Infection and Disease

SBL101

James Gomes
School of Biological Sciences
Indian Institute of Technology Delhi

All Figures in this Lecture are taken from

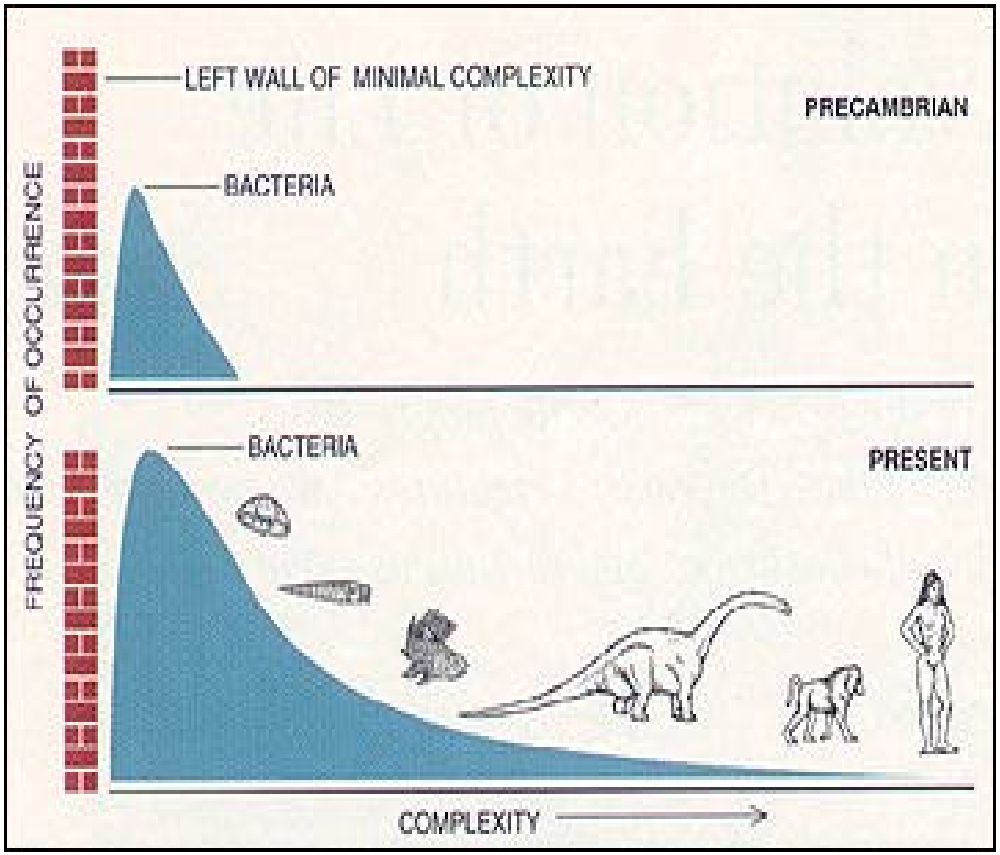
1. Molecular biology of the cell / Bruce Alberts et al., 5th ed.

2. Research papers as cited

- Lectures will be in 3 parts

1. Background to understanding basic elements
2. Defense mechanisms in humans
3. Understanding interactions between pathogens and hosts

All we known is that life on this planet began over three and half billion years ago



Infection and human life

- More than a third of all human deaths is caused by infectious diseases
  - More that the deaths caused by all the cancers
- Burden of old diseases and well as new ones
  - OLD →Tuberculosis and Malaria
  - NEW → AIDS a world wide epidemic
- Spread unequally across the planet
  - Poorer nations suffer more, poor public health and sanitation conditions
  - Urbanization problems – infection through AC vents
- Infection, disease and death is as old as the human civilization

Infectious diseases

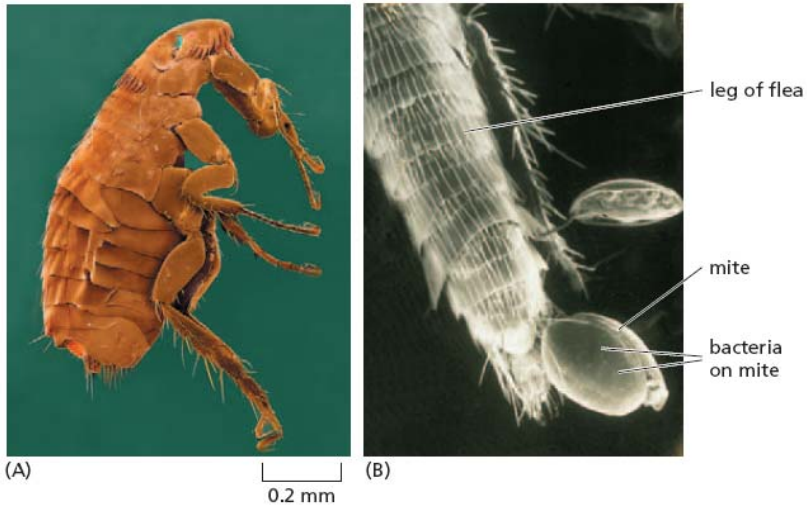
Definition:

- **Agents that cause infectious diseases are collectively called *Pathogens***
- **Pathogens exploit the attributes of the host cell to infect them**
  - **Need to understand the mechanisms of infection to treat disease/design drugs**
  - **Cross barriers – skin, mucus, chemical defenses**
  - **Cells aggressively degrade double-stranded RNA (signature of viral infection)**

Pathogens

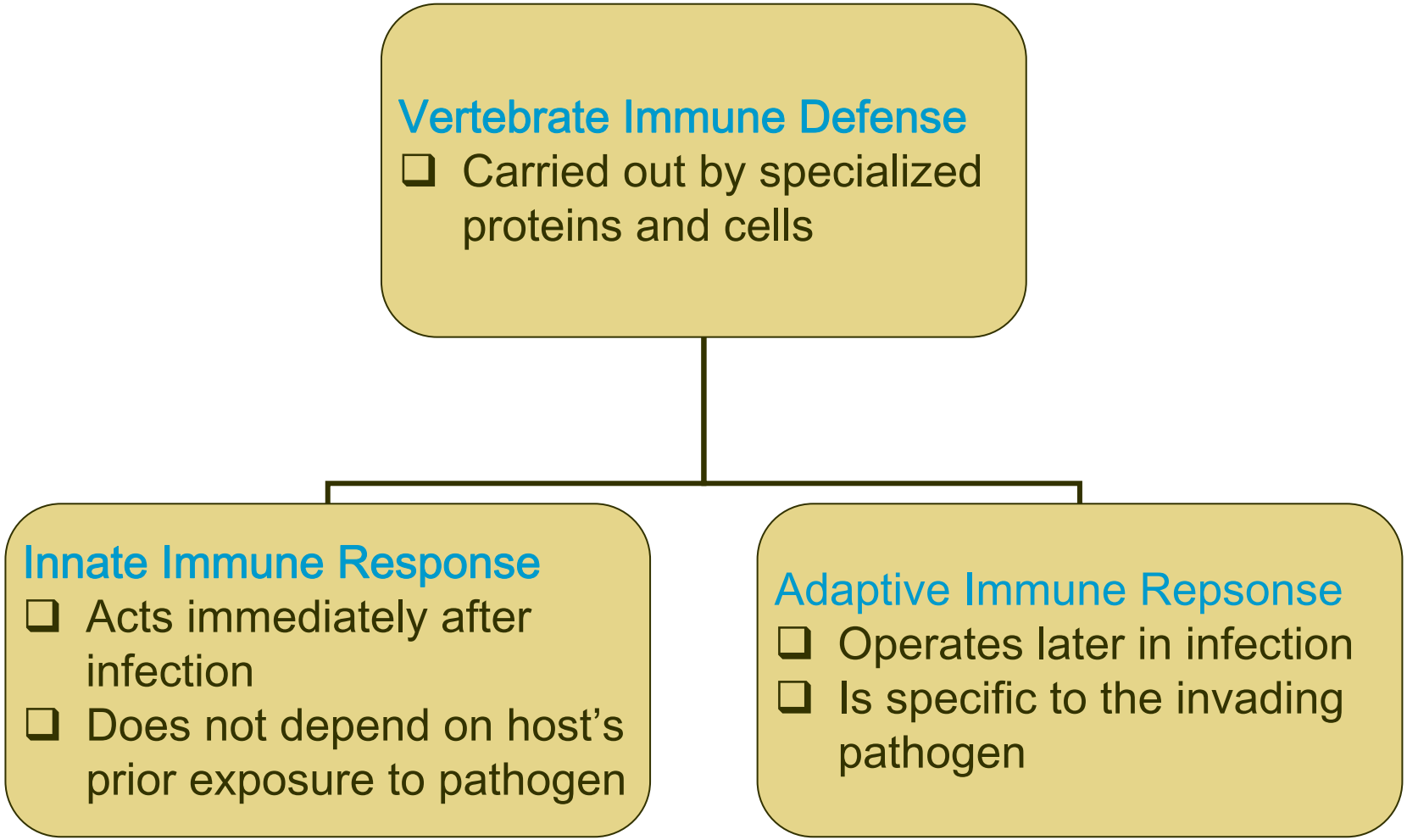
- Pathogens, like other living organisms, are fulfilling their biological directive – live and procreate
- The human host is a rich source for proliferation
  - Many microorganisms have evolved to the ability to survive and reproduce in this environment

Scanning electron micrograph of a flea



1. A common parasite of mammals—including dogs, cats, rats, and humans, flea bites can cause the spread bubonic plague by passing the pathogenic bacterium *Yersinia pestis* from the bloodstream of one infected host to that of another
2. A close-up view of this flea's leg reveals that it also has a parasite, a type of mite. The mite, in turn, is covered with bacteria. It is likely that bacteriophages, which are bacterial viruses that parasitize these bacteria.
3. Jonathan Swift reported a similar observation in 1733: a flea has smaller fleas that on him prey;And these have smaller still to bite 'em; and so proceed ad infinitum.

Immune Defense



Pathogen-host interaction

- **The human body is a complex ecosystem**
  - **Contains 10^{13} cells and in addition 10^{14} bacterial, fungal and protozoan cells**
  - **These commensal microbes are the *Normal flora*– they are not free-loading but help is digestion and combating disease-causing microoganisms**
  - **The human cells live in harmony with these cells**
- **Why do certain microbes cause illness and death?**

Examples of Primary Pathogens

Primary pathogens are distinctly different from the *normal flora* and cause disease in a healthy person

- **Historically important**
  - **Bubonic plague and small pox**
- *Mycobacterium tuberculosis* which cause causes the life threatening lung disease may remain dormant in the host for years
- Where does one draw the line been "persistent infection" and "commensalism"
- *There is constant battle between the hosts defense and the pathogens*

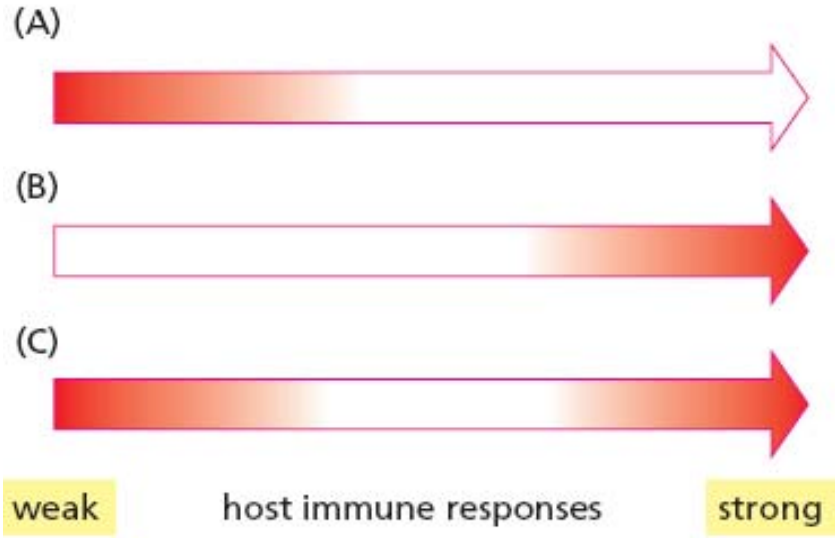
Evolution of pathogens

In order to survive and multiply in a host, a successful pathogen must be able to

- colonize the host
- find a nutritionally compatible niche in the host's body
- avoid, subvert, or circumvent the host's innate and adaptive immune responses
- replicate, using host resources
- exit and spread to a new host

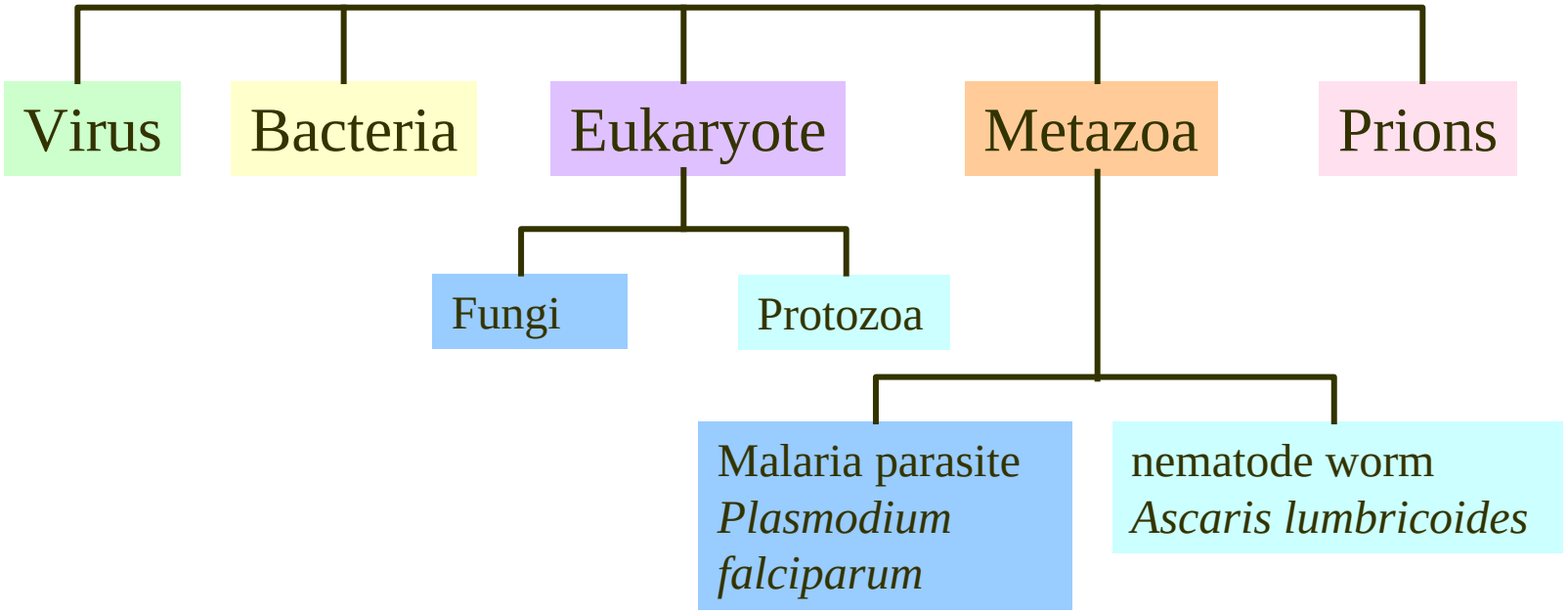
Under severe selective pressure to induce host responses that help to accomplish these tasks, pathogens have evolved mechanisms that maximally exploit the biology of their host organisms

Pathogen-immune reponse

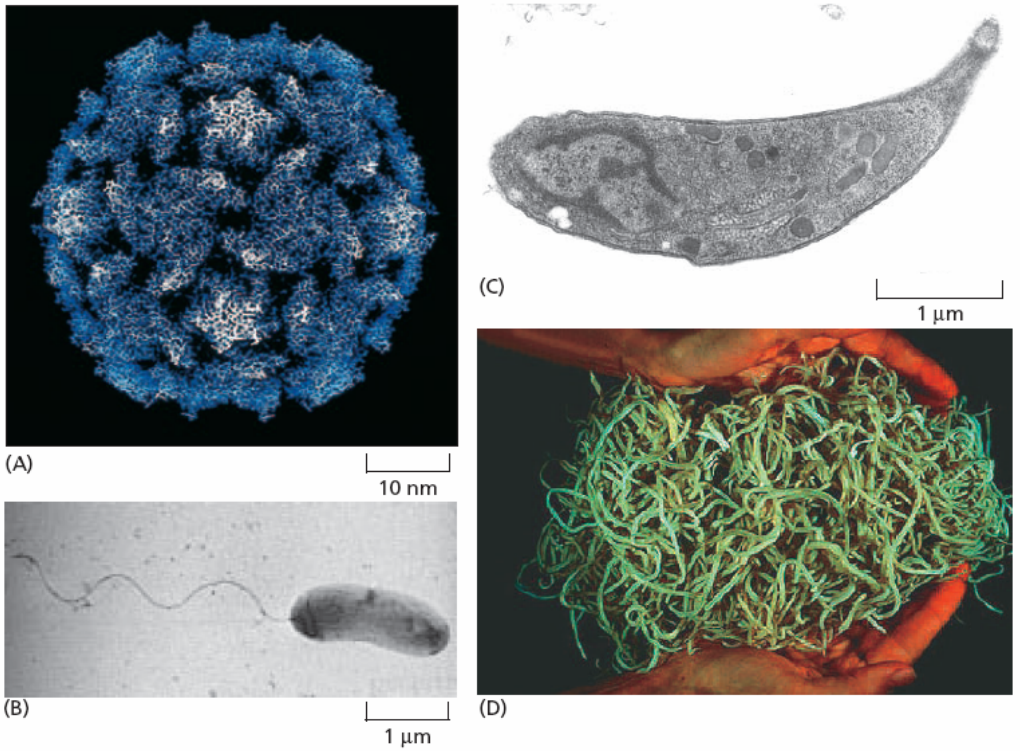


- Normal flora cause disease only when the immune system is abnormally weak
- In some diseases the immune reponse is responsible for tissue damage (sexually transmitted diease caused by *Chamlydia trachomatis*)
- For certain pathogen colonization, very strong or very weak response can cause damage (*Mycobacterium tuberculosis, Aspergillus* sp.)

Pathogens are phylogenetically diverse



Example of different pathogens



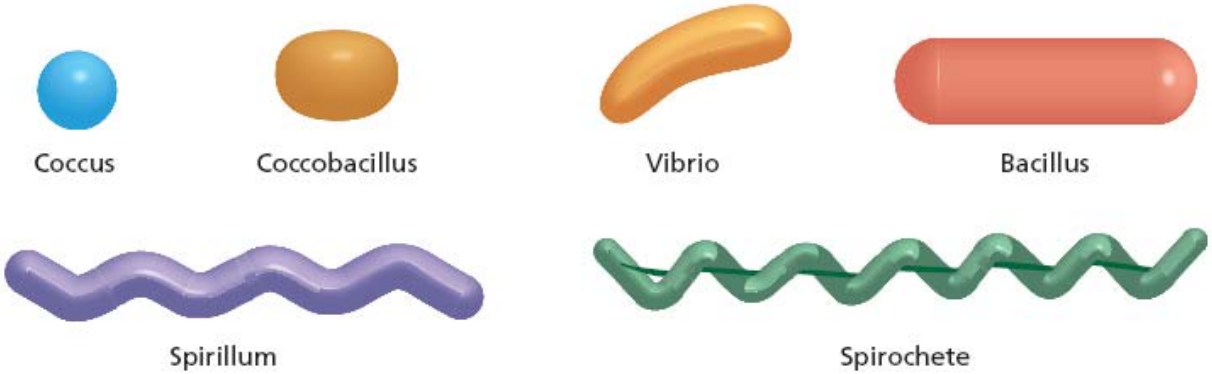
A. The structure of the protein coat, or capsid, of poliovirus
   - This virus was once a common cause of paralysis, but the disease (poliomyelitis) has been nearly eradicated by widespread vaccination.

B. The bacterium *Vibrio cholerae*,
   - epidemicdiarrheal disease cholera.

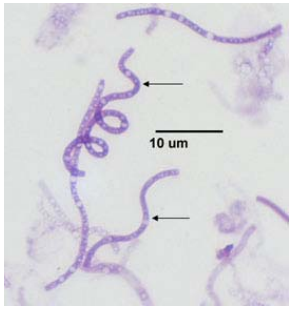
C. The protozoan parasite *Toxoplasma gondii*
   - The definitive hosts for this organism are cats, ranging in size from housecats to tigers, but it also can cause serious infections in the muscles and brains of immuno-compromised people with AIDS.

D. This clump of Ascaris nematodes
   - removed from the obstructed intestine ofa two-year-old boy.

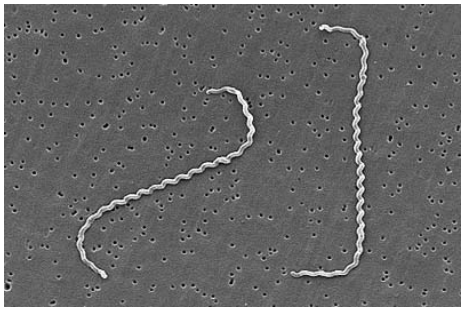
Bacteria classification by shape



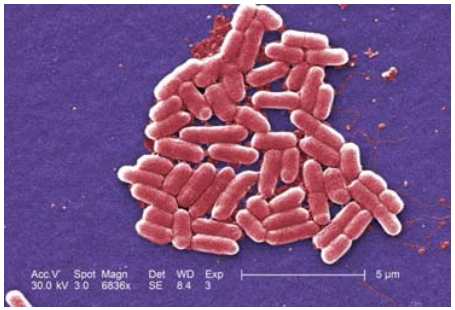
Electromicrogram of *Staphylococcus aureus*



Micrograph of *Spirillum sp.*

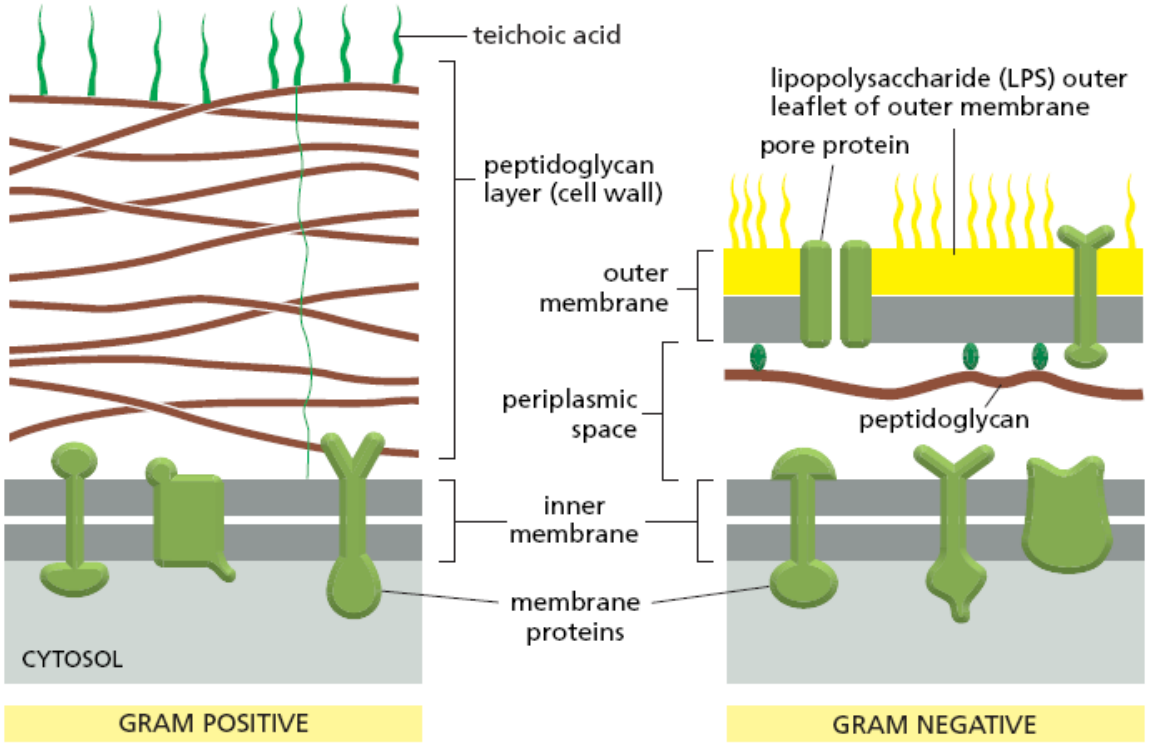


Scanning Electron Micrograph of *Leptospira interrogans*



Scanning Electron Micrograph of *Escherichia coli* O157H7

Classification according to Gram staining



- Bacteria such as *Streptococcus* and *Staphylococcus* have a single membrane and a thick cell wall made of cross-linked peptidoglycan. They retain the violet dye used in the Gram staining procedure and are thus called Gram-positive

- Gram-negative bacteria such as *Escherichia coli (E. coli)* and *Salmonella* have two membranes, separated by a periplasmic space. The peptidoglycan layer in the cell wall of these organisms is located in the periplasmic space and is thinner than in Gram-positive bacteria; they therefore fail to retain the dye in the Gram staining procedure
  - The inner membrane of Gram-negative bacteria is a phospholipid bilayer
  - inner leaflet of the outer membrane is also made primarily of phospholipids
  - the outer leaflet of the outer membraneis composed of a unique glycosylated lipid called lipopolysaccharide

Pathogens

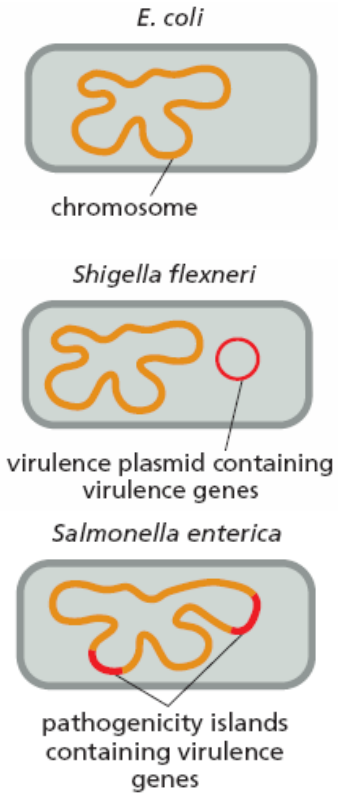
- Obligate
  - Only a small minoroty of bacteria are capable of infecting humans
  - They possess the capacity to live and proliferate in humans
- Facultative/opportunistic
  - Normally harmless but possess the latent ability of infecting the host if immunity/defense is compromised
  - For example normal flora can cause severe infections in AIDS afflicted people

Virulence genes/factors

Definition:

Genes that contribute to the ability of an organism to cause disease are called virulence genes, and the proteins they encode are called virulence factors.

- **Virulence genes are frequently clustered together, either in groups on the bacterial chromosome called pathogenicity islands or on extrachromosomal virulence plasmids**
- **Example - If these three organisms were being named today based on molecular techniques, they would be classified in the same genus, if not in the same species. The chromosome of *S. flexneri* differs from that of *E. coli* at only a few loci; most of the genes required for pathogenesis (virulence genes) are carried on an extrachromosomal virulence plasmid. The chromosome of *S. enterica* carries two large inserts (pathogenicity islands) not found in the *E. coli* chromosome**

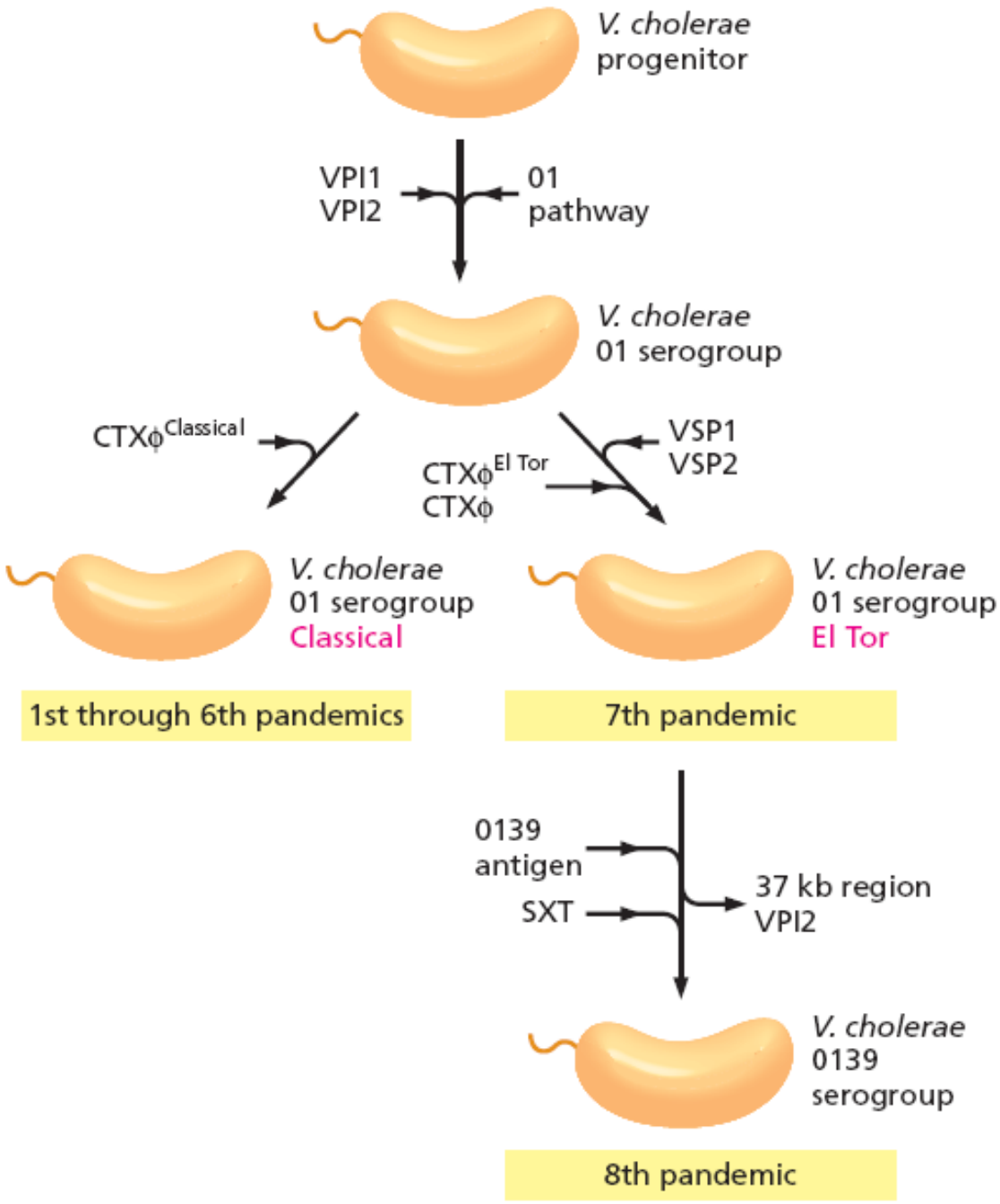


Case Study: Cholera

Definiton

***Vibrio cholerae*—the Gram-negative bacterium that causes the epidemic diarrheal disease cholera. The genes encoding the two subunits of the toxin that cause the diarrhea are carried on a mobile bacteriophage**

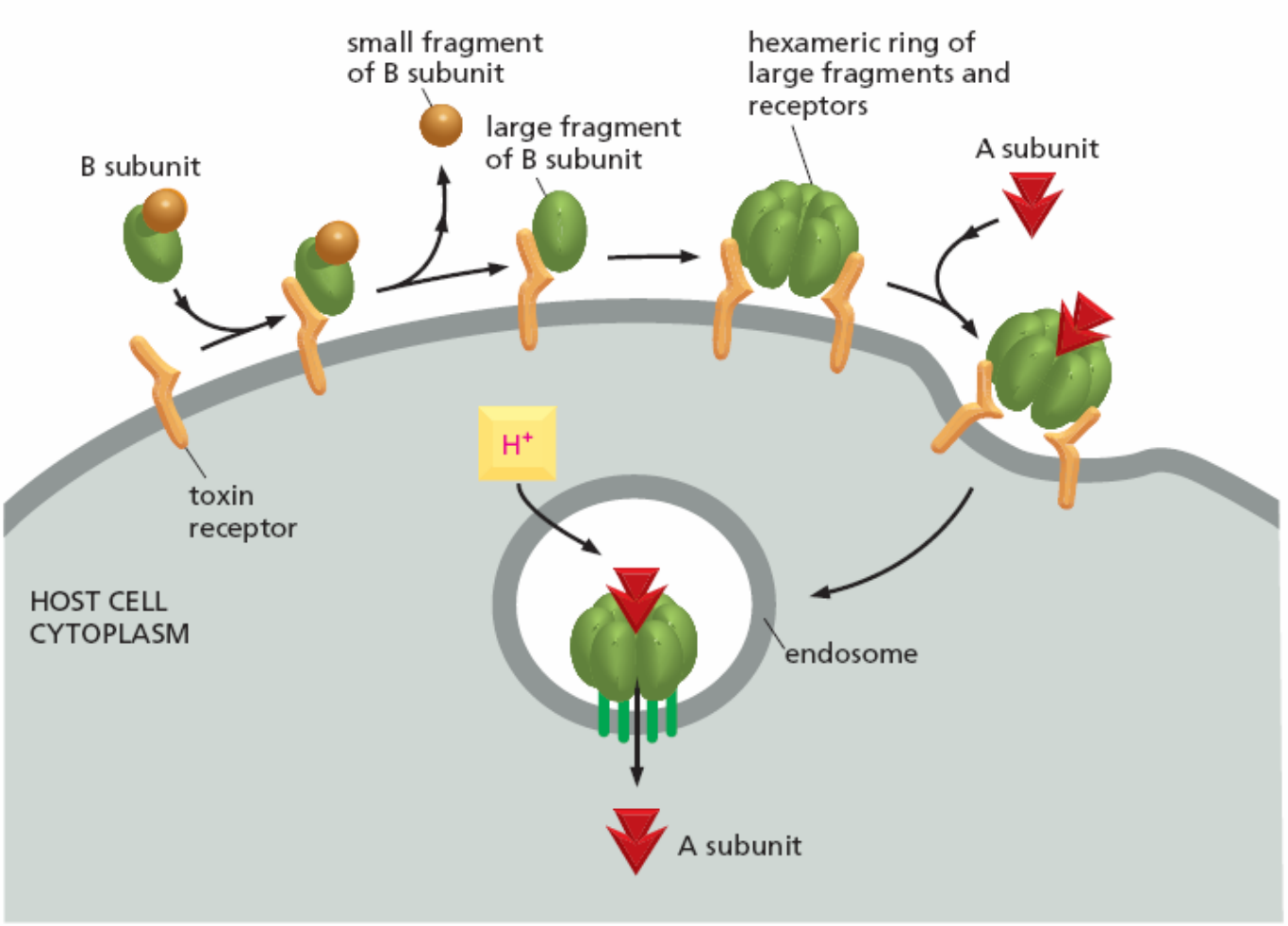
- **Since 1817, there have been 8 pandemics of cholera**
  - **The first six were the same andBesides the toxins encoded by the bacteriophage and pathogenicity islands, the Classical strains also shared a similar primary carbohydrate surface antigen, called O1, which is part of the lipopolysaccharide that makes up the outer leaflet of the outer membrane**
  - **In 1961, the seventh pandemic occurred and was caused by a variant E1 Tor**
  - **In 1991, the eight pandemic occurred; those who previously suffered cholera were not immune because the O1 carbohydrate surface antigen was modified by a newly acquired cassettte**


V. cholerae
progenitor
VPI1
VPI2
01
pathway
V. cholerae
01 serogroup
CTXϕ Classical
CTXϕ El Tor
CTXϕ
VSP1
VSP2
V. cholerae
01 serogroup
Classical
V. cholerae
01 serogroup
El Tor
1st through 6th pandemics
7th pandemic
0139
antigen
SXT
37 kb region
VPI2
V. cholerae
0139
serogroup
8th pandemic

Mechanism of Cholera

- Two virulence genes carried by the *Vibrio cholerae* phage encode two subunits of cholera toxin
- The B subunit of this secreted, toxic protein binds to a glycolipid component of the plasma membrane of the epithelial cells in the gut of a person who has consumed contaminated water
- The B subunit transfers the A subunit through the plasma membrane into the epithelial cell cytoplasm.
- The A subunit is an enzyme that catalyzes the transfer of an ADP-ribose moiety from NAD+ to the trimeric G protein Gs, which normally activates adenylyl cyclase to make cyclic AMP
- ADP-ribosylation of the G protein results in an overaccumulation of cyclic AMP and an ion imbalance, leading to the massive watery diarrhea associated with cholera.

Case Study - Anthrax

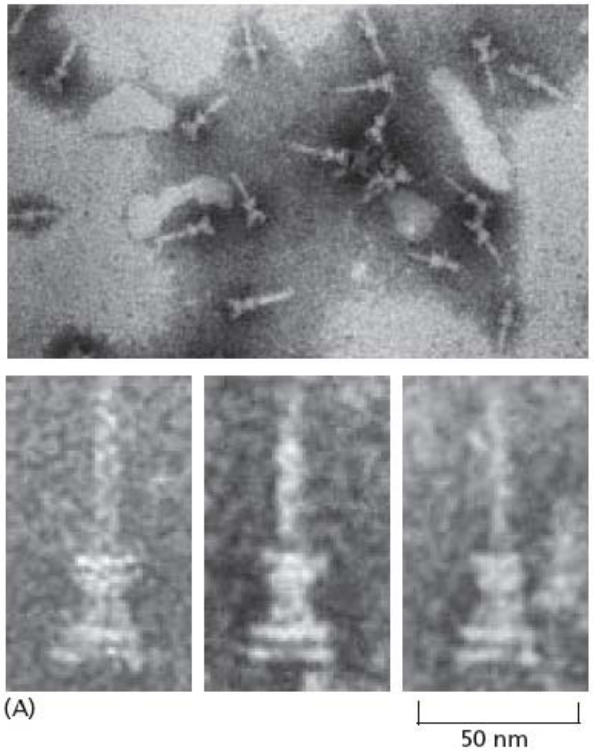


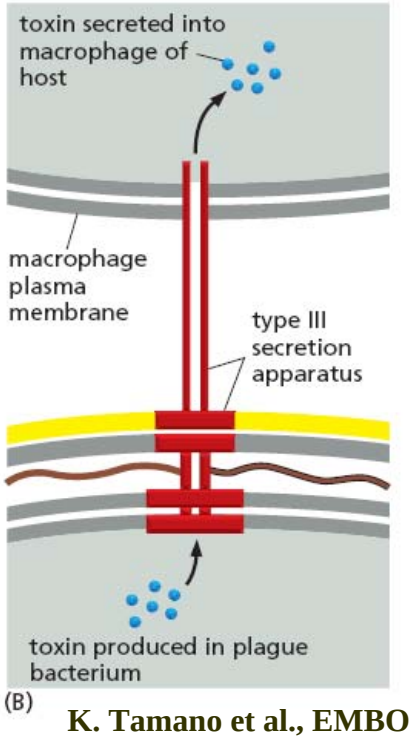
Pathology of Anthrax

Definiton

- Caused by contact with spores of the Gram-positive bacterium *Bacillus anthracis*. Dormant spores can survive in soil for long periods and are highly resistant to adverse environmental conditions, including heat, ultraviolet and ionizing radiation, pressure, and chemical agents. After the spores are inhaled, ingested, or rubbed into breaks in the skin, the spores germinate, and the bacteria begin to replicate.
- The bacteria secrete two toxins, called *lethal toxin* and *edema toxin*, either of which is sufficient to cause signs of infection.
- The B subunit is identical in the two anthrax toxins, and it binds to a host cell-surface receptor protein to transfer the two different A subunits into host cells.
- The A subunit of edema toxin is an adenylyl cyclase that directly converts host-cell ATP into cyclic AMP, leading to an ion imbalance that can cause an accumulation of extracellular fluid (edema) in the infected skin or lung. The A subunit of lethal toxin is a protease that cleaves several members of the MAP kinase family
- Injection of lethal toxin into the bloodstream of an animal causes shock (a fall in blood pressure) and death.
- The molecular mechanisms leading to death in anthrax remain uncertain.

Type III secretion system

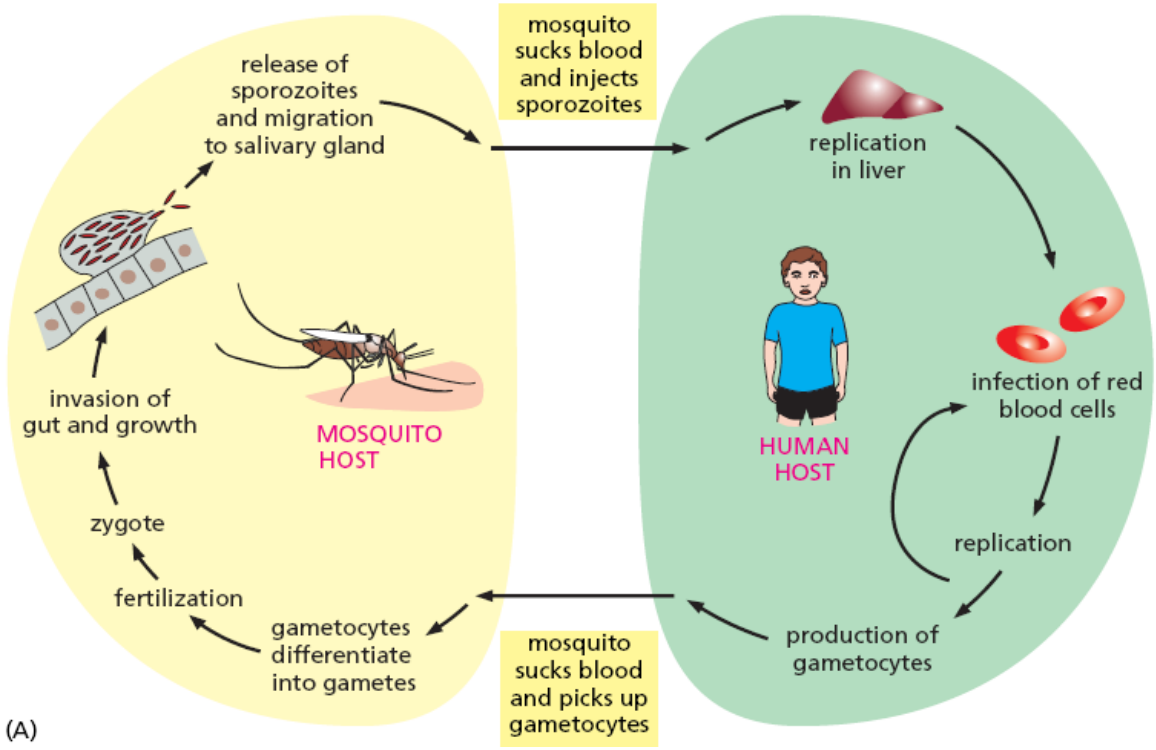




K. Tamano et al., EMBO J. 19:3876–3887

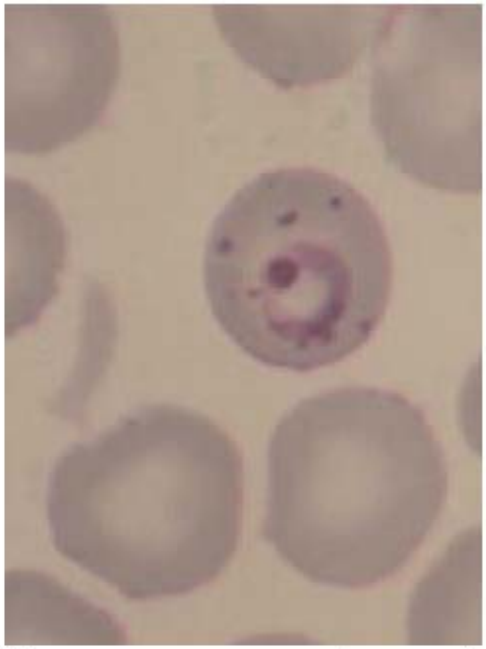
- The large lower ring is embedded in the bacterial plasma membrane, and the smaller upper ring is embedded in the bacterial outer membrane
- During infection, contact of the hollow tube tip with the plasma membrane of a host cell triggers secretion into the host cell
- Cartoon shows how plague bacterium, *Yersinia pestis*, delivers toxins to a macrophage

Case Study - Malaria

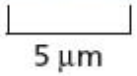


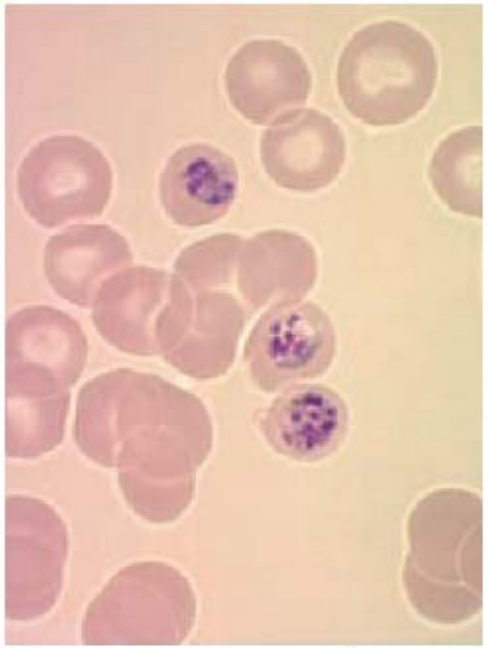
- **Malaria** is the most common protozoal disease, infecting 200–300 million people every year and killing 1–3 million of them. It is caused by four species of *Plasmodium*, which are transmitted to humans by the bite of the female of any of 60 species of *Anopheles* mosquito
- The sexual cycle of *Plasmodium* requires passage between a human host and an insect host.

Forms of parasite in blood

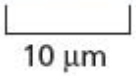


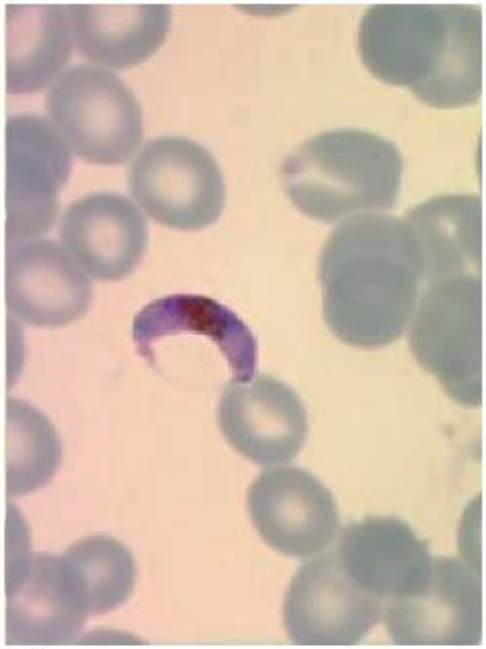
Ring stage



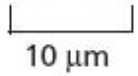


Schizont stage



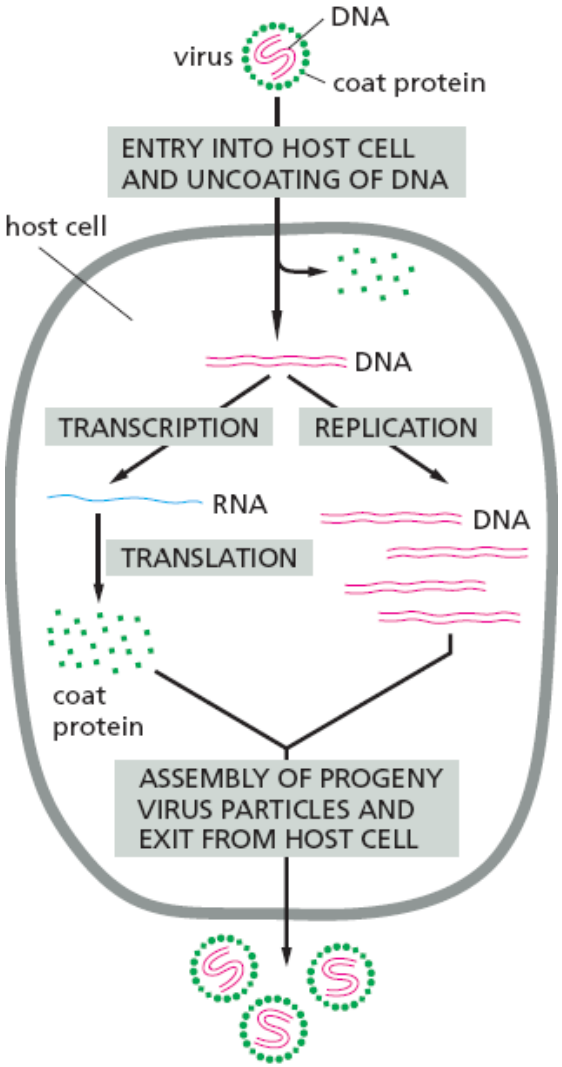


gametocyte

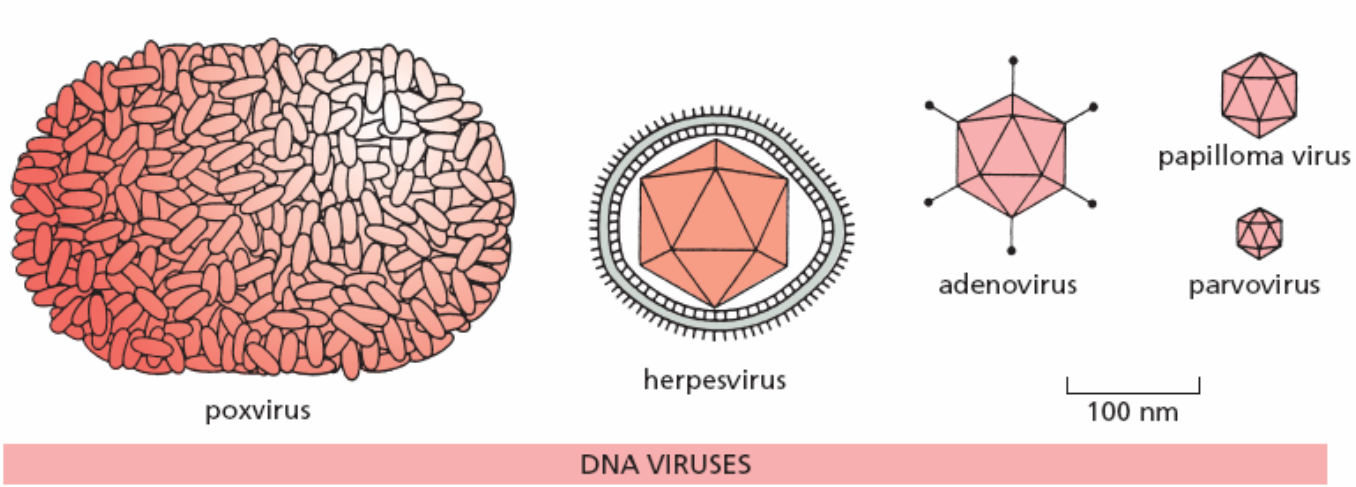


Viral Propagation

- Viruses do not possess their own molecular machinery, but only information
- Viruses have a small genome, made up of a single nucleic acid type—either DNA or RNA—which, in either case, may be single-stranded or double-stranded.
- The genome is packaged in a protein coat, which in some viruses is further enclosed by a lipid envelope.

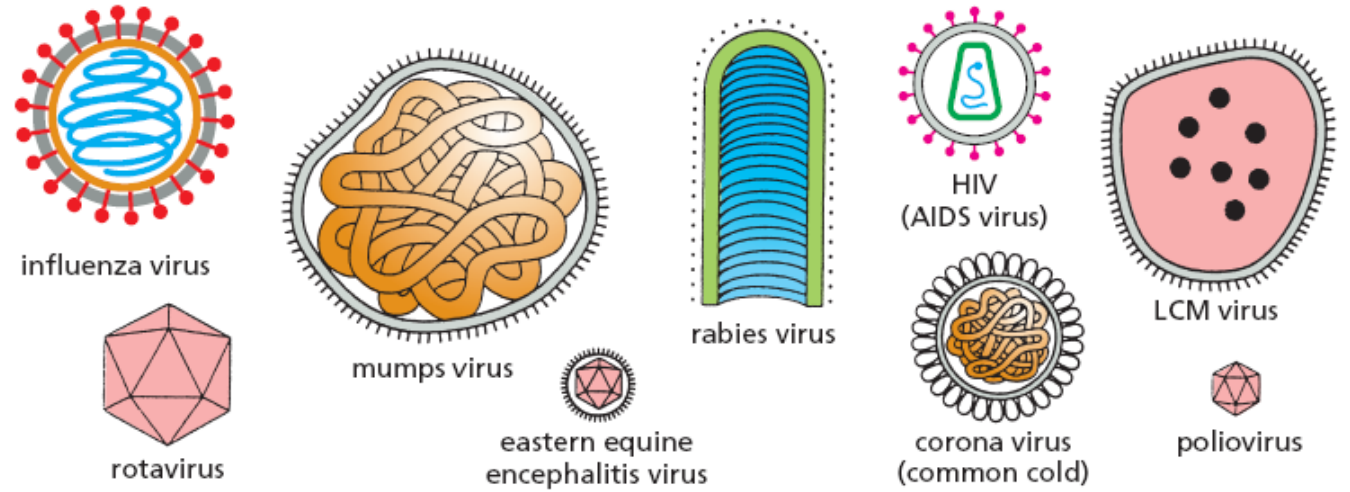


DNA Viruses



- **A single virus particle, *a virion*, can range from 20nm (parvovirus) to 450 nm (poxvirus)**
- The **capsid** that encloses the viral genome is made of one or several proteins, arranged in regularly repeating layers and patterns; the viral genome together with the capsid is called a **nucleocapsid**.

RNA Viruses

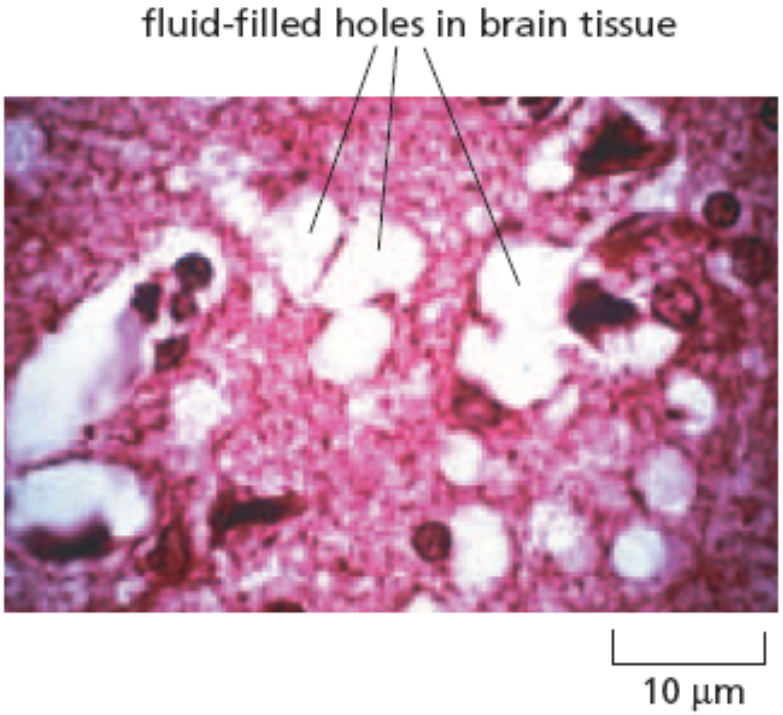

influenza virus
rotavirus
mumps virus
eastern equine encephalitis virus
rabies virus
HIV (AIDS virus)
corona virus (common cold)
LCM virus
poliovirus
RNA VIRUSES

Infectious Proteins

- **Prions** are infectious agents that replicate in the host by copying an aberrant protein structure
- They cause neurodegenerative diseases in mammals
  - *bovine spongiform encephalopathy* (BSE, or mad cow disease)
  - Kuru in humans
- They have been found in yeast, sea slugs, cattle and humans

Mechanism

- The host makes the infectious prion protein
- Prion'samino acid sequence is identical to that of a normal host protein
  - The prion and normal forms of the protein are indistinguishable in their post-translational modifications
  - Only difference between them appears to be in their folded three-dimensional structure
  - The misfolded prion protein tends to aggregate to form regular helical fibers called *amyloid*
  - misfolded prion form has the remarkable capacity to cause the normal protein to adopt its misfolded prion conformation and thereby to become infectious



Kuru is a human prion disease, very similar to BSEThe large fluidfilled holes are places where neuronshave died. These characteristic holes are why prion-based neurological diseases are called spongiform encephalopathies.

Part II

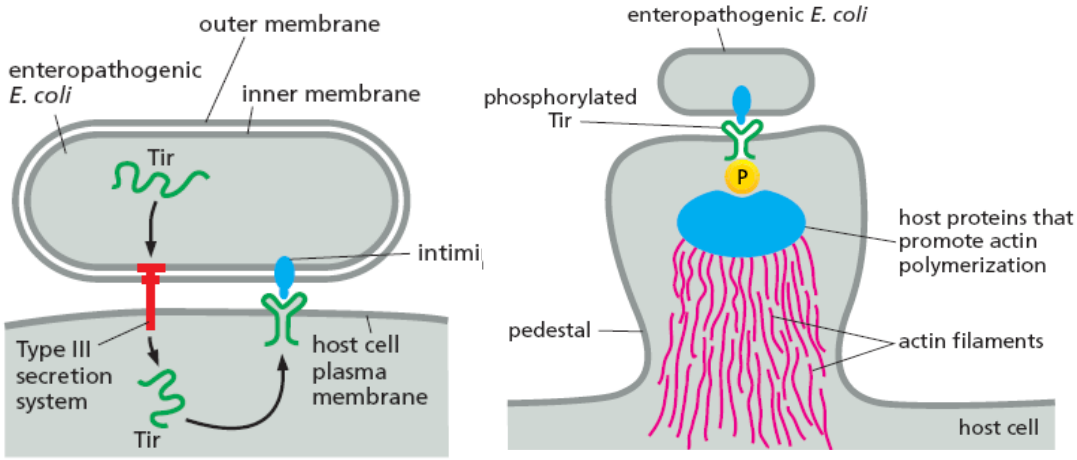
Host defense and how it is by-passed by pathogens

Infection of epthelial cells

- Epithelial cells act as barriers
- The colonize the normal flora
- Covered with protective mucus lining
- Possess cilia that sweeps away debris and bacteria

- Adhere to cell lining with specific proteins called adhesins
- Create a micro-environment that is conducive to its survival
- Alters protein function/metabolism to make the host cell ineffective against it

Example of *E.coli* colonization



- Enteropathogenic *E. coli*,which causes diarrhea in young children
  - uses a type III secretion systemto deliver its own bacterially produced receptor protein (called Tir) into its host cell
  - Tir inserts into the host cell's plasma membrane
  - The host's tyrosine kinase phosphorylates the Tir receptor protein on tyrosines
  - The phosphorylated Tir recruits a GTPases, which promotes actin polymerization through a series of intermediate steps
  - The polymerized actin then forms a unique cell-surface protrusion, called a *pedestal*, that pushes the tightly adherent bacteria up about 10 mm from the host cell surface

Intracellular mechanisms of entering the host cell

- **The first step is binding – but no cell has evolved to allow and invader to bind**

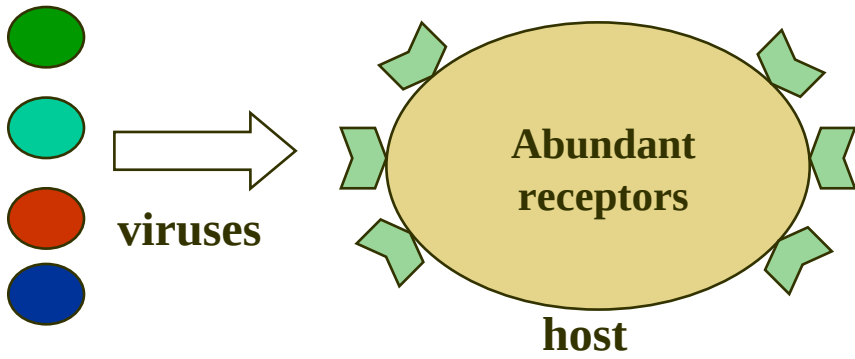
→ The host cell is tricked

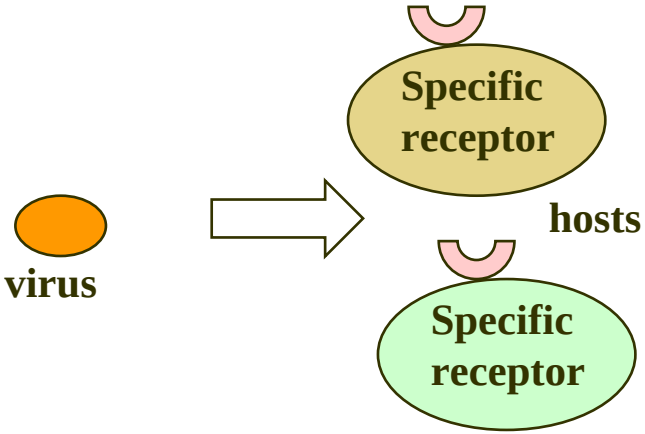
Virus binding

- Viruses seek out surface receptors → trick the host cells → and invade it
- The targets could those receptors that are abundant (influenza)

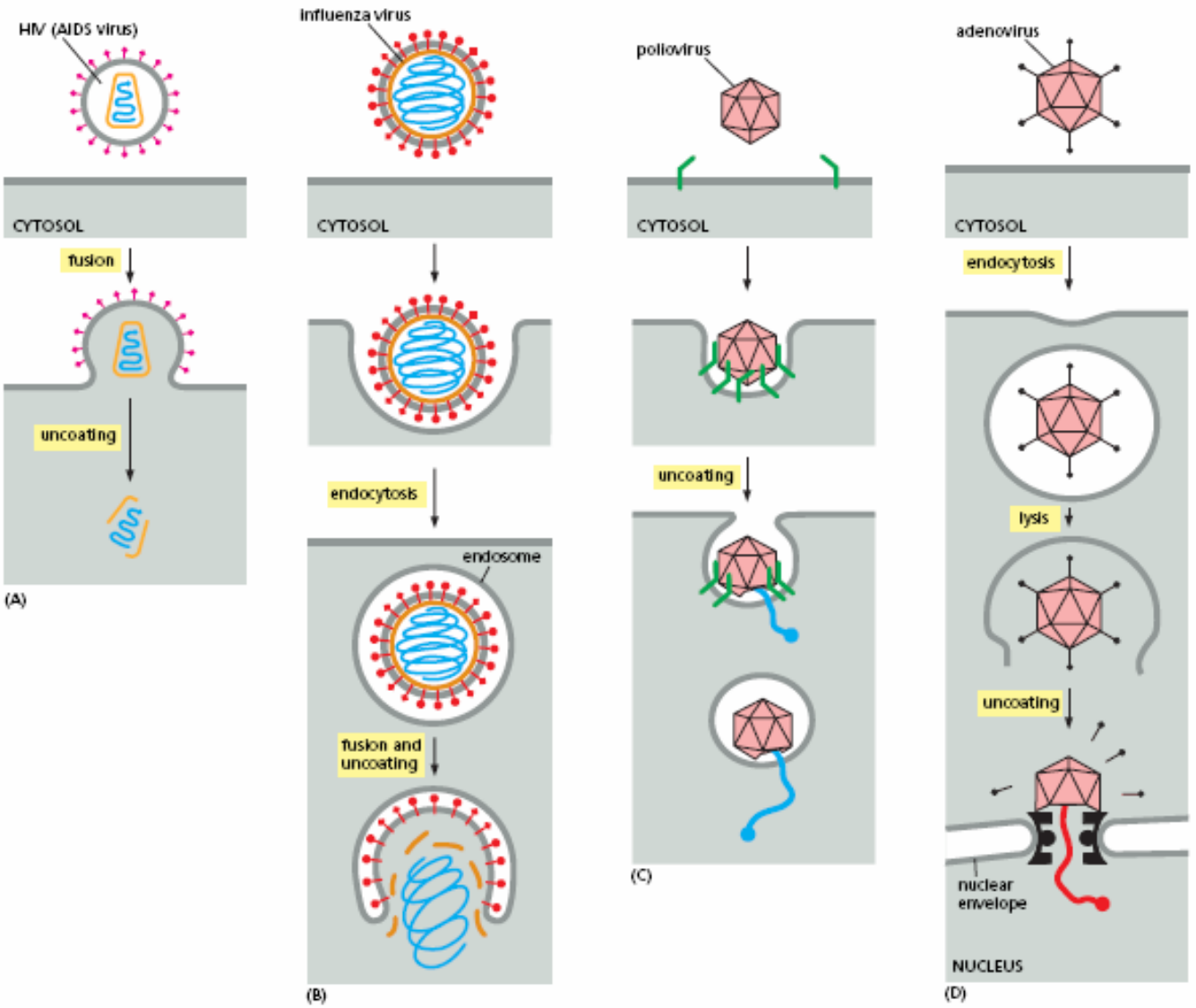
OR

- Those that are specific (hepatitis viruses)





Virus uncoating strategies



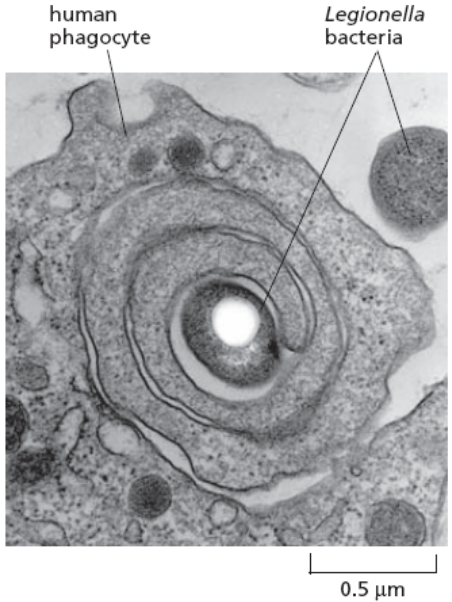
Bacteria invade by phagocytosis

- Bacteria are much larger than viruses and cannot be endocytosed – they are phagocytosed
  - Phagocytosis is the normal cellular defense by which macrophages which patrol the tissue of the body identify and ingest and destroy unwanted microbes
  - Some bacteria have developed the ability to survive and grow within macrophages and use this defense mechanism to invade cells

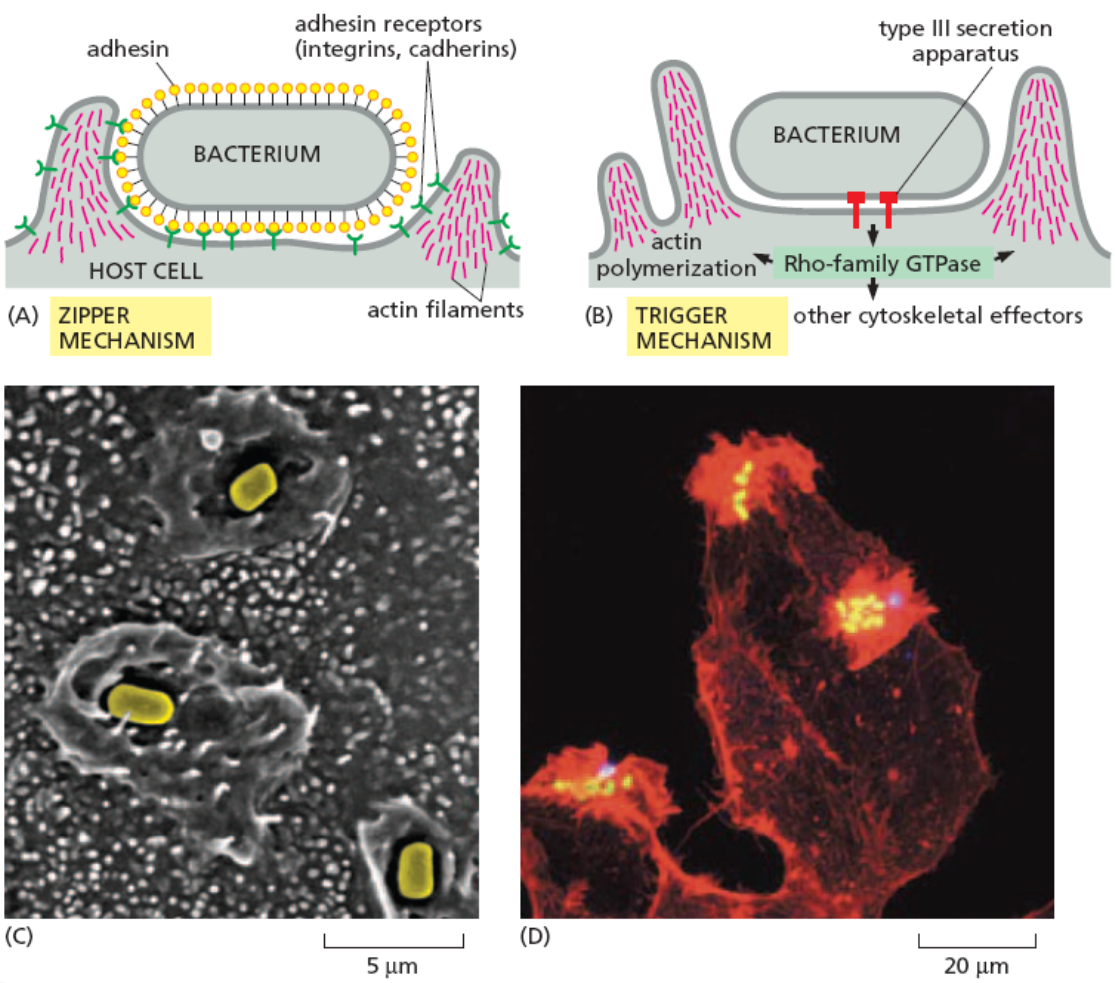
Example

- **Tuberculosis**
  - **Enters the lungs by phagosytosis**
  - **The infection is contained in a lesion called a tubercle (easily seen in X-ray)**
  - **The bacteria can survive for decades in this state**
  - **When immmunity of the host drops, infection spreads**

- **Legionnaire's Disease**
  - *Legionella pneumophila* is normally a parasite of freshwater amoebae, which take it up by phagocytosis
  - Aerosols – supermarket sprays, fountains, ACs are primary source
  - The bacteria can invade and live inside alveolar macrophages
  - This infection causes pneumonia

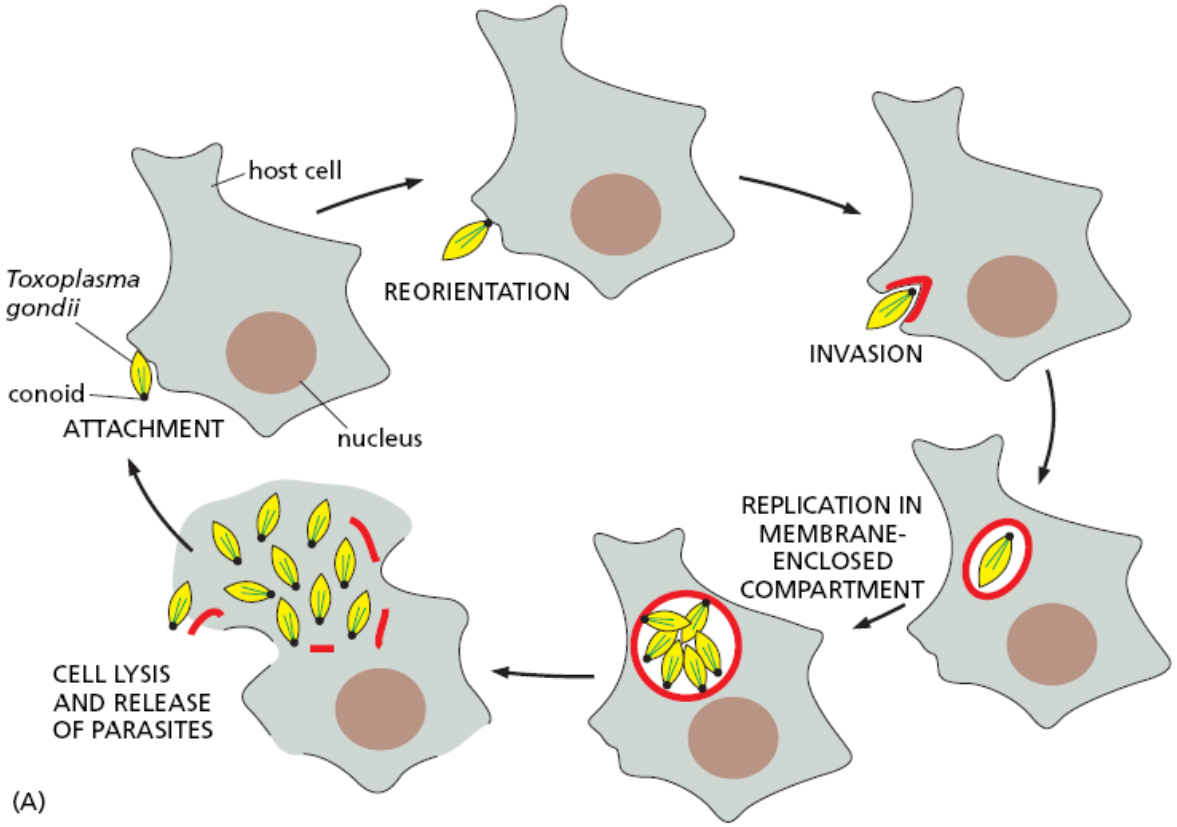


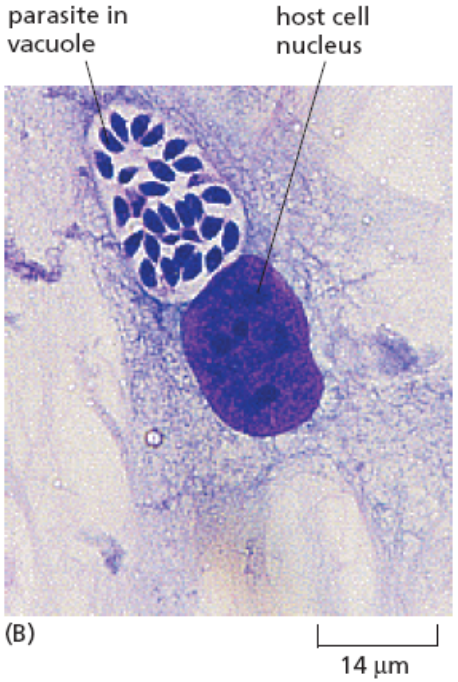
Nonphagocytic Invasion



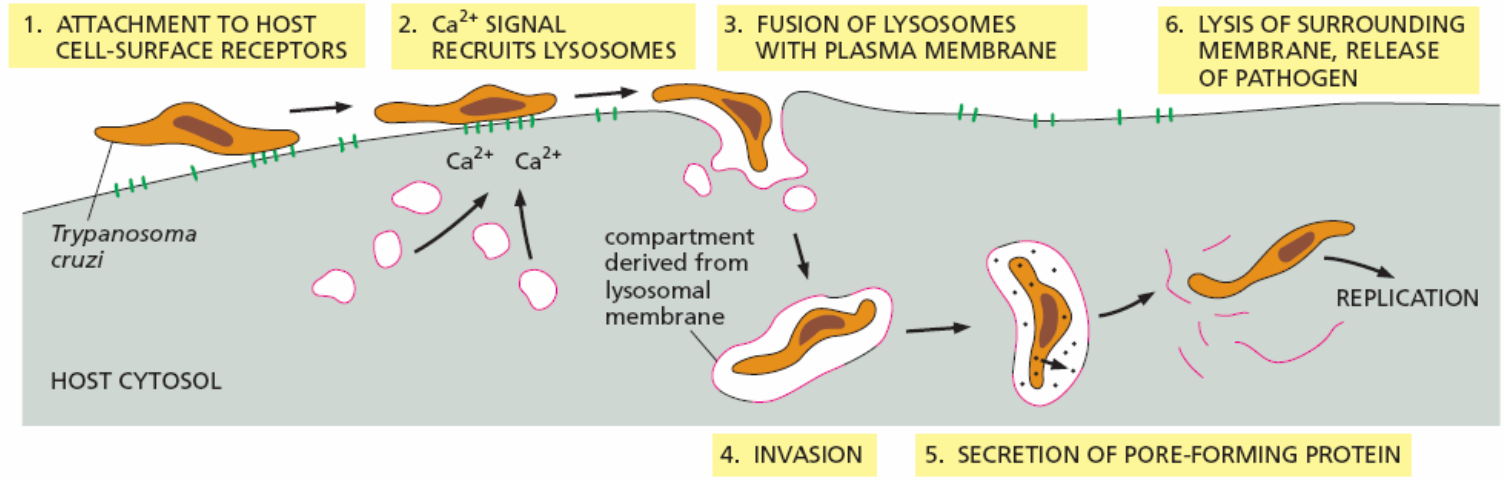
A. Zipper mechanism
B. Trigger mechanisms
C. A scanning electron micrograph showing a very early stage of *Salmonella enterica* invasion by the trigger mechanism
D. Fluorescence micrograph showing that the large ruffles that engulf the *Salmonella bacteria* are actin-rich. Thebacteria are labeled in green and actin filaments in red; because of the color overlap, the bacteria appear yellow.

The life cycle of the intracellular parasite *Toxoplasma gondii*



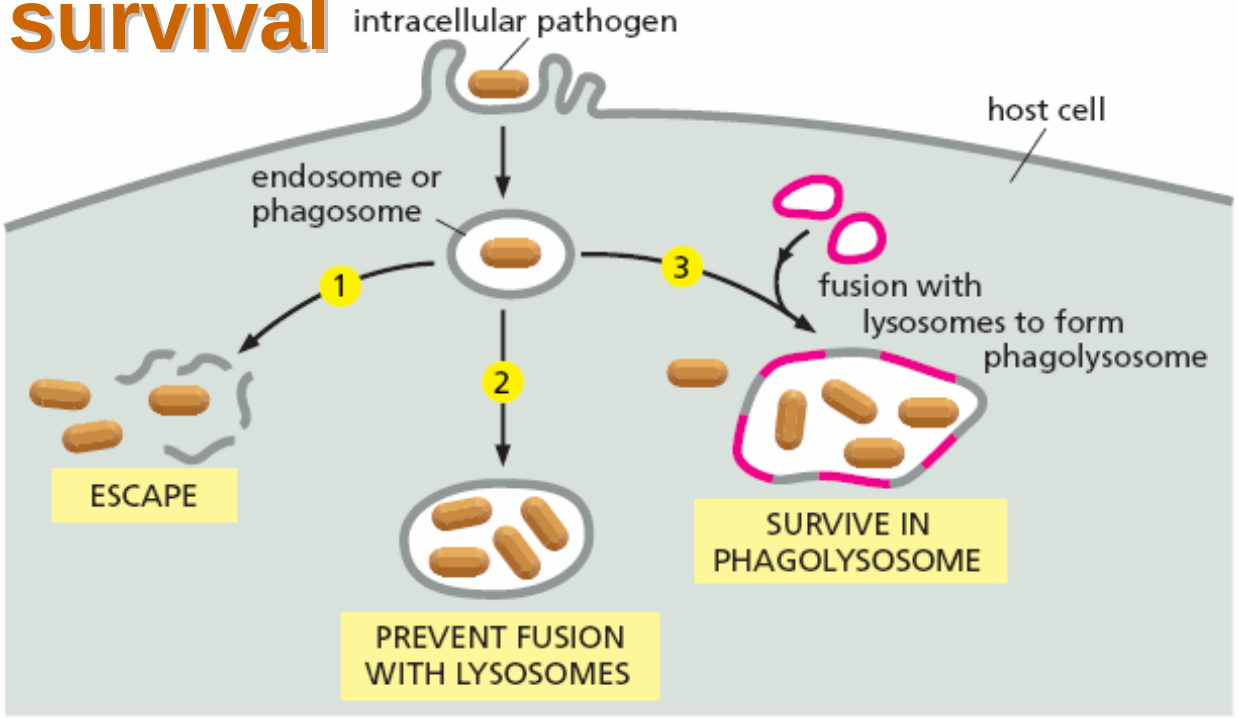


Trypanosoma cruzi



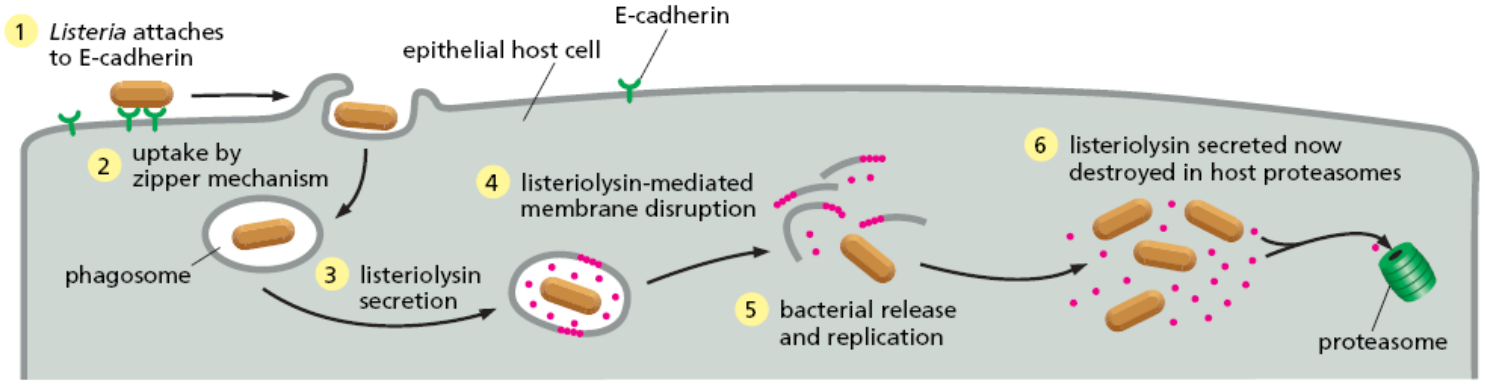
- Causes Chagas Disease related to the African trypanosome that causes sleeping sickness
- Disease is spred by insect bites
- Fever, malaise, swelling of one eye
  - Cardiomyopathy and swelling of lymphs

Intracellular pathogenesis – strategies for survival



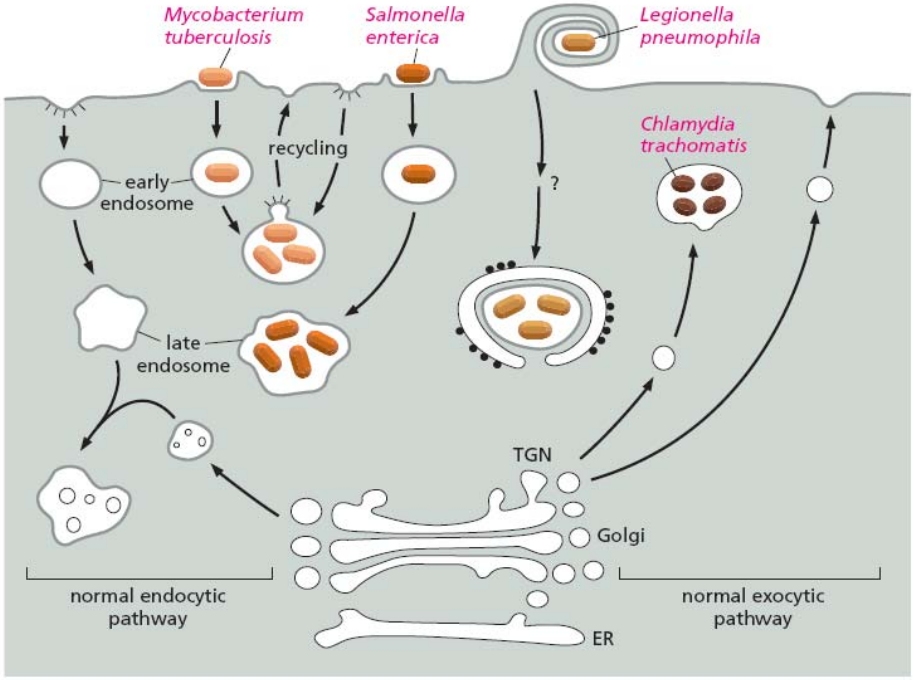
- Followed by all viruses
- Followed by *Mycobacterium tuberculosis*, *Salmonella enterica*, *Legionella pneumophila*, and *Chlamydia trachomatis*
- Followed by *Coxiella burnetii* and *Leishmania*.

Modification of lyposomal membrane



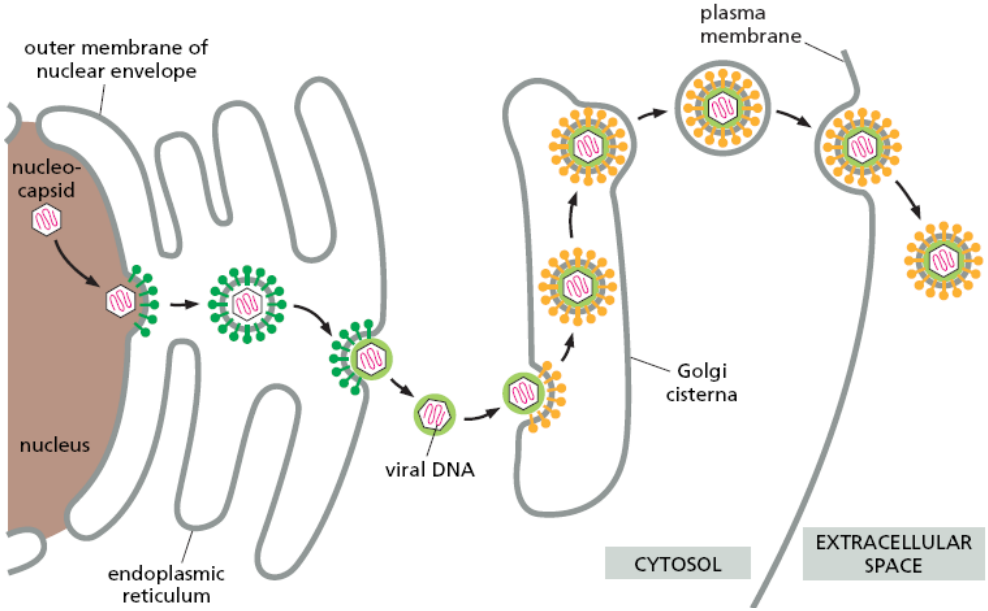
- *L. monocytogenes* attaches to E-cadherin on the surface of epithelial cells and induces its own uptake by the zipper mechanism
- Within the phagosome, the bacterium secretes the hydrophobic protein listeriolysin O, which forms oligomers in the host cell membrane, thereby creating large pores and eventually disrupting the membrane.
- Once in the host cell cytosol, the bacteria begin to replicate and continue to secrete listeriolysin O. Because the listeriolysin O in the cytosol is rapidly degraded by proteasomes, the host cell's plasma membrane remains intact.

Modifying host cell membrane



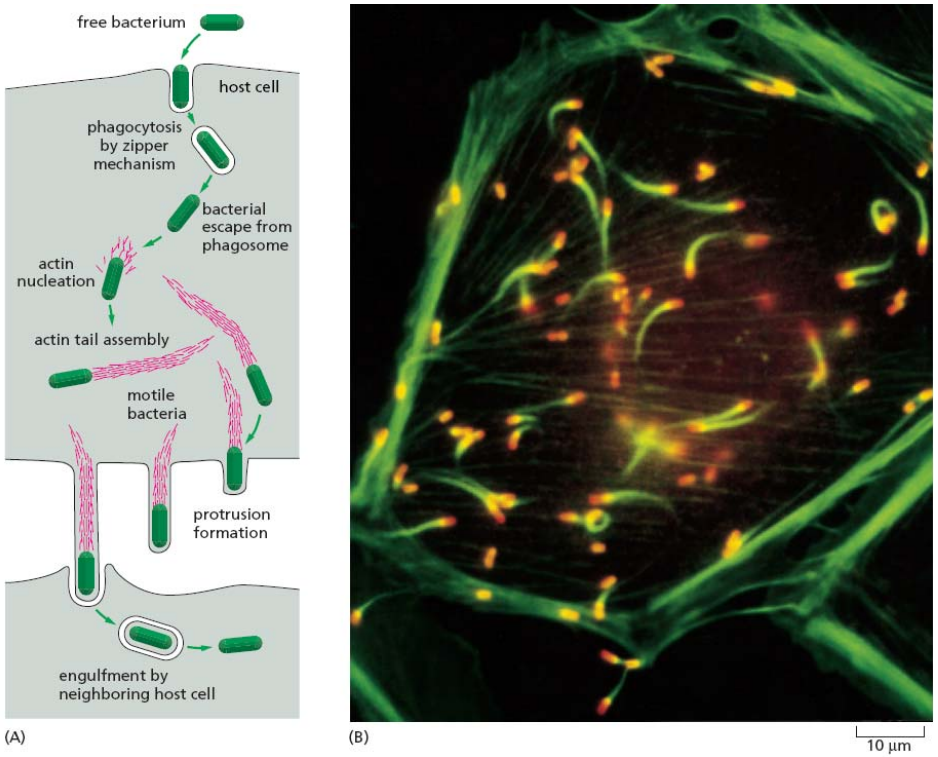
- *M. tuberculosis* remains in a compartment that has early endosomal markers and continues to communicate with the plasma membrane via transport vesicles.
- *S. enterica* replicates in a compartment that has late endosomal markers and does not communicate with the plasma membrane
- *L. pneumophila* replicates in an unusual compartment that is wrapped in several layers of rough endoplasmic reticulum (ER) membrane;
- *C. trachomatis* replicates in an exocytic compartment that fuses with vesicles coming from the trans Golgi network

Acquiring viral envelope



- Herpes virus nucleocapsids assemble in the nucleus and then bud through the inner nuclear membrane into the space between the inner and outer nuclear membranes, acquiring a membrane coat
- The virus particles then apparently lose this coat when they fuse with the outer nuclear membrane to escape into the cytosol.
- Subsequently, the nucleocapsids bud into the Golgi apparatus and bud out again on the other side, acquiring two new membrane coats.
- The virus then buds from the cell with a single membrane when its outer membrane fuses with the plasma membrane.

Actin-based movement



- These bacteria induce the assembly of actin-rich tails in thehost cell cytoplasm, enabling the bacteria to move rapidly.
  - Motile bacteria spread from cell to cell by forming membrane-enclosed protrusions that are engulfed by neighboring cells.
- Fluorescence micrograph of the bacteria moving in a cell that has been stained to reveal bacteria in red and actin filaments in green.

The continuing battle

- **Pathogens can alter the behavior of the host organism to facilitate the spread of the pathogen**
- **Pathogens evolve rapidly**
  - humans and chimpanzees have acquired a 2% difference in genome sequences over about 8 million years of divergent evolution, poliovirus manages a 2% change in its genome in 5 days
- **Antigenic variation in pathogens occurs by multiple ways**
- **Error prone replication dominates viral evolution**
  - Untreated HIV infection may eventually produce HIV genomes with every possible point mutation
- **Pathogens acquire drug resistance**
  - Once a pathogen has chanced upon an effective drug-resistance strategy, the newly acquired or mutated genes that confer the resistance are frequently spread throughout the pathogen population