Pathogenicity and virulence of microrganisms

The Nature of Bacterial Host-Parasite Relationships

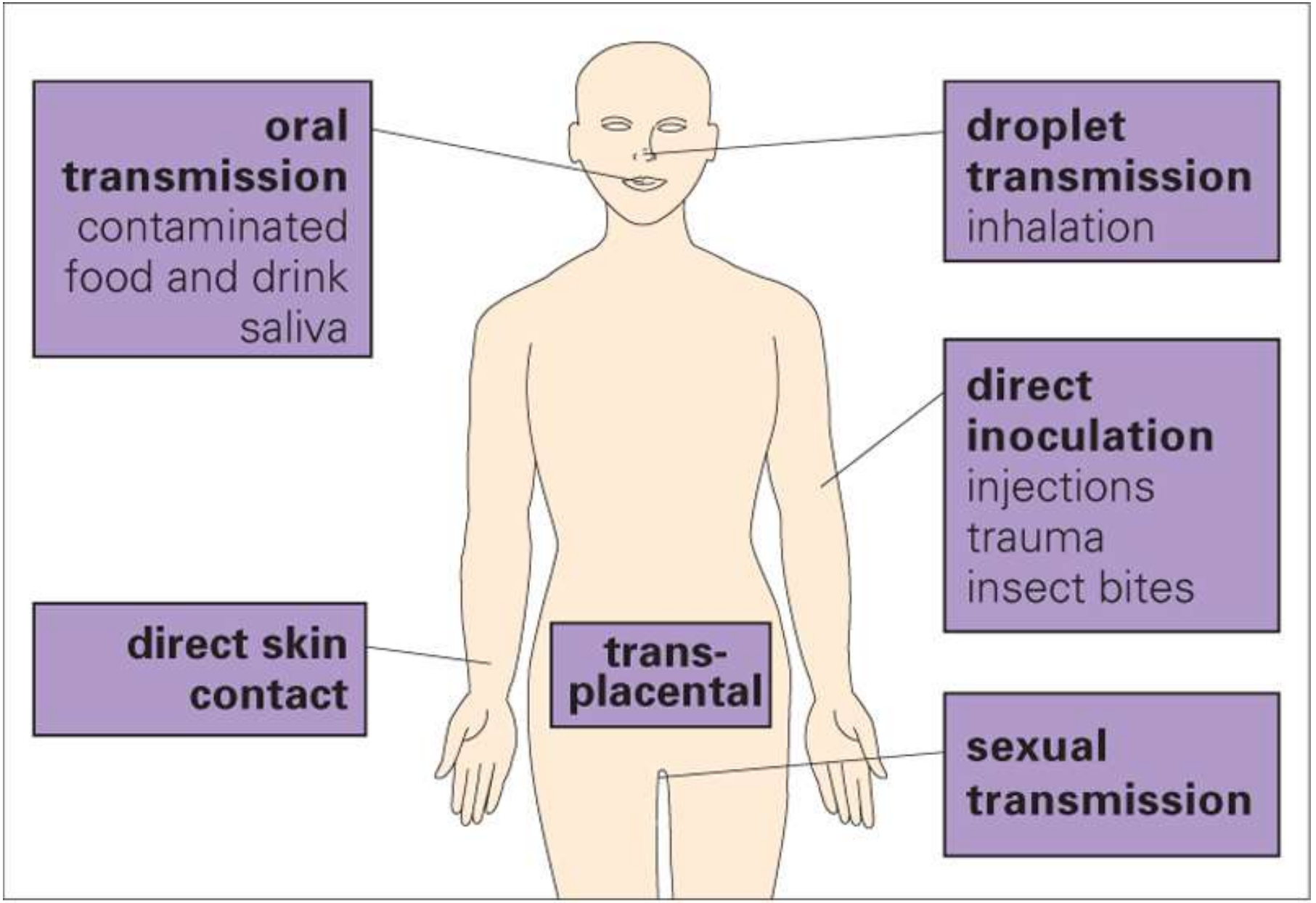
- Bacteria are consistently associated with the body surfaces of animals (Bacteria > Animal cells
- Peaceful association with an animal = normal flora (symbiosis or **indigenous microbiota**)
- Harmful association = infection and disease
  - Who is guilty?
    - The bug or the host = not all that clear….
    - Depends on the action of the bug and reaction of the host…

Table 3 | **Different types of microbial damage**

Type of damage	Mechanism	Example
Molecular	Mutation	Retroviral integration
	Pump dysfunction	Cholera toxin
	Antigenic mimicry	Rheumatic fever
	Ig cleavage	IgA protease
Cellular	Necrosis	Herpes encephalitis
	Oncogenesis	EBV-related lymphoma
	Morphological	*Bacillus anthracis* oedema toxin
	Apoptosis	Shigellosis
Tissue	Inflammation	Schistosomiasis
	Malignancy	Hepatocellular carcinoma
	Fibrosis	Mediastinal fibrosis
	Cytokine dysregulation	Septic shock
Organ	Ductal obstruction	Worm infestation
	Psychosis	Rheumatic fever
Organism	Behavioural	Loss of predator fear, hydrophobia

EBV, Epstein–Barr virus; Ig, immunoglobulin.

Routes of 'infection'



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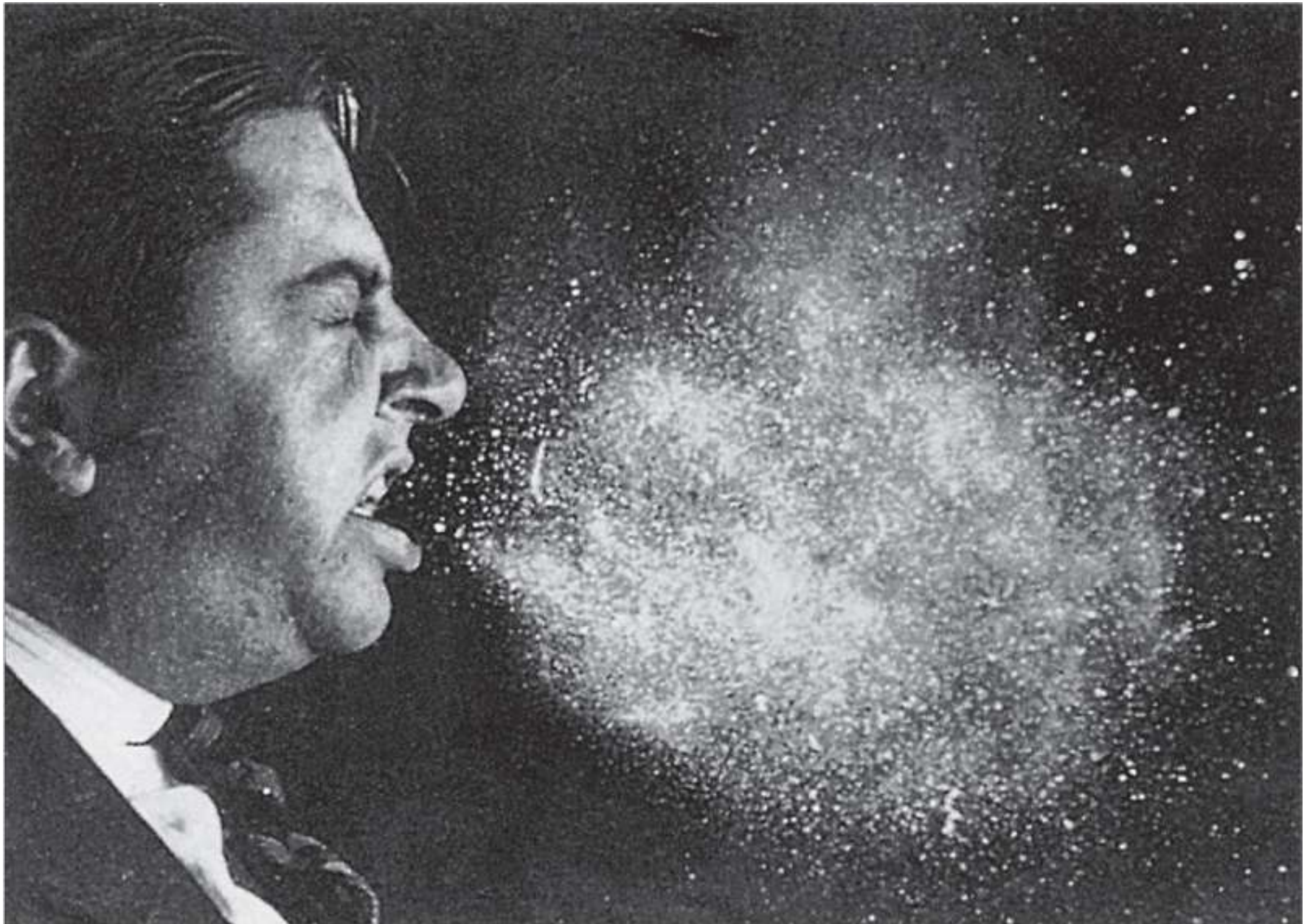
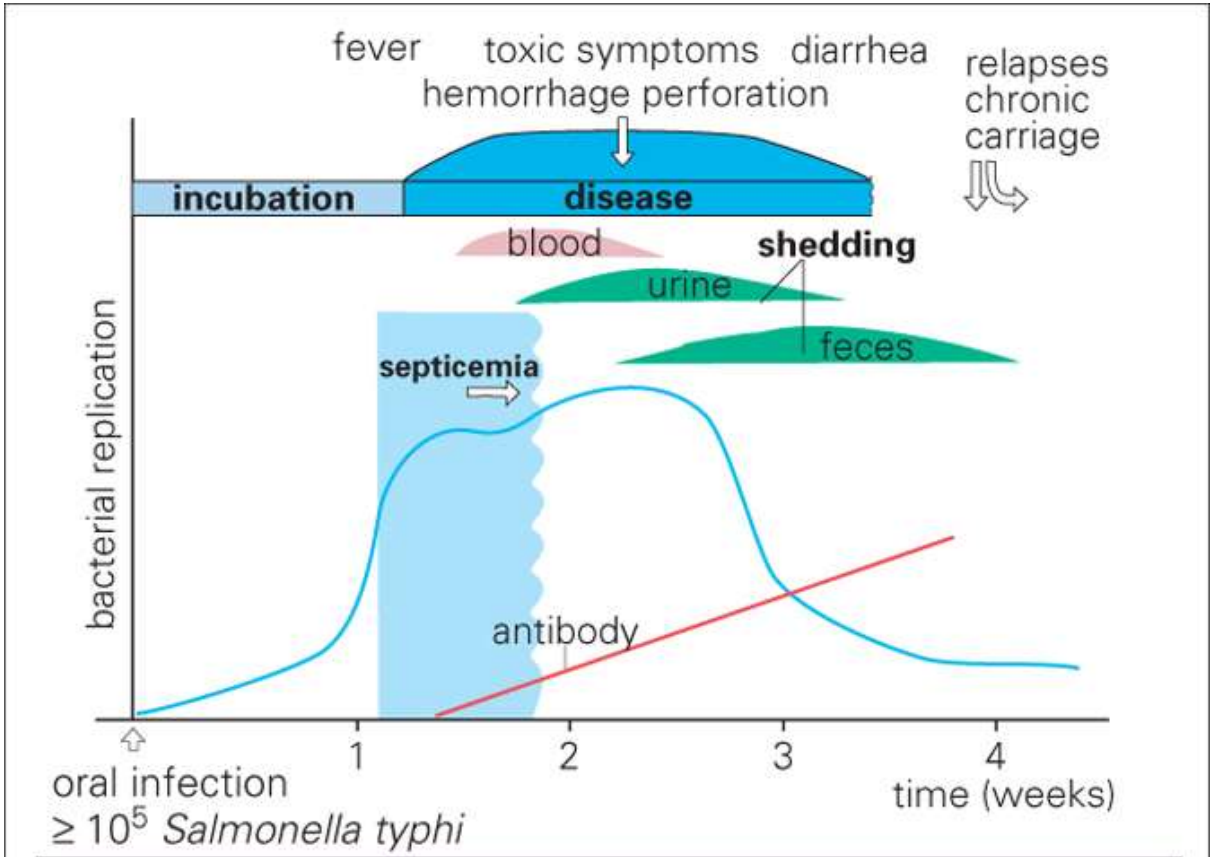
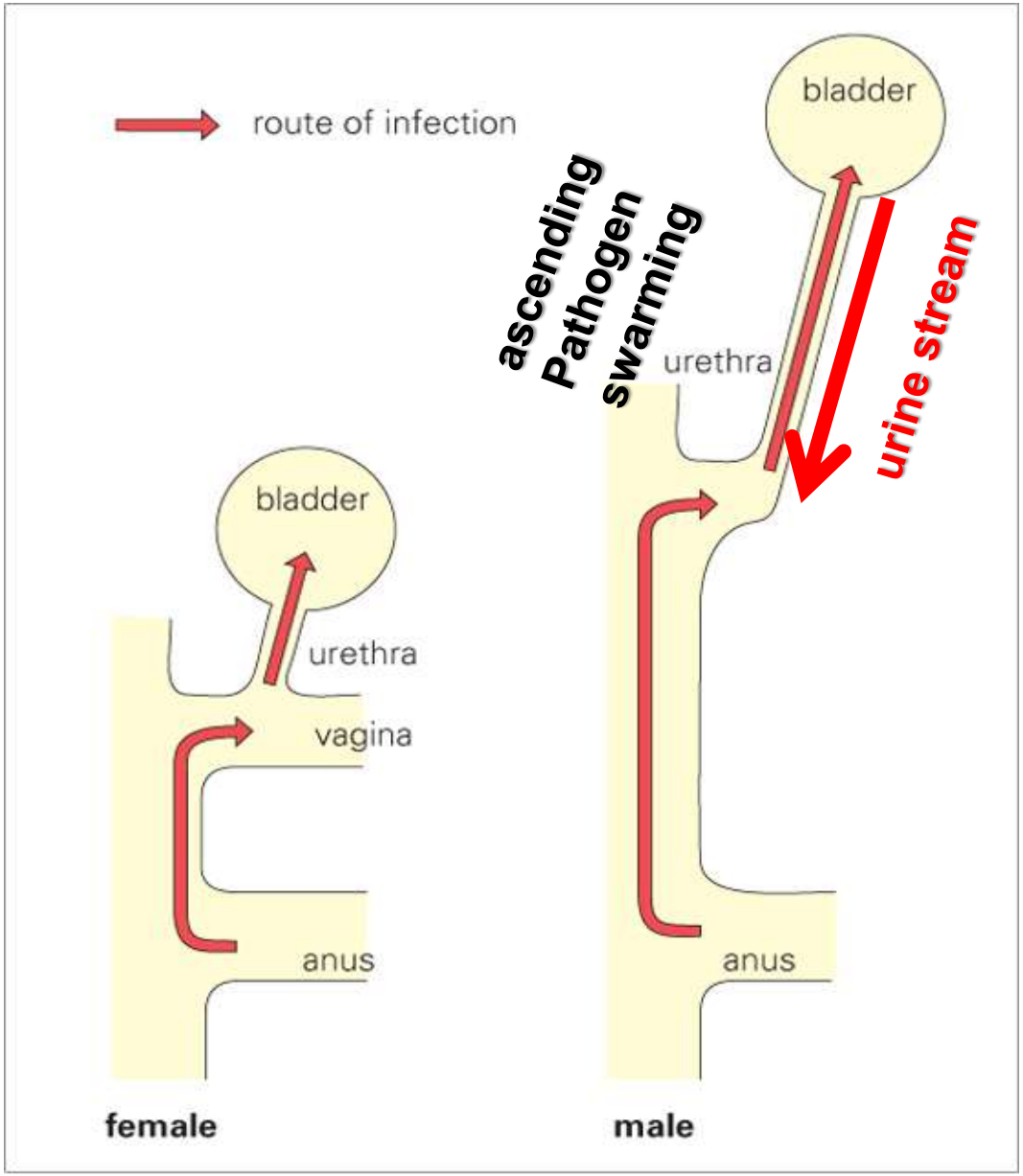


Salmonella: Classics



week	invasion of:
1	small intestine, lymphatics, spread to blood (bacteremia)
2	reticuloendothelial system (liver, spleen and bone marrow macrophages)
3	kidney, gall bladder, gut (Peyer's patches)

Why 50% of females experience an urinary tract infection in their life



The potentially uropathogenic E. coli from feces have much harder time to ascend the longer urethra of penis in males than in females

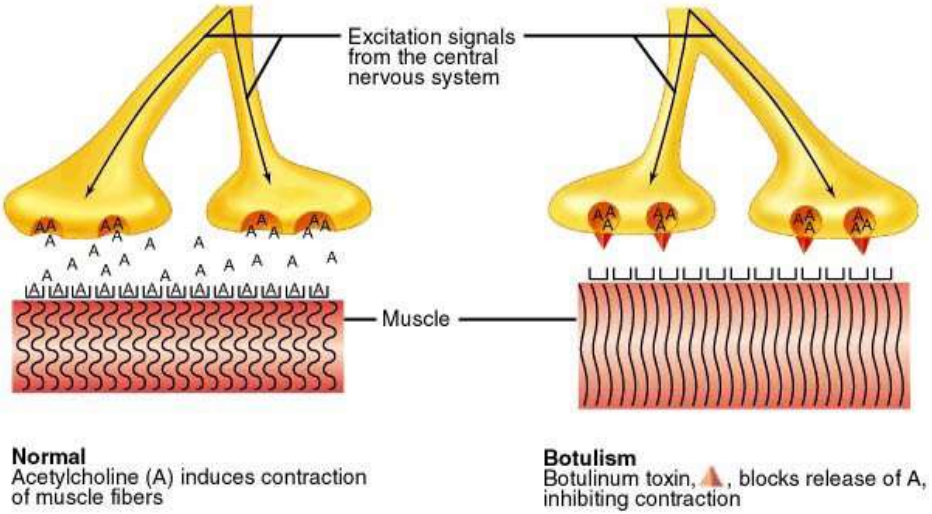
They have to resist the washing/cleaning action of very fast urine stream

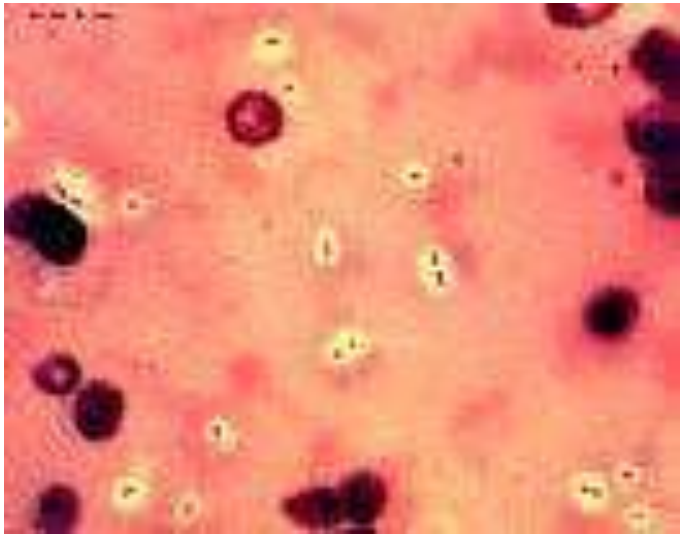
Basic terms in bacteriology

- **colonization** – persistence in a particular body site of bacteria that does not cause disease (resident microflora. e.g. commensal *E. coli* in the gut)
- **asymptomatic carriage** – if the host defences are adequate, a person can be infected without any sign of symptoms (→ spreading the infection, e.g. *N. meningitis* in nasopharynx)
- **infection** – pathogenic bacterium capable of causing disease becomes established in the body, e.g. latent *M. tuberculosis* infection - in alveolar macrophages
- **disease** – infection that produces symptoms / cough in tuberculosis or pertussis, fever, inflammation, diarrhea etc

Inavsinevess and toxogenicity

- **Invasinevess:** **Ability of bacteria to infiltrate sterile tissues of the host organism and then enforce spread to a new host by causing damage**
- **Toxogenicity:** **Ability of bacteria to produce toxic substances toxic for host organism**

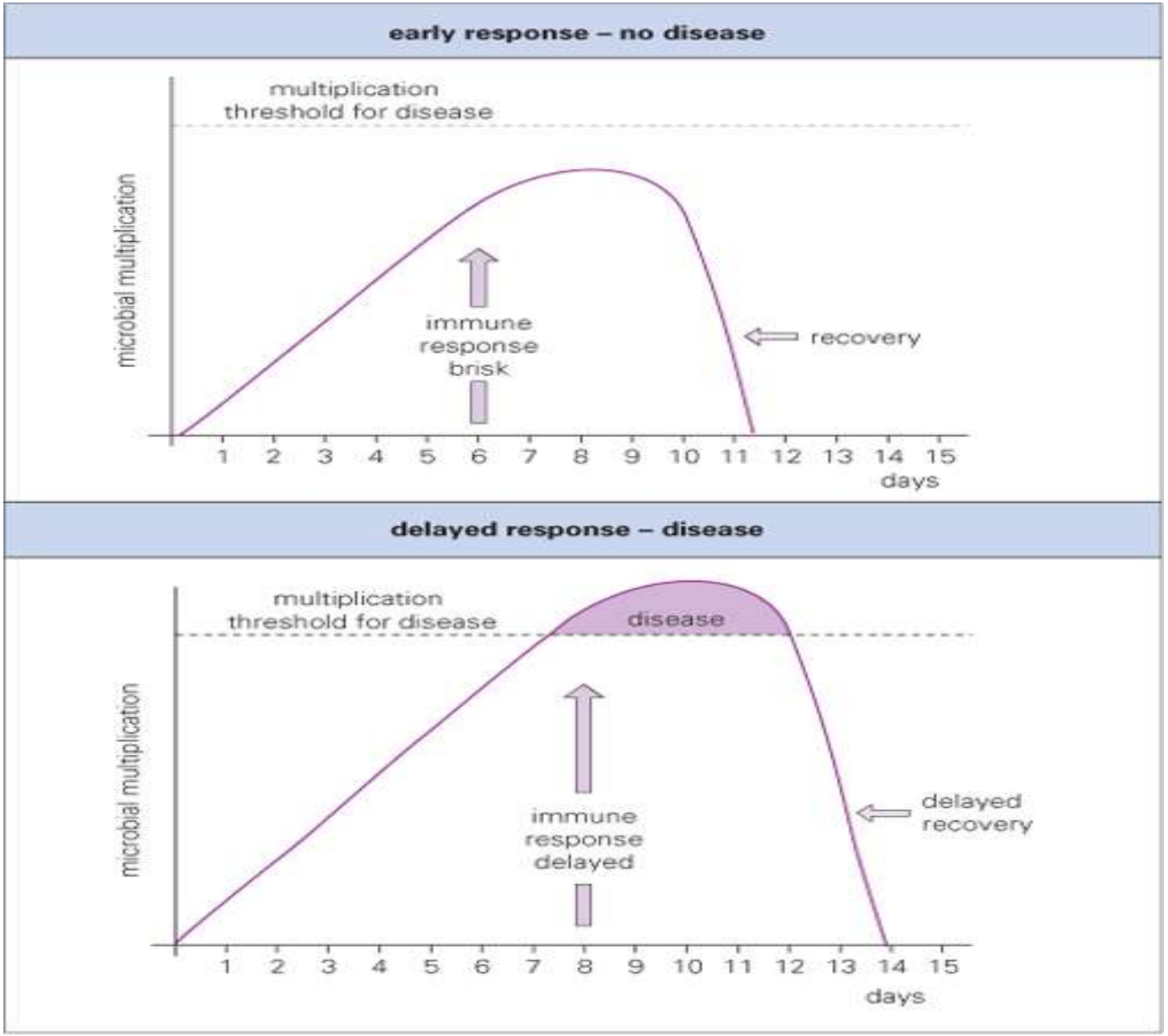




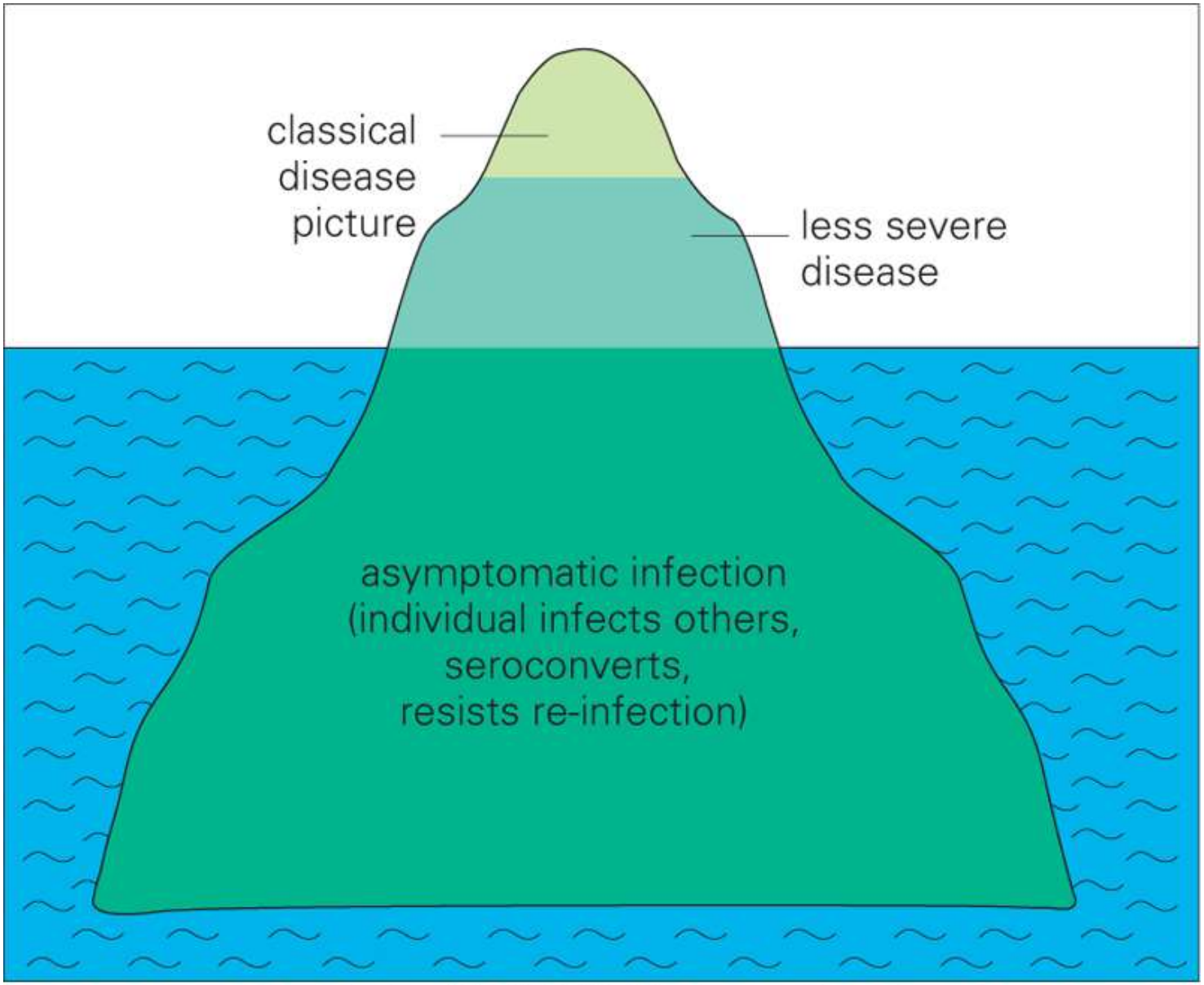
Clostridium botulinum – toxin
noninvasive

Streptococcus pneumoniae invasive

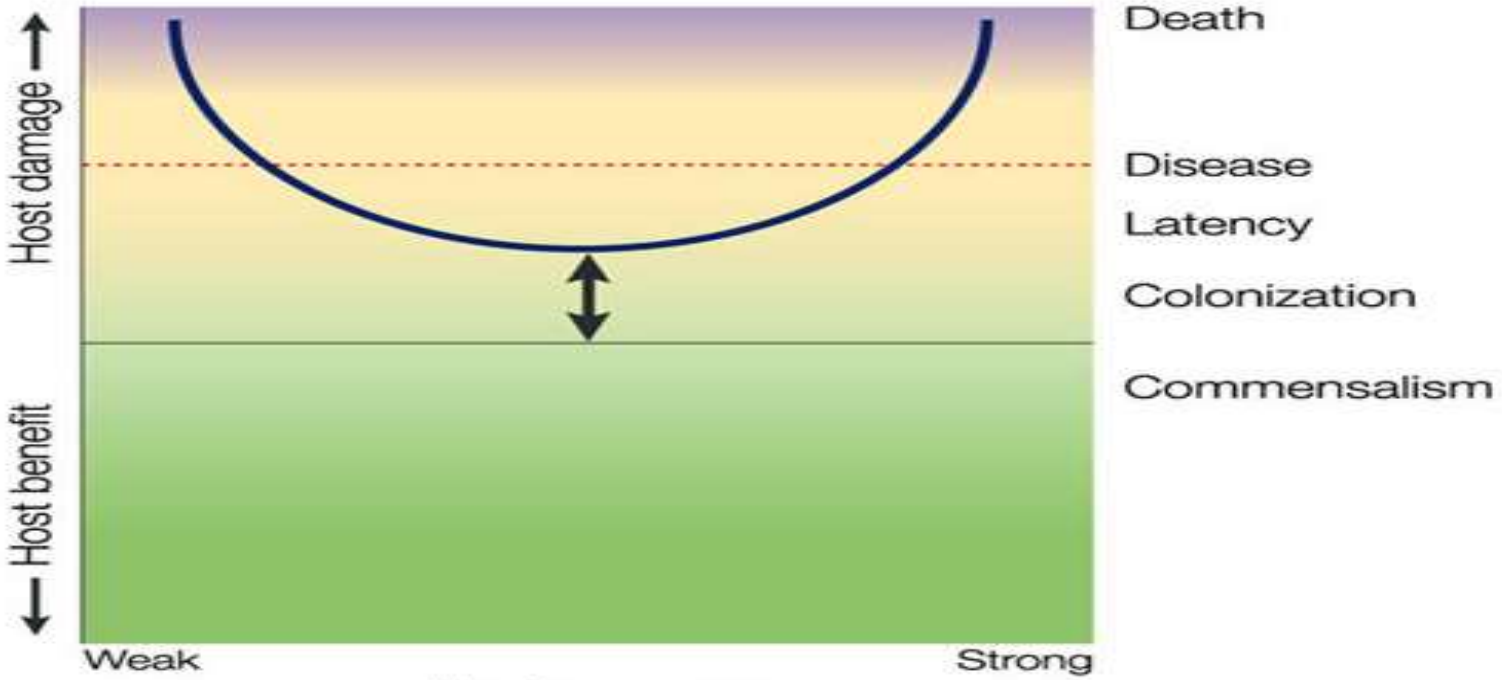
The historical view of infectious disease race …



Disease is the tip of the iceberg



It is more complicated, inadequate reaction can make the host really sick…



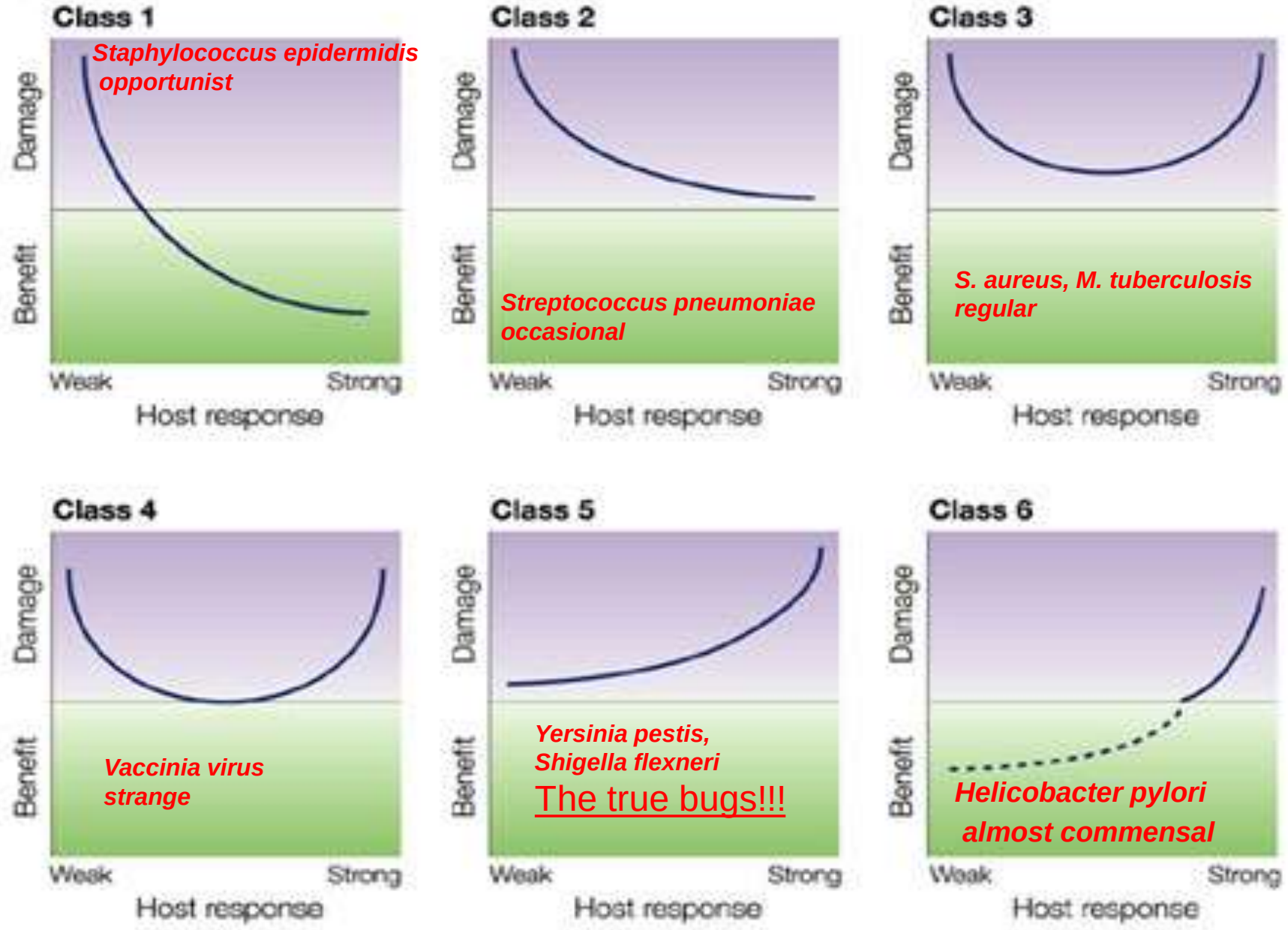
Basic parabolic curve of the damage-response framework.

The arrow indicates that the position of the curve is variable, and depends on the particular host–microorganism interaction.

Classes of pathogens according to the damage-response framework:

1	**Only cause disease if host response is weak**	**e.g. *Staphylococcus epidermidis - opportunist***
2	**Cause disease at weak or normal host response**	**e.g. *Streptococcus pneumoniae* - *occasional***
3	**Can cause disease despite appropriate response**	**e.g. *M. tuberculosis – regular***
4	**disease at very weak or very strong response**	**e.g. *Vaccinia virus – strange***
5	**Cause disease always – need it for transmission**	**e.g. Yersinia pestis, Shigella flexneri – true**
6	**Cause disease only if response is strong**	**e.g. *Helicobacter pylori – almost commensal***

Classes of pathogens according to damage caused in function of host response



Class 1 microorganisms can reproduce in normal hosts, whereby the host response prevents significant damage and commensalism confers a benefit to the host. Examples of pathogens classified by the six damage-response curves are listed in Table 2. We have previously suggested that *Helicobacter pylori* should be placed in Class 6 (Ref. 2). The dashed line below the *x*-axis in the panel for Class 6 reflects recent evidence that *H. pylori* can confer a benefit in certain hosts, based on the observation of an inverse correlation between colonization with this organism and reflux oesophagitis27. Modified with permission from Ref. 1 © (1999) AmericanSociety for Microbiology. **b** | The figure denotes a situation where a microbial factor is entirely responsible for host damage — for example, a toxin that causes damage irrespective of the host response because toxin action is so rapid and/or the amount of toxin is insufficient to trigger an immune response. Previously, we have proposed that toxin-producing microorganisms are a variant of Class 3 where the curve is flat at both ends1, but here we suggest that this type of interaction might be unique and warrants a separate panel. As shown here, the damage-response classification scheme is flexible and makes it possible to postulate the existence of pathogens for which there are no known examples at present. Such pathogens could be recognized in the future as 'emerging' pathogens as shown in **c** and **d**. **c** | The Class 4 curve is extended below the *x*-axis. Such a theoretical microorganism would be a commensal in the setting of intermediate host responses, but pathogenic in hosts with either weak or strong responses. **d** | The inverted parabola represents a putative host–microorganism interaction that induces damage over a narrow and limited range of responses, but not in the presence of either strong or weak host responses. One example of such a phenomenon would be an antibody response to a hypothetical microorganism, whereby host damage is caused by antigen–antibody complexes. Although we cannot think of a specific microorganism that fits this description at this time, examples of this type of host damage are the host–microorganism interactions characterized by the Herxheimer reaction following treatment of syphilis, the similar reaction that can occur after the initiation of therapy for *Pneumocystis carinii* pneumonia, and serum sickness following the injection of foreign protein.

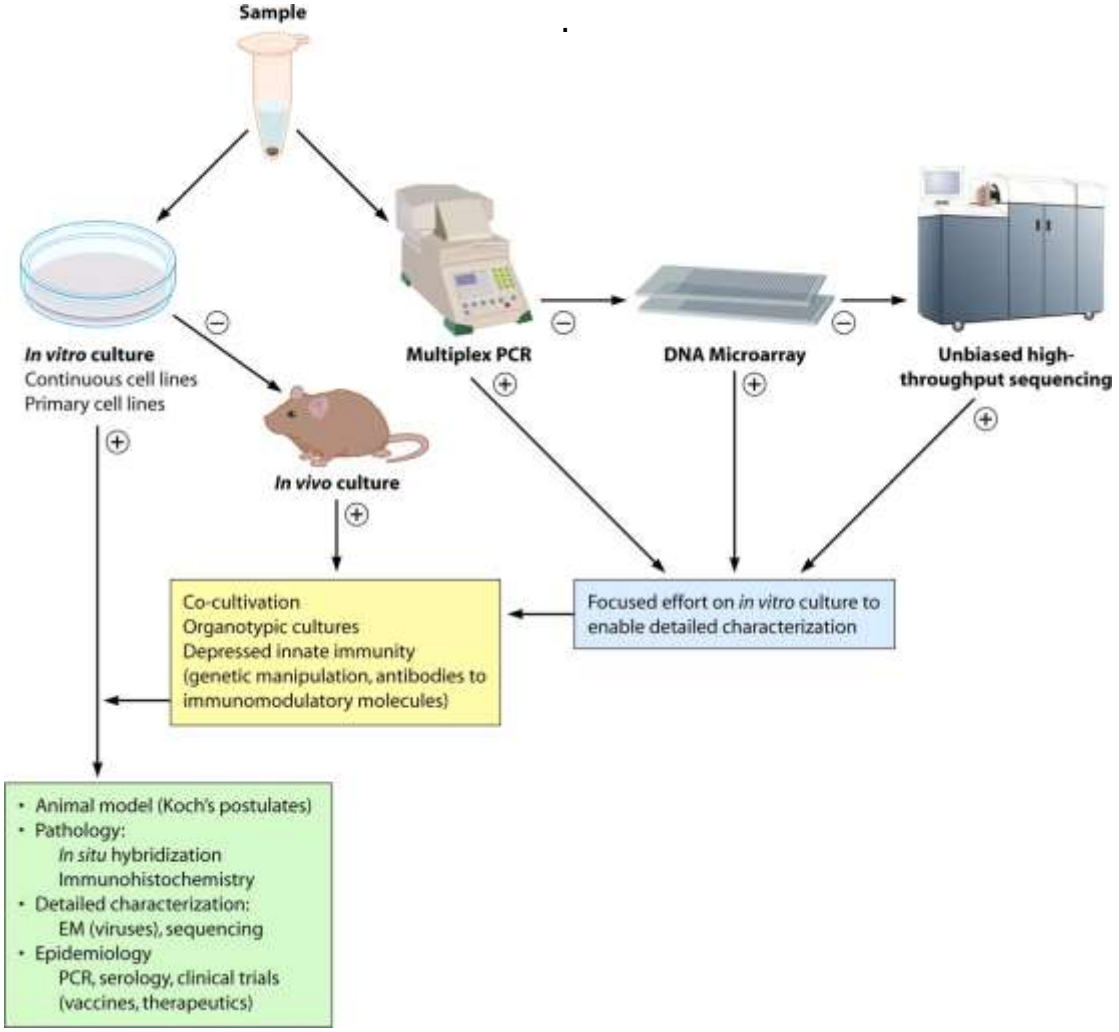
Term	Definition(s) from the literature	Proposed definition
Pathogen	A microbe capable of causing disease growing in living tissue and causing disease depending on capacity to replicate and persist on or within another species by actively overcoming barriers parasite capable of producing some disturbance	**capable of causing host damage = classical pathogens and opportunistic pathogens; host damage immune response …**
Pathogenicity	The capacity of a microbe to produce disease	**The capacity to cause damage in a host**
Virulence	Degree of pathogenicity Virulence 1/resistance Strength of the pathogenic activity Relative capacity to overcome available defenses Disease severity as assessed by reductions in host fitness following infection Percent of death per infection A synonym for pathogenicity Property of invasive power capacity of a microorganism to infect or damage a host Relative capacity to enter and multiply in a given host	**relative capacity to cause damage in a host**
Virulence factor (or determinant)	A component of a pathogen that when deleted specifically impairs virulence but not viability (33) products that permit a pathogen to cause disease	**component of a pathogen that damages the host; e.g. components essential for viability including modulins**

Evolution of Koch`s postulates of causality between infection by an agent agent and disease

Koch (1890)	Rivers (1936)	Fredericks and Relman (1996)
		However, many agents cannot be cultivated…
		There is a very thin line between a commensal and pathogen…
The bacterium should be found in all people with the disease, and the bacterium or its products should be found in parts of the body affected by the disease	A virus must be associated with a disease with a degree of regularity	Candidate **sequences should be present in most cases of disease** and at sites of disease pathology
The bacterium **should be isolated from the lesions** of an infected person and maintained **in pure culture**	The association cannot be incidental	Few or **no sequences** should be **present in host or tissue without disease**
The pure culture**, when inoculated into** a susceptible human volunteer or experimental **animal**, should produce the symptoms of the disease	Rivers predicted that methods for proving causal relationships would evolve with improvements in technology; at the time of his writing, he invoked seroconversion and experimental inoculations but acknowledged the limitations of both	Sequences should diminish in frequency with resolution of disease and increase with relapse
The same bacterium should be reisolated in pure culture from the intentionally infected animal or human		Sequences should be present prior to the onset of disease

Staged strategy for pathogen discovery and proof of causation:

In the molecular era of pathogen discovery, culture and molecular methods are pursued in parallel until an agent is detected, isolated, and characterized. +, positive result; –, negative result.



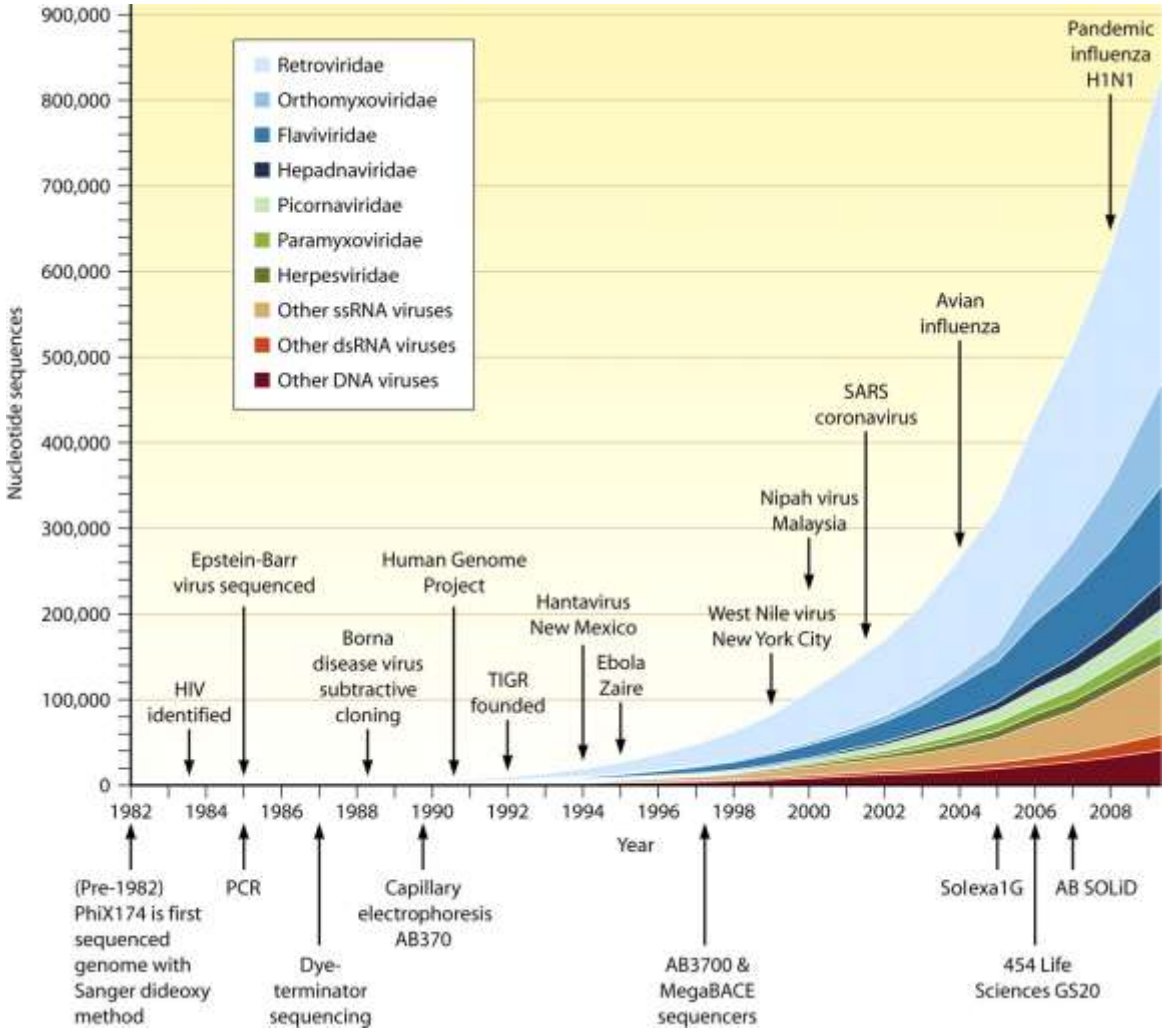
Lipkin, W. I.. 2010. Microbiol. Mol. Biol. Rev. 74(3):363-377

Proving implication of an infectious agent: a four-step process

- ***Step 1. Detect an agent or its footprints in association with disease***
  - **Isolate and propagate the agent** in culture or in animal models
  - **Image the agent or its gene products** *in situ* by using immunological (immunohistochemistry) or molecular (*in situ* hybridization) methods
  - **Demonstrate an agent-specific adaptive immune response** (e.g., IgM or an increase in IgG titer, cell-mediated immunity)
- ***Step 2. Provide a plausible mechanism for an explanation of disease***
  - **Demonstrate the presence of the agent**, a gene product of the agent (e.g., a toxin), or a host product attributable to infection (e.g., a cross-reactive antibody or cytotoxic T cell) at site(s) of pathology
  - **Demonstrate that the** same or a similar **agent can induce disease in a model system**
- ***Step 3. Demonstrate that modulation of the agent concentration, or of a factor that can be attributed to the presence of the agent (e.g., an antibody), influences the presence or severity of disease***

- ***Step 4. Demonstrate that preventing infection prevents disease***

Growth of the viral sequence database mapped to seminal discoveries and improvements in sequencing technology



Lipkin, W. I.. 2010. Microbiol. Mol. Biol. Rev. 74(3):363-377

Virulence (pathogenicity)

- ability of a bacterium to cause infection

- **<u>Virulence assessment:</u>**

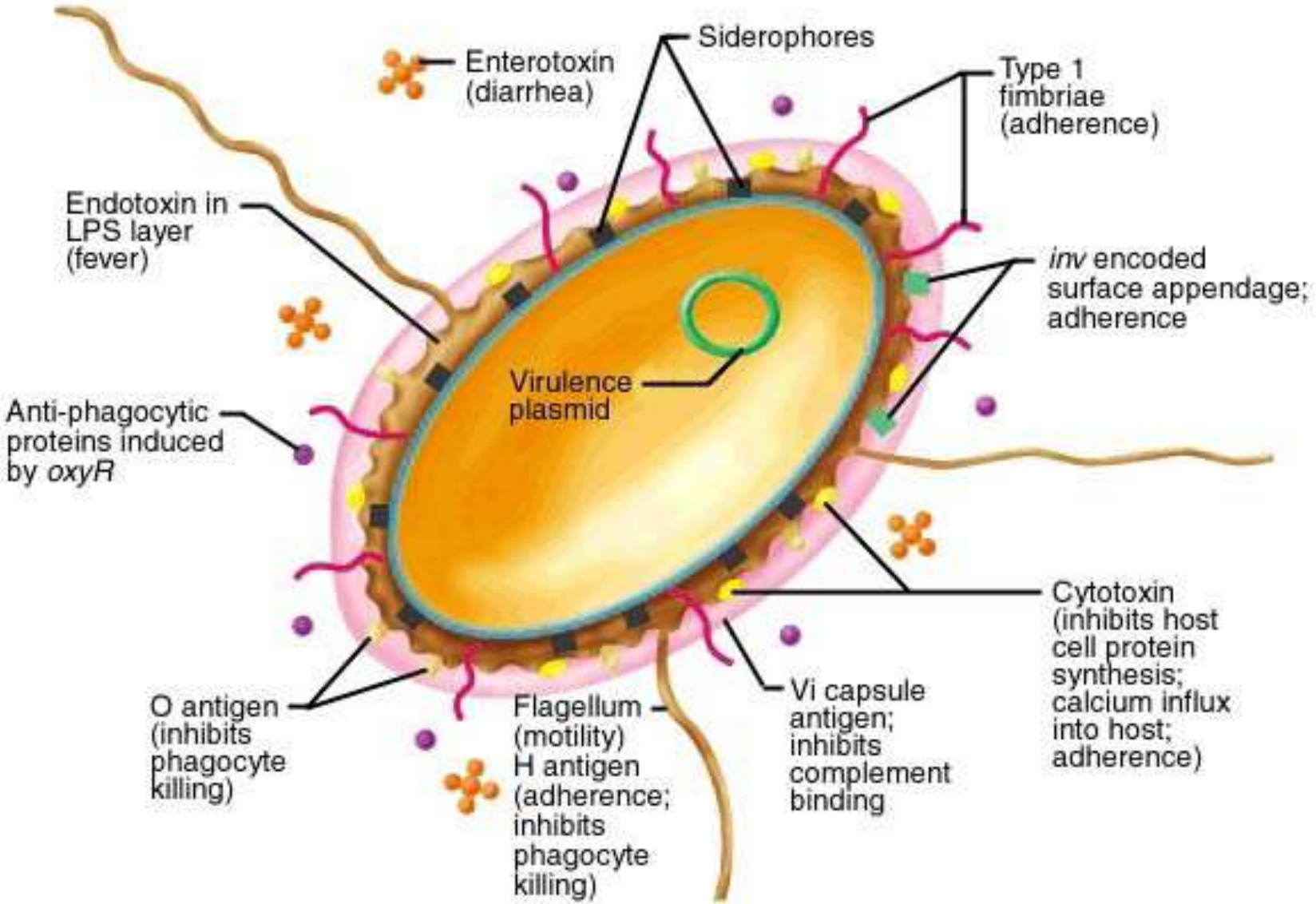
  LD_{50}
  (lethal dosis – number of bacterial cells/animal → 50 % killed)

  ID_{50}
  (infection dosis – number of bacterial cells/animal → 50 % infected

Defense	Site of action	Function
Transferrin	Blood/tissue	Limits iron availability
PMNs	Blood/tissue (attracted to infection site)	Ingest and kill bacteria
Monocytes	Blood (attracted to infection site)	Weakly phagocytic, produce cytokines
Macrophages	Tissue (especially lymph nodes, spleen, liver, lungs)	Actively phagocytic; produce cytokines; present antigens to T cells on MHC I (intracellular pathogens) or MHC II (extracellular pathogens)
Complement	Blood/tissue (inactive unless activated by foreign invader)	C3a activated phagocytes; C5a attracts phagocytes; C3b opsonizes bacteria; C5b-C9 form MAC (kills G- bact.)
Mannose binding protein	Produced by liver	Binds to bacterial surface and activated complement
T cells	Blood (attracted to infection site)	T helper cells (CD4+) stimulate B cells to produce antibodies (Th2) or produce IFN-gamma to activate macrophages (Th1); Cytotoxic T cells (CD8+) kill infected host cells; cytokine production
B cells	Blood (attracted to infection site)	Produce antibodies
Antigens (IgG, IgM)	Blood	Opsonize bacteria (IgG); activate complement (IgM >IgG); neutralize toxins

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

Virulence factors of *Salmonella???*



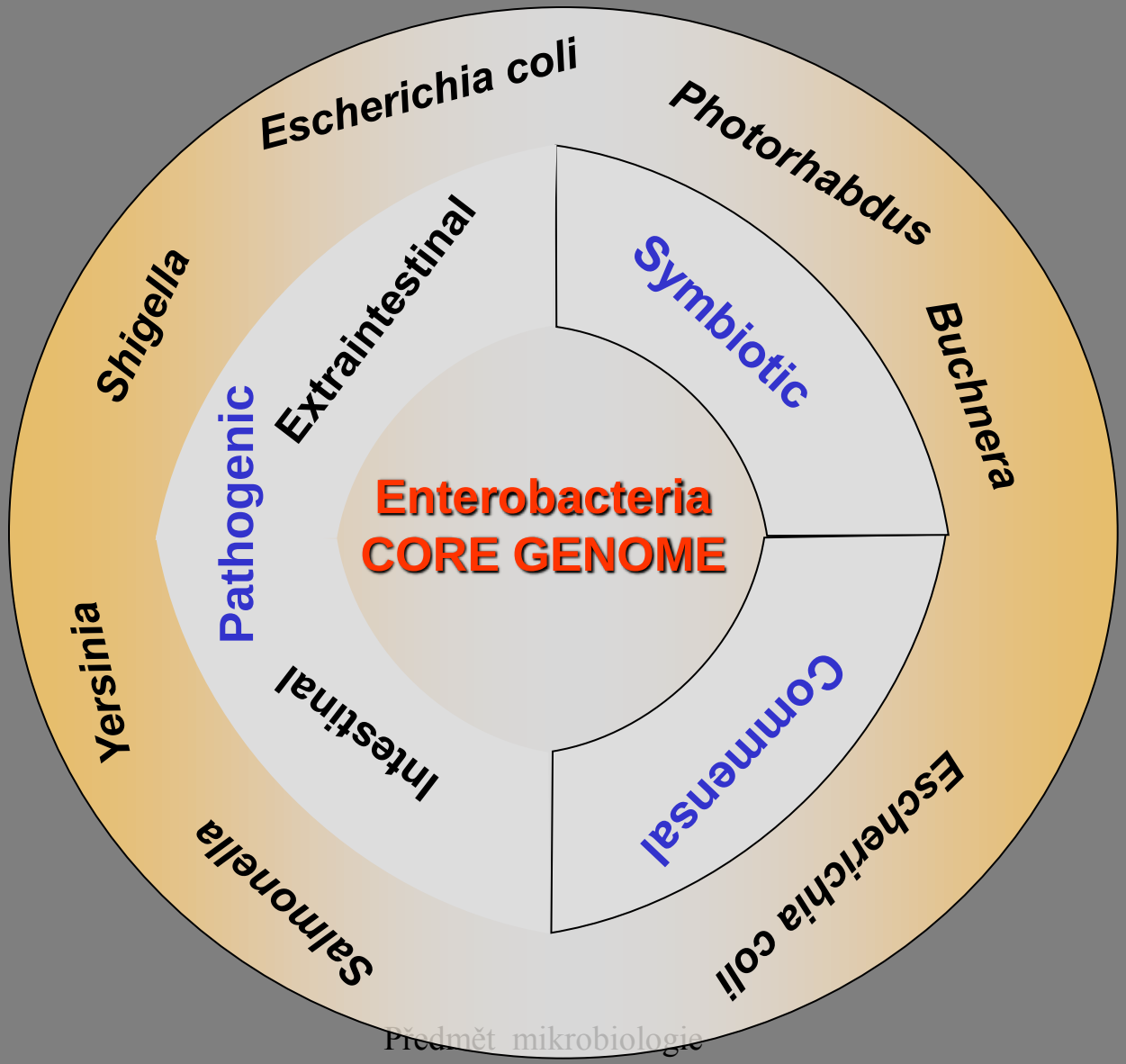
Confusion: fitness or virulence factor???

- What should be considered as a virulence factor?
  - traits and products enabling adherence, toxic protein prduction, directly linked to infection process???
  - traits like energy from sugar fermentation – housekeeping functions, but still essential for infection???
  - virulence factors can be used as a target of a vaccine or therapeutic strategy

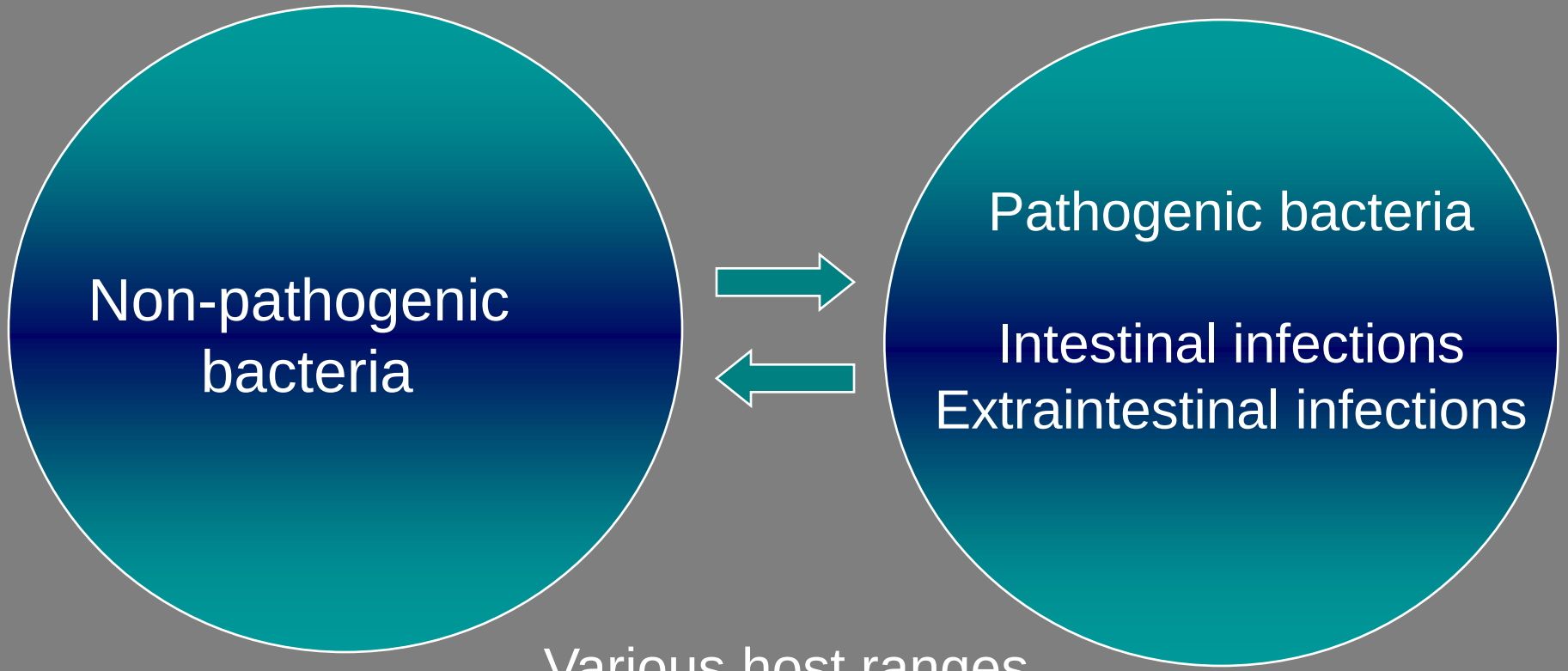
Core *E. coli* genome is about 3500 genes, additional 3500 *E. coli* genes are in mobile genomic islands, serving for adaptation to a given niche, some involved in pathogenicity…

To me, a virulence factor is a bacterial product or strategy that contributes to virulence or pathogenicity and is <u>really</u> absent in the commensal variants of the same organism…

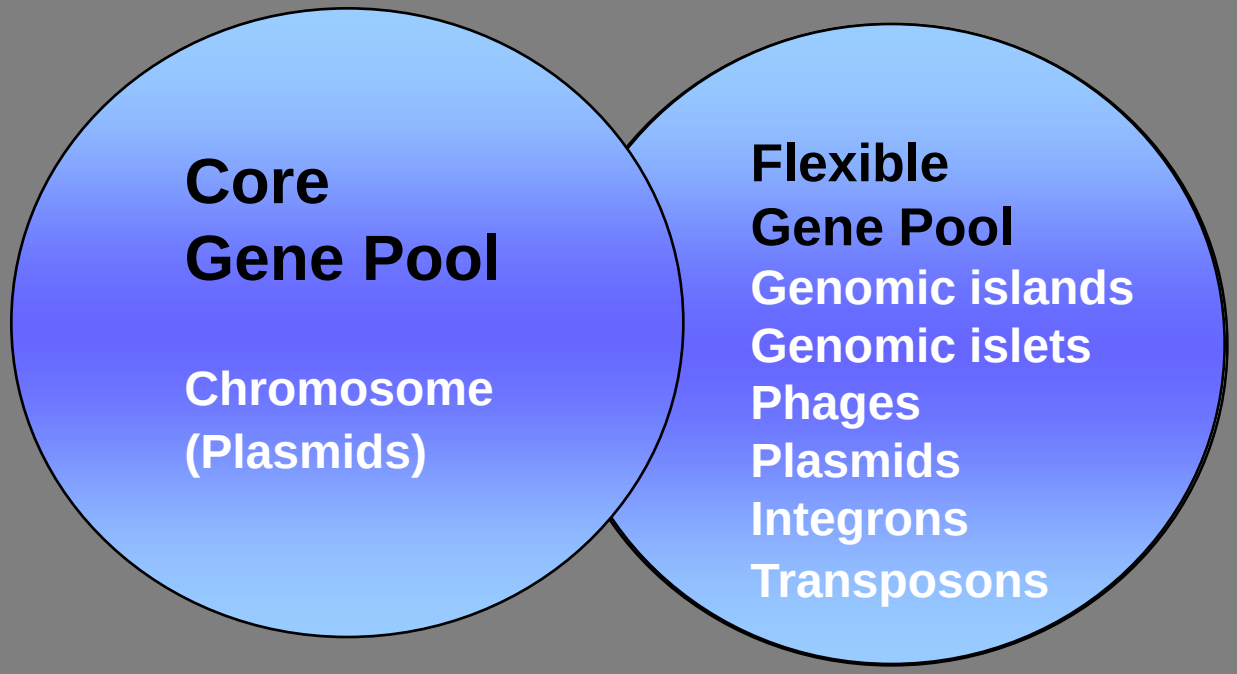
Enterobacteria have many faces



Escherichia coli



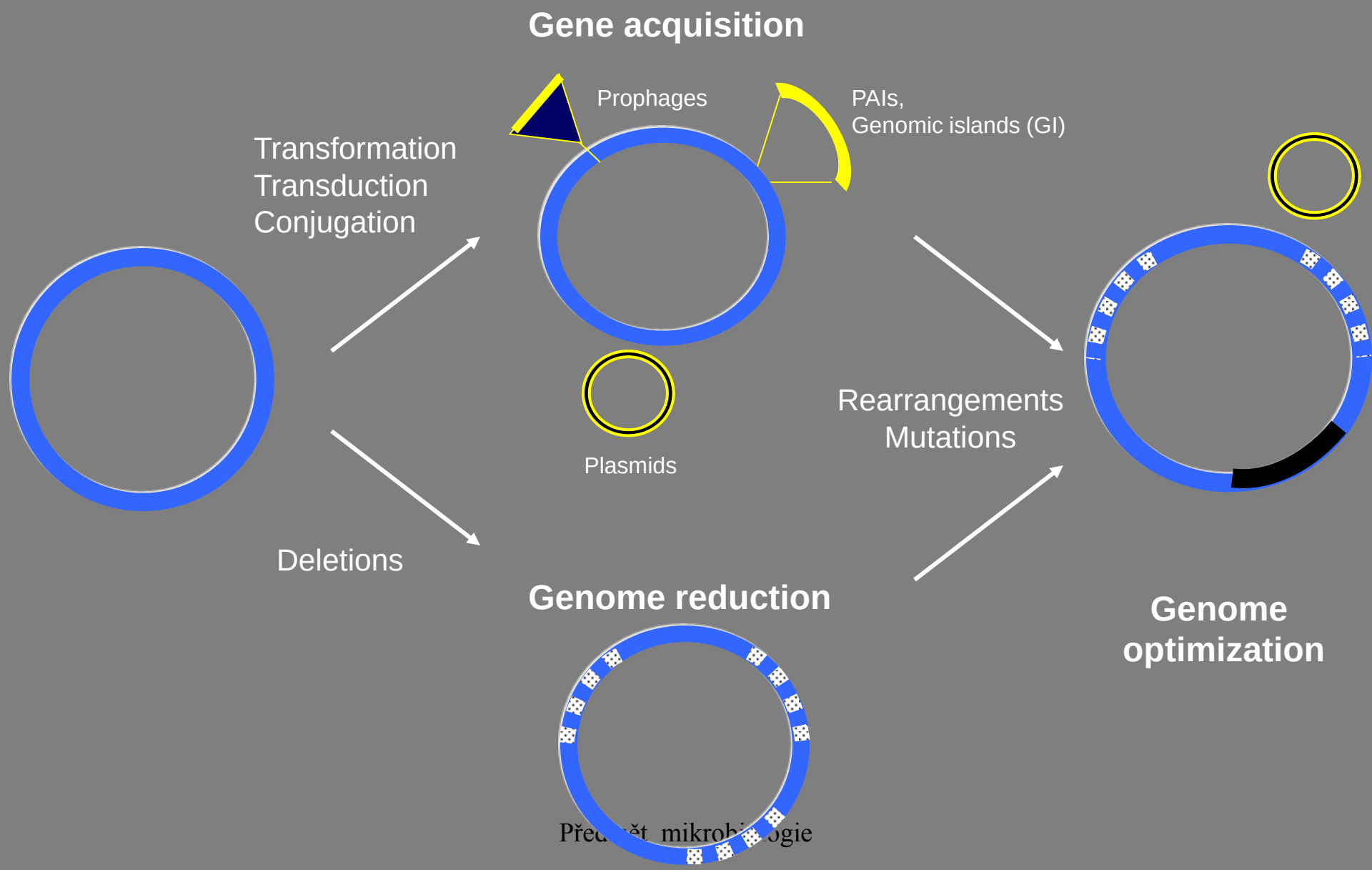
Various host ranges



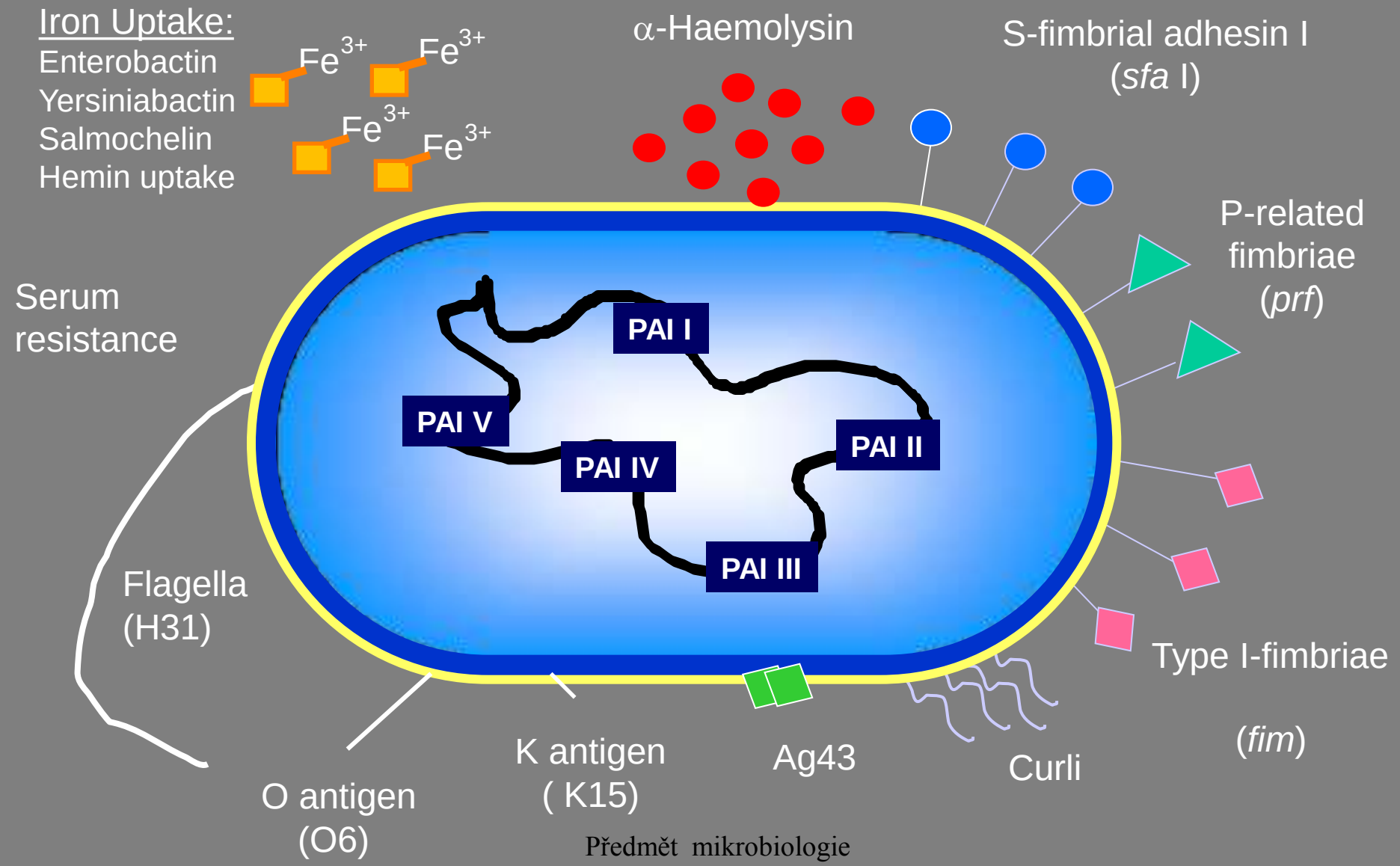
- Ribosomes
- Cell structure
- Basic metabolic pathways
- DNA-Replication
- Nucleotide turnover
- ...

- Pathogenicity
- Resistance to antibiotics
- Secretion
- Symbiosis
- Degradation
- Secondary metabolism
- Restriction/ modification
- Transposases/integrases
- :

Evolution of Genomes



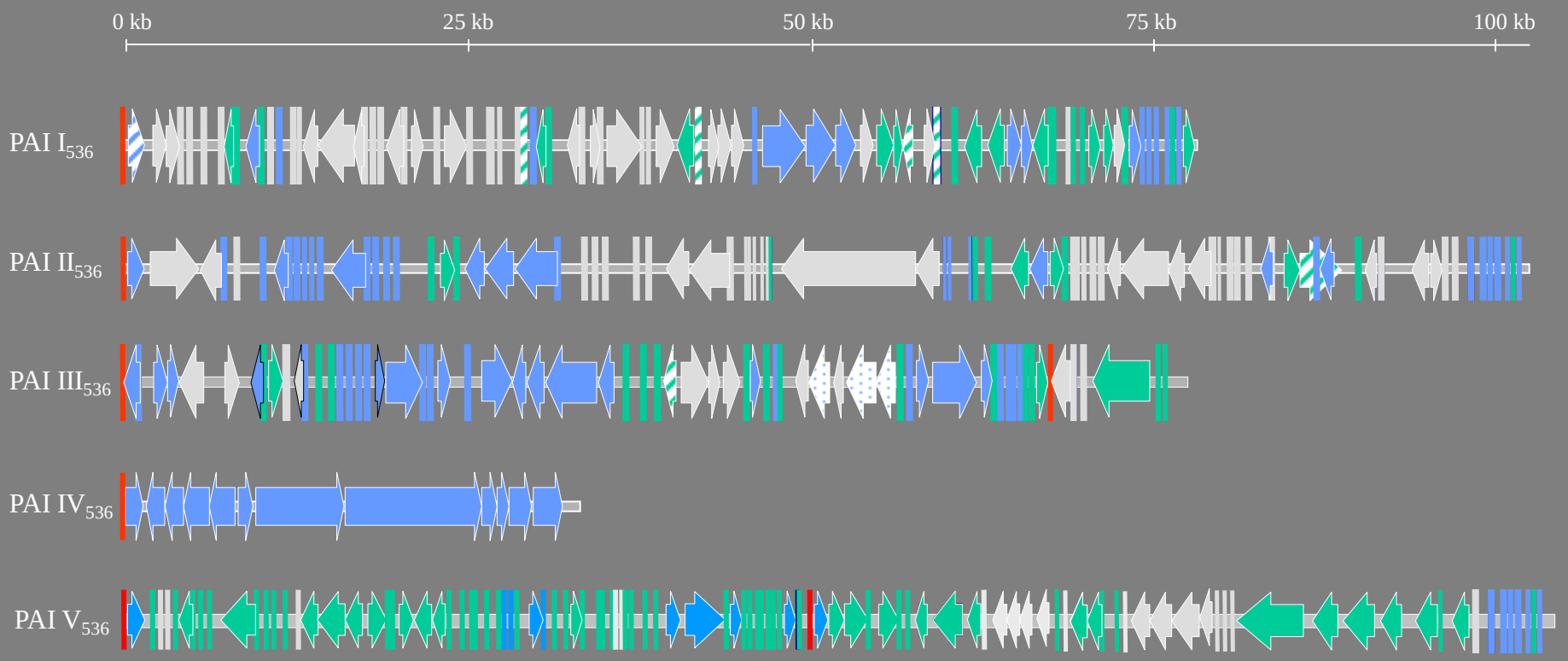
Pathogenicity factors of the uropathogenic *E. coli* strain 536



Main features of PAIs of *E. coli* strain 536

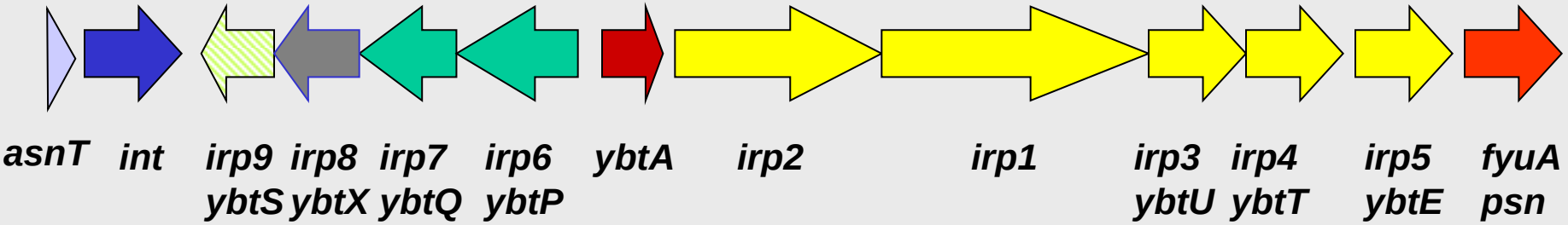
Designation	Insertion site (unit)	Target tRNA	Size (kb)	Virulence genes encoded	Integrase *(kryptic)	Boundary
PAI I	82	*selC*	76.8	α-haemolysin different fimbriae?	CP4-like	DR
PAI II	97	*leuX*	102.2	α− haemolysin P-fimbriae Heat resistant agglutinin	P4	DR
PAI III	5	*thrW*	(68.1) 75.8	S-fimbriae Ag43 *iro* siderophore system Hemoglobin protease	SfX	(DR)
PAI IV	44	*asnT*	30	Yersiniabaktin	P4-like*	-
PAI V	64	*pheV*	(48.7) 105	Capsule, Ag43 P-fimbriae?	P4-like	(DR)

PAI I_{536}-V_{536} of *E. coli* strain 536



Předmět mikrobiologie

Distribution of the High Pathogenicity Island (HPI) among enterobacteria

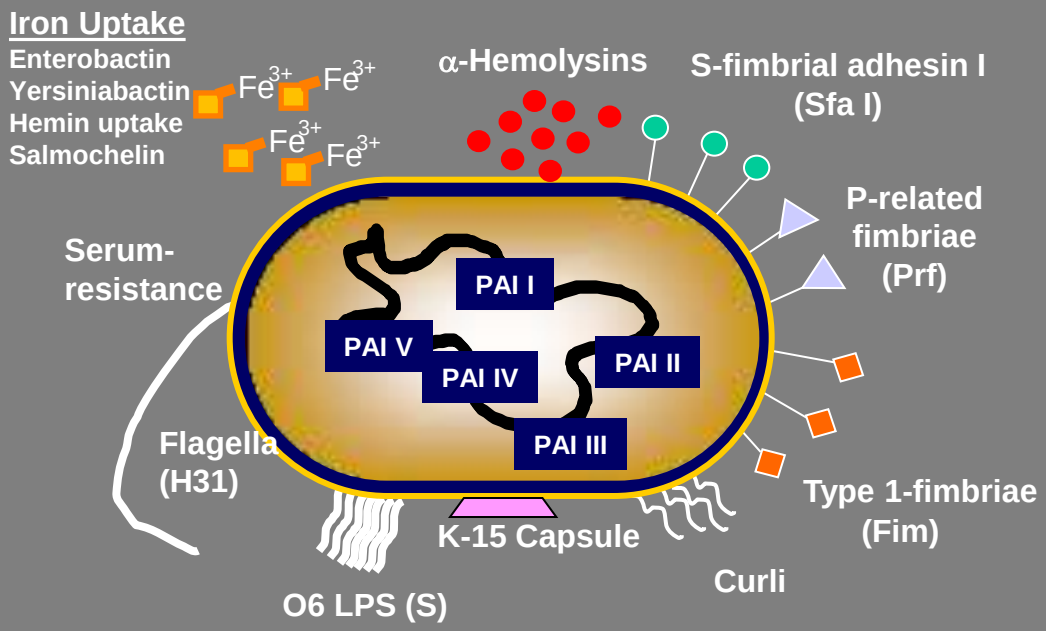


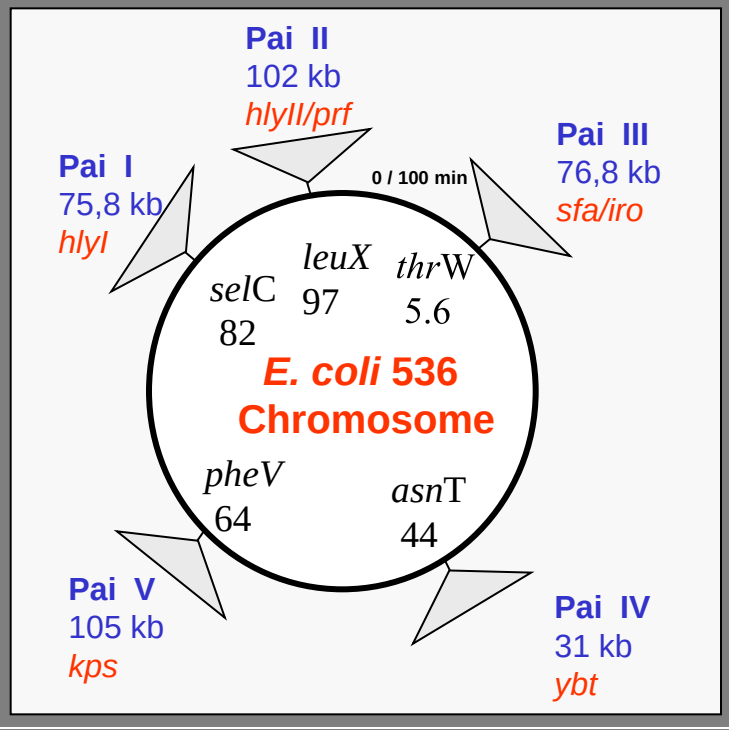
Commensal

E. coli - commensals
Klebsiella rhinoscleromatis
Klebsiella ozaenae
Klebsiella planticola
Klebsiella oxytoca
Salmonella enterica subspecies IIIa, IIIb, VI
Photorhabdus spp.

Pathogenic

Pathogenic *E. coli*
Pathogenic *Yersinae*
Citrobacter diversus
Klebsiella pneumoniae
Enterobacter spec.


Iron Uptake
Enterobactin
Yersiniabactin
Hemin uptake
Salmochelin
Fe3+
α-Hemolysins
S-fimbrial adhesin I
(Sfa I)
P-related
fimbriae
(Prf)
Serum-
resistance
PAI I
PAI V
PAI IV
PAI II
PAI III
Flagella
(H31)
K-15 Capsule
Type 1-fimbriae
(Fim)
Curli
O6 LPS (S)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Pai III
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sfa/iro
Pai I
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thrW
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selC
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E. coli 536
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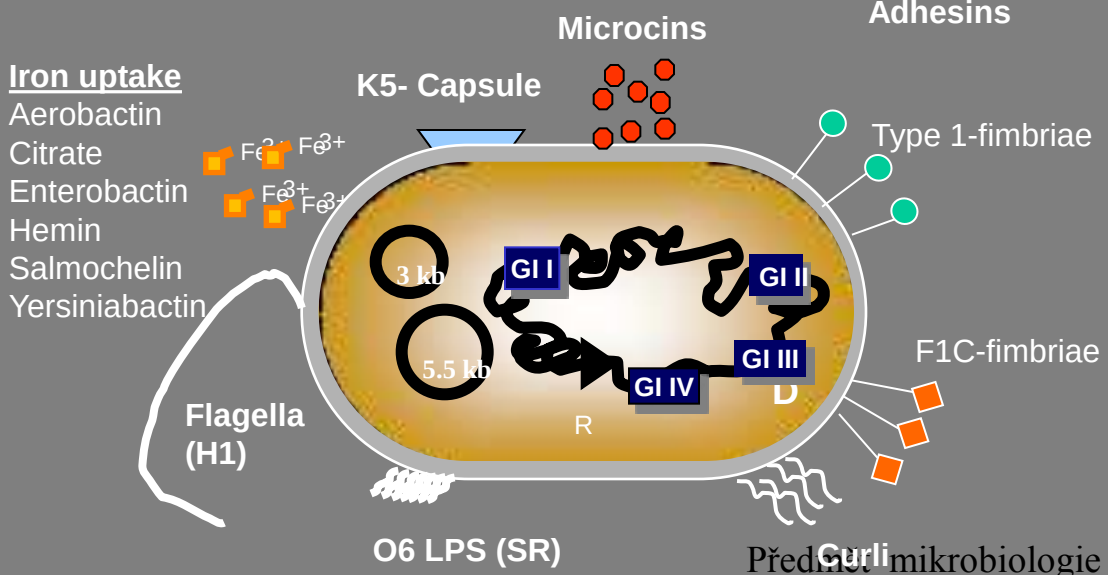
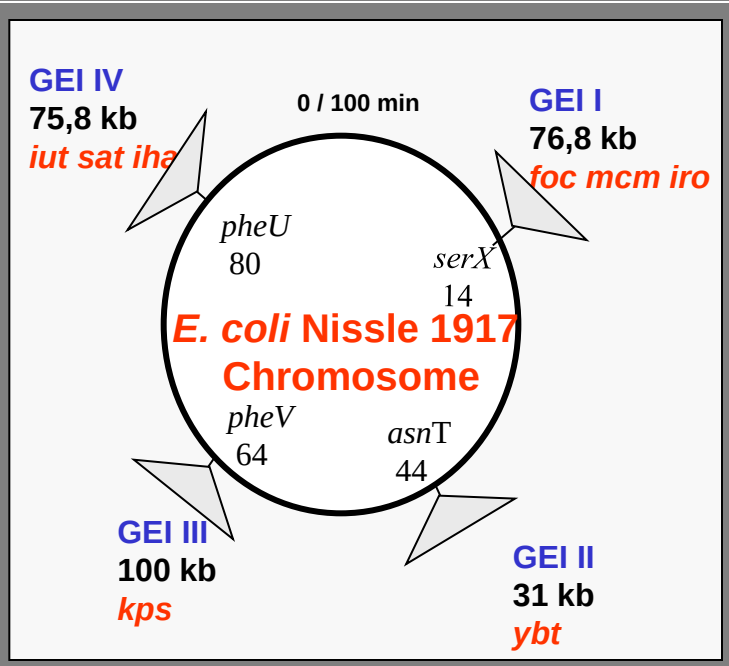

Iron uptake
Aerobactin
Citrate
Enterobactin
Hemin
Salmochelin
Yersiniabactin
Microcins
Adhesins
K5- Capsule
Type 1-fimbriae
Fe3+
3 kb
GI I
GI II
5.5 kb
GI III
GI IV
R
D
F1C-fimbriae
Flagella
(H1)
O6 LPS (SR)
Curli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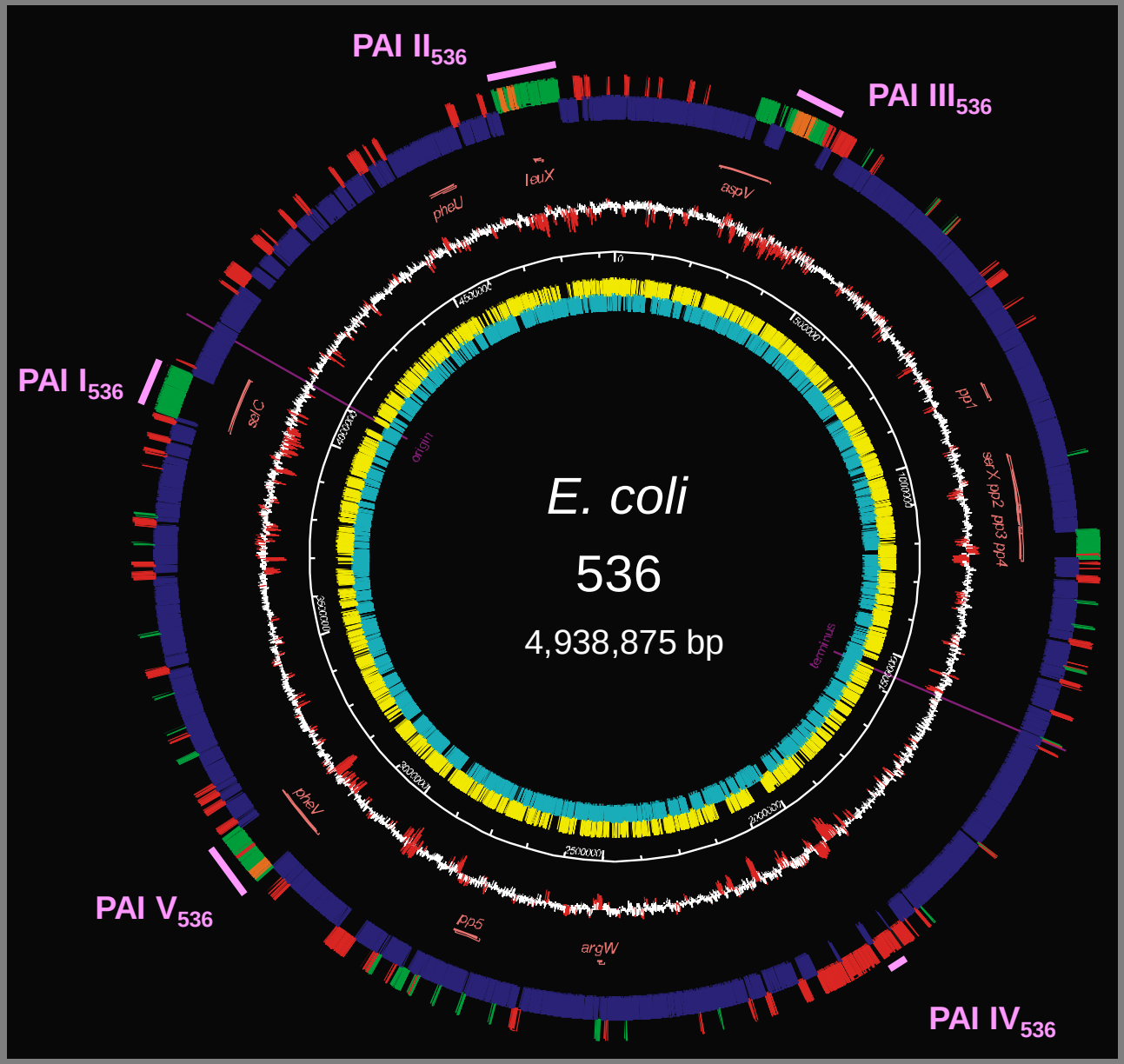
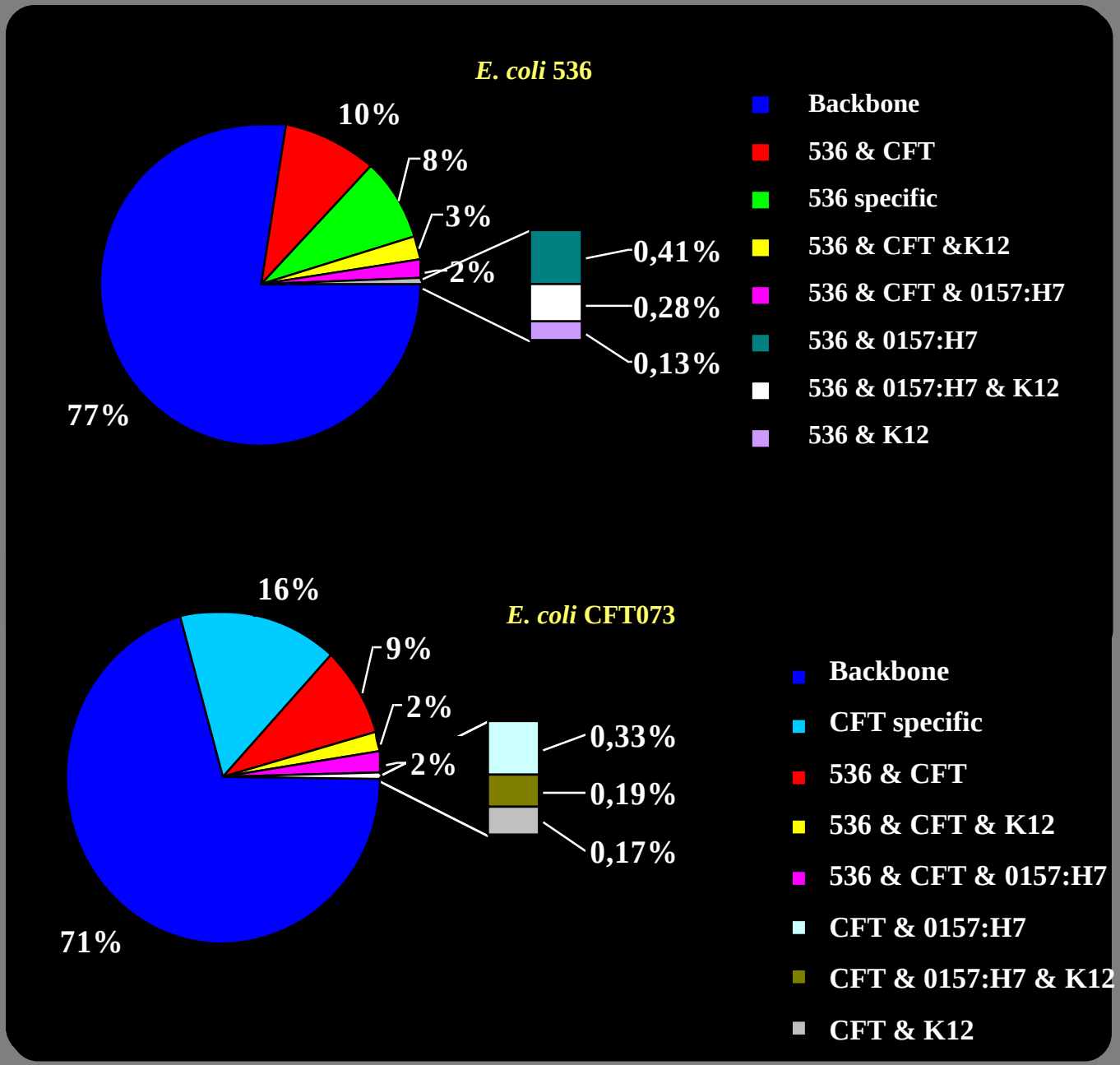
iut sat iha
0 / 100 min
GEI I
76,8 kb
foc mcm iro
pheU
80
serX
14
E. coli Nissle 1917
Chromosome
pheV
64
asnT
44
GEI III
100 kb
kps
GEI II
31 kb
ybt

Genome comparison of *E. coli* cystitis isolate 536 (O6:K15:H31) and *E. coli* urosepsis isolate CFT073 (O6:K2(?):H1)



Genome comparison of four *E. coli* genomes (2)



Conclusions

- Genetic diversity among different *E. coli* isolates is high
- The ability to acquire and “collect“ virulence-associated genes determines the virulence potential of individual *E. coli* strains
- Different virulence potentials result not only from different “arsenals“ of virulence-associated genes, but also from differences in virulence gene expression
- Pathotyping and risk assessment of ExPEC is difficult as they lack a major virulence factor
- “Virulence genes“ and genomic islands may be also present in genomes of non-pathogens
- The encoded gene products contribute to / increase bacterial adaptability, competitiveness, colonization efficiency

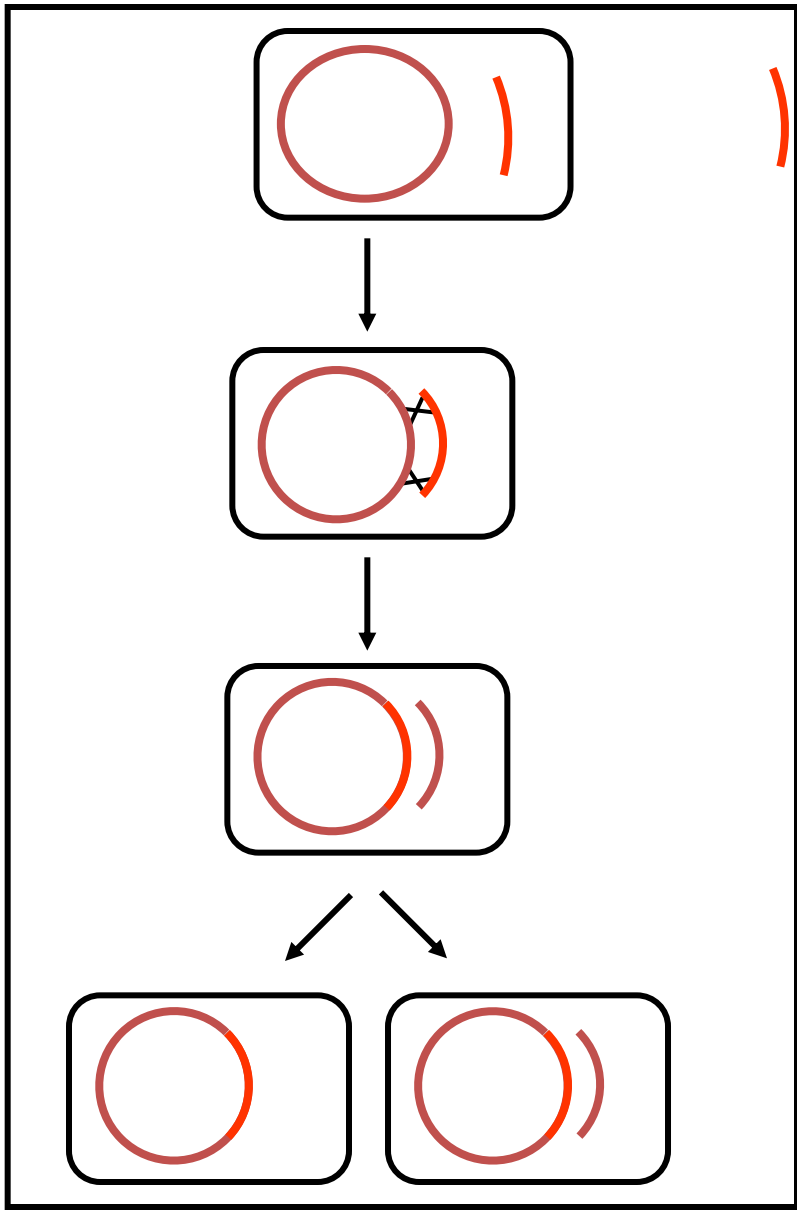
Horizontal gene transfer and Exchange of Genetic Information

Transformation

- Definition: Gene transfer resulting from the uptake of DNA from a donor.
- Factors affecting transformation
  - DNA size and state
    - Sensitive to nucleases
  - Competence of the recipient (*Bacillus, Haemophilus, Neisseria, Streptococcus*)
    - Competence factors
    - Induced competence

Transformation

– Recombination
  - Legitimate, homologous or general
  - recA, recB and recC genes



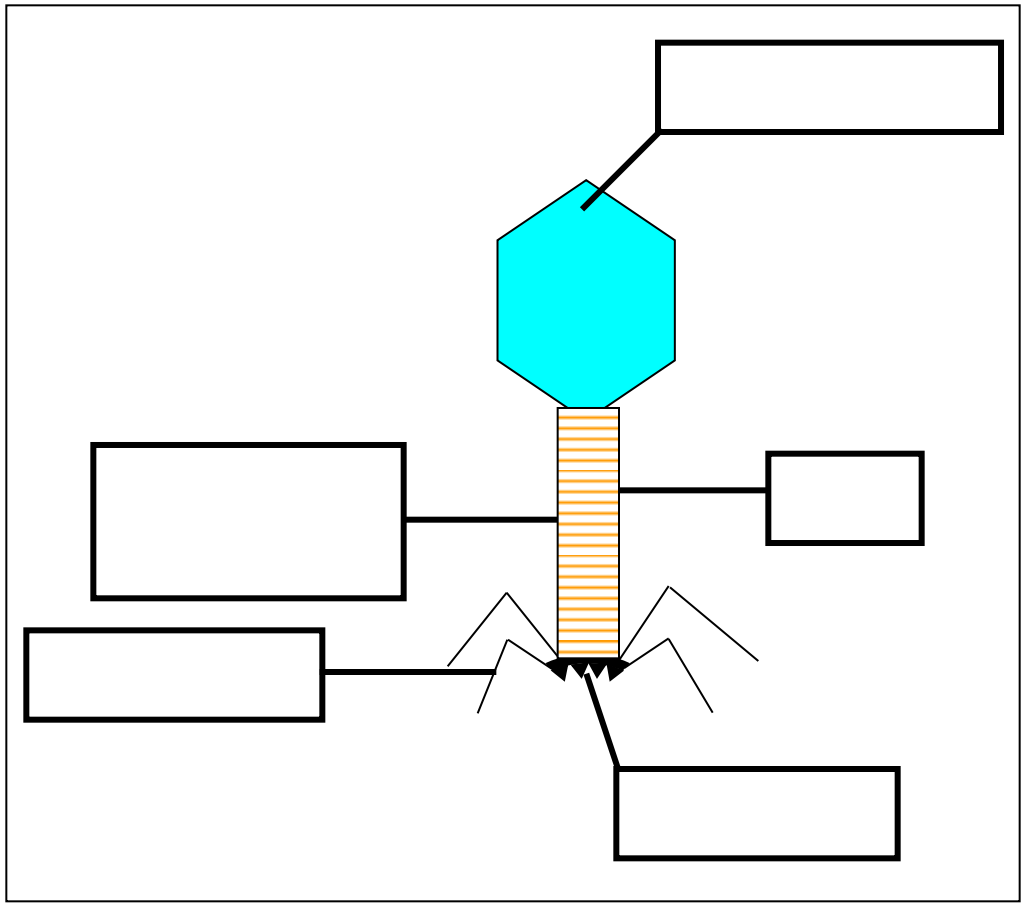
Transduction

- Definition: Gene transfer from a donor to a recipient by way of a bacteriophage

- Bacteriophage (phage): A virus that infects bacteria

Phage Composition and Structure

- Composition
  - Nucleic acid
    - Genome size
    - Modified bases
  - Protein
    - Protection
    - Infection

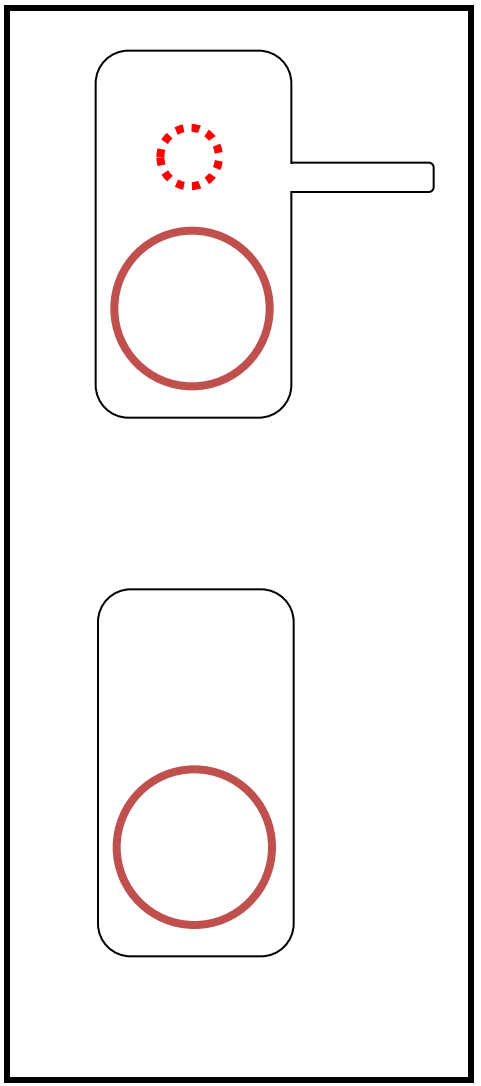


Transduction

- Definition
- Types of transduction
- Significance
  - Common in Gram+ bacteria
  - Lysogenic (phage) conversion
    - e.g. Corynebacterium diptheriae toxin

Conjugation

- Definition: Gene transfer from a donor to a recipient by direct physical contact between cells
- Mating types in bacteria
  - Donor
    - F factor (Fertility factor)
      - **F (sex) pilus**

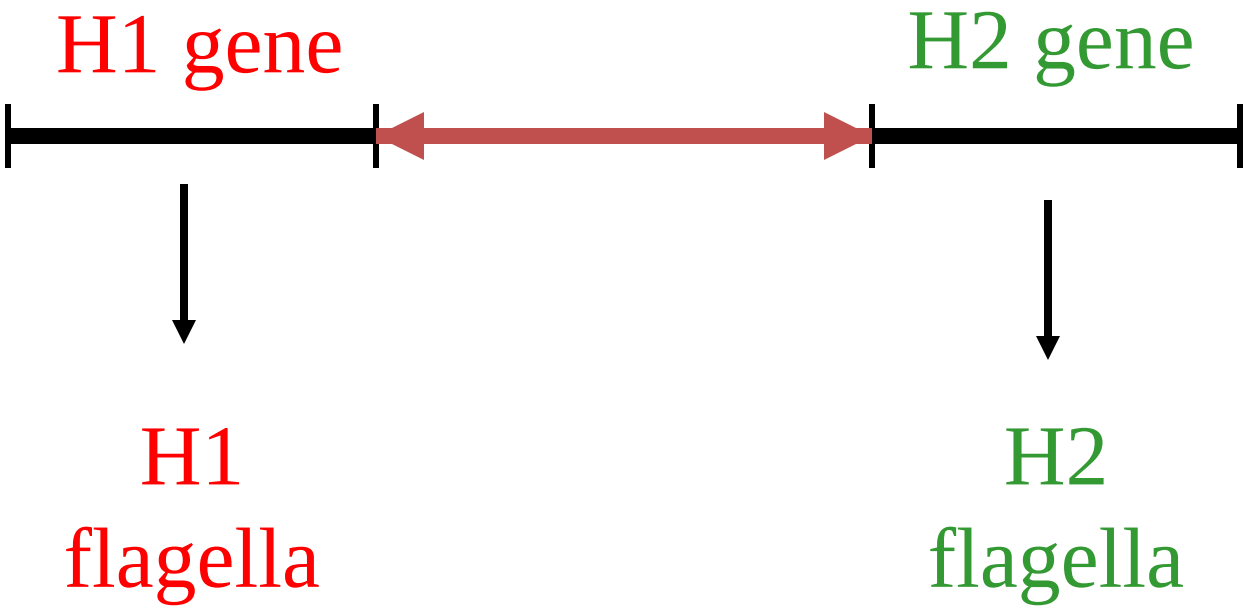


Conjugation

- Significance
  - Gram - bacteria
    - Antibiotic resistance
    - Rapid spread
  - Gram + bacteria
    - Production of adhesive material by donor cells

Transposable Genetic Elements

- Definition: Segments of DNA that are able to move from one location to another
- Properties
  - "Random" movement
  - Not capable of self replication (not a replicon)
  - Transposition mediated by site-specific recombination
    - Transposase
  - Transposition may be accompanied by duplication


H1 gene
H2 gene
H1
flagella
H2
flagella

Types of Transposable Genetic Elements

- Transposons (Tn)
  - Definition: Elements that carry other genes except those involved in transposition
  - Nomenclature - Tn10
  - Structure
    - Composite Tns

Plasmids

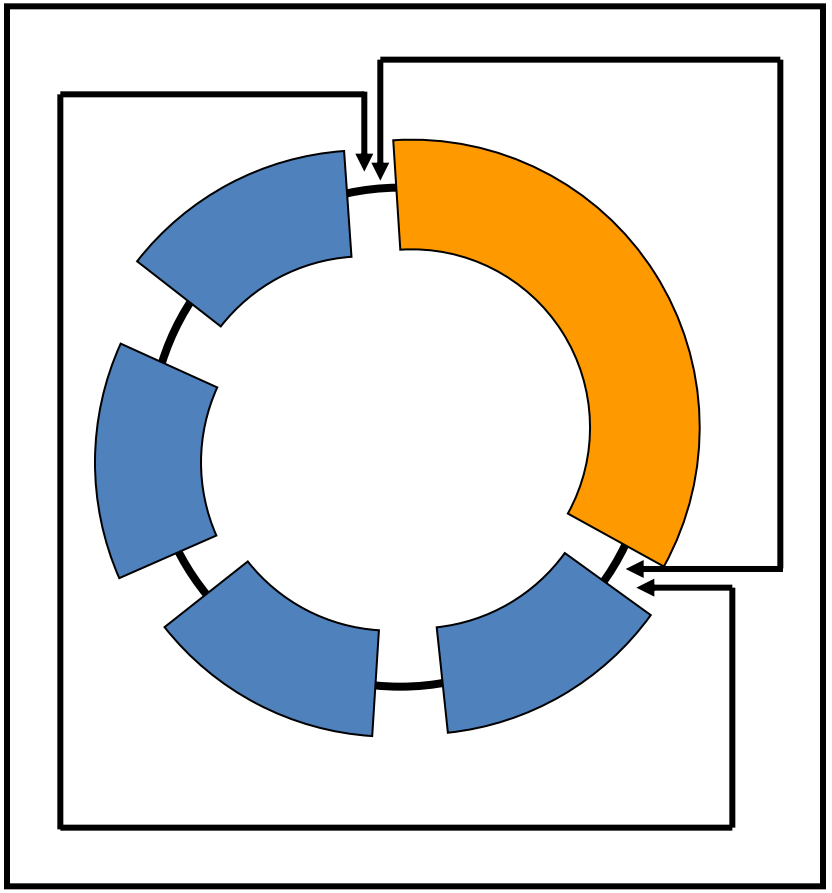
- Definition: Extrachromosomal genetic elements that are capable of autonomous replication (replicon)
- Episome - a plasmid that can integrate into the chromosome

Classification of Plasmids

- Transfer properties
  - Conjugative
  - Nonconjugative
- Phenotypic effects
  - Fertility
  - Bacteriocinogenic plasmid
  - Resistance plasmid (R factors)

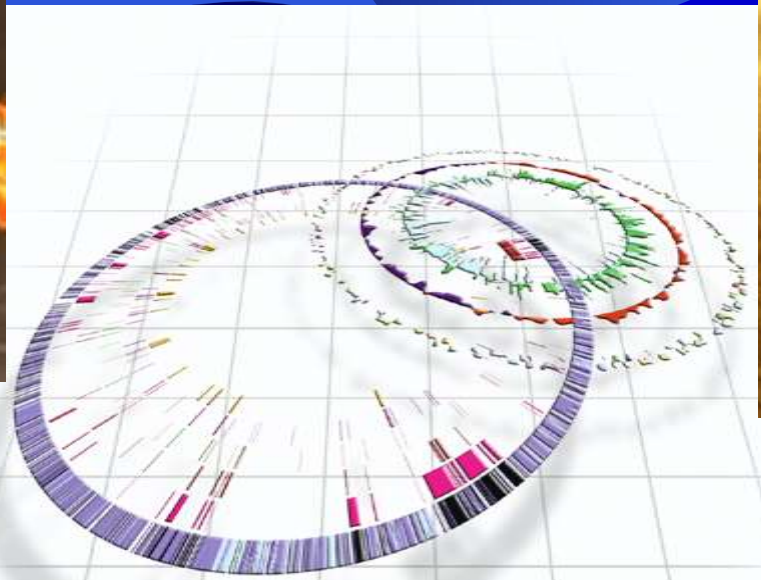
Structure of R Factors

- RTF
  - Conjugative plasmid
  - Transfer genes

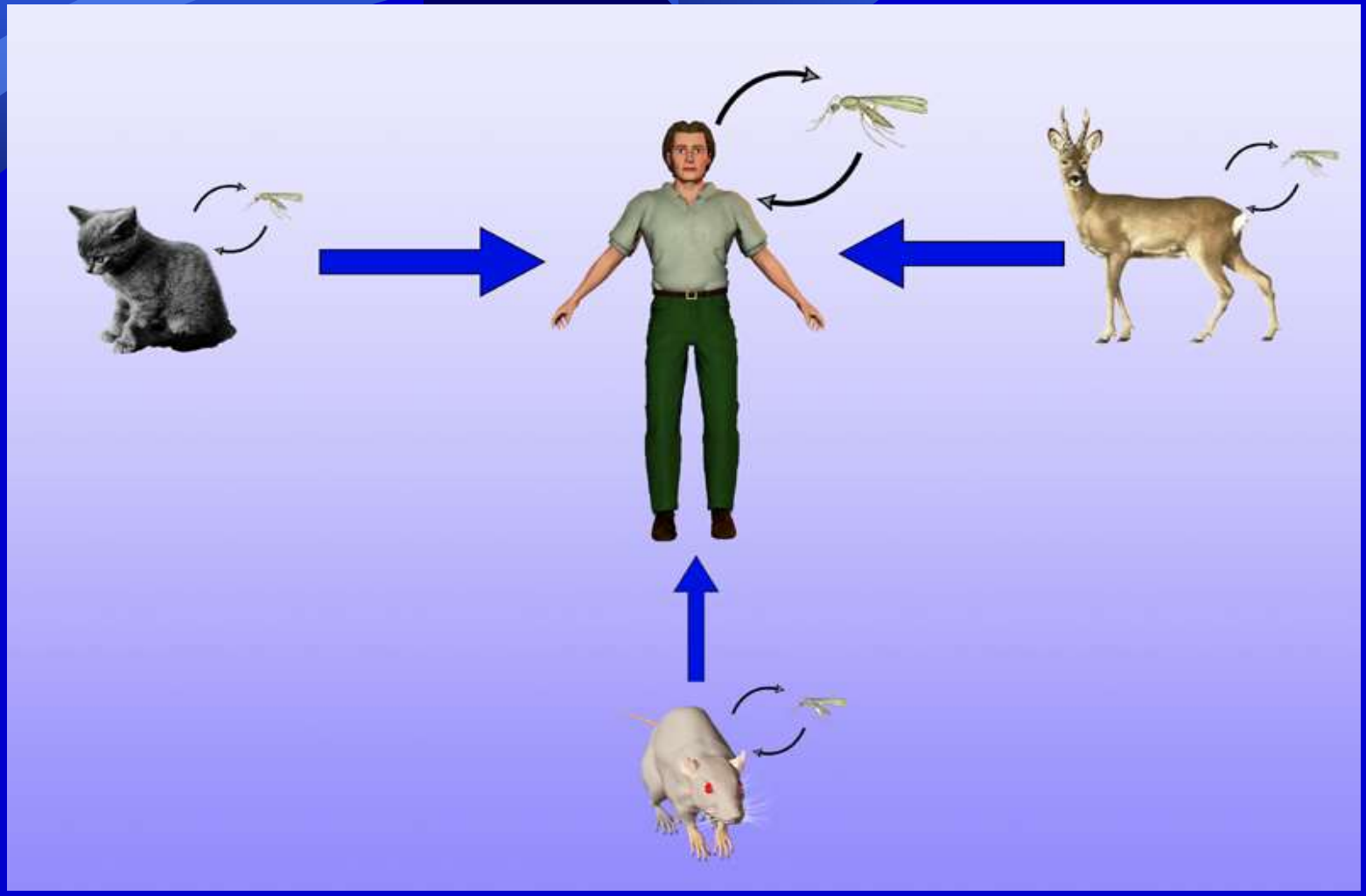


The other extreme: reductive evolution of highly specialized – exclusively human pathogens

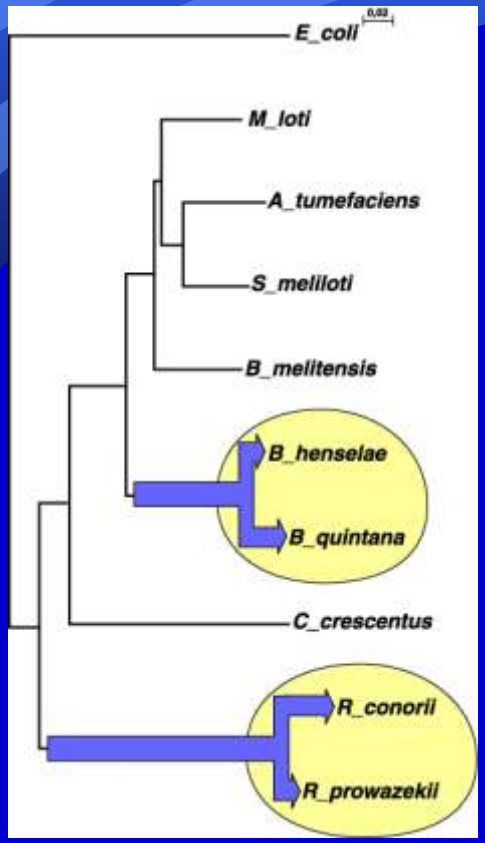
Comparative Genome Analyses: *Rickettsia* and *Bartonella*



Single versus Multihost Pathogens



Comparative Genomics of α-Proteobacteria



Completed Genomes

• *Rickettsia prowazekii*	1.1 Mb	Uppsala 98
• *Rickettsia conorii*	1.3 Mb	France 01
• *Bartonella quintana*		1.6 Mb
Uppsala 03		
• *Bartonella henselae*	1.9 Mb	Uppsala 03
• *Brucella melitensis*	3.3 Mb	Scranton 02
• *Brucella suis*	3.3 Mb	TIGR 02
• *Caulobacter melitensis*	4.0 Mb	TIGR 01
• *Agrobacterium tumefaciens*	5.6 Mb	Wash 01
• *Sinorhizobium meliloti*	6.7 Mb	EU/Can 01
• *Mesorhizobium loti*	7.7 Mb	Kazusa 00
• *Bradyrhizobium japonicum*	9.1 Mb	Kazusa 02

Large variations in genome size and genome structure

Rhizobacteria

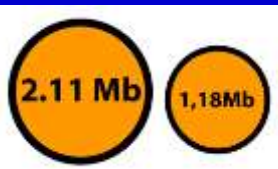
Bartonella

B.quintana

B.henselae

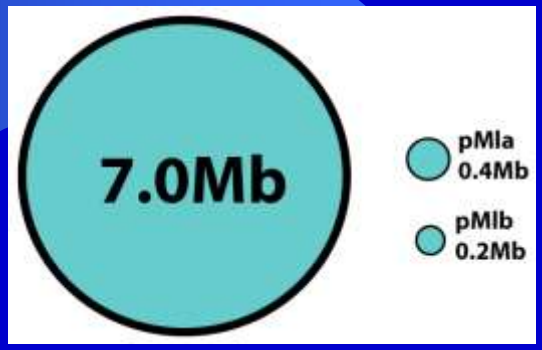
Brucella

B.melitensis



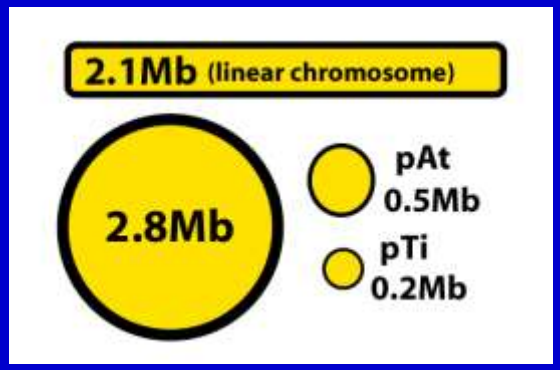
B.suis biovar 1

Mesorhizobium loti



Sinorhizobium meliloti

Agrobacterium tumefaciens



12 November 1998

International weekly journal of science

nature

www.nature.com

Typhus pathogen genome

Superconductivity The paramagnetic Meissner effect

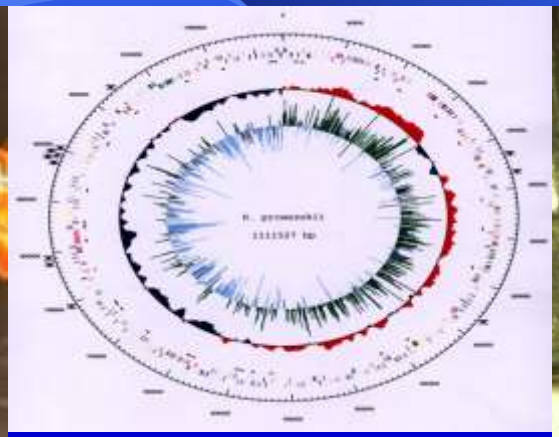
Nematode sex Counting on X chromosomes

Supernovae A young nearby remnant

Science

June 2001

R. prowazekii and *R. conorii*



Rickettsia prowazekii

- Epidemic Typhus
- Transmitted by lice
- 1.1 Mb; 834 Genes
- 24% junk DNA

Rickettsia conorii

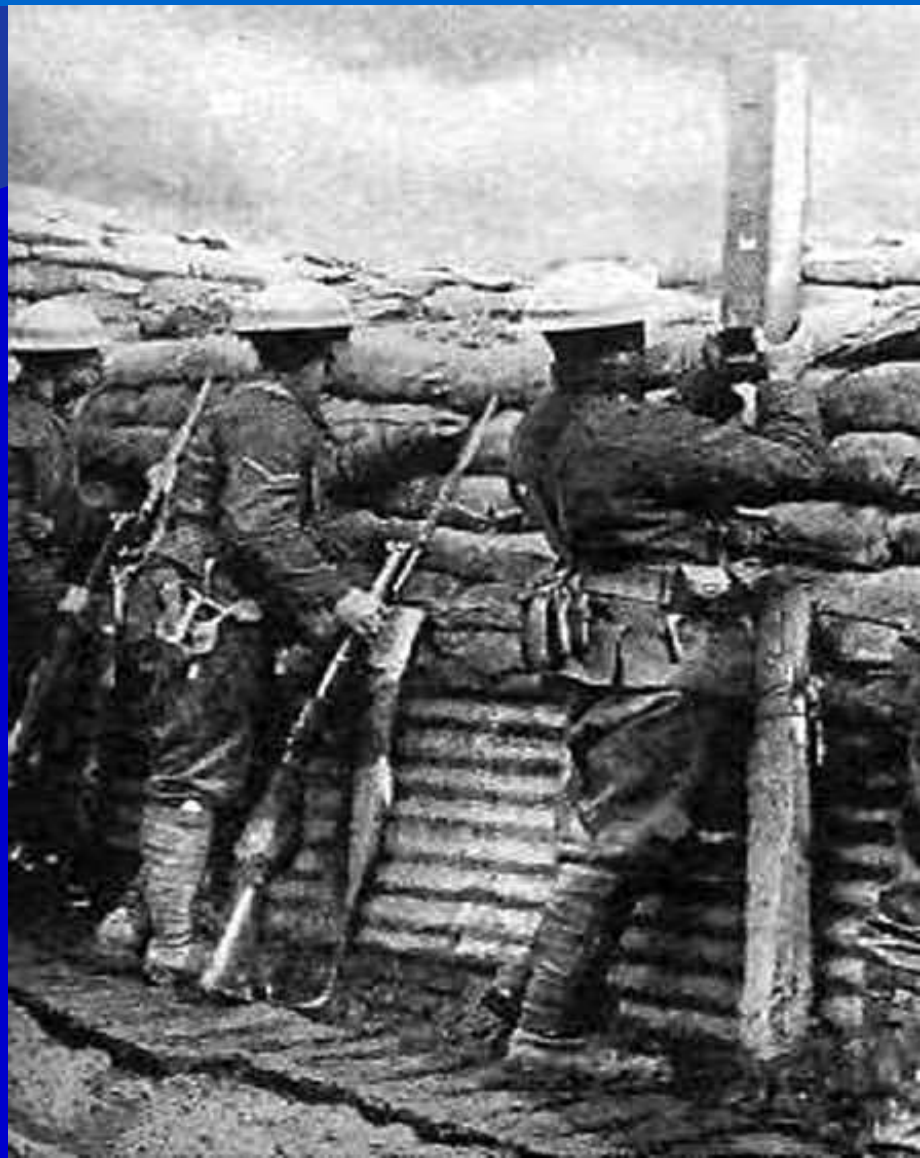
- Mediterranean Spotted Fever
- The tick is the reservoir
- 1.3 Mb; <1374 Genes
- 20% junk DNA

Bartonella quintana

1.58 Mb; 1138 Genes

- **Trench Fever**
- 5-day fever, "quintan fever"
- >1 million soldiers affected during World War I (II)
- Re-emerged among homeless and alcoholics in urban areas

- **Single-host pathogen:** Transmitted by the body louse
- Humans are the sole host

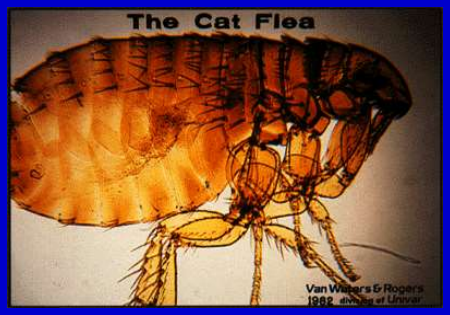


Bartonella henselae

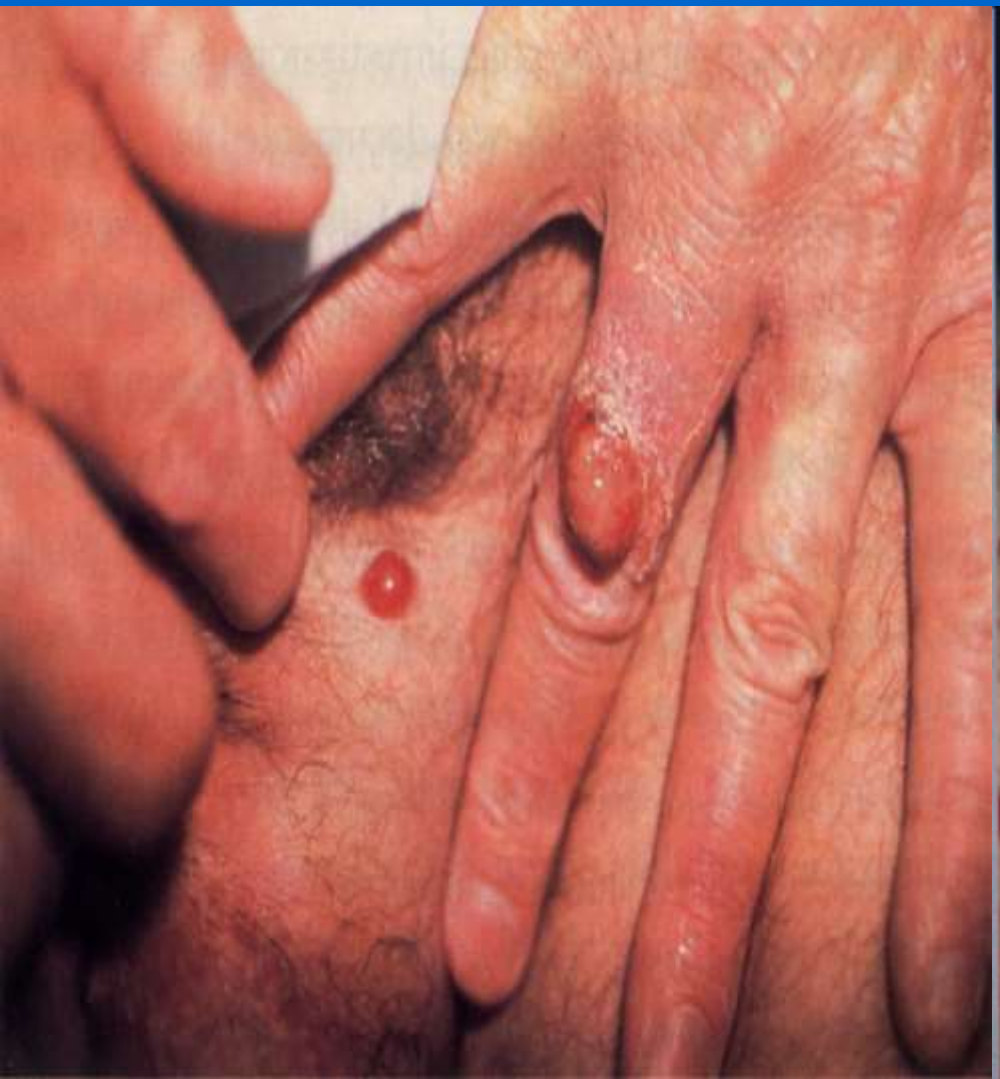
1.93 Mb; 1493 Genes

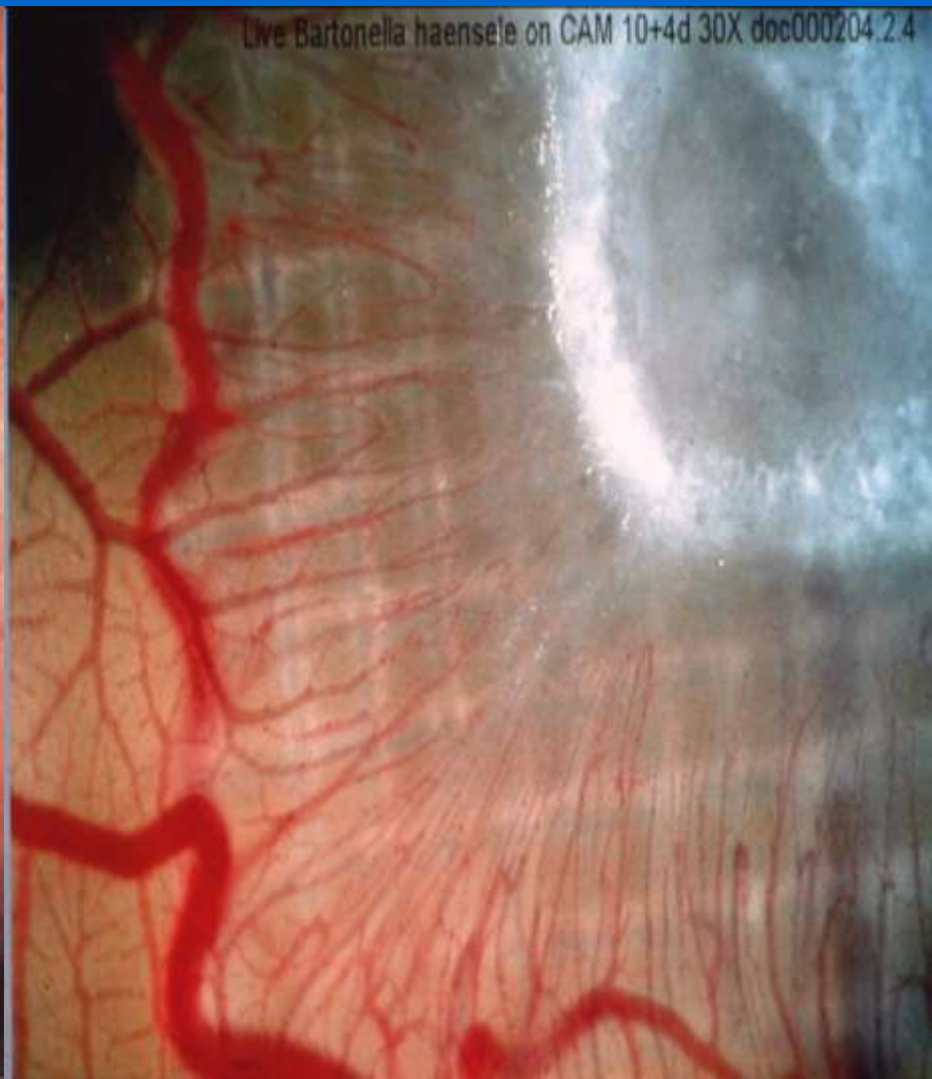
- **Cat-Scratch Disease**
- Lymphadenopathy and fever
- 24.000 cases per year in USA
- Isolated 1992, HIV-patient

- **Multi-Host Pathogen**
- The cat is the reservoir
- 30-60% of cats infected
- Transmitted by the cat flea

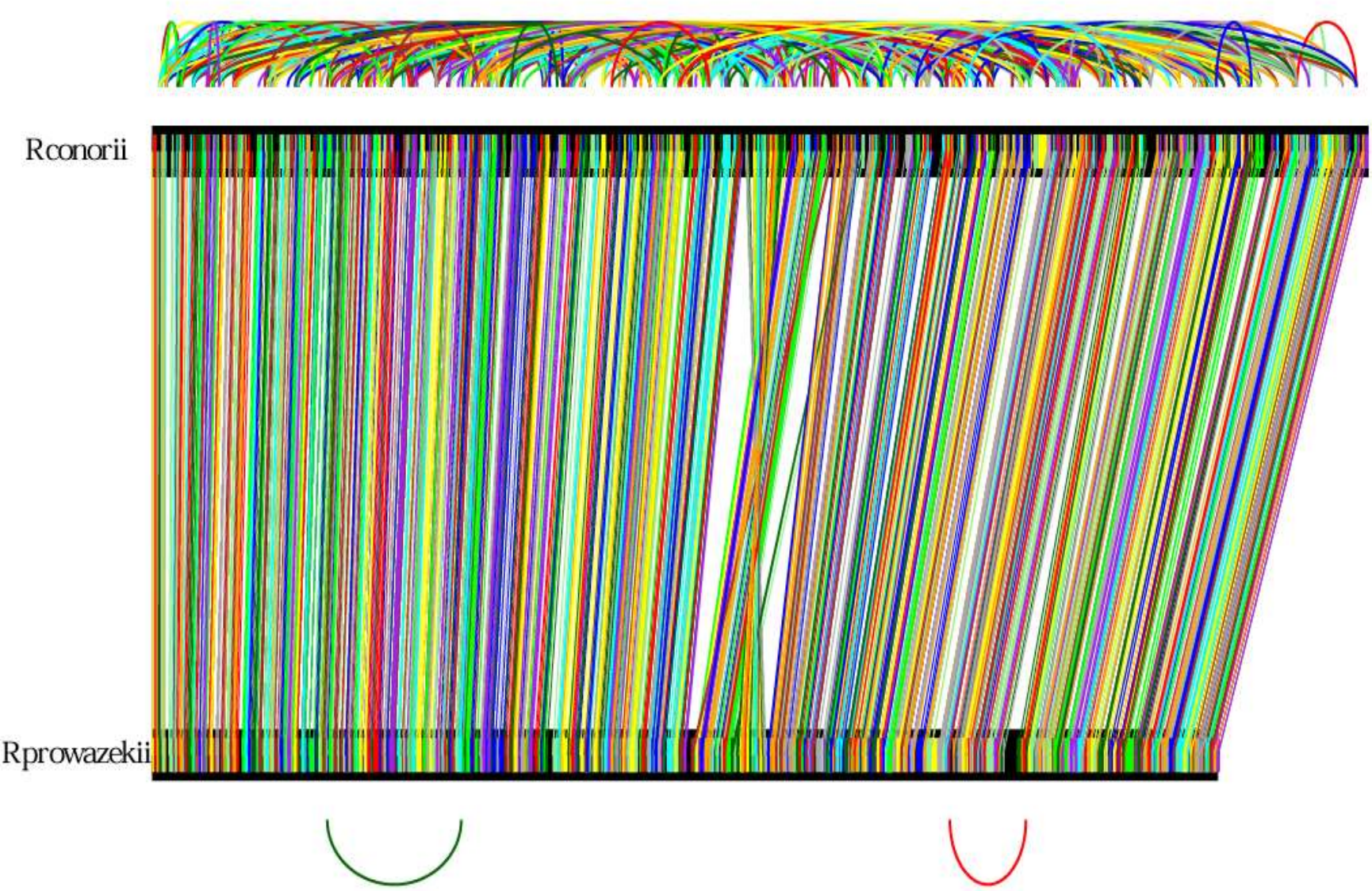


Bacillary Angiomatosis in HIV+ patients



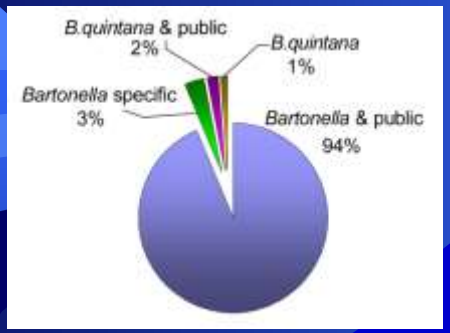


Genome Architecture of *Rickettsia*
R. prowazekii and *R. conorii*

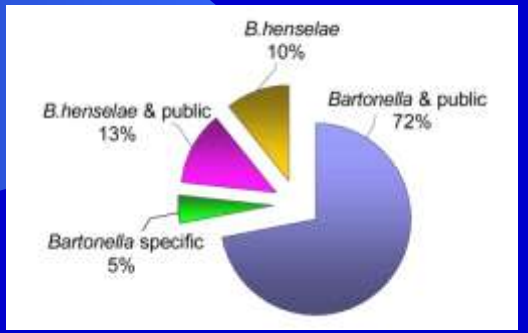


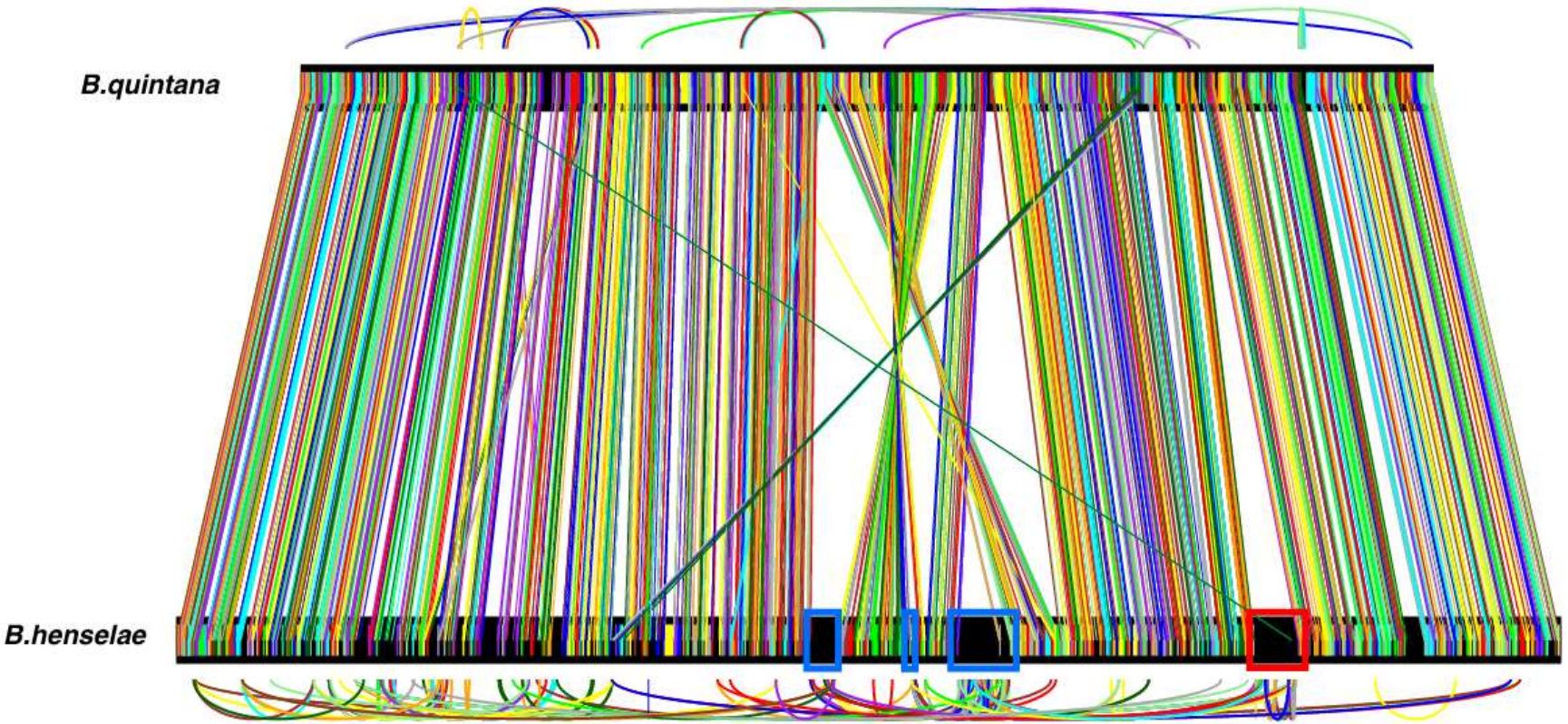
Bartonella

B.quintana (1138 genes)

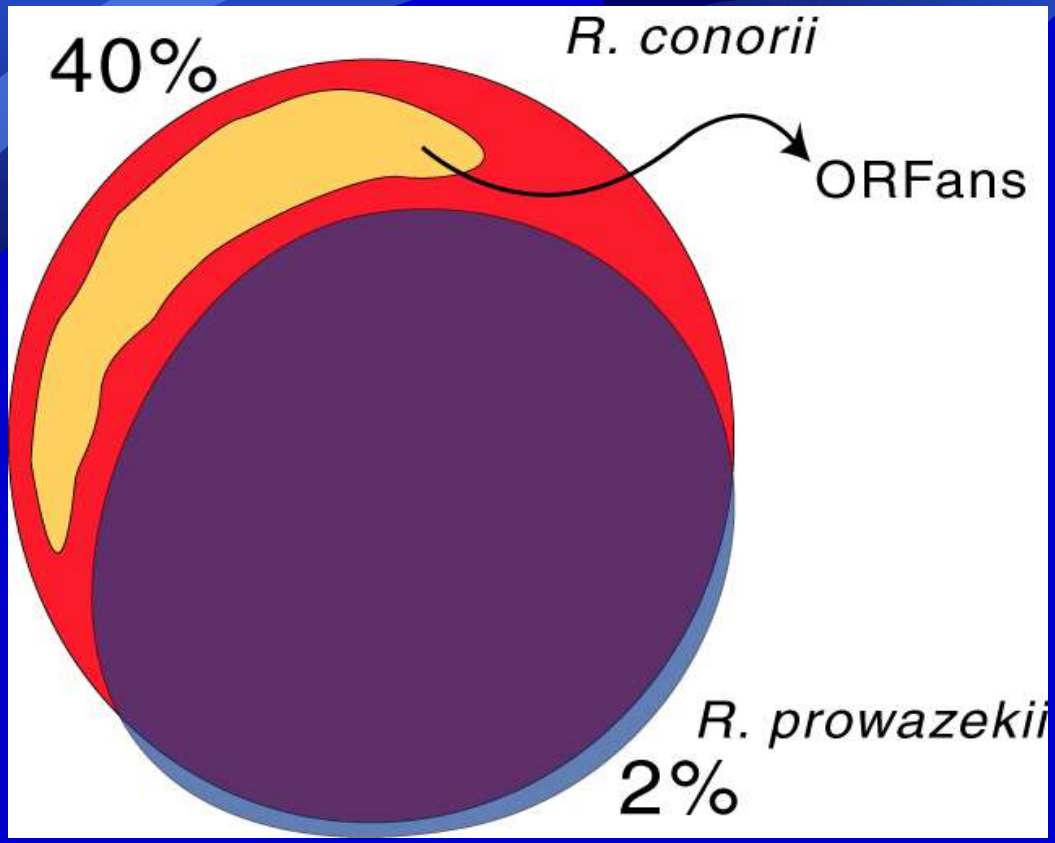


B.henselae (1493 genes)





Gene Content of *R. prowazekii* and *R. conorii*



R. prowazekii:

1.1 Mb, 834 Genes

- 30 Species-specific
- 80% ORPHANS

R. conorii:

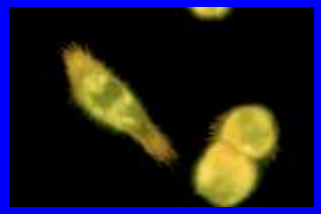
1.3 Mb, 1374 Genes

- 554 Species-specific
- 80% ORPHANS

Sensing of environment and proper regulation of gene expression is crucial!!!

‘better loose energy than control!

Biological cycle



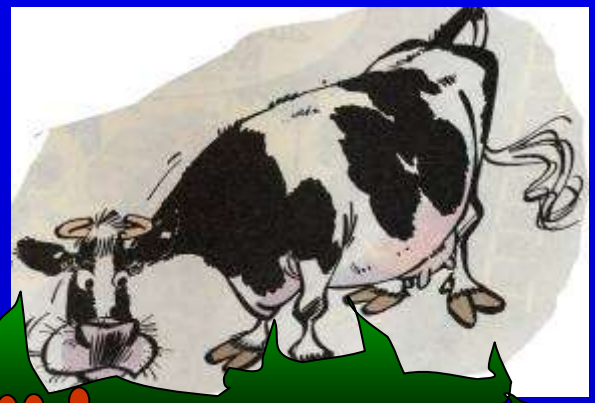
Germination

Bacilli multiplication

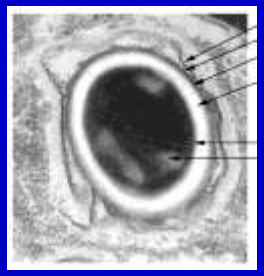
Toxins

Capsule

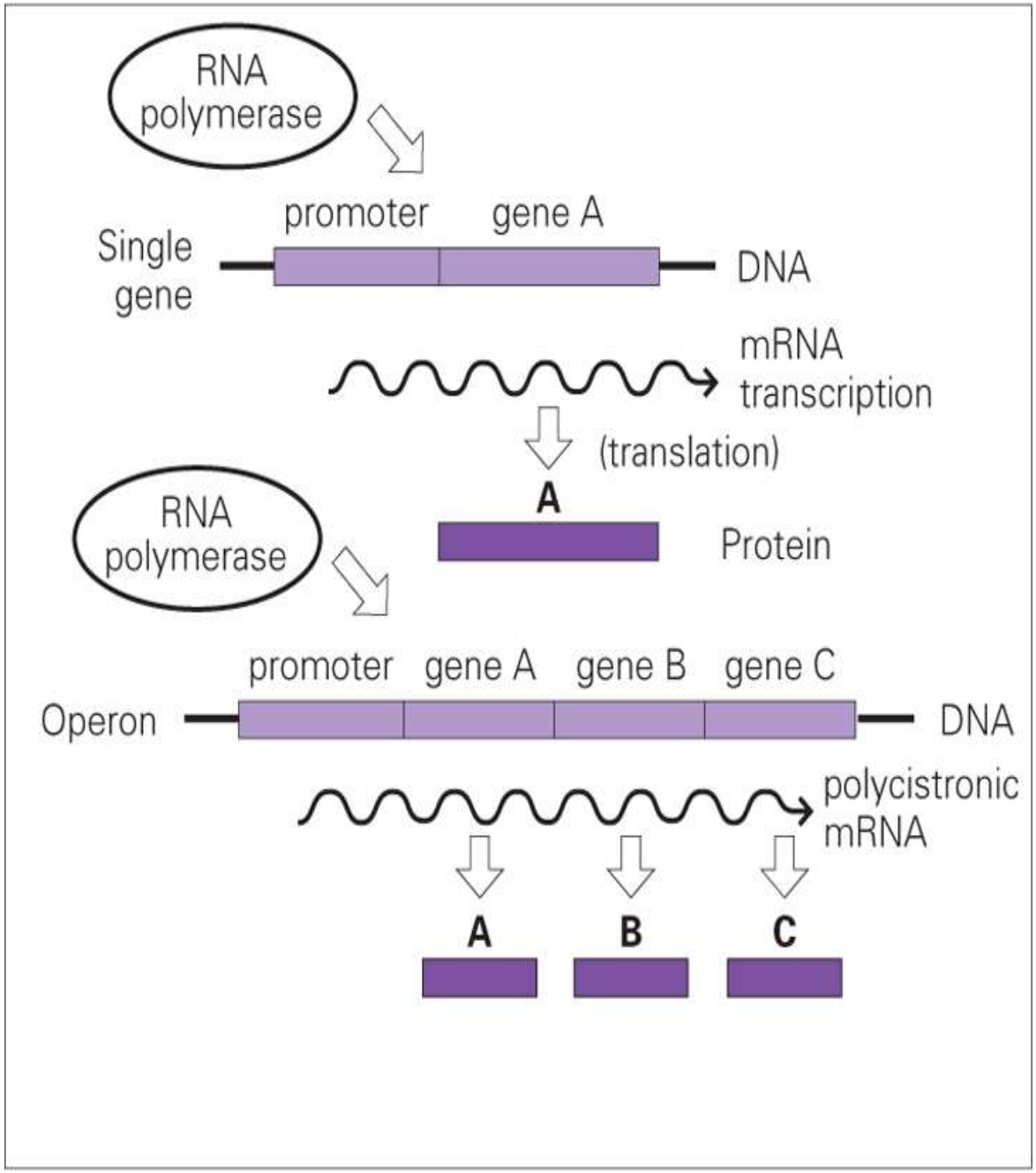
Death

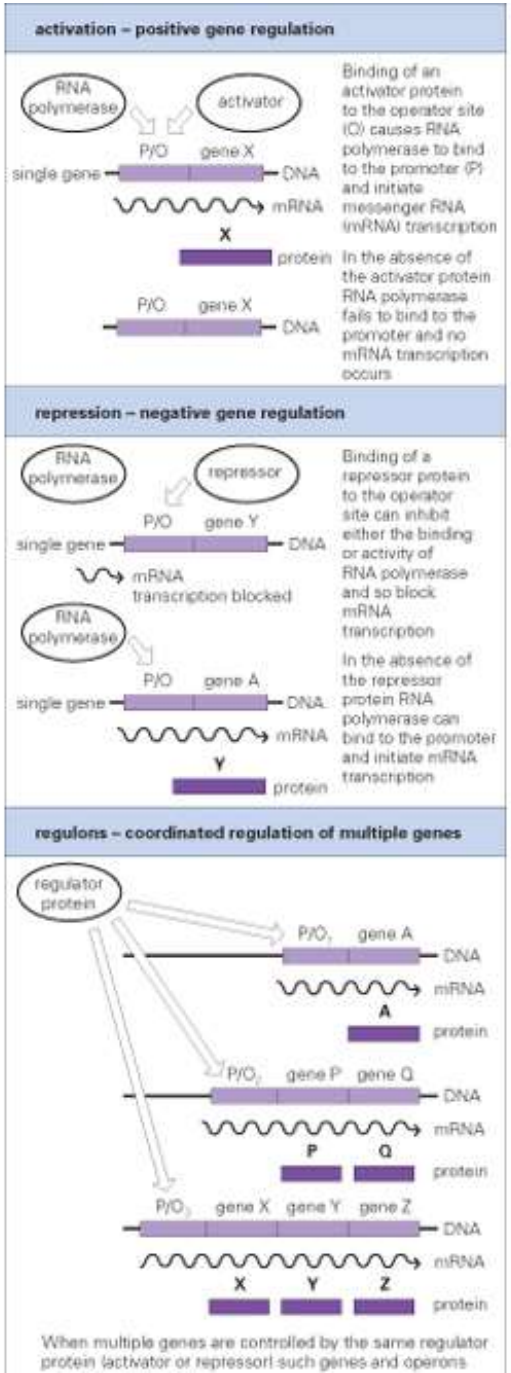


Spores

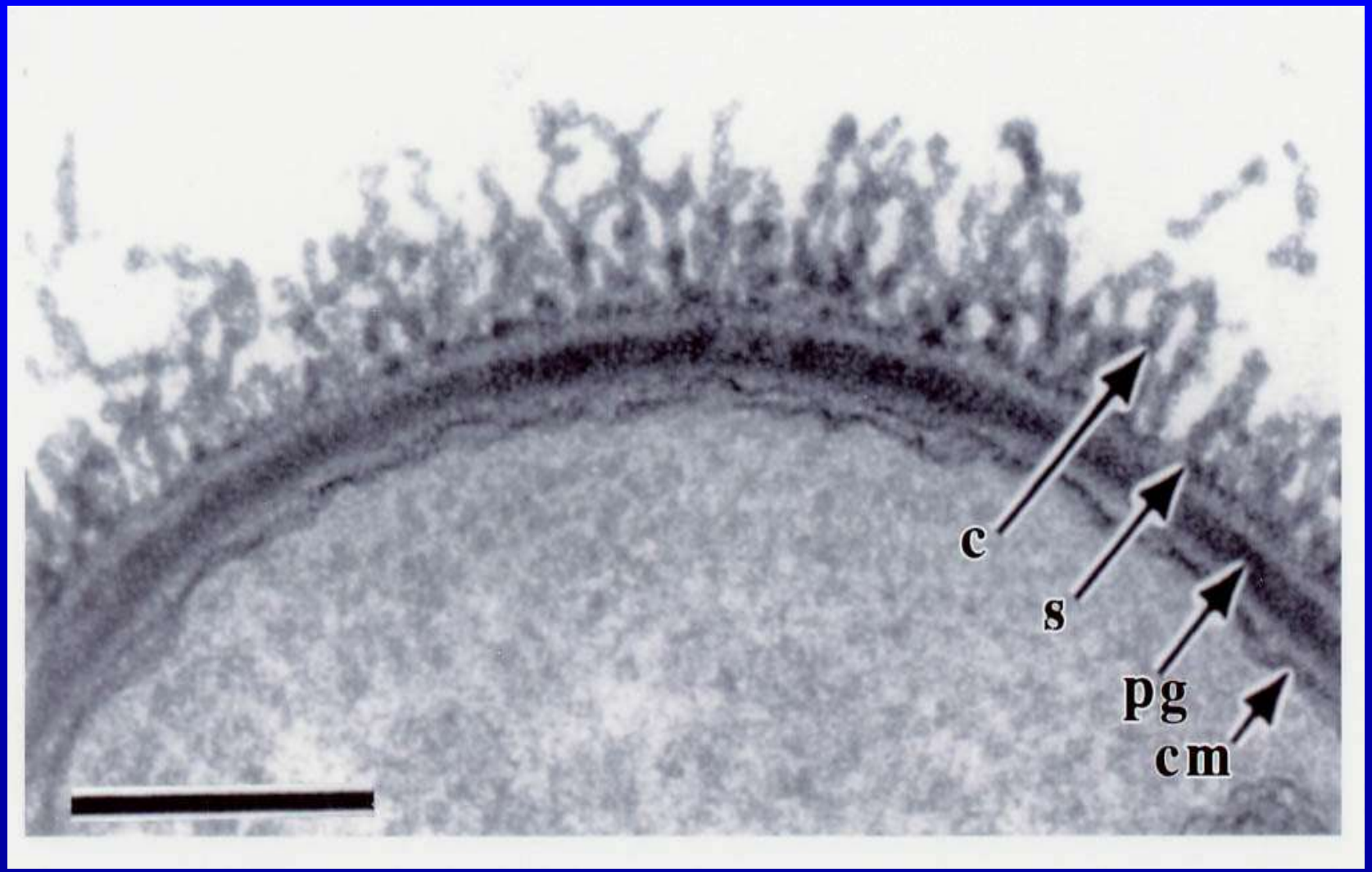


Sporulation

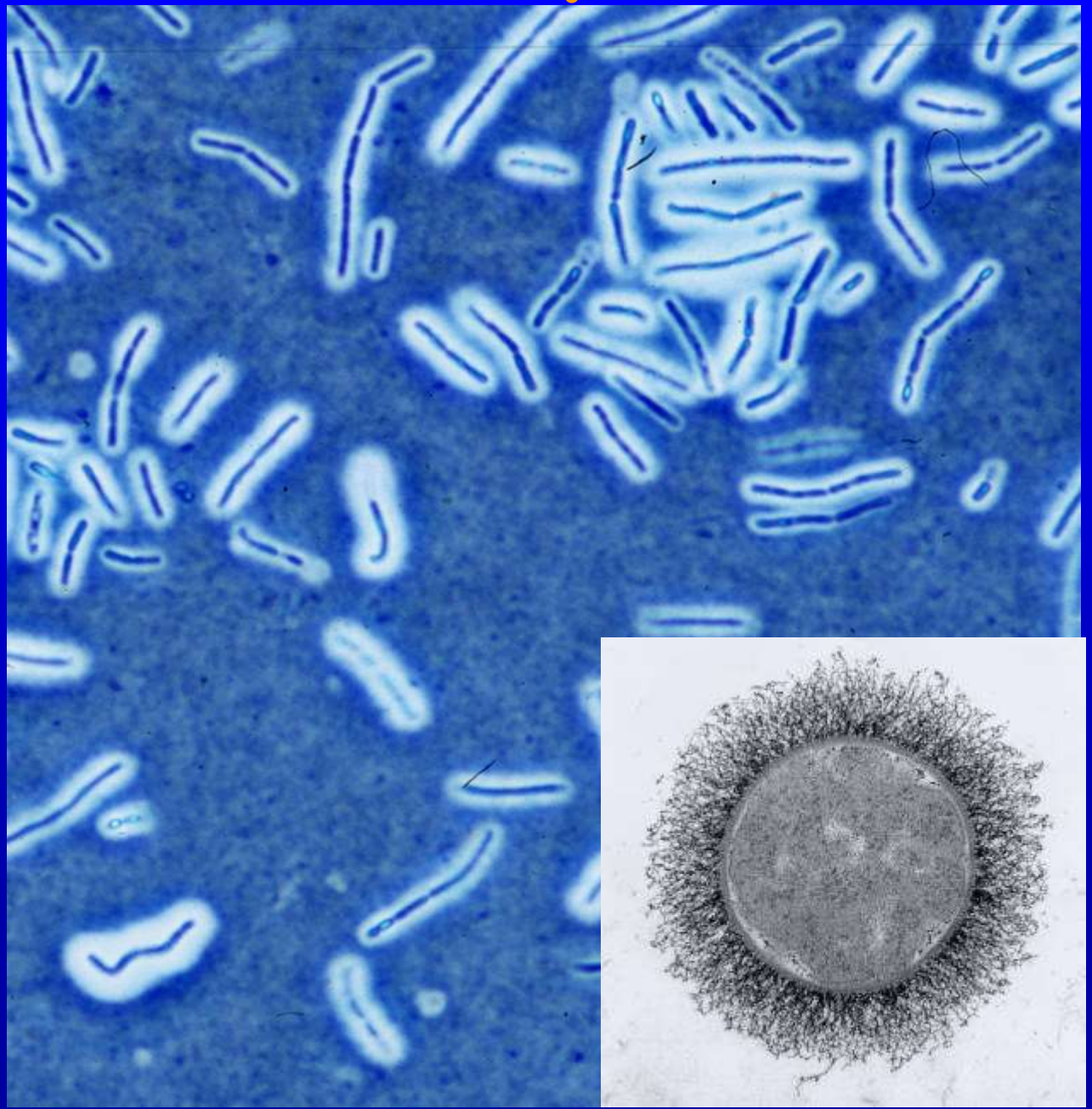




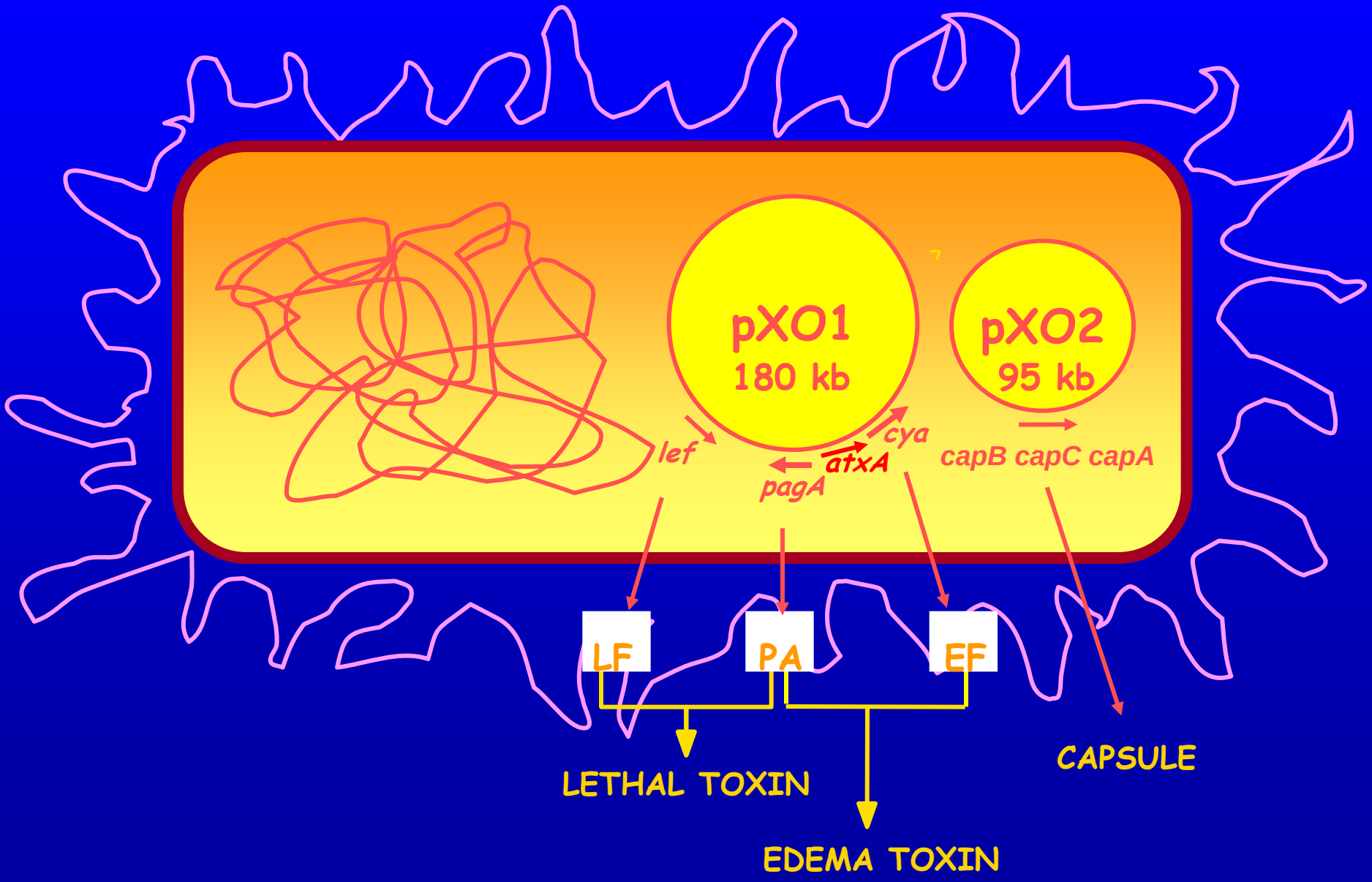
B. anthracis surface structure



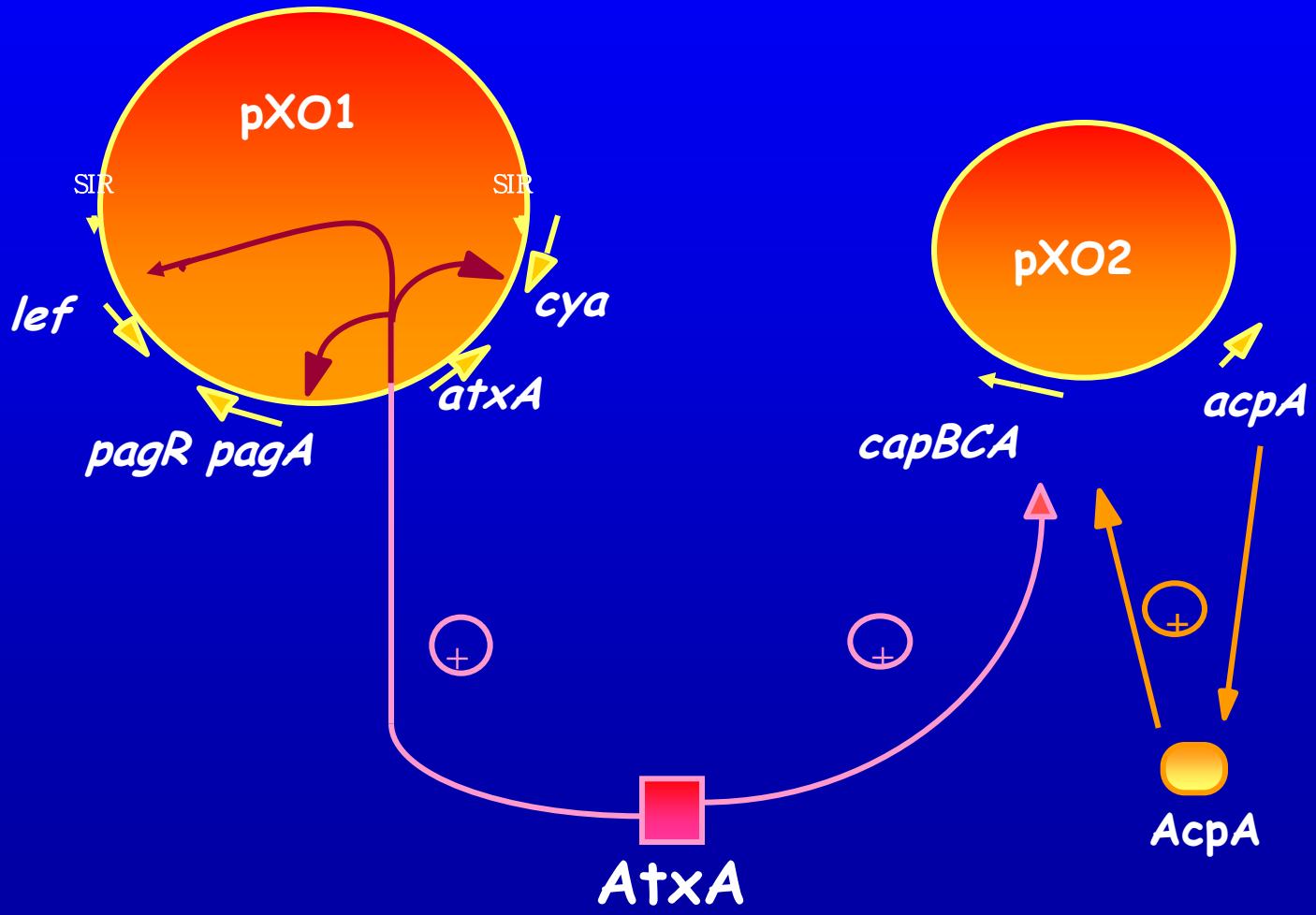
The capsule



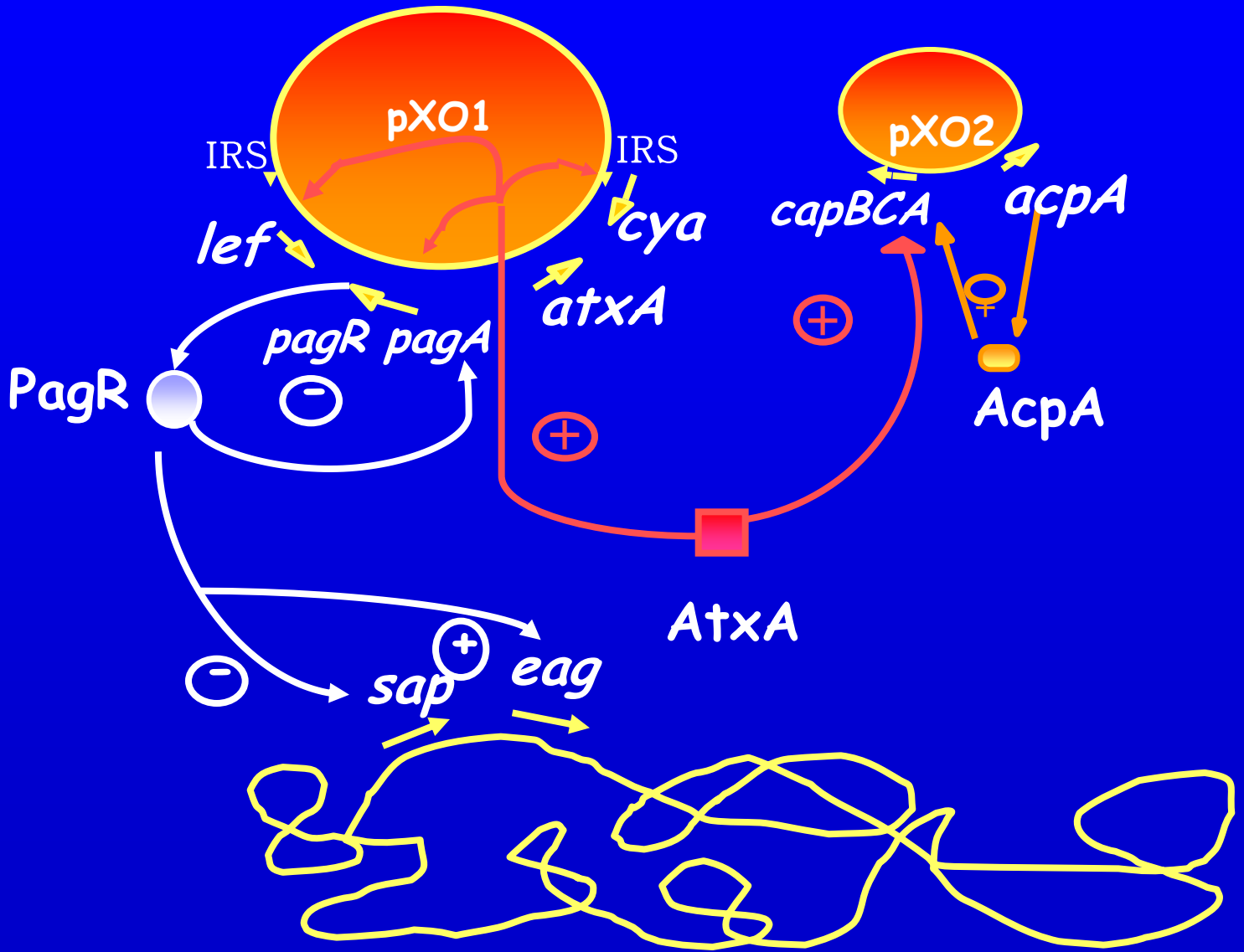
Virulence factors



AtxA : a central transcriptional activator on pXO1



The plasmid-encoded regulator AtxA couples the synthesis of toxins and surface structures



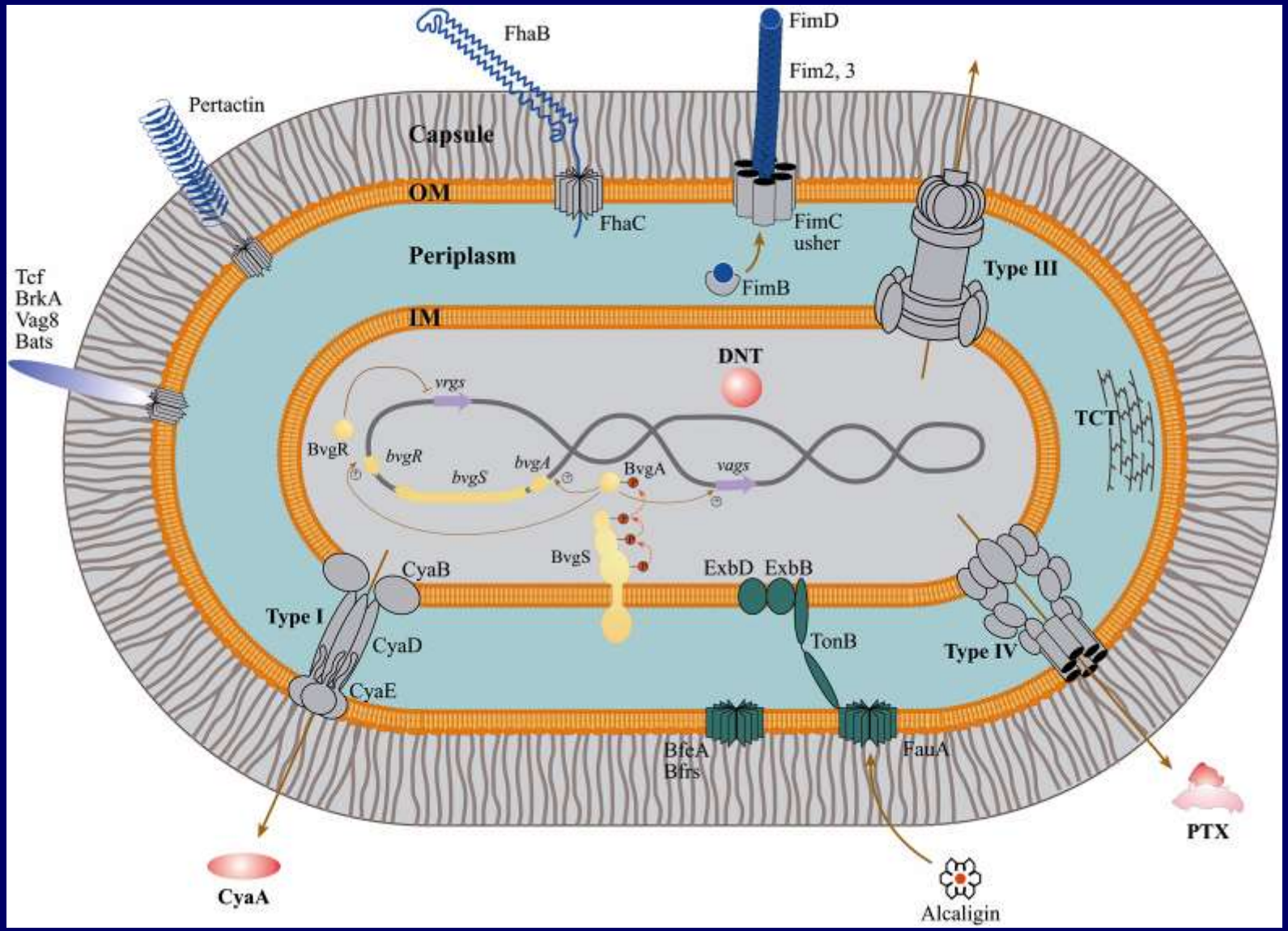
B. pertussis adhesins and toxins

Adhesins

- Filamentous haemagglutinin (FHA)
- Fimbriae
- Pertactin
- Tracheal colonisation factor (TCF)

Toxins

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


FhaB
FimD
Fim2, 3
Pertactin
Capsule
OM
FhaC
FimC
usher
Periplasm
FimB
Type III
Tcf
BrkA
Vag8
Bats
IM
DNT
vrgs
BvgR
bvgR
bvgS
bvgA
BvgA
vags
TCT
BvgS
CyaB
ExbD
ExbB
Type I
CyaD
TonB
Type IV
CyaE
BfeA
Bfrs
FauA
PTX
CyaA
Alcaligin

Bordetella pertussis phenotypic modulation

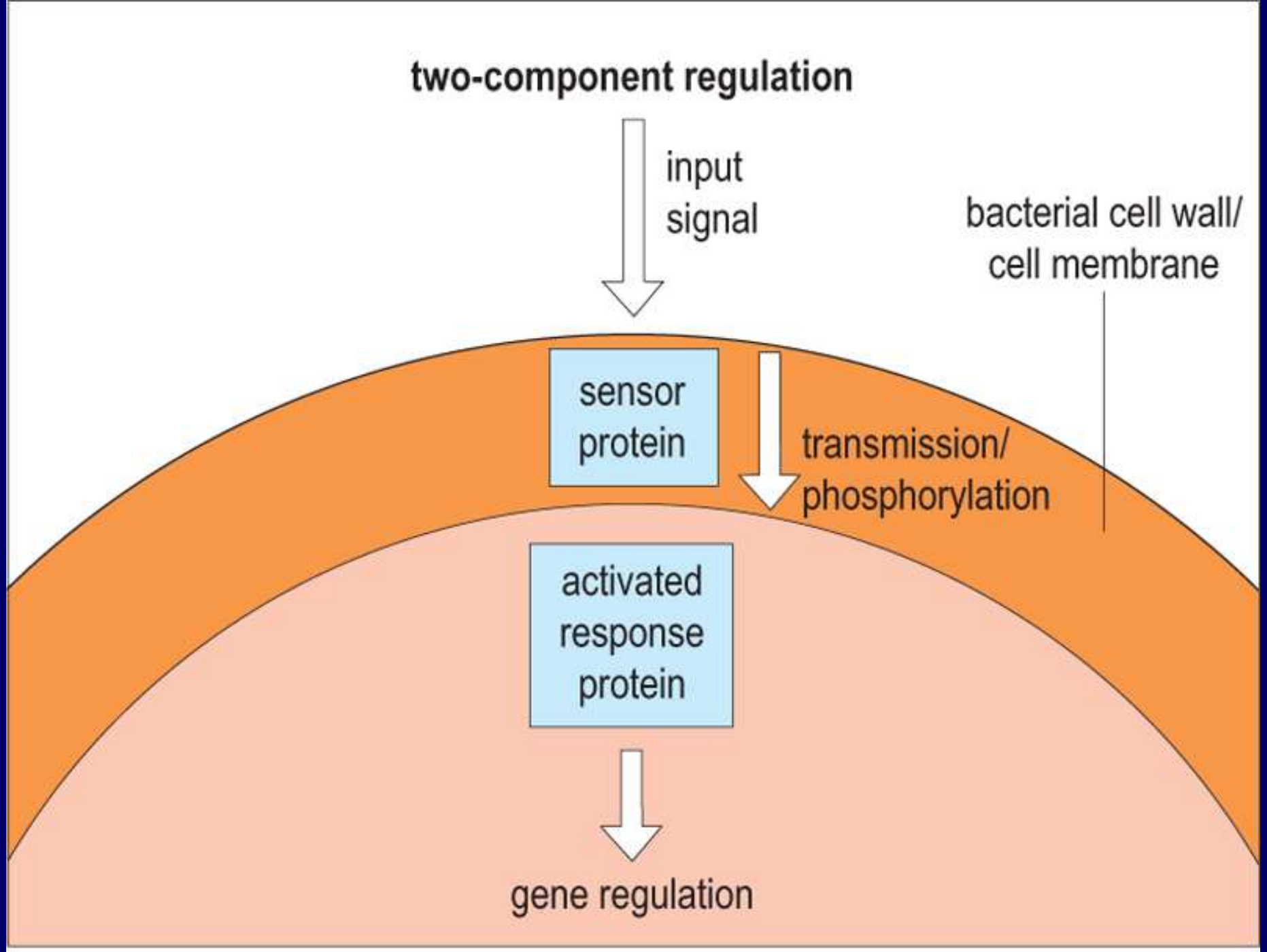
C mode

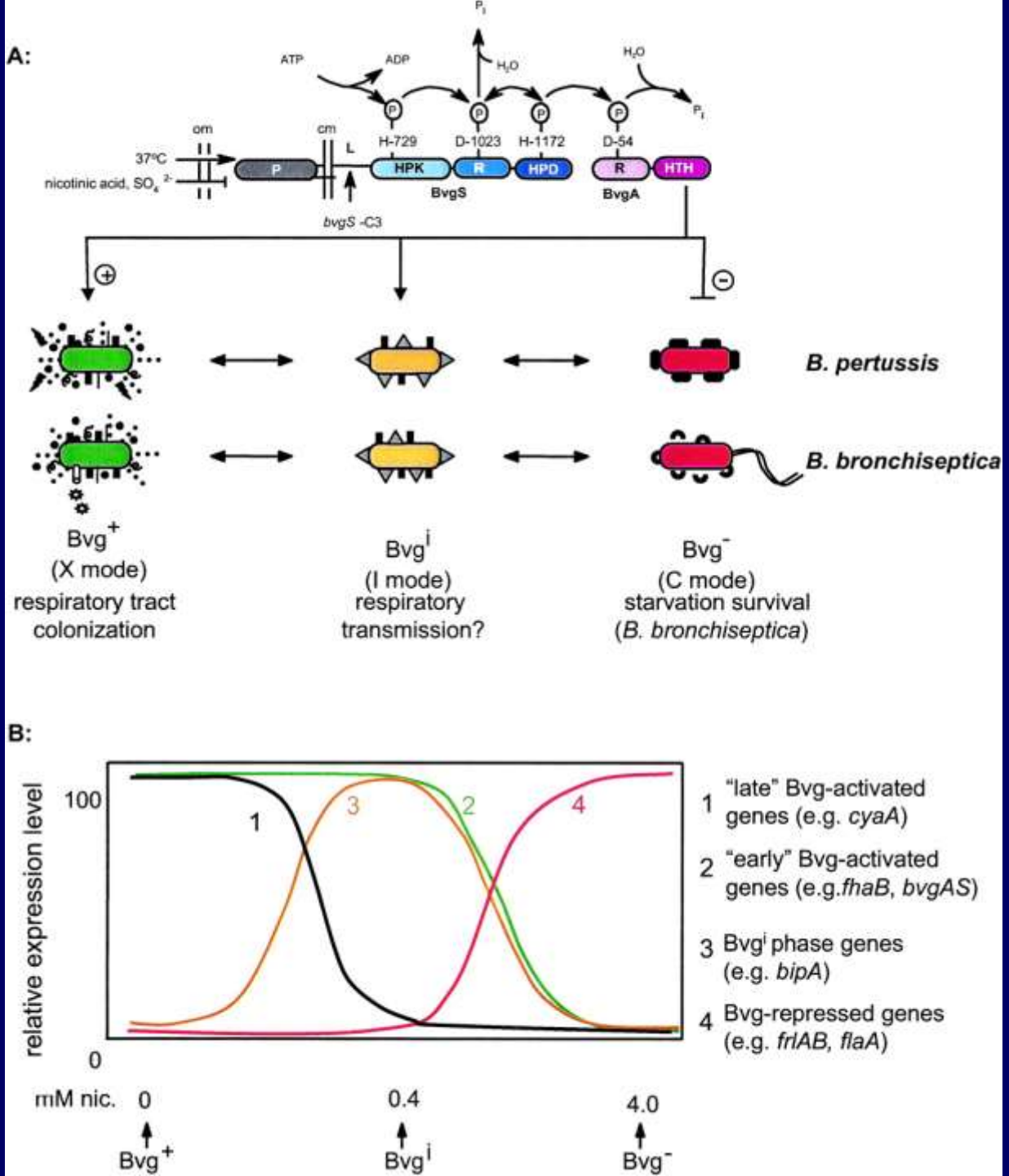
Vrgs

X mode

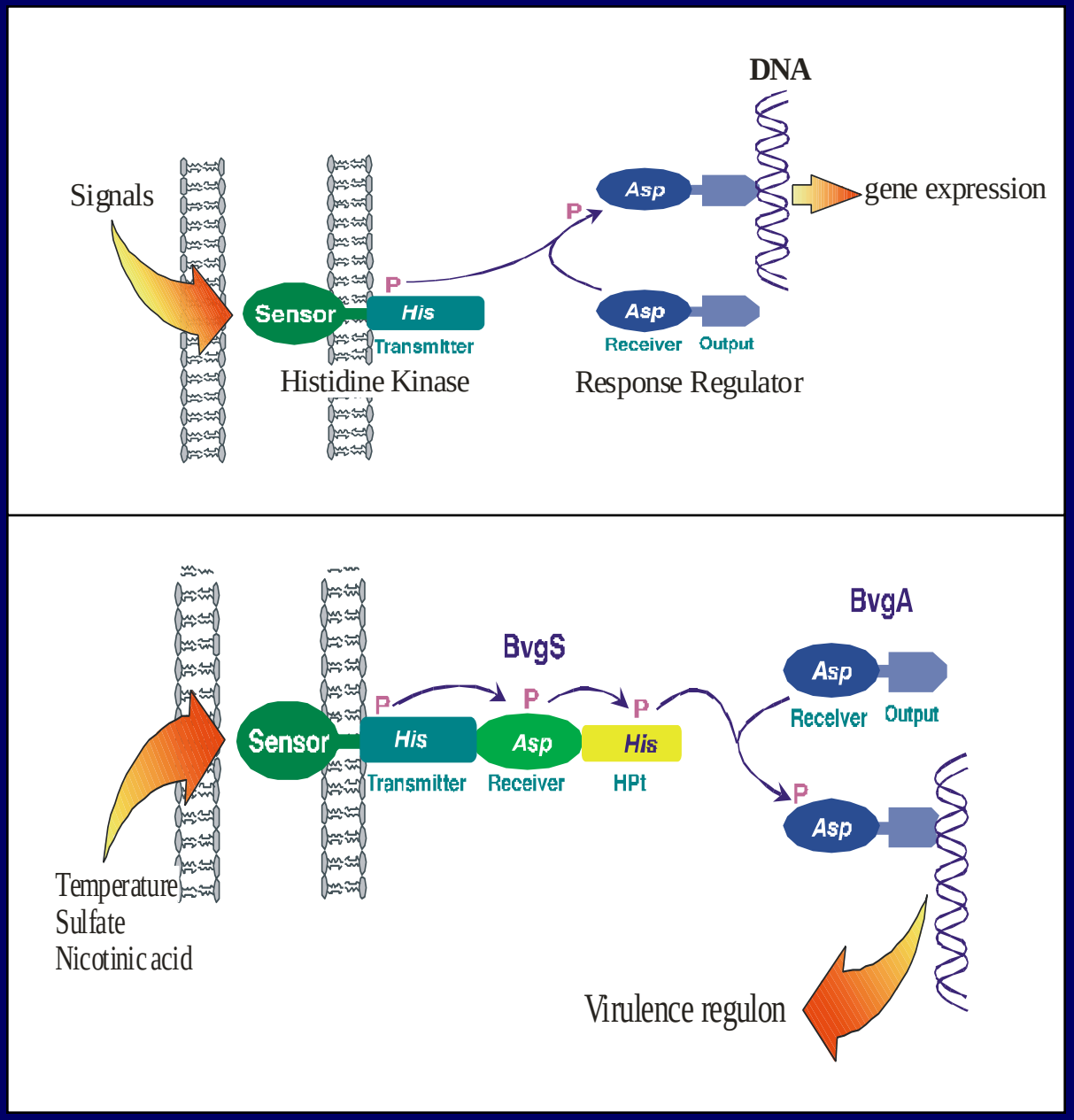
Vags

PTX
FHA
AC
pertactin

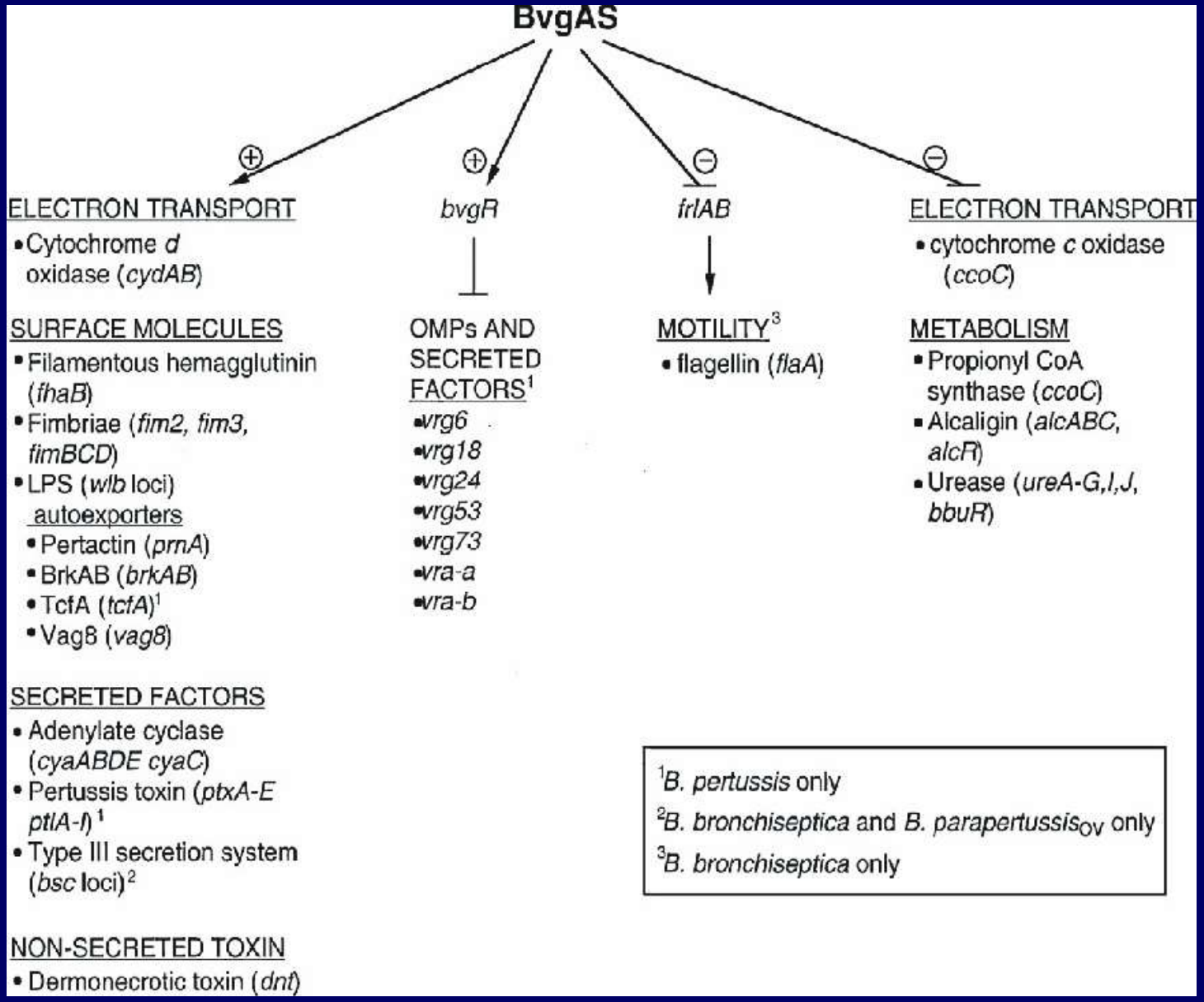



A:
ATP
ADP
P_i
H_2O
H_2O
P_i
om
cm
L
H-729
D-1023
H-1172
D-54
37°C
nicotinic acid, SO_4^{2-}
P
HPK
R
HPD
R
HTH
BvgS
BvgA
bvgS -C3
⊕
⊖
B. pertussis
B. bronchiseptica
Bvg+
(X mode)
respiratory tract
colonization
Bvgi
(I mode)
respiratory
transmission?
Bvg-
(C mode)
starvation survival
(B. bronchiseptica)
B:
relative expression level
100
0
1
3
2
4
mM nic.
0
0.4
4.0
Bvg+
Bvgi
Bvg-
1 "late" Bvg-activated genes (e.g. cyaA)
2 "early" Bvg-activated genes (e.g.fhaB, bvgAS)
3 Bvgi phase genes (e.g. bipA)
4 Bvg-repressed genes (e.g. frlAB, flaA)

Classical and unconventional two-component regulatory systems

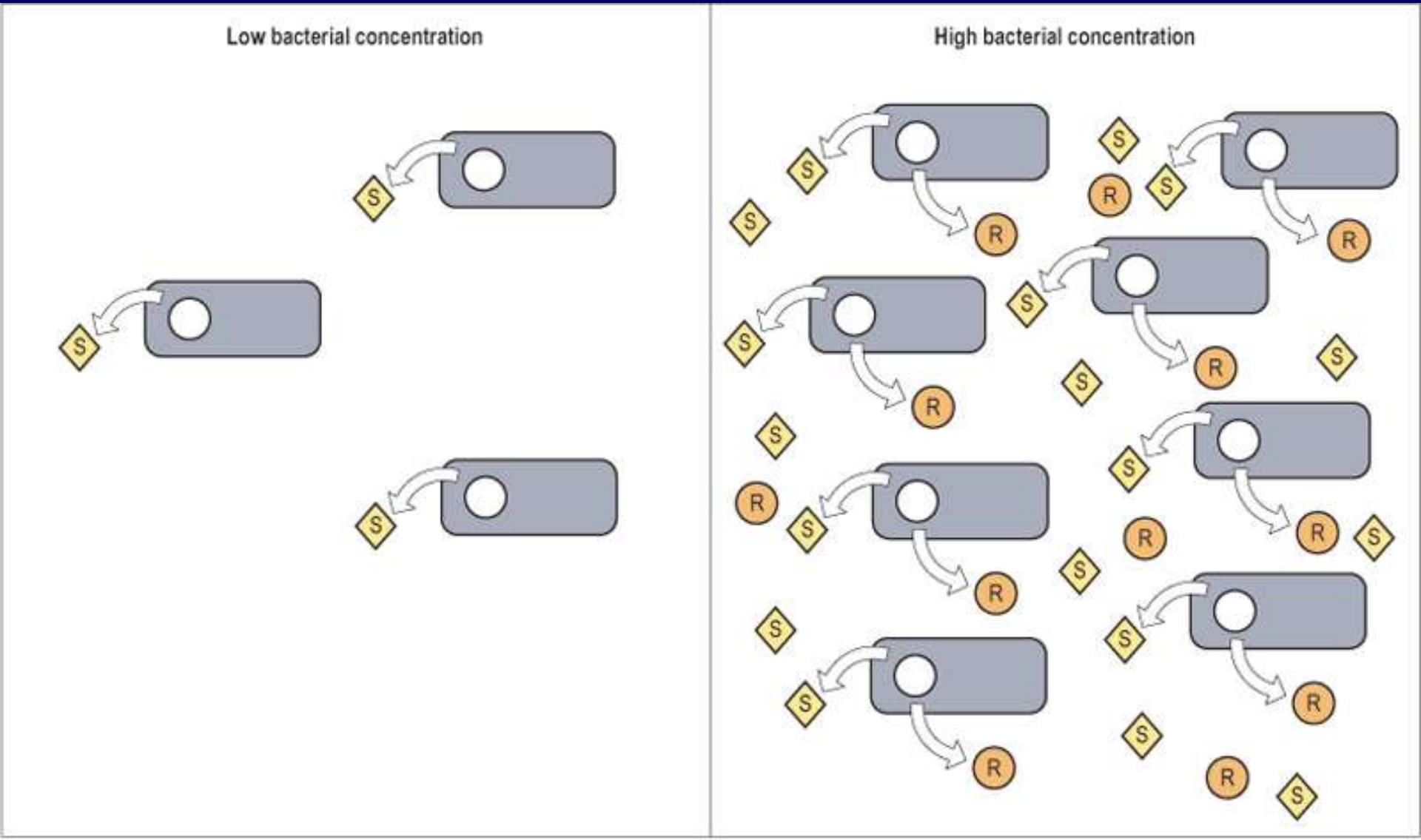


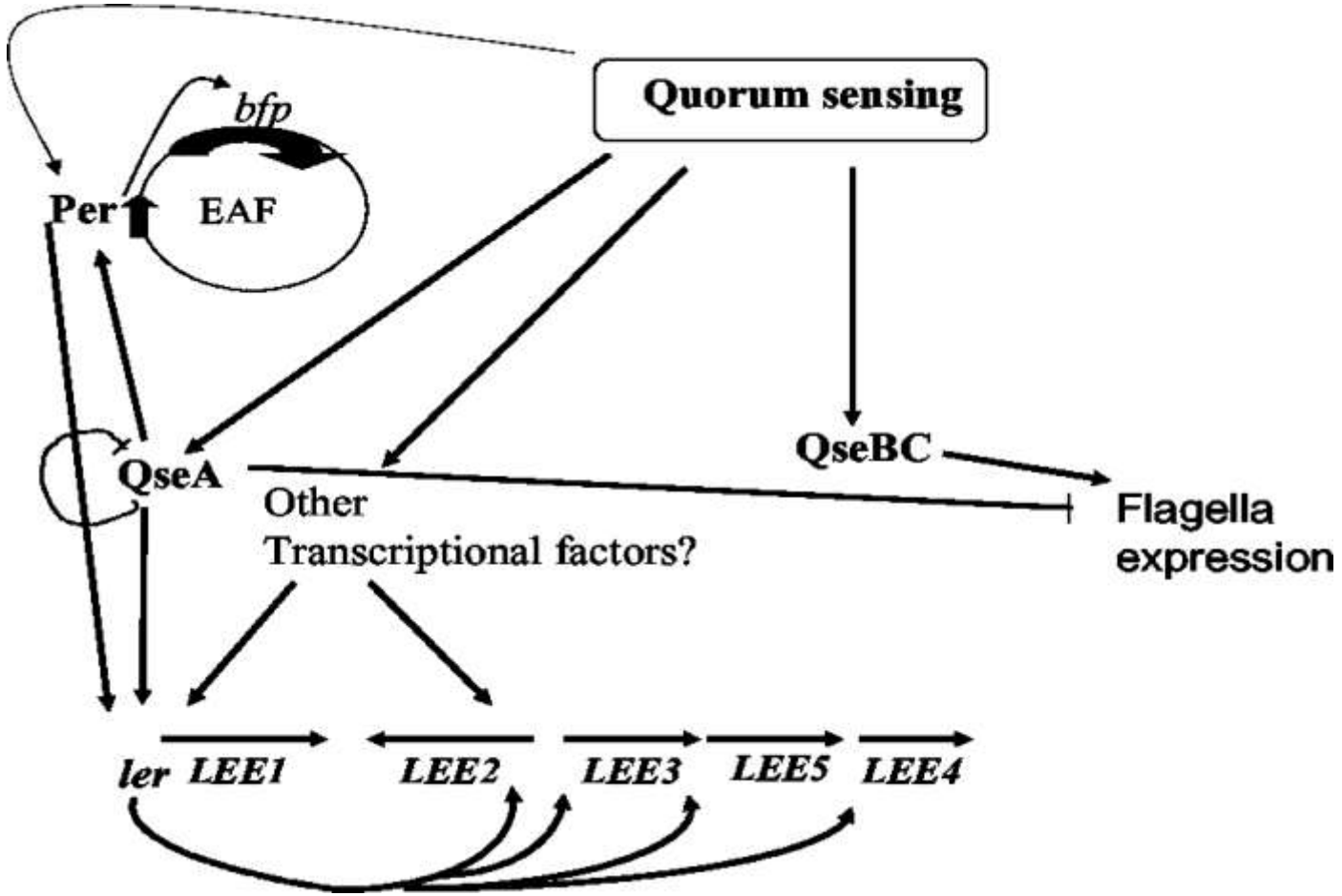
The Bvg-regulon of *Bordetella* species



Cotter and DiRita 2001

Quorum sensing





Schematic representation of virulence gene regulation by quorum sensing in EPEC. Ler activates transcription of the LEE genes. Transcription of *ler* is activated by quorum sensing through QseA and other, yet-unidentified transcriptional factors. QseA autorepresses its own transcription. Transcription of *bfp* is activated by Per, and transcription of *per* is autoactivated by Per and positively modulated by QseA. Quorum-sensing regulation of the flagellar regulon is activated by QseBC and repressed by QseA.

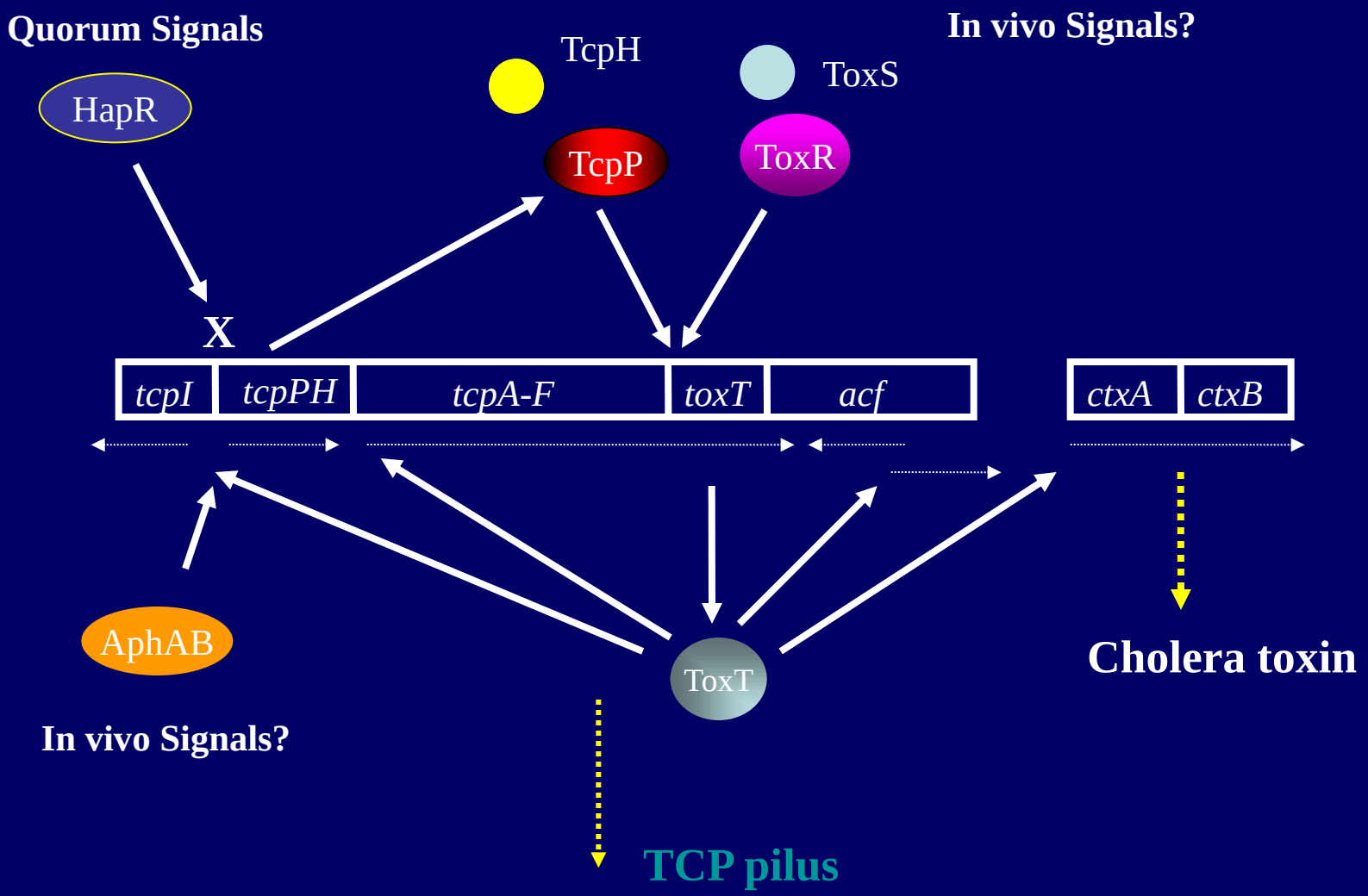
Quorum-sensing regulation is different in EPEC and EHEC.

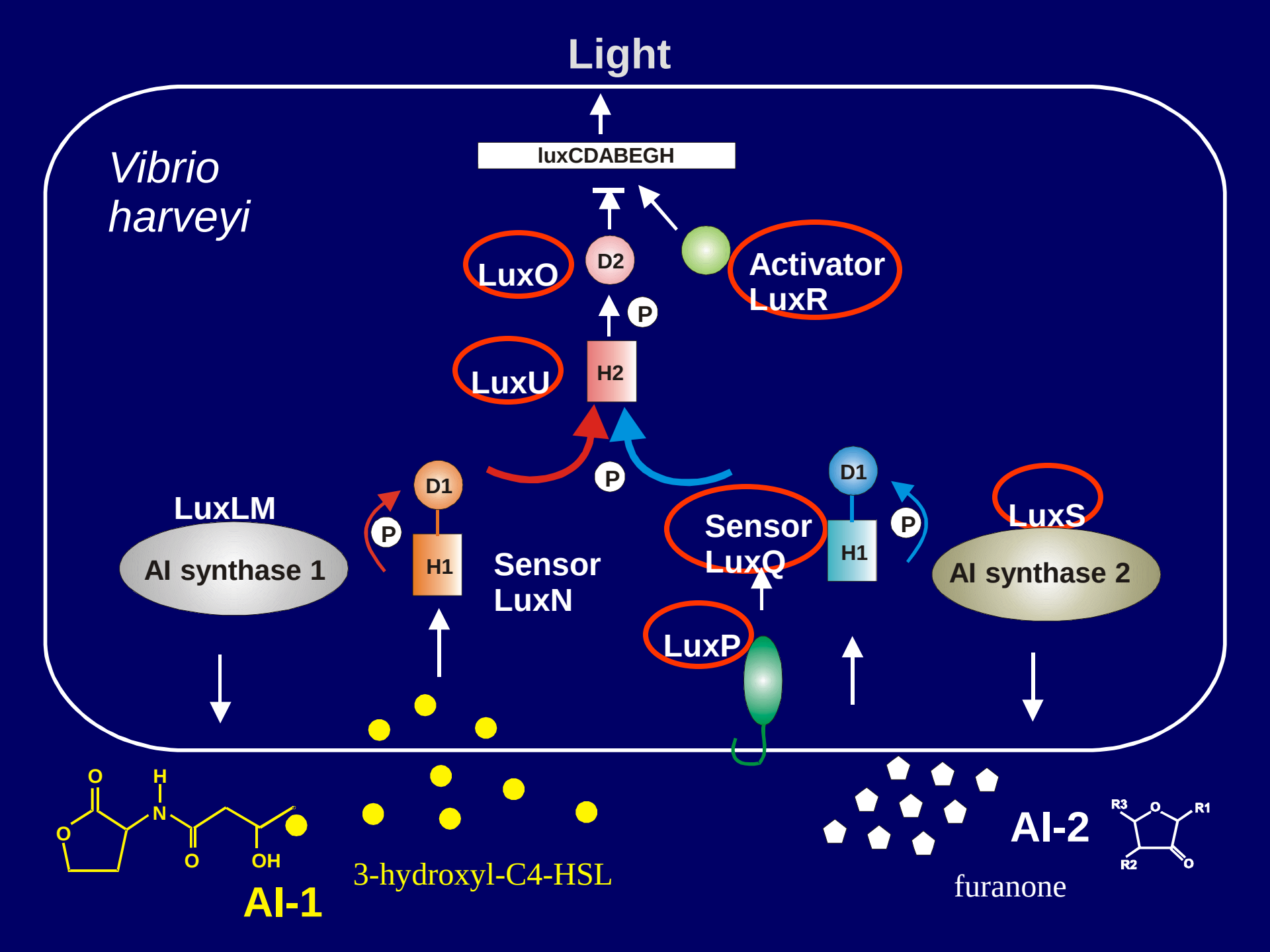
EPEC colonizes the proximal small intestine, which is thought to have very little or no resident flora. Therefore, while quorum sensing is primarily an ***interspecies*** signaling system during EHEC infection, it seems to be used for ***intraspecies*** signaling during EPEC infection.

Another remarkable difference between EPEC and EHEC regarding quorum-sensing regulation involves QseA. In EHEC, QseA activates transcription of the **LEE genes** but is not involved in the regulation of the **flagellar regulon**. **In EPEC, QseA** clearly **represses flagellation and motility.**

This difference may also be reflective of the differential role played by flagella in EPEC and EHEC pathogenesis. While flagella in EHEC are used mostly for swimming, they are involved in adherence and microcolony formation in EPEC, thus causing the need to coordinate transcription of the LEE genes with flagellation.

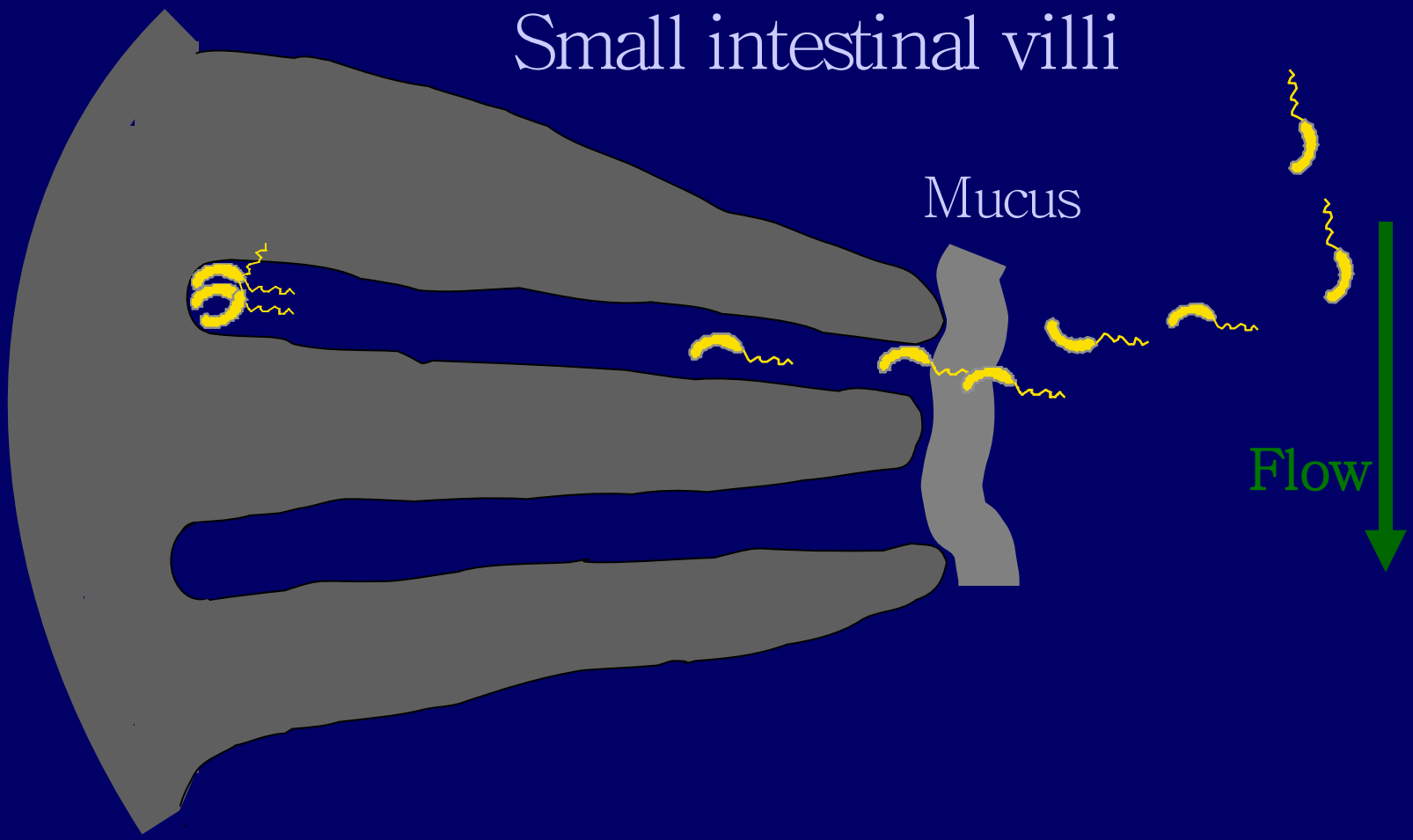
Regulatory Pathways Influencing the Expression of the Virluence Genes in *V. cholerae*



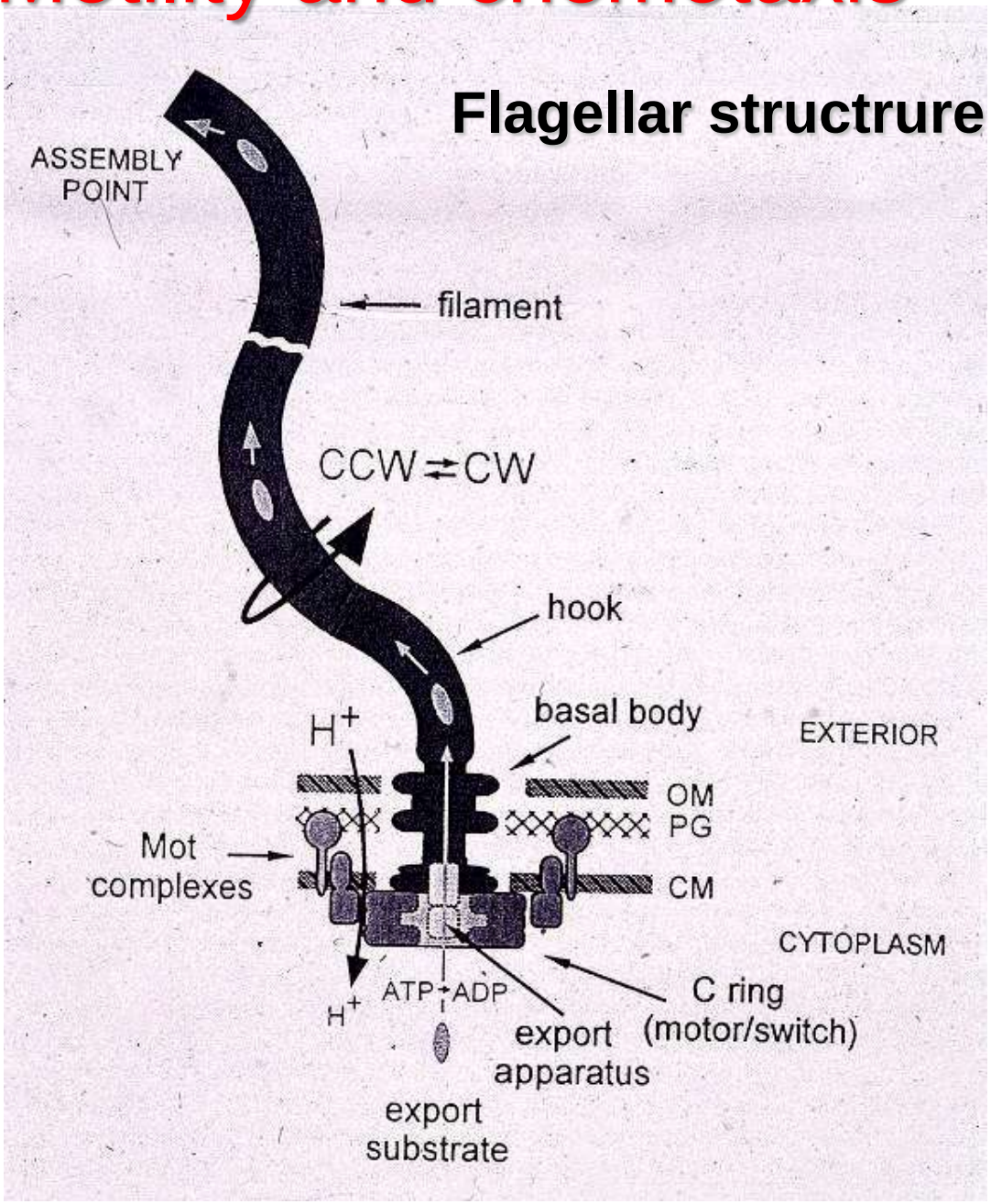

Light
luxCDABEGH
Vibrio harveyi
LuxO
D2
P
Activator LuxR
LuxU
H2
P
D1
H1
LuxLM
AI synthase 1
Sensor LuxN
D1
H1
P
Sensor LuxQ
LuxP
LuxS
AI synthase 2
O
H
N
O
O
OH
AI-1
3-hydroxyl-C4-HSL
AI-2
furanone
R3
O
R1
R2
O

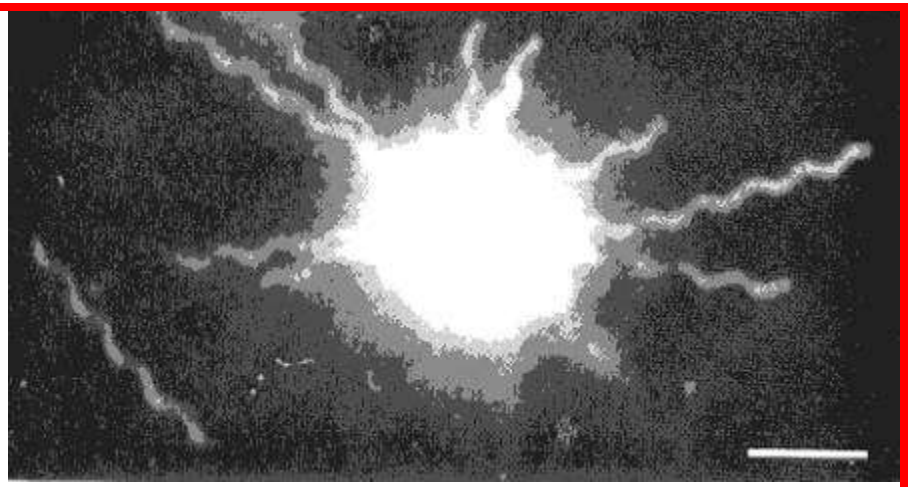
- Quorum sensing down-regulates CT and TCP by a HapR-dependent repression of TcpPH
- Quorum sensing up-regulates HA protease which can cause detachment from the epithelial surface
- Thus, quorum sensing may occur in vivo after ToxT-dependent biofilms have formed, and facilitate exit from the host
- Could *Vibrio* autoinducers be used as therapeutics to down regulate ToxT regulated virulence properties? Engineer banana for LuxS expression?

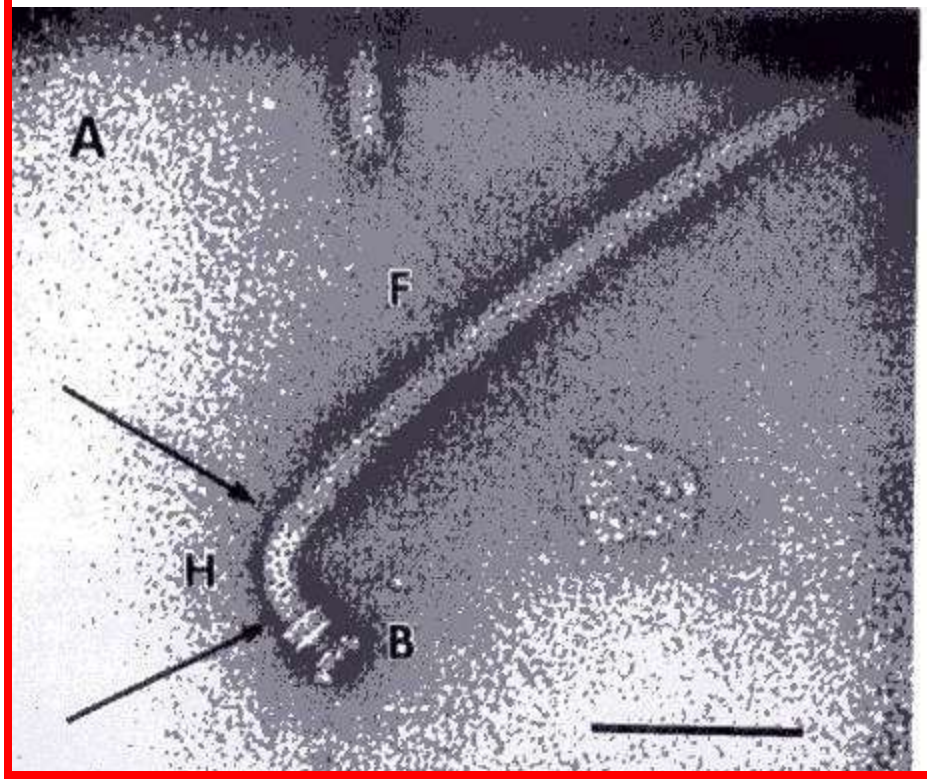
Motility and chemotaxis is crucial for V. Cholerae

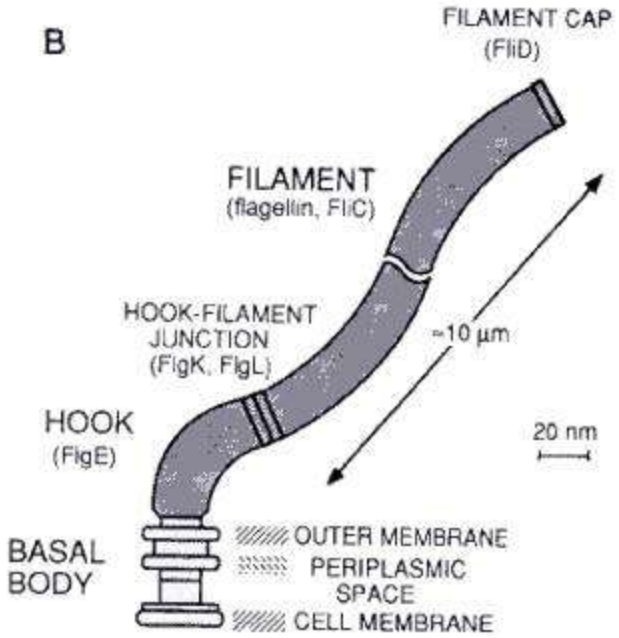


Motility and chemotaxis





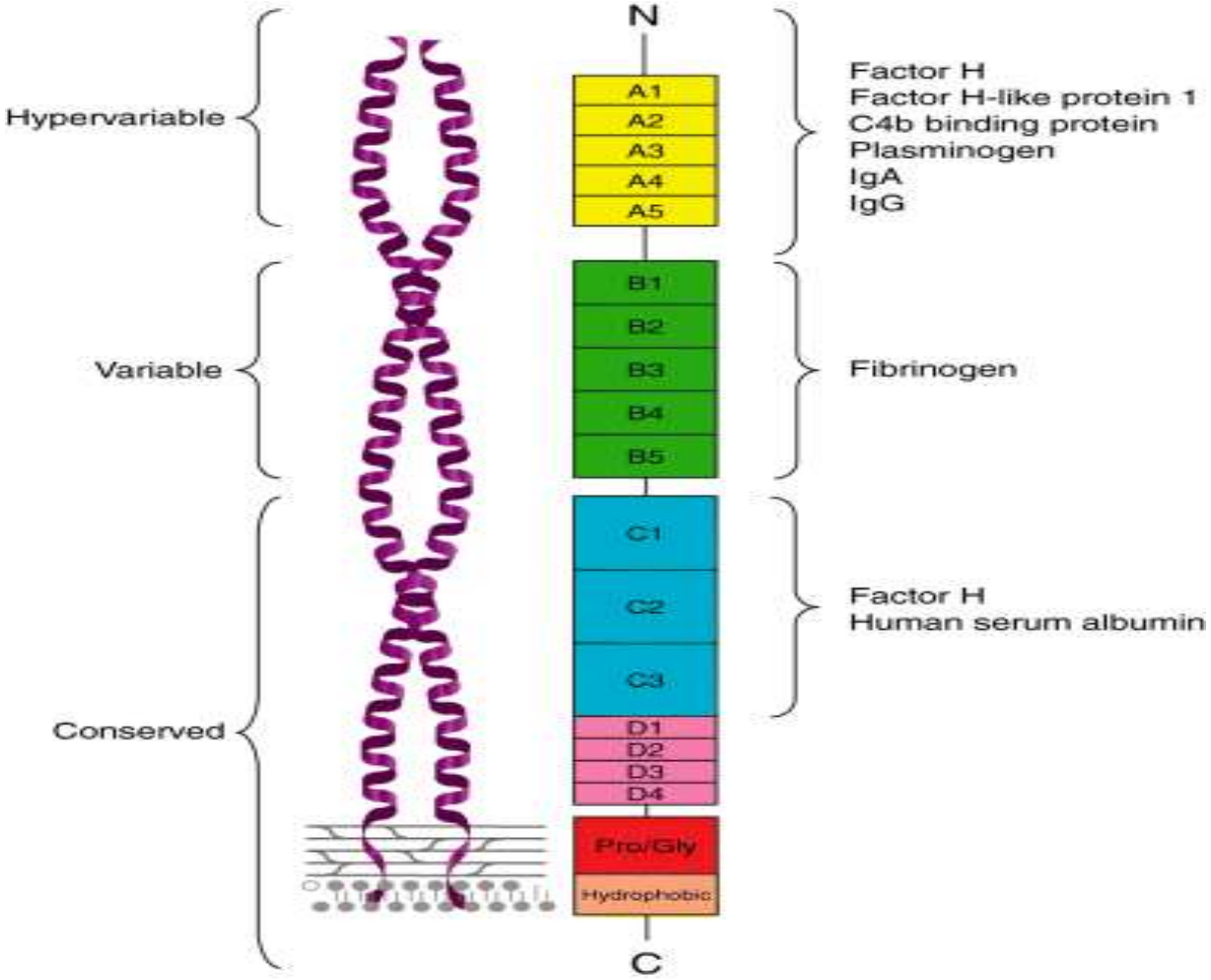

A
F
H
B


B
FILAMENT CAP
(FliD)
FILAMENT
(flagellin, FliC)
HOOK-FILAMENT
JUNCTION
(FlgK, FlgL)
~10 µm
HOOK
(FlgE)
20 nm
BASAL
BODY
OUTER MEMBRANE
PERIPLASMIC
SPACE
CELL MEMBRANE

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

Evading antibody responses

Stealth technology I: looking as ‘self’



Structural and functional characteristics of streptococcal M and M-like proteins. The molecule is anchored in the group A streptococcal cell wall via the C terminus (Pro/Gly rich and hydrophobic domains). M protein consists of A-, B-, C- and D-repeats with capacity to bind several human plasma proteins as indicated (right]). Adapted from Fischetti (2000).

Streptococcus pyogenes (group A streptococcus) colonizes skin and throat tissues resulting in a range of benign and serious human diseases.

Opsonization and phagocytosis are important defence mechanisms employed by the host to destroy group A streptococci. Antisera against the cell-surface M protein, of which over 150 different types have been identified, are opsonic and contribute to disease protection.

Human plasma proteins bound to M5 protein B- and C-repeats were shown to block opsonization.

Stealth technology I: PrtF1/SfbI – Fibronectin binding proteins of *Streptococci* - looking 'self'

binding 30 kDa N-term domain of fibronectin

- invisible for antibodies and complement

•**triggers internalization through integrins**

Formation of focal complex (signaling cascade involving FAK, Rho fam. Proteins) – cytoskeleton rearrangement – **phagocytic vacuole**

Can lead to resting or multiplication of the bacteria

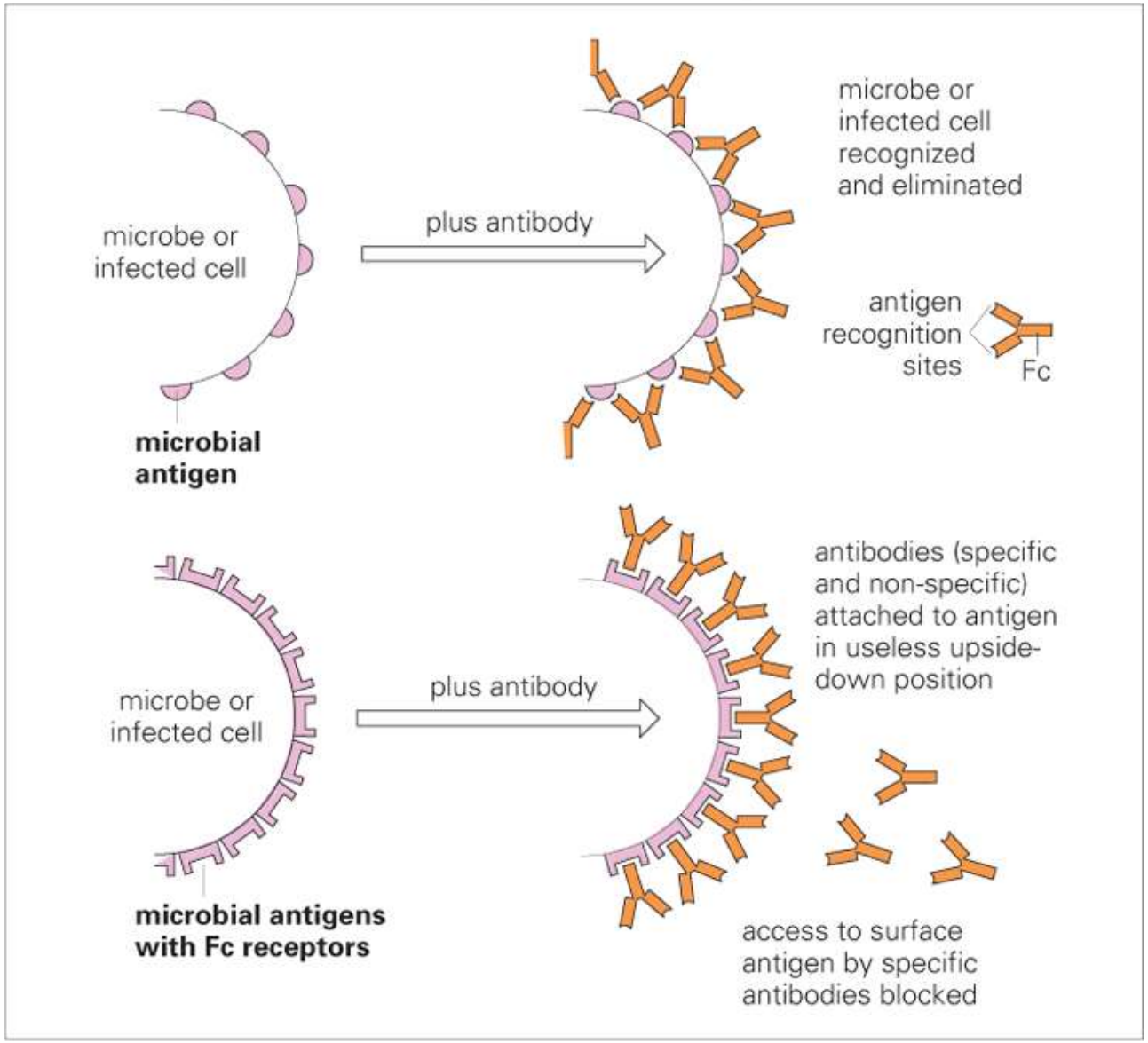
Prerequisite for Group A *Streptococci* persistance - hiding in epithelial cells

Stealth technolog I: Coating by Plasmin(ogen): crucial host factor for invasive GAS infection

- GAS interacts with plasminogen to acquire surface plasmin, that can not be regulated by alpha-antiplasmin
  - Mechanism to hijack the host fibrinolytic system
  - Can be the cause of human specificity of GAS
- Plasminogen-binding proteins
  - PAM - Plasminogen-binding group A streptococcal M-like protein
  - SEN – surface alpha enolase
  - Plr/SDH/GAPDH
  - Streptokinase

Chhatwal GS, McMillan DJ, Tr Mol Med, Vol11 No4 april 2005
Wlaker MJ et al, Tr Microbiol Vol 13 No7 July 2005

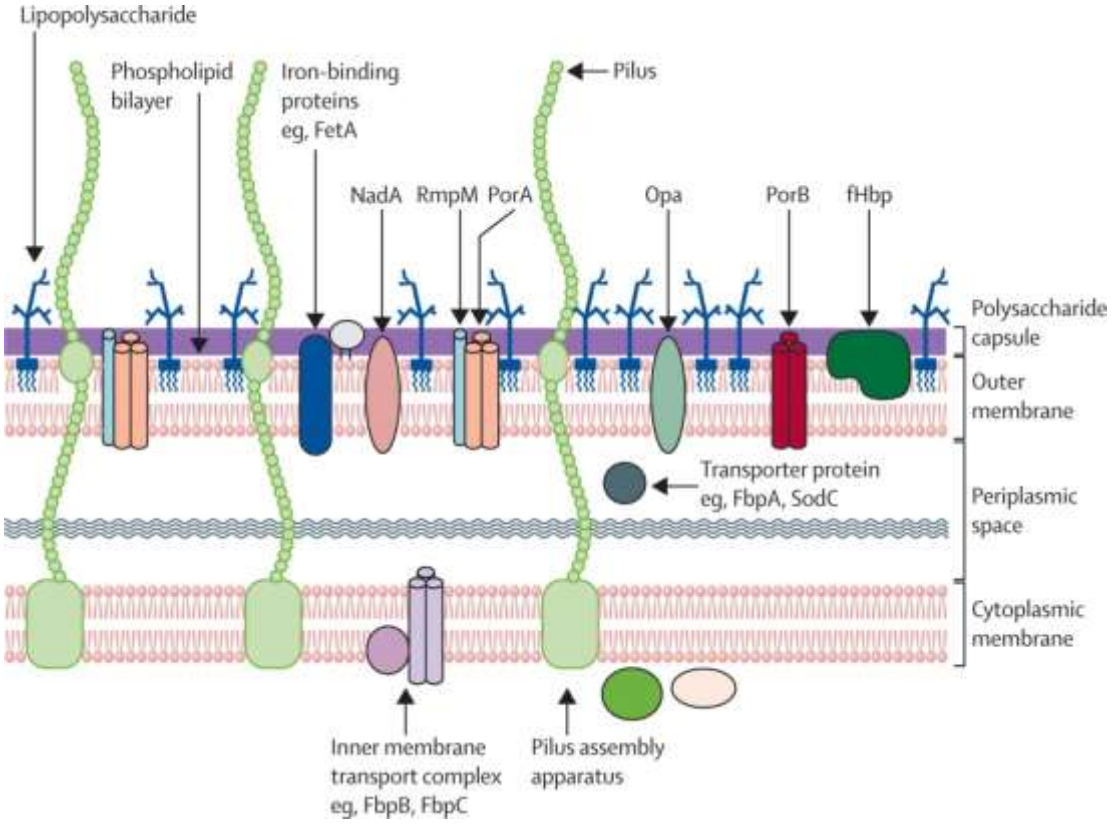
Stealth technology II: protein A or protein G bind IgG through Fc fragments…



Stealth technology III: Non-immunogenic capsule

The key component of the *Neisseria meningitidis* serogroup B capsule is an α2–8-linked sialic acid homopolymer, which contains epitopes that are cross-reactive with the polysialylated form of the neural cell adhesion molecule.

Anticapsular antibodies would crossreact with host antigens and contribute to autoimmune disease.



Stealth technology III: Molecular mimicry between *Helicobacter pylori* and the host

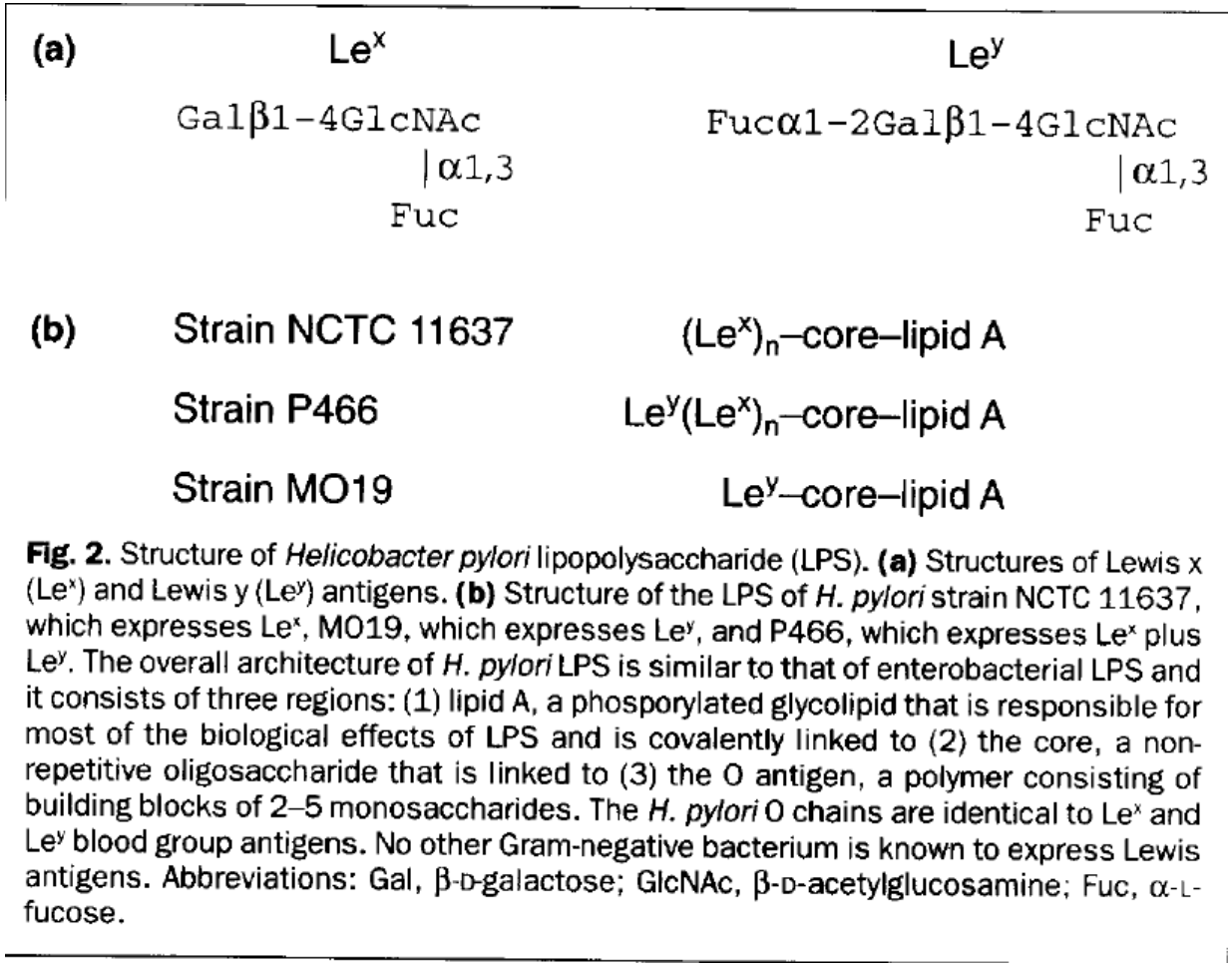
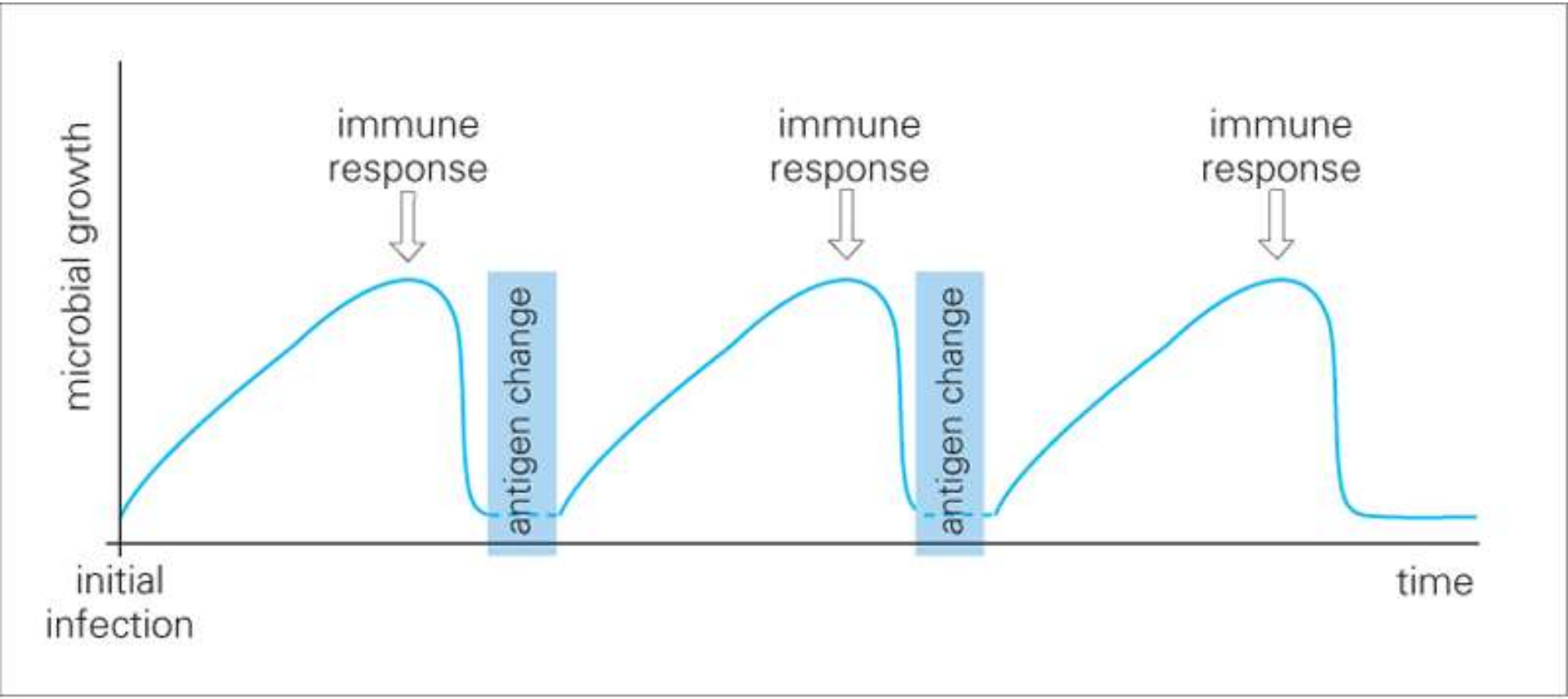


Fig. 2. Structure of *Helicobacter pylori* lipopolysaccharide (LPS). **(a)** Structures of Lewis x (Le^x) and Lewis y (Le^y) antigens. **(b)** Structure of the LPS of *H. pylori* strain NCTC 11637, which expresses Le^x, MO19, which expresses Le^y, and P466, which expresses Le^x plus Le^y. The overall architecture of *H. pylori* LPS is similar to that of enterobacterial LPS and it consists of three regions: (1) lipid A, a phosporylated glycolipid that is responsible for most of the biological effects of LPS and is covalently linked to (2) the core, a non-repetitive oligosaccharide that is linked to (3) the O antigen, a polymer consisting of building blocks of 2–5 monosaccharides. The *H. pylori* O chains are identical to Le^x and Le^y blood group antigens. No other Gram-negative bacterium is known to express Lewis antigens. Abbreviations: Gal, β-D-galactose; GlcNAc, β-D-acetylglucosamine; Fuc, α-L-fucose.

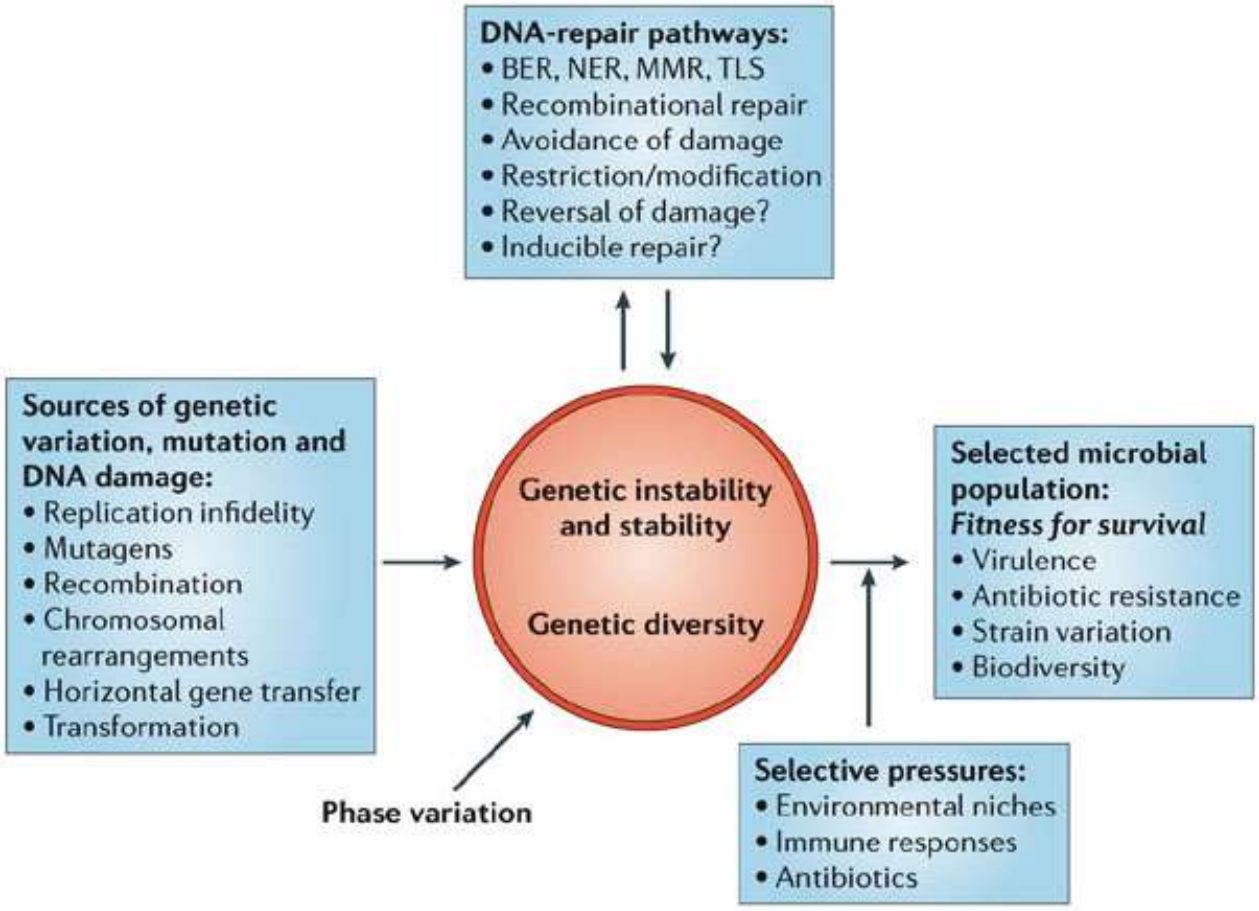
Helicobacter pylori lipopolysaccharide (LPS) expresses Lewis x and Lewis y blood group antigens that are identical to those occurring in the human gastric mucosa.

Antibodies, against LPS, which bind to host Lewis antigens may cause autoimmune inflammation.

Stealth technology IV: antigenic variation



A champion of antigenic variation: the meningococcus



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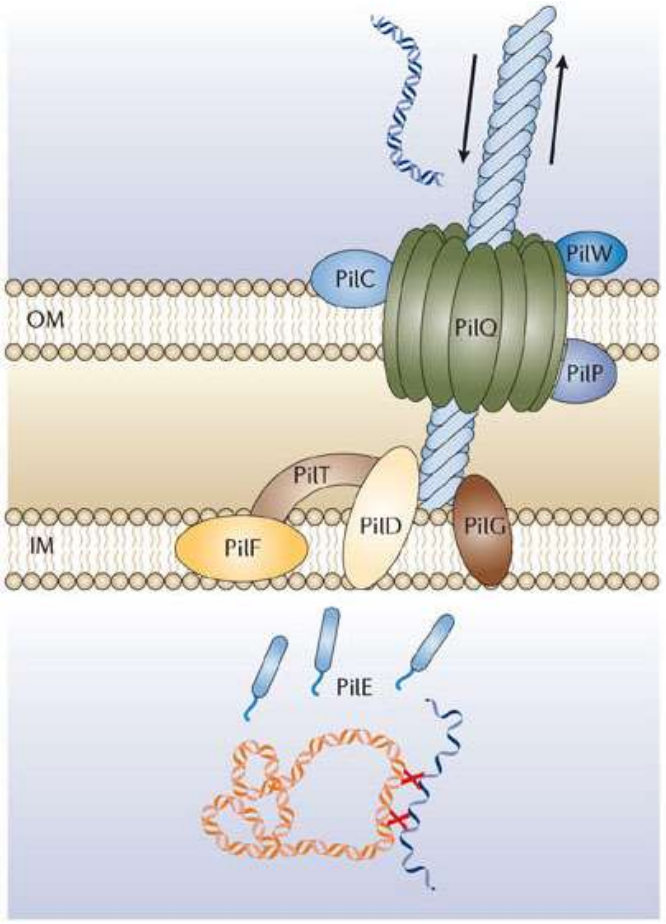
DNA repair and selective pressures promote genetic diversity and fitness of meningococci. **Mutations and DNA damage** that will often be harmful, but simultaneously represent a **source of genomic variation**. **Horizontal gene transfer and recombination direct genomic diversification** by incorporating new genetic information and shuffling the genome. **Phase variation and antigenic variation produce diverse antigens** in important surface-associated virulence factors, and constitute an important pathway for the **avoidance of host immune systems**. The meningococcus therefore **relies on frequent genetic diversification** to produce **a surplus of spontaneous variants**. Although genomic variation is a prerequisite for meningococcal fitness and survival, the result of an 'ever-changing' genome is genomic instability. Selective pressures in the meningococcus habitat, such as environmental characteristics, immune responses and antibiotics, influence meningococcal fitness for survival. BER, base excision repair; MMR, mismatch repair; NER, nucleotide excision repair; TLS, translesion synthesis.

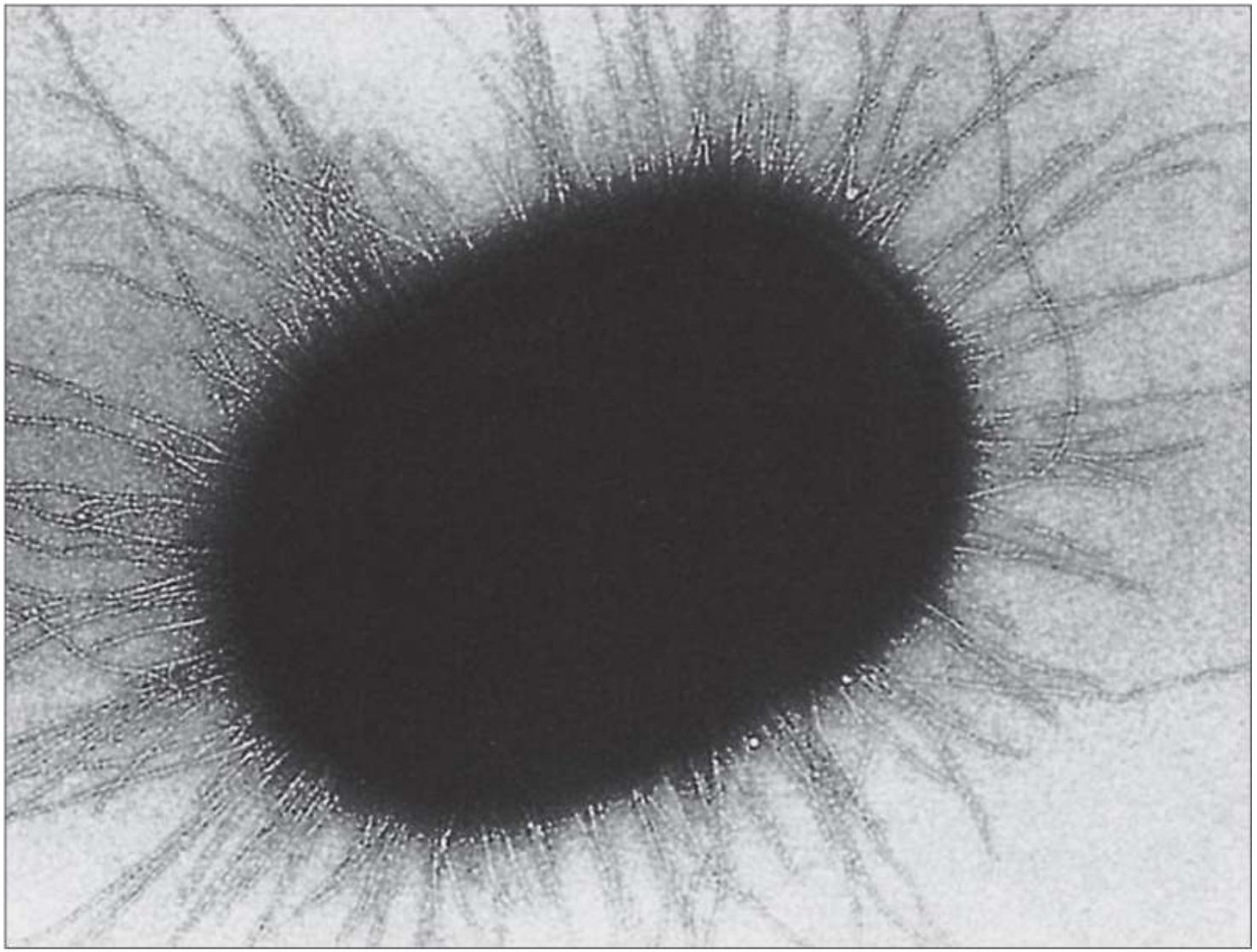
Horizontal gene transfer by transformation of exogenous DNA.



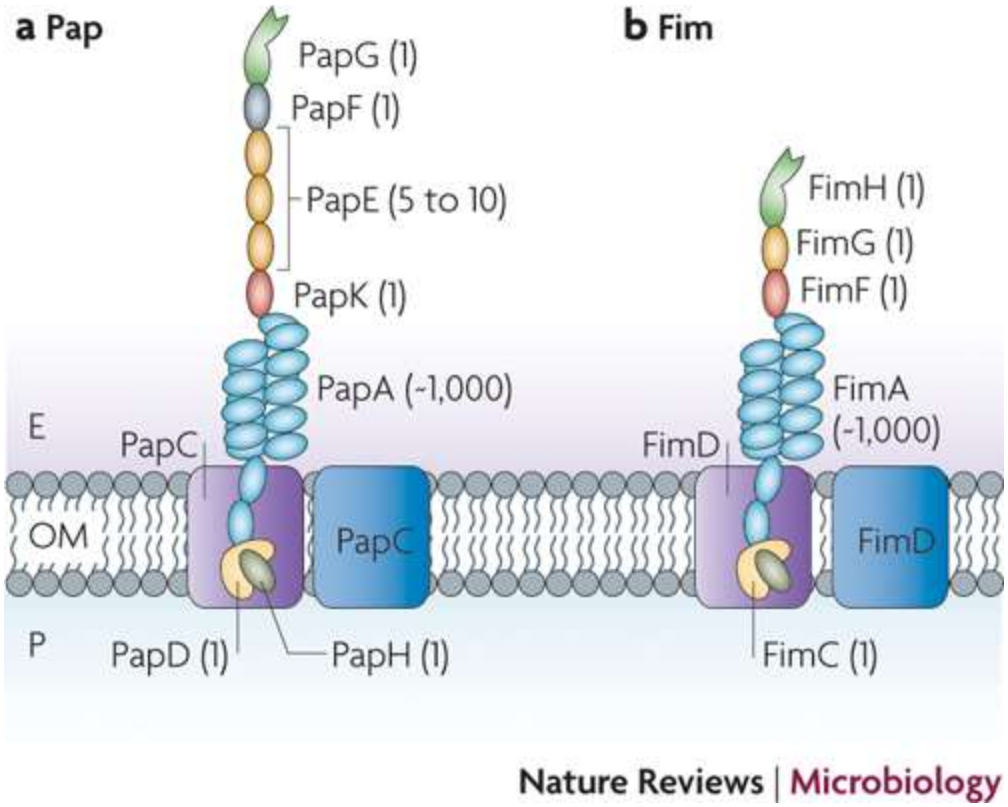
DNA in the environment can be entangled by retracting type IV pili and introduced into the cell through the outer (OM) and inner membranes (IM). Components of the type IV pilus biogenesis apparatus in Neisseria meningitidis are shown. In the cytoplasm, the incoming DNA (blue) is integrated into the genome (red) by homologous recombination127.

Adhesion *versus* evasion of antibody response…

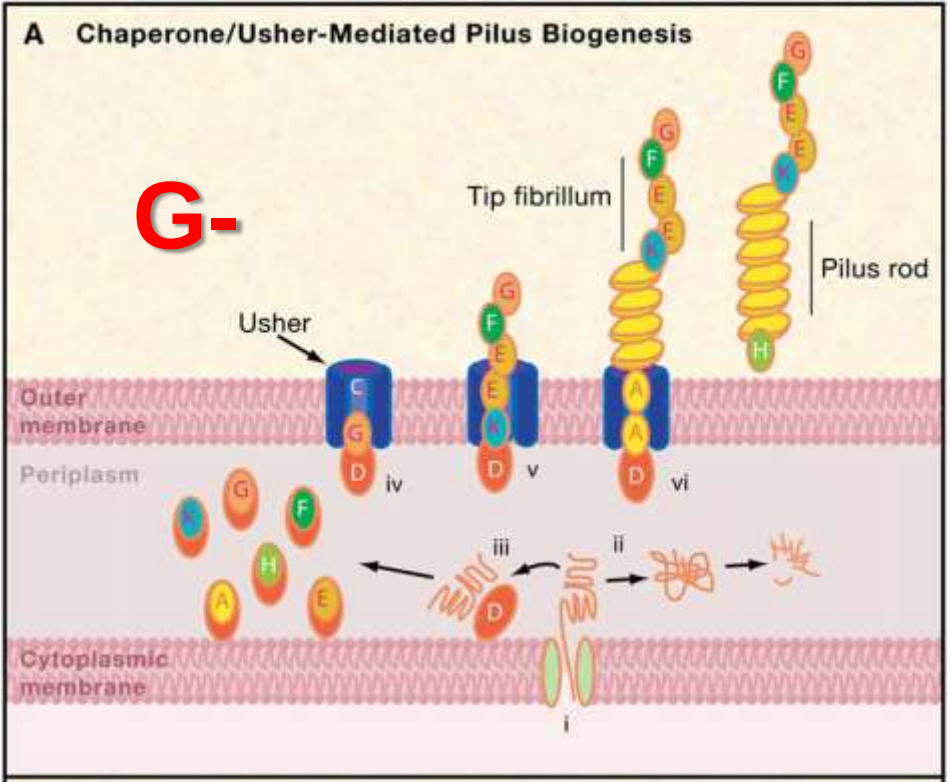
Pili and fimbriae:
Major adhesins = major antigens…

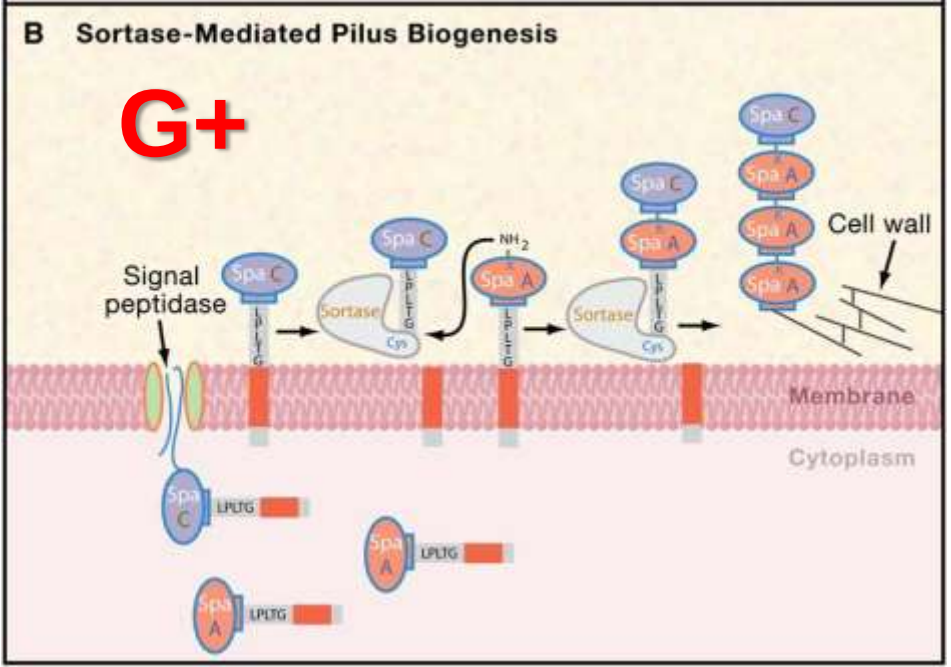


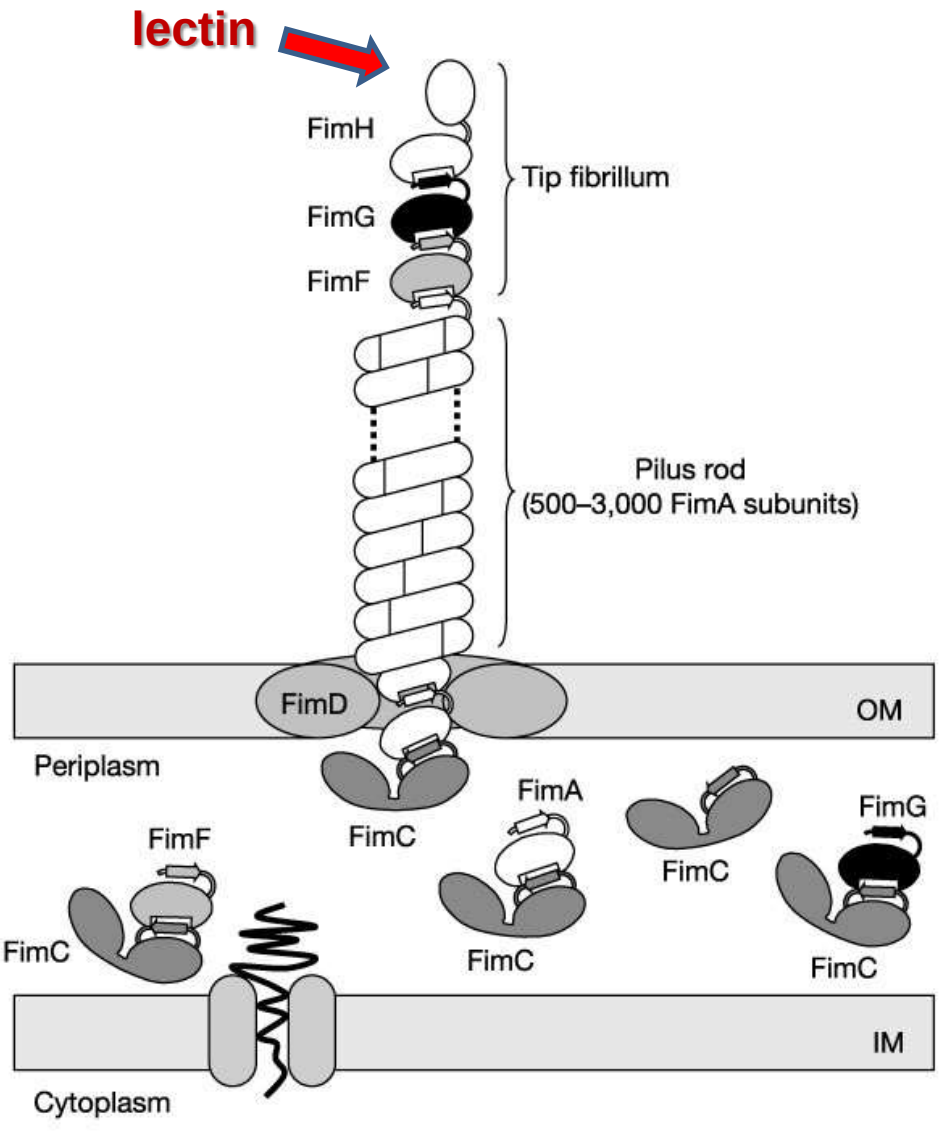
P and type 1 pili/fimbriae



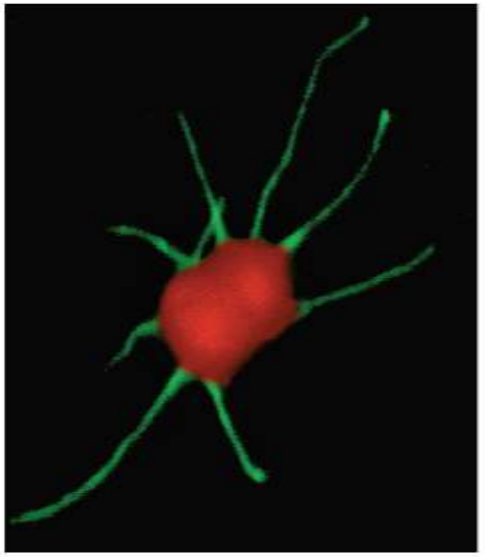
A schematic of **P** (part a) and **type 1** (part b) pili, represented by the Pap and Fim systems, respectively. Numbers indicate the number of copies of each subunit in the pilus. The chaperones attached to the last subunit to be incorporated into each pilus are shown in yellow. P pili are terminated at the outer membrane (OM) by the termination subunit, PapH. No such subunit is known in the Fim system. The usher dimers are indicated in purple and blue. E, extracellular space; P, periplasm.


A Chaperone/Usher-Mediated Pilus Biogenesis
G-
Tip fibrillum
Pilus rod
Usher
Outer membrane
Periplasm
Cytoplasmic membrane
iv
v
vi
i
ii
iii

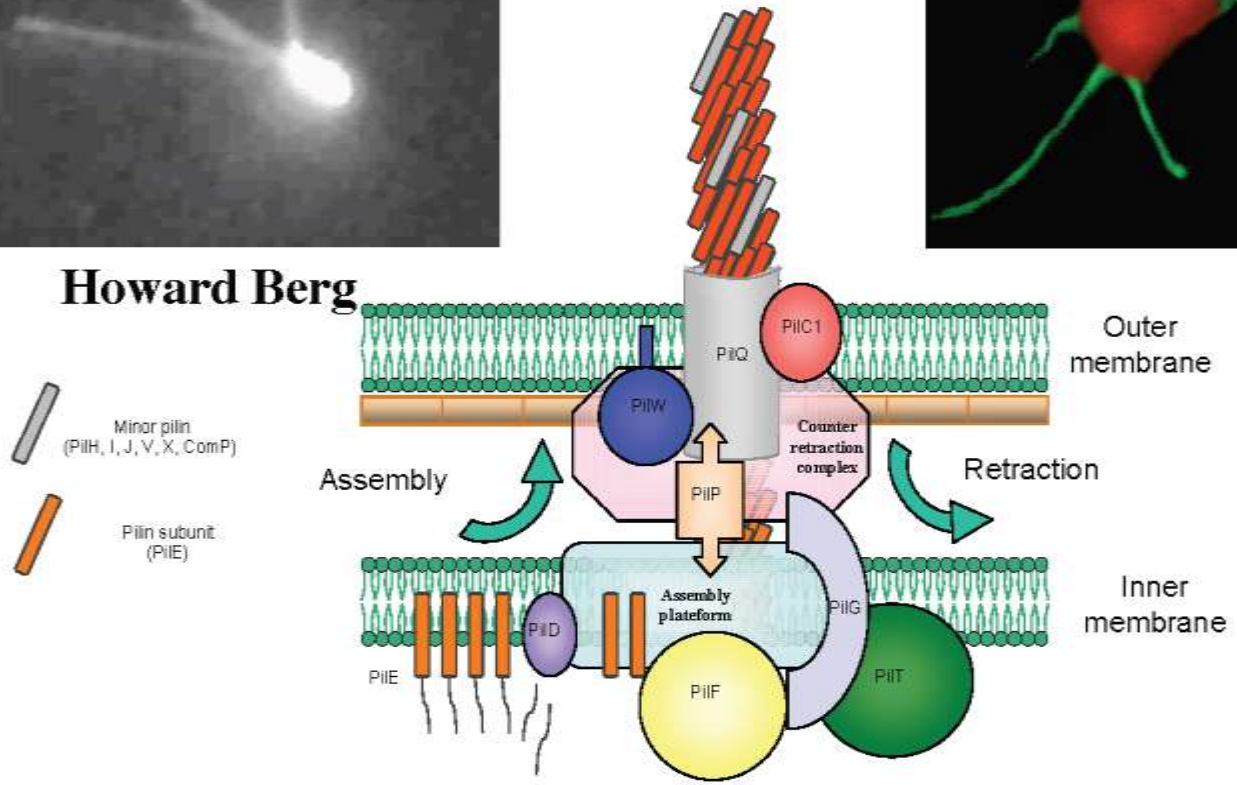

B Sortase-Mediated Pilus Biogenesis
G+
Signal peptidase
Sortase
Cell wall
Membrane
Cytoplasm
LPLTG


lectin
FimH
FimG
FimF
Tip fibrillum
Pilus rod
(500–3,000 FimA subunits)
FimD
OM
Periplasm
FimC
FimA
FimG
IM
Cytoplasm

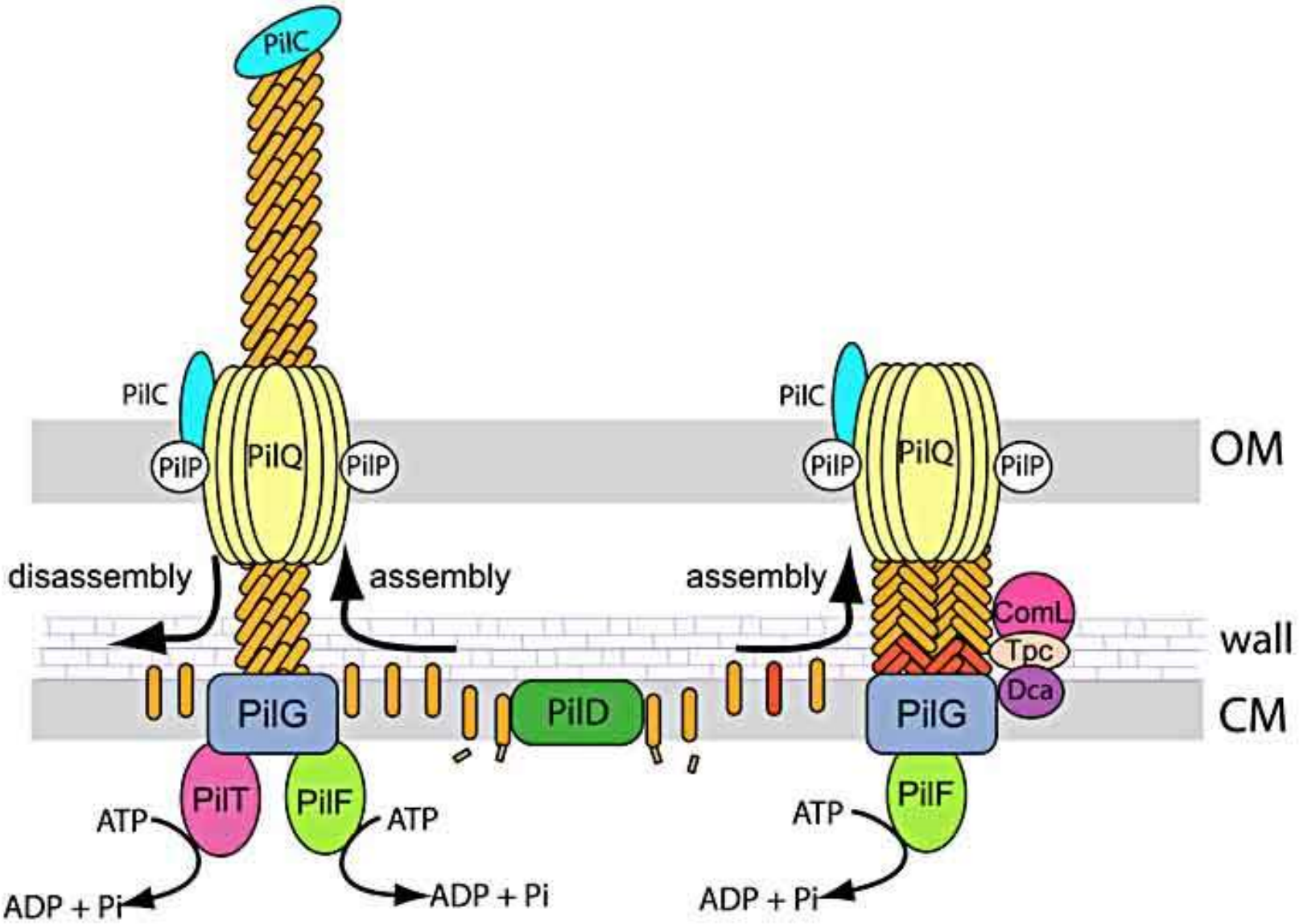
Type IV pili:
a role in adhesion and twitching motility



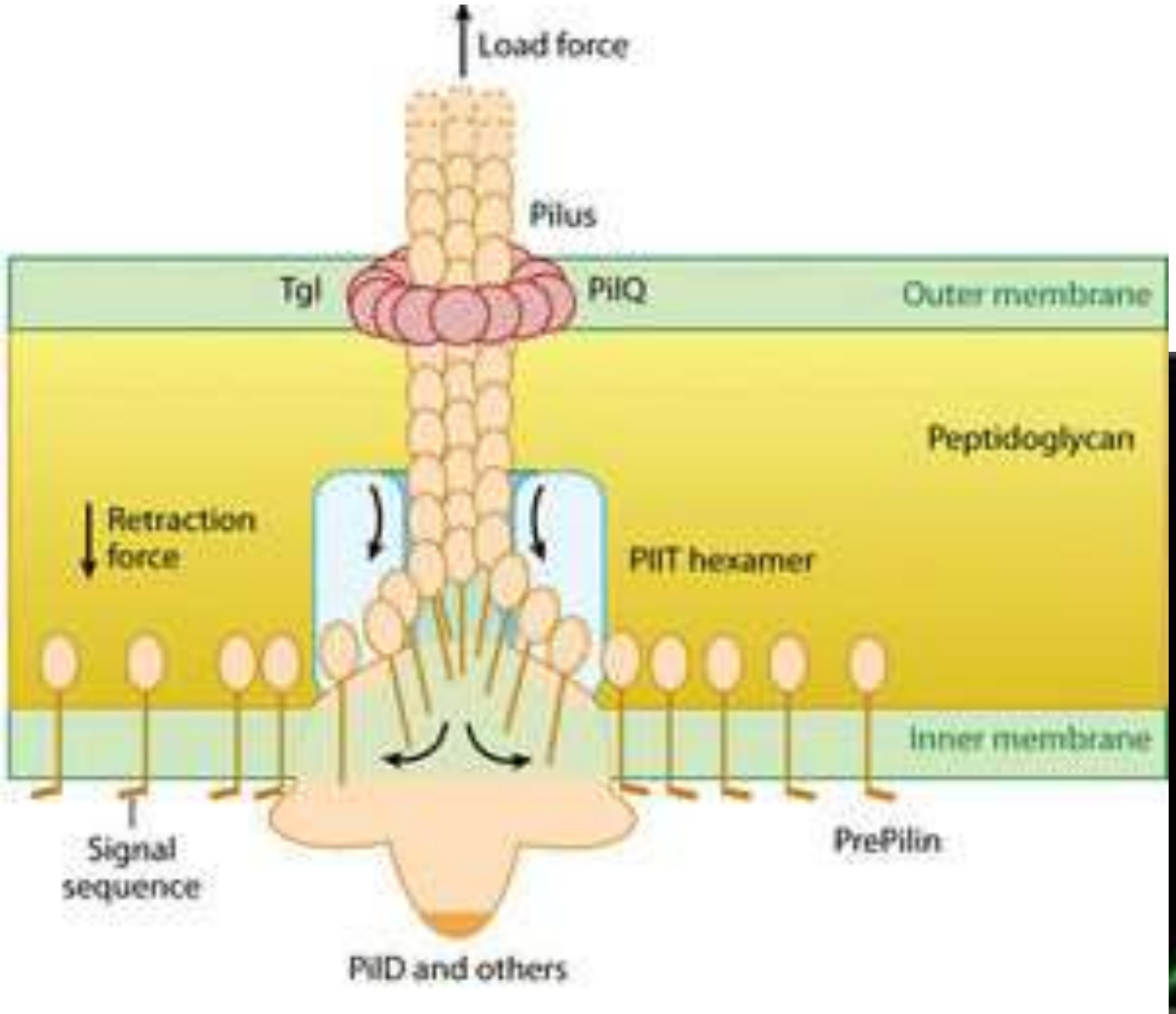
Howard Berg



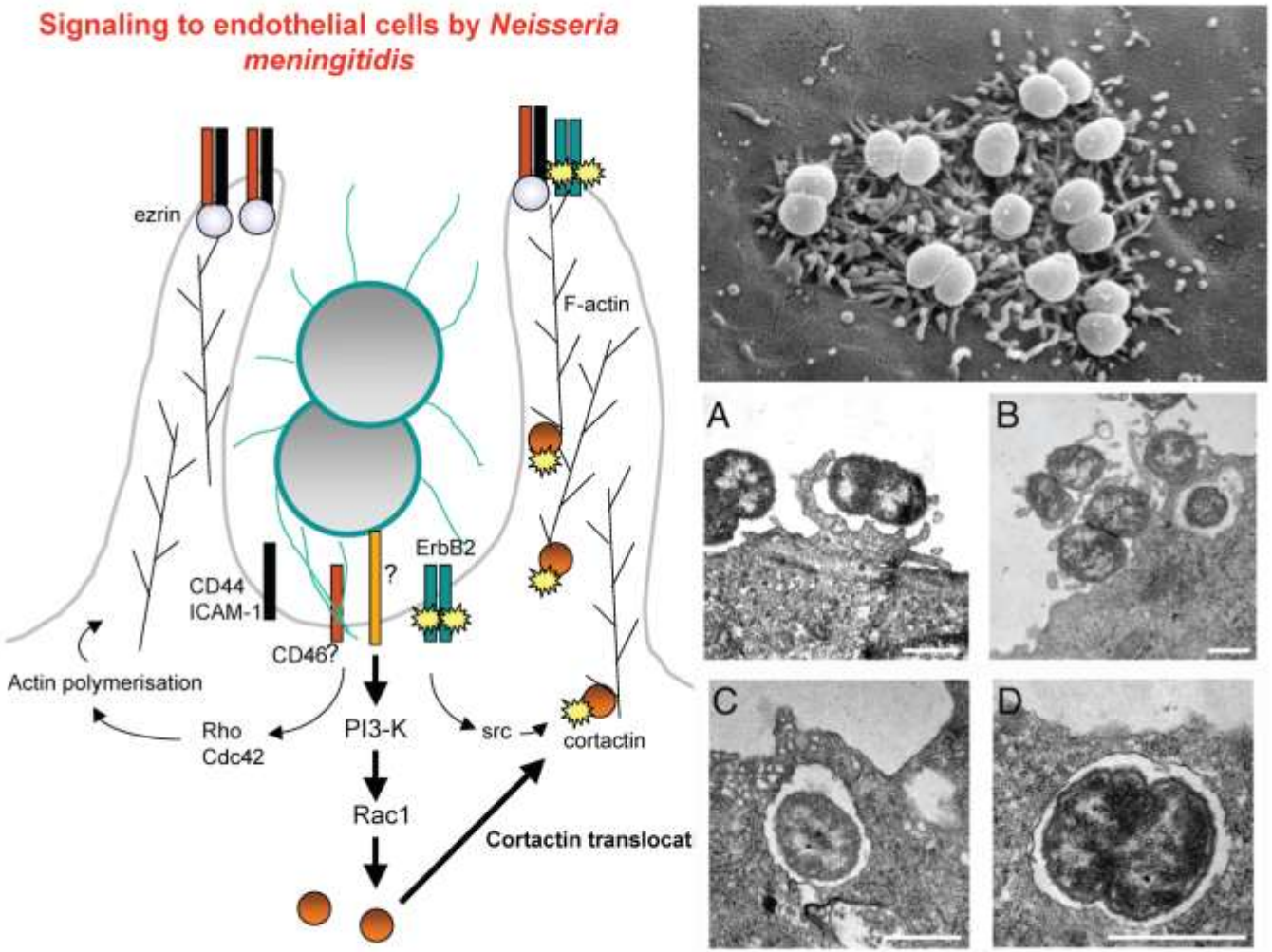
Type IV pili biogenesis



Type IV pili retraction

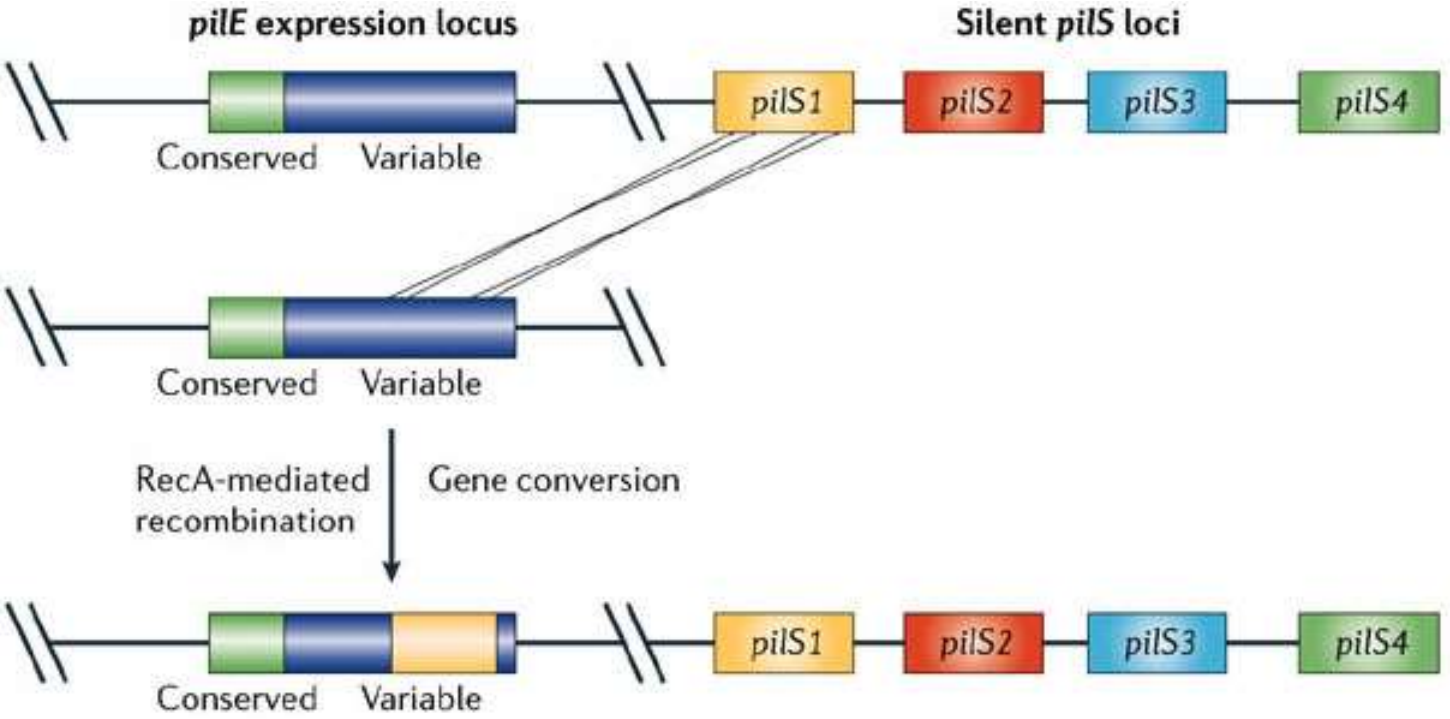


Type IV pili signaling triggers internalization of meningococci



Changing coats :

Antigenic variation due to recombination between 1 expressed and 10 silent and hypervariable alleles over 10^{11} possible variants / Frequency: 10^{-3} per cell per generation – no way to catch up with antibodies...



Meningococcal pilin antigenic variation is mediated by gene conversion. Pili consist of thousands of pilin (PilE) subunits polymerized into long fibres. The PilE protein contains a highly conserved N-terminal domain and a variable C-terminal domain, the latter determining the antigenicity of the pili. The variable region is the result of a non-reciprocal transfer of DNA from one of many silent partial pilS loci to the single pilE expression locus. The silent loci, which are sometimes present several hundred bp away from the pilE expression locus, can donate a stretch of nucleotides, on the basis of short sequence homology. The genetic mechanism proceeds through a form of gene conversion that requires RecA and several crossover events during recombination.

Long-range adherence through pili or fimbriae is often followed by tight adherence through afimbrial adhesins, such as through Opa proteins

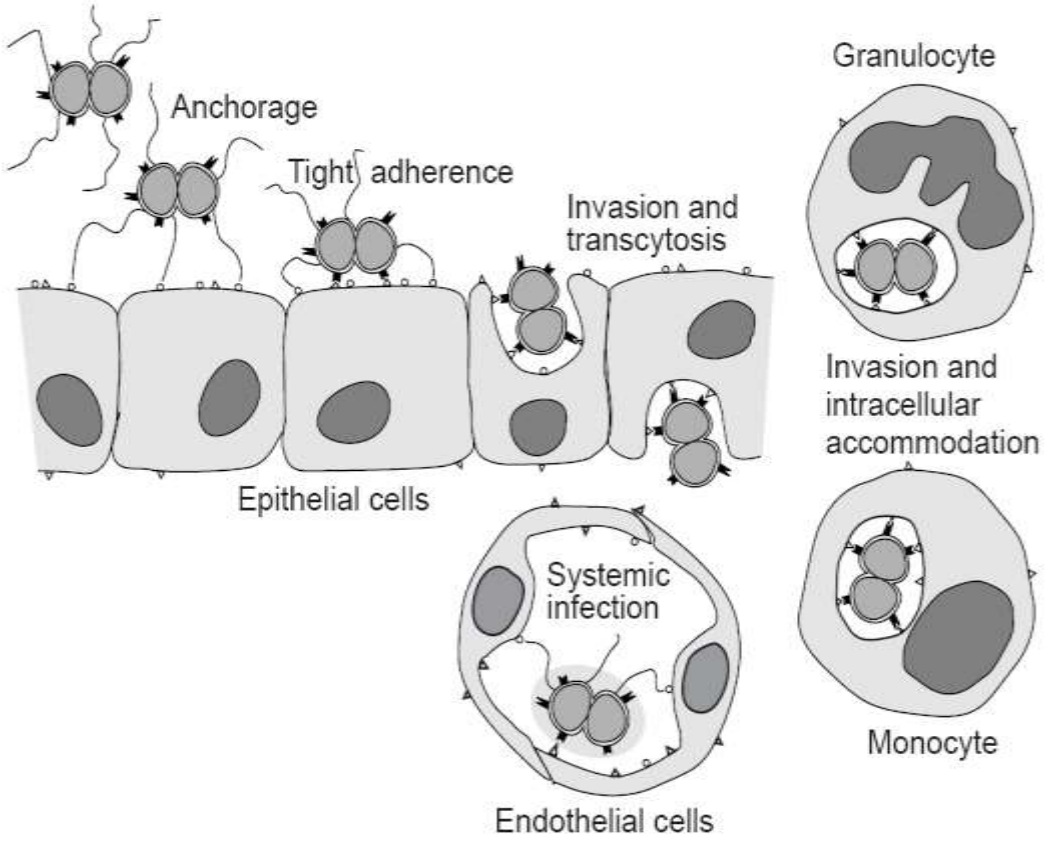


Fig. 1. Model of the sequential interactions between pathogenic *Neisseria* and host cells during the colonization of a mucosal tissue. Primary adherence to epithelial cells probably occurs via pili. The bacteria can then establish an intimate contact with the host cells via their Opa protein(s), an interaction that might allow their transcytotic passage to subepithelial tissues. Pilus- or Opa-mediated interactions between bacteria and professional phagocytes (e.g. granulocytes and/or monocytes) leads to the opsonin-independent uptake of bacteria. Neisserial interactions with the endothelia might result in its entry into the bloodstream and subsequent systemic dissemination. Expression of capsule by meningococci or sialylation of lipopolysaccharide renders bacteria resistant to killing by serum, while these structures might mask integral outer membrane proteins, such as Opa.

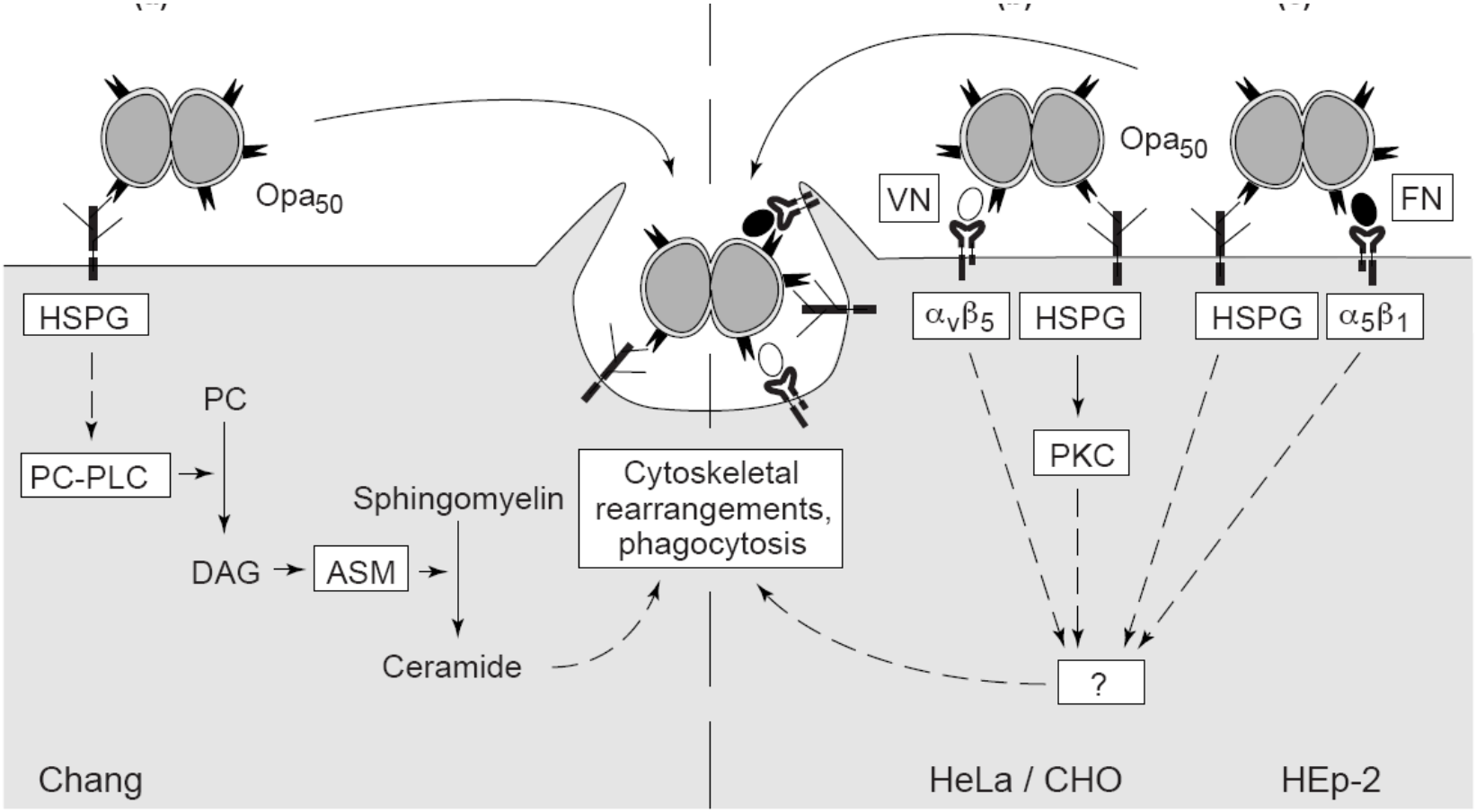


Fig. 2. Distinct mechanisms of Opa_{50}-mediated internalization into different epithelial cells lines. **(a)** In Chang conjunctiva epithelial cells, heparan sulphate proteoglycan (HSPG)-dependent internalization involves the activation of phosphatidylcholine-dependent phospholipase C (PC-PLC), which results in the generation of the second messenger diacylglycerol (DAG) from phosphatidylcholine (PC). DAG activates the acidic sphingomyelinase (ASM), which then generates ceramide from sphingomyelin. By an unknown process, ceramide is implicated in mediating cytoskeletal reorganization and bacterial uptake by a mechanism that resembles conventional phagocytosis. **(b)** Efficient bacterial uptake into HeLa cervical carcinoma cells and Chinese hamster ovary (CHO) cells also relies on the ability of Opa_{50} to mediate binding to the extracellular matrix protein vitronectin (VN) and, thereby, to co-ligate HSPGs and α_v integrin-containing VN receptors, including $\alpha_v\beta_5$. This internalization process appears to be dependent on the activity of protein kinase C (PKC). **(c)** In HEp-2 larynx carcinoma cells, efficient bacterial uptake of Opa_{50}-expressing gonococci requires binding of the extracellular matrix protein fibronectin (FN), which results in a co-ligation of HSPGs and the FN receptor $\alpha_5\beta_1$ integrin; however, the mechanism of entry is still poorly understood.

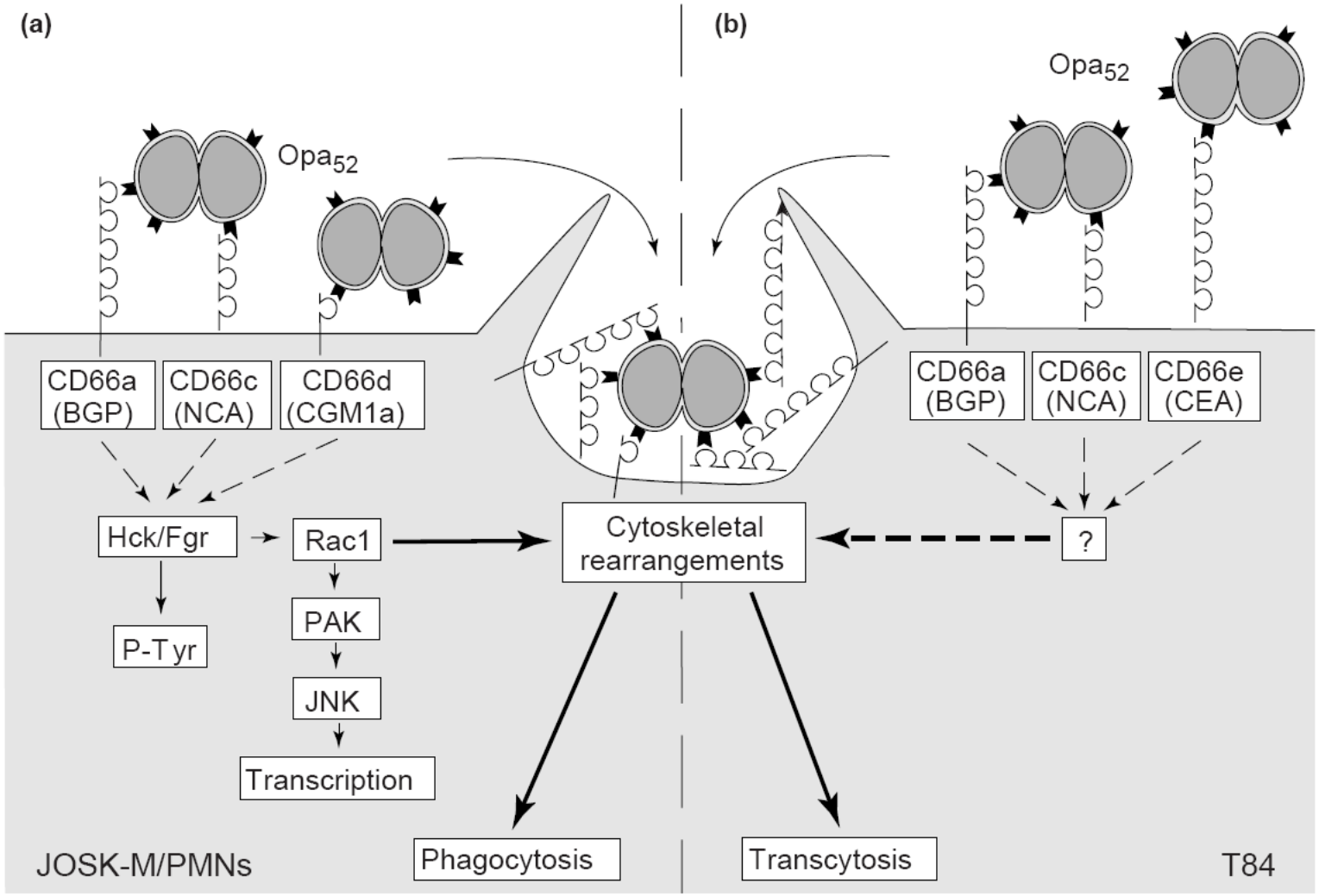
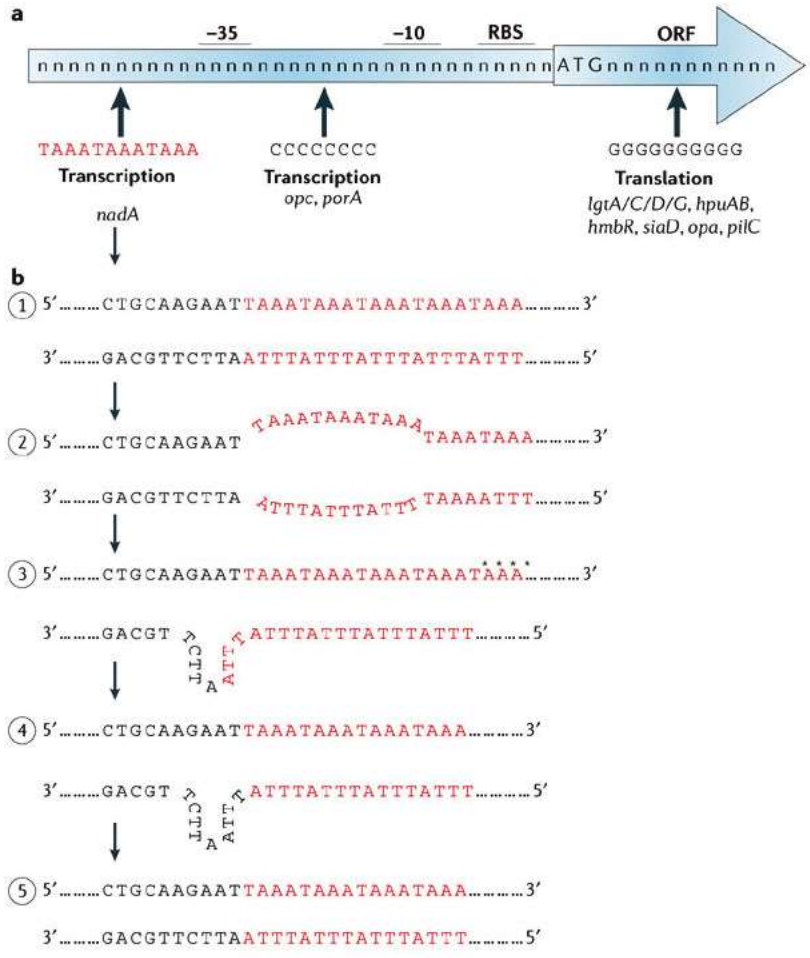


Fig. 3. Opa-mediated interactions with CD66 receptors expressed by **(a)** phagocytic cells or **(b)** polarized T84 epithelial cells. Opa_{52} is shown as a representative of the CD66-binding Opa protein family. **(a)** In the myelomonocytic cell line JOSK-M and in polymorphonuclear neutrophils (PMNs), Opa-mediated binding to surface-expressed CD66 receptors [either CD66a (biliary glycoprotein, BGPa), CD66c (non-specific cross-reacting antigen, NCA) or CD66d (CEA gene family member 1, CGM1a)] results in the activation of the Src-family non-receptor protein tyrosine kinases Hck and Fgr. This results in an increased cellular protein tyrosine phosphorylation and the activation of the small G protein Rac1, which is implicated in the cytoskeletal rearrangements ultimately leading to the phagocytic uptake of bound bacteria. Rac1 also activates the p21-activated protein kinase (PAK) and Jun-N-terminal kinase (JNK), probably leading to a subsequent activation of nuclear transcription. **(b)** Transcytosis of Opa_{52}-expressing gonococci through a monolayer of polarized T84 epithelial cells is mediated via binding to surface-expressed CD66a (BGPa), CD66c (NCA) or CD66e (CEA) receptors by an unknown mechanism.

On/Off switching of Opa and other antigen production: mechanism of phase variation at a frequency ~10^{-6} *per* cell *per* generation:

a) loss of transcription

b) loss of translation



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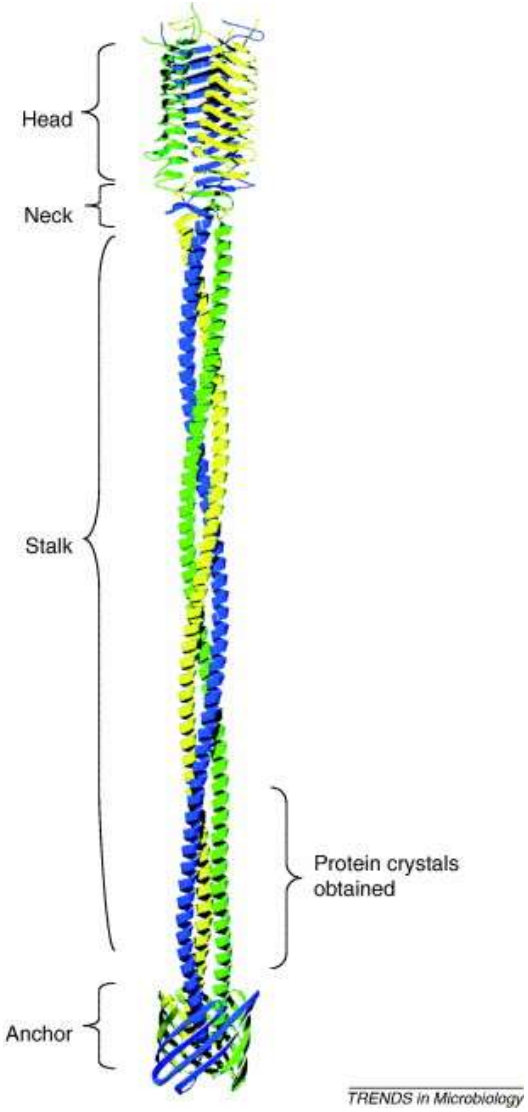
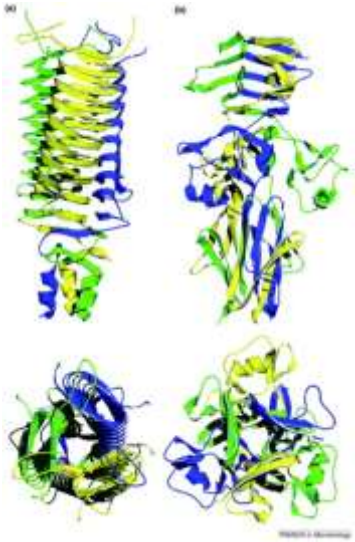
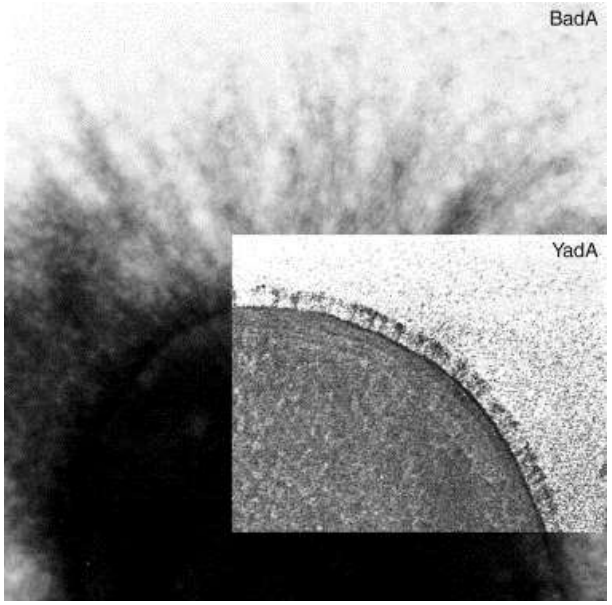
The molecular mechanism that most often mediates meningococcal phase variation is **RecA-independent slipped-strand mispairing of repeats during DNA replication** or repair. a | **Tandem repeats** are found either **in the promoter region** of the gene, affecting the binding of the RNA polymerase and therefore transcription, or in the open reading frame (ORF), affecting translation. These repeats can be homopolymeric or can be composed of tetranucleotides. The different meningococcal loci altered by phase variation and the corresponding position of tandem repeats are listed. b | The mutational mechanism responsible for phase variation of the meningococcal outer-membrane protein and adhesin NadA is shown. (1) When **5 TAAA repeats are present upstream of the promoter region,** the expression level of NadA is high51. (2) The AT-rich sequence is prone to strand separation. (3) Subsequent mispairing of the TAAA repeats can occur during re-annealing due to slippage of the DNA strands. Slippage in the 5' to 3' direction as shown leaves an unpaired copy of TAAA (indicated by the asterisks). (4) The unpaired TAAA is deleted, and the single-stranded loop is the target of excision-repair processes. (5) With a reduction in the TAAA repeat number to four, the expression level of NadA is low51. Additionally, recombination by a mode of gene conversion (Fig. 5) can lead to phase variation when premature stop codons are removed or introduced into genes, resulting in functional or non-functional proteins, respectively. RBS, ribosomal binding site. Figure part b modified with permission from Ref. 68 © (1994) Elsevier.

Trimeric autotransporter adhesins





Structure of TAA heads (side and top views).



Length of YadA and BadA shown by negative stain electron microscopy. Note the difference between YadA (23 nm) and BadA (300 nm), which is mainly caused by the length of their stalks

Trimeric autotransporter adhesins (TAAs) are important virulence factors in Gram-negative pathogens. Despite the variety of hosts ranging from plants to mammals and the specialized regulation of TAAs, their molecular organization follows surprisingly simple rules: they form trimeric surface structures with a head–stalk–anchor architecture. The head and stalk are composed of a small set of domains, building blocks that are frequently arranged repetitively.

Iron acquisition

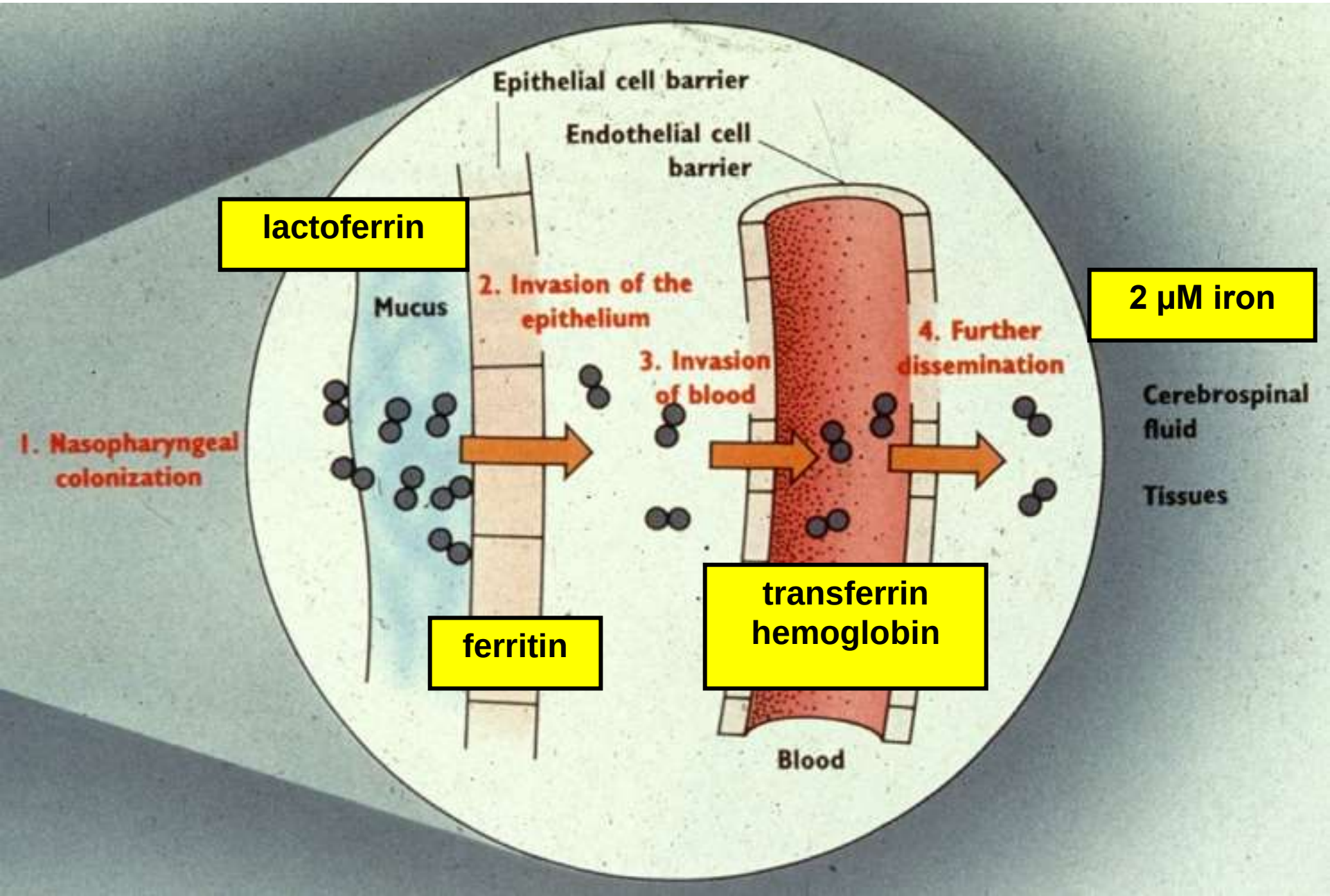
IRON AVAILABILITY IN THE HUMAN HOST:

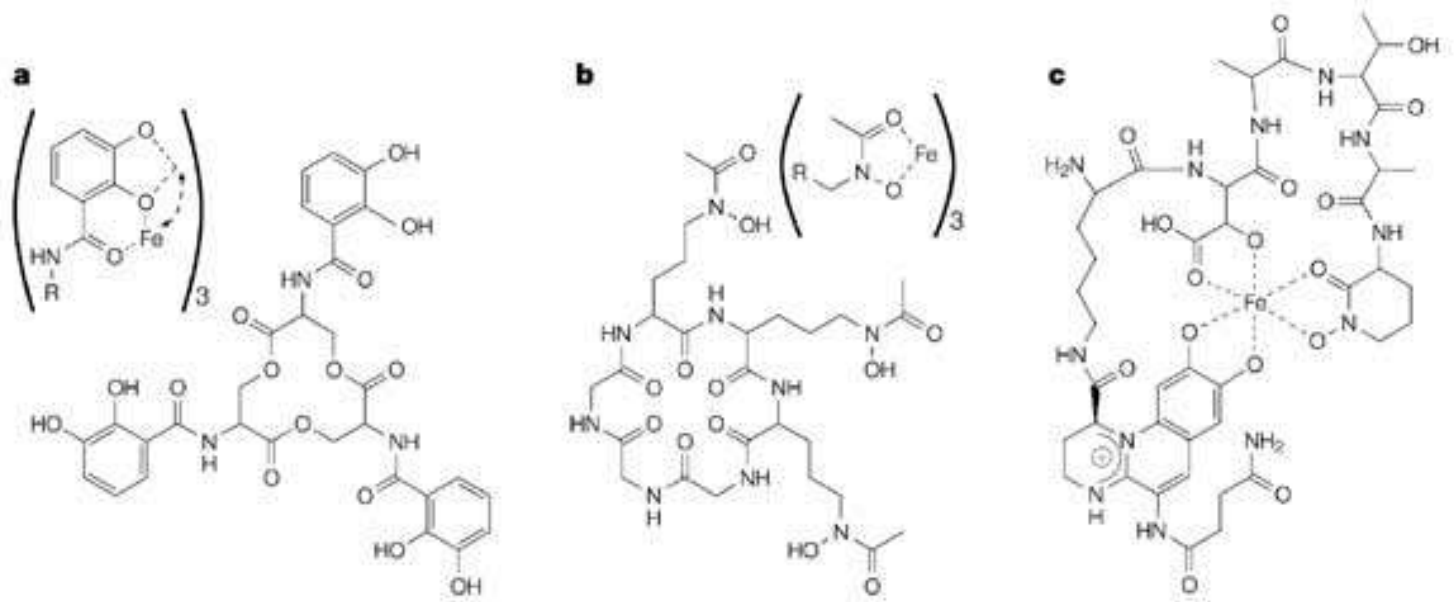
- free iron concentrations are low in aqeous environment, in aerobic conditions, iron exists in the oxidized, ferric form (Fe^{III}), which at pH 7, soluble to $1.4x10^{-9}$ M
- erroneously it used to be given as 10^{-18} M, however, the at pH 7 the principal ionic species is polymeric $Fe(OH)^{+}_{2}$ not $Fe(OH)_3$
- For invasion and proliferation bacteria need to induce specific pathways capable of scavenging iron from the host to about 10^{14} iron atoms per bacterium
- free iron concenration in host is about 10 x lower that in media
- **In human body fluids more than 99,9% of iron is bound to transport (transferrin, lactoferrin) and storage proteins (ferritin, heme-containing compounds) with extremely** high binding constants of 10^{36}, 30-40% saturated :
  - transferrin in plasma and lymph
  - lactoferrin in milk and secretions
  - Another source of iron is hemoglobin

    (i.e. meningococci are capable of using haptoglobin-hemoglobin and free heme as sources of iron)

- **iron concentration tells the pathogen it is inside the host**
- **Two general mechanisms of iron acquisition in bacteria have been described:**
  - **siderophore-mediated iron acquisition by cognate receptors**
  - **receptor-mediated iron acquisition from host iron-binding proteins**

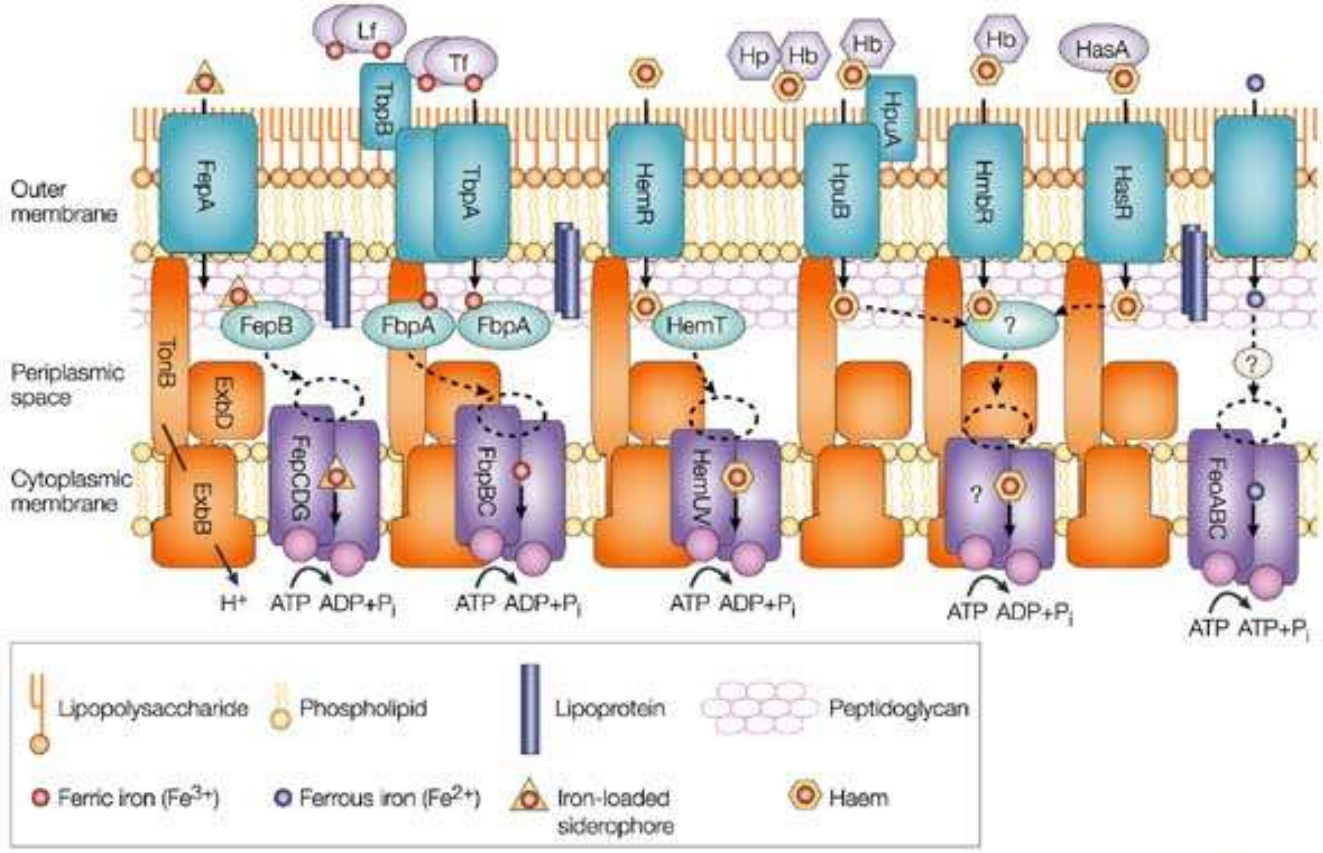
Iron availability in the human host





Siderophores are low-molecular-weight molecules that are synthesized by bacteria and fungi and are excreted into the medium to sequester and make soluble ferric iron (Fe3+).

- capture iron from proteins such as haemoglobin or transferrin.
- iron-loaded siderophores are transported into the bacterial cell, where iron is released
-siderophores chelate ferric iron with high affinity
- siderophores can be divided into hydroxamates and catecholates
- Enterobactin (a) is a 669-Da catecholate (*Escherichia coli* and enterobacteria)
- Ferrichrome (b) is a 740-Da hydroxamate-type siderophore of fungi scavenged by bacteria, -
- Mycobactins, which are synthesized by mycobacteria, are distinct from other siderophores
- Pyoverdins, such as pseudobactin B10 (c), are the most prevalent siderophores of *Pseudomonas*.
- Siderophore–antibiotic conjugates can be used to deliver antimicrobial agents into the cell, which reduces the minimal inhibitory concentration several-hundredfold.



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Under aerobic conditions, bacteria rely on high-affinity surface receptor proteins (blue) that bind Fe3+-containing proteins (light purple), such as iron-loaded siderophores or haem, and that subsequently facilitate their translocation into the periplasmic space. This process is activated by the Ton complex (TonB–ExbBD). Periplasmic-binding proteins (light blue) and ATP-driven transporters (purple) that are in the cytoplasmic membrane are used to ensure further transport into the cell (the same is true for Gram-positive species, except that in these bacteria the binding proteins are membrane-anchored).

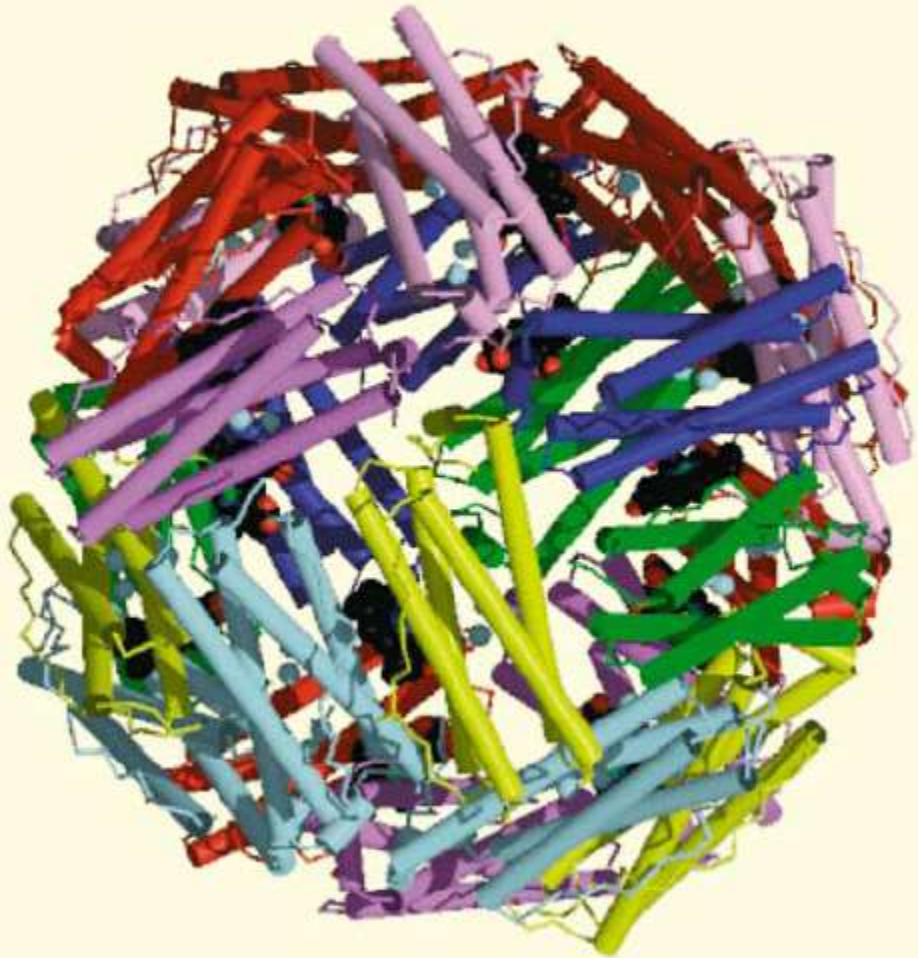
Proteins involved in bacterial iron storage in menigococci

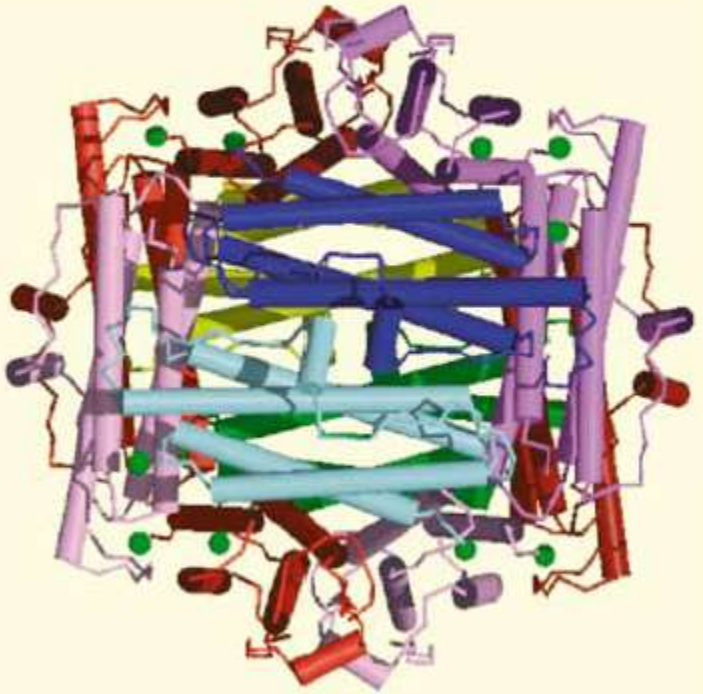
- Free iron in presence of oxygen can form free radicals which are toxic to the cell.
- Storage of iron in nontoxic form is very important!
- Two types of iron storage proteins have been identified in bacteria.
  - bacterioferritin - heme iron and nonheme iron
    ferritin - only iron and not heme

- In presence of iron
  - *bfrA* - up-regulated more than 11 times
    bfrB - up-regulated nearly 8 times
- In presence of desferal
  - putative ferredoxin - up-regulated 2.4 times

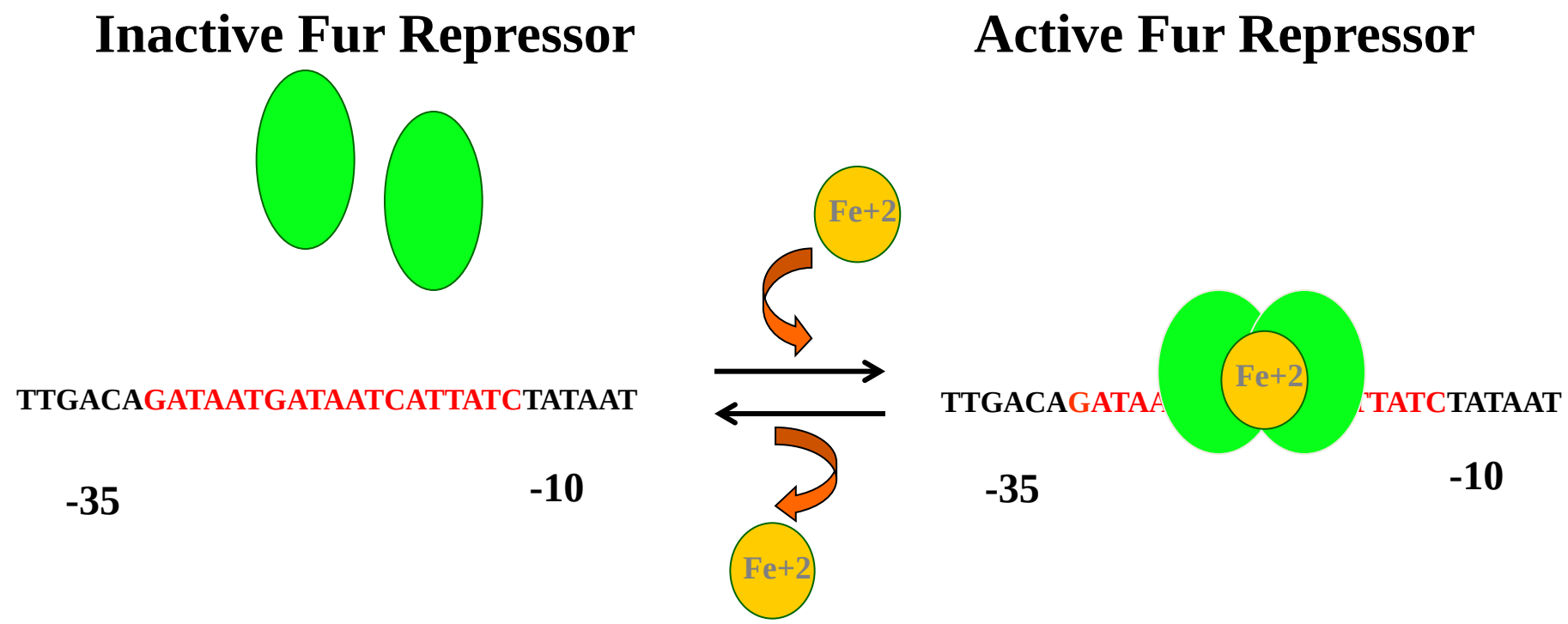
Structures of iron storage proteins from *E. coli*

Bfr	**Dps**
500 kDa, 2000-3000 iron atoms/24-mer	250 kDa, 500 iron atoms/12-mer

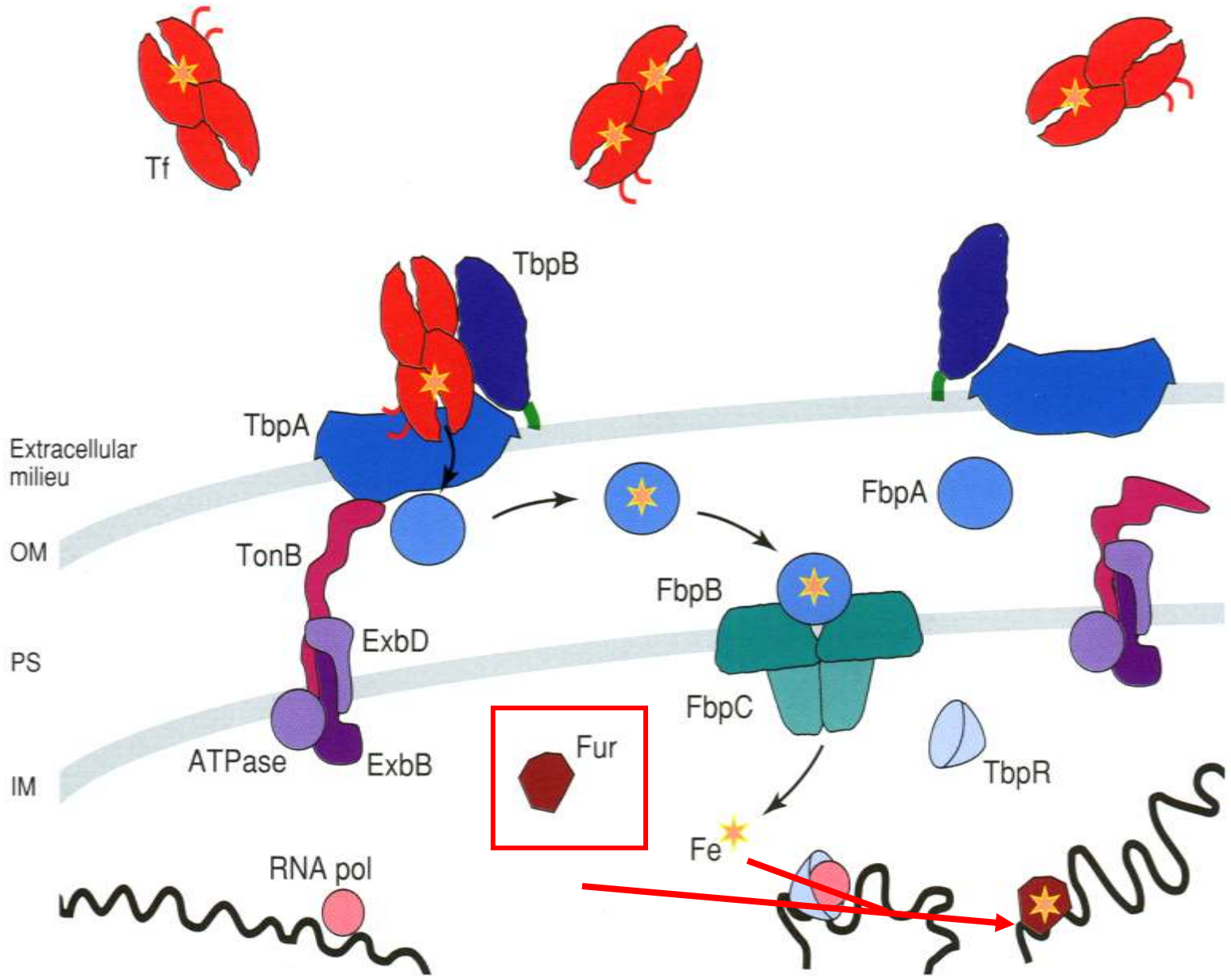




MECHANISM OF Fur REGULATION



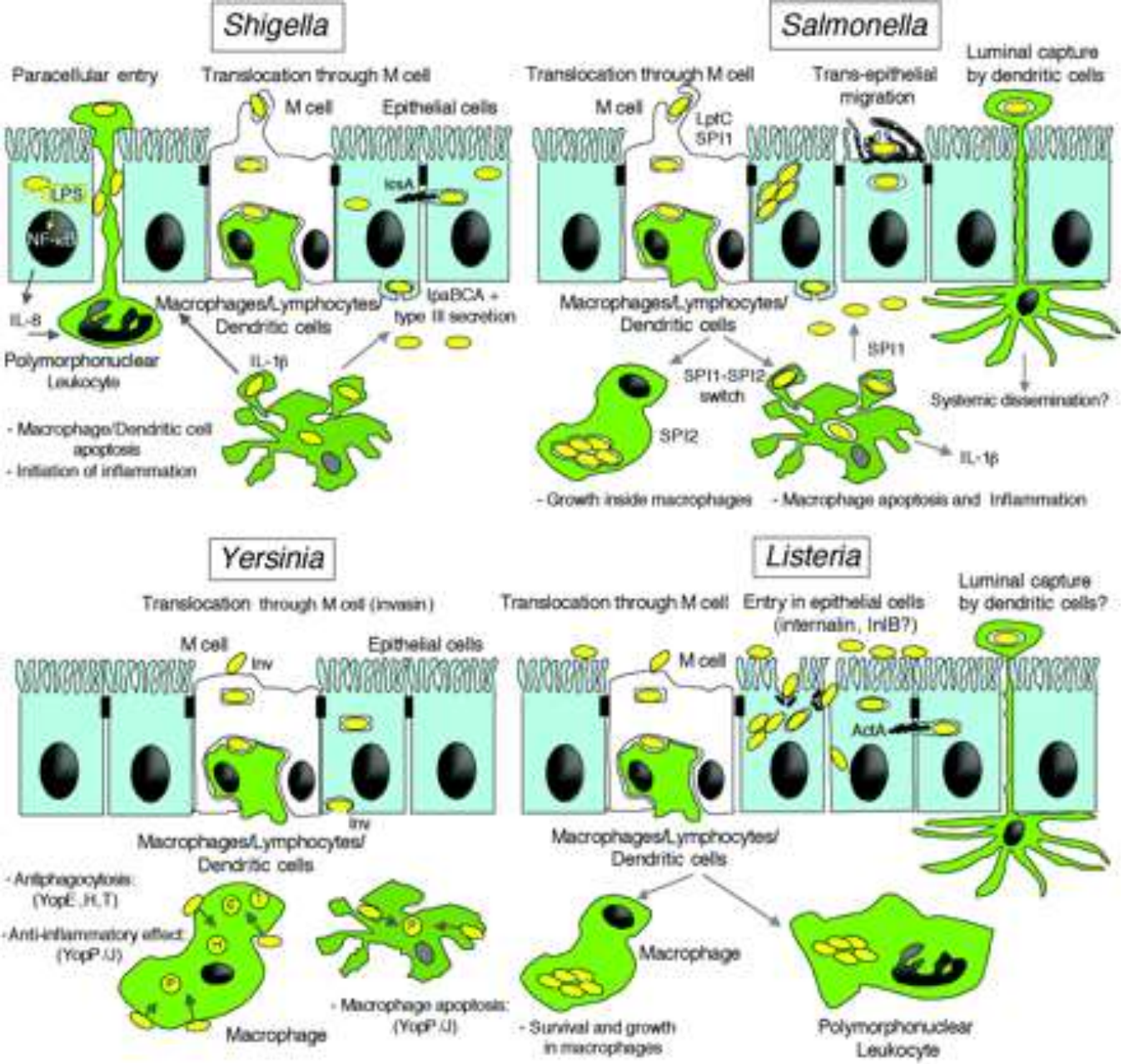
(Courtessy of R. Rappuoli – Chiron Vaccines S.p.A, Siena Italy)



(Courtessy of R. Rappuoli – Chiron Vaccines S.p.A, Siena Italy)

Strategies for hiding inside host cells

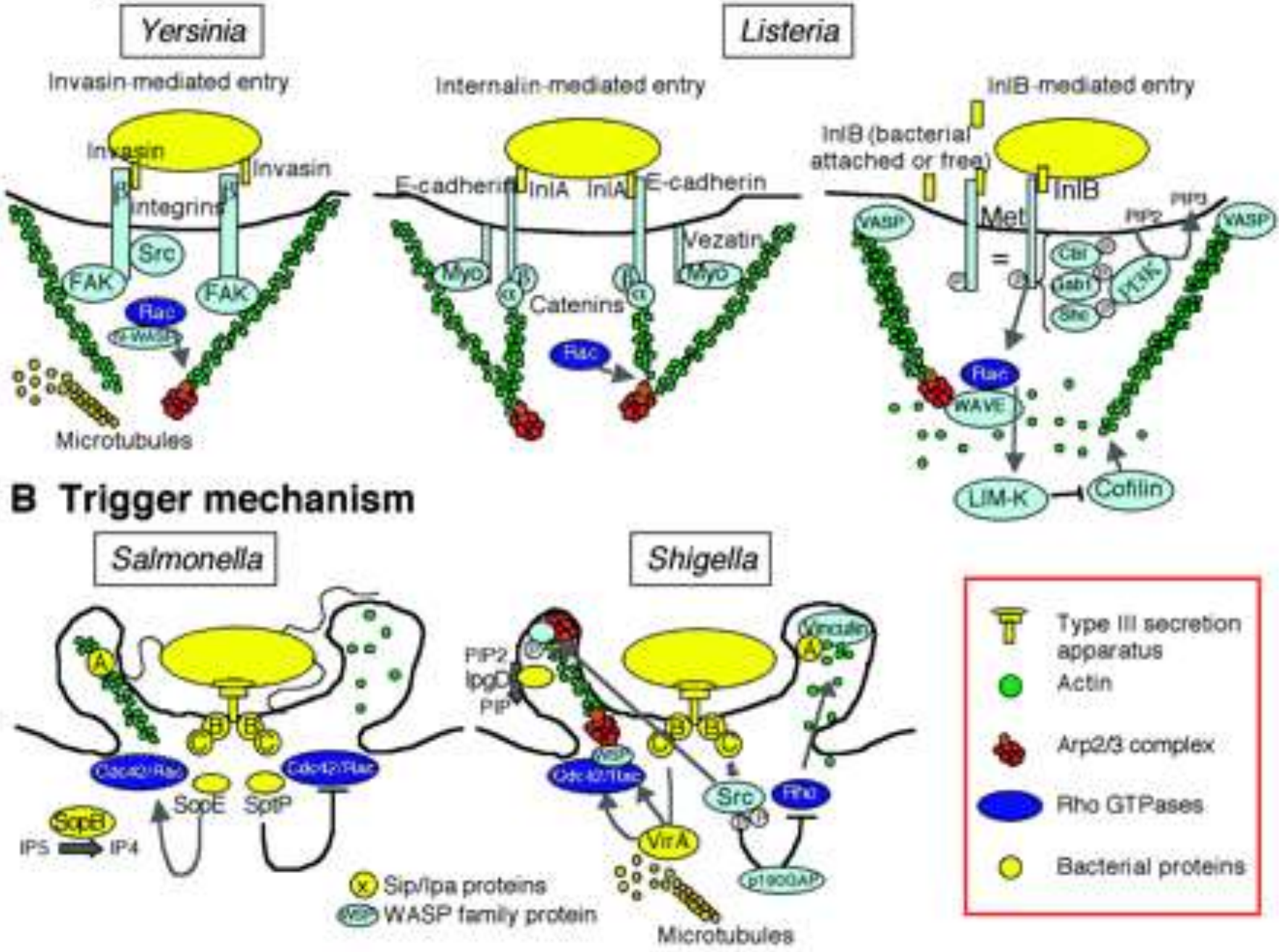
escape to complement and antibody action, plenty of nutrients…

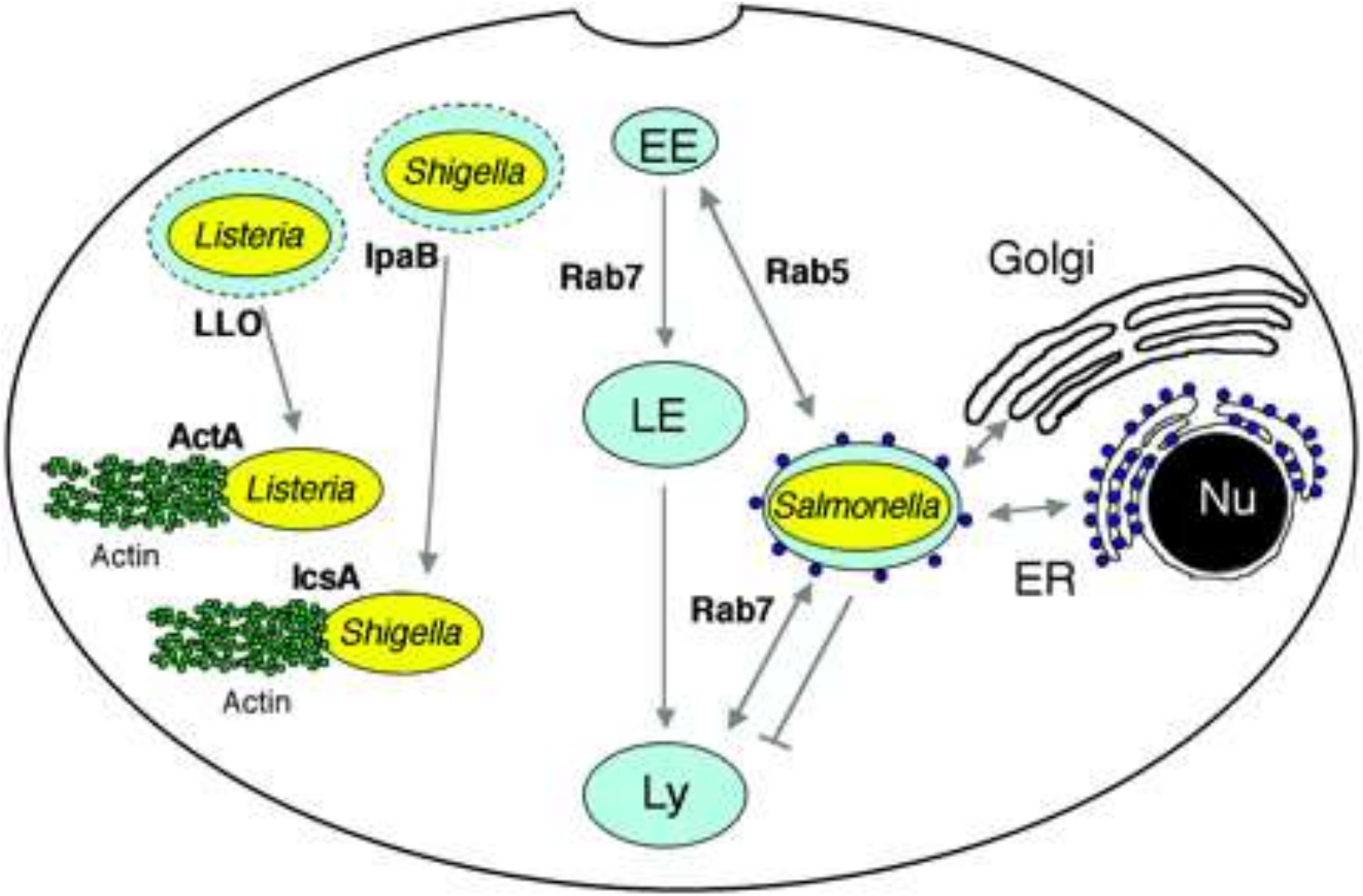


The invasive strategies of enteroinva-sive pathogens.

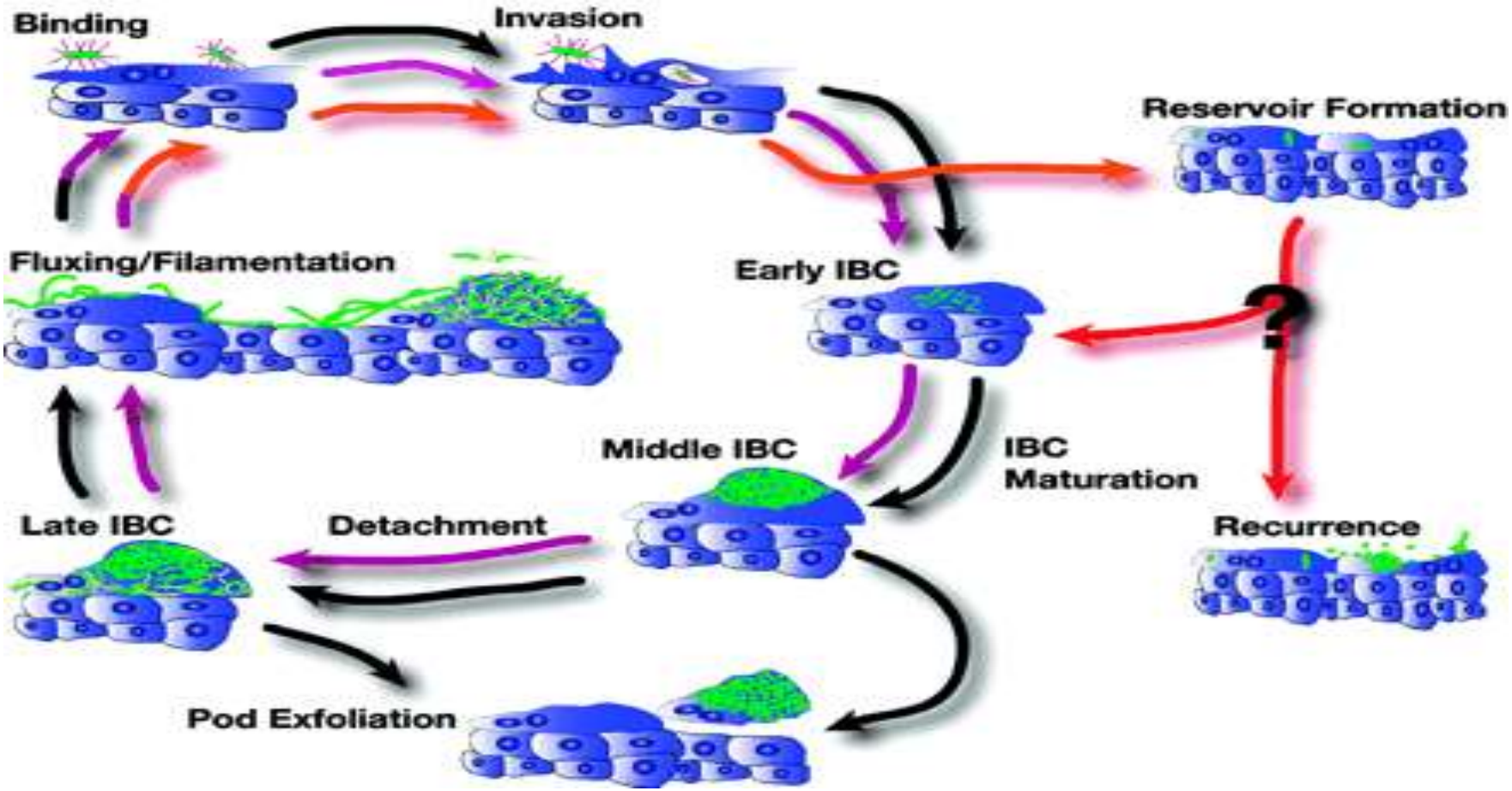
Intestinal epithelial cells (IECs) maintain a physical barrier against commensal flora, although specialized sites such as the follicle-associatedepithelium (FAE) allow constant sampling of the luminal flora through M cells.

Invasive pathogens take advantage of this route to cross the epithelial barrier. Once translocated, bacteria must survive attack by macrophages. The four bacterial species considered have solved this issue differently: ***L. monocytogenes*** are phagocytosed but escape into the cytoplasm, and thus avoid being killed in lysosomal compartments. *Yersinia* adopt an antiphagocytic strategy by intracellular injection of YopE, H, and T that inactivate the actin cytoskeleton. ***Shigella*** not only cause apoptosis of macrophages and monocytes, thus ensuring their own survival, but also trigger early mucosal inflammation through the release of mature IL-1ß and IL-18, which disrupts epithelial impermeability and facilitates bacterial spread at a distance. Finally, ***Salmonella*** remodel their phagosomes, thus avoiding its transition to a lysosome and creating an intracellular niche that allows their efficient replication.


A Zipper mechanism
Yersinia
Invasin-mediated entry
Invasin
Invasin
β1 integrins
Src
FAK
FAK
Rac
c-WASP
Microtubules
Listeria
Internalin-mediated entry
E-cadherin
InlA
InlA
E-cadherin
Vezatin
Myo
Myo
Catenins
Rac
InlB-mediated entry
InlB (bacterial attached or free)
InlB
Met
PIP2
PIP3
VASP
VASP
Cbl
Lab1
Shc
PI3K
Rac
WAVE
LIM-K
Cofilin
B Trigger mechanism
Salmonella
SopE
SptP
SopB
IP5
IP4
Shigella
PIP2
IpgD
PIP
Vinculin
Src
Rho
VirA
p190GAP
Microtubules
Sip/Ipa proteins
WASP family protein
Type III secretion apparatus
Actin
Arp2/3 complex
Rho GTPases
Bacterial proteins



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



Urinary tract infection by pathogenic *E. coli* cascade model. . Bacteria (green) bind to and invade into superficial umbrella cells via type 1 pili (purple). Within the terminally differentiated umbrella cells of the bladder epithelium, UPEC is trafficked into CD63-positive compartments. UPEC persists within CD63-positive compartments, forming quiescent intracellular reservoirs that may serve as a source for later recurrent acute infections. UPEC can break out of its membrane-bound compartment and multiply within the cytosol of host umbrella cells, forming large intracellular bacterial communities (IBCs. The first round of the developmental process (black arrows) directly leads to the second round (magenta arrows) that completes at the time of massive exfoliation of the superficial umbrella cells. At this time, the reservoir is established (orange arrows). Exfoliation of epithelial cells occurs as a mechanism of the innate immune system (gray arrows). Events that lead to recurrence and the mechanism of bacterial growth during recurrence are unclear (red arrows and question mark); these events may include reentry into the characterized cycle at the point of early IBC

Class of mechanism	Specific products
Acidification	pH=~3.5–4.0, bacteriostatic or bactericidal
Toxic oxygen-derived products	Superoxide O_2^-, hydrogen peroxide H_2O_2, singlet oxygen $^1O_2^*$ hydroxyl radical $^*OH$, hypohalite OCl^-
Toxic nitrogen oxides	Nitric oxide NO
Antimicrobial peptides	Defensins and cationic proteins
Enzymes	Lysozyme—dissolves cell walls of some Gram-positive bacteria. Acid hydrolases—further digest bacteria
Competitors	Lactoferrin (binds Fe) and vitamin B_{12}-binding protein

Figure 2-9 Immunobiology, 7ed. (© Garland Science 2008)

- Bactericidal agents produced or released by phagocytes on the ingestion of microorganisms

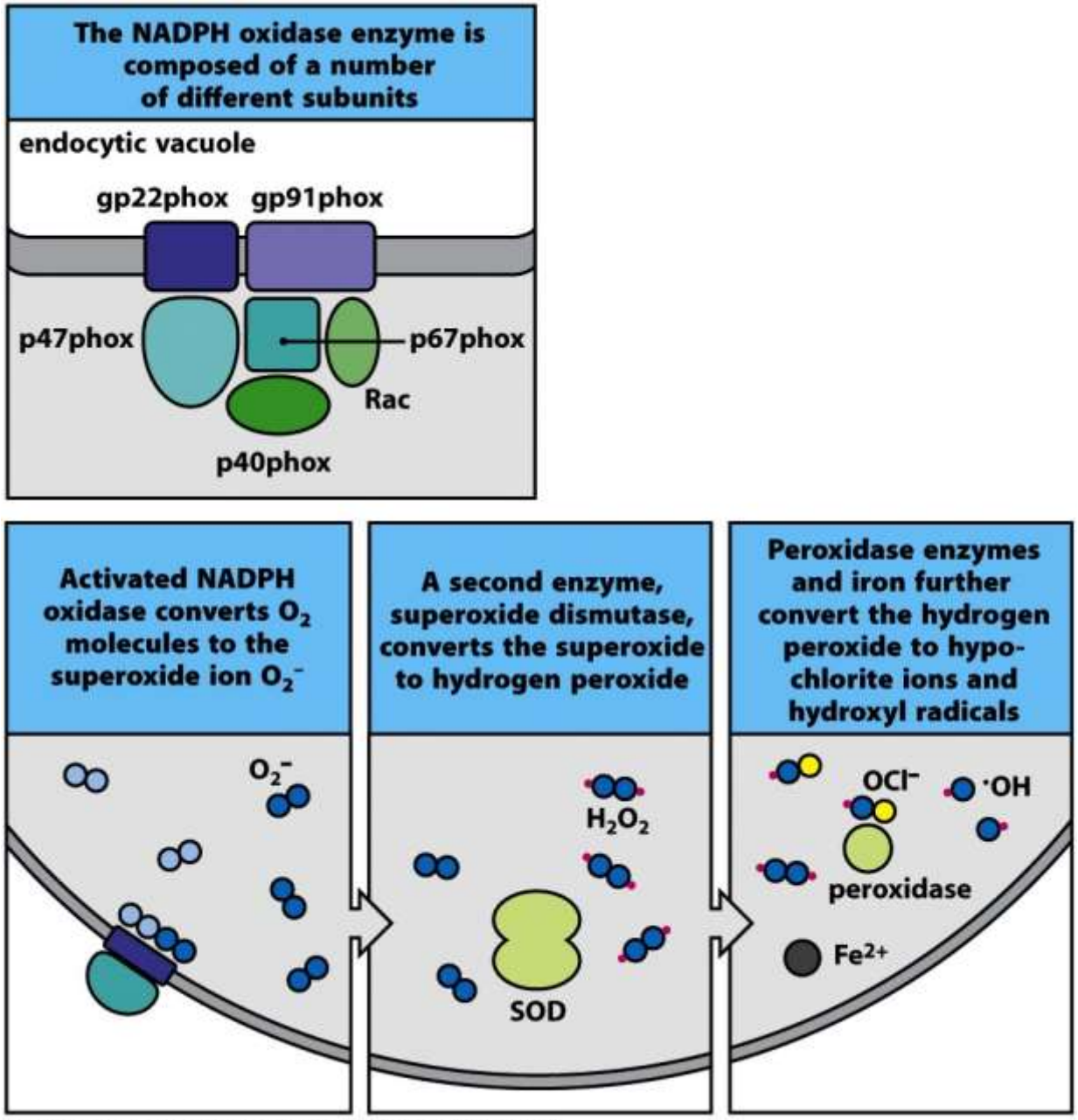


Figure 2-10 Immunobiology, 7ed. (© Garland Science 2008)

- The respiratory burst in macrophages and neutrophils is caued by a transient increase in oxygen consumption during the production of microbicidal oxygen metabolites

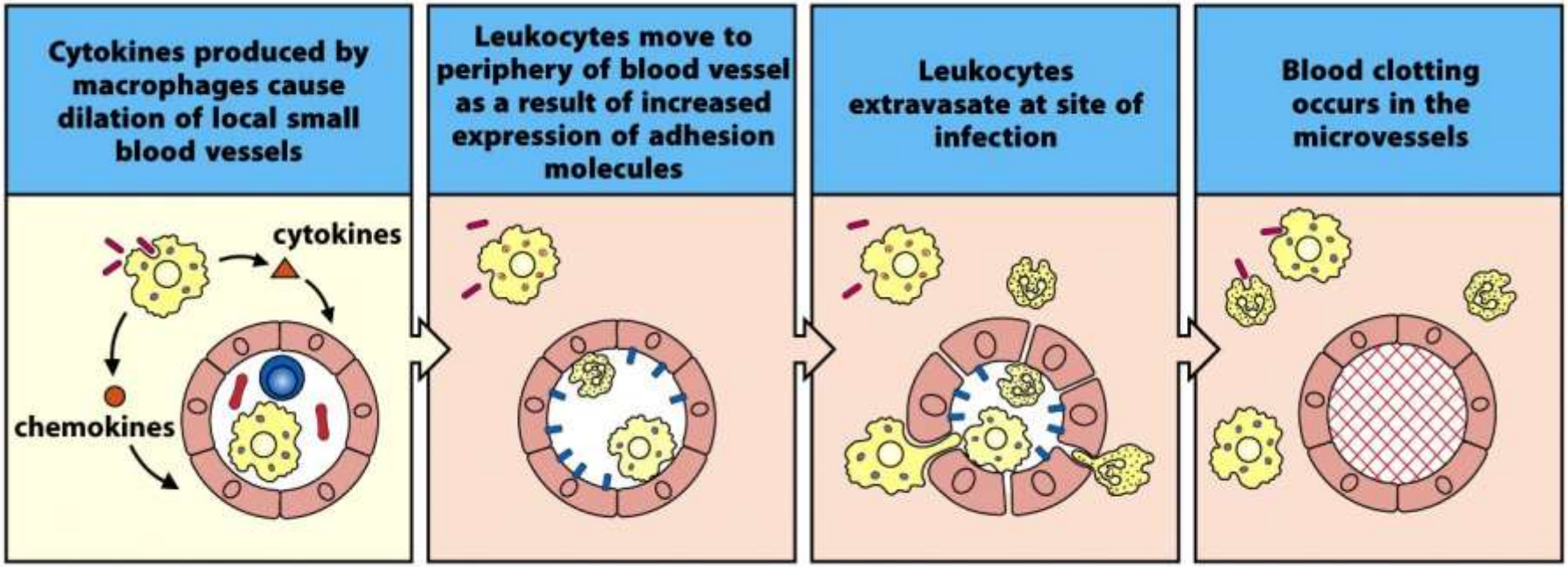


Figure 2-11 Immunobiology, 7ed. (© Garland Science 2008)

- Infection stimulates macrophages to release cytokines nad chemokines that initiate an inflammatory response

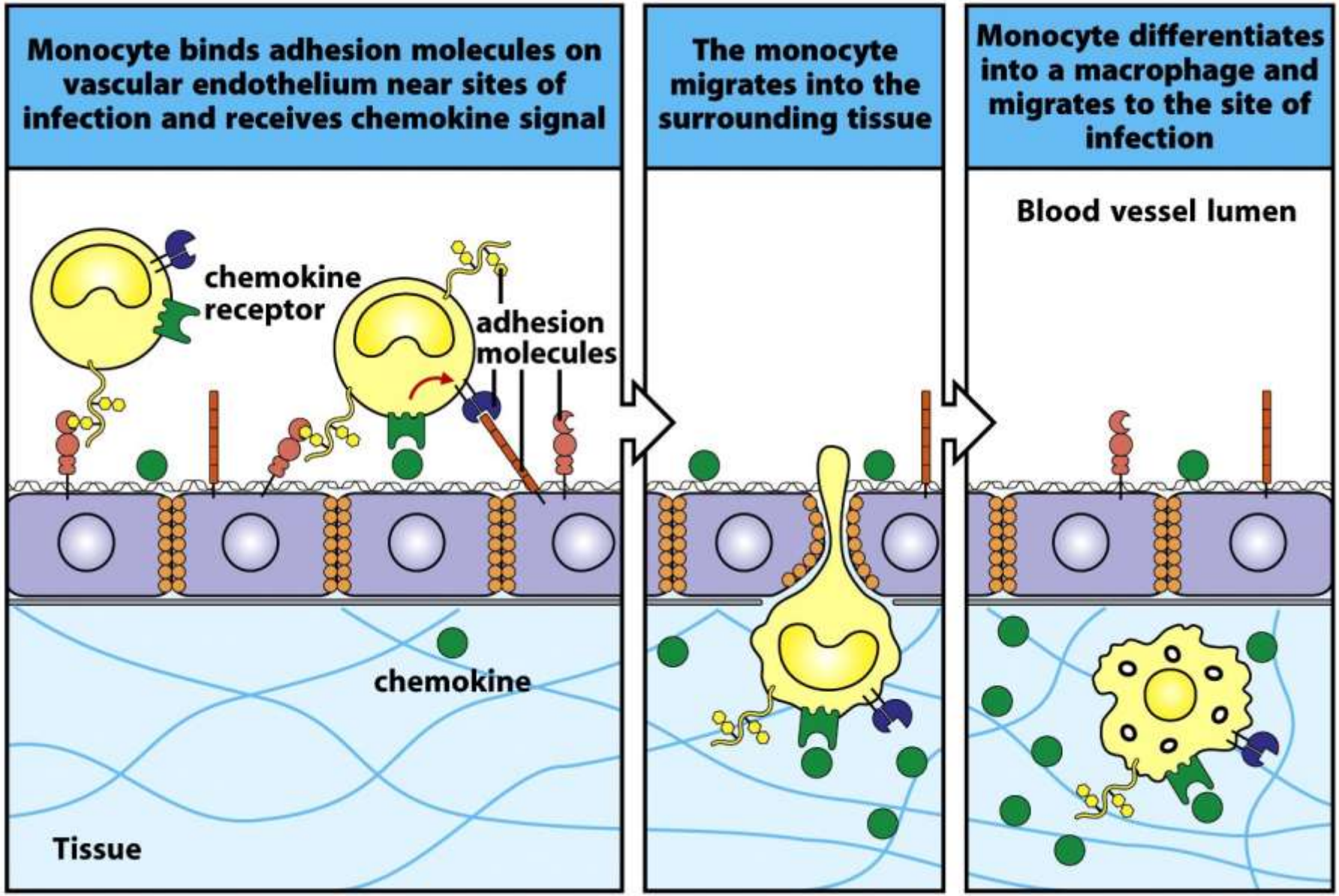


Figure 2-12 Immunobiology, 7ed. (© Garland Science 2008)

- Monocytes circulating in the blood leave the bloodstream to migrate toward sites of infection and inflammation

host defense mechanisms

- **1. Innate Defenses -** Defenses common to all healthy animals
  - **they are inherent to the host**.
    - anatomical and structural barriers
    - inflammation
    - phagocytosis
    - presence of a normal bacterial flora

- **2. Inducible Defenses**
  - mechanisms that **must be induced or turned on by host exposure to a pathogen**
  - not immediately ready to come into play
  - synonymous with **acquired or adaptive immunity**
  - generally quite specifically directed against an invading pathogen
- **active immunity - the host undergoes an immunological response:** antibodies and/or immuno-reactive lymphocytes
- **passive immunity - acquisition by a host of immune factors which were produced in another animal**

- Infection stimulates macrophages to release cytokines and chemokines that initiate an inflammatory response

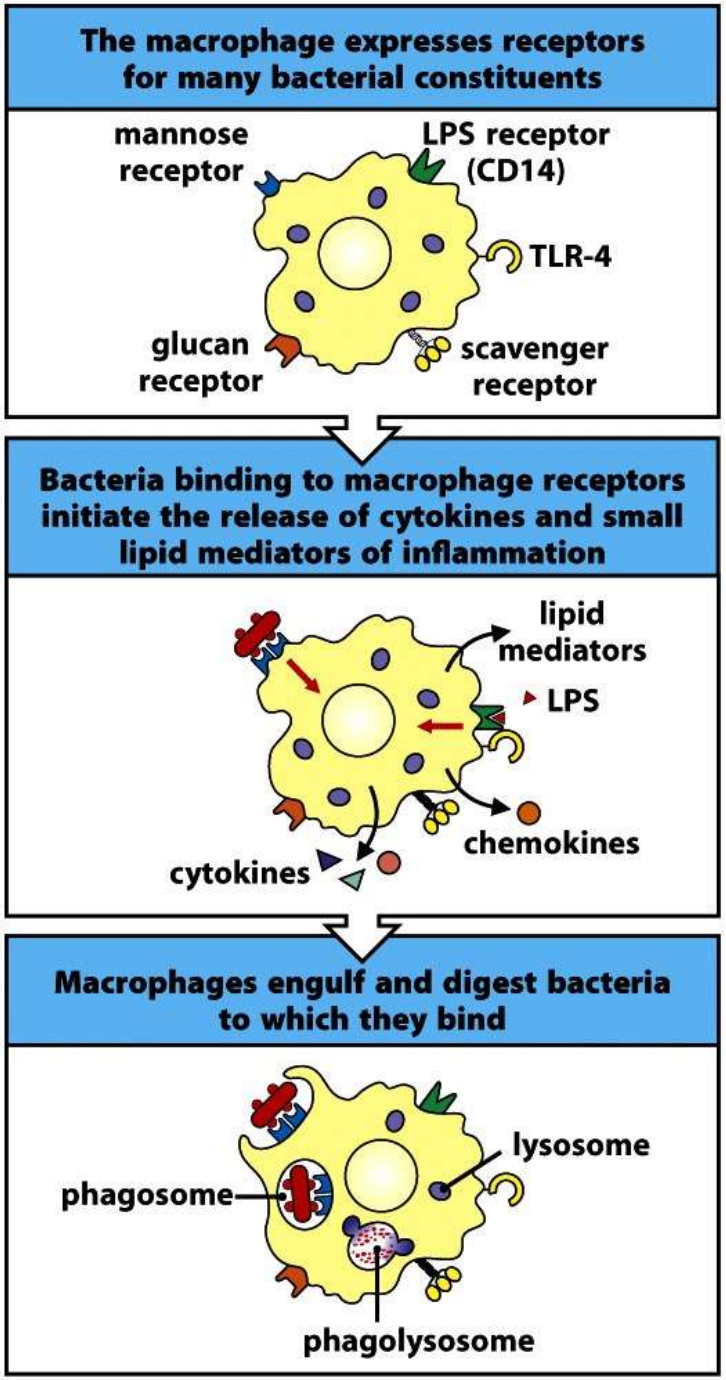


Figure 2-8 Immunobiology, 7ed. (© Garland Science 2008)