Chapter 47

VIRUS GENETICS: PLANT AND ANIMAL VIRUSES

Lecturer—*J. LEDERBERG*

PRE-LECTURE ASSIGNMENT

1. Quickly review notes for the previous lecture.
2. Suggested readings:
   a. General genetics textbooks
      Sinnott, Dunn, and Dobzhansky: Chap. 27, pp. 377-378.
      Srb and Owen: Chap. 19, pp. 400-402.
   b. Additional references
      Burnet, F. M., and Stanley, W. M. Editors, 1959. The viruses. Vol. 1, General virology. 609 pp. Vol. 2, Plant and bacterial viruses. 408 pp. Vol. 3, Animal viruses. 428 pp. New York: Academic Press.
      Fraenkel-Conrat, H., and Williams, R. C. 1955. Reconstitution of active tobacco mosaic virus from its inactive protein and nucleic acid components. Proc. nat. Acad. Sci., U.S., 41: 690-698. Reprinted in: "Classic papers in genetics", J. A. Peters, Ed. 1959. Englewood Cliffs, N.J.: Prentice-Hall, Inc.
      Luria, S. E. 1953. General virology. 427 pp. New York: John Wiley & Sons, Inc.

LECTURE NOTES

A. Plant and animal viruses are
   1. of great economic and medical significance.
   2. difficult to study for technical reasons.

B. Determination of an infective unit
   1. This is a chief difficulty.
   2. Plant virus
      a. To titrate a virus attacking leaves, a sample is rubbed on the leaf surface.
      b. Only a small fraction of the virus particles find and penetrate susceptible cells and give a demonstrable lesion.
   3. Poliomyelitis virus
      a. When propagated on intact host animals, quantitation of particles is expensive and time-consuming.
      b. Samples to be titrated may be plated onto agar layers seeded with susceptible tissue culture cells. Clearing plaques are produced as by bacteriophage. This kind of technique is very useful.
   4. Unfortunately, many viruses (like influenza virus) do not produce sufficient cytopathic effect to produce detectable plaques on such agar plates. For these viruses, the techniques of limit-dilution must still be used.
   5. Influenza virus
      a. This has been adapted to grow on cells lining the fluid cavities of the chick embryo.
      b. A sample of virus to be tested is sufficiently diluted, and then aliquots innoculated into a series of eggs.
      c. After 48 hours or so, the eggs are harvested to determine the fraction which contained a virus particle.
      d. If near-limit dilutions are used (so the probability is low that an aliquot contains a virus particle), one can estimate the virus content of the entire sample.
      e. At near-limit dilutions, the virus particles harvested from an egg are probably from one clone.

C. Life cycle of animal viruses
   1. Influenza virus cycle is used as an example, whose general features may apply also to other animal and, to some extent, plant viruses.
   2. The mammalian host cell (Fig. 47-1) has
      a. a flexible shape,
      b. an ambiguous margin, and
      c. an outermost mucoid coat which acts as

a substrate for an enzyme located on the virus surface. This coat constitutes, therefore, a virus receptor.

3. The influenza virus has
   a. an inner core of RNA genetic material, and
   b. an outer protein coat containing the mucin-reacting enzyme.
4. The virus cannot attach if the mucoid coat is stripped by specific enzyme or periodate treatment.
5. After attachment, the particle enters the cell, perhaps by being engulfed via the cell's normal pseudopodial activity.
6. Once inside the cell, the particle enters an eclipse phase (see Chap. 46) and multiplies vegetatively, at which time the cell's RNA content increases.
7. After some hours intact particles are gradually liberated.
   a. Evidence indicates that the influenza viral coat is added during emergence from the host.
   b. This coat contains some material made (by the host -- therefore host cell-specific) before infection and some made after infection (by host and virus together).

D. Consequences of mixed infections
   1. Burnet and others used MEL and WSE strains of influenza virus which differ in their markers.
      a. Serologically, WSE is W and MEL is A.
      b. WSE is inactivated by ovomucin (c) while MEL is not (C).
      c. WSE is pathogenic when placed on the egg's chorio-allantoic membrane (e) while MEL is not (E).
   2. Egg membranes are multiply-infected with mixtures of the two strains.
   3. Phenotypic mixing (Burnet)
      a. From such mixed infections, daughter particles are neutralized almost perfectly efficiently by antiserum for either strain.
      b. The coats show this phenotypic mixing even though the genomes within them are either WSE or MEL.
      c. This effect, then, is not due to genetic recombination.
   4. Heterozygosis (Burnet)
      a. The virus from mixed infections is harvested and clones obtained via limit-dilution (see B5).
      b. Some clones contain more than one genetic type.

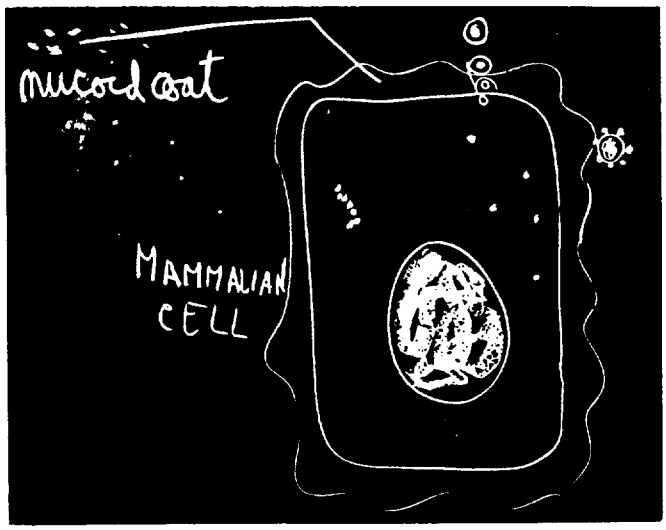

Figure 47-1

      c. This heterozygosis may be explained either by adhesion of two, whole, genetically-different particles which act as a unit on limit-dilution, or by one particle containing two different genomes.
      d. Since there has been no exchange of parts of the genetic material in forming a stable clone, this also is not a complete phenomenon of genetic recombination.
   5. Genetic recombination
      a. Influenza virus
         Mixed infections give particles which yield pure clones that are stable recombinants (A c or W C).
      b. Vaccinia virus (Fenner)
         Similar evidence was obtained, for this more complex virus, in experiments involving markers for hemagglutinins, heat resistance, virulence, and pock character.

E. Poliomyelitis viruses
   1. No evidence has been obtained so far for genetic recombination in these viruses.
   2. If found, recombination would greatly accelerate the deletion of the specific adaptation to the human host which these viruses possess.
   3. Present work is limited to mutational studies.

F. Chemical separation of viral components
   1. Aqueous phenol solution destroys the protein but leaves the nucleic acid intact (Schramm, using animal viruses).
   2. Tobacco mosaic virus protein is removed,

almost unchanged, by moderate alkalinity.

G. Infection by nucleic acid
   1. RNA, minus demonstrable protein, has been shown to be capable of infecting tobacco and mammalian cells.
   2. After vegetative multiplication, the mature particles formed have the protein coats that would be specified by this RNA when introduced by virus.
   3. Thus virus protein plays no part in replication either of the genetic material or of itself.
   4. As compared to nucleic acid in virus, purified nucleic acids have infectivities up to several percent.
   5. Isolated RNA is more susceptible to ribonuclease, temperature, and pH, and less susceptible to detergents, than is intact virus.
   6. Recent evidence suggests isolated DNA from bacteriophage is infective.

H. Reconstitution experiments with tobacco mosaic virus (TMV)
   1. TMV is a long linear particle; the outside is protein, built up of stacks of monomeric blocks; the inside is spiralled RNA.
   2. Fraenkel-Conrat has reconstituted essentially the original virus by mixing, under certain conditions, its separate protein and RNA.
   3. Using two strains of TMV, the standard (TMV) and Holmes ribgrass (HR), he was able to construct virus with TMV RNA and HR protein.
      a. The behavior of these particles was somewhat like that of animal viruses showing phenotypic mixing.
      b. However, particles have such low infectivity in plants that mixed infections, which could produce phenotypic mixing, do not occur.
      c. Such a reconstituted particle is inactivated not by anti-TMV serum but by anti-HR serum. The progeny of such a particle are typical TMV.
      d. Corresponding results were obtained from reconstituted particles having HR RNA and TMV coats.

I. Biologically-active nucleic acids have not yet been synthesized in the laboratory.
   1. The success of systems using DNA as a primer for synthesizing more DNA (Kornberg; see Chap. 41) is a giant step in this direction.
   2. The genetic behavior of RNA motivates attempts to replicate it in vitro.

J. "It should be stressed that DNA and RNA are not unique materials. They have the same relationship to the information contained in them as carbon black does to the words in a dictionary."

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

47. 1. What are the comparative advantages and disadvantages of influenza and poliomyelitis viruses as material for genetic investigation?

47. 2. Why is the WSE strain of tobacco mosaic virus called an egg-adapted strain?

47. 3. Is the technique of limit-dilution used in assaying tobacco mosaic virus? Explain.

47. 4. In what respects do the host cells of bacteriophage, on the one hand, and of plant and animal viruses, on the other hand, differ from each other with respect to virus?

47. 5. What evidence was presented that genetically recombinant clones of influenza virus are stable and pure?

47. 6. How can the different consequences of mixed infections with influenza virus be distinguished from each other?

47. 7. Specifically what would you do experimentally, in order to benefit mankind, if the phenomenon of genetic recombination was discovered in the poliomyelitis viruses?

47. 8. What evidence does Fraenkel-Conrat's experiments furnish that RNA is the sole or primary determinant of the coat protein of the tobacco mosaic virus?

47. 9. Cite evidences for the exact replication of RNA.

47.10. What genetic attributes does RNA share with DNA?

47.11. In what respects have genetic investigations using plant and animal viruses been more fruitful, to date, than those using bacteriophages?

47.12. How would you proceed to determine whether a nucleic acid synthesized in vitro was biologically active?

47.13. In what respects has our understanding of the genetics of higher organisms been aided by the genetic study of microorganisms?

47.14. Discuss the meaning of Lederberg's concluding statement, quoted in J in the lecture notes.