ANALYSIS OF INTERACTION BETWEEN MOLECULES OF BOMBYX MORI NUCLEOPOLYHEDROVIRUS IE-2 USING A YEAST TWO-HYBRID SYSTEM

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Summary. – Baculovirus IE-2 protein is one of well-known transactivators. In this report, we demonstrate that Bombyx mori nucleopolyhedrovirus (BmNPV) IE-2 interacts with itself. Several clones were obtained from a yeast two-hybrid screening system using IE-2 as bait and were found to encode IE-2 protein. Nucleotide sequencing of these clones showed that they contained C-terminal regions in common. Further analyses suggest that BmNPV IE-2 protein interacts with itself through 80 amino acid residues of coiled-coil domain in C-terminus.

Key words: Bombyx mori nucleopolyhedrovirus ; IE-2 transactivator; yeast two-hybrid system; coiled-coil domain

The amino acid sequence of BmNPV IE-2 protein shows 73% identity to that of Autographa californica multicapsid nucleopolyhedrovirus (AcMNPV) IE-2 protein. Furthermore, all the functional domains found in AcMNPV IE-2 were also well conserved in BmNPV IE-2 except for a substitution of repeated glutamines by glutamic acids (Gomi *et al.*, 1997). Compared to BmNPV IE-2, AcMNPV IE-2 has been analyzed in more detail. AcMNPV IE-2 has been reported to transactivate the 39 K promoter together with another baculovirus transactivator, IE-1 (Carson *et al.*, 1988). In addition, IE-2 was also reported to stimulate replication of plasmid DNA in transfection assays by transactivating the expression of genes required for replication indirectly (Kool *et al.*, 1995). Although both IE-2 of AcMNPV and BmNPV do not seem to be essential for virus replication, both viruses containing deletions in IE-2 gene display delay in viral DNA synthesis and a reduced production of budded virus or occlusion bodies (Gomi *et al.*, 1997; Prikhod'ko *et al.*, 1999). A recent study also describes that transient expression of IE-2 blocked cell cycle progression (Prikhod'ko *et al.*, 1998). Although the function of IE-2 has been well characterized, little is known about the mechanism of IE-2 function. As the first step to address this question, we performed a yeast two-hybrid screening to find IE-2 interacting proteins. In this report, we describe interaction between IE-2 and itself and provide evidence indicating the domain involved in this interaction.

To identify interacting IE-2 proteins, we performed a yeast two-hybrid screening. Two-hybrid experiments (PROQUEST™ Two-Hybrid System, Gibco-BRL) were performed by following the manufacturer's instruction. To do this, the IE-2 gene was fused with the DNA binding domain of GAL4 as bait (the pDB-ie2 vector was obtained) and a cDNA library was constructed by using mRNA

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Abbreviations: AcMNPV = Autographa californica multicapsid nucleopo-lyhedrovirus; BmNPV = Bombyx mori nucleopolyhedrovirus; p.i. = post infection; SDS-PAGE = polyacrylamide gel electro-phoresis in the presence of sodium dodecyl sulfate; STAT = signal transducers and activators of transcription; TAF-1 = template activating factor 1

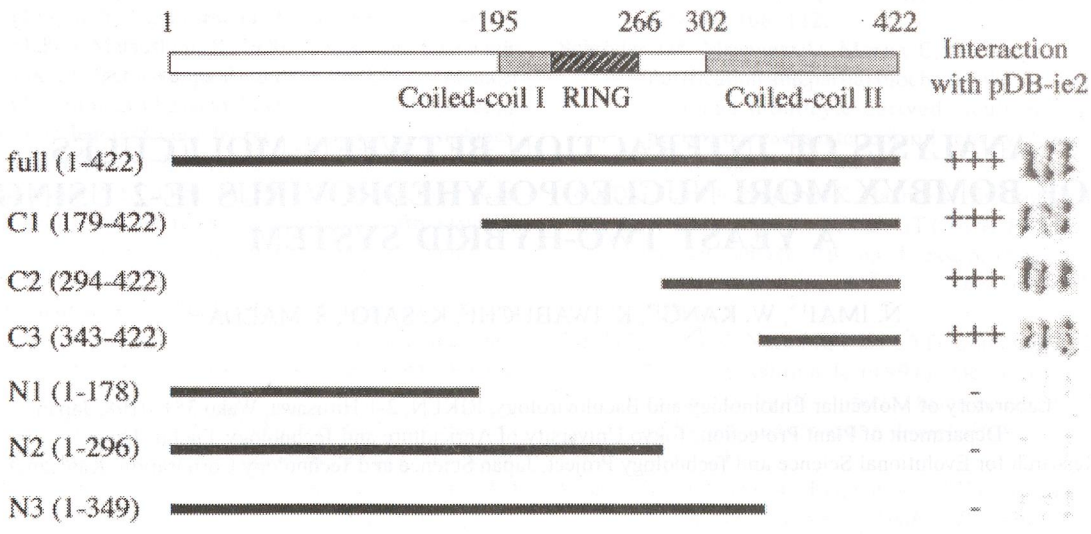

Fig. 1

The structure of BmNPV IE-2 and results of yeast 2-hybrid screening using pDB-ie2

The box on the top indicates the structure of BmNPV IE-2. The predicted two coiled-coil domains and the known RING finger domain are also shown. There is overlap of 3 amino acids between the coiled-coil domain I and the RING finger. Solid lines below the box show the portions of IE-2 obtained from the yeast two-hybrid screening (full to C3) or present in deletion constructs (N1 to N3). The results of the β-galactosidase assay are shown on the right. The level of interaction is defined by comparing with control strains provided from the manufacturer (Gibco-BRL). Controls A, B, C, D, and E (shown in Fig. 2) show no (–), weak (+), moderately strong (+), strong (++), and very strong (+++) interactions, respectively.

isolated from BmNPV-infected BmN cells at 2 hrs post infection (p.i.) and the pPC86 vector which allowed the expression of cDNA-encoded proteins was fused with the activation domain of GAL4. Both vectors were introduced into yeast MaV203 strain, 5 x 10^5 transformants were plated on a medium lacking tryptophan, leucine (to select for both bait-expressing and cDNA library-containing plasmids) and histidine but containing 10 mmol/l 3-amino-triazole (to select for interactions between IE-2 and cDNA library-encoded proteins). Out of 24 positive clones obtained 20 showed positive results when assayed for β-galactosidase activity (second selection marker) and grew on the medium lacking uracil (third marker). Then, we reconfirmed the interaction by reintroducing plasmid DNA (purified from the clones and amplified in *Escherichia coli*) together with pDB-ie2 into yeast MaV203 strain. The nucleotide sequence of one positive clone was found to contain the IE-2 gene, suggesting that the IE-2 protein interacts with itself. Twenty out of 21 clones were found to encode IE-2 protein by Southern blot analysis using the IE-2 gene as a probe (data not shown).

The size of each insert in cDNA clones encoding IE-2 protein seemed to differ from each other. Therefore, we selected 4 clones as representatives to determine the region for interaction. The nucleotide sequencing showed that the pPC-ie2 clone encoded full length of IE-2 (422 amino acids (aa)), while the clones pPC-ie2C1, pPC-ie2C2, and pPC-ie2C3 encoded only a part of IE-2 (aa 179-422, 294-422, and 343-422, respectively) (Fig. 1). This result showed that these clones contained a C-terminal region in common, suggesting that at least 80 amino acids of C-terminus are necessary for the IE-2 interaction.

To exclude the possibility that the N-terminal region is also involved in this interaction, we constructed three clones, in which each clone contained only the N-terminal region which was absent in the clones pPC-ie2C1, C2, or C3. Thus, the pPC-ie2N1 clone contained aa 1-178, the pPC-ie2N2 clone aa 1-296, and the pPC-ie2N3 clone aa 1-349. These C-terminal deletion clones failed to interact with full-length IE-2 in the yeast two-hybrid assay (Fig. 1). The clone pPC-ie2N3 lacked only 73 amino acids in C-terminus of IE-2, however, it did not interact with pDB-ie2.

To further examine whether the C-terminal 80 amino acids are necessary for the interaction, the insert from the pPC-ie2C3 clone was transferred to pDB vector and used for the yeast two-hybrid assay. The pairs of full-length plus full-length or full-length plus C3 showed strong interactions (Fig. 2). Moreover, only a weak interaction was observed between the clones pDB-ie2C3 and pPC-ie2C3, suggesting that the C-terminal 80 amino acids are necessary but not sufficient for self-interaction of IE-2. This may suggest that the other regions are also required for maintaining the IE-2/IE-2 interaction stable.

A

	DB	AD	Selection Marker -his+3AT	X-Gal	-ura
1	full	full	++	+++	+++
2	C3	no	-	-	-
3	C3	C3	±	+	±
4	C3	full	++	+++	+++
5	no	C3	-	-	-
6	no	full	-	-	-

B

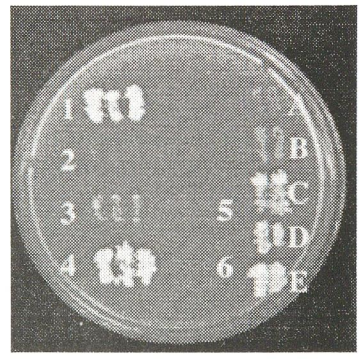

Fig. 2
Analysis of interaction domain of IE-2

(A) Comparative analyses of interactions between full-length IE-2 and its C-terminal 80 amino acids. "no" indicates vector with no insert (negative control). Growth on plates with various selection markers (–his +3AT, X-Gal, and –ura) was checked. The level of interaction is described in Fig. 1. (B) Assay of interaction on the –his + 3AT plates.

To verify the IE-2 interaction observed in the yeast two-hybrid system, a full-length IE-2 was expressed as a His-tagged protein in *E. coli* and purified by using His-Bind resin (Novagen). Then, the purified His-tagged IE-2 was cross-linked with glutaraldehyde as described by Zoog *et al.* (1999) and analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) (6%) followed by silver staining. IE-2 multimers were observed in the presence of cross-linker (Fig. 3). The His-tagged IE-2 showed the expected size (54 K) in 10 % SDS-PAGE (data not shown). The size of the multimers was estimated between 105 K and 160 K, suggesting that IE-2 could form a dimer or a trimer. Taken together, we conclude that IE-2 interacts with IE-2 itself.

Two coiled-coil domains in IE-2 were predicted from the COILS analysis (Lupas *et al.*, 1991) with high probabilities (Fig. 1). The C-terminal coiled-coil domain contains the region for IE-2 interaction identified by the yeast two-hybrid assay. A coiled-coil structure has been reported to participate in protein-protein interactions.

Signal transducers and activators of transcription (STAT) proteins are transcription factors (Darnell *et al.*, 1994; Schindler and Darnell, 1995) which are known to interact with IRF9 using its coiled-coil region (Horvath *et al.*, 1996). Our result also suggests that the IE-2/IE-2 interaction might occur via coiled-coil region. Baculovirus IE-1 is well known transactivator and also forms a homodimer through a helix-loop-helix domain (Rodems *et al.*, 1997), suggesting that the IE-1/IE-1 interaction is required for transactivation of IE-1. A host factor, the template activating factor-1 (TAF-1) is also reported to activate DNA replication of adenovirus by forming a dimer via a coiled-coil structure of N-terminus (Miyaji-Yamaguchi *et al.*, 1999). Such oligomerization is considered reasonable since this structure could stabilize the binding activity to other components, such as nucleotides or proteins. IE-2 may stabilize the binding activity to other substances by forming similar structure.

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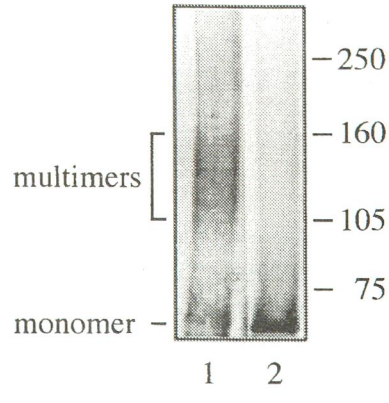

Fig. 3
Cross-linking of IE-2 multimers

His-tagged IE-2 was treated with (lane 1) or without (lane 2) 0.1% glutaraldehyde and subjected to SDS-PAGE (6% gel) followed by silver staining. The approximate sizes of marker proteins (Rainbow marker, Amersham) are indicated on the right (in M_r).

References

Carson DD, Guarino LA, Summers MD (1988): Functional mapping of an AcNPV immediately early gene which augments expression of the IE-1 trans-activated 39K gene. *Virology* **162**, 444–451.

Darnell JE Jr, Kerr IM, Stark GR (1994): Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**, 1415–1421.

Gomi S, Zhou CE, Yih W, Majima K, Maeda S (1997): Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. *Virology* **230**, 35–47.

Horvath CM, Stark GR, Kerr IM, Darnell JE Jr (1996): Interactions between STAT and non-STAT proteins in the interferon-stimulated gene factor 3 transcription complex. *Mol. Cell Biol.* **16**, 6957–6964.

Kool M, Ahrens CH, Vlak JM, Rohrmann GF (1995): Replication of baculovirus DNA. *J. Gen. Virol.* **76**, 2103–2118.

Lupas A, Van Dyke M, Stock J (1991): Predicting coiled coils from protein sequences. *Science* **252**, 1162–1164.

Miyaji-Yamaguchi M, Okuwaki M, Nagata K (1999): Coiled-coil structure-mediated dimerization of template activating factor-I is critical for its chromatin remodeling activity. *J. Mol. Biol.* **290**, 547–557.

Prikhod'ko EA, Lu A, Wilson JA, Miller LK (1999): In vivo and in vitro analysis of baculovirus ie-2 mutants. *J. Virol.* **73**, 2460–2468.

Rodems SM, Pullen SS, Friesen PD (1997): DNA-dependent transregulation by IE1 of Autographa californica nuclear polyhedrosis virus: IE1 domains required for trans-activation and DNA binding. *J. Virol.* **71**, 9270–9277.

Schindler C, Darnell JE Jr (1995): Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu. Rev. Biochem.* **64**, 621–651.

Zoog SJ, Bertin J, Friesen PD (1999): Caspase inhibition by baculovirus P35 requires interaction between the reactive site loop and the b-sheet core. *J. Biol. Chem.* **274**, 25995–26002.