi protein targets.



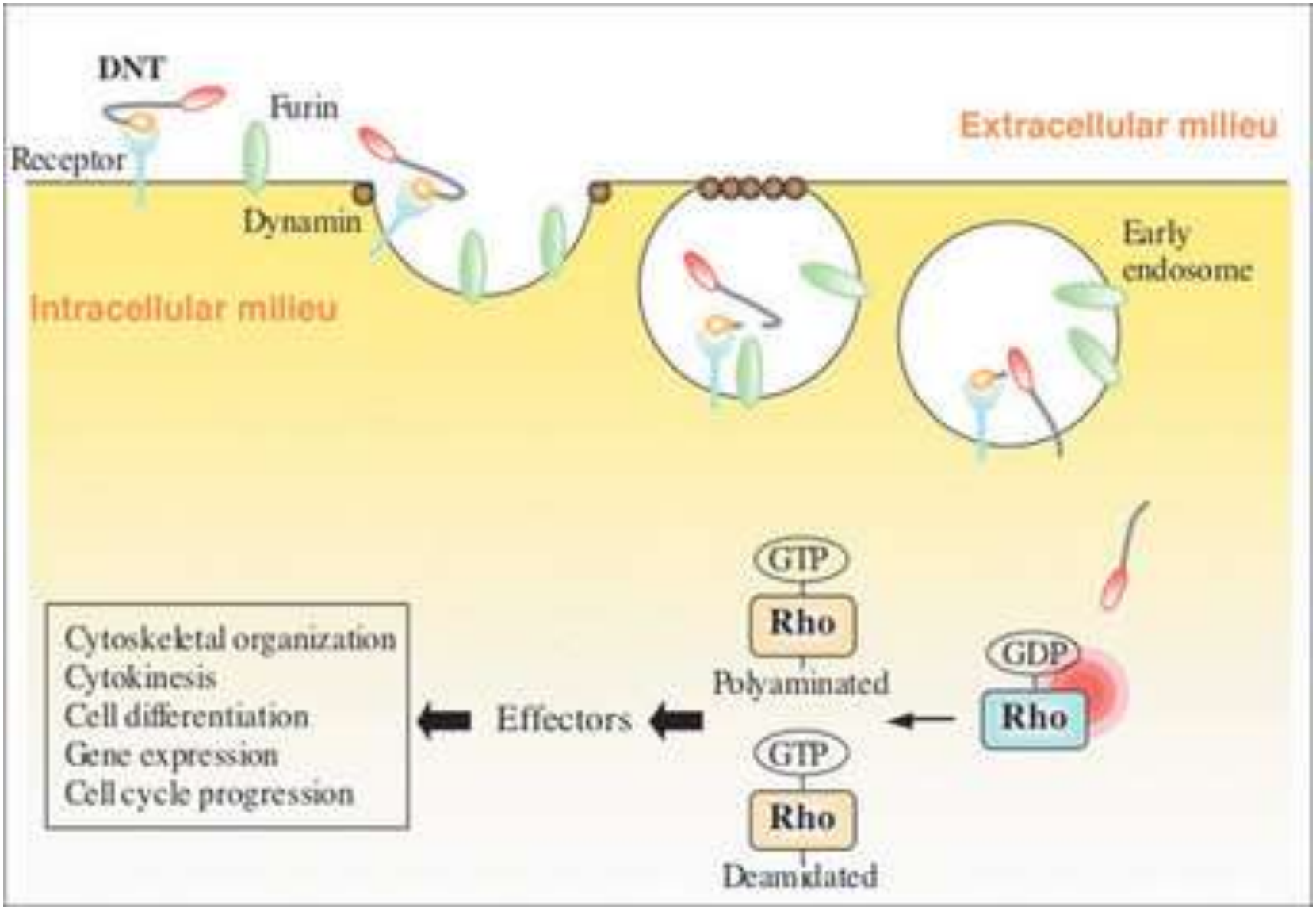




Pertussis toxin catalyzes ADP-ribosylation of Gi proteins

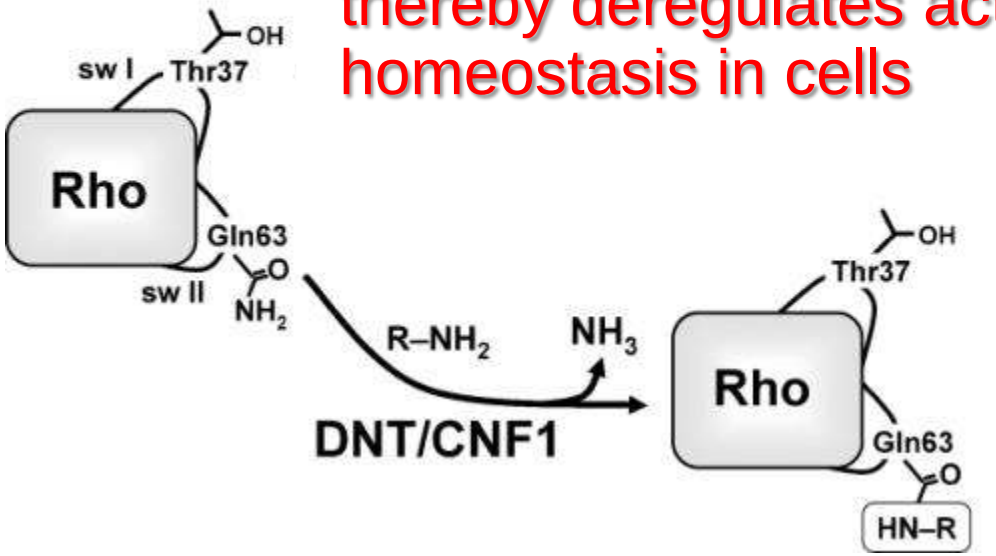


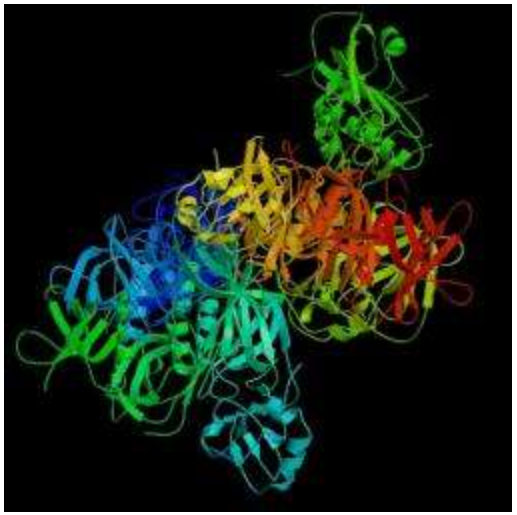
Dermonecrotic toxin is released by lysis of bacteria and causes deregulation of actin cytoskeleton homeostasis

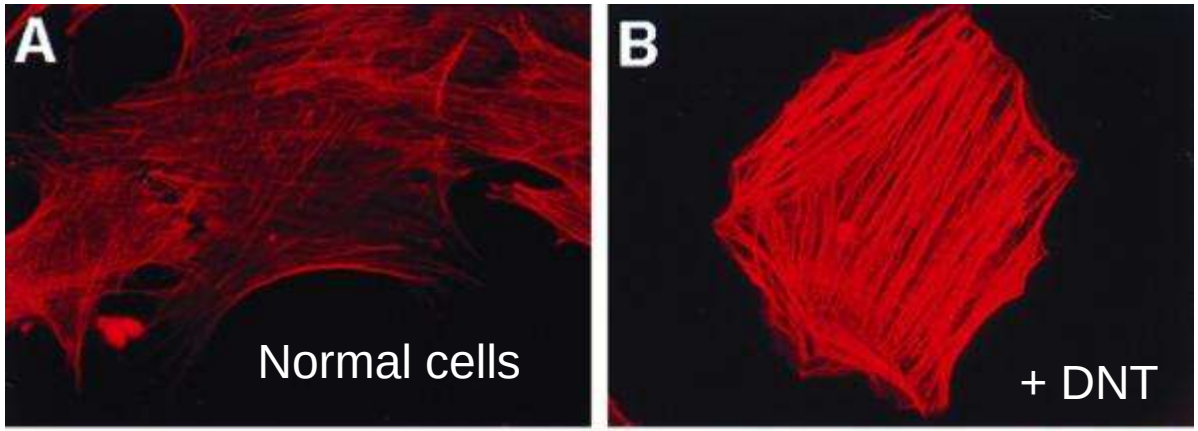


Bordetella Dermonecrotic toxin action

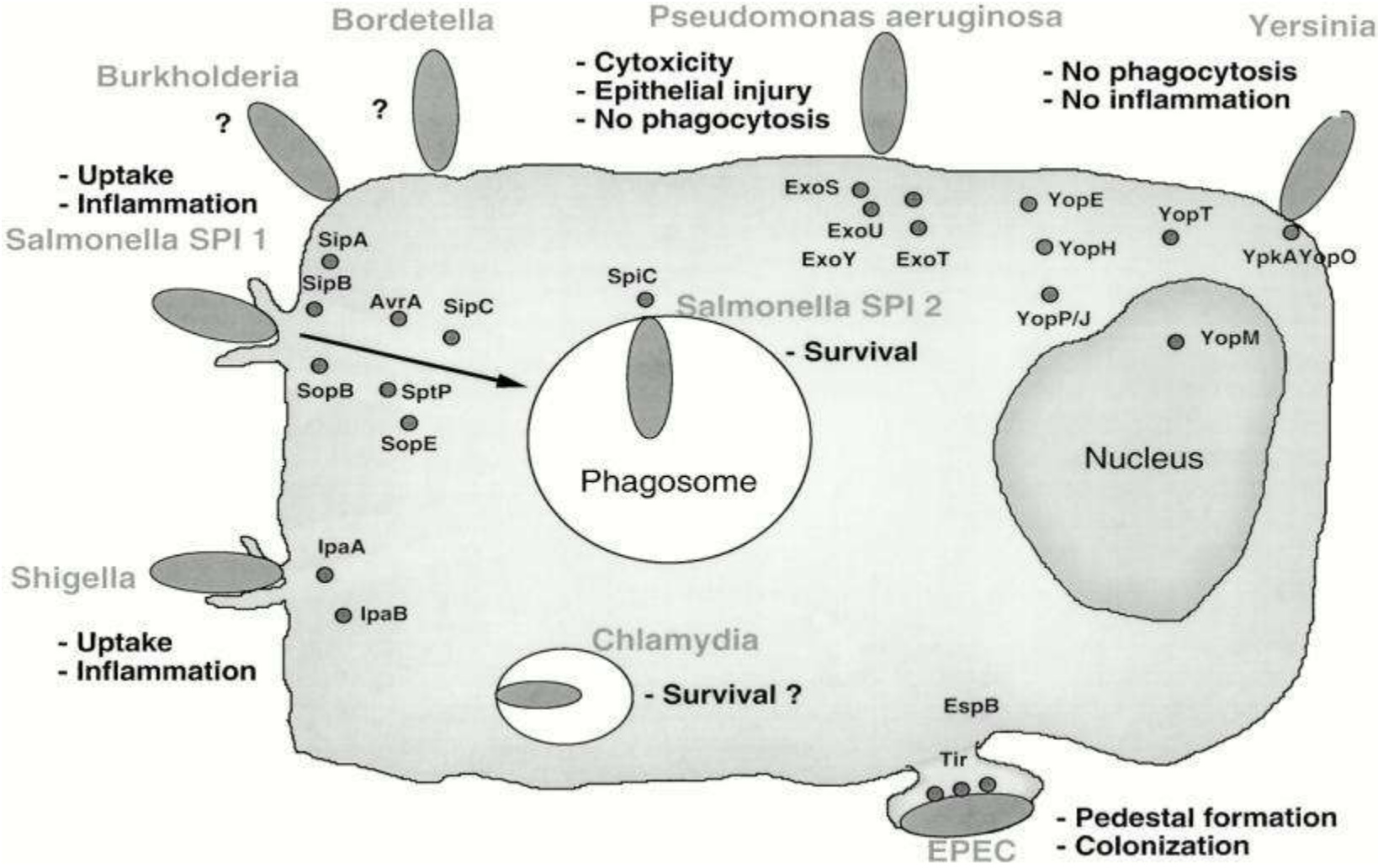
Causes deamination of Glutamine 63 of the small GTP-ase Rho and thereby deregulates actin homeostasis in cells





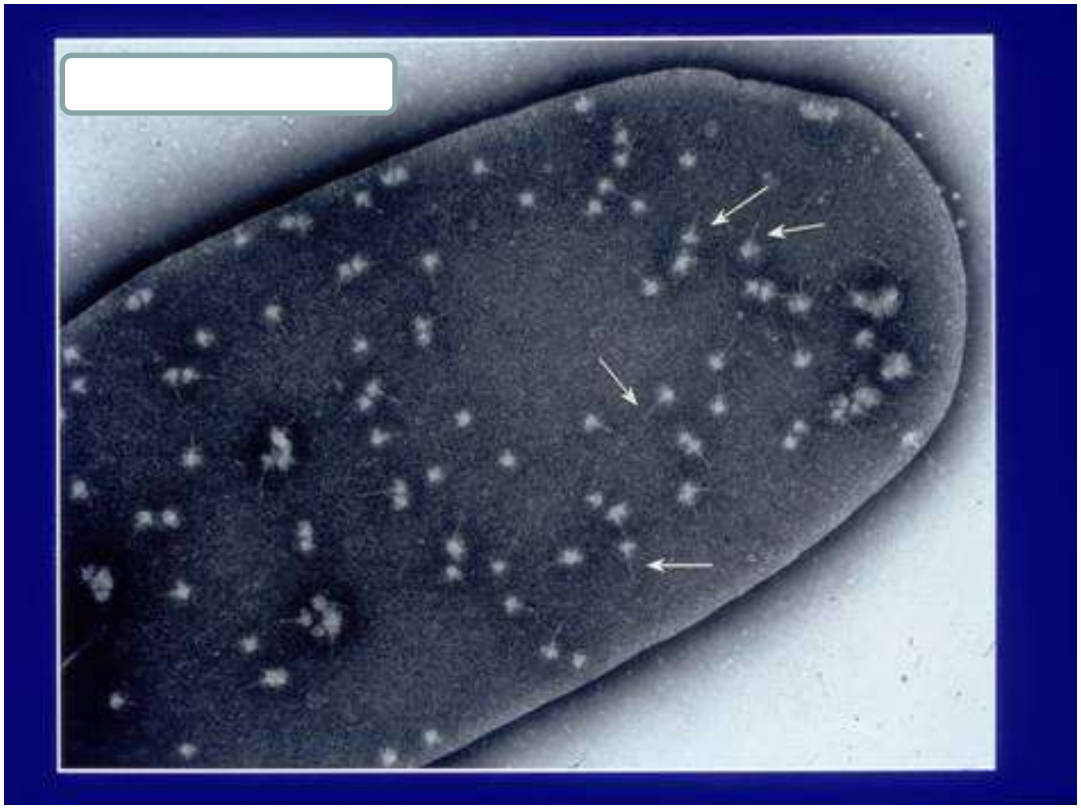


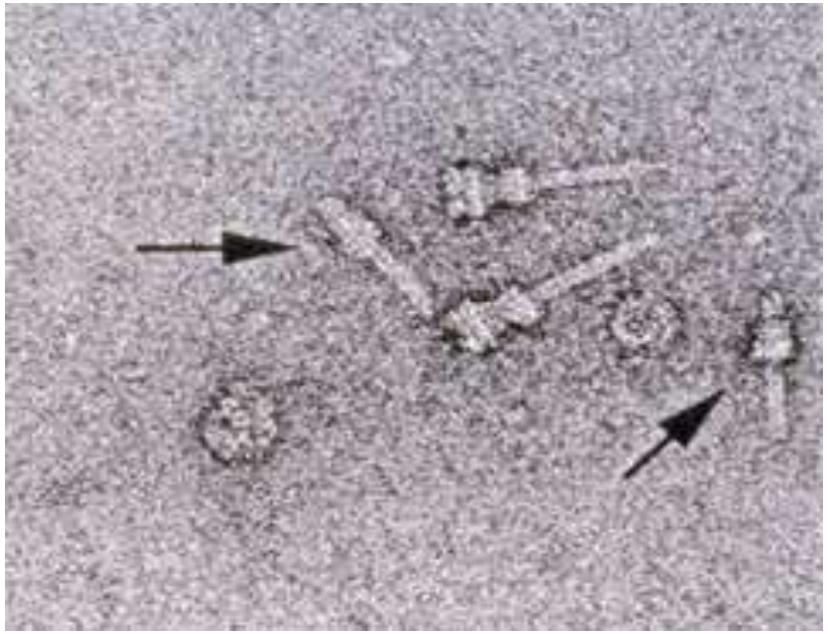
Type III secretion systems in mammalian pathogens



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

Bordetella pertussis expresses a functional type III secretion system that subverts protective innate and adaptive immune responses.





TTSS effectors, Bsp22, BopN, BteA and BopD, were identified as TTSS substrates in B. pertussis 12743.

Bsp22 is expressed in a significant proportion of clinical isolates but not in common laboratory-adapted strains of B. pertussis.

Fennely et al. Infect Immun. 2008 Mar;76(3):1257-66.

INFECTION AND IMMUNITY, Mar. 2008, p. 1257–1266

Bordetella pertussis Expresses a Functional Type III Secretion System That Subverts Protective Innate and Adaptive Immune Responses

Neil K. Fennelly,[...] and Kingston H. G. Mills

secretion of the *Bordetella TTSS* effector, Bsp22, by a significant portion of **low-passage clinical isolates** of *B. pertussis,*

But not by common laboratory-adapted strains, such as Tohama I and Wellcome 28.

Mutation of *bscN* abolished *in vitro* secretion of TTSS substrates by a clinical isolate of *B. pertussis,*

reduced ability to colonize the respiratory tracts of mice,
enhanced local inflammatory and antigen-specific cellular and humoral immune

Han HJ, Kuwae A, Abe A, Arakawa Y, Kamachi K.
PLoS One. 2011 Mar 10;6(3):e17797.

Differential Expression of Type III Effector BteA Protein Due to IS481 Insertion in Bordetella pertussis.

- The T3ISS effector BteA is responsible for host cell death in B. bronchiseptica infections.
- The cytotoxic effector **BteA protein is expressed at higher levels in B. pertussis nonvaccine-type strains** than in vaccine-type strains.
- This type-dependent expression is due to an insertion of IS481 in *B. pertussis* clinical strains, suggesting that **augmented expression of BteA protein might play a key role in the type shift of B. pertussis.**

J. Exp. Med. Vol. 206, No. 13, 3073-3088 (2009)

Bordetella evades the host immune system by inducing IL-10 through a type III effector, BopN

Kanna Nagamatsu,1 Asaomi Kuwae,1 Tadashi Konaka,1 Shigenori Nagai,3,4
Sei Yoshida,3,4 Masahiro Eguchi,2 Mineo Watanabe,2 Hitomi Mimuro,5
Shigeo Koyasu,3,4,6 and Akio Abe1

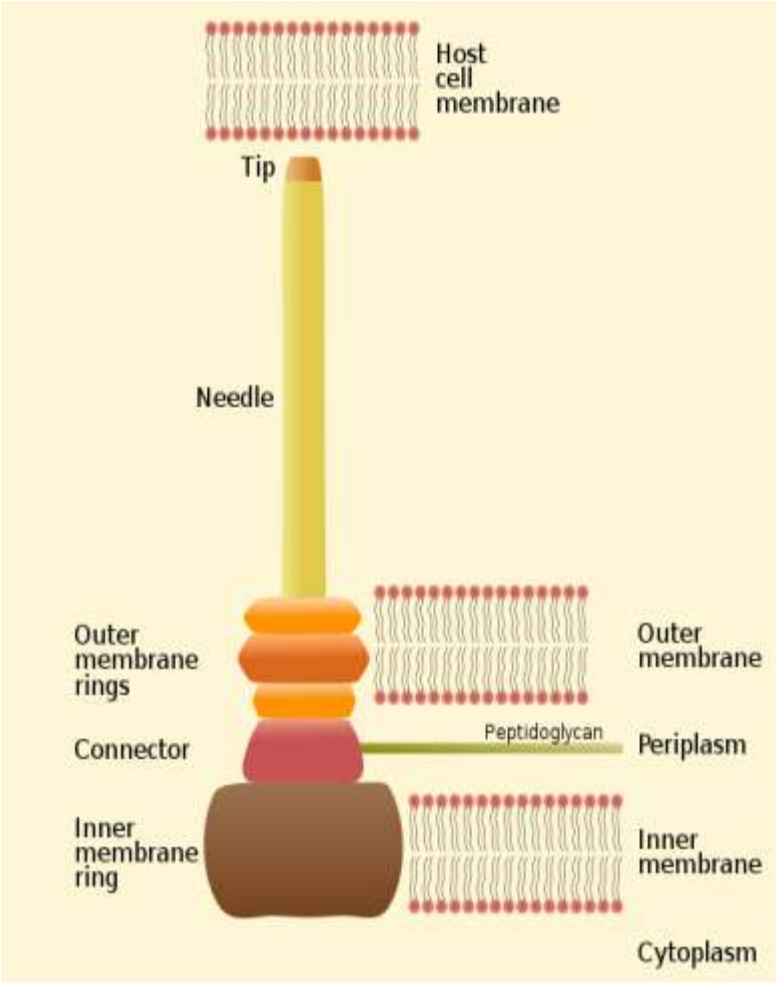
The inflammatory response is one of several host alert mechanisms that recruit neutrophils from the circulation to the area of infection.

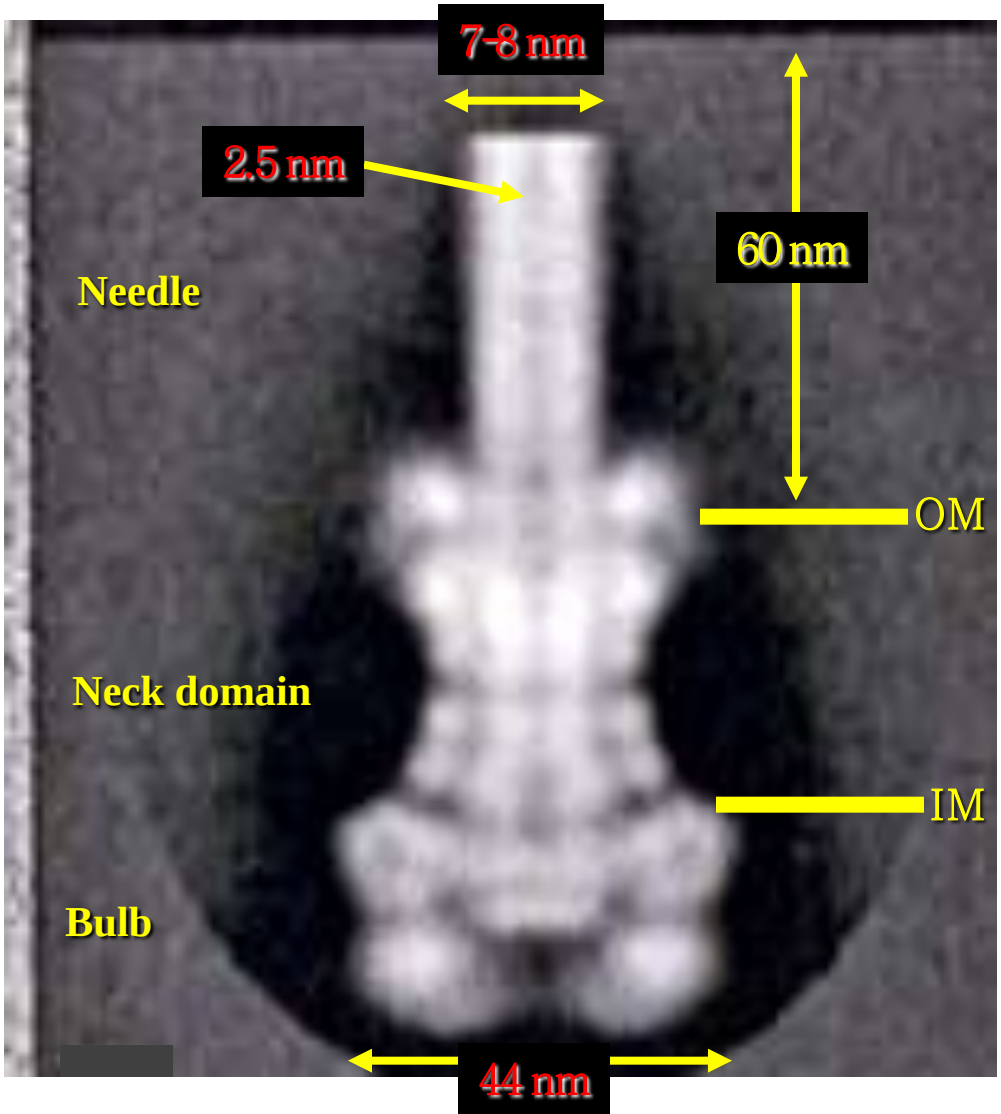
a Bordetella effector, **BopN, that is translocated into the host cell via the type III secretion system,** where it induces enhanced production of IL-10.

A BopN-deficient strain was unable to induce IL-10 production in mice, resulting in the elimination of bacteria via neutrophil infiltration into the pulmonary alveoli.

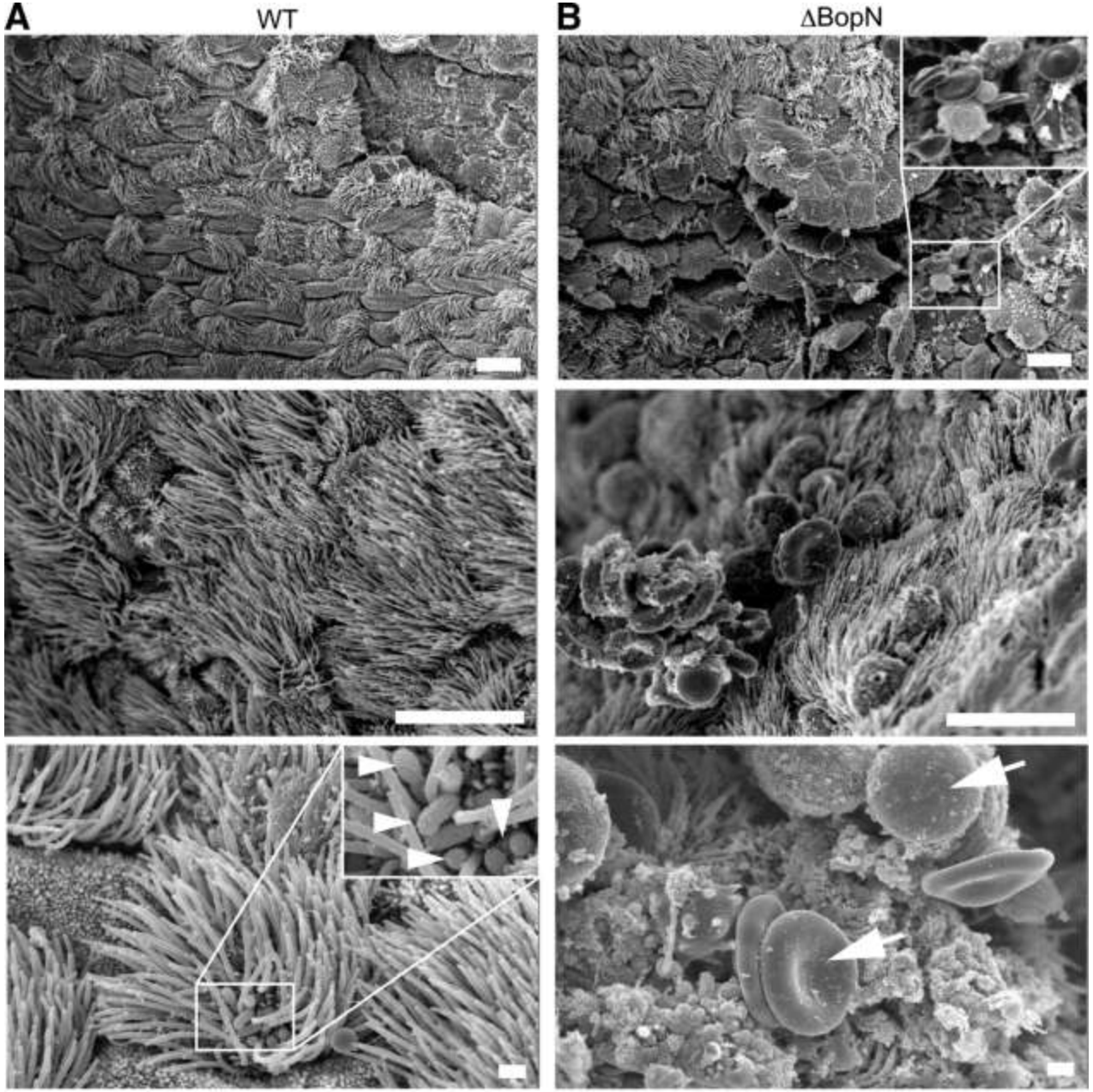
These results explain the ability of Bordetella species to avoid induction of the inflammatory response.

Bordetella evades the host immune system by Inducing IL-10 through a type III effector, BopN





BopN suppresses inflammatory responses at the bacterial-colonized area



(A and B) Scanning electron micrographs of mouse tracheas infected with WT (A) and ΔBopN *Bordetella bronchiseptica (B).*

C57BL/6J mice were infected intranasally with 5×10^6 WT and ΔBopN B. *ronchiseptica,* and tracheal sections were obtained 2 d after infection.

Note that **extensive cell-surface disruption,** including **increased unciliated cells** as well as **infiltration of inflammatory cells** and erythrocytes, is observed in mice infected **with ΔBopN but not WT.**

INFECTION AND IMMUNITY, Sept. 2003, p. 4936–4942

Bordetella pertussis Acquires Resistance to Complement-Mediated Killing In Vivo

Elizabeth J. Pishko, David J. Betting,† Christina S. Hutter,‡ and Eric T. Harvill*

Bordetella pertussis *l*acks O antigen and i**s sensitive to naıve serum *in vitro***, yet it also efficiently colonizes the respiratory tract.

serum concentration and growth conditions can greatly alter the observed level of sensitivity to complement

***B. pertussis* rapidly acquires increased resistance in vivo (cultured on blood)**

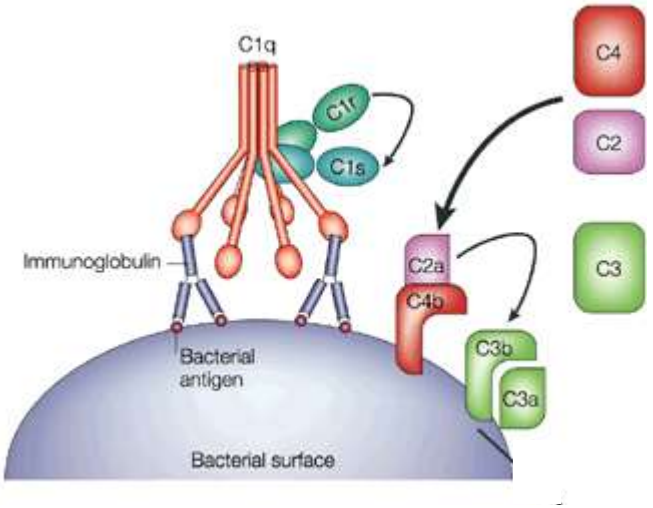
a novel O antigen-independent method by which B. pertussis evades complement-mediated killing.

Complement resistance

Complement system

- inhibition of complement-induced phagocytosis - **BrkA (*Bordetella* resistence to killing A)**

→ interference with the classical pathway of complement activation



http://www.nature.com/nri/journal/v2/n5/fig_tab/nri800_F1.html

- interfers with the deposition of C4b, C2a, and C3b → preventing phagocytosis and killing by neutrophils
- binds and recruits the major inhibitors (C4b-binding protein and human C1 esterase inhibitor)

INFECTION AND IMMUNITY, Mar. 2010, p. 907–913 Vol. 78, No. 3

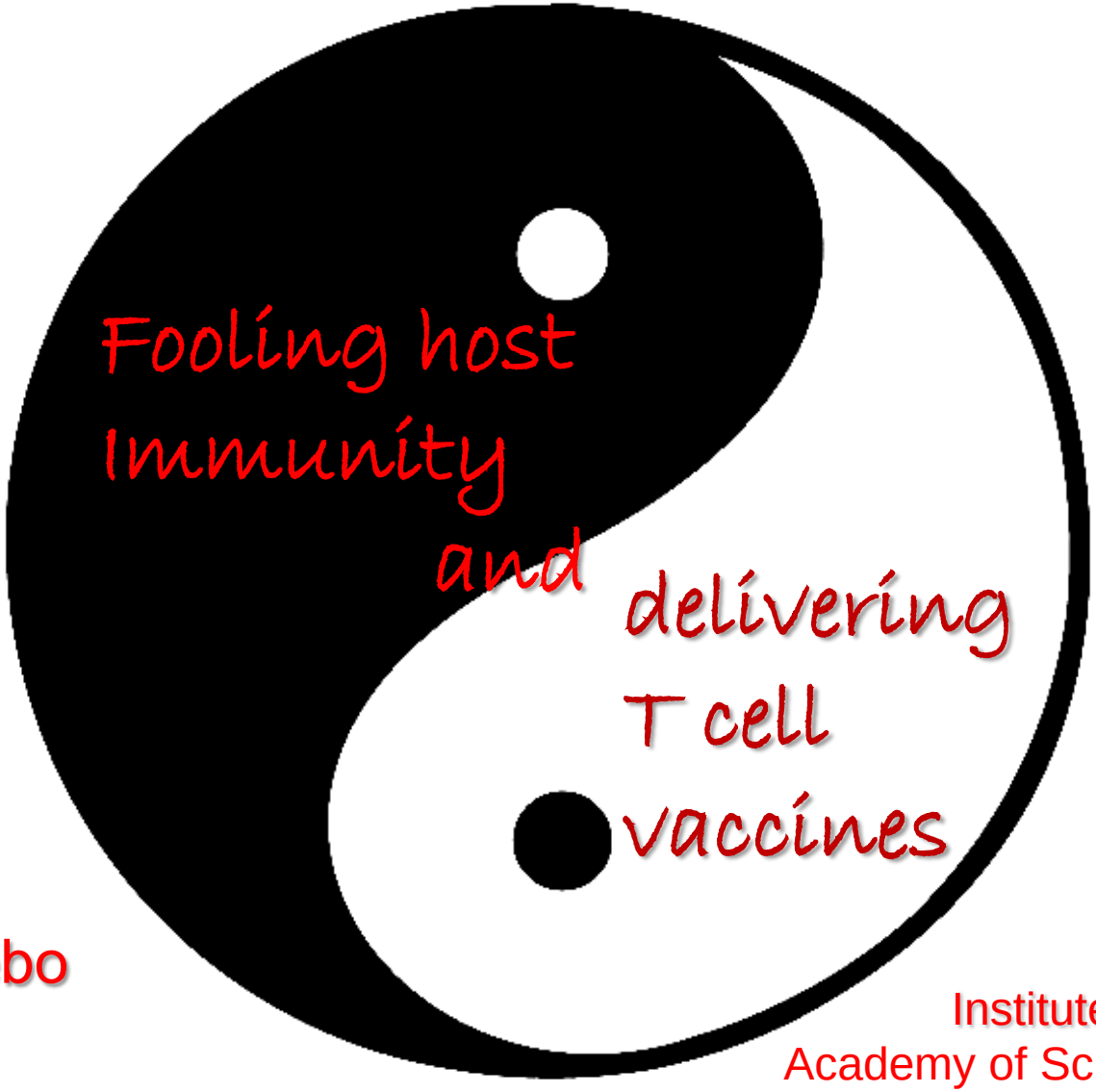
Intracellular Trafficking of *Bordetella pertussis* in Human Macrophages

Yanina A. Lamberti,1 Jimena Alvarez Hayes,1 Maria L. Perez Vidakovics,1†
Eric T. Harvill,2 and Maria Eugenia Rodriguez1*

Although *Bordetella pertussis* has been observed to survive inside macrophages, its ability to resist or evade degradation in phagolysosomes has not been defined.

roughly one-fourth of the bacteria taken up evade initial killing … *Viable bacteria accumulated* within phagosomal compartments positive for the early endosomal marker Rab5 but not the late endosomal marker LAMP.
Moreover, ***B. pertussis-containing phagosomes acquired exogenously added transferrin,*** *indicating* that intracellular bacteria have access to extracellular components and essential nutrients via the hostcell recycling pathway. Overall, these results suggest that *B. pertussis* survives and eventually replicates in compartments with characteristics of early endosomes, potentially contributing to its extraordinary ability to persist within hosts and populations.

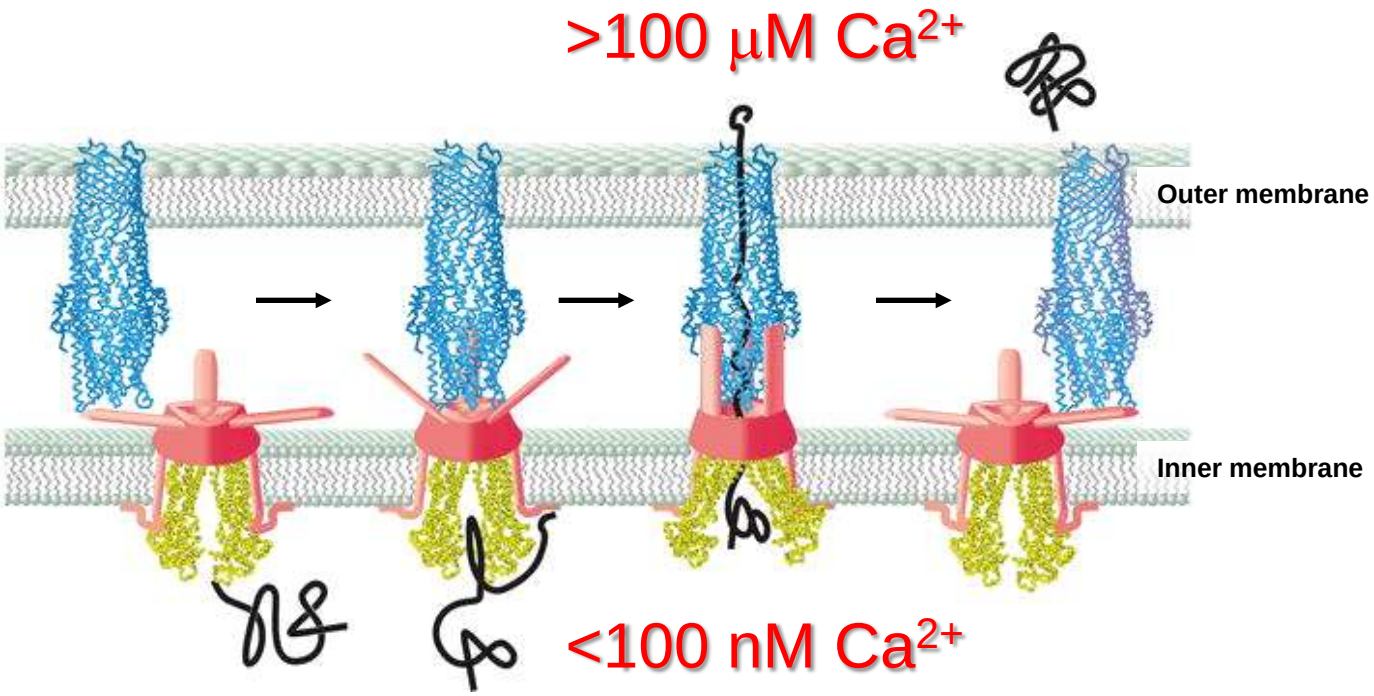
THE YIN AND YANG OF A BACTERIAL TOXIN



P. Šebo

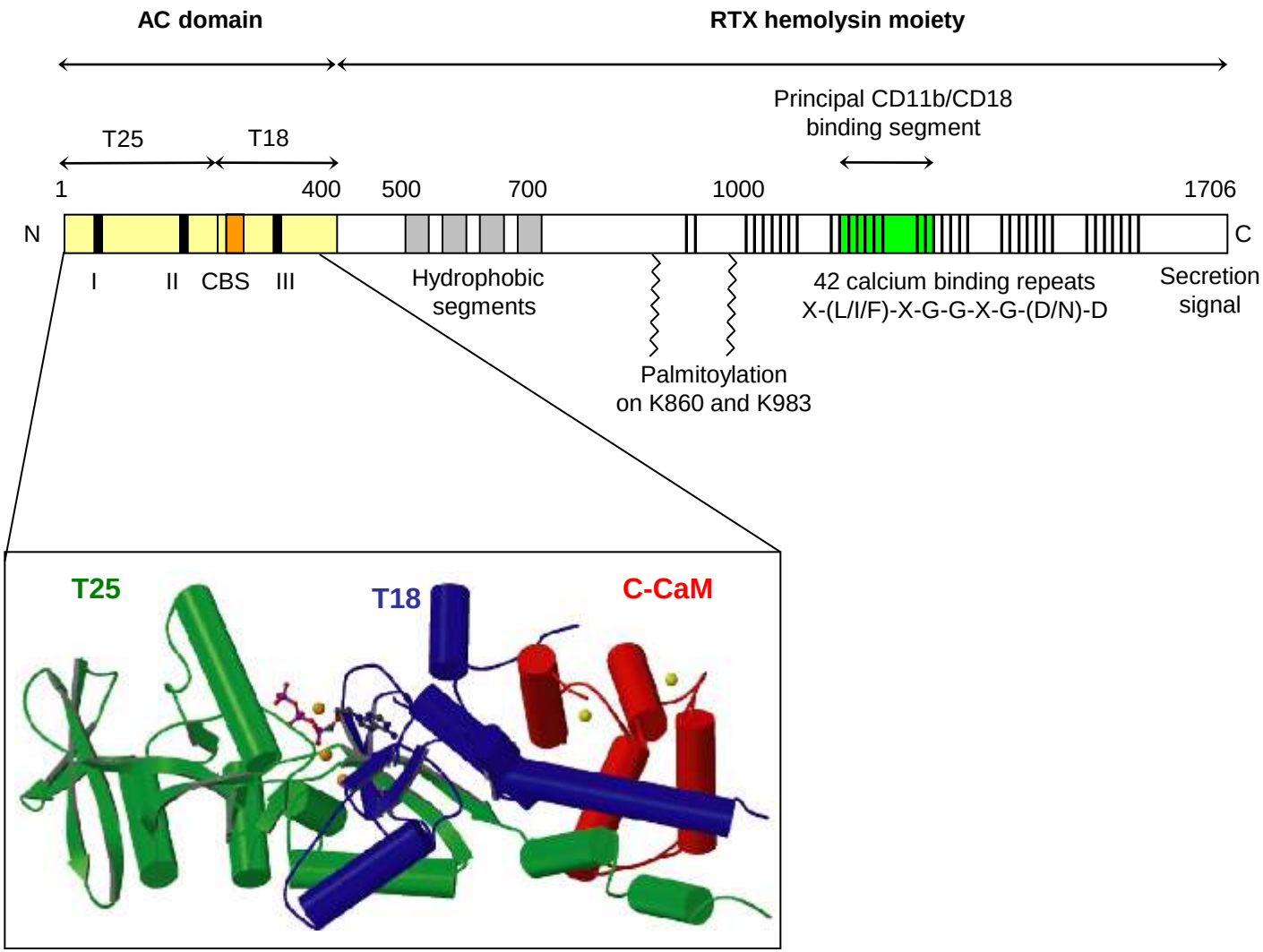
PRAGUE
Institute of Microbiology
Academy of Sciences of the CR

RTX proteins are secreted by a type I system…



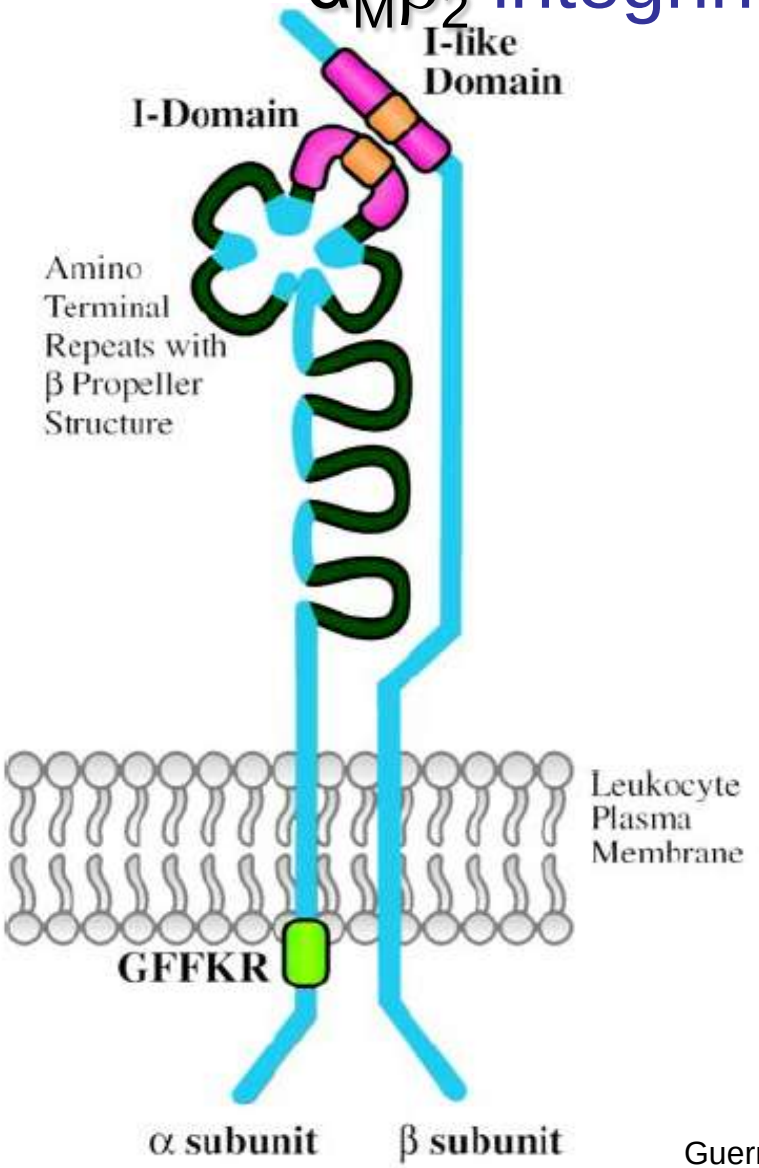
Need to unfold and refold
on the way to target…

Adenylate cyclase toxin - cytolysin



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

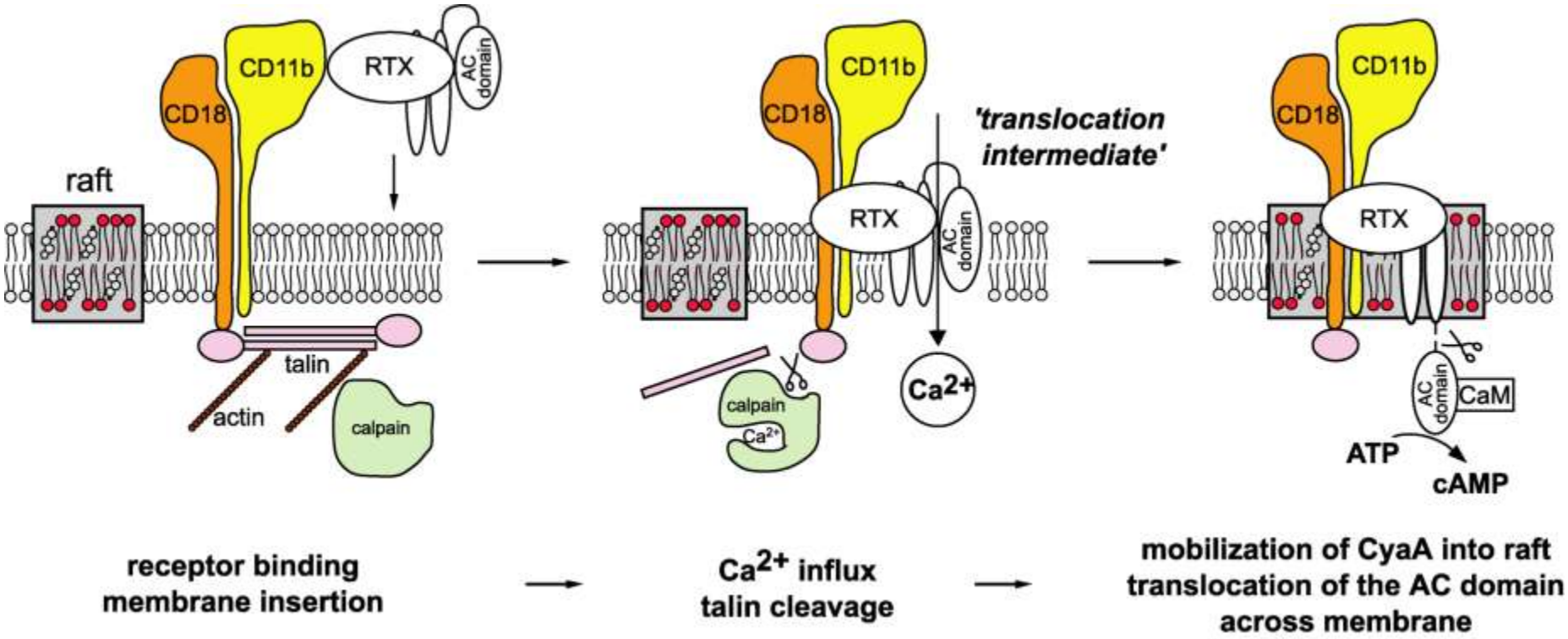
ACT targets myeloid phagocytes bearing $\alpha_M\beta_2$ integrin CD11b/CD18



- 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

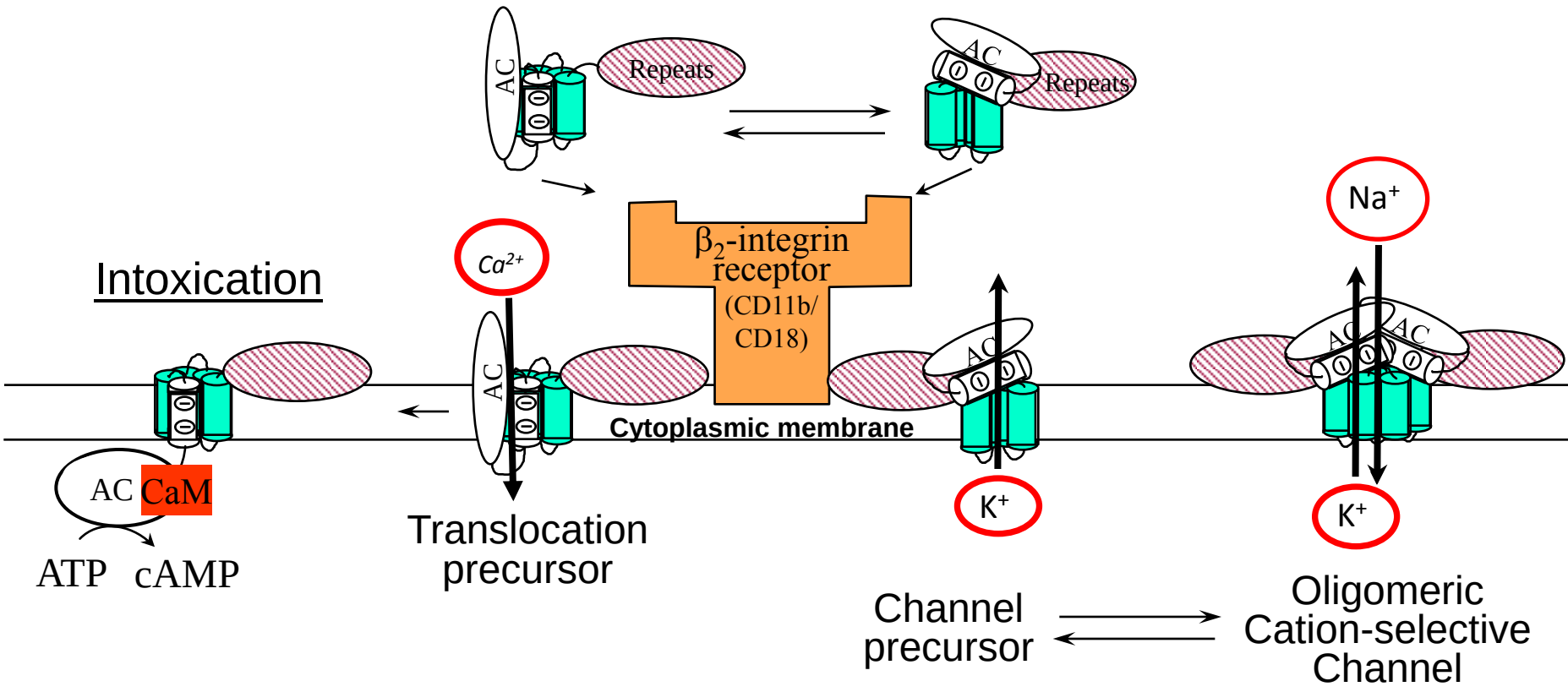
Guermonprez et al. 2001, J Exp. Med.

Bordetella adenylate cyclase toxin hijacks its β_2 integrin receptor into lipid rafts to accomplish membrane translocation in two steps



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

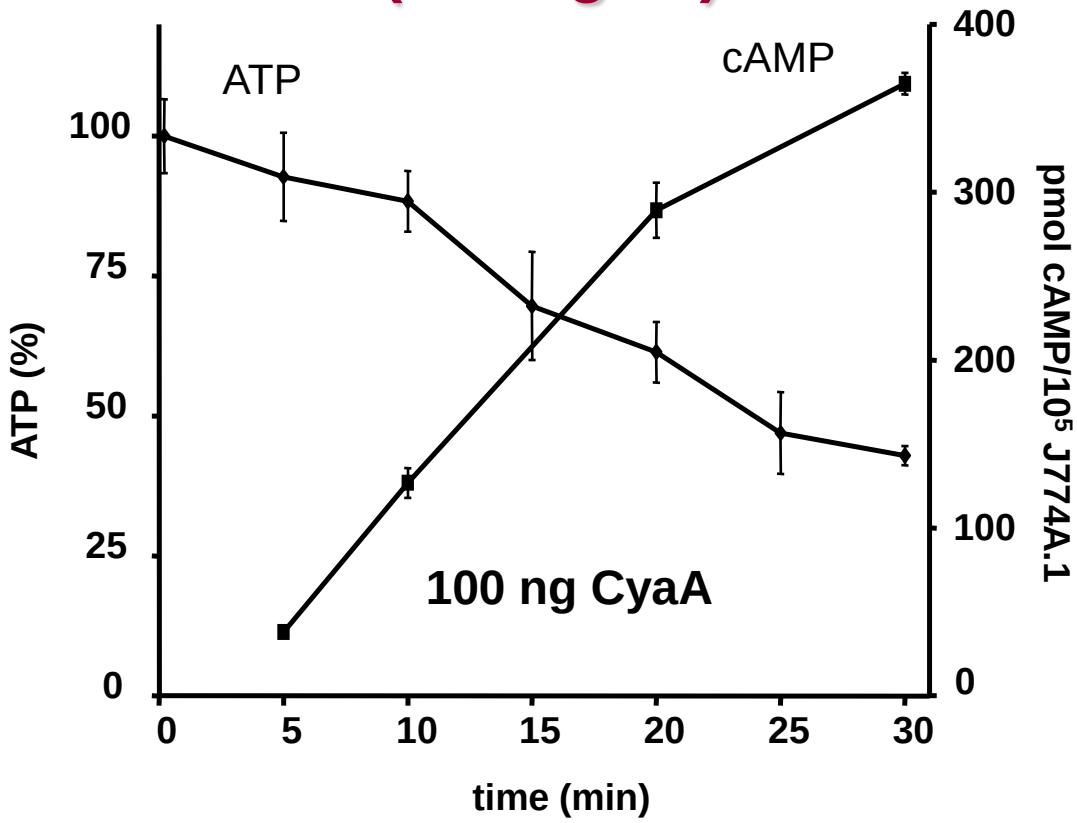
Mode of action of ACT adenylate cyclase toxin – **Cytolysin**



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

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

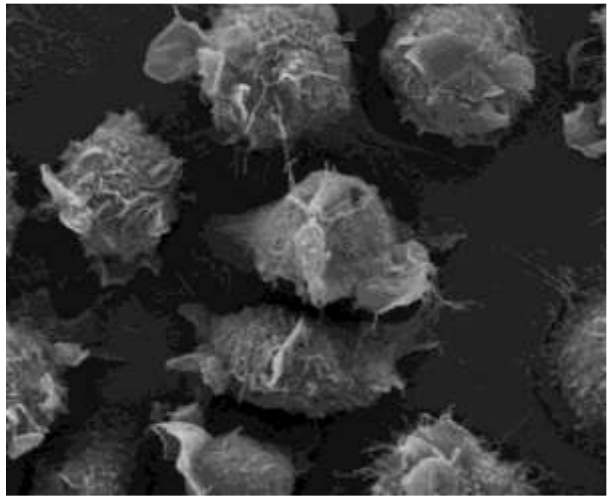
Basler *et al*., 2006, *Infect. Immun.*, 74, 2207-2214. .

CyaA-induced morphological rearrangements

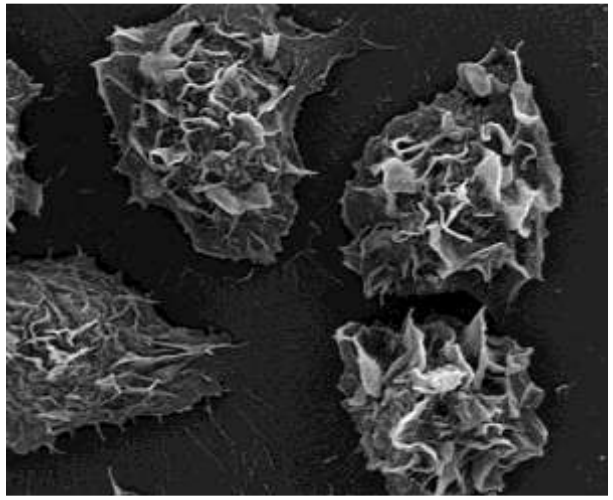
Mouse macrophage-like cell line J774 A.1:

Kamanova *et al.* (2008)
J. Immunol. 181: 5587–5597

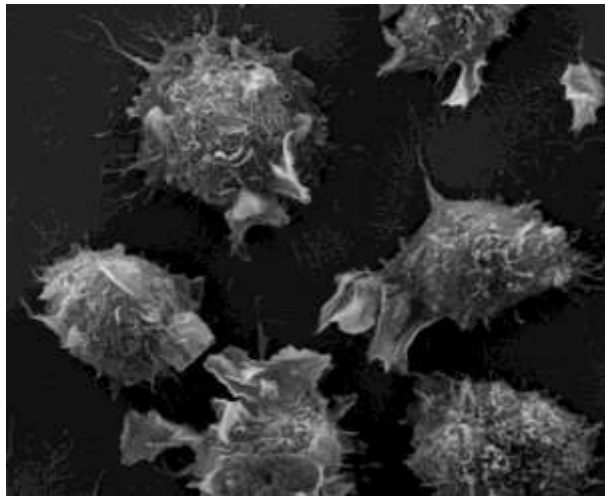
Buffer, 5 min



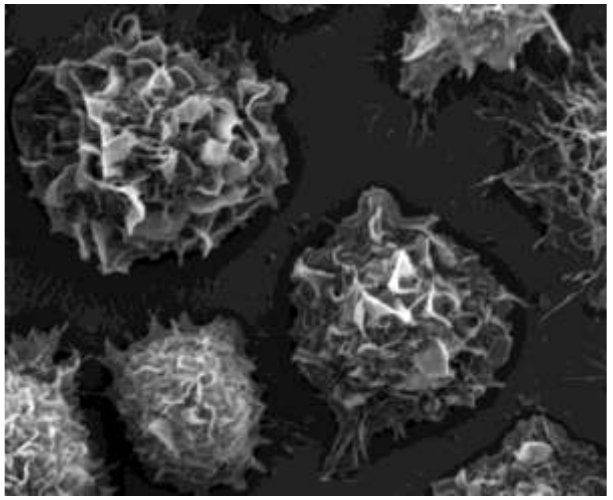
CyaA, 10 ng/ml, 5 min



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

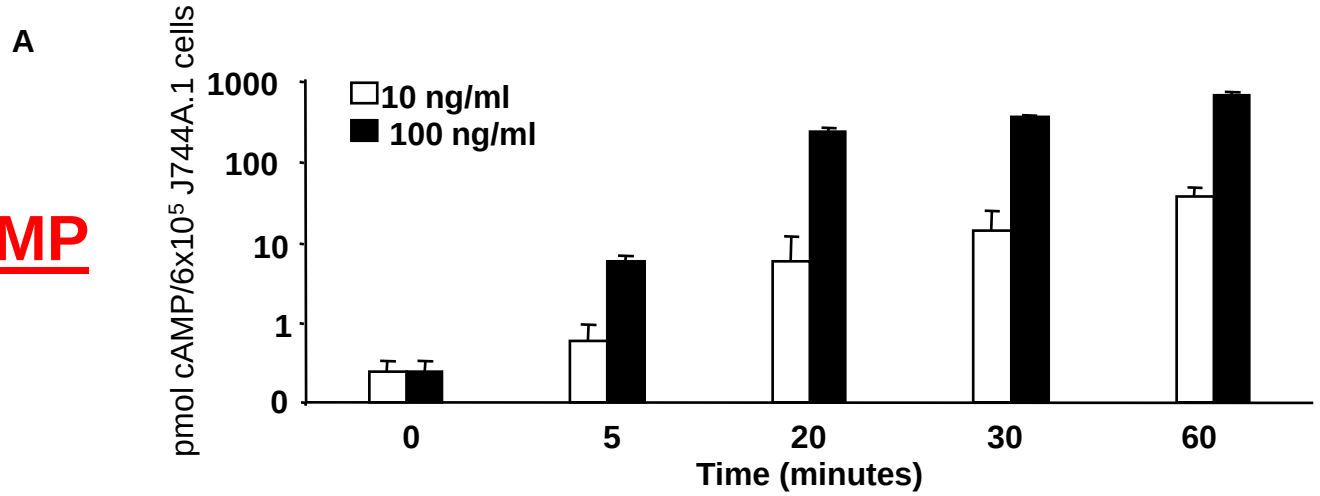


db-cAMP, 2mM, 10 min

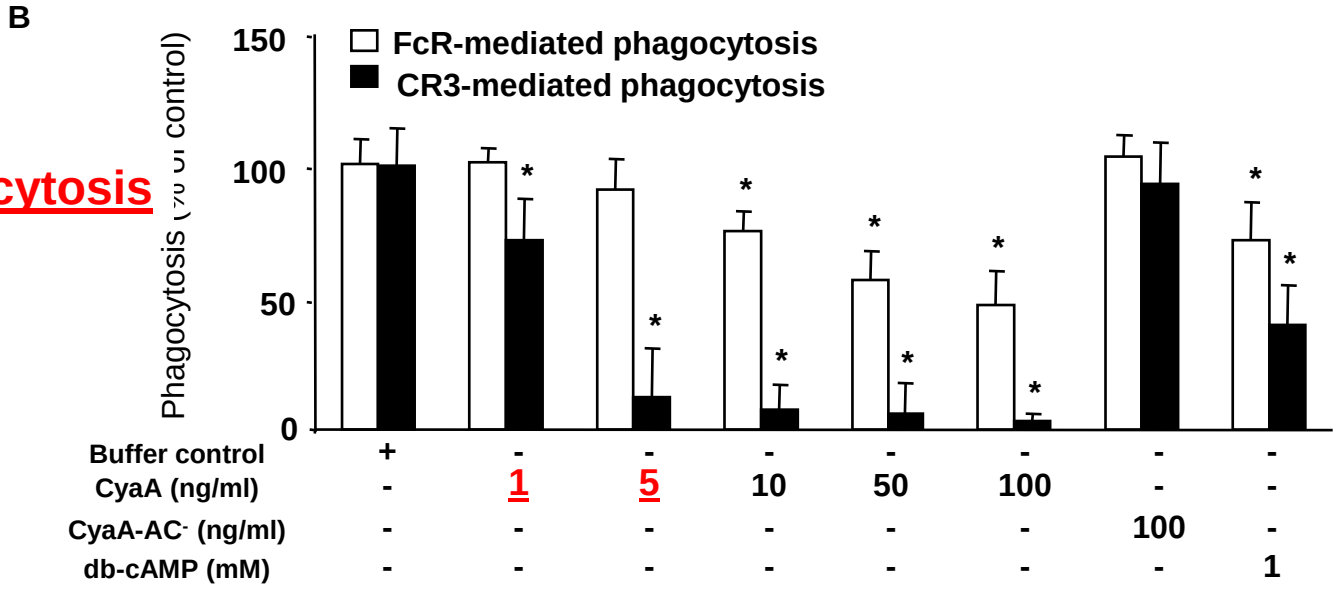


ACT at low doses ablates complement-mediated phagocytosis

cAMP



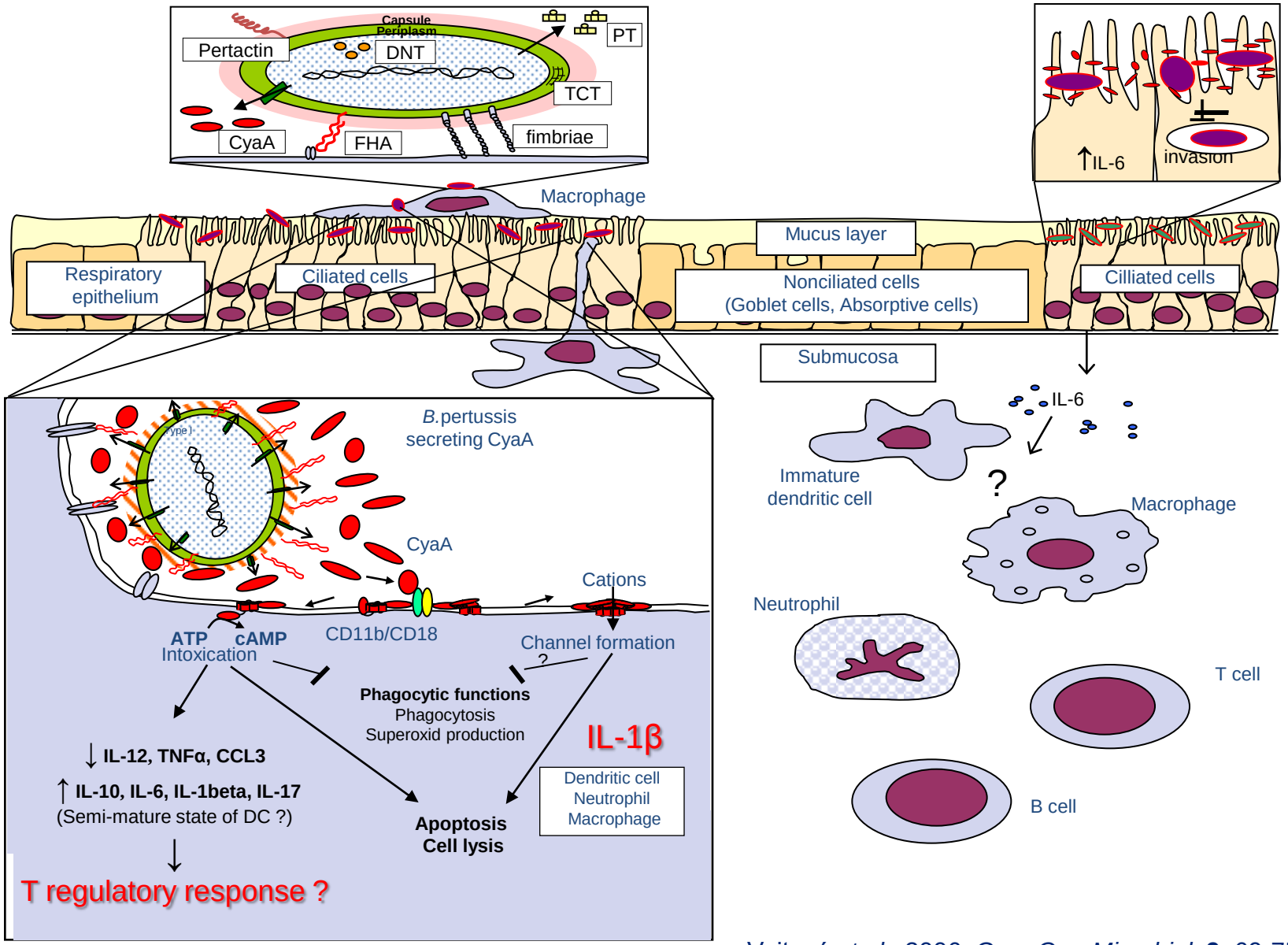
phagocytosis



Kamanova *et al.* (2008) J. Immunol. 181: 5587–5597

the Yang: ACT as a SWIFT SABOTEUR

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

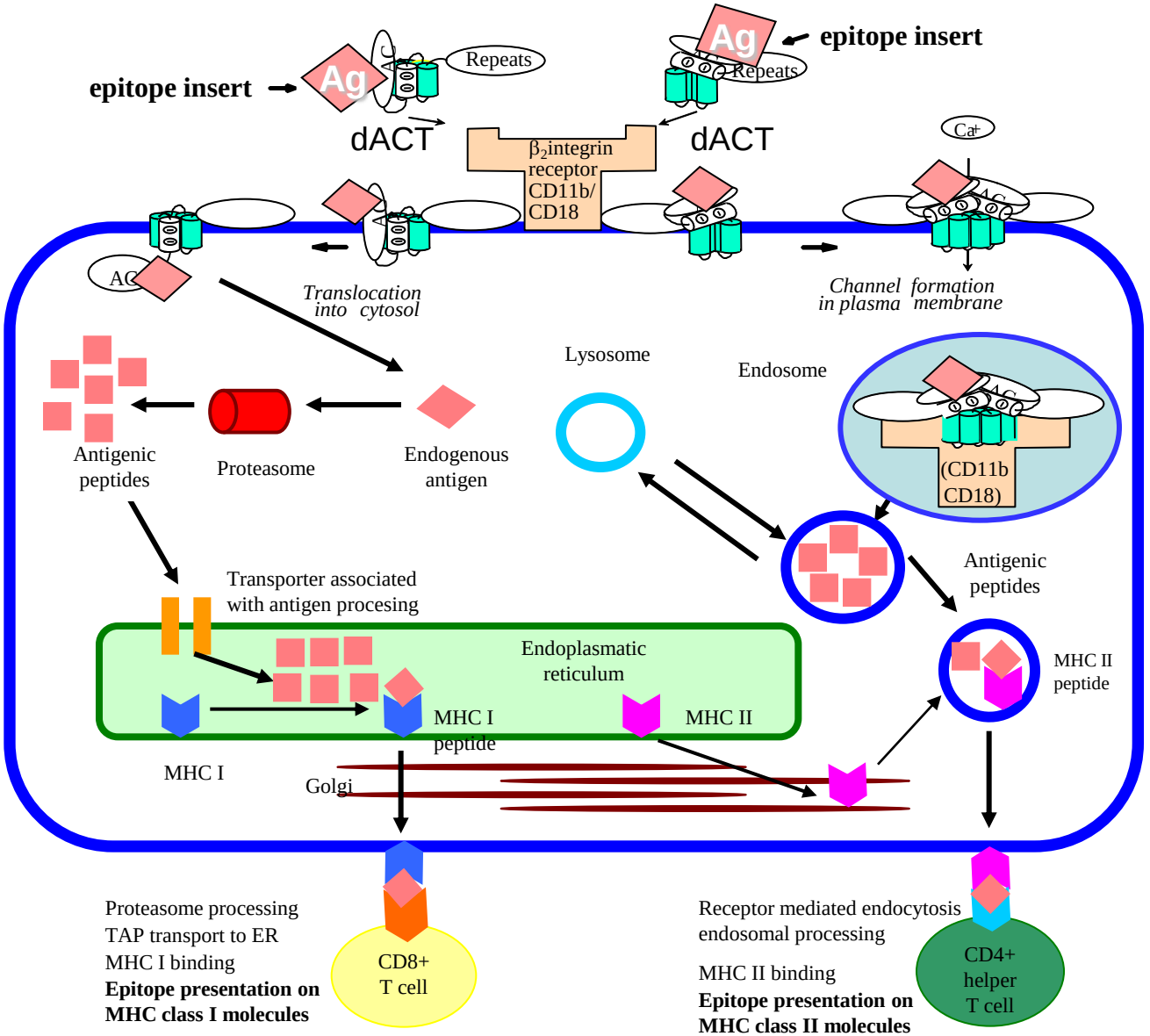


Osičková *et al.,* (1999) J. Biol. Chem. **274**, 37644)

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

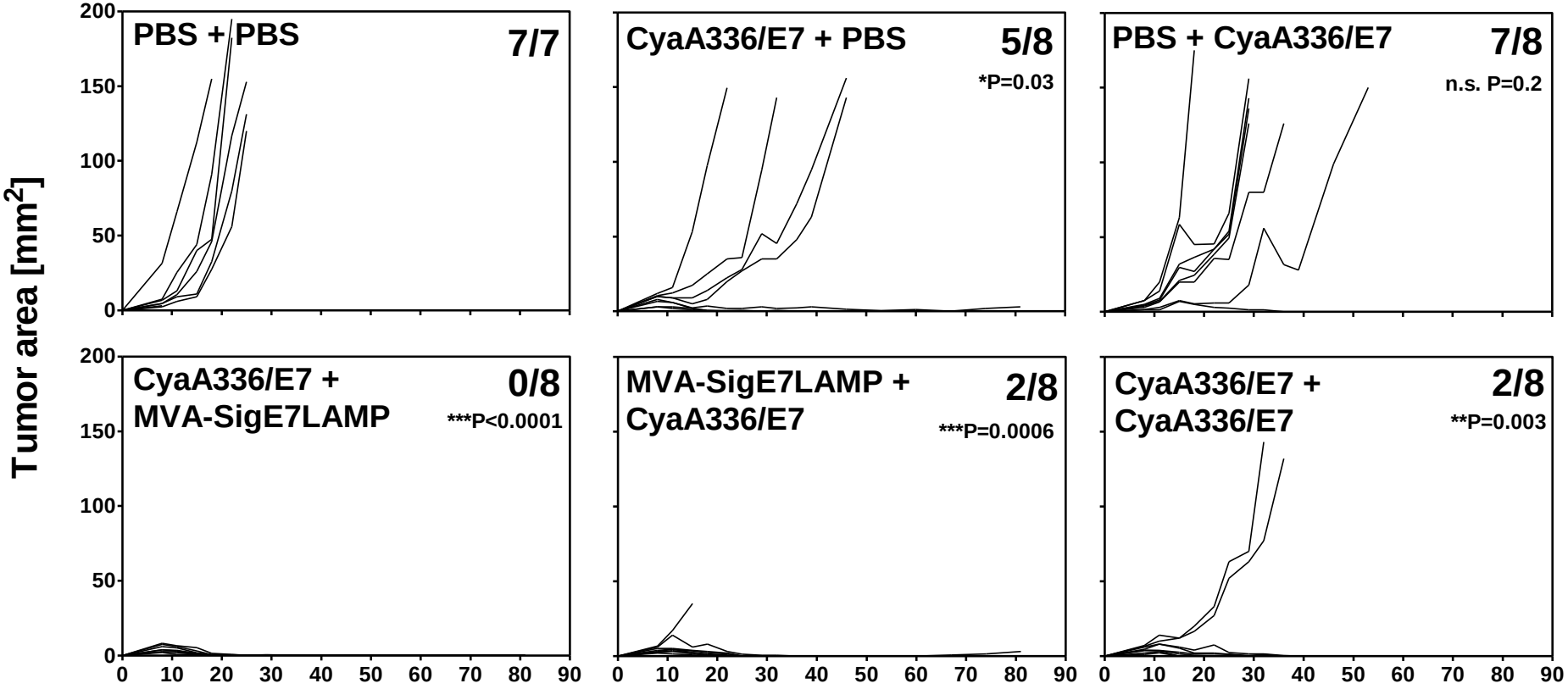
THE YIN OF A BACTERIAL TOXIN

dACT as a novel antigen delivery tool



Simsova *et al.* (2004) Int. J. Med. Microbiol. 293, 1 -6

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

GENTICEL has selected the prevention of cervical cancer as the medical target to demonstrate the safety and efficacy of its unique therapeutic vaccine platform, the Adenylate Cyclase (CyaA)

The strategic goal of the company is to ensure that a vaccine solution can be offered to all women in order to prevent cervical cancers.

Preventive HPV vaccines are indicated for individuals, mainly teenage girls and young women, who have not yet been exposed to oncogenic Human Papillomavirus (HPV). However, these prophylactics are not effective once one is already infected (Hildesheim et al., 2007; Hung et al., 2008) and because at any given time, approximately 13% of sexually active women bear HPV (De Sanjosé et. al., 2007), Genticel is developing products that are part of a new class of vaccines, 'therapeutic vaccines', which remain active after infection and therefore complement the current preventive vaccines.

For more information go to our web page "THE HUMAN PAPILLOMAVIRUS (HPV)"

News

- **Approval for clinical trial of ProCervix**
  July, 23 2010
  Genticel's therapeutic vaccine, ProCervix, aimed at preventing cervical cancer in patients already infected by human papillomavirus (HPV), receives clearance to start a Phase I clinical trial.
  Press release

- **Le vaccin ProCervix autorisé en essai clinique**
  July, 23 2010
  Genticel annonce que ProCervix, son vaccin thérapeutique destiné à prévenir le cancer du col de l'utérus chez les patientes infectées par le virus du papillome humain (HPV), a reçu l'autorisation d'entrer en essai clinique de Phase I.
  Communiqué de presse

- **Genticel secures EUR 13.1M (USD 17.7M) in capital funding**
  March, 09 2010
  Press Release

Current status of dACT-antigen delivery technology

(developed at Institut Pasteur, Paris through collaboration of the team of C. Leclerc in collaboration with teams of D. Ladant and P. Sebo)

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)*)

- preclinical data *in vivo*
- Toxicological studies
- GMP batches released

- Phase I clinical trial for HPV16/18-induced cervical carcinoma started July 7, 2010 by Genticel S.A. Toulouse France
- Phase I/II clinical trial in melanoma patients starts soon (EU 6. FP consortium THERAVAC)