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Handouts: 16 A -- Plasmid vs Fragment & Integration of Fragment.
16 B -- Complementation

I. Wrap up of Prokaryotic Regulation (Operons)

A. Operons in general -- common features (1-3 were discussed in detail last time)

1. Co-ordinate Control. Genes coding for proteins of related function are controlled together -- the level of synthesis of the corresponding proteins is coordinated. All the (structural) genes involved are clustered -- the genes are next to each other on the DNA and are controlled by a single promoter; therefore transcription → single polycistronic mRNA.

2. Transcriptional Control. The level of protein synthesis is controlled by controlling the level of transcription of the gene coding for the protein. The production of mRNA is the only step that is regulated. There is no direct control of translation -- no control of use or degradation of mRNA.

3. The 2-Part Switch. There's a switch (controlling transcription) with two parts -- A DNA sequence or site (the operator) and an allosteric protein (repressor) to bind to the site.

4. Negative Control. Regulatory system is "negative" -- meaning a protein (repressor) must function properly to turn transcription of the system **off**. If the repressor protein is missing or does not work, transcription is stuck in the "on" position. Doesn't matter if operon is inducible or repressible -- same thing happens!

B. Induction vs. Repression

1. What are the characteristics of an Operon (either type)?

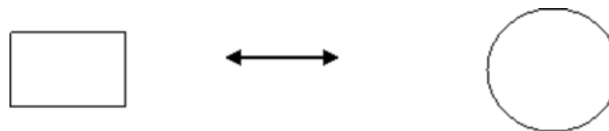
a. Repressor protein binds to operator and shuts off transcription of operon

b. Repressor protein has two forms. (See Becker fig. 23-5)

"Rectangle form" of rep. protein = "sticky" form that binds to O

"Circle form" = form that doesn't bind to O

Rectangle ↔ Circle



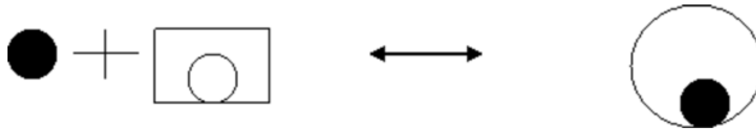
c. Effector molecule (inducer or co-repressor) binds to one form of repressor protein and shifts above equilibrium to right or to left.

2. What are the characteristics of an Inducible Operon? (See Becker fig. 23-4 or Sadava fig. 16.11 (13.19).

a. Overall: Effector molecule (inducer) that binds to repressor protein **prevents** repressor from binding to operator -- decreases supply of rectangles by converting them to circles.

b. **Empty** form of repressor protein (without effector) = rectangle; sticks to operator

c. Rectangle form (empty) + effector ↔ Circle form (full)



d. Effector (Inducer) shifts reaction above to **right**.

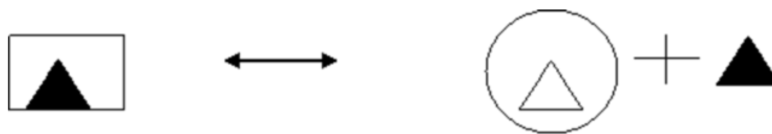
e. On handout, inducer = ●

3. What are the characteristics of a Repressible Operon? (See Becker fig. 23-6 or Sadava 13.20 in 8th ed)

a. Overall: Effector molecule (co-repressor) that binds to repressor protein **promotes** repressor binding to operator -- increases supply of rectangles by converting circles to rectangles.

b. Full form of repressor protein (effector-protein complex) = rectangle; sticks to operator.

c. Rectangle form (full) ↔ Circle form (empty) + effector



d. Effector (co-repressor) shifts reaction above to **left**.

e. On handout, co-repressor = ▲

f. To compare to induction, can also write the reaction:

Circle form (empty) + effector ↔ rectangle form (full)

Effector shifts equilibrium of reaction (written as in f) to the **right.**

For an animation of repression (the trp operon) go to http://highered.mcgraw-hill.com/olc/dl/120080/bio26.swf

4. How do repressible and inducible operons compare? See 2 & 3 above, and problems below.

It may help to make a table for yourself comparing induction & repression. Some questions to consider:
(1) Which form, empty or full, sticks to the DNA?
(2) When protein is made, is it 'sticky'?
(3) How (in which direction) does the effector shift the equilibrium between sticky and non-sticky forms?

5. Reminder: The repressor protein of each operon is unique, and binds only to its respective operator (& effector). It is important to remember that not all rectangles (or circles) are the same. Each unique repressor protein is allosteric and has 2 forms -- "sticky" and "nonsticky." For comparison, on the handouts, all "sticky" forms are drawn as rectangles and all "nonsticky" forms are drawn as circles. However, each repressor protein is different. The only thing all "rectangles" have in common is that they all stick to their respective operators.

To review how operons work, do problems 12-0, 12-1 and 12-2 A-B. To compare repression and induction, do 12-2 C, and 12-7. To review the differences between repression and feedback inhibition, try problem 12-2, esp. part D, and 12R-4.

C. Regulation in general (FYI). This will not be discussed in detail in class but is included here as a summary and to help you get the big picture. It will be discussed in detail next term when we get to regulation of eukaryotic protein synthesis.

1. All models of regulation are based on knowledge of operons. Why? Because operons were the first systems of regulation of protein synthesis to be understood.

2. Features of operons to consider -- see above

a. Transcriptional Control.

b. The 2-Part Switch.

c. Negative Control.

d. Co-ordinate Control.

3. Are these features universal? Does Regulation of protein synthesis always work the same way? Is what is true of *E. coli* true of the elephant? (Monod, one of the originators of the operon model, liked to think so.)

a. Transcriptional control is common. It is the primary way, but not the only way, to regulate protein synthesis. In eukaryotes, other steps are often controlled as well.

b. Two part switches, consisting of a protein and DNA site are very, very common. The situation is often more complex than the one described above, especially in eukaryotes. For example: Either half of the switch can be made of RNA. Often there are multiple sites and/or multiple regulatory proteins (which can interact with each other as well as with DNA) that can affect transcription of a particular gene. Details will be discussed next term.

c. Negative control is not universal. Negative control is very common in prokaryotes; positive control (where a protein is needed to turn ON a gene) is more common in multicellular eukaryotes.

d. Co-ordinate control is common, but the mechanism is different in different organisms. Genes of related function are generally clustered in prokaryotes, and share a common "switch (P, O etc.)." Genes that code for multiple enzymes of the same pathway are generally NOT clustered in eukaryotes. Since each gene in the set is located in a different place, each gene has its own "switch." (But all the switches are tripped coordinately.) So there is generally no poly-cistronic mRNA in multicellular eukaryotes.

II. How is bacterial DNA passed on? Asexual Reproduction

A. Introduction to cell division -- How does 1 cell make 2?

1. How do you double cell contents? Consider the central dogma -- we've covered it all -- how to double DNA, RNA and protein, and how to regulate protein synthesis. Once you double the protein (enzymes), that allows doubling of everything else, like carbos, lipids, etc. So suppose you double everything in the cell. How do you get 2 cells from 1?

2. Why distribution of DNA is the critical issue -- Making two cells from one comes down to "once the program is doubled, how are the two copies distributed to daughter cells?" Stuff that is not part of the program (not part of the genetic material) need not be divided exactly, but because of the chicken and egg problem, there must be **some** of the other material in each daughter cell. (Need some ribosomes, RNA polymerase etc. in each cell. But as long as you have some, and the genetic material, you can always make more ribosomes, enzymes etc.)

B. How do prokaryotes do it? binary fission -- regular segregation of circular chromosome attached to membrane

1. What does the DNA (genetic information) of a bacterium look like? Each bacterium has one, circular, double stranded DNA molecule = chromosome; the chromosome is attached to the cell membrane.

2. How the Chromosomal DNA is distributed.

a. To start, you have one cell with one double stranded DNA circle attached to membrane.

b. DNA replicates by birectional DNA replication (two forks start from a single origin) → two double stranded circles, both attached to membrane. (See Becker fig. 19-4 (19-5))

c. Circles grow apart as membrane is laid down between the attachment points of DNA to membrane → two circles pushed to opposite ends of cell. (There is also an active process, other than growth of membrane, that pushes the two origins of DNA replication apart. This has only been recently discovered.)

d. To end, you need only to lay down a membrane (and wall) between the two halves of cell, each containing one circle (= complete double stranded chromosome). This → 2 complete cells.

e. Note this is not mitosis OR meiosis; it is a different process (binary fission). Mitosis and meiosis occur only in eukaryotes; they will be discussed later.

f. How will the genetic material in the two daughter cells compare? If there are no mutations it will be the same, and all descendants will be identical. All the descendants produced in this way (by asexual reproduction of a single founder) are called a clone. (Doesn't matter if "founder" is a cell, molecule, or organism.) Is there any way (besides mutation) to get new combinations of genes? To mix genes from separate clones? That requires bacterial sex.

III. Introduction to Bacterial Sex & Recombination

A. What is the biological definition of sex? Any method for exchanging genes and/or passing DNA around from organism to organism. Mutation produces variants; sexual reproduction (re)shuffles them and produces new combinations.

B. How do bacteria get 'extra' DNA? *Three basic ways to be explained in greater below:*

- *Transformation* -- DNA released from one bacterium is taken up by another.
- *Conjugation* -- DNA is passed by cell-cell contact (mating -- forming a bridge)
- *Transduction* -- DNA is carried by a virus from one host to the next.

For pictures, see Sadava fig. 12.26 & 12.27 (13.12 to 13.14) or Becker 20-18 to 20-20 (20-19 to 20-21).

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

C. Haploid & Diploid -- Terminology Review

1. Haploid = A cell (or organism) with one copy of each chromosome. Therefore one copy of each gene. Example: bacteria.

2. Diploid = A cell (or organism) with two copies of each chromosome (usually one copy from each parent). Therefore 2 copies of each gene. Examples: mammals, higher plants.

3. Partial Diploid = A cell (or organism) that is basically haploid, but has two copies of a few genes. This can happen in nature, or as a result of lab manipulations. (See B above.) How are extra copies passed on? See below.

D. 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 above.)

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

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

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

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

3. Is the Extra DNA Passed on? Plasmids vs Fragments (For a diagram, see handout 16A.)

a. Plasmids are inherited -- Progeny get copies of the chromosome **and** the added piece (the plasmid). Therefore progeny are partial diploids or have added extra genes. 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.; this is discussed in detail below.)

b. Fragments are not inherited -- Added genes on fragments are only passed on to all progeny if they have been integrated into the chromosome (details below). Therefore progeny are haploids.

IV. Fates of Transferred DNA -- Details for Plasmids vs Fragments

A. What Good are Plasmids?

1. Importance of Origins. Plasmids have origins of DNA replication. Therefore they are replicated and passed on to almost all descendants. (Fragments of DNA, on the other hand, are generally not replicated, and are lost and/or degraded fairly rapidly as explained below.)

2. Plasmids can carry extra copies of chromosomal genes.

a. A partial diploid has two copies of a DNA section -- an "extra" copy on the plasmid and the original copy on the chromosome.

b. The two copies in a partial diploid can be compared. The two copies do not have to be exactly the same -- one can be normal and one mutant, or they can both be different mutants. For example, suppose a bacterium has two copies of the lactose operon. Suppose one copy is constitutive and the other is inducible, or suppose both are constitutive. What should happen 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).

3. 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.

4. Why plasmids tend to get lost. Control of duplication and distribution of plasmids may not be as tight as for chromosomes, and/or the consequences of loss are not as serious. (Consider: What happens to a cell that gets no chromosome? No plasmid?) Because of errors in replication and/or distribution of plasmids, a descendant sometimes does not get a copy of the plasmid. As the bacteria grow, the number of bacteria without plasmids gradually increases. Loss is a very rare event, but plasmids tend to get lost over ***long*** periods of time (many generations) -- UNLESS there is selection against loss of the plasmid.

5. Details of Selection.

a. Basic Idea. Selection means there is an advantage (to the bacterium) of retaining the plasmid (*or* a disadvantage to losing it) so that bacteria with the plasmid tend to survive and bacteria without the plasmid tend to die off. The selective conditions (such as presence of an antibiotic, absence of an amino acid, etc.) **do not** affect the chance that an individual cell will **lose** a plasmid, but the selective conditions **do** affect the chance of **survival** of a bacterium that has lost its plasmid.

b. An example. Consider resistance to antibiotics. How does the environment select for resistance, or what is 'most fit?'

(1). What happens if antibiotic is present? When bacteria (carrying the resistance genes on a

plasmid) are grown in the presence of an antibiotic, there is selection *against* bacteria that are not resistant because they have lost their plasmids. The bacteria without plasmids die and only the bacteria that have retained their plasmids grow. So virtually all the bacteria in the culture have plasmids, generation after generation. Selection for bacteria carrying drug resistant plasmids has accidentally been carried out (very efficiently) in hospitals.

(2). What if there is no antibiotic present? If there is no antibiotic around, there is no selection against cells that have lost their plasmid (they will continue to grow) and the bacteria in the culture will gradually lose their plasmids. There is now no selection ***against*** the bacteria that lose their plasmids. There may even be selection ***for*** loss of plasmids -- in absence of drug, cells without plasmids may grow faster.

(3). What is most fit? This is not absolute -- it depends on the environment -- whether antibiotic is present or not.

To review plasmids, do problem 11-2.

B. Recombination -- What happens to fragments of DNA that are transferred/passed around?

1. The Question -- What good are fragments? Plasmids can be replicated and passed on to all descendants (see above) but what happens to fragments?

a. Replicated? Fragments generally do not have an origin of replication, so they are not replicated. (See handout 16A -- "plasmid vs fragment.")

b. Degraded? In addition, linear fragments are generally degraded by enzymes, so they are not only not replicated -- they are degraded and the nucleotides are recycled. So what good are fragments?

2. The Answer -- Recombination. Parts of a fragment can be integrated into the DNA of the chromosome and replace the equivalent (homologous) piece. (See handout 16A.) This process is called "crossing over" or "genetic recombination" -- it produces a chromosome with a new combination of genes. The new chromosome or bacterium is thus called a "recombinant." See Sadava fig. 12.26 (13.12)

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. Homology. DNA's must be homologous in order to pair up so crossing over can occur.

(1). 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.

(2). Examples:

(a). 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.

(b). Hemoglobin differences. Consider the gene (section of the DNA) that codes for the beta chain of hemoglobin. One version of the gene (β_A) carries the information to make the beta chain of hemoglobin A (glutamic in position 6) while another version of the same gene (β_S) carries the information to make the beta chain of hemoglobin S (valine in position 6). These two versions of the gene (β_A and β_S) are homologous. They 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.)

(3). 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.
- For example, β_A and β_S are two different alleles of the same gene.
- On the diagram at the bottom of 16A, "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 enzyme, say, β-galactosidase; B and b could code for two different versions of another enzyme, and so on.
- The two forms of the enzyme coded for by "D" and "d' must be very similar in amino acid sequence; they may or may not be very similar in function. (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. More on this when we get to eukaryotic genetics.)

(4). Why is homology required?

- 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.
- How it works: Proteins of recombination must bind to homologous DNA's and align them before cutting and rejoining can occur.

c. Enzymes. Enzymes that help pair, cut, and rejoin DNA's are required for recombination. Note that it takes two cut and rejoin events to switch a section on the fragment for a section on the chromosome.

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.

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

A.Transformation (also called Transfection, especially in eukaryotes)

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 (say a PS+ -- see lecture 10) convert or transform another cell, say from PS- into PS+?

2. Basic Process -- See Sadava fig. 13. 13 (a) in 8th ed. or Becker 20-18a [20-19a]

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.

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 16A bottom -- "integration of fragment.")

Note that transformation (as shown here) involves the transfer of linear fragments of DNA as vs transfer of a plasmid. Plasmids can be transferred by transformation in lab experiments, but probably aren't often transferred this way in nature.

c. Detection: How is integration (successful transformation) detected? By a change in

phenotype of the recipient. In example on 16A, 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 colonies and/or the ability to cause disease (as for PS- to PS+) etc.

To review transformation, see problem 11-1.

B. Conjugation -- a kind of mating between two types of bacteria. See Sadava fig. 12.26 & 12.27 (13.12 & 13.14) or Becker fig. 20-19 & 20-20 (20-20 & 20-21).

1. Cell to cell contact is required. Conjugation, unlike transformation, requires cell-cell contact and the DNA (a copy -- see 3 below) is passed across a bridge that forms temporarily between the pair of mating cells.

2. 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. 20-19 (20-20) or Sadava 12.26 (a) (13.11).

3. Copy of DNA, not original, is transferred. If the one and only copy of the DNA is transferred, that could be suicidal. So a copy is transferred, either a copy of a plasmid or a copy of part of the chromosome. Therefore recipient can get a fragment (from a copy of part of the donor's chromosome) or a plasmid. See Becker fig. 20-20 (20-21).

4. What's the difference between an F+ and an Hfr?

a. F+. The genes required for transfer are usually on a plasmid. A cell with that plasmid is called an F+, and the plasmid is called an F factor.

b. Hfr. In some strains, the plasmid has crossed over with the chromosome, making one big circle. See Becker fig. 20-20 b (20-21b) and 6 below. When the F factor becomes part of the chromosome, the cells with an integrated F factor are called Hfr.

5. What is transferred?

a. F+ vs HFr: An F+ cell can transfer copies of the plasmid. An Hfr cell can transfer copies of parts of the chromosome. See Becker 20-20 (20-21). .

b. Frequency: Copies of plasmids are transferred more often than copies of parts of the chromosome, so genes on plasmids are relatively easily transferred from one bacterium to another. This 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).

6. How do plasmids pick up genes, such as those that code for drug resistance? Probably by crossing over with the chromosome. 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). It is thought that this process (of joining and then un-joining the two circles) is what transfers genes 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 as in (3) above.

The results of a typical mating experiment are presented in problem 11-9. 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.)

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

C. Transduction & the Viral Life Cycle: How do viruses facilitate bacterial sex? This will be discussed in detail next time (& handout will be provided). For the viral life cycle, see Sadava 16.4 (13.3). For transduction see Becker fig. 20-18b (20-19b). Here is the short version:

1. What is a virus? A virus particle contains genetic information inside a protein coat.

2. How do viruses reproduce? Viruses are intracellular parasites -- they reproduce only inside living cells.

3. How do viruses transfer bacterial DNA? When a virus reproduces inside a cell, it can (accidentally) pick up a piece of bacterial DNA and package it inside its protein coat. The virus can then carry the bacterial fragment to the next cell that the virus infects.

4. What is transduction? When a virus acts as an unwitting agent of bacterial DNA transfer; the virus transfers a fragment of bacterial DNA from one cell to another. (Sadava 13.13 (b) [8th ed only])

VI. Complementation and Recombination -- the consequences of having "extra" DNA.

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. This could occur as a result of genetic engineering, conjugation, transformation, etc., and the "extra" piece could be a plasmid or a fragment. (So the partial diploidy could be a permanent or a temporary state.) The partial diploid has one copy of most genes (on the chromosome) but has two copies of a few genes. For these few genes, there is one copy of the gene(s) on the chromosome and one copy on the extra DNA.

2. The question: Now suppose each copy of the DNA that is diploid (present in two copies) has a mutation, so 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 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. Are the two mutations in the same place? In the same gene?

See handout 16B 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 it 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: 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 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.)

For more details on how to tell complementation and recombination apart, see chart on handout 16B. For problems, try 11-5, 11-6 & 11-11. Wait on the problems involving viruses until next time.

3. Terminology. This may 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 one 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).

Next time: Wrap up of viral and bacterial genetics; then on to restriction enzymes and genetic engineering.

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