UN2005/UN2401 '18 -- Lecture 18 -- Last Edited 11/13/17. (Problems to do are indicated in **red bold.**)

You need handouts 17A, 17B, & 17C. (Copies will be provided in class. Extra paper copies will be in the boxes on 7th floor of Mudd after the pm class). If you need a pdf, and handouts are not yet posted on the handout list, see handout list of 2017.

Reminder: Selected problems are indicated in **red bold**. We suggest doing these problems as soon as possible to consolidate your understanding of the material covered in each lecture. (However, most people need to look over the relevant section of the notes before trying the problems.) Use these problems to learn from; do not use them to test yourself.
Important Reminder: These are NOT the only problems you should do -- you should do ALL of them. For a complete list of problems corresponding to each lecture, see the problems to do page.

Topics I & II are covered in Notes of Lecture #17, pp. 14-15.

I. How is Bacterial DNA Passed On? Asexual Reproduction (See topic VI of lecture #17)

II. Introduction to Bacteria Sex I. (See topic VII of Lecture #17, parts A & B.)

A. How do bacteria get 'extra' DNA? *Three basic ways.*

1. DNA Transformation -- DNA from one bacterium is released into the medium and taken up by another bacterium.** (This is what happens in nature. In labs, DNA from any source can be added to the medium and taken up by living bacteria.) ** Discovery of this phenomenon led to the conclusion that DNA must be the genetic material. (See lecture #11.)

2. Conjugation -- DNA is passed by cell-cell contact (mating -- forming a bridge). A copy of the DNA, not the original, is passed along. This is a major method of transfer of plasmids (really copies) from one cell to another.

3. Viral Transduction -- DNA is carried by a virus from one host to the next. How?

a. What's a virus? A virus particle contains genetic information inside a protein coat.

b. Viruses are intracellular parasites -- they reproduce only inside living cells.

c. Viruses as agents for DNA transfer: When a DNA virus reproduces inside a cell, it can (accidentally) pick up a piece of bacterial DNA instead of all or part of its own DNA. The piece of host DNA ends up inside the virus's protein coat, and is carried to the next cell that the virus infects.

** Note: DNA transfer from cell to cell in eukaryotes is not usually called 'transformation' because the term 'transformation' is used instead to refer to cancerous transformation -- the transformation of a normal cell into a cancer cell. When speaking of DNA transfer in eukaryotes, the term 'transfection' is usually used instead of the term transformation. (How the DNA is passed from one eukaryotic cell to another will be discussed later in the term. It does not necessarily involve viruses.)

For pictures, see Sadava fig. 12.21 & 12.22 (12.23 & 12.24) or Becker 25-18 to 25-20 (20-18 to 20-20).

Problem 11-1, experiments (1) to (3), gives examples of all three methods of bacterial DNA transfer. (You have to figure out which is which.)

B. Results of Bacterial Sex

1. How much DNA is transferred? -- recipient gets some 'extra' DNA only.

a. Recipient of transferred DNA is not a complete diploid. The recipient cell gets only a few extra genes, either permanently or transiently. The recipient does not get a complete set of genes or chromosomes from each parent. (See 'haploid & diploid' terminology from last time.)

b. What is the 'extra' DNA? Transferred ('extra') DNA can carry new genes or additional copies of genes normally present only once on the chromosome

2. Where is the Extra DNA? Two possibilities for the location of the 'extra genes'

a. Fragments = short linear DNAs with (virtually always) no origin of replication.

b. Plasmids = small circular mini-chromosomes with their own origin of replication.

3. Is the Extra DNA Passed on? Plasmids vs Fragments (See Diagrams on 17A, top)

a. Fragments are <u>not</u> inherited

- Fragments generally do not have an origin of replication, so they are not replicated. (See handout 17A -- "plasmid vs fragment.")
- Fragments are usually degraded by enzymes, so they are not only not replicated -- they are degraded and the nucleotides are recycled.
- Added genes on fragments are passed on to all progeny only if the genes have been integrated into the chromosome. (Details of how integration works below).
- Progeny are haploids.

b. Plasmids <u>are</u> inherited

- Plasmids are generally replicated and passed on to all progeny, like the regular chromosome. (Some descendants may lack plasmids due to inefficient replication, distribution, etc.) **
- Progeny get copies of the chromosome and the added piece (the plasmid).
- Progeny are partial diploids or have added extra genes.

**Note: It is possible to set up conditions so that only bacteria that have inherited a plasmid will grow. In other words, it is possible to select <u>against</u> cells that have not inherited a plasmid. Selection does not <u>cause</u> loss (or retention) of the plasmid, but it prevents cells without plasmids from growing. In the absence of selection, the proportion of the population that has no plasmids will gradually increase.
(See problem 11-2.) How selection works to cause changes in the makeup of a population of bacteria is discussed at length in lecture 18 of 2017, pp 10-11.

C. What Use is the Extra DNA?

1. What use is a partial diploid with extra DNA on a plasmid?

a. The two copies in a partial diploid can be compared. For example, consider the constitutive mutants described last time -- what should happen in a bacterium that has two copies of the lactose operon -- one copy constitutive and the other copy inducible. When you put the two operons together will both be constitutive? Both inducible?

If you haven't done them yet, try problems 12-4 & 12-9 (12-8 in older editions).

b. Plasmids can carry 'new genes'. These are genes that are not on the chromosome. Having these 'extra' genes can change the phenotype of the bacterium. For example, many genes that confer resistance to antibiotics (by coding for proteins that destroy the antibiotics, prevent their uptake, etc.) are found on plasmids, not on the chromosome.

2. What good are fragments?

a. Fragments are not inherited. Plasmids can be replicated and passed on to all descendants (see above) but fragments cannot.

b. The information in a fragment can be passed on -- if there is recombination.

c. What is recombination? Parts of a fragment can be integrated into the DNA of the chromosome and replace the equivalent (homologous) piece. (See bottom of handout 17A.) This process is called "crossing over" or 'recombination.'

d. Results of recombination/crossing over -- it produces a chromosome with a new combination of genes.

e. Terminology: The new chromosome or bacterium with a new combo of genes is called a "recombinant." See Sadava fig. 12.21 (12.23)

3. Details on Recombination.

a. How does recombination work? It requires two things.

- Enzymes to pair up, cut, and rejoin the two DNA's involved.
- Homology between the two DNA's.

b. Why Homology. An extensive description of homology, with examples, is below (see III) for reference.

- DNA's must be homologous (that is, equivalent, but not necessarily identical) in order to pair up, so crossing over can occur.
- The pieces that are exchanged must contain genetic information that is equivalent -- codes for the same proteins, same regulatory sites, etc.

- Why it makes sense: Crossing over between non-homologous genes would scramble the genetic information; crossing over between homologous genes does not, because it exchanges equivalent pieces of information.

c. Why Enzymes. Enzymes are required to help pair, cut, and rejoin DNA's .

- Enzymes/Proteins of recombination must bind to homologous DNA's and align them before cutting and rejoining can occur
- A single cross over or recombination event involves enzymes for cutting of both homologous DNAs and for rejoining crosswise.
- Note that it takes two such cut and rejoin events to switch a section on a fragment for a section on a chromosome.
- Terminology: The rejoining of two DNA ends during crossing over (recombination) is sometimes called 'splicing.' This term, splicing, is now usually reserved for rejoining of two ***RNA*** ends during removal of introns in eukaryotes. (Eukaryotic RNA processing, including splicing, will be explained in detail next term.)

d. When does recombination occur?

(1). In bacteria, # of copies of the DNA is limiting. Enzymes for repair of the DNA are probably always present and can be used to carry out recombination at any time. However, recombination does not normally take place because bacteria are haploid -- there is usually only one copy of the DNA per cell. Recombination only occurs if "extra" DNA is present due to transformation, transduction, etc.

(2). In eukaryotes, the enzymes needed are limiting. The enzymes used for recombination are only present in cells that produce gametes (eggs and sperm) and only at certain times in the life cycle of the cells (during meiosis). Eukaryotic cells are diploid -- they normally have two homologous copies of the DNA, but only cells of the germ line make the enzymes that allow crossing over to occur.

III. Homology Details -- Definitions, Terminology, and Examples

A. Definition: What does homology mean?

- It means very similar but not necessarily the same.
- DNA's that carry the same genes (that code for the same proteins) are called homologous.
- The homologous DNA's carry the same genes in the same order (say for beta-galactosidase, or tryptophan synthetase, etc.) but not necessarily the same ***versions*** of the genes.

- Different versions of the same gene are called **alleles**, as explained in detail below.

- In an evolutionary context, homology implies common origin as well.

B. Examples:

1. Enzyme Differences.

One DNA can carry the information to make (for example) one form of trp synthetase or β-galactosidase and the homologous DNA can carry the information to make a slightly different version of the same enzyme, with, say, a few amino acids different out of a total of several hundred. The two forms of the DNA will be almost, but not exactly the same, and the two forms of the protein will be very similar as well. The two forms of the protein will catalyze the same reaction, but differ in K_m, heat sensitivity, V_{max}, etc. Sometimes the difference in properties is large enough that one form of the enzyme is enzymatically active, and the other is essentially inactive.

2. Protein Differences (for a protein that is not an enzyme).

One DNA can carry the information to make (for example) one form of the beta chain of hemoglobin, say, the beta chain of hemoglobin A (glutamic in position 6), and the homologous DNA can carry the info to make a slightly different version of the beta chain, namely the beta chain of hemoglobin S (valine in position 6). These two alleles or two versions of the gene for the beta chain (β_A and β_S) are homologous. Different versions of the same gene (different alleles) do not code for two different proteins -- the two DNA's code for two different versions of the **same** protein -- two different types of beta chains that differ in only one or two amino acids out of hundreds. Note: bacteria do not make hemoglobin; this example was used because HbA and HbS have been previously discussed.

C. Alleles.

- Different alternative versions of the same gene are known as alleles. Alleles code for variant forms of the same protein, not for different proteins.

- On the diagram at the bottom of 17A, "D" and "d" represent two alleles of the "Dee" gene, "B" and "b" two alleles of the "Bee" gene, and so on.

- D and d could code for two different version of some protein, say the enzyme, β-galactosidase; B and b could code for two different versions of another enzyme, and so on.

- The two forms of the protein coded for by "D" and "d' must be very similar in amino acid sequence.

- The two forms of the protein are usually very similar in function, although sometimes one form is active ('works' well) and the other is not.

- If one version of the enzyme is active and one is not, it is customary to use 'D' for the allele coding for active enzyme and 'd' for the allele coding for inactive enzyme.

- Another example: β_A and β_S are two different alleles of the same eukaryotic gene.

D. How do you detect the presence of new alleles or extra DNA? The usual method is by checking out the phenotype -- some examples on next page.

1. Change in shape or color of colonies For example, conversion of C- (not colored) to C+ (colored). This was the phenotype described earlier when discussing transformation as proof DNA is the genetic material. (In the original experiments, a different phenotype was used.)

2. Change in ability of colonies to grow on a particular medium For example, conversion of trp- (unable to make trp therefore unable to grow on medium w/o trp) to trp+ (able to make trp so able to grow on medium w/o trp) or conversion of drug sensitive (Drug ^{S}) to drug resistant (Drug ^{R}),

3. In cases 1 & 2, what extra DNA was transferred to give the new phenotype? (C- to C+ or trp- to trp+ or Drug ^{S} to Drug ^{R}.) What information did the 'extra' DNA contain? Be sure you can explain this in terms of genes, alleles, enzymes, & pathways.

IV. Complementation and Recombination -- how to analyze the results of having "extra" DNA. (See handout 17B)

A. The Setup for Bacteria.

1. The physical setup: You need two copies of the genes you want to test. A normal bacterial cell is haploid -- it has one copy of each gene or stretch of DNA. Therefore you need a partial diploid with some "extra" DNA.

a. Only a few genes will be diploid. The partial diploid will have one copy of most genes (on the chromosome) but have two copies of a few genes. For these few genes, there will be one copy of the gene(s) on the chromosome and one copy on the "extra" DNA.

b. Where did the extra DNA come from? The partial diploid could have extra DNA as a result of genetic engineering, conjugation, transformation, etc. How the extra DNA is picked up will be discussed in more detail below.

c. What is the "extra" piece? It could be a plasmid or a fragment.

d. How long will the extra piece last? The partial diploidy could be a permanent state (if the extra piece is on a plasmid or integrated into the chromosome) or a temporary state (if the extra piece is a fragment).

2. The question(s): Now suppose each copy of the DNA that is diploid (present in two copies) has a mutation, so it is unable to carry out the function we are talking about. In other words, neither DNA alone has correct, working genetic information to do some function. (That is, neither DNA can code for the proteins and/or RNA's needed to carry out some function). Therefore a cell with either copy of the DNA has a particular (mutant) phenotype.

a. Will function be restored? Will the cell with the two different copies be able to carry out the function we are talking about? Will the cell with both copies have a normal phenotype or a mutant one?

b. How will you know if function is restored? The usual method is by checking out the phenotype – such as ability to form colonies on a particular medium or change in shape of color of colonies, etc. Do you have normal phenotype?

c. Why bother with this? If function is (or isn't) restored, what does it mean? The results can help you answer the Q: Where are the 2 mutations? Are the two mutations in the same place? In the same gene? How will we know?

d. Possibilities: See handout 17B for 4 possible cases, A to D. In A and C there is only one gene to consider (or the number of genes is irrelevant); in cases B and D there are two genes to consider.

B. How could you Restore Function?

1. By Recombination.

a. How recombination works: If crossing over can occur between the two DNA's, then you can regenerate a DNA that has no mutations by cutting and rejoining the two DNA's. This will work as long as the two mutations are in different places on the DNA (non-overlapping) as in cases A, B and D. It doesn't matter if the two mutations are in the same gene or not -- as long as they are non-overlapping, crossing over can produce a normal recombinant with no mutations.

b. When do you need recombination (as vs complementation) to restore function?

- Recombination is usually the only way to restore function (long term) if you have one mutation on the chromosome and one on a fragment.
- The crossing over must generate a non-mutant chromosome. (It doesn't help to have a non-mutant fragment, as the fragment will be lost if it isn't integrated.)
- Note that it may take more than one cut and rejoin event to generate a recombinant chromosome.

c. Frequency.

- If the 2 mutations are close to each other on the DNA, crossing over **between them** will be rare. (More on the frequency of crossing over in a lecture or two.)
- However, it is usually possible to select & detect even very rare recombinants by setting up conditions where only the recombinant will grow.
- Note that complementation does not depend on a rare event.

2. By Complementation.

a. How it works (to restore function): If both DNA's can remain in the cell, and each one has a mutation in a different functional unit (case B) then the cell with the two mutant DNA's should be able to function normally. In other words, if each DNA has a mutation in a different gene, then the two DNA's between them have at least one good copy of each gene, can make all necessary RNA's and peptides, and can do the job that needs to be done. In this case, the two mutant DNA's are said to complement each other. (The top left gene "covers" for the bottom left one, which is mutant, and the bottom right gene covers for the top right, which is mutant.)

b. When it doesn't work: If two DNA's are present, but both DNAs have mutations in the same gene (not necessarily in the same place), as in case D, then complementation will not restore function. The cell has two defective copies (and no good copies) of one gene and the corresponding job won't get done -- phenotype will be mutant.

c. Frequency: A rare event is not required here -- as long as both DNA's are present, and have complementary defects, function will be restored.

d. How long does complementation last? As long as both copies of the DNA remain in the cell. If there is no recombination (only complementation), then both DNA's (chromosomal and plasmid) remain defective and function will be maintained only as long as both DNA's remain. (Phenotype is normal, but both genotypes are defective.) Function/normal phenotype will be lost if either DNA is lost.

- With a plasmid: Complementation in bacteria usually occurs between a mutation on the chromosome and a mutation on a plasmid because both DNA's can remain indefinitely -- the plasmid and the chromosome can be replicated and transmitted to the progeny.

- With a fragment: ***Transient*** complementation can occur between a mutation on the chromosome and a mutation on a fragment of added DNA. Complementation with a fragment works for the recipient cell, but the added genes aren't transmitted to the progeny, because the fragment is degraded and/or not replicated. (Transient complementations are not usually done with bacteria, but are common in non-growing eukaryotic cells in laboratories.) Q: Will transient complementation in bacteria generate colonies with a normal phenotype?

3. Implications -- What do you learn from distinguishing complementation & recombination?

For what you learn, and more details about how to do it, see chart on handout 17B, and try problem 11-5. Wait on the problems involving viruses, and how you transfer the 'extra' DNA, until you have covered the material below.

4. Terminology. This is for reference. It will not be covered in class, but is helpful in understanding complementation.

a. Cistron. The term "gene" is used in more than one way. The term "cistron" is used (as a more specific term than gene) to mean a stretch of DNA that codes for a single component of function (one component = one polypeptide, or one tRNA, etc.) The term 'cistron' is derived from the way complementation tests were originally done -- the method was called a "cis/trans" test, and therefore mutations that didn't complement were said to lie in the same 'cistron' or unit of function.

All the mutations that do NOT complement each other are assigned to the same "complementation group" (see d below) and must be located in the same cistron. If two mutations complement each other, as in case B, they are said to lie in different cistrons.

b. Genotype and Phenotype. Two mutants may have the same appearance and/or function (say, both be his- or unable to make his) and have different mutations in their DNA. Therefore you have to distinguish between genotype (state of the DNA) and phenotype (state of function and/or appearance). The tests described here allow you to get information about the genotypes of 2 mutants with the same phenotype (say his- for bacteria, or failure to form plaques in the case of viruses). Do the mutants have defects in the same gene? If so, are the defects in the same place in the DNA? See the problems for examples.

c. Complementation. The term "complementation" is usually used to refer to restoration of function when two separate mutant copies of the DNA are present in a single cell. (This is how it is used in all the problems.) However, the term is sometimes used to refer to a slightly different situation in which function is restored by adding additional (normal) DNA to a cell with a mutation. In these cases, various different pieces of DNA are added, to see which one(s) "complement" or restore function. Only pieces of added DNA with good copies of the mutant gene will "cover for" or "complement" the mutation. This sort of experiment is used to identify a piece of DNA carrying a normal copy of a gene.

d. Complementation Groups. Mutants with the same phenotype are often assigned to "complementation groups." All mutants that do not complement each other are assigned to the same group. All the mutants in one complementation group must have mutations in the same gene, and usually have a defect in the same polypeptide. This allows you to figure out how many genes/polypeptides it takes to carry out a particular function. Suppose you have a lot of mutants with a particular phenotype -- all are defective in the same general function, but not necessarily in the same step. (For example, you have many his^- mutants -- all are unable to synthesize histidine.) You assign the mutants to complementation groups, and from the number of groups you can figure out how many genes/polypeptides it takes to carry out the function (ability to synthesize his).

Genetic complementation is not covered in Sadava or Becker. See the end of this lecture for links to text book explanations of complementation. Alternatively, try Google.

V. How DNA is Transferred from one Bacterium to Another -- A closer Look at all 3 methods

A. DNA Transformation (also called Transfection, especially in eukaryotes) – this section will not be discussed in class, but is included for Reference.

1. Historical Significance. Explained much earlier in course (lecture 10) that transformation occurs; transformation by DNA was one of the first lines of evidence that DNA is the genetic material. How does DNA from a one cell convert or transform another cell, say from trp- into trp+ or colorless into pigmented? (fig. 13-1 & 13-2.)

2. Basic Process -- See Becker 25-18a (20-18a). For transfection (Eukaryotes) see Sadava fig. 13. 5 (10th ed).

a. Release of DNA: One cell (the donor) dies and releases its chromosomal DNA (which is broken into linear fragments). The DNA is released and broken either naturally or by the scientist doing the experiment. (But see d.)

b. Recombination/Integration: Another cell (the recipient) takes up the released DNA from the medium, so the recipient has a complete chromosome plus a fragment. (See handout 17A bottom -- "integration of fragment.")

c. Detection: How is integration (successful transformation) detected? By a change in phenotype of the recipient. In example on 17A, donor DNA carries the B allele and original recipient had the b allele. If transformation occurs, recipient will be converted from b to B. For transformation to be detected, change in genotype from b to B must cause some change in phenotype that can be measured -- for example, it must confer the ability to grow under new conditions, or the ability to form different shaped (or colored) colonies, and/or the ability to cause disease, etc.

d. Transformation with plasmids: Note that transformation (as described above) involves the uptake of linear fragments of DNA as vs uptake of a plasmid. This is probably what usually happens in nature, leading to transfer of linear fragments from one cell to another. However, in lab experiments, cells can take up plasmids which have been constructed by the experimenters. Whenever DNA is added to cells, this is known as transformation (or transfection in eukaryotes). More on this when we get to genetic engineering.

To review transformation, see problem 11-1.

B. Conjugation -- a kind of mating between two types of bacteria. See Sadava fig. 12.21 & 12.22 (12.23 & 12.24) or Becker figs. 25-19 & 25-20 (20-19 & 20-20).

1. Cell to cell contact is required. Conjugation, unlike transformation, requires cell-cell contact and the DNA (a copy, not the original, see 4 below) is passed across a bridge that forms temporarily between the pair of mating cells.

2. What allows mating?

a. Genes Involved: A special set of genes is required for copying and transferring of DNA during conjugation.

(1). These genes are different than the ones involved in ordinary DNA replication.

(2). DNA replication during conjugation is unidirectional, not bidirectional.

b. F+. A cell with the genes for conjugation on a plasmid is called an F+, and the plasmid is called an F factor.

c. FYI only: Hfr. In some strains, the plasmid has crossed over with the chromosome, making one big circle. See Becker fig. 25-20B (20-20 b) and 6 below. When the F factor becomes part of the chromosome, the cells with an integrated F factor are called Hfr.

(1). Mechanism: How an Hfr transfers DNA during conjugation will not be discussed in class.

(2). Significance: Conjugation with Hfr strains is of great historical interest -- it allowed mapping of the bacterial chromosome and led to the discovery that the bacterial chromosome is circular.

(3). For students in 2501 lab: You will probably do a conjugation experiment with an Hfr strain. (See problem 11-9 and the note below.)

Note: The results of a typical mating experiment are presented in problem 11-9. (This problem is included for those doing interrupted conjugation experiments in bio lab.) Remember that in an Hfr, copying of the chromosome starts in the middle of the integrated F, and goes in one direction. The chromosomal gene that is copied (and transferred) first, is determined by where the F factor is integrated into the chromosome. The order of copying and transfer depends on the orientation of the F in the chromosome. (The F can be inserted "facing" either way. In terms of the rubber tubing model, the arrow in the middle of the F can point clockwise or counterclockwise around the big circle.)

3. Transfer is in one direction only. Donor is called F+ (or Hfr); recipient is called F-. DNA is passed from donor to recipient. {Q&A}. Note that transfer is always from F+ (or Hfr) to F-, never the other way around or from F+ to F+, F- to F- etc. For pictures see Becker fig. 25-19 (20-19) or Sadava 12.21 (A) [12.23 (A)].

4. What is transferred?

a. Copy of DNA, not original, is transferred. If the only copy of a DNA is transferred, the information is lost. So a copy is transferred, not the original.

(1). F+ to F-: Recipient gets a complete circular plasmid (a copy of the donor's plasmid). Recipient becomes F+. Note: In this course we will consider transfer of plasmids from F+ to F-. We will ignore transfer of chromosomal genes by Hfr strains, a phenomenon with is largely of historical interest. See texts if you are interested.

(2). FYI: Hfr to F-. Recipient gets a fragment (a copy of part of the donor's chromosome). Recipient almost never gets all the genes necessary for conjugation and remains F-. See Becker fig. 25-20 (20-20).

b. Significance

(1). Genes for antibiotic resistant are usually on plasmids, and copies of plasmids are relatively easily transferred from one bacterium to another.

(3). Spread of drug resistance. Transfer of plasmids (carrying genes for antibiotic resistance) has contributed in a major way to the spread of drug resistance among bacteria, especially in areas such as hospitals where antibiotics are wide spread (so there is strong selection for growth of bacteria containing drug resistance genes).

5. FYI: How do plasmids pick up genes, such as those that code for drug resistance? Probably by crossing over with the chromosome.

a. Mechanism: A single cut and rejoin event between two circles (such as the bacterial chromosome and a plasmid) generates one big circle. This type of recombination does occur, joining the two circles. The process can be reversed, regenerating the two individual circles. If mistakes are made during the "reverse" cut and "un-join" event, some of the DNA that used to be on the chromosome will end up on the plasmid (or vice versa).

b. Significance: It is thought that this process (of joining and then un-joining the two circles) is what transfers genes (such as the ones for drug resistance) from the chromosome to the plasmid. Conjugation can then transfer a copy of the plasmid (with the added genes), passing the added genes from bacterium to bacterium.

To review plasmids, do problem 11-2.

To compare transformation and conjugation, try problem 11-3. An additional problem involving transformation is 11-15, parts A & B.

C. Transduction

How do viruses facilitate bacterial sex? When a DNA virus reproduces inside a cell, it can (accidentally) pick up a piece of bacterial DNA instead of all or part of its own DNA. The piece of host DNA ends up inside the virus's protein coat, and is carried to the next cell that the virus infects. When a virus acts as an unwitting agent of bacterial DNA transfer in this way, it is called transduction. For evolutionary background on viruses (FYI) see Sadava section 25.4 (26.4). For pictures of various virus particles, see Becker fig. 4-27 (4-26) or Sadava fig 25.22 (26.23).For transduction see Becker fig. 25-18B (20-18b). For how transduction fits into the viral lifecycle, see below.

VI. Viral Life Cycle & Viral Genetics

A. Transduction & the (Lytic) Viral Life Cycle: For viral life cycle, see handout 17C. For details, see Sadava 16-11 to 16-13 (16.12 to 16.14). For transduction see Becker fig. 25-18B (20-18b). The major points to notice about the viral life cycle are as follows:

1. Structure & Inertness --

- Viruses have one type of nucleic acid (either DNA ***or*** RNA, single or double stranded) that serves as genetic information.
- The nucleic acid is enclosed in a protein shell (or head).
- Viruses have genetic information but no means to express it -- no ribosomes, no way to generate ATP.
- Examples: Flu, enteroviruses (polio, D68), TMV (a plant virus), and Ebola are RNA viruses; the viruses that cause Herpes and chicken pox are DNA viruses.

2. Host Specificity depends on shell protein -- Virus attachment to host cell surface requires match between complementary structures on viral surface protein and cell surface; which cells a virus will attack is largely determined by these interactions.

Note: Virions (virus particles) come in many shapes. For pictures, see Sadava fig. 25.22 (26.23) or Becker fig. 4-27 (4-26). For TMV, see Becker fig. 2-19 (2-20). In viruses that have both a 'head' and a 'tail,' like the model virus shown in class (or in Sadava fig. 13.3), it is usually the tail, not the head, of the virion that binds to the cell surface. (See also pictures in Sadava ch. 16.)

3. How Virus takes Over. See Sadava fig. 16.11 (16.13) for how a bacteriophage (bacterial virus) takes over; figs. 16.13 (16.16) for cycle of a more complex human virus (HIV).

a. What enters the cell?

(1). Nucleic Acid. In all viruses, viral nucleic acid enters the host cell.

(2). Any viral Protein? In some cases, some viral proteins (made in the previous cycle of infection) may also enter the cell to assist in transcription & translation of viral genetic material.

b. What happens inside the infected cell?

(1). Use of host materials: The viral nucleic acid uses (primarily or exclusively) enzymes (and the ATP) of the host to assist in transcription and translation of its own genetic information.

(2). Use of Viral materials: The viral genetic info directs synthesis of materials (mostly proteins) to favor its own reproduction at expense of the host's reproduction.

(3). Additional Viral Proteins: Some, but not all, viruses make proteins that break up the DNA of the host, or that help lyse (break open) the host cell, etc.

4. Assembly Line Reproduction. Viruses generally reproduce in an assembly line manner -- they build up supplies of all parts (nucleic acid, & structural proteins) and then assemble them as a last step. They don't double in size and then divide in half the way cells do. This method of self assembly means that recombinant viruses can be assembled in a test tube from separate sources of proteins + nucleic acid (modified in a lab).

5. Lytic Cycle & Plaques.

a. Virus reproduction is a cycle. One virus particle infects a cell, and the virus reproduces inside the cell. Many viruses lyse (break open) and release the progeny virus particles, killing their host cells; others do not kill the host cell but cause it to shed virus particles. In either case, the infected cell releases progeny virus particles that infect neighboring cells, and so on.

b. Lysis. When the host cells lyse (break open) and release progeny virus particles, the resulting cycle is called the lytic cycle. If the viruses are growing on top of a solid layer of bacteria ("a lawn"), the result is a plaque -- a hole in the lawn that is full of progeny viruses.

- A single bacterium on a Petri dish forms a colony (a lump).
- A single bacterial virus landing on a lawn of bacteria forms a plaque (hole).
- All the organisms in a colony or a plaque = a clone.

c. Discovery of 'Phage'. Bacterial viruses were first discovered by their ability to form plaques. They were named "bacteriophage" because they were "eating" holes in the lawn of bacteria. (Note: the term bacteriophage -- or phage for short -- is used only for bacterial viruses. Viruses that infect other organisms are called viruses**, not** phages.)

6. Transduction --Because of the assembly line method of viral reproduction, pieces of bacterial nucleic acid can sometimes accidentally get packaged inside a viral coat. Such a virus is a 'phony phage' -- it is really a virus coat containing bacterial, not viral DNA. If such a (phony) virus particle infects another host, it delivers the bacterial DNA to the second host and does not destroy the recipient cell. This is a case of transduction -- the virus has acted as an unwitting agent of bacterial DNA transfer. (Sadava 8th ed. fig 13.13 (b))

To review the lytic cycle, see problem 11-4.

B. Lysogenic Cycle See 17C, Sadava fig. 16.12 (16.14) or Becker fig. 16-3 esp. (d) [Box 18A, esp. fig. 18A-4].

1. Integration. Some viruses can become part of the host chromosome by crossing over between the viral DNA and the bacterial DNA -- the process is parallel to the way a plasmid such as the F factor joins the bacterial chromosome. (Viruses, like plasmids, can pick up bacterial genes by the reverse of this process.)

2. Lysogeny. The integrated virus can remain dormant for long periods of time. This dormant state is known as lysogeny (in bacteria) , and a bacterium with an integrated, dormant, virus is said to be lysogenic (capable of entering the lytic cycle).

3. Mechanism: What keeps the virus from making viral proteins and entering the lytic cycle? A repressor protein made by the virus itself.

- This repressor protein is not allosteric; it must be destroyed to be inactivated. The degradation of repressor protein allows the virus to leave the dormant state and enter the lytic cycle.

- Jacob, Lwoff, & Monod received the Nobel Prize in Physiology in 1965 for figuring out how repressors control both operons and lysogeny.

C. Retroviruses.

1. Need for Reverse Transcriptase:

a. Genetic Material in the virus particle (virion) is RNA.

b. RNA is copied into DNA in the host.

(1). Function of Reverse Transcriptase -- When the RNA enters the cell, it uses a special enzyme made by the virus, reverse transcriptase, to make a DNA copy of the RNA.

(2). Where does reverse transcriptase come from? The reverse transcriptase is carried into the host inside the viral particle. It was made in the *previous* host cell.

c. The DNA then inserts into the host chromosome and remains dormant, in much the same way as a lysogenic virus.

2. Significance of Reverse transcriptase: Reverse transcriptase obtained from retroviruses is used in the lab as an important tool to make DNA copies of RNA. (Examples will be discussed next time.) For HIV life cycle see Sadava fig. 16.13 (16.16) or Becker fig. 18-4 (Box 21-A). The 1975 Nobel Prize in Physiology was awarded to Dulbecco, Temin & Baltimore in 1975 for the discovery of reverse transcriptase in tumor-causing viruses.

3. An Important Retrovirus -- HIV, the virus that causes AIDS, is a human retrovirus.

4. Not all RNA viruses are retroviruses -- some RNA viruses replicate without a DNA intermediate (for example, Ebola).

D. Viral Crosses -- Complementation & Recombination can occur with viruses as well as with bacteria. If a cell is simultaneously infected with two variants (mutants) of the same virus, then crossing over and/or complementation can occur between the two viruses during the course of infection. See above and handout 17B for more details on complementation (& how to distinguish complementation and recombination).

For an example of crosses with viruses, see problem 11-8. (For more problems involving complementation & recombination in viruses, see 11-10 to 11-13.)

For text book descriptions of how complementation works, see one of these sources from the PubMed Bookshelf:

From Suzuki: http://www.ncbi.nlm.nih.gov/books/NBK21989/

From Alberts: http://www.ncbi.nlm.nih.gov/books/NBK26818/#A1625

From Lodish: http://www.ncbi.nlm.nih.gov/books/NBK21735/#A1905

See Becker fig. 25-17 (20-17) for recombination in viruses. (Re-assortment may also occur in the case of flu virus, which has an RNA genome segmented into 8 pieces. See CDC page for more details.

Next Time: Anything above we don't get to, and then restriction enzymes & genetic engineering. Blots, Probes, & how you make a recombinant plasmid and find a cell carrying it.