Baculovirus Controls Host Catapillars by Manipulating Host Physiology and Behavior

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1. Introduction

Baculoviridae is a large family of pathogens that infect insects, particularly the order Lepidoptera. Baculoviruses have a large, circular, supercoiled, and double-stranded DNA genome packaged into rod-shaped virions. Baculoviruses are divided phylogenetically into four genera: Alphabaculovirus, Betabaculovirus, Gammabaculovirus, and Deltabaculovirus (Jehle et al. 2006). Alphabaculoviruses can be further subdivided into group I and II nucleopolyhedroviruses (NPVs), based on phylogenetic studies (Herniou et al. 2001). NPVs produce two types of virions during their infection cycle to bring about efficient viral replication within infected larvae and to spread the virus among insects. Occlusion-derived viruses (ODVs) are occluded in occlusion bodies (OBs) (Figs. 1, 2), which are composed primarily of a single polypeptide known as polyhedrin. ODVs transmit the virus from insect to insect via oral infection, whereas budded viruses (BVs) are utilized to spread infection to neighboring cells (Keddie et al. 1989). At the late stage of infection, a markedly enhanced locomotion behavior of infected larvae is observed (Goulson 1997), which is followed by a dramatic degradation of the host cadaver.

The Bombyx mori NPV (BmNPV), one of the well-characterized baculoviruses, is a virus specifically pathogenic for the domesticated silkworm B. mori. DNA sequencing revealed that BmNPV potentially encodes 136 putative proteins. Mutagenesis experiments have discovered the viral proteins that control host catapillars at cellular and/or organismal levels: ecdysteroid UDP-glucosyltransferase inactivates an insect molting hormone ecdysone, protein tyrosine phosphatase is involved in wandering behavior at the late stage of infection, fibroblast growth factor induces host cell chemotaxis, and chitinase and cathepsin are required for postmortem host liquefaction. Furthermore, comparative analyses of the genomes of B. mori and baculoviruses have revealed that the modern lepidopteran baculoviruses may have acquired and modified several genes from an ancestral host insect to control the physiology of their own hosts and to increase the efficiency of virus transmission in nature.
animal.

The Maeda’s group has also determined the complete sequence of BmNPV (Gomi et al. 1999) and unraveled the roles of each BmNPV gene. For example, this group identified anti-apoptotic gene (Kamita et al. 1993), a gene for post-mortem host degradation (Ohkawa et al. 1994), a DNA-binding protein gene (Mikhailov et al. 1998), a gene required for host range determination (Kamita and Maeda 1993) and a gene inducing host wandering behavior (Kamita et al. 2005) from the BmNPV genome. When I was a master course student at The University of Tokyo, I joined his team at RIKEN in 1996 and started my research on BmNPV genes and development of a novel transfer vector. In this review, I introduce my research on BmNPV, especially focusing on the molecular dissection of baculoviral manipulation of host physiology and behavior.

2. Identification and preliminary characterization of host homologs encoded by baculoviruses

Comparative studies of genome sequences from large nuclear and cytoplasmic DNA viruses of eukaryotes revealed that most of them have acquired from other organisms (Iyer et al. 2006). In some cases, there is experimental evidence that these viral gene products can disrupt host signaling pathways and thus are advantageous to the virus (Liu et al. 2013). Phylogenetic analyses support the hypothesis that some sets of genes have been acquired from the host genome by potential horizontal transfers (Iyer et al. 2006).

Phylogenetic analysis of the complete genomes of baculoviruses suggested that two baculoviral genes, inhibitor of apoptosis (iap) and ec dysoideroid UDP-glucosyltransferase (egt), were likely to be derived from the insect host by horizontal gene transfer (Hughes and Friedman 2003). However, due to a lack of the complete genome sequences of lepidopteran insects, genome-wide survey of baculoviral gene homologs in the lepidopteran host insect genome had not been performed. Using the draft genome sequence of B. mori (The International Silkworm Genome Consortium 2008), we performed a detailed genome-wide search of B. mori homologs encoded by BmNPV. The BmNPV genome was 128,413 nucleotides long and contained 136 putative open reading frames (ORFs) (Gomi et al. 1999). By BLAST analyses using amino acid sequences of all BmNPV proteins as the queries, we found that 15 of 136 putative BmNPV proteins (11%) show significant similarity to the B. mori proteins (Katsuma et al. 2008c).

We examined functions of these host homologs by generating a series of gene-knockout BmNPV mutants with a conventional hsp70 promoter-lacZ cassette insertion. We showed that none of these host homologs are dispensable, but six genes are essential for virus production at least in a silkworm ovary-derived cell line, BmN4. Among the six essential genes, dnapol, pk1, and desmoplakin have been shown to be required for virus replication of the closely related NPV, Autographa californica NPV (AcMNPV). AcMNPV dnapol encodes a DNA polymerase that is directly involved in viral DNA replication (Vanarsdall et al. 2005). AcMNPV pk1 encodes a serine/threonine protein kinase and is essential for nucleocapsid assembly (Liang et al. 2013). AcMNPV desmoplakin (ac66) is
required for virion production, but not for virus repli-

Fig. 3. Phylogenetic tree of FGFs from NPVs, insects, and mammals. Neighbor-joining tree generated from the con-
served domain of FGFs are shown. One thousand bootstrap pseudoreplicates were performed, and bootstrap support
greater than 50% is indicated. Sequences used are as fol-

Fig. 4. Tissue distribution of Bmnbnl. RT-PCR analysis of
Bmnbnl and Bmbnl (see Fig. 23) in various tissues from day 3
fifth instar larvae or BmN cells. actin3 was used as a load-
ing control. BR, brain; PG, prothoracic gland; SG, salivary

gland; FB, fat body; TR, trachea; IC, hemocyte; TES, tes-
tis; OV, ovary; ASG, anterior silk gland; MSG, middle silk
gland; PSG, posterior silk gland; FG, foregut; MG, midgut;

hormone ecdysonde (O’Reilly and Miller 1989), pro-
tein tyrosine phosphatase (FTP) is involved in wan-
dering behavior at the late stage of infection (Kamita
et al. 2005; Katsuma et al. 2012b), fibroblast growth
factor (vFGF) induces host cell chemotaxis (Detvisitsakun
et al. 2005; Katsuma et al. 2006b), and chitinase (V-CHIA)
and cathepsin (V-CATH) are re-

suggested BmNPV bacmid system and identified these three
auxiliary genes, iap1 (BmNPV
orf18), v-ubi2 (orf26), and vgt (orf33) and observed that the

Fig. 4. 5–27, 2015 3

required for post-mortem host degradation (Ohkawa
et al. 1994; Hawtin et al. 1997). We performed deletion
analysis of other three auxiliary genes, iap1 (BmNPV
orf18), v-ubs (orf26), and vgt (orf33) and observed that the

mutant viruses took a longer time to kill B. mori
larvae than wild-type virus (Katsuma et al. 2008a,
2008c, 2011b), indicating that these genes are viral
pathogenicity factors for B. mori larvae. However, we did
not observe the effect of methyltransferase (orf57)
deletion on BmNPV virulence in B. mori

Although vFGF is present in most lepidopteran
baculoviruses, some orthologs found in group II NPVs
show low similarity with BmNPV vFGF (Fig. 3). This
suggests that vFGF might have specifically evolved in
each host insect. In Drosophila, an fgf homolog,
branchless (bnl) is required for tracheal development
(Sutherland et al. 1996). However, an fgf homolog from
lepidopteran insects had not been cloned. In order to
acquire further evidence on the possibility of horizon-
tal transfer of vfgfs from the host, we attempted to find
lepidopteran fgfs from the B. mori genome. In a ho-

mology search with BmNPV vFGF as the query, we
identified one fgf-related sequence in the Bomby x
genome and named this gene Bomby x branchless, Bmbnl
(Fig. 3). Expression profiling showed that Bmbnl
is ubiquitously expressed in various tissues in B. mori
larvae (Fig. 4). Phylogenetic analysis using conserved
FGF core domains of vFGFs, insect FGFs, and mam-
nalian FGFs revealed that Bomby x and Drosophila
FGFs are more closely related to vFGFs than other
FGFs, supporting a hypothesis of horizontal transfer of vfgfs from the host insect (see Subsection 6.3 for more detail).

A ptp knockout BmNPV (BmPTPD) cannot induce wandering of B. mori larvae (Kamita et al. 2005). B. mori gene, Bmptp-h, showed significant homology to baculoviral ptp genes. When Bmptp-h was inserted into the genome of BmPTPD, wandering was partially recovered, suggesting that Bmptp-h is a functional homolog of BmNPV ptp (see Section 4 for more detail). In contrast, we revealed that B. mori chitinase BmChi-h (Daimon et al. 2005), which shows a marked homology to and a specific cross-immune reactivity with baculoviral chitinase BmChi-h (Daimon et al. 2006) (Fig. 5) (see Section 3 for more detail). These results suggest that baculoviruses selectively maintain or modify functions of the captured host genes to obtain evolutionary advantages.

3. Baculovirus-encoded proteins required for post-mortem host degradation

In AcMNPV and BmNPV, two auxiliary genes, cathepsin (v-cath) and chitinase (v-chiA), have been shown to be required for post-mortem host degradation by investigating mutant viruses lacking functional v-cath and v-chiA (Ohkawa et al. 1994; Slack et al. 1995; Hawtin et al. 1997; Katsuma et al. 2004b; Daimon et al. 2007) (Fig. 6). The v-cath and v-chiA genes encode cysteine protease and chitinase, respectively. The substrate specificity of V-CATH is closer to that of cathepsin B than cathepsin L, although the sequence of V-CATH is more closely similar to that of cathepsin L. V-CHIA possesses both exo- and endochitinolytic activities (Hawtin et al. 1995; Daimon et al. 2007). The two baculoviral enzymes cooperate to disrupt host insects after death (Hawtin et al. 1997), enhancing the dissemination of progeny viruses away from the host.

We and other groups showed that inactivation of v-chiA results in the accumulation of insoluble V-CATH within virus-infected cells (Hom and Volkman 2000; Daimon et al. 2007), proposing an additional role for V-CHIA as a molecular chaperone during pro-V-CATH processing. Also, V-CATH activity was not detected in v-chiA-inactivated NPV-infected cells (Hom and Volkman 2000; Katsuma et al. 2004b; Daimon et al. 2007), strongly suggesting that reduced host degradation by infection with a v-chiA inactivated virus is mainly due to the loss of V-CATH activity, but not to the loss of chitinase activity of V-CHIA.

The V-CATH proteins of AcMNPV and BmNPV have been shown to be modified with N-linked glycans (Slack et al. 1995; Hom and Volkman 2000; Katsuma et al. 2009a). When Sf-9 cells infected with AcMNPV were treated with tunicamycin to block N-linked glycosylation, no proteolytic processing of pro-V-CATH was observed (Hom and Volkman 2000; Katsuma et al. 2009a). This non-glycosylated form of pro-V-CATH was detected in the insoluble fraction of virus-infected cells, as was the case with pro-V-CATH.
produced in v-chiA-inactivated AcMNPV-infected cells. Together with these results, it was proposed that the putative V-CHIA-V-CATH interaction might be mediated by N-linked glycans of V-CATH, although the positions of N-linked glycans on V-CATH have not been determined.

Amino acid sequence analysis of V-CATHs from Group I NPVs shows that V-CATH includes two consensus N-linked glycosylation sites, asparagine 65 and 158, whereas BmNPV has two sites in the pro-region, and one in the mature region. We generated a series of recombinant BmNPVs expressing mutant V-CATHs, and identified two residues, asparagine 38 and 65, as the glycosylation sites of BmNPV V-CATH (Katsuma et al. 2009a). We also showed that AcMNPV V-CATH has a single N-glycosylation site (asparagine 65) in the pro-region (Katsuma et al. 2009a). Biochemical experiments showed that N-linked glycans are required for the proper production and activation of BmNPV V-CATH (Fig. 7). We also revealed that mutation of either of two of BmNPV V-CATH glycosylation sites results in the formation of insoluble V-CHIA, although v-chiA is intact (Katsuma et al. 2009a) (Fig. 7). This suggests that non-glycosylated V-CATH inhibits the function of V-CHIA, probably due to co-aggregation of pro-V-CATH and V-CHIA.

V-CHIA remains in the infected cells due to its C-terminal KDEL endoplasmic reticulum (ER) retention motif. Hodgson et al. demonstrated direct evidence that ER-resident V-CHIA is needed for the folding of pro-V-CATH (Hodgson et al. 2011). They showed that the N-terminal 22 amino acids of AcMNPV prepro-V-CATH are essential for the entry of pro-V-CATH into the ER. Using fluorescent protein-fused constructs, they successfully observed the direct interaction of V-CHIA with pro-V-CATH in the ER. This was confirmed by immunoprecipitation experiments using His-tagged proteins. They also showed that deletion of the V-CHIA KDEL motif resulted in the secretion of premature V-CATH.
CHIA as well as premature pro-V-CATH from the virus-infected cells. These data clearly indicate that these two proteins interact directly with each other and that this interaction aids the cellular retention of pro-V-CATH.

During the course of study on few polyhedra (FP) mutants possessing mutations in the fp25K gene (see below), we accidentally found that infection with each FP mutant resulted in reduced post-mortem host degradation (Katsuma et al. 1999b) (Fig. 6). The degree to which liquefaction was blocked corresponded to the degree of V-CATH secretion of the fp25K gene product and to the extent to which OB production was reduced. Electron microscopy revealed that the basal lamina of fat body tissue was not destroyed by infection and accumulations of virions occurred along the membrane (Katsuma et al. 1999b). Rosas-Acosta et al. reported that AcMNPV fp25K mutants also showed the liquefaction-defective phenotype in Trichoplusia ni larvae (Rosas-Acosta et al. 2001). Our comparative experiments revealed that a less liquefied phenotype of B. mori larvae infected with BmNPV fp25K deletion mutant can be rescued by introduction of fp25K genes from AcMNPV and Spodoptera litura NPV (SpltMNPV, group II NPV), but that from Xestia c-e nigrum GV (XecnGV) does not eliminate the defect (Nakanishi et al 2010) (Fig. 8). Taken together with these results, FP25K proteins of lepidopteran NPVs are considered as an additional protein involved in post-mortem host degradation.

How FP25K works in the post-mortem degradation process of virus-infected hosts remained unknown. We found that the activity of V-CATH is severely reduced in the hemolymph of B. mori larvae infected with a fp25K-mutated BmNPV, which probably results in inhibition of host degradation after death (Katsuma et al. 2004b). This is because inactivation of BmNPV fp25K causes a defect in V-CATH secretion in the hemolymph of BmNPV-infected larvae (Katsuma et al. 2009b) (Fig. 9). Further experiments showed that disruption of BmNPV fp25K attenuates the expression of v-cath at a late stage of infection, and thus reduces the secretion of its product V-CATH (Katsuma et al. 2009b). Collectively, these results suggest that BmNPV FP25K is essential for the proper transcriptional regulation of v-cath and efficient secretion of V-CATH, and a steady-state level of v-cath expression during the period of V-CATH secretion is crucial for post-mortem host degradation in B. mori larvae.

4. Behavioral control of host insects by baculoviruses: Wipfelkrankheit

Viruses are known to alter the behavior of their hosts for their own benefit. This type of behavior modification by animal viruses is widely observed in arthropod hosts (Hurd 2003). One of the earliest documented examples of such behavior modification is a baculoviral disease, Wipfelkrankheit (Goulson 1997). A hallmark of this disease is enhanced locomotory activity (ELA) that causes the virus-infected larvae to migrate to the...
upper foliage of the host plant where they die and liquify after death (Goulson 1997; Kamita et al. 2005; Hoover et al. 2011). This behavior is believed to lead to the dispersal of progeny OBs over a larger surface area, thus improving the chance of virus transmission to other larvae.

In order to identify the viral genes involved in ELA, Kamita et al. (2005) performed behavioral screening of B. mori larvae against a library of gene knockout mutants of BmNPV. They found that this typical behavior is not observed in B. mori larvae that are infected with a mutant BmNPV (BmPTPD) lacking its protein tyrosine phosphatase (ptp) gene. Biochemical studies using recombinant proteins showed that baculovirus PTP has the ability to remove phosphate groups from protein and RNA substrates (Sheng and Charbonneau 1993; Takagi et al. 1998; Gross and Shuman 1998; Katsuma et al. 2012b). These results suggested that NPV-induced ELA involves the dephosphorylation of an unknown protein or RNA target by baculovirus PTP. However, how PTP works within the cells and insect bodies to induce ELA remained unknown.

In order to reveal the role of PTP in NPV-induced ELA, we generated BmPTP-C119S, a mutant BmNPV that expressed PTP that was nearly deficient in protein phosphatase activity (Katsuma et al. 2012b). Unexpectedly, BmPTP-C119S induced a typical ELA in B. mori larvae in a manner similar to that observed in wild-type virus-infected larvae (Fig. 10). This indicated that the phosphatase activity of PTP is not required for BmNPV-induced ELA in B. mori larvae. Further
behavioral assays using two BmNPV mutants carrying a ptp gene with a premature stop codon showed that they were both unable to induce ELA in B. mori larvae (Fig. 10), indicating that the PTP protein itself is required for the induction of ELA.

We performed a yeast two-hybrid screening using cDNA libraries from BmNPV-infected BmN4 cells, and identified a baculoviral WAP-like protein ORF1629 as an interacting partner of PTP. Immunoprecipitation experiments clearly verified that PTP interacts strongly with ORF1629 protein in BmNPV-infected BmN4 cells. Because ORF1629 is a capsid structural protein, PTP is expected to be also a virion-associated structural protein. Western blot analysis showed that PTP is mainly localized in the BV envelope (Fig. 11). Phenotypic analysis of BmPTPD revealed a marked decrease in GP64 and ORF1629 accumulation in BVs, demonstrating that ptp disruption results in the formation of structurally abnormal BVs with potentially reduced virus infectivity (Fig. 11).

In order to understand the mechanism underlying the phenotypic defects observed in BmPTPD-infected B. mori larvae, we compared the expression of viral genes in 16 tissues that were isolated from wild-type- and BmPTPD-infected larvae. qRT-PCR experiments showed that relative expression levels of late/very late genes were much lower in BmPTPD-infected larvae than in wild-type BmNPV-infected larvae (Fig. 12). The expression of polh in the brain of BmPTPD-infected larvae at 4 days postinfection (dpi) showed the most dramatic reduction (Fig. 12). These findings indicate that the reduction in virus replication was most pronounced in the larval brain.

Our experiments using the BmNPV-silkworm system revealed that the PTP protein functions to induce ELA as a viral structural protein and not likely as an enzyme. Notably, we found that virus propagation was markedly reduced in brain tissues when ptp was inactivated. Collectively, we conclude that PTP augments ELA as a viral structural protein and not likely as an enzyme. We also showed that the active site of the AcMNPV PTP enzyme is re-arranged by the new partner, which prevents interaction with protein serine/threonine phosphatase. Notably, we found that virus propagation was markedly reduced in brain tissues when ptp was inactivated. Collectively, we conclude that PTP augments ELA and the polh gene is required for the induction of ELA.

Recently, van Houte et al. (2012) reported that AcMNPV induces ELA in Spodoptera exigua larvae and the ptp deletion mutant of AcMNPV did not induce ELA larvae, suggesting that the AcMNPV ptp gene also plays a key role in the induction of ELA (van Houte et al. 2012). However, surprisingly, they also showed that the active site of the AcMNPV PTP enzyme is required for stimulation of ELA in the larval brain. They speculated that the difference in behavioral phenotype between the ptp catalytic mutant in the BmNPV-B. mori system and the AcMNPV-S. exigua system may imply that distinct mechanisms underlie hyperactivity in these two systems. More recently, the same group reported that AcMNPV ptp is not required for climbing behavior (van Houte et al. 2014), indicating that ELA and Wipfelkrankheit (tree-top disease) induced by AcMNPV in S. exigua larvae are established by independent mechanisms.

Another gene that is involved in NPV-induced behavior modification encodes an ecldosteroid UDP-
Fig. 13. Putative origin of auxiliary genes in the modern baculovirus. Ancestral baculoviruses are likely to have obtained DNA sequences from ancestral hosts by horizontal gene transfer during replication. The ancestral host-derived genes have been retained during evolution probably because they confer selective advantages to the baculovirus. The authentic biological functions or activities of the genes or their products have been retained, modified or lost during evolution. In the case of BmNPV PTP, the protein is essential for the induction of ELA behaviour as a structural component of the BV envelope but phosphatase activity associated with the protein appears unnecessary.
glucosyltransferase (EGT). As described above, EGT inactivates the major insect molting hormone ecdysone, inhibiting larval molting (O’Reilly and Miller 1989). Gypsy moth larvae usually spend the daytime in the soil and only come out to feed at night. This behavior is controlled by the ecdysone titer. However, larvae infected with Lymantria dispar NPV (LdMNPV) climb to the top of the trees, die and finally liquefy to disseminate OBs on the vegetation below. Hoover et al. (2011) observed that gypsy moth larvae infected with an egt knockout LdMNPV exhibits a reduced climbing behavior (Hoover et al. 2011), indicating that a baculovirus-encoded enzyme controls host climbing behavior by manipulating the hormone levels in the host. Our study, however, clearly showed that a series of egt-inactivated BmNPVs induce a typical ELA in B. mori larvae (Katsuma et al. 2012a). Taken together with the fact that both ptp and egt are the host-derived genes, the modern lepidopteran baculoviruses have evolved multiple strategies to manipulate host behavior (i.e. ELA and Wipfelkrankheit), presumably by hijacking different host signaling pathways.

5. Genes involved in tissue tropism of a baculovirus-infected host

Lepidopteran NPVs show a unique tissue tropism in host insects. Most insect viruses propagate only in the midgut, which is the primary target tissue in oral infection. In contrast, lepidopteran NPVs establish a transient infection in the midgut, without OB production, and then the infection spreads to most of the larval tissues. Recent studies using recombinant NPVs expressing marker genes have allowed us to visually observe the sites where NPVs propagate during the infection (Engelhard et al. 1994; Barrett et al. 1998; Rahman and Gopinathan 2004), but the tissue tropism of NPV-infected larvae has not been quantitatively examined yet.

We measured the expression levels of early, late and very late viral genes in 16 tissues from BmNPV-infected B. mori larvae and found an obvious tissue tropism (Katsuma et al. 2012a). As shown in Fig. 14, viral gene expression in hemocytes was detected by 1 dpi, peaked at 2 dpi, and then gradually declined. In most tissues, the expression was first detected at 2 dpi and peaked at 3 dpi. This expression pattern is consistent with our study using a recombinant BmNPV expressing GFP (Katsuma et al. 2008b). Notably, we found that the silk glands, midgut, and Malpighian tubule were much less infected than the other tissues (Fig. 14), indicating the existence of clear tissue tropism in BmNPV-infected B. mori larvae.

In order to identify the viral genes involved in the tissue tropism of BmNPV infections, we screened a library of gene knockout BmNPV mutants where one gene was functionally disrupted by the insertion of a lacZ cassette. We found that polh expression was abnormally enhanced in the middle silk glands (MSGs) of larvae infected with three mutants. They had a deletion in one of three tandemly arrayed open reading frames (ORFs) (Bm7 (egt), Bm8 (bv/odv-e26), and Bm9). Microscopic observations revealed that these three mutants produced large amounts of OBs around the tracheas of MSGs, whereas OB production was rarely seen in the MSGs of wild-type-infected larvae (Fig. 15).

We next asked if all three genes are essential for proper expression of polh in the MSGs by generating...
seven additional mutants in which one or two genes were partially deleted without inserting a \textit{lacZ} cassette. 

Functional disruption of \textit{Bm7} or \textit{Bm9} did not result in increased OB production in the MSGs. However, Bm8D-1 or Bm8D-2, in which only Bm8 is partially deleted, showed a phenotype similar to that observed in \textit{B. mori} larvae infected with Bm7D, Bm8D, or Bm9D, indicating that the Bm8 protein is solely required for \textit{polh} expression (i.e. inhibition of OB production) in the MSGs of \textit{B. mori} larvae. Further experiments also revealed that a \textit{lacZ} cassette insertion, but not a partial deletion, in \textit{Bm7} or \textit{Bm9} resulted in enhanced expression of Bm8. These results strongly suggest that either enhanced or reduced expression of Bm8 results in abnormal activation for \textit{polh} in the MSGs.

Our results demonstrate that the group I NPV-specific protein BV/ODV-E26 is crucial for the suppression of virus growth in the MSGs of host lepidopteran insects. To our knowledge, this is the first report to identify a gene(s) determining the NPV’s tissue tropism. In order to obtain hints of how this protein functions in virus-infected cells, we screened host proteins that potentially interact with Bm8 using a yeast two-hybrid system (Kang et al. 2012). We identified 6 host clones as Bm8-interacting partners from cDNA libraries prepared from BmN cells or \textit{B. mori} larvae. Our assays showed that the N-terminal region of Bm8 is crucial for the interaction with most host clones and that two of them can associate with IE1, an immediate early viral gene product that is co-localized with Bm8 (Imai et al. 2004). Our experiments to generate recombinant BmNPVs overexpressing these host genes also identified a gene that potentially functions as a negative factor for BmNPV infection (Kang et al. 2012). Further detailed analyses of Bm8-interacting host proteins will explore the role of Bm8 in tissue tropism of host insects.

The role of insect hemocytes in NPV infection has not fully been investigated. Hemocytes of \textit{Helicoverpa zea}, a semipermissive insect for AcMNPV, remove the virus from the hemolymph and participate in the encapsulation of infection foci (Trudeau et al. 2001; Washburn et al. 2003). On the other hand, we identified a viral protein, vFGF, which is one of the key factors facilitating BmNPV infection of hemocytes (Katsuma et al. 2004a). vFGF allows BVs to infect hemocytes efficiently and to establish a systemic infection in \textit{B. mori} larvae, which accelerates a larval death (Katsuma et al. 2006c, 2008b). Collectively, lepidopteran larval hemocytes play either positive or nega-

Fig. 15. OB production in MSGs of BmNPV-infected \textit{B. mori} larvae. (A) MSGs of \textit{B. mori} larvae infected with 50 µl (1 × 10⁵ pfu) of a viral suspension of T3 (wild-type), Bm7D (Bm7 deletion virus), Bm8D (Bm8 deletion virus), and Bm9D (Bm9 deletion virus) at 4 dpi. (B) Light microscopic observations of MSGs of BmNPV-infected larvae (1 × 10⁵ pfu per larva) at 4 dpi. High-magnification images are shown below. The dark blobs are OB-laden nuclei of multinucleated giant cells in MSGs. Bars, 0.2 mm. Reprinted with permission from \textit{J. Virol.}, 86, Katsuma et al., Baculovirus-encoded protein BV/ODV-E26 determines tissue tropism and virulence in lepidopteran insects, 2545–2555, Fig. 3, © 2012a, American Society for Microbiology.

Fig. 16. GFP expression in hemocytes of \textit{B. mori} larvae infected with a mutant BmNPV expressing GFP. BVs (1 × 10⁵ pfu) were injected into the body cavity of 5th-instar \textit{B. mori} larvae. At 48 and 72 hpi, hemocytes were collected and GFP expression was observed. Abbreviations: PR, prohemocytes; GR, granulocytes; SP, spherulocytes; OE, oenocytoids; PL, plasmatocytes. Reprinted from \textit{J. Invertebr. Pathol.}, 112, Hori et al., Silkworm plasmatocytes are more resistant than other hemocyte morphotypes to \textit{Bombyx mori} nucleopolyhedrovirus infection, 102–104, © 2013, with permission from Elsevier.
Fig. 17. Effects of inhibitors of MAPK pathways on OB production. (A) Light microscopy observations of BmNPV-infected BmN cells (MOI = 5) at 72 hpi after treatment at 0 hpi with DMSO, U0126 (10 µM), PD98059 (30 µM), SB203580 (10 µM), and SP600125 (10 µM). (B) Quantification of OB production at 72 hpi in BmNPV-infected BmN cells (MOI = 5) treated at 0 hpi with DMSO, U0126, PD98059 (PD), SB203580 (SB), and SP600125 (SP). *p < 0.05 versus DMSO-treated control. (C) SDS-PAGE analysis of polyhedrin synthesis at 72 hpi in BmNPV-infected BmN cells (MOI = 5) treated at 0 hpi with DMSO, U0126, PD98059, SB203580, and SP600125. The gel was stained with Coomassie brilliant blue. Reprinted with permission from J. Virol., 81, Katsuma et al., ERK- and JNK-dependent signaling pathways contribute to Bombyx mori nucleopolyhedrovirus infection, 13700–13709, Fig. 1, © 2007, American Society for Microbiology.
Fig. 18. Activation of ERK and JNK by BmNPV in BmN cells. BmN cells were mock infected or infected with BmNPV at an MOI of 5. Activation of ERK and JNK at 0, 2, 4, 8, 12, and 24 hpi was assessed by Western blotting using phospho-ERK (pERK)- and phospho-JNK (pJNK)-specific antibodies, respectively. Total levels of ERK and JNK were examined by anti-ERK and anti-JNK antibodies, respectively. Reprinted with permission from J. Virol., 81, Katsuma et al., ERK- and JNK-dependent signaling pathways contribute to Bombyx mori nucleopolyhedrovirus infection, 13700–13709, Fig. 5. © 2007, American Society for Microbiology.

tive roles in NPV infection. However, differences in the viral susceptibility of multiple insect hemocyte morphotypes have not been investigated to date. We generated a BmNPV derivative possessing a Drosophila hsp70 promoter-driven GFP gene and investigated NPV tropism of B. mori larval hemocytes. Our experiments clearly revealed that there were fewer GFP-positive plasmatocytes than those observed in other types of hemocytes, such as granulocytes, oenocytoids, and spherulocytes (Hori et al. 2013) (Fig.
Fig. 19. Effects of dsRNA-mediated knockdown of BmErk and BmJnk on OB and BV production in BmNPV-infected BmN cells. (A) dsRNA-mediated knockdown of BmErk and BmJnk. BmN cells were transfected with dsRNA for BmErk (dsERK), BmJnk (dsJNK), and egfp (dsGFP). Twenty-four hours after transfection, the expression of BmERK, BmJNK, and actin was examined by Western blot analysis using anti-ERK, anti-JNK, and anti-actin antibodies, respectively. (B) Effects of dsRNA-mediated knockdown of BmErk and BmJnk on OB production. Twenty-four hours after transfection, BmN cells were infected with BmNPV at an MOI of 5, and OB production was assessed at 72 hpi. (C) Effects of dsRNA-mediated knockdown of BmErk and BmJnk on BV production. Twenty-four hours after transfection, BmN cells were infected with BmNPV at an MOI of 5, and BV production at 72 hpi was assessed by plaque assay. *p < 0.05 versus egfp dsRNA-treated control. Reprinted with permission from J. Virol., 81, Katsuma et al., ERK- and JNK-dependent signaling pathways contribute to Bombyx mori nucleopolyhedrovirus infection, 13700–13709, Fig. 8, © 2007, American Society for Microbiology.
Fig. 20. Effect of MG-132 on BmNPV infection in BmN cells. (A) BV production. BV titers in BmNPV-infected BmN cells (MOI = 5) treated with DMSO or MG-132 (5 µM) at 0 h p.i. were measured by plaque assay. (B) Light microscopy observations of mock- or BmNPV-infected BmN cells (MOI = 5) at 72 hpi after treatment at 0 hpi with DMSO or MG-132 (5 µM). (C) Expression of viral gene products and accumulation of ubiquitylated proteins. BmNPV-infected BmN cells (MOI = 5) were treated with DMSO or MG-132 (5 µM) at 0 hpi, lysed and immunoblotted with antibodies against ubiquitin (Ub), DNA-binding protein (DBP), viral chitinase (v-CHIA), polyhedrin (POLH) or actin. Reprinted with permission from J. Gen. Virol., 92, Katsuma et al., Role of the ubiquitin-proteasome system in Bombyx mori nucleopolyhedrovirus infection, 699–705, Fig. 1, © 2011b, Society for General Microbiology.
indicating the existence of the NPV tropism within silkworm larval hemocyte subsets, i.e. silkworm plasmatocytes are more resistant than other hemocyte morphotypes to BmNPV infection.

6. Hijacking the signaling pathways of host cells by baculovirus infection

6-1. MAPK pathways

Viral infection causes the deregulation of various host signaling pathways, some of which reflect cellular responses to infection, while others are the result of viral modification of cellular environments. A common strategy that a virus uses to facilitate its propagation in host cells is to take advantage of these altered signaling pathways. For example, modulation of mitogen-activated protein kinase (MAPK) pathways is known to be essential for replication of hepatitis B virus, Epstein-Barr virus, and vaccinia virus (de Magalhaes et al. 2001; Zheng et al. 2003; Gao et al. 2004).

In order to identify the signaling pathways induced by baculovirus infection, we examined the involvement of host MAPK pathways on BmNPV infection (Katsuma et al. 2007). We first assessed the effects of specific inhibitors on three components of the MAPK pathways: extracellular signal-regulated kinase (ERK) kinase (inhibitors U0126 and PD98059), c-Jun NH2-terminal kinase (JNK) (inhibitor SP600125), and p38 (inhibitor SB203580). Among these inhibitors, those for ERK kinase and JNK significantly reduced BmNPV propagation (Fig. 17). Further pharmacological experiments showed that MAPK pathway inhibitors reduce BmNPV propagation at the late stage of infection. We observed that these inhibitors markedly repressed or deregulated the expression of delayed early, late, and very late viral gene products (Katsuma et al. 2007).

Western blot analysis using phospho-MAPK-specific antibodies showed that ERK and JNK were activated at the late stage of BmNPV infection (Fig. 18) and this BmNPV-induced ERK and JNK activation depends on viral dose (Katsuma et al. 2007). In order to verify the effects of the inhibitors for ERK kinase and JNK on BmNPV propagation, we performed RNA interference (RNAi) experiments against BmErk and BmJnk, encoding B. mori ERK and JNK, respectively. Virus propagation was significantly reduced in knocked down cells compared to the control cells, indicating that that BmERK and BmJNK are involved in BmNPV infection (Fig. 19). Taking together with the results obtained in the chemical inhibitor experiments, we conclude that BmNPV takes advantage of the ERK- and JNK-dependent signaling pathways to facilitate its propagation in silkworm cells. A similar approach was used to identify the signaling pathways involved in AcMNPV infection. Xiao et al. reported that the PI3K-Akt signaling pathway is required for efficient AcMNPV replication in Spodoptera frugiperda cells (Xiao et al. 2009).
6-2. Ubiquitin–proteasome pathway

The ubiquitin–proteasome system plays a central role in the degradation of intracellular proteins. Using proteasome inhibitors such as MG-132, viruses belonging to several different families have been shown to utilize or modulate this system to their advantage during their infection cycles (Satheshkumar et al. 2009; Teale et al. 2009; Raaben et al. 2010). We investigated the effects of MG-132 on BmNPV propagation to ask if the ubiquitin–proteasome system is required for efficient BmNPV infection (Katsuma et al. 2011b). The treatment significantly reduced BmNPV propagation, especially BV production and delayed or reduced expression of early, late and very late gene products of BmNPV (Fig. 20).

BmNPV (Fig. 20).

Homologues of ubiquitin (U-VUBI) are found in most lepidopteran baculoviruses (Katsuma et al. 2008c). V-UBI is 75% identical to eukaryotic ubiquitin and appears to be present on the inner surface of viral envelopes (Guanino et al. 1995). AcMNPV V-UBI is involved in the formation of viral particles (Reilly and Guarino 1996), whereas deletion of v-ubi does not affect BV and OB production in BmNPV-infected BmN4 cells (Katsuma et al. 2011b), suggesting that the common role of v-ubi in baculovirus infection remains unclear to date.

BmNPV is known to encode three proteins, IAP2, PE38 and IE2, that have E3 ubiquitin ligase activity (Imai et al. 2003). Imai et al. showed by transient expression experiments that IE2 is actively degraded by the ubiquitin–proteasome system, and that the degradation process is seemingly regulated by its own E3
ligase activity (Imai et al. 2005). These results indicate that IE2 is being auto-ubiquitylated and degraded during BmNPV infection. In order to know the role of E3 ligase activity of IE2 in more detail, we generated a mutant virus, BmIE2CS, expressing an E3 ligase activity-deficient IE2 derivative and examined its properties. Expectedly, IE2 foci in BmIE2CS-infected cells were brighter compared with those in T3-infected cells. In addition, foci in BmIE2CS-infected cells were clearly detected even at the time when those in wild-type virus-infected cells were rarely detected (Katsuma et al. 2011b) (Fig. 21). This result confirms the degradation process of BmNPV IE2 by its own E3 ligase activity. Further experiments also revealed that V-UBI is not required for IE2 degradation in BmNPV-infected cells (Katsuma et al. 2011b).

### 6-3. FGF signaling

The FGFs appear to play important roles in both developing and adult tissues of vertebrates and invertebrates. Sequence analysis has revealed the existence of FGF gene homologs (vfgf) in the NPV genome (Ayres et al. 1994; Gomi et al. 1999). vfgf is present in almost all lepidopteran baculoviruses, but is absent in the genome of a dipteran NPV (Herniou et al. 2003), suggesting that vFGF may be required for baculovirus infection to lepidopteran hosts, but not to dipteran hosts. We for the first time cloned and characterized vfgf from BmNPV (Katsuma et al. 2004a). Our experiments showed that vfgf is one of the baculovirus early genes, and its product vFGF is a glycosylated protein that is efficiently secreted from BmNPV-infected cells (Katsuma et al. 2004a) (Fig. 22). Biochemical studies using recombinant viruses expressing a series of vFGF derivatives revealed that the two residues, asparagine
44 and 171, are the glycosylation sites of BmNPV vFGF (BmFGF) and N-linked glycans of BmFGF are required for its secretion (Katsuma et al. 2006a) (Fig. 22). Interestingly, these two residues are not conserved in closely related AcMNPV-encoded vFGF (AcFGF). Western blot analysis indicated that AcFGF is not glycosylated and is poorly secreted. A mutated AcFGF derivative with two N-linked glycosylation sites was shown to be secreted more abundantly than that which occurred for wild-type AcFGF (Katsuma et al. 2006a). NPV-encoded vFGFs other than AcFGF possess at least one or more putative N-linked glycosylation sites, suggesting that modification with N-linked glycans is a common feature of vFGFs. Therefore, the poor secretion of AcFGF may be indicative of the unique evolution of AcFGF and may possess a specific function(s).

VFGF is a secreted protein, suggesting that it may act as an extracellular ligand as observed in the vertebrate FGF signaling cascade. FGFs are known to function by binding heparin or heparan sulfate proteoglycans to form oligomers, and this complex interacts specifically with cell-surface FGF receptors (FGFRs). FGFRs are the receptor-type tyrosine kinases that are activated upon FGF binding. The binding leads to receptor dimerization and auto-phosphorylation, and the activated FGFR then stimulates signal transduction pathways (Powers et al. 2000). In order to investigate the signaling cascade triggered by vFGF, we attempted to identify the vFGF receptor. Since vFGF has a high homology to Drosophila Branchless/FGF, which is a ligand for Breathless (Btl) (Sutherland et al. 1996), we speculated that the B. mori Btl ortholog might be a receptor for vFGF. We successfully cloned B. mori and S. frugiperda Btl genes (Bmbtl and Sfbtl, respectively) and showed that these genes encode the functional receptors for vFGF (Katsuma et al. 2006b) (Fig. 23). BmFGF stimulation phosphorylated Bmbtl (Fig. 24) and enhanced the migration activity in cultured cells (Fig. 25). These results strongly suggest that vFGF induces host cell chemotaxis via the host cell-surface receptor, Bmbtl.

In order to investigate the importance of vfgf in virus infection, we generated a BmNPV mutant lacking functional vfgf and characterized it in BmN4 cells and B. mori larvae. We observed that virus production was reduced in vfgf mutant-infected BmN4 cells and B. mori larvae. We observed that virus production was reduced in vfgf mutant-infected BmN4 cells and B. mori larvae (Katsuma et al. 2006c). The larval bioassays also revealed that deletion of vfgf took 20 h longer to kill B. mori larvae than wild-type virus, when tested either by BV injection or by OB ingestion (Table 1). These results suggest that BmNPV vfgf is involved in efficient virus growth in both BmN4 cells and B. mori larvae. In order to uncover the role of vFGF during systemic infection of B. mori larvae, we further generated a vfgf deletion BmNPV derivative possessing an ie-1 promoter-driven gfp gene. Intraheemocoelic and oral infection experiments revealed that the loss of functional vFGF reduces viral infectivity in B. mori hemocytes (Katsuma et al. 2008b) (Fig. 26). Our results suggest that BmNPV vFGF is required for efficient systemic infection, presumably by a chemotactic effect that allows budded virus to infect hemocytes.

Fig. 26. GFP expression in hemocytes of B. mori larvae intraheemocoelicly infected with BmNPV mutants. Hemolymph was collected from BmNPV-infected fifth-instar B. mori larvae (1 × 10³ pfu per larva) at 6, 12, 24, 48, and 72 hpi, and GFP-positive cells were counted using a fluorescence microscope. BmIEGFP, a BmNPV mutant possessing an ie-1 promoter-driven egfp at the immediately upstream region of polh; BmIEGFP/FgFD, a BmIEGFP derivative in which vfgf is partially deleted from the genome of BmIEGFP. *p < 0.05. Reprinted from Virus Res., 137, Katsuma et al., The fibroblast growth factor homolog of Bombyx mori nucleopolyhedrovirus enhances systemic virus propagation in B. mori larvae, 80–85, © 2008b, with permission from Elsevier.
Surprisingly, a vfgf mutated AcMNPV exhibits a markedly different phenotype observed in a BmNPV vfgf mutant. Detvisitsakun et al. (2006, 2007) reported that no obvious differences were observed in cultured cells between the mutant and wild-type AcMNPVs. In contrast, deletion of vfgf delayed the time to death in S. frugiperda and T. ni larvae when the virus was delivered by feeding but not by intrahemocoelic injection. These results suggest that AcFGF may play a role in dissemination of the virus from the midgut in the host larvae. The same group showed that AcFGF is associated with virions (Lehiy et al. 2009) and involved in remodeling of basal lamina lining tracheal cells as-

Fig. 28. Comparison of OB production levels in BmN cells infected with BmNPVs expressing fp25K derivatives. (A) Light microscopic observations of BmNPV-infected BmN cells at 5 dpi (MOI = 5). (B) OB production. BmNPV-infected BmN cells (MOI = 5) at 3 dpi were gently scraped using a rubber policeman, and total OB production was measured as in Fig. 17B. *p < 0.05 by one-way ANOVA and Dunnett’s post tests using T3 as a control. The abbreviations for virus names are the same as in Fig. 8. Reprinted with permission from J. Virol., 84, Nakanishi et al., Comparative studies of a lepidopteran baculovirus-specific protein FP25K: Development of a novel Bombyx mori nucleopolyhedrovirus-based vector with a modified fp25K gene, 5191–5200, Fig. 2A and 2B, © 2010, American Society for Microbiology.
production of foreign proteins in larvae and pupae of *B. mori* (Maeda 1989). In order to improve this system, our group has been attempting to identify the genes modifying the promoter activity of *polh*. We isolated and characterized a series of OB morphology *BmNPV* mutants generated by mutagenesis with 5-bromo-2′-deoxyuridine (BrdU) (Katsuma et al. 1999a, 1999b, 2000). Most of the mutants showing aberrant size and number of OBs possessed mutations in the *polh* coding region. An OB deficient mutant, #24, which produces neither polyhedrin protein nor *polh* transcripts in virus-infected *B. mori* cells had a single nucleotide mutation in the five very conserved nucleotides of the *polh* promoter (from ATAA to ATAA), but not in the *polh* coding region, verifying the importance of the *polh* promoter region in the hyperexpression of polyhedrin protein (Shimada et al. 1994; Katsuma et al. 1999a).

Serial passage of NPVs often generates mutant viruses called few-polyhedron (FP) mutants. They produce fewer OBs in which few or no ODVs are occluded associated with the intestine (Means and Passarelli 2010). These results strongly support the above-mentioned hypothesis that AcFGF possesses unique and specific properties in virus infection.

7. Identification of genes required for efficient *polh* expression: development of a novel *BmNPV*-based expression vector

The baculovirus expression vector system (BEVS) has been used to produce many proteins, such as vaccines and veterinary medicine. BEVS has been greatly improved in terms of host range expansion (Mori et al. 1992), development of a bacmid system (Luckow et al. 1993), and stabilization of the foreign products by deletion of *v-cath* and *v-chiA* from the viral genome (Suzuki et al. 1997; Kaba et al. 2004). Although a strong promoter of *polh* is a key factor for the expression of a huge amount of foreign proteins in insect cultured cells or larvae, little genetic modification has been performed to increase the amount of foreign products or enhance the activity of the *polh* promoter in the BEVS.

Unlike the AcMNPV-based BEVSs, the *BmNPV*-based BEVSs are particularly suitable for the mass production of foreign proteins in larvae and pupae of *B. mori* (Maeda 1989). In order to improve this system, our group has been attempting to identify the genes modifying the promoter activity of *polh*. We isolated and characterized a series of OB morphology *BmNPV* mutants generated by mutagenesis with 5-bromo-2′-deoxyuridine (BrdU) (Katsuma et al. 1999a, 1999b, 2000). Most of the mutants showing aberrant size and number of OBs possessed mutations in the *polh* coding region. An OB deficient mutant, #24, which produces neither polyhedrin protein nor *polh* transcripts in virus-infected *B. mori* cells had a single nucleotide mutation in the five very conserved nucleotides of the *polh* promoter (from ATAA to ATAA), but not in the *polh* coding region, verifying the importance of the *polh* promoter region in the hyperexpression of polyhedrin protein (Shimada et al. 1994; Katsuma et al. 1999a).

Serial passage of NPVs often generates mutant viruses called few-polyhedron (FP) mutants. They produce fewer OBs in which few or no ODVs are occluded...
Mapping analysis has located a single gene, \( fp25K \), encoding a 25 kDa protein involved in the FP phenotype of AcMNPV (Beames and Summers 1989). Sequencing and comparative studies of baculovirus genomes have revealed that the gene product FP25K is a lepidopteran baculovirus-specific protein (Ikeda et al. 2006). We isolated five BrdU-induced FP mutants of BmNPV, which exhibited a typical FP phenotype. Sequence analysis revealed nucleotide substitutions in the coding region of \( fp25K \) that are required for the FP phenotype of these BmNPV mutants (Katsuma et al. 1999b). We also performed comparative studies of the \( fp25K \) genes by generating recombinant BmNPVs in which \( fp25K \) was replaced with the corresponding gene from AcMNPV, SpltMNPV, or Xestia c-nigrum granulovirus (XecnGV). We found that Bm25KD-Ac, a BmNPV possessing AcMNPV \( fp25K \) instead of BmNPV \( fp25K \), produces more OBs in BmN4 cells than the wild-type virus through enhanced \( polh \) expression (Fig. 28). In order to determine the region required for AcMNPV-type OB production, we generated four recombinant BmNPVs expressing chimeric FP25K proteins and revealed that the N-terminal half of AcMNPV FP25K is required for enhanced expression of OBs (Nakanishi et al. 2010).

According to the above-mentioned experimental results, we attempted to generate a novel BmNPV-based expression vector by inserting AcMNPV \( fp25K \) into the \( fp25K \) locus of BmNPV. We used mouse interleukin-3 (mIL-3) as a foreign gene, because mIL-3 was shown to be abundantly expressed in a BmNPV expression system (Miyajima et al. 1987). We found that the introduction of AcMNPV \( fp25K \) into the BmNPV genome accelerates secretion of mIL-3 into \( B. mori \) larval hemolymph (Nakanishi et al. 2010). In order to quantify the expression of recombinant proteins accurately, we further examined luciferase activities in BmN4 cells infected with recombinant BmNPVs expressing firefly luciferase. Luciferase expression was significantly higher when AcMNPV \( fp25K \) was introduced into the BmNPV genome (Fig. 29) (Nakanishi et al. 2010). These results indicate that a BmNPV-based vector with AcMNPV \( fp25K \) has great potential for increasing the production of recombinant proteins in cultured cells and shortening the time for obtaining the secreted recombinant proteins from larval hemolymph.

In order to find a new gene affecting the \( polh \) transcription, we screened the library of BmNPV mutants, in which each gene was functionally disrupted by the insertion of a \( lacZ \) cassette. We found that a mutant BmORF34D, in which \( Bm34 \) was disrupted, produced far fewer OBs in BmN4 cells as compared with wild-type BmNPV (Fig. 30) (Katsuma and Shimada 2009).\( Bm34 \), a BmNPV homolog of \( Ac43 \), encodes a small nuclear protein (a molecular mass of approximately 10.5 kDa), which is composed of 78 amino acid residues and shows no homology to any known proteins. Expression analysis of viral genes and reporter assays showed that a marked decrease in \( polh \) transcription observed in BmORF34D-infected cells might result from down-regulation of two genes \( fp25K \) and \( vlf-1 \), both of which are involved in very late gene expression (Harrison et al. 1996; Yang and Miller 1999). These results demonstrate that \( Bm34 \) is a novel gene required for efficient expression of late and very late genes and production of OBs.\( Bm34 \) protein plays an important role in the transcription of \( vlf-1 \) and \( fp25K \), and indirectly controls \( polh \) expression. By exploring the molecular mechanisms by which \( Bm34 \) enhances the \( polh \) transcription in more detail, we will develop a novel BEVS with a modified \( polh \) promoter regulation.
8. Conclusion

In this review, I describe the progress of the baculovirus biology especially from our experimental results. However, at present, it remains largely unknown how a baculovirus can establish its highly tuned gene expression that enables the virus to control host physiology and behavior. In order to understand how transcriptional regulation operates in NPV-infected host cells, we and other groups have performed genome-wide expression analyses of NPV and/or host genes using DNA microarrays (Fig. 31) (Yamagishi et al. 2003; Iwanaga et al. 2004; Katsuma et al. 2005; Jiang et al. 2006) or RNA sequencing by a next-generation sequencer (Chen et al. 2013). This technology, however, cannot discriminate between the variable-length baculovirus transcripts that commonly overlap more than one ORF (Smith 2007). In order to obtain a precise overview of the population of “transcriptional units” expressed from an NPV genome, we generated and characterized a full-length-enriched cDNA library from BmNPV-infected BmN cells (Katsuma et al. 2011a). This attempt showed for the first time a precise landscape of transcriptional units expressed from an NPV genome. Surprisingly, we discovered a number of novel as-yet-uncharacterized tran-

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Fig. 32. Identification of putative regulatory ncRNAs. Bold arrows indicate putative regulatory ncRNAs found in the polh (a) and pif-1 (b) loci. Late (TAAG) and early (CAGT/CAGA) motifs are shown at the end of each ncRNA. Arrows show all transcriptional units identified in this region. Reprinted with permission from J. Gen. Virol., 92, Katsuma et al., Mass identification of transcriptional units expressed from the Bombyx mori nucleopolyhedrovirus genome, 200–203, Fig. 3, © 2011a, Society for General Microbiology.
scripts, including long non-coding RNAs (ncRNAs) (Fig. 32). To date, baculovirus researchers have focused almost exclusively on the protein-coding genes predicted from the genome information. However, our results suggest that more attention should be paid in future to all of the transcriptional units produced from the viral genome. Further studies on putative regulatory ncRNAs will provide new insights into the complex transcriptional regulation of an NPV genome.

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