Chapter 44

BACTERIAL GENETICS: SEXUAL REPRODUCTION

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PRE-LECTURE ASSIGNMENT

1. Quickly review notes for the previous lecture.
2. Suggested readings:
   a. General genetics textbooks
      Altenburg: Chap. 22, pp. 377-385, 392-393.
      Sinnott, Dunn, and Dobzhansky: Chap. 23, pp. 315-318.
      Snyder and David: Chap. 26, pp. 407-409.
      Winchester: Chap. 23, pp. 326-327.
   b. Additional references
      Jacob, F., and Wollman, E. L. 1958. Genetic and physical determination of chromosomal segments in Escherichia coli. Sympos. Soc. exper. Biol., 12: 75-92.
      Lederberg, J. 1947. Gene recombination and linked segregations in Escherichia coli. Genetics, 32: 505-525. Reprinted in "Papers in microbial genetics", selected by J. Lederberg. 1951. Madison: University of Wisconsin Press.
      Lederberg, J. 1956. Conjugal pairing in Escherichia coli. J. Bact., 71: 497-498.
      Lederberg, J. 1959. Bacterial reproduction. Harvey Lect., 53: 69-82.
      Wollman, E. L., and Jacob, F. 1956. Sexuality in bacteria. Scient. Amer., 195: 109-118.

LECTURE NOTES

A. Genetic recombination in bacteria
   1. Genetic analysis based only upon mutations in asexually reproducing lines is severely limited (see also Chap. 6).
   2. The infrequency of sexual processes shows their detection under the microscope is ordinarily improbable.
   3. The search for sexuality is greatly expedited by the selective isolation of specific genotypes, using mutants for different nutritional defects.
      a. The wild-type Escherichia coli strain K-12 is nutritionally sufficient, i.e., is a prototroph (M^+ T^+) (see central part of Fig. 44-1).
      b. Two nutritionally-dependent mutants, or auxotrophs, were obtained -- one requiring the amino acid methionine (M^- T^+), and the other requiring the amino acid threonine (M^+ T^-).
      c. Neither auxotroph can produce colonies when plated separately on a basal, minimal culture medium containing neither amino acid.
      d. If the two mutant strains are mixed and genetic recombination produces M^+ T^+ prototrophs, these will be the only cells forming colonies when plated on agar containing the minimal medium.
      e. Such evidence for genetic recombination was first obtained by Lederberg and Tatum (1946).
   4. The test for prototrophs derived from different auxotrophs is very efficient for detecting genetic recombination.

B. The fertilization process
   1. Early experiments used mating type F^+, and gave a very low frequency of recombination.
   2. Later, other strains were found giving very high frequencies of recombination, hence called Hfr strains, which were useful in learning the details of fertilization.
   3. As demonstrated, a ♂ (Hfr) cell is seen under the microscope to form a conjugation bridge with a ♀ (F^-) cell once these make a random contact (see also left side of Fig. 44-1).

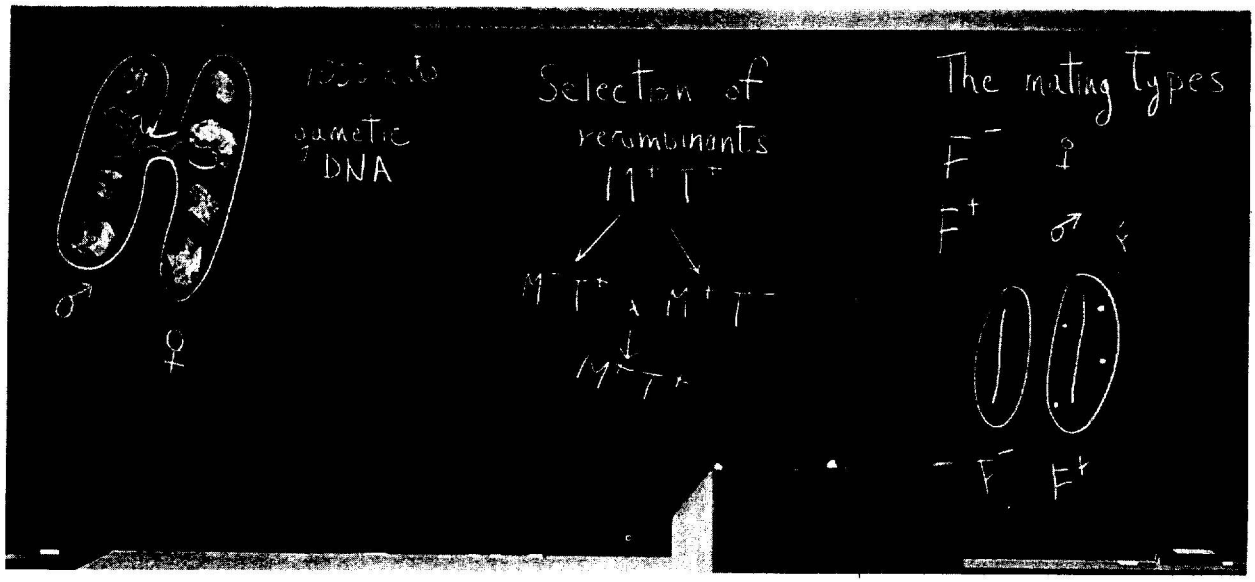

Figure 44-1

4. When Wollman and Jacob used a Waring blender to separate the cells at different times after mating started, they found
   a. bacteria so separated were viable.
   b. there was a progressive linear transfer of genetic markers from ♂ to ♀.
      1) This transfer required 100 minutes for completion. (The total DNA length transferred is about 22μ, or 5 to 10 times the bacteria's length.)
      2) The DNA to be transferred must have unwound.
      3) One end of the DNA string was preferentially transferred first.
   c. Our most detailed map of bacterial genes was made this way.
5. The rate of transfer is 1,000 nucleotide units, or n'its, per second (6×10^6 n'its in 100 minutes).
6. The genetic unit (cistron) specifying a typical protein is about 2,000 n'its.
   a. Levinthal and Garen studied a variety of mutants defective for alkaline phosphatase, using procedures of this sort.
   b. They found it required about 2,000 n'its to code this enzyme, which contains about 400 amino acids.
   c. Such evidence supports a 4:1 (n'it:amino acid) coding ratio (see Chap. 41).
7. The injected ♂ DNA synapses with that in the ♀.
8. Recombination between the ♂ and ♀ DNA occurs, producing recombinant strands with markers from both parents. The mechanism for this is not known, and could result from
   a. breakage and cross-unions between parental strands.
   b. a copy-choice mechanism, in which the daughter DNA alternates in using maternal and paternal DNA as a template.

C. Markers for genetic recombination
   1. Usually 6 to 12 markers are studied in a single cross.
   2. The top center plate in Fig. 43-1 shows, using two markers, how recombinants typically are detected (see also Chap. 43 F2a).
      a. A loop of streptomycin was brushed vertically on the agar, then four clones were streaked horizontally across this region.
      b. Suppose one parental type (top colony in Fig. 43-1 top center plate) was lactose-negative (light colored) and streptomycin-sensitive (interrupted streak).
      c. Suppose the other parental type (third colony down on this plate) was lactose-positive (dark colored) streptomycin-resistant (uninterrupted streak).
      d. The other two colonies would be recombinants -- lactose-positive streptomycin-sensitive (bottom one), and lactose-negative streptomycin-resistant (colony next to top).
   3. Traits of mutants may involve their nutrition, bacteriophages, specific antigens, motility, etc.

D. Basis for sexual differentiation
   1. Male sexuality is an infectious phenomenon

a. One F^+ (♂) cell can rapidly convert all F^- (♀) cells in a culture to F^+.
b. The new F^+ cells transmit this trait to their progeny.
c. The infective factor must multiply at least twice as fast as the typical cell.
d. This factor, called F, is extra-chromosomal and not isolable as a cell-free virus particle (see right side of Fig. 44-1, where the chromosome is diagrammed as a single line).

2. Properties of the F particle
a. It is transferred from ♂ to ♀ in a transient mating.
b. Such matings are more unstable but more frequent than matings which involve chromosome transfer.
c. The dye, acridine orange, inhibits reproduction of F but has no apparent effect on chromosomal genes. Treatment with this dye results in converting F^+ to F^- cells.

E. Mating types
1. F^+ and F^- types have been described already.
2. The F particle
a. must modify the ♂ cell wall so as to recognize and react with a ♀ cell it contacts,
b. must form a bridge between ♂ and ♀,
c. probably confers motility to the F^+ ♂ chromosome by attaching to it at least temporarily (Fig. 44-2, small circle attached to chromosome in F^+ cell).
3. Hfr mating type carries F on the chromosome end transmitted last in fertilization (Fig. 44-2).

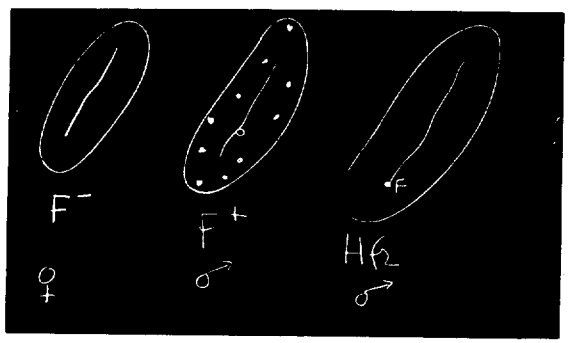

Figure 44-2

a. Hfr strains do not transmit F particles contagiously.
b. All Hfr strains are derived from F^+ cells (which may have come from F^- cells).
c. Many Hfr strains are unstable and revert to F^+ type, simultaneously losing their high fertility and gaining the property of infectious F particles.
d. The ♂ character of Hfr is not inhibited by acridine orange.

F. Episomal cycle refers to the facultative participation of a factor as an extra-chromosomal or as a chromosomal element.
1. F is an episome
a. In F^+ cells, F is normally extra-chromosomal. It is suggested that the low fertility of F^+ is due to a transient connection of F with the chromosome.
b. In Hfr cells F has a chromosomal locus.
2. The episome lambda (see also Chaps. 45 and 46)
a. E. coli strain K-12 normally carries a symbiotic bacteriophage, lambda, to which it is relatively insensitive.
b. A sensitive mutant, which had lost lambda, was found. This strain is used to test for lambda.
c. In the normal strain, lambda is temperate since it does not cause conspicuous lysis.
d. Since the normal insensitive strain can potentially produce infective lambda, it is said to be lysogenic.
e. Crosses between lysogenic and sensitive bacteria showed that lambda, as a prophage, has a definite locus on the chromosome, Lp, closely linked to Gal, a locus responsible for galactose fermentation.
f. This close linkage was observed in the haploid segregants from diploid exceptional clones of genotype Gal^+ Lp^+/ Gal^- Lp^S. (That is, ability to use galactose, Gal^+, was closely linked to capacity to produce bacteriophage, Lp^+, as was Gal^- to Lp^S).
g. Occasionally the Lp^+ chromosomal factor enters a cycle of vegetative multiplication in the cytoplasm, after which it matures as intact virus.
This occurs quite frequently when Lp^+ cells are treated with ultraviolet light (Lwoff).
h. Once Lp^+ forms mature virus the cell lyses and free virus is liberated.
i. The free lambda can enter other cells either to multiply as a parasitic virus, or to reenter the chromosome to again produce the lysogenic state.

3. Other particles in E. coli also act as episomes.
4. It is possible that cytoplasmic factors in other organisms are due to genic factors which can enter the cytoplasm.

POST-LECTURE ASSIGNMENT

1. Read the notes immediately after the lecture or as soon thereafter as possible, making additions to them as desired.
2. Review the reading assignment.
3. Be able to discuss or define orally or in writing the items underlined in the lecture notes.
4. Complete any additional assignment.

QUESTIONS FOR DISCUSSION

44. 1. What kinds of genetic information obtained from sexually reproducing organisms cannot be obtained from organisms reproducing only asexually?

44. 2. Why is it futile to search cytologically for mating couples in an ordinary culture of *E. coli* strain K-12 in which F^+ fertilizes F^- once per million bacteria?

44. 3. How do you suppose it was possible to prove that the results from mixing auxotrophs were not the consequence of mutation but rather of genetic recombination?

44. 4. Suppose an Hfr clone has the normal genes R O T S V while an F^- clone is mutant for these markers.

The clones are mixed at 10 a.m. At the times specified below the "happy couples" were separated, and analysis showed the normal genes indicated there had been transported into the F^- cells.

10:02 a.m. -- none
10:05 a.m. -- T
10:15 a.m. -- O T
10:25 a.m. -- S O T V
10:35 a.m. -- V S O T

Make a genetic map for the marker genes which is as complete as the data allow.

44. 5. If the DNA in a bacterial nucleus which is transferred is 22μ long, approximately how much of this is transferred 10 minutes after fertilization begins?

Approximately how long does it take for an average gene to be transferred in a bacterial fertilization?

44. 6. Several independently-arising, auxotrophic, mutants for the same trait were obtained and crossed to each other in pairs.

What conclusions could you reach from the results following, from which mutational events have been excluded, on the supposition that bacteria can be mated and can be separated instantaneously?

a. Mutant A x B never gave prototrophs, even when fertilization was permitted to be completed.

b. Mutant A x C never gave prototrophs when the pairs were separated before 1,000 seconds, but gave increasing percentages of prototrophs up to 1,002 seconds, after which this maximum frequency remained unchanged.

c. Mutant C x D gave no prototrophs before 1,001 seconds, but reached its maximum frequency beginning 1,002 seconds after mating.

44. 7. Draw a suitably labeled diagram showing a daughter DNA strand and the maternal and paternal DNA strands from which it was produced by a copy-choice mechanism.

44. 8. List as many differences as you can between F^+ and F^- cells.

44. 9. List as many differences as you can between F^+ and Hfr cells.

44.10. Specify the kinds of particulate genetic matter which may be transferred from one bacterium to another.

44.11. In bacteria, is sex type determined genetically? chromosomally? Explain.

44.12. Describe how one might obtain experimental evidence that F is transferred extra-chromosomally from F^+ to F^- cells.

44.13. What would you predict about the chemical composition of the F particle? Justify your answer.

44.14. Describe how you would proceed to obtain a Gal^+ Lp^+ strain from a Gal^- Lp^+ strain.

How would you test to show the desired genotype was obtained?

44.15. What are the similarities and differences between F and lambda?

44.16. What experimental evidence can you give in support of the schematic diagram at the left of Fig. 44-1?

44.17. Which cytoplasmic factors discussed in Chapters 34 and 35 might be episomes?