VIROLOGY - CHAPTER TWO

BASIC VIROLOGY: REPLICATION OF VIRUSES

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TEACHING OBJECTIVES

An overall view of the replication cycle of viruses

PRINCIPAL EVENTS INVOLVED IN REPLICATION

Adsorption

The first step in infection of a cell is attachment to the cell surface. Attachment is via ionic interactions which are temperature-independent. The viral attachment protein recognizes specific receptors, which may be protein, carbohydrate or lipid, on the outside of the cell. Cells without the appropriate receptors are not susceptible to the virus.

Penetration

The virus enters the cell in a variety of ways according to the nature of the virus.

Enveloped viruses

Entry by fusing with the plasma membrane
ome enveloped viruses fuse directly with the plasma membrane. Thus, the internal components of the virion are immediately delivered to the cytoplasm of the cell (figure 1).

Entry via endosomes at the cell surface (figure 2)
Some enveloped viruses require an acid pH for fusion to occur and are unable to fuse directly with the plasma membrane. These viruses are taken up by invagination of the membrane into endosomes. As the endosomes become acidified, the latent fusion activity of the virus proteins becomes activated by the fall in pH and the virion membrane fuses with the endosome membrane. This results in delivery of the internal components of the virus to the cytoplasm of the cell

Non-enveloped viruses

Non-enveloped viruses may cross the plasma membrane directly or may be taken up into endosomes. They then cross (or destroy) the endosomal membrane.

Uncoating

Nucleic acid has to be sufficiently uncoated that virus replication can begin at this stage. When the nucleic acid is uncoated, infectious virus particles cannot be recovered from the cell - this is the start of the ECLIPSE phase - which lasts until new infectious virions are made.

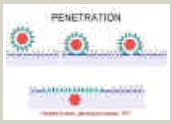

Figure 1. Fusion of a virus with the plasma membrane after attachment to a cell surface receptor

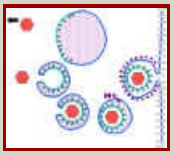
Figure 2. Fusion of a virus with the membrane of an endosome


Figure

3. Transmission electron micrograph of HIV-1, budding and free CDC

Figure 4. HIV budding from human lymph tissue (TEM x133,335) © Dennis Kunkel Microscopy, Inc. Used with permission

Synthesis of viral nucleic acid and protein

Many strategies are used, some will be discussed in later chapters.

Assembly/maturation

New virus particles are assembled. There may be a maturation step that follows the initial assembly process.

Release

Virus may be released due to cell lysis, or, if enveloped, may bud from the cell. Budding viruses (figures 3 and 4) do not necessarily kill the cell. Thus, some budding viruses may be able to set up persistent infections. Not all released viral particles are infectious. The ratio of non-infectious to infectious particles varies with the virus and the growth conditions.

STRUCTURAL VERSUS NON-STRUCTURAL PROTEINS

All proteins in a mature virus particle are said to be structural proteins - even if they make no contribution to the morphology or rigidity of the virion - non-structural proteins are those viral proteins found in the cell but not packaged into the virion.

EFFECT OF VIRUSES ON HOST MACROMOLECULAR SYNTHESIS

Many viruses inhibit host RNA, DNA or protein synthesis (or any combination of these). The mechanisms by which the virus does this vary widely.

CYTOPATHIC EFFECT (CPE)

Figure 5.
A plaque assay. Serial dilutions of virus have been plated on confluent monolayer cultures of cells. The cells are stained after a period of time in which a single virus infects a cell, produces new virus particles and infects surrounding cells. The white areas show areas of the culture in which the cells have been killed. Each "plaque" is the result of the presence of one original infectious virus particle.

The presence of the virus often gives rise to morphological changes in the host cell. Any detectable changes in the host cell due to infection are known as a cytopathic effect. Cytopathic effects (CPE) may consist of cell rounding, disorientation, swelling or shrinking, death, detachment from the surface, etc.

Many viruses induce apoptosis (programmed cell death) in infected cells. This can be an important part of the host cell defense against a virus - cell death before the completion of the viral replication cycle may limit the number of progeny and the spread of infection. (Some viruses delay or prevent apoptosis - thus giving themselves a chance to replicate more virions.)

Some viruses affect the regulation of expression of the host cell genes which this can have important results both for the virus's ability to grow, and in terms of the effect on the host cell.

The cytopathic effects produced by different viruses depend on the virus and the cells on which it is grown. This can be used in the clinical virology laboratory to aid in identification of a virus isolate.

Assays for plaque-forming units

The CPE effect can be used to quantitate infectious virus particles by the plaque-forming unit assay (figure 5).

Cells are grown on a flat surface until they form a monolayer of cells covering a plastic bottle or dish. They are then infected with the virus. The liquid growth medium is replaced with a semi-solid one so that any virus particles produced as the result of an infection cannot move far from the site of their production. A plaque is produced when a virus particle infects a cell, replicates, and then kills that cell. Surrounding cells are infected by the newly replicated virus and they too are killed. This process may repeat several times. The cells are then stained with a dye which stains only living cells. The dead cells in the plaque do not stain and appear as unstained areas on a colored background. Each plaque is the result of infection of one cell by one virus followed by replication and spreading of that virus. However, viruses that do not kill cells may not produce plaques.

Other assays for viruses

Some methods (e.g. electron-microscopy) enable every virion to be counted but are not informative about infectivity. Other methods (e.g. hemagglutination) are a less sensitive measure of how much virus is present, but again are not informative about infectivity. Other methods, e.g. plaque assay, measure the number of infectious virus particles.

WEB RESOURCES

Virusworld

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