Identification, characterization and functional studies of serine/threonine kinase of *Spodoptera litura* nucleopolyhedrovirus – I (*Splt*NPV-I)

**ABSTRACT**

An open reading frame (ORF) of 819 nt to code for a 272 amino acids protein is identified in the genome of *Spodoptera litura* nucleopolyhedrovirus (*Splt*NPV-I). Nucleotide and nucleotide sequence derived amino acid sequence analysis of this ORF suggested it to be a eukaryotic type protein kinase having conserved I-XI subdomains of Hanks kinase. In addition to kinase catalytic domains, this putative protein has two bromo-domains which could play regulatory role in transcription. The ORF expressed as ~31 kDa apoprotein in *E. coli* and ~33 kDa glycoprotein in S/9 cells, the expressed protein is designated as *Splt*NPV-I pk1 or pk1. The protein is localized in the nucleus of the *Splt*NPV-I infected permissive cell line NIV-HA-197. The recombinant protein has auto-phosphorylation and substrate phosphorylation (MBP and Histone H1) activities in presence of Mn$^{2+}$ or Mg$^{2+}$, and these activities are inhibited by staurosporine. Mutation of Lys-50 to Met but not Lys-44 to Gln of the protein abolished its kinase activity. Kinetics of pk1 showed that rate of phosphorylation of *Splt*NPV-I pk1> MBP> Histone H1, and both MBP and Histone H1 have the $K_m$ of 3 μM. Analysis of phosphorylated protein showed the phosphorylation of serine and threonine residues but not tyrosine. All these results suggested that identified *Splt*NPV-I ORF codes for a serine/threonine kinase.

*Polyhedrin (polh)* and *p10* are the two hyper-expressed very late genes of nucleopolyhedroviruses. Alpha amanitin resistant transcription from *Splt*NPV-I *polh* promoter occurred with virus infected nuclear extract of NIV-HA-197 cells but not with that from uninfected nuclear extract. Anti-pk1 antibody inhibited the transcription and the inhibition reversed on addition of pk1, however, pk1 mutant protein, K50M having no phosphorylation activity did not overcome the
transcription inhibition. Chromatin immuno-precipitation assays with viral anti-pk1 antibody showed the interaction of pk1 with the polh while electrophoretic mobility shift assays indicated the strong binding affinity (\(K_a \sim 5.5 \times 10^{11}\)) of purified pk1 with the polh promoter.

Very late gene transcription initiation complex of polh promoter of Autographa californica nucleopolyhedrovirus (AcNPV) was isolated by DNA affinity chromatography of nuclear extract of virus infected Sf9 cells. SDS-PAGE analysis of transcription initiation complex of polh promoter showed 32 kD AcNPV pk1 as its major component when probed with anti-pk1 antibody of AcNPV. Incubation of polh transcription initiation complex in kinase buffer with [\(^{γ-32}\)P]ATP indicated the phosphorylation of a 102 kD lef-8 like protein of the complex and auto-phosphorylation of pk1. Phosphorylation of 102 kD lef-8 like protein was progressively inhibited on addition of increasing amount of pk1 antibody confirming the phosphorylation of 102 kD protein by pk1.

The AcNPV pk1 was post-transcriptionally silenced in wild type AcNPV infected Sf9 cells when transfected with DNAzymes E1 and E2. The dose dependent inhibition of pk1 mRNA in E1 and E2 treated virus infected cells indicated the down regulation of pk1. Interestingly down regulation of pk1 by its specific DNAzymes also down regulated the AcNPV polh gene expression as well as polyhedrin promoter driven expression of green fluorescence protein (GFP) even though these DNAzymes did not cleave polyhedrin transcript in vitro.

All the above results suggested that the viral encoded pk1 acts as a transcription factor and regulate the transcription of baculovirus very late genes.