Molecular mechanisms of bacterial virulence

or

Molecular tricks of host-pathogen interactions

Sebolab…

Institute of Microbiology AS CR v.v.i.

sebo@biomed.cas.cz

every Tuesday 1 pm

I need your e-mail…

Resources

- Co jsem zač: http://www.linkedin.com/profile/view?id=64793525&trk=nav_responsive_tab_profile_pic
- Moje laborka: http://l125lsx.mbu.cas.cz/Lab125/
- My lectures… https://l125lsx.mbu.cas.cz/BacPatho/
- http://www.ceskapozice.cz/domov/veda-vzdelavani/studium-line-trend-ktery-muze-zmenit-planetarni-vzdelanost
- https://www.coursera.org/
- https://www.edx.org/
- http://gsbs.utmb.edu/microbook/toc.htm
- http://www.textbookofbacteriology.net/
- http://www-micro.msb.le.ac.uk/MBChB/Merralls/Merralls.html
- http://www.natur.cuni.cz/~konop/

- Abigail A. Salyers, Dixie D. Whitt : Bacterial Pathogenesis: A Molecular Approach, ASM Press; 2nd edition (2002), **ISBN-10:** 155581171X, **ISBN-13:** 978-1555811716
- Janeway's Immunobiology, Seventh Edition (Immunobiology: The Immune System (Janeway), Garland Science; 7 edition (November 27, 2007), **ISBN-10:** 0815341237, **ISBN-13:** 978-0815341239
- Mims' Medical Microbiology, 4th Edition by Richard Goering , MD , PhD , Hazel Dockrell , MD , Ivan Roitt , MA , DSc(Oxon) , Hon FRCP(Lond) , FRCPath , FRS , Mark Zuckerman , MB , BS(London) , MSc , BSc , MRCP , MRCPath and Derek Wakelin , BSc , PhD , DSc & FRCPath **ISBN: 9780323044752** | Copyright 2007 | Paperback, ELSEVIER

Synopsis of the course (22 hours in total):

0. the Concepts…
1. Molecular mechanisms of anti-infectious immunity
2. Principles of action and category of virulence factors
3. Genetic equipment and gene expression regulation of bacterial pathogens
4. Experimental systems for virulence genes identification and host-pathogen interaction studies
5. Protective mechanisms enabling bacteria to overcome innate immunity
6. Cell biology and mechanisms of bacterial toxin action
9. Cellular microbiology - intracellular bacterial pathogens
10. Vaccines as tool for modulation of host response and protection from infection
11. Antibiotics as anti-infectious therapeutics and resistance to antibiotics
12. Intestinal microflora and commensals of the mammalian gut

The general concepts

13. Most studied bacterial pathogens - an overview:
13. Corynebacterium diphteriae, Streptococcus pyogenes a Staphylococcus aureus
14. Clostridium botulinum, perfringens, tetani, B. cereus, Helicobacter pylori
15. Ricketsia, Fracisella, Coxiella, Yersinia
16. Salmonella, Shigella,
18. Campylobacter jejuni, Vibrio cholerae
17. Escherichia coli ? EPEC, ETEC, UPEC, NMEC
17. Bordetella, Mycobacterium, Neisseria,
18. Listeria, Borelia, Mycoplasma, Chlamydia
13. *Corynebacterium diphteriae, Streptococcus pyogenes a Staphylococcus aureus*
14. *Clostridium botulinum, perfringens, tetani, B. cereus, Helicobacter pylori*
15. *Ricketsia, Fracisella, Coxiella, Yersinia*
16. *Salmonella, Shigella,*
18. *Campylobacter jejuni, Vibrio cholerae*
17. *Escherichia coli – EPEC, ETEC, UPEC, NMEC*
17. *Bordetella, Mycobacterium, Neisseria,*
18. *Listeria, Borelia, Mycoplasma, Chlamydia*

Specific examples

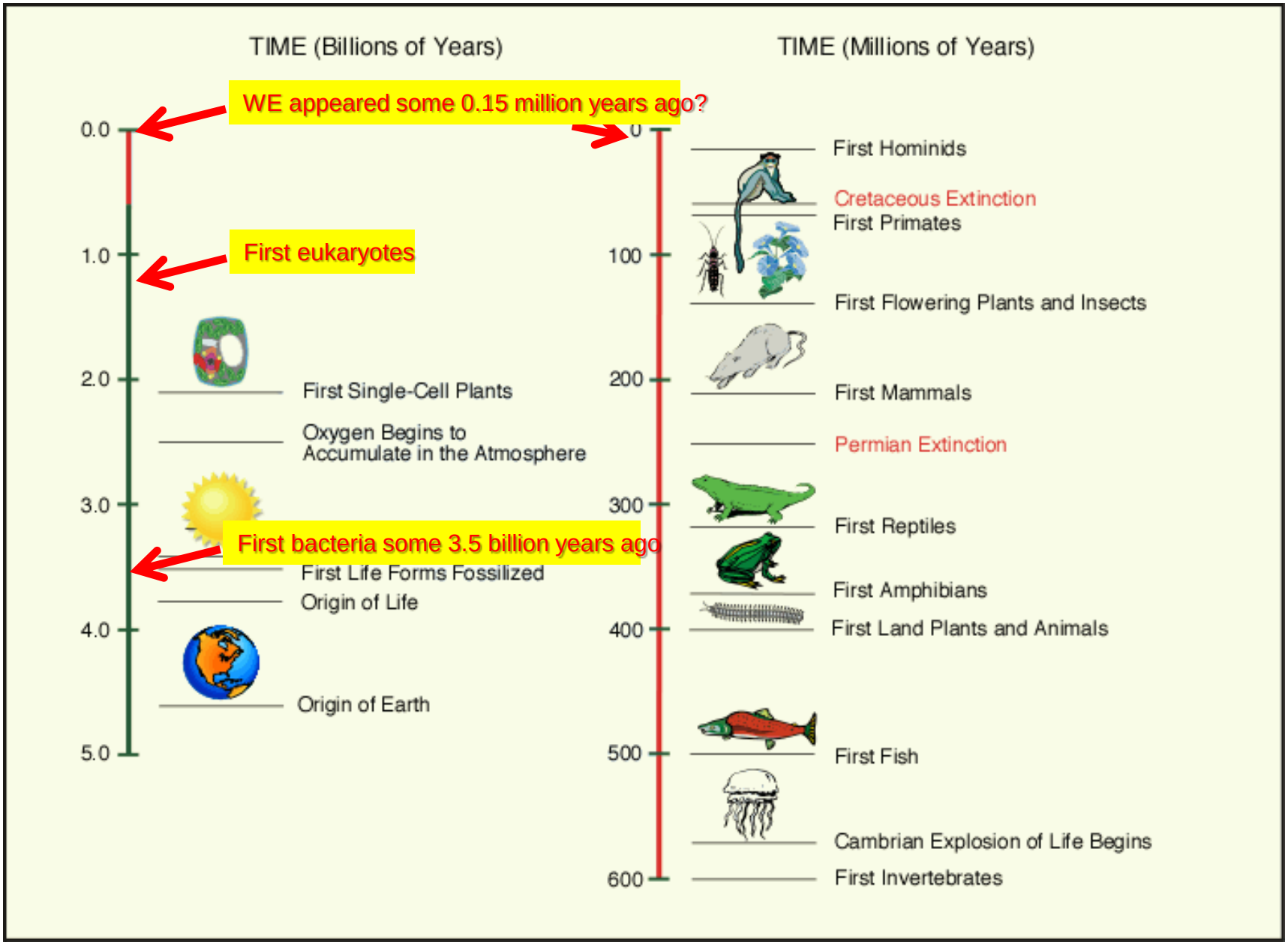
The concept:

- Bacteria evolved for hundreds of millions of years with the ancestral and present hosts –
  - **The war for survival/transmission and food…**

::::**Commensals and pathogens evolved unbelieveable tools to manipulate hosts and evade immune response**

- many of the tricks evolved to allow colonization of protozoa, worms, insects (**lived here before us…)**
  - Now used to colonize vertebrates and humans
  - Many mechanisms can be studies in worms, insects and protoza
    - *amenable to genetic manipulation …*

We as humans, we are an anecdote of Nature…



Presence of commensal flora in the human body
Microorganisms colonize most of the epithelial surfaces exposed to the environment („microbial organ“)

SKIN 10^{12}
Staph. aureus
Staph. epidermidis
Ps. aeruginosa
Propionibacterium acnes
Anaerobes

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Staph. aureus
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Diphtheroids
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Por. Gingivalis plus
Some 700 other
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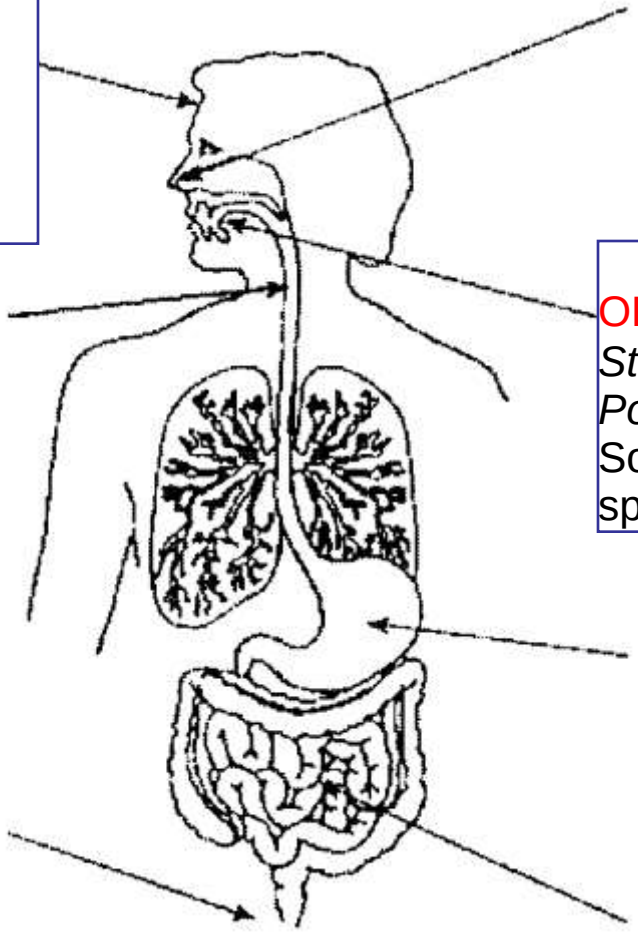

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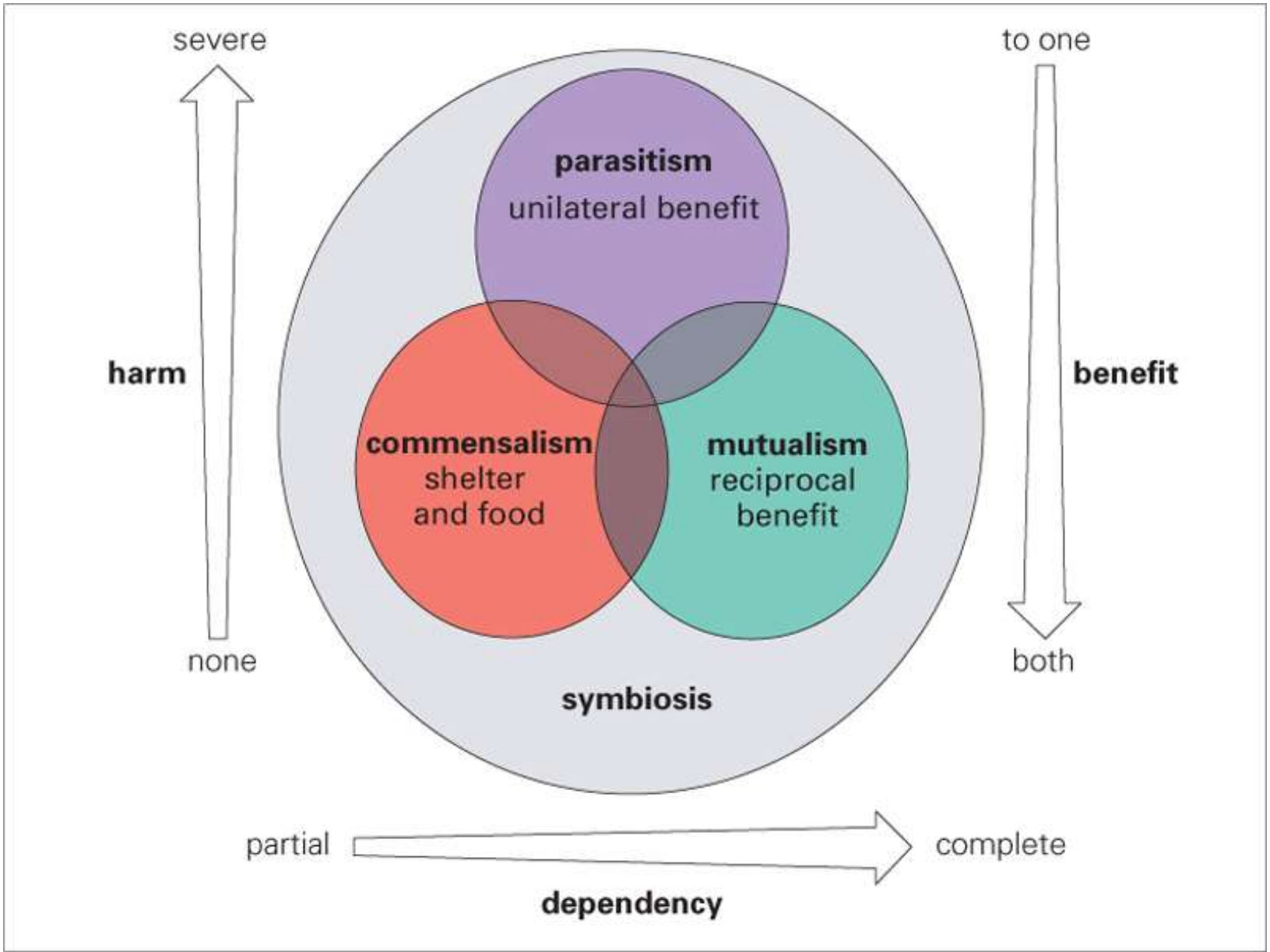
Hel. pylori

URETHRA AND VAGINA 10^{8}
Staph. epidermidis
Streptococci
Lactobacilli
Veillonella
etc.

INTESTINES 10^{14} ~220g
Bacteroides spp.
Bifidobacter spp.
Bacillus spp.
Eubacteria
Ruminococcus albus
etc.

(Wilson et al, 2002)

Nature has many flavors: by no means “on sizes fits all” would apply
host pathogen interactions have many facets,
it is all about food, survival and transmission…



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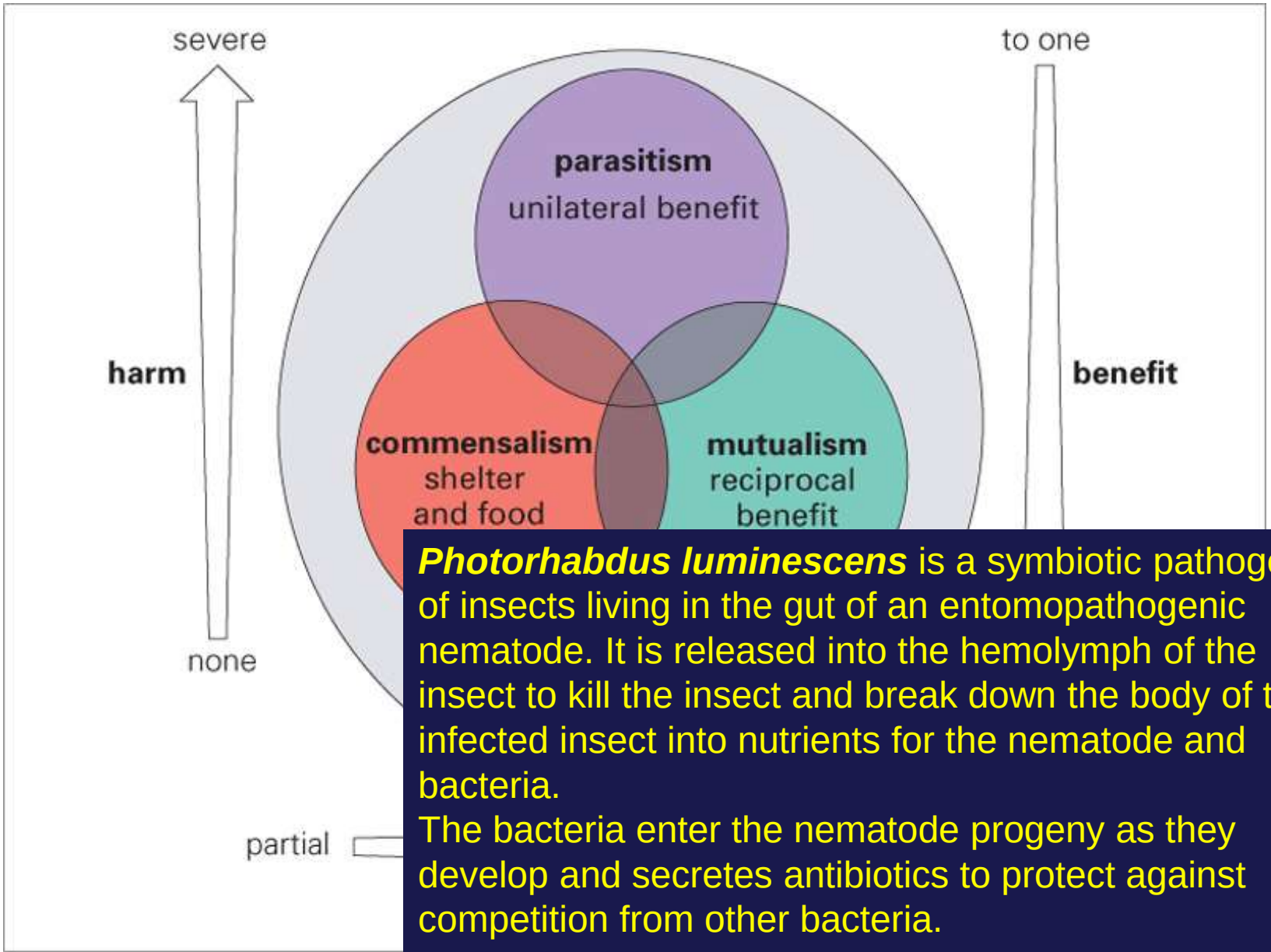

Photorhabdus luminescens is a symbiotic pathogen of insects living in the gut of an entomopathogenic nematode. It is released into the hemolymph of the insect to kill the insect and break down the body of the infected insect into nutrients for the nematode and bacteria.

The bacteria enter the nematode progeny as they develop and secretes antibiotics to protect against competition from other bacteria.

Silly use of antibiotics drives evolution of multidrug-resistant nosocomial pathogens

Forsberg *et al.* **The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens**
Science 31 August 2012: Vol. 337, pp. 1107-1111, DOI: 10.1126/science.1220761

Soil microbiota represent one of the ancient evolutionary origins of antibiotic resistance and have been proposed as a reservoir of resistance genes available for exchange with clinical pathogens. Using a high-throughput functional metagenomic approach in conjunction with a pipeline for the de novo assembly of short-read sequence data from functional selections (termed PARFuMS), we provide evidence for recent exchange of antibiotic resistance genes between environmental bacteria and clinical pathogens**. We describe multidrug-resistant soil bacteria containing resistance cassettes against five classes of antibiotics (β-lactams, aminoglycosides, amphenicols, sulfonamides, and tetracyclines) that have perfect nucleotide identity to genes from diverse human pathogens.** This identity encompasses noncoding regions as well as multiple mobilization sequences, offering not only evidence of lateral exchange but also a mechanism by which antibiotic resistance disseminates.

The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens

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Table 1. Nonredundant antibiotic resistance genes with 100% identity to known human pathogens.

Gene name	GenBank ID	Number of selections*	Antibiotic class	Annotation [mechanism]	Pathogens hit (GI number)
AB95_PI_68.1	JX009363	4	β-lactam	blaP1 [enzymatic degradation]	*A. baumannii* (94960156), *K. pneumoniae* (114147191), *P. aeruginosa* (117321883), *S. typhimurium* (12719011), *P. mirabilis* (157674381)†
AB95_CH_13.1	JX009364	1	Amphenicol	Chloramphenicol efflux [efflux]	*A. baumannii* (169147133), *P. aeruginosa* (260677483)
AB95_TE_2.2	JX009366	3	Tetracycline	tetA(G) [efflux]	*A. baumannii* (169147133), *S. typhimurium* (12719011)
AB95_TE_1.1	JX009365	3	Tetracycline	tetA [efflux]	A. baumannii (169147133), *E. coli* (312949035), *K. pneumoniae* (290792160), *S. typhimurium* (37962716)†
AB95_GE_3.3	JX009367 JX009373	2	Aminoglycoside	aadB [covalent modification]	*E. cloacae* (71361871), *K. pneumoniae* (206731403), *P. aeruginosa* (37955767), *S. typhimurium* (17383994)†
AB95_GE_3.1	JX009368 JX009374	2	Sulfonamide	sul1 [target modification]	*C. diptheriae* (323714042) *E. cloacae* (71361871), *K. pneumoniae* (206731403), *P. aeruginosa* (37955767), *S. typhimurium* (17383994), *Yersinia pestis* (165913934)†
AB95_CH_21.1	JX009369	1	Aminoglycoside	aacA4 [covalent modification]	*A. baumannii* (164449567), *K. pneumoniae* (238865601), *P. aeruginosa* (219872982), *S. typhi* (34014739)†

*Number of selections in which the entirety of a given gene was captured. †More pathogens exist for which 100% nucleotide identity was observed than listed

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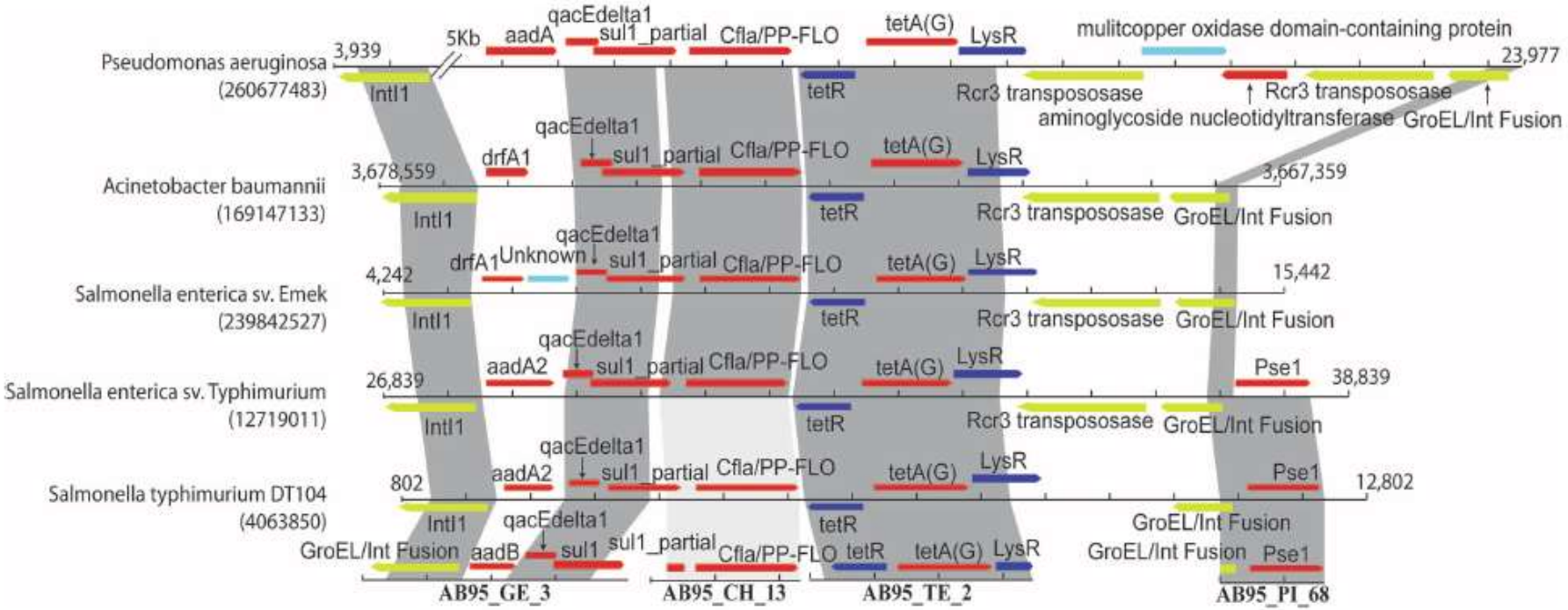
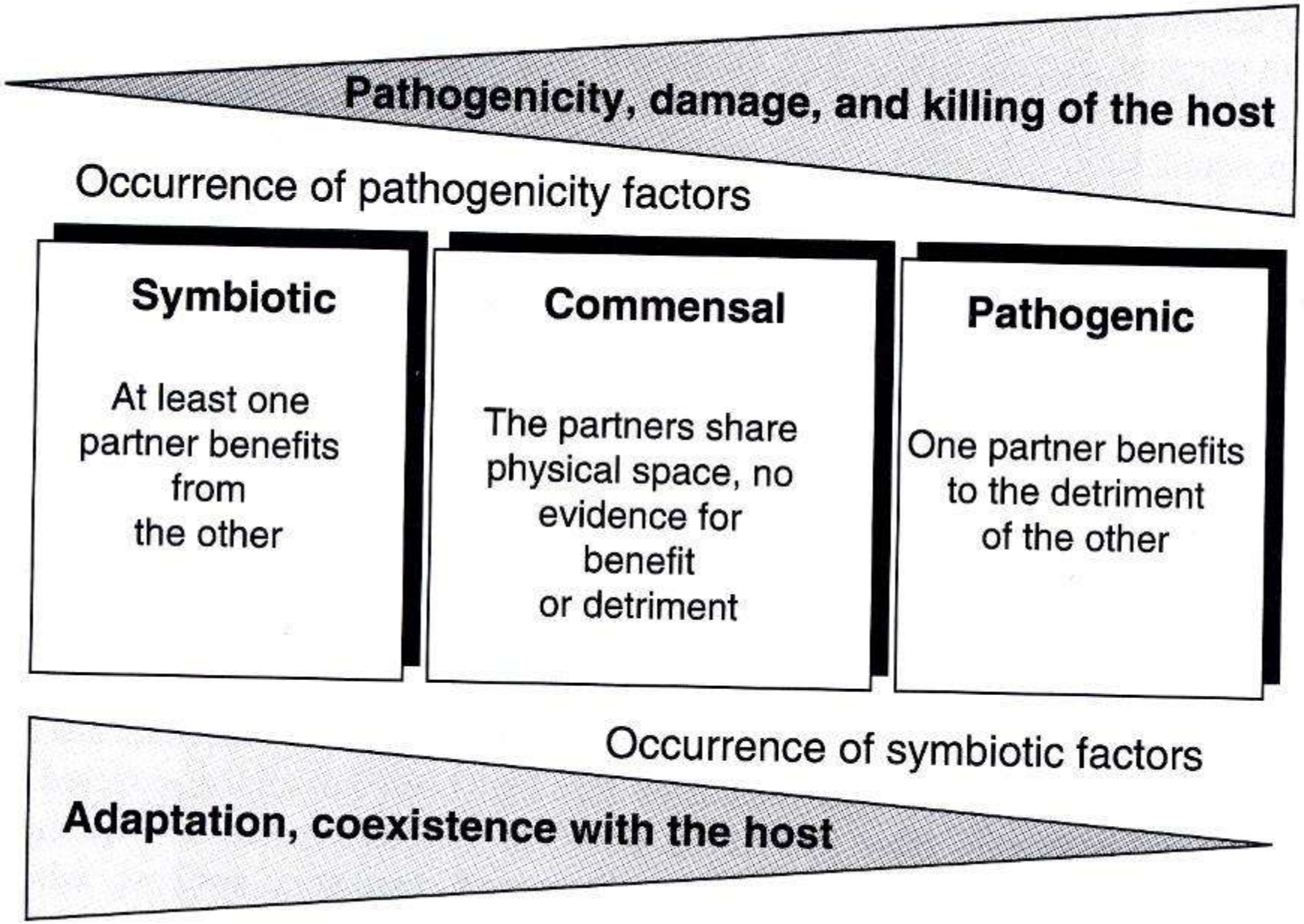


Fig. 3. Comparison of four AB95-derived resistance fragments to five human pathogenic isolates. The four fragments are depicted along the bottom, and shading indicates high nucleotide identity between the fragments and pathogens (NCBI GenInfo numbers identify each pathogenic isolate). Dark gray shading indicates >99% identity; light gray shading indicates ~88% identity. Base-pair coordinates flank pathogenic sequences, and the distance between each tick mark is 800 bp. Red ORFs represent resistance genes, yellow represents mobility elements, dark blue represents resistance-associated regulatory elements, and light blue represents other functions.


Pathogenicity, damage, and killing of the host
Occurrence of pathogenicity factors
Symbiotic
At least one partner benefits from the other
Commensal
The partners share physical space, no evidence for benefit or detriment
Pathogenic
One partner benefits to the detriment of the other
Occurrence of symbiotic factors
Adaptation, coexistence with the host

Presence of commensal flora in the human body
Microorganisms colonize most of the epithelial surfaces exposed to the environment („microbial organ“)

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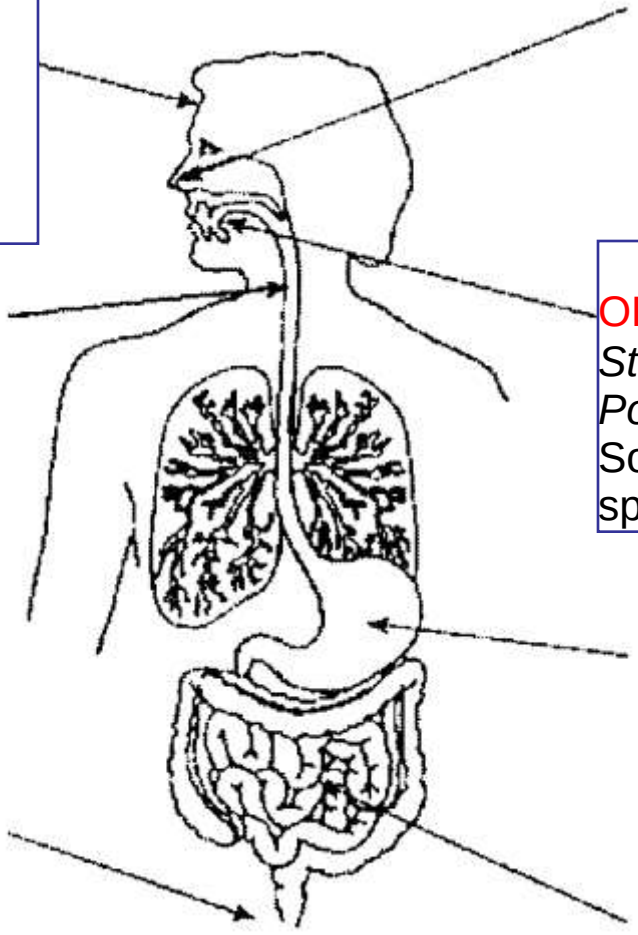

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Bifidobacter spp.
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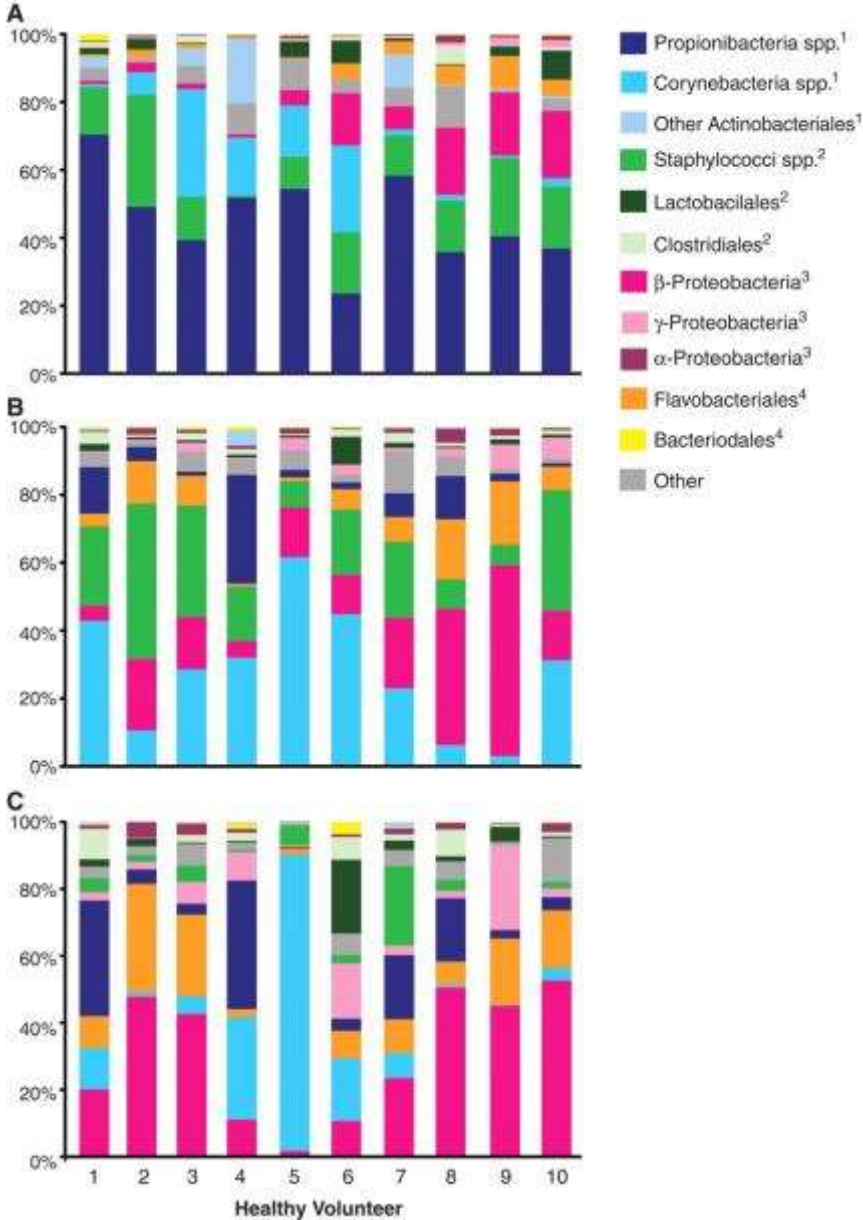
Eubacteria
Ruminococcus albus
etc.

(Wilson et al, 2002)

Metagenomics nowadays helps to explore the „ecosystem“ of the human „microbial organ“

Science 29 May 2009:
Vol. 324. no. 5931, pp. 1190 – 1192
Topographical and Temporal Diversity of the Human Skin Microbiome

Human skin is a large, heterogeneous organ that protects the body from pathogens while sustaining microorganisms that influence human health and disease. The analysis of 16*S* ribosomal RNA gene sequences obtained from 20 distinct skin sites of healthy humans revealed that physiologically comparable sites harbor similar bacterial communities. The complexity and stability of the microbial community are dependent on the specific characteristics of the skin site. This topographical and temporal survey provides a baseline for studies that examine the role of bacterial communities in disease states and the microbial interdependencies required to maintain healthy skin



June 2012: Nature special issue on human „Microbiomics…

CURRENT RESEARCH IN *NATURE*

Gut microbiota composition correlates with diet and health in the elderly **FREE**
Marcus J. Claesson *et al.*
doi:10.1038/nature11319 (09 August 2012)

A framework for human microbiome research
Barbara A. Methé *et al.*
doi:10.1038/nature11209 (14 June 2012)

Structure, Function and Diversity of the Healthy Human Microbiome
The Human Microbiome Project Consortium
doi:10.1038/nature11234 (14 June 2012)

Human gut microbiome viewed across age and geography **FREE**
Tanya Yatsunenko *et al.*
doi:10.1038/nature11053 (14 June 2012)

Dietary fat-induced taurocholic acid production promotes pathobiont and colitis in IL-10$^{-/-}$ mice **FREE**
Suzanne Devkota *et al.*
doi:10.1038/nature11225 (14 June 2012)

RECENT CONTENT IN *NATURE*

Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling **FREE**
Christoph Reinhardt *et al.*
Nature **483**, 627-631 (29 March 2012)

Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity
Jorge Henao-Mejia *et al.*
Nature **482**, 179-185 (09 February 2012)

Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination
Kerstin Berer *et al.*
Nature **479**, 538-541 (24 November 2011)

Peripheral education of the immune system by colonic commensal microbiota
Stephanie K. Lathrop *et al.*
Nature **478**, 250-254 (13 October 2011)

Antibiotic overuse: Stop the killing of beneficial bacteria
Martin Blaser
Nature **476**, 393-394 (25 August 2011)

Ecology drives a global network of gene exchange connecting the human microbiome
Chris S. Smillie *et al.*
Nature **480**, 241-244 (08 December 2011)

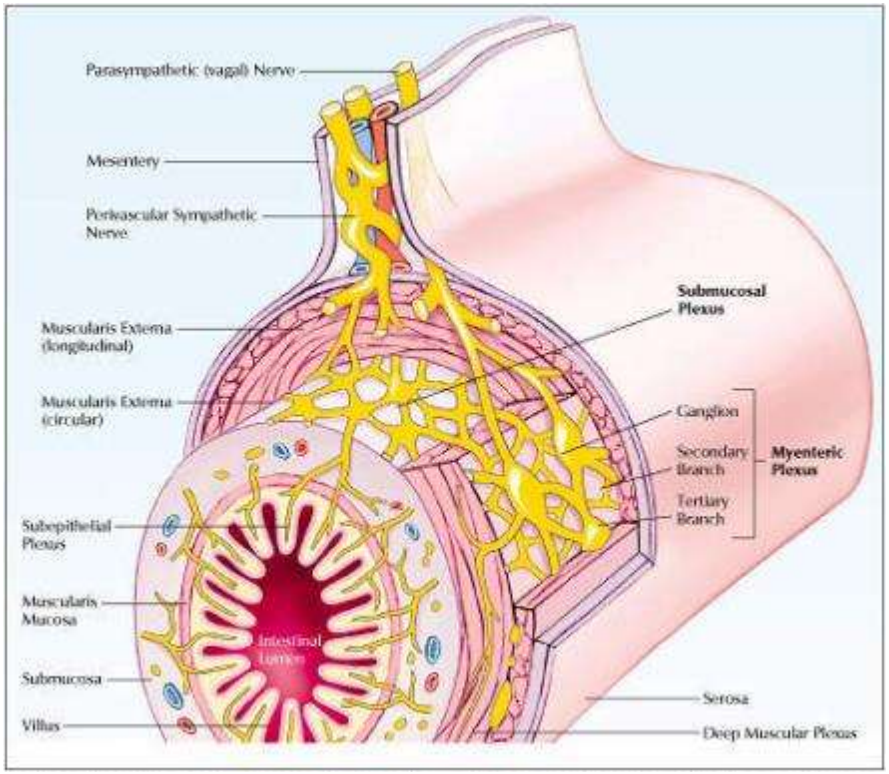
Human nutrition, the gut microbiome and the immune system
Andrew L. Kau *et al.*
Nature **474**, 327-336 (16 June 2011)

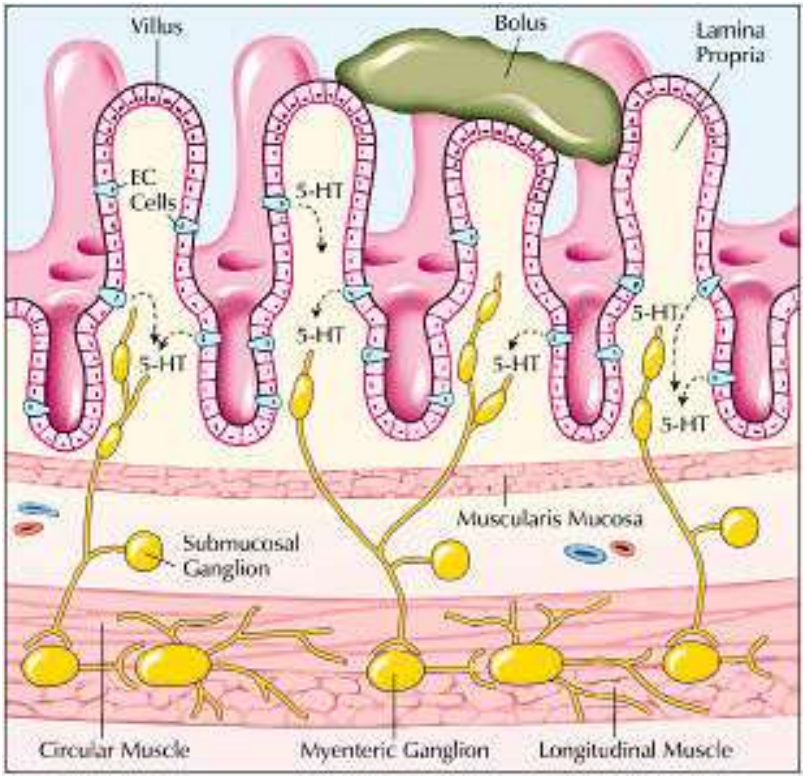
Enterotypes of the human gut microbiome
Manimozhiyan Arumugam *et al.*
Nature **473**, 174-180 (12 May 2011)

Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease

Enteric nervous system

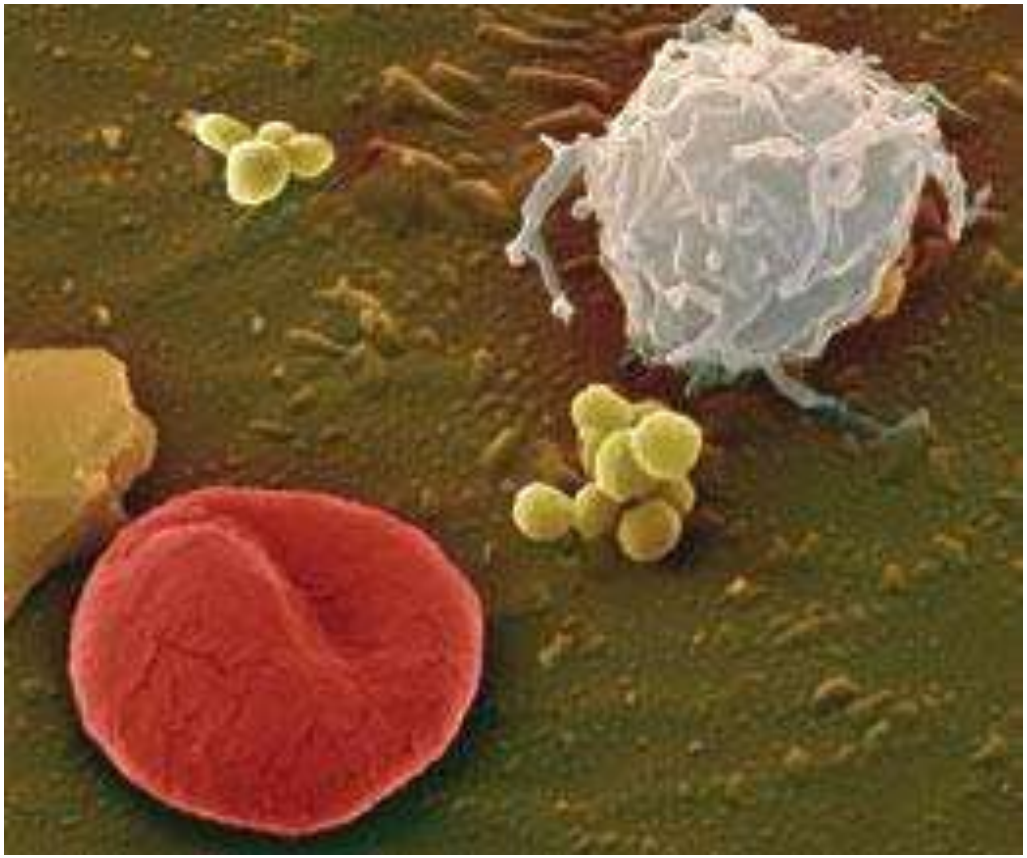
- Over 500 million neurons in the intestinal tract which is as much as the spinal cord.





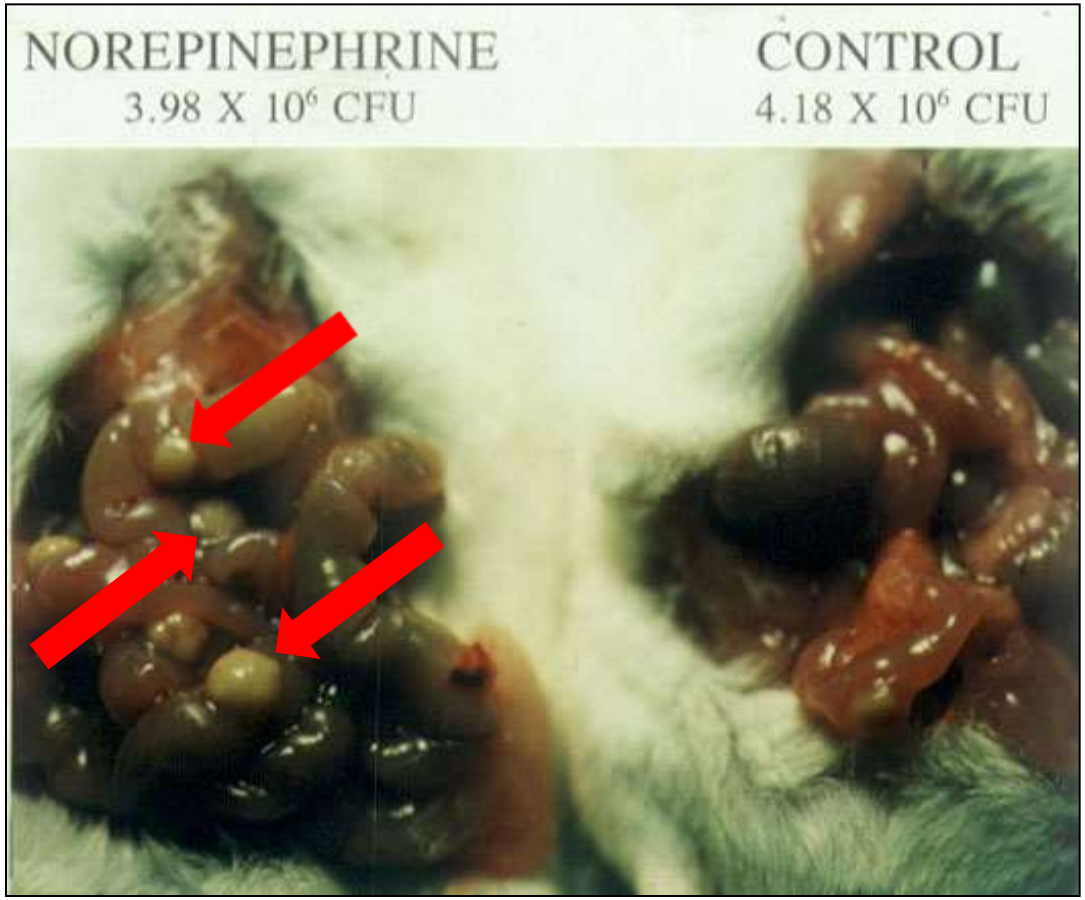
Gershon, Hosp. Pract. 34:31, 1999

Who's listening in?
Staphylococcus bacteria between two body cells..



Some infectious bacteria intercept the human stress-response hormone noradrenaline and use it as a cue to escalate their growth
— perhaps explaining why stressed animals are more likely to die of infection…
QseC, a bacterial receptor that detects a quorum-sensing signal called autoinducer 3 (AI-3), is also activated by the mammalian hormones adrenaline and noradrenaline[3]. Both cause the bacterium Escherichia coli to express virulence genes

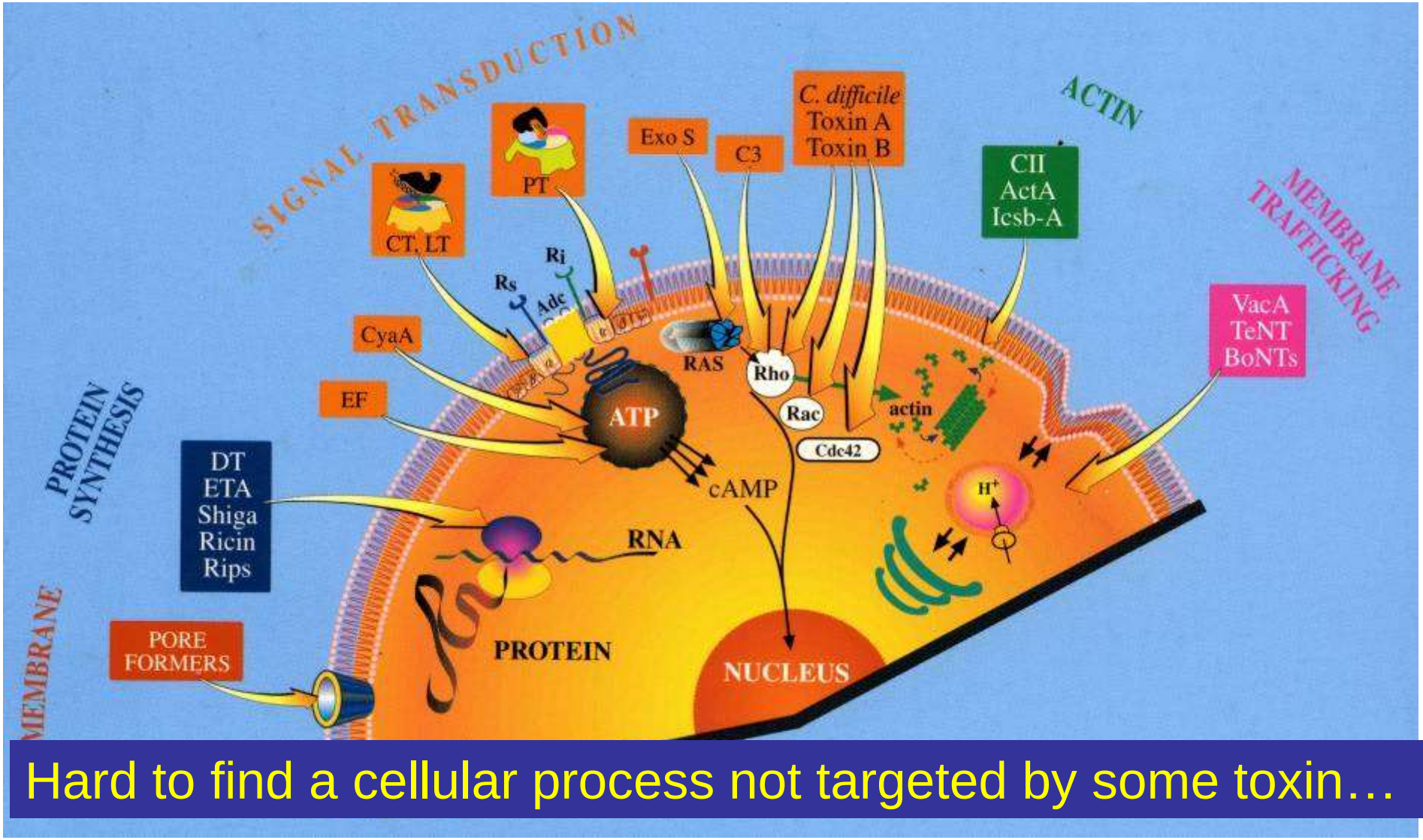
Pre-treatment with norepinephrine increases virulence of *Y. enterocolitica*



One week post-challenge *Y. enterocolitica* pre-treated with norepinephrine 50,000X more infective.

Virulence factor	Strategy involved in virulence
Pili	Adherence to mucosal surfaces
Nonfimbrial adhesins	Tight binding to host cell
Bacterial triggering of actin rearrangement in host cells	Forced phagocytosis of bacteria by normally nonphagycytic host cells; movement of bacteria within host cells or from one host cell to another
Binding to and entry of M cells	M cells used as natural port of entry into underlying tissue
Motility and chemotaxis	Reaching mucosal surfaces (especially areas with fast flow)
sIgA proteases	Prevent trapping of bacteria in mucin
Siderophores, surface proteins that bind transferrin, lactoferrin, ferritin, or hemin	Iron acquisition
Capsules (usually polysaccharides)	Prevent phagocytic uptake; reduce complement activation
Altered LPS O antigen	MAC not formed; serum resistance
C5a peptidase	Interferes with signaling function of complement
Toxic proteins	Kill phagocytes; reduce strength of oxidative burst
Variation in surface antigens	Evade antibody response

Among the most sophisticated bacterial tools are Proteins toxins tha are “smart, pretty and usefull”



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

Paradigm – use lower animals as models…

e.g. Caenorhabditis elegans as host to study bacterial toxins

host

toxin

Whole genome siRNA screen

host -1 gene

toxin

resistant

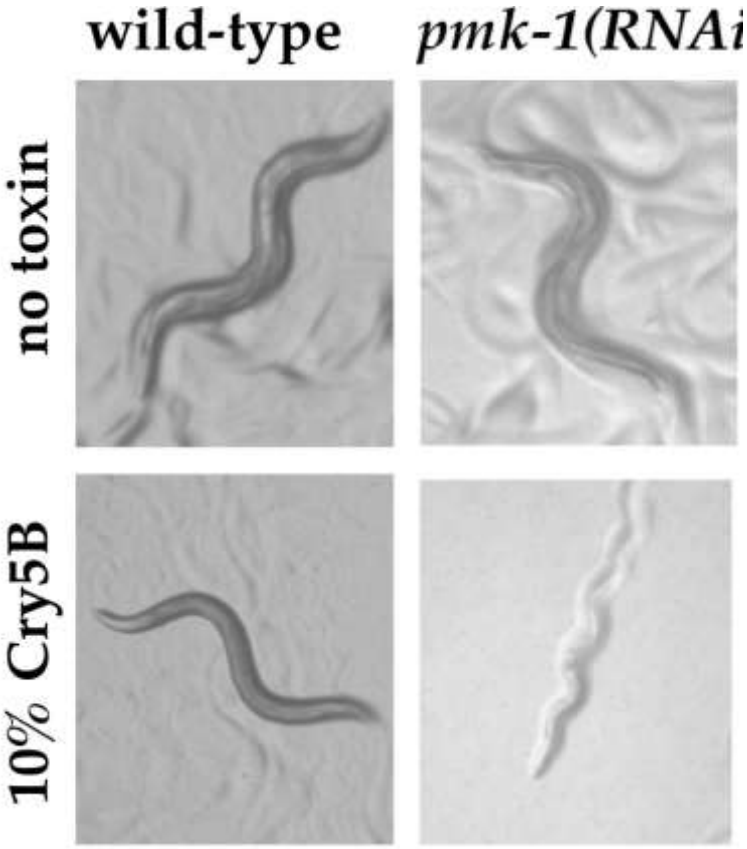
= intoxicating host gene

host -1 gene

hypersensitive

= defending host gene

hypersensitive and resistance can be recapitulated by RNAi feeding



Animal fed vector alone on Cry5B toxin plate

Animal fed *bre-5* dsRNA on Cry5B toxin plate

Microorganisms colonize our skin and most of the epithelial surfaces exposed to the environment - making a „microbial organ“

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Staph. epidermidis
Ps. aeruginosa

NOSE 10^{8}
Staph. aureus
Staph. epidermidis

The peaceful coexistence with our microflora is occasionally perturbed by intruders

pathogenic microorganisms

specifically equipped for manipulation of our immune system

If overwhelming - cause us problems

URETHRA AND VAGINA 10^{8}
Staph. epidermidis
Streptococci
Lactobacilli
Veillonella
etc.

(Wilson et al, 2002)

INTESTINES 10^{14} ~220g
~**1200 species…**
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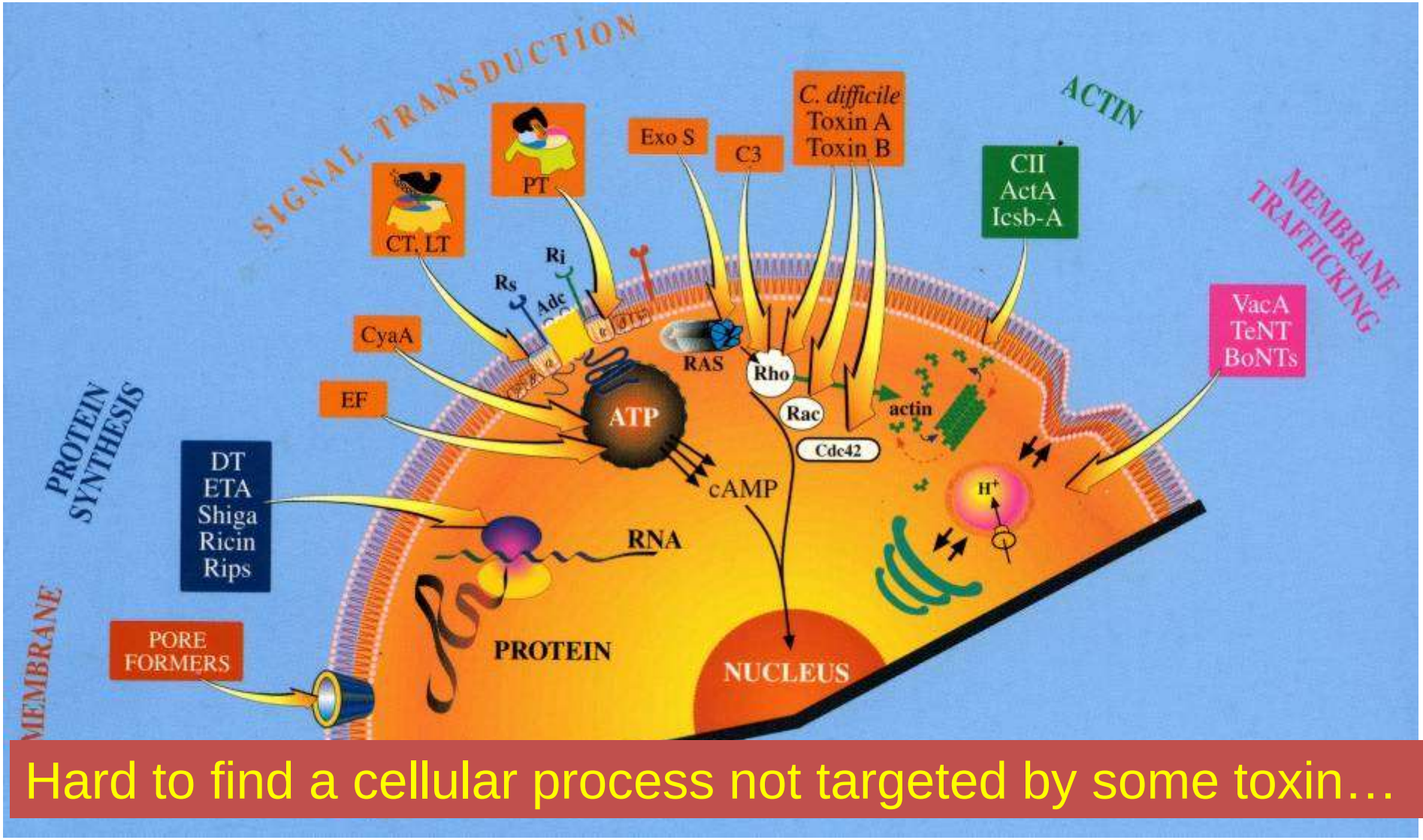
Knowledge is useful:

How bacterial toxins manipulate us and How we use them

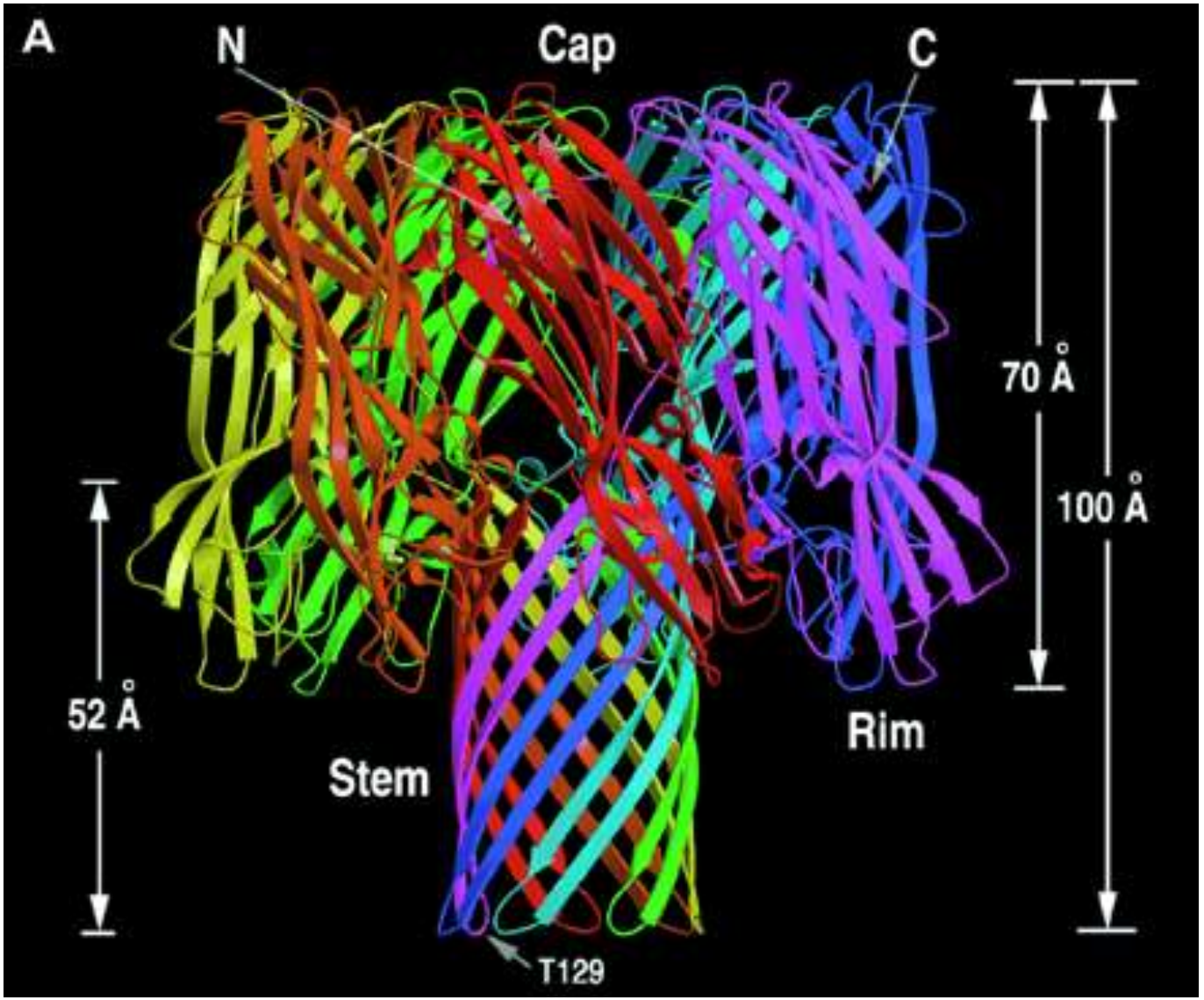
Central to bacterial virulence and immunomodulation are **PROTEIN TOXINS**

That are “smart, pretty and useful”



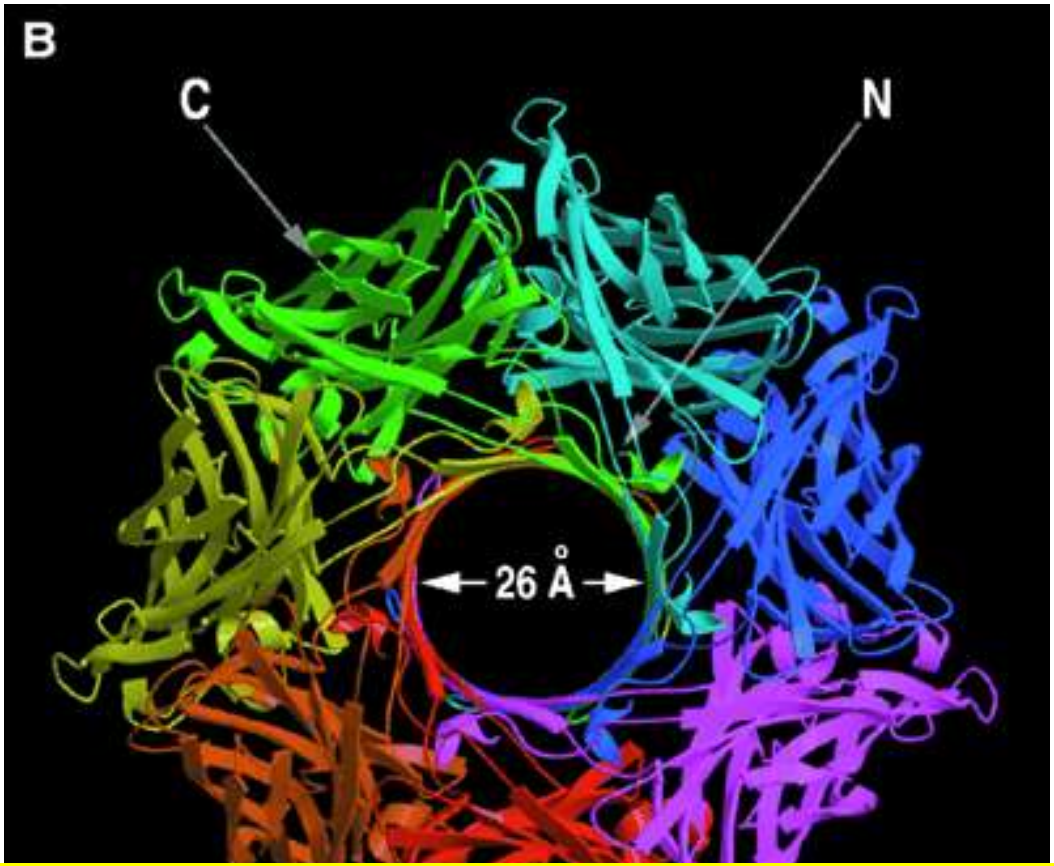
Hard to find a cellular process not targeted by some toxin…

Toxins are pretty



***Stapylococcus aureus* α-toxin heptamer**

Toxins can be very usefull…



Electrical potential gradient on a typical cell membrane is 50 000 V/cm

Millions of ions per second make …

Genetically modified toxins as molecular gatekeepers for drug and cryoprotectant delivery, biosensors etc..

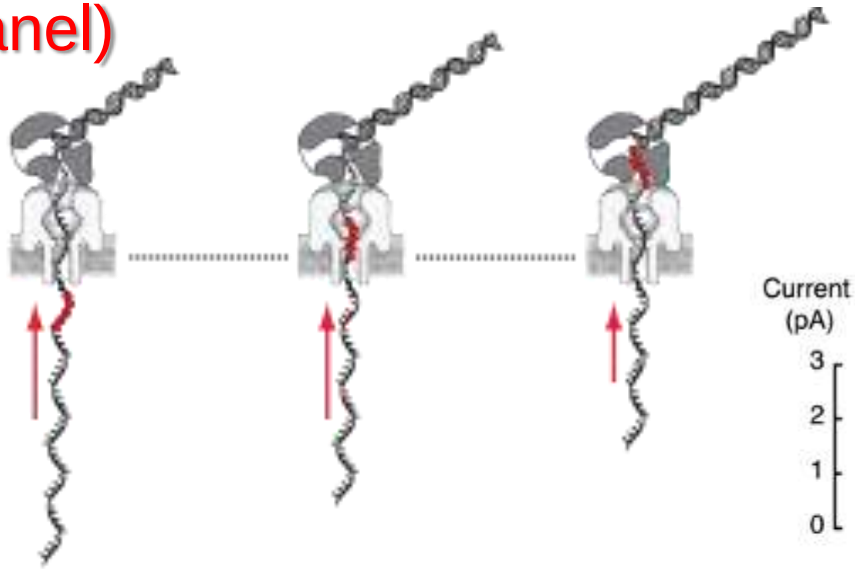
Bayley H, Cremer PS. Stochastic sensors inspired by biology. Nature, 413, 226-230 (2001)

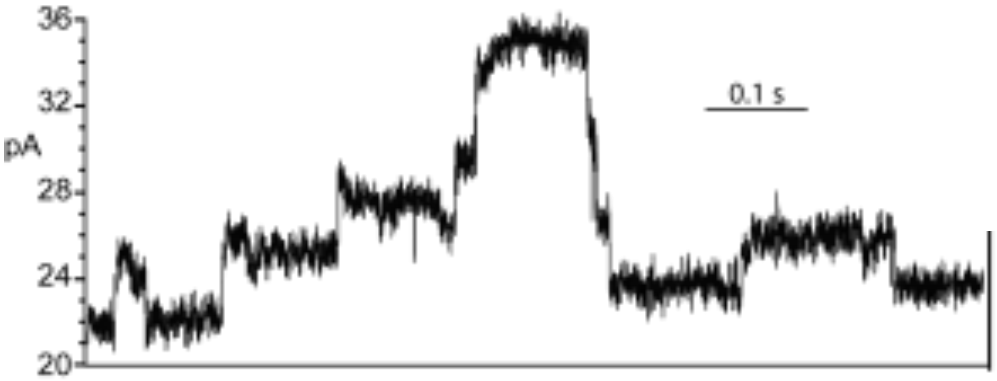
Staphylococcus aureus α-Toxin in a next generation sequencer…!!!

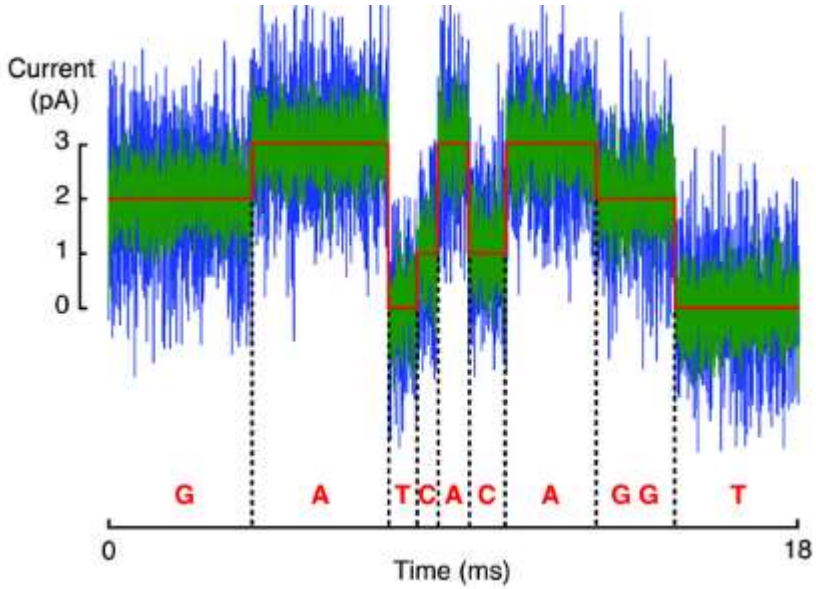
$900 USB stick

Oxford Nanopore's MinION sequencer can read DNA fragments up to 10 kilobases long

The rate of movement of a DNA strand through the nanopore can be controlled by the use of an enzyme, such as a DNA polymerase (shown in dark grey, upper panel)



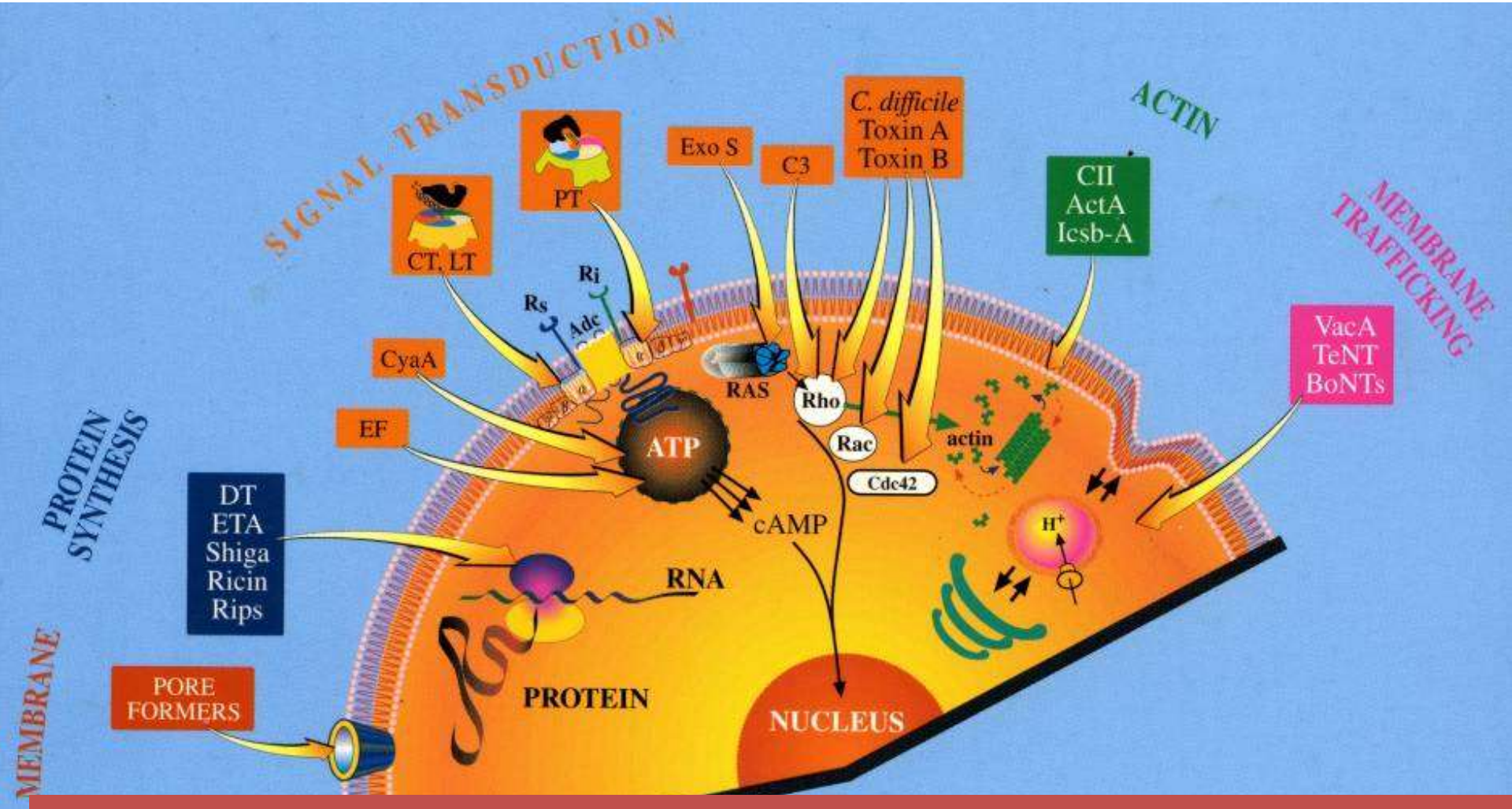




Oxford Nanopore MinION Data from E.Coli K-12 Genome is here

September 10, 2014 by nextgenseek · Leave a Comment

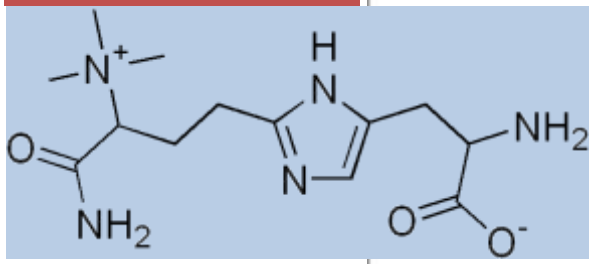
Bacterial toxins are “smart, pretty and usefull”

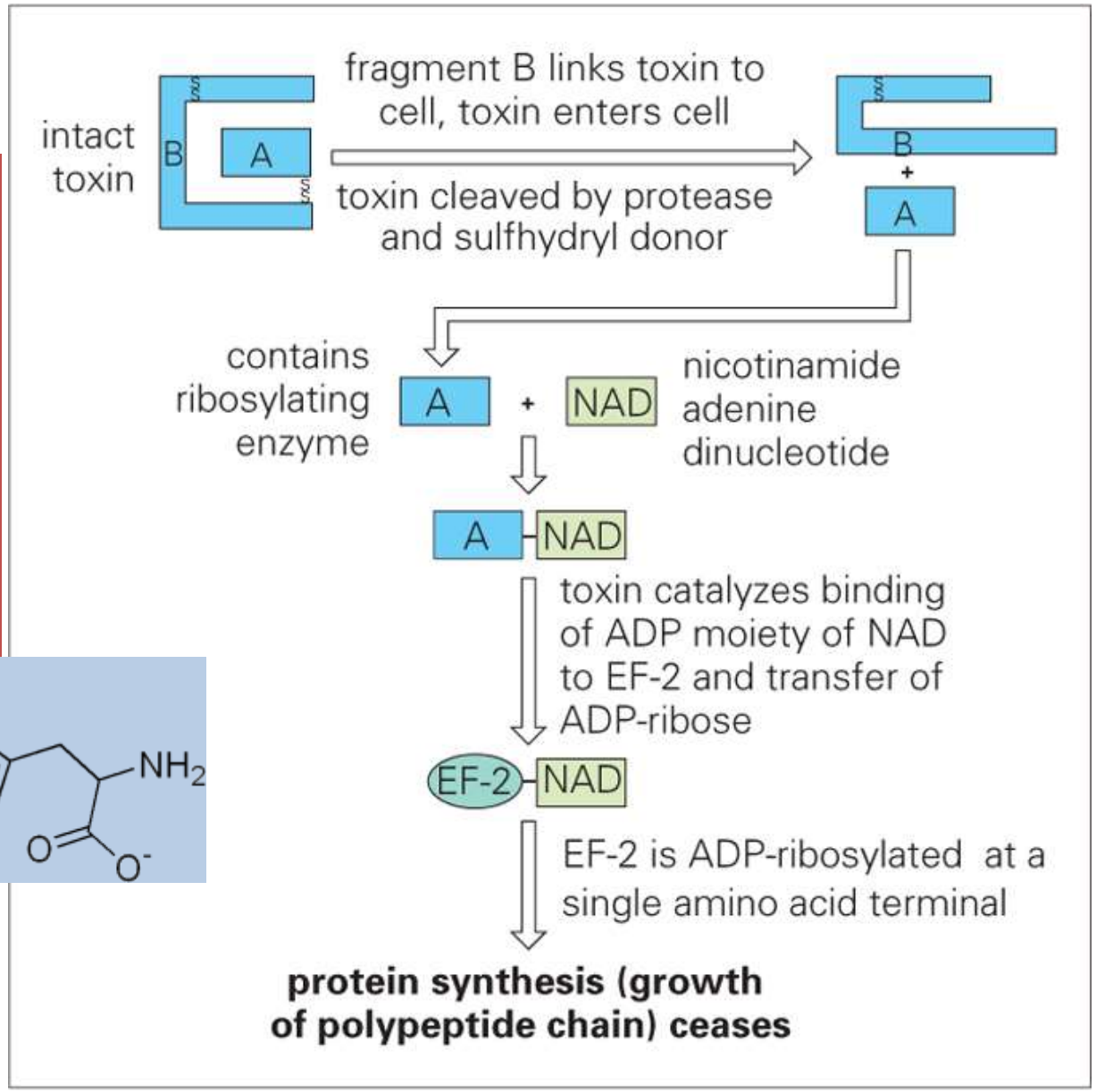


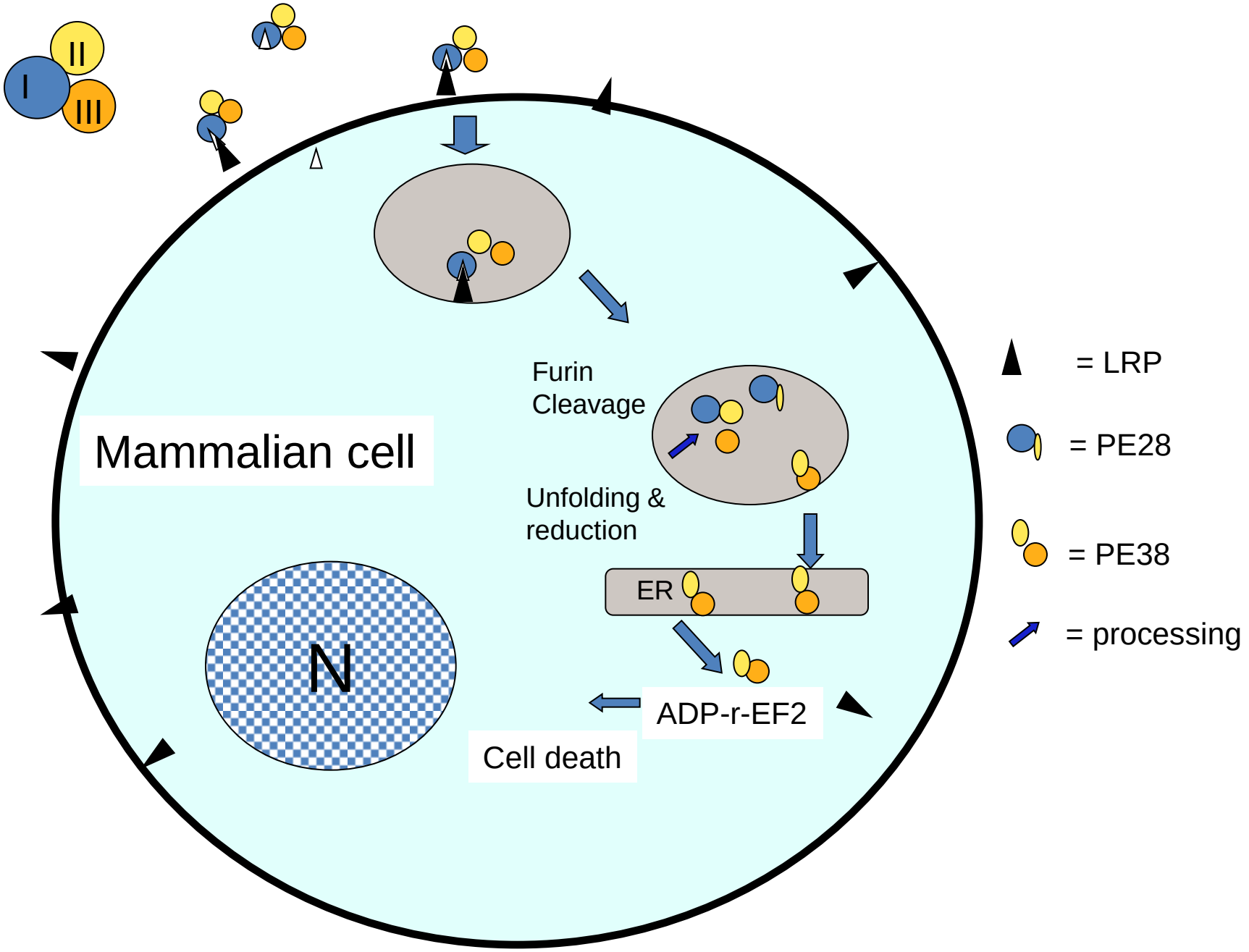
Hard to find a cellular process not targeted by some toxin…

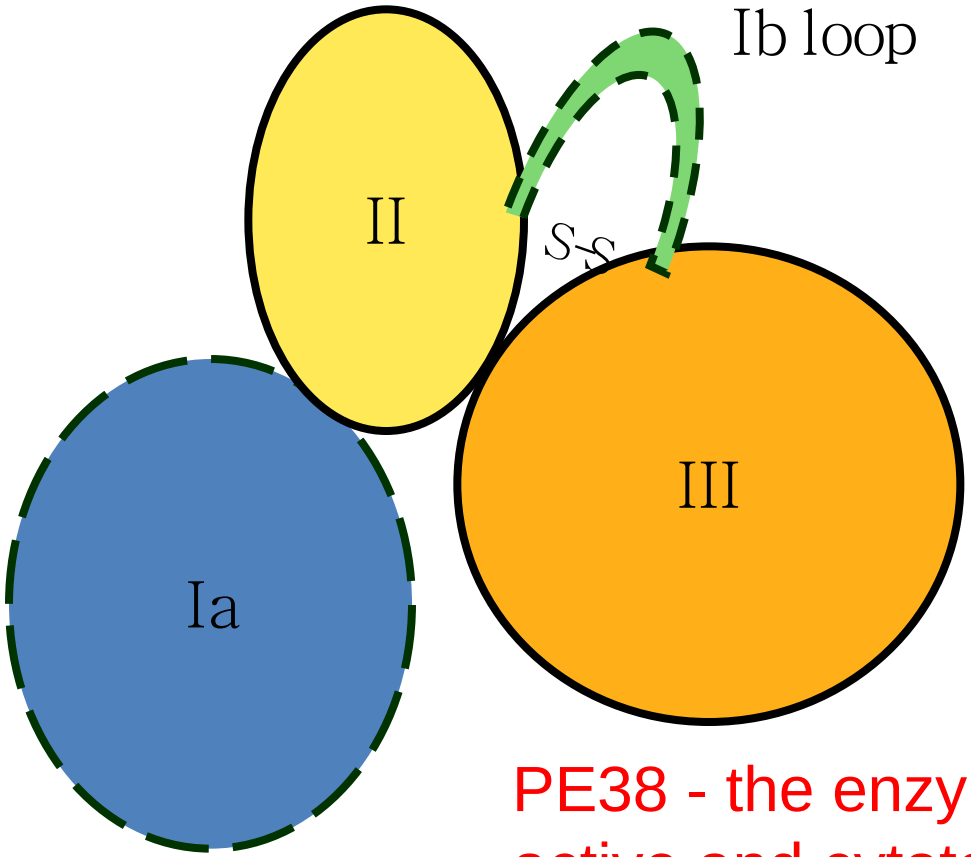
Diphteria or ExoA toxin action

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.



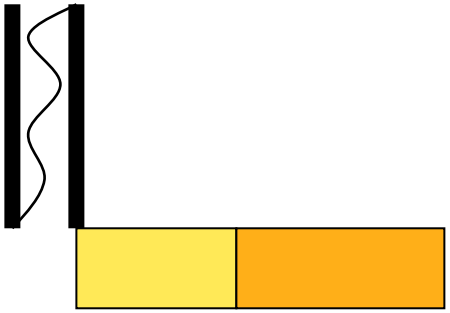



I
II
III
Mammalian cell
Furin
Cleavage
Unfolding &
reduction
ER
N
ADP-r-EF2
Cell death
= LRP
= PE28
= PE38
= processing

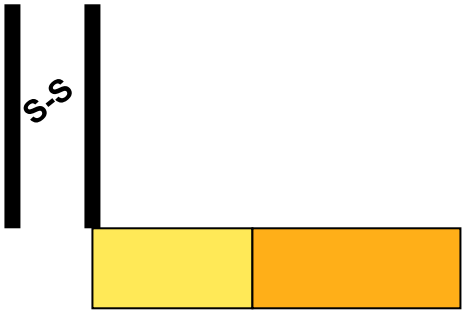


PE38 - the enzymatically active and cytotoxic domain

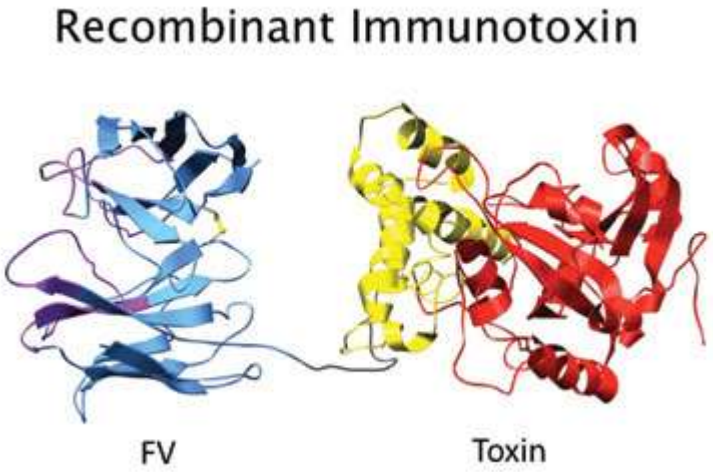
Antibody-toxin fusions - recombinant Immunotoxins

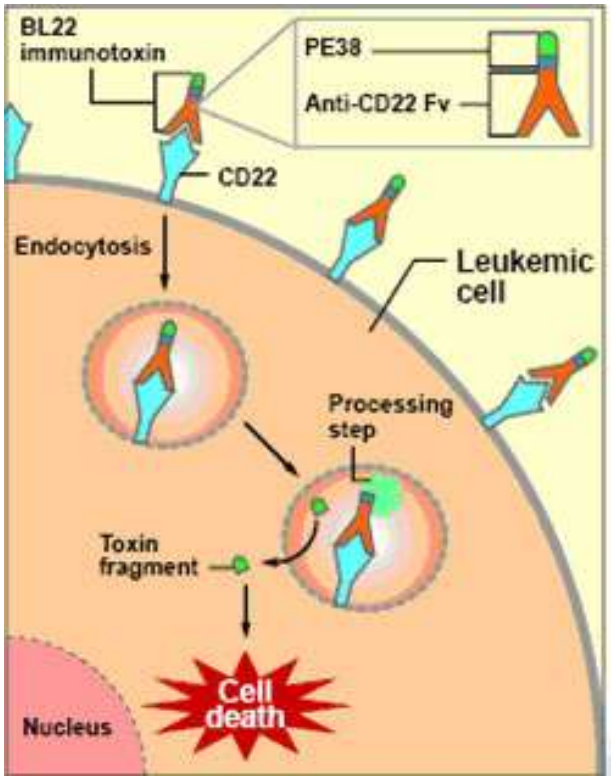


scFv-PE38



dsFv-PE38

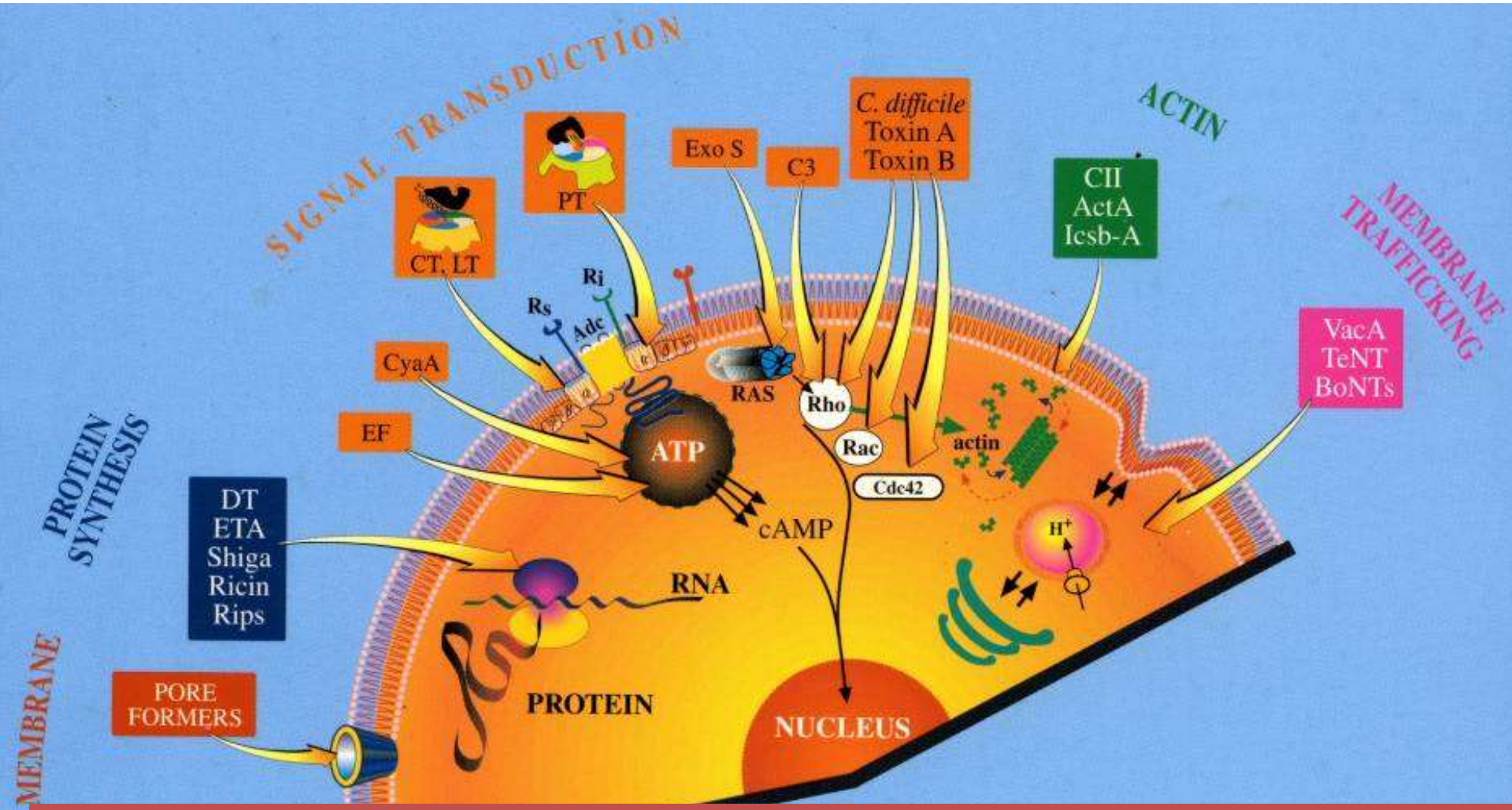




On 16 May 2013, AstraZeneca started Phase III clinical trials with CAT-8015 that targets various CD22 positive B-cell malignancies:

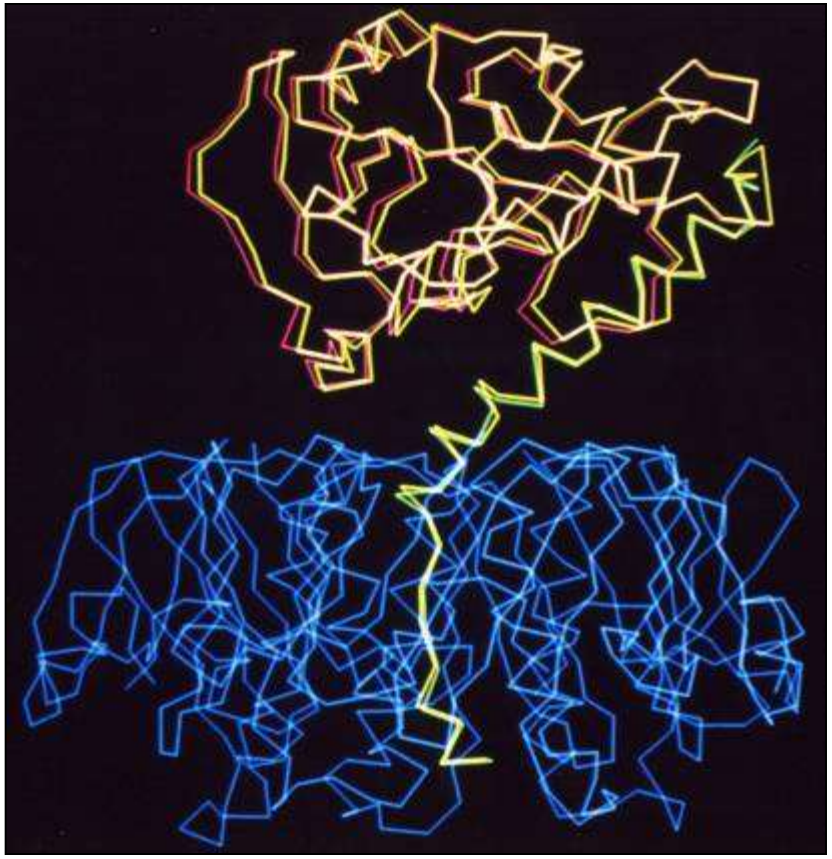
Non-Hodgkin lymphoma
chronic lymphocytic leukemia
Hairy cell leukemia
acute lymphoblastic leukemia (pediatric)

Bacterial toxins are “smart, pretty and usefull”

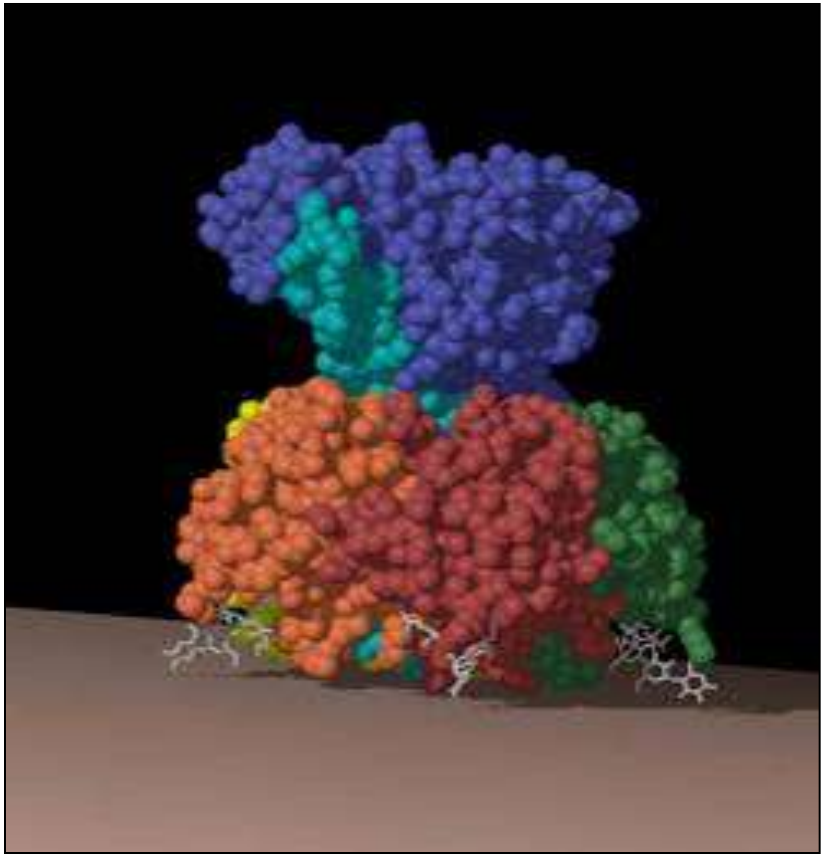


Hard to find a cellular process not targeted by some toxin…

Termo-labile toxin of *V. cholerae* or *E. coli* can make you really sick…

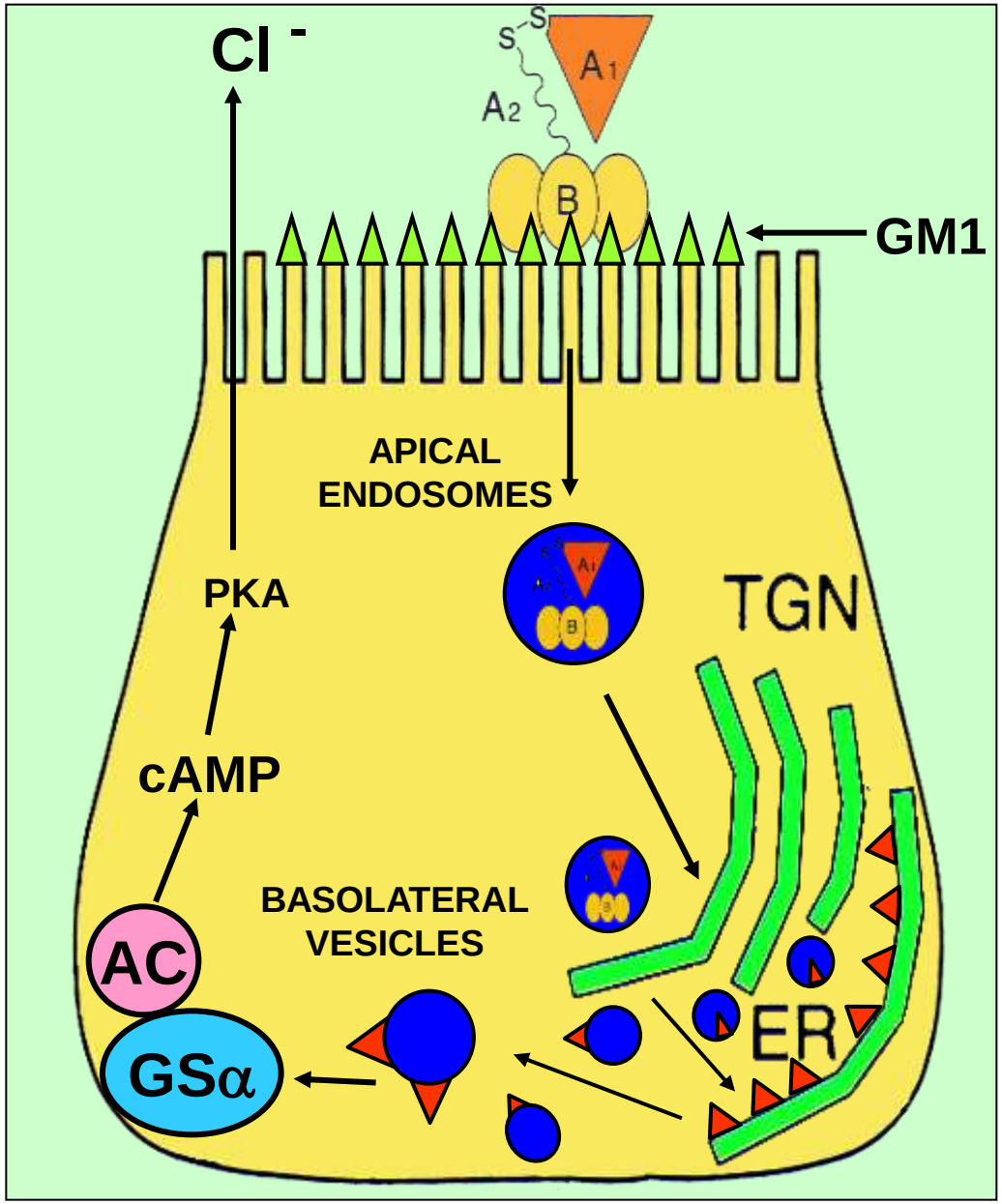


Sixma *et al.*, Nature 351: 371-377, 1991



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

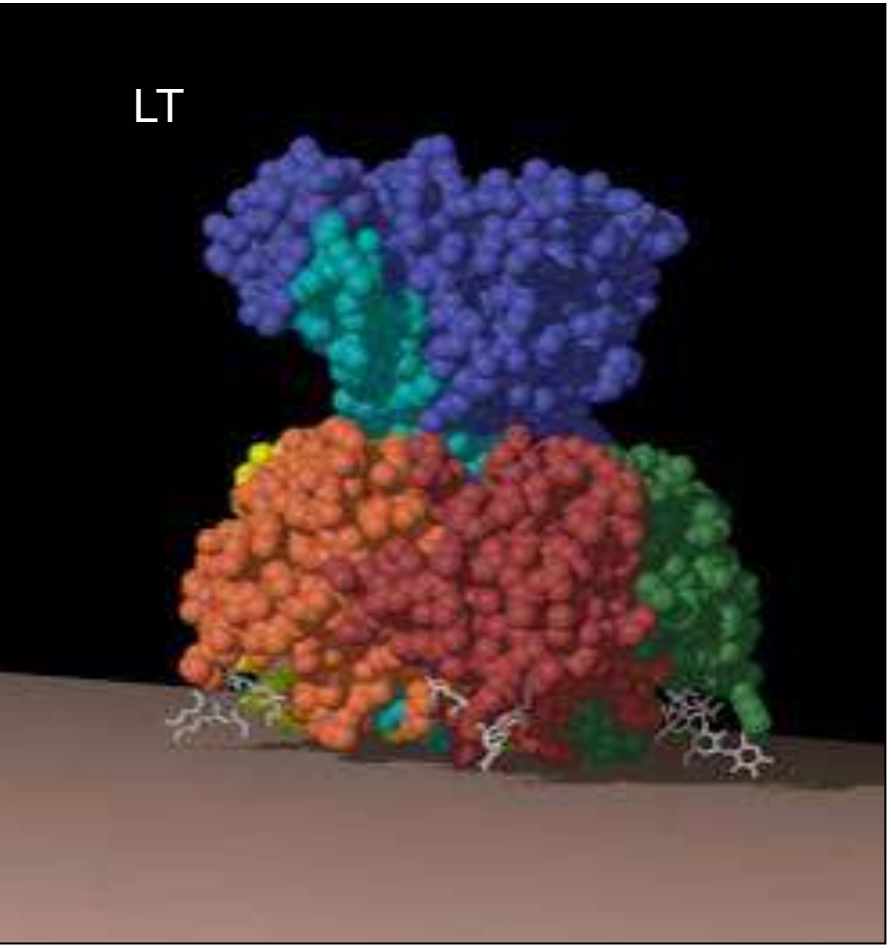
Action of cholera toxin and related enterotoxins

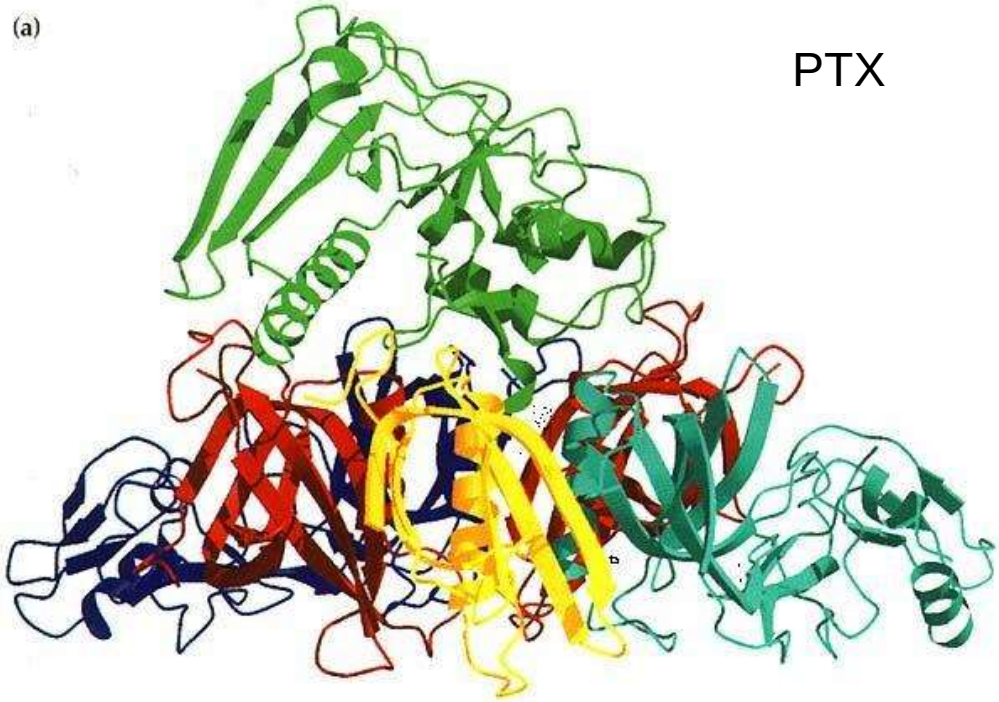


A cholera patient of this size can produce up to 80 L of “rice water stool” per day….

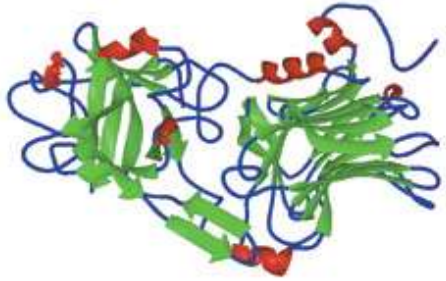
VACCINE antigens and adjuvants

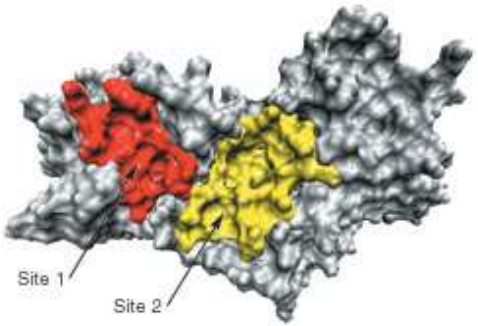
Toxoid (chemically or genetically inactivated toxin)



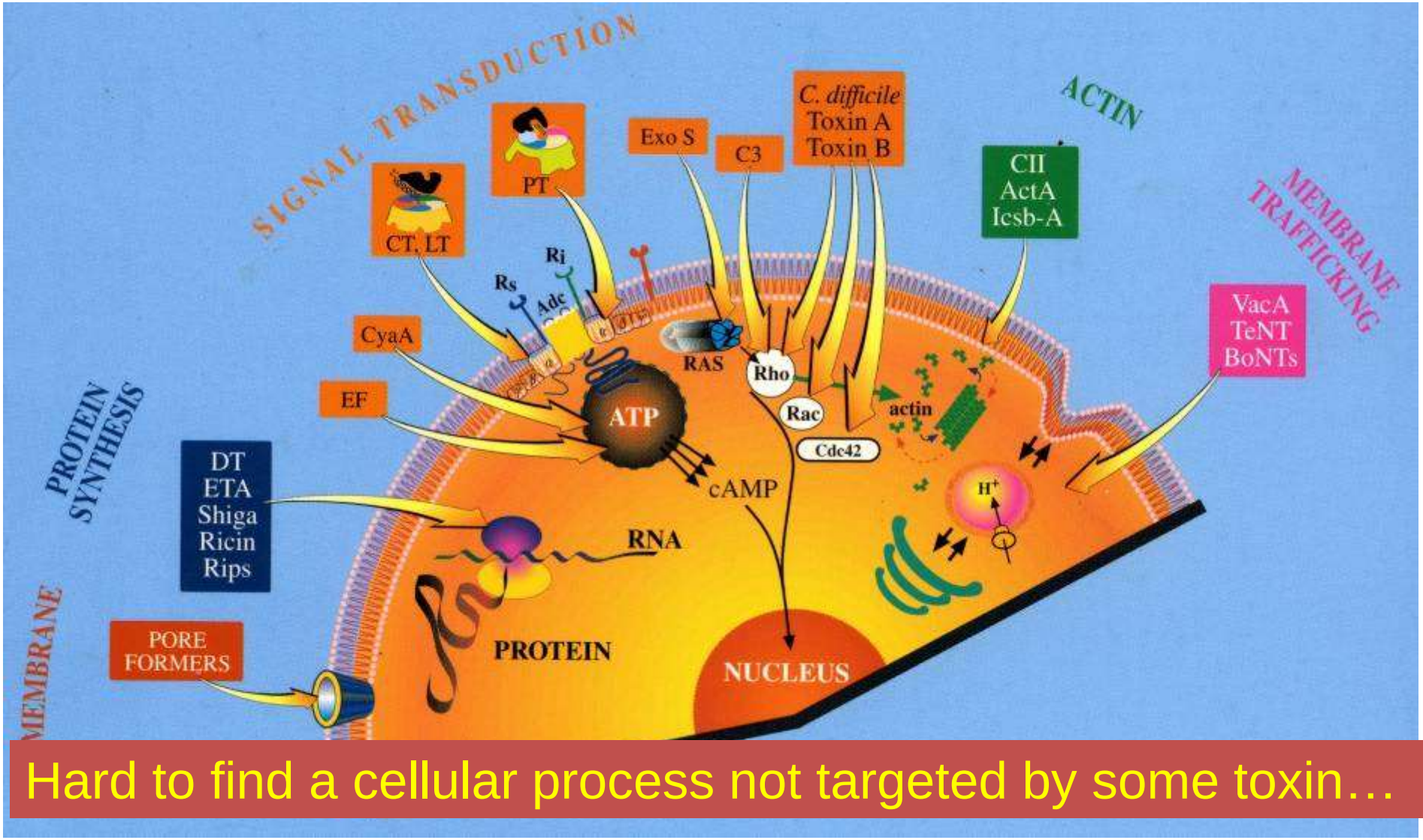


DT



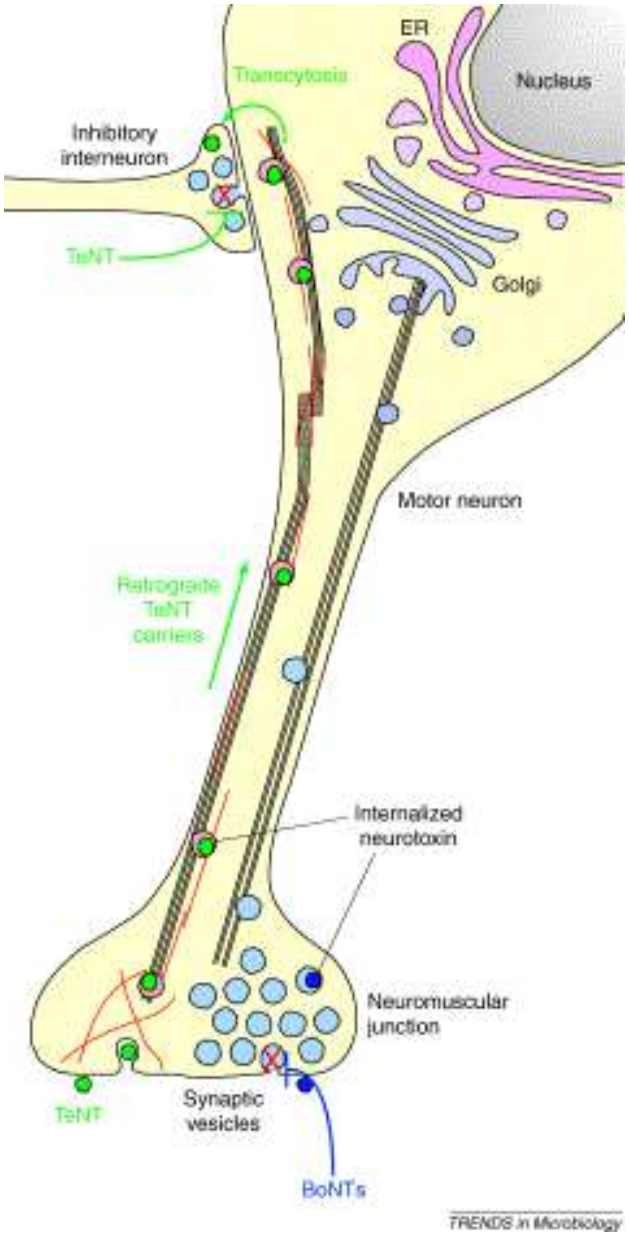


Bacterial toxins are “smart, pretty and usefull”



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

Clostridial neurotoxin trafficking



The tetanus (TeNT; green) and botulinum neurotoxins (BoNTs; blue) act on mammalian motor neurons and an interacting spinal inhibitory interneurons.

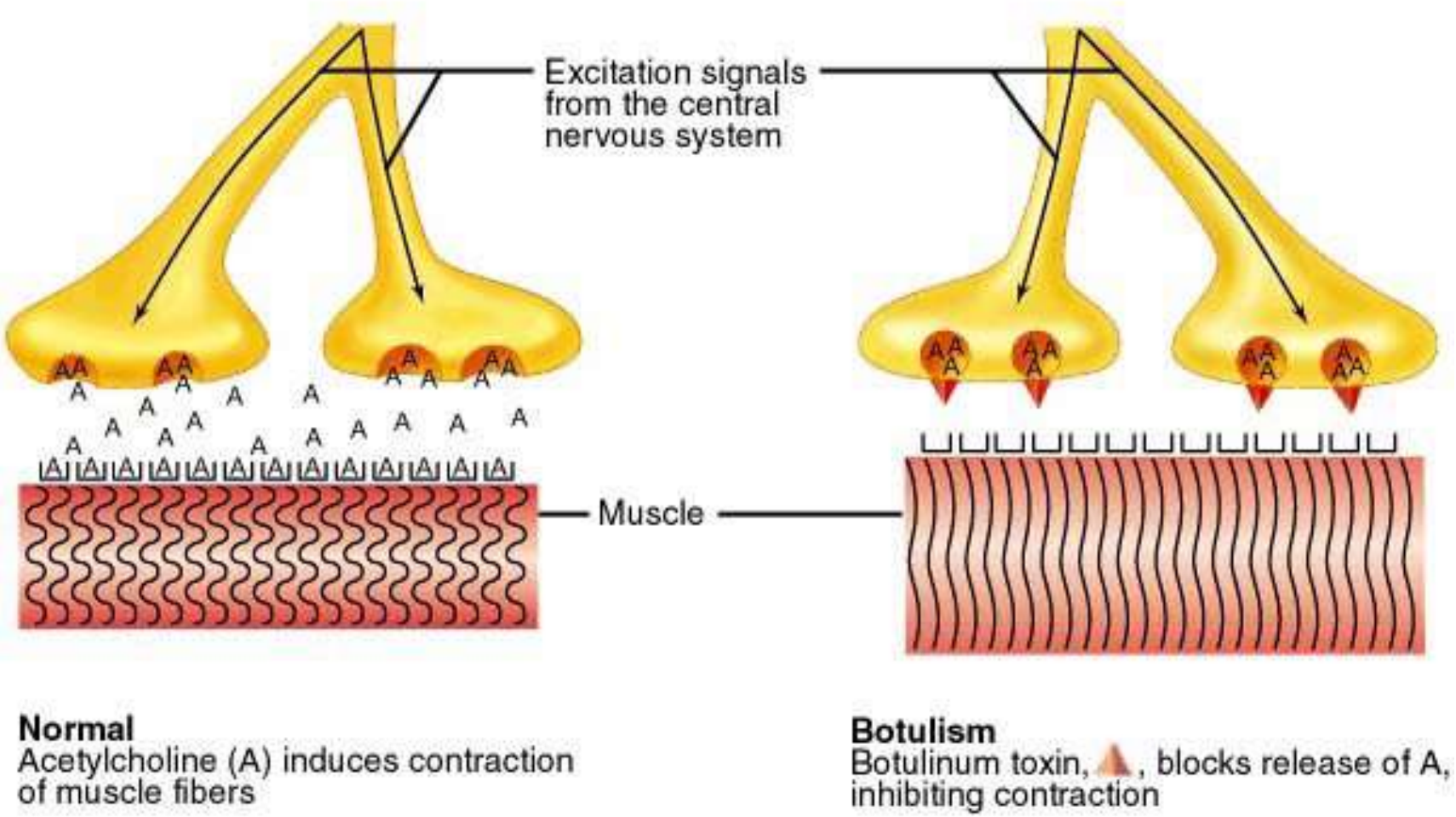
Clostridium tetani toxin (tetanospasmin)

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

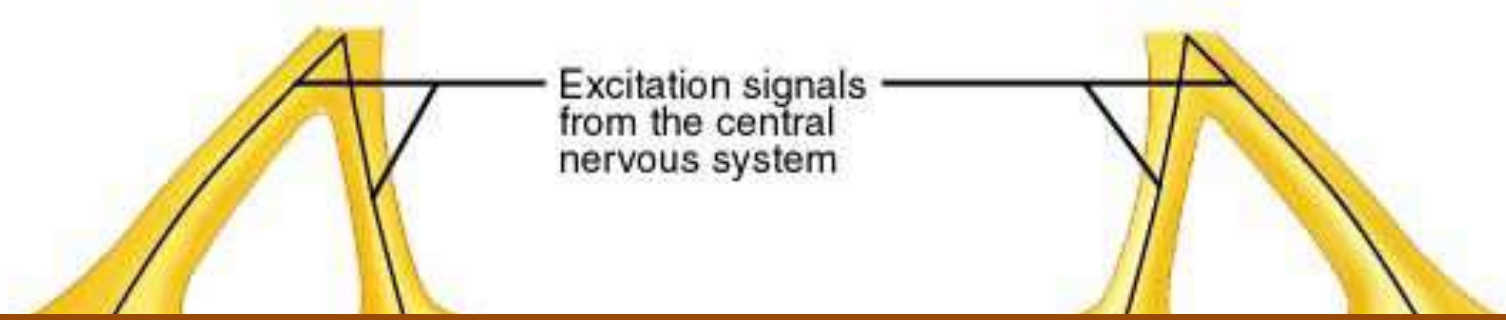
Clostridium botulinum toxin (botulismus)

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.

Acetylcholine (A) induces contraction of muscle fibers

Botulinum toxin, , blocks release of A, inhibiting contraction

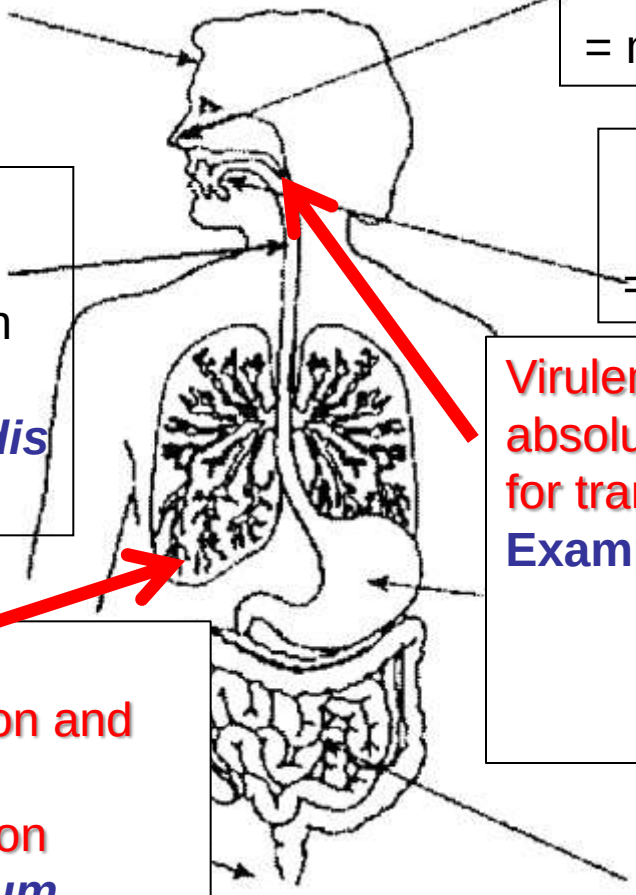
Virulence is a matter of niche and lifestyle…

No virulence needed
for transmission
real commensal

No virulence needed
for transmission
= mostly commensals

No virulence needed
for transmission
= commensal/patogen
Example 1:
Neisseria meningitidis

No virulence needed
for transmission
= mostly commensals



Virulence=cough
absolutely needed
for transmission
Example 2: *Bordettella pertussis*

Virulence = pathology
Provoking tissue destruction and
cough absolutely needed
for survival and transmission
Example 3: *Mycobacterium tuberculosis*

Example 1: The smartest bacteria are the commensals that make their host happy to facilitate survival and transmission

Neisseria gonorrhoeae ⟷ ***Neisseria meningitidis***

↗ Asymptomatic colonization of the genitourinary tract

↗ Localized inflammation

↗ **Asymptomatic colonization of the nasopharynx**

↗ **Localized inflammation (pneumoniae)**

↗ **Invasive disease cross the blood brain barrier**

Neisseria lactamica

↗ **Commensal (< 5 years)**

↗ **Non pathogenic**

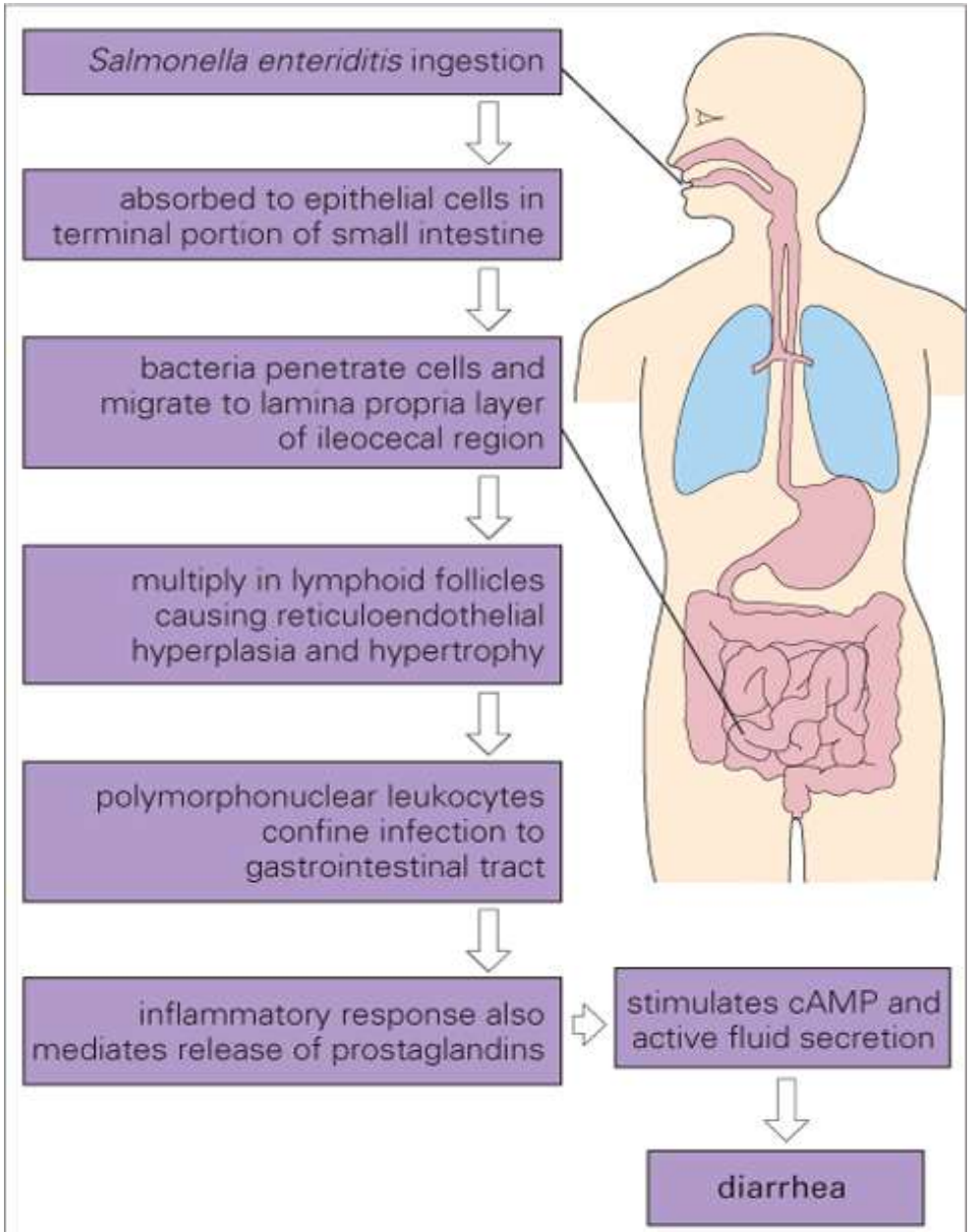
Example 1: The smartest bacteria are the commensals that make their host happy to facilitate survival and transmission

-Efficient adaptation to host mucosa environment:

- Several adhesive mechanisms (pili, Opa, fha)
- Phase variation (On-Off switchiong of antigens 10^{-6}/generation)
- Antigenic hypervariability – 10^{11} pili variants
- Natural competence for DNA take-up – high genome flexibility due to recombination
- iron acquisition systems – siderophores and transferrin receptor
- serum (complement) resistance – polysaccharide capsule
- IgA inactivating protease
- „tight“ adhesion to mucosal epithelia
- Antiapoptotic and cell-cycle supprting signaling - „Make your host cell happy“

-10% healthy carriers of Neisseria menigitidis…., up to 30% among recruits and freshmen at university entry

Example of a pathogen?

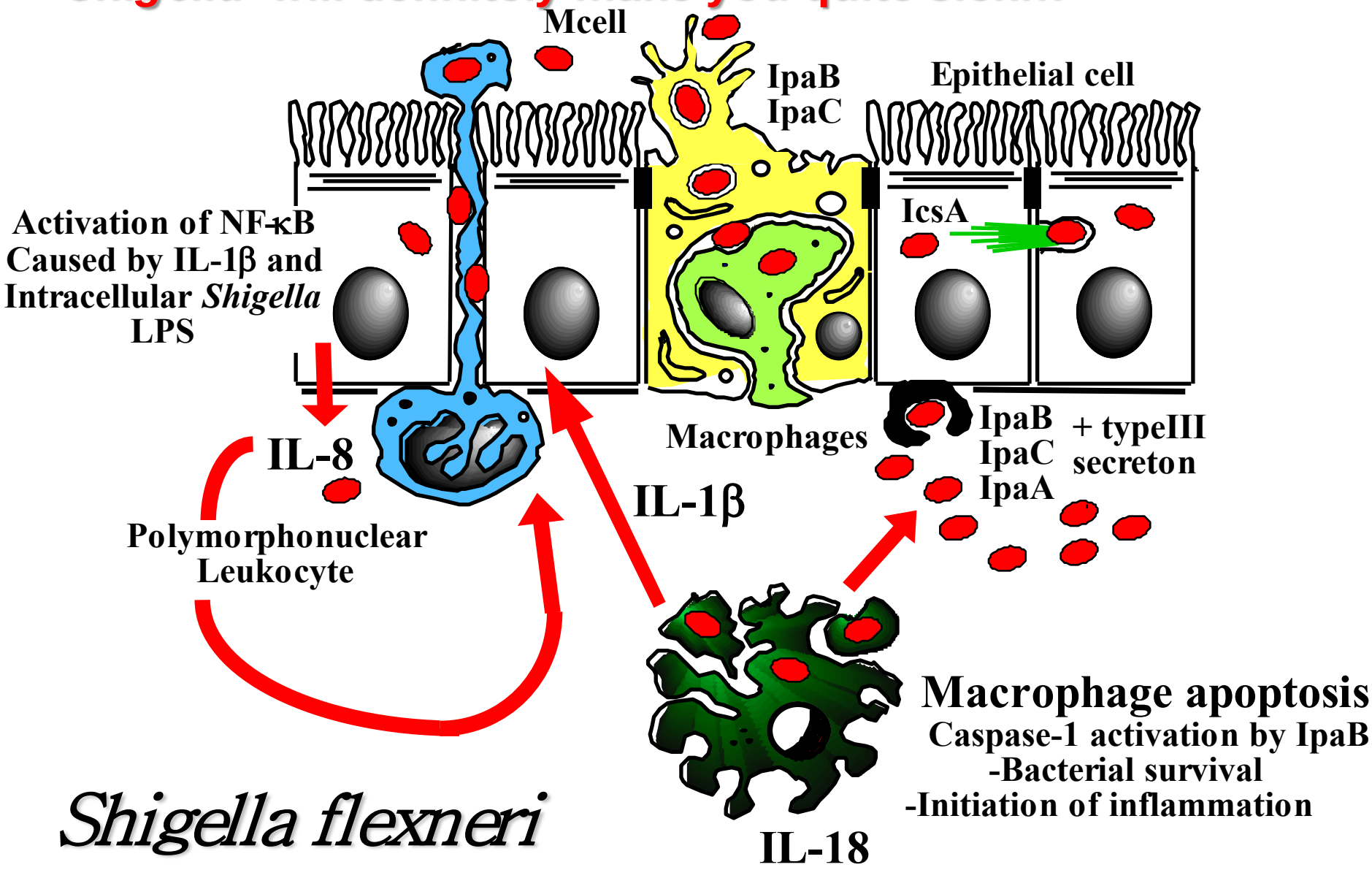


Salmonella does not really need to make you sick to spread…

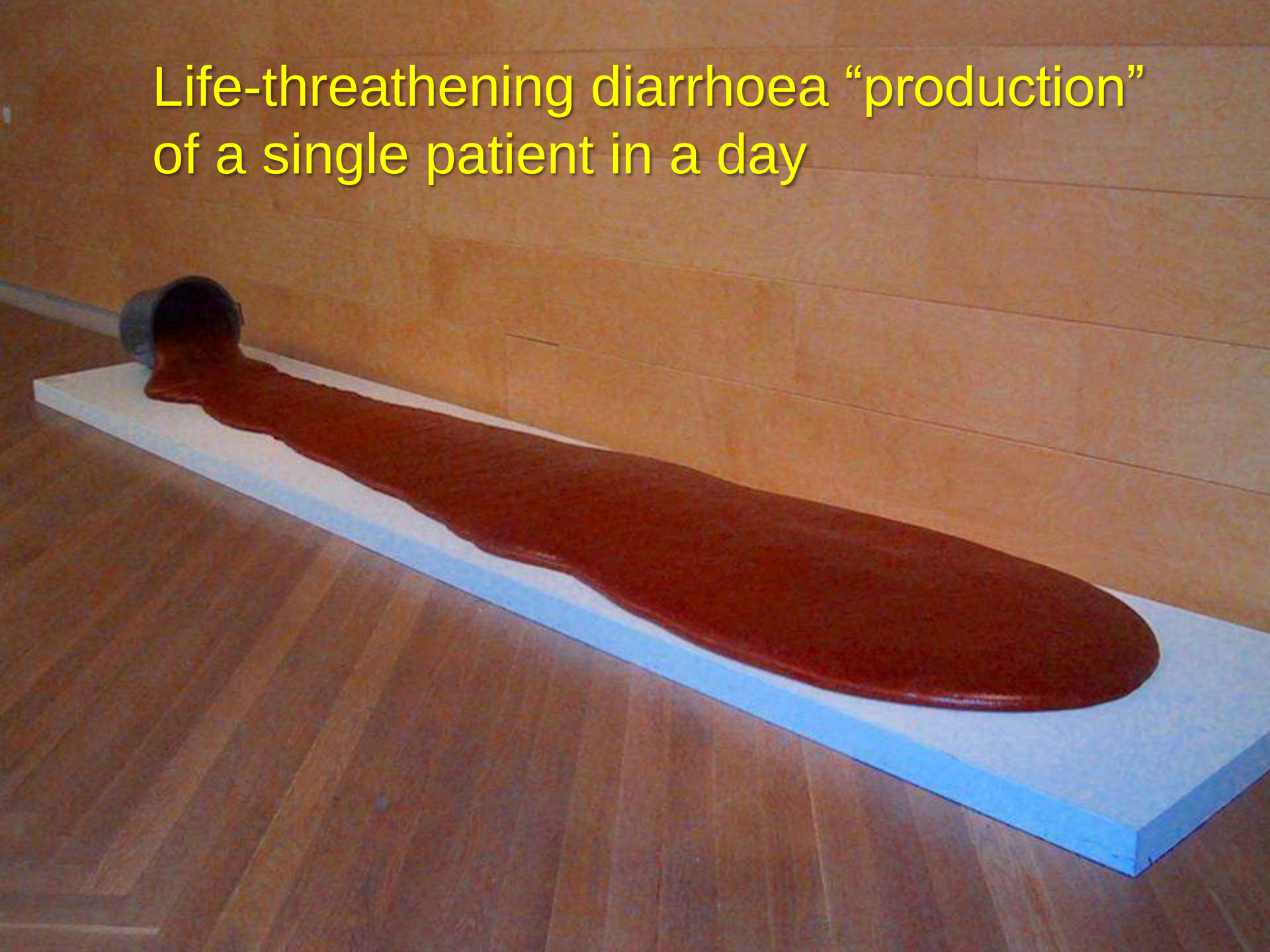
Depending on the host genotype and immune status, Salmonelosis can range from asymptomatic to life-threatening systemic disease

You may be shedding *Salmonella* for years without taking note…

Shigella will definitely make you quite sick…



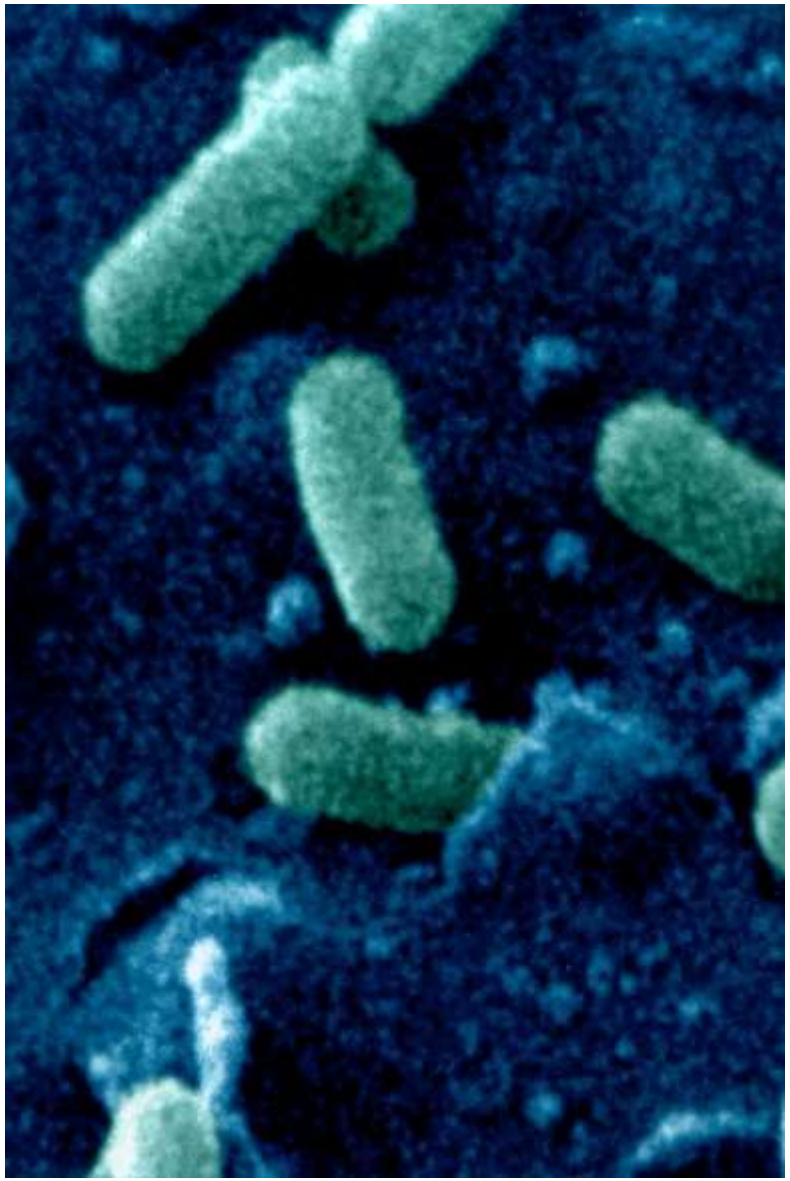
Life-threathening diarrhoea “production” of a single patient in a day

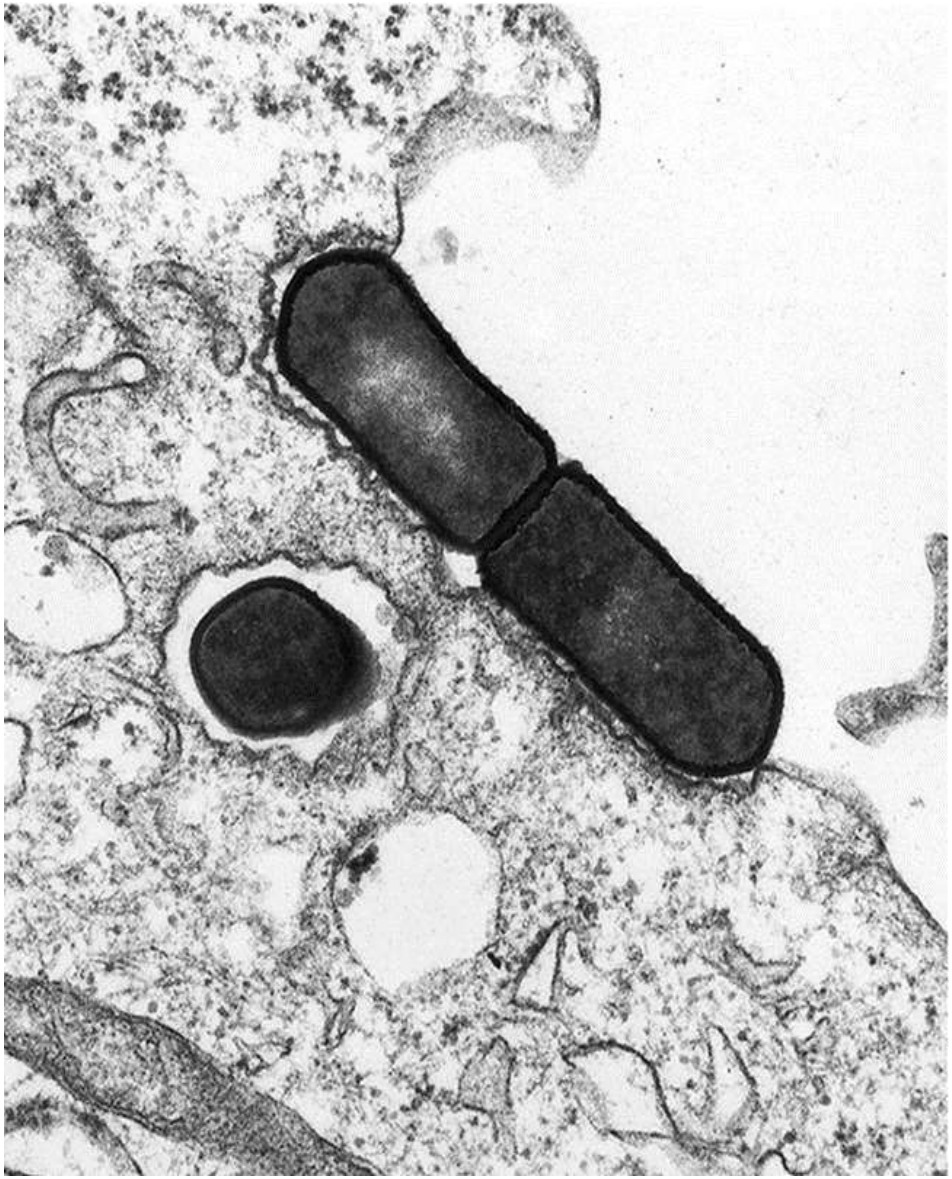


Strategies for hiding inside host cells

escape to complement and antibody action, plenty of nutrients…

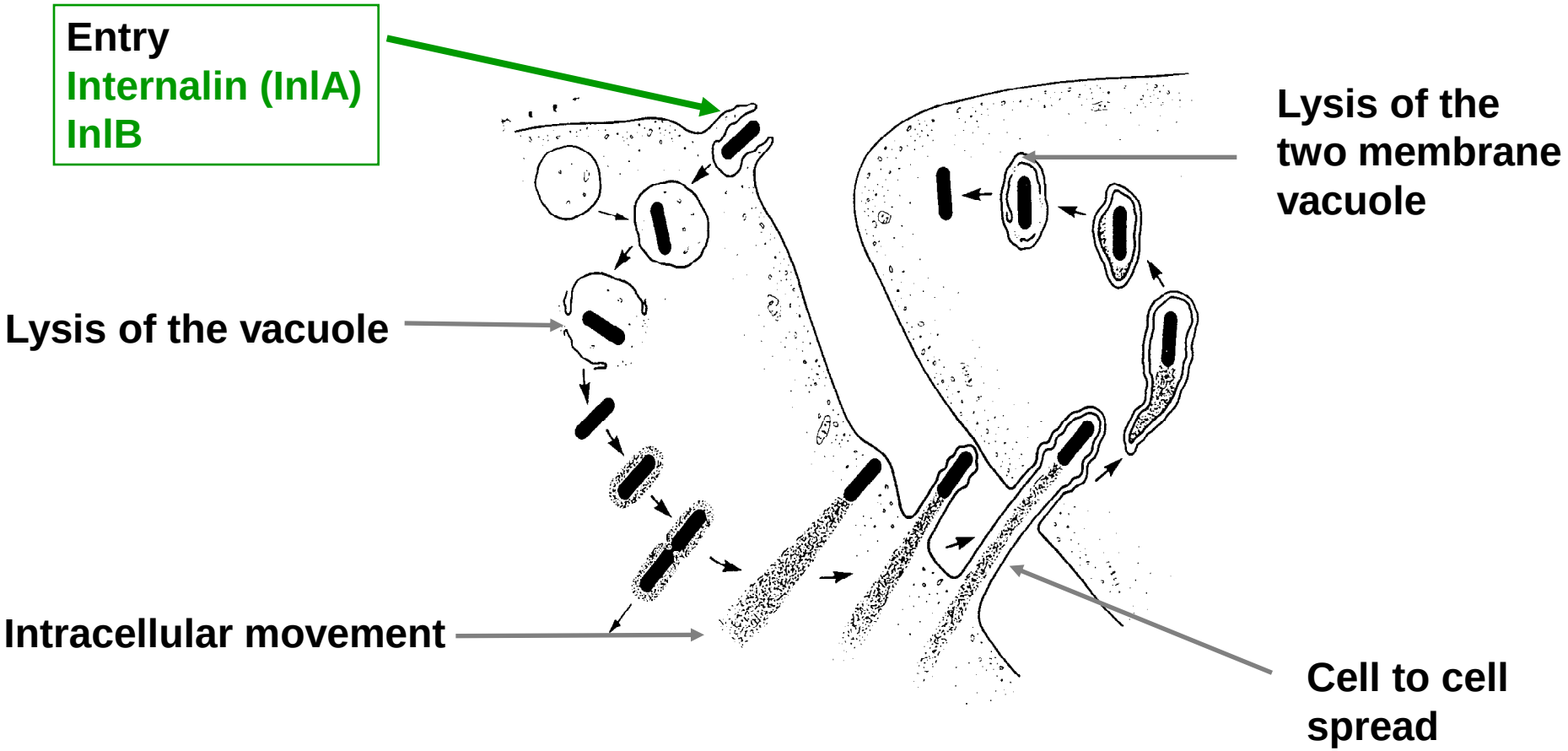
Entry into non phagocytic cells





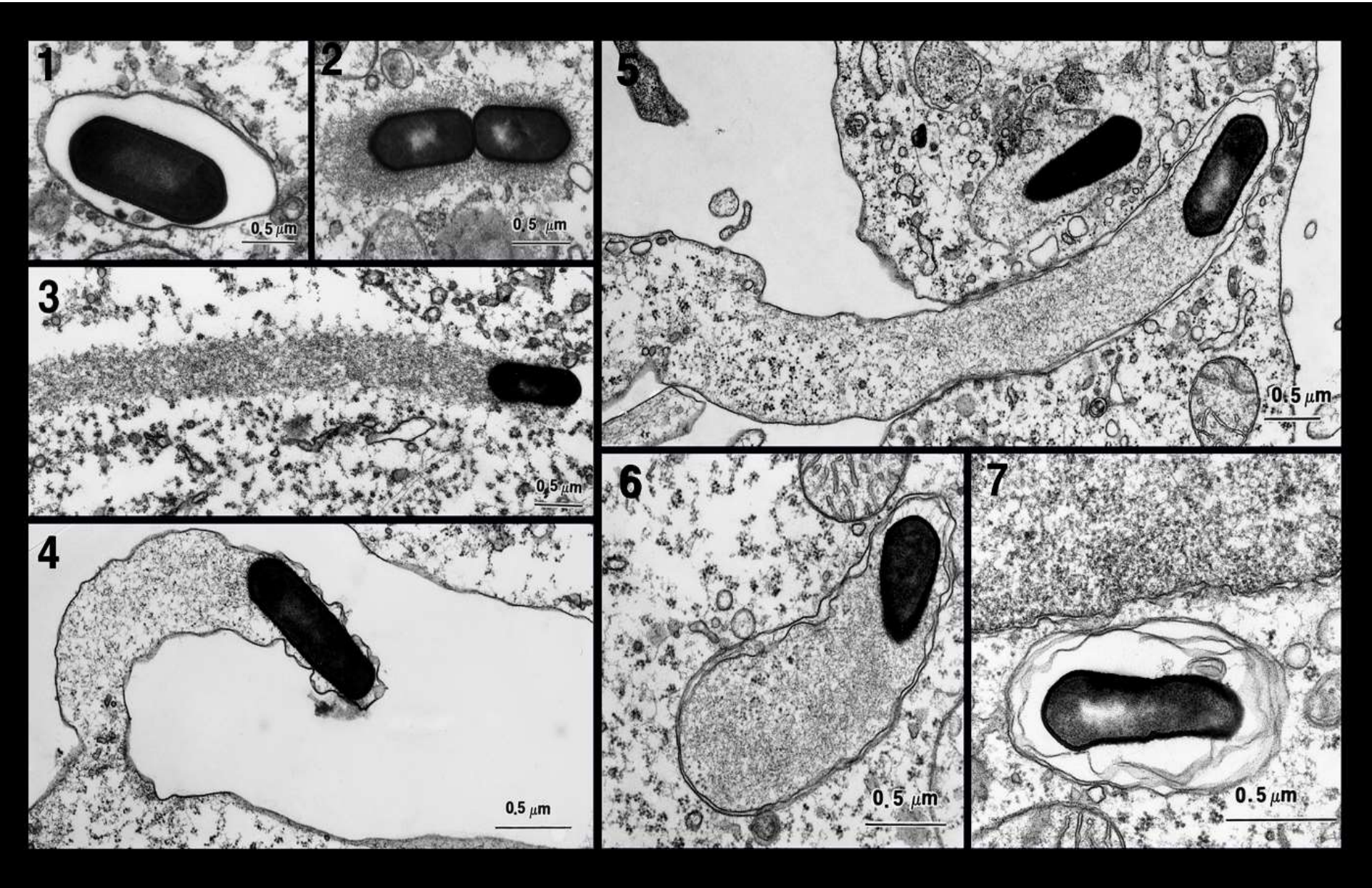
Successive steps of the cell infectious process

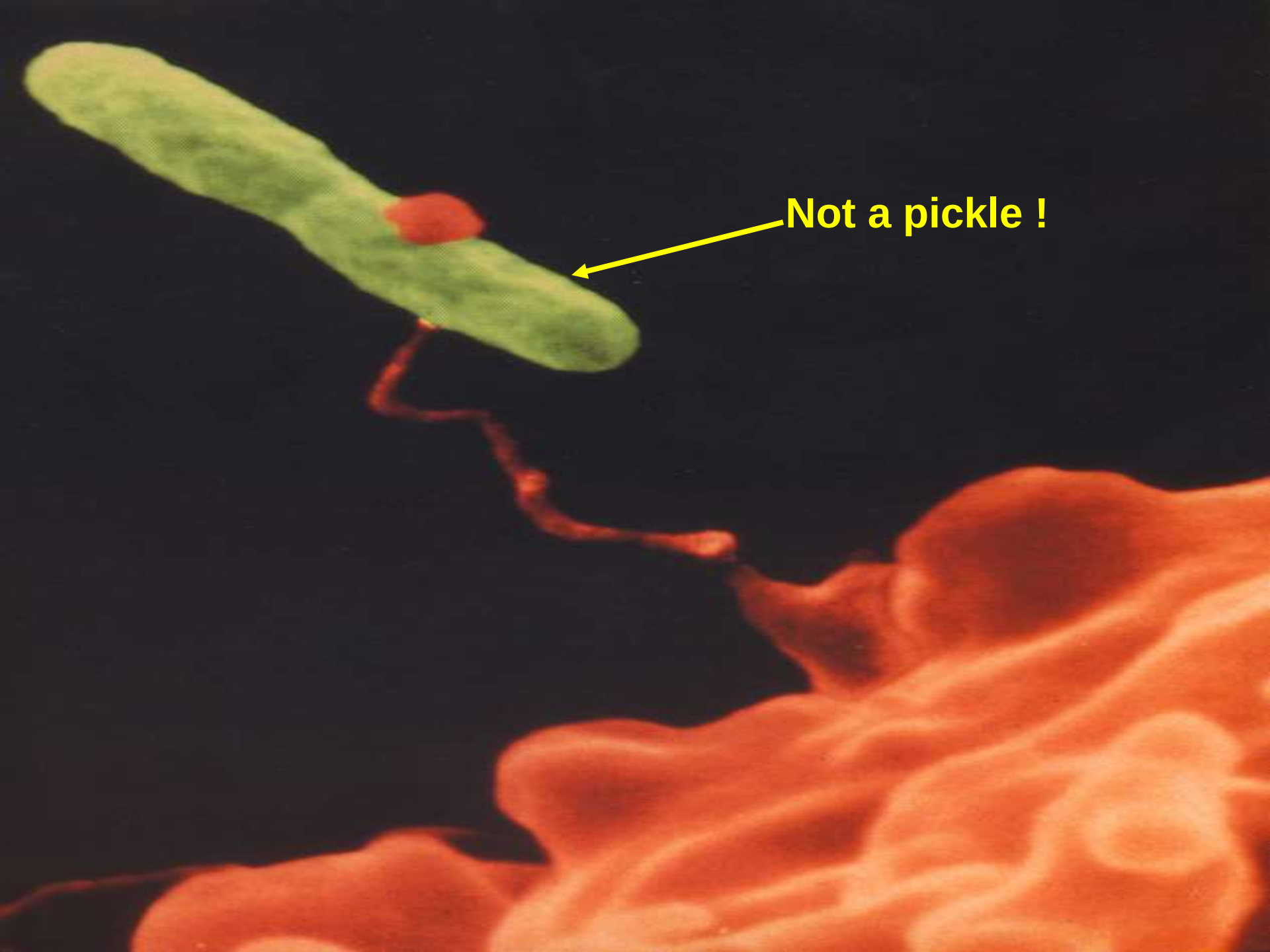
bacterial factors

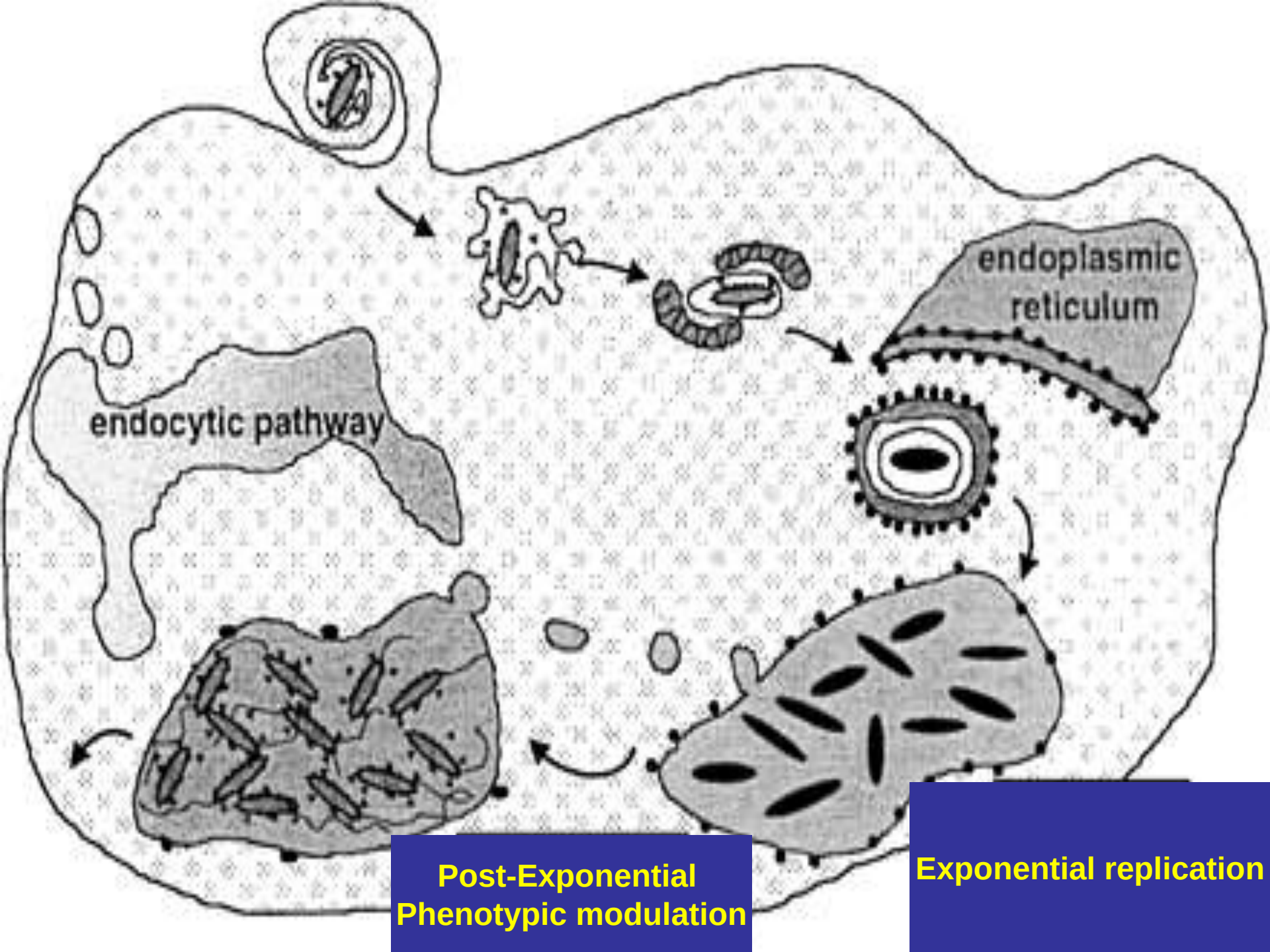


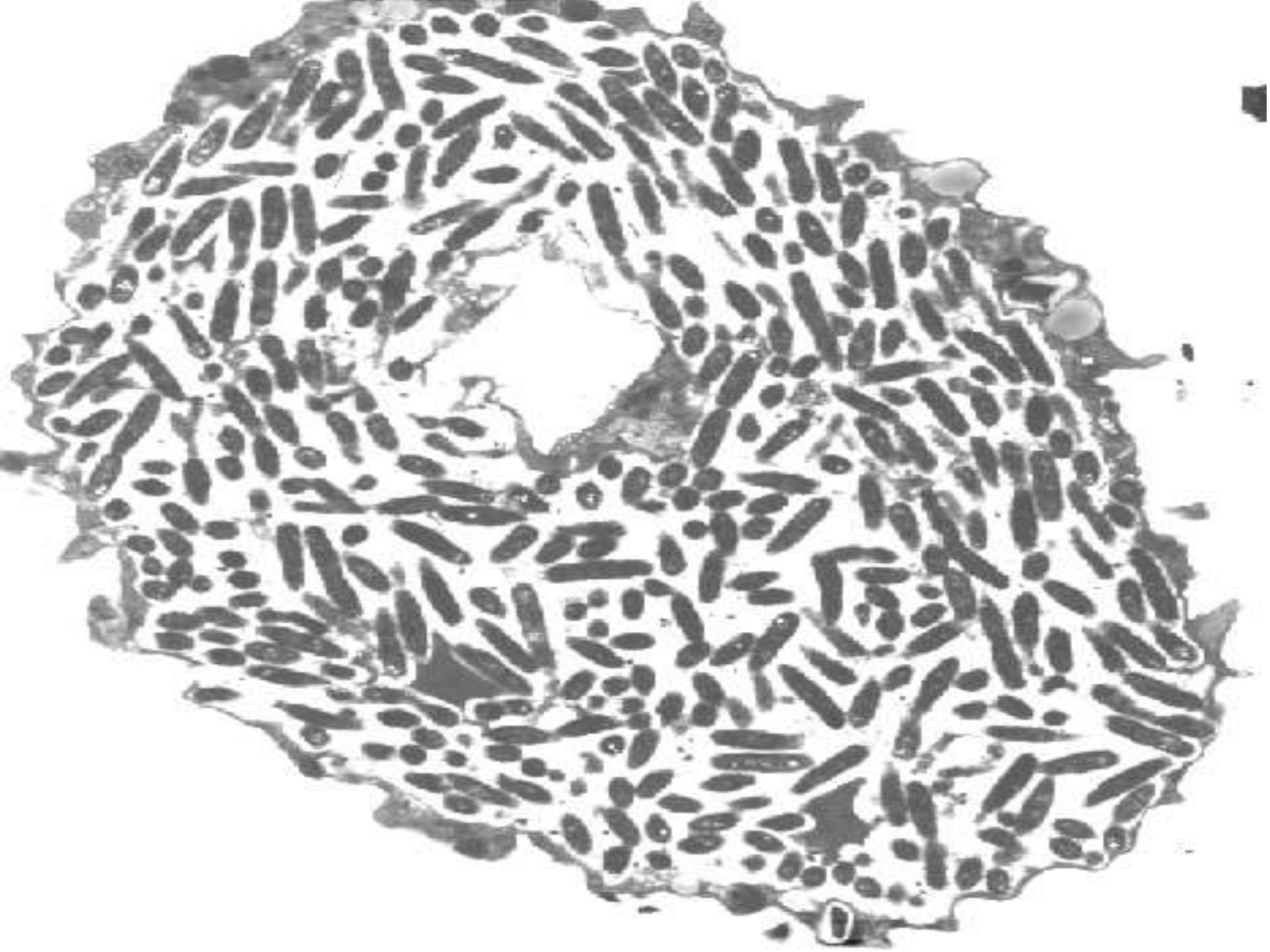
Adapted from L.G. Tilney and D.A. Portnoy J.Cell.Biol. (1989) 109, 1597

Successive steps of the cell infectious process

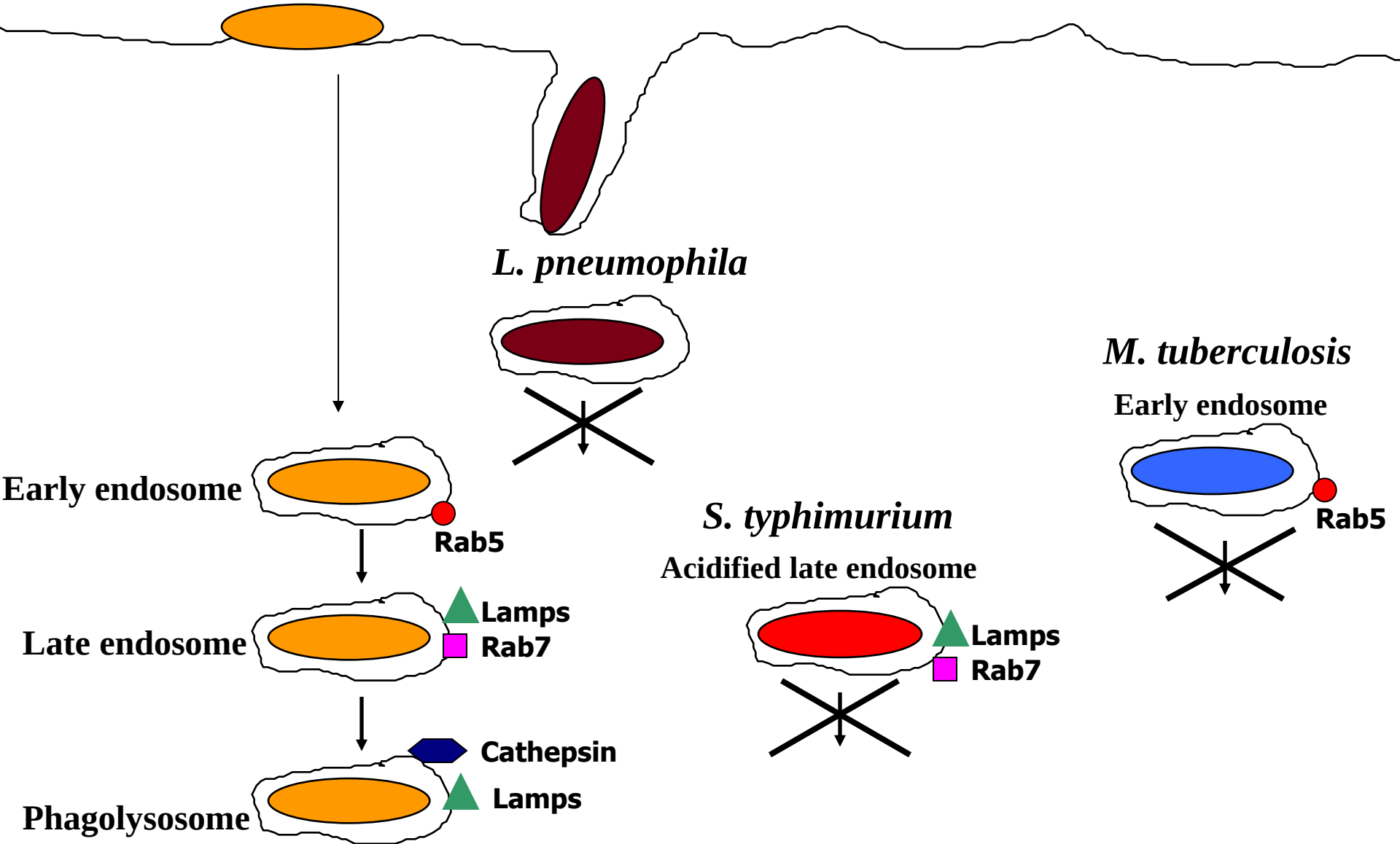


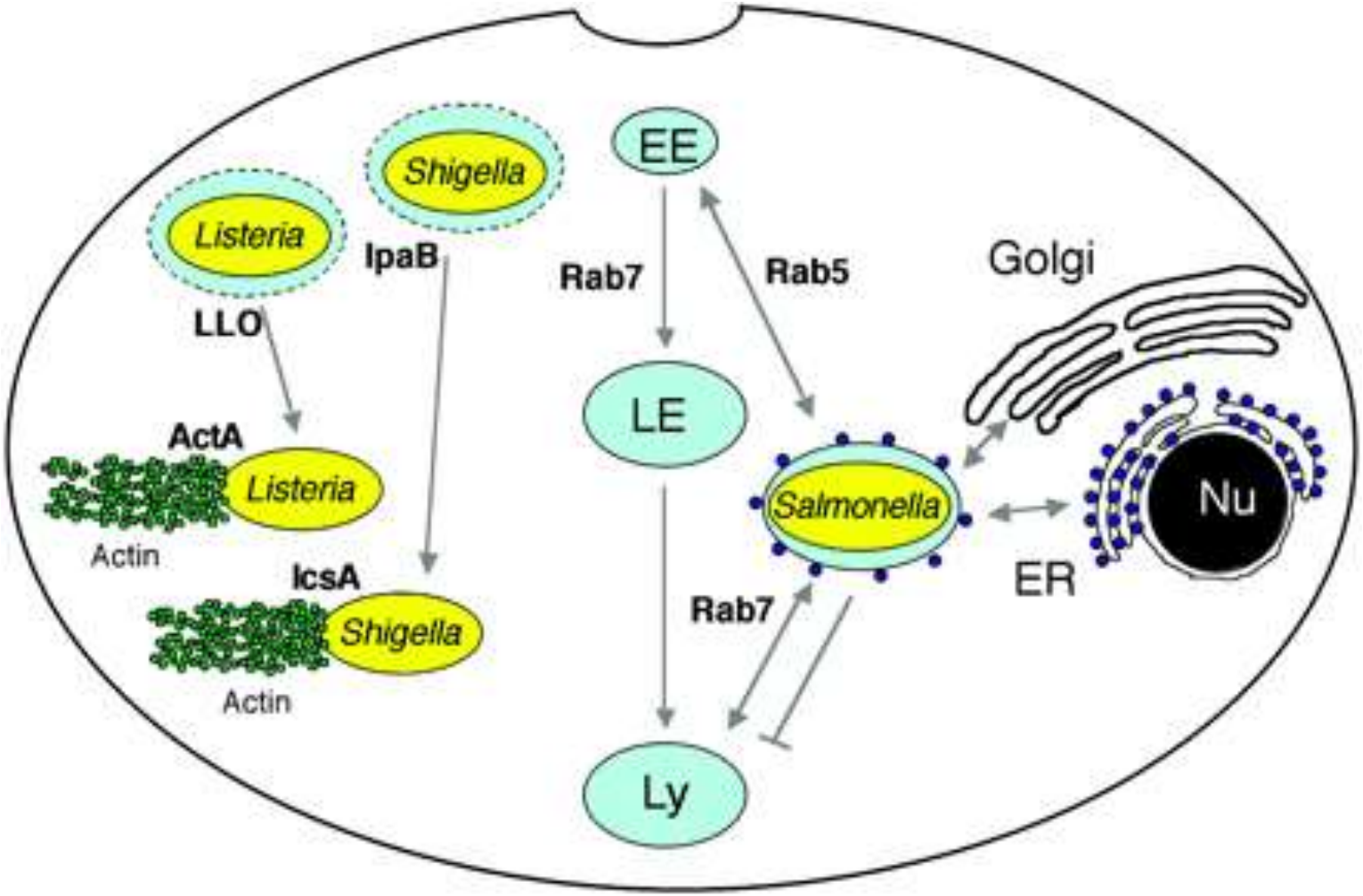

Not a pickle !


endocytic pathway
endoplasmic reticulum
Post-Exponential
Phenotypic modulation
Exponential replication



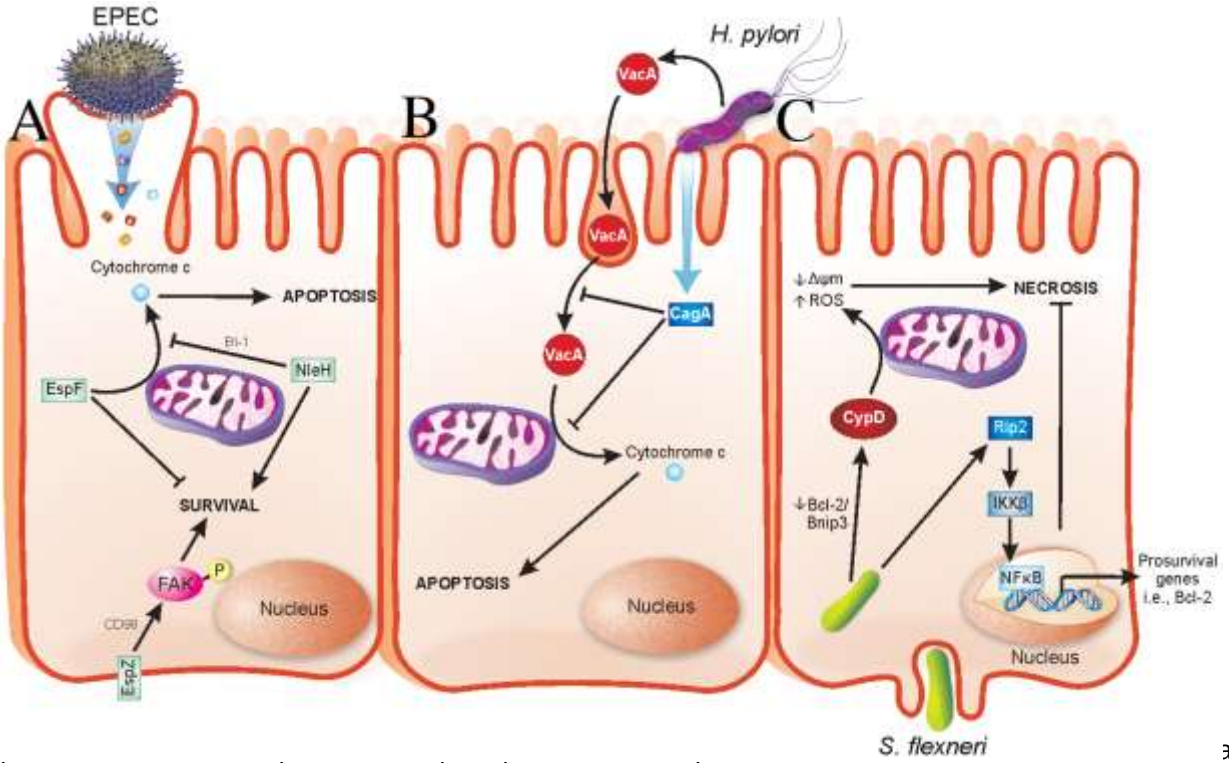
Vacuolar intracellular pathogens





Intracellular life-styles. Schematic representation of the *Salmonella*-containing vacuole (see text). *Listeria* and *Shigella* lyse the vacuole and move in the cytosol by an actin-based motility process mediated by ActA or IcsA/VirG, which interact with Arp2/3 or N-WASP and Arp2/3, respectively. EE: early endosome; LE: late endosome; Ly: lysosome; ER: endoplasmic reticulum

Strategies evolved by bacterial pathogens to restrain virulence



(A) EPEC injects effector . ase of cytochrome *c* into the host cell cytosol, which results in apoptotic death of the host cells. NleH interacts with Bax inhibitor-1 (BI-1), which inhibits release of cytochrome *c* from mitochondria. EspZ interacts with CD98, which then stimulates phosphorylation of focal adhesion kinase (FAK) to promote survival. Localization of NleH and EspZ in host cells during early stages of EPEC infection is unclear and has been portrayed as shown for simplicity. (B) *H. pylori* injects virulence factors into gastric epithelial cells via a type IV secretion system in addition to secreting soluble toxins. VacA is an *H. pylori*–secreted toxin that enters cells by pinocytosis and penetrates intracellular endosome trafficking pathways. VacA causes release of cytochrome *c* from mitochondria of infected cells, thus mediating host cell apoptosis. CagA is a T4S virulence factor, which prevents both pinocytosis/trafficking and cytochrome *c* release by VacA. Functions of CagA are dependent on its phosphorylation state, not depicted here. (C) *S. flexneri* enters IECs from their basolateral surface and then resides in the cell cytoplasm. Prosurvival signaling is initiated by Nod1 activation of Rip2 signaling, which terminates in expression of pro-survival genes, including Bcl-2, via NFκB activation and nuclear translocation. Conversely, *S. flexneri* facilitates a decrease in the Bcl-2/Bnip3 ratio, which leads to CypD-mediated disruption of mitochondria and oxidative stress-induced necrotic cell death.

doi:10.1371/journal.ppat.1001057.g001