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# Control of Baculovirus Polyhedrin Gene Expression by Very Late Factor 1

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*vlf-1* is a baculovirus gene that regulates very late gene expression (J. R. McLachlin and L. K. Miller, *J. Virol.,* 68, 7746–7756, 1994) and also plays a crucial role in the replication of the budded form of *Autographa californica* nuclear polyhedrosis virus (AcMNPV) (S. Yang and L. K. Miller, ''Expression and mutational analysis of the baculovirus very late factor 1 (*vlf-1*) gene.'' *Virology,* 245, 99–109, 1998). To examine the influence of *vlf-1* expression on baculovirus infection, we constructed recombinant viruses that expressed only low levels of VLF-1 and recombinants with *vlf-1* under the control of different promoters. Viruses with mutant alleles of *vlf-1* that produced low levels of VLF-1 replicated the budded form of the virus normally but produced no occlusion bodies. Thus, a higher concentration of VLF-1 was needed to activate very late gene expression than was needed to support budded virus production. By altering the level and/or timing of *vlf-1* expression, the timing of polyhedrin gene (*polh*) expression, which normally occurs very late in infection, could be advanced or delayed. Early overexpression of *vlf-1* increased the level of expression from the *polh* promoter but caused premature cellular disintegration. The data indicate that VLF-1 is the limiting factor in very late gene expression and that the level of VLF-1 controls the onset of occlusion. 

## INTRODUCTION

Infection of insect cells by *Autographa californica* nuclear polyhedrosis virus (AcMNPV) proceeds in a cascade of early gene expression, initiation of viral DNA replication, late gene expression, and very late gene expression (reviewed by Friesen, 1997; Lu and Miller, 1997; Lu *et al.,* 1997). Two types of virions are produced during the infection: budded virions (BVs), which are released from the cell during the late phase, and occluded virions (OVs), which are embedded in a crystalline matrix in the nucleus to form occlusion bodies (OBs) during the very late phase. While many early gene products regulate downstream events in infection, virus structural proteins such as polyhedrin, a very late gene product and the major component of the crystalline matrix of OBs, are generally encoded by the late and very late genes. During the very late phase of infection, the polyhedrin gene (*polh*) and another very late gene, *p10*, undergo a burst of transcription, leading to the massive accumulation of their products in the cell.

Promoters of late and very late genes are similar in that they use a TAAG sequence as the transcription initiation site, which, together with a few surrounding nucleotides, is recognized directly or indirectly by a virus-induced RNA polymerase (reviewed by Lu and Miller, 1997). However, a very late promoter possesses a "burst sequence" in addition to the TAAG motif (Morris and Miller, 1994; Ooi *et al.,* 1989) and needs at least one more viral factor (very late factor 1 or VLF-1) than a late promoter to achieve high-level expression during the very late phase. *vlf-1* was identified by marker rescue (McLachlin and Miller, 1994) of a temperature-sensitive mutant, *ts*B837, which is defective in OB formation but produces BVs normally at the nonpermissive temperature (Lee and Miller, 1979). *ts*B837 contains a cysteine to tyrosine mutation at amino acid 202 in VLF-1, causing a dramatic decrease in *polh* transcription and a moderate decrease in *p10* transcription in *ts*B837-infected cells at the nonpermissive temperature while the transcription of late genes, e.g., *vp39* and *603 ORF*, is not affected (McLachlin and Miller, 1994). Thus, the influence of VLF-1 on transcription is specific to very late promoters. This specific effect is also observed in transient expression assays in which expression from the very late *polh* or *p10* promoters but not that from the late *vp39* or *p6.9* promoters is affected by VLF-1 (Todd *et al.,* 1996).

In addition to stimulation of transcription of very late genes, VLF-1 has another function that is important in the propagation of AcMNPV based on the observation that a *vlf-1*–null virus is either not viable or extremely impaired in its ability to replicate (Yang and Miller, 1998) even though *polh* and *p10* are nonessential for replication of the BV. Sequence similarity of VLF-1 to the lambda phage integrase/resolvase family suggested VLF-1 may possess integrase activity (McLachlin and Miller, 1994; Nunes-Duby *et al.,* 1998). One of the three highly con-

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served residues of this family, the tyrosine, is especially important for the integrase/resolvase activity because it is required to form a covalently bonded DNA–protein intermediate that is essential to the recombination reaction (Pargellis *et al.,* 1988; Landy, 1993; Evans *et al.,* 1990; Kwon *et al.,* 1997). Recombinant baculoviruses with a mutation in any one of the three VLF-1 residues that are rigorously conserved by other members of the integrase/resolvase family were either nonviable or nonisolatable. However, a VLF-1 mutant with the conserved tyrosine mutated to a phenylalanine was still able to stimulate transcription from the *polh* promoter in transient expression assays, suggesting that the putative integrase activity of VLF-1 defines the essential function but not the transactivation function of VLF-1 (Yang and Miller, 1998).

VLF-1 may be present throughout the infection process because it is a component of the nucleocapsids of both BV and OV (Yang and Miller, 1998). However, *vlf-1* is expressed primarily as a late gene. Although several transcripts are produced in the *vlf-1* region (McLachlin and Miller, 1994), translation of *vlf-1* mRNA is directed by transcription initiated from a TAAG motif 71 bp upstream of the *vlf-1* open reading frame (ORF) (Yang and Miller, 1998). By placing different promoters between this TAAG motif and the *vlf-1* ORF, the expression pattern of *vlf-1* can be changed. In the current report, we study the effects of altering the expression level and/or timing of *vlf-1* on expression from the *polh* promoter and on virus replication.

## RESULTS

### Cells infected with v6.9vlf1 or vpolhvlf1 have an unusual OB formation pattern

Previously we constructed a recombinant virus, vTGCGpolhvlf1, with the TAAG sequence of the *vlf-1* promoter mutated to TGCG and the *polh* promoter inserted immediately upstream of the *vlf-1* ORF (Yang and Miller, 1998). This recombinant virus displays delayed OB formation following infection of SF-21 cells. Because OB is mainly composed of polyhedrin whose production is affected by *vlf-1*, we suspected that the altered *vlf-1* expression caused this unusual OB formation phenotype. To further examine whether the timing of OB formation is determined by the *vlf-1* expression pattern, we constructed two recombinant viruses, v6.9vlf1 and vpolhvlf1, which contain the *p6.9* promoter or the *polh* promoter between the *vlf-1* ORF and the *vlf-1* promoter, respectively (Fig. 1). Effectively, this placed *vlf-1* under the control of either the very strong late *p6.9* promoter or the very strong very late *polh* promoter.

As shown in Fig. 2, tiny OBs began to appear in wild-type (wt) AcMNPV-infected cells by 24 h p.i. while almost all v6.9vlf1-infected cells contained large, essentially full-sized OBs by the same time. In contrast, OBs were not observed at 24 h p.i. in vpolhvlf1-infected cells and were observed only in a small portion of these cells even by 48 h p.i.. Thus, OB formation was advanced in v6.9vlf1-infected cells and delayed in vpolhvlf1-infected cells compared with wt AcMNPV-infected cells. Furthermore, OBs produced in v6.9vlf1-infected cells were larger than wt OBs and were usually cubic in shape. It was also observed that by 72 h p.i., more than half of the v6.9vlf1-infected cells had shrunk or lost their cytoplasm while virtually all wt AcMNPV- or vpolhvlf1-infected cells were intact, indicating that v6.9vlf1-infected cells lost cell integrity earlier than wt AcMNPV- or vpolhvlf1-infected cells.

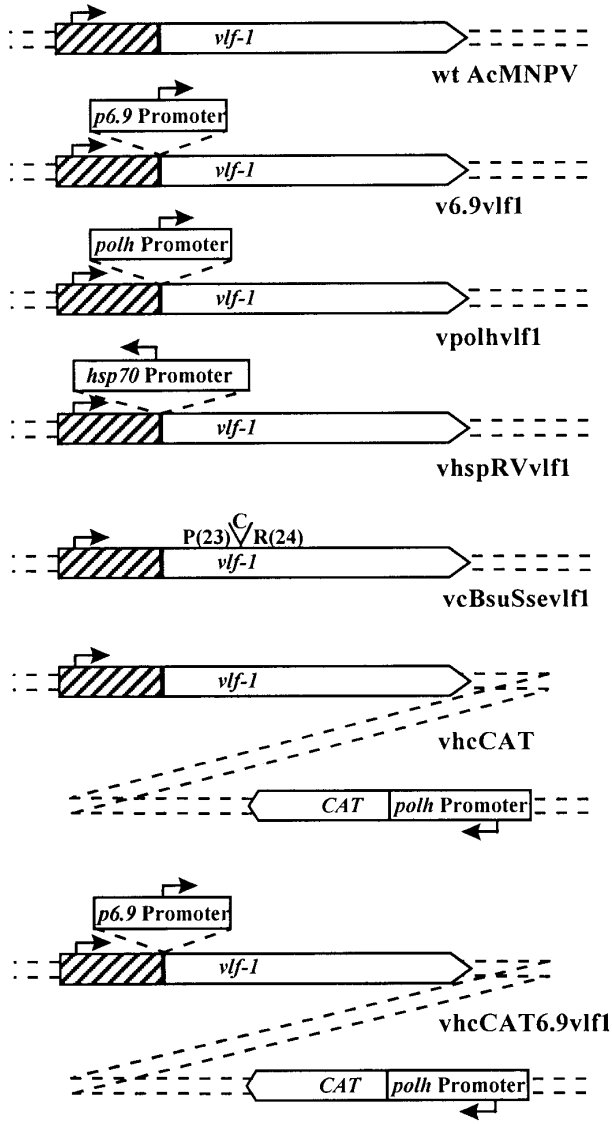


FIG. 1. Schematic illustration of structures of recombinant viruses. The striped boxes represent the wt *vlf-1* promoter. Additional promoters inserted between the *vlf-1* promoter and the open reading frame (ORF) are indicated by boxes above the lines with the direction of transcription indicated by an arrow. An additional cysteine residue is present in the *vlf-1* of vcBsuSsevlf1. The *polh* gene has been replaced by a CAT gene in vhcCAT and vhcCAT6.9vlf1.

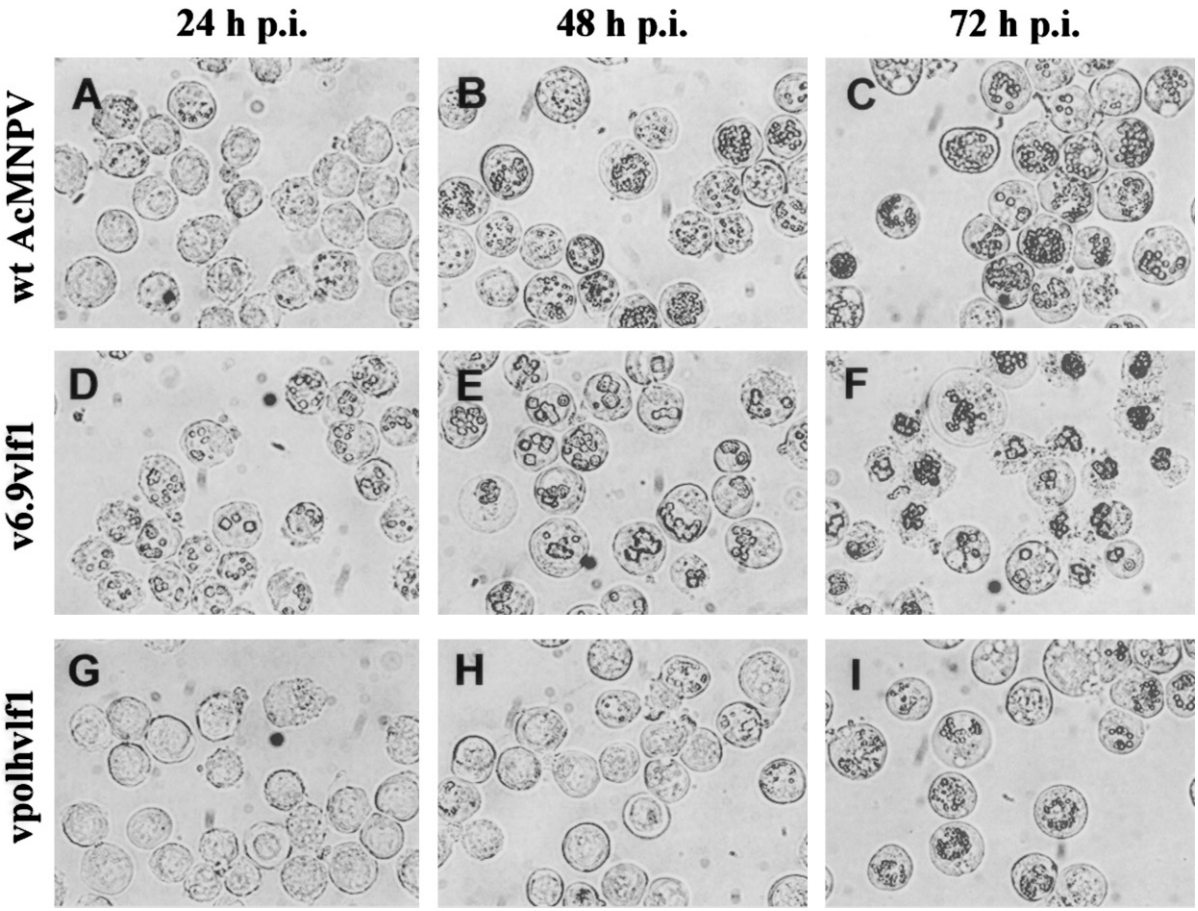


FIG. 2. Time course of occlusion body formation in wt or recombinant virus-infected cells. SF-21 cells were infected with wt AcMNPV (A, B, and C), v6.9vlf1 (D, E, and F), or vpolhvlf1 (G, H, and I) at a multiplicity of infection (m.o.i.) of 20 plaque forming units (PFU) per cell and visualized under the light microscope at 24 h p.i. (A, D, and G), 48 h p.i. (B, E, and H) and 72 h p.i. (C, F, and I).

## Polyhedrin expression is tightly coupled to VLF-1 expression

To investigate whether the unusual OB formation patterns were correlated with changes in polyhedrin synthesis, we first examined the kinetics of protein synthesis in v6.9vlf1- or vpolhvlf1-infected cells. As shown in Fig. 3, polyhedrin was being rapidly synthesized in v6.9vlf1-infected cells at 18 h p.i. but was not observed in wt Ac*M*NPV- or vpolhvlf1-infected cells at the same time. Strong expression of polyhedrin was detected 6 h later, at 24 h p.i., in wt AcMNPV-infected cells but not in vpolhvlf1-infected cells. By 48 h p.i., rapid polyhedrin synthesis was observed in vpolhvlf1-infected cells. Thus, active polyhedrin synthesis occurs earlier in v6.9vlf1-infected cells and later in vpolhvlf1-infected cells than in wt AcMNPV-infected cells.

Notably, the synthesis kinetics of VLF-1 also differed in cells infected with different viruses (Fig. 3, the upper arrowhead). The synthesis rate of VLF-1 appeared to be the highest at 18 h p.i. in v6.9vlf1-infected cells but declined thereafter. In vpolhvlf1-infected cells, VLF-1 synthesis was not observed at 24 h p.i. but was substantial at 48 and 60 h p.i.. The fact that VLF-1 was not readily detected in wt AcMNPV-infected cells indicates that the *vlf-1* promoter is much weaker than the *p6.9* promoter and *polh* promoter.

Immunoblot analysis showed that the timing and steady-state levels of both VLF-1 and polyhedrin were altered in v6.9vlf1- and vpolhvlf1-infected cells (Fig. 4). In wt AcMNPV-infected cells, VLF-1 was detected at 15 h p.i. and declined after 24 h p.i.. In v6.9vlf1-infected cells, VLF-1 was already abundant as early as 12 h p.i. and continued to accumulate to a much higher level than that in wt AcMNPV-infected cells with a sharp drop only by 72 h p.i. (Fig. 4A). In contrast, in vpolhvlf1-infected cells, VLF-1 was detected 18 h p.i., remained at a level lower than that in wt AcMNPV-infected cells until 48 h p.i., and then increased between 48 and 72 h p.i. (Fig. 4B). Interestingly, polyhedrin was detected earlier in v6.9vlf1-infected cells (at 12 h p.i.) than in wt AcMNPV-infected cells (at 18 h p.i.) (Fig. 4A) and did not accumulate in vpolhvlf1-infected cells to a detectable level until 36 h p.i. even though a low level of VLF-1 was observed from 18 h p.i.. Taken together, these expression patterns revealed a

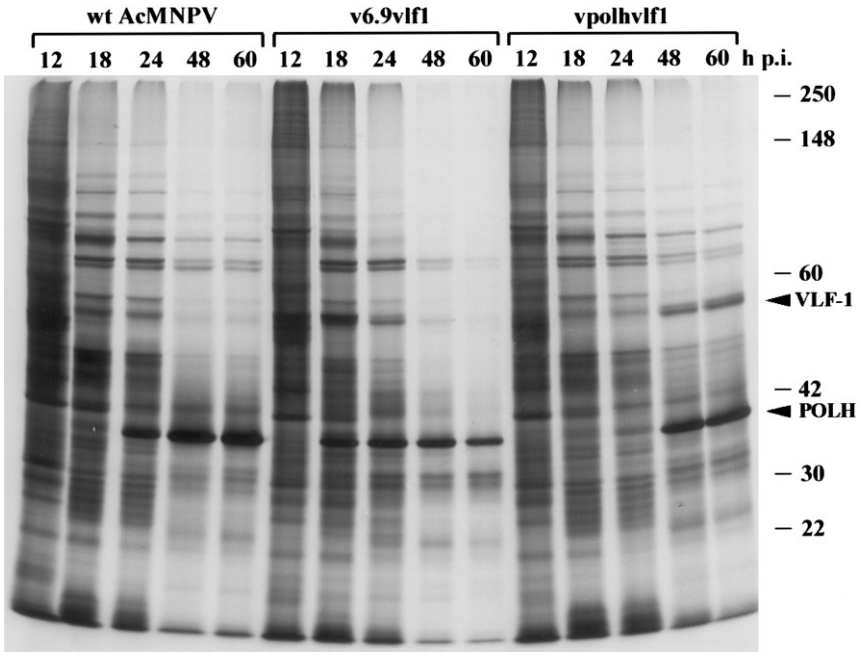


FIG. 3. Kinetics of protein synthesis in wt or recombinant virus-infected cells. SF-21 cells were infected with wt AcMNPV, v6.9vlf1, or vpolhvlf1 at a m.o.i. of 20 PFU per cell, labeled with [$^{35}$S]methionine and cysteine for 1 h at the times after infection indicated above each lane and lysed. Proteins were separated by SDS-PAGE and visualized by fluorography. VLF-1 and polyhedrin (POLH) are indicated by arrowheads and positions of protein standards are marked (in kilodaltons) on the right.

correlation between the timing of VLF-1 expression and the timing and/or level of polyhedrin synthesis.

To further verify this relationship, we accelerated the timing and raised the level of *vlf-1* expression in vpolhvlf1-infected cells by transfecting cells with pXA76.9, a plasmid containing a *p6.9* promoter-driven *vlf-1*, prior to infection (Fig. 5). As expected, in pXA76.9-transfected cells, VLF-1 accumulated more rapidly and to a higher level due to the expression from pXA76.9 than in the control cells transfected with pXA76.9d, which is identical to pXA76.9 except that pXA76.9d contains a frameshift mutation in the *vlf-1* ORF and thus can not express a full-length VLF-1. Accordingly, polyhedrin was detected at 18 h p.i. in pXA76.9-transfected cells, at least 6 h earlier than the cells expressing VLF-1 only from the viral genome. These results demonstrate that the delay in polyhedrin synthesis in vpolhvlf1-infected cells is indeed

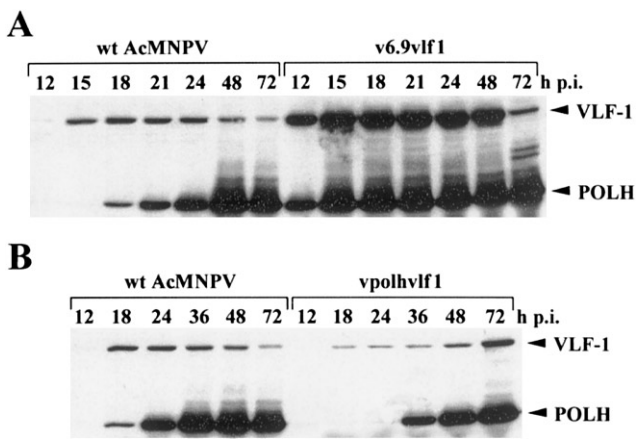


FIG. 4. Western blots of VLF-1 and polyhedrin accumulation in wt or recombinant virus-infected cells. SF-21 cells were infected with wt AcMNPV, v6.9vlf1, or vpolhvlf1 at a m.o.i. of 20 PFU per cell and harvested at the times after infection indicated above each lane. Cell lysates were analyzed for the presence of VLF-1 and polyhedrin by immunoblotting. VLF-1 and polyhedrin (POLH) are indicated by arrow heads on the right. (A) Cells infected with wt AcMNPV or v6.9vlf1. (B) Cells infected with wt AcMNPV or vpolhvlf1.

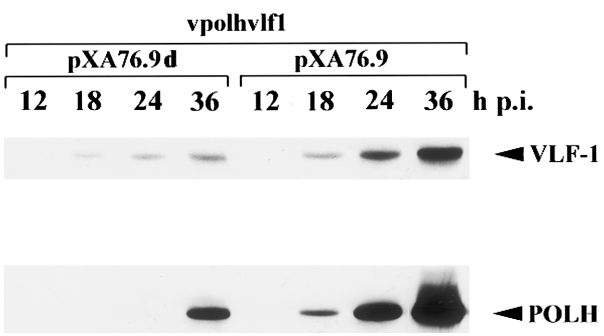


FIG. 5. Polyhedrin synthesis is accelerated by higher levels of VLF-1. SF-21 cells ($10^6$) were first transfected with 2.5 $\mu$g of pXA76.9 d or pXA76.9 DNA, plasmids expressing mutant or wt *vlf-1*, respectively, under the *p6.9* promoter control. After incubation at 27°C for 4 h, the cells were infected with vpolhvlf1 at a m.o.i. of 20 PFU per cell. At the times indicated above each lane, the cells were lysed and analyzed by immunoblotting to reveal the presence and levels of VLF-1 and polyhedrin (POLH).

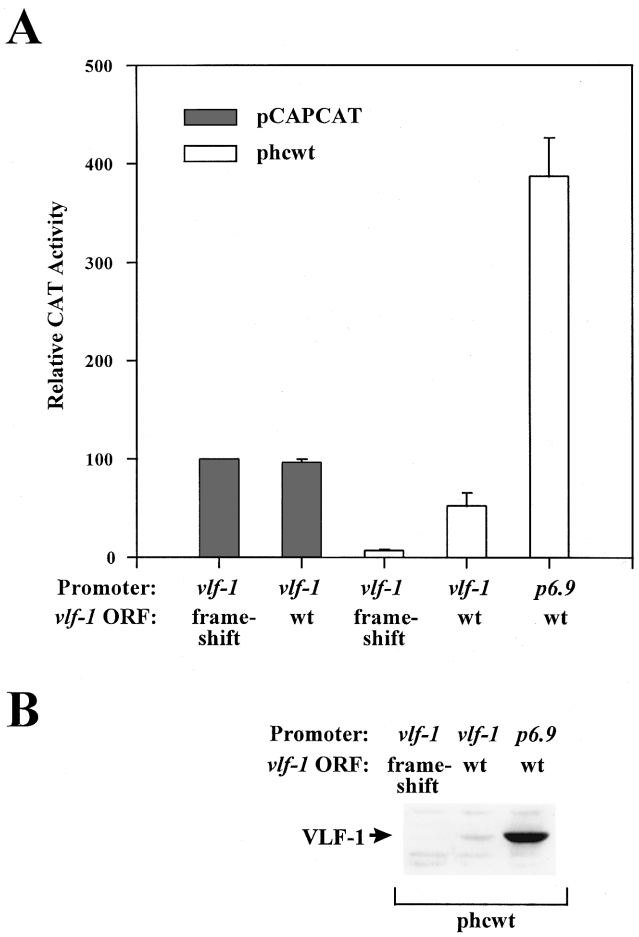


FIG. 6. Elevated VLF-1 levels enhance expression from the *polh* promoter in transient expression assays. (A) Influence of *vlf-1* driven by different promoters on CAT expression from *polh* promoter. A reporter plasmid (either pCAPCAT or phcwt, which contain the late *vp39* promoter or very late *polh* promoter-driven CAT gene, respectively) was transfected into cells with genomic clones collectively containing all *lef*s but not *vlf-1*. *vlf-1* was supplied on plasmid pXA7dBSA containing *vlf-1* frameshift driven by the *vlf-1* promoter, pXA7 containing wt *vlf-1* driven by the *vlf-1* promoter or pXA76.9 containing *vlf-1* driven by the *p6.9* promoter in the transfection as indicated. The CAT activity of the transfection involving pCAPCAT and pXA7dBSA was arbitrarily set to be 100% as a standard for other transfections. Triplicate data were used to calculate the standard errors. (B) Immunoblot examination of VLF-1 levels in the transfections in (A) involving phcwt.

caused by low VLF-1 level and/or delayed VLF-1 accumulation.

We also assessed the effect of altering the VLF-1 level on expression of the CAT reporter gene under the control of the *polh* promoter in transient expression assays (Fig. 6). A reporter plasmid containing a *vp39* promoter-driven CAT gene (pCAPCAT) or a *polh* promoter-driven CAT gene (phcwt) was transfected into cells with plasmid pSDEM2 containing *lef3* and an AcMNPV genomic library lacking clones PstH5 and HC9 containing *lef3* and *vlf-1* (Todd *et al.,* 1995, 1996). Thus, collectively, all the late gene expression factor genes (*lef*s) necessary to activate expression from late or very late promoters were included in the transfection. An additional plasmid was included to supply either a wt or frameshifted version of *vlf-1* driven by its natural promoter or by the *p6.9* promoter. Consistent with previous results, addition of the wt *vlf-1* did not affect expression from the late *vp39* promoter (pCAPCAT; Fig. 6A). However, *polh* promoter-driven CAT gene expression (phcwt; Fig. 6A) was stimulated sevenfold by the wt *vlf-1* driven by its own promoter compared with the *vlf-1* frameshift. Stimulation of CAT gene expression by the *p6.9* promoter-driven *vlf-1* was 55 times higher than that by the *vlf-1* frameshift and approximately 8 times higher than that by the wt *vlf-1* driven by it own promoter (Fig. 6A). As expected, VLF-1 was found to be much more abundant in cells transfected with *vlf-1* under *p6.9* promoter control than *vlf-1* promoter control (Fig. 6B). These results provide additional evidence that the level of VLF-1 is important to expression from the *polh* promoter.

## Effects of over-expressing *vlf-1* on the level of expression from the *polh* promoter in infected cells

We also addressed the question of whether over-expressing *vlf-1* could lead to a higher level of a heterologous protein expressed from the *polh* promoter in very late phase. vhcCAT6.9vlf1, a recombinant virus in which the *polh* is replaced by the CAT gene and *vlf-1* is over-expressed from the *p6.9* promoter (Fig. 1), was constructed for this purpose. As a control, we used vhcCAT (Ooi *et al.,* 1989) which is identical to vhcCAT6.9vlf1 except that vhcCAT has the *vlf-1* driven by its natural promoter. In vhcCAT6.9vlf1-infected cells, the burst of expression from the CAT gene was observed between 12 and 24 h p.i. instead of between 24 and 48 h p.i. as observed with vhcCAT; by 24 h p.i., the CAT activity level in these cells was already similar to that in vhcCAT-infected cells at 48 h p.i.. From 24 to 48 h p.i., CAT expression in vhcCAT6.9vlf1-infected cells increased an additional 48% (Fig. 7). Thus, the influence of over-expressing *vlf-1* is manifested primarily in the timing of expression from the *polh* promoter. The overall level of heterologous gene product can be increased but, owing to premature cell disintegration after 48 h p.i., the benefit is minor.

## Less VLF-1 is needed for virus propagation than for polyhedrin production

In the course of our work, we inserted a 0.7-kb DNA fragment containing the *hsp70* promoter between the *vlf-1* ORF and its natural promoter but with the *hsp70* promoter in opposite orientation to the *vlf-1* promoter. The resulting recombinant virus, vhspRVvlf1 (Fig. 1), was normal in BV production. Because *vlf-1* is essential for the replication of BV, the viability of vhspRVvlf1 predicts that VLF-1 is produced by this recombinant. Immunoblot examination confirmed that, in vhspRVvlf1-infected cells,

VLF-1 was present but at a much lower level than that in wt AcMNPV-infected cells (Fig. 8). This is probably because some *vlf-1* transcription still occurs and these transcripts direct low-level expression of *vlf-1*. Furthermore, vhspRVvlf1 is defective in OB formation (data not shown) and polyhedrin was undetectable in vhspRVvlf1-infected cells throughout the infection (Fig. 8). The defect in expression of *polh* in vhspRVvlf1-infected cells could be compensated by transfecting cells with plasmid pXA76.9, which expresses *vlf-1* from the *p6.9* promoter in a viral infection-dependent manner (Fig. 8, lane 7), indicating that the defect was caused by inadequate expression of VLF-1. Taken together, these results indicate that a higher level of VLF-1 is required to support active polyhedrin synthesis than to support the replication of BV.

Another recombinant virus vcBsuSsevlf1, which has a cysteine inserted in the *vlf-1* ORF between the proline at residue 23 and the arginine at residue 24 (Fig. 1), displays the same phenotype of low-level expression of *vlf-1* and a defect in polyhedrin production (Fig. 8, lane 8 through lane 12). This recombinant also produces BV at a normal level but is occlusion negative (data not shown). Unlike vhspRVvlf1, the greatly decreased VLF-1 level in vcBsuSsevlf1-infected cells is probably due to instability of VLF-1 caused by the inserted cysteine.

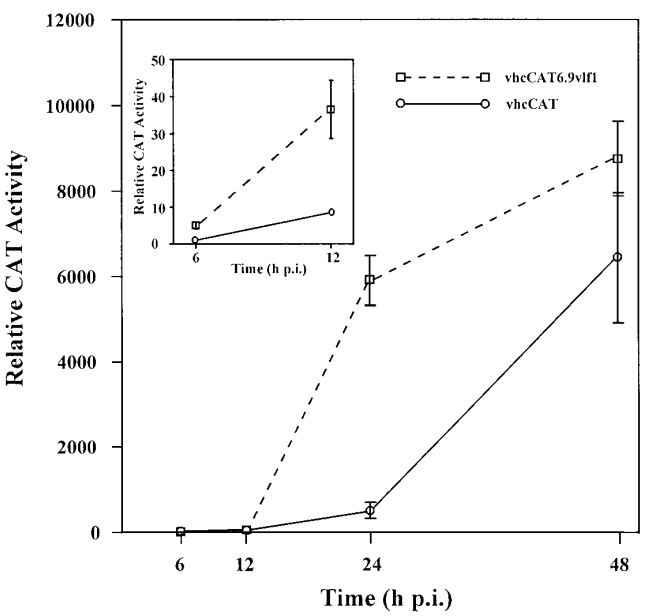


FIG. 7. Time course of expression of CAT gene from the *polh* promoter in vhcCAT- or vhcCAT6.9vlf1-infected cells. SF-21 cells ($10^6$) were infected with vhcCAT or vhcCAT6.9vlf1 at a m.o.i. of 40 PFU per cell and assayed for CAT activity at 6, 12, 24, and 48 h p.i. The CAT activity in vhcCAT-infected cells at 6 h p.i. was arbitrarily set to be 1%. The relative CAT activities of other infections were determined based on this standard. The standard errors were obtained from triplicate data. The inset is a closer look at the CAT activity at 6 and 12 h p.i..

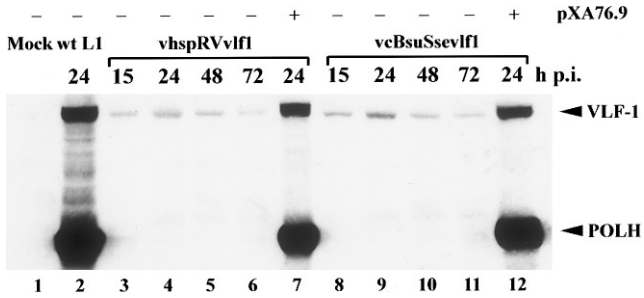


FIG. 8. Expression of VLF-1 and polyhedrin in vhspRVvlf1 or vcBsuSsevlf1-infected cells. Cells were infected with wt AcMNPV, vhspRVvlf1 or vcBsuSsevlf1 at m.o.i. of 20 PFU per cell and harvested at the times indicated at the top of each lane. The cell lysates were analyzed for the presence of VLF-1 and polyhedrin (POLH) by immunoblotting. In lanes 7 and 12, cells were also transfected with 5 $\mu$g of plasmid pXA76.9, which contains a *p6.9* promoter-driven *vlf-1*, prior to infection.

## DISCUSSION

We have altered the expression pattern of VLF-1 by linking *vlf-1* to a strong late *p6.9* promoter or a strong very late *polh* promoter and found that the timing of *polh* expression is correlated with *vlf-1* expression, e.g., accelerated VLF-1 production results in premature polyhedrin synthesis. The authenticity of this relationship is supported by the observation that the delayed polyhedrin synthesis in cells infected with a recombinant with delayed *vlf-1* expression, vpolhvlf1, could be rescued by enhanced plasmid-directed VLF-1 production. Although it can not be excluded that both the timing of *vlf-1* expression and the level of VLF-1 in the cell may be important to the regulation of the timing of *polh* expression, the requirement for the level of VLF-1 is more obvious based on the fact that low levels of VLF-1 are not able to initiate potent synthesis of polyhedrin as observed in infections with recombinants expressing only low levels of VLF-1 (e.g., vhspRVvlf1 or vcBsuSsevlf1). Also, VLF-1 was found in trace amounts as early as 9 h p.i. (data not shown) if a monoclonal anti-FLAG antibody, which is more sensitive than the polyclonal anti-VLF–1 antibody used in this study, was used to detect FLAG-tagged VLF-1 in cells infected with vcFgvlf1, a recombinant virus with a FLAG epitope inserted in the C-terminus of VLF-1 (Yang and Miller, 1998). Such a low level of VLF-1, which may be attributed to earlier, low-level *vlf-1* expression and/or introduction of VLF-1 carried in by the virions, has little or no effect on polyhedrin expression, suggesting a threshold level of VLF-1 is necessary to activate the *polh* promoter.

Late and very late promoters are transcribed from a TAAG motif recognized directly or indirectly by a virus-induced RNA polymerase (reviewed by Lu and Miller, 1997) with the strength of the TAAG motifs in different promoters varying according to the sequence context. The poor TAAG motif of the *polh* promoter probably determines that *polh* is at a disadvantage in competing

with other promoters for RNA polymerase, resulting in relatively weak expression during the late phase (Morris and Miller, 1994). VLF-1 appears to be the limiting factor in the burst transcription of *polh* as implicated by the sensitivity of *polh* expression to the level and/or timing of VLF-1 expression. During infection, the burst of *polh* transcription is probably not activated until the concentration of VLF-1 reaches a threshold level. Although *vlf-1* is mainly transcribed during late phase (Yang and Miller, 1998), its stability (unpublished data) enables VLF-1 to be present at a level high enough to support active transcription of *polh* throughout the very late phase.

In addition to its very late gene transactivation function, VLF-1 also plays a crucial role in efficient replication of BV (Yang and Miller, 1998). However, fulfillment of this function requires only a low level of VLF-1 compared with the level required for normal expression of *polh.* This was demonstrated in the cases of vhspRVvlf1 and vcBsuSsevlf1, which produce enough VLF-1 to support production of BV but not enough to activate synthesis of polyhedrin. Having a gene with both an essential and nonessential function is a novel strategy for a virus to ensure the maintenance of the nonessential function, in this case, the occlusion phase. Relying on the relative levels of the gene product in the cell appears to be the means by which the two functions of VLF-1 are controlled.

The stimulating effect of VLF-1 on the transcription from the *polh* promoter was demonstrated before in transient expression assays. A 6- to 10-fold stimulation was achieved when *vlf-1* driven by its natural promoter was used (Todd *et al.,* 1996). This stimulating effect is much less than that in infection. One of the underlying reasons for this observation may be that the *vlf-1* promoter is not activated rapidly enough or is not strong enough to make sufficient VLF-1 in transfected cells. Indeed, pXA76.9, a plasmid containing *vlf-1* driven by the strong late *p6.9* promoter produced much more VLF-1 and stimulated expression of the reporter gene by 55 fold (Fig. 6).

Because expression of *polh* responds to expression of *vlf-1*, VLF-1 can provide a means of regulating baculovirus expression systems employing the *polh* promoter to drive foreign gene expression. Although overexpressing VLF-1 accelerates cell disintegration and increases the overall yield marginally, earlier activation of the *polh* promoter may be beneficial in optimizing the posttranslational modification of expressed foreign proteins (reviewed by Jarvis, 1997). A cell line we have constructed that overexpresses VLF-1 in an infection-dependent manner can restore and advance polyhedrin synthesis when infected with a recombinant virus such as vhspRVvlf1, which is defective in polyhedrin production in normal cell lines due to reduced expression of *vlf-1* (unpublished data). Such *vlf-1*–expressing cell lines may find their place in applications such as producing baculovirus pesticides, which are occlusion-defective when propagated in larvae and in field situations due to lowered expression of *vlf-1*. Another possible application of *vlf-1*–expressing cell lines is the expression of toxic foreign proteins from *vlf-1*–diminished expression vectors propagated in normal cell lines and activated for toxin production in VLF-1–expressing cells.

## MATERIALS AND METHODS

### Cell line

The *Spodoptera frugiperda* (fall armyworm) IPLB-SF–21 (SF-21) cell line (Vaughn *et al.,* 1977) was grown at 27°C in TC-100 medium (GIBCO/BRL) supplemented with 10% fetal bovine serum (Intergen, Purchase, NY) and 0.26% tryptose broth (O'Reilly *et al.,* 1992).

### Construction of plasmids and recombinant viruses

pXA7 (McLachlin and Miller, 1994), pXA7dBSA (Todd *et al.,* 1996), phcwt (Rankin *et al.,* 1988), pCAPCAT (Thiem and Miller, 1990), pXA76.9 (Yang and Miller, 1998), and pXA7polh (Yang and Miller, 1998) were described previously.

Plasmid pXA76.9d containing a frameshift mutation in *vlf-1* under the control of the *p6.9* promoter was constructed by digestion of pXA76.9 with *Bst*EII, blunt-ending and self-ligation. pXA7hspRV was constructed by inserting a *Bgl*II and *Not*I fragment from pHSP70PL containing the *Drosophila melonogaster* heat shock protein gene (*hsp70*) (Clem and Miller, 1994) into the *Bsu*36I site of pXA7nBsu (Yang and Miller, 1998) in an orientation so that the *hsp70* promoter does not transcribe the *vlf-1* ORF. The *Bsu*36I site of pXA7nBsu is located 3 bp upstream of the *vlf-1* translation start codon. pXA7cBsuSse was constructed by site-directed mutagenesis (Deng and Nickoloff, 1992) to introduce a *Bsu*36I site (using oligomer BSU36I: 5′-CCCGACGAACCTGAGGAATTGCGATTATG-3′) 37 bp upstream of the stop codon of the *vlf-1* ORF and a Sse8387I site (using oligomer SSE8387: 5′-GGACTCGAACCTGCAGGGAGCGGATTG-3′) 57 bp downstream of the start codon of the *vlf-1* ORF into pXA7.

Recombinant viruses v6.9vlf1, vpolhvlf1, vhspRVvlf1, and vcBsuSsevlf1 were generated by cotransfecting 2 $\mu$g of pXA76.9, pXA7polh, pXA7hspRV, or pXA7cBsuSse DNA, respectively, with 1 $\mu$g of *Bsu36*I-linearized vncBsuvlf1 (Yang and Miller, 1998) DNA into $10^6$ SF-21 cells. Virus structure was verified by restriction digestion and PCR analysis. Cotransfection of v6.9vlf1 DNA and phcwt DNA resulted in a recombinant, vhcCAT6.9vlf1, with occlusion body negative plaques. This recombinant was confirmed to have the *polh* gene substituted by the gene encoding chloramphenicol acetyltransferase (CAT). vhcCAT was described before (Ooi *et al.,* 1989).

### Metabolic labeling

Metabolic labeling with [$^{35}$S]methionine and PPO fluorography were performed as previously described (O'Reilly *et al.,* 1992).

### Immunoblotting analysis

Proteins of cell lysates were separated on SDS-10% polyacrylamide gels (Laemmli, 1970) and transferred to nylon membranes (Millipore), which were subsequently blocked in TBST buffer (10 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.1% Tween 20) with 5% nonfat dried milk. A 1:2500 dilution polyclonal antibody against VLF-1 (Yang and Miller, 1998) and a 1:10,000 dilution horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G in TBST buffer were used as the first and the secondary antibody, respectively. Blots were visualized with the enhanced chemiluminescence Western blot Kit (Amersham). After detection of VLF-1, membranes were probed again with a 1:20,000 dilution of polyclonal antibody against polyhedrin and the same secondary antibody to reveal polyhedrin.

### Transient expression assay

Transient expression assays to assess the ability of VLF-1 mutants to transactivate reporter gene expression were described before (Todd *et al.,* 1996) with minor modification (Yang and Miller, 1998). Cells were lysed 72 h p.i. and assayed for CAT activity (Gorman *et al.,* 1982). A portion of the whole cell lysates were also examined for VLF-1 expression by immunoblot analysis.

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