S1.In *E. coli*, the gene *bioD* encodes an enzyme involved in biotin synthesis, and *galK* encodes an enzyme involved in galactose utilization. An *E. coli* strain that contained wild-type versions of both genes was infected with P1, and then a P1 lysate was obtained. This lysate was used to transduce a strain that was *bioD*$^-$ and *galK*$^-$. The cells were plated on media containing galactose as the sole carbon source for growth to select for transduction of the *galK* gene. These plates also were supplemented with biotin. The colonies were then restreaked on plates that lacked biotin to see if the *bioD* gene had been cotransduced. The following results were obtained:

Selected Gene	Non-selected Gene Frequency	Number of Colonies That Grew On: Galactose + Biotin	Galactose – Biotin	Cotrans-duction
galK	*bioD*	80	10	0.125

How far apart are these two genes?

Answer: We can use the cotransduction frequency to calculate the distance between the two genes (in minutes) using the equation:

$$\text{Cotransduction frequency} = (1 - d/2)^3$$

$$0.125 = (1 - d/2)^3$$

$$1 - d/2 = \sqrt[3]{0.125}$$

$$1 - d/2 = 0.5$$

$$d/2 = 1 - 0.5$$

$$d = 1.0 \text{ minute}$$

The two genes are approximately 1 minute apart on the *E. coli* chromosome.

S2. By conducting mating experiments between a single *Hfr* strain and a recipient strain, Wollman and Jacob mapped the order of many bacterial genes. Throughout the course of their studies, they identified several different *Hfr* strains in which the F factor DNA had been integrated at different places along the bacterial chromosome. A sample of their experimental results is shown in the following table:

Hfr **Bacterial Genes**		**Order of Transfer of Several Different**								
strain	**Origin**	**First**								**Last**
H	O	*thr*	*leu*	*azi*	*ton*	*pro*	*lac*	*gal*	*str*	*met*
1	O	*leu*	*thr*	*met*	*str*	*gal*	*lac*	*pro*	*ton*	*azi*
2	O	*pro*	*ton*	*azi*	*leu*	*thr*	*met*	*str*	*gal*	*lac*
3	O	*lac*	*pro*	*ton*	*azi*	*leu*	*thr*	*met*	*str*	*gal*
4	O	*met*	*str*	*gal*	*lac*	*pro*	*ton*	*azi*	*leu*	*thr*
5	O	*met*	*thr*	*leu*	*azi*	*ton*	*pro*	*lac*	*gal*	*str*
6	O	*met*	*thr*	*leu*	*azi*	*ton*	*pro*	*lac*	*gal*	*str*
7	O	*ton*	*azi*	*leu*	*thr*	*met*	*str*	*gal*	*lac*	*pro*

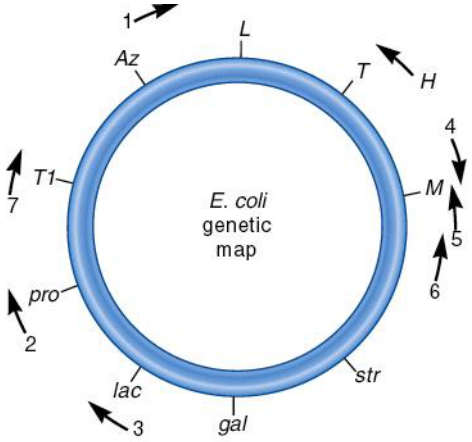
A. Explain how these results are consistent with the idea that the bacterial chromosome is circular.

B. Draw a map that shows the order of genes and the locations of the origins of transfer among these different *Hfr* strains.

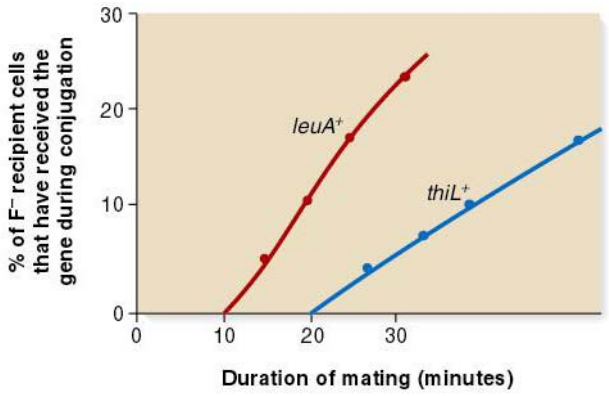
Answer:

A. In comparing the data among different *Hfr* strains, the order of the nine genes was always the same or the reverse of the same order. For example, *HfrH* and *Hfr1* have the same order of genes but are reversed relative to each other. In addition, the *Hfr* strains showed an overlapping pattern of transfer with regard to the origin. For example, *Hfr1* and *Hfr2* had the same order of genes, but *Hfr1* began with *leu* and ended with *azi* while *Hfr2* began with *pro* and ended with *lac.* From these findings, Wollman and Jacob concluded that the segment of DNA that was the origin of transfer had been inserted at different points within a circular *E. coli* chromosome in different *Hfr* strains. They also concluded that the origin can be inserted in either orientation, so that the direction of gene transfer can be clockwise or counterclockwise around the circular bacterial chromosome.

B. A genetic map that is consistent with these results is shown here.



S3. An *Hfr* strain that is *leuA*$^+$ and *thiL*$^+$ was mated to a strain that is *leuA*$^-$ and *thiL*$^-$. In the data points shown here, the mating was interrupted and the percentage of recombinants for each gene was determined by streaking on plates that lacked either leucine or thiamine. The results are shown.



What is the map distance (in minutes) between these two genes?

Answer: This problem is solved by extrapolating the data points to the x-axis to determine the time of entry. For *leuA*$^+$, they extrapolate back to 10 minutes. For *thiL*$^+$, they extrapolate back to 20 minutes. Therefore, the distance between the two genes is approximately 10 minutes.

S4. Genetic transfer via transformation can also be used to map genes along the bacterial chromosome. In this approach, fragments of chromosomal DNA are isolated from one bacterial strain and used to transform another strain. The experimenter examines the transformed bacteria to see if they have incorporated two or more different genes. For example, the DNA may be isolated from a donor *E. coli* bacterium that has functional copies of the *araB* and *leuD* genes. Let's call these genes *araB*$^+$ and *leuD*$^+$ to indicate that the genes are functional. These two genes are required for arabinose metabolism and leucine synthesis, respectively. To map the distance between these two genes via transformation, a recipient bacterium would be used that is *araB*$^-$ and *leuD*$^-$. Following transformation, the recipient bacterium may become *araB*$^+$ and *leuD*$^+$. This phenomenon is called cotransformation because two genes from the donor bacterium have been transferred to the recipient via transformation. In this type of experiment, the recipient cell is exposed to a fairly low concentration of donor DNA, making it unlikely that the recipient bacterium will take up more than one fragment of DNA. Therefore, under these conditions, cotransformation is likely only when two genes are fairly close together and are found on one fragment of DNA.

In a cotransformation experiment, a researcher has isolated DNA from an *araB*$^+$ and *leuD*$^+$ donor strain. This DNA was transformed into a recipient strain that was *araB*$^-$ and *leuD*$^-$. Following transformation, the cells were plated on media containing arabinose and leucine. On these plates, only bacteria that are *araB*$^+$ can grow. The bacteria can be either *leuD*$^+$ or *leuD*$^-$ because leucine is provided in the plates. Colonies, which grew on these plates, were then restreaked on plates that contained arabinose but lacked leucine. Only *araB*$^+$ and *leuD*$^+$ cells could grow on these second-ary plates. Following this protocol, a researcher obtained the following results:

Number of colonies growing on arabinose plus leucine plates: 57

Number of colonies that grew when restreaked on arabinose plates without leucine: 42

What is the map distance between these two genes? Note: This problem can be solved using the strategy of a cotransduction experiment except that the researcher must determine the average size of DNA fragments that are taken up by the bacterial cells.
This would correspond to the value of *L* in a cotransduction experiment.

Answer: As mentioned, the basic principle of gene mapping via cotransformation is identical to the method of gene mapping via cotransduction described in chapter 6. One way to calculate the map distance is to use the same equation that we used for cotransduction data, except that we substitute cotransformation frequency for cotransduction frequency.

$$\text{Cotransformation frequency} = (1 - d/L)^3$$

(Note: Cotransformation is not quite as accurate as cotransduction because the sizes of chromosomal pieces tend to vary significantly from experiment to experiment, so that the value of *L* is not quite as reliable. Nevertheless, cotransformation has been used extensively to map the order and distance between closely linked genes along the bacterial chromosome.)

The researcher needs to experimentally determine the value of *L* by running the DNA on a gel and estimating the average size of the DNA fragments. Let's assume that they are about 2% of the bacterial chromosome, which, for *E. coli*, would be about 80,000 base pairs in length. So *L* equals 2 minutes, which is the same as 2%.

$$\text{Cotransformation frequency} = (1 - d/L)^3$$

$$42/57 = (1 - d/2)^3$$

$$d = 0.2 \text{ minutes}$$

The distance between *araB* and *leuD* is approximately 0.2 minutes.

S5. In our discussion of transduction via P1 or P22, the life cycle of the bacteriophage sometimes resulted in the packaging of many different pieces of the bacterial chromosome. For other bacteriophages, however, transduction may only involve the transfer of a few specific genes from the donor cell to the recipient. This phenomenon is known as specialized transduction. The key event that causes specialized transduction to occur is that the lysogenic phase of the phage life cycle involves the integration of the viral DNA at a single specific site within the bacterial chromosome. The transduction of particular bacterial genes involves an abnormal excision of the phage DNA from this site within the chromosome that would carry adjacent bacterial genes. For example, a bacteriophage called lambda (l) that infects *E. coli* specifically integrates between two genes designated gal^+ and bio^+ (required for galactose utilization and biotin synthesis, respectively). Either of these genes could be packaged into the phage if an abnormal excision event occurred. How would specialized transduction be different from generalized transduction?

Answer: Generalized transduction can involve the transfer of any bacterial gene, while specialized transduction can transfer only genes that are adjacent to the site where the phage integrates. As mentioned, a bacteriophage that infects *E. coli* cells, known as lambda (l), provides a well-studied example of specialized transduction. In the case of phage lambda, the lysogenic life cycle results in the integration of the phage DNA at a site that is called the attachment site. This is described in chapter 17. The attachment site is located between two bacterial genes, gal^+ and bio^+. An *E. coli* strain that is lysogenic for phage lambda will have the lambda DNA integrated between these two bacterial genes. On occasion, the phage may enter the lytic cycle and excise its DNA from the bacterial chromosome. When this occurs normally, the phage excises its entire viral DNA from the bacterial chromosome. The excised phage DNA is then replicated and becomes packaged into newly made phages. However, an abnormal excision does occur at a low rate (i.e., about one in a million). In this abnormal event, the phage DNA is excised in such a way that an adjacent bacterial gene is included and some of the phage DNA is not included in the final product. For example, the abnormal excision may yield a fragment of DNA that includes the gal^+ gene and some of the lambda DNA but is missing part of the lambda DNA. If this DNA fragment is packaged into a virus, it is called a defective phage because it is missing some of the phage DNA. If it carries the gal^+ gene, it is designated *ldgal* (the letter *d* designates a defective phage). Alternatively, an abnormal excision may carry the bio^+ gene. This phage is designated *ldbio.* Defective lambda phages can then transduce the gal^+ or bio^+ genes to other *E. coli* cells.