

1  *Microbial Models: Genetics of Viruses and Bacteria*

Chapter 18

2  *Discovery of Viruses*

- Tobacco mosaic disease is an infectious disease
- A. Mayer successfully transmitted the disease but couldn't find a bacterium 1883
- Ivanowsky proposed it was caused by a bacterium too small to be trapped by filters or by a toxin too small to be filtered
- Beijerinck proposed the disease was caused by a reproducing particle that was smaller and simpler than a bacterium

3  *Demonstrating an Infectious Agent and not a toxin causes the disease*

- To do this, Beijerinck demonstrated that the agent could reproduce
- Plants were sprayed with filtered sap from the diseased plant
- The sprayed plants developed tobacco mosaic disease
- Sap from newly infected plants was used to infect others
- The experiment was repeated for multiple generations

4  *Unlike bacteria...*

- The pathogen reproduced only within the host it infected
- It could not be cultured on media
- It could not be killed by alcohol
- Stanley crystallized the infectious particle now known as Tobacco Mosaic Virus (TMV) in 1935

5  *General Viral Structure*

- Viruses were first seen w electron microscopes
- The virion is nucleic acid enclosed by a protein coat

6  *Protein Capsids and Envelopes*

- Capsid: protein may be rod shaped, polyhedral or complex
- Protein subunits made from only one or a few types of protein
- Envelope- membrane that cloaks some viral capsids
- Envelope helps viruses infect host undetected as the membrane is derived from host cell membrane which is virus modified and contains proteins of viral origin
- Bacteriophages, bacterial viruses, have the most complex capsids

7  *Virus life cycle*

- Viruses are obligate intracellular parasites
- Each virus has a specific host range
- The host cell is recognized by a complementary fit between the virus proteins and the cell surface receptor sites
- Once the virus recognizes the cell, the viral genome is inserted in the cell
- Host resources are used to replicate the viral genome and capsid proteins

- New viruses are assembled and released

8  *Viral Genomes*

- May be double or single stranded DNA or RNA
- The nucleic acid molecules may be linear or circular
- It may be as limited as 4 genes or as complex as several hundred genes

9  *3 patterns of viral genome replication*

- DNA to DNA: if db stranded, DNA replication resembles that of cellular DNA using host DNA polymerase
- RNA to RNA: most RNA viruses have a gene that codes for RNA replicase, which uses viral RNA as a template to produce complementary RNA
- RNA → DNA → RNA: RNA viruses that use this mode encode reverse transcriptase; an enzyme that transcribes DNA from an RNA template

10  *Diverting host resources for viral replication*

- Viral genes use the host cell's enzymes, ribosomes, tRNAs, amino acids, ATP and other resources to produce viral components
- New viral nucleic acid and capsids self assemble into hundreds or thousands of virions which then leave as the cell lyses to parasitize new hosts
- Capsid proteins and nucleic acids held together by weak bonds

11  *Viruses have 2 reproductive cycles*

- Virulent phages replication cycle results in the death or LYSIS of the host cell: lytic cycle
- Temperate viruses coexist w their hosts by incorporating their genome into the host's genome and remain dormant until they initiate a lytic cycle; these cycle is the lysogenic cycle

12  *Lytic Cycle*

- Virus attaches to cell surface: cell recognition
- Virus contracts sheath and injects DNA: uses ATP
- Hydrolytic enzymes destroy host cell's DNA: uses restriction enzymes
- Virus genome directs the host cell to produce viral components
- Cell lyses and releases virus particles: lysosomes attack the cell membrane; osmotic swelling lyses cell

13  *Attacks and Defenses*

- Lytic cycles can complete in 20-30 minutes at 37C, body temperature
- Released viruses attack other cells
- Bacteria mutations change receptor sites to prevent recognition
- Bacteria (& other organisms) have restriction enzymes of their own which attack foreign DNA
- Bacteria can chemically alter their own DNA so it is not destroyed by their restriction enzymes

- 14  *Lysogenic Cycle*
- Temperate viruses have two modes of reproductive cycles: lysogenic and lytic cycle
  - Virus binds to surface of host cell
  - Virus injects its DNA into the bacterial host
  - Viral DNA inserts into the bacterial genome at a specific site and becomes a prophage
  - When the viral DNA is excised from the bacterial genome, a lytic cycle will begin
  - While latent, the viral DNA will be copied when bacterium divides, both daughter cells are infected
- 15  *Prophage*
- Most prophage genes are inactive
  - One active prophage gene codes for the production of repressor protein which switches off the other prophage genes
  - A prophage may be carried in the host cell's chromosomes for many generations
- 16  *Activation of Prophage Genes*
- May be spontaneous or caused by environmental factors e.g. radiation, temperature, pH
  - Virions produced during the lytic cycle may begin either a lytic or lysogenic cycle in new host cells
- 17  *Lysogenic Cells*
- These are host cells harboring a temperate virus that could lyse if a lytic cycle begins
  - Some prophage genes in a lysogenic cell may be expressed and change the cell's phenotype in a process called lysogenic conversion
  - Lysogenic conversion occurs in bacteria that cause diphtheria, botulism and scarlet fever. Disease is caused by the toxins coded for by the prophage genes.
- 18  *Replication by viruses w envelopes*
- Membrane covering virus has glycoprotein spikes
  - Enter by receptor-mediated endocytosis.
  - Cell enzymes uncoat the genome
  - Viral enzymes are required to replicate RNA genome and transcribe mRNA (RNA polymerase/transcriptase)
  - Viral glycoproteins are synthesized by ribosomes attached to ER; Golgi vesicles transport to membrane
  - Virions bud from host membrane w glycoproteins
- 19  *Herpes Viruses*
- Double stranded DNA
  - Viral envelope is derived from host nuclear membrane
  - Reproduce within the host cell's nucleus
  - Use both viral and cellular enzymes to replicate and transcribe their genomic DNA
  - Integrate as provirus

20  *mononucleosis*

- Caused by Epstein-Barr virus (EBV), a member of the herpes virus family
- Antibody levels (called "titers") against the EBV virus reveal if there is a current or prior infection from the virus. Also, there is usually an increased number of white blood cells called lymphocytes.
- Most people have been infected w EBV at some point in their lives and have had few symptoms.
- EBV remains dormant or latent in a few cells in the throat and blood for the rest of the person's life.
- When it reactivates, it is usually symptomless.

21  *Herpes simplex*

- Genital herpes is a sexually transmitted disease (STD) caused by the herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2).
- Although the infection can stay in the body indefinitely, the number of outbreaks tends to decrease over a period of years.
- HSV-1 can cause genital herpes, but it more commonly causes infections of the mouth and lips, "fever blisters."
- Herpes can make people more susceptible to HIV infection, and it can make HIV-infected individuals more infectious.
- Lysogenic cells are found in trigeminal & sacral nerve ganglia.

22  *RNA Viruses*

- Classified by the relationship of mRNA to their genome
- mRNA is the plus (+) strand that codes for proteins
- Minus (-) strand is a template for synthesis of a plus strand; it is complementary to the plus strand

23  *Animal RNA viruses: Class III RNA viruses*

- db strand RNA; minus strand template for mRNA
- reo is an acronym for respiratory enteric orphan
- mild infections of the upper respiratory and gastrointestinal tract of humans
- Reovirus selectively kills cells with an activated Ras pathway. Infection of a single cancer cell by a single reovirus particle can generate a thousand progeny. Normal cells are spared.

24  *Animal RNA viruses: Class IV RNA viruses*

- Single plus strand genome; plus can function directly as mRNA but is also template for synthesis of minus RNA
- Viral enzymes are required for RNA synthesis from RNA templates
- Picornavirus (18-30 nm icosahedrals); togavirus (10-12 Kb)
- aseptic meningitis syndrome (the most common acute viral disease of the CNS), colds, febrile illness with rash, aseptic meningitis syndrome (the most common acute viral disease of the CNS), colds, febrile illness with rash, conjunctivitis, muscle infection, heart infection, hepatitis A, poliomyelitis virus are caused by picornavirus

- Togavirus causes Rubella (German measles)
- Flavivirus: Hepatitis C

25  *Animal RNA viruses: Class V RNA viruses*

- Single minus strand; mRNA transcribe directly from genomic RNA
- Rhabdovirus: bullet shaped, rabies
- Paramyxovirus: helical nucleocapsid, measles, mumps, parainfluenza
- Orthomyxovirus: spherical, influenza

26  *Animal RNA viruses: Class VI RNA viruses*

- Single plus strand genome; the plus strand is a template for complementary DNA synthesis
- Reverse transcriptase catalyzes this reverse transcription from RNA to DNA to mRNA
- Retroviruses
- HIV (human immunodeficiency syndrome), tumors

27  *Animal Viruses: Class I*

- Double stranded DNA
- Adenoviruses (respiratory tract),
- Herpes type I, II
- Human papillomaviruses (warts)
- Pox viruses-small pox

28  *Animal Viruses: Class II*

- Single stranded DNA: may encapsulate both plus and minus strands individually or may just be minus strand
- parvoviruses

29  *Animal Viruses: Class VII*

- Partially double stranded DNA and single stranded DNA with longer than genome length ssRNA intermediates
- Envelope is detergent sensitive
- Hepadnaviruses: hepatitis B

30  *Retrovirus*

- RNA virus that uses reverse transcriptase to transcribe DNA from viral RNA genome
- Has the most complicated reproductive cycle
- Following entry and uncoating of viral RNA, reverse transcriptase catalyzes rx to produce minus strand DNA
- New db strand DNA enters chromosomal DNA as a provirus
- Proviral DNA is transcribed into mRNA & translated into proteins

- If oncogenes present, may transform host cell to cancerous cell

31  *Why do you feel bad?*

- How disease symptoms occur is not clear
- Some viruses kill or damage cells; lysosomes may release hydrolytic enzymes
- Virus may be toxic or infected cell may release toxins
- Degree of cell damage depends on tissue type's ability to regenerate-respiratory tract can and nervous tissue can't (poliovirus)
- Fever, aches & inflammation result from immune systems response

32  *Medical Interventions for Viral Infections*

- Vaccines: harmless variants or derivatives of pathogenic microbes that mobilize a host's immune system
- Edward Jenner developed first vaccine for smallpox in 1796
- Vaccines exist for polio, rubella, measles, mumps, and several other viral diseases
- Antiviral drugs are a recent development
- Some are analogs of purine nucleosides that interfere with viral nucleic acid synthesis

33  *Emergent Viruses*

- Emerging viruses arise from existing viruses when
- It evolves and causes disease in individuals who have immunity only to the ancestral virus (influenza virus)
- Spreads from one host species to another (monkeypox spread to humans, bird flu spread to humans)
- Disseminates from a small population to become more widespread (hantavirus, AIDS)

34  *Environmental factors contribute to emergent viruses*

- Traffic on new roads into previously undisturbed areas can spread viruses among previously isolated populations
- Deforestation activities brings humans into contact with animals that may host a virus capable of infecting humans

35  *Some Viruses Cause Cancer*

- Cells infected with tumor viruses may transform to a cancerous state
- For example: retroviruses, papovavirus, adenovirus, and herpes viruses
- Retrovirus → adult leukemia
- Herpesvirus → Epstein-Barr → Burkitt's lymphoma
- Papovirus → human warts → cervical cancer
- Hepatitis B virus → chronic hepatitis → liver cancer

36  *Tumor Viruses are not proviruses*

- They never excise; the insertion into the host cell DNA is permanent

37  *Oncogenes*

- These genes may be found in viral genomes or as a part of the normal eukaryotic genome
- They can trigger transformation of the cell to a cancerous state
- Oncogenes code for cellular growth factors or for proteins involved in the function of growth factors

- Oncogenes are found in the normal cells of many species

38  *Oncogene activation*

- Some tumor viruses transform cells by activating cellular oncogenes
- More than one oncogene must usually be activated to completely transform a cell
- Tumor viruses are effective only in combination with other events such as exposure to carcinogens
- Carcinogens probably act by turning on cellular oncogenes

39  *Plant viruses*

- Serious agricultural pests
- Are RNA viruses with rod-shaped capsids with capsomeres arranged in a spiral
- Horizontal transmission: organism receives the virus from an external source such as another plant, contaminated garden implements, or insect vectors
- Vertical transmission: organism inherits a viral infection from its parent
- Pass through plasmodesmata

40  *viroids*

- Smaller and simpler than viruses
- Naked RNA molecules with only several hundred nucleotides
- Disrupt normal plant metabolism, development and growth by causing errors in regulatory systems that control gene expression
- Affect many commercially important plants such as coconut palms, chrysanthemums, potatoes, and tomatoes
- May be escaped introns; similar to self-splicing introns found in normal eukaryotic genes, including rRNA genes
- Self-direct their own replication and are not diluted in transmission

41  *Prions*

- Misshaped or malformed proteins
- Pathogenic and cause normal proteins to become malformed
- Cause scrapie in sheep and spongy encephalitis-mad cow disease
- Prions trigger chain reactions that increase their numbers and allow them to spread through a host population without dilution

42  *Viruses Living?*

- May have evolved from other mobile genetic elements
- Cannot reproduce independently
- Have a genome w same genetic code as living organisms
- Can mutate and evolve
- May have evolved after the first cells, from fragments of cellular nucleic acid that were mobile genetic elements
- Evidence to support this: genetic material similar to hosts', some viral genes are identical to cellular genes (oncogenes in retroviruses), viruses of eukaryotes are similar in genomic structure to their cellular hosts than to bacterial viruses, viral genomes are similar to cellular genetic elements such as plasmids and transposons-which are called mobile genetic elements

43  *Bacterial genomes*

- Prokaryotic genomes are about 0.01 of a eukaryotic genome
- The main double stranded DNA circular chromosome is called a chromosome
- Some bacteria have extrachromosomal DNA in plasmids which is also a ring
- mutations are rare events but they impact genetic diversity in bacteria because of their rapid reproductive rate
- Mutation is a major source of genetic variation in bacteria but not in more slowly reproducing organisms which have sexual reproduction

44  *3 natural processes of genetic recombination in bacteria*

- Transformation
- Transduction
- conjugation

45  *transformation*

- Bacterial cells assimilate foreign DNA from surroundings
- Some can take up naked DNA
- foreign DNA may be integrated into the bacterial chromosome by recombination
- Progeny carry the new combination of genes
- Many bacteria have surface proteins that recognize and import naked DNA from closely related bacterial species
- Some bacteria may be induced to take up foreign DNA by incubating bacteria in a medium rich in Ca ions or by heat shock (insulin and human growth hormone made this way by bacteria)

46  *transduction*

- Gene transfer from one bacterium to another by a bacteriophage
- Generalized transduction: random pieces of host genome are packaged within a phage capsid during lytic cycle
- When phage particle infects a new host cell, the donor cell DNA recombines w recipient cell DNA
- Specialized transduction: transduction that occurs when a prophage excises from bacterial chromosome and carries w it some host genes adjacent to the excision site (restricted transduction)
- Transduced bacterial genes are restricted to those adjacent to the prophage insertion site in specialized transduction

47  *conjugation*

- Conjugation is the direct transfer of gene between two cells that are temporarily joined
- DNA donating cell extends external appendages called sex pili
- Sex pili attach to a DNA receiving cell
- A cytoplasmic bridge forms through which DNA transfer occurs
- Ability to form sex pili conferred by genes in a plasmid called the F plasmid (fertility plasmid)

48  *plasmids*

- Small double stranded ring of DNA that carries extrachromosomal genes in some bacteria
- Have only a few genes which are not required for survival and reproduction
- May be beneficial in stressful environments
- F plasmid- fertility plasmid
- R plasmid- resistance to antibiotics

49  *Plasmid replication*

- Some plasmids replicate in synchrony w the bacterial chromosome

- Some replicate on their own schedule, so the number of plasmids in the cell at any point in time may vary to as few as 1 to as many as 100
- Some plasmids can reversibly insert into the cell's chromosomes; called episomes

50  *episomes*

- Genetic elements that can replicate either independently as free molecules in the cytoplasm or as integrated parts of the main bacterial chromosome
- Episomes may be plasmids or phage genomes
- Plasmids are usually beneficial and virus genomes are usually harmful to host cell

51  *F plasmid*

- Has about 25 genes
- Bacterial cells which contain the F factor and can donate DNA (male) are called F<sup>+</sup> cells
- F factor replicates in synchrony w chromosomal DNA ; F<sup>+</sup> factor is inheritable
- Cells w/o F factor are designated F<sup>-</sup> (female)

52  *Rolling circle replication of plasmids*

- 5' end of the copy peels off the circular plasmid and is transferred in linear form
- This episome may integrate into bacterial chromosome
- These bacteria cells are called Hfr (high frequency of recombination)
- If conjugation is incomplete, resulting cells are different from both parent cells and produce bacterial colony that genetically differs
- Incomplete conjugation used to map bacterial plasmid chromosomes

53  *R plasmids*

- Some bacterial cells may carry as many as 10 different R plasmids
- During conjugation, some mobilize their own transfer to nonresistant cells
- Increased antibiotic use has selected for antibiotic resistant bacterial strains carrying the R plasmid
- R plasmids can transfer resistance genes to bacteria of different species, including pathogenic strains resulting in resistant strains of pathogens (superbugs)

54  *transposons*

- DNA sequences that can move from one chromosomal site to another
- Occur in both prokaryotic and eukaryotic organisms
- Barbara McClintock deduced their existence in maize in the 1940's (work went unrecognized until 1983 when she rec'd Nobel prize)
- Two patterns of transposition: conservative and replicative

55  *Transposons have no specific target*

- May scatter certain genes throughout the genome with no apparent single, specific site
- All other mechanisms of genetic recombination require homologous strand exchange; meiotic crossing over in eukaryotes and transformation, transduction, and conjugation in prokaryotes
- Insertion of episomes into chromosomes is also site specific, even though it does not require an extensive stretch of DNA homologous to the plasmid

- 56  *Conservative transposition*
- Movement of preexisting genes from one genomic location to another
  - The transposon's genes are not replicated prior to the move so the number of gene copies is conserved
- 57  *Replicative transposition*
- Movement of gene copies from their original site of replication to another location in the genome
  - the transposon's genes are inserted at some new site without being lost from the original site
- 58  *Insertion sequences*
- These are the simplest transposons
  - Contain only the genes necessary for the process of transposition
  - Has two essential types of DNA: sequence coding for transposase and inverted repeats
  - Transposase is the enzyme that catalyzes insertion of transposons into new chromosomal sites
  - Inverted repeats are noncoding sequences of DNA that are repeated in reverse order on opposite ends of a transposon
  - Inverted repeats are recognition sites for transposase
- 59  *Insertion of transposon*
- Transposase binds to the inverted repeats and holds them close together
  - Cuts and reseals DNA required for insertion of the transposon at the new site
  - DNA polymerase will fill in the staggered cuts made by transposase in DNA
  - Filled in areas are called direct repeats-which are characterized by their identical DNA sequences in the same molecule
- 60  *Alteration of the cell's phenotype*
- Due to transposons may cause mutations by interrupting coding sequences for proteins
  - Increase or decrease a protein's production by inserting within regulatory regions that control transcription rates
  - In bacteria, could be a major source of bacterial evolution
  - Insertion sequences cause 1 mutation in every  $10^6$  generations
- 61  *Complex transposons*
- These transposons contain additional genetic information besides that required for transposition – may have one or two genes
  - Can insert into almost any stretch of DNA since their insertion is not dependent upon DNA sequence homology
  - Generate genetic diversity in bacteria by moving genes from chromosome to chromosome or from one species to another
  - Ex. Antibiotic resistance, F factor, R plasmids, DNA version of retrovirus gene
- 62  *Bacterial control of metabolism*
- Regulation of enzyme activity
  - Regulation of gene expression

- 63  *operon*
- Regulated cluster of adjacent structural genes w related functions
  - Has a single promoter region, so RNA polymerase will transcribe all the genes on an all or none basis
  - Transcription produces a single polycistronic mRNA w coding sequences for all enzymes in a metabolic pathway
- 64  *Polycistronic mRNA*
- A large mRNA molecule that is a transcript of many genes
  - Is translated into separate polypeptides
  - Contains stop and start codons for the translation of each polypeptide
- 65  *Polycistronic mRNA*
- A large mRNA molecule that is a transcript of several genes
  - Is translated into separate polypeptides
  - Contains stop and start codons for the translation of each polypeptide
- 66  *Advantages of operons*
- By grouping structural genes the expression of these genes can be coordinated
  - When a cell needs the product of a metabolic pathway, all the necessary enzymes are synthesized at one time
  - The entire operon can be controlled by a single operator
- 67  *operator*
- A DNA segment between an operon's promoter and structural genes which controls access of RNA polymerase to structural genes
  - Acts as an on/off switch for movement of RNA polymerase and transcription of the operon's structural genes
  - The operator is "on" unless switched "off" by a repressor protein
- 68  *Repressor*
- Specific protein that binds to an operator and blocks transcription of the operon
  - Blocks attachment of RNA polymerase to the promoter
  - Like an enzyme, it has an active site with a specific conformation
  - Binds reversibly to DNA
  - May have an allosteric site in addition to DNA binding site
  - Repressors are encoded (DNA code to make repressor) by regulatory genes
- 69  *Regulatory genes*
- Genes that code for repressor or regulators of other genes
  - Often located some distance away from the operons they control
  - Are involved in switching on or off the transcription of structural genes
  - Continually transcribed, activity depends on how efficient their promoters are in binding RNA polymerase

- 70  *How regulatory genes work*
- Transcription of the regulatory gene PRODUCES
  - mRNA TRANSLATED INTO
  - Regulatory protein BINDS TO
  - Operator REPRESSES OR ACTIVATES
  - Transcription of operon's structural genes
- 71  *Regulation of trp operon in E.coli*
- 72  **How does trp activate the repressor protein?**
- Repressor protein normally has low affinity for operator
  - Has DNA binding site plus an allosteric site specific for tryptophan
  - When tryptophan binds to allosteric site, it activates the repressor
  - The activated repressor binds to the operator
  - Trp operon gets switched off
  - Tryptophan is a corepressor
  - Corepressor are usually metabolites
- 73  *Two types of NEGATIVE Gene Regulation*
- Repressible enzymes: enzymes have their synthesis inhibited by a metabolite-tryptophan
  - Inducible enzymes: enzymes which have their synthesis induced by specific metabolites – allolactose
- 74  *Lac operon*
- Lac repressor is innately active, so it is usually attached to the operon preventing transcription
  - Lactose in the cell is cleaved into glucose and galactose by  $\beta$ -galactosidase
  - To make more enzymes, need to switch on lac operon
- 75  *Regulation of lac operon*
- 76  *Lac operon has dual regulation*
- Negative control by the repressor
  - Positive control by CAP which determines rate of transcription
  - Recall that CAP is an activator of many operons that program catabolic pathways
- 77  *CAP regulation of lac operon*