Graduation Spring 2025!

UNLV | School of LIFE SCIENCES

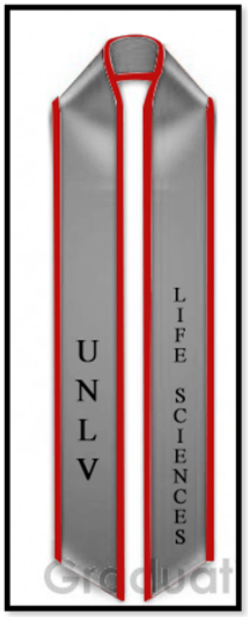
2025

Graduation Reception

AT THE SCIENCE & ENGINEERING BUILDING

Friday
May 16

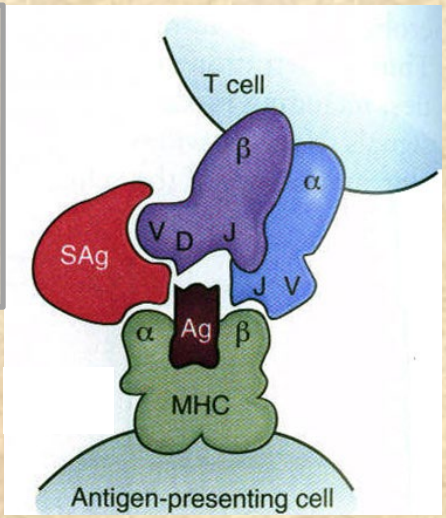
5:00 PM - 6:30PM
at SEB First Floor Lobby,
4505 S Maryland Pkwy
Las Vegas, NV 89154



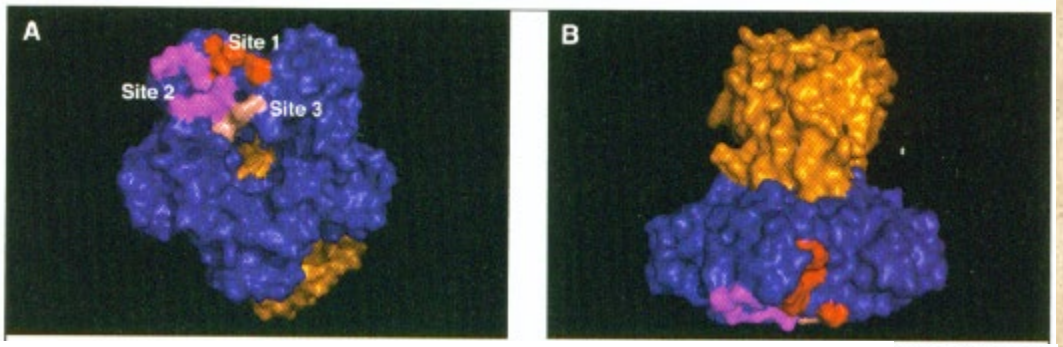
Graduating Students that RSVP AND attend the Graduation Reception receive a School of Life Sciences Stole to wear during their graduation ceremony

Lecture 24 & 25: Bacterial Toxins (Chapter 12)

- **Toxicity versus Invasiveness**
- **Mechanisms and Mysterious purposes**
- **Nomenclature** **LECT 24**
- **Class versus Type**



- Class III AB toxins exotoxins eg. Diphtheria and Botulinum
- Class II act on eukaryotic cells phospholipases, pore forming toxins, membrane disrupting
- Class I superantigens
- Those that act in the extracellular matrix – wound infections
- Injected into host cells (type III secretion) cytotoxin
- Endotoxin



Structure of Shiga toxin 1. Stx1A subunit, orange; and Stx2 B-pentamer, blue. Amino acid regions associated with binding to the mammalian receptor, Gb3, shown in shades of red; Site 1 (red), Site 2 (magenta), and Site 3 (salmon). A, Bottom view of the B-pentamer; B, Side view.

Toxin Types

- Class I

– bind target on cell surface not translocated into the cell eg. Superantigens

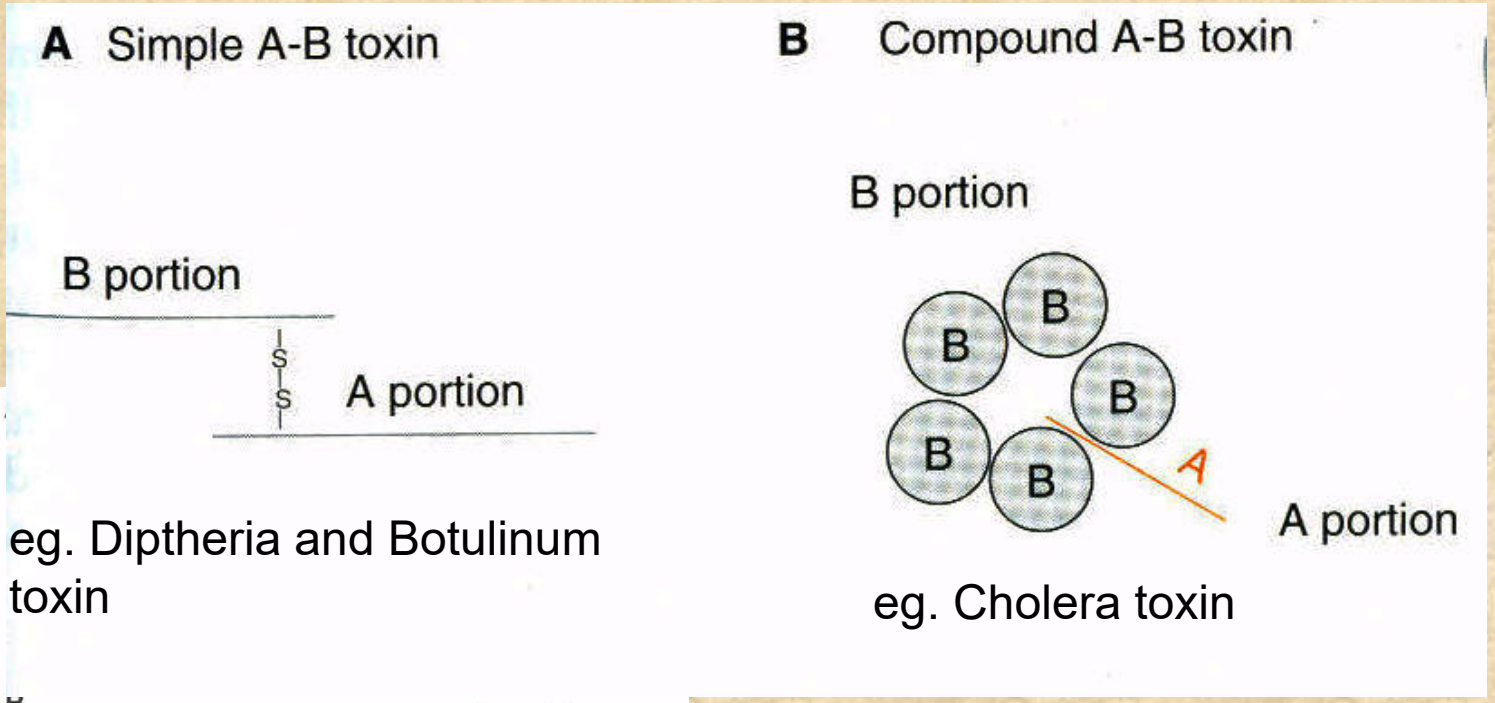
- Class II

- destroy integrity of eukaryotic cell membranes eg. Pore-forming cytotoxins, haemolysins, phospholipases

- Class III

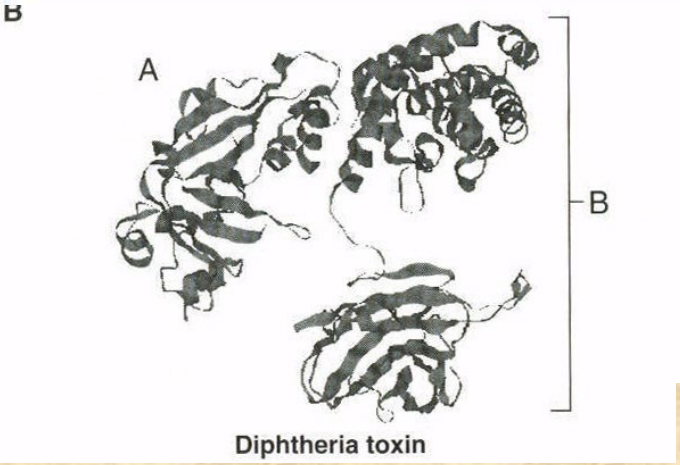
– A-B toxins, Simple or Complex eg. Diphtheria and Botulinum toxin

Simple and Complex Class III toxins



eg. Diptheria and Botulinum toxin

eg. Cholera toxin



Translocation of simple Class III toxins

DT toxin, a simple toxin has two subunits encoded by a single gene. Subunits are cleaved from each other but are held together by a disulfide bond. The toxin is endocytosed after receptor binding. Upon acidification of the endocytic vesicle a dramatic conformational change occurs whereby it inserts into the vesicle's membrane. This results in the translocation of the A subunit to the cytoplasm where it ADP ribosylates its intracellular target, EF-2 (a protein that plays an essential role in host protein synthesis)

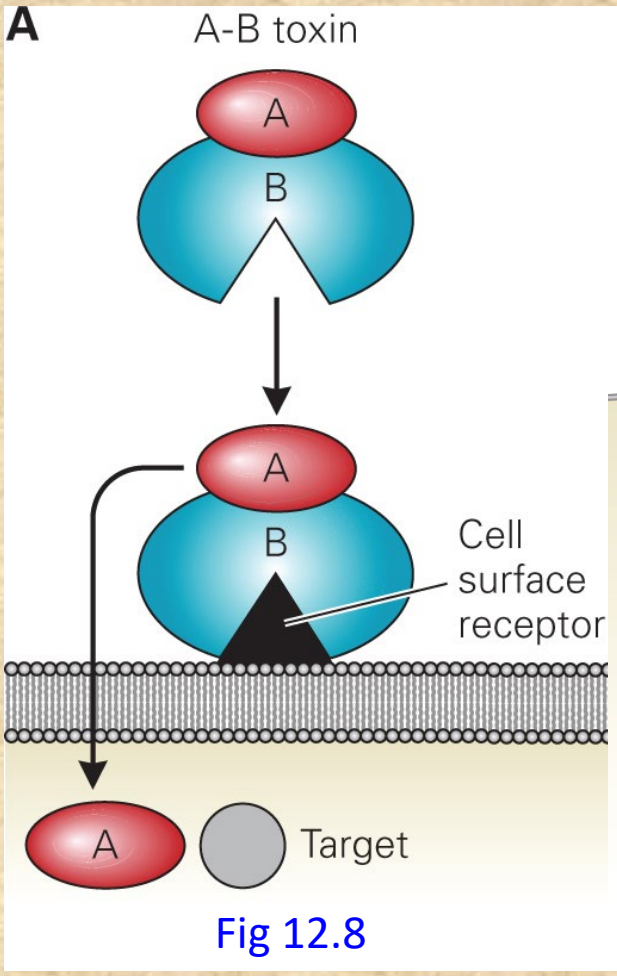


Fig 12.8

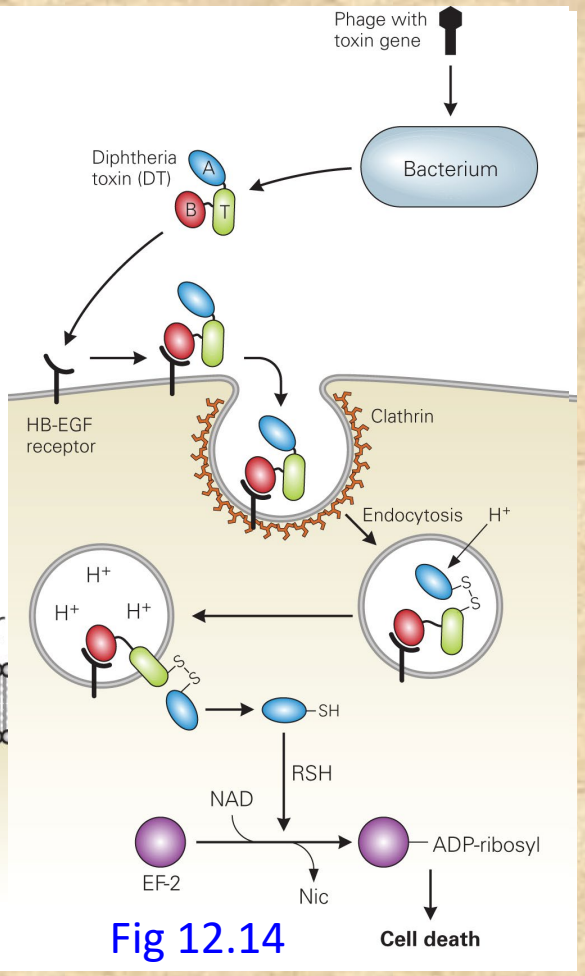
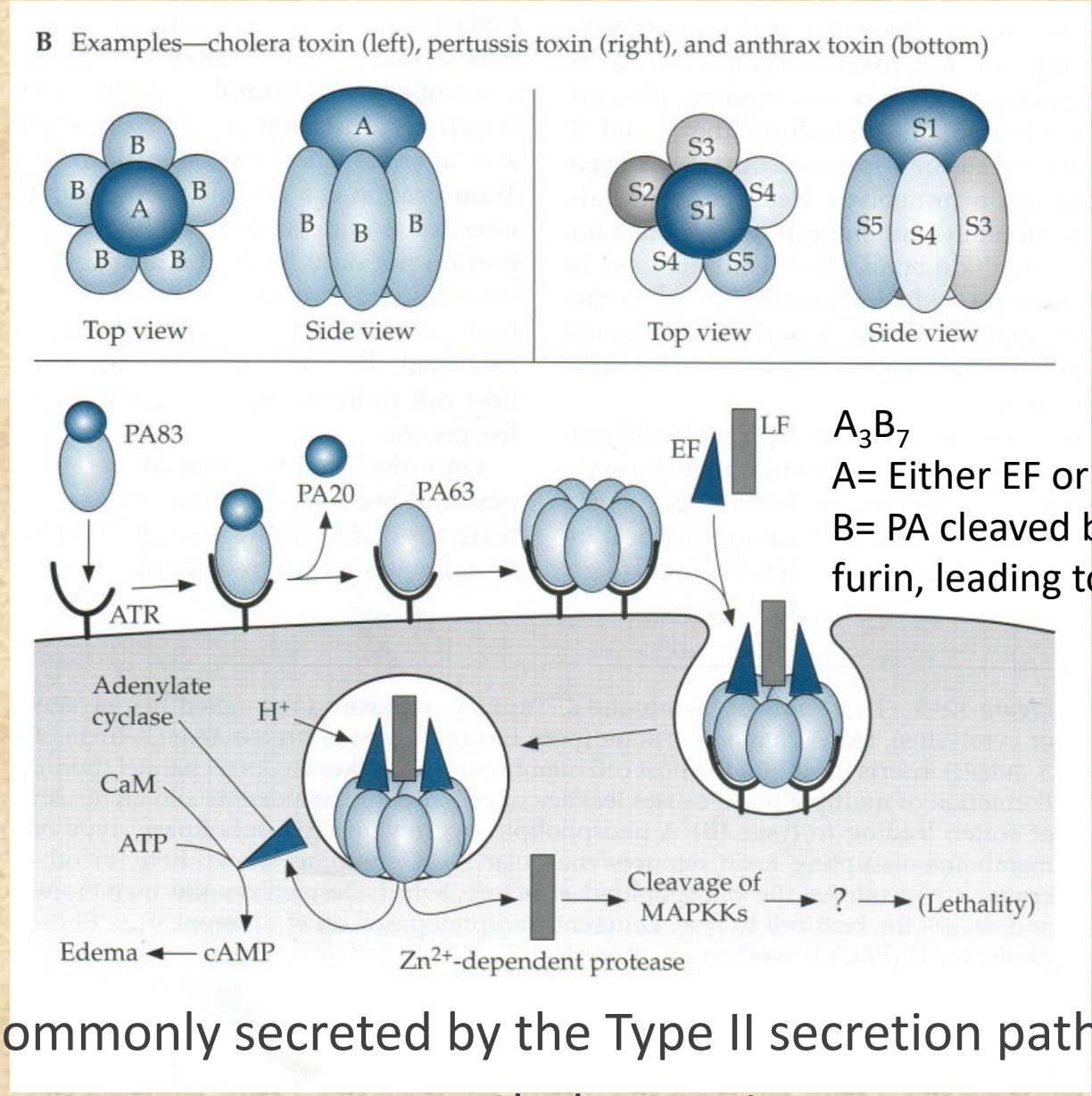


Fig 12.14

HB-EGF= Heparin Binding Epidermal Growth Factor receptor

Complex or multi-subunit Class III toxins



A_3B_7

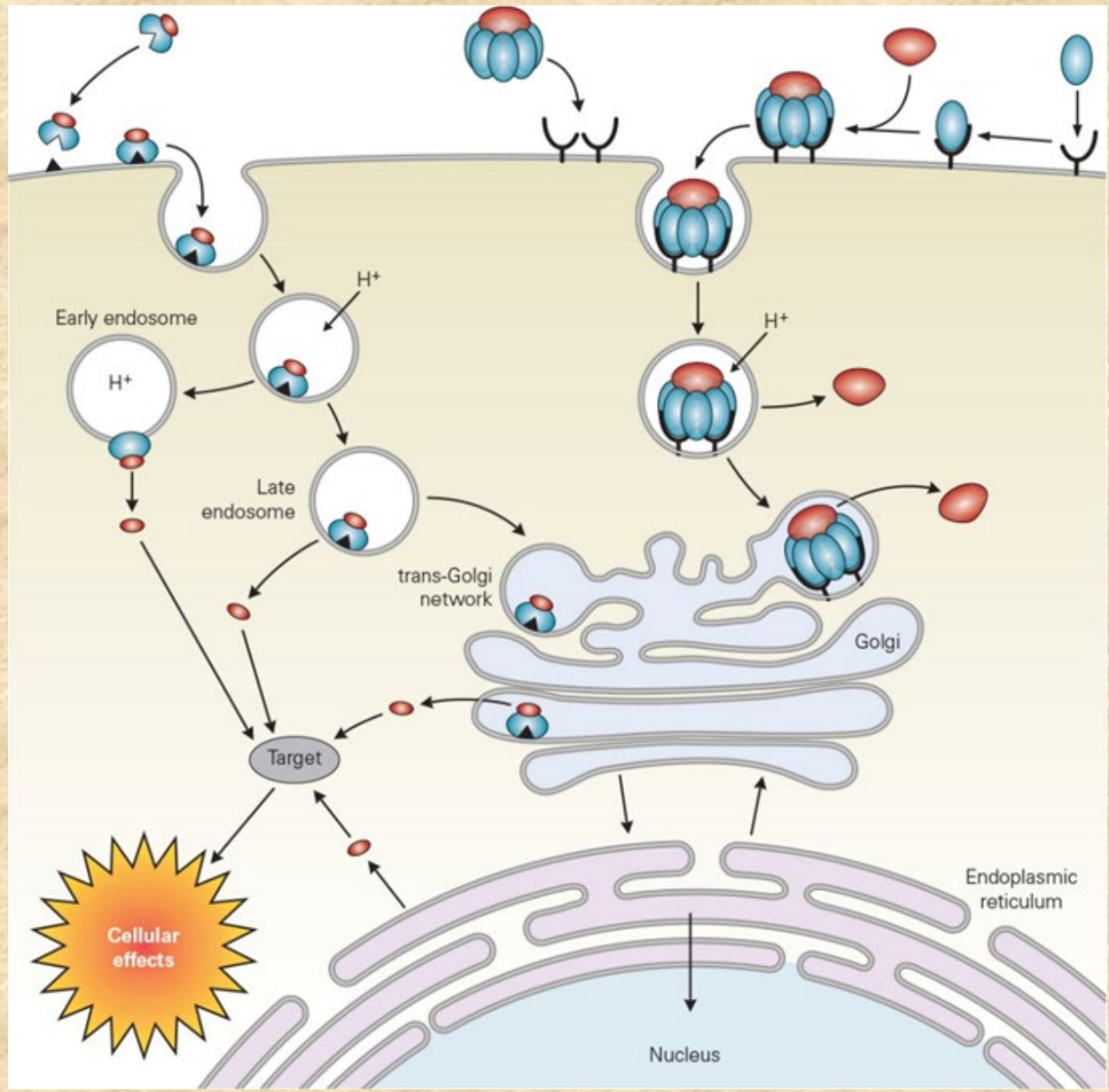
A= Either EF or LF

B= PA cleaved by host protein, furin, leading to heptamerization

Commonly secreted by the Type II secretion pathways

Eg. Cholera toxin

Translocation of Complex Class III toxins



What does B bind to?

Class III toxins
Modes of action

Diphtheria toxin (toxoid D)
An ADP-ribosylase

- Mode of action
- Toxin receptor is HB-EGF (heparin binding epidermal growth factor precursor)
- Receptor is found on many cell types

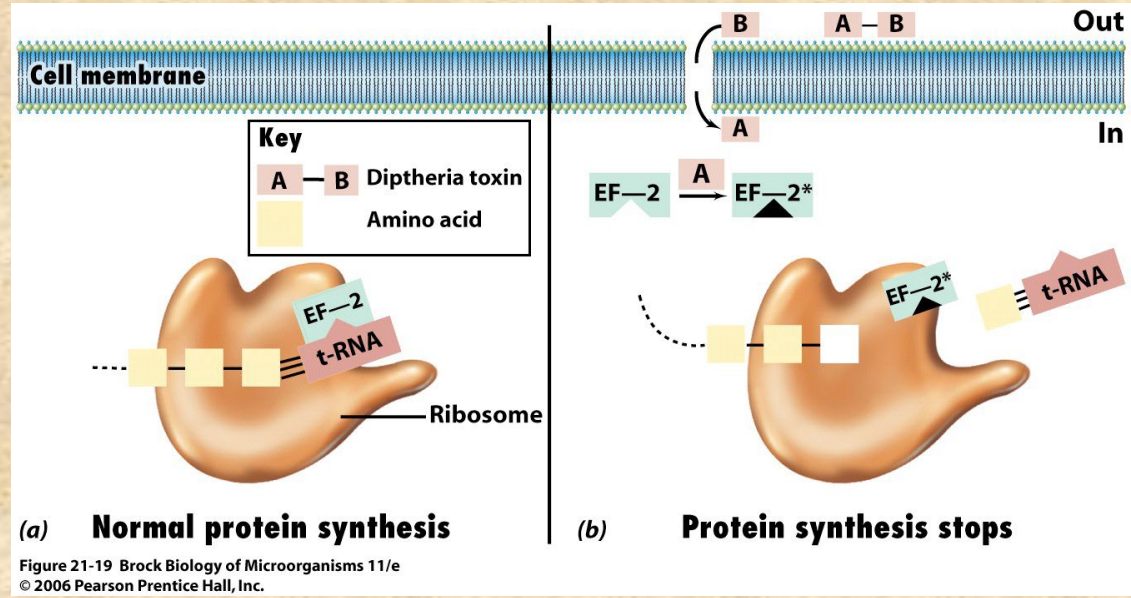


Figure 21-19 Brock Biology of Microorganisms 11/e
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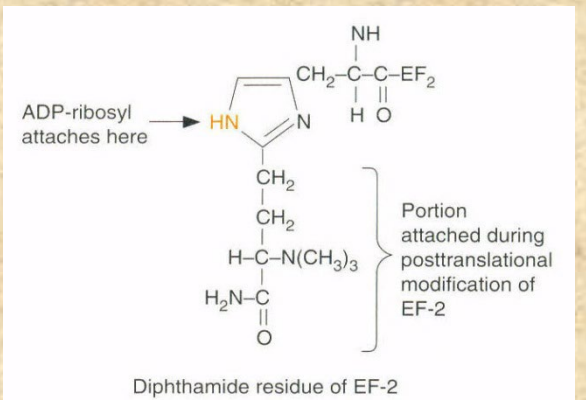
- Translocated through pH dep process after endocytosis
- ADP-ribosylates the diphthamide residue of Elongation factor-2



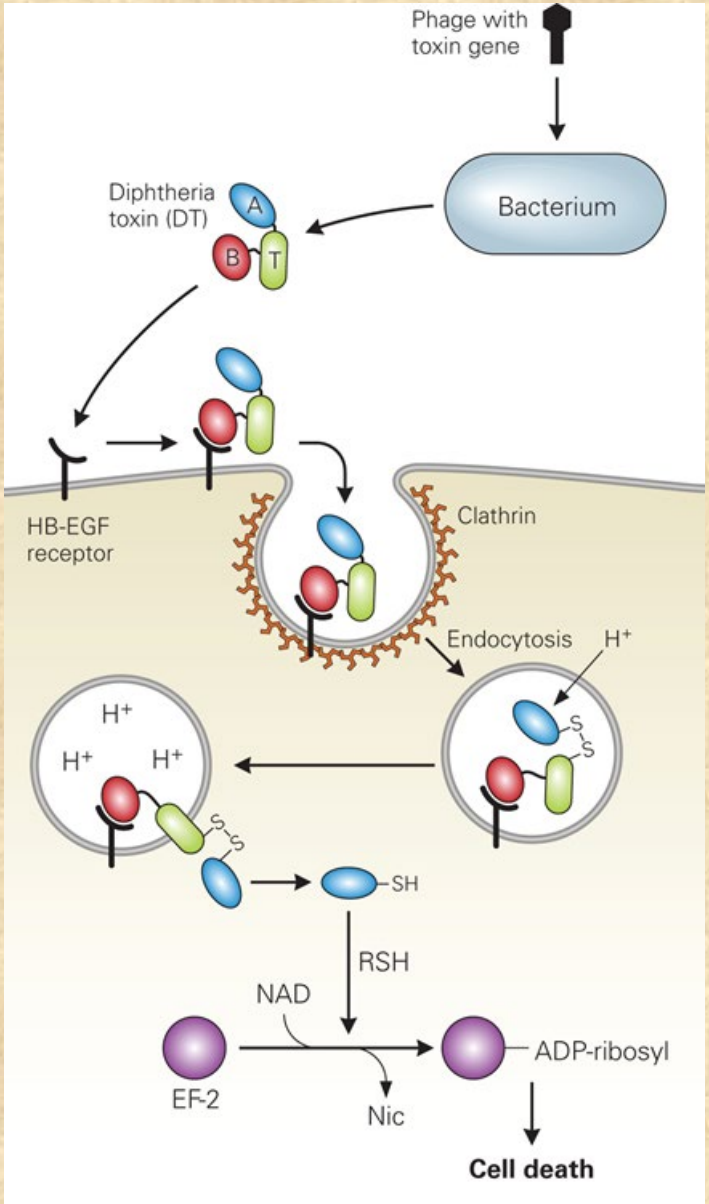
Diphthamide residue of EF-2

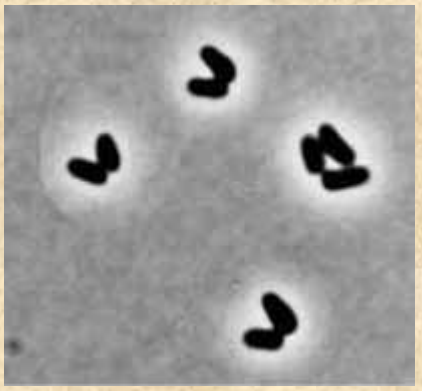
ADP ribosylation of EF-2 by diphtheria toxin

$$\text{EF-2} + \text{NAD}^+ \longrightarrow \text{ADP-ribosylated EF-2} + \text{nicotinamide} + \text{H}^+$$

Diphtheria toxin is extremely toxic a single molecule can kill a cell!

Translocation of Diphtheria toxin





Cholera toxin – an enterotoxin which is also an ADP-ribosylase

Eg. Secreted by a Type II secretion system

- ADP ribosylates an arginine residue on G protein that controls cyclic AMP levels in the host cell.
- ADP-ribosylation prevents the enzyme from being turned off.
- This causes the host cell to lose control of ion flow and results in massive water loss, which manifests itself as watery diarrhea (50-100 liters per day!)

ADP ribosylation of G protein by cholera toxin

G protein + NAD^+ ⟶ ADP-ribosylated G protein + Nicotinamide + H^+

G protein exists in two conformations:
GTP-bound "on" and GDP-bound "off"
GTP-bound form activates adenylate cyclase,
ADP-ribosylation locks G proteins in the "on" conformation

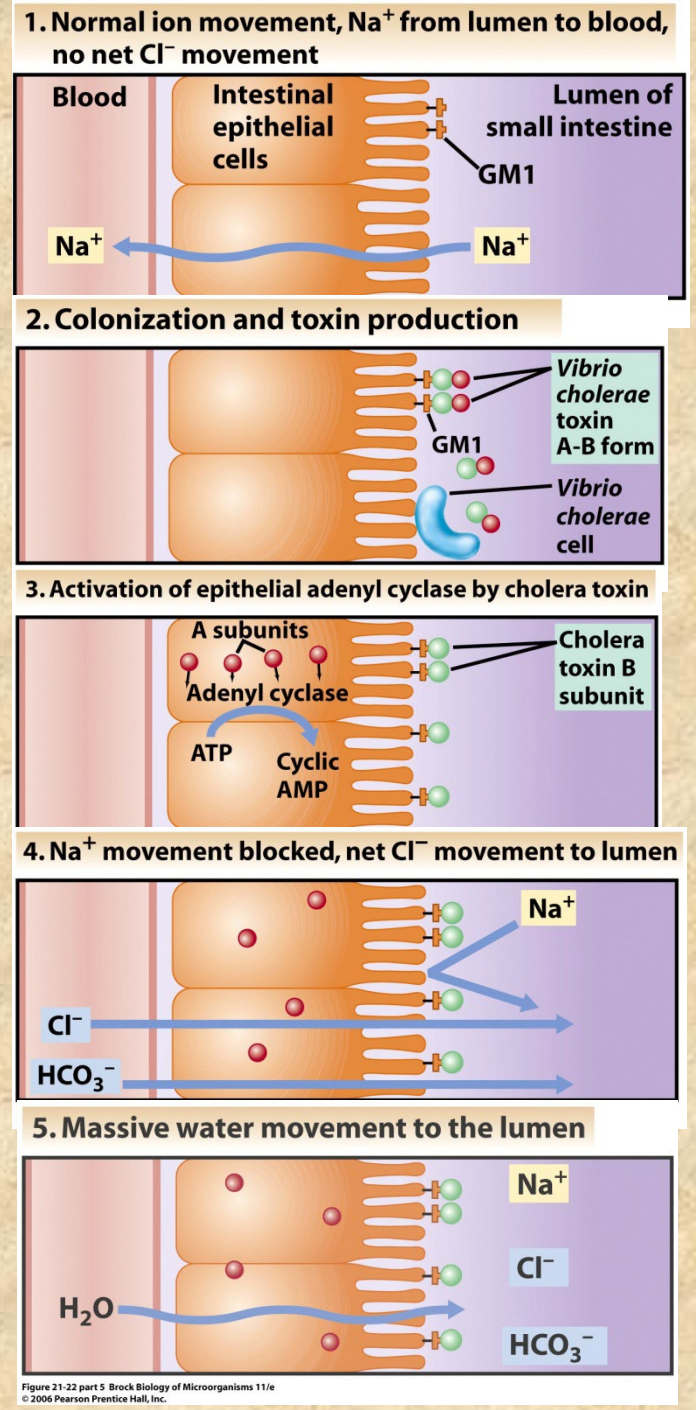


Figure 21-22 part 5 Brock Biology of Microorganisms 11/e
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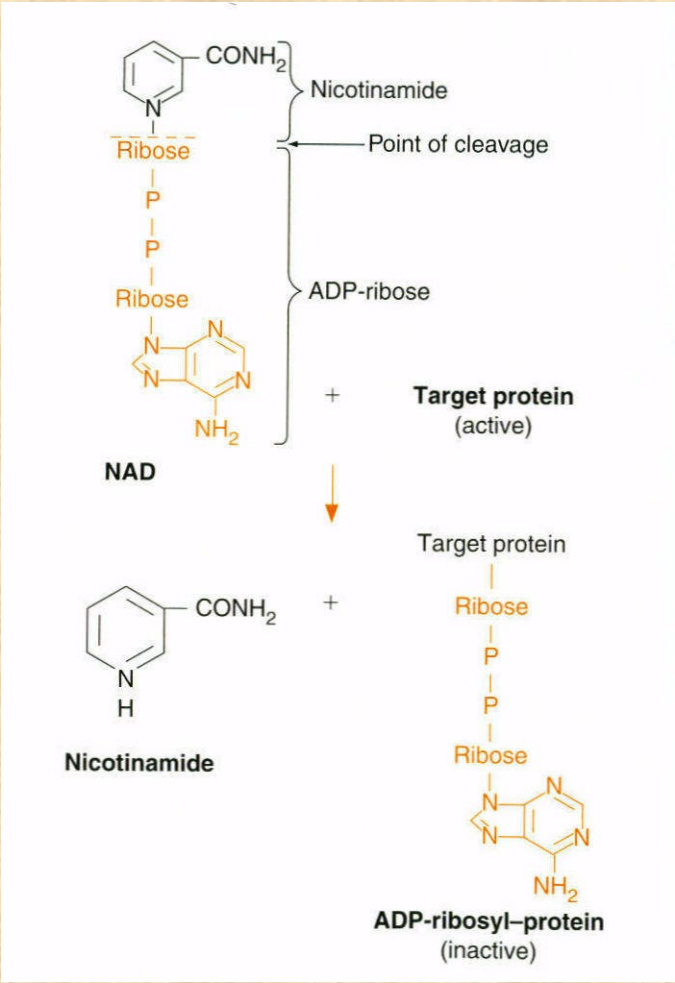
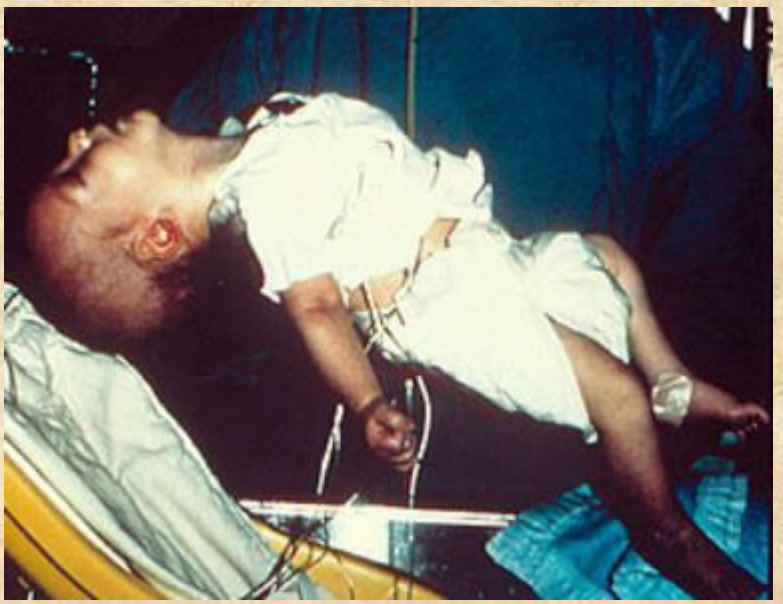
ADP-ribosylation of host proteins



Shiga toxin – an enterotoxin
A ribonuclease (targets rRNA)

- Most, but not all, AB toxin ADP-ribosylate their substrate
- Shiga-toxin cleaves host cell rRNA. This results in the shutdown of protein synthesis



A young child suffering from botulism (a flaccid paralysis)

A painting of a gentleman suffering from Tetanus experiencing a spastic paralysis

Both cleave synaptobrevins

Similar toxin different symptoms

The symptom observed is determined by different cell specificities of the binding regions

-Botulinum toxin target peripheral neurons

-Tetanus toxins acts on the CNS

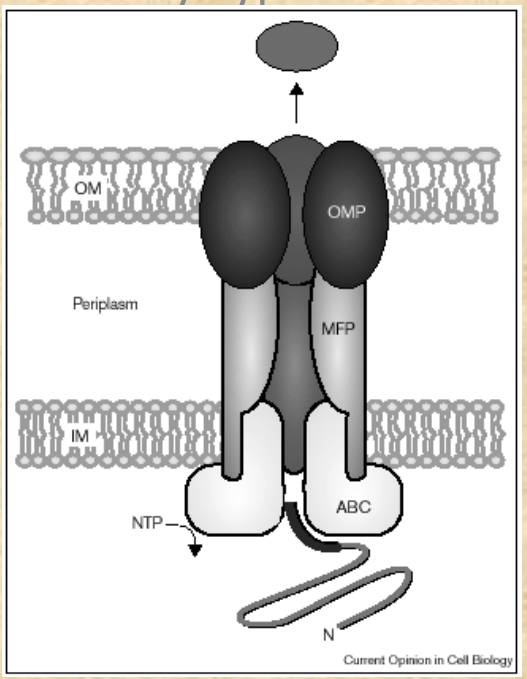
Reading assignment
Botulism & Tetanus C12, Pg319-323

Class II toxins– membrane disrupting

aka Hemolysin/Cytolysins

Hemolysis: hemolytic activity of different Streptococci on blood agar plates. Alpha e.g. *S. pneumoniae,* Beta *S. pyogenes,* Gamma rarely cause disease *S. epidermidis*

Commonly secreted by Type I bacterial secretion pathways



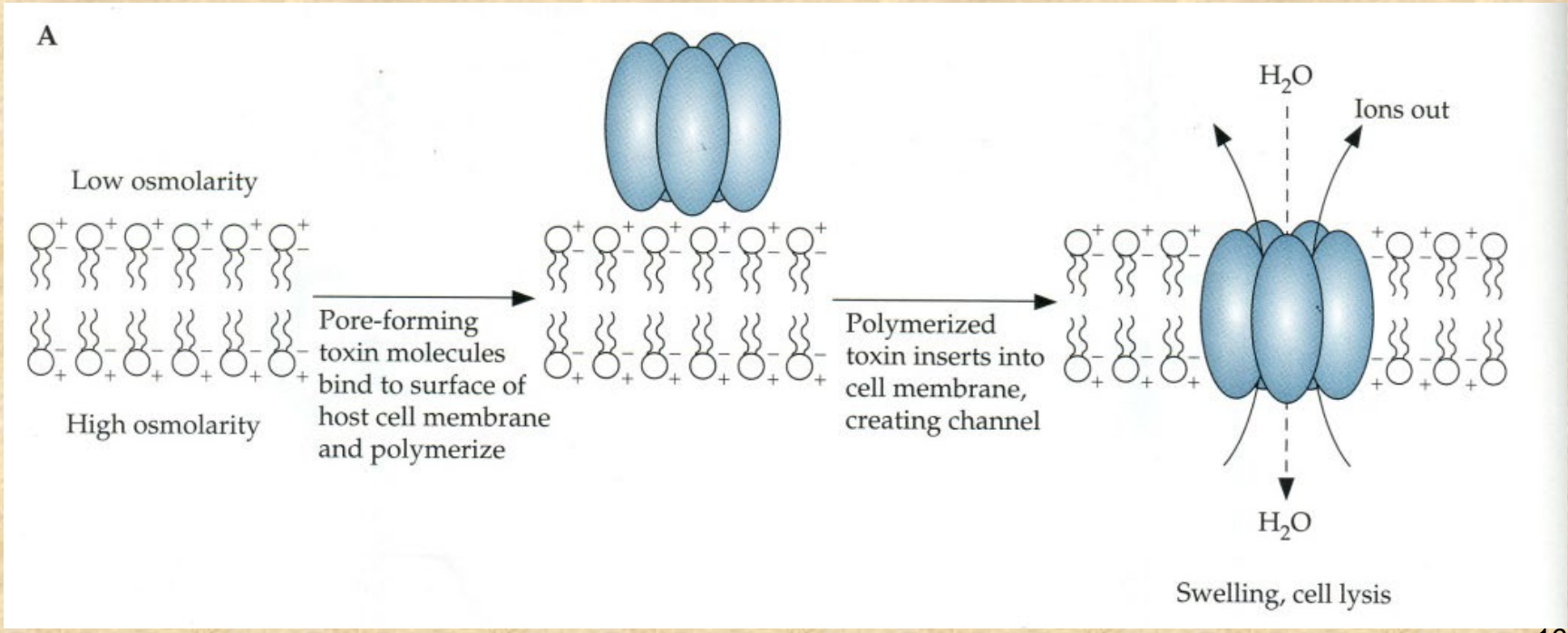
eg. *E. coli* hemolysin HlyA
Serratia marcescens HasA

Class II toxins
Mode of Action

Eg. Listeriolysin O*, L. monocytogenes*

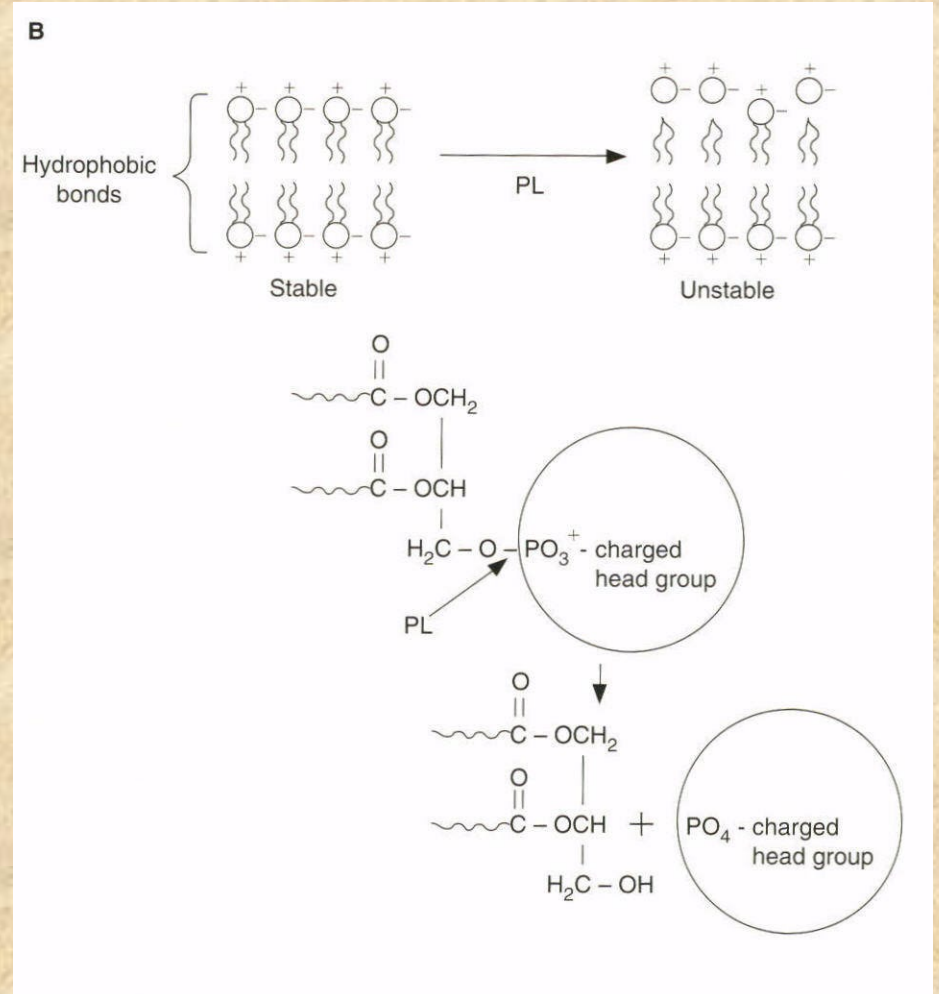
Streptolysin O from *S. pyogenes (see last lecture)*

- Eg. Alpha toxin *Staphylococcus aureus*



Class II toxins
Mode of Action

- Eg. Phospholipase C & D (see Table 12-1)



Class I toxins
- superantigens

Mode of action

- Mostly associated with gram-positive pathogens Secretion: GSP pathway
- *Streptococcus* and *Staphylococcus*

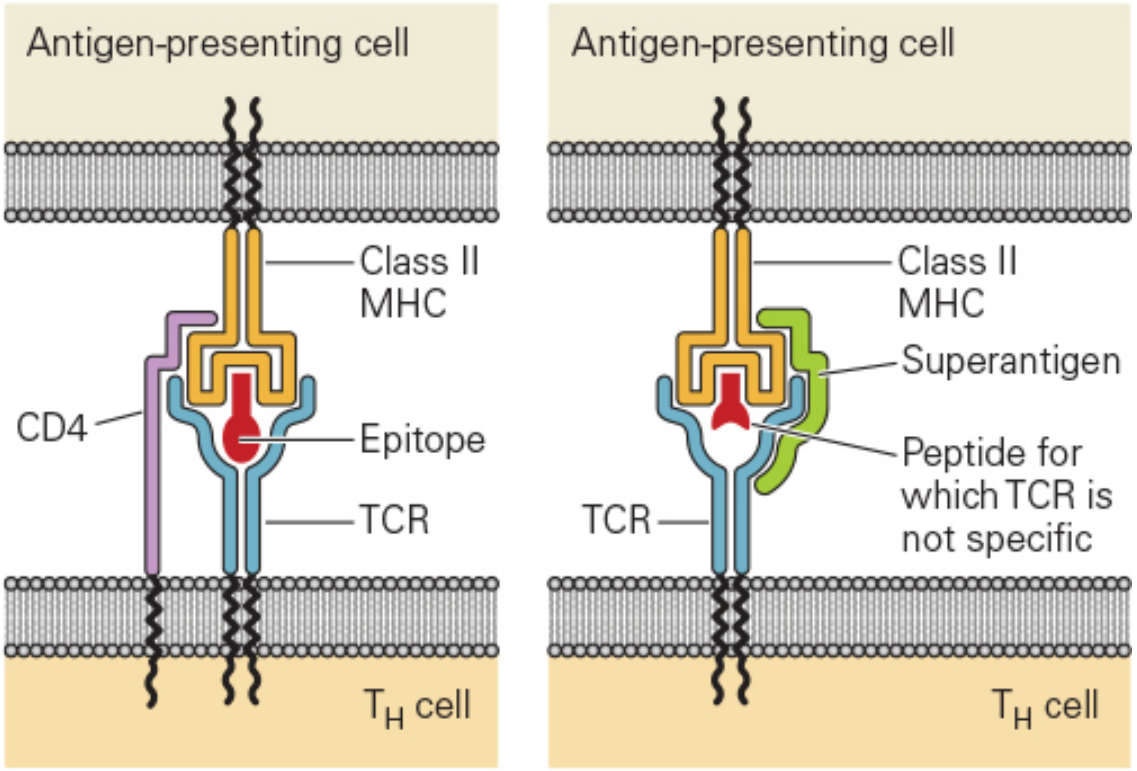
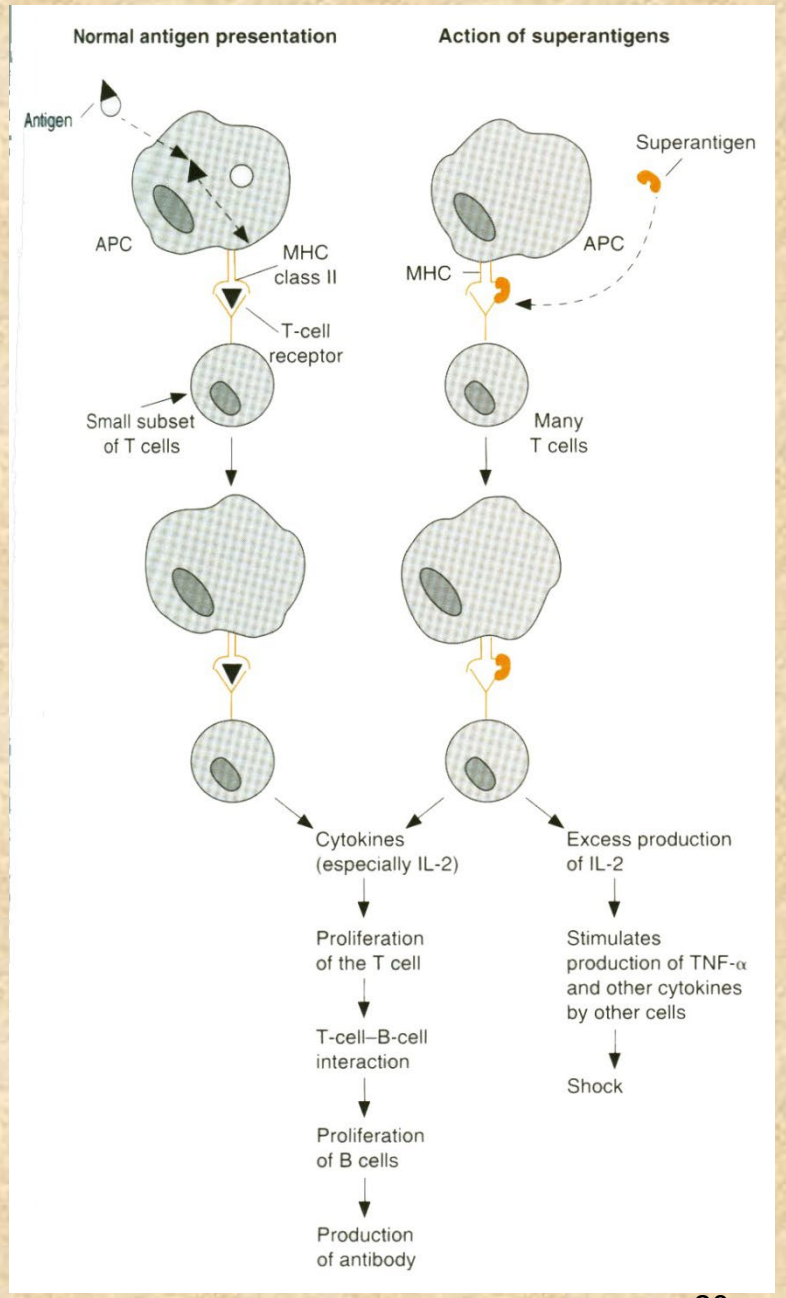


Figure 12–4 Normal interaction of an APC and a T helper cell (left) compared with the interaction of an APC and a T helper cell mediated by a superantigen (right). Superantigens form a bridge complex between the MHC molecules on APCs and T-cell receptors with or without the peptide antigen present, which in turn overstimulates the T cells to release cytokines (especially IL-2), resulting in T-cell and B-cell proliferation and toxic shock.

Superantigens cause shock

Eg. Toxic shock syndrome

- Superantigens activate T cells independent of antigen
- During normal infection 1 in 10,000 T cells are activated by macrophages bearing antigen
- In the presence of superantigen as many as 1 in 5 T cells are stimulated
- This results in a massive release of the cytokine IL-2, which leads to nausea, vomiting and fever, and may lead to death

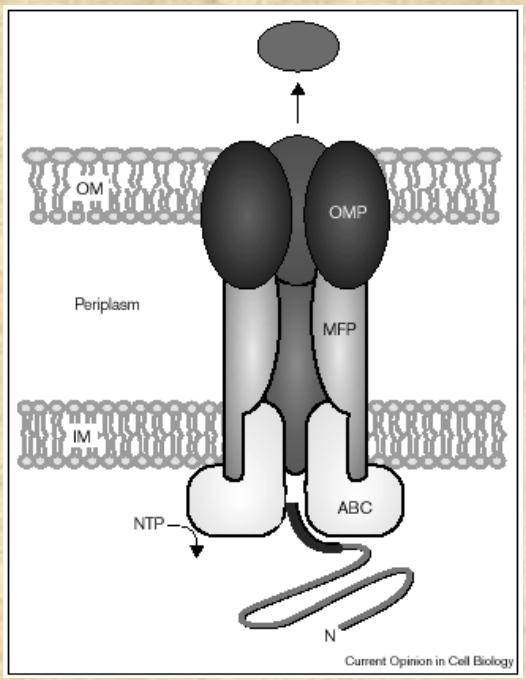


Secretion of toxins

- Many pathways are used to deliver/secrete proteins – many deliver important virulence proteins
- Common themes can be drawn

Type I secretion

- Pore forming cytotoxins – as these would disrupt the bacterial membranes too

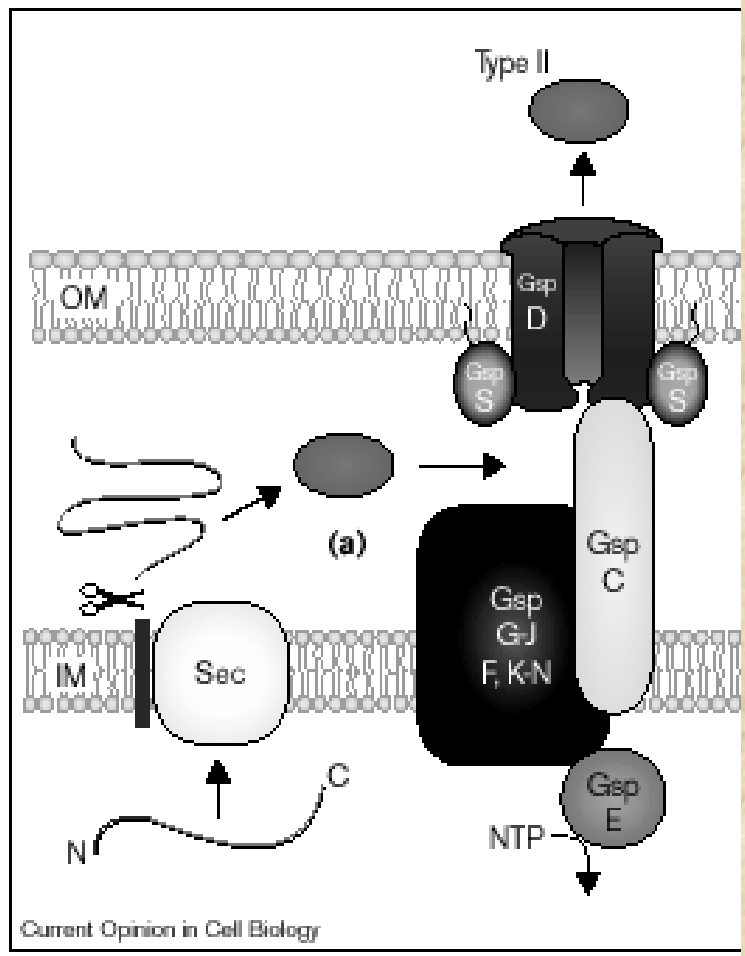


Type II secretion

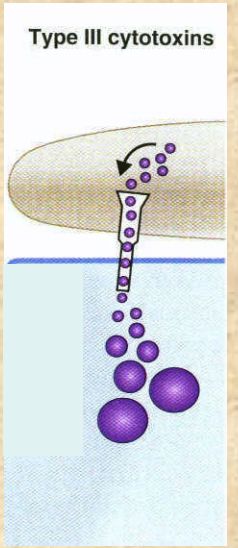
- A-B toxins
- Folding and disulfide bond formation in the periplasm
- Some A-B toxins form the A-B conformation in the periplasm.
- Some are secreted
- Others AB toxins are only released during cell lysis

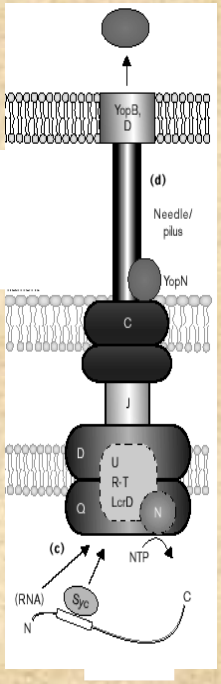
Eg. Shiga toxin, Cholera toxin

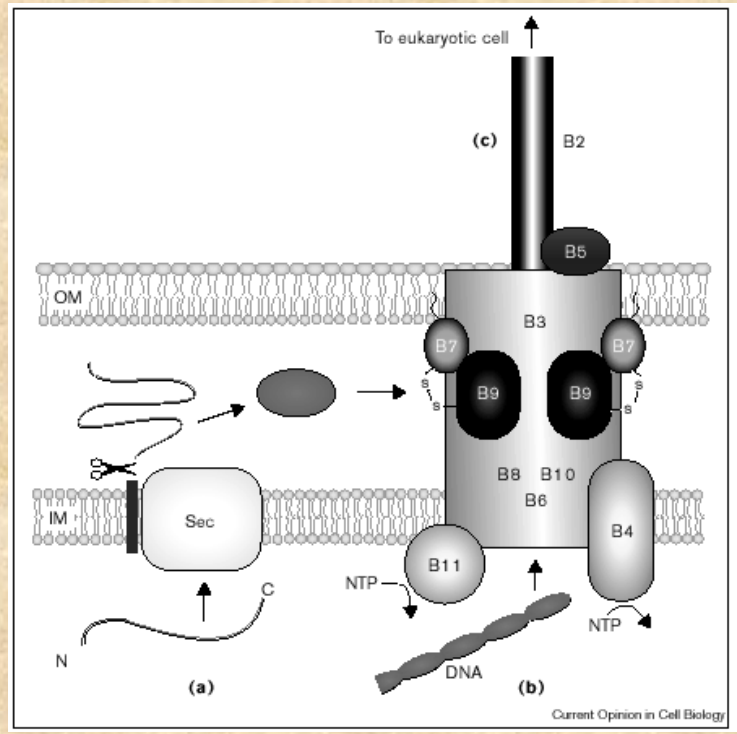
<u>Problem:</u> Antibiotic treatment



Type III & IV secretion



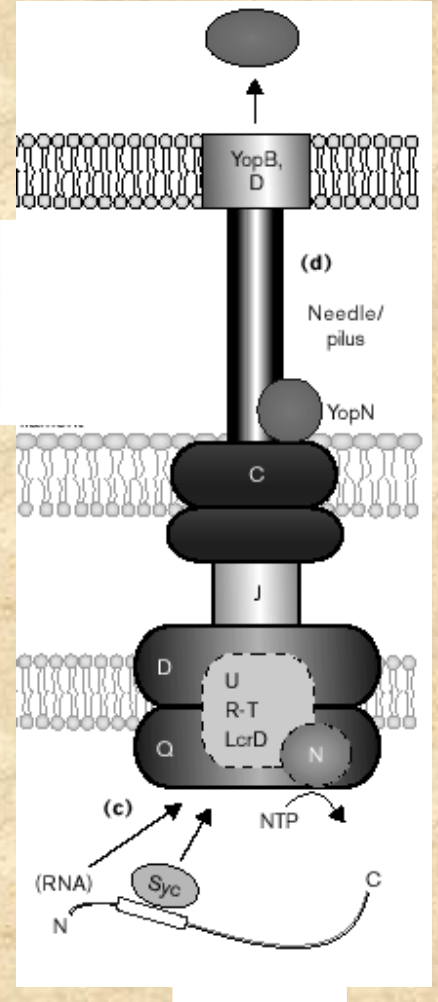




- Phospholipases delivered directly to the host cell membrane by type III secretion – no chance for antibody neutralization
- Other toxins are injected directly into the host cell by Type IV secretion systems

Type III secreted cytotoxins

- Some pathogenic bacteria have the ability to secrete cytotoxins directly into the host cell cytoplasm
- Known to be contact dependent but signal is not known
- On contact produces type III secretion needle
- Delivers cytotoxin effectors to the host
- Numerous bacteria use this strategy including *Salmonella, Shigella, Pseudomonas, Yersinia,* cholera bacilli
- Vary in their modes of action, but often interfere with ability to respond to infection by direct killing of the host cell or modulation of the actin cytoskeleton of the host cell
- A functional actin cytoskeleton is required for efficient phagocytosis



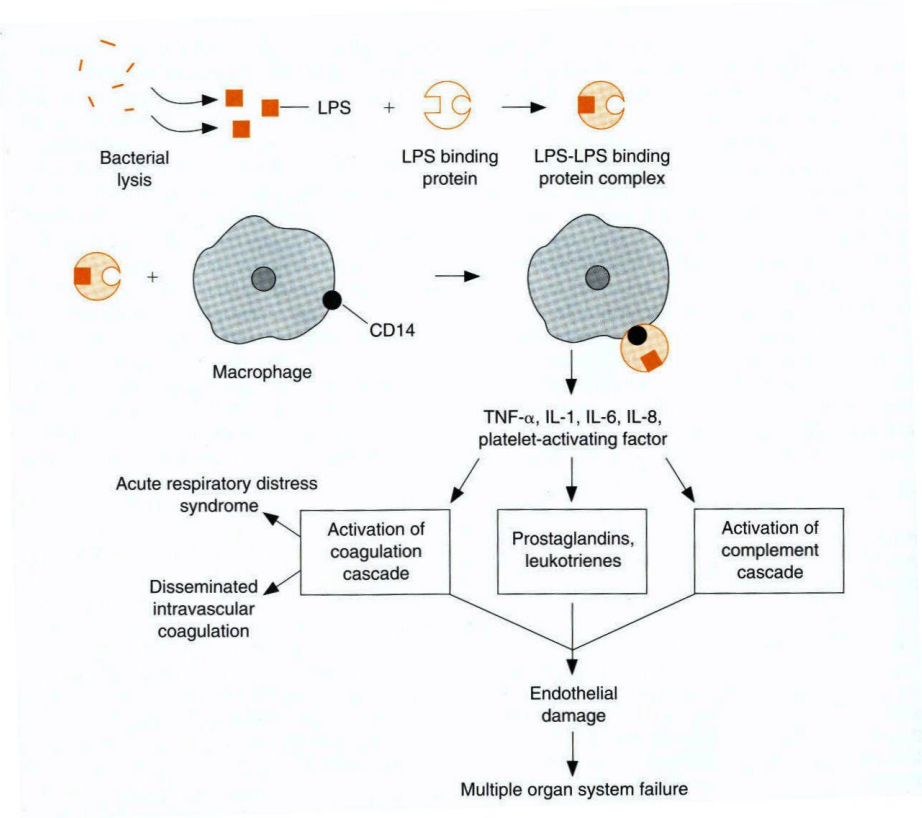
Toxins that act on the extracellular matrix - Wound infections

- Hyaluronidase Eg. *Streptococcus pyogenes*
- "The bacterium that ate my face"
- Necrotizing fasciitis – release of nutrients & dead tissue can be a safe haven for bacterial pathogens

- Pla *Yersinia pestis*
- Activates plasminogen – plasmin
- Plasmin attacks fibrin. Fibrin important component of cell junctions, hence eliminates barriers that might interfere with invasion.
- Plasmin also activates collagenases and elastases

Endotoxin or LPS

- Endotoxin is LPS
- At low concentrations LPS elicits a series of alarm reactions
- At high concentrations LPS leads to shock and even death
- Endotoxin is NOT internalized
- LPS binds TLR on macrophages & neutrophils etc. and this triggers cytokine release
- This leads to fever, activation of complement, activation of macrophages, stimulation of B lymphocytes
- May lead to shock when levels are high mediated by IL-1 and TNF-a



Lecture 25 & 26: Antimicrobial compounds (Chapter 15)

- Importance of antimicrobial compounds
- Bactericidal versus Bacteriostatic
- Reminder of pre-antibiotic era
- Antiseptics, Disinfectants,
  - Antiseptic and Disinfectants – mechanism of action resistance ?
- Antibiotics
  - Distribution of drug in body – antiseptic v antibiotics
  - Pharmacokinetics
  - Side effects
  - Antibiotics - characteristics
  - Antibiotic discovery (see lecture 2 or 3?)
  - Economics
  - Targets

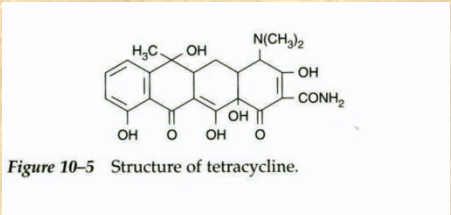


Figure 10–5 Structure of tetracycline.

Cell wall=peptidoglycan layer; pos and neg, Steps, b-lactams; glycopeptides; others

Protein synthesis inhibitors – ribosome structure A sites Aminoglycosides; tetracyclines; macrolides; lincosamide; streptogramins

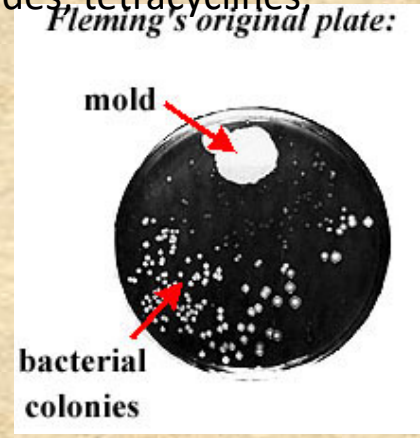
Rifampicin- targeting beta subunit RNA polymerase

DNA replication- DNA gyrase quinolones

Trimethoprin & Sulfonamides – folic acid

Metronidazole- interferes with DNA replication

Newest – include oxazolidones eg. Zyvox like macrolides



Importance of Antimicrobial compounds

- Control of microbial growth: experimental and clinical importance
- Antimicrobial compounds include: disinfectants, antiseptics and antibiotics

Pre-antibiotic era

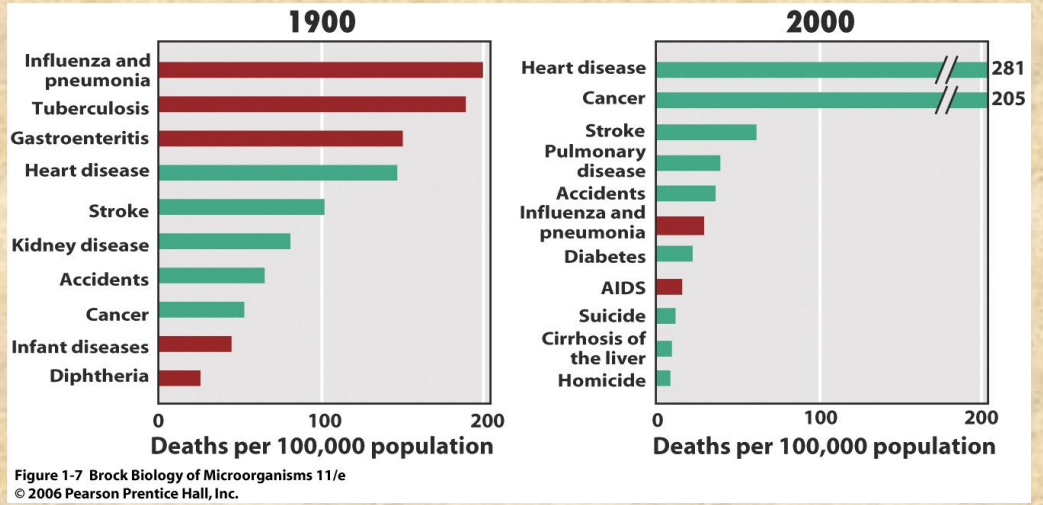
Staphylococcus aureus infections were fatal more than 80% of the time.

Appendicitis killed 3000 people every year in the UK

In dentistry, infections most commonly treated by tooth extraction.

Streptococcus pyogenes caused half of all post-birth deaths and was a major cause of death from burns.

Tuberculosis and pneumonia bacteria were famous killers.



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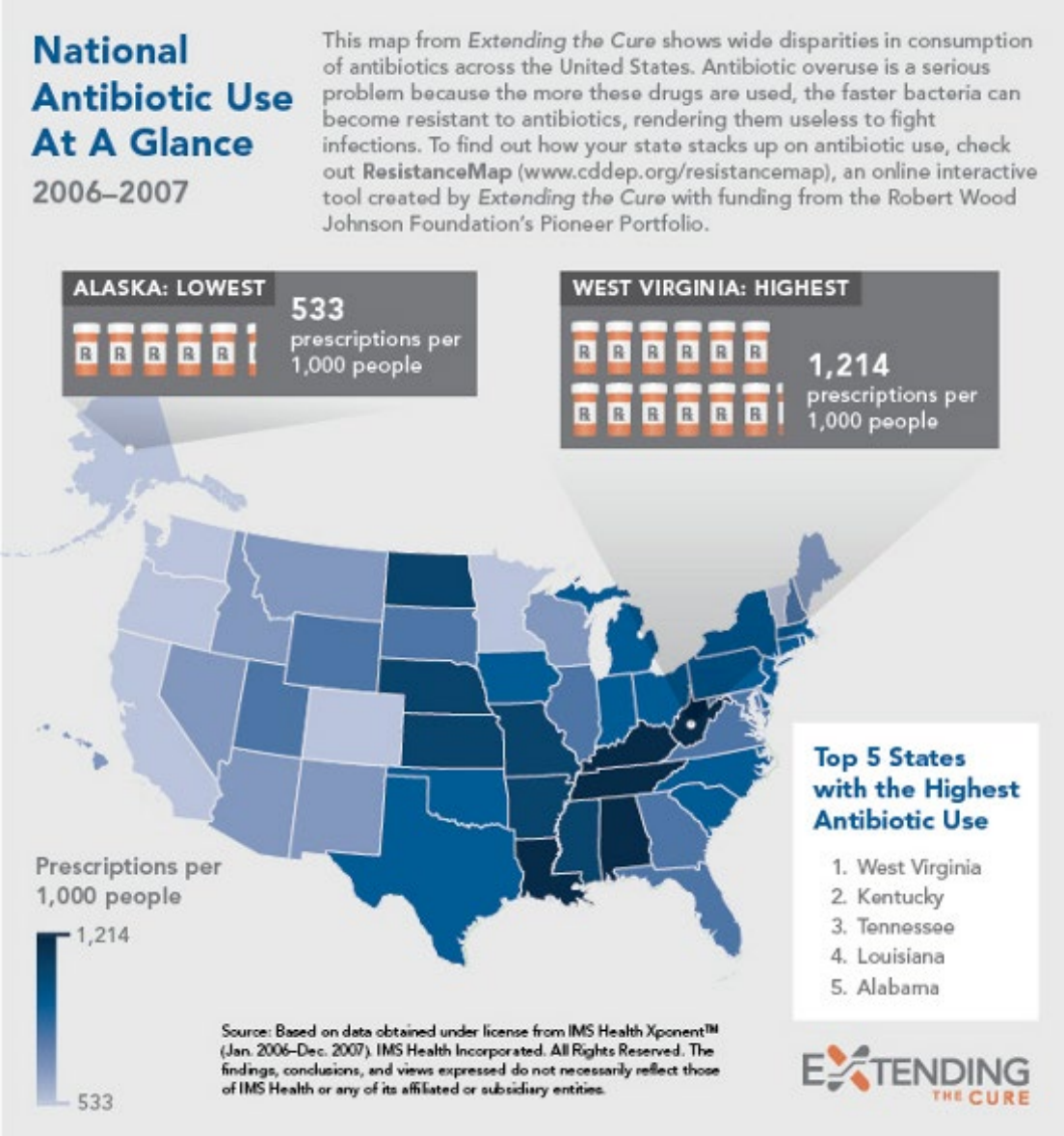
Use of Antibiotics in the US



- http://the-scientist.com/2011/11/17/mapping-antibiotic-use-and-resistance/