JOURNAL OF VIROLOGY, Mar. 1992, p. 1442–1448
0022-538X/92/031442-07$02.00/0

Vol. 66, No. 3

Differential Expression of Hz-1 Baculovirus Genes during Productive and Persistent Viral Infections

YU-CHAN CHAO,[1]* H. ALAN WOOD,[2] CHERNG-YUI CHANG,[1,3] HAN-JONG LEE,[1] WEI-CHIANG SHEN,[1] AND HUNG-TU LEE[3]

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan 11529,[1] and Institute of Life Science, National Tsing Hua University, Hsinchu, Taiwan 30043,[3] Republic of China, and Boyce Thompson Institute for Plant Research, Tower Road, Ithaca, New York 14853[2]

Received 20 August 1991/Accepted 19 November 1991

Hz-1 viral RNA transcription was studied during productive and persistent infections. The RNAs were localized to 10- to 30-kb regions within the viral genome, and the timing of their expression was determined. During productive infections, we detected 101 virus-specific transcripts that could be grouped into three categories by time of appearance. At 2 h postinoculation (p.i.), a total of 34 virus-specific transcripts were detected. An additional 51 and 16 virus-specific transcripts appeared between 4 and 6 h p.i. and at 8 h p.i., respectively. After 8 h, no new transcripts were found. Under conditions of persistent infection, we detected only one viral persistency-associated transcript (PAT1). The region of the viral DNA which encodes PAT1 was cloned. During productive infections, three transcripts were derived from this region. Each had the same polarity as PAT1. One of them was of the same size as PAT1 and had similar, if not identical, 3′ and 5′ ends. This report provides detailed and very useful information concerning sequentially expressed transcripts of the Hz-1 baculovirus.

The nonoccluded Hz-1 virus belongs to subgenus C of the family *Baculoviridae*. It was isolated from persistently infected IMC-Hz-1 tissue culture cells by Granados and coworkers (9). The Hz-1 viral genome is a 228-kb covalently closed circular double-stranded DNA molecule (5, 6, 10). This virus can establish both persistent and productive infections in several lepidopteran insect cell lines. Once a persistent infection is established, the insect cells are resistant to superinfection with homologous and heterologous baculoviruses (2, 13, 16, 18).

Defective virus particles containing genomic deletions have been observed in some Hz-1 viral isolates (5, 6, 10). Burand et al. (5) showed that defective virus particles are generated following serial passage of the standard virus in vitro and are required for establishment of persistent infections. However, standard virus was needed as a helper virus for replication of defective virus particles. Defective virus particles, in turn, have been shown to interfere with infection and replication of standard virus particles. Thus, these defective viruses were termed defective interfering particles (4).

Hz-1 viral replication in cell culture has been studied (3, 16). Infectious extracellular virus is first detected 12 h postinoculation (p.i.) (3, 16). At this time, virus-infected TN368 cells appeared round and 20 to 30% of the cells were lysed, indicating that the complete replication cycle of the Hz-1 virus matures before 12 h (3). Studies of DNA replication and virus-induced protein synthesis have been conducted (4, 5). On the basis of the appearance of the viral proteins, the replication cycle of the virus was divided into three stages: early (0 to 4 h p.i.), intermediate (4 to 8 h p.i.), and late (8 to 13 h p.i.) (3). A physical map of the Hz-1 genome has recently been constructed with five restriction enzymes. With this map, the regions of the genome deleted in some defective interfering particles have been identified (6).

Persistent viral infection in insects is suspected to be a common phenomenon. However, very little research has been conducted in this area (2, 15). To elucidate the nature of persistent and productive viral infections, we investigated the regulation of viral transcription during persistent and productive infections. The data in this report identified approximately 100 Hz-1 viral transcripts under conditions of productive replication, whereas only a single major viral transcript was detected in persistently infected cells. This transcript was found to have a constitutively expressed early RNA analog transcribed during productive viral replication. This is the first report on the persistency-associated gene in insect virology. In addition, it is a key discovery in uncovering the mysterious mechanisms of persistent baculoviral infection.

MATERIALS AND METHODS

Persistently infected cells. The *Trichoplusia ni* TN368 and *Spodoptera frugiperda* SF21 cell lines were maintained at 26°C in modified TNMFH medium as described by Burand et al. (4). Standard virus was plaque purified from an Hz-1 B1 viral isolate (5). The persistently infected *S. frugiperda* SFP2 and *T. ni* TNP3 cell lines were established as described by Burand et al. (4). Ninth-passage SFP2 and 175th-passage TNP3 cells were used in this study.

Isolation of total RNAs. SF21 tissue culture cells were infected with Hz-1 standard virus as described by Burand and Wood (4). A total of 4×10^7 SF21 cells were dispensed into a six-well culture plate, inoculated with 10 PFU of standard virus per cell, and incubated at 26°C. The cells were harvested at various times by being scraped into phosphate-buffered saline and pelleted by centrifugation. Cells were suspended in disruption buffer (5 M guanidinium isothiocyanate, 20 mM Tris hydrochloride [pH 7.4], 1 mM EDTA, and 8.8 μl of β-mercaptoethanol per ml) at 1.15 ml per plate. An

* Corresponding author.

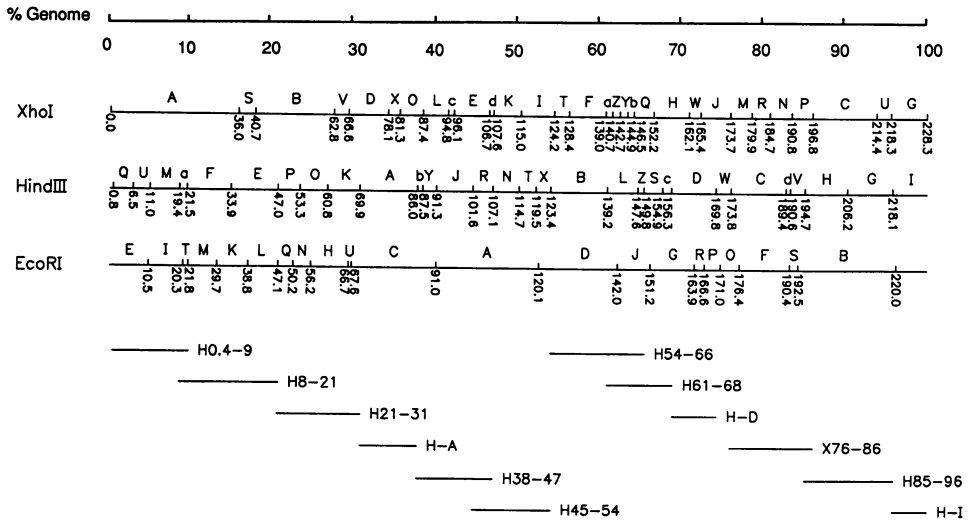

FIG. 1. Physical map of the viral genome and coordinates of the probes used for Northern hybridizations. The probes used included H-A, H-D, H-I, H0.4-9, H8-21, H21-31, H38-47, H45-54, H54-66, H61-68, H85-96, and X76-86. H-A, H-D, and H-I were viral *Hin*dIII A, D, and I fragments. The remaining probes were cosmid clones containing viral inserts. The H and X notations indicate that those clones were derived from *Hin*dIII and *Xho*I cosmid libraries, respectively. Numbers after H or X denote the coordinates (%genome) that that particular clone spans. The circular viral genome was linearized at the junction of *Xho*I fragments A and G for ease of presentation (6). Restriction maps for three enzymes, *Xho*I, *Hin*dIII, and *Eco*RI, are included. Numbers under the individual restriction sites of the physical map are cumulative sizes, in kilobases, of the viral genome.

equal volume of 5 M cesium chloride was then added, and the mixture was layer onto a 0.5-ml volume of a cesium chloride solution containing 5 M cesium chloride, 20 mM Tris-hydrochloride (pH 7.4), and 1 mM EDTA. The samples were centrifuged in a Beckman SW60 rotor at 40,000 rpm for 16 h at 20°C. The pelleted RNAs were dissolved in diethyl pyrocarbonate-treated water. Total RNA was also isolated from persistently infected SFP2 and TNP3 cells as described above.

Agarose gel electrophoresis and Northern (RNA) blotting. Total cellular RNA samples were incubated at 50°C for 1 h in 1 M glyoxal (Sigma Chemical Co., St. Louis, Mo.)–10 mM $NaPO_4$ (pH 6.5). Samples containing 3 μg of total RNAs were fractionated by electrophoresis through 1% agarose gels containing 10 mM $NaPO_4$, pH 6.5, with continuous buffer circulation and transferred to GeneScreen (NEN Research Products, Inc., Boston, Mass.) nylon membrane filters by capillary action. Equal amounts of RNA samples were confirmed by identical ethidium bromide staining of rRNA.

Preparation of isotopically labeled probes. Cosmid clones containing viral DNA fragments (6) were used as probes (Fig. 1). Viral fragments which are not found in cosmids, the *Hin*dIII A, D, and I fragments, were isolated following agarose gel fractionation of the *Hin*dIII-restricted viral genome. Cosmids, isolated fragments, and total viral DNA were [α-^{32}P]CTP radiolabeled by using random primers as described by Feinberg and Vogelstein (8). Strand-specific, single-stranded (ss) RNA probes were synthesized from pBluescript plasmid KSM+ (Stratagene Cloning System, Inc., La Jolla, Calif.) containing viral inserts as described by Melton and coworkers (14).

Hybridization of Northern blots. Northern blotted filters were prehybridized for 4 h at 42°C and then hybridized with random-primer-labeled DNA or strand-specific RNA probes. The prehybridization and hybridization solutions contained 50% formamide, 1 M NaCl, 1× Denhardt's solution, 0.1 mg of calf thymus DNA per ml, 50 mM Tris-HCl (pH 7.5), 0.1% $Na_4P_2O_7$, 1% sodium dodecyl sulfate (SDS), 1 mM EDTA, and 10% dextran sulfate. After hybridization, the filters were washed twice at room temperature in 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate) containing 0.1% SDS and then 0.1× SSC containing 0.1% SDS at 65°C for 30 min. The filters were air dried, and autoradiograms were prepared by exposure to XAR-5 film at −70°C with an intensifying screen.

RNase protection assay. Total RNAs (10 μg) purified from either productively or persistently infected cells were hybridized to the B and C + F ssRNA antisense probes (see Fig. 5). They were digested with RNase A and T1 as described by Ausubel et al. (1). The protected fragments were fractionated through 1% agarose and detected by autoradiography.

DNA purification and hybridization. Purification of both viral and cellular DNAs (SF21, TN368, SFP2, and TNP3) was performed as described by Chao et al. (6). Five micrograms of each of these DNAs was digested with restriction enzyme *Eco*RI and fractionated through a 1% agarose gel. This gel was blotted onto a GeneScreen nylon membrane filter and hybridized with ^{32}P-labeled standard viral DNA as described previously (6).

RESULTS

Mapping of the virus-specific transcript. Since some Hz-1 viral transcripts may not be polyadenylated (11, 12), total RNAs were isolated at 2, 4, 6, 8, 10, and 12 h p.i. of SF21 cells. Total cellular RNAs were also isolated from SFP2 and TNP3 cells persistently infected with the Hz-1 virus. Northern blots (Fig. 2) of these RNA samples were probed with labeled DNA fragments (6) covering 98% of the Hz-1 viral genome, as illustrated in Fig. 1.

During productive viral infection, a total of 101 virus-specific transcripts which differed in expression pattern,

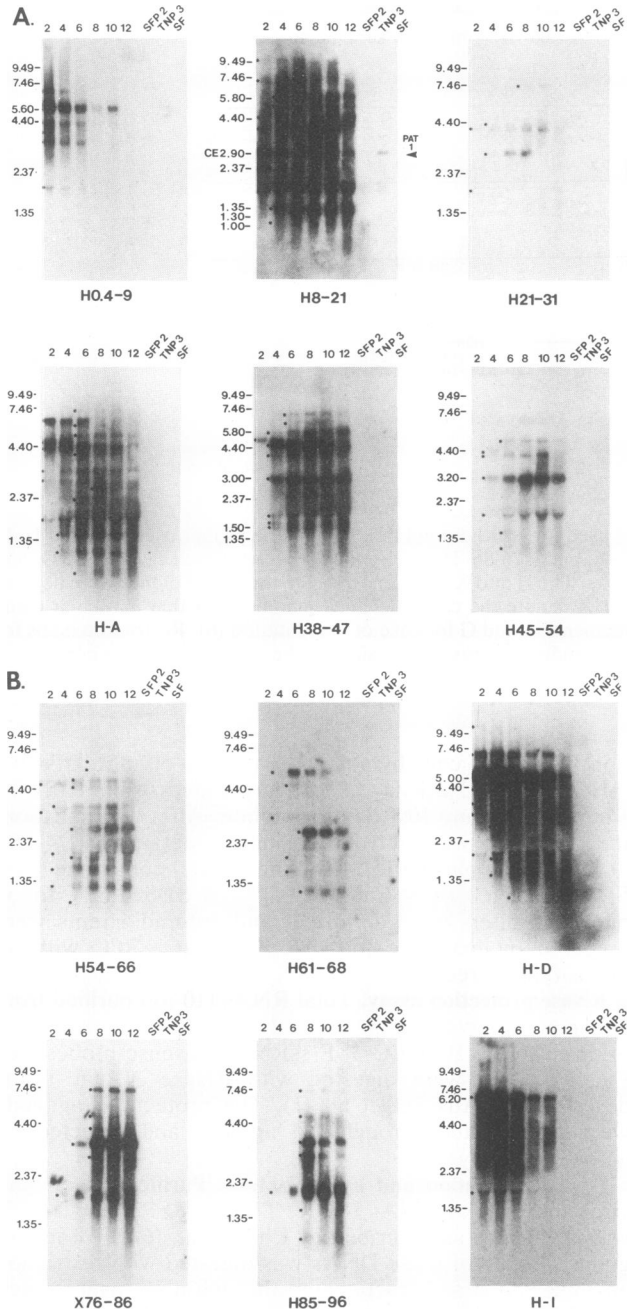

FIG. 2. Northern blot hybridizations with subgenomic probes. The probe used is indicated under each panel. Each panel was composed as follows. Total RNAs were extracted from the infected SF21 cell line 2, 4, 6, 8, 10, and 12 h after productive viral infection, as indicated over the first six lanes of each panel. Total RNAs were also extracted from two persistently infected cell lines, SFP2 and TNP3, for comparison. The last lane was the total RNA extracted from the uninfected SF21 cell line as a control. Each blot was hybridized by ^{32}P-labeled probes with the same total count and similar specific activities. All blots were exposed for 20 h, except the blot hybridized by the probe derived from the cosmid H0.4-9 insert. Since no new bands were detected after 20 h of exposure, this filter was exposed for 10 h for better visualization of the weaker bands. The onset (earliest detection time) of each transcript is marked with a dot. Molecular size standards are shown on the left of each panel in kilobases. PAT1 is indicated by an arrowhead.

location, and size were estimated. Approximately a third of the transcripts were expressed transiently. Thirty-four virus-specific transcripts were detected at 2 h p.i. The most notable transcripts were the 5.0-kb RNA detected in the *Hin*dIII-D (H-D) region, the 6.2-kb RNA detected in the *Hin*dIII-I (H-I) region, and the 5.6-kb RNA detected in the H0.4-9 cosmid viral insert region (Fig. 2). These RNAs were highly expressed at 2 h p.i.; however, only small amounts of these transcripts were detectable at 12 h p.i. At 4, 6, and 8 h p.i., 30, 21, and 16 additional viral transcripts, respectively, were detected. No new transcript was detected after 8 h p.i. The sizes of the detected viral RNAs ranged from 0.8 kb to larger than 9.5 kb.

The pattern of the expressed RNAs in Fig. 2 illustrates that the Hz-1 viral genome has predominant early and late gene regions. At 2 h p.i., only three transcripts were detectable in the region between map units 38 and 68. All of the other transcripts were transcribed after 2 h p.i. This region covers the viral inserts of the H38-47, H45-54, H54-66, and H61-68 cosmids. In addition, transcripts detected in the region between map units 76 and 96, including viral inserts of the X76-86 and H85-96 cosmids, were predominantly late. Only three different transcripts from this region were detected before 8 h p.i. All of the remaining transcripts were detected after 6 h p.i. Most transcripts from the remainder of the genome, which includes the regions between 68 and 74 (H-D), 96 and 100 (H-I), and 0 and 38 (including H0.4-9, H8-21, H21-31, and H-A) map units, were predominantly early. For those transcripts that appeared at 2 h p.i. (for example, the 5.6-, 5.0-, and 6.20-kb RNAs from the H0.4-9, H-D, and H-I cosmids, respectively), the levels detected always decreased gradually throughout the infection cycle.

During persistent viral infection, only one virus-specific transcript was detected. It hybridized to the H8-21 probe (Fig. 2A). This transcript, termed persistency-associated transcript 1 (PAT1), is a 2.9-kb RNA species detected in total RNAs isolated from persistently infected SFP2 and TNP3 cells. The size of PAT1 corresponds to a constitutively expressed (CE) 2.9-kb (CE2.90) transcript detected during 2 to 12 h p.i. (Fig. 2A; see also Fig. 4B). To detect additional viral transcripts in these persistently infected cells, total viral DNA was labeled and used to probe the same Northern blots. The 2.9-kb PAT1 RNA was still the only transcript detected in the persistently infected cell lines (Fig. 3).

Strand-specific RNA probe hybridizations. The H8-21 cosmid, which hybridized to PAT1, contains *Hin*dIII a, F, and E fragments (Fig. 1 and 4A). Further experiments revealed that only the probe from *Hin*dIII-F, but not the flanking *Hin*dIII a or E fragment, hybridized to both the CE 2.9-kb and PAT1 RNAs (data not shown). The *Hin*dIII F fragment was further mapped and found to contain 4.2-kb *Hin*dIII-*Eco*RI, 7.3-kb *Eco*RI-*Eco*RI, and 0.3-kb *Eco*RI-*Hin*dIII DNA fragments (Fig. 4A). The 4.2-kb *Hin*dIII-*Eco*RI fragment did not hybridize to the CE 2.9-kb or PAT1 RNA (data not shown). The 7.3-kb *Eco*RI-*Eco*RI and contiguous 0.3-kb *Eco*RI-*Hin*dIII fragments in the original viral genomic order, were subcloned into the pBluescript vector. The resulting plasmid was named pHzE-M (Fig. 4A). Labeled strand-specific ssRNA transcripts from both strands of this cloned viral DNA in plasmid pHzE-M were synthesized in vitro by using T3 and T7 bacteriophage RNA polymerases. The probes transcribed by the T3 and T7 polymerases were arbitrarily designated plus- and minus-strand transcripts, respectively (Fig. 4A). The plus- and minus-strand RNA probes were hybridized separately to two identical

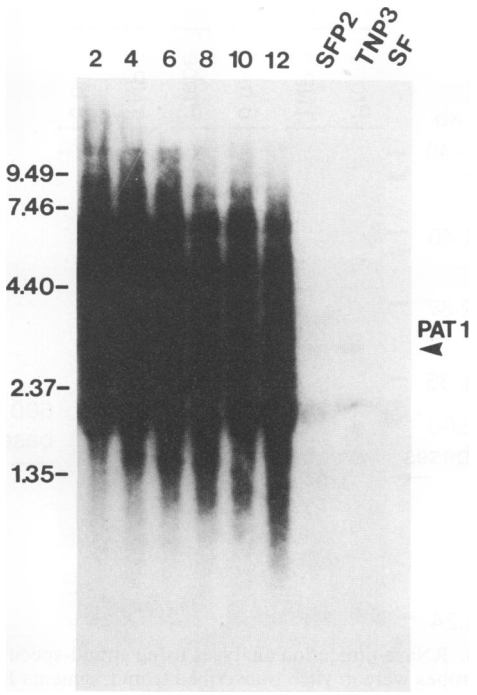

FIG. 3. Northern analysis using entire Hz-1 viral DNA as a probe. A Northern blot identic to that in Fig. 2 was probed. PAT1 is indicated by an arrowhead.

Northern blots. Only the plus-strand probe hybridized to the CE 2.9-kb and PAT1 RNAs (Fig. 4B and data not shown). These results indicate that these two transcripts have the same direction.

The viral DNA insert in plasmid pHzE-M was further mapped to identify more precisely the region from which the CE 2.9-kb and PAT1 RNAs were transcribed. Six viral restriction fragments, A, B, E, D, C, and F, were generated from pHzE-M by using restriction enzymes *Eco*RI and *Kpn*I. These fragments were subcloned into pBluescript separately. ^{32}P-labeled ssRNAs antisense to both the CE 2.9-kb and PAT1 RNAs were transcribed in vitro from all of these subclones and used to probe Northern blots (Fig. 5). Northern blotting was performed with total RNA samples harvested from productively infected SF21 cells at 8 h p.i., the persistently infected TNP3 cell line, and healthy SF21 control cells. ssRNA probes derived from fragments B, E, D, and C + F hybridized to both the CE 2.9-kb and PAT1 RNAs; those from fragments A and F did not (Fig. 5B). These results further refined the previous data and indicated that during productive and persistent infections, both the CE 2.9-kb and PAT1 RNAs are transcribed from the *Kpn*I-*Kpn*I B fragment to the *Kpn*I-*Eco*RI C fragment (Fig. 5B; see also Fig. 7).

It was notable that the ssRNA plus-strand probe transcribed in vitro from the pHzE-M plasmid (Fig. 4A) hybridized to abundant multiple viral transcripts on all RNAs derived from productive viral infections (Fig. 4B, lanes 2 to 12). Similar dark, abundant, multiple viral transcripts were also detected by using a probe transcribed in vitro from subcloned fragment A (Fig. 5B, fragment A, lane 1), but few or none were detected with probes derived from the subcloned B, E, D, C + F, and F fragments (Fig. 5B), revealing that multiple transcripts must be transcribed across fragment

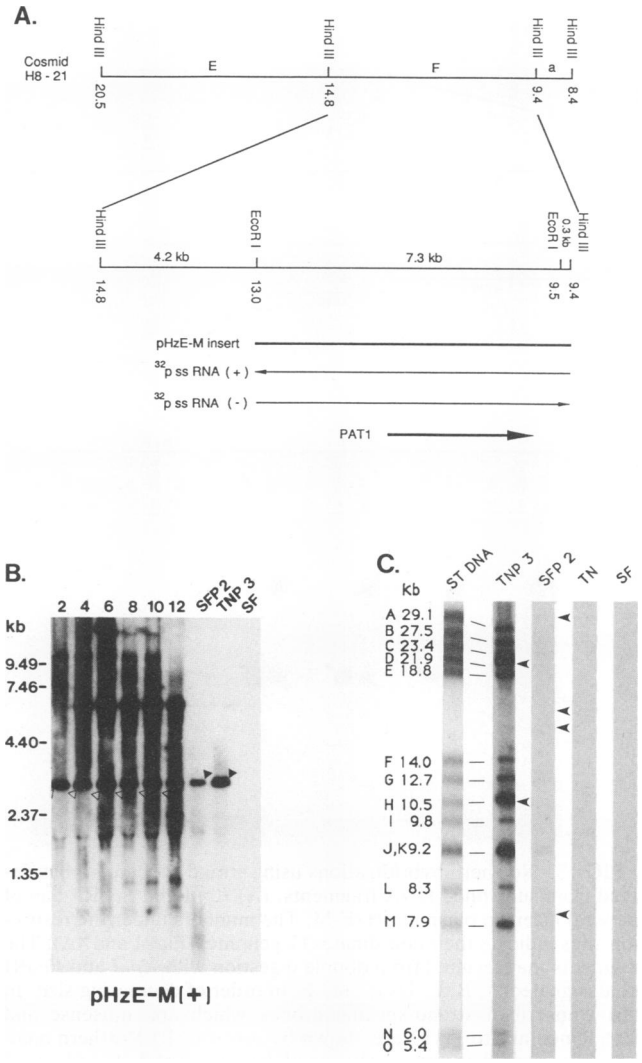

FIG. 4. (A) Genomic map of the PAT1-coding region. The numbers under the lines are the coordinates (%genome) of the restriction sites. Cosmid clone H8-21, which hybridized to PAT1, contains *Hin*dIII fragments E, F, and a. An *Eco*RI-*Hin*dIII fragment spanning the region between map units 9.4 and 13.0 was subcloned into pBluescript KSM+ to produce plasmid pHzE-M. The plus- and minus-strand ^{32}P-labeled ssRNA probes synthesized from this plasmid are indicated. The orientation of PAT1 and the region it covers are also illustrated. (B) Northern analysis using the ssRNA probe. A Northern blot identical to that in Fig. 2 was hybridized with the plus-strand ssRNA [pHzE-M(+)] probe, as indicated in Fig. 4A. CE 2.9-kb RNAs are marked with empty arrowheads, and PAT1 RNAs are marked with filled arrowheads. (C) Southern analysis of the *Eco*RI-digested genomic DNAs of TNP3, SFP2, TN368 (TN), and SF21 (SF) cells using ^{32}P-labeled standard viral DNA as the probe. Extra or stronger bands are indicated by arrowheads. ST DNA stands for standard viral DNA which was ^{32}P labeled in vivo. After radioisotope labeling, the viral DNA was purified, digested with *Eco*RI, fractionated through the agarose gel, and exposed on an X-ray film as previously described (6).

A and most of them were terminated before fragment B, because these transcripts were not evident when ssRNA probes derived from fragments other than A were used. Such abundant multiple transcripts created a dark background,

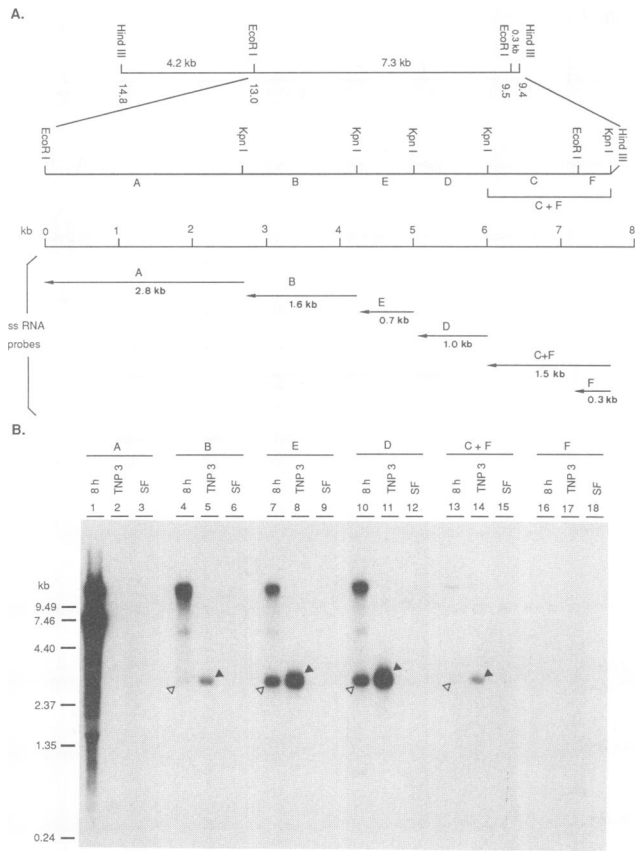

FIG. 5. Northern hybridizations using strand-specific probes derived from subcloned DNA fragments. (A) *Kpn*I and *Eco*RI map of the viral insert in plasmid pHzE-M. The numbers under the restriction sites indicate their coordinates (%genome) (Fig. 1 and 4A). The fragments that resulted from double digestion with *Kpn*I and *Eco*RI are designated A, B, C, D, E, and F, in order of decreasing size. In vitro-transcribed strand-specific probes which are antisense and complementary to PAT1 are shown by arrows. (B) Northern analyses using strand-specific probes as shown in panel A. Six identical Northern blots were probed. From left to right, each blot contained total RNA extracted from the productively infected SF21 cell line at 8 h p.i. (8 h), the persistently infected TNP3 cell line (TNP3), and an uninfected SF21 cell line (SF), as a control. The RNAs were fractionated through agarose gels and hybridized with strand-specific probes as shown at the top of each blot. CE 2.9-kb RNA is indicated with open arrowheads, and PAT1 is indicated by filled arrowheads.

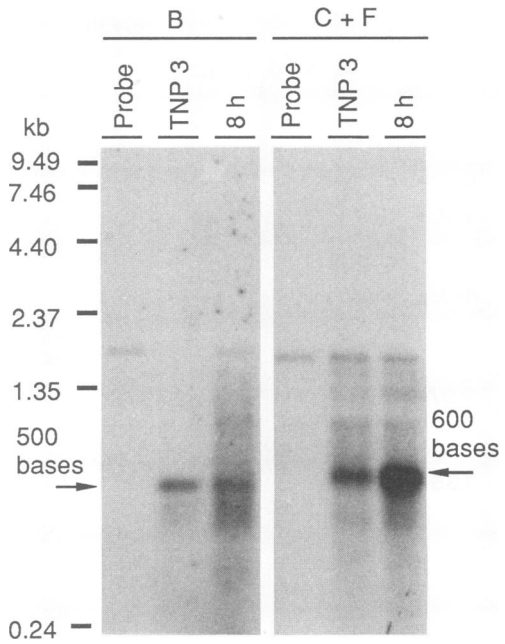

FIG. 6. RNase protection analyses using strand-specific probes. ssRNA probes were in vitro transcribed from fragments B and C + F as described in the legend to Fig. 5A. Lanes labeled TNP3 and 8 h represent the protected B and C + F ssRNA probes using total RNAs from TNP3 cell RNA and RNA extracted at 8 h p.i. from uninfected SF21 cells, respectively. The sizes of the major protected fragments were indicated.

making identification of other discrete bands difficult (Fig. 5B, fragment A, lane 1).

During productive viral infection, distinct 6.6- and >9.5-kb transcripts were detected with ssRNA probes derived from subcloned fragments B, E, D, and C + F (Fig. 5). RNase protection experiments using fragment C + F as a probe indicated that all three virus-specific RNAs, the >9.5-, 6.6-, and CE 2.9-kb transcripts which were detected during productive viral infection, and PAT1, which was the only virus-specific RNA species detected during persistent viral infection, were coterminated at adjacent, if not identical, 3′ sites (Fig. 6 and 7). In each case, only single major bands were protected from total RNAs extracted from both productively and persistently infected cells, and the sizes of the major protected bands from both phases were the same (Fig. 6). The 3′ termination site was calculated to be in the C fragment, 600 bases from the junction between fragments D and C (Fig. 7). By using ssRNA derived from fragment B, the 5′ ends of the CE 2.9-kb and PAT1 RNAs were mapped to the B fragment, 500 bases away from the junction between fragments B and E (Fig. 6 and 7). On the basis of these data, both the CE 2.9-kb and PAT1 RNAs are obviously transcripts derived from the same gene.

The >9.5-kb transcript is longer than the viral insert in plasmid pHzE-M; therefore, its 5′ end is likely to be situated upstream from the A fragment. The dark mutiple transcripts in the A fragment region (Fig. 5B, fragment A, lane 1) render mapping of the 5′ terminus of the 6.6-kb transcript difficult.

Detection of Hz-1 viral DNA in persistently infected cell lines. The existence of viral DNA in persistently infected

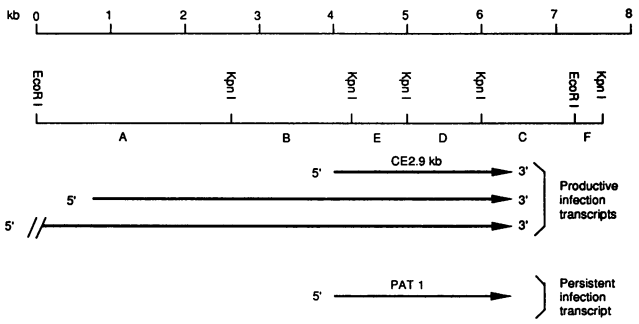

FIG. 7. Summary of the data in Fig. 5 and 6. Transcripts in the PAG1 region detected during productive and persistent infections are shown. The orientations of the transcripts are indicated.

host cells was examined. Total genomic DNAs from SFP2, TNP3, SF21, and TN368 cells were purified and digested with restriction enzyme *Eco*RI. They were then fractionated through an agarose gel and blotted onto a GeneScreen filter. Southern hybridization revealed that persistently infected cell lines SFP2 and TNP3 both contained viral DNA, whereas healthy control cell lines SF21 and TN368 did not. The amounts of Hz-1 viral DNA in these two lines differed. Not only did the persistently infected cells contain standard viral DNA, but the emergence of extra or stronger bands on the Southern blot (Fig. 4C) indicated that they may also have contained deleted viral DNA (5, 6).

DISCUSSION

We have identified the major Hz-1 viral transcripts in both the productive and the persistent viral infection phases. Individual restricted fragments isolated from either viral DNA or cosmid clones which represent 98% of the viral genome were used as probes to assign viral transcripts to regions of 10 to 30 kb of viral DNA. Since the sizes of the viral transcripts ranged from 0.8 kb to larger than 9.5 kb, it is possible that some transcripts, especially the larger ones, hybridized to adjacent DNA probes.

The temporal expression patterns for most transcripts are well defined. Three distinct classes of transcripts are produced 2, 4 to 6, and 8 h after productive viral infection. These classes may correspond to different temporal gene classes. Burand et al. (3, 4) examined the synthesis of Hz-1 virus-specific intracellular and structural proteins. On the basis of the viral growth cycle, the pattern of DNA synthesis, and the appearance of these proteins, the replication of Hz-1 virus was divided into the following three stages: (i) the early stage, 0 to 4 h p.i.; (ii) the intermediate stage, 4 to 8 h p.i.; and (iii) the late stage, which includes all virus-specific events occurring after 8 h p.i. Although it is not easy to correlate the appearance of tremendous amounts of viral proteins and mRNAs, the appearance of classes of discrete RNA or protein species was evident. On the basis of the sequential appearance of discrete viral transcripts, we propose that those transcripts that begin to be detectable before 2 h p.i. be referred to as early transcripts, those that appear after 2 h p.i. but before 6 h p.i. be referred to as intermediate transcripts, and those that appear after 6 h p.i. be referred to as late transcripts.

Early and late transcripts of *Autographa californica* nuclear polyhedrosis virus have been mapped by Erlandson and Carstens (7) and Vlak and Van Der Krol (17). Since viral DNA replication of *A. californica* nuclear polyhedrosis virus occurs at 6 h p.i., transcripts detected before this time were referred to as early and those detected after this time point were referred to as late. At the early stage, although specific regions were predominantly transcribed, transcripts from several regions of the *A. californica* nuclear polyhedrosis virus genome were found. After the onset of viral DNA synthesis, predominant cytoplasmic transcripts represented more dispersed areas of the genome, particularly those regions which were only weakly transcribed early p.i. Similar, although more localized, transcription patterns were evident in Hz-1 virus. It is notable that a continuous 30-kb viral genomic region is covered by viral *Hin*dIII fragment I (H-I) and the cosmid H0.4-9 insert, which gives rise only to transcripts that are initiated within 2 h p.i. and persist to later times. These transcripts may correspond to the immediate-early or early gene products. No new transcripts were detected at later times, even after prolonged exposure. Regions of late transcription were spread more widely than those of early transcription. These late regions of the Hz-1 viral genome were detected primarily in viral inserts covered by cosmid clones H85-96, H54-66, H61-68, and X76-86.

Our data suggest that the CE 2.9-kb and PAT1 RNAs are very similar transcripts, because they have the same size and direction and share similar 5′ and 3′ ends. However, the CE 2.9-kb RNA is an early gene product out of the PAT1-encoding region during productive viral infection. More interestingly, the CE 2.9-kb RNA is the only virus-specific RNA species that was detected at a constant level from 2 to 12 h during productive viral infection (Fig. 2A and 4B).

It is interesting that the two persistently infected cell lines, SFP2 and TNP3, may contain both standard and deleted viral DNAs. Although the amounts and patterns of the viral DNAs are different in these two lines, both of them contain the viral *Eco*RI M fragment, the region that encodes PAT1. Therefore, this evidence strongly suggests that there are viral template sources for PAT1 transcription in persistently infected insect host cells.

All of the above observations indicate that PAT1 is a unique transcript and may play a role in the establishment and/or maintenance of persistent viral infection. It may also be responsible for the viral resistance observed in persistently infected cells (2). Further studies are needed to explore these possibilities.

ACKNOWLEDGMENTS

We thank Show-Shan Sheu and Meng-Chi Chang for excellent technical assistance and A. L. Wang, L. T. Chow, S. R. Roffler, and G. W. P. Joshua for critical review of the manuscript.

This work was supported by grant NSC 79-0203-B001-15, Molecular Basis of Cellular Responses to Stress, from the National Science Council, Taiwan, Republic of China.

REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology, p. 4.7.1–4.7.8. John Wiley & Sons, Inc., New York.
2. **Burand, J. P., C. Y. Kawanishi, and Y. S. Huang.** 1986. Persistent baculovirus infections, p. 159–175. *In* R. R. Granados and B. A. Federici (ed.), The biology of baculovirus. CRC Press, Inc., New York.
3. **Burand, J. P., B. Stiles, and H. A. Wood.** 1983. Structural and intracellular proteins of the nonoccluded baculovirus Hz-1. J. Virol. **46**:137–142.
4. **Burand, J. P., and H. A. Wood.** 1986. Intracellular protein synthesis during standard and defective Hz-1 virus replication. J. Gen. Virol. **67**:167–173.
5. **Burand, J. P., H. A. Wood, and M. D. Summers.** 1983. Defective particles from a persistent baculovirus infection in *Trichoplusia ni* tissue culture cells. J. Gen. Virol. **64**:391–398.
6. **Chao, Y. C., M. Hamblin, and H. A. Wood.** 1990. Physical map of Hz-1 baculovirus genome from standard and DI particles. J. Gen. Virol. **71**:1265–1270.
7. **Erlandson, M. A., and E. B. Carstens.** 1983. Mapping early transcription products of *Autographa californica* nuclear polyhedrosis virus. Virology **126**:398–402.
8. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA to high specific activity. Anal. Biochem. **132**:6–13.
9. **Granados, R. R., T. Nguyen, and B. Cato.** 1978. An insect cell line persistently-infected with a baculovirus-like particle. Intervirology **10**:309–317.
10. **Huang, Y.-S., M. Hedberg, and C. Y. Kawanishi.** 1982. Characterization of the DNA of a nonoccluded baculovirus, Hz-1V. J. Virol. **43**:174–181.
11. **Jun-Chuan, Q., and R. F. Weaver.** 1982. Capping of viral RNA

in cultured *Spodoptera frugiperda* cells infected with *Autographa californica* nuclear polyhedrosis virus. J. Virol. **43:** 234–240.

12. **Lübbert, H., and W. Doerfler.** 1984. Mapping of early and late transcripts encoded by the *Autographa californica* nuclear polyhedrosis virus genome: is viral RNA spliced? J. Virol. **50:**497–506.
13. **McIntosh, A. H., and C. M. Ignoffo.** 1981. Establishment of a persistent baculovirus infection in a lepidopteran cell line. J. Invertebr. Pathol. **38:**395–403.
14. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage Sp 6 promoter. Nucleic Acids Res. **12:**7035–7056.
15. **Podgwaite, J. D., and H. M. Mazzone.** 1986. Latency of insect viruses. Adv. Virus Res. **31:**293–320.
16. **Ralston, A. L., Y.-S. Huang, and C. Y. Kawanishi.** 1981. Cell culture studies with the IMC-Hz-1 nonoccluded virus. Virology **115:**33–44.
17. **Vlak, Y. M., and S. Van Der Krol.** 1982. Transcription of the *Autographa californica* nuclear polyhedrosis virus genome: location of late cytoplasmic mRNA. Virology **123:**222–228.
18. **Wood, H. A., and J. P. Burand.** 1986. Persistent and productive infections with the Hz-1 baculovirus. Curr. Top. Microbiol. Immunol. **131:**119–134.