Host Range Expansion by Recombination of the Baculoviruses *Bombyx mori* Nuclear Polyhedrosis Virus and *Autographa californica* Nuclear Polyhedrosis Virus

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Received 3 December 1990/Accepted 28 March 1991

The mechanisms of host specificity of nuclear polyhedrosis viruses (NPVs) (*Baculoviridae*) were analyzed after coinfection of *Bombyx mori* NPV (BmNPV) and one of four distinct groups of *Spodoptera litura* NPV (SINPV), including an *Autographa californica* NPV (AcNPV) variant (S. Maeda, Y. Mukohara, and A. Kondo, J. Gen. Virol. 71:2631–2639, 1990), into various lepidopteran cell lines. Replication of BmNPV in nonpermissive cells (TN-386, SF-21, and CLS-79) was induced by coinfection with AcNPV but not with the other three SINPV groups. These induced progeny NPVs were plaque purified in BmN cells, which are susceptible to only BmNPV, and characterized. Most of these isolates did not replicate in the cell lines in which they were produced, indicating the existence of a helper function of AcNPV for BmNPV replication in nonpermissive cells. Some of these isolates, however, were able to replicate in cell lines nonpermissive to BmNPV, indicating the appearance of a new virus with wider host specificity. DNA restriction endonuclease analysis showed that the isolates exhibiting wider host range were recombinant viruses between the parents, AcNPV and BmNPV, resulting from various types of crossovers of relatively large areas of their genomes. Expansion of host range was also observed in larvae.

Baculoviruses are enveloped viruses having a circular double-stranded DNA genome. They are specific to arthropods and have potential for use as insecticides (6). Viruses of the nuclear polyhedrosis virus (NPV) subgroup are also used as efficient vectors for the expression of foreign genes in established cell lines and whole insects (for reviews, see references 11, 13, and 18).

Studies on baculovirus host specificity have shown that these viruses possess relatively narrow host ranges both in vivo and in vitro (6). Recent advances in the molecular biology of baculoviruses and development of an in vitro replication system have revealed some important mechanisms of baculovirus replication (for reviews, see references 1 and 18). However, the exact mechanisms of host range determination in baculoviruses are still unknown. In some vertebrate viruses, the initial attachment of a virus to a host cell receptor is considered an important process for host specificity (for a review, see reference 10). In the infection process of NPVs, an envelope protein (gp64) plays a role in the attachment of viral particles to the host cell surface (21). It has been shown that a polypeptide produced by a granulosis virus can enhance the infectious activity of an NPV by increasing the NPV's efficiency of attachment to the host cell surface (22) or by disruption of a peritrophic membrane, the barrier organ inside the insect midgut (5). However, it is questionable whether these molecules play important roles in the mechanisms of baculovirus host specificity in general. It has also been shown that the baculovirus *Autographa californica* NPV (AcNPV) can penetrate the nucleus of nonpermissive insect cells and whole insects (2), indicating

that the mechanisms of host range determination of AcNPV seem to lie mainly in the expression of the viral genome.

Recently, we isolated four distinct viral groups from wild stocks of *Spodoptera litura* NPV (SINPV), including an AcNPV variant (17). The four groups displayed in vitro host range specificities that were different from each other as well as from that of *Bombyx mori* NPV (BmNPV). In this report, we describe the helper function of an SINPV isolate (an AcNPV variant) in the replication of BmNPV and the isolation of recombinant viruses with a host range phenotype wider than that of these two viruses.

MATERIALS AND METHODS

Viruses. The BmNPV T3 isolate (12, 15) was propagated in BmN cells. The four distinct isolates of SINPV, CC5 (SINPV-A group), FC3 (SINPV-B group), OC12 (SINPV-C group), and OT2 (an AcNPV variant) (17), were propagated in CLS-79 or SF-21 cells.

Cell lines and plaque assays. Four cell lines established from lepidopteran insects were used: TN-368, established from *Trichoplusia ni* (8); CLS-79 and SF-21, established from *Spodoptera frugiperda* (9, 20); and BmN, established from *B. mori* (14). TN-368 was maintained with TNM-FH, CLS-79 was maintained with IPL-41, and SF-21 and BmN were maintained with TC-100 as described previously (17). Plaque assays were carried out with SeaPlaque agarose (FMC, Rockland, Maine) as described previously (12, 17).

Propagation of viruses in cell lines. Confluent cell monolayers were infected by addition of viral suspension (about 200 μ l per 60-mm dish or 50 μ l per 35-mm dish) to obtain a viral titer of 5 PFU per cell. After incubation for 1 h at 27°C with rocking every 15 min, the infection medium was removed and the cells were washed twice with fresh culture medium. Then 4 ml (or 1.5 ml per 35-mm dish) of fresh medium was added, and the cells were incubated at 27°C.

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This time point was designated as 0 h postinfection (p.i.). At appropriate times after infection, 50 μ l of the supernatant was collected, diluted 10-fold with culture medium, and stored at -80°C until use. For coinfection, 5 PFU per cell as calculated in susceptible cell lines of each of the appropriate viruses (BmNPV and one of the SINPVs) was used for inoculation.

DNA restriction endonuclease analysis. Budded viruses in culture fluid were used for viral DNA purification. The infected culture fluid prepared as described previously (14) was layered onto 4 ml of a 40% (wt/wt) sucrose cushion or onto a 10 to 40% (wt/wt) sucrose gradient in an SW28 tube (Beckman, Palo Alto, Calif.) and centrifuged at $83,000 \times g$ for 1.5 h at 15°C . Viral particles in a pellet under the cushion or in a band in the gradient were collected and suspended in 10 mM Tris-HCl-1 mM EDTA (pH 7.5). The viral DNA was extracted after treatment with proteinase K in the presence of 1% sodium dodecyl sulfate (SDS) (14) and digested with the restriction endonucleases *Eco*RI, *Hind*III, *Pst*I, *Bam*HI, *Kpn*I, and *Sma*I under the conditions recommended by the manufacturer. The cleaved DNA fragments were electrophoresed on a 0.7% agarose gel using Tris-acetate buffer.

Southern blot analysis of the polyhedrin gene. The *Hpa*I-*Hind*III fragment of BmNPV (15), containing the entire polyhedrin gene and 580 bp of the 5' and 448 bp of the 3' flanking sequences (about 1.8 kb total), was used as a probe after labeling by nick translation. *Eco*RI-digested viral DNA electrophoresed on a 0.7% agarose gel was transferred to a nitrocellulose filter and hybridized to the probe as described previously (17).

Infection of larvae with viruses. Infection by subcutaneous injection of *S. litura* or *B. mori* larvae was carried out by using a modification of methods described previously (15). A 20- μ l sample of the virus suspension (infected culture fluid) containing 2×10^5 to 5×10^5 PFU was infected into the body cavity of fifth (*B. mori*)- or sixth (*S. litura*)-instar larvae. Infected larvae were reared on artificial diets.

Oral infection with viruses was carried out by using second-instar *Heliothis virescens* larvae and first-instar *B. mori* larvae. Precipitated BmN cells containing about 10^4 polyhedral inclusion bodies (PIBs) per larva were applied on a small pellet of artificial diet. After all of the diet containing the PIBs was ingested, the larvae were cultured at 25°C ; viral infection was monitored for at least 2 weeks.

RESULTS

More than 100 plaque-purified isolates from wild stocks of SINPV collected in Japan were classified into four distinct virus groups according to in vitro host range, growth pattern, polyhedral protein characteristics, and DNA homology (17). The four groups were (i) SINPV-A (the *Spodoptera littoralis* NPV-A group of Cherry and Summers [3]), (ii) SINPV-B (the *S. littoralis* NPV-B group of Cherry and Summers [3]), (iii) SINPV-C (specific to *S. litura* in Japan), and (iv) AcNPV (an AcNPV variant). Since the in vitro host ranges of the four groups were completely different from that of BmNPV (Table 1), BmNPV and each of the four SINPVs were used to elucidate the mechanisms of host specificity by coinfection into various cell lines.

BmNPV replication after coinfection of BmNPV with each SINPV isolate in three different cell lines. BmNPV and each of the SINPV isolates (OT2, FC3, OC12, and CC5) were separately coinfecting into the cell lines TN-368, SF-21, and CLS-79. As shown in Table 1 (17), TN-368 is susceptible to only SINPV OT2 (hereafter referred as AcNPV, AcNPV

TABLE 1. Host specificities of the NPVs used^a

Virus group	Isolate	Host specificity ^b			
		TN-368	SF-21	CLS-79	BmN
AcNPV	OT2	+++	+++	+++	—
SINPV-B	FC3	—	+++	+++	—
SINPV-C	OC12	—	+	+++	—
SINPV-A	CC5	—	—	+++	—
BmNPV	T3	—	—	—	+++

^a Data taken from Maeda et al. (17).

^b +++, $>10^7$ PFU/ml at maximum titer; +, 10^5 to 10^6 PFU/ml at maximum titer; —, no replication.

OT2, or OT2), SF-21 is susceptible to only three of the SINPVs (OT2, FC3, and OC12), and CLS-79 is susceptible to only the SINPV isolates. Viral growth of BmNPV after coinfection was determined by plaque assay using BmN, which is susceptible to only BmNPV. In TN-368, replication of BmNPV was not detected (above background levels) after infection with only BmNPV (Fig. 1A), as expected. Coinfection of BmNPV with AcNPV in TN-368 showed a slight increase of BmNPV growth at 24 h p.i. and reached a titer of 5×10^5 PFU/ml at 48 h p.i. This was about 20-fold higher than that detected after single infection with BmNPV (Fig. 1A). In contrast, coinfection of BmNPV with one of the three remaining groups (CC5, FC3, and OC12) showed no increased viral growth of BmNPV. The titers of these coinfections were around 10^4 PFU/ml, which were considered to represent viruses remaining in the medium after washing. These results indicate the existence of some mechanism, such as a helper function, for increased replication of BmNPV in nonpermissive cells by coinfection with AcNPV. The lack of any detectable effects of coinfection with the CC5, FC3, and OC12 isolates on BmNPV replication may be explained by the nonsusceptibility of TN-368 to these viruses.

When SF-21 was infected with the same combination of viruses as in the TN-368 infections, no detectable BmNPV replication was observed at 24 h p.i. (Fig. 1B). At 48 h p.i., however, increased BmNPV titers were detected (about fivefold higher) only in cells coinfecting with AcNPV.

When CLS-79 was infected with the combination of viruses used in the SF-21 and TN-368 infections, coinfection with BmNPV and AcNPV produced roughly a 5-fold-higher

FIG. 1. Coinfection of BmNPV and each of the four SINPVs in cells nonpermissive to BmNPV. Cell monolayers of TN-368 (A), SF-21 (B), and CLS-79 (C) in 35-mm dishes were singly infected or coinfecting with 5 PFU of each of the viruses per cell. Virus titers in the culture fluids after single infection with BmNPV T3 (▲) and coinfection of BmNPV with SINPV CC5 (□), FC3 (○), OC12 (■), and OT2 (●) were plaque assayed on BmN cells.

FIG. 2. Effect of coinfection on the replication of AcNPV OT2 and BmNPV T3 in TN-368 (A), SF-21 (B), and CLS-79 (C). Cell monolayers in 60-mm dishes were singly infected with BmNPV or AcNPV or coinfecting with BmNPV and AcNPV. The culture fluid was plaque assayed on BmN cells (susceptible to BmNPV) or the original cells (susceptible to AcNPV) used for coinfection. Symbols: ▲, single infection of BmNPV into TN-368 (A), SF-21 (B), and CLS-79 (C) and assayed on BmN; ●, single infection of AcNPV into TN-368 (A), SF-21 (B), and CLS-79 (C) and assayed on TN-368 (A), SF-21 (B), and CLS-79 (C); △, coinfection into TN-368 (A), SF-21 (B), and CLS-79 (C) and assayed on BmN; ○, coinfection into TN-368 (A), SF-21 (B), and CLS-79 (C) and assayed on TN-368 (A), SF-21 (B), and CLS-79 (C).

replication of BmNPV at 24 h p.i. and a 20-fold-higher replication at 48 h p.i. (Fig. 1C). Coinfection with BmNPV and either of the remaining three SINPVs (CC5, FC3, and OC12) produced no detectable increase in viral growth.

In the coinfection experiments using these three cell lines, increased viral growth of BmNPV in nonpermissive cell lines was observed only when AcNPV was used for coinfection. AcNPV and BmNPV were thus used for further experiments.

Replication after coinfection of AcNPV and BmNPV in various cell lines. The growth curves of AcNPV and BmNPV in single infections and coinfections were analyzed in detail by using cell lines TN-368, SF-21, and CLS-79. In TN-368, single infection with AcNPV showed a typical growth curve (Fig. 2A). Single infection with BmNPV in TN-368 showed no viral replication at 72 h p.i. (the titer was about 3×10^3 PFU/ml). When AcNPV and BmNPV were coinfecting, AcNPV showed a growth curve quite similar to that of single infection with AcNPV. This finding indicates no interfering or enhancing effects of BmNPV on the AcNPV replication upon coinfection. When the same coinfection supernatant was plaque assayed by using BmN cells, viral growth of BmNPV was also detected (Fig. 2A), as expected from the preliminary experiments (Fig. 1A). At 72 h p.i., coinfection produced a titer about a 500-fold higher than that obtained upon single infection with BmNPV.

When these coinfection experiments were repeated with SF-21, similar results were obtained (Fig. 2B). The viral growth curves of AcNPV in both single infection and coinfection were almost identical to each other. When the same coinfecting samples were plaque assayed on BmN, replication of BmNPV was about 200-fold higher than for BmNPV singly infected at 72 h p.i.

When CLS-79 was infected with the same combination of viruses, viral growth of AcNPV was observed in both single infection and coinfection. The growth of AcNPV coinfecting with BmNPV, however, was two- to fourfold lower than upon single infection with AcNPV, indicating interference by BmNPV in CLS-79. When the coinfection supernatant was plaque assayed on BmN, replication of BmNPV was

about 100-fold higher than upon infection with BmNPV alone at 72 h p.i.

These results showing increased BmNPV replication in all three nonpermissive (to BmNPV) cell lines lead to two possible hypotheses: (i) AcNPV provides a helper function for BmNPV replication in nonpermissive cells to BmNPV and (ii) the cultures contain a recombinant virus between AcNPV and BmNPV which possesses a wider host range (susceptible to both BmN and other cell lines). To test these hypotheses, pure clones from the coinfecting cultures were plaque purified and analyzed.

Restriction endonuclease analysis of the genomes of isolates from AcNPV- and BmNPV-coinfecting cultures. During the coinfection experiments described above, heterogeneity in plaque morphology of the coinfecting samples was observed. Plaques produced on BmN monolayers from the supernatant of SF-21 coinfecting cells at 72 h p.i. had the most easily recognized plaque heterogeneity (Fig. 3). This type of heterogeneity was not observed from the supernatants of SF-21 infected with only BmNPV. Furthermore, the numbers of heterogeneous plaques seemed to increase according to the time of incubation after infection. This phenomenon strongly suggests the appearance of new viruses, which were presumably produced by recombination between the BmNPV and AcNPV genomes.

To examine the possibility of recombination, seven isolates from three independent SF-21 cultures (S1, S2, and S3) coinfecting with BmNPV and AcNPV were randomly collected from separate plaques produced on a BmN cell monolayer. Viral DNAs of these isolates as well as the parent viruses were cleaved with *Eco*RI and electrophoresed on a 0.7% agarose gel. The patterns of all seven isolates were different from those of the parent viruses and from each other (Fig. 4), indicating that they were recombinant viruses. When the restriction fragment patterns of each of the isolates were compared with those of the parent viruses, some fragments were indistinguishable from or identical to that of BmNPV while some were identical to that of AcNPV. However, the overall patterns of the isolates were more similar to those of BmNPV than to those of AcNPV. For example, all of the cloned isolates had an identical *Eco*RI A or B fragment (20 to 21 kb) found only in BmNPV. No common fragment was found which corresponded only to an AcNPV fragment. Furthermore, the pattern of one isolate (S1-B) was almost identical to that of BmNPV except for two fragments (*Eco*RI C [14.5 kb] and *Eco*RI M [1.3 kb] of BmNPV). Also, the growth rate of AcNPV in the coinfecting SF-21 cultures when measured on SF-21 was about 10 times higher than that of BmNPV when measured on BmN. This finding implies that the recombination rate between the AcNPV and BmNPV genomes occurs at a high frequency (at least 10% of the produced viruses in the coinfecting SF-21 supernatant at 72 h p.i.) in BmNPV-nonpermissive cells.

Plaque purification of isolates with wider host range from coinfecting cultures. Since recombination seemed to be involved in increasing the titers of the isolates from the coinfecting cultures in nonpermissive cells, the host ranges of possible recombinant viruses were analyzed. To reduce the possibility of plaque purifying isolates with multiple recombinations, cultures collected during an early stage of infection (26 h p.i.) were used in the following experiments.

Viruses from BmNPV- and AcNPV-coinfecting cultures of TN-368, SF-21, and CLS-79 at 26 h p.i. were plaque purified and examined to determine whether they acquired a wider in vitro host range (in BmN and other cell lines). From TN-368 coinfecting cells, 48 clones were plaque purified on BmN

FIG. 3. Heterogeneous plaques produced on a BmN cell monolayer from BmNPV- and AcNPV-coinfected SF-21 culture fluid at 72 h p.i. Arrowheads indicate plaques indistinguishable from original BmNPV plaques. These plaques are about 2 mm in diameter.

cells. None of these isolates showed cytopathic effects (CPE) in TN-368. This finding indicates that the increase in viral titer detected on BmN after coinfection was mainly the result of a helper function from the coinfecting AcNPV rather than the appearance of recombinant viruses with wider host range. From a similar coinfecting culture of SF-21, 17 of 70 isolates plaque purified on BmN were able to show CPE and grow in SF-21 cells. Expansion of the *in vitro* host range of the 17 isolates was confirmed by repeated plaque purification of pure clones and reinfection using BmN and SF-21 cells. From a similar coinfecting culture of CLS-79, 2 of 48 clones plaque purified on BmN cells showed CPE in CLS-79 cells.

In all three coinfections at 26 h p.i., the viral titers increased at least 10-fold when detected on BmN cells; however, isolates with a wider host range comprised only 24% of the SF-21 isolates, 4% of the CLS-79 isolates, and 0% (less than 2%) of the TN-368 isolates. This finding strongly suggested that (i) titers of the coinfections at 26 h p.i. increased primarily as a result of a helper function of AcNPV and (ii) new viruses with expanded host range were also produced. These new viruses were most likely recombinant viruses between AcNPV and BmNPV and presumably played some role in increasing growth, especially late in infection. Four different isolates with expanded host range from SF-21 (S-7 and S2-2) and CLS-79 (C-8 and C-38) were chosen for further analysis.

Viral growth of isolates with wider host range in four established cell lines. The growth curves of the four newly

isolated viruses (S-7, S2-2, C-8, and C-38) with wider host range were examined in four different cell lines (BmN, TN-368, SF-21, and CLS-79), with the parent viruses, AcNPV and BmNPV, used as controls. Replication of the viruses was determined by plaque assay on BmN cells at the appropriate times p.i. On BmN cells, all of the isolates and BmNPV showed typical growth curves similar to each other (Fig. 5A). The viral titers of the recombinant viruses at 48 h p.i. were also similar to that of the parent, BmNPV T3. AcNPV infection on BmN cells, which was determined by plaque assay on TN-368, showed no viral replication, as expected from the previous results.

On TN-368, the shapes of growth curves of the parent AcNPV and the recombinant viruses S-7, S2-2, and C-38 were typical; however, C-38 and S2-2 showed growth rates 1/5 and 1/20, respectively, that of AcNPV (and S-7) at 72 h p.i. (Fig. 5B).

On SF-21, the S-7 and C-38 infections showed growth curves similar to that of AcNPV but about 10-fold lower (Fig. 5C). The C-8 and S2-2 infections showed very poor growth and reached only about 10^5 PFU/ml at 72 h p.i. However, CPE and viral replication were apparent.

On CLS-79, C-38 showed a typical growth curve similar to that observed in AcNPV infection albeit at a slightly lower rate (two- to sixfold) (Fig. 5D). S-7 showed a lower growth curve and reached only 1/50 that of AcNPV at 72 h p.i. S2-2 and C-8 did not replicate in CLS-79.

Restriction endonuclease analysis of the newly isolated

FIG. 4. Restriction endonuclease analysis of isolates plaque purified on BmN cells from a SF-21 culture coinfecting with BmNPV and AcNPV. Three independent cultures (S1, S2, and S3) were coinfecting with 5 PFU each of AcNPV and BmNPV per cell, and the culture fluid was collected at 72 h p.i. Purified viral DNAs of these isolates as well as the parent viruses, BmNPV and AcNPV, were cleaved with *EcoRI* and electrophoresed on a 0.7% agarose gel. S1-A and S1-B were isolated from the S1 culture, S2-A and S2-B were isolated from the S2 culture, and S3-A, S3-B, and S3-C were isolated from the S3 culture. Lane M, lambda DNA cleaved with *HindIII*. The size (in kilobases) of each lambda fragment is shown at the left.

viruses with wider host range. To analyze the genetic relatedness of the viruses with expanded host ranges, their genomic DNAs were extracted and their restriction patterns were compared. The DNAs of the four isolates with wider host range and the two parent viruses were digested with six endonucleases (*EcoRI*, *HindIII*, *PstI*, *BamHI*, *SmaI*, and *KpnI*) and electrophoresed on a 0.7% agarose gel (Fig. 6). When the *EcoRI* fragments of each of these recombinant viral DNAs were compared with those of AcNPV and BmNPV, the C-8 isolate had 6 bands indistinguishable from those of BmNPV and 11 bands indistinguishable from those of AcNPV (Fig. 6). The C-38 isolate had 8 bands indistinguishable from those of BmNPV and 14 bands indistinguishable from those of AcNPV; S-7 had 9 and 11, respectively, and S2-2 had 8 and 12. The patterns of the viral DNAs generated upon digestion with the other five endonucleases were between those of the parents, BmNPV and AcNPV, indicating that the new viruses were recombinant viruses between BmNPV and AcNPV. No submolar bands were detected, and most of the digested fragments belonged to either AcNPV or BmNPV. The restriction patterns of these viruses were unchanged after several passages in BmN cells (data not shown).

The restriction fragment patterns of recombinant viruses differed greatly from those of the parent viruses, AcNPV and BmNPV. A few fragments, however, were common to the four recombinant viruses; e.g., the *BamHI* 3.2- and 2.9-kb fragments were found in all recombinant viruses and AcNPV. To analyze the origins of the cleaved fragments of

FIG. 5. Viral replication of various recombinant isolates with wider host range in BmN (A), TN-368 (B), SF-21 (C), and CLS-79 (D). Cell monolayers in 60-mm dishes were infected at 5 PFU per cell with S-7 (○), S2-2 (△), C-8 (■), or C-38 (□) or with the parent virus AcNPV OT2 (●) or BmNPV T3 (▲). Viral titers were plaque assayed on BmN.

the recombinant viruses, each fragment was mapped by comparison with the corresponding fragment of the parents BmNPV T3 (16) and AcNPV OT2. The restriction pattern of OT2 was similar to those of AcNPV HR3 and AcNPV L-1 (4) (data not shown). Figure 7 maps the areas that each of the recombinants acquired from the parents for the various endonucleases. Although the mapping patterns of each of the four isolates were distinct, there were common areas corresponding to only one of the parent viruses in these recombinants. Positions around 22, 40, and 80 map units were derived from AcNPV, while positions around 62 and 98 map units were from BmNPV. The mapping patterns also indicated that recombination occurred in relatively large genomic areas (approximately 30 to 70% of the entire genome) and that recombination occurred more than once.

The location of the polyhedrin gene in these recombinant viruses was examined by Southern blot analysis using the polyhedrin gene of BmNPV as a probe. When *EcoRI*-digested fragments were hybridized with this probe, the 10.5-kb fragment of S-7, C-8, S2-2, and BmNPV T3 and the 7.2-kb fragment of C-38 and AcNPV were hybridized (data not shown). These results are consistent with the mapping results obtained by comparison of the restriction endonuclease patterns shown in Fig. 7.

In vivo host range analysis of the recombinant viruses. Recombinant viruses with wider host range were injected

FIG. 6. Restriction endonuclease analysis of various recombinant viruses with wider host ranges. Viral DNA was cleaved with *EcoRI*, *HindIII*, *PstI*, *BamHI*, *SmaI*, or *KpnI* and electrophoresed on a 0.7% agarose gel. Lane M, lambda DNA cleaved with *HindIII*. The size (in kilobases) of each lambda fragment is shown at the right.

into the body cavities of *B. mori* and *S. litura* larvae to examine host specificity in vivo. Injection rather than oral infection was initially chosen because of the poor production of PIBs in some isolates and to avoid any secondary effects of the complex process of viral transmission through the midgut tissues. After injection with the recombinant viruses, all fifth-instar *B. mori* larvae showed typical CPE and died similarly to BmNPV T3-injected larvae. Five days p.i. the hemolymph turned whitish, which is typical of PIBs produced and released from infected fat body cells after degradation of the tissues. Fewer polyhedra were produced by C-38 and S-7 infection in both *B. mori* larval tissues and

BmN cells than by C-8, S2-2, and wild-type BmNPV infection.

The same isolates failed to show typical CPE when injected into sixth-instar *S. litura* larvae; however, all larvae died by viral infection after pupation. AcNPV OT2 also showed minimal CPE in *S. litura*, probably because this insect is not the natural host of OT2 (17). Production of polyhedra in fat bodies was observed by light microscopy in all isolates. BmNPV showed no signs of viral infection in *S. litura* larvae.

Oral infectivity of each of the four recombinant viruses was also examined. When first-instar *B. mori* larvae and

FIG. 7. Mapping analysis of various recombinant viruses with wider host ranges. Open bars indicate fragments originating from the AcNPV OT2 genome, and shaded bars indicate fragments originating from the BmNPV T3 genome. The origins of the fragments were determined by comparison of the mobilities of the recombinant and parental viral DNA fragments in 0.7% agarose gels as shown in Fig. 6. The arrowhead indicates the location of the polyhedrin gene.

second-instar *H. virescens* larvae were infected per os with polyhedra of each of the recombinants propagated in BmN, more than 75% of the larvae of both species died of nuclear polyhedrosis within 10 days.

DISCUSSION

In our study of baculovirus host specificity, we showed a helper function of AcNPV for BmNPV replication in non-permissive cells and isolated recombinant baculoviruses with wider host range. These phenomena have not been reported in previous studies of baculovirus host specificity. Our studies were helped greatly by the use of viruses with sensitive assay systems (plaque assay) and by the use of cell lines susceptible to specific viruses. In the four coinfection combinations (BmNPV and one of the four distinct groups of SINPVs), only one (AcNPV and BmNPV), with high genome homology (17), showed a helper function of one virus for another.

The helper effect of AcNPV for BmNPV replication in cells nonpermissive to BmNPV indicates that viral products of AcNPV can be used as functional molecules effective for synthesis of progeny BmNPV. However, all tested BmNPV isolates which replicated in nonpermissive cells (SF-21) by the AcNPV helper function were not the original, wild-type BmNPV but recombinant viruses. These results suggest that wild-type BmNPV cannot replicate with only the helper function of the AcNPV products, while recombinant BmNPV containing the appropriate AcNPV DNA fragment(s) can replicate with the helper function of AcNPV. This finding indicates that *cis* activation in the recombined BmNPV genome may be involved in viral replication by the AcNPV helper function. *cis* activation has been shown to be an important process in AcNPV replication (7).

The results of the restriction endonuclease analysis showing various BmNPV and AcNPV recombinant viruses indicate (i) a high rate of recombination in the infected cell nucleus between these two viruses and (ii) the existence of numerous recombination possibilities between the two viruses, which allow viral replication. It has been shown that progeny viruses produced by coinfection with AcNPV and its variants can be used for mapping of the polyhedral protein and other viral structural genes by comparing their restriction endonuclease patterns and mobilities by SDS-polyacrylamide gel electrophoresis (19). Our data showed the extensive possibility for replacement of various genes (or regions) between two completely different NPVs. In preliminary experiments, the polyhedral protein of these viruses was analyzed by SDS-polyacrylamide gel electrophoresis. The polyhedral protein of C-8, S-7, and S2-2 migrated to the identical 29-kDa position of BmNPV, while the polyhedral protein of C-38 migrated to the same 31-kDa position of AcNPV. These data were consistent with the result of the hybridization experiments showing that of the four recombinant viruses, only C-38 had a polyhedrin gene fragment of the same size as AcNPV. The recombinant viruses seemed to have acquired the polyhedrin gene from one or the other of the parent viruses. Other genes related to phenotypical characterization between BmNPV and AcNPV can also be mapped in the viral genome by using a similar approach.

We also tried to isolate similar recombinant viruses with wider host range from BmN cells coinfecting with AcNPV and BmNPV; however, no recombinant viruses were obtained (data not shown). Furthermore, BmNPV replication in BmN seemed to be blocked by coinfection with AcNPV. Single infection of BmN with AcNPV also showed a unique

CPE despite its inability to replicate. These observations indicate that the mechanisms of expanded host range in our studies may be related to the elimination of the CPE of AcNPV toward BmN cells.

The recombinant virus S2-2 grew well in TN-368, very poorly in SF-21, and not at all in CLS-79. This type of host range specificity was not found in the screening of many isolates of *S. litura* NPV. In previous studies (17; Table 1), all the SINPV isolates, which could replicate in TN-368, could also replicate very rapidly in CLS-79 and SF-21. This finding indicates that the regions in the viral genome responsible for determination of host specificity are likely to be unique for different host cells. The restriction endonuclease patterns of the recombinant viruses with expanded host range possessed patterns quite different from those of their parents, BmNPV and AcNPV. Furthermore, the patterns of these viruses differed widely from each other. Preliminary analysis of the physical maps of the four recombinant viruses revealed that a common region related to host range expansion may span a relatively large area of the genome. By repetition of the recombination experiments and DNA analysis, we are currently attempting to map a specific region responsible for host range expansion.

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