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4. Early events in infection: Virus transcription

Transcriptional activators, enhancers and the host RNA polymerase

An underlying feature of gene expression in eukaryotic cells is that relatively few genes are concurrently transcribed and the population of genes that are expressed may change during development. Critical for regulating gene expression is a somewhat scarce set of proteins that comprise the transcriptional apparatus. The heart of this complex is an enzyme, RNA polymerase, that synthesizes RNA. However, for RNA polymerase to selectively transcribe specific genes, a variety of transcription factors are required. These factors have different functions and can include: selecting the genes that will be transcribed; attracting and stabilizing the RNA polymerase complex; and facilitating RNA synthesis. Another category of critical factors are transcriptional activators that elevate the levels of specific RNA synthesis. These often function in conjunction with enhancers that are DNA sequences that bind transcription factors and bring them in close proximity to the RNA polymerase complex, thereby elevating the levels of RNA synthesis. Collectively these DNA sequences, enzymes, and factors regulate transcription by influencing the rate at which RNA polymerase is recruited to, or stabilized, at a specific promoter and its subsequent ability to efficiently transcribe RNA. In higher eukaryotes, different transcriptional activators can cause the expression of ordered sets of genes at each stage of development, or can lead to the transcription of tissue-specific genes in differentiated tissues.

Viruses destined for productive infections are confronted with a dilemma upon entry into a cell: they must compete with the cell for control of its well-organized and programmed transcriptional machinery that is strictly regulated for carrying out cellular processes. Without special mechanisms to take control of the cell and focus its replicative machinery on the synthesis of viral products, the viral genome would languish in the cell and could be overwhelmed by competition from cellular genes. To outcompete the cell and focus transcription on the viral genome, viruses rely on several mechanisms. They can selectively inhibit host cell transcription or eliminate host mRNA. They also can encode their own set of transcriptional activators and enhancer sequences, which facilitate the assembly of the host cell transcription complex at the site of RNA initiation of viral genes on the viral genome, rather than elsewhere, e.g., the host genome. Transcriptional activators can coordinate both the assembly of the transcription complex, and by interaction with enhancer sequences, they can bring enhancer-bound cellular transcription factors into close proximity to a gene promoter region. In some cases, this can elevate expression levels of viral genes several thousand-fold, thereby allowing the virus to redirect cellular processes for its own replication. Many DNA viruses use this process to cause a transcriptional cascade upon infection. In this process, one of the first viral genes synthesized is a transcription factor that activates a set of genes including a second transcription factor. This second factor activates transcription of a second set of genes

and so on until the transcription cascade is complete. This produces a regulated progression through a transcription program of categories of early and late genes that carry out the functions necessary for each stage of viral replication. In the case of herpes simplex virus, the transcriptional program is initiated by a transcriptional activator (vp16) that is a structural component of the virion and is transported into the nucleus during the initiation of infection. Other nuclear replicating viruses, e.g., adenoviruses, express a transcriptional activator early after infection, thereby initiating the transcriptional cascade. Throughout the transcriptional programs employed by these two viruses, the virus is dependent upon regulating the host RNA polymerase to transcribe its genes and thereby carry out its replication.

Baculovirus infection: selective effects on host cell gene expression

Early investigations using pulse labeling with ^{35}S methionine to examine protein synthesis in baculovirus infected cells indicated that as the infection proceeded, the host cell proteins faded and the viral proteins became more prominent (1). This was subsequently correlated with the down regulation of host cell mRNA levels (2, 3). It could also be a result of a phenomenon called translational arrest (4). Although the precise mechanism is not known, since the virus is dependent on a variety of cell functions including RNA polymerase II, a generalized inhibition of all host protein expression is unlikely in a permissive infection. More recent experiments have indicated that a number of host genes are up regulated during infection. For example, certain mitogen activated protein kinases (MAPKs) appear to be required for BmNPV gene expression. Inhibition of the expression of some MAPK genes results in reduced virus production (5), suggesting that any inhibition of host gene expression that occurs is selective or that the virus is able to up regulate certain categories of host genes. During AcMNPV infection of *T. ni* cells, the majority of host transcripts decrease between 6 to 48 hr post infection (pi) 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 hr pi 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 (6). Using 2-D gel comparisons in combination with proteomic analysis, a comparison of protein expression in cells permissive, semi- and non-permissive for AcMNPV infection was reported (7). It has also been observed that in *S. exigua* larvae infected with AcMNPV, some host genes are up-regulated while others are down regulated (8) suggesting that in whole insects the general shut down of host genes does not occur. It has also been shown that baculovirus DNA replication can trigger both apoptosis and translational arrest, thereby influencing the expression of host genes (9). The conflicting evidence regarding levels of host protein synthesis could be due to the level of expression of the up-regulated genes. Many of the most abundant host proteins could be shut down, while other genes, particularly those with an essential enzymatic function, might be up regulated, but are not evident because their expression is relatively low. For additional discussion of the regulation of RNA polymerase II, see the section Shutoff of Early Transcription below.

The baculovirus transcription cascade: the evolution of a novel strategy

Baculoviruses begin their infection cycle similar to other large DNA viruses and employ enhancers and transcriptional activators to exploit the host transcriptional apparatus. This early program is focused on establishing the infection and producing the components necessary for initiating viral DNA replication and other early functions. However, whereas these early events are dependent on genes transcribed by the host RNA polymerase, the later genes are transcribed by a baculovirus-encoded RNA polymerase (Figure 1). Consequently, early in infection transcription is carried out by the host RNA polymerase, whereas the viral RNA polymerase is involved late in infection. Although some bacterial viruses, e.g., T7, also exploit their host's RNA polymerase

early and employ their own RNA polymerase later in infection, baculoviruses are the only nuclear replicating DNA viruses of eukaryotes that employ this combination of cellular and viral polymerases.

Other eukaryotic DNA viruses that encode their own RNA polymerases, such as pox viruses, replicate in the cytoplasm and do not have ready access to the cellular transcriptional apparatus because of their separation from the nucleus. The evolutionary logic for baculoviruses to encode their own RNA polymerase is unclear. However, it may have originally been a major advantage for the virus to be able to regulate its own polymerase in the context of cells that were undergoing the shock of virus infection. This advantage could include the mitigation of possible effects caused by low levels of host RNA polymerase that might be normally present or caused by the infection (see Chapter 6).

Transcriptional enhancers

Similar to other viruses, enhancers and transcriptional activators play a major role in the early events in baculovirus infections. Enhancers are specific DNA sequences (Figure 2) that are able to bind and concentrate transcription factors and can deliver them to the regulatory region of a gene, thereby facilitating transcriptional initiation (Figure 3). They can be located near or at some distance from the site of transcriptional initiation. It is thought that, when distant, the enhancer DNA with its associated transcription factors can bend around and be positioned in close proximity to the site of transcriptional initiation (Figure 3). Bending of the DNA can be facilitated by transcriptional activators that bind to both the enhancer sequence, and to the RNA polymerase complex. In this process, the RNA polymerase complex would bind to the gene promoter and the activator could then bind to the enhancer region and bring it and its associated transcription factors into close proximity to the RNA polymerase-promoter complex (Figure 3). Whereas one might consider an enhancer sequence distant from a promoter, activators binding to the enhancer located on the same DNA molecule as a promoter would likely be in much closer proximity to the promoter than if they were floating free in the cell.

Baculovirus enhancers:*hrs* (homologous regions)

A characteristic feature of most baculovirus genomes is the presence of homologous regions, or *hrs*. In AcMNPV, *hrs* are comprised of repeated units of about 70-bp with an imperfect 30-bp palindrome near their center (Figure 2). They are present at eight locations in the genome with 2 to 8 repeats at each site. They are highly variable, and although they are closely related within a genome, they may show very limited homology between different viruses. For example, in the CpGV genome, tandem repeated sequences are not evident, although a 75-bp imperfect palindrome is present at 13 different locations on the genome (10). In addition, in the TnNPV (group II) and several other genomes, *hr*-like sequences were not found (11), reviewed in (12).

Hrs have been implicated both as transcriptional enhancers and origins of DNA replication for a number of baculoviruses (13-18). In AcMNPV, they bind the transcriptional activator IE1 (Ac147) (19-21) and this binding can elevate the levels of IE1 transactivation up to 1000-fold (13). In addition, *hr* binding may cause IE1 to localize to sites that may be a prelude to replication foci (22). *Hrs* contain cAMP and 12-O-tetradecanoylphorbol 13-acetate (TPA) response elements (CRE and TRE)-like sequences located between the palindromes Figure 2). A survey of baculovirus genomes found that these elements were preferentially concentrated within the *hr* sequences. In AcMNPV, although *hrs* comprise less than 3% of the total genome sequence, they contain 94% (48 of 51) of the CRE and 62% (13 of 21) of the TRE motifs. One to two of these elements are found between each pair of *hr* palindromes (Figure 2). These are evolutionarily conserved throughout the lepidopteran NPVs, but are not found in GVs, or dipteran and hymenopteran NPVs. In other systems, these elements bind cellular transcription factors and stimulate RNA polymerase II dependent transcription. In AcMNPV, the CRE and TRE were found to bind to host cell proteins and activate transcription in transient assays (23). Despite the concentration of these motifs in *hrs*, their ability to activate transcription appears to be adapted to each virus, probably due to a reduction in the affinity of IE1 from one virus to the *hrs* of another. For example, AcMNPV

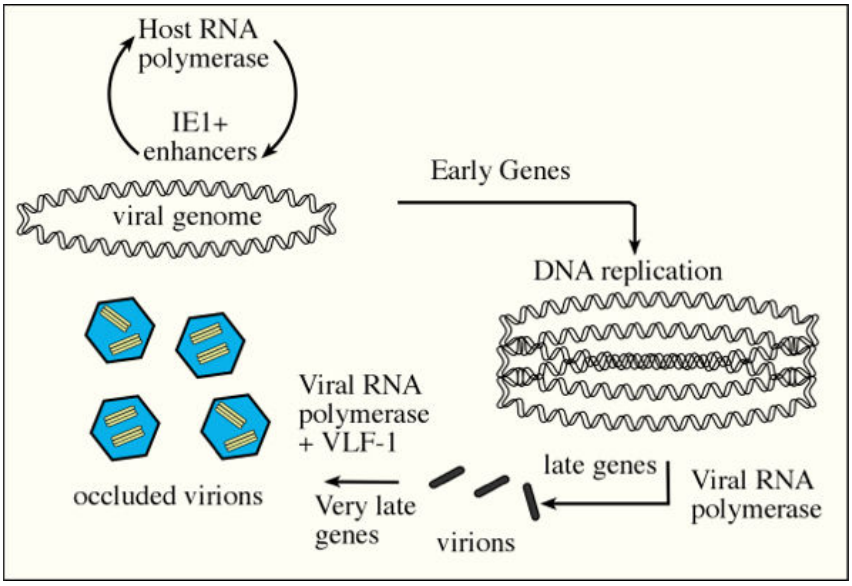

Figure 1. The baculovirus transcriptional cascade showing the interrelationship of host and viral RNA polymerases and DNA replication and VLF-1.

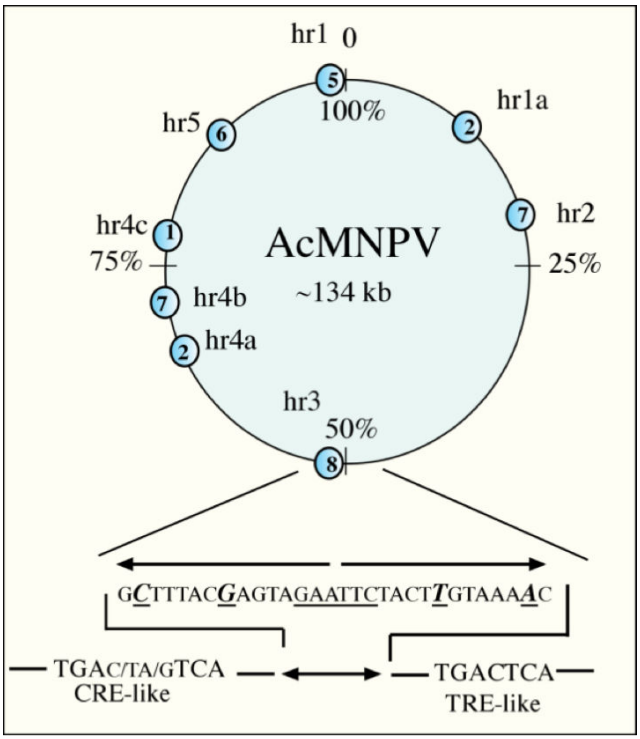

Figure 2. Diagram of the AcMNPV genome showing *hrs*. The circled numbers indicate the number of repeats in each *hr*. Below is shown a representative palindrome with mismatches shown as underlined, italicized larger type. The EcoRI site at the center of the palindrome is also underlined. At the bottom is a schematic showing the CRE- and TRE-like sequences that are located between the major palindromes.

IE1 is much more efficient in activating a reporter gene linked to an AcMNPV *hr* than is OpMNPV IE1 (24). The consensus sequences of the *hr* palindromes between the two viruses are about 57% (17/30) identical (25).

It has been shown that deletion of individual or combinations of two *hrs* did not appear to affect AcMNPV replication in cultured cells (26). This is probably due to the ability of transcriptional activators to interact with

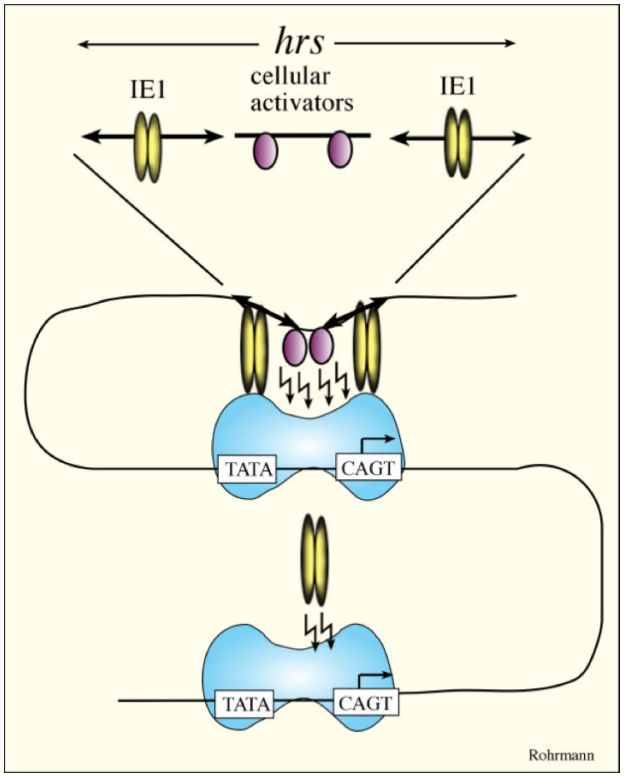

Figure 3. Activation of baculovirus early gene transcription by *hrs* and IE1. This diagram shows two possible mechanism by which IE1 activates transcription. Transcription might be activated directly by IE1 as shown in the bottom of the diagram, or it can interact with both RNA polymerase II (in blue) and *hr* sequences thereby bringing *hr*-bound transcriptional activators in close proximity to RNA polymerase II as shown in the top of the figure.

hrs in other locations of the genome, allowing them to be brought into close proximity to the promoters being activated by bending of the DNA (Figure 3). However, the removal of up to five *hrs* reduced BV titers about 10-fold and the removal of all eight AcMNPV *hrs* caused a reduction in BV production of over 10,000-fold, and the number of cells producing polyhedra was reduced to a few percent compared to wt infection. In addition, the expression levels of LEF3, GP64 and VP39(capsid) was greatly reduced when eight *hrs* were removed (27). Since *hr* sequences serve are origins of DNA replication in transient assays (see Chapter 5), the severe effects of removing all the AcMNPV *hrs* could be due to a reduction in DNA replication or transcriptional activation or a combination of both.

Transactivation of early genes.

There are five genes that are expressed as immediate early genes and they all have been reported to have transactivator function. They include *ie0*, *ie1*, *ie2*, *me53*, and *pe38*. An analysis of all five genes indicated that elevated levels of IE gene expression is related to the activation of this set of genes. However, IE0 has a repressor function caused by its interaction with IE1 and the role of Me53 is unclear (28).

A major transcriptional activator of early genes, immediate early gene 1 (IE1, Ac147). To start the transcriptional cascade that initiates the baculovirus replication cycle, many baculoviruses employ the transcriptional activator, IE1. IE1 was originally identified because of its ability to transactivate early promoters of AcMNPV (29). It is transcribed early in infection (hence the name: immediate early gene 1, *ie1*) and continues to be transcribed through the late phase (30). Orthologs of IE1 have been identified in all Group I and II NPV genomes sequenced. They also appear to be present in GV genomes, but the similarity is very low, e.g., XcGVorf9 and Ac147 are about 10% identical. However, the orientation and position of XcGV orf9 relative to more

conserved orfs is similar to Ac147, which suggests that Ac147 and XcGV orf9 are related. Part of the IE1 population is called IE0 and is translated from a larger spliced mRNA which is the only major spliced transcript described for baculoviruses (see below Ac147-0). In AcMNPV, either IE1 or IE0 can support viral replication (see below). AcMNPV IE1 contains two separate domains: an acidic activation domain located in the N-terminal region, and a DNA binding domain present in the C-terminal region (31, 32). It also contains a dimerization domain that is associated with a predicted helix-loop-helix sequence near the C terminus (21, 33). A positively charged domain adjacent to the dimerization domain is involved in nuclear import and is dependent on IE1 dimerization (34). A conserved 10-aa basic region (aa 152-161-KIKLRPKYKK) separating two acidic activation domains was found to be required for DNA binding. It was suggested that this basic region neutralizes the adjacent acidic activation domains. In this model, when the basic region interacts with DNA, the acidic domains are exposed, allowing them to participate in activation (35). IE1 from a related virus, OpMNPV, has activation domains similar to AcMNPV IE1 (36) and a region in the 65 N-terminal amino acids that is involved in both transcriptional activation and DNA replication. The replication domain was specific to the virus and other sequences that supported transactivation did not support replication in an OpMNPV transient assay system (37). The ability of IE1 to transactivate transient transcription is greatly enhanced when the activated gene is linked to *hrs* (14). IE1 may also participate in the negative regulation of some genes (38) (see below). IE1 is required for transient DNA replication (39, 40) and is an essential gene as a bacmid deleted for this gene was not viable (41). Similar results have been reported for BmNPV (42).

Binding of IE1 to *hr* sequences

Although IE1 has been extensively investigated, purification of biologically active IE1 has not been reported. This suggests that it might be unstable and inactivated during purification procedures or that it is only active in combination with another protein(s). However, IE1 has been shown to bind to *hr* sequences independent of insect cell proteins. This was accomplished by transcribing the *ie1* gene in vitro, and then translating the resulting RNA in a rabbit reticulocyte system. The product was able to specifically bind to *hr* sequences indicating that additional insect cell proteins are not required for binding to occur (19, 43).

Many proteins, including type II restriction enzymes and numerous transcription factors, recognize and bind to palindrome sequences. Such proteins are normally found as homodimers and interact in such a way that the DNA binding site of each monomer recognizes the identical sequence, but on opposite sides of the palindrome. IE1 appears to follow this pattern in its interaction with the palindromes of *hr* sequences, and both halves of a palindrome are necessary for activation (21). Footprinting analyses have not been straightforward, which may be due to the presence of two types of binding to DNA. If mixtures of these two types of binding occur, especially when mutants are being examined, the actual pattern of binding may be obscured. However, foot printing of wt sequences suggests that IE1 interacts with both halves of the palindrome, but on opposite DNA strands as described above. Although altering a palindrome half-site by mutation prevents activation, IE1 still binds to the remaining site. However, the binding properties are changed, such that IE1 binds to both strands of the remaining half site (44). It was suggested that this could explain the mechanism by which IE1 negatively regulated the expression of certain genes that have an *hr* half-site sequence located between their TATA promoter and mRNA start site motif (38). By binding in this region, IE1 could prevent the binding and function of RNA polymerase II, thereby preventing transcription of the gene.

Despite their apparently critical role as transcriptional enhancers and probable role as origins of DNA replication, it is not clear how *hrs* are recognized by the proteins involved in these functions. It was suggested that they may form cruciform structures in the DNA that would provide landmarks for the binding of transcriptional activators. This has been observed in other palindrome-like structures, e.g., the high affinity binding of the cyclic AMP response element binding protein (CREB) to enkephalin enhancers (45). However, studies in vitro were unable to detect the formation of cruciform-like structures by AcMNPV *hr*-like imperfect

palindromes, although such structures were formed by perfect palindromes in which mismatches in the predicted *hr* structure were eliminated. In addition, IE1 was able to bind to both these structures (46).

Splicing in AcMNPV; Ac147-0 (ie0) is a major spliced gene

Part of the IE1 population is called IE0 and is translated from a larger spliced mRNA which was the only major spliced transcript described for baculoviruses, and in AcMNPV results in an additional 54 amino acids at the N-terminus of IE1 for a total of 636 amino acids (47). In a survey of the AcMNPV transcriptome in infected *T. ni* cells, 11 spliced mRNAs in addition to IE1 were identified (48). However, only 3 of these sites were located within coding regions, the rest being in the 5' or 3' UTRs. For *ie1*, the spliced form is abundant early and comprised over 80% of the ie1 mRNA at 6 hpi, but declined to 2% by 48 hpi. Other splice sites in reading frames included p47 that resulted in a reading frame change and in exon 0, however these were minor components. With the exception of IE1, most of the spliced mRNAs are minor components of the transcript population, however, the majority of the SOD and orf114 transcripts have spliced 3' UTRs that are present throughout the infection.

Another example of a spliced mRNA was reported from OpMNPV, in which a 2.8 kb intron was removed. This resulted in the fusion of Op18 mRNA downstream of its stop codon with Op21 downstream of the Op21 ATG. This mRNA would include all the Op18 orf and its stop codon and would be unlikely to produce a novel protein because of the location of a stop codon upstream of the splice site (49).

AcMNPV IE1 is present as a homodimer but also can form a heterodimer with IE0 that has been reported to suppress IE1 activation (28). Either IE1 or IE0 can support the production of infectious virus; however, there are subtle differences in the timing of events and of BV and polyhedra production depending on which gene is being expressed, suggesting that both IE0 and IE1 are required for wt levels of infection (41). In contrast, in LdMNPV, only the spliced form is able to transactivate transient transcription and DNA replication (50).

Two additional transcriptional activators

AcMNPV appears to encode two additional factors that may be involved in activating transcription. It is likely that both these proteins form homodimers, but unlike IE1, specific DNA binding sites have not been identified, suggesting that their activation may be based on their interaction with other proteins.

Ac151 (ie2/ie-n). Homologs of *ie2* are limited to Group I lepidopteran NPVs. IE2 contains a predicted RING finger domain indicating that it likely interacts with other proteins, and in *B. mori* has been found to interact with itself (51). IE2 was found to augment activation by IE1 (52-54) and was required for optimal origin-specific plasmid DNA replication when transfected into Sf-21 cells, but had little effect in Tn-368 cells (55). AcMNPV mutants deleted for *ie2* show a delay in infection in Sf-21. However, this delay was not observed in *T. ni* cells. In larvae of *T. ni* and *S. frugiperda*, the mutant viruses were significantly less infectious than wt which appeared to be due to the lack of virions in the occlusion bodies produced by the mutants. However, the intrahemocelic injection of mutant BV caused infections similar to wt (56). IE-2 may also be involved in cell cycle regulation (57).

Ac153 (pe38). The other potential transcriptional activator in AcMNPV is *Ac153*. Homologs of Ac153 have an unusual distribution being found in all Group I NPV and at least four GV sequenced genomes. Ac153 was originally identified because of its early transcription profile and the presence of predicted zinc finger and leucine zipper motifs. It was named after a cDNA clone that hybridized to a PstI/EcoRI fragment (58). Although it was not found to activate transient late transcription in one assay (59), evidence from OpMNPV indicated that it is a transactivator (60). When AcMNPV *pe38* was deleted, although the expression of several genes was unaffected, a number of others were delayed (61). This appears to delay the infection and results in a 99% reduction in BV production, reduced levels of DNA synthesis, and a reduction in oral infectivity in larvae (62). In addition, it appears to activate DNA replication in transient assays (39).

RNA polymerase II signals regulating early virus gene transcription

Upstream of baculovirus early genes are insect cell regulatory sequences including promoters that are recognized by the host cell RNA polymerase II and the associated transcriptional apparatus. The insect RNA polymerase II is able to initiate transcription from baculovirus promoters and even promoters that normally are transcribed by vertebrate cells such as the adenovirus major late promoter (63). Two major sequences have been identified in baculovirus early genes including a TATA promoter motif and a transcriptional initiation consensus sequence, CAGT. Transient transcription of a minimal gp64 promoter was eliminated when either of these sequences was mutated, suggesting that they both are necessary for transcription (64). The CAGT sequence is apparently required for positioning the location where the mRNA sequence is started and can also act independently as an initiator. Initiators facilitate the assembly of the RNA polymerase II preinitiation complex and can promote transcription in the absence of a TATA sequence. This has also been demonstrated in baculoviruses for the *ie1* gene (65). Many baculovirus early genes have TATA promoter sequences along with consensus CAGT (or CATT — see below) transcriptional initiation sequences located about 30 bp downstream. These consensus sequences are two of the most prevalent motifs found in regulatory regions of genes from *Drosophila* (66). Several sequences have been identified both upstream and downstream of the promoter region that elevate early gene transcription; however, the mechanism underlying their function has not been characterized (67).

Genome-wide analysis of baculovirus promoters

A survey of predicted baculovirus promoter sequences was conducted on 26 baculovirus genomes (68). In this investigation, sequence patterns were characterized for the 300 bp of DNA upstream of the ATG initiation codons of baculovirus orfs. The significance of the prevalence of these predicted promoter sequences was determined by comparing their frequency in the 300-bp downstream of the ATG. There was a strong correlation for TATA sequences upstream of an ORF. Particularly significant were sequences such as TATAAGG that contain both TATA and late promoter sequences (ATAAG) in combination. These were about 7 times as frequent in the upstream location. Furthermore, about 70% of the TATA sequences present in the genome are clustered in the 100-bp upstream of the ATG. TATA sequences combined with mRNA start site consensus sequences CAGT or CATT separated by ~30-bp were 3 times as frequent in the upstream region. Similarly, putative initiator sequences (69) in combination with a TCGT motif ~30-bp downstream were almost 5 times more prevalent upstream of the ATG. Some other sequences such as GTAG, AGTC and TAGG were about 20 times more frequent in the promoter (upstream) region. They were often present in combination with early or late promoter elements, suggesting that they are involved in optimizing transcription from these sequences.

In a study of the transcriptome from AcMNPV infected *T. ni* cells (48), the sequences near the early transcription start site (TSS) had the following frequency: CAGT, 15%; CATT, 9%; and CAAT, 8.7%. In addition, the TSS was located ~32 nt downstream of the TATAA sequence.

For more information on transcriptional initiation sites, see Chapter 6.

Temporal expression of early and late genes (see Chapter 6).

How are baculovirus early genes activated?

IE1 can activate transcription, and the level of activation is increased many fold in the presence of *hr* enhancer sequences. In addition, *hr* sequences appear to be capable of binding to cellular factors that activate transcription. Since IE1 can activate transcription in the absence of *hrs*, it likely has the ability to interact with the RNA polymerase II-containing transcription complex and facilitate either the recruitment of other factors, or to elevate the levels of transcription itself. Since *hrs* appear to bind both cellular transcription factors and IE1,

IE1 could recruit the *hr* bound factors to the RNA polymerase II complex (Figure 3). In this manner it would appear to be able to influence the rate at which RNA polymerase is recruited to or stabilized at a specific promoter and its subsequent ability to efficiently transcribe RNA.

Shutoff of early transcription

A feature of most baculovirus early genes is that they appear to be transiently expressed and are shut off at late times post-infection. The mechanism for this shutoff is not understood. Some early genes avoid being shut off early by having late in addition to early promoter elements. These genes can be continuously expressed through both early and late times post-infection. In addition, some genes such as *ie1* appear to be transcribed throughout infection by an undetermined mechanism (30).

A mechanism for the regulation of RNA polymerase II entails the addition or removal of phosphates to its carboxyl terminal domain (CTD), and CTD phosphatases are involved in this process. Ac98 is a core gene and has homology to CTD phosphatases and is required for viral viability (70), may stimulate both early and late gene expression in transient assays (71) and dephosphorylates the p6.9 DNA binding protein which allows it to be packaged with the viral DNA (72). Dephosphorylation of the CTD can negatively regulate RNA polymerase II by inhibiting RNA elongation (73, 74). Although Ac98 may stimulate early transcription in a transient assay, this needs to be investigated in the context of viral infections as Ac98 itself could be regulated to selectively stimulate baculovirus early genes.

Caveats and qualifications

Despite the complexity of the structure of baculovirus virions and the presence of nonstructural proteins that are likely packaged with budded and occluded virions, it is possible that none of these proteins are required for the initiation of infection once the DNA is uncoated in the nucleus. Consequently, although many proteins have been reported to be associated with baculovirus virions, none have been shown to be required for the initiation of gene expression or DNA replication. Additional evidence for independence from virion proteins is the observation that purified viral DNA devoid of proteins is capable of initiating baculovirus infection when transfected into susceptible cells (75-78). Therefore, no viral proteins are required to initiate a baculovirus infection; however, this observation does not rule out the possibility that they facilitate the efficiency of the infection. In fact, IE1 was found to be associated with BV but not ODV of OpMNPV (79). In addition, a low level of a variety of late transcripts was observed at early times in the transcriptome of AcMNPV infected *T. ni* cells suggesting that they may have been transferred with the virion during infection (48). It is not clear whether they contribute to the initiation of the infection.

Another caveat regarding the molecular events involved in viral transcription and replication is that almost all this information is derived from AcMNPV, and to a lesser extent, the closely related BmNPV. Whereas much of this information is likely applicable to most baculoviruses, there are major differences, not only in gene content, but also in gene sequence homology between AcMNPV and many other baculoviruses. In addition, there are major differences in the number and distribution of *hr* enhancer sequences. All these factors likely have major, but currently unknown, influences on these events in other baculoviruses.

References

1. Kelly DC, Lescott T. Baculovirus replication: protein synthesis in *Spodoptera frugiperda* cells infected with *Trichoplusia ni* nuclear polyhedrosis virus. Microbiologica. 1981;4:35–47.
2. Nobiron I, O'Reilly DR, Olszewski JA. Autographa californica nucleopolyhedrovirus infection of Spodoptera frugiperda cells: a global analysis of host gene regulation during infection, using a differential display approach. J Gen Virol. 2003;84:3029–39. PubMed PMID: 14573808.

3. Ooi BG, Miller LK. Regulation of host RNA levels during baculovirus infection. Virology. 1988;166:515–523. PubMed PMID: 2459844.
4. Thiem SM. Baculovirus genes affecting host function. In Vitro Cell Dev Biol Anim. 2009;45:111–26. PubMed PMID: 19247726.
5. Katsuma S, Mita K, Shimada T. ERK- and JNK-dependent signaling pathways contribute to Bombyx mori nucleopolyhedrovirus infection. J Virol. 2007;81:13700–9. PubMed PMID: 17913811.
6. 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.
7. Popham HJ, Grasela JJ, Goodman CL, McIntosh AH. Baculovirus infection influences host protein expression in two established insect cell lines. J Insect Physiol. 2010;56:1237–45. PubMed PMID: 20362582.
8. 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.
9. Schultz K, Friesen P. Baculovirus DNA replication-specific expression factors trigger apoptosis and shutoff of host protein synthesis during infection. J Virol. 2010;83:11123–32. PubMed PMID: 19706708.
10. Luque T, Finch R, Crook N, O'Reilly DR, Winstanley D. The complete sequence of the Cydia pomonella granulovirus genome. J Gen Virol. 2001;82:2531–47. PubMed PMID: 11562546.
11. Willis LG, Siepp R, Stewart TM, Erlandson MA, Theilmann DA. Sequence analysis of the complete genome of Trichoplusia ni single nucleopolyhedrovirus and the identification of a baculoviral photolyase gene. Virology. 2005;338:209–26. PubMed PMID: 15951000.
12. Thumbi DK, Eveleigh RJ, Lucarotti CJ, Lapointe R, Graham RI, Pavlik L, Lauzon HA, Arif BM. Complete sequence, analysis and organization of the Orgyia leucostigma nucleopolyhedrovirus genome. Viruses. 2011;3:2301–27. PubMed PMID: 22163346.
13. Guarino LA, Gonzalez MA, Summers MD. Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1986;60:224–229. PubMed PMID: 16789259.
14. Guarino LA, Summers MD. Interspersed homologous DNA of *Autographa californica* nuclear polyhedrosis virus enhances delayed-early gene expression. J Virol. 1986;60:215–223. PubMed PMID: 16789258.
15. Leisy DJ, Rohrmann GF. Characterization of the replication of plasmids containing *hr* sequences in baculovirus-infected *Spodoptera frugiperda* cells. Virology. 1993;196:722–730. PubMed PMID: 8372444.
16. Pearson MN, Bjornson RM, Pearson GD, Rohrmann GF. The *Autographa californica* baculovirus genome: Evidence for multiple replication origins. Science. 1992;257:1382–1384. PubMed PMID: 1529337.
17. Pearson MN, Bjornson RM, Ahrens C, Rohrmann GF. Identification and characterization of a putative origin of DNA replication in the genome of a baculovirus pathogenic for *Orgyia pseudotsugata*. Virology. 1993;197:715–725. PubMed PMID: 8249294.
18. Pearson MN, Rohrmann GF. *Lymantria dispar* nuclear polyhedrosis virus homologous regions: characterization of their ability to function as replication origins. J Virol. 1995;69:213–221. PubMed PMID: 7983712.
19. Choi J, Guarino LA. The baculovirus transactivator IE1 binds to viral enhancer elements in the absence of insect cell factors. J Virol. 1995;69:4548–4551. PubMed PMID: 7769721.
20. Leisy DJ, Rasmussen C, Kim H-T, Rohrmann GF. The *Autographa californica* nuclear polyhedrosis virus homologous region 1a: Identical sequences are essential for DNA replication activity and transcriptional enhancer function. Virology. 1995;208:742–752. PubMed PMID: 7747446.
21. Rodems SM, Friesen PD. Transcriptional enhancer activity of *hr*5 requires dual-palindrome half sites that mediate binding of a dimeric form of the baculovirus transregulator IE1. J Virol. 1995;69:5368–5375. PubMed PMID: 7636981.
22. Nagamine T, Kawasaki Y, Iizuka T, Matsumoto S. Focal Distribution of Baculovirus IE1 Triggered by Its Binding to the hr DNA Elements. JVirol. 2005;79:39–46. PubMed PMID: 15596799.

23. Landais I, Vincent R, Bouton M, Devauchelle G, Duonor-Cerutti M, Ogliastro M. Functional analysis of evolutionary conserved clustering of bZIP binding sites in the baculovirus homologous regions (hrs) suggests a cooperativity between host and viral transcription factors. Virology. 2006;344:421–31. PubMed PMID: 16198391.
24. Theilmann DA, Stewart S. Identification and characterization of the IE-1 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. Virology. 1991;180:492–508. PubMed PMID: 1989381.
25. Ahrens CH, Russell R, Funk CJ, Evans JT, Harwood SH, Rohrmann GF. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. Virology. 1997;229:381–399. PubMed PMID: 9126251.
26. Carstens EB, Wu Y. No single homologous repeat region is essential for DNA replication of the baculovirus Autographa californica multiple nucleopolyhedrovirus. J Gen Virol. 2007;88:114–22. PubMed PMID: 17170443.
27. Bossert M, Carstens EB. Sequential deletion of AcMNPV homologous regions leads to reductions in budded virus production and late protein expression. Virus Res. 2018;256:125–133. PubMed PMID: 30121325.
28. Ono C, Sato M, Taka H, Asano S, Matsuura Y, Bando H. Tightly regulated expression of Autographa californica multicapsid nucleopolyhedrovirus immediate early genes emerges from their interactions and possible collective behaviors. PLoS One. 2015;10:e0119580. PubMed PMID: 25816136.
29. Guarino LA, Summers MD. Functional mapping of a trans-activating gene required for expression of a baculovirus delayed-early gene. J Virol. 1986;57:563–57l. PubMed PMID: 3944847.
30. Kovacs GR, Guarino LA, Graham BL, Summers MD. Identification of spliced baculovirus RNAs expressed late in infection. Virology. 1991;185:633–643. PubMed PMID: 1962442.
31. Kovacs GR, Choi J, Guarino LA, Summers MD. Functional dissection of the *Autographa californica* nuclear polyhedrosis virus immediate-early 1 transcriptional regulatory protein. J Virol. 1992;66:7429–7437. PubMed PMID: 1433524.
32. Slack JM, Blissard GW. Identification of two independent transcriptional activation domains in the Autographa californica multicapsid nuclear polyhedrosis virus IE1 protein. J Virol. 1997;71:9579–87. PubMed PMID: 9371622.
33. Olson VA, Wetter JA, Friesen PD. Oligomerization mediated by a helix-loop-helix-like domain of baculovirus IE1 is required for early promoter transactivation. J Virol. 2001;75:6042–51. PubMed PMID: 11390606.
34. Olson VA, Wetter JA, Friesen PD. Baculovirus transregulator IE1 requires a dimeric nuclear localization element for nuclear import and promoter activation. J Virol. 2002;76:9505–15. PubMed PMID: 12186932.
35. Olson VA, Wetter JA, Friesen PD. The highly conserved basic domain I of baculovirus IE1 is required for hr enhancer DNA binding and hr-dependent transactivation. J Virol. 2003;77:5668–77. PubMed PMID: 12719559.
36. Forsythe IJ, Shippam CE, Willis LG, Stewart S, Grigliatti T, Theilmann DA. Characterization of the acidic domain of the IE1 regulatory protein from Orgyia pseudotsugata multicapsid nucleopolyhedrovirus. Virology. 1998;252:65–81. PubMed PMID: 9875318.
37. Pathakamuri JA, Theilmann DA. The acidic activation domain of the baculovirus transactivator IE1 contains a virus-specific domain essential for DNA replication. J Virol. 2002;76:5598–604. PubMed PMID: 11991988.
38. Leisy DJ, Rasmussen C, Owusu O, Rohrmann GF. A mechanism for negative gene regulation in *Autographa californica* multicapsid nuclear polyhedrosis virus. J Virol. 1997;71:5088–5094. PubMed PMID: 9188574.
39. Kool M, Ahrens C, Goldbach RW, Rohrmann GF, Vlak JM. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. Proc Natl Acad Sci USA. 1994;91:11212–11216. PubMed PMID: 7972036.
40. Lu A, Miller LK. The roles of eighteen baculovirus late expression factor genes in transcription and DNA replication. J Virol. 1995;69:975–982. PubMed PMID: 7815565.

41. Stewart TM, Huijskens I, Willis LG, Theilmann DA. The Autographa californica multiple nucleopolyhedrovirus ie0-ie1 gene complex is essential for wild-type virus replication, but either IE0 or IE1 can support virus growth. J Virol. 2005;79:4619–29. PubMed PMID: 15795248.
42. Gomi S, Zhou CE, Yih W, Majima K, Maeda S. Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. Virology. 1997;230:35–47. PubMed PMID: 9126260.
43. Rodems SM, Pullen SS, Friesen PD. DNA-dependent transregulation by IE1 of Autographa californica nuclear polyhedrosis virus: IE1 domains required for transactivation and DNA binding. J Virol. 1997;71:9270–9277. PubMed PMID: 9371585.
44. Leisy DJ, Rohrmann GF. The Autographa californica nucleopolyhedrovirus IE-1 protein complex has two modes of specific DNA binding. Virology. 2000;274:196–202. PubMed PMID: 10936100.
45. McMurray CT, Wilson WD, Douglass JO. Hairpin formation within the enhancer region of the human enkephalin gene. Proc Natl Acad Sci USA. 1991;88:666–670. PubMed PMID: 1988963.
46. Rasmussen C, Leisy DJ, Ho PS, Rohrmann GF. Structure-function analysis of the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus homologous region palindromes. Virology. 1996;224:235–245. PubMed PMID: 8862418.
47. Chisholm GE, Henner DJ. Multiple early transcripts and splicing of the *Autographa californica* nuclear polyhedrosis virus IE-1 gene. J Virol. 1988;62:3193–3200. PubMed PMID: 3043024.
48. Chen YR, Zhong S, Fei Z, Hashimoto Y, Xiang JZ, Zhang S, Blissard GW. The transcriptome of the baculovirus Autographa californica Multiple Nucleopolyhedrovirus (AcMNPV) in Trichoplusia ni cells. J Virol. 2013;87:6391–405. PubMed PMID: 23536684.
49. Pearson MN, Russell RLQ, Rohrmann GF. Transcriptional mapping of two genes encoding baculovirus envelope-associated proteins. J Gen Virol. 2002;83:937–943. PubMed PMID: 11907344.
50. Pearson MN, Rohrmann GF. Splicing is required for transactivation by the immediate early gene 1 of the Lymantria dispar multicapsid nuclear polyhedrosis virus. Virology. 1997;235:153–165. PubMed PMID: 9300047.
51. Imai N, Kang W, Iwabuchi K, Sato K, Maeda S. Analysis of interaction between molecules of Bombyx mori nucleopolyhedrovirus IE-2 using a yeast two-hybrid system. Acta Virol. 2000;44:199–202. PubMed PMID: 11155366.
52. Carson DD, Guarino LA, Summers MD. Functional mapping of an AcNPV immediate early gene which augments expression of the IE-1 trans-activated 39K gene. Virology. 1988;162:444–451. PubMed PMID: 3277328.
53. Carson DD, Summers MD, Guarino LA. Molecular analysis of a baculovirus regulatory gene. Virology. 1991;182:279–286. PubMed PMID: 2024466.
54. Yoo S, Guarino LA. The *Autographa californica* nuclear polyhedrosis virus *ie2* gene encodes a transcriptional regulator. Virology. 1994;202:746–753. PubMed PMID: 8030237.
55. 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.
56. Prikhod'ko EA, Lu A, Wilson JA, Miller LK. In vivo and in vitro analysis of baculovirus ie-2 mutants. J Virol. 1999;73:2460–8. PubMed PMID: 9971831.
57. Prikhod'ko EA, Miller LK. Role of baculovirus IE2 and its RING finger in cell cycle arrest. J Virol. 1998;72:684–92. PubMed PMID: 9420274.
58. Krappa R, Knebel-Mörsdorf D. Identification of the very early transcribed baculovirus gene PE-38. J Virol. 1991;65:805–812. PubMed PMID: 1987375.
59. Passarelli AL, Miller LK. Three baculovirus genes involved in late and very late gene expression: ie-1, ie-n, and lef-2. J Virol. 1993;67:2149–2158. PubMed PMID: 8445724.
60. Wu X, Stewart S, Theilmann DA. Alternative transcriptional initiation as a novel mechanism for regulating expression of a baculovirus *trans* activator. J Virol. 1993;67:5833–5842. PubMed PMID: 8371344.
61. Jiang SS, et al. Temporal transcription program of recombinant Autographa californica multiple nucleopolyhedrosis virus. J Virol. 2006;80:8989–99. PubMed PMID: 16940511.

62. Milks ML, Washburn JO, Willis LG, Volkman LE, Theilmann DA. Deletion of pe38 attenuates AcMNPV genome replication, budded virus production, and virulence in Heliothis virescens. Virology. 2003;310:224–34. PubMed PMID: 12781710.
63. Hoopes RR Jr, Rohrmann GF. *In vitro* transcription of baculovirus immediate early genes: Accurate mRNA initiation by nuclear extracts from both insect and human cells. Proc Nat Acad Sci USA. 1991;88:4513–4517. PubMed PMID: 2034691.
64. Blissard GW, Kogan PH, Wei R, Rohrmann GF. A synthetic early promoter from a baculovirus: Roles of the TATA box and conserved mRNA start site CAGT sequence in basal levels of transcription. Virology. 1992;190:783–793. PubMed PMID: 1519359.
65. Pullen SS, Friesen PD. The CAGT motif functions as an initiator element during early transcription of the baculovirus transregulator ie-1. J Virol. 1995;69:3575–83. PubMed PMID: 7745705.
66. Ohler U, Liao GC, Niemann H, Rubin GM. 2002. Computational analysis of core promoters in the Drosophila genome. Genome Biol 3:0087.1.
67. Friesen PD. 1997. Regulation of baculovirus early gene expression. *In* Miller LK (ed), The Baculoviruses. Plenum, NY.
68. Xing K, Deng R, Wang J, Feng J, Huang M, Wang X. Analysis and prediction of baculovirus promoter sequences. Virus Res. 2005;113:64–71. PubMed PMID: 15908030.
69. Hariharan MJ, Driver DA, Townsend K, Brumm D, Polo JM, Belli BA, Catton DJ, Hsu D, Mittelstaedt D, McCormack JE, Karavodin L, Dubensky TW Jr, Chang SM, Banks TA. DNA immunization against herpes simplex virus: enhanced efficacy using a Sindbis virus-based vector. J Virol. 1998;72:950–8. PubMed PMID: 9444987.
70. Wu W, Lin T, Pan L, Yu M, Li Z, Pang Y, Yang K. Autographa californica multiple nucleopolyhedrovirus nucleocapsid assembly is interrupted upon deletion of the 38K gene. J Virol. 2006;80:11475–85. PubMed PMID: 16987976.
71. Hefferon KL. ORF98 of Autographa californica nucleopolyhedrosisvirus is an auxiliary factor in late gene expression. Can J Microbiol. 2003;49:157–63. PubMed PMID: 12795401.
72. Lai Q, Wu W, Li A, Wang W, Yuan M, Yang K. The 38K-Mediated Specific Dephosphorylation of the Viral Core Protein P6.9 Plays an Important Role in the Nucleocapsid Assembly of Autographa californica Multiple Nucleopolyhedrovirus. J Virol. 2018.:92. PubMed PMID: 29444944.
73. Yeo M, Lin PS. Functional characterization of small CTD phosphatases. Methods Mol Biol. 2007;365:335–46. PubMed PMID: 17200573.
74. Yeo M, Lin PS, Dahmus ME, Gill GN. A novel RNA polymerase II C-terminal domain phosphatase that preferentially dephosphorylates serine 5. J Biol Chem. 2003;278:26078–85. PubMed PMID: 12721286.
75. Burand JP, Summers MD, Smith GE. Transfection with baculovirus DNA. Virology. 1980;101:286–290. PubMed PMID: 18631639.
76. Carstens EB, Tjia ST, Doerfler W. Infectious DNA from *Autographa californica* nuclear polyhedrosis virus. Virology. 1980;101:311–314. PubMed PMID: 16945844.
77. Potter KN, Miller LK. Transfection of two invertebrate cell lines with DNA of *Autographa californica* nuclear polyhedrosis virus. J Invertebr Pathol. 1980;36:431–432.
78. Kelly DC, Wang X. 1981. The infectivity of nuclear polyhedrosis virus DNA. Annals of Virology (Institute Pasteur) 132 E:247-259.
79. Theilmann DA, Stewart S. Analysis of the *Orgyia pseudotsugata* multcapsid nuclear polyhedrosis virus trans-activators IE-1 and IE-2 using monoclonal antibodies. J Gen Virol. 1993;74:1819–1826. PubMed PMID: 8376961.

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