VIROLOGICA SINICA, August 2009, 24 (4):315-322
DOI 10.1007/s12250-009-3054-z

CLC number: R373 Document code: A Article ID: 1674-0769 (2009) 04-0315-08

Molecular Dissection of *Bombyx mori* Nucleopolyhedrovirus *orf8* Gene

WonKyung Kang**

(Molecular Entomology Laboratory, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan)

Abstract: Viruses including baculoviruses are obligatory parasites, as their genomes do not encode all the proteins required for replication. Therefore, viruses have evolved to exploit the behavior and the physiology of their hosts and often coevolved with their hosts over millions of years. Recent comparative analyses of complete genome sequences of baculoviruses revealed the patterns of gene acquisitions and losses that have occurred during baculovirus evolution. In addition, knowledge of virus genes has also provided understanding of the mechanism of baculovirus infection including replication, species-specific virulence and host range. The Bm8 gene of *Bombyx mori* nucleopolyhedrovirus (NPV) and its homologues are found only in group I NPV genomes. The *Autographa californica* NPV Ac16 gene is a homologue of Bm8 and, encodes a viral structural protein. It has been shown that Bm8/Ac16 interacts with baculoviral and cellular proteins. Bm8/Ac16 interacts with baculoviral IE1 that is facilitated by coiled coil domains, and the interaction with IE1 is important for Bm8 function. Ac16 also forms a complex with viral FP25 and cellular actin and associates with membranes via palmitoylation. These data suggested that this gene family encodes a multifunctional protein that accomplishes specific needs of group I NPVs.

Key words: Group I NPV; Bm8; Early gene; Structural protein; Nuclear localization

The *Baculoviridae* is a large family of viruses that infect arthropods, particularly insects of the order Lepidoptera, Diptera, and Hymenoptera. Baculoviruses contain circular double-stranded DNA genomes of 80 to 180 kb, which are contained within an enveloped, rod-shaped virion. The family *Baculoviridae* is divided into four genera based on the infected host larva: the *alphabaculovirus* are lepidopteran-specific nucleopolyhedroviruses (NPVs), the *betabaculovirus* are lepidopteran-specific granuloviruses, the *gammabaculovirus* are hymenopteran-specific NPVs and the *deltabaculovirus* are dipteran-specific NPVs The lepidopteran NPVs can be further subdivided into groups I and II based on phylogenetic studies (15). The group I NPVs include *Autographa californica* multiple NPV (AcMNPV) and *Bombyx mori* NPV (BmNPV). Both viruses are models for studies on basic baculovirology and application of baculoviruses as gene expression vectors and insecticide.

Since the genome sequence of AcMNPV was reported

Received: 2009-01-31, Accepted: 2009-05-07
** Corresponding author.
Phone: +81-48-467-9584, Fax: +81-48-462-4678,
E-mail: wkkang@riken.jp

for the first among baculoviruses, the number of complete sequences has grown rapidly and current database contains the sequences of 48 baculovirus genomes including 12 group I NPVs. These demonstrated that there are 30 conserved core genes present in all baculoviruses and 17 genes present only in group I NPVs (16). A signature gene among these unique to group I NPV is *gp64/gp67*. A distinguishing difference between group I and II is that group II NPVs utilize an ancient fusion homologue for cell-to-cell spread and this fusion activity is replaced by *gp64* in group I NPVs. Therefore, it has been suggested that acquisition of these unique genes promoted the diversification of group I NPVs and may contribute to baculovirus speciation by causing alterations in host range (16). The *orf8* gene (Bm8) of BmNPV is one of such gene found in only and all group I NPVs sequenced to date. In this review, I summarize current knowledge of this gene family that was obtained mostly from AcMNPV and BmNPV.

CHARACTERISTICS AND DIVERSITY OF Bm8 GENE AND ITS HOMOLOGUES

Table 1 summarizes the information on Bm8 and its homologues from group I NPVs. The deduced amino acid sequence of Bm8 showed significantly high identity (93 to 96 %) to that of AcMNPV, *Rachiplusia ou* (Ro) MNPV, and *Plutella xylostella* (Plxy) NPV homologues. On the other hand, it showed lower identity (approximately 30 %) to the rest. A phylogenetic tree of deduced amino acid sequences of this gene family also showed that Bm8 was closely related to the homologues from AcMNPV, BmNPV, RoMNPV, *Maruca vitrata* (Mavi) MNPV, and to lesser extent to those from the other viruses (Fig. 1). This phylogenetic tree showed two monophyletic clades; Bm8 was found in a clade B including well-characterized species such as AcMNPV and RoMNPV. The genes in this clade seem to be very closely related to each other except for the homologue from Mavi-MNPV. The other clade A included *Epiphyas postvittana*

Table 1. List of BmNPV *orf8* and its homologues

Virus	ORF no.	aa	Identity (%) to		Coiled coil[a]	Sequence accession Number	References
			Bm8	Op15			
AcMNPV	16	225	96	33	+	L22858	2
BmNPV	8	229		30	+	L33180	11
MaviNPV	8	209	70	34	+	EF125867	8
PlxyMNPV	16	74	95	21	-	DQ457003	14
RoMNPV	15	225	93	36	+	AY145471	13
AgMNPV	17	215	31	59	+	DQ813662	28
AnpeNPV[b]	134 (15)	197	33	59	+	EF207986	10
						(DQ486030)	(26)
AnpeNPV-S		181	33	58	+	AY846867[c]	
CfDEFNPV	15	215	31	62	+	AY327402	22
CfMNPV	15	197	28	70	+?	AF512031	9
EppoMNPV	13	204	33	58	+	AY043265	17
HycuNPV	137	127	25	66	+	AP009046	18
OpMNPV	15	197	30		+	U75930	1

[a] Coiled coil domain in N-terminal region. [b] There are two genomic sequences of AnpeNPV Liaoning strain reported. The amino acid sequence of orf134 (EF207986) is identical to that of orf15 (DQ486030). [c] This is a partial sequence of AnpeNPV Shenyang strain.

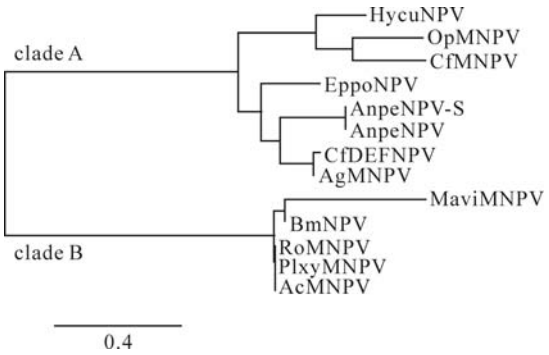

Fig. 1. Phylogeny of Bm8 and its homologues.

(Eppo) NPV, *Choristoneura fumiferana* (Cf) MNPV, *C. fumiferana* DEF (CfDEF) MNPV, and *Orgyia pseudotsugata* (Op) MNPV as prominent species. The amino acid identity between the genes in this clade was not so high (50 to 70 %), suggesting that the genes in clade A are more diverse than the genes in clade B.

Most of Bm8 homologues were predicted to encode proteins of approximately 200 amino acids (aa) except for Plxy16 and Hycu137 (Table 1). The Plxy16 ORF contains only 74 aa and was approximately 150 aa smaller than others. Harrison and Lynn (14) suggested that this truncation was due to a single base mutation and that the virus with this truncation is maintained in larvae. In addition, the predicted amino acid sequences of flaking small ORFs did not exhibit any similarity to other known proteins including Bm8 (14). On the other hand, Hycu137 includes 127 aa, which are approximately 100 aa shorter than the other homologues. This truncation was due to a single base insertion that was confirmed by re-sequencing of amplified Hycu137 region from larval stock (personal communication with M. Ikeda). However, the flanking ORF (Hycu136 containing 72 aa) also showed significant similarity to the C-terminal region of Bm8 (18). Therefore, there is a possibility that Hycu136 corresponds to the truncated C-terminus of Hycu137.

It has been shown that N-terminal region of Bm8 is important for its function and contains a coiled coil domain (19, 20). Prediction programs of coiled coil domains revealed that most of Bm8 homologues contain a coiled coil domain in N-terminal region (between 60 to 110 aa) except for Plxy16 (Table 1). The Plxy16 ORF may be too short to contain this motif since it encodes only 74 aa. The Cf15 ORF exhibited a low level of probabilities for coiled coil prediction, however, the protein encoded by Cf15 may contain a coiled coil domain because the sequence of around this region in Cf15 was aligned well with others.

CONFLICTING OBSERVATIONS SURROUNDING Ac16 AND Bm8

The analyses on this gene family were obtained mainly from AcMNPV and BmNPV, but included conflicting observations regarding phenotypes of mutant viruses and the feature of protein encoded by Bm8 or Ac16. It was initially shown that Ac16 played a role in late gene expression (12). However, another report demonstrated that the disruption of Ac16 did not delay the expression of late genes and that Ac16 was not essential for AcMNPV replication (29). There was some doubt when it was suggested that Bm8 was essential for viral replication since a null mutant virus was not isolated (19). Recently, Nie *et al.* demonstrated that the deletion of Ac16 does not affect either BV production or viral DNA replication but does affect the relative expression level of IE0 to IE1 using knockout and repair bacmids (25). Therefore, it appears that Bm8 is essential for BmNPV replication while Ac16 is not essential for AcMNPV replication even though these two share 96 % identity. This may be true and the difference may be due to adaptation to

different hosts. In support of this, PlxyNPV seem to replicate productively with truncated Plxy16. This also implies that Ro15 may be not be essential either since these three viruses are very closely related (Fig. 1). Even though I believe that the deletion mutagenesis of Bm8 was properly done, further studies including construction of knockout bacmids of Bm8 and other homologues should provide a clear more definitive answers.

The Ac16 gene was reported to encode an envelope protein of BV and ODV while it was shown that Bm8 was not a structural protein either of BV or ODV (3, 19). However, recent report settled this question. Burks *et al.* pointed out that all the conclusions about Ac16 and Bm8 proteins were based on antibody-generated data and there was a notable difference in the production of these antibodies, that is, the source of antigen. The Ac16 protein was expressed in baculovirus infected cells while recombinant Bm8 protein was expressed in *E. coli*, and consequently the antiserum against each antigen was capable of recognizing different unique epitope. They actually demonstrated that multiple isoforms of Ac16-encoded protein are present in infected cells, and these could be discriminated either by different antibodies raised against virally or bacterially expressed Ac16 (7). Therefore, the conflicting observations on Ac16 and Bm8 proteins is now considered a matter of detection ability of each antiserum, suggesting that Bm8 may encode an envelope protein of BV and ODV.

EXPRESSION OF Bm8 AND Ac16 DURING INFECTION

It was initially reported that the Ac16 gene is transcribed as an early gene (29). Primer extension analysis demonstrated that transcripts of Ac16 are abundant between 6 and 12 h p.i. and are also present at 48 and 72 h p.i., suggesting that Ac16 is transcribed both early and late during AcMNPV infection. In support of this, the 5' ends of the Ac16 transcripts were mapped to the regions containing early/late gene motifs, CAGT and TAAG (7). I also noticed that the Bm8 gene can be expressed solely by the host transcription machinery and that the expression can be enhanced by the presence of IE1 from the transient expression assay using the reporter gene *cat* under the control of Bm8 gene promoter region (unpublished data).

Analyses using antiserums revealed that both Bm8 and Ac16 encode a protein of 26 kDa, consistent with the predicted molecular mass of Ac16 and Bm8 (25 909 and 26 199 Da, respectively). Western blot analysis using anti-ORF8 antiserum raised against bacterially expressed Bm8 protein detected a 26 kDa protein by 4 h p.i. The Bm8 protein was detected only from the nuclear fraction of BmNPV infected cells and maintained at relatively constant levels from 4 to 24 h p.i. (19). On the other hand, the antibodies against virally expressed Ac16 (anti-E26) predominantly recognized a polypeptide of 26 kDa and other proteins (18 to 34 kDa) at later infection times (3). Burks *et al.* verified that at least two forms of Ac16- encoded protein are present in AcMNPV infected cells (7). One form migrates at 26 kDa and is recognized only by anti-E26. The expression of this form occurs by 4 h p.i., accumulates to high levels by 16 h p.i. and remains at high levels through 96 h p.i. In addition, it was shown that this 26 kDa protein is a structural protein of the envelope of BV and ODV, so it was named BV/ODV-E26 (3). The other form of Ac16-

encoded protein migrates at 33 kDa, is recognized by antiserum against bacterially produced Ac16 and is also expressed from early stage of infection. It was also shown that anti-ORF8 antiserum was able to detect the 33 kDa form, but not the 25 kDa form (7).

INTRACELLULAR LOCALIZATION OF Bm8 AND Ac16

As described above, Bm8 is a nuclear protein. It localizes specifically in the nucleus and accumulates as distinct foci in the early phase. The foci increase in size as infection progresses and eventually occupying most of the nucleus in the late phase. Moreover, Bm8 colocalizes with IE1 in these distinct nuclear foci throughout infection (19). Further investigation using *gfp* fused with Bm8 demonstrated that a direct interaction between Bm8 and IE1 is essential for mediating the nuclear localization of Bm8 and that the localization of Bm8 to specific nuclear sites requires *hr* elements similar to IE1 (20).

On the other hand, the localization of Ac16 changes temporally during AcMNPV infection. At early stage (1-6 h p.i.), Ac16 protein distributes in punctuate foci both in the cytoplasm and nucleus that may represent chromatin-like regions and membrane vesicles. At 8-12 h p.i., Ac16 accumulates within distinct foci in the nucleus and this pattern of localization resembles that of Bm8. At 16 h p.i., the dominant pattern changes again from virogenic stroma to cytoplasmic and nuclear membranes and foci of viral induced microvesicles (3, 7). This change in localization is due to the presence of multiple isoforms, the 26 kDa isoform may be responsible for the localization in membranes and microvesicles while the 33 kDa isoform may be responsible for the accumulation in virogenic stroma. Furthermore, the isoform of 26 kDa associates with intracellular membranes and this association is mediated via palmitoylation. Burks *et al.* suggested that palmitoylation enables the Ac16 protein go through dynamic alterations including membrane association property or different subcellular localizations (7).

INTERACTION WITH OTHER PROTEINS

The Ac16 or Bm8 proteins interact with other proteins that seem to be important for their function. The Bm8 interacts with IE1 and Ac16 also interacts with IE1 and/or IE0 (5, 20, 25). The region in Bm8 or IE1 protein that is responsible for the interaction has been investigated. Using a yeast two-hybrid assay, it was demonstrated that the N-terminal region containing the coiled-coil domain of Bm8 is required for the interaction with IE1. Furthermore, this region is sufficient for the localization to specific nuclear sites in the presence of IE1 and *hr3*, suggesting that N-terminus containing coiled coil domain is important for Bm8 function. On the other hand, Ac16 binding domain was mapped to amino acid residues 126-153 and 72-99 of IE0 or IE1 respectively and this Ac16 binding domain of IE0 or IE1 also contains a coiled coil domain. These suggest that coiled coil domains facilitate the interaction between Bm8/Ac16 and IE0 or IE1.

In addition to IE0/IE1, other proteins were identified as Ac16 interacting partners. Immunoprecipitation and yeast two-hybrid assays demonstrated that Ac16 and FP25 (Ac61) of AcMNPV are capable of forming a complex and cellular actin is also a component of this complex (3). In addition, Braunagel *et al.* demonstrated that Ac16 and FP25 associate with the viral

inner nuclear membrane sorting motif derived from envelope protein ODV-E66 (Ac46) (4). The cellular protein importin-α-16 was detected from an immunoprecipitation assay using antibodies raised against bacterially expressed Ac16 (31). In addition, Ac16 showed colocalization with importin-α-16 (6). However, the interaction between these two still remains unclear since it is likely that Ac16 and importin-α-16 only share epitopes (31).

POSSIBLE FUNCTIONS OF Bm8/Ac16 AND REMAINING QUESTIONS

Although there is yet disagreement on the phenotypes of null mutants, accumulated data to date suggest that Bm8/Ac16 protein may be a multifunctional protein which plays important roles in baculovirus infection. Firstly, the colocalization with IE1 suggests that Bm8/Ac16 may play a role in viral DNA replication and/or transcription. Some reports from our lab and others suggest that transcription and replication of baculoviruses take place in specific regions of nucleus as other eukaryotic DNA viruses (23, 24, 27). Okano *et al.* demonstrated that DNA replication of BmNPV occurs at specific sites in the nucleus of BmN cells and that IE1 initially forms foci in the nucleus, some other replication factors like DBP and LEF3 then associate with these foci that gradually enlarge as the infection proceeds (27). Therefore, the colocalization with IE1 suggests that Bm8 may be involved in DNA replication. In addition, Bm8 colocalizes with IE1 from 4 h p.i., before the onset of DNA replication, until 20 h p.i. which is after DNA replication ceases (19). The replication factor, DBP does not colocalize with IE1 either before or after the DNA replication while transcriptional factor such as LEF3 colocalizes with IE1 after DNA replication but not before the onset of replication (27). Baculovirus IE1 is essential not only for viral DNA replication but also for transcription of viral genes (21, 30), thus it is possible that Bm8 is also involved in transcription. In support of this, there are some observations that Ac16 functions to regulate gene late gene expression in cooperation with Ac18 in AcMNPV and that Ac16 may be involved in regulation of IE0 expression (12, 25). The ability of Bm8 to bind nucleic acids also supports a role for Bm8 in DNA replication or transcription. However, the specific role in DNA replication and transcription still remains to be determined.

The Ac16 has a second functional activity that is associated with membranes. During infection, ODV envelope proteins integrate into endoplasmic reticulum membranes and traffic to the inner nuclear membrane (INM) where microvesicles are induced. These virally induced intranuclear microvesicles eventually become the viral envelope. The N-terminal amino acid sequence sufficient to direct proteins to the INM has been identified from viral envelope protein ODV-E66 and named the viral inner nuclear membrane sorting motif (INM-SM) (4). Chemical crosslinking studies showed that Ac16 and FP25 are associated with the viral INM-SM derived from ODV-E66 (4). Moreover, Ac16 is a structural protein of BV and ODV, associates with intracellular membranes via palmitoylation, and forms a complex with FP25 and cellular actin. Therefore, it was suggested that Ac16 facilitates the trafficking of some ODV envelope proteins to the nuclear envelope by interacting with cellular motors (4, 6, 30).

Studies on Bm8 and Ac16 to date have revealed that Bm8 gene family encodes a multifunctional protein which accomplishes specific needs of group I

NPVs. However, the specific roles and mechanism in suggested functions still remain unclear. Further analyses including construction of knockout bacmids of Bm8 and other homologues, identification of some other interacting proteins, and analyses of interaction with such proteins *in vivo* and *in vitro* should advance better understanding of Bm8 gene family.

References

1. **Ahrens C H, Russell R L, Funk C J, *et al.*** 1997. The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. **Virology,** 229: 381-399.
2. **Ayres M D, Howard S C, Kuzio J, *et al.*** 1994. The complete DNA sequence of *Autographa californica* nuclear polyhedrosis virus. **Virology**, 202: 586-605.
3. **Beniya H, Braunagel S C, Summers M D.** 1998. *Autographa californica* nuclear polyhedrosis virus: subcellular localization and protein trafficking of BV/ODV-E26to intranuclear membranes and viral envelopes. **Virology,** 240 (1): 64-75.
4. **Braunagel S C, Williamson S T, Saksena S, *et al.*** 2004. Trafficking of ODV-E66 is mediated via a sorting motif and other viral proteins: facilitated trafficking to the inner nuclear membrane. **Proc Natl Acad Sci USA,** 101: 8372-8377.
5. **Braunagel S C, Summers M D.** 2007. Molecular biology of the baculovirus occlusion-derived virus envelope. **Curr Drug Targets,** 10: 1084-1095.
6. **Braunagel S C, Cox V, Summers M D.** 2009. Baculovirus data suggest a common but multifaceted pathway for sorting proteins to the inner nuclear membrane. **J Virol,** 83: 1280-1288.
7. **Burks J K, Summers M D, Braunagel S C.** 2007. BV/ODV-E26: a palmitoylated, multifunctional structural protein of *Autographa californica* nucleopolyhedrovirus. **Virology,** 361 (1): 194-203.
8. **Chen Y R, Wu C Y, Lee S T, *et al.*** 2008. Genomic and host range studies of *Maruca vitrata* nucleopolyhedrovirus. **J Gen Virol,** 89: 2315-2330.
9. **de Jong J G, Lauzon H A, Dominy C, *et al.*** 2005. Analysis of the *Choristoneura fumiferana* nucleopolyhedrovirus genome. **J Gen Virol,** 86: 929-943.
10. **Fan Q, Li S, Wang L, *et al.*** 2007. The genome sequence of the multinucleocapsid nucleopolyhedrovirus of the Chinese oak silkworm *Antheraea pernyi*. **Virology,** 366: 304-315.
11. **Gomi S, Majima K, Maeda S.** 1999. Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. **J Gen Virol,** 80: 1323-1337.
12. **Guarino L A, Summers M D.** 1988. Functional mapping of *Autographa californica* nuclear polyhedrosis virus genes required for late gene expression. **J Virol,** 62: 463-471.
13. **Harrison R L, Bonning B C.** 2003. Comparative analysis of the genomes of *Rachiplusia ou* and *Autographa californica* multiple nucleopolyhedroviruses. **J Gen Virol,** 84: 1827-1842.
14. **Harrison R L, Lynn D E.** 2007. Genomic sequence analysis of a nucleopolyhedrovirus isolated from the diamondback moth, *Plutella xylostella*. **Virus Genes,** 35: 857-873.
15. **Herniou E A, Luque T, Chen X, *et al.*** 2001. Use of whole genome sequence data to infer baculovirus phylogeny. **J Virol,** 75: 8117-8126.
16. **Herniou E A, Olszewski J A, Cory J S, *et al.*** 2003. The genome sequence and evolution of baculoviruses. **Annu Rev Entomol,** 48: 211-234.
17. **Hyink O, Dellow R A, Olsen M J, *et al.*** 2002. Whole genome analysis of the *Epiphyas postvittana* nucleopolyhedrovirus. **J Gen Virol,** 83: 957-971.
18. **Ikeda M, Shikata M, Shirata N, *et al.*** 2006. Gene organization and complete sequence of the *Hyphantria cunea* nucleopolyhedrovirus genome. **J Gen Virol,** 87: 2549-2562.
19. **Imai N, Kurihara M, Matsumoto S, *et al.*** 2004. *Bombyx mori* nucleopolyhedrovirus *orf8* encodes a nucleic acid binding protein that colocalizes with IE1 during infection. **Arch Virol,** 149 (8): 1581-1594.
20. **Kang W-K, Imai N, Kawasaki Y, *et al.*** 2005. IE1 and *hr* facilitate the localization of *Bombyx mori* nucleopolyhedrovirus ORF8 to specific nuclear sites**. J Gen Virol,** 86: 3031-3038.
21. **Kool M, Ahrens C H, Goldbach R W, *et al.*** 1994. Identification of genes involved in DNA replication of the *Autographa californica* baculovirus. **Proc Natl Acad Sci USA,** 91: 11212-11216.
22. **Lauzon H A, Jamieson P B, Krell P J, *et al.*** 2005. Gene

organization and sequencing of the *Choristoneura fumiferana* defective nucleopolyhedrovirus genome. **J Gen Virol,** 86: 945-961.

23. **Mainz D, Quadt I, Knebel-Morsdorf D.** 2002. Nuclear IE-2 structures are related to viral DNA replication sites during baculovirus infection. **J Virol,** 76: 5198-5207.
24. **Maul G G.** 1998. Nuclear domain 10, the site of DNA virus transcription and replication. **Bioessays,** 20: 660-667.
25. **Nie Y, Fang M, Theilmann D A.** 2009. AcMNPV Ac16 (da26, BV/ODV-E26) regulates the levels of IE0 and IE1 and binds to both proteins via a domain located within the acidic transcriptional activation domain. **Virology,** (in press)
26. **Nie Z M, Zhang Z F, Wang D, *et al.*** 2007. Complete sequence and organization of *Antheraea pernyi* nucleopolyhedrovirus, a dr-rich baculovirus. **BMC Genomics,** 8: 248.
27. **Okano K, Mikhailov V S, Maeda S.** 1999. Colocalization of baculovirus IE-1 and two DNA-binding proteins, DBP and LEF-3, to viral replication factories. **J Virol,** 73: 110-119.
28. **Oliveira J V, Wolff J L, Garcia-Maruniak A, *et al.*** 2006. Genome of the most widely used viral biopesticide: *Anticarsia gemmatalis* multiple nucleopolyhedrovirus. **J Gen Virol**, 87: 3233-3250.
29. **O'Reilly D R, Passarelli A L, Goldman I F, *et al.*** 1990. Characterization of the DA26 gene in a hypervariable region of the *Autographa californica* nuclear polyhedrosis virus genome. **J Gen Virol,** 71: 1029-1037.
30. **Rodems S M, Pullen S S, Friesen P D.** 1997. DNA-dependent transregulation by IE1 of *Autographa californica* nuclear polyhedrosis virus: IE1 domains required for transactivation and DNA binding**. J Virol,** 71: 9270-9277.
31. **Saksena S, Summers M D, Burks J K, *et al.*** 2006. Importin-alpha-16 is a translocon-associated protein involved in sorting membrane proteins to the nuclear envelope. **Nat Struct Mol Biol,** 13: 500-508.