NOTES

Apparent In Vivo Pathway of Granulosis Virus Invasion and Infection

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The invasion and ensuing replication of an insect granulosis virus in *Trichoplusia ni* is described.

Virus invasion and subsequent replication processes have been widely studied with certain bacteriophages and vertebrate viruses by using tissue culture methods. However, with animal viruses very little is known about such phenomena as they occur in vivo. This paper will describe the invasion and ensuing replication of an insect granulosis virus (GV) in *Trichoplusia ni* as observed by in vivo electron microscopy studies.

The insect granuloses are deoxyribonucleic acid rod-shaped viruses; they have a lipoprotein envelope and are occluded in protein crystals (1). In the case of *T. ni* granulosis virus (GV) they have been shown (with few exceptions) to infect only fat-body tissue (6). GV invasion and infection processes are most interesting because these viruses must be liberated from a crystalline inclusion and pass midgut cells of the alimentary canal without detection (3) en route to the fat-body cells where infected cells are easily detected (3, 6).

It is generally accepted that the insect GV gain entrance to their hosts and susceptible cells via the gut, but the mode and mechanisms of such invasion processes have remained obscure (5). In comparison, the entry of certain vertebrate viruses into cells has been studied extensively

(8). The results of electron microscopic studies have suggested that enveloped virions gain entry into host cells by a process of phagocytosis or by a fusion of opposing lipoprotein membranes of the virus and host cell.

To study the GV invasion process, fourth instar larvae of *T. ni* were infected per os with a highly purified capsule preparation (7). At post infection periods of 2, 6, 12, 20, 24, and 48 hr, midgut tissue was removed and prepared for electron microscopy by methods similar to those of Arnott and Smith (1). Ultrathin sections were observed on an RCA-3F electron microscope.

After introducing the capsules into the gut lumen, dislocation of the capsule protein first occurs adjacent to the virus particle (Fig. 1), and, in general, the capsule separates at each end to release the virion still enclosed in its lipoprotein envelope. Presumably, dissociation of the capsule protein is effected by high pH conditions of the gut contents (2). The envelope of the virus may also disappear during this process (Fig. 1), but this was rarely observed in this particular study.

Within 2 hr, many of the liberated virus particles are observed to be associated with the microvilli of the midgut cells (Fig. 2, 4). Phagocytosis of the viruses was not observed in this

FIG. 1. Dislocation of the capsule protein to release the virions as the process occurs in the gut lumen. Arrow designates the release of a virion, apparently without its lipoprotein envelope. Bar represents 0.5 μ m.

FIG. 2-4. Enveloped virions in the gut lumen, some of which are intimately associated with microvilli of the columnar cells. Arrows designate virions apparently inside microvilli. Bars represent 0.5 μ m in Fig. 2 and 4, 0.2 μ m in Fig. 3.

FIG. 5. Cross section observation of enveloped virion (arrow) and virion inside the microvillus. Note the absence of the lipoprotein membrane of the virus inside the microvillus. Bar represents 0.2 μ m.

FIG. 6 and 7. Virions inside microvilli after entry into the cell. Note the absence of the lipoprotein envelope of the virus. Bar in Fig. 6 represents 0.2 μ m, 0.5 μ m in Fig. 7.

FIG. 8 and 9. Apparent end-on interaction of the viruses with the pore of the nuclear envelope. Arrows designate empty tubules believed to be the empty inner membrane of the virus particle. N = nucleus. Bars represent 0.5 μ m.

FIG. 10. Virus particles observed in membrane vesicles in the cytoplasm of the columnar cells after breakdown of the nuclear envelope. Bar represents 1.0 μ m.

study; instead, there appears to be a disruption or fusion (Fig. 3, 4) of the virus envelope with the microvillar membrane. The mechanism of entry is still obscure, but it is believed that the virus envelope is lost at the cell surface since the virions are observed in the microvilli without the envelope (Fig. 5-7).

Approximately 2 to 6 hr after infection, virions are observed in an apparently nonspecific association with the nuclear envelope, but some appear directly associated end-on with the nuclear pore (Fig. 8). Structures similar to empty inner membranes (6) of the virus are also associated in a like manner with nuclear pores (Fig. 9). This suggests that the virus genome is released into the nucleus without the virion entering the nuclear region — a mechanism perhaps similar to phage infection of bacteria.

From approximately 6 to 20 hr after infection, changes in the nucleoplasm suggest eclipse-phase activity, and by 24 hr virus progeny are observed. The nuclear envelope loses its structural integrity, thus establishing continuity with the cytoplasm, and some of the virus particles are engulfed in vesicles of unknown origin (Fig. 10). It is suspected that by this means the virus is "transported" through the columnar cells to the basement membrane. U-shaped virus particles were also seen in these vesicles, and these results are similar to those of Harrap and Robertson (4), as observed in the nuclear polyhedrosis virus infection pathway of *Aglaia urticae*. The process by which the viruses are released into the hemocoel is still obscure, but some observations suggest that the vesicle with the virus passes through the basement membrane.

These preliminary in vivo observations indicate an unique and complicated series of events involved in *T. ni* GV invasion and infection of a host. (i) Phagocytosis of enveloped virions was not observed. This is in contrast to other studies which have shown both phagocytosis and fusion phenomena associated with virus entry (8). (ii) Virus interaction with the nuclear pores suggests that the virus genome may be released into the nucleus by this process, and that uncoating of the virion in the cytoplasm is not necessary. (iii) Viral replication processes appear to be confined to the nuclear region of the midgut

cells, whereas replication occurs in both nuclear and cytoplasmic regions of the fat body cells (*unpublished data*). (iv) Virus progeny in midgut cells are not occluded in proteinaceous crystals as is observed in the developmental sequence in fat-body cells (1). Instead, some virus particles are caught up in vesicles and passed on to the base of the cell. This nonocclusion phenomenon in the midgut cells is due perhaps to unknown properties of the membrane vesicles which contain the viruses. A more complete electron microscopic study of these lipoprotein membranes and their association with the virus developmental sequence has been conducted by M. D. Summers and H. J. Arnott (*J. Ultrastruct. Res.*, *in press*).

A more extensive evaluation of these preliminary observations will be reported. Of considerable interest is the observation that virus entry apparently occurs without phagocytic processes being involved. Studies by using other methods to characterize the basic factors underlying these biological interactions are necessary for a more complete understanding of the mechanisms involved; they are in progress.

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