SUMMARY

It was previously shown that the Autographa californica nuclear polyhedrosis virus (AcNPV) polyhedrin gene TATA-less "initiator" promoter specifically binds a 30-kDa host factor, polyhedrin promoter binding protein or PPBP, through an octanucleotide motif (TAAGTATT) and a hexanucleotide motif (AATAAAA) spanning the transcription start site. PPBP exhibits unusual affinity, specificity and stability in binding to its cognate sequence motifs. A number of indirect evidences pointed to the possibility of its involvement in polyhedrin transcription. This study is an extended investigation on the mechanism of polyhedrin basal and activated transcription with respect to some of the host factors involved and their cognate sequence recognition motifs.

It was demonstrated that the host factor, PPBP can also specifically bind to another very late baculovirus promoter, the p10 promoter suggesting a similar mode of regulation for the baculovirus very late promoters. These two very late and hyperactive promoters were classified as "initiator" promoters as they require sequences around the transcription start point for basal promoter function and do not require upstream TATA box element. In addition, they show considerable similarity to the initiator consensus sequence, Py Py A+1 T/A N Py Py. PPBP exhibits significant differences in its binding to the 5' untranslated leader sequences of the two genes pointing to the importance of PPBP in very late burst of transcription, characteristic for such promoters. Further, the host factor binding the p10 promoter does not display ssDNA binding restricted to the coding strand alone unlike the polyhedrin promoter. A protein of much higher molecular mass could also bind to the sequence 5' to the p10 initiator.

In direct 'knock-out' experiments it was demonstrated that binding of PPBP to its cognate sequences was essential for transcription in vivo. Complementary oligonucleotides spanning the polyhedrin promoter with mutated PPBP cognate motifs, where either the hexa-motif was mutated to CCGCCC or both the hexa- and octa-motifs were mutated (GCCTGCGG in place of TAAGTATT), were synthesized
and cloned in appropriate plasmid vectors to drive the expression of the luciferase reporter gene. The mutant derivatives of the promoter failed to generate a PPBP-promoter complex in gel mobility shift assays. The recombinant plasmid constructs were used in a Lipofectin mediated luciferase-based \textit{in vivo} transient expression assay where luciferase expression levels were compared with the unmutated promoter control. Both the hexa-octa mutation construct and the hexa-mutation construct did not express luciferase above the cut-off limit as detected by the very sensitive luminometric assay, demonstrating that in the absence of binding of PPBP, due to binding sequence knock-outs, expression of polyhedrin driven reporter gene is not detected \textit{in vivo}. Fine mapping of the hexanucleotide motif by mutation analysis and its corresponding effect on expression was studied by focusing on the four bases (TAAA) of the hexa-motif, shared between polyhedrin and p10 promoters. Each of these bases were individually mutated and mutated versions were cloned to drive the luciferase gene. All of these mutations affected PPBP binding in a gel retardation assay. There was a drastic reduction in luciferase expression \textit{in vivo} with any of the mutations as compared to the unmutated promoter construct.

It was further demonstrated that PPBP binding to the initiator region of the polyhedrin promoter is independent of the TATA binding protein (TBP), a ubiquitous transcription factor which is known to directly bind the promoter and thus aid in transcription initiation complex assembly. However, TBP activity was identified in insect cell nuclear extracts which could specifically bind to a synthetic TFIID consensus sequence and could not be competed for by the PPBP binding sequence motifs. \textit{Sf9} TBP:TFIID complex had a distinct mobility than the classical PPBP complex in a gel retardation assay. Furthermore, affinity purified PPBP could not bind the polyhedrin initiator region. \textit{Sf9} TBP had a molecular mass of \textasciitilde30-kDa as seen in a Western blot using polyclonal antiserum against the highly conserved C-terminal domain of TBP. These experiments suggested that PPBP is an initiator binding protein and TBP, though distinct from PPBP, may nonetheless be involved in transcription through possible TBP-PPBP interaction(s).
The importance of context sequences on promoter function was also investigated. Plasmid constructs with increasing lengths of sequences upstream to the polyhedrin promoter displayed enhancement of luciferase expression over and above the basal expression construct lacking the upstream sequences, in transient expression assays. The absence of the hexa-motif within the initiator region could be buffered to a limited extent by upstream sequences. A GC-rich 30-mer stretch was identified within this upstream sequence which shared considerable homology to Sp1 consensus sequence which binds the eukaryotic cellular transcription activator, Sp1, known to be involved in initiator-mediated transcription initiation. An Sp1-like activity was identified in the insect cell nuclear extracts which could specifically bind to the consensus Sp1 sequence and also to the GC-rich region upstream to the polyhedrin promoter in the viral genome. This activity was distinct from PPBP and could not bind to PPBP cognate motifs. These results clearly point to the additional involvement of Sp1-like general transcription factor which binds to upstream sequences and consequently regulates transcription from the polyhedrin promoter possibly through an initiator independent fashion.

Summarizing the above data, a model involving PPBP, TBP and Sp1 in polyhedrin transcription was proposed. The initiator is recognized by the initiator binding protein PPBP to which TBP binds leading to the formation of the initiation complex assembly. Sp1 via tethering factors recognizes TBP at the initiation complex and acts as a coactivator of transcription. In absence of the initiator motif and consequently the initiator binding protein, Sp1 helps in recruiting TBP functionally at the start site and allows for basal levels of transcription.