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Characterization of LEF4 ligand binding property and its role as part of baculoviral transcription machinery

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Abstract Late expression factor 4 (LEF4) is one of the four identified subunits of *Autographa californica* nucleopolyhedrosis virus (*Ac*NPV) encoded RNA polymerase that carries out transcription from viral late and very late promoters. This 464-amino acid baculovirus-encoded protein also harbors 5′ mRNA capping activity that includes RNA 5′ triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities. Hydrolysis of 5′ triphosphate RNA and free NTPs is metal ion dependent property of the protein. In the present communication, we describe the structural changes in the recombinant LEF4 protein following ligand binding. Metal ion binding causes some alteration in the conformation around aromatic amino acids whereas there is no effect on tryptophan fluorescence on GTP binding in absence and presence of metal ion. It is found that GTP and divalent cation cofactor produce some prominent changes in the secondary structure of the protein. Electrophoretic mobility shift assay (EMSA) shows that LEF4 is the probable factor that acts as anchor to dock the viral RNA polymerase on the very late *polyhedrin* promoter (Ppolh) facilitated by other factors.

Keywords LEF4 · *Ac*NPV · Ligand binding · EMSA · *Polyhedrin* promoter

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Introduction

The infection cycle of the *Autographa californica* nucleopolyhedrosis virus (*Ac*NPV) can be subdivided into three major phases of viral transcription: early, late, and very late [1, 2]. Early gene expression occurs after uncoating of the viral genome in the host cell nucleus and is mediated by host RNA polymerase II. By contrast, late transcription is mediated by an α-amanitin resistant, virus-encoded RNA polymerase that is detected after DNA replication begins [3, 4]. The baculovirus late and very late gene promoters are the initiator promoters where a short conserved sequence serves both as a promoter and as an initiator element. This conserved sequence element, (A/T/G) TAAG, along with the encompassing 18 bp region has been shown to be the minimal promoter determinant for basal transcription.

Several reports are available that describe the interplay between host and viral factors in regulating transcription from late and very late gene promoters. Burma et al. [5] identified and affinity-purified a 30-kDa cellular factor from *Sf*21 cells, *polyhedrin* promoter binding protein (PPBP) that specifically binds to transcriptionally important sequence motifs, AATAAA and TAAGTATT of the very late gene *polyhedrin* promoter (Ppolh). PPBP was

shown to exhibit single-stranded DNA-binding activity to the coding strand of the promoter that was transcriptionally important for the virus [6, 7]. Another host factor identified was the 38 kDa homologous region-1 (hr1) binding protein (hr1BP) that binds at multiple sites within the *Ac*NPV hr1 which acts as an enhancer for transcription and origin of replication [8–10]. Recently, Landais et al. [11] have found conserved binding sites for cellular bZIP family of transcription factors in the hr sequences. Cellular Sp family-like protein factors were reported to modulate transcription from Ppolh through a possible crosstalk with TFIID complex [12, 13]. Thus, these findings add weight to the emerging realization that host factors are indeed a critical component of transcription regulation of the Ppolh.

The viral RNA polymerase complex was suggested to consist of mainly four subunits LEF4, LEF8, LEF9, and p47 [14]. The inter-subunit interactions between different subunits of *Ac*NPV RNA polymerase were defined by Crouch et al. [15]. LEF8 and LEF9, each has predicted amino acid sequence motifs found in RNA polymerases, but no functional data are available for LEF8, LEF9, or p47 proteins [16, 17]. Studies have shown that the product of *lef4* is involved in 5′ RNA capping activity that involves several enzymatic reactions: (1) the removal of the terminal phosphate group from the triphosphate moiety at the 5′ end of mRNA by an RNA triphosphatase, (2) guanylation of mRNA 5′ end with GMP by GTP:RNA guanylyltransferase, and (3) methylation of guanine by guanine-7-methyltransferase [18]. It has been found that LEF4 protein has both RNA 5′ triphosphatase and guanylyltransferase activities in vitro [19–21]. LEF4 hydrolyses the β–γ phosphoanhydride bond of both the terminal phosphate group of RNA as well as of free NTPs. Recently another baculoviral protein with RNA triphosphatase activity called PTP/BVP is reported [22]. Apart from 5′ RNA capping activity associated with LEF4, recently our group has shown LEF4 to be a very stable and robust protein based on denaturation studies [23]. The comprehensive biophysical analysis of LEF4 demonstrates the highly stable nature of this protein, which is suggestive of one of the several strategies adapted by the virus to survive under very adverse environmental and physiological conditions.

Herein, we employ the biophysical approach to study the ligand binding properties of LEF4 protein as an attempt to study the structure–function relationship. This helps to study the ligand binding pockets on the protein molecule and the changes in the conformation of the protein that follow. Evidences are also presented to postulate that viral RNA polymerase is recruited to the promoter by LEF4 subunit.

Materials and methods

Materials

Magnesium chloride and manganese chloride were purchased from Sigma-Aldrich. GTP was from Amersham-Pharmecia. All other reagents were of analytical grade.

Cloning, expression, and purification of His-tagged LEF4

Lef4 gene was cloned under T7 promoter in pET28a vector (Invitrogen) and expressed with His-tag in *E. coli* Bl21 (DE3) cells [23]. The recombinant LEF4 was purified to homogeneity on TALON column (Clontech) in 20 mM Tris (pH 8.0) and 100 mM NaCl and used for the study.

Fluorescence studies

Fluorescence spectra were recorded with a Cary Eclipse-Fluorescence Spectrophotometer. For monitoring tryptophan fluorescence, protein excitation was done at 280 nm, and fluorescence emission was measured between 300 and 400 nm with 10 and 5 nm slit widths for excitation and emission, respectively. For the experiments, protein was taken at a concentration of 0.46 and 1.84 μM in 20 mM Tris (pH 8.0) and 50 mM NaCl.

Circular dichroism

CD studies were done on Jasco J-810 spectropolarimeter. Each spectrum is a result of five scans. Temperature was maintained at 30°C. Far-UV CD spectra were taken in the wavelength range of 200 and 250 nm at a protein concentration of 3.70 μM in a pathlength of 1 mm. Mean residual ellipticity (MRE) was calculated as shown by Hackeng et al. [24] using following equation:

$$\mathrm{MRE} = \theta_{\mathrm{obs}}/n \times l \times c \times 10$$

where θ_{obs} is observed ellipticity in millidegrees, n is number of aminoacid residues, l is pathlength in centimeters, and c is molar concentration.

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was performed as described earlier [12, 13] using purified recombinant LEF4 protein. Oligos containing the overlapping domains A, B, and C spanning 92 bp Ppolh were synthesized as described by Ghosh et al. [7].

Results and discussion

Based on sequence alignment studies of baculoviral LEF4 to the guanylyltransferases from fungi, chlorella virus, and African swine fever virus, it was inferred that LEF4 is a virus-encoded capping enzyme. The phosphate hydrolyzing activity of LEF4 was found to be metal-ion dependent [20, 21]. The homology model of *Bm*NPV LEF4, a counterpart of *Ac*NPV LEF4 which shares 97% homology with *Ac*NPV LEF4, is known [25]. It is shown that the polypeptide folds to give rise to two separate catalytic domains (N- and C-terminal) responsible for the triphosphatase and guanylyltransferase activities, respectively. It is known from the model that the inter-domain region contains most of the conserved residues and the GTP-binding motif. In view of the RNA 5′ triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities of LEF4 subunit of *Ac*NPV RNA polymerase, we have investigated the metal ion and GTP binding properties of the purified recombinant LEF4 protein using fluorescence and circular dichroism.

Metal ion binding to LEF4

The intrinsic fluorescence of a protein contributed by the aromatic residues is a good probe to measure the changes in the protein micro-environment. Quenching of intrinsic fluorescence can be used as a probe to check the relative exposure of fluorescent residues in a protein under a set of conditions. The 464-amino acid baculovirus LEF4 protein is a bi-functional mRNA capping enzyme with triphosphatase and guanylyltransferase activities. The hydrolysis of 5′ triphosphate RNA and free NTPs by LEF4 is intrinsically dependent on divalent cation cofactor, Mg^{2+} or Mn^{2+} [26]. In order to elucidate the nature of metal ion binding to LEF4, we had employed the endogenous tryptophan fluorescence to precisely quantitate the interaction of metal ions with the enzyme. A typical titration experiment using Mg^{2+} as the ligand is shown in Fig. 1. Here changes in the intrinsic fluorescence of LEF4 protein as a function of increasing concentration of Mg^{2+} were recorded in the absence and presence of 5 mM EDTA. It can be seen from the figure that addition of Mg^{2+} to LEF4 in the absence of the chelating agent did not lead to any significant change in fluorescence intensity. However, when LEF4 protein was extensively dialyzed against 5 mM EDTA and then studied for the effect of increasing concentration of Mg^{2+} ion, approximately 75% quenching of the intrinsic fluorescence was observed at 25 mM metal ion concentration (Fig. 1). It is to be noted that metal ion binding resulted in a decrease in fluorescence intensity but no effect was seen on the emission maximum of the protein (data not shown). This significant decrease in emission

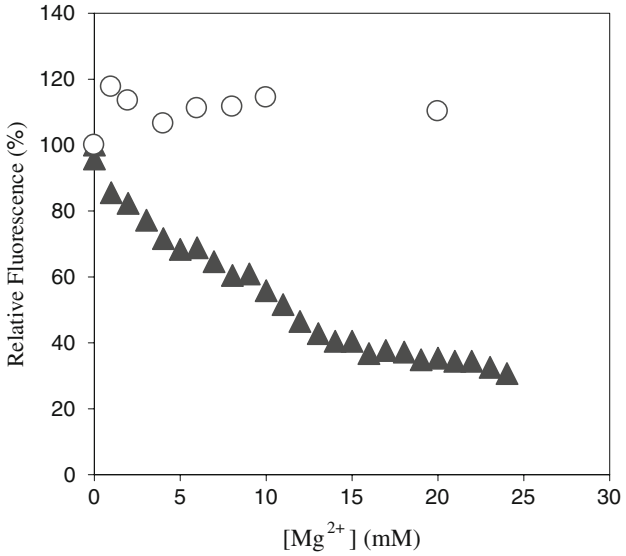

Fig. 1 LEF4 shows binding to metal ion. Changes in intrinsic fluorescence of LEF4 at 340 nm with increasing concentration of Mg^{2+} (0–25 mM) in absence (*open circle*) and presence (*filled triangle*) of 5 mM EDTA

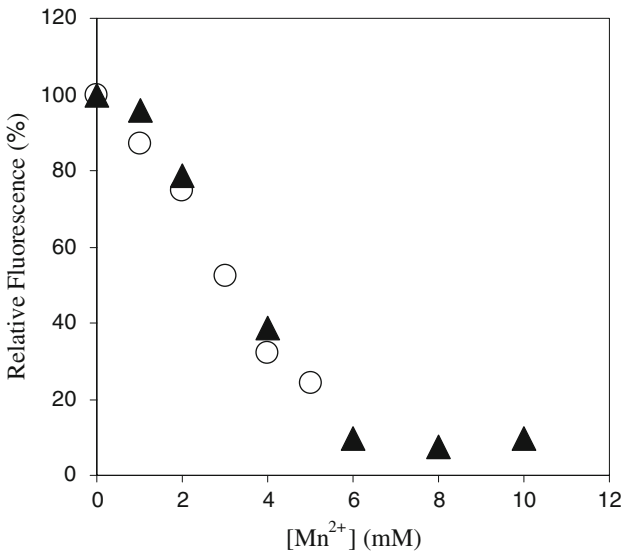

Fig. 2 Binding of Mn^{2+} is independent of Mg^{2+} binding to LEF4. Changes in intrinsic fluorescence of LEF4 at 340 nm with increasing concentration of Mn^{2+} (0–10 mM) in absence (*open circle*) and presence (*filled triangle*) of 0.5 mM Mg^{2+}

fluorescence intensity observed may be either due to a direct quenching from the aromatic side chains and/or due to altered microenvironment of aromatic residues (mostly tryptophan) in the protein on metal ion binding. Martins and Shuman [26] showed that the autonomous triphosphatase catalytic domain encompasses N-terminal 236 residues of 464 amino acid polypeptide encoded by the *Ac*NPV *lef*4 gene. There are two conserved glutamate-rich motifs A and C in the triphosphatase domain that are

involved in metal ion binding. These two conserved motifs consist of alternating glutamate residues interdigitated with alternating aliphatic or aromatic residues. These essential residues are conserved across baculoviral LEF4 orthologs including *Orgyia pseudotsugata* NPV (OpNPV), *Helicoverpa armigera* NPV (HaNPV), *Lymantria dispar* NPV (LdNPV), *Xestia c-nigrum granulovirus* (XcGNV), and *Plutella xylostella* granulovirus (PxGNV). This explains our observation that on metal ion binding to *Ac*NPV LEF4 there was a direct effect (quenching) on tryptophan fluorescence intensity (Figs. 1, 2). Moreover, it is clear that protein when purified in a metal ion bound form, additional metal ion cofactors did not lead to fluorescence quenching. Fluorescence quenching was observed only when protein was treated with the chelating agent before addition of metal ion cofactor. Here after all the experiments were done with EDTA-treated protein preparations. Similar effect on the intrinsic fluorescence due to the association of metal ion with the enzyme was observed in the case of Hepatitis C virus RNA polymerase [27].

RNA triphosphatase activity is optimal at pH 7.5 with either magnesium or manganese, however, NTP hydrolysis at neutral pH is activated only by manganese or cobalt [26]. Mapping of the triphosphatase active site of LEF4 by Martins and Shuman [28] suggests a two-metal mechanism of γ-phosphate hydrolysis. Hence, effect of increasing concentration of Mn^{2+} binding on EDTA-treated LEF4 protein fluorescence was examined in an analogous manner in the absence and presence of 0.5 mM Mg^{2+} (Fig. 2). For this LEF4 protein was extensively dialyzed against 5 mM EDTA and then studied for the effect of increasing concentration of Mn^{2+} ion in absence and presence of 0.5 mM Mg^{2+}. As clearly shown in the figure significant quenching of the intrinsic protein fluorescence was observed for Mn^{2+} ions, both in absence and presence of Mg^{2+} suggesting that Mg^{2+} does not interfere with the binding of Mn^{2+} to the protein. These results are in accordance with the earlier model [28] suggesting mutually exclusive binding sites for each metal ion.

To further analyze the conformational changes that may result due to interaction of metal ions with the protein, far-UV CD spectra of LEF4 in the absence and presence of metal ions were recorded. Circular dichroism measurements in the far-UV region (200–250 nm) yield data that may be equated with the general secondary folding of the protein molecule, i.e., amount of α-helix, β-sheets, β-turns, or aperiodic structure. Changes in far-UV CD spectrum were used to observe the effect of metal ion binding on secondary structural features of LEF4. In the far-UV region, LEF4 showed a CD spectrum indicating the presence of substantial secondary structure primarily β-sheets besides α-helices, turns, and aperiodic regions. Percent alpha helix evaluated is 14% of the total secondary conformations [23]. Figures 3 and 4 show a decrease in the MRE values at 208 nm as well as 217 nm for LEF4 on addition of increasing concentrations of Mg^{2+} (0–10 mM) (Fig. 3) and Mn^{2+} (0–4 mM) (Fig. 4) ions. Analysis of the far-UV CD spectra revealed that the binding of metal ions to LEF4 protein induces a modification of the secondary structure component of the protein. These modifications may reflect major domain rearrangements required for the formation of catalytically active site.

Nucleotide binding to LEF4

Having shown earlier guanylyltransferase activity and presence of GTP-binding site in the inter-domain region of LEF4 protein, effect on the relative fluorescence emission intensity of LEF4 protein in the presence of increasing molar ratio of GTP:protein was herein studied. As can be seen from the figure, an insignificant decrease in the fluorescence emission intensity was observed upto 4 M excess of GTP over protein (Fig. 5), however, no shift in wavelength of maximum emission was observed (data not shown). Moreover, when the protein was preincubated with 2 mM Mg^{2+} or Mn^{2+} followed by addition of GTP, minimal changes were observed in fluorescence intensity (data not shown). Thus, binding of cofactor GTP to the protein did not lead to gross conformational changes in the vicinity of aromatic amino acids.

To further characterize the interaction between GTP and LEF4 far-UV CD spectra were recorded both in the absence and presence of the nucleotide cofactor (Fig. 6). Addition of nucleotide (0–500 μM) to LEF4 reflected similar trend as metal ion.

Overall, the CD spectra suggest that rather than undergoing a subtle conformational change, the protein undergoes a radical modification of protein architecture upon binding

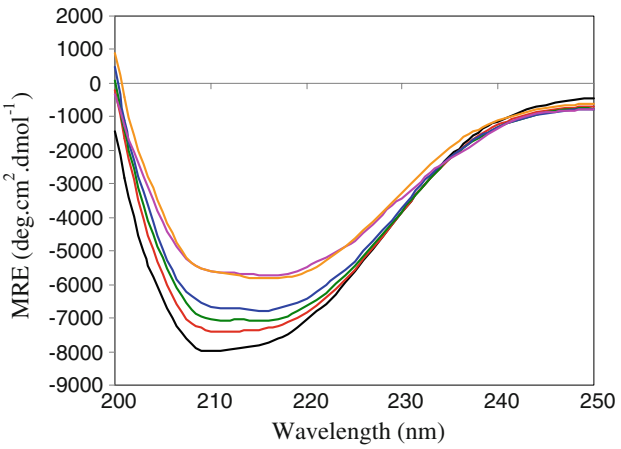

Fig. 3 Mg^{2+} induces changes in the secondary structure. Far-UV CD spectra of LEF4 protein with increasing concentration of Mg^{2+} [0 mM (*black*), 2 mM (*red*), 4 mM (*green*), 6 mM (*blue*), 8 mM (*pink*), and 10 mM (*orange*)]

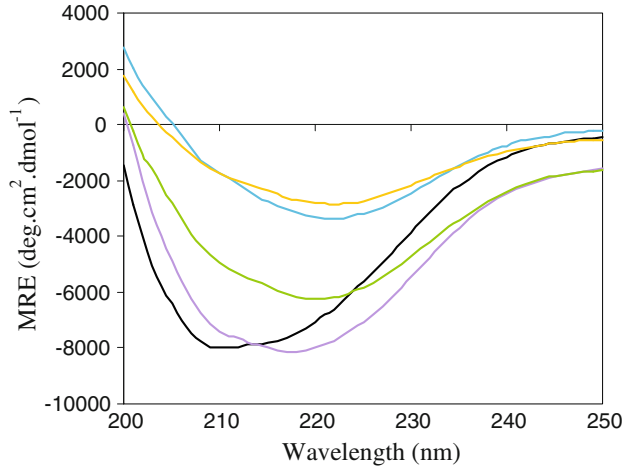

Fig. 4 Mn^{2+} induces changes in the secondary structure of LEF4. Far-UV CD spectra of LEF4 protein with increasing concentration of Mn^{2+} [0 mM (*black*), 0.5 mM (*purple*), 1 mM (*green*), 1.5 mM (*blue*), and 4 mM (*yellow*)]

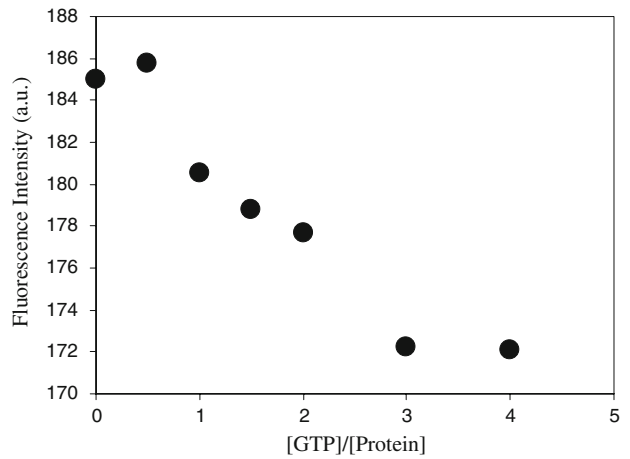

Fig. 5 Changes in the intrinsic fluorescence of LEF4 at 340 nm on GTP binding

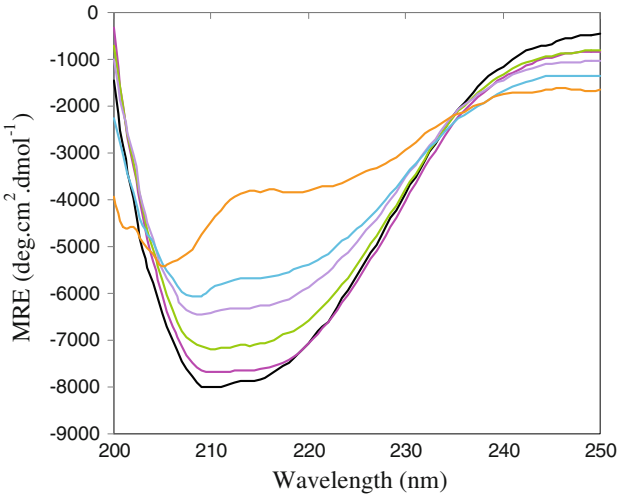

Fig. 6 GTP induces conformational changes. Far-UV CD spectra of LEF4 protein with increasing concentration of GTP [0 (*black*), 20 (*pink*), 50 (*green*), 100 (*purple*), 200 (*blue*), and 500 μM (*orange*)]

to metal ions or nucleotide. These conformational changes may possibly be required for the enzymatic activity of the protein. Analysis of the far-UV CD spectra reveals that the binding of Mg^{2+}/Mn^{2+}/GTP induces a significant modification of the secondary structure component of the protein. Six nucleotidyltransferase structural motifs form a GTP binding pocket in the crystal structure of Chlorella virus guanylyltransferase [29]. Assuming a similar pocket existed in LEF4, the profound effect of GTP binding on its secondary structure can be explained.

LEF4 recruits the viral RNA polymerase to the promoter

The only factor reported till date to specifically recognize the minimal Ppolh is the host factor—PPBP [5, 6]. It binds to AT-rich sequence motifs spanning Ppolh that are transcriptionally important. It binds to the hexanucleotide and octanucleotide motifs present in the minimal promoter. It is suggested to bind to specific sequences within the promoter, on one hand, and make contacts with the rest of the transcription machinery, on the other hand. This may help in the recruitment of RNA polymerase to the promoter. Lately a virus encoded protein kinase (pk-1) is reported as an integral component of the very late gene transcription initiation complex that associates with *polyhedrin* promoter and phosphorylates LEF8 RNA polymerase subunit as a mechanism to regulate transcription initiation [30]. However, additional factors may also participate in the recruitment of virus-encoded RNA polymerase to the promoter and its regulation. Till date no direct functional role has been defined for LEF4 as part of viral RNA polymerase complex. Thus, recombinant LEF4 was checked for any such role in Ppolh activation. The three domains [7] spanning the promoter: A (−93 to −52), B (−63 to −32) and C (−43 to −2) were checked for the binding with LEF4 factor by EMSA. It was observed that LEF4 binds very strongly to domain A, however, weak binding with domain B and C was observed (data not shown). Figure 7 shows LEF4-domain A complex on 5% native PAGE (Lane 2). Cold competition with all the three domains showed that the affinity of the factor for domain A is highest followed by that for domain C and least for domain B (Lanes 3–5). The specificity of DNA–protein complex was evident by the observation that no competition was observed when non-specific competitor pUC18 was used (Lane 6). Hence LEF4 may be the missing link between the promoter and the *Ac*NPV RNA polymerase. This highlights the essential central role of LEF4 factor in late gene expression as shown

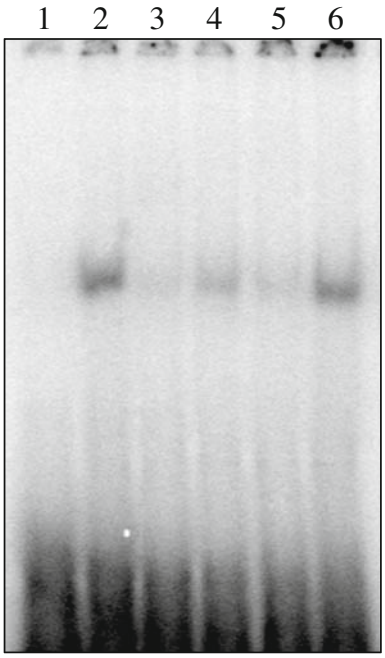

Fig. 7 LEF4 binds to *polyhedrin* promoter. Recombinant LEF4 protein binding to very late gene *polyhedrin* promoter of *Ac*NPV. Lane 1 is the free probe (p29A domain). Lane 2 is protein-DNA complex between recombinant LEF4 and p29A domain. Lanes 3–6 are cold competitions with ×200 excess of p29A, p29B, p29C, and pUC18

by the *lef4* gene knockout study by Knebel et al. [31]. Crouch et al. [15] suggest that the two LEF4 subunits in the RNA polymerase complex may have another function associated with transcription apart from RNA capping that positions the two subunits at different locations within the complex. Present study suggests that LEF4 being one of the RNA polymerase subunits may play a role to dock the transcription machinery on the promoter with the help of PPBP. Interaction of PPBP and LEF4 with specific motifs in Ppolh may be a strategy adopted by the virus for specific and strict transcription regulation.

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References

1. Glocker B, Hoopes RR Jr, Hodges L, Rohrmann GF (1993) In vitro transcription from baculovirus late gene promoters: accurate mRNA initiation by nuclear extracts prepared from infected *Spodoptera frugiperda* cells. J Virol 67:3771–3776
2. Huh NE, Weaver RF (1990) Identifying the RNA polymerase that synthesize specific transcripts of *Autographica californica* nuclear polyhedrosis virus. J Gen Virol 71:195–199
3. Fuchs LY, Woods MS, Weaver RF (1983) Viral transcription during AcNPV infection: a novel RNA polymerase induced in infected *Spodoptera frugiperda* cells. J Virol 48:641–646
4. Beniya H, Funk CJ, Rohrmann GF, Weaver RF (1996) Purification of a virus-encoded RNA polymerase from *Autographica californica* nuclear polyhedrosis virus-infected *Spodoptera frugiperda* cells that accurately initiates late and very late transcription in vitro. Virology 216:12–19
5. Burma S, Mukherjee B, Jain A, Habib S, Hasnain SE (1994) An unusual 30-kDa protein binding to the polyhedrin gene promoter of *Autographa californica* nuclear polyhedrosis virus. J Biol Chem 269:2750–2757
6. Mukherjee B, Burma S, Hasnain SE (1995) The 30-kDa protein binding to the "Initiator" of the baculovirus polyhedrin promoter also binds specifically to the coding strand. J Biol Chem 270: 4405–4411
7. Ghosh S, Jain A, Mukherjee B, Habib S, Hasnain SE (1998) The host factor polyhedrin promoter binding protein (PPBP) is involved in transcription from the baculovirus polyhedrin gene promoter. J Virol 72:7484–7493
8. Habib S, Hasnain SE (1996) A 38-kDa host factor interacts with functionally important motifs within the *Autographa californica* multinucleocapsid nuclear polyhedrosis virus homologous region (hr1) DNA sequence. J Biol Chem 271:28250–28258
9. Habib S, Pandey S, Chatterji U, Burma S, Ahmad R, Jain A, Hasnain SE (1996) Bifunctionality of the homologous region sequence (hr1); enhancer and ori functions have different sequence requirements. DNA Cell Biol 15:737–747
10. Viswanathan P, Venkaiah B, Kumar MS, Rasheedi S, Vrati S, Bashyam MD, Hasnain SE (2003) The homologous region sequence (hr1) of *Autographa californica* multinucleocapsid polyhedrosis virus can enhance transcription from non-baculoviral promoters in mammalian cells. J Biol Chem 278:52564–52571
11. Landais I, Vincent R, Bouton M, Devauchelle G, Duonor-Cerutti M, Ogliastro M (2006) Functional analysis of evolutionary conserved clustering of bZIP binding sites in the baculovirus homologous regions (hrs) suggests a cooperativity between host and viral transcription factors. Virology 344:421–431
12. Ramachandran A, Jain A, Arora P, Bashyam MD, Chatterjee U, Ghosh S, Parnaik VK, Hasnain SE (2001) Novel Sp family-like transcription factors are present in adult insect cells and are involved in transcription from the polyhedrin gene initiator promoter. J Biol Chem 276:23440–23449
13. Rasheedi S, Ramachandran A, Ehtesham NZ, Hasnain SE (2007) Biochemical characterization of *Sf*9 Sp-family-like protein factors reveals interesting features. Arch Virol 152:1819–1828
14. Guarino LA, Xu B, Jin J, Dong W (1998) A virus-encoded RNA polymerase purified from baculovirus-infected cells. J Virol 72:7985–7991
15. Crouch EA, Cox LT, Morales KG, Passarelli AL (2007) Intersubunit interactions of the *Autographa californica* M nucleopolyhedrovirus RNA polymerase. Virology 367:265–274
16. Passarelli AL, Todd JW, Miller LK (1994) A baculovirus gene involved in late gene expression predicts a large polypeptide with a conserved motif of RNA polymerases. J Virol 68:4673–4678
17. Lu A, Miller LK (1994) Identification of three late expression factor genes within the 33.8- to 43.4-map-unit region of *Autographica californica* nuclear polyhedrosis virus. J Virol 68:6710–6718
18. Shuman S (2000) Structure, mechanism, and evolution of the mRNA capping apparatus. Prog Nucleic Acid Res Mol Biol 66:1–40
19. Guarino LA, Jin J, Dong W (1998) Guanylyltransferase activity of the LEF-4 subunit of baculovirus RNA polymerase. J Virol 72:10003–10010
20. Jin J, Dong W, Guarino LA (1998) The LEF4 subunit of baculovirus RNA polymerase has RNA 5′-triphosphatase and ATPase activities. J Virol 72:10011–10019
21. Gross CH, Shuman S (1998) RNA 5′-triphosphatase, nucleoside triphosphatase, and guanylyltransferase activities of baculovirus LEF-4 protein. J Virol 72:10020–10028

22. Li Y, Guarino LA (2008) Roles of LEF-4 and PTP/BVP RNA triphosphatases in processing of baculovirus late mRNAs. J Virol 82:5573–5583
23. Rasheedi S, Suragani M, Haq SK, Ghosh S, Ehtesham NZ, Hasnain SE (2009) Biophysical characterization and unfolding of LEF4 factor of RNA polymerase from *Ac*NPV. Biopolymers 91:574–582
24. Hackeng TM, Fernández JA, Dawson PE, Kent SBH, Griffin JH (2000) Chemical synthesis and spontaneous folding of a multi-domain protein: anticoagulant microprotein S. Proc Natl Acad Sci USA 97:14074–14078
25. Sehrawat S, Srinivasan N, Gopinathan KP (2002) Functional characterization and structural modelling of late gene expression factor 4 from *Bombyx mori* nucleopolyhedrovirus. Biochem J 368:159–169
26. Martins A, Shuman S (2001) Mutational analysis of baculovirus capping enzyme Lef4 delineates an autonomous triphosphatase domain and structural determinants of divalent cation specificity. J Biol Chem 276:45522–45529
27. Bougie I, Charpentier S, Bisaillon M (2003) Characterization of the metal ion binding properties of the Hepatitis C virus RNA polymerase. J Biol Chem 278:3868–3875
28. Martins A, Shuman S (2003) Mapping the triphosphatase active site of baculovirus mRNA capping enzyme LEF4 and evidence for a two-metal mechanism. Nucleic Acids Res 31:1455–1463
29. Hakansson K, Doherty AJ, Shuman S, Wigley DB (1997) X-ray crystallography reveals a large conformational change during guanyl transfer by mRNA capping enzymes. Cell 89:545–553
30. Mishra G, Chadha P, Das RH (2008) Serine/threonine kinase (pk-1) is a component of *Autographa californica* multiple nucleopolyhedrovirus (*Ac*MNPV) very late gene transcription complex and it phosphorylates a 102 kDa polypeptide of the complex. Virus Res 137:147–149
31. Knebel-Mőrsdorf D, Quadt I, Li Y, Montier L, Guarino LA (2006) Expression of baculovirus late and very late genes depends on LEF-4, a component of the viral RNA polymerase whose guanyltransferase function is essential. J Virol 80:4168–4173