Identification of an enhancer-like element in the polyhedrin gene upstream region of *Bombyx mori* nucleopolyhedrovirus

Asha Acharya and Karumathil P. Gopinathan

Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore 560012, India

A series of deletions in the upstream region of the gene encoding polyhedrin (*polh*) of *Bombyx mori* nucleopolyhedrovirus (BmNPV) were generated in plasmid constructs and tested for transcription. In transient transfection assays in *Bombyx mori*-derived BmN cells with firefly luciferase as the reporter gene, a 293 bp fragment located 1–0 kb upstream with respect to the +1 ATG of *polh* showed 10-fold enhancement in expression from the minimal promoter. This increase in reporter activity was observed only when the fragment was positioned *in cis* with respect to the promoter and not *in trans*. The stimulation of reporter gene expression was independent of the orientation of the fragment and was due to increased transcription from the promoter. When placed upstream of another promoter, the viral very late gene *p10* promoter, the enhancer brought about a 2-fold increase in expression. The region encompassing the enhancer was itself transcriptionally active, and transcripts corresponding to both of the encoded ORFs (N-terminal regions of ORF453 and ORF327, located in opposite orientations) were detected. Two AP1 sites (TGACTCG) in the 293 bp fragment did not appear to contribute to the enhancer function. Since repeat motifs, the hallmark of conventional enhancer sequences, were absent from this fragment, it is designated as an enhancer-like element. The influence of this region of the *polh* upstream sequence on expression from strong, very late viral promoters has not been reported previously.

**Introduction**

The baculoviruses comprise a large family of occluded DNA viruses with covalently closed, circular, double-stranded genomes that primarily infect the holometabolous insects (Blissard & Rohrmann, 1990; Hayakawa et al., 2000). Among these, *Autographa californica* multinucleocapsid nuleopolyhedrovirus (AcMNPV), a virus of the alfalfa looper, is by far the best studied at the molecular level and hence serves as the prototype baculovirus. Parallel investigations of several other baculoviruses, such as *Bombyx mori* nucleopolyhedrovirus (BmNPV), *Lymnantria dispar* MNPV (LdMNPV) and *Orgyia pseudotsugata* MNPV (OpMNPV), have been carried out and their full genomic sequences have been reported (Ayres et al., 1994; Gomi et al., 1999; Kuzio et al., 1999; Ahrens et al., 1997). A distinctive feature of the baculovirus life cycle is the synthesis of two forms of infectious particles, which is temporally regulated via a cascade of gene expression pathways (Keddie et al., 1989). The budded or extracellular virus is released from the cell between 8 and 12 h post-infection (p.i.) and requires viral DNA replication and synthesis of structural proteins. As the infection proceeds into the very late phase (20–72 h p.i.), the virions are occluded into large polyhedral bodies inside the nucleus. A 29 kDa polypeptide known as polyhedrin constitutes the major component of these occlusion bodies. Polyhedrin, despite being non-essential for virus propagation *in vitro*, is produced abundantly at late times. Other very late, non-essential genes like *p10* are also expressed to very high levels. The occluded virion can survive adverse environmental conditions and has evolved to mediate horizontal transmission of the pathogen. The replacement of the gene encoding polyhedrin (*polh*) with a foreign gene of interest to generate recombinant viruses has formed the basis of the baculovirus-based overexpression systems. The analysis of regulation of expression from this promoter is therefore significant.

An interesting feature of the late and very late viral promoters is that transcription from these promoters is carried out by an RNA polymerase distinct from the host-cell polymerases, made up entirely of four virus-encoded subunits, LEF8, LEF9, LEF4 and p47 (Guarino et al., 1998). The AcMNPV *polh* promoter has been studied extensively by using both deletion (Possee & Howard, 1987) and linker-scan
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Fig. 1. Plasmid constructs generated in the study. The plasmid constructs generated that harbour different lengths of the BmNPV polh 5' upstream region are shown schematically. The reporter luciferase gene (luc), containing an 80 bp leader sequence at the 5' end and the polyadenylation signals at the 3' end, was placed immediately downstream of the polh promoter (cloning site at −3 nt with respect to +1 ATG of polyhedrin and 50 nt downstream of the TAAG transcription start site motif). Plasmid construction was carried out essentially as described in Sambrook et al. (1989). For details of the individual plasmid constructs, see Methods. The 3' and 5' ends of the different constructs shown extend into the vector pBS KS+ sequences. The symbols + and − indicate the presence of the insert in one or other orientation, while * indicates the primer site used in generation of the PCR subclones of the 293 bp RsaI–MluI fragment. The 101 bp (R−*) fragment harboured two AP1 sites and the 198 bp (−M) fragment harboured one AP1 site. Restriction sites indicated are: E, EcoRI; M, MluI; R, R' and R"; RsaI; S, SalI; and X, XhoI.

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(Rankin et al., 1988; Ooi et al., 1989) mutational analysis. These studies identified a conserved TAAG core sequence that serves both as an integral promoter element and as the transcription start point. Besides the core sequence, linker-scan studies also demonstrated that the region between the core sequence and the translational initiation site greatly affects the steady-state level of polh transcript (Ooi et al., 1989). However, in addition to these proximal promoter elements, polh transcription is also influenced by distal enhancer elements in AcMNPV. Inter-spersed throughout the length of the baculovirus genome are homologous repeat (hr) sequences that are composed of several (two to eight) ~ 30 bp imperfect palindromic sequences. Transient in vitro plasmid-based replication assays have shown that the hr sequences function as origins of viral DNA replication (Pearson et al., 1992; Leisy & Rohmann, 1993). The hr sequences have also been shown to act as enhancers. For instance, the AcMNPV hr5 functions as a transcriptional enhancer of the delayed-early gene 39k (Guarino & Summers, 1986) and the transactivator protein IE-1 is a component of the DNA–protein complexes in this hr5-mediated enhancer function (Guarino & Dong, 1994). The AcMNPV hr1 enhances reporter expression from the polh promoter (Habib et al., 1996). Likewise, the BmNPV hr3 acts as an enhancer for the B. mori cytoplasmic actin gene promoter in vitro, augmenting reporter expression in transfected cells by two orders of magnitude (Lu et al., 1997). This was stimulated further more than 1000-fold on supplementation of transfected cells with the BmNPV transactivator protein IE-1.

In an attempt to characterize further the polh promoter of BmNPV, we have analysed the far-upstream region, beyond the core sequence element. A comparison of this region between the genomes of AcMNPV (Ayres et al., 1994) and BmNPV (Gomi et al., 1999) revealed an overall conservation of sequences as well as some marked differences. Starting with a reporter plasmid construct containing as much as 2.3 kb of the upstream region of the polh promoter, a series of deletions were generated. Using the minimal polh promoter construct harbouring the core sequence TAAG as the reference plasmid, our studies identified a 293 bp fragment located approximately 1.0 kb upstream of the promoter that enhanced transcription from the minimal polh promoter over 10-fold in a position- and orientation-independent fashion. This region, however, encoded the N-terminal segments of ORF453 and ORF327, located in opposite orientations, and both were actively transcribed.

Methods

- **Generation of plasmid constructs.** The recombinant plasmids generated in the study are presented in Fig. 1. A 2.3 kb XhoI–EcoRI fragment corresponding to the BmNPV genomic sequences of the polh upstream region was mobilized from the plasmid pBmΩ300 (Maeda, 1989) into pBS KS+. The reporter gene, firefly luciferase (luc; a 1.8 kb DNA fragment containing an 80 bp untranslated leader sequence as well as a polyadenylation signal at the 3' end) was cloned downstream of the promoter as a BamH1 fragment to generate the construct pBmpolh. A minimal promoter construct, pBmMpolh, containing 192 bp of the polh immediate 5' upstream sequences and encompassing the consensus core promoter motif TAAG, was made by subcloning the PCR-amplified SalI–EcoRI fragment of pBmΩ30 in pBS KS+ and inserting luc downstream of the promoter. From the parental construct, pBmpolh, deletion of a 1.6 kb SalI fragment followed by self-ligation generated pBmpolhAS, while deletion of a 665 bp MluI fragment gave rise to the construct pBmpolhAM. A 1.2 kb SalI fragment and the 665 bp MluI fragment were mobilized separately into the SalI site in pBmMpolh to generate pBmMpolhAS and pBmMpolhAM, respectively. The MluI fragment was cloned in both orientations with respect to the polh promoter (after blunt-ending both the vector and the insert with Klenow DNA polymerase) to provide pBmMpolhAM+.

Subclones of the 665 bp MluI fragment were made by partial digestion of this fragment with SalI and cloning the digested fragments at the SalI site of pBmMpolh after blunt-end ligating with Klenow DNA polymerase. The full fragment was represented in four sub-clones generated in this way, pBmMpolhR92+, pBmMpolhR9−, pBmMpolhR3+ and pBmMpolhR3−. pBmMpolhR92+ contained 188 and 114 bp fragments in inverse orientations while pBmMpolhR9− had only the 188 bp fragment in the proper orientation. Plasmids pBmMpolhR3+ and pBmMpolhR3− contained the 293 bp Rsal–MluI fragment cloned in both orientations. This fragment was split further into two fragments of 101 bp and 198 bp by PCR amplification (using primers P1a, 5' GCGTCGACGGTTGACCTCGGG 3', and P1b, 5' GCGTCGACGGTTGACCTCGGG 3' for the 101 bp fragment and P2a, 5' GCGTCGACGGTTGACCTCGGG 3', and P2b, 5' GCGTCGACGGTTGACCTCGGG 3' for the 198 bp fragment) and subcloned individually in pBmMpolh to generate pBmMpolhRFl and pBmMpolhRP2, respectively. In order to analyse the influence of the MluI fragment on transcription from other promoters, it was mobilized upstream of the AcMNPV p10 promoter in the plasmid construct pUW1 (Sriram et al., 1997), generating pUW1M. Yet another plasmid construct, pTZS1, harbouring the 1.2 kb SalI fragment (encompassing ORF453, ORF327 and the N-terminal half of LEF-2) in pTZ18R, was generated in the study are presented in Fig. 1. A 2.3 kb XhoI–EcoRI fragment corresponding to the BmNPV genomic sequences of the polh upstream region was mobilized from the plasmid pBmΩ300 (Maeda, 1989) into pBS KS+. The reporter gene, firefly luciferase (luc; a 1.8 kb DNA fragment containing an 80 bp untranslated leader sequence as well as a polyadenylation signal at the 3' end) was cloned downstream of the

- **Cell culture and transfections.** The B. mori-derived cell line BmN was maintained at 27 °C in TC-100 medium supplemented with 10% foetal bovine serum (Gibco BRL). Transfections with various plasmid DNA constructs were carried out in 6-well microtitre plates (106 cells per well in 2 ml medium). The cells were infected with wild-type BmNPV at an m.o.i. of 10 for all the transient transfections (Palhan et al., 1995). The virus stocks were maintained and titres were determined according to standard protocols (O'Reilly et al., 1992). The cells were transfected with plasmid constructs (2.5 µg covalently closed, circular DNA) using lipofectin (Gibco BRL) in serum-free medium for 8 h, followed by virus infection at an m.o.i. of 10 (Palhan et al., 1995). The cells were incubated at 27 °C for 48 h, harvested and washed with PBS (10 mM K2HPO4, 2 mM Na2HPO4, 140 mM NaCl and 25 mM KCl). Luciferase activity in the cells was quantified (non-invasive assay) by using a luminometer (Palhan et al., 1995; Sriram et al., 1997).

- **RNA isolation and slot-blot hybridization.** Total RNA was isolated from transfected cells by the guanidinium isothiocyanate method (Chomczynski & Sacchi, 1987) and treated with RNase-free DNase. RNA slot-blot analysis was carried out with 5 µg total RNA in 50% formamide, 6% formaldehyde and 1 x SSC (0.15 M NaCl and 0.1 M sodium citrate, pH 7.4), blotted on to a nylon membrane (Amersham) and probed using radiolabelled luc and RNA probes. Hybridization was carried out at
42 °C in the presence of 50% formamide and the blots were washed at a final stringency of 0.1 × SSC and 0.1% SDS at 65 °C for 30 min.

■ RNase protection assays. The transcription status of ORF453 and ORF327 was analysed by an RNase protection assay using corresponding antisense RNA probes. For ORF453, the plasmid pBmMinpolhR3, encompassing the N-terminal region of the gene, was linearized by digestion with MluI and transcribed in vitro using T3 RNA polymerase in the presence of radiolabelled [α-32P]UTP. The 32P-labelled probe for ORF327 was generated similarly from plasmid pBmMinpolhR3. The antisense riboprobes (15 × 10^6 c.p.m.) were co-precipitated with 20 μg total RNA, isolated from both uninfected and infected cells at various times p.i. in the presence of 200 mM NaCl and 20 μg carrier RNA using 2.5 vol. ethanol. Following hybridization at 50 °C overnight in the presence of 50% formamide, an RNase digestion mixture containing RNase A (2 U) and RNase T1 (1 U) was added and the samples were precipitated in the presence of 10 μg yeast tRNA and 2.5 vol. ethanol. The RNase-protected samples were analysed by electrophoresis on 8 M urea–6% acrylamide gels and visualized by autoradiography.

Results

Analysis of the 5’ flanking region of polh

We have analysed the effect of the 5’ upstream region of polh on transcription from the polh promoter. The AcMNPV and BmNPV promoters function equally well in the host cells Sf21 and BmN on infection with AcMNPV and BmNPV, respectively (Palhan et al., 1995; Sriram et al., 1997). A comparison of the genomic regions up to 2.3 kb upstream of the polh promoter of AcMNPV and BmNPV is presented in Fig. 2. This region encompasses AcMNPV ORFs 4–7, of which Ac4, Ac5 and Ac6 show greater than 92% identity to BmNPV counterparts at the amino acid level. Ac6, commonly referred to as lef-2 (late gene expression factor-2), is essential for both viral DNA replication and late gene expression in AcMNPV, BmNPV and OpMNPV (Merrington et al., 1996; Sriram & Gopinathan, 1998; Ahrens & Rohrmann, 1995; Sriram, 1998). The functions of ORF453 (Ac4) and ORF327 (Ac5) remain unidentified in all the baculoviruses characterized so far. The major differences were in ORF603 (Ac7) and the bro-e gene of BmNPV (Fig. 2). ORF603, located immediately upstream of the polh promoter in AcMNPV, is absent from the BmNPV genome (Gomi et al., 1999). This ORF is a non-essential gene in AcMNPV, since its disruption had no effect on virus infectivity or polyhedron production (Gearing & Possee, 1989). The gene designated bro-e, present at about 2.0 kb upstream in BmNPV, is absent in AcMNPV and belongs to the highly repeated baculovirus-repeated ORF (bro) gene family (Kang et al., 1999). There are five copies of bro (bro a–e) in BmNPV, whereas AcMNPV encodes only a single copy (Ac2, a homologue of BmNPV bro-d) that is located elsewhere in its genome. The encoded products of bro genes have been shown to bind nucleic acids and are involved in nucleosome organization (Zemskov et al., 2000). The presence of bro-e in the upstream region of polh of BmNPV compensates for the absence of ORF603 to locate hr1, which has an enhancer function (see the following section), at the same position with respect to the polh +1 ATG in both the viruses.

A 293 bp fragment from the polh upstream region enhances expression

In order to achieve the high levels of expression observed from the AcMNPV promoter, the virus hr1 sequences located about 4 kb upstream of the polh +1 ATG site have been shown to function as an enhancer (Habib et al., 1996). The BmNPV polh upstream region also harbours a similar hr sequence at the same distance (4.2 kb). In order to study the effect of cis elements other than the hr sequence from the polh upstream region, we generated a series of plasmid constructs harbouring luc as the reporter placed under the polh promoter covering different regions of the 5’ upstream sequence, up to 2.3 kb (Fig. 1). The parental plasmid construct pBmpolh, harbouring the entire 2.3 kb 5’ sequence of the BmNPV T3 strain (nt 126087–128413 on the BmNPV genome, GenBank accession no. L33180), was derived from the transfer vector pBM030 (Maeda, 1989). The minimal polh promoter plasmid (pBmMinpolh), containing up to 192 nt upstream of the polh ATG, was used as the reference to study the influence of other regulatory elements. Reporter gene (luc) expression was 25-fold higher compared with the minimal promoter construct (Fig. 3) when the additional 2.1 kb was added (plasmid pBmpolh). Deletion of a 1.6 kb Sau3A fragment (nt 126625–128227) that removed the whole of ORF327, ORF453 and parts of the lef-2 and bro-e sequences (plasmid pBmpolhAS) reduced reporter expression to the same level as that of pBmMinpolh, suggesting the presence of a positive regulatory element in this region.
This reduction in activity was not due to the deletion of lef-2, as the following studies show. When the 665 bp MluI fragment (nt 126799–127464) was deleted from pBmpolh to give construct pBmpolhΔM (Fig. 1), the luciferase levels were comparable to those from the minimal promoter or pBmpolh (harbouring the entire 2.5 kb upstream region). Since the MluI fragment encompassed the complete ORF453 together with its 150 bp upstream region, this result suggested that the DNA fragment behaved like an enhancer element. The extent of stimulation, however, was limited to 10-fold compared with the 25-fold stimulation seen with pBmpolh (harbouring the entire 2.3 kb upstream sequence). Since the MluI fragment encompassed the complete ORF453 together with its 150 bp upstream region (with respect to its +1 ATG) as well as the N-terminal region of ORF327, the possibility of activation in trans by the corresponding encoded proteins could not be ruled out. To test this, plasmid pTZSt (harbouring the 1.2 kb Sall fragment with both the ORFs cloned in plasmid pTZ18R) was co-transfected together with minimal promoter or pBmpolhAM construct. No stimulation of the luciferase activity was seen (Fig. 3), ruling out trans-activation.

In order to narrow down the cis-acting element further, the 665 bp MluI region was partially digested with Rsal and the individual fragments were cloned into pBmMinpolh to generate four additional subclones, pBmMinpolhR9⁺, pBmMinpolhR9⁻, pBmMinpolhR3⁺ and pBmMinpolhR3⁻ (Fig. 1). pBmMinpolhR9⁺ had a 188 bp Rsal–MluI fragment (nt 126800–126988 on the BmNPV genome) cloned in the right orientation whereas pBmMinpolhR9⁻ carried the same 188 bp fragment as well as the 114 bp fragment (nt 126800–127102) in the inverse orientation. The 293 bp Rsal–MluI region (nt 127170–127463), encompassing the N-terminal regions of ORF453 and ORF327, was cloned into both orientations, giving pBmMinpolhR3⁺ and pBmMinpolhR3⁻. When the above constructs were transfected into BmN cells, pBmMinpolhR9⁺ and pBmMinpolhR9⁻ failed to activate luc expression. On the other hand, both plasmids pBmMinpolhR3⁺ and pBmMinpolhR3⁻ stimulated expression from the minimal polh promoter to the same extent as the
parental construct pBmMinpolhM (Fig. 3). This activation was thus independent of the orientation of the insert, further substantiating the enhancer model.

Since the 293 bp fragment contained two AP1 sequence motifs, we examined whether the enhancing effect of this fragment on transcription from the polh promoter was due to the presence of these motifs. For this purpose, the 293 bp region was split into two fragments, of 101 bp (nt 127168–127269 on the BmNPV genome) and 198 bp (nt 127256–127464), with an overlap of 16 nt between them. The 101 bp fragment contained two AP1 sequence motifs (TGACTCG) separated by 40 nt, whereas the 198 bp fragment retained only one of the AP1 sites, at the extreme 5′ end of the fragment. These two fragments were generated by PCR amplification from genomic DNA using appropriate primers and cloned individually into pBmMinpolh to give constructs pBmMinpolhRP1 and pBmMinpolhRP2 (harbouring the 101 and 198 bp fragments, respectively; Fig. 1). Neither of the constructs showed stimulation of luciferase expression over and above the minimal promoter construct. The minimal enhancer element could thus be narrowed down only to the 293 bp Rsal–Mhhl fragment presented in Fig. 4.

**The increase in luc expression was due to enhanced transcription**

In order to determine whether the increase observed in luciferase activity in the presence of the Rsal–Mhhl fragment was indeed due to an increase in luc transcript levels, the transcripts were quantified (Fig. 5). The levels of transcripts were normalized with respect to an endogenous transcript from the host cell (tRNA Gly, levels of which do not change drastically following virus infection; Sharma et al., 1997), as well as to the amounts of transfected DNA (Fig. 5d). A clear increase in the level of luc transcripts was detected in all the samples that were transfected with plasmids containing the enhancer in cis, compared with those lacking it or the minimal promoter construct.

**Transcription status of the enhancer fragment**

In order to check whether the region containing the enhancer element (nt 127170–127463 on the BmNPV genome) was itself transcriptionally active, RNase protection assays were carried out using antisense riboprobes corresponding to the individual ORFs contained within this
A 192 nt protected fragment corresponding to the ORF453 transcript, which followed a delayed expression pattern (the transcript first appearing at 36 h p.i. and peaking between 48 and 60 h p.i.), was clearly seen (Fig. 6a). The distal late transcription start site TAAG, mapping at −40 nt from the +1 ATG of ORF327, is common to lef-2 and ORF327 (Sriram & Gopinathan, 1998). A 163 nt protected fragment of ORF327 was seen when the corresponding antisense probe was used (Fig. 6b). Thus, both ORF453 and ORF327 were transcribed in the course of virus infection.

**Activation from heterologous promoters**

Since enhancer sequences are known to bring about activation of heterologous promoters as well, the effect of this enhancer-like element on another very late gene promoter (p10 promoter) was analysed. The enhancer-containing MluI fragment was cloned upstream of the AcMNPV p10 promoter in the transfer vector pUW1, carrying the reporter gene luc (pUW1M). Expression from this construct showed only a 2-fold stimulation of luciferase activity (Fig. 3, right panel). However, expression from the p10 promoter itself (in the parental plasmid pUW1) was very high, almost 20 times that observed in the case of pBmpolh.

**Discussion**

We have identified a region in the upstream flanking sequences of polh of BmNPV, encompassing ORF453 and ORF327, that enhances polh promoter-based expression. The 293 bp fragment was found to satisfy the following criteria for an enhancer element: (i) it augmented transgene expression directed by the polh promoter in a position- and orientation-independent manner, (ii) no stimulation of the polh promoter was observed in trans, (iii) increased expression of the reporter gene, concomitant with increased transcription levels, was observed and (iv) expression was stimulated without promoter specificity. The enhancement of transcriptional activity was only 10-fold, compared with the 1000-fold stimulation observed from the hr-based enhancers. The enhancer-harboring fragments alone could not restore the minimal promoter activity back to that of the parental plasmid pBmpolh, suggesting that even the remaining regions upstream of polh are important in fostering maximal levels of transcription. No repeat elements characteristic of enhancer elements could be identified in the enhancer region analysed here. However, two AP1 sites (TGACTCG), separated from each other by 40 nt, were identified in this region. Subfragments of the enhancer region containing one or both the AP1 motifs, when placed upstream of the minimal promoter, did not stimulate expression from the polh minimal promoter. Although the 101 bp subfragment, harbouring two AP1 sites, formed a specific complex with nuclear extracts from infected BmN cells (but not with uninfected cell extracts) in gel mobility-shift assays, the complex was not chased out with an excess of AP1 sequences (data not shown). Taken together, these results imply that the AP1 sites may not be involved in the enhancer function. The minimal enhancer element constituted the N-terminal coding region of ORF453 and ORF327, which belong to the class of uncharacterized genes in all known baculoviruses. The region encompassing the enhancer is actively transcribed (Fig. 6a, b) from both strands, a feature so far not reported in other conventional enhancers. The significance, if any, of the transcription of this enhancer region is not clear. We therefore define this newly identified region as an enhancer-like element, to distinguish it from conventional enhancers.

The enhancer fragment identified here has been tested with two very late viral promoters, polh and p10. Although these promoters are transcribed exclusively by the virus-encoded polymerase induced late in the infection (Guarino et al., 1998), host factors have also been reported to play a role in late gene
transcription (Burma et al., 1994; Mukherjee et al., 1995). However, since extracts from uninfected cells failed to yield a complex with a segment of the enhancer fragment (data not shown), the involvement of early or late virus factors serving as activators through this enhancer element is more likely. For the highest levels of expression from the polh promoter containing the entire 4·0 kb 5′ upstream region of the promoter (as in pVL1392), the optimal placement of the foreign gene is at +35 nt with respect to the +1 ATG (Luckow & Summers, 1989). In vector pBM030, the reporter gene is located at −3 nt in relation to the polh ATG, and the expression levels are much lower (10–20% of the former; Sriram et al., 1997). Even though the p10 promoter in vector pUW1 contains only 300 nt upstream of the +1 ATG, it still provides an expression level of 60% compared with the optimized polh promoter-based vector, with its full-length 5′ upstream region. Activity from the minimal AcMNPV polh promoter (harbouring up to 550 nt of 5′ upstream sequences) was much lower (20%) compared with p10-based expression (Sriram et al., 1997; Sriram & Gopinathan, 1998; Palhan & Gopinathan, 2000).

Although both p10 and polh are transcribed as very late genes by the same virus-encoded polymerase, the requirements for cis elements appear to be somewhat different. Earlier studies on expression from these two promoters suggested competition for one or more components (Chaabihi et al., 1993; van Oers et al., 1992). Deletion of the p10 promoter boosted polh gene expression, presumably through the liberation of one or more components involved in polh transcription. In contrast, no transcriptional enhancement from the p10 promoter was seen when the polh gene was deleted, suggesting the requirement for p10-specific factors that are limiting. The activation of p10 occurs a few hours earlier than that of polh and the maximum expression levels attained are much lower (Roelvink et al., 1992). Furthermore, except for the consensus core sequence TAAG motif at the transcriptional initiation site, the leader sequences of polh and p10 share only limited nucleotide sequence similarity, implying different requirements for transcription factors. This may be the basis for differential regulation of the two very late viral promoters, despite being transcribed by the same virus RNA polymerase. We extend this hypothesis to the case of the enhancer-like region identified here, since the extent of stimulation observed for the two promoters was not the same. Although the 2·3 kb polh 5′ upstream regions of BmNPV and AcMNPV differed significantly (absence of ORF603 and presence of bro-e in BmNPV), the 293 bp region was present in both baculoviruses and showed more than 95% identity. However, the 293 bp region was located at different distances, 0·9–1·2 kb upstream of the +1 ATG of polh in BmNPV and 1·6–1·9 kb upstream in AcMNPV. In both viruses, the lr1 sequences are located about 4 kb upstream of the polyhedrin start site. The very high level of expression from the polh promoter achieved in vivo is likely to be the combined effect of the enhancer-like element(s) and the lr sequences.

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