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Baculovirus Host-Range

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Abstract Baculoviruses are used as microbial insecticides, protein expression vectors, epitope display platforms, and most recently as vectors for gene therapy. Understanding the mechanisms that control baculovirus host-range and tissue tropisms are important for assessing their safety and for improving their properties for these biotechnology applications. In the past two decades some progress has been made and several baculovirus genes that influence host-range have been identified. Despite this progress, our understanding of the underlying mechanisms that restrict baculovirus host-range is still limited. Here we review what is currently known about baculovirus genes that influence virus host-range.

Key words: Apoptosis; Translation arrest; p35; hrf-1; p143

Baculoviruses are double-stranded DNA viruses that infect invertebrates, primarily insects (8, 33, 89). They are characterized by enveloped rod-shaped nucleocapsids that are occluded in a proteinaceous matrix. Granuloviruses (GVs) generally contain a single enveloped nucleocapsid per occlusion body, whereas nucleopolyhedroviruses (NPVs) contain numerous nucleocapsids that are enveloped singly (S) or multiply (M), and distributed throughout the polyhedral occlusion body (OB) matrix. Baculoviruses have genome sizes ranging between 80 and 180 kbp, and they replicate in the nuclei of infected cells. There are currently four recognized genera in the family *Baculoviridae* that infect three insect orders (61, 66). These include GVs that infect the larval stages of insects in the order Lepidoptera, and NPVs that infect larvae in the orders Lepidoptera, Hymenoptera, or Diptera. Because they are the most extensively studied this review will focus on the NPVs infecting Lepidoptera, genus: alphabaculovirus, and unless stated otherwise the term baculovirus and the abbreviation NPV will refer to these NPVs. There is a long-standing interest in baculovirus host-range stemming from the practical perspective of using baculoviruses as microbial insecticides in agriculture and forestry, and more recently for assessing the safety of baculoviruses for applications in biotechnology. Although there are exceptions, notably *Autographa californica* M NPV (AcMNPV), most baculoviruses have relatively

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narrow host-ranges. Since the early 1990s, several baculovirus genes that influence host-range have been identified, but their specific functions and the underlying mechanisms that control baculovirus host-range are still poorly understood.

Studies describing baculovirus isolation from specific insect species and subsequent cross-infection studies date to the early days of microbiology prior to a full understanding of the nature of viruses (3). Interpretation of studies in the earlier literature is difficult owing to a lack of reliable methods for confirming the identities of input and output viruses to satisfy Koch's postulates. In many cases, reported cross-infections do not hold up to scrutiny and can be attributed to either inadvertent infection with heterogeneous test viruses or activation of latent infections in the test insects. The rise of improved technologies for virus characterization such as electron microscopy, serology, and eventually molecular genetics made more accurate virus identification possible and facilitated better assessments of virus host-specificity. Moreover it became possible to enhance the insecticidal properties of baculoviruses through genetic engineering driving a resurgence of interest in baculovirus host-range and impetus for understanding the molecular mechanisms responsible. In the 1980s and 1990s this renewed interest was driven by the prospect of developing baculovirus insecticides with enhanced potency that could target multiple pest species and to assure the safety of modified viruses for non-target species (7, 60).

BACULOVIRUS HOST-RANGE IN INSECTS

Reviews of literature on baculovirus host-specificity studies conducted up to the mid 1960s (57) and from the mid 1960s through the early 1980s (42) indicated that baculoviruses were only rarely transmitted across families and that the host-ranges of GVs were found to be narrower than NPVs. However, the earlier review cataloged reports of three successful transmissions across orders (57). It should be noted that the author of this review accepted the interpretations of host-specificity presented in the original papers. The subsequent application of molecular tools to characterize baculoviruses suggests that these early reports of cross-order infectivity are probably not valid.

The use of molecular identification methods for validating cross-infection is exemplified by an extensive study of *Mamestra brassicae* NPV (MbNPV) host-range (26). In this study DNA dot blots and restriction enzyme digests were used to verify infections. Although OBs were observed in *Neodiprion sertifer* (Hymenoptera: Symphyta) this apparent cross-order transmission was not confirmed as MbNPV by dot blot. No signs of infection were observed in other species in the orders Hymenoptera, Coleoptera, or Neuroptera that were tested. But MbNPV had a surprisingly broad hostrange in Lepidoptera particularly among Noctuidae although infection was restricted to three families. Moreover all infections outside of the Noctuidae required higher doses of virus to initiate infection.

AcMNPV and several closely related viruses infect the broadest range of insect species, although as observed for MbNPV (26), these species vary in their susceptibility to infection. In the Gröner review AcMNPV was reported to infect 33 lepidopteran species in 10 families (42). A few other baculoviruses that are closely related to AcMNPV also exhibit relatively broad host-ranges. For example, *Anagrapha falcifera* NPV (AfNPV), later shown to be the previously

described *Rachiplusia ou* MNPV (RoMNPV) (49), was found to infect 31 lepidopteran species in 10 families (56, 114). Several of these species had not been previously reported to be susceptible to AcMNPV, and one species, *Helicoverpa zea*, was significantly more susceptible to AfNPV than to AcMNPV (56, 114). In contrast, *Bombyx mori* NPV (BmNPV) another close relative of AcMNPV has a narrower host-range, reportedly infecting insects in seven lepidopteran families (42). Although comprehensive host-range studies validated by unequivocal virus identification are lacking for most baculoviruses, it appears that in general baculoviruses have relatively restricted host-ranges. Our current understanding is that baculovirus host-range is restricted to order and that within a single order, most are restricted to a single family and many to a single species or a few closely related species.

CELLULAR HOST-RANGE

Virus host-range is determined by the ability of a virus to enter the cells of an organism, replicate its genome, assemble new virus particles, and release infectious progeny. Studies into these processes are facilitated by the availability of cultured cells that support the entire life cycle of the virus. For baculoviruses robust replication in cell culture has been achieved for NPVs infecting Lepidoptera (39, 54, 116, 121) and to a limited extent for GVs (40, 77, 98, 133). Although infection in cell culture can provide important insights into the molecular determinants of baculovirus host-range, it does not necessarily reflect a true organismal host-range. Virus host-range is less restricted in cultured cells and it is not uncommon for NPVs to replicate in cells derived from non-host species. Moreover, cell culture studies do not account for physical barriers to infection, immune defenses, or even limitations to infection due to tissue tropisms in an animal.

Understanding what determines the host-ranges of lepidopteran NPVs is further complicated by their bi-phasic lifecycles. During their lifecycle two morphological forms of virus are produced that have different functions and properties (8, 33, 34). Occlusion derived virus (ODV), embedded in polyhedral OBs, spreads the virus horizontally and is orally infectious for caterpillars. OBs are ingested by caterpillars and dissolve in the highly alkaline midgut releasing the enveloped virions. The virus envelope fuses with cellular membranes at the microvillar surfaces of midgut epithelial cells in a process that is poorly understood, but novel in that optimal fusion occurs at pH greater than eight (55). Following cell entry, nucleocapsids are transported to the nucleus where they uncoat and replicate. Budded virus (BV) is produced following the primary infection and spreads the virus to other cells within the infected caterpillar. Infection of tracheal cells facilitates BV movement across the basal lamina from the sites of primary infection in midgut epithelial cells to the haemocoel and to other tissues (30, 32). BV acquires its envelope from infected cell membranes and bears virally encoded fusion proteins, not found on the envelopes of ODV. BV enters cells by receptor mediated endocytosis (118, 119). The fusion proteins induce membrane fusion in the endosome at low pH, releasing the nucleocapsid (9, 58, 82, 101, 118, 119). BV are highly infectious for cultured cells and are used almost exclusively for host-range studies employing cell lines. ODV are much less infectious for cultured insect cells than BV (122), but are able to infect some cell lines (84).

VIRUS ENTRY

The first critical step in a productive infection is entering a host cell. This involves virus binding to a cellular receptor. Therefore the presence or absence of an appropriate virus receptor is often a major determinant for virus host-range. In the case of NPVs little is known about the specific virus receptors. Only a few binding studies have been conducted on ODV. These studies suggest that ODV binds to specific receptors and that different viruses may have different receptors (46, 47, 55, 99). Specific binding of ODV to midgut receptors was first demonstrated for *Lymantria dispar* NPV (LdNPV) in binding studies using *L. dispar* brush border membrane vesicles (BBMV) (55). Subsequent to this study several NPV genes were identified that encode proteins required for oral infectivity. These *per os* infectivity factors (pifs) P74, PIF1, PIF2 and PIF3 (72, 75, 99, 103) are all components of the ODV envelope and all but PIF3 are involved in receptor binding (46, 99, 109). Interestingly, oral infection with a *pif*-deletion mutant could not be rescued by co-infection with another virus bearing the gene, suggesting that all four proteins must be components of the same virus envelope to initiate infection (109). AcMNPV ODV from wt virus bound *Heliothis virescens* midgut epithelium more efficiently than ODV from p74 deletion viruses, but there were no differences in membrane fusion. The three-fold differences in binding efficiencies between the viruses could not account for the absence of oral infectivity by the P74 deletion virus, suggesting additional roles for P74 in initiating primary infection (46). AcMNPV is infectious for *Spodoptera frugiperda*, but only at high dosages most likely because of inefficient initiation of a primary infection (45). When the binding of AcMNPV and *S. frugiperda* NPV (SfMNPV) ODV to *S. frugiperda* midgut epithelial cells were compared, AcMNPV ODV bound at much lower levels that SfMNPV, but once bound there was little difference in membrane fusion between the viruses (47). AcMNPV did not compete with SfMNPV for binding in co-inoculation studies with labeled virus suggesting the two viruses bind to different receptors. These studies implicate specific midgut receptors for oral infection by NPV, but it is still unknown if they have a role in determining baculovirus host-range. Indeed, the p74 deletion virus study suggests that events subsequent to binding may be more important for initiating a productive infection (46). One report suggested that P74 might be a host-range determinant because replacement of the AcMNPV p74 gene with the *Spodoptera litura* multicapsid nucleopolyhedrovirus (SpltMNPV) p74 gene abolished AcMNPV oral infectivity for *Argyrogramma agnata* larvae (134). However a role for P74 in host-specificity has not been independently confirmed and there may be other explanations for these results, for example, it is possible that SpltMNPV P74 is incompatible with the other AcMNPV pifs (109).To date there have been no comparative studies of ODV binding of an NPV to midgut epithelial cells from insect species with different levels of resistance to oral infection. Identification of *pif* genes involved in virus attachment should facilitate the identification of specific receptors for ODV to help resolve these questions.

Although a specific receptor has yet to be identified, AcMNPV BV can enter diverse cells, including mammalian cells, and express a reporter gene (11, 23, 93, 94). These studies indicate that the receptor for BV is

highly conserved and the block to baculovirus infection in cultured cells, at least for AcMNPV, occurs subsequent to entry and transport to the nucleus. While these observations indicate that BV receptor binding has little influence on baculovirus specificity at the cellular level, there is a complication. Two types of fusion proteins are associated with the BVs, GP64 and F-protein. AcMNPV BV bears GP64 (117, 120). F-protein is the ancestral fusion protein, whereas GP64 was acquired more recently and is related to the fusion proteins of *Thogotovirus*, an orthomyxovirus (58, 96, 101, 102). The GP64 type of fusion protein is used by a subset of baculoviruses, termed group I NPVs (52, 137), which are phylogenetically related to AcMNPV. The others, the group II NPVs, bear F-protein on the BV envelope. Both GP64 and F-proteins promote membrane fusion in a similar manner and replacing AcMNPV gp64 with F-proteins from LdMNPV, *S. exigua* NPV (SeNPV), or *Heliothis armigera* NPV (HearNPV) resulted in pseudotyped AcMNPV BV that could initiate membrane fusion in Sf9 cells (78, 82). Competitive binding studies between group I and group II NPVs demonstrated that they bind to different receptors (130, 131). However, the ability of AcMNPV pseudotyped with different F-proteins to fuse with cells that are normal hosts for AcMNPV but not hosts for LdMNPV or HearNPV, the sources of the F-protein genes, suggests that specific receptor binding does not play a key role in restricting the host-ranges of type II viruses.

While it is tempting to rule out viral entry as a factor affecting baculovirus host-range, the ability of BV from few baculoviruses, other than AcMNPV, to bind to and enter diverse cell lines have been investigated using sensitive techniques such as reporter genes. One such study implicates GP64 as a host-range factor for BmNPV (71). As noted above, AcMNPV has a broader host-range than BmNPV, yet they are closely related group I NPVs, with common genes that share over 90% nucleotide and amino acid identity (35). The difference in host-range extends to cultured cells, making AcMNPV, BmNPV, and the cells that support their replication ideal models for investigating the molecular basis of baculovirus host-range. This study investigated the ability of BmNPV to infect seven non-permissive or semi-permissive insect cell lines, using a virus expressing a fluorescent reporter gene driven by the *ie-1* promoter (71). Fluorescence was observed in only a small percentage of infected Sf9 and BTI-Tn 5B1-4 (TnHi5) cells, but the percentage of fluorescing cells increased and detectable viral progeny were produced when viral DNA was transfected into cells. Localization of the virus via immuno-cytochemistry showed that the virus trafficked to the region surrounding the nucleus, but failed to enter it in the majority of infected cells. When the BmNPV GP64 was replaced with AcMNPV GP64, the pseudotyped BmNPV could then enter the nucleus and complete its lifecycle in TnHi5 cells, but not in Sf9 cells. This suggested that the BmNPV GP64 could not efficiently mediate fusion in the endosome to release the nucleocapsids, raising the possibility that fusion proteins may indeed have a role in host-range, albeit subsequent to cellular entry. Previous studies that examined BmNPV infection in Sf9, Sf21, and Tn368 cells found either limited infection, or minimal DNA replication and an extremely low level of progeny virus produced, consistent with productive infection in only a small subset of infected cells (86), as was observed in this study. These results

are also consistent with the low levels of virus gene transcription observed in Sf9 cells infected with BmNPV or transfected with BmNPV DNA (65). A puzzling aspect about this study arises when comparing it with several earlier studies on BmNPV and AcMNPV host-range. Specifically, it is hard to reconcile the inability of BmNPV to enter the nuclei of Sf9 with studies in which recombination between AcMNPV and BmNPV occurred in Sf21 cells since it would require both viral genomes in the nucleus (73). See discussion of *p143*, below. One possibility is that Sf9 and Sf21 differ in this respect and in these co-infection studies AcMNPV provided a "helper function", which allowed BmNPV to enter Sf9 cells. This possibility is consistent with the study of Rahman and Gopinathan in which they observed no BmNPV replication in BmNPV-infected Sf9 cells unless they were co-infected with AcMNPV (105). The Katou *et al.* study implicates AcMNPV gp64 as providing the putative "helper function" (71). AcMNPV and BmNPV gp64 proteins are highly conserved, sharing 95% amino acid identity. However, they reported more efficient membrane protein fusion by AcMNPV gp64 than BmNPV gp64 in syncia forming assays. But it is unclear how gp64 could facilitate nuclear entry in trans, in the co-infection studies (105). This study also illustrates the diversity amongst the numerous cell lines support AcMNPV replication. When the block to nuclear entry was relieved by pseudotyping BmNPV with AcMNPV gp64, BmNPV could replicate efficiently in TnHi5 but not Sf9 cells, indicating another block to infection. The additional block for infection in Sf9 cells may be related to trafficking and function of p143, p35, or both in these cells (5). See below.

BACULOVIRUS GENES THAT AFFECT HOST-RANGE

With the exception of the study reported above, most available evidence indicates that the restriction to baculovirus replication in cultured insect cells occurs subsequent to virus entry. However, infection is blocked at different steps in the replication cycle depending on the specific combination of virus and cell line (44, 71, 86, 88, 94, 123). These steps, that include transport to the site of replication, viral gene expression, and generation of viral progeny, require complex interactions between the virus and its host cell. Hundreds of cell lines have been established from lepidopteran cells, (37, 38, 83) and have the potential to provide a wealth of knowledge regarding the molecular bases of baculovirus host-range. However this resource is largely untapped. As described in the following paragraphs, studying the interactions of baculoviruses in a few of these cell lines has identified six genes that affect host-range. Several of these were confirmed to alter baculovirus host-range in insects.

Studies of NPV replication in cultured cells identified *p35*, the viral DNA helicase *p143*, *lef7*, *ie-2*, *hcf-1*, and *hrf-1* as virus genes that affect baculovirus host-range or tissue tropism. The majority of these were identified in AcMNPV. Among these, *p143* is a core gene found in all baculoviruses that is required for viral DNA replication. The rest are auxiliary genes found in only a few baculoviruses or groups of baculoviruses. All but *hrf-1* encode AcMNPV late expression factors (*lefs*), proteins required for late gene expression with roles in late gene transcription, DNA replication, or both (80, 107, 113). In addition to changing host-range in cultured cells, changes in the AcMNPV *p143* coding sequence or insertion of the

hrf-1 gene into the AcMNPV genome expanded AcMNPV host-range in insects. In contrast, deleting *p35*, *lef7*, or *hcf-1* reduced the host-range of AcMNPV in cultured cells. Deleting *p35* also reduced AcMNPV host-range in insects. These genes appear have very different functions, but with the exception of p35, our understanding of how the products of these genes affect host-range is still limited. The effects of these genes on baculovirus host-range were reviewed previously (90, 110), therefore we will briefly recap what was previously reported and present the results of subsequent research. In addition, we introduce another example of a baculovirus cell culture system currently under investigation that may reveal additional baculovirus host-range factor genes. These co-infection studies of *Thysanoplusia orichalcea* M NPV (ThorMNPV) and AcMNPV suggest that AcMNPV encodes a factor, or alternatively induces expression of a host factor, that expands the host-range of ThorMNPV.

The viral DNA helicase gene, *p143*, was the first baculovirus gene to be associated with virus host-range. The involvement of *p143* in host-range was revealed when BmNPV and AcMNPV were used to co-infect TN368 cells and Sf21 cells, which support replication of AcMNPV but not BmNPV. Recombinant viruses arose that were also infectious for BmN cells, a cell line that does not normally support productive AcMNPV replication (73). These viruses had broader host-range in cultured cells, replicating in BmN as well as in Tn368 and Sf21 cells. They were also infectious for *B. mori* larvae when BV was injected and when larvae were orally infected with OBs (73). Repeated backcrossing of recombinants, by co-infecting with AcMNPV, resulted in a virus nearly identical to AcMNPV, called eh2-AcNPV that maintained the expanded host-range phenotype for BmN cells. These viruses had a small insert of BmNPV genomic DNA that mapped to a 600 bp region of *p143* (85). A role for *p143* in AcMNPV host-range expansion in cultured cells and larvae was confirmed independently with different *B. mori* cell lines and a different isolate of AcMNPV (2, 24). In these studies, recombinant viruses were isolated following co- transfection of Sf9 cells with AcMNPV DNA and BmNPV restriction fragments. These studies further refined the requisite changes in AcMNPV p143 amino acid sequence, to that of BmNPV p143 sequence, to three residues, Leu556, Asn564 and Leu577 for infection of cultured *B. mori* cells (24). The latter two amino acid changes (Asn564 and Leu577) were sufficient for AcMNPV to infect and kill *B. mori* larvae when BV was injected (2). These changes in the amino acid sequence of AcMNPV p143 were thought to reduce cytotoxicity caused by AcMNPV replication in the *B. mori* cells (68). When BmN cells are infected with AcMNPV, or co-infected with BmNPV and AcMNPV, a cytopathic effect is observed in which translation of both host and virus genes are attenuated. This effect was not observed when the cells were co-infected with BmNPV and eh2-AcNPV. It should be noted that not all isolates of AcMNPV induce the same degree of cytopathic effect in BmN cells. For example the AcMNPV L1 isolate did not inhibit BmNPV replication in co-infection experiments (68). It is possible that the observed cytotoxicity is the result of aberrant DNA replication or other activities of p143, as *p143* is expressed at higher levels in AcMNPV-infected BmN cells than in Sf9 cells (65). Although the changes in AcMNPV p143 sequence allow

AcMNPV to replicate in *B. mori* cells and larvae as well as in Sf9 cells when infected at high multiplicity of infection (moi), the altered p143 may not be fully functional in Sf9 cells since eh2- AcMNPV infection aborts at an early stage in the majority of cells when infected at a low moi (69).

BmNPV does not replicate in Sf9 or Sf21 cells, but it does not cause the type of cytotoxic effect that is observed when AcMNPV infects BmN cells (73, 86, 105). It is likely that the lack of cytotoxicity is because of low virus activity. BmNPV genes are expressed very poorly in Sf9 and Sf21 cells and there is little evidence of DNA replication (65, 73, 86, 105). These results are consistent with the findings of Katou *et al*. that indicated that transport of BmNPV to the nucleus was blocked in most but not all Sf9 cells. Yet, BmNPV failed to replicate efficiently in Sf9 cells even when the problem of transport was solved (71). Given the ability of a few amino acid changes in AcMNPV p143 to expand its host-range to *B. mori* larvae and cell lines, might problems with BmNPV p143 function be responsible for its inability to replicate in the Sf9 cells? A study that examined the ability of BmNPV *lefs* to functionally substitute for AcMNPV *lefs* in transient assays suggests this is the case (5). Nineteen AcMNPV *lef* genes were identified using a transient assay in which cloned fragments of AcMNPV genomic DNA were assayed for their ability to activate expression of a reporter gene driven by a late AcMNPV promoter (100, 113). In this study BmNPV *p143* was one of only three BmNPV *lefs*, out of the 19 *lefs*, that could not functionally substitute for its AcMNPV counterpart in Sf21 cells in this assay (5). The others *ie-2* and *p35* have also been implicated in baculovirus host-range (see below). They found that the BmNPV p143 protein was not transported to the nucleus. Because lef-3 has been shown to interact with p143 and facilitate p143 nuclear transport (135), plasmids expressing Bm *p143* and Bm *lef3* were substituted together. Although Bm *lef3* alone successfully replaced Ac *lef3* in the transient expression assay, co-transfecting Bm *lef3* and Bm *p143* abolished reporter gene expression and Bm p143 was not transported to the nucleus. Mutating the AcMNPV p143 sequence to the BmNPV p143 sequence from Ser to Asn at residue 564 or Phe to Leu at 577 (2, 24), individually, restored late gene expression when the altered AcMNPV *p143* was expressed with either AcMNPV or BmNPV *lef3* (5). However, AcMNPV *p143* with both mutations had little activity when expressed with either AcMNPV or BmNPV *lef3*, and less with BmNPN *lef3* than AcMNPV *lef3*. AcMNPV p143 with the double mutation physically associated with both BmNPV and AcMNPV *lef3* and localized to the nucleus. Bm p143 with reciprocal mutations, to the AcMNPV sequence, e.g., Asn to Ser at residue 564 or Leu to Phe at 577, or at both residues, were unable to substitute for AcMNPV p143, with either BmNPV or AcMNPV lef3, to activate reporter gene expression. Moreover, the modified BmNPV p143 did not physically interact with either lef3 and did not localized to the nucleus. No reporter gene activity was observed when Sf21 cells were transfected with all BmNPV *lefs*, and AcMNPV *p35*. Low level reporter gene expression was observed when BmNPV *p143* was replaced with AcMNPV *p143* in these assays. These observations support the idea that the inability of BmNPV p143 to properly function in Sf21 cells contributes to, but is probably not the sole reason, for the inability of BmNPV to replicate in Sf21 cells.

AcMNPV p35 functions as a host-range factor because deleting the *p35* gene reduces the ability of AcMNPV to infect Sf21 cells (20, 53) and *S. frugiperda* larvae (21, 22). For larval infections the route of infection was important. *S. frugiperda* larvae were 1000 fold less susceptible to Δp35AcMNPV ODV delivered orally via OBs than to injected BV (22). This may be due to elimination of infected midgut cells during primary infection before the virus can spread systemically (124, 126, 128, 138). The ability of Δp35AcMNPV to infect Sf21 cells was restored when it carried a different apoptotic suppressor gene, inhibitor of apoptosis (*iap*) from the *Cydia pomonella* GV (25), suggesting that the role of p35 in AcMNPV host-range is preventing virus-induced apoptosis in *S. frugiperda* cells and larvae. When tissues of AcMNPV-infected *S. frugiperda* larvae were analyzed for apoptosis by TUNEL assay, those injected with Δp35AcMNPV showed higher numbers of TUNEL positive cells relative to a revertant virus, further supporting this idea (18). Similar studies have not been done using an oral route of infection. Few other baculoviruses carry *p35* or the related apoptotic suppressor *p49* (27, 139), but most carry one or more copies of *iap*. Among the 48 baculoviruses, of all genera, sequenced to date seven carry *p35* and four carry *p49*. Possible roles for the various apoptotic suppressors in virus host-range have not been extensively studied. P35 may be one of the factors that contribute towards the narrow host-range of BmNPV relative to AcMNPV as BmNPV p35 does not appear to be functional in Sf21 cells (5), was not essential for BmNPV replication in BmN cells or larvae (36, 70), and is less effective than AcMNPV p35 in blocking apoptosis in mammalian cells (92). The roles of *p35* and two *iaps* carried by AcMNPV were tested in six cell lines, Sf21, Tn368 and four additional cell lines. Deleting both *iaps* had no effect on AcMNPV infection in any cell line. Whereas deleting *p35* resulted in apoptosis in AcMNPV-infected Ld652Y and Sl2 cells, two cell lines that do not support AcMNPV replication, and had no effect in *M. brassicae* or *Panolis flammea* cells (41). Beyond these reports, to our knowledge, roles for the apoptotic suppressors of other baculoviruses in determining their host- ranges have not been investigated.

Lef7 genes have been found only in baculoviruses and were identified in ten out of the 44 fully sequenced baculoviruses, all of them type I NPVs. *Lef7* was identified in transient assay screens for AcMNPV genes required for enhancing late gene expression in Sf21 cells (95). However when the *lefs* required for late gene expression in Sf21 cells were assayed in Tn368 cells *lef7* was non-essential, as were *p35*, and *ie-2* (79). Further support for the role of *lef7* as a host-range factor was provided when deletion of *lef7* was identified as the defect in two AcMNPV mutants that were defective for AcMNPV replication in Sf21 cells but not Tn368 cells (14). Sf21 cells infected with AcMNPV lacking *lef7* have dramatically reduced titers, produce few if any OBs, and DNA replication is barely detectable (14). The effects of *lef7* deletion on AcMNPV infectivity in *S. frugiperda* and *T. ni* larvae have not been investigated. *Lef7* is also non-essential for BmNPV replication in BmN cells (36). In BmN cells infected with *lef7* mutated BmNPV, viral DNA replication was reduced and late transcription was enhanced relative to wt BmNPV, but there was no noticeable effect on BV production. Moreover, deleting *lef7* did not impair BmNPV

infectivity for *B. mori* larvae when BV was injected (36). Together these studies suggest that *lef7* functions as a cell or tissue specific factor required either directly or indirectly for viral DNA replication.

Hcf-1 is a gene from AcMNPV that is necessary for it to infect *Trichoplusia ni* cell lines (79, 81). When Tn368 cells were transfected with the AcMNPV *lef* library in transient expression assays as described above, reporter gene expression was weak. This suggested that additional *lefs* were required for AcMNPV late gene expression in Tn368 cells. Further screening revealed a single additional gene required for gene expression in Tn368 cells. This gene corresponded to AcMNPV ORF70 and was renamed host cell factor 1, *hcf-1* (79). *Hcf-1* has no homologues outside of baculovirus, and a paucity of motifs that might suggest a function. The predicted protein is rich in cysteine residues and has a putative C4C4 RING finger motif (1, 48). RING motifs coordinate zinc ions and are associated with various cellular functions including, protein-protein interaction and ubiquitin ligase activity (10, 67, 108). Interestingly, *hcf-1* is one of only three *lef* genes found in the AcMNPV genome, but missing from the BmNPV genome (35). In fact *hcf-1* has been identified in only 4 of the 44 completely sequenced baculovirus genomes, AcMNPV (L22858), RoMNPV (AY145471), *Plutella xylostella* NPV (PlxyNPV) (DQ457003), and *Clanis bilineata* NPV (ClbiNPV) (DQ504428). PlxyNPV and RoMNPV are type I NPVs, closely related to AcMNPV, and *hcf-1* shares 99 and 84% amino acid identity and 99 and 91% similarity with AcMNPV *hcf-1*, respectively. In contrast, ClbiNPV is a type II NPV and shares only 24% identity, and 43% similarity with AcMNPV hcf-1.

AcMNPV lacking *hcf-1* replicates in Sf21 cells but its replication is impaired in *T. ni* cell lines (81). However, the effects are much more severe in Tn368 cells than in TnHi5 cells. In TnHi5 cells production of OBs were delayed by approximately 24 h and although OBs were observed in most cells by 72 h pi, they differed morphologically from those in wt AcMNPV-infected cells. In contrast, no OBs were observed in Tn368 cells infected with *hcf-1* deletion virus. A slight increase in BV titer was observed over time in Tn368 cells infected by an *hcf-1* deletion mutant, but translation of virus and host genes arrested by 18 h pi. Moreover viral DNA replication was barely detectable and the viral capsid protein could not be detected on Western blots. Deleting *hcf-1* had no effect on AcMNPV oral infectivity for *T. ni* larvae, although it did reduce its virulence. However when *hcf-1* mutant virus BV was injected there was a significant reduction in infectivity for both *S. frugiperda* and *T. ni* larvae, as well as reduced virulence in *T. ni*, relative to wt AcMNPV. Thus *hcf-1* functions more like a virulence factor than a host-range determinant for AcMNPV in *T. ni* larvae, and appears to have an important role in cell or tissue tropism within the insect. Whether it might have a more definitive effect on larval host-range in another host species has not been determined. It would be interesting to determine if the addition of *hcf-1* to the BmNPV genome would affect its ability to infect *T. ni* cells. Although the specific role for *hcf-1* in infection is still unclear, its activity appears to require self-association and nuclear localization (51). Hcf-1 nuclear staining is diffuse in transfected cells but shows distinct punctate foci in infected cells, suggesting that it may localize to promyelocytic leukemia (PML) nuclear bodies (132). This suggests it

could play a role in viral DNA replication or possibly immune response (4, 31). AcMNPV ie-2 and PE38, immediate early transcription factors also localize to these nuclear regions in transfected Sf21 cells (74), and associate with PML in mammalian cells (97). Like hcf-1, ie-2 and PE38 have RING fingers but they are not the C4C4 type found in hcf-1. Mutational analysis of hcf-1 function in AcMNPV infection implicates the RING-finger domain in its ability to support virus replication and to self-associate (132).

AcMNPV *ie-2*, also referred to as ie-N, is a transcription factor that stimulates transcription by the essential early transcription factor ie-1 (12). It was identified as an AcMNPV *lef* gene in Sf21 cells in transient expression assays where it stimulates late gene expression and plays a role in viral DNA replication (80, 100). In contrast to Sf21 cells, *ie-2* was not necessary for late gene expression in Tn368 or Hi5 cells in transient expression assays, suggesting it was a host-range gene (79). The *ie-2* gene is found in approximately one quarter of sequenced baculovirus genomes, all type I NPVs. It has no known homologues outside of the baculoviruses and is characterized by the presence of a RING finger. The RING finger confers the ability to arrest the cell cycle in Sf21 and Tn368 cells, but is not involved in its ability to stimulate transcription (104). Mutant AcMNPV in which *ie-2* is deleted or mutated in domains involved in either transcriptional activation or cell cycle arrest were viable in Sf21, Tn368, and TnHi5 cells. All of these mutants exhibited some level of deficiency in DNA replication, transcription, or production of BV and OBs in Sf21, Tn368, and TnHi5 cells, although the deficiencies were more severe in Sf21 cells than either *T. ni* cell line. All of these *ie-2* mutant AcMNPV viruses exhibited reduced oral infectivity against *S. frugiperda* and *T. ni* larvae, but no differences in infectivity, relative to wt AcMNPV, when BV was injected. The reduction in oral infectivity could be attributed to a reduction in the number of ODV imbedded in OBs produced by mutant virus infections, rather than to functional deficiencies of ie-2 protein. BmNPV *ie-2* mutants were also viable and produced normal levels of BV, however DNA replication was delayed and reduced relative to wt virus (36). These studies suggest that *ie-2* is somewhat more important for AcMNPV replication in Sf21 cells than in *T. ni* cell lines. These cell line specific effects suggest that it may be involved in cell or tissue tropism, but it does not appear to play a significant role in baculovirus host-range. Based on the BmNPV studies, the defect in infections by *ie-2* deletion mutants is reduced viral DNA replication. How *ie-2* influences the number of ODV embedded in polyhedra is unknown, but it could simply be due to a reduction in the number of enveloped virions present in the nucleus during the occlusion process.

A baculovirus gene, *hrf-1*, was identified in a genetic screen for baculovirus host-range factors from *L. dispar* M NPV (LdMNPV) (112). This screen was based on the observation that AcMNPV progeny are not produced in Ld652Y, but the block to AcMNPV replication could be relieved by super-infecting AcMNPV-infected Ld652Y cells with LdMNPV (87, 88). The *hrf-1* gene encodes a 26 kDa protein with no known motifs to suggest its function (112). To date, only one *hrf-1* homolog has been identified and it is found in another baculovirus, *Orgyia pseudotsugata* M NPV (OpMNPV), which also replicates in Ld652Y cells. OpMNPV encodes a carboxy-terminal-truncated

version of the LdMNPV hrf-1 with low sequence identity. It is 78 amino acids long compared to the 218 amino acids comprising LdMNPV hrf-1 (59, 112). AcMNPV infection of Ld652Y cells induces a cytopathic effect and all temporal classes of virus genes are transcribed, viral DNA replication is observed, but translation of both virus and host proteins arrests by approximately 12 h post infection (44, 88, 94). AcMNPV recombinant viruses bearing *hrf-1* productively infect Ld652Y cells and translation arrest is rescued (28, 112). Moreover AcMNPV bearing *hrf-1* was orally infectious *L. dispar* larvae, which are not susceptible to AcMNPV infection (13). In contrast, the OpMNPV *hrf-1* homolog did not rescue translation arrest or support AcMNPV replication in Ld652Y cells. AcMNPV bearing a chimeric *hrf-1* comprised of the OpMNPV *hrf-1* fused to the carboxy-terminal coding region of the LdMNPV *hrf-1* also failed to replicate in Ld652Y cells or rescue translation arrest (59). Because the OpMNPV *hrf-1* is not functional in the context of AcMNPV-infected Ld652Y cells it is unclear if it is a functional homolog of LdMNPV *hrf-1*. However, it could play a role in the ability of OpMNPV to replicate in Ld652Y cells.

Neither the cause of translation arrest in Ld652Y cells, nor the mechanisms by which *hrf-1* rescues translation arrest and expands the host-range of AcMNPV are understood. Hrf-1 does not function as an apoptotic suppressor. AcMNPV *p35* is expressed and functions to prevent apoptosis in AcMNPV-infected Ld652Y cells (29). Hrf-1 could not prevent apoptosis in the absence of *p35*, although it did prevent global translation arrest in apoptotic cells prior to their final destruction (29). There does, however, appear to be a connection between p35 activity and translation arrest. In recombinant AcMNPV-infected Ld652Y cells, the presence of *p35* in the absence of *hrf-1* correlated with global translation arrest, suggesting that the activity of p35 might trigger translation arrest. In cells treated with the DNA synthesis inhibitor aphidicolin, translation is attenuated rather than fully arrested, but strikingly, is only observed in Ld652Y cells infected with AcMNPV expressing *p35* but not *hrf-1 (29, 111)*. Infection with a AcMNPV *p143* mutant, a temperature sensitive replication mutant, had a similar effect as aphidicolin on translation arrest in wt AcMNPV-infected Ld652Y cells (111). These two observations suggest that early activity of p35 plays some role in initiating translation arrest, but an event associated with DNA replication or other event in the late stage of the virus replication is what drives global translation arrest. Apoptosis in Sf21 cells infected with *p35* deletion mutants exhibits an identical pattern, early initiation but a dramatic increase at the onset of DNA replication in the late stage of infection (76).

The anti-apoptotic function of p35 clearly plays a role in inducing translation arrest either directly or indirectly (29, 111). Infecting Ld652Y cells with AcMNPV expressing p35 with functional mutations that do not block apoptosis (6), does not induce global translation arrest (6, 111). Moreover, AcMNPV expressing other baculovirus apoptotic suppressors, OpMNPV iap3, CpGV iap3, or SlNPV p49, in lieu of p35 induce global translation arrest in a manner identical to p35. Surprisingly, treating cells with peptide caspase inhibitors to block apoptosis in Ld652Y cells infected with the AcMNPV *p35* deletion mutant did not result in translation arrest. The inability of peptide inhibitors of apoptosis to induce translation arrest in

AcMNPV-infected Ld652Y cells, while effectively inhibiting apoptosis, suggests that baculovirus apoptotic suppressors have additional functions beyond simply preventing apoptosis. The nature of this function is puzzling since two very different types of apoptotic suppressor proteins, P35-like and IAP, that prevent apoptosis by different mechanisms (19), have the same effect on translation in the AcMNPV-infected Ld652Y cells. One possibility is that the viral proteins not only prevent apoptosis, but stimulate viral DNA replication, late gene transcription, or other activity late in the virus replication cycle, in a way that the caspase inhibitors cannot fully mimic. These late events in the virus replication cycle might then in turn drive or enhance the translation arrest mechanism. Given the differences amongst the different types of baculovirus apoptotic suppressors, it is unclear how each might stimulate late events in the AcMNPV replication cycle.

Mutagenesis of *hrf-1* provided few clues to its function (59). Truncating the protein at either the amino- or the carboxyl-termini of the protein and two-amino acid insertional mutations throughout the length of the protein abolished its ability to rescue translation arrest. As noted above chimeric hrf-1 in which the amino terminus was replaced with hrf-1 from OpMNPV did not prevent translation arrest, suggesting the amino terminus of hrf-1 is important for its function. The mutant proteins also appeared to be unstable in mutant AcMNPV-infected Ld652Y cells, but not in Sf21 cells. Although lacking any characteristic functional motifs, hrf-1 possesses one highly acidic domain. Some of the mutations in this region reduced or abolished translation arrest whereas others had little effect. These results suggested the structure of hrf-1 was important for its function and stability and that the acidic region might play a functional role in rescuing translation arrest. Reasons for the differences in expression and/or stability of hrf-1 mutant proteins in Ld652Y relative to Sf21 cells are unknown.

Regardless of how *hrf-1* functions it appears to be a critical virus factor for infecting Ld652Y cells. Although Ld652Y cells are permissive for LdMNPV and Op-MNPV replication, they do not support the replication of a number of other baculoviruses. In screens of Ld652Y cells with seven different baculoviruses, including AcMNPV, apoptosis was induced to some degree by *Hyphantia cunea* NPV (HycuNPV), *Spodoptera exigua* NPV (SeMNPV), *Spodoptera littoralis* NPV (SpltNPV), BmNPV, and OpMNPV (63). Apoptosis was most pronounced in HycuNPV-infected cells and the least in the OpMNPV-infected cells. In a subsequent study treating HycuNPV-infected Ld652Y cells with peptide apoptotic suppressors prevented apoptosis, but progeny virus was not produced (62). As in AcMNPV-infected Ld652Y cells, translation arrested in HycuNPV-infected Ld652Y cells by 12 h pi. However, when Ld652Y cells were infected with a recombinant HycuNPV bearing *hrf*-1 progeny BV were produced and translation arrest was prevented. Even with the addition of peptide caspase inhibitors no OBs were observed and no polyhedrin protein was produced in Ld652Y cells infected with HycuNPV bearing *hrf-1*. No virus progeny were produced in BmNPV-, SeMNPV-, or SpltNPV- infected Ld652Y cells. Expressing *hrf-1* from a transfected plasmid promoted productive BmNPV and SeMNPV, but not SpltNPV, infections. In contrast to HycuNPV-infected cells,

both BV and OBs were produced. This study suggested that translation arrest could be a cellular mechanism for limiting baculovirus infection by Ld652Y cells. In the case of HycuNPV there is an additional block to a very late events in infection preventing OB production, but the nature of that block is currently unknown. Surprisingly, when *hrf-1* was incorporated into BmNPV genome, it was less effective in promoting BmNPV replication in Ld652Y cells than *hrf-1* supplied in trans (64). The reason for this difference is unclear, but could possibly be due to lower levels of *hrf-1* expression in the context of virus infection, because pre-transfecting *hrf-1*-expressing plasmids increased the expression of late virus proteins.

A NEW BACULOVIRUS CELL LINE COMBINATION

Thysanoplusia orichalcea M NPV (ThorMNPV) is a recently discovered NPV that exhibits a relatively narrow host-range in insect cell lines and larvae (17, 123). Phylogenetic analysis using *polh*, *lef-8* and *lef-9* genes predicted that ThorMNPV is clustered with AcMNPV in the group I NPV (17). ThorMNPV replicates in TnHi5 cells very well and high BV titers can be obtained (17, 123). Using a recombinant Thor-MNPV containing a GFP-reporter controlled by the AcMNPV polyhedrin promoter, vThGFP, eight of 28 cell lines tested were permissive at different degrees for vThGFP as determined by green fluorescence (123). In contrast to TnHi5 cells in which over 95% of cells fluoresced and produced OBs by 3 days p.i., only a few Sf21 cells (<1%) were infected by 3 days p.i.

Co-infection studies of vThGFP and vAcRed, a recombinant AcMNPV expressing the red fluorescent protein (RFP) gene from the polyhedrin promoter, in Sf21 cells demonstrated that AcMNPV could promote ThorMNPV replication in the Sf21 cells. In the co-infected cells, 65% of the cells showed only green fluorescence indicating ThGFP replication, 33% of the cells showed red fluorescence indicating vAcRed replication, and about 2% of cells showed yellow when GFP and RFP channel images were merged, indicating replication of both vThGFP and vAcRed in the same cells. OBs were formed in majority of cells expressing GFP by vThGFP. It was surprising that so few cells expressed both GFP and RFP, indicating that replication of one virus inhibited replication of the other virus (16, 123).

There are at least three possible explanations for the enhanced infectivity of vThGFP by vAcRed in Sf21 cells: recombination between the viruses, an AcMNPV gene product acting in *trans*, or secretion of a cellular (or virus) factor from the AcMNPV-infected cells. Enhanced infectivity of vThGFP does not appear to be the result of recombination with vAcRed, since no recombinant virus could be isolated from co-infected Sf21cells (16, 123). The possibility that AcRed provided trans-activation factors that enhanced vThGFP infection in Sf21 also appears unlikely because co-transfecting Sf21 cells with an AcMNPV cosmid library failed to identify any gene(s) that could enhance vThGFP infection of Sf21 cells. However, centrifuged media from AcMNPV-infected Sf21 cells enhanced vThGFP infection of Sf21 cells 15-fold (123), supporting the third hypothesis. At the present time the secreted factor from AcMNPV-infected Sf21 cells has not been identified.

At the organismal level, ThorMNPV is highly virulent to three species, *T. orichalcea*, *T. ni,* and *Pseudoplusia includens*. Other insects such as *S. frugi-*

perda, *S. exigua,* and *H. zea* are resistant to Thor-MNPV (17). When the larvae of resistant species were fed vThGFP OBs, no mortality was observed and all larvae developed into pupa and then adults. However, hemocytes, trachea and Malpighian tubules showed GFP fluorescence demonstrating secondary spread of infection from the midgut, although no OBs were observed in any of these tissues. GFP expression was not detected in fat bodies from any of the three resistant insects and may be the reason oral infection with vThGFP OBs could not kill these hosts because fat body is the major tissue of NPV replication in native hosts. Levels of infection in the other tissues where fluorescence was observed, hemocytes, tracheal and Malpighian tubules, may not be sufficient to kill the insect or impede development (123). Intrahemocoelic injection of vThGFP BVs to these nonsusceptible insects (*S. frugiperda, S. exigua* and *H. zea*) produced high mortality, which suggested that although BV was produced in the midgut in orally infected insects and was able to spread to a limited number of tissues, the levels of BV were not sufficient to initiate a robust secondary infection (123).

FUTURE PERSPECTIVES

All of the host-range genes identified, to date, were isolated functionally using virus infected insect cells in culture. Each relied on the availability of systems of baculoviruses and cell lines with suitable properties for identifying a host-range determinant, such as the closely related AcMNPV and BmNPV with distinctly different host-ranges in cultured cells. With advances in rapid DNA sequencing technology coupled with decreasing costs, genomic analysis promises to be an important and powerful tool for increased understanding of how baculovirus host-range is determined and for identifying the genes responsible (52, 115). If the currently known host-range genes are reasonable examples, additional baculovirus host-range genes are likely to be found amongst the unique or less widely distributed auxiliary baculovirus genes.

Understanding the role of insect immunity in restricting baculovirus host-range at the organismal level is among the most intriguing questions still remaining. Research to date suggests that the relative ability of the host insect to resist or eliminate virus infection plays an important role in determining baculovirus host-range. *In vivo* studies using marked viruses and immune suppressors demonstrated that highly resistant species could be infected if their immune systems were compromised (125, 127). Genetic studies with susceptible and resistant *B. mori* strains reveal a dominant gene or set of linked genes to be responsible for *B. mori* resistance to AcMNPV infection, but are not yet identified or cloned (43). In this study, AcMNPV BV was injected into the larvae, so it is also not clear whether or not the gene(s) affect *B. mori* resistance to AcMNPV oral infection. It is also not known if *B. mori* resistance to AcMNPV is part of an immune response. As discussed above, cellular responses such as apoptosis and translation arrest can prevent or reduce productive baculovirus infection in a cell-line-specific manner. But how the cell senses that it is infected and signals for a response is unknown. Both responses also appear to be important at the organismal level. Apoptosis is considered an immune response against virus infection and at least for AcMNPV, the ability to prevent apoptosis in *S. frugiperda* due to the activity of p35 broadens its host-range (18, 21, 22). It is likely that specific apoptotic

suppressors borne by other baculoviruses are responsible for their abilities to infect specific insect species, tissues, or cells. Translation arrest is associated with abortive baculovirus infection in cell culture and has been observed in AcMNPV-infected BmN cells, AcMNPV *hcf-1* deletion mutant-infected Tn368 cells, and Ld652Y cells infected with AcMNPV, HycuNPV, or BmNPV (44, 62, 68, 81). Translation arrest occurs as part of the innate immune response to virus infection in vertebrates, as a component of the interferon response signaled through Toll-like receptors (TLRs) (106), but it is unclear if it plays a similar role in insect defenses against virus infection. Recent advances in understanding insect immune responses to virus infection, that to date are limited to RNA viruses, and a few insect species and no Lepidoptera, suggests that the Toll- and Jak/Stat signaling pathways are involved in insect responses to virus infection (50, 136). The completion of the *B. mori* genome and identification of conserved genes involved in immune response in *Drosophila*, *Aedes aegypti*, and *Anopheles gambiae* may help to identify the genes responsible for lepidopteran immune responses to virus infection (15, 91, 129).

References

1. **Albert T K, Hanzawa H, Legtenberg Y I A, *et al.*** 2002. Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. **Embo J,** 21 (3): 355-364.
2. **Argaud O, Croizier L, Lopez-Ferber M, *et al.*** 1998. Two key mutations in the host-range specificity domain of the p143 gene of *Autographa californica* nucleopolyhedrovirus are required to kill *Bombyx mori* larvae. **J Gen Virol,** 79: 931-935.
3. **Benz G A**. 1986. Introduction: Historical perspectives. In: **The biology of baculoviruses** (Granados R R, Federici B A, ed.), vol.1. Biological properties and molecular biology. Boca Raton: CRC Press.
4. **Bernardi R, Pandolfi P P.** 2007. Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. **Nat Rev Mol Cell Biol,** 8 (12): 1006-1016.
5. **Berretta M F, Deshpande M, Crouch E A, *et al.*** 2006. Functional characterization of *Bombyx mori* nucleopolyhedrovirus late gene transcription and genome replication factors in the non-permissive insect cell line SF-21. **Virology,** 348 (1): 175-189.
6. **Bertin J, Mendrysa S M, LaCount D J, *et al.*** 1996. Apoptotic suppression by baculovirus P35 involves cleavage by and inhibition of a virus-induced CED-3/ ICE-like protease. **J Virol,** 70 (9): 6251-6259.
7. **Black B C, Brennan L A, Dierks P M, *et al.*** 1997. Commercialization of baculovirus insecticides, p. 341-387. In: **The Baculoviruses** (Miller L K,ed.),. New York: Plenum Press.
8. **Blissard G W.** 1996. Baculovirus-insect cell interactions. **Cytotechnology,** 20 (1-3): 73-93.
9. **Blissard G W, Wenz J R.** 1992. Baculovirus gp64 envelope glycoprotein is sufficient to mediate pH-dependent membrane-fusion. **J Virol,** 66 (11): 6829-6835.
10. **Borden K L B.** 2000. RING domains: Master builders of molecular scaffolds? **J Mol Biol,** 295 (5): 1103-1112.
11. **Boyce F M, Bucher N L R.** 1996. Baculovirus-mediated gene transfer into mammalian cells. **Proc Natl Acad Sci U S A,** 93 (6): 2348-2352.
12. **Carson D D, Guarino L A, Summers M D.** 1988. Functional mapping of an AcNPV immediately early gene which augments expression of the ie-1 trans-activated 39k-gene. **Virology,** 162 (2): 444-451.
13. **Chen C J, Quentin M E, Brennan L A, *et al.*** 1998. *Lymantria dispar* nucleopolyhedrovirus *hrf-1* expands the larval host range of *Autographa californica* nucleopolyhedrovirus. **J Virol,** 72 (3): 2526-2531.
14. **Chen C J, Thiem S M.** 1997. Differential infectivity of two *Autographa californica* nucleopolyhedrovirus mutants on three permissive cell lines is the result of *lef-7* deletion. **Virology,** 227 (1): 88-95.
15. **Cheng T C, Zhang Y L, Liu C, *et al.*** 2008. Identification and analysis of Toll-related genes in the domesticated silkworm, *Bombyx mori*. **Dev Comp Immunol,** 32 (5): 464-475.
16. **Cheng X-W, Lynn D E**. 2009. Virus-virus interaction *in vitro* and *in vivo*. **Adv Appl Microbiol**, 68:218-239.

17. **Cheng X W, Carner G K, Lange M, *et al.*** 2005. Biological and molecular characterization of a multicapsid nueleopolyhedrovirus from *Thysanoplusia orichalcea* (L.) (Lepidoptera : Noctuidae). **J Invertebr Pathol,** 88 (2): 126-135.
18. **Clarke T E, Clem R J.** 2003. *In vivo* induction of apoptosis correlating with reduced infectivity during baculovirus infection. **J Virol,** 77 (3): 2227-2232.
19. **Clem R J.** 2007. Baculoviruses and apoptosis: A diversity of genes and responses. **Current Drug Targets,** 8 (10): 1069-1074.
20. **Clem R J, Fechheimer M, Miller L K.** 1991. Prevention of apoptosis by a baculovirus gene during infection of insect cells. **Science,** 254 (5036): 1388-1390.
21. **Clem R J, Miller L K.** 1993. Apoptosis reduces both the invitro replication and the invivo infectivity of a baculovirus. **J Virol,** 67 (7): 3730-3738.
22. **Clem R J, Robson M, Miller L K.** 1994. Influence of infection route on the infectivity of baculovirus mutants lacking the apoptosis-inhibiting gene P35 and the adjacent gene P94. **J Virol,** 68 (10): 6759-6762.
23. **Condreay J P, Kost T A.** 2007. Baculovirus expression vectors for insect and mammalian cells. **Curr Drug Targets,** 8:1126-1131.
24. **Croizier G, Croizier L, Argaud O, *et al.*** 1994. Extension of *Autographa-californica* nuclear polyhedrosis-virus host-range by interspecific replacement of a short DNA-sequence in the P143 helicase gene. **Proc Natl Acad Sci USA,** 91 (1): 48-52.
25. **Crook N E, Clem R J, Miller L K.** 1993. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. **J Virol,** 67 (4): 2168-2174.
26. **Doyle C J, Hirst M L, Cory J S, *et al.*** 1990. Risk assessment studies-Detailed host range testing of wild-type cabbage moth, *Mamestra-Brassicae* (Lepidoptera, Noctuidae), Nuclear Polyhedrosis-Virus. **Appl Environ Microbiol,** 56 (9): 2704-2710.
27. **Du Q S, Lehavi D, Faktor O, *et al.*** 1999. Isolation of an apoptosis suppressor gene of the *Spodoptera littoralis* nucleopolyhedrovirus. **J Virol,** 73 (2): 1278-1285.
28. **Du X L, Thiem S M.** 1997. Characterization of host range factor 1 (*hrf-1*) expression in *Lymantria dispar* M nucleo-polyhedrovirus-and recombinant *Autographa californica* M nucleopolyhedrovirus-infected IPLB- Ld652Y cells. **Virology,** 227 (2): 420-430.
29. **Du X L, Thiem S M.** 1997. Responses of insect cells to baculovirus infection: Protein synthesis shutdown and apoptosis. **J Virol,** 71 (10): 7866-7872.
30. **Engelhard E K, Kammorgan L N W, Washburn J O, *et al.*** 1994. The insect tracheal system - a conduit for the systemic spread of *Autographa-californica*-M nuclear polyhedrosis-virus. **Proc Natl Acad Sci USA,** 91 (8): 3224-3227.
31. **Everett R D, Chelbi-Alix M K.** 2007. PML and PML nuclear bodies: Implications in antiviral defence. **Biochimie,** 89 (6-7): 819-830.
32. **Flipsen J T M, Martens J W M, Vanoers M M, *et al.*** 1995. Passage of *Autographa-californica* nuclear poly-hedrosis-virus through the midgut epithelium of *Spodoptera-exigua* larvae. **Virology,** 208 (1): 328-335.
33. **Friesen P D**. 2007. Insect Viruses, In: **Fields Virology** (Knipe D M, Howley P M. ed.), 5th ed, vol. 1. Phi-ladelphia, Lippincott Williams & Wilkins. p707-736.
34. **Funk C J, Braunagel S C, Rohrmann G F**. 1997. Baculovirus structure, In: **The Baculovirus** (Miller L K,ed.), New York: Plenum Press. p7-32.
35. **Gomi S, Majima K, Maeda S.** 1999. Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. **J Gen Virol,** 80: 1323-1337.
36. **Gomi S, Zhou C E, Yih W Y, *et al.*** 1997. Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. **Virology,** 230 (1): 35-47.
37. **Goodman C L, El Sayed G N, McIntosh A H, *et al.*** 2001. Establishment and characterization of insect cell lines from 10 lepidopteran species. **In Vitro Cell Dev Biol-Anim,** 37 (6): 367-373.
38. **Goodman C L, Wang A A, Nabli H, *et al.*** 2004. Development and partial characterization of heliothine cell lines from embryonic and differentiated tissues. **In Vitro Cell Dev Biol-Anim,** 40 (3-4): 89-94.
39. **Goodwin R H, Vaughn J L, Adams J R, *et al.*** 1970. Replication of a nuclear polyhedrosis virus in an established insect cell line. **J Invertebr Pathol,** 16 (2): 284-288.
40. **Granados R R, Derksen A C G, Dwyer K G.** 1986. Replication of the *Trichoplusia-ni* granulosis and nuclear polyhedrosis viruses in cell-cultures. **Virology,** 152 (2):

472-476.

41. **Griffiths C M, Barnett A L, Ayres M D, *et al.*** 1999. *In vitro* host range of *Autographa californica* nucleopolyhedrovirus recombinants lacking functional p35, iap1 or iap2. **J Gen Virol,** 80: 1055-1066.
42. **Gröner A**. 1986. Specificity and safety of baculoviruses, In:**The biology of baculoviruses** (Granados R R, Federici B A,ed.), vol. 1. Biological properties and molecular biology. Boca Raton:CRC Press. p177-202.
43. **Guo T Q, Wang S P, Guo X Y, *et al.*** 2005. Productive infection of *Autographa californica* nucleopolyhedrovirus in silkworm *Bombyx mori* strain Haoyue due to the absence of a host antiviral factor. **Virology,** 341 (2): 231-237.
44. **Guzo D, Rathburn H, Guthrie K, *et al.*** 1992. Viral and host cellular transcription in *Autographa-californica* nuclear polyhedrosis virus-infected gypsy-moth cell-lines. **J Virol,** 66 (5): 2966-2972.
45. **Haas-Stapleton E J, Washburn J O, Volkman L E.** 2003. Pathogenesis of *Autographa californica* M nucleopolyhedrovirus in fifth instar *Spodoptera frugiperda*. **J Gen. Virol,** 84: 2033-2040.
46. **Haas-Stapleton E J, Washburn J O, Volkman L E.** 2004. P74 mediates specific binding of *Autographa californica* M nucleopolyhedrovirus occlusion-derived virus to primary cellular targets in the midgut epithelia of *Heliothis virescens* larvae. **J Virol,** 78 (13): 6786-6791.
47. **Haas-Stapleton E J, Washburn J O, Volkman L E.** 2005. *Spodoptera frugiperda* resistance to oral infection by *Autographa californica* multiple nucleopolyhedrovirus linked to aberrant occlusion-derived virus binding in the midgut. **J Gen Virol,** 86: 1349-1355.
48. **Hanzawa H, de Ruwe M J, Albert T K, *et al.*** 2001. The structure of the C4C4 RING finger of human NOT4 reveals features distinct from those of C3HC4 RING fingers. **J Biol Chem,** 276 (13): 10185-10190.
49. **Harrison R L, Bonning B C.** 1999. The nucleopolyhedroviruses of *Rachiplusia ou* and *Anagrapha falcifera* are isolates of the same virus. **J Gen Virol,** 80: 2793-2798.
50. **Hedges L M, Johnson K N.** 2008. Induction of host defence responses by *Drosophila* C virus. **J Gen Virol,** 89: 1497-1501.
51. **Hefferon K L.** 2003. Characterization of HCF-1, a determinant of *Autographa californica* multiple nucleopolyhedrovirus host specificity. **Insect Mol Biol,** 12 (6): 651-658.
52. **Herniou E A, Olszewski J A, Cory J S, *et al.*** 2003. The genome sequence and evolution of baculoviruses. **Annu Rev Entomol,** 48: 211-234.
53. **Hershberger P A, Dickson J A, Friesen P D.** 1992. Site-specific mutagenesis of the 35-kilodalton protein gene encoded by *Autographa-californica* nuclear polyhedrosis-virus-cell line-specific effects on virus-replication. **J Virol,** 66 (9): 5525-5533.
54. **Hink W F.** 1970. Established insect cell line from cabbage looper, *Trichoplusia-ni*. **Nature,** 226 (5244): 466-467.
55. **Horton H M, Burand J P.** 1993. Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane-fusion. **J Virol,** 67 (4): 1860-1868.
56. **Hostetter D L, Puttler B.** 1991. A new broad host spectrum nuclear polyhedrosis-virus isolated from a celery looper, *Anagrapha-falcifera* (Kirby), (Lepidoptera, Noctuidae). **Environ Entomol,** 20 (5): 1480-1488.
57. **Ignoffo C M.** 1968. Specificity of insect viruses. **Bull Entomol Soc Am,** 14: 265-276.
58. **Ijkel W F J, Westenberg M, Goldbach R W, *et al.*** 2000. A novel baculovirus envelope fusion protein with a proprotein convertase cleavage site. **Virology,** 275 (1): 30-41.
59. **Ikeda M, Reimbold E A, Thiem S M.** 2005. Functional analysis of the baculovirus host range gene, *hrf-1*. **Virology,** 332: 602-613.
60. **Inceoglu A B, Kamita S G, Hinton A C, *et al.*** 2001. Recombinant baculoviruses for insect control. **Pest Management Science,** 57 (10): 981-987.
61. **International Committee on Taxonomy of Viruses.** 2008. **Virus Taxonomy 2008**. http://www.ictvonline.org/virusTaxonomy.asp?version=2008&bhcp=1.
62. **Ishikawa H, Ikeda M, Alves C A F, *et al.*** 2004. Host range factor 1 from *Lymantria dispar* nucleopolyhedrovirus (NPV) is an essential viral factor required for productive infection of NPVs in IPLB-Ld652Y cells derived from *L. dispar*. **J Virol,** 78 (22): 12703-12708.
63. **Ishikawa H, Ikeda M, Yanagimoto K, *et al.*** 2003. Induction of apoptosis in an insect cell line, IPLB-

Ld652Y, infected with nucleopolyhedroviruses. **J Gen Virol,** 84: 705-714.

64. **Ishikawa H, Ogasawara T, Ikeda M, *et al.*** 2006. A recombinant *Bombyx mori* nucleopolyhedrovirus possessing *hrf-1* gene replicates in nonpermissive *Lymantria dispar* IPLB-Ld652Y cell line. **J Insect Biotechnol Sericology,** 75 (1): 31-38.
65. **Iwanaga M, Takaya K, Katsuma S, *et al.*** 2004. Expression profiling of baculovirus genes in permissive and nonpermissive cell lines. **Biochem Biophys Res Commun,** 323 (2): 599-614.
66. **Jehle J A, Blissard G W, Bonning B C, *et al.*** 2006. On the classification and nomenclature of baculoviruses: A proposal for revision. **Arch Virol,** 151 (7): 1257-1266.
67. **Joazeiro C A P, Weissman A M.** 2000. RING finger proteins: Mediators of ubiquitin ligase activity. **Cell,** 102 (5): 549-552.
68. **Kamita S G, Maeda S.** 1993. Inhibition of *Bombyx-mori* nuclear polyhedrosis-virus (NPV) replication by the putative DNA helicase gene of *Autographa-californica* NPV. **J Virol,** 67 (10): 6239-6245.
69. **Kamita S G, Maeda S.** 1996. Abortive infection of the baculovirus *Autographa californica* nuclear polyhedrosis virus in Sf-9 cells after mutation of the putative DNA helicase gene. **J Virol,** 70 (9): 6244-6250.
70. **Kamita S G, Majima K, Maeda S.** 1993. Identification and characterization of the P35-gene of *Bombyx- mori* nuclear polyhedrosis-virus that prevents virus-induced apoptosis. **J Virol,** 67 (1): 455-463.
71. **Katou Y, Ikeda M, Kobayashi M.** 2006. Abortive replication of *Bombyx mori* nucleopolyhedrovirus in Sf9 and High Five cells: Defective nuclear transport of the virions. **Virology,** 347 (2): 455-465.
72. **Kikhno I, Gutierrez S, Croizier L, *et al.*** 2002. Characterization of pif, a gene required for the per os infectivity of *Spodoptera littoralis* nucleopolyhedrovirus. **J Gen Virol,** 83: 3013-3022.
73. **Kondo A, Maeda S.** 1991. Host range expansion by recombination of the baculoviruses *Bombyx-mori* nuclear polyhedrosis-virus and *Autographa- californica* nuclear polyhedrosis-virus. **J Virol,** 65 (7): 3625-3632.
74. **Krappa R, Roncarati R, Knebelmorsdorf D.** 1995. Expression of PE38 and IE2, viral members of the C3HC4 finger family, during baculovirus infection-PE38 and IE2 localize to distinct nuclear regions. **J Virol,** 69 (9): 5287-5293.
75. **Kuzio J, Jaques R, Faulkner P.** 1989. Identification of P74, a gene essential for virulence of baculovirus occlusion bodies. **Virology,** 173 (2): 759-763.
76. **LaCount D J, Friesen P D.** 1997. Role of early and late replication events in induction of apoptosis by baculoviruses. **J Virol,** 71 (2): 1530-1537.
77. **Lery X, Giannotti J, Taha A, *et al.*** 1997. Multiplication of a granulosis virus isolated from the potato tuber moth in a new established cell line of *Phthorimaea operculella*. ***In Vitro* Cell Dev Biol-Anim,** 33 (8): 640-646.
78. **Long G, Westenberg M, Wang H L, *et al.*** 2006. Function, oligomerization and N-linked glycosylation of the *Helicoverpa armigera* single nucleopolyhedrovirus envelope fusion protein. **J Gen Virol,** 87 839-846.
79. **Lu A, Miller L K.** 1995. Differential requirements for baculovirus late expression factor genes in 2 cell-lines. **J Virol,** 69 (10): 6265-6272.
80. **Lu A, Miller L K.** 1995. The roles of 18 baculovirus late expression factor genes in transcription and DNA-replication. **J Virol,** 69 (2): 975-982.
81. **Lu A, Miller L K.** 1996. Species-specific effects of the *hcf-1* gene on baculovirus virulence. **J Virol,** 70 (8): 5123-5130.
82. **Lung O, Westenberg M, Vlak J M, *et al.*** 2002. Pseudotyping *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV): F proteins from group II NPVs are functionally analogous to AcMNPV GP64. **J Virol,** 76 (11): 5729-5736.
83. **Lynn D E.** 2001. Novel techniques to establish new insect cell lines. ***In Vitro* Cell Dev Biol-Anim,** 37 (6): 319-321.
84. **Lynn D E.** 2003. Comparative susceptibilities of insect cell lines to infection by the occlusion-body derived phenotype of baculoviruses. **J Invertebr Pathol,** 83 (3): 215-222.
85. **Maeda S, Kamita S G, Kondo A.** 1993. Host-range expansion of *Autographa-californica* nuclear polyhedrosis-virus (NPV) following recombination of a 0.6- Kilobase-pair DNA fragment originating from *Bombyx-mori* NPV. **J Virol,** 67 (10): 6234-6238.
86. **Martin O, Croizier G.** 1997. Infection of a *Spodoptera frugiperda* cell line with *Bombyx mori* nucleopoly hedrovirus.

Virus Res, 47 (2): 179-185.

87. **McClintock J T, Dougherty E M.** 1987. Superinfection of baculovirus-infected gypsy-moth cells with the nuclear polyhedrosis viruses of *Autographa-californica* and *Lymantria-dispar*. **Virus Res,** 7 (4): 351-364.
88. **McClintock J T, Dougherty E M, Weiner R M.** 1986. Semipermissive replication of a nuclear polyhedrosis-virus of *Autographa-californica* in a gypsy-moth cell-line. **J Virol,** 57 (1): 197-204.
89. **Miller L K**. 1997. **The Baculoviruses**. New York: Plenum Press,
90. **Miller L K, Lu A**. 1997. The molecular basis of baculovirus host range, In:**The Baculoviruses** (Miller L K,ed.), New York: Plenum Press. p217-235.
91. **Mita K, Kasahara M, Sasaki S, *et al.*** 2004. The genome sequence of silkworm, Bombyx mori. **Dna Research,** 11 (1): 27-35.
92. **Morishima N, Okano K, Shibata T, *et al.*** 1998. Homologous p35 proteins of baculoviruses show distinctive anti- apoptotic activities which correlate with the apoptosis- inducing activity of each virus. **FEBS Lett,** 427 (1): 144-148.
93. **Morris T D, Miller L K.** 1992. Promoter influence on baculovirus-mediated gene-expression in permissive and nonpermissive insect cell-lines. **J Virol,** 66 (12): 7397-7405.
94. **Morris T D, Miller L K.** 1993. Characterization of productive and nonproductive AcMNPV infection in selected insect-cell lines. **Virology,** 197 (1): 339-348.
95. **Morris T D, Todd J W, Fisher B, *et al.*** 1994. Identification of *Lef-7* - a baculovirus gene affecting late gene-expression. **Virology,** 200 (2): 360-369.
96. **Morse M A, Marriott A C, Nuttall P A.** 1992. The glycoprotein of Thogoto virus (a tick-borne Orthomyxo-like virus) is related to the baculovirus glycoprotein gp64. **Virology,** 186 (2): 640-646.
97. **Murges D, Quadt I, Schroer J, *et al.*** 2001. Dynamic nuclear localization of the baculovirus proteins IE2 and PE38 during the infection cycle: The promyelocytic leukemia protein colocalizes with IE2. **Exp Cell Res,** 264 (2): 219-232.
98. **Naser W L, Miltenburger H G, Harvey J P, *et al.*** 1984. *In vitro* replication of the *Cydia-pomonella* (codling moth) granulosis-virus. **Fems Microbiol Lett,** 24 (1): 117-121.
99. **Ohkawa T, Washburn J O, Sitapara R, *et al.*** 2005. Specific binding of *Autographa californica* M nucleopoly-hedrovirus occlusion-derived virus to midgut cells of Heliothis virescens larvae is mediated by products of *pif* genes Ac119 and Ac022 but not by Ac115. **J Virol,** 79 (24): 15258-15264.
100. **Passarelli A L, Miller L K.** 1993. 3 Baculovirus genes involved in late and very late gene- expression - *IE-1*, *IE-N*, and *Lef-2*. **J Virol,** 67 (4): 2149-2158.
101. **Pearson M N, Groten C, Rohrmann G F.** 2000. Identification of the *Lymantria dispar* nucleopoly-hedrovirus envelope fusion protein provides evidence for a phylogenetic division of the Baculoviridae. **J Virol,** 74 (13): 6126-6131.
102. **Pearson M N, Rohrmann G F.** 2002. Transfer, incorporation, and substitution of envelope fusion proteins among members of the Baculoviridae, Orthomyxoviridae, and Metaviridae (insect retrovirus) families. **J Virol,** 76 (11): 5301-5304.
103. **Pijlman G P, Pruijssers A J P, Vlak J M.** 2003. Identification of pif-2, a third conserved baculovirus gene required for per os infection of insects. **J Gen Virol,** 84:2041-2049.
104. **Prikhod'ko E A, Lu A, Wilson J A, *et al.*** 1999. *In vivo* and *in vitro* analysis of baculovirus ie-2 mutants. **J Virol,** 73 (3): 2460-2468.
105. **Rahman M, Gopinathan K P.** 2003. Analysis of host specificity of two closely related baculoviruses in permissive and nonpermissive cell lines. **Virus Res,** 93 (1): 13-23.
106. **Randall R E, Goodbourn S.** 2008. Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures. **J Gen Virol,** 89:1-47.
107. **Rapp J C, Wilson J A, Miller L K.** 1998. Nineteen baculovirus open reading frames, including LEF-12, support late gene expression. **J Virol,** 72 (12): 10197-10206.
108. **Saurin A J, Borden K L B, Boddy M N, *et al.*** 1996. Does this have a familiar RING? **Trends Biochem Sci,** 21 (6): 208-214.
109. **Song J J, Wang R R, Deng F, *et al.*** 2008. Functional studies of per os infectivity factors of *Helicoverpa armigera* single nucleocapsid nucleopolyhedrovirus. **J Gen Virol,** 89: 2331-2338.

110. **Thiem S M.** 1997. Prospects for altering host range for baculovirus bioinsecticides. **Curr Opin Biotechnol,** 8 (3): 317-322.

111. **Thiem S M, Chejanovsky N.** 2004. The role of baculovirus apoptotic suppressors in AcMNPV-mediated translation arrest in Ld652Y cells. **Virology,** 319 (2): 292-305.

112. **Thiem S M, Du X L, Quentin M E, *et al.*** 1996. Identification of a Baculovirus gene that promotes *Autographa californica* nuclear polyhedrosis virus replication in a nonpermissive insect cell line. **J Virol,** 70 (4): 2221-2229.

113. **Todd J W, Passarelli A L, Lu A, *et al.*** 1996. Factors regulating baculovirus late and very late gene expression in transient-expression assays. **J Virol,** 70 (4): 2307-2317.

114. **Vail P V, Hoffmann D F, Streett D A, *et al.*** 1993. Infectivity of a Nuclear Polyhedrosis-Virus isolated from *Anagrapha-falcifera* (Lepidoptera, Noctuidae) against production and postharvest pests and homologous cell-lines. **Environ Entomol,** 22 (5): 1140-1145.

115. **van Oers M M, Vlak J M.** 2007. Baculovirus genomics. **Curr Drug Targets,** 8:1051-1068.

116. **Vaughn J L, Goodwin R H, Tompkins G J, *et al.*** 1977. Establishment of 2 cell lines from insect *Spodoptera-frugiperda* (Lepidoptera-Noctuidae). In: **Vitro-Journal of the Tissue Culture Association,** 13 (4): 213-217.

117. **Volkman L E, Goldsmith P A.** 1984. Budded *Autographa-californica* NPV-64k protein-Further biochemicalanalysis and effects of postimmunoprecipitation sample preparation conditions. **Virology,** 139 (2): 295-302.

118. **Volkman L E, Goldsmith P A.** 1985. Mechanism of neutralization of budded *Autographa-californica* Nuclear Polyhedrosis-Virus by a Monoclonal-antibody-Inhibition of entry by adsorptive endocytosis. **Virology,** 143 (1): 185-195.

119. **Volkman L E, Goldsmith P A, Hess R T.** 1986. Alternate pathway of entry of budded *Autographa-californica* Nuclear Polyhedrosis-Virus-Fusion at the plasma-membrane. **Virology,** 148 (2): 288-297.

120. **Volkman L E, Goldsmith P A, Hess R T, *et al.*** 1984. Neutralization of budded Autographa-californica NPV by a monoclonal-antibody-Identification of the target antigen. **Virology,** 133 (2): 354-362.

121. **Volkman L E, Knudson D L**. 1986. *In vitro* replication of baculoviruses, In: **The biology of baculoviruses** (Granados R R, Federici B A,ed.), Boca Raton: CRC Press. vol 1: p109-127.

122. **Volkman L E, Summers M D, Hsieh C H.** 1976. Occluded and nonoccluded Nuclear Polyhedrosis-Virus grown in *Trichoplusia-ni* - Comparative neutralization, comparative infectivity, and Invitro growth studies. **J Virol,** 19 (3): 820-832.

123. **Wang L, Salem T Z, Lynn D E, *et al.*** 2008. Slow cell infection, inefficient primary infection and inability to replicate in the fat body determine the host range of *Thysanoplusia orichalcea* nucleopolyhedrovirus. **J Gen Virol,** 89: 1402-1410.

124. **Washburn J O, Chan E Y, Volkman L E, *et al.*** 2003. Early synthesis of budded virus envelope fusion protein GP64 enhances *Autographa californica* multicapsid nucleopolyhedrovirus virulence in orally infected *Heliothis virescens*. **J Virol,** 77 (1): 280-290.

125. **Washburn J O, Haas-Stapleton E J, Tan F F, *et al.*** 2000. Co-infection of *Manduca sexta* larvae with polydnavirus from *Cotesia congregata* increases susceptibility to fatal infection by *Autographa californica* M Nucleopolyhedrovirus. **J Insect Physiol,** 46 (2): 179-190.

126. **Washburn J O, Kirkpatrick B A, Volkman L E.** 1995. Comparative pathogenesis of *Autographa-californica* M Nuclear Polyhedrosis-Virus in larvae of *Trichoplusia-ni* and *Heliothis-virescens*. **Virology,** 209 (2): 561-568.

127. **Washburn J O, Kirkpatrick B A, Volkman L E.** 1996. Insect protection against viruses. **Nature,** 383 (6603): 767-767.

128. **Washburn J O, Trudeau D, Wong J F, *et al.*** 2003. Early pathogenesis of *Autographa californica* multiple nucleopolyhedrovirus and Helicoverpa zea single nucleo-polyhedro virus in *Heliothis virescens*: a comparison of the "M" and "S" strategies for establishing fatal infection. **J Gen Virol,** 84: 343-351.

129. **Waterhouse R M, Kriventseva E V, Meister S, *et al.*** 2007. Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. **Science,** 316: 1738-1743.

130. **Westenberg M, Uijtdewilligent P, Vlak J M.** 2007. Baculovirus envelope fusion proteins F and GP64 exploit

distinct receptors to gain entry into cultured insect cells. **J Gen Virol,** 88: 3302-3306.

131. **Wickham T J, Shuler M L, Hammer D A, *et al.*** 1992. Equilibrium and kinetic-analysis of *Autographa-californica* Nuclear Polyhedrosis-Virus attachment to different insect cell- lines. **J Gen Virol,** 73: 3185-3194.
132. **Wilson J A, Forney S D, Ricci A M, *et al.*** 2005. Expression and mutational analysis of *Autographa californica* nucleopolyhedrovirus HCF-1: Functional requirements for cysteine residues. **J Virol,** 79 (22): 13900-13914.
133. **Winstanley D, Crook N E.** 1993. Replication of *Cydia-pomonella* Granulosis-Virus in cell-cultures. **J Gen Virol,** 74: 1599-1609.
134. **Wu W W, Wang J W, Xie F, *et al.*** 2003. Baculovirus p74 gene is a species-specific gene. **Acta Biochim Biophys Sin,** 35 (9): 834-840.
135. **Wu Y T, Carstens E B.** 1998. A baculovirus single-stranded DNA binding protein, LEF-3, mediates the nuclear localization of the putative helicase P143. **Virology,** 247 (1): 32-40.
136. **Xi Z, Ramirez J L, Dimopoulos G.** 2008. The *Aedes aegypti* Toll Pathway Controls Dengue Virus Infection. **PLoS Pathogens,** 4 (7): e1000098.
137. **Zanotto P M D, Kessing B D, Maruniak J E.** 1993. Phylogenetic interrelationships among Baculoviruses - Evolutionary rates and host associations. **J Invertebr Pathol,** 62 (2): 147-164.
138. **Zhang J H, Washburn J O, Jarvis D L, *et al.*** 2004. *Autographa californica* M nucleopolyhedrovirus early GP64 synthesis mitigates developmental resistance in orally infected noctuid hosts. **J Gen Virol,** 85: 833-842.
139. **Zoog S J, Schiller J J, Wetter J A, *et al.*** 2002. Baculovirus apoptotic suppressor P49 is a substrate inhibitor of initiator caspases resistant to P35 *in vivo*. **Embo J,** 21 (19): 5130-5140.