Chapter 46

VIRUS GENETICS: BACTERIOPHAGE

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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. 386-393.
      Colin: Chap. 12, pp. 216-220.
      Sinnott, Dunn, and Dobzhansky: Chap. 16, pp. 225-226; Chap. 23, pp. 317-318; Chap. 27, p. 373.
      Snyder and David: Chap. 27, pp. 412-413.
      Srb and Owen: Chap. 14, pp. 282-283.
      Winchester: Chap. 23, pp. 327-328.
   b. Additional references
      Anfinsen, C. B. 1959. The molecular basis of evolution. 230 pp. New York: John Wiley & Sons, Inc.
      Brenner, S. 1959. Physiological aspects of bacteriophage genetics. Adv. Virus Res., 6: 138-158.
      Burnet, F. M., and Stanley, W. M. Editors, 1959. The viruses. Vol. 1, General virology. 609 pp. New York: Academic Press.
      Luria, S. E. 1953. General virology. 427 pp. New York: John Wiley & Sons, Inc.

LECTURE NOTES

A. Nucleotide pairs (n'its) in different organisms

Mouse	5 billion per cell
Drosophila	80 million per cell
Bacterium	6 million per cell

If 2,000 n'its specify an average protein (see Chap. 44), bacteria could have about 3,000 different enzymes specified by its DNA content. It is surprising they could be free-living with so low a number.

B. Bacteriophages like T2 or lambda contain about 100,000 n'its, which, on this view, permit the manufacture of only about 50 different proteins.
   1. It is not surprising, then, these are not free-living organisms.
   2. The host cell provides most of the accessory metabolism for synthesizing new virus.
   3. The virus, in adding elements unique to its structure,
      a. competes with the metabolic systems of its host, and
      b. produces the protective structures enclosing it when outside its host.

C. Smaller viruses
   1. These include animal viruses causing encephalitic diseases and the exceptional phage ϕX174.
   2. These have only about 5,000 n'its, which could encompass only one or a few genes.
   3. Their nucleic acid is one-stranded.
      a. Most small viruses of plants and animals contain RNA.
      b. ϕX174 is exceptional in containing one-stranded (otherwise typical) DNA.
   4. It is remarkable that by probably producing but a few distinctive proteins these viruses can divert the host's metabolism so that virus nucleic acid and protein are made rather than the host's normal metabolic products.

D. Bacteriophage structure
   1. These bacteria-attacking viruses are .1 to .2μ long, about one-tenth the bacterial diameter.
   2. They have a somewhat-crystalline protein coat within which is packed double-stranded DNA (about 34μ long when extended).
   3. The protein coat has a tail, covered by a spiral protein, with tail tip fibers.

E. Lytic cycle of a bacteriophage
   1. Phage attaches to the bacterium tail first.

2. The DNA enters the bacterium leaving a shell of protein outside.
   a. Hershey and Chase labeled the DNA by P32 and the protein by S35.
   b. The empty shells, containing all or almost all the phage protein, can be sheared off the bacteria without changing the fate of the infected cells.
   c. The bacterium was innoculated, therefore, not with whole phage but with phage's genetic element.
3. An eclipse period follows during which no infective phage can be demonstrated in the recently infected bacterium.
   a. During the first several minutes there is replication of the phage DNA.
   b. This vegetative phage forms a pool of DNA units.
4. From time to time this pool is sampled, in that a fraction of it undergoes condensation and is surrounded by a new skin (head and tail) formed from a cycle of protein synthesis and organization; as a consequence infective phage is produced.
5. Phage-infected bacteria produce endolysins which, 20-40 minutes after infection, lyse the cell wall and liberate infective phage in the medium.
6. The free infective phage can attach and penetrate a sensitive bacterium, then
   a. repeat its lytic cycle,
   b. or, as in the case of lambda (Chaps. 44 and 45), establish a relationship with the bacterial chromosome, so that the bacterium is lysogenic and has the property of having some of its descendents produce some lytic phage.

F. Methods for assaying phage
   1. One method is to determine the time required for complete lysis of a liquid culture of sensitive bacteria.
   2. If a few phage are added on top of an agar medium recently seeded heavily with sensitive bacteria,
      a. the bacterial clones will form a continuous and somewhat opaque lawn.
      b. each phage particle that enters a bacterium will lyse it and release up to several hundred daughter particles which will attack bacteria near the original burst. This cycle will result in a progressively increasing zone of lysis which appears as a clearing or plaque in the bacterial lawn.
      c. each plaque will be a phage colony derived from one ancestral particle.
      d. Genetically different phages may produce different types of plaques.

G. What is unique to phage DNA?
   1. It must be able to stop bacteria from making their own protein and DNA.
   2. Even-numbered phages, T2, T4, and T6, have hydroxymethyl-cytosine in their DNA instead of cytosine.
   3. But in T1 or lambda there is no such peculiarity in base composition,
      a. yet T1 is virulent (causes lysis),
      b. and lambda can act either temperately (in a lysogenic bacterium) or lytically (upon ultraviolet induction).
   4. The complete answer is still unknown.

H. Genetic recombination in phage
   1. Given one phage strain mutant for both host range, h, and plaque type, r, and one strain that is wild-type.
   2. Sensitive bacteria are exposed to a mixture of such large numbers of the two mutants that some will have been multiply-infected.
   3. Some doubly-infected cells will carry both phage types.
   4. The daughter phage from such mixedly-infected cells may be of parental (h r and h^+ r^+) and recombinational (h^+ r and h r^+) types.

I. Fine structure analysis of T4
   1. Since T4 contains about 100,000 n'its, there could be that number of mutational sites.
   2. One segment, r, is about 1% of the total map length, or about 1,000 n'its long.
   3. The wild-type, r^+, grows on both strains B and K-12 of E. coli, while the r mutants grow on B but not K-12.
   4. Benzer made mixed infections with different r mutants, then easily detected the rare (1 in 10^6 or 10^8) recombinant particles capable of growing on K-12.
   5. Of 1,500 spontaneous r mutants Benzer tested, 100 were different. Thus there are at least 100 sites for mutation in the r segment.
   6. Certain mutants must have recurred frequently, indicating certain sites in r were "hot spots" of spontaneous mutation.
   7. The frequency of least recombination observed between two mutants was .0001. Since there are about 10 exchanges (morgans) per phage per generation (lytic cycle) over its whole linkage map, the total number of crossover sites in phage should be

10/.0001 or 100,000.

8. Thus, crossover site number equals n'it number; the genetic unit of recombination equals the smallest meaningful chemical unit -- the interval between two successive n'its.
9. Benzer also found phenotypic interaction between different r mutants growing in the same bacterium.
   a. By itself neither r mutant can lyse K-12.
   b. The vegetative phage of both together, in the same cell, can cause lysis.
   c. Since the phage liberated were usually of parental type, this phenotypic cooperation did not require genetic recombination.
   d. The r region can be divided into A and B sections or cistrons. Only a mixed infection including one mutant from A and one from B results in phenotypic cooperation.
   e. It is concluded, therefore, r governs the production of two separate protein elements.
   f. Mutants which behave as deletions for different regions in r have been obtained. It is possible to arrange the spontaneous point mutations in only one order which will give a compatible superposition of the various deletion types.
   g. Even in the utmost details of its fine structure the genetic material of phage T4 is organized in a linear fashion.

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

46. 1. What functions are attributed to the spiral protein and tip fibers of the tail of a bacteriophage?

46. 2. Distinguish a virus from a bacteriophage.

46. 3. Defend the statement: "Viruses are not genes."

46. 4. What do you consider the most remarkable and important feature of phage øX174? Why?

46. 5. What evidence proves or suggests that a transducing virus cannot

a. carry a whole bacterial chromosome?
b. simultaneously carry a viral genome in addition?

46. 6. Design an experiment which would demonstrate the "eclipse period" of phage.

46. 7. Discuss the phenotypic interactions between viral and bacterial genes.

46. 8. Are phages harmful to a bacterial species as a whole? Explain.

46. 9. Discuss the statement: "The holes in a bacterial lawn are contagious."

46.10. Why is it so important to learn the chemical nature of the proteins specified by phage?

46.11. From what is known about the chemical nature of the hereditary material in different organisms, discuss the evolutionary changes which the gene itself may have undergone.

46.12. What preliminary experiments had to be performed and what results obtained, do you suppose, before Benzer accepted an $\underline{r}^+$ genotype as the result of phage recombination?

46.13. Using different r mutants of phage T4, indicate the requirements and procedures necessary, in each case, to obtain

a. genetic recombination without phenotypic cooperation.
b. both phenotypic cooperation and genetic recombination.
c. spontaneous reverse mutations to $\underline{r}^+$.

46.14. What hypotheses can you offer to explain the occurrence of "hot spots" for spontaneous mutation in the r chromosome segment of E. coli?

46.15. Of what significance is the fact that crossover percentages lower than .01% were not found between r mutants, even though lower values would have been detected readily?

46.16. What bearing has Benzer's work to our understanding of the gene as a unit?

46.17. Briefly describe the evidence that phage DNA is organized linearly.

46.18. What attributes are common to all viruses?

46.19. Which do you suppose came first in evolution, bacterial-like or viral-like organisms? Explain.

46.20. Compare viruses and antibiotics.