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8. Host resistance, susceptibility, and the effect of viral infection on host molecular biology

Viruses are dependent on a variety of cellular functions to successfully infect an organism. For a productive infection, cellular structures and molecular pathways must be compatible with the virus for all the major events in virus replication including attachment, entry, uncoating, replication, assembly and exit. Insects, like many other organisms, have evolved methods to inhibit or block virus infections. Several methods by which organisms inhibit virus infection are summarized below. For a more detailed review of some of the topics in this chapter, see (1).

The insect immune system: hemocytes, melanization and encapsulation

Although lacking an adaptive immune system similar to vertebrates, insects have a variety of methods to resist infection by pathogens. Many of these defense mechanisms are similar to those of higher organisms and include physical barriers along with local and systemic responses. The latter includes the production of antimicrobial peptides and highly specialized cells called hemocytes. Hemocytes are circulating cells found in the hemolymph of insects and other arthropods that are similar to neutrophils and carry out roles in immunity such as phagocytosis, encapsulation and melanization as a first line of defense after injury or invasion by microorganisms. Although not employed by mammals, melanization causes a blackening of a wound site, which results from the synthesis and deposition of melanin. It is an important defense against infections because it can encapsulate and isolate pathogens similar to the blood clotting system in vertebrates. In addition, the intermediates of the melanization reaction can involve the production of reactive oxygen species that can be directly toxic to pathogenic microbes. In arthropods, an inactive form of phenoloxidase (prophenoloxidase) is synthesized and secreted into the hemolymph. Melanization is regulated by a cascade of serine proteases that cleave and activate prophenoloxidase (to phenoloxidase) that is then able to catalyze the oxidation of phenols (e.g., tyrosine) to non-aromatic ring compounds called quinones, which then polymerize and form melanin (2). It has been suggested that phenol oxidase may have an antiviral effect in the plasma of insects in addition to its role in encapsulation (3). The Toll pathway is another component of the innate immune response in many organisms. It acts by the recognition of structurally conserved molecules associated with pathogens such as viruses that are not present in the host organism. This recognition activates a cellular immune response. In *Drosophila*, melanization requires activation of the Toll pathway and is dependent on the removal of a serine protease inhibitor (Serpin27A), thereby allowing the cleavage of prophenoloxidase. The Toll pathway has been shown to be required for the efficient inhibition of Drosophila X virus. Inactivation of this system led to rapid death of infected insects and elevated virus titers (4).

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Although most of the characterization of components of insect immune systems has been described from *Drosophila*, investigations have been conducted on baculovirus infection of Lepidoptera that suggest that they have similar defense systems. *Heliothis virescens* (tobacco bud worm) and *Helicoverpa zea* (corn earworm) are both members of the Noctuidae, but *H. zea* is 1,000-fold less susceptible to AcMNPV infection than *H. virescens*. Larvae of the two species are similar in their susceptibility to primary infection of the midgut and secondary infections of the tracheal epidermis. However, in *H. zea,* the foci of infections in the tracheal epidermis become melanized and encapsulated, and hemocytes appear to be resistant to infection and are capable of removing the virus from the hemolymph. Therefore, AcMNPV infection of *H. zea* appears to be able to activate the host melanization and encapsulation responses that reduce viral titers in the hemolymph and inhibit the progression of the infection. In contrast, in *H. virescens* this pathway appears to be much less active (5). *Spodoptera littoralis* is also highly resistance to AcMNPV infection and has been shown to be capable of efficiently encapsulating the virus in tracheoblast cells that serve the midgut columnar cells. These viral foci were removed during molting thereby eliminating the infection (6).

A sequence related to lepidopteran serpins was found in the genome of a baculovirus of *Hemileuca* sp. (HespNPV) (7). Serpins, (serine protease inhibitors), were named because of their ability to inhibit chymotrypsin-like serine proteases. Serpins bind serine proteases and then undergo cleavage. This results in a major change in the conformation of the serpin and the formation of a covalent linkage with the protease leading to its inactivation. In cowpox virus, a serpin, crmA, has been implicated in mitigating both the inflammatory and apoptotic responses. Although crmA is a member of the serpin group, in contrast to other serpins, it is capable of inhibiting cysteine caspases (8). Similar to serpins, crmA binds the active caspases as a pseudosubstrate and has been shown to irreversibly bind to caspase-1 and is also capable of inhibiting caspases 4, 5, 9 and 10, reviewed in (9). The baculovirus serpin was able to inhibit a subset of serine proteases and also inhibited phenol oxidase activation in vitro indicating that it is a functional serpin. When expressed in AcMNPV it accelerated BV production and reduced the dose to produce an LD50 in T. ni larvae 4 fold (10).

RNA interference

Viral RNAi. RNA interference (RNAi) has been shown to inhibit the infection of insects by some RNA viruses. In *Drosophila*, evidence suggests that virus-specific dsRNA replication intermediates of RNA viruses are released upon cell lysis and taken up and processed by other cells, thereby generating systemic virus-specific RNAi based interference (11). However, some viruses can also interfere with this response. In one well-studied example, Flock house virus encodes a protein called B2 that suppresses RNAi by inhibiting the ability of Dicer to cleave long dsRNAs, thereby preventing the generation of siRNAs. In addition, by binding to siRNAs, B2 prevents their incorporation into the RISC complex that is dependent on their incorporation to identify and cleave homologous RNA sequences, thereby blocking the cleavage of target RNAs (12). Although it was originally thought that flies were not able to generate a systemic RNAi response, it was shown that an uptake pathway for dsRNA exists in *Drosophila*. This pathway is essential for antiviral immunity in adult flies. Mutants defective for this system had up to a 100-fold increase in viral titers (11).

Baculovirus and cellular RNAi. Similar to other viruses, baculoviruses encode microRNAs (13) that are involved in the regulation of both host and viral genes. In one study, four miRNAs were predicted from analyses of the BmNPV genome (14). The data suggested that they were targeted against 8 viral and 64 cellular genes. The viral targets included genes involved in replication, transcription (e.g., dbp, DNA polymerase, and lef-8). Two different bro genes, chitinase, and several other genes were also targeted. Some of the cellular targets involved host defense pathways that are likely involved in resisting the virus infection. These include prophenoloxidase, which is integrally involved in the melanization response described above. Hemolin, another antiviral compound, also was predicted to be targeted. It is involved in pattern recognition, hemocyte aggregation, and phagocytosis. Several proteins involved in the pre-miRNA response and mRNA initiation were also identified. In another investigation of host and viral miRNAs of BmNPV and *B. mori*, it was found that BmNPV encodes a

miRNA (miR-1) that down regulates the host GTP-binding protein RAN. RAN is an essential component of the Exportin-5 mediated transport machinery that is involved in the transport of small RNA molecules from the nucleus to the cytoplasm. Blocking BmNPV miR-1 results in elevated expression of RAN and a decrease in BmNPV replication. In contrast blocking a host miRNA (bmo-miR-8) that is targeted at BmNPV IE-1 transcripts resulted in a 3 fold increase in *ie-1* transcripts and an about 8 fold increase of BmNPV DNA accumulation in fat body tissue (15). It has also been reported that BmNPV-mir-3 down regulates the DNA binding protein, p6.9 (16). It also has imperfect matches with several other baculovirus late genes. It was suggested that this may delay viral maturation in order to allow the virus to escape host defenses which may become less effective later in the infection (13). AcMNPV and BmNPV mir-3 do not apper to be related. In contrast to BmNPV mir-3 that regulates p6.9, AcMNPV mir-3 is encoded on the opposite strand of Ac101 and appears to be involved in its down regulation and may be involved in regulating BV and ODV production (17).

In another report it would found that miRNAs appeared to be conserved between *S.frugiperda* and *B. mori* and were down regulated during baculovirus infection (18). In contrast to the implication of small RNAs in host cell defense, in *Helicoverpa armigera* larvae infected with HaSNPV, a large population of small RNAs were found that mapped to a variety of viral structural and replication genes. The authors suggest that the down regulation of these genes may make the infection more efficient by preventing the over replication and premature death of the cell (19). It has been found in AcMNPV that the open reading frame of ODV-E25 (Ac94) encodes a miRNA that down regulates ODV-E25 expression. Evidence suggested that this miRNA causes a reduction in infectious virus production and may be involved in the shift from budded virus to occluded virus production (20). The BmNPV ie-1 gene also appears to be a target of B. mori bmo-miR-2819 (21).

In another investigation characterizing baculovirus miRNA, SpliNPV infection of Sf21 cells was examined. At 12 hpi >95% of the host proteins were stable, but by 72 hpi, over half the host proteins were down regulated by 2-fold or more. This was reflected in the down regulation of translation, transcription, protein export and oxidative phosphorylation pathways. 117 host genes were identified that were possible targets of 10 viral miRNAs. Two miRNAs were examined experimentally and 9 host genes were identified that were down regulated by these molecules (22).

It was also observed that components of the RNAi system such as Dicer-2, R2D2, and Ago2 were upregulated in the T. ni midgut in apparent response to AcMNPV infection (23). It is not clear whether these are used by a viral or host RNAi response.

Additional mechanisms of virus and host resistance

Antimicrobial peptides. Antimicrobial peptides have also been implicated in protection against viral infection. One of these, gloverin, a cationic protein with antibacterial properties was found to be induced by AcMNPV infection. The addition of gloverin to infected cells was found to reduce AcMNPV BV production to 8 to 27% of untreated samples (24). However, when cDNA sequences were examined in infected cells, it was found that AcMNPV infection of *Spodoptera exigua* larvae down regulated three forms of gloverin along with attacin, another antimicrobial peptide (25). In addition, a member of the atlastin family, BmAtlastin-n, was shown to have an antiviral effect against BmNPV in Bombyx mori larva (26). It was observed to be upregulated in T. ni larvae infected with AcMNPV (23).

Developmental resistance. It was also observed that *H. virescens* larvae demonstrate increasing resistance to fatal AcMNPV infections as they age. In one investigation it was found that the progression of infection is much slower in fifth- compared to fourth-instar larvae that had been orally infected. It was suggested that resistance was caused by different physiology of the midgut or tracheal cells. In particular, it was noted that midgut cells might undergo major changes since their composition is altered late in the fifth instar as the gut develops as part of metamorphosis to adult cell types. It was also suggested that midgut cells may be sloughed at a higher rate in later larval instars (27). Similar patterns of resistance to baculovirus infection has been observed in *Lymantria*

dispar. Newly molted fourth-instar larvae showed lower LD_{50} titers compared to larvae infected at 2–3 days post molting. Also, the 2–3-day post-molt insects showed a higher number of foci of infection that had been melanized and a reduced number of infected haemocytes (28, 29).

Inactivation of superoxides, Ac31, superoxide dismutase (SOD). Insect hemocytes are phagocytic cells (see above) and can destroy invading pathogens by the production of reactive oxygen such as superoxide (30). SOD can inactivate superoxide by converting it to hydrogen peroxide, which is also toxic, but can itself be inactivated with catalase to yield water and O_2. Many baculoviruses may infect hemocytes and in this manner can spread an infection throughout an insect. Orthologs of SOD are found in the genomes of almost all lepidopteran baculovirus. Ac31 (SOD) is closely related to SOD from a variety of insects including *B. mori* (E = 8E-49). The expression of viral SOD might mitigate the effects of superoxide production by hemocytes. However, AcMNPV deleted for *sod* replicated normally in cultured cells and insect larvae. The *sod*-deleted viruses showed no reduction in replication when grown in the presence of paraquat, a superoxide anion inducer (31). Whereas these data suggest that SOD of baculoviruses may be involved in some other role in virus biology, it could also indicate the conditions used to investigate its activity were not sensitive enough to determine its role. In contrast to AcMNPV, in BmNPV it was found that *sod* (Bm23) was essential for replication in BmN cells (32).

Other inhibitors of viral replication. In field populations of *Cydia pomonella* exposed to the granulovirus (CpGV), some populations have been identified with up 10,000-fold resistance. Using several approaches, changes in the innate immune systems, and altered peritrophic membranes and midgut receptors were ruled out as causing resistance. Using Q-PCR to examine viral replication in different tissues, no viral DNA was detected in the fat body, hemocytes or midgut cells of the tissues of resistant insects suggesting that the virus was unable to replicate in these cells. Furthermore, resistance could not be conferred by the transfusion of hemolymph from a resistant to a susceptible insect. This led the authors to conclude that resistance occurs within cells and must inhibit a very early step in viral replication (33).

Host resistance to baculovirus infection in the midgut

The peritrophic matrix (PM). In addition to protection from external invasion by pathogens that is provided by the chitin-containing exoskeleton, an example of another physical barrier unique to insects that might influence resistance to viral infection is the PM. The ability of a virus to access the midgut epithelium would be one of the first problems confronting a virus when it is initiating infection. In some insects that are resistant to infection it was found that differing features of the PM can influence the susceptibility of an insect to viral infection. In *Anticarsia gemmatalis*, it was observed that insects that were more resistant to infection had a relatively thicker PM, a higher chitin content, and bound wheat germ agglutinin more intensely than the more susceptible insects (34) (35).

Midgut interactions. It has also been demonstrated that the oral infectivity of a virus could be influenced by the ability of the ODV to bind to the midgut of insects. It was observed that compared to SfMNPV, AcMNPV ODV had a reduced affinity for midgut cells of *S. frugiperda* larvae. Evidence was also presented suggesting that SfMNPV might bind to a different receptor(s) on columnar epithelial cells and this could contribute to the efficiency of its ability to initiate infection (36).

Other factors influencing Baculovirus host range

A variety of phenomena that govern the selective infectivity of baculoviruses has been covered previously and there is little to add to their review (37). However, there have been recent contributions to understanding specific genes that have been observed to affect host range in insects. Most investigations on the molecular basis or host range specificity of baculoviruses have been done using BmNPV, AcMNPV, and LdMNPV. All of these viruses can be grown in cell culture and BmNPV and AcMNPV are closely related with homologous orfs showing ~90% nt and ~93% aa sequence identity (38). In contrast, LdMNPV is a member of the Group II baculoviruses and its

orfs show about 41% aa identity with their AcMNPV homologs (39). Despite the similarity of AcMNPV and BmNPV, AcMNPV infects a much more diverse set of insects and insect cell lines than BmNPV (40). Whereas some of the limits on host range that were initially observed in cultured cells extend through to infection of the host insects, other host range effects are limited to a cell line and are not as restrictive in other cell lines from the same insects or in insect larvae of the species from which the cell lines were derived. This section will focus on investigations of host range in AcMNPV and BmNPV.

Investigations on BmNPV and AcMNPV host range in *B. mori* and *S. frugiperda* cells

Although the baculoviruses of BmNPV and AcMNPV are closely related, they differ significantly in their infectivity spectrum. For example, BmNPV replicates in *B. mori* (BmN) cells, but not *S. frugiperda* (Sf) cells. Conversely, AcMNPV replicates in Sf, but not BmN cells. Although the two viruses do not appear to productively infect the heterologous cell line, their patterns of gene expression differ in the nonpermissive cell lines. For example, almost all of the AcMNPV genes were found to be expressed in both BmN and Sf9 cells, although peak levels were delayed by about 12 hr in the nonpermissive BmN cells and polyhedrin and p10 expression were substantially reduced. In contrast, although almost all of the BmNPV genes were expressed in BmN cells, their expression in Sf9 cells was greatly reduced (41). Several different laboratories have investigated the factors responsible for the inability of these viruses to replicate in the heterologous cells. Some of these studies are summarized below.

Implication of DNA helicase in specifying host range in BmNPV and AcMNPV. By characterizing mixed infections of BmNPV and AcMNPV, a variant of BmNPV was isolated that was able to replicate in both BmN and Sf-21 cells. A 572 bp fragment of the BmNPV DNA helicase gene was found to be responsible for this altered host range (42). Further characterization of this region identified a single amino acid in the helicase orf (Asn564Ser) responsible for this change (43). Using a similar approach, other investigators found that altering three closely spaced amino acids in the AcMNPV helicase gene with the amino acids from BmNPV located at these positions, i.e., Val556Leu, Ser564Asn, Phe577Leu, allowed AcMNPV to replicate in *B. mori* cells (44). When AcMNPV mutants selected for their ability to replicate in BmN cells were passed through *B. mori* larvae, two amino acid changes (Ser564Asn, Phe577Leu) were found to be required to cause death of the larvae (45). It is unclear what role helicase plays in governing the inability of the virus to replicate in the heterologous cell lines. However, co-infection of BmN cells with wt AcMNPV and wt BmNPV causes premature cessation of both viral and host protein synthesis, although viral transcription appears to be normal. This effect is not observed when BmN cells are infected with both the recombinant AcMNPV and wt BmNPV. It has been suggested that since these few changes in the helicase gene have such a major effect on infection in BmN cells, the wt AcMNPV helicase gene may be toxic to the cell by eliciting an antiviral defense mechanism, by interacting with a host cell protein or nucleic acid, or by interfering with the translational apparatus (46). A wild silkworm, *B. mandarina* was found that had a host range allowing it to replicate in Bm5, Sf9, and T. ni cells. The BoMANPV helicase exhibited two mutations Asp291Asn and Asp300Asn. However, due a variety of other differences in many other genes, the significance of these changes remains to be determined (47).

BmNPV may be infectious for Sf-9 and other cells, but at a low level. In contrast to the reports described above, other laboratories using viral constructs expressing reporter genes have detected replication of BmNPV and AcMNPV in heterologous cell lines. In one study, a BmNPV construct expressing the LacZ gene under the polyhedrin promoter in Sf-9, Sf-21 and Hi-5 cells was examined. Compared with BmNPV infection in Bm5 cells, the BmNPV infections in the other cell lines were delayed with DNA replication detectable 3 to 5 days after being observed in BmN cells. In addition, the viral titers were much lower, varying from 0.7 to 7% (10^6 to 10^7 pfu/ml) the level in BmN cells (48). Since this report utilized LacZ expression to trace the virus, it may be more sensitive than previous studies that utilized the visualization of polyhedron production to monitor viral replication. Indeed, these authors detected either very few or no polyhedra in the heterologous infections using a

wt BmNPV with an intact polyhedrin gene. In another investigation, using an AcMNPV construct that expressed the firefly luciferase gene under the *Drosophila* heat shock promoter, luciferase activity was detected in both virus infected larvae and in larvae of the next generation (49). Therefore, both these studies indicate that viral replication is occurring in the heterologous cell lines. Another report described AcMNPV DNA replication in BmN cells, but budded virions were not produced. In contrast, BmNPV DNA replication and BV production only occurred in Sf cells when they were superinfected with AcMNPV (50).

Implication of a host factor in specifying host range in BmNPV and AcMNPV (51). In studies conducted by the intrahemocoelic injection of AcMNPV into 31 different strains of *B. mori* larvae, 14 permissive insect strains were identified. A series of genetic crosses implicated a dominant host gene or set of linked genes that prevented AcMNPV infection in the resistant insects, but that are not present or do not interact with the virus in a negative manner in the susceptible insects.

GP64: an AcMNPV and BmNPV host range determinant. The one study that is difficult to reconcile with the investigations described above examined the replication of BmNPV in Sf-9 and Tn-5 (Hi-5) cells. This investigation suggested that the barrier to infection was caused by the inability of BmNPV to be translocated to the nuclei of the Sf-9 or Tn-5 cells. It was found that BmNPV constructs that lacked the BmNPV *gp64* envelope fusion protein gene, but contained the AcMNPV *gp64* gene, were able to be translocated to nuclei. Although this process resulted in a productive infection in Hi-5 cells, replication in Sf-9 cells was still compromised. There are 22 codons that are different between these two *gp64* genes. There is also the potential that the BmNPV *gp64* orf may encode 19 additional amino acids at the N-terminus in transcripts initiated from late promoter elements (40). This study indicated that abortive replication was caused by the inability of BmNPV GP64 to facilitate fusion with the endosomal membrane. This would suggest that BmNPV GP64 has a major difference in biological properties compared to AcMNPV GP64, which has been demonstrated to allow entry into a wide array of vertebrate cells (52). Recent evidence suggests that a single amino is the host range determinant in AcMNPV GP64. It is HIS155 and the equivalent amino acid in BmNPV is TYR153. Changing AcMNPV gp64 to HIS155TYR and that of BmNPV to TYR153HIS decreased and increased the replication and cell spread of the respective viruses (53). One possible difference between GP64 expression in the two viruses is the observation that, in contrast to the AcMNPV *gp64* gene, which is shut off late in infection of Sf9 cells, in BmNPV infected BmN cells, *gp64* is not shut off late in infection (54).

Global protein synthesis shutdown.

Global protein synthesis shutdown has been observed in a variety of combinations of baculoviruses and cell types, reviewed in (55) and can involve either transcriptional or translational shut down. This phenomenon appears to be a host response to viral infection in some cell lines and consequently viruses have developed mechanisms to counteract it. Helicase has been implicated in this process (see above), but there are also several other genes that act as host range factors because they allow the virus to replicate in certain cells that otherwise would be able to shut down the virus infection. Several such genes are described below.

Host cell-specific factor-1 (*hcf-1*, Ac70): AcMNPV specificity for *T. ni* cells. The AcMNPV gene host cell-specific factor-1 (hcf-1, Ac70) has been implicated in global protein synthesis shutdown. Homologs of hcf-1 are present in a few other baculoviruses. Two are close relatives of AcMNPV and their HCF-1 orfs are 99% (PlxyNPV) and 84% (RoMNPV) identical to that of AcMNPV, whereas the homolog in ClbiNPV is more distantly related (21% identical). HCF-1 was found to be required for transient expression of a late promoter-reporter gene by a late expression factor library in Tn368 cells, but not SF-21 cells (56). AcMNPV with null mutations of *hcf-1* appeared to replicate normally in both Sf-21 cells and *S. frugiperda* larvae. However, in Tn-368 cells replication was impaired, including defects in DNA replication, late gene transcription, and virus production. This was reflected in the global shutdown of both host and viral protein synthesis. Such severe effects were not observed in another *T. ni* cell line. In *T. ni* larvae the oral infectivity of the null mutant was relatively normal, although the insects died more slowly than when infected with wt (57). This would suggest

that HCF-1 is required for the productive infection of some, but not all cell types in *T. ni* larvae. Therefore, whereas AcMNPV deleted for *hcf*-1 was unable to replicate in cells similar to Tn-368, it can replicate in other types of *T. ni* cells, thereby allowing infection of larvae that contain a variety of different cell types.

Host range factor-1 (*hrf-1*): An LdMNPV gene that allows AcMNPV to replicate in *L. dispar* cells and insects. The Ld652Y cell line is semi-permissive for AcMNPV replication and all categories of viral genes are transcribed, but both viral and host mRNA translation is blocked late in infection (58). Cotransfection of LdMNPV and AcMNPV DNA into Ld652Y cells results in the production of budded virus that can replicate in Sf cells, suggesting that AcMNPV had been altered such that it could replicate in Ld652Y cells. To determine the LdMNPV gene responsible for this change, AcMNPV DNA was co-transfected with cloned segments of the LdMNPV genome, and an LdMNPV gene was identified, *hrf-1*, that permitted AcMNPV to replicate in Ld652Y cells (59). This recombinant AcMNPV was also able to infect *L. dispar* larvae in concentrations similar to wt AcMNPV in permissive insects (60). It was found that wt AcMNPV infection of *L. dispar* cells resulted in a shutdown of protein synthesis late in the infection (61). This protein synthesis inhibition was at the level of mRNA translation and could be rescued with tRNA from uninfected cells. This suggested that infection of *L. dispar* cells with wt AcMNPV results in the depletion or blockage of tRNA synthesis (62). It was also found that AcMNPV, lacking the apoptotic inhibitor p35 did not cause translational arrest, suggesting that the inhibition of apoptosis, which normally prevents cell death, in this case induces translational arrest (63). It was found that HRF-1 facilitated the replication of two other viruses in *L. dispar* cells (64).

Apoptosis and the specificity of baculovirus infections. Insect cells have different abilities to detect the presence of a virus infection and initiate an apoptotic program. In some cells, virus infection will induce apoptosis, whereas in others, virus replication is unaffected. In addition, baculoviruses are able to interfere with apoptosis by the expression of apoptotic inhibitors. This has led to advances in understanding of apoptotic pathways and to the identification of baculovirus proteins that can block this process. It has also led to an understanding of the role apoptosis can play in determining the host range of a virus. An overview of apoptosis with relation to baculoviruses is included in Chapter 7.

The reaction of host cells to baculovirus infection: The challenge of interpreting data from proteomics, microarrays, and expression analyses

During AcMNPV infection of *T. ni* cells, the majority of host transcripts decrease between 6 h pi – 48 hpi such that at the latter time they comprise about 10% of the total. However, about 6% of host genes are upregulated from 0-6 hr pi and then decrease for the remainder of the infection. In addition, a small group of genes related to metabolism and stress response were elevated at 18-24 hpi and then declined. Concomitant to the decline in host RNA, the viral RNA increased such that it comprised over 50% of the total by about 13 hr pi infection and continued increasing thereafter (65). The challenge of providing an overview of the response of insect cells to baculovirus infection is that when the expressed transcripts are compartmentalized into broad categories, the categories are often so large that as many transcripts are up-regulated as down-regulated. For example, in AcMNPV infected T. ni cells at 6 hr pi, transcripts of genes that have catalytic activity comprised 31% of those upregulated, but also 24% of those down regulated (65). Therefore, these investigations have major value when an individual has a specific question in mind and then accesses the data and can hopefully obtain the desired information. An example of this was described in Chapter 5, where it was found that host genes that are required for DNA replication (DNA ligase and topoisomerase) but that are not encoded the AcMNPV, were up-regulated and stabilized during the initial stages of infection. Also, in some instances when authors have a particular interest they will parse and interpret their data for publication. Examples of this are cell transcripts involved in cell entry, midgut expression and the ESCRT and NSF pathways (66-69). Similar generalization regarding the changes in the proteome of baculovirus infected cells are also challenging, for example at 72 hpi, similar sized

categories of the same type of proteins are both constant or down regulated (22). Therefore, although the data is there, it is up to the individual to find and interpret the relevant information.

References

1. Thiem SM. Baculovirus genes affecting host function. In Vitro Cell Dev Biol Anim. 2009;45:111–26. PubMed PMID: 19247726.
2. Lemaitre B, Hoffmann J. The host defense of Drosophila melanogaster. Annu Rev Immunol. 2007;25:697–743. PubMed PMID: 17201680.
3. Popham HJ, Shelby KS, Brandt SL, Coudron TA. Potent virucidal activity in larval Heliothis virescens plasma against Helicoverpa zea single capsid nucleopolyhedrovirus. J Gen Virol. 2004;85:2255–61. PubMed PMID: 15269366.
4. Zambon RA, Nandakumar M, Vakharia VN, Wu LP. The Toll pathway is important for an antiviral response in Drosophila. Proc Natl Acad Sci U S A. 2005;102:7257–62. PubMed PMID: 15878994.
5. Trudeau D, Washburn JO, Volkman LE. Central role of hemocytes in Autographa californica M nucleopolyhedrovirus pathogenesis in Heliothis virescens and Helicoverpa zea. J Virol. 2001;75:996–1003. PubMed PMID: 11134313.
6. Rivkin H, Kroemer JA, Bronshtein A, Belausov E, Webb BA, Chejanovsky N. Response of immunocompetent and immunosuppressed Spodoptera littoralis larvae to baculovirus infection. J Gen Virol. 2006;87:2217–25. PubMed PMID: 16847117.
7. Rohrmann GF, Erlandson MA, Theilmann DA. The genome of a baculovirus isolated from Hemileuca sp. encodes a serpin ortholog. Virus Genes. 2013;47:357–364. PubMed PMID: 23852342.
8. Ekert PG, Silke J, Vaux DL. Caspase inhibitors. Cell Death Differ. 1999;6:1081–6. PubMed PMID: 10578177.
9. Turner S, Kenshole B, Ruby J. Viral modulation of the host response via crmA/SPI-2 expression. Immunol Cell Biol. 1999;77:236–41. PubMed PMID: 10361255.
10. Ardisson-Araujo DM, Rohrmann GF, Ribeiro BM, Clem RJ. Functional characterization of hesp018, a baculovirus-encoded serpin gene. J Gen Virol. 2015;96:1150–60. PubMed PMID: 25573886.
11. Saleh MC, Tassetto M, van Rij RP, Goic B, Gausson V, Berry B, Jacquier C, Antoniewski C, Andino R. Antiviral immunity in Drosophila requires systemic RNA interference spread. Nature. 2009;458:346–50. PubMed PMID: 19204732.
12. Venter PA, Schneemann A. Recent insights into the biology and biomedical applications of Flock House virus. Cell Mol Life Sci. 2008. PubMed PMID: 18516498.
13. Tang Q, Qiu L, Li G. Baculovirus-Encoded MicroRNAs: A Brief Overview and Future Prospects. Curr Microbiol. 2018. doi: 10.1007/s00284-018-1443-y. PubMed PMID: 29487989.
14. Singh J, Singh CP, Bhavani A, Nagaraju J. Discovering microRNAs from Bombyx mori nucleopolyhedrosis virus. Virology. 2010;407:120–128. PubMed PMID: 20800868.
15. Singh CP, Singh J, Nagaraju J. A baculovirus-encoded MicroRNA (miRNA) suppresses its host miRNA biogenesis by regulating the exportin-5 cofactor Ran. J Virol. 2012;86:7867–79. PubMed PMID: 22593162.
16. Singh CP, Singh J, Nagaraju J. bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in Bombyx mori. Insect Biochem Mol Biol. 2014;49:59–69. PubMed PMID: 24698834.
17. Jiao Y, Wang J, Deng R, Yu X, Wang X. AcMNPV-miR-3 is a miRNA encoded by Autographa californica nucleopolyhedrovirus and regulates the viral infection by targeting ac101. Virus Res. 2019. doi: 10.1016/j.virusres.2019.05.004. PubMed PMID: 31077766.
18. Mehrabadi M, Hussain M, Asgari S. MicroRNAome of Spodoptera frugiperda cells (Sf9) and its alteration following baculovirus infection. J Gen Virol. 2013;94:1385–97. PubMed PMID: 23407421.
19. Jayachandran B, Hussain M, Asgari S. RNA interference as a cellular defense mechanism against the DNA virus baculovirus. J Virol. 2012;86:13729–34. PubMed PMID: 23055564.

20. Zhu M, Wang J, Deng R, Xiong P, Liang H, Wang X. A MicroRNA Encoded by Autographa californica Nucleopolyhedrovirus Regulates Expression of Viral Gene ODV-E25. J Virol. 2013;87:13029–34. PubMed PMID: 24027316.
21. Wu P, Shang Q, Dweteh OA, Huang H, Zhang S, Zhong J, Hou Q, Guo X. Over expression of bmo-miR-2819 suppresses BmNPV replication by regulating the BmNPV ie-1 gene in Bombyx mori. Mol Immunol. 2019;109:134–139. PubMed PMID: 30947109.
22. Nayyar N, Kaur I, Malhotra P, Bhatnagar RK. Quantitative proteomics of Sf21 cells during Baculovirus infection reveals progressive host proteome changes and its regulation by viral miRNA. Sci Rep. 2017;7:10902. PubMed PMID: 28883418.
23. Shrestha A, Bao K, Chen W, Wang P, Fei Z, Blissard GW. Transcriptional responses of the Trichoplusia ni midgut to oral infection by the baculovirus Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2019. doi: 10.1128/JVI.00353-19. PubMed PMID: 31043536.
24. Moreno-Habel DA, Biglang-awa IM, Dulce A, Luu DD, Garcia P, Weers PM, Haas-Stapleton EJ. Inactivation of the budded virus of Autographa californica M nucleopolyhedrovirus by gloverin. J Invertebr Pathol. 2012;110:92–101. PubMed PMID: 22401766.
25. Choi JY, Roh JY, Wang Y, Zhen Z, Tao XY, Lee JH, Liu Q, Kim JS, Shin SW, Je YH. Analysis of genes expression of Spodoptera exigua larvae upon AcMNPV infection. PLoS One. 2012;7:e42462. PubMed PMID: 22860129.
26. Liu TH, Dong XL, Pan CX, Du GY, Wu YF, Yang JG, Chen P, Lu C, Pan MH. A newly discovered member of the Atlastin family, BmAtlastin-n, has an antiviral effect against BmNPV in Bombyx mori. Sci Rep. 2016;6:28946. PubMed PMID: 27353084.
27. Kirkpatrick BA, Washburn JO, Volkman LE. AcMNPV pathogenesis and developmental resistance in fifth instar Heliothis virescens. J Invertebr Pathol. 1998;72:63–72. PubMed PMID: 9647703.
28. McNeil J, Cox-Foster D, Slavicek J, Hoover K. Contributions of immune responses to developmental resistance in Lymantria dispar challenged with baculovirus. J Insect Physiol. 2010;56:1167–77. PubMed PMID: 20350551.
29. McNeil J, Cox-Foster D, Gardner M, Slavicek J, Thiem S, Hoover K. Pathogenesis of Lymantria dispar multiple nucleopolyhedrovirus in L. dispar and mechanisms of developmental resistance. J Gen Virol. 2010;91:1590–600. PubMed PMID: 20164260.
30. Bergin D, Reeves EP, Renwick J, Wientjes FB, Kavanagh K. Superoxide production in Galleria mellonella hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. Infect Immun. 2005;73:4161–70. PubMed PMID: 15972506.
31. Tomalski MD, Eldridge R, Miller LK. A baculovirus homolog of a Cu/Zn superoxide dismutase gene. Virology. 1991.:149–161. PubMed PMID: 1871962.
32. Katsuma S, Kawaoka S, Mita K, Shimada T. Genome-wide survey for baculoviral host homologs using the Bombyx genome sequence. Insect Biochem Mol Biol. 2008;38:1080–6. PubMed PMID: 19280699.
33. Asser-Kaiser S, Radtke P, El-Salamouny S, Winstanley D, Jehle JA. Baculovirus resistance in codling moth (Cydia pomonella L.) caused by early block of virus replication. Virology. 2011;410:360–7. PubMed PMID: 21190707.
34. Levy SM, Falleiros AM, Moscardi F, Gregorio EA. Susceptibility/resistance of Anticarsia gemmatalis larvae to its nucleopolyhedrovirus (AgMNPV): Structural study of the peritrophic membrane. J Invertebr Pathol. 2007;96:183–6. PubMed PMID: 17568609.
35. Levy SM, Falleiros AM, Moscardi F, Gregorio EA. The role of peritrophic membrane in the resistance of Anticarsia gemmatalis larvae (Lepidoptera: Noctuidae) during the infection by its nucleopolyhedrovirus (AgMNPV). Arthropod Struct Dev. 2012;40:429–34. PubMed PMID: 21775204.
36. Haas-Stapleton EJ, Washburn JO, Volkman LE. Spodoptera frugiperda resistance to oral infection by Autographa californica multiple nucleopolyhedrovirus linked to aberrant occlusion-derived virus binding in the midgut. J Gen Virol. 2005;86:1349–55. PubMed PMID: 15831946.
37. Miller LK, Lu A. 1997. The molecular basis of baculovirus host range. *In* Miller LK (ed), The Baculoviruses. Plenum, New York.

38. Gomi S, Majima K, Maeda S. Sequence analysis of the genome of Bombyx mori nucleopolyhedrovirus. J Gen Virol. 1999;80:1323–37. PubMed PMID: 10355780.
39. Kuzio J, Pearson MN, Harwood SH, Funk CJ, Evans JT, Slavicek J, Rohrmann GF. Sequence and analysis of the genome of a baculovirus pathogenic for *Lymantria dispar*. Virology. 1999;253:17–34. PubMed PMID: 9887315.
40. Katou Y, Ikeda M, Kobayashi M. Abortive replication of Bombyx mori nucleopolyhedrovirus in Sf9 and High Five cells: defective nuclear transport of the virions. Virology. 2006;347:455–65. PubMed PMID: 16412489.
41. Iwanaga M, Takaya K, Katsuma S, Ote M, Tanaka S, Kamita SG, Kang W, Shimada T, Kobayashi M. Expression profiling of baculovirus genes in permissive and nonpermissive cell lines. Biochem Biophys Res Commun. 2004;323:599–614. PubMed PMID: 15369793.
42. Maeda S, Kamita SG, Kondo A. 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. 1993;67:6234–6238. PubMed PMID: 8396678.
43. Kamita SG, Maeda S. Sequencing of the putative DNA helicase-encoding gene of the Bombyx mori nuclear polyhedrosis virus and fine-mapping of a region involved in host range expansion. Gene. 1997;190:173–9. PubMed PMID: 9185864.
44. Croizier G, Croizier L, Argaud O, Poudevigne D. 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 Sc USA. 1994;91:48–52. PubMed PMID: 8278405.
45. Argaud O, Croizier L, Lopez-Ferber M, Croizier G. 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. 1998;79(Pt 4):931–5. PubMed PMID: 9568990.
46. Kamita SG, Maeda S. Inhibition of *Bombyx mori* nuclear polyhedrosis virus (NPV) replication by the putative DNA helicase gene of *Autographa californica* NPV. J Virol. 1993;67:6239–6245. PubMed PMID: 7690422.
47. Xu YP, Gu LZ, Lou YH, Cheng RL, Xu HJ, Wang WB, Zhang CX. A baculovirus isolated from the wild silkworm encompasses the host ranges of Bombyx mori NPV and Autographa californica MNPV in cultured cells. J Gen Virol. 2012;93:2480–9. PubMed PMID: 22855783.
48. Woo SD, Roh JY, Choi JY, Jin BR. Propagation of Bombyx mori Nucleopolyhedrovirus in nonpermissive insect cell lines. J Microbiol. 2007;45:133–8. PubMed PMID: 17483798.
49. Mori H, Yamao M, Nakazawa H, Sugahara Y, Shirai N, Matsubara F, Sumida M, Imamura T. Transovarian transmission of a foreign gene in the silkworm, Bombyx mori, by Autographa californica nuclear polyhedrosis virus. Biotechnology (N Y). 1995;13:1005–7. PubMed PMID: 9636278.
50. Rahman MM, Gopinathan KP. Analysis of host specificity of two closely related baculoviruses in permissive and nonpermissive cell lines. Virus Res. 2003;93:13–23. PubMed PMID: 12727338.
51. Guo T, Wang S, Guo X, Lu C. Productive infection of Autographa californica nucleopolyhedrovirus in silkworm Bombyx mori strain Haoyue due to the absence of a host antiviral factor. Virology. 2005;341:231–7. PubMed PMID: 16087208.
52. Hofmann C, Sandig V, Jennings G, Rudolph M, Schlag P, Strauss M. Efficient gene transfer into human hepatocytes by baculovirus vectors. Proc Natl Acad Sci U S A. 1995;92:10099–103. PubMed PMID: 7479733.
53. Katou Y, Yamada H, Ikeda M, Kobayashi M. A single amino acid substitution modulates low-pH-triggered membrane fusion of GP64 protein in Autographa californica and Bombyx mori nucleopolyhedroviruses. Virology. 2010;404:204–14. PubMed PMID: 20627345.
54. Rahman MM, Gopinathan KP. Characterization of the gene encoding the envelope fusion glycoprotein GP64 from Bombyx mori nucleopolyhedrovirus. Virus Res. 2003;94:45–57. PubMed PMID: 12837556.
55. Ikeda M, Yamada H, Hamajima R, Kobayashi M. Baculovirus genes modulating intracellular innate antiviral immunity of lepidopteran insect cells. Virology. 2013;435:1–13. PubMed PMID: 23217611.

56. Lu A, Miller LK. Differential requirements for baculovirus late expression factor genes in two cell lines. J Virol. 1995;69:6265–6272. PubMed PMID: 7666527.
57. Lu A, Miller LK. Species-specific effects of the hcf-1 gene on baculovirus virulence. J Virol. 1996;70:5123–30. PubMed PMID: 8764020.
58. Guzo D, Rathburn H, Guthrie K, Dougherty E. Viral and host cellular transcription in Autographa californica nuclear polyhedrosis virus-infected gypsy moth cell lines. J Virol. 1992;66:2966–72. PubMed PMID: 1560533.
59. Thiem SM, Du X, Quentin ME, Berner ML. Identification of a baculovirus gene that promotes *Autographa californica* nuclear polyhedrosis virus replication in a nonpermissive insect cell line. J Virol. 1996;70:2221–2229. PubMed PMID: 8642646.
60. Chen CJ, Quentin ME, Brennan LA, Kukel C, Thiem SM. *Lymantria dispar* nucleopolyhedrovirus *hrf*-1 expands the larval host range of *Autographa californica* nucleopolyhedrovirus. J Virol. 1998;72:2526–2531. PubMed PMID: 9499118.
61. Du X, Thiem SM. Responses of insect cells to baculovirus infection: protein synthesis shutdown and apoptosis. J Virol. 1997;71:7866–7872. PubMed PMID: 9311875.
62. Mazzacano CA, Du X, Thiem SM. Global protein synthesis shutdown in Autographa californica nucleopolyhedrovirus-infected Ld652Y cells is rescued by tRNA from uninfected cells. Virology. 1999;260:222–31. PubMed PMID: 10417257.
63. Thiem SM, Chejanovsky N. The role of baculovirus apoptotic suppressors in AcMNPV-mediated translation arrest in Ld652Y cells. Virology. 2004;319:292–305. PubMed PMID: 14980489.
64. Ishikawa H, Ikeda M, Alves CA, Thiem SM, Kobayashi M. 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. 2004;78:12703–8. PubMed PMID: 15507661.
65. Chen YR, Zhong S, Fei Z, Gao S, Zhang S, Li Z, Wang P, Blissard GW. Transcriptome responses of the host Trichoplusia ni to infection by the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Virol. 2014;88:13781–97. PubMed PMID: 25231311.
66. Hodgson JJ, Buchon N, Blissard GW. Identification of insect genes involved in baculovirus AcMNPV entry into insect cells. Virology. 2018;527:1–11. PubMed PMID: 30445201.
67. Shrestha A, Bao K, Chen YR, Chen W, Wang P, Fei Z, Blissard GW. Global Analysis of Baculovirus Autographa californica Multiple Nucleopolyhedrovirus Gene Expression in the Midgut of the Lepidopteran Host Trichoplusia ni. J Virol. 2018.:92. PubMed PMID: 30209166.
68. Yue Q, Yu Q, Yang Q, Xu Y, Guo Y, Blissard GW, Li Z. Distinct Roles of Cellular ESCRT-I and ESCRT-III Proteins in Efficient Entry and Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29046462.
69. Guo Y, Yue Q, Gao J, Wang Z, Chen YR, Blissard GW, Liu TX, Li Z. Roles of Cellular NSF Protein in Entry and Nuclear Egress of Budded Virions of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2017.:91. PubMed PMID: 28747507.

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