Basic introduction to bacterial pathogenicity and virulence factors

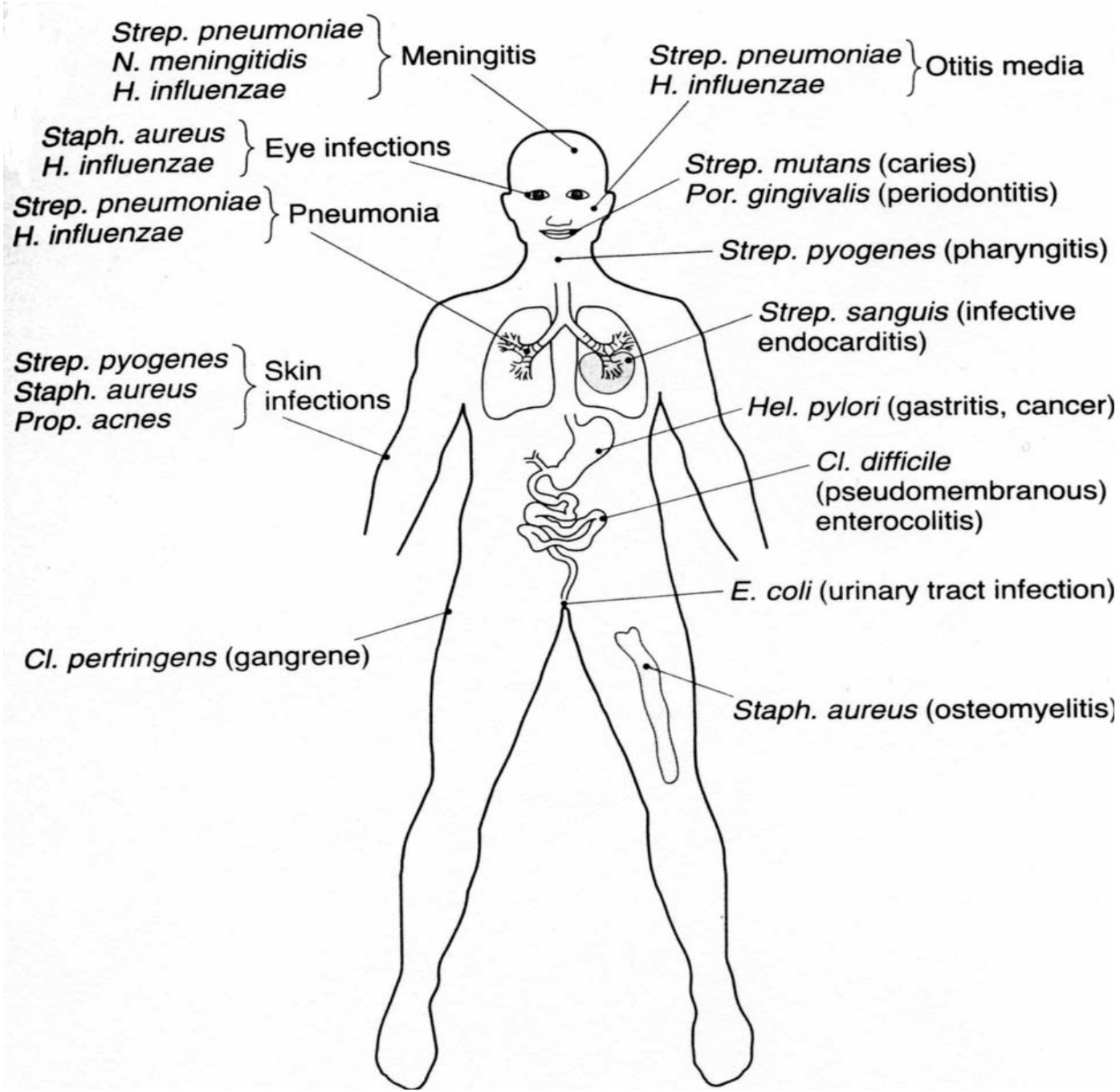
Learning objectives

You should be able to:

(1) Define a virulence factor

(2) Give examples of different types of virulence factors

(3) Show how knowledge of virulence factors can lead to new therapies


Strep. pneumoniae
N. meningitidis
H. influenzae
Meningitis
Strep. pneumoniae
H. influenzae
Otitis media
Staph. aureus
H. influenzae
Eye infections
Strep. mutans (caries)
Por. gingivalis (periodontitis)
Strep. pneumoniae
H. influenzae
Pneumonia
Strep. pyogenes (pharyngitis)
Strep. sanguis (infective endocarditis)
Strep. pyogenes
Staph. aureus
Prop. acnes
Skin infections
Hel. pylori (gastritis, cancer)
Cl. difficile (pseudomembranous) enterocolitis)
E. coli (urinary tract infection)
Cl. perfringens (gangrene)
Staph. aureus (osteomyelitis)

Normal flora (or indigenous biota)

	Microflora (grams wet weight)
Eyes	1
Nose	10
Mouth	20
URT	20
Vagina	20
Skin	200
Intestines	1000

Environmental factors

Chemical	Macronutrients, vitamins/growth factors, trace elements, pH, oxygen
Physical	Temperature, redox potential, osmotic and hydrostatic pressures
Biological	Host defence, presence other bacteria (nutrients/waste products), host secretions
Mechanical	Mechanical and fluid shear forces

Microbe human associations

- Encounter may be:
  ENDgenous or EXOgenous

- Factors affecting progress of a pathogen:
  - circumstantial
  - organism-related
  - host-related

Endogenous - caused by normal flora

Pneumonia following viral pneumonia:	*H. influenzae* (HI) *S. pneumoniae* (SPN)
Meningitis:	*Neisseria meningitidis* HI, SPN
Otitis media:	HI, SPN

Opportunistic (endogenous) infections arise:

- Presence of a foreign body e.g. catheter
- Transfer to sites where not normal flora e.g. NTHI
- Suppression of the immune system e.g. AIDS
- Disruption of normal flora by antibiotics
- Unknown factors…

Exogenous infections

- *Bordetella pertussis*
- *Mycobacterium tuberculosis*
- *Treponema pallidum*
- *Bacillus anthracis*

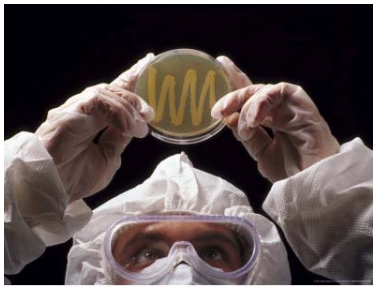
Not normally resident microflora (but TB!)

Establishing a specific microbe as the cause of an infective disease:

The Koch - Henle postulates:

1. Isolate the organism from all cases
2. Grow in pure culture *in vitro*
3. Inject the organism into a suitable recipient and reproduce disease
4. Re-isolate the organism from infection

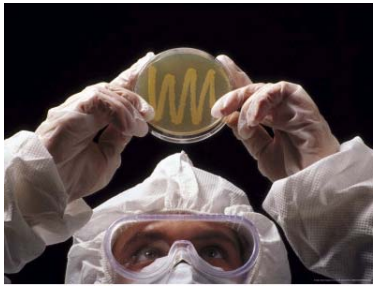
OK for major acute diseases e.g. plague, smallpox, typhoid



Isolate MO

Infection in a suitable animal model

Reproduce disease



Isolate MO

Confirm identity

But...

- chronic or minor conditions
- multiple causes
- pathogen can't be grown
- no suitable model

How would you define a virulence factor (determinant)?

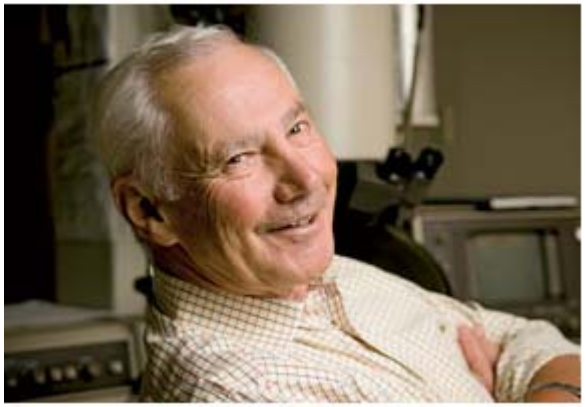
Any specific attribute of an organism whose loss decreases virulence

or

A component of the pathogen that damages the host: this can also include components essential for viability

Molecular Koch's postulates

Experimental criteria to show that a gene is a virulence factor



Falkow S (1988) *Rev Infect Dis* **10**(suppl 2):S274-S276.

1. Identify gene (or gene product) responsible for the virulence determinant

2. Show gene present in genus/species/ strains of bacteria that cause the disease

3. Not present in avirulent strains

4. Disrupting the gene reduces virulence

5. Introduction of cloned gene into avirulent strain confers virulence

6. The gene is expressed in vivo

7. Specific immune response to gene protects

But…

- Genetic manipulation may not be possible
- Lack of animal models
- What defines a genus or a species?

- Adhesin:

  Enables binding to a host tissue

- Invasin:

  Enables invasion of a host cell/tissue

- Impedin:

  Enables avoidance one or more of the host's defence mechanisms e.g. capsule

- Aggressin:

  Causes damage to the host directly
  e.g. exotoxins, enzymes

- Modulin:

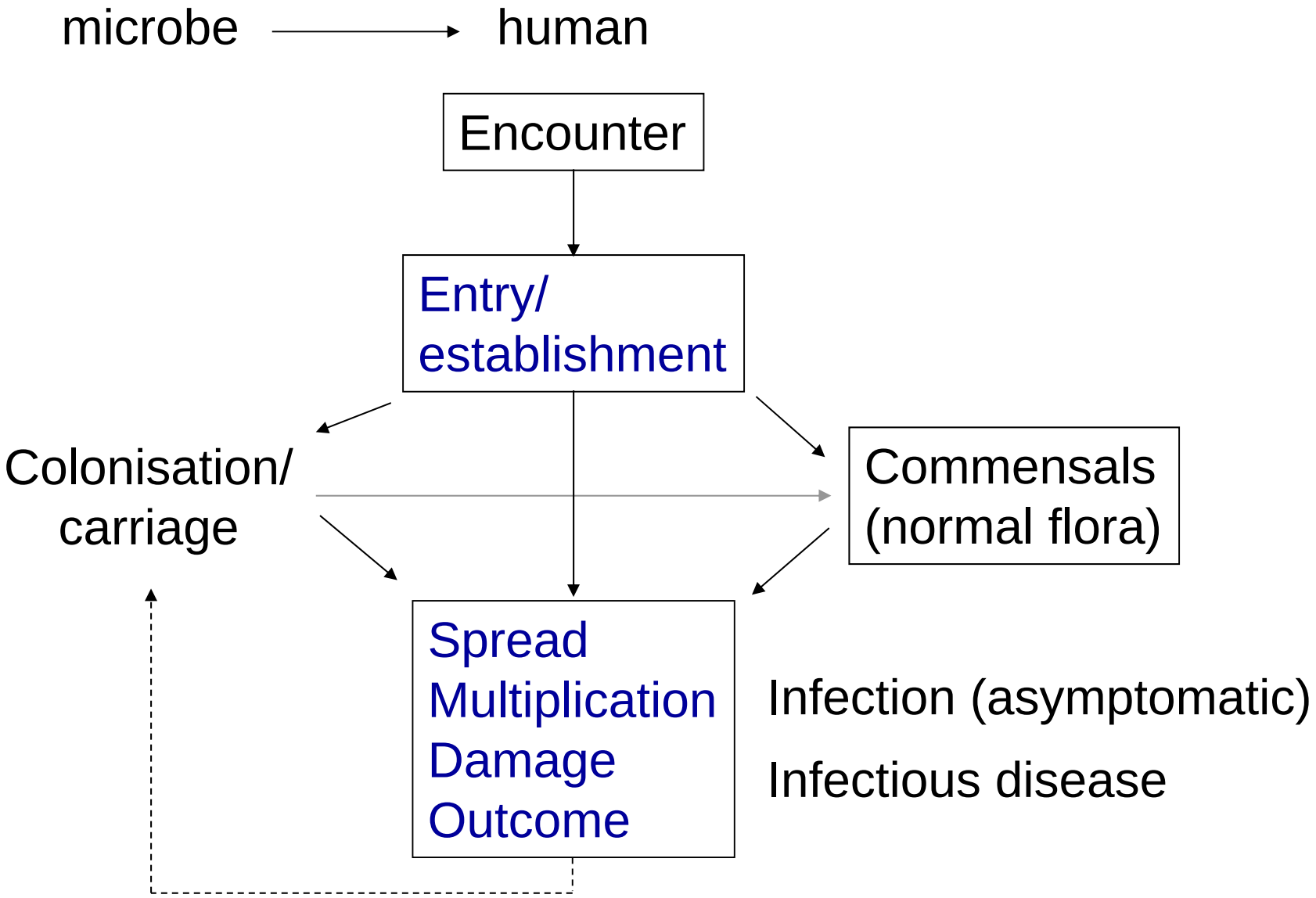
  Induces damage in the host indirectly by perturbing cytokine networks e.g. LPS

Why do we need to know?

Proof of involvement is important:

- Scientifically
- Identify new targets for:

  antibiotics,
  microbiocides,
  vaccines
  anti-virulence therapy etc…


microbe
human
Encounter
Entry/
establishment
Colonisation/
carriage
Commensals
(normal flora)
Spread
Multiplication
Damage
Outcome
Infection (asymptomatic)
Infectious disease

“Virulence factors” can be related to the stages of infection

But…

some may be required for multiple stages in an infection

Entry/establishment

Attachment via pili or non-fimbrial adhesins

Motility and chemotaxis

Invasion of epithelial cells

sIgA proteases

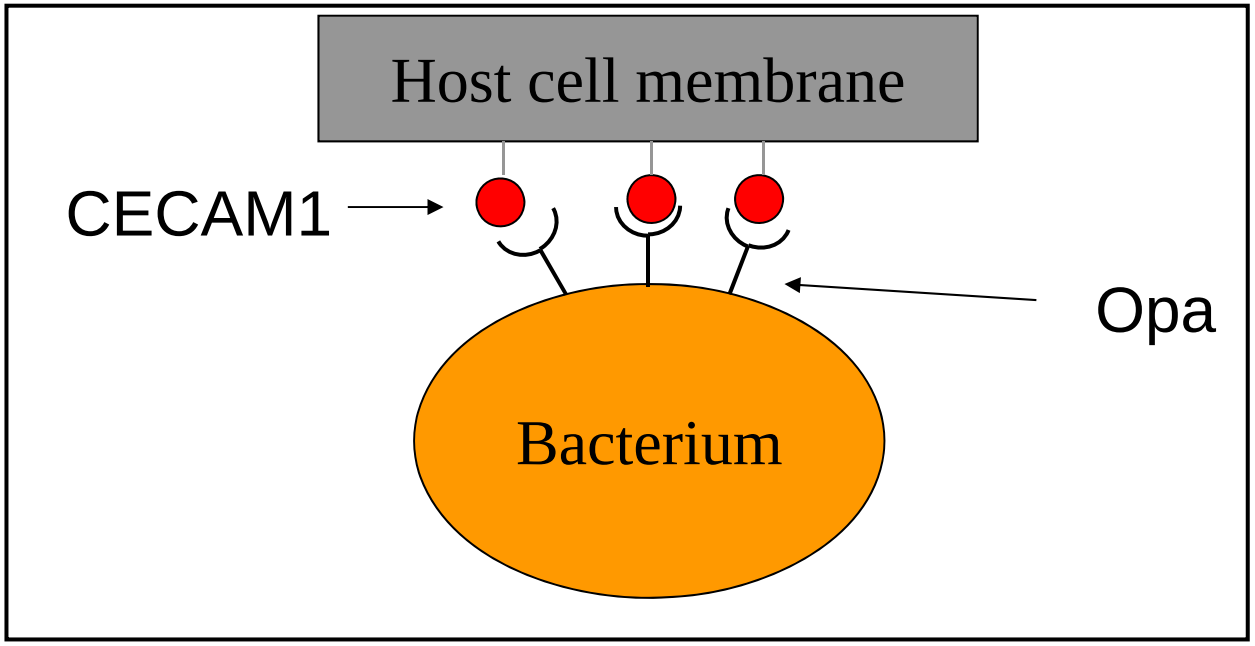
Capsules

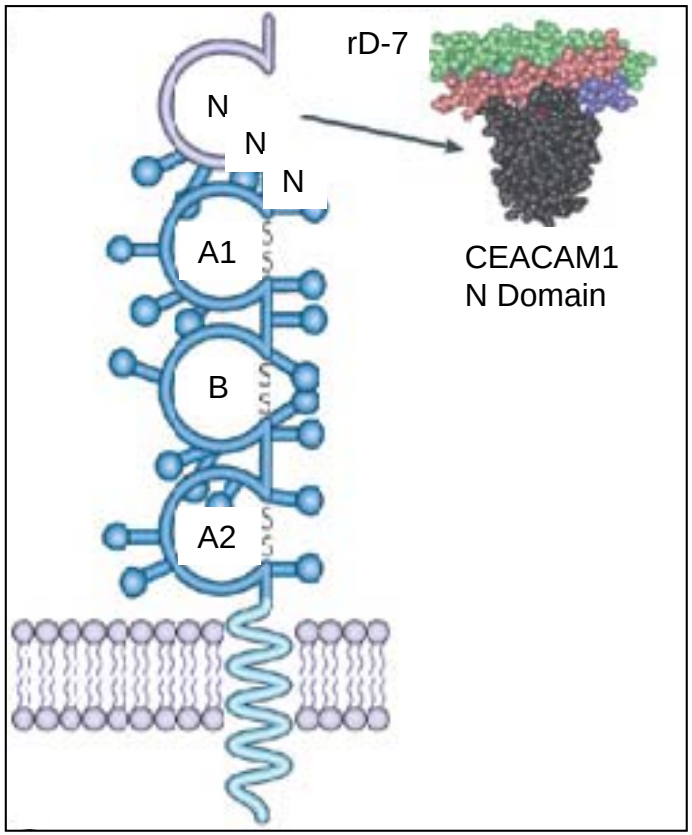
Survival inside phagocytes

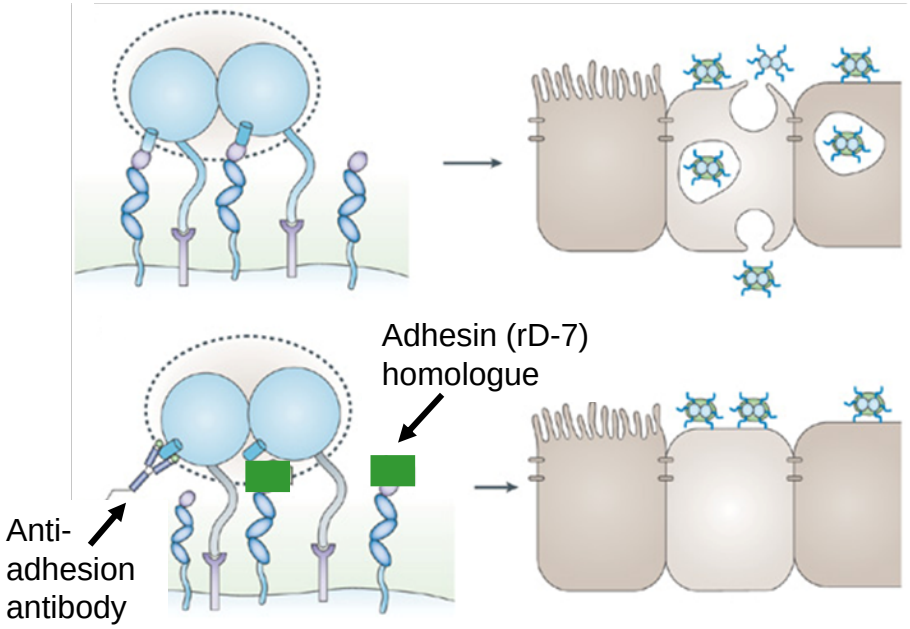
Host colonisation and invasion

Adherence

e.g. *N. meningitidis* Opa proteins bind to Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1)

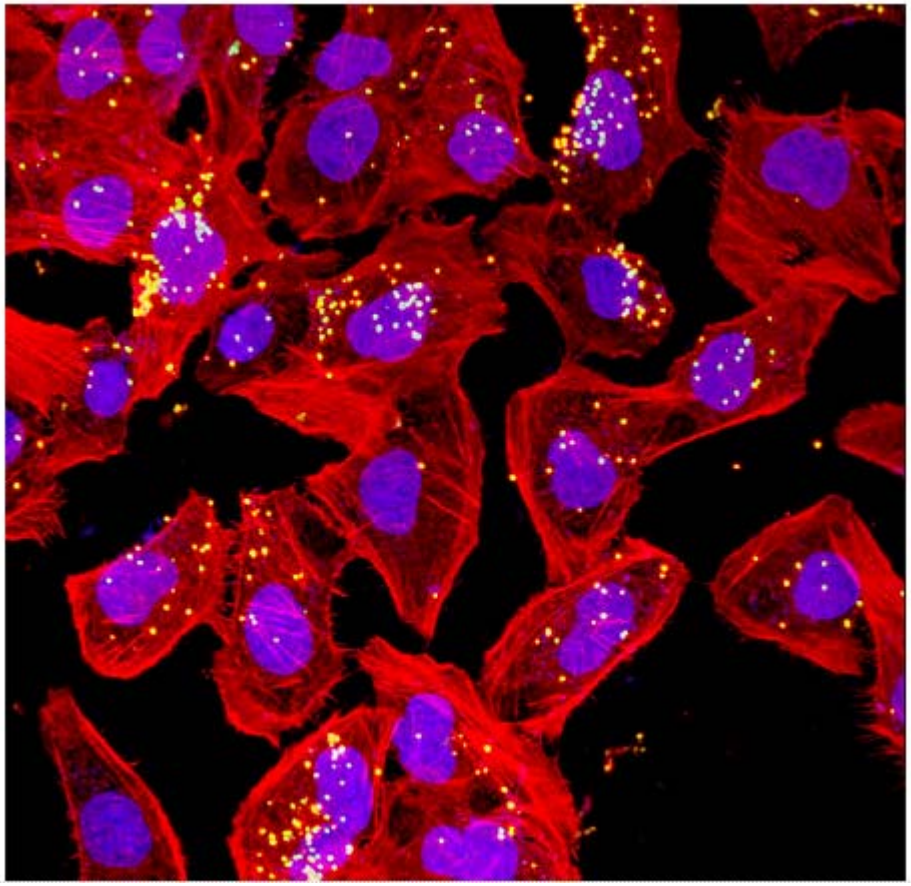


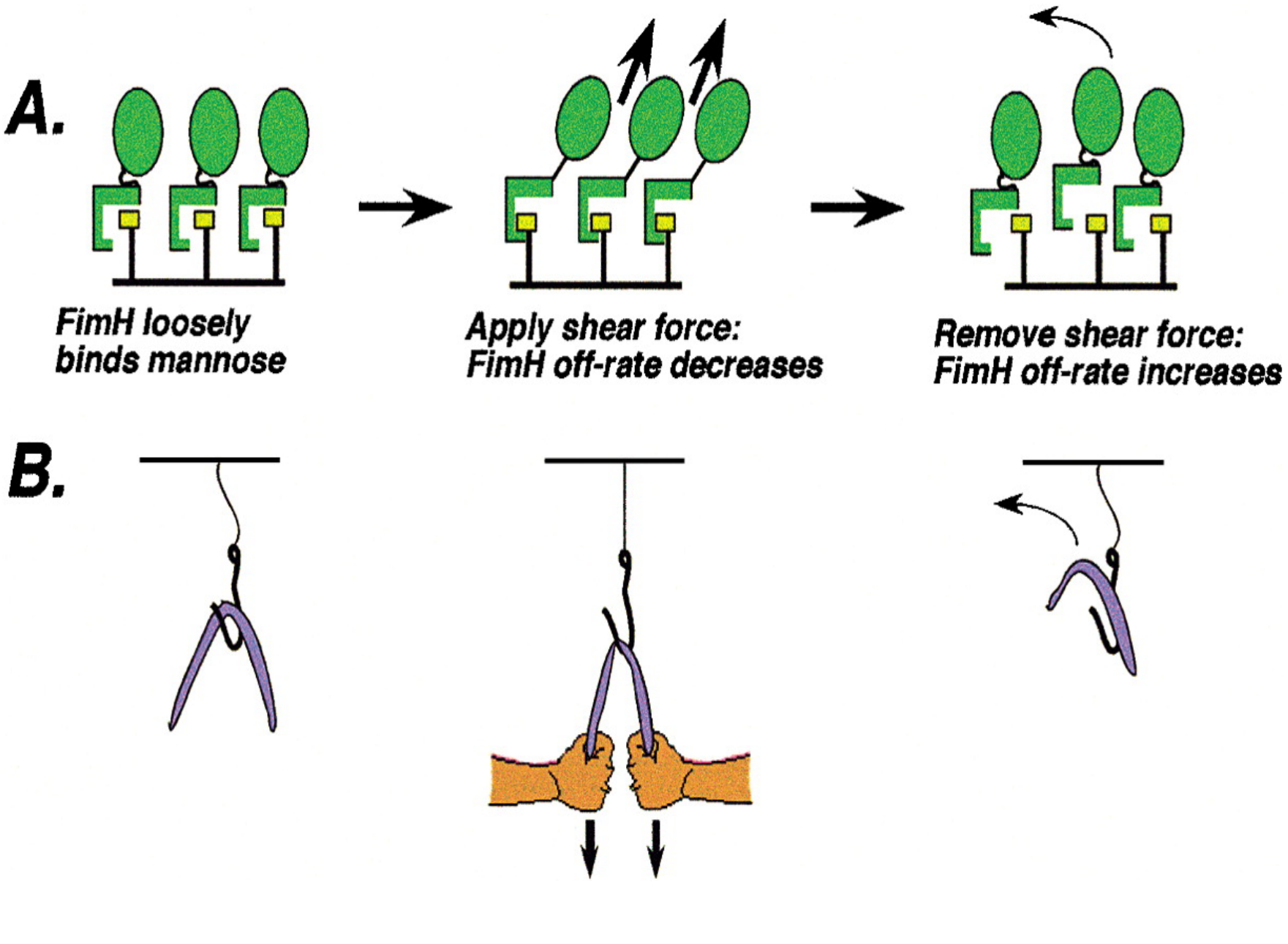


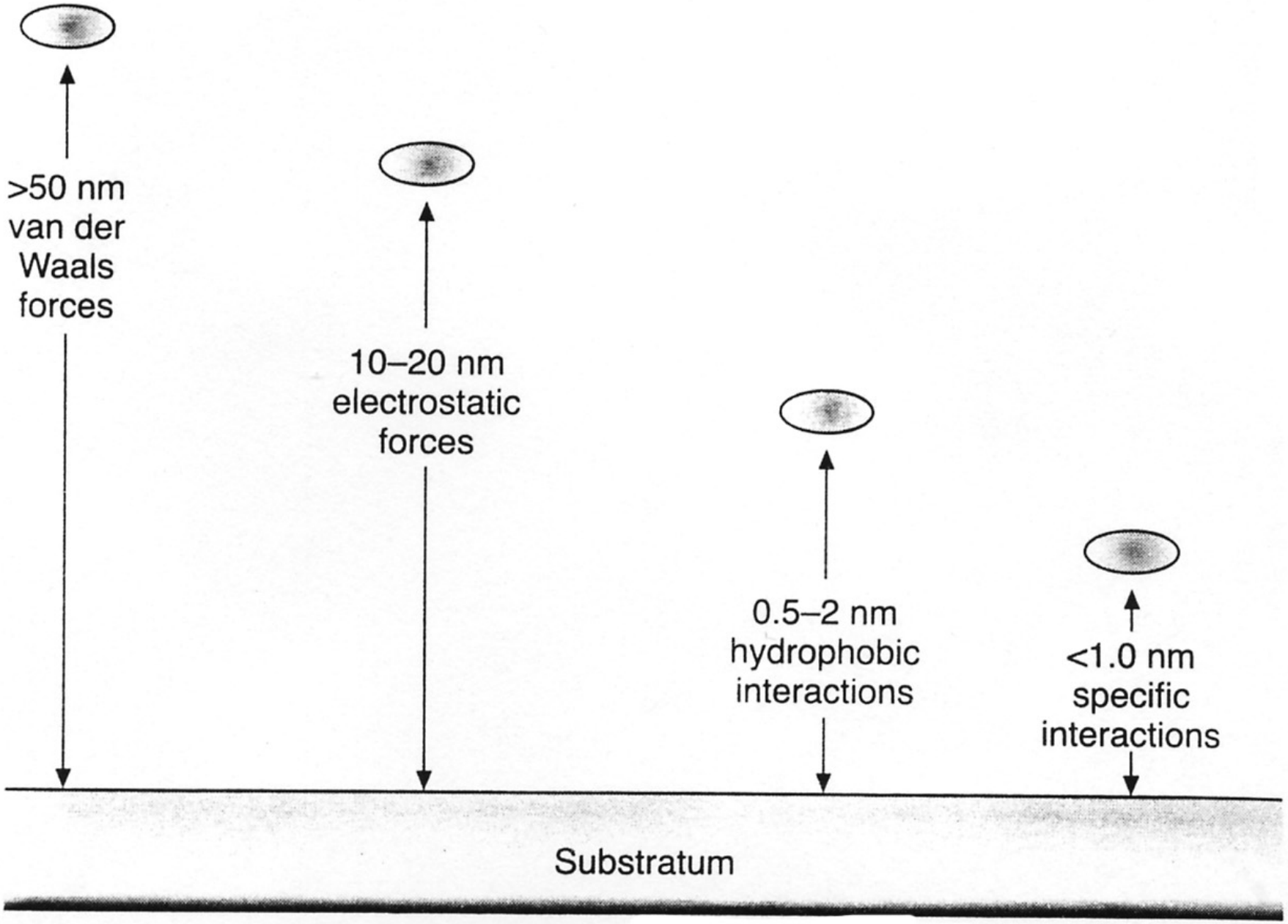


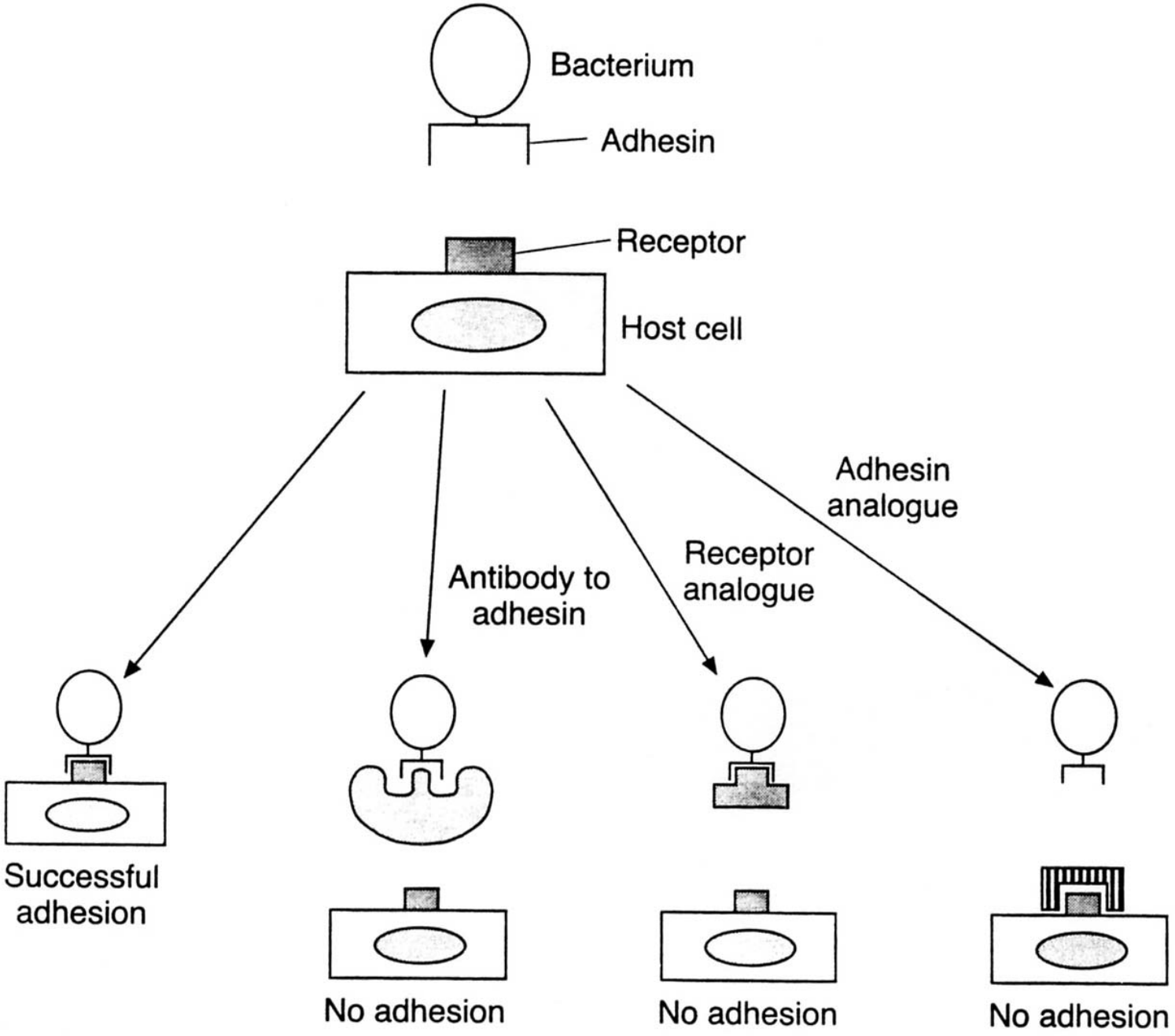
Virji M (2009) Nature Reviews Microbiology 7:274-286

N. meningitidis adhering to bronchial epithelial cells



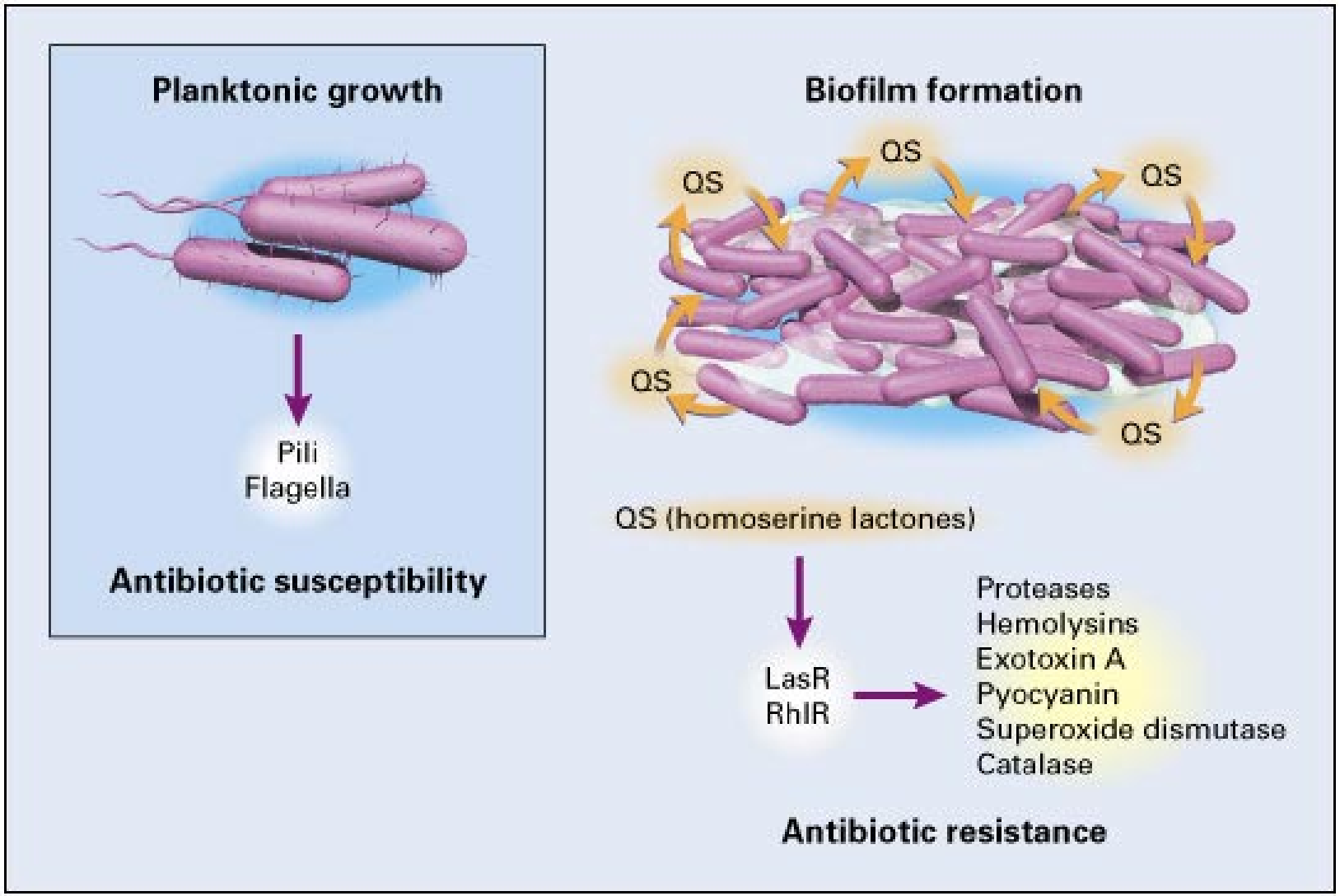

A.
FimH loosely binds mannose
Apply shear force: FimH off-rate decreases
Remove shear force: FimH off-rate increases
B.


>50 nm
van der
Waals
forces
10–20 nm
electrostatic
forces
0.5–2 nm
hydrophobic
interactions
<1.0 nm
specific
interactions
Substratum


Bacterium
Adhesin
Receptor
Host cell
Adhesin analogue
Receptor analogue
Antibody to adhesin
Successful adhesion
No adhesion
No adhesion
No adhesion

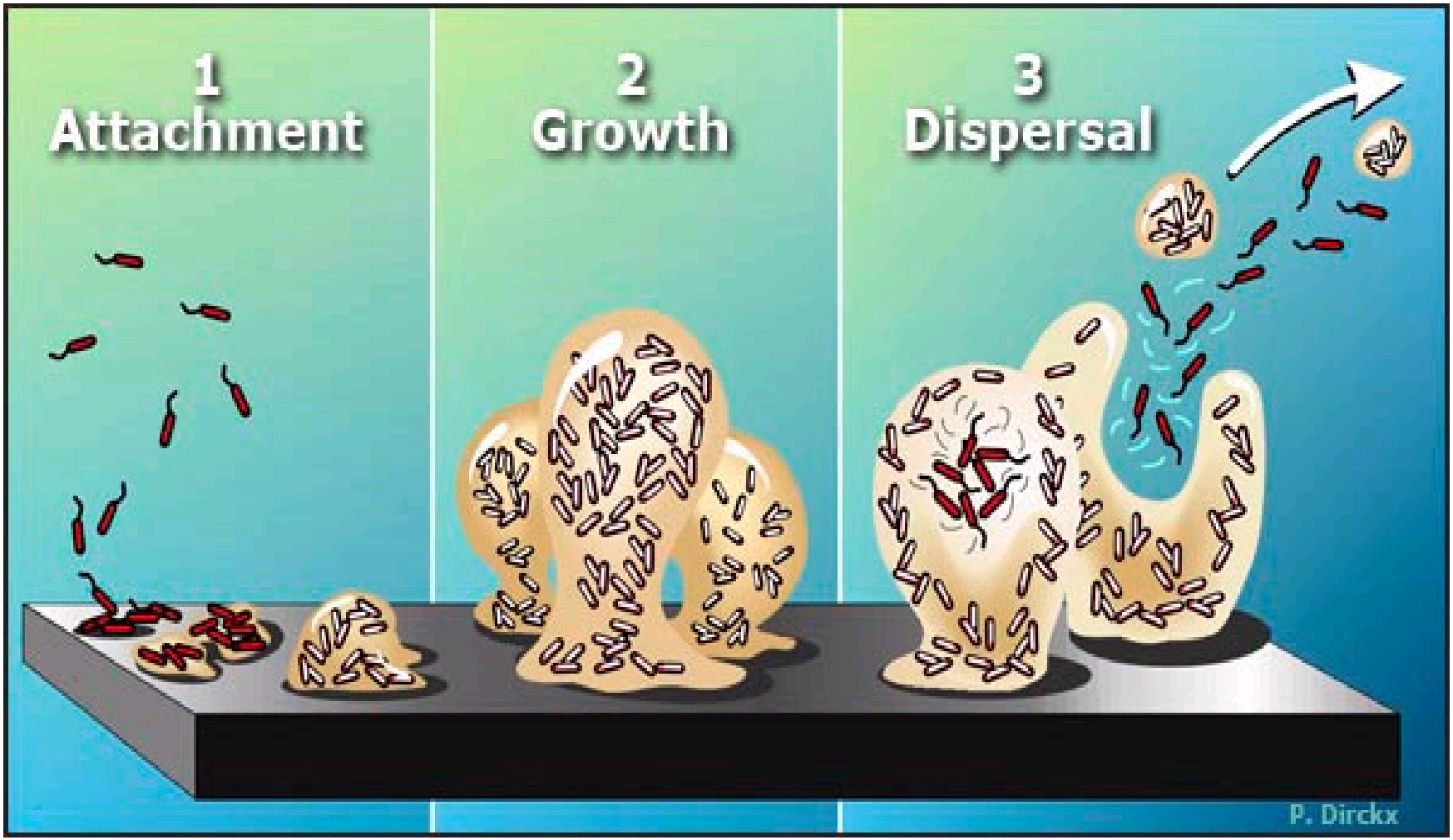
Biofilms - an adherence mechanism

- 3D structure
- Forms at interfaces
- Exhibits spatial heterogeneity
- Marked decrease in susceptibility to antimicrobial agents and host defences
  (cf to planktonic counterparts)



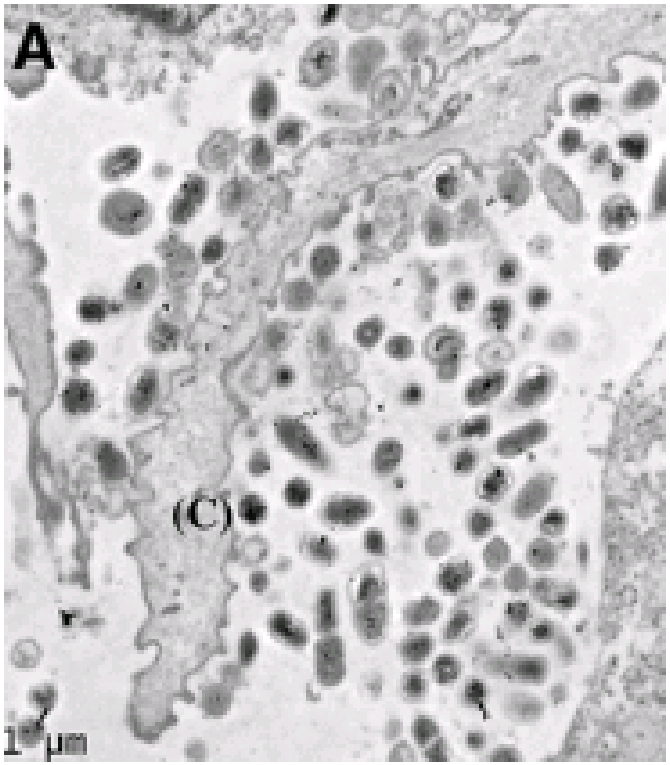
e.g. *Pseudomonas aeruginosa* Cystic fibrosis

Haemophilus influenzae NT Otitis media


1
Attachment
2
Growth
3
Dispersal
P. Dirckx

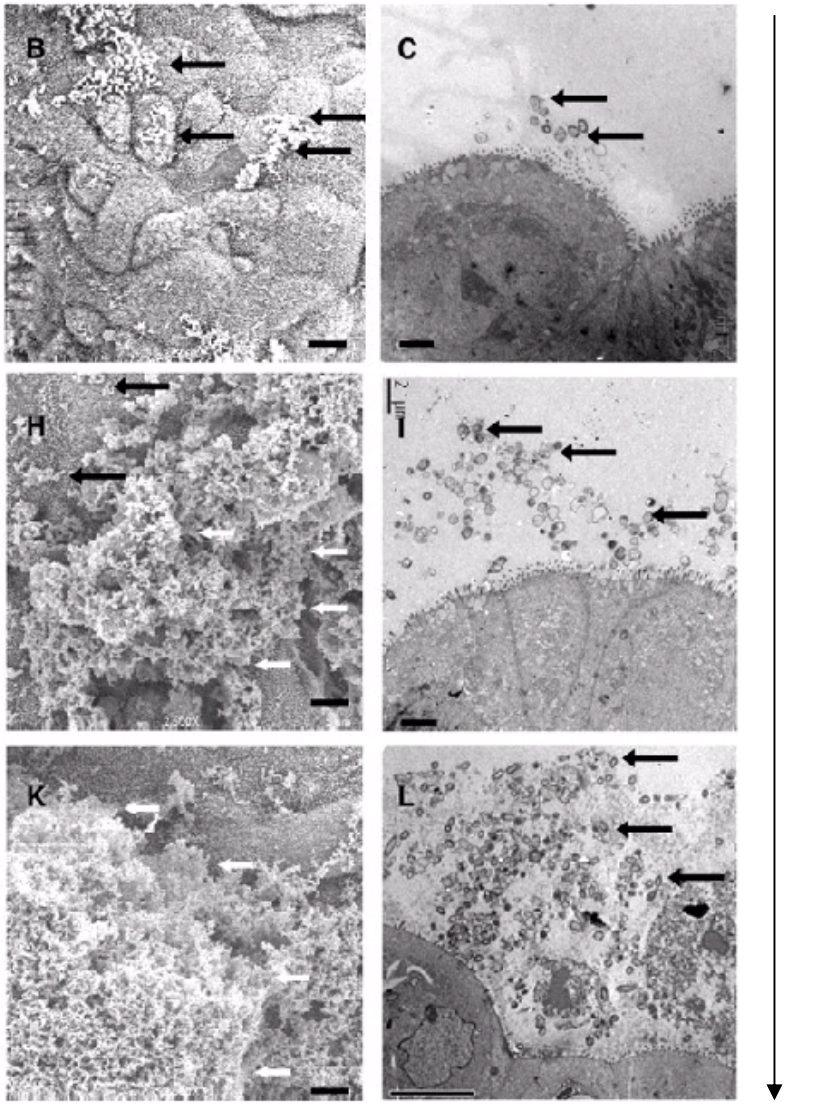
Non-typeable *H. influenzae*

Starner et al. (2007) AJRCCM 174:213-220



CF lung

time



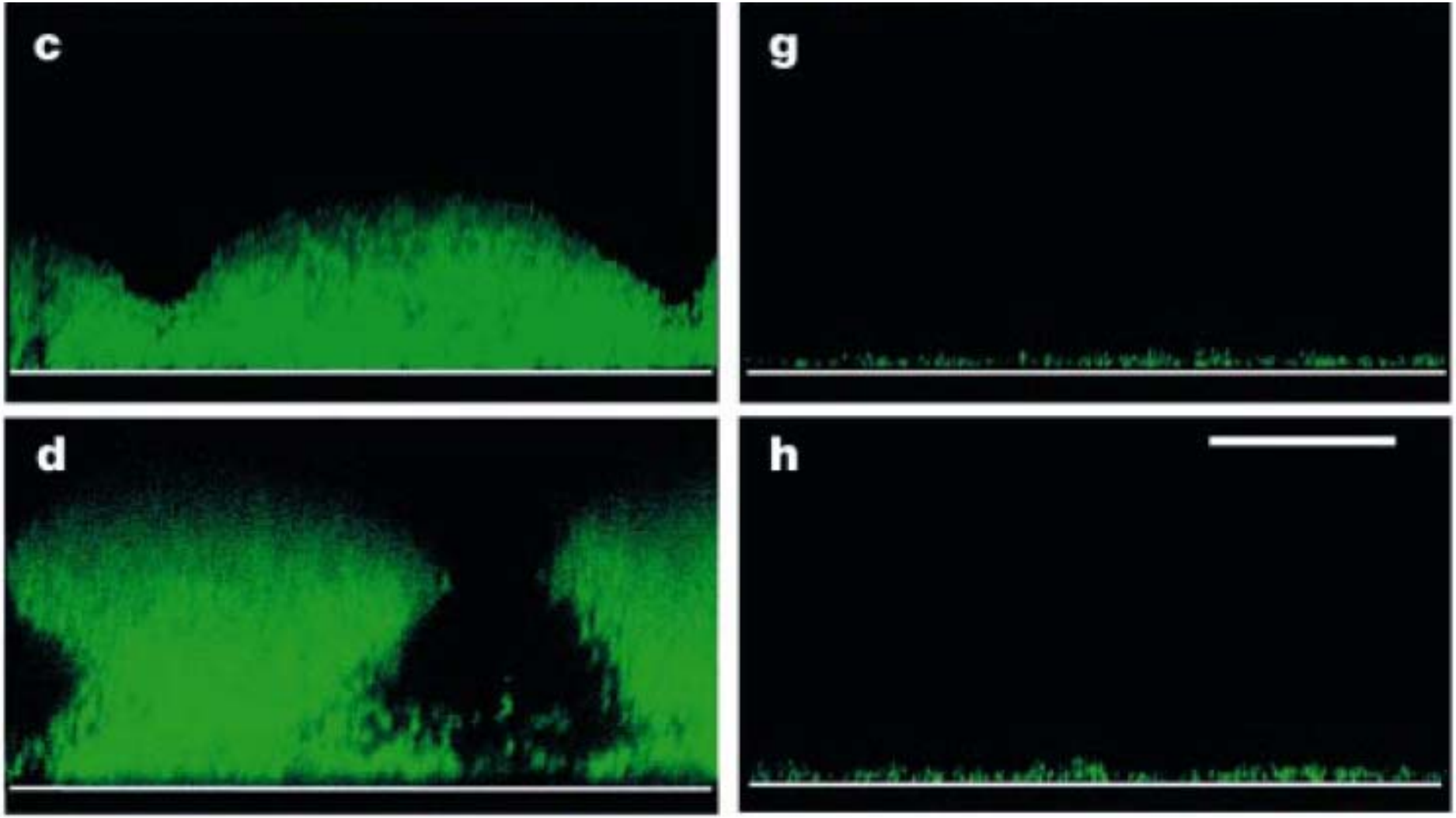
lung epithelial cells

Lactoferrin blocks biofilm development by Ps. aeruginosa

- LF + iron → stimulates bacterial “twitching”
- Bacteria wander across the surface instead of forming cell clusters and biofilms

Singh PK et al. (2002) Nature 417:552-555

Play movies



No lactoferrin

+ lactoferrin

Antimicrobial resistance and biofilms

- Binding to biofilm matrix
- Inactivation by enzymes in matrix
- Reduced growth rate of bacteria
- Altered microenviroment (pH, oxygen) leading to reduced activity
- Altered gene expression of biofilm leading to resistance

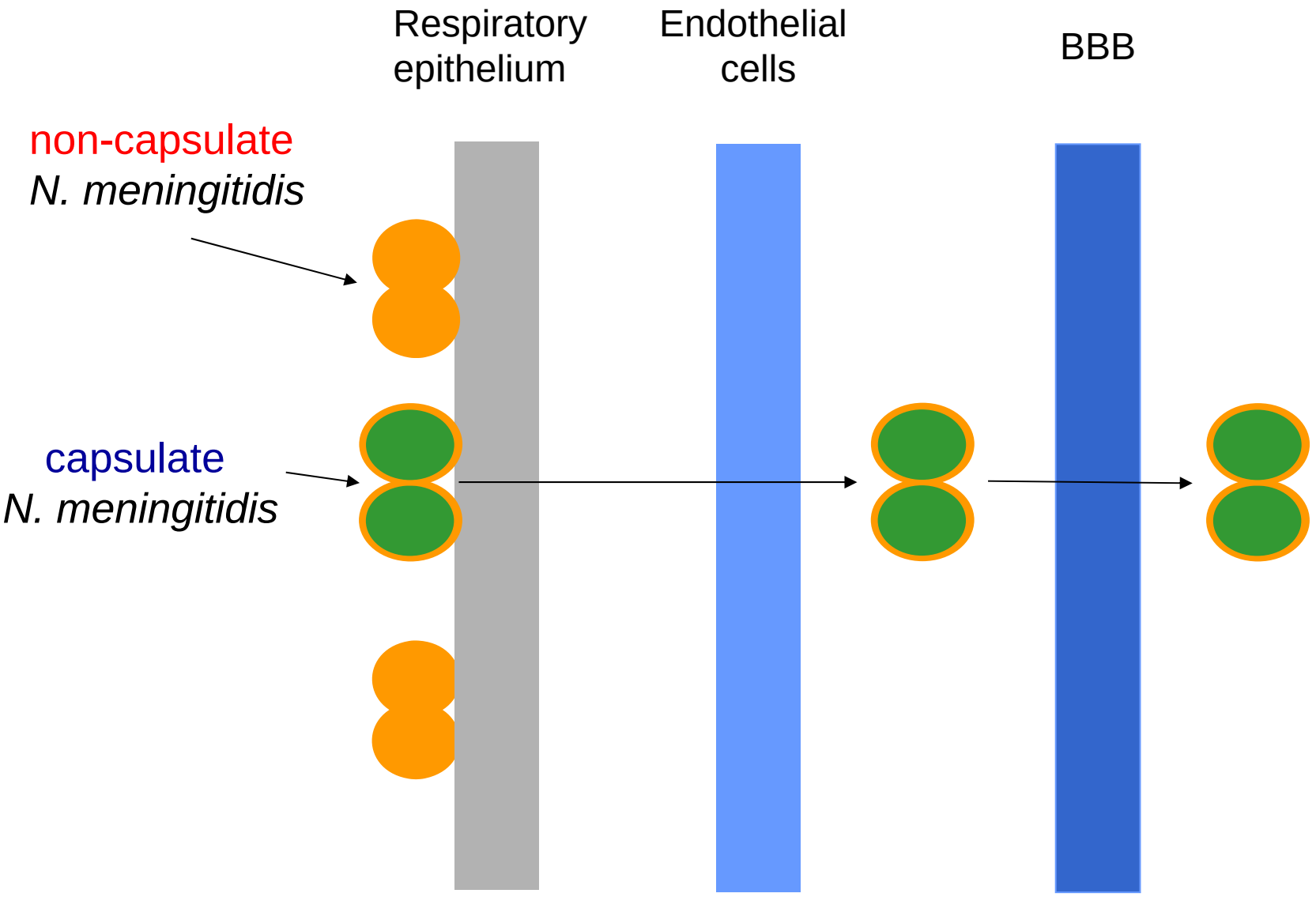
NB Also more resistant to phagocytosis and C’ killing

2. Spread

Tissue breakdown by enzymes or toxins e.g. phospholipases

Penetration to bloodstream (via endothelial cells) or lymphatics

Dissemination in circulating cells e.g. macrophages, PMNs


Respiratory epithelium
Endothelial cells
BBB
non-capsulate
N. meningitidis
capsulate
N. meningitidis

3. Multiplication

Nutrient acquisition

- especially Fe (siderophores, TBPs, LBPs)
- immune evasion (e.g. molecular mimicry)

4. Damage

Toxins

Host "over-reaction" (immune-mediated hypersensitivity)

Fe-acquisition by pathogens

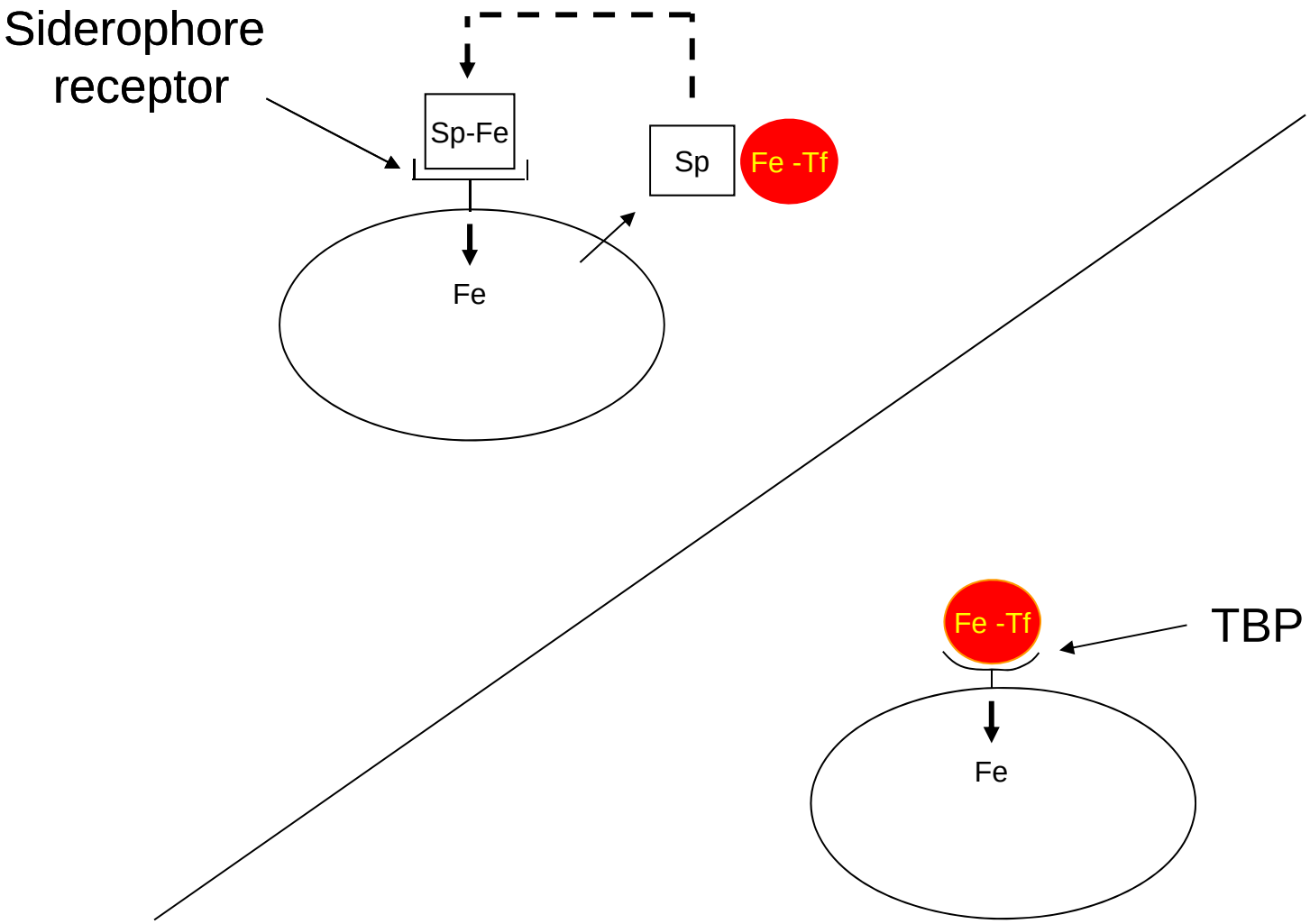
Need Fe for growth

↓

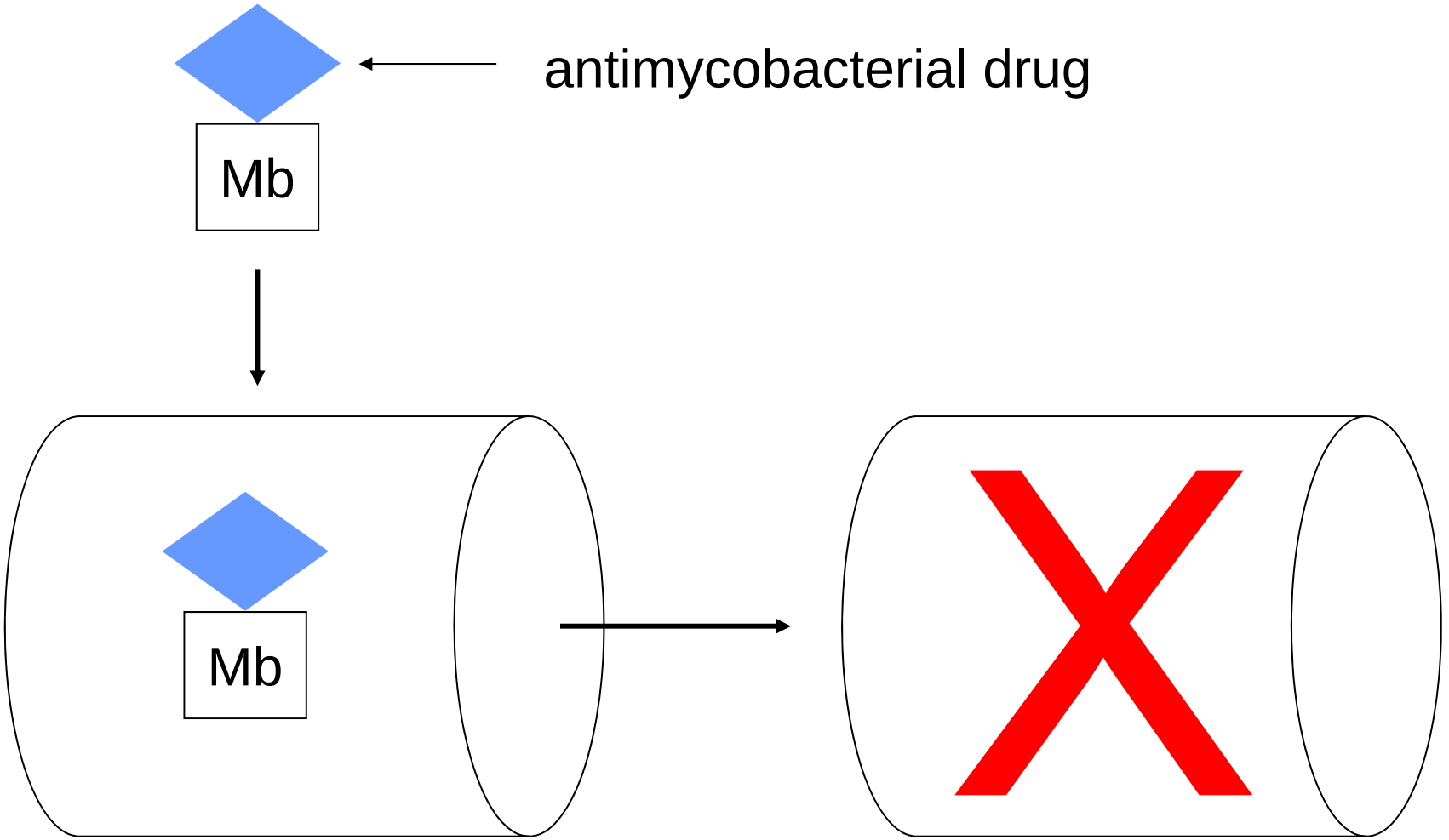
Host Fe bound by transferrin, lactoferrin, haemoglobin

↓

Developed mechanisms to bind host Fe

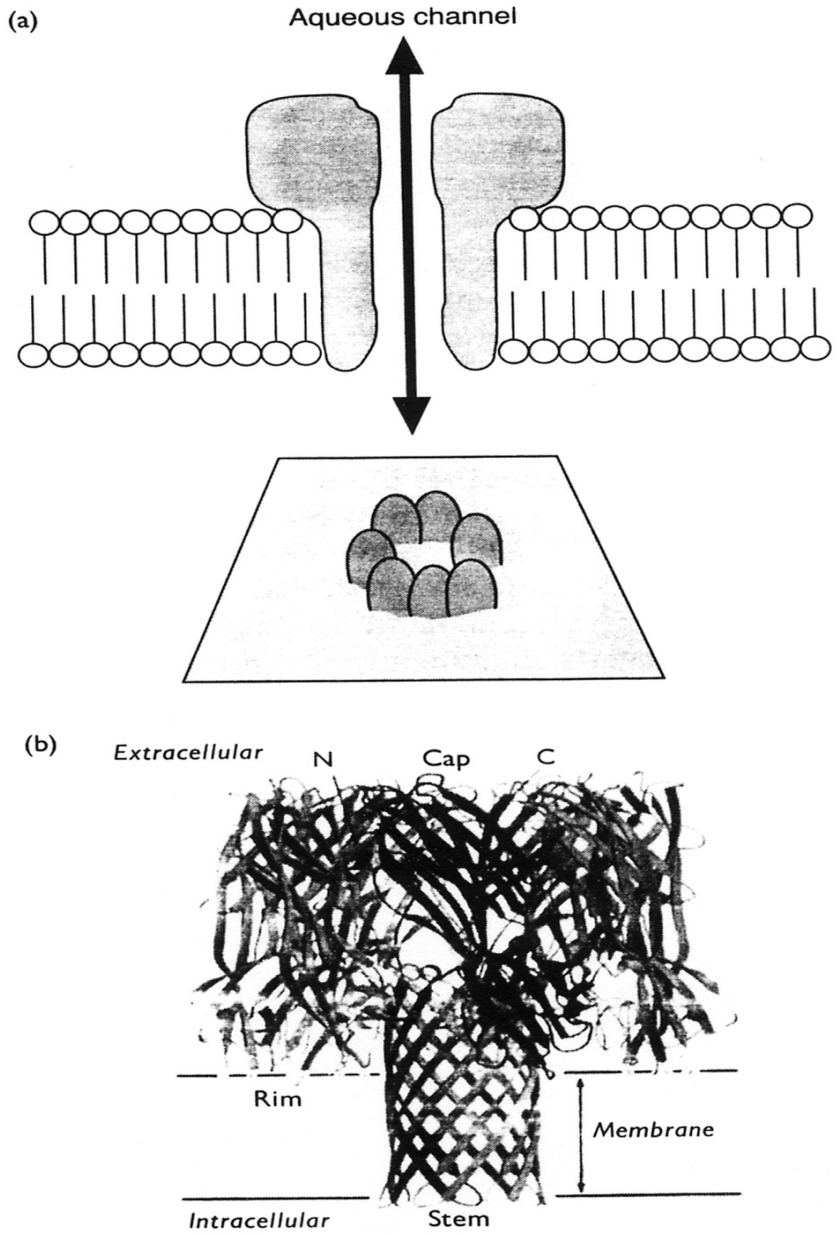

Siderophore receptor
Sp-Fe
Sp
Fe -Tf
Fe
Fe -Tf
TBP
Fe

Mycobacterial siderophores = mycobactins (Mb)



Toxins

Strep pneumoniae	Pneumolysin
	Streptolysin O
Staph aureus	Alphatoxin
B. pertussis	CyaA
C. diphtheriae	Diphtheria toxin
	(ADP-ribosylation)
V. cholerae	Cholera toxin
E. coli	Haemolysin



Pore formers

S. aureus alpha-toxin

Diphtheria toxin

Catalytic

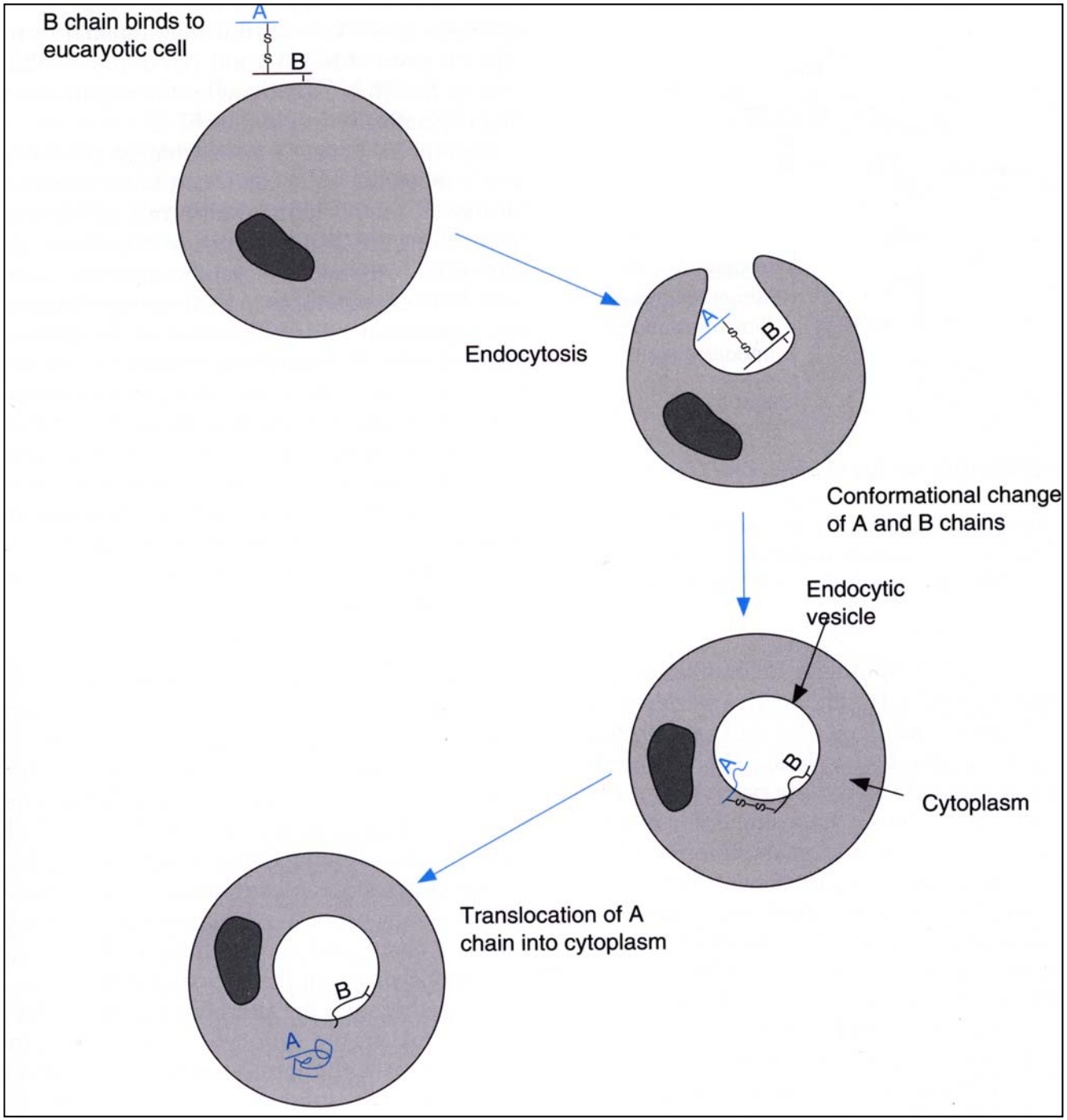
Receptor
Translocation

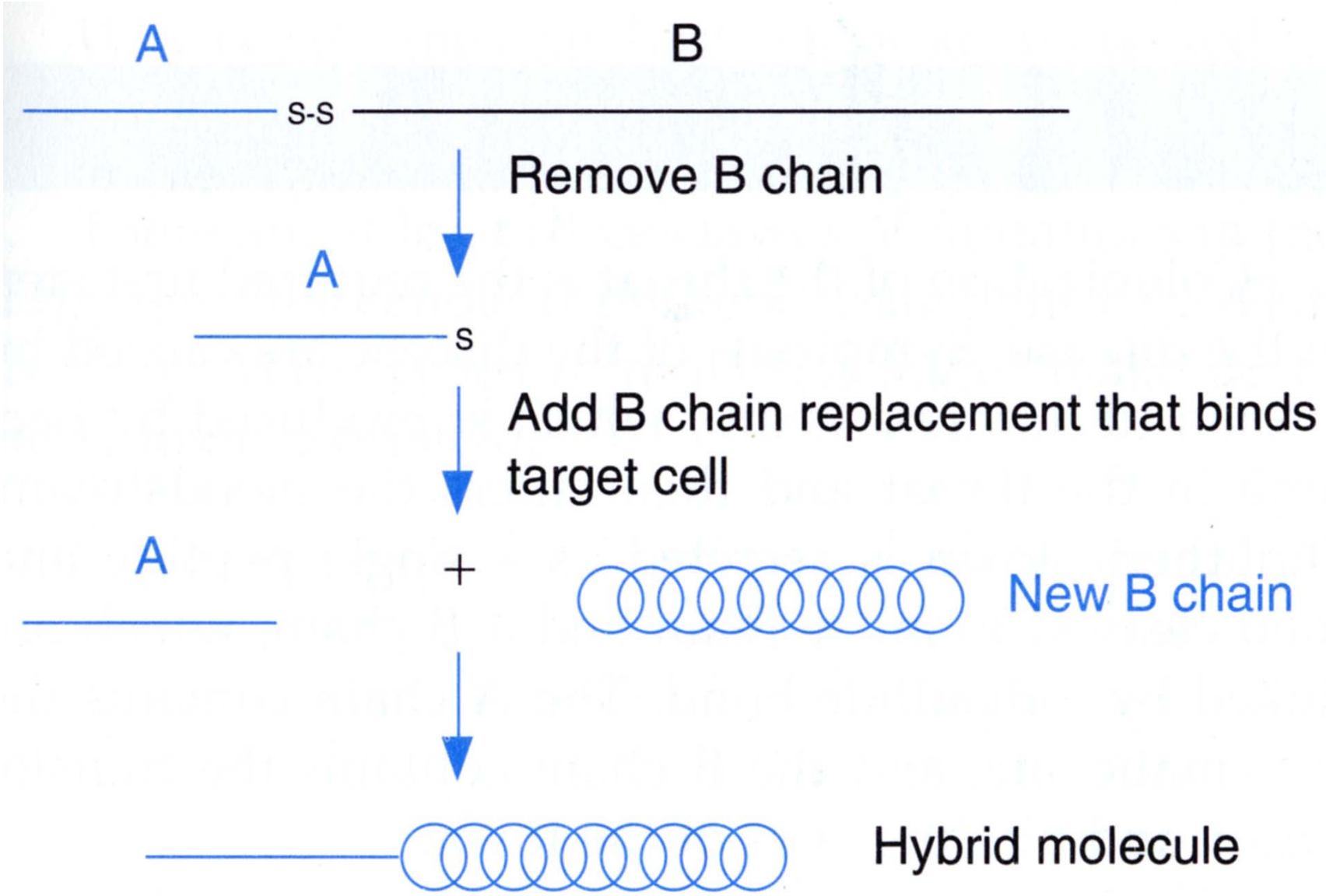
- DT is secreted
- ADP ribosylation

  NAD + EF-2

  → ADP-ribosyl-2 + nicotinamide + H^+
- Inhibition of elongation factor 2 (EF-2)

  → cell death


B chain binds to
eucaryotic cell
A
S
S
B
Endocytosis
A
B
Conformational change
of A and B chains
Endocytic
vesicle
A
B
Cytoplasm
Translocation of A
chain into cytoplasm
B
A


A
B
S-S
Remove B chain
A
S
Add B chain replacement that binds target cell
A
+
New B chain
Hybrid molecule

Can use the knowledge for good

Proposed use	Substitute for B chain
Kill erythroleukemia cells	Single-chain antibody to transferrin receptor
Kill peripheral blood mononuclear cells from patients with chronic lymphocytic leukemia	Single-chain antibody against interleukin-2 receptor (FDA license)

Potal S *et al.* (2008) Drug Discovery Today 13:807-814

Messages

- Infection is a multifactorial process – all these could contribute to virulence
- But proof of involvement is important
- Scientifically - allows targets for treatment and prevention to be identified

Antibiotics/Vaccines/Therapy