Contributed paper. Thursday, 14:45, **226**

The postfusion 3D-structure of the Spodoptera exigua multiple nucleopolyhedrovirus envelope fusion protein F

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Baculoviruses utilize envelope fusion proteins (F or GP64) on the surface of budded virions for low-pH dependent membrane fusion to enter insect cells. Baculovirus F and GP64 proteins belong to Class I and Class III envelope fusion proteins, respectively. The baculovirus F protein may represent the archetype F protein, whereas GP64 has been acquired much later by (Group I) alphabaculoviruses. Cell entry via the F protein requires proteolytic cleavage of F into F1 and F2 in order to become fusogenic. To understand the function and mode of action of baculovirus F proteins the 3D-structure is important to complement the current biochemical and genetic studies. The 3D-structure of GP64 confirmed that it belongs to the Class III fusion proteins (Kadlec et al., 2008). Here we report the 3-D structure of the post-fusion ectodomain of baculovirus F protein (*Spodoptera exigua* nucleopolyhedrovirus). This protein lacking the transmembrane anchor domain and the C-terminal cytoplasmic tail, and containing a trypsin cleavage site downstream of the furin cleavage site was expressed in *Drosophila* S2 cells. Purified F ectodomains were deglycosylated with PNGase-F and treated with trypsin and acidic pH allowing conformational rearrangement into the postfusion trimeric state. The crystals were subjected to X-ray crystallography and diffracted to 2.7-3.4 °A resolution. This 3D-structure confirmed computational predictions that the baculovirus F protein adopts a Class I fusion protein fold and is homologous to the mammalian paramyxovirus F protein. The baculovirus F protein, from a DNA virus, possibly is the archetype F protein of vertebrate RNA virus F proteins. The results imply interesting evolutionary links between DNA and RNA viruses and their hosts.

Contributed paper. Thursday, 15:00, **227**

A new system for studies of viral envelope protein trafficking in insect cells

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Trafficking of viral envelope proteins to the plasma membrane is critical for egress of most enveloped viruses. For many orally-acquired viruses of insects, progression to a systemic infection requires polarized trafficking of envelope proteins to basal membranes in the infected midgut cells, followed by viral egress from the basal membranes. Because the cellular trafficking pathways are not well defined, identification of the pathway proteins will be important for strategies to interrupt virus transmission, or to enhance beneficial viruses. To identify trafficking pathway proteins, we developed *Drosophila*-based systems for studies in cell lines and midgut cells. To examine general trafficking pathways in cell lines, we developed a baculovirus-based transduction system for transient expression of AcMNPV GP64 or VSV G protein. dsRNA-mediated RNAi is used to deplete candidate cellular proteins and to identify those required for transport of GP64 or VSV G to the plasma membrane. We first focused on Rab GTPases which are involved in vesicular trafficking of cellular proteins. To study polarized trafficking, we engineered *Drosophila* fly lines for midgut-specific expression of GP64 or VSV G, and found that they traffic to basal membranes of the gut epithelia. This provides evidence that each viral protein encodes information for polarized trafficking. Crosses of these lines with fly lines expressing dominant negative constructs of cellular trafficking proteins, permits screening and identification of cellular proteins required for polarized transport. Combined, these cell and fly-based systems permit identification of trafficking proteins and pathways important for success of insect-vectored and insect-pathogenic viruses.

Contributed paper. Thursday, 15:15, **228**

Rescue of the entry of AcMNPV fusion-defective mutants by low-pH triggering: higher fusion activity is required for GP64-mediated entry into mammalian cells compared to insect cells?

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AcMNPV can transduce a variety of mammalian cells low efficiently, and a low-pH trigger significantly improves the entry efficiency of AcMNPV in mammalian cells. To explore the mechanism, a series of GP64s with mutations in D295/D301 localized within the central helix stalk were made. The expression and infection of the most mutants are similar to that of wild type (WT) GP64. However, syncytium formation assay showed that while D295E and -H slightly reduced fusion activity of GP64, the others (D301E, -H, D295/301H, D295/301E) showed dramatic reduction in fusogenicity, with only 3-10% of WT level remained, implying that D301 is a crucial residue for low-pH dependent conformational change of GP64. Mammalian cell transduction reveals a perfect correlation between the entry efficiency and fusion activity of the mutants. For example, the transduction rate of D295E and D301E is ˜80% and ˜9% of WT, as their fusion activity is ˜88% and ˜7% of WT, respectively. Interestingly, while the low fusion activity of D301E mutant could be improved with pH gradually decreasing, the transduction efficiency of D301E was efficiently rescued by low-pH triggering after virus binding (84% of WT at pH4.8). Our major findings are: 1) the AcMNPV mutants harboring low-fusogenic GP64, although infected insect cells as efficiently as WT virus, their ability to transduce mammalian cells were greatly impaired; 2) low-pH trigger significantly rescue the entry of the mutants in mammalian cells, probably via improving the fusogenicity of GP64s. We propose that higher fusogenicity of GP64 may be required for AcMNPV to enter mammalian cells than insect cells, and this may explain why low-pH triggered direct fusion as a high efficient route for the entry of AcMNPV in mammalian cells.

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Extra genomic DNA elements found in an entomopoxvirus

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Entomopoxviruses (EPVs) possess a single linear double stranded DNA genome. The genome of Adoxophyes honmai entomopoxvirus (AHEV) was extracted from purified virus particles and sequenced fully. Apart from the 228,750 bp viral genome the sequence revealed the presence of two extra genomic DNA elements of 11,449 and 12,085 bp. These elements, predicted to encode 22 ORFs including a capsid protein, a cysteine protease