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The One-Step Growth Cycle

One important objective of research in virology is to understand how viruses enter individual cells, replicate, and assemble new infectious particles. These studies are usually carried out with cell cultures rather than with animals, because cell cultures provide a much simpler and more homogeneous experimental system. More important, cell cultures can be infected in such a way as to ensure that a single replication cycle occurs synchronously in every infected cell. The idea that one-step growth analysis can be used to study the single-cell life cycle of viruses was first set forth by Ellis and Delbrück in 1939, in their studies with bacteriophages described in Chapter 1. Synchronous infection, the key to the one-step growth cycle, is accomplished by infecting cells with a sufficient number of virus particles to ensure that most of the cells are infected rapidly. Most one-step growth experiments are conducted at a **multiplicity of infection** (Box 2.4) of 5 to 10 PFU per cell to ensure that almost all cells receive at least 1 infectious unit.

One-step growth analysis begins with removal of the medium from the cell monolayer and addition of virus in

BOX 2.4

Multiplicity of infection (MOI)

Infection depends on the random collision of cells and virus particles. When susceptible cells are mixed with a suspension of virus, some cells are uninfected and other cells receive one, two, three, etc., particles. The distribution of virus particles per cell is best described by the Poisson distribution:

$$P(k) = \frac{e^{-m}m^k}{k!}$$

In this equation, $P(k)$ is the fraction of cells infected by k virus particles. The multiplicity of infection, m, is calculated from the proportion of uninfected cells, $P(0)$, which can be determined experimentally. If k is made 0 in the above equation, then

$$P(0) = e^{-m} \text{ and } m = -\ln P(0)$$

The fraction of cells receiving 0, 1, and more than one virus particle in a culture of 10^6 cells infected with an MOI of 10 can be determined as follows.

Fraction of cells that receive 0 particles:

$$P(0) = e^{-10} = 4.5 \times 10^{-5}$$

and in a culture of 10^6 cells this equals 45 uninfected cells.

Fraction of cells that receive 1 particle:

$$P(1) = 10 \times 4.5 \times 10^{-5} = 4.5 \times 10^{-4}$$

and in a culture of 10^6 cells, 450 cells receive 1 particle.

Fraction of cells that receive >1 particle:

$P(>1) = 1 - e^{-m}(m + 1)^*$ = 99.95% of cells receive more than 1 particle

For MOI = 0.001:

$P(0)$ = 99.99%
$P(1)$ = 0.0999% (for 10^6 cells, 10^4 are infected)
$P(>1) = 10^{-6}$

The MOI required to infect 99% of the cells in a cell culture dish:

$P(0)$ = 1% = 0.01
m = –ln (0.01) = 4.6 PFU per cell

*Obtained by subtracting from 1 (the sum of all probabilities for any value of k) the probabilities $P(0)$ and $P(1)$.

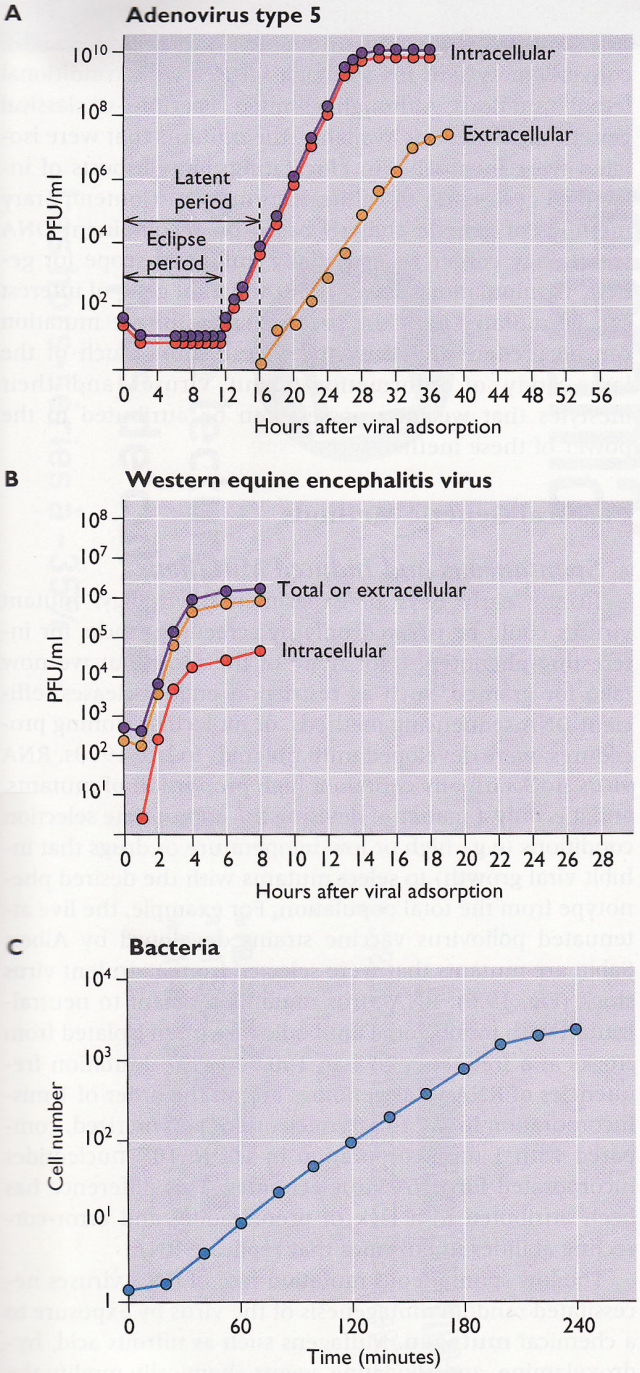

Figure 2.15 One-step growth curves. (A) Growth of a nonenveloped virus, adenovirus type 5. The titers of extracellular virus (yellow), intracellular virus (red), and the sum of both (purple) are plotted as a function of the number of hours after adsorption. The yield of infectious virus per cell can be calculated by subtracting the residual infectivity observed during the eclipse period from the total number of infectious viruses produced and dividing this number by the number of cells in the culture. (B) Growth of an enveloped virus, western equine encephalitis virus, a member of the *Togaviridae*. The total replication cycle is short, as are the eclipse, latent, and synthetic periods. This virus acquires infectivity after maturation at the plasma membrane, and therefore little intracellular virus can be detected. The small amounts observed at each time point probably represent released virus contaminating the cell extract. (C) Growth curve for a bacterium. The number of bacteria is plotted as a function of time. One bacterium is added to the culture at time zero; after a brief lag, the bacterium begins to divide. The number of bacteria doubles every 20 min until nutrients in the medium are depleted and the growth rate decreases. (A and B) Adapted from B. D. Davis et al., *Microbiology* (J. B. Lippincott Co., Philadelphia, Pa., 1980), with permission. (C) Adapted from B. Voyles, *The Biology of Viruses* (McGraw-Hill, New York, N.Y., 1993), with permission.

a small volume to promote rapid adsorption. After approximately 1 h, the unadsorbed inoculum is removed, the cells are washed, and fresh medium is added. At different times after infection, samples of the cell culture supernatant are collected, and the virus titer is determined.

The kinetics of intracellular virus production can be monitored by removing the medium containing extracellular particles, scraping the cells into fresh medium, and lysing them by repeated cycles of freeze-thawing. A cell-free extract is prepared after removal of cellular debris by centrifugation, and the virus titer in the extract is measured.

When the results of a one-step growth experiment are plotted graphically, a number of important features about viral replication are revealed. In the example shown in Fig. 2.15A, the first 11 h after infection constitute the **eclipse period**, during which the viral nucleic acid is uncoated from its protective shell and no infectious virus can be detected inside cells. The low level of infectivity detected during this period probably results from adsorbed virus that was not uncoated. Beginning at 12 h after adsorption, the quantity of intracellular infectious virus begins to increase, marking the onset of the synthetic phase, during which new virus particles are assembled. During the **latent period** no extracellular virus can be detected. At 18 h after adsorption, virus is released from cells and found in the extracellular medium. Ultimately, virus production plateaus as the cells become metabolically and structurally incapable of supporting additional replication.

The yield of infectious virus per cell can be calculated from the data collected during a one-step growth experiment (Fig. 2.15). This value varies widely among different viruses and with different virus-host cell combinations. For many viruses, increasing the multiplicity of infection above a certain point does not increase the yield. Cells have a finite capacity to produce new virus particles.

The nature of the one-step growth curve can vary dramatically among different viruses. For example, enveloped viruses that mature by budding from the plasma membrane, as discussed in Chapter 13, generally become infectious only as they leave the cell, and therefore little intracellular infectious virus can be detected (Fig. 2.15B). The first one-step growth curves of viruses were prepared for bacteriophages, and the results surprised scientists who had expected that they would resemble the growth curves of bacteria or cultured cells. After a short lag, bacterial cell growth becomes exponential (i.e., each progeny cell is capable of dividing) and follows a straight line (Fig. 2.15C). Exponential growth continues until the nutrients in the medium are exhausted. The one-step growth curves of viruses are very different: they begin with a lag period (eclipse) during which no virus growth is observed, followed by the sudden appearance of new infectious virus. We now know that during the eclipse period the components of new virus particles are being synthesized and assembled. The curve shown in Fig. 2.15A illustrates the pattern observed for a DNA virus with the long latent and synthetic phases typical of many DNA viruses, some retroviruses, and reovirus. For small RNA viruses, the entire growth curve is complete within 6 to 8 h, and the latent and synthetic phases are correspondingly shorter. Counterintuitively, polyomavirus, with one of the smallest genomes of the DNA viruses, has a very long latent period. The basis for these differences is related to the various strategies of gene expression and genome replication, to be discussed in Chapter 3.

One-step growth curve analysis can provide quantitative information about different virus-host systems. It is frequently employed to study mutant viruses to determine what parts of the replication cycle are affected by a particular genetic lesion. It is also valuable for studying the multiplication of a new virus or viral replication in a new virus-host cell combination.

When cells are infected at a low multiplicity of infection, several cycles of viral replication may occur. Growth curves established under these conditions can also provide useful information. For example, if a mutation fails to have an obvious effect on viral replication in a one-step growth curve, the defect may become obvious following a low-multiplicity infection. Because the effect of a mutation in each cycle is multiplied over several cycles, a small effect can be amplified. Defects in the ability of viruses to spread from cell to cell may also be revealed when multiple cycles of replication occur.