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6. Baculovirus late transcription

Activation of baculovirus late genes

A common feature of many viruses is the division of transcription into early and late categories. These divisions may be further separated into immediate early, early, late and very late. The separation between early and late transcription is always the onset of DNA replication. Despite this being a common feature of viruses, the mechanism underlying the linkage of DNA replication to the activation of late gene transcription is unclear. Two major features of newly replicated DNA may be involved in this process. Immediately after DNA replication there may be a transient period when proteins are not bound to the DNA and this might expose late promoters and facilitate their activation. In poxviruses, a set of transcriptional activators that facilitate the expression of late genes are expressed from newly replicated (naked) DNA (1). In baculoviruses, the generation of DNA free of proteins might facilitate the activation of late promoters until p6.9 or other DNA binding proteins accumulate to such a level that all viral DNA is coated and transcription is inhibited. Another major feature of DNA in the process of being replicated is the numerous nicks and RNA-DNA junctions generated by the synthesis of Okazaki fragments during lagging strand synthesis. In viruses, e.g., T-4, it has been suggested that the unligated junctions of Okazaki fragments may serve as transient enhancers of late transcription by acting as loading sites for late gene activators that move along the DNA until late promoters are recognized (2). Once DNA replication is completed, these loading sites for late gene activators might be eliminated by the removal of the RNA primers and ligation of the lagging strand, thereby terminating late transcription. In baculoviruses, essential activators of late gene transcription have not been identified in in vitro analyses, however, several additional factors have been characterized in transient assays that may be essential for late transcription (see below).

Baculovirus late promoter elements

Initially, because the sequence of the AcMNPV polyhedrin gene region revealed a conventional-appearing TATA sequence upstream of the reading frame, it was suggested that baculovirus late genes were transcribed using a conventional RNA polymerase II promoter (3). However, the OpMNPV polyhedrin gene lacked this sequence, and the mapping of its mRNA start site indicated that it initiated at an ATAAG sequence. When several other baculovirus late genes were sequenced (4-7), they were all found to have this sequence. This led to the proposal that ATAAG was the baculovirus late promoter sequence and that mRNA initiated within this sequence (8). Subsequently, this was demonstrated experimentally (9) and eventually it was determined that the core sequence is normally ATAAG, GTAAG, or TTAAG, but that CTAAG is apparently not used. A survey of predicted baculovirus promoter sequences was conducted on 26 baculovirus genomes (10) (see Chapter 5). The purpose of this investigation was to determine whether baculovirus promoter elements could be correlated with their position relative to the ATG translation initiation codons. They found a number of clear correlations. There was a strong correlation of TAAG sequences upstream of the ATG, particularly when combined with a TATA early promoter, e.g., TATAAGG. These were about 7 times as frequent in the upstream location and reflect genes that are transcribed by both the host RNA polymerase II and the baculovirus RNA polymerase and are expressed both early and late in infection. TAAG sequences were almost 6 times more prevalent in the promoter (upstream) region. Of the TAAG sequences, ATAAG and GTAAG sequences were most prevalent, followed by TTAAG. In contrast, CTAAG, which does not appear to function as a late promoter element, was only slightly more prevalent in the upstream promoter regions.

In comprehensive investigations using genome wide analyses, strand-specific RNAseq technology, and the sequencing of 5' RACE PCR products (11), 218 transcriptional start sites (TSS) were mapped for 156 AcMNPV orfs from infected *Trichoplusia ni* cells. The majority of the TSS (126) were located at consensus later promoter motifs (with the sequence TAAG) upstream of 101 orfs, or about 65% of the AcMNPV orfs. In addition, 92 non late promoter TSS were identified of which 89 were located immediately upstream of 77 predicted orfs, or about half of the orfs. There were 21 orfs identified that had multiple late TSS and another 21 that had both early and late TSS.

Late TSS always initiated at the second nt of the TAAG late promoter element. About 13% of the orfs have more than one late TSS. Although no additional consensus nt were identified near the TAAG sequence, they were located in the context of an AT-rich sequence, with the 6-7 nt upstream and downstream of the TAAG being about 75% AT, vs 59% for the genome as a whole. In addition, no other consensus nucleotides were identified that were associated with the relative strength of the late promoters (11).

An implication of the novel promoter and mRNA start site of late and very late baculovirus genes is that their transcripts would all begin with the sequence AAG (Figure 1). Whether this serves as a signal for selective sorting or transport of these mRNAs remains to be determined.

Temporal gene expression

In the characterization of the transcriptome of AcMNPV infected *T. ni* cells, it was observed that at 6 hpi, the majority of transcripts were from early genes and comprised about 3% of the total mRNA in the cell. The genes expressed included some involved in DNA replication (lef-2 – the primase accessory factor, lef-3 – the SSB), anti-apoptosis (p35), and gp64, the envelope fusion protein. Other abundantly expressed genes included dbp, pp31(39K), and lef6. For genes that have both early and late promoters, such as gp64 and pp31, the late promoters were expressed more highly than the early promoters at 6 hpi. This suggests that early promoter activity might facilitate access to the late promoter by the viral RNA polymerase or enhance its activity in some other manner. At 12 hpi, which corresponds to the beginning of DNA replication, the viral mRNA population greatly expanded to comprise 38% of the total mRNA in the cells and likely reflects the amplification of the templates and also the accessibility of the late promoter elements possibly due to their nascent synthesis. Several transcripts encoding virion associated structures including p6.9, odv-ec27, odv-e18 were 2 – 3-fold higher in concentration than other transcripts. By 24 hpi, polyhedrin and p10 were the most abundant mRNAs and by 48 hpi, they were up to 4-fold higher than the mRNA of the next highest group that included p6.9, odv-e18 and odv-ec27. At this time polyhedrin and p10 mRNA comprised 24% and 7.5% of the total RNA of the cell, respectively. Overall, genes that had conventional early promoters (TATAA and CAGT-like sequences) were expressed highest at 6-12 hpi, whereas those with a later promoter element were expressed the highest at 12-18 hpi or later (11).

Insect virus RNA polymerases and occlusion body protein hyperexpression

Baculoviruses encode a novel RNA polymerase composed of four subunits that transcribes late and very late genes and that recognizes the unique promoter consensus sequence described above. It is not clear why a virus

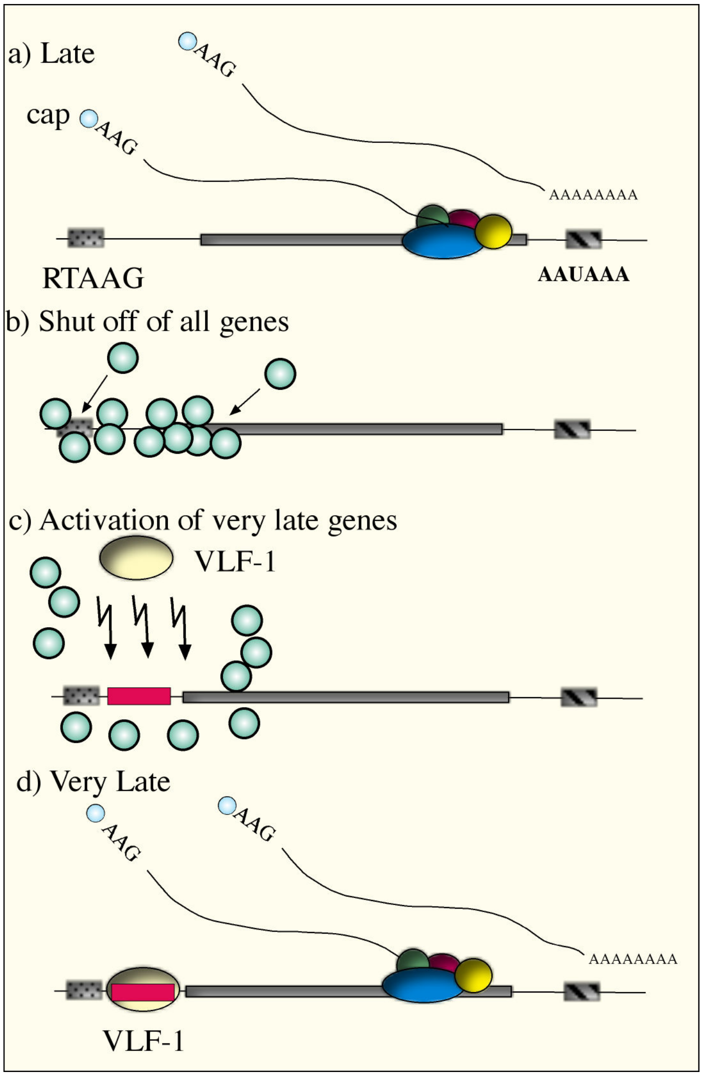

Figure 1. Comparison of late and very late gene transcription. Shown is: (a) late gene transcription; (b) a hypothetical mechanism for the shutoff of late transcription; and (c and d) activation of very late gene transcription involving VLF-1 interacting with the burst sequence (shown as a red rectangle). Late transcription initiates within the late promoter element. Therefore, all late messages likely begin with the sequence AAG.

that replicates in the nucleus would encode its own RNA polymerase, since many such viruses depend on exploiting the host enzyme for transcribing all their genes. The remarkable ability of baculoviruses to hyper express very late genes might be considered the impetus for the evolution of a system that is independent of host transcription. Although several families of cytoplasmic RNA and DNA viruses, such as the Poxviridae and Reoviridae, encode their own RNA polymerases, most lineages of these viruses do not hyper express genes. However, both these families have occluded genera, the cypoviruses and entomopox viruses respectively, that are pathogenic for insects and express occlusion body proteins at very high levels reminiscent of baculovirus very late genes, e.g., entomopox spheroidin, can comprise 30-40% of total protein (12). These viruses are able to accomplish this utilizing RNA polymerases related to those of other members of their viral family that do not hyper express genes, indicating that the evolution of the polymerase might not be specifically linked to its ability to hyper express genes. However, the independence of transcription from the host cell RNA polymerase could have facilitated the evolution of the extraordinary levels of gene expression of the polymerases of the occluded viruses. In the case of baculoviruses, the independence from the host enzyme may have initially provided regulatory advantages, such as independence from low levels of RNA transcription factors in nondividing cells. This could have provided the enzymatic platform for the evolution of their ability to hyper express very late genes involved in occlusion body formation. The combination of factors that likely influence high levels of baculovirus gene expression is discussed in detail in Chapter 10.

The baculovirus RNA polymerase

Hints of the presence of a novel baculovirus RNA polymerase had been suggested by the observation that the expression of baculovirus late genes was α-amanitin resistant (13, 14). Alpha-amanitin is a fungal toxin that inhibits RNA polymerase II and hence mRNA synthesis at very low concentrations. The resistance to α-amanitin, along with the observation that baculovirus late genes employed a novel late promoter element, suggested that there was a distinct RNA polymerase involved in the expression of baculovirus late genes. Using a transient assay system that was dependent upon transcription from a late promoter element to express a reporter gene led to the identification of baculovirus genes that are involved in late gene transcription (15, 16). This assay eventually implicated 19 genes in late transcription; however, since the assay was dependent upon DNA replication, it included genes that were involved in that process. The DNA replication genes were identified using a separate assay (see Chapter 5), and the genes involved in late transcription were inferred by subtracting the replication genes. Subsequently, a protein complex of about 560 kDa was isolated that was able to support in vitro transcription from late promoter-containing DNA templates. This complex includes late expression factors (LEF) -4, -8, -9, and p47 with predicted molecular masses of 54, 102, 55 and 47 kDa, respectively. The combined predicted molecular mass of these four proteins is about 260 kDa. Because they appear to be present in equimolar amounts, it was suggested that the baculovirus RNA polymerase complex contains two molecules of each peptide (17). The significance of this is unclear, as such a subunit composition has not been reported for other RNA polymerases. Homologs of these gene products are present in all baculovirus genomes that have been sequenced. Two of these subunits (LEF-8 and -9) have significant levels of similarity to the two largest subunits found in bacterial and eukaryotic polymerases, respectively (Figure 2). These are called β and β' subunits, but they can vary greatly in size and consequently their size may be independent of their lineage.

LEF-9 is related to the large RNA polymerase subunits. LEF 9 contains a 7-amino acid motif (NTDCDGD) similar to the Mg++ binding sequence (NADFDGD) found in the catalytic center in the large RNA polymerase subunits of DNA-dependent RNA polymerases (18) (Figure 2). The D residues are critical components of the sequences because they coordinate the binding of Mg++ that is necessary for the activity of the polymerase. These three residues are conserved in all the orthologous large subunit sequences. AcMNPV LEF-9, at 516 amino acids, is much smaller than the other large RNA polymerase subunits, e.g., that of *Drosophila* is almost 1900 amino acids. Alignments indicate that LEF-9 is most closely related to the N-terminal 60% of the larger orthologous subunits. However, the homology is very low and other domains found conserved in this subunit from bacteria through eukaryotes and vaccinia virus (19) have not been reported in the baculovirus protein.

LEF-8 is related to the second largest RNA polymerase subunit. LEF-8 shows limited homology with the second largest subunit of a number of other organisms at the conserved 13-amino acid sequence GDKXX(s/g)RHG(q/n)KG(v/i/t). This homology is shown in Figure 2 and compared to the orthologous *D. melanogaster* subunit and has been proposed to be part of the catalytic site (20, 21) (Figure 3).

P47, a unique subunit. The third subunit, P47, was originally identified as the site of a ts mutation that caused a defect in late gene expression (22, 23). It has not been convincingly shown to be related to RNA polymerase subunits from other organisms.

LEF-4 is an RNA capping enzyme. The 5' RNA cap is a novel feature of mRNAs from eukaryotic cells and viruses that is not present in bacteria and archaea, because they lack the necessary enzymes for its synthesis. The cap is added to the 5' end of mRNA and consists of a guanine nucleotide linked to the mRNA with a 5' to 5' triphosphate linkage. The guanosine is methylated at the N-7 position by an enzyme called methyl transferase. It can be further modified by an additional methylation of the 2' hydroxyl groups of the ribose sugars at the 5' end of the mRNA (**Figure 4**). This results in the RNA resembling the 3' end of an RNA molecule -- the 5' carbon of the cap ribose is blocked whereas the 3' position is free. Capping involves an RNA 5' triphosphatase that removes the terminal phosphate of the RNA, a guanylyl transferase to add the guanine, and two different methylases. The first two activities are present in LEF-4 and are also present on a single protein located at the N- and C-termini, respectively, in metazoans and plants. Although having similar activity, LEF-4 is unrelated to this category of capping enzyme, but is a member of the metal dependent group of capping enzymes found in fungi and protozoa (24). The enzymes that are involved in the two methylase reactions are not known, although AcMNPV does encode a methyltransferase homolog (Ac69) that would be capable of carrying out the second methylase reaction (see below).

The 5' cap structure is thought to have several major roles associated with mRNA function. These include: i) nuclear export: it binds a protein that is recognized by the nuclear pore complex and is exported; ii) protection from exoribonucleases: this can be important because the export process can take significant amounts of time during which the RNA is subjected to exonuclease exposure; iii) promotion of translation: the cap serves to recruit initiation factors, which in turn recruit ribosomes (25). Capping can also be involved in intron excision; however, this may not be a major role in baculoviruses because of the limited amount of splicing that occurs.

Capping in eukaryotes involves an enzyme that associates with the highly repetitive carboxyl terminal domain (CTD) of the β' subunit of RNA polymerase II. Because the baculovirus polymerase lacks a similar domain, it is likely that it evolved to include the enzyme as part of the RNA polymerase complex. Similarly, vaccinia virus also lacks a CTD on its β' subunit, and its capping enzyme is also associated with the RNA polymerase complex, and capping occurs during transcription when the nascent RNA oligomer is about 30 nt long (26); however, it does not appear to be an integral component of the polymerase enzyme.

Assuming that in vitro transcription reactions are free from exoribonuclease, it is not clear why LEF-4 is required for transcription in vitro assays. These assays monitor RNA transcripts that would not need to be capped in order to be detected. This suggests that LEF-4 may play a structural role in the organization of the polymerase subunits or it may have some other function. Furthermore, inactivation of both the LEF-4 RNA triphosphatase domain and the RNA triphosphatase encoded by Ac orf1 did not affect the viability of a double mutant virus. Therefore, if capping is required in cultured cells, these data suggest that a host enzyme can carry out the triphosphatase reaction. In addition, the chemical composition of the baculovirus cap structure does not appear to be identical to the conventional motif (27). However, variant cap structures have been described in at least one other virus (28). Complicating an interpretation of the role of LEF-4 in capping is evidence indicating that the 5' untranslated region of p10 mRNA facilitates translation in a cap independent manner (29). Since commonly used internal ribosome entry sites are apparently not active in certain insect cell systems, the p10 5' untranslated region has been used as a translational enhancer of uncapped mRNA in investigations employing insect cells (30).

Baculovirus protein involved in RNA capping

Ac69, a methyltransferase (MTase). Ac69 encodes a methyltransferase and it is found in most Group I NPV, about one-half the Group II NPV and in the NeseNPV genomes. Ac69 was found to stimulate late gene transcription in a transient assay (31). The gene encodes a protein with RNA Cap (nucleoside-2-O)-methyltransferase activity. AcMNPV, with a null mutation of the gene, replicated normally in cell culture (32). Similar results were observed for a knockout of the homolog (Bm57) in BmNPV (33). Therefore, it is not clear whether a host gene can carry out this function or the viral gene may not be necessary for viral replication in cell culture.

Ac38—a baculovirus decapping enzyme? Ac38 is an ADP-ribose pyrophosphatase (ADPRase), a member of the subfamily of nudix pyrophosphatases. Purified Bm29 also showed ADPRase activity (34). Orthologs are found in all lepidopteran NPV and GV genomes. Proteins of the nudix superfamily are common in all organisms and have been reported in other viruses, including T4 bacteriophage, African swine fever virus (family :

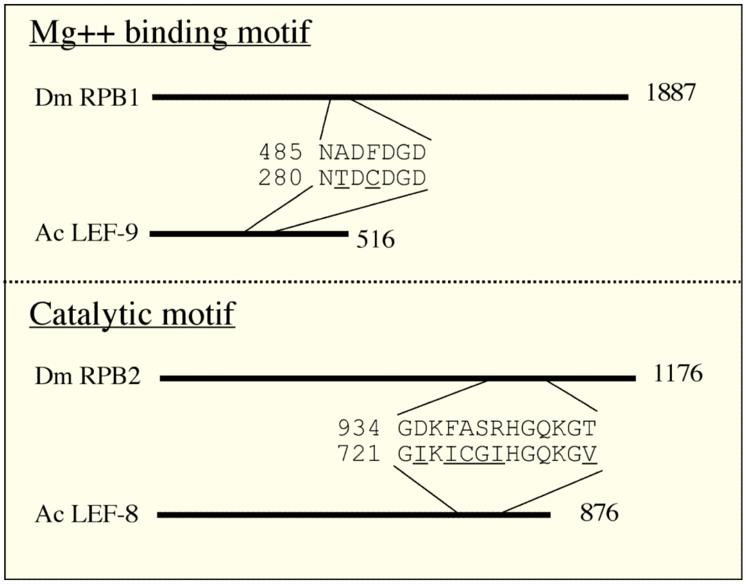

Figure 2. Homology of baculovirus RNA polymerase subunits. The similarity of the LEF-8 and -9 subunits to the two largest RNA polymerase subunits of *Drosophila melanogaster* RNA polymerase II is shown. The numbers at the end of the lines indicate the size of each protein in amino acids. The numbers before the sequences indicate the location of the domain within the sequence. The underlined amino acids are not conserved.

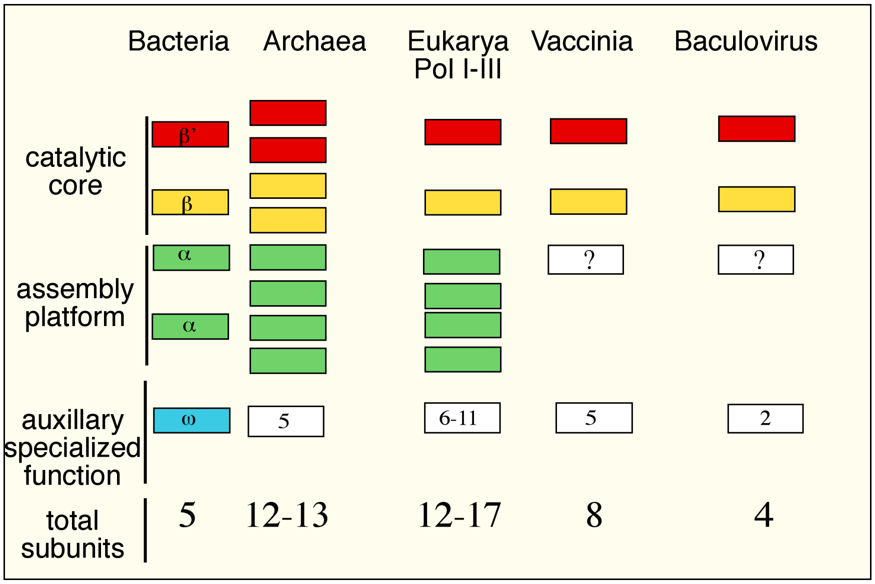

Figure 3. Relationships of RNA polymerase subunits. The large subunits, β' and β, in some archaea have undergone fission and are present as either 3 or 4 components. The subunits are color-coded to indicate similar function. This figure is based on the data of (38, 42, 44, 83, 84). For description of baculovirus homology, see text.

Asfarviridae), and poxvirus. An AcMNPV Ac38 deletion mutant was severely compromised and produced BV at 1% the level of wt (35). Vaccinia virus also encodes a nudix protein and it may be important in negatively regulating gene expression by acting as a decapping enzyme (36) by removing the 7-methylguanosine diphosphate. Deletion of the gene in vaccinia resulted in smaller plaques and lower virus yield (37), similar to the *Ac38*-deleted AcMNPV.

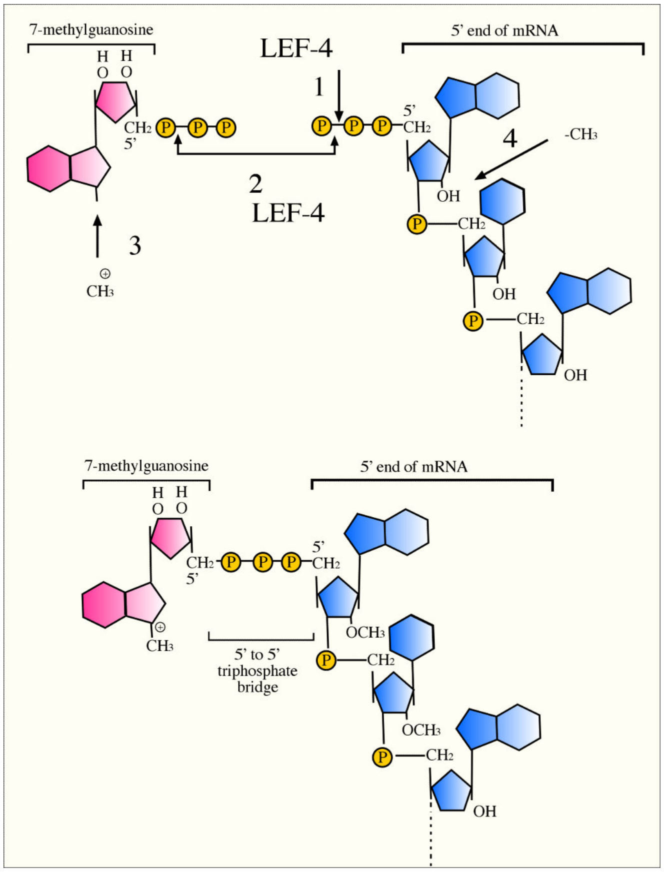

Figure 4. Capping baculovirus mRNA. The four modifications that occur during capping are summarized in the following steps: 1) LEF-4 has the potential to dephosphorylate the 5' end of mRNA; 2) and to transfer guanosine to the 5' end of the mRNA; 3) the guanosine is methylated--enzyme is unknown; 4) The 2' OH group of the terminal A is methylated by an unknown methyltransferase. The final structure is shown at the bottom.

The categories of RNA polymerases

There are four major lineages of RNA polymerases that appear to be unrelated. These include: i) RNA-dependent RNA polymerases of RNA viruses, ii) the DNA-dependent RNA polymerases of some phage such as T7, iii) primases that produce short RNA transcripts for priming DNA replication, and iv) the DNA-dependent RNA polymerases of eubacteria, archaea, and eukaryotic cells. The DNA-dependent RNA polymerases of bacteria and eukaryotes are highly conserved enzymes and are composed in most organisms of 5 to 15 subunits. Four of these correspond to α, β, β' and ω subunits of bacteria and form the conserved core, and orthologs are present in all the cellular enzymes (Figure 3). The β and β' subunits interact with each other, and the active site is formed by their interface [(38) and references therein]. The β' subunit contains a catalytic site and has three invariant aspartate residues that interact with a Mg2+ ion and the α-phosphate of the NTP during polymerization (39). Subunits related to the β and β' polypeptides are also encoded by the genomes of a number of families of large DNA viruses that are pathogenic for eukaryotes (40). The α subunit is involved in the initiation of RNA polymerase assembly and sequence specific protein-DNA interactions that result in promoter recognition, and is also a target for transcriptional activation (41). Two identical α subunits are present in bacterial RNA polymerase and four homologs of the α subunit are found in the polymerases of archaea and eukarya (Figure 3). Surprisingly, a homolog of the α subunit has not been described for the vaccinia RNA polymerase (42).

In addition to the subunits that comprise the RNA polymerase, transcription of genes often requires other additional factors. Bacterial RNA polymerases recognize specific promoter elements by interacting with sigma factors that specify transcription of specific categories of genes. However, transcription from the eukaryotic RNA polymerases involves a variety of transcription factors, some of which are composed of multiple subunits. Perhaps as a consequence of the size and complexity of eukaryotic polymerases, the promoters to which the polymerases bind and the location of the transcription start sites are separated by 30 bases or more. Although not as complex, the promoter and start site region of bacterial polymerases encompasses up to 35 bp. Finally, the RNA polymerases of phage such as T7 are composed of a single subunit unrelated to those described above, and have a compact polymerase binding RNA start site of about 20-bp (43). In contrast, as described above, both the baculovirus RNA polymerase promoter and mRNA start site are very compact and located at TAAG sequences.

The relationship of baculovirus RNA polymerase to other RNA polymerases

Although there is homology to the β and β' subunits of RNA polymerase, as summarized above, the degree of relatedness is very limited making it difficult to determine the lineage of the baculovirus polymerase. In addition, no other RNA polymerase appears to have a promoter similar in sequence to that of late baculovirus genes (see above). The initiator sequences of RNA polymerase II (Chapter 4) are similar in that transcription initiates at the promoter sequence. However, the extent of the interaction of the baculovirus RNA polymerase with promoter sequences has not been determined, possibly because the complex is so unstable that it cannot be footprinted using conventional techniques. Analysis of the RNA polymerase subunits of bacteria, archaea and eukaryotes found that they almost all encode four subunits related to the bacterial β and β', α and ω (38). The RNA polymerase of the poxvirus, vaccinia, is similar to those of eukarya as it has a complex subunit composition with at least eight subunits, seven of which appear to be orthologs of RNA polymerase II subunits β and β' plus RPB 5, 6, 7, and 10 and the transcription factor SII (42, 44) (Figure 3). The baculovirus RNA polymerase is distinct from these other major DNA dependent RNA polymerases in that it is composed of four subunits. Since the catalytic cleft is located on the two largest peptides, and baculoviruses possess orthologs of these two subunits, the basic enzyme core is retained. However, based on current analyses, the phylogenetic origin of the baculovirus RNA polymerase is unclear.

In vitro transcription assays

It should be noted that much of the information regarding baculovirus RNA polymerase has been derived from in vitro transcription assays (17, 45) and these systems involve the addition of DNA templates containing late promoter elements. Since this DNA is purified and presumably protein free, it is not clear how well these systems reflect late transcription in infected cells. Future analysis of transcription from templates complexed with proteins may yield information on additional proteins involved in the activation of late transcription. In addition, whereas the baculovirus RNA polymerase complex is limited to four proteins, a number of other proteins are required for late transcription in transient assays (see below).

Termination and polyadenylation of early and late mRNAs

In the study of AcMNPV transcripts in cultured *T. ni* cells (11), it was observed that most early and late orfs have a single polyadenylation site (PAS) located 18-22 nt downstream of a consensus AAUAAA consensus sequence. About 10% of the ORFs had multiple PAS. Also, a number of sets of tandem orfs oriented in the same direction shared the same PAS such that their transcripts, although initiating at different locations, overlapped one or more downstream transcripts, all of which terminated at the same location. For example, there are 7 sets of 5 or more orfs that share a common PAS. The PAS are located in an AT rich region and the 40 nt surrounding the PAS was 80% AT compared to the AcMNPV genome average of (59%). Whereas 3' processing of early transcripts is carried out by the host cell system resulting in polyadenylated transcripts, the situation with late genes has been unclear. It was suggested that late genes transcribed using purified baculovirus RNA polymerase in a cell free system have 3'ends formed by the presence of T-rich sequences that destabilize the late transcription complex (46). However, in cultured cells, most (87%) of the transcripts reflected conventional 3' processing by the host cell downstream of an AAUAAA sequence (Figure 1) (11).

Very late gene expression

Very late factor 1 (VLF-1, Ac77). A novel feature of baculoviruses is their ability to express genes at high levels very late in infection. This phenomenon has been exploited in the development of baculoviruses as expression vectors. Two highly expressed very late genes have been characterized, polyhedrin and p10. Polyhedrin is the occlusion body protein, whereas the role of p10 is not clear, although it appears to form fibrillar structures that may be involved in the assembly of the polyhedron envelope during the maturation of polyhedra (47) (see Chapter 2, Figure 2) and may influence the lysis of terminally infected nuclei (48). Because these genes appear to be involved in polyhedron morphogenesis, which is a very late step in the baculovirus life cycle that occurs after virions destined for occlusion have been assembled, it is likely that they are transcribed from DNA that does not become packaged as virion genomes. Consequently, it remains accessible to the very late RNA polymerase complex. Both polyhedrin and p10 genes contain an A/T-rich sequence downstream of a late promoter sequence that is involved in their high level expression (49). This sequence was called the 'burst sequence' because it caused a burst of transcription very late in infection. VLF-1 was originally identified because it influences the hyperexpression of very late genes (50). Subsequently, it was found that VLF-1 interacts with the burst sequence in gel shift assays (51) and the presence of this sequence stimulates the level of VLF-1. In vitro transcription assays suggest that VLF-1 can stimulate very late transcription about 10-fold. In addition, when the burst sequence is removed, the level of stimulation is reduced about fourfold (52) (Figure 1). Homologs of VLF-1 are found in all sequenced baculovirus genomes, and they belong to a family of proteins that includes lambda integrase. Integrases are a large group of site-specific DNA recombinases that catalyze DNA rearrangements and are found in a variety of organisms including viruses where they are involved in the integration and excision of viral genomes and decatenation of newly replicated chromosomes. Evidence suggests that VLF-1 may also be involved in the processing or packaging of baculovirus genomes (see Chapter 5).

LEF-2 (Ac6) and PK-1 (Ac10). In addition to VLF-1, LEF-2 (Ac6) appears to be involved in very late transcription. Although it is an essential replication gene where it apparently functions as a primase accessory factor (see Chapter 5), LEF-2 mutants of AcMNPV have been characterized that appear to affect very late transcription, indicating that it may have roles in both replication and transcription (53). In BmNPV, LEF-2 has also been shown to activate late transcription (54) independent of its influence on DNA replication. It has been suggested that Ac10, which encodes a serine/threonine kinase (called PK-1), also influences very late gene expression. Orthologs of Ac10 are found in all lepidopteran baculovirus genomes. The inhibition of PK-1 expression by either a ts mutation (55) or by DNAzyme technology causes a reduction in the expression from the polyhedrin promoter (56). PK-1 also may be associated with a very late transcription complex and be involved in the phosphorylation of LEF-8 (57). PK1 of SpltNPV-I and AcMNPV may interact with the polyhedrin promoter, suggesting that it might act as a very late gene transcription factor (58) (59).

Other genes involved in late transcription

With the removal of the six essential replication genes and the four genes encoding the late RNA polymerase complex from the total of 19 genes that have been implicated in influencing late gene transcription (16), the roles of nine genes are left unexplained. These genes are required for or stimulatory for late transcription in the transient assay system.

P35, LEF-7, LEF-11, and IE2. P35 blocks apoptosis and therefore, by preserving the viability of cells, promotes both viral DNA replication and late transcription. LEF-7 is stimulatory for DNA replication and deletion of *lef-7* from the AcMNPV genome led to a major reduction in late gene expression and a 100-fold reduction in infectious virus production. It was suggested that LEF-7 is involved in the regulation of the host cell DNA damage response (DDR) and diverts host DDR proteins from cellular chromatin, so that they can be exploited for viral DNA replication (60) (see Chapter 5). LEF-11 was not identified as being involved in DNA replication in the transient assay system, however a *lef-11* knockout construct is defective in DNA synthesis (see Chapter 5). IE-2 may influence late transcription because of its role in the activation of early transcription (see Chapter 4). Therefore, LEF-7 and IE-2 could affect late gene transcription by influencing DNA replication. This leaves five genes that may specifically influence late gene transcription. These are described below.

Lef-5 (Ac99), a possible ortholog of TFIIS. Orthologs of *lef-5* are found in all baculoviruses and are also present in nudiviruses (61). LEF-5 was originally identified as being required for transient late gene transcription (62). It was demonstrated to interact with itself and to contain a domain similar to that of the RNA polymerase II elongation factor TFIIS (63). Subsequent investigations indicated that LEF-5, although highly stimulatory for in vitro transcription, did not enable the baculovirus polymerase to transit pause sites, and it was concluded that it functions as an initiation factor, rather than an elongation factor (64). Deletion is probably lethal as interrupted mutants of this gene in BmNPV (Bm83) could not be isolated (65).

Lef-6 (Ac28), a possible mRNA export factor. Homologs of *lef-6* are found in the genomes of all lepidopteran NPVs and GVs. It was originally identified because it was required for transient transcription of late genes (66). A bacmid deleted for lef-6 was infectious, but the infection was delayed and the titer was reduced to about 10% of wt. The major effect appeared to be reflected in a delay in the onset of late transcription (67). LEF-6 is not particularly well conserved. Although AcMNPV and LdMNPV showed only 27% amino acid sequence identity, using the HHpred program described above, both showed over 80% probability of encoding a region that is related to the RNA binding domain of a factor called TAP that is involved in the export of mRNA out of nuclei through their interaction with nuclear pore proteins (68, 69). If LEF-6 performs a similar function, it might compensate for the nuclear transport pathway if it is compromised by the infection. It might also be essential for the transport of the high levels of mRNA produced by very late genes. BmNPV LEF-6 is highly phosphorylated during infection (70).

Lef-10 (Ac53a); a prion? This orf was named Ac53a because it was not identified in the original AcMNPV genome sequence because it is a small orf encoding 78 aa and about half the coding region at the 3' end overlaps the 5' region of Ac 54. Homologs of lef-10 are found in all Group I and most Group II NPV and GV genomes. Lef-10 was originally identified because it was required for late gene expression (18). It is likely an essential gene as an insertion mutant in the BmNPV homolog (Bm42a) could not be isolated (65). When linked to EGFP, Lef-10 formed punctate spots (71). It has been reported that LEF10 behaves as a prion (72). The full-length protein or its predicted prion-forming domain can functionally replace the prion domain of the prion, Sup35, of yeast. A high multiplicity of infection can lead to conversion of LEF10 to an aggregated state that inhibits late gene expression (71, 72).

Ac41 (Lef-12). Lef-12 is found in about half the Group I and Group II NPV genomes sequenced. Although 18 genes were originally identified as being involved in transient expression from a late promoter (15), when a set of these genes were individually cloned, they failed to support late transcription. Because of its close proximity to Ac 40 (p47), Ac41 (*lef-12*) had not been identified in the initial screen. It was subsequently demonstrated to be required for transient late gene transcription *in S. frugiperda* cells (16, 31), but not required for late transcription in *T. ni* cells (16). Mutants with *lef-12* interrupted by insertional mutagenesis or by mutation of the ATG translation initiation codon were viable in both *S. frugiperda* and *T. ni* cells, although reduced yields of BV were observed (20-40% of wt) in both cell lines, and the infection cycle appear to be slowed (73). It was suggested that *lef-12* may be functionally redundant in the AcMNPV genome, and therefore it is not essential for late transcription when the rest of the virus genome is present (73). Analysis by Hhpred (74, 75) predicts that Lef-12 may be structurally related to TFIIA with a probability of about 70%. TFIIA is involved in transcriptional initiation of RNA polymerase II (76).

pp31 (39K -Ac36). Pp31 was originally identified because it contains an early promoter that is stimulated by IE-1 (77). Homologs are present in all lepidopteran NPV and GV genomes. It is phosphorylated and localizes to the virogenic stroma of infected cells, and is capable of binding to DNA, but is not a virion structural protein (78). Purified PP31 binds to single-stranded and double-stranded DNA with equal affinities and inhibited transcription in vitro (79). Phosphorylation of PP31 is a dynamic process (80). Several basic regions appeared to be involved in nuclear localization, and one of these regions is involved in DNA binding (81). It was found to stimulate late gene transcription in a transient transcription assay (15). Deletion of the *pp31* homolog in BmNPV (Bm27) resulted in virus that, although viable, showed a reduction in late gene transcription, a 100-fold reduction in BV production, and improper formation of the virogenic stroma (65). Similar results were obtained for an AcMNPV bacmid deleted for *pp31,* and it was observed that the deletion resulted in a significant decrease of the transcription of several late genes (82). It is not clear whether this gene acts directly on late transcription or may be a structural protein of the virogenic stroma and is required for the optimal organization of this structure, thereby indirectly influencing late transcription.

How can baculoviruses express very late genes at such high levels?

There is no evidence that the baculovirus RNA polymerase has an intrinsic ability for high level gene expression. In fact, it may not bind to the very late promoter region with high levels of tenacity as reflected in the fact that the footprint of the polymerase on genomic DNA has never been reported despite an extensive effort by at least one laboratory (Rohrmann, unpublished).

It is likely that high levels of gene expression are influenced by several features of baculovirus biology. These include: i) the amplification of genes by DNA replication (see Chapter 5); ii) the shutoff of most late transcription, possibly by DNA binding proteins that coat the DNA and thereby make RNA polymerase available for very late transcription; iii) the efficiency of the late polymerase and VLF-1 in recognizing and initiating from very late promoter elements; iv) the efficiency of LEF-4 in capping the mRNA (see Figure 4), and v) a possible role for LEF-2 and PK-1. As mentioned above, the 5' untranslated region of p10 mRNA appears to be capable of facilitating cap-independent translation, which may reduce the reliance of these transcripts on LEF-4 activity (29). Other factors that might enhance translation of very late expressed mRNAs have not been identified. For a detailed discussion of the factors influencing very late transcription, see Chapter 10.

References

1. Keck JG, Baldick CJ, Moss B. Role of DNA replication in vaccinia virus gene expression: A naked template is required for transcription of three late trans-activator genes. Cell. 1990;61:801–809. PubMed PMID: 2344616.
2. Brody EN, Kassavetis GA, Ouhammouch M, Sanders GM, Tinker RL, Geiduschek EP. Old phage, new insights: two recently recognized mechanisms of transcriptional regulation in bacteriophage T4 development. FEMS Microbiol Lett. 1995;128:1–8. PubMed PMID: 7744235.
3. Hooft van Iddekinge BJL, Smith GE, Summers MD. Nucleotide sequence of the polyhedrin gene of *Autographa californica* nuclear polyhedrosis virus. Virology. 1983;131:561–565. PubMed PMID: 18639177.
4. Kuzio JD, Rohel Z, Curry CJ, Krebs A, Carstens EB, Faulkner P. Nucleotide sequence of the p10 polypeptide gene of *Autographa californica* nuclear polyhedrosis virus. Virology. 1984;139:414–418. PubMed PMID: 18639833.
5. Iatrou K, Ito K, Witkiewicz H. Polyhedrin gene of Bombyx mori nuclear polyhedrosis virus. J Virol. 1985;54:436–445. PubMed PMID: 3886932.
6. Chakerian R, Rohrmann GF, Nesson MH, Leisy DJ, Beaudreau GS. The nucleotide sequence of the Pieris brassicae granulosis virus granulin gene. J Gen Virol. 1985. PubMed PMID: 3891914.
7. Akiyoshi D, Chakerian R, Rohrmann GF, Nesson MH, Beaudreau GS. Cloning and sequencing of the granulin gene from the *Trichoplusia ni* granulosis virus. Virology. 1985;141:328–332. PubMed PMID: 4082501.

8. Rohrmann GF. Polyhedrin structure. J Gen Virol. 1986;67:1499–1513. PubMed PMID: 3525744.
9. Rankin C, Ooi BG, Miller LK. Eight base pairs encompassing the transcriptional start point are the major determinant for baculovirus polyhedrin gene expression. Gene. 1988;70:39–49. PubMed PMID: 3071492.
10. 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.
11. 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.
12. Li Y, Hall RL, Yuan SL, Moyer RW. High-level expression of Amsacta moorei entomopoxvirus spheroidin depends on sequences within the gene. J Gen Virol. 1998;79(Pt 3):613–22. PubMed PMID: 9519842.
13. Grula MA, Buller PL, Weaver RF. Alpha amanitin-resistant viral RNA synthesis in nuclei isolated from nuclear polyhedrosis virus-infected *Heliothis zea* larvae and *Spodoptera frugiperda* cells. J Virol. 1981;38:916–921. PubMed PMID: 16789208.
14. Fuchs YL, Woods MS, Weaver RF. Viral transcription during Autographa californica nuclear polyhedrosis virus infection: A novel RNA polymerase induced in infected Spodoptera frugiperda cells. J Virol. 1983;48:641–646. PubMed PMID: 16789246.
15. Todd JW, Passarelli AL, Miller LK. Eighteen baculovirus genes, including *lef-11, p35, 39K*, and *p47*, support late gene expression. J Virol. 1995;69:968–974. PubMed PMID: 7815564.
16. Rapp JC, Wilson JA, Miller LK. Nineteen baculovirus open reading frames, including LEF-12, support late gene expression. J Virol. 1998;72:10197–206. PubMed PMID: 9811761.
17. Guarino LA, Xu B, Jin J, Dong W. A virus-encoded RNA polymerase purified from baculovirus-infected cells. J Virol. 1998;72:7985–7991. PubMed PMID: 9733837.
18. Lu A, Miller LK. Identification of three late expression factor genes within the 33.8- to 43.4-map-unit region of *Autographa californica* nuclear polyhedrosis virus. J Virol. 1994;68:6710–6718. PubMed PMID: 8084003.
19. Broyles SS, Moss B. Homology between RNA polymerases of poxviruses, prokaryotes, and eukaryotes: nucleotide sequence and transcriptional analysis of vaccinia virus genes encoding 147-kDa and 22-kDa subunits. Proc Natl Acad Sci U S A. 1986;83:3141–5. PubMed PMID: 3517852.
20. Passarelli AL, Todd JW, Miller LK. A baculovirus gene involved in late gene expression predicts a large polypeptide with a conserved motif of RNA polymerases. J Virol. 1994;68:4673–4678. PubMed PMID: 8207843.
21. Titterington JS, Nun TK, Passarelli AL. Functional dissection of the baculovirus late expression factor-8 gene: sequence requirements for late gene promoter activation. J Gen Virol. 2003;84:1817–26. PubMed PMID: 12810876.
22. Partington S, Yu H, Lu A, Carstens EB. Isolation of temperature sensitive mutants of Autographa californica nuclear polyhedrosis virus: phenotype characterization of baculovirus mutants defective in very late gene expression. Virology. 1990;175:91–102. PubMed PMID: 2408231.
23. Carstens EB, Lu AL, Chan HLB. Sequence, transcriptional mapping, and overexpression of p47, a baculovirus gene regulating late gene expression. J Virol. 1993;67:2513–2520. PubMed PMID: 8474157.
24. Gross CH, Shuman S. RNA 5'-triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities of baculovirus LEF-4 protein. J Virol. 1998;72:10020–8. PubMed PMID: 9811740.
25. Shuman S. What messenger RNA capping tells us about eukaryotic evolution. Nat Rev Mol Cell Biol. 2002;3:619–25. PubMed PMID: 12154373.
26. Hagler J, Shuman S. A freeze-frame view of eukaryotic transcription during elongation and capping of nascent mRNA. Science. 1992;255:983–6. PubMed PMID: 1546295.
27. Li Y, Guarino LA. Roles of LEF-4 and PTP/BVP RNA triphosphatases in processing of baculovirus late mRNAs. J Virol. 2008;82:5573–83. PubMed PMID: 18385232.
28. Ogino T, Banerjee AK. Formation of guanosine(5')tetraphospho(5')adenosine (GppppA) cap structure by an unconventional mRNA capping enzyme of vesicular stomatitis virus. J Virol. 2008;82:7729–34. PubMed PMID: 18495767.

29. Scheper GC, Vries RG, Broere M, Usmany M, Voorma HO, Vlak JM, Thomas AA. Translational properties of the untranslated regions of the p10 messenger RNA of Autographa californica multicapsid nucleopolyhedrovirus. J Gen Virol. 1997;78:687–96. PubMed PMID: 9049423.
30. Kohl A, Hart TJ, Noonan C, Royall E, Roberts LO, Elliott RM. A bunyamwera virus minireplicon system in mosquito cells. J Virol. 2004;78:5679–85. PubMed PMID: 15140965.
31. Li L, Harwood SH, Rohrmann GF. Identification of additional genes that influence baculovirus late gene expression. Virology. 1999;255:9–19. PubMed PMID: 10049816.
32. Wu X, Guarino LA. Autographa californica nucleopolyhedrovirus orf69 encodes an RNA cap (nucleoside-2'-O)-methyltransferase. J Virol. 2003;77:3430–40. PubMed PMID: 12610118.
33. Ono C, Kamagata T, Taka H, Sahara K, Asano S, Bando H. Phenotypic grouping of 141 BmNPVs lacking viral gene sequences. Virus Res. 2012;165:197–206. PubMed PMID: 22421381.
34. Chen HQ, Zhou YJ, Chen KP, Yu Q. Expression and functional validation of Bombyx mori nucleopolyhedrovirus ORF29, a conserved Nudix motif protein. Acta Virol. 2013;57:442–6. PubMed PMID: 24294958.
35. Ge J, Wei Z, Huang Y, Yin J, Zhou Z, Zhong J. AcMNPV ORF38 protein has the activity of ADP-ribose pyrophosphatase and is important for virus replication. Virology. 2007;361:204–11. PubMed PMID: 17174373.
36. Parrish S, Resch W, Moss B. Vaccinia virus D10 protein has mRNA decapping activity, providing a mechanism for control of host and viral gene expression. Proc Natl Acad Sci U S A. 2007;104:2139–44. PubMed PMID: 17283339.
37. Parrish S, Moss B. Characterization of a vaccinia virus mutant with a deletion of the D10R gene encoding a putative negative regulator of gene expression. J Virol. 2006;80:553–61. PubMed PMID: 16378957.
38. Iyer LM, Koonin EV, Aravind L. Evolutionary connection between the catalytic subunits of DNA-dependent RNA polymerases and eukaryotic RNA-dependent RNA polymerases and the origin of RNA polymerases. BMC Struct Biol. 2003;3:1. PubMed PMID: 12553882.
39. Wang D, Bushnell DA, Westover KD, Kaplan CD, Kornberg RD. Structural basis of transcription: role of the trigger loop in substrate specificity and catalysis. Cell. 2006;127:941–54. PubMed PMID: 17129781.
40. Iyer LM, Balaji S, Koonin EV, Aravind L. Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. Virus Res. 2006;117:156–84. PubMed PMID: 16494962.
41. Ebright RH, Busby S. The Escherichia coli RNA polymerase alpha subunit: structure and function. Curr Opin Genet Dev. 1995;5:197–203. PubMed PMID: 7613089.
42. Knutson BA, Broyles SS. Expansion of poxvirus RNA polymerase subunits sharing homology with corresponding subunits of RNA polymerase II. Virus Genes. 2008;36:307–11. PubMed PMID: 18264749.
43. Turingan RS, Liu C, Hawkins ME, Martin CT. Structural confirmation of a bent and open model for the initiation complex of T7 RNA polymerase. Biochemistry. 2007;46:1714–23. PubMed PMID: 17253774.
44. Broyles SS. Vaccinia virus transcription. J Gen Virol. 2003;84:2293–303. PubMed PMID: 12917449.
45. Glocker B, Hoopes RR Jr, Hodges L, Rohrmann GF. In vitro transcription from baculovirus late gene promoters: accurate mRNA initiation by nuclear extracts prepared from infected *Spodoptera frugiperda* cells. J Virol. 1993;67:3771–3776. PubMed PMID: 8510206.
46. Jin J, Guarino LA. 3'-end formation of baculovirus late RNAs. J Virol. 2000;74:8930–8937. PubMed PMID: 10982336.
47. Russell RLQ, Pearson MN, Rohrmann GF. Immunoelectron microscopic examination of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus-infected *Lymantria dispar* cells: time course and localization of major polyhedron-associated proteins. J Gen Virol. 1991;72:275–283. PubMed PMID: 1993872.
48. van Oers MM, Vlak JM. The baculovirus 10-kDa protein. J Invertebr Pathol. 1997;70:1–17. PubMed PMID: 9217463.
49. Ooi BG, Rankin C, Miller LK. Downstream sequences augment transcription from the essential initiation site of a baculovirus polyhedrin gene. J Mol Biol. 1989;210:721–736. PubMed PMID: 2693741.

50. McLachlin JR, Miller LK. Identification and characterization of vlf-1, a baculovirus gene involved in very late gene expression. J Virol. 1994;68:7746–7756. PubMed PMID: 7966564.
51. Yang S, Miller LK. Activation of baculovirus very late promoters by interaction with very late factor 1. J Virol. 1999;73:3404–9. PubMed PMID: 10074194.
52. Mistretta TA, Guarino LA. Transcriptional activity of baculovirus very late factor 1. J Virol. 2005;79:1958–60. PubMed PMID: 15650223.
53. Merrington CL, Kitts PA, King LA, Possee RD. An Autographa californica nucleopolyhedrovirus lef-2 mutant: consequences for DNA replication and very late gene expression. Virology. 1996;217:338–348. PubMed PMID: 8599220.
54. Sriram S, Gopinathan KP. The potential role of a late gene expression factor, lef2, from Bombyx mori nuclear polyhedrosis virus in very late gene transcription and DNA replication. Virology. 1998;251:108–22. PubMed PMID: 9813207.
55. Fan X, Thirunavukkarasu K, Weaver RF. Temperature-sensitive mutations in the protein kinase-1 (*pk-1*) gene of the *Autographa californica* nuclear polyhedrosis virus that block very late gene expression. Virology. 1996;224:1–9. PubMed PMID: 8862393.
56. Mishra G, Chadha P, Chaudhury I, Das RH. Inhibition of Autographa californica nucleopolyhedrovirus (AcNPV) polyhedrin gene expression by DNAzyme knockout of its serine/threonine kinase (pk1) gene. Virus Res. 2008;135:197–201. PubMed PMID: 18353480.
57. Mishra G, Chadha P, Das RH. Serine/threonine kinase (pk-1) is a component of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) very late gene transcription complex and it phosphorylates a 102kDa polypeptide of the complex. Virus Res. 2008;137:147–9. PubMed PMID: 18577405.
58. Mishra G, Gautam HK, Das RH. Serine/Threonine kinase dependent transcription from the polyhedrin promoter of SpltNPV-I. Biochem Biophys Res Commun. 2007;358:942–7. PubMed PMID: 17512903.
59. Liang C, Su X, Xu G, Dai X, Zhao S. Autographa californica multiple nucleopolyhedrovirus PK1 is a factor that regulates high-level expression of very late genes in viral infection. Virology. 2017;512:56–65. PubMed PMID: 28915406.
60. Mitchell JK, Byers NM, Friesen PD. Baculovirus F-Box Protein LEF-7 Modifies the Host DNA Damage Response To Enhance Virus Multiplication. J Virol. 2013;87:12592–9. PubMed PMID: 24027328.
61. Wang Y, Kleespies RG, Huger AM, Jehle JA. The genome of Gryllus bimaculatus nudivirus indicates an ancient diversification of baculovirus-related nonoccluded nudiviruses of insects. J Virol. 2007;81:5395–406. PubMed PMID: 17360757.
62. Passarelli AL, Miller LK. Identification of genes encoding late expression factors located between 56.0 and 65.4 map units of the Autographa californica nuclear polyhedrosis virus genome. Virology. 1993;197:704–714. PubMed PMID: 8249293.
63. Harwood SH, Li L, Ho PS, Preston AK, Rohrmann GF. AcMNPV late expression factor-5 interacts with itself and contains a zinc ribbon domain that is required for maximal late transcription activity and is homologous to elongation factor TFIIS. Virology. 1998;250:118–134. PubMed PMID: 9770426.
64. Guarino LA, Dong W, Jin J. In vitro activity of the baculovirus late expression factor LEF-5. J Virol. 2002;76:12663–75. PubMed PMID: 12438592.
65. 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.
66. Passarelli AL, Miller LK. Identification and transcriptional regulation of the baculovirus lef-6 gene. J Virol. 1994;68:4458–4467. PubMed PMID: 8207819.
67. Lin G, Blissard GW. Analysis of an Autographa californica multicapsid nucleopolyhedrovirus lef-6-null virus: LEF-6 is not essential for viral replication but appears to accelerate late gene transcription. J Virol. 2002;76:5503–14. PubMed PMID: 11991978.
68. Liker E, Fernandez E, Izaurralde E, Conti E. The structure of the mRNA export factor TAP reveals a cis arrangement of a non-canonical RNP domain and an LRR domain. Embo J. 2000;19:5587–98. PubMed PMID: 11060011.

69. Matzat LH, Berberoglu S, Levesque L. Formation of a Tap/NXF1 Homotypic Complex Is Mediated through the Amino-Terminal Domain of Tap and Enhances Interaction with Nucleoporins. Mol Biol Cell. 2008;19:327–38. PubMed PMID: 17978099.
70. Shobahah J, Xue S, Hu D, Zhao C, Wei M, Quan Y, Yu W. Quantitative phosphoproteome on the silkworm (Bombyx mori) cells infected with baculovirus. Virol J. 2017;14:117. PubMed PMID: 28629377.
71. Xu X, Zhou X, Nan H, Zhao Y, Bai Y, Ou Y, Chen H. Aggregation of AcMNPV LEF-10 and Its Impact on Viral Late Gene Expression. PLoS One. 2016;11:e0154835. PubMed PMID: 27152613.
72. Nan H, Chen H, Tuite MF, Xu X. A viral expression factor behaves as a prion. Nat Commun. 2019;10:359. PubMed PMID: 30664652.
73. Guarino LA, Mistretta TA, Dong W. Baculovirus lef-12 is not required for viral replication. J Virol. 2002;76:12032–43. PubMed PMID: 12414945.
74. Soding J. Protein homology detection by HMM-HMM comparison. Bioinformatics. 2005;21:951–60. PubMed PMID: 15531603.
75. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. A Completely Reimplemented MPI Bioinformatics Toolkit with a New HHpred Server at its Core. J Mol Biol. 2018;430:2237–2243. PubMed PMID: 29258817.
76. Louder RK, He Y, Lopez-Blanco JR, Fang J, Chacon P, Nogales E. Structure of promoter-bound TFIID and model of human pre-initiation complex assembly. Nature. 2016;531:604–9. PubMed PMID: 27007846.
77. 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.
78. Guarino LA, Dong W, Xu B, Broussard DR, Davis RW, Jarvis DL. The baculovirus phosphoprotein pp31 is associated with the virogenic stroma. J Virol. 1992;66:7113–7120. PubMed PMID: 1433508.
79. Guarino LA, Mistretta TA, Dong W. DNA binding activity of the baculovirus late expression factor PP31. Virus Res. 2002;90:187–95. PubMed PMID: 12457973.
80. Broussard DR, Guarino LA, Jarvis DL. Dynamic phosphorylation of Autographa californica nuclear polyhedrosis virus pp31. J Virol. 1996;70:6767–74. PubMed PMID: 8794314.
81. Broussard DR, Guarino LA, Jarvis DL. Mapping functional domains in AcMNPV pp31. Virology. 1996;222:318–31. PubMed PMID: 8806516.
82. Yamagishi J, Burnett ED, Harwood SH, Blissard GW. The AcMNPV pp31 gene is not essential for productive AcMNPV replication or late gene transcription but appears to increase levels of most viral transcripts. Virology. 2007;365:34–47. PubMed PMID: 17467768.
83. Werner F. Structure and function of archaeal RNA polymerases. Mol Microbiol. 2007;65:1395–404. PubMed PMID: 17697097.
84. Minakhin L, Bhagat S, Brunning A, Campbell EA, Darst SA, Ebright RH, Severinov K. Bacterial RNA polymerase subunit omega and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. Proc Natl Acad Sci U S A. 2001;98:892–7. PubMed PMID: 11158566.

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