Chapter 45

BACTERIAL GENETICS: GENETIC TRANSDUCTION

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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. 392-393, 396-397, 398-399.
      Sinnott, Dunn, and Dobzhansky: Chap. 23, pp. 318-319; Chap. 28, pp. 384-386.
      Snyder and David: Chap. 26, pp. 410-413.
      Winchester: Chap. 23, pp. 324-326, 322-324.
   b. Additional references
      Lederberg, E. M., and Lederberg, J. 1953. Genetic studies of lysogenicity in Escherichia coli. Genetics, 38: 51-64.
      Lederberg, J. 1956. Genetic transduction. Amer. Scient., 44: 264-280.
      Lederberg, J. 1959. A view of genetics. Stanford Med. Bull., 17: 120-132. This Nobel Prize lecture is published also in "Science".
      Lederberg, J. 1959. Bacterial reproduction. Harvey Lect., 53: 69-82.
      Zinder, N. D. 1958. "Transduction" in bacteria. Scient. Amer., 199: 38-43.

LECTURE NOTES

A. Chap. 44 described a sexual mechanism in Escherichia coli involving the mediation of F which, like fertilization in higher forms,
   1. involved intact cells as participants, and
   2. had an entire genome as the unit of transfer.
   Relatives of E. coli and some filamentous bacteria also show sexual processes.

B. Genetic transductions refer to processes of fragmentary genetic exchange. One type, discussed at length here, involves bacteriophage.

C. Salmonella typhimurium
   1. causes mouse typhoid and is an agent in human food poisoning.
   2. Like its close relative E. coli, it is cultured on simple medium.
   3. Zinder and Lederberg found genetic recombination, of erratic pattern, between certain strains.
      a. Mixing an M^+ T^- strain with an M^- T^+ strain produced a filterable, heat-resistant agent with M^+ activity.
      b. This agent made prototrophs when added to an M^- indicator strain.
      c. The agent, smaller than a bacterium, could not be isolated from a pure M^+ T^- culture although this must have donated the M^+ factor when mixed with the M^- T^+ strain.
      d. Yet a drop of filtrate from the mixed culture added to fresh M^+-carrying cells evoked more filterable M^+ factor.
      e. Two activities were involved -- evoking and transferring M^+.

D. Bacteriophage as the evoking agent
   1. The phage P22 is lysogenic in the M^- T^+ strain.
   2. A stock suspension of P22 grown on the M^+ T^- strain yields particles with M^+ activity.
   3. The indicator strain M^- was derived from the M^- T^+ strain and is, therefore, lysogenic for P22.

E. Bacteriophage as the transferring agent
   1. P22 acquires fragments of genetic material from the host on which it is grown.
   2. Evidences for the association of bacteriophage with the genetic transferring capacity of the phage suspension -- the transductional capacity -- include:
      a. both show the same temperature inactivation pattern;
      b. both have the same susceptibility to an

antiserum that blocks phage attachment to cells;

c. both attach to susceptible cells simultaneously;

d. size and mass of both are the same, as determined by filtration and sedimentation tests.

3. It is strongly suspected that phage that carries part of a bacterial genome is defective for virus genome -- that there is a replacement of the latter by the former.

F. Example of transduction experiment in Salmonella

1. Phage 22 is grown on bacteria genetically $M^+ T^+ X^+ Y^- Z^-$.
2. Part of the crop of phage harvested is then tested on suitable indicator strains (M^-, T^-, X^-, Y^-, Z^-) one at a time.
3. This is done to show that the phage filtrate has the same range of activity as the bacteria on which it was grown.
4. Another part of the crop is now grown on a new bacterial strain, for example, $M^+ T^- X^+ Y^+ Z^-$.
   a. The new crop of phage harvested has now lost T^+ and gained Y^+.
   b. The phage is passive with respect to the content of the genes it transduces.
5. To harvest the phage, the liquid culture is centrifuged and the supernate heated at 60°C for 20 to 30 minutes (to kill any remaining bacteria).
6. To detect transduction of M^+, phage is grown on M^+ bacteria, harvested as described, mixed with M^- bacteria and plated on agar containing methionine-deficient medium.
   a. Phage attaches and injects its DNA into the M^- bacterium (see Chap. 46).
   b. If the bacterium survives this attack and if it acquires the M^+ fragment from the phage a clone will be formed.
   c. This transduction can by symbolized:

$$M^+ \xrightarrow{P22} X \quad M^- \longrightarrow M^+$$

G. Genetic scope of transduced material

1. Usually a single bacterial marker is transduced.
   a. $M^+ T^+ X^+ \xrightarrow{P22} X \quad M^- T^- X^-$
   b. The latter bacteria are grown on different media -- one which selects for M^+, another for T^+, and a third for X^+.
   c. When the M^+ clones are further typed they are still $T^- X^-$. Similarly, T^+ clones are still $M^- X^-$, and X^+ clones are still $M^- T^-$.
2. In contrast, in sexual recombination, large blocks of genes are transmitted together from Hfr to F^- cells.
3. Several markers may be transduced together in linked transduction or co-transduction.
   a. Demerec has shown, using transduction, that the genes for the biosynthetic sequence: anthranilic acid to indol to tryptophan (see Chap. 40), are closely linked to each other.
   b. Different mutants involved in defecting a particular enzyme are even more closely linked.
   c. Histidine biosynthesis is also controlled by a cluster of genes.
   d. This correspondence between biosynthetic and genetic association, though it does not apply to some higher organisms (e.g. Neurospora) may be adaptive in providing a mechanism for turning on or off a whole series of enzymes.
4. Any locus in Salmonella is transducable by P22.

H. Co-transduction in E. coli

1. From bacterial crosses, Lp and Gal are known to be closely linked (Chap. 44).
2. When lambda is harvested from $Gal^+ Lp^+$ prophage cells, with the aid of ultraviolet light (Chap. 44), it has Gal^+ transducing activity.
3. Gal is the only marker known to be transduced by lambda. The typical rate is one transduction per 100,000 phage particles.
4. The transduced Gal^- strain is a heterogenote (partial heterozygote), having one complete and unchanged chromosome of the host ($Gal^- Lp$) and a fragment (perhaps attached to the chromosome), carried over with lambda, containing $Gal^+ Lp^+$.
5. Heterogenotes can undergo reduction during which the Gal^+ may exchange places with Gal^-.
6. Nearly every lambda obtained from a heterogenote contains Gal.

   This was demonstrated by obtaining lambda from a Gal^+ heterogenote and cross-brushing it over a Gal^- clone streaked on galactose-deficient nutrient agar. The Gal^+-carrying lambda particles can be counted by the number of Gal^+ colonies that grow at the zone of intersection.

7. About one hundredth of the bacterial DNA is transduced at one time.

I. Recombination mechanisms in bacteria may
   1. involve whole nuclei (sexuality).
      a. Heterokaryosis, in certain filamentous fungi, involves concurrence of nuclei in common cytoplasm and leads to
      b. heterozygosis, as in E. coli K-12.
   2. involve fragments of genomes (transduction) via
      a. bacteriophages, like P22,
      b. episomes (see Chap. 44), like F and lambda, and
      c. purified DNA, in bacterial transformation (see Chap. 40).

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

45. 1. Is the infective F particle (Chap. 44) appropriately placed under the heading of genetic transduction? Explain.

45. 2. What evidence can you cite that the genetic recombination observed in Salmonella is not accomplished by a sexual process?

45. 3. Describe how you would perform an experiment to transduce the $\underline{X^-}$ $\underline{Z^-}$ loci present in a given strain of Salmonella.

45. 4. What evidence would you accept as proof that phage P22 is passive with respect to the genes it transduces?

45. 5. How would the results in G of the lecture notes be changed if co-transduction occurred

a. between $\underline{M}$ and $\underline{T}$ only?
b. between $\underline{T}$ and $\underline{X}$ only?

45. 6. Should lambda be called a virus or a segment of a bacterial chromosome? Explain.

45. 7. Compare P22 with lambda, as to similarities and differences.

45. 8. How is it possible to estimate the proportion of the total bacterial chromosome which can be carried in a transducing phage?

45. 9. Of all the types of transduction, what is unique to bacterial transformation?

45.10. Compare the genetic behavior of E. coli and S. typhimurium.

45.11. Learn what colicins are from the suggested readings. Have they any bearing upon genetic recombination in bacteria? Explain.

45.12. Do you suppose transduction occurs also in higher organisms? Explain.

45.13. What are the possible advantages and disadvantages of transduction as compared with sexuality?

45.14. Do bacteria obey Mendel's laws of inheritance? Justify your answer.