MINIREVIEW

Transfer, Incorporation, and Substitution of Envelope Fusion Proteins among Members of the *Baculoviridae*, *Orthomyxoviridae*, and *Metaviridae* (Insect Retrovirus) Families†

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Recent research suggests that members of the *Baculoviridae* family can be divided into two groups on the basis of their envelope fusion proteins (31). One group utilizes proteins related to GP64. Homologs of GP64 are also used by the thogotoviruses (27), a genus of the *Orthomyxoviridae* family. Members of the other group of baculoviruses utilize envelope fusion proteins related to a protein called LD130. LD130 has been shown to be related to the envelope protein of insect retroviruses in the genus *Errantivirus* (family *Metaviridae*) (24, 36). In this review, the evidence for these data is outlined and possible pathways of transfer, incorporation, and substitution are discussed.

The *Baculoviridae* is a large and diverse family of occluded viruses, with double-stranded DNA genomes, that are pathogenic for insects. They have been divided into two genera on the basis of their occlusion body morphology: the nucleopolyhedroviruses (NPVs) (34), which have large occlusion bodies containing numerous virions, and the granuloviruses (GVs) (48), which normally have single virions occluded within small granular occlusion bodies. In addition, baculoviruses are characterized by the development of two types of virions during their replication cycle, differing in the origin and composition of their envelopes (44). The budded virus (BV) type is enveloped with a modified host cell membrane as it exits from the infected cell. In contrast, the occluded virion (OV) type is enveloped in a membrane that is assembled in the nucleus prior to being surrounded by a proteinaceous occlusion body. The occluded form of the virus is highly stable and can persist for years outside the host insect. It is normally transmitted between insects via contamination of their diet. The BV type enters cells by an endocytic mechanism facilitated by the presence in its envelope of a virus-encoded, low-pH-activated envelope fusion protein. In contrast, the OV type lacks the envelope fusion protein of the BV type, and the mechanism by which it infects insect midgut cells is not well characterized but may be independent of low pH. Furthermore, studies on the BV type of *Autographa californica* multinucleocapsid NPV (AcMNPV) have shown that it is over 1,000-fold more

infectious for cultured insect cells than the OV type of this virus (44) but is over 1,000-fold less infectious than the OV type when fed to insects (43). Therefore, the two types of baculovirus virions have envelopes derived from different sources and have different infectivity patterns, and they may enter cells by different mechanisms.

In earlier investigations of baculovirus biology, a protein called GP64 was identified as the envelope fusion protein of AcMNPV and its close relatives (2, 3, 42, 47). Subsequently, it was discovered that GP64 is closely related (~30% identity at the amino acid level) to the envelope fusion protein encoded by members of the genus *Thogotovirus* (family *Orthomyxoviridae*), which are pathogenic for ticks and can also infect vertebrates (22, 27, 29, 33). This indicated that an envelope fusion protein either had been transferred between a minus-sense RNA virus and a double-stranded DNA virus or was acquired independently by each of these viruses from another source, such as a conserved host gene (29) (Fig. 1).

MANY BACULOVIRUSES LACK GP64 AND LIKELY USE LD130 HOMOLOGS AS THEIR ENVELOPE FUSION PROTEIN

The first three baculovirus genomes that were sequenced were all found to contain a gene encoding GP64 (reviewed in references 14 and 15). However, the subsequently obtained complete genome sequences of both NPVs and GV were all found to lack genes encoding GP64 homologs (1, 5, 12, 13, 17, 21). Analysis of the *Lymantria dispar* (gypsy moth) multicapsid NPV (LdMNPV) genome, which does not encode a *gp64* homolog, led to the identification of a single open reading frame (ORF), called *ld130*, encoding a protein with the predicted properties of a membrane protein, including signal and transmembrane domains and N-linked glycosylation sites (21). Subsequent analyses demonstrated that LD130 behaves as an envelope fusion protein in that it localized to the membranes of cells infected with *Lymantria dispar* multinucleocapsid NPV or transfected with *ld130*, and in the latter instance, a reduction in pH resulted in membrane fusion (31). Similar data were obtained for a homolog of *ld130*, *se8*, from a multinucleocapsid NPV of *Spodoptera exigua* (18). Members of this group of envelope fusion proteins have many of the properties of fusion proteins of a number of viruses that infect vertebrates. They appear to be cleaved by a cellular enzyme at a furin-like cleavage site (18, 46), and they are predicted to contain a coiled-coil

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FIG. 1. Distribution of envelope fusion protein homologs. Three virus families and a retrotransposon are indicated. Below each viral family are listed the genera in the family, and below that is shown the category of the envelope fusion protein. The category encompassing baculovirus LD130, SE8, OP21, AC23, etc., is called the F protein group in accordance with the convention of Westenberg et al. (46). A schematic representation of the phylogenetic relationships (not to scale) of the genera is shown at the bottom of the figure. The horizontal dashed arrows show possible pathways of envelope fusion protein exchange. The GP64 arrow is not connected, reflecting the possibility that gp64 entered a baculovirus lineage during a coinfection with a baculovirus and a thogotovirus or that it entered both lineages independently via recombination with a host-encoded gp64 homolog. Abbreviations: HA, hemagglutinin protein; HEF, hemagglutinin-esterase-fusion protein.

region (36), suggesting that they may form structures of higher order similar to those found in many other viral fusion proteins (see, e.g., references 7 and 37). They have been called the baculovirus F (fusion) proteins (46).

GP64-CONTAINING BACULOVIRUSES ALSO ENCODE HOMOLOGS OF LD130 (BACULOVIRUS F PROTEIN)

Surprisingly, analysis of the completed baculovirus genome sequences has revealed that they all encode homologs of the baculovirus F protein, including those that appear to utilize GP64 as their envelope fusion protein. In addition, the baculovirus GP64 homologs are all closely related (>74% amino acid sequence identity), whereas the baculovirus F protein family is very diverse (20 to 40% sequence identity) and is found in both NPVs and GVs (Fig. 1). Therefore, it has been suggested that *gp64* was incorporated relatively recently into the genome of a baculovirus, where it displaced the envelope fusion function of the baculovirus *F* homolog (31) (Fig. 1). This likely contributed to the evolution of viruses such as AcMNPV and its close relatives, which depend on GP64 as their envelope fusion protein. However, the fact that baculovirus F protein homologs are retained by *gp64*-containing viruses, but appear to be unable to act as fusion proteins, suggests that they perform other essential functions (32). In addition, attempts to delete the entire coding region of one such gene were unsuccessful (G. F. Rohrmann, unpublished data). We have distinguished these protein types as baculovirus Fa proteins (fusion competent) and Fb proteins (not fusion competent).

Further evidence for the adaptability of baculoviruses was provided by a recent report showing that a baculovirus lacking *gp64* could utilize the envelope fusion protein of vesicular stomatitis virus as its fusion protein. Therefore, the ability of baculoviruses to enter cells appears to be accommodated by a variety of different envelope proteins (25).

BACULOVIRUS F PROTEIN HOMOLOGS ARE THE ENVELOPE PROTEINS OF INSECT RETROVIRUSES

The similarity between long terminal repeat (LTR) retrotransposons and retroviruses has long been noted (6, 39, 49). A major difference between these retroelements is that the former do not encode an envelope protein. Consequently, although retrotransposons can move and amplify within organisms, their ability to be transmitted to other organisms is limited, and they are not infectious. Recently, two reports have provided evidence that a baculovirus *F* progenitor was the source of the envelope protein gene that resulted in the conversion of a *gypsy*-like noninfectious retrotransposon of insects into a retrovirus (24, 36). These insect retroviruses have been named the errantiviruses (4), and 13 species have been identified (40). One of these viruses has been shown to be infectious (20, 38), and when the gene encoding its predicted envelope protein was incorporated into the Moloney murine leukemia virus genome, that virus was able to infect *Drosophila* cells (41). The relatedness of the insect retrovirus *env* gene and the baculovirus *F* homologs likely reflects more than a fortuitous relationship between these two viruses. This is indicated in a study showing that an insect retrovirus called TED integrates into a baculovirus genome (8, 26). TED is present as a midrepetitive element (about 50 copies/genome) in an AcMNPV host, *Trichoplusia ni* (9). It is capable of transposition from the insect into the baculovirus genome; it encodes a full complement of retrovirus ORFs, including an *env* gene (30) that is related to the baculovirus *F* gene; and it is able to produce virus-like particles (23). A key feature of this relationship likely evolved because of the remarkable ability of baculoviruses to express genes at very high levels. This feature of their life cycle forms the basis of their widespread utility as expression vectors and appears to be due to the fact that they encode an RNA polymerase (11). This polymerase recognizes

a novel promoter sequence (DTAAG) (35) that is found in the TED LTR as a palindrome. Late in the baculovirus infection cycle, mRNA is expressed from these LTRs at high levels (9). Therefore, integration into a baculovirus genome may reflect a strategy to exploit baculovirus late-gene expression to hyper-express the integrated retrovirus genome. This could result in the production of a mixture of retrovirus particles and occluded baculoviruses containing integrated retroviruses. It has been suggested that in insects with fatal baculovirus infections, this may provide retroviruses such as TED with a two-pronged method of escape: they could survive by integrating into baculovirus genomes or as infectious virus particles, similar to those observed for other gypsy-like elements (36). The evolution of this relationship between a baculovirus and a primordial LTR-type retrotransposon provides a clear pathway, via DNA recombination, for the transposable element to incorporate the baculovirus *F* homolog into its genome, thereby converting it into an infectious retrovirus. Indeed, the evolutionary analyses of the envelope proteins of the errantiviruses suggest that the incorporation of the envelope protein gene into a transposable element was a monophyletic event (24). Furthermore, such an interaction may not be confined to the *Baculoviridae*, as it has been suggested that it may have occurred between transposable elements and other enveloped viruses on several occasions (7a, 24).

It may be significant that evidence for transfer and incorporation of the homologs of *gp64* and baculovirus *F* genes is found among viruses pathogenic for invertebrates (ticks and insects). It is likely that invertebrate organisms, their viruses, and transposable elements proliferated and developed ancient relationships that predate the evolution of most vertebrate lineages. As vertebrates evolved, they would have provided new hosts for existing invertebrate viruses. However, whereas related patterns of structure and function have been suggested for viral envelope fusion proteins from different vertebrate viruses (7, 10, 16, 19, 28, 37, 45), clear evidence of such relatedness from primary sequence data appears to be confined to viruses that are pathogenic for arthropods. The preservation of primary sequence similarity may result from the absence of an adaptive immune response in the invertebrate hosts. In vertebrate viruses, the exposed position of envelope fusion proteins on the virion surface and the critical role they often play in virion attachment and cell entry make them prime targets for the adaptive immune response. This has resulted in a continuous and intense selective pressure on the proteins to retain their critical function yet evade the host immune response. This can be accomplished by changes in the primary sequence that preserve function but alter antigenicity. In contrast, arthropods lack adaptive immunity, and many insect hosts are short lived, resulting in an absence of this type of selective pressure. This may have led to the preservation of the primary sequence similarity, which allows determination of the relatedness of these proteins among different viruses of invertebrates.

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