CHAPTER III

BINDING OF THE UNUSUAL 30-kDa HOST FACTOR TO THE TRANSCRIPTIONALLY IMPORTANT MOTIFS WITHIN THE BACULOVIRUS VERY LATE p10 GENE PROMOTER

1. A host factor with cognate sequence recognition motifs similar to PPBP binds to the p10 promoter
2. The same 30-kDa factor binds to the p10 promoter.
3. Another host factor with a molecular weight of ~80-kDa binds to regions upstream to the p10 initiator
4. PPBP binds with different affinities to the 5' untranslated leader regions of p10 and p29 genes
5. The p10 initiator:PPBP has a half-life similar to that of p29 initiator:PPBP complex
6. The p10 5' leader:PPBP complex has a longer half-life than the p29 5' leader:PPBP complex
7. PPBP exhibits ssDNA binding activity with respect to the p10 promoter
The p10 promoter regulating transcription of another very late gene encoding a 10-kDa protein, also used for foreign gene expression (Vlak et al., 1990, Weyer et al., 1990), is similarly hyper transcribed by an α-amanitin resistant form of RNA polymerase (Huh and Weaver, 1990). The p10 promoter exhibits sequence homology to the p29 promoter around the mRNA initiation site (Rohrmann, 1986; Fig. 3.1). Like the p29 promoter, this promoter is also A-T rich with the untranslated leader region not only required for promoter activity but also for the very late burst of transcription (Weyer and Possee, 1988; Qin et al., 1989; Weyer and Possee, 1989). Though essentially very late in their time of activation post infection, subtle differences between these two promoters have been reported in terms of precise activation time and relative strength (Roelvink et al., 1992). There are suggestions that despite the above dissimilarities, these two baculovirus very late gene promoters might follow a common regulatory pattern. Studies were initiated to ascertain the involvement of host factor(s), if any, in p10 gene transcription.

1. A Host Factor with Cognate Sequence Recognition Motifs Similar to PPBP Binds to the p10 Promoter

The polyhedrin and the p10 promoter sequences were aligned with respect to the "TAAG" motif which is present at the transcription initiation point of both late and very late promoters. It was evident that the two sequences were fairly homologous around the regions encompassing the transcription start points but were not so in the other regions i.e. the sequences upstream to the transcription start site and the 5' untranslated leaders. This sequence alignment was used to design p10 promoter specific oligonucleotides (Fig. 3.1). The p10 promoter was divided into three domains A (-101 to -76), B (-83 to -42), and C (-41 to +1) on similar lines as described earlier for the polyhedrin promoter (Burma et al., 1994) keeping the transcription start point as the reference. The A domain refers to sequences upstream to the mRNA start site, B domain corresponds to the region surrounding the transcription initiation and the C domain is the 5' untranslated leader region. Complementary oligonucleotides corresponding to these three domains were synthesized, annealed, end-labeled and
used in electrophoretic mobility shift assays using nuclear extracts prepared from uninfected Sf9 insect cells as well as that infected with AcNPV 51 h pi (Hasnain et al., 1995). To begin with, binding reactions were carried out with the B domain of the p10 promoter since it displayed the maximum amount of homology with the polyhedrin promoter. Assays carried out with the B domain revealed a DNA-protein complex showing a similar mobility on a 5% native polyacrylamide gel using both the uninfected (Fig. 3.2, lane 2) and infected (lane 8) insect cell nuclear extracts, suggesting that a host factor is binding the p10 promoter. The specificity of this DNA-protein complex was checked in a homologous cold competition assay using a 25-fold molar excess of unlabeled p10 B domain oligonucleotide (lanes 3 and 9). The complex could also be competed out in the presence of unlabeled oligonucleotide representing the polyhedrin B domain (lanes 4 and 10). However, mutated versions of the polyhedrin B domain carrying sequences different from the polyhedrin PPBP-cognate motifs (CCGCCC in place of AATAAA and GCCTGCGG in place of TAAGTATT) could not compete for binding to the p10 B domain (lanes 5, 6, 11 and 12) thus confirming that the factor which binds the p10 promoter recognizes the same sequence motif as that recognized by the polyhedrin promoter binding protein, PPBP (Burma et al., 1994). Non-specific competitor such as pUC18 failed to compete for complex formation (lanes 7 and 13). These results clearly indicated that the host factor which binds to transcriptionally important motifs of the p10 promoter is similar to the host factor PPBP in terms of cognate sequence recognition motifs.

2. The Same 30-kDa Factor Binds to the p10 Promoter

To further establish that the p10 promoter binding factor is the same as PPBP which binds to the p29 promoter in terms of molecular mass, UV cross-linking analyses was performed with radio labeled oligonucleotides (Fig. 3.3 A, B, C, and D, lane 1) representing the "initiator" (B domain) and the 5' leader region (C domain) of both genes. As expected, a cross-linked complex with a molecular mass of about 30-kDa (Burma et al., 1994) was obtained with labeled p29 B domain oligonucleotide (Fig. 3.3 A, lane 4). Labeled p10 B domain also revealed a complex having a molecular
Fig. 3.1 Sequence alignment of the nucleotide sequences of the AcNPV polyhedrin (p29) and p10 gene promoters (Possee et al., 1991; Weyer and Possee, 1989). The sequences are aligned with respect to the TAAG motif. The transcription start point, marked with an extending arrow, is at -50 for the p29 promoter and at -63 for the p10 promoter. Common bases within the two sequences are shown in bold. The relative boundaries (indicated by numbers) of domains A, B, and C for both the promoters are demarcated above and below the p29 and p10 sequences.

Fig. 3.2 Binding of the host factor PPBP to the p10 gene promoter.
End-labeled domain B of the p10 promoter was incubated alone (lane 1), with 4 µg (lanes 2-7) of nuclear extract from uninfected Sf9 cells, or with 4 µg (lanes 8-13) of nuclear extract from Sf9 cells infected with AcNPV. The complex obtained with the uninfected and infected extract (lanes 2 and 8) was competed with 25 ng of unlabeled p10 B (lanes 3 and 9), p29 B (lanes 4 and 10), mutOct (lanes 5 and 11), mutHex (lanes 6 and 12), or pUC18 (lanes 7 and 13). M refers to the labeled φX/Tag1 molecular size marker.
mass of 30-kDa (Fig. 3.3 B, lane 4). These complexes could be competed away with an excess of the respective unlabeled oligonucleotides (Fig. 3.3 A, B, lane 5) but were unaffected in the presence of an excess of pUC18 DNA (Fig. 3.3 A, B, lane 6) confirming the specificity of the cross-linked complex. A cross-linked complex with a molecular mass of ~30-kDa was once again evident for both the p29 C and p10 C domains (Fig. 3.3 C, D, lane 4) using nuclear extracts from uninfected Sf9 cells. This region represents the untranslated leader regions of the two genes. This complex could also be specifically competed out in a homologous cold competition assay with a 25-fold excess of the respective unlabeled oligonucleotide (lane 5) but not by a non-specific competitor, pUC18 (lane 6) thus, confirming its specificity. In control experiments (lanes 1 to 3) DNA-protein complex was not obtained with the probe alone (lane 1), even after UV irradiation (lane 2) or when the probe and the nuclear extract were not irradiated (lane 3). These results demonstrate that a host factor with a molecular mass of ~30-kDa, similar to PPBP, binds both the polyhedrin and p10 promoters. Furthermore this host factor also has the ability to independently contact the initiator and the 5' untranslated leader region.

3. Another Host Factor with a Molecular Weight of ~80-kDa Binds to Regions Upstream to the p10 Initiator

UV crosslinking analyses was carried out using nuclear extracts from Sf9 cells with the oligonucleotides corresponding to sequences upstream to the p29 initiator region (p29 A domain; Fig. 3.4 A) and the p10 initiator region (p10 A domain; Fig. 3.4 B) to determine if the same host factor contacts this region too as is the case for the p29 (Burma et al., 1994). A 30-kDa factor was crosslinked with the p29 A domain (Fig. 3.4 A, lane 4) which could be specifically competed in a homologous cold competition assay in the presence of unlabeled p29 A domain (lane 5) but could not be competed with a 25-fold excess of non specific DNA such as pUC18 (lane 6). Surprisingly, we observed the specific crosslinking of a factor ~70-80 kDa with the p10 A domain (Fig. 3.4 B, lane 4). This factor again could be competed in a
Fig. 3.3 The same 30-kDa factor binds both the very late promoters. Labeled p29 B (panel A), or p10 B (panel B), or p29 C (panel C), or p10 C (panel D) was either not subjected to UV irradiation (lane 1) or UV irradiated alone without nuclear extract (lane 2) or with 2 μg of nuclear extract without irradiation (lane 3) to serve as negative controls. In experimental sets the incubation mixture was irradiated for 30 min without any competitor DNA (lane 4). Competition of the cross-linked complex was performed with 25 ng of the respective homologous oligonucleotide (lane 5) or 25 ng of pUC18 (lane 6). Protein molecular size markers (in kDa) are indicated on the right.
Fig. 3.4 A host factor with a molecular weight of ~80-kDa binds regions upstream to the p10 initiator. Labeled p29 A (panel A), or p10 A (panel B) was either not subjected to UV irradiation (lane 1) or UV irradiated alone without nuclear extract (lane 2) or with 2 µg of nuclear extract without irradiation (lane 3) to serve as negative controls. In experimental sets the incubation mixture was irradiated for 30 min without any competitor DNA (lane 4). Competition of the cross-linked complex was performed with 25 ng of the respective homologous oligonucleotide (lane 5) or 25 ng of pUC18 (lane 6). Protein molecular size markers (in kDa) are indicated on the right.
homologous cold competition assay (lane 5) but not in a heterologous cold competition assay with nonspecific pUC18 DNA (lane 6). No crosslinked complex in the 30-kDa range was detected though the experiment was repeated several times with different preparations of the nuclear extracts. These results in conjunction with the previous observations indicate that the same host factor of molecular weight ~30-kDa contacts both the p10 and p29 promoters utilizing similar motifs for binding. However, the molecular weight of the factor binding the domain upstream to the p10 initiator is much greater indicating either the presence of associated factor(s) and/or a degree of post-translational modification of the binding factor.

4. PPBP Binds with Different Affinities to the 5' Untranslated Regions of p10 and p29 Genes

Having demonstrated that a PPBP-like host factor also binds to the 5' untranslated region of the p10 gene, we designed experiments to examine if PPBP displayed differences in binding to this region so important for promoter function as well as late burst of transcription. This was also interesting in light of the fact that this region does not share the extent of sequence homology with the p29 promoter apparent for the initiator region. EMSAs coupled with cross cold competition analyses were performed with the C domains (Fig. 3.5) of the polyhedrin (-43 to -1) and the p10 promoter (-41 to +1). It was apparent that the 29C:PPBP complex (panel A, lane 2) could be specifically competed by a 25-fold molar excess of 29 C, 29 B, 10 C or 10 B domains (lanes 3, 4, 5 and 6) pointing to a somewhat similar affinity of this factor for these four domains. Excess of non-specific DNA such as pUC18 could not compete for complex formation (lane 9) indicating that this complex was specific. Further, mutated versions of the p29 promoter B domain (Burma et al., 1994), viz., mutHex (lane 7) and mutOct (lane 8) could not compete for complex formation confirming the authenticity of the PPBP complex. In a complementary experiment, the 10C:PPBP complex (panel B, lane 2) could be specifically competed by a 25-fold molar excess of unlabeled 10 C (lane 3), 10 B (lane 4) and 29 B domains (lane 6). However, a 25-fold
Fig. 3.5 PPBP binding to the 5' untranslated region of the very late genes. Labeled p29 C domain (panel A) was either incubated alone (lane 1) or in presence of 4 μg of Sf9 nuclear extract (lanes 2-9). The DNA-protein complex obtained (lane 2) was competed in presence of 25 ng of p29 C (lane 3), or p29 B (lane 4), or p10 C (lane 5), or p10 B (lane 6), or mutHex (lane 7), or mutOct (lane 8), or pUC18 (lane 9). Labeled domain p10 C 5' untranslated leader region (panel B) was either incubated alone (lane 1) or in the presence of 4 μg of Sf9 nuclear extract (lanes 2-9). The DNA-protein complex obtained (lane 2) was competed in presence of 25 ng of p10 C (lane 3), or p10 B (lane 4), or p29 C (lane 5), or p29 B (lane 6), or mutOct (lane 7), or mutHex (lane 8), or pUC18 (lane 9). M is the DNA molecular size marker.
molar excess of unlabeled 29 C domain (lane 5) could not compete for the 10 C-complex to the same extent as compared to the homologous 10 C competitor. This complex again was not affected by competition using the mutated versions of the polyhedrin promoter or excess of nonspecific competitor DNA (lanes 7, 8 and 9). These results clearly indicated that PPBP could bind to the 10 C domain relatively strongly as compared to 29 C domain possibly as a result of a higher affinity for the p10 5' non-coding region than the corresponding sequence of the p29 gene.

5. The p10 Initiator:PPBP has a half-life similar to that of p29 Initiator:PPBP Complex

It was earlier reported that the half-life of polyhedrin B domain-PPBP complex was ~15 min (Burma et al., 1994). p10 B domain shares a high degree of sequence homology with the p29 B domain (Fig. 3.1). Therefore, it was ascertained if PPBP binds with the same affinity to the p10 B domain as to the p29 B domain. In an earlier experiment it was demonstrated that the DNA-protein complex formed with the p10 B domain was competed out with a 25-fold molar excess of p29 B domain (Fig. 3.2, lanes 4 and 10). The complex obtained with the labeled p29 B domain could also be competed out to the same extent using a 25-fold excess of p29 B or the p10 B domain, respectively (Burma et al., 1994). This indicated that in contrast to the C domains, PPBP bound with a similar affinity to both the B domains of the p29 and p10 promoters. This was further confirmed by calculating the half-life of PPBP with respect to the two domains (Choo and Klug, 1993; Mukherjee et al., 1995a; Fig. 3.6). Preformed p29B:PPBP (panel A) or p10B:PPBP complexes (panel B) were challenged with an excess of unlabeled respective domains and the reactions were loaded onto a running gel over a time period extending from 0 to 60 min. The extent of loss of radioactivity from the original complexes was quantitated by phosphor image analysis (Molecular Imager BIO-RAD, USA, model-GS250) and % maximal binding was plotted against time (panel C). As expected, the half-life of p29 B domain:complex was estimated to be ~15 min. Interestingly, the half-life of p10 B domain:complex was
Fig. 3.6 PPBP has a similar half-life for the initiator region of the p10 and p29 promoters. Preformed p29B:PPBP complex (panel A) and p10B:PPBP complex (panel B) were challenged with 30 ng of unlabeled homologous domain. The reactions were loaded onto a running gel over a time period of 0 min to 60 min (shown over each lane). The dissociation of the original complex was plotted as % maximal binding vs. time (panel C).
Fig. 3.7 PPBP has a higher half-life for the p10 5' untranslated region of the p10 promoter as compared to the p29 leader. Preformed p29C:PPBP complex (panel A) and p10C:PPBP complex (panel B) were challenged with 30 ng of unlabeled homologous domain. The reactions were loaded onto a running gel over a time period of 0 min to 60 min (shown over each lane). The dissociation of the original complex was plotted as % maximal binding vs. time (panel C).
also estimated to be ~15 min. These data demonstrated that the p10 and p29 promoters which shared a high sequence homology around the transcription start sites also bind the host transcription factor, PPBP with the same affinity within this region.

6. The p10 5' Leader:PPBP Complex has a longer half-life than the p29 5' Leader:PPBP Complex

To confirm the observed PPBP affinity differences within the 5' non-coding leader domains described above in section 4, the half life of PPBP with respect to the C domains of the p29 and p10 promoters was determined (Fig. 3.7). As described earlier, preformed p29C:PPBP (panel A) or p10C:PPBP complexes (panel B) were challenged with an excess of unlabeled p29 or p10 untranslated leader sequences, respectively, and the reactions were loaded onto a pre-running gel over a time period extending from 0 to 60 min. The extent of loss of radioactivity from the original complexes as quantitated by phosphor image analysis and % maximal binding was plotted against time (panel C). The half-life of p29 untranslated leader:PPBP complex was estimated to be only ~10 min whereas that of the p10 untranslated leader:PPBP complex was ~30 min. These results demonstrated that the PPBP:p10 leader sequence displayed a longer half-life than the p29 leader further pointing to the possible involvement of this host factor in late burst of transcription characteristically associated with the p10 and p29 leader sequences.

7. PPBP Exhibits ssDNA Binding Activity with Respect to the p10 Promoter

PPBP exhibits single-stranded DNA-binding activity for the polyhedrin "initiator" region which is restricted to its coding strand only (Mukherjee et al., 1995a). It has been suggested that such an activity would allow PPBP to maintain its position even when the DNA helix melts during the initiation of transcription and would enable repeated rounds of transcription from this promoter. Since the two promoters share conspicuous homology in this region and are also suggested to follow a similar transcription profile (Roelvink et al., 1992) single-stranded binding activity, if any, for
Fig. 3.8 PPBP also has a ssDNA binding activity with respect to the p10 promoter. Labeled p10 B duplex promoter (panel A) or p10 B coding strand (panel B) was either incubated alone (lane 1) or in presence of 2 µg of Sf9 nuclear extract (lanes 2-9, panel A and lanes 2-11, panel B). The DNA-protein complex obtained with the duplex promoter (panel A, lane 2) was competed in presence of 25 ng of p10 duplex (lane 3), or p10 coding (lane 4), or p10 non-coding (lane 5), or p29 duplex (lane 6), or p29 coding (lane 7), or p29 non-coding (lane 8), or pUC18 (lane 9). The DNA-protein complex obtained with the coding strand (panel B, lane 2) was competed in presence of 25 ng of p10 coding (lane 3), or p29 coding (lane 4), or p10 non-coding (lane 5), or p29 non-coding (lane 6), or p10 duplex (lane 7), or p29 duplex (lane 8), or mutHex (lane 9), or mutOct (lane 10), or pUC18 (lane 11). Labeled p10 B non-coding strand was either incubated alone (panel C, lane 1) or in presence of 2 µg of Sf9 nuclear extract (lane 2).
the p10 "initiator" was investigated. EMSAs were carried out with labeled p10 B duplex domain (Fig. 3.8, panel A) and with the labeled p10 B coding strand (panel B). As expected in control lanes the p10 duplex:PPBP complex (panel A, lane 2) could be specifically competed with a 25-fold molar excess of p10 duplex DNA (lane 3) and with the p29 duplex DNA (lane 6) as shown earlier. It could also be competed with the coding strand of the p29 initiator (lane 7) but not with the non-coding strand of p29 (lane 8) thus confirming previous results (Mukherjee et al., 1995a) that PPBP specifically contacts the template strand of the polyhedrin promoter. The complex could also not be competed with an excess of non-specific DNA such as pUC18 (lane 9). Surprisingly, the DNA-protein complex was competed away to the same extent with both the coding (lane 4) and the non-coding strands (lane 5) of the p10 promoter. In binding reactions using labeled p10 coding strand (panel B) the DNA-protein complex (lane 2) could be specifically competed with a 25-fold excess of p29 duplex (lane 8) or p10 duplex (lane 7) DNA as well as the p29 coding strand (lane 4) or p10 coding strand (lane 3). The complex however, could also be competed with the p10 noncoding (lane 5) but not the p29 noncoding strand (lane 6). The complex could also not be competed with an excess of mutated PPBP-binding motifs, mutHex (lane 9) and mutOct (lane 10), or in presence of non-specific DNA (lane 11). To further confirm these observations direct binding experiments with the labeled p10 noncoding strand (panel C) were carried out. It was observed that PPBP could bind the p10 noncoding strand (lane 2) and no binding was observed in the absence of the Sf9 nuclear extract (lane 1). These results unequivocally demonstrate that PPBP can also bind the ssDNA in case of the p10 promoter without any preference for the template or the non-template strand and it utilizes similar motifs for binding as those used in case of the p29 promoter.

DISCUSSION

POSSIBLE MECHANISM OF ACTION OF BACULOVIRUS VERY LATE PROMOTERS

The baculovirus very late p10 gene promoter besides having a similar activation profile exhibits conspicuous similarities to the p29 initiator promoter
sequence and also to the consensus "initiator" sequence (Fig. 1.1 and 3.1) as described by Javahery et al. (1994). By definition the p10 promoter, like the p29 promoter also belongs to the class of initiator promoters (Smale and Baltimore, 1989). In light of these observations, it is possible that these two baculovirus very late promoters share some basic steps in transcription activation. We previously showed that a host factor binds to transcriptionally important motifs within the p29 promoter (Burma et al., 1994; Mukherjee et al., 1995a; 1995b; Hasnain et al., 1995). Recent data from our laboratory unequivocally demonstrate that PPBP-p29 interaction is critical and crucial for transcription from this promoter in vivo. We showed that transcription from the p29 promoter in vitro is abolished when PPBP is sequestered out but is restored upon the addition of purified PPBP. These results demonstrate the requirement of a host factor in specific transcription from the baculovirus p29 promoter. PPBP is an initiator binding protein (IBP) which binds the initiator. Comparison of the two promoters for PPBP cognate motifs shows that the acta-motif (T AAGT ATT) is nearly 90% similar and the hexa-motif (AA T AAA) shares the last four bases (T AAA) between the two promoters. These four bases are critical for PPBP binding and the resulting reporter gene expressions in vitro (Chapter IV). Thus, the requirements for PPBP binding at the transcription initiation site are very much similar between the two baculovirus very late promoters. Since p29 and p10 promoters share a lot of similarities and p10 promoter is also an initiator promoter, it will therefore be logical to expect a similar mode of regulation for the two very late promoters including the requirement of a host factor as an initiator binding protein at the p10 promoter.

The ATAAG motif of the p29 and p10 promoters have been considered to have originated from the host genome (Friesen et al., 1986) which can possibly explain why this motif is recognized by a host factor. Binding of PPBP to the p10 promoter also is therefore, not surprising as similar reports exist in other systems. For example, the same host factor which regulates transcription from the herpes virus VP5 late gene (Chen et al., 1992) could also bind to other HSV promoters having similar recognition sequences. According to a possible model, PPBP as an IBP first
contacts the "initiator" in both the p29 and p10 promoters, an event which then triggers off the assembly of the remaining transcription initiation complex which possibly includes a battery of identified late expression factors or lefs.

IN Volvement OF CeLLuLar FaCTORS IN TrAnsCRiPTION OF OTHER ViRAL SYSTEMS

Involvement of cellular factors in conjunction with viral factors in transcriptional activation has been well documented for several other viral systems. The well studied among these include HCF and YY1 in HSV (Thompson and Mcknight, 1992; Chen et al., 1992), TRP-185 (Sheline et al., 1991), TDP-43 (Ou et al., 1995), TAK (Herrmann and Rice, 1995) and Tat-SF (Zhou and Sharp, 1995) in human immunodeficiency virus 1 (HIV-1), several factors in rous sarcoma virus (Boulden and Sealy, 1990) and CBF1 which along with virally encoded EBNA2 regulates transcription in the epstein barr virus (Henkel et al., 1994). Parvoviruses are also dependent on cellular factors (Momoeda et al., 1994) for their replication because of the limited size of their genome. YY1, a common cellular transcriptional regulator which is also an initiator binding protein, is involved in transcription activation from the parvovirus P5 promoter. Adenoviruses make use of the same host cell transcriptional complex as the herpes simplex virus to initiate its infectious cycle. Influenza virus makes use of host factor(s) for its replication and transcription machinery (Toyoda et al., 1994). In most cases the viral factors are recruited to the transcription complex by either directly binding weakly to the DNA or by binding weakly to cellular factor(s) through protein-protein interactions or to cellular factor(s) which weakly bind to the promoter or by altering the activity of one or more cellular transcription factors which directly contact the promoter both in uninfected and infected extracts as seen in the case of VP5 gene promoter of herpes simplex virus. The HSV protein VP16 does nucleate complex formation but only in the presence of two cellular proteins, Oct1 and the host cell factor HCF. VP16 alone cannot bind DNA efficiently instead its interaction with HCF primes it for further association on the VP16 cis-regulatory target. In case of transcription from the AcNPV very late promoters too, PPRP, a host cell transcription
factor, plays an important role but the transcription process definitely requires viral or virally-modified factors since it has been demonstrated that infection with the wild type AcNPV is an important step in very late promoter-mediated expression as seen in transient transfection experiments as well as in in vitro transcription experiments (Xu et al., 1995). At the same time PPBP constitutes an essential component since mutations in the PPBP binding motifs abolishes PPBP binding and consequent reporter gene expression (Chapter IV).

IMPORTANCE OF 5' UNTRANSLATED REGIONS IN CONTEXT OF PROMOTER FUNCTION

The untranslated leader regions of the baculovirus very late genes have been described to be essential for the very late burst in transcription (Possee and Howard, 1987; Weyer and Possee, 1988; Ooi et al., 1989). This region also contributes to the relative difference in the efficiency of expression between these two very late promoters (Roelvink et al., 1992) and also between the late and very late promoters (Weyer and Possee, 1988; Morris and Miller, 1994). Morris and Miller (1994) have proposed a model wherein they suggest that it is the affinity of the TAAG sequence, for certain yet unidentified late transcription factors, present at the transcription initiation site of late and very late promoters which determines the time difference between the two. The very late promoters have lower affinity TAAGs than the late promoters and thus they are not activated at late times post infection. They further suggest that the p10 TAAG has a somewhat higher affinity than the p29 TAAG and therefore, it is activated earlier than the polyhedrin promoter. In absence of any available data demonstrating direct binding of any late expression factors (lefs) to late or very late promoters, this model lacks supporting experimental evidence. While the affinity of PPBP to the TAAG and the surrounding sequences of both p10 and p29 promoters is similar (half-life of ~15 min), nonetheless it shows striking differences in terms of binding to the 5' untranslated leader sequences. Therefore, it is tempting to propose that the binding of PPBP with a higher affinity (half-life ~30 min) to the p10
5' non-translated leader sequence probably allows it to reach its activation peak earlier than the polyhedrin promoter.

SPECULATED ROLE OF PPBP ds AND ssDNA BINDING ACTIVITIES AT THE BACULOVIRUS INITIATOR ELEMENTS

The initiator element, in case of initiator promoters, is the critical component for transcription initiation (Weis and Reinberg, 1992). Initiator elements are susceptible to DNA melting as one of the initial steps in the process of onset of transcription (Goodrich and Tjian, 1994). In the case of the p29 initiator, PPBP specifically contacts the coding strand of the promoter. Mukherjee et al. (1995a) suggested that this activity must be important for promoter activation since it allows PPBP to maintain its position at the initiator inspite of promoter melting. They went on to further suggest PPBP as a novel transcriptional regulator since it probably regulates transcription through another level of control which involves regulatory proteins with dual ds and ssDNA binding activities. There are several examples, in literature, of transcription factors with dual ds and ssDNA binding activities. Some examples are the estrogen receptor element (Lannigan and Notides, 1989), muscle factor 3 and myoD (Santoro et al., 1991) and sterol regulatory element-binding factor (Stark et al., 1992). Some of these factors specifically bind a particular strand of DNA as in case of the p29 promoter, whereas such preference does not exist for others.

We have shown that the p10 initiator, though homologous to the p29 initiator, binds PPBP or PPBP-like host factor in both its single stranded and duplex form. This binding is also sequence specific since random sequence motifs cannot compete for the binding of the host factor. There are marked differences between the p29 and p10 activation profiles (Roelvink et al., 1992) though both follow the same general pattern in their expression kinetics. We have also observed variations with respect to PPBP affinity in their 5' non-coding regions which might be responsible for certain differences in the activation of the two promoters. However, the host factor's binding to the non-template strand may well be due to differences in the mechanisms involved in the activation of the two genes. It is possible that this
difference exists because of the interactive action of certain other factor(s). We have also observed the binding of another host factor of a much higher molecular weight to the regions upstream to the p10 initiator whereas the same 30-kDa factor binds the sequences upstream to the p29 initiator.

A similar situation exists in case of the class III 5S gene of *Xenopus* (Lassar et al., 1983). Factor A makes contacts with phosphate and guanine residues primarily on the non-coding strand of the promoter. This leads to the suggestion that factor A might transiently shift to the non-coding strand during transcription and this interaction is enhanced by factor C which thereby induces a stable "open" complex on the gene. Further, various classes of 5S RNA genes in *Xenopus* are differentially expressed since they are subject to regulation by both common and gene-specific (factor A) components. Exactly how these genes are differentially regulated is unclear, but it has been demonstrated (Bogenhagen et al., 1982) that this reflects the selective establishment and maintenance of stable complexes on one class of genes in somatic cells that contain limiting amounts of factor A. It is possible that there are some structural differences (bulky or charged groups) between the p10 and p29 initiators (p10 initiator is closer to the initiator consensus than the p29 initiator) which allow PPBP to specifically bind the coding strand on the p29 promoter and either of the two strands on the p10 promoter. Once PPBP has maintained the initiator region in the "melted" state, repeated rounds of transcription can take place at both the promoters of the two highly expressed genes.

Binding of PPBP to the non-coding strand is not a very peculiar phenomenon. Studies with the T7 RNA polymerase contributes substantially to the emerging picture that the non-template strand is an important element of the transcription elongation complex (Zhou et al., 1995). The melting of duplex DNA is accomplished by the formation of the transcription bubble, which is maintained throughout the entire transcription process (Yager and von Hippel, 1987). One component of this bubble is the non-template strand, but its role in transcription was not well understood. Several studies (Chamberlin and Berg, 1964; Milligan et al., 1987) suggested that it was dispensable for transcription. Recently, however, a role for the non-coding strand in
the regulation of elongation was suggested in the lambda phage (Ring and Roberts, 1994). In case of the T7 system, the non-template strand plays an important role in transcription bypass of template gaps i.e. the non-template strand appears to function as a guide to establish the coding register for a non contiguous template strand. These kind of gaps may also be the result of groups which hinder polymerase movement. It is suggested that such an interaction between the polymerase and the non-template strand may be a key component for efficient elongation for RNA polymerases in general.

We show that a PPBP or PPBP-like 30-kDa cellular factor also binds the p10 promoter and this binding involves motifs similar to the PPBP cognate sequences. Significant differences exist in the affinity and turnover of PPBP with respect to the 5' untranslated leader sequence of the two very late genes. Further, though both promoters exhibit duplex as well as single stranded DNA binding activities, marked differences in strand preference exist which are probably a reflection of their differential regulation. The observations presented in this chapter add yet another dimension to the spectrum of novelties of PPBP wherein it can distinguish between promoter differences and subsequently utilize different mechanisms to aid in open complex formation.