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The Genetics of Bacteria and Their Viruses

Key Concepts

- Bacteria take advantage of several mechanisms by which DNA sequences can move from one DNA molecule to another, from one cell to another, or even from one bacterial species to another; these mechanisms have led to the evolution of multiple antibiotic-resistant bacteria.
- Some bacteria are capable of DNA transfer and genetic recombination.
- In *E. coli*, the F (fertility) plasmid can mobilize the chromosome for transfer to another cell in the process of conjugation.
- Some types of bacteriophages can incorporate bacterial genes and transfer them into new host cells in the process of transduction.
- DNA molecules from related bacteriophages that are present in the same host cell can undergo genetic recombination.
- Some bacteriophages are able to integrate their DNA into that of the host cell, where it replicates along with the host DNA and is transmitted to progeny cells.

Key Terms

1. site-specific recombinase
2. mobile DNA
3. conjugative plasmid
4. cointegrate
5. R plasmid
6. Hfr cells
7. counterselected marker
8. minimal medium
9. auxotroph
10. frequency of cotransduction
11. plaque
12. phage repressor

Concepts in Action

7.1. The new cassette is immediately adjacent to the promoter.

7.2. For a mean of 1 phage per cell, the expected proportion of uninfected cells is given by the Poisson distribution as $e^{-1} = 0.368$. If you are not familiar with the Poisson distribution, you can work out the answer as follows. The probability that a particular bacteria is infected by a particular phage is 1×10^{-6}; hence, the probability of a particular bacteria escaping a particular phage is 0.999999. The probability of a particular bacteria escaping all 1,000,000 phages is therefore $(0.999999)^{1,000,000} = 0.368$, which equals the expected proportion of uninfected cells.

7.3. Rolling-circle replication is the type of replication, and it originates at a site within the F factor.

7.4. Yes, as long as it has a replication origin.

7.5. The original titer was $36 \times 100 \times 100 \times 100 \times 100 \times 10 \times 10 = 36 \times 10^{10} = 3.6 \times 10^{11}$ viable phage per mL.

7.6. One wants 50 cells in 0.1 mL, so the concentration in the final dilution should be 5×10^2 viable cells per mL. Dilution of the original 5×10^7 to a final 5×10^2 would require two dilutions of 100-fold each and one dilution of tenfold.

7.7. In the undiluted suspension, the titer was $42 \times 100 \times 100 \times 10 \times 10 \times 10 = 42 \times 10^7 = 4.2 \times 10^8$ viable cells/mL.

7.8. *E. coli* has a genome size of 4.6×10^6 bp and there are 100 minutes in the map; hence, the length of one minute in the map is 4.6×10^4 bp = 46 kb. The genetic length of a lambda prophage is therefore approximately one minute.

7.9. The selected marker is originally present in the Hfr parent and is necessary for recombinant F progeny to survive. The counterselected marker is presenting the F parent and prevents survival of the Hfr parent. Without selection and counterselection, the small number of recombinant cells could not be identified among the large number of Hfr and nonrecombinant F parental cells.

7.10. Apparently *h* and *tet* are closely linked, so recombinants that contain the h^+ allele of the Hfr tend also to contain the *tet-s* allele of the Hfr, and these recombinants are eliminated by the counterselection for *tet-r*.

7.11. All receive the a^+ allele, but whether a particular b^+ *str-r* recombinant contains a^+ depends on the positions of the genetic exchanges.

7.12. The attachment site, *att*, must be between genes *f* and *g*.

7.13. Of the four genes, *b* and *c* must be farthest apart because they are the only pair that is not cotransduced. Gene *a* is closer to *b* than gene *d* is to *b* (29% vs. 1% cotransduction). Gene *d* is closer to *c* than gene *a* is to *c* (50% vs. 2% cotransduction). The order of the genes is therefore *b—a—d—c*.

7.14. The three possible orders are: (1) *pur-pro-his*; (2) *pu-his-pro*; and (3) *pro-pur-his*. The predictions of the three orders are as follows: (1) Virtually all pur^+ his^- transductants should be pro^-, but this is not true. (2) Virtually all pur^+ pro^- transductants should be his^- but this is not true. (3) Some pur^+ pro^- transductants will be his^- and some pur^+ his^- transductants will be pro^- (depending on the locations of the exchanges). Therefore, order (3) is the only one that is not contradicted by the data.

7.15. The principle to be applied is that, under ideal circumstances, a mutation in a gene coding for any enzyme in a metabolic pathway will be able to grow on medium supplemented with an intermediate that comes *after* the step catalyzed by the enzyme, but will be unable to grow on medium supplemented with an intermediate that comes *prior* to that step. Hence, the data in the table indicate the following pathway:

$$D \xrightarrow{a1} B \xrightarrow{a2} C \xrightarrow{a3} E \xrightarrow{a4} A$$
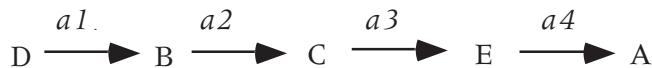
7.16. Any phage transduces one small, contiguous piece of DNA. Cotransduction therefore indicates very close linkage. Hence, *G* and *H* are close, and *G* and *I* are close, but *H* and *I* are not close (because they do not cotransduce), so the order of these three genes must be *I G H* (or the reverse). The location of the other three genes can be deduced similarly: *A* is close to *H*, *B* is close to *I*, and *T* must be close to *A* but not close to *H*. Hence, the gene order is *B I G H A T* or, equivalently, *T A H G I B*.

7.17. **(a)** The *cos* sites that allow circularization of the phage are at the ends, and recombination takes place at the *att* site. Hence, the prophage gene order is *gal —att int xis N CI O P Q S R A B C D E att —bio*. **(b)** In the mutant phage, the attachment site must be inverted relative to the ends of the phage chromosome. **(c)** The superinfecting phage can enter into either the left or the right *att* site, yielding either the arrangement *gal —att int xis . . . D E Z —att int xis . . . D E att —bio* or the arrangement *gal— att int xis . . . D E—att int xis . . . D E Z att—bio*.

7.18. The map implied by the time-of-entry data is below.
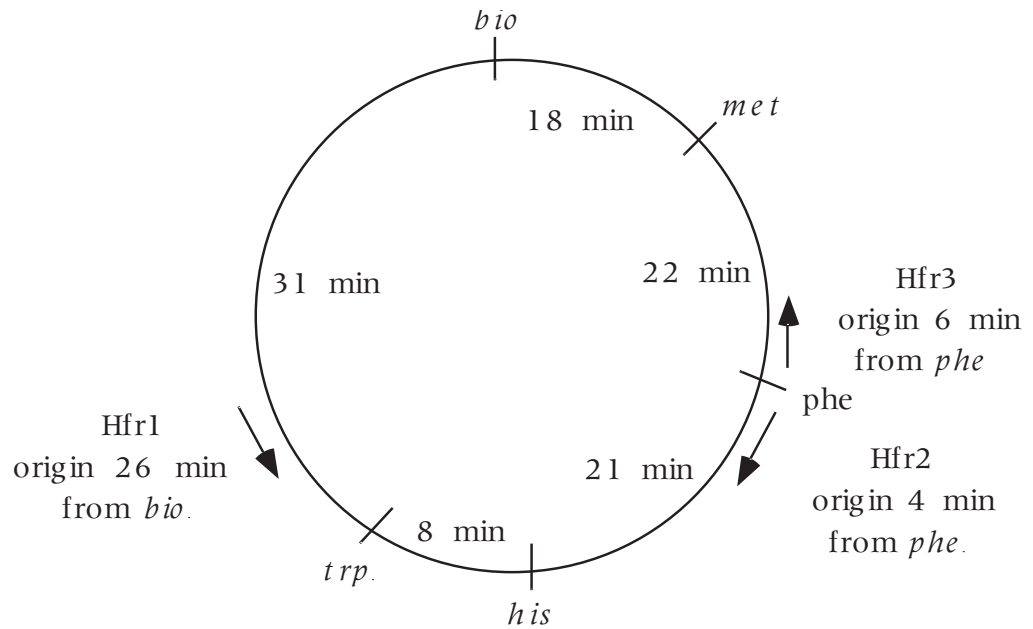
The entries that correspond to the question marks should be, for *his* in Hfr1, 87 minutes; for *phe* in Hfr2, 4 minutes; at 35 minutes in Hfr3, *trp*; and for *bio* in Hfr3, 66 minutes.

7.19. **(a)** The genetic maps are the same but the distances differ. The map order in both cases is *pro–met–arg–ile*. In the *E. coli* Hfr, the distances between adjacent markers are 16, 2, and 6 minutes, respectively, whereas in the *S. enterica* Hfr these are 25, 4, and 14 minutes, respectively. **(b)** Of these four markers, the *E. coli* Hfr transfers *pro* first and *ile* last, whereas the *S. enterica* Hfr transfers *ile* first and *pro* last. **(c)** The *S. enterica* Hfr transfers DNA about half as fast as the *E. coli* Hfr.

7.20. The times of entry correspond to the x-intercepts in the accompanying graph. For the genes *a, b, c,* and *d* the times of entry are 10, 15, 20, and 30 minutes, respectively.
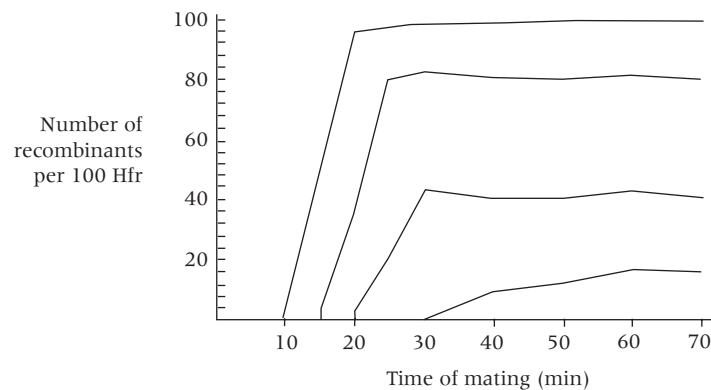
Study Questions

7.S1. You are given a suspension of bacteria and told that it contains 4×10^6 viable cells per mL. How many tenfold serial dilutions would you carry out so that 0.1 mL of the final dilution would contain approximately 40 viable cells?

A) 1

B) 2

C) 3

D) 4

E) 5

7.S2. A temperate bacteriophage has gene order DEFattABC. What is the gene order in the prophage?

A) ABCattDEF

B) attABCDEatt

C) attABCattDEatt

D) attABCDE

E) attABCDEFatt

7.S3. In the specialized transduction of a strain lac⁻pro⁻ of *E. coli* using bacteriophage l from a lac⁺pro⁺ lysogen, what medium would select for lac⁺ transductants without selecting for pro⁺?

A) Minimal medium + lactose + proline

B) Minimal medium + lactose

C) Minimal medium + proline

D) Minimal medium

7.S4. Which of the following statements are true regarding temperate bacteriophages?

A) They may transmit host DNA to other cells.

B) Integration forms a bacterial strain called a lysogen.

C) They can be induced to enter a lytic cycle.

D) They have terminal cohesive ends to form circles.

E) Phage capsids enter the recipient during transduction.

7.S5. In *E. coli*, the F (fertility) plasmid can mobilize the chromosome for transfer to another cell in the process of _____.

7.S6. In the process of transduction, a bacterial DNA fragment is transferred from one bacterial cell to another by a _____ containing the bacterial DNA.

7.S7. Cotransformation of two genes at a frequency substantially greater than the product of the single-gene transformations implies that the two genes are _____ in the bacterial chromosome.

7.S8. Like many transposable elements in eukaryotes, insertion sequences possess inverted-repeat sequences at their termini, which are used by the _____ for recognizing and mobilizing IS elements.

7.S9. In a cross between Hfr met + kan-s × F⁻ met⁻ kan-r, what medium should the mating pairs be plated to select for met + kan-r recombinants? Which are the selected and counterselected markers? Which strain is a prototrophic for methionine production and which is auxotrophic?