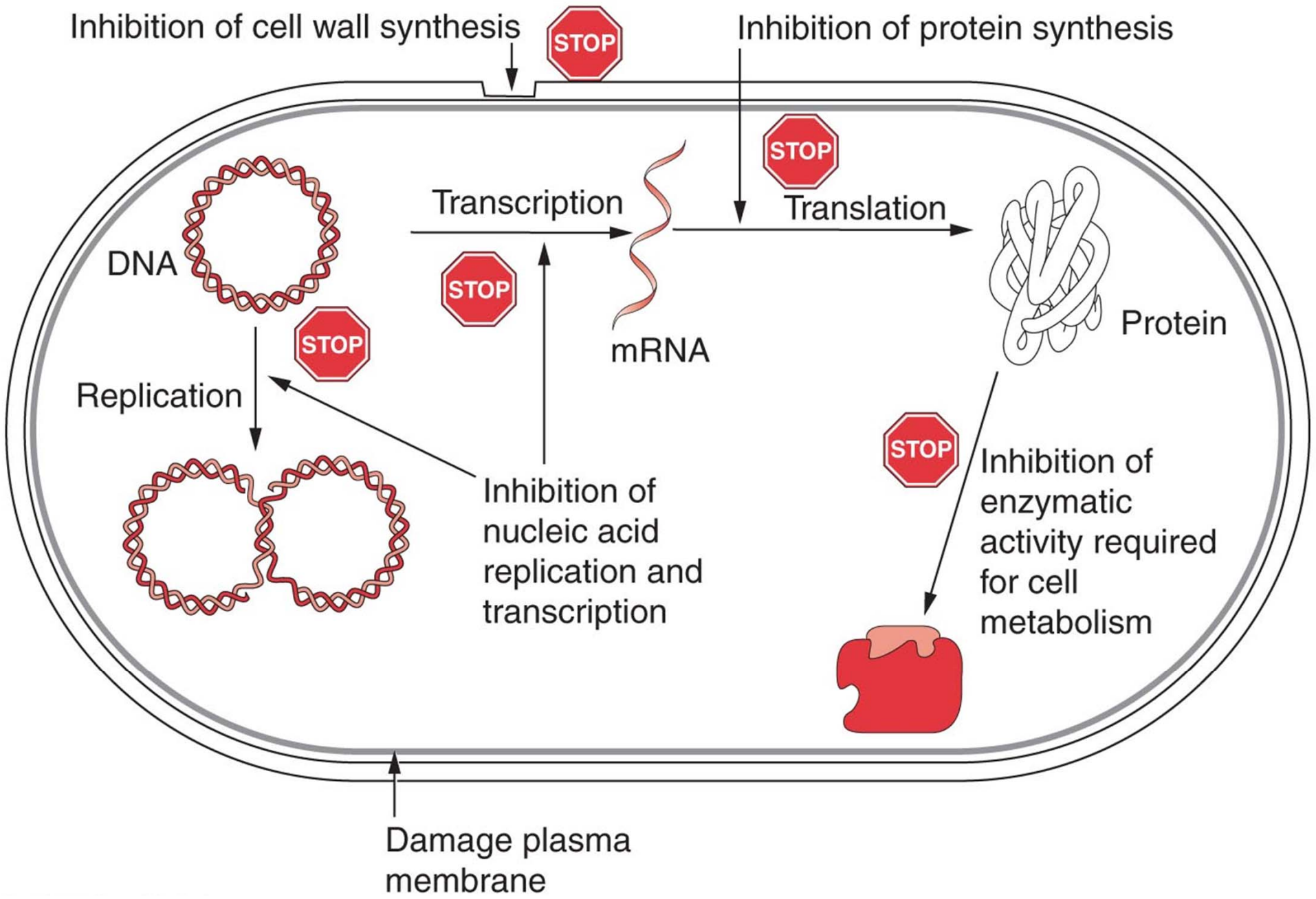
Previous class

- Gram staining of microbes
- Microorganisms as tools
  - Industries : food, beverage, textiles, detergents, etc
  - Molecular biology: recombinant DNA technology (transformation, fusion proteins, reporter genes, etc)
  - Glycolysis, fermentation (lactic acid and alcohol)
  - Therapeutics

Antibiotics

- Produced by microbes that inhibit the growth of other microbes
- 1928 discovery of penicillin by Alexander Fleming
  - Colonies of mold *Penicillium notatum* inhibited growth of bacterium *Staphylococcus aureus*
- Majority are produced by bacteria, and inhibit the growth of other bacteria
- Act in a few key ways
  - Prevent replication
  - Kill directly
  - Damage cell wall or prevent its synthesis


Inhibition of cell wall synthesis
STOP
Inhibition of protein synthesis
STOP
Transcription
Translation
DNA
STOP
mRNA
Protein
STOP
Replication
STOP
Inhibition of enzymatic activity required for cell metabolism
Inhibition of nucleic acid replication and transcription
Damage plasma membrane

Antibiotic Resistant Strains

- How do antibiotic resistant strains arise?
  - Improper and over use of antibiotics
- Antibiotic resistant strains of *S. aureus, P. aeruginosa, S. pneumoniae, M. tuberculosis* and many other human pathogens have already been detected
- Resistance to one antibiotic often leads to resistance to many other drugs
- Antimicrobial drugs harmful for bacteria in different ways need to be developed

How can studying bacterial pathogens lead to new drugs?

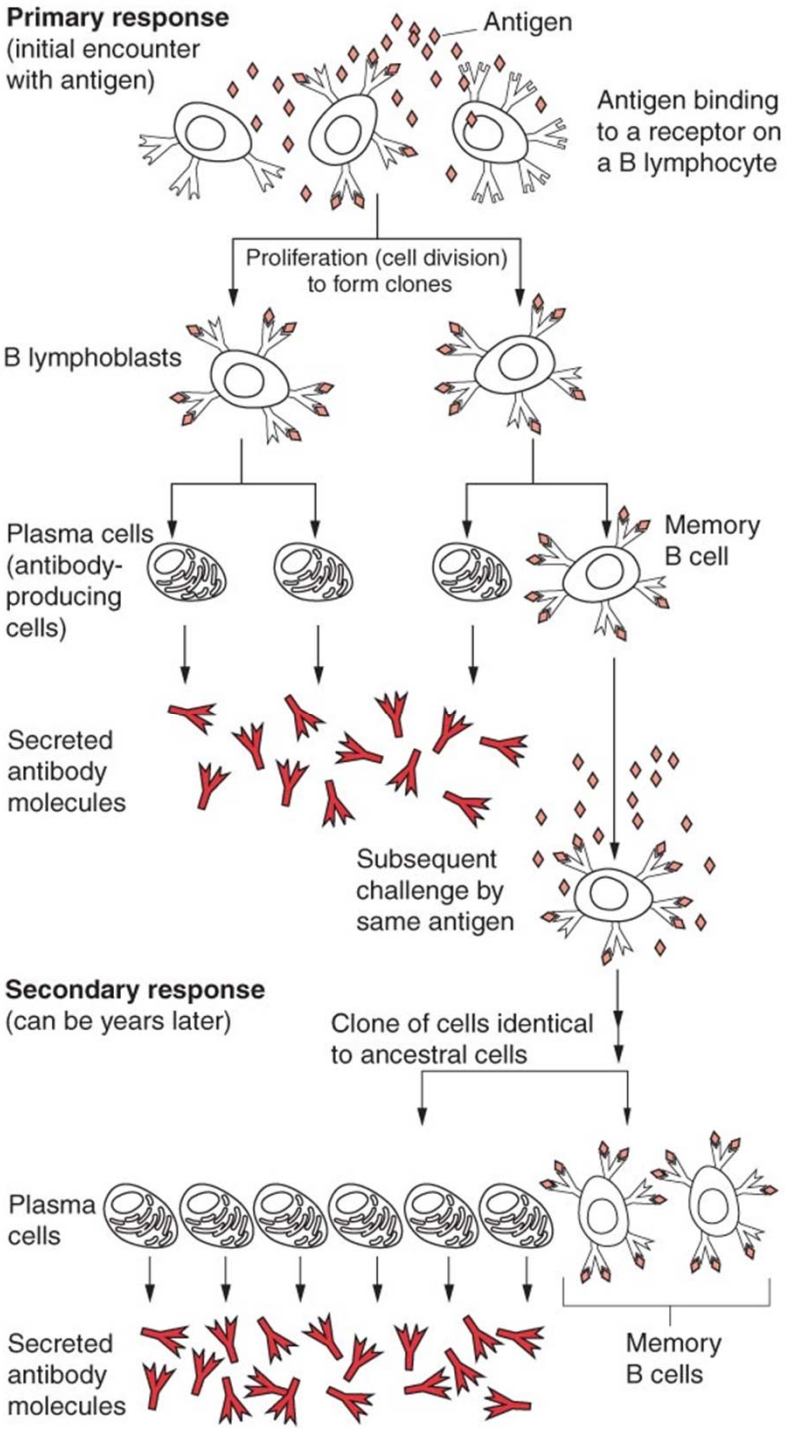
- Identify toxins and properties that disease – causing bacteria use
- For example,
  - Recombinant form of *S. mutans* is proposed for reducing tooth decay
  - Naturally occurring *S. mutans* in oral cavity metabolize sugars to produce lactic acid
  - Lactic acid dissolves tooth enamel and dentin in teeth, leading to cavities and tooth decay
  - Recombinant *S. mutans* can not do this
  - Replace natural *S. mutans* with recombinant microbe

Vaccines

- First vaccine developed in 1796 by Edward Jenner
  - Used live cowpox virus to vaccinate against smallpox
  - **Vaccination** : using infectious agents to provide immune protection against illness.
  - DPT - diphtheria, pertussis, and tetanus
  - MMR – measles, mumps, and rubella
  - OPV - oral polio vaccine

Immune System and Antibodies

- **Antigens** are foreign substances that stimulate an immune response
  - Whole bacteria, fungi, and viruses
  - Proteins, lipids, or carbohydrates
- Immune system responds to antigens by producing antibodies
  - Called **antibody-mediated immunity**
  - **B cells** (B lymphocytes, type of white blood cells (WBCs) or leukocytes), with the help of **T cells** (T lymphocytes), recognize and bind to the antigen
  - B cells then develop to form **plasma cells** (that produce antibodies) and “memory cells”



Antigen binding to receptor on B-cell

Plasma cells and Memory B-cells are formed

Antibodies are secreted

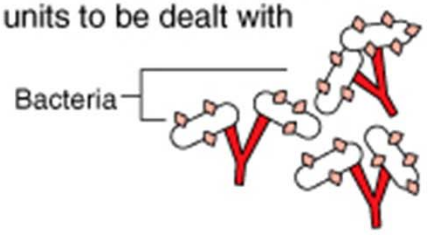
Memory B-cells remain for secondary response

Immune System and Antibodies

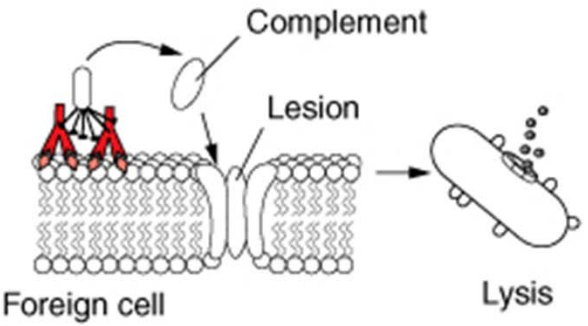
– Antibodies are very specific
  - Bind to the antigen
  - Macrophage can then recognize the antigens coated with antibodies and “eat” them – phagocytosis.
  - Macrophages engulf antigen covered with antibody; organelles in the macrophage called lysosomes unleash digestive enzymes that degrade the antigen.

Protective mechanism of binding antibodies to antigens

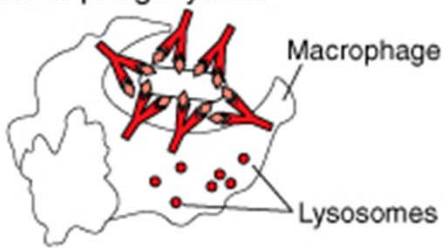
Agglutination (Clumping)
Enhances phagocytosis and reduces number of infectious units to be dealt with



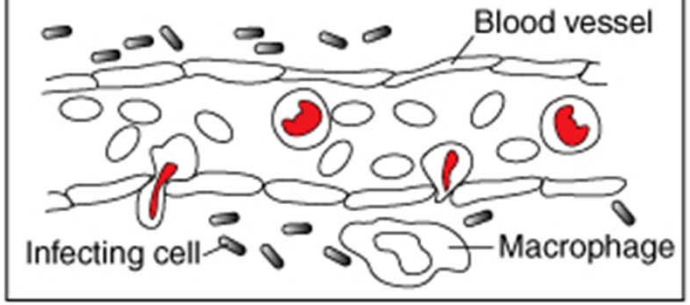
Activation of complement (Complement fixation)



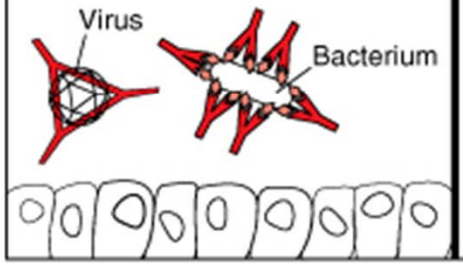
Opsonization
Coating antigen with antibody enhances phagocytosis



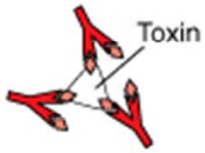
Inflammation
Disruption of cell by complement/reactive protein attracts phagocytic and other defensive immune system cells



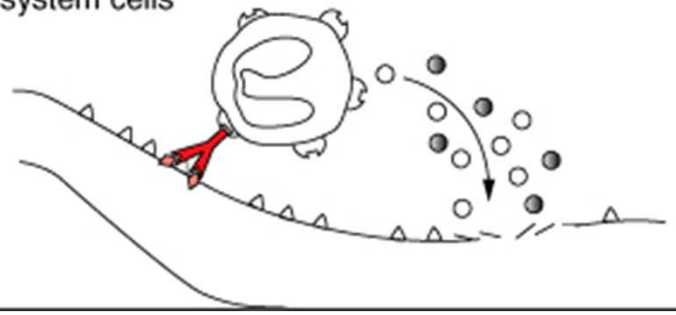
Neutralization
Blocks adhesion of bacteria and viruses to body cells



Blocks active site of toxin



Antibody-dependent cell-mediated cytotoxicity
Antibodies attached to target cell cause destruction by non-specific immune system cells



- Sometimes our natural production of antibodies is not enough to protect us from pathogens
- For example, smallpox, viruses causing hepatitis, HIV causing AIDS.
- Vaccines may be useful in some cases

Vaccines

- **Vaccines** – parts of a pathogen or whole organisms that can be given to humans or animals by mouth or by injection to stimulate the immune system against infection by those pathogens
- Mostly vaccines are **preventative** or **prophylactic** (by providing protection against a pthogen should you be exposed) and not *therapeutic*

Vaccine production

- Three Major Strategies to Make Vaccines
  - **Subunit vaccines** are made by injecting portions of viral or bacterial structures
    - Eg, hepatitis B, tetanus, anthrax ,and meningococcal diesase
  - **Attenuated vaccines** use live bacteria or viruses that have been weakened through aging or by altering their growth conditions to prevent replication
    - Eg, polio, MMR, tuberculosis, cholera, and chickenpox
  - **Inactivated (killed) vaccines** are made by killing the pathogen and using the dead or inactivated microorganism for the vaccine
    - Eg, polio, rabies, DPT, influenza etc.
  - **DNA-based vaccines**

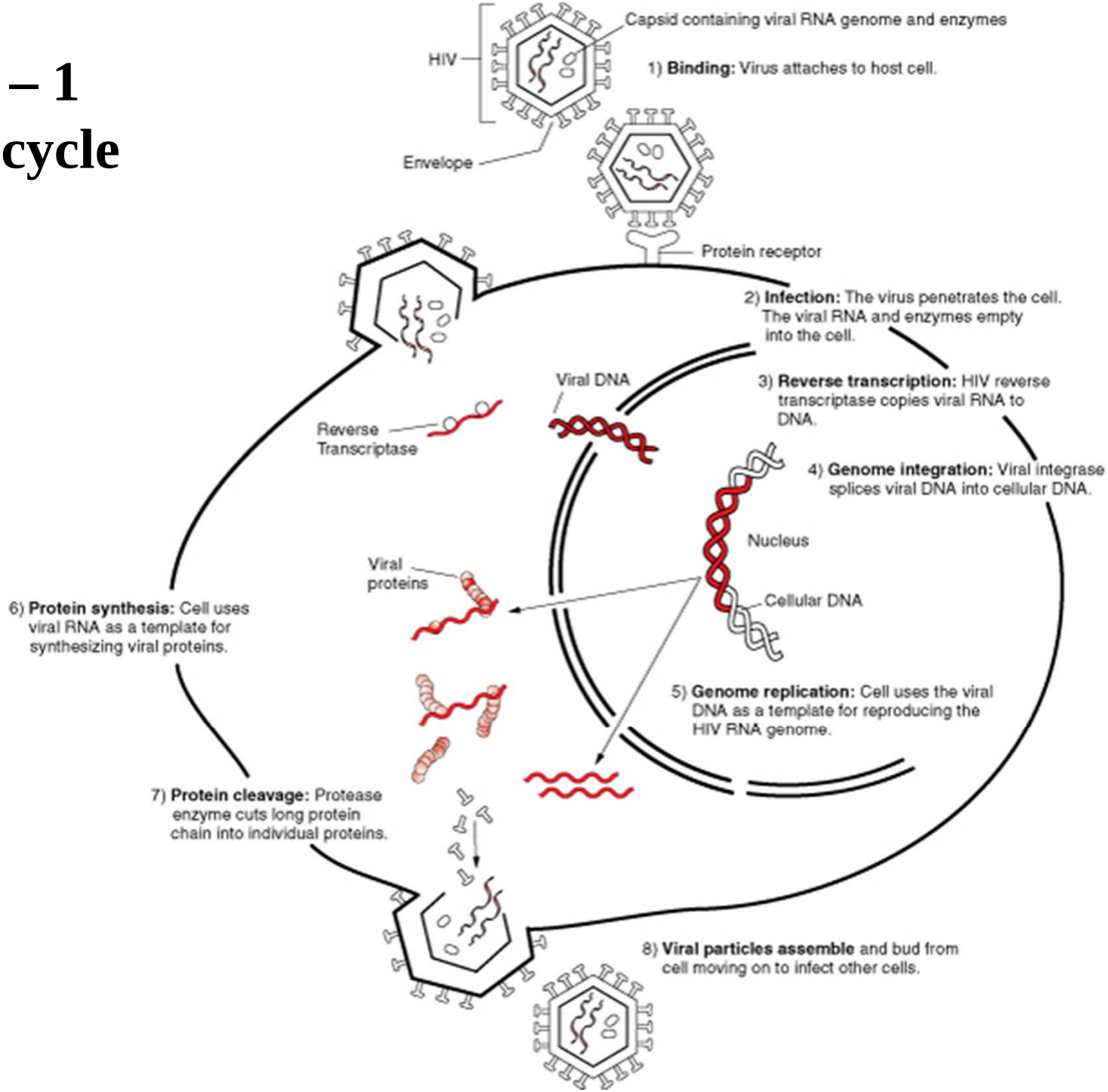
Vaccines

- Immunity from vaccinations can fade with time, particularly for inactivated vaccines
- So immunization booster shots are required to re-stimulate the immune system
- Eg, DPT, tetanus, flu etc.
- Hepatitis B vaccine was earlier prepared by isolating the virus from the blood of infected patients and purifying viral proteins.
- Now recombinant subunit vaccine is produced by cloning genes for proteins on outer surface of the virus into plasmids and transforming yeast to produce fusion proteins.

HIV – 1 (human immunodeficiency virus – 1)

- Causative agent of AIDS
- Infects human immune cells by binding to it and injecting its RNA genome
- Reverse transcriptase converts HIV RNA genome to DNA
- HIV is a retrovirus (which transcribe their RNA genomes into DNA)

HIV – 1
Life cycle



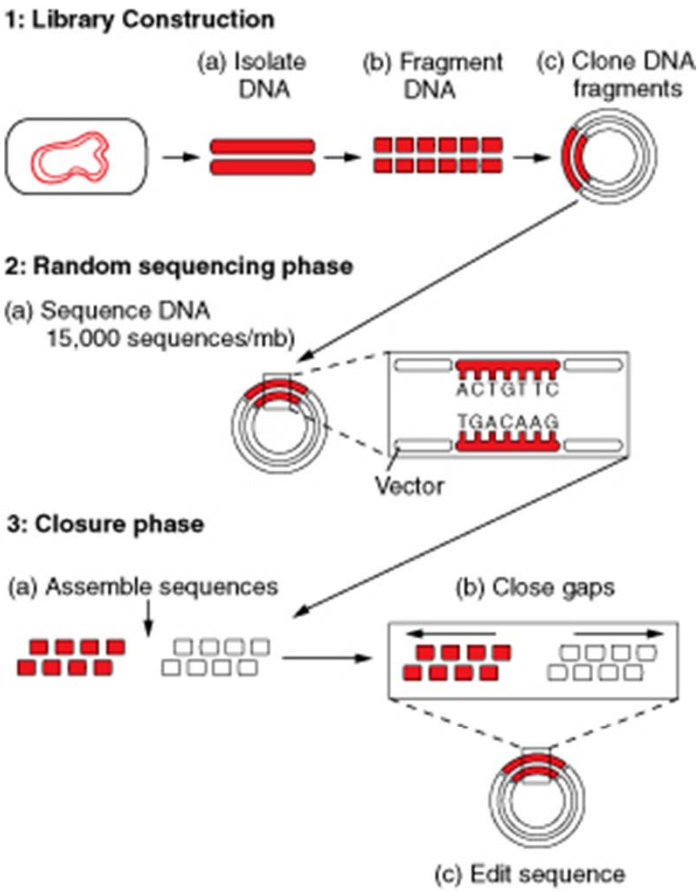
Vaccines Targets

- Influenza
  - Flu viruses mutate rapidly
  - Avian flu (H5N1) – infect chicken – use 2 viral surface proteins hemagglutinin and neuraminidase
  - Bird-to-pig transmission, and pig strain is mutated to produce strain infecting human
  - Swine flu (H1N1)
- Tuberculosis
  - Very adept at evolving new strains that are resistant to treatment
- Malaria
- HIV
  - High mutation rate

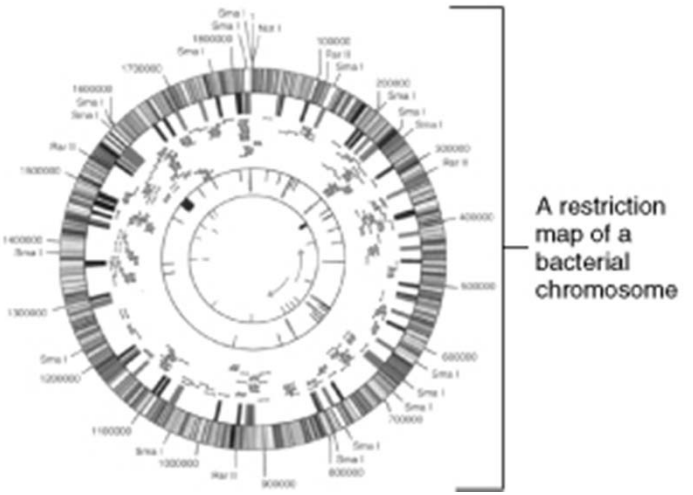
Microbial Genomes

- 1994 Microbial Genome Program (MGP)
  - To sequence the entire genomes of microorganisms that have potential applications in environmental biology, research, industry, and health as well as genomes of protozoan pathogens

Genome Sequencing Strategy



4: Annotation and publication



A restriction map of a bacterial chromosome

Microbial Genomes

- Why sequence microbial genomes?
  - *Streptococcus pneumoniae*, which causes ear and lung infections, kills 3 million children worldwide each year
    - Many of the vaccines are ineffective in children
    - In 2001 the genome was sequenced and many genes encoding proteins on the surface of the bacteria were discovered
    - Could lead to new treatments, including gene therapy

Microbial Genomes

- Why sequence microbial genomes?
  - Identify genes involved in bacterial cell metabolism, cell division, and genes that cause human and animal illnesses
  - Of the microbes sequences to date, approx. 45% of genes have unknown function, and approx. 25% genes are unique

Table 5.3 SELECTED MICROBIAL GENOMES

Bacterium	Human Disease Condition	Approximate Genome Size (megabases, mB)	Approximate Number of Genes
Bacillus anthracis	Anthrax	5.23	5,000
Borrelia burgdorferi	Lyme disease	1.44	853
Chlamydia trachomatis	Eye infections, genitourinary tract infections (e.g., pelvic inflammatory disease)	1.04	896
Escherichia coli 0157:H7	Severe foodborne illness (diarrhea)	4.10	5,283
Haemophilus influenzae	Serious infections in children (eye, throat, and ear infections, meningitis)	1.83	1,746
Helicobacter pylori	Stomach (gastric) ulcers	1.66	1,590
Listeria monocytogenes	Listeriosis (serious foodborne illness)	2.94	2,853
Mycobacterium tuberculosis	Tuberculosis	4.41	3,974
Neisseria meningitidis (MC58)	Meningitis and blood infections	2.27	2,158
Pseudomonas aeruginosa	Pneumonia, chronic lung infections	6.30	5,570
Rickettsia prowazekii	Typhus	1.11	834
Rickettsia conorii	Mediterranean spotted fever	1.30	1,374
Streptococcus pneumoniae	Acute (short-term) respiratory infection	2.16	2,236
Yersinia pestis	Plague	4.65	4,012
Vibrio cholerae	Cholera (diarrheal disease)	4.00	3,885

Sources: Sawyer, T. K. (2001). Genes to Drugs. *Biotechniques* 30(1): 164–168. TIGR Microbial Database (www.tigr.org/tdb/mdb/mdbcomplete) and Gold: Genomes OnLine Database (wit.integratedgenomics.com/GOLD).

Metagenomics

- Sequencing of genomes for entire community of microbes
- Study different environments – water, soil, air, oceans, glaciers, mines, human body…
- < 1% of total microbial population is known
- Most are unculturable
- A very important advancement in biotech – metagenomics
- Sequencing of unculturable microbes can be done

Human Microbiome Project

- 2008 NIH announced plans for the Human Microbiome Project
  - 5-year project to sequence 600 to 1000 genomes of microorganisms (bacteria, viruses, and yeasts) that live on and inside humans
  - Microbes comprise 1 – 2 % of human body outnumbering human cells by 10 to 1.
  - Goals of HMP:
    - Core human microbiome
    - Maintaining microbial communities
    - Effect of alterations in microbiome on human health
    - Developing new bioinformatics tools
    - Address ethical, legal, and social implications raised by human microbiome research